A NEW ANTIPSEUDOMONAL CEPHALOSPORIN CP6162 AND ITS CONGENERS†

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The synthesis and biological activity of a series of 3-[2-(5-hydroxy-4-pyridon-2-yl)ethenyl]cephalosporin derivatives are described. They showed very potent activity against Gram-negative bacteria, especially *Pseudomonas aeruginosa*. (6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methyl)-ethoxyiminoacetamido]-3-[(*Z*)-2-(1,5-dihydroxy-4-pyridon-2-yl)ethenyl]ceph-3-em-4-carboxylic acid, CP6162 (**8e**), was selected for further evaluation as an antipseudomonal chemotherapeutic agent.

The opportunistic infections caused by various Gram-negative bacteria including *Pseudomonas aeruginosa*, have progressively increased and become a serious problem in chemotherapy. Previously, we reported a novel cephalosporin derivative with a 1,5-dihydroxy-4-pyridone-2-carbonyl group, MT0703*S*, which has an excellent antibacterial activity, especially strong antipseudomonal activity[1,2]. In recent years, it was reported that aminothiazolyloxyiminocephalosporins having the dihydroxy aromatic moiety like a catechol at C-3 position exhibited potent activity against *P. aeruginosa*[3,4]. We presumed that the 1,5-dihydroxy-4-pyridone moiety might act as a catechol isoster with respect to some biological properties. In continuation of synthetic studies on the cephalosporin derivatives with the pyridone moiety at C-3 or C-7 position, we found that aminothiazolyloxyiminocephalosporins possessing 2-(5-hydroxy-4-pyridon-2-yl)ethenyl groups as the C-3 side chain showed strong activity against Gram-negative bacteria including *P. aeruginosa*. Some aminothiazolyloxyiminocephalosporins having the ethenyl side chain at C-3 were also reported, but their antipseudomonal activity was not found[5~7].

In this paper, we describe the synthesis and biological acivity of a series of 3-(5-hydroxy-4-pyridon-2-yl)ethenylcephalosporins leading to CP6162 (**8e**) and its related compounds. Detailed antibacterial evaluations and pharmacokinetics of CP6162 will be reported in a separate paper[8].

Chemistry

Preparation of various aminothiazolylacetamido derivatives having 2-(1,5-dihydroxy-4-pyridon-2-yl)ethenyl group at C-3 position is illustrated in Scheme 1. *p*-Methoxybenzyl (6*R*,7*R*)-7-phenylacetamido-3-(chloromethyl)ceph-3-em-4-carboxylate[9] (**1**) was treated with Ph_3P and NaI in acetone to give the phosphonium salt (**2**). Wittig reaction of **2** with 2-formyl-5-*p*-methoxybenzyloxy-4-diphenylmethoxypyridine *N*-oxide (**3**) was carried out in a heterogeneous system of CH_2Cl_2 - H_2O at room temperature in the

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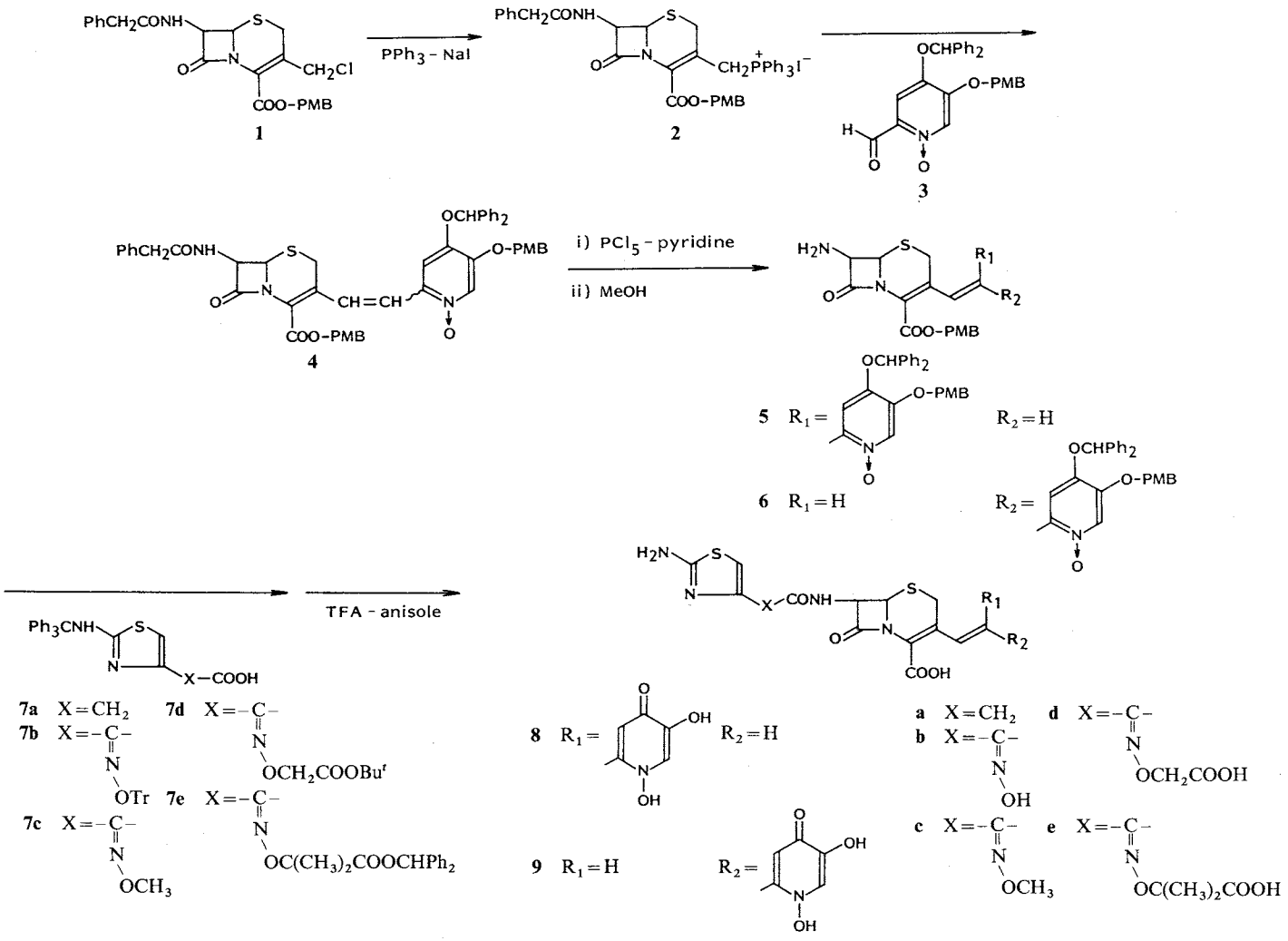
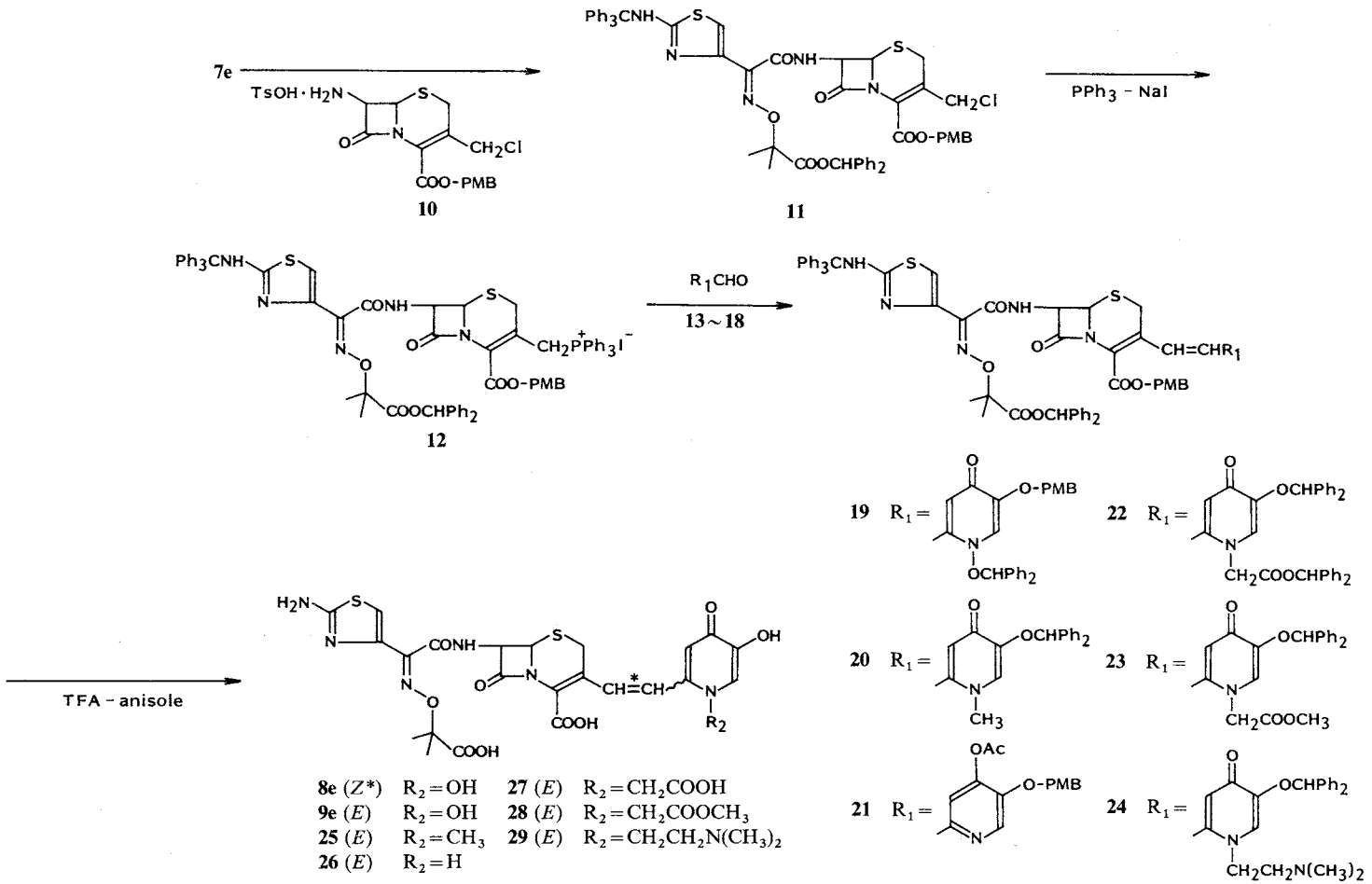
Scheme 1. Synthesis of 2-(1,5-dihydroxy-4-pyridon-2-yl)ethenyl compounds.



Scheme 2. Synthesis of 2-(1-substituted-5-hydroxy-4-pyridon-2-yl)ethenyl compounds.



presence of $NaHCO_3$ and followed by silica gel column chromatography to give the olefin derivative (**4**) in 78.0% yield from **1**. Compound **4** was a 2:1 mixture of *Z* and *E* isomers in regard to olefin group at C-3 position, judging from the ^1H NMR spectrum and TLC. Since the separation of these isomers at this stage was difficult, **4** was used for the next step without separation. The phenylacetyl side chain of **4** was cleaved by known imino-chloride method[10] and followed by silica gel column chromatography to afford amino esters **5** and **6** in 32.8% and 19.6% yield, respectively. The ^1H NMR spectrum of *Z* isomer (**5**) showed each doublet at δ 6.59 and 6.78 ($J=12$ Hz) assigned to olefin protons, whereas those of *E* isomer (**6**) were observed at lower field with larger coupling constant (δ 7.39 and 7.54, $J=16$ Hz). Various aminothiazolylacetic acids **7** were condensed with **5** or **6** using $POCl_3$ and followed by deprotection with TFA-anisole.

Table 1. Formation ratio of *Z* and *E* isomers on Wittig reaction.

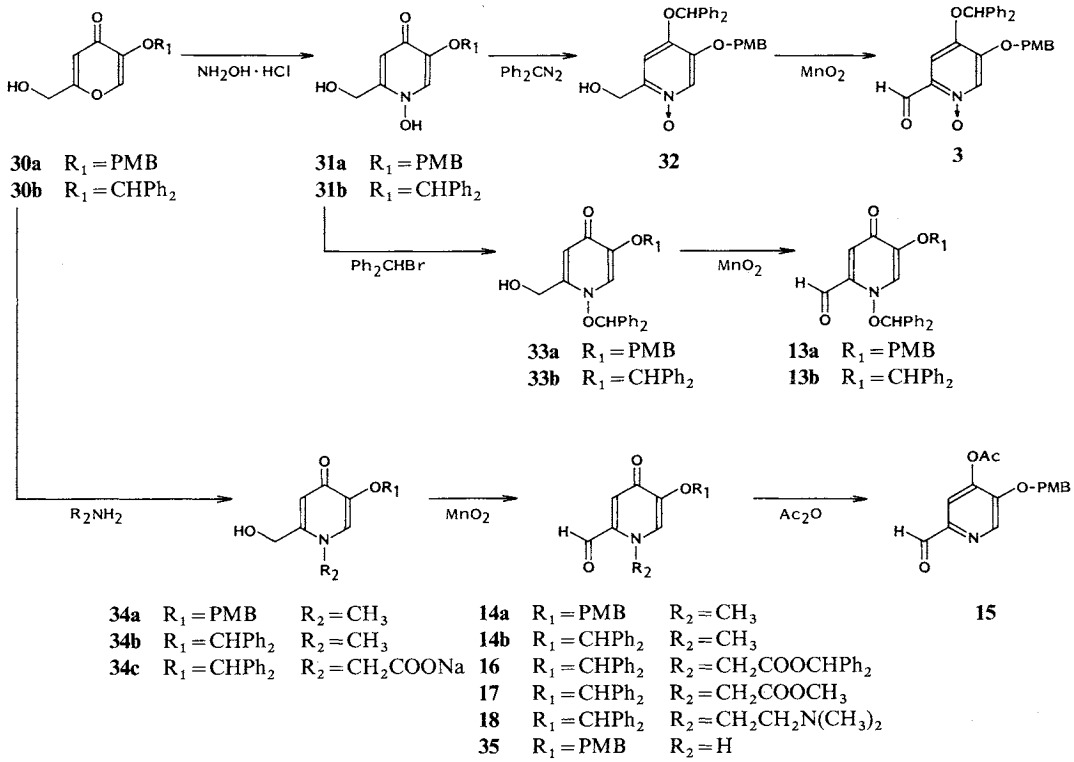
Run	Starting material		*Z*-*E* ratio of product
	Phosphonium salt	RCHO (eq)	
1	**2**	**3** (4.5)	2:1
2	**2**	**13a** (1.1)	5:1
3	**2**	**13b** (1.1)	4:1
4	**12**	**3** (4.5)	2:1
5	**12**	**13a** (1.1)	5:2
6	**12**	**13b** (1.1)	2:1
7	**12**	**14a** (1.1)	1:3
8	**12**	**14b** (1.1)	1:4

3-[2-(1-Substituted-5-hydroxy-4-pyridon-2-yl)ethenyl]cephalosporins having (*Z*)-2-(2-aminothiazol-4-yl)-2-(1-carboxy-1-methyl)ethoxyiminoacetamido group as the C-7 side chain were prepared as shown in Scheme 2. Compound **7e** which was prepared from allyl (*Z*)-2-(2-tritylaminothiazol-4-yl)-2-hydroxyiminoacetate[11], was condensed with TsOH salt of *p*-methoxybenzyl (6*R*,7*R*)-7-amino-3-(chloromethyl)ceph-3-em-4-carboxylate[12] (**10**) using $POCl_3$ to afford **11**. Compound **11** was converted to the phosphonium salt **12** and reacted with respective aldehydes (**13**~**18**) to give olefin derivatives (**19**~**24**) in a similar manner as described above. As **19** was a 5:2 mixture of *Z* and *E* isomers, after treating with TFA-anisole, *Z* (**8e**) and *E* isomers (**9e**) were separated by Diaion HP-20 column chromatography. Contrary to **19**, compounds **20**~**24** were predominant in the *E* isomer. Removal of protective groups of **20**~**24** with TFA-anisole afforded pure *E* isomers, **25**~**29**. Through Wittig reactions described above, the ratio of *Z* and *E* isomers was found to be influenced by 7-acylamido side chain and by nature of aldehydes. The results are shown in Table 1. The *Z*-*E* ratio was determined by the integration of peaks of ^1H NMR spectrum. Reaction of **2** with **13a** or **13b** afforded *Z* isomer predominantly but the reaction of the same aldehydes with **12** gave a non-specific result. Interestingly, the reaction of **12** with **14a** or **14b** gave mainly *E* isomer without the influence of the OH protecting group at 5-position of pyridone.

Aldehydes **3** and **13**~**18** were prepared from protected pyrone **30** as shown in Scheme 3. Compounds **3**, **13a** and **13b** were easily obtained by the oxidation[13] of corresponding alcohols **32** and **33**[2] with manganese dioxide. Similarly, *N*-substituted derivatives (**14a**, **14b**, **16**, **17** and **18**) were prepared from **30** *via* **34** by the reaction of pyrone with amine to give pyridone[14].

A practical synthesis of **8e** (CP6162) was performed from **2** as shown in Scheme 4. After the Wittig reaction of **2** with **13a**, *Z* isomer **36** was isolated by crystallization. Cleavage of 7-acyl side chain of **36** by imino-chloride method afforded **37**. Condensation of **37** with **7e** by $POCl_3$-pyridine or dicyclohexylcarbodiimide method was unsuccessful, even with silylated **37** prepared by *bis*(trimethylsilyl)acetamide. Finally, the desired compound **38** could be obtained by Schotten-Baumann acylation of **37** in aq THF. Compound **8e** was obtained in a 15.0% over all yield from **1**.

Scheme 3. Synthesis of pyridone aldehydes.



Scheme 4. Practical synthesis of CP6162.

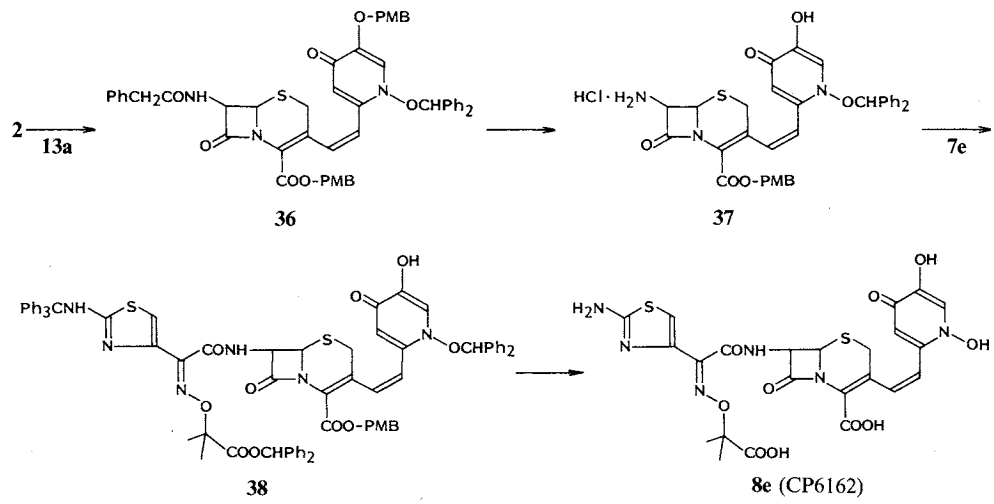


Table 2. Antibacterial activity of compounds **8** and **9**.

Test organism	MIC (μg/ml)						
	8a	**8b**	**8c**	**9c**	**8d**	**8e**	**9e**
Staphylococcus aureus FDA 209P JC-1	25	25	50	25	>100	>100	>100
Bacillus subtilis ATCC 6633	1.56	6.25	6.25	6.25	100	50	100
Escherichia coli No. 29	0.20	0.05	0.05	<0.025	0.20	0.10	0.10
Klebsiella pneumoniae GN69[a]	100	0.78	0.05	<0.025	0.05	0.05	<0.025
Proteus vulgaris GN76[b]	>100	0.39	0.05	<0.025	0.10	<0.025	<0.025
Salmonella typhi 0-901-W	0.20	0.05	<0.025	<0.025	0.05	0.05	<0.025
Citrobacter freundii GN346[b]	>100	1.56	6.25	0.10	25	6.25	3.13
Enterobacter cloacae G-0008	3.13	0.78	1.56	0.20	6.25	3.13	3.13
Serratia marcescens No. 1	1.56	0.20	0.20	<0.025	0.20	0.10	0.05
Pseudomonas aeruginosa GN10362[b]	>100	12.5	1.56	0.39	0.39	0.20	0.10
P. aeruginosa E-2	>100	1.56	0.20	0.10	0.05	<0.025	<0.025

[a] Penicillinase-producing strain.
[b] Cephalosporinase-producing strain.

Biological Activity

The *in vitro* antibacterial activity of several aminothiazolylacetamidocephalosporin derivatives having 2-(1,5-dihydroxy-4-pyridon-2-yl)ethenyl group at C-3 position are shown in Table 2. All of them, except **8a**, exhibited potent activity against Gram-negative bacteria, but were virtually devoid of activity against Gram-positive bacteria. It is known that introduction of a carboxyl group to the oxyimino moiety enhances the antipseudomonal activity[11,15~17]. Compounds **8e** and **9e** having (1-carboxy-1-methyl)ethoxyimino moiety also showed the most strong activity against *P. aeruginosa*. Effect of stereochemistry of **8e** (*Z* isomer) and **9e** (*E* isomer) on the antibacterial activity was not significant, but **9c** (*E* isomer) with methoxyimino moiety showed stronger activity than **8c** (*Z* isomer) against most of Gram-negative bacteria.

MICs of 3-[2-(1-substituted-5-hydroxy-4-pyridon-2-yl)ethenyl] derivatives against β-lactamase producing bacteria determined at two inoculum sizes[18], 10^6 and 10^8 cfu/ml, are shown in Table 3. Compound **9c** having a methoxyimino group was devoid of antibacterial activity at the high inoculum against almost all the organisms tested. But, **8d**, **8e** and **9e** showed potent activity against a wide variety of β-lactamase producing bacteria. Activity of **8e** at 10^8, as well as 10^6, was equivalent to **9e** and was superior to **8d**. Activities of **25**, **27** and **28** were approximately equal to that of **8e** at 10^6, but **26** and **29** were somewhat inferior to them. In comparison of activity of **8e** and **25**~**29** against *P. aeruginosa* at the high inoculum, **25**, **26** and **29** were less active than **8e** and others.

Three candidates **8e**, **27** and **28** were selected and further evaluated. The urinary recovery of **8e** in mice was higher than those of **27** and **28**, as shown in Table 4. Compounds **8e** and **27** did not show any toxicity after a single iv administration of 2.0 g/kg in mice, but **28** was more toxic (Table 4). The *in vivo* activities against systemic infections with *P. aeruginosa* in mice are shown in Table 5. Compound **8e** showed superior *in vivo* activity compared to other compounds tested. From the above mentioned results, CP6162 (**8e**) was chosen as an antipseudomonal candidate for further evaluations.

Experimental

NMR spectra were recorded at 400 MHz on a Jeol GX-400 NMR spectrometer and at 90 MHz on a Hitachi 90H NMR spectrometer using TMS as an internal standard. IR spectra were recorded on a

Table 3. Antibacterial activity of compounds **8d**, **8e**, **9c**, **9e** and **25**~**29** against β-lactamase-producing bacteria and *Pseudomonas aeruginosa*.

Test organism	MIC (μg/ml)									
	9c		**8d**		**8e**		**9e**		**25**	
	10^{6a}	10^8	10^6	10^8	10^6	10^8	10^6	10^8	10^6	10^8
Staphylococcus aureus 606[b]	50	50	>100	>100	>100	>100	100	>100	100	100
Escherichia coli 255[c]	0.05	>50	0.05	1.56	0.05	12.5	<0.025	3.13	0.05	25
Klebsiella pneumoniae GN69[b]	0.025	0.39	0.05	0.10	0.05	0.39	<0.025	0.78	0.05	0.39
Proteus vulgaris GN76[c]	0.025	>50	0.10	0.20	<0.025	0.20	<0.025	0.20	<0.025	0.10
Morganella morganii 1510[c]	0.10	>50	0.39	12.5	0.10	12.5	0.10	12.5	0.20	6.25
Citrobacteri freundii GN346[c]	0.10	>50	25	25	6.25	25	3.13	50	3.13	>100
Enterobacter cloacae GN7471[c]	0.05	>50	1.56	12.5	0.39	12.5	3.13	12.5	0.20	6.25
Serratia marcescens GN629[c]	0.05	>50	0.20	3.13	0.10	6.25	0.10	3.13	0.10	0.39
P. aeruginosa GN10362[c]	0.39	>50	0.39	3.13	0.20	0.78	0.10	0.78	0.20	0.78
P. aeruginosa M-0148[b]	0.39	>50	0.39	1.56	0.05	1.56	0.05	1.56	0.20	25
P. aeruginosa M1 Rms139[b]	0.05	3.13	0.05	0.39	0.05	0.05	<0.025	0.20	0.05	0.10
P. aeruginosa E-2	0.10	>50	0.05	0.39	<0.025	0.10	<0.025	0.05	0.05	0.20

Test organism	MIC (μg/ml)									
	26		**27**		**28**		**29**		CAZ	
	10^{6a}	10^8	10^6	10^8	10^6	10^8	10^6	10^8	10^6	10^8
S. aureus 606[b]	50	50	>100	>100	50	>100	100	>100	6.25	12.5
E. coli 255[c]	0.20	50	0.10	12.5	0.10	6.25	0.05	6.25	25	50
K. pneumoniae GN69[b]	0.05	0.39	0.10	1.56	0.10	0.20	0.10	0.39	0.10	0.39
P. vulgaris GN76[c]	<0.025	0.10	0.05	0.20	0.025	0.39	0.05	0.20	0.05	0.05
M. morganii 1510[c]	0.78	100	0.05	0.39	0.20	3.13	0.20	3.13	0.05	6.25
C. freundii GN346[c]	12.5	>100	0.39	>100	6.25	100	6.25	>100	25	100
E. cloacae GN7471[c]	0.78	12.5	6.25	12.5	0.39	3.13	0.78	6.25	3.13	12.5
S. marcescens GN629[c]	0.10	0.78	0.20	1.56	0.20	1.56	0.20	1.56	0.20	0.39
P. aeruginosa GN10362[c]	0.39	>100	0.20	0.78	0.10	0.78	0.39	1.56	1.56	1.56
P. aeruginosa M-0148[b]	0.20	>100	<0.025	0.78	0.10	0.39	0.39	1.56	1.56	3.13
P. aeruginosa M1 Rms139[b]	0.10	0.39	<0.025	0.05	0.05	0.20	0.20	0.39	0.78	1.56
P. aeruginosa E-2	0.05	0.20	<0.025	0.05	0.05	0.05	0.10	0.39	0.39	0.78

[a] Inoculum size (cfu/ml).
[b] Penicillinase-producing strain.
[c] Cephalosporinase-producing strain.

Table 4. Urinary recovery and acute toxicity in mice.

Compound	8e	27	28
Urinary recovery (%) ($n=3$, 25 mg/kg, sc)	54.3	26.5	15.2
Acute toxicity, survivals ($n=3$, 2 g/kg, iv)	3	3	2

Table 5. Therapeutic efficacy against systemic infections in mice.

Test organism	Challenge dose[a] (cfu/mouse)	Compound[b]	ED_{50} (mg/kg)	MIC (μg/ml)
Pseudomonas aeruginosa E-2	1.2×10^5	**8e**	7.5	<0.025
		27	15.0	<0.025
P. aeruginosa GN10362	1.0×10^5	**8e**	18.5	0.20
		27	15.0	0.20
		28	86.5	0.10

[a] Treated intraperitoneally twice, 1 and 3 hours after bacterial challenge.
[b] Subcutaneously.

Jasco IR-1 spectrometer. MS were taken on a Hitachi M-80B mass spectrometer. MP's were measured using a Mitamura micro melting point apparatus and are uncorrected.

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 at two inoculum sizes of 10^6 and 10^8 cfu/ml.

The *in vivo* antibacterial activity was tested using male mice (Jcl: ICR, 4 weeks old). Each of eight mice in a group was challenged intraperitoneally with about 10^5 cfu of the bacterial suspension in 0.5 ml of saline containing 2.5% gastric mucin (Difco Laboratories). The animals were treated subcutaneously with test compounds 1 and 3 hours after challenge. ED_{50} values (mg/kg) were calculated by probit analysis from the number of survivals 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 drug concentrations in urine collected at 0 to 4 hours after administration. Concentrations were determined by bioassay using *Escherichia coli* K-12 HW8236 as a test organism.

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

2-Formyl-5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine *N*-Oxide (**3**)

2-Hydroxymethyl-5-(*p*-methoxybenzyloxy)-4-diphenylmethoxypyridine *N*-oxide[2] (**32**, 3.32 g) was dissolved in CH_3CN (200 ml) at 50°C, followed by addition of activated MnO_2 (17.0 g, Aldrich Chemical Company, Inc.) and stirred for 1.5 hours at the same temperature. The insoluble material was filtered off, and the filtrate was evaporated. The residue was dissolved in EtOAc, washed with H_2O and dried over $MgSO_4$. The organic layer was concentrated and crystallized from CH_2Cl_2-Et_2O (1:2) to afford **3** (2.54 g, 76.9%): MP 160~162°C; IR (KBr) cm^{-1} 3400, 3050, 1685, 1605, 1510, 1420, 1240, 1170; ^1H NMR ($CDCl_3$) δ 3.83 (3H, s), 5.14 (2H, s), 6.31 (1H, s), 6.93 (2H, d), 7.22 (1H, s), 7.2~7.5 (12H, m), 7.87 (1H, s), 10.41 (1H, s); FD-MS *m/z* 441 (M^+).

Anal Calcd for $C_{27}H_{23}NO_5$: C 73.46, H 5.25, N 3.17.
Found: C 73.05, H 5.34, N 3.19.

5-(*p*-Methoxybenzyloxy)-1-diphenylmethoxy-4-pyridone-2-aldehyde (**13a**)

To a suspension of 2-hydroxymethyl-5-(*p*-methoxybenzyloxy)-1-diphenylmethoxy-4-pyridone[2] (**33a**, 6.0 g) in MeOH (300 ml) was added MnO_2 (15.0 g), and the mixture was refluxed for 30 minutes. After removal of the insoluble material by filtration, the solution was evaporated. The residue was dissolved in EtOAc, washed with H_2O, dried and concentrated. The crystals formed were collected and dried to afford **13a** (4.66 g, 78.0%): MP 135~136°C; IR (KBr) cm^{-1} 3400, 3050, 1740, 1700, 1570, 1240; ^1H NMR ($CDCl_3$)

δ 1.50 (1.5H, t, J=7 Hz), 2.04 (1.5H, s), 3.80 (3H, s), 4.12 (1H, q, J=7 Hz), 4.91 (2H, s), 5.96 (1H, s), 6.71 (1H, s), 6.89 (2H, d, J=9 Hz), 6.96 (1H, s), 7.23 (2H, d, J=9 Hz), 7.2~7.5 (10H, m), 9.65 (1H, s); FD-MS m/z 441 (M^+).

Anal Calcd for $C_{27}H_{23}NO_5 \cdot \frac{1}{2}$EtOAc: C 71.74, H 5.60, N 2.88.
Found: C 71.15, H 5.30, N 2.95.

1,5-Bis(diphenylmethoxy)-4-pyridone-2-aldehyde (**13b**)

The compound was prepared similarly as **13a** from **33b** in 75.6% yield: MP 91~93°C, IR (KBr) cm^{-1} 3000, 1700, 1590, 1570, 1280, 1100; ^{1}H NMR ($CDCl_3$) δ 5.81 (1H, s), 6.46 (1H, s), 6.65 (1H, s), 7.10 (1H, s), 7.1~7.5 (20H, m), 9.55 (1H, s); FD-MS m/z 487 (M^+).

Anal Calcd for $C_{32}H_{25}NO_4$: C 78.83, H 5.17, N 2.87.
Found: C 78.08, H 5.16, N 2.77.

1-Methyl-5-diphenylmethoxy-4-pyridone-2-aldehyde (**14b**)

To a solution of kojic acid (28.0 g) in MeOH (600 ml) was added diphenyldiazomethane (78.0 g) under ice-cooling. The mixture was stirred at room temperature for 36 hours and concentrated. The crystals formed were collected and dried to give 2-hydroxymethyl-5-diphenylmethoxy-4-pyrone (**30b**, 49.8 g, 80.8%): ^{1}H NMR (DMSO-d_6) δ 4.23 (2H, d, J=6 Hz), 5.64 (1H, t, J=6 Hz), 6.32 (1H, s), 6.48 (1H, s), 7.2~7.5 (10H, m), 8.03 (1H, s); FD-MS m/z 308 (M^+).

To a solution of **30b** (3.08 g) in MeOH (20 ml) was added 40% aq $MeNH_2$ (50 ml). The mixture was stirred for 4 hours at room temperature and concentrated to 15 ml. The crystals formed were collected, washed with H_2O and EtOAc and dried to give 2-hydroxymethyl-1-methyl-5-diphenylmethoxy-4-pyridone (**34b**, 2.04 g, 63.6%): ^{1}H NMR (DMSO-d_6) δ 3.46 (3H, s), 4.27 (2H, d, J=5 Hz), 5.44 (1H, t, J=5 Hz), 6.19 (1H, s), 6.72 (1H, s), 7.1~7.5 (10H, m); FD-MS m/z 321 (M^+).

To a solution of **34b** (1.73 g) in MeOH (350 ml) was added MnO_2 (10.4 g), and the mixture was stirred for 2 hours at room temperature. The insoluble material was removed by filtration. The filtrate was evaporated and the residue was dissolved in $CHCl_3$. This was washed with sat $NaHCO_3$, dried over $MgSO_4$ and concentrated. The crystals formed were collected and dried to give **14b** (1.42 g, 82.6%): MP 188~189°C; IR (KBr) cm^{-1} 3400, 3050, 1705, 1605, 1580, 1300, 1120; ^{1}H NMR ($CDCl_3$) δ 3.74 (3H, s), 6.82 (1H, s), 6.90 (1H, s), 6.94 (1H, s), 7.1~7.5 (10H, m), 9.54 (1H, s); FD-MS m/z 319 (M^+).

Anal Calcd for $C_{20}H_{17}NO_3$: C 75.21, H 5.36, N 4.39.
Found: C 74.97, H 5.42, N 4.24.

1-Methyl-5-(*p*-methoxybenzyloxy)-4-pyridone-2-aldehyde (**14a**)

The compound was prepared similarly as **14b** from **30a** through **34a** in 52.8% yield: MP 148~149°C; IR (KBr) cm^{-1} 3400, 1700, 1600, 1510, 1300, 1120; ^{1}H NMR ($CDCl_3$) δ 3.80 (3H, s), 3.86 (3H, s), 5.18 (2H, s), 6.87 (2H, d, J=9 Hz), 6.95 (1H, s), 6.97 (1H, s), 7.33 (2H, d, J=9 Hz), 9.61 (1H, s); EI-MS m/z 273 (M^+).

Anal Calcd for $C_{15}H_{15}NO_4$: C 65.92, H 5.53, N 5.12.
Found: C 65.53, H 5.41, N 5.04.

4-Acetoxy-5-(*p*-methoxybenzyloxy)pyridine-2-aldehyde (**15**)

5-*p*-Methoxybenzyloxy-4-pyridone-2-aldehyde (**35**, 1.30 g) which was obtained from **30a** using conc NH_3 by a similar procedure to **14b**, was dissolved in pyridine (30 ml) and added acetic anhydride (0.59 g) to the solution. The mixture was stirred for 2 hours at room temperature and evaporated. The residue was dissolved in CH_2Cl_2, washed with H_2O, 5% aq $KHSO_4$ and sat $NaHCO_3$, dried over $MgSO_4$ and concentrated to give **15** as colorless crystals (1.18 g, 78.4%): MP 137~138°C; IR (KBr) cm^{-1} 3400, 3050, 1765, 1700, 1570, 1490, 1250, 1180; ^{1}H NMR ($CDCl_3$) δ 2.30 (3H, s), 3.82 (3H, s), 5.22 (2H, s), 6.93 (2H, d, J=9 Hz), 7.33 (2H, d, J=9 Hz), 7.72 (1H, s), 8.50 (1H, s), 9.95 (1H, s); FD-MS m/z 301 (M^+).

Anal Calcd for $C_{16}H_{15}NO_5$: C 63.78, H 5.02, N 4.65.
Found: C 63.56, H 4.98, N 4.63.

Diphenylmethyl (2-Formyl-5-diphenylmethoxy-4-pyridon-1-yl)acetate (**16**)

Sodium salt of (2-hydroxymethyl-5-diphenylmethoxy-4-pyridon-1-yl)acetic acid (**34c**, 2.0 g) which was

obtained from **30b** using glycine in a similar manner as described above, was dissolved in MeOH (200 ml). To this solution was added MnO_2 (12.0 g) and the mixture was stirred for 2 hours at room temperature. After removal of the insoluble material by filtration, the filtrate was concentrated to 50 ml. The precipitate formed was filtered off and the filtrate was evaporated. The residue was suspended in CH_2Cl_2 (200 ml) and H_2O (50 ml). Diphenyldiazomethane (1.21 g) was added to this suspension and the mixture was adjusted to pH 3 with sat $KHSO_4$ under ice-cooling. After stirring for 2 hours at the same temperature, the separated organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography (benzene - EtOAc, 1 : 2) to afford **16** as colorless crystals (1.86 g, 68.1%): MP 171~174°C (dec); IR (KBr) cm^{-1} 3400, 1750, 1700, 1605, 1570, 1190; ^1H NMR ($CDCl_3$) δ 4.82 (1H, s), 6.68 (1H, s), 6.82 (2H, s), 6.86 (1H, s), 7.1~7.5 (20H, m), 9.33 (1H, s), FD-MS *m/z* 529 (M^+).

Anal Calcd for $C_{34}H_{27}NO_5$: C 77.11, H 5.14, N 2.64.
Found: C 76.84, H 5.16, N 2.53.

Methyl (2-Formyl-5-diphenylmethoxy-4-pyridon-1-yl)acetate (**17**)

To a suspension of **34c** (3.1 g) in CH_2Cl_2 (60 ml) and H_2O (60 ml) was added diphenyldiazomethane (3.02 g), and the mixture was adjusted to pH 3 with 3 N HCl and stirred at room temperature for 1 hour. The separated organic layer was dried and evaporated. The residue was oxidized with MnO_2 in MeOH by a similar procedure as described above to give **17** as colorless crystals (1.50 g, 49.8%): MP 173~175°C (dec); IR (KBr) cm^{-1} 3400, 3050, 1755, 1700, 1610, 1590, 1200; ^1H NMR ($CDCl_3$) δ 3.67 (3H, s), 4.75 (2H, s), 6.71 (1H, s), 6.84 (1H, s), 6.90 (1H, s), 7.1~7.5 (10H, m), 9.43 (1H, s); FD-MS *m/z* 377 (M^+).

Anal Calcd for $C_{22}H_{19}NO_5$: C 70.02, H 5.07, N 3.71.
Found: C 69.68, H 5.08, N 3.64.

1-(*N*,*N*-Dimethylaminoethyl)-4-diphenylmethoxy-4-pyridone-2-aldehyde (**18**)

The compound was obtained from **30b** by a similar procedure using *N*,*N*-dimethylethylenediamine, instead of $MeNH_2$ in **14b** in 40.4% yield: MP 123~125°C; IR (KBr) cm^{-1} 3400, 1690, 1600, 1580, 1110; ^1H NMR ($CDCl_3$) δ 2.05 (6H, s), 2.31 (2H, t, J=6 Hz), 4.14 (2H, t, J=6 Hz), 6.73 (1H, s), 6.85 (1H, s), 6.98 (1H, s), 7.1~7.5 (10H, m), 9.43 (1H, s); FD-MS *m/z* 376 (M^+).

Anal Calcd for $C_{23}H_{24}N_2O_3$: C 73.38, H 6.43, N 7.44.
Found: C 73.10, H 6.25, N 7.14.

Compounds **5** and **6**

To the solution of **1** (1.95 g) in acetone (50 ml) were added NaI (660 mg) and Ph_3P (1.15 g). The mixture was stirred at room temperature for 1 hour and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (30 ml), and to the solution **3** (7.94 g) and 5% aq $NaHCO_3$ (20 ml) were added. After stirring at room temperature for 3 hours, the organic layer was washed with brine, dried over $MgSO_4$ and concentrated to 50 ml. Ether (60 ml) was added to this solution and the crystals formed were filtered off. The filtrate was evaporated, and the residue was purified on silica gel column chromatography ($CHCl_3$ - MeOH, 100 : 1) to afford **4** (2.73 g, 78.0%).

To a solution of **4** (2.54 g) in CH_2Cl_2 (40 ml) were added pyridine (1.06 ml) and PCl_5 (1.21 g) at −20°C. After being stirred at 0~5°C for 1 hour, the reaction mixture was poured into MeOH (40 ml) at −20°C, and stirred at room temperature for 1 hour. The reaction mixture was partitioned between CH_2Cl_2 (120 ml) and brine (120 ml) under ice-cooling and stirred for 30 minutes. The separated organic layer was washed with brine and sat $NaHCO_3$, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography with $CHCl_3$ - MeOH (50 : 1) to give **5** as a foam (720 mg, 32.8%) and crystalline **6** (430 mg, 19.6%). **5**: IR (KBr) cm^{-1} 3400, 1770, 1720, 1610, 1510, 1240, 1170; ^1H NMR ($CDCl_3$) δ 1.65 (2H, br s), 3.24 (2H, ABq, J=18 Hz), 3.81 (3H, s), 3.83 (3H, s), 4.86 (1H, br s), 4.89 (1H, d, J=5 Hz), 5.08 (2H, s), 5.24 (2H, ABq, J=12 Hz), 6.24 (1H, s), 6.59 (1H, d, J=12 Hz), 6.74 (1H, s), 6.78 (1H, d, J=12 Hz), 6.87 (2H, d, J=9 Hz), 6.91 (2H, d, J=9 Hz), 7.15~7.5 (15H, m), 7.94 (1H, s); FD-MS *m/z* 757 (M^+).

Anal Calcd for $C_{43}H_{39}N_3O_8S$: C 68.15, H 5.19, N 5.54.
Found: C 67.95, H 5.12, N 4.98.

6: MP 145~149°C (dec); IR (KBr) cm^{-1} 3400, 1770, 1750, 1700, 1600, 1510, 1160; ^1H NMR ($CDCl_3$) δ 1.80 (2H, br s), 3.70 (2H, ABq, J=18 Hz), 3.77 (3H, s), 3.82 (3H, s), 4.75 (1H, br s), 4.96 (1H, d, J=5 Hz),

5.07 (2H, s), 5.31 (2H, ABq, $J=12$ Hz), 6.29 (1H, s), 6.91 (4H, m), 7.00 (1H, s), 7.2~7.5 (14H, m), 7.39 (1H, d, $J=16$ Hz), 7.54 (1H, d, $J=16$ Hz), 7.90 (1H, s); FD-MS m/z 757 (M^+).

Anal Calcd for $C_{43}H_{39}N_3O_8S$: C 68.15, H 5.19, N 5.54.
Found: C 67.62, H 5.09, N 5.26.

Compound **8c**

To a mixture of (*Z*)-2-(2-tritylaminothiazol-4-yl)-2-methoxyiminoacetic acid (**7c**, 222 mg) and **5** (379 mg) in CH_2Cl_2 (10 ml) were added pyridine (0.16 ml) and $POCl_3$ (51 μl) at -20°C. After stirring for 1 hour, the reaction mixture was diluted with EtOAc, washed with brine, dried over $MgSO_4$ and evaporated to give the crude protected product of **8c**: IR (KBr) cm^{-1} 3400, 1780, 1720, 1670, 1510, 1240, 1165; ^1H NMR ($CDCl_3$) δ 3.22 (2H, ABq, $J=18$ Hz), 3.80 (3H, s), 3.90 (3H, s), 4.90 (1H, d, $J=5$ Hz), 5.09 (2H, s), 5.24 (2H, ABq, $J=12$ Hz), 5.91 (1H, q, $J=5$ and 9 Hz), 6.16 (1H, s), 6.67 (1H, d, $J=12$ Hz), 6.73 (1H, s), 6.80 (1H, d, $J=12$ Hz), 6.89 (2H, d, $J=9$ Hz), 6.90 (1H, s), 6.91 (2H, d, $J=9$ Hz), 7.1~7.6 (30H, m), 8.03 (1H, s); SI-MS m/z 1,183 $(M+H)^+$.

The crude product was dissolved in anisole (1.09 ml) and added TFA (3.08 ml) under ice-cooling. The reaction mixture was stirred for 1 hour at the same temperature and poured into isopropyl ether (15 ml). The resulting precipitate was collected by filtration, dissolved in 5% aq $NaHCO_3$, adjusting to pH 7.5, and purified by Diaion HP-20 column chromatography. Appropriate fractions eluted with H_2O were collected, concentrated and lyophilized to give **8c** as the sodium salt (158 mg, 54.5%). See Table 6.

Compounds **8a**, **8b**, **8d**, **8e**, **9c** and **9e**

They were prepared by a similar procedure using **5** or **6** with corresponding acids (**7a**~**7e**). See Table 6.

Compound **25**

To a solution of **7e** (681 mg) in CH_2Cl_2 (10 ml) were added **10** (540 mg) and pyridine (0.4 ml) at -10°C. The mixture was stirred at the same temperature for 30 minutes and added $POCl_3$ (0.1 ml) at -20°C. After stirring for 1 hour, the reaction mixture was diluted with EtOAc, washed with brine, dried over $MgSO_4$ and evaporated to afford **11**. By a similar procedure as **4**, compound **11** was converted into phosphonium salt **12**, reacted with 1-methyl-5-diphenylmethoxy-4-pyridone-2-aldehyde (**14b**, 350 mg) and followed by silica gel column chromatography to afford **20** (740 mg, 56.9%): IR (KBr) cm^{-1} 3350, 1780, 1720, 1680, 1570, 1510, 1300, 1200, 1170; ^1H NMR ($CDCl_3$) δ 1.67 (3H, s), 1.70 (3H, s), 3.42 (3H, s), 3.44 (2H, s), 3.81 (3H, s), 4.95 (1H, d, $J=5$ Hz), 5.25 (2H, d, $J=12$ Hz), 5.95 (1H, q, $J=5$ and 9 Hz), 6.36 (1H, d, $J=16$ Hz), 6.58 (1H, s), 6.62 (1H, s), 6.76 (1H, s), 6.85 (1H, s), 6.90 (1H, s), 6.91 (2H, d, $J=9$ H), 7.1~7.5 (39H, m), 7.38 (1H, d, $J=16$ Hz); SI-MS m/z 1,299 $(M+H)^+$.

To a solution of **20** (650 mg) in anisole (1.09 ml) was added TFA (3.08 ml) and stirred for 1 hour under ice-cooling. The reaction mixture was poured into isopropyl ether, and the precipitate formed was filtered and dried. This was dissolved in 5% aq $NaHCO_3$, adjusted to pH 7.5 and chromatographed on Diaion HP-20. Appropriate fractions eluted with H_2O and followed by 10% aq MeOH were collected, concentrated and lyophilized to give **25** as the sodium salt (192 mg, 59.3%). See Table 6.

Compounds **26**~**29**

They were similarly prepared from **12** and corresponding aldehydes **15**~**18** instead of **14b** through **21**~**24**. See Table 6.

Compound **36**

To a mixture of **2** prepared from **1** (19.46 g) and **13a** (19.4 g) in CH_2Cl_2 (200 ml) was added 5% aq $NaHCO_3$ (200 ml). The mixture was stirred for 3 hours at room temperature, and organic layer was separated, washed brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from CH_2Cl_2 and EtOAc (2:3) to afford **36** (25.3 g, 65.7%): MP 116~118°C; IR (KBr) cm^{-1} 3400, 1780, 1720, 1680, 1605, 1560, 1510, 1240, 1170; ^1H NMR ($CDCl_3$) δ 1.26 (3H, t, $J=7$ Hz), 2.04 (3H, s), 3.03 (2H, ABq, $J=18$ Hz), 3.63 (2H, s), 3.78 (3H, s), 3.79 (3H, s), 4.12 (2H, q, $J=7$ Hz), 4.76 (2H, br s), 4.94 (1H, d, $J=5$ Hz), 5.12 (2H, ABq, $J=12$ Hz), 5.86 (1H, q, $J=5$ and 9 Hz), 5.89 (1H, s), 6.20

Table 6. IR, mass and ^{1}H NMR data.

Compound	IR ν_{max} (KBr) (cm^{-1})	SI-MS (m/z)	^{1}H NMR (D_2O) δ (ppm)					
			2-CH_2 (2H, ABq, J=18 Hz)	6-H (1H, d, J=5 Hz)	7-H (1H, d, J=5 Hz)	Thiazole-H (1H, s)	3-Olefin-H, H (each of 1H, d, J=12[a] or 16[b] Hz)	Pyridone-H, H (each of 1H, s)
8a	1750, 1660, 1600, 1520	536 $(M+H)^+$ as 2Na salt	3.26	5.20	5.67	6.89	6.51, 6.71[a]	6.59, 7.59
8b	1750, 1660, 1610, 1520	521 $(M+H)^+$	3.35	5.32	5.87	7.00	6.53, 6.71[a]	6.56, 7.57
8c	1760, 1660, 1600, 1530	579 $(M+H)^+$ as 2Na salt	3.35	5.31	5.85	7.05	6.53, 6.74[a]	6.61, 7.61
8d	1760, 1660, 1610, 1530	645 $(M+H)^+$ as 3Na salt	3.33	5.29	5.83	7.03	6.49, 6.69[a]	6.52, 7.51
8e	1760, 1650, 1580, 1530	673 $(M+H)^+$ as 3Na salt	3.34	5.32	5.86	7.03	6.53, 6.74[a]	6.64, 7.61
9c	1750, 1650, 1600, 1520	579 $(M+H)^+$ as 2Na salt	3.86	5.35	5.89	7.08	7.08, 7.28[b]	6.94, 7.57
9e	1755, 1650, 1580, 1530	673 $(M+H)^+$ as 3Na salt	3.84	5.33	5.88	7.03	7.06, 7.28[b]	6.92, 7.55
25	1760, 1660, 1600, 1550	605 $(M+H)^+$	3.86	5.39	5.91	7.07	6.75, 7.29[b]	6.86, 7.66
26	1760, 1660, 1600, 1560	635 $(M+H)^+$ as 2Na salt	3.78	5.32	5.88	7.03	6.58, 7.33[b]	6.73, 7.60
27	1760, 1660, 1620, 1540	715 $(M+H)^+$ as 3Na salt	3.75	5.31	5.87	7.01	6.46, 7.25[b]	6.81, 7.61
28	1750, 1660, 1610, 1540	707 $(M+H)^+$ as 2Na salt	3.72	5.31	5.87	7.02	6.51, 7.20[b]	6.76, 7.59
29	1760, 1650, 1600, 1530	706 $(M+H)^+$ as 2Na salt	3.79	5.32	5.84	7.04	6.65, 7.23[b]	6.65, 7.55

(1H, s), 6.25 (1H, d, $J=12$ Hz), 6.69 (1H, d, $J=12$ Hz), 6.80 (1H, s), 6.86 (4H, d, $J=8$ Hz), 7.2~7.7 (19H, m); FD-MS m/z 876 $(M+H)^+$.

Anal Calcd for $C_{51}H_{45}N_3O_9S \cdot EtOAc$: C 68.52, H 5.54, N 4.36.
Found: C 67.82, H 5.27, N 4.39.

Compound **37**

To a solution of **36** (25.3 g) in CH_2Cl_2 (450 ml) were added pyridine (10.52 ml) and PCl_5 (12.02 g) at $-20°C$, and the mixture was stirred at 0~5°C for 1 hour. The reaction mixture was poured into MeOH (375 ml) at $-20°C$ and stirred at room temperature for 1 hour. The reaction mixture was partitioned between CH_2Cl_2 (1.2 liters) and brine (800 ml) under ice-cooling and stirred for 1 hour. The separated organic layer was washed with brine, dried over $MgSO_4$ and concentrated to 200 ml. To the concentrate EtOAc (200 ml) was added. The precipitate formed was collected by filtration and dried to give **37** (15.6 g, 88.1%) as the hydrochloride: IR (KBr) cm^{-1} 3400, 2950, 1780, 1720, 1610, 1510, 1240, 1170; ^1H NMR (DMSO-d_6) δ 3.16 (2H, ABq, $J=17$ Hz), 3.75 (3H, s), 5.10 (1H, d, $J=5$ Hz), 5.10 (2H, br s), 5.20 (1H, d, $J=5$ Hz), 5.96 (1H, s), 6.46 (1H, d, $J=12$ Hz), 6.52 (1H, s), 6.71 (1H, d, $J=12$ Hz), 6.92 (2H, d, $J=9$ Hz), 7.31 (2H, d, $J=9$ Hz), 7.3~7.6 (10H, m), 7.75 (1H, s); SI-MS m/z 638 $(M+H)^+$.

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methyl)ethoxyiminoacetamido]-3-[(*Z*)-2-(1,5-dihydroxy-4-pyridon-2-yl)ethenyl]ceph-3-em-4-carboxylic Acid (**8e**, CP6162)

To a mixture of DMF (1.74 ml) and CH_2Cl_2 (16.2 ml) was added $POCl_3$ (2.06 ml) at 0°C, and the mixture was stirred at the same temperature for 1 hour to prepare Vilsmeier reagent[19]. To a solution of **7e** (13.6 g) in THF (200 ml) was added the reagent at $-20°C$ and the mixture was stirred for 1 hour to prepare an acid chloride solution. The hydrochloride of **37** (13.47 g) was dissolved in a mixture of THF (200 ml) and H_2O (40 ml) and added dropwise the acid chloride solution at $-10°C$ maintaining the pH at 6.5~7.0 with TEA. After stirring for 1 hour at this temperature, the reaction mixture was acidified to pH 2 with 6 N HCl and extracted with EtOAc. The separated organic layer was washed with brine, dried and concentrated under reduced pressure. The residue was dissolved in benzene (200 ml) and added isopropyl ether (200 ml). The precipitate formed was collected by filtration and dried to give **38** (23.5 g): IR (KBr) cm^{-1} 3350, 1780, 1720, 1680, 1510, 1240, 1170; ^1H NMR ($CDCl_3$) δ 1.67 (3H, s), 1.71 (3H, s), 3.01 (2H, ABq, $J=18$ Hz), 3.80 (3H, s), 5.00 (1H, d, $J=5$ Hz), 5.12 (2H, ABq, $J=12$ Hz), 5.94 (1H, q, $J=5$ and 9 Hz), 6.06 (1H, s), 6.20 (1H, s), 6.22 (1H, d, $J=12$ Hz), 6.62 (1H, s), 6.80 (1H, d, $J=12$ Hz), 6.87 (2H, d, $J=9$ Hz), 6.89 (1H, s), 7.14 (1H, s), 7.1~7.5 (37H, m); SI-MS m/z 1,301 $(M+H)^+$.

To suspension of **38** (21.2 g) and anisole (35.1 ml) was added dropwise TFA (99.5 ml) under ice-cooling. The mixture was stirred for 1 hour at the same temperature and poured into isopropyl ether (400 ml). The precipitate was collected by filtration, dried, then dissolved in 5% aq $NaHCO_3$ and purified by Diaion HP-20 column chromatography to afford **8e** as the sodium salt (3.15 g, 26.0% from **37**).

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