AstraZeneca

Dockets Management Branch
WA-105
Food and Drug Administration
5630 Fishers Lane
Rm. 1061
Rockville, MD 20852

Re: **Docket No. 99N-3088**
RIN 0910-AB33
Marketing Exclusivity and Patent Provisions for Certain Antibiotic Drugs

Dear Sir or Madam:

Reference is made to the FDA's proposed rule, published **in the** Federal Register **of** January 24, 2000, to exempt marketing applications for certain antibiotic drug products from the regulatory provisions governing marketing exclusivity, The proposal applies to all marketing applications for drug products containing an antibiotic received by the FDA before November 21, 1997, the effective date of the Food and Drug Administration Modernization Act of 1997.

Reference is also made to our January 24, 2001 submission to the above referenced Docket supporting our position that MERREM® I.V. (meropenem for injection), NDA No. 50-706, should be classified as an "anti-infective" and not an antibiotic as defined previously by Section 507 [357](a) of the Federal Food, Drug and Cosmetic Act.

Finally, reference is made to a February 14, 2001 telephone conversation between the FDA's Ms. Frances Turner and the undersigned, during which Ms. Turner notified AstraZeneca that our January 24, 2001 submission could not be released to the public because of the confidentially statement included in the cover letter,

The purpose of this submission is to notify the Dockets Management Branch that our January 24, 200 1 submission can be released to the public. An additional copy of this submission is attached for your convenience.

US Regulatory Affairs
AstraZeneca LP
1800 Concord Pike PO Box 8355 Wilmington DE 19803-8355

AZ0010 (8/00)

99N-3088

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AstraZeneca

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Dockets Management Branch
HFA-305
Food and Drug Administration
5630 Fishers Lane
Rm. 1061
Rockville, MD 20852

JAN 24 2001

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Re: Docket No. 99N-3088
RIN 0910-AB33
Marketing Exclusivity and Patent Provisions for Certain Antibiotic Drugs

Dear Sir or Madam:

Reference is made to the FDA's proposed rule published in the Federal Register of January 24, 2000, to exempt marketing applications for certain antibiotic drug products from the regulatory provisions governing marketing exclusivity. The proposal would apply to marketing applications for drug products containing an antibiotic drug that was subject of a marketing application received by the FDA before November 21, 1997, the effective date of the Food and Drug Administration Modernization Act of 1997.

Reference is also made to a January 22, 2001 telephone conversation between the FDA's Mr. Wayne Mitchell and the undersigned regarding a submission that AstraZeneca made to the FDA's Division of Anti-Infective Drug Products on January 22, 1999. In that submission, AstraZeneca Pharmaceuticals LP indicated that our drug product, MERREM® I.V. (meropenem for injection), NDA No. 50-706, should be classified as an "anti-infective" and not an antibiotic as defined previously by Section 507 [357](a) of the Federal Food, Drug and Cosmetic Act. Mr. Mitchell indicated that FDA is in the process of finalizing the proposed rule, and he requested that AstraZeneca submit a copy of our January 22, 1999 document to the above-referenced Docket for consideration as soon as possible.

Accordingly, attached is a copy of our January 22, 1999 submission to the FDA's Division of Anti-Infective Drug Products regarding the classification of MERREM® I.V. As noted in the document cover letter, microorganisms do not produce the structural component of the active ingredient of MERREM® I.V. that imparts the capacity to inhibit or destroy microorganisms. The synthesis of meropenem, and all intermediates in the synthetic pathway, cannot be manufactured by fermentation and is described in the MERREM® I.V., NDA No. 50-706 and in Sumitomo's DMF #10322. The January 22, 1999 submission also provided a review article by S. Coulton and E. Hunt (Progress in Medicinal Chemistry 1996; 33:99-145) that discusses the chemistry and biology of carbapenems. In this article, meropenem is characterized as a totally synthetic non-natural carbapenem, providing further evidence that MERREM® I.V. does not meet the strict definition of an antibiotic.

US Regulatory Affairs
AstraZeneca Pharmaceuticals LP
1800 Concord Pike PO Box 8355 Wilmington DE 1985043355

AZ0010 (8/00)

99N-3088

The confidentiality of this submission, and all information contained herein, is claimed by AstraZeneca under all applicable laws and regulations. Disclosure of any such information is not authorized without the prior written authorization of AstraZeneca.

I trust this information is helpful. If you have any questions or comments, please do not hesitate to contact me, or in my absence, Ms. Darci Bertelsen at (302) 886-7355.

Sincerely,

Barry D. Sickels
Director, Regulatory Affairs
(302) 886-5895
(302) 886-2822 (fax)

BDS/DLB/mrsc
Enclosure

ZENECA
Pharmaceuticals
A Business Unit of Zeneca Inc.

1800 Concord Pike
PO Box 15437
Wilmington, DE 19850-5437

SENT VIA UNITED PARCEL SERVICE

JAN 2 2 1999

Gary K. Chikami, M.D.
Director
Division of Anti-Infective
Drug Products
Office of Drug Evaluation IV
Center for Drug Evaluation and Research
Food and Drug Administration
HFD No. 520, Document Control Room
920 1 Corporate Boulevard
Rockville, MD 20850

Dear Dr. Chikami:

Re: MERREM® I.V. (meropenem for injection)
NDA SO-706
Request for Drug Classification

Zeneca is writing in regards to an unresolved issue from 1993. At the request of Zeneca Pharmaceuticals (ZENECA), FDA pre-assigned an NDA number to the MERREM? I.V. (meropenem for injection) NDA on May 7, 1993. The MERREM I.V. NDA was inadvertently assigned a 50-series number (NDA 50-706). The original NDA for MERREM I.V. was submitted on October 28, 1993 by ZENECA under section 505(b) of the Federal Food, Drug and Cosmetic Act.

During a conversation initiated by Dr. Kathleen A. Creedon, (Microbiologist, Division of Anti-Infective Drug Products) on November 3, 1993, she noted that the NDA was submitted under section 505. Dr. Creedon commented that the NDA number was incorrectly cited since 50-series numbers are reserved for antibiotics, as defined by section 507.[357](a) of the Act, and that drugs which are submitted under section 505 should be designated with 20-series NDA numbers. In the November 4, 1993 telephone conversation between Dr. Creedon and ZENECA, Dr. Creedon stated if MERREM I.V. was submitted under section 505 it should be referred to as an anti-infective, and that the NDA number for MERREM I.V. could be reassigned to the 20-series numbers upon the submission of documentation which proved that MERREM I.V. does not fit the definition of an antibiotic.

Section 507.[357](a) of the Federal Food, Drug and Cosmetic Act defined an antibiotic as: "*any drug (except drugs for use in animals other than humans) composed wholly or partly of any kind of penicillin, streptomycin, chlortetracycline, chloramphenicol, bacitracin, or any other drug intendedfor use by man containing any quantify of any chemical substance which is produced by a micro-organism and which has fhe capacity to inhibit or destroy micro-organisms in dilute solution (including the chemically synthesized equivalent of any such substance) or any derivative thereof.*" ZENECA is aware that section 507 has been repealed; however, the repeal of this section did not change the definition for antibiotic classification (see FDA Guidance for Industry and Reviewers: Repeal of section 507 of the Federal Food, Drug and Cosmetic Act [May 1998] referring to new section 201(j)(j) of the Act).

The structural component of the active ingredient of MERREM I.V. that imparts the capacity to inhibit or destroy micro-organisms is not produced by micro-organisms. The synthesis of meropenem, along with all intermediates in the synthetic pathway, cannot be manufactured by fermentation and is described in the MERREM I.V. NDA 50-706 and in the Sumitomo meropenem Drug Master File (DMF #10322). In addition, the attached review article written by S. Coulton discusses the chemistry and biology of carbapenems. In this article meropenem is characterized as a totally synthetic non-natural carbapenem. These documents provide evidence that MERREM I.V. does not meet the strict definition to be classified as an antibiotic.

Correspondence to FDA, dated December 3, 1993 requested that the MERREM I.V. NDA number be reassigned to a non 50-series NDA number, and the NDA be filed under section 505(b). Subsequently, the NDA was filed by FDA under section 507, and no further communication regarding this issue has been received.

By means of this correspondence, ZENECA again respectfully requests that the MERREM I.V. NDA be re-assigned a 20-series NDA number and be classified as an anti-infective drug.

We will be contacting the FDA Project Manager shortly to discuss this matter. If you have any questions *or* comments, please do not hesitate to contact me.

Sincerely,

Gerald L. Limp
Manager, Marketed Products Group
Drug Regulatory Affairs Department
(302) 886-8017
(302) 886-2822 (fax)

GLL/DLB/jr
Enclosure

Desk Copy: Ms. Maureen P. Dillon-Parker, HFD No. 520

AN 43521

Progress in Medicinal Chemistry 1996;33:99-145
Editors: Ellis GP & Luscombe DK

Progress in Medicinal Chemistry – Vol. 33. edited by G.P. Ellis and D.K. Luscombe
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3 Recent Advances in the Chemistry and Biology of Carbapenem Antibiotics

S. COULTON, Ph.D., C.Chem., F.R.S.C. and
E. HUNT, Ph.D., C.Chem.F.R.S.C

Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey, RX3 7AJ, U.K.

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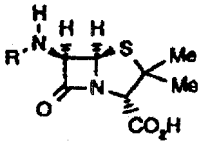
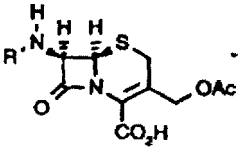
INTRODUCTION

The discovery of the antibacterial properties of a culture of the fungus *Penicillium notatum* by Alexander Fleming in 1929 [1] was a milestone in modem medicine. However, it was not until 1940 that a research team at Oxford University. kd by Abraham, Chain and Florey, isolated penicillin G (I) in pure form and identified its immense potential for the treatment of a wide range of life threatening bacterial infections [2].



(1) R = $PhCH_2CO$; Penicillin G
(2) R = H; 6-APA



(3) R = $H_2NCH(CO_2H)CH_2CH_2CH_2CO$; Cephalosporin C

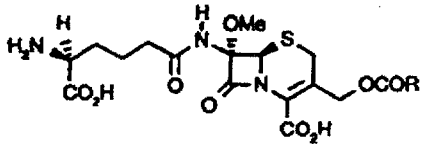
(4) R = H: 7-ACA

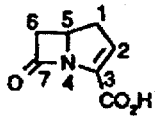
Antibacterial chemotherapy received a further impetus in 1959, when Beecham scientists recognized 6-aminopenicillanic acid (2) (6-APA) as a precursor of the bioauive penicillins during the fermentation of *Penicillium chrysogenum* [3]. These results prompted the production of 6-APA on a large scale, principally by the enzymatic deacylation of penicillin G. Chemical reacylation of 6-APA then led to the preparation of many new penicillins and the clinical use of compounds such as methicillin, ampicillin, amoxycillin, carbenicillin, ticarcillin, and flucloxacillin [4].

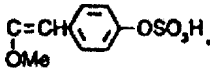
The successful development of semi-synthetic 6-APA derivatives also prompted considerable effort in cephalosporin research. Cephalosporin C (3) had been isolated by Abraham and colleagues from a strain of *Cephalosporium acremonium* supplied by Professor Brotzu [5]. Prior to the discovery of 6-APA, Brotzu had suggested that from micro-organisms associated with sewage outflows, one could expect to obtain compounds antagonistic to sewage bacteria; *Cephalosporium acremonium* was indeed such an organism. Chemical deacylation of cephalosporin C, followed by reacylation of the cephalosporin nucleus, 7-ACA (4), led to the clinical development of antibiotics such as cephalothin, cephaloridine and cephalexin, the so-called first generation cephalosporins [6, 7].

By the mid-1960's, with the widespread use of penicillins and cephalosporins, bacterial resistance became a major concern in β-lactam chemotherapy. During their early studies on the purification and evaluation

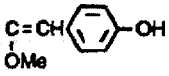
of penicillin, Abraham and Chain recognized that bacteria could produce an enzyme that was capable of inactivating the antibiotic [8]. This enzyme, which they named penicillinase, is one of a class of hydrolytic enzymes now referred to as β-lactamases.





(5) R = NH_2 , 

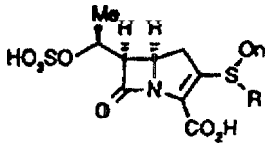
(6)

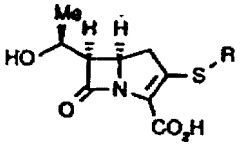


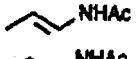
The year 1971 proved to bc another landmark in β-lactam research when the β-lactamase-stable cephamycins (5) were reported simultaneously by Lilly [9] and Merck [IO] workers. This discovery was of particular significance because these 7α-methoxy-cephalosporin derivatives were isolated from *Streptomyces* species*; all naturally occurring β-lactam antibiotics had previously been produced by fungi. Since then, extensive screening of soil micro-organisms by many researchers kd to the discovery of many new 'non-classical' β-lactams, differing widely in structure from penicillins and cephalosporins. These include a novel family of β-lactam antibiotics based upon the carbapen-2-em-3-carboxylic acid (6) nucleus*+ and are represented by the olivanic acids (7-13) [1 1–14] and the thienamycins (14-16) [15]. Their structures are distinct from the classical β-lactam antibiotics in that (a) they have a highly strained 4, 5-bicyclic ring system consisting of an unsaturated five-membered ring in which a

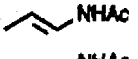
* *Streptomyces* are Gram-positive, aerobic bacteria of the Actinomycetales. They differ from fungi in that they are procaryotic, have bacterial-type cell walls and are inhibited by antibacterial agents. The streptomycetes form long chain spores, many species are pigmented and are found in soil.

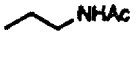
* based upon the 'trivial' carbapenem nomenclature. The systematic nomenclature defines this ring system as 7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-arboxylic acid.

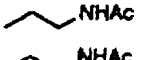


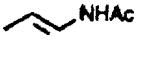


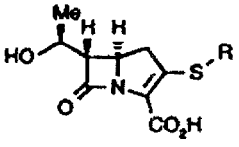
(7) R =  , n = 0; MM 13902

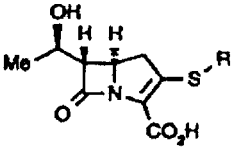
(8) R =  , n = 1; MM 4550

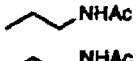
(9) R =  , n = 0; MM17880

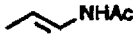
(10) R =  ; MM 22380

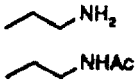
(11) R =  ; MM 22382

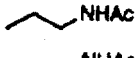


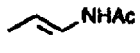


(12) R =  ; MM 22381

(13) R =  ; MM 22383

(14) R =  ; Thienamycin

(15) R =  ; N-Acetylthienamycin

(16) R =  ; N-Acetyldehydro-thienamycin

methylene replaces the sulphur atom at position 1, and (b) the C-6(7) acylamino-substituent of the bicyclic β-lactam ring is replaced by a carbon substituent, which invariably is an a-hydroxyethyl group. The so-called 'carbapenem' family of antibiotics display potent activity against a broad range of Gram-positive and Gram-negative bacteria, including β-lactamase-producing organisms. Furthermore, several members of this class of β-lactam antibiotics are potent inhibitors of β-lactamase enzymes.

These developments prompted considerable effort directed towards the chemical modification of these natural products, as well as their total syntheses. Much of this work has been the subject of numerous reviews [16–19] and will be discussed only briefly on this occasion. The present review focuses upon the development of the newer generation of totally synthetic carbapenem derivatives with improved metabolic stability.

β-LACTAM ANTIBIOTICS

MODE OF ACTION

The bacterial cell requires a rigid structure external to its cell membrane in order to maintain the integrity of the organism in hypotonic environments. This framework is provided by peptidoglycan, which is synthesized by all bacteria. β-Lactam antibiotics exert their lethal effect by inhibiting the synthesis of the bacterial peptidoglycan, thereby disrupting the morphology of the cell and eventually resulting in cell lysis and cell death [20–23].

Peptidoglycan is synthesized by all bacterial cells, but not by eukaryotic cells. and it is this property which provides the β-lactam antibiotics with their selective toxicity, *i.e.* their ability to kill bacterial cells without damaging mammalian cells.

The cell wall peptidoglycan is a network structure and all the peptidoglycans are built on the same general pattern [22, 24–27]. Linear glycan strands of alternative β, 1-4 linked *N*-acetylglucosamine (G) and *N*-acetylmuramic acid (M) pyranoside residues are substituted through the D-lactyl group of *N*-acetylmuramic acid, by L-Ala-γ-D-Glu-L-Xaa-D-Ala tetrapeptide units, where L-Xaa is most often a diamino acid, such as *meso*-diaminopimelic acid in *Escherichia coli* (see *Figure 3*. I)+. Peptide units substituting adjacent glycan strands are linked together by means of bridges that extend from the C-terminal D-alanine of one peptide to the ω-amino group of the diamino residue of another peptide.

Of the various discrete stages that have been identified for the biosynthesis of the bacterial cell wall, the final stage concerns the incorporation of newly formed disaccharide peptide units into pre-existing wall peptidoglycan; new peptide bonds must be made between nascent (uncross-linked) glycan strands and existing peptidoglycan. This reaction is called transpcptidation and is carried out by serine proteases, known as transpeptidases. Only when cross-links are formed does peptidoglycan become an insoluble matrix capable of maintaining the structural integrity of the wall. The sequence of events leading to the formation of cross-links in *E. coli* has several distinct stages. The transpeptidases operate by an acyl-enzyme mechanism, involving a serine residue at the active site. Initial formation of a non-covalent Michaelis complex between the enzyme and the terminal D-alanyl-D-alanine unit of a pentapeptide chain linked to muramic

* In Gram-positive bacteria, such as *Staphylococcus aureus*, the dibasic amino acid is L-Lysine and the amide cross-link has a Gly, peptide linker.

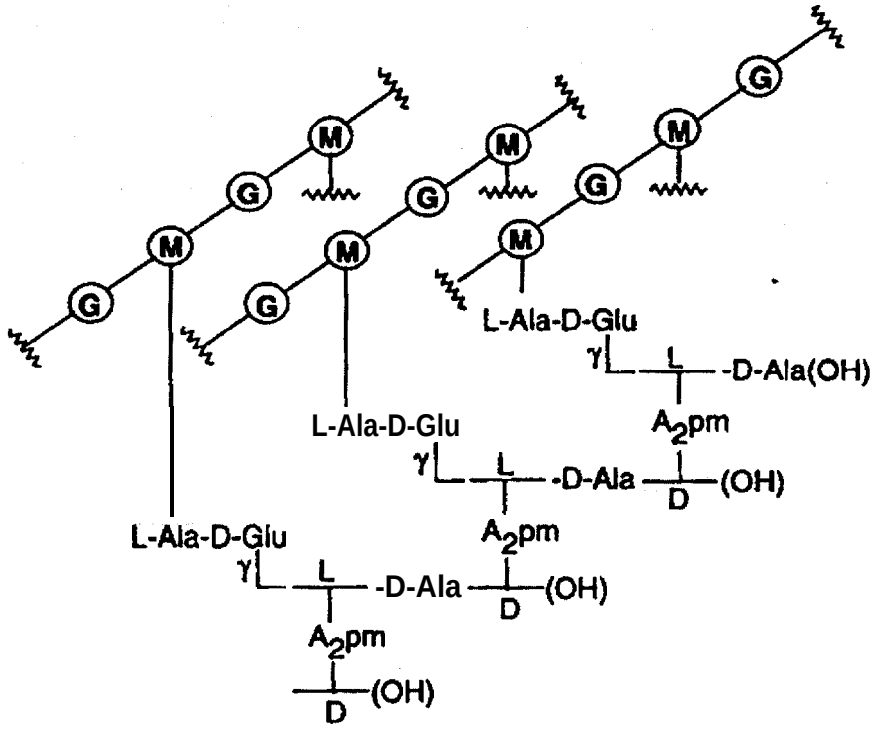


Figure 3.1. Wall peptidoglycan in E. coli showing cross-linking [24, 26]; G = N-acetylglucosamine, M = N-acetylmuramic acid, A_2pm = meso-diaminopimelic acid.

acid an a nascent glycan strand is followed by the formation of a covalent acyl-enzyme and elimination of the terminal D-alanine. Attack at the acyl-enzyme ester bond by a suitably positioned ω-amino group of a *meso* diamino-pimelic acid residue on an adjacent glycan strand provides the cross-link between the nascent glycan and the existing peptidoglycan of the wall; similar transpeptidations serve to cross-link the glycan strands at other points and firmly link the network together (*Figure* 3.2).

In *E. coli*, only 20-30% of the peptidoglycan is cross-linked; the remaining peptides which are not cross-linked are all tetrapeptides, lacking the fifth amino acid. An enzyme called D, D-carboxypeptidase is responsible for removing the terminal D-alanine from the pentapeptides on nascent glycan chains, The activity of this enzyme is similar to that of the transpeptidase, in that it bin& to the D-alanine-D-alanine portion, cleaves the peptide linkage

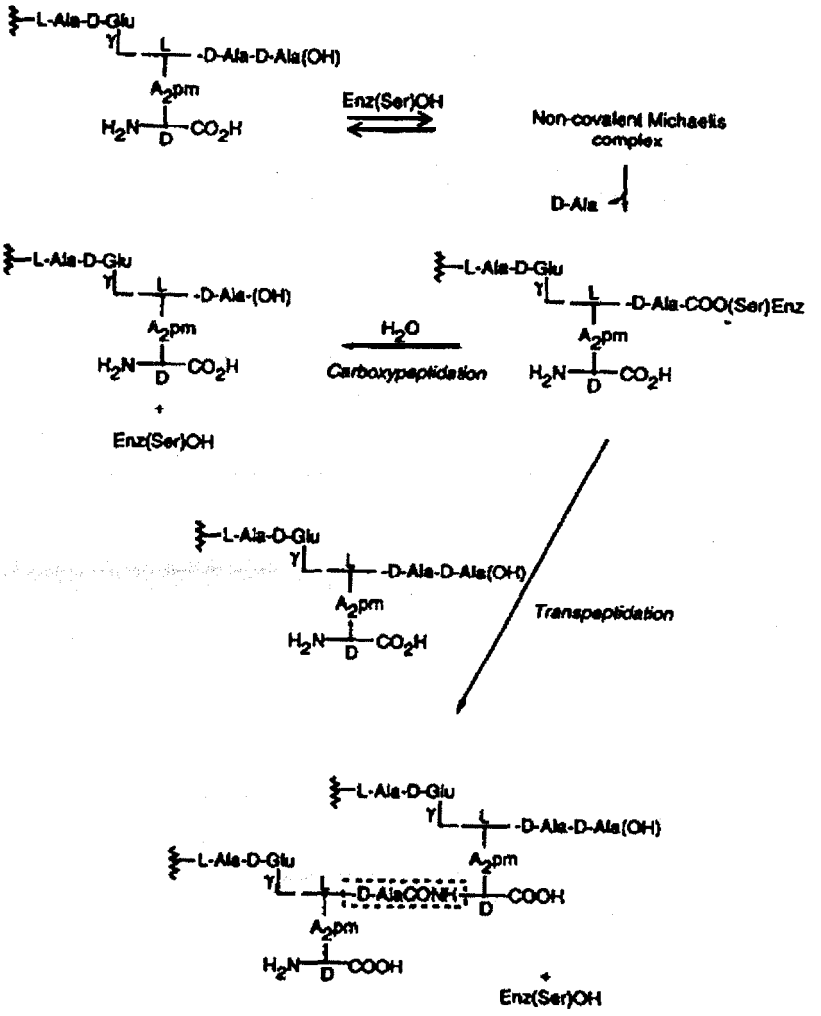
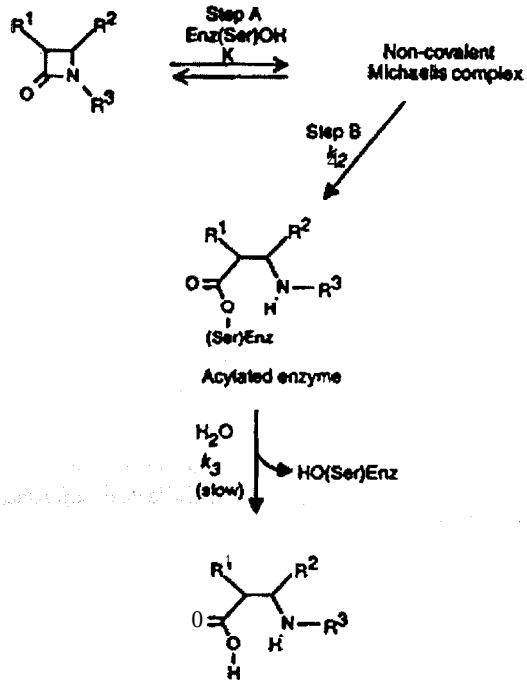


Figure 3.2. Transpeptidation and carboxypeptidation catalysed by D,D-peptidases in E. coli [24-27]

and releases the terminal D-alanine (*Figure* 3.2). However, instead of carrying out the transpeptidation, the carboxypeptidase leaves the peptide as an uncross-linked tetrapeptide. Organisms such as *Staphylococcus aureus* which have highly cross-linked peptidoglycan have very low carboxypepti-



Scheme 3.1. Interaction between a β-lactam antibiotic and a serine transpeptidase [24, 38]

dase activity. It therefore seems that the enzymes combine to control cross-linking.

Another D, D-peptidase involved in peptidoglycan biosynthesis is the D, D-endopeptidase enzyme. This enzyme breaks the cross-links in the peptidoglycan strands; that is, it reverses the action of transpeptidases. This activity is believed to provide local sites for peptidoglycan expansion and wall extension, which may be important at the point of cell division.

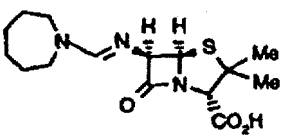
β-Lactam antibiotics exert their antibacterial activity by irreversibly inhibiting the serine transpeptidases; they simulate the D-Ala-D-Ala portion of the pentapeptide substrate and acylatc the serine hydroxyl group themselves. As in the interaction of the transpeptidase with the nascent pentapeptide, the first stage is the formation of a non-covalent Michaelis complex (*Scheme* 3.1; Step A). This is followed by the formation of an acylated enzyme (*Scheme* 3.1; Step B). Because the amide bond that is cleaved is endocyclic, there is no loss of a fragment corresponding to D-Ala. The resulting complex is very stable and resistant to attack by an external nucleophile (H_2O or RNH_2). Consequently, k_3 has a very small value and

the reaction effectively stops at this stage. The net effect is that the transpeptidase is prevented from exercising its normal function of completing the peptide cross-linkages in the cell wall. The cell wall weakens as the cell grows and eventually bursts (lysis), resulting in cell death (24,251.

β-Lactams bind not only with transpeptidases, but also with D, D-carboxypeptidases and D, D-endopeptidases. These enzymes are therefore collectively referred to as penicillin-binding proteins (PBPs). Each bacterial species has its own specific assortment of PBPs, which are numbered in order of decreasing molecular mass. The several PBPs present in a single bacterial cell may fulfil distinct cellular functions; they do not exhibit the same degree of essentiality and they show widely varying sensitivity to β-lactam antibiotics.

In *E. coli*, the most studied bacterium, seven PBPs are consistently found in the cytoplasmic membrane, ranging in molecular weights of 91,000 to 40,000 (PBP 1A, PBP 1B, PBPs 2-6). Two further PBPs with molecular weights 32,000 (PBP-7) and 29,000 (PBP-8) are occasionally detected [25]. The higher molecular weight PBPs, PBP 1 A, 1B, 2 and 3 are believed to function as transpeptidases and are vital to cell viability; hence they are often referred to as essential PBPs. Each of these enzymes has a distinct role in peptidoglycan biosynthesis. PBPs 1A and 1 B are believed to play vital parts in maintaining the integrity of the cell and possibly controlling the extension of the cell walls during growth. PBP 2 is involved in controlling the shape of the cell and PBP 3 is involved in cell division. The most abundant PBPs are those of lowest molecular weight, that is PBPs 4, 5, and 6. They have D, D-carboxypeptidase activity and, whilst they possibly control the degree of cross-linking of the' peptidoglycan, they are not considered to be essential for the viability of the cells.

β-Lactam antibiotics differ in their affinities for penicillin binding proteins. Acylaminopenicillins and cephalosporins exert their antibacterial effect on Gram-negative bacteria by biding to PBPs 1 and 3. Amidinopenicillins such as mecillinam (17) bind only to PBP 2; this results in the formation of large spheroplasts which lyse at antibiotic concentrations that are considerably higher than the minimum inhibitory concentrations



(17) Mecillinam

(MICs). Thienamycin and imipenem (*N*-formimidoylthienamycin) meanwhile have been found to bind with highest affinity to PBP 2, and then to PBP 1A and 1B [28–30]. This leads to the formation of spheroplasts which rapidly lyse and bactericidal concentrations are similar to inhibitory concentrations. In contrast to β-lactams which bind to PBP 3, no filaments arc produced.

These differences in mode of action between the carbapenem, imipenem, and other β-lactam antibiotics may explain the observation that imipenem, in contrast to penicillins and cephalosporins, has a post-antibiotic effect (PAE) when used against Gram-negative bacteria [31, 32]. This means that when a bacterial culture is exposed to imipenem, which is then removed, growth will not resume until after a lag-period. If clinically relevant, this would permit the USC of longer dose intervals even if the drug concentration at the site of infection falls below inhibitory levels.

BACTERIAL RESISTANCE TO β-LACTAM ANTIBIOTICS

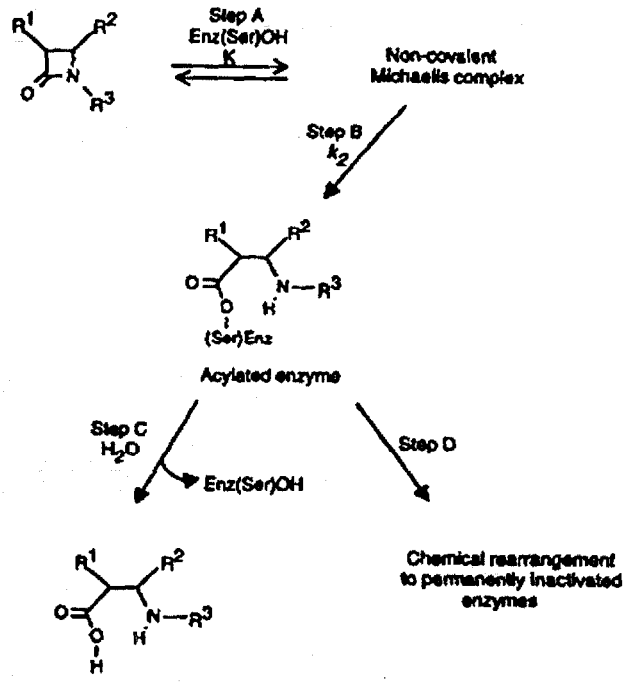
The discovery and *development* of penicillin revolutioniscd the therapy and *control* of bacterial infections. Yet, even before penicillin was used in the clinic, there was evidence that the potential value of the drug was under threat from bacterial resistance. The existence of a major resistance mechanism. inactivation by a bacterial enzyme, was described soon after the first report of the successful isolation of penicillin [2]. Within a decade, the majority of strains of *Staph. aureus* isolated in hospitals were resistant to penicillin G.

The discovery of the penicillin nucleus,- which made possible the preparation of the semi-synthetic penicillins and which also provided the impetus for cephalosporin research, kd to the introduction into the clinic of a variety of β-lactam antibiotics with differing antibacterial and pharmacological properties. Many of these agents were designed with the specific aim of being effective against bacteria which were resistant to existing penicillins and ccphalosporins. The development of each new agent was however soon followed by the appearance of strains of bacteria possessing one or more resistance mechanisms [33]. As a consequence, the development of resistance seriously threatened the clinical usefulness of β-lactam antibiotics in both hospital and community practice.

Bacteria may exhibit resistance to β-lactam antibiotics by one or more mechanisms [33]. One mechanism is the production, by Gram-negative and Gram-positive bacteria, of modified target sites with reduced affinities for β-lactam antibiotics (modified PBPs). Another way by which bacteria may display resistance is modification of the cell wall, resulting in the reduction

in the rate of passage of the antibiotic into the cell and to the target site, This is of importance in the case of Gram-negative bacteria only, as the cell walls of Gram-positive bacteria lack the permeability barriers to β-lactam antibiotics. The major mechanism of bacterial resistance however is due to the production of bacterial β-lactamases [34–36].

With the exception of one rather uncommon class of zinc-requiring β-lactamases [37], initial non-covalent biidiig at the active site frequently leads to acylation of a serine hydroxyl group by the β-lactam (Scheme 3.3, step B) which, irrespective of the fate of the protein, results in destruction of the β-lactam. A β-lactam is a substrate for the β-lactamase if acylation is followed by hydrolysis of the serine ester linkage (*Scheme 3.2*, step C), which regenerates the active enzyme and at the same time a ring-opened form of the β-lactam, which is antibacterially inactive; hence the term β-lactamase. Alternatively, formation of the acylated enzyme may be followed by



Scheme 3.2. Interaction of β-lactams with serine β-lactamases [38]

chemical rearrangement co a permanently inactivated β-lactamase (*Scheme* 3.2, step D); in this situation the β-lactam agent acts as an inhibitor of the β-lactamase. In combination with β-lactamase-susceptible antibiotics, β-lactamase inhibitors protect the antibiotic from inactivation by the β-lactamase enzyme, thereby producing a synergistic effect against β-lactamase producing bacteria [38].

NATURALLY OCCURRING CARBAPENEM ANTIBIOTICS

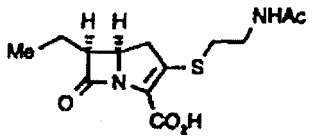
ISOLATION

Application of a screen, designed by Beecham scientists to discover naturally occurring inhibitors of bacterial β-lactamases, resulted in the isolation of a group of β-lactamase inhibitors named the olivanic acids from soil micro-organisms [I1]. This assay, which came to be known as the KAG assay, involved seeding an agar plate containing penicillin G with a β-lactamase-producing strain of *Klebsiella aerogenes*. The test solution was then introduced into wells in the agar and the plate was incubated overnight at 37°C. Solutions containing a diffusible β-lactamase inhibitor gave zones of inhibition around the wells, resulting from protection of the penicillin present in the agar. in the absence of β-lactamase inhibitor, bacterial growth occurred as a result of the inactivation of the penicillin by the β-lactamase.

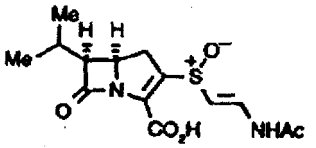
The first β-lactamase inhibitors to be discovered using this assay were the olivanic acids, MM 13902 (7), MM 4550 (8) and MM 17880 (9), so named as they were produced by a strain of *Streptomyces olivaceus* [11–13]. Not only arc they potent β-lactamase inhibitors, but are also powerful antibacterial agents. The discovery of these sulphate esters was soon followed by the identification of the closely related hydroxy compounds, MM 22380 (IO), MM 22382 (11), MM 22381 (12) and MM 22383 (13) [14].

At the same time, Merck researchers isolated the thienamycin* family of antibiotics (14- 16) from *Streptomyces cattleya*, using an assay designed to detect inhibitors of peptidoglycan synthesis [15, 39]. Subsequently, a whole series of carbapenem derivatives, represented by PS-5 (18) [40], carpetimycin A (19) [41], and asparenomycin A (20) [42] have been isolated from *Streptomyces* spp. Many arc potent inhibitors of β-lactamases, as well as antibacterial agents, Their occurrence and biological properties have been the subject of several recent reviews [16–19].

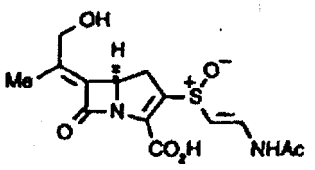
* named to denote the then novel β-thioenamine chromophore.



(18) PS-5



(19) Carpelimycin A



(20) Asparenomycin A

BIOLOGICAL ACTIVITY OF THE CARBAPENEM ANTIBIOTICS

The carbapeaems are a family of extremely potent naturally occurring antibiotics, with thienamycin being the most active. They display potent activity against a broad range of Gram-positive and Gram-negative bacteria, including penicillin-resistant strains of *Staph. aureus* and *Haemophilus influenzae* and organisms that are often resistant to other *β*-lactam antibiotics, such as indok-positive *Proteus*, *Enterobacter* spp., *Serratia marcescens* and the anacrobe *Bacteroides fragilis*. The antibacterial properties of the olivanic acids (7-1 3) are shown in Table 3. *1*, whilst those of thienamycin (14) and *N*-acetylthienamycin (15) are outlined in Tub/e 3.2. The antibacterial activities of some othn representative carbapenems arc given in Table 3.3.

The major structural difference between the olivanic acids and the thienamycins is the absolute stereochemistry of the chiral centre at C-8, which is (*S*) for the olivanic acids and (A) for the thienamycins. In the olivanic acid series, it is those compounds with the *cis* orientated *β*-lactam (i.e. 5*R*, 6*R*), as in MM 13902 (7), MM 17880 (9), MM 22380 (10) and MM 22382 (1 1), that arc most potent.* Whilst the corresponding (8*S*) hydroxyethyl compounds with the *trans β*-lactam (Le. 5*R*, 6*S*), *MM 22381* (12) and MM 22383 (13), also have a broad spectrum of activity, they are less potent than the *cis* compounds.

* The decreased activity of the *cis* carbapenem sulphoxide, MM 4550 (8) has been attributed to its chemical instability [43].

Table 3.1. ANTIBACTERIAL SPECTRUM OF SEVEN OLIVANIC ACID DERIVATIVES; MEAN MIC (mg/l) [43]

Organism	*MM 4550*	*MM 13902*	*MM 17880*	*MM* 22380	*MM* 22381	*MM* 22382	*MM* 22383
Escherichia coli	12.5	0.2	0.2	0.2	6.2	0.2	12.5
Escherichia coli •	2s	1.6	1.6	25	6.2	25	12.5
Klebsiella aerogenes	12.5	0.4		0.8	6.2	0.4	6.2
Klebsiella aerogenes •	50	0.2	9::	50.8	6.2	100	12.5
Proteus mirabilis	12.5	0.2	.04	0.8	12.5	0.2	12.5
Proteus mirabilis •	12.5		0.4	1.6	12.5	0.2	12.5
Proteus rettgeri	25	0.4	0.8	3.1	12.5	0.8	25
			0.8	...		1.6	12.5
Proteus *vulgaris*	12.5	0.4	0.8	1.6	6.2	0.8	6.2
Enterobacter aerogenes	25	3.1	3.1	1.6	6.2	1.6	12.5
Enterobacter cloacae	100	12.5	6.2	3.1	12.5	3.1	25
Serratia marcescens	25	3.1	3.1	3.1	12.5	3.1	12.5
Pseudomonas aeruginosa	>100	25–50	1 o	o	>100	>100	>100 >100
Haemophilus influenzae	6.2	0.1	0 2	0.2	6.2	0.1	6.2
Haemophilus influenzae •	6.2	0.1	0'2		6.2	0.5	6.2
	3.1	1.6	...	1.0	3.1	0.4	3.1
Staphylococcus aureus	25	1.6	0.4	0.4	3.1	0.4	6.2
Staphylococcus aureus •	50	...	1.6	0.8	3.1	0.4	6.2
Staphylococcus aureus • ⊠	100	12.5	12.5	6.2	100	6.2	100
Streptococcus pyogenes	6.2	0.2	0.1	0.1	0.8	0.05	1.6
Streptococcus faecalis	50	4.2	6.2	1.6	25	1.6	50

*β-lactamase producing strains
** methicillin-resistant strains

Table 3.2. ANTIBACTERIAL ACTIVITIES OF N-ACETYLTHIENAMYCIN AND THIENAMYCIN; MIC VALUES (mg/l) [43]

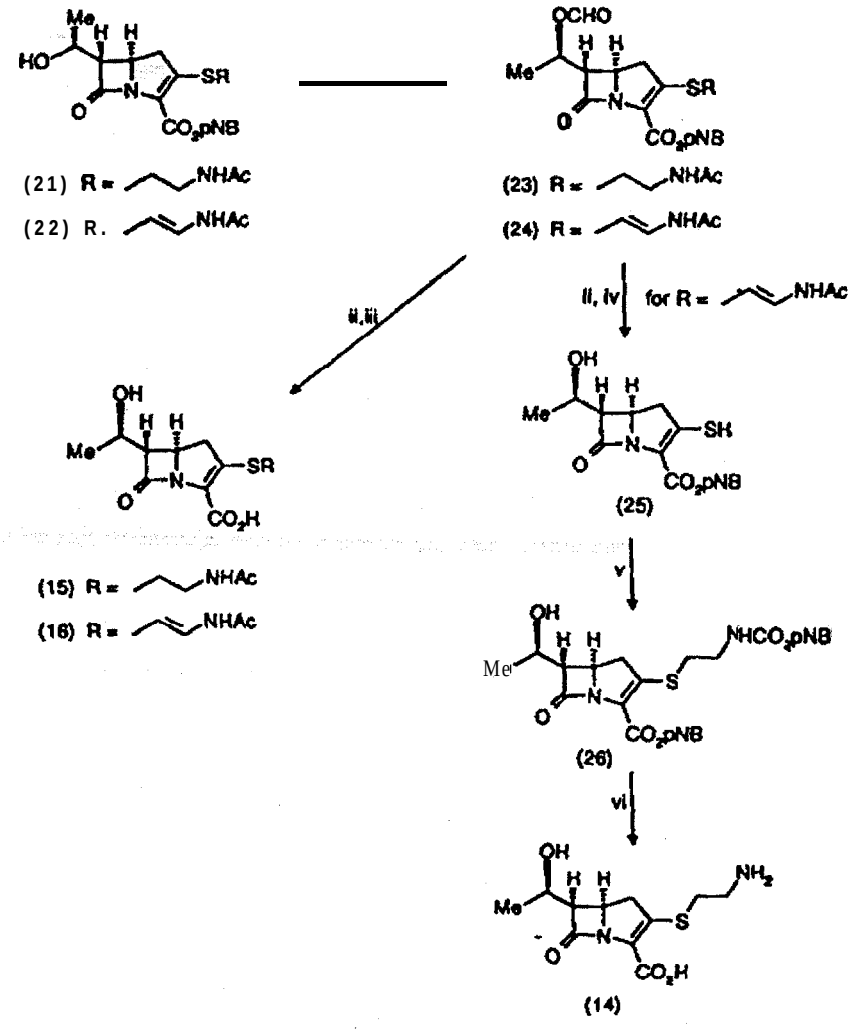
Organism	*N-Acetylthienamycin*	*Thienamycin*
Escherichia coli (0111)	0.8	0.2
Escherichia coli JT39 (RTEM)	0.8	a.4
Klebsiella aerogenes A	0.4	0.4
Klebsiella aerogenes (RTEM)	0.8	0.4
Proteus mirabilis 977	3.1	3.1
Proteus rettgeri WM16	3.1	3.1
Enterobacter cloacae T753	3.1	3.1
Serratia marcescens US20	3.1	1.6
Pseudomonas aeruginosa NCTC 10662	25	3.1
Bacteroides fragilis BC16	NT	0.4
Staphylococcus aureus Russell	0.2	0.04
Streptococcus faecalis I	6.2	1.6
Streptococcus pyogenes CN10	0.05	0.01

Thienamycin (14) and *N*-acetylthienamycin (15) have the same *trans* β-lactam orientation as MM 22381 (12) and MM 22383 (13) but have the (*R*) configuration at C-8. They have much improved antibacterial potency compared to MM 22381 and MM 22383, being similar to the c&-isomer, whilst retaining the stability of the (5*R*, 6*S*, 8*S*) compound8 towards R-TEM β-lactamases. Furthermore, thienamycin (14) displays exceptional activity against *Pseudomonas aeruginosa* and this is attributed to the basic amino group of the C-2 cystcaminyl side-chain. This combines to make thienamycin one of the most potent, broad spectrum antibacterial agents to be isolated from natural sources. Thienamycin was shown to have pronounced activity against *E. coli, Proteus mirabilis, Klebsiella pneumon&e, Serratia marcescens, Streptococcus pyogenes,* penicillin G sensitive and penicillin G resistant *Staph. aureus ad Enterobacter* spp., as well as *Ps. aeruginosa* [47]. A number of studies have shown it to be superior to other semi-synthetic, as well as naturally occurring penicillins and cephalosporins, including amoxycillin, carbenicillin, ticarcillin, apalcillin, mezlocillin, azlocillin, piperacillin, cephalothin, cefoxitin and cefotaxime [48–52].

Table 3.3. *IN VITRO* ACTIVITIES (MIC, mg/l) OF PS-5 (18) [44], CARPETIMYCIN *A* (19) [45] AND ASPARENOMYCIN *A* (20) [46]

Organism	*Strain*	*PS-5 (18)*	*Strain*	*Carpetimycin A (19)*	*Strain*	*Asparenomycin A (20)*
Escherichia coli	K-12	1.56	JC-2	0.05	JC-2	1.56
Escherichia coli •			EC-1	0.2	377	0.39
Klebsiella pneumoniae	K2	3.13	PCI-602	0.2	SRL-1	0.78
Klebsiella pneumoniae *			25	0.78	363	0.78
Proteus mirabilis	P-6	6.25	2 1 1 0 0	1.56	PR-4	3.13
Proteus mirabilis *			TN 265	2 . 5		
Proteus morganii			IFO 3168	0.39		
Proteus rettgeri	P-7	625				
Proteus vulgaris	109	12.5	IID 874	0.39	CN-329	12.5
Proteus vulgaris *	P-5	625	69	0.78		
Enterobacter cloacae	45	625	IID 977	0.78	233	1.56
Enterobacter cloacae *	E16	3.13	3	3.13		
Serratia marcescens	S-18	3.13	NHL	0.2	13880	12.5
Serratia marcescens *			4	3.13		
Pseudomonas aeruginosa	NC5	>100	10490	6.25	25619	25
Staphylococcus aureus	209P	0.02	209P	0.39	JC-1	1.56
Staphylococcus aureus •	Russell	0.20			C-14	1.56
Streptococcus pyogenes	NY5	0.08			C-203	1 . 5 6

* β-lactamase producing strain



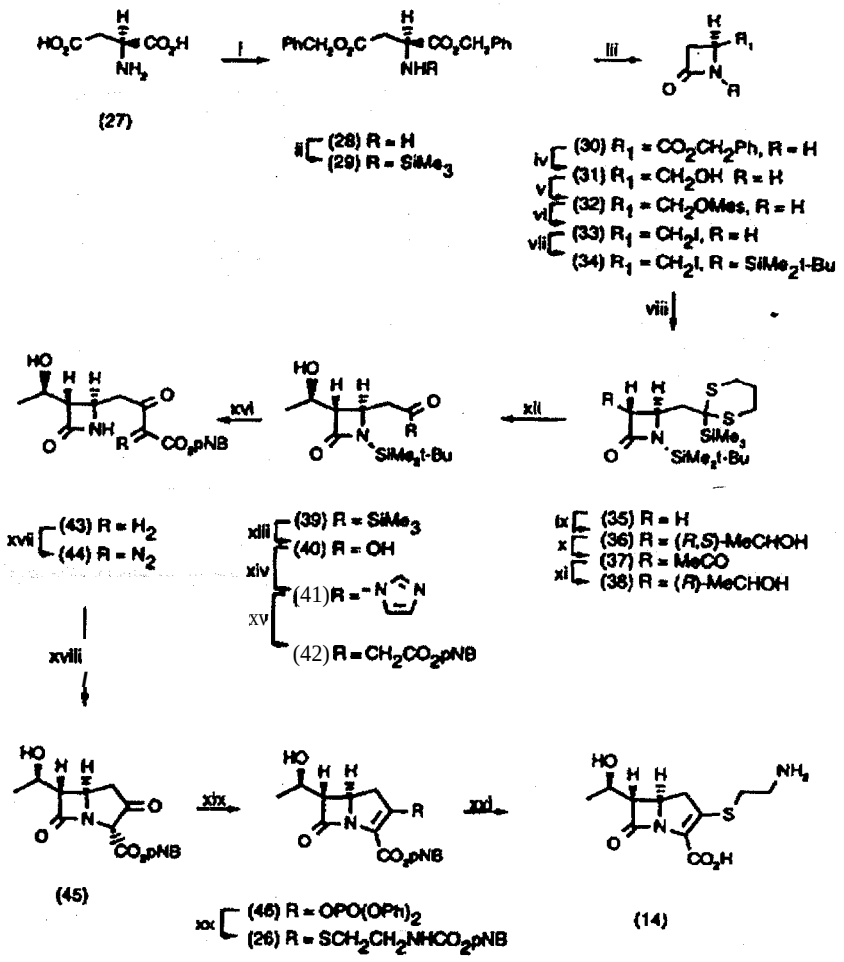
Scheme 3.3. Interconversion of the olivanic acids and the thienamycins [53].
Reagents: (i) Diethyl azodicarboxylate, PPh_3, HCO_2H; (ii) NaOH, aq. 1,4-dioxan; (iii) (a) H_2, 5% Pd/C, aq. 1,4-dioxan, (b) $NaHCO_3$; (iv) N-bromoacetamide, aq. 1,4-dioxan; (v) 2-p-nitrobenzyloxycarbonylaminoethyl bromide, K_2CO_3, DMF; (vi) H_2, 5% Pd/C, aq. 1,4-dioxan, pH 7.0 phosphate buffer.

INTERCONVERSION OF THE OLIVANIC ACIDS AND THE THIENAMYCINS

Structure-activity studies revealed that the stereochemical configuration required for optimum antibacterial activity was that found in the thicnamycin series, that is (5*R*, 6*S*, 8*R*). The olivanic acids, MM 22381 (12) and MM 22383 (13) differ from *N*-acetylthienamycin (15) and *N*-acetyldehydrothienamycin (16) respectively by virtue of the stereochemical configuration at C-8. In order to inter-relate the two series, the configuration of the a-hydroxyethyl side-chain of MM 22381 and MM 22383 was inverted by way of a Mitsunobu reaction sequence [53]. Thus, treatment of the *p*-nitrobenzyl esters (21) and (22) of MM 22381 and MM 22383 with diethyl azodicarboxylate, triphenylphosphine and formic acid provided the formate esters (23) and (24), respectively. Alkaline hydrolysis of the formate esters, followed by hydrogenolysis provided *N*-acetylthienamycin (15) and *N*-acetyldehydrothienamycin (16) (*Scheme* 3.3). The combination of this inversion process with a procedure developed for the variation of the C-2 atkylthio side-chain in the olivanic acids [54] provided a preparation of thicnamycin (14) from MM 22383 (13). Addition of hypobromous acid to the double bond of the C-2 acetamidoethenylthio substituent in MM 22383, as well as MM 22382, resulted in the formation of a bromohydrin, which fragmented to provide the C-2 thiol (25); subsequent alkylation or addition to propiolate esters provided a wide range of C-2 substituents with either the (5*R*, 6*S*, 8*S*), (5*R*, 6*R*, 8*S*) or (5*R*, 6*S*, 8*R*) stereochemistry, depending on whether MM 22383, MM 22382 or *N*-acetyl-dehydrothienamycin was chosen as starting material [55, 56]. Thus, thienamycin was prepared from MM 22383 by elaboration to the thiol (25) and alkylation with 2-*N*-*p*-nitrobenzyloxycarbonylaminoethyl bromide, followed by hydrogcnolysis of the resulting ester (26).

TOTAL SYNTHESIS OF (+)-THIENAMYCIN

Owing to the chemical instability of the carbapenem antibiotics at high concentrations, fermentation procedures failed to provide sufficient quantities of natural products for clinical evaluation and production. For Merck scientists, the solution to this problem was to devise an efficient, high yielding and cost-effective synthetic route to thienamycin and related clinically important compounds. Initial efforts were directed towards the synthesis of racemic thicnamycin [16, 18]. In their first chiral synthesis of (+)-thienamycin, which proceeded from L-aspartic acid (27), the pivotal reaction was the carbene insertion reaction to provide the bicyclic ring system by formation of the C-3–N-4 bond [57] (*Scheme 3.4*).



Scheme 3.4. Synthesis of (+)-Thienamycin (14) from L-Aspartic Acid [57].
Reagents: (i) $PhCH_2OH$, ptsa.H_2O, benzene, reflux; (ii) Me_3SiCl, Et_3N, Et_2O; (iii) t-BuMgCl, Et_2O; (iv) $NaBH_4$, MeOH; (v) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (vi) NaI, acetone, reflux; (vii) t-$BuMe_2SiCl$, Et_3N, DMF, (viii) 2-lithio-2-(trimethylsilyl)-1,3-dithiane, THF, -78°C; (ix) MeCHO, LDA, THF, -78°C; (x) TFAA-Me_2SO, Et_3N, CH_2Cl_2, -78°C; (xi) K-selectride, KI, ether; (xii) $HgCl_2$, HgO, aq. MeOH, reflux; (xiii) H_2O_2, aq. MeOH; (xiv) carbonyldiimidazole, THF; (xv) Mg salt of mono-p-nitrobenzyl malonate, THF; (xvi) methanolic HCl; (xvii) p-carboxybenzenesulphonyl azide, Et_3N, MeCN; (xviii) rhodium (II) acetate, toluene, 80°C; (xix) $ClP(O)(OPh)_2$, cat. DMAP, i-Pr_2NEt, MeCN; (xx) N-p-nitrobenzyloxycarbonylcysteamine, i-Pr_2NEt, MeCN, -5°C; (xxi) H_2, 10% Pd/C, 40 psi.
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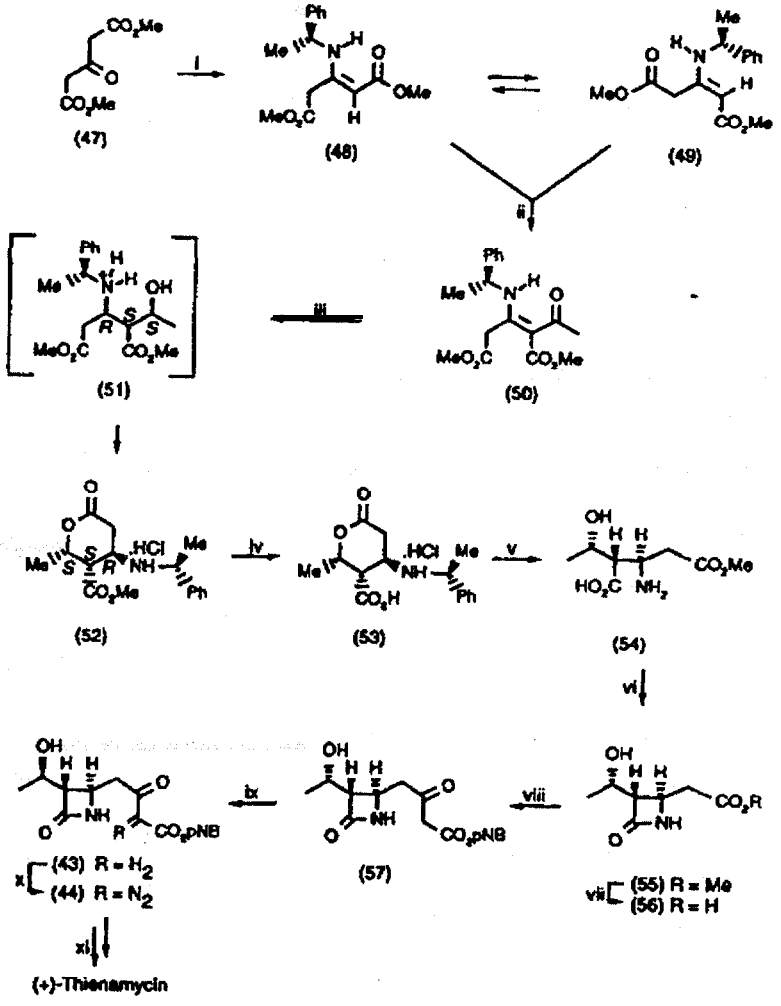
Sodium borohydride reduction of the azetidinone ester (30), obtained by *t*-butyl magnesium chloride-mediated cyclization of the *N*-trimethylsilyl derivative of dibenzyl aspartate (29), provided alcohol (31), which was elaborated to the iodomethyl derivative (33), *via* the mesylate (32). *N*-Silylation of (33). followed by reaction with 2-lithio-2-trimethylsilyl-1,3-dithiane gave the substituted dithiane (35). Condensation of the lithium enolate derived from (35) with acetaldehyde gave a 1:1 mixture of *trans-(R)* and *trans-(S)* alcohols (36). Oxidation of the diastereoisomeric mixture of alcohols to the *trans*-acetyl derivative (37), followed by potassium selectride reduction gave predominantly the *trans-(R)* alcohol (38). Dithiane hydrolysis provided the trimethylsilyl ketone (39), which underwent Baeyer-Villiger oxidation CO the acid (40). The requisite ketoester intermediate (42) was then obtained by condensing the imidazole derivative (41) with the magnesium salt of mono-*p*-nitrobenzyl malonate. Removal of the *N*-silyl protecting group followed by diazo exchange from *p*-carboxybenzenesulphonyl azide provided the cyclization precursor (44). Thermolysis of (44) in the presence of rhodium acetate then provided the bicyclic ketocstcr (45). Introduction of the cysteamine side-chain was accomplished by converting ketoester (45) to the enol phosphate (46) and reaction with *N*-(*p*-nitrobenzyloxycarbonyl)-cysteamine. Finally, hydrogenolytic removal of the *p*-nitrobenzyl protecting groups of (26) provided (+)-thienamycin (14).

A commercially viable, enantioselective route was subsequently developed by the Merck Process Research group [58, 59]. This approach, which is also based upon the carbene insertion reaction, is characterized by the early introduction of the hydroxyethyl group through the formation of a highly functionalized acyclic derivative with three contiguous asymmetric centres (*Scheme* 3.5).

Condensation of dimethyl 1,3-acetonedicarboxylate (47) with (*R*)-(+)-α-methyl- benzylamine provided an equilibrium mixture of enamines (48) and (49) in nearly quantitative yield, without the need for purification. Acylation of this equilibrium mixture using ketene then furnished the enamino ketone (50) in excess of 95% yield. This product (SO) displayed a rigid structure by virtue of an internal hydrogen bond, thus setting the stage for a stereoselective reduction. Hydrogenation of (SO) in acetic acid containing 2 molar equivalents of phosphoric acid and 5% platinum-on-carbon catalyst, followed by lactonization of alcohol (51) gave the triply asymmetric, chiral lactone (52) in 80-90% yield, as the hydrochloride salt. The relative stereochemistry of (52) had already been established by conversion of racemic intermediates to model azetidinones [60] and was confirmed by X-ray crystallography. Unfortunately, the relative stereochemistry at the methyl substituted carbon was incorrect and necessitated



Scheme 3.5. Practical synthesis of (+)-thienamycin (14) from dimethyl acetonedicarboxylate and (+)-α-methylbenzylamine [59].

Reagents: (i) (R)-(+)-α-methylbenzylamine, toluene, AcOH (6 mol %); (ii) ketene, toluene; (iii) H_3PO_4 (2 mol equiv.), AcOH, 5% Pd/C, H_2, 90-1000 psi., 20°C; (iv) conc. aq. HCl, 85° C; (v) (a) MeOH, H_2, Pd/C, 40 psi., 60°C; (b) n-Bu_3N, CH_2Cl_2; (vi) DCCI, aq. MeCN; (vii) aq. NaOH; (viii) (a) carbonyldi-imidazole; (b) Mg salt of mono p-nitrobenzyl malonate; (ix) (a) diethyl azodicarboxylate, PPh_3, HCO_2H; (b) dil aq. HCl; (x) p-dodecylbenzenesulphonyl azide; (xi) see Scheme .4.

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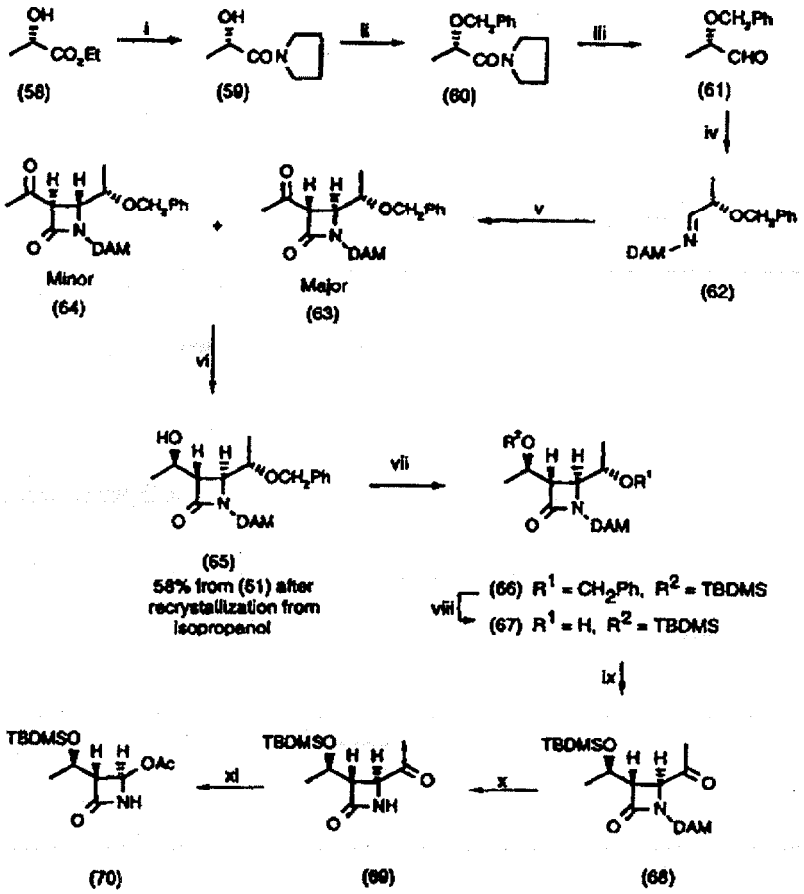
on inversion step at a later stage in the synthetic route. Hydrolysis of (52) to the carboxylic acid (53), followed by hydrogenolysis of the α-methylbenzyl group and methanolysis of the lactone ring then provided the highly functionalized, chiral amino acid (54) in high yield [> 90% from (52); 50% overall yield] and excellent purity with the need for only one purification stage. Amino acid (54) was then cyclized in high yield to azetidinone (55) using dicyclohexylcarbodi-imide. Saponification provided the sodium salt of acid (56), which was converted to ketoester (57) by the previously described method. Inversion of the I-hydroxycthyl group was then achieved by treating azetidinone (57) with formic acid in the presence of triphenylphosphine and diethyl azodicarboxylate; hydrolysis of the resulting inverted formate ester provided (43). Diazo transfer from *p*-dodecylbenzenesulphonyl azide yielded the diazo ketoester (44), which was converted to (+)-thienamycin by the previously described methodology.

Numerous asymmetric syntheses of thienamycin and structurally related carbapenems have since been reported. Most strategies have focusscd upon the elaboration of the correct stereochemistry at the three chiral centres of a monocyclic precursor [61]. Subsequent progression has usually been by way of the diazo-ketone approach and the hydroxycthyl side-chain has often been introduced by way of a stereoselective aldol condensation [62]. In other cases, the substituent has been incorporated before construction of the β-lactam ring using chiral building blocks derived from o-glucose [63], L-threonine [64, 65], D-allothreonine [66], and (*S*)-(-)-hydroxyethylbutyrate [67, 68]. Functionalized β-lactams derived from the penicillin nucleus have also been elaborated to (+)-thienamycin and related compounds [69–71].

A particularly versatile and much used intermediate for asymmetric carbapenem syntheses is (3*R*, 4*R*)-4-acetoxy-3-[(*R*)-1-(*t*-butyldimethylsilyloxy)ethyl]-2-azetidinone (70). A practical synthesis of this key intermediate has been reported by Ito and co-workers (Schemc3.6) [72]. The [2 + 2] cycloaddition reaction of diketene with the chiral imine (62), derived from the inexpensive (*S*)-ethyl lactate (Sg), proceeded in a highly stereoselective manner to provide the desired 3, 4-*trans*-3-acetylazetidinone (63) as the major product (diastereoselectivity 7 ~ 10:1). Potassium triethylborohydride reduction of the crude diastereoisomeric mixture of azetidinones (63) and (64) gave the pure 3-[(1*R*)-hydroxyethyl]azetidin-2-one (65) in good yield after recrystallization from isopropanol. The alcohol (65) was converted to the ketone (68) by way of the silyl ether (66) and the alcohol (67). Removal of the di-*p*-anisylmethyl group in ketone (68) was cleanly effected by treatment with sodium peroxydisulphate in the presence of disodium hydrogen phosphate buffer; oxidation of (69) with *m*-chloroperbenzoic acid furnished the chiral 4-acetoxy azetidinone derivative (70). The elaboration

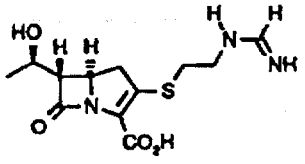


Scheme 3.6. Chiral synthesis of the key intermediate, (3R, 4R)-4-Acetoxy-3-[(R)-1-(t-butyldimethylsilyloxy)ethyl]azetidin-2-one (70) from (S)-Ethyl lactate [72].
Reagents: (i) pyrrolidine; (ii) benzyl chloride, NaH; (iii) $NaAl(OCH_2CH_2OMe)_2H_2$, toluene; (iv) di-p-anisylmethylamine ($DAM-NH_2$), $MgSO_4$, toluene; (v) Diketene (4 equiv.), CH_2Cl_2, -35°C, 3 days; (vi) $KBEt_3H$, THF, -78°C; (vii) TBDMSCl (t-$BuMe_2SiCl$), DMAP, DMF; (viii) H_2, Pd/C, EtOAc; (ix) N-chlorosuccinimide, Me_2S, Et_3N, toluene; (x) $Na_2S_2O_4$, Na_2HPO_4, aq. acetone; (xi) m-chloroperbenzoic acid, EtOAc.
Reprinted from Tetrahedron, 45, 5767-5790, Ito, Y., Kobayashi, Y., Kawabata, T., Takase, M. and Terashima, S., 1989, with kind permission from Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington OX5 1GB, UK.

of this versatile intermediate to a variety of optically active carbapenem derivatives is dimmed in later sections of this review.

IMIPENEM (*N*-FORMIMIDOYLTHIENAMYCIN, MK 0787)

The unique structure and exceptional antibacterial potency, particularly against *Pseudomonas spp.*, created considerable interest in thienamycin. Unfortunately, its novel, highly strained carbapenem nucleus, together with the basic aminoethylthio side-chain contributed to its chemical instability. It is believed that this concentration-dependent instability, which made thicnamycin less attractive as a parenterally administered drug, is due to the intermolecular aminolysis of the β-lactam ring by the cysteamine side-chain. Merck scientists therefore investigated the derivatization of the amino group of thicnamycin and found that the *N*-formimidoyl derivative (71) (imipcncm, MK 0787), prepared by the reaction of thienamycin with methyl formimidatc in aqueous solution at pH 8.2, was S-fold more stable than thienamycin in concentrated aqueous solution [73]. Furthermore, the crystalline *N*-formimidoylthienamycin (71) retained the antibacterial spectrum of thienamycin and exhibited enhanced potency against *Ps. aeruginosa.*

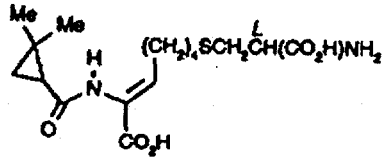


(71) Imipenem

PRIMAXIN (IMIPENEM IN COMBINATION WITH CILASTATIN)

In view of the improved chemical stability, excellent antimicrobial activity, stability to bacterial β-lactamases and accessibility in large quantities by total synthesis, *N*-formimidoylthienamycin (71) was selected for clinical evaluation. However, as in the case of other naturally occurring carbapenem antibiotics, only a Low recovery of imipenem was evident in the urine after parenteral administration in animal models, as well as man [74]. Subsequently, detaikd studies revealed that this class of antibiotics is extensively degraded by the renal dipeptidase enzyme (DHP-1) [75, 76] located in the brush borders of the kidney [77] (*Table 3.4*). Enzymatic turnover of imipcncm *in vivo* therefore posed a serious threat to its clinical efficacy and inhibitors of this zinc-containing hydrolytic enzyme were therefore sought. Indeed, coadministration of imipenem with a potent, competitive inhibitor of the DHP-1 enzyme, cilastatin (MK 0791, 72) (78, 79], in a 1:1 ratio

resulted in a 10-fold improvement in the urinary recovery of imipenem in chimpanzees and humans [77, 80]. Furthermore, the nephrotoxic potential of the antibiotic was significantly reduced. This led to an intensive evaluation of the imipenem-cilastatin combination [81, 82], which was ultimately marketed as Primaxin, an injectable product for the treatment of lower respiratory tract, intra-abdominal, genito-urinary, gynaecological, bone and joint and skin and soft tissue infections, septicaemia and prophylaxis of post-operative infections.



(72) Cilastatin

Most of the research since the discovery of Primaxin has concentrated upon the development of a single compound that possesses all, or most of the properties of the imipenem/cilastatin combination. This work is the subject of the following sections of the present review.

Tabk3.4. RELATIVE SUSCEPTIBILITY TO DHP-1 OF NATURALLY OCCURRING CARBAPENEM ANTIBIOTICS AND ITS CORRELATION WITH LOW URINARY RECOVERY [77]

Antibiotic	*Susceptibility to DHP-1 (hog renal) relative to thienamycin*	*% Dose recovered in urine (mice)*
Thienamycin	1.0	25
N-Formimidoylthienamycin	0.9	28
N-Acetylthienamycin	4.2	Il.4
MM 22380	12	1.0
MM22381	20	ND
MM22383	51	ND
MM 13902	30	<1.0
MM 17880	8.3	NO
PS 5	43	ND

ND = Not determined

TOTALLY SYNTHETIC NON-NATURAL CARBAPENEMS

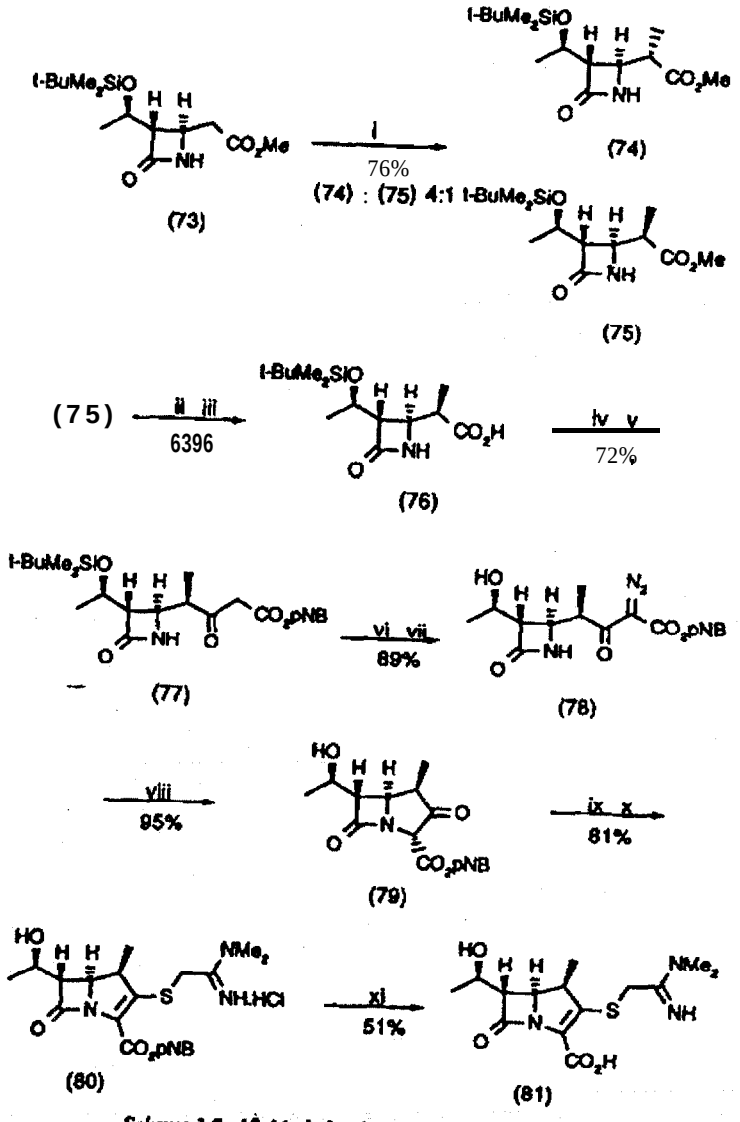
By the time that imipenem was under development, the scope for semi-synthetic modification of natural carbapenems appeared to have been practically exhausted. The next phase of carbapenem research was therefore directed at the total synthesis of non-natural carbapenem structures, largely based on systematic variations of the thitnamycin nucleus. Over the past decade a considerable effort has been devoted to this task. A major objective of this research was the discovery of a carbapenem with the antimicrobial spectrum and potency of imipenem, but with significantly 'enhanced chemical and metabolic stability.

MODIFICATIONS AT C-3, C-5, AND C-6

Analogues of thienamycin in which the 3-carboxy group has been replaced by a 3-(5-tetrazolyl) [83] or a 3-(methylphosphonyl) group [84] have much improved stability to DHP-1, but greatly diminished antibacterial activity; the 5-tetrazolyl compound has about one-tenth of the potency of thienamycin, and the methylphosphonyl derivative is even less active.

Racemic 5-methylthienamycin and a number of C-2 modified analogues were prepared and, in comparison with the 5-unsubstituted carbapenems, were found to have improved chemical stability and much greater stability to mouse kidney homogenate [85]. Unfortunately, the improved stability had been gained at the cost of significantly reduced antibacterial activity. For racemic *N*-acetyl-5-methylthienamycin, the various epimers at C-6 and C-8 were also prepared, and the same order of activity relative to stereochemisty was observed as in the 5-unsubstituted series: 8*R*, 6*S* > 8*S*, 6*R* > 8*S*, 6*S* > 8*R*, 6*R*.

A variety of small alkyl groups, sometimes substituted by a hydroxy group or sulphatcd hydroxy group, arc found at C-6 in natural carbapenems, with both 6*R* and 6*S* stereochemistries being known; introduction of larger or more functionalised 6-alkyl substituents generally resulted in reduced potency [86–92]. (6*R*)-[(*R*)-1-Fluoroethyl] carbapenems have improved water solubility over their 6-(1-hydroxyethyl) couterparts, but similar antibacterial activity and stability to DHP-1 [93, 94]. 6-Aminomethyl- and 6-[(*R*)-1-aminoethyl)]- 1β-methylcarbapenems show good activity against Gram-negative organisms *in vitro*, but have relatively poor chemical stability [87]. 6-Acylamino- [95, 96) and 6-carbamoyl-1β-methylcarbapenems [97] also have poor chemical stability, and consequently showed rather weak activity in MIC testing. Disubstitution at C-6, as for example in 6-hydroxy and 6-methoxy *epi*-PS-5 [98], resulted in



Scheme 3.7. 1β-Methylcarbapenem synthesis [99]
Reagents: (i) lithium diisopropylamide (2 equiv.); MeI; (ii) 2.5 M NaOH, MeOH; (iii) 6 M HCl; (iv) carbonyl di-imidazole; (v) magnesium p-nitrobenzyl malonate; (vi) 6 M HCl; (vii) dodecylbenzenesulphonyl azide, Et_3N; (viii) rhodium(II) octanoate; (ix) $(PhO)_2POCl$, $(iPr)_2NEt$; (x) $HSCH_2C(NMe_2)NH.HCl$, $(iPr)_2NEt$; (xi) H_2, Pd/C, $NaHCO_3$ (1 equiv.).

increased chemical and metabolic stability, but at the expense of drastically reduced antibacterial activity.

In conclusion, modifications at C-3, C-5, and C-6 have so far failed to give any overall improvement on the properties of the natural carbapcnems in terms of stability and antibacterial activity. At these positions, the thienamycin structure is thcrcforc regarded as being optimal. Modifications at the remaining positions, C-l and C-Z, arc discussed in the following sections.

1β-METHYL AND OTHER I-SUBSTITUTED CARBAPENEMS

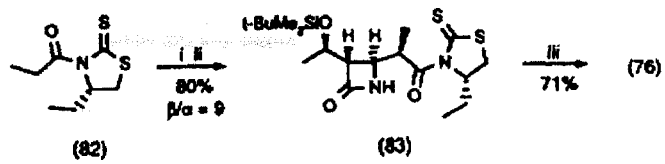
The Merck group described the first synthesis of a 1β-methylcarbapenem, starting with the optically active ester (73), an intermediate in the chiral synthesis of imipenem (*Scheme* 3.7) [99]. The resulting 1β-methylcarbapenem (81), which also contains the novel 2-dimethylamino-2-iminoethylthio side-chain at C-2, is chemically stable, resistant to DHP-I , and ret&s the potent antibacterial activity of imipenem (*Table* 3.5). The introduction of la-methyl, 1β-ethyl, la- and 1β-hydroxy, and 1α- and 1β-methoxy groups also increased stability to DHP-I over that of the 1-unsubstituted carbapcnems, but antibacterial activity was diminished [86].

The improved stability imparted to the carbapenem nucleus by introduction of a 1β-methyl group aroused considerable interest. Consequently, the stereoselective synthesis of the later-stage intermediates (76) - (79) in the

Table 3.5. RELATIVE IN VITRO ANTIBACTERIAL POTENCY AND DHP-I SUSCEPTIBILITY OF IMIPENEM (71) 'AND 1β-METHYLCARBAPENEM (81) IN COMPARISON WITH THIENAMYCIN (14) [99]

Organism (n)	*Thienamycin Mean MIC (mgll)*	*Imipenem Potency*[a]	*Compound (81) Potency*[a]
			0.5
Enterococcus (2)*ureus* (5)	3.80	1.2	0.8
Escherichia call (7)	0.36	1.6	3.5
Enterobacter spp. (6)	2.04	2.1	8.0
Klebsiella spp. (5)	1.77	1.2	3.5
Serratia spp. (2)	2.50	2.6	7.0
*Proteus spp.*5)	7.08	1.5	4.6
Pseudomonas aeruginosa (5)	9.13	3.5	5.3
DHP-I susceptibility[b]	1.0	0.90	0.026

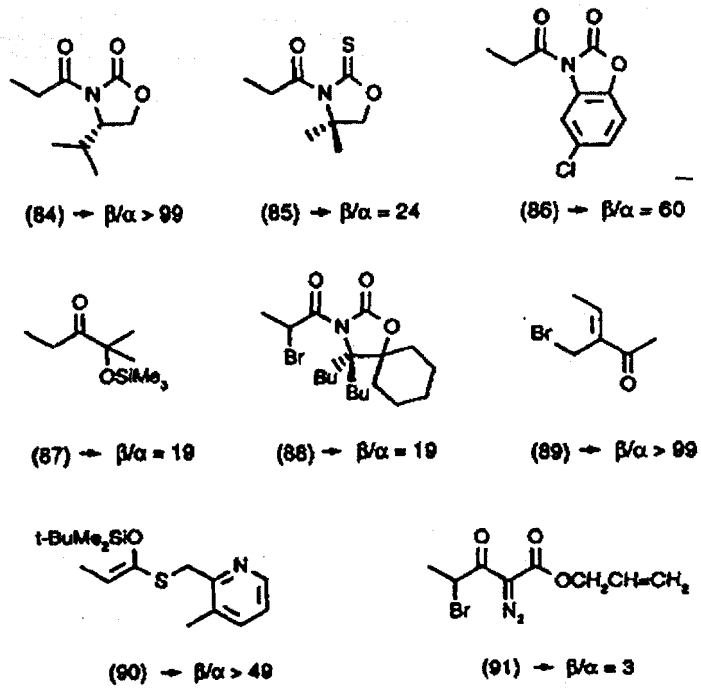
[a] Antibacterial potency relative to thienamycin = I
[b] Relative susceptibility to hog renal DHP-1 [77]



Scheme 3.8. Stereoselective synthesis of the Merck carboxylic acid (76) [100]. Reagents: (i) $Sn(OSO_2CF_3)_2$, N-ethylpiperidine, THF -50°C; (ii) azetidinone (71): (iii) 1M NaOH, THF.

Merck synthesis (*Scheme* 3.7) became an important goal in carbapenem research.

Aldol-type condensations between the chiral acetoxy-azetidinone (70) and enolates of propionic acid derivatives have been used by several research groups seeking stereoselective syntheses of the acid (76). Nagao *et al.* [100,101] used the tin enolate of the chiral *N*-propionylthiazolidinthione (82) to give the 4-substituted azetidinone (83) which was hydrolysed to the

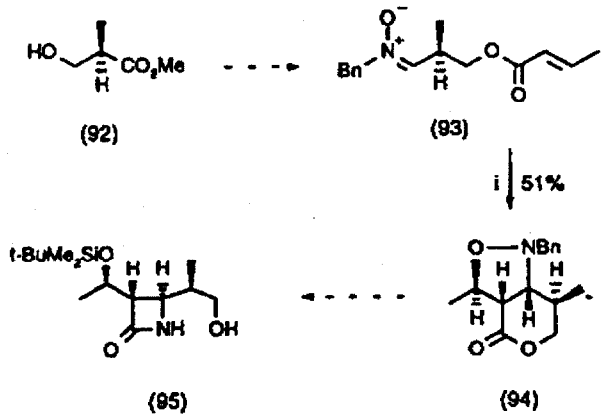


carboxylic acid (76) (*Scheme 3.8*). The Merck group [102] similarly used the boron enolate of the chiral oxazolidinone (84) (giving $\beta/\alpha > 99$). A number of achiral derivatives have been used to equally good effect, providing stereoselective syntheses of intermediates which arc convertable into the acid (76): Bristol-Myers researchers used the tin cnolatc of the oxazolidinthione (85) [103,104] and zirconium enolates of various *S*-alkyl and *S*-aryl thiopropionates [105, 106]; Sankyo scientists used the boron enolate of the oxazolidinone (86) [107]; and Shirai and Nakai used the tin cnolatc of the α-silyloxy-ketone (87) [108]. In a similar vein, stereoselective Reformatsky-type reactions have been used: Ito *et al.* used the α-bromopropionate (88) [109]; and Mori and Oida used the allylic bromide (89) [110]. Thiolesters of the acid (76) have been prepared by Lewis acid-mcdiatcd condensation of (70) with 0-silyl enol ethers of thiopropionatcs [111-113], and in this respect the ether (90), which gave a high yielding (90%) and stereoselective ($\beta/\alpha >$ 49) condensation, proved particularly useful [112].

Stereoselective aldol-type reactions have also been used to convert the azetidinone (70) directly to β-ketoester intermediates analogous to (77) and (78). Metal salt-mediated condensations beween (70) and the dianion or bis-silyl enol ether of methyl 3-oxopentanoate gave the methyl ester corresponding to (77) [114]. Although the yield and stereochemical outcome in these reactions could be independently optimised by varying the metal salt, no method emerged from this study in which both were high. Better results were obtained using the tin enolate derived from (91). which gave, after *O*-desilylation, the ally1 ester corresponding to (78) (> 98% pure; 45% overall yield) [115].

Using the aldol condensation, a number of practical, stereoselective methods have been developed for the routine preparation of 1β-methylcarbapenems. The literature contains many more excellent routes to these compounds (116–139], but not all of these will be described in detail; a few examples will be given by way of illustrating the variety of methods that are now available.

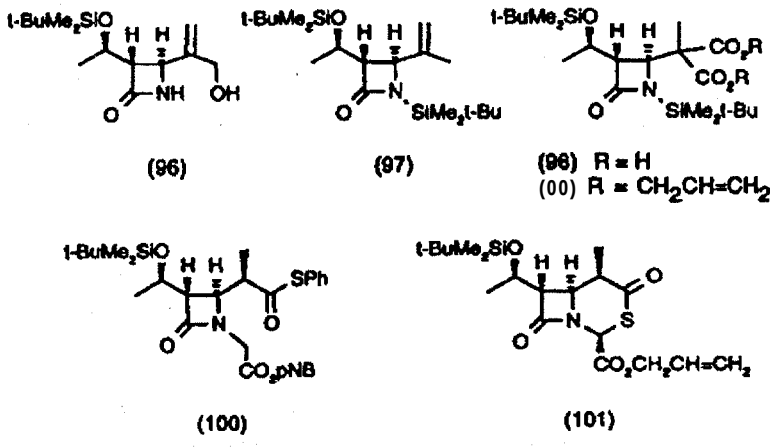
Ihara *et al.* [116] used the (*R*)-2-methylhydroxypropionate (92) to construct the nitrone (93) which underwent 8 completely sterospecific, intramolecular oycloaddition to give the isoxazolidine (94) (*Scheme* 3.9). Compound (94) was converted into the alcohol (95), which could bc oxidized to the acid (76). The alcohol (95) has also been prepared from the azetidinone (70), by way of stereoselective hydrogenation of the olefin (96) over a ruthenium catalyst [117], and by stereoselective hydroboration-oxidation of the olefin (97) [118]. Two high-yielding and stereoselective syntheses of (76) from (70) have been developed using malonic acid derivatives: the diacid (98) was decarboxylated to (76) in refluxing formic



Scheme 3.9. Nitrone cycloaddition route to 1β-methylcarbapenems [114]. Reagents: (i) refix in t-amyl alcohol. Bn = benzyl.

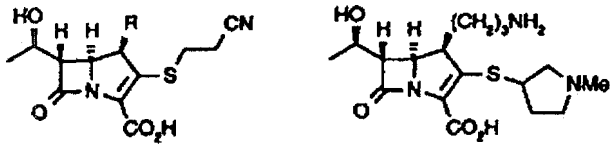
acid/ethyl acetate [119, 120]; and the diallyl ester (99) was converted into (76) in a palladium mediated deallylation-decarboxylation reaction [121].

Although for the most part, the methods outlined in *Scheme 3.7* have been used to complete the synthesis of 1β-methylcarbapenems from intermediatts such as (76), some studies have been described on alternative procedures for carrying out these later synthetic steps, For example, the Sumitomo group prepared the thiolester (100) and used this in a Dieckmann cyclization to prepare the β-ketoester (79) [MO]; and the Tanabe group converted the acid (76) into the thiazinone (101) and used the Eschenmoser



sulphide contraction to convert this into various 1β-methylcarbapenem ally] esters [141].

In addition to the considerable efforts devoted to 1β-methylcarbapenems, a few studies have been described on I-substituted-akyl carbapenems, with the object of obtaining compounds that possess the favourable stability properties of the l-methyl derivatives but improved antibacterial properties. The Shionogi group prepared 1α- and I&-substituted (fluoro, cyano, hydroxy, and acetoxy)-methyl carbapenems [142]. The 1β-fluoromethyl derivatives showed the best antibacterial activity, but overall offered littk improvement over their I/?-methyl couterparts. The Bristol-Meyers Squibb group undertook a more detailed study of 1β-substituted-alkyl carbapenems [143–147]. Incorporation of larger hydrocarbon groups (ethyl, n-propyl, vinyl, allyl) in place of the 1β-methyl group in (102) had a detrimental effect on antibacterial activity. In an attempt to overcome the loss of activity associated with increased stcric bulk, alkyl groups carrying various polar substituents were introduced. Hydroxy, ether, thioether, azido, cyano, formyl (and derivatives), and carboxy (and derivatives) substitucnts had little effect on antimicrobial activity; however, amino substituents, as in (103), did restore activity against Gram-negative organisms to the level seen in the 1β-methyl analogue and showed particularly good anti-pseudomonal activity [143]. This activity was even more pronounced in analogues



(102) R = Me
(103) R = $(CH_2)_2NH_2$

(104)

carrying an aminoalkylthio substituent at C-2; compound (104), for example, showed good activity against Gram-positive and Gram-negative organisms and better activity than imipencm against *Ps. aeruginosa*. Although these compounds have useful antibacterial activity and stability to DHP-1, they have only limited chemical stability in solution at physiological pH. In order to overcome this poor stability, various mono- and di-peptidc derivatives of the 1β-aminoethyl compound (103) were prepared [145]. These showed much increased chemical stability, but reduced activity in *vitro*. However, in *vivo* the peptide derivatives showed similar or slightly improved activity compared with the parent amine (1 03). suggesting that they were acting as prodrugs of (103) [145]. The 1β-aminoalkyl carbapen-

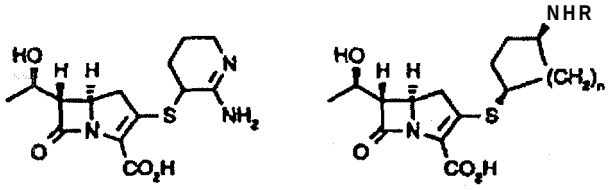
ems are an interesting development, although it still remains to be seen whether their weakness in terms of chemical instability can be overcome,

Introduction of the l&methyl group into the carbapenem nucleus gave the desired improvements in chemical and metabolic stability, and a number of practical syntheses of this class of compound have been developed. The next stage in finding an improvement on the imipeaem-cilastatin combination was the optimisation of the C-2 substituent for antibacterial potency.

MODIFICATION AT C-2

The availability of the ketoesters (45) and (79) has enabled the synthesis of a wide variety of carbapenems with thio-linked substituents at C-2. Early structure-activity studies had indicated that for potent, broad spectrum activity, and especially for activity against *Ps. aeruginosa*, a basic or positively charged group was required in the C-2 substituent, and consequently many of the compounds described in the literature are of this type.

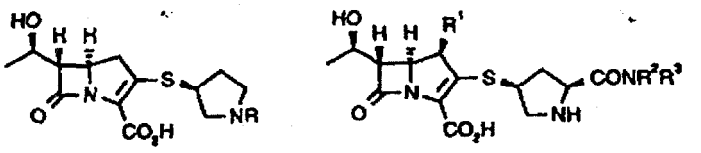
Compounds containing an amidinoalkylthio group in place of the cysteamine side-chain of thienamycin have excellent antibacterial activity, comparable to that of imipenem [86, 148]; compound (81) (*Scheme 3.7*), which also contains a 1β-methyl group, is an example of this type. Compounds in which the amidino group is incorporated in a five- or six-membered ring, as in (105), also have excellent activity [149].



(105) (106) n = 1 or 2
R = H or CH=NH

The Bristol-Myers group [150] investigated the effect of replacing the flexible ethyl chain in the cysteamine moiety by a more rigid cyclopentyl or cylohexyl ring, as in (106). Overall, the introduction of a rigid ring structure between the nucleus and the basic functionality did not significantly change the antibacterial potency. The Sankyo group [151] have also investigated the effect of using a ring system between the amino group and the nucleus, but in this case the amino function was incorporated in a five-membered ring; the amino compound (107) and its amidino derivative (108) are more active

than thienamycin against many Gram-negative organisms, including *Ps. aeruginosa*. Compound (108), known as panipenem, has recently been launched in Japan as a broad spectrum, injectable antibiotic. Although panipenem has better stability than imipenem towards renal DHP-I, it is trill extensively metabolized by this enzyme *in vivo*, and is therefore used in a 1:1 combination with *N*-benzoyl *β*-alanine (betamipron), an inhibitor of renal tubular uptake which prevents nephrotoxicity [152].



(107) R - H

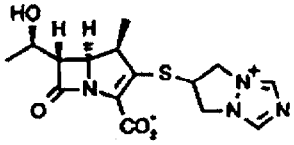
(108) R = CMe=NH; Panipenem

11091 R^1 = H; R^2 and R^3 = various (153)

(110) R^1 = R^2 = R^3 =Me: Meropenem

The 2-pyrrolidinothiocarbapenem series was developed further by the Sumitomo group, who investigated the effects of adding an aminocarbonyl group at the pyrrolidine 5'-position [153]. Compounds (109, R^2 = R^3 = H and R^2 = R' = Me) and their (3'*R*, 5'*S*), (3'*R*, 5'*R*), and (3'S, 5'*R*) isomers were studied to determine the effect of stereochemistry on activity. All were highly active antibacterials, but the *cis* compounds had the better anti-pseudomonal activity, with the (3'*S*,5'*S*) isomer (109) being the most active, Activity in the (3'*S*, 5'*S*) series was also dependent on the nature of the aminocarbonyl substituent, with the dimethyl compound being one of the most active. Finally, the effect of a 1*β*-methyl group was investigated; in comparison with (109, R^2 = R^3 = Me), compound (1 10) has improved activity against Gram-negative bacteria and greatly improved stability to DHP-1 (half-life in the presence of hog renal DHP-I was increased from 16 to 310 minutes) [153]. Compound (110). known as meropenem, is an extremely potent, broad spectrum antibacterial [154]; activity against Gram-negative organisms is better than- that of imipenem, whereas that against Gram-positive organisms is slightly worse (*Table* 3.6). Meropenem was developed jointly by Sumitomo and ICI, and has recently been launched as a parenteral antibiotic for treatment of severe bacterial infections; unlike imipenem, meropenem has sufficient stability to DHP-1 to be used without co-administration of a DHP-1 inhibitor.

C-2 Heterocyclyl-alkylthio carbapenems carrying a highly basic [155] or quaternised nitrogen or sulphur [156] are also potent antibacterials. Studies on these series revealed a correlation between the basicity of the C-2



(111) Biapenem

side-chain and activity against certain organisms, especially *Ps. aeruginosa*. Likewise, compounds containing a quaternised nitrogen or sulphur have much better anti-pseudomonal activity than the corresponding unquaternised derivatives. One notable compound of this type is the triazolium derivative (111) [101, 157, 158], known as biapcnem, which is currently being developed by Lederle as a broad spectrum. DHP-I stable carbapenem. Biapenem has better stability to DHP-I than either imipenem or meropenem [159], and appears to fall beween these two antibiotics in terms of antibacterial activity (*Table* 3.6).

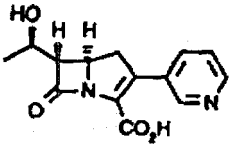
To date, panipenem, meropenem, and biapcnem represent the ultimate developments in terms of C-2 thio-substituted carbapenems; the latter two

Table 3.6. COMPARATIVE IN VITRO ANTIBACTERIAL ACTIVITIES FOR IMIPENEM (71), MEROPENEM (I 10), AND BIAPENEM (1 I I) [157]

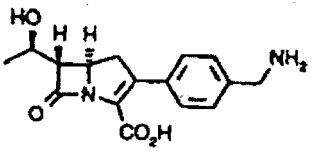
Organism (n)	*MIC90 (mgll)*		
	Imipenem	*Meropenem*	*Biapenem*
Staphylococcus aureus (19) (methicillin sensitive)	0.03	0.12	0.06
Staphylococcus aureus (27) (methicillin resistant)	16	8	8
Coagulese-negative staphylococci (26)	0.5	2	2
Streptococcus pyogenes (20)	≤0.008	≤0.008	≤0.008
Streptococcus pneumoniae (20)	1	0.06	0.03
Enterococcus faecalis (20)	0.25	4	4
Escherichia coli (24)		0.03	0.25
Klebsiella spp. (24)	0.25	0.06	0.25
Enterobacter spp. (24)	1	0.12	0.25
Serratia marcescens (22)	I	0.12	2
Proteus mirabilis (24)	2	0.12	2
Morganella morganii (26)	2	0.12	2
Pseudomonas aeruginosa (25)	8	8	4
Xanthomonas maltophilia (18)	>128	128	>128
Haemophilus influenzae (34)	2	0.12	
Moraxella catarrhalis (25)	0.06	≤0.008	A.03
Bacteriodes fagilis (31)	0.5	I	0.5

derivatives also incorporating the 1β-methyl modification. Interestingly, all these compounds contain the S-C-C-N arrangement in the C-2 group, in common with the natural carbapenem thicnamycin and its derivative imipenem.

Although thio-linked substitucnts have been the major area of study in terms of C-2 modified carbapenems, carbon-linked substituents have also attracted some attention. The Merck group [160- 162) prepared a number of 2-aryl carbapenems, such as (I 12). These compounds have good antibacterial activity (except against *Ps. aeruginosa*), and, more importantly,



(112)



(113)

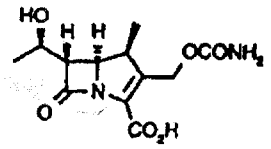
they showed better stability to DHP-1 than thicnamycin (*Table 3.7*). The antibacterial potency and stability to DHP-1 were increased by addition of a basic or quaternised nitrogen substitutnt, as in (113) (*Table 3.7*), but anti-pseudomonal activity was still less than that of thienamycin [163]. The effects of 1α- and 1β-methyl substituents in these 2-aryl derivatives were also studied [164], but, counter to expectations, these modifications resulted in a reduction in both antimicrobial activity and stability to DHP-I.

Table 3.7. RELATIVE IN VITRO ANTIBACTERIAL POTENCY AND DHP-1 SUSCEPTIBILITY OF 2-ARYL-CARBAPENEMS (112) AND (113) IN COMPARISON WITH THIENAMYCIN (14) [164]

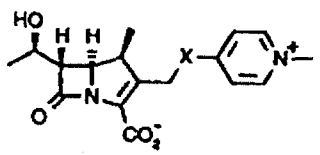
Organism (n)	*Thienamycin Mean MIC (mg/l)*	*Compound (112) Potency*[a]	*Compound (113) Potency*[a]
Staphylococcus aureus (5)	0.04	1.5	1.5
Enterococcus (3)	5	6	9.2
Escherichia coli (5)	0.3	9	3.5
Enterobacter spp. (6)	1.4	20	11
Klebsiella spp. (5)	1.3	7	3.2
Serratia spp. (2)	2.5	28	5.3
Proteus spp. (5)	7.5	17	6.5
Pseudomonas aeruginosa (5)	5.8	0.05	0.3
DHP-1 susceptibility[b]	1.0	0.4	0.02

[a] Antibacterial potency relative to thienamycin = 1
[b] Relative susceptibility to hog renal DHP-1 [77]

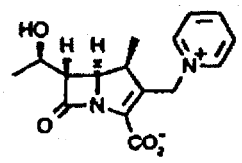
Variously substituted 2-alkyl carbapenems have also been investigated. Fujimoto *et* al. [165] prepared dcthiathienamycin and found it to be about half as potent as thienamycin. Merck workers [166] prepared a number of 2-(*O*-functionalized methyl)-l/I-methyl- carbapenems; the earbamate (I 14) is the most active of these and exceeds the activity of thienamycin against most organisms, except *Ps. aeruginosa*. The Shionogi group [167, 168] prepared a series of 2-(*S*-functionalized methyl) derivatives, and ICI Pharma [169] prepared a series of 2-(*N*-functionalised methyl) carbapenems; the 4-pyridinium compounds (115) and (116) were the best antibacterials in these series, although, like the *O*-functionalized-methyl compounds, they had weaker anti-pseudomonal activity than imipenem. Scientists at Shionogi [170] also prepared 2-(*N*-functionalised methyl)- and 2-(*N*-functionalised vinyl)-carbapenems containing a quaternised nitrogen substituent. Compounds (117) and (I 18) have the best activity, being similar to imipenem, including activity against *Ps. aeruginosa*.



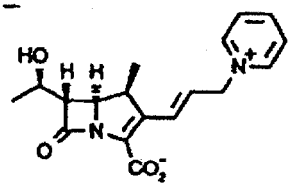
(114)



(116) X = S
(116) X - N H



(117)

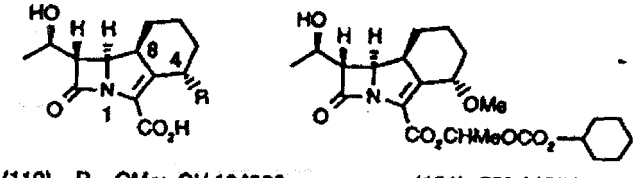


(118)

Carbapenems with carbon-linked substituents at C-2 have not received the same attention as those with a C-2 sulphur-linked substituent. Nevertheless, work in this series has resulted in compounds with. broad-spectrum activity comparable to sulphur-linked derivatives such as imipenem. An advance over the sulphur-linked compounds is provided by the 2-aryl carbapenems which have good stability to DHP-I without the addition of a I/-methyl group.

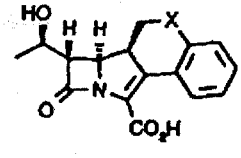
TRI- AND TETRA-CYCLIC CARBAPENEMS

A natural progression from carbapenems carrying carbon-linked substituents at C-1 and C-2 was to join these groups together so as to form a further ring structure. Glaxo scientists [171] have described a series of such tricyclic carbapenem structure, known as tribactams. Compound (119), GV 104326,



(119) R = OMe; GV 104326
(120) R = H, SMe, SOMe, NHMe

(121) GV 118819



(122) X = CH_2
(123) X = S

was found to be substantially mom active than its C-4 and C-8 stereoisomers, and, in terms of its overall antibacterial activity and stability to DHP-1, was also preferred to its analogues (120) [172]. This tribactam has broad-spectrum activity (excluding *Ps. aeruginosa*) (*Table 3.8*) and is completely stable to DHP-1; its hexetil prodrug ester (121), GV 118819, is orally absorbed in mice (bioavailability of GV 104326 = 77%) and shows good efficacy in murine infection models [174]. Recently, the pharmacokinetics of GV 104326 have been determined following single intravenous and oral (as its prodrug GV 118819) administration in man [175]. The kinetics of GV 104326 were linear up to doses of 1 g (ix) and 0.5 g (p.o.), oral bioavailability was about 40%, and the compound was well tolerated in both studies.

Scientists at Hoechst [176] and Bayer [177] prepared a number of tetracyclic carbapenems, and found that, as in the tribactams, the β-stereochemistry is required at the carbapenem C-1 position for optimal antibacterial activity. Compounds (122) and (123) are two of the more

active members of this series, and show good activity against Gram-positive bacteria, but only moderate activity against Gram-negative organisms and no activity against *Ps. aeruginosa*. The pivaloyloxymethyl ester of (123) was orally absobed in mice [177].

Tri- and tetra-cyclic structures are an interesting development in the area of non-natural carbapenems. These compounds have good stability to DHP-I, but to date have not shown the potent, truly broad-spectrum activity that distinguishes carbapenems such as imipenem and meropenem. However, the demonstration of good oral absorption by way of pro-drug esters is a notable advance.

CLINICAL AND COMMERCIAL ASPECTS

Since its introduction into clinical use in 1985, the imipenem-cilastatin combination (Primaxin) has largely lived up to its initial promise. Imipenem has the broadest spectrum of antibacterial activity of all antibiotics

Table 3.8. COMPARATIVE IN VITRO ANTIBACTERIAL ACTIVITIES FOR IMIPENEM (71) AND TRIBACTAM GV 104326 (119) [173]

organism (n)	MIC_{90} (mg/l)	
	Imipenem (71)	GV 104326 (119)
Staphylococcus aureus (27) (methicillin sensitive)	SO.015	0.12
Staphylococcus aureus (15) (methicillin resistant)	4	16
Staphylococcus epidermidis (12)	50.015	0.006
Streptococcus pyogenes (9)	0.003	0.015
Streptococcus pneumoniae (11)	0.003	0.007
Enterococcus faecalis (10)	2	4
Escherichia coli (10)	0.25	0.5
Klebsiella pneumoniae (11)	0.5	2
Enterobacter cloacae (10)	1	2
Serratia marcescens (I 0)	>32	16
Proteus mirabilis (1 I)	4	2
Proteus vulgaris (9)	8	I
Morganella morganii (11)	4	4
Haemophilus influenzae (11)	1	0.25
Haemophilus parainfluenzae (7)	I	I
Moraxella catarrhalis (8)	0.25	0.5

available for *systemic* use in humans. It is used as initial monotherapy of a variety of moderate to severe infections, with a clear role in empirical treatment of mixed infection. Administration is generally in 500mg to 750mg intravenous doses every 6 hours. The daily cost of imipenem is one of the highest of the currently available parenteral antibiotics, although this is approximately the same as that of most double-antibiotic regimens and less than that of most conventional triple-antibiotic regimens. In 1993, Primaxin was the 10th best selling antibiotic worldwide and the total audited saks exceeded £277 million [178].

Imipenem is not, however, without its drawbacks. The most common adverse effects arc nausea and vomiting, which are often associated with over-rapid intravenous infusion, and seizures, the incidence of which is unacceptably high for patients with underlying neurological disease or impaired renal function. Imipenem is not recommended for therapy of meningitis. Another shortcoming of imipenem is in the treatment of infections involving *Ps. aeruginosa*, where clinical failures have occurred as a result of development of resistance (*i.e.* selection of insensitive strains) during therapy.

Meropenem addresses at least some of these deficiencies. It is reported to be less epileptogenic than imipenem and has better activity against *Ps. aeruginosa* [154]. Imipenem is a powerful inducer of chromosomal β-lactamase, and moderate level resistance in *Ps. aeruginosa* has been attributed to slow hydrolysis by β-lactamase combined with reduced permeability; the overall result of this being that, at therapeutic concentrations, insufficient number of molecules reach the target PBPs [179]. Meropenem, being a poorer inducer of β-lactamase, is subject to less hydrolysis in strains with reduced permeability, and is thus better able to combat moderately imipenem-resistant pseudomonas. Highly imipenem-resistant *Ps. aeruginosa*, however, are also highly resistant to meropenem.

Although imipcncm and meropenem are resistant to most serine β-lactamases, they are subject to rapid hydrolysis by a number of bacterial zinc metallo-β-lactamase enzymes. Such enzymes have been found in *Xanthomonas maltophila*, *Aeromonas hydrophila*, and *Bacteroides fragilis* (180]. All strains of *Xanthomonas maltophila* produce a metallo-β-lactamase, and are consequently resistant to carbapenems. The possible emergence of further strains which produce carbapenem-hydrolysing enzymes represents a potential threat to the future use of these otherwise extremely potent antibiotics.

SUMMARY

The discovery of the olivanic acids and thienamycin aroused considerable interest amongst medicinal chemists and microbiologists around the world, The susceptibility of these agents to metabolic degradation has, however, been a major obstacle in their development. For many years the only notabk success from such intensive research was the combination of imipenem with cilastatin, an inhibitor of the renal dipeptidase enzyme DHP-1. The enormous success of Primaxin for the treatment of a range of life-threatening bacterial infections provided the impetus for the discovery of totally synthetic, non-natural carbapenem derivatives that combine the broad spectrum of antimicrobial activity with stability to enzymatic degradation. This has indeed been realised in the development of meropenem; it possesses the broad spectrum of activity and resistance to β-lactamases that arc embodied in imipenem as well as displaying increased stability to human dehydropeptidases.

Most recent research has focused upon the development of carbapenem antibiotics which combine broad spectrum antimicrobial activity and metabolic stability with oral absorption, for the treatment of community-acquired infections. Indeed, the pro-drug esters of the tricyclic carbapenems represent the first significant advance in this respect.

However, the increased use of carbapenem antibiotics would undoubtedly accelerate the emergence of carbapenem-hydrolysing enzymes. The ultimate *challenge* could therefore be the design and synthesis of carbapenem derivatives that are resistant to these metallo-β-lactamases.

Due to the enormous probkms encountered in the development of the carbapenem antibiotics, this area of research has, in the past, been described as a battlefield that did not bode well for the future [181]. Primaxin and meropenem proved however that these problems were not insurmountable, and are therefore a testimony to the persistence and dedication of those scientists in their war against bacterial infection.

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