SEMISYNTHETIC β-LACTAM ANTIBIOTICS

I. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 7β-[2-ARYL-2-(AMINOACETAMIDO)ACETAMIDO]-CEPHALOSPORINS

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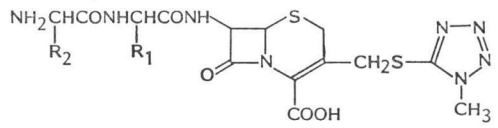
The synthesis and *in vitro* structure-activity relationships of cephalosporins having dipeptides substituted with various aryl groups as the side chain at the C-7 position have been outlined. Of these compounds, 2-aminothiazol derivatives showed a broad spectrum of enhanced antibacterial activity, and 7β-[DL-2-(D-aminopropionamido)-2-(2-aminothiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (**9**) was the most balanced of these active derivatives with respect to both Gram-positive and Gram-negative strains.

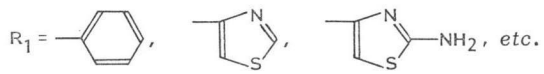
In recent years, the search for newer cephalosporin antibiotics has made remarkable progress, but the management of infections caused by multiple antibiotic-resistant bacteria and opportunistic pathogens, *e.g. Pseudomonas* and *Serratia* species, is still a serious therapeutic problem. For this reason, many research groups, world wide, have been searching for more effective β-lactam antibiotics.

This paper deals with the first stage of an attempt to identify new β-lactam antibiotics with enhanced activity against bacteria over an expanded spectrum and with greater β-lactamase stability. It describes the synthesis of new compounds represented by the general structure **I** (Fig. 1) and the antibacterial effects of the substituents R_1 and R_2 on the *in vitro* activity.

Fig. 1.



I



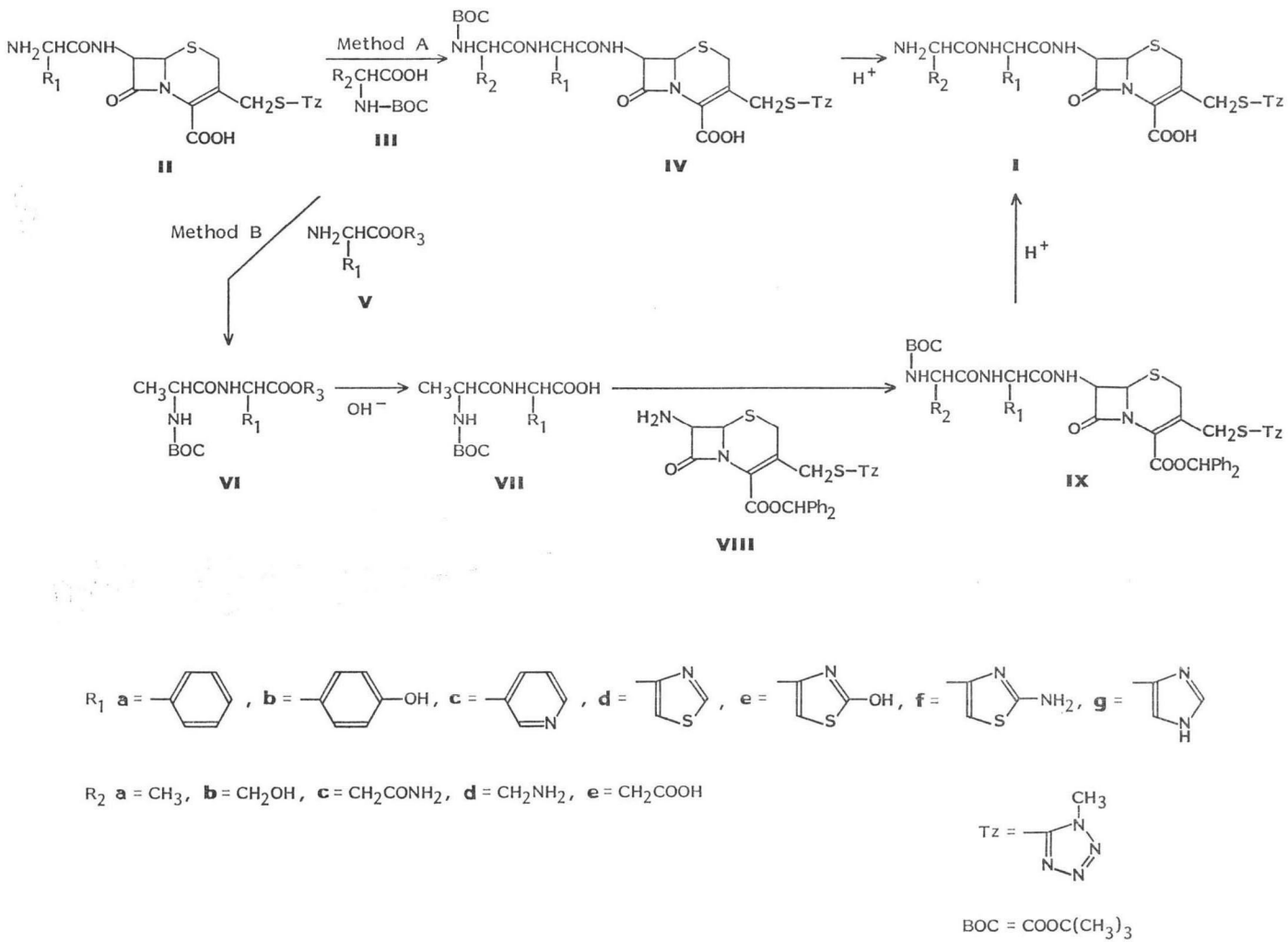
$R_2 = CH_3$, CH_2NH_2, CH_2COOH, *etc.*

It is well known that β-lactam antibiotics have a similar conformation to that of D-alanyl-D-alanine, which is the terminal dipeptide of *N*-acetyl-glucosamyl-pentapeptide, and consequently reacted with D-alanine-carboxypeptidase and glycopeptide transpeptidase.[1] As the natural substrates have pentapeptide structures incorporating D-alanyl-D-alanine, cephalosporin derivatives substituted with this dipeptide at the C-7 position of the cephem nucleus may, by analogy, have enhanced affinity to the target enzymes, consequently, may have enhanced antibacterial activity. This reasoning led us to synthesize compounds with the general formula **I**.

Chemistry

The cephalosporins listed in Table 1 were prepared by the two general methods, A and B, outlined

Scheme 1.


Method A
Method B
H+
OH−
II
III
IV
I
V
VI
VII
VIII
IX
R1 a = , b = , c = , d = , e = , f = , g =
R2 a = CH3, b = CH2OH, c = CH2CONH2, d = CH2NH2, e = CH2COOH
Tz =
BOC = COOC(CH3)3

in Scheme 1.

Method A was adopted to introduce two amino acids, stepwise, into the cephem nucleus. The desired cephalosporins (**I**) were derived by peptide coupling of an aliphatic amino acid (**III**) with aromatic glycylcephalosporins (**II**), which were prepared as previously reported,[2~5] or using the mixed anhydride or active ester formed from the appropriate *N-tert*-butoxycarbonyl amino acid (**III**). Following this, the protecting group was removed by use of trifluoroacetic acid-anisole. Method B involved the preliminary preparation of dipeptide (**VII**), followed by coupling with the cephem nucleus (**VIII**). Conversion of the aromatic glycyl ester (**V**) into the dipeptide ester (**VI**) with the mixed anhydride of **III** was followed by ester hydrolysis to give the dipeptides (**VII**). The coupling reaction of **VII** with **VIII** into cephem derivatives (**IX**) was achieved by the mixed anhydride method. Deprotection by a procedure similar to that used in Method A afforded the final product, **I**.

Some derivatives having a methyl group as the substituent R_2 were synthesized by both methods, A and B, but no advantage of Method B over Method A was found. Therefore, Method A was employed for the synthesis of other cephalosporin derivatives; it was more convenient for the synthesis of many similar derivatives.

D-Amino acid (**III**), rather than the L-isomer, was chosen as the terminal amino acid in the dipeptide side chain of **I** to avoid decomposition with amidase *in vivo*. Optical resolution of (*dl*)-*N*-(*tert*-butoxycarbonyl)-2-(thiazol-4-yl)glycine (**IIId**), which was one of the starting materials of **I**, was accomplished with optically active ephedrine.

The coupling reaction of 2-hydroxythiazol-4-yl derivatives (**VIIe**) with **VIII** by the mixed anhydride method using isobutyl chloroformate did not give the expected product (**IX**), but the ester deriva-

Scheme 2.

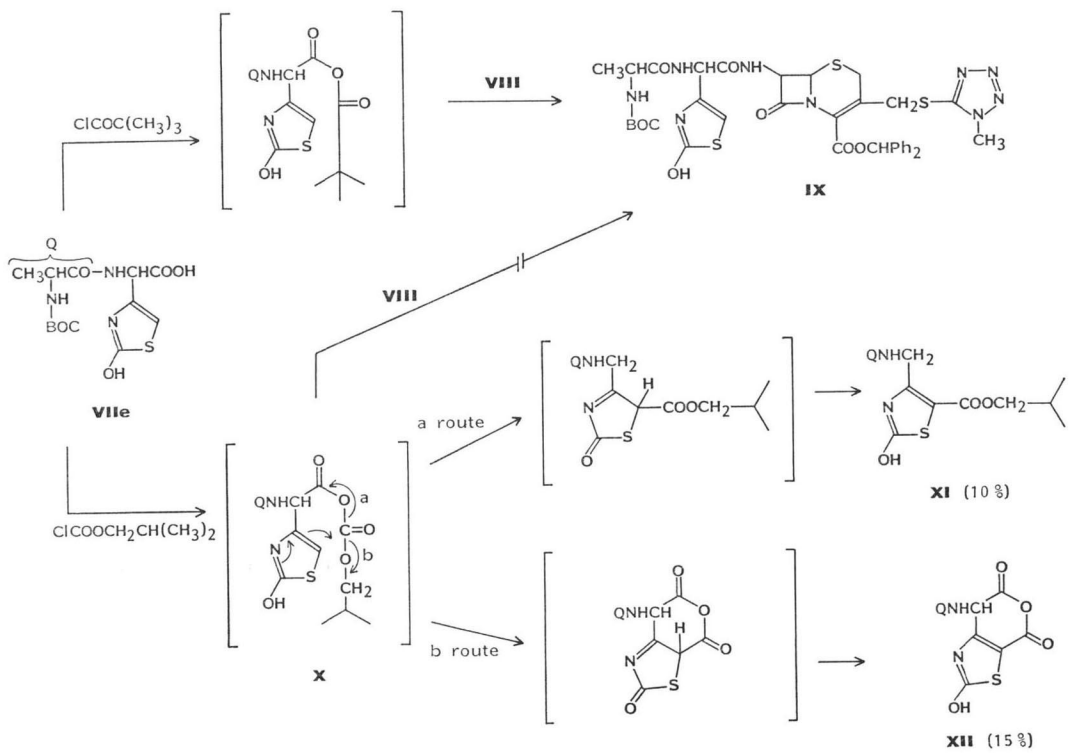
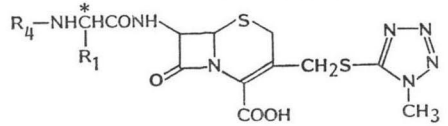
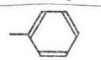


Table 1. Antibacterial activity (MIC, μg/ml) of cephalosporins (I).

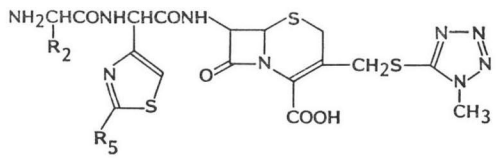


Compound	Structure R_1	Structure R_4	Optical activity*	Synthetic route	*S.a.*	*S.p.*	*E.c.*	*P.m.*	*K.p.*	*En.c.*
1		H	*dl*	—	25	12.5	3.13	12.5	50	>100
2		$COCHCH_3$ | NH_2	*dl*	A	3.13	1.56	0.78	1.56	50	12.5
3		$COCHCH_3$ | NH_2	*dl*	A, B	1.56	0.78	0.78	3.13	25	6.25
4		$COCHCH_3$ | NH_2	*dl*	A	1.56	0.78	1.56	6.25	25	12.5
5		H	*dl*	—	3.13	6.25	6.25	25	25	>100
6		$COCHCH_3$ | NH_2	*dl*	A, B	0.78	0.39	0.78	0.78	0.78	0.78
7		$COCHCH_3$ | NH_2	*dl*	A, B	3.13	3.13	6.25	6.25	25	12.5
8		H	*dl*	—	6.25	1.56	0.78	1.56	1.56	12.5
9		$COCHCH_3$ | NH_2	*dl*	A, B	1.56	0.39	0.20	0.39	0.39	0.39
10		$COCHCH_3$ | NH_2	*dl*	A	12.5	1.56	6.25	6.25	6.25	12.5
11		H	*d*	—	1.56	6.25	6.25	25	25	>100
12		H	*l*	—	25	12.5	25	50	50	>100
13		$COCHCH_3$ | NH_2	*d*	A	1.56	0.39	0.78	0.78	0.78	0.78
14		$COCHCH_3$ | NH_2	*l*	A	3.13	0.39	0.78	1.56	0.78	0.78

* Optical rotation of 2-aromatic-glycine.

Abbreviations: *S.a.*; *Staphylococcus aureus* 209P, *S.p.*; *Streptococcus pyogenes* G-36, *E.c.*; *Escherichia coli* NIHJ, *P.m.*; *Proteus mirabilis* 1287, *K.p.*; *Klebsiella pneumoniae* Type 1, *En.c.*; *Enterobacter cloacae* 12001.

Table 2. Antibacterial activity (MIC, μg/ml) of cephalosporins (I).



Compound	Structure		*S.a.**	*S.p.*	*E.c.*	*P.m.*	*K.p.*	*En.c.*	*P.a.***
	R_2	R_5							
6	CH_3	H	1.56	0.39	0.78	0.78	0.78	0.78	>100
15	CH_2OH	H	3.13	0.78	0.78	0.78	0.78	3.13	>100
16	CH_2CONH_2	H	6.25	3.13	1.56	0.78	1.56	6.25	>100
17	CH_2NH_2	H	6.25	0.39	0.78	1.56	6.25	3.13	>100
18	H	NH_2	1.56	0.20	0.20	0.39	0.39	0.78	>100
9	CH_3	NH_2	1.56	0.20	0.20	0.39	0.39	0.20	>100
19	CH_2OH	NH_2	3.13	0.39	0.20	0.20	0.20	0.78	>100
20	CH_2CONH_2	NH_2	3.13	0.39	0.20	0.20	0.20	0.20	>100
21	CH_2NH_2	NH_2	6.25	0.39	0.20	0.39	0.39	0.39	>100
22	CH_2COOH	NH_2	6.25	0.78	0.20	0.20	0.20	0.20	>100
Cefotaxime			1.56	0.39	0.20	0.20	0.20	0.20	12.5

* See footnote in Table 1.

** *P.a.*; *Pseudomonas aeruginosa* 2092.

tive (**XI**) and the acid anhydride derivative (**XII**) in 10% and 15% yields, respectively. However, the reaction using pivaloyl chloride did give the expected compound **IX**. The following mechanism appears to account for the formation of compounds **XI** and **XII** as shown in Scheme 2. The C-5 position of the thiazole ring was intensely activated by a hydroxyl group at the 2-position, and consequently, an intramolecular reaction of the mixed anhydride **X** (a and b routes) proceeded instead of an intermolecular acylation between **X** and the amino group of cephem **VIII**. When pivaloyl chloride was employed, these side reactions were not observed because of steric hindrance by the bulky pivaloyl radical.

Antibacterial Activity and Discussion

The minimum inhibitory concentration (MIC) values of this series of cephalosporins against selected strains of Gram-positive and Gram-negative bacteria were determined by the standard serial two-fold agar dilution method.[6] Effects of the second amino acid (R_4) and the aromatic group (R_1) on the *in vitro* antibacterial activity are shown in Table 1. The introduction of thiazol-4-yl (**6**) and 2-aminothiazol-4-yl (**9**) groups as R_1 led to increased activity, especially against Gram-negative bacteria, to a greater extent than that of phenyl (**2**), hydroxyphenyl (**3**), pyridyl (**4**), 2-hydroxythiazolyl (**7**) and imidazolyl (**10**) groups.

POLACEK[7] reported that the L-isomer of 7-(α-ureido-2-amino-4-thiazolylacetyl)cephalosporin was more active than the corresponding D-isomer. In our study, cephalosporin modified with the thiazol-4-yl group at the C-7 position exhibited a distinct difference of activity between optical isomers (**11** vs. **12**). However, the activities of the optically active dipeptide compounds (**13** vs. **14**) were almost equal. This finding is in interesting contrast with the POLACEK report.

The effects on antibacterial activity of the terminal amino acid linked to thiazol-4-yl and 2-aminothiazol-4-yl glycine at the C-7 position are shown in Table 2. Introduction of an amino group (**9**, **19**~**22**) into the 2-position of the thiazole ring yielded increased potency for all substituent groups at R_2

against Gram-negative strains except *Pseudomonas*, but did not affect significantly the activity against Gram-positive strains.

Introduction of all of the hydrophilic groups (Table 2), which are neutral (**19**, **20**), basic (**21**) and acidic (**22**), led to a decrease of the activity against Gram-positive strains, and to an increase of that against Gram-negative strains. This was especially marked in the case of the acidic group, and was considered to be related to the penetration of the bacterial cell membranes by the cephalosporins.

Some of cephalosporin derivatives (**9**, **20**) prepared in this study exhibited antibacterial activity comparable to that of cefotaxime[5,9] (Tables 1 and 2), with the exception of its anti-pseudomonal activity. When R_1 in structure I was the 2-aminothiazol-4-yl, the antibacterial activity was enhanced. The compounds (**9**, **18**~**20**) substituted with neutral groups R_2 showed a good balance between activity against Gram-positive and Gram-negative strains.

Experimental

Melting points were determined using a Yanagimoto MP-1 micro melting point apparatus and are uncorrected. IR spectra were taken on a Hitachi 285 spectrophotometer. NMR spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-20B and at 200 MHz on a Varian XL-200 spectrometer using TMS or sodium 2,2-dimethyl-2-silapentane-1-sulfonate (DSS) as an internal standard. Optical rotations were determined with Perkin-Elmer 141 polarimeter. Organic solvents were dried over anhydrous Na_2SO_4 and all concentrations by evaporation were carried out *in vacuo*.

7β-[DL-2-Amino-2-(imidazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**IIg**)

1) Preparation of DL-2-(*tert*-Butoxycarboxamido)-2-(1-*tert*-butoxycarbonylimidazol-4-yl)acetic Acid: A mixture of DL-2-amino-2-(imidazol-4-yl)acetic acid[10] (1.41 g, 10 mmol), 2-*tert*-butoxycarbonyloxyimino-2-phenylacetonitrile (7.41 g, 30 mmol) and triethylamine (3.03 g, 30 mmol) in dioxane (30 ml) and H_2O (20 ml) was stirred for 3 hours at room temp. The reaction mixture was poured into H_2O and washed with EtOAc. The separated aqueous layer was acidified (pH 4) with 10% citric acid and extracted with EtOAc. The extract was washed with H_2O, dried and evaporated to give a colorless powder (1.72 g, 50.4%): NMR ($CDCl_3$) δ 1.47 (9H, s, *tert*-Bu), 1.64 (9H, s, *tert*-Bu), 5.40 (1H, d, glycine-CHCO), 7.52 (1H, s, imidazole 5-H), 8.24 (1H, s, imidazole 2-H).

2) Preparation of 7β-[DL-2-(*tert*-Butoxycarboxamido)-2-(1-*tert*-butoxycarbonylimidazol-4-yl)-acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid: To an ice-cold solution of the above acetic acid (1.70 g, 5 mmol) and *N*-hydroxysuccinimide (0.575 g, 5 mmol) in THF (15 ml) was added *N*,*N*-dicyclohexylcarbodiimide (1.03 g, 5 mmol), and the mixture was stirred for 3 hours while being ice-cooled. The solid was filtered off, and the filtrate was added dropwise to an ice-cold solution of 7β-amino-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (1.64 g, 5 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (6 ml) in CH_2Cl_2 (20 ml), and the reaction mixture was stirred for 1 hour with ice-cooling and for an additional 3 hours at room temp. After removing the solvent, the residue was dissolved with EtOAc and washed with 10% citric acid. The organic layer was separated, washed with H_2O, dried and evaporated to give a colorless powder (0.942 g, 28.9%): MP 150~155°C (dec); IR (KBr) 1785, 1760, 1740, 1720, 1395 cm^{-1}; NMR ($CDCl_3$) δ 1.41 (9H, s, *tert*-Bu), 1.59 (9H, s, *tert*-Bu), 3.5~3.8 (2H, br s, C2-CH_2), 3.90 (3H, s, tetrazole-CH_3), 4.2~4.6 (2H, br s, C3-H), 4.8~5.1 (1H, br s, C6-H), 5.39 (1H, d, J=7 Hz, glycine-CH), 5.4~5.5 (1H, br s, C7-H), 7.44 (1H, s, imidazole 5-H), 8.17 (1H, s, imidazole 2-H).

3) Preparation of Compound **IIg**: A solution of the above (1.30 g, 2 mmol) in TFA (12 ml) and anisole (1.8 ml) was stirred at room temp for 1 hour. The solution was added to Et_2O (80 ml) with stirring. The resulting precipitates were filtered off and washed with Et_2O to give trifluoroacetate as a powder. The solution of this powder in H_2O (50 ml) was adjusted to pH 5.4 with Amberlite IRA-45 (OH^-) and filtered. The filtrate was evaporated and the residue was triturated with Et_2O to afford the

title compound as a powder (0.495 g, 54.8%): MP 154~164°C (dec); IR (KBr) 1765, 1680, 1590, 1610 cm^{-1}.

Anal Calcd for $C_{15}H_{17}N_9O_4S_2 \cdot H_2O$: C 38.37, H 4.24, N 26.85.
Found: C 38.92, H 4.33, N 26.48.

General Procedure for the Preparation of **I** (Method A): A Typical Procedure is Described for the Preparation of 7β-[DL-2-(D-2-Aminopropionamido)-2-(thiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**6**)

1) Preparation of 7β-[DL-2-(D-2-*tert*-Butoxycarboxamidopropionamido)-2-[(thiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**IV**; R_1=**d**, R_2=**a**): To a cooled (−15°C) solution of **IIIa** (378 mg, 2 mmol) and *N*-methylmorpholine (202 mg, 2 mmol) in THF (15 ml) was added a solution of isobutyl chloroformate (273 mg, 2 mmol) in THF (22 ml) and the mixture was stirred for 30 minutes to give a mixed anhydride solution. To this solution was added dropwise an ice-cooled solution of **IId**[4] (937 mg, 2 mmol) and *N*-methylmorpholine (202 mg, 2 mmol) in 50% aq THF (10 ml). The reaction mixture was stirred for 1 hour with ice-cooling and for another 2 hours at room temp. After removal of the solvent, the residue was dissolved in H_2O and washed with EtOAc. The aqueous layer was adjusted to pH 2 with 10% citric acid and extracted with EtOAc. The organic layer was washed with H_2O, dried and evaporated. Trituration of the residue with Et_2O and EtOAc afforded the desired compound (620 mg, 48.5%): MP 150~157°C (dec); IR (KBr) 1780, 1685, 1510 cm^{-1}; NMR (DMSO-d_6) δ 1.20 and 1.22 (3H, 2×d, J=7 Hz, glycine-CH_3), 1.39 (9H, s, *tert*-Bu), 3.64 and 3.70 (1H, br s, C2-CH_2), 3.97 (3H, s, tetrazole-CH_3), 4.30 (2H, br s, C3-CH_2), 5.04 and 5.09 (1H, 2×d, J=5 Hz, C6-H), 5.5~6.0 (2H, m, thiazole-CHCO and C7-H), 7.6~7.7 (1H, br s, thiazole 5-H).

Anal Calcd for $C_{23}H_{29}N_9O_7S_3$: C 43.18, H 4.57, N 19.71.
Found: C 43.50, H 4.73, N 19.37.

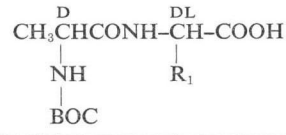
2) Removal of Protecting Group: To a cooled (−15°C) mixture of **IV** (R_1=**d**, R_2=**a**) (1.28 g, 2 mmol) and anisole (1 ml) was added trifluoroacetic acid (7 ml). The mixture was stirred for 1 hour in ice and for another 30 minutes at room temp. The solvent was evaporated and the residue was dissolved in H_2O and then adjusted to pH 7 with aq $NaHCO_3$. This solution was subjected to column chromatography on a non-ionic adsorption resin (Diaion HP-20). The column was washed with H_2O and eluted with 30% MeOH. The eluates containing the product were combined, evaporated to remove MeOH and lyophilized to afford the desired compound **6** (0.608 g, 56.3%): MP 170~185°C (dec).

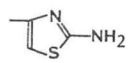
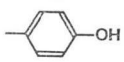
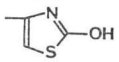
General Procedure for the Preparation of **I** (Method B): A Typical Procedure is Described for the Preparation of 7β-[DL-2-(D-Aminopropionamido)-2-(2-aminothiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**9**)

1) Preparation of Ethyl 2-(2-*tert*-Butoxycarboxamidopropionamido)-2-(2-aminothiazol-4-yl)acetate (**VI**; R_1=**f**, R_3=Et): To a cooled (−14°C) solution of *N*-*tert*-butoxycarbonyl-D-alanine (1.14 g, 6 mmol) and *N*-methylmorpholine (0.607 g, 6 mmol) in THF (20 ml) was added isobutyl chloroformate (0.82 g, 6 mmol) with stirring. After 20 minutes of stirring at the same temp, a solution of ethyl (2-aminothiazol-4-yl)glycinate[5] (1.20 g, 6 mmol) in 50% aq THF (20 ml) was added dropwise to this mixture. The mixture was stirred for 1 hour at the same temp and for another 30 minutes at room temp. The reaction mixture was concd. The residue was diluted with EtOAc, washed successively with 10% citric acid, 4% $NaHCO_3$, and brine, and then dried. After evaporation of the solvent, trituration with petroleum ether-Et_2O give a solid (1.93 g, 86.4%): MP 81~85°C (dec); IR (KBr) 1740, 1670, 1510, 1160 cm^{-1}; NMR ($CDCl_3$) δ 1.36 (3H, d, J=7 Hz, glycine-CH_3), 1.45 (9H, s, *tert*-Bu), 3.75 (3H, s, OCH_3), 4.0~4.4 (1H, m, glycine-CH), 5.1~5.4 (1H, br s, NH), 5.48 (1H, d, J=8 Hz, thiazole-CHCO), 5.97 (2H, br s, NH_2), 6.49 (1H, s, thiazole 5-H).

2) Preparation of 2-(2-*tert*-Butoxycarboxamidopropionamido)-2-(2-aminothiazol-4-yl)acetic Acid (**VIIf**): The above solid (1.86 g, 5 mmol) was stirred with 1 N NaOH (5.5 ml) in 50% aq EtOH at room temp for 2 hours. The mixture was adjusted to pH 6.8 with aq citric acid with cooling, and then concd. It was next extracted with EtOAc, dried, and evaporated. Trituration with Et_2O give colorless crystals (0.46 g, 26.7%): MP 107~110°C (dec); IR (KBr) 1720, 1680, 1630, 1510 cm^{-1}; NMR (DMSO-d_6) δ

Table 3. Melting point, IR and NMR data of dipeptides (VII).



R_1	MP (°C (dec))	IR (KBr) cm^{-1}	NMR (DMSO-d_6) δ
	102~105	1720, 1630	1.21 (3H, d), 1.39 (9H s), 3.6~3.7 (1H, m), 5.69 (1H, d), 7.65 (1H, d), 9.05 (1H, d)
	107~110	1720~1680	1.19 (3H, d), 1.39 (9H, s), 3.8~4.2 (1H, m), 5.20 (1H, d), 6.51 (1H, s), 7.01 (1H, br s)
	115~120	1720~1660	1.20 (3H, d), 1.40 (9H, s), 4.09 (1H, t), 5.10 (1H, d), 6.76 (2H, d), 7.24 (2H, d)
	127~130	1720, 1665	1.22 (3H, d), 1.40 (9H, s), 3.9~4.2 (1H, m), 5.24 (1H, s), 6.24 ($\frac{1}{2}$H, s), 6.30 ($\frac{1}{2}$H)

1.19 (3H, d, J=7 Hz, glycine-CH_3), 1.39 (9H, s, *tert*-Bu), 3.8~4.2 (1H, m, glycine-CH), 5.20 (1H, d, J=8 Hz, thiazole-CHCO), 6.51 (1H, s, thiazole 5-H), 7.01 (2H, br s, NH_2), 8.07 (1H, d, J=8 Hz, NH).

Various dipeptide derivatives (**VII**), synthesized according to Method B and bearing different aromatic rings are listed in Table 3.

3) Preparation of 7β-[DL-2-(D-2-*tert*-Butoxycarboxamidopropionamido)-2-(2-aminothiazol-4-yl) acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid Diphenylmethyl Ester (**IX**; R_1=**f**, R_2=**a**): Isobutyl chloroformate (273 mg, 2 mmol) was added to a cooled (−18°C) solution of dipeptide **VIIf** (689 mg, 2 mmol) and triethylamine (202 mg, 2 mmol) in a mixture of THF (8 ml), dioxane (2 ml) and DMF (4 ml) with stirring, and the solution was further stirred for 30 minutes at −15°C. To this solution was added dropwise a cooled (0°C) solution of **VIII** (1.02 g, 2 mmol) in THF (2 ml), and then continuous stirring was maintained at the same temp for 2 hours, and at room temp for 1 hour. After removal of the solvent, the residue was dissolved in H_2O and extracted with EtOAc. The organic layer was washed with 10% citric acid, 4% $NaHCO_3$ and brine successively and dried. After evaporation of the solvent, the residue was subjected to silica gel chromatogram which was eluted with $CHCl_3$. The fractions containing the desired compound were evaporated and the residue was triturated with Et_2O to afford the title compound (1.18 g, 71.9%): MP 123~135°C (dec); IR (KBr) 1780, 1700, 1510 cm^{-1}; NMR ($CDCl_3$) δ 1.40 (9H, s, *tert*-Bu), 3.69 (2H, br s, C2-CH_2), 3.96 (3H, s, tetrazole-CH_3), 4.0~4.9 (3H, m, C3-CH_2 and glycine-CH_3), 5.00 (1H, d, J=5 Hz, C6-H), 5.2~6.3 (4H, br s, thiazole-CHCO and C7-H), 6.50 (1H, s, thiazole 5-H), 7.03 (1H, s, diphenyl-CH), 7.42 (10H, s, phenyl).

Anal Calcd for $C_{36}H_{40}N_{10}O_7S_3 \cdot H_2O$: C 51.54, H 5.05, N 16.70.
Found: C 51.81, H 5.22, N 16.38.

4) Removal of Protecting Group of **IX**: The removal reaction was carried out as described for the Method A to give **9**: MP 180~186°C (dec).

Anal Calcd for $C_{18}H_{22}N_{10}O_5S_3 \cdot 1\frac{1}{2}H_2O$: C 37.17, H 4.33, N 24.08.
Found: C 36.98, H 4.05, N 24.48.

The IR and ^1H NMR data of compound **I** are listed in Table 4.

Reaction of DL-2-(2-*tert*-Butoxycarboxamidopropionamido)-2-(2-hydroxythiazol-4-yl)acetic Acid (**VIIe**) with Isobutyl Chloroformate

To a cooled (−18°C) mixture of **VIIe** (691 mg, 2 mmol) and *N*-methylmorpholine (202 mg, 2 mmol) in THF (8 ml) was added isobutyl chloroformate (273 mg, 2 mmol) with stirring, and the solution was kept for 30 minutes at room temp. To this solution was added dropwise a cooled (0°C) solution of **VIII** (657 mg, 2 mmol) and triethylamine (203 mg, 2 mmol) in 50% THF (10 ml). After the resulting solution was stirred for 1 hour, the solvent was removed. The glass-like substance obtained was sub-

Table 4. IR and ^{1}H NMR data of cephalosporins (**I**).

Compound	IR (KBr) (cm^{-1}) β-Lactam	^{1}H NMR δ value (D_2O)[a]						
		C2-CH_2 2H, ABq J=18 Hz	C3-CH_2 2H, ABq J=13 Hz	C6-H 1H, d J=5 Hz	C7-H 1H, d J=5 Hz	CH 1H, s	N-CH_3 3H, s	Other protons
2	1760	3.60	4.24	4.92	5.57	5.64	3.90	1.39 (3H, d, J=7 Hz), 7.2~7.6 (5H, m)
3	1770	3.43	4.21	5.05	5.78	5.60	4.01	1.78 (3H, s), 7.00 (2H, d, J=8 Hz), 7.48 (2H, d, J=8 Hz)
4	1770	3.70	4.35	5.29	5.85	5.88	4.05, 4.01 (2×s)	1.72, 1.84 (3H, 2×d, J=7 Hz), 7.5~9.0 (4H, br s)
6	1775	3.65	4.30	5.04, 5.09 (2×d)	5.56, 5.66 (2×d)	5.86	4.06	1.55, 1.65 (3H, 2×d, J=7 Hz), 7.74, 7.79 (1H, 2×d, J=2 Hz)
7	1760	3.67	[b]	5.14	5.59, 5.67 (2×d)	5.49	4.08	1.59 (3H, d, J=7 Hz), 6.52, 6.57 (1H, 2×s)
9	1765	3.70	4.25	5.10, 5.12 (2×d)	5.61, 5.69 (2×d)	5.49	4.09	1.57, 1.63 (3H, 2×d, J=7 Hz), 6.78, 6.80 (1H, 2×s)
10	1760	3.60	4.25	5.13	5.65	5.63	4.06	1.54, 1.60 (3H, m), 7.33 (1H, br s), 7.91 (1H, d, J=2 Hz)
11	1760	3.50	4.21	5.05	5.71	5.60	4.03	8.00 (1H, d, J=2 Hz), 9.15 (1H, d, J=2 Hz)
12	1760	3.53	4.23	5.10	5.69	5.63	4.05	7.98 (1H, d, J=7 Hz), 9.16 (1H, d, J=2 Hz)
13	1765	3.62	4.30	5.06	5.49	5.86	4.04	1.65 (3H, d, J=7 Hz), 7.98 (1H, d, J=2 Hz), 9.09 (1H, d, J=2 Hz)
14	1760	3.64	[b]	5.11	5.59	5.83	4.06	1.55 (3H, d, J=7 Hz), 7.74 (1H, d, J=2 Hz), 9.08 (1H, J=2 Hz)
15	1770	3.60	4.20	5.05, 5.10 (2×d)	5.60, 5.66 (2×d)	5.88	4.05	3.9~4.5 (2H, br s), 7.77, 7.79 (1H, 2×d, J=2 Hz), 9.09 (1H, br d, J=2 Hz)
16	1765	3.55	4.25	5.07	5.64, 5.69 (2×d)	5.85	4.02	2.6~3.0 (2H, br s), 7.79 (1H, d, J=2 Hz), 9.09 (1H, d, J=2 Hz)
17	1770	3.45	[b]	5.08	5.69	5.72	4.05	3.1~3.8 (2H, br s), 7.81 (1H, d, J=2 Hz), 9.10 (1H, d, J=2 Hz)
18	1770	3.55	[b]	5.05	5.67	5.78	4.04	3.96 (2H, s), 7.76 (1H, br s), 6.66 (1H, s)
19	1765	3.63	4.20	5.07, 5.14 (2×d)	5.68, 5.72 (2×d)	5.58	4.02	3.9~4.5 (2H, m), 6.75 (1H, br s)
20	1770	3.62	4.15	5.01	5.55, 5.63 (2×d)	5.41, 5.47 (2×s)	4.00	2.7~3.0 (2H, br s), 6.68 (1H, s)
21	1765	3.62	[b]	5.05	5.59	5.62	4.03	3.1~3.7 (2H, br s), 6.5~6.8 (1H, br s)
22	1765	3.55	4.35	5.05	5.63	5.48	4.00	2.6~3.0 (2H, br s), 6.71, 6.77 (1H, 2×s)

[a] In NMR descriptions, 2×s or 2×d shows that singlet or doublet is isolated by DL. Only compound **2** was recorded with DMSO-d_6 as solvent.
[b] It was difficult to read the δ value because the signals overlapped with those of H_2O or other protons.

jected to silica gel column chromatography. The fraction eluted with EtOAc - benzene (1 : 9) gave brown glass materials (**XI**; 78 mg, 10%); and the fraction eluted with EtOAc - benzene (4 : 6) gave brown needles (**XII**; 11 mg, 15%). Compound **XI**: IR (KBr) 1715, 1680, 1660 cm^{-1}; NMR ($CDCl_3$) δ 0.69 (6H, d, J=7 Hz, CH_3 of *iso*-Bu), 1.43 (9H, s, *tert*-Bu), 1.3~2.3 (1H, m, CH of *iso*-Bu), 4.2 (2H, d, J=7 Hz, CH_2 of *iso*-Bu), 4.61 (2H, s, CH_2), 1.43 (3H, d, J=6 Hz, glycine-CH_3). Compound **XII**: MP 205~208°C (dec); IR (KBr) 1740, 1710, 1690 cm^{-1}; NMR ($CDCl_3$) δ 1.31 (3H, d, J=6 Hz, glycine-CH_3), 1.38 (9H, s, *tert*-Bu), 4.68 (1H, s, CH), 4.3~4.7 (1H, q, J=6 Hz, glycine-CHCO).

7β-[DL-2-(D-2-*tert*-Butoxycarboxamidopropionamido)-2-(2-hydroxythiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid Diphenylmethyl Ester (**IX**) R_1=**e**, R_2=**a**)

To a cooled (−10°C) solution of **VIIe** (1.38 g, 4 mmol) and pyridine (0.32 ml, 4 mmol), *N*-ethylpiperidine (453 mg, 4 mmol) in $CHCl_3$ (8 ml) was added pivaloyl chloride (480 mg, 4 mmol). After this solution was stirred for 10 minutes, a solution of **VIII** (1.978 g, 4 mmol) and triethylamine (404 mg, 4 mmol) in $CHCl_3$ (10 ml) was added at the same temp. After being stirred for 30 minutes at room temp, the resulting solution was washed successively with 1% HCl, 4% $NaHCO_3$, and brine, and then the solvent was removed. The residue was chromatographed over silica gel. Elution with 3% MeOH - $CHCl_3$ gave the title compound (1.42 g, 43.2%): MP 124~129°C (dec); IR (KBr) 1780, 1660~1680, 1505, 1360~1380, 1240 cm^{-1}; NMR (DMSO-d_6) δ 1.21 (3H, d, J=7 Hz, glycine-CH_3), 1.39 (9H, s, *tert*-Bu), 3.90 (3H, s, tetrazole-CH_3), 4.3 (2H, br s, C3-CH_2), 5.18 (1H, d, J=5 Hz, C6-H), 5.4~5.8 (2H, m, glycine-CHCO and C7-H), 6.90 (1H, s, diphenyl-CH), 6.25 (1H, s, thiazole 5-H), 7.3~7.7 (10H, phenyl).

7β-[(*d*) and (*l*)-2-Amino-2-(thiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**11** and **12**)

1) Preparation of Optically Active 2-(*tert*-Butoxycarboxamido)-2-(thiazol-4-yl)acetic Acid: The salt of (*dl*)-2-(*tert*-butoxycarboxamido)-2-(thiazol-4-yl)acetic acid[4] with optically active (*d*)-ephedrine was recrystallized from EtOAc and *n*-hexane, and then the less soluble salt obtained was dissolved in EtOAc, washed successively with 10% citric acid and brine. The organic layer was dried and evaporated. The residue was recrystallized from EtOAc - *n*-hexane to give (*d*)-isomer. $[\alpha]_D^{22}$ +117° (*c* 1.0, MeOH); mp 130~131.5°C (dec).

In a similar manner, (*l*)-isomer was obtained from the experiment using (*l*)-ephedrine. (*l*)-Isomer: $[\alpha]_D^{22}$ −117° (*c* 1.0, MeOH); mp 127~130°C (dec).

2) 7β-[(*d*) and (*l*)-2-(*tert*-Butoxycarboxamido)-2-(thiazol-4-yl)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid were prepared from the above acetic acid in a method similar to that described in the ref.[5]

(*d*)-Compound was prepared from (*d*)-isomer: MP 130~135°C (dec); IR (KBr) 1775, 1700, 1490~1510 cm^{-1}; NMR ($CDCl_3$) δ 1.46 (9H, s, *tert*-Bu), 3.70 (2H, br s, C2-CH_2), 3.95 (3H, s, tetrazole-CH_3), 4.41 (2H, br s, C3-CH_2), 4.98 (1H, d, J=5 Hz, C6-H), 5.5~6.1 (2H, m, C7-H and thiazole-CHCO), 6.35 (1H, d, J=6 Hz, NH), 7.53 (1H, d, J=2 Hz, thiazole 5-H), 7.91 (1H, d, J=8 Hz, NH), 8.99 (1H, d, J=2 Hz, thiazole 2-H).

Anal Calcd for $C_{20}H_{24}N_8O_6S$: C 42.24, H 4.25, N 19.71.
Found: C 42.18, H 4.21, N 19.36.

(*l*)-Compound was prepared from (*l*)-isomer: MP 125~140°C (dec); IR (KBr) 1770, 1700, 1500 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (9H, s, *tert*-Bu), 3.70 (2H, br s, C2-CH_2), 3.93 (3H, s, tetrazole-CH_3), 4.40 (2H, br s, C3-CH_2), 5.00 (1H, d, J=5 Hz, C6-H), 5.63 (1H, s, thiazole-CHCO), 5.71 (1H, d, J=5 Hz, C7-H), 7.50 (1H, s, thiazole 5-H), 8.91 (1H, d, J=1.5 Hz, thiazole 2-H).

Anal Calcd for $C_{20}H_{24}N_8O_6S_3$: C 42.24, H 4.25, N 19.71.
Found: C 42.23, H 4.23, N 19.52.

3) Removal of Protecting Group: Removing reaction was carried out as described for the Method A to give **11** and **12**.

(*d*)-Compound (**11**) was prepared from the above (*d*)-isomer: MP 159~165°C (dec); IR (KBr) 1760, 1680~1690, 1590 cm^{-1}; NMR (D_2O) δ 3.50 (2H, ABq, J=18 Hz, C2-CH_2), 4.03 (3H, s, tetrazole-CH_3), 4.21 (2H, br s, C3-CH_2), 5.05 (1H, d, J=5 Hz, C6-H), 5.60 (1H, s, thiazole-CHCO), 5.71 (1H, d,

J=5 Hz, C7-H), 8.00 (1H, d, J=2 Hz, thiazole 5-H), 9.15 (1H, d, J=2 Hz, thiazole 2-H).

(*l*)-Compound (**12**) was prepared from (*l*)-isomer: MP 155~165°C (dec); IR (KBr) 1770, 1690, 1600 cm^{-1}; NMR (D_2O) δ 3.53 (2H, ABq, J=18 Hz, C2-CH_2), 4.05 (3H, s, tetrazole-CH_3), 4.23 (2H, br s, C3-CH_2), 5.10 (1H, d, J=5 Hz, C6-H), 5.63 (1H, s, thiazole-CHCO), 5.69 (1H, d, J=5 Hz, C7-H), 7.98 (1H, d, J=2 Hz, thiazole 5-H), 9.16 (1H, d, J=2 Hz, thiazole 2-H).

The IR and ^1H NMR data of cephalosporins (I) were listed in Table 4.

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