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Synthesis and Antibacterial Activity of Novel 1β-Methyl Carbapenems with Cycloalkylamine Moiety at the C-2 Position

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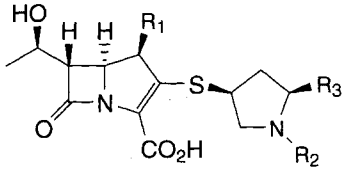
Novel 1β-methyl carbapenems with a cycloalkylamine moiety as a side chain were synthesized and their structure-activity relationships were studied. These carbapenems showed potent antibacterial activities against a wide range of Gram-positive and Gram-negative bacteria, and moderate urinary recovery when administered intraperitoneally in mice.

Since the discovery of thienamycin,[1,2] a lot of carbapenem derivatives has been prepared in search of highly potent carbapenem antibiotics.[3] So far, two 1-H carbapenems, imipenem[4,5] and panipenem,[6~8] and one 1β-methyl carbapenem, meropenem,[9] have been launched on the market. Although 1-H carbapenems are highly stable against serine β-lactamases, they are unstable to hydrolysis by human dehydropeptidase-I (DHP-I). However, 1β-methyl carbapenems have high stability against hydrolysis by DHP-I due to the steric hindrance of the β-methyl group at the C-1 position.[10,11] Therefore, since discovery of 1β-methyl carbapenem, its derivatives have been mainly studied around the world for about fifteen years. Recently, several analogues such as BO-2727,[12,13] S-4661,[14] E-1010,[15,16] IH201[17] are at the clinical or preclinical stage.

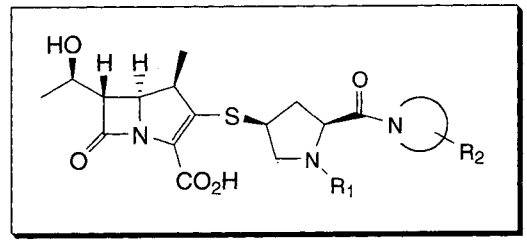
It is well known that carbapenems with a pyrrolidine moiety as a side chain have very potent antibacterial activity as it can been seen from the SAR studies for panipenem (**1**) and meropenem (**2**). In a previous paper, we also reported on 1β-methyl carbapenem derivatives with pyrrolidine moiety containing a quarternary ammonium.[18] In order to design a new parenteral carbapenem antibiotic that has potent activity against a wide spectrum of bacteria, our attention is focused on novel carbapenem derivatives with various cyclic amine moieties including pyrrolidine.

We have synthesized a series of 1β-methyl carbapenems with pyrrolidin-3-ylthio groups substituted with various cyclic amino carbonyl moieties at the C-5 position in the



panipenem (**1**) $R_1 = H$ $R_2 = C(=NH)CH_3$ $R_3 = H$

meropenem (**2**) $R_1 = CH_3$ $R_2 = H$ $R_3 = CON(CH_3)_2$



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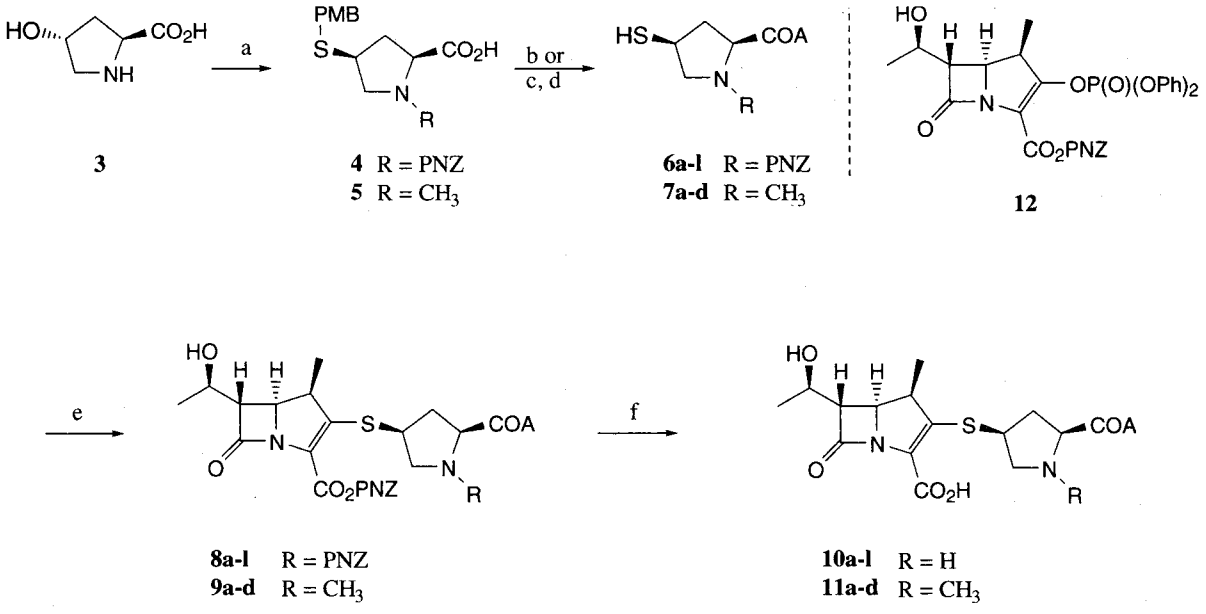
Scheme 1.



3

4 R = PNZ
5 R = CH_3

6a-l R = PNZ
7a-d R = CH_3

12

8a-l R = PNZ
9a-d R = CH_3

10a-l R = H
11a-d R = CH_3

Reagents and conditions: a) ref. 18 ; b) CDI, 3-aminoazetidine / CN_3CN then PNZCl, *i*-PrN(Et)$_2$ / CH_2Cl_2 (for only **6a** and **7a**); c) CDI, amine (AH, see Fig.1) / CH_3CN or PivCl, Et_3N, amine (AH) / THF-CH_3CN; c) TfOH, anisole / TFA; d) **12**, DIPEA / CH_3CN; e) H_2, 10% Pd/C / THF-H_2O

pyrrolidine ring and evaluated the antibacterial activity and other biological properties of their derivatives. We have found that a series of 1β-methyl carbapenem derivatives with a 5-[(substituted pyrrolidinyl)carbonyl]pyrrolidin-3-ylthio moiety show a potent antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* and also moderate urinary recovery.

In this paper, we describe the synthesis and structure-activity relationships of the above mentioned 1β-methyl carbapenem derivatives with cycloalkylamine moiety.

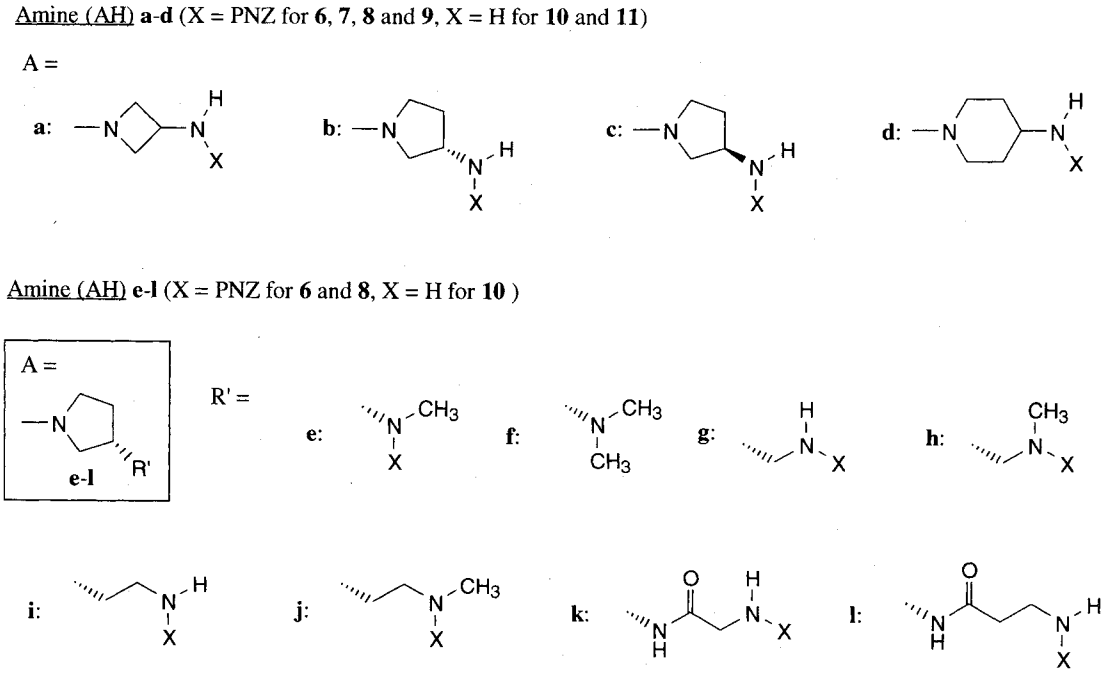
Chemistry

A series of carbapenem derivatives with cycloalkylamine moiety was synthesized by common procedures as shown in Scheme 1. The side chains were synthesized from L-hydroxyproline (**3**) as starting material. Proline derivatives **4** and **5**, which were prepared by known methods[19] from **3**, were used to give thiol **6a~l** and **7a~d** *via* amidation with various amines (Figure 1) using 1,1′-carbonyldiimidazole (CDI) or mixed anhydride with pivaloyl chloride.

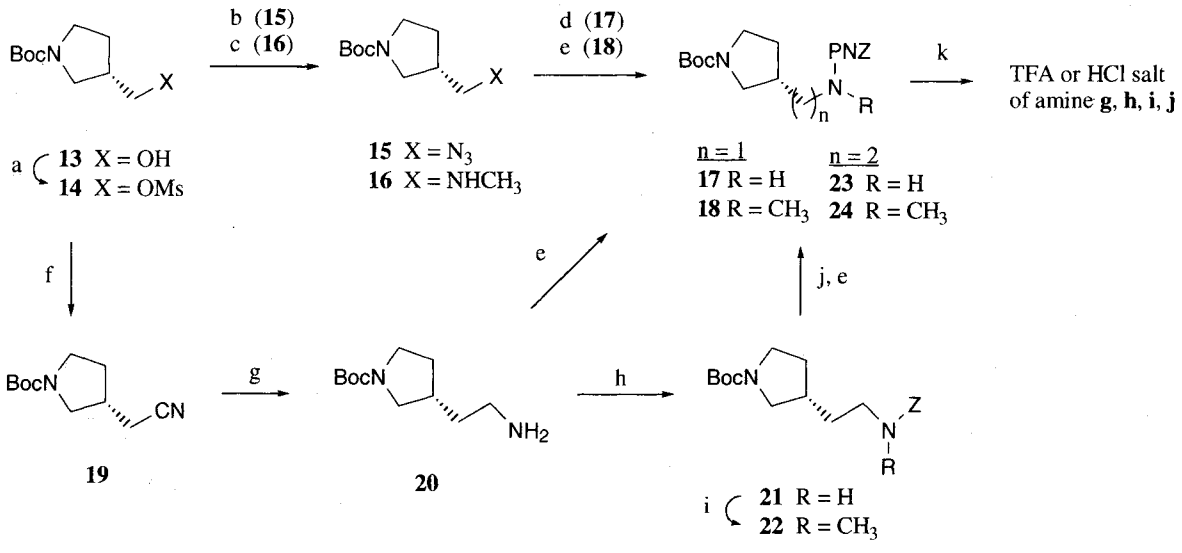
In particular, amines **g**, **h**, **i** and **j** were synthesized from **14**[20] as they were commercially unavailable (Scheme 2). Amine **g** was prepared by azidation, reductive degradation of the azide group and sequential protection with a 4-nitrobenzyloxycarbonyl (PNZ) group, and amine **h** was synthesized by amination with methylamine at a high temperature and pressure and sequential protection with PNZ group. Amines **i** and **j** were also prepared from **14**. The cyanation of **14** followed by hydrogenation in the presence of PtO_2 as a catalyst gave amine **20**. Amine **20** was protected with PNZ group to give **23**, and **24** was prepared *via* methylation of **21** with sodium hydride and methyl iodide since methylation of **23** under the same condition was unsuccessful.

Condensation of amines **a~l** and proline derivatives **4** or **5**, and sequential deprotection of the 4-methoxybenzyl (PMB) group using trifluoromethanesulfonic acid (TfOH) and anisole, gave thiol **6a~l** and **7a~d**. Then, a condensation reaction of these compounds with phosphate intermediate **12** under basic conditions afforded protected carbapenems **8a~l** and **9a~d**. The deprotection of the

Fig. 1. Various amines for the synthesis of carbapenem derivatives.

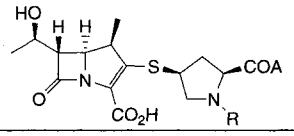


Scheme 2.

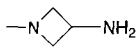
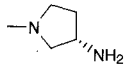
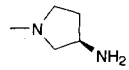
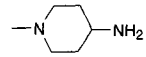


Reagents and conditions: a) ref. 20; b) NaN_3 / DMF; c) methylamine/MeOH; d) Ph_3P / CH_3CN then 1M NaOH, PNZCl ; e) PNZCl, $EtN(i\text{-}Pr)_2$, CH_3CN; f) acetone cyanohydrin, DBU / CH_3CN, reflux; g) PtO_2, NH_4OAc / EtOH; h) Z-Cl, $EtN(i\text{-}Pr)_2$ / CH_3CN; i) NaH, MeI, DMF; j) 7.5% Pd/C, H_2 / EtOH; k) TFA / CH_2Cl_2 or HCl / EtOAc

Table 1. Antibacterial activity (MIC, μg/ml) of novel carbapenems **10a~d** and **11a~d**.



10a-d: R = H
11a-d: R = CH_3

A =								
	10a	**11a**	**10b**[a]	**11b**	**10c**	**11c**	**10d**	**11d**
Staphylococcus aureus 209P	≤0.01	0.02	≤0.01	≤0.01	0.02	≤0.01	≤0.01	≤0.01
S. aureus 56R	0.05	0.1	0.02	0.05	0.02	0.1	0.05	0.1
S. aureus 535 (MRSA)	3.1	3.1	3.1	1.5	3.1	3.1	3.1	3.1
Enterococcus faecalis 681	0.8	0.8	0.8	0.2	0.8	0.4	0.8	0.4
Escherichia coli NIHJ	≤0.01	≤0.01	≤0.01	≤0.01	0.02	≤0.01	0.02	≤0.01
E. coli 609	0.02	0.02	0.02	0.02	0.05	0.02	0.05	0.02
Salmonella entericidis	≤0.01	≤0.01	0.02	≤0.01	0.05	≤0.01	0.05	0.02
Klebsiella pneumoniae 806	0.02	0.02	0.02	≤0.01	0.02	0.02	0.02	0.02
Enterobactor cloacae 963	0.05	0.02	0.02	0.02	0.05	0.02	0.05	0.02
Serratia marcescens 1184	≤0.01	≤0.01	0.02	≤0.01	0.05	≤0.01	0.05	≤0.01
Proteus vulugaris 1420	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
Morganella morganii 1510	0.2	0.1	0.2	0.4	0.2	0.4	0.2	0.4
Pseudomonas aeruginosa 1001	0.1	1.5	0.05	0.4	0.2	0.4	0.2	0.4
P. aeruginosa N07	0.4	1.5	0.2	0.4	0.4	0.8	0.4	0.4
P. aeruginosa 3719	0.4	6.2	0.2	0.8	0.2	1.5	0.2	0.8
Urinary recovery (%)	65.6	73.0	29.5	50.4	33.5	56.4	31.6	57.9

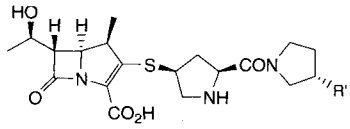
[a] this compound was prepared as an HCl salt

4-nitrobezyl (PNB) group and PNZ group in these precursors was carried out in hydrogen atmosphere in the presence of 10% Pd/C to give carbapenem derivatives **10a~l** and **11a~d**. These compounds were purified by reverse phase column chromatography and lyophilized.

Biological Properties

In vitro antibacterial activity and urinary recovery of novel carbapenems are shown in Table 1 and Table 2. First, we investigated the ring size of aminocycloamines connected to 3-thiopyrrolidine moiety by an amide bond. As shown in Table 1, all of the derivatives with azetidine, pyrrolidine and piperidine, showed potent antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria. In general, N(H)-carbapenem derivatives (**10a~d**) exhibited greater antipseudomonal activity than N(Me)-carbapenem derivatives (**11a~d**) but the urinary recovery of **10a~d** was obviously lower than that of **11a~d**. Although azetidine derivatives (**10a** and **11a**) showed both potent antibacterial activity and exellent urinary recovery, their plasma half lives of **10a** and **11a** were too short to evaluate them further as clinical candidates. For the other derivatives, the antibacterial activity of four pyrrolidine derivatives (**10b**, **10c**, **11b** and **11c**) was higher than that of piperidine derivatives (**10d** and **11d**). However there were no significant differences of urinary recovery between pyrrolidine derivatives and piperidine derivatives (**10b**, **10c** and **10d** or **11b**, **11c** and **11d**). As for the stereochemistry of the pyrrolidine moiety, compared with carbapenems (**10c** and **11c**) containing the (*R*)-isomer, carbapenems (**10b** and **11b**) containing the (*S*)-isomer had slightly higher antibacterial activity. In particular, **10b** had strong antipseudomonal activity and **11b** had strong antibacterial activity against *S. aureus* 56R, *S. aureus* 535 (MRSA), *E. faecalis* 681 and *K. pneumoniae* 806. In view of the potent antibacterial activity against a wide range of bacteria and high rate of urinary recovery, **10b** and **11b**, which have (*S*)-pyrrolidine moiety, were selected as lead compounds for further investigation.

Table 2. Antibacterial activity (MIC, μg/ml) of novel carbapenems **10e~l**.



R' =									MEPM[b]
	10e	**10f**	**10g**	**10h**	**10i**	**10j**	**10k**	**10l**	
S. aureus 209P	≤0.01	≤0.01	≤0.01	≤0.01	≤0.012	≤0.012	≤0.01	≤0.01	0.02
S. aureus 56R	0.05	0.05	0.02	0.02	0.05	0.05	0.1	0.05	0.05
S. aureus 535 (MRSA)	3.1	6.2	3.1	3.1	3.13	3.13	3.1	6.2	6.2
E. faecalis 681	0.8	0.8	0.8	0.8	0.78	0.78	0.8	0.8	1.5
E. coli NIHJ	≤0.01	≤0.01	≤0.01	≤0.01	≤0.012	0.025	0.02	≤0.01	≤0.01
E. coli 609	0.05	0.05	0.05	0.02	0.05	0.025	0.05	0.02	0.02
S. entericidis	0.05	0.02	0.02	0.02	0.025	0.025	0.02	0.02	0.02
K. pneumoniae 806	0.02	0.02	≤0.01	0.02	0.025	0.025	0.02	0.02	0.02
E. cloacae 963	0.05	0.05	0.1	0.1	0.1	0.1	0.1	0.1	0.05
S. marcescens 1184	0.02	0.05	0.02	0.02	0.025	0.025	0.02	0.02	0.02
P. vulugaris 1420	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.05
M. morganii 1510	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.1	0.1
P. aeruginosa 1001	0.2	0.2	0.1	0.05	0.1	0.05	0.2	0.1	0.2
P. aeruginosa N07	0.4	0.8	0.1	0.05	0.05	0.1	0.2	0.05	0.4
P. aeruginosa 3719	0.4	1.5	0.1	0.2	0.2	0.2	0.4	0.2	6.2
Urinary recovery (%)	33.1	N.T.[a]	22.0	37.4	19.1	6.7	34.0	24.9	29.2

[a] Not tested [b] meropenem

Furthermore, in order to explore carbapenems with better antipseudomonal activity, we focused on pyrrolidinylthio carbapenems containing a 3-substituted pyrrolidine moiety. The 3-substituted pyrrolidine moiety in **10b**, which has the highest antipseudomonal activity, was modified by the addition of the one or more methyl or methylene groups.

The antibacterial activities of these carbapenems (**10e~10l**) are shown in Table 2. All of these carbapenems clearly showed the potent antipseudomonal activity in comparision with meropenem, only clinically available 1β-methyl carbapenem. The conversion of 3-aminopyrrolidine into 3-methylamino or 3-dimethylaminopyrrolidine reduced its activity against Gram-negative bacteria. Although the addition of one or two methylene groups at the C-3 position in pyrrolidine ring showed slightly better antipseudomonal activity (**10g~10j**), introduction of two methylene groups on the pyrrolidine ring reduced the urinary recovery (**10i** and **10j**). Lengthening the methylene chain by adding an amide group did not influence the antipseudomonal activity nor urinary recovery.

Among these compounds, in particular, the 3-(*N*-methyl)aminomethylpyrrolidine derivative (**10h**) showed the most potent antipseudomonal activity and moderate urinary recovery.

Through these experiments, novel carbapenem **10h**, which has antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria, and moderate urinary recovery, was selected as the most promising compound for further evaluation.

Conclusion

We designed and synthesized novel 1β-methyl carbapenem derivatives with cycloalkylamine moiety at the C-2 position and investigated their antibacterial activity and urinary recovery. We found (*S*)-3-(*N*-methyl)aminomethylpyrrolidine derivative (**10h**), which showed antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria, and moderate urinary

recovery.

Experimental

General Methods

IR spectra were recorded on a Jasco FT-IR 8300 or Jasco FT-IR 8900 spectrometer. NMR spectra were determined on a JEOL GX-270 (270 MHz) or GX-400 (400 MHz) spectrometer using tetramethylsilane (TMS) or sodium 3-(trimethylsilyl)-propionate-d_4 (TSP) as the internal standard. Mass spectra were recorded on JEOL HX-100, SX-102A or AX-505H mass spectrometer. Optical rotations were measured with a Jasco DIP-370 polarimeter. Column chromatography was carried out on a Silica gel 60 (230~400 mesh, Art. 9385, Merck) or a Cosmosil 75C_{18} PREP (75 μm, Nacalai Tesque, Inc.) column.

Preparation of (1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[3-Amino azetidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**10a**)

(2*S*,4*S*)-4-Mercapto-2-[3-(4-nitrobenzyloxycarbonyl-amino)azetidin-1-ylcarbonyl]-1-(4-nitrobenzyloxycarbonyl)-pyrrolidine (**6a**)

To a solution of **4** (2.05 g) in CH_3CN (20 ml) was added *N*,*N*′-carbonyldiimidazole (0.78 g) at room temperature and the resulting mixture was stirred at 40°C for 1 hour. To the solution, 3-aminoazetidine dihydrochloride (1.0 g) and diisopropylethylamine (2.40 ml) in MeOH (10 ml) were added under ice cooling and the mixture was stirred at room temperature for 1 hour. The solvent was removed under reduced pressure, and the resulting residue was diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude oil was purified by silica gel column chromatography (EtOAc : MeOH=3 : 2) to give (2*S*,4*S*)-2-(3-aminoazetidin-1-ylcarbonyl)-4-(4-methoxybenzylthio)-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (2.56 g) as an amorphous powder. IR (KBr) cm^{-1} 1708, 1660, 1609, 1513, 1442, 1404, 1346, 1248; ^1H NMR (270 MHz, $CDCl_3$) δ 1.88~2.04 (1H, m), 2.38~3.20 (4H, m), 3.25~3.34 (1H, m), 3.67~4.62 (6H, m), 3.72 (2H, s), 3.78, 3.79 (3H, s ×2), 5.09~5.37 (2H, m), 6.84 (2H, d, J=8.3 Hz), 7.22 (2H, d, J=8.8 Hz), 7.45 (2H, d, J=8.8 Hz), 8.21 (2H, d, J=8.8 Hz).

To the solution of (2*S*,4*S*)-2-(3-aminoazetidin-1-ylcarbonyl)-4-(4-methoxybenzylthio)-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (0.81 g) in CH_2Cl_2 (20 ml) were added diisopropylethylamine (0.32 g) and 4-nitrobenzyl chloroformate (0.40 g) under ice cooling and the resulting mixture was stirred for 1 hour. The reaction mixture was diluted with EtOAc, and the organic layer was washed with aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$, and evaporated under reduced pressure. The crude oil was purified by silica gel column chromatography (EtOAc : MeOH=99 : 1) to give (2*S*,4*S*)-4-(4-methoxybenzylthio)-2-[3-(4-nitrobenzyloxycarbonylamino)-azetidin-1-ylcarbonyl]-1-(4-nitrobenzyloxycarbonyl)-pyrrolidine (0.64 g) as an amorphous powder. IR (KBr) cm^{-1} 1725, 1662, 1608, 1521, 1440, 1347, 1250; ^1H NMR (270 MHz, $CDCl_3$) δ 1.94~2.04 (1H, m), 2.31~2.48 (1H, m), 3.04~3.12 (1H, m), 3.26~3.34 (1H, m), 3.72 (2H, s), 3.79 (3H, s), 3.81~3.99 (1H, m), 4.08~4.87 (6H, m), 5.09~5.72 (5H, m), 6.82~6.87 (2H, m), 7.23 (2H, d, J=8.3Hz), 7.45, 7.50 (4H, d ×2, J=8.8 Hz), 8.22, 8.23 (2H, d ×2, J=8.8 Hz). MS (FAB) *m/z*: 680 $(M+H)^+$.

To the solution of (2*S*,4*S*)-4-(4-methoxybenzylthio)-2-[3-(4-nitrobenzyloxycarbonylamino)azetidin-1-ylcarbonyl]-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (0.59 g) in trifluoroacetic acid (3.32 ml) were added anisole (0.94 ml) and trifluoromethanesulfonic acid (0.15 ml) under ice cooling and the mixture was stirred at room temperature for 1 hour. The solvent was removed under reduced pressure, and the residue was washed with hexane to remove the anisole. To the residue was added Et_2O and the precipitate was washed several times by decantation. The solidified product was filtered and the filtrate was dissolved in EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give **6a** (0.44 g) as an amorphous powder. IR (KBr) cm^{-1} 1709, 1656, 1521, 1440, 1405, 1347, 1257; ^1H NMR (270 MHz, DMSO-d_6) δ 1.62~1.85 (1H, m), 2.56~2.69 (1H, m), 3.05~3.25 (2H, m), 3.70~4.55 (8H, m), 5.08~5.28 (4H, m), 7.55~7.65 (4H, m), 8.11~8.25 (5H, m).

4-Nitrobenzyl (1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[3-(4-Nitro-benzyloxycarbonylamino)azetidin-1-ylcarbonyl]-1-(4-nitrobenzyloxycarbonyl)pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**8a**)

To the solution of 4-nitrobenzyl (1*R*,5*S*,6*S*)-2-diphenylphosphoryloxy-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapenam-3-carboxylate (**12**, 0.46 g) in CH_3CN (4 ml) were added diisopropylethylamine (0.14 ml) and a solution of **6a** (0.43 g) in CH_3CN (4 ml) under ice cooling and the resulting mixture was stirred for 3 hours. The solvent was removed under reduced pressure, and the resulting residue was diluted with EtOAc. The organic layer was washed

with water, aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude oil was purified by silica gel column chromatography (EtOAc : MeOH=98 : 2) to give **8a** (224 mg) as a powder. IR (KBr) cm^{-1} 1772, 1713, 1659, 1608, 1522, 1453, 1403, 1347; ^1H NMR (270 MHz, DMSO-d_6+D_2O) δ 1.06~1.27 (6H, m), 1.66~1.89 (1H, m), 2.64~2.85 (1H, m), 3.08~3.37 (2H, m), 3.41~4.59 (11H, m), 5.07~5.52 (6H, m), 7.51~7.78 (6H, m), 8.23~8.25 (6H, d, J=8.8 Hz). MS (FAB) m/z: 904 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-(3-Aminoazetidin-1-ylcarbonyl)-pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**10a**)

The solution of **8a** (3.63 g) in THF (120 ml) and water (80 ml) was hydrogenated under H_2 atmosphere in the presence of 10% Pd/C (5.5 g) at 30°C for 1.5 hours. The catalyst was filtered away, and the filtrate was washed with Et_2O. The aqueous layer was evaporated under reduced pressure, and the concentrated solution (10 ml) was purified by reverse phase column chromatography (H_2O : CH_3CN= 100 : 0 to 94 : 6) The desired fraction was concentrated under reduced pressure and lyophilized to give **10a** (0.90 g) as a powder. IR (KBr) cm^{-1} 1755, 1642, 1594, 1464, 1387; ^1H NMR (270 MHz, D_2O) δ 1.22 (3H, d, J=7.2 Hz), 1.29 (3H, d, J=6.3 Hz), 1.75~1.85 (1H, m), 2.70~2.83 (1H, m), 3.14~3.21 (1H, m), 3.35~3.47 (3H, m), 3.82~3.92 (1H, m), 3.96~4.03 (1H, m), 4.05~4.18 (2H, m), 4.19~4.28 (3H, m), 4.34~4.43 (1H, m), 4.56~4.65 (1H, m). MS (FAB) m/z: 411 $(M+H)^+$.

Preparation of (1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-Amino-pyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid Hydrochloride (**10b**)

(2*S*,4*S*)-4-Mercapto-2-[(*S*)-3-(4-nitrobenzyloxycarbonyl-amino)pyrrolidin-1-ylcarbonyl]-1-(4-nitrobenzyloxy-carbonyl)pyrrolidine (**6b**)

To the solution of **4** (1.43 g) in THF (10 ml) were added dropwise Et_3N (0.41 ml) and pivaloyl chloride (0.41 g) under ice cooling and the resulting mixture was stirred for 30 minutes. To this were added (*S*)-3-(4-nitrobenzyloxycarbonylamino)pyrrolidine trifluoroacetate (1.50 g) and diisopropylethylamine (1.15 ml) in CH_3CN (7 ml) under ice cooling and the mixture was stirred at room temperature for 2.5 hours. The solvent was removed under reduced pressure, and the resulting residue was diluted with EtOAc. The organic layer was washed with water, aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude oil was purified by silica gel column chromatography (EtOAc : CH_2Cl_2 : CH_3CN= 4 : 4 : 1) to give (2*S*,4*S*)-4-(4-methoxybenzylthio)-2-[(*S*)-3-(4-nitrobenzyloxycarbonyl amino)pyrrolidin-1-ylcarbonyl]-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (1.47 g) as a powder. IR (KBr) cm^{-1} 1716, 1625, 1609, 1519, 1346, 737; ^1H NMR (270 MHz, DMSO-d_6, TMS) δ 1.54~1.65 (1H, m), 1.72~1.86 (1H, m), 2.57~2.69 (1H, m), 2.99~3.13 (1H, m), 3.71, 3.73 (3H, s ×2), 3.15~4.15 (12H, m), 4.36~4.58 (1H, m), 5.00~5.23 (4H, m), 6.87 (1H, d, J=8.3 Hz), 7.26 (1H, d, J=8.8 Hz), 7.46~7.62 (4H, m), 7.70~7.80 (1H, m), 8.15~8.25 (4H, m). MS (FAB) m/z: 694 $(M+H)^+$.

To the solution of (2*S*,4*S*)-4-(4-methoxybenzylthio)-2-[(*S*)-3-(4-nitrobenzyloxycarbonylamino)pyrrolidin-1-ylcarbonyl]-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (1.47 g) in trifluoroacetic acid (12 ml) were added anisole (2.3 ml) and trifluoromethanesulfonic acid (0.38 ml) under ice cooling and the mixture was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure, and the residue was washed with hexane to remove the anisole and dissolved in EtOAc (100 ml). The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give **6b** (1.26 g) as a powder. IR (KBr) cm^{-1} 1710, 1522, 1347, 854, 738; ^1H NMR (270 MHz, DMSO-d_6, TMS) δ 1.60~2.20 (2H, m), 2.62~2.75 (1H, m), 3.08~4.13 (11H, m), 4.37~4.59 (1H, m), 5.02~5.26 (4H, m), 7.47~7.81 (4H, m), 8.16~8.26 (4H, m).

4-Nitrobenzyl (1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-(4-Nitrobenzyloxycarbonylamino)pyrrolidin-1-ylcarbonyl]-1-(4-nitrobenzyloxycarbonyl)pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**8b**)

To the solution of 4-nitrobenzyl (1*R*,5*S*,6*S*)-2-diphenylphosphoryloxy-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapenam-3-carboxylate (**12**, 1.20 g) in CH_3CN (6 ml) were added diisopropylethylamine (0.38 ml) and a solution of **6b** (1.26 g) in CH_3CN (6 ml) under ice cooling and the resulting mixture was stirred for 3 hours. The solvent was removed under reduced pressure, and the resulting residue was diluted with EtOAc. The organic layer was washed with water, aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude oil was purified by silica gel column chromatography (EtOAc : MeOH=96 : 4) to give **8b** (1.01 g) as a powder. IR (KBr) cm^{-1} 3377, 1774, 1713, 1648, 1607, 1521, 1346, 852, 736; ^1H NMR (270 MHz, DMSO-d_6, TMS) δ 1.15~1.81 (6H, m), 1.70~2.20 (2H, m), 2.77~2.85 (1H, m), 3.12~4.27

(14H, m), 4.49~4.64 (1H, m), 5.05~5.49 (7H, m), 7.48~7.82 (6H, m), 8.17~8.25 (6H, m). MS (FAB) *m/z*: 918 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-Aminopyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid Hydrochloride (**10b**)

To the solution of **8b** (1.0 g) in THF (20 ml) and water (10 ml) was added 1 M aqueous HCl (1.0 ml) and the resulting mixture was hydrogenated under H_2 atmosphere in the presence of 10% Pd/C (1.5 g) at room temperature for 2 hours. The catalyst was filtered away, and the filtrate was washed with Et_2O. The aqueous layer was evaporated under reduced pressure, and the residue was purified by reverse phase column chromatography with water as an eluent. The desired fraction was concentrated under reduced pressure and lyophilized to give **10b** (0.17 g) as a powder. IR (KBr) cm^{-1} 3397, 1758, 1653, 1587, 1465, 1386; ^1H NMR (270 MHz, D_2O) δ 1.21 (3H, d, J=7.3 Hz), 1.29 (1H, d, J=6.4 Hz), 1.97~2.19 (1H, m), 2.21~2.29 (1H, m), 2.36~2.60 (1H, m), 3.02~3.14 (1H, m), 3.32~3.43 (1H, m), 3.45~3.53 (2H, m), 3.57~3.90 (5H, m), 3.98~4.17 (2H, m), 4.20~4.29 (2H, m), 4.63~4.82 (1H, m). MS (FAB) *m/z*: 425 $(M+H)^+$.

10c~10l were prepared in a similar manner as that described for the preparation of **10b**. However, **10d** and **10g~10l** were prepared as a free form without the addition of aqueous HCl during hydrogenation.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*R*)-3-Aminopyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**10c**)

IR (KBr) cm^{-1} 3370, 2967, 1756, 1634, 1594, 1454, 1387, 1261; ^1H NMR (270 MHz, D_2O) δ 1.22 (3H, d, J=7.3 Hz), 1.30 (3H, d, J=6.3 Hz), 1.65~1.83 (1H, m), 2.00~2.25 (1H, m), 2.27~2.52 (1H, m), 2.71~2.88 (1H, m), 3.08~3.17 (1H, m), 3.23~3.48 (3H, m), 3.51~3.92 (5H, m), 3.94~4.30 (4H, m). MS (FAB) *m/z*: 425 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[4-Aminopiperidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**10d**)

IR (KBr) cm^{-1} 3397, 1758, 1653, 1587, 1465, 1386; ^1H NMR (270 MHz, D_2O) δ 1.22 (3H, d, J=7.3 Hz), 1.30 (1H, d, J=6.4 Hz), 1.47~1.68 (4H, m), 2.07~2.23 (2H, m), 2.67~2.92 (2H, m), 3.03~3.57 (6H, m), 3.77~3.88 (1H, m), 3.94~4.07 (1H, m), 4.12~4.33 (2H, m), 4.43~4.57 (1H, m). MS (FAB) *m/z*: 439 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-Methylaminopyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid Hydrochloride (**10e**)

IR (KBr) cm^{-1} 3380, 1766, 1660, 1552, 1458, 1379; ^1H NMR (270 MHz, D_2O) δ 1.21 (3H, d, J=7.3 Hz), 1.27 (3H, d, J=6.4 Hz), 2.19~2.36 (3H, m), 2.55~2.76 (1H, m), 2.94~2.98 (3H, m), 3.05~3.33 (2H, m), 3.46~3.55 (2H, m), 3.60~3.70 (2H, m), 3.75~4.15 (6H, m), 4.14~4.31 (1H, m). MS (FAB) *m/z*: 439 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-Dimethylaminopyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid Hydrochloride (**10f**)

IR (KBr) cm^{-1} 3385, 1764, 1656, 1553, 1466, 1375; ^1H NMR (270 MHz, D_2O) δ 1.22 (3H, d, J=7.3 Hz), 1.28 (3H, d, J=6.4 Hz), 1.95~2.10 (1H, m), 2.15~2.35 (3H, m), 2.52~2.73 (1H, m), 2.96~2.97 (6H, m), 3.00~3.15 (1H, m), 3.37~3.43 (1H, m), 3.46~3.52 (2H, m), 3.56~3.70 (2H, m), 3.73~4.11 (6H, m), 4.15~4.30 (1H, m). MS (FAB) *m/z*: 453 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*R*)-3-Aminomethylpyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**10g**)

IR (KBr) cm^{-1} 1759, 1637, 1599, 1455, 1386, 1312, 1283; ^1H NMR (400 MHz, D_2O) δ 1.22 (3H, d, J=7.3 Hz), 1.30 (3H, d, J=6.4 Hz), 1.55~1.86 (2H, m), 2.16~2.35 (1H, m), 2.55~2.76 (2H, m), 3.02~4.05 (12H, m), 4.18~4.28 (2H, m). MS (FAB) *m/z*: 439 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*R*)-3-(Methylaminomethyl)-pyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**10h**)

IR (KBr) cm^{-1} 1757, 1634, 1598, 1456, 1386, 1311, 1284; ^1H NMR (400 MHz, D_2O) δ 1.22 (3H, d, J=7.2 Hz), 1.30 (3H, d, J=6.4 Hz), 1.58~1.87 (2H, m), 2.16~2.31 (1H, m), 2.58~2.80 (2H, m), 2.76 (3H, s), 3.03~4.07 (12H, m), 4.18~4.29 (2H, m). MS (FAB) *m/z*: 453 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-Aminoethylpyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**10i**)

IR (KBr) cm^{-1} 3389, 1755, 1629, 1453, 1387, 1312; ^1H NMR (400 MHz, D_2O) δ 1.21 (3H, d, J=7.2 Hz), 1.30 (3H, d, J=6.4 Hz), 1.55~1.90 (4H, m), 2.07~2.45 (2H, m), 2.70~2.82 (1H, m), 3.00~3.28 (5H, m), 3.32~3.87 (6H,

m), 3.95~4.07 (1H, m), 4.19~4.30 (2H, m). MS (FAB) *m/z*: 453 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-(Methylaminoethyl)-pyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**10j**)

IR (KBr) cm^{-1} 3318, 1758, 1632, 1604, 1455, 1385, 1314; ^1H NMR (400 MHz, D_2O) δ 1.22 (3H, d, J=7.1 Hz), 1.30 (3H, d, J=6.4 Hz), 1.53~1.91 (4H, m), 2.04~2.43 (3H, m), 2.73 (3H, s), 3.00~3.21 (4H, m), 3.33~3.89 (7H, m), 3.91~4.03 (1H, m), 4.17~4.30 (2H, m). MS (FAB) *m/z*: 467 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-(Glycylamino)pyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**10k**)

IR (KBr) cm^{-1} 3270, 2966, 1755, 1634, 1598, 1462, 1387, 1285; ^1H NMR (400 MHz, D_2O) δ 1.21, 1.22 (3H, d ×2, J=7.1 Hz), 1.30 (3H, d, J=6.4 Hz), 1.66~1.76 (1H, m), 1.88~2.11 (1H, m), 2.17~2.38 (1H, m), 2.75~2.88 (3H, m), 3.12~3.20 (1H, m), 3.27~3.77 (6H, m), 3.70, 3.72 (2H, s ×2), 3.84~3.93 (2H, m), 4.11~4.18 (1H, m), 4.19~4.30 (2H, m), 4.42~4.55 (1H, m). MS (FAB) *m/z*: 482 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-(Aminoethylcarbonyl-amino)pyrrolidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**10l**)

IR (KBr) cm^{-1} 3270, 2966, 1757, 1638, 1459, 1386, 1255; ^1H NMR (400 MHz, D_2O) δ 1.22 (3H, d, J=7.2 Hz), 1.30 (3H, d, J=6.3 Hz), 1.55~1.72 (1H, m), 1.91~2.10 (1H, m), 2.17~2.35 (1H, m), 2.63~2.82 (3H, m), 3.03~3.13 (1H, m), 3.17~3.28 (1H, m), 3.24 (2H, t, J=6.8 Hz), 3.35~3.90 (7H, m), 3.97~4.10 (1H, m), 4.18~4.30 (2H, m), 4.37~4.50 (1H, m). MS (FAB) *m/z*: 496 $(M+H)^+$.

Preparation of (1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-(3-aminoazetidin-1-ylcarbonyl)-1-methylpyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**11a**)

(2*S*,4*S*)-4-Mercapto-1-methyl-2-[3-(4-nitrobenzyloxy-carbonylamino)azetidin-1-ylcarbonyl]pyrrolidine (**7a**)

To the solution of **5** (1.62 g) in CH_3CN (20 ml) was added *N*,*N*′-carbonyldiimidazole (1.02 g) at room temperature and the resulting mixture was stirred at 40°C for 1 hour. To this solution were added 3-aminoazetidine dihydrochloride (1.0 g) and diisopropylethylamine (2.40 ml) in MeOH (10 ml) under ice cooling and the mixture was stirred at room temperature for 1.5 hours. The solvent was removed under reduced pressure, and the resulting residue was purified by reverse phase column chromatography (MeOH : H_2O=7 : 3) to give (2*S*,4*S*)-2-(3-aminoazetidin-1-ylcarbonyl)-4-(4-methoxybenzylthio)-1-methylpyrrolidine (1.21 g) as a colorless oil. IR (KBr) cm^{-1} 1618, 1510, 1465, 1246, 1176; ^1H NMR (270 MHz, $CDCl_3$) δ 1.77~2.00(3H, m), 2.30 (3H, s), 2.45~2.56 (2H, m), 2.88~2.95 (1H, m), 3.00~3.16 (2H, m), 3.65~4.06 (10H, m), 4.23~4.33 (1H, m), 4.50~4.68 (1H, m), 6.81~6.87 (2H, m), 7.18~7.24 (2H, m).

To the solution of (2*S*,4*S*)-2-(3-aminoazetidin-1-ylcarbonyl)-4-(4-methoxybenzylthio)-1-methylpyrrolidine (0.60 g) in CH_2Cl_2 (18 ml) were added diisopropylethyl-amine (0.38 ml) and 4-nitrobenzyl chloroformate (0.46g) under ice cooling and the resulting mixture was stirred for 30 minutes. The reaction mixture was diluted with EtOAc, and dried over $MgSO_4$, and evaporated under reduced pressure. The crude oil was purified by silica gel column chromatography (EtOAc : MeOH=95 : 5) to give (2*S*,4*S*)-4-(4-methoxybenzylthio)-1-methyl-2-[3-(4-nitrobenzyloxy-carbonylamino)azetidin-1-ylcarbonyl]pyrrolidine (0.85 g) as an amorphous powder. IR (KBr) cm^{-1} 1725, 1637, 1610, 1512, 1463, 1346, 1251; ^1H NMR (270 MHz, $CDCl_3$) δ 1.77~1.95 (1H, m), 2.32, 2.36 (3H, s ×2), 2.46~2.66 (2H, m), 2.98~3.14 (3H, m), 3.69 (2H, s), 3.80 (3H, s), 3.73~3.94 (1H, m), 4.11~4.83 (4H, m), 5.15~5.25 (2H, m), 5.41~5.51 (1H, m), 6.81~6.87 (2H, m), 7.18~7.22 (2H, m), 7.51 (2H, d, J=8.8 Hz), 8.22 (2H, d, J=8.8 Hz). MS (FAB) *m/z*: 515 $(M+H)^+$.

To the solution of (2*S*, 4*S*)-4-(4-methoxybenzylthio)-1-methyl-2-[3-(4-nitrobenzyloxycarbonylamino)azetidin-1-ylcarbonyl]-pyrrolidine (0.73 g) in trifluoroacetic acid (7.25 ml) was added anisole (1.53 ml) and trifluoromethane-sulfonic acid (0.25 ml) under ice cooling and the mixture was stirred at room temperature for 1.5 hours. The solvent was removed under reduced pressure, and the residue was washed with hexane to remove the anisole and dissolved in EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure to give **7a** (0.42 g) as an amorphous powder. IR (KBr) cm^{-1} 1721, 1638, 1609, 1522, 1460, 1347, 1258; ^1H NMR (270 MHz, $CDCl_3$) δ 1.85~2.12 (2H, m), 2.35, 2.37 (3H, s ×2), 2.63~2.82 (2H, m), 3.00~3.10 (2H, m), 3.30 (1H, br s), 3.86~3.96 (1H, m), 4.08~4.79 (4H, m), 5.21 (2H, s), 5.40~5.62 (1H, m), 7.51 (2H, d=8.8 Hz), 8.22 (2H, d, J=8.8 Hz).

4-Nitrobenzyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-[(2*S*,4*S*)-1-methyl-2-[3-(4-nitrobenzyloxycarbonylamino)azetidin-1-ylcarbonyl]pyrrolidin-4-ylthio]-1-carbapen-2-em-3-carboxylate (**9a**)

To the solution of 4-nitrobenzyl (1*R*,5*S*,6*S*)-2-diphenylphosphoryloxy-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapenam-3-carboxylate (**12**, 0.70 g) in CH_3CN (10 ml) were added diisopropylethylamine (0.18 ml) and a solution of **7a** (0.43 g) in CH_3CN (10 ml) under ice cooling and the resulting mixture was stirred for 2 days. The solvent was removed under reduced pressure, and the resulting residue was diluted with EtOAc. The organic layer was washed with water, aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The crude oil was purified by silica gel column chromatography (EtOAc : MeOH=95 : 5) to give **9a** (0.25 g) as a powder. IR (KBr) cm^{-1} 1771, 1723, 1641, 1608, 1522, 1455, 1347; ^1H NMR (270 MHz, $CDCl_3+D_2O$) δ 1.27, 1.28 (3H, d ×2, J=7.1 Hz), 1.36 (3H, d, J=5.9 Hz), 1.85~2.04 (1H, m), 2.33, 2.37 (3H, s ×2), 2.67~2.80 (2H, m), 3.03~3.39 (4H, m), 3.65~3.73 (1H, m), 3.90~3.95 (1H, m), 4.10~4.83 (6H, m), 5.09~5.52 (4H, m), 7.47~7.67 (4H, m), 8.15~8.26 (4H, m). MS (FAB) *m/z*: 739 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-(3-Aminoazetidin-1-ylcarbonyl)-1-methylpyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**11a**)

The solution of **9a** (0.25 g) in THF (3 ml) and water (2 ml) was hydrogenated under H_2 atmosphere in the presence of 10% Pd/C (0.25 g) at 30°C for 1.5 hours. The catalyst was filtered away, and the filtrate was washed with Et_2O. The aqueous layer was evaporated under reduced pressure, and the concentrated solution (2 ml) was purified by reverse phase column chromatography (H_2O : CH_3CN=100 : 0 to 95 : 5). The desired fraction was concentrated under reduced pressure and lyophilized to give **11a** (0.05 g) as a powder. IR (KBr) cm^{-1} 1755, 1641, 1598, 1462, 1386, 1284, 1255; ^1H NMR (270 MHz, D_2O) δ 1.21 (3H, d, J=7.3 Hz), 1.30 (3H, d, J=6.4 Hz), 1.75~1.85 (1H, m), 2.46, 2.47 (3H, s ×2), 2.79~2.89 (1H, m), 2.97~3.07 (1H, m), 3.22~3.53 (4H, m), 3.90~4.06 (1H, m), 4.13~4.29 (4H, m), 4.35~4.44 (1H, m), 4.54~4.84 (1H, m). MS (FAB) *m/z*: 425 $(M+H)^+$.

In a similar manner as that described for the preparation of **10b**, **11b**~**11d** were prepared from **5** instead of **4** and as a free form without the addition of aqueous HCl in hydrogenation.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-Aminopyrrolidin-1-ylcarbonyl]-1-methylpyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**11b**)

IR (KBr) cm^{-1} 3390, 1760, 1655, 1599, 1467, 1374; ^1H NMR (270 MHz, D_2O) δ 1.21 (3H, d, J=7.3 Hz), 1.29 (3H, d, J=6.4 Hz), 1.95~2.30 (1H, m), 2.30~2.70 (2H, m), 2.95, 2.97 (3H, s ×2), 3.15~3.27 (1H, m), 3.27~3.40 (1H, m), 3.46~3.49 (1H, m), 3.50~4.35 (10H, m), 4.45~4.65 (1H, m). MS (FAB) *m/z*: 439 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*R*)-3-Aminopyrrolidin-1-ylcarbonyl]-1-methylpyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**11c**)

IR (KBr) cm^{-1} 3382, 2966, 1758, 1634, 1594, 1453, 1384, 1257; ^1H NMR (270 MHz, D_2O) δ 1.20 (3H, d, J=7.2 Hz), 1.30 (3H, d, J=6.5 Hz), 1.67~1.79 (1H, m), 2.01~2.21 (1H, m), 2.27~2.49 (1H, m), 2.39, 2.40 (3H, s ×2), 2.80~2.92 (1H, m), 2.94~3.02 (1H, m), 3.17~3.22 (1H, m), 3.31~3.41 (1H, m), 3.43 (1H, dd, J=2.4 Hz), 3.51~3.94 (6H, m), 3.86~4.01 (1H, m), 4.20 (1H, dd, J=2.4 Hz), 4.22~4.28 (1H, m). MS (FAB) *m/z*: 439 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[4-Aminopiperidin-1-ylcarbonyl]-1-methylpyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid (**11d**)

IR (KBr) cm^{-1} 3388, 2965, 1756, 1633, 1599, 1455, 1384, 1247; ^1H NMR (270 MHz, D_2O) δ 1.21 (3H, d, J=7.3 Hz), 1.30 (1H, d, J=6.4 Hz), 1.40~1.73 (4H, m), 2.04~2.22 (2H, m), 2.29, 2.32 (3H, s ×2), 2.74~2.92 (2H, m), 3.05~3.52 (5H, m), 3.54~3.62 (1H, m), 3.78~3.91 (1H, m), 4.14~4.31 (2H, m), 4.20~4.29 (2H, m), 4.44~4.59 (1H, m). MS (FAB) *m/z*: 453 $(M+H)^+$.

Preparation of (*R*)-1-*tert*-Butoxycarbonyl-3-(4-nitrobenzyloxycarbonylaminomethyl)pyrrolidine (**17**) from **14**

To the solution of **14** (1.83 g) in DMF (20 ml) was added NaN_3 (1.26 g) and the resulting mixture was stirred at 80°C for 1.5 hours. The reaction mixture was diluted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane : EtOAc=70 : 30) to give (*S*)-3-azidomethyl-1-*tert*-butoxycarbonylpyrrolidine (**15**, 1.39 g) as an oil. To the solution of **15** (1.23 g) in CH_3CN (13 ml) was added Ph_3P (1.50 g) and the resulting mixture was refluxed for 1 hour. To the reaction mixture, 4-nitrobenzyl chloroformate

(1.52g) and 1 M aqueous NaOH solution (7 ml) were successively added under ice cooling and stirred at room temperature for 30 minutes. The reaction mixture was diluted with water and then extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane : EtOAc=40 : 60) to give **17** (1.97 g) as a powder. $[\alpha]_D^{25}=-14.3$ (c=1.0, $CHCl_3$); IR (neat) cm^{-1} 3326, 1727, 1683, 1524, 1413, 1348, 1250; ^1H NMR (270 MHz, $CDCl_3$) δ 1.45 (9H, s), 1.55~1.66 (1H, m), 1.92~2.03 (1H, m), 2.35~2.45 (1H, m), 2.95~3.55 (6H, m), 5.18 (1H, br s), 5.20 (2H, s), 7.51 (2H, d, J=8.6 Hz), 8.22 (2H, d, J=8.6 Hz). MS (FAB) m/z: 380 $(M+H)^+$.

Preparation of (*R*)-1-*tert*-Butoxycarbonyl-3-[*N*-methyl-*N*-(4-nitrobenzyloxycarbonyl)aminomethyl]pyrrolidine (**18**) from **14**

To the solution of **14** (1.16 g) in MeOH was added a solution of 40% methylamine (1.26 g) in MeOH and the resulting mixture was heated in an autoclave at 100°C for 4 hours. The reaction mixture was evaporated under reduced pressure to obtain crude amine (**16**, 1.32 g). To the solution of crude amine (**16**, 1.32 g) in CH_3CN, *N*,*N*-diisopropylethylamine (1.4 ml) and 4-nitrobenzyl chloroformate were added under ice cooling and the mixture was stirred for 2 hours. The reaction mixture was diluted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane : EtOAc=40 : 60) to give **18** (1.42 g) as a powder. $[\alpha]_D^{25}=-6.9$ (c=1.0, $CHCl_3$); IR (neat) cm^{-1} 1696, 1608, 1523, 1480, 1455, 1404, 1366, 1347, 1293, 1255; ^1H NMR (270 MHz, $CDCl_3$) δ 1.45 (9H, s), 1.56~1.68 (1H, m), 1.89~2.01 (1H, m), 2.45~2.55 (1H, m), 2.98 (3H, s), 2.98~3.10 (1H, m), 3.27~3.57 (5H, m), 5.23 (2H, s), 7.51 (2H, d, J=8.6 Hz), 8.23 (2H, d, J=8.6 Hz). MS (FAB) m/z: 394 $(M+H)^+$.

Preparation of (*S*)-1-*tert*-Butoxycarbonyl-3-(4-nitrobenzyloxycarbonylaminoethyl)pyrrolidine (**23**) from **14**

To the solution of **14** (29.9 g) in CH_3CN (300 ml) was added acetone cyanohydrin (24.5 ml) followed by a dropwise addition of a solution of DBU (40.1 ml) in CH_3CN (75 ml) at the room temperature over 20 minutes. The resulting mixture was refluxed for 13 hours. The solvent was removed under reduced pressure, and the resulting residue was diluted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane : CH_2Cl_2 : EtOAc=60 : 20 : 20) to give (*R*)-1-*tert*-butoxycarbonyl-3-cyanomethylpyrrolidine (**19**, 21.4 g) as a powder. ^1H NMR (270 MHz, $CDCl_3$) δ 1.46 (9H, s), 1.70~1.80 (1H, m), 2.05~2.20 (1H, m), 2.35~2.59 (3H, m) 3.05~3.15 (1H, m), 3.30~3.42 (1H, m), 3.42~3.56 (1H, m), 3.56~3.67 (1H, m). MS (FAB) m/z: 211 $(M+H)^+$.

The solution of **19** (6.04 g) and ammonium acetate (4.42 g) in EtOH (121 ml) was hydorogenated under H_2 atmosphere in the presence of PtO_2 (3.02 g) at room temperature for 2 hours. The catalyst was filtered away, and the filtrate was evaporated under reduced pressure. To the residue was added 2 M aqueous HCl solution and the aqueous layer was washed with EtOAc. The aqueous solution was made alkaline condition with K_2CO_3 and extracted with EtOAc. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to obtain crude amine (**20**, 6.91 g). To the solution of crude amine (**20**, 0.90 g) in DMF was added *N*,*N*-diisopropylethylamine (1.20 ml) and 4-nitrobenzyl chloroformate (0.90 g) under ice cooling and stirred at same temperature for 2 hours. The reaction mixture was diluted with EtOAc and the organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane : EtOAc=50 : 50) to give **23** (0.78 g) as a powder. IR (KBr) cm^{-1} 3330, 1725, 1682, 1518, 1411, 1347, 1252; ^1H NMR (270 MHz, $CDCl_3$) δ 1.46 (9H, s), 1.95~2.22 (3H, m), 2.81~2.97 (1H, m), 3.16~3.33 (3H, m), 3.43~3.63 (2H, m), 4.85 (1H, br s), 5.19 (2H, s), 7.51 (2H, d, J=8.6 Hz), 8.22 (2H, d, J=8.6 Hz). MS (FAB) m/z: 394 $(M+H)^+$.

Preparation of (*S*)-1-*tert*-Butoxycarbonyl-3-[*N*-methyl-*N*-(4-nitrobenzyloxycarbonyl)aminoethyl]pyrrolidine (**24**) from **20**

To the solution of crude amine **20** (7.0 g), which was described in the preparation for **23**, in CH_3CN (150 ml) were added *N*,*N*-diisopropylethylamine (3.0 ml) and benzyl chloroformate (3.59 g) under ice cooling and the reaction mixture was stirred for 1 hour. The reaction mixture was diluted with EtOAc and the organic layer was washed with aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane : EtOAc=75 : 25) to give (*S*)-1-*tert*-butoxycarbonyl-3-(benzyloxycarbonylaminoethyl)pyrrolidine (**21**, 3.50 g) as an oil. IR (neat) cm^{-1} 3332, 2976, 1697, 1409, 1247, 1171, 1137; ^1H NMR (400 MHz, $CDCl_3$) δ 1.42~1.67 (3H, m), 1.45 (9H, s), 1.93~2.19 (2H, m), 2.79~2.94 (1H, m), 3.13~3.32 (3H, m), 3.35~3.62 (2H, m), 4.76 (1H, br s), 5.10 (2H, s), 7.27~7.39 (5H, m). MS (FAB) m/z: 349

$(M+H)^+$.

To the solution of **21** (1.20 g) in DMF (30 ml) were added 50% NaH in mineral oil (660 mg) and MeI (1.29 ml) under ice cooling and the reaction mixture was stirred for 3 hours at room temperature. To the mixture was added aq. NH_4Cl and EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane : EtOAc=75 : 25) to give (*S*)-1-*tert*-butoxycarbonyl-3-[*N*-methyl-*N*-(benzyloxycarbonyl)aminoethyl]pyrrolidine (**22**, 1.20 g) as an oil. IR (neat) cm^{-1} 2930, 1699, 1405, 1174; ^1H NMR (400 MHz, $CDCl_3$) δ 1.38~1.71 (3H, m), 1.46 (9H, s), 1.87~2.15 (2H, m), 2.78~2.94 (1H, m), 2.92 (3H, s), 3.13~3.62 (5H, m), 5.13 (2H, s), 7.27~7.39 (5H, m) MS (FAB) *m/z*: 363 $(M+H)^+$.

The solution of **22** (1.20 g) in EtOH (20 ml) was hydorogenated under H_2 atmosphere in the presence of 7.5% Pd-C (0.50 g) at room temperature for 1 hour. The catalyst was filtered off, and the filtrate was evaporated under reduced pressure. The redidue (0.83 g) was diluted with THF, and then *N*,*N*-diisopropylethylamine (0.6 ml) and 4-nitrobenzyl chloroformate (0.74 g) were added to the solution under ice cooling. The reaction mixture was stirred for 2 hours at 0°C and diluted with EtOAc. The organic layer was washed with aq. $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (Hexane : EtOAc=50 : 50) to give **24** (1.50 g) as an oil. IR (neat) cm^{-1} 2976, 1697, 1524, 1405, 1174; ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (9H, s), 1.47~1.69 (3H, m), 1.92~2.15 (2H, m), 2.81~2.94 (1H, m), 2.95 (3H, s), 3.16~3.61 (5H, m), 5.22 (2H, s), 7.53 (2H, d, *J*=8.5 Hz), 8.22 (2H, d, *J*=8.5 Hz). MS (FAB) *m/z*: 408 $(M+H)^+$.

Measurement of Antibacterial Activity

Bacteria of 10^7 cfu/ml were inoculated on nutrient agar (Eiken Chemical Co., Ltd.) and the MIC was measured by the two-fold serial dilution method.

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