Synthesis and Structure-activity Relationships of 1β-Methylcarbapenems with Quaternary Ammonium Side Chains

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(Received for publication May 8, 1998)

The synthesis and antibacterial activity of 1β-methylcarbapenems with quaternary ammonium groups at the C-2 position have been studied. Two types of new carbapenem derivatives have been synthesized. These 1β-methylcarbapenems, one type having a (2*S*,4*S*)-2-[1,1-dimethyl-2-(1-piperazinyl)carbonyl]pyrrolidinio-4-ylthio group and the other type having a (2*S*,4*S*)-2-(4-carbamoylmethyl-4-methylhomopiperazinio-1-ylcarbonyl)pyrrolidin-4-ylthio group, show potent and well balanced antibacterial activity as well as high stability against dehydropeptidase-I. The *in vivo* potency of these two carbapenems was compared with that of meropenem. The structure-activity relationships leading to these carbapenems are also described.

Since the discovery of thienamycin,[1,2] many carbapenem derivatives have been synthesized and intensive studies on carbapenem antibiotics are continuing today.[3] Two 1-H carbapenem antibiotics imipenem/cilastatin[4,5] and panipenem/betamipron[6~8] are being used clinically. It is known that the introduction of 1β-methyl group onto carbapenem skeleton increases the stability to renal dehydropeptidase-I (DHP-I).[9~11] Recently meropenem[12], a 1β-methylcarbapenem antibiotic, has been launched.

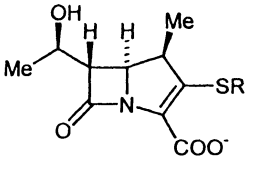
In this study, our attention is focused on the quaternarization of the pyrrolidinylthio group instead of the *N*-acetimidoylpyrrolidinylthio side chain of panipenem. Especially, synthesis of 1β-methylcarbapenems with quaternary ammonium side chains is of interest in connection with the stability to DHP-I and enhancement of antipseudomonal activity. For this purpose, a convenient synthesis of versatile side chain intermediates for carbapenem antibiotics has been reported.[13] The synthesis of a quaternary ammonium derivative of meropenem and other piperazinio derivatives has been studied.[14~17]

We have synthesized two types of new carbapenem derivatives with a quaternary ammonium group at the C-2 side chain and evaluated these derivatives for antibacterial activity and other biological properties. We have found that 1β-methylcarbapenems having a (2*S*,4*S*)-2-[1,1-dimethyl-2-(1-piperazinyl)carbonyl]pyrrolidinio-4-ylthio group or a (2*S*,4*S*)-2-(4-carbamoylmethyl-4-methylhomopiperazinio-1-ylcarbonyl)pyrrolidin-4-ylthio group show a potent and well balanced antibacterial activity against Gram-positive and Gram-negative bacteria, including *Pseudomonas aeruginosa* and also show a high urinary recovery.

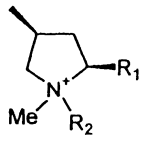
In this paper, we describe the synthesis and structure-activity relationships of the above mentioned two types of 1β-methylcarbapenems with quaternary ammonium groups.

Chemistry

The type I carbapenem derivatives were synthesized according to the general procedures as shown in Schemes 1~3. The preparation of (*S*)-1,1-dimethyl-3-mercaptopyrrolidinium fluorosulfonate was carried out *via* (*S*)-3-



Type I R =



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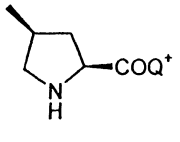
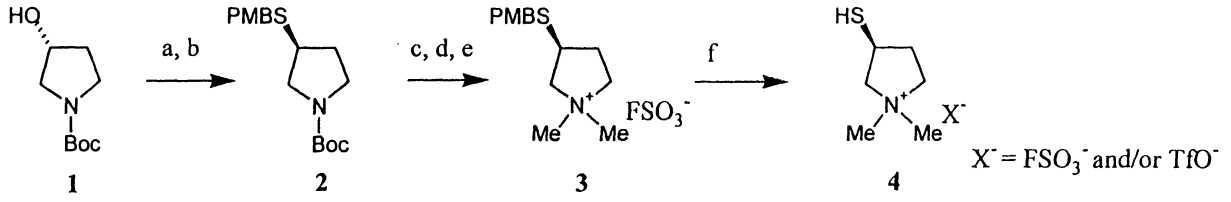
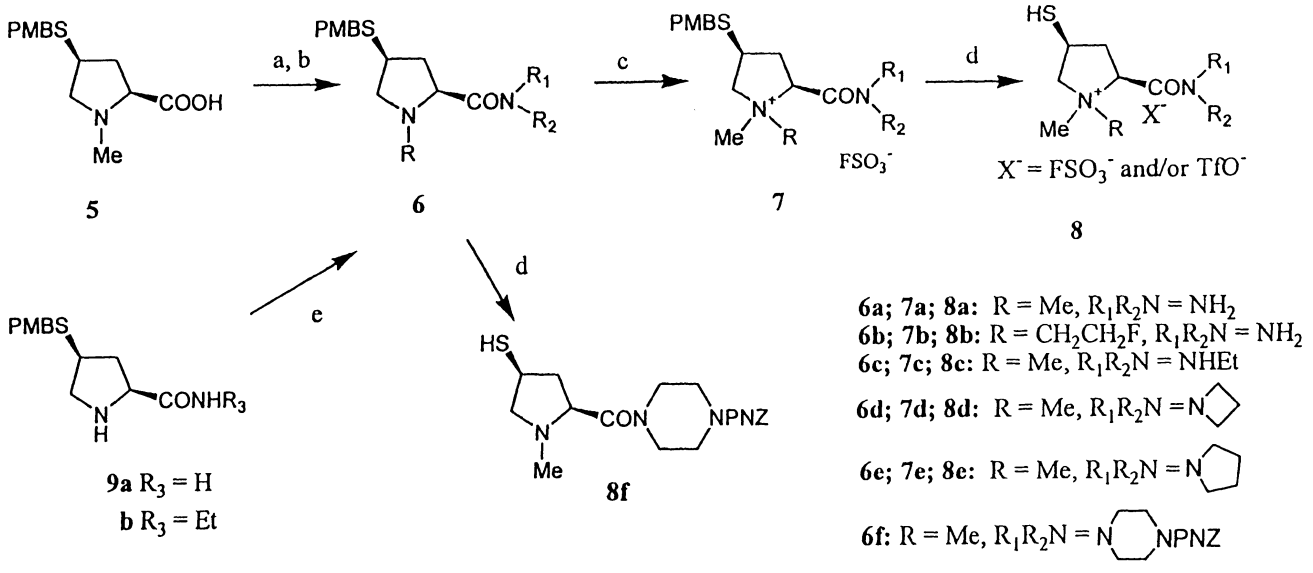


Scheme 1.



Reagents: (a) MsCl, Et_3N; (b) PMBSH, NaH; (c) 4 N HCl-AcOEt; (d) 35% aq. HCHO, $NaCNBH_3$; (e) FSO_3Me; (f) TfOH, TFA, anisole

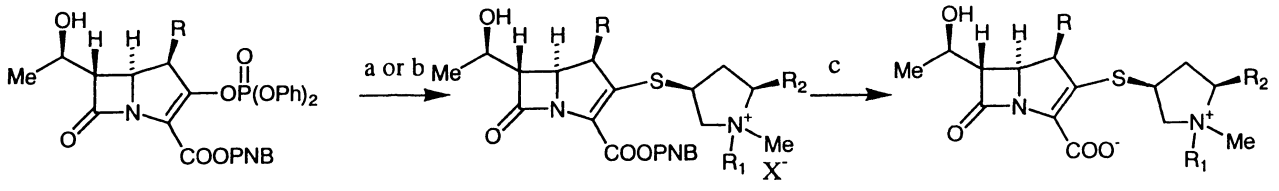
Scheme 2.



Reagents: (a) Im_2CO; (b) R_1R_2NH; (c) FSO_3CH_3; (d) TfOH, TFA, anisole; (e) $BrCH_2CH_2F$, $NaHCO_3$

(4-methoxybenzylthio)-1-methylpyrrolidine (Scheme 1). Mesylation of (*R*)-1-*t*-butoxycarbonyl-3-hydroxypyrrolidine followed by reaction with sodium 4-methoxybenzylthiolate afforded sulfide **2**. Deprotection of the *t*-butoxycarbonyl group of **2** with 4 N ethyl acetate solution of hydrogen chloride and successive *N*-methylation with 35% formaldehyde and sodium cyanoborohydride provided a *N*-methyl compound. A quaternary ammonium moiety was introduced to the *N*-methyl compound with methyl fluorosulfonate to give pyrrolidinium derivative **3**. Subsequently, deprotection of the 4-methoxybenzyl (PMB) group of **3** with trifluoromethanesulfonic acid (TfOH) in the presence of anisole and trifluoroacetic acid (TFA) afforded desired mercaptan 4. Alternatively, 2-substituted 4-mercaptopyrrolidine derivatives were prepared from (2*S*,4*S*)-4-(4-methoxybenzylthio)-1-methyl-2-pyrrolidinecarboxylic acid **5** according to the route in Scheme 2. The carboxylic acid **5** was allowed to react with 1,1'-carbonyldiimidazole and the subsequent reaction with amines provided amides **6d**~**f**. Ethylcarbamoyl compound **6c** was prepared from **9b** and methyl fluorosulfonate. Also, 1-fluoroethyl compound **6b** was prepared with **9a** and 2-fluoro-1-bromoethane. Quaternarization of **6a**~**e** with methyl fluorosulfonate provided **7a**~**e**. Deprotection of the PMB group of **7a**~**e** afforded mercaptans **8a**~**e**. The amide **6f** was directly converted into mercaptan **8f**. Condensation of 1β-methylcarbapenem-2-yl diphenylphosphates **10b**[10] with mercaptans **4** or **8a**—**e** afforded esters **11a** and **11c**—**g**. The mercaptan **8f** was treated with **10b** and subsequent quaternarization with methyl fluorosulfonate provided **11h**. 1-H Carbapenem ester **11b** was also prepared from phosphate **10a** and **8a**. Deprotection of the 4-nitrobenzyl (PNB) group and/or the 4-nitrobenzyl-

Scheme 3.

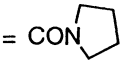


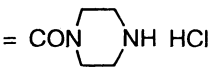
10a R = H
10b R = Me

11a ~ h $X^- = FSO_3^-$ and/or TfO^- **12a ~ h**

11a; 12a: R = Me, R_1 = Me, R_2 = H
11b; 12b: R = H, R_1 = Me, R_2 = $CONH_2$
11c; 12c: R = Me, R_1 = Me, R_2 = $CONH_2$
11d; 12d: R = Me, R_1 = CH_2CH_2F, R = $CONH_2$
11e; 12e: R = Me, R_1 = Me, R_2 = CONHEt

11f; 12f: R = Me, R_1 = Me, R_2 = 

11g; 12g: R = Me, R_1 = Me, R_2 = 

11h; 12h: R = Me, R_1 = Me, R_2 = 

Reagents: (a) mercaptan **4** or **8a~e**, $(iPr)_2NEt$; (b) (i) mercaptan **8f**, $(iPr)_2NEt$, (ii) FSO_3CH_3; (c) H_2, 10% Pd-C

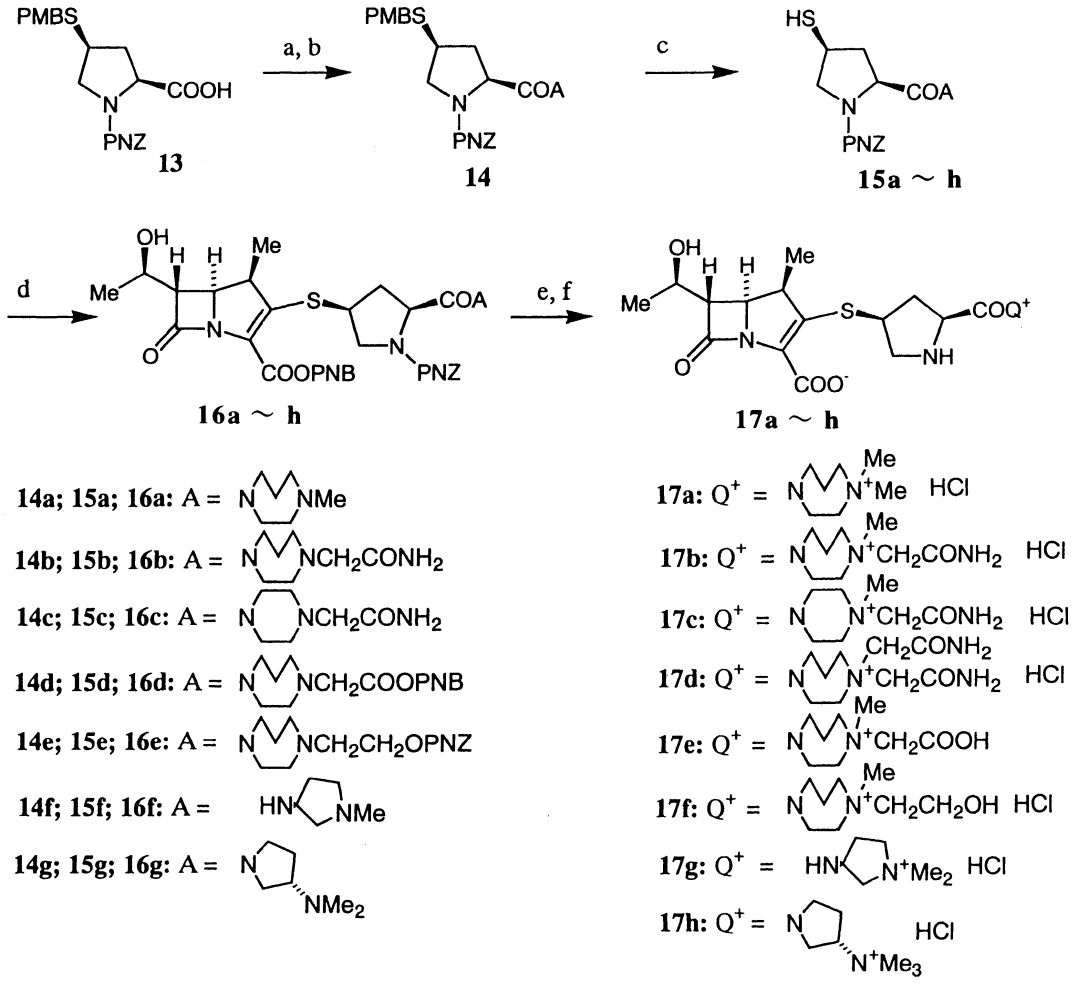
oxycarbonyl (PNZ) group of **11a~h** was carried out by the hydrogenation in the presence of 10% Pd-C to give carbapenems **12a~h**. These carbapenems **12a~g** were purified by reverse phase column chromatography and then lyophilized. The carbapenem **12h** was purified by ion exchange column chromatography and reverse phase column chromatography and then lyophilized.

On the other hand, type II carbapenems were synthesized as shown in Scheme 4. The carboxylic acid **13** was converted into amides **14a~g** by using 1,1'-carbonyldiimidazole and amines. Similarly, the deprotection of the PMB group of **14a~g** afforded mercaptans **15a~g**. Condensation of **10b** with **15a~g** afforded carbapenem esters **16a~g**. Quaternarization of **16a~g** with methyl fluorosulfonate followed by deprotection of the PNB and PNZ groups by hydrogenation in the presence of 10% Pd-C provided 1β-methylcarbapenems **17a~c** and **17e~h**. Alternatively, quaternarization of **16b** with 2-iodoacetamide followed by deprotection afforded carbapenem **17d**. These 1β-methylcarbapenems were purified by ion exchange column chromatography and reverse phase column chromatography and then lyophilized. In the case of **17e**, diastereoisomers A and B which have a chiral nitrogen at the homopiperazine ring were separated by reverse phase column chromatography.

Biological Properties

The antimicrobial activities (MICs) of the type I and II carbapenems are shown in Table 1 and 2, respectively. First of all, the antibacterial activity of 2-carbamoylpyrrolidinio-4-ylthio 1β-methylcarbapenem **12c** was compared with that of unsubstituted pyrrolidinio carbapenem **12a**. Carbapenem **12c** was more active against Gram-positive and Gram-negative bacteria, especially against MRSA and *P. aeruginosa*, than **12a**. Furthermore, the antibacterial activity of 1-H carbapenem **12b**, having a substituent group similar to 1β-methylcarbapenem **12c**, was lower against Gram-negative bacteria, including *P. aeruginosa* than that of **12c**. The urinary recoveries of **12b** and **12c** in mice after sc administration were 26% and 82%, respectively. The low urinary recovery of **12b** was probably due to the degradation by DHP-I. Compared to **12c**, the fluoroethyl carbapenem **12d** which was modified at the 1 position of the pyrrolidine moiety showed a lower activity against the majority of Gram-positive and Gram-negative bacteria. The ethylaminocarbonyl and azetidinylcarbonyl compounds **12e** and **12f** exhibited higher activity against *P. aeruginosa*. The pyrrolidinylcarbonyl analogue **12g**, however, showed a lower activity against *P. aeruginosa* than **12c**. The piperazinylcarbonyl analogue **12h** exhibited a well balanced and potent antibacterial

Scheme 4.



Reagents: (a) Im_2CO; (b) amine (AH); (c) TfOH, TFA, anisole; (d) **10b**, $(iPr)_2NEt$; (e) FSO_3CH_3 or ICH_2CONH_2; (f) H_2, 10% Pd-C

activity against both Gram-positive and Gram-negative bacteria. Notably, the activity of **12h** against MRSA and *P. aeruginosa* was much greater than that of **12c**. In addition, the urinary recovery of **12h** was 98%, which was the highest recovery among both type I and type II carbapenems.

The type II carbapenems differ from the type I carbapenems in having a quaternary ammonium moiety at the 2 position of the pyrrolidine ring. The dimethylhomopiperazinio compound **17a** showed excellent activity against Gram-positive and Gram-negative bacteria, especially, MRSA and *P. aeruginosa*, but it was toxic in mice (LD_{50}: <1500 mg/kg). In order to find a low toxicity compound, a variety of substituted alkyl groups were introduced to the ammonium moiety. The carbamoylmethyl derivative **17b** exhibited a low toxicity (LD_{50}: >1500 mg/kg) and a well balanced and potent antibacterial activity against Gram-positive and Gram-negative bacteria. In the comparison of **17b** with **17a** and the piperazinio analogue **17c**, **17b** had a higher activity against Gram-positive bacteria, especially, MRSA, and the urinary recovery of **17b** (46%) was similar to that of **17a** and **17c**. The bis(carbamoylmethyl) and 2-hydroxyethyl derivatives **17d** and **17f** had a slightly lower activity against Gram-negative bacteria than did **17b**. The carboxymethyl derivative, particularly diastereomer **17eA**, exhibited the most potent anti-pseudomonal activity but had inferior activity against Gram-positive bacteria, including MRSA. **17g** and **17h**, carbapenems having a pyrrolidinio and an ammonio moiety, respectively, showed slightly lower activity against Gram-positive bacteria, including MRSA, than **17b**. In order to clarify the *in vivo* activity of the type I and II carbapenems, the protective effects of **12h** and **17b** against

Table 1. Antibacterial activity (MIC, μg/ml)[a] of carbapenems **12a～h** and urinary recovery (%)[b] in mice.

	12a	**12b**	**12c**	**12d**	**12e**	**12f**	**12g**	**12h**
Staphylococcus aureus 209P	0.02	≤0.01	≤0.01	0.02	0.02	0.02	0.05	≤0.01
S. aureus 56R	0.05	≤0.01	≤0.01	0.05	0.05	0.1	0.1	0.05
S. aureus 535 (MRSA)	25	25	12.5	25	12.5	25	25	6.2
Enterococcus faecalis 681	3.1	0.8	3.1	1.5	3.1	3.1	6.2	1.5
Escherichia coli NIHJ	0.05	0.05	0.05	0.05	0.05	0.05	0.1	0.1
E. coli 609	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.2
Salmonella enteritidis	0.1	0.1	0.05	0.05	0.1	0.05	0.1	0.1
Klebsiella pneumoniae 806	0.1	0.1	0.05	0.1	0.1	0.1	0.1	0.1
K. pneumoniae 846	0.05	0.1	0.05	0.1	0.1	0.1	0.2	0.05
Enterobacter cloacae 903	0.4	0.8	0.2	0.8	0.2	0.4	0.4	0.4
Serratia marcescens 1184	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.1
Proteus vulgaris 1420	0.8	0.4	0.2	0.2	0.2	0.2	0.4	0.2
Morganella morganii 1510	1.5	1.5	0.8	0.8	0.4	0.8	0.8	0.8
Pseudomonas aeruginosa 1001	3.1	3.1	1.5	1.5	0.8	0.8	3.1	0.4
Urinary Recovery (%) (s.c.)	65	26	82	60	68	84	62	98

[a] MIC was determined by agar dilution method with an inoculum of 10^7 cfu/ml.
[b] Urinary recovery (%) was determined by disk method using *Bacillus subtilis* ATCC 6633 as a test strain after sc administration of compound (50 mg/kg) in SPF *dd*Y mice (n=5, 0～24 hours).

Table 2. Antibacterial activity (MIC, μg/ml)[a] of carbapenems **17a～h** and meropenem and urinary recovery (%)[b] in mice.

	17a	**17b**	**17c**	**17d**	**17eA**[c]	**17eB**[c]	**17f**	**17g**	**17h**	Meropenem
Staphylococcus aureus 209P	≤0.01	≤0.01	≤0.01	0.05	0.1	0.1	≤0.01	0.02	0.02	0.02
S. aureus 56R	0.05	0.05	0.1	0.1	0.2	0.2	0.05	0.1	0.1	0.05
S. aureus 535 (MRSA)	6.2	3.1	6.2	6.2	12.5	12.5	3.1	6.2	6.2	6.2
Enterococcus faecalis 681	0.8	0.8	0.8	1.5	3.1	3.1	0.8	1.5	1.5	1.5
Escherichia coli NIHJ	≤0.01	≤0.01	0.02	0.02	0.02	0.02	0.02	0.05	0.02	≤0.01
E. coli 609	0.05	0.05	0.1	0.05	0.05	0.05	0.05	0.1	0.05	0.02
Salmonella enteritidis	≤0.01	0.02	0.02	0.05	0.02	0.02	0.02	0.05	0.05	0.02
Klebsiella pneumoniae 806	0.02	0.02	0 .02	0.05	0.02	0.02	0.02	0.05	0.05	0.02
K. pneumoniae 846	≤0.01	≤0.01	≤0.01	0.02	≤0.01	0.02	≤0.01	0.02	0.02	≤0.01
Enterobacter cloacae 903	0.1	0.05	0.2	0.2	0.1	0.2	0.1	0.2	0.02	≤0.01
Serratia marcescens 1184	0.05	0.02	0.05	0.05	0.02	0.05	0.05	0.1	0.2	0.02
Proteus vulgaris 1420	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.05	0.05
Morganella morganii 1510	0.4	0.2	0.4	0.4	0.2	0.2	0.4	0.8	0.4	0.1
Pseudomonas aeruginosa 1001	0.2	0.4	0.4	0.2	0.1	0.2	0.4	1.5	0.2	0.2
Urinary Recovery (%) (s.c.)	41	46	49	27	48	43	39	55	40	29

[a] MIC was determined by agar dilution method with an inoculum of 10^7 cfu/ml.
[b] Urinary recovery (%) was determined by disk method using *Bacillus subtilis* ATCC 6633 as a test strain after sc administration of compound (50 mg/kg) in SPF *dd*Y mice ($n=5$, 0～24 hours).
[c] Diastereomers A and B at the chiral nitrogen of homopiperazine ring were separated.

Table 3. Protective effect of 1β-methylcarbapenems **12h** and **17b** with a quaternary ammonium moiety against experimental infection in mice.

Organism	ED_{50} (mg/kg)[a]		
	12h	**17b**	Meropenem
S. aureus Smith[b]	0.36	0.96	2.31
E. coli 704	1.37	0.75	0.58
P. aeruginosa 1008	1.00	0.43	1.15

[a] 50% effective sc dose.
[b] Challenged with 5% mucin.

experimental infections in mice were compared to those of meropenem. Both **12h** and **17b** showed good protection against *S. aureus* Smith, *E. coli* 704 and *P. aeruginosa* 1008, and especially **17b** exhibited an approximately 2 times greater efficacy *in vivo* against *S. aureus* and *P. aeruginosa* than meropenem.

Conclusion

The structure-activity relationships of two types of carbapenems with a quaternary ammonium moiety were clarified. 1β-Methylcarbapenem **12h** and **17b** showed the expected higher *in vitro* activity against Gram-positive bacteria, including MRSA, and showed higher *in vivo* efficacy against *S. aureus* and *P. aeruginosa* compared to meropenem. Also, both compounds exhibited markedly low acute toxicity in mice. Other pharmacological evaluations of both compounds are underway.

Experimental

General Methods

IR spectra were recorded on a Nicolet NIC FT-IR (5SXC) spectrometer. NMR spectra were determined on a Varian EM-360L (60 MHz), Jeol GX-270 (270 MHz) or GX-400 (400 MHz) spectrometer using tetramethylsilane (TMS) as an internal or external standard and sodium 3-(trimethylsilyl)-propionate-d_4 (TSP) as an internal standard. The mp was determined using a Yanagimoto micro-melting point apparatus and was not corrected. UV spectra were recorded on a Shimadzu UV-3100 spectrometer. Column chromatography was carried out on Silica gel 60 (230~400 mesh, Art.9385, Merck), Cosmosil 75C_{18} PREP (75 μm, Nacalai Tesque, Inc.) or MCI GEL CHP-20P (75~150 μm, Mitsubishi Kasei Corporation).

Preparation of (*S*)-1,1-dimethyl-3-(4-methoxybenzylthio)pyrrolidinium fluorosulfonate (3)

To a solution of (*R*)-1-*t*-butoxycarbonyl-3-hydroxypyrrolidine **1** (25 g) in THF (250 ml) were added triethylamine (16.9 ml) and methanesulfonyl chloride (9.4 ml) under ice-cooling, and then the mixture was stirred for 30 minutes. The mixture was then further stirred at 15°C for 30 minutes. The mixture was diluted with EtOAc and washed with water and brine. The EtOAc layer was dried (Na_2SO_4) and concentrated by evaporation under reduced pressure to give (*R*)-1-*t*-butoxycarbonyl-3-methanesulfonyloxypyrrolidine as a colorless oil (31 g): ^{1}H NMR (60 MHz, $CDCl_3$, TMS) δ 1.48 (9H, s), 1.91~2.45 (2H, m), 3.04 (3H, s), 3.26~3.82 (4H, m), 6.10~6.44 (1H, m).

To a solution of 4-methoxybenzyl mercaptan (16.9 ml) in DMF (200 ml) was added sodium hydride (5.32 g, 55% w/w dispersion in mineral oil) under ice-cooling and then the mixture was stirred at room temperature for 30 minutes. To this mixture was added a solution of (*R*)-1-*t*-butoxycarbonyl-3-methanesulfonyloxypyrrolidine (31 g) in DMF (50 ml) under ice-cooling and then the mixture was stirred under ice-cooling for 30 minutes and allowed to stand overnight at room temperature. The mixture was poured into ice-water and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane-EtOAc, 5:1) to give

(*S*)-1-*t*-butoxycarbonyl-3-(4-methoxybenzylthio)pyrrolidine **2** as a pale-brown oil (28 g): ^{1}H NMR (60 MHz, $CDCl_3$, TMS) δ 1.46 (9H, s), 1.50~2.35 (2H, m), 2.81~3.88 (5H, m), 3.70 (2H, s), 3.79 (3H, s), 6.83, 7.27 (4H, A_2B_2, J=9.0 Hz).

To a solution of **2** (27.5 g) in EtOAc (100 ml) was added 4 N hydrogen chloride in EtOAc (106 ml) under ice-cooling, and the mixture was stirred at 0~5°C for 30 minutes and then at 25°C for 2 hours. The mixture was diluted with diisopropyl ether (200 ml), and the crystals which precipitated from the mixture were filtered and dried to give (*S*)-3-(4-methoxybenzylthio)pyrrolidine hydrochloride as colorless crystals (20.8 g): mp 125~126°C; IR (KBr) cm^{-1} 1510, 1246, 1174; ^{1}H NMR (60 MHz, D_2O, TMS) δ 1.52~2.53 (2H, m), 2.91~3.70 (5H, m), 3.63 (2H, s), 3.67 (3H, s), 6.80, 7.16 (4H, A_2B_2, J=9.0 Hz).

To a solution of (*S*)-3-(4-methoxybenzylthio)pyrrolidine (750 mg) in acetonitrile (15 ml), which was prepared by neutralization of (*S*)-3-(4-methoxybenzylthio)pyrrolidine hydrochloride (900 mg) with $NaHCO_3$, were added 35% formaldehyde (1.44 ml) and sodium cyanoborohydride (338 mg) at room temperature, and then the mixture was stirred for 15 minutes. To the mixture was added an excess amount of acetic acid and the mixture was then stirred at room temperature for 2.5 hours. The mixture was poured into EtOAc (200 ml) and the mixture was washed with 2 N NaOH and brine and dried (K_2CO_3). The mixture was concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc-MeOH, 3:1) to give (*S*)-3-(4-methoxybenzylthio)-1-methylpyrrolidine as a colorless oil (349 mg): ^{1}H NMR (60 MHz, $CDCl_3$, TMS) δ 1.40~3.49 (7H, m), 2.33 (3H, s), 3.69 (2H, s), 3.78 (3H, s), 6.86, 7.25 (4H, A_2B_2, J=9.0 Hz).

To a solution of (*S*)-3-(4-methoxybenzylthio)-1-methylpyrrolidine (340 mg) in methylene chloride (20 ml) was added methyl fluorosulfonate (118 μl) under ice-cooling and the mixture was stirred at 0~5°C for 30 minutes and then stirred at room temperature for 3.5 hours. The mixture was concentrated by evaporation under reduced pressure and the residue was washed by decantation with diethyl ether and dried to afford the title compound **3** as a colorless powder (0.5 g): ^{1}H NMR (270 MHz, D_2O, TMS) δ 1.86~2.05 (1H, m), 2.34~2.56 (1H, m), 2.90 (3H, s), 3.01 (3H, s), 2.98~3.73 (5H, m), 3.65 (3H, s), 3.67 (2H, s), 6.82, 7.17 (4H, A_2B_2, J=8.6 Hz).

Preparation of (2*S*,4*S*)-2-Carbamoyl-1,1-dimethyl-4-(4-methoxybenzylthio)pyrrolidinium Fluorosulfonate (**7a**)

To a solution of (2*S*,4*S*)-2-carbamoyl-4-(4-methoxybenzylthio)-1-methylpyrrolidine (320 mg) in methylene chloride (7 ml) was added methyl fluorosulfonate (123 μl) under ice-cooling and the mixture was stirred at 0~5°C for 20 minutes and then stirred at room temperature for 2 hours. The mixture was concentrated by evaporation under reduced pressure and the residue was washed by decantation with diethyl ether and dried to afford the title compound **7a** as an oil (525 mg): ^{1}H NMR (270 MHz, D_2O, TMS) δ 2.01~3.68 (5H, m), 3.02 (3H, s), 3.03 (3H, s), 3.65 (3H, s), 3.68 (2H, s), 4.07 (1H, dd, J=8.4, 7.7 Hz), 6.81, 7.16 (4H, A_2B_2, J=8.8 Hz).

Preparation of (2*S*,4*S*)-2-Carbamoyl-1-(2-fluoroethyl)-1-methyl-4-(4-methoxybenzylthio)pyrrolidinium Fluorosulfonate (**7b**)

To a solution of (2*S*,4*S*)-2-carbamoyl-4-(4-methoxybenzylthio)pyrrolidine **9** (1.2 g) in DMF (12 ml) were added 1-bromo-2-fluoroethane (0.4 ml), sodium iodide (3.83 g) and sodium bicarbonate (0.38 g) under ice-cooling and the mixture was stirred at room temperature for 20 minutes and then at 40°C for 20 hours. The mixture was poured into a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$) and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc) to give (2*S*,4*S*)-2-carbamoyl-1-(2-fluoroethyl)-4-(4-methoxybenzylthio)pyrrolidine **6b** as a colorless powder (838 mg): mp 122~123°C; IR (KBr) cm^{-1} 1636, 1610, 1510; ^{1}H NMR (60 MHz, $CDCl_3$, TMS) δ 1.55~3.40 (8H, m), 3.69 (2H, s), 3.79 (3H, s), 4.47 (2H, dt, J=47.0, 6.0 Hz), 4.78 (1H, br s), 7.02 (4H, A_2B_2, J=9.0Hz), 6.95~7.50 (1H, br s).

The title compound **7b** (850 mg) was prepared from **6b** (630 mg) and methyl fluorosulfonate (0.17 ml) by a similar manner as that described for the preparation of **7a**: ^{1}H NMR (270 MHz, D_2O, TMS) δ 1.84~4.73 (10H, m), 3.18 (3H, s), 3.65 (3H, s), 3.68 (2H, s), 6.79~7.19 (4H, m).

Preparation of (2*S*,4*S*)-1,1-Dimethyl-2-ethylcarbamoyl-4-(4-methoxybenzylthio)pyrrolidinium Fluorosulfonate (**7c**)

The title compound **7c** was prepared as an oil from **9b** by *N*-methylation with 35% formaldehyde and sodium cyanoborohydride followed by quaternarization

with methyl fluorosulfonate as that described for the preparation of **4**: IR (KBr) cm^{-1} 1680, 1510, 1240; ^1H NMR (270 MHz, D_2O, TMS) δ 0.94 (3H, t, $J=7.3$ Hz), 1.95~2.20 (1H, m), 2.60~2.98 (1H, m), 2.96 (3H, s), 3.00 (3H, s), 3.02~3.70 (5H, m), 3.64 (3H, s), 3.67 (2H, s), 3.97 (1H, t, $J=7.9$ Hz), 6.81, 7.01 (4H, A_2B_2, $J=$ 8.8 Hz).

Preparation of (2*S*,4*S*)-2-(1-azetidinylcarbonyl)-1,1-dimethyl-4-(4-methoxybenzylthio)pyrrolidinium fluorosulfonate (**7d**)

To a suspension of (2*S*,4*S*)-4-(4-methoxybenzylthio)-1-methyl-2 pyrrolidinecarboxylic acid (**5**, 0.6 g) in acetonitrile (15 ml) was added 1,1'-carbonyldiimidazole (0.42 g) at room temperature and the mixture was stirred at 35~40°C for 1 hour. To the resulting solution was added azetidine (0.22 ml) at room temperature and the mixture was stirred at the same temperature for 1.5 hours and was allowed to stand overnight. The mixture was concentrated and diluted with EtOAc and washed with brine and dried ($MgSO_4$). The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (chloroform - MeOH, 9 : 1) to give the compound **6d** as a colorless oil (735 mg): IR ($CHCl_3$) cm^{-1} 1610, 1510, 1465, 1440; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 1.80~1.91 (1H, m), 2.20~2.34 (2H, m), 2.30 (3H, s), 2.43~2.57 (2H, m), 2.86~3.15 (3H, m), 3.70 (2H, s), 3.80 (3H, s), 3.98~4.10 (2H, m), 6.84, 7.21 (4H, A_2B_2, $J=8.8$ Hz).

The title compound **7d** (586 mg) was prepared from **6d** (450 mg) and methyl fluorosulfonate (0.12 ml) by a similar manner as that described for the preparation of **7a**: IR ($CHCl_3$) cm^{-1} 1655, 1610, 1585, 1510, 1460, 1440; ^1H NMR (270 MHz, D_2O, TMS) δ 1.96~2.23 (3H, m), 2.61~2.76 (1H, m), 2.99 (6H, s), 3.40~3.56 (3H, m), 3.65 (3H, s), 3.68 (2H, s), 3.83~4.24 (5H, m), 6.28, 7.17 (4H, A_2B_2, $J=8.8$ Hz).

Preparation of (2*S*,4*S*)-1,1-Dimethyl-4-(4-methoxybenzylthio)-2-(1-pyrrolidinylcarbonyl)pyrrolidinium Fluorosulfonate (**7e**)

The title compound **7e** was prepared as an oil from **5** by a similar manner as that described for the preparation of **7d**: ^1H NMR (270 MHz, D_2O, TMS) δ 1.68~1.85, 1.94~2.10, 2.69~3.85 (7H, m), 2.99 (3H, s), 3.03 (3H, s), 3.65 (3H, s), 3.67 (2H, s), 4.41 (1H, dd, $J=8.1$, 6.6 Hz), 6.81, 7.17 (4H, A_2B_2, $J=8.6$ Hz).

Preparation of (2*S*,4*S*)-4-(4-Methoxybenzylthio)-1-methyl-2-[4-(4-nitrobenzyloxycarbonyl)piperazin-1-ylcarbonyl]pyrrolidine (**6f**)

To a suspension of **5** (24 g) in acetonitrile (200 ml) was added 1,1'-carbonyldiimidazole (16.6 g) at room temperature and the mixture was stirred at 35°C for 45 minutes. To the resulting solution was added piperazine (14.7 g) in acetonitrile (200 ml) at 30~35°C and the mixture was stirred at the same temperature for 30 minutes. To the mixture was added 4-nitrobenzyl chloroformate (36.8 g) in acetonitrile (100 ml) under ice-cooling and the mixture was stirred at room temperature for 1 hour. The mixture was concentrated and diluted with EtOAc and washed with 10% aq $NaHCO_3$ and brine and dried ($MgSO_4$). The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc - MeOH, 10 : 1) to give the title compound **6f** as a colorless powder (27.7 g): IR (melted film) cm^{-1} 1706, 1648, 1609, 1463, 1434, 1346; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 1.73~1.87 (1H, m), 2.32 (3H, s), 2.45~2.58 (2H, m), 3.02~3.21 (3H, m), 3.34~3.85 (7H, m), 3.70 (2H, s), 3.80 (3H, s), 3.95~4.16 (1H, m), 5.24 (2H, s), 6.84 (2H, d, $J=8.8$ Hz), 7.20 (2H, d, $J=8.8$ Hz), 7.52 (2H, d, $J=8.8$ Hz), 8.23 (2H, d, $J=8.8$ Hz).

(1*R*,5*S*,6*S*)-2-[(*S*)-1,1-Dimethylpyrrolidinio-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**12a**)

To a suspension of **7a** (453 mg) in anisole (1.40 ml) were added TFA (4.97 ml) and TfOH (0.249 ml) under ice-cooling and the mixture was stirred at the same temperature for 2 hours. The mixture was concentrated by evaporation under reduced pressure and the residue was washed by decantation with diethyl ether and dried to afford mercaptan **8a** as an oil (250 mg).

To a solution of 4-nitrobenzyl (1*R*,5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-oxo-1-carbapenam-3-carboxylate (250 mg) in acetonitrile (2 ml) were added diphenylphosphoryl chloride (150 μl) and *N*,*N*-diisopropylethylamine (126 μl) under ice-cooling and the mixture was stirred at the same temperature for 1 hour. To the mixture were added *N*,*N*-diisopropylethylamine (145 μl) and **8a** in acetonitrile (4 ml) under ice-cooling and the mixture was stirred for 2 hours and was allowed to stand in a refrigerator for 2 days. The mixture was concentrated by evaporation under reduced pressure and the residue was washed by decantation with diethyl ether and dried to afford **11a**. The crude product **11a** was dissolved in THF (20 ml) and 0.1 M phosphate buffer (pH 7.0, 20 ml)

and hydrogenated at room temperature for 2.5 hours. in the presence of 10% Pd-charcoal (300 mg). The catalyst was removed from the reaction mixture by filtration with Celite, and the filtrate was washed twice with diethyl ether. The aqueous layer was then concentrated by evaporation under reduced pressure and the resulting residue was chromatographed through MCI GEL CHP-20 (Mitsubishi Kasei Corporation, 75~150 μm, 50 ml) developed with 5% aq acetone. The desired fraction was concentrated to 10 ml and then lyophilized to give a crude product as a powder. This crude product was purified by Lobar column chromatography (Merck Co., LiChroprep RP-8, size B; 10~15% aq MeOH) to afford the title compound **12a** as a colorless powder (90 mg): UV (H_2O) λ_{max} nm 297.3; ^{1}H NMR (270 MHz, D_2O, TMS) δ 1.02 (3H, d, J=7.3 Hz), 1.09 (3H, d, J=6.6 Hz), 1.96~2.14 (1H, m), 2.52~2.70 (1H, m), 3.00 (3H, s), 3.09 (3H, s), 3.04~3.18 (1H, m), 3.28 (1H, dd, J=6.1, 2.8 Hz), 3.38~3.67 (3H, m), 3.80 (1H, dd, J=12.5, 7.7 Hz), 3.91~4.11 (3H, m).

(5*R*,6*S*)-2-[(2*S*,4*S*)-2-Carbamoyl-1,1-dimethylpyrrolidinio-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-carbapen-2-em-3-carboxylate (**12b**)

To a suspension of **7b** (520 mg) in anisole (1.45 ml) were added TFA (5.14 ml) and TfOH (0.13 ml) under ice-cooling and the mixture was stirred at the same temperature for 2 hours. The mixture was concentrated by evaporation under reduced pressure and the residue was washed by decantation with diethyl ether and dried to afford mercaptan **8b** as an oil (420 mg).

To a solution of 4-nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-oxo-1-carbapenam-3-carboxylate (400 mg) in acetonitrile (5 ml) were added diphenylphosphoryl chloride (250 μl) and diisopropylethylamine (210 μl) under ice-cooling and the mixture was stirred at the same temperature for 1 hour. To the mixture were added *N*,*N*-diisopropylethylamine (240 μl) and **8b** (447 mg) in acetonitrile under ice-cooling and stirred for 4 hours, and then the mixture was allowed to stand in a refrigerator for 2 days. The mixture was concentrated by evaporation under reduced pressure and the residue was washed by decantation with diethyl ether and dried to afford **11b**. The crude product **11b** was dissolved in THF (30 ml) and 0.1 M phosphate buffer (pH 7.0, 30 ml) and hydrogenated at room temperature for 2.5 hours in the presence of 10% Pd-charcoal (450 mg). The catalyst was removed by filtration with Celite from the reaction mixture and the filtrate was washed twice with diethyl ether. The aqueous layer was then concentrated by evaporation under reduced pressure and the resulting residue was chromatographed through MCI GEL CHP-20 (Mitsubishi Kasei Corporation, 75~150 μm, 75 ml) developed with water. The desired fraction was concentrated to 10 ml and then lyophilized to give a crude product as a powder. This crude product was purified by Lobar column chromatography (Merck Co., LiChroprep RP-8, size B; 5% aq MeOH) to afford the title compound **12b** as a colorless powder (208 mg): UV (H_2O) λ_{max} nm 298; ^{1}H NMR (270 MHz, D_2O, TMS) δ 1.09 (3H, d, J=6.2 Hz), 2.01~2.18 (1H, m), 2.53~2.72 (1H, m), 2.89~3.04 (2H, m), 3.01 (3H, s), 3.09 (3H, s), 3.23 (1H, dd, J=6.1, 2.8 Hz), 3.36 ~3.67 (3H, m), 3.85 (1H, dd, J=12.6, 8.2 Hz), 3.93~4.09 (3H, m).

The following carbapenems **12c~g** were prepared as colorless powder from **7a~e** by a similar manner as that described for the synthesis of **12a**.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-Carbamoyl-1,1-dimethylpyrrolidinio-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**12c**): UV (H_2O) λ_{max} nm 297; ^{1}H NMR (270 MHz, D_2O, TMS) δ 1.02 (3H, d, J=7.3 Hz), 1.10 (3H, d, J=6.2 Hz), 2.20~2.31 (1H, m), 2.87~3.22 (2H, m), 3.10 (3H, s), 3.14 (3H, s), 3.28 (1H, dd, J=6.1, 2.8 Hz), 3.67~3.73 (1H, m), 3.86~4.08 (4H, m), 4.22 (1H, dd, J=9.4, 7.5 Hz).

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-Carbamoyl-1-(2-fluoroethyl)-1-methylpyrrolidinio-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**12d**): UV (H_2O) λ_{max} nm 297; ^{1}H NMR (270 MHz, D_2O, TMS) δ 1.03 (3H, d, J=7.3 Hz), 1.10 (3H, d, J=6.6 Hz), 2.27~2.35 (1H, m), 2.92~3.12 (2H, m), 3.19 (3H, s), 3.29 (1H, dd, J=6.1, 2.8 Hz), 3.67~3.78 (2H, m), 3.91~4.09 (5H, m), 4.37 (1H, dd, J=10.8, 7.5 Hz), 4.69~4.91 (2H, m).

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-1,1-Dimethyl-2-ethylcarbamoylpyrrolidinio-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**12e**): UV (H_2O) λ_{max} nm 297; ^{1}H NMR (270 MHz, D_2O, TMS) δ 0.95 (3H, t, J=7.2 Hz), 1.02 (3H, d, J=7.3 Hz), 1.10 (3H, d, J=6.2 Hz), 2.17~2.30 (1H, m), 2.81~3.18 (2H, m), 3.05 (3H, s), 3.10 (2H, q, J=7.3 Hz), 3.11 (3H, s), 3.28 (1H, dd, J=6.1, 2.8 Hz), 3.69 (1H, dd, J=12.1, 5.5 Hz), 3.84~4.16 (5H, m).

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-(1-Azetidinylcarbonyl)-1,1-dimethylpyrrolidinio-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**12f**): UV (H_2O) λ_{max} nm 296.6; IR (KBr) cm^{-1} 1708, 1654, 1608, 1583,1513; ^{1}H NMR (270 MHz, D_2O, TMS) δ 1.01 (3H, d, J=7.3 Hz), 1.09 (3H, d, J=6.2 Hz), 2.11~2.24 (3H, m), 2.79~2.93 (1H, m), 3.00~3.17 (1H, m), 3.08 (3H,

s), 3.10 (3H, s), 3.28 (1H, dd, J=6.2, 2.9 Hz), 3.66 ~4.10 (7H, m), 4.18~4.29 (3H, m).

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-1,1-Dimethyl-2-(1-pyrrolidinylcarbonyl)pyrrolidinio-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**12g**): UV (H_2O) λ_{max} nm 298; ^{1}H NMR (270 MHz, D_2O, TMS) δ 1.02 (3H, d, J=7.0 Hz), 1.10 (3H, d, J=6.2 Hz), 1.65~1.84 (4H, m), 2.14~2.24 (1H, m), 2.92~3.04 (1H, m), 3.10 (3H, s), 3.12 (3H, s), 3.20~3.57 (5H, m), 3.74~4.11 (6H, m), 4.47~4.63 (1H, m).

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-1,1-Dimethyl-2-(1-piperazinylcarbonyl)pyrrolidinio-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate hydrochloride (**12h**)

To a solution of **6f** (13.8 g) in anisole were added TFA (130 ml) and TfOH (4.26 ml) under ice-cooling and the solution was stirred at the same temperature for 30 minutes. The mixture was concentrated by evaporation under reduced pressure and the residue was washed by decantation with diethyl ether and dried to afford mercaptan **8f** as a colorless powder (13.9 g): IR (KBr) cm^{-1} 1695, 1643, 1518, 1446, 1345; ^{1}H NMR (270 MHz, DMSO-d_6, TMS) δ 1.73~1.85 (1H, m), 2.80, 2.82 (together 3H, s×2), 2.94~3.04 (1H, m), 3.31~3.79 (12H, m), 4.59~4.68 (1H, m), 5.26 (2H, s), 7.65, 8.24 (4H, A_2B_2, J=8.8 Hz).

To a solution of 4-nitrobenzyl (1*R*,5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-oxo-1-carbapenam-3-carboxylate (8.6 g) in acetonitrile (120 ml) were added diphenylphosphoryl chloride (7.0 g) and *N*,*N*-diisopropylethylamine (3.4 g) under ice-cooling and the mixture was stirred at the same temperature for 1 hour. To the mixture were added *N*,*N*-diisopropylethylamine (6.7 g) and **8f** (13.9 g) in acetonitrile (120 ml) under ice-cooling. The mixture was stirred for 4 hours and allowed to stand in a refrigerator for 1 day. The mixture was concentrated by evaporation under reduced pressure and the residue was diluted with EtOAc and washed with water and brine and dried ($MgSO_4$). The EtOAc layer was concentrated and the residue was purified by silica gel column chromatography (EtOAc - MeOH, 9 : 1) to give 4-nitrobenzyl (1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-1-methyl-2-[4-(4-nitrobenzyloxycarbonyl)-1-piperazinylcarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate **11h** (7.0 g) as a colorless powder: IR (KBr) cm^{-1} 1771, 1706, 1647, 1606, 1521, 1461, 1436, 1346; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 1.27 (3H, d, J=7.3 Hz), 1.37 (3H, d, J=6.4 Hz), 1.80~1.95 (1H, m), 2.37 (3H, s), 2.61~2.80 (2H, m), 3.10~3.82 (13H, m) 4.21~4.31 (3H, m), 5.20~5.52 (4H, m), 7.22 (2H,d, J=8.8Hz), 7.24 (2H, d, J=8.8 Hz), 7.53 (2H, d, J=8.8Hz), 7.66 (2H, d, J=8.8 Hz).

To a solution of **11h** (5 g) in acetonitrile was added methyl fluorosulfonate (0.57 ml) under ice-cooling. The mixture was stirred at 0~5°C for 30 minutes and then the mixture was concentrated by evaporation under reduced pressure. The residue was dissolved in 50% aq THF (50 ml) and 1 N hydrochloric acid (3.8 ml). The mixture was hydrogenated at room temperature for 2 hours in the presence of 10% Pd-charcoal (5 g). The catalyst was removed by filtration with Celite from the reaction mixture and the filtrate was washed twice with diethyl ether. The aqueous layer was then concentrated by evaporation under reduced pressure and the resulting residue was chromatographed through DOWEX 1-X4 (Cl^- type) column developed with water. The desired fraction was concentrated to 100 ml and then lyophilized to give a crude product as a powder. This crude product was purified by reverse phase column chromatography (Merck Co., LiChroprep RP-8; 1.5% aq MeOH) to afford the title compound **12h** as a colorless powder (1.8 g):

Anal Calcd for $C_{21}H_{32}N_4O_5SCl\cdot 2H_2O$:
C 48.04, H 6.91, N 10.67, S 6.10, Cl 6.75.
Found: C 47.84, H 7.17, N 10.66, S 5.83, Cl 7.03.

UV (H_2O) λ_{max} nm 296.2; IR (KBr) cm^{-1} 1756, 1653, 1600, 1606, 1455, 1436, 1383; ^{1}H NMR (270 MHz, D_2O, TMS) δ 1.05 (3H, d, J=7.3Hz), 1.10 (3H, d, J=6.4 Hz), 2.15~2.26 (1H, m), 2.90~3.22 (4H, m), 3.10 (3H, s), 3.18 (3H, s), 3.30 (1H, dd, J=6.1, 2.7 Hz), 3.60~4.11 (10H, m), 4.78 (1H, t, J=7.8 Hz).

Preparation of (2*S*,4*S*)-4-(4-Methoxybenzylthio)-2-(4-methyl-1-homopiperazinylcarbonyl)-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (**14a**)

To a solution of (2*S*,4*S*)-4-(4-methoxybenzylthio)-1-(4-nitrobenzyloxycarbonyl)-2-pyrrolidinecarboxylic acid **13** (2.68 g) in acetonitrile (30 ml) was added 1,1'-carbonyldiimidazole (1.17 g) at room temperature and the mixture was stirred at 40°C for 30 minutes. To the resulting solution was added 1-methylhomopiperazine (1.12 ml) at the same temperature and the mixture was stirred at 40°C for 3 hours. The mixture was concentrated, diluted with EtOAc, washed with brine and dried ($MgSO_4$). The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc - MeOH, 7 : 3) to give the compound **14a** as a pale yellow oil (2.6 g): IR (liq) cm^{-1} 1710, 1650, 1513; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 1.73~2.04 (3H, m), 2.23~2.77 (8H, m),

3.33~4.08 (6H, m), 3.70, 3.72 (together 2H, s×2), 3.79, 3.80 (together 2H, s×2), 4.15~4.63 (1H, m), 5.01~5.34 (2H, m), 6.85 (2H, d, J=8.8 Hz), 7.24 (2H, d, J=8.8 Hz), 7.45, 7.47 (together 2H, δ×2, J=8.8 Hz), 8.19, 8.23 (together 2H, δ×2, J=8.8 Hz).

Preparation of (2*S*,4*S*)-2-(4-carbamoylmethyl-1-homopiperazinylcarbonyl)-4-(4-methoxybenzylthio)-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (**14b**)

To a solution of **13** (15.0 g) in acetonitrile (120 ml) was added 1,1'-carbonyldiimidazole (6.55 g) at room temperature and the mixture was stirred at room temperature for 3 hours. To the resulting solution were added 1-carbamoylmethylhomopiperazine 2TFA (15.6 g) and *N*,*N*-diisopropylethylamine (22 ml) in acetonitrile (100 ml) at room temperature and the mixture was stirred at 45~50°C for 20 hours. The mixture was concentrated, diluted with EtOAc, washed with brine and dried ($MgSO_4$). The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc - MeOH, 9 : 1) to give the compound **14b** as a colorless powder (16 g): IR (KBr) cm^{-1} 1707, 1684, 1651, 1609, 1584, 1512; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 1.75 (1H, m), 2.01~2.33 (1H, m), 2.40~4.75 (16H, m), 3.74 (2H, s), 3.79 (3H, s), 5.07~5.29 (2H, m), 6.05 (1H, br s), 6.83~6.94 (2H, m), 7.17~7.33 (2H, m), 7.40~7.55 (2H, m), 8.16~8.27 (2H, m), 8.67 (1H, br s).

The following compounds **14c~g** were prepared as colorless powder from **13** by a similar manner as that described for the preparation of **14b**.

(2*S*,4*S*)-2-(4-Carbamoylmethyl-1-piperazinylcarbonyl)-4-(4-methoxybenzylthio)-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (**14c**): IR (KBr) cm^{-1} 1708, 1654, 1608, 1583, 1513; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 1.72~1.83 (1H, m), 2.02~2.10 (1H, m), 2.34~2.72 (5H, m), 2.99~3.18 (3H, m), 3.30~3.64 (4H, m), 3.73 (2H, s), 3.79, 3.80 (together 3H, s×2), 3.82~4.13 (1H, m), 4.52~4.77 (1H, m), 5.01~5.36 (2H, m), 5.56 (1H, br s), 6.77, 6.88 (together 1H, br s×2), 6.84, 6.85 (together 2H, δ×2, J=8.8 Hz), 7.22, 7.23 (together 2H, δ×2, J=8.8 Hz), 7.43, 7.47 (together 2H, δ×2, J=8.8 Hz), 8.19, 8.23 (together 2H, δ×2, J=8.8 Hz).

(2*S*,4*S*)-4-(4-Methoxybenzylthio)-2-[4-(4-nitrobenzyloxycarbonylmethyl)-1-homopiperazinylcarbonyl]-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (**14d**): IR (liq) cm^{-1} 1748, 1704, 1650, 1608, 1520, 1429; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 1.71~2.03 (3H, m), 2.42~3.17 (6H, m), 3.32~4.08 (8H, m), 3.73 (2H, s), 3.80, 3.82 (together 3H, s×2), 4.49~4.63 (1H, m), 5.02~5.35 (4H, m), 6.85 (2H, d, J=8.3 Hz), 7.23 (2H, d, J=8.3 Hz), 7.43~7.52 (4H, m), 8.15~8.25 (4H, m).

(2*S*,4*S*)-4-(4-Methoxybenzylthio)-2-[4-[2-(4-nitrobenzyloxycarbonyloxy)ethyl]-1-homopiperazinylcarbonyl]-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (**14e**): IR (KBr) cm^{-1} 1748, 1709, 1650, 1608, 1521, 1347; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 1.71~1.89 (2H, m), 2.42~2.86 (7H, m), 3.01~3.13 (1H, m), 3.32~3.88 (8H, m), 3.72 (2H, s), 3.78, 3.80 (together 3H, s×2), 4.01~4.27 (3H, m), 4.51~4.61 (1H, m), 5.00~5.33 (4H, m), 6.84 (2H, d, J=8.3 Hz), 7.23 (2H, d, J=8.3 Hz), 7.41~7.56 (4H, m), 8.16~8.24 (4H, m).

(2*S*,4*S*)-4-(4-Methoxybenzylthio)-2-[(*S*)-1-methyl-3-pyrrolidinylaminocarbonyl]-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (**14f**): IR (KBr) cm^{-1} 1713, 1648, 1523, 1346; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 1.87~3.71 (16H, m), 3.79 (3H, s), 4.15~4.50 (2H, m), 5.10~5.30 (2H, m), 6.82~6.87 (2H, m), 7.19~7.23 (2H, m), 7.49 (2H, d, J=8.8 Hz), 8.22 (2H, d, J=8.3 Hz).

(2*S*,4*S*)-2-[(*S*)-3-Dimethylamino-1-pyrrolidinylcarbonyl]-4-(4-methoxybenzylthio)-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (**14g**): IR (KBr) cm^{-1} 1710, 1654, 1512, 1345; ^1H NMR (270 MHz, DMSO-d_6, TMS) δ 1.49~3.31 (15H, m), 3.35~3.57 (2H, m), 3.71~4.00 (6H, m), 4.44~4.56 (1H, m), 5.00~5.21 (2H, m), 6.88 (2H, d, J=8.8 Hz), 7.27 (2H, d, J=8.3 Hz), 7.51~7.61 (2H, m), 8.19~8.26 (2H, m).

Synthesis of (1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-(4,4-Dimethyl-1-homopiperaziniocarbonyl)pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate hydrochloride (**17a**)

To a suspension of **14a** (620 mg) in anisole (1.23 ml) were added TFA (6.2 ml) and TfOH (200 μl) under ice-cooling and then the mixture was stirred at room temperature for 1 hour. The mixture was concentrated by evaporation under reduced pressure and the residue was washed by decantation with diethyl ether and dried to afford mercaptan **15a** as an oil (640 mg): IR (liq) cm^{-1} 1701, 1608, 1524, 1347; ^1H NMR (270 MHz, DMSO-d_6, TMS) δ 1.66~1.78 (1H, m), 1.96~2.13 (2H, m), 2.67~2.86 (5H, m), 2.99~4.07 (2H, m), 4.64~4.83 (1H, m), 5.02~5.26 (2H, m), 7.49~7.65 (2H, m), 8.23 (2H, d, J=8.8 Hz).

To a solution of 4-nitrobenzyl (1*R*,5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-oxo-1-carbapenam-3-carboxylate (340 mg) in acetonitrile (3.5 ml) were added diphenylphosphoryl chloride (210 μl) and *N*,*N*-diisopropylethylamine (180 μl) under ice-cooling and the mixture was stirred at the same temperature for 1 hour. To the mixture

were added *N,N*-diisopropylethylamine (580 μl) and **15a** (640 mg) in acetonitrile (5 ml) under ice-cooling and stirred for 2 hours and the mixture was allowed to stand overnight in a refrigerator. The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc - MeOH, 5 : 1) to afford **16a** as a colorless powder (247 mg): IR (KBr) cm^{-1} 1773, 1711, 1654, 1606, 1521, 1345; ^1H NMR (270 MHz, DMSO-d_6, TMS) δ 1.15 (3H, d, J=6.4 Hz), 1.16 (3H, d, J=7.3 Hz), 1.57~1.66 (1H, m), 2.05~2.38 (5H, m), 2.12, 2.20 (together 3H, s×2), 2.81~2.89 (1H, m), 3.11~3.63 (7H, m), 3.79~4.28 (4H, m), 4.76, 4.85 (together 1H, t×2, J=7.8 Hz), 5.05~5.48 (4H, m), 7.55, 7.65 (together 2H, d×2, J=8.8 Hz), 7.72 (2H, d, J=8.8 Hz), 8.22, 8.23 (together 4H, d×2, J=8.8 Hz).

To a solution of **16a** (1.36 g) in methylene chloride (13 ml) was added methyl fluorosulfonate (283 μl) under ice cooling and then the mixture was stirred at room temperature for 1 hour. The mixture was concentrated by evaporation under reduced pressure and the residue was dissolved in 50% aq THF (60 ml) and hydrogenated at room temperatire for 3 hours in the presence of 10% Pd-charcoal (1.2 g). The catalyst was removed by filtration with Celite from the reaction mixture and the filtrate was washed twice with diethyl ether. The aqueous layer was then concentrated to 20 ml by evaporation under reduced pressure and then lyophilized to give a crude product as a powder (920 mg). This crude product was chromatographed through DOWEX 1-X4 (Cl^- type) column developed with water. The desired fraction was concentrated and purified by Lobar column chromatography (Merck Co. LiChroprep RP-8, size B; 5% aq MeOH) to afford the title compound **17a** as a colorless powder (480 mg): ^1H NMR (270 MHz, D_2O, TMS) δ 1.02 (3H, d, J=7.3 Hz), 1.10 (3H, d, J=6.3 Hz), 1.72~1.89 (1H, m), 2.15 (2H, br s), 2.75~2.96 (1H, m), 3.02, 3.03 (together 6H, s×2), 3.11~3.25 (1H, m), 3.26~3.34 (2H, m), 3.41~3.92 (10H, m), 4.02~4.11 (2H, m), 4.63~4.69 (1H, m).

Synthesis of (1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-(4-carbamoylmethyl-4-methyl-1-homopiperaziniocarbonyl)pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate Hydrochloride (**17b**)

To a suspension of **14b** (15.6 g) in anisole (29 ml) were added TFA (78 ml) and TfOH (3.5 ml) under ice-cooling and then the mixture was stirred at room temperature for 1 hour. The mixture was concentrated by evaporation under reduced pressure and the residue was washed by decantation with diethyl ether and dried. The resulting powder was neutralized with aq $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried (Na_2SO_4) and concentrated by evaporation under reduced pressure to afford mercaptan **15b** as a colorless powder (13 g): IR (KBr) cm^{-1} 1698, 1655, 1608, 1522, 1437, 1409; ^1H NMR (400 MHz, $CDCl_3$]D_2O, TMS) δ 1.73~2.22 (3H, m), 2.55~2.88 (4H, m), 2.96~3.08 (2H, m), 3.17~4.16 (9H, m), 4.60~4.72 (1H, m), 5.05~5.40 (2H, m), 7.51, 8.23 (4H, A_2B_2, J=8.7 Hz).

To a solution of 4-nitrobenzyl (1*R*,5*R*,6*S*)-2-diphenylphosphoryloxy-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapenem-3-carboxylate (13.5 g) in acetonitrile (130 ml) were added **15b** (12.9 g) in acetonitrile (50 ml) and *N,N*-diisopropylethylamine (3.9 ml) under ice-cooling and the mixture was stirred for 6 hours and was allowed to stand overnight in a refrigerator. The mixture was concentrated by evaporation under reduced pressure and the residue was purified by a reverse phase column chromatography (nacalai tesque, Cosmosil 75C_{18}-PREP, 50% aqueous acetonitrile) to afford **16b** as a colorless powder (11.2 g): IR (KBr) cm^{-1} 1772, 1707, 1652, 1606, 1521, 1495, 1433, 1405; ^1H NMR (400 MHz, DMSO-d_6, TMS) δ 1.10~2.02 (6H, m), 1.57~1.97 (3H, m), 2.25~3.40 (10H, m), 3.41~3.73 (4H, m), 3.77~4.05 (2H, m), 4.10~4.30 (2H, m), 4.71, 4.80 (together 1H, t×2, J=7.7 Hz), 5.06~5.50 (5H, m), 7.05~7.25 (2H, m), 7.52~7.74 (4H, m), 8.14~8.27 (4H, m).

To a solution of **16b** (4.92 g) in acetonitrile (50 ml) was added methyl fluorosulfonate (0.72 ml) under ice cooling and then the mixture was stirred at 0~5°C for 1 hour. The mixture was concentrated by evaporation under reduced pressure, the residue was dissolved in THF (130 ml) and water (100 ml) and hydrogenated at room temperature for 3 hours in the presence of 10% Pd-charcoal (10 g). The catalyst was removed from the reaction mixture by filtration with Celite and the filtrate was washed twice with diethyl ether. The aqueous layer was then concentrated by evaporation under reduced pressure. The residue was chromatographed through DOWEX 1-X4 (Cl^- type) column developed with water. The desired fraction was concentrated and purified by a riverse phase column chromatography (nacalai tesque, Cosmosil 75C_{18}-PREP, water) to afford the title compound **17b** as a colorless powder (1.45 g):

Anal Calcd for $C_{23}H_{36}N_5O_6SCl \cdot 2H_2O$:
C 47.46, H 6.93, N 12.03, S 5.51, Cl 6.09.
Found: C 47.85, H 7.30, N 12.24, S 5.33, Cl 6.35.

UV (H_2O) λ_{max} nm 296.6; IR (KBr) cm^{-1} 1756, 1695, 1652, 1594, 1459, 1383; ^1H NMR (400 MHz, D_2O, TSP)

δ 1.22 (3H, d, J=7.2 Hz), 1.29 (3H, d, J=6.4 Hz), 1.96~2.08 (1H, m), 2.26~2.46 (2H, m), 3.00~3.14 (1H, m), 3.33~3.42 (1H, m), 3.38 (3H, s), 3.45~3.53 (2H, m), 3.64~4.14 (10H, m), 4.20~4.32 (4 H, m), 4.81~4.92 (1H, m).

The following carbapenems **17c** and **17e~h** were prepared as colorless powder from **14c~g** by a similar manner as that described for the synthesis of **17a**.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-(4-Carbamoylmethyl-4-methyl-1-piperaziniocarbonyl)pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate hydrochloride (**17c**): IR (KBr) cm^{-1} 1754, 1694, 1660, 1600, 1453, 1386; ^1H NMR (270 MHz, D_2O, TMS) δ 1.02 (3H, d, J=7.3Hz), 1.10 (3H, d, J=6.4 Hz), 1.72~1.83 (1H, m), 2.76~2.88 (1H, m), 3.13~3.30 (2H, m), 3.26 (3H, s), 3.43~3.88 (11H, m), 4.02~4.15 (4H, m), 4.51~4.58 (1H, m).

Synthesis of (1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[4,4-bis(carbamoylmethyl)-1-homopiperaziniocarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate Hydrochloride (**17d**)

To a solution of **16b** in acetonitrile (20 ml) was added 2-iodoacetamide (2.3 g) and the mixture was stirred at 70°C for 18 hours and then heated at reflux for 4 hours. The mixture was cooled to 25°C, concentrated by evaporation under reduced pressure, and the residue was washed by decantation with diethyl ether and dried to give a colorless powder (2.9 g). The resulting powder (1.3 g) was dissolved in THF (20 ml) and water (18 ml) and hydrogenated in the presence of 10% Pd-charcoal (1.8 g) and treated by a similar manner as that described for the synthesis of **17a** to give **17d** as colorless powder (164 mg): IR (KBr) cm^{-1} 1756, 1695, 1652, 1597, 1451, 1385; ^1H NMR (270 MHz, D_2O, TSP) δ 1.21 (3H, d, J=7.3 Hz), 1.28 (3H, d, J=6.4 Hz), 1.94~2.10 (1H, m), 2.23~2.46 (2H, m), 2.98~3.16 (1H, m), 3.30~3.57 (3H, m), 3.62~4.35 (12H, m), 4.56 (2H, s), 4.58 (2H, s), 4.80~4.95 (1H, m).

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-(4-Carboxymethyl-4-methyl-1-homopiperaziniocarbonyl)pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**17eA** and **17eB**): fast eluted isomer (**17eA**): IR (KBr) cm^{-1} 1761, 1660, 1455, 1382; ^1H NMR (270 MHz, D_2O, TSP) δ 1.22 (3H, d, J=7.3 Hz), 1.29 (3H, d, J=6.4 Hz), 1.97~2.08 (1H, m), 2.20~2.45 (2H,m), 3.02~3.14 (1H, m), 3.30~3.58 (3H, m), 3.32, 3.36 (together 3H, s×2), 3.61~4.09 (12H, m), 4.21~4.30 (2H, m), 4.70~4.89 (1H, m).

Slow eluted isomer (**17eB**): IR (KBr) cm^{-1} 1752, 1634, 1464, 1386; ^1H NMR (270 MHz, D_2O, TMS) δ 1.22 (3H, d, J=7.3 Hz), 1.30 (3H, d, J=6.4 Hz), 1.96~2.06 (1H, m), 2.27~2.50 (2H,m), 2.96~3.12 (1H, m), 3.28~3.56 (3H, m), 3.33, 3.35 (together 3H, s×2), 3.59~4.12 (12H, m), 4.21~4.30 (2H, m), 4.72~4.89 (1H, m).

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[4-(2-Hydroxyethyl)-4-methyl-1-homopiperaziniocarbonyl)pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate hydrochloride (**17f**): IR (KBr) cm^{-1} 1768, 1707, 1638, 1521, 1345; ^1H NMR (270 MHz, D_2O, TSP) δ 1.21 (3H, d, J=6.9 Hz), 1.28 (3H, d, J=6.3 Hz), 1.95~2.08 (1H, m), 2.34 (2H, br s), 3.03~3.14 (1H, m), 3.24 (3H, s), 3.31~3.43 (1H, m), 3.44~3.53 (3H, m), 3.55~4.16 (13H, m), 4.20~4.29 (2H, m), 4.83~4.92 (1H, m).

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-1,1-Dimethyl-3-pyrrolidinioaminocarbonyl]pyrrolidin-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate hydrochloride (**17g**): IR (KBr) cm^{-1} 1758, 1683, 1595, 1562, 1452, 1384; ^1H NMR (270 MHz, D_2O, TSP) δ 1.21 (3H, d, J=7.3 Hz), 1.29 (3H, d, J=6.4 Hz), 2.10~2.20 (1H, m), 2.25~2.40 (1H, m), 2.70~2.85 (1H, m), 2.85~3.00 (1H, m), 3.24 (3H, s), 3.28 (3H, s), 3.35 (1H, dd, J=9.3, 7.3 Hz), 3.40~4.10 (9H, m), 4.20~4.30 (2H, m), 4.48 (1H, dd, J=9.3, 5.9 Hz).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-[(2*S*,4*S*)-2-[(*S*)-3-trimethylammonio-1-pyrrolidinylcarbonyl]pyrrolidin-4-ylthio]-1-carbapen-2-em-3-carboxylate hydrochloride (**17h**): IR (KBr) cm^{-1} 1756, 1656, 1599, 1479, 1373; ^1H NMR (270 MHz, D_2O, TSP) δ 1.21 (3H, d, J=6.8 Hz), 1.28 (3H, d, J=6.4Hz), 1.95~2.10 (1H, m), 2.40~2.65 (2H, m), 3.00~3.15 (1H, m), 3.21 (6H, s), 3.23 (3H, s), 3.37 (1H, dd, J=9.3, 7.3 Hz), 3.40~4.20 (9H, m), 4.20~4.30 (2H, m), 4.65~4.75 (1H, m).

Measurement of Antibacterial Activity

MICs were measured on Nutrient agar (Eiken Chemical Ltd.) by the twofold dilution method. The inoculum size of the bacteria was one-loopful of 10^7 cfu/ml.

Urinary Recovery of Carbapenems in Mice

Carbapenems **12a~h** and **17a~h** (dose: 50 mg/kg) were dissolved in water and then subcutaneously administered to mice (n=5, SPF ddY strain). Urine was collected at 8 hours and 24 hours after administration. Excretion as the parent carbapenem was determined by bioassay using *Bacillus subtilis* ATCC 6633. Urinary recovery (%, 0~24 hours) was calculated based on the

excretion and the initial dose.

Therapeutic Effect on Systemic Mouse Infections

Overnight cultures of organisms grown at 37°C in Trypto-soy broth (Eiken Chemical Co., Ltd., Tokyo, Japan) were diluted according to their virulence. The diluted cultures, if necessary, were mixed with the same amount of 5% gastric mucin (Tokyokasei-kogyo Co., Ltd., Tokyo, Japan). Seven male SPF *dd*Y mice in each group were infected intraperitoneally with 0.2 ml portions of these bacterial mixtures. β-Lactam antibiotics were administered subcutaneously at 0 and 4 hours after infection. The ED_{50} of mice were calculated by the probit method according to the survial rate after 5 days.

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