Total Synthesis of 6-Hydroxy-*epi*-PS 5 and 6-Methoxy-*epi*-PS 5

Azuma WATANABE,* Yasuo FUKAGAWA, Tomoyuki ISHIKURA, and Takeo YOSHIOKA
SANRAKU INC., Central Research Laboratories, 9-1 Johnan 4 Chome, Fujisawa, 251
(Received November 13, 1986)

The stabilizing effect of the 6-methoxyl or 6-hydroxyl substituent on dehydropeptidase I sensitivity of PS 5 was examined by a new total synthesis of 6-methoxy-*epi*-PS 5 and 6-hydroxy-*epi*-PS 5 from dimethyl benzyloxycarbonylaminomalonate. The Wittig reaction of α-(*t*-butoxycarbonyl)propylidenetriphenylphosphorane with a pyrrolidine derivative predominantly afforded a *trans*-α,β unsaturated ester, which was epoxidated, *N*-deprotected and cyclized to give a bicyclic carbapenam with a C-6 hydroxyl group. After the 6-hydroxyl group was methylated, the 2-(acetamido)ethylthio side chain was introduced at C-3 of the carbapenam by benzeneselenenylation of the geminal diester, followed by eliminatlon of the seleninyl group. The resulting carbapenam was converted to 6-methoxy-*epi*-PS 5. Furthermore, 6-hydroxy-*epi*-PS 5 was synthesized by the use of tetrahydropyranyl and trimethylsilyl ethers for protection of the 6-hydroxyl group in a process similar to that for synthesis of 6-methoxy-*epi*-PS 5.

Although the carbapenem antibiotic PS 5[1] and related compounds[2] have very broad spectra of potent antimicrobial activity and high resistance to β-lactamase, they are unexpectedly sensitive to renal dehydropeptidase I (DHP-I), resulting in poor therapeutic effects in vivo. In a continued effort to improve the DHP-I stability of PS 5 to a clinically acceptable level, a variety of C-3 side chains have been introduced into PS 5,[3] but no PS 5 derivative with satisfactory DHP-I stability has yet been prepared.

Considered from the observations that the stability of cephalosporins to β-lactamase is improved by introduction of the 7α-methoxyl group[4] and that the stereochemistry of C-6 of carbapenems is not critically important for antimicrobial activity,[5] it is interesting to examine whether the introduction of the 6α-methoxyl or 6α-hydroxyl substituent serves to stabilize carbapenem against DHP-I or not. For this purpose, a new route of total synthesis of carbapenem has been developed using dimethyl benzyloxycarbonylaminomalonate as starting material. This paper describes the stereoselective total synthesis of 6-methoxy-*epi*-PS 5 (**1**) and 6-hydroxy-*epi*-PS 5 (**2**) together with their enzymological and antimicrobial evaluation.

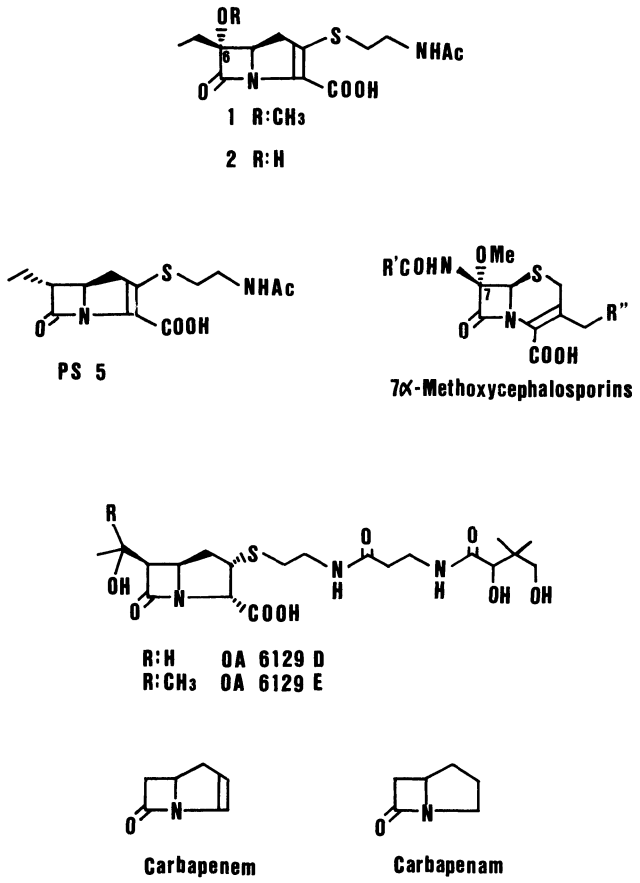

Fig. 1.

Results and Discussion

Carbapenam compounds such as OA 6129D and E[6] are physicochemically very stable and easily convertible to carbapenems. In hitherto known routes of synthesis of carbapenems, the β-lactam ring is formed before the 5-membered pyrrolidine ring is closed, whereas the reverse route has least been reported.[7] Thus it is interesting to develop a new route to synthesize 6-methoxy-*epi*-PS 5 (**1**). This comprises the initial closure of the pyrrolidine ring, the susequent formation of the β-lactam ring and the final conversion of carbapenam to carbapenem, starting from dimethyl (*N*-protected amino)malonate **H** according to the retrosynthetic strategy as shown below (Fig. 2).

The key points to the successful synthesis seem to be in the introduction of a leaving group X at C-2 (derivative **B**), followed by conversion to carbapenem **A**, and in the stereoselective preparation of a *trans*-α,β-unsaturated ester by the Wittig reaction of a pyrrolidine derivative **G** with a stable ylid **F**. If this route of synthesis is operative as described above, an optically active compound **B** will be obtained from D- or L-glutamic acid.[8]

A key intermediate carbapenam **9** for 6-methoxy-*epi*-PS 5 (**1**) was prepared as shown above. The Michael addition of dimethyl benzyloxycarbonylaminomalonate (**3**) (readily preparable from dimethyl malonate)

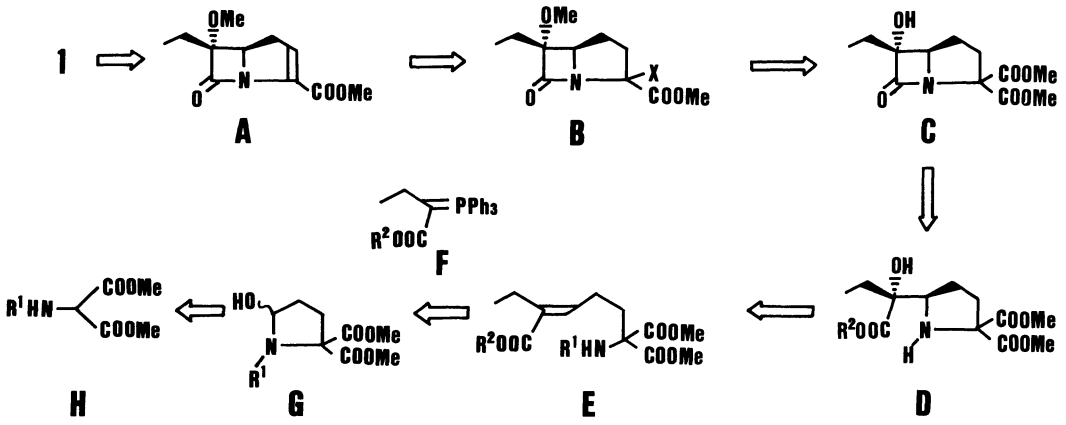

Fig. 2.

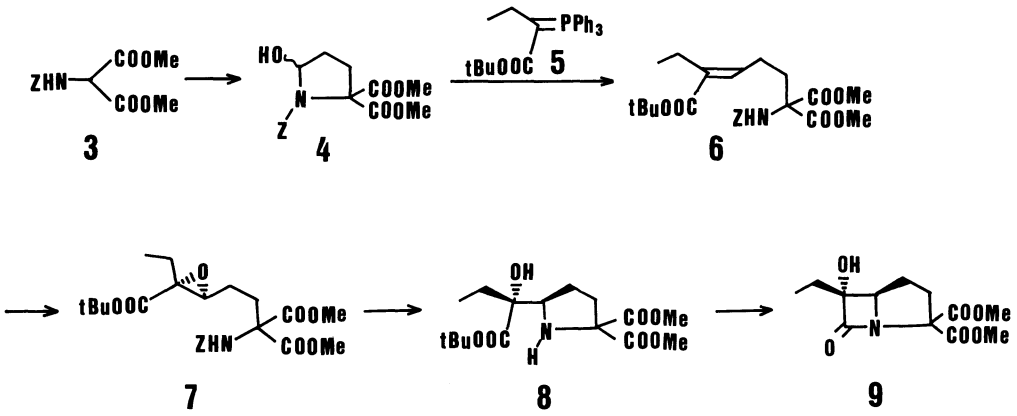

Fig. 3.

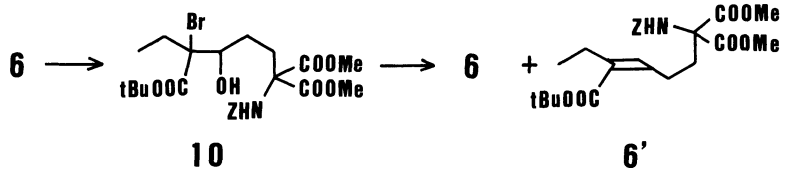

Fig. 4.

with acrylaldehyde in the presence of sodium methoxide catalyst afforded 5-hydroxypyrrolidine **4** in 85% yield. The Wittig reaction of **4** with α-(*t*-butoxycarbonyl)propylidenetriphenylphosphorane (**5**) predominantly provided a *trans*-α,β-unsaturated ester **6** in good yield under reflux in benzene for 16 h. The geometrical isomerism of **6** was examined by transformation to the cis isomer **6'** according to the following scheme (Fig. 4):

The treatment of the unsaturated ester **6** with *N*-bromoacetamide in aqueous acetone gave bromohydrins **10**, which were subjected to reduction with tributyltin hydride, methylsulfonylation with methanesulfonyl chloride and triethylamine, and β-elimination with 1,6-diazabicyclo[5.4.0]undec-7-ene, affording geometrical isomers **6** and **6'**. The cis isomer **6'** was separated by preparative silica-gel thin-layer chromatography (TLC). The NMR spectrometric comparison of these isomers by the chemical shift of the vinyl proton clearly showed that the isomerism of the predominant compound **6** was trans (δ=6.44; δ=5.64 for **6'**).[9] The oxidation of **6** with *m*-chloroperbenzoic acid in dichloromethane at 40 °C for 4 h afforded an epoxide **7** (90% yield). The cleavage of the *N*-benzyloxycarbonyl group from **7** by hydrogenation gave a pyrrolidine derivative **8** in 89% yield. After the *t*-butyl ester group was hydrolyzed in 4 mol dm^{-3} HCl in dioxane, the resulting β-amino acid was cyclized at 60 °C for 4 h with dicyclohexylcarbodiimide in acetonitrile to provide the bicyclic β-lactam **9** in 70% yield.

The methylation of the hydroxyl group of **9** with methyl iodide in the presence of silver oxide in *N*,*N*-dimethylformamide resulted in a methoxycarbapenam **11** (90% yield). The carbapenam **11** was transformed to a carbapenem **14** by selenoxide elimination which was operative under milder conditions than sulfoxide elimination. Benzeneselenenyl derivatives **13a** and **13b** are preparable from the diester **11** by two routes.

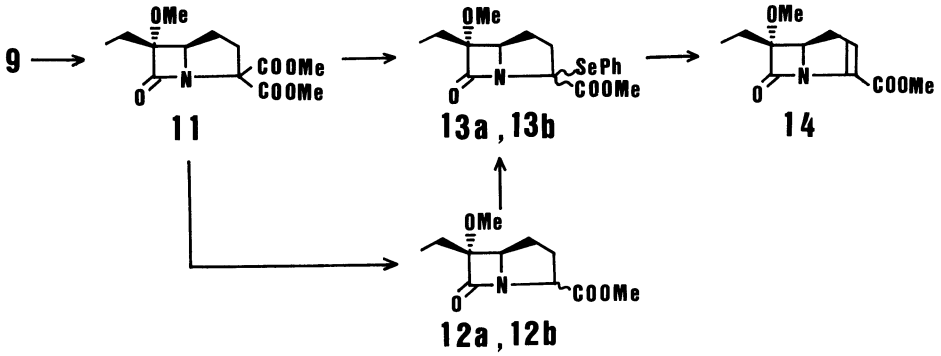

Fig. 5.

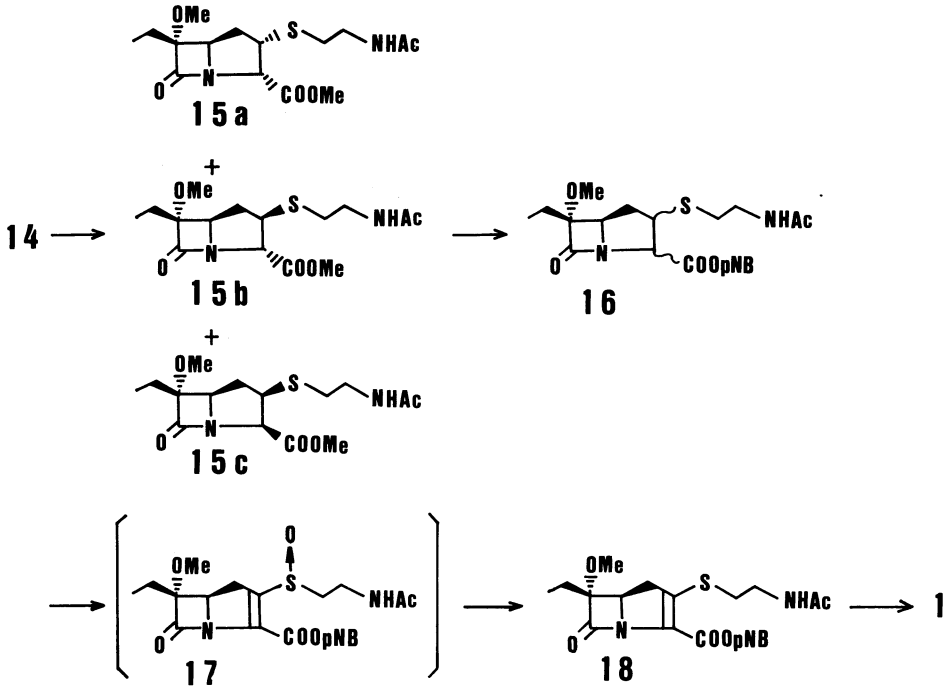

Fig. 6

First, the diester **11** was converted to the benzeneselenenyl compounds **13a** and **13b** via mono(methoxycarbonyl) derivatives **12a** and **12b**. More particularly, the diester **11** was hydrolyzed in 1 mol dm^{-3} NaOH and then heated at 100 °C in pyridine to give a diastereoisomeric mixture of the mono(methoxycarbonyl) derivatives **12a** and **12b** in 52% yield. After treatment with lithium diisopropylamide in tetrahydrofuran, the mixture was allowed to react with benzeneselenenyl chloride, leading to the benzeneselenenyl compounds **13a** and **13b** (a mixture of the two diastereoisomers) in a total yield of 61% from **12a** and **12b**.

Then the direct route of benzeneselenenylation was investigated by replacing sulfur by selenium in the procedure of Asaoka et al.[10] which was used to sulfenylate a geminal diester by demethoxycarbonylation. The reaction of 1 mol of the diester **11** with 1 mol of diphenyl diselenide in the presence of 2 mol of NaI in hexamethylphosphoric triamide at 80 °C for 2 h gave a mixture of **13a** and **13b** in 50% yield together with 31% of the unreacted diester **11**. The treatment of the isomeric mixture of the selenides **13a** and **13b** with *m*-chloroperbenzoic acid at −30 °C, followed by triethylamine addition, produced the carbapenem **14** in 78% yield via selenoxide.

The introduction of a 2-(acetamido)ethylthio side chain at C-3 of the carbapenem **14** in the presence of triethylamine in *N,N*-dimethylformamide give a diastereoisomeric mixture of **15a**, **15b**, and **15c** in a good yield of 95%. They were separated from each other by silica-gel column chromatography. ^{1}H NMR analysis indicated that the molar ratio of **15a** (δ=4.72, $J_{2,3}$=8.0 Hz), **15b** (δ=4.33, $J_{2,3}$=6.0 Hz), and **15c** (δ=4.19, $J_{2,3}$=8.0 Hz) was 2 : 5 : 3.[11]

The mixture **15a**, **15b**, and **15c** was hydrolyzed in 0.1 mol dm^{-3} NaOH and subjected to ester exchange with *p*-nitrobenzyl bromide to give a carbapenam *p*-nitrobenzyl ester **16** in 72% yield. The ester **16** was converted to a carbapenem **18** via a sulfoxide **17** in 54% yield by the method of Bateson et al.[12] 6-Methoxy-*epi*-PS 5 (**1**)

(sodium salt) was obtained from the *p*-nitrobenzyl ester (**18**) in 70% yield by hydrogenolytic removal of the *p*-nitrobenzyl group, followed by column chromatographic purification on QAE-Sephadex A-25 and Diaion CHP-20P.

Generally speaking, penicillins and cephalosporins are sensitive to β-lactamase and insensitive to DHP-I, whereas carbapenems are sensitive to DHP-I and insensitive to β-lactamase. The introduction of the 7α-methoxyl or 7α-hydroxyl group to cephalosporins improves the resistance to β-lactamase, but accompanies a reduction in antimicrobial activity. Thus it is interesting to compare the 6-methoxyl and 6-hydroxyl substituents of carbapenems to give useful information on the structure-activity relationship of carbapenems in antimicrobial activity, β-lactamase sensitivity, and DHP-I resistance. For this purpose, 6-hydroxy-*epi*-PS 5 (**2**) was prepared using a similar procedure.

Without protecting the 6-hydroxyl group, the alcohol **9** was converted directly to a benzeneselenenyl derivative **19** in a poor yield, which was then converted to a 6-hydroxycarbapenem **20**, also in a poor yield by oxydation, followed by elimination of the benzeneseleninyl group. For improvement of the reaction yields, the 6-hydroxyl group of **9** was protected with a tetrahydropyranyl (THP) group which survived the subsequent treatment.

Fig. 7.

The alcohol **9** was treated with 3,4-dihydro-2*H*-pyran in the presence of *p*-toluenesulfonic acid to yield an anomeric *O*-THP derivative mixture **21**, which was led to a carbapenam **25** in four steps in the same manner as described for 6-methoxy-*epi*-PS 5. The carbapenam **25** was convertible to the corresponding carbapenem, but the deprotection of the *O*-THP group without breakdown of the β-lactam ring was unsuccessful. Therefore the *O*-THP group of the carbapenam **25** was removed by treatment with pyridinium *p*-toluenesulfonate (PPTS)[13] in ethanol to provide an alcohol **26**. As no direct conversion to the corresponding carbapenem was successful, the alcohol **26** was reprotected with the trimethylsilyl (TMS) group which was readily deprotectable under mild conditions to give an *O*-TMS carbapenam **27**. The carbapenam **27** was transformed to a carbapenem **28** and the hydrolytic cleavage of the TMS ether **28** in 0.001 mol dm^{-3} HCl-ethanol (1 : 1) at 0 °C for 30 min gave a 6-hydroxycarbapenem **29** in 87% yield. The *p*-nitrobenzyl ester **29** was hydrogenated to yield 6-hydroxy-*epi*-PS 5 (**2**), as described above for 6-methoxy-*epi*-PS 5 (**1**). It is worth noting that the TMS group is useful for protection of hydroxyl groups on the carbapenem system.

Table 1 shows the comparative antimicrobial activities of 6-methoxy-*epi*-PS 5 (**1**), 6-hydroxy-*epi*-PS 5 (**2**), PS 5 and cefazolin. It seems highly probable that the introduction of the methoxyl or hydroxyl group at C-6 of PS 5 results in a significant reduction of the antimicrobial potency, as is reported for 7α-methoxy- and 7α-hydroxycephalosporins.[14] It is interesting to note that the 6-hydroxyl group is more influential than the 6-methoxyl in reduction of the antimicrobial activity.

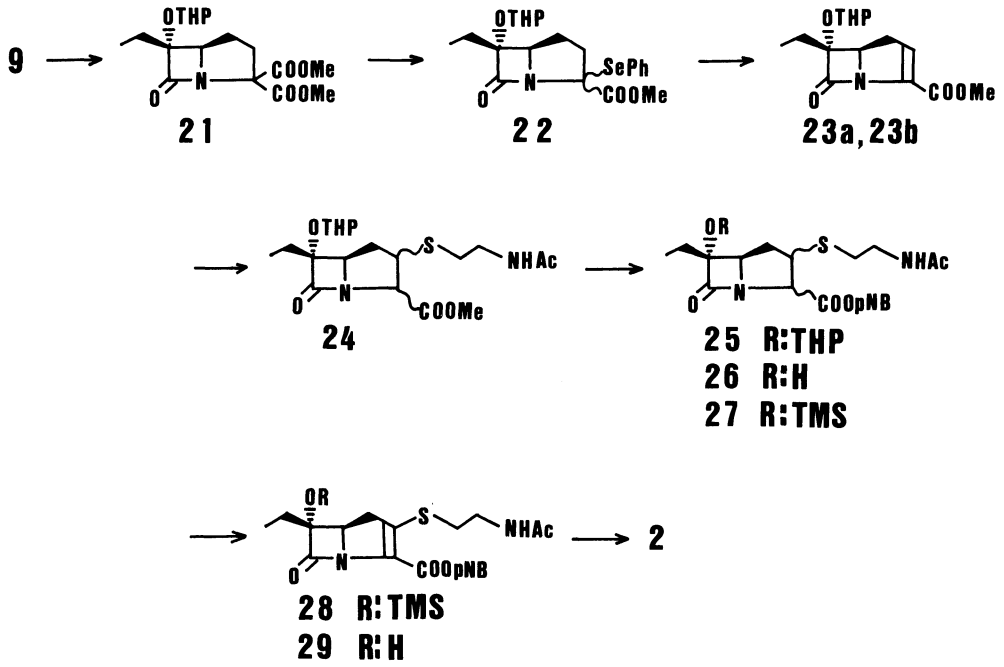

Fig. 8.

Table 1. Comparative Antimicrobial Activities of 6-Methoxy-*epi*-PS 5 (**1**), 6-Hydroxy-*epi*-PS 5 (**2**), PS 5 and Cefazolin (MIC in $\mu g\,ml^{-1}$)

Microorganism	1	2	PS 5	Cefazolin
Bacillus subtilis ATCC 6633	6.25	50	0.10	0.10
Staphylococcus aureus FDA209P	0.78	50	0.024	0.05
Staphylococcus aureus Smith	3.13	25	0.20	0.20
Citrobacter freundii GN346	>50	>50	1.56	>100
Comamonas terrigena IFO12685	0.78	—	0.012	0.10
Enterobacter cloacae 45	>50	>50	3.13	>100
Escherichia coli K-12	25	6.25	1.56	0.78
Proteus vulgaris GN76	>50	>50	12.5	>100
Pseudomonas aeruginosa NCTC10490	>50	>50	25	>100
Salmonella gallinarum ATCC9184	25	25	1.56	1.56
Serratia marcescens IFO3736	>50	>50	6.25	>100
Shigella sonnei EW33	50	>50	3.13	3.13

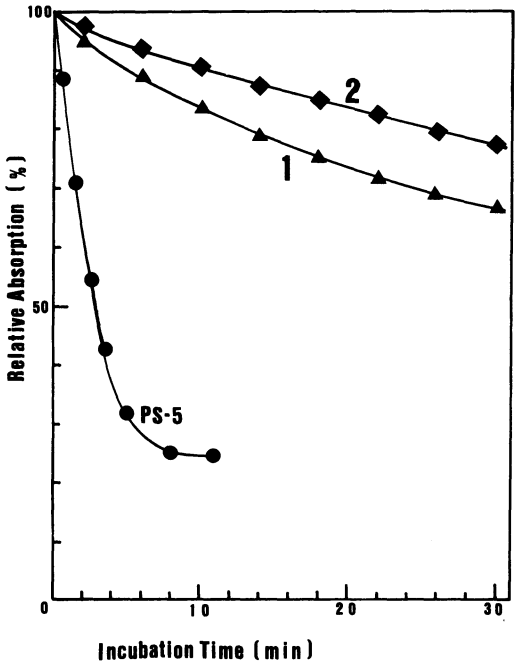

Fig. 9. Comparative DHP-I stabilities.

The comparative DHP-I stabilities of the three carbapenem derivatives are compared in Fig. 9, using the time course curves of hydrolysis by DHP-I on the relative UV absorptions at 300 nm. The DHP-I enzyme used in these assays was a partially purified preparation from hog kidney. The 6-hydroxyl substituent seems more effective than the 6-methoxyl in stabilization of carbapenem against DHP-I.

In conclusion, the introduction of the 6α-methoxyl or 6α-hydroxyl group in carbapenem significantly improves the DHP-I stability as expected, but with drastic reduction in antimicrobial activity.

Experimental

General. All mp's were measured with a YANACO apparatus and are uncorrected. Silica-gel thin-layer chromatography (TLC) and column chromatography were carried out on precoated TLC plates silica gel 60 F-254 (E. Merck) and silica gel 60 (70—230 mesh, E. Merck), respectively. Infrared (IR), ultraviolet (UV), ^{1}H NMR and ^{13}C NMR spectra were recorded with a Hitachi 260-30 infrared spectrophotometer, a Hitachi 200-20 spectrophotometer, a Varian EM-390 (90 MHz) spectrometer and a JEOL FX-100 spectrometer, respectively. Chemical shifts are given in ppm from internal TMS in $CDCl_3$ or TSP [sodium 3-(trimethylsilyl)propionate] in D_2O. Mass spectrometric data were collected on a Hitachi EM-80 spectrometer. Antimicrobial activity and DHP-I stability were measured as previously described.[15]

1-Benzyloxycarbonyl-2,2-bis(methyoxycarbonyl)-5-hydroxypyrrolidine (4). To a solution of 28.1 g (0.10 mol) of dimethyl benzyloxycarbonylaminomalonate **3** in 250 ml of benzene, 0.5 g of sodium methoxide, and 10 ml (0.20 mol) of acrylaldehyde were added and stirred for 1 h at room temperature. The reaction mixture was poured into 100 ml of cold 0.1 mol dm^{-3} HCl and the aqueous layer was separated and extracted twice with 100 ml each of benzene. The organic layer and the benzene washes were combined and rinsed with brine, dried (Na_2SO_4) and concentrated in vacuo to give a liquid. The liquid was chromatographed on a silica-gel column (250 g) using benzene-acetone (7:1) as eluent to provide 28.9 g of **4** as an oil in 85% yield: IR ($CHCl_3$) 1750, 1740, and 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ=1.75—2.25 (2H, m), 2.25—2.85 (2H, m), 3.53 (3H, s), 3.75 (3H, s), 4.02 (1H, s), 5.10 (1H, s), 5.17 (1H, s), 5.67 (1H. br) and 7.30 (5H, s); MS (EI) m/z 319 (M^+-H_2O).

***trans*-2-Benzyloxycarbonylamino-6-(*t*-butoxycarbonyl)-2-methoxycarbonyl-5-octenoic Acid Methyl Ester (6).** A mixture of 1.1 g (3.3 mmol) of 1-benzyloxycarbonyl-2,2-bis-(methoxycarbonyl)-5-hydroxypyrrolidine (**4**) and 1.73 g (4.3 mmol) of α-(*t*-butoxycarbonyl)propylidenetriphenylphosphorane (**5**) in 50 ml of benzene was refluxed for 16 h and concentrated to dryness in vacuo. Purification by silica-gel column (50 g) chromatography with benzene-acetone (10:1) resulted in isolation of 1.20 g of **6** as needles (80% yield) (recrystallized from benzene-hexane): mp 93—94 °C; IR ($CHCl_3$) 1740, 1720, and 1700 cm^{-1}; ^{1}H NMR ($CHCl_3$) δ=0.94 (3H, t, J=7.0 Hz), 1.47 (9H, s). 1.85—2.55 (6H, m), 3.73 (6H, s), 5.08 (2H, s), 6.15 (1H, s), 6.44 (1H, t, J=7.5 Hz), and 7.30 (5H, s).

Found: C, 62.45; H, 7.28; N, 3.01%; M^++1, 464. Calcd for $C_{24}H_{33}NO_8$: C, 62.19; H, 7.18; N, 3.02%; M+1, 464.

6' (isomer of **6**): IR ($CHCl_3$) 1740 and 1715 cm^{-1}; ^1H NMR δ=0.99 (3H, t, J=7.5 Hz), 1.47 (9H, s), 2.18 (2H, q, J=7.5 Hz), 2.2—2.5 (4H, m), 3.77 (6H, s), 5.09 (2H, s), 5.64 (1H, t, J=7.5 Hz), 6.27 (1H, br), and 7.35 (5H, s).

Found: m/z 464.2270. Calcd for $C_{24}H_{34}NO_8$: M+1, 464.2281.

2-Benzyloxycarbonylamino-6-(*t*-butoxycarbonyl)-5,6-epoxy-2-(methoxycarbonyl)octanoic Acid Methyl Ester (7). *m*-Chloroperbenzoic acid (2.80 g, 16.2 mmol) was added to a solution of 3.83 g (8.3 mmol) of *trans*-2-benzyloxycarbonylamino-6-(*t*-butoxycarbonyl)-2-(methoxycarbonyl)-5-octenoic acid methyl ester **6** in 50 ml of dichloromethane, and stirred for 4 h at 40 °C. After quenching with 1.15 ml (8.0 mmol) of triethylamine, the reaction mixture was washed with aqueous saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated to dryness. The residue was purified by silica-gel column (140 g) chromatography using benzene-ethyl acetate (10 : 1), providing 3.56 g of **7** as needles (recrystallized from benzene-hexane) (90% yield): mp 61—62 °C; IR ($CHCl_3$) 1740 and 1725 cm^{-1}; ^1H NHR $CDCl_3$) δ=1.01 (3H, t, J=7.5 Hz), 1.47 (9H, s), 1.2—2.05 (4H, m), 2.35—2.6 (2H, m), 3.04 (1H, t, J=6.0 Hz), 3.73 (6H, s), 5.08 (2H, s), 6.14 (1H, s) and 7.34 (5H, s).

Found: C, 60.07; H, 6.94; N, 2.94; M^+, 479. Calcd for $C_{24}H_{33}NO_9$: C, 60.11; H, 6.94; N, 2.92; M, 479.

2,2-Bis(methoxycarbonyl)-5-[1-(*t*-butoxycarbonyl)-1-hydroxypropyl]pyrrolidine (8). A suspension of the epoxide **7** (9.24 g, 19.3 mmol) and 10% Pd-charcoal (2.0 g) in 200 ml of dioxane was agitated at room temperature for 5 h at 1 atm under hydrogen, and then filtered. After the dioxane was evaporated off, the residue was chromatographed on silica gel with benzene-ethyl acetate (5 : 1) as eluent. From the eluate 5.90 g of **8** (89% yield) was provided as needles (recrystallized from benzene-hexane): mp 105—105.5 °C; IR ($CHCl_3$) 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.87 (3H, t, J=7.0 Hz), 1.46 (9H, s), 1.6—2.65 (6H, m), 3.08 (1H, d, J=5.0 Hz), 3.41 (1H, s), 3.53 (1H, m), 3.73 (3H, s), and 3.78 (3H, s); ^{13}C NMR ($CDCl_3$) δ=7.7 (q), 26.1 (t), 27.9 (q), 30.0 (t), 32.9 (t), 52.7 (q), 52.9 (q), 65.2 (d), 71.9 (s), 78.9 (s), 82.3 (s), 170.1 (s), 172.7 (s), and 173.5 (s).

Found: C, 55.73; H, 7.92; N, 4.00; M^++1, 346.1865. Calcd for $C_{16}H_{27}NO_7$: C, 55.64; H, 7.88; N, 4.06; M+1, 346.1864.

2,2-Bis(methoxycarbonyl)-6-ethyl-6-hydroxy-1-azabicyclo-[3.2.0]heptane (9). The *t*-butyl ester **8** (439 mg, 1.3 mmol) in 10 ml of 4 mol dm^{-3} HCl-dioxane was kept for 4 h at room temperature and the solution was concentrated to dryness in vacuo. The residue was subjected to Sephadex LH-20 column chromatography using methanol to give 336 mg of a β-amino acid as an amorphous solid (81% yield): IR (KBr) 1750 cm^{-1}; ^1H NMR (D_2O) δ=0.92 (3H, t, J=7.5 Hz), 1.6—3.0 (6H, m), 3.90 (6H, s), and 4.25 (1H, dd, J=8.0 and 9.5 Hz).

Dicyclohexylcarbodiimide (42 mg, 0.20 mmol) was added to a solution of 59 mg (0.18 mmol) of the β-amino acid in 5 ml of acetonitrile and stirred for 10 min. After 37 μl (0.27 mmol) of triethylamine was dropped into the solution, stirring was continued at 60 °C for 4 h. Concentration under reduced pressure gave a solid, which was taken into 4 ml of ethyl acetate and filtered. The ethyl acetate was removed by evaporation. The residue was applied on a silica-gel column (4 g) and developed with benzene-ethyl acetate (2 : 1), providing 43 mg of a bicyclic β-lactam **9** (88% yield): IR ($CHCl_3$) 1770 and 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.02 (3H, t, J=7.5 Hz), 1.65—2.2 (6H, m), 2.62 (2H, dd, J=4.5 and 9.0 Hz), 3.64 (1H, s), 3.80 (6H, s), and 3.83 (1H, m); MS (FD) m/z 271 (M^+).

2,2-Bis(methoxycarbonyl)-6-ethyl-6-methyoxy-1-azabicyclo-[3.2.0]heptan-7-one (11). A suspension of 50 mg (0.19 mmol) of the alcohol **9**, 215 mg (0.93 mmol) of silver dioxide and 0.23 ml (3.8 mmol) of methyl iodide in 5 ml of *N,N*-dimethylformamide was heated at 40 °C for 4.5 h and diluted with 20 ml of ethyl acetate. After filtration, the filtrate was evaporated to give an oil, which was purified by silica-gel column chromatography with benzene-ethyl acetate (5 : 1). A colorless oil of a methyl ether **11** (48 mg, 90% yield) was obtained from the eluate: IR ($CHCl_3$) 1765 and 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.95 (3H, t, J=7.5 Hz), 1.5—2.15 (4H, m), 2.60 (2H, dd, J=7.0 and 9.0 Hz), 3.47 (3H, s), 3.80 (6H, s), and 3.91 (1H, dd, J=7.5 and 8.5 Hz); ^{13}C NMR ($CDCl_3$) δ=6.8 (q), 23.8 (t), 25.5 (t), 39.7 (t), 53.3 (q), 53.4 (q), 68.5 (d), 70.8 (s), 84.6 (s), 167.4 (s), 168.9 (s), and 173.8 (s).

Found: C, 52.88; H, 6.28; N, 5.10; M^+, 285. Calcd for $C_{12}H_{17}NO_6$: C, 53.13; H, 6.32; N, 5.16; M, 285.

6-Ethyl-6-methoxy-2-methoxycarbonyl-1-azabicyclo[3.2.0]-heptan-7-ones (12a and 12b). A solution of 130 mg (0.46 mmol) of the diester **11** in 10 ml of acetone was mixed at 0 °C with 4.6 ml of 0.1 mol dm^{-3} NaOH, and stirred for 1 h. After the acetone was removed by evaporation, the concentrate was diluted with 26 ml of ethyl acetate and then carefully acidified to pH 2 with 0.1 mol dm^{-3} HCl (about 4.7 ml). The organic layer was separated, washed with brine, dried (Na_2SO_4), and concentrated to dryness in vacuo to afford 123 mg of a monocarboxylic acid. The crude monocarboxylic acid was dissolved in 10 ml of pyridine and heated at 100 °C for 7 h. Evaporation of the solvent gave a residue, which was dissolved in 1 ml of dichloromethane and chromatographed on 8 g of silica gel with benzene-ethyl acetate (5 : 1) to provide 45 mg (43% yield) of **12a** and 9 mg (9% yield) of **12b**. From the silica-gel column, 40 mg (31%) of the monocarboxylic acid was recovered with methanol. **12a**: IR ($CHCl_3$) 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.98 (3H, t, J=7.0 Hz), 1.4—2.35 (6H, m), 3.49 (3H, s), 3.73 (3H, s), 3.97 (1H, t, J=6.5 Hz), and 4.44 (1H, t, J=6.0 Hz); MS (FD) m/z 227 (M^+).

12b: IR ($CHCl_3$) 1755 and 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.98 (3H, t, J= 7.0 Hz), 1.6—2.0 (4H, m), 2.15—2.5 (2H. m), 3.46 (3H, s), 3.74 (3H, s) and 3.7—4.0 (2H, m); MS (FD) m/z 227 (M^+), 212 (M^+-CH_3).

6-Ethyl-6-methoxy-2-phenylseleno-7-oxo-1-azabicyclo-[3.2.0]heptane-2-carboxylic Acid Methyl Esters (13a and 13b). A solution of 6.6 mmol of butyllithium in 2.83 ml of hexane was added at −30 °C under nitrogen to a solution of 0.94 ml (6.6 mmol) of diisopropylamine in 30 ml of THF and cooled to −70 °C. A solution of 285 mg (1.3 mmol) of an isomeric mixture of **12a** and **12b** was added dropwise to the reaction mixture within 5 min. After the temperature of the solution was raised to −30 °C, 1.27 g (1.3 mmol) of benzeneselenenyl chloride in 3 ml of THF was added to the mixture and stirred for 30 min below −30 °C. Ammonium chloride (0.5 g) was added to the reaction mixture and then poured in 300 ml of ethyl acetate. The ethyl acetate was separated, rinsed with water, dried (Na_2SO_4) and concentrated to dryness in vacuo. The residue was subjected to silica-gel column chromatography with benzene-ethyl acetate (10 : 1) to afford a mixture of **13a** and **13b** (300 mg, 61% total yield), and a portion of the mixture was subjected to preparative silica-gel TLC (benzene-ethyl acetate 5 : 1) to give **13a** and **13b**, respectively.

13a: IR ($CHCl_3$) 1760 and 1735 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.92 (3H, t. J=7.5 Hz), 1.65—2.0 (4H, m), 2.25—2.75 (2H, m), 3.33 (3H, s), 3.42 (1H, t, J=7.5 Hz), 3.82 (3H, s), 7.3—7.5 (3H, m), and 7.6—7.8 (2H, m); MS (EI) m/z 384, 383, and 382.

13b: IR ($CHCl_3$) 1755 and 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.00 (3H, t, J=7.5 Hz), 1.65—2.1 (2H, m), 2.4—2.85 (2H, m), 3.49 (3H, s), 3.57 (3H, s), 3.90 (1H, dd, J=6.5 and 10.0 Hz), 7.3—7.45 (3H, m), and 7.6—7.8 (2H, m): MS (EI) m/z 384, 383, 382, and 381.

Direct Derivation of 13a and 13b from 11. A mixture of 242 mg (0.85 mmol) of the methyl ester **11**, 253 mg (1.70 mmol) of sodium iodide and 265 mg (0.85 mmol) of diphenyl diselenide in 2 ml of hexamethylphosphoric triamide was heated at 80 °C for 2 h, poured into 5 ml of ice-water, and extracted 3 times with 10 ml each of benzene. The combined benzene extracts were washed with brine, dried (Na_2SO_4), and concentrated in vacuo, giving an oil. The oil was chromatographed on silica gel using benzene-acetone (10 : 1) to provide 95 mg of **13a** and 55 mg of **13b** (50% total yield) together with 75 mg of the methyl ester **11** (31% yield).

6-Ethyl-6-methoxy-7-oxo-1-azabicyclo[3,2,0]hept-2-ene-2-carboxylic Acid Methyl Ester (14). Into a solution of the isomeric mixture of the selenides **13a** and **13b** (45 mg, 0.12 mmol) in 8 ml of dichloromethane was dropped a solution at −30 °C of 20.3 mg (0.12 mmol) of *m*-chloroperbenzoic acid in 1 ml of dichloromethane and stirred at −30 °C for 15 min. The reaction mixture was quenched with 17 μl (0.16 mmol) of triethylamine and diluted with 50 ml of dichloromethane. The organic layer was separated, washed twice with 25 ml each of aqueous saturated $NaHCO_3$ and once with 25 ml of brine, dried (Na_2SO_4), and concentrated to dryness under reduced pressure. The residue was purified by preparative TLC (benzene-acetone 5 : 1), affording 20 mg of a carbapenem **14** as a colorless oil (76% yield): UV (CH_2Cl_2) 282 nm (ε 5000); IR ($CHCl_3$) 1775 and 1728 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.02 (3H. *t*, J=7.0 Hz), 1.65—2.05 (2H, m), 2.81 (2H, dd, J=3.0 and 9.8 Hz), 3.53 (3H, s), 3.83 (3H, s), 4.45 (1H, t, J=9.8 Hz), and 6.37 (1H, t, J=3.0 Hz).

3-[2-(Acetamido)ethylthio]-6-ethyl-6-methoxy-7-oxo-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Methyl Esters (15a—c). Triethylamine (127 μl, 0.92 mmol), and then a solution of 94 mg (0.79 mmol) of *N*-acetylcysteamine in 1 ml of *N*,*N*-dimethylformamide were added at −30 °C to 130 mg (0.61 mmol) of the carbapenem **14** in 12 ml of *N*,*N*-dimethylformamide, warmed to 25 °C, and stirred for 1 h at the same temperature. After the solvent was removed by evaporation under reduced pressure, the residue was purified by Bio-Beads S-X3 (200—400 mesh) column (100 ml) chromatography using benzene and by silica-gel column chromatography with benzene-acetone (2 : 1), giving an isomeric mixture (193 mg, 95% total yield) which was further subjected to silica-gel column chromatography for separation of each isomer (**15a**, **15b**, or **15c**) under delicately controlled development conditions:

15a: IR ($CHCl_3$) 1760 and 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.06 (3H, t, J=7.0 Hz), 1.3—2.6 (4H. m), 2.00 (3H, s), 2.77 (2H, m), 3.1—3.6 (3H, m), 3.50 (3H, s), 3.79 (3H, s), 4.18 (1H, dd, J=3.0 and 8.0 Hz), 4.72 (1H, d, J=8.0 Hz), and 6.0—6.4 (1H, br); MS (EI) m/z 344 (M^+) and 316.

15b: IR ($CHCl_3$) 1760 and 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.98 (3H, t, J=7.0 Hz), 1.4—3.2 (6H, m), 2.01 (3H, s), 3.2—3.6 (2H, m), 3.50 (3H, s), 3.6—4.1 (2H, m), 3.81 (3H, s), 4.33 (1H, d, J=6.0 Hz), and 6.0—6.5 (1H, br); MS (EI) m/z 344 (M^+) and 316.

15c: IR ($CHCl_3$) 1760, 1740, and 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.97 (3H, t, J=7.0 Hz), 1.5—2.4 (4H, m), 2.00 (3H, s), 2.5—3.0 (2H, m), 3.2—3.5 (2H, m), 3.47 (3H, s), 3.6—4.0 (2H, m), 3.80 (3H, s), 4.19 (1H, d, J=8.0 Hz), and 5.9—6.4 (1H, br); MS (EI) m/z 344 (M^+) and 316.

3-[2-(Acetamido)ethylthio]-6-ethyl-6-methoxy-7-oxo-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid *p*-Nitrobenzyl Ester (16). A tenth mol dm^{-3} NaOH (476 μl) was dropped at 0 °C into a solution of 15.8 ml (48 μmol) of the methyl ester isomers **15a—c** in 3 ml of acetone and agitated for 1.5 h. The reaction mixture was mixed at 0 °C with 240 μl of 0.1 mol dm^{-3} HCl and concentrated to dryness by repeated azeotropic evaporation with benzene. The residue was dissolved in 1.5 ml of *N*,*N*-dimethylformamide. *p*-Nitrobenzyl bromide (15.4 mg, 71 μmol) and triethylamine (17 μl, 120 μmol) were added at 0 °C to the solution, and stirred for 12 h at room temperature. The reaction mixture was diluted with 20 ml of ethyl acetate and washed twice with 7 ml each of brine. Drying (Na_2SO_4) and evaporation in vacuo give an oil, which was purified by preparative silica-gel TLC with benzene-acetone (1 : 1) to afford a mixture of *p*-nitrobenzyl esters **16** (15.3 mg, 72% yield): IR ($CHCl_3$) 1760 and 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.96 (3H, t, J=7.5 Hz), 1.4—3.1 (6H, m), 2.0 (3H, s), 3.2—3.6 (2H, m), 3.50 (3H, s), 3.6—4.1 (2H, m), 4.38 (1H, d, J=6.0 Hz), 5.3 (2H, s), 5.8—6.2 (1H, br), 7.55 (2H, d, J=9.0 Hz), and 8.27 (2H, d, J=9.0 Hz).

Found: m/z 466.1629. Calcd for $C_{21}H_{28}N_3SO_7$: M^++1, 466.1644.

3-[2-(Acetamido)ethylthio]-6-ethyl-6-methoxy-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid *p*-Nitrobenzyl Ester (18). To a solution of 15 mg (32 μmol) of the carbapenam **16** in 2 ml of dichloromethane was added dropwise at 0 °C first 13 μl (160 μmol) of pyridine containing 0.57 μl (32 μmol) of water, and then 23 mg (81 μmol) of (dichloroiodo)benzene in 0.5 ml of dichloromethane. After stirring for 30 min, 12 μl (81 μmol) of triethylamine was added to the mixture and was stirred for 15 min. further 30 min. The reaction mixture was mixed with 8 μl (33 μmol) of tributyl phosphine at 0 °C and stirred for 15 min. Silica-gel column chromatography using benzene-acetone mixtures of (10 : 1), (5 : 1), (2 : 1), and (1 : 1) gave 8.0 mg (54% yield) of a carbapenem **18**: UV ($CHCl_3$) 271 (ε 10200) and 324 nm (11400); IR ($CHCl_3$) 1772, 1700, and 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ= 1.03 (3H, t, J=7.5 Hz), 1.7—2.05 (2H, m), 1.98 (3H, s), 2.8—3.55 (6H, m), 3.50 (3H, s), 4.40 (1H, t, J=10.0 Hz), 5.22 (1H, d, J=14.0 Hz), 5.51 (1H, d, J=14.0 Hz), 6.17 (1H, br), 7.67 (2H, d, J=9.0 Hz), and 8.20 (2H, d, J=9.0 Hz).

Found: m/z 463.1401. Calcd for $C_{21}H_{25}N_3SO_7$: M, 463.1410.

3-[2-(Acetamido)ethylthio]-6-ethyl-6-methoxy-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (6-Methoxy-epi-PS 5 Na Salt: 1). A solution of 95 mg (0.20 mmol) of the *p*-nitrobenzyl ester **18** was dissolved in 12 ml of a 2 : 1 mixture of tetrahydrofuran and 0.1 mol dm^{-3} phosphate buffer (pH 7.6) and hydrogenated at room temperature for 3 h at a hydrogen pressure of 4 kg cm^{-2} in the presence of platinum dioxide (100 mg). The filtrate was subjected to column chromatographic purification on QAE-Sephadex A-25 (Pharmacia Fine Chemicals AB, Sweden) and on Diaion CHP-20P (Mitsubishi Chemical Industries Ltd., Japan). Eluate fractions which indicated a UV absorption maximum

at 305.5 nm were combined and lyophilized to provide a white powder of the sodium salt of **1** in 70% yield (50 mg): UV (H_2O) 305.5 nm (ε 8000); IR (KBr) 1752, 1665, and 1608 cm^{-1}; ^1H NMR (D_2O) δ=0.96 (3H, t, J=7.5 Hz), 1.87 (2H, q, J=7.5 Hz), 1.97 (3H, s), 2.7—3.5 (6H, m), 3.50 (3H, s), and 4.43 (1H, t, J=10.0 Hz); ^{13}C NMR (D_2O, Dioxane) δ=7.1 (q), 22.8 (q), 23.9 (t), 31.7 (t), 35.8 (t), 40.4 (t), 54.2 (d), 60.4 (q), 92.6 (s), 128.3 (s), 130.8 (s), 168.5 (s), 175.2 (s), and 177.1 (s).

2,2-Bis(methoxycarbonyl)-6-ethyl-6-tetrahydropyranyloxy-1-azabicyclo[3.2.0]heptan-7-one (21). 3,4-Dihydro-2*H*-pyran (3.87 ml, 43 mmol) and anhydrous *p*-toluenesulfonic acid (0.15 g, 0.85 mmol) were added at 0 °C to a solution of 2.30 g (8.5 mmol) of **9** in 60 ml of ethyl acetate and stirred for 2 h at room temperature. After quenching with 0.18 ml (1.3 mmol) of triethylamine, the reaction solution was diluted with 100 ml of ethyl acetate, washed with aqueous saturated $NaHCO_3$ and brine, and dried (Na_2SO_4). The solvent was eliminated by evaporation and the residue was purified by silica-gel column chromatography with benzene-ethyl acetate mixtures of (20 : 1), (10 : 1), (5 : 1), and (3 : 1) as eluents to afford 2.91 g (97% yield) of an anomeric mixture of **21**: ^1H NMR ($CDCl_3$) δ=0.85—1.2 (3H, m), 1.25—2.25 (10H, m), 2.25—2.75 (2H, m), 3.3—4.4 (3H, m), 3.70 (6H, s), and 4.8—5.25 (1H, m), MS (FD) m/z 356 (M^++1).

6-Ethyl-7-oxo-2-phenylseleno-6-tetrahydropyranyloxy-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Methyl Ester (22). A solution containing 2.85 g (8.0 mmol) of **21**, 2.53 g (8.0 mmol) of diphenyl diselenide, and 2.41 g (16 mmol) sodium iodide in 15 ml of hexamethylphosphoric triamide was heated at 80 °C for 4 h. As described for **13a** and **13b**, a mixture of the four isomers of **22** (2.22 g, 61% yield) was obtained together with unreacted **21** (0.64 g): IR ($CHCl_3$) 1755 and 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.80—1.25 (3H, m), 1.3—2.25 (10H, m), 2.25—2.8 (2H, m), 3.3—4.45 (6H, m), 4.8—5.25 (1H, m), 7.2—7.5 (2H, m), and 7.6—7.9 (2H, m).

6-Ethyl-7-oxo-6-tetrahydropyranyloxy-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylic Acid Methyl Esters (23a and 23b). The treatment described for **14** was repeated with the isomeric mixture **22**, giving a 1 : 1 mixture of two anomers **23a** and **23b** in 80% yield. **23a**: IR ($CHCl_3$) 1780, 1737, and 1618 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.07 (3H, t, J=7.5 Hz), 1.35—2.05 (8H, m), 2.65—2.95 (2H, m), 3.3—4.2 (2H, m), 3.80 (3H, s), 4.72 (1H, t, J=10.0 Hz), 5.00 (1H, br), and 6.32 (1H, t, J=3.0 Hz); MS (FD) m/z 295 (M^+).

23b: IR ($CHCl_3$) 1780, 1737, and 1618 cm^{-1}; ^1H NMR ($CDCl_3$) δ=1.10 (3H, t, J=7.5 Hz), 1.35—2.1 (8H, m), 2.65—2.9 (2H, m), 3.4—4.2 (2H, m), 3.84 (3H, s), 4.52 (1H, t, J=10.0 Hz), 5.18 (1H, br), and 6.33 (1H, t, J=3.0 Hz); MS (FD) m/z 295 (M^+).

3-[2-(Acetamido)ethylthio]-6-ethyl-7-oxo-6-tetrahydropyranyloxy-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid Methyl Ester (24). Under reaction conditions similar to those described for **15a**, **15b**, and **15c**, 4.4 g (84% yield) of an isomeric mixture of **24** was obtained from 3.7 g of the mixture of **23a** and **23b**: IR ($CHCl_3$) 1758 and 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.8—1.2 (3H, m), 1.3—2.15 (9H, m), 2.00 (3H, s), 2.15—3.0 (3H, m), 3.0—4.8 (7H, m), 3.77 (3H, m), 4.8—5.25 (1H, m), and 6.20 (1H, br); MS (FD) m/z 415 (M^++1).

3-[2-(Acetamido)ethylthio]-6-ethyl-7-oxo-6-tetrahydropyranyloxy-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid *p*-Nitrobenzyl Ester (25). Treatment of 134 mg (0.32 mmol) of **24** with 0.1 mol dm^{-3} NaOH and *p*-nitrobenzyl bromide gave 131 mg (76% yield) of **25**: IR ($CHCl_3$) 1750 and 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.85—1.2 (3H, m), 1.3—2.15 (9H, m), 2.15—3.0 (3H, m), 3.2—3.65 (3H, m), 3.65—4.05 (2H, m), 4.15 (1H, t, J=7.0 Hz), 4.33 (1H, d, J=6.0 Hz), 4.8—5.2 (1H, m), 5.35 (2H, s), 6.00 (1H, br), 7.50 (2H, d, J=9.0 Hz), and 8.13 (2H, d, J=9.0 Hz); MS (FD) m/z 536 (M^++1).

3-[2-(Acetamido)ethylthio]-6-ethyl-6-hydroxy-7-oxo-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid *p*-Nitrobenzyl Ester (26). Pyridinium *p*-toluenesulfonic acid (0.71 g, 2.8 mmol) was added to a solution of 6.0 g (11 mmol) of the tetrahydropyranyl ether **25** in 120 ml of ethanol and stirred at 50 °C for 5 h. After dilution with 600 ml of ethyl acetate, the reaction mixture was washed successively with saturated aqueous $NaHCO_3$ and with brine, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on a silica-gel column with benzene-acetone (2 : 1), providing 4.1 g (81% yield) of an alcohol **26**: IR ($CHCl_3$) 1745 and 1665 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.85—1.2 (3H, m), 1.45—2.0 (3H, m), 1.94 (3H, s), 2.2—2.75 (3H, m), 3.2—3.6 (3H, m), 3.6—4.0 (2H, m), 4.0—4.8 (2H, m), 5.25 (2H, s), 6.22 (1H, br), 7.45 (2H, d, J=9.0 Hz), and 8.12 (2H, d, J=9.0 Hz); MS (FD) m/z 451 (M^+).

3-[2-(Acetamido)ethylthio]-6-ethyl-7-oxo-6-trimethylsilyloxy-1-azabicyclo[3.2.0]heptane-2-carboxylic Acid *p*-Nitrobenzyl Ester (27). The alcohol **26** (2.61 g, 5.8 mmol), triethylamine (16.0 ml, 120 mmol), and trimethylchlorosilane (13.9 ml, 110 mmol) were dissolved in 100 ml of tetrahydrofuran and stirred for 12 h at room temperature. The reaction mixture was diluted with 300 ml of ethyl acetate, washed with brine, dried (Na_2SO_4), and concentrated to give a residue. The residue was purified by silica-gel column chromatography with benzene-acetone (3 : 1) to give 2.22 g of **27** (73% yield): IR ($CHCl_3$) 1755 and 1668 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.20 (9H, s), 0.8—1.15 (2H, m), 1.3—2.0 (3H, m), 1.96 (3H, s), 2.25—2.9 (3H, m), 3.2—3.55 (2H, m), 3.55—4.0 (2H, m), 4.3—4.75 (1H, m), 5.27 (2H, s), 6.51 (1H, br), 7.48 (2H, d, J=9.0 Hz), and 8.13 (2H, d, J=9.0 Hz); MS (FD) m/z 523 (M^+).

3-[2-(Acetamido)ethylthio]-6-ethyl-7-oxo-6-trimethylsilyloxy-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid *p*-Nitrobenzyl Ester (28). The treatment of the carbapenam **27** as described for **18** gave a carbapenem **28**: IR ($CHCl_3$) 1770, 1700, and 1678 cm^{-1}; UV ($CHCl_3$) 271 (ε 10700) and 325 nm (12000); ^1H NMR ($CDCl_3$) δ=0.27 (9H, s), 1.07 (3H, t, J=7.5 Hz), 1.78 (2H, q, J=7.5 Hz), 1.99 (3H, s), 2.8—3.7 (6H, m), 4.20 (1H, t, J=10.0 Hz), 5.22 (1H, d, J=14.0 Hz), 5.47 (1H, d, J=14.0 Hz), 6.15 (1H, br), 7.63 (2H, d, J=9.0 Hz), and 8.16 (2H, d, J=9.0 Hz), MS (FD) m/z 521 (M^+).

3-[2-(Acetamido)ethylthio]-6-ethyl-6-hydroxy-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid *p*-Nitrobenzyl Ester (29). Twenty milliliter of 0.001 mol dm^{-3} HCl was added dropwise at 0 °C to a solution of 46 mg (88 μmol) of the trimethylsilyl ether **28** in 20 ml of ethanol and agitated for 30 min. The solution was mixed with 200 ml of ethyl acetate, washed with cold 5% $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated to dryness. The residue was chromatographed on silica gel with benzene and acetone (1 : 1) to give 34.5 mg (87% yield) of **29** as plates (recrystallized from benzene): mp 74—75 °C: IR (KBr) 1760 and 1690 cm^{-1}; UV (CH_2Cl_2) 271 (ε 11000) and 325.5 nm (11600); ^1H NMR ($CDCl_3$) δ=1.08 (3H, t, J=7.5 Hz), 1.62 (1H, br), 1.82 (2H, q, J=7.5 Hz), 1.96 (3H, s), 2.7—3.85 (6H, m), 4.23 (1H, t, J=10 Hz), 5.18 (1H, d, J=14.0 Hz), 5.45 (1H, d, J=14.0 Hz), 5.90 (1H, br), 7.60 (2H, d, J=9.0 Hz), and 8.12 (2H, d, J=9.0 Hz).

3-[2-(Acetamido)ethylthio]-6-ethyl-6-hydroxy-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (6-Hydroxy-*epi*-PS 5 Na Salt: 2). Under the same conditions as for **1**, the *p*-nitrobenzyl ester **29** was hydrogenated to give a white powder of **2**: IR (KBr) 1750 and 1600 cm^{-1}; UV (H_2O) 304.5 nm (ε 8900); ^1H NMR (D_2O) δ=1.00 (3H, t, J=7.3 Hz), 1.82 (2H, q, J=7.3 Hz), 2.00 (3H, s), 2.7—3.5 (6H, m), and 4.22 (1H, t, J=9.6 Hz).

The authors are grateful to Professor Yasuji Yamada, Tokyo College of Pharmacy, for his valuable advice throughout this study, and to Mrs. Michiko Sakamoto and Miss Fumito Satoh for the antimicrobial and enzymological evaluations.

References

1) K. Okamura, S. Hirata, Y. Okumura, Y. Fukagawa, Y. Shimauchi, K. Kouno, T. Ishikura, and J. Lein, *J. Antibiot.*, **31**, 480 (1978).

2) N. Shibamoto, A. Koki, M. Nishino, K. Nakamura, K. Kiyoshima, K. Okamura, M. Okabe, R. Okamoto, Y. Fukagawa, Y. Shimauchi, T. Ishikura, and J. Lein, *J. Antibiot.*, **33**, 1128 (1980).

3) K. Yamamoto, T. Yoshioka, Y. Kato, K. Isshiki, M. Nishino, F. Nakamura, Y. Shimauchi, and T. Ishikura, *Tetrahedron Lett.*, **23**, 897 (1982).

4) C. M. Cimarusti, *J. Med. Chem.*, **27**, 247 (1984).

5) Y. Fukagawa, K. Kubo, K. Okamura, and T. Ishikura, "Trends in Antibiotic Research: Genetics, Biosyntheses, Actions & New Substances," ed by H. Umezawa, A. Demain, T. Hata, and C. R. Hutchinson, Japan Antibiot. Research Association, Tokyo (1982), p. 248.

6) T. Yoshioka, A. Watanabe, I. Kojima, Y. Shimauchi, M. Okabe, Y. Fukagawa, and T. Ishikura, *J. Antibiot.*, **37**, 211 (1984).

7) M. Miyashita, N. Chida, and A. Yoshikoshi, *J. Chem. Soc., Chem. Commun.*, **1984**, 195.

8) M. D. Bachi, R. Breiman, and H. Meshulam, *J. Org. Chem.*, **48**, 1439 (1983).

9) T. H. Kinstle and B. Y. Mandanas, *J. Chem. Soc., Chem. Commun.*, **1968**, 1699.

10) M. Asaoka, K. Miyake, and H. Takei, *Bull. Chem. Soc. Jpn.*, **51**, 3008 (1978).

11) J. H. Bateson, R. I. Hickling, P. M. Roberts, T. C. Smale, and R. Southgate, *J. Chem. Soc., Chem. Commun.*, **1980**, 1084.

12) J. H. Bateson, P. M. Roberts, T. C. Smale, and R. Southgate, *J. Chem. Soc., Chem. Commun.*, **1980**, 185.

13) M. Miyashita, A. Yoshikoshi, and P. A. Grieco, *J. Org. Chem.*, **42**, 3772 (1977).

14) J. D. Hood, A. G. Brown, A. L. Elson, M. L. Gilpin, and R. Holmes. "Recent Advances in the Chemistry of Beta-Lactam Antibiotics," ed by A. G. Brown and S. M. Roberts, The Royal Society of Chemistry, Burlington House, London (1985), p. 52.

15) T. Yoshioka, K. Isshiki, F. Nakamura, K. Yamamoto, Y. Shimauchi, M. Sakamoto, N. Shibamoto, Y. Fukagawa, and T. Ishikura. Abstracts of the 24th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D. C., October 1984, Abstr. No. 132.