NEW AMINOTHIAZOLYLGLYCYLCEPHALOSPORINS WITH A 1,5-DIHYDROXY-4-PYRIDONE-2-CARBONYL GROUP

II. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF MT0703 AND ITS DIASTEREOMERS

HIROKO OGINO, KATSUYOSHI IWAMATSU, KIYOAKI KATANO, SATORU NAKABAYASHI, TAKASHI YOSHIDA, SEIJI SHIBAHARA, TAKASHI TSURUOKA*, SHIGEHARU INOUYE and SHINICHI KONDO†

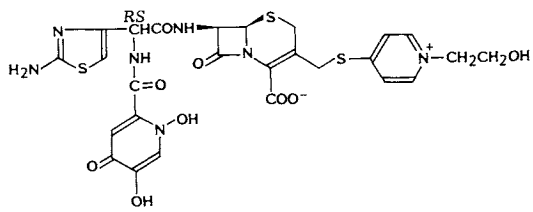
Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd.,
760 Morooka-cho, Kohoku-ku, Yokohama 222, Japan

(Received for publication October 5, 1989)

A practical synthetic method for large scale production of MT0703, (6*R*,7*R*)-7-[(*RS*)-2-(2-aminothiazol-4-yl)-2-(1,5-dihydroxy-4-pyridone-2-carboxamido)acetamido]-3-[[1-(2-hydroxyethyl)pyridinium-4-yl]thiomethyl]ceph-3-em-4-carboxylate, was established. Its two diastereomers on configuration of the aminothiazolylglycyl moiety were synthesized using chemico-enzymatic method. The *S*-isomer of MT0703 was found to be more active against Gram-positive and Gram-negative bacteria including β-lactamase-producing strains than the *R*-isomer.

In a previous paper[1], we have reported that a novel cephalosporin derivative MT0703 (**1**) (Fig. 1) possessing a 1,5-dihydroxy-4-pyridone-2-carbonyl group at the α-amino group of the aminothiazolylglycyl side chain and a 1-(2-hydroxyethyl)pyridiniumthiomethyl substituent at C-3 has an excellent antibacterial activity, especially strong anti-pseudomonal activity. As **1** was a mixture of diastereomers derived from an asymmetric α carbon of the aminothiazolylglycyl moiety, we have synthesized two diastereomers, MT0703*R* (**1*R***) and MT0703*S* (**1*S***).

Fig. 1. MT0703 (**1**).



In this paper, the practical synthetic method for large scale production of diastereomeric mixture MT0703 (**1**) and the synthesis and antibacterial activity of its diastereomers are described.

Chemistry

For establishment of a practical synthetic method of **1**, the aminothiazolylglycyl moiety (**11**) bearing a 1,5-dihydroxy-4-pyridone-2-carbonyl group was prepared by the method outlined in Schemes 1 and 2. Kojic acid (**2**) protected by a *p*-methoxybenzyl (PMB) group was treated with hydroxylamine hydrochloride to convert into *N*-hydroxypyridone **3**. The reaction of **3** with diphenyldiazomethane afforded pyridine *N*-oxide **4** and pyridone **5** (approximately 3 : 1) in the presence of Et_3N. On the other hand, treatment of **3** with diphenylmethyl bromide gave only **5**. The structures of **4** and **5** were confirmed by the ^1H and ^{13}C NMR spectra. The ^{13}C NMR chemical shifts of relievant carbons of **4** and **5** are presented in Table 1.

† Present address: Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan.

The C-4 carbon of **5** was assigned as a carbonyl carbon signal at 170.9 ppm. Compound **4** was smoothly oxidized with nickel peroxide in aqueous CH_3CN to afford **6** in an overall yield of 24% from **2**. Compound **5**, however, was not easily oxidized to give **7** by the similar procedure owing to its insolubility.

Next, key-intermediate (**11**) was prepared from a trityl derivative of ethyl (*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetate[2] (**8**) in three steps (Scheme 2). Reduction of **8** with zinc powder in aqueous acetic acid gave an aminothiazolylglycyl derivative (**9**) as a racemic mixture. Condensation of **6** with **9** by the *N*,*N*-dicyclohexylcarbodiimide (DCC) method was not accomplished, but it was completed by the active ester method using 3-chlorobenzisothiazoline 1,1-dioxide (saccharin chloride) to afford **10**, followed by hydrolysis to convert into **11** in an overall yield of 78% from **8**.

Table 1. ^{13}C NMR data[a] of **4** and **5**.

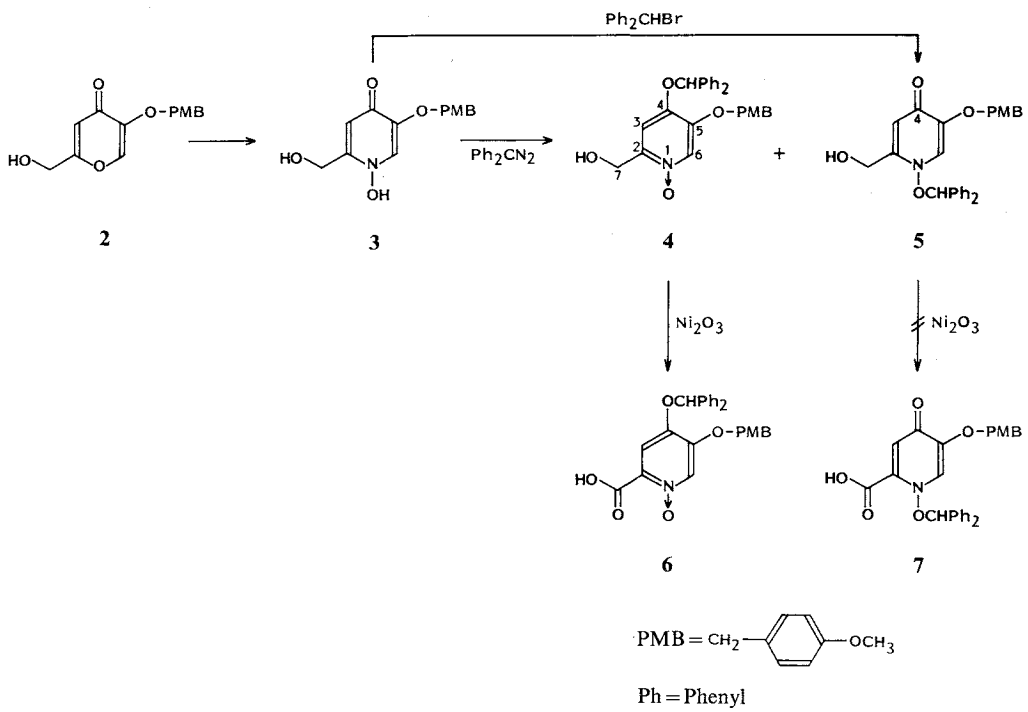
Carbon	4	5
2	144.8	148.1
3	108.8	110.9
4	146.4	170.9
5	145.2	145.8
6	128.2	123.6
7	58.7	56.9

[a] δ (ppm) in DMSO-d_6.

As shown in Scheme 2, **11** was condensed with TsOH salt of PMB (6*R*,7*R*)-7-amino-3-(chloromethyl)-ceph-3-em-4-carboxylate (**12**) by the acid chloride method using $POCl_3$, followed by substitution at C-3 methylene with 1-(2-hydroxyethyl)-4(1*H*)-pyridinethione and removal of the protecting groups with trifluoroacetic acid to give **1** in an overall yield of 35% from **11**.

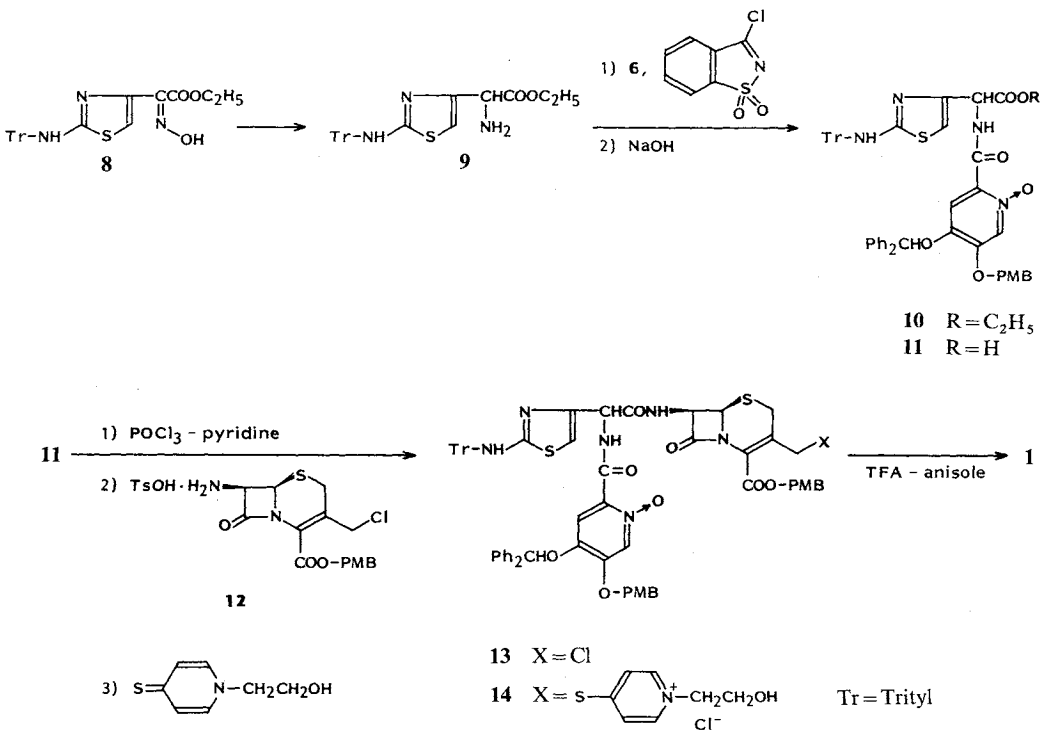
Two diastereomers (**1*S*** and **1*R***) were synthesized from optically active aminothiazolylglycyl derivatives (**20*S*** and **20*R***) obtained by the chemico-enzymatic method using L-aminoacylase through the route shown in Scheme 3. Racemic aminothiazolylglycine **18**, in which its α-amino group was protected by a chloroacetyl

Scheme 1.

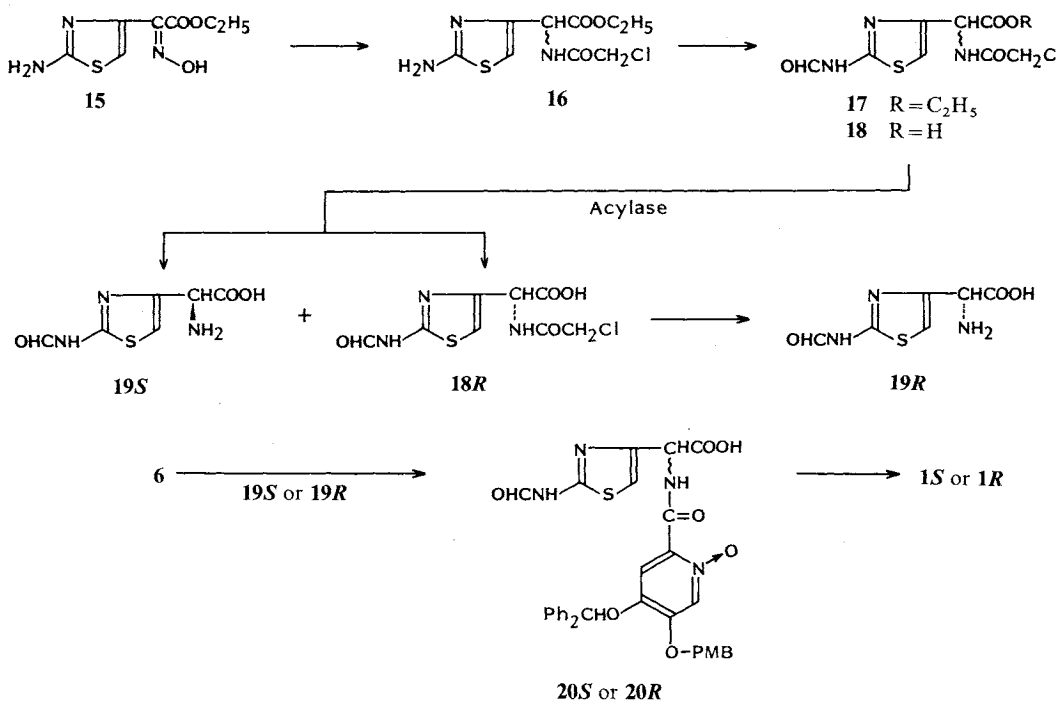


2 3 4 5 6 7

Scheme 2.



Scheme 3.



group, was easily prepared from ethyl (*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetate[2] (**15**). Compound **18** was incubated with acylase, followed by extraction with EtOAc to give D-isomer **18*R***, and from the aqueous layer to afford L-amino acid **19*S***. D-Amino acid **19*R*** was obtained by deprotection of **18*R*** with thiourea. Optical purity of **19*S*** and **19*R*** was determined by HPLC analysis. The optically active **20*S*** and **20*R*** were prepared through condensation of sililated compounds of **19*S*** and **19*R*** with **6** by Vilsmeier method, followed by the similar procedures in the case of preparation of **1** to afford **1*S*** and **1*R***, respectively.

Antibacterial Activity

In vitro and *in vivo* antibacterial activities of *R*- and *S*-isomers (**1*R*** and **1S**) are listed in Tables 2 and 3, respectively. **1*S*** had more potent *in vitro* activity against most of the test organisms, especially against Gram-negative bacteria including β-lactamase-producing strains, than **1*R***. (Table 2) Their anti-pseudomonal activities were superior to those of ceftazidime (CAZ), cefoperazone (CPZ) and cefsulodin (CFS). As shown in Table 3, comparing with **1*R***, **1*S*** was more effective against experimental infection

Table 2. Antibacterial activity of MT0703*R* (**1*R***), MT0703*S* (**1*S***) and other cephalosporins.

Test organism	MIC (μg/ml)				
	1*R*	**1*S***	CAZ	CPZ	CFS
Staphylococcus aureus 209P JC-1	6.25	3.13	6.25	0.78	3.13
S. epidermidis ATCC 14990	6.25	3.13	6.25	1.56	3.13
Bacillus subtilis ATCC 6633	3.13	6.25	3.13	0.39	12.5
Escherichia coli No. 29	0.39	0.05	0.10	0.20	50
E. coli GN206[a]	3.13	0.20	1.56	0.78	25
Klebsiella pneumoniae GN69[a]	0.78	0.05	0.10	3.13	100
K. pneumoniae PCI 602	0.20	<0.025	<0.025	0.20	50
Salmonella typhi 0-901-W	0.10	<0.025	0.05	0.20	25
Morganella morganii 1510[a]	25	0.20	6.25	0.78	>100
Providencia rettgeri GN624[a]	25	0.39	0.39	12.5	50
Enterobacter cloacae GN7471[a]	50	1.56	1.56	12.5	50
Serratia marcescens No. 1	6.25	0.20	<0.025	1.56	50
Pseudomonas aeruginosa GN10362[a]	0.78	0.20	0.78	6.25	3.13
P. aeruginosa MB-3833	0.39	0.05	0.78	3.13	1.56
P. aeruginosa E-2	0.05	0.05	0.78	3.13	1.56
P. aeruginosa ML Rms139[a]	0.39	0.20	0.78	50	50
P. cepacia M-0527	0.20	<0.025	0.10	3.13	50
Xanthomonas maltophilia M-0627	>100	6.25	50	50	>100

[a] β-Lactamase-producing strain.

Table 3. Therapeutic efficacy of MT0703*R* (**1*R***), MT0703*S* (**1*S***) and CAZ in systemic infection of mice.

Test organism	Challenge dose (cfu/mouse)[a]	Compound[b,c]	ED_{50} (mg/mouse)	MIC (μg/ml)
Escherichia coli No. 29	2.8×10^6	**1*R***	0.021	0.39
		1*S*	<0.004	0.05
		CAZ	0.032	0.10
Pseudomonas aerignosa E-2	2.6×10^6	**1*R***	0.10	0.05
		1*S*	0.20	0.05
		CAZ	1.61	0.78

[a] Intraperitoneally.
[b] Subcutaneously.
[c] Administration: 1 hour after infection with *E. coli* No. 29. 1 and 3 hours after infection with *P. aeruginosa* E-2.

of *Escherichia coli* No. 29, and was almost equally active against the infection with *Pseudomonas aeruginosa* E-2 as expected from MIC values.

This result indicates that *S*-configuration effects an increase in the antibacterial activity against Gram-negative bacteria and the stability to β-lactamase. A similar configurational effect on the antibacterial activity was reported in the aminothiazolylcephems having a carbamoyl group[3] or 3,4-dihydroxybenzoyl group[4] at the α-amino group in the C-7 substituent, while it was known that the *R*-isomer showed stronger antibacterial activity than the *S*-isomer in the case of phenylglycylcephalosporins[5].

Experimental

MP's were determined using a Yanaco MP-1 micro melting points apparatus and are uncorrected. IR spectra were recorded on a Jasco A-202 IR spectrophotometer. ^{1}H NMR spectra were recorded at 400 MHz on a Jeol GX-400 NMR spectrometer using TMS as an internal standard. All chemical shifts are reported in δ ppm. Mass spectra were taken on a Hitachi M-80B mass spectrometer. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Biological Activity

In vitro and *in vivo* antibacterial activities were determined by the methods described in a previous paper[1].

1-Hydroxy-2-hydroxymethyl-5-(*p*-methoxybenzyloxy)-4-pyridone (3)

To a solution of 2-hydroxymethyl-5-(*p*-methoxybenzyloxy)-4-pyrone[1] (**2**) (50 g) in 760 ml of pyridine was added hydroxylamine hydrochloride (66.4 g) at 50°C. The reaction mixture was stirred at 75°C for 2.5 hours and concentrated under reduced pressure. The residue was dissolved in 250 ml of H_2O and acidified to pH 2.0~2.5 with 4 N HCl at 0°C and stirred for 30 minutes. The crystals formed were collected, washed with H_2O and dried to afford **3** (20 g, 37.8%): IR (KBr) cm^{-1} 3370, 2940, 1620, 1520, 1390, 1260, 1180; ^{1}H NMR (DMSO-d_6) δ 3.76 (3H, s), 4.46 (2H, s), 5.03 (2H, s), 6.86 (1H, s), 6.93 (2H, d), 7.37 (2H, d), 7.97 (1H, s); FD-MS *m/z* 278 $(M+H)^+$.

Anal Calcd for $C_{14}H_{15}NO_5$: C 60.65, H 5.45, N 5.05.
Found: C 60.36, H 5.27, N 5.26.

2-Hydroxymethyl-5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine *N*-Oxide (4)

To a suspension of **3** (20 g) in 170 ml of 2-methoxyethanol were added dropwise Et_3N (15.1 ml) and diphenyldiazomethane (21.05 g) in 2-methoxyethanol (50 ml) at room temperature. The reaction mixture was stirred at 60°C for 5 hours and evaporated. To the residue was added 90 ml of a mixture soln of EtOAc and isopropyl ether (1 : 1). The crystals formed were filtered, washed with isopropyl ether and dried. The crystals were dissolved in 220 ml of CH_2Cl_2 and an insoluble material was removed by filtration (**5**, 6.65 g, 20.8%). The CH_2Cl_2 soln was concentrated under reduced pressure and the residue was crystallized from CH_2Cl_2 and EtOAc (1 : 1) to afford **4** (19.95 g, 62.4%): IR (KBr) cm^{-1} 3330, 1620, 1520, 1255, 1180; ^{1}H NMR (DMSO-d_6) δ 3.77 (3H, s), 4.40 (2H, s), 5.16 (2H, s), 5.49 (1H, m), 6.63 (1H, s), 6.97 (2H, d), 7.11 (1H, s), 7.28 (2H, t), 7.36 (4H, t), 7.41 (2H, d), 7.47 (4H, t), 8.13 (1H, s); FD-MS *m/z* 443 (M^+).

Anal Calcd for $C_{27}H_{25}NO_5$: C 73.12, H 5.68, N 3.16.
Found: C 73.18, H 5.52, N 3.11.

2-Hydroxymethyl-5-(*p*-methoxybenzyloxy)-1-diphenylmethoxy-4-pyridone (5)

To a suspension of **3** (27.7 g) in 200 ml of DMF were added *tert*-BuOK (11.2 g) and diphenylmethyl bromide (24.7 g) under ice-cooling. The mixture was stirred at room temperature for 2.5 hours, concentrated under reduced pressure and poured into a mixture soln of EtOAc and H_2O (2 : 1) at 0°C. The precipitate formed was collected by filtration and dried to give **5** (36.45 g, 82.3%): IR (KBr) cm^{-1} 3080, 1610, 1565, 1520, 1260, 1230; ^{1}H NMR (DMSO-d_6) δ 3.77 (3H, s), 4.25 (2H, s), 4.66 (2H, s), 5.57 (1H, m), 6.05 (1H, s), 6.51 (1H, s), 6.94 (2H, d), 7.25 (2H, d), 7.38 (1H, s), 7.40~7.45 (10H, m); FD-MS *m/z* 443 (M^+).

Anal Calcd for $C_{27}H_{25}NO_5$: C 73.12, H 5.68, N 3.16.
Found: C 72.80, H 5.35, N 3.10.

2-Carboxy-5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine *N*-Oxide (**6**)

To a suspension of **4** (25 g) in 620 ml of 50% aq CH_3CN was added 50 g of nickel peroxide at room temperature, and the reaction mixture was stirred at room temperature for 1.5 hours. An insoluble material was filtered off and the filtrate was concentrated and acidified to pH 1.8 with 4 N HCl under ice-cooling. The precipitate formed was collected, washed with H_2O and dried to afford **6** (18 g, 69.8%): IR (KBr) cm^{-1} 3460, 1620, 1525, 1440, 1250; ^1H NMR ($CDCl_3$) δ 3.84 (3H, s), 5.19 (2H, s), 6.45 (1H, s), 6.93 (2H, d), 7.2~7.5 (12H, m), 7.80 (1H, s), 7.92 (1H, s); FD-MS *m/z* 458 $(M+H)^+$.

Anal Calcd for $C_{27}H_{23}NO_6$: C 70.89, H 5.07, N 3.06.
Found: C 70.50, H 4.94, N 3.20.

Ethyl (*RS*)-2-(2-Tritylaminothiazol-4-yl)-2-[5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine-*N*-oxide-2-carboxamido]acetate (**10**)

To a solution of 20.7 g of ethyl (*Z*)-2-hydroxyimino-2-(2-tritylaminothiazol-4-yl)acetate hydrochloride (**8**) in 250 ml of a mixture soln of THF and MeOH (2:1) was added 85% AcOH (70.6 ml) and then was added 20.6 g of zinc powder over 1 hour at 0~5°C. After stirring at 0~5°C for 1 hour, the precipitate was removed by filtration. The filtrate was evaporated and extracted with EtOAc. The extract was washed with aq Na_2CO_3 and brine, dried over $MgSO_4$ and concentrated under reduced pressure to obtain a concd soln of **9**.

To a suspension of **6** (17.3 g) in 150 ml of DMF were added Et_3N (5.8 ml) and saccharin chloride (8.2 g) under −10°C. The mixture was stirred at −10°C for 1 hour. To this active ester was added dropwise the EtOAc soln containing **9** at −15~−10°C and stirred at −10°C for 1 hour. The reaction mixture was extracted with EtOAc, washed with aq $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated under reduced pressure. The residue was crystallized from EtOAc and isopropyl ether (4:1) to afford **10** (37 g): IR (KBr) cm^{-1} 3400, 1735, 1660, 1610, 1520, 1500, 1250; ^1H NMR ($CDCl_3$) δ 1.20 (3H, t), 3.82 (3H, t), 4.20 (2H, q), 5.11 (2H, s), 5.60 (1H, d), 6.31 (1H, s), 6.40 (1H, s), 6.70 (1H, s), 6.91 (2H, d), 7.2~7.5 (27H, m), 7.82 (1H, s), 7.89 (1H, s), 12.22 (1H, d); FD-MS *m/z* 883 $(M+H)^+$.

(*RS*)-2-(2-Tritylaminothiazol-4-yl)-2-[5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine-*N*-oxide-2-carboxamido]acetic Acid (**11**)

To a solution of **10** (37 g) in 160 ml of a mixture of THF and EtOH (1:1) was added dropwise 1 N NaOH (66 ml) under room temperature. The reaction mixture was stirred at 20°C for 30 minutes, concentrated, acidified to pH 2 with 1 N HCl under ice-cooling and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The residue was crystallized from EtOAc and isopropyl ether (1:2) to give **11** (28 g, 86.5% from **6**): IR (KBr) cm^{-1} 3400, 1655, 1610, 1520, 1250; ^1H NMR ($CDCl_3$) δ 3.81 (3H, s), 5.10 (2H, s), 5.63 (1H, d), 6.18 (1H, s), 6.37 (1H, s), 6.91 (2H, d), 7.2~7.5 (28H, m), 7.80 (1H, s), 7.95 (1H, s), 12.30 (1H, d); FD-MS *m/z* 856 $(M+2H)^+$.

Sodium (6*R*,7*R*)-7-[(*RS*)-2-(2-Aminothiazol-4-yl)-2-(1,5-dihydroxy-4-pyridone-2-carboxamido)-acetamido]-3-[[1-(2-hydroxyethyl)pyridinium-4-yl]thiomethyl]ceph-3-em-4-carboxylate (**1**)

(a): To a solution of 27.6 g of **11** in 280 ml of CH_2Cl_2 were added **12** (15.7 g) and pyridine (12.9 ml) at −10°C. **12** was prepared from PMB (6*R*,7*R*)-7-phenylacetamido-3-(chloromethyl)ceph-3-em-4-carboxylate[6] (Otsuka Chemical Co., Ltd.) by conventional work-up using PCl_5. The mixture was stirred at −10°C for 30 minutes. Then 3.3 ml of $POCl_3$ was added at −20~−15°C and the mixture was stirred for 30 minutes. The reaction mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$ and evaporated to give PMB (6*R*,7*R*)-7-[(*RS*)-2-(2-trithylaminothiazol-4-yl)-2-[5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine-*N*-oxide-2-carboxamido]acetamido]-3-(chloromethyl)ceph-3-em-4-carboxylate (**13**): IR (KBr) cm^{-1} 3400, 1790, 1725, 1670, 1610, 1515, 1500, 1250; ^1H NMR ($CDCl_3$) δ 3.10 and 3.49 (1H, d), 3.35 and 3.58 (1H, d), 3.79 and 3.81 (3H, s), 3.82 (3H, s), 4.40 and 4.54 (1H, d), 4.41 (1H, ABq), 4.91 and 4.92 (1H, d), 5.12 (2H, s), 5.23 (2H, s), 5.65 and 5.72 (1H, d), 5.79 and 5.93 (1H, q), 6.20 and 6.28 (1H, s), 6.40 and 6.41 (1H, s), 6.76 (1H, s), 6.90 (4H, m), 7.2~7.5 (29H, m), 7.80 and 7.81 (1H, s),

7.89 and 7.90 (1H, s), 7.96 and 8.32 (1H, d), 12.30 and 12.32 (1H, d); FD-MS *m/z* 1,205 (M^+).

(b): To a solution of **13** obtained in (a) in 80 ml of DMSO was added 4.9 g. of 1-(2-hydroxyethyl)-4(1*H*)-pyridinethione. The mixture was stirred at 20°C for 1 hour and extracted with CH_2Cl_2, The extract was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was poured into a mixture soln of EtOAc and isopropyl ether (1 : 2). The precipitate formed was collected, washed with a mixture soln of EtOAc and isopropyl ether (2 : 1) and dried to afford PMB (6*R*,7*R*)-7-[(*RS*)-2-(2-tritylaminothiazol-4-yl)-2-[5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine-*N*-oxide-2-carboxamido]acetamido]-3-[[1-(2-hydroxyethyl)pyridinium-4-yl]thiomethyl]ceph-3-em-4-carboxylate chloride (**14**) (30 g, 76.0% from **12**): IR (KBr) cm^{-1} 3400, 1780, 1720, 1630, 1610, 1515, 1495, 1250; ^1H NMR ($CDCl_3$) δ 3.45 and 3.50 (1H, d), 3.72 and 3.73 (3H, s), 3.76 (1H, m), 3.78 and 3.79 (3H, s), 3.83 (2H, br s), 4.35 (2H, br s), 4.53 (2H, br s), 5.12 and 5.14 (1H, d), 5.21 (2H, ABq), 5.29 (2H, s), 5.50 (1H, m), 5.73 and 5.75 (1H, d), 6.39 and 6.43 (1H, s), 6.73 (1H, s), 6.88 (2H, m), 6.99 (3H, m), 7.1～7.6 (29H, m), 7.65 and 7.66 (1H, s), 7.94 (2H, d), 8.29 and 8.30 (1H, s), 8.71 (2H, d), 9.01 and 9.07 (1H, d), 11.82 and 11.83 (1H, d); FD-MS *m/z* 1,326 $(M+H)^+$.

(c): To a mixture of anisole (49.3 ml) and TFA (136 ml) was added 30 g of **14** at 0°C, and the reaction mixture was stirred at 20°C for 1 hour and poured into isopropyl ether under ice-cooling. The precipitate formed was filtered and dried. This product was dissolved in 5% aq $NaHCO_3$, adjusted to pH 8.3 with satd aq $NaHCO_3$ and purified by Diaion HP-20 column chromatography. Appropriate fractions eluted with 10% aq MeOH were collected and lyophilized to afford **1** as a diastereomeric mixture (7.0 g, 45.5%): IR (KBr) cm^{-1} 3400, 1760, 1660, 1625, 1600, 1515, 1495; ^1H NMR (D_2O) δ 3.37 and 3.43 (1H, d), 3.63 and 3.68 (1H, d), 4.01 (2H, t), 4.14 and 4.17 (1H, d), 4.37 and 4.40 (1H, d), 4.53 (2H, m), 5.07 and 5.11 (1H, d), 5.60 (1H, s), 5.61 and 5.70 (1H, d), 6.73 and 6.77 (1H, s), 7.29 and 7.30 (1H, s), 7.52 and 7.54 (1H, s), 7.79 (2H, m), 8.43 (2H, m); SI-MS *m/z* 698 $(M+H)^+$.

(*RS*)-2-Chloroacetamido-2-(2-formamidothiazol-4-yl)acetic Acid (**18**)

(a): To a solution of 30.0 g of ethyl (*Z*)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetate[2] (**15**) in 100 ml of MeOH were added H_2O (100 ml) and formic acid (100 ml), and then was added zinc powder (30 g) carefully at 0～5°C. After stirring at 0～5°C for 1 hour, the insoluble material was removed by filtration and washed with 50% aq MeOH (150 ml). The filtrate was concentrated to a volume of 100 ml and H_2O (50 ml) was added. Then, to the mixture solution was added dropwise 30 g of chloroacetic anhydride in THF (100 ml) at 0°C maintaining the pH at 8～8.5 with 10 N NaOH. The reaction mixture was stirred for 30 minutes and extracted with EtOAc. The extract was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated under reduced pressure. The crystals formed were collected by filtration and dried to give ethyl (*RS*)-2-(2-aminothiazol-4-yl)-2-chloroacetamidoacetate **16** (30.7 g, 79.0%): IR (KBr) cm^{-1} 3430, 3340, 2900, 1730, 1670, 1625, 1570, 1525; ^1H NMR ($CDCl_3$) δ 1.26 (3H, t), 4.08 (2H, ABq), 4.25 (2H, m), 5.27 (2H, br s), 5.47 (1H, d), 6.55 (1H, s), 7.61 (1H, d); EI-MS *m/z* 277 (M^+).

Anal Calcd for $C_9H_{12}N_3O_3SCl$: C 38.92, H 4.36, N 15.13.
Found: C 39.07, H 4.21, N 15.08.

(b): A mixture of acetic anhydride (20 g) and formic acid (11.8 g) was stirred at 40～45°C for 1 hour. To this mixture was added 13.5 g of **16** at room temperature. The reaction mixture was stirred for 1 hour and concentrated under reduced pressure. The residue was dissolved in EtOAc (200 ml), washed with satd aq $NaHCO_3$ and brine and dried over $MgSO_4$. The organic layer was evaporated under reduced pressure and the residue was crystallized from EtOAc and Et_2O (2 : 1) to give ethyl (*RS*)-2-chloroacetamido-2-(2-formamidothiazol-4-yl)acetate **17** (13.96 g, 93.9%): IR (KBr) cm^{-1} 3340, 3280, 3000, 1745, 1685, 1650, 1550, 1525; ^1H NMR ($CDCl_3$) δ 1.24 (3H, t), 4.10 (2H, ABq), 4.24 (2H, m), 5.65 (1H, s), 7.05 (1H, s), 7.73 (1H, d), 8.66 (1H, s), 10.12 (1H, br s); EI-MS *m/z* 305 (M^+).

Anal Calcd for $C_{10}H_{12}N_3O_4SCl$: C 39.29, H 3.96, N 13.74.
Found: C 39.68, H 4.05, N 13.85.

(c): To a solution of 13.7 g of **17** in a mixture of EtOH (90 ml) and THF (45 ml) was added 1 N NaOH (112.5 ml) at room temperature, and the mixture was stirred at room temperature for 1 hour. The reaction mixture was evaporated under reduced pressure, acidified to pH 2.0 with 6 N HCl under ice-cooling and

extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$ and concentrated. The crystals formed were collected by filtration and dried to afford **18** (9.4 g, 68.7%): IR (KBr) cm^{-1} 3300, 3030, 2970, 1720, 1690, 1645, 1570, 1530; ^1H NMR (DMSO-d_6) δ 4.18 (2H, s), 5.44 (1H, d), 7.22 (1H, s), 8.49 (1H, s), 8.81 (1H, d), 12.35 (1H, s), 13.02 (1H, br s); EI-MS *m/z* 277 (M^+).

Anal Calcd for $C_8H_8N_3O_4SCl$: C 34.60, H 2.90, N 15.13.
Found: C 34.57, H 2.67, N 14.80.

Enzymatic Reaction of **18** with Acylase

18 (5.55 g) suspended in 40 ml of H_2O was dissolved with 1 N NaOH (22 ml). 0.1 M KH_2PO_4 (20 ml), 0.1 M $CoCl_2 \cdot 6H_2O$ (0.67 ml) and 1.40 g of L-aminoacylase (acylase "Amano" from *Aspergillus melleus*, Amano Pharmaceuticals, Co., Ltd., Tokyo) were added (pH 8.0). Then, the mixture was incubated at 30~33°C for 30 hours. The insoluble material was filtered off, and the filtrate was acidified to pH 2.0 with 6 N HCl and extracted with EtOAc (150 ml × 3). The extract was washed with H_2O, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from EtOAc to afford (*R*)-2-chloroacetamido-2-(2-formamidothiazol-4-yl)acetic acid (**18*R***, 2.13 g, 38.4%): MP 167°C; IR (KBr) cm^{-1} 3300, 1690, 1655, 1560, 1545; $[\alpha]_D^{27}$ −166.1° (*c* 1.0, MeOH).

Anal Calcd for $C_8H_8N_3O_4SCl$: C 34.60, H 2.90, N 15.13.
Found: C 34.65, H 2.80, N 14.88.

The aqueous layer was adjusted to pH 3.0, evaporated to remove EtOAc under reduced pressure and purified by Diaion HP-20 column chromatography with elution by H_2O. The desired eluate was concentrated under reduced pressure, and the crystals formed were collected by filtration and dried to obtain (*S*)-2-amino-(2-formamidothiazol-4-yl)acetic acid (**19*S***, 1.39 g, 34.6%, optical purity 91%): MP 167~168°C (dec); IR (KBr) cm^{-1} 3380, 3100~2800, 1685, 1655, 1580, 1510, 1450, 1380, 1290; $[\alpha]_D^{27}$ +107.5° (*c* 1.0, 0.05 N HCl); ^1H NMR (DMSO-d_6) δ 4.37 (1H, s), 7.16 (1H, s), 8.49 (1H, s); FD-MS *m/z* 201 (M^+).

Anal Calcd for $C_6H_7N_3O_3S \cdot \frac{1}{2}H_2O$: C 34.28, H 3.83, N 19.99.
Found: C 34.57, H 4.09, N 20.25.

(*R*)-2-Amino-2-(2-formamidothiazol-4-yl)acetic Acid (**19*R***)

Thiourea (1.37 g) and AcONa (1.48 g) were added to a solution of **18*R*** (1.67 g) in 36 ml of MeOH. The mixture was stirred at room temperature for 15 hours and concentrated, and THF was added to the residue. The precipitate formed was filtered and dried. This product was dissolved in H_2O, adjusted to pH 3 and purified by a column chromatography on Diaion HP-20. The desired eluate with H_2O was concentrated under reduced pressure, and the crystals formed were collected by filtration and dried to obtain D-amino acid **19*R*** (715 mg, 59.3%, optical purity 96%): MP 167~168°C (dec); IR (KBr) cm^{-1} 3570, 3480, 3100~2800, 1680, 1655, 1575, 1515, 1445, 1380, 1290; $[\alpha]_D^{27}$ −112.7° (*c* 1.0, 0.05 N HCl).

Anal Calcd for $C_6H_7N_3O_3S \cdot \frac{1}{2}H_2O$: C 34.28, H 3.83, N 19.99.
Found: C 34.31, H 3.64, N 20.07.

Conditions of HPLC analysis for optical purity of **19*R*** and **19*S***: (Column: Crownpak CR (+), 0.4 i.d. × 15 cm (Daiseru Chemical, Co., Ltd.); mobile phase: aq $HClO_4$ (pH 2.0); detection: UV 270 nm at 27°C).

(*S*)-2-(2-Formamidothiazol-4-yl)-2-[5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine-*N*-oxide-2-carboxamido]acetic Acid (**20*S***)

To a suspension of **6** (1.83 g) in 45 ml of THF was added 4 ml of Vilsmeier reagent (prepared from DMF (0.87 ml) and $POCl_3$ (1.03 ml) in 8.1 ml of CH_2Cl_2 at 0°C for 1 hour) at −15~−20°C, and the mixture was stirred at the same temperature for 1 hour. On the other hand, *N,O*-bis(trimethylsilyl)acetamide (5.0 ml) was added to a suspension of 0.88 g of **19*S*** in EtOAc (20 ml) at room temperature. The mixture was stirred for 15 minutes at room temperature and then at 45°C for 30 minutes. Next, this mixture containing sililated **19*S*** was added dropwise to the acid chloride obtained above at −15°C. After stirring for 1 hour, EtOAc (150 ml) and satd aq NaCl (100 ml) were added. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by a column chromatography on silica gel ($CHCl_3$-MeOH, 30:1~10:1) and crystallized from EtOAc and isopropyl

ether (1 : 2) to afford **20*S*** (2.14 g, 83.6%): MP 149°C; IR (KBr) cm^{-1} 1670, 1610, 1560, 1515, 1505, 1250; $[\alpha]_D^{27}$ +76.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 3.77 (3H, s), 5.06 (2H, ABq), 5.76 (1H, d), 6.36 (1H, s), 6.86 (2H, d), 6.97 (1H, s), 7.2~7.5 (13H, m), 7.75 (1H, s), 8.02 (1H, s), 8.49 (1H, s), 12.10 (1H, d); FD-MS *m/z* 641 $(M+H)^+$.

Anal Calcd for $C_{33}H_{28}N_4O_8S$: C 61.87, H 4.40, N 8.75.
Found: C 61.60, H 4.66, N 8.49.

Sodium (6*R*,7*R*)-7-[(*S*)-2-(2-Aminothiazol-4-yl)-2-(1,5-dihydroxy-4-pyridone-2-carboxamido)acetamido]-3-[[1-(2-hydroxyethyl)pyridinium-4-yl]thiomethyl]ceph-3-em-4-carboxylate (**1*S***)

(a): To a solution of **20*S*** (640 mg) in 20 ml of CH_2Cl_2 were added **12** (540 mg), pyridine (0.5 ml) and $POCl_3$ (0.126 ml) at −15°C. The reaction mixture was stirred at −15°C for 1 hour and EtOAc (100 ml) was added. The organic layer was washed twice with satd aq NaCl (50 ml), dried over $MgSO_4$ and evaporated under reduced pressure to obtain PMB (6*R*,7*R*)-7-[(*S*)-2-(2-formamidothiazol-4-yl)-2-[5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine-*N*-oxide-2-carboxamido]acetamido]-3-(chloromethyl)-ceph-3-em-4-carboxylate (1.05 g): IR (KBr) cm^{-1} 1780, 1720, 1690, 1670, 1610, 1540, 1515, 1500, 1245; ^1H NMR ($CDCl_3$) δ 2.82 (1H, d), 3.31 (1H, d), 3.78 (3H, s), 3.80 (3H, s), 3.89 (1H, d), 4.63 (1H, d), 4.82 (1H, d), 5.17 (4H, m), 5.86 (1H, br s), 5.90 (1H, q), 6.38 (1H, s), 6.55 (1H, br s), 6.85 (2H, d), 6.91 (2H, d), 7.2~7.5 (14H, m), 7.61 (1H, s), 7.93 (1H, s), 8.55 (1H, s), 9.06 (1H, br s), 10.96 (1H, br s), 12.11 (1H, d).

(b): A mixture of 3-chloromethylcephalosporin derivative (840 mg) obtained in (a) and 1-(2-hydroxyethyl)-4-(1*H*)-pyridinethione (155 mg) in 2.5 ml of DMSO was stirred at room temperature for 1 hour. The reaction mixture was extracted with CH_2Cl_2 (90 ml), washed with 15% aq NaCl (30 ml) and evaporated under reduced pressure. The residue was crystallized from EtOAc to give PMB (6*R*,7*R*)-7-[(*S*)-2-(2-formamidothiazol-4-yl)-2-[5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine-*N*-oxide-2-carboxamido]acetamido]-3-[[1-(2-hydroxyethyl)pyridinium-4-yl]thiomethyl]ceph-3-em-4-carboxylate chloride (**21*S***, 585 mg, 63.8% from **12**): IR (KBr) cm^{-1} 1780, 1685, 1665, 1630, 1610, 1540, 1515, 1495, 1245; ^1H NMR (DMSO-d_6) δ 3.53 (1H, d), 3.73 (3H, s), 3.75 (1H, d), 3.79 (3H, s), 3.81 (2H, br s), 4.33 (2H, br s), 4.51 (2H, br s), 5.17 (1H, d), 5.19 (2H, ABq), 5.28 (2H, s), 5.66 (1H, q), 5.78 (1H, d), 6.74 (1H, s), 6.86 (2H, d), 7.00 (2H, d), 7.15 (1H, s), 7.2~7.5 (14H, m), 7.69 (1H, s), 7.94 (2H, d), 8.30 (1H, s), 8.43 (1H, s), 8.70 (2H, br s), 9.33 (1H, d), 12.17 (1H, d), 12.41 (1H, s).

(c): To a solution of **21*S*** (515 mg) in anisole (0.98 ml) was added TFA (2.77 ml) and the mixture was stirred at room temperature for 1 hour. The reaction mixture was poured into isopropyl ether (15 ml). The precipitate formed was collected, dried and dissolved in MeOH (6 ml). Next, to this solution was added concd HCl (0.38 ml) and the mixture was stirred at room temperature for 2 hours. Isopropyl ether (12 ml) was added, and the precipitate formed was collected by filtration and dried. The product was dissolved in aq $NaHCO_3$ and purified by Diaion HP-20 column chromatography with elution by 5% aq MeOH. Appropriate fractions were collected and lyophilized to afford **1*S*** (195 mg, 62.2%, optical purity 88%): IR (KBr) cm^{-1} 3400, 1765, 1630, 1610, 1495; ^1H NMR (D_2O) δ 3.45 (1H, d), 3.70 (1H, d), 4.03 (2H, m), 4.18 (1H, d), 4.45 (1H, d), 4.54 (2H, m), 5.10 (1H, d), 5.59 (1H, s), 5.60 (1H, d), 6.74 (1H, s), 7.34 (1H, s), 7.58 (1H, s), 7.84 (2H, d), 8.47 (2H, d); SI-MS *m/z* 698 $(M+H)^+$.

Conditions of HPLC analysis for optical purity of the diastereomers: (Column: Cosmosil $5C_{18}$, 4.6 i.d. × 150 mm (Nacalai Tesque, Inc.); mobile phase: 0.01 M $NH_4H_2PO_4$ - MeOH (9 : 1, pH 2.5); detection: UV 305 nm at 27°C).

Sodium (6*R*,7*R*)-7-[(*R*)-2-(2-Aminothiazol-4-yl)-2-(1,5-dihydroxy-4-pyridone-2-carboxamido)-acetamido]-3-[[1-(2-hydroxyethyl)pyridinium-4-yl]thiomethyl]ceph-3-em-4-carboxylate (**1*R***)

1*R* was obtained from **19*R*** by a similar procedure for preparation of **1*S*** as described above. Optical purity 92%; IR (KBr) cm^{-1} 3420, 1765, 1630, 1610, 1510; ^1H NMR (D_2O) δ 3.37 (1H, d), 3.64 (1H, d), 4.02 (2H, m), 4.15 (1H, d), 4.38 (1H, d), 4.53 (2H, m), 5.06 (1H, d), 5.60 (1H, s), 5.70 (1H, d), 6.77 (1H, s), 7.39 (1H, s), 7.64 (1H, s), 7.80 (2H, d), 8.43 (2H, d); SI-MS *m/z* 698 $(M+H)^+$.

Acknowledgment

The authors wish to thank Dr. R. OKAMOTO for kind advice on the biological study and Ms. S. MIKI for the mass

spectral data.

References

1) OGINO, H.; K. IWAMATSU, K. KATANO, S. NAKABAYASHI, T. YOSHIDA, T. TSURUOKA, S. INOUYE & S. KONDO: New aminothiazolylglycylcephalosporins with a 1,5-dihydroxy-4-pyridone-2-carbonyl group. I. Synthesis and biological activity of cephalosporin derivatives leading to MT0703. J. Antibiotics 43: 174~188, 1990
2) OCHIAI, M.; A. MORIMOTO, Y. MATSUSHITA & T. OKADA: Synthesis and structure-activity relationships of 7β-[2-(2-aminothiazol-4-yl)acetamido]cephalosporin derivatives. IV. Synthesis of 2-(2-aminothiazol-4-yl)-2-methoxyiminoacetic acid derivatives and related compounds. J. Antibiotics 34: 160~170, 1981
3) POLACEK, I. & B. STARKE: Diastereomeric 7-α-ureidoacetyl cephalosporins. V. Antimicrobial activity, β-lactamase stability and pharmacokinetics of 7-(α-ureido-2-amino-4-thiazolylacetyl)-cephalosporins. J. Antibiotics 33: 1031~1036, 1980
4) MOCHIDA, K.; C. SHIRAKI, M. YAMASAKI, T. HIRATA, K. SATO & R. OKACHI: Aminothiazolylglycyl derivatives of carbacephems. I. Synthesis and antibacterial activity of novel carbacephems with substituted aminothiazolyl groups. J. Antibiotics 40: 14~21, 1987
5) MATSUBARA, N.; S. MINAMI, T. MURAOKA, I. SAIKAWA & S. MITSUHASHI: *In vitro* antibacterial activity of cefoperazone (T-1551), a new semisynthetic cephalosporin. Antimicrob. Agents Chemother. 16: 731~735, 1979
6) TORII, S.; H. TANAKA, N. SAITOH, T. SIROI, M. SASAOKA & J. NOKAMI: Penicillin-cephalosporin conversion III. A novel route to 3-chloromethyl-Δ^3-cephems. Tetrahedron Lett. 1982: 2187~2188, 1982