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

I. SYNTHESIS AND BIOLOGICAL ACTIVITY OF CEPHALOSPORIN DERIVATIVES LEADING TO MT0703

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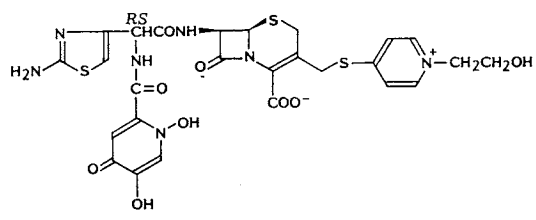
(Received for publication August 21, 1989)

A series of new aminothiazolylglycylcephalosporins with a mono- or dihydroxypyridonecarbonyl group at the α-amino group of the C-7 substituent have been prepared and antibacterial activity of these compounds was investigated. Among them, the compounds having a 1,5-dihydroxy-4-pyridone-2-carbonyl group showed excellent anti-pseudomonal activity. In particular, (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 (MT0703, **7g**) was found to be a well balanced compound with respect to antibacterial activity.

In a previous paper[1], we reported that the new aminothiazolyloxyiminocephalosporins with an *N*-alkylcyclopentano[*b*]pyridiniumthiomethyl group at C-3 showed enhanced antibacterial activity, especially against β-lactamase-producing strains, and that introduction of a carboxyl group to the C-7 substituent influenced the anti-pseudomonal activity, solubility and toxicity. In recent years, it was reported that penicillin and cephalosporin derivatives[2,3] having a catechol moiety exhibit strong activity against *Pseudomonas aeruginosa*, however its anti-pseudomonal activity is lost through *O*-methylation of the catechol moiety by catechol-*O*-methyltransferase (COMT, EC 2.1.1.6)[4]. Taking these points into account, we designed new aminothiazolylglycylcephalosporins bearing a mono- or dihydroxypyridone instead of a catechol connected at the α-amino group through an amide linkage, to improve the anti-pseudomonal activity and the stability to COMT. Recently, MOCHIDA *et al.* reported on the aminothiazolylglycylcephem compounds KT-4697 and KT-4788 with the hydroxypyridone moiety, which had a good activity against Gram-positive and Gram-negative bacteria including *P. aeruginosa*[5].

In this paper, we describe the synthesis and the antibacterial activity of new cephalosporins that have various mono- or dihydroxypyridonecarbonyl groups at the α-amino group of the aminothiazolylglycyl side chain, leading to a diastereomeric mixture MT0703 (**7g**) (Fig. 1) possessing a 1,5-dihydroxy-4-pyridone-2-carbonyl group in the C-7 substituent and a 1-(2-hydroxyethyl)pyr-

Fig. 1. Structure of MT0703 (**7g**).



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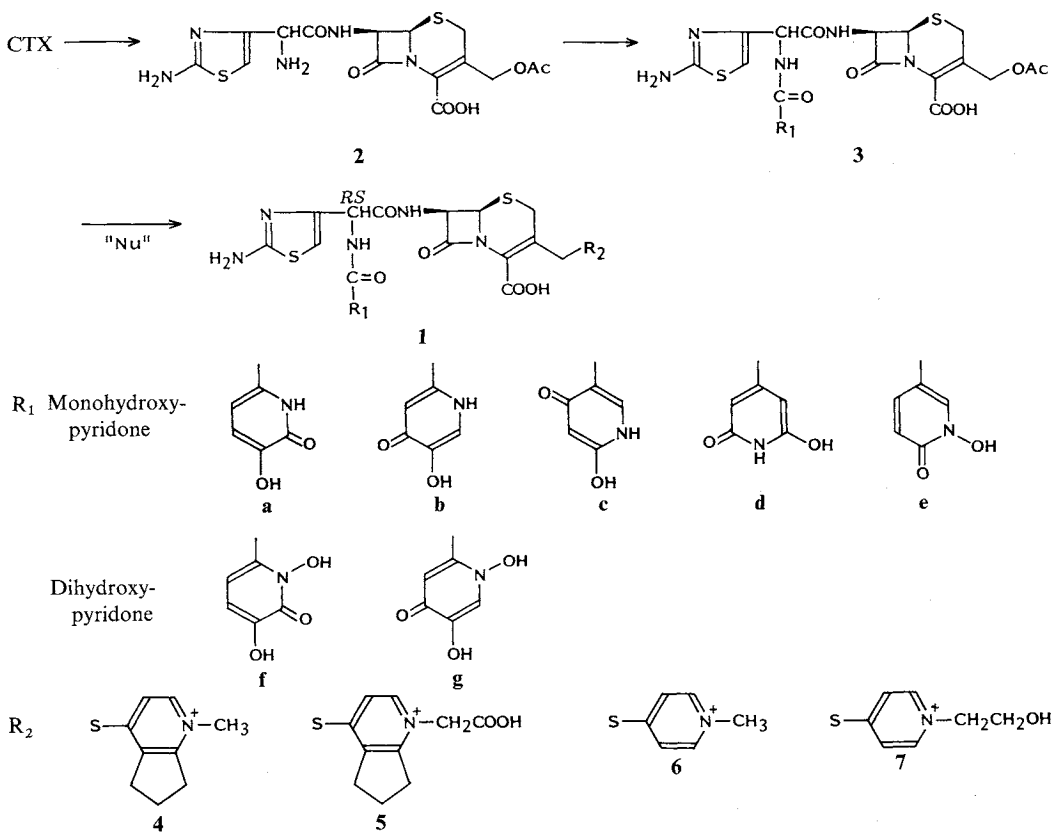
idiniumthiomethyl group at C-3 as a candidate for clinical use. The synthesis and the antibacterial activity of each diastereomer of **7g** will be reported in the next paper[6].

Chemistry

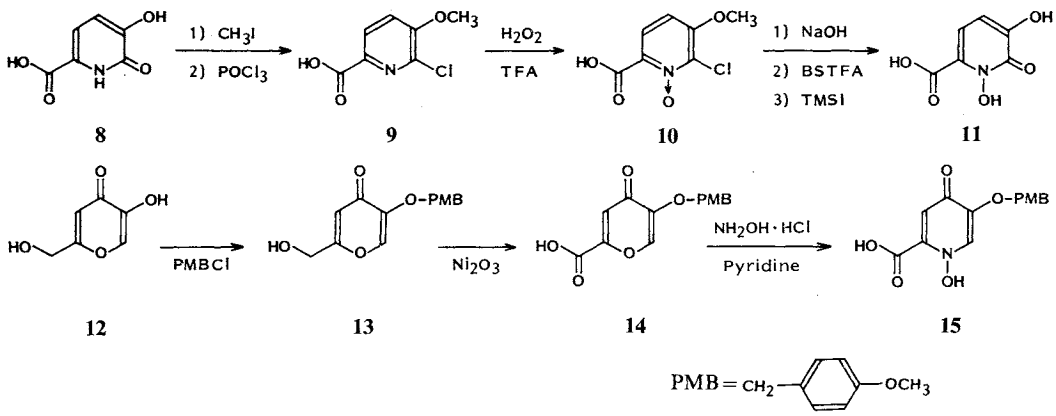
Aminothiazolylglycylcephalosporins (**1**) with various mono- or dihydroxypyridonecarbonyl groups were prepared by the method outlined in Scheme 1. An aminothiazolylglycyl derivative (**2**) was obtained as a 1:1 mixture of diastereomers by reduction[7] of the methoxyimino group of cefotaxime (CTX) with zinc powder in aqueous HCOOH. Various mono- and dihydroxypyridonecarboxylic acids were condensed with **2** by the acid chloride or *N*,*N*-dicyclohexylcarbodiimide (DCC) method, followed by removal of protecting groups, if necessary, to afford corresponding compounds **3a**~**3g**. Compounds **3** were converted into desired products **1** by displacement of the acetoxy group at C-3 methylene with various nucleophiles. Thus, 44 new cephalosporin derivatives have been synthesized as a mixture of diastereomers. Only compounds **3b** and **6b** (KT-4788) were already synthesized and characterized by MOCHIDA *et al.*[5].

New *N*-hydroxypyridone substituents, 1,3-dihydroxy-2-pyridone-6-carboxylic acid (**11**) and 1,5-dihydroxy-4-pyridone-2-carboxylic acid protected as the *p*-methoxybenzyl ether (**15**), were prepared according to the route shown in Scheme 2. Compound **11** was prepared from 3-hydroxy-6-pyridone-2-carboxylic acid (**8**) which is easily obtained from D-glucaro-1,5-lactam[8]. The phenolic hydroxyl group of **8** was protected by methylation, and the methylated compound was treated with phosphoryl chloride to convert it into chloropyridine **9**. Compound **9** was oxidized with hydrogen peroxide in trifluoroacetic acid

Scheme 1.



Scheme 2.



8 9 10 11

12 13 14 15

to afford pyridine *N*-oxide **10**. Hydrolysis and demethylation of **10** gave **11** in an overall yield of about 33% from **8**. Condensation of **11** with **2** by the DCC method was not accomplished without protecting the two hydroxyl groups of **11**, but it was completed after protection of them with 2-methoxyethoxymethyl groups.

Compound **15** was prepared from kojic acid (**12**). Its phenolic hydroxyl group was protected by a *p*-methoxybenzyl group to afford **13**, followed by oxidation with nickel peroxide[9] to give carboxylic acid **14**. Compound **14** was treated with hydroxylamine hydrochloride in pyridine to convert it into *N*-hydroxypyridone **15** in an overall yield of 25% from **12**.

Effects of Mono- and Dihydroxypyridone Moiety on the Antibacterial Activity

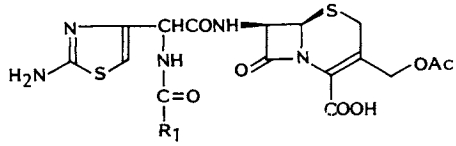
The MICs of the new cephalosporins having mono- or dihydroxypyridonecarbonyl groups are listed in Tables 1, 2 and 3. Among monohydroxypyridone derivatives having an acetoxymethyl group at C-3, **3a** and **3b** showed potent activity against Gram-positive and Gram-negative bacteria including *P. aeruginosa*. 1,5-Dihydroxy-4-pyridone derivative (**3g**) exhibited stronger activity than **3b** against *Pseudomonas*. 1,3-Dihydroxy-2-pyridone derivative (**3f**), however, was less active than **3a** (Table 1).

As shown in Table 2, replacement of the C-3 acetoxymethyl group of anti-pseudomonal compounds **3** by the *N*-methylcyclopentano[*b*]pyridiniumthiomethyl group improved the antibacterial activity against almost all the organisms tested. The effects of the pyridone moieties in compounds **4** were similar to those in **3**. Among compounds **4**, anti-pseudomonal activity of **4b** and **4g** were superior to that of ceftazidime.

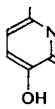
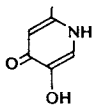
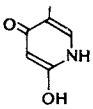
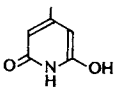
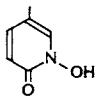
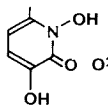
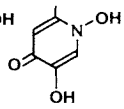
Comparison of the biological activities of the compounds having a 5-hydroxy-4-pyridone-2-carbonyl group (type **b**) with those of the compounds bearing a 1,5-dihydroxy-4-pyridone-2-carbonyl group (type **g**), as shown in Table 3, demonstrated that type **g** compounds (**5g**, **6g** and **7g**) have activity superior to type **b** compounds (**5b**, **6b** and **7b**) and catechol derivatives (**16** and **17**) against *Pseudomonas* and other Gram-negative bacteria. The activity of type **g** compounds against Gram-positive bacteria was approximately equal or somewhat inferior to that of type **b** compounds.

Concerning solubility in water, type **g** compounds were more soluble than type **b** compounds and were suitable as injectable drugs. For instance, the solubility of **6g** was more than 30% w/v, while that of **6b** was less than 10% w/v.

Table 1. Antibacterial activity of compounds **3**.

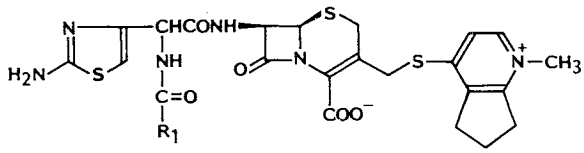


3

Test organism	MIC (μg/ml)						
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**
R_1:							
Staphylococcus aureus 209P JC-1	0.78	3.13	6.25	12.5	1.56	6.25	12.5
Bacillus subtilis ATCC 6633	1.56	3.13	3.13	6.25	1.56	3.13	6.25
Escherichia coli NIHJ JC-2	0.20	0.20	0.39	0.78	0.39	0.39	0.20
Klebsiella pneumoniae GN69[a]	0.39	0.05	0.20	0.39	0.78	0.39	0.05
K. pneumoniae PCI 602	0.10	<0.025	0.20	0.39	0.20	0.20	<0.025
Providencia rettgeri GN624[a]	12.5	3.13	6.25	3.13	25	50	6.25
Enterobacter cloacae G-0008	0.39	0.39	0.39	0.78	0.78	1.56	0.78
Serratia marcescens No. 1	0.39	0.78	3.13	3.13	12.5	3.13	0.78
Pseudomonas aeruginosa GN10362[a]	12.5	0.39	12.5	>50	>50	25	0.05
P. aeruginosa E-2	0.78	0.10	6.25	>50	>50	3.13	<0.025
P. cepacia M-0527	0.20	0.05	3.13	12.5	12.5	0.20	<0.025

[a] β-Lactamase-producing strain.

Table 2. Antibacterial activity of compounds **4**.



4

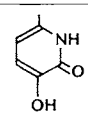
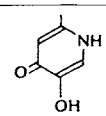
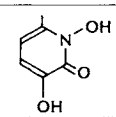
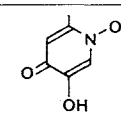
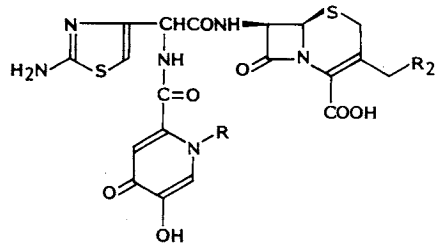
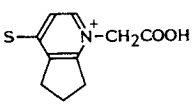
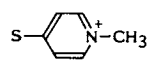
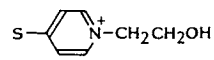
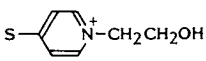
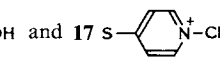
Test organism	MIC (μg/ml)				
	4a	**4b**	**4f**	**4g**	
R_1:					Ceftazidime
Staphylococcus aureus 209P JC-1	0.20	0.39	3.13	0.78	6.25
Bacillus subtilis ATCC 6633	0.78	1.56	12.5	3.13	3.13
Escherichia coli NIHJ JC-2	0.10	0.20	0.39	0.05	0.20
Klebsiella pneumoniae GN69	0.10	0.10	0.39	0.05	0.10
K. pneumoniae PCI 602	0.05	0.05	0.39	<0.025	<0.025
Providencia rettgeri GN624	0.78	0.20	6.25	1.56	0.39
Enterobacter cloacae G-0008	0.20	0.20	0.78	0.10	0.20
Serratia marcescens No. 1	0.20	0.20	1.56	0.20	<0.025
Pseudomonas aeruginosa GN10362	12.5	0.39	12.5	0.20	0.78
P. aeruginosa E-2	0.39	0.10	1.56	0.05	0.78
P. cepacia M-0527	0.05	0.05	0.20	0.05	0.10

Table 3. Antibacterial activity of compounds **5**, **6**, **7**, **16** and **17**.



Test organism	MIC (μg/ml)							
	5b	**5g**	**6b**	**6g**	**7b**	**7g**	**16**[a]	**17**[a]
R:	H	OH	H	OH	H	OH		
R_2:								
Staphylococcus aureus 209P JC-1	3.13	12.5	0.78	0.78	0.78	3.13	0.78	3.13
Bacillus subtilis ATCC 6633	3.13	12.5	1.56	3.13	1.56	3.13	3.13	1.56
Escherichia coli NIHJ JC-2	0.10	0.10	0.20	0.05	0.20	0.10	0.39	0.10
Klebsiella pneumoniae GN69	0.10	0.05	0.20	0.05	0.39	0.10	0.39	0.39
K. pneumoniae PCI 602	<0.025	<0.025	0.05	<0.025	0.05	<0.025	0.10	0.05
Providencia rettgeri GN624	0.39	0.10	1.56	0.39	0.39	0.20	0.39	0.39
Enterobacter cloacae G-0008	0.39	0.39	0.20	0.10	0.39	0.20	1.56	0.78
Sterratia marcescens No. 1	0.20	0.10	0.20	0.20	0.39	0.20	0.78	0.39
Pseudomonas aeruginosa GN10362	1.56	0.10	1.56	0.20	1.56	0.20	6.25	1.56
P. aeruginosa E-2	0.05	0.05	0.20	0.05	0.78	0.05	1.56	0.78
P. cepacia M-0527	0.10	<0.025	0.10	<0.025	0.05	<0.025	<0.025	<0.025

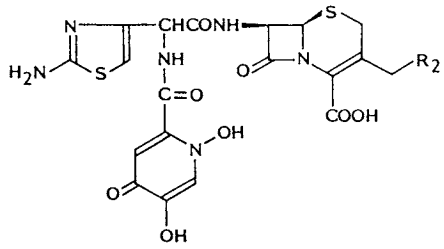
[a] R_1: Catechol moiety, R_2: **16**  and **17**  .

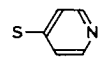
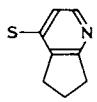
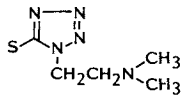
It was presumed that a hydroxyl group introduced at 1-N of the 5-hydroxy-4-pyridone-2-carbonyl group acted like an acidic substituent such as carboxyl group and this could contribute much to the increase in anti-pseudomonal activity and solubility in water. Thus, we selected the 1,5-dihydroxy-4-pyridone-2-carbonyl group (type **g**) as the preferred acyl moiety attached to the α-amino group of the aminothiazolylglycyl side chain.

Effects of C-3 Substituents on the Antibacterial Activity

In a series of compounds having the 1,5-dihydroxy-4-pyridone-2-carbonyl group, effects of various substituents at C-3 on the antibacterial activity were examined. Among the derivatives (**18**～**32**) containing various heterocyclic thiomethyl groups, compounds **18**, **19** (pyridyl), **23** (1,3,4-triazinyl), **25** (1,2,3-thiadiazolyl) and **31** (benzothiazolyl) had a well balanced activity against Gram-positive and Gram-negative bacteria, as shown in Table 4. On the other hand, among the derivatives (**4g**～**7g**, **33**～**44**) containing various *N*-alkylpyridiniumthiomethyl or pyridiniummethyl groups, compounds **4g**, **6g**, **7g**, **38**, **40** and **44** with neutral substituents on the 1-N of the pyridine nucleus showed more potent activity than others against Gram-negative bacteria including *Pseudomonas*, as shown in Tables 2, 3 and 5. Compounds

Table 4. Antibacterial activity of compounds **18**~**32**.



Test organism	MIC (μg/ml)				
	18	**19**	**20**	**21**	**22**
	R_2: 				
Staphylococcus aureus 209P JC-1	1.56	1.56	6.25	12.5	6.25
Bacillus subtilis ATCC 6633	3.13	3.13	3.13	12.5	6.25
Escherichia coli NIHJ JC-2	0.10	0.20	0.10	0.20	0.20
Klebsiella pneumoniae GN69	0.05	0.05	0.05	0.20	0.10
K. pneumoniae PCI 602	<0.025	0.05	0.05	0.10	<0.025
Providencia rettgeri GN624	3.13	0.20	1.56	1.56	6.25
Enterobacter cloacae G-0008	0.78	0.39	0.39	0.39	1.56
Serratia marcescens No. 1	0.20	0.39	0.20	0.39	0.78
Pseudomonas aeruginosa GN10362	0.39	0.20	0.39	0.78	0.78
P. aeruginosa E-2	0.20	<0.025	0.05	0.10	0.10
P. cepacia M-0527	0.05	<0.025	0.05	0.05	0.10

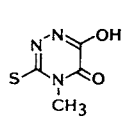
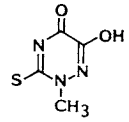
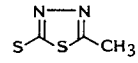
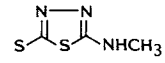
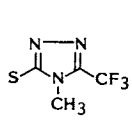
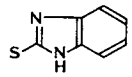
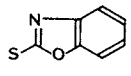
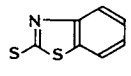
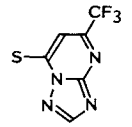
Test organism	MIC (μg/ml)				
		24	**25**	**26**	**27**
	R_2: 				
Staphylococcus aureus 209P JC-1	3.13	25	3.13	12.5	12.5
Bacillus subtilis ATCC 6633	3.13	3.13	3.13	6.25	6.25
Escherichia coli NIHJ JC-2	0.10	0.10	0.20	0.20	0.20
Klebsiella pneumoniae GN69	0.05	0.10	0.05	0.10	0.10
K. pneumoniae PCI 602	<0.025	0.05	0.05	0.05	0.05
Providencia rettgeri GN624	0.20	0.78	3.13	50	6.25
Enterobacter cloacae G-0008	0.20	0.39	0.78	0.78	0.78
Serratia marcescens No. 1	0.20	0.39	0.20	1.56	1.56
Pseudomonas aeruginosa GN10362	0.20	0.39	0.39	6.25	0.39
P. aeruginosa E-2	0.10	0.10	0.05	0.78	0.20
P. cepacia M-0527	0.05	0.05	0.05	0.10	0.10

Table 4. (Continued)

Test organism	MIC (μg/ml)				
	28	**29**	**30**	**31**	**32**
	R_2: 				
Staphylococcus aureus 209P JC-1	25	6.25	12.5	3.13	6.25
Bacillus subtilis ATCC 6633	12.5	6.25	6.25	3.13	6.25
Escherichia coli NIHJ JC-2	0.20	0.20	0.39	0.20	0.20
Klebsiella pneumoniae GN69	0.10	0.05	0.20	0.05	0.05
K. pneumoniae PCI 602	0.05	0.05	0.10	0.05	0.05
Providencia rettgeri GN624	0.20	12.5	25	3.13	0.05
Enterobacter cloacae G-0008	1.56	1.56	3.13	0.78	0.39
Serratia marcescens No. 1	0.78	0.39	0.78	0.20	0.39
Pseudomonas aeruginosa GN10362	0.39	0.20	0.39	0.20	0.20
P. aeruginosa E-2	0.10	0.05	0.20	0.05	0.10
P. cepacia M-0527	0.05	<0.025	0.10	<0.025	<0.025

(**33**~**36**) bearing a pyridiniummethyl group showed inferior activity against most of Gram-negative bacteria to the derivatives with an *N*-alkylpyridiniumthiomethyl group (Table 5). Introduction of an acidic substituent such as carboxymethyl (**5g** and **37**) or sulfoethyl (**43**) group in the C-3 substituent reduced the activity against Gram-positive bacteria.

Among these derivatives, we selected some candidates (**4g**, **6g**, **7g**, **19**, **25**, **31** and **40**) for further evaluation on the basis of anti-pseudomonal activity and solubility in water. The urinary recovery rates in mice by subcutaneous administration of the derivatives (**4g**, **6g**, **7g** and **40**) possessing an *N*-alkylpyridiniumthiomethyl group were 67~90% and higher than those of compounds (**19**, **25** and **31**) with a heterocyclic thiomethyl group, as shown in Table 6. In comparison with **6g** having an *N*-methylpyridiniumthiomethyl group, compound **4g** having an *N*-methylcyclopentano[*b*]pyridiniumthiomethyl group showed a high urinary recovery rate but was less soluble in water and more toxic. Compounds **4g**, **19** and **31** revealed toxicity at a dose of 2.0 g/kg in mice after intravenous injection. However, no toxicity was observed for **7g** at a dose of 4.0 g/kg.

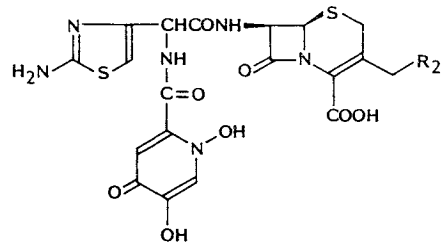
Antibacterial Activity *In Vivo*

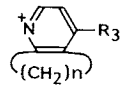
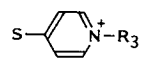
The activities *in vivo* of 1,5-dihydroxy-4-pyridone derivatives **6g**, **7g** and **40** are listed in Table 7. These three compounds were active in mice against an experimental infection with *Escherichia coli* GN206. Compound **7g** showed strong activity *in vivo* against the infection with *P. aeruginosa* GN10362 through only one administration as expected from the MIC value.

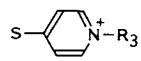
As shown in Table 8, the antibacterial activity *in vivo* of **7g** (MT0703) against Gram-negative bacteria was compared with that of ceftazidime and cefoperazone. MT0703 showed strong activity *in vivo* against *P. aeruginosa* E-2 and GN10362, *E. coli* No. 29 and *Klebsiella pneumoniae* PCI 602 as well as *in vitro*, and was more potent than ceftazidime and cefoperazone in all of the infections tested.

Based on these results, MT0703 (**7g**), containing a [1-(2-hydroxyethyl)pyridinium-4-yl]thiomethyl group

Table 5. Antibacterial activity of compounds **33**~**44**.



Test organism	MIC (μg/ml)						
	33	**34**	**35**	**36**	**37**	**38**	**39**
R_2:							
n:	3	0	3	0			
R_3:	H	H	SCH_3	SCH_3	CH_2COOH	CH_2CONH_2	$CH_2CONHOH$
Staphylococcus aureus 209P JC-1	0.78	6.25	3.13	3.13	6.25	1.56	3.13
Bacillus subtilis ATCC 6633	3.13	6.25	6.25	6.25	6.25	3.13	3.13
Escherichia coli NIHJ JC-2	0.39	0.78	0.39	0.20	0.05	0.10	0.20
Klebsiella pneumoniae GN69	0.39	0.39	0.39	0.20	0.10	0.10	0.20
K. pneumoniae PCI 602	0.10	0.10	0.05	0.05	<0.025	<0.025	0.10
Providencia rettgeri GN624	3.13	12.5	1.56	1.56	0.20	0.20	0.20
Enterobacter cloacae G-0008	0.78	6.25	1.56	0.78	0.20	0.20	0.78
Serratia marcescens No. 1	0.39	3.13	0.39	0.39	0.20	0.20	0.39
Pseudomonas aeruginosa GN10362	0.78	1.56	1.56	0.78	0.20	0.20	1.56
P. aeruginosa E-2	0.10	0.20	0.20	0.10	0.05	0.05	0.20
P. cepacia M-0527	0.10	0.10	0.05	0.10	<0.025	<0.025	0.10

Test organism	MIC (μg/ml)				
	40	**41**	**42**	**43**	**44**
R_2:					
R_3:		CH_2CH_2OH[a]	CH_2CH_2F	CH_2SO_3H	$CH_2SO_2NH_2$
Staphylococcus aureus 209P JC-1	3.13	3.13	3.13	12.5	1.56
Bacillus subtilis ATCC 6633	3.13	6.25	6.25	6.25	3.13
Escherichia coli NIHJ JC-2	0.10	0.20	0.10	0.10	0.10
Klebsiella pneumoniae GN69	0.10	0.05	0.10	0.10	0.05
K. pneumoniae PCI 602	<0.025	0.05	<0.025	<0.025	<0.025
Providencia rettgeri GN624	0.10	0.39	0.20	0.20	0.20
Enterobacter cloacae G-0008	0.20	0.39	0.20	0.20	0.20
Serratia marcescens No. 1	0.20	0.39	0.20	0.10	0.39
Pseudomonas aeruginosa GN10362	0.39	0.39	0.20	0.20	0.39
P. aeruginosa E-2	0.10	0.10	0.10	<0.025	0.05
P. cepacia M-0527	<0.025	<0.025	0.05	<0.025	<0.025

[a] Pyridinium-3-yl.

Table 6. Urinary excretion in mice.

Compound	**4g**	**6g**	**7g**	**19**	**25**	**31**	**40**
Urinary recovery[a] (%)	90.2	79.1	67.2	10.2	29.9	11.3	77.2

[a] After 25 mg/kg injection subcutaneously.

Table 7. Therapeutic efficacy in experimental infections in mice.

Test organism	Challenge dose (cfu/mouse)[a]	Compound[b]	ED_{50} (mg/kg)	MIC (μg/ml)
Escherichia coli GN206[c]	9.5×10^6	**6g**	13.5	0.05
		7g	6.0	0.20
		40	6.5	0.20
		Ceftazidime	>50	1.56
Pseudomonas aeruginosa GN10362[c]	8.8×10^5	**6g**	>200	0.20
		7g	145	0.20
		40	>200	0.39
		Ceftazidime	>400	1.56

[a] Intraperitoneally.
[b] Subcutaneously.
[c] β-Lactamase-producing strain.

Table 8. Therapeutic efficacy of MT0703 (**7g**) and other cephalosporins in systemic infections in mice.

Test organism	Challenge dose (cfu/mouse)[a]	Compound[b,c]	ED_{50} (mg/kg)	MIC (μg/ml)
Escherichia coli No. 29	3.5×10^6	MT0703	0.4	0.05
		Ceftazidime	1.8	0.10
Klebsiella pneumoniae PCI 602	4.4×10^3	MT0703	0.3	<0.025
		Ceftazidime	9.2	<0.025
Pseudomonas aeruginosa E-2	5.2×10^6	MT0703	12.5	0.05
		Ceftazidime	76	0.78
		Cefoperazone	>400	3.13
P. aeruginosa GN10362[d]	6.8×10^5	MT0703	12	0.20
		Ceftazidime	40.5	0.78
		Cefoperazone	>400	6.25

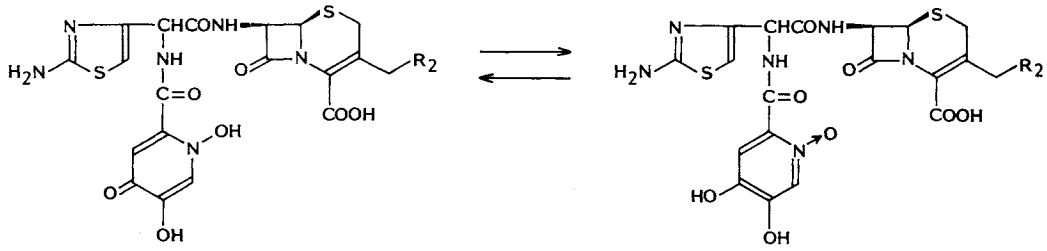
[a] Intraperitoneally.
[b] Subcutaneously.
[c] Administration: 1 and 3 hours after infections with *P. aeruginosa*.
[d] β-Lactamase-producing strain.

at C-3, was found to have excellent antibacterial activity, especially against *Pseudomonas*, and the most favorable solubility, pharmacokinetics and low toxicity among the candidates.

The Stability to COMT

As the type **g** acyl moiety is able to exist as dihydroxypyridone (**1g**) and dihydroxypyridine *N*-oxide (**1g'**) tautomers as shown in Scheme 3, we think it is a catechol analogue. By enzymatic reaction with COMT for 2 hours, 18% of **17**, which has a 3,4-dihydroxybenzoyl group, was *O*-methylated. However, type **g** compounds **7g** and **37** were not methylated. This suggests that the 1,5-dihydroxy-4-pyridone-2-carbonyl group at the α-amino group of the aminothiazolylglycyl side chain is stable to COMT and contributes to the potent antibacterial activity against *Pseudomonas* strains.

Scheme 3.



1g 1g'

Experimental

NMR spectra were recorded at 60 MHz on a Varian T-60A NMR spectrometer and at 400 MHz on a Jeol GX-400 NMR spectrometer using TMS as an internal standard. All chemical shifts are reported in δ ppm. IR spectra were recorded on a Jasco A-202 IR spectrophotometer. MS were taken on a Hitachi M-80B mass spectrometer (mainly SI-MS).

Biological Evaluation

MICs (μg/ml) were determined by the 2-fold agar dilution method using sensitivity disk agar (Nissui Seiyaku, Co., Ltd.) after incubation at 37°C for 20 hours with an inoculum size of about 10^6 cfu/ml.

Antibacterial activity *in vivo* was tested using male mice (Jcl:ICR, 4 weeks old). Each of eight mice in a group was challenged intraperitoneally with 10^3 to 10^6 cfu of the bacteria suspended in 0.5 ml of saline containing 2.5% gastric mucin (Difco Laboratories). The animals were treated subcutaneously with test compounds 1 hour after challenge. ED_{50} values (mg/kg) were calculated by the probit method from the number of mice surviving 7 days after infection.

Urinary excretion was tested using male mice (Jcl:ICR, 4 weeks old). The test compounds were administered subcutaneously to three mice at a dose of 25 mg/kg. Urinary recovery rates (%) were calculated from the urinary concentrations of drugs at 0 to 4 hours after administration. Concentrations were determined by bioassay using *E. coli* K-12 HW8236 as a test organism.

The acute toxicity was determined by the survival rate of male mice (Jcl:ICR, 5 weeks old, three per group) 2 weeks after intravenous injection of the test compounds.

Enzymatic Reaction with COMT

To a solution of 0.2 ml of substrate (**7g**, **17** or **37**, 1 mg/ml), 0.2 ml of 1 mM *S*-adenosylmethionine, 0.2 ml of 1 mM $MgCl_2$ and 0.2 ml of 0.05 M phosphate buffer (pH 7.9) was added 0.2 ml of porcine liver COMT (500 U/ml, Sigma). All materials were dissolved in 0.05 M phosphate buffer at pH 7.9 and the mixture was incubated at 37°C with gentle shaking. Since compound **16** was insoluble in the phosphate buffer at pH 7.9, its activity could not be tested. The methylation was monitored by HPLC (column: Cosmosil $5C_{18}$, 4.6 i.d. × 150 mm (Nacalai Tesque, Inc.), mobile phase: 0.05 M $NH_4H_2PO_4$-CH_3CN-MeOH (85:10:5, pH 2.5), UV detection at 305 nm, 29°C).

3-Hydroxy-2-pyridone-6-carboxylic Acid (**8**)

To a suspension of potassium salt of D-glucaro-1,5-lactam[8] (60 g) in 500 ml of pyridine was added 250 ml of acetic anhydride, and the reaction mixture was stirred at 70~75°C for 4 days. The insoluble product was collected by filtration, washed with ether, crystallized from H_2O and dried to afford 3-acetoxy-2-pyridone-6-carboxylic acid as the potassium salt (52 g). A mixture of the potassium salt (52 g) and NaOH (30 g) in 500 ml of H_2O was stirred at 60°C for 20 minutes and acidified to pH 1.8 with 5 N HCl. The crystals were collected by filtration and dried to afford **8** (31 g, 76%): IR (KBr) cm^{-1} 3060, 1685, 1630, 1610, 1555, 1285; ^1H NMR (DMSO-d_6) δ 6.73 (1H, d), 6.90 (1H, d), 9.97 (1H, br s); EI-MS *m/z* 155 (M^+).

Anal Calcd for $C_6H_5NO_4$: C 46.46, H 3.25, N 9.03.
Found: C 46.48, H 3.19, N 8.96.

6-Carboxy-2-chloro-3-methoxypyridine *N*-Oxide (**10**)

(a): To a solution of **8** (31 g) and K_2CO_3 (55.2 g) in 500 ml of H_2O were added dioxane (150 ml) and CH_3I (50 ml). The reaction mixture was stirred at 70°C for 7 hours and the precipitate was collected. The precipitate was dissolved in water and acidified to pH 1.5 with 6 N HCl. The crystals were collected by filtration, washed with H_2O and dried to give 3-methoxy-2-pyridone-6-carboxylic acid (25 g, 74%): ^{1}H NMR (DMSO-d_6) δ 3.77 (3H, s), 6.83 (1H, d), 6.96 (1H, d).

3-Methoxy-2-pyridone-6-carboxylic acid (16.9 g) was suspended in 150 ml of phosphoryl chloride, and the reaction mixture was refluxed for 20 hours. The precipitate was filtered off and the filtrate was evaporated. To the residue was added ice-water. After this mixture had been stirred for 1 hour, the product formed was collected by filtration, dissolved in H_2O at pH 10 and treated with charcoal. The charcoal was filtered off and the filtrate was acidified to pH 2 with 5 N HCl. The crystals were collected by filtration and recrystallized from a mixture of $CHCl_3$ and MeOH to afford 2-chloro-3-methoxy-6-pyridinecarboxylic acid (**9**) (15 g, 80%): ^{1}H NMR (DMSO-d_6) δ 3.95 (3H, s), 7.64 (1H, d), 8.05 (1H, d); FD-MS *m/z* 188 $(M+H)^+$.

(b): To a solution of **9** (14.8 g) in 150 ml of TFA was added 75 ml of 30% hydrogen peroxide. The reaction mixture was stirred at 80°C for 2 hours and concentrated under reduced pressure. To the residue was added 45 ml of MeOH. The crystals were collected by filtration and dried to afford **10** (15.6 g, 97%): IR (KBr) cm^{-1} 1705, 1590, 1470, 1370, 1240, 1075; ^{1}H NMR (DMSO-d_6) δ 4.06 (3H, s), 7.66 (1H, d), 8.23 (1H, d); SI-MS *m/z* 204 $(M+H)^+$.

1,3-Dihydroxy-2-pyridone-6-carboxylic Acid (**11**)

To the suspension of **10** (15.5 g) in 100 ml of H_2O was added 28.56 g of NaOH, and the mixture was refluxed for 1.5 hours. The mixture was cooled with an ice bath and acidified to pH 1.5 with conc HCl. The crystals were collected by filtration, washed with ice-water and hot MeOH, and dried to give 1-hydroxy-3-methoxy-2-pyridone-6-carboxylic acid (14.9 g): ^{1}H NMR (DMSO-d_6) δ 3.75 (3H, s), 6.76 (1H, d), 6.85 (1H, d); FD-MS *m/z* 185 (M^+).

To a suspension of 1-hydroxy-3-methoxy-2-pyridone-6-carboxylic acid (3.16 g) in 100 ml of 1,2-dichloroethane was added 12 ml of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). After the reaction mixture had been stirred at room temperature for 1.5 hours, 7.2 ml of trimethylsilyl iodide (TMSI) was added. The reaction mixture was stirred at 50°C for 48 hours and diluted with 100 ml of CH_2Cl_2. After addition of 4 ml of ice-water, the mixture was stirred for 15 minutes. The precipitate was collected by filtration, washed with CH_2Cl_2, Me_2CO and ice-water successively and dried to afford **11** (1.61 g, 58% from **10**): IR (KBr) cm^{-1} 3390, 2800, 1685, 1620, 1560, 1460, 1280; ^{1}H NMR (D_2O+$NaHCO_3$) δ 6.87 (1H, d), 7.09 (1H, d); FD-MS *m/z* 171 (M^+).

Anal Calcd for $C_6H_5NO_5 \cdot \frac{1}{2}H_2O$: C 40.01, H 3.63, N 7.78.
Found: C 40.90, H 3.40, N 7.77.

5-(*p*-Methoxybenzyloxy)-4-pyrone-2-carboxylic Acid (**14**)

To a suspension of kojic acid (**12**) (42.6 g) and K_2CO_3 (82.8 g) in 350 ml of DMF was added dropwise 55.0 g of *p*-methoxybenzyl chloride at 70°C. The reaction mixture was stirred for 1.5 hours at 75°C and concentrated under reduced pressure. The residue was poured over ice-water, and the precipitate was collected, washed with H_2O and EtOAc, and dried to give 2-hydroxymethyl-5-(*p*-methoxybenzyloxy)-4-pyrone (**13**) as colorless crystals (61.6 g, 78%): ^{1}H NMR (DMSO-d_6) δ 3.80 (3H, s), 4.43 (2H, s), 4.96 (2H, s), 6.50 (1H, s), 6.88 (2H, d), 7.30 (2H, d), 7.51 (1H, s).

To a solution of **13** (26.2 g) in a mixture of THF (125 ml), H_2O (62.5 ml) and 1 N NaOH (125 ml) was added 78 g of nickel peroxide at 0°C, and the mixture was stirred at 10~15°C for 2.5 hours. The insoluble material was filtered off, and the filtrate was concentrated and acidified to pH 2 with 6 N HCl. The crystalline product was collected, washed with H_2O and $CHCl_3$, and dried to afford **14** (20.5 g, 74%): IR (KBr) cm^{-1} 3400, 2850, 1720, 1610, 1580, 1550, 1250, 1210; ^{1}H NMR (DMSO-d_6) δ 3.77 (3H, s), 4.90 (2H, s), 6.92 (1H, s), 6.96 (2H, d), 7.37 (2H, d), 8.34 (1H, s); FD-MS *m/z* 276 (M^+).

1-Hydroxy-5-(*p*-methoxybenzyloxy)-4-pyridone-2-carboxylic Acid (**15**)

A mixture of **14** (10.35 g) and hydroxylamine hydrochloride (13 g) in 150 ml of pyridine was stirred

at 75°C for 1.5 hours. The reaction mixture was cooled and evaporated. The residue was dissolved in water and acidified to pH 2.0~2.5 with 4N HCl at 0°C. The precipitate was collected, washed with H_2O and dried to afford **15** (4.7g, 43%): IR (KBr) cm^{-1} 3430, 1610, 1530, 1515, 1380, 1290, 1240; ^1H NMR (DMSO-d_6) δ 3.80 (3H, s), 5.22 (2H, s), 7.00 (2H, d), 7.43 (2H, d), 7.59 (1H, s), 8.59 (1H, s); FD-MS *m/z* 292 $(M+H)^+$.

Anal Calcd for $C_{14}H_{13}NO_6$: C 57.73, H 4.50, N 4.81.
Found: C 57.27, H 4.39, N 4.70.

General Procedure for the Acylation of **2**

Method A: To a solution of 1 mmol of **2** in a mixture of 5ml of THF and 5ml of H_2O at pH 8 adjusted with Et_3N was added 1.1 to 1.2mmol of acid chloride or active ester of mono- or dihydroxypyridonecarboxylic acid at 0°C. The reaction mixture was stirred at 0°C for 2 hours, adjusted to pH 6.5, evaporated to remove THF and acidified to pH 2 with 1N HCl. The residue was purified by column chromatography on Diaion HP-20 eluted with aq Me_2CO to give **3**. When the phenolic hydroxyl group was protected, removal of protecting group was accomplished with TFA in anisole. The product was dissolved in 10% aq $NaHCO_3$ after being adjusted to pH 7.0~7.5, and was chromatographed on Diaion HP-20 eluted with 50% aq MeOH to afford **3** as the sodium salt.

Method B: To a solution of 1 mmol of **2** and 1.2mmol of Et_3N in 8ml of DMF was added 1.2mmol of active ester of mono- or dihydroxypyridonecarboxylic acid at 0°C. The reaction mixture was stirred at room temperature for 2.5 hours and evaporated under reduced pressure. The product was purified by a similar procedure to that described in Method A to obtain **3**.

The IR, mass and ^1H NMR data for compounds **3** are listed in Table 9.

(6*R*,7*R*)-7-[(*RS*)-2-(2-Aminothiazol-4-yl)-2-(1,3-dihydroxy-2-pyridone-6-carboxamido)acetamido]-3-(acetoxymethyl)ceph-3-em-4-carboxylic Acid (**3f**)

(a): To a solution of 1.344g of **11** in 20ml of DMF was added 1.2g of 60% sodium hydride at 0°C, and the mixture was stirred for 30 minutes. To the reaction solution was added 3.5ml of 2-methoxyethoxymethyl chloride at 0°C. The reaction mixture was stirred at room temperature for 3 hours. After addition of MeOH (0.5ml), the reaction mixture was diluted with EtOAc, washed with H_2O, dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified on silica gel column chromatography with $CHCl_3$-MeOH (100:3), to afford 2-methoxyethoxymethyl 1,3-bis(2-methoxyethoxymethoxy)-2-pyridone-6-carboxylate (1.867g, 54%): ^1H NMR ($CDCl_3$) δ 3.38 (9H, br s), 3.4~3.65 (6H), 3.7~4.1 (6H), 5.34 (4H, s), 5.52 (2H, s), 6.76 (1H, d), 7.00 (1H, d).

To a solution of 1.25g of 2-methoxyethoxymethyl 1,3-bis(2-methoxyethoxymethoxy)-2-pyridone-6-carboxylate in a mixture of THF (4ml) and MeOH (1ml) was added 1N NaOH (4ml). The mixture was stirred at room temperature for 1 hour and evaporated under reduced pressure. To the residue was added brine, and the mixture was adjusted to pH 2 and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ amd Molecular Sieves 4A, and evaporated to give 1,3-bis(2-methoxyethoxymethoxy)-2-pyridone-6-carboxylic acid (865mg, 87%): ^1H NMR ($CDCl_3$) δ 3.37 (6H, s), 3.43~3.68 (4H), 3.7~4.2 (4H), 5.38 (4H, s), 6.85 (1H, d), 7.07 (1H, d).

(b): To a solution of 392mg of the product obtained in (a) in a mixture of CH_2Cl_2 (3ml) and DMF (1ml) were added 245mg of DCC and 153mg of 1-hydroxybenzotriazole. The mixture was stirred at room temperature for 1 hour and filtered. The filtrate was added to a mixture of the HCl salt of **2** (577mg) and Et_3N (0.26ml) in DMF (6ml). The reaction mixture was stirred at room temperature for 2.5 hours and evaporated under reduced pressure. The residue was purified by column chromatography on Diaion HP-20 to give (6*R*,7*R*)-7-[(*RS*)-2-(2-aminothiazol-4-yl)-2-[1,3-bis(2-methoxyethoxymethoxy)-2-pyridone-6-carboxamido]acetamido]-3-(acetoxymethyl)ceph-3-em-4-carboxylic acid as the Et_3N salt (483mg, 49%): ^1H NMR (D_2O) δ 1.29 (9H, t), 2.11 (3H, s), 3.22 (6H, q), 3.31 (3H), 3.34 (3H, s), 3.3~3.9 (10H), 4.65~4.95 (2H), 5.13 and 5.17 (1H, d), 5.30 (1H), 5.42 (4H), 5.65 (1H), 6.81~6.9 (2H), 7.26 (1H, d).

(c): To a suspension of 465mg of the product obtained in (b) in 2ml of anisole was added 4ml of TFA at 0°C and the reaction mixture was stirred from 0°C to room temperature for 4.5 hours, and poured into 60ml of isopropyl ether. The precipitate was collected by filtration and dissolved in 10% aq $NaHCO_3$ after being adjusted to pH 7.5, and chromatographed on Diaion HP-20 eluted with H_2O. The appropriate

Table 9. IR, mass and ^1H NMR data for new cephalosporins.

Compound	IR ν_{max} (KBr) (cm^{-1})	SI-MS (m/z)	^1H NMR (D_2O) δ (ppm)
3a	1760, 1640, 1610	587 $(M+H)^+$ as mono Na salt	2.12, 2.13 (3H, s), 3.35, 3.42 (1H, d), 3.63, 3.68 (1H, d), 4.72, 4.74 (1H, d), 4.89, 4.90 (1H, d), 5.14, 5.18 (1H, d), 5.60 (1H, s), 5.70, 5.77 (1H, d), 6.77, 6.82 (1H, s), 7.03 (1H, d), 7.15 (1H, dd)
3c	1760, 1655, 1615	609 $(M+H)^+$ as di Na salt	2.12, 2.13 (3H, s), 3.34, 3.41 (1H, d), 3.64, 3.68 (1H, d), 4.72, 4.74 (1H, d), 4.90, 4.91 (1H, d), 5.14, 5.18 (1H, d), 5.59, 5.60 (1H, s), 5.68, 5.76 (1H, d), 6.75, 6.80 (1H, s), 8.13 (2H, s)
3d	1765, 1670, 1610		2.14 (3H, s), 3.39, 3.46 (1H, d), 3.66, 3.71 (1H, d), 4.74, 4.76 (1H, d), 4.90, 4.91 (1H, d), 5.15, 5.19 (1H, d), 5.58, 5.59 (1H, s), 5.5~5.8 (1H), 5.72, 5.79 (1H, d), 6.7~6.85 (1H), 6.79, 6.83 (1H, s)
3e	1765, 1660, 1610		2.12, 2.13 (3H, s), 3.35, 3.43 (1H, d), 3.65, 3.69 (1H, d), 4.72, 4.75 (1H, d), 4.89, 4.91 (1H, d), 5.14, 5.18 (1H, d), 5.60 (1H, s), 5.69, 5.77 (1H, d), 6.69 (1H, d), 6.78, 6.83 (1H, s), 8.08 (1H, d), 8.21 (1H, br s)
4f	1765, 1615, 1515	686 $(M+H)^+$	2.26 (2H, m), 2.90 (2H, m), 3.21 (2H, m), 3.37, 3.43 (1H, d), 3.62, 3.66 (1H, d), 4.01 (3H, s), 4.10, 4.12 (1H, d), 4.35, 4.39 (1H, d), 5.05, 5.07 (1H, d), 5.57 (1H, s), 5.58, 5.68 (1H, d), 6.75, 6.78 (1H, s), 6.80, 7.14 (1H, d), 7.52 (1H, m), 8.15 (1H, m)
6g	1765, 1660, 1630, 1500, 1495	646 $(M+H)^+$	3.54, 3.58 (2H, ABq), 4.29, 4.32 (2H, ABq), 4.22 (3H, s), 5.09, 5.13 (1H, d), 5.62 (1H, s), 5.62, 5.73 (1H, d), 6.76, 6.81 (1H, s) 7.39, 7.40 (1H, s), 7.63, 7.64 (1H, s), 7.81 (2H, d), 8.40 (2H, d)
7g	1760, 1660, 1625, 1600, 1515, 1495	698 $(M+H)^+$ as mono Na salt	3.37, 3.43 (1H, d), 3.63, 3.68 (1H, d), 4.01 (2H, t), 4.14, 4.17 (1H, d), 4.37, 4.40 (1H, d), 4.53 (2H, m), 5.07, 5.11 (1H, d), 5.60 (1H, s), 5.61, 5.70 (1H, d), 6.73, 6.77 (1H, s), 7.29, 7.30 (1H, s), 7.52, 7.54 (1H, s), 7.79 (2H, m), 8.43 (2H, m)
25	1760, 1660, 1600, 1515	683 $(M+H)^+$ as di Na salt	3.34, 3.40 (1H, d), 3.67, 3.71 (1H, d), 3.90, 3.92 (1H, d), 4.32, 4.37 (1H, d), 5.06, 5.09 (1H, d), 5.59, 5.60 (1H, s), 5.59, 5.68 (1H, d), 6.73, 6.78 (1H, s), 7.37 (1H, s), 7.61 (1H, s), 8.68, 8.69 (1H, s)
31	1760, 1660, 1600, 1515, 1500	732 $(M+H)^+$ as di Na salt	3.30, 3.38 (1H, d), 3.63, 3.68 (1H, d), 4.02, 4.06 (1H, d), 4.49, 4.53 (1H, d), 4.96, 5.01 (1H, d), 5.56 (1H, s), 5.56, 5.66 (1H, d), 6.70, 6.73 (1H, s), 7.27, 7.31 (1H, s), 7.36 (1H, m), 7.46 (1H, m), 7.53, 7.55 (1H, s), 7.76 (1H, d), 7.81 (1H, d)
40	1765, 1660, 1625, 1600, 1495	672 $(M+H)^+$	1.30 (4H, m), 3.36, 3.42 (1H, d), 4.38, 4.42 (1H, d), 5.05, 5.09 (1H, d), 5.57 (1H, s), 5.58, 5.68 (1H, d), 6.73, 6.77 (1H, s), 7.37, 7.38 (1H, s), 7.59, 7.60 (1H, s), 7.76 (2H, m), 8.52 (2H, d)

fractions were collected, concentrated and lyophilized to give **3f** as the sodium salt (185 mg, 54%): IR (KBr) cm^{-1} 1760, 1660, 1605, 1515; ^1H NMR (D_2O) δ 2.08 and 2.09 (total 3H, each s), 3.31 and 3.37 (1H, d), 3.61 and 3.64 (1H, d), 4.76~4.90 (2H), 5.10 and 5.14 (1H, d), 5.54 and 5.57 (1H, s), 5.63 and 5.74 (1H, d), 6.75~6.85 (2H), 7.23 (1H, d); SI-MS m/z 603 $(M+H)^+$.

(6*R*,7*R*)-7-[(*RS*)-2-(2-Aminothiazol-4-yl)-2-(1,5-dihydroxy-4-pyridone-2-carboxamido)acetamido]-3-(acetoxymethyl)ceph-3-em-4-carboxylic Acid (**3g**)

(a): To a solution of 7.1 g of **15** and 4.4 ml of Et_3N in 100 ml of THF was added 5.33 g of PCl_5 at -10~-15°C, and the reaction mixture was stirred for 1 hour. This acid chloride solution was added to a solution of 20 mmol of **2** in a mixture of THF (100 ml) and H_2O (200 ml) at pH 8.0~8.5 adjusted with

Et_3N under ice-cooling. After stirring at 0°C for 1 hour maintaining the pH at 8.0~8.5 with Et_3N, the reaction mixture was concentrated and acidified to pH 2 with 1 N HCl. The precipitate was collected, washed with H_2O and dried to give crude (6*R*,7*R*)-7-[(*RS*)-2-(2-aminothiazol-4-yl)-2-[1-hydroxy-5-(*p*-methoxybenzyloxy)-4-pyridone-2-carboxamido]acetamido]-3-(acetoxymethyl)ceph-3-em-4-carboxylic acid (9.2 g).

(b): To a suspension of 9.2 g of the product obtained in (a) in anisole (22 ml) was added dropwise 97 ml of TFA at 0°C. The reaction mixture was stirred at room temperature for 30 minutes, and poured into 700 ml of isopropyl ether. The precipitate was collected by filtration, dried, then dissolved in satd aq $NaHCO_3$ and purified by Diaion HP-20 column chromatography with elution by H_2O and 5% aq Me_2CO to afford **3g** as the sodium salt (3.34 g, 27.7% from **2**): IR (KBr) cm^{-1} 1760, 1670, 1610, 1510; ^1H NMR (D_2O) δ 2.11 and 2.12 (3H, s), 3.48 and 3.55 (1H, ABq), 4.79 and 4.82 (1H, ABq), 5.11 and 5.15 (1H, d), 5.60 and 5.62 (1H, s), 5.65 and 5.75 (1H, d), 6.75 and 6.80 (1H, s), 7.48 (1H, s), 7.60 (1H, s); SI-MS *m/z* 603 $(M+H)^+$.

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

To a solution of 600 mg of **3g** in 10 ml of 50% aq CH_3CN was added NaI (1.5 g) and 1-methylcyclopentano[*b*]pyridine-4-thione (250 mg). The reaction mixture was stirred at 70°C for 3 hours, cooled to room temperature and poured into 50 ml of Me_2CO. The precipitate was collected by filtration, and purified by column chromatography on Diaion HP-20 with elution by 5~10% aq Me_2CO and on Sephadex LH-20 with elution by 50% aq MeOH. The appropriate fractions were collected, evaporated and lyophilized to provide **4g** (150 mg, 21.9%): IR (KBr) cm^{-1} 1765, 1660, 1615, 1515, 1500; ^1H NMR (D_2O) δ 2.28 (2H, m), 3.88 (2H, m), 3.21 (2H, m), 3.55 and 3.60 (1H, ABq), 4.04 (3H, s), 4.26 and 4.28 (1H, ABq), 5.09 and 5.13 (1H, d), 5.62 and 5.72 (1H, d), 5.63 (1H, s), 6.75 and 6.79 (1H, s), 7.26 and 7.31 (1H, s), 7.53 (1H, d), 7.54 and 7.57 (1H, s), 8.15 (1H, d); SI-MS *m/z* 686 $(M+H)^+$.

By a similar procedure as described for the preparation of **4g**, the desired compounds **4**~**7** and **18**~**44** were obtained from **3** by substitution of the acetoxy group at C-3 methylene with various kinds of nucleophiles. The IR, mass and ^1H NMR data of typical compounds are summarized in Table 9.

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.

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