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THE UNIVERSITY OF ALBERTA

STUDIES ON BETA-LACTAM ANTIBIOTICS

BY

KAZUHARU NOGUCHI

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy.

IN

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled STUDIES ON BETA-LACTAM ANTIBIOTICS submitted by KAZUHARU NOGUCHI in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in PHARMACEUTICAL SCIENCES (Medicinal Chemistry).

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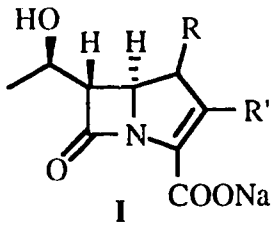
ABSTRACT

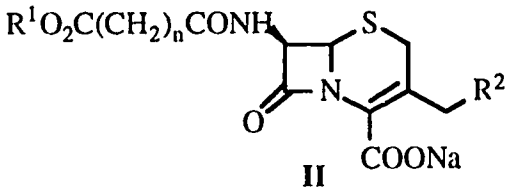
The carbapenem class of β-lactam antibiotics is one of the most promising classes of antimicrobial agents because of its exceptionally high efficacy against a wide range of bacteria. In search of a new carbapenem derivative with an improved profile, studies were conducted towards the syntheses of the 1-substituted carbapenem derivatives (I). Two approaches were employed for the most crucial step in the carbapenem synthesis, the construction of the carbapenem nucleus. One was by the carbene insertion reaction to form a C_3-N_4 connection and another was by Wittig-type reaction to form a C_2-C_3 connection. Attempts were made to introduce several heterocyclic groups into the 1-position of the carbapenem nucleus, however, no carbapenem derivative with a 1-heterocylic substituent was successfully synthesized. During these studies, 1-unsubstituted, 1-α-phenyl and 1-α-methyl carbapenem derivatives (**I**, R=H, α-Ph, α-Me, R'=SPh) were synthesized and their antimicrobial activity was tested. The 1-α-methyl derivative was found to be less active than the unsubstituted derivative and the 1-α-phenyl substituted derivative was found to be even less active than the 1-α-methyl derivative.



I

As a second part of the research project, penicillin and cephalosporin derivatives with an extra carboxyl group at the C_6/C_7 side chain were synthesized as possible cephalosporinase inhibitors and their biological activity was assessed. In general, the malonamates (n=1) were found to be

the strongest inhibitors of the cephalosporinase, the oxamates (n=0) to be the next strongest and the succinamates (n=2) to be the weakest inhibitors in both cephalosporin (**II**) and penicillin derivatives (**III**). The penicillin derivatives showed stronger inhibition than the corresponding cephalosporin derivatives. Introduction of a dithiolanylidyl group at the methylene position of the penicillin malonamate (**IV**) resulted in a dramatic increase in cephalosporinase inhibitory activity. Penicillin sulfones (**III** and **IV**, m=2) showed a diminished inhibition compared to the corresponding penams (**III** and **IV**, m=0). All the penicillin and cephalosporin derivatives showed very weak, if any, inhibitory activity against *Bacillus cereus* penicillinase and TEM2 enzyme. 6-*C*-Substituted penicillins (**V**) were also synthesized. Selected compounds were tested against β-lactamase-producing intact microbes in combination with ampicillin or cephalothin for the evaluation of their synergism. Synergism was observed for some combinations.



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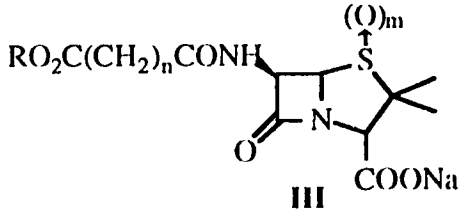

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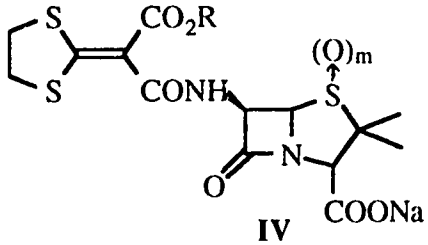

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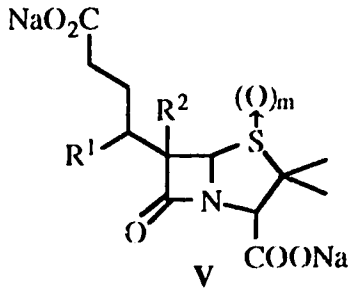

V

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Mr. Andrew Jodhan	FAB MS
Ms. Joanne McLernon (SynPhar Labs.)	Elementary analysis
Mr. Kevin Atchison (SynPhar Labs.)	*In Vitro* enzyme inhibition assay
Ms. Anita Gandhi (SynPhar Labs.)	Antimicrobial assay

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LIST OF ABBREVIATIONS

α	Alpha
Ac	Acetyl
7-ACA	7-Aminocephalosporanic acid
7-ADCA	7-Amino-3'-desacetoxycephalosporanic acid
Ala	Alanine
6-APA	6-Aminopenicillanic acid
aq.	Aqueous
β	Beta
BBN	Borabicyclo[3.3.1]nonane
Bu	Butyl
Bzl	Benzyl
°C	Degree Celsius
CDI	Carbonyldiimidazole
δ	Delta
DCC	1,3-Dicyclohexylcarbodiimide
DHP	Dehydropeptidase
DMF	Dimethylformamide
Enz	Enzyme
γ	Gamma
g	Gram
h	Hour(s)
Hz	Hertz
IR	Infra Red
LDA	Lithium diisopropylamide
Lys	Lysine

m	Meta
M	Molar
mg	Milligram
μg	Microgram
MIC	Minimum Inhibitory Concentration
min	Minute(s)
mmol	Millimole
mol	Mole
MRSA	Methicillin-resistant *S. aureus*
NMR	Nuclear Magnetic Resonance
o	Ortho
ONB	*o*-Nitrobenzyl
p	Para
PBP	Penicillin Binding Protein
Ph	Phenyl
PNB	*p*-Nitrobenzyl
PPTS	Pyridinium *p*-toluenesulfonate
Ser	Serine
TBDMS	*tert*-Butyldimethylsilyl
tert	Tertiary
Tf	Trifluoromethylsulfonyl(triflyl)
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	a) Trimethylsilyl (in chemical structures)
	b) Tetramethylsilane (as internal standard for NMR)
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Ts	*p*-Toluenesulfonyl

	Name of the Companies
Beecham	Beecham Pharmaceuticals Research division
Eli Lilly	Eli Lilly and Company
Merck	Merck Sharp & Dohme Research, Merck & Co., Inc.
Sankyo	Sankyo., Ltd.
Squibb	The Squibb Institute for Medical Research
Sumitomo	Sumitomo Chemical Co., Ltd.
Takeda	Takeda Chemical Industries, Ltd.

Chapter 1
INTRODUCTION

1.1 β-LACTAM ANTIBIOTICS

β-Lactam antibiotics are antimicrobial agents with a chemical 'β-lactam' moiety in their structures. Because of their high efficacy and safety profile, β-lactam antibiotics have been the most widely used chemotherapeutic agents during the last few decades to prevent and treat infectious diseases caused by bacteria.

1.1.1 Penicillins

The history of β-lactam antibiotics starts in 1928 when Fleming observed that *Penicillium notatum* inhibited the growth of bacteria in an experimental culture dish.[1] In 1940 Chain *et al.* extracted the active substance from the microorganism[2] and in 1941 Abraham *et al.* demonstrated that this substance, named penicillin G, was impressively effective in treating bacterial infections.[3]

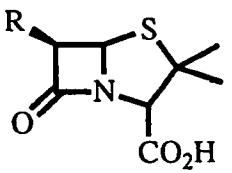
This naturally occurring penicillin G (1) was highly effective initially and hence was used widely to treat infections. However, very soon it was found that an increasing number of resistant microbes, primarily *Staphylococcus aureus*, were generated.[4] Even as early as 1940, Abraham recognized the presence of enzymes, named "penicillinases", which were capable of hydrolyzing penicillin G.[5] Subsequently many more β-lactam-hydrolyzing enzymes, which are now collectively called "β-lactamases", have been discovered in various bacteria and have been found to possess a variety of substrate specificities.[6] Later, the rapid development of penicillin-

resistant *S. aureus* strains has been shown to be due to the rapid transfer of a plasmid penicillinase-encoding gene, from one microbe to another.[7]

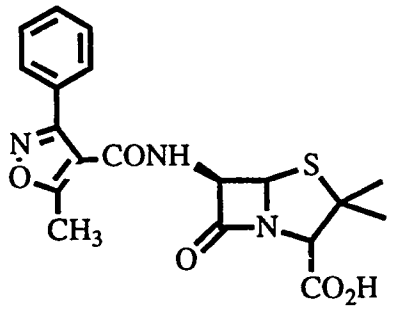
When 6-aminopenicillanic acid (6-APA) (**2**) became available in large quantities in the late 1950s by an enzymatic procedure[8], enormous efforts were poured into the syntheses of semisynthetic penicillins in search of new derivatives with extended antibacterial spectrum and improved stability against β-lactamases (Figure 1-1).

The earliest success in chemical modification led to oxacillin[9] (**3**) and methicillin[10] (**4**). Presumably due to the bulkiness of the acyl side chains, these compounds acquired stability against β-lactamases, and consequently regained activities against some β-lactamase-producing microbes, most notably against *S. aureus* strains of bacteria. However their intrinsic spectra were just comparable to that of penicillin G. Namely, they inhibited mainly Gram-positive bacteria and showed limited activity against Gram-negative bacteria.

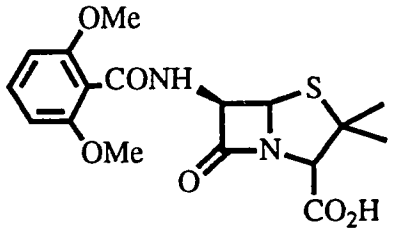
Ampicillin[11] (**5**), a penicillin G analogue with an amino group at the benzylic position, although as susceptible to β-lactamases as penicillin G, was found to have a broader spectrum of activity, particularly against Gram-negative bacteria including *Escherichia coli*, *Hemophilus influenza* and *Proteus* species. On the other hand, carbenicillin[12] (**6**) and sulbenicillin[13] (**7**), which had acidic substituents instead of an amino group at the benzylic position of penicillin G, also acquired activity against Gram-negative bacteria including problem microbes such as *Pseudomonas aeruginosa*,[14] and these activities were partly attributed to their stability against β-lactamases produced by Gram-negative bacteria. Unlike ampicillin, carbenicillin and sulbenicillin lost some activity against Gram-positive bacteria.



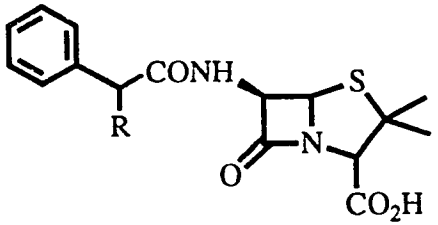
R=$PhCh_2CONH$ Penicillin G (**1**)
R=H_2N 6-Aminopenicillanic acid (**2**)



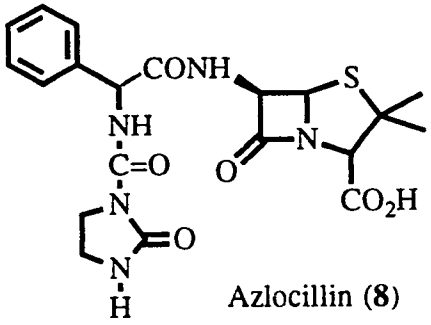
Oxacillin (**3**)



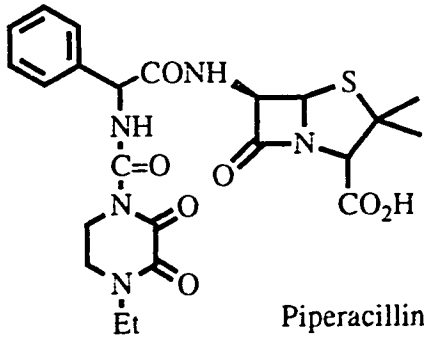
Methicillin (**4**)



R=NH_2 Ampicillin (**5**)
R=CO_2H Carbenicillin (**6**)
R=SO_3H Sulbenicillin (**7**)



Azlocillin (**8**)



Piperacillin (**9**)

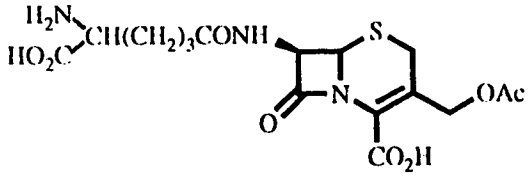
Figure 1-1 Structures of penicillins.

The introduction of an acyl group at the amino moiety of ampicillin resulted in broader spectrum penicillins, such as azlocillin[15] (**8**) and piperacillin[16] (**9**). These derivatives showed comparable efficacy against *P. aeruginosa* with aminoglycosides, and improved activity against *Klebsiella pneumoniae*, *Serratia* species and *Bacteroides* species relative to the other penicillin derivatives. This type of penicillin has been reported to be relatively susceptible to β-lactamases.

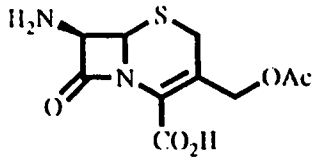
1.1.2 Cephalosporins

In 1953 cephalosporin C (**10**) was isolated from *Acremonium chrysogenum* (previously known as *Cephalosporium acremonium*) by Newton and Abraham.[17] Although cephalosporin C had an apparent advantage over penicillins in that it was resistant to degradation by penicillinase produced by *S. aureus*, its antibacterial activity was not strong enough in comparison with the penicillins.[18] The large scale production of 7-aminocephalosporanic acid (7-ACA) (**11**) was achieved in 1962 by a research group in Eli Lilly, using a chemical method and consequently it became possible to synthesize semisynthetic cephalosporins.[19] Since cephalosporins have two easily modifiable sites, *viz.* the 3-position and the 7-position, a larger variety of derivatives have been generated chemically with the cephalosporins than the penicillins and they have been classified into several generations according to their structures and biological characteristics (Figure 1-2).

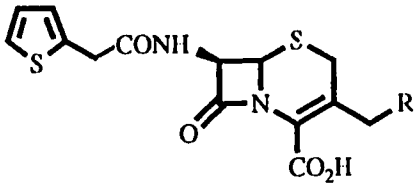
Early derivatives, such as cephalothin[20] (**12**) and cephaloridine[21] (**13**), are called first generation cephalosporins. As common characteristics, they are susceptible to some β-lactamases, especially to cephalosporinases produced by Gram-negative bacteria. Although first generation cephalo-



Cephalosporin C (**10**)

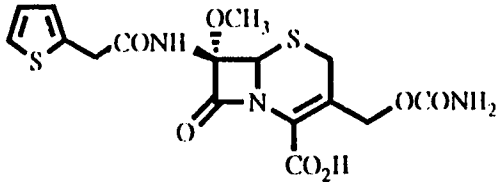


7-Aminocephalosporanic acid (**11**)

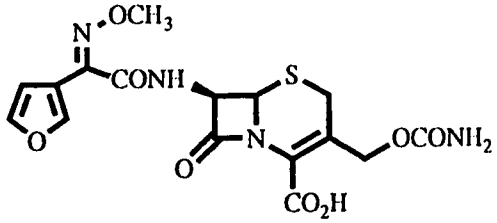


R=OAc Cephalothin (**12**)

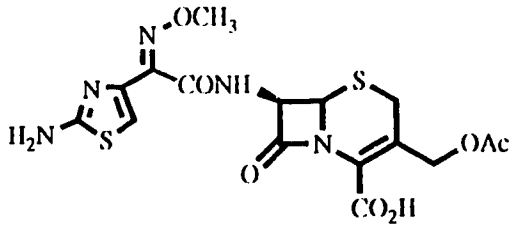
R= Cephaloridine (**13**)



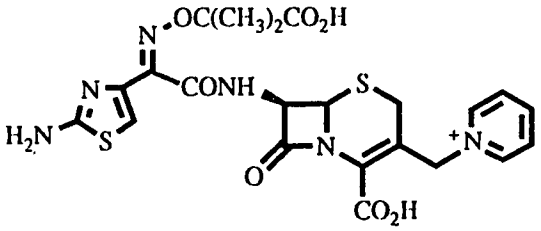
Cefoxitin (**14**)



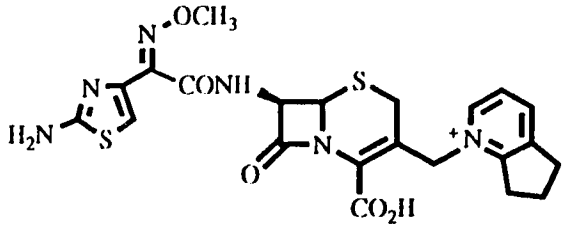
Cefuroxime (**15**)



Cefotaxime (**16**)



Ceftazidime (**17**)



Cefpirome (**18**)

Figure 1-2 Structures of cephalosporins.

sporins had a broader spectrum of activity than penicillins, then available, and were used as penicillin substitutes for patients who were allergic to penicillins, their activities against Gram-negative bacteria were not satisfactory.

In 1969, cephamycins, a new type of cephalosporin analogue with an α-methoxy group at the 7-position, was isolated from *Streptomyces lipmanii.*[22] Interestingly, they showed a high degree of stability to a wide range of β-lactamases.[23] Cefoxitin[24] (**14**) was obtained from structure optimization of this type of compound. On the other hand, at almost the same time, cefuroxime[25] (**15**), which has a *syn*-methoxyimino group at the 7-position side chain, was synthesized and found to be resistant to β-lactamases. These drugs, cefoxitin and cefuroxime, were indeed proven to be effective against some β-lactamase-producing Gram-negative bacteria as well. Together with several others, these cephalosporins have been classified into the second generation cephalosporins.

Further improvement led to a series of third generation cephalosporins. Most third generation cephalosporins, *e.g.* cefotaxime[26] (**16**) and ceftazidime[27] (**17**), possess an aminothiazolyl group and the *syn*-alkoxy-imino group at the 7-position side chain. These cephalosporins acquired impressively broad overall antibacterial activity because of the combination of high stability against most β-lactamases and strong inhibition of the target PBP enzymes, but they lost some activity against *S. aureus* in exchange.

Cefpirome[28] (**18**), which is now under clinical evaluation and regarded as one of the fourth generation cephalosporins, was reported not only to be resistant to β-lactamases but also to have lower affinity to β-lactamases than its predecessors did. Another unique feature of cefpirome is that unlike all

the other penicillins and cephalosporins it possesses high potency against both *S. aureus* and *P. aeruginosa* at the same time.

1.1.3 Carbapenems

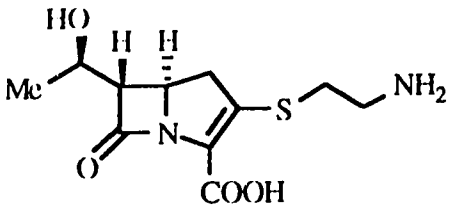
During the 1970s, progress in chromatographic and microanalytical techniques together with advances in microbiology made it possible to isolate and identify tiny amounts of unstable antibiotics from fermentation broths.

Carbapenems, a new class of β-lactam antibiotics, were discovered independently in the mid 1970s by research groups in Merck and Beecham. These were named thienamycin and olivanic acids, respectively (Figure 1-3). Subsequently around fifty new natural carbapenems have been reported.

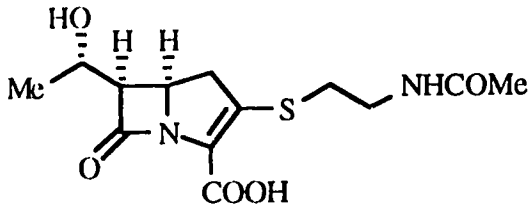
Thienamycin (**19**) was isolated from *Streptomyces cattleya* and found to possess exceptionally potent antibacterial activity against a wide range of bacteria including clinically problematic microbes such as *P. aeruginosa* and some methicillin-resistant *S. aureus*.[29] Olivanic acids, *e.g.* olivanic acid MM22380 (**20**), were isolated from *Streptomyces olivaceus* by researchers in Beecham while they were screening for β-lactamase inhibitors. Although the antibacterial activity of the olivanic acids was inferior to that of thienamycin, they showed a high degree of β-lactamase inhibitory activity.[30]

The carbapenems discovered so far can be divided into three major groups according to their 6-substitution pattern. These are trans-carbapenems represented by thienamycin; cis-carbapenems represented by olivanic acids; and ene-carbapenems such as asparenomycins[31] (**21**). The structure-activity relationships of the known carbapenems have revealed that trans-carbapenems generally show superior antimicrobial activity over the other types of compounds. Among trans-carbapenems, the derivatives with the

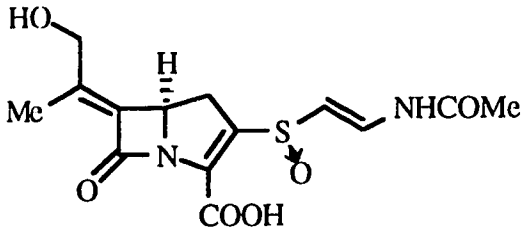
same 6-substituent as thienamycin usually demonstrate better antimicrobial activity than other derivatives.[32]



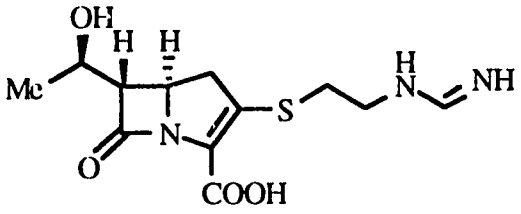
Thienamycin (**19**)



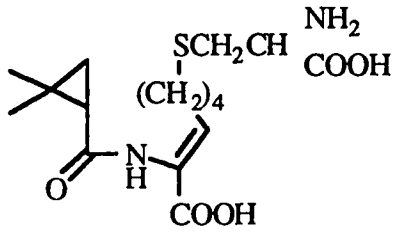
Olivanic acid MM22380 (**20**)



Asparenomycin A (**21**)



Imipenem (**22**)



Cilastatin (**23**)

Figure 1-3 Structures of carbapenem antibiotics.

Despite its potency, thienamycin exhibited several weak points to be corrected before it could be commercialized. First, thienamycin was extremely unstable chemically due to its highly strained bicyclic system, a common feature of carbapenems, and furthermore the amino group on the 2-position of the side chain of thienamycin rendered the intrinsically unstable

bicyclic system even more unstable, especially in a concentrated solution or in the solid state.[29] Second, unlike the penicillins and cephalosporins, the carbapenems were produced by microorganisms in very low yields and, therefore to supply carbapenems in large quantities, they had to be synthesized chemically.[33]

The first weak point was overcome by chemical modification. Imipenem (**22**), the *N*-formimidoyl derivative of thienamycin, was synthesized as a crystallizable compound and found to be more stable than thienamycin, especially in a concentrated solution.[34] A practical manufacturing procedure, the second problem to be solved, was eventually resolved after intensive synthetic studies by Merck and many other groups. However, another problem emerged when impenem was studied further using animals. Imipenem was metabolized extensively in the body, consequently, giving a very low urinary recovery.[35] The renal enzyme, dehydropeptidase I (DHP-I), was identified as the causative enzyme in hydrolyzing imipenem.[36] Scientists at Merck finally solved this problem by synthesizing a selective DHP-I inhibitor, named cilastatin (**23**), and combining it with imipenem.[37] A one to one combination improved the pharmacokinetic profile of imipenem and has been successfully marketed.[38]

Imipenem's high degree of activity has been considered to be due to its high reactivity with the target PBP enzymes, stability to most β-lactamases, and an efficient distribution to the target site. Especially strong activity against *P. aeruginosa* was partly attributed to its high penetrating ability into the periplasmic space where its target enzymes reside. Imipenem was demonstrated to utilize additional special channels, called the D2 protein channel, to pass through the outer membrane[39] whereas other β-lactams use only non-specific pores formed by proteins called porins. In recent years, an

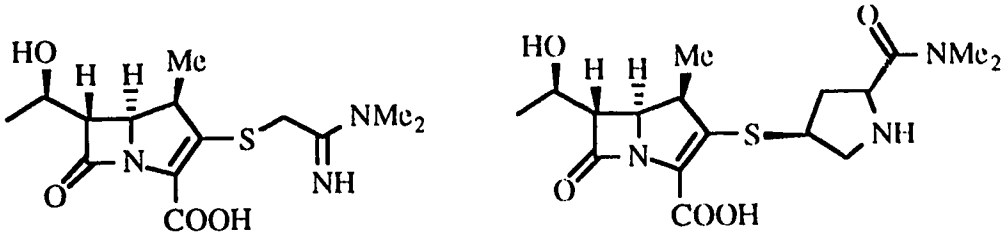
increasing number of imipenem-resistant *P. aeruginosa* are emerging, and many of them have been found to be D2-protein-deficient mutants.[40]

In general, carbapenems are highly resistant to commonly occurring β-lactamases.[41] However some bacteria, e.g. *Pseudomonas maltophilia* and *Fusobacterium odoratum*,[42] produce metalloenzymes which can hydrolyze carbapenems. Most carbapenems are not only stable to β-lactamases, but also inhibit them in a moderate to high degree.[43]

Imipenem is currently regarded as one of the 'last line' drugs in the treatment of infectious diseases caused by highly resistant bacteria. Therefore its usage should be controlled with great care to prevent emergence of more resistant microbes. Furthermore, there is another reason why it has to be used carefully. Many Gram-negative bacteria carry cephalosporinase-encoding genes in their chromosome, and these genes are usually under the control of regulatory genes. Imipenem has been found to be an extremely good inducer of these cephalosporinases by stimulating gene expression through yet-undefined mechanisms.[44] Once cephalosporinases are produced in large quantity, even β-lactamase stable cephalosporins and penicillins are affected.[45]

In 1984 Merck researchers reported the synthesis of the 1-β-methyl-substituted carbapenem derivative (**24**) and it was found to be very stable to porcine renal dehydropeptidase I with high antibacterial potency.[46] This result stimulated many other groups to synthesize 1-methyl-substituted carbapenem derivatives. Among many synthesized analogues, meropenem (**25**), synthesized by Sumitomo researchers, has emerged as one of the most potent and enzymatically stable compounds[47] and is now under clinical evaluation as a single drug. Meropenem has been found to show superior activity against many Gram-negative bacilli in comparison to imipenem.[48]

Interestingly meropenem was suggested to have different PBP targets from those of imipenem.[49]



24 Meropenem (25)

Figure 1-4 Structures of 1 β-methylcarbapenems.

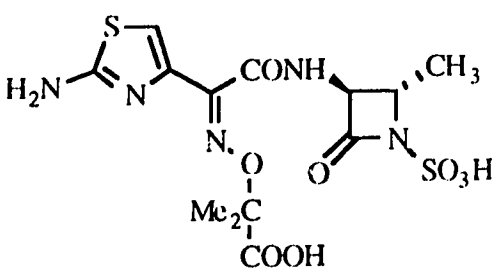
Despite their several drawbacks, carbapenem antibiotics are regarded as one of the most promising classes of chemotherapeutic agents for the future because of their exceptional antimicrobial activity.

1.1.4 Monobactams

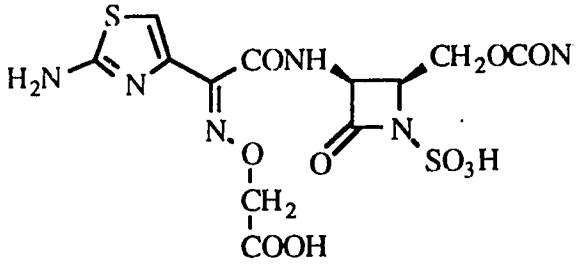
Yet another class of β-lactam antibiotics with an entirely new basic structure was discovered independently in 1981 by research groups in Squibb[50] and Takeda[51]. Their structures were determined as unique *N*-sulfonated monocyclic azetidinones with an amide group at the 3-position. Another atypical characteristic of this type of β-lactam is that they were isolated from bacteria, not from fungi or actinomycetes like other β-lactam antibiotics. Sykes named this class of compound "monobactam" after "monocyclic bacteria-originated β-lactam".[50]

Unlike other β-lactam antibiotics, monobactams showed activity exclusively against Gram-negative bacteria. Squibb and Takeda workers independently developed β-lactamase-stable monobactams, *viz.* aztreonam[52] (**26**) and carumonam[53] (**27**) respectively, as narrow-spectrum chemotherapeutic agents (Figure 1-5). Both aztreonam and carumonam achieved a

moderately high level of activity against most Gram-negative bacteria including *P. aeruginosa*.



Aztreonam (**26**)



Carmonam (**27**)

Figure 1-5 Structures of monobactams.

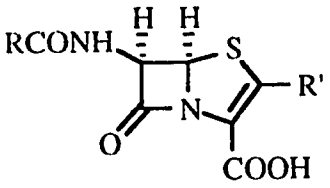
1.1.5 Other β-lactam and γ-lactam analogues

While microbiologists were looking for new naturally occurring antibiotics, many medicinal and synthetic chemists were trying to find new types of β-lactam-antibiotic-like antibacterial agents by means of drug design and organic synthesis.

Woodward and coworkers synthesized the first penem derivative, which was designed as a structural hybrid of penicillin and cephalosporin.[54] Initially they synthesized the 6-β-acylaminopenem (**28**) and found it to be less active than either penicillins or cephalosporins. Later a hydroxyethyl group, which was based on the thienamycin structure, was introduced at the 6-α-position of penems and the activities of these derivatives were found to improve greatly.[55] Recent penems such as FCE-22101[56] (**29**) show comparable activity to those of carbapenems except against *P. aeruginosa*.

More recently Eli Lilly workers demonstrated that the β-lactam structure is not really essential for the antimicrobial activity of this type of antibiotic. They synthesized biologically active γ-lactam analogues (**30**) of

β-lactam antibiotics, but the overall antimicrobial activities of these γ-lactam derivatives were much weaker than those of their β-lactam counterparts.[57]



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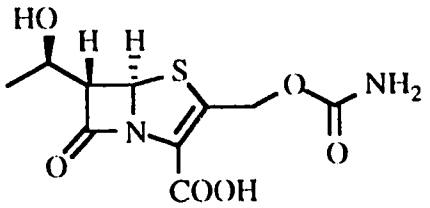
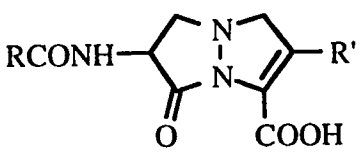
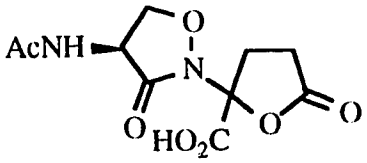


FCE-22101 (**29**)



γ-Lactams **30**



Lactivicin (**31**)

Figure 1-6 Structures of penems and γ-lactams.

Also, a group in Takeda discovered the naturally occurring γ-lactam analogue, lactivicin[58] (**31**). They demonstrated that lactivicin bound to the essential PBPs, the target enzyme of β-lactams, in a fashion similar to that of β-lactam antibiotics. Lactivicin was also found to be susceptible to β-lactamases.[59]

1.2 INTERACTIONS OF β-LACTAM ANTIBIOTICS WITH BACTERIA

In nature, bacterial cells are always exposed to a dynamically changing environment. To protect themselves from disruption caused by mechanical insult and osmotic pressure, virtually all the bacterial cells possess rigid

outer coats outside their cytoplasmic membrane. These coats are called the 'cell wall'. β-lactam antibiotics have been demonstrated to exert their bactericidal activity by disturbing the bacterial cell wall synthesis, leading to cell lysis.

1.2.1 Structure and biosynthesis of bacterial cell walls

Traditionally bacteria have been classified into two groups, *viz.* Gram-positive bacteria and Gram-negative bacteria, based on their response to the Gram stain technique. Detailed studies on cell structures have revealed that the differences in response to the Gram staining technique are mainly due to the considerable differences in their cell wall structures.[60] In Gram-positive bacteria, the major component of the cell wall is a thick peptidoglycan which has a multi-layered and highly cross-linked structure. On the other hand, Gram-negative bacteria have a thin layer of peptidoglycan, and in addition they possess an additional hydrophobic membrane, called the outer membrane, outside of the peptidoglycan layer. The space between the peptidoglycan layer and the outer membrane in Gram-negative bacteria is called the periplasmic space. In both cases, the peptidoglycan layers are responsible for the mechanical strength of the bacterial cells and maintenance of their cell shape (Figure 1-7).

Although there are some differences, the structures of the peptidoglycans of Gram-positive and Gram-negative bacteria are fundamentally similar. Peptidoglycans are composed of linear mucopolysaccharides and short peptide chains, which serve as backbones and hands for cross linking respectively. Mucopolysaccharides consist of two alternating sugars; *N*-acetylglucosamine and *N*-acetylmuramic acid, and peptide chains that are attached to the 4-position of each *N*-acetylmuramic acid (Figure 1-8).

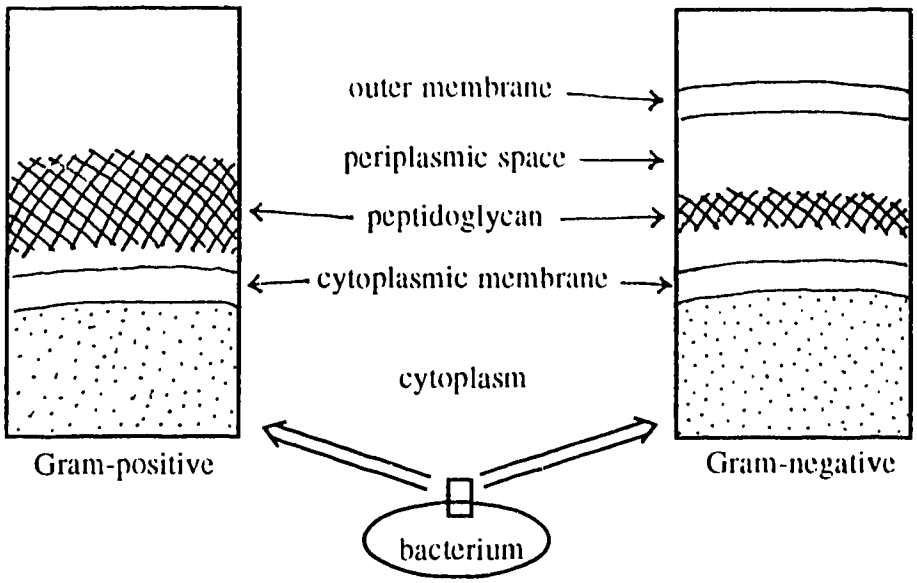


Figure 1-7 The cell wall structures of Gram-positive and Gram-negative bacteria.

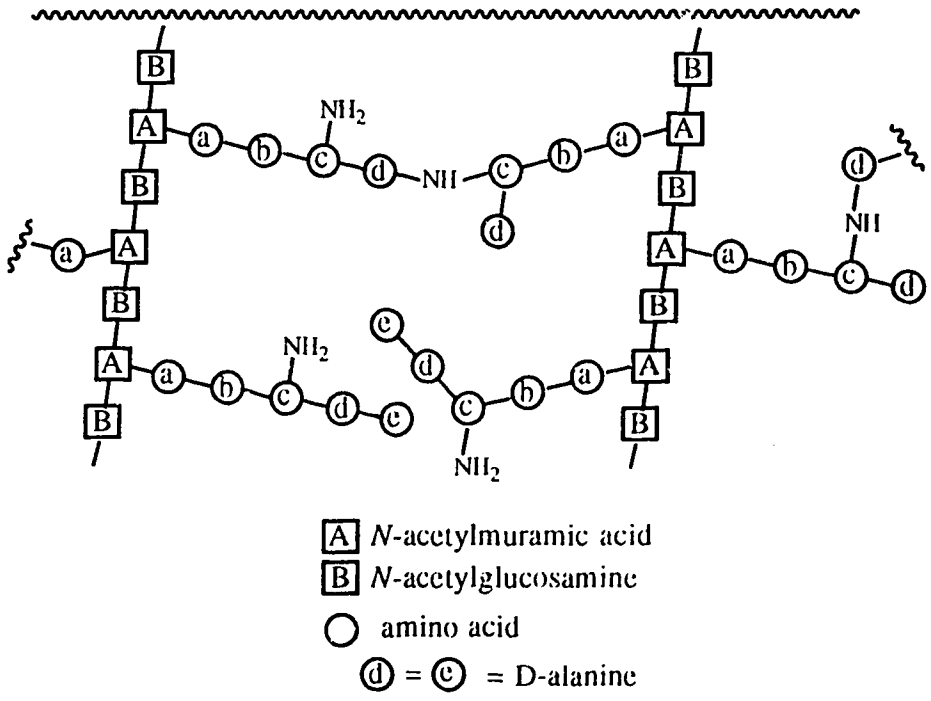


Figure 1-8 Structure of peptidoglycan.

When bacteria grow, the building blocks of peptidoglycan are synthesized in the cytoplasm and transported to the surface of the cytoplasmic membrane with the assistance of a special carrier molecule called bactoprenol.[60] There, a small part of the peptidoglycan network is cut and opened by the action of enzymes called autolysins, and newly synthesized building blocks are integrated into the existing network of the peptidoglycan layer. For the latter process, two types of reactions take place. One is a sugar-backbone elongation reaction called transglycosylation, and the other is a cross-linking reaction between two adjacent peptide chains, called transpeptidation. Both of these reactions are catalyzed by a group of enzymes called penicillin-binding-proteins (PBPs). The transpeptidation reaction is represented by the following reaction scheme.

$$\text{R-D-Ala-D-Ala} + \text{H}_2\text{N-R}' \longrightarrow \text{R-D-Ala-NH-R}' + \text{H}_2\text{N-D-Ala}$$

First, the PBPs recognize the terminal D-Ala-D-Ala structure and break the peptide linkage to form the acyl enzyme. Subsequent reaction of the acyl enzyme with an amino group on the other peptide chain concludes the transpeptidation reaction by forming a cross-linkage and releasing the free form of the PBP. β-Lactam antibiotics are believed to inhibit PBP's catalytic activity of transpeptidation by forming stable acyl-enzyme complexes with them. In fact, the conformations of D-Ala-D-Ala and β-lactam antibiotics have been found to be remarkably similar.[61]

1.2.2 Penicillin-binding proteins (PBPs)

Penicillin-binding proteins (PBPs) comprise a group of enzymes which act as transpeptidases, carboxypeptidases, endopeptidases and transglycosylases. As their name implies, these enzymes were so termed after their ability

to bind with penicillin G covalently.[62] PBPs are the cytoplasmic membrane-bound proteins with molecular weights ranging from 90,000 to 12,000, that catalyze peptidoglycan modifying reactions at the outer surface of the cytoplasmic membrane.[63] Each bacterial cell carries several sets of PBPs and their constitution and composition vary from one species to another and from strains to strain.

In *E. coli*, at least seven PBPs have been identified, *viz.* PBPs 1A, 1B, 2, 3, 4, 5, and 6.[64] These PBPs have been numbered in the order of decreasing molecular weight. Although their functions in peptidoglycan biosynthesis are still not precisely understood, the effect of each PBP on bacterial morphology has been described by Spratt, as follows.[65] PBPs 1A and 1B are the bifunctional enzymes with transpeptidase and transglycosylase activity. Selective inhibition of these enzymes results in inhibition of cell wall elongation and causes rapid cell lysis. PBP 2 has transpeptidase activity and appears to be responsible for the maintenance of cell shape. Selective inhibition of PBP 2 results in formation of oval shape bacteria. PBP 3 is also a transpeptidase/transglycosylase bifunctional enzyme. It catalyzes the formation of the cross-wall in cell division. Consequently, by selective inhibition of PBP 3, formation of long, filamentous cells are observed. PBPs 4, 5, and 6 have been found to have mainly carboxypeptidase activities.

The antibacterial activities of β-lactam antibiotics are mediated by inhibition of one or more of the PBPs 1A, 1B, 2, and 3, whereas inhibition of PBPs 4, 5, and 6 seems to have no significant effect on bacterial growth and morphology.[61] Different β-lactam antibiotics usually show different affinities to each of the PBPs. Imipenem, for instance, binds all the PBPs, but shows stronger affinity for PBPs 1 and 2.[66] Mecillinam, with an unusual amidine side chain at 6-position, on the other hand, possesses affinity

exclusively for PBP 2.[67] In general, the more PBPs the β-lactam antibiotic inhibits simultaneously, the stronger its bactericidal activity becomes. Because of their biological functions and molecular sizes, PBPs 1-3 are sometimes referred to as essential PBPs or high-molecular-weight PBPs.

Production of unusual PBPs has been observed in methicillin-resistant *S. aureus* (MRSA).[68] The uniqueness of this enzyme, termed penicillin-binding protein 2' (PBP 2') or penicillin-binding protein 2a (PBP 2a), is that it is not inhibited by β-lactam antibiotics but has the capability to catalyze necessary peptidoglycan biosynthesis for bacterial growth and division when other PBPs are inactivated by β-lactam antibiotics.[69] This feature makes MRSA extremely difficult to be treated with usual β-lactam antibiotics. Methicillin-sensitive *S. aureus*, on the other hand, apparently lack the gene for PBP 2a expression.

1.2.3 Distribution of β-lactam antibiotics to the target site in Gram-negative bacteria

Unlike Gram-positive bacteria, Gram-negative bacteria do not allow β-lactam antibiotics free access to their target site because of their cell wall structure. Before reaching the periplasmic space of Gram-negative bacteria, β-lactam antibiotics have to somehow penetrate through the hydrophobic outer membrane.[70] Most commonly, hydrophilic β-lactams cross the outer membrane through water filled channels called "porins".[71] In *E. coli*, three types of porins, *viz.* Omp F, Omp C, and Pho E, have been identified.[72] Of these, the first two are considered to be the most important for uptake of β-lactams in physiological conditions.[73] Exclusion limits for these porins were experimentally determined to be 600-800.[74] The rate of influx of β-lactam antibiotics through the porins is influenced by such factors as molecular size,

hydrophilicity and net charge of molecule. In general, smaller and more hydrophilic compounds penetrate more readily through the porins.[75] Additional positive charges on mono anionic β-lactam antibiotics largely enhance the rate of permeation, whereas additional negative charges lower their permeation.[76]

Some strains of *P. aeruginosa* generally shows high MICs due to its high resistance to β-lactam permeation through the outer membrane.[77] Studies of porins of this microorganism, however, showed surprising results. Contrary to many researchers' expectations, *P. aeruginosa* was found to express large quantities of Omp F.[78] Furthermore, *in vitro* experiments indicated that these porins had much higher exclusion limits (2,000-3,000) than those of *E. coli* porins.[78] Recently, these contradictory observations have been explained by the finding that the majority of porins had narrower pore sizes than previously expected from *in vitro* studies.[79]

In addition to porins, there are several other ways in which β-lactam antibiotics are transported through the outer membrane of Gram-negative bacteria. Imipenem, for instance, utilizes basic-peptide-specific channels formed by proteins called D2 proteins as described earlier.[39] Some catechol-substituted β-lactam antibiotics are actively transported into the periplasmic space using the so called ton-B dependent iron-transport system.[80]

1.2.4 β-Lactamases

The β-lactamases are a class of enzymes which are capable of hydrolyzing β-lactam antibiotics.[6] Although β-lactamases had already been present in nature before the use of penicillins, many years of heavy usage of β-lactam antibiotics has undoubtedly contributed to the world wide spread of β-lactamase-producing bacteria and has accelerated the evolution of β-

lactamases by selective pressure. It is thought that virtually all Gram-negative bacteria and most Gram-positive bacteria are potentially capable of producing one or more β-lactamases, and no β-lactam antibiotic is totally free from degradation by β-lactamases.

Genetically, the β-lactamase-encoding genes are located either on the chromosome[81] or on plasmids. When the β-lactamase-encoding gene is on a plasmid, gene transfer may occur quite readily between strains and sometimes between species. Some cases of the prevalence of β-lactamase-producing microbes, e.g. *S. aureus* during 1950s, were associated with β-lactamase-encoding gene transfer.

Many β-lactamases, including the plasmid-mediated penicillinases of staphylococci and the chromosomally-mediated cephalosporinases of Gram-negative bacteria, are known to be inducible.[82] Following exposure to certain β-lactam antibiotics such as imipenem, the production of β-lactamase increases dramatically. A similar type of hyperproduction of β-lactamase is observed among mutant microbes. By mutations of genes which regulate expression of β-lactamases, these microbes stably produce large amounts of β-lactamase constitutively in the absence of inducers. This phenomenon is called 'derepression' and is commonly seen in Gram-negative bacteria such as *Enterobacter* and *Citrobacter*.[83] The population of these mutant microbes is generally small, however during exposure to β-lactam antibiotics, β-lactamase-hyperproducing microbes are gradually selected out.

β-Lactamases of Gram-positive and Gram-negative bacteria behave quite differently. β-Lactamases produced by Gram-positive bacteria are secreted from the cell to hydrolyze β-lactam antibiotics in the extracellular space, whereas β-lactamases synthesized by Gram-negative bacteria do not penetrate the outer membrane and accumulate in the periplasmic space.[84]

Therefore when β-lactamases are produced at a high level by induction or stable derepression, the concentration of β-lactamases could reach extremely high levels.[83] For instance, when cephalosporinase production is derepressed in Gram-negative bacteria, the concentration of cephalosporinase reaches such a high level that even cephalosporinase-stable β-lactams, such as the third generation cephalosporins, cannot reach their target enzymes in sufficient amount.[85]

In 1973, Richmond and Sykes reviewed the β-lactamase literature thoroughly, and presented a classification scheme for β-lactamases from Gram-negative bacteria as follows:[6]

Class I: Enzymes predominantly active against cephalosporins.

Class II: Enzymes predominantly active against penicillins.

Class III: Enzymes with approximately equal activity against penicillins and cephalosporins, but which are sensitive to cloxacillin inhibition and resistant to *p*-chloromercuribenzoate.

Class IV: Enzymes of similar substrate profile to those of class III, but which are resistant to cloxacillin and sensitive to p-chloromercuribenzoate.

Class V: Enzymes that have a penicillinase profile which includes cloxacillin and which are resistant to sulfhydryl agents.

In this classification, inducible, chromosomally mediated cephalosporinases were classified into class I. Class III includes clinically important plasmid-mediated β-lactamases, *e.g.* the TEM and SHV enzymes and class V includes some other plasmid-mediated β-lactamases, *e.g.* the PSE and OXA enzymes.

In 1980, another type of classification, based upon homology in amino acid sequence of the active-site, was proposed by Ambler.[86] β-Lactamases,

for which active-site sequences have been determined, were classified into two groups, namely class A and class B. Class A enzymes were found to be active-site serine enzymes and class B enzymes were identified as metalloenzymes. Later this scheme was expanded by Jaurin and Grundström to include the class C enzymes.[87] Class C enzymes are also active-site serine enzymes, but they have amino acid sequences distinct from those of class A β-lactamases. Interestingly, all the class C β-lactamases hydrolyze cephalosporins predominantly, whereas class A enzymes have substrate profiles indicating either penicillinase or broad-spectrum activity.

More recently, Bush updated the review of β-lactamases and proposed a new classification scheme in which β-lactamases were divided into four groups based upon the substrate and inhibitor profile as follows:[6]

Group 1: Enzymes that preferentially hydrolyze cephalosporins and are not inhibited by 10 μM clavulanic acid.

Group 2: Enzymes which are inhibited by clavulanic acid.

Group 2a enzymes are the classical penicillinases.

Group 2b enzymes are the traditional broad-spectrum β-lactamases.

Group 2b' enzymes are the group 2b related enzymes, but with the ability to hydrolyze the "extended-spectrum" β-lactam antibiotics, such as cefotaxime, ceftazidime, or aztreonam.

Group 2c enzymes are penicillinases that hydrolyze carbenicillin.

Group 2d enzymes are the penicillinases that hydrolyze cloxacillin.

Group 2e enzymes preferentially hydrolyze cephalosporins.

Group 3: Enzymes that require a metal ion for enzymatic activity and are not inhibited by clavulanic acid.

Group 4: Enzymes that preferentially hydrolyze penicillins and are not inhibited by clavulanic acid.

Among these, the group 2b' enzymes, called "extended-spectrum β-lactamase",[88] have gained considerable attention because of their clinical significance. The amino acid sequencing studies revealed that these new enzymes were derived from commonly occurring plasmid-mediated β-lactamases such as TEM and SHV enzymes by point mutations. Cefotaxime-hydrolyzing enzyme, TEM-3 for instance, was found to have only two differences in amino acid residues from TEM-2,[89] but shows significant differences in kinetics.[90] Since these enzymes are encoded on plasmids, they are prone to be transferred to other bacteria.[91] In fact, these enzymes have spread to a variety of enterobacteria and their prevalence is reported to be increasing in France.[88] One fortunate thing is that many of these β-lactamases are inhibited by inhibitors such as clavulanic acid, sulbactam, and tazobactam.[92]

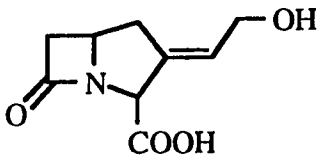
1.2.5 β-Lactamase inhibitors

There are two approaches to overcome the problems caused by bacterial β-lactamases. One is by finding new synthetic or naturally occurring β-lactam analogues which are not inactivated by β-lactamases. This has been a major approach since the modification of penicillin started. Consequently, many β-lactams have been synthesized and claimed to be β-lactamase-stable. However, as history indicates, the introduction of any β-lactams to clinical use is almost invariably followed by the emergence and prevalence of β-lactamases which can hydrolyze these β-lactams. Another approach is to find β-lactamase inhibitors which can be used concomitantly with β-lactam antibiotics.[43,93]

Attempts to find β-lactamase inhibitors were made from as early as the 1940s. In the early 1960s, some semisynthetic penicillins, *e.g.* cloxacillin (**3**)

and methicillin (**4**), were found to possess β-lactamase inhibitory activity.[94] These compounds showed some synergy with certain β-lactam antibiotics, however none of them were successfully developed.[94]

In 1976, olivanic acids, *e.g.* MM22380 (**20**) and clavulanic acid[95] (**32**) were discovered by workers in Beecham from a screening program for naturally occurring β-lactamase inhibitors . Olivanic acids were found to be ineffective *in vivo* due to rapid metabolism in the body and low penetration through the outer membrane of Gram-negative bacteria. Clavulanic acid was proved to be effective and marketed in 1981 as a combination drug with amoxicillin, which is known as Augmentin.[96] Clavulanic acid inhibits many enzymes including penicillinases and broad-spectrum β-lactamases, but does not inhibit class I enzymes, namely inducible, chromosomally mediated cephalosporinases produced by Gram-negative bacteria.



Clavulanic acid (32)

Figure 1-9 Structure of clavulanic acid.

The mechanism of enzyme inhibition by clavulanic acid has been postulated as shown in Figure 1-10.[97] In the first stage, the enzyme makes a complex with clavulanic acid by non-covalent interactions. Second, the active-site serine-OH attacks the β-lactam carbonyl to form the acylated-enzyme **A**. Third, instead of being hydrolyzed, the acylated-enzyme **A** either undergoes tautomerization to yield the enamine **B**, or reacts with the intracellular amine group to form another type of enamine **C**. Since the

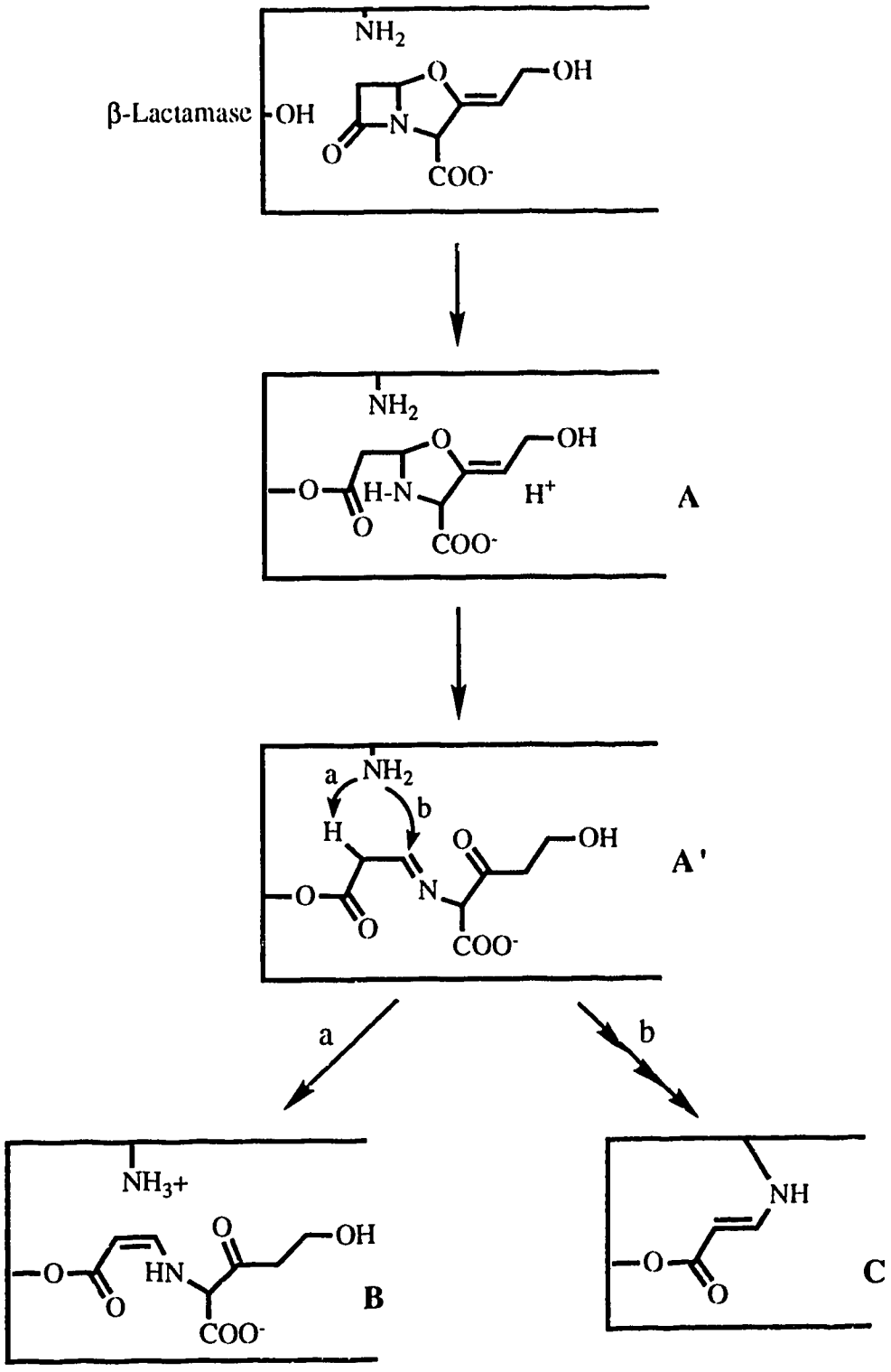
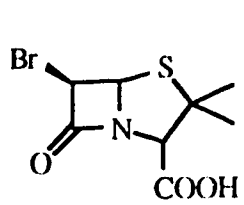
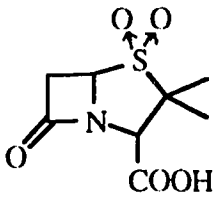


Figure 1-10 Interaction of clavulanic acid with β-lactamase.

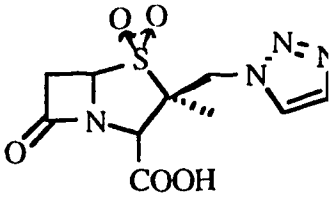
enamines **B** and **C** are chemically much more resistant to hydrolysis than **A**, the enzyme is irreversibly inactivated or undergoes "turn over" very slowly.



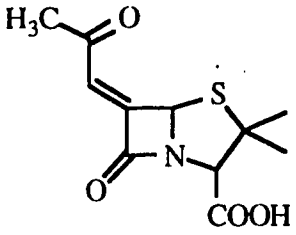
6-β-Bromopenicillanic acid (**33**)



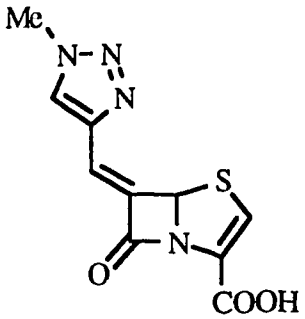
Sulbactam (**34**)



Tazobactam (**35**)



Ro 15-1903 (**36**)



BRL-42715 (**37**)

Figure 1-11 Strucutres of post-clavulanic acid β-lactamase inhibitors.

The success of clavulanic acid prompted work in this field to yield more potent β-lactamase inhibitors. Such inhibitors include 6-β-bromopenicillanic acid[98] (**33**), sulbactam[99] (**34**), tazobactam[100] (**35**). Ro 15-1903[101] (**36**), and BRL-42715[102] (**37**) (Figure 1-11). All of these inhibitors are believed to form the stable acylated-enzyme after formation of the acylated enzyme followed by rearrangement. Sulbactam is currently marketed in combination with ampiciilin. Tazobactam, an improved version of sulbactam, in combination with piperacillin is in the approval process in many countries. Sulbactam and tazobactam also showed selective inhibition to penicillinases and broad-spectrum β-lactamases as clavulanic acid did. BRL-42715 was

found to possess exceptionally strong *in vitro* inhibitory activity against almost all the active-site serine β-lactamases including class I cephalosporinases. However because of problems such as chemical instability, Beecham has recently abandoned further development of this compound.

Since class I cephalosporinases produced by Gram-negative bacteria are clinically problem-causing β-lactamases for most β-lactam antibiotics, including the third generation cephalosporins, and also since there are no practically promising cephalosporinase inhibitors that have been reported, the discovery of potent cephalosporinase inhibitors is currently of importance.

1.2.6 Relationships between penicillin-binding proteins (PBPs) and β-lactamases

Although PBPs and β-lactamases function in quite different manners in bacterial cells, they have been suspected to have an evolutionally similar origin.[103] In fact, this view has been supported by recently available amino acid sequences of many PBPs and β-lactamases.[104] Similarity of amino acid sequences, however, suggests that each of these group are quite distantly separated in one superfamily. Nevertheless, the active site of the enzymes (-Ser-X-X-Lys-) and some other portions of the enzymes which come close to the active site are highly conserved. Furthermore, X-ray crystallography of the DD-peptidase from *Streptomyces* R61 (a soluble low-molecular-weight PBP) and class A β-lactamases from *Bacillus licheniformis* and *Bacillus cereus* have revealed that the distribution of the secondary elements in the three-dimensional structures share striking similarity.[105]

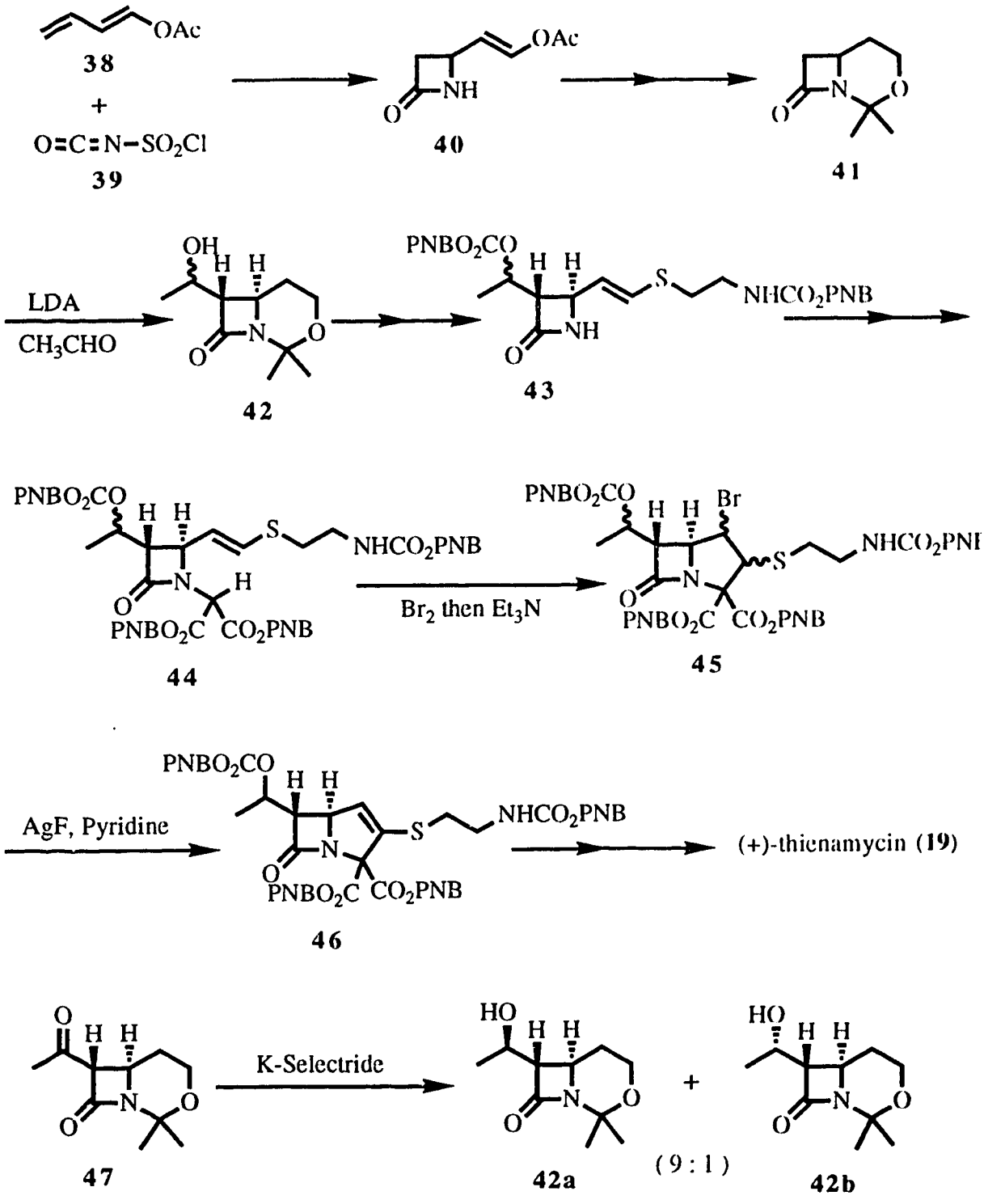
1.3 SYNTHESES OF CARBAPENEMS

Among three types of carbapenems, *viz.* trans-carbapenems, cis-carbapenems, and ene-carbapenems, trans-carbapenems have been the most extensively studied because of their high potency and practical potential as useful chemotherapeutic agents. Accordingly, extensive work has been undertaken by many groups on the total or partial synthesis of thienamycin and related compounds. Also, the syntheses of other naturally occurring carbapenems have been reported and many new synthetic analogues have been created.[106]

There are always two major problems in the synthesis of carbapenems. One is the control of absolute and relative stereochemistry and the other is the construction of the bicyclic ring system. Since the resulting bicyclic ring system is very unstable due to its highly strained structure, vigorous reaction conditions cannot be used during the cyclization and after the formation of the bicyclic ring system. In most cases, the cyclization reaction is carried out at a later stage of the synthesis after all of the necessary stereochemistry is established.

1.3.1 First total synthesis of carbapenem antibiotic

The first total synthesis of thienamycin (**19**) was reported in 1978 by a group in Merck.[107] Starting from 1-acetoxy-1,3-butadiene (**38**), the 4-substituted-azetidinone **40** was synthesized *via* a [2+2] cycloaddition reaction with chlorosulfonyl isocyanate (**39**). After conversion into the bicyclic compound **41**, the introduction of a hydroxyethyl moiety at the 3-position of the azetidinone **41** was carried out by lithiation of the azetidinone with LDA, followed by the reaction with acetaldehyde to afford an epimeric mixture of **42**. After elaboration of the side chain at the 4-position and


OAc
38
+
O=C=N–SO2Cl
39
OAc
NH
O
40
N
O
41
LDA
CH3CHO
OH
H H
N
O
O
42
PNBO2CO
H H
S
NHCO2PNB
NH
O
43
PNBO2CO
H H
S
NHCO2PNB
N
H
O
PNBO2C CO2PNB
44
Br2 then Et3N
PNBO2CO
H H
Br
S
NHCO2PNB
N
O
PNBO2C CO2PNB
45
AgF, Pyridine
PNBO2CO
H H
S
NHCO2PNB
N
O
PNBO2C CO2PNB
46
(+)-thienamycin (19)
O
H H
N
O
O
47
K-Selectride
HO
H H
N
O
O
42a
+
(9 : 1)
HO
H H
N
O
O
42b

Scheme 1-1

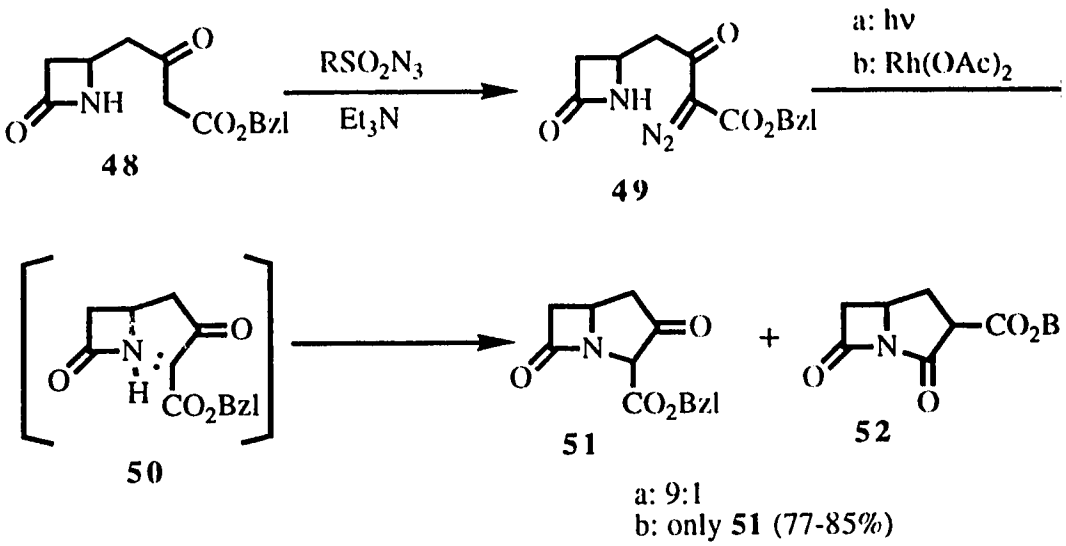
introduction of the dialkyl malonate moiety at the 1-position, **44** was subjected to a cyclization reaction by successive treatment with bromine and triethylamine to give the desired carbapenam **45**. Final conversion to thienamycin was accomplished in 3 steps from **46**. During this study, the *p*-nitrobenzyl (PNB) group, which could be removed readily by hydrogenolysis, was found to be a suitable protecting group for the synthesis of carbapenem (Scheme 1-1).

Later, stereoselective synthesis of the 6-hydroxyethyl-substituted derivative **42b** was achieved by the stereoselective reduction of the corresponding ketone **47**, which was obtained by oxidation of the alcohol **42** or by the direct acetylation of **41**. K-selectride and potassium iodide were used as the reducing agent to yield the desired 8*R* isomer in a ratio of 9:1.[108]

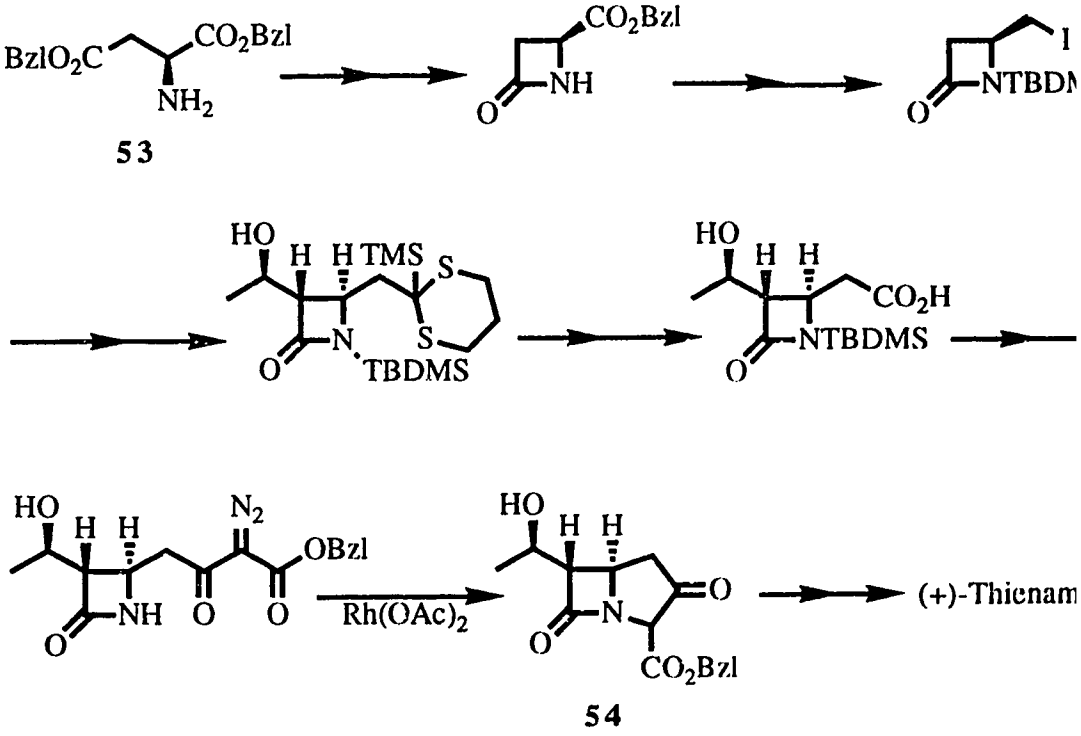
1.3.2 Bicyclic ring formation

Although many procedures to construct the carbapenem bicyclic ring system have been reported so far, only a few of them have practical use.

Ratcliffe *et al.* in Merck reported the formation of the bicyclic nucleus by a carbene insertion reaction (Scheme 1-2).[109] By treating the diazo compound **49**, which was obtained from the acetoacetate **48** by the diazo exchange reaction with *p*-carboxylbenzenesulfonyl azide, either photochemically or by rhodium (II) acetate, the desired bicyclic product **51** was obtained. Interestingly, another bicyclic compound **52** was formed only when the carbene **50** was produced photochemically. Saltzmann and coworkers elegantly synthesized thienamycin from dibenzyl aspartate (**53**) using the carbene insertion reaction as a key step as shown in Scheme 1-3.[110] One of the advantages of this method is that various alkylthio groups can be



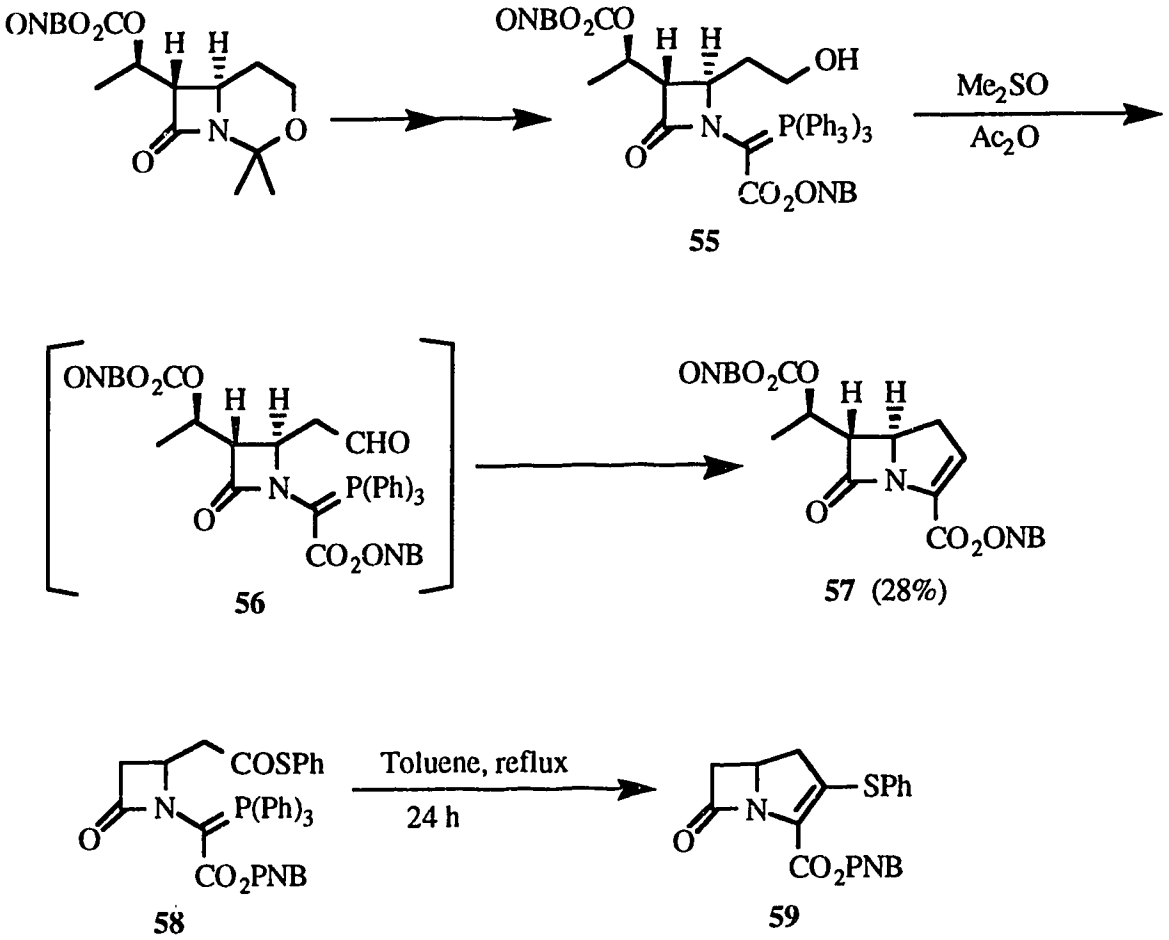
Scheme 1-2



Scheme 1-3

readily introduced at the 2-position, after the formation of the ketoester **54**.[111]

The intramolecular Wittig reaction, which was first established by Woodward *et al.* for the penem synthesis[54], was successfully applied to the carbapenem synthesis by several groups. Cama *et al.* demonstrated that the aldehyde **56**, produced by oxidation of the alcohol **55**, spontaneously reacted

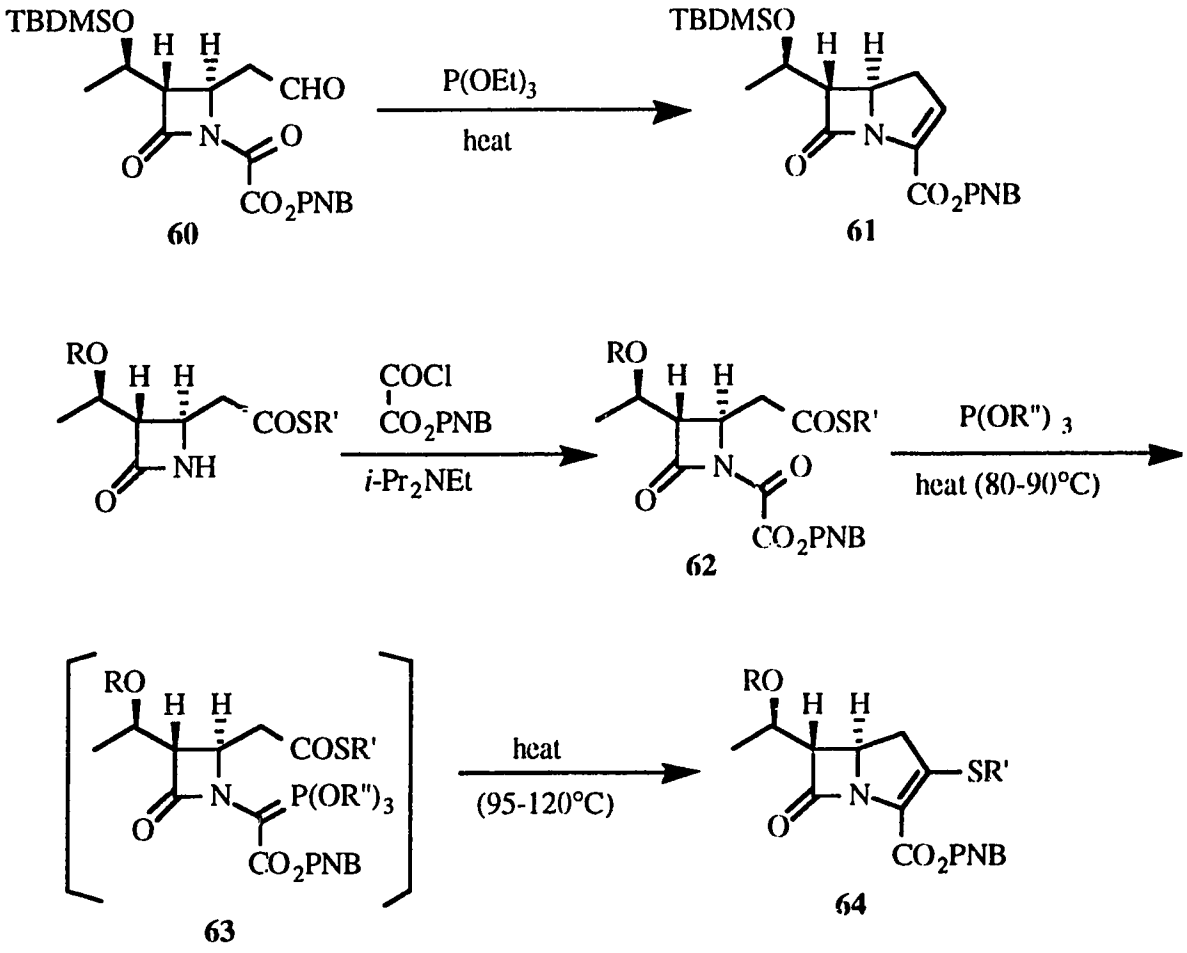


ONB: *o*-nitrobenzyl
PNB: *p*-nitrobenzyl

Scheme 1-4

with intramolecular triphenylphospholane group to give the desired carbapenem **57**.[112] Beecham researchers also showed that some thioesters such as **58**, which was much less reactive than the aldehyde, gave the cyclized product **59** when it was heated in toluene (Scheme 1-4).[113]

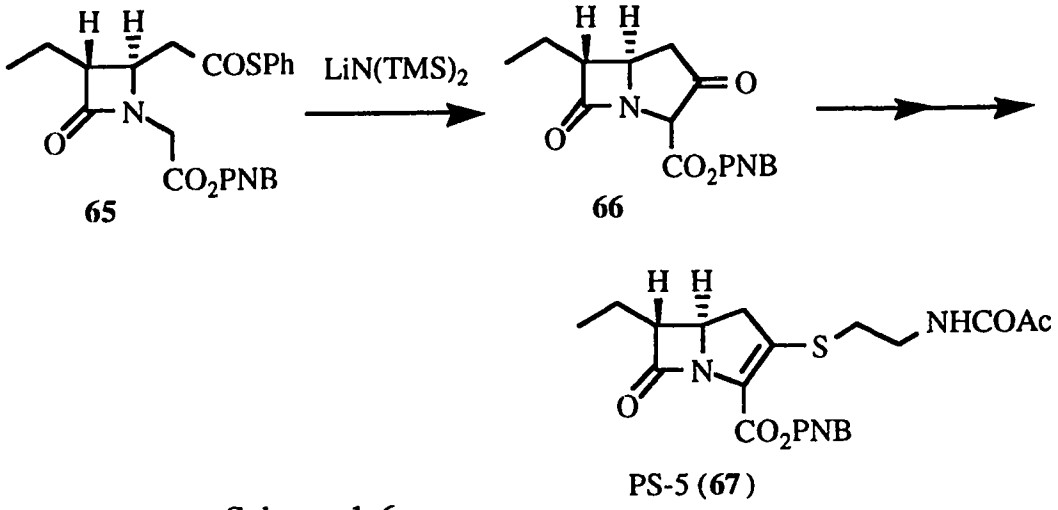
Another type of intramolecular Wittig reaction was independently reported from two groups. The Farmitalia-Calro Erba group succeeded in the reductive cyclization of the dicarbonyl compound **60** upon treatment with triethylphosphite to synthesize carbapenem **61**.[114] The Sankyo researchers



Scheme 1-5

synthesized a series of 2-alkylthio-substituted carbapenems **64** using a similar reaction (**62**→**64**), but they demonstrated that this reaction proceeded *via* an intramolecular Wittig reaction by isolating the trialkyloxyphospholane **63**, as an intermediate.[115] It was claimed that the trialkyloxyphospholanes were more reactive than the corresponding triphenylphospholanes in this intramolecular Wittig cyclization (Scheme 1-5).

Hatanaka and coworkers synthesized (±)-PS-5 (**67**) *via* the azetidinone **65** using the intramolecular Dieckmann-type condensation reaction to construct the bicyclic ring system **66**.[116] As a base, lithium bis(trimethylsilyl)amide was successfully used (Scheme 1-6).



Scheme 1-6

1.3.3 Syntheses of key intermediates

The stereoselective syntheses of the key intermediates such as the azetidinone-4-acetic acid derivative **68** and azetidinone-4-acetoacetate **69** have been extensively studied by many research groups and consequently a variety of methodologies has been established.

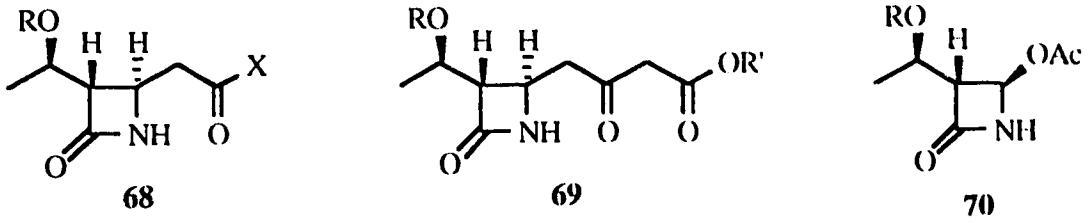
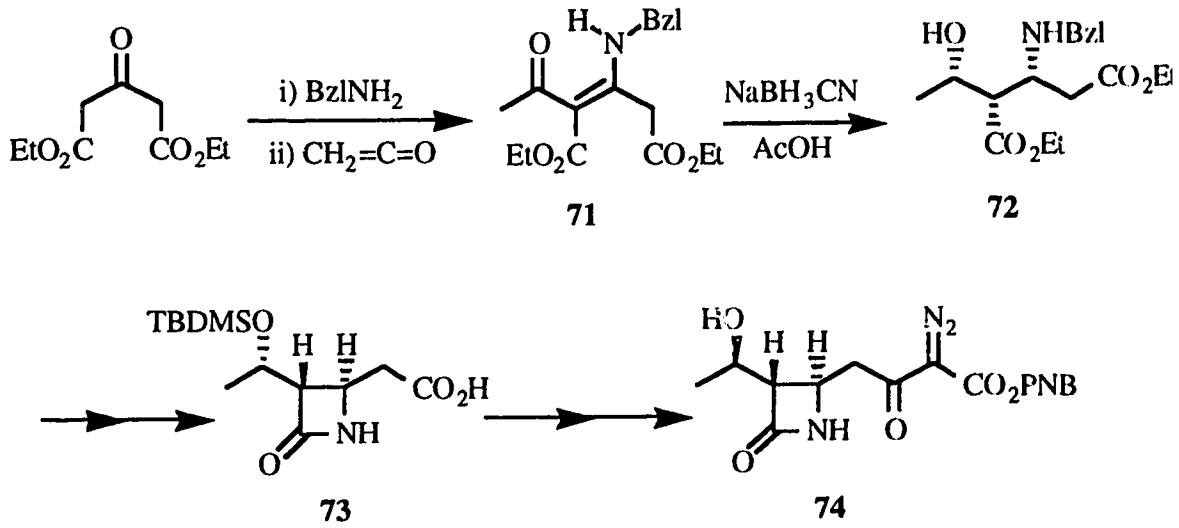


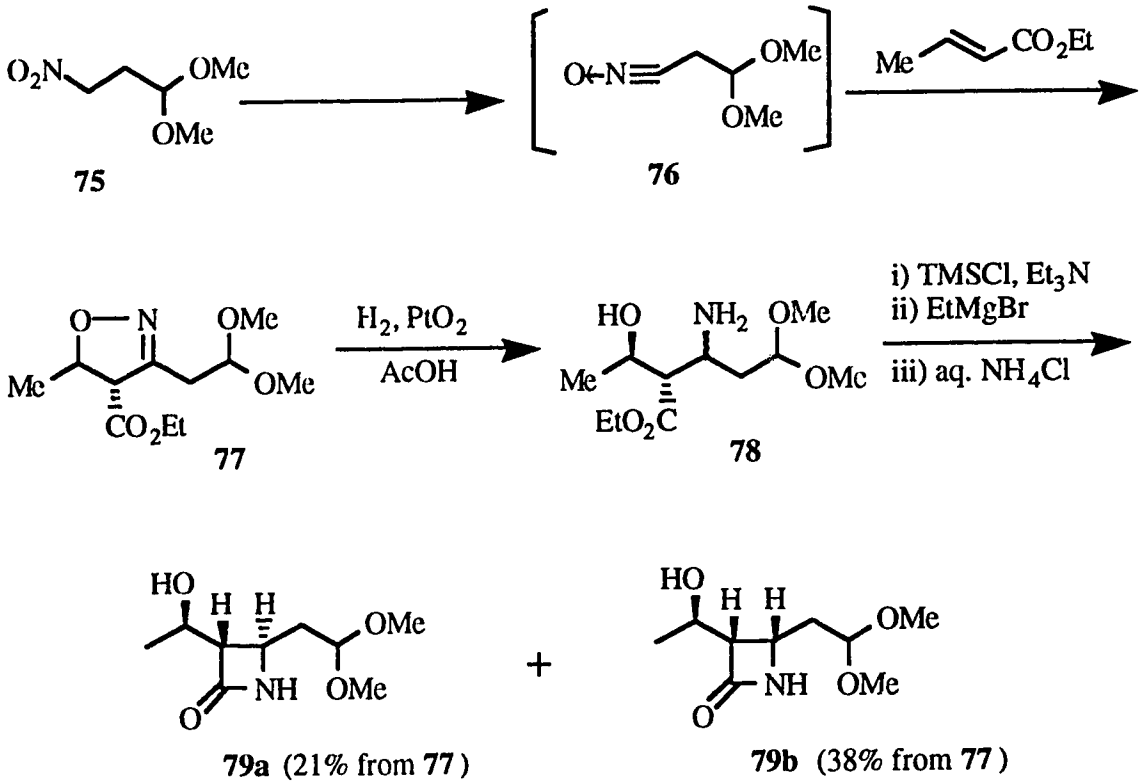
Figure 1-12 Structures of key intermediates for the carbapenem synthesis.

Mellillo and coworkers in Merck developed the first practical procedure to produce the azetidinone-4-acetoacetate **74** using a stereoselective reduction of the enamine **71** as a key reaction as shown in scheme 1-7.[117] With this reduction, only one stereoisomer **72** was reported to be formed. Subsequent elaboration led to the azetidinone **73** which was then converted into the acetoacetate **74** by side chain elongation and inversion of the hydroxy group (Scheme 1-7).



Scheme 1-7

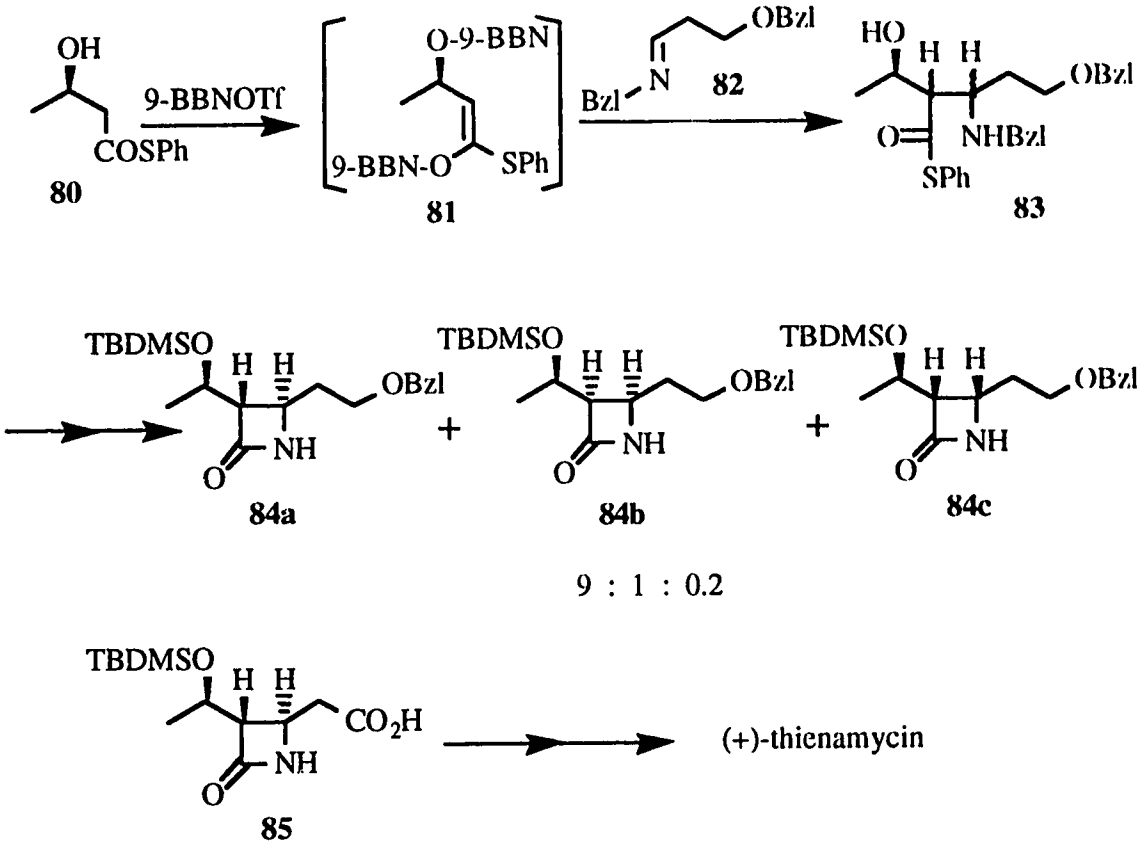
Kametani *et al.* constructed the azetidinone-4-acetaldehyde dimethyl-acetals **79a** and **79b** *via* the *trans*-isoxazolidine-ester **77**, which was formed by the 1,3-dipolar cycloaddition of the nitrile oxide **76** and ethyl crotonate.[118] The catalytic hydrogenation of **77** over platinum oxide gave the aminoester **78**, which was converted into the azetidinone **79a** and **79b** by *N*-trimethylsilylation and cyclization with Grignard reagent, followed by desilylation (Scheme 1-8).



Scheme 1-8

Iimori and coworkers prepared the optically active azetidinones **84a-c** utilizing the aldol-type condensation of the boron enolate **81** and the imine **82** as a key step.[119] The desired stereoisomer **84a** was obtained as a major

product among three isolated stereoisomers after cyclization of **83** (Scheme 1-9).

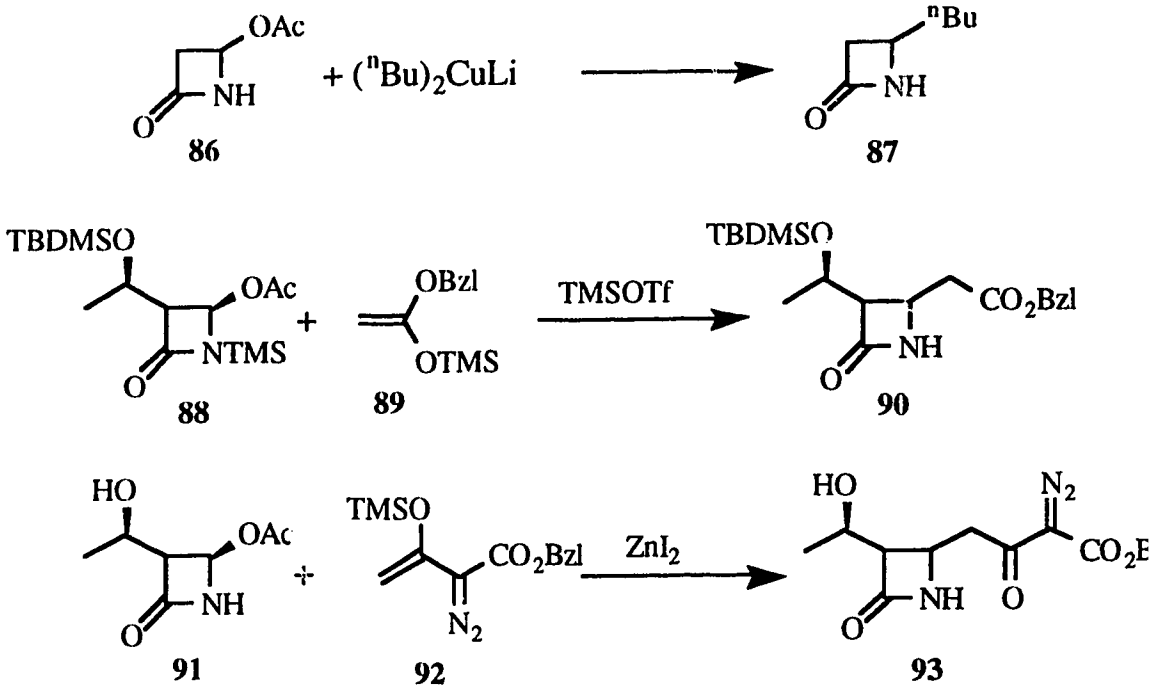


Scheme 1-9

D-Glucosamine and D-Glucose were also used as chiral starting materials to synthesize the optically active thienamycin intermediates such as **68** and **69** by many groups working independently.[120]

4-Acetoxyazetidinone **70** was recognized as an extremely versatile intermediate for the synthesis of trans-carbapenems, when it was found that the acetoxy group at the 4-position could be readily displaced by a variety of *C*-substituents. The displacement reaction can be achieved by two different ways. One is by using an anionic reagent such as Grignard reagents, lithium

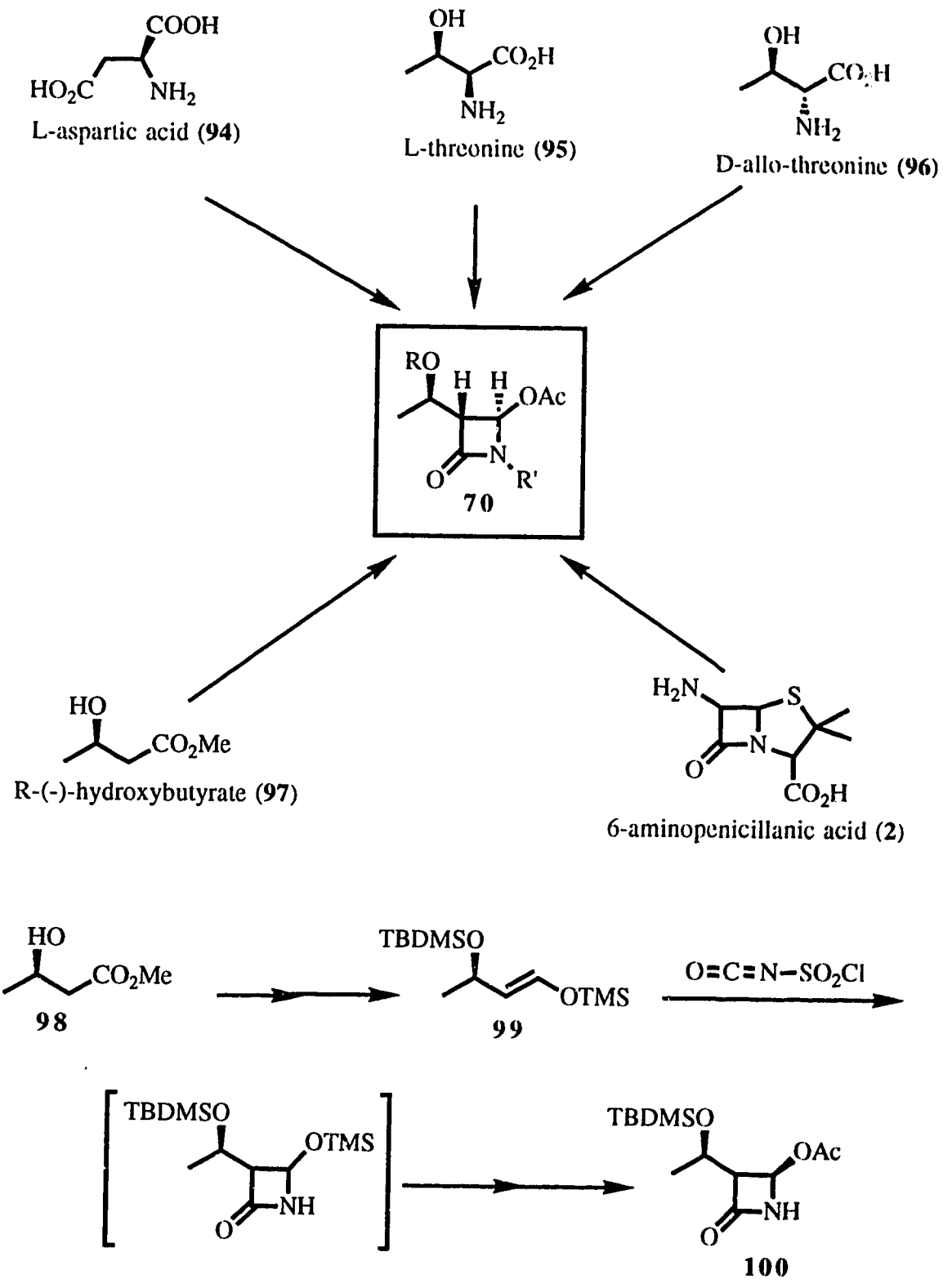
dialkylcuprates (**86**→**87**), and metal enolates.[121] Another is by the Lewis acid-catalyzed reaction of silylenol ether with acetoxyazetidinone. Using the latter method the azetidinone-4-acetate[122] **90** (from **88** and **89**) and azetidinone-4-acetoacetate[123] **93** (from **91** and **92**) have been readily prepared (Scheme 1-10).



86 **87** **88** **89** **90** **91** **92** **93**

Scheme 1-10

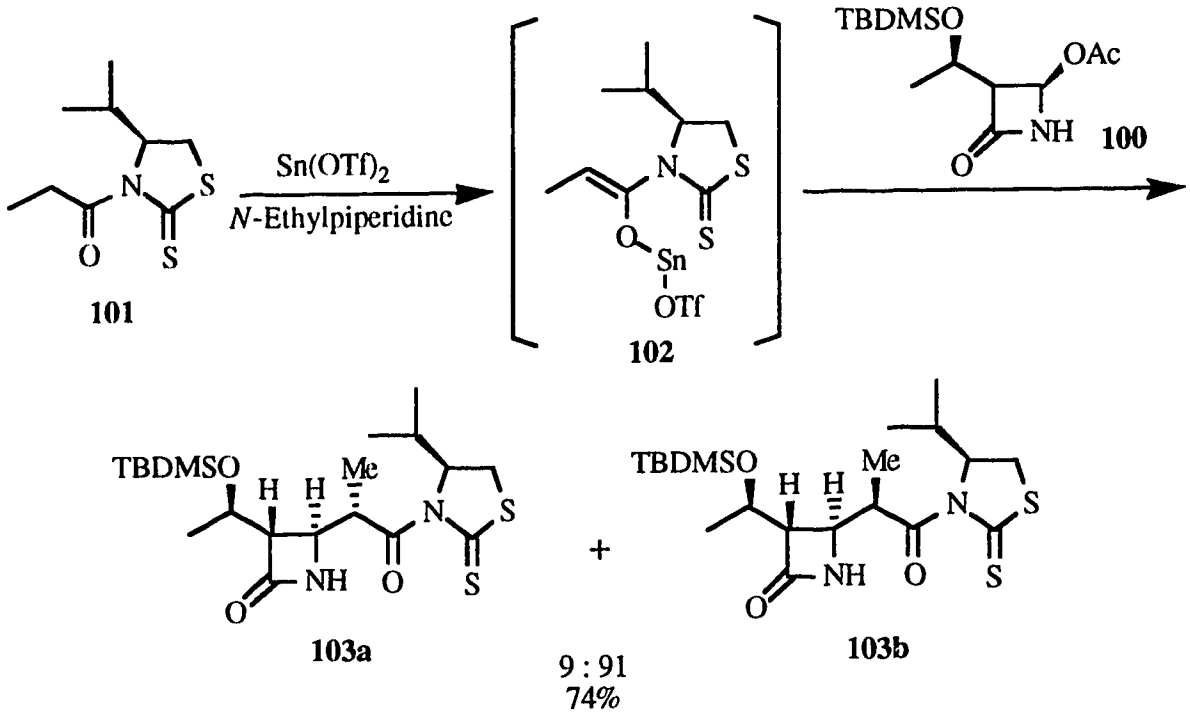
The enantioselective syntheses of the 4-acetoxyazetidinone **70** have been accomplished from various starting materials including L-aspartic acid[123] (**94**), L-threonine[124] (**95**), D-allothreonine[125] (**96**), *R*-(-)-hydroxy-butyrate[126] (**97**), and 6-aminopenicillanic acid[127] (6-APA) (**2**). Recently a group in Kanegafuchi Kagaku developed a practical large scale procedure to manufacture the 4-acetoxyazetidinone **100** starting from methyl *R*-3-


L-aspartic acid (94)
L-threonine (95)
D-allo-threonine (96)
70
R-(-)-hydroxybutyrate (97)
6-aminopenicillanic acid (2)
98
99
100

Scheme 1-11

hydroxybutanoate **98**, using the [2+2] cycloaddition reaction of the silylenol ether **99** and chlorosulfonyl isocyanate as a key reaction (Scheme 1-11).[128]

4-Acetoxyazetidinone **100** has also been used as an intermediate to synthesize 1-β-methylcarbapenems. Nagao and coworkers reported that β-methyl adduct **103b** was stereoselectively obtained in a ratio of 9:1 by the condensation reaction of the azetidinone **100** and the tin enolate **102** (Scheme 1-12).[129]



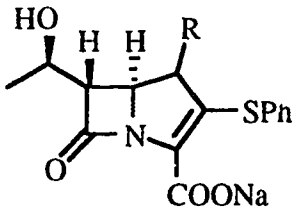
Scheme 1-12

Chapter 2
OBJECTIVES

The primary objective of the research covered in this thesis was to study the syntheses and biological properties of β-lactam compounds, which would, (a) possess broad anti-microbial spectra, or (b) inhibit β-lactamases to protect broad spectrum-β-lactam antibiotics from hydrolytic degradation.

During the last few decades, great advances have been made in the treatment of bacterial infectious diseases, and many classes of antibacterial drugs, including β-lactams, quinolones, aminoglycosides, macrolides, and others, have been commercialized. The *in vitro* and *in vivo* activities of these drugs indicate that almost all bacterial infections could be treated successfully if one or more of these drugs was properly chosen and used. Nevertheless, in practice, many bacterial infections are found to be life-threatening because of the following reasons: 1) Serious infectious diseases have to be treated as soon as possible, sometimes even before the causative bacteria are identified and it is difficult to choose the proper drug(s) without knowing the pathogens; 2) during the treatment, bacteria often acquire or develop resistance against the antibacterial agents used. In the long term, the number of drug-resistant bacteria increases due to the selective pressure by the use of the antibacterial agents; 3) because of the advances in medical science, immunologically compromised individuals, *e.g.* elderly people and patients with other serious diseases, live longer and become more susceptible to infections. Because of these reasons, new antibacterial agents with broader antibacterial spectra and a higher degree of antibacterial activity together with less susceptibility to bacterial resistance continue to be in great demand.

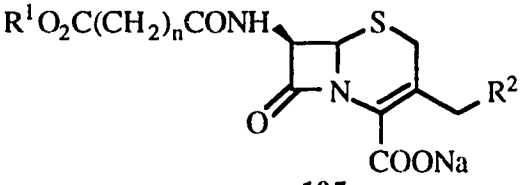
Since the discovery of thienamycin, the carbapenem class of β-lactam antibiotics has been regarded as the most promising group of β-lactam antibiotics in terms of antibacterial spectrum and activity. Extensive chemical modifications have been carried out at the 2- and 6-positions of carbapenems by many research groups, whereas the modification of the 1-position has been left relatively untouched despite the interesting finding that the introduction of a 1-β-methyl group made many carbapenems enzymatically more stable against the mammalian renal enzyme, dehydropeptidase-I. Therefore, it was anticipated that the synthesis of 1-substituted-carbapenems would possibly result in new carbapenems with improved antibacterial activity and pharmacokinetic properties. Based on this consideration, the objective of the first part of this study was the investigation of methods of synthesizing 1-substituted-carbapenems **104**. The substituents selected for introduction at the 1-position were small heterocyclic moieties such as the triazole(thio) and the tetrazole(thio) groups. These candidate substituents are not only relatively small in their molecular size, but are also expected to alter the nature of the carbapenem because of their polar nitrogen-rich systems. As models for the investigation of the synthetic route, other substituents including methyl, phenyl(thio), chloro and bromo were selected.



104

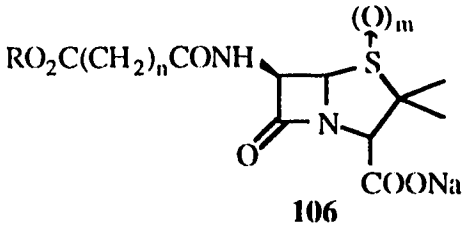
R = Heterocyclic(thio) substitutents
Me, Ph, PhS, Cl, Br

The second part of the study consisted of the syntheses and biological evaluation of cephems **105** and penams **106**, **107**, and **108** as β-lactamase inhibitors.



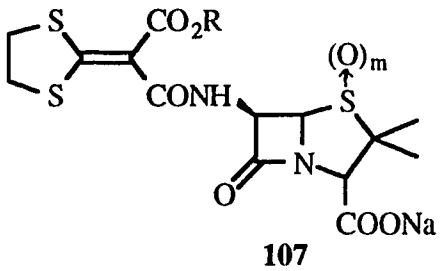
105

R^1 = Allyl or Na
R^2 = OAc or H
n = 0, 1, 2



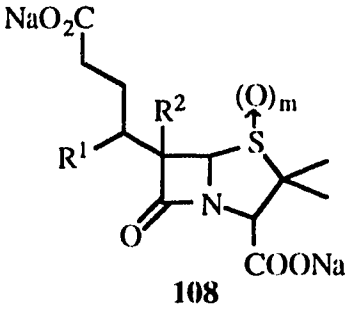
106

R = Me, Et, or Na
n = 0, 1, 2
m = 0, 2



107

R = Me, Et, or Na
m = 0, 2



108

a) R^1 = OH and R^2 = α-H
b) R^1 and R^2 = bond
m = 0, 2

Currently available β-lactamase inhibitors, *e.g.* clavulanic acid, sulbactam, and tazobactam, inhibit many penicillinases and broad-spectrum β-lactamases strongly and are regarded as excellent synergistic partners for the penicillin-type antibiotics. However, since these compounds are weak inhibitors of cephalosporinases, they are not considered to be clinically useful inhibitors with the cephalosporin-type antibiotics. Recent problems with cephalosporins are the hyper-production of cephalosporinases by Gram-

negative bacteria and the emergence and prevalence of extended-spectrum β-lactamases. This has necessitated the discovery of new β-lactamse inhibitors which could be combined with cephalosporins to overcome these problems. Based on this need, the second objective of the research was directed toward a study of β-lactamase inhibitors which would inhibit such enzymes as cephalosporinases, broad-spectrum β-lactamases, and extended-spectrum β-lactamases. The primary emphasis was on cephalosporinases, since the other two types of β-lactamases are known to be inhibited by other potent inhibitors, whereas inhibitors of cephalosporinases have not yet been reported.

For this work, the effect of an (un)protected carboxyl group or its equivalent in the 7-position side chain of cephems and the 6-position side chain of penams on their β-lactamase inhibitory activities was systematically studied.

Chapter 3

RESULTS AND DISCUSSION - 1

SYNTHESES OF 1-SUBSTITUTED CARBAPENEMS

In this chapter, various attempts at the synthesis of 1-substituted carbapenems are described. Also, the comparative antimicrobial activity of some 1-substituted carbapenems will be mentioned.

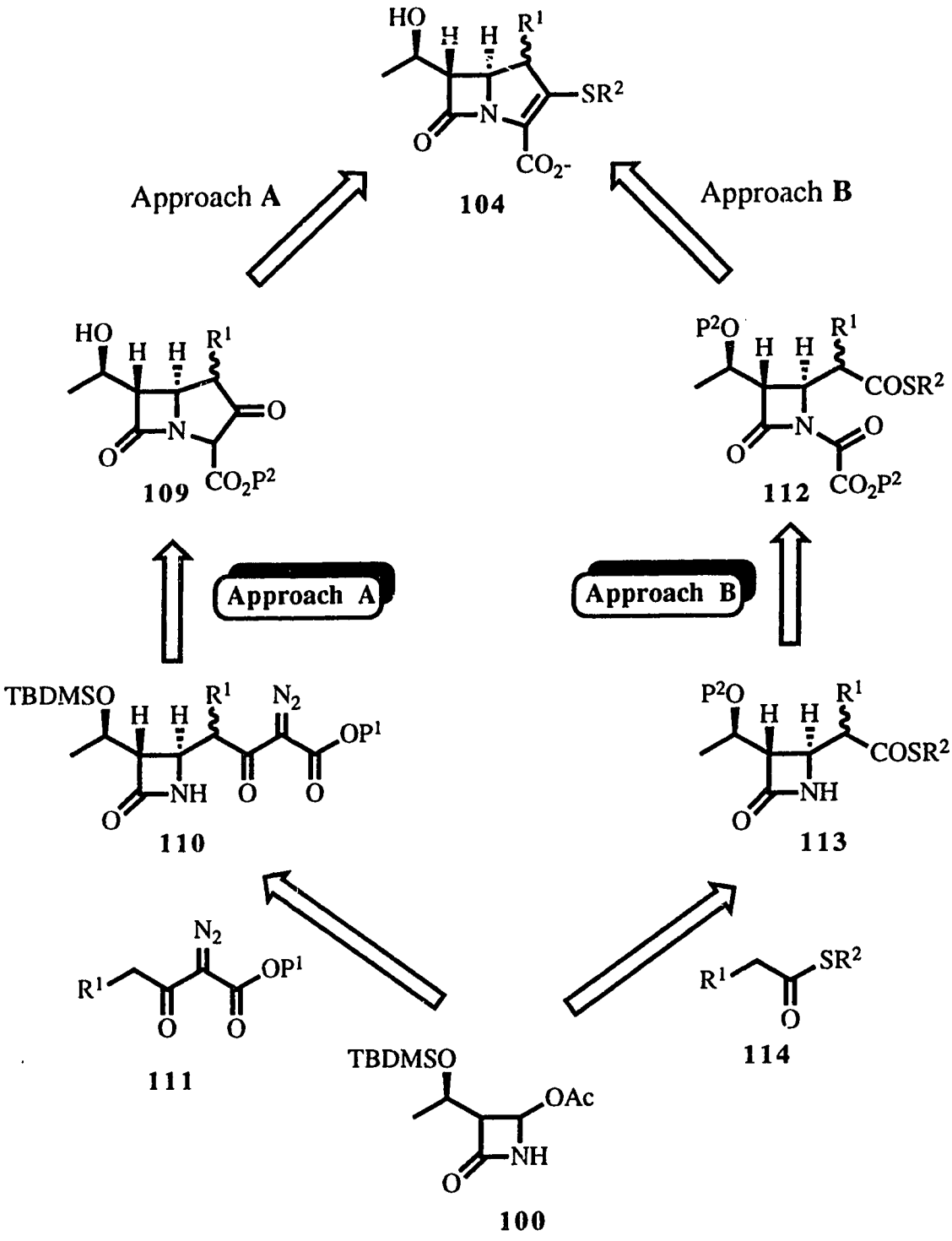
3.1 Synthetic strategy

Scheme 3-1 summarizes the strategy for the syntheses of the 1-substituted carbapenems **104**. Two approaches, which have been the most extensively studied and appear to be the most feasible, have been selected for this study. In this scheme, P^1 and P^2 represent suitable protecting groups.

In approach **A**, the carbene insertion reaction was used as the key step for the construction of the carbapenem bicyclic ring system (**110**→**109**). The precursor **110** for the carbene insertion reaction was derived from the condensation reaction of the known 4-acetoxyazetidinone **100** with a suitable 4-substituted 2-diazoacetoacetate **111**. The advantage of this approach is that a variety of substituted-alkylthio groups may be introduced at the 2-position of the carbapenem **109** once the bicyclic ketoester is formed.

In approach **B**, the carbapenem skeleton was constructed directly by an intramolecular Wittig-type cyclization reaction by heating the oxalimide derivative **112** with a trialkylphosphite in toluene or xylene. The compound **112** was readily prepared from the azetidinone **113**, which was synthesized by the condensation reaction of the 4-acetoxyazetidinone **100** and an α-substituted thioester **114**.

In both approaches, the 4-acetoxyazetidinone **100** served as a key intermediate.


HO
H
H
R1
SR2
N
O
CO2-
104
Approach A
Approach B
HO
H
H
R1
O
N
O
CO2P2
109
P2O
H
H
R1
COSR2
N
O
O
CO2P2
112
Approach A
Approach B
TBDMSO
H
H
R1
N2
OP1
NH
O
O
O
110
P2O
H
H
R1
COSR2
NH
O
113
N2
R1
OP1
O
O
111
R1
SR2
O
114
TBDMSO
OAc
NH
O
100

Scheme 3-1

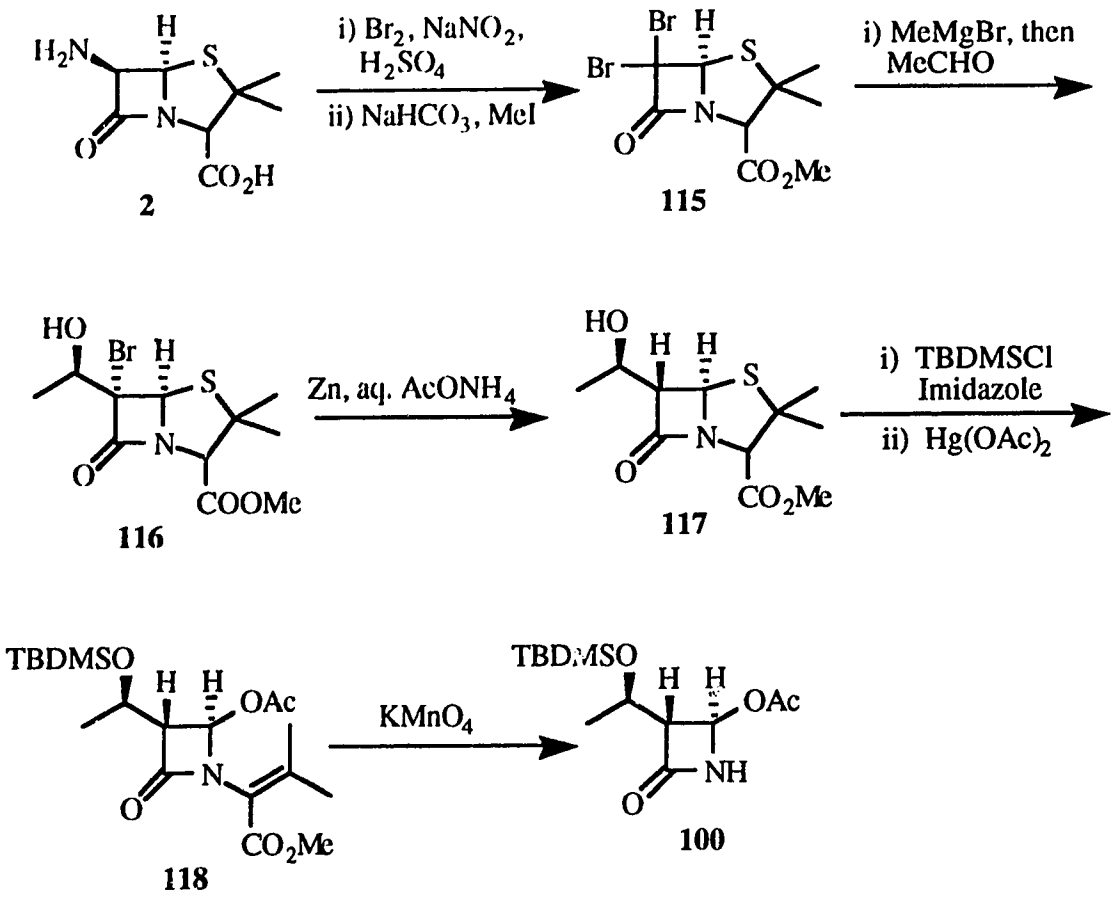
3.2 Synthesis of the 4-acetoxyazetidinone 100

As described earlier (Section 1.3.3), various methodologies for the synthesis of 4-acetoxyazetidinone derivatives have been reported by several groups. Of these, the procedure described by Leanza *et al.* was chosen for the synthesis of the common key intermediate **100** as shown in scheme 3-2, because of its simplicity.[127c]

Thus 6-aminopenicillanic acid (**2**) was first converted into the 6,6-dibromopenicillanate **115**. Introduction of a hydroxyethyl group at the 6-position of the penicillanate and successive debromination afforded the desired 6-α-(*R*)-hydroxyethyl-substituted penam **117** as the major product. Protection of the hydroxy group with a *tert*-butyldimethylsilyl (TBDMS) group, followed by ring cleavage using mercuric acetate in refluxing acetic acid gave the azetidinone **118**, and the final oxidative removal of the *N*-substituent using potassium permanganate gave the desired key intermediate, the 4-acetoxyazetidinone **100**.

3.3 Syntheses of acetoacetate derivatives

The 4-substituted-2-diazoacetoacetate derivatives, other key intermediates in approach **A**, were prepared by different methods, depending on the nature of the 4-substituting groups. At this stage, proper selection of the ester groups was essential, since these esters have to be cleaved at the last stage of the synthesis, and deprotection reactions have to be executed under very mild conditions to keep the labile carbapenem nucleus intact. The *p*-nitrobenzyl (PNB)[107a] and allyl groups[130] are the most commonly used for the protection of the carbapenem carboxyl groups.



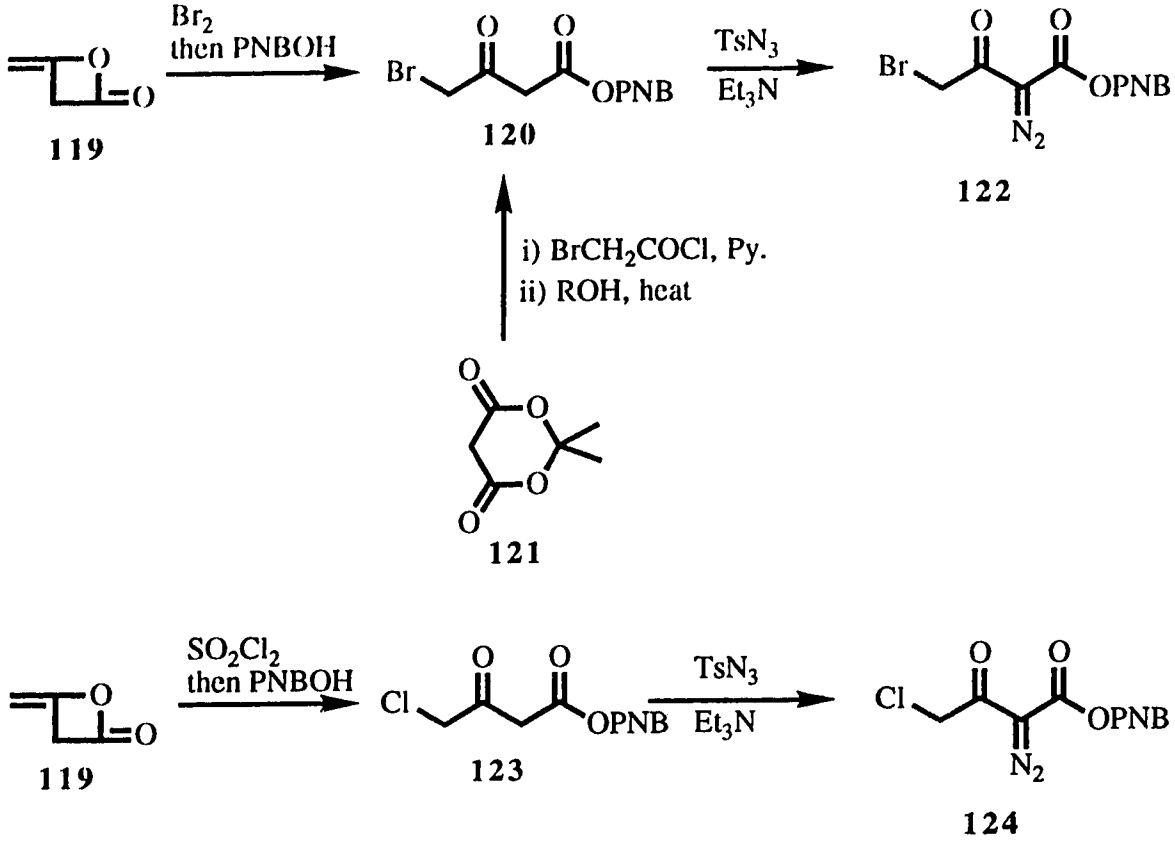
Scheme 3-2

The synthesis of the *p*-nitrobenzyl 4-bromoacetoacetate (**120**) was carried out by using two different methods. Bromination of diketene (**119**) with one molar equivalent of bromine at -78°C in dichloromethane, followed by the addition of *p*-nitrobenzyl alcohol afforded the desired bromoacetoacetate **120** in 68%, after flash silica gel column purification.[131] In this reaction, the order of the addition of reagents was found to be important. When bromine was added to the mixture of diketene and *p*-nitrobenzyl alcohol at -78°C, a complex mixture including the desired product **120**, *p*-nitrobenzyl acetoacetate and many other minor products were formed, whereas when *p*-nitrobenzyl alcohol was added at the end, the

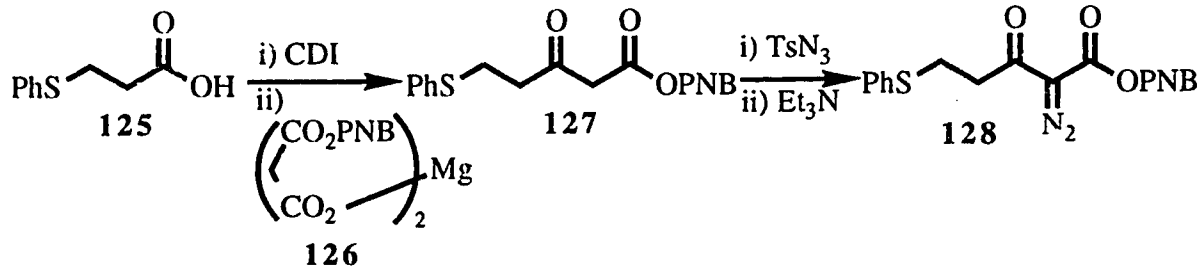
desired bromide **120** was obtained as a substantially single product. The bromoacetoacetate **120** was somewhat unstable at room temperature. Analogously, *p*-nitrobenzyl 4-chloroacetoacetate (**123**) was synthesized from diketene and sulfuryl chloride in 68% yield. Another procedure to synthesize the bromoacetoacetate **120** involved the bromoacetylation of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (**121**) and the thermolysis of the acylated Meldrum's acid in the presence of *p*-nitrobenzyl alcohol.[132] By this process, the bromoacetoacetate **120** was obtained in 42% yield from Meldrum's acid (**121**). The 4-bromo- and 4-chloro-acetoacetates **120** and **123** were converted into the 2-diazo derivatives **122** and **124** in 80% and 74% yields, respectively, by the diazo exchange reaction with *p*-toluenesulfonyl azide and triethylamine in dichloromethane.[133] This reaction was largely affected by solvent. For instance, when chloroform or toluene was used as solvent , no reaction took place (Scheme 3-3).

The synthesis of the 5-(phenylthio)ketoester **127** was achieved by the chain elongation reaction developed by Masamune.[134] β-(Phenylthio)-propionic acid (**125**) was first converted into the acylimidazole by the reaction with carbonyldiimidazole, then treated with magnesium *p*-nitrobenzyl malonate (**126**) to yield the ketoester **127** in 64% yield, which was then diazotized in a manner similar to that described above to give the diazo compound **128** in 79% yield (Scheme 3-4).

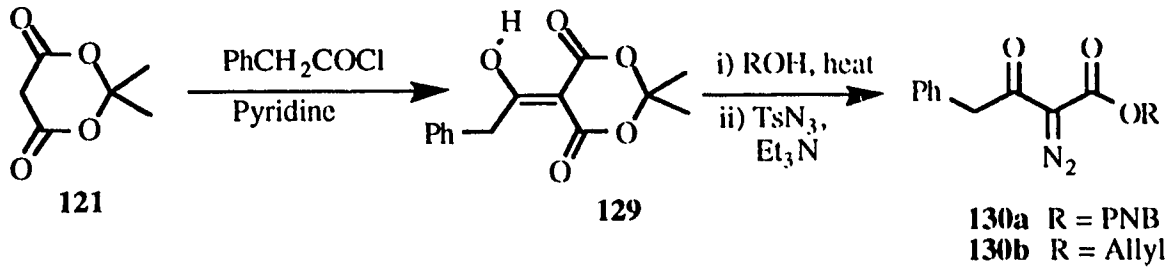
The 2-diazo-4-phenylacetoacetate derivatives **130a** and **130b** were synthesized from phenylacetyl chloride and Meldrum's acid in 57% and 66% overall yield, respectively, by a similar procedure utilized for the synthesis of the bromide (**121**→**122**) (Scheme 3-5).



Scheme 3-3



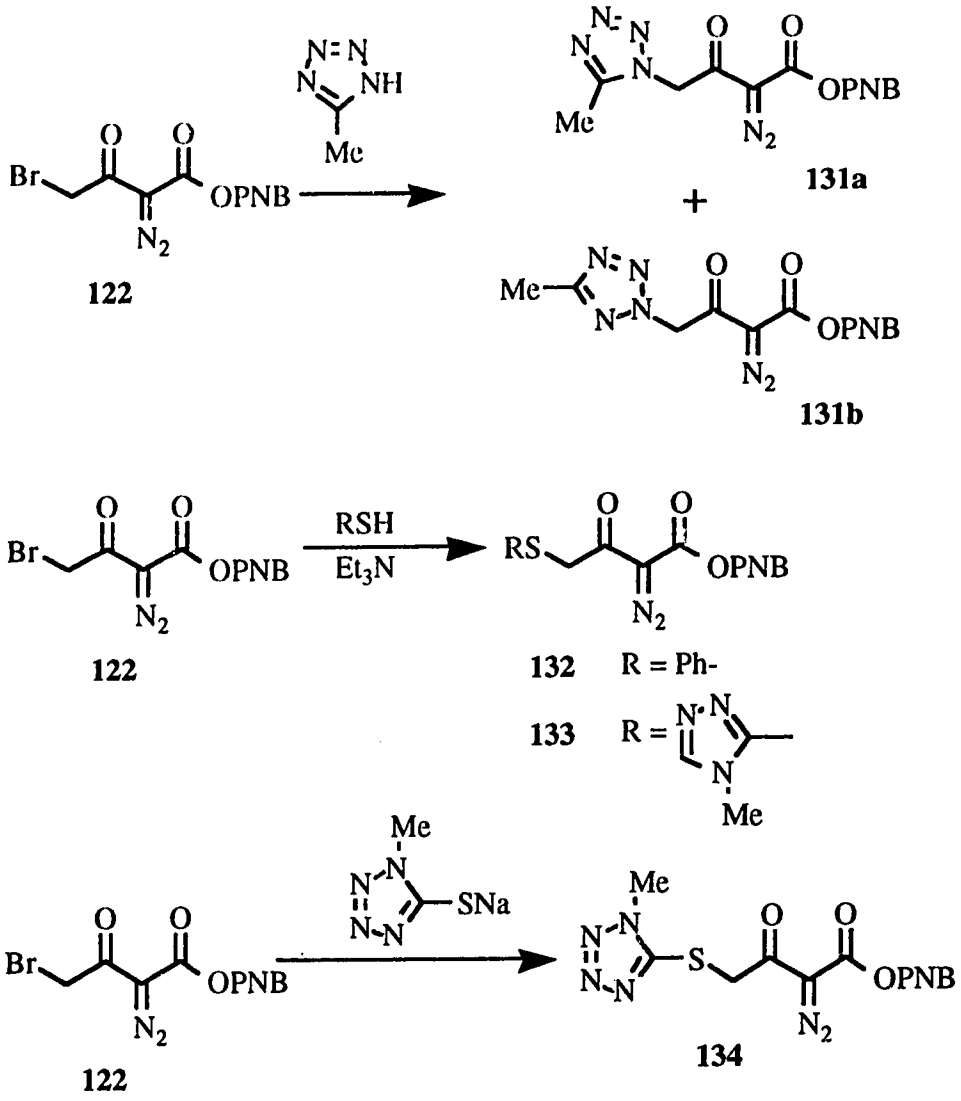
Scheme 3-4



Scheme 3-5

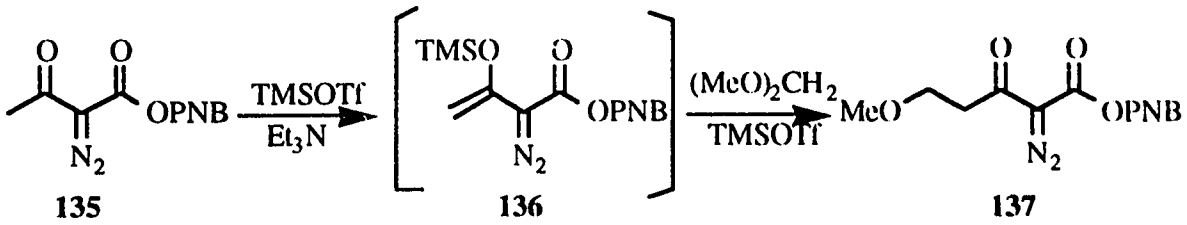
Various groups were introduced at the 4-position of the 2-diazoacetoacetate by nucleophilic displacement reactions. Treatment of *p*-nitrobenzyl 4-bromo-2-diazoacetoacetate (**122**) with 5-methyltetrazole and triethylamine in dichloromethane afforded a mixture of the *N*-substituted-tetrazole-acetoacetate derivatives, which were separated by silica gel column chromatography to give the *N*-1 isomer **131a** and the *N*-2 isomer **131b** in 52% and 32% yield, respectively. Determination of the regiochemistry of these two isomers was based on a comparison of the ^{1}H NMR spectra and the behavior on silica gel TLC with those of the known 5-methyltetrazole-acetic acid derivatives[135] (Scheme 3-6).

The 2-diazo-4-phenylthioacetoacetate **132** was prepared in 94% yield from the bromoacetoacetate **122** by treatment with thiophenol and triethylamine in dichloromethane. Similarly, the 4-(4-methyl-4-*H*-1,2,4-triazolylthio)-substituted derivative **133** was obtained in 95% yield from the bromide **122** and 3-mercapto-4-methyl-1,2,4-triazole. The 2-diazo-4-(1-methyltetrazole-thio)acetoacetate **134** was prepared in 90% yield by reacting the bromide **122** with the sodium 1-methyltetrazole-5-thiolate in tetrahydrofuran (Scheme 3-6).



Scheme 3-6

The methoxy derivative **137** was synthesized from *p*-nitrobenzyl 2-diazoacetoacetate (**135**) by the chain extension reaction. Treatment of **135** with triethylamine and trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave the silyl enol ether **136**, which was then treated with excess dimethoxymethane under Lewis acid (TMSOTf) catalytic condition to afford the desired ketoester **137** in 51% yield (Scheme 3-7).



135 136 137

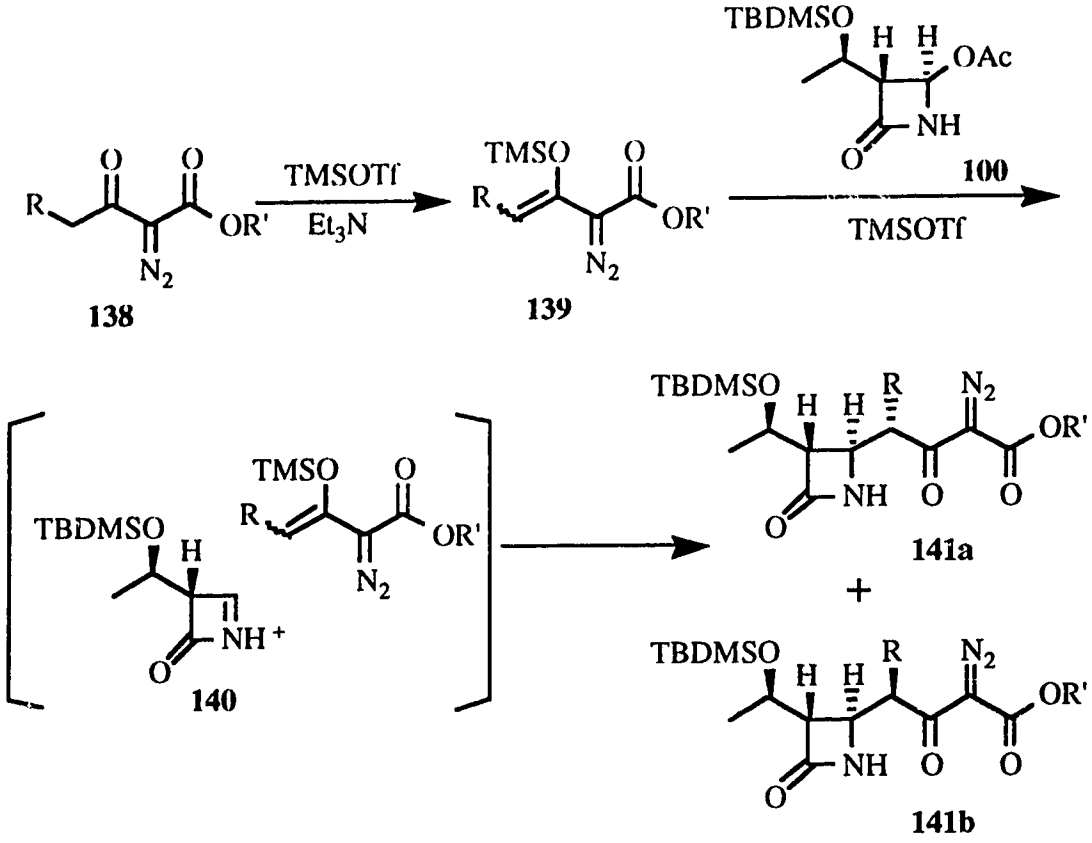
Scheme 3-7

3.4 Syntheses of azetidinone-4-acetoacetate derivatives.

The condensation reaction of the 2-diazo-4-substituted-acetoacetate derivatives with the 4-acetoxyazetidinone **100** was used to synthesize the azetidinone-4-acetoacetate derivatives. The modified procedure described by Karady *et al.* was utilized for this type of reaction[136].

In general, the diazoacetoacetate **138** was first treated with triethylamine and TMSOTf to form the silyl enol ether **139**, which was then treated with the 4-acetoxyazetidinone **100** under Lewis acid catalytic conditions to afford a stereoisomeric mixture of the 4-substituted azetidinones **141a** and **141b**. TMSOTf was found to be a good Lewis acid catalyst for this condensation reaction. This reaction is believed to proceed *via* an aldol-type condensation between the reactive iminium intermediate **140** and the silyl enol ether **139** (Scheme 3-8).

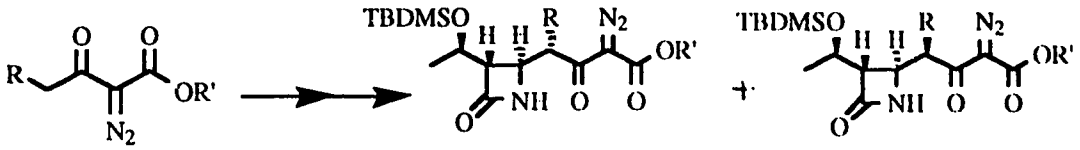
When the acetoacetate derivatives **122**, **124**, **130**, **131**, **133** were used as starting materials, the condensation reaction proceeded smoothly to give a mixture of desired products **142a,b**, **143a,b**, **144a,b**, **145a,b**, and **146a,b**, respectively. In all cases, 3,4-*cis*-substituted product was not detected in the reaction mixture. This result indicated that the nucleophilic attack of the silyl enol ether **139** had taken place stereoselectively from the less hindered β-face of the iminium intermediates **140**.

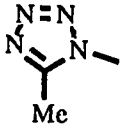
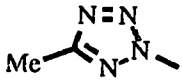
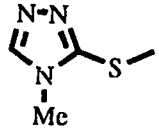
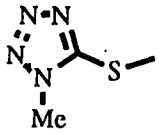


Scheme 3-8

Table 3-1 summarizes the results of the condensation reactions. No significant stereoselectivity for the formation of the α- and β-isomers was observed. The reaction yield (α-isomer + β-isomer), based on the 4-acetoxyazetidinone **100**, was found to be affected considerably by the amount of the diazoacetoacetate derivatives used for trimethysilylation. Thin layer chromatography of the reaction indicated that a considerable amount of the *N*-1-trimethylsilyl substituted azetidinone **147** was formed during the reaction. This result implied that the trimethylsilyl group had migrated from the silyl enol ether **139** to the azetidinone during the condensation reaction, resulting in the consumption of the reactive silyl enol ether (**139→148**). This

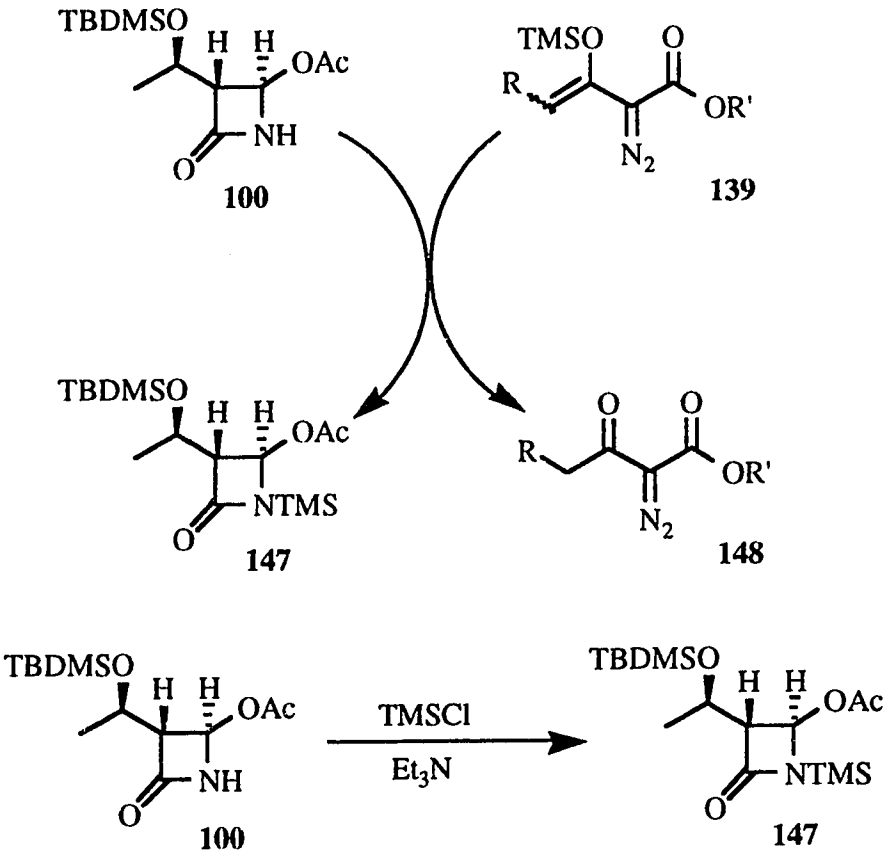
Table 3-1 Results of the condensation reaction of the acetoacetates and the 4-acetoxyazetidinone.



Starting compounds			Products and Yields (%)*	
No.	R	R'	α-isomer	β-isomer
122	Br	PNB	142a (42)	142b (30)
124	Cl	PNB	143a (35)	143b (37)
130	Ph	PNB	144a (33)	144b (22)
131	Ph	Allyl	145a (30)	145b (40)
133	PhS	PNB	146a (34)	146b (32)
132a		PNB	(0)	(0)
132b		PNB	(0)	(0)
134		PNB	(0)	(0)
135		PNB	(0)	(0)

* Yields are based on the 4-acetoxyazetidinone **100**.

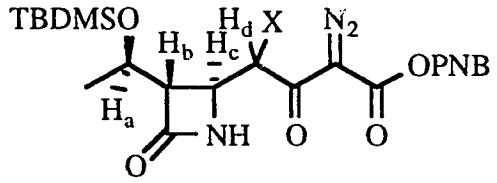
problem was solved either by using excess silylated diazoacetoacetate or by protecting the amide group of the azetidinone with a trimethylsilyl group prior to the condensation reaction (**100**→**147**). For practical purposes, the former method was used because of the simplicity of the operation, and ease of recovery of the excess diazoacetoacetate from the reaction mixture by silica gel column chromatography (Scheme 3-9).



Scheme 3-9

The stereochemistry of the condensation products has been assigned by careful comparison of the ^1H NMR spectra of both isomers. ^1H NMR data are summarized in Table 3-2. From molecular model studies, it was expected that the α-stereoisomer **141a** and β-isomer **141b** were likely to take

Table 3-2 ^{1}H NMR data of the azetidinone-4-acetoacetates.



		Chemical shifts:δ (coupling constants:Hz)				
No.	X	CH_3	H_a	H_b	H_c	H_d
142a	α-Br	1.31 (6)	4.27-4.33	3.04 (2)	4.36 (2, 10)	5.17 (10)
142b	β-Br	1.19 (6)	4.18-4.28*	3.04 (2, 4)	4.18-4.28*	5.57 (4)
143a	α-Cl	1.24 (6)	4.22-4.29*	3.06 (2)	4.22-4.29*	5.11 (9)
143b	β-Cl	1.18 (6)	4.15-4.25	3.11 (2)	4.13 (2, 4)	5.49 (4)
144a	α-Ph	0.22 (6)	4.00-4.06	2.80 (2)	4.31 (2, 10)	4.64 (10)
144b	β-Ph	1.16 (6)	4.04-4.16	2.66 (2, 4)	4.22 (2, 6)	4.90 (6)
146a	α-SPh	1.29 (6)	4.20-4.30	2.99 (2)	4.27 (2, 10)	4.82 (10)
146b	β-SPh	1.10 (6)	4.10-4.22	2.99	4.07 (2, 7)	5.00 (7)

* Overlapping peaks

conformations **A** and **B**, respectively, as a result of an intramolecular hydrogen bonding and the steric interactions between vicinal substituents (Figure 3-1). The dihedral angles of Hc-C-C-Hd in conformations **A** and **B** were measured as approximately 150°-180° and 30°-60° respectively, therefore, the expected coupling constants were calculated to be approximately 8-10 Hz for the α-isomers and 2-6 Hz for the β-isomers.[137] Based on this consideration, compounds **142a, 143a, 144a, 145a, 146a**, with coupling constants of 9-10 Hz, were assigned as the α-isomers, and isomers **142b, 143b, 144b, 145b, 146b**, with coupling constants 4-7 Hz, were assigned as the β-isomers. To confirm these assignments, NOE studies were carried out using **142a** and **142b**. Thus, Hd was irradiated and the NOE effect on Hc was determined. As expected from the comformations **A** and **B** in Figure 3, a stronger NOE effect was observed in **142b** than in **142a**. Furthermore, the unusually high chemical shift of the methyl group in one of the phenyl-substituted derivatives **144a** could be attributed to an anisotropic effect by the phenyl group which supported the above assignment, since an anisotropic effect was reasonably expected only for the α-isomer in conformation **A**.

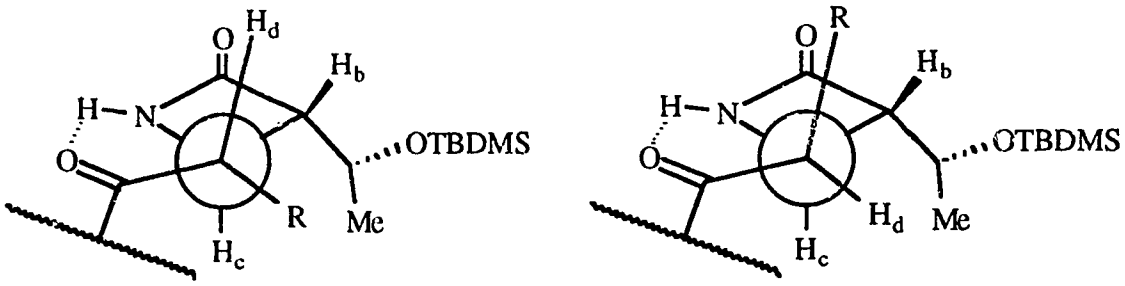
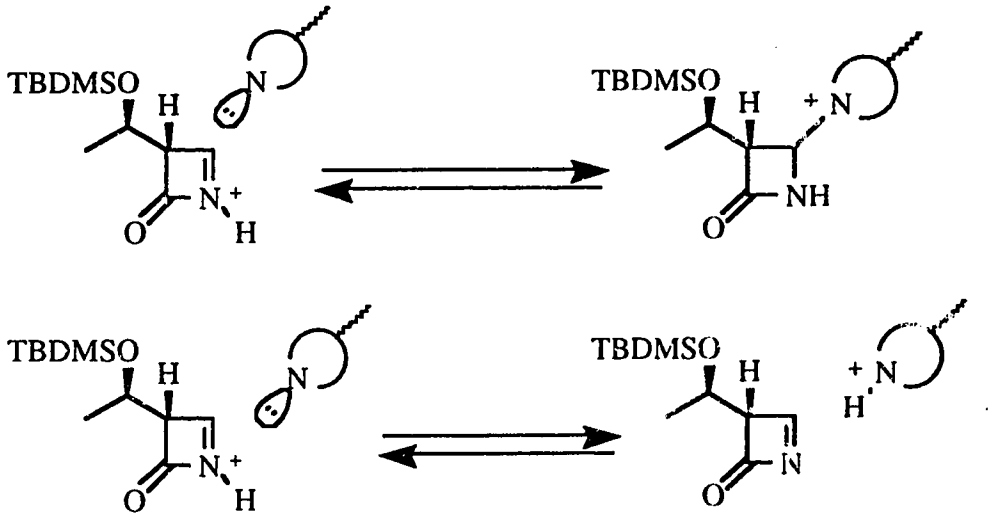


Figure 3-1 Conformations of azetidinone-4-acetoacetate stereoisomers.

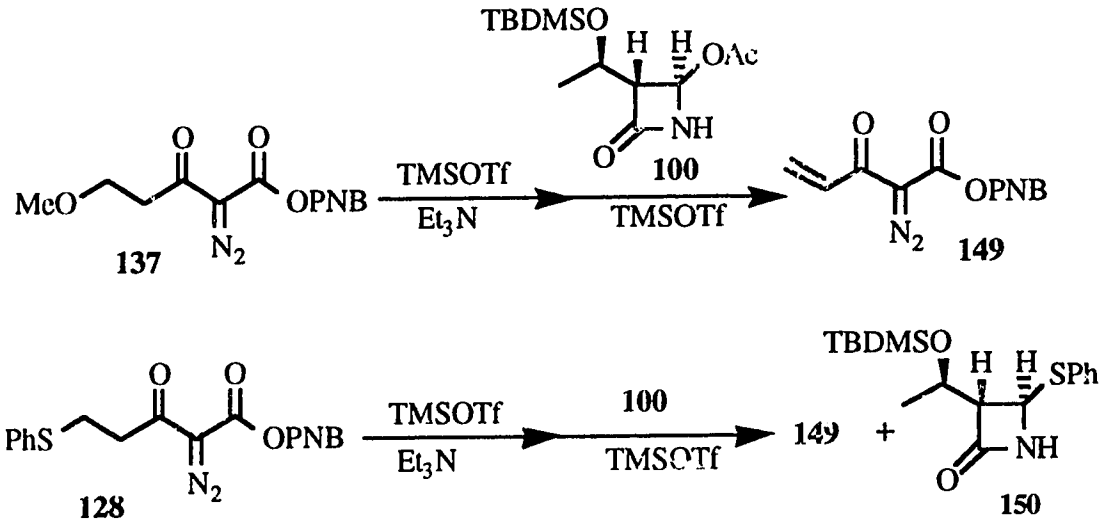
Unlike the above successful results, when the acetoacetate derivatives, **132a**, **132b**, **134**, and **135** were used as starting materials, the condensation reactions failed. After the reaction, most of the starting acetoacetate derivatives were recovered unchanged, while the acetoxyazetidinone **100** was extensively decomposed. Since all of these acetoacetate derivatives possess a nitrogen-containing heterocyclic group in their structures, the following reasons have been considered as possible causes of their unreactivity: 1) The Lewis acid (TMSOTf) interacts with an electron lone pair on the heterocyclic nitrogen, and consequently, the catalyst is inactivated. 2) The heterocyclic nitrogen interacts with the iminium intermediate, making the intermediate **140** unavailable for reaction with the silyl enol ethers. 3) An acidic proton in the iminium moiety is abstracted by the nitrogen containing heterocycle making the azetidinone intermediate less reactive (Scheme 3-10).



Scheme 3-10

The first possibility was shown to be unlikely, because no improvement of the reaction was observed when an excess of the Lewis acid was used. In addition, other Lewis acids, such as tin (IV) chloride, titanium (IV) chloride, zinc (II) chloride, and boron trifluoride etherate were also utilized, however no reaction was observed.

When the ketoester **137** was used as a starting material, no condensation reaction proceeded, instead, the α,β-unsaturated ketoester **149** was formed as a result of elimination of methanol. Similarly, from the phenylthio-substituted ketoester **128**, the α,β-unsaturated ketoester **149** was obtained. Additionally, the 4-(phenylthio)azetidinone **150** was isolated from the reaction mixture (Scheme 3-11).



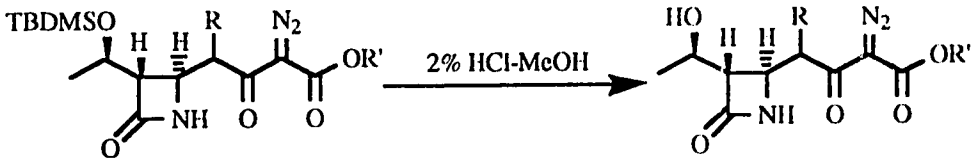
Scheme 3-11

3.5 Deprotection of TBDMS group

The azetidinone-4-acetoacetate derivatives **142a,b**, **143a,b**, **144a,b**, **145a,b**, and **146a,b**, whose syntheses are described in the preceding section, were then converted into the hydroxy compounds **151a,b**, **152a,b**, **153a,b**,

154a,b, and **155a,b**, by deprotection of the TBDMS group. Preliminary experiments revealed that this deprotection reaction could be successfully carried out by several procedures, including i) treatment with boron trifluoride etherate in acetonitrile[138] ii) treatment with trifluoroacetic acid in various solvents, or iii) treatment with 2% aq. HCl in methanol. Because of simplicity and high conversion yield, the third method was chosen to convert the above derivatives to the related alcohols. Thus the TBDMS-protected compounds were dissolved in methanol, then the appropriate amount of concentrated HCl solution was added dropwise. The results of the deprotection reactions are summarized in Table 3-3.

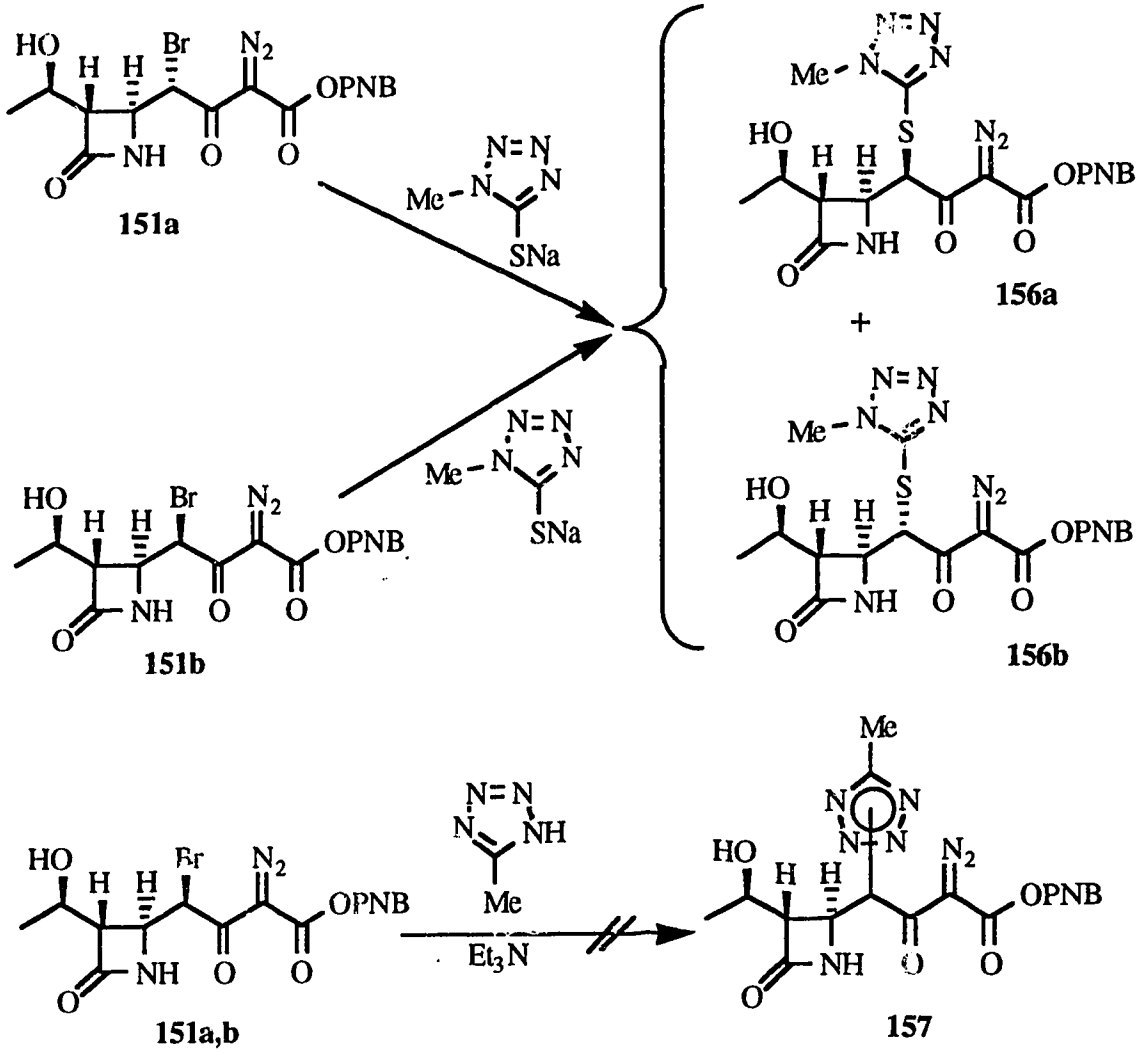
Table 3-3 Results of the desilylation reaction.



Starting compounds			Products
No.	R	R'	Yields (%)
142a	α-Br	PNB	151a (83)
142b	β-Br	PNB	151b (79)
143a	α-Cl	PNB	152a (90)
143b	β-Cl	PNB	152b (76)
144a	α-Ph	PNB	153a (84)
144b	β-Ph	PNB	153b (84)
145a	α-Ph	Allyl	154a (74)
145b	β-Ph	Allyl	154b (51)
146a	α-PhS	PNB	155a (86)
146b	β-PhS	PNB	155b (87)

3.6 Modification of the 4-position side chain of azetidinone

As described in Section 3.4, the synthesis of the heterocyclic-substituted azetidinone derivatives by the condensation reaction was found to be unsuccessful. Therefore, to synthesize these derivatives, another approach involving a nucleophilic substitution reaction was attempted.



Scheme 3-12

Upon treatment of the α-bromo-substituted azetidinone **151a** with sodium 1-methyltetrazole-5-thiolate in tetrahydrofuran, all the starting material was converted into the desired product. However due to rapid epimerization during the reaction, the obtained product was a stereoisomeric mixture of two isomers **156a** and **156b**. Although these isomers could not be separated, the NMR spectrum indicated that the ratio of these isomers was approximately 1:1. Also from the β-isomer **151b**, the same products with approximately the same ratio were obtained (Scheme 3-12).

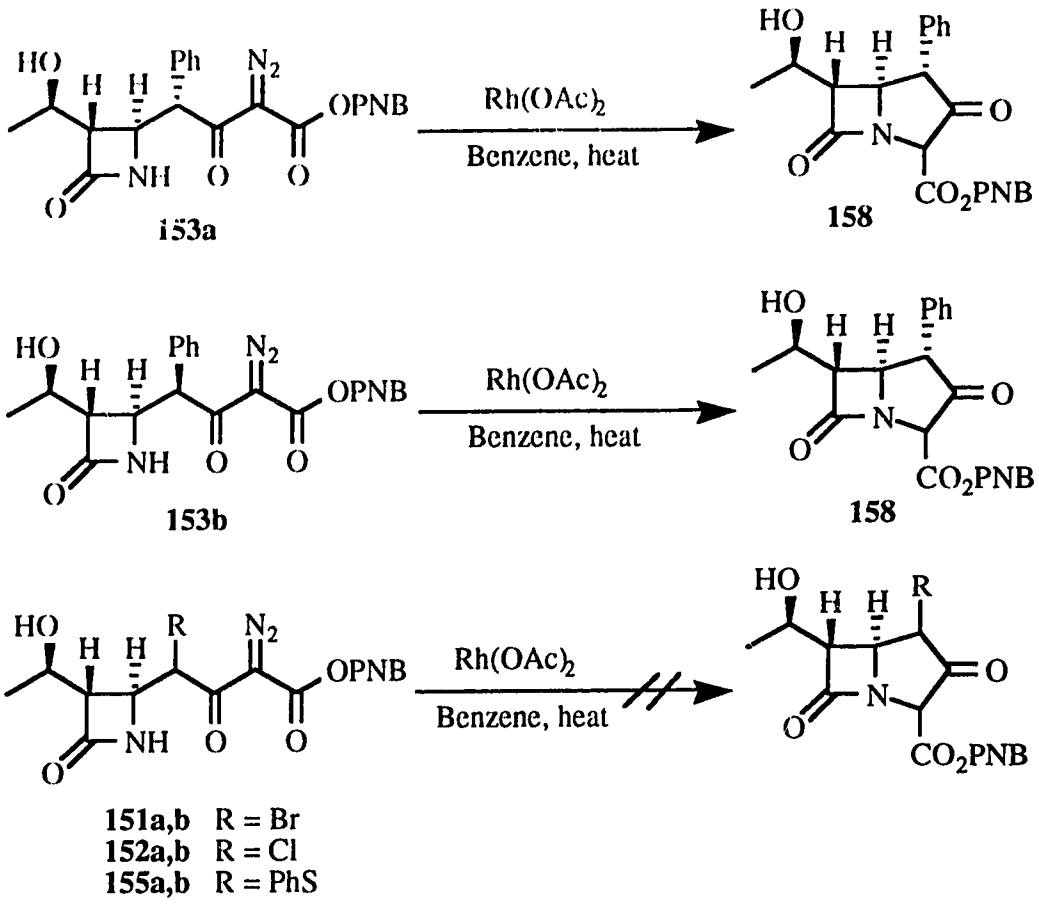
Attempts to introduce a tetrazole group (**151a,b→157**) by the same type of substitution reaction were not successful.

3.7 Carbene insertion reaction

In the next step, the conversion of the diazo intermediates, obtained in the above reactions, into the bicyclic ketoesters **109** was attempted using the carbene insertion reaction[109], in the next step.

Cyclization of the α-phenyl diazo compound **153a** was successfully carried out by treating **153a** with a catalytic amount of rhodium (II) acetate in refluxing benzene to give the desired bicyclic compound **158** in 67% yield. Similar treatment of the β-isomer **153b** also resulted in the cyclized product in 72% yield, however this product was found not to be the desired 1-β-phenyl derivative, but the epimerized 1-α-phenyl derivative **158** (Scheme 3-13).

On the other hand, the other diazo derivatives **151a,b**, **152a,b**, and **155a,b**, upon treatment with rhodium (II) acetate in various solvents and under various conditions, did not afford the desired cyclized products. From TLC and NMR studies, extensive decomposition was observed in these cases.



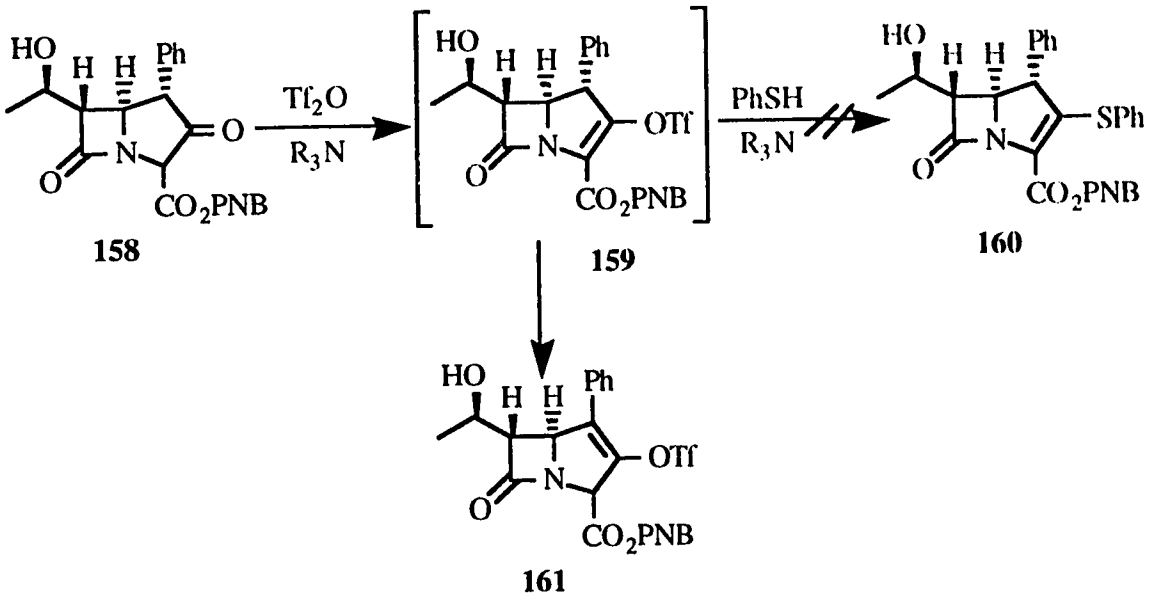
Scheme 3-13

3.8 Attempts to synthesize 1-phenyl-2-substituted-carbapenem

Conversion of the 1-α-phenyl-carbapenem ketoester **158** into the 2-substituted-carbapenem **160** was attempted in the next step. For this purpose, the phenylthio group was selected as a group to be introduced at the 2-position of carbapenem.

Thus the keto ester **158** was first treated with a base (triethylamine or diisopropylethylamine) and trifluoromethanesulfonic anhydride at -30°C to form the activated enol ester **159**, which was then treated with thiophenol and a base.[139] The addition-elimination reaction was expected to yield the desired 2-phenylthio derivative **160**. However only the double bond-

migrated product **161** was isolated from the reaction mixture. Although various conditions were studied, the desired product was not isolated (Scheme 3-14).



Scheme 3-14

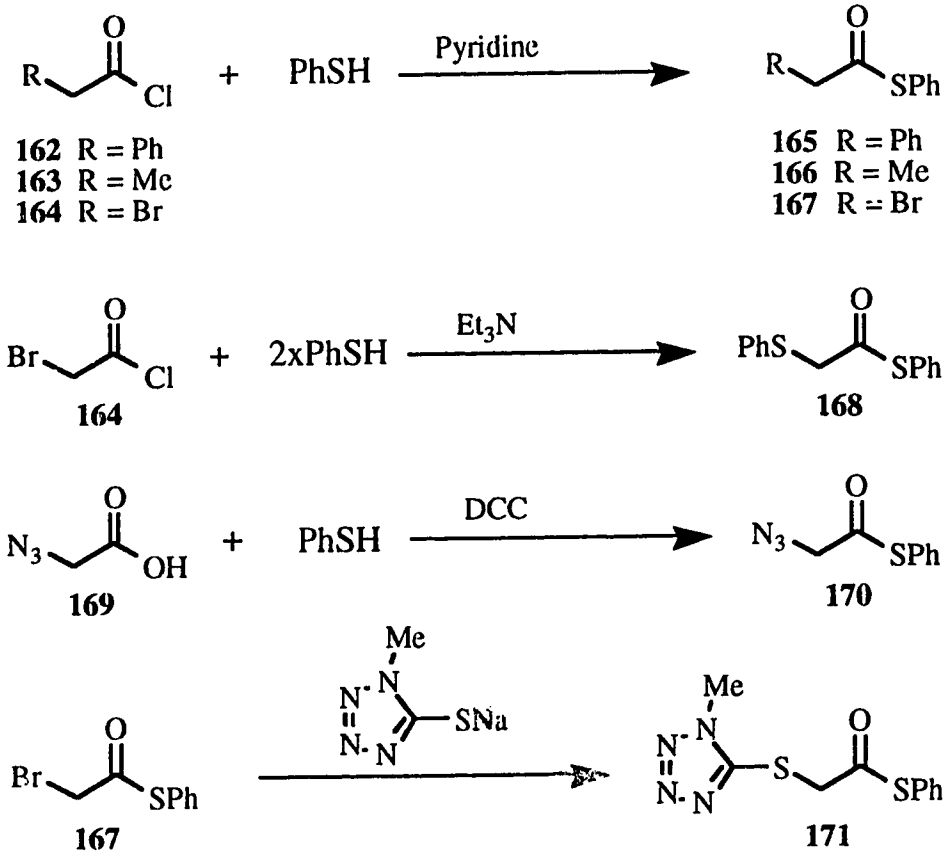
As described so far, attempts to synthesize the 1-substituted carbapenems **104** by approach **A** have met with many difficulties, therefore, it was decided to study approach **B**.

3.9 Syntheses of α-substituted thioesters

Scheme 3-15 summarizes the syntheses of the various α-substituted thioesters.

The thioesters **165**, **166** and **167** were prepared in quantitative yields by the reaction of the corresponding acyl chloride **162**, **163**, and **164** with thiophenol in the presence of pyridine. The α-phenylthio-substituted derivative **168** was obtained by the treatment of bromoacetyl chloride with

two molar equivalents of thiophenol and two molar equivalents of triethylamine. The azido derivative **170** was prepared from azidoacetic acid (**169**) and thiophenol using *N,N'*-dicyclohexylcarbodiimide as a coupling agent. The nucleophilic substitution of the bromide **167** with the sodium 1-methyltetrazole-5-thiolate gave the thioester **171** in 89% yield.

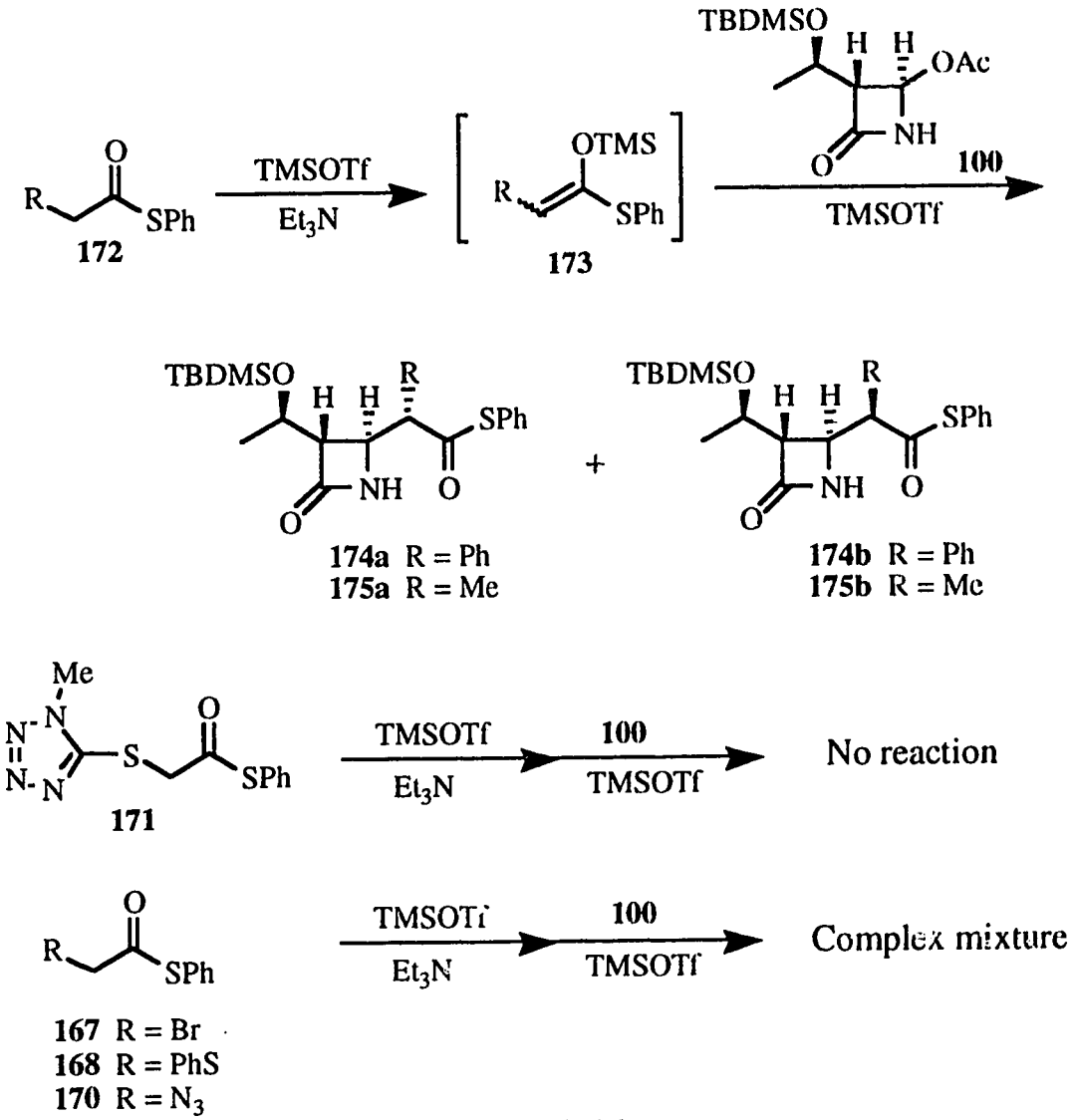


Scheme 3-15

3.10 Syntheses of azetidinone-4-thioacetate derivatives

The azetidinone-4-thioacetate derivatives were synthesized by condensation reactions similar to those described for the syntheses of the azetidinone-4-acetoacetate derivatives (see Section 3.4). The α-substituted

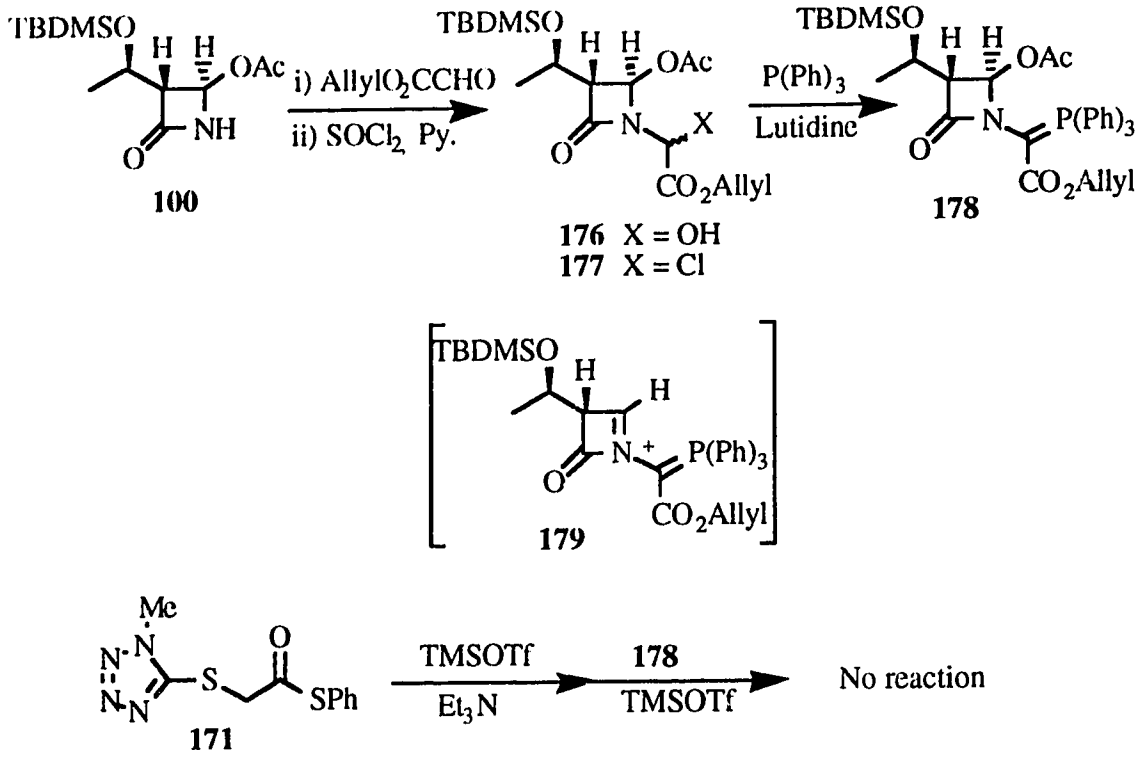
thioacetate **172** was first converted into the silyl enol ether **173** by treatment with triethylamine and TMSOTf, then reacted with the 4-acetoxyazetidinone **100** under Lewis acid catalytic conditions to give the expected compounds (Scheme 3-16).



Scheme 3-16

As expected, a stereoisomeric mixture of the desired condensation products **174a,b** and **175a,b** was obtained in good yields when the thioesters **165** and **166** were used as starting materials (details will be described in the

following sections). Again no reaction was observed from the tetrazole-substituted derivative **171**. When the reaction was carried out using the thioesters **167**, **168**, and **170**, a complex mixture resulted (Scheme 3-16).



Scheme 3-17

Speculating that the *N*-substituted iminium intermediate **179** would possess higher reactivity toward nucleophiles, the *N*-substituted 4-acetoxy-azetidinone derivative **178** was synthesized and treated with the silyl enol ether obtained from the thioester **171**. Thus, the 4-acetoxyazetidinone **100**, allyl glyoxalate, and silica gel were reacted in refluxing toluene with concomitant elimination of water using a Dean-Stark apparatus to give the alcohol **176**. Without silica gel, the reaction was observed to proceed more slowly. Chlorination of the hydroxy group of **176** with thionyl chloride and pyridine gave the chloride **177**, which was treated with triphenylphosphine

in DMF, in the presence of 2,6-lutidine as a base, to afford the desired product **178**. The condensation reaction between the azetidinone **178** and the tetrazolethioacetate **171**, however, did not give the desired product (Scheme 3-17).

3.11 Syntheses of 1-α-phenylcarbapenem-3-carboxylates

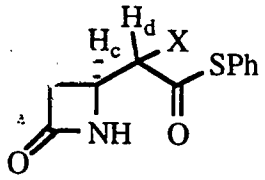
The synthesis of 1-substituted carbapenems was first attempted utilizing the phenyl substituted derivatives.

As mentioned in the preceding section, a mixture of stereoisomers **174a** and **174b** was synthesized by the condensation reaction between the thioacetate and the acetoxyazetidinone **100**, according to the general procedure. Separation of these isomers was achieved by a combination of silica gel column chromatography and recrystallization to give the α-isomer **174a** and β-isomer **174b** in 23% and 28% yield, respectively. The stereochemistry of these isomers was established from the NMR spectra in a similar manner as described for the azetidinone-4-acetoacetate derivatives. The more polar isomer which showed a coupling constant of 10Hz for H_c and H_d was assigned as the α-isomer **174a** and the less polar isomer with a coupling constant of 7Hz was assigned as the β-isomer **174b**. Table 3-4 summarizes the NMR spectra of **174a** and **174b** and related compounds.

Treatment of the isomers **174a** and **174b** with triethylamine and allyl oxalyl chloride yielded the oxalimides **180a** and **180b** in almost quantitative yield after flash silica gel column purification (Scheme 3-18).

The intramolecular Wittig-type cyclization was successfully carried out by treating the α-phenyl-substituted oxalimide **180a** with five molar equivalents of trimethylphosphite in refluxing toluene[115] to yield the desired carbapenem **181** in 78% yield. On the contrary, no cyclized product was

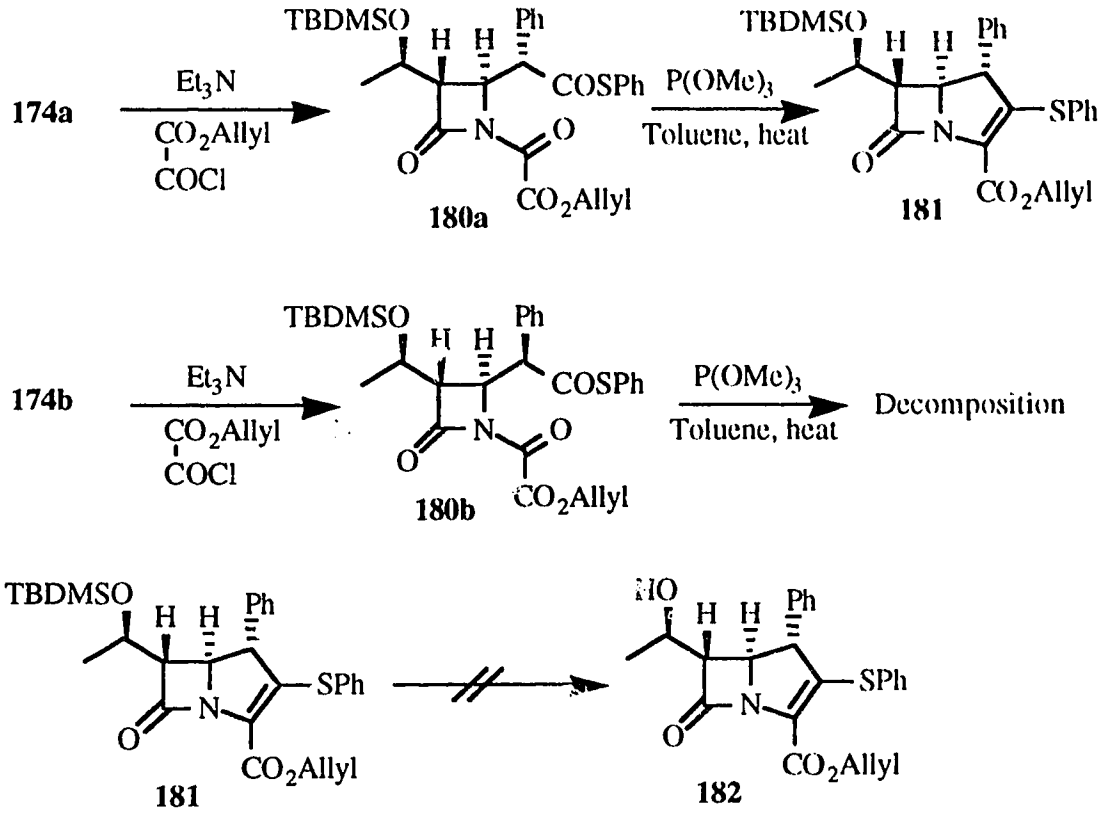
Table 3-4 ^{1}H NMR data of the azetidinone-4-thioacetates.



			Chemical shifts:δ (coupling constants:Hz)				
No.	R	X	CH_3	H_a	H_b	H_c	H_d
174a	TBDMS	α-Ph	0.28 (6)	4.05 (2,6)	2.75 (2)	4.24 (2, 10)	3.92 (10)
174b	TBDMS	β-Ph	1.17 (6)	4.22 (4, 6)	2.86 (3, 6)	4.28 (3, 7)	3.98 (7)
183a	TMS	α-Ph	0.43 (6)	3.98-4.04	2.76 (2, 3)	4.18 (2, 10)	3.91 (10)
183b	TMS	β-Ph	1.23 (6)	4.15-4.25*	2.94 (2, 5)	4.15-4.25*	3.97 (8)
186a	TMS	α-Me	1.30 (6)	4.10-4.16	2.80-2.90	4.09 (2, 10)	2.85 (10)
186b	TMS	β-Me	1.11 (6)	4.25-4.31	2.95 (2, 5)	4.25 (2, 5)	2.87 (5)

* Overlapping peaks

obtained from the reaction of the β-isomer **180b** with trimethylphosphite in refluxing toluene or xylene (Scheme 3-18).



Scheme 3-18

The differences in reactivity of these two isomers **180a** and **180b** may be explained by a thermodynamic consideration of the transition states **A** and **B** as shown in Figure 3-2. In transition state **A**, the phosphorane group can approach the carbonyl group in a thermodynamically favored conformation, whereas in transition state **B**, an approach of the phosphorane to the carbonyl group is restricted because of the unfavored steric interaction between the phenyl group and the β-lactam ring.

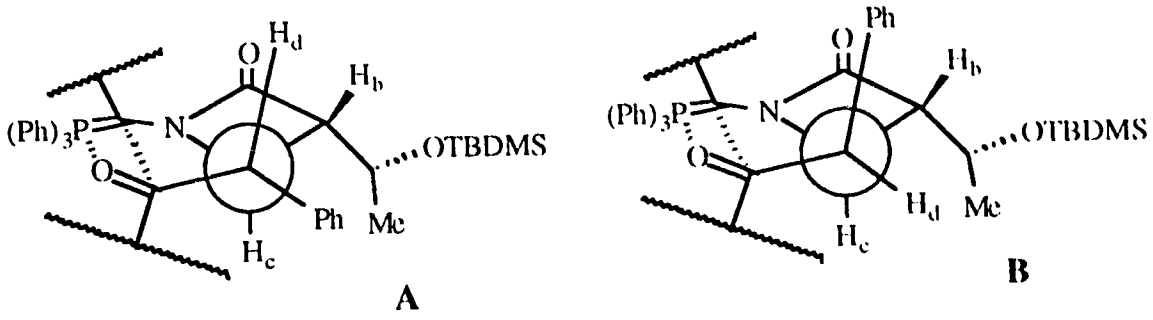


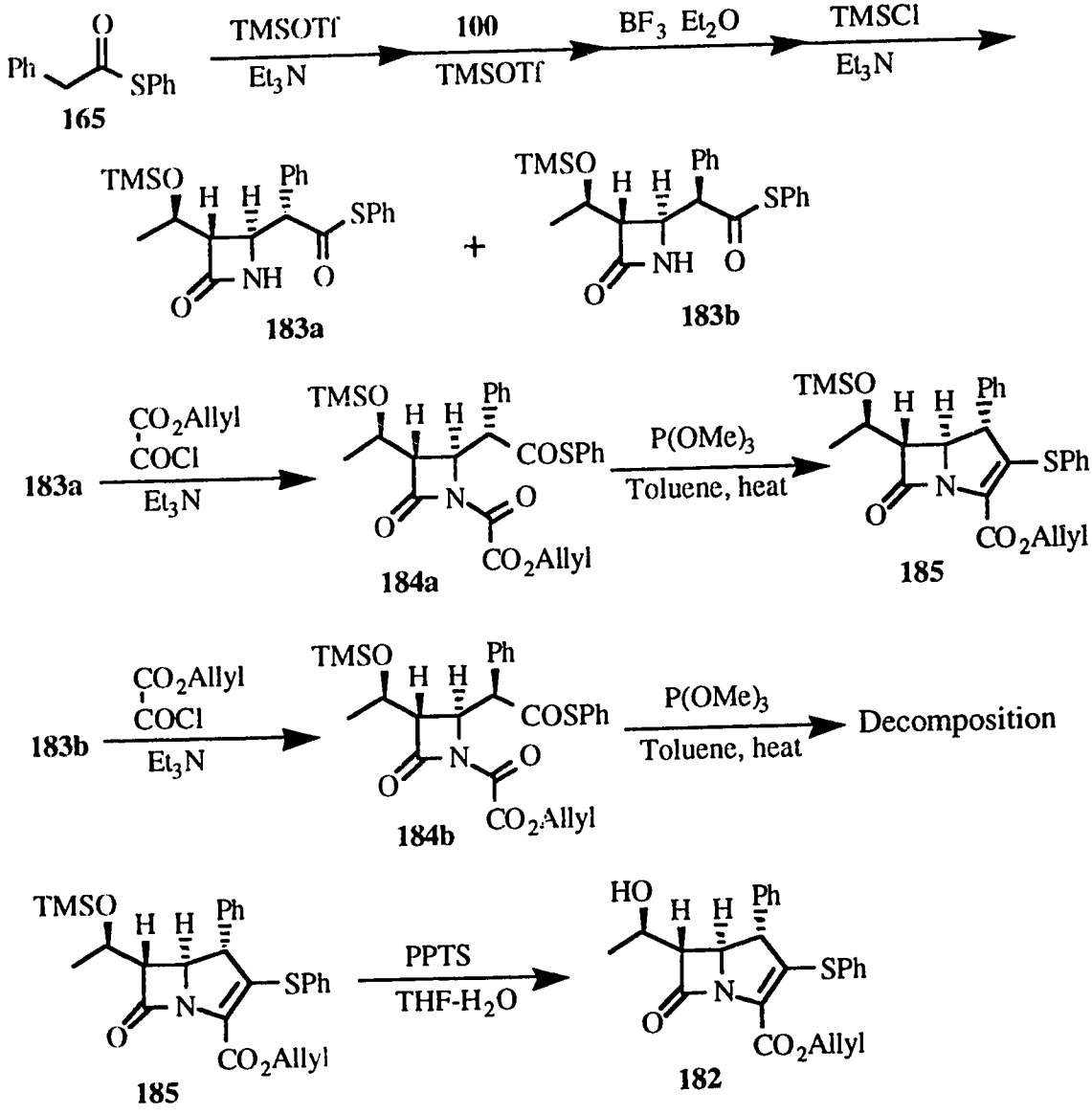
Figure 3-2 Transition states of the stereoisomeric intermediates in intramolecular Wittig reaction.

Next, the desilylation of the protected carbapenem **181** to obtain the alcohol **182** was attempted using the following conditions: 1) Treatment with tetrabutylammonium fluoride in THF with or without acetic acid.[140] 2) Treatment with trifluoroacetic acid in dichloromethane or in dichloromethane/methanol mixture. 3) Treatment with boron trifluoride etherate in dichloromethane or acetonitrile. 4) Treatment with hydrochloric acid in methanol. In all cases, however, decomposition took place before any deprotection was observed.

In order to synthesize the hydroxy compound **182**, it was hence necessary to exchange the protecting group from the TBDMS group to a more readily removable group prior to the bicyclic ring formation. For this purpose, a trimethylsilyl (TMS) group was selected as an alternative protecting group.

Conversion of a mixture of the TBDMS protected derivatives **174a** and **174b** into the TMS protected derivatives **183a** and **183b** was accomplished in two consecutive steps, *i.e.* deprotection of the TBDMS group with boron trifluoride in acetonitrile, followed by trimethylsilylation. Since the β-lactam NH group was also silylated under the same reaction conditions, more than two molar equivalents of the reagents (TMSCl/Et_3N) were required to

complete the trimethylsilylation of the hydroxy group. The resulting mixture was separated by silica gel column chromatography to afford the isomers **183a** and **183b** in 34% and 47% overall yields, respectively, based on the acetoxyazetidinone **100**. During the chromatography, the *N*-TMS group was spontaneously cleaved (Scheme 3-19).



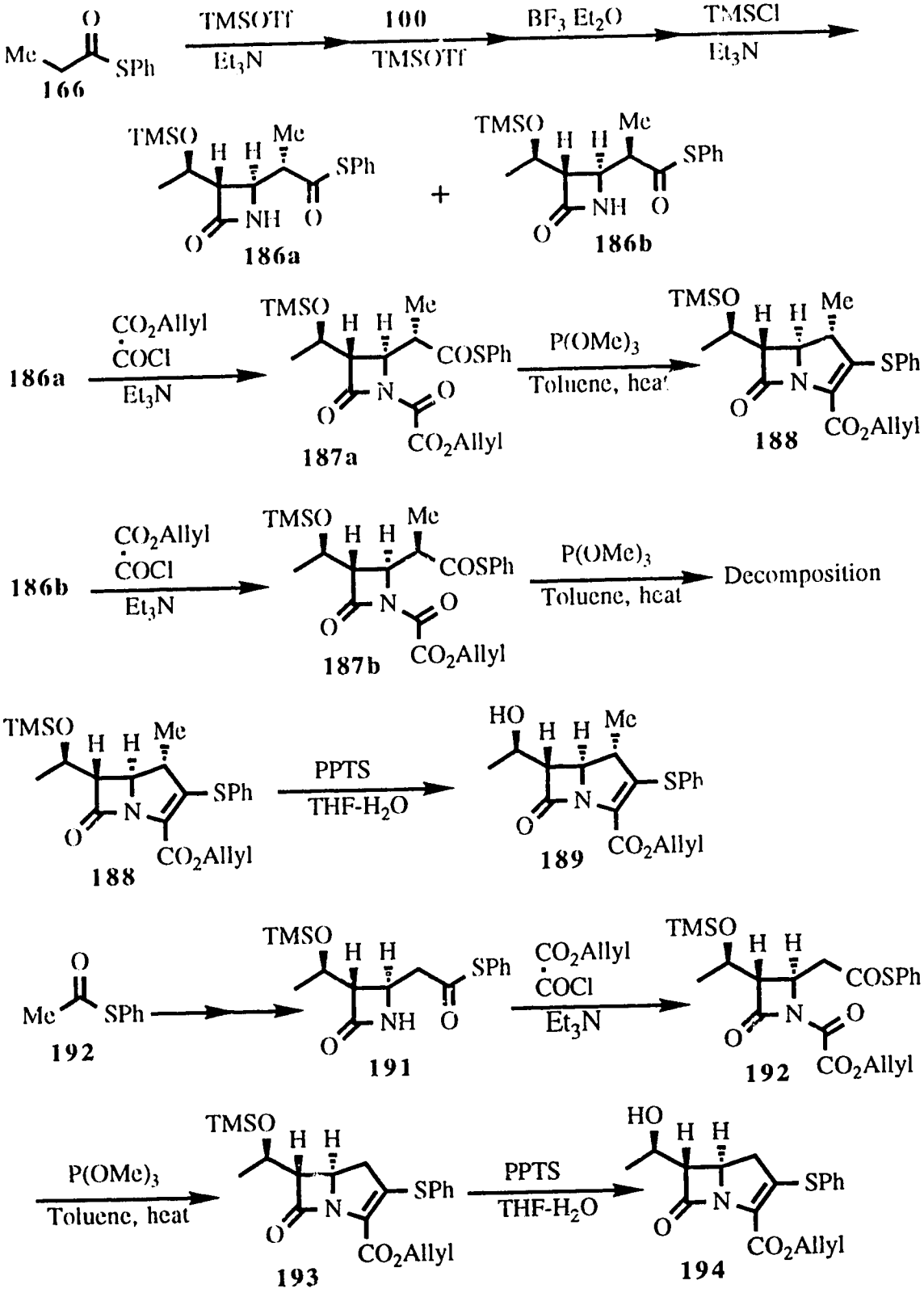
Scheme 3-19

The syntheses of the oxalimides **184a** and **184b** were carried out by the reaction of the azetidinone **183a** and **183b** with triethylamine and allyl oxalyl chloride. Treatment of the oxalimides **184a** and **184b** with trimethylphosphite in a similar manner as described above for the TBDMS protected derivatives yielded the desired cyclized product **185** in 47% and the decomposed product, respectively. Desilylation of the *O*-TMS of the carbapenem **185** was successfully performed by treating **185** with a catalytic amount of pyridinium *p*-toluenesulfonate in aqueous tetrahydrofuran to give the hydroxy compound **182** in 80% yield (Scheme 3-19).

12 Syntheses of 1-unsubstituted and 1-methyl substituted carbapenem-3-carboxylates

To carry out a comparative evaluation of the biological activity of the 1-α-phenyl-substituted carbapenem, the 1-methyl substituted and unsubstituted carbapenems were also synthesized using the intramolecular Wittig-type cyclization.

The condensation reaction of *S*-phenyl thiopropionate **163** with the acetoxyazetidinone **100**, followed by exchange of the TBDMS to the TMS group, in a manner similar to that described above for the phenyl-substituted derivatives, gave the α-methyl and β-methyl isomers **186a** and **186b** in 35% and 50% overall yield, respectively, after silica gel column separation. Unlike all the other condensation products described so far, the β-methyl isomer was found to be more polar than the α-methyl isomer. As was observed for the phenyl substituted derivatives, the α-methyl isomer **186a** was successfully converted into 1-α-methylcarbapenem-3-carboxylate **188** *via* the oxalimide **187a** in 69% yield, whereas the 1-β-methyl derivative was


Me
SPh
166
TMSOTf
Et3N
100
TMSOTf
BF3 Et2O
TMSCl
Et3N
TMSO
H
H
Me
SPh
NH
O
186a
+
186b
186a
CO2Allyl
COCl
Et3N
COSPh
187a
CO2Allyl
P(OMe)3
Toluene, heat
188
186b
187b
Decomposition
PPTS
THF-H2O
HO
189
192
191
193
194

Scheme 3-20

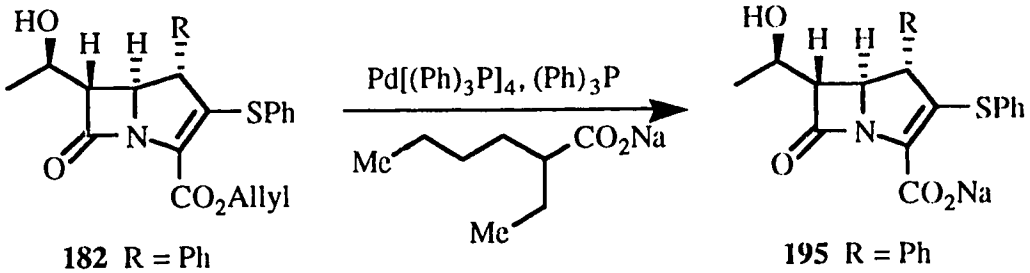
not obtained from the p-methyl oxamimide **187b**. Desilylation of **188** provided the desired hydroxy compound **189** in 62% (Scheme 3-20).

Analogously, the 1-unsubstituted carbapenem-3-carboxylate **193** was synthesized from the *S*-phenyl thioacetate **190** and the acetoxyazetidinone **100** in 33% yield *via* **191** and **192** and desilylation gave the hydroxy compound **194** in 83% yield (Scheme 3-20).

3.13 Syntheses of sodium carbapenem-3-carboxylate derivatives

Final deprotection of the allyl esters and conversion to the sodium salts was attempted according to the procedure described by Jeffrey and McCombie.[130]

Thus the allyl esters **182**, **189**, and **194** were treated with sodium 2-ethyl-hexanoate in the presence of tetrakis(triphenylphosphine)palladium (0) to yield the sodium carboxylates **195**, **196**, and **197**, in 42%, 54%, and 51% yields, respectively (Scheme 3-21).



182 R = Ph
189 R = Me
194 R = H

195 R = Ph
196 R = Me
197 R = H

Scheme 3-21

3.14 Antibacterial activities of the prepared carbapenems

Table 3-5 summarizes the antibacterial activities of the 1-α-phenyl, 1-α-methyl and 1-unsubstituted carbapenem derivatives **195**, **196** and **197** in comparison with those of imipenem **22**. The phenyl derivative **195** showed

MIC values of >128 μg/ml against most microbes. The methyl derivative **196** showed moderate antibacterial activities, however, its activities were several times lower than those of the 1-unsubstituted derivative **197** in most cases. From these results, it is clear that when a larger group is introduced at the 1-α-position of carbapenem, the antibacterial activities decrease accordingly. This decrease in activities is possibly attributed to the decrease permeability through the outer membrane, in the cases of Gram-negative bacteria, or to the decreased affinities of the compound to the target enzymes, PBPs.

No significant differences in chemical stability were observed among obtained three compounds, **195**, **196** and **197**, during synthesis and storage, however, there was an impression that the 1-unsubstituted derivative **197** was slightly less stable than other derivatives during the synthetic process.

Table 3-5 Antibacterial activity of the carbapenem derivatives.

	MIC* (μg/mL)			
Microbes	**195**	**196**	**197**	Imipenem
E. coli (S-63)	>128	32	4	0.12
E. coli (CT-70)	>128	16	4	0.12
E. cloacae (S-11)	>128	>128	32	0.5
P. aeruginosa (S-67)	>128	>128	64	2
K. pneumoniae (S-80)	>128	128	4	1
P. rettgeri (S121)	>128	8	1	0.5
E. faecalis (S-141)	>128	64	2	0.5
S. aureus (S-127)	8	0.25	<0.06	0.008
S. aureus (S-127M)	>128	32	32	>8
S. aureus (CT-5)	>128	64	32	>8

*Microbroth dilution method, MHB; inoculum, $5x10^5$ cfu/mL; incubation, 35°C, 18h.

Chapter 4

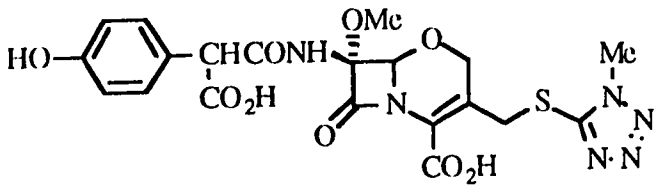
RESULTS AND DISCUSSION - 2

STUDIES ON β-LACTAMASE INHIBITORS

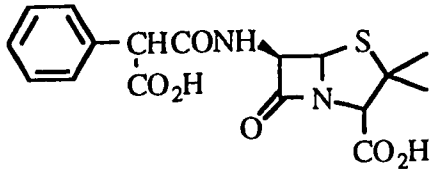
In a search for β-lactamase inhibitors which would protect cephalosporin-type antibiotics from cephalosporinases, a series of cephalosporins and penicillins with an (un)protected carboxyl substituent on 7- or 6-position side chain were synthesized as potential inhibitors and their biological activities were checked.

Moxalactam (**198**), one of the oxacephem derivatives, has been known not only to be very stable to class 1 cephalosporinases, but also to inhibit these enzymes.[141] A research group at Shionogi clearly demonstrated that the introduction of a carboxyl group on the side chain at the 7-position of the oxacephem was responsible for the stabilization of the substrate against degradation by cephalosporinases.[142] Incidentally, carbenicillin (**6**), a penicillin derivative with a similar type of carboxyl-substituted side chain at the 6-position, has been known to be resistant to degradation by chromosomally mediated cephalosporinases.[143] Although it is possible that the steric effect of the carboxylic acid contributes towards their stability to cephalosporinases and inhibition of the enzymes, the fact that some β-lactam antibiotics, *e. g.* piperacillin, with a similar degree of steric hinderance at the same position on the side chain, are more susceptible to cephalosporinases implies that the role of the carboxylic acid moiety may be more than just a steric hinderance. From these findings, it was natural to infer that the carboxyl group was playing an important role in the interaction of these substrates with cephalosporinases. However, there has been no detailed study in regard to this aspect. Therefore the second part of this study was

directed toward the systematic syntheses of cephalosporins and penicillins that possess an (un)protected carboxyl group on their 7- or 6-position side chain to investigate the effect of the carboxyl-substituent on the β-lactamase inhibitory activity, primarily against cephalosporinases.



Moxalactam (198)



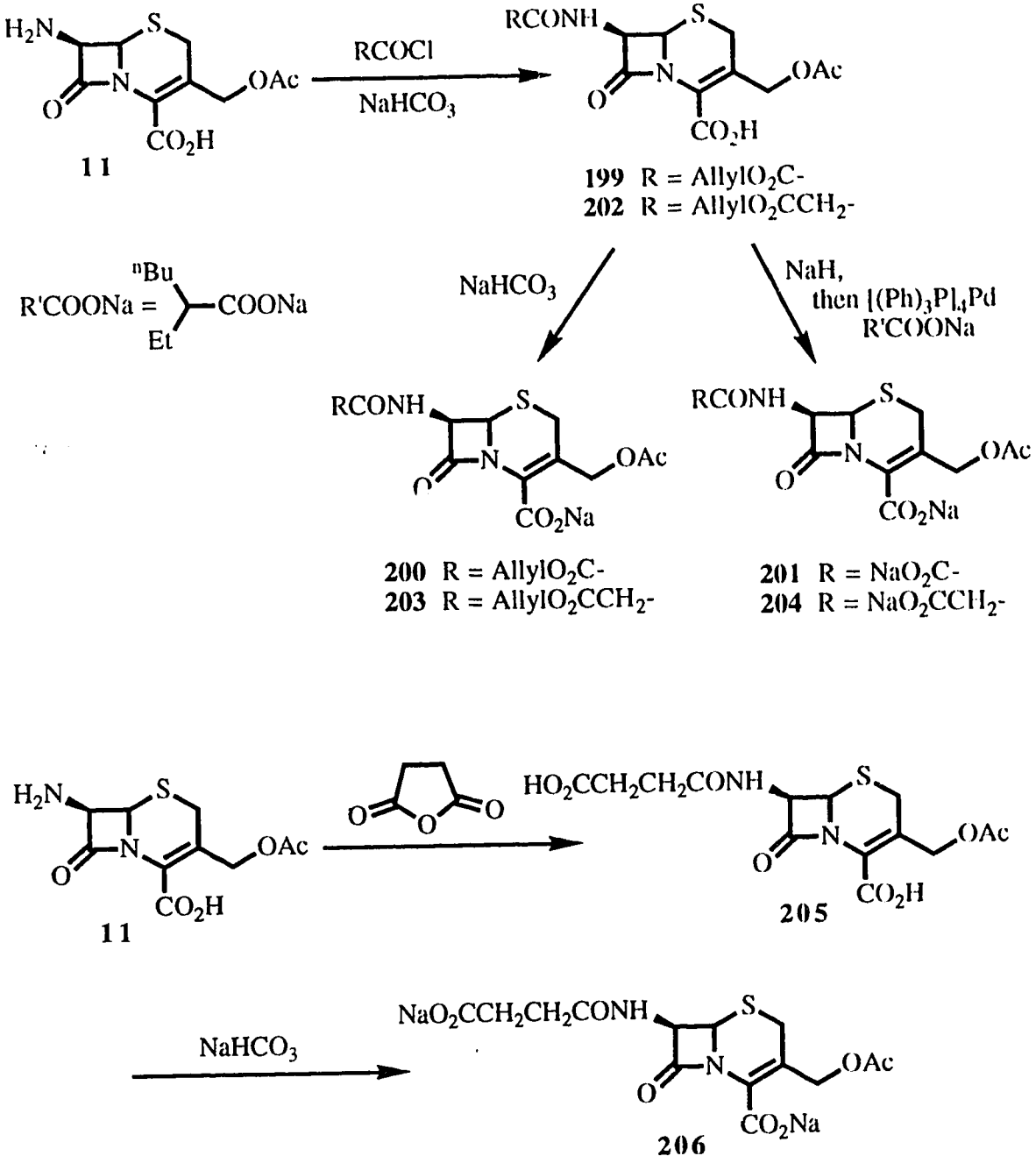
Carbenicillin (6)

Figure 4-1 Structures of moxalactam and carbenicillin.

4.1 Syntheses and β-lactamase inhibitory activities of 7-substituted cephalosporins

A series of cephalosporin derivatives with an (un)protected carboxyl group at various relative positions were prepared and their β-lactamase inhibitory activities were determined, in order to investigate whether the carboxyl group is really contributing to the β-lactamase inhibitory activity or not, and if so, in which position in the β-lactam nucleus has the most contribution to the inhibition. Since the primary interest was cephalosporinases, the cephems were chosen as the basic nucleus for the initial study.

Scheme 4-1 summarizes the syntheses of the 7-substituted cephalosporin derivatives. Reaction of 7-aminocephalosporanic acid (7-ACA) (**11**) with allyl oxalyl chloride in a bi-phasic mixture (THF/aqueous sodium bicarbonate solution), followed by acidification and extraction gave the allyl oxamate **199** in 25% yield, after silica gel column purification. The low yield


RCOCl
NaHCO3
11
199 R = Allyl O2C-
202 R = Allyl O2CCH2-
R'COONa =
nBu
Et
COONa
NaHCO3
NaH,
then [(Ph)3P]4Pd
R'COONa
200 R = Allyl O2C-
203 R = Allyl O2CCH2-
201 R = NaO2C-
204 R = NaO2CCH2-
HO2CCH2CH2CONH
205
NaHCO3
NaO2CCH2CH2CONH
206

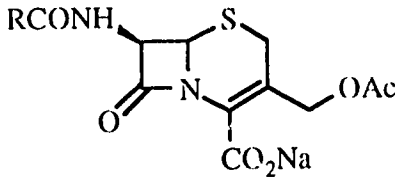
Scheme 4-1

in this reaction was attributed to the rapid hydrolysis of allyl oxalyl chloride under these conditions. The obtained carboxylic acid compound **199** was then converted into the 4-sodium carboxylate **200**, in almost quantitative yield, by treatment with 1 molar equivalent of sodium bicarbonate in a small amount of water followed by lyophilization. The disodium salt **201** was prepared in 94% yield by treating **199** first with 1 molar equivalent of sodium hydride in DMF, then with sodium 2-ethylhexanoate in the presence of tetrakis(triphenylphosphine)palladium (0). Similarly, the malonamate derivative **202** was synthesized in 20% yield from 7-ACA and allyl malonyl chloride, which was prepared from allyl hydrogen malonate on treatment with oxalyl chloride in dichloromethane. Compound **202** was then converted into the sodium carboxylate **203** and the disodium dicarboxylate **204** in almost quantitative yield and 55% yield, respectively, in a manner similar to that described above. Synthesis of the succinamic acid derivative **205** was accomplished in 18% yield by adding a solution of succinic anhydride in acetone to a bicarbonate solution of 7-ACA, followed by acidification and extraction with ethyl acetate. Treatment of **205** with 2 molar equivalent of sodium bicarbonate in water afforded the desired disodium salt **206** in almost quantitative yield.

The 7-substituted cephalosporins, obtained in the above reactions, were tested against three different classes of β-lactamases, *viz.* penicillinase, broad-spectrum β-lactamase (TEM enzyme), and cephalosporinase. The β-lactamase inhibitory activity was measured by a spectrophotometric method with penicillin G as a substrate for penicillinase, and with cephaloridine as a substrate for cephalosporinase and the TEM enzyme.

Table 4-1 summarizes the β-lactamase inhibitory activities of the 7-substituted cephalosporin derivatives as compared with those of tazobactam (**35**).

Table 4-1 β-Lactamase inhibitory activities of the 7-substituted cephalosporins.



Compound No.	R	IC_{50} (μM) Pen-ase*	TEM2**	Ceph-ase***
200	AllylO_2C-	100	100	1.5
201	NaO_2C-	>100	>100	1.5
203	AllylO_2CCH_2-	>100	>100	4.0
204	NaO_2CCH_2-	>100	>100	1.0
206	$NaO_2CCH_2CH_2$-	>100	>100	30
Tazobactam		0.31	0.076	2.4

* Penicillinase from *Bacillus cereus*.

** TEM2 from *Escherichia coli*.

*** Cephalosporinase from *Enterobacter cloacae*.

Although all of the prepared compounds showed no inhibitory activity against penicillinase and TEM enzyme, they were found to possess considerable levels of inhibitory activity against cephalosporinase as determined initially. The relative position of the carboxyl group on the side chain was observed to have an influence on the inhibitory activity. When there were no or one intervening methylene group between the carboxyl

group and the amide group, *i.e.* **200**, **201**, **203** and **204**, a similar degree of cephalosporinase inhibitory activity (IC_{50} = 1~4 μM) was observed, whereas, when there were two intervening methylene groups, *i.e.* **206**, inhibitory activity against the cephalosporinase decreased. There were no significant differences in inhibitory activities between the allyl esters **200** and **203** and the corresponding sodium carboxylates **201** and **204**.

4.2 Syntheses and β-lactamase inhibitory activities of 7-substituted 3'-desacetoxycephalosporins

As described in the preceding section, some 7-substituted cephalosporin derivatives were demonstrated to possess cephalosporinase inhibitory activity. However, their mode of inhibition (whether they inhibit the enzyme by simple non-covalent interaction or by forming the stable acyl-enzyme intermediate) is not yet known.

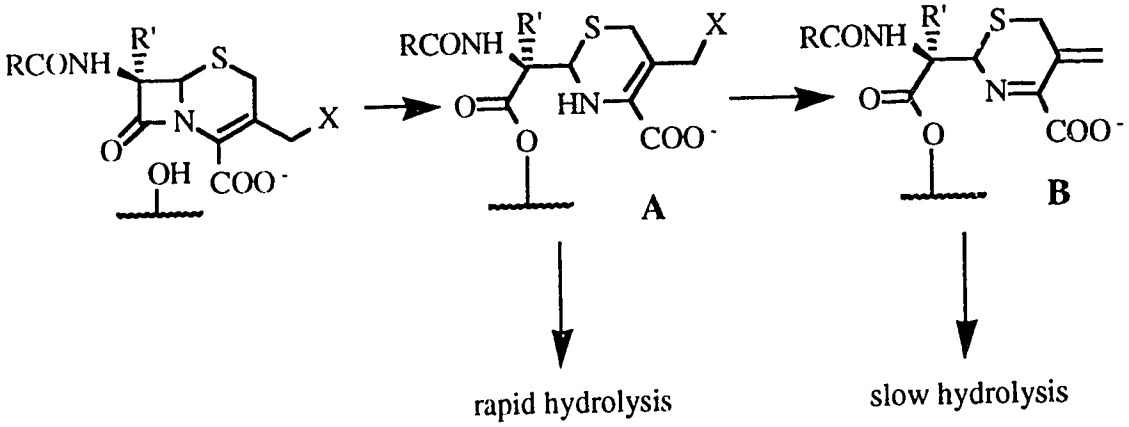


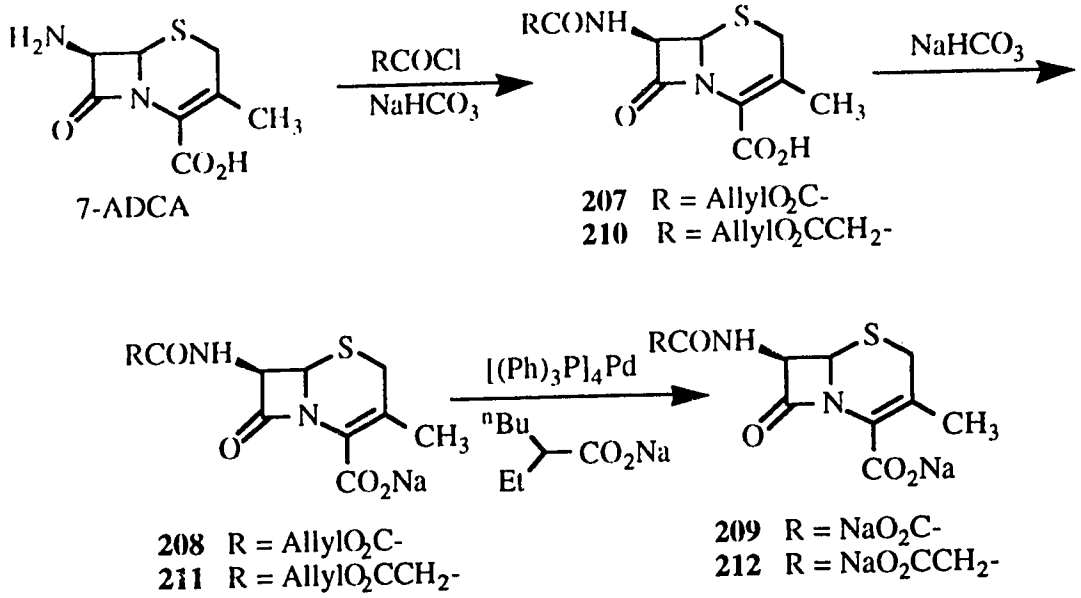
Figure 4-2 Hydrolysis of cephalosporins.

It has been reported that cephalosporins with a good leaving group at the 3'-position transiently inhibit some enzymes covalently by acylation of the enzyme to form the unstable acyl-enzyme **A** followed by elimination of

the 3'-leaving group to form a relatively stable acyl-enzyme complex **B**; whereas, without a good leaving group at the 3'-position, the cephalosporins form unstable acyl-enzyme intermediates, which are hydrolyzed rapidly (Figure 4-2).[144] Therefore it was considered that a comparison of the cephalosporin derivatives with their 3'-desacetoxycephalosporin analogues in their β-lactamase inhibitory activities could afford some insight into their interactions with β-lactamases.

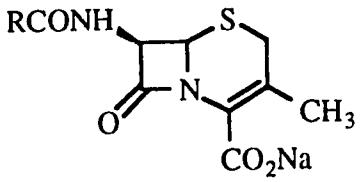
The 3'-desacetoxycephalosporin derivatives were synthesized as shown in Scheme 4-2. The 7-allyloxalamido- and 7-allylmalonamido-3'-desacetoxy-cephalosporanic acids **207** and **210** were synthesized from 3'-desacetoxy-cephalosporanic acid (7-ADCA) in 59% and 43% yields, respectively, in a manner similar to that utilized for the 3'-acetoxy analogues. Treatment of **207** and **210** with sodium bicarbonate yielded the sodium carboxylates **208** and **211** in almost quantitative yields. Preparation of the disodium salts **209** and **212** was accomplished by the reaction of **208** and **211** with sodium 2-ethylhexanoate in the presence of palladium (0) catalyst in 62% and 86% yields, respectively.

Table 4-2 shows the β-lactamase inhibitory activities of the prepared compounds. Although compounds **211** and **212** showed some inhibitory activity against cephalosporinase, the overall inhibitory activity of the desacetoxy derivatives were much weaker than those of the acetoxy-substituted counterparts. These results may imply that the prepared cephalosporin derivatives, the acetoxy and desacetoxy compounds, are likely to be interacting with the β-lactamases covalently by forming acyl-enzyme intermediates, which are subsequently hydrolyzed at different rates. This result agrees with the finding that moxalactam inhibits cephalosporinases by the formation of the covalent acyl-enzyme complex.[147]



Scheme 4-2

Table 4-2 β-Lactamase inhibitory activities of the 7-substituted 3'-desacetoxycephalosporins.



Compound No.	R	IC_{50} (μM) Pen-ase*	TEM2**	Ceph-ase**
208	AllylO_2C-	100	>100	>100
209	NaO_2C-	>100	>100	>100
211	AllylO_2CCH_2-	>100	>100	13.6
212	NaO_2CCH_2-	>100	>100	85.4
Tazobactam		0.31	0.076	2.4

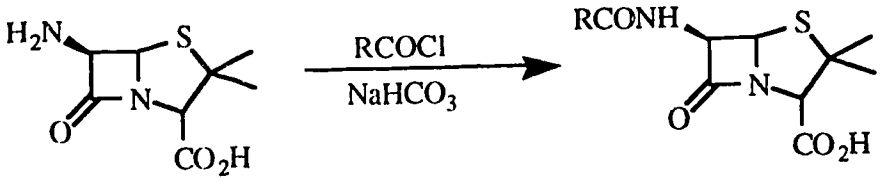
For footnotes see Table 4-1.

4.3 Syntheses and β-lactamase inhibitory activities of 6-substituted penicillins

Although cephalosporinases are known to hydrolyze cephalosporin antibiotics, *e.g.* cephalothin and cephaloridine, faster than penicillins, *e.g.* penicillin G and ampicillin, it does not necessarily mean that cephalosporinases have a higher affinity for cephalosporin derivatives than for penicillins. Frère *et al.* have studied the kinetic parameters of class C cephalosporinases with relation to a variety of β-lactam antibiotics and revealed that cephalosporinases, in fact, do have higher affinities (Km) for penicillins such as penicillin G and ampicillin, than for some cephalosporins, *e.g.* cephaloridine and cephalothin, but because of a much faster processing rate (k_{cat}), the cephalosporins are apparently hydrolyzed faster than penicillins.[146] Since high affinity (Km) to enzyme is one of the most important characteristics for enzyme inhibitors, it was therefore considered to be of interest to modify the penicillin nucleus with similar types of carboxyl substituted side chains, to those which were utilized for modification of the cephalosporin derivatives, to achieve higher degrees of enzyme inhibition.

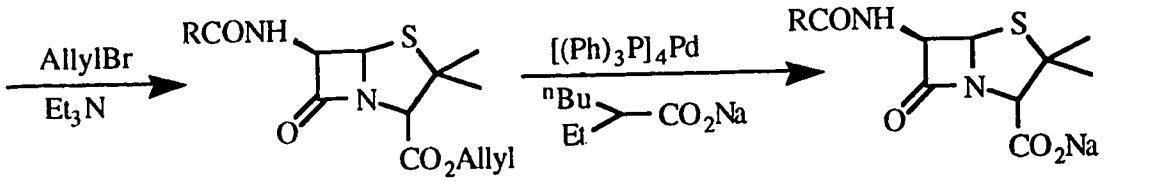
The syntheses of the 6-substituted penicillin derivatives are summarized in Scheme 4-3. The introduction of the allyloxalyl group to the amino moiety of 6-aminopenicillanic acid (6-APA) (**2**) was carried out in a similar fashion as described for the cephalosporin derivative **199**, except that allyloxalyl chloride was dissolved in toluene instead of ether, to afford the crude acid **216**. Less decomposition of allyloxalyl chloride was observed when toluene was used as a solvent than when diethyl ether or THF were used. Purification of **216** by silica gel column chromatography, however, was not successful due to its chemical instability. To obtain pure product, therefore,

the carboxylic compound **216** was first converted into the more stable and more readily purifiable diallyl ester **217** in 32% overall yield by treatment with triethylamine and allyl bromide in DMF, then deprotected to give the disodium carboxylate **218** in 94% yield. The methyl oxamate compound **214** was prepared analogously in 59% overall yield from 6-APA in two steps, then converted into the sodium carboxylate **215** in 71% yield. The malonamates **220**, **223**, and **226** were prepared in 32%, 51%, and 46% yields, respectively, by the reaction of 6-APA with the corresponding malonyl chlorides, followed by esterification with triethylamine and allyl



6-APA (**2**)

213 R = MeO_2C-
216 R = $AllylO_2C$-
219 R = MeO_2CCH_2-
222 R = EtO_2CCH_2-
225 R = $AllylO_2CCH_2$-
228 R = $AllylO_2CCH_2CH_2$-



214 R = MeO_2C-
217 R = $AllylO_2C$-
220 R = MeO_2CCH_2-
223 R = EtO_2CCH_2-
226 R = $AllylO_2CCH_2$-
229 R = $AllylO_2CCH_2CH_2$-

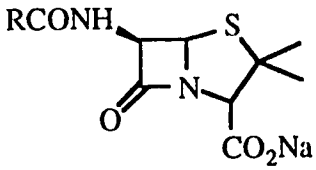
215 R = MeO_2C-
218 R = NaO_2C-
221 R = MeO_2CCH_2-
224 R = EtO_2CCH_2-
227 R = NaO_2CCH_2-
230 R = $NaO_2CCH_2CH_2$-

Scheme 4-3

bromide. The methyl ester derivatives were found to be much less stable than the other two analogues. Palladium (0) catalyzed deesterification of **223**, **226**, and **226** with sodium 2-ethylhexanoate afforded the sodium carboxylates **221**, **224**, and **227** in 88%, 91%, and 94% yields, respectively. The allyl succinamate **229** was prepared in 42% yield from 6-APA after acylation and esterification. Deprotection of **229** afforded the desired sodium carboxylate **230** in 33% yield.

Table 4-3 summarizes the β-lactamase inhibitory activities of the prepared penicillin derivatives. Interestingly, overall inhibitory activity of the penicillin derivatives against cephalosporinase were found to be higher than those of the cephalosporin derivatives.

Table 4-3 β-Lactamase inhibitory activities of the 6-substituted penicillins.



Compound No.	R	IC_{50} (μM) Pen-ase*	TEM2**	Ceph-ase**
215	MeO_2C-	>100	>100	0.78
218	NaO_2C-	>100	>100	1.0
221	MeO_2CCH_2-	>100	>100	0.107
224	EtO_2CCH_2-	>100	>100	0.082
227	NaO_2CCH_2-	>100	50	0.072
230	$NaO_2CCH_2CH_2$-	>100	>100	2.8
Tazobactam		0.31	0.076	2.4

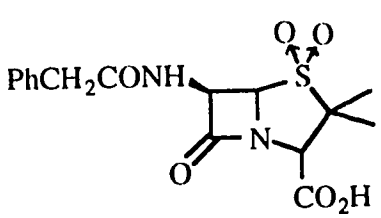
For footnotes see Table 4-1.

Again, the succinamate **230** showed much less inhibitory activity than the oxamate and malonamate derivatives **218** and **227** against the cephalosporinase. The oxamates **215** and **218** showed comparable activity to their cephalosporin counterparts, **200** and **201**. However, interestingly, the malonamate derivatives **221**, **224** and **227** showed much stronger cephalosporinase inhibitory activity than the corresponding cephalosporin derivatives, **203** and **204**. Similar to the cephalosporin derivatives, only small differences in inhibitory activity between the esters and the corresponding sodium carboxylates were observed. None of the prepared penicillin derivatives showed inhibitory activity against penicillinase and TEM enzyme except for the disodium malonamate **227**, which weakly inhibited the TEM enzyme.

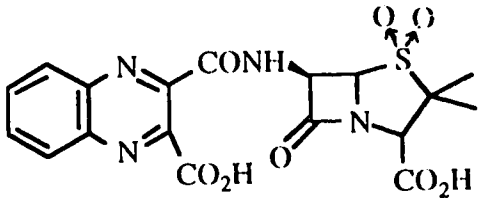
4.4 Syntheses and β-lactamase inhibitory activities of 6-substituted penicillin sulfones

Most of the potent β-lactamase inhibitors, including sulbactam (**34**) and tazobactam (**35**), are penicillin sulfone derivatives. Their mode of inhibition involves the initial formation of an acyl-enzyme intermediate, followed by transformation of this intermediate to the more stable tautomerized acyl-enzyme. For this transformation, the sulfone moiety is considered to be playing an essential role as a leaving group. Therefore, the oxidation of the penicillin derivative to its corresponding penicillin sulfone is speculated to potentiate its β-lactamase inhibitory property. However, it should be noted that penicillin G sulfone (**231**) is a poor inhibitor and that quinacillin, which is a poor β-lactamase substrate, on oxidation, produces the sulfone **232** which inhibits β-lactamases similar to sulbactam (Figure 4-3). It is not clear

yet what effect the oxidation of penam has on its interaction with β-lactamases.

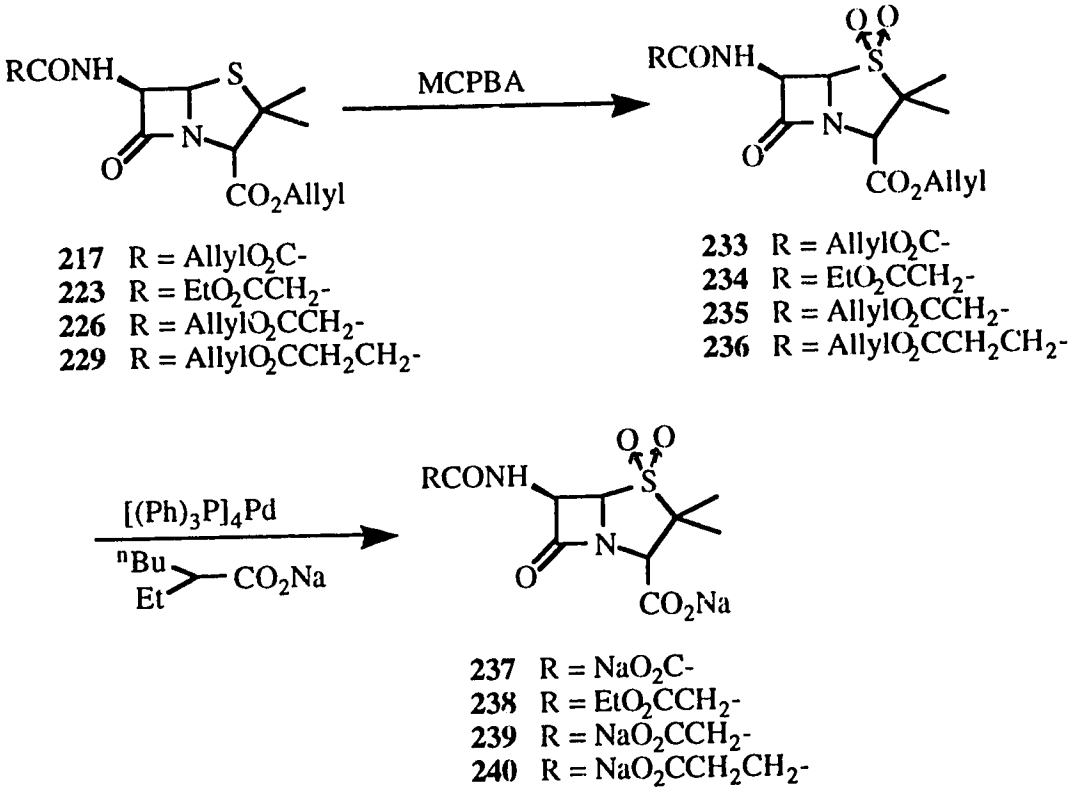


231



232

Figure 4-3 Structures of penicillin sulfones.



217 R = $AllylO_2C$-
223 R = EtO_2CCH_2-
226 R = $AllylO_2CCH_2$-
229 R = $AllylO_2CCH_2CH_2$-

233 R = $AllylO_2C$-
234 R = EtO_2CCH_2-
235 R = $AllylO_2CCH_2$-
236 R = $AllylO_2CCH_2CH_2$-

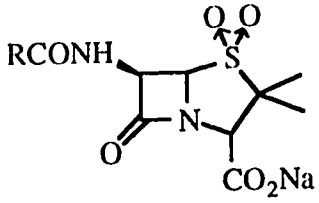
237 R = NaO_2C-
238 R = EtO_2CCH_2-
239 R = NaO_2CCH_2-
240 R = $NaO_2CCH_2CH_2$-

Scheme 4-4

With these facts in mind, the sulfone analogues of the 6-substituted penicillins were synthesized as shown in Scheme 4-4. Thus oxidation of the

penams **217**, **223**, **226**, and **229** with *m*-chloroperbenzoic acid in dichloromethane afforded the desired sulfones **233**, **234**, **235**, and **236** in 32%, 57%, 41%, and 15% yields, respectively. Extensive decomposition was observed during the oxidation of the succinamate **229**. Deprotection of the allyl esters **233**, **234**, **235**, and **236** was performed by the palladium (0) mediated reaction to yield **237**, **238**, **239**, and **240** in 98%, 95%, 48%, and 33% yields, respectively.

Table 4-4 β-Lactamase inhibitory activities of the 6-substituted penicillin sulfones.



Compound No.	R	IC_{50} (μM) Pen-ase*	TEM2**	Ceph-ase**
237	NaO_2C-	100	>100	40.0
238	EtO_2CCH_2-	>100	16.3	0.95
239	NaO_2CCH_2-	>100	>100	1.6
240	$NaO_2CCH_2CH_2$-	>100	>100	>100
Tazobactam		0.31	0.076	2.4

For footnotes see Table 4-1.

The β-lactamase inhibitory activities of the obtained penam sulfones **237**, **238**, **239**, and **240** were found to be much weaker than those of the corresponding penams **218**, **224**, **227**, and **230** (Table 4-4). An approximately 10-40 times decrease in the inhibitory activity was observed, in these cases. Although the specific reason for this decrease in inhibitory

activity cannot be determined, a possible explanation is that the penam sulfones have lower affinity to the enzyme relative to the corresponding penams and/or their acyl-enzyme complexes are not stable.

4.5 Syntheses and β-lactamase inhibitory activities of 6-dithiolane substituted penicillins and penicillin sulfones

Since the 6-malonamidopenam derivatives **221**, **224**, and **227** showed the most interesting β-lactamase inhibitory activity against cephalosporinase among a series of cephalosporin and penicillin derivatives prepared so far, further modification on this type of derivatives was pursued. Expecting to obtain more potent β-lactamase inhibitors, the dithiolane substituted compounds **241** were designed and synthesized. This type of side chain structure was designed based on the structural similarity to those of the β-lactamase-stable β-lactam antibiotics, *e.g.* methicillin (**4**), oxacillin (**3**), and the newer generation of cephalosporins such as cefotaxime (**16**), which are also known to be good β-lactamase inhibitors against some cephalosporinases (Figure 4-4). In addition, cephalosporin derivatives with similar types of side chains have been reported to possess a high degree of inhibitory activity against cephalosporinases produced by *Proteus* species.

The dithiolane-substituted derivatives **242**, **243**, and **244** were synthesized from the corresponding methylene compounds **220**, **223**, and **226** in 88%, 65%, and 58% yields, respectively, by treatment with 2 molar equivalents of sodium hydride and 1.5 molar equivalents of 1,2-dibromoethane in DMF in the presence of a large excess of carbon disulfide. Palladium catalyzed deesterification of **242**, **243**, **244** with sodium 2-ethylhexanoate afforded the desired sodium carboxylates **245**, **246**, and **247** in 84%, 92%, and 87% yields, respectively. Analogously, modification of

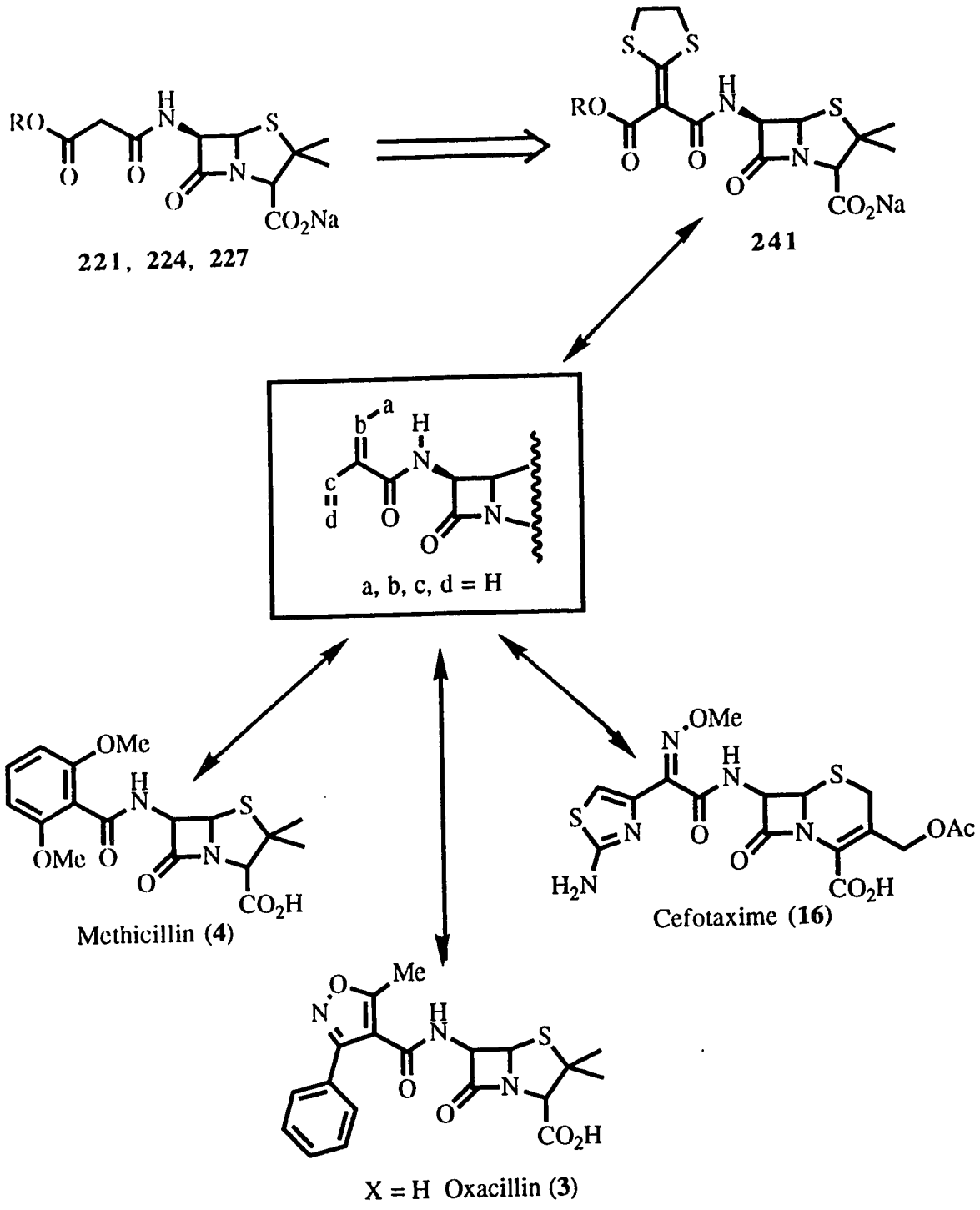
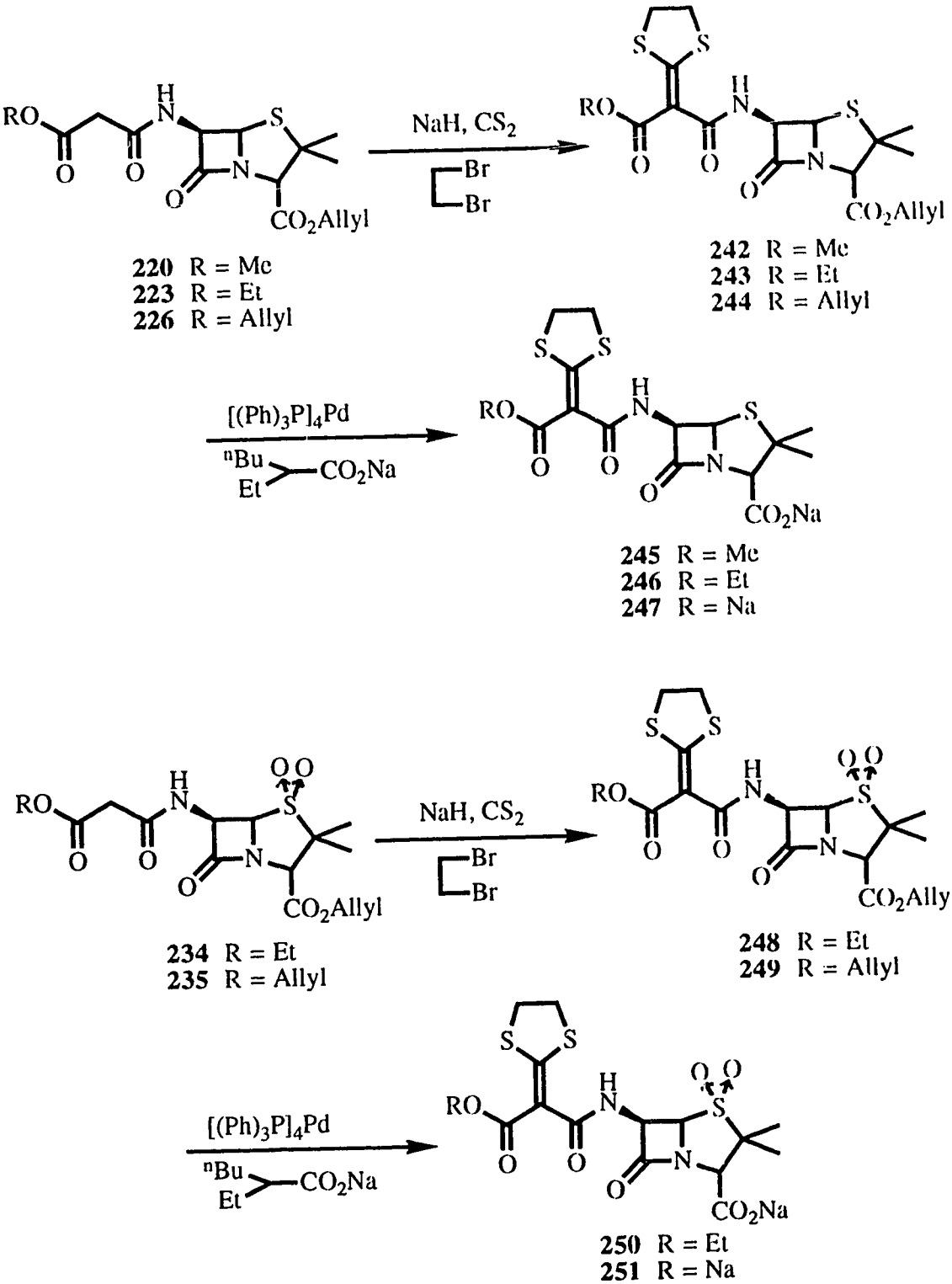


Figure 4-4 Structure design of cephalosporinase inhibitors.



220 R = Me
223 R = Et
226 R = Allyl

242 R = Me
243 R = Et
244 R = Allyl

245 R = Me
246 R = Et
247 R = Na

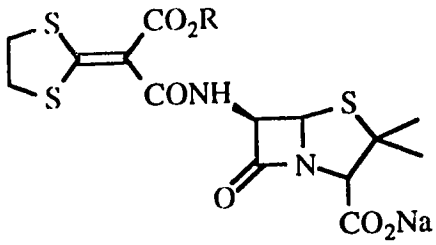
234 R = Et
235 R = Allyl

248 R = Et
249 R = Allyl

250 R = Et
251 R = Na

Scheme 4-5

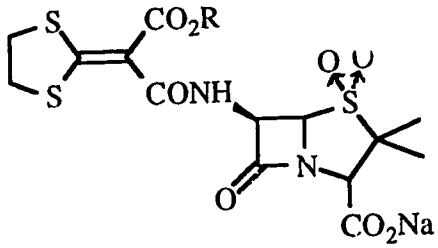
Table 4-5 β-Lactamase inhibitory activities of the 6-dithiolane-substituted penicillins.



Compound No.	R	IC_{50} (μM) Pen-ase*	TEM2**	Ceph-ase**
245	Me-	>100	14.4	0.00061
246	Et-	>100	7.3	0.00049
247	Na-	>100	9.8	0.0091
Tazobactam		0.31	0.076	2.4

For footnotes see Table 4-1.

Table 4-6 β-Lactamase inhibitory activities of the 6-dithiolane-substituted penicillin sulfones.



Compound No.	R	IC_{50} (μM) Pen-ase*	TEM2**	Ceph-ase**
250	Et	>100	53.1	0.15
251	Na	>100	34	0.226
Tazobactam		0.31	0.076	2.4

For footnotes see Table 4-1.

the penam sulfones **234** and **235** provided the dithiolane substituted penam sulfones **248** and **249** in 53% and 69% yields, which were then converted into the sodium carboxylate **250** and **251** in 80% and 90% yields, respectively (Scheme 4-5).

As summarized in Table 4-5, the β-lactamase inhibitory activities of the dithiolane substituted penicillin derivatives against cephalosporinase were remarkably improved relative to the corresponding methylene compounds. The ester groups at the side chain did not make a large difference in inhibitory activity of **245** and **246**. However, as opposed to most of the other cases studied, the free carboxylate compound **247** in this series showed a much weaker inhibition (more than 10 times) compared to its ester counterparts. Additionally, all of these derivatives also inhibited TEM enzyme, although the activities were quite low. Again, the sulfone derivatives **250** and **251** were found to possess much lower activities against the cephalosporinase than the corresponding penam derivatives **246** and **247** (Table 4-6).

4.6 Structure - activity relationships

Although the precise mode of interaction of carboxyl-substituted cephalosporins and penicillins with β-lactamases is not clear, from the data accumulated so far, the following structure-activity relationships are drawn.
1) Most carboxyl substituted cephalosporins and penicillins inhibited the enzymatic activity of the cephalosporinase. Considering that the 6-malonamidopenam **221** and penicillin G (**1**) interact with the cephalosporinase in a quite different manner, namely the former acts as a potent inhibitor and the latter as a good substrate, while they both occupy quite similar space in a three dimensional structure, it is apparent that the

carboxyl group is indeed playing an important role in stabilizing the substrate-enzyme complex.

2) The substrate-enzyme complex can be either non-covalently complexed or covalently linked in a relatively stable acyl-enzyme complex. Comparison of the cephalosporin and 3'-desoxycephalosporin derivatives in their cephalosporinase inhibitory activities suggests that these derivatives are apparently interacting with the enzyme by a covalent interaction. The findings that most cephalosporinase-stable β-lactam antibiotics such as aztreonam, moxalactam, and the third generation cephalosporins inhibit cephalosporinases by forming the stable acyl-enzyme complex[146,147,148] also strongly support the above conclusion.

3) When the same side chain was introduced, the penicillin analogues showed higher inhibitory activity against the cephalosporinase than cephalosporin analogues did. This phenomenon is possibly explained either by a stronger affinity of the penicillin derivatives for the cephalosporinase or by the formation of a more stable acyl-enzyme complex by the penicillin derivatives, or by both these explanations.

4) The relative position of the carboxyl group in the side chain structure affected the inhibitory activity of the substance against the cephalosporinase. In general, stronger inhibitory activity was observed with the order $RO_2CCH_2CONH- > RO_2CCONH- > RO_2CCH_2CH_2CONH-$.

5) In most cases, the ester derivatives showed substantially similar degrees of inhibitory activity as the corresponding sodium carboxylate derivatives.

6) The penam sulfone derivatives showed much lower activity against the cephalosporinase than the corresponding penam analogues.

7) Introduction of a dithiolanylidene group at the methylene position of the malonamidopenams enhanced their cephalosporinase inhibitory activity

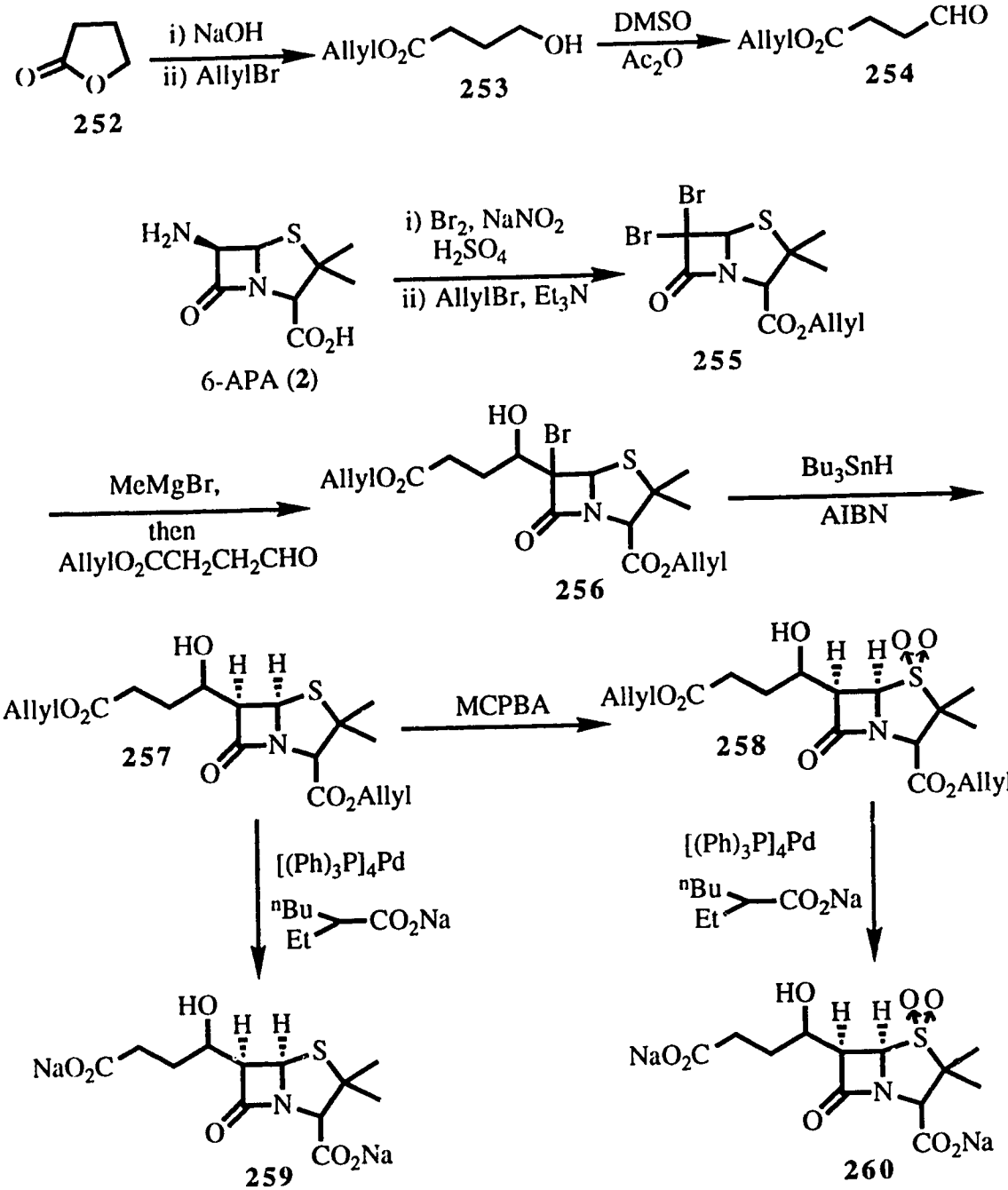
dramatically. This improvement in the activity may be attributed to the synergism of two independent substitution effects of the carboxyl group and the bulkiness of the side chain structure which leads to the further stabilization of the acyl-enzyme intermediate.

4.7 Syntheses and β-lactamase inhibitory activities of 6-*C*-substituted penicillin derivatives

With the success in the preparation of potent cephalosporinase inhibitors with a series of carboxyl substituted β-lactam derivatives, the syntheses of 6-*C*-substituted analogues of the carboxyl-substituted penicillins were investigated next. The compounds **259** and **260** were selected to be synthesized based on the similarity in the relative position of the carboxyl group to that of the 6-malonamidopenam **227**. In addition, the dehydrated products **264** and **265** were also synthesized.

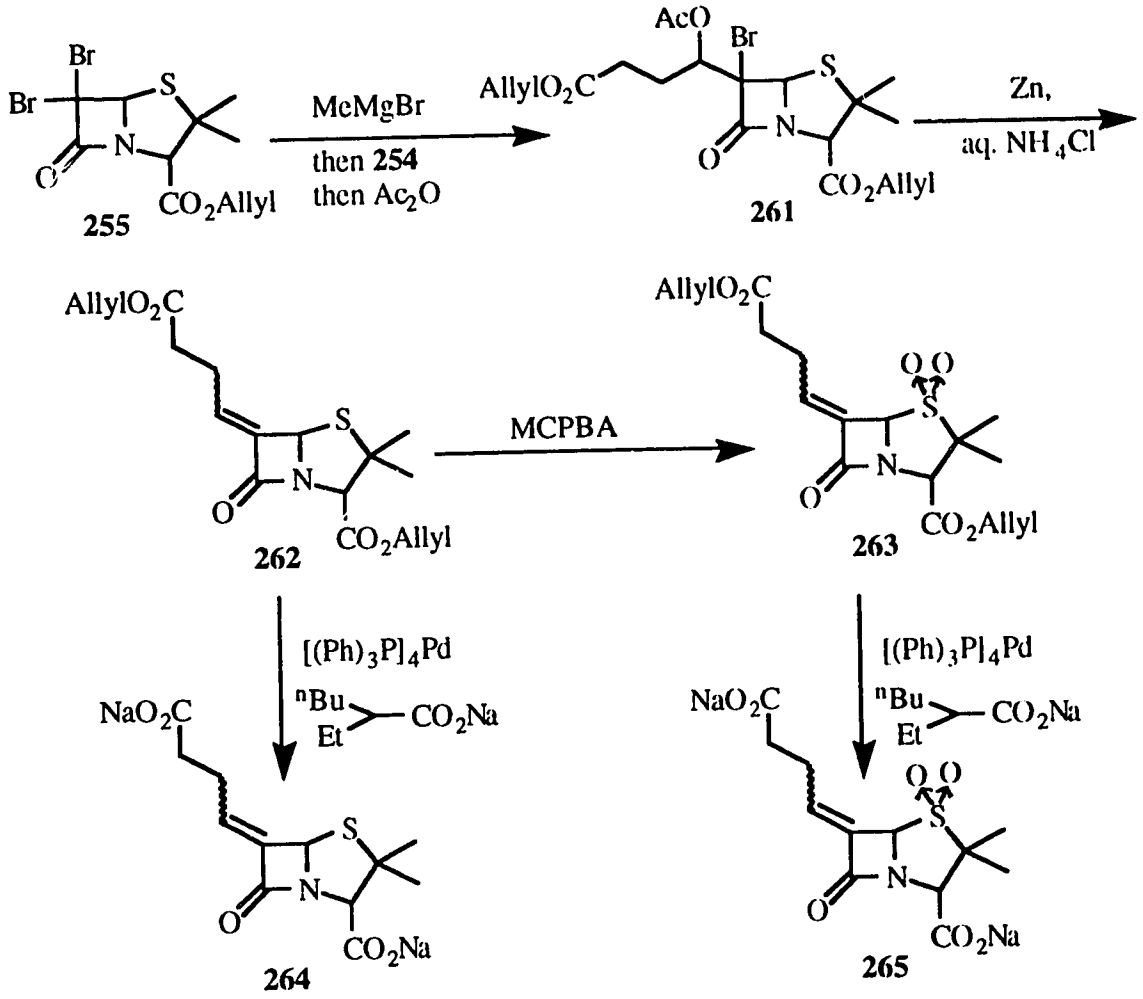
Scheme 4-6 summarizes the syntheses of **259** and **260**.

Allyl 6,6-dibromopenicillanate (**255**), which was readily prepared from 6-APA (**2**), was first treated with methyl magnesium bromide at -78°C, then trapped with the aldehyde **254** to afford a stereoisomeric mixture of the hydroxyalkyl adduct **256**. The aldehyde **254** was prepared from butyrolactone (**252**) through the hydrolysis and esterification, followed by Swern's oxidation.[149] The stereoselective reduction of the mixture **256** with tributyltin hydride in benzene afforded the desired alcohol **257** in 40% yield (based on **255**) as a major product.[150] The *cis*-configuration between C5-C6 was evident from the coupling constant of 5 Hz, which is typical of 3,4-*cis*-substituted azetidinones. Oxidation of **257** with *m*-chloroperbenzoic acid in dichloromethane provided the corresponding sulfone **258** in 53% yield.


i) NaOH
ii) AllylBr
AllylO2C
OH
DMSO
Ac2O
CHO
252
253
254
H2N
S
N
O
CO2H
6-APA (2)
i) Br2, NaNO2
H2SO4
ii) AllylBr, Et3N
Br
CO2Allyl
255
MeMgBr,
then
AllylO2CCH2CH2CHO
HO
256
Bu3SnH
AIBN
257
MCPBA
258
[(Ph)3P]4Pd
nBu
Et
CO2Na
NaO2C
259
260

Scheme 4-6

Conversion of the allyl esters **257** and **258** to the corresponding sodium salts **259** and **260** was achieved in 82% and 91% yields, respectively.

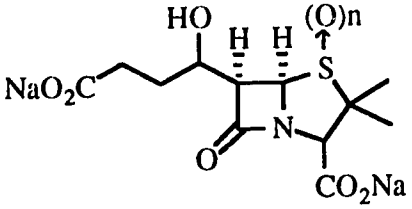


Scheme 4-7

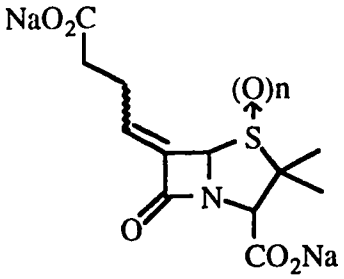
Scheme 4-7 depicts the syntheses of **264** and **265**. Allyl 6,6-dibromopenicillanate (**255**) was converted into a mixture of the hydroxyalkyl adducts, which was then acetylated with acetic anhydride *in situ* to give the acetoxy bromide **261**. The reductive elimination of the acetoxy-bromide with zinc powder in a bi-phasic mixture of THF / aq.NH4Cl solution afforded the olefinic product **262** in 21% yield (based on **255**). Oxidation of the penam

262 with *m*-chloroperbenzoic acid gave the sulfone **263** in 61% yield. Conversion of **262** and **263** to the corresponding sodium carboxylates **264** and **265** was carried out by the deesterification reaction with sodium 2-ethylhexanoate in the presence of palladium (0) catalyst in 82%, 91%, 56%, and 82% yields, respectively.

Table 4-7 β-Lactamase inhibitory activities of the 6-*C*-substituted penicillins and penicillin sulfones.



259 and **260**



264 and **265**

Compound No.	n	IC50 (μM) Pen-ase*	TEM2**	Ceph-ase**
259	0	>100	53	5.3
260	2	>100	16.4	0.35
264	0	>100	>100	>100
265	2	17	0.83	5.3
Tazobactam		0.31	0.076	2.4

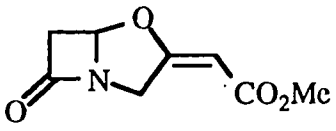
For footnotes see Table 4-1.

Table 4-7 summarizes the β-lactamase inhibitory activities of the prepared compounds. The hydroxyalkylpenam **259** showed some activities against TEM enzyme and cephalosporinase, while it did not inhibit penicillinase. Contrary to the 6-amido-substituted penam derivatives, the penam sulfone **260** inhibited both TEM enzyme and cephalosporinase more

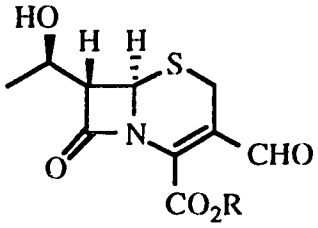
strongly than the penam **259**. The ene-penam **264** showed no inhibitory activities against any of the three enzymes, whereas its sulfone analogue **265** inhibited all three enzymes strongly. The fact that the penam sulfones **260** and **265** possess stronger inhibitory activity relative to the corresponding penams may imply that the sulfone derivatives inhibit these enzymes by a branched pathway mechanism as in the case of clavulanic acid and sulbactam.

4.8 Syntheses and β-lactamase inhibitory activities of 7-substituted methyl cephalosporanates and *N*-penicillanylglycine sulfone

Generally, β-lactam antibiotics and β-lactamase inhibitors require anionic groups, primarily a carboxylic group, at certain positions of the molecule to inhibit PBPs or β-lactamases. Penicillins and carbapenems, for instance, carry their carboxylic group at the 3-position of the nucleus, and cephalosporins at the 4-position. Although monobactams possess unique structures, they also have an anionic group, such as SO_3^-, on the N1-position of the azetidinone nucleus. However, there are some exceptions. BRL 19378 **266**, an oxapenam derivative without any substituent at the 3-position, for instance, has been found to inhibit class I cephalosporinases.[151]



BRL 19378 (**266**)



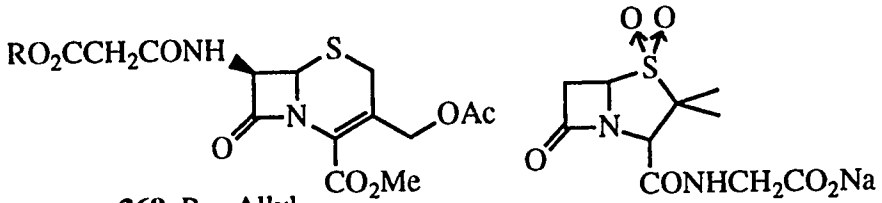
267 R = Allyl
268 R = Na

Figure 4-5 Structures of cephalosporinase inhibitors.

More recently, the allyl cephalosporanate **267** has been reported to show stronger inhibition than the corresponding sodium carboxylate **268** against some class I cephalosporinases (Figure 4-5).[152] These findings suggested that the anionic group, which was considered to be indispensable in most cases, might not be necessary in the inhibition of certain cephalosporinases. To investigate this consideration, the cephalosporin derivatives **269**, **270**, and sulbactam derivative **275** were synthesized and biologically evaluated.

Scheme 4-8 summarizes the syntheses of **269**, **270** and **275**. The methyl cephalosporanate **269** was prepared in 89% yield from the carboxylic compound **202** by treatment with *N,N'*-dicyclohexylcarbodiimide (DCC) in the presence of excess methanol. Palladium (0) mediated allyl-sodium exchange of **202** afforded the desired sodium carboxylate **270** in 95% yield.

Table 4-8 β-Lactamase inhibitory activities of the cephem esters and the *N*-penicillanylglycine sulfone.

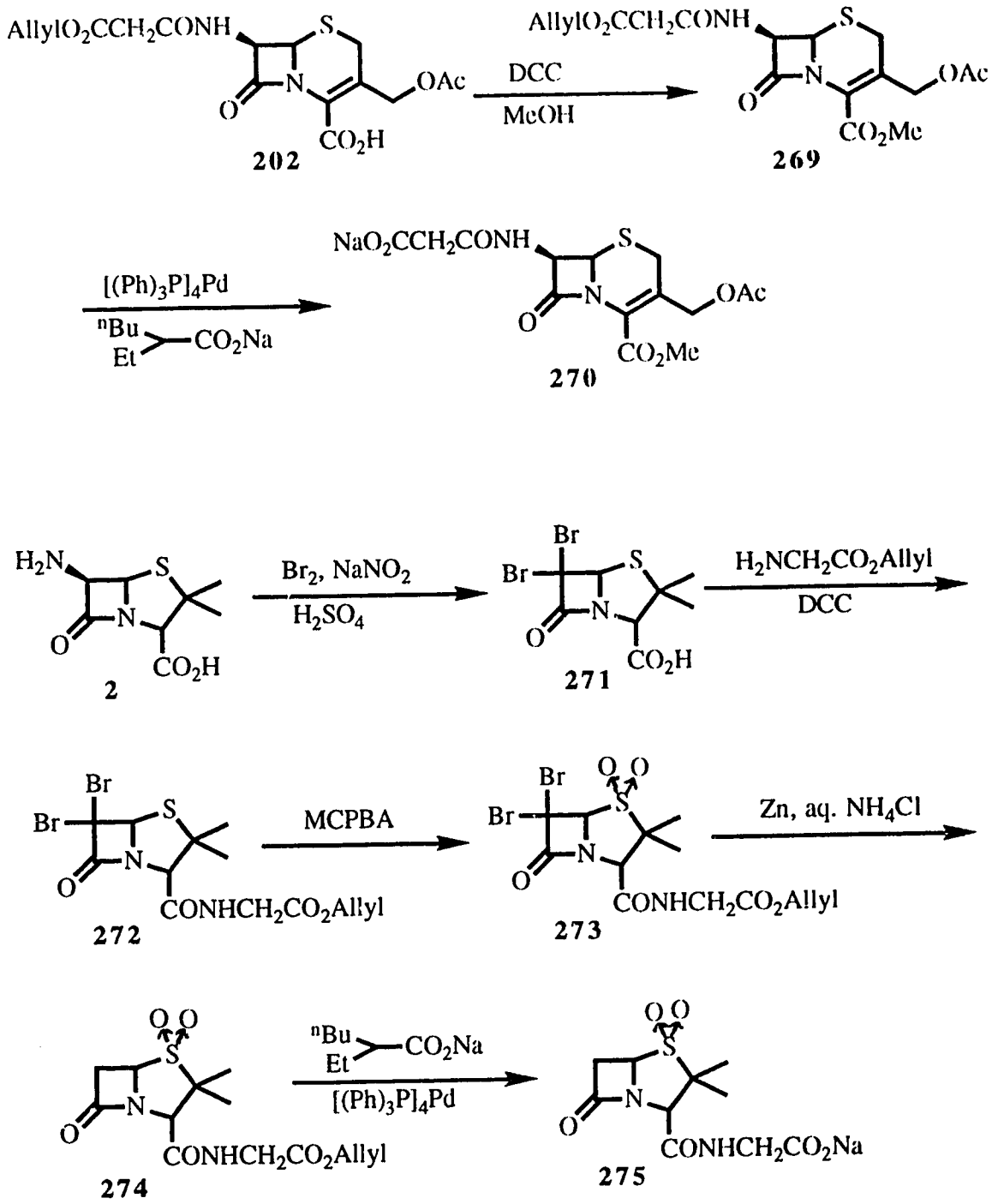


269 R = Allyl
270 R= Na

275

Compound No.	IC50 (μM) Pen-ase*	TEM2**	Ceph-ase**
269	>100	>100	>100
270	>100	>100	49.7
275	>100	>100	>100
Tazobactam	0.31	0.076	2.4

For footnotes see Table 4-1.


AllylO2CCH2CONH
OAc
CO2H
202
DCC
MeOH
CO2Me
269
[(Ph)3P]4Pd
nBu
Et
CO2Na
NaO2CCH2CONH
270
H2N
2
Br2, NaNO2
H2SO4
Br
271
H2NCH2CO2Allyl
DCC
MCPBA
CONHCH2CO2Allyl
272
273
Zn, aq. NH4Cl
274
275
CONHCH2CO2Na

Scheme 4-8

Preparation of the amide **272** was accomplished in 18% yield by the treatment of 6,6-dibromopenicillanic acid **271** with glycine allyl ester and DCC in THF. Oxidation of **272** with *m*-chloroperbenzoic acid afforded the sulfone **273** in 60% yield, which was reduced with zinc to give the debrominated product **274** in 68% yield. Final conversion of **274** into the sodium carboxylate **275** was carried out as usual with palladium (0) catalyst in 85% yield.

Table 4-8 indicates the β-lactamase inhibitory activities of **269**, **270**, and **275**. Although **270** showed weak inhibition against cephalosporinase, the others did not inhibit any of the three enzymes.

4.9 Synergistic activities of carboxyl substituted cephalosporins and penicillins with β-lactam antibiotics

Several (un)protected carboxyl substituted cephalosporin derivatives **200** and **201** and penicillin derivatives **218**, **221**, **227**, **239**, **245**, and **247**, which showed a high level of β-lactamase inhibitory activity against the isolated enzymes, were selected for synergistic activity testing. Ampicillin and cephalothin were selected as the β-lactam antibiotics to be combined with these inhibitors. Various β-lactamase producing microbes were used for the testing and the minimum inhibitory concentrations (MICs) determined by the microbroth dilution assay, for the evaluation of the synergistic effect. The concentration of inhibitors were fixed at 5 μg/mL.

Tables 4-9 and 4-10 show the synergistic activities of **200**, **201**, **218**, **239**, **221**, **227**, **245**, and **247** with ampicillin. Tables 4-11 and 4-12 summarize the synergistic activities of **200**, **201**, **218**, **239**, **221**, **227**, **245**, and **247** with cephalothin. The MICs of all the inhibitors alone were found to be more than 128 mg/mL except for **227** (64 μg/mL against *E. coli* S103).

No synergistic effect was observed when inhibitors **201**, **218**, **239**, **221**, were combined with either ampicillin or cephalothin. Some synergy was observed for the combination of **200** with ampicillin and cephalothin against *E. coli* S102 and *C. freundii* S40. A strong synergistic effect was observed when combinations of inhibitors **245** and **247** with ampicillin and cephalothin were tested against cephalosporinase producing microbes, *viz. E. aerogenes* S95 and *C. freundii* S40. These results reflected the *in vitro* β-lactamase inhibitory activity, however, the overall synergistic activity was somewhat lower than expected. Nikaido *et al.* studied diffusion rates of various β-lactam antibiotics through OmpF and OmpC porin channels of *E. coli* K-12[71] and found that there was a dependence of the penetration rate on the hydrophobicity of the molecule. For instance, in the case of the classical monoanionic β-lactams, 10-fold increase in the octanol-water partition coefficient of the uncharged molecule decreased the penetration rate by a factor of 5 to 6. Since most Gram-negative bacteria share a similar outer membrane structure, the observed low synergy against Gram-negative bacteria can be possibly attributed to the decreased penetration of those inhibitors through the outer membrane.

Table 4-9 Synergistic activity of inhibitors with ampicillin (A).

Microbes	MIC (μg / mL)									
	A	200	200+A	201	201+A	218	218+A	239	239+A	A+TZB
E. coli S101	>128	>128	>128	>128	>128	>128	>128	>128	>128	2
E. coli S102	>128	>128	32	>128	>128	>128	>128	>128	>128	4
E. coli S103	>128	>128	>128	>128	>128	>128	>128	>128	>128	8
E. coli S104	>128	>128	>128	>128	>128	>128	>128	>128	>128	128
E. cloacae S11	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
E. aerogenes S95	>128	>128	64	>128	64	>128	64	>128	64	64
F. odoratum S90	64	>128	64	>128	64	>128	64	>128	64	64
C. freundii S40	128	>128	2,4,16	>128	128	>128	128	>128	128	2,8,32
K. pneumoniae S80	64	>128	64	>128	64	>128	64	>128	64	4
S. marcescens S126	>128	>128	128	>128	128	>128	128	>128	128	128
P. aeruginosa S67	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
S. epidermidis S128	128	>128	64	>128	128	>128	128	>128	128	8

Microbroth Dilution Method, MHB; Inoculum, $5x10^5$ cfu/mL; Incubation, 16h/35°C;
Concentration of inhibitors = 5 μg/mL (fixed); TZB, tazobactam.

Table 4-10 Synergistic activity of inhibitors with ampicillin (A).

Microbes	MIC (μg / mL)									
	A	221	221+A	227	227+A	245	245+A	247	247+A	A+TZB
E. coli S101	>128	>128	>128	>128	>128	>128	>128	>128	>128	2
E. coli S102	>128	>128	>128	>128	>128	>128	>128	>128	>128	4
E. coli S103	>128	>128	>128	64	>128	>128	>128	>128	>128	8
E. coli S104	>128	>128	>128	>128	>128	>128	>128	>128	>128	128
E. cloacae S11	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
E. aerogenes S95	>128	>128	128	>128	128	>128	64	>128	128	32
F. odoratum S90	64	>128	64	>128	64	>128	64	>128	64	64
C. freundii S40	128	>128	128	>128	128	>128	2	>128	2	4
K. pneumoniae S80	64	>128	128	>128	128	>128	64	>128	128	4
S. marcescens S126	>128	>128	>128	>128	>128	>128	>128	>128	>128	128
P. aeruginosa S67	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
S. epidermidis S128	128	>128	>128	>128	>128	>128	>128	>128	>128	8

Microbroth Dilution Method, MHB; Inoculum, $5x10^5$ cfu/mL; Incubation, 16h/35°C;
Concentration of inhibitors = 5 μg/mL (fixed); TZB, tazobactam.

Table 4-11 Synergistic activity of inhibitors with cephalothin (C).

Microbes	MIC (μg / mL)									
	C	200	200+C	201	201+C	218	218+C	239	239+C	C+TZB
E. coli S101	16	>128	8	>128	8	>128	8	>128	8	2
E. coli S102	16	>128	4	>128	16	>128	8	>128	8	2
E. coli S103	64	>128	64	64	64	>128	64	>128	32	2
E. coli S104	8	>128	8	>128	16	>128	16	>128	8	8
E. cloacae S11	>256	>128	256	>128	>256	>128	>256	>128	>256	>256
E. aerogenes S95	256	>128	256	>128	256	>128	>256	>128	>256	>256
F. odoratum S90	>256	>128	>256	>128	>256	>128	>256	>128	>256	>256
C. freundii S40	256	>128	2,32	>128	8,32,128	>128	256	>128	256	8
K. pneumoniae S80	8	>128	8	>128	8	>128	16	>128	8	4
S. marcescens S126	>256	>128	>256	>128	>256	>128	>256	>128	>256	>256
P. aeruginosa S67	>256	>128	>256	>128	>256	>128	>256	>128	>256	>256
S. epidermidis S128	64	>128	32	>128	64	>128	64	>128	64	32

Microbroth Dilution Method, MHB; Inoculum, $5x10^5$ cfu/mL; Incubation, 16h/35°C;
Concentration of inhibitors = 5 μg/mL (fixed); TZB, tazobactam.

Table 4-12 Synergistic activity of inhibitors with cephalothin (C).

Microbes	MIC (μg / mL)									
	C	221	221+C	227	227+C	245	245+C	247	247+C	C+TZB
E. coli S101	16	>128	16	>128	8	>128	8	>128	8	2
E. coli S102	16	>128	8	>128	16	>128	8	>128	8	2
E. coli S103	64	>128	64	64	64	>128	64	>128	64	2
E. coli S104	8	>128	8	>128	8	>128	8	>128	8	8
E. cloacae S11	>256	>128	>256	>128	>256	>128	>256	>128	>256	>256
E. aerogenes S95	>256	>128	>256	>128	>256	>128	8	>128	4	>256
F. odoratum S90	>256	>128	>256	>128	>256	>128	>256	>128	>256	>256
C. freundii S40	256	>128	256	>128	64	>128	4	>128	2	8
K. pneumoniae S80	8	>128	8	>128	8	>128	4	>128	16	4
S. marcescens S126	>256	>128	>256	>128	>256	>128	>256	>128	>256	>256
P. aeruginosa S67	>256	>128	>256	>128	>256	>128	>256	>128	>256	>256
S. epidermidis S128	64	>128	64	>128	64	>128	64	>128	64	32

Microbroth Dilution Method, MHB; Inoculum, $5x10^5$ cfu/mL; Incubation, 16h/35°C;
Concentration of inhibitors = 5 μg/mL (fixed); TZB, tazobactam.

EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. The IR spectra were recorded on a Nicolet DX-FTIR spectrometer. NMR spectra were recorded on a Bruker AM-300 spectrometer using tetramethylsilane as an internal standard. Elementary analyses were performed with a Carlo Erba 1108 CHN analyzer. High resolution mass spectra were recorded on a Kratos MS50 and FAB mass spectra were recorded on a AEI MS9. Some of the compounds described below have neither elementary analysis data nor Mass spectrum result, usually due to their chemical unstability. However, these compounds showed satisfactory result with other spectra to support their structure assignment. For TLC analysis, glass TLC plates (Sigma, T-6270, with 254 nm fluorescent indicater, 250 μm layer thickness) were used and detected with a UV detecter, Model UVG-54 (wavelength 254 nm). THF was dried by distillation over benzophenone and sodium metal. Benzene, dichloromethane and toluene were distilled over phosphorus pentoxide to make them anhydrous before usage.

5.1 Experiments for Chapter 3

p-Nitrobenzyl 4-Bromo-3-oxobutanoate (120).

Method A. A solution of bromine (16.0 g, 100 mmol) in 20 mL of anhydrous dichloromethane was added to a stirred solution of diketene (8.41 g, 100 mmol) in 200 mL of anhydrous dichloromethane at -78°C under a nitrogen atmosphere. After 15 min of stirring, *p*-nitrobenzyl alcohol (15.3 g, 100 mmol) was added to the solution and the mixture allowed to warm up to

room temperature. After 1 h of stirring at room temperature, the reaction mixture was washed sequentially with water, saturated $NaHCO_3$ solution and saturated NaCl solution, then dried over Na_2SO_4. After removal of the solvent, the residue was chromatographed on silica gel with chloroform as the eluting solvent to yield the bromoacetoacetate **120** (20.8 g, 68 %) as a brown solid, which was recrystallized from toluene-hexane to give light brown crystals: m.p. 48-50°C; IR ($CHCl_3$) 1750, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.81 (2H, s, C_2-$\underline{H}_2$), 4.01 (2H, s, C_4-$\underline{H}_2$), 5.28 (2H, s, $OC\underline{H}_2$), 7.52 and 8.23 (each 2H, d, J=9Hz, aromatic H); HR-MS calcd for $C_{13}H_{12}N_2O_3$ *m/z* 244.0848, found *m/z* 244.0849.

Method B. Meldrum's acid (1.44 g, 10 mmol) was dissolved in 30 mL of anhydrous dichloromethane and treated with 2,6-lutidine (2.14 g, 20 mmol) and bromoacetyl chloride (1.57 g, 10 mmol) at 0°C. The reaction mixture was stirred for 1 h in an ice bath under a nitrogen atmosphere. After the addition of 10 mL of 1N HCl solution, the organic layer was separated, washed with water and saturated NaCl solution, and dried over Na_2SO_4. Removal of the solvent gave the crude acylated Meldrum's acid which was used for the next reaction without purification.

The crude residue obtained above was dissolved in 20 mL of toluene, *p*-nitrobenzyl alcohol (1.07 g, 7.0 mmol) was added and the mixture refluxed for 2 h under a nitrogen atmosphere. The solution was concentrated, and the residue subjected to silica gel chromatography with chloroform as the eluant, to give the bromoacetoacetate **120** (1.34 g, 42 %).

***p*-Nitrobenzyl 4-Chloro-3-oxobutanoate (123).**

Sulfuryl chloride (8.03 mL, 100 mmol) was added to a solution of diketene (8.41 g, 100 mmol) in anhydrous dichloromethane under a nitrogen

atmosphere at -78°C. The solution was stirred for 15 min, treated with sulfuryl chloride (8.03 g, 100 mmol), then allowed to warm to room temperature. After stirring for an additional 3 h, the reaction mixture was washed sequentially with water, saturated $NaHCO_3$ solution, and saturated NaCl solution, then dried over Na_2SO_4. After concentration, the residue was chromatographed on silica gel with chloroform as the eluant to give the chloroacetoacetate **123** (18.4 g, 68 %) as a brown oil: IR ($CHCl_3$) 1745, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.76 (2H, s, C_2-$\underline{H}_2$), 4.19 (2H, s, C_4-$\underline{H}_2$), 5.27 (2H, s, $OC\underline{H}_2$), 7.52 and 8.22 (each 2H, d, J=9Hz, aromatic H).

***p*-Nitrobenzyl 4-Bromo-2-diazo-3-oxobutanoate (122).**

Triethylamine (1.4 mL, 10 mmol) and *p*-toluenesulfonyl azide (1.97 g, 10 mmol) were added to a stirred solution of the bromoacetoacetate **120** (3.04 g, 10 mmol) in 30 mL of anhydrous dichloromethane. The reaction mixture was stirred at room temperature for 3 h. The resulting precipitate was filtered off and the filtrate concentrated under reduced pressure. The residue was purified by silica gel column chromatography with chloroform as the eluant to afford the diazoacetoacetate **122** (2.63 g, 80 %) as a yellow solid, which was recrystallized from toluene-hexane to give light yellow crystals: m.p.117-118°C; IR ($CHCl_3$) 2150, 1720, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.37 (2H, s, C_4-$\underline{H}_2$), 5.37 (2H, s, $OC\underline{H}_2$), 7.54 and 8.25 (each 2H, d, J=9Hz,$C_6\underline{H}_4$).

***p*-Nitrobenzyl 4-Chloro-2-diazo-3-oxobutanoate (124).**

The title compound was obtained as light yellow crystals in 74 % yield from the corresponding chloroacetoacetate **123** according to the procedure described above for **122**: m.p. 120-121°C; IR ($CHCl_3$) 2150, 1720, 1670 cm^{-}

1; ^1H NMR ($CDCl_3$) δ 4.58 (2H, s, C_4-$\underline{H}_2$), 5.36 (2H, s, $OC\underline{H}_2$), 7.53 and 8.24 (each 2H, d, J=9Hz, aromatic H).

_p_-Nitrobenzyl 5-Phenylthio-3-oxopentanoate (125).

Carbonyldiimidazole (3.69 g, 22.8 mmol) was added to a stirred solution of the carboxylic acid **123** (3.95 g, 21.7 mmol) in 20 mL of anhydrous tetrahydrofuran under a nitrogen atmosphere. Immediately, vigorous evolution of carbon dioxide was observed. After 30 min of stirring at room temperature, the solution was mixed with a suspension of magnesium *p*-nitrobenzyl malonate (**124**) (10.9 mmol) in 10 mL of anhydrous tetrahydrofuran, and stirred overnight at ambient temperature. The reaction mixture was diluted with ethyl acetate and washed sequentially with diluted HCl solution, water, saturated $NaHCO_3$ solution,and saturated NaCl solution. After drying over Na_2SO_4, the solvent was evaporated *in vacuo* to give a thick oil which was purified by silica gel column chromatography using chloroform as the eluant to give the desired product **125** (4.54 g, 6 %) as a yellow solid, which was recrystallized from toluene-hexane to afford light yellow crystals: m.p. 74-75°C; IR ($CHCl_3$) 1745, 1715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.85 (2H, t, J=7Hz, C_4-$\underline{H}_2$), 3.14 (2H, t, J=7Hz, C_5-$\underline{H}_2$), 3.53 (2H, s, C_2-$\underline{H}_2$), 5.24 (2H, s, $OC\underline{H}_2$), 7.15-7.34 (5H, m, $C_6\underline{H}_5$), 7.49 and 8.20 (each 2H, J=9Hz, $C_6\underline{H}_4$). Anal. Calcd for $C_{18}H_{17}NO_5S$: C, 60.16; H, 4.77; N, 3.90. Found: C, 60.02, H, 4.68; N, 4.01.

_p_-Nitrobenzyl 2-Diazo-3-oxo-5-phenylthiopentanoate (126).

The title compound was obtained in 79 % yield as light yellow crystals from the oxopentanoate **125** (4.54 g, 12.6 mmol) according to the procedure described for **122**: m.p. 89-90°C; IR ($CHCl_3$) 2145, 1720, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.15-3.25 (4H, m, $SC\underline{H}_2C\underline{H}_2CO$), 5.32 (2H, s, $OC\underline{H}_2$), 7.12-

7.36 (5H, m, $C_6\underline{H}_5$), 7.49 and 8.23 (each 2H, d, J=9Hz, $C_6\underline{H}_4$). Anal. Calcd for $C_{18}H_{15}N_3O_5S$: C, 56.10; H, 3.92; N, 10.90. Found: C, 56.18, H, 3.63; N, 10.42.

_p_-Nitrobenzyl 2-Diazo-3-oxo-4-phenylbutanoate (130a).

Pyridine (31.6 g, 400 mmol) and phenylacetyl chloride (30.9 g, 200 mmol) were added to a stirred solution of Meldrum's acid (28.8 g, 200 mmol) in 400 mL of anhydrous dichloromethane at 0°C. The reaction mixture was stirred at 0°C for 30 min under a nitrogen atmosphere, then 200 mL of 1N HCl solution added. The organic layer was separated, washed sequentially with water and saturated NaCl solution. After drying over Na_2SO_4, the solution was concentrated under reduced pressure.

To this residue, *p*-nitrobenzyl alcohol (30.6 g, 200 mmol) was added and the mixture heated to 140°C. The stirring was continued for 1 h *in vacuo* using water aspiration. After cooling, the mixture was dissolved in 400 mL of anhydrous dichloromethane and treated with *p*-toluenesulfonyl azide (39.4 g, 200 mmol) followed by triethylamine (20.2 g, 100 mmol). The solution was stirred at room temperature for 1 h. The precipitate was filtered off and the filtrate concentrated under vacuum. The residue was chromatographed on silica gel with chloroform as the eluant to give the diazoacetoacetate **130a** (38.6 g, 57 %) as a yellow solid, which was recrystallized from toluene-hexane: m.p. 82-83°C; IR ($CHCl_3$) 2150, 1720, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.17 (2H, s, C_4-$\underline{H}_2$), 5.35 (2H, s, $OC\underline{H}_2$), 7.20-7.35 (5H, m, $C_6\underline{H}_5$), 7.51 and 8.24 (each 2H, d, J=9Hz, $C_6\underline{H}_4$). Anal. Calcd for $C_{17}H_{13}N_3O_5$: C, 60.18; H, 3.86; N, 12.38. Found: C, 60.06, H, 3.57; N, 12.18.

Allyl 2-Diazo-3-oxo-4-phenylbutanoate (130b).

In a manner similar to that described above for **130a**, the title compound **130b** was prepared from Meldrum's acid (14.4 g, 100 mmol) in 66 % yield as a yellow oil: IR ($CHCl_3$) 2145, 1710, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.19 (2H, s, C_4-$\underline{H}_2$), 4.70-4.78 (2H, m, -$C\underline{H}_2$-CH=CH_2), 5.27-5.39 (2H, m, -CH_2-CH=$C\underline{H}_2$), 5.87-6.01 (1H, m, -CH_2-$C\underline{H}$=CH_2), 7.20-7.38 (5H, m, aromatic H); HR-MS calcd for $C_{13}H_{12}N_2O_3$ *m/z* 279.0743, found *m/z* 279.0746.

***p*-Nitrobenzyl 2-Diazo-4-(5-methyltetrazol-2-yl)-3-oxobutanoate (132a) and *p*-Nitrobenzyl 2-Diazo-4-(5-methyltetrazol-1-yl)-3-oxobutanoate (132b).**

5-Methyltetrazole (0.303 g, 3.6 mmol) and triethylamine (0.334 g, 3.3 mmol) was added to a solution of the bromide **122** (1.03 g, 3 mmol) in 10 mL of anhydrous dichloromethane. The reaction mixture was stirred at room temperature for 3 h, then washed sequentially with 1N HCl solution, water, saturated $NaHCO_3$ solution, and saturated NaCl solution. The solution was dried over Na_2SO_4. Solvent removal gave a thick oil which was chromatographed on silica gel. The *N*-2 isomer **132b** (0.330 g, 32 %) was obtained from chloroform-ethyl acetate (85:15) eluting fractions as yellow crystals: m.p. 138-140°C (recrystallized from toluene); IR ($CHCl_3$) 2150, 1720, 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.55 (3H, s, $C\underline{H}_3$), 5.41 (2H, s, $OC\underline{H}_2$), 5.85 (2H, s, C_4-$\underline{H}_2$), 7.56 and 8.26 (each 2H, d, J=9Hz, aromatic H). The *N*-1 isomer **132a** (0.539 g, 52 %) was obtained from chloroform-ethyl acetate (60:40) eluting fractions as yellow crystals: m.p. 158-159°C (recrystallized from toluene); IR ($CHCl_3$) 2160, 1725, 1675 cm^{-1}; ^1H NMR

($CDCl_3$) δ 2.24 (3H, s, CH3), 5.20 (2H, s, C4-H2), 5.44 (2H, s, OCH2), 7.37 and 8.01 (each 2H, d, J=9Hz, aromatic H).

_p_-Nitrobenzyl 2-Diazo-3-oxo-4-phenylthiobutanoate (133).

Thiophenol (3.31 g, 30 mmol) was added to a cooled solution of the bromide **122** (10.26 g, 30 mmol) in 60 mL of anhydrous dichloromethane followed by triethylamine (4.6 mL, 3.3 mmol) under a nitrogen atmosphere at 0°C. The reaction mixture was stirred for 30 min at 0°C. The mixture was washed sequentially with water, saturated $NaHCO_3$ solution and saturated NaCl solution, and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel using chloroform as the eluant to give the desired diazoacetoacetate **133** (10.5 g, 94 %) as colorless crystals after recrystallization from toluene-hexane: m.p. 91°C-92°C; IR ($CHCl_3$) 2145, 1715, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.11 (2H, s, C_4-$\underline{H}_2$), 5.36 (2H, s, $OC\underline{H}_2$), 7.18-7.31 (5H, m, $C_6\underline{H}_5$), 7.53 and 8.24 (each 2H, d, J=9Hz, $C_6\underline{H}_4$). Anal. Calcd for $C_{17}H_{13}N_3O_5S$: C, 54.98; H, 3.53; N, 11.31. Found: C, 54.64, H, 3.14; N, 11.01.

_p_-Nitrobenzyl 2-Diazo-4-(4-methyl-4-*H*-1,2,4-triazole-3-thio)-3-oxo-butanoate (134).

4-Methyl-4*H*-1,2,4-triazole-3-thiol (288 mg, 2.5 mmol) and triethylamine (0.28 mL, 2 mmol) were added to a solution of the bromide **122** (684 mg, 2 mmol) in 6 mL of anhydrous dichloromethane under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1 h, washed sequentially with water, saturated $NaHCO_3$ solution, and saturated NaCl solution, then dried over Na_2SO_4. The solution was concentrated, and the residue chromatographed on silica gel with chloroform-ethyl acetate (50:50) as the eluting solvent system to give the

desired product **134** (712 mg, 95 %) as yellow crystals after recrystallization from toluene: m.p.154-155°C; IR ($CHCl_3$) 2145, 1715, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.66 (3H, s, N-C<u>H</u>$_3$), 4.53 (2H, s, C_4-<u>H</u>$_2$), 5.36 (2H, s, OC<u>H</u>$_2$), 7.55 and 8.25 (each 2H, d, J=9Hz, aromatic H), 8.12 (1H s, triazolyl C_5-<u>H</u>). Anal. Calcd for $C_{14}H_{12}N_6O_5S$: C, 44.68; H, 3.21; N, 22.33. Found: C, 44.93, H, 3.03; N, 21.94.

_p_-Nitrobenzyl 2-Diazo-4-(1-methyltetrazole-5-thio)-3-oxobutanoate (135).

5-Mercapto-1-methyltetrazole sodium salt (345 mg, 2.5 mmol) was added to a stirred solution of the bromide **122** (684 mg, 2 mmol) in 6 mL of anhydrous tetrahydrofuran under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 1 h, diluted with ethyl acetate, then washed sequentially with water and saturated NaCl solution. The extract was dried over Na_2SO_4, and the solution was concentrated under reduced pressure. The residue was purified by silica gel column chromatography with acetone as the eluant to afford the desired compound **135** (680 mg, 90 %) as light yellow crystals: m.p. 129-130°C (recrystallized from toluene); IR ($CHCl_3$) 2150, 1720, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.97 (3H, s, N-C<u>H</u>$_3$), 4.68 (2H s, C_4-<u>H</u>$_2$), 5.38 (2H, s, OC<u>H</u>$_2$), 7.56 and 8.25 (each 2H, d, J=9Hz, aromatic H). Anal. Calcd for $C_{13}H_{11}N_7O_5S$: C, 41.38; H, 2.94; N, 25.98. Found: C, 41.37, H, 2.80; N, 25.64.

_p_-Nitrobenzyl 2-Diazo-5-methoxy-3-oxopentanoate (137).

Triethylamine (1.4 mL, 10 mmol) and trimethylsilyl trifluoromethanesulfonate (2.44 g, 11 mmol) were added successively to a solution of the diazoacetoacetate **135** (2.63 g, 10 mmol) in 30 mL of anhydrous dichloromethane at 0°C under a nitrogen atmosphere. The reaction mixture

was stirred in ice bath for 30 min and then treated with dimethoxymethane (2.65 g, 30 mmol). The mixture was allowed to warm to room temperature, and the stirring continued for 1 h. The solution was poured into ice water. The organic layer was separated, washed sequentially with saturated $NaHCO_3$ solution and saturated NaCl solution, and dried over Na_2SO_4. After removal of the solvent, the residue was chromatographed on silica gel with chloroform as the eluant to give the desired diazo compound **137** (1.56 g, 51 %) as yellow crystals: m.p. 62-63°C (recrystallized from toluene-hexane); IR ($CHCl_3$) 2145, 1720, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.11 (2H, t, J=6, C_4-$\underline{H}_2$), 3.33 (3H, s, $OC\underline{H}_3$), 3.70 (2H, t, J=6Hz, C_5-$\underline{H}_2$), 5.34 (2H, s, $OC\underline{H}_2$), 7.52 and 8.23 (each 2H, d, J=9Hz, aromatic H).

(3*S*, 4*S*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(1*R*)-1-bromo-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxopropyl]azetidin-2-one (142a) and (3*S*, 4*S*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(1*S*)-1-bromo-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxopropyl]azetidin-2-one (142b).

Triethylamine (1.21 g, 12 mmol) was added to a cooled solution of the diazoketoester **122** (4.45 g, 13 mmol) in 25 mL of anhydrous dichloromethane under a nitrogen atmosphere at 0°C, followed by trimethylsilyl trifluoromethanesulfonate (2.67 g, 12mmol). The reaction mixture was stirred in an ice bath for 30 min. To this solution, the 4-acetoxyazetidinone **100** (2.87 g, 10 mmol) was added portionwise, followed by trimethylsilyl trifluoromethanesulfonate (0.267 g, 1.2 mmol). The reaction mixture was stirred for 30 min at 0 °C under a nitrogen atmosphere , and 25 mL of water containing $NaHCO_3$ (0.42 g, 5 mmol) was added. The organic layer was separated, and the water layer was extracted with 2x10 mL

of dichloromethane. The combined organic layer was washed sequentially with diluted HCl solution, water, saturated $NaHCO_3$ solution, and saturated NaCl solution. After drying over Na_2SO_4, the solution was concentrated under reduced pressure to give a thick brown oil which was chromatographed on silica gel. From chloroform-ethyl acetate (95:5) eluting fractions, the β-isomer **142b** (17.2 g, 30 %) was obtained as colorless crystals: m.p. 123-124°C (recrystallized from toluene-hexane); IR ($CHCl_3$) 2150, 1770, 1725, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.06 and 0.07 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.86 (9H, s, $SiC(C\underline{H}_3)_3$), 1.19 (3H, d, J=6, $CHC\underline{H}_3$), 3.04 (1H, dd, J=2 and 4Hz, C_3-$\underline{H}$), 4.04 (1H, dd, J=2 and 4Hz, C_4-$\underline{H}$), 4.18-4.28 (1H, m, $C\underline{H}CH_3$), 5.35-5.45 (2H, s, $OC\underline{H}_2$), 5.57 (1H, d, J=4Hz, C_4-$C\underline{H}Br$), 6.11 (1H, brs, $N\underline{H}$), 7.56 and 8.27 (each 2H, d, J=9Hz, $C_6\underline{H}_4$). Anal. Calcd for $C_{22}H_{29}BrN_4O_7Si$: C, 46.40; H, 5.13; N, 9.84. Found: C, 45.94, H, 4.89; N, 9.53. Another product, the α-isomer, **142a** (2.37.g, 42 %) was obtained from chloroform-ethyl acetate (90:10) eluting fractions as colorless crystals: m.p. 121-122°C (recrystallized from toluene-hexane); IR ($CHCl_3$) 2150, 1765, 1725, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.07 and 0.09 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.88 (9H, s, $SiC(C\underline{H}_3)_3$), 1.31 (3H, d, J=6Hz, $CHC\underline{H}_3$), 3.04 (1H, t, J=2Hz, C_3-$\underline{H}$), 4.27-4.33 (1H, m, $C\underline{H}CH_3$), 4.36 (1H, dd, J=2 and 10Hz, C_4-$\underline{H}$), 5.17 (1H, d, J=10Hz, C_4-$C\underline{H}Br$), 5.38-5.45 (2H, m, $OC\underline{H}_2$), 5.87 (1H, brs, $N\underline{H}$), 7.56 and 8.27 (each 2H, d, J=9Hz, $C_6\underline{H}_4$). Anal. Calcd for $C_{22}H_{29}BrN_4O_7Si$: C, 46.40; H, 5.13; N, 9.84. Found: C, 46.03, H, 4.91; N, 9.85.

(3*S*, 4*S*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(1*R*)-1-chloro-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxopropyl]azetidin-2-one (143b) and (3*S*, 4*S*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(1*S*)-1-

chloro-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxopropyl]azetidin-2-one (143a).

The title compounds **143b** (1.94 g, 37 %) and **143a**.(1.85 g, 35.2 %) were prepared from the chlorodiazoketoester **124** (3.87 g, 13 mmol) and the 4-acetoxyazetidinone **100** (2.87 g, 10 mmol) as colorless crystals using the similar procedure described above for **142b** and **142a**. The β-isomer **143b**: m.p. 123-124°C (recrystallized from toluene-hexane); IR ($CHCl_3$) 2150, 1770, 1725, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05 and 0.06 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.84 (9H, s, $SiC(C\underline{H}_3)_3$), 1.18 (3H, d, J=6, $CHC\underline{H}_3$), 3.11 (1H, t, J=2Hz, C_3-$\underline{H}$), 4.13 (1H, dd, J=2 and 4Hz, C_4-$\underline{H}$), 4.15-4.25 (1H, m, $C\underline{H}CH_3$), 5.38 (2H, s, $OC\underline{H}_2$), 5.49 (1H, d, J=4Hz, C_4-$C\underline{H}SPh$), 6.04 (1H, brs, N$\underline{H}$), 7.49 and 8.24 (each 2H, d, J=9Hz, $C_6\underline{H}_4$). The α-isomer **143a**: m.p. 128-129°C (recrystallized from toluene-hexane); IR ($CHCl_3$) 2150, 1770, 1725, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05 and 0.07 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.86 (9H, s, $SiC(C\underline{H}_3)_3$), 1.24 (3H, d, J=6, $CHC\underline{H}_3$), 3.06 (1H, t, J=2Hz, C_3-$\underline{H}$), 4.22-4.29 (2H, m, $C\underline{H}CH_3$ and C_4-$\underline{H}$), 5.11 (1H, d, J=9Hz, C_4-$C\underline{H}Cl$), 5.33-5.43 (2H, m, $OC\underline{H}_2$), 5.82 (1H, brs, N$\underline{H}$), 7.55 and 8.26 (each 2H, d, J=9Hz, $C_6\underline{H}_4$). Anal. Calcd for $C_{22}H_{29}ClN_4O_7Si$: C, 50.33; H, 5.57; N, 10.67. Found: C, 50.39, H, 5.36; N, 10.46.

(3*S*, 4*R*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(1*R*)-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxo-1-phenylpropyl]azetidin-2-one (144b) and (3*S*, 4*R*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(1*S*)-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxo-1-phenylpropyl]azetidin-2-one (144a).

Triethylamine (2.8 mL, 20 mmol) and trimethylsilyl trifluoromethanesulfonate (4.25 mL, 22 mmol) were added to a solution of the diazoketoester

130 (6.8 g, 20 mmol) in 50 mL of anhydrous dichloromethane under a nitrogen atmosphere at 0°C. After stirring for 30 min in an ice bath, the 4-acetoxyazetidinone **100** (2.87 g, 10 mmol) was added to the reaction mixture. The reaction mixture was stirred in an ice bath for an additional 1 h, and added 10 mL of 0.5N $NaHCO_3$ solution. The organic layer was separated, washed sequentially with water and saturated NaCl solution, then dried over Na_2SO_4. The solvent was evaporated *in vacuo*. The resulting thick oil was chromatographed on silica gel. The chloroform-ethyl acetate (95:5) eluting fractions afforded one of the isomers, the β-isomer, **144b** (1.24 g, 22 %) as a yellow foam: IR ($CHCl_3$) 2145, 1760, 1725, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.03 and 0.05 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.85 (9H, s, $SiC(C\underline{H}_3)_3$), 1.16 (3H, d, J=6, $CHC\underline{H}_3$), 2.66 (1H, dd, J=2 and 4Hz, C_3-$\underline{H}$), 4.04-4.16 (1H, m, $C\underline{H}CH_3$), 4.22 (1H, dd, J=2 and 6Hz, C_4-$\underline{H}$), 4.90 (1H, d, J=6Hz, C_4-$C\underline{H}Ph$), 5.20-5.33 (2H, m, $OC\underline{H}_2$), 5.88 (1H, brs, $N\underline{H}$), 7.24-7.34 (5H, m, $C_6\underline{H}_5$), 7.42 and 8.20 (each 2H, d, J=9Hz, $C_6\underline{H}_4$). And the chloroform-ethyl acetate (90:10) eluting fractions gave another isomer, the α-isomer, **144a** (1.85 g, 33 %) as colorless crystals: m.p. 137-138°C (recrystallized from toluene-hexane); IR ($CHCl_3$) 2145, 1760, 1730, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ –0.06 and -0.03 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.22 (3H, d, J=6, $CHC\underline{H}_3$), 0.82 (9H, s, $SiC(C\underline{H}_3)_3$), 2.80 (1H, t, J=2Hz, C_3-$\underline{H}$), 4.00-4.06 (1H, m, $C\underline{H}CH_3$), 4.31 (1H, dd, J=2 and 10Hz, C_4-$\underline{H}$), 4.64 (1H, d, J=10Hz, C_4-$C\underline{H}Ph$), 5.18-5.31 (2H, m, $OC\underline{H}_2$), 5.92 (1H, brs, $N\underline{H}$), 7.24-7.35 (5H, m, $C_6\underline{H}_5$), 7.41 and 8.20 (each 2H, d, J=9Hz, $C_6\underline{H}_4$); FAB-MS *m/z* 567 (MH^+).

(3*S*, 4*R*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(1*R*)-3-diazo-3-allyloxycarbonyl-2-oxo-1-phenylpropyl]azetidin-2-one (145b) and

(3*S*, 4*R*)-3-[(1*S*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(1*S*)-3-diazo-3-allyloxycarbonyl-2-oxo-1-phenylpropyl]azetidin-2-one (145a).

The title compounds **145b** (3.75 g, 40 %) and **145a** (2.85 g, 30 %) were obtained from the corresponding diazoketoester **131** (9.77 g, 40 mmol) and the acetoxyazetidinone **100** (5.75 g, 20 mmol) as a yellow paste and colorless crystals, respectively, according to the procedure described above for **144b** and **144a**. The β-isomer **145b**: IR ($CHCl_3$) 2145, 1760, 1725, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05 and 0.06 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.86 (9H, s, $SiC(C\underline{H}_3)_3$), 1.17 (3H, d, J=6, $CHC\underline{H}_3$), 2.68 (1H, dd, J=2 and 4Hz, C_3-$\underline{H}$), 4.04-4.22 (1H, m, $C\underline{H}CH_3$), 4.23 (1H, dd, J=2 and 6Hz, C_4-$\underline{H}$), 4.55-4.72 (2H, m, $OC\underline{H}_2CH=CH_2$), 4.95 (1H, d, J=6Hz, C_4-$C\underline{H}Ph$), 5.24-5.34 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.80-5.92 (2H, m, $OCH_2C\underline{H}=CH_2$ and N$\underline{H}$), 7.24-7.36 (5H, m, $C_6\underline{H}_5$). The α-isomer **145a**: m.p. 133-134°C (recrystallized from toluene-hexane); IR ($CHCl_3$) 2145, 1760, 1725,1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ –0.06 and -0.02 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.23 (3H, d, J=6, $CHC\underline{H}_3$), 0.82 (9H, s, $SiC(C\underline{H}_3)_3$), 2.81 (1H, t, J=2Hz, C_3-$\underline{H}$), 4.00-4.10 (1H, m, $C\underline{H}CH_3$), 4.32 (1H, dd, J=2 and 10Hz, C_4-$\underline{H}$), 4.50-4.70 (2H, m, $OC\underline{H}_2CH=CH_2$), 4.68 (1H, d, J=6Hz, C_4-$C\underline{H}Ph$), 5.20-5.30 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.80-5.95 (2H, m, $OCH_2C\underline{H}=CH_2$ and N$\underline{H}$), 7.25-7.35 (5H, m, $C_6\underline{H}_5$); FAB-MS *m/z* 472 (MH^+).

(3*S*, 4*S*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(1*R*)-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxo-1-(phenylthio)propyl]azetidin-2-one (146b) and (3*S*, 4*S*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(1*S*)-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxo-1-(phenylthio)propyl]-azetidin-2-one (146a).

The title compounds **146b** (3.78 g, 32 %) and **146a** (4.07 g, 34 %) were prepared from the corresponding diazoketoester (9.65 g, 26 mmol) and the acetoxyazetidinone **100** (5.74 g, 20 mmol) as light colorless crystals and a light yellow foam, respectively, using a similar procedure described above for **142b** and **142a**. The β-isomer **146b**: m.p. 128-129°C (recrystallized from toluene-hexane); IR ($CHCl_3$) 2145, 1765, 1725, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.01 (6H, s, $Si(C\underline{H}_3)_2$), 0.82 (9H, s, $SiC(C\underline{H}_3)_3$), 1.10 (3H, d, J=6, $CHC\underline{H}_3$), 2.97-3.01 (1H, m, C_3-$\underline{H}$), 4.07 (1H, dd, J=2 and 7Hz, C_4-$\underline{H}$), 4.10-4.22 (1H, m, $C\underline{H}CH_3$), 5.00 (1H, d, J=7Hz, C_4-$C\underline{H}SPh$), 5.29 (2H, s, $OC\underline{H}_2$), 6.07 (1H, brs, $N\underline{H}$), 7.20-7.44 (5H, m, $C_6\underline{H}_5$), 7.49 and 8.24 (each 2H, d, J=9Hz, $C_6\underline{H}_4$). The α-isomer **146a**: IR ($CHCl_3$) 2145, 1765, 1725, 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.03 and 0.07 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.85 (9H, s, $SiC(C\underline{H}_3)_3$), 1.29 (3H, d, J=6, $CHC\underline{H}_3$), 2.99 (1H, t, J=2Hz, C_3-$\underline{H}$), 4.27 (1H, dd, J=2 and 10Hz, C_4-$\underline{H}$), 4.20-4.30 (1H, m, $C\underline{H}CH_3$), 4.82 (1H, d, J=10Hz, C_4-$C\underline{H}SPh$), 5.28-5.36 (2H, m, $OC\underline{H}_2$), 5.72 (1H, brs, $N\underline{H}$), 7.24-7.50 (5H, m, $C_6\underline{H}_5$), 7.52 and 8.25 (each 2H, d, J=9Hz, $C_6\underline{H}_4$).

(3*R*, 4*R*)-4-Acetoxy-3-[(1*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]-1-(trimethylsilyl)azetidin-2-one (147).

Triethylamine (152 mg, 1.5 mmol) was added to a solution of the acetoxyazetidinone **100** (287 mg, 1 mmol) in 2 mL of anhydrous dichloromethane under a nitrogen atmosphere at 0°C, followed by chlorotrimethylsilane (141 mg, 1.3 mmol). The reaction mixture was stirred at room temperature for 1 h. Hexane (10 mL) was added to the reaction mixture and the resulting precipitates were filtered off. Concentration of the filtrate gave the desired silylated compound **147** in almost quantitative yield as a yellow oil: ^1H NMR ($CDCl_3$) δ 0.02 and 0.04 (each 3H, each s,

$OSi(^tBu)(C\underline{H}_3)_2$), 0.24 (9H, s, $Si(C\underline{H}_3)_3$), 0.84 (9H, s, $C(C\underline{H}_3)_3$), 1.20 (3H, d, J=6Hz, $CHC\underline{H}_3$), 2.06 (3H, s, $OCOC\underline{H}_3$), 3.11 (1H, dd, J=1 and 3Hz, C_3-$\underline{H}$), 4.17 (1H, dq, J=3 and 6Hz, $C\underline{H}CH_3$), 6.10 (1H, d, J=1Hz, C_4-$\underline{H}$).

***p*-Nitrobenzyl 2-Diazo-3-oxo-4-pentenoate (149).**

Triethylamine (0.28 mL, 2 mmol) and trimethylsilyl trifluoromethanesulfonate (0.425 mL, 2.2 mmol) were added to a stirred solution of the diazoketoester **137** (614 mg, 2 mmol) in 5 mL of anhydrous dichloromethane under a nitrogen atmosphere at 0°C. The mixture was stirred for 30 min at 0°C, then treated with the 4-acetoxyazetidinone **100** (574 mg, 2 mmol). The resulting solution was stirred for an additional 30 min at 0°C. A 1 mL of 0.5N $NaHCO_3$ solution was added to the mixture and the organic layer separated, washed with saturated NaCl solution and dried over Na_2SO_4. The solution was concentrated and the residue was chromatographed on silica gel with chloroform-ethyl acetate (98:2) as the eluant to afford the olefinic compound **149** (225 mg, 41 %) as yellow crystals: m.p. 87-88°C (recrystallized from toluene-hexane); IR ($CHCl_3$) 2140, 1720, 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.36 (2H, s, $OC\underline{H}_2$), 5.74 (1H, dd, J=2 and 10Hz, $CH=C\underline{H}_{cis}H_{trans}$), 6.45 (1H, dd, J=2 and 17Hz, $CH=CH_{cis}\underline{H}_{trans}$), 7.37 (1H, dd, J=10 and 17Hz, $C\underline{H}=CH_{cis}H_{trans}$), 7.53 and 8.24 (each 2H, d, J=9Hz, aromatic H).

(3*S*, 4*R*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-(phenylthio)-azetidin-2-one (150).

Triethylamine (1.18 mL, 8.43 mmol) and trimethylsilyl trifluoromethanesulfonate (1.79 mL, 9.28 mmol) were added to a solution of the diazoketoester **128** (3.25 g, 8.43 mmol) in 20 mL of anhydrous dichloromethane under a nitrogen atmosphere at 0°C. After the reaction

mixture was stirred for 30 min in an ice bath, the acetoxyazetidinone **100** (2.42 g, 8.43 mmol) was added, and the stirring was continued for an additional 30 min at 0°C. The reaction mixture was washed with 0.5N $NaHCO_3$ solution and saturated NaCl solution, then dried over Na_2SO_4. After concentration, the residue was chromatographed on silica gel. From the chloroform eluting fractions, the olefinic compound **149** (0.542 g, 23 %) was obtained. From the chloroform-ethyl acetate (90:10) eluting fractions, the 4-phenyl-thioazetidinone **150** (1.08 g, 38 %) was obtained as colorless crystals: m.p. 118-119°C (recrystallized from toluene-hexane); ^1H NMR ($CDCl_3$) δ 0.04 and 0.05 (each 3H, each s, $Si(C\underline{H}_3)_2$), 0.86 (9H, s, $SiC(C\underline{H}_3)_3$), 1.19 (3H, d, J=6, $CHC\underline{H}_3$), 3.02 (1H, dd, J=2 and 3Hz, C_3-$\underline{H}$), 4.21 (1H, dq, J=3 and 6 Hz, $C\underline{H}CH_3$), 5.07 (1H, d, J=2Hz, C_4-$\underline{H}$), 7.30-7.48 (5H, m, aromatic H). Anal. Calcd for $C_{17}H_{27}NO_2SSi$: C, 60.49; H, 8.06; N, 4.15. Found: C, 60.25, H. 8.30; N, 4.17.

(3*S*, 4*S*)-4-[(1*R*)-1-Bromo-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxo-propyl]-3-[(1*R*)-1-hydroxyethyl]azetidin-2-one (151b).

The silyl ether **142b** (1.72 g, 3.02 mmol) was dissolved in 17 mL of methanol and treated with 1 mL of concentrated HCl solution. The mixture was stirred at room temperature for 2 h. After addition of 1 mL of pyridine, the solution was concentrated. The residue was taken up in 50 mL of ethyl acetate, and washed with water and saturated NaCl solution. After drying over Na_2SO_4, the solution was concentrated *in vacuo* and the resulting residue was chromatographed on silica gel with chloroform-methanol (95:5) as the eluant to give the desired alcohol **151b** (1.09 g, 79 %) as a light yellow foam: IR ($CHCl_3$) 3410, 2150, 1770, 1725, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (3H, d, J=6Hz, $CHC\underline{H}_3$), 2.97 (1H, dd, J=1,7Hz, C_3-$\underline{H}$), 4.09

(1H, dd, J=2 and 7Hz, C4-H), 4.10-4.25 (2H, m, C<u>H</u>CH3), 5.32-5.44 (2H, m, OC<u>H</u>2), 5.45 (1H, d J=7Hz, C4-C<u>H</u>Br), 6.28 (1H, brs, N<u>H</u>), 7.55 and 8.26 (each 2H, d, J=9Hz, aromatic H).

(3*S*, 4*S*)-4-[(1*S*)-1-Bromo-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxo-propyl]-3-[(1*R*)-1-hydroxyethyl]azetidin-2-one (151a).

The title compound **151a** was obtained from the corresponding silyl ether **142a** in 83 % yield as light brown crystals after recrystallization from toluene, according to the procedure described above for **151b**: m.p. 124-125°C (decomp.); IR ($CHCl_3$) 3410, 2150, 1770, 1725, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37 (3H, d, J=6Hz, CHC<u>H</u>3), 2.55 (1H, brs, OH), 3.15 (1H, dd, J=2 and 5Hz, C3-<u>H</u>), 4.20-4.30 (2H, m, C<u>H</u>CH3 and C4-<u>H</u>), 5.26 (1H, d J=6Hz, C4-C<u>H</u>Br), 5.32-5.44 (2H, m, OC<u>H</u>2), 6.29 (1H, brs, N<u>H</u>), 7.55 and 8.25 (each 2H, d, J=9Hz, aromatic H); FAB-MS *m/z* 455 and 457 (MH^+).

(3*S*, 4*S*)-4-[(1*R*)-1-Chloro-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxo-propyl]-3-[(1*R*)-1-hydroxyethyl]azetidin-2-one (152b).

The title compound **152b** was obtained from the corresponding silyl ether **143b** in 76 % yield as a yellow foam according to the procedure described above for **151b**: IR ($CHCl_3$) 3410, 2150, 1770, 1725, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (3H, d, J=6Hz, CHC<u>H</u>3), 3.04-3.07 (1H, m, C3-<u>H</u>), 4.10-4.26 (2H, m, C<u>H</u>CH3 and C4-<u>H</u>), 5.38 (2H, s, OC<u>H</u>2), 5.42 (1H, d J=6Hz, C4-C<u>H</u>Cl), 6.25 (1H, brs, N<u>H</u>), 7.54 and 8.26 (each 2H, d, J=9Hz, aromatic H); FAB-MS *m/z* 411 (MH^+).

(3*S*, 4*S*)-4-[(1*S*)-1-Chloro-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxo-propyl]-3-[(1*R*)-1-hydroxyethyl]azetidin-2-one (152a).

The title compound **152a** was obtained from the corresponding silyl ether **143a** in 90 % yield as colorless crystals according to the procedure described above for **151b**: m.p. 135-136°C (decomp.); IR ($CHCl_3$) 3410, 2150, 1770, 1725, 1655 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (3H, d, J=6Hz, $CHC\underline{H}_3$), 3.05 (1H, dd, J=2 and 5Hz, C_3-$\underline{H}$), 4.05-4.16 (2H, m, $C\underline{H}CH_3$ and C_4-$\underline{H}$), 5.20 (1H, d J=8Hz, C_4-$C\underline{H}Cl$), 5.25-5.36 (2H, m, $OC\underline{H}_2$), 6.84 (1H, brs, $N\underline{H}$), 7.48 and 8.17 (each 2H, d, J=9Hz, aromatic H); FAB-MS *m/z* 411 (MH^+).

(3*S*, 4*R*)-3-[(1*R*)-1-Hydroxyethyl]-4-[(1*R*)-3-diazo-3-*p*-nitrobenzyloxy-carbonyl-2-oxo-1-phenylpropyl]azetidin-2-one (153b).

The title compound **153b** was prepared from the silyl ether **144b** in 84 % yield as light yellow crystals according to the procedure described above for **151b** m.p. 75-77°C (recrystallized from toluene); IR ($CHCl_3$) 3410, 2150, 1765, 1725, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (3H, d, J=6Hz, $CHC\underline{H}_3$), 2.79 (1H, dd, J=1 and 8Hz, C_3-$\underline{H}$), 4.07-4.17 (2H, m, $C\underline{H}CH_3$ and C_4-$\underline{H}$), 4.74 (1H, d, J=9Hz, C_4-$C\underline{H}Ph$), 5.22 and 5.29 (each 1H, d, J=13Hz, $OC\underline{H}_a\underline{H}_bC_6H_4NO_2$), 5.70 (1H, s, $N\underline{H}$), 7.20-7.38 (5H, m, $C_6\underline{H}_5$), 7.32 and 8.20 (each 2H, d, J=9Hz, $C_6\underline{H}_4$); FAB-MS *m/z* 453 (MH^+).

(3*S*, 4*R*)-3-[(1*R*)-1-Hydroxyethyl]-4-[(1*S*)-3-diazo-3-*p*-nitrobenzyloxy-carbonyl-2-oxo-1-phenylpropyl]azetidin-2-one (153a).

The title compound **153a** was prepared from the silyl ether **144a** in 84 % yield as a white foam according to the procedure described above for **151b**: IR ($CHCl_3$) 3410, 2145, 1765, 1730, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.56 (3H, d, J=6Hz, $CHC\underline{H}_3$), 2.90 (1H, dd, J=1 and 8Hz, C_3-$\underline{H}$), 3.95-4.05

(1H, m, C<u>H</u>CH$_3$), 4.26 (1H, dd, J=2 and 10Hz, C$_4$-<u>H</u>), 4.68 (1H, d, J=10Hz, C$_4$-C<u>H</u>Ph), 5.21 and 5.29 (each 1H, d, J=13Hz, OC<u>H$_a$H$_b$</u>C$_6$H$_4$NO$_2$), 6.10 (1H, s, N<u>H</u>), 7.20-7.40 (5H, m, C$_6$<u>H</u>$_5$), 7.41 and 8.20 (each 2H, d, J=9Hz, C$_6$<u>H</u>$_4$); FAB-MS *m/z* 453 (MH$^+$).

(3*S*, 4*R*)-3-[(1*R*)-1-Hydroxyethyl]-4-[(1*R*)-3-allyloxycarbonyl-3-diazo-2-oxo-1-phenylpropyl]azetidin-2-one (154b).

The title compound **154b** was prepared from the silyl ether **145b** in 51 % yield as a white foam according to the procedure described above for **151b**: IR (CHCl$_3$) 3410, 2150, 1765, 1725, 1630 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.29 (3H, d, J=6Hz, CHC<u>H</u>$_3$), 2.77 (1H, dd, J=2 and 8Hz, C$_3$-<u>H</u>), 4.00-4.15 (1H, m, C<u>H</u>CH$_3$), 4.12 (1H, dd, J=2 and 9Hz, C$_4$-<u>H</u>), 4.53-4.70 (2H, m, OC<u>H</u>$_2$CH=CH$_2$), 4.77 (1H, d, J=9Hz, C$_4$-C<u>H</u>Ph), 5.22-5.30 (2H, m, OCH$_2$CH=C<u>H</u>$_2$), 5.75-5.90 (2H, m, OCH$_2$C<u>H</u>=CH$_2$ and N<u>H</u>), 7.20-7.36 (5H, m, C$_6$<u>H</u>$_5$); FAB-MS *m/z* 358 (MH$^+$).

(3*S*, 4*R*)-3-[(1*R*)-1-Hydroxyethyl]-4-[(1*S*)-3-allyloxycarbonyl-3-diazo-2-oxo-1-phenylpropyl]azetidin-2-one (154a).

The title compound **154a** was prepared from the silyl ether **145a** in 74 % yield as a white foam according to the procedure described above for **151b**: IR (CHCl$_3$) 3410, 2150, 1760, 1720, 1640 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.52 (3H, d, J=6Hz, CHC<u>H</u>$_3$), 2.89 (1H, dd, J=2 and 4Hz, C$_3$-<u>H</u>), 3.95-4.05 (1H, m, C<u>H</u>CH$_3$), 4.28 (1H, dd, J=2 and 10Hz, C$_4$-<u>H</u>), 4.54-4.70 (2H, m, OC<u>H</u>$_2$CH=CH$_2$), 4.72 (1H, d, J=10Hz, C$_4$-C<u>H</u>Ph), 5.22-5.31 (2H, m, OCH$_2$CH=C<u>H</u>$_2$), 5.78-5.92 (1H, m, OCH$_2$C<u>H</u>=CH$_2$ and N<u>H</u>), 6.38 (1H, brs, N<u>H</u>), 7.20-7.36 (5H, m, C$_6$<u>H</u>$_5$); FAB-MS *m/z* 358 (MH$^+$).

(3*S*, 4*S*)-3-[(1*R*)-1-Hydroxyethyl]-4-[(1*R*)-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxo-1-(phenylthio)propyl]azetidin-2-one (155b).

The title compound **155b** was prepared from the silyl ether **146b** in 87 % yield as brown paste using the same procedure described above for **151b**: IR ($CHCl_3$) 3410, 2145, 1765, 1720, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (3H, d, J=6Hz, $CHC\underline{H}_3$), 2.83-2.87 (1H, m, C_3-$\underline{H}$), 3.80 (1H, dd, J=3 and 9Hz, C_4-$\underline{H}$), 3.97-4.08 (1H, m, $C\underline{H}CH_3$), 4.74 (1H, d, J=9Hz, C_4-$C\underline{H}SPh$), 5.30-5.40 (2H, m, $OC\underline{H}_2$), 6.27 (1H, brs, N$\underline{H}$), 7.25-7.44 (5H, m, $C_6\underline{H}_5$), 7.53 and 8.25 (each 2H, d, J=9Hz, $C_6\underline{H}_4$); FAB-MS *m/z* 485 (MH^+).

(3*S*, 4*S*)-3-[(1*R*)-1-Hydroxyethyl]-4-[(1*S*)-3-diazo-3-*p*-nitrobenzyloxycarbonyl-2-oxo-1-(phenylthio)propyl]azetidin-2-one (155a).

The title compound **155a** was prepared from the silyl ether **146a** in 86 % yield as yellow foam using the similar procedure described above for **151b**: IR ($CHCl_3$) 3410, 2150, 1765, 1725, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (3H, d, J=6Hz, $CHC\underline{H}_3$), 3.12 (1H, dd, J=2 and 6Hz, C_3-$\underline{H}$), 4.04 (1H, dd, J=2 and 9Hz, C_4-$\underline{H}$), 4.10-4.22 (1H, m, $C\underline{H}CH_3$), 4.82 (1H, d, J=9Hz, C_4-$C\underline{H}SPh$), 5.30-5.40 (2H, m, $OC\underline{H}_2$), 6.00 (1H, brs, N$\underline{H}$), 7.25-7.55 (5H, m, $C_6\underline{H}_5$), 7.52 and 8.24 (each 2H, d, J=9Hz, $C_6\underline{H}_4$); FAB-MS *m/z* 485 (MH^+).

Stereoisomeric mixture of (3*S*, 4*S*)-3-[(1*R*)-1-Hydroxyethyl]-4-[(1*S*)-3-diazo-1-(1-methyltetrazole-5-thio)-3-*p*-nitrobenzyloxycarbonyl-2-oxopropyl]azetidin-2-one (156b) and (3*S*, 4*S*)-3-[(1*R*)-1-Hydroxyethyl]-4-[(1*R*)-3-diazo-1-(1-methyltetrazole-5-thio)-3-*p*-nitrobenzyloxycarbonyl-2-oxopropyl]azetidin-2-one (156a).

5-Mercapto-1-methyltetrazole sodium salt (207 mg, 1.5 mmol) was added to a solution of the bromide **151a** (455 mg, 1 mmol) in 5 mL of anhydrous tetrahydrofuran under a nitrogen atmosphere. The reaction

mixture was stirred at room temperature for 2 h, diluted with ethyl acetate, and washed with water and saturated NaCl solution. The organic phase was dried over Na_2SO_4. Solvent removal gave an oil, which was chromatographed on silica gel eluting with chloroform-methanol (95:5) to give a stereoisomeric mixture of the desired compounds **156a** and **156b** in almost quantitative yield with an approximate ratio of 1:1 as a yellow form.

Starting from another isomeric bromide **151b** (455 mg, 1 mmol), the similar result (stereoisomeric mixture of the desired products **156a** and **156b** in approximately 1:1 ratio in almost quantitative yield) was obtained: IR ($CHCl_3$) 3410, 2150, 1770, 1720, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20-1.32 (3H, m, $CHC\underline{H}_3$), 3.20-3.30 (1H, m, C_3-$\underline{H}$), 3.93 and 3.94 (each 1.5H, each s, N-$C\underline{H}_3$), 4.10-4.20 (1H, m), 4.23-4.30 (1H, m), 5.36 and 5.38 (each 1H, $OC\underline{H}_2$), 5.73-5.78 (1H, m), 6.60 and 6.73 (each 0.5H, s, N$\underline{H}$), 7.56 and 8.24 (each 2H, d, J=9, aromatic H).

_p_-Nitrobenzyl (1*S*, 5*R*, 6*S*)-6-[(1*R*)-1-Hydroxyethyl]-2-oxo-1-phenyl-carbapenam-3-carboxylate (158).

The diazo compound **153a** (452 mg, 1 mmol) was dissolved in 10 mL of anhydrous benzene and treated with 5 mg of rhodium (II) acetate. The mixture was heated to reflux for 30 min under a nitrogen atmosphere. After cooling, solvent was removed under reduced pressure and the residue was passed through short column with ethyl acetate as the solvent. Concentration of the elutant yielded the bicyclic compound **158** (283 mg, 67 %) as a brown foam: IR ($CHCl_3$) 1770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (3H, d, J=6Hz, $CHC\underline{H}_3$), 2.10 (1H, d, J=4Hz, O$\underline{H}$), 3.40 (1H, dd, J=2 and 6Hz, C_6-$\underline{H}$), 3.55 (1H, d, J=8Hz, C_1-$\underline{H}$), 4.23 (1H, dd, J=2 and 8Hz, C_5-$\underline{H}$), 4.24-4.35 (1H, m,

C<u>H</u>CH3), 4.96 (1H, s, C3-<u>H</u>), 5.25-5.36 (2H, m, OC<u>H</u>2), 7.08-7.40 (5H, m, C6<u>H</u>5), 7.51 and 8.19 (each 2H, d, J=9Hz, C6<u>H</u>4).

In a similar manner, the same bicyclic compound **153b** (304 mg, 71.6 %) was obtained from another isomeric diazo compound **158** (452 mg, 1 mmol).

***p*-Nitrobenzyl (5*S*, 6*S*)-6-[(1*R*)-1-Hydroxyethyl]-1-phenyl-2-(trifluoromethanesulfonyloxy)carbapen-1-em-3-carboxylate (161).**

The ketone **158** (424 mg, 1 mmol) was dissolved in 3 mL of anhydrous dichloromethane and cooled down to -30 °C, then treated with triethylamine (101 mg, 1 mmol), followed by trifluoromethanesulfonic anhydride (282 mg, 1mmol). After the mixture was stirred at -30°C for 30 min, a solution of triethylamine (101 mg, 1 mmol) and thiophenol (110 mg, 1 mmol) in 1 mL of anhydrous dichloromethane was added to this mixture. The reaction mixture was stirred at -30°C for 30 min, washed with water and saturated NaCl solution. The organic phase was dried over Na2SO4 and solvent removal gave a brown oil, which was chromatographed on silca gel eluting with chloroform-ethyl acetate (80:20) to give the olefinic compound **161** (154 mg, 28 %) as a thick brown oil: IR ($CHCl_3$) 1790, 1770 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (3H, d, J=6Hz, CHC<u>H</u>3), 3.45 (1H, dd, J=2 and 8 Hz, C6-<u>H</u>), 4.35-4.48 (1H, m, C<u>H</u>CH3), 4.82-4.86 (1H, m, C5-<u>H</u>), 5.32 (2H, m, OC<u>H</u>2), 5.55 (1H, d, J=4, C3-<u>H</u>), 7.05-7.75 (5H, m, C6<u>H</u>5), 7.54 and 8.26 (each 2H, d, J=9, C6<u>H</u>4).

***S*-Phenyl Phenylthioacetate (165).**

Pyridine (9.70 mL, 120 mmol) was added to a ice-cooled solution of thiophenol (11.0 g, 100 mmol) in 250 mL of anhydrous dichloromethane, followed by phenylacetyl chloride (17.0 g, 110 mmol). The reaction mixture

was stirred for 30 min at 0°C under a nitrogen atmosphere and poured into ice water. The organic layer was separated, washed with saturated $NaHCO_3$ solution and saturated NaCl solution, then dried over Na_2SO_4. The solution was concentrated under reduced pressure and the residue was passed through short column using toluene as the solvent to give the desired thioester **165** in almost quantitative yield as a yellow oil: IR ($CHCl_3$) 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.94 (2H, s, $PhC\underline{H}2$), 7.30-7.45 (10H, m, aromatic H).

_S_-Phenyl Thiopropionate (166).

Using similar procedure described above for **165**, the title compound **166** was prepared from thiophenol (11.0 g, 100 mmol) and propionyl chloride (17.0 g, 110 mmol) in almost quantitative yield as a yellow oil: IR ($CHCl_3$) 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (3H, t, J=7Hz, $CH_2C\underline{H}_3$), 2.68 (2H, q, J=7Hz, $C\underline{H}_2CH_3$), 7.37-7.43 (5H, m, aromatic H).

_S_-Phenyl Bromothioacetate (167).

Bromoacetyl chloride (15.7 g, 100 mmol) was dissolved in 250 mL of anhydrous dichloromethane and treated with pyridine (8.70 g, 110 mmol) at 0°C. Thiophenol (11.0 g, 100 mmol) was slowly added to the solution under ice-bath cooling. The reaction mixture was stirred at 0°C, washed sequentially with diluted HCl solution, water, saturated $NaHCO_3$ solution and saturated NaCl solution. The organic phase was dried over Na_2SO_4 and the solution concentrated. The resulting residue was passed through short column using toluene as the eluant to give the bromothioacetate **167** in almost quantitative yield as a light brown oil: IR ($CHCl_3$) 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.29 (2H, s, $BrC\underline{H}_2$), 7.35-7.50 (5H, m, aromatic H).

S-Phenyl (Phenylthio)thioacetate (168).

Bromoacetyl chloride (7.87 g, 50 mmol) was added dropwise to a mixed solution of thiophenol (11.0 g, 100 mmol) and triethylamine (10.1 g, 100 mmol) in 250 mL of anhydrous dichloromethane at 0°C and the reaction mixture was stirred at 0°C for 30 min under a nitrogen atmosphere. The mixture was washed with water and saturated NaCl solution. After drying over Na_2SO_4, the solution was concentrated *in vacuo* to give a residue, which was chromatographed on silica gel with toluene as the eluant to give the desired product **168** (5.43 g, 42 %) as a brown oil: IR ($CHCl_3$) 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.91 (2H, s, $SC\underline{H}_2$), 7.20-7.52 (10H, m, aromatic H).

S-Phenyl Azidothioacetate (170).

Azidoacetic acid (**169**) (5.05 g, 50 mmol) and thiophenol (5.51 g, 50 mmol) were dissolved in 100 mL of dichloromethane and treated with dicyclohexylcarbodiimide (DCC) (10.3 g, 50 mmol). Immediately after addition of DCC, the precipitation of white solid was observed. The reaction mixture was stirred at room temperature over night. The precipitate was filtered off. The filtrate was concentrated and purified with silica gel column chromatography with chloroform as the eluant to give the desired product **170** (7.61 g, 74 %) as a colorless oil: IR ($CHCl_3$) 2115, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.11 (2H, s, $C\underline{H}_2$), 7.40-7.52 (5H, m, aromatic H). Anal. Calcd for $C_8H_7N_3OS$: C, 49.73; H, 3.65; N, 21.75. Found: C, 49.53, H, 3.54; N, 21.64.

S-Phenyl (1-Methyltetrazole-5-thio)thioacetate (171).

The bromothioacetate **167** (2.31 g, 10 mmol) was dissolved in 20 mL of anhydrous tetrahydrofuran and treated with 5-mercapto-1-methyltetrazole sodium salt (1.66 g, 12 mmol). The reaction mixture was stirred at room temperature for 2 h under a nitrogen atmosphere. After dilution with toluene,

the mixture was washed with water and saturated NaCl solution, and dried over Na_2SO_4. Concentration of the solution gave the crude product, which was subjected to the silica gel column chromatography eluting with chloroform-ethyl acetate (60:40) to yield the desired product **171** (2.25 g, 89 %) as a yellow oil: IR ($CHCl_3$) 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.97 (3H, s, N-C<u>H</u>$_3$), 4.44 (2H, s, SC<u>H</u>$_2$), 7.40-7.44 (5H, m, aromatic H).

(3*S*, 4*S*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(*R*)-phenyl-(phenylthiocarbonyl)methyl]azetidin-2-one (174b) and (3*S*, 4*S*)-3-[(1*R*)-1-(*tert*-Butyldimethylsilyloxy)ethyl]-4-[(*S*)-phenyl(phenylthiocarbonyl)-methyl]-azetidin-2-one (174a).

Triethylamine (2.79 mL, 20 mmol) and trimethylsilyl trifluoromethanesulfonate (4.25 mL, 22 mmol) were successively added to a cooled solution of the thioester **165** (4.57 g, 20 mmol) in 40 mL of anhydrous dichloromethane under a nitrogen atmosphere at 0°C. The reaction mixture was stirred at 0°C for 30 min, and then treated with the acetoxyazetidinone **100** (2.87 g, 10 mmol). The mixture was stirred for an additional 30 min in an ice bath. A 10 mL volume of 0.5N $NaHCO_3$ solution was added to the mixture and the organic layer separated, washed with saturated NaCl solution. The organic phase was dried over Na_2SO_4, and solvent removed under reduced pressure. The residue was chromatographed on silica gel. The chloroform-ethyl acetate (95:5) eluting fractions were collected to yield one of the desired isomers, the β-isomer, **174b** (1.28 g, 28 %) as a yellow solid, which was recrystallized from hexane to give colorless crystals: m.p. 111-112°C; IR ($CHCl_3$) 3410, 1765, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05 and 0.06 (each 3H, s, Si(C<u>H</u>$_3$)$_2$), 0.86 (9H, s, SiC(C<u>H</u>$_3$)$_3$), 1.17 (3H, d, J=6Hz, CHC<u>H</u>$_3$), 2.86 (1H, dd, J=3 and 6Hz, C$_3$-<u>H</u>), 3.98 (1H, d, J=7Hz, C<u>H</u>Ph),

4.22 (1H, dq, J=4 and 6Hz, C<u>H</u>CH$_3$), 4.28 (1H, dd, J=2 and 7Hz, C$_4$-<u>H</u>), 5.66 (1H, s, N<u>H</u>), 7.30-7.48 (10H, m, aromatic H). Anal. Calcd for $C_{25}H_{33}NO_5SSi$: C, 65.89; H, 7.30; N, 3.07. Found: C, 65.64, H, 7.55; N, 3.15. Another isomer, the α-isomer, **174a** (1.06 g, 23 %) was obtained from the chloroform-ethyl acetate (90:10) eluting fractions as colorless crystals: m.p. 188-189°C (recrystallized from hexane); IR ($CHCl_3$) 3410, 1765, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ -0.05 and 0.01 (each 3H, s, Si(C<u>H</u>$_3$)$_2$), 0.28 (3H, d, J=6, CHCH3), 0.80 (9H, s, SiC(C<u>H</u>$_3$)$_3$), 2.75 (1H, t, J=2Hz, C$_3$-<u>H</u>), 3.92 (1H, d, J=10Hz, C<u>H</u>Ph), 4.05 (1H, dq, J=2 and 6Hz, C<u>H</u>CH$_3$), 4.24 (1H, dd, J=2 and 10Hz, C$_4$-<u>H</u>), 6.05 (1H, s, N<u>H</u>), 7.28-7.75 (10H, m, aromatic H). Anal. Calcd for $C_{25}H_{33}NO_5SSi$: C, 65.89; H, 7.30; N, 3.07. Found: C, 65.91, H, 7.37; N, 3.05.

(3*R*, 4*R*)-4-Acetoxy-1-[(allyloxycarbonyl)(triphenylphosphoranylidene)-methyl]-3-[(1*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (178).

A mixture of the acetoxyazetidinone **100** (2.87 g, 10 mmol), allyl glyoxylate monohydrate (1.45 g, 11 mmol) and 5 g of silica gel in 20 mL of toluene was heated to reflux for 2 h with the use of a Dean-Stark water separator. The reaction mixture was then cooled and filtered to get rid of silica gel. The solvent removal gave a stereoisomeric mixture of the alcohols **176** as a thick oil which was used for the next reaction without purification.

To a solution of the crude hydroxy compound in 50 mL of anhydrous tetrahydrofuran at -30°C under a nitrogen atmosphere was added 2,6-lutidine (1.75 mL, 15 mmol) followed by thionyl chloride (1.09 mL, 15 mmol) dropwise. The reaction mixture was stirred for 45 min at -30°C. The mixture was diluted with ethyl acetate, washed sequentially with water, saturated $NaHCO_3$ solution and saturated NaCl solution, then dried over Na_2SO_4. The

solvent removal gave an isomeric mixture of the chlorides **177**, which were used directly in the next reaction without further purification.

The crude mixture of chlorides was dissolved in 30 mL of sieve-dried dimethylformamide and treated with triphenylphosphine (3.93 g, 15 mmol) and 2,6-lutidine (1.75 mL, 15 mmol). The reaction mixture was stirred at 70°C for 3 h under a nitrogen atmosphere. After cooling, the mixture was diluted with ethyl acetate and washed several times with water, then with saturated NaCl solution.The organic phase was dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was chromatographed on silica gel with chloroform-ethyl acetate (90:10) as the eluting solvent to give the desired ylide compound **178** (4.81 g, 75 %) as a yellow paste: IR ($CHCl_3$) 1755, 1625 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.45-7.80 (15H, m, aromatic H); FAB-MS *m/z* 646 (MH^+).

(3*S*, 4*S*)-1-Allyloxalyl-3-[(1*R*)-(*tert*-butyldimethylsilyloxy)ethyl]-4-[(*R*)-phenyl(phenylthiocarbonyl)methyl]azetidin-2-one (180b).

The lactam **174b** (1.82 g, 4 mmol) was dissolved in 20 mL of anhydrous dichloromethane and treated with triethylamine (0.84 mL, 6 mmol) and allyl oxalyl chloride (891 mg, 6 mmol) at 0°C under a nitrogen atmosphere. The mixture was stirred for 1 h under ice-bath cooling, washed with water and saturated NaCl solution, then dried over Na_2SO_4. The solution was concentrated under vacuum, and the resulting residue was passed through short column with chloroform as the eluant to give the oxalimide **180b** in almost quantitative yield as a yellow oil: IR ($CHCl_3$) 1810, 1750, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ -0.06 (6H, s, $Si(C\underline{H}_3)_2$), 041 (3H, d, J=6, $CHC\underline{H}_3$), 0.77 (9H, s, $SiC(C\underline{H}_3)_3$), 3.77 (1H, t, J=3Hz, C_3-$\underline{H}$), 4.14-4.18 (1H, m, $C\underline{H}CH_3$), 4.58-4.62 (1H, m, C_4-$\underline{H}$), 4.78-4.82 (2H, m,

$OC\underline{H}_2CH=CH_2$), 4.93 (1H, d, J=5, C_4-C<u>H</u>Ph), 5.29-5.44 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.89-6.02 (1H, m, $OCH_2C\underline{H}=CH_2$), 7.30-7.45 (10H, m, aromatic H).

(3*S*, 4*S*)-1-Allyloxalyl-3-[(1*R*)-(*tert*-butyldimethylsilyloxy)ethyl]-4-[(*R*)-phenyl(phenylthiocarbonyl)methyl]azetidin-2-one (180a).

Using the procedure described above for **180b**, the title compound **180a** was obtained from the corresponding lactam **174a** in almost quantitative yield as a yellow oil: IR ($CHCl_3$) 1810, 1755, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ -0.06 and -0.02 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.66 (3H, d, J=6, $CHC\underline{H}_3$), 0.80 (9H, s, $SiC(C\underline{H}_3)_3$), 3.04-3.08 (1H, m, C_3-<u>H</u>), 4.10-4.22 (1H, m, C<u>H</u>CH_3), 4.38 (1H, d, J=7, C_4-C<u>H</u>Ph), 4.75-4.82 (2H, m, $OC\underline{H}_2CH=CH_2$), 4.95 (1H, dd, J=3 and 7Hz, C_4-<u>H</u>), 5.26-5.44 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.86-6.02 (1H, m, $OCH_2C\underline{H}=CH_2$), 7.30-7.45 (10H, m, aromatic H).

Allyl (1*S*, 5*S*, 6*S*)-6-[(1*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]-1-phenyl-2-(phenylthio)carbapen-2-em-3-carboxylate (181).

The oxalimide **180a** (2.27 g, 4.0 mmol) was dissolved in 30 mL of anhydrous toluene and treated with trimethylphosphite (2.36 mL, 20 mmol). The reaction mixture was heated to reflux under a nitrogen atmosphere and stirred for 3 h. After cooling, the solvent was removed under reduce pressure. The resulting residue was chromatographed on silica gel using chloroform as the eluting solvent to afford the desired carbapenem **181** (1.67 g, 78 %) as a yellow paste: IR ($CHCl_3$) 1775, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ -0.29 and -0.10 (each 3H, s, $Si(C\underline{H}_3)_2$), 0.62 (9H, s, $SiC(C\underline{H}_3)_3$), 1.16 (3H, d, J=6, $CHC\underline{H}_3$), 3.23 (1H, dd, J=3 and 8Hz, C_3-<u>H</u>), 3.83 (1H, dd J=3 and 8Hz, C_5-<u>H</u>), 4.00-4.06 (1H, m, C<u>H</u>CH_3), 4.18 (1H, d, J=7Hz, C_1-<u>H</u>), 4.74-

4.92 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.25-5.60 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.95-6.08 (1H, m, $OCH_2C\underline{H}=CH_2$), 6.60-7.20 (10H, m, aromatic H).

(3*S*, 4*S*)-4-[(*R*)-Phenyl(phenylthiocarbonyl)methyl]-3-[(1*R*)-1-(trimethylsilyloxy)ethyl]azetidin-2-one (183b) and (3*S*, 4*S*)-4-[(*S*)-Phenyl-(phenylthio-carbonyl)methyl]-3-[(1*R*)-1-(trimethylsilyloxy)ethyl]-azetidin-2-one (183a).

Triethylamine (2.79 mL, 20 mmol) and trimethylsilyl trifluoromethanesulfonate (4.25 mL, 22mmol) were added to a cooled solution of the thioester **165** (4.57 g, 20 mmol) in 40 mL of anhydrous dichloromethane under a nitrogen atmosphere at 0°C. After 30 min of stirring at 0°C, the acetoxyazetidinone **100** (2.87 g, 10 mmol) was added to the solution. The mixture was stirred for an additional 30 min at 0°C. The solution was washed with water and saturated NaCl solution, dried over Na_2SO_4, and concentrated *in vacuo*.

The residue was dissolved in 20 mL of sieve-dried acetonitrile and treated with boron trifluoride etherate (1.84 mL, 15 mmol). The mixture was stirred at 0°C for 2 h, then poured into ice water and extracted with dichloromethane several times. The combined organic layer was washed with saturated $NaHCO_3$ solution, saturated NaCl solution, and dried over Na_2SO_4. The solvent removal gave a crude mixture of alcohols as an oil.

The obtained oil was dissolved in 40 mL of anhydrous dichloromethane and treated with triethylamine (4.2 mL, 30 mmol), followed by chlorotrimethylsilane (3.17 mL, 25 mmol). The reaction mixture was stirred at room temperature for 3 h, wahed sequentially with diluted HCl solution, water, saturated $NaHCO_3$ solution, and saturated NaCl solution. The solution was dried over Na_2SO_4 and concentrated under reduced pressure. The

resulting oil was chromatographed on silica gel. One of the isomers, the β-isomer, **183b** (1.93 g, 47 %) was obtained from the chloroform-ethyl acetate (95:5) eluting portions as a yellow viscous oil: IR ($CHCl_3$) 3410, 1765, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.12 (9H, s, $Si(C\underline{H}_3)_3$), 1.23 (3H, d, J=6, $CHC\underline{H}_3$), 2.94 (1H, dd, J=2 and 5Hz, C_3-$\underline{H}$), 3.97 (1H, d, J=8Hz, PhC$\underline{H}$), 4.15-4.25 (2H, m, C$\underline{H}CH_3$ and C_4-$\underline{H}$), 5.64 (1H, s N$\underline{H}$), 7.30-7.50 (5H, m, aromatic H). Another isomer, the α-isomer, **183a** (1.42 g, 34 %) was obtained from the chloroform-ethyl acetate (90:10) eluting fractions as colorless crystals: m.p. 156-157°C (recrystallized from hexane); IR ($CHCl_3$) 3410, 1765, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.01 (9H, s, $Si(C\underline{H}_3)_3$), 0.43 (3H, d, J=6, $CHC\underline{H}_3$), 2.76 (1H, dd, J=2 and 3Hz, C_3-$\underline{H}$), 3.91 (1H, d, J=10Hz, PhC$\underline{H}$), 3.98-4.04 (1H, m, C$\underline{H}CH_3$), 4.18 (1H, dd, J=2 and 10Hz, C_4-H), 6.07 (1H, s, N$\underline{H}$), 7.28-7.43 (5H, m, aromatic H). Anal. Calcd for $C_{22}H_{27}NO_3SSi$: C, 63.89; H, 6.58; N, 3.39. Found: C, 64.21, H, 6.59; N, 3.38.

(3*S*, 4*S*)-1-Allyloxalyl-4-[(*R*)-phenyl(phenylthiocarbonyl)methyl]-3-[(1*R*)-(trimethylsilyloxy)ethyl]azetidin-2-one (184b).

Using a similar procedure described above for **180b**, the title compound **184b** was obtained from the correspondind azetidinone **183b** (1.24 g, 3 mmol) in almost quantitative yield as a yellow oil: ^1H NMR ($CDCl_3$) δ -0.02 and 0.05 (total 9H, s, $Si(C\underline{H}_3)_3$), 0.50 and 0.66 (total 3H, d, J=6, $CHC\underline{H}_3$), 3.76 and 3.80 to(total 1H, t, J=2, C_3-$\underline{H}$), 4.07-4.17 (1H, m, C$\underline{H}CH_3$), 4.55-4.61 (1H, m, C_4-$\underline{H}$), 4.76-4.83 (2H, m, $OC\underline{H}_2CH{=}CH_2$), 4.87-4.93 (1H, m, PhC$\underline{H}$), 5.28-5.50 (2H, m, $OCH_2CH{=}C\underline{H}_2$), 5.88-6.04 (1H, m, $OCH_2C\underline{H}{=}CH_2$), 7.31-7.47 (10H, m, aromatic H).

(3*S*, 4*S*)-1-Allyloxalyl-4-[(*S*)-phenyl(phenylthiocarbonyl)methyl]-3-[(1*R*)-(trimethylsilyloxy)ethyl]azetidin-2-one (184a).

Using a similar procedure described above for **180b**, the title compound **184a** was obtained from the azetidinone **183a** (414 mg, 1 mmol) in almost quantitative yield as a yellow oil: IR ($CHCl_3$) 1810, 1750, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.02 (9H, s, $Si(C\underline{H}_3)_3$), 0.67 (3H, d, J=6, $CHC\underline{H}_3$), 3.04 (1H, t, J=2, C_3-$\underline{H}$), 4.13 (1H, dd J=2 and 6Hz, $C\underline{H}CH_3$), 4.35 (1H, d, J=8Hz, PhCH), 4.76-4.81 (2H, m, $OC\underline{H}_2CH=CH_2$), 4.94 (1H, dd, J=2 and 8Hz, C_4-$\underline{H}$), 5.27-5.43 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.78-6.02 (1H, m, $OCH_2C\underline{H}=CH_2$), 7.31-7.47 (10H, m, aromatic H).

Allyl (1*S*, 5*S*, 6*S*)-1-Phenyl-2-phenylthio-6-[(1*R*)-1-(trimethylsilyloxy)-ethyl]carbapen-2-em-3-carboxylate (185).

The title compound **185** (702 mg, 47 %) was obtained as a yellow oil from the oxalimide **184a** (4 mmol), using the procedure described above for **181**: IR ($CHCl_3$) 1785, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.11 (9H, s, $Si(C\underline{H}_3)_3$), 1.43 (3H, d, J=6Hz, $CHC\underline{H}_3$), 3.53 (1H, dd, J=3 and 7Hz, C_6-$\underline{H}$), 4.07 (1H, dd, J=3 and 7Hz, C_5-$\underline{H}$), 4.26-4.32 (1H, m, $C\underline{H}CH_3$), 4.45 (1H, d, J=7Hz, PhC$\underline{H}$), 5.00-5.20 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.53-5.83 (2H, m, $OCH_2CH=C\underline{H}_2$), 6.23-6.38 (1H, m, $OCH_2C\underline{H}=CH_2$), 6.89-7.53 (10H, m, aromatic H).

Allyl (1*S*, 5*S*, 6*S*)-6-[(1*R*)-1-Hydroxyethyl]-1-phenyl-2-(phenylthio)-carbapen-2-em-3-carboxylate (182).

The silyl ether **185** (580 mg, 1.17 mmol) was dissolved in 10 mL of tetrahydrofuran and treated with a solution of pyridinium *p*-toluenesulfonate in 10 mL of distilled water. The bi-phasic mixture was stirred at room temperature for 2 h and diluted with ethyl acetate. The organic layer was

separated, washed with water and saturated NaCl solution, then dried over Na_2SO_4. The solvent was removed *in vacuo* at <30°C and the residue was passed through a short silica gel column eluting with chloroform, to give the alcohol **182** (395 mg, 80 %) as a light brown form: IR ($CHCl_3$) 1780 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (3H, d, J=6Hz, $CHC\underline{H}_3$), 3.49 (1H, dd, J=3 and 6Hz, C_6-$\underline{H}$), 4.08 (1H, dd, J=3 and 7Hz, C_5-$\underline{H}$), 4.26-4.32 (1H, m, $C\underline{H}CH_3$), 4.37 (1H, d, J=7Hz, $PhC\underline{H}$), 4.85-5.06 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.38-5.70 (2H, m, $OCH_2CH=C\underline{H}_2$), 6.10-6.25 (1H, m, $OCH_2C\underline{H}=CH_2$), 6.75-7.50 (10H, m, aromatic H).

(3*S*, 4*S*)-4-[(1*S*)-1-(Phenylthiocarbonyl)ethyl]-3-[(1*R*)-1-(trimethylsilyloxy)ethyl]azetidin-2-one (186b) and (3*S*, 4*S*)-4-[(1*R*)-1-(Phenylthiocarbonyl)ethyl]-3-[(1*R*)-1-(trimethylsilyloxy)ethyl]azetidin-2-one (186a).

According to the procedure described for **183b** and **183a**, the title compounds **186a** (2.45 g, 35 %) and **186b** (3.51 g, 50 %) were prepared as colorless crystals, from the thioester **166** (6.65 g, 40 mmol) and the acetoxyazetidinone **100** (5.75 g, 20 mmol). The α–isomer **186a**: m.p. 90-95°C (recrystallized from hexane); IR ($CHCl_3$) 3420, 1765, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.13 (9H, s, $Si(C\underline{H}_3)_3$), 1.30 (3H, d, J=6, C_3-$CHC\underline{H}_3$), 1.38 (3H, d, J=7, C_4-$CHC\underline{H}_3$), 2.80-2.90 (2H, m, C_3-$\underline{H}$ and C_4-$C\underline{H}CH_3$), 3.68 (1H, dd, J=2 and 10Hz, C_4-$\underline{H}$), 4.10-4.16 (1H, m, C_3-$C\underline{H}CH_3$), 6.00 (1H, s, $N\underline{H}$), 7.38-7.50 (5H, m, aromatic H). The β-isomer **186b**: m.p. 74-78°C (recrystallized from hexane); IR ($CHCl_3$) 3420, 1765, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.00 (9H, s, $Si(C\underline{H}_3)_3$), 1.11 (3H, d, J=6, C_3-$CHC\underline{H}_3$), 1.20 (3H, d, J=7, C_4-$CHC\underline{H}_3$), 2.84-2.90 (1H, m, C_4-$C\underline{H}CH_3$), 2.95 (1H, dd, J=2 and 5Hz, C3-H), 3.76 (1H, dd, J=2 and 5Hz, C_4-$\underline{H}$), 4.25-4.31 (1H, m, C_3-$C\underline{H}CH_3$), 5.79 (1H, s, $N\underline{H}$), 7.27-7.35 (5H, m, aromatic H).

(3*S*, 4*S*)-1-Allyloxalyl-4-[(1*S*)-1-(phenylthiocarbonyl)ethyl]-3-[(1*R*)-(trimethylsilyloxy)ethyl]azetidin-2-one (187a).

The title compound **187a** was obtained in almost quantitative yield as a white foam from the azetidinone **186a** (2.28 g, 6.49 mmol), using the procedure described above for **180b**: IR ($CHCl_3$) 1810, 1750, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.00 (9H, s, $Si(C\underline{H}_3)_3$), 1.07 (3H, d, J=6, C_3-$CHC\underline{H}_3$), 1.21 (3H, d, J=7, C_4-$CHC\underline{H}_3$), 3.17 (1H, t, J=3Hz, C_3-$\underline{H}$), 3.36-3.42 (1H, m, C_4-$C\underline{H}CH_3$), 4.15-4.25 (1H, m, C_3-$C\underline{H}CH_3$), 4.54 (1H, dd, J=3 and 6Hz, C_4-$\underline{H}$), 4.70-4.75 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.20-5.38 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.83-5.95 (1H, m, $OCH_2C\underline{H}=CH_2$), 7.30-7.38 (5H, m, aromatic H).

(3*S*, 4*S*)-1-Allyloxalyl-4-[(1*R*)-1-(phenylthiocarbonyl)ethyl]-3-[(1*R*)-(trimethylsilyloxy)ethyl]azetidin-2-one (187b).

The title compound **187b** was obtained in almost quantitative yield as a white foam from the corresponding azetidinone **186b**, using the procedure described above for **180b**: IR ($CHCl_3$) 1810, 1750, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.07 (9H, s, $Si(C\underline{H}_3)_3$), 1.18 (3H, d, J=6, C_3-$CHC\underline{H}_3$), 1.32 (3H, d, J=7, C_4-$CHC\underline{H}_3$), 3.55 (1H, t, J=4Hz, C_3-$\underline{H}$), 3.57-3.70 (1H, m, C_4-$C\underline{H}CH_3$), 4.17-4.24 (1H, m, C_3-$C\underline{H}CH_3$), 4.35-4.39 (1H, m, C_4-$\underline{H}$), 4.73-4.82 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.28-5.45 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.88-6.01 (1H, m, $OCH_2C\underline{H}=CH_2$), 7.35-7.45 (5H, m, aromatic H).

Allyl (1*S*, 5*S*, 6*S*)-1-Methyl-6-[(1*R*)-1-(trimethylsilyloxy)ethyl]-2-(phenylthio)carbapen-2-em-3-carboxylate (188).

The title compound **188** (1.92 g, 69 %) was obtained as a yellow oil from the corresponding oxalimide **187a** (6.49 mmol), according to the procedure described above for **181**: ^1H NMR ($CDCl_3$) δ 0.09 (9H, s, $Si(C\underline{H}_3)_3$), 0.90 (3H, d, J=7Hz, C_1-$C\underline{H}_3$), 1.24 (3H, d, J=6Hz, C_6-$CHC\underline{H}_3$),

3.14 (1H, dd, J=3 and 7Hz, C_6-H), 3.20-3.26 (1H, m, C_1-H), 3.60 (1H, dd, J=3 and 7 Hz, C_5-H), 4.09-4.15 (1H, m, C_6-CHCH$_3$), 4.68-4.85 (2H, m, OCH$_2$CH=CH$_2$), 5.20-5.50 (2H, m, OCH$_2$CH=CH$_2$), 5.91 -6.04 (1H, m, OCH$_2$CH=CH$_2$), 7.30-7.50 (5H, m, aromatic H).

Allyl (1*S*, 5*S*, 6*S*)-6-[(1*R*)-1-Hydroxyethyl]-1-methyl-2-(phenylthio)-carbapen-2-em-3-carboxylate (189).

In a manner similar to that described above for **182**, the title compound **189** (935 mg, 62 %) was prepared from the silyl ether **188** (1.80 g, 4.17 mmol) as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, d, J=7Hz, C_1-CH$_3$), 1.29 (3H, d, J=6Hz, C_6-CHCH$_3$), 3.19 (1H, dd, J=3 and 6Hz, C_6-H), 3.22-3.28 (1H, m, C_1-H), 3.71 (1H, dd, J=3 and 7 Hz, C_5-H), 4.17-4.23 (1H, m, C_6-CHCH$_3$), 4.60-4.90 (2H, m, OCH$_2$CH=CH$_2$), 5.20-5.50 (2H, m, OCH$_2$CH=CH$_2$), 5.90 -6.10 (1H, m, OCH$_2$CH=CH$_2$), 7.20-7.60 (5H, m, aromatic H).

(3*S*, 4*R*)-4-(Phenylthiocarbonyl)methyl-3-[(1*R*)-1-(trimethylsilyloxy)-ethyl]azetidin-2-one (191).

The title compound **191** (3.04 g, 20 mmol) was obtained in almost quantitative yield as light brown crystals, from *S*-phenyl thioacetate (3.04 g, 20 mmol) and the acetoxyazetidinone **100** (2.87 g, 10 mmol), according to the procedure described above for **183b** and **183a**: m.p. 75-76°C (recrystallized from toluene-hexane); ^{1}H NMR ($CDCl_3$) δ 0.05 (9H, s, Si(CH$_3$)$_3$), 1.28 (3H, d, J=6, C_3-CHCH$_3$), 2.91 (1H, dd, J=2 and 6Hz, C3-H), 2.93-3.12 (2H, m, C_4-CH$_a$H$_b$CO), 3.95-4.03 (1H, m, C_4-H), 4.10-4.20 (1H, m, CHCH$_3$), 6.27 (1H, s, NH), 7.37-7.44 (5H, m, aromatic H).

Allyl (5*R*, 6*S*)-6-[(1*R*)-1-(Trimethylsilyloxy)ethyl]-2-(phenylthio)-carbapen-2-em-3-carboxylate (193).

Using the procedures described above for **180a** and **181**, the title compound **193** (1.38 g, 33 %) was prepared from the azetidinone **191** (3.38 g, 10 mmol) as a white foam: ^{1}H NMR ($CDCl_3$) δ 0.00 (9H, s, $Si(C\underline{H}_3)_3$), 1.13 (3H, d, J=6Hz, C_6-$CHC\underline{H}_3$), 2.46-2.64 (1H, m, C_1-$\underline{H}_a\underline{H}_b$), 2.93 (1H, dd, J=3 and 7Hz, C_6-$\underline{H}$), 3.89 (1H, dt, J=3 and 9Hz, C_5-$\underline{H}$), 4.01-4.07 (1H, m, C_6-$C\underline{H}CH_3$), 4.62-4.81 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.15-5.45 (2H, m, $OCH_2C\underline{H}=CH_2$), 5.85-5.98 (1H, m, $OCH_2CH=C\underline{H}_2$), 7.28-7.50 (5H, m aromatic H).

Allyl (5*R*, 6*S*)-6-[(1*R*)-1-Hydroxyethyl]-2-(phenylthio)carbapen-2-em-3-carboxylate (194).

Using the procedure described above for **182**, the title compound **194** (946 mg, 83 %) was prepared from the silyl ether **193** (3.38 g, 10 mmol) as a white foam: IR ($CHCl_3$) 1775, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, d, J=6Hz, C_6-$CHC\underline{H}_3$), 1.60 (1H, s, OH), 2.63-2.67 (1H, m, C_1-$\underline{H}_a\underline{H}_b$), 3.06 (1H, dd, J=3 and 7Hz, C_6-$\underline{H}$), 4.06 (1H, dt, J=3 and 9Hz, C_5-$\underline{H}$), 4.08-4.20 (1H, m, C_6-$C\underline{H}CH_3$), 4.69-490 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.24-5.50 (2H, m, $OCH_2C\underline{H}=CH_2$), 5.93-6.06 (1H, m, $OCH_2CH=C\underline{H}_2$), 7.34-7.57 (5H, m aromatic H).

Sodium (1*S*, 5*S*, 6*S*)-6-[(1*R*)-1-Hydroxyethyl]-1-phenyl-2-(phenylthio)-carbapen-2-em-3-carboxylate (195).

A 1.6 mL of 0.5M sodium 2-ethylhexanoate in ethyl acetate solution, 15 mg of triphenylphosphine and 15 mg of tetrakis(triphenylphosphine)-palladium(0) were added to a solution of the carbapenem allyl ester **182** (337 mg, 0.8 mmol) in 2 mL of anhydrous dichloromethane. The mixture was

stirred at room temperature for 1 h under a nitrogen atmosphere. The disappearance of the starting material was observed by TLC. The solvent was evaporated, and the residue was dissolved in 1 mL of ethyl acetate. Diethyl ether 5 mL was added dropwise over the period of 30 min, and during that period yellow solids started to precipitate out. The precipitate was separated by filtration and washed several times with diethyl ether to give the desired sodium salt **195** (169 mg, 42 %) as a light brown powder: ^{1}H NMR (D_2O) δ 0.90 (3H, d, J=6Hz, $CHC\underline{H}_3$), 3.44-3.52 (1H, m, C_6-$\underline{H}$), 3.97-4.05 (1H, m, $C\underline{H}CH_3$), 4.33 (1H, d, J=8Hz, C_1-$\underline{H}$), 6.80-7.40 (10H, m, aromatic $\underline{H}$).

Sodium (1*S*, 5*S*, 6*S*)-6-[(1*R*)-1-Hydroxyethyl]-1-methyl-2-(phenylthio)-carbapen-2-em-3-carboxylate (196).

The allyl ester **189** (826 mg, 2.23 mmol) was dissolved in 4.5 mL of 0.5 M sodium 2-ethylhexanoate / ethy acetate solution and treated with 44 mg of triphenylphosphine and 44 mg of tetrakis(triphenylphosphine)palladium(0). The reaction mixture was stirred at room temperature for 30 min under a nitrogen atmosphere. The resulting precipitates were collected and washed with total 10 mL of ethyl acetate, then with diethyl ether to give the desired carbapenem sodium salt **196** (412 mg, 54 %) as a white powder: ^{1}H NMR (D_2O) δ 0.83 (3H, d, J=7Hz, C_1-$C\underline{H}_3$), 1.10 (3H, d, J=6Hz, C_6-$CHC\underline{H}_3$), 3.14-3.20 (1H, m, J=7Hz, C_1-$\underline{H}$), 3.31 (1H, dd, J=2 and 5Hz, C_6-$\underline{H}$), 3.62 (1H, dd, J=2 and 7Hz, C_5-$\underline{H}$), 4.04-4.10 (1H, m, C_6-$C\underline{H}CH_3$), 7.15-7.60 (5H, m, aromatic H).

Sodium (5*R*, 6*S*)-6-[(1*R*)-1-Hydroxyethyl]-2-(phenylthio)carbapen-2-em-3-carboxylate (197).

In a manner similar to that described above for **196**, the title compound **197** (450 mg, 51 %) was prepared from the corresponding allyl ester **194** (930 mg, 2.69 mmol) as a white powder: ^{1}H NMR (D_2O) δ 1.07 (3H, d, J=6Hz, C_6-CHC<u>H</u>$_3$), 2.43-2.68 (2H, m, C_1-<u>H</u>$_a$<u>H</u>$_b$), 3.31 (1H, dd, J=2 and 5Hz, C_6-<u>H</u>), 3.62 (1H, dd, J=2 and 7Hz, C_5-<u>H</u>), 4.04-4.10 (1H, m, C_6-C<u>H</u>CH$_3$), 7.15-7.60 (5H, m, aromatic H).

5.2 Experiments for Chapter 4

3-Acetoxymethyl-7β-(allyloxalamido)ceph-3-em-4-carboxylic acid (199).

7-Aminocephalosporanic acid (**11**) (5.44g, 20 mmol) and sodium bicarbonate (5.04 g, 60 mmol) were suspended in 50 mL of H_2O at 0°C and the mixture was stirred for 15 min. Vigorous evolution of carbon dioxide was observed and all the solids were gradually dissolved. To this mixture, a solution of allyl oxalyl chloride (2.97 g, 20 mmol) in 30 mL of tetrahydrofuran was added dropwise. The two phase mixture was stirred at 0°C for 2 h. Diethyl ether was added and the aqueous layer was separated. The organic layer was extracted twice with small amount of water. The combined aqueous layer was washed with diethyl ether, acidified with concentrated HCl solution to pH 2, then extracted with ethyl acetate several times. The combined organic layer was washed with small amount of water and then with saturated NaCl solution. The extract was dried over Na_2SO_4 and evaporated to give a thick oil, which was chromatographed on silica gel eluting with chloroform-methanol (95:5) to afford the desired oxalamide **199** (1.92 g, 25 %) as a thick yellow oil: IR ($CHCl_3$) 1800, 1755, 1720 cm^{-1}; ^{1}H

NMR ($CDCl_3$) δ 2.03 (3H, s, $OCOC\underline{H}_3$), 3.36 and 3.53 (each 1H, d, J=18Hz, C2-$\underline{H}_a\underline{H}_b$), 4.69-4.75 (2H, m, $OC\underline{H}_2CH=CH_2$), 4.85 and 5.08 (each 1H, d, J=13Hz, C_3-$C\underline{H}_2$), 5.00 (1H, d, J=5Hz, C_6-$\underline{H}$), 5.25-5.40 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.73 (1H, dd, J=5 and 9Hz, C_7-$\underline{H}$), 5.84-6.00 (1H, m, $OCH_2C\underline{H}=CH_2$), 8.14 (1H, d, J=9Hz, N$\underline{H}$).

Sodium 3-Acetoxymethyl-7β-(allyloxalamido)ceph-3-em-4-carboxylate (200).

The acid **199** (384 mg, 1 mmol) was dissolved in 1 mL of diethyl ether and treated with a 2 mL of 0.5N $NaHCO_3$ solution. The two phase mixture was stirred at 0°C until the evolution of carbon dioxide had entirely ceased (approximately 30 min.). The aqueous layer was separated and washed several times with diethyl ether, then lyophilized to yield the desired sodium salt **200** in almost quantitative yield as a light yellow powder: ^1H NMR (D_2O) δ 1.97 (3H, s, $OCOC\underline{H}_3$), 3.28 and 3.54 (each 1H, d, J=18Hz, C2-$\underline{H}_a\underline{H}_b$), 4.55-4.78 (4H, m, $OC\underline{H}_2CH=CH_2$ and C_3-$C\underline{H}_2$), 5.06 (1H, d, J=5Hz, C_6-$\underline{H}$), 5.14-5.34 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.61 (1H, d, J=5Hz, C_7-$\underline{H}$), 5.80-6.00 (1H, m, $OCH_2C\underline{H}=CH_2$); FAB-MS(negative) *m/z* 383 ($M-Na^+$).

Disodium 3-Acetoxymethyl-7β-(carboxylatocarboxyamido)ceph-3-em-4-carboxylate (201).

A 2 mL volume of 0.5N sodium 2-ethylhexanoate/ethyl acetate solution and sodium hydride (40 mg, 1 mmol) were added to a solution of the allyl ester **199** (384 mg, 1 mmol) in a mixture of 2 mL of anhydrous tetrahydrofuran and 2 mL of sieve-dried dimethylformamide at 0°C under a nitrogen atmosphere. The reaction mixture was stirred at 0°C for 30 min, then treated with 30 mg of triphenylphosphine and 30 mg of tetrakis(triphenyl-phosphine)palladium(0). After 15 min of stirring, the

mixture became turbid with fine precipitates. The precipitates were collected by filtration and washed with ethyl acetate and diethyl ether successively to give the desired disodium salt **201** (365 mg, 94 %) as a yellow powder: ^{1}H NMR (D_2O) δ 1.97 (3H, s, OCOC$\underline{H}_3$), 3.27 and 3.53 (each 1H, d, J=18Hz, C_2-$\underline{H}_a\underline{H}_b$), 4.56-4.80 (2H, m, C_3-C$\underline{H}_2$), 5.03 (1H, d, J=5Hz, C_6-$\underline{H}$), 5.52 (1H, d, J=5Hz, C_7-$\underline{H}$); FAB-MS(negative) *m/z* 365 ($M-Na^+$).

3-Acetoxymethyl-7β-[(allyloxycarbonyl)acetamido]ceph-3-em-4-carboxylic acid (202).

Oxalyl chloride (1.92 mL, 22 mmol) was added to a solution of allyl hydrogen malonate (2.88 g, 20 mmol) in 20 mL of anhydrous dichloromethane. The mixture was stirred at room temperature for 2 h under a nitrogen atmosphere. The solvent removal gave the crude allyl malonyl chloride, which was used for next reaction without purification.

A solution of allyl malonyl chloride (20 mmol), prepared by the procedure described above for **199**, in 30 mL of diethyl ether was added to a solution of 7-aminocephalosporanic acid (5.44 g, 20 mmol) and sodium bicarbonate (5.04 g, 60 mmol) in 50 mL of H_2O. The mixture was stirred at 0°C for 2 h. The aqueous layer was separated, washed with diethyl ether, then acidified with concentrated HCl solution to pH 2. Ethyl acetate was added and the insoluble material was filtered off. The organic layer was washed with water and saturated NaCl solution, and dried over Na_2SO_4. After evaporation of the solvent, the resulting thick oil was chromatographed on silica gel using chloroform-methanol (95:5) as the eluant to give the desired amide **202** (1.60 g, 20 %) as a yellow form: IR ($CHCl_3$) 1790, 1730, 1690 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.10 (3H, s, C$\underline{H}_3$CO), 3.43 and 3.60 (each 1H, d, J=19Hz, C_2-$\underline{H}_a\underline{H}_b$), 3.45 (2H, s, COC$\underline{H}_2$CO), 4.63-4.68 (2H, m,

$OC\underline{H}_2CH=CH_2$), 4.94 and 5.12 (each 1H, d, J=14Hz, C_3-$C\underline{H}_a\underline{H}_b$-O), 5.03 (1H, d, J=5Hz, C_6-$\underline{H}$), 5.25-5.39 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.83-5.98 (2H, m, $OCH_2C\underline{H}=CH_2$ and C_7-$\underline{H}$), 8.09 (1H, d, J=9Hz).

Sodium 3-Acetoxymethyl-7β-[(allyloxycarbonyl)acetamido]ceph-3-em-4-carboxylate (203).

In a manner similar to that described above for **200**, the title compound **203** was obtained from the carboxylic acid **202** (398 mg, 1 mmol) in almost quantitative yield as a yellow powder: ^1H NMR (D_2O) δ 1.98 (3H, s, $OCOC\underline{H}_3$), 3.28 and 3.54 (each 1H, d, J=18Hz, C_2-$\underline{H}_a\underline{H}_b$), 4.48-4.62 (4H, m, $OC\underline{H}_2CH=CH_2$ and C_3-$C\underline{H}_2$), 4.57-4.78 (2H, m, C_3-$C\underline{H}_a\underline{H}_b$-O), 5.03 (1H, d, J=5Hz, C_6-$\underline{H}$), 5.15-5.30 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.56 (1H, d, J=5Hz, C_7-$\underline{H}$), 5.79-5.94 (1H, m, $OCH_2C\underline{H}=CH_2$); FAB-MS(negative) *m/z* 397 ($M-Na^+$).

Disodium 3-Acetoxymethyl-7β-(carboxylatoacetamido)ceph-3-em-4-carboxylate (204).

The allyl ester **202** (398 mg, 1 mmol) was dissolved in a mixture of 8 mL of dimethylformamide, 6 mL of ethyl acetate and 2 mL of 0.5N sodium 2-ethylhexanoate/ethyl acetate solution, and treated with sodium hydride (40 mg, 1 mmol) at 0°C under a nitrogen atmosphere. The mixture was stirred at 0°C for 30 min. Triphenylphosphine (30 mg) and 30 mg of tetrakis(triphenylphosphine)-palladium(0) were added to the solution and the reaction mixture was stirred for an additional 2 h at room temperature. The resulting precipitate was collected by filtration, washed with ethyl acetate and diethyl ether, and dried under vacuum, to give the desired disodium salt **204** (223 mg, 55 %) as a brown powder: ^1H NMR (D_2O) δ 2.00 (3H, s, $OCOC\underline{H}_3$), 3.16 (2H, s, $COC\underline{H}_2CO$), 3.29 and 3.55 (each 1H, d, J=18Hz, C_2-

H_aH_b), 5.03 (1H, d, J=5Hz, C_6-H), 5.60 (1H, d J=5Hz, C_7-H; FAB-MS (negative) *m/z* 379 ($M-Na^+$)).

3-Acetoxymethyl-7β-(3-carboxypropionamido)ceph-3-em-4-carboxylic Acid (205).

A solution of succinic anhydride (1.0 g, 10 mmol) in 25 mL of acetone was added dropwise to a mixed solution of 7-aminocephalosporanic acid (2.72 g, 10 mmol) and sodium bicarbonate (2.10 g, 25 mmol) in 25 mL of H_2O at 0°C. The reaction mixture was stirred at 0°C for 2 h. After concentrating to half of its original volume, the solution was acidified with concentrated HCl solution to pH 2-3. The resulting acidic solution was washed several times with dichloromethane, then extracted with 3x25 mL of ethyl acetate. The combined ethyl acetate solution was washed with small amount of saturated NaCl solution, and dried over Na_2SO_4. Solvent removal gave the desired diacid **205** (673 mg, 18 %) as a yellow foam: ^1H NMR ($CDCl_3$+DMSO-d_6) δ 2.19 (3H, s, $OCOCH_3$), 2.23 (4H, s, $COCH_2CH_2CO$), 3.02 and 3.22 (each 1H, d, J=18, C_2-H_aH_b), 4.44-4.76 (2H, m, C_3-CH_2O), 4.64 (1H, d, J=5Hz, C_6-H), 5.40 (1H, dd, J=5 and 8Hz, C_7-H), 8.28 (1H, d, J=8Hz, NH).

Disodium 3-Acetoxymethyl-7β-(3-carboxylatopropionamido)ceph-3-em-4-carboxylate (206).

The carboxylic acid **205** (372 mg, 1 mmol) was dissolved in 1 mL of ethyl acetate and treated with 2 mL of 1N $NaHCO_3$ solution. The two phase solution was stirred vigorously at room temperature for 30 min. The aqueous phase was separated and washed with diethyl ether. Lyophilization gave the desired disodium salt **206** in almost quantitative yield as a light yellow powder: ^1H NMR (D_2O) δ 1.98 (3H, s, $OCOCH_3$), 2.32-2.50 (4H, m,

$COCH_2CH_2CO$), 3.27 and 3.53 (each 1H, d, J=18Hz, C_2-H_aH_b), 4.54-4.76 (2H, m, C_3-CH_2), 5.99 (1H, d, J=5Hz, C_6-H), 5.54 (1H, d, J=5Hz, C_7-H; FAB-MS(negative) *m/z* 393 ($M-Na^+$)).

7β-(Allyloxalamido)-3-methylceph-3-em-4-carboxylic Acid (207).

7-Amino-3-desacetoxycephalosporanic acid (4.28 g, 20 mmol) and sodium bicarbonate (5.04 g, 60 mmol) was dissolved in 50 mL of H_2O. To this mixture, a solution of allyl oxalyl chloride (2.97 g, 20 mmol) in 30 mL of toluene was added and the resulting two phase mixture was stirred vigorously at room temperature for 30 min. The aqueous layer was separated, washed with dichloromethane, and acidified with concentrated HCl solution to pH 2. The mixture was extracted with ethyl acetate three times. The combined organic layer was washed with water and saturated NaCl solution. The organic solution was dried over Na_2SO_4 and evaporated to give the residue, which was purified by silica gel column chromatography with chloroform-methanol (95:5) as the eluant, to give the desired oxalamide **207** (3.85 g, 59 %) as a yellow foam: IR ($CHCl_3$) 1790, 1715 cm^{-1}; ^1H NMR ($CDCl_3$ + 2drops of DMSO-d_6) δ 2.09 (3H, s, C_3-CH_3), 3.20 and 3.39 (each 1H, d, J=18Hz, C_2-H_aH_b), 4.68-4.73 (2H, m, $OCH_2CH=CH_2$), 4.95 (1H, d, J=5Hz, C_6-H), 5.19-5.38 (2H, m, $OCH_2CH=CH_2$), 5.62 (1H, dd, J=5 and 9Hz, C_7-H), 5.82-5.96 (1H, m, $OCH_2CH=CH_2$), 8.11 (1H, d, J=9Hz, NH).

Sodium 7β-(allyloxalamido)-3-methylceph-3-em-4-carboxylate (208).

The carboxylic acid **207** (3.30 g, 10.1 mmol) was dissolved in 10 mL of diethyl ether and treated with 10 mL of H_2O. The two phase mixture was stirred vigorously at room temperature for 30 min. The aqueous layer was separated and washed with diethyl ether. The aqueous solution was

lyophylized to afford the desired sodium salt **208** (3.41 g, 97 %) as a brown powder: ^{1}H NMR (D_2O) δ 1.86 (3H, s, C_3-$C\underline{H}_3$), 3.19 and 3.51 (each 1H, J=18Hz, C_2-$\underline{H}_a\underline{H}_b$), 4.74-4.77 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.07 (1H, d, J=5Hz, C_6-$\underline{H}$), 5.27-5.40 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.56 (1H, d, J=5Hz, C_7-$\underline{H}$), 5.87-6.02 (1H, m, $OCH_2C\underline{H}=CH_2$); FAB-MS(negative) *m/z* 325 ($M-Na^+$).

Disodium 7β-(carboxylatocarboxyamido)-3-methylceph-3-em-4-carboxylate (209).

The allyl ester **208** (348 mg, 1 mmol) was dissolved in a mixture of 2 mL of dimethylformamide and 2 mL of 0.5N sodium 2-ethylhexanoate/ethyl acetate solution, and treated with 30 mg of triphenylphosphine and 30 mg of tetrakis(triphenylphosphine)palladium(0). The reaction mixture was stirred at room temperature for 30 min under a nitrogen atmosphere. At the end of the reaction, white precipitate was observed, which was collected by filtration. The precipitate was washed several times with ethyl acetate and several times with diethyl ether, dried under vacuum to give the desired disodium salt **209** (205 mg, 62 %) as a yellow powder: ^{1}H NMR (D_2O) δ 1.85 (3H, s, C_3-$C\underline{H}_3$), 3.18 and 3.51 (each 1H, d, J=18Hz, C_2-$\underline{H}_a\underline{H}_b$), 5.04 (1H, d, J=5Hz, C_6-$\underline{H}$), 5.49 (1H, d, J=5Hz, C_7-$\underline{H}$); FAB-MS(negative) *m/z* 307 ($M-Na^+$).

7β-[(Allyloxycarbonyl)acetamido]-3-methylceph-3-em-4-carboxylic acid (210).

A solution of allyl malonyl chloride (10 mmol), prepared according to the procedure described above for **202**, in 15 mL of toluene was added to a solution of 7-amino-3-desacetoxycephalosporanic acid (2.14 g, 10 mmol) and sodium bicarbonate (2.52 g, 30 mmol) in 25 mL of H_2O at 0°C under a nitrogen atmosphere. The two phase mixture was stirred at room temperature

for 30 min. Using the same work-up procedure as described above for **202**, the title carboxylic acid **210** (1.46 g, 43 %) was obtained as a yellow oil: IR ($CHCl_3$) 1785, 1735, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.19 (3H, s, C_3-$C\underline{H}_3$), 3.26 and 3.54 (each 1H, d, J=18Hz, C_2-$\underline{H}_a\underline{H}_b$), 3.45 (2H, s, $COC\underline{H}_2CO$), 4.55-4.70 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.01 (1H, d, J=5Hz, C_6-$\underline{H}$), 5.20-5.40 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.78-6.00 (2H, m, $OCH_2C\underline{H}=CH_2$ and C_7-$\underline{H}$), 8.06 (1H, d, J=9Hz, N$\underline{H}$).

Sodium 7β-[(Allyloxycarbonyl)acetamido]-3-methylceph-3-em-4-carboxylate (211).

The carboxylic acid **210** (681 mg, 2 mmol) was dissolved in 2 mL of ethyl acetate and treated with 2 mL of 1N $NaHCO_3$ solution. The two phase mixture was stirred vigorously at room temperature for 30 min. The aqueous layer was separated, and washed with diethyl ether, then lyophilized to give the desired sodium salt **211** (665 mg, 92 %) as a yellow powder: ^1H NMR (D_2O) δ 1.84 (3H, s, C_3-$C\underline{H}_3$), 3.16 and 3.52 (each 1H, J=18Hz, C_2-$\underline{H}_a\underline{H}_b$), 4.60-4.75 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.02 (1H, d, J=4Hz, C_6-$\underline{H}$), 5.20-5.32 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.52 (1H, d, J=5Hz, C_7-$\underline{H}$), 5.80-5.98 (1H, m, $OCH_2C\underline{H}=CH_2$); FAB-MS(negative) *m/z* 339 ($M-Na^+$).

Disodium 7β-(Carboxylatoacetamido)-3-methylceph-3-em-4-carboxylate (212).

According to the procedure described above for **209**, the title compound **212** (312 mg, 86 %) was obtained from the allyl ester **211** (362 mg, 1 mmol) as a brown powder: ^1H NMR (D_2O) δ 1.84 (3H, s, C_3-$C\underline{H}_3$), 3.16 and 3.51 (each 1H, J=18Hz, C_2-$\underline{H}_a\underline{H}_b$), 5.02 (1H, d, J=4Hz, C_6-$\underline{H}$), 5.56 (1H, d, J=4Hz, C_7-$\underline{H}$); FAB-MS(negative) *m/z* 321 ($M-Na^+$).

Allyl 6β-(Methoxalamido)-2,2-dimethylpenam-3-carboxylate (214).

6-Aminopenicillanic acid (**2**) (4.32 g, 20 mmol) and sodium bicarbonate (4.20 g, 50 mmol) were dissolved in 40 mL of H_2O and treated with a solution of methyl oxalyl chloride (2.45 g, 20 mmol) in 20 mL of toluene at 0°C. The reaction mixture was stirred vigorously at room temperature for 30 min. The aqueous layer was separated, washed with dichloromethane, and acidified with concentrated HCl solution to pH 2. The mixture was extracted with ethyl acetate three times and the combined organic layer was washed with water and saturated NaCl solution. The extract was dried over Na_2SO_4 and evaporated to give the crude oxamate **213**, which was used for the next reaction without further purification.

Triethylamine (2.79 mL, 20 mmol) and allyl bromide (2.16 mL, 25 mmol) were added to a stirred solution of the crude oxamate **213** in 20 mL of sieve-dried dimethylformamide under a nitrogen atmosphere. The reaction mixture was stirred at room temperature over night. The mixture was concentrated *in vacuo* at <30°C, and the residue was taken up in dichloromethane, washed with water and saturated NaCl solution. The extract was dried over Na_2SO_4 and evaporated to give a thick oil, which was chromatographed on silica gel eluting with chloroform and ethyl acetate (95:5) to afford the desired penam **213** (4.02 g, 59 %) as a yellow oil: ^1H NMR ($CDCl_3$) δ 1.51 and 1.67 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.92 (3H, s, $OC\underline{H}_3$), 4.49 (1H, s, C_3-$\underline{H}$), 4.60-4.70 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.20-5.42 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.58 (1H, d, J=4Hz, C_5-$\underline{H}$), 5.65 (1H, dd, J=4 and 9Hz, C_6-$\underline{H}$), 5.85-5.98 (2H, m, $OCH_2C\underline{H}=CH_2$), 7.72 (1H, d, J=9Hz, N$\underline{H}$). Anal. Calcd for $C_{14}H_{18}N_2O_6S$: C, 49.11; H, 5.30; N, 8.18. Found: C, 49.35, H, 5.46; N, 8.20.

Sodium 6β-(Methoxalamido)-2,2-dimethylpenam-3-carboxylate (215).

A 0.5N sodium 2-ethylhexanoate/ethyl acetate solution (2 mL), triphenylphosphine (30 mg), and tetrakis(triphenylphosphine)palladium(0) (30 mg) were added to a solution of the allyl ester **214** (342 mg, 1 mmol) in 2 mL of sieve-dried ethyl acetate under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 30 min, and filtered to collect the precipitate. The collected precipitate was washed with ethyl acetate and diethyl ether sequentially, and dried under vacuum to give the desired sodium salt **215** (231 mg, 71 %) as a light yellow powder: ^{1}H NMR (D_2O) δ 1.41 and 1.53 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.79 (3H, s, $OC\underline{H}_3$), 4.20 (1H, s, C_3-$\underline{H}$), 5.44 and 5.49 (each 1H, each d, J=4Hz, C_5-$\underline{H}$, and C_6-$\underline{H}$); FAB-MS(negative) *m/z* 301 ($M-Na^+$).

Allyl 6β-(Allyloxalamido)-2,2-dimethylpenam-3-carboxylate (217).

In a manner similar to that described above for **214**, the title compound **217** (3.53 g, 46 %) was prepared from 6-aminopenicillanic acid (2.88 g, 20 mmol) as a yellow oil: IR ($CHCl_3$) 1790, 1745, 1710 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.53 and 1.68 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 4.51 (1H, s, C_3-$\underline{H}$), 4.66-4.82 (4H, m, $OC\underline{H}_2CH=CH_2$), 5.29-5.47 (4H, m, $OCH_2CH=C\underline{H}_2$), 5.60 (1H, d, J=4Hz, C_5-$\underline{H}$), 5.67 (1H, dd, J=4 and 9Hz, C_6-$\underline{H}$), 5.86-6.05 (2H, m, $OCH_2C\underline{H}=CH_2$), 7.73 (1H, d, J=9Hz, N$\underline{H}$).

Disodium 6β-(Carboxylatocarboxyamido)-2,2-dimethylpenam-3-carboxylate (218).

A 0.5N sodium 2-ethylhexanoate (4 mL), triphenylphosphine (30 mg) and tetrakis(triphenylphosphine)palladium(0) (30 mg) were added to a solution of the diallyl ester **217** (368 mg, 1 mmol) in 2 mL of dimethylformamide under a nitrogen atmosphere. The reaction mixture was

stirred at room temperature for 30 min. The resulting precipitate was collected by filtration, washed with ethyl acetate and diethyl ether, and dried *in vacuo* to yield the desired disodium salt **218** (313 mg, 94 %) as a white powder: ^{1}H NMR (D_2O) δ 1.41 and 1.51 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 4.16 (1H, s, C_3-$\underline{H}$), 5.38 and 5.49 (each 1H,each d, J=4Hz, C_5-$\underline{H}$ and C_6-$\underline{H}$); FAB-MS(negative) *m/z* 309 ($M-Na^+$).

Allyl 6β-[(Methoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate (220).

Malonic acid monomethyl ester (2.95 g, 25 mmol) was dissolved in 25 mL of anhydrous benzene and treated with 2.5 mL of oxalyl chloride and 2 drops of dimehylformamide. The reaction mixture was stirred at room temperature for 3 h. Evaporation of the solvent gave the crude methyl malonyl chloride, which was used for the next reaction without purification.

A solution of methyl malonyl chloride (25 mmol) in 25 mL of toluene was added to a solution of 6-aminopenicillanic acid (5.41 g, 25 mmol) and sodium bicarbonate (5.25 g, 62.5 mmol) in 50 mL of H_2O at 0°C. The reaction mixture was stirred at room temperature for 30 min. The aqueous layer was separated, washed with dichloromethane, and acidified with concentrated HCl solution to pH 2. The mixture was extracted with ethyl acetate several times, and the combined organic layer was washed with saturated NaCl solution. After drying, the extract was concentrated *in vacuo* to give the crude carboxylic acid **219**, which was subjected to the esterification reaction immediately.

The carboxylic acid **219** was dissolved in 20 mL of sieve-dried dimethylformamide, and treated with triethylamine (3.5 mL, 25 mmol) and allyl bromide (2.60 mL, 30 mmol). The reaction mixture was stirred at room

temperature over night under a nitrogen atmosphere. Solvent removal gave the residue, which was taken up in dichloromethane, and washed with water and saturated NaCl solution, then dried over Na_2SO_4. After concentration, the resulting residue was chromatographed on silica gel eluting with chloroform-ethyl acetate (95:5) to give the desired penam **220** (2.82 g, 32 %) as a white foam: IR ($CHCl_3$) 1790, 1740, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.51 and 1.67 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.30-3.45 (2H, m, $COC\underline{H}_2CO$), 3.76 (3H, s, $OC\underline{H}_3$), 4.48 (1H, s, C_3-$\underline{H}$), 4.64-4.68 (4H, m, $OC\underline{H}_2CH=CH_2$), 5.25-5.41 (4H, m, $OCH_2CH=C\underline{H}_2$), 5.56 (1H, d, J=4Hz, C_5-$\underline{H}$), 5.75 (1H, dd, J=4 and 9Hz, C_6-$\underline{H}$), 5.84-5.98 (2H, m, $OCH_2C\underline{H}=CH_2$), 8.07 (1H, d, J=9Hz, N$\underline{H}$).

Sodium 6β-[(Methoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate (221).

To a mixed solution of allyl ester **220** (356 mg, 1 mmol) in ethyl acetate (2mL) and a 0.5N sodium 2-ethylhexanoate/ethyl acetate solution (2 mL) were added 30 mg of triphenylphosphine and 30 mg of tetrakis(tripheny-phosphine)palladium(0). The reaction mixture was stirred at room temperature for 30 min, and to this mixture, diethyl ether (10 mL) was added dropwise over 30 min. The solids were collected, washed with diethyl ether, and dried under vacuum, to give the desired sodium salt **221** (298 mg, 88 %) as a light yellow powder: ^1H NMR (D_2O) δ 1.43 and 1.55 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.67 (3H, s, OCH_3), 4.16 (1H, s, C_3-$\underline{H}$), 5.43 and 5.49 (each 1H, each d, J=4Hz, C_5-$\underline{H}$ and C_6-$\underline{H}$).

Allyl 6β-[(Ethoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate (223).

In a manner similar to that described above for **220**, the title compound **223** (9,35 g, 51 %) was obtained from malonic acid monoethyl ester (6.61 g, 50 mmol) and 6-aminopenicillanic acid (10.8 g, 50 mmol) as colorless crystals: m.p. 68-69°C (recrystallized from toluene); IR ($CHCl_3$) 1790, 1745, 1725, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (3H, t, J=7Hz, $CH_2C\underline{H}_3$), 1.50 and 1.67 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.37 (2H, s, $COC\underline{H}_2CO$), 4.21 (2H, q, J=7Hz, $C\underline{H}_2CH_3$), 4.48 (1H, s, C_3-$\underline{H}$), 4.64-4.70 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.25-5.40 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.56 (1H, d, J=4Hz, C_5-$\underline{H}$), 5.75 (1H, dd, J=4 and 9Hz, C_6-$\underline{H}$), 5.84-6.00 (1H, m, $OCH_2C\underline{H}=CH_2$), 8.11 (1H, d, J=9Hz, N$\underline{H}$). Anal. Calcd for $C_{16}H_{22}N_2O_6S$: C, 51.88; H, 5.99; N, 7.56. Found: C, 51.93, H, 6.24; N, 7.59.

Sodium 6β-[(Ethoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate (224).

Using the similar procedure described above for **221**, the title compound **224** (319 mg, 91 %) was prepared from the allyl ester **223** (370 mg, 1 mmol) as a white powder: ^1H NMR (D_2O) δ 1.16 (3H, t, J=7Hz, $CH_2C\underline{H}_3$), 1.41 and 1.52 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 4.04-4.15 (3H, m, $OC\underline{H}_2CH_3$ and C_3-$\underline{H}$), 5.30-5.50 (2H, m, C_5-$\underline{H}$ and C_6-$\underline{H}$); FAB-MS (negative) *m/z* 329 ($M\text{-}Na^+$).

Allyl 6β-[(Allyloxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate (226).

In a manner similar to that described for **220**, the title compound **226** (3.53 g, 46 %) was prepared from malonic acid monoallyl ester (2.88 g, 20 mmol) and 6-aminopenicillanic acid (4.33 g, 20 mmol) as colorless crystals:

m.p. 59-60°C (recrystallized from toluene); IR ($CHCl_3$) 1790, 1730, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.51 and 1.67 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.35-3.47 (2H, m, $COC\underline{H}_2CO$), 4.48 (1H, s, C_3-$\underline{H}$), 4.60-4.70 (4H, m, $OC\underline{H}_2CH{=}CH_2$), 5.22-5.42 (4H, m, $OCH_2CH{=}C\underline{H}_2$), 5.56 (1H, d, J=4Hz, C_5-$\underline{H}$), 5.75 (1H, dd, J=4 and 9Hz, C_6-$\underline{H}$), 5.84-6.00 (2H, m, $OCH_2C\underline{H}{=}CH_2$), 8.03 (1H, d, J=9Hz, N$\underline{H}$).

Disodium 6β-(Carboxylatoacetamido)-2,2-dimethylpenam-3-carboxylate (227).

Triphenylphosphine (30 mg) and tetrakis(triphenylphosphine)-palladium(0) (30 mg) were added to a mixed solution of the diallyl ester **226** (382 mg, 1 mmol) in 4 mL of sieve-dried dimethylformamide and 2 mL of 0.5N sodium 2-ethylhexanoate/ethyl acetate solution. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 30 min. The resulting precipitate was collected by filtration, washed sequentially with ethyl acetate and diethyl ether, and dried under vacuum to yield the desired disodium salt **227** (324 mg, 94 %) as a white powder: ^1H NMR (D_2O) δ 1.41 and 1.52 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 414 (1H, s, C_3-$\underline{H}$), 5.36-5.48 (2H, m, C_5-$\underline{H}$ and C_6-$\underline{H}$).

Allyl 6β-(3-Allyloxycarbonylpropionamido)-2,2-dimethylpenam-3-carboxylate (229).

Following the procedure described above for **220**, the title compound **229** (4.98 g, 42 %) was prepared from succinic acid monoallyl ester (4.74 g, 30 mmol) and 6-aminopenicillanic acid (6.29 g, 30 mmol) as a yellow oil: IR ($CHCl_3$) 1790, 1735, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 and 1.64 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 2.50-2.75 (4H, m, $COC\underline{H}_2C\underline{H}_2CO$), 4.42 (1H, s, C_3-$\underline{H}$), 4.55-4.70 (4H, m, $OC\underline{H}_2CH{=}CH_2$), 5.19-5.40 (4H, m, $OCH_2CH{=}C\underline{H}_2$), 5.52

(1H, d, J=4Hz, C_5-H), 5.69 (1H, dd, J=4 and 9Hz, C_6-H), 5.80-5.98 (2H, m, $OCH_2CH=CH_2$), 6.58 (1H, d, J=9Hz, NH).

Disodium 6β-(3-Carboxylatopropionamido)-2,2-dimethylpenam-3-carboxylate (230).

According to the procedure described above for **221**, the title compound **230** (120 mg, 33 %) was prepared from the ester **229** (396 mg, 1 mmol) as a white powder: ^1H NMR (D_2O) δ 1.41 and 1.53 (each 3H, s, C_2-$(CH_3)_2$), 2.30-2.65 (4H, m, $COCH_2CH_2CO$), 4.12 (1H, s, C_3-H), 5.30-5.45 (2H, m, C_5-H and C_6-H); FAB-MS(negative) *m/z* 337 ($M-Na^+$).

Allyl 6β-(Allyloxalamido)-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (233).

3-Chloroperoxybenzoic acid, 80 % (1.73 g, 8 mmol) was added to a stirred solution of the sulfide **217** (1.47 g, 4 mmol) in 20 mL of anhydrous dichloromethane at 0°C. The reaction mixture was stirred at room temperature for 18 h under a nitrogen atmosphere. After removal of the solvent, toluene (20 mL) was added and the mixture was stirred for 10 min. The suspending solids were filtered off, and the filtrate was washed sequentially with 10 % $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution and saturated NaCl solution. The extract was dried over Na_2SO_4, and evaporated to afford a yellow oil, which was chromatographed on silica gel using chloroform-ethyl acetate (90:10) as the eluant to yield the desired sulfone **233** (508 mg, 32 %) as a white powder: IR ($CHCl_3$) 1820, 1760, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 and 1.62 (each 3H, s, C_2-$(CH_3)_2$), 4.53 (1H, s, C_3-H), 4.65-4.85 (4H, m, $OCH_2CH=CH_2$) ,4.80 (1H, d, J=5Hz, C_5-H), 5.30-5.45 (4H, m, $OCH_2CH=CH_2$), 5.84-6.01 (2H, m, $OCH_2CH=CH_2$), 6.03 (1H, dd,

J=5 and 11Hz, C6-H), 8.38 (1H, d, J=11Hz, NH). Anal. Calcd for $C_{16}H_{20}N_2O_8S$: C, 48.00; H, 7.30; N, 5.03. Found: C, 47.75, H, 7.55; N, 5.10.

Allyl 6β-[(Ethoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (234).

3-Chloroperoxybenzoic acid, 80 % (4.31 g, 20 mmol) was added portionwise to a cooled solution of the sulfide **223** (3.70 g, 10 mmol) in 50 mL of anhydrous dichloromethane at 0°C. The mixture was stirred at room temperature under a nitrogen atmosphere for 16 h. After addition of 50 mL of toluene, the mixture was concentrated *in vacuo* to about 50 mL. The mixture was filtered to get rid of precipitated 3-chlorobenzoic acid, and the filtrate was washed sequentially with 10 % $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution, and saturated NaCl solution, and then dried over Na_2SO_4. Solvent removal gave a residue, which was purified by silica gel column chromatography eluting with chloroform-ethyl acetate (90:10) to afford the sulfone **234** (2.30 g, 57 %) as yellow crystals: m.p. 114-115°C (recrystallized from toluene); IR ($CHCl_3$) 1815, 1755, 1730, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (3H, t, J=7Hz, CH_2CH_3), 1.40 and 1.61 (each 3H, s, C_2-$(CH_3)_2$), 3.38 (2H, s, $COCH_2CO$), 4.22 (2H, q, J=7Hz, CH_2CH_3), 4.52 (1H, s, C_3-H), 4.60-4.76 (2H, m, $OCH_2CH=CH_2$), 4.78 (1H, d, J=5Hz, C_5-H), 5.30-5.42 (2H, m, $OCH_2CH=CH_2$), 5.84-6.00 (1H, m, $OCH_2CH=CH_2$), 6.16 (1H, dd, J=5 and 10Hz, C_6-H), 8.61 (1H, d, J=10Hz, NH). Anal. Calcd for $C_{16}H_{22}N_2O_8S$: C, 47.75; H, 5.51; N, 6.96. Found: C, 47.69, H, 5.55; N, 6.90.

Allyl 6β-[(Allyloxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (235).

According to the procedure described above for **234**, the title sulfone **235** (843 mg, 41 %) was obtained from the sulfide **226** (1.91 g, 5 mmol) as colorless crystals: m.p. 104-106°C (recrystallized from toluene); IR ($CHCl_3$) 1815, 1755, 1730, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 and 1.61 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.42 (2H, s, $COC\underline{H}_2CO$), 4.52 (1H, s, C_3-$\underline{H}$), 4.64-4.72 (4H, m, $OC\underline{H}_2CH=CH_2$) ,4.78 (1H, d, J=5Hz, C_5-$\underline{H}$), 5.24-5.43 (4H, m, $OCH_2CH=C\underline{H}_2$), 5.83-5.99 (2H, m, $OCH_2C\underline{H}=CH_2$), 6.16 (1H, dd, J=5 and 10Hz, C_6-$\underline{H}$), 8.56 (1H, d, J=10Hz, N$\underline{H}$). Anal. Calcd for $C_{17}H_{22}N_2O_8S$: C, 49.27; H, 5.35; N, 6.76. Found: C, 49.38, H, 5.39; N, 6.50.

Allyl 6β-[3-(Allyloxycarbonyl)propionamido]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (236).

Using the procedure described above for **234**, the title compound **236** (328 mg, 15 %) was obtained from the sulfide **229** (1.98 g, 5 mmol) as colorless crystals: m.p. 89-90°C (recrystallized from toluene); IR ($CHCl_3$) 1810, 1755, 1735, 1695 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 and 1.62 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 2.57-2.75 (4H, m, $COC\underline{H}_2C\underline{H}_2CO$), 4.52 (1H, s, C_3-$\underline{H}$), 4.58-4.75 (4H, m, $OC\underline{H}_2CH=CH_2$) ,4.78 (1H, d, J=5Hz, C_5-$\underline{H}$), 5.20-5.45 (4H, m, $OCH_2CH=C\underline{H}_2$), 5.85-6.00 (2H, m, $OCH_2C\underline{H}=CH_2$), 6.15 (1H, dd, J=5 and 10Hz, C_6-$\underline{H}$), 6.98 (1H, d, J=10Hz, N$\underline{H}$). Anal. Calcd for $C_{18}H_{24}N_2O_8S$: C, 50.46 H, 5.65; N, 6.54. Found: C, 50.47, H, 6.01; N, 6.56.

Disodium 6β-(Carboxylatocarboxyamido)-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (237).

The title compound **237** (178 mg, 98 %) was prepared from the penam sulfone **233** (200mg, 0.5 mmol) according to the procedure described above

for **215** as a white powder: ^{1}H NMR (D_2O) δ 1.33 and 1.47 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 4.18 (1H, s, C_3-$\underline{H}$), 5.38 (1H, d, J=4Hz, C_5-$\underline{H}$), 5.83 (1H, d, J=4Hz, C_6-$\underline{H}$).

Sodium 6β-[(Ethoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (238).

Using the similar procedure described above for **215**, the title compound **238** (365 mg, 95 %) was obtained from the corresponding allyl ester **234** (402 mg, 1 mmol) as a white powder: ^{1}H NMR (D_2O) δ 1.15 (3H, t, J=7Hz, $CH_2C\underline{H}_3$), 1.32 and 1.46 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 4.03-4.16 (2H, m, $OC\underline{H}_2CH_3$), 4.12 (1H, s, C_3-$\underline{H}$), 5.09 (1H, d, J=4Hz, C_5-$\underline{H}$), 5.81 (1H, d, J=4Hz, C_6-$\underline{H}$); FAB-MS(negative) *m/z* 361 ($M-Na^+$).

Disodium 6β-(Carboxylatoacetamido)-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (239).

Using the procedure described above for **227**, the title compound **239** (183 mg, 48 %) was prepared from the corresponding ester **235** (207 mg, 0.5 mmol) as a white powder: ^{1}H NMR (D_2O) δ 1.30 and 1.45 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 4.23 (3H, m, C_3-$\underline{H}$), 5.07 (1H, d, J=4Hz, C_5-$\underline{H}$), 5.86 (1H, dd, J=4 and 8Hz, C_6-$\underline{H}$).

Disodium 6β-(3-Carboxylatopropionamido)-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (240).

According to the procedure described above for **227**, the title compound **240** (190 mg, 97 %) was obtained from the corresponding ester **236** (223 mg, 0.5 mmol) as a white powder: ^{1}H NMR (D_2O) δ 1.29 and 1.44 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 2.25-2.60 (4H, m, $COC\underline{H}_2C\underline{H}_2CO$), 4.22 (1H, s, C_3-$\underline{H}$), 5.02-5.05 (1H, m, C_5-$\underline{H}$), 5.70-5.76 (1H, m, C_6-$\underline{H}$).

Allyl 6β-[(1,3-dithiolan-2-ylidene)(methoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate (242).

Carbon disulfide (1 mL) and then sodium hydride, 60 % (160 mg, 4 mmol) were added portionwise to a stirred solution of the penam **220** (713 mg, 2 mmol) in 2 mL of sieve-dried dimethylformamide at 0°C. The reaction mixture was stirred at 0°C for 10 min under a nitrogen atmosphere, treated with dibromoethane (413 mg, 2.2 mmol), and stirred at 0°C for 30 min. The mixture was diluted with toluene, washed with water and saturated NaCl solution, and dried over Na_2SO_4. The solution was concentrated to give a brown oil, which was chromatographed on silica gel eluting with chloroform-ethyl acetate (95:5) to yield the desired olefinic product **242** (811 mg, 88 %) as a yellow foam: IR ($CHCl_3$) 1790, 1750, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50 and 1.67 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.33 (4H, s, $SC\underline{H}_2C\underline{H}_2S$), 3.88 (3H, s, $CO_2C\underline{H}_3$), 4.48 (1H, s, C_3-$\underline{H}$), 4.64-4.68 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.25-5.42 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.58 (1H, d, J=4, C_5-$\underline{H}$), 5.83 (1H, dd, J=4 and 9Hz, C_6-$\underline{H}$), 5.85-6.00 (1H, m, $OCH_2C\underline{H}=CH_2$), 8.97 (1H, d, J=9Hz, N$\underline{H}$).

Allyl 6β-[(1,3-dithiolan-2-ylidene)(ethoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate (243).

Carbon disulfide (1.5 mL), 1,2-dibromoethane (845 mg, 4.5 mmol) and sodium hydride, 60 % (240 mg, 6 mmol) were added sequentially to a solution of the penam **223** (1.11 g, 3 mmol) in 3 mL of dimethylformamide at 0°C. The reaction mixture was stirred at 0°C for 30 min under a nitrogen atmosphere. The mixture was diluted with toluene, washed with water and saturated NaCl solution, and dried over Na_2SO_4. After concentration, the residue was chromatographed on silica gel with chloroform-ethyl acetate

(95:5) as the eluant to give the desired product **243** (926 mg, 65 %) as yellow crystals: m.p. 116-117°C (recrystallized from ethyl acetate); IR ($CHCl_3$) 1790, 1750, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (3H, t, J=7Hz, $CH_2C\underline{H}_3$), 1.49 and 1.66 (each 3H, s, C_2-($C\underline{H}_3$)2), 3.32 (4H, s, $SC\underline{H}_2C\underline{H}_2S$), 4.30-4.40 (2H, m, $C\underline{H}_2CH_3$), 4.46 (1H, s, C_3-$\underline{H}$), 4.62-4.68 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.26-5.40 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.57 (1H, d, J=4, C_5-$\underline{H}$), 5.83 (1H, dd, J=4 and 9Hz, C_6-$\underline{H}$), 5.83-6.00 (1H, m, $OCH_2C\underline{H}=CH_2$), 9.05 (1H, d, J=9Hz, N$\underline{H}$). Anal. Calcd for $C_{19}H_{24}N_2O_6S_3$: C, 48.29; H, 5.12; N, 5.93. Found: C, 48.33, H, 5.00; N, 5.94.

Allyl 6β-[(allyloxycarbonyl)(1,3-dithiolan-2-ylidene)acetamido]-2,2-dimethylpenam-3-carboxylate (244).

According to the procedure described above for **242**, the title compound **244** (843 mg, 58 %) was prepared from the corresponding penam **226** (1.15 g, 3 mmol) as yellow crystals: m.p. 76-77°C (recrystallized from ethyl acetate); IR ($CHCl_3$) 1790, 1750, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 and 1.65 (each 3H, s, C_2-($C\underline{H}_3$)2), 3.33 (4H, s, $SC\underline{H}_2C\underline{H}_2S$), 4.46 (1H, s, C_3-$\underline{H}$), 4.60-4.82 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.24-5.44 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.57 (1H, d, J=4, C_5-$\underline{H}$), 5.82 (1H, dd, J=4 and 9Hz, C_6-$\underline{H}$), 5.82-6.08 (1H, m, $OCH_2C\underline{H}=CH_2$), 8.99 (1H, d, J=9Hz, N$\underline{H}$). Anal. Calcd for $C_{20}H_{24}N_2O_6S_3$: C, 49.57; H, 4.99; N, 5.78. Found: C, 49.50, H, 4.90; N, 5.81.

Sodium 6β-[(1,3-dithiolan-2-ylidene)(methoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate (245).

The allyl ester **242** (459 mg, 1 mmol) was dissolved in 0.25N sodium 2-ethylhexanoate/ethyl acetate solution (4 mL), and treated with triphenylphosphine (30 mg) and tetrakis(triphenylphosphine)palladium(0) (30 mg). The reaction mixture was stirred at room temperature for 2 h under a

nitrogen atmosphere. The mixture was filtered, and the collected precipitate was washed with ethyl acetate and diethyl ether, dried *in vacuo* to yield the desired sodium salt **245** (368 mg, 84 %) as a light yellow powder: ^{1}H NMR (D_2O) δ 1.40 and 1.50 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.25-3.45 (4H, m, $SC\underline{H}_2C\underline{H}_2S$), 3.69 (3H, s, $OC\underline{H}_3$), 4.13 (1H, s, C_3-$\underline{H}$), 5.45-5.52 (2H, m, C_5-$\underline{H}$ and C_6-$\underline{H}$); FAB-MS(negative) *m/z* 417 ($M\text{-}Na^+$).

Sodium 6β-[(1,3-dithiolan-2-ylidene)(ethoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate (246).

In a manner similar to that described above for **245**, the [illegible]pound **246** (418 mg, 92 %) was prepared from the ester **243** (473 [illegible] mmol) as a brown powder: ^{1}H NMR (D_2O) δ 1.21 (3H, t, J=7Hz, $CH_2C\underline{H}_3$), 1.41 and 1.50 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.25-3.40 (4H, m, $SC\underline{H}_2C\underline{H}_2S$), 4.12 (1H, s, C_3-$\underline{H}$), 4.10-4.20 (2H, m, $OC\underline{H}_2CH_3$), 5.45-5.55 (2H, m, C_5-$\underline{H}$ and C_6-$\underline{H}$; FAB-MS(negative) *m/z* 431 ($M\text{-}Na^+$)).

Disodium 6β-[carboxylato(1,3-dithiolan-2-ylidene)acetamido]-2,2-dimethylpenam-3-carb[illegible]late (247).

The penam este[illegible] mg, 1 mmol) was dissolved in a mixture of dimethylformamide (2 [illegible] ethyl acetate [illegible]2 mL) and 0.5N sodium 2-ethylhexanoate/ethyl acetate solution (4 mL), and treated with triphenylphosphine (30 mg) and tetrakis(triphenylphosphine)palladium(0) (30 mg). The reaction mixture was stirred at room temperature for 1 h under a nitrogen atmosphere. The mixture was filtered. The collected precipitate was washed successively with ethyl acetate and diethyl ether, dried under vacuum to afford the desired product **247** (388 mg, 87 %) as a light yellow powder: ^{1}H NMR (D_2O) δ 1.31 and 1.36 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.30-3.50

(4H, m, SCH_2CH_2S), 3.96 (1H, s, C_3-H), 5.98 (1H, d, J=5Hz, C_6-H); FAB-MS(negative) *m/z* 425 ($M-Na^+$).

Allyl 6β-[(1,3-dithiolan-2-ylidene)(ethoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (248).

According to the procedure described for **243**, the title compound **248** (535 mg, 53 %) was prepared from the penam sulfone **234** (804 mg, 2 mmol) as light yellow crystals: m.p. 150-153°C (decomp.) (recrystallized from ethyl acetate); IR ($CHCl_3$) 1810, 1755, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (3H, t, J=7Hz, CH_2CH_3), 1.39 and 1.60 (each 3H, s, C_2-$(CH_3)_2$), 3.33 (4H, s, SCH_2CH_2S), 4.30-4.43 (2H, m, CH_2CH_3), 4.51 (1H, s, C_3-H), 4.60-4.76 (2H, m, $OCH_2CH=CH_2$), 4.79 (1H, d, J=5, C_5-H), 5.30-5.42 (2H, m, $OCH_2CH=CH_2$), 5.85-6.00 (1H, m, $OCH_2CH=CH_2$), 6.31 (1H, dd, J=5 and 10Hz, C_6-H), 9.44 (1H, d, J=10Hz, NH).

Allyl 6β-[(allyloxycarbonyl)(1,3-dithiolan-2-ylidene)acetamido]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (249).

The title compound **249** (708 mg, 69 %) was obtained from the corresponding sulfone **235** (829 mg, 2 mmol) as a yellow foam, using the procedure described for **243**: IR ($CHCl_3$) 1810, 1755, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 and 1.60 (each 3H, s, C_2-$(CH_3)_2$), 3.34 (4H, s, SCH_2CH_2S), 4.51 (1H, s, C_3-H), 4.60-4.85 (5H, m, $OCH_2CH=CH_2$ and C_5-H), 5.24-5.42 (4H, m, $OCH_2CH=CH_2$), 5.85-6.08 (2H, m, $OCH_2CH=CH_2$), 6.30 (1H, dd, J=5 and 10Hz, C_6-H), 9.47 (1H, d, J=10Hz, NH). Anal. Calcd for $C_{20}H_{24}N_2O_8S_3$: C, 46.50; H, 4.68; N, 5.42. Found: C, 46.54, H, 4.54; N, 5.45.

Sodium 6β-[(1,3-dithiolan-2-ylidene)(ethoxycarbonyl)acetamido]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (250).

In a manner similar to that described above for **245**, the title compound **250** (391 mg, 80 %) was obtained from the ester **248** (505 mg, 1 mmol) as a brown powder: ^{1}H NMR (D_2O) δ 1.19 (3H, t, J=7Hz, $CH_2C\underline{H}_3$), 1.32 and 1.46 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.25-3.40 (4H, m, $SC\underline{H}_2C\underline{H}_2S$), 4.10-4.25 (2H, m, $OC\underline{H}_2CH_3$), 4.23 (1H, s, C_3-$\underline{H}$), 5.13 (1H, d, J=4Hz, C_5-$\underline{H}$), 5.99 (1H, d, J=4Hz, C_6-$\underline{H}$); FAB-MS(negative) *m/z* 463 ($M-Na^+$).

Disodium 6β-[carboxylato(1,3-dithiolan-2-ylidene)acetamido]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (251).

According to the procedure described above for **247**, the title compound **251** (433 mg, 90 %) was prepared from the ester **249** (517 mg, 1 mmol) as a light yellow powder: ^{1}H NMR (D_2O) δ 1.30 and 1.45 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.18-3.30 (4H, m, $SC\underline{H}_2C\underline{H}_2S$), 4.21 (1H, s, C_3-$\underline{H}$), 5.09 (1H, d, J=4Hz, C_5-$\underline{H}$), 5.98 (1H, d, J=4Hz, C_6-$\underline{H}$).

Allyl 3-Formylpropionate (254).

A 2N aqueous NaOH solution (50 mL) was added dropwise to a solution of butyrolactone (8.61 g, 100 mmol) in 100 mL of methanol at 0°C. After stirring at room temperature for 1 h, the solution was concentrated *in vacuo*. The resulting white solid was suspended in 100 mL of anhydrous dimethylformamide and treated with allyl bromide (13.3 g, 110 mmol). The mixture was stirred at room temperature over night. At the end of the reaction, the mixture became a clear solution. The solution was concentrated under reduced pressure to about half of the original volume, and diluted with a mixture of toluene and ethyl acetate. The mixture was washed with water several times and then with saturated NaCl solution, dried over Na_2SO_4, and

evaporated to give the crude alcohol **253** (10.75g), which was used for the next reaction without further purification.

Oxalyl chloride (12.7 g, 100 mmol) was added to a mixture of anhydrous dimethylsulfoxide (15.6 g, 200 mmol) and dichloromethane (200 mL) at 0°C, and the resulting solution was stirred at 0°C for 15 min. A solution of the crude alcohol, obtained above, in 20 mL of dichloromethane was added to the mixture, and the resulting solution was stirred at 0°C for 15 min under a nitrogen atmosphere. Finally, triethylamine (20.2 g, 200 mmol) was added, and the reaction mixture was stirred at 0°C for 30 min. The mixture was washed with water and saturated NaCl solution, dried over Na_2SO_4, and concentrated to give an oil, which was chromatographed on silica gel with chloroform as the eluant, to afford the desired aldehyde **254** (8.98 g, 63 %) as a yellow oil: ^1H NMR ($CDCl_3$) δ 2.55-2.85 (4H, m, $C\underline{H}_2C\underline{H}_2$), 4.50-4.65 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.16-5.34 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.80-6.00 (1H, m, $OCH_2C\underline{H}=CH_2$), 9.81 (1H, s, $C\underline{H}O$).

Allyl 6β-[3-(Allyloxycarbonyl)-1-hydroxypropyl]-2,2-dimethylpenam-3-carboxylate (257).

The dibromopenam **255** (3.99 g, 10 mmol) was dissolved in 25 mL of anhydrous tetrahydrofuran and cooled to -78°C. 3M Methylmagnesium bromide in ether (3.5 mL, 10.5 mmol) was added and the mixture was stirred at -78°C for 10 min. A solution of the aldehyde **254** (1.71 g, 12 mmol) in 5 mL of tetrahydrofuran was added and stirring was continued at -78°C for 30 min. The reaction was quenched with saturated NH_4Cl solution, and extracted with ethyl acetate. The organic layer was washed with water and saturated NaCl solution, dried over Na_2SO_4, and evaporated to give an oil, which was purified using a short silica gel column and eluting with

chloroform-ethyl acetate (80:20) to give a stereoisomeric mixture of bromohydrins **256** (3.26 g).

The obtained mixture of bromohydrins **256** was dissolved in 30 mL of anhydrous benzene, and treated with tributyltin hydride (2.91 g, 10 mmol) and catalytic amount of AIBN. The reaction mixture was heated to reflux under a nitrogen atmosphere for 30 min. After cooling, the mixture was concentrated, and the residue was chromatographed on silica gel using chloroform-ethyl acetate (70:30) as the eluting solvent to yield the desired alcohol **257** (1.54 g, 40 %) as a colorless oil: IR ($CHCl_3$) 1770, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 and 1.66 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 1.76-1.92 (2H, m, C_6-$CHC\underline{H}_2CH_2$), 2.44-2.65 (2H, m, C_6-$CHCH_2C\underline{H}_2$), 3.54 (1H, dd, J=7 and 13Hz, C_6-$\underline{H}$), 4.12-4.24 (1H, m, C_6-$C\underline{H}$), 4.43 (1H, s, C_3-$\underline{H}$), 4.55-4.70 (4H, m, $OC\underline{H}_2CH=CH_2$), 4.79 (1H, d, J=5, C_5-$\underline{H}$), 5.20-5.46 (5H, m, $OCH_2CH=C\underline{H}_2$ and C_5-H), 5.85-6.00 (2H, m, $OCH_2C\underline{H}=CH_2$).

Allyl 6β-[3-(Allyloxycarbonyl)-1-hydroxypropyl]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (258).

3-Chloroperoxybenzoic acid, 80 % (431 mg, 2 mmol) was added to a solution of the sulfide **257** (767 mg, 2 mmol) in 10 mL of anhydrous dichloromethane at 0°C and the reaction mixture was stirred at room temperature over night. The mixture was filtered to eliminate the precipitated 3-chlorobenzoic acid and the filtrate washed sequentially with 10 % $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution and saturated NaCl solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was chromatographed on silica gel with chloroform-ethyl acetate (70:30) as the eluant to afford the sulfone **258** (438 mg, 53 %) as colorless crystals: m.p. 79-83°C (recrystallized from toluene);

IR ($CHCl_3$) 1800, 1755, 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37 and 1.56 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 1.75-1.95 (2H, m, C_6-$CHC\underline{H}_2CH_2$), 2.42-2.68 (2H, m, C_6-$CHCH_2C\underline{H}_2$), 3.02 (1H, s, O<u>H</u>), 3.80 (1H, dd, J=5 and 9Hz, C_6-<u>H</u>), 4.45 (1H, s, C_3-<u>H</u>), 4.50-4.80 (6H, m, $OC\underline{H}_2CH{=}CH_2$ and C_5-<u>H</u> and C_6-C<u>H</u>), 5.17-5.42 (4H, m, $OCH_2CH{=}C\underline{H}_2$), 5.85-6.00 (2H, m, $OCH_2C\underline{H}{=}CH_2$). Anal. Calcd for $C_{18}H_{25}NO_8S$: C, 52.04; H, 6.06; N, 3.37. Found: C, 51.64, H, 5.76; N, 3.40.

Disodium 6β-[3-Carboxylato-1-hydroxypropyl]-2,2-dimethylpenam-3-carboxylate (259).

Triphenylphosphine (30 mg) and tetrakis(triphenylphosphine)-palladium(0) (30 mg) were added to a mixture of the diallyl ester **257** (383 mg, 1 mmol), ethyl acetate (4 mL) and a 0.5N sodium 2-ethylhexanoate /ethyl acetate solution (4 mL). The reaction mixture was stirred at room temperature for 30 min under a nitrogen atmosphere. Diethyl ether (20 mL) was then added to the mixture slowly over the period of 30 min. The resulting precipitate was filtered, and washed with diethyl ether to give the desired disodium salt **259** (285 mg, 82 %) as a yellow powder: ^1H NMR (D_2O) δ 1.39 and 1.53 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 1.90-2.45 (4H, m, C_6-$CHC\underline{H}_2C\underline{H}_2$), 3.58 (1H, dd, J=4 and 10Hz, C_6-<u>H</u>), 4.10 (1H, s, C_3-<u>H</u>), 5.31 (1H, d, J=4Hz, C_5-<u>H</u>).

Disodium 6β-[3-Carboxylato-1-hydroxypropyl]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (260).

According to the procedure described above for **259**, the title compound **260** (172 mg, 91 %) was obtained from the sulfone **258** (208 mg, 0.5 mmol) as a yellow powder: ^1H NMR (D_2O) δ 1.29 and 1.42 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 1.55-1.70 (2H, m, C_6-$CHC\underline{H}_2CH_2$), 2.10-2.30 (2H, m, C_6-

CHCH2CH2), 3.02 (1H, s, OH), 3.87 (1H, dd, J=5 and 10Hz, C6-H), 4.13 (1H, s, C3-H), 4.36-4.45 (1H, m, C6-CH), 4.98 (1H, d, J=5Hz, C5-H); FAB-MS(negative) *m/z* 356 ($M-Na^+$).

Allyl 6-[3-(Allyloxycarbonyl)propylidene]-2,2-dimethylpenam-3-carboxylate (262).

The dibromopenam **255** (7.98 g, 20 mmol) was dissolved in 50 mL of anhydrous tetrahydrofuran and cooled to -78°C. 3M Methylmagnesium bromide in ether (7 mL) was added, and the mixture was stirred at -78°C for 10 min. A solution of the aldehyde **254** (3.42 g, 24 mmol) in 10 mL of tetrahydrofuran was added and stirring was continued at -78°C for 30 min. Acetic anhydride (2.45 g, 24 mmol) was added and the reaction mixture was gradually warmed to room temperature. The mixture was diluted with toluene, washed with saturated NH4Cl solution and saturated NaCl solution, and dried over Na2SO4. Concentration of the extract gave the crude acetoxybromopenam **261**, which was used for the next reaction without further purification.

The crude **261** was dissolved in 50 mL of tetrahydrofuran and added dropwise to a suspension of zinc (2.61 g, 40 mmol) in 50 mL of 1N NH4Cl solution at 0°C. The reaction mixture was stirred at 0°C for 1 h. After passing through celite, the organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with saturated NH4Cl solution and saturated NaCl solution. The extract was dried over Na2SO4 and evaporated *in vacuo* to give a brown oil, which was chromatographed on silica gel, eluting with chloroform-ethyl acetate (95:5) to yield the desired olefinic product **262** (1.52 g, 21 %) as a colorless oil: IR ($CHCl_3$) 1770, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 and 1.58 (each 3H, s,

C_2-($C\underline{H}_3$)$_2$), 2.42-2.60 (4H, m, C_6=CHC$\underline{H}_2C\underline{H}_2$), 4.49 (1H, s, C_3-$\underline{H}$), 4.56-4.70 (4H, m, $OC\underline{H}_2CH=CH_2$), 5.20-5.45 (4H, m, $OCH_2CH=C\underline{H}_2$), 5.84-6.00 (2H, m, $OCH_2C\underline{H}=CH_2$), 6.25 (1H, t, J=7Hz, C_6=C$\underline{H}$).

Allyl 6-[3-(Allyloxycarbonyl)propylidene]-2,2-dimethylpenam-3-carboxylate 1,1-Dioxide (263).

In a manner similar to that described above for 258, the title sulfone **263** (241 mg, 61 %) was prepared from the sulfide 262 (73[illegible] mg, 2 mmol) as a brown oil: IR ($CHCl_3$) 1795, 1740 cm^{-1}; ^1H NMR ($C[illegible]_3$) δ 1.44 and 1.59 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 2.50-2.70 (4H, m, C_6=CHC$\underline{H}_2C\underline{H}_2$), 4.43 (1H, s, C_3-$\underline{H}$), 4.55-4.75 (4H, m, $OC\underline{H}_2CH=CH_2$), 5.20-5.45 (5H, m, $OCH_2CH=C\underline{H}_2$ and C_5-$\underline{H}$), 5.85-6.00 (2H, m, $OCH_2C\underline{H}=CH_2$), 6.66 (1H, t, J=7Hz, C_6=C$\underline{H}$).

Disodium 6-[3-Carboxylatopropylidene]-2,2-dimethylpenam-3-carboxylate (264).

The title compound **264** (183 mg, 56 %) was obtained from the ester **262** (365 mg, 1 mmol) as a yellow powder, using the procedure described for **259**: ^1H NMR (D_2O) δ 1.40 and 1.47 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 2.18-2.40 (4H, m, C_6=CHC$\underline{H}_2C\underline{H}_2$), 4.13 (1H, s, C_3-$\underline{H}$), 5.73 (2H, s, C_5-$\underline{H}$), 6.29 (1H, t, J=7Hz, C_6=C$\underline{H}$).

Disodium 6-[3-Carboxylatopropylidene]-2,2-dimethylpenam-3-carboxylate 1,1-dioxide (265).

The title compound **265** (295 mg, 82 %) was prepared from the ester **263** (397 mg, 1 mmol) as a yellow powder, using the procedure described for **259**: ^1H NMR (D_2O) δ 1.38 and 1.48 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 2.22-2.45 (4H, m, C_6=CHC$\underline{H}_2C\underline{H}_2$), 4.11 (1H, s, C_3-$\underline{H}$), 5.59 (1H, m, C_5-$\underline{H}$), 6.73 (1H, t, J=7Hz, C_6=C$\underline{H}$); FAB-MS(negative) *m/z* 338 ($M-Na^+$).

Methyl 3-Acetoxymethyl-7β-[(allyloxycarbonyl)acetamido]ceph-3-em-4-carboxylate (269).

To a solution of the carboxylic acid **202** (1.99 g, 5 mmol) and methanol (1mL) in anhydrous dichloromethane was added 1,3-dicyclohexyl-carbodiimide (1.24 g, 6 mmol) portionwise. The reaction mixture was stirred at room temperature for 16 h. The mixture was filtered and the filtrate was concentrated to give an oil, which was chromatographed on silica gel with chloroform as the eluting solvent to yield the desired methyl ester **269** (1.84 g, 89 %) as a brown oil: ^{1}H NMR ($CDCl_3$) δ 2.07 (3H, s, $OCOC\underline{H}_3$), 3.39 and 3.57 (each 1H, d, J=19Hz, C_2-$C\underline{H}_a\underline{H}_b$), 3.42 (2H, s, $COC\underline{H}_2CO$), 3.86 (3H, s, $OC\underline{H}_3$), 4.57 and 4.71 (2H, m, $OC\underline{H}_2CH=CH_2$), 4.82 and 5.09 (each 1H, d, J=13Hz, C_3-$C\underline{H}_a\underline{H}_b$-O), 5.00 (1H, d, J=5Hz, C_6-$C\underline{H}$), 5.25-5.37 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.80-5.98 (2H, m, $OCH_2C\underline{H}=CH_2$ and C_7-$\underline{H}$), 7.99 (1H, d, J=9Hz, $N\underline{H}$). Anal. Calcd for $C_{17}H_{20}N_2O_8S$: C, 49.51; H, 4.89; N, 6.79. Found: C, 49.64, H, 5.00; N, 7.17.

Sodium 3-Acetoxymethyl-7β-[(allyloxycarbonyl)acetamido]ceph-3-em-4-carboxylate (270).

The allyl ester **269** (412 mg, 1 mmol) was dissolved in 2 ml of 0.5N sodium 2-ethylhexanoate/ethyl acetate solution and additional 2 mL of ethyl acetate, and treated with triphenylphosphine (30 mg) and tetrakis-(triphenylphosphine)palladium(0) (30 mg). The reaction mixture was stirred at room temperature for 30 min. To this mixture 10 mL of diethyl ether was added slowly. The precipitation of solids was observed. The mixture was filtered to collect the resulting precipitate and the collected precipitate was washed with diethyl ether and dried under vacuum to afford the desired sodium salt **270** (376 mg, 95 %) as a yellow powder: ^{1}H NMR (D_2O) δ 2.00

(3H, s, OCO$C\underline{H}_3$), 3.44 and 3.62 (each 1H, d, J=19Hz, C_2-$C\underline{H}_a\underline{H}_b$), 3.78 (3H, s, $OC\underline{H}_3$), 4.77 and 4.93 (each 1H, d, J=13Hz, C_3-$C\underline{H}_a\underline{H}_b$-O), 5.07 (1H, d, J=5Hz, C_6-C$\underline{H}$), 5.64 (1H, d, J=5Hz, C_7-$\underline{H}$).

_N_-Allyloxycarbonylmethyl-6,6-dibromo-2,2-dimethylpenam-3-carboxyamide (272).

6,6-Dibromopenicillanic acid (**271**) (18.0 g, 50 mmol) and glycine allyl ester (5.76 g, 50 mmol) were dissolved in 100 mL of anhydrous tetrahydrofuran and treated with 1,3-dicyclohexylcarbodiimide (10.3 g, 50 mmol). The reaction mixture was stirred at room temperature for 3 h under a nitrogen atmosphere. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The resulting residue was chromatographed on silica gel with chloroform as the eluant to give the desired amide **272** (8.34 g, 18 %) as a brown oil: IR ($CHCl_3$) 1810, 1750, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.53 and 1.69 (each 3H, s, C_2-($C\underline{H}_3$)2), 3.93-4.21 (2H, m, NH$C\underline{H}_2$), 4.37 (1H, s, C_3-$\underline{H}$), 4.60-4.65 (2H, m, $OC\underline{H}_2CH{=}CH_2$), 5.22-5.36 (2H, m, $OCH_2CH{=}C\underline{H}_2$), 5.69 (1H, s, C_5-$\underline{H}$), 5.80-5.95 (1H, m, $OCH_2C\underline{H}{=}CH_2$), 6.80 (1H, brs, N$\underline{H}$).

_N_-Allyloxycarbonylmethyl-6,6-dibromo-2,2-dimethylpenam-3-carboxyamide 1,1-Dioxide (273).

3-Chloroperoxybenzoic acid, 80 % (4.75 g, 22 mmol) was added portionwise to a cooled solution of the sulfide **272** (5.0 g, 11.0 mmol) in 30 mL of anhydrous dichloromethane. The reaction mixture was stirred at room temperature for 4 h under a nitrogen atmosphere, then filtered to get rid of the precipitated 3-chlorobenzoic acid. The filtrate was washed sequentially with 10 % of $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution, saturated NaCl solution, and dried over Na_2SO_4. Evaporation of the solvent gave an oil,

which was purified by silica gel column chromatography using chloroform-ethyl acetate (95:5) as the eluant to give the desired sulfone **273** (3.20 g, 60 %) as a colorless oil: IR ($CHCl_3$) 1830, 1750, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.46 and 1.68 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.80-4.32 (2H, m, $NHC\underline{H}_2$), 4.38 (1H, s, C_3-$\underline{H}$), 4.64-4.67 (2H, m, $OC\underline{H}_2CH=CH_2$), 5.05 (1H, s, C_5-$\underline{H}$), 5.26-5.38 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.83-5.98 (1H, m, $OCH_2C\underline{H}=CH_2$), 6.86 (1H, brs, $N\underline{H}$).

_N_-Allyloxycarbonylmethyl-2,2-dimethylpenam-3-carboxyamide 1,1-Dioxide (274).

A solution of the dibromopenam **273** (3.0 g, 6.15 mmol) in 20 mL of tetrahydrofuran was added to a suspension of zinc (1.61 g, 24.6 mmol) in 20 mL of 1N NH_4Cl solution at 0°C. The two phase mixture was stirred at room temperature for 30 min, and filtered through a packed celite column. The filtrate was extracted with ethyl acetate three times. The combined organic layer was washed with saturated NH_4Cl solution and saturated NaCl solution, dried over Na_2SO_4, and concentrated *in vacuo* to give an oil, which was chromatographed on silica gel eluting with chloroform-ethyl acetate (80:20) to afford the desired reduction product **274** (1.39 g, 68 %) as a colorless viscous oil: IR ($CHCl_3$) 1815, 1750, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 and 1.62 (each 3H, s, C_2-($C\underline{H}_3$)$_2$), 3.40-3.55 (2H, m, C_6-$\underline{H}_a\underline{H}_b$), 3.83-4.24 (2H, m, $NHC\underline{H}_2$), 4.19 (1H, s, C_3-$\underline{H}$), 4.59-4.65 (3H, m, $OC\underline{H}_2CH=CH_2$ and C_5-$\underline{H}$), 5.20-5.35 (2H, m, $OCH_2CH=C\underline{H}_2$), 5.79-5.93 (1H, m, $OCH_2C\underline{H}=CH_2$), 7.09-7.13 (1H, brs, $N\underline{H}$).

Sodium *N*-Carboxylatomethyl-2,2-dimethylpenam-3-carboxyamide (275).

The allyl ester **274** (330 mg, 1 mmol) was dissolved in 2 ml of 0.5N sodium 2-ethylhexanoate/ethyl acetate solution, and treated with triphenylphosphine (30 mg) and tetrakis(triphenylphosphine)palladium(0). The reaction mixture was stirred at room temperature for 30 min under a nitrogen atmosphere and filtered to collect the resulting precipitate. The collected precipitate was washed with ethyl acetate, then with diethyl ether and dried under vacuum to afford the desired sodium salt **275** (264 mg, 85 %) as a yellow powder: ^{1}H NMR (D_2O) δ 1.31 and 1.45 (3H, s, C_2-($C\underline{H}_3$)$_2$), 3.28-3.70 (2H, m, C_6-$\underline{H}_a\underline{H}_b$), 3.65 (2H, s, $C\underline{H}_2CO_2Na$), 4.27 (1H, s, C_3-$\underline{H}$), 4.92-5.02 (1H, m, C_5-$\underline{H}$); FAB-MS(negative) *m/z* 289 ($M\text{-}Na^+$).

5.3 Biological section

5.3.1 *In Vitro* β-Lactamase Inhibitory Assays

The following three enzymes were selected for testing: (1) Penicillinase (penicillinase type I from *Bacillus cereus*, purchased from Sigma). (2) Cephalosporinase (penicillinase type IV from *Enterobacter cloacae*, purchased from Sigma). (3) Broad spectrum TEM enzyme (purchased from Boehringer).

The IC_{50} values were determined using the following spectroscopic method: 2.875 mL of a phosphate buffer (pH 7.0), 0.015 mL of enzyme/buffer solution, and 0.050 mL of inhibitor/buffer solution were introduced into a 3 mL cuvette (quartz). The mixture was incubated at 30°C for 10 min, and 0.060 mL of substrate/buffer solution then added. The rate of decrease in absorption (A/min) at 233 nm (for penicillin G) or 260 nm (for cephaloridine) was measured over 130 sec. The data obtained between 10

and 130 sec were used for the calculation. The amount of enzyme and the final concentration of substrate are listed in Table 5-1. The inhibitor was added in a range of concentrations(x), including zero as a control. Inhibition (I) was calculated by the equation; $I=100x(A_0-A_x)/A_0$, where A_0 and A_x represent the rate of decrease in absorption with inhibitor concentration 0 and x respectively. Inhibition (I) was plotted against inhibitor concentration (x), and from the resulting calibration curve, the IC_{50} value was determined. As a representative example, the calibration curve used for the calculation of IC_{50} value of **247** against the cephalosporinase is shown in the appendix at the end of the thesis. The upper limit of inhibitor concentration was fixed at 100 μM, which is almost the same concentration as the substrates.

Table 5-1 The amount of enzyme and the concentration of substrate.

Enzyme	Amount	Substrate	Concentration
Penicillinase	0.2 Unit*	Penicillin G	0.2 mM
Cephalosporinase	0.003 Unit*	Cephaloridine	0.1 mM
TEM enzyme	0.14 Unit*	Penicillin G	0.2 mM

* 1 Unit of enzyme hydrolyzes 1 μmole of substrate per minute at pH 7.0 at 25°C.

5.3.2 Antimicrobial Activities and Synergistic Effect

Antimicrobial activities (MIC values) were determined by the microbroth dilution assay as follows.

(1) Preparation of antibiotic solution. Mueller Hinton (M.H.) Broth was prepared and sterilized according to the manufacturer's directions. A series of M.H.Broth samples (100 μL) containing decreasing concentrations of β-lactam antibiotics (256-2.0 μg/mL) and fixed concentrations of β-lactamase

inhibitor (0 or 20 μg/mL) were prepared by the serial two fold dilution method.

(2) Preparation of inocula. Microbes of interest were taken from a frozen stock culture and streaked on Blood Agar Plates (tryptic soy agar with 5% sheep blood, purchased from PML microbiologicals). After overnight incubation, a few isolated colonies were picked up from the plate and suspended in 0.9% saline. The turbidity of the suspension was controlled by means of spectroscopic absorption at 600 nm to 0.1-0.11. This turbidity corresponds to approximately 10^8 CFU/mL of microbes. The resulting suspension was diluted 100 fold with M.H.Broth to make the 10^6 CFU/mL inoculum.

(3) Determination of MICs. The inoculum (100 μL) of each microbe was dispensed in a series of antibiotic/inhibitor solutions (100 μL). The final concentration was 128-1 μg/ml (a common concentration range for MIC determination) for antibiotics and 0 or 10 μg/mL for inhibitors. The mixtures were mixed gently and incubated at 35°C for 16 h. The lowest concentration at which the microbe failed to grow was recorded as the MIC (minimum inhibitory concentration) value.

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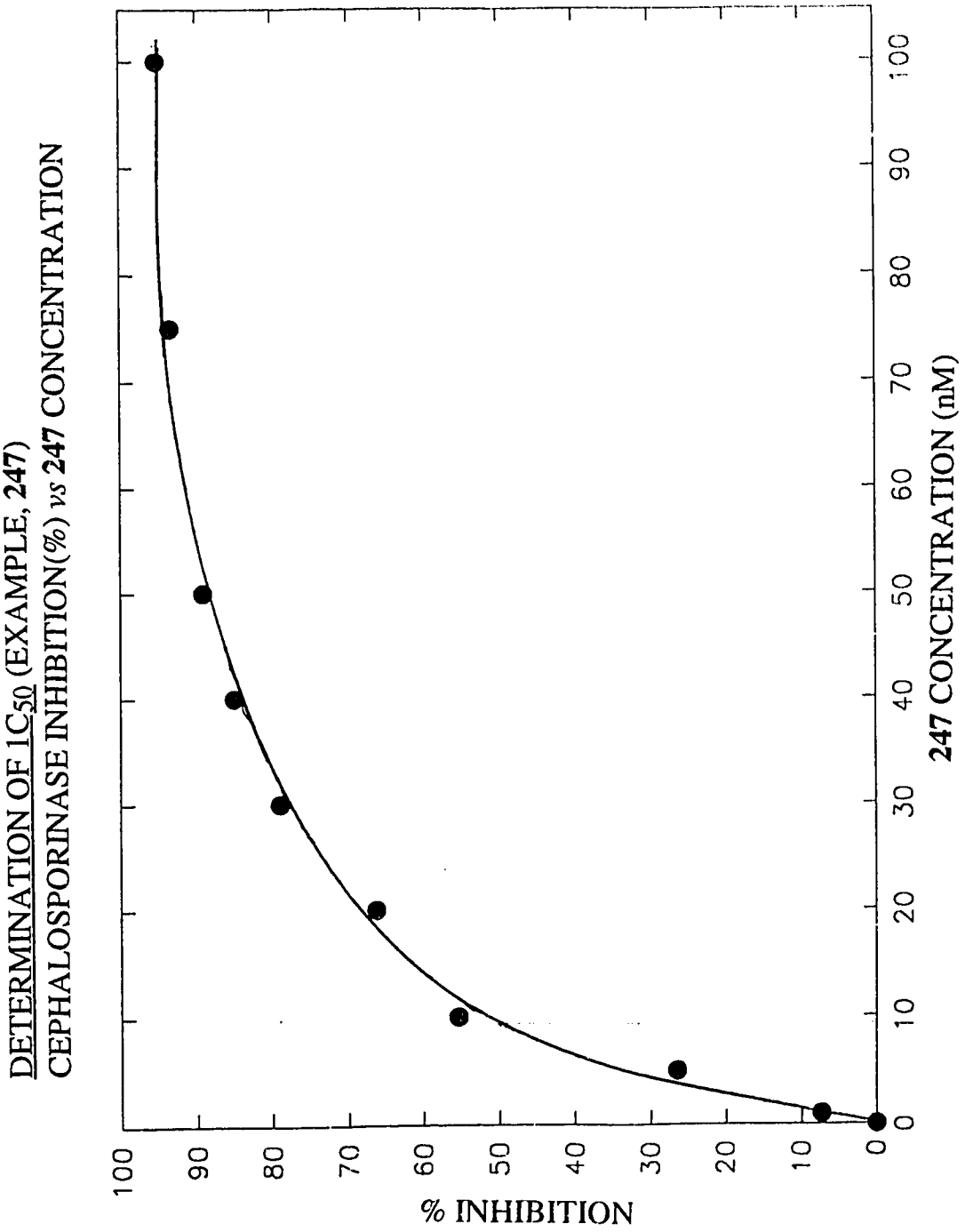
APPENDIX


DETERMINATION OF IC_{50} (EXAMPLE, 247)
CEPHALOSPORINASE INHIBITION(%) *vs* 247 CONCENTRATION
% INHIBITION
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
247 CONCENTRATION (nM)