Synthesis and Biological Evaluation of 3-Chloro-1-carbacephem Compounds

Ikuo MATSUKUMA,[a] Shigeo YOSHIIYE,[a] Kenichi MOCHIDA,[a] Yukio HASHIMOTO,[a] Kiyoshi SATO,[b] Ryo OKACHI,[b] and Tadashi HIRATA*,[a]

Kyowa Hakko Kogyo Co., Ltd., Tokyo Research Laboratories,[a] Machida-shi, Tokyo 194, Japan and Pharmaceutical Research Laboratories,[b] Mishima-shi, Shizuoka 411, Japan. *Received August 2, 1988*

The 3-chloro-1-carbacephem nucleus was prepared for the first time from a 3*H*-1-carbacephem compound through a sequence of reactions involving addition of thiophenol, oxidation of sulfide to sulfoxide, and α-chlorination of the sulfoxide, followed by elimination of phenylsulfinic acid. The 2-β-methyl analog was similarly prepared, but the 2α-methyl analog was not obtained.

Optical resolution of the 3-chloro-1-carbacephem compound was achieved by the employment of penicillin acylase. That is, the 7-phenylacetamido derivative was enantioselectively hydrolyzed to afford the optically active 7-amino-3-chloro-1-carbacephem compound. Carbacefaclor, the carbacephem analog of cefaclor, was directly and efficiently prepared by enzymatic phenylglycylation of the racemic 7-amino-3-chloro-1-carbacephem compound by using immobilized penicillin acylase. Carbacefaclor thus prepared exhibited comparable antibacterial activity against most gram positive bacteria tested and higher activity against typical gram negative bacteria as compared with cefaclor. Moreover, carbacefaclor possessed remarkably high chemical stability.

Keywords β-lactam; nuclear analog; carbacephem; 3-chloroderivative; addition-elimination; Pummerer reaction; enzymatic reaction; penicillin acylase; antimicrobial activity; chemical stability

During the course of extensive studies on 1-carbacephem compounds we have developed an efficient synthesis of the 3*H*-1-carbacephem nucleus, and promising antimicrobial activities of its acyl derivatives were found.[1] To uncover further potential usefulness of this novel nuclear analog of cephalosporin we have attempted the conversion of this nucleus to a 3-chloro-1-carbacephem compound. The orally administered cephem antibiotic cefaclor, a 3-chlorocephalosporin, already plays an important role in current chemotherapy for infectious disease, so it would be of great interest to synthesize the corresponding compound, carbacefaclor, and compare its biological activity with that of cefaclor.

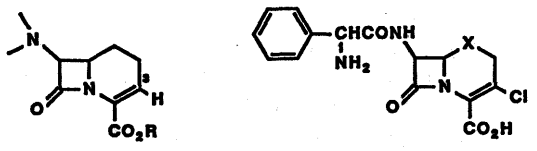


Chart 1

Synthesis of 3-Chloro-1-carbacephem Compounds 3-Chlorocephem compounds have been efficiently prepared by chlorination of 3-hydroxy-cephems.[2] So we firstly attempted to prepare 3-hydroxy-carbacephem compound **5** as a possible precursor.

After the completion of this work[3] Uyeo and Ona reported an unsuccessful attempt at a similar conversion.[4] The 3*H*-1-carbacephem **1**, when treated with thiophenol in the presence of a base such as piperidine, gave the phenylsulfide **2** almost quantitatively. The product is a single isomer with a sharp melting point. This is in contrast with the analogous thiol addition to a carbapenem, in which three stereoisomers were isolated with the 2α-SR-3α-CO_2Bzl isomer as the major product.[5] The stereochemistry of the sulfide at C-2 and C-3 is not certain but can be assigned as

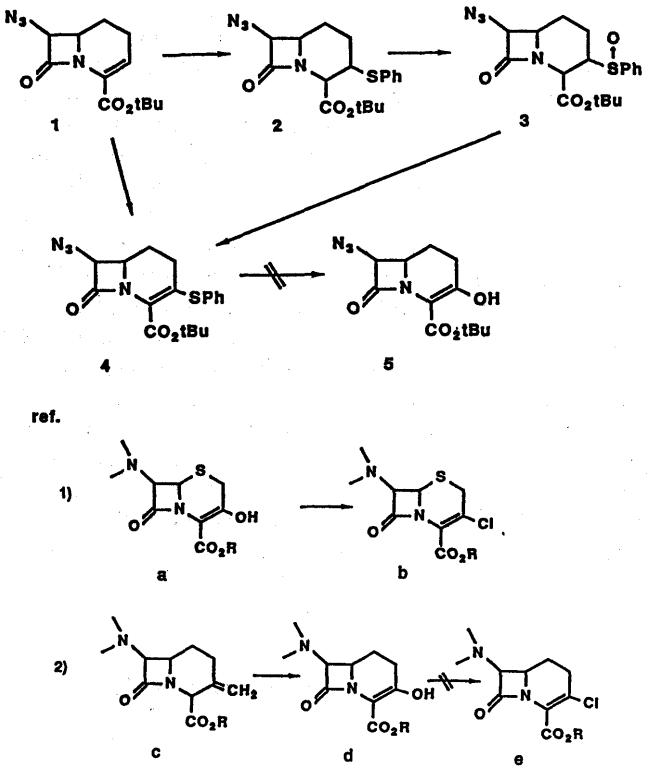


Chart 2

3α-SPh-4α-$CO_2{}^tBu$ with a twisted chair form of the tetrahydropyridine ring as judged from the almost null coupling constant of C_3H–C_4H in the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum and the presumed favorable addition of phenylsulfide anion from the less hindered α-face. Attempted hydrolysis of **2** with various mercuric salts failed to give the 3-OH compound. Dehydrogenation of **2** also failed to afford the phenyl sulfide **4**. Very facile oxidation of **2** was effected with either *m*-chloroperbenzoic acid (*m*-CPBA) or hydrogen peroxide to give rise

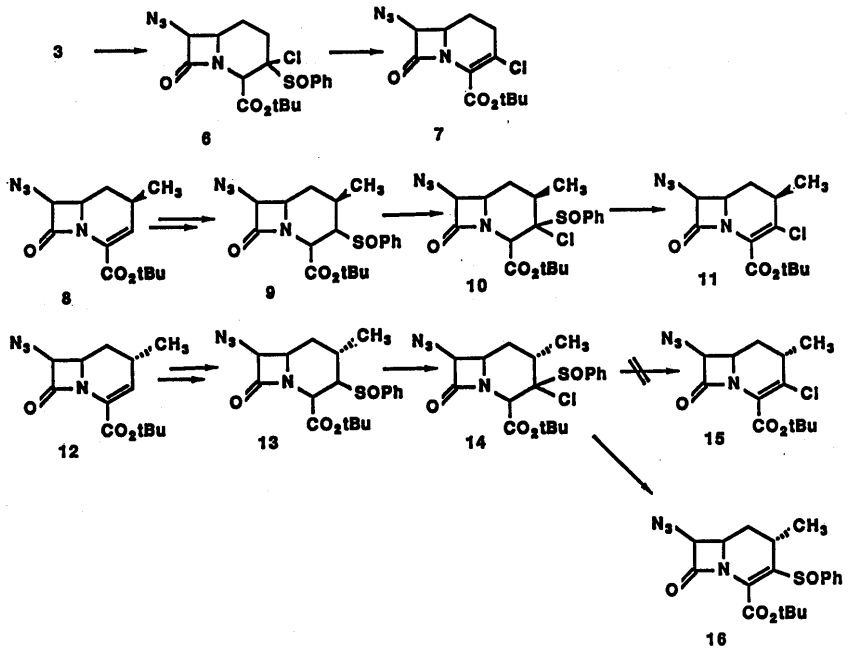


Chart 3

to the phenylsulfinyl compound **3**. Treatment of excess peracid resulted in the concomitant formation of the sulfone. The Pummerer reaction was applied to the sulfoxide **3** using acetic anhydride at elevated temperature, but the only product detected was the starting compound **1** formed by elimination of phenylsulfinic acid. The reaction proceeded successfully to give compound **4** by employing trifluoroacetic anhydride[6] instead of acetic anhydride. The phenyl sulfide **4** was also prepared directly from **1** with phenylsulfenyl chloride in moderate yield. However, attempted hydrolysis of **4** to the objective 3-OH compound **5** failed under various reaction conditions.

Then we turned our attention to α-chlorination of the phenylsulfinyl compound **3** followed by elimination of phenylsulfinic acid to lead to the 3-chlorocarbacephem **7**. Attempted chlorination of **3** with *N*-chlorosuccinimide or *p*-toluenesulfonyl chloride was unsuccessful, but Pummerer-type chlorination proceeded smoothly with sulfuryl chloride[7] to afford the α-chloro sulfoxide **6** which, on brief heating at reflux in carbon tetrachloride, gave the objective chloro compound **7**, liberating sulfinic acid.

As shown in Chart 3, the 2β-methyl analog was subjected to an analogous sequence of reactions to afford the 2β-methyl-3-chloro compound **11**. In the 2α-methyl series on the contrary, the α-chloro sulfoxide **14** did not give the 3-chloro compound **15** even at elevated temperature. Prolonged heating of **14** in toluene with pyridine afforded the phenylsulfinyl compound **16**.

The reaction mechanism can be presumed to be as follows. In all cases, thiophenol attacks C-3 of the olefin **1**, **8** or **12** from the less hindered α-face to form the 3α-phenylthio compound, which is oxidized to 3α-phenylsulfinyl compound **3**, **9** or **13**.

In the 2H or 2β-methyl series, subsequent chlorination with sulfuryl chloride occurs again from the less hindered α-side of the presumed sulfinium intermediate f to form the α-chloro-β-phenylsulfonyl compound **6** or **10**, which in turn affords the 3-chloro compound **7** or **11** by facile *syn*-

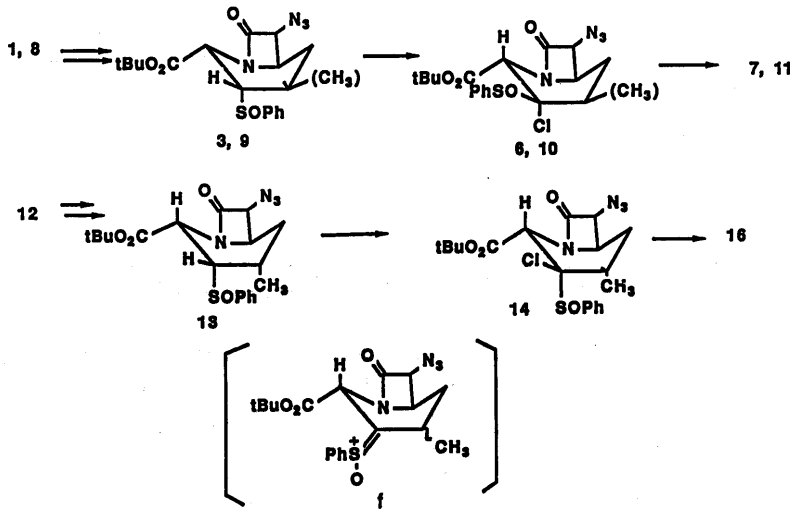


Chart 4

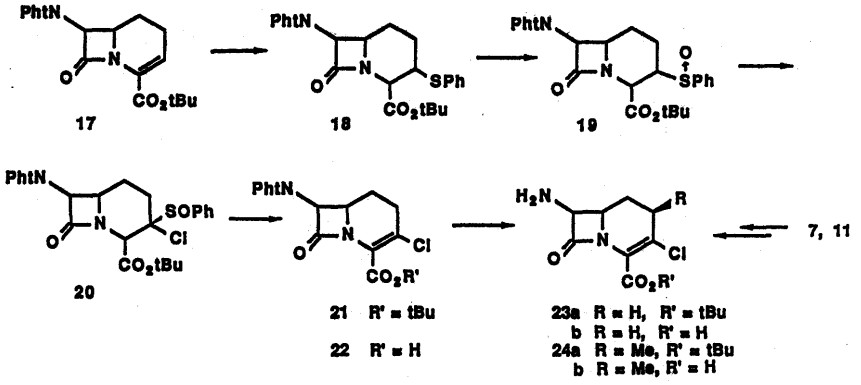
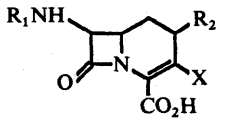


Chart 5

TABLE I. Comparative Antimicrobial Activity of 3-Cl-1-Carbacephems



MIC (μg/ml)

R_1	R_2	X	Compd. No.	S.a. 209P	E.c. NIHJJC-2	K.p. 8045	S.m. T-55	P.mir. 1289	P.v. 6897	P.ret. 4289	P.a. #1
ATM (±)	H	H		12.5	≦0.01	≦0.01	0.1	≦0.01	≦0.01	≦0.01	25
	H	Cl	**25**	50	0.78	≦0.05	1.56	0.1	0.4	0.1	100
	β-CH_3	Cl	**26**	100	6.25	0.78	12.5	0.4	0.4	100	>100
ATM	H	H		3.13	≦0.01	≦0.01	0.02	≦0.01	≦0.01	≦0.01	6.25
	H	Cl	**29**	6.25	0.1	0.05	0.78	0.01	0.02	0.02	100
PG	H	H		0.4	3.13	0.78	12.5	12.5	25	100	>100
	H	Cl	**30** KT3777	0.1	1.56	0.2	6.25	3.13	100	100	>100
		Cefaclor		0.05	3.13	0.1	12.5	3.13	12.5	100	>100
HPG	H	H		0.4	3.13	0.78	6.25	50	100	12.5	>100
	H	Cl	**31**	0.4	1.56	0.78	6.25	3.12	100	100	>100

Agar dilution method, inoculum size 10^6 cfu/ml. PG, D-phenylglycyl; ATM, 2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetyl; HPG, *p*-hydroxy-D-phenylglycyl. S.a., *Staphylococcus aureus*; E.c., *Escherichia coli*; K.p., *Klebsiella pneumoniae*; S.m., *Serratia marcescens*; P.mir., *Proteus mirabilis*; P.v., *Proteus vulgaris*; P.ret., *Proteus rettgeri*; P.a., *Pseudomonas aeruginosa*.

elimination of phenylsulfinic acid.[8] On the contrary, in the 2α-methyl series chloride attacks C-3 from the β-face due to steric hindrance of the 2β-methyl group, leading to the 3β-chloro-3α-phenylsulfinyl compound **14** which is apparently resistant to *syn*-elimination of phenylsulfinic acid; instead, base-catalyzed elimination of hydrochloride gave compound **16**. The configurations of the 3-phenylthio and 3-phenylsulfinyl compounds are consistent with the observed coupling constants, $J_{3H,4H}$ in the ^{1}H-NMR spectra of these compounds, as mentioned previously.

Since an efficient preparative route to the 3-chloro-1-carbacephem nucleus has now been developed, we applied this method to the 7-phthalimide congener as a more practical procedure for large scale preparation. That is, the 7-phthalimide compound **17** was employed as the starting compound.[9] Thiophenol addition *m*-CPBA oxidation of the phenylsulfide, and α-chlorination of the phenylsulfoxide, followed by dephenylsulfination afforded the 3-chloro compound **21** in an overall yield of 56%. Throughout this process no difficulty was encountered in isolation and purification of each product.

With a fairly large quantity of the 3-chloro-1-carbacephem nucleus in hand, we tried to prepare several derivatives furnished with a typical 7-acyl group as seen in cephalosporin antibiotics. 7-Amino-1-carbacephem-2-car-

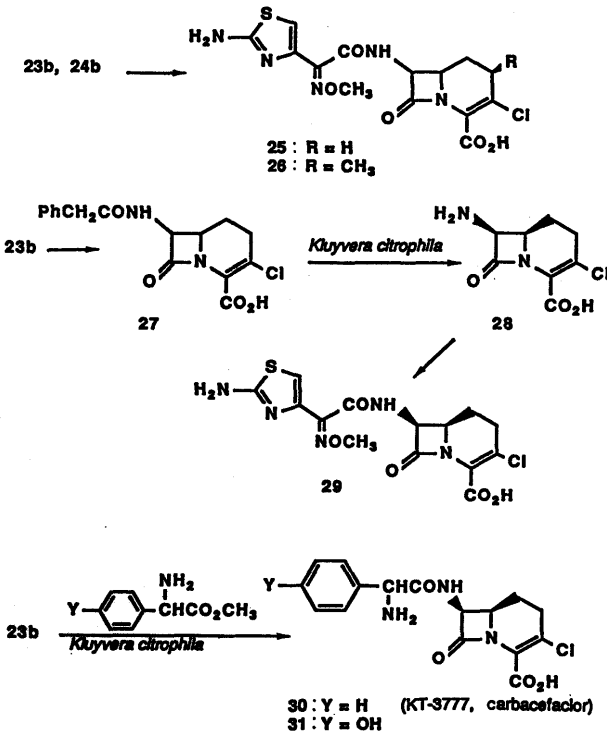


Chart 6

boxylic acids **23b** and **24b** were prepared from compounds **7** and **11** by catalytic hydrogenation of the azido group followed by acidic cleavage of the *tert*-butyl group.

Dephthaloylation of compound **22** by careful hydrazinolysis was also successful.[10] The 2-aminothiazol-2-(*Z*)-methoxyimino-acetyl group (heinafter abbreviated as ATM) was introduced to **25** and **26** with racemic carbacephem nuclei. The corresponding optically active compound **29** was prepared by employing the procedure developed and applied for 3*H*-1-carbacephem compounds.[1] Namely the phenylacetamido compound **27** was enantioselectively deacylated with β-lactamase-deficient penicillin acylase produced by *Kluyvera citrophila* to afford the optically pure 3-chloro-1-carbacephem nucleus **28** which was then furnished with an acyl group to give **29**. 3-Chloro-1-carbacephem **30**, carbacefaclor and its *p*-hydroxy analog **31** were directly prepared efficiently by enzymatic acylation of the racemic nucleus **23b** enantioselectively by passing the substrate solution along with methyl phenylglycynate repeatedly through a column charged with penicillin acylase immobilized on the surface of a porous ceramic.

Biological Activities Comparative antimicrobial activities of 3-chloro-1-carbacephem compounds are shown in Table I. In the series of compounds with the ATM group, introduction of chlorine at C-3 did not improve the antimicrobial activity against any of the microbes tested. Optical resolution again improved the potency significantly, as is evident from the comparison of compound **29** with **25**. Among compounds with the 7-phenylglycyl group, the 3-chloro compound **30** or KT3777 showed antimicrobial potency and a breadth of antimicrobial spectrum far superior to those of the 3*H* compound and almost equivalent to those of cefaclor, the corresponding cephem analog. Addition of a *p*-hydroxy group to the phenyl ring did not alter the activity significantly. Antimicrobial activities of KT3777 or carbacefaclor against various clinical isolates were compared with those of cefaclor (Table II). Both compounds showed almost the same degree of activity against gram positive bacteria, but carbacefaclor was almost twice as active as cefaclor against typical gram negative bacteria, *e.g.*, *Escherichia coli* and *Klebsiella pneumoniae*.

Chemical stability is an important feature of an antibiotic for practical use. As is clearly shown in Table III, KT3777 unexpectedly demonstrated remarkably good stability, that is, at physiological pH no decomposition product was detected after incubation for 22 h at 37 °C, whereas cefaclor showed a much shorter half life. This excellent stability is reflected in favorable pharmacokinetics of this carbacefaclor, KT3777.[11]

TABLE II. Antibacterial Activity against Various Clinical Isolates

Organism (No.)	Antibiotic	Range	MIC_{50}
S. aureus	KT3777	0.39—>100	1.56
(51)	Cefaclor	0.39—>100	1.56
S. pyogenes	KT3777	0.1 —0.39	0.2
(19)	Cefaclor	0.1 —0.12	0.2
H. influenzae	TK3777	0.78—1.56	1.56
(11)	Cefaclor	0.78—3.13	1.56
E. coli	KT3777	0.2 —>100	0.78
(54)	Cefaclor	0.39—>100	1.56
K. pneumoniae	KT3777	0.2 —>100	0.39
(54)	Cefaclor	0.2 —>100	0.78

Experimental

Infrared (IR) spectra were measured with a JASCO IR-810, and ^{1}H-NMR spectra were measured at 60 MHz on a Varian T-60 spectrometer and at 100 MHz on a JEOL GNM PS-100. ^{13}C-NMR spectra were measured on a JEOL FX-100 spectrometer. Optical rotations were measured on a Perkin Elmer model 141 polarimeter.

For column chromatography, silica gel (Wako C-200) was used unless otherwise specified. Thin layer chromatography (TLC) was performed on Silica gel 60 F_{254} plates (Merck). All organic solvent extracts were dried over anhydrous sodium sulfate.

***tert*-Butyl (6*R**,7*S**)-3-Phenylthio-7-azido-8-oxo-1-azabicyclo[4.2.0]-octan-2-carboxylate (2)** Thiophenol (0.2 ml) and piperidine (0.2 ml) were added to a solution of 528 mg of **1** in 15 ml of absolute benzene. The reaction mixture was stirred at room temperature for 2 h, washed with 10% citric acid solution and brine, dried and evaporated under reduced pressure. The oily residue was chromatographed on silica gel. Elution with hexane–AcOEt (4:1) gave **2** (720 mg, 96.3%). mp 77.5—78 °C. MS *m/z*: 374 (M^+). IR (KBr): 2110, 1765, 1745, 1155 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.28—7.67 (5H, m), 4.78 (1H, d, *J*=4.8 Hz), 4.33 (1H, s), 3.78—3.98 (1H, m), 3.81 (1H, br s), 1.50—2.34 (4H, m), 1.42 (9H, s).

***tert*-Butyl (6*R**,7*S**)-3-Phenylsulfinyl-7-azido-8-oxo-1-azabicyclo-[4.2.0]octan-2-carboxylate (3)** *m*-CPBA (240 mg) was added to a solution of 480 mg of **2** in 50 ml of distilled $CHCl_3$ under ice cooling. After being stirred at 0 °C for 30 min, the reaction mixture was washed with saturated $NaHCO_3$ solution and brine, dried and evaporated to afford **3** (500 mg, 99.9%). mp 95.5—96.5 °C. IR (KBr): 2120, 2100, 1780, 1735, 1160 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.55—7.91 (5H, m), 4.87 (1H, d, *J*=4.0 Hz), 4.05 (1H, s), 3.90—4.10 (1H, m), 3.10 (1H, br s), 1.70—2.84 (4H, m), 1.30 (9H, s). The 2β-methyl analog **9** and 2α-methyl analog **13** were prepared similarly from **8** and **12**, respectively, *via* the corresponding sulfides.

9: IR (KBr): 2400, 2120, 1778, 1743 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.53—8.05 (5H, m), 4.80 (2H, d, *J*=4.6 Hz), 4.06 (1H, s), 3.90—4.11 (1H, m), 3.26 (1H, s), 1.8—2.2 (3H, m), 1.54, 1.41 (3H, d, *J*=4.4 Hz), 1.31 (9H, s).

13: IR (KBr): 2130, 2110, 1790, 1725 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.38—7.96 (5H, m), 5.02 (2H, m), 4.03 (1H, s), 3.9—4.2 (1H, m), 3.10 (1H, m), 1.80 (2H, m), 1.33 (9H, s), 1.13 (3H, d, *J*=7.2 Hz).

TABLE III. Comparative Chemical Stability

	pH 1.0 NaOAc–HCl buffer 37 °C, 27 h 600 μg/ml	pH 7.2 Phosphate buffer 37 °C 600 μg/ml	pH 10.3 Borate–Na_2CO_3 buffer 37 °C	
			600 μg/ml	600 μg/ml
30	Residual (%)	Decomposition rate		
KT3777	98	No decomposition after 22 h	k, 1.14×10^{-2} h^{-1} $t_{1/2}$, 26.4 h	k, 1.08×10^{-2} h^{-1} $t_{1/2}$, 27.8 h
Cefaclor	86	k, 2.61×10^{-1} h^{-1} $t_{1/2}$, 1.05 h	k, 6.19×10^{-1} h^{-1} $t_{1/2}$, 0.49 h	k, 4.73×10^{-1} h^{-1} $t_{1/2}$, 0.64 h

HPLC: Nucleosil C_{18} 4.6×250 mm, 40% MeOH–phosphate buffer pH 3. Detection: UV at 250 nm.

***tert*-Butyl (6*R**,7*S**)-3-Phenylthio-7-azido-9-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylate (4)** Method A: 2,6-Lutidine (0.88 ml) and trifluoroacetic anhydride (0.80 g) were added to a solution of the sulfoxide **3** (370 mg) in 5.7 ml of CH_3CN. The mixture was stirred at 60 °C for 30 min, then 6 ml of $NaHCO_3$ solution and 10% citric acid solution were added to adjust the pH *ca.* 4. The resulting mixture was extracted with AcOEt and then dried. The solvent was evaporated off *in vacuo* and the residue was subjected to chromatography (SiO_2, 7 g; *n*-hexane : AcOEt = 10 : 1) to obtain 197 mg (55.8%) of **4**. IR (KBr): 2120, 1790, 1750 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.17—7.57 (5H, m), 4.88 (1H, d, J = 5.0 Hz), 3.72 (1H, m), 1.70—2.33 (4H, m), 1.57 (9H, s). MS *m/z*: 372 (M^+), 316.

Method B: Benzenesulfenyl chloride (43 mg) was added to a solution of compound **1** (53 mg) in 1 ml of CH_2Cl_2 at −78 °C. The mixture was stirred at −78 °C to room temperature for 2 h then washed with $NaHCO_3$ solution. The solution was evaporated *in vacuo* and the residue was subjected to chromatography (SiO_2, 5 g; *n*-hexane : AcOEt = 20 : 1—4 : 1) to obtain 40 mg (53.8%) of **4**. The properties of the compound agreed with those of the product obtained by method A.

***tert*-Butyl (6*R**,7*S**)-3-Chloro-3-phenylsulfinyl-7-azido-8-oxo-1-azabicyclo[4.2.0]octan-2-carboxylate (6)** A suspension of 109 mg of **3** and 23.5 mg of CaO in 1 ml of CH_2Cl_2 was treated with 27 μl of SO_2Cl_2 at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, washed successively with 10% citric acid solution, saturated $NaHCO_3$ solution and brine, dried, and evaporated. The residue was chromatographed on silica gel, eluting with hexane–AcOEt (5 : 1), to give **6** as an oil (66.5 mg, 56.1%). MS *m/z*: 424 (M^+). IR ($CHCl_3$): 2120, 1770, 1735, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.53—8.00 (5H, m), 4.90 (1H, d, J = 5.2 Hz), 4.43 (1H, s), 4.15—4.35 (1H, m), 1.83—2.85 (4H, m), 1.38 (9H, s).

The 2β-methyl analog **10** and 2α-methyl analog **14** were similarly prepared from **9** and **13**, respectively.

10: IR (KBr): 2120, 1776, 1738 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.28—7.88 (5H, m), 4.73 (1H, d, J = 5.0 Hz), 4.59 (1H, br s), 4.0 (1H, s), 2.95 (1H, m), 1.8—2.0 (2H, m), 1.36 (9H, s), 1.16 (3H, d, J = 6.4 Hz).

14: IR (KBr): 2160, 2120, 1785, 1740 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.4—8.1 (5H, m), 4.86 (1H, d, J = 4.8 Hz), 4.40 (1H, br s), 4.2 (1H, m), 2.3—3.1 (2H, m), 1.8 (1H, m), 1.39 (9H, s), 1.26 (3H, d, J = 6.2 Hz).

***tert*-Butyl (6*R**,7*S**)-3-Chloro-7-azido-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylate (7)** Compound **6** (1.3 g) was dissolved in 100 ml of CCl_4 and the solution was refluxed for 6 h. After cooling, the reaction mixture was evaporated. The product was purified by column chromatography on silica gel with *n*-hexane–AcOEt (5 : 1) to give **7** (596 mg, 65.2%). mp 96.0—97.0 °C. MS *m/z*: 298 (M^+). IR (KBr): 2120, 1765, 1735, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.93 (1H, d, J = 5.1 Hz), 3.82 (1H, ddd, J = 4.2, 5.1, 10.7 Hz), 2.56—2.70 (2H, m), 1.86—2.32 (2H, m), 1.55 (9H, s). The 2β-methyl analog **11** was prepared similarly except that the temperature was raised to the reflux temperature of toluene.

11: IR (KBr): 2110, 1785, 1733 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.91 (1H, d, J = 4.4 Hz), 3.67—4.31 (1H, m), 2.23—2.90 (2H, m), 1.93—2.23 (1H, m), 1.54 (9H, s), 1.33 (3H, m).

***tert*-Butyl (4*S**,6*R**,7*S**)-3-Phenylsulfinyl-4-methyl-7-azido-8-oxo-1-azabicyclo[4.2,0]oct-2-en-2-carboxylate (16)** Pyridine (0.2 ml) was added to a solution of **14** (704 mg) in 70 ml of toluene. The mixture was refluxed for 37 h 40 min. After cooling to room temperature, the reaction mixture was washed with saturated NaCl solution, dried and evaporated *in vacuo*. The residue was subjected to chromatography (SiO_2, 75 g; *n*-hexane : AcOEt = 2 : 1) to obtain 37.9 mg (58.6%) of **16**. IR (KBr): 2130, 2110, 1790, 1725, 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.92—7.40 (5H, m), 5.03 (1H, d, J = 5.0 Hz), 4.32—3.85 (1H, m), 2.90—2.10 (3H, m), 1.60 (9H, s), 1.39 (3H, d, J = 8.0 Hz).

***tert*-Butyl (6*R**,7*S**)-3-Phenylthio-7-phthalimido-8-oxo-1-azabicyclo-[4.2.0]oct-2-en-2-carboxylate (18)** Thiophenol (2.83 ml) and 0.5 ml of piperazine were added to a solution of 9.20 g of **17** in 100 ml of $CHCl_3$. The reaction mixture was stirred for 3 h and concentrated. The residue was chromatographed on silica gel with *n*-hexane–AcOEt (2 : 1) to afford **18** as colorless crystals (9.26 g, 77.5%). IR (KBr): 1800, 1785, 1775, 1770, 1750, 1740, 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.2—7.8 (9H, m), 5.50 (1H, d, J = 5.2 Hz), 4.53 (1H, s), 3.8—4.1 (1H, m), 3.82 (1H, br s), 1.5—2.3 (4H, m), 1.47 (9H, s).

***tert*-Butyl (6*R**,7*S**)-3-Chloro-7-phthalylimido-8-oxo-1-azabicyclo-[4.2.0]oct-2-en-2-carboxylate (21)** A solution of 4.78 g of **18** in 100 ml $CHCl_3$ was treated with 2.37 g of *m*-chloroperbenzoic acid at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and 1 ml of 10% sodium thiosulfate solution was added. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried and evaporated. The resultant white solid was dissolved in 50 ml of CH_2Cl_2 and 1.45 ml of SO_2Cl_2 was added to the solution under ice cooling. The reaction mixture was stirred for 1 h at 0 °C, diluted with 50 ml of CH_2Cl_2, washed successively with 10% citric acid solution, $NaHCO_3$, and brine, dried, and evaporated. The colorless powder thus obtained was dissolved in 80 ml of toluene. The solution was refluxed for 2 h and evaporated. The product was chromatographed over silica gel with *n*-hexane–AcOEt (1 : 1) to afford **21** as colorless crystals (3.50 g, 87%). mp 177.6 °C. IR (KBr): 1795, 1785, 1760, 1750, 1730, 1715, 1605 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.7—7.9 (4H, m), 5.58 (1H, d, J = 6.0 Hz), 3.85—4.1 (1H, m), 2.5—2.7 (2H, m), 1.8—2.4 (2H, m), 1.52 (9H, s). ^{13}C-NMR ($CDCl_3$) δ: 167.3 (phthalimide carbonyl), 160.4 (C-8), 159.4 (CO_2-), 134.7, 131.5, 123.8 (phenyl), 126.9 (C-3), 125.4 (C-2), 83.3 (CMe_3), 56.8 (C-7), 53.0 (C-6), 31.5 (C-4), 28.0 (CH_3). *Anal.* Calcd for $C_{20}H_{19}ClN_2O_5$: C, 59.63; H, 4.75; Cl, 8.80; N, 6.95. Found: C, 59.91; H, 4.86; Cl, 8.73; N, 6.67.

(6*R,7*S**)-3-Chloro-7-phthalylimido-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (22)** A solution of 1.7 g of **21** and 8 ml of trifluoroacetic acid (TFA) was stirred at 0 °C for 1 h. The reaction mixture was concentrated under high vacuum to give a brown oil. Trituration with ether afforded 1.1 g (71.8%) of **22**. IR (KBr): 1785, 1770 (sh), 1755, 1720, 1710, 1610 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 7.92 (4H, br s), 5.70 (1H, d, J = 5.0 Hz), 3.95—4.2 (1H, m), 2.5—2.75 (2H, m), 1.8—2.15 (2H, m). High MS Calcd for $C_{16}H_{10}ClN_2O_5$ $(CH_3)_3Si$: 418.0750 (^{35}Cl), 420.0720 (^{37}Cl). Found: 418.0709 (^{35}Cl), 420.0584 (^{37}Cl).

***tert*-Butyl (6*R**,7*S**)-3-Chloro-7-amino-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylate (23a)** The 7-azido compound **7** (350 mg) was subjected to hydrogenolysis with 10% Pd–C catalyst (70 mg) in 100 ml of EtOH and 1.2 ml of 1 N HCl for 3 h at room temperature under atmospheric pressure. The catalyst was filtered off and washed with EtOH. The combined filtrate was evaporated. The residual solid was dissolved in water and washed with ether. The aqueous layer was adjusted to pH 8 with $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with brine, dried, and evaporated to give **23a** as a colorless powder (218 mg, 68.4%). IR (KBr): 1770, 1720, 1620 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.43 (1H, d, J = 4.8 Hz), 3.52—3.90 (1H, m), 2.52—2.72 (2H, m), 2.22 (2H, br s), 1.87—2.17 (2H, m), 1.55 (9H, s).

(6*R,7*S**)-3-Chloro-7-amino-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (23b)** Method A: The *tert*-butyl ester **23a** (102 mg) was treated with 1 ml of CF_3CO_2H for 30 min at room temperature. The reaction mixture was evaporated and the residue was triturated with ether to afford **23b** trifluoroacetate as a yellow powder (75.5 mg, 60.9%). IR (KBr): 1795, 1630, 1550 cm^{-1}. ^{1}H-NMR (D_2O + NaOD) δ: 4.48 (1H, d, J = 5.1 Hz), 3.88 (1H, m), 2.57—2.71 (2H, m), 1.80—2.18 (2H, m).

The 2β-methyl analog **24b** was similarly prepared.

24b: IR (KBr): 1795, 1780, 1760, 1635 cm^{-1}. ^{1}H-NMR (D_2O): δ 4.52 (1H, d, J = 5.0 Hz), 3.80 (1H, m), 1.9—2.9 (3H, m), 1.32 (3H, d, J = 9.1 Hz).

Method B: A cold aqueous solution of **22** (10.96 g in 150 ml H_2O) was treated with 1.85 eq of hydrazine hydrate and the solution was adjusted to pH 7.5—7.7 with 0.2 N NaOH. After 3 h of stirring at 5 °C the reaction mixture was warmed to 35 °C, acidified to pH 0.8 with 1 N HCl and kept standing for 4 h. A precipitate of phthalazide was removed by filtration. The filtrate was concentrated to 93 ml and kept standing overnight at room temperature. The precipitate was collected by filtration and dried to afford 5.54 g (purity 83.5%, yield 70.6%) of **23b** as a crude powder. This was purified by chromatography on Dianion HP-10 with H_2O to afford **23b** as colorless crystals. The physical data were identifical with those of **23b** prepared by method A. High MS Calcd for $C_8H_7ClN_2O_3$ $2(CH_3)_3Si$: 360.1090. Found: 360.1090.

(6*R,7*S**)-3-Chloro-7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyimino-acetylamino]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (25)** 2-(2-Chloroacetamidothiazol-4-yl)-2-(*Z*)-methoxyiminoacetic acid (122.6 mg) was dissolved in 2.5 ml of anhydrous CH_2Cl_2. Then, 68 μl (0.49 mmol) of triethylamine was dissolved therein and 92.0 mg of PCl_5 was added under ice cooling. The mixture was stirred at that temperature for 1 h, after which 5 ml of *n*-hexane was added. The reaction mixture was stirred under ice cooling for an additional 15 min. An oily material was obtained by removing *n*-hexane by decantation. The oily material was dissolved in 4 ml of tetrahydrofuran (THF) to prepare an acid chloride solution. In a separate vessel, compound **23b** (121.7 mg) was dissolved in 5 ml of 50% aqueous THF and 0.2 ml of triethylamine. To this solution, the acid chloride solution prepared above was added with stirring under ice cooling. The mixture was stirred at that temperature for 1 h and adjusted to pH 3 with 1 N HCl. Water was then added and the mixture was extracted with EtOAc. The extract was washed with saturated NaCl and dried. The solvent was distilled off to yield 53.9 mg (30.5%) of the

chloroacetyl derivative of **25** as a powder. IR (KBr): 1770, 1680, 1555, 1045 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 9.38 (1H, d, J=8.0 Hz), 7.37 (1H, s), 5.45 (1H, q, J=5.0, 8.0 Hz), 4.35 (2H, s).

The chloroacetamido compound (51.2 mg) was dissolved in 1 ml of dimethylacetamide and 16.3 mg (0.22 mmol) of thiourea was added. The reaction was carried out by stirring the mixture at room temperature for 14 h. Then, 7 ml of ether was added to the reaction mixture and stirring was continued for an additional 10 min. A separated oily material obtained by removing the ether by decantation was dissolved in a small amount of dimethylsulfoxide and chromatographed (10 ml of HP-10, water–MeOH) to obtain 15.2 mg of **25** as an amorphous powder. mp: 185.0—188.0 °C (dec.). IR (KBr): 1765, 1670, 1630, 1540, 1040 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 9.28 (1H, d, J=8.8 Hz), 7.17 (2H, s), 6.75 (1H, s), 5.44 (1H, q, J=5.3, 8.8 Hz), 3.84 (3H, s), 1.24—2.52 (4H, m). The 2β-methyl analog **26** was similarly prepared as a powder.

26: IR (KBr): 1770(sh), 1755, 1690(sh), 1670 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 9.28 (1H, d, J=8.6 Hz), 7.19 (2H, br s), 6.77 (1H, s), 5.45 (1H, dd, J=8.6, 5.3 Hz), 3.95 (3H, s), 2.75 (1H, m), 1.56—2.19 (2H, m), 1.22 (3H, d, J=6.8 Hz).

(6*R,7*S**)-3-Chloro-7-phenylacetamido-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (27)** The CF_3CO_2H salt of **23b** (150 mg) was suspended in a mixed solvent of 2 ml of water and 2 ml of acetone, and 134 mg of $NaHCO_3$ was added thereto to make a homogeneous solution. Next, a solution of 84.2 mg of phenylacetyl chloride in 0.5 ml of acetone was added dropwise under ice cooling over 1 h. The mixture was stirred for 3 h, adjusted to pH 2 with 1 N HCl and extracted with five 2 ml portions of EtOAc. The extract was concentrated under reduced pressure and the residue was dried to obtain 80 mg of **27** as a white powder. IR (KBr): 1790, 1705, 1630, 1560 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 7.29 (5H, s), 5.36 (1H, d, J=5.0 Hz), 3.79—3.99 (1H, m), 2.56—2.75 (2H, m), 1.17—2.02 (2H, m).

(6*R*,7*S*)-3-Chloro-7-amino-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (28) *Kluyvera citrophila* KY7844 was inoculated into 10 ml of a seed medium containing 1% polypeptone, 1% yeast, 0.5% meat extract, 0.5% sodium glutamate and 0.25% NaCl, and cultured at 30 °C for 24 h. The whole seed broth was then inoculated into 300 ml of a culture medium having the same composition as that of the seed medium, and the culturing was carried out with shaking at 30 °C for 24 h. The culture broth was subjected to centrifugation to obtain cell bodies. The cells were washed twice with 50 ml of 0.9% saline solution and suspended at a concentration of 40 mg/ml (dry weight) in 1/30 M phosphate buffer solution (pH 8.0). Compound **27** (200 mg) was added to 9 ml of 1/30 M phosphate buffer (pH 8.0), then 2 N NaOH was added in small portions and the mixture was adjusted to pH 8.0 to dissolve the compound. Deionized water was added to make 10 ml of solution.

The disrupted cell suspension (10 ml) mentioned above was added to the substrate solution and the enzyme reaction was carried out at a temperature of 40 °C for 80 min.

After the completion of the reaction, cells were removed by centrifugation from the reaction solution. The supernatant was concentrated under reduced pressure to make 5 ml of solution. The solution was charged on a column (1.75 cm width, 42 cm height) packed with Diaion HP-10. Elution was carried out with deionized water. The desired compound was eluted from 90 to 120 ml. These fractions were concentrated under reduced pressure to make 2 ml of solution and the solution was adjusted to pH 3.5 with 1 N HCl to deposit crystals. The crystals were recovered by filtration, washed with a small amount of MeOH and dried to obtain 38 mg (59.0%) of a white powder. IR (KBr): 3200, 1800, 1790(sh), 1640(sh), 1630, 1555 cm^{-1}. ^{1}H-NMR (D_2O) δ: 4.47 (1H, d, J=5.1 Hz), 3.88 (1H, m), 2.64 (2H, m), 1.93 (2H, m). $[\alpha]_D^{25}$: −2.7° (c=0.24, 1 M phosphate buffer, pH 7.0).

(6*R,7*S**)-3-Chloro-7-[2-(2-aminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetamido]-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (29)** A solution of 920 mg of 2-(2-tritylaminothiazol-4-yl)-2-(*Z*)-methoxyiminoacetic acid and 0.282 ml of triethylamine in 30 ml of THF was treated with 420 mg of PCl_5 at −20 °C. The reaction mixture was stirred at −20 °C for 1 h to prepare an acid chloride solution. The acid chloride solution was added to a solution of 450 mg of **28** in 20 ml of THF and 20 ml of water under ice cooling, keeping the pH at 7.0 with triethylamine. After stirring for 1 h, the solution was saturated with NaCl and the resulting aqueous layer was extracted twice with THF. The combined organic layers were concentrated *in vacuo* and the residue was treated with 40 ml of 50% acetic acid at 50 °C for 1 h. The reaction mixture was concentrated and shaken well with 40 ml each of EtOAc and water. The aqueous layer was concentrated and the residue was chromatographed on Diaion HP-10, eluting with methanol–water (1 : 2) to give **29** as a white powder. (340 mg, 40.9%). SIMS m/z: 400 $(M+1)^+$. $[\alpha]_D^{21}$: +21.0° (c=0.2, 1/30 M phosphate buffer pH 7). The ^{1}H-NMR and IR spectra were identical with those of **25**.

(6*R*,7*S*)-3-Chloro-7-(*R*)-phenylglycinamido-8-oxo-1-azabicyclo[4.2.0]oct-2-en-2-carboxylic Acid (30) Porous ceramic (*ca.* 1 mm diameter, Corning Glass Works) was treated with γ-aminopropyltriethoxysilane and then with glutaraldehyde. The crude enzyme preparation, an extract of disrupted cells of *Kluyvera citrophila* KY-7844, was reacted with this pretreated porous ceramic to form an immobilized enzyme. Compound **23b** (9.73 g) was dissolved in 580 ml of phosphate buffer (pH 6.75) and 51 g of methyl phenylglycinate was added. The solution mixture was passed through the column of immobilized enzyme (185 ml). The inner temperature of the column was kept at 20 °C. The eluent was recycled through the column another three times. The total eluate of time required was 8.5 h. The final eluate provided 6.15 g of **30**, KT3777, in 78.2% yield. mp 205—215 °C (dec.). IR (KBr): 1788, 1754, 1690, 1607 cm^{-1}. ^{1}H-NMR (DCl–D_2O, pD 0.84 measured at 400 Hz on a Bruker AM400 spectrometer) δ: 7.43—7.51 (6H, m, phenyl protons), 5.36 (1H, d, J=4.9 Hz, C-7H), 5.18 (1H, s, C<u>H</u>–NH_2), 3.92 (1H, ddd, J=11.8, 4.9, 3.7 Hz, C-6H), 2.60 (1H, ddd, J=19.6, 11.5, 6.1 Hz, C-2αH), 2.49 (1H, ddd, J=19.6, 5.9, 1.8 Hz, C-2βH), 1.64 (1H, dddd, J=13.1, 6.1, 3.7, 1.8 Hz, C-1αH), 1.25 (1H, dddd, J=13.1, 11.8, 11.5, 5.9 Hz, C-1βH). ^{13}C-NMR (DCl–H_2O, pD 0.84 measured at 100 Hz on a Bruker AM400 spectrometer) δ: 170.0 (CONH), 164.5 (CO_2H), 166.5 (β-lactam CO), 134.3 (C-3), 132.5 (phenyl C-1′), 131.5 (phenyl C-4′), 130.6 (phenyl-3′,5′), 129.0 (phenyl-2′,6′), 123.6 (C-2), 58.6 (C-7), 57.5 (CH–NH_2), 53.6 (C-6), 31.9 (C-4), 22.0 (C-5). The chemical shifts were assigned by CH-COSY and COLOC. $[\alpha]_D^{21}$: +34.0° (c=0.35, H_2O). *Anal.* Calcd for $C_{16}H_{16}ClN_2O_4 \cdot H_2O$: C, 52.25; H, 4.93; N, 11.42. Found: C, 52.11; H, 4.77; N, 11.63. The *p*-hydroxyphenylglycyl derivative **31** was similarly prepared as a white powder.

31: IR (KBr): 1765, 1695, 1615 cm^{-1}. ^{1}H-NMR (D_2O) δ: 7.36 (2H, d, J=8.8 Hz), 6.96 (2H, d, J=8.8 Hz), 5.36 (1H, d, J=4.6 Hz), 5.11 (1H, s), 3.81—4.00 (1H, m), 2.42—2.58 (2H, m), 1.59—1.77 (1H, m), 1.17—1.48 (1H, m). $[\alpha]_D^{20}$: +44.0° (c=0.25, 1 M phosphate buffer, pH 7.0).

Determination of Minimal Inhibitory Concentrations (MICs) MICs were determined by means of the usual twofold serial dilution method with Mueller Hinton agar (Difco). One loopful of diluted overnight culture in Mueller Hinton broth (Difco) of each test organism (about 10^6 colony-forming units per ml) was inoculated onto assay media containing graded concentrations of the test drug. MICs were determined after incubation at 37 °C for 18 h. Clinical isolates of various species of bacteria were provided from several university hospitals in Japan.

References and Notes

1) *a*) T. Hirata, T. Ogasa, H. Saito, S. Kobayashi, A. Sato, Y. Ono, Y. Hashimoto, S. Takasawa, K. Sato, and K. Mineura, Abstracts of Papers, 21st Intersci. Conf. on Antimicrob. Agents and Chemother., Chicago, Ill., September 1981; *b*) T. Hirata, T. Ogasa, H. Saito, and N. Nakamizo, Japan. Patent 79128591 (1979) [*Chem. Abstr.*, **93**, 150115*a* (1980)] and Japan. Patent 8049375 (1980) [*Chem. Abstr.*, **93**, 168137*u* (1980)]; *c*) T. Ogasa, H. Saito, Y. Hashimoto, K. Sato, and T. Hirata, *Chem. Pharm. Bull.*, **37**, 315 (1989).
2) R. R. Chauvette and P. A. Pennington, *J. Am. Chem. Soc.*, **96**, 4986 (1976).
3) *a*) T. Hirata, I. Matsukuma, Japan. Patent 8087791 (1980) [*Chem. Abstr.*, **93**, 239247*t* (1980)]; *b*) T. Hirata, I. Matsukuma, S. Yoshiie, K. Satoh, and Y. Ohhashi, Japan. Patent 8116491 (1981) [*Chem. Abstr.*, **94**, 121355*u* (1981)].
4) S. Uyeo and H. Ona, *Chem. Pharm. Bull.*, **28**, 1563 (1980).
5) J. H. Bateson, P. M. Roberts, T. C. Smale, and R. Southgate, *J. Chem. Soc., Chem. Commun.*, **1980**, 185.
6) H. Sugihara, R. Tanikaga, and A. Kaji, *Synthesis*, **1978**, 881.
7) K.-C. Tin and T. Durst, *Tetrahedron Lett.*, **1970**, 4643.
8) V. Reutrakul and P. Thamnusan, *Tetrahedron Lett.*, **1979**, 617.
9) An efficient and practical synthesis of this compound will be presented in a separate paper.
10) A paper is under preparation.
11) The *in vitro* and *in vivo* antimicrobial activities will be reported in *J. Antibiot.*, in press.