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An Approach to Broad-Spectrum Cephalosporins

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An approach to broad-spectrum cephalosporins

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Based on our view that cephalosporins with potent activities share active hydrogen(s) on the α-carbon of the side chain acyl, we undertook to introduce β-ketoacid moieties onto the cephalosporin structure. Thus, starting with deacetylcephalosporin C (DCPC), first made available in quantities by our own fermentation technique, 7-amino-3′-*O*-acetoacetyldeacetylcephalosporanic acid (7-AACA) was made accessible. Acylation of 7-AACA with various β-ketoacids followed by substitution at the 3′-position led to 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thiomethyl]ceph-3-em-4-carboxylic acid (SCE-963, cefotiam), a potent broad-spectrum cephalosporin. Further elaboration of the structure of cefotiam led to an extended broad-spectrum cephalosporin, SCE-1365. These two classes of cephalosporins, together with our previously reported antipseudomonal cephalosporin (SCE-129, cefsulodin), could control a wide range of pathogenic bacteria.

Introduction

The last decade has witnessed a very rapid and prosperous progress in the research of cephalosporins.

When we started studies some 10 years ago, the reasons for our undertaking the research were as follows:

Structure: the cephalosporin structure provides a wider choice for chemical modifications as compared with penicillins and by this characteristic there would be possibilities for discovering antibiotics with superior therapeutic efficacies.

Economy: the economic aspects for the production of cephalosporins could be improved either by innovative fermentation technologies or by the rapidly increasing knowledge of the chemical transformation of penicillin to cephalosporin.

Stability: the cephalosporin nucleus, the fused β-lactam-dihydrothiazine, possesses a higher stability than the penicillin nucleus, the fused β-lactam-thiazolidine, and this stability would confer cephalosporins with a great advantage over penicillins, particularly when compounds are produced on a large scale or finally developed into drugs.

DCPC: a new starting material

Deacetylcephalosporin C (**2**, DCPC) was first described by Jeffery *et al.* (1961) as obtainable by an enzymic and also by a chemical hydrolysis of cephalosporin C (**1**, CPC) under mild conditions.

A large-scale production of DCPC was made feasible in our laboratories by fermentation with a *Cephalosporium* strain (Fujisawa *et al.* 1973) followed by a sequence of separation processes. DCPC turned out to be fairly stable and can be handled without lactone formation under

ordinary conditions. After the conventional protection of the amino group on the side chain acyl, the hydroxyl on the 3′-position was readily acylated with various acylating agents (Tsushima *et al.* 1976) including diketene (figure 1). Among these 3′-*O*-acylated cephalosporins, 7-amino-3′-*O*-acetoacetyldeacetylcephalosporanic acid (**6**, 7-AACA) was readily obtained by the conventional side chain cleavage of *N*-protected-3′-*O*-acetoacetyl-DCPC (**4**), and 7-AACA thus obtained turned out to be a useful key intermediate throughout our cephalosporin research. Subsequent substitution of the 3′-acetoacetoxyl group by various nucleophiles proceeded at much faster rates and in higher yields compared with that of the corresponding 3′-acetoxyl group as shown in figure 2 (Tsushima *et al.* 1979).

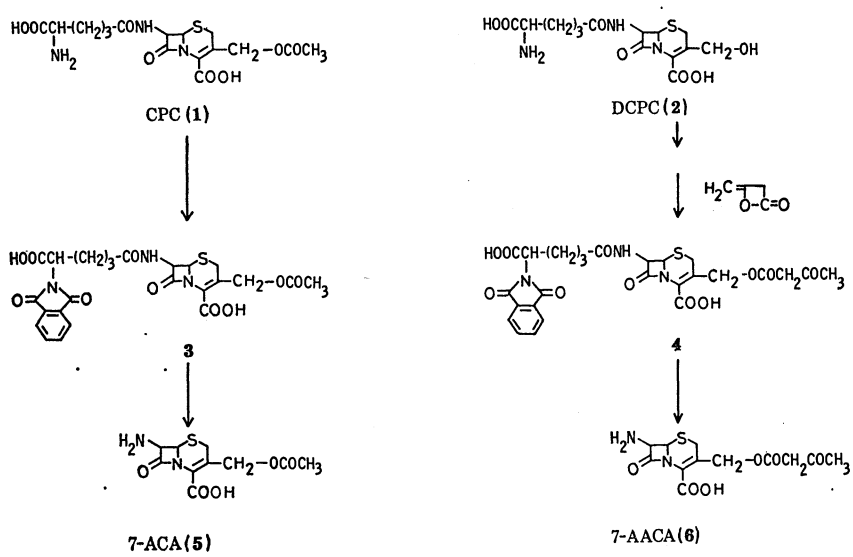


FIGURE 1. 7-ACA and 7-AACA.

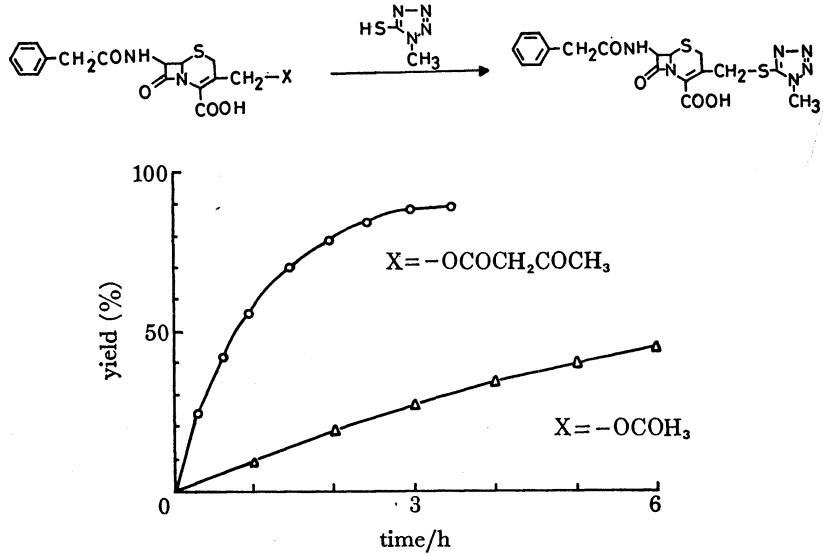
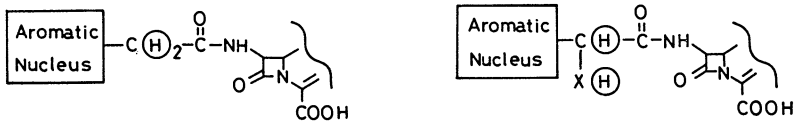


FIGURE 2. Comparative rates of 3′-nucleophilic substitution. Temperature, 47 °C; solvent, 0.24 M phosphate buffer of pH 6; initial concentration, 1.25 M.

Active hydrogen

On the other hand, a strategy of synthetic work starting mainly with 7-AACA was carried out based on rationale rather than random synthesis and screening.

First of all, we did a careful investigation on the structure–activity relations and we paid attention to the fact that cephalosporins in clinical use or with potent activity have 'active hydrogen' on the α-carbon of the side chain acyl at the 7-position. Although the definition of 'active hydrogen' is somewhat vague, it appeared to us that the α-hydrogen(s) of the side chain acyls of cephalothin, cephaloridine, cefazolin, cefamandole, and other cephalosporins are activated by the adjacent carbonyl and an aromatic six π-electron system (figure 3). The only exception appears to be the cefuroxime molecule, and this particular case will be discussed later.

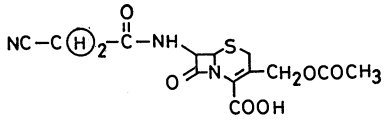


7

cephalothin
cephaloridine
cefazolin
cephapirin, etc.

8

cefamandole, X=O
cephalexin, X=NH
cephaloglycin, X=NH



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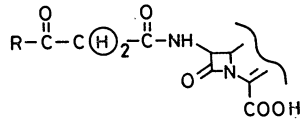
cephacetrile



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'target' for our studies

Figure 3. Active hydrogen(s) on the α-carbon of side chain acyls of cephalosporins.

In this connection, it should be mentioned that cephacetrile, a Ciba-Geigy cephalosporin, possesses two active hydrogens in the cyanoacetyl side chain. Therefore, we thought that introduction of β-ketoacyl moieties bearing activated hydrogen(s) on the α-carbon would deserve investigation.

To our knowledge, no systematic investigation has been made to synthesize cephalosporins with β-ketoacyl moieties in the side chain and this seems to be due to the fact that the availability of β-ketoacids is somewhat limited and, moreover, most β-ketoacids are labile and prone to decarboxylation; hence the introduction into the 7-amino of cephalosporins does not appear to be an easy task by conventional methods.

It has been known that antibacterial activity of β-lactam antibiotics can be correlated with the chemical reactivity (Woodward 1949; Sweet & Dahl 1970) of the β-lactam carbonyl. In fact, Tipper & Strominger (1965), with a Gram-positive bacterium, and later Izaki *et al.* (1966), with a Gram-negative bacterium, have proposed that an irreversible acylation of a nucleophilic receptor in the cavity of the enzyme is involved in the inhibitory mechanism of the bacterial cell-wall synthesis by β-lactam antibiotics. Also, Martin *et al.* (1976) reported that the rate constant of the aminolysis of benzylpenicillin with ethylenediamine, a difunctional base, is

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30 times greater than that with a monoamine of the same basicity and that the reaction would involve an 'intramolecular general-base catalysis'. A simpler and typical example of the reaction can be illustrated as in figure 4 (Tillet & Wiggins 1971).

In the light of the aforementioned arguments, it appeared to us that, in a transition state of the irreversible acylation of the enzyme by a cephalosporin molecule with an active α-hydrogen on the side chain acyl, abstraction of the active hydrogen by a base in the enzyme would take place first and then an intramolecular general-base catalysis involving a nucleophilic receptor site could occur as depicted in figure 5, whereby the rate of reaction of the β-lactam and hence the antibacterial activity would be enhanced.

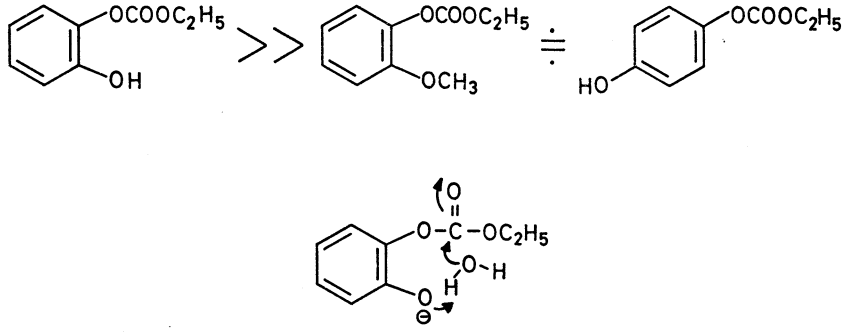

FIGURE 4. The hydrolysis rate of carbonate esters at pH 10. (From Tillet & Wiggins (1971).)

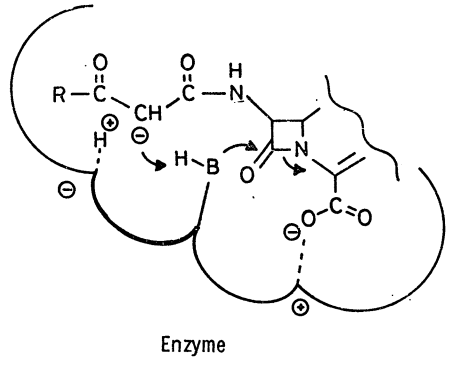

FIGURE 5. A proposed 'intramolecular general-base catalysis' for irreversible acylation of enzyme. B denotes a nucleophile.

SCE-963

Based on these speculative ideas, we attempted to synthesize cephalosporins with β-ketoacid moieties in the side chain. For this purpose, we have prepared a variety of β-ketoacids by the method reported by Stiles (1959). By this method, β-ketoacids were produced by the carboxylation of methylketones in the presence of magnesium methyl carbonate in DMF. In general, β-ketoacids do not give the acid chlorides by conventional methods; however, treatment of the acids with *n*-butyllithium afforded the anhydrous lithium salts, which upon further treatment with isobutyl chlorocarbonate resulted in the corresponding mixed anhydrides in reasonable yields. Finally, the reaction of the mixed anhydrides with 7-aminocephalosporins led to 7-β-ketoacylaminocephalosporins (Numata *et al.* 1978*a*). Contrary to our expectations, however,

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the compounds thus obtained and the analogues that were derived by subsequent substitution at the 3′-position with nucleophiles showed only moderate activities. When an ε-methylthioacetoacetyl group was introduced onto the 7-amino of 7-ACA, the compound (**13**) thus obtained showed an activity as potent as that of cephalothin (figure 6) (Numata *et al.* 1978*b*).

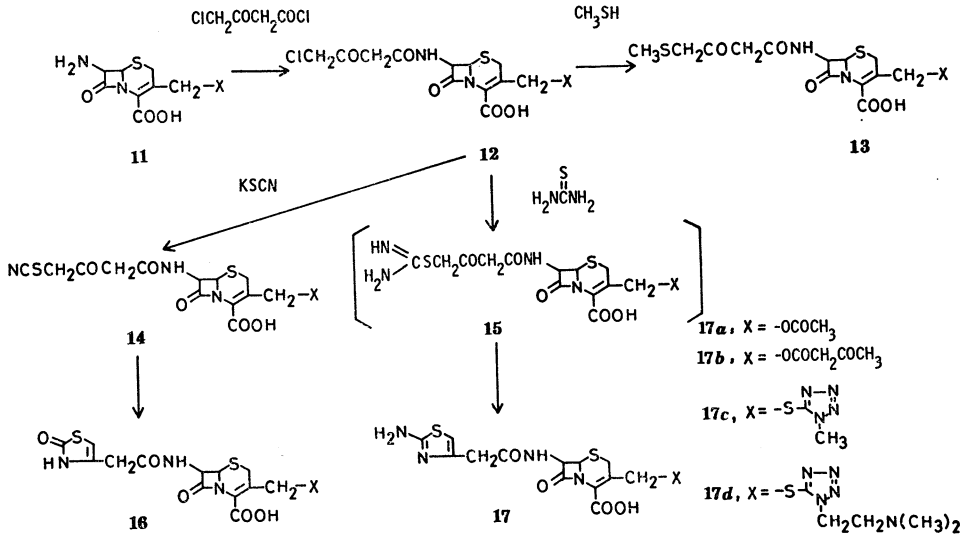

FIGURE 6. Synthesis of 2-aminothiazol-4-ylacetyl cephalosporins.

The above-mentioned finding encouraged us to synthesize the analogues more extensively. We tried to replace the chlorine of ε-chloroacetoacetyl cephalosporins (**12**) by treating with thiocyanate. When the thin-layer chromatogram of an aliquot of the reaction mixture was run, a new spot was detected on the thin-layer plate; however, when the experiment was repeated several days later, it was found that the specimen had changed into another substance which showed a definitely faster mobility on the chromatoplate. Therefore, the experiment was conducted under more drastic conditions. Eventually, a hitherto unknown compound was isolated in good yields and the structure was established as 2-oxo-4-thiazolin-4-ylacetyl cephalosporin (**16**). When thiourea was used in place of thiocyanate, the corresponding 2-amino-thiazol-4-ylacetyl cephalosporins (**17**) were obtained in good yields. The 2-aminothiazol-4-ylacetyl cephalosporins thus obtained turned out to have a remarkable antibacterial activity (Numata *et al.* 1977, 1978*c*).

Although spectroscopic and physico-chemical data indicate that the 2-iminothiazoline structure seems to be favoured rather than the 2-aminothiazole, 2-amino structure will be adopted, following the conventions in the chemistry of heterocycles (Katritzky & Lagowsky 1963) hereafter in the present paper.

Subsequent chemical modifications were straightforward, and among many analogues thus synthesized we finally selected 7β-[2-(2-aminothiazol-4-yl)acetamido]-3-[[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-yl]thiomethyl]ceph-3-em-4-carboxylic acid (coded as SCE-963 or cefotiam) (Numata *et al.* 1977) for further detailed investigations. In fact, cefotiam was found to have outstanding *in vitro* and *in vivo* activities and excellent therapeutic efficacy against a wide range of Gram-positive and Gram-negative bacteria in clinical trials (Tsuchiya *et al.* 1978*b*) (table 1).

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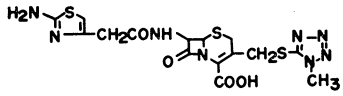
Obviously, antibacterial activity depends upon factors such as enzyme recognition, cell permeability, susceptibility to β-lactamases as well as intrinsic acylating ability.

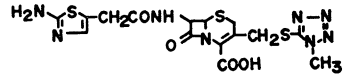
In fact, a position isomer (**18**) of a cefotiam congener (**17c**) showed a lower degree of activity as shown in table 2. A reasonable explanation for the difference of the activity between the two isomers will have to await future studies.

TABLE 1. COMPARISON OF THE MINIMUM INHIBITORY CONCENTRATIONS (micrograms per millilitre) OF CEFOTIAM AND CEFAZOLIN

organism	cefotiam (**17d**)	cefazolin
S. aureus 209P	0.39	0.39
S. aureus 1840	1.56	3.13
E. coli NIHJ JC-2	0.2	1.56
E. coli O-111	0.05	1.56
E. coli T-7	3.13	100
K. pneumoniae DT	0.1	1.56
P. vulgaris IFO 3988	0.78	6.25
P. morganii IFO 3168	0.39	> 100
P. rettgeri GN 4733	0.78	100
C. freundii GN 99	0.78	> 100
S. marcescens TN 1774	25	> 100

TABLE 2. COMPARISON OF THE MINIMUM INHIBITORY CONCENTRATIONS (micrograms per millilitre) OF POSITION ISOMERS **17c** AND **18**





organism	**17c**	**18**
S. aureus 209P	0.39	0.39
S. aureus 1840	0.78	0.78
E. coli NIHJ JC-2	0.39	3.13
E. coil O-111	0.1	0.78
E. coli T-7	6.25	> 100
K. pneumoniae DT	0.2	1.56
P. vulgaris IFO 3988	0.39	1.56
P. morganii IFO 3168	1.56	> 100

SCE-1365

Researchers of Eli Lilly & Co. discovered several years ago that introduction of an α-hydroxyl into the phenylacetamido side chain confers cephalosporins with a profound activity. This enabled the company to succeed in developing the well-reputed cephalosporin, cefamandole (Wick *et al.* 1972). Similarly, introduction of a D-phenylglycin moiety into the cephalosporin structure led to an orally active cephalosporin (Ryan *et al.* 1969). Also, quite recently Glaxo's group succeeded in the development of cefuroxime (Cherry *et al.* 1977), in which a unique *syn*-methoxyimino moiety was introduced onto the α-carbon of furylacetamido side chain.

In recent years, the Merck (Cama *et al.* 1972) and Eli Lilly (Nagarajan *et al.* 1971) scientists revealed that methoxy group introduced on to the 7α-position of the cephalosporin skeleton markedly increased the resistance of the cephalosporin to β-lactamases. It is therefore interesting to investigate if an adequate combination of the aforementioned chemical modifications would lead to compounds with further enhanced activity and resistance to β-lactamases.

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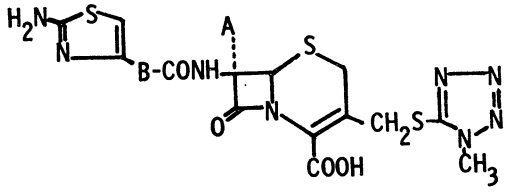
Thus, the first step that we undertook was to introduce an amino group to the α-position of the 2-aminothiazol-4-ylacetamido side chain with an expectation of obtaining orally active cephalosporins. In addition to this, we carried out a sequence of reactions to introduce α-hydroxyl, α-methyl, α,α-dimethyl and other groups into the 2-aminothiazol-4-ylacetyl side chain by various means (Ochiai *et al.* 1978).

TABLE 3. THE MINIMUM INHIBITORY CONCENTRATIONS (micrograms per millilitre) OF 2-AMINOTHIAZOLYLACETAMIDOCEPHALOSPORIN DERIVATIVES AGAINST β-LACTAMASE-PRODUCING STRAINS



	17c	**19**	**20**	**21**	**22**	**23**	SCE-1365(24)
A	H	H	H	H	H	OCH_3	H
B	–CH– | H	–CH– | OH	–CH– | CH_3	CH_3 | –C– | CH_3	–CH– | NH_2	–CH– | H	–C– ‖ N \ (Z) OCH_3
organism		(DL)	(DL)		(DL)		
S. aureus 1840	0.78	1.56	3.13	50	12.5	3.13	3.13
E. coli T-7	6.25	1.56	25	> 100	3.13	6.25	0.78
S. marcescens TN 24	100	25	12.5	> 100	50	6.25	0.20
P. vulgaris GN 4413	> 100	> 100	> 100	> 100	> 100	6.25	0.39
E. cloacae TN 1282	> 100	6.25	100	> 100	25	> 100	1.56

TABLE 4. HYDROLYSIS OF SCE-1365 AND THE RELATED COMPOUNDS BY β-LACTAMASES

	relative rates of hydrolysis, with cephaloridine = 100				
source of enzyme ...	*S. aureus*† 1840	*E. coli* T-7	*S. marcescens* TN 24		*P. vulgaris* GN 4413	*E. cloacae* TN 1282
substrate	(PCase)	(PCase)	(PCase)	(CSase)	(CSase)	(CSase)
cephaloridine	0.14	100	100	100	100	100
SCE-1365	< 0.01	0.32	0.31	< 0.1	44.9 (0.39)‡	0.08
cefuroxime	0.05	0.34	0.35	0.6	232	0.13
cefoxitin	< 0.01	< 0.01	< 0.01	< 0.1	< 0.01 (6.25)‡	0.31

The rate of hydrolysis was determined by the ultraviolet method.
† Relative rates of hydrolysis, with penicillin G = 100.
‡ Minimum inhibitory concentration in micrograms per millilitre.

When we finally synthesized the desired α-amino compound (**22**) and its congeners, they showed a fairly high activity. But, when we synthesized a 2-aminothiazol-4-yl-2-methoxyimino-acetamido derivative (**24**) (Ochiai *et al.* 1977), the compound showed a potency and broad-spectrum activity never before encountered, together with an excellent resistance to various β-lactamases as shown in tables 3 and 4.

It is interesting to note that the α,α-dimethyl derivative (**21**), in sharp contrast with other derivatives possessing hydrogen on the α-carbon, showed a greatly diminished activity.

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The stereochemistry of the side chain structure of a more potent isomer (**24**) was soon established to be *syn* by analogy with cefuroxime.

As already pointed out, cefuroxime is unique in that the compound, while lacking the active hydrogen on the α-carbon of the side-chain acyl, shows a potent antibacterial activity together with a profound resistance to β-lactamases. The highly resistant nature of *syn*-methoxyimino-acetyl cephalosporins, including SCE-1365 and others, to β-lactamases seems to be due to steric hindrance of the *syn*-methoxyimino group. On the other hand, potent antibiotic activity

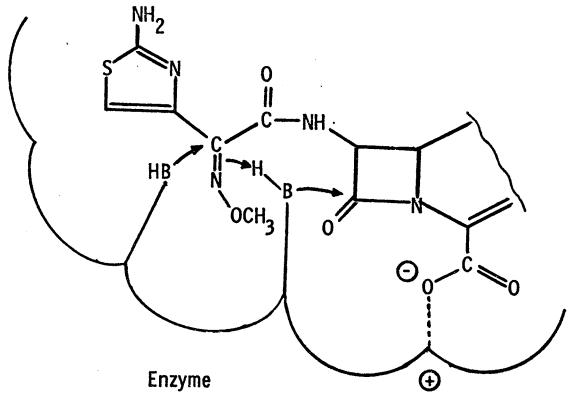

FIGURE 7. Extension of an 'intramolecular general-base catalysis' mechanism to α-methoxyiminoacetyl cephalosporins.

TABLE 5. COMPARATIVE *IN VITRO* ACTIVITY OF SCE-1365 AND CEFOTIAM: CONCENTRATION (micrograms per millilitre) REQUIRED TO INHIBIT 50 % OF STRAINS

organism (number of strains)	SCE-1365	cefotiam
S. aureus (105)	1.56	0.78
S. pyogenes (52)	0.013	0.05
E. coli (104)	0.1	0.2
K. pneumoniae (75)	0.1	0.2
H. influenzae (69)	0.013	0.78
P. mirabilis (107)	0.05	0.39
indole-positive *Proteus* (231)	0.1	25
C. freundii (80)	0.39	1.56
E. cloacae (78)	0.39	3.13
S. marcescens (104)	0.2	6.25
P. aeruginosa (108)	12.5	> 100
B. fragilis (4)	3.13	> 100

of the compounds might be explained by the notion of steric assistance. These two accounts appear more or less self-contradictory and therefore we thought that a more reasonable and less contradictory explanation would be needed. Thus, we thought that a methoxyimino-cephalosporin, once at a proper site, would be attacked by a base in the enzyme at the α-carbon of the side chain acyl to cause the electron displacement in the C=N double bond. Thus, a whole picture of possible 'intra-molecular general-base catalysis' can be depicted in figure 7.

Synthesis of a number of analogues with the 2-aminothiazol-4-yl-2-*syn*-methoxyiminoaceta-mido side chain on the 7-position and bearing a variety of heterocyclicthiomethyl substituents on the 3-position was again straightforward. Finally, we selected a compound coded as SCE-1365 (Ochiai *et al.* 1978), which has 1-*N*-methyltetrazolylthiomethyl on the 3-position for further development and ultimate clinical trials.

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At almost the same time, researchers of Roussel-Uclaf (Bucourt *et al.* 1977) in France and those of Fujisawa (Takaya *et al.* 1978) in Japan synthesized the compounds of the same side chain structure, and claimed that these compounds share highly potent and broad-spectrum activity comparable with that of SCE-1365.

As shown in table 5, SCE-1365 exhibits a potent and extraordinarily extended antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria. It should be noted that the spectral advantage of SCE-1365 encompasses the so-called 'problem' pathogenic bacteria such as indole-positive *Proteus*, *Serratia marcescens*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Bacteroides fragilis*, both *in vitro* and in experimental infections in animals.

Some years ago, we reported our new cephalosporin, SCE-129 (cefsulodin) (Nomura *et al.* 1974), which exhibited a unique and profound antipseudomonal activity comparable to, or in some cases, superior to aminoglycosides including gentamicin, dibekacin and others (Tsuchiya & Kondo 1978*a*; Kondo & Tsuchiya 1978). Because the antibacterial activities of SCE-129 and SCE-963 or SCE-1365 are complementary to cover a wide range of bacteria, and cephalosporins are far less toxic in nature compared to aminoglycosides, we believe that combinations of these cephalosporins could well control a wide range of pathogenic bacteria.

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