A NEW AMINOTHIAZOLYLCEPHALOSPORIN HAVING 1-CARBOXYETHOXYIMINO GROUP, ME1228†

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Aminothiazolylacetamidocephalosporins having 1-carboxyethoxyimino groups were synthesized and found to have excellent antibacterial activities including anti-pseudomonal activity and low toxicities. Among these cephalosporins, ME1228 having (*S*)-1-carboxyethoxyimino substituent and being combined with an (*N*-ethyl-4-pyridinio)thiomethyl group at C-3 showed marked therapeutic effects against systemic infections in mice and was selected as the best candidate for further evaluation.

Emergence of resistant bacteria to aminothiazolylcephalosporins is important in chemotherapy. In the course of our studies on new aminothiazolylcephalosporins possessing β-lactamase stability, ME1228, (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-((*S*)-1-carboxyethoxyimino)acetamido]-3-[(1-ethylpyridinium-4-yl)thiomethyl]ceph-3-em-4-carboxylate, was found to show an excellent activity against Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*.[1,2] In this paper, we wish to report the synthesis of ME1228 and related analogues, and their antibacterial activities and toxicities.

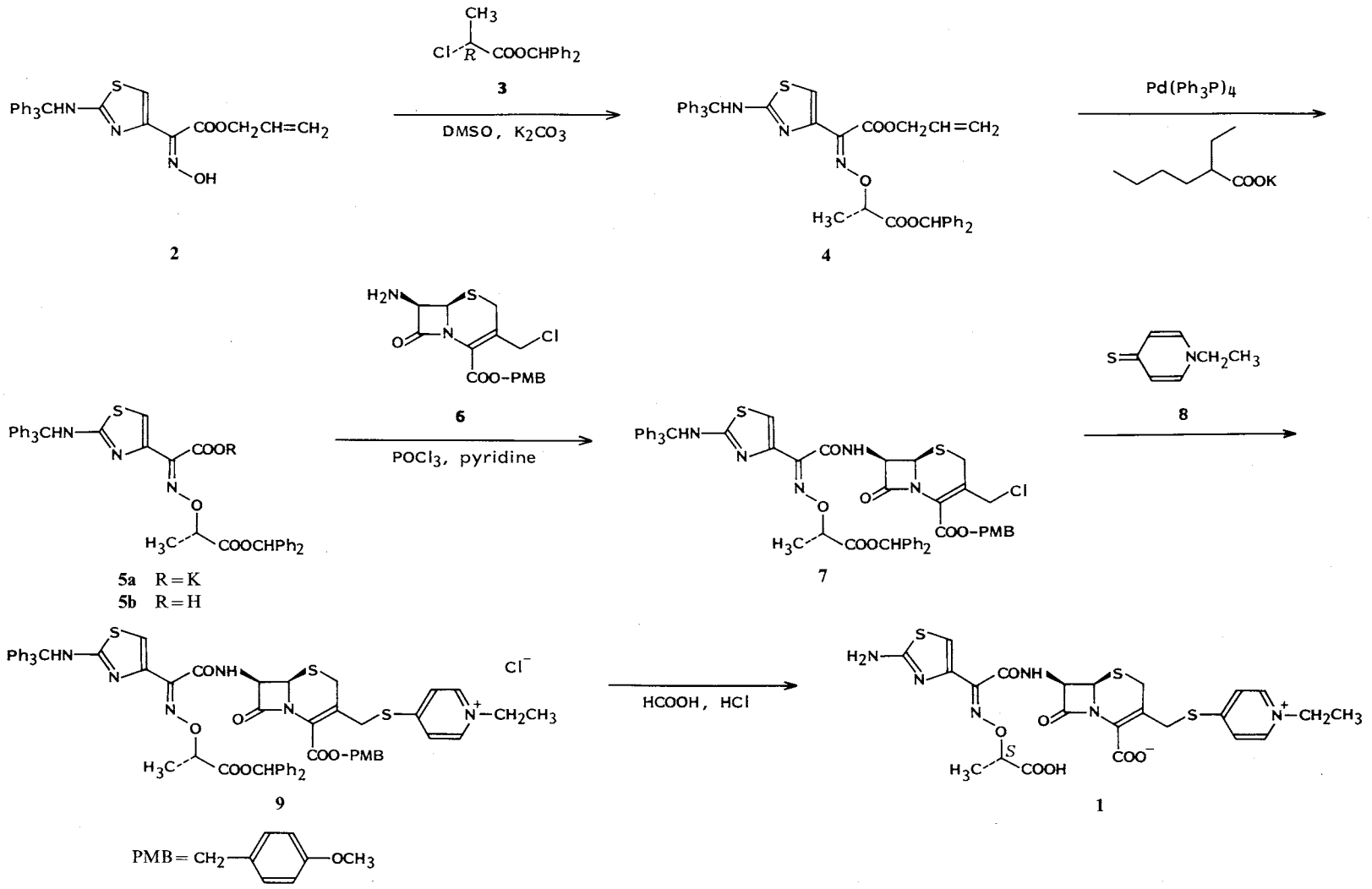
Chemistry

Several aminothiazolylacetamidocephalosporins having 1-carboxyalkoxyimino substituents[3] on the acetamido group and (*N*-alkyl-4-pyridinio)thiomethyl substituents[4] at C-3 were synthesized. The best compound, ME1228 (**1**) was prepared as shown in Scheme 1. Alkylation[3] of the hydroxyimino moiety of allyl 2-(2-tritylaminothiazol-4-yl)-2-hydroxyiminoacetate[5] (**2**) with diphenylmethyl (*R*)-2-chloropropionate (**3**) in the presence of finely powdered potassium carbonate in dimethyl sulfoxide at room temperature afforded **4**. The allyl protecting group of **4** was removed by palladium catalyzed reaction[6] with $Pd(Ph_3P)_4$ in the presence of potassium 2-ethylhexanoate in ethyl acetate to give the potassium salt (**5a**), followed by treatment with dilute acid to give the chiral acid (**5b**) in 48% yield from **2**. *p*-Methoxybenzyl (6*R*,7*R*)-7-amino-3-chloromethylceph-3-em-4-carboxylate[7] (**6**) was reacted with **5b** and phosphoryl chloride in the presence of pyridine in dichloromethane at $-10°C$ to afford **7** in 87% yield. Compound **7** was reacted with 1-ethylpyrid-4-thione[8] (**8**) in dimethyl sulfoxide at room temperature to give **9** in 85% yield. Deprotection of **9** was performed with formic acid containing hydrochloric acid in the presence of anisole to give **1** in 78% yield. Compound **1** was crystallized as the stable dihydrochloride in fine needles from aqueous acetone containing 6 equivalents of hydrochloric acid.

Compound **3** was derived from diphenylmethyl (*S*)-2-hydroxypropionate (**10**) by reaction with sulfuryl chloride in *N*,*N*-dimethylformamide at $-40°C$ in 78% yield (Scheme 2). Diphenylmethyl (*S*)-2-

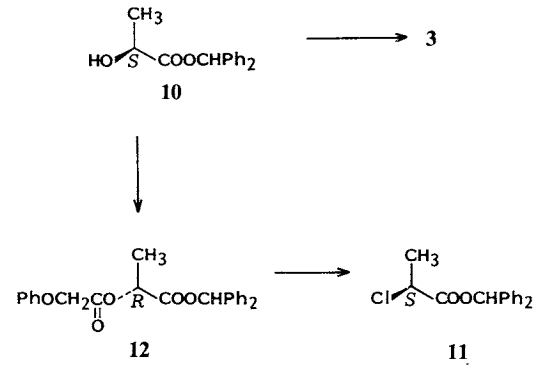
† Most of this work was presented at the 27th Intersci. Conf. on Antimicrob. Agents Chemother. held in New York on Oct. 6, 1987.[1]

Scheme 1.


Ph3CHN
COOCH2CH=CH2
N
OH
2
CH3
Cl
R
COOCHPh2
3
DMSO, K2CO3
H3C
4
Pd(Ph3P)4
COOK
H2N
O
Cl
COO-PMB
6
POCl3, pyridine
COOR
5a R=K
5b R=H
CONH
7
S
NCH2CH3
8
Cl⁻
+N-CH2CH3
9
PMB= CH2 OCH3
HCOOH, HCl
COO⁻
COOH
1

chloropropionate (**11**), the optical isomer of **3** was prepared from **10** through diphenylmethyl (*R*)-2-phenoxyacetoxypropionate (**12**) in a good yield. Compound **10** was condensed with phenoxyacetic acid by MITSUNOBU reaction[9] to give oily **12** and followed by hydrolysis with dilute ammonium hydroxide to give diphenylmethyl (*R*)-2-hydroxypropionate, which was converted into **11** by chlorination.

Scheme 2.

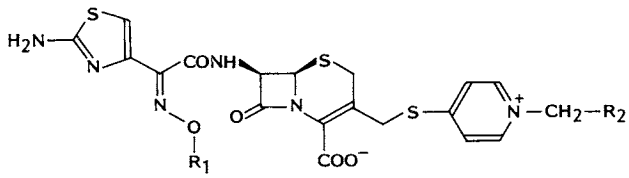


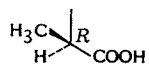
Compounds **13** and **16** (Fig. 1) were obtained from **11** by the aforementioned reaction sequence. 1-Methyl-4-pyridinio analogues (**15**~**18**) were prepared similarly with 1-methylpyrid-4-thione instead of **8**. 1-Carboxy-1-methylethoxyimino compounds (**14** and **17**) were prepared by a known method.[10,11] The demethyl analogue **18** is a known compound.[4]

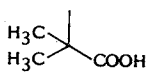
Antibacterial Activity and Toxicity

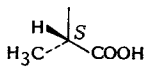
The *in vitro* antibacterial activity and acute toxicity in mice are shown in Table 1. Aminothiazolylacetamidocephalosporins having 1-carboxy-1-methylethoxyimino group[3,10] including ceftazidime (CAZ) are known to have potent anti-pseudomonal activity. In this series the 1-carboxy-1-methylethoxyimino compounds (**14** and **17**) showed also good anti-pseudomonal activity, but possessed relatively high toxicity (LD_{50} in mice, iv; <1 g/kg). The demethyl analogue **18** markedly lowered toxicity (LD_{50} >3 g/kg), but decreased anti-pseudomonal activity. The 1-caboxyethoxyimino compounds

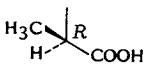
Fig. 1. Cephalosporin derivatives synthesized.

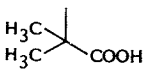


13 $R_1=$  $R_2=CH_3$

14 $R_1=$  $R_2=CH_3$

15 $R_1=$  $R_2=H$

16 $R_1=$  $R_2=H$

17 $R_1=$  $R_2=H$

18 $R_1=$  $R_2=H$

(**1**, **13**, **15** and **16**) showed promising activity against β-lactamase producers including *Escherichia coli* and *P. aeruginosa*, and displayed low toxicity ($LD_{50}>3$ g/kg). The stereochemistry of the chiral 1-carboxyethoxyimino moiety influenced the activity, and the *S* isomers (**1** and **15**) appeared superior to the *R* isomers (**13** and **16**). The *N*-alkyl group of the pyridinium moiety also had some influence on the activity, and *N*-ethyl compounds were selected for further studies. Compound **1** showed better activity against both Gram-positive and Gram-negative bacteria, compared to CAZ. The anti-pseudomonal activity of **1** was as potent as that of CAZ, and **1** had far better activity against β-lactamase-producing Gram-negative bacteria, *E. coli*, *Klebsiella oxytoca*, *Morganella morganii* and *Enterobacter cloacae*, compared to CAZ. Furthermore, the antibacterial spectrum of **1** covered anaerobic bacteria including *Clostridium* and *Bacteroides* as shown in Table 2. The acute intravenous toxicity of **1** in mice was very low ($LD_{50}>5$ g/kg), whereas the corresponding 1-carboxy-1-methylethoxyimino compounds **14** and **17** were more toxic ($LD_{50}<1$ g/kg). It is noteworthy that the difference of one methyl group between the (*S*)-1-carboxyethoxyimino and 1-carboxy-1-methylethoxyimino substituents in this series influences both the activity and toxicity.

Therapeutic Effect in Mice

The subcutaneous administration of **1** exhibited marked therapeutic activity against systemic infections in mice, as shown in Table 3. The therapeutic effect of **1** against all infections tested with *E. coli*,

Table 1. The *in vitro* antibacterial activity and acute toxicity.

Test organism	MIC (μg/ml)							
	1	**13**	**14**	**15**	**16**	**17**	**18**	CAZ
Staphylococcus aureus 606[a]	3.13	6.25	3.13	3.13	6.25	3.13	3.13	6.25
S. aureus FDA 209P JC-1	1.56	3.13	1.56	1.56	3.13	3.13	3.13	3.13
Escherichia coli W3630 RGN14[a]	0.10	0.20	0.20	0.10	0.10	0.20	0.025	0.20
E. coli 255[b]	0.20	0.39	0.78	0.78	0.39	3.13	0.78	25
Klebsiella oxytoca F-0100[a,b]	0.05	0.10	0.20	0.20	0.20	0.20	0.05	0.20
Proteus vulgaris GN76/C-1[b]	0.05	0.10	0.10	0.05	0.10	0.10	0.39	0.05
Morganella morganii 1510[b]	0.78	0.78	1.56	1.56	0.78	6.25	1.56	1.56
Citrobacter freundii GN346[b]	6.25	6.25	12.5	6.25	6.25	25	6.25	25
Enterobacter cloacae G-0005[b]	0.05	0.20	0.39	0.05	0.10	0.39	0.10	0.10
Serratia marcescens GN629[b]	0.39	1.56	1.56	0.39	0.78	1.56	1.56	0.78
Pseudomonas aeruginosa M-0148[a]	0.78	1.56	1.56	0.78	1.56	1.56	6.25	1.56
P. aeruginosa IAM 1007	0.78	1.56	1.56	0.78	1.56	1.56	3.13	1.56
P. aeruginosa M1 Rms139[a]	0.78	1.56	1.56	0.78	1.56	1.56	3.13	0.78
LD_{50} (g/kg mice, iv)	>5	>3	<1	>3	>3	<1	>3	—

[a] Penicillinase producer. [b] Cephalosporinase producer.

Table 2. The *in vitro* antibacterial activity against anaerobic bacteria.

Test organism	MIC (μg/ml)		Test organism	MIC (μg/ml)	
	1	CAZ		**1**	CAZ
Staphylococcus saccharolyticus ATCC 14953	0.39	1.56	*C. sporogenes* No. 1	6.25	25
Streptococcus parvulus Moore 5229	6.25	25	*Bacteroides fragilis* GM-7000	6.25	12.5
Propionibacterium acnes ATCC 6919	0.39	3.13	*B. fragilis* C-2	12.5	12.5
Clostridium tetani G-41	1.56	6.25	*B. praeacutus* ATCC 25539	0.39	0.78
			B. furcosus ATCC 25662	0.78	3.13

Table 3. The therapeutic effects of ME1228 (**1**) and CAZ.

Test organism	Challenge dose (cfu/mouse, ip)	Compound (sc)	ED_{50} (mg/kg)	MIC (μg/ml)
Escherichia coli No. 29	2.8×10^6	**1**	0.35	0.10
		CAZ	1.6	0.20
E. coli GN206[a]	9.5×10^6	**1**	18	0.20
		CAZ	>50	1.56
Klebsiella pneumoniae PCI 602	4.4×10^3	**1**	4.0	0.05
		CAZ	9.2	0.20
Pseudomonas aeruginosa E-2	2.6×10^6	**1**	62	0.78
		CAZ	80.5	1.56
P. aeruginosa GN10362[a]	6.8×10^6	**1**	19.5	0.78
		CAZ	40.5	0.78

[a] Cephalosporinase producer.

K. pneumoniae and *P. aeruginosa* was superior to that of CAZ.

Experimental

General Methods

MP's were determined in capillaries and uncorrected. ^{1}H NMR spectra were recorded on a Jeol JNM-GX400 (400 MHz) or on a Hitachi R-90 (90 MHz) spectrometer. IR spectra were taken on a Hitachi 260-10 spectrophotometer.

Diphenylmethyl (*R*)-2-Chloropropionate (**3**)

To a stirred solution of diphenylmethyl (*S*)-2-hydroxypropionate (**10**, 4.3 g) in DMF (25 ml) at -40°C was added sulfuryl chloride (1.62 ml) dropwise. The solution was kept for 1 hour at room temperature. The reaction mixture was partitioned between EtOAc (40 ml) and ice-water (90 ml) containing $NaHCO_3$ (8 g). The organic layer was separated and washed with NaCl-satd water (40 ml × 2) and water (40 ml). The separated organic layer was dried over $MgSO_4$, filtered and concentrated to give crystalline **3** (3.6 g, 78%): MP 57~59°C; $[\alpha]_D^{25}$ +7.86° (*c* 5.0, $CHCl_3$); ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.70 (3H, d, J=7.03 Hz), 4.48 (1H, q, J=7.03 Hz), 6.89 (1H, s), 7.20~7.40 (10H, m).

Diphenylmethyl (*S*)-2-Chloropropionate (**11**)

To a stirred solution of **10** (4.0 g), phenoxyacetic acid (2.49 g) and triphenylphosphine (4.9 g) in THF (100 ml) at 0°C was added diethyl azodicarboxylate (3.45 ml) in one portion. The solution was stirred at room temperature overnight and concentrated to give an oily residue. The residue was extracted with ether (100 ml) and filtered. The extract was concentrated and the conc was purified by silica gel column chromatography (Wakogel C-100, Wako Pure Chem. Ind., 300 g) with toluene as an eluent to give **12** as an oil (5.7 g, 94%), $[\alpha]_D^{25}$ +29.7° (*c* 5.0, $CHCl_3$). Compound **12** was dissolved in 50 ml of MeOH and treated with 0.7 ml of 25% NH_4OH at room temperature with stirring. After 1 hour, the reaction mixture was adjusted to pH 7.0 with 0.1 N HCl and evaporated. The residue was partitioned between EtOAc (50 ml) and H_2O (50 ml). The organic layer was concentrated and the residue was purified by silica gel column chromatography with toluene - EtOAc (10 : 1) as an eluent to give diphenylmethyl (*R*)-2-hydroxypropionate (2.7 g, 73%), mp 80~82°C, $[\alpha]_D^{25}$ +9.10° (*c* 5.0, $CHCl_3$). It was converted into **11** by the method used for *R* isomer **3**, in 80% yield, mp 56~59°C, $[\alpha]_D^{25}$ -7.62° (*c* 5.0, $CHCl_3$).

Allyl 2-(2-Tritylaminothiazol-4-yl)-2-hydroxyiminoacetate (**2**)

Ethyl 2-(2-tritylaminothiazol-4-yl)-2-hydroxyiminoacetate[5] (3.0 g) was suspended in 36 ml of allyl alcohol and heated at 60°C with stirring. To the suspension was added 200 mg of sodium ethoxide dissolved in 1 ml of allyl alcohol. The mixture was sitrred at 60°C for 3 hours and then cooled to room temperature. After neutralization with 0.15 ml of 2 N HCl, the mixture was concentrated and triturated with 10 ml of

isopropyl ether to give the crude powder (2.6 g), which was partitioned between 15 ml of $CHCl_3$ and 10 ml of H_2O. The organic layer was separated, dried over $MgSO_4$ and concentrated to 7 ml. Addition of 10 ml of EtOAc to the conc afforded **2** as a crystalline compound (2.42 g, 79%): MP 187~189°C; FD-MS *m/z* 469 (M^+); ^1H NMR (90 MHz, $CDCl_3$) δ 4.73 (2H, m), 5.15 (1H, m), 5.33 (1H, m), 5.90 (1H, m), 6.30 (1H, s), 7.10~7.40 (15H, m).

Anal Calcd for $C_{27}H_{23}N_3O_3S$: C 69.06, H 4.94, N 8.95.
Found: C 68.63, H 4.76, N 9.04.

2-(2-Tritylaminothiazol-4-yl)-2-[(*S*)-1-(diphenylmethoxycarbonyl)ethoxyimino]acetic Acid (**5b**)

To a solution of **2** (3.1 g) in DMSO (16.5 ml) was added **3** (2.0 g) and K_2CO_3 (powdered, 1.82 g) at room temperature with stirring. The mixture was stirred overnight and partitioned between EtOAc (60 ml) and ice-water (30 ml). The organic layer was separated and washed with NaCl-satd aq solution (30 ml) containing 0.13 ml of 2 N HCl (pH 7.0). The organic layer was separated, dried over $MgSO_4$, filtered and concentrated to 6 ml. The conc containing **4** was dissolved in CH_2Cl_2 (40 ml) and treated with triphenylphosphine (180 mg) and tetrakis(triphenylphosphine)palladium (34 mg). To the stirred solution potassium 2-ethylhexanoate (1.26 g) in EtOAc (13 ml) was added under N_2 atmosphere and stirred for 2 hours. After trituration with isopropyl ether (45 ml), the potassium salt (**5a**) was obtained by filtration. The potassium salt was partitioned between EtOAc (65 ml) and ice-water (26 ml) and adjusted to pH 2.8 with 2 N HCl (3.4 ml). The organic layer was separated, dried over $MgSO_4$ and concentrated to give crystalline **5b** (2.13 g, 48% from **2**): MP 134~137°C (dec); $[\alpha]_D^{25}$ −10.2° (*c* 5.0, $CHCl_3$); ^1H NMR (90 MHz, $CDCl_3$) δ 1.50 (3H, d, J=7.03 Hz), 5.05 (1H, q, J=7.03 Hz), 6.62 (1H, s), 6.90 (1H, s), 7.00~7.40 (26H, m).

Anal Calcd for $C_{40}H_{33}N_3O_5S$: C 71.95, H 4.98, N 6.29.
Found: C 71.43, H 4.85, N 6.23.

2-(2-Tritylaminothiazol-4-yl)-2-[(*R*)-1-(diphenylmethoxycarbonyl)ethoxyimino]acetic Acid (*R* Isomer of **5b**)

The compound was prepared from **11** by the method used for **5b**, mp 135~137°C, $[\alpha]_D^{25}$ +10.2° (*c* 5.0, $CHCl_3$).

p-Methoxybenzyl (6*R*,7*R*)-7-[2-(2-Tritylaminothiazol-4-yl)-2-[(*S*)-1-(diphenylmethoxycarbonyl)ethoxyimino]acetamido]-3-chloromethylceph-3-em-4-carboxylate (**7**)

To a suspension of *p*-methoxybenzyl (6*R*,7*R*)-7-amino-3-chloromethylceph-3-em-4-carboxylate[7] (**6**, 1.32 g, 3.6 mmol) and **5b** (2.4 g, 3.6 mmol) in CH_2Cl_2 (40 ml) was added pyridine (1.45 ml) at 0°C. The mixture was stirred for 1 hour to give a solution, cooled to −10°C and followed by addition of phosphoryl chloride (0.35 ml, 3.96 mmol). After 1 hour, the mixture was concentrated, diluted with EtOAc (54 ml) and washed with NaCl-satd water (30 ml). The organic layer was separated, dried over $MgSO_4$ and concentrated to give **7** as a foam (3.2 g, 87%). The product was used without further purification for the next step. ^1H NMR (400 MHz, $CDCl_3$) δ 1.60 (3H, d, J=7.18 Hz), 3.36 and 3.54 (2H, ABq, J=18.2 Hz), 3.80 (3H, s), 4.41 and 4.59 (2H, ABq, J=11.8 Hz), 4.95 (2H, d, J=4.87 Hz), 5.16 (1H, q, J=7.18 Hz), 5.18 and 5.25 (2H, ABq, J=11.8 Hz), 5.90 (1H, dd, J=8.47 and 4.87 Hz), 6.70~7.50 (32H, m), 8.07 (1H, d, J=8.47 Hz).

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-[(*S*)-1-carboxyethoxyimino]acetamido]-3-[(1-ethylpyridinium-4-yl)thiomethyl]ceph-3-em-4-carboxylic Acid (**1**) Dihydrochloride

Compound **7** (3.70 g, 3.6 mmol) and 1-ethylpyrid-4-thione (**8**, 0.5 g, 4.07 mmol) were dissolved in DMSO (11 ml) and stirred at room temperature for 1 hour. To the reaction mixture was added $CHCl_3$ (23 ml), EtOAc (10 ml) and NaCl-satd water (20 ml). The organic layer was separated, washed with H_2O (10 ml × 2), dried over $MgSO_4$ and concentrated to give **9** (3.6 g, 85%). To a solution of **9** in anisole (5 ml) was added 88% formic acid (4 ml) and concd HCl (2 ml). The solution was stirred at room temperature for 4 hours, diluted with isopropyl alcohol (100 ml) and adjusted to pH 3.5 with 25% NH_4OH with ice cooling. After stirring at room temperature overnight the resulting ppt was collected and dried to give **1** as a free amine (1.6 g, 78% from **9**). The free amine was dissolved in H_2O (8 ml) and followed by addition of concd HCl (1.4 ml, 6 equiv) at 5°C. The solution was diluted with acetone (15 ml) and stirred overnight at 5°C to give the crystalline dihydrochloride of **1**, which was washed with acetone (5 ml) and

dried: MP 180~183°C (dec); $[\alpha]_D^{25}$ −34.3° (*c* 1.0, H_2O); UV $\lambda_{max}^{H_2O}$ nm (ε) 231 (25,390), 259 (19,620), 304 (32,150); IR ν_{max} (KBr) cm^{-1} 1770, 1709, 1625; ^1H NMR (400 MHz, D_2O) δ 1.57 (3H, d, *J*=6.92 Hz, $OCCH_3$), 1.59 (3H, t, *J*=7.44 Hz, $NCCH_3$), 3.58 and 3.79 (2H, ABq, *J*=17.9 Hz, 2-H_2), 4.39 and 4.43 (2H, ABq, *J*=13.3 Hz, 3'-H_2), 4.47 (2H, q, *J*=7.44 Hz, NCH_2C), 4.98 (1H, q, *J*=6.92 Hz, OCHC), 5.25 (1H, d, *J*=4.62 Hz, 6-H), 5.82 (1H, d, *J*=4.62 Hz, 7-H), 7.23 (1H, s, thiazole-H), 7.83 and 8.51 (4H, ABq, *J*=7.18 Hz, pyridine-H_4).

Anal Calcd for $C_{23}H_{24}N_6O_7S_3 \cdot 2HCl \cdot H_2O$: C 40.41, H 4.13, N 12.29, S 14.07, Cl 10.37, H_2O 2.63.
Found: C 39.93, H 4.15, N 12.24, S 14.0, Cl 10.1, H_2O 3.0.

Compounds **13**~**17**

These compounds (Fig. 1) were prepared similarly as **1** and purified by column chromatography on Diaion HP-20 resin (Mitsubishi Chemical Ind. Ltd.) eluted with H_2O-MeOH (10:1) to obtain as purified free amines. **13**: ^1H NMR (400 MHz, D_2O) δ 1.44 (3H, d, *J*=7.2 Hz, $OCCH_3$), 1.54 (3H, t, $NCCH_3$), 3.51 and 3.71 (2H, ABq, *J*=18.0 Hz, 2-H_2), 4.23 and 4.35 (2H, ABq, *J*=13.9 Hz, 3'-H_2), 4.43 (2H, q, NCH_2C), 4.63 (1H, q, *J*=7.2 Hz, OCHC), 5.19 (1H, d, *J*=4.5 Hz, 6-H), 5.73 (1H, d, *J*=4.5 Hz, 7-H), 6.96 (1H, s, thiazole-H), 7.84 and 8.43 (4H, ABq, *J*=5.6 Hz, pyridine-H_4). **14**: ^1H NMR (90 MHz, D_2O) δ 1.53 (3H, t, $NCCH_3$), 1.59 (6H, s, $OCCH_3 \times 2$), 3.57 (2H, ABq, *J*=17.5 Hz, 2-H_2), 4.39 (2H, br, 3'-H_2), 4.45 (2H, q, NCH_2C), 5.29 (1H, d, 6-H), 5.83 (1H, d, 7-H), 6.97 (1H, s, thiazole-H), 7.91 and 8.51 (4H, ABq, pyridine-H_4). **15**: ^1H NMR (90 MHz, D_2O) δ 1.40 (3H, d, $OCCH_3$), 3.39 and 3.70 (2H, ABq, 2-H_2), 4.10 (3H, s, NCH_3), 4.21 (2H, br, 3'-H_2), 4.60 (1H, q, OCHC), 5.41 (1H, d, 6-H), 5.87 (1H, d, 7-H), 6.85 (1H, s, thiazole-H), 7.72 and 8.25 (4H, ABq, pyridine-H_4). **16**: ^1H NMR (90 MHz, D_2O) δ 1.38 (3H, d, $OCCH_3$), 3.44 and 3.80 (2H, ABq, 2-H_2), 4.08 (3H, s, NCH_3), 4.10 (2H, br, 3'-H_2), 4.55 (1H, q, OCHC), 5.25 (1H, d, 6-H), 5.62 (1H, d, 7-H), 6.75 (1H, s, thiazole-H), 7.75 and 8.21 (4H, ABq, pyridine-H_4). **17**: ^1H NMR (400 MHz, D_2O) δ 1.54 (3H, s, $OCCH_3$), 1.55 (3H, s, $OCCH_3$), 3.51 and 3.75 (2H, ABq, *J*=17.8 Hz, 2-H_2), 4.22 and 4.41 (2H, ABq, *J*=13.8 Hz, 3'-H_2), 4.24 (3H, s, NCH_3), 5.25 (1H, d, 6-H), 5.82 (1H, d, 7-H), 7.01 (1H, s, thiazole-H), 7.87 and 8.42 (4H, ABq, *J*=6.2 Hz, pyridine-H_4).

Antibacterial Activity

According to the method of Japan Society of Chemotherapy, MICs were determined in Mueller-Hinton agar medium by the standard 2-fold dilution method with 10^6 cfu/ml inoculum size after incubation at 37°C for 20 hours. In the case of anaerobic bacteria, GAM Agar Medium (Nissui) in an anaerobic chamber (Forma Scientific Ltd.) with a condition of N_2-CO_2-H_2O (80:10:10) was used.

Acute Toxicity

LD_{50} were determined with three ICR mice (5-week old) at each dose level. Compounds dissolved in physiological saline solution were injected intravenously, and mice were observed for 14 days.

Therapeutic Effect in Mice

Male ICR mice (4-week old) were infected intraperitoneally (challenge doses are shown in Table 3). Five mice at each dose level were given the test compounds subcutaneously 1 hour after the bacterial challenge in case of *E. coli* and *K. pneumoniae*. In case of *P. aeruginosa*, mice were treated with antibiotics twice, 1 and 3 hours after the challenge. The ED_{50} was calculated by the method of LITCHFIELD and WILCOXON,[12] from survival rate recorded on day 7.

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