SEMISYNTHETIC β-LACTAM ANTIBIOTICS

III. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 7β-[2-(2-AMINOTHIAZOL-4-YL)-2-(SUBSTITUTED CARBAMOYLMETHOXYIMINO)ACETAMIDO]CEPHALOSPORINS

MASAHIRO ARIMOTO, TAKESHI HAYANO, TSUNEHIKO SOGA,
TOSHIYUKI YOSHIOKA, HIROAKI TAGAWA
and MINORU FURUKAWA

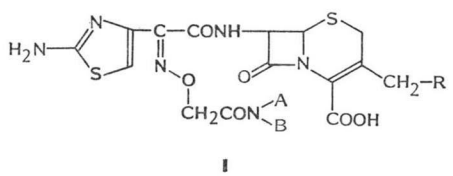
Research Institute, Daiichi Seiyaku Company, Ltd.,
1-16-13 Kita-kasai, Edogawa-ku, Tokyo 134, Japan

(Received for publication April 25, 1986)

Syntheses of cephalosporins modified with a 7β-[2-(2-aminothiazol-4-yl)-2-(substituted carbamoylmethoxyimino)acetamido] group at the C-7 position and with various hetero aromatics at the C-3 position are described. The effects of substituents on the carbamoyl group in the 7-side chain were investigated in order to improve antibacterial activity. Some of these compounds exhibited high antibacterial activity against Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa*, as well as good resistance to β-lactamase.

In recent years, several new cephalosporin antibiotics with a broad spectrum of activity and increased activity against bacteria producing β-lactamase have been developed.[1~3] In the course of our extensive research on the modification of cephalosporin, our efforts have been focused on synthesizing new cephalosporins with enhanced activity against a variety of Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*. In the preceding paper,[4] we reported the synthesis and the antibacterial activity of 7-[2-(2-aminothiazol-4-yl)-2-(acylamino)acetamido]cephalosporin derivatives, in which the cephalosporins having a basic acyl group showed a broad spectrum and enhanced antibacterial activity, but their activities against *Pseudomonas aeruginosa* and their stabilities to β-lactamase were inferior to the oxyimino compounds, such as cefotaxime. As an extension of our program to improve these shortcomings, we studied cephalosporins bearing a basic carbamoyl methoxyimino group instead of a neutral methoxyimino group in the 7-side chain of cefotaxime.

Fig. 1.



I

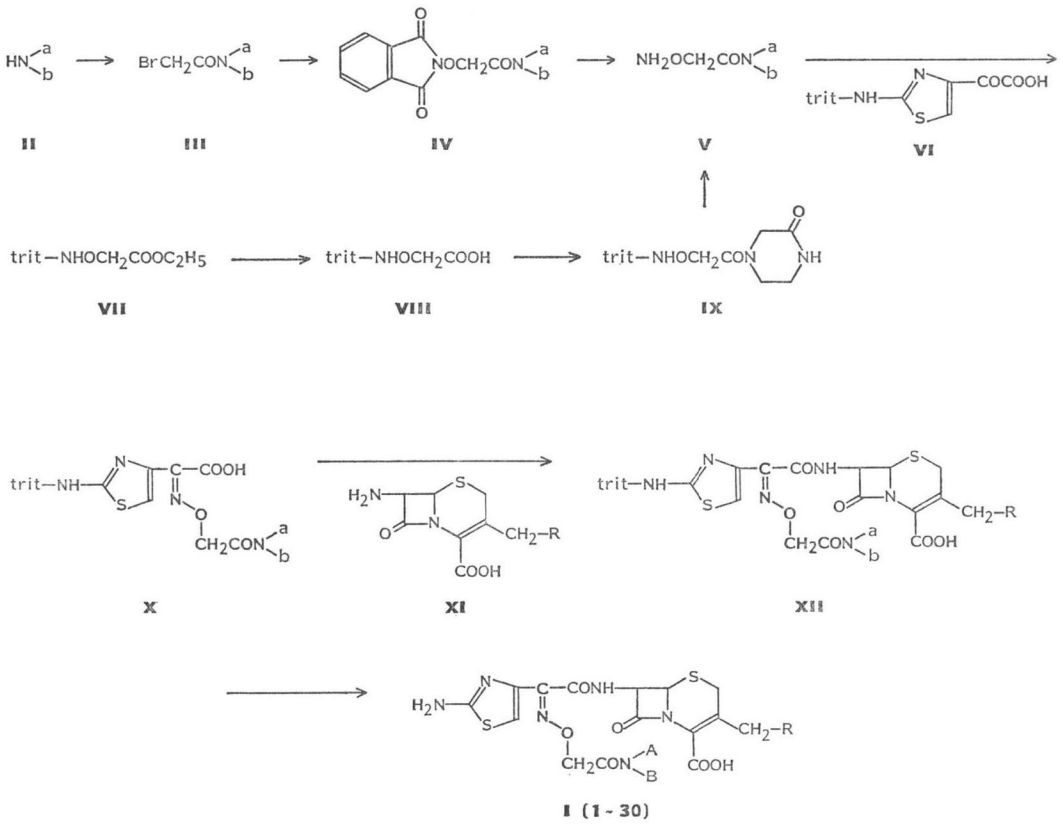
This paper describes the synthesis of the new cephalosporins represented by formula **I** (Fig. 1) and their *in vitro* antibacterial activities.

Chemistry

Most 7β-[2-(2-aminothiazol-4-yl)-2-(substituted carbamoylmethoxyimino)acetamido]cephalosporins (**I**) were prepared according to the synthetic route shown in Scheme 1.

Acylation of the appropriate amino derivative (**II**), shown in Table 5, with bromoacetyl bromide to its bromoacetamide derivatives (**III**), followed by coupling with *N*-hydroxyphthalimide in the presence of a base afforded the derivatives **IV**. The treatment of **IV** with hydrazine hydrate gave the cor-

Scheme 1.



trit; Triphenylmethyl

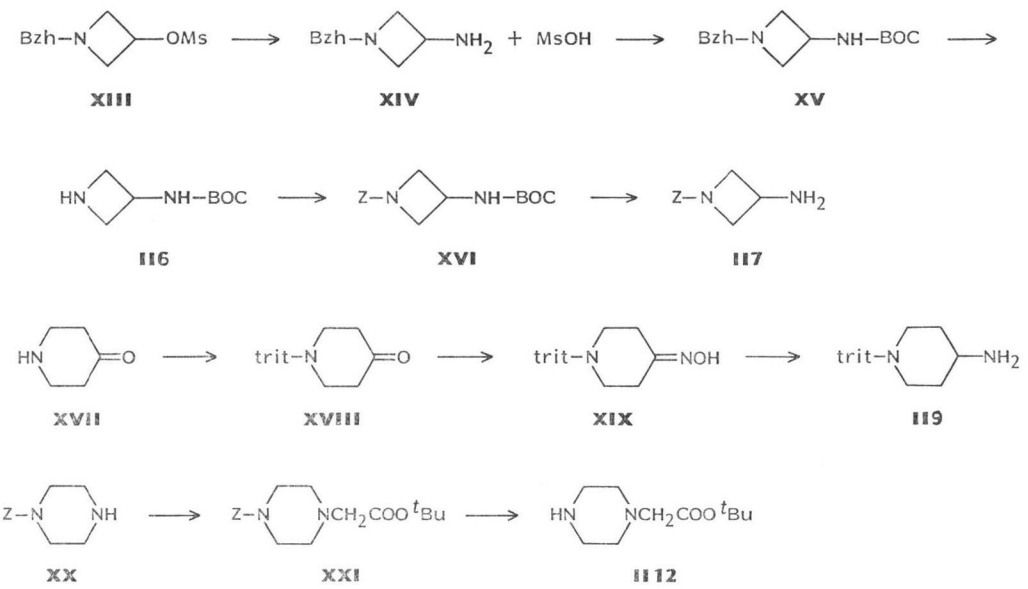
responding carbamoylmethoxyamine compounds (V). The compound **V20** alone, having a 2-ketopiperazinyl group as the carbamoyl group, was prepared by a different synthetic route from **VII**: the acid compound **VIII**, which was made by hydrolysis of the ester (**VII**), was coupled to 2-ketopiperazine by the activated ester method, followed by the removal of the trityl group of **IX** with hydrochloric acid to afford the desired compound (**V20**). Conversion of the resulting **V** into α-carbamoylmethoxyiminoacetic acid derivatives (**X**) were performed smoothly by the reaction of **V** with α-keto acid (**VI**). The *syn*-isomer, alone, was isolated in all case, presumably due to the steric hindrance of the bulky carbamoyl moiety. The compounds **IV** and **X** are listed in Tables 5 and 6.

The coupling of the α-oxyiminoacetic acid derivatives (**X**) with 7-amino cephalosporin compounds (**XI**) was accomplished *via* their activated esters (formed from *N,N*-dicyclohexylcarbodiimide and 1-hydroxybenztriazole) or their acid chlorides (formed with VILSMEYER reagent[5]) to give the protected final compound at ice-bath temperature. The protecting groups of these compounds (**XII**), were generally removed with formic acid or with trifluoroacetic acid and anisole. In the case of the compound **XII7**, which was protected with a benzyloxycarbonyl group, a more powerful reagent, such as trifluoroacetic acid and thioanisole, was needed.

Nucleophilic displacement of the 3-acetoxy group of the compound **24** with pyridine was performed in the usual way[6] to afford the 3-pyridinium methyl compound **30**.

The starting materials (**II6, II7, II9, II12**) are new and their preparations are outlined in Scheme

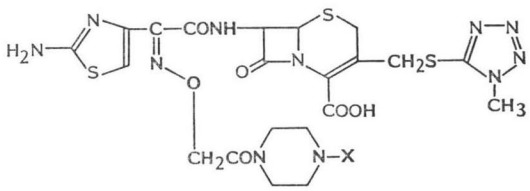
Scheme 2.



trit; Triphenylmethyl, BOC; *tert*-butoxycarbonyl, Bzh; benzhydryl, Z; carbobenzyloxy.

2. 3-(*tert*-Butoxycarbonylamino)azetidine (**II6**) and 1-(carbobenzyloxy)-3-aminoazetidine (**II7**) were obtained as follows. Amination of 1-benzhydryl-3-mesyloxyazetidine (**XIII**)[7,8] with ammonia - ethanol gave the aminoazetidine mesylate (**XIV**), but only in low yield. After protection of the amino group of **XIV** by using 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile, hydrogenation in the presence of Pd-C at low pressure to remove the benzhydryl group gave **II6**. This azetidine was carbobenzyloxylated to **XVI**; this was followed by removal of the *tert*-butoxycarbonyl group with trifluoroacetic acid to give 3-amino-1-protected azetidine (**II7**).

Fig. 2.



10 X=H
17 X=$CONH_2$
18 X=$COCH_3$
19 X=$COCONH_2$

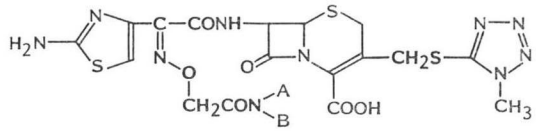
Conversion of the piperidone (**XVII**) to its trityl derivative (**XVIII**), followed by oximation and reduction by lithium aluminium hydride gave 4-amino-1-tritylpiperizine (**II9**). *tert*-Butyl 4-(carbobenzyloxy)piperazinylacetate (**XXI**) prepared from *tert*-butyl bromoacetate and 1-(carbobenzyloxy)piperazine (**XX**) was hydrogenated over Pd-C to give *tert*-butyl piperazinylacetate (**II12**).

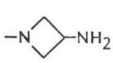
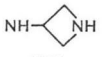
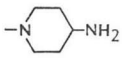
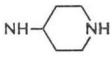
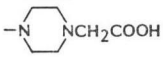
Acylpiperazine derivatives (**17**, **18**, **19**) were synthesized from compound **10** (Fig. 2). When **10** was treated with potassium cyanate in acetic acid and water, the by product, **18**, was obtained together with the desired carbamoyl derivative (**17**). These two cephalosporins were isolated by the HPLC method. In addition, compound **19** was prepared by acylation of **10** with oxamic acid chloride.

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-

Table 1. Structure and antibacterial activity (MIC, μg/ml) of cephalosporins (I).



Compound	$N\begin{matrix}A\\B\end{matrix}$	*S.a.* 209P	*S.a.* Smith	*E.c.*	*En.c.*	*P.a.* 2092	*P.a.* 2131
1	$NHCH_2CH_2OH$	12.5	3.13	0.19	1.56	25	25
2		12.5	3.13	0.78	0.78	25	25
3		25	3.13	0.39	0.78	25	50
4	$NHCH_2CH_2NH_2$	3.13	1.56	0.39	1.56	6.25	6.25
5	$NHCH_2CH_2NHCH_3$	3.13	3.13	0.78	3.13	12.5	12.5
6		6.25	1.56	0.19	0.78	6.25	6.25
7		6.25	1.56	0.78	0.39	25	25
8		6.25	1.56	0.78	1.56	6.25	6.25
9		3.13	3.13	0.39	3.13	12.5	12.5
10		3.13	1.56	0.39	0.78	3.13	3.13
11		50	12.5	0.10	0.78	12.5	25
12		25	12.5	0.39	0.78	50	50
Cefotaxime		1.56	1.56	0.10	0.10	12.5	12.5

Abbreviations: *S.a.* 209P; *Staphylococcus aureus* 209P, *S.a.* Smith; *Staphylococcus aureus* Smith, *E.c.*; *Escherichia coli* NIHJ, *En. c.*; *Enterobacter cloacae* 12005, *P.a.* 2092; *Pseudomonas aeruginosa* 2092, *P.a.* 2131; *Pseudomonas aeruginosa* 2131.

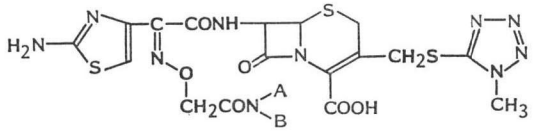
fold agar dilution method.[9]

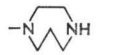
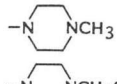
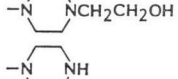
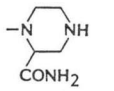
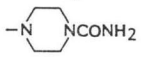
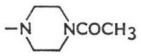
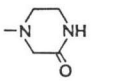
Firstly, the C-3 position of the cephalosporins was substituted with 1-methyltetrazolylthiomethyl, and the oxime moiety of the side chain at the C-7 position was modified with several types of neutral, basic and acidic acetamido groups (refer Table 2). The effects of these substituents on the *in vitro* antibacterial activity of the cephalosporin were examined. The structures and activities are shown in Table 1.

Cephalosporins having the oxime moiety substituted with a neutral group (**1**~**3**) showed moderate activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

On the other hand, the activity of cephalosporins having a basic oxime moiety (**4**~**10**) against *S. aureus* and *P. aeruginosa* was markedly improved, but no significant change in activity was observed against Gram-negative strains except *P. aeruginosa*. It is very interesting that cephalosporins having a primary amine showed higher anti-pseudomonal activity than did compounds with a secondary amine at the terminus of the oxime moiety (**4** vs. **5**, **6** vs. **7**, **8** vs. **9**). Even so, among the compounds with basic oxyimino substituents, that with a piperazine substituent (**10**) (although it has no terminal primary

Table 2. Structure and antibacterial activity (MIC, μg/ml) of cephalosporins (**I**).



Compound	N^{A}_{B}	*S.a.* 209P	*S.a.* Smith	*E.c.*	*En.c.*	*P.a.* 2092	*P.a.* 2131
13		12.5	3.13	0.78	1.56	12.5	12.5
14		12.5	1.56	0.39	0.78	25	25
15		12.5	1.56	0.19	1.56	50	50
16		12.5	3.13	0.78	0.78	6.25	6.25
17		25	6.25	0.39	1.56	25	25
18		25	3.13	0.78	0.78	50	100
19		25	6.25	0.78	6.25	50	50
20		25	3.13	0.39	0.78	12.5	12.5
Cefotaxime		1.56	1.56	0.10	0.10	12.5	12.5

Abbreviations: See footnote in Table 1.

amine function) gave the cephalosporin with the highest activity.

Introduction of acidic substituents (**11**, **12**) into the acetamide moiety caused a significant decrease of the activity against *S. aureus* and *P. aeruginosa*.

Because of the excellent antibacterial activity, compound **10** was further modified with respect to the piperazine substituent. The results are shown in Table 2.

Introduction of the carbon chain into the piperazine ring (**13**~**16**) caused a decrease of the anti-staphylococcal and anti-pseudomonal activity. However, non-basic compounds, which were acylated at the basic nitrogen of piperazine (**17**~**19**) or had ketone at the 3-position of piperazine (**20**), were far less active than their parent compounds (**10**), especially against *S. aureus* and *P. aeruginosa*. This effect on activity was attributed to the increase of lipophilicity and/or the decrease of basicity.

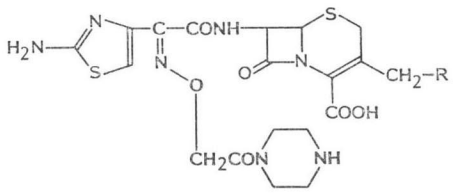
In this study, the compound **10** exhibited the best antibacterial activity against Gram-positive and Gram-negative strains including *P. aeruginosa*.

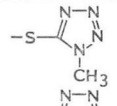
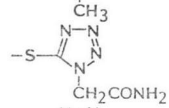
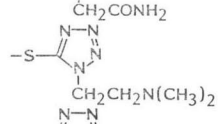
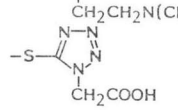
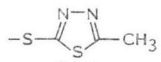
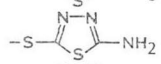
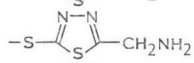
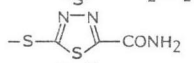
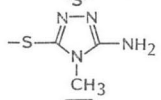
The stabilities of the typical compound **10** to a few β-lactamases were examined. The results shown in Table 4 indicate that this compound has good stability.

This prompted us to make further modification of the substituent at the 3-position of compound **10** in order to improve activity. Commonly used heterocyclic thiols were introduced at the 3-position. The antibacterial activities and structure are listed in Table 3.

In this modification, the (1-methyltetrazol-5-yl) thio substituent of compound **10** was replaced with

Table 3. Structure and antibacterial activity (MIC, μg/ml) of cephalosporins (I).



Compound	R	*S.a.* 209P	*S.a.* Smith	*E.c.*	*En.c.*	*P.a.* 2092	*P.a.* 2131
10		3.13	1.56	0.39	0.78	3.13	3.13
21		25	12.5	0.39	1.56	6.25	6.25
22		25	6.25	0.39	0.78	25	12.5
23		100	25	0.19	1.56	25	12.5
24	$OCOCH_3$	3.13	3.13	0.19	0.19	6.25	6.25
25		12.5	3.13	1.56	6.25	12.5	12.5
26		6.25	3.13	0.39	0.39	3.13	3.13
27		6.25	1.56	0.39	0.78	3.13	6.25
28		6.25	3.13	0.39	0.78	6.25	6.25
29		12.5	12.5	0.19	0.19	6.25	6.25
30		3.13	1.56	0.39	0.39	1.56	3.13
Cefotaxime		1.56	1.56	0.10	0.10	12.5	12.5

Abbreviations: See footnote in Table 1.

neutral, basic and acidic substituents, namely, carbamoylmethyl- (**21**), dimethylaminoethyl- (**22**) and carboxymethyl- (**23**) tetrazoylthiol, respectively. In all cases, a significant decrease of activity against *S. aureus* and *P. aeruginosa* was observed.

Replacement of tetrazole by a few other hetero-aromatics, thiadiazole or triazole analogs (**25**~**29**) as an isomer did not show marked improvement of the activity in comparison with the 1-methyl-1*H*-tetrazole compound **10**.

Further, compared with compound **10**, the acetoxymethyl derivatives (**24**) exhibited improved activity against Gram-negative strains other than *P. aeruginosa*, and similar activity against Gram-positive strains.

The compound **30**, in which pyridiniomethyl was substituted at the 3-position, exhibited better

Table 4. Stability of compounds **10** and **30** to β-lactamase.

β-Lactamase		Relative rate of hydrolysis				
Type[a]	Source	CER[b]	CPZ[c]	CTX[d]	Compound	
					10	**30**
Ia	*E. cloacae* GN7471	100	4.3	0.8	0.5	0.4
Ib	*E. coli* 1154	100	6.9	0.3	0.1	0.1
Ic	*P. vulgaris* GN76	100	16	0.3	6.2	5.8
Id	*P. aeruginosa* 2006	100	4.6	1.0	0.4	0.5

[a] Enzyme classification according to RICHMOND and SYKES.[10]
[b] Cephaloridine. [c] Cefoperazone. [d] Cefotaxime.

antibacterial activity against a variety of Gram-positive and Gram-negative strains, including *P. aeruginosa*, than compound **10**. It is well known that introduction of pyridiniomethyl at the 3-position in the cephem nucleus increases the activity of the cephem derivatives against *P. aeruginosa*. The strong antibacterial activity of compound **30** seemed to be caused by the presence of two basic substituents, namely, 3-pyridiniomethyl and basic acetamide moiety in the 7-side chain. It can therefore be presumed that the basicity of cephem derivatives facilitates the penetrability of Gram-negative and Gram-positive bacterial membranes by cephem antibiotics. Another favorable property of compound **30** is its good β-lactamase stability, which may be derived from the steric effect of the bulky acetamide moiety in the 7-side chain. On the basis of these results, compound **30**, with 3-pyridiniomethyl, was selected as a lead compound for further modification.

Experimental

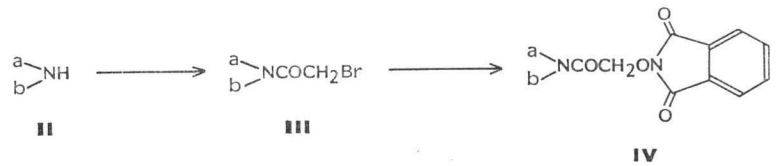
Melting points were determined using a Yanagimoto MP-1 micro melting apparatus and are uncorrected. IR spectra were taken on a Hitachi 285 spectrophotometer. NMR spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-20B and at 200 MHz on a Varian XL-200 spectrometer using TMS or sodium 2,2-dimethyl-2-silapentane-1-sulfonate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

General Procedure for the Preparation of **I**: A Typical Procedure is Described for the Preparation of 7β-[2-(2-Aminothiazol-4-yl)-2-(piperazino-carbonylmethoxyimino)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**10**)

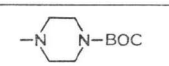
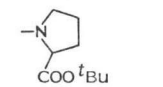
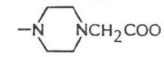
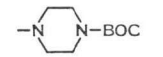
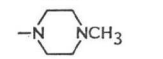
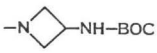
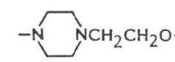
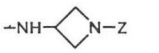
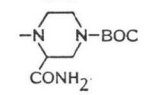
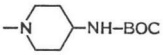
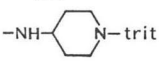
1) Preparation of 1-(Bromoacetyl)-4-(*tert*-butoxycarbonyl)piperazine (**III10**): To a stirred cold (0°C) solution of 1-*tert*-butoxycarbonylpiperazine (**II10**; 7.44 g, 40 mmol) and dimethylaniline (4.85 g, 40 mmol) in Me_2CO (150 ml), 8.0 g of bromoacetyl bromide (40 mmol) was added dropwise. After stirring for 1 hour at room temp, the mixture was evaporated and the residue was diluted with EtOAc, washed successively with 10% citric acid, 5% $NaHCO_3$ and brine, then dried and evaporated to afford the title compound as a brown oil (10.8 g, 87.9%), which was used for the next step without further purification. NMR ($CDCl_3$) δ 1.50 (9H, s, *tert*-Bu), 3.5~3.8 (8H, br s, piperazine CH_2), 3.86 (2H, s, CH_2CO).

2) Preparation of 1-(*tert*-Butoxycarbonyl)-4-phthalimidoyloxyacetylpiperazine (**IV10**): A solution of **III10** (12.3 g, 40 mmol) in CH_3CN (30 ml) was added to an ice cold solution of *N*-hydroxyphthalimide (6.52 g, 40 mmol) and triethylamine (4.04 g, 40 mmol) in CH_3CN (100 ml). The mixture was stirred for 4 hours at room temp. After evaporation of the solvent, the residue was extracted with EtOAc, and the extract was washed successively with 5% $NaHCO_3$ and brine, and was then dried and concd. The residue was triturated with Et_2O to give a colorless powder (10.7 g, 69.0%): MP 177~178°C; IR (KBr) 1780, 1730, 1680 cm^{-1}; NMR ($CDCl_3$) δ 1.50 (9H, s, *tert*-Bu), 3.6 and 3.7 (each 4H, br s, piperazine CH_2), 4.87 (2H, s, CH_2CO), 7.85 (4H, s, phenyl).

Table 5. Melting points and yields of intermediate **IV1**~**IV16** (yield from **III1**~**II16**).



II **III** **IV**

Compound No. of IV	a, b	Yield (%)	MP (°C)	Compound No. of IV	a, b	Yield (%)	MP (°C)
1	$NHCH_2CH_2O^tBu$	48.0	74~77	10		58.6	177~178
2		62.4	133~135	11		62.7	99~101
3		50.4	173~174	12		38.9	Oil
4	$NHCH_2CH_2$–BOC	52.2	157~158	13		73.1	Oil
5	$N(CH_3)CH_2CH_2N(CH_3)$–BOC	39.8	Oil	14		66.2	Oil
6		86.5	173~175	15		36.7	60~62
7		88.8	172~176	16		68.4	233~236
8		67.5	138~141				
9		62.0	191~194				

Various phthalimidoyloxyacetylamine derivatives **IV** are listed in Table 5.

3) Preparation of 1-(Aminooxyacetyl)-4-(*tert*-butoxycarbonyl)piperazine (**V10**): To a suspension of **IV10** (19.5 g, 50 mmol) in EtOH (150 ml) was added hydrazine hydrate (3.0 g, 60 mmol), and the mixture was refluxed for 30 minutes. The cooled reaction mixture was filtered to remove the precipitate formed, and the filtrate was concd and dissolved in EtOAc, and washed successively with 5% $NaHCO_3$, brine; it was then dried and concd to give colorless crystals (9.22 g, 71.1%): MP 128~129°C; IR (KBr) 1680, 1640 cm^{-1}; NMR ($CDCl_3$) δ 1.50 (9H, s, *tert*-Bu), 3.48 (8H, br s, piperazine CH_2), 4.40 (2H, s, CH_2CO), 5.96 (2H, br s, NH_2).

4) 2-(2-Tritylaminothiazol-4-yl)-2-(4-*tert*-butoxycarbonylpiperazino-carbonylmethoxyimino)acetic Acid (**X10**): A mixture of **V10** (2.59 g, 10 mmol) and 2-(2-tritylaminothiazol-4-yl)glyoxylic acid (**VI**; 4.14 g, 10 mmol) in EtOH (200 ml) was stirred for 5 hours at room temp. The reaction mixture was concd, made acidic with 10% citric acid and extracted with $CHCl_3$. The extract was washed with H_2O, dried and evaporated. Trituration with Et_2O afforded colorless crystals (6.44 g, 98.2%): MP 165~167°C (dec).

The IR and NMR data of various acetic acid (**X**) are listed in Table 6.

5) 7β-[2-(2-Tritylaminothiazol-4-yl)-2-(4-*tert*-butoxycarbonylpiperazino-carbonylmethoxyimino)-acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**XII10**): *N*,*N*-Dicyclohexylcarbodiimide (1.03 g, 5 mmol) was added to a mixture of **X10** (3.28 g, 5 mmol) and 1-hydroxybenztriazole (0.676 g, 5 mmol) in DMF (40 ml). After 5 hours of stirring at room temp, the mixture was filtered and the filtrate was added to a stirred solution of 7β-amino-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (**XI**; 1.64 g, 5 mmol) and triethylamine (1.4 ml) in DMF (10 ml). The mixture was stirred for 12 hours at room temp. After evaporation of the solvent, the residue was diluted with H_2O, made acidic with 10% citric acid, and extracted with EtOAc. The organic layer was separated, washed with H_2O, dried and evaporated. Trituration of the residue with Et_2O afforded the title compound as a powder (4.60 g, 95.3%). The crude product was used

Table 6. Spectra and physical properties of compound **X1**~**X 20**.

Compound No. of X	MP (°C)	^{1}H NMR δ value	Solvent A: $CDCl_3$ B: DMSO-d_6	IR (KBr) (cm^{-1})
1	185~190	1.13 (9H, s), 3.41 (4H, s), 4.70 (2H, s), 6.73 (1H, s), 7.3 (15H, s)	B	1680, 1630, 1580
2	134~137	1.7~2.2 (4H, m), 3.2~3.6 (4H, m), 4.87 (2H, s), 6.90 (1H, s), 7.3 (15H, s), 9.10 (1H, br s)	A	1740, 1600, 1530
3	170~171	3.25~4.85 (8H, m), 5.01 (2H, s), 6.91 (1H, s), 7.45 (15H, s), 7.7 (1H, m)	A	1630, 1600
4	177~179	1.43 (9H, s), 3.06 (4H, br s), 4.40 (2H, s), 6.78 (1H, s), 7.33 (15H, s), 8.66 (1H, br s)	B	1680, 1600, 1530
5	124~125	1.41 (9H, s), 2.78 (3H, s), 3.20 (4H, br s), 4.66 (2H, s), 6.78 (1H, s), 7.33 (15H, s)	A	1690
6	140~145	1.42 (9H, s), 3.95 (1H, br s), 4.2~4.5 (4H, m), 4.74 (2H, s), 5.9 (1H, br s), 6.80 (1H, s), 7.33 (15H, s)	A	1705, 1630, 1520
7	179~183	3.84 (2H, dd), 4.23 (2H, t), 4.7 (1H, m), 4.72 (2H, s), 5.06 (2H, s), 6.87 (1H, s), 7.33 (20H, br s)	B	1700, 1650
8	133~137	1.2~2.2 (4H, m), 1.45 (9H, s), 2.7~4.7 (5H, m), 5.02 (2H, s), 6.93 (1H, s), 7.33 (15H, s)	A	1710, 1615
9	185~188	1.3~1.8 (6H, m), 3.0 (2H, br s), 3.4 (1H, m), 4.41 (2H, br s), 6.90 (1H, s), 7.4 (30H, br s)	B	1610, 1530
10	165~167	1.46 (6H, s), 3.3~3.7 (8H, m), 5.15 (2H, s), 6.97 (1H, s), 7.36 (15H, s)	A	1745, 1690, 1600
11	136~140	1.40 (9H, s), 1.85~2.00 (4H, m), 3.50 (2H, m), 4.73 (2H, s), 6.86 (1H, s), 7.30 (15H, s)	B	1720, 1630
12	130~133	1.46 (9H, s), 2.5~2.8 (4H, m), 3.20 (2H, s), 3.3~3.5 (2H, m), 3.5~3.8 (2H, m), 4.98 (2H, s), 6.87 (1H, s), 7.30 (15H, s)	A	1730, 1630
13	125~130	1.46 (9H, s), 1.7~2.0 (2H, m), 3.3~3.7 (8H, m), 5.00 (2H, s), 6.90 (1H, s), 7.30 (15H, s)	A	1740, 1680, 1600
14	192~194	2.52 (3H, s), 2.77 (4H, m), 3.62 (4H, m), 4.67 (2H, s), 6.71 (1H, s), 7.32 (15H, s), 8.65 (1H, s)	B	1660, 1610, 1520
15	162~164	3.15 (2H, m), 3.43 (4H, m), 4.10 (6H, m), 4.75 (2H, m), 6.83 (1H, s), 7.35 (15H, s)	B	1640, 1530, 1490
16	158~164	1.40 (9H, s), 4.37 (1H, m), 4.75 (1H, m), 4.85 (1H, m), 6.85 (1H, s), 7.32 (15H, s), 8.80 (1H, s)	B	1680, 1600
20	181~184	3.2~3.9 (4H, m), 3.9~4.2 (2H, br s), 4.68 (2H, s), 6.68 (1H, s), 7.3 (15H, s)	A	1720, 1650

without further purification.

6) Preparation of **10**: A mixture of **XII10** (1.9 g, 2 mmol) and TFA (20 ml) and anisole (0.3 ml) was stirred for 2 hours at room temp. After evaporation of TFA, Et_2O was added to the residue. The precipitates were collected by filtration, washed with Et_2O, dissolved in a 5% $NaHCO_3$ solution to adjust the pH to 6, and then subjected to column chromatography on a non-ionic adsorption resin (Diaion HP-20). The column was washed with H_2O and eluted with 10% aq MeOH, followed by lyophilization of fractions containing the desired product, to afford the title compound.

Tritylaminooxyacetic acid (**VIII**)

A solution of ethyl tritylaminooxyacetate (**VII**; 2.17 g, 6 mmol) in EtOH (20 ml) and 1 N NaOH (20 ml) was stirred for 5 hours at room temp. After evaporation of the solvent, the residue was diluted

Table 7. Spectral data of compound I.

Compound	^{1}H NMR δ value (D_2O+DCl)							IR (KBr) (cm^{-1})
	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	Thiazole 5-H 1H, s	OCH_2CO 2H, s	Other protons	
1	3.68	4.20	5.23	5.83	7.15	4.78	3.43 (2H, t), 3.70 (2H, t), 4.06 (3H, s)	1770, 1630
2	[a]	4.31	5.28	5.88	7.16	4.96	2.02 (4H, m), 3.51 (4H, m), 4.13 (3H, s)	1760, 1620
3	3.67	4.20	5.02	5.82	7.12	5.00	3.69 (8H, m), 4.09 (3H, m)	1760, 1630
4	3.81	4.22	5.27	5.85	7.29	4.88	3.20 (2H, m), 3.58 (2H, m), 4.10 (3H, m)	1780, 1670
5	3.79	4.22	5.29	5.87	7.39	4.88	2.74 (3H, s), 3.25 (2H, t), 3.65 (2H, t), 4.10 (3H, s)	1770, 1660
6	3.79	[a]	5.28	5.84	7.29	4.92	4.2~4.8 (5H, m), 4.10 (3H, s)	1770, 1630
7	3.80	[a]	5.29	5.86	7.30	4.88	4.10 (3H, s), 4.2~4.5 (5H, m)	1770, 1660
8	3.76	4.22	5.27	5.83	7.28	5.10	1.60, 2.15 (each 2H, m), 2.90, 3.30, 3.5, 4.0, 4.50 (each 1H, m), 4.09 (3H, s)	1775, 1630
9	3.78	4.22	5.38	5.83	7.39	4.80	1.78 (2H, m), 2.16 (2H, m), 3.15 (2H, m), 3.50 (2H, m), 4.09 (3H, s), 4.40 (1H, m)	1770, 1630
10	3.80	4.25	5.28	5.83	7.30	5.15	3.19 (4H, m), 3.75 (4H, m)	1775, 1670
11	3.67	4.21	5.22	5.82	7.10	4.93	1.8~2.4 (4H, m), 3.55~3.62 (2H, m), 4.05, (3H, s), 4.38 (1H, s)	1760, 1590
12	3.66	4.20	5.11	5.82	7.10	5.00	2.70 (4H, m), 3.14 (2H, s), 3.60 (4H, s), 4.05 (3H, s)	1760, 1600
13	3.78	4.22	5.28	5.82	7.28	5.15	2.22 (2H, m), 3.44 (4H, m), 3.71 (4H, m), 4.10 (3H, s)	1775, 1675
14	3.70	4.20	5.26	5.83	7.28	5.12	2.96 (3H, s), 3.20 (4H, m), 3.63 (4H, m), 4.08 (3H, s)	1780, 1640
15	3.86	4.22	5.14	5.82	7.28	5.14	3.29 (6H, m), 3.74 (2H, t), 3.96 (4H, m), 4.10 (3H, s)	1775, 1630
16	[a]	4.23	5.28	5.85	7.30	5.23	3.2~4.0 (8H, m), 4.50 (3H, s), 5.47 (1H, s)	1770, 1675
17	[a]	4.20	5.22	5.82	7.12	5.01	3.4~3.7 (8H, m), 4.05 (3H, s)	1760, 1600
18	3.68	4.21	5.23	5.82	7.13	5.03	2.14 (3H, s), 3.62 (4H, m), 3.66 (4H, m), 4.06 (3H, s)	1760, 1620
19	[a]	4.35	5.02	5.85	7.21	4.98	3.2~3.8 (8H, m), 3.92 (3H, s)	1765, 1630
20	3.65	4.25	5.09	5.82	7.11	5.20	3.3~3.6 (2H, m), 3.7~3.9 (2H, m), 4.0~4.5 (2H, m), 4.05 (3H, s)	1770, 1650
21	3.64	4.30	5.26	5.82	7.26	5.12	3.36 (4H, m), 3.86 (4H, m), 5.36 (2H, s)	1775, 1690
22	3.86	4.24	5.30	5.84	7.28	5.15	3.05 (6H, s), 3.38 (4H, m), 3.86 (6H, m)	1770, 1680
23	3.74	4.30	5.28	5.85	7.28	5.14	3.36 (4H, m), 3.86 (4H, m), 5.24 (2H, s)	1780, 1680
24	3.60	4.94	5.29	5.91	7.16	5.05	2.14 (3H, s), 3.2 (4H, m), 3.8 (4H, m)	1770, 1730, 1630
25	3.70	4.32	5.28	5.80	7.30	5.14	2.74 (3H, s), 3.36 (4H, m), 3.85 (4H, m)	1775, 1630
26	3.72	4.21	5.28	5.83	7.28	5.12	3.36 (4H, m), 3.91 (4H, m)	1765, 1660
27	3.79	4.41	5.30	5.85	7.31	5.18	3.40 (4H, m), 3.88 (4H, m), 4.74 (2H, s)	1770, 1660
28	3.61	[a]	5.12	5.85	7.12	5.02	2.85 (4H, m), 3.52 (4H, m)	1760, 1670
29	3.72	4.10	5.26	5.82	7.26	5.12	3.36 (4H, m), 3.90 (4H, m), 3.56 (3H, s)	1770, 1670
30	3.48	4.51	5.32	5.94	7.18	5.06	3.2~3.54 (4H, m), 3.8~4.0 (4H, m), 8.13 (2H, t), 8.62 (1H, t), 8.99 (2H, d)	1770, 1670

[a] It was difficult to read the δ value because the signals overlapped with those of H_2O or other protons.

with EtOAc, washed with 10% citric acid, H_2O, dried and concd to dryness. The residue was triturated with Et_2O-petroleum ether and collected by filtration to give colorless crystals (1.92 g, 96.1%): MP 200~203°C (dec); IR (KBr) 1705, 1260 cm^{-1}; NMR ($CDCl_3$) δ 4.23 (2H, s, CH_2), 7.32 (15H, s, trityl).

4-(Tritylaminooxyacetyl)-2-oxopiperazine (IX)

To an ice-cooled solution of tritylaminooxyacetic acid (VIII; 0.667 g, 2 mmol) and *N*-hydroxysuccinimide (0.23 g, 2 mmol) in THF (20 ml) was added *N*,*N*-dicyclohexylcarbodiimide (0.412 g, 2 mmol) and stirred for 2 hours at room temp. The precipitate formed was filtered off, and the filtrate was added a solution of 2-oxopiperazine (0.20 g, 2 mmol) in DMF (5 ml) and stirred for 2 hours. The mixture was concd and extracted with EtOAc, washed with 5% $NaHCO_3$, H_2O, dried and evaporated. The residue was triturated with Et_2O to give crystals (0.36 g, 43.4%): MP 228~230°C; IR (KBr) 1680, 1645 cm^{-1}; NMR (DMSO-d_6) δ 2.9~3.5 (4H, br s, piperazine CH_2), 3.87 (2H, s, piperazine CH_2), 4.40 (2H, s, OCH_2), 7.29 (15H, s, trityl), 7.7~8.1 (2H, br s, NH).

2-(2-Tritylaminothiazol-4-yl)-2-(3-oxopiperazino-carbonylmethoxyimino)acetic Acid (X20)

A suspension of **IX** (0.415 g, 1 mmol) in 6 N HCl (4 ml) and THF (4 ml) was stirred for 2 hours at room temp. After evaporation of the solvent, the residue was dissolved in H_2O and washed with EtOAc. The aqueous layer was concd, adjusted to pH 6 with the ion exchange resin IRA-45 (OH^-), and filtered. The filtrate was concd to give a residue containing the oxyamine compound **V20**, which was collected and used for the next step without further purification. This residue was suspended in 2-(2-tritylaminothiazol-4-yl)glyoxylic acid (VI; 0.207 g, 0.5 mmol) in EtOH (5 ml), and stirred overnight at room temp. After concentration, the residue was chromatographed over silica gel. Elution with $CHCl_3$ - MeOH (2: 1) gave **X20** as colorless crystals (0.088 g, 30.2%): MP 181~184°C (dec).

3-Amino-1-benzhydrylazetidine Methansulfonate (XIV)

A solution of 1-benzhydryl-3-methansulfonylazetidine[7,8] (XIII; 2.78 g, 8.8 mmol) in 16% ammonia-MeOH solution (40 ml) was stood for 4 days at room temp. After removing the solvent, the residue was triturated with EtOAc to give colorless crystals (0.80 g, 27.3%): MP 160~165°C (dec); NMR (D_2O+DCl) δ 2.83 (3H, s, CH_3), 4.53 (5H, s, azetidine CH_2 and CH), 5.85 (1H, s, benzhydryl CH) 7.50 (10H, s, phenyl)

1-Benzhydryl-3-(*tert*-butoxycarbonylamino)azetidine (XV)

A mixture of **XIV** (2.01 g, 6 mmol), 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (1.63 g, 6.6 mmol) and triethylamine (1 ml) in dioxane (60 ml) and H_2O (10 ml) was stirred for 2.5 hours at room temp. The reaction mixture was evaporated and the residue was dissolved with EtOAc, washed with H_2O, dried and evaporated to give colorless prisms (1.91 g, 94.1%): MP 168~170°C; IR (KBr) 3440, 1705, 1525 cm^{-1}; NMR ($CDCl_3$) δ 1.42 (9H, s, *tert*-Bu), 2.81 (2H, m, CH_2), 3.51 (2H, m, CH_2), 4.25 (1H, m, azetidine CH), 4.28 (1H, s, benzhydryl CH), 4.85 (1H, br s, NH), 7.2~7.4 (10H, m, phenyl).

3-*tert*-Butoxycarbonylaminoazetidine (II6)

A mixture of **XV** (1.69 g, 5 mmol) and 10% Pd-C (0.4 g) in 1 N HCl (5.1 ml) and 80% EtOH (100 ml) was hydrogenated at low pressure (initial 3.85 kg/cm^2). After the theoretical amount of H_2 had been absorbed, the catalyst was filtered off. The filtrate was concd and the residue was made alkaline with NaOH solution, and extracted with $CHCl_3$. The extract was washed with H_2O, dried and evaporated to give the title compound as a colorless powder (0.82 g, 95.2%): MP 75~78°C; IR (KBr) 3330, 1690 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (9H, s, *tert*-Bu), 2.06 (1H, s, azetidine NH), 3.50 (2H, m, CH_2), 3.85 (2H, m, CH_2), 4.5 (1H, m, azetidine CH), 5.2 (1H, br s, NH).

1-Benzyloxycarbonyl-3-(*tert*-butoxycarbonylamino)azetidine (XVI)

To an ice-cold solution of **II6** (0.62 g, 3.6 mmol) in CH_2Cl_2 (20 ml) was added dropwise triethylamine (0.6 ml) and carbobenzoxy chloride (0.68 g, 4 mmol). After stirring for 1.5 hours at room temp, the mixture was washed with 10% citric acid, brine, and was dried and evaporated to give an oil. This oil was triturated with *n*-hexane to give colorless crystals (0.92 g, 83.4%): MP 103~105°C; IR (KBr) 3350, 1705, 1665 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (9H, s, *tert*-Bu), 3.85 (2H, m, azetidine CH_2), 4.2~4.5 (3H, m, azetidine CH_2 and CH), 5.10 (2H, s, $COOCH_2$), 5.2 (1H, br s, NH), 7.35 (5H, s, phenyl).

3-Amino-1-benzyloxycarbonylazetidine (**II7**)

A solution of **XVI** (0.90 g, 2.9 mmol) in TFA (10 ml) was stirred for 30 minutes at room temp. The mixture was evaporated and the residue was diluted with Et_2O and extracted with H_2O. The aqueous layer was separated, made alkaline with NaOH solution and extracted with CH_2Cl_2, washed with brine, dried and evaporated to give a colorless oil (0.55 g, 92.0%): IR (KBr) 3350, 3300, 1700 cm^{-1}; NMR ($CDCl_3$) δ 1.64 (2H, s, NH_2), 3.6~3.9 (3H, m, azetidine CH_2 and CH), 4.2 (2H, m, azetidine CH_2), 5.10 (2H, s, $COOCH_2$), 7.36 (5H, s, phenyl).

1-Trityl-4-piperidone (**XVIII**)

To a mixture of 4-piperidone hydrochloride monohydrate (**XVII**; 4.61 g, 30 mmol) and trityl chloride (9.2 g, 33 mmol) in 10% aq THF (60 ml), triethylamine (6.67 g, 66 mmol) was added dropwise. After stirring for 3 hours at room temp, the mixture was evaporated. The residue was diluted with EtOAc and washed successively with H_2O, 10% citric acid, 5% $NaHCO_3$, brine; then it was dried and evaporated. The residue was crystallized from EtOH to give **XVIII** as colorless needles (9.02 g 88.1%): MP 212~214°C; IR (KBr) 3240, 1590 cm^{-1}; NMR ($CDCl_3$) δ 2.55 (8H, s, CH_2), 7.2~7.6 (15H, m, trityl).

1-Trityl-4-piperidone Oxime (**XIX**)

To a solution of **XVIII** (7.65 g, 22.4 mmol) in EtOH (150 ml) was added a solution of hydroxylamine hydrochloride (2.34 g, 34 mmol) and potassium hydroxide (1.9 g, 34 mmol) in H_2O (30 ml). After refluxing for 30 minutes, the solution was concd and diluted with EtOAc, washed successively with H_2O, brine, and then dried and evaporated. The residue was triturated with *n*-hexane to afford 1-trityl-4-piperidone oxime as colorless prisms (6.42 g, 80.4%): MP 243~245°C; IR (KBr) 3450, 1710 cm^{-1}; NMR ($CDCl_3$) δ 2.4 (6H, m, CH_2), 2.7 (2H, m, CH_2), 7.2~7.6 (15H, m, trityl).

4-Amino-1-tritylpiperidine (**II9**)

A solution of **XIX** (5.35 g, 15 mmol) in THF (50 ml) was added dropwise to a suspension of $LiAlH_4$ (1.14 g, 30 mmol) in THF (100 ml) and refluxed for 1 hour. After this solution was chilled a mixture of H_2O (1.14 ml) and THF (10 ml) was added dropwise to it, and this was followed by the addition of 15% NaOH (1.14 ml) and H_2O (3.42 ml). The precipitate was filtered off, and the filtrate was concd. The residue was extracted with EtOAc, and extract was washed with H_2O, dried and evaporated to give an oil (4.86 g, 94.6%). IR (KBr) 3400, 1735, 1590 cm^{-1}; NMR ($CDCl_3$) δ 1.4~1.8 (8H, m, CH_2 and NH_2), 2.5 (1H, m, CH), 3.0 (2H, m, CH_2), 7.2~7.6 (15H, m, trityl).

4-Benzyloxycarbonyl-1-*tert*-butoxycarbonylmethylpiperazine (**XXI**)

To a solution of 1-benzyloxycarbonylpiperazine (**XX**; 4.4 g, 20 mmol) and triethylamine (2.7 ml), *tert*-butyl bromoacetate (3.67 g, 19 mmol) was added dropwise with ice-cooling. The mixture was stirred for 1 hour at room temp and concd. The residue was dissolved in EtOAc, washed with H_2O, dried and evaporated to give an oil (5.8 g, 91.3%). NMR ($CDCl_3$) δ 1.47 (9H, s, *tert*-Bu), 2.55 (4H, t, J=5 Hz, piperazine CH_2), 3.14 (2H, s, CH_2COO), 3.57 (4H, t, J=5 Hz, piperazine CH_2), 5.14 (2H, s, $COOCH_2$), 7.36 (5H, s, phenyl).

1-*tert*-Butoxycarbonylmethylpiperazine (**II12**)

A solution of **XXI** (3.3 g, 10 mmol) in EtOH (60 ml) was hydrogenated over 10% Pd-C (1.0 g). After the theoretically estimated amount of H_2 had been absorbed, the catalyst was filtered off. The filtrate was concd to give an oil (1.8 g, 93.9%). NMR ($CDCl_3$) δ 1.49 (9H, s, *tert*-Bu), 2.8~3.0 (4H, m, piperazine CH_2), 3.19 (2H, s, CH_2COO), 3.1~3.4 (4H, m, piperazine CH_2).

7β-[2-(2-Aminothiazol-4-yl)-2-(4-carbamoylpiperazino-carbonylmethoxyimino)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**17**) and 7β-[2-(2-Aminothiazol-4-yl)-2-(4-acetylpiperazino-carbonylmethoxyimino)acetamido]-3-(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**18**)

To a solution of **10** (0.374 g, 0.6 mmol) in AcOH (7 ml) and H_2O (1 ml) was added a solution of KCNO (0.075 g, 0.78 mmol) in H_2O (2 ml). After stirring for 2 hours at room temp, more KCNO

(0.041 g, 0.5 mmol) was added, the mixture was stirred for an additional hour, evaporated, diluted with H_2O, and filtered, and the filtrate was evaporated. Trituration of the residue with Et_2O to afford a hygroscopic solid. This was dissolved in 5% $NaHCO_3$ and adjusted to pH 6 and then subjected to chromatography on a Diaion HP-20 column. The fraction containing the desired compounds was lyophilized and subjected to HPLC to give the two title compounds: **17** (0.072 g); mp 175~190°C (dec) and **18** (0.053 g); mp 175~180°C (dec).

Compound **17**

Anal Calcd for $C_{22}H_{26}N_{12}O_7S_3 \cdot H_2O$: C 38.59, H 4.12, N 24.55.
Found: C 38.48, H 4.10, N 24.17.

Compound **18**

Anal Calcd for $C_{23}H_{27}N_{11}O_7S_3 \cdot H_2O$: C 40.40, H 4.28, N 22.54.
Found: C 40.82, H 4.26, N 22.35.

7β-[2-(2-Aminothiazol-4-yl)-2-(4-carbamoylketopiperazino-carbamoylmethoxyimino)acetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**19**)

Oxamic acid chloride (0.12 g, 1.1 mmol) was added to a solution of **10** (0.3 g, 0.48 mmol) and bis(trimethylsilyl)acetamide (1.2 ml) in CH_3CN (12 ml) at −40°C under stirring. The mixture was stirred for another 1 hour at room temp. After evaporation of solvent, trituration with Et_2O - MeOH gave a solid, which was dissolved in H_2O and adjusted to pH 6 with 5% $NaHCO_3$ and subjected to chromatography using a Diaion HP-20 column. The fraction containing the desired compound was lyophilized to give the title compound (0.066 g): MP 180~190°C (dec).

7β-[2-(2-Aminothiazol-4-yl)-2-(piperazino-carbonylmethoxyimino)acetamido]-3-pyridiniomethylceph-3-em-4-carboxylic Acid (**30**)

To a solution of **24** (1.135 g, 2 mmol) in H_2O (2 ml) were added sodium iodide (3 g, 2 mmol), $NaHCO_3$ (168 mg, 2 mmol) and pyridine (1 ml), and the mixture was stirred for 1 hour at 80°C. After cooling, the mixture was poured into Me_2CO (50 ml). The precipitate formed was collected by suction and subjected to column chromatography (Diaion HP-20). Lyophilization of the product-containing fractions afforded the title compound (470 mg): MP 165~190°C (dec).

The IR and NMR data of various cephalosporins (**I**) are listed in Table 7.

Acknowledgment

The authors wish to thank Dr. Y. OSADA and the staff of the Laboratory of Microbiology and of the Analytical Section of the Research Institute of Daiichi Seiyaku Co., Ltd.

References

1) BUCOURT, R.; R. HEYMES, A. LUTZ, L. PENASSE & J. PERRONNET: Propriétés antibiotiques inattendues dans le domaine des céphalosporines. C. R. Acad. Sci. Paris, Series D 284: 1847~1849, 1977
2) OCHIAI, M.; O. AKI, A. MORIMOTO, T. OKADA & Y. MATSUSHITA: New cephalosporin derivatives with high antibacterial activities. Chem. Pharm. Bull. 25: 3115~3117, 1977
3) KAMIMURA, T.; Y. MATSUMOTO, N. OKADA, Y. MINE, M. NISHIDA, S. GOTO & S. KUWAHARA: Ceftizoxime (FK-749), a new parenteral cephalosporin: *In vitro* and *in vivo* antibacterial activities. Antimicrob. Agents Chemother. 16: 540~548, 1979
4) ARIMOTO, M.; A. EJIMA, T. WATANABE, H. TAGAWA & M. FURUKAWA: Semisynthetic β-lactam antibiotics. II. Synthesis and antibacterial activity of 7β-[2-(acylamino)-2-(2-aminothiazol-4-yl)acetamido]-cephalosporins. J. Antibiotics 39: 1236~1242, 1986
5) ZAORAL, M. & Z. ARNORD: A novel peptide synthesis. Tetrahedron Lett. 1960: 9~12, 1960
6) MURPHY, C. F. & J. A. WEBBER: Alteration of the dihydrothiazine ring moiety. Cephalosporins and penicillins. *In* Chemistry and Biology. Chapter 4. *Ed.*, E. H. FLYNN, pp. 135~171, Academic Press, New York, 1972
7) ANDERSON, Jr., A. G. & R. LOK: The synthesis of azetidine-3-carboxylic acid. J. Org. Chem. 37: 3953~3955, 1972
8) OKUTANI, T.; T. KANEKO & K. MASUDA: Studies on azetidine derivatives. I. Synthesis of 3-substituted azetidine derivatives. Chem. Pharm. Bull. 22: 1490~1497, 1974

9) Japan Society of Chemotherapy: Determination method of MIC. Chemotherapy (Tokyo) 23: Color pages 1~2, 1975
10) RICHMOND, M. H. & R. B. SYKES: β-Lactamases of gram-negative bacteria and their possible physiological role. *In* Advances in Microbial Physiology. Vol. 9. *Eds.* A. H. ROSE & D. W. TEMPEST, pp. 31~85, Academic Press, New York, 1973