

RECENT ADVANCES IN THE CHEMISTRY OF
 **β -Lactam
Antibiotics**

EDITED BY A.G.BROWN
AND S.M.ROBERTS

Third International Symposium
1984

Recent Advances in the Chemistry of β -Lactam Antibiotics

The Proceedings of the Third International Symposium,
arranged by the Fine Chemicals and Medicinals Group
of the Industrial Division of The Royal Society of Chemistry

Cambridge, England, 2nd—4th July 1984

Edited by

A. G. Brown

Beecham Pharmaceuticals Research Division

S. M. Roberts

Glaxo Group Research Ltd.



Y078425

The Royal Society of Chemistry
Burlington House, London, W1V 0BN



Copyright ©,1985
The Royal Society of Chemistry

All rights reserved

No part of this book may be reproduced or transmitted in any form or by any means—graphic, electronic, including photocopying, recording, taping or information storage and retrieval systems — without written permission from The Royal Society of Chemistry

British Library Cataloguing in Publication Data

Recent advances in the chemistry of β -lactam antibiotics: the proceedings of the third international symposium arranged by the Fine Chemicals and Medicinals Group of the Industrial Division of the Royal Society of Chemistry: Cambridge, England, 2nd—4th July 1984.— (Special publication; no. 52)

1. Beta lactamases 2. Antibiotics

I. Brown, A. G. II. Roberts, S. M. III. Royal

Society of Chemistry IV. Series

615'.329 RM666.C379

ISBN 0-85186-955-6

Printed in Great Britain by
Whitstable Litho Ltd., Whitstable, Kent

Preface

Worldwide sales of anti-bacterial substances amount to ca. £6,000M per annum. About 60% of this figure can be allocated to the β -lactam antibiotics and it can be estimated that β -lactams account for about 10% of total drug sales.

Not surprisingly, therefore, many laboratories in academia and the pharmaceutical industry are continuing the search for new and improved anti-bacterial substances based on the β -lactam template. The 3rd International Conference on the Chemistry of the β -Lactam Antibiotics held at Churchill College, Cambridge in July 1984 allowed a timely review of this area of research. This book is a collection of articles written by the various contributors at the Conference. In the main the articles contain the results of research accomplished since the last Conference in this series in 1980.

In this period of time new cephalosporins have been found with highly impressive anti-bacterial activity especially against Gram-negative organisms. Equally, potent penicillins have been developed with improved β -lactamase stability. Penems and carbapenems (structures brought to the attention of a wide audience in the first Conference in this series in 1976) have been synthesized which display anti-bacterial activity against a wide range of organisms. The more recently discovered monocyclic β -lactams (monobactams) have been subjected to close scrutiny and new chemical routes to these compounds have been devised. The low cost of some β -lactams (e.g. clavulanic acid, simple penicillins and cephalosporins) makes these materials suitable synthons for the preparation of new entities retaining the β -lactam ring. In all of these areas the authors disclose fascinating new chemistry.

Further progress has been made in elucidating the mechanism of interaction of β -lactam antibiotics with key enzymes engaged in bacterial cell-wall synthesis and with the β -lactamase enzymes. X-Ray crystallographic data have been obtained on some of these enzymes with and without a β -lactam substrate at the active site. Much more information of this type is needed before sensible de novo design of anti-bacterial (non- β -lactam?) substances can begin.

Thus this text book summarizes some of the recent advances in β -lactam research conducted in Europe, the U.S.A., and Japan. We thank all the authors for their prompt preparation of the manuscripts and Mr Alan Cubitt (Royal Society of Chemistry) for invaluable help in the final preparation of the text.

August 1984

London

A.G. Brown

S.M. Roberts

Contents

1	The Synthesis and Biological Properties of Recent Cephalosporins	1
	By C.E. Newall	
2	The Mechanisms of Reactions of Cephalosporins and the Synthesis of Non- β -Lactam Analogues	18
	By D. Agathocleous, S. Buckwell, P. Proctor, and M.I. Page	
3	Recent Advances in the Chemistry and Biology of Penicillins	32
	By R.J. Ponsford, M.J. Basker, G. Burton, A.W. Guest, F.P. Harrington, P.H. Milner, M.J. Pearson, T.C. Smale, and A.V. Stachulski	
4	Studies on the Methoxylation of Cephalosporins and Penicillins with Cell-free Enzyme Preparations from <u>Streptomyces</u> Species	52
	By J.D. Hood, A.G. Brown, A.L. Elson, M.L. Gilpin, and R. Holmes	
5	Recent Advances in Penicillin Biosynthesis	62
	By J.E. Baldwin	
6	Structure-Activity Relationships Among Some Totally Synthetic Carbapenems	86
	By A. Andrus, F. Baker, F.A. Bouffard, L.D. Cama, B.G. Christensen, R.N. Guthikonda, J.V. Heck, D.B.R. Johnston, W.J. Leanza, R.W. Ratcliffe, T.N. Salzmann, S.M. Schmitt, D.H. Shih, N.V. Shah, K.J. Wildonger, and R.R. Wilkening	
7	Synthesis and Some Antibacterial Properties of 2-Oxypenems	100
	By M.D. Cooke, K.W. Moore, B.C. Ross, and S.E. Turner	

8	Cycloaddition Reactions of Some Olivanic Acid Derivatives	116
	<i>By J.H. Bateson, D.F. Corbett, and R. Southgate</i>	
9	Synthesis and Antibacterial Activity of Asparenomycins and Related Carbapenem and Penem Antibiotics	131
	<i>By S. Uyeo</i>	
10	β -Lactam Synthesis from 2-Pyridone Photoisomers	151
	<i>By C.J. Ashcroft, J. Brennan, W.J. Norris, and C.A. Robson</i>	
11	The OA-6129 Group of Carbapenems and the Biosynthesis of Carbapenem Antibiotics	163
	<i>By Y. Fukagawa, M. Okabe, T. Yoshioka, and T. Ishikura</i>	
12	Synthesis of Novel Bicyclic β -Lactam Derivatives	183
	<i>By R.J. Stoodley</i>	
13	6-Diazopenicillanate Chemistry and Some Aspects of Penam Rearrangements	193
	<i>By D.I. John</i>	
14	Do β -Lactam Antibiotics Require a β -Lactam Ring?	209
	<i>By G. Lowe and S. Swain</i>	
15	The Chemistry of Clavulanic Acid and Its Derivatives	222
	<i>By G. Brooks, G. Bruton, M.J. Finn, J.B. Harbridge, M.A. Harris, T.T. Howarth, E. Hunt, I. Stirling, and I.I. Zomaya</i>	
16	YTR-830 and Related Active β -Lactamase Inhibitors	242
	<i>By T.W. Hall, S.N. Maiti, R.G. Micetich, P. Spevak, S. Yamabe, N. Ishida, M. Kajitani, M. Tanaka, and T. Yamasaki</i>	
17	On the Design of New β -Lactamase Inhibitors	255
	<i>By C.D. Foulds, M. Kosmirak, A.C. O'Sullivan, and P.G. Sammes</i>	
18	Synthesis of Penem Antibiotics	266
	<i>By A. Afonso, A.K. Ganguly, V. Girijavallabhan, and S. McCombie</i>	
19	Mechanisms of Action of β -Lactamases	280
	<i>By R. Bicknell, S.J. Cartwright, E.L. Emanuel, G.C. Knight, and S.G. Waley</i>	

- 20 The Chiral Synthesis of 3-Acylamino-2-oxoazetidines
with Various N-1 Functionality 293
*By C.M. Cimarusti, T.H. Denzel, R. Fox, G.A. Jacobs,
T.P. Kissick, W.H. Koster, J.L. Moniot, and R.H. Mueller*
- 21 Synthesis, Chemical, and Biological Properties of
Substituted N-Hydroxy-2-azetidinones 305
By A. Biswas, M.J. Miller, M.A. Krook, S.R. Woulfe
- 22 X-Ray Structure of Transpeptidase Enzyme and Its
 β -Lactam Binding Site 318
By J.B. Bartolone, G.J. Hite, J.A. Kelly, and J.R. Knox

Posters Presented at the Symposium

- ¹
Substitution Reactions on 3-Bromomethyl-3-cephems and
Their Sulphone Analogues with Acyloxy Residues 331
*By M. Botta, F. De Angelis, F. Giannessi, M. Marzi,
and R. Nicoletti*
- Synthesis of 3-Substituted Cephalosporins from
Penicillins 336
By J.F. Peyronel, C. Moutonnier, and B. Plau
- Reaction of Cephalosporin Derivatives with Cerium(IV)
Ammonium Nitrate 342
*By R.A. Fletton, D.C. Humber, S.M. Roberts, and
J.L. Wright*
- The Synthesis of 4-Amino-5-thiazolyl- and 5-Amino-4-
thiazolyl-acetamidocephalosporins 346
By B.E. Looker and I.L. Paternoster
- Synthesis and Structure-Activity Relationships of
Acylureido-oxacephem Derivatives 350
By W. Hartwig, D. Habich, P. Naab, and K. Metzger
- New Syntheses of Biologically Active Penems with
Extensive Utilization of the Penam Framework Elements 357
*By C. Battistini, M. Alpegiani, A. Bedeschi, E. Perrone,
C. Scarafile, and G. Franceschi*
- Penems from 2-Thiacephem 361
*By E. Perrone, M. Alpegiani, R. Battaglia, A. Bedeschi,
and G. Franceschi*

Synthesis of Fluorinated Carbapenems: Reactions of Monocyclic and Bicyclic β -Lactams with Diethylamino-sulfur Trifluoride	366
<i>By C.P. Mak and K. Wagner</i>	
A 'Biogenetically Patterned' Preparation of β -Lactams	371
<i>By J. Nally, N.H.R. Ordsmith, and G. Procter</i>	
6-Bromopenicillanic Acid Sulphoxides: Preparation Isomerization, and Synthetic Utilization	375
<i>By W. von Daehne, E.T. Hansen, and N. Rastrup-Andersen</i>	
A Re-examination of the Kinetic Parameters of the <u>Bacillus cereus</u> Type I β -Lactamase	381
<i>By Y. Fukagawa, M. Sakamoto, and T. Ishikura</i>	
Stereocontrolled Annelation of Imines to Enantiomeric β -Lactams	387
<i>By A.K. Bose, M.N. Manhas, J.M. van der Veen, S.S. Bari, D.R. Wagle, and V.R. Hegde</i>	

The Synthesis and Biological Properties of Recent Cephalosporins

By C. E. Newall

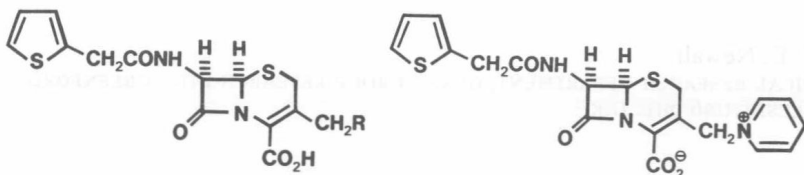
CHEMICAL RESEARCH DEPARTMENT, GLAXO GROUP RESEARCH LTD., GREENFORD, MIDDLESEX UB6 0HE, U.K.

In the eight years since the first Symposium¹ many novel β -lactam antibiotics have been described; most of them, for example clavulanic acid, the carbapenems and the mono-bactams, were first discovered as natural products. Others, such as the penems first described by Woodward at the 1976 Symposium, and the oxacephamycins (e.g. moxalactam) are based on ring systems not found in Nature. This Meeting will provide us with a feast of novel chemistry but this afternoon you will have to be content with a more pedestrian affair in which I shall review the clinical requirements which led to the development of the recent broad-spectrum cephalosporins, the methods by which they may be made and, finally, their biological properties.

As we all know, the β -lactam story began with the penicillins, which in the 1940s provided the first really safe and effective chemotherapy for serious bacterial infections. The early compounds possessed a narrow spectrum of activity, confined largely to Gram-positive bacteria, and resistance caused by penicillinases appeared all too rapidly. In the 1950s the use of 6-aminopenicillanic acid as a synthetic intermediate afforded compounds such as ampicillin with a broader spectrum of activity including some important Gram-negative pathogens such as E.coli. Narrow spectrum antibiotics, e.g. methicillin, which resisted hydrolysis by penicillinases were also developed.

The isolation of cephalosporin C, from a strain of Cephalosporium acremonium collected by Brotzu in 1945, opened a new vista in β -lactam chemistry, which by 1965 had afforded two broad-spectrum antibiotics, the closely related cephalothin and cephaloridine. Cephalothin, which carries the 3-acetoxymethyl group present in the parent cephalosporin C, is somewhat unstable in the presence of mammalian esterases, being partially converted into the less active desacetyl metabolite in the body. In cephaloridine the acetoxy group is replaced by a pyridinium group, which is not metabolised.

Scheme 1



Cephalothin R=OAc

Desacetylcephalothin R=OH

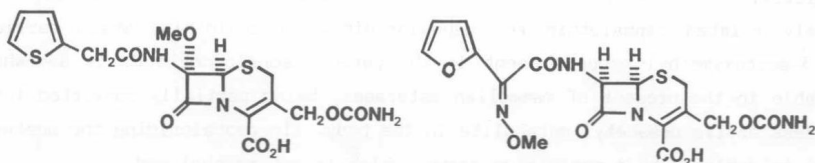
Cephaloridine

Although cephalothin and cephaloridine were stable to Gram-positive penicillinases their widespread use soon revealed the presence of β -lactamases produced by Gram-negative bacteria and by 1970 resistance attributable to these enzymes had become a serious problem. Furthermore, although the early cephalosporins had a broader-spectrum of activity than the penicillins then available, they were not effective against some clinically important bacteria, including *Pseudomonas* and some *Proteus* species.

Resistance caused by β -lactamases may be prevented by co-administering a lactamase inhibitor, such as clavulanic acid, or by using compounds intrinsically stable to these enzymes. Examples of the second approach are the cefoxitin and cefuroxime.

Cefoxitin derives its stability to β -lactamases from the presence of the characteristic cephamycin 7 α -methoxy group; the stability of cefuroxime has been attributed to the *syn*-oxime function in the side-chain. Both compounds are effective against lactamase producing strains of many common bacteria.

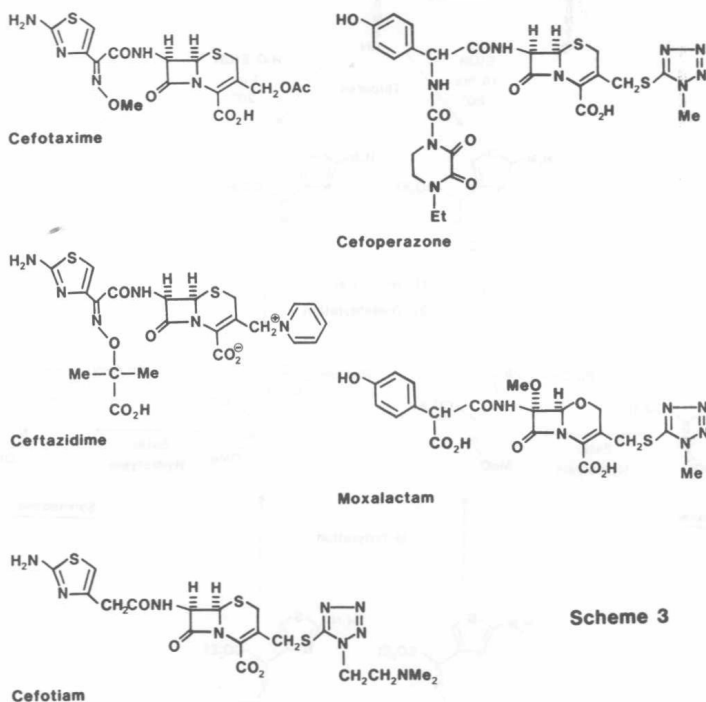
Scheme 2



Cefoxitin

Cefuroxime

Despite the improved spectrum of these compounds, further improvements were still possible and by 1980 three new groups of broad-spectrum antibiotics had emerged. These were the 2-aminothiazol-4-ylacetamido cephalosporins, exemplified by cefotaxime and ceftazidime, the ureidophenylglycyl penicillins and cephalosporins, such as azlocillin and cefoperazone, and the desthia-oxacephamycins, for example moxalactam. The 7-acyl group of cefotaxime bears a marked resemblance to that of cefuroxime and those of cefoperazone and moxalactam to the 7-acyl groups of the penicillins piperacillin and carbenicillin, respectively.



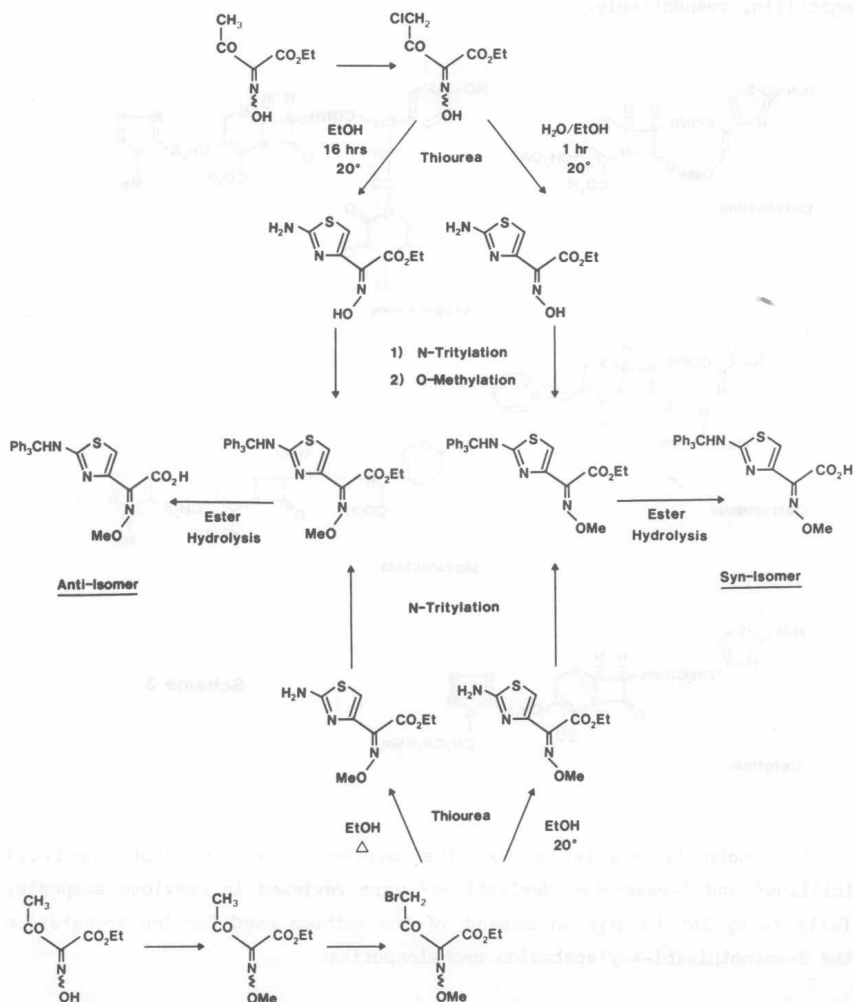
Scheme 3

The methods available for the synthesis of ureidophenylglycyl penicillins² and 1-oxacephem derivatives³ were reviewed in previous symposia; it falls to my lot to give an account of the methods used for the preparation of the 2-aminothiazol-4-ylacetamido cephalosporins.

In this group the simplest side-chain is derived from the very readily available 2-aminothiazol-4-ylacetic acid; one clinical candidate, cefotiam⁴,

carries this 7-substituent. In all other cases the 7-acyl substituent contains an alkoximino group, often a methoximino group. A number of methods for preparing these side-chain acids have been reported; the work of Bucourt and his colleagues provides good examples of the synthesis of the lower alkoximes⁵.

Scheme 4



Thus, chlorination of ethyl 2-hydroxyimino-3-oxobutyrate with sulphuryl chloride, as described by Hatanaka and Ishimaru⁶, followed by reaction of the resulting 4-chloro ester with thiourea in dry ethanol for 16 hours at room temperature affords the anti-isomer in about 60% yield. However, the same reactants provide a similar yield of the corresponding syn-isomer after one hour at room temperature in aqueous ethanol. It would seem that both the syn and anti-isomers are formed in the reaction and that a slow isomerisation of the former to the latter occurs under the reaction conditions in dry ethanol. This isomerisation, which is probably catalysed by the hydrochloric acid liberated during the cyclisation process, may be accelerated by heating the reaction mixture and largely be prevented by the addition of a base. Tritylation of the anti and syn oximes proceeds smoothly and the resulting N-protected oximes may then be O-alkylated, for example with dimethyl sulphate, and saponified to give the corresponding N-tritylated 2-methoxyimino side-chain acids.

In cases where the group used to alkylate the oxime function is a methyl group an alternative procedure has been described. Thus, methylation of ethyl 2-hydroxyimino-3-oxobutyrate followed by bromination gave the 4-bromo-2-methoxyimino ester; this cyclised with thiourea in refluxing dry ethanol to give the anti-isomer. On the other hand cyclisation at room temperature gave the syn-isomer, which could be converted into the anti-isomer by treatment with hydrogen bromide in aqueous ethanol.

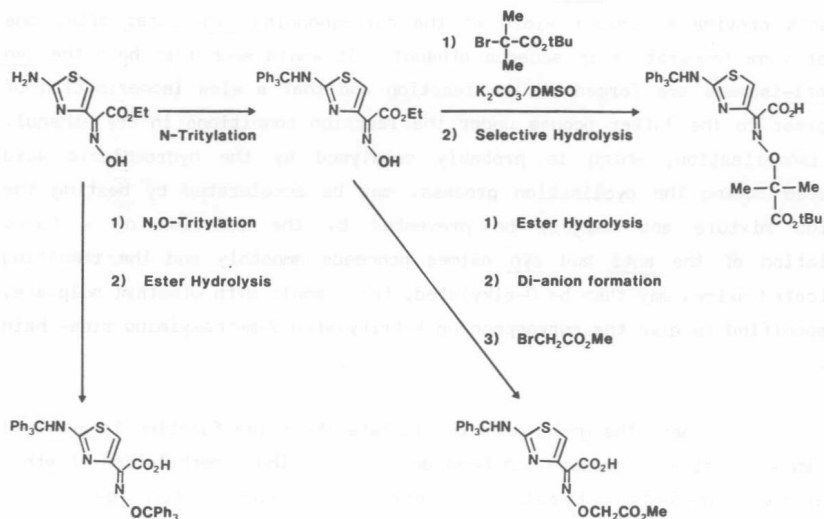
In this and other cases where both the syn- and anti-isomers are available they may often be distinguished by the position of the thiazole 5-proton. Thus, in the case of the tritylated methoxime esters the thiazole 5-proton of the syn-isomer resonates at 6.47 ppm and that of the anti-isomer resonates at 7.32 ppm in deuteriochloroform.

Although convenient for the synthesis of some alkoximes, alkylation of the hydroxime prior to formation of the thiazole ring is not generally useful as it may restrict the possibilities for later modification of the molecule. As the thiazole amino group may readily be protected, the syn-hydroxime ester provides a generally useful intermediate for the alkylation step.

Thus, the hydroxime may be O-alkylated and the ester group saponified to provide the N-protected side-chain acid ready for coupling with 7-ACA or one of its derivatives. A method for the preparation of the side-chain of ceftazidime illustrates this procedure; note that in the last stage of this

sequence the ethyl ester group is selectively hydrolysed in the presence of the more sterically hindered t-butyl ester.

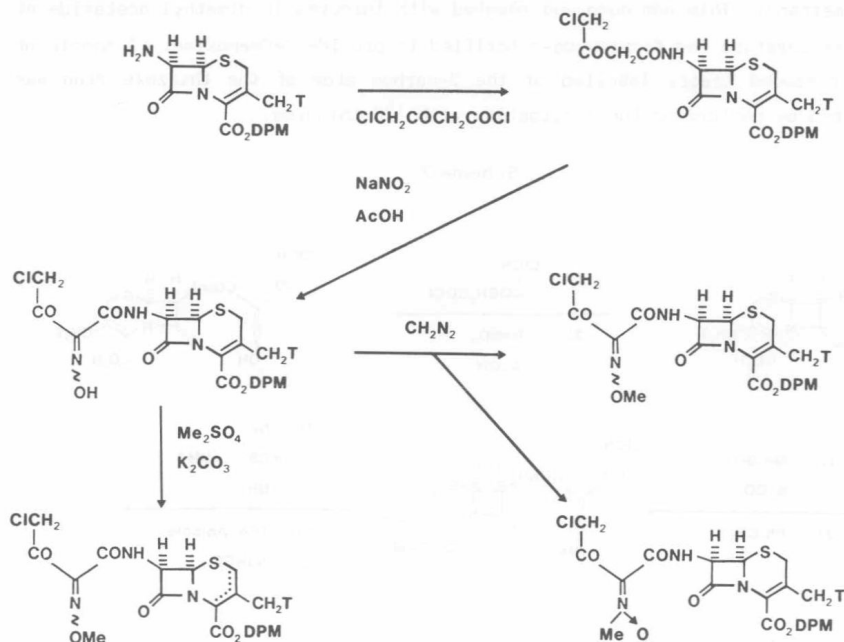
Scheme 5



However, when the gem-dimethyl groups flanking the t-butyl ester are absent considerable hydrolysis of both ester groups occurs and a mixture of the mono-esters and the di-acid results. In such cases a 'di-anion' alkylation may be used. Thus, the tritylated hydroxime ethyl ester may be hydrolysed to the acid, which is treated with the appropriate alkyl halide in the presence of two or more equivalents of base. Alkylation occurs preferentially at the oxime oxygen and the side-chain acid is formed directly.

Complete tritylation of the oxime ester under more vigorous conditions provides the bis-trityl ester, which may be saponified to give the side-chain acid with the oximino group protected. This may be coupled with a 7-aminocephalosporin and the trityl groups removed simultaneously to afford the hydroximino cephalosporin. Attempts to couple the hydroximino acid with 7-amino cephalosporins tend to give poor yields, most of the side-chain acid being converted into a nitrile.

Although the synthesis of the side-chain will normally be complete, apart from minor adjustments of functionality, before it is coupled with the cephalosporin nucleus, this need not always be the case.



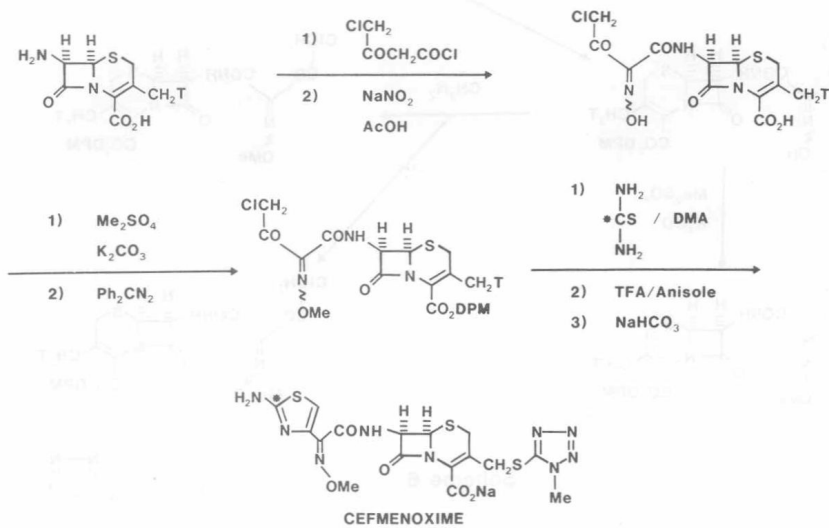
Scheme 6

For example, Ochiai and his co-workers⁷ at Takeda reacted the diphenylmethyl (DPM) ester of a 7-aminocephalosporin with 4-chloro-3-oxobutyryl chloride (prepared by the addition of chlorine to diketene) to give the 7-chloroacetylacetamido cephalosporin ester. Oxymination with sodium nitrite afforded the oxime, which on methylation with diazomethane provided a mixture of the desired methoxime and a nitron. On the other hand, methylation with dimethyl sulphate in the presence of potassium carbonate gave a mixture of the methoxime and its Δ^2 -isomer but none of the nitron.

Now, the isomerisation of a Δ^3 -cephalosporin ester to a Δ^2 -cephalosporin ester is known to occur readily in the presence of base, but the corresponding acids do not usually isomerise under these conditions. An obvious modification to the reaction was to react the cephalosporanic acid with dimethyl sulphate and potassium carbonate, thus avoiding these undesirable reactions. Scheme 7 illustrates the reaction scheme which successfully provided the chlorinated open-chain methoximino cephalosporin ester after esterification with diphenyl

diazomethane. This compound was reacted with thiourea in dimethyl acetamide at room temperature and finally de-esterified to provide cefmenoxime. A sample of this compound tracer labelled at the 2-carbon atom of the thiazole ring was prepared by performing the cyclisation with ^{14}C thiourea.

Scheme 7



Once the desired side-chain acid has been prepared in a suitably protected form it may be coupled with the appropriate derivative of 7-ACA. However, before considering the preparation of the latter we should look at the methods available for the coupling reaction.

All the coupling methods traditionally used in cephalosporin and penicillin chemistry may be used. Thus an activated derivative of the side-chain acid is reacted with the desired 7-aminocephalosporin or one of its esters. The activated derivative may be an acid halide, an anhydride or an active ester and may be formed *in situ*. Scheme 8 illustrates the use of an acid chloride in the coupling reaction from a synthesis of ceftriaxone - note that no protection of the cephalosporin carboxyl group was necessary⁸.

A commonly used method for coupling involves the use of a condensing agent, such as dicyclohexyl carbodiimide (DCC). This may be added to a solution of the side-chain acid, forming a symmetrical anhydride with which the