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Contributions to the chemistry of β-lactam antibiotics: 1-oxa nuclear analogs of naturally occurring β-lactam antibiotics

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Abstract — Contributions to the chemistry of β-lactam antibiotics which have been performed for the past fifteen years are summarized. The work comprises syntheses of 1-oxa nuclear analogues of naturally occurring β-lactam antibiotics in relation with structure-activity relationships. As the result of these studies several promising compounds of the 1-oxacephem series were found. Among these, latamoxef **18** and flomoxef **33g** proved to have excellent biological properties and are currently used clinically as the third and the fourth generation β-lactam antibiotics respectively. Unstable 1-oxapenem derivatives **61a, b, c** were also synthesized and tested for their biological properties. It was demonstrated that these compounds were the strong cephalosporinase inhibitors like *epi*-thienamycin B rather than clavulanic acid. Highly strained tricyclic 1-oxacephem derivative **86** was also synthesized. Disappointingly this unique β-lactam compound showed only weak activity against gram-positive bacteria. In addition to this 1-oxacephem derivatives, a practically useful synthetic method for 3'-nor-cephalosporin nucleus was established and applied to the production of an oral β-lactam antibiotic, ceftibuten **11**, which was recently discovered in Shionogi Research Laboratories.

INTRODUCTION

As is well known, the so-called semisynthetic penicillins and cephalosporins, which are chemically derived from the corresponding naturally occurring β-lactams such as penicillin G **1** and cephalosporin C **2**, have played a principal role in treating infectious diseases for a long time owing to their dramatic efficacy and little adverse effect. The situation has not been altered even at the present time, rather the excitement of and interest in the β-lactam antibiotics have been raised to a peak by the recent discovery of the so-called non-classical β-lactam antibiotics such as clavulanic acid **3**, thienamycin **4** and monobactam (sulfazecin) **5**, whose biological properties have been found conspicuously unique.

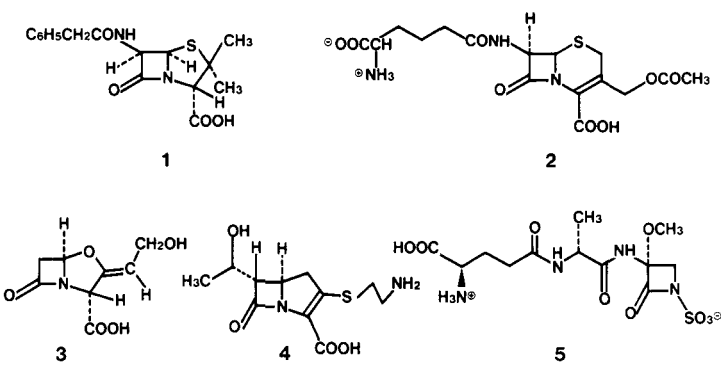


CHART 1

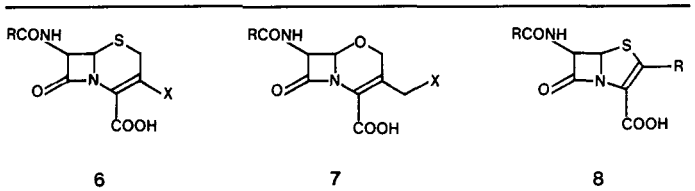


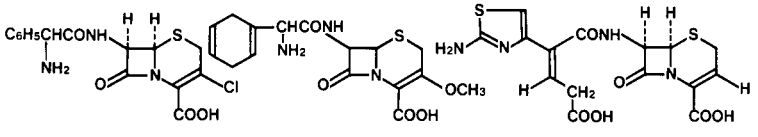
CHART 2

It has been remarkably recognized that chemical modifications of penicillins or cephalosporins to obtain β-lactams exhibiting better biological properties had been carried out mostly at the side chain parts of the mother compounds until early in the 1970's, and, to our knowledge, only a few instances at the nuclear parts at the time when we started β-lactam research in 1974. We suspected that the side chain modifications had been worked out by the precedent other research groups and that we were too late to obtain a new and useful side chain in our hand. We, therefore, decided to explore the possibility of nuclear modification. This strategy seemed to be appropriate and even effective from the view point that remarkable progress had been made in the action mechanism and in the structure-activity relationships of β-lactam antibiotics. The plans included: (1) synthesis of the

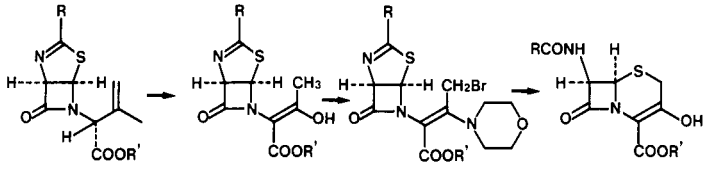
3'-nor cephalosporin nucleus **6**, (2) synthesis of the 1-oxa-1-dethia cephalosporin nucleus **7**, and (3) synthesis of the penem nucleus **8**. The last plan was later cancelled, because there was a rumor that Professor R. B. Woodward (deceased, July 8, 1979) accomplished synthesis of a penem compound which was found very labile.

3'-NORCEPHALOSPORINS, CEFTIBUTEN AND ITS SYNTHESIS

Soon after we began the synthesis, we noticed that two orally effective β-lactams of the second generation had been developed, the one being cefaclor (CCL) **9** discovered in Eli Lilly Laboratories and the other being cefroxadine (CXD) **10** in Woodward Institute in Basle. Both compounds belong to the 3'-nor type cephalosporin as illustrated and good synthetic methods were also reported to be developed in each laboratories. Our efforts had been centered also upon developement of our own efficient, practical synthetic method which was achieved by the end of 1974 (ref. 1). The key steps in the synthesis is functionalization at the terminal methyl group in **13** to obtain **14** which on acid hydrolysis can be converted into the 3'-norcephalosporin **15** very smoothly. This functionalization was effected by treating the thiazolidinoazetidinone **13** successively with $MsCl/Et_3N/THF$, morpholine and bromine at a low temperature. In another project a practically useful method was developed for ester deprotection which comprises the use of $AlCl_3$ in the presence of anisole at a low temperature (ref. 2) and the method was successfully applied to the present synthesis.



9 10 11



12 13 14 15

(R = $C_6H_5CH_2-$ or $C_6H_5OCH_2-$ R' = $-CH_2-$ $-NO_2$ or $-CH(C_6H_5)_2$)

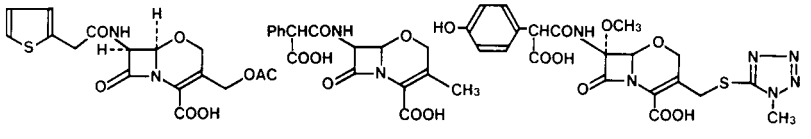
CHART 3

It is worthy to add that very recently a new oral cephalosporin **11** named ceftibuten (ref. 3) was discovered in our laboratories. Unusually high absorbability in oral administration is characteristic of this cephalosporin and this compound is now under clinical evaluation as a third generation oral cephalosporin. The synthetic method just shown in this chart is now nicely applied to the production of ceftibuten on an industrial scale.

1-OXACEPHEMS (1), LATAMOXEF (MOXALACTAM)

In parallel with the project described above, considerable efforts had also been made to synthesize 1-oxa-1-dethia-3-cephem-4-carboxylic acid (abbreviated as 1-oxacephem in the present paper). However, we felt completely frustrated when we noticed the successful syntheses reported independently by S. Wolfe et al. (ref. 4) of the Queen's University, Canada, and L. D. Cama and B. G. Christensen (ref. 5) of the Merck Research Laboratories in early 1975. In particular the work of the latter group deserves admiration, since they showed for the first time that 1-oxacephalothin 16, though in a racemic form, exhibited antibacterial activity as high as its 1-thia congener, cephalothin, and that 1-sulfur atom in cephalosporins was not essential for exhibiting the activity.

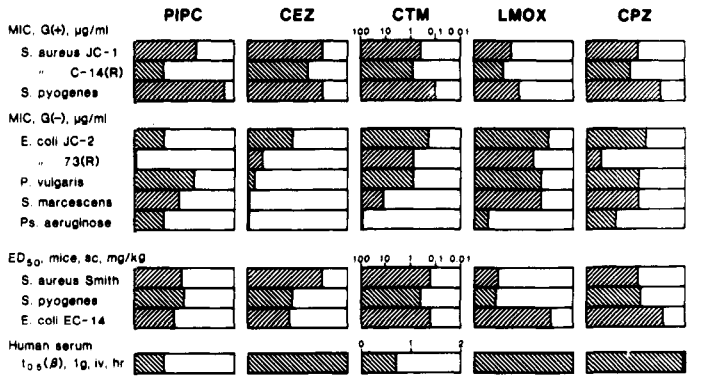
Despite this discouragement we still continued the study expecting that there would be some possibility of finding a promising compound in this new class of β-lactam antibiotics. In the meantime we succeeded also in synthesizing 1-oxa analogs of desacetyl cephalosporin derivatives and subsequently 1-oxacephalothin **16** in an optically active form. We were then encouraged a great deal by the finding that our sample of **16** showed activity several times higher than that reported by the Merck group and were further excited by the fact that 1-oxacephem derivative **17** was found to be eight to sixteen times as active as the corresponding 1-thia congener. A number of 1-oxacephems were then prepared and subjected to a primary assay program. As a result we succeeded in discovering latamoxef (moxalactam) (ref. 6) **18**, the biological activities of which were thoroughly investigated by our biologists showing its extraordinary high and broad activity against gram-negative bacteria including some *Pseudomonas* sp., its efficacy against resistant strains and its excellent pharmacological properties. After extensive clinical studies, this compound has been highly evaluated as one of the so-called third generation β-lactam antibiotics and used for



16 17 18 (LMOX)

CHART 4

DIAGRAM 1. Profile of marketed, parenteral β-lactam antibiotics



treating infectious diseases since 1982. The chemotherapeutic profile is shown in Diagram 1 in comparison with that of piperacillin (PIPC), a newly developed penicillin, cefazolin (CEZ), a typical first generation cephalosporin, cefoperazone (CPZ) and cefotaxime (CTX), two other third generation cephalosporins of different structure. MIC values of each antibiotic against gram-positive and gram-negative bacteria are shown in the first and second rows, respectively. Protective effect (ED_{50}) and half life in human serum are illustrated in the third and forth rows, respectively. This illustration clearly shows that latamoxef is uppermost as a chemotherapeutic agent at least for treating infectious diseases caused by pathogenic gram-negative bacteria.

Although we thus attained the initial purpose to get a promising compound in hand, there seemed little prospect of commercial success, because the structural complexity of latamoxef made it almost impossible to establish an industrially feasible synthetic route. Indeed, it was clear that our first synthetic method was never practical and we had to challenge this serious problem which was standing in our way to the ultimate success. Fortunately our efforts were rewarded with establishing a well sophisticated and industrially feasible synthetic route. These achievements and the further improved processes have already been published (ref. 7) and, therefore, I would like only to refer to the most exciting experiences in the synthesis.

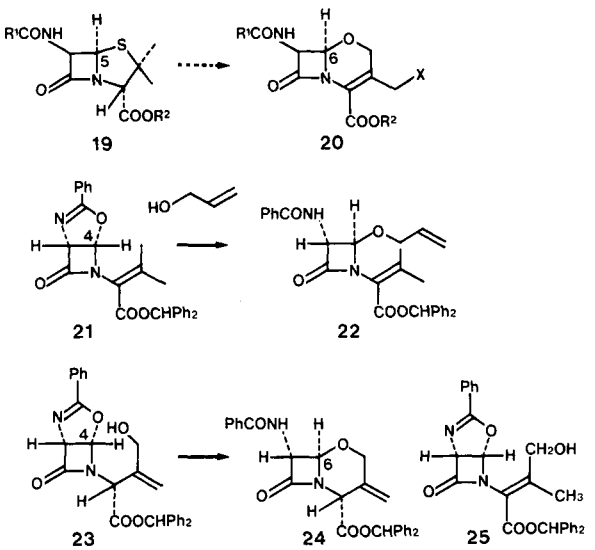


CHART 5

Obviously the most important and difficult step was how to convert the C_5-S bond in **19** into the C_6-O bond in **20** with retention of the C_5 (or C_6) stereochemistry. The first step for the solving of this problem was provided by an observation that *epi*-oxazolidinoazetidinone **21** was nicely converted into **22** by the substitution reaction with allyl alcohol in the presence of an acid. In this conversion allyl alcohol was introduced into the C_4 position in **21** with more than 98% inversion of its stereochemistry. This intermolecular substitution reaction could be transferred to an intramolecular cyclization of **23** to obtain **24**. In this cyclization substitution at C_4 was expected to take place similarly with high inversion and, therefore, the method was thought quite favorable for the present purpose. Actually this was the case. Compound **23** was smoothly cyclized to **24** with *p*-toluene sulfonic acid or BF_3·Et_2O demonstrating the usefulness of this approach. The second step in solving the stereochemical problem which involved transformation of **19** into **20** was how to derive the substrate **23**, and not **25**, from an appropriate penicillin intermediate. We believed that, for several reasons, an isomeric substrate **25** was not a suitable one. Fortunately, this second problem was nicely solved by the observation that heating *epi*-penicillin S-oxide **26** in the presence of triphenylphosphin allowed smooth formation of the desired *epi*-oxazolidinoazetidinone **28** with little or no contamination from the isopropylidene isomer.

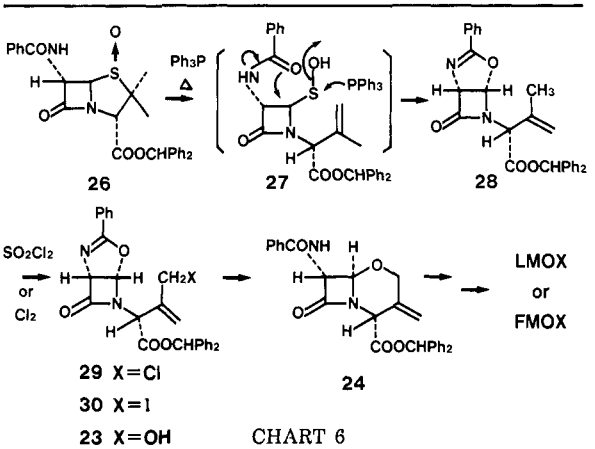


CHART 6

Finally we encountered difficulties in direct allylic oxidation of **28** at the terminal methyl in the side chain. All attempted oxidations using known methods failed. This low reactivity was unexpected and probably due to steric hindrance exerted by the *epi*-oxazolidine ring. This difficulty, though indirectly, was overcome by the finding that allylic chlorination took place nicely by simple addition of chlorine or sulfuryl

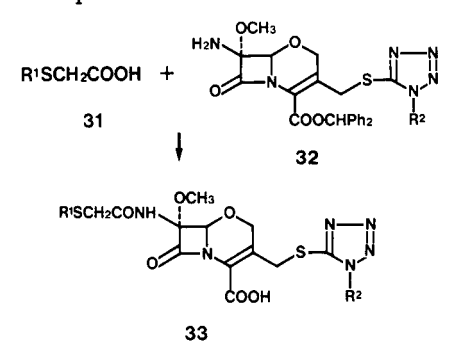
chloride giving **29** in good yield. A number of useful synthetic methods were developed for converting this chloride **29**, *via* the allyl alcohol **23** and 3-exomethylene 1-oxacepham **24**, into either latamoxef (LMOX) or flomoxef (FMOX) and the latter compound will be a topic in the following section.

The synthetic method was further developed by the people of the process research and development section (Production Department) for large scale production. The procedures thus developed are now carried out at our Kanegasaki plant where latamoxef and flomoxef were produced on multi ton scales (photo-picture of Kanegasaki plant). This success in large scale production made it possible to supply latamoxef in abundance, meaning that latamoxef was now qualified for a therapeutic agent. In fact, it has been broadly used clinically for treating infectious diseases. It should be pointed out that, latamoxef is quite unique in its history in that it is the first and the only β-lactam antibiotic with an unnatural nuclear structure.

OXACEPHEMS (2), FLOMOXEF AND THE RELATED β-LACTAM ANTIBIOTICS

In the previous section, I have mentioned that latamoxef has been broadly used clinically, as one of the representative third generation β-lactam antibiotics. However, despite its excellent activity against gram-negative bacteria, latamoxef, as other third generation β-lactam antibiotics, has a drawback of weak activity against *Staphylococcus aureus*. Therefore, our next target was to find a new oxacephem antibiotic exhibiting strong activity not only against gram-negative but also gram-positive bacteria, in other words, activity of the new antibiotic should be well balanced against both gram-positive and gram-negative bacteria.

In the beginning we adopted a strategy which comprised, (1) search for an excellent cephalosporin 7β-side chain **31** in the literature, and (2) introduction of this side chain into the 1-oxacephamycin nucleus **32** (7α-methoxy-1-oxacephem). This particular nucleus was selected as the best from the viewpoints of activity against various kinds of bacteria, stability to β-lactamases, pharmacology, and adverse reactions. Literature inspection suggested that certain alkylthio- or substituted alkylthio-acetamido side chains, R^1SCH_2CONH; might be promising. For example, trifluoromethylthio-acetamido or cyanomethylthioacetamido groups were used respectively for cefazaflur (ref. 8) or cefmethazole (ref. 9) which were reported to exhibit enhanced gram-positive activity. These groups were now introduced into the nucleus **32** ($R^2 = CH_3$) to obtain **33a**, **33b** and **33c**. Among these, **33b** bearing the trifluoromethylthio-acetamido side chain was found to be most active against gram-positive and gram-negative bacteria and exhibit even a 4-63 fold enhanced activity as compared with that of 1-thia congener. Unfortunately, however, preparation of this side chain, that is, the corresponding trifluorothio-acetic acid **31** ($R^1 = CF_3$) seemed to be laborious. Thus, according to the literature (ref. 8), this acid could only be prepared by replacement of methyl iodoacetate with silver trifluoromethylsulfide followed by alkali hydrolysis. Moreover, preparation of the latter reagent, $AgSCF_3$, required heating silver fluoride and carbon disulfide in an autoclave, the procedure being never industrial, as you can see from the equation in Chart 7. We therefore planned to substitute difluoromethyl for trifluoromethyl, since difluoromethylthioacetic acid **31** ($R^1 = CHF_2$) could be more easily obtained by reaction of thioglycolic acid ester with difluorocarbene, which was generated *in situ* by treatment of cheap chlorodifluoromethane (Freon 22) with base, and by subsequent alkaline hydrolysis as shown in Chart 7. In accord with our expectation, the difluoromethylthioacetamido-1-oxacephem **33d** was found to exhibit antibacterial activity comparable with that of trifluoro congener **33b**. Next, we prepared **33e**, **33f**, and **33g** in which cyanoethyl, carbamoyl methyl or hydroxyethyl was substituted for N_1-methyl in the tetrazole ring in **33d**. The purpose of this modification was to avoid a disulfiram-like adverse reaction which was assumed to be ascribed to the N_1-methyl tetrazolylthio moiety. The 1-oxacephem nucleus bearing the N_1-substituted tetrazolylthio group at $C_{3'}$ was first prepared and coupled with difluoromethylthioacetic acid in the usual way. The antibacterial activity of the three oxacephems, **33e**, **33f**, and **33g** was very high and compared well with each other and also with the original N_1-methyl analog **33d**. The activity of the compound **33g** bearing the N_1-hydroxyethyl group was shown to be 4-16 times higher than that of the corresponding 1-thia congener. This oxacephem **33g** called flomoxef was selected for further development as a candidate of a new therapeutic agent (ref. 10). Very recently this compound was approved for marketing and launched a few days ago as the second antibacterial agent of the 1-oxacephem series. The biological profile of flomoxef is illustrated schematically in Diagram 2 in comparison with latamoxef (LMOX) **18**, and the newly developed three other antibiotics, ceftazidime (CAZ), cefuzonam (CZON), and imipenem (IPM). Antibacterial activity (MIC) against gram-positive and gram-negative bacteria, protecting effect (ED_{50}), and half life in human



a $R^1 = C_2H_5$, $R^2 = CH_3$
b $R^1 = CF_3$, $R^2 = CH_3$
c $R^1 = NCCH_2$, $R^2 = CH_3$
d $R^1 = CHF_2$, $R^2 = CH_3$
e $R^1 = CHF_2$, $R^2 = CH_2CH_2CN$
f $R^1 = CHF_2$, $R^2 = CH_2CONH_2$
g $R^1 = CHF_2$, $R^2 = CH_2CH_2OH$ *(flomoxef)*

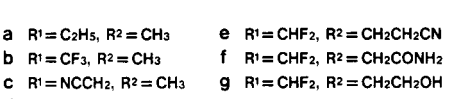
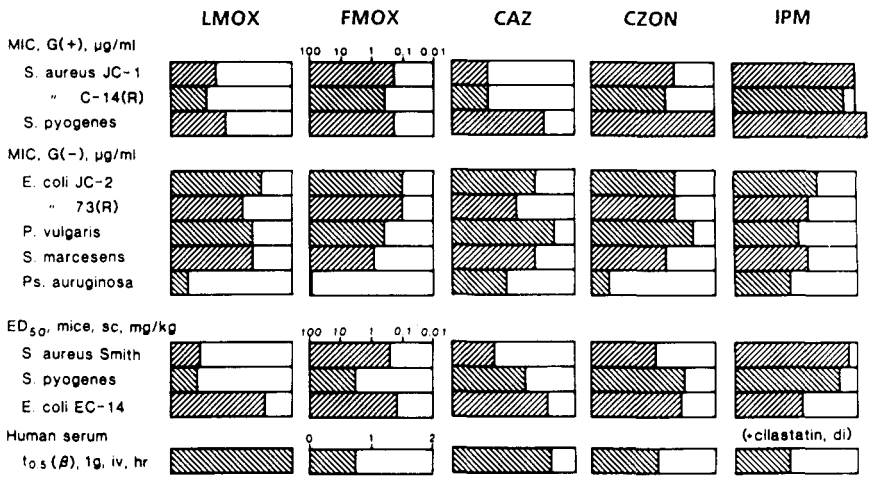


CHART 7

DIAGRAM 2. Profile of new parenteral β-lactam antibiotics



serum are shown by shadow bars in the first, the second, the third, and the fourth rows. As can be seen from this diagram, while ceftazidime (CAZ), like latamoxef (LMOX), exhibit only low activity against *Staphylococcus aureus*, flomoxef (FMOX), cefuzonam (CZON), and imipenem (IPM) exhibit well-balanced high activity against various species of bacteria, although activity of the former two antibiotics against *Pseudomonas* species is low. Flomoxef is characterized also by its high activity against methicillin resistant *Staphylococci* (MRSA). Judging from this unique character and its still high activity against gram-negative bacteria, we may say that flomoxef does complement latamoxef well. However, it should be pointed out that half-life of flomoxef in human serum is significantly shorter than that of latamoxef.

In view of this unfavorable property of flomoxef, we continued chemical modification further to obtain a better β-lactam antibiotic. It was known to us that *ortho* chlorophenylthioacetamido side chain **34** brought about extremely enhanced gram-positive activity and conversely much lowered gram-negative activity. We suspected that *cis* chlorovinylthioacetamido side chain obtained by breaking the benzene ring as indicated by the dotted line in **34** might give rise to marked enhancement of the gram-negative activity and the resulting β-lactam antibiotic would have well-balanced activity. This prediction was based upon an assumption that this side chain is less lipophilic as compared with the *ortho* chlorophenyl group.

Synthesis of the acid **39** corresponding to the side chain **35** was thus carried out (ref. 11). Treatment of methyl chloroethylthioacetate **37** with DBU followed by chlorination gave dichlorothioacetate **38**. When this ester was subjected to dehydrochlorination by heating with lithium chloride followed by alkaline hydrolysis, we could obtain *cis* chlorovinylthioacetic acid **39** as a major product. In contrast, the synthesis of the fluoro analog **43** was not easy. The starting methyl fluoroethylthioacetate **40** was first chlorinated and the resulting dichloroderivative was oxidized with *m*-chloroperbenzoic acid to obtain the dichloro-S-oxide **41**. Pummerer reaction of this S-oxide was successful only by the use of triflic acid anhydride and 4-methyl-2,6-di-*tert*-butyl pyridine giving, to our surprise, exclusively the desired *cis* fluorovinylthio derivative **42**, which was then dechlorinated with zinc and acetic acid, and

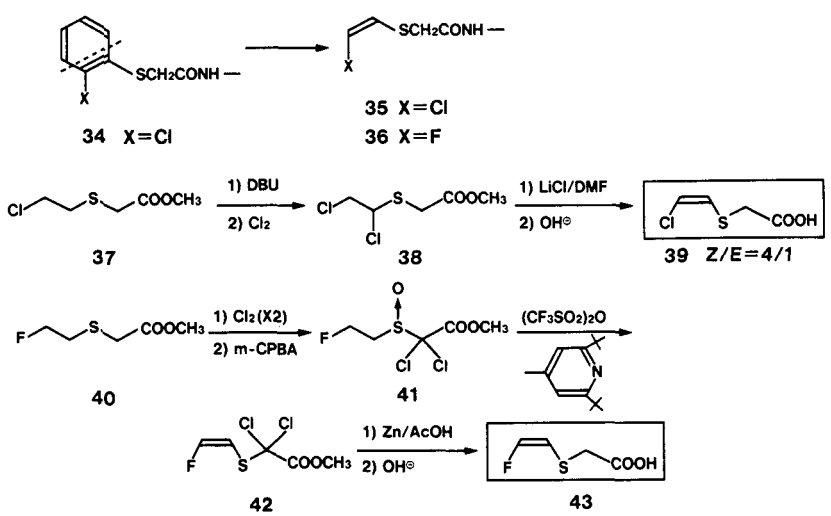
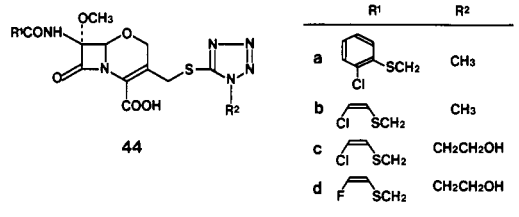


CHART 8

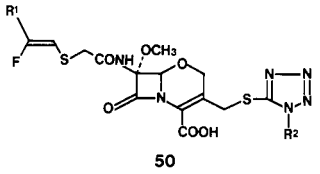
TABLE 1 Comparative Activity (MIC : μg/ml) of cis-halovinylthioacetamido-oxacephems



44

	44a	44b	44c	44d	LMOX	CEZ
S. aureus JC-1	0.05	0.1	0.1	0.2	6.3	0.2
S. aureus C-14(R)	0.2	0.1	0.2	0.2	6.3	0.4
E. Coli JC-2	6.3	0.02	0.02	0.05	0.1	1.6
E. Coli 73(R)	12.5	0.1	0.2	0.1	0.4	25
P. mirabilis PR-4	3.1	0.1	0.1	0.1	0.1	3.1
P. vulgaris CN-329	6.3	0.2	0.2	0.2	0.2	50
(R : Resistant Strains)						

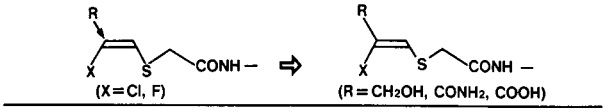
TABLE 2 Plasma levels and half lives of cis-fluorovinylthioacetamido-oxacephems in monkeys (Dose : 20mg/kg, i.v.)



50

	R¹	R²
44e	H	CH_2CONH_2
44d	H	CH_2CH_2OH
50a	H_2NCO-	CH_2CONH_2
50b	H_2NCO-	CH_2CH_2OH (2355-S)

	44e	50a	44d	50b (2355-S)
AUC (μg·ml⁻¹·hr)	30	54	20	42
Half Life (min.)	35	50	27	39



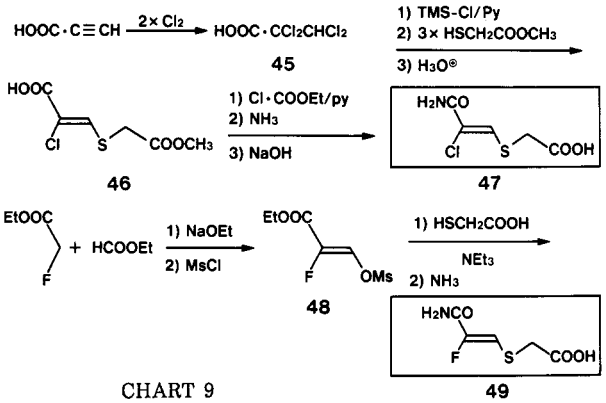


CHART 9

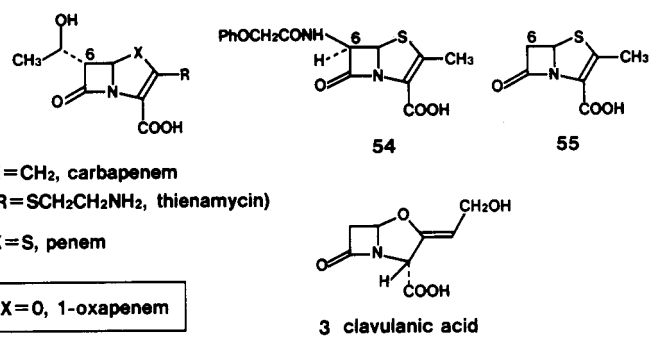
subsequently hydrolyzed to **43** (ref. 12). It is note-worthy that *cis* isomers were formed predominantly or exclusively in these elimination reactions. Being interested in this stereochemical outcome we investigated the mode of this reaction. The results will be published elsewhere.

We first prepared the *cis*-chlorovinylthioacetamido 1-oxacephem **44b** conventionally by acylation of the 7β-amino 1-oxacephem nucleus with the *cis* acid of **39**. Antibacterial activity of **44b** is compared with its *ortho*-chlorophenylthio congener **44a** and other reference antibiotics, latamoxef and cefazolin. As we predicted, compound **44b** exhibited very high gram-negative activity, that is 30-120 times as active as the chlorophenyl congener **44a**, without loss of the very high gram-positive activity of the latter. Moreover, the data clearly showed that its gram-positive and gram-negative activity was comparable with that of cefazolin and latamoxef, respectively. Encouraged by this result we prepared a number of 1-oxacephems of this series. As in the case of flomoxef, substitution of hydroxyethyl for methyl at the tetrazol ring was also carried out to obtain **44c**. As can be seen from Table 1, this compound and its fluoro analog **44d** exhibited similarly high activity with **44b** and, therefore, were subjected to further biological evaluation. Unfortunately, however, these oxacephems had unsatisfactory pharmacological properties, i.e., the relatively low blood levels and the short half-lives, and, thus, further structural modification was necessary (ref. 11). We suspected that introduction of certain hydrophilic substituents, such as hydroxylmethyl, carbamoyl, and carboxyl, at the vinyl part of the side chain might be effective for this purpose and the synthesis along this line was carried out. The acids, for example **47** and **49**, required for this synthesis were prepared according to the methods given in Chart 9. The syntheses involved no particular reaction to be commented, but I would like only to point out that, in the conversion of **45** into **46**, dechlorination with and addition of methyl thioglycolate, and elimination of hydrogen chloride took place at once.

Carboxyl-, carbamoyl-, and hydroxymethyl-*cis*-chlorovinylthioacetamido-oxacephems were prepared and their antibacterial activity was compared. Among these, the carbamoyl derivative was found to exhibit the highest and the most balanced activity. Therefore, two carbamoyl substituted fluoro counterparts **50a** and **50b** were prepared and tested for their antibacterial activity and also for pharmacological behaviour. Both exhibited satisfactory antibacterial activity and spectra. It was further shown that the carbamoyl substitution actually improved the pharmacology significantly as can be seen from Table 2. The AUC values and the half-lives for **50a** and **50b** are larger than those for the non-substituted congeners **44e** and **44d**. The one containing the hydroxyethyltetrazolethio substituent at C_3' was selected for development with the code number of 2355-S. Further investigation of its antibacterial profile using clinical bacterial isolates showed that the antibacterial patterns of 2355-S and flomoxef are almost superimposable. It was also shown that 2355-S had the distinct advantage of superior pharmacological properties, that is, its AUC value and half-life were, respectively, one and a half times and twice as large as those of flomoxef when administered through intravenous drip infusion to normal volunteers.

β-LACTAM ANTIBIOTICS LACKING ACYLAMIDO SIDE CHAIN (1) 1-OXAPENEMS

Isolation of thienamycin from fermentation broth of *Streptomyces* cattleya and the subsequent structure elucidation gave a strong impetus to the β-lactam research, since this new antibiotic had an unusual structure lacking a β-acylamido side chain and, instead, having hydroxyethyl with an α-configuration. This side chain is not necessarily essential, but most favorable for exhibiting antibacterial activity. There have been some arguments (ref. 13) that this side chain may stabilize the labile carbapenem nucleus, and/or, have a special binding affinity to a target enzyme, but to our knowledge, its role on mode of action as an antibiotic is still obscure. It is known that this particular side chain is also favorable for penems which represent another class of artificial β-lactam antibiotics. In his pioneering studies on penems, the late Professor R. B. Woodward (ref. 14) noted that, the penem **54** bearing 6β-phenoxyacetamido group which was essential for classical β-lactam antibiotics, was quite labile and consequently exhibited only a weak activity, while the penem **55** having no substituent at C_6 possessed a very strong activity. He further showed that the penem **52** (R = H) bearing 6α-hydroxyethyl at C_6 was relatively stable and again strongly active. It has been recognized that there have been close relationships between the chemical reactivity of the β-lactam ring and the antibacterial activity, that is, as the reactivity of the β-lactam ring is increased, the biological activity becomes higher. However, this relationship is valid only in a range of levels of β-lactam reactivity. In cases where reactivity is beyond this level range, even above the upper limit, antibacterial activity will be weak or nonexistent. This means that if a β-lactam is far too reactive, it will encounter and randomly react with numerous nucleophiles with the result that it never reaches its target enzyme. In this light, it may be pertinent now to compare the three penems, **54**, **55**, and **52** (R = H) with respect to the relation between reactivity or lability and biological activity. While the increased reactivity due to the intrinsic fused ring strain is common among the three penems, there is a possibility for **54** to undergo intramolecular nucleophilic attack by participation of the 6β-acid amido group and, thus, this penem is most labile and only weakly active. The β-lactam ring in **55** is most exposed to nucleophilic attack due to the absence of a C_6-substituent and thus its stability is estimated as intermediary. For the penem **52** (R = H) the situation may be most favourable, because it may be moderately stable owing the the existence of the neighbouring hydroxyethyl substituent.



51 X=CH_2, carbapenem (R=$SCH_2CH_2NH_2$, thienamycin)

52 X=S, penem

53 X=O, 1-oxapenem

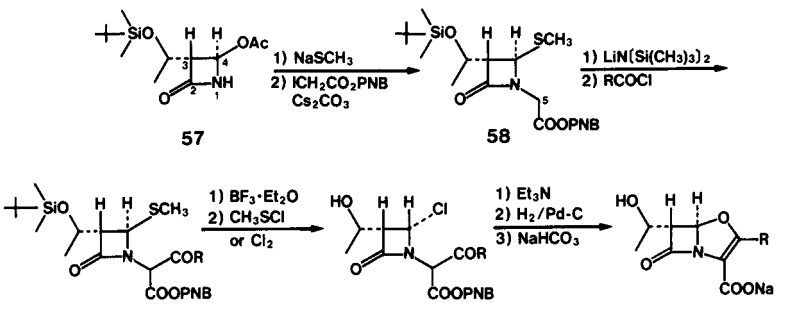
54 **55**

3 clavulanic acid

CHART 10

On the basis of the above consideration we undertook the synthesis of the 1-oxapenem **53** bearing hydroxyethyl at 6α. It should be noted that studies on 1-oxapems are very few, although there have been abundant reports on the 1-oxapenams in relation to clavulanic acid. Moreover, no report has been published on the synthesis of 1-oxapenem bearing the hydroxyethyl substituent. This situation may be reasonable if one presumes that 1-oxapenem is extremely unstable.

The syntheses were carried out principally according to the method reported by T. Kametani et al. (ref. 15). The reaction sequence is given in Chart 11. Commercially available, optically active azetidinone **57** was converted into **58** which was acylated with various acid chlorides to obtain β-keto-



57 **58**

59a, b, c, **60a, b, c,** **61a, b, c,**

Stability ($\tau_{1/2}$ min. PH 7.0, 37°)

61a	R=CH_3	43
61b	R=C_6H_5	24
61c	R=$CH(CH_3)_2$	200

CHART 11

TABLE 3. Inhibitory Activity of β-Lactamase Inhibitors

Source of β-lactamase [1]	Type [2]	Minimum effective concentration (μg/ml) [3]				
		61a	61b	61c	epi-Thienamycin B	Clavulanic acid
E. coli 6	C	0.002	0.032	0.016	0.016	250
P. morganii 7	C	0.016	0.008	0.008	0.032	>250
P. inconstans 31	C	0.25	0.016	0.032	0.25	>250
E. cloacae 92	C	0.008	0.032	0.016	0.016	>250
P. vulgaris 31	C	0.5	8	1	4	1
K. oxytoca 696	P	32	63	>63	>63	0.063
E. coli RTEM	P	63	16	16	8	0.063
E. coli RGN 238	P	0.125	0.063	0.063	0.125	4

1) Enzymes were crude extracts
2) C: Cephalosporinase; P: Penicillinase
3) Minimum effective concentration was determined by spot test using Nitrocefin

esters **59a, b, c**. These azetidino-ketoesters after desilylation were chlorinated to **60a, b, c** which underwent intramolecular cyclization by treatment with triethylamine and the subsequent ester deprotection by catalytic hydrogenation to obtain the desired 1-oxacephems **61a, b, c**. Their half-lives at pH 7.0 and 37°C were determined to be 43, 24, and 200 min, respectively. While the first two compounds were unstable as anticipated, the last compound was surprisingly stable as judged from its half-life value of 200 min. In accord with their stability the former two oxapenems **61a** and **b** were virtually inactive, whereas the 2-isopropyloxapenem **61c** exhibited activity which was weak but a little bit better than clavulanic acid. It was quite interesting, however, that all these three oxapenems were found to have very strong β-lactamase inhibitory activity as listed n Table 3. This 1-oxapenem inucleus is equipped with both of the 1-oxygen atom of clavulanic acid and the 2,3-double bond of carbapenem. These structural elements are closely associated with the biological activity of the two classes of β-lactam compound. Now what would be the effect of incorporating both of the structural elements within the same molecule? As can be seen from this table, all the cephalosporinases tested were sensitive to 1-oxapenems. Their inhibitory spectrum is similar to that of *epi*-thienamycin B and not of clavulanic acid. It should be noted that this fact might be interesting from the view-point of the mode of action.

β-LACTAM ANTIBIOTICS LACKING ACYLAMIDO SIDE CHAIN (2) 1-OXACEPHEMS

It is a matter of common knowledge that there are two elemental factors controlling the activity of β-lactam antibiotics, that is, the chemical reactivity of the β-lactam ring to acylate the peptidoglycan transpeptidase and the shape of the β-lactam molecule which determines the binding-affinity to the enzyme. As discussed previously, the shape of the molecule is also related to the reactivity of the β-lactam ring, when the molecule possesses ring strain. In a quantitative sense, as the reactivity is increased, the antibacterial activity becomes stronger at least within certain limits. We centered our attention firstly on increasing the chemical reactivity electronically and, secondly, on making up the molecule in a bent form.

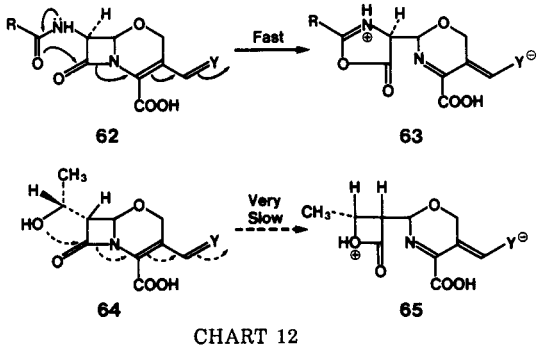


CHART 12

As I referred to already, in the classical β-lactam antibiotics there is a limitation to increasing the reactivity electronically by introducing an electron-withdrawing substituent at an appropriate position, because such a modification results in the decomposition of the β-lactam ring by intramolecular incorporation of the neighboring acyl amido side-chain. Thus, oxacephem **62**, for example, would be readily decomposed to **63**, if C = Y is a strong electronegative group. We suspected that, in contrast, oxacephem **64** bearing hydroxyethyl instead of the acyl amido group might undergo degradation only slowly, even if degradation occurred, and the C = Y group of the strongest electrowithdrawing rank could be substituted.

Based upon this consideration we synthesized a number of oxacephems of type **64** starting from 4β-acetoxy azetidinone **57**. The reaction sequence is shown in Chart 13. The key intermediate was the Woodward type phosphorane **67** which underwent smooth cyclization by heating and the subsequent Swern oxidation to give **68** or **69**. The aldehyde **68** was then converted into **70a, b,** and **c** and these compounds and **69**, after desilylation, were ester-deprotected to obtain **71a, b, c** and **d**.

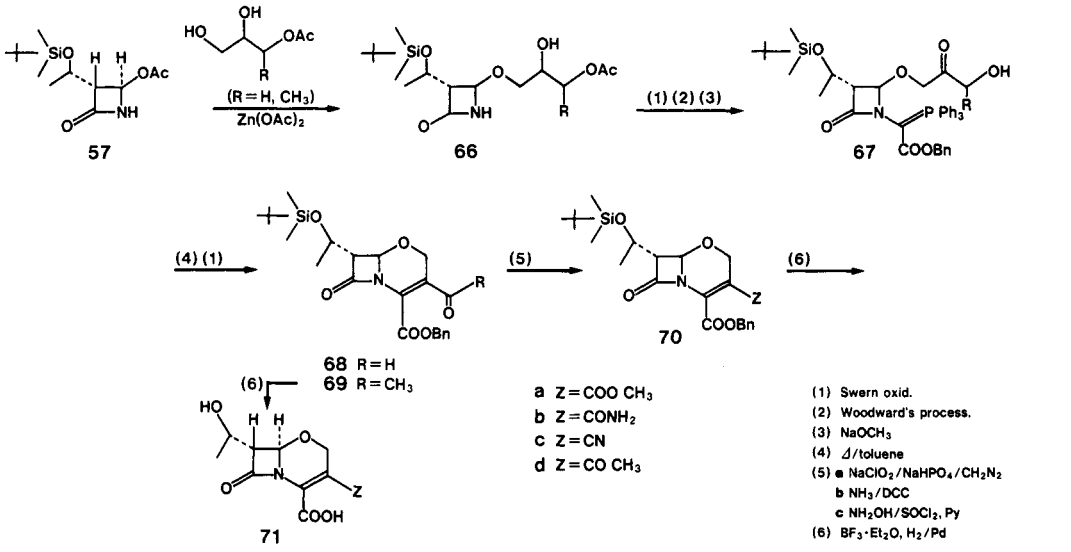


CHART 13

Antibacterial assay showed that these compounds were actually active, as predicted, but the activity was, in a quantitative sense, quite disappointing. The nitrile **71c**, which was strongest among them, showed MIC values of 12.5-25 μg/ml against various *Staphylococcus aureus* strains and 50-100 μg/ml against *E. coli*, *Klebsiella* and *Enterobacter* species.

β-LACTAM ANTIBIOTICS LACKING ACYLAMIDO SIDE CHAIN (3) BRIDGED 1-OXACEPHEMS

We finally challenged the synthesis of a bent 1-oxacephem which might have a good binding affinity to the target enzyme and at the same time a constraint imposed in such a molecule will increase the β-lactam reactivity as I referred to in the foregoing discussions. Now, what is the way to construct such a bent molecule which still contains the 1-oxacephem ring? In Chart 15 are given perspective views of representative β-lactam antibiotics. While the molecules of cephalosporin **72a** and 1-oxacephalosporin **73a** are rather flat, those of penicillin 1 and thienamycin **74a** are greatly folded. As discussed previously, as the folding is greater, the activity becomes stronger. There was a formalism for determining the degree of the folding, that is, for defining the critical departure from coplanarity to be formed by the amide resonance. A trigonal pyramid is imagined in A, the corners of whose base are C_3, C_5, and C_7, while N-4 is at its apex; the altitude h from the apex to the base, will be larger as the departure from coplanarity increases. The h values have been determined for various β-lactam antibiotics: those were 0.20, 0.23, 0.40 and 0.49 Å for cephalosporin **72a**, 1-oxacephem **73a**, penicillin **1**, and carbapenem **74a**, respectively, the values being in good agreement with their activities in a qualitative sense.

We suspected that the way yielding a large h value comparable to that of carbapenem was to connect the C_2 and C_7 with one or two carbons at the β-side to form **75**, for example. This idea was not unreasonable, since there were reports (ref. 16) that introduction of a methyl group at the 2β position in 1-oxacephem and at the 1β position in carbapenem did not reduce, the original activity. Moreover, lack of two methyl groups at C_2 in penicillin was reported to lower the activity. On the contrary, dialkylation at C_6 in penem was reported to result in a complete loss of the activity suggesting evidence

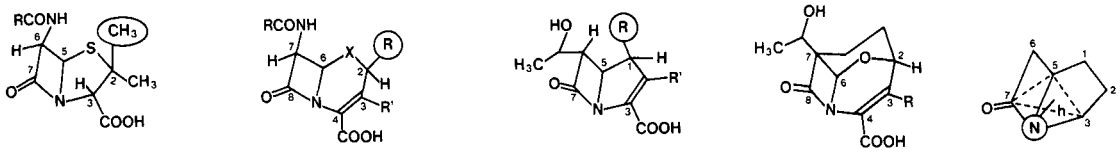


CHART 14

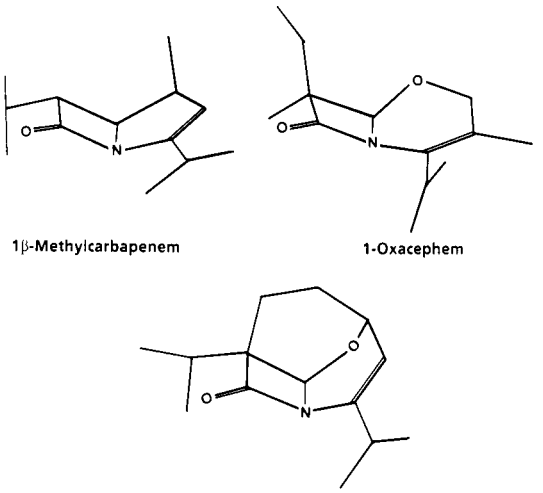


Figure 1 Bridged 1-Oxacephem (4,6,6-Tricyclic Ring System)

against our idea. However, we were optimistic at this point, because, in a rigid ring system like **75**, steric hindrance towards the β-lactam carbonyl might be reduced as in case of camphor whose carbonyl reactivity retained in spite of disubstitution at the neighbouring bridge-head.

Before carrying out the synthesis, the stereostructure of **75** was compared with the stereographs of 1β-methylcarbapenem **74b** and 1-oxacephem **73a** with the aid of computer graphics. The three stereographs were determined by Dr. Ezumi using the AM-I method and is shown in Fig. 1. The bridged 1-oxacephem **75**, 1β-methylcarbapenem **74b**, and 1-oxacephem are drawn by red, yellow, and blue lines respectively with the common axis of the nitrogen and C_6. It was clear that the molecules of the bridged 1-oxacephem and 1β-methyl carbapenem were roughly superimposed, whereas the 1-oxacephem molecule was largely apart.

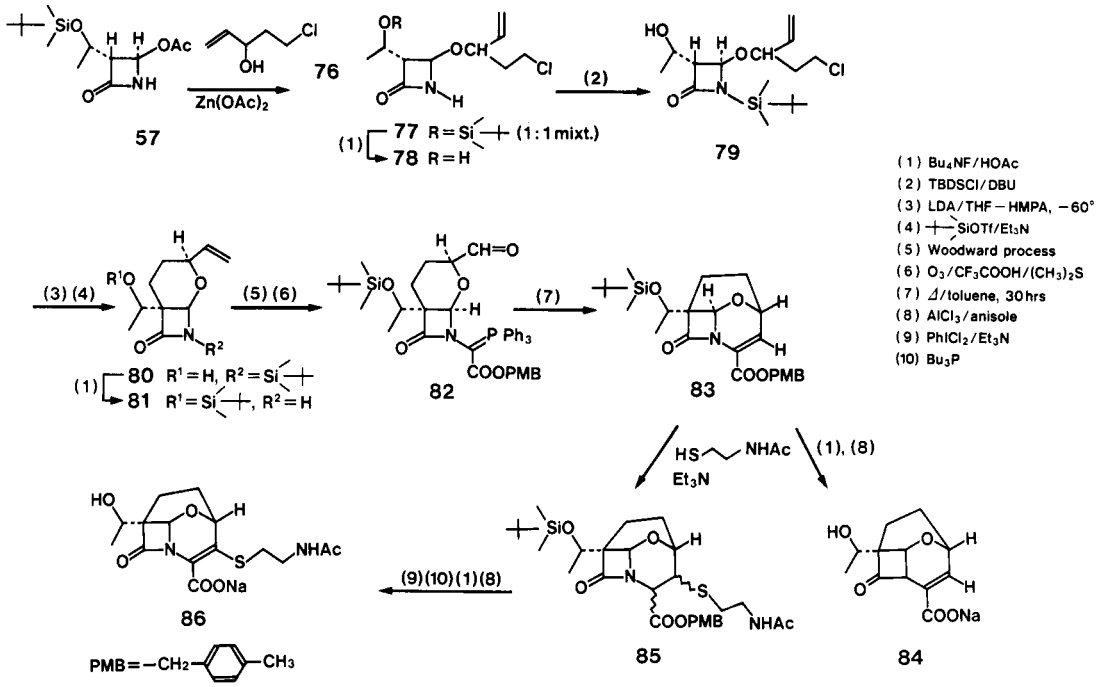


CHART 15

Encouraged by this result we undertook the synthesis of the bridged 1-oxacephem **75** of the 4,6,6 tricyclic ring system. Reaction of 4β-acetoxyazetidinone **57** with racemic 5-chloro-3-hydroxy-1-pentene gave a 1:1 mixture of **77** which was separated into two etherealisomers in 46 and 45% yield respectively. In order to effect intramolecular cyclization, compound **77** was converted into **79** via **78** as shown in Chart 15. This is because that cyclization of O,N-bissilyl compound failed giving mainly an olefinic compound and it was expected that the carbanion-alkoxide dianion from the hydroxyethyl compound would be less vulnerable to the elimination of the hydroxy functionality. Indeed, the hydroxyethyl compound **79** derived from the more polar isomer of **77** was cyclized smoothly by treatment with LDA at −60°C to obtain the bicyclic compound **80**. The configuration of this intermediate was confirmed by X-ray crystallography. This was converted into a Woodward phosphorane **82** which was then cyclized to the tricyclic compound **83** in 90% yield. Astonishingly, this cyclization required an unusually high temperature and a long reaction time, suggesting that a highly strained intermediate is induced in the transition state. This was either desilylated and subsequently ester deprotected to obtain **84** or transformed via **85** into **86**, the desired target compound.

X-Ray crystalography

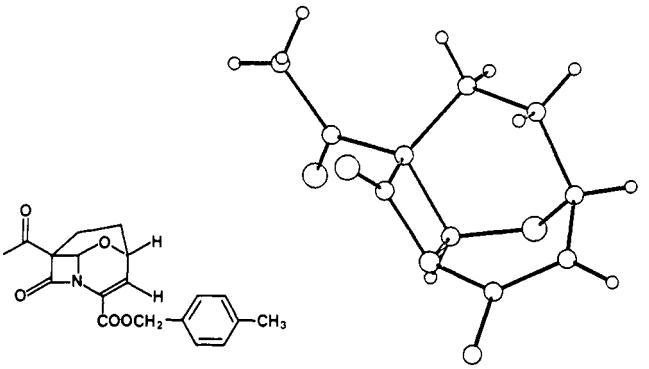


Figure 2 Bridged 1-Oxacephem (4,6,6-Tricyclic Ring System)

The structure of this compound **86** was confirmed by X-ray analysis of the tricyclic ketone *p*-methylbenzyl ester derived from desilylation and subsequent Swern oxidation of **83**. The three dimensional structure of the keto ester is given in Fig. 2. According to the data obtained by this analysis, the *h* value was determined as 0.4 Å which was a little smaller than the value of 0.51 Å estimated by the computer graphics.

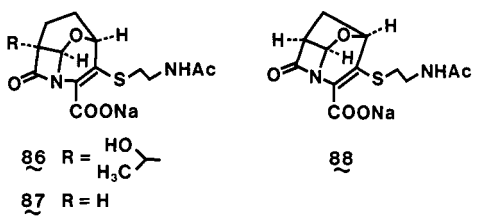


CHART 16

The antibacterial activity of the tricyclic acid sodium salt **86** was quite disappointing; its MIC values were only in the order of 25-50 μg/ml against *Staphylococcus aureus* including resistant strains. However, we believe, there will be still possibilities that the des-hydroxyethyl derivative **87** or the corresponding compound **88** with tricyclic 4, 5, 6 fused ring system would exhibit an excellent antibacterial activity. The work along this line remains to be explored.

In conclusion, we believe, we have made significant contributions to the chemistry and the biology of β-lactam antibiotics by exploring the 1-oxanuclear analogs of naturally occurring β-lactam antibiotics, which comprise a new class of the antibiotics and are available only artificially. In this sense, synthetic chemistry was recognized as a very important tool both in precision and usefulness and has been developed extensively. As a result we were successful not only in elucidating the biological properties of 1-oxacephems, but also in producing two useful injectable antibiotics of this type which are currently applied for treating infectious diseases.

In closing my lecture, I would like to mention that the work has been done cooperatively by a number of scientists in Shionogi Research Laboratories. The most recent work concerning with the syntheses of 1-oxa nuclear analogs lacking the acylamido side chain was performed with the earnest and skilled cooperation of Dr. T. Aoki, Mr. M. Murakami, and Mr. M. Matsuura to whom I would like to express my most sincere thanks. I also appreciate very much Dr. M. Yamakawa, Dr. K. Ezumi, Mr. H. Nakai, and Dr. M. Shiro for their performing the computer graphics and X-ray crystallography. I also would like to extend my gratitude to Professor S. Takano of Tohoku University for his kind to help. The relatively newer work including syntheses of flomoxef, 2355-S and the related substances were achieved in close association and cooperation of Dr. M. Yoshioka, Dr. T. Tsuji, Dr. M. Narisada, Dr. Y. Nishitani, Dr. T. Aoki, Dr. S. Yamamoto and their collaborators. Other earlier work including the discovery of latamoxef, the establishment of its industrially feasible synthetic method and its improved procedures, and finally the earliest work, that is, development of the synthetic method for 3'-norcephalosporin nucleus were achieved by devoted efforts of Dr. Y. Hamashima, Dr. S. Uyeo, Dr. H. Matsumura, Dr. I. Kikkawa and their collaborators in addition to the chemists given above. Biological studies were carried out by Dr. T. Yoshida, Dr. S. Matsuura, and Dr. Y. Komatsu and their collaborators. I would like to take this opportunity to express my sincere gratitude to these scientists for their stimulating cooperation. Finally I am grateful to Dr. K. Takeda for his encouraging me to accept the invitation of the organizing committee to present you with the present lecture.

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