SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NOVEL 3-SUBSTITUTED CARBACEPHEMS

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(Received for publication August 23, 1988)

A series of novel 3-heterocyclothio substituted carbacephems having phenylglycyl side chain have been prepared starting from 3-H carbacephem. The compounds exhibit better chemical stability than the corresponding cephalosporin and strong activity against Gram-negative and Gram-positive organisms including *Enterococcus faecalis*.

In a field of cephalosporin, cefaclor (**1**)[1] and cefroxadine[2] are well known antibiotics in which a heteroatom is attached directly at the 3-position. These compounds have stronger antibacterial activity than cephalexin, which is the 3-methyl analog, because the cephem nucleus is activated by the effects of the substituent at the 3-position.

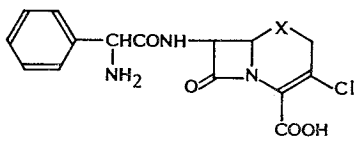
We previously reported the synthesis and antibacterial activity of KT-3777 (**2**)[3,4] which is a carbacephem with a chlorine atom at the 3-position. KT-3777 showed comparable antibacterial activity and pharmacokinetics in mouse and rat with cefaclor, and was far superior in chemical stability. In this paper, we described the synthesis and antibacterial activity of the compounds in which a heteroatom, particularly the heterocyclic thio group, was introduced at the 3-position by taking advantage of the excellent stability of carbacephem nucleus. The substituent of cephalosporin at the 10-position not only affects the antibacterial activity, but often modifies pharmacokinetics and also contributes to the stability against β-lactamase[5]. In this respect, the direct substitution of a heteroatom at the 3-position instead of the 10-position would be of great interest.

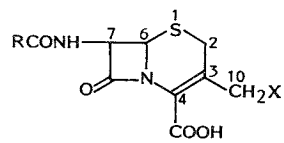
Chemistry

An important intermediate **7** was prepared by the method outlined in Scheme 1 starting from **3** which is available in large quantity through the development of a practical synthetic procedure[6].

Protected 3-H carbacephem **3** was reacted with one equivalent of *N*-bromosuccinimide in water

Fig. 1. Cefaclor and KT-3777, and numbering of cephem nucleus.



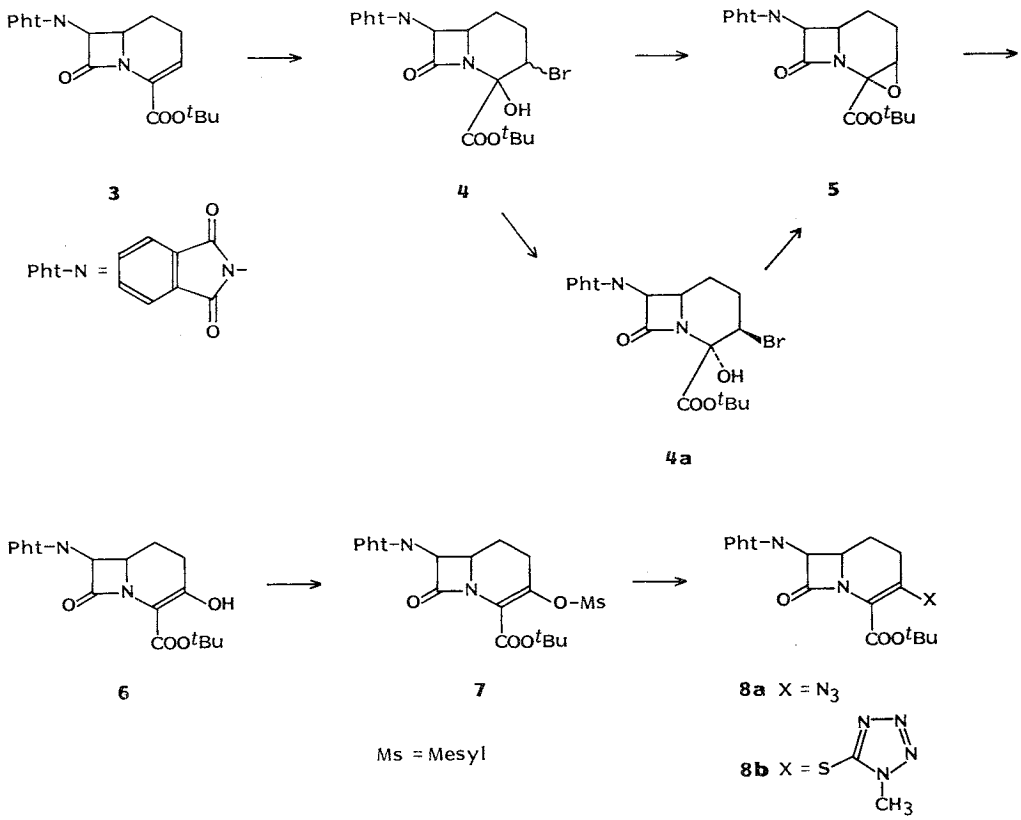


Cefaclor (**1**) X=S
KT-3777 (**2**) X=CH_2

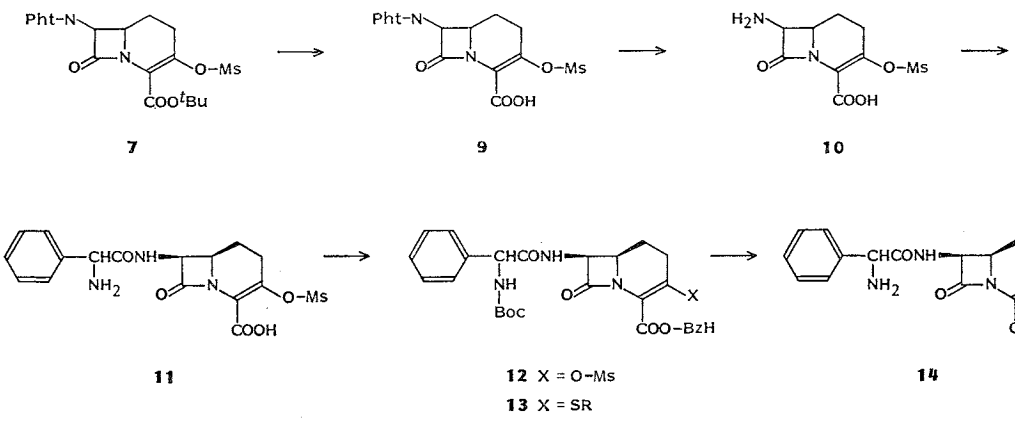
and THF to afford a mixture of three components. The mass spectra of three compounds showed the same molecular ion peak at *m/z* 408 and 410, and the chemical shift of C-3 carbon of each compound appeared around 50 ppm in ^{13}C NMR, the substituent of the 3-position was considered to be bromine in all compounds. According to above data, three compounds were stereoisomers of bromohydrin **4**. Interestingly enough, treatment of this mixture with catalytic to equivalent amount of triethylamine did not give epoxide **5**, but converted it into one isomer **4a** which was the minor component of three isomers. The stereochemistry of **4a** could not be decided from its analytical data. However, we postulated **4a** as 3-β-Br and 4-α-OH in twisted chair form of tetrahydropyridine ring judged from the fact that the coupling pattern of 3-H was different from those of other two isomers in ^1H NMR, and presumed favorable attack of bromonium ion from less hinderd α-face.

Epoxidation of **4a** was smoothly effected using 1,8-diazabicyclo[5.4.0]undecen to give **5** as single product in almost quantitative yield. The stereochemistry of **5** was considered to be α-epoxide from the configuration of **4a**. Epoxide **5** was also obtained as single crystals from a mixture of three isomers **4**. Isomerization of expoxide to ketone was performed by treating **5** with catalytic amount of TsOH in toluene at 110°C to give **6** in 73% yield. The 3-hydroxy derivative of carbacephem has been prepared and reported to be extremely unstable[7]. Surprisingly 3-hydroxy carbacephem **6** turned out to be very stable, and formed mesylate **7** in good yield. Recently, the alternative synthetic procedure of 3-hydroxy carbacephem was reported[8~10]. The mesylate **7** reacted readily with various

Scheme 1.



Scheme 2.



BzH=Benzhydryl, Boc=*tert*-butoxycarbonyl.

Scheme 3.

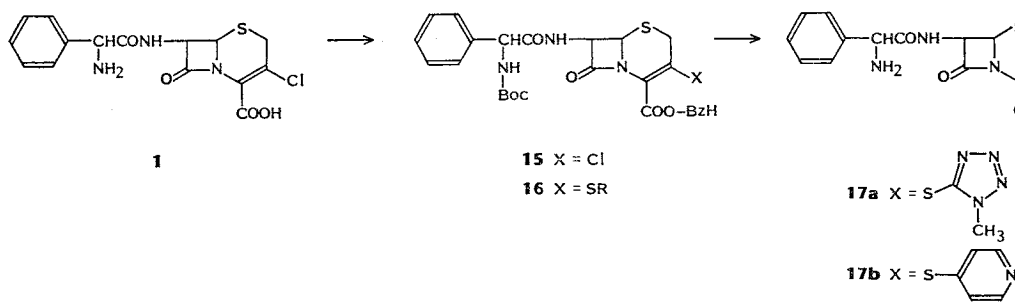


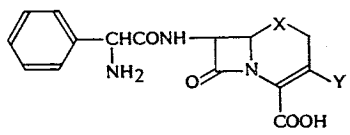
Table 1. Mass, ^{1}H NMR and IR spectal data of **14**.

	Mass (*m/z*)	IR (KBr) (cm^{-1})	^{1}H NMR (solvent, δ)
14a	430 $(M+1)^+$	1743, 1681, 1621	(D_2O) 7.40 (5H, s), 5.33 (1H, d), 5.08 (1H, s), 3.93 (3H, s), 3.8 (1H, m), 2.2~1.4 (4H, m)
14b	425 $(M+1)^+$	1765, 1685, 1542	(D_2O) 8.33 (2H, d), 7.92 (2H, d), 7.40 (5H, s), 5.32 (1H, d), 5.10 (1H, s), 3.8 (1H, m), 2.4~1.4 (4H, m)
14d	446 $(M+1)^+$	1780, 1695, 1605	(D_2O - DCl) 7.40 (5H, s), 5.38 (1H, d), 5.13 (1H, s), 3.8 (1H, m), 2.69 (3H, s), 2.2~1.4 (4H, m)
14e	432 $(M+1)^+$	1770, 1695, 1575	(CD_3OD) 8.53 (1H, s), 7.40 (5H, s), 5.38 (1H, d), 5.13 (1H, s), 3.8 (1H, m), 2.4~1.3 (4H, m)
14f	447 $(M+1)^+$	1751, 1692, 1605	(D_2O) 7.40 (5H, s), 5.32 (1H, d), 5.10 (1H, s), 3.8 (1H, m), 2.2~1.4 (4H, m)

nucleophile to produce 3-substituted carbacephem compounds **8**. For example, treatment of **7** with NaN_3 in DMF gave 3-azido derivative **8a** in 87% yield. As a typical heterocyclic thio group, 5-mercapto-1-methyltetrazole was selected and reacted with **7** in the presence of NaH in DMF to afford **8b** in 58% yield. A phenylglycyl group was selected as an acyl group to the 7-amino group to measure the antibacterial activity. The phenylglycyl derivatives **14** with optically active nucleus were prepared by the efficient route shown in Scheme 2. Treatment of **7** with trifluoroacetic acid gave carboxylic acid **9**.

Dephthalization of **9** was accomplished with methylhydrazine in an aqueous media[11] to afford deprotected zwitterion **10** in 68% yield. Racemic mixture of zwitterion **10** was incubated with immobilized enzyme, penicillin acylase obtained from *Kluyvera citrophila*[12], and D-phenylglycine methyl ester at 30°C adjusting to pH 7.5 with 1 N KOH to give rise to **11** in 38% yield. In ^{1}H NMR **11** showed a signal of one of the two kinds of diastereomer which were formed by chemical phenylglycylation of **10**. This suggested that enzymatic phenylglycylation proceeds stereoselectively in these carbacephem nucleus. Protected product **12** was obtained in 87% yield by protecting the amino group with *tert*-butoxycarbonyl group and carboxylic moiety with benzhydryl group. The compound **12** reacted with thiolate as readily as **7** to give **13** followed by deprotection using trifluoroacetic acid and anisole to obtain **14** in 30 to 55% yield and Table 1 shows the physical data of **14**.

Table 2. Chemical stability of 3-substituted carbacephems and cephems.



	X	Y	$T_{1/2}$ (hour) at 30°C
14a	CH_2		Stable (pH 7.4)
17a	S		<0.5 (pH 6.6)
14b	CH_2		Stable (pH 7.4)
17b	S		0.6 (pH 6.6)

Monitored by HPLC.

Several corresponding cephalosporins were prepared applying the reported procedure[13] as shown in Scheme 3 to compare antibacterial activity and chemical stability. That is cefaclor (**1**) was protected in the same way as carbacephem to give **15**. The reaction of **15** with thiol compound in the presence of $NaHSO_3$ in DMF to afford **16**. Deprotection of **16** using trifluoroacetic acid and anisole, and adjusting pH to 3.5 gave **17**.

Antibacterial Activity

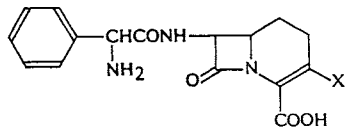
As shown in Table 2, cephalosporins **17a** and **17b** were extremely unstable, and decomposed rapidly under even neutral condition, while the corresponding carbacephem **14** showed excellent stability. This result suggests that compounds with a heterocyclothio group at the 3-position are taking full advantage of the good stability of carbacephem nucleus. The comparative antibacterial activities of carbacephem compounds **14** and cephalosporins **17** are summarized in Tables 3 and 4. Most compounds were more active against Gram-positive and Gram-negative organisms than cefaclor and 3-H carbacephem compound, the mother compound. In paticular, **14f** was active against indole positive *Proteus* with the MIC of 1.56 to 3.13 μg/ml. As shown in Table 4, these compounds were strongly active against *Enterococcus faecalis* which is resistant to most cephalosporins including cefaclor, especially **14e** was very potent against most clinical isolate strains of *E. faecalis* tested.

We found interesting properties in carbacephems with heterocyclothio group at the 3-position and further studies are in progress to evaluate these compounds.

Experimental

^{1}H 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

Table 3. Antibacterial activity (MIC, μg/ml) of **14**.



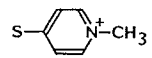
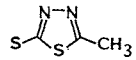
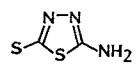
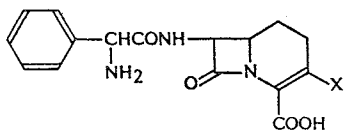
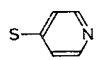
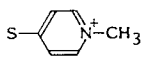
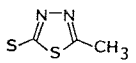
	X: H	Cl							
		2	**14a**	**14b**	**17b** (Cephem)	**14c**	**14d**	**14e**	**14f**
Staphylococcus aureus 209-P	1.56	0.2	0.2	0.1	50	0.05	0.1	0.1	0.05
S. aureus Smith	3.13	0.78	0.39	0.2	50	0.39	0.39	0.2	0.2
S. epidermidis	6.25	3.13	0.78	0.39	100	0.39	0.39	0.39	0.2
Escherichia coli NIHJ JC-2	3.13	1.56	1.56	0.39	100	1.56	0.78	0.39	0.39
Klebsiella pneumoniae 8045	1.56	0.39	0.78	0.1	100	0.78	0.39	0.1	0.2
Proteus mirabilis 1287	6.25	1.56	3.13	3.13	100	0.1	1.56	0.78	0.2
P. vulgaris 6879	100	100	100	50	100	3.13	50	12.5	3.13
Providencia rettgeri 4289	12.5	50	100	100	100	100	100	100	1.56
Enterobacter cloacae F1510	6.25	3.13	25	25	100	12.5	25	12.5	50
Citrobacter freundii F1528	1.56	1.56	6.25	0.78	100	0.78	3.13	1.56	1.56

Table 4. Antibacterial activity (MIC, μg/ml) of carbacephems **14**.



	X: H	Cl						Cefaclor (**1**)
		2	**14a**	**14b**	**14c**	**14d**	**14e**	
Enterococcus faecalis ATCC 10541	100	25	3.13	1.56	6.25	1.56	0.39	25
E. faecalis KT-5698	100	25	3.13	1.56	6.25	1.56	0.78	25
E. faecalis J 112*	100	100	25	6.25	25	12.5	6.25	100
E. faecalis J 116*	100	100	12.5	6.25	12.5	6.25	6.25	50
E. faecalis F 3557*	100	50	12.5	3.13	12.5	3.13	3.13	50
E. faecalis F 3558*	6.25	6.25	1.56	0.39	0.39	0.2	0.39	1.56
E. faecalis F 3560*	100	100	100	100	100	50	12.5	100
Streptococcus pyogenes S23	0.78	0.2	0.05	0.01	0.01	0.01	0.01	0.1
S. pyogenes Cook	0.78	0.2	0.05	0.01	0.01	0.01	0.01	0.1
S. pneumoniae I	0.78	0.39	0.2	0.1	0.1	0.02	0.05	0.39
S. pneumoniae 8102	12.5	3.13	0.2	0.2	0.39	0.05	0.1	1.56

* Clinical isolate.

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 spectra (SI-MS). Estimation of purity of analogues was greater than 95% by analytical HPLC.

Antibiotic Susceptibility

All antibacterial activity data are given as the 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 about 10^6 cfu/ml.

tert-Butyl (±)-*cis*-7-Phthalimido-3-bromo-2-hydroxy-1-azabicyclo[4.2.0]octan-8-oxo-2-carboxylate (**4a**)

To a suspension of *tert*-butyl (±)-*cis*-7-phthalimido-1-azabicyclo[4.2.0]oct-2-en-8-oxo-2-carboxylate (**3**, 3.68 g) in 80 ml of THF and 20 ml of water was added portionwise 1.66 g of *N*-bromosuccinimide (NBS) over 1 hour. The reaction mixture was stirred for 2 hours at room temperature. The reaction mixture was extracted twice with EtOAc and the organic layer was washed with water and brine and evaporated under reduced pressure. The residue was dissolved in 100 ml of $CHCl_3$ and 0.5 ml of triethylamine was added to the solution. The reaction mixture was stirred for overnight at room temperature, washed with water, dilute HCl and brine. The solvent was evaporated to give the residue, which was crystallized from hexane - EtOAc (1 : 1) to afford **4a** as colorless crystals (3.39 g, 73.1%): ^{1}H NMR ($CDCl_3$) δ 7.8 (4H, m), 5.28 (1H, d), 4.65 (1H, dd), 3.9 (1H, m), 2.4~1.5 (4H, m), 1.56 (9H, s); IR (KBr) cm^{-1} 1777, 1753, 1743, 1705, 1689, 1662, 1611; MS *m/z* 409, 407 (M−*tert*-Bu)$^+$.

tert-Butyl (±)-*cis*-7-Phthalimido-2,3-epoxy-1-azabicyclo[4.2.0]octan-8-oxo-2-carboxylate (**5**)

To a suspension of 18.4 g of **3** in 400 ml of THF and 100 ml of water, was added portionwise 8.3 g of NBS over 1 hour with vigorous stirring. The reaction mixture was stirred for 2 hours at room temperature. After addition of 400 ml of EtOAc, the organic layer was separated, washed with water and brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved in 300 ml of $CHCl_3$ and 7.0 ml of 1,8-diazabicyclo[5.4.0]undecen (DBU) was added to the solution under ice-cooling. The reaction mixture was stirred for 2 hours at room temperature and washed with water, 10% citric acid solution and brine. The solvent was evaporated to give the residue, which was crystallized from hexane - EtOAc (1 : 1) to afford **5** as colorless crystals (12.2 g, 63.5%): ^{1}H NMR ($CDCl_3$) δ 7.8 (4H, m), 5.55 (1H, d), 3.7 (1H, m), 3.65 (1H, m), 2.5~1.6 (4H, m), 1.52 (9H, s); IR (KBr) cm^{-1} 1795, 1790, 1775, 1735, 1720.

tert-Butyl (±)-*cis*-7-Phthalimido-3-hydroxy-1-azabicyclo[4.2.0]oct-2-en-8-oxo-2-carboxylate (**6**)

A suspension of 12.2 g of **5** and catalytic amount of *p*-TsOH in 245 ml of toluene was heated for 20 minutes at 110°C. After cooling to room temperature, the reaction mixture was diluted with EtOAc, washed with water, saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated to afford **6** as yellow solid (11.6 g, 95.1%): ^{1}H NMR ($CDCl_3$) δ 7.8 (4H, m), 7.18 (1H, s), 5.52 (1H, d), 3.9 (1H, m), 2.8~1.8 (4H, m), 1.56 (9H, s); IR (KBr) cm^{-1} 1785, 1765, 1730, 1720.

tert-Butyl (±)-*cis*-7-Phthalimido-3-mesyloxy-1-azabicyclo[4.2.0]oct-2-en-8-oxo-2-carboxylate (**7**)

To a solution of 11.6 g of **6** in 230 ml of CH_2Cl_2 were added 2.8 ml of methansulfonylchloride and 5.0 ml of triethylamine at −50°C, and the reaction mixture was stirred for 1 hour at room temperature. The reaction mixture was diluted with $CHCl_3$, washed with water, 10% citric acid solution, water and saturated $NaHCO_3$ solution successively, dried over Na_2SO_4 and evaporated. The residue was crystallized from hexane - EtOAc (1 : 1) to afford 9.2 g of **7** (89.3%): ^{1}H NMR ($CDCl_3$) δ 7.8 (4H, m), 5.60 (1H, d), 4.0 (1H, m), 3.27 (3H, s), 2.8~1.8 (4H, m), 1.57 (9H, s); IR (KBr) cm^{-1} 1800, 1790, 1775, 1725.

tert-Butyl (±)-*cis*-7-Phthalimido-3-azido-1-azabicyclo[4.2.0]oct-2-en-8-oxo-2-carboxylate (**8a**)

To a solution of 600 mg of **7** in 10 ml of DMF was added 150 mg of sodium azide. The reaction mixture was stirred for 1 hour at room temperature and evaporated. The residual solid was dissolved in EtOAc and the solution was washed with water, saturated $NaHCO_3$ solution and brine, dried over

Na_2SO_4 and evaporated. The residue was subjected to column chromatography on silica gel eluting with hexane - EtOAc (1 : 1) to give **8a** as yellow crystals (472 mg, 89%): ^1H NMR ($CDCl_3$) δ 7.8 (4H, m), 5.60 (1H, d), 3.9 (1H, m), 2.7~1.8 (4H, m); IR (KBr) cm^{-1} 2120, 1795, 1780, 1740, 1725.

tert-Butyl (±)-*cis*-7-Phthalimido-3-(1-methyl-1,2,3,4-tretazol-5-yl)thio-1-azabicyclo[4.2.0]oct-2-en-8-oxo-2-carboxylate (**8b**)

To a suspension of 360 mg of sodium hydride (50%) in 10 ml of dried DMF were added 1.2 g of 1-methyl-5-mercapto-1,2,3,4-tetrazole and 1.2 g of **7** under ice cooling. After stirring for 18 hours at room temperature, the reaction mixture was concentrated to dryness. The residue was dissolved in 100 ml of $CHCl_3$, and the solution was washed with water, dried over Na_2SO_4 and evaporated. The product was purified by column chromatography on silica gel, eluting with $CHCl_3$ to afford 0.89 g (69%) of **8b** as colorless powder: ^1H NMR ($CDCl_3$) δ 7.8 (4H, m), 5.67 (1H, d), 4.10 (3H, s), 3.9 (1H, m), 2.9~1.8 (4H, m), 1.56 (9H, s).

(±)-*cis*-7-Amino-3-mesyloxy-1-azabicyclo[4.2.0]oct-2-en-8-oxo-2-carboxylic Acid (**10**)

Mesylate **7** (4.6 g) was dissolved in 10 ml of trifluoroacetic acid and 10 ml of CH_2Cl_2 and reacted for 1 hour at room temperature. The reaction mixture was concentrated to dryness under reduced pressure. The residual solid was suspended in 220 ml of water and the pH was adjusted to 7 with saturated $NaHCO_3$ solution. To the suspension was added dropwise 0.83 ml of methylhydrazine over 45 minutes under ice cooling. The reaction mixture was stirred for 3 hours at room temperature and acidified to pH 1 with conc HCl. After removal of precipitates by filtration, the filtrate was adjusted to pH 3.5 with suturated $NaHCO_3$ solution and concentrated to 50 ml. The solution was stood for over night in refrigerator to give **10** as colorless crystals (1.82 g, 66.2%): ^1H NMR (D_2O - NaOD) δ 4.52 (1H, d), 3.89 (1H, m), 3.25 (3H, s), 2.7~2.6 (2H, m), 1.8~1.2 (2H, m); IR (KBr) cm^{-1} 1800, 1650, 1620, 1560, 1545.

(6*R*,7*S*)-7-[(*R*)-Phenylglycylamino]-3-mesyloxy-1-azabicyclo[4.2.0]oct-2-en-8-oxo-2-carboxylic Acid (**11**)

To a solution of 460 mg of **10** and 1.5 g of phenylglycine methyl ester hydrochloride in 20 ml of 1/10 M phosphate buffer (pH 7.0) was added 10 ml of immobolized enzyme having a penicillin acylase activity. The reaction mixture was incubated at 30°C for 1.5 hours while adjusting the mixture to pH 7.0 with 1 N KOH. The immobilized enzyme was removed by filtration, and the filtrate was adjusted to pH 3.0 with 1 N HCl, and concentrated to about 5 ml. After removal of precipitate by filtration, the filtrate was chromatographed on Diaion HP-10 with eluting by 30% aqueous MeOH to give **11** as colorless powder (210 mg, 62%): ^1H NMR (D_2O) δ 7.55 (5H, s), 5.41 (1H, d), 3.9 (1H, m), 3.30 (3H, s), 2.7~2.3 (2H, m), 1.8~1.1 (2H, m); IR (KBr) cm^{-1} 1770, 1700, 1630, 1610; SI-MS *m/z* 410 $(M+1)^+$.

Benzhydryl (6*R*,7*S*)-7-[2-(*R*)-*tert*-Butoxycarbonylamino-2-phenylacetamido-3-mesyloxy-1-azabicyclo[4.2.0]oct-2-en-8-oxo-2-caboxylate (**12**)

To a solution of 1.36 g of **11** in 20 ml of THF and 20 ml of water adjusting to pH 7.0 with aq $NaHCO_3$ was added dropwise 1.5 g of di-*tert*-butyl-dicarbonate in 4 ml of THF. After stirring for 2 hours at room temperature, 50 ml of EtOAc was added to the reaction mixture. The aqueous layer was acidified with 1 N HCl to pH 1.9 and extracted twice with EtOAc. The organic layer was collected, washed with water and brine, dried over Na_2SO_4 and evaporated. The residue was dissolved in 20 ml of $CHCl_3$, and 1.5 g of diphenyldiazomethane was added to the solution. The reaction mixture was stirred 30 minutes and evaporated. The product was purified by column chromatography on silica gel to afford **12** as colorless powder (1.42 g, 63.3%): ^1H NMR ($CDCl_3$) δ 7.5~7.2 (15H, m), 6.90 (1H, s), 6.8 (1H, d), 5.65 (1H, d), 5.37 (1H, dd), 5.16 (1H, d), 3.8 (1H, m), 2.83 (3H, s), 2.7~2.4 (2H, m), 1.38 (9H, s), 1.9~1.3 (2H, m).

General Procedure for the Synthesis of **14**

To a solution of 1 mmol of NaH and 1 mmol of thiol in 5 ml of DMF was added 0.5 mmol of **12**. The reaction mixture was stirred for 2 to 16 hours at room temperature to 60°C and evaporated under

reduced pressure. The residue was dissolved in EtOAc and the solution was washed with water and brine. After evaporation to dryness, the yellow powder was dissolved in 5 ml of CH_2Cl_2 and 0.5 ml of anisole, and 0.5 ml of TFA was added to the solution under ice cooling. The reaction mixture was stirred for 1 hour at 0°C, evaporated and solidified with ether. The solid was dissolved in 2 ml of water and chromatographed on Diaion HP-10 to afford **14**. The physical data of **14** were shown in Table 1.

(6*R*,7*S*)-7-[(*R*)-Phenylglycylamino]-3-(1-methylpyridinium-4-yl-thio)-1-azabicyclo[4.2.0]oct-2-en-8-oxo-2-carboxylic Acid (**14c**)

To a solution of 570 mg of **12** in 10 ml of DMF were added 220 mg of 4-mercaptopyridine and 0.2 ml of triethylamine. The reaction mixture was stirred for 4 hours at 50°C and evaporated. The residue was dissolved in EtOAc and washed with water and brine. After removal of solvent, the residual yellow solid was dissolved in 5 ml of CH_2Cl_2 and 0.2 ml of methyliodide was added to the solution. The reaction mixture was stirred overnight and evaporated to dryness. To the residue were added 5 ml of CH_2Cl_2, 0.5 ml of anisole and 5 ml of TFA under ice cooling. The solution was allowed to react for 1 hour and evaporated. The product was subjected to column chromatography on Diaion HP-10 to afford **14c** as brown powder (140 mg, 31.6%): ^{1}H NMR (D_2O) δ 8.40 (2H, d), 7.66 (2H, d), 7.51 (5H, s), 5.49 (1H, d), 5.28 (1H, s), 4.12 (3H, s), 3.9 (1H, m), 2.5~1.4 (4H, m); IR (KBr) cm^{-1} 1771, 1687, 1634; SI-MS *m/z* 439 $(M+1)^+$.

Benzhydryl 7-[2-(*R*)-*tert*-Butoxycarbonylamino-2-phenylacetamido]-3-chloro-3-cephem-2-carboxylate (**15**)

To a solution of 500 mg of cefaclor (**1**) in 50 ml of THF and 50 ml of water adjusting to pH 7.5 with triethylamine was added 840 mg of di-*tert*-butyl-dicarbonate under ice cooling. After stirring for 4 hours, the reaction mixture was acidified with 1 N HCl to pH 2.5 and extracted twice with EtOAc. The organic layer was collected, washed with brine and evaporated. The residual solid was dissolved in 20 ml of THF, and 750 mg of diphenyldiazomethane was added to the solution. The reaction mixture was stirred 1 hour and evaporated. The product was purified by column chromatography on silica gel to give **15** as colorless powder (800 mg, 89.2%): ^{1}H NMR ($CDCl_3$) δ 7.47 (15H, s), 6.98 (1H, s), 5.75 (1H, s), 5.23 (1H, d), 4.98 (1H, d), 3.72 (1H, d), 3.34 (1H, d), 1.41 (9H, s).

7-[(*R*)-Phenylglycylamino]-3-(4-pyridylthio)-3-cephem-2-carboxylic Acid (**17b**)

To a solution of 500 mg of **15** in 10 ml of DMSO were added 4 equivalents of 4-mercaptopyridine and 2 equivalents of $NaHSO_3$. After stirring for 1 hour, 2 equivalents of $NaHSO_3$ was added to the solution, and the stirring was continued for 4 hours at room temperature. EtOAc and water were added to the reaction mixture. The organic layer was separated, washed twice with water and brine, and evaporated. The residue was dissolved in 8 ml of CH_2Cl_2 and 0.4 ml of anisole, and 8 ml of TFA was added to the solution under ice cooling. The reaction mixture was stirred for 30 minutes at 0°C, diluted with 100 ml of toluene and evaporated. The residue was solidified with ether and dissolved in 4 ml of water adjusting to pH 4.8 with triethylamine. The precipitates formed by addition of 4 ml of acetone was chromatographed on Diaion HP-10 to afford **17a** (94 mg, 30.7%): ^{1}H NMR (D_2O - DCl) δ 8.46~8.38 (2H, m), 7.68~7.58 (2H, m), 7.49 (5H, s), 5.81 (1H, d), 5.28 (1H, d), 5.21 (1H, s), 3.83 (1H, d), 3.46 (1H, d); IR (KBr) cm^{-1} 1795, 1770, 1705, 1600.

N-Methyltetrazolylthio derivative **17a** was obtained by a similar procedure as described above (yield 21.0%): ^{1}H NMR (CD_3OD - DCl) δ 7.54~7.46 (5H, m), 5.85 (1H, d), 5.19 (1H, d), 4.09 (3H, s), 3.62 (1H, d), 3.30 (1H, d); IR (KBr) cm^{-1} 1790, 1785, 1700, 1680, 1605.

References

1) NEU, H. C. & K. P. FU: Cefaclor: In vitro spectrum of activity and beta-lactamase stability. Antimicrob. Agents Chemother. 13: 584~588, 1978
2) YASUDA, K.; S. KURASHIGE & S. MITSUHASHI: Cefroxadine (CGP-9000), an orally active cephalosporin. Antimicrob. Agents Chemother. 18: 105~110, 1980
3) HIRATA, T.; I. MATSUKUMA, K. MOCHIDA & K. SATO: KT3777 (LY163892), a new oral carbacephem

antibiotic; synthesis and chemistry. Program and Abstracts of the 27th Intersci. Conf. on Antimicrob. Agents Chemother., No. 1187, p. 304, New York, Oct. 4~7, 1987

4) MATSUKUMA, I.; S. YOSHIIE, K. MOCHIDA, Y. HASHIMOTO, K. SATO, R. OKACHI & T. HIRATA: Synthesis and Biological Evaluation of 3-chloro-1-carbacephem compounds. Chem. Pharm. Bull., in press
5) ARISAWA, M.; J. OHSHIMA & H. B. MARUYAMA: Effect of 3-substitution in oxyiminocephalosporins on the stability to and the inhibition of various β-lactamases. Chem. Pharm. Bull. 30: 3333~3339, 1982
6) HIRATA, T.; T. OGASA, H. SAITO, S. KOBAYASHI, A. SATO, Y. ONO, Y. HASHIMOTO, S. TAKASAWA, K. SATO & K. MINEURA: Antimicrobial activity of carbacephem compounds, novel cephalosporin-like β-lactams. Program and Abstracts of the 21st Intersci. Conf. on Antimicrob. Agents Chemother., No. 557, Chicago, Nov. 4~6, 1981
7) UYEO, S. & H. ONA: Synthesis of 1-carbacephem derivatives. Chem. Pharm. Bull. 28: 1563~1577, 1980
8) HATANAKA, M. & T. ISHIMARU: A simple synthesis of ($\pm$)-1-carbacephem derivatives. Tetrahedron Lett. 24: 4837~4838, 1983
9) EVANS, D. A. & E. B. SJOGREN: The asymmetric synthesis of β-lactam antibiotics-I. Application of chiral oxazolidones in the Staudinger reaction. Tetrahedron Lett. 26: 3783~3786, 1985
10) EVANS, D. A. & E. B. SJOGREN: The asymmetric synthesis of β-lactam antibiotics-II. The first enantioselective synthesis of the carbacephalosporin nucleus. Tetrahedron Lett. 26: 3787~3790, 1985
11) KUKOLJA, S. & S. R. LAMMERT: Azetidinone antibiotics. XIV. Removal of a phthaloyl protective group from acid and base sensitive compounds. J. Am. Chem. Soc. 97: 5582~5583, 1975
12) HASHIMOTO, Y.; S. TAKASAWA, T. OGASA, H. SAITO, T. HIRATA & K. KIMURA: Enzymatic synthesis of optically pure 1-carbacephem compounds. *In* Enzyme Engineering 7. Vol. 434. pp. 206~209, The New York Academy of Sciences, New York, 1984
13) SCARTAZZINI, R.; P. SCHNEIDER & H. BICKEL: Neue Beta-Lactam Antibiotika, uber die Funktionalisierung der Cephem-3-Stellung mittle Schwefer order Stickstoff. Helv. Chem. Acta 58: 262~273, 1975