AMINOTHIAZOLYLGLYCYL DERIVATIVES OF CARBACEPHEM ANTIBIOTICS

II. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NOVEL AMINOTHIAZOLYL CEPHEM COMPOUNDS WITH HYDROXYPYRIDONE MOIETY

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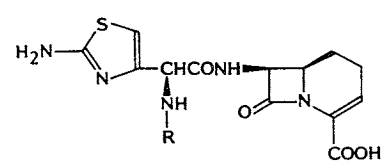
The synthesis and antimicrobial activity of novel carbacephem antibiotics which have amido moiety of (*S*)-aminothiazolylglycyl side chain are described. Among them, the compound having 5-hydroxy-4-pyridon-2-carboxyl group (KT-4697) showed exceptionally strong activity against *Pseudomonas aeruginosa* as well as Gram-negative bacteria. A cephalosporin with this acyl group namely KT-4788 with methylpyridiniumthiomethyl group at C-3 was found to be the most active against Gram-positive and Gram-negative strains including *P. aeruginosa*.

In the previous paper[1], we reported the antibacterial activity of 7-[2-(2-aminothiazol-4-yl)-2-acylamidoacetyl]carbacephem antibiotics having hydroxyl substituents on benzoyl moiety. Among these compounds, 3,4-dihydroxy benzoyl derivative bearing (*S*)-configuration of aminothiazolylglycyl moiety (**1**, KT-4380) exhibited the highest activity against *Pseudomonas aeruginosa* as well as Gram-negative strains. *In vivo*, however, KT-4380 showed weaker activity than expected from *in vitro* anti-pseudomonal activity. This was explained by the fact that catechol moiety was methylated *in vivo* by catechol *O*-methyltransferase (COMT) and loose its anti-pseudomonal activity. Therefore we continued the screening of new anti-pseudomonal acyl group other than that has catechol moiety. Recently synthesis and antimicrobial activity of ureido-penicillins bearing catechol moiety was reported[2,3]. In this paper, we wish to describe the synthesis and the antimicrobial activity of compounds (**3**) which have an acyl moiety attached to the aminothiazolylglycyl side chain.

Fig. 1.



KT-4380 (**1**) R = —CO— (OH, OH)

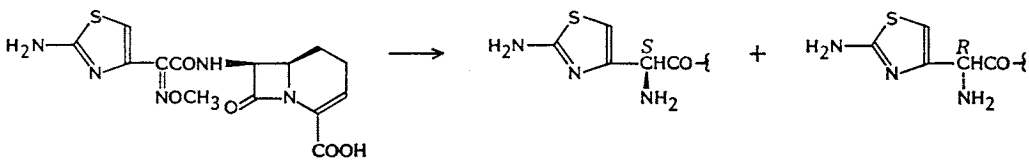
2 R = H

3 R = COAr

Chemistry

The stereo-selective reduction of methoxyimino derivative (**4**, KT-3767) was studied first, since as previously reported that (*S*)-configuration compound is more active against Gram-negative strains than (*R*)-isomer, and we designed to use (*S*)-isomer of aminothiazolylglycyl carbacephem compound **2** as a

Table 1. Stereo-selective reduction of KT-3767 (**4**).

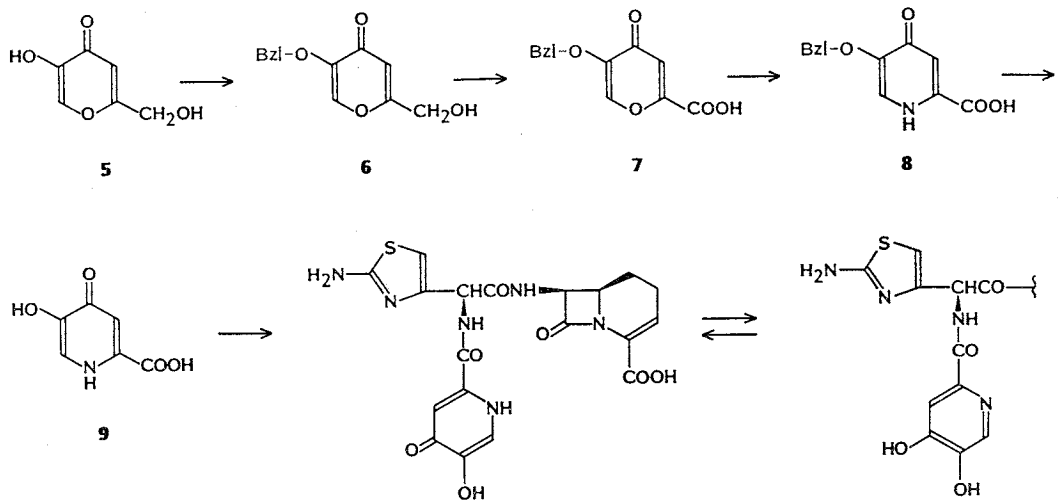


4

Temp (°C)	Time (minutes)	S/R[a]	Yield of (*S*)-isomer (%)[b]
25	30	62/38	40
10	45	65/35	50
0	90	68/32	58
−5	240	73/27	64

[a] Monitored by HPLC.
[b] After purification by column chromatography on Diaion HP-10.

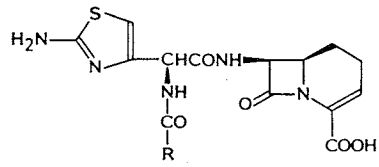
Scheme 1.

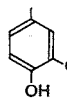
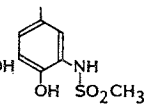
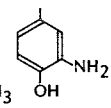
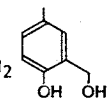
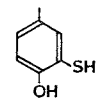
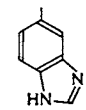
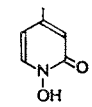


Bzl=Benzyl.

starting material in this study. We found that reduction of KT-3767 by zinc in acetic acid increased the formation of (*S*)-isomer with decreasing of temperature as shown in Table 1. In addition, (*S*)-isomer was obtained at high yield taking advantage of the fact that only (*S*)-amino diastereomer forms crystals by chelating with zinc. A bioisostere[4,5] of catechol (a compound which is equivalent to catechol biologically) was selected as the acyl group linked to this (*S*)-isomer. Other substituted heterocyclic compounds were selected as acyl groups. The compounds were condensed with **2** using several procedures acid chloride, mixed anhydride, DCC or active ester method, followed by cleavage of protecting group if necessary to give desired products **3**. 5-Hydroxy-4-pyridon-2-carboxylic acid (**9**),[6] a typical heterocyclic acyl group, was prepared through the route shown in Scheme 1. Kojic acid **5** was selected as a starting material, and its enolic hydroxy group was protected by benzylation to afford **6**. Compound **6** was converted into carboxylic acid **7** by Jones oxidation, and treated with concentrate NH_4OH

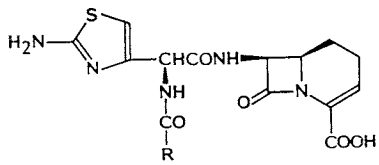
Table 2. Antimicrobial activity of catechol bioisostere (MIC, μg/ml).

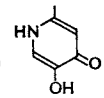
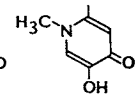


R:	KT-4380	3a	3b	3c	3d	3e	3f
							
Staphylococcus aureus 209-P	12.5	6.25	6.25	6.25	12.5	6.25	25
S. epidermidis	12.5	12.5	12.5	12.5	50	6.25	6.25
Escherichia coli Juhl	0.05	0.1	0.05	0.05	0.78	0.2	0.05
Klebsiella pneumoniae 8045	0.02	0.1	0.05	0.05	0.2	0.2	0.02
Serratia marcescens T-26	0.78	3.13	12.5	6.25	12.5	25	12.5
Proteus mirabilis 1287	0.1	0.2	0.1	0.2	0.1	0.78	0.01
Enterobacter cloacae F 1510	0.01	0.01	0.2	0.2	0.78	0.78	0.39
Citrobacter freundii F 1526	0.1	0.2	0.2	0.2	1.56	0.39	0.2
Pseudomonas aeruginosa #1	0.78	50	12.5	25	12.5	50	12.5
P. aeruginosa 145	0.39	100	12.5	50	12.5	100	12.5

Mueller-Hinton agar dilution method, 10^6 cfu/ml.

Table 3. Antimicrobial activity of heterocyclic compounds (MIC, μg/ml).



R:	KT-4380	3g	3h	3i	3j	3k	3l
							
Staphylococcus aureus 209-P	12.5	50	12.5	25	25	25	25
S. epidermidis	12.5	25	12.5	25	12.5	25	12.5
Escherichia coli Juhl	0.05	0.2	0.05	0.1	0.1	0.05	0.1
Klebsiella pneumoniae 8045	0.02	0.2	0.02	0.05	0.01	0.02	0.1
Serratia marcescens T-26	0.78	12.5	25	6.25	0.78	0.78	3.13
Proteus mirabilis 1287	0.1	0.39	0.2	0.1	0.1	0.1	0.1
Enterobacter cloacae F 1510	0.01	1.56	0.39	0.39	0.02	0.05	0.1
Citrobacter freundii F 1526	0.1	0.78	0.2	0.2	0.1	0.1	0.39
Pseudomonas aeruginosa #1	0.78	25	50	50	0.39	3.13	25
P. aeruginosa 145	0.39	50	100	100	0.2	6.25	25

to form pyridone **8**. The deprotection of **8** with concentrate HCl gave **9** in over all yield of 45% from **5**.

Antibacterial Activity

The minimum inhibitory concentration (MIC) values of the amido-carbacephem antibiotics (**3**)

Table 4. Enzymatic reaction with COMT. Ratio of *O*-methylated product.

	30 minutes	60 minutes
KT-4380	20%	28%
KT-4697	Trace	2%

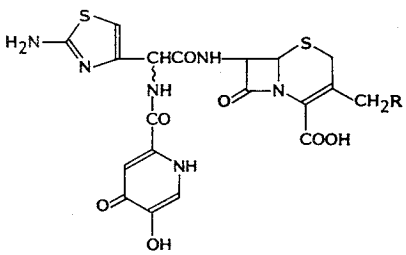
Monitored by HPLC. Conditions, see Experimental section.

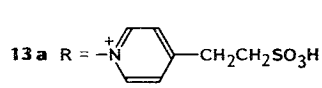
against Gram-positive and Gram-negative strains are listed in Tables 2 and 3. No compound which was a bioisostere of catechol showed anti-pseudomonal activity. This is in contrast with the fact that some strong β-adrenostimulants have catechol bioisosteres such as those of compounds **3a**, **3e** and **3f**[7,8]. Only **3j** (KT-4697) having 5-hydroxy-4-pyridon-2-carbonyl group, which is a more distant bioisostere[9] of catechol, showed strong anti-pseudomonal activity. It is worthwhile to note that **3k** which is a regioisomer of **3j**, demonstrated only moderate activity against *Pseudomonas* sp. These acyl group have hydroxypyridone-pyridine diol tautomerism as shown in Scheme 1. We conducted various studies to determine which tautomer was involved in KT-4697, but spectrometrically (UV, IR and NMR) could not come to a conclusion. As shown in Table 4, however, KT-4697 unlike KT-4380 was only slightly methylated by the enzymatic reaction of catechol *O*-methyltransferase *in vitro*. So it is expected to demonstrate strong activity against *Pseudomonas* sp. *in vivo*. As shown in Table 3, KT-4697 had strong and broad activity against Gram-negative bacteria including *P. aeruginosa*, while it showed week activity against Gram-positive bacteria. In order to enhance the activity against Gram-positive bacteria, especially against *Staphylococcus aureus*, we prepared cephalosporin analog which had same 7-acyl group with KT-4697 and various substituents at 10-position.

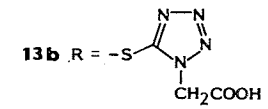
Preparation and Antibacterial Activity of Cephalosporin Analogs

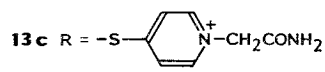
The compounds listed in Table 5 were prepared by method outlined in Scheme 2. Reduction of methoxyimino group of cefotaxime (**10**) gave aminothiazolylglycyl derivative (**11**) as 1 : 1 mixture of

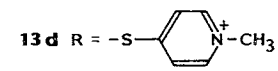
Table 5. Antimicrobial activity of cephalosporins (MIC, μg/ml).





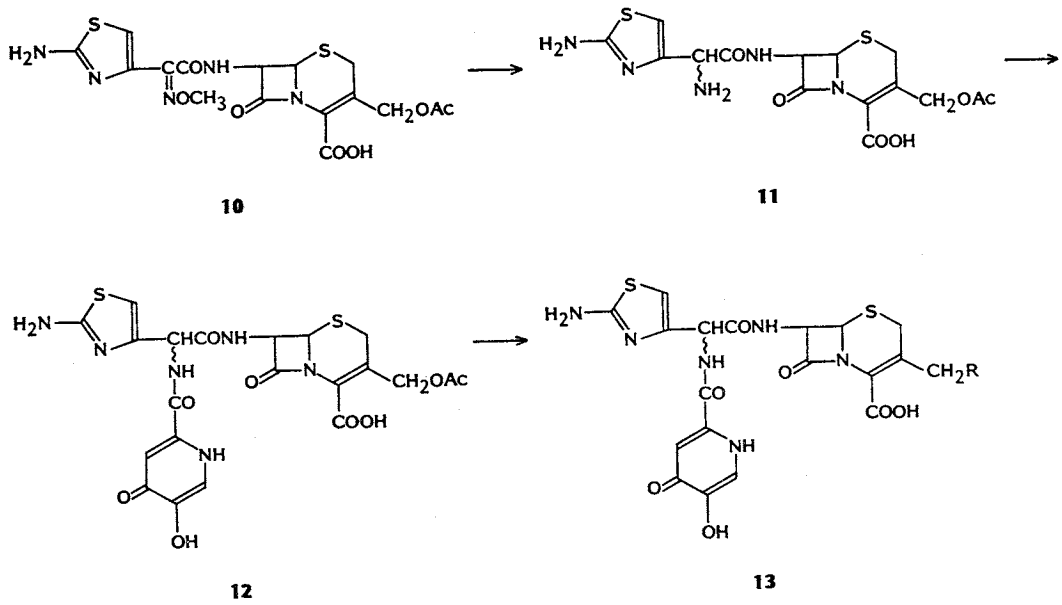






	KT-4697	**12**	**13a**	**13b**	**13c**	**13d**	Cefoperazone
Staphylococcus aureus 209-P	25	12.5	12.5	50	1.56	0.78	1.56
S. epidermidis	12.5	12.5	50	50	3.13	1.56	3.13
Escherichia coli Juhl	0.1	0.1	0.39	0.39	0.1	0.1	0.1
Klebsiella pneumoniae 8045	0.01	0.02	0.1	0.05	0.1	0.05	0.1
Serratia marcescens T-26	0.78	12.5	6.25	0.78	12.5	6.25	6.25
Proteus mirabilis 1287	0.1	0.39	1.56	0.2	3.13	0.78	0.78
Enterobacter cloacae F 1510	0.02	0.05	0.1	0.02	0.05	0.02	0.2
Citrobacter freundii F1526	0.1	0.1	0.2	0.1	0.2	0.1	0.39
Pseudomonas aeruginosa #1	0.39	0.39	0.2	0.39	1.56	0.39	3.13
P. aeruginosa 145	0.2	0.39	0.39	0.2	0.78	0.78	6.25

Scheme 2.



diastereomers. Then 5-hydroxy-4-pyridon-2-carboxylic acid (**9**) was condensed with **11** by active ester method employed in the case of carbacephem compounds. Substitution at 10-position of **12** by various kind of nucleophile was accomplished in the usual way to afford **13**. The results of *in vitro* antibacterial evaluation are summarised in Table 5. Cephalosporins with this acyl group showed strong anti-pseudomonal activity. Among these compounds, **13d** (KT-4788) having methylpyridiniumthio group at 10-position was significantly active against Gram-positive bacteria as well as Gram-negative bacteria including *P. aeruginosa*. Thus we have found 5-hydroxy-4-pyridon-2-carbonyl moiety attached to aminothiazolylglycyl side chain is a good alternative to catechol moiety in β-lactam antibiotics. The application of this acyl group to cephalosporin has thus culminated in KT-4788 which has a broad antimicrobial spectrum extended to Gram-positive bacteria.

Experimental

NMR spectra were recorded at 90 MHz on a Varian EM-390 NMR spectrometer and at 100 MHz on a Jeol FX-100 NMR spectrometer using TMS or DSS as an internal standard. All chemical shifts are reported in δ ppm. IR spectra were taken on a Jasco IR-810 IR spectrometer. Mass spectra were recorded on a Hitachi M-80B mass spectrometer (secondary ion mass spectrometry (SI-MS)). Estimation of purity of compounds was greater than 95% by analytical HPLC.

Antibiotic Susceptibility

All antimicrobial activity data are given as the minimum inhibitory concentration (MIC) in μg/ml. MICs were determined by the agar dilution method using Mueller-Hinton agar after incubation at 37°C for 20 hours with an inoculum size of 10^6 cfu/ml.

Stereo-selective Reduction of KT-3767

To a solution of KT-3767 (**4**, 1.0 g) in 18 ml of acetic acid was added portionwise 710 mg of zinc dust over 30 minutes at various temperature (when reaction temperature was below 10°C, MeOH was added to the solution as an anti-freeze). The reaction mixture was stirred until the starting

Table 6. ^{1}H NMR, IR and mass data of **3** and **13**.

	Mass (*m/z*)	IR (KBr) ν_{max} (cm^{-1})	^{1}H NMR (δ, ppm)
3a	551 $(M+1)^+$	1741, 1663, 1633, 1569	(D_2O); 7.82 (1H, d), 7.70 (1H, dd), 7.03 (1H, d), 6.76 (1H, s), 6.23 (1H, m), 5.62 (1H, s), 5.37 (1H, d), 3.93 (1H, m), 2.6~1.3 (4H, m)
3b		1740, 1665, 1630, 1610	(D_2O); 7.32 (1H, s), 7.22 (1H, m), 6.98 (1H, m), 6.72 (1H, s), 6.23 (1H, s), 5.62 (1H, s), 5.35 (1H, d), 3.88 (1H, m), 2.6~1.3 (4H, m)
3c		1740, 1706, 1646, 1637	(D_2O); 7.8 (2H, m), 6.58 (1H, m), 6.67 (1H, s), 6.18 (1H, m), 5.60 (1H, s), 5.31 (1H, d), 4.64 (2H,s), 3.84 (1H, m), 2.6~1.3 (4H, m)
3d	489 $(M+1)^+$	1751, 1696, 1685, 1637, 1630	(DMSO - CD_3OD); 8.07 (1H, d), 7.67 (1H, dd), 6.88 (1H, d), 6.45 (1H, s), 6.30 (1H, m), 5.53 (1H, s), 5.32 (1H, s), 2.2~2.5 (2H, m), 1.6~1.9 (2H, m)
3e	482 $(M+1)^+$	1757, 1654, 1636	(DMSO); 8.88 (1H, d), 8.40 (1H, d), 8.70 (1H, s), 8.20 (1H, d), 7.77 (1H, dd), 7.62 (1H, d), 6.97 (2H, s), 6.49 (1H, s), 6.30 (1H, m), 5.58 (1H, d), 5.33 (1H, dd), 3.8 (1H, m), 2.6~1.3 (4H, m)
3f	497 $(M+1)^+$	1741, 1730, 1652, 1637, 1618	(D_2O); 7.95 (1H, d), 6.97 (1H, d), 6.77 (1H, dd), 6.73 (1H, s), 6.20 (1H, m), 5.57 (1H, s), 5.34 (1H, d), 3.86 (1H, m), 2.6~1.3 (4H, m)
3g	477 $(M+2)^+$	1749, 1733, 1718, 1686, 1651, 1608	(D_2O); 8.30 (1H, s), 6.61 (1H, s), 6.10 (1H, m), 5.43 (1H, s), 5.22 (1H, d), 3.9 (1H, m), 2.3 (2H, m), 2.2~1.5 (2H, m)
3h	459 $(M+1)^+$	1743, 1665, 1617	(DMSO); 8.84 (1H, d), 8.45 (1H, d), 8.11 (1H, d), 7.89 (1H, dd), 6.93 (2H, s), 6.42 (1H, s), 6.31 (1H, d), 6.29 (1H, m), 5.47 (1H, d), 5.33 (1H, dd), 3.78 (1H, m), 2.6~1.3 (4H, m)
3i	476 $(M+1)^+$	1752, 1684, 1670, 1637	(DMSO - CD_3OD); 8.02 (1H, s), 7.01 (1H, s), 6.55 (1H, s), 6.46 (1H, m), 5.55 (1H, d), 3.8 (1H, m), 2.5~1.4 (4H, m)
3k	497 $(M+1)^+$	1746, 1680, 1656, 1620	(D_2O); 7.16 (1H, d), 6.65 (1H, s), 6.50 (1H, d), 6.2 (1H, m), 5.55 (1H, s), 5.33 (1H, d), 3.9 (1H, m), 2.6~1.5 (4H, m)
3l	511 $(M+1)^+$	1765, 1700, 1660, 1635	(DMSO - CD_3OD); 7.43 (1H, s), 6.53 (1H, s), 6.33 (1H, s), 6.3 (1H, m), 5.47 (1H, s), 5.32 (1H, d), 3.8 (1H, m), 3.67 (3H, s), 2.4 (2H, m), 2.0~1.4 (2H, m)
13a		1780, 1660, 1620	(D_2O); 8.80 (2H,d), 8.1 (2H, m), 7.71 (1H, s), 7.17 (1H, s), 6.71 (1H, s), 5.9~5.0 (3H, m), 5.56 (1H, s), 3.8~3.0 (2H, m), 3.35 (4H, s)
13b	709 $(M+1)^+$	1770, 1620, 1560	(D_2O); 7.75 (1H, s), 7.19 (1H, s), 6.78 and 6.74 (1H, s), 5.8~5.5 (1H, m), 5.59 (1H, s), 5.2~4.9 (1H, m), 5.00 (2H, s), 4.5~3.9 (2H, m), 3.9~3.2 (2H, m)
13c		1765, 1690, 1630	(D_2O); 8.28 (2H, m), 7.72 (2H, m), 7.65 (1H, s), 7.14 and 7.13 (1H, d), 6.73 and 6.70 (1H, s), 5.68 and 5.58 (1H, d), 5.56 (1H, s), 5.07 (1H, m), 4.5~4.0 (2H, m), 3.9~3.1 (2H, m)

material was disappeared on HPLC. After filtration, the ratio of (*S*) and (*R*)-diastereomer produced was measured as the ratio of area under the peak of HPLC (Nucleosil $10C_{18}$, 10% MeOH - 1/20 M phosphate buffer at pH 3). The filtrate was concentrated and the residue was dissolved in 5 ml of water. (*S*)-Diastereomer **2** chelated with zinc was crystallized and passed through the column packed with Diaion HP-10. During this procedure, the chelate with zinc was removed and free **2** was obtained as colorless powder. The result was listed in Table 2. *In vivo* activity will be reported in another paper.

In Vitro Enzymatic Reaction of KT-4380 and KT-4697 with COMT

To a solution of 0.2 ml of substrate (KT-4380 or KT-4697, 1 mg/ml), 0.2 ml of cofactor (*S*-adenosyl methionine, 10 mM), 0.2 ml of $MgCl_2$ (1 mM) and 0.2 ml of 1/20 M phosphate buffer (pH 7.9) was added 0.2 ml of porcine liver COMT (1,000 U/2 ml, Sigma). All materials were dissolved in 1/20 M phosphate buffer at pH 7.9. The mixture was incubated at 37°C with gentle shaking. The methylation reaction was monitored by HPLC (column; Nucleosil $10C_{18}$, mobile phase; 25% MeOH - phosphate buffer (pH 3, at 40°C). The result was listed in Table 4.

General Procedure for the Acylation of **2**

To a solution of 0.5 mmol of **2** in 2 ml of THF and 2 ml of H_2O at pH 8 with NEt_3 was added portionwise 0.6~1.0 mmol of acid chloride or active ester of acyl moiety at 0°C. The reaction mixture was stirred at room temp for 2 hours, acidified to pH 2 with 1 N HCl and concentrated. The residue was purified by column chromatography on Diaion HP-10, eluting with an aq MeOH, to give **3**. In the case of the product with acetoxy group, removal of protecting group was accomplished by ammonolysis in MeOH. The product was chromatographed on Diaion HP-10 at pH 2 (1 N HCl) or pH 8 (aq $NaHCO_3$) to afford **3** as free acid or sodium salt, respectively. The physical data of **3** are listed in Table 6.

5-Benzyloxy-4-pyridon-2-carboxylic Acid (**8**)

To a solution of 5-benzyloxy-2-(hydroxymethyl)pyran-4-one[10] (**6**) (4.3 g) in 250 ml of acetone was added dropwise 12.5 ml of Jones reagent at 0°C. The reaction mixture was stirred for 2.5 hours, and 1 ml of 2-PrOH was added. After being stirred at room temp for 1 hour, the reaction mixture was filtered and concentrated under reduced pressure to afford **7** as yellow crystals (2.8 g, 61%): ^{1}H NMR (DMSO-d_6) δ 7.93 (1H, s), 7.3 (6H, m), 5.01 (2H, s). Two g of **7** was dissolved in 12 ml of conc NH_4OH and the solution was heated at 90°C in a sealed tube for 2 hours. The reaction mixture was cooled and evaporated. The residue was dissolved in H_2O and adjusted to pH 2.5 with 1 N HCl. The precipitates were filtered, washed with water and dried to give **8** as a colorless crystals (96%): IR (KBr) cm^{-1} 1654, 1643, 1573; ^{1}H NMR (D_2O - NaOD) δ 7.53 (1H, s), 7.36 (5H, s), 7.00 (1H, s), 5.03 (2H, s).

5-Hydroxy-4-pyridon-2-carboxylic Acid (**9**)

The suspension of **8** and 10 ml of conc HCl was heated at 90°C for 2 hours. After cooling, the reaction mixture was evaporated and residual solid was dissolved in 5 ml of H_2O. Adjusting pH at 2 with saturated $NaCO_3$ solution yielded **8** as a brown crystals (580 mg, 93.2%): IR (KBr) cm^{-1} 3592, 1653, 1584, 1559; ^{1}H NMR (DMSO-d_6 - CD_3OD) δ 7.73 (1H, s), 7.30 (1H, s).

(6*R*,7*S*)-7-[(*S*)-2-(2-Aminothiazol-4-yl)-2-(5-hydroxy-4-pyridon-2-carboxyamido) acetamido]-1-aza-bicyclo[4,2,0]oct-2-en-8-oxo-2-carboxylic Acid (**3j**, KT-4697)

To a solution of **9** (310 mg) and 1-hydroxybenzotriazole (260 mg) in 4 ml of DMSO was added 410 mg of dicyclohexylcarbodiimide. After being stirred at room temp for 1 hour, the reaction mixture containing active ester was added to a solution of 370 mg of **2** and 0.3 ml of NEt_3 in 2 ml of DMSO, and stirring was continued for 2 hours. The precipitates formed were filtered off, and 5 ml of H_2O was added to the filtrate. The solution was adjusted to pH 2 with 1 N HCl and chromatographed on Diaion HP-10 with elution by 50% aq MeOH to afford 260 mg of **3j** as colorless powder (52.9%): IR (KBr) cm^{-1} 1755, 1670, 1650, 1600, 1570; ^{1}H NMR (D_2O - NaOD) δ 7.67 (1H, s), 7.23 (1H, s), 6.73 (1H, s), 6.2 (1H, m), 5.60 (1H, s), 5.34 (1H, d, J=5 Hz), 3.9 (1H, m), 2.4 (2H, m), 2.2~1.5 (2H, m); SI-MS *m/z* 475 $(M+1)^+$.

7-[2-(2-Aminothiazol-4-yl)-2-(5-hydroxy-4-pyridon-2-carboxyamido)acetamido]-3-cephem-4-carboxylic Acid Sodium Salt (**12**)

To a solution of 5-hydroxy-4-pyridon-2-carboxylic acid (**9**, 1.0 g) and *N*-hydroxy succinimide (750 mg) in 4.3 ml of DMSO was added dicyclohexyl carbodiimide (1.34 g). After stirring for 1 hour at room temp, the reaction mixture was added to a solution of 7-[2-(2-aminothiazol-4-yl)-2-amino-acetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid hydrochloride[11] (**11**, 2.0 g) and 1.2 ml of

NEt_3 in 6.4 ml of DMSO. The mixture was stirred for 3 hours at room temp and adjusted to pH 2 with 1 N HCl. The precipitates formed were filtered off, and the solution was chromatographed on Diaion HP-10 with eluting by 30% aq MeOH to give **12** as free acid (1.08 g). Free acid **12** (770 mg) was dissolved in aq $NaHCO_3$ solution and passed through the column of Diaion HP-10 to afford **12** as sodium salt (470 mg, 26.0%): IR (KBr) cm^{-1} 1770, 1660, 1610, 1520; ^1H NMR (D_2O) δ 7.73 (1H, s), 6.75 and 6.72 (1H, s), 5.7~5.5 (1H, m), 5.59 (1H, s), 5.1~5.0 (1H, m), 4.9~4.5 (2H, m), 3.7~3.1 (2H, m), 2.08 and 2.04 (3H, s); SI-MS *m/z* 587 $(M+1)^+$.

7-[2-(2-Aminothiazol-4-yl)-2-(5-hydroxy-4-pyridon-2-carboxyamido)acetamido]-3-(1-methyl-4-pyridiniothiomethyl)-3-cephem-4-carboxylate (**13d**, KT-4788)

To a solution of 318 mg of $NaHCO_3$ and 663 mg of 1-methylpyrido-4-thione in 38 ml of H_2O was added 2.13 g of **12**. The reaction mixture was stirred at 80°C for 3.5 hours and cooled to room temp. The product was purified by column chromatography on Diaion HP-10. The appropriate fraction eluted by 30% aq MeOH were combined and evaporated under reduced pressure to provide **13d** as yellow powder (730 mg, 31%): IR (KBr) cm^{-1} 1780, 1670, 1640, 1610, 1560; ^1H NMR (D_2O-NaOD) δ 8.2 (2H, m), 7.6 (2H, m), 7.59 and 7.58 (1H, s), 7.09 and 7.07 (1H, s), 6.71 and 6.68 (1H, s), 5.66 and 5.56 (1H, d), 5.54 (1H, s), 5.0 (1H, m), 4.08 (3H, s), 3.8~3.0 (2H, m); SI-MS *m/z* 629 $(M+1)^+$.

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