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Synthesis and Structure-activity Relationships of Novel Parenteral Carbapenems, CS-023 (R-115685) and Related Compounds Containing an Amidine Moiety

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In order to design a new parenteral 1β-methylcarbapenem antibiotic which has a broad antibacterial spectrum and improved plasma half-life, a series of 1β-methylcarbapenems with 5-substituted pyrrolidine-3-ylthio groups including an amidine moiety at the C-2 position have been synthesized and structure-activity relationships were investigated. Among those carbapenem derivatives, CS-023 (R-115685) showed a broad spectrum and excellent antibacterial activity against Gram-positive and Gram-negative bacteria. This compound also showed sufficient dehydropeptidase-I (DHP-I) stability and high urinary recovery in animals after subcutaneous administration without cilastatin, a DHP-I inhibitor. Based on these characteristics, CS-023 was selected for further study.

Carbapenems are one of the most potent types of antibacterial agents and are among those used as last resort against infections in the clinical field. Two 1-H carbapenems, imipenem[1,2] and panipenem,[3~5] and three 1β-methylcarbapenems, meropenem,[6] biapenem,[7,8] ertapenem[9] have been marketed so far. In particular, since it was revealed that 1β-methylcarbapenems showed not only a broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria but also high stability to human renal DHP-I,[10,11] a lot of 1β-methylcarbapenem derivatives have been prepared and their biological properties were studied in the last two decades.[12] In fact, several carbapenem derivatives have been developed such as S-4661,[13] E-1010,[14,15] IH201,[16] and J-111,347[17] and are under clinical or preclinical studies since the launch of meropenem.

A distinctive advantage of carbapenem antibiotics is their potent activity against *Pseudomonas aeruginosa*. In recent years, however, a number of clinical *P. aeruginosa* isolates have been reported to be resistant to carbapenems.[18~20] In general, Gram-negative bacteria including *P. aeruginosa* have an outer membrane, which covers the whole cell and prevents hydrophilic molecules from penetrating into the bacterial cell. However, Gram-negative bacteria can incorporate nutrients from the environment through porin protein channels. In *P. aeruginosa*, the OprD porin protein channel is known to facilitate the preferential incorporation of basic amino acids, such as lysine, arginine, histidine and ornithine, and peptides containing these basic amino acids and structurally related compounds, such as carbapenems and penems.[19,21] Imipenem, panipenem and meropenem have been shown to penetrate the outer membrane mainly through OprD channels[22] and the antipseudomonal activity of these carbapenems was influenced by the concentration of basic amino acids.[23] These facts suggest that carbapenem derivatives as mimics of basic amino acids could increase the antipseudomonal activity.

On the other hand, from SAR studies of panipenem and meropenem, it was found that carbapenems with a pyrrolidine moiety among various cyclic amines as a side chain had very potent antibacterial activity. In a previous paper, we also reported on potent carbapenems having a pyrrolidine moiety.[24]

Based on these findings, we designed and synthesized

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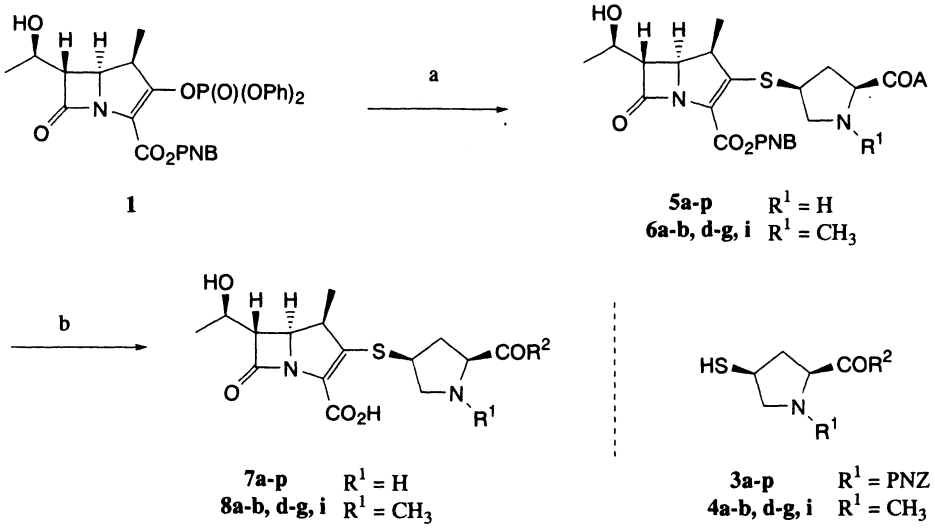
Scheme 1.



Reagents and conditions: a) **3** or **4**, DIPEA / CH_3CN; b) H_2, 10% Pd/C / THF-H_2O

novel 5-substituted pyrrolidine-3-ylthio-1β-methyl-carbapenems with an amidine moiety, which are comparatively more basic, in order to discover the most potent antipseudomonal carbapenem that also has a broad spectrum of activity. Here, we report the synthesis and the structure-activity relationships of CS-023 and related compounds having an amidine moiety and the DHP-I stability of CS-023.

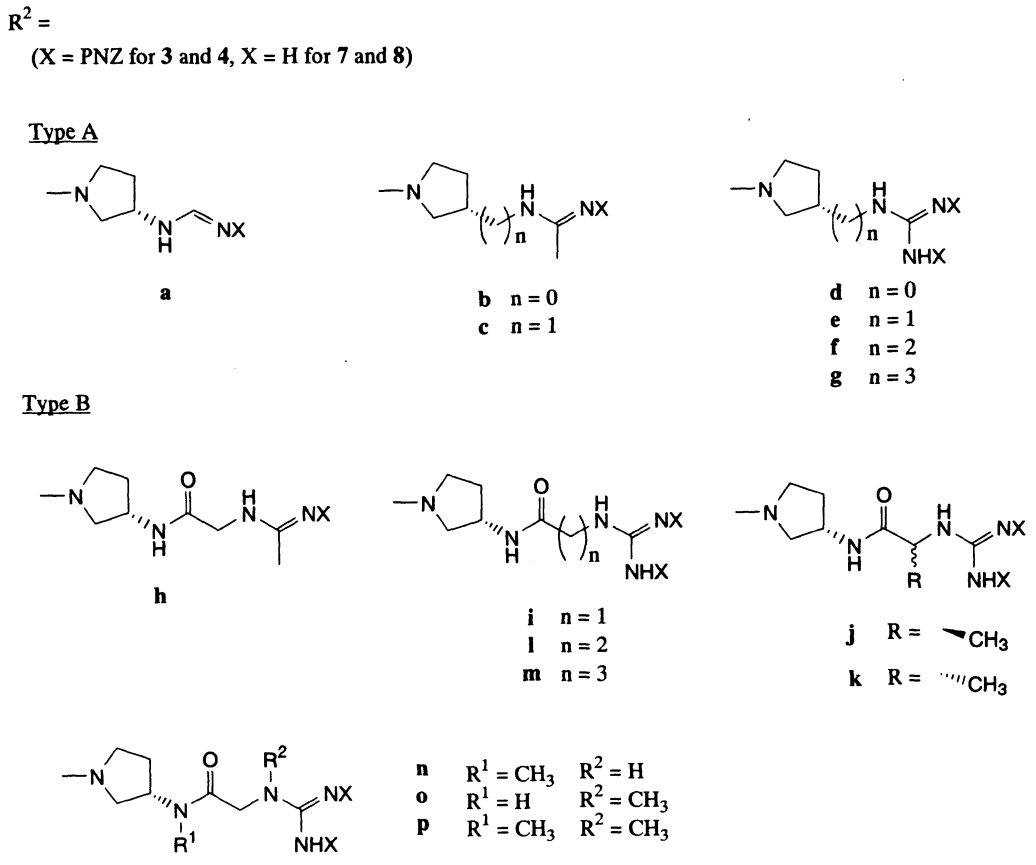
Chemistry

A series of carbapenem derivatives was synthesized by conventional procedures as shown in Scheme 1. The thiols **3** and **4**, which were prepared by known methods,[24~26] with various 3-substituted pyrrolidines (Figure 1) led to carbapenem precursor **5** and **6**, respectively, *via* a coupling reaction with carbapenem enol phosphate **1**. Subsequent deprotection of these precursors by hydrogenation gave the crude carbapenems and purification by column chromatography yielded the desired carbapenem derivatives as solids after lyophilization.

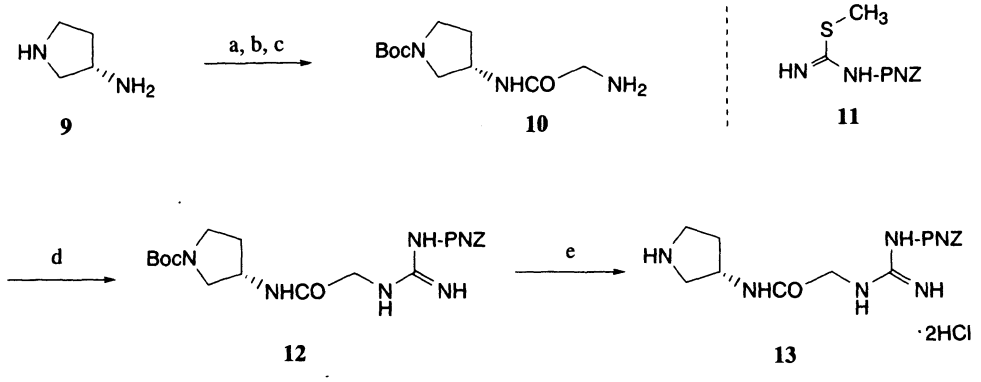
The practical synthesis of CS-023 is described in Scheme 2 and Scheme 3. Scheme 2 shows the preparation of the 3-aminopyrrolidine moiety which includes a guanidine group. Aminopyrrolidine **9** was converted to amine **10** by protection of the secondary amine, chloroacetylation and subsequent amination using aqueous ammonia solution in the presence of NaI. The guanidation of amine in the synthesis of the side chain was carried out with a 1*H*-pyrazole-1-carboxyamidine reagent[27] or isothiourea reagent[28] that was protected by two PNZ groups. However, it was found that guanidation was also effective with isothiourea reagent **11** protected by one PNZ group. The stability of the synthetic intermediates was improved by changing to this new method because mono PNZ intermediate **12** was more stable than the corresponding bis PNZ intermediate. After introduction of the guanidine moiety, deprotection of the resulting compound **12** with 4 M HCl-EtOAc gave an HCl salt of desired pyrrolidine **13**.

Next, the preparation of proline derivative **16** is shown in Scheme 3. L-Hydroxyproline was converted into allyl ester **15** by reductive amination with formaldehyde and esterification. Mesylation of **15** using a standard procedure was followed by thioacetylation with thioacetic acid and Cs_2CO_3 to give allyl ester **16**. Deprotection of allyl ester **16** with a Pd catalyst effectively led to carboxylic acid **17**, which is another important intermediate in the side chain synthesis. The condensation of **16** and **13** *via* a mixed anhydride afforded the completed side chain **18**. Deacetylation of **18** under alkaline condition followed by coupling reaction with enol phosphate **1** provided CS-023 precursor **19**. In the last step, the deprotection of **19** with 7.5% Pd/C under a hydrogen atmosphere and recrystallization afforded CS-023 (**8i**) as a stable crystal

Fig. 1. Various side chains of novel carbapenem derivatives.

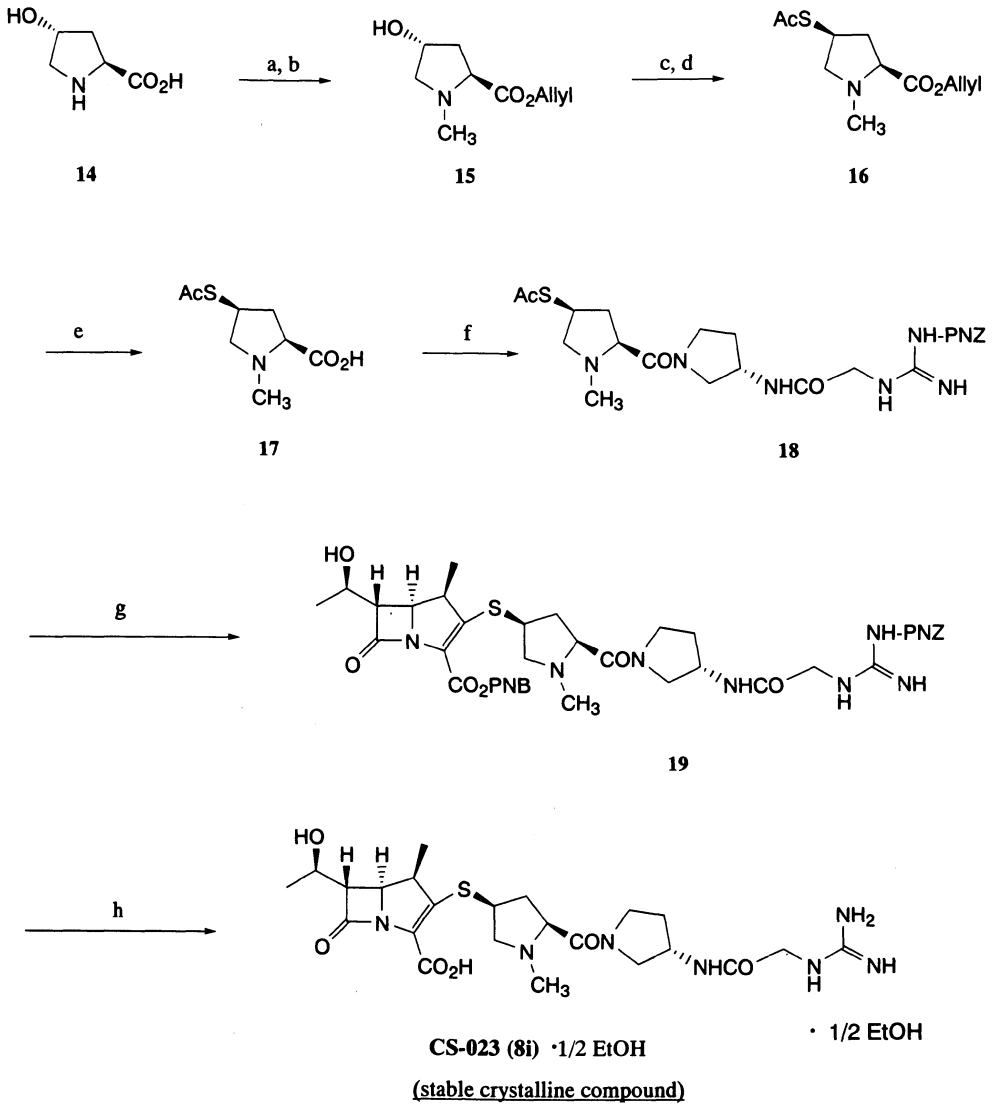


Scheme 2.



Reagents and conditions: a) Boc_2O / MeOH, 79%; b) chloroacetyl chloride, Et_3N / CH_2Cl_2, quant.; c) 28% aq. NH_3, NaI / MeOH, 87%; d) **11** / MeOH, 69%; e) 4M HCl-EtOAc / CH_3CN, 95%.

Scheme 3.



CS-023 (8i) ·1/2 EtOH

(stable crystalline compound)

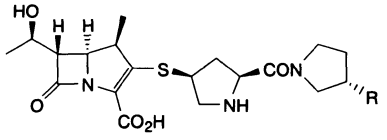
Reagents and conditions: a) H_2, aq. HCHO, PtO_2 / H_2O-AcOH, 93%; b) $SOCl_2$ / allyl alcohol, quant.; c) MsCl, Et_3N / THF, 77%; d) AcSH, Cs_2CO_3 / dimethylacetamide, 70%; e) $Pd(PPh_3)_4$, PPh_3, dimedone, 82%; f) PivCl, *i*-Pr_2NEt / CH_3CN then **13**, *i*-Pr_2NEt, 62%; g) NaOMe / MeOH then **1**, *i*-Pr_2NEt / DMF, 63%; h) H_2, 7.5% Pd-C / THF-H_2O then recrystallization from H_2O-EtOH, 61%.

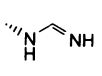
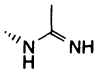
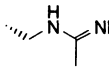
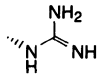
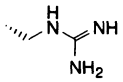
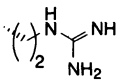
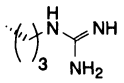
containing a half molar equivalent of ethanol.

Biological Properties

In vitro antibacterial activity and urinary recovery of novel carbapenems with an amidine moiety are shown in Table 1 to Table 5. We already found that a series of 5-[(substituted pyrrolidinyl)carbonyl]pyrrolidin-3-ylthio-carbapenems showed potent antibacterial activity against a wide range of Gram-positive and Gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*.[24] Furthermore, we investigated the most suitable substituted pyrrolidine moiety, which improved the antibacterial activity and urinary recovery, at the end of the C-2 side chain. In this study, the introduction of an amidine moiety, which has high basicity and low nucleophilicity, was examined in order to improve drug permeability through the outer

Table 1. Antibacterial activity (MIC, μg/ml) and urinary recovery of novel carbapenems.



R =							
	7a	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**
Staphylococcus aureus 209P	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.012	≤0.012
S. aureus 56R	0.02	0.02	0.02	0.02	≤0.01	≤0.012	≤0.012
S. aureus 535 (MRSA)	3.1	3.1	3.1	1.5	1.5	1.56	1.56
Enterococcus faecalis 681	0.4	0.8	0.8	0.4	0.4	0.20	0.39
Escherichia coli NIHJ	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.012	≤0.012
E. coli 609	0.02	0.02	0.05	0.02	0.02	≤0.012	0.025
Salmonella enteritidis	0.02	0.02	0.02	≤0.01	≤0.01	≤0.012	≤0.012
Klebsiella pneumoniae 806	0.02	0.02	0.02	≤0.01	≤0.01	≤0.012	≤0.012
Enterobacter cloacae 963	0.05	0.02	0.1	0.02	0.1	0.05	0.10
Serratia marcescens 1184	0.02	0.02	0.02	0.02	≤0.01	≤0.012	≤0.012
Proteus vulgaris 1420	0.1	0.1	0.4	0.1	0.2	0.10	0.10
Morganella morganii 1510	0.2	0.2	0.1	0.2	0.1	0.05	0.10
Pseudomonas aeruginosa 1001	0.05	0.05	0.1	0.05	0.05	0.025	0.10
P. aeruginosa N07	0.2	0.2	0.1	0.1	0.05	0.025	0.10
P. aeruginosa 3719	0.2	0.2	0.2	0.2	0.1	0.10	0.39
Urinary recovery (%)	21.6	21.6	18.2	13.0	10.1	1.0	1.1

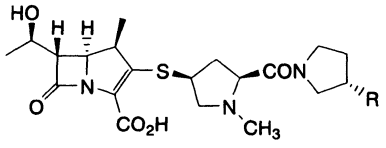
membrane (especially the OprD channel). Imipenem and panipenem are well-known carbapenems having an amidine moiety.

In our last paper,[24] we found that carbapenem derivatives with an (*S*)-3-aminopyrrolidine or (*R*)-3-aminomethyl-pyrrolidine moiety showed exellent antibacterial activity and significant urinary recovery. First, we introduced amidino moieties into these derivatives and elongated the methylene chain at the end of the pyrrolidine moiety. The antibacterial activity of these carbapenems **7a**~**7g** is shown in Table 1. As shown in Table 1, carbapenem **7d** having a guanidine moiety showed a broad spectrum and potent antibacterial activity compared with carbapenems **7a** and **7b**. In short, among the three kinds of amidine moiety (**7a**, **7b** and **7d**), the guanidine moiety gave the best result. However, although an elongation of the methylene chain of the 3-substituted pyrrolidine at the end of the side chain (**7d**, **7e**, **7f** and **7g**) was effective in improving the antibacterial activity against Gram-negative bacteria, the urinary recovery of these derivatives dropped considerably.

Next, we carried out further modifications to increase the level of urinary recovery without decreasing the antibacterial activity. It was found that the introduction of a methyl group to the pyrrolidine moiety next to the carbapenem core raised the level of urinary recovery.[24] Therefore, we introduced a methyl group on the nitrogen atom of the 3,5-disubstituted pyrrolidine moiety in the side chain. The results are shown in Table 2. The antibacterial activity of all of the N–Me derivatives (**8a**~**8g**) against both Gram-positive and Gram-negative bacteria was maintained. Unfortunately, the antipseudomonal activity of carbapenems **8a**~**8g** was 2~16 fold lower than that of N–H derivatives (**7a**~**7g**) and the increase in urinary recovery of carbapenems **8a**~**8g** was also not satisfactory.

After many trials, we found that carbapenem derivatives with a 3-amino pyrrolidine moiety tethered by an amidino acetyl group at the end of the side chain improved urinary recovery. As shown in Table 3, carbapenems **7h**, **7i**, **7l** and **7m**, which are derivatives with an amide functional group inserted at the end of the pyrrolidine side chain of **7c**, **7e**, **7f**

Table 2. Antibacterial activity (MIC, μg/ml) and urinary recovery of novel carbapenems.



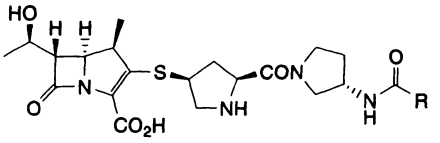
	R =						
		8a	**8b**	**8d**	**8e**	**8f**	**8g**
Staphylococcus aureus 209P		0.02	≤0.01	≤0.01	≤0.01	≤0.012	≤0.012
S. aureus 56R		0.1	0.1	0.05	0.05	0.025	0.05
S. aureus 535 (MRSA)		3.1	3.1	1.5	3.1	1.56	1.56
Enterococcus faecalis 681		0.4	0.2	0.2	0.4	0.10	0.20
Escherichia coli NIHJ		≤0.01	≤0.01	≤0.01	≤0.01	≤0.012	≤0.012
E. coli 609		0.02	0.02	0.02	0.05	≤0.012	0.05
Salmonella enteritidis		≤0.01	≤0.01	≤0.01	≤0.01	≤0.012	0.025
Klebsiella pneumoniae 806		≤0.01	0.02	≤0.01	≤0.01	≤0.012	0.025
Enterobacter cloacae 963		0.02	0.02	0.02	0.05	0.05	0.10
Serratia marcescens 1184		≤0.01	≤0.01	≤0.01	0.02	≤0.012	0.025
Proteus vulgaris 1420		0.2	0.1	0.1	0.2	0.10	0.20
Morganella morganii 1510		0.4	0.4	0.2	0.2	0.10	0.20
Pseudomonas aeruginosa 1001		0.2	0.4	0.4	0.4	0.39	0.78
P. aeruginosa N07		0.4	0.4	0.4	0.8	0.39	1.56
P. aeruginosa 3719		0.4	0.8	0.8	1.5	0.78	1.56
Urinary recovery (%)		35.9	38.4	31.7	31.2	19.7	9.3

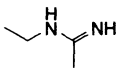
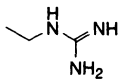
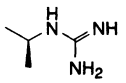
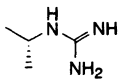
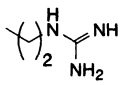
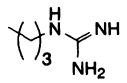
and **7g**, respectively, showed potent antibacterial activities and significant improvement of urinary recoveries (**7e** (10.1%)~**7i** (20.1%), **7f** (1.0%)~**7l** (17.6%), **7g** (1.1%)~**7m** (10.3%)). On the other hand, the introduction of a chiral methyl group into the guanidinoacetyl moiety was not effective in improving both the antibacterial activity and urinary recovery (**7j** and **7k**). At this stage, **7i** was selected as a promising lead compound for further investigation due to its well-balanced and potent antibacterial activity against both Gram-positive and Gram-negative bacteria and improved urinary recovery.

In the next stage, we carried out the synthesis of some N–Me derivatives of **7i** for further improvement of urinary recovery without decreasing the antibacterial activity (Table 4). As shown in Table 4, with respect to mono- or di-methylated derivatives **7n**, **7o** and **7p**, the antibacterial activities and urinary recoveries were not improved. However, it was revealed that carbapenem **8i**, in which a methyl group was introduced into the pyrrolidine moiety of carbapenem **7i**, showed excellent urinary recovery that was enough to make up for the disadvantage of the decrease in antipseudomonal activity and its antibacterial activity was superior to that of meropenem. Moreover, carbapenem **8i** showed not only a four-fold higher antibacterial activity against *S. aureus* 535 (MRSA), *E. faecalis* 681 and *P. aeruginosa* 3719, which are clinically important species, but also a two-fold higher urinary recovery than meropenem.

Next, our attention was focused on the antibacterial profile of stereoisomers of **8i** (Table 5). Surprisingly, the antibacterial activity of each of the isomers depended on the stereochemistry of the two pyrrolidine moieties in the side chain. In particular, the stereochemistry of the 3,5-disubstituted pyrrolidine moiety (particularly the 3-position) influenced the antibacterial activity. Among the isomers, although carbapenem **8q**, which had a stereochemistry similar to **8i** in regard to the 3,5-disubstituted pyrrolidine moiety and showed good

Table 3. Antibacterial activity (MIC, μg/ml) and urinary recovery of novel carbapenems.



R =						
	7h	**7i**	**7j**	**7k**	**7l**	**7m**
Staphylococcus aureus 209P	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01
S. aureus 56R	0.1	0.02	0.05	0.05	0.02	0.05
S. aureus 535 (MRSA)	6.2	1.5	3.1	3.1	6.2	3.1
Enterococcus faecalis 681	0.8	0.4	0.4	0.8	0.8	0.4
Escherichia coli NIHJ	0.02	≤0.01	≤0.01	≤0.01	0.02	≤0.01
E. coli 609	0.05	0.02	0.02	0.02	0.02	0.02
Salmonella enteritidis	0.02	≤0.01	0.02	≤0.01	0.02	0.02
Klebsiella pneumoniae 806	0.02	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01
Enterobacter cloacae 963	0.2	0.05	0.1	0.1	0.1	0.05
Serratia marcescens 1184	0.05	≤0.01	0.02	≤0.01	0.02	≤0.01
Proteus vulgaris 1420	0.4	0.1	0.2	0.2	0.2	0.1
Morganella morganii 1510	0.2	0.1	0.1	0.2	0.1	0.1
Pseudomonas aeruginosa 1001	0.2	0.05	0.1	0.2	0.05	0.05
P. aeruginosa N07	0.2	0.05	0.1	0.2	0.05	0.05
P. aeruginosa 3719	0.4	0.1	0.4	0.8	0.4	0.4
Urinary recovery (%)	22.5	20.1	13.6	23.9	17.6	10.3

antibacterial activity equal to that of **8i**, the antipseudomonal activity of **8q** was slightly lower than that of **8i**. The relationship between the urinary recovery and the stereochemistry of isomers was not clear.

The antibacterial activity of **8i**, imipenem and meropenem against another series of Gram-positive and Gram-negative bacteria observed in the real clinical setting is listed in Table 6. It was found that carbapenem **8i** also showed potent antibacterial activity against these strains. In particular, it was revealed that the antibacterial activity of this compound against drug-resistant strains such as *S. aureus* 123-1 (MRSA), *S. pneumoniae* 10675 (PRSP) and three kinds of *P. aeruginosa*, was markedly higher than that of imipenem and meropenem.

The stability against hydrolysis by dehydropeptidase-I (DHP-I) of **8i**, imipenem and meropenem was tested. As shown in Table 7, the order of hydrolysis rates of these carbapenems by human renal DHP-I was imipenem> meropenem>**8i**>biapenem. This result demonstrated that carbapenem **8i**, like meropenem, was also sufficient stable for dosing without a DHP-I inhibitor.

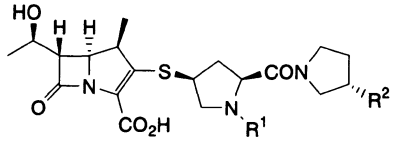
In addition, very low accumulation of **8i** was reported in the rabbit renal cortex after intravenous administration and therefore, nephrotoxicity as low as that of meropenem could be expected.[29]

Due to its potent antibacterial activity and high enough DHP-I stability, carbapenem **8i** was selected as the most promising compound for further evaluation. The preliminary results of the biological and pharmacological study of this compound, which had already been reported,[30~33] suggest an elongated half-life in human plasma. Results of advanced studies will be forthcoming.

Conclusion

We designed and synthesized novel 1β-methyl carbapenem derivatives with an amidine moiety at the C-2

Table 4. Antibacterial activity (MIC, μg/ml) and urinary recovery of novel carbapenems.



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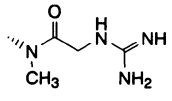
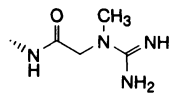
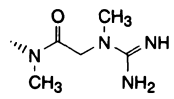
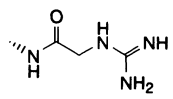
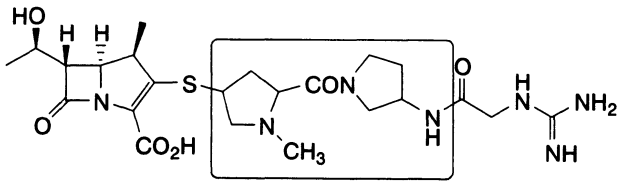
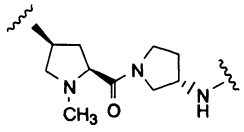
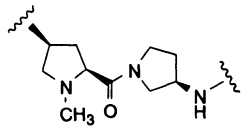
R^2 =					Meropenem
	7n	**7o**	**7p**	**8i (CS-023)**	
Staphylococcus aureus 209P	≤0.01	≤0.01	≤0.01	≤0.012	0.02
S. aureus 56R	0.05	0.05	0.05	0.10	0.05
S. aureus 535 (MRSA)	3.1	3.1	6.2	1.56	6.2
Enterococcus faecalis 681	0.8	0.4	0.8	0.39	1.5
Escherichia coli NIHJ	≤0.01	≤0.01	0.02	≤0.012	≤0.01
E. coli 609	0.05	0.02	0.05	≤0.012	0.02
Salmonella enteritidis	0.02	0.02	0.02	≤0.012	0.02
Klebsiella pneumoniae 806	0.02	≤0.01	0.02	≤0.012	0.02
Enterobacter cloacae 963	0.1	0.1	0.2	0.05	0.05
Serratia marcescens 1184	0.02	0.02	0.02	≤0.012	0.02
Proteus vulgaris 1420	0.2	0.2	0.4	0.20	0.05
Morganella morganii 1510	0.2	0.2	0.2	0.20	0.1
Pseudomonas aeruginosa 1001	0.2	0.1	0.2	0.20	0.2
P. aeruginosa N07	0.2	0.05	0.2	0.20	0.4
P. aeruginosa 3719	0.4	0.4	0.4	0.39	6.2
Urinary recovery (%)	14.4	23.3	15.8	52.8	29.2

Fig. 2. Stereoisomers of CS-023.

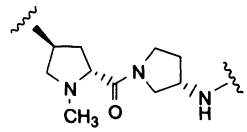




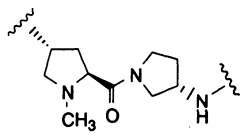
8i (CS-023)



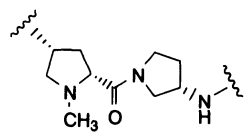
8q



8r

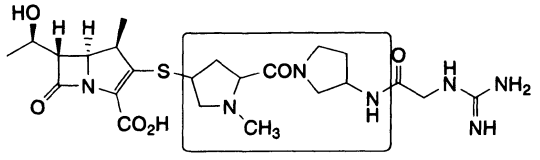


8s



8t

Table 5. Antibacterial activity (MIC, μg/ml) and urinary recovery of stereoisomers of **8i**.



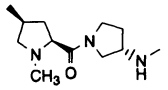
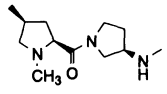
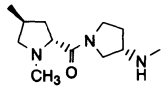
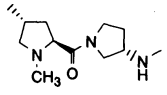
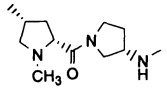
R =					
	8i (CS-023)	**8q**	**8r**	**8s**	**8t**
Staphylococcus aureus 209P	≤0.012	≤0.012	≤0.012	0.025	≤0.012
S. aureus 56R	0.10	0.10	0.05	0.10	0.10
S. aureus 535 (MRSA)	1.56	3.13	6.25	25	25
Enterococcus faecalis 681	0.39	0.39	3.13	6.25	0.78
Escherichia coli NIHJ	≤0.012	≤0.012	0.20	0.78	0.10
E. coli 609	≤0.012	0.025	0.39	1.56	0.20
Salmonella enteritidis	≤0.012	≤0.012	0.20	0.78	0.10
Klebsiella pneumoniae 806	≤0.012	≤0.012	0.20	0.78	0.10
Enterobacter cloacae 963	0.05	0.10	1.56	12.5	0.78
Serratia marcescens 1184	≤0.012	0.025	0.39	1.56	0.20
Proteus vulgaris 1420	0.20	0.39	0.78	1.56	3.13
Morganella morganii 1510	0.20	0.20	0.78	3.13	1.56
Pseudomonas aeruginosa 1001	0.20	0.78	1.56	12.5	25
P. aeruginosa N07	0.20	0.39	1.56	12.5	25
P. aeruginosa 3719	0.39	0.78	6.25	12.5	12.5
Urinary recovery (%)	52.8	36.5	32.3	62.9	53.0

Table 6. Antibacterial activity (MIC, μg/ml)[a] of CS-023, imipenem and meropenem.

	CS-023 (**8i**)	Imipenem	Meropenem
Staphylococcus aureus 209P JC-1	0.05	0.012	0.10
S. aureus 505 (MRSA)	0.78	0.10	1.56
S. aureus 123-1 (MRSA)	6.25	50	25
Streptococcus pneumoniae 2132 (PRSP)	≤0.012	≤0.006	≤ 0.012
S. pneumoniae 10675 (PRSP)	0.05	0.10	0.20
Enterococcus faecalis 10785	1.56	0.78	6.25
Escherichia coli NIHJ JC-2	0.025	0.20	0.025
E. coli 609	0.05	0.78	0.05
Klebsiella pneumoniae 806	0.025	0.20	0.025
Enterobacter cloacae 963	0.05	0.78	0.05
Serratia marcescens IAM 1184	0.025	0.05	≤0.006
Morganella morganii 1510	0.20	3.13	0.10
Haemophilus influenzae 11260	0.05	0.39	0.05
H. influenzae 9787	0.05	0.78	0.20
Pseudomonas aeruginosa PAO1	0.20	1.56	0.39
P. aeruginosa OCR1	0.39	0.78	1.56
P. aeruginosa MR08	1.56	12.5	3.13

a) MIC was measured on Mueller-Hinton agar by two-fold dilution method.

position and evaluated their antibacterial activity, urinary recovery and DHP-I stability. Among them, a novel carbapenem, CS-023 (**8i**), with a (2-guanidinoacetylamino)pyrrolidine moiety showed potent antibacterial activity against Gram-positive and Gram-negative bacteria, high urinary recovery and sufficient stability to allow dosing without a DHP-I inhibitor. CS-023 (**8i**) was therefore selected as a potential drug candidate.

Table 7. Hydrolysis rates of carbapenems by DHP-I in human renal homogenate.

Compound	Hydrolysis Rate[a] (nmol/min/mg-protein)
Imipenem	0.931 ± 0.638
Meropenem	0.130 ± 0.189
CS-834 (**8i**)	0.108 ± 0.023
Biapenem	0.000

a) Substrate conc.: 1 mM
Protein conc.: 10.5 mg/ml.
The rate of hydrolysis by DHP-I was defined as the difference between the rates with and without cilastatin (10 mM). Data are represented as the mean ±S.D. (n=3)

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), GX-400 (400 MHz) or Varian Mercury (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. Column chromatography was carried out on Silica gel 60 (Merck, Art. 9385), Silica gel 60 N (Kanto Chemical, spherical, neutral) or Cosmosil 75C_{18} PREP (Nacalai Tesque).

(*S*)-3-(2-Aminoacetyl)amino-1-(*tert*-butoxycarbonyl)-pyrrolidine (**10**)

To a solution of **9** (10.0 g, 116 mmol) in MeOH (50 ml) was added a solution of Boc_2O (20.3 g, 92.9 mmol) in MeOH (100 ml) under ice cooling and the resulting mixture was stirred at 0°C for 3 hours. The reaction mixture was evaporated under reduced pressure and the residue was diluted with aqueous HCl (0.6 M, 400 ml). The aqueous layer was washed with CH_2Cl_2 (400 ml) to remove the bis Boc compound, made alkaline with K_2CO_3, and extracted with CH_2Cl_2 (400 ml×3) after NaCl was added. The organic layer was dried over $NaSO_4$ and evaporated under reduced pressure to give (*S*)-3-amino-1-(*tert*-

Table 8. Column-switching HPLC conditions for determination of hydrolysis rates of carbapenems by DHP-I.

	Imipenem	Meropenem	CS-023 (**8i**)	Biapenem
Column 1	PROTEIN KW-604S[a]	PROTEIN KW-604S[a]	TSK-gel G2000SW[b]	PROTEIN KW-604S[a]
Mobile phase 1	20 mM MOPS (pH 6.0)	20 mM MOPS (pH 6.0)	20 mM MOPS (pH 6.0)	20 mM MOPS (pH 6.0)
Column 2	YMC-A-312[c]	YMC-A-312[c]	YMC-A-312[c]	YMC-A-312[c]
Mobile phase 2	20 mM MOPS (pH 7.0), 5 mM SOS[d] / MeOH = 90 / 10 (V/V)	20 mM MOPS (pH 7.0) / MeOH = 85 / 15 (V/V)	20 mM MOPS (pH 7.0) / MeOH = 85 / 15 (V/V)	20 mM MOPS (pH 7.0), 5 mM SOS[d] / MeOH = 87 / 13 (V/V)
Flow rate	1.0 ml / min	1.0 ml / min	1.0 ml / min	1.0 ml / min
UV detection	280 nm	280 nm	304 nm	294 nm
Injection volume	10 μl	10 μl	10 μl	10 μl
Internal standard	Nicotinamide	Nicotinamide	p-Acetamidophenol	p-Acetamidophenol

a) Showa Denko Co., Ltd. b) Tosoh Co. c) YMC Co., Ltd. d) Sodium 1-octanesulfonate

butoxycarbonyl)pyrrolidine (13.4 g, 78%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (2H, br s), 1.46 (9H, s), 1.57~1.72 (1H, m), 1.99~2.09 (1H, m), 2.93~3.10 (2H, m), 3.30~3.59 (4H, m). MS (FAB) *m/z* 187 $(M+H)^+$.

To a solution of (*S*)-3-amino-1-(*tert*-butoxycarbonyl)-pyrrolidine (5.50 g, 29.5 mmol) in CH_2Cl_2 (55 ml) were added Et_3N (3.29 g, 32.5 mmol) and chloroacetyl chloride (3.67 g, 32.5 mmol) under ice cooling and the resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over $NaSO_4$ and evaporated under reduced pressure to give (*S*)-3-chloroacetylamino-1-(*tert*-butoxycarbonyl)pyrrolidine (7.75 g, 100%) as a powder. IR (KBr) cm^{-1} 3328, 2978, 1671, 1547, 1421, 1367, 1159, 1112; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (9H, s), 1.82~1.97 (1H, m), 2.18~2.41 (1H, m), 3.19~3.34 (1H, m), 3.38~3.57 (2H, m), 3.66 (1H, dd, *J*=11.5, 6.3 Hz), 4.11 (2H, s), 4.43~4.52 (1H, m), 6.66 (1H, br). MS (FAB) *m/z* 263 $(M+H)^+$.

To a solution of (*S*)-3-chloroacetylamino-1-(*tert*-butoxycarbonyl)pyrrolidine (0.20 g, 0.76 mmol) in MeOH (4 ml) were added NaI (0.34 g, 2.28 mmol) and 28% aqueous ammonia (4.0 ml) and the resulting mixture was stirred at room temperature for 20 hours. The solvent was removed under reduced pressure and the resulting residue was diluted with aq. K_2CO_3 (10 ml) and brine (10 ml). The aqueous layer was extracted six times with CH_2Cl_2 (20 ml). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give **10** (0.16 g, 87%) as an oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.46 (9H, s), 1.73~1.93 (1H, br), 2.08~2.26 (1H, m), 3.08~3.57 (3H, m), 3.35 (2H, s), 3.57~3.70 (1H, m), 4.40~4.56 (1H, m). MS (FAB) *m/z* 244 $(M+H)^+$.

S-Methyl-*N*-(4-nitrobenzyloxycarbonyl)isothiourea (**11**)

To a solution of methylisothiourea sulfate (15.0 g, 53.9 mmol) in CH_2Cl_2 (200 ml) was added saturated aqueous $NaHCO_3$ (200 ml) and the solution of 4-nitrobenzyl chloroformate (16.3 g, 75.4 mmol) in CH_2Cl_2 (200 ml) under ice cooling and the resulting mixture was stirred at 0°C for 2 hours. After the organic layer was separated, the aqueous layer was extracted with CH_2Cl_2 (200 ml×2). The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. Recrystallization of the crude product from CH_2Cl_2 gave **11** (16.1 g, 79%) as a solid. IR (KBr) cm^{-1} 3424, 3269, 2976, 1661, 1571, 1521, 1352, 1245, 1067, 1129, 810; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.49 (3H, s), 5.25 (2H, s), 7.57 (2H, d, *J*=8.7 Hz), 8.22 (2H, d, *J*=8.7 Hz). MS (FAB) *m/z* 270 $(M+H)^+$.

(*S*)-1-(*tert*-Butoxycarbonyl)-3-[2-[3-(4-nitrobenzyloxy-carbonyl)guanidino]acetylamino]pyrrolidine (**12**)

To a solution of **10** (0.12 g, 0.50 mmol) in methanol (1.0 ml) was added **11** (0.16 g, 0.60 mmol) and the resulting mixture was heated until it became a clear solution. After the solvent was removed, the residue was stirred at 80°C for 4 hours. The residue was purified by silica gel column chromatography (EtOAc : MeOH=90 : 10) to give **12** (0.16 g, 69%) as a powder. IR (KBr) cm^{-1} 3393, 3316, 2976, 1662, 1524, 1415, 1347, 1291, 1166, 1129, 1110; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (9H, s), 1.89~1.95 (1H, m), 2.02~2.18 (1H, m), 3.17~3.30 (1H, m), 3.31~3.46 (3H, m), 3.57 (1H, d, *J*=10.4, 6.1 Hz), 3.81~3.99 (2H, m), 4.23~4.41 (1H, m), 5.12 (2H, s), 7.41 (1H, br), 7.53 (2H, d, *J*=8.6 Hz), 8.20 (2H, d, *J*=8.6 Hz). MS (FAB) *m/z* 465 $(M+H)^+$.

(*S*)-3-[2-[3-(4-Nitrobenzyloxycarbonyl)guanidino]-acetylamino]pyrrolidine dihydrochloride (**13**)

To a solution of **12** (11.8 g, 25.4 mmol) in CH_3CN (95 ml) was added 4 M HCl/EtOAc (95 ml) and the resulting mixture was stirred at room temperature for 1.5 hours. The solvent was removed under reduced pressure. The resulting residue was diluted with EtOAc and the precipitate was isolated by filtration to give **13** (10.6 g, 95%) as a powder. IR (KBr) cm^{-1} 3361, 3035, 2733, 1742, 1695, 1630, 1555, 1519, 1345, 1255, 1233, 1183; ^{1}H NMR (400 MHz, D_2O) δ 2.02~2.12 (1H, m), 2.34~2.44 (1H, m), 3.30 (1H, dd, *J*=12.6, 4.8 Hz), 3.38~3.52 (2H, m), 3.60 (1H, dd, *J*=12.6, 7.1 Hz), 4.18 (2H, s), 4.48~4.55 (1H, m), 5.42 (2H, s), 7.65 (2H, d, *J*=8.7 Hz), 8.28 (2H, d, *J*=8.7 Hz). MS (FAB) *m/z* 365 (free form) $(M+H)^+$.

Allyl (2*S*,4*R*)-4-Hydroxy-1-methyl-2-pyrrolidine-carboxylate (**15**)

To a solution of L-hydroxyproline **14** (10.0 g, 76.3 mmol) in water (50 ml) were added acetic acid (85 ml) and 37% aqueous formaldehyde (6.6 ml). The resulting mixture was stirred under H_2 atmosphere in the presence of PtO_2 (0.87 g) at 30°C for 5.5 hours. The catalyst was filtered away, and the filtrate was evaporated under reduced pressure. The residue was diluted with acetone and the precipitate was isolated by filtration to give (2*S*,4*R*)-4-hydroxy-1-methyl-2-pyrrolidinecarboxylic acid (10.3 g, 93%) as a powder. ^{1}H NMR (400 MHz, D_2O) δ 2.19~2.29 (1H, m), 2.45~2.56 (1H, m), 3.18 (1H, d, *J*=12.5 Hz), 3.95 (1H, dd, *J*=12.5, 4.8 Hz), 4.18 (1H, dd, *J*=11.0, 7.3 Hz), 4.60~4.69 (1H, m). MS *m/z* 145 (M^+). To a suspension of (2*S*,4*R*)-4-hydroxy-1-methyl-2-pyrrolidinecarboxylic acid (2.0 g, 13.8 mmol) in allylalcohol (24.0 ml) were added

dimethylformamide (1.0 ml) and thionyl chloride (4.9 g, 41.1 mmol) and the resulting mixture was refluxed for 8 hours. The solvent was removed under reduced pressure. Brine and aqueous $NaHCO_3$ were added to the residue, and then the aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give **15** (2.56 g, 100%) as an oil. IR (neat) cm^{-1} 3388, 2949, 1744, 1669, 1455, 1273, 1194, 1090; ^1H NMR (400 MHz, $CDCl_3$) δ 1.83 (1H, br s), 2.05~2.12 (1H, m), 2.22~2.31 (1H, m), 2.39 (1H, dd, J=10.3, 4.4 Hz), 2.44 (3H, s), 3.39 (1H, t, J=8.1 Hz), 3.47 (1H, dd, J=10.3, 5.9 Hz), 4.46~4.52 (1H, m), 4.62~4.66 (2H, m), 5.22~5.38 (1H, m), 5.87~5.99 (1H, m). MS (FAB) m/z 186 $(M+H)^+$.

Allyl (2*S*,4*S*)-4-Acetylthio-1-methyl-2-pyrrolidinecarboxylate (**16**)

To a solution of **15** (3.0 g, 16.2 mmol) in THF (60 ml) were added Et_3N (3.38 g, 33.4 mmol) and methanesulfonyl chloride (1.91 g, 16.7 mmol) under ice cooling and the resulting mixture was stirred at the same temperature for 2 hours. 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 Na_2SO_4, and evaporated under reduced pressure. The crude oil was purified by silica gel column chromatography (Hexanes : EtOAc=1 : 1) to give allyl (2*S*,4*R*)-4-methanesulfonyloxy-1-methyl-2-pyrrolidinecarboxylate (3.33 g, 77%) as an oil. IR (KBr) cm^{-1} 2946, 1743, 1357, 1174, 968, 898; ^1H NMR (400 MHz, $CDCl_3$) δ 2.44~2.49 (2H, m), 2.51 (3H, s), 2.78 (1H, dd, J=11.0, 4.4 Hz), 3.08, (3H, s), 3.47, (1H, t, J=7.7 Hz), 3.62 (1H, dd, J=11.0, 6.6 Hz), 4.46~4.72 (1H, m), 5.25~5.35 (1H, m), 5.28~5.42 (2H, m), 5.92~6.03 (1H, m). MS (FAB) m/z 264 $(M+H)^+$.

To a solution of allyl (2*S*,4*R*)-4-methanesulfonyloxy-1-methyl-2-pyrrolidinecarboxylate (1.68 g, 6.38 mmol) in DMF (60 ml) were added Cs_2CO_3 (1.56 g, 4.79 mmol) and thioacetic acid (0.73 g, 9.56 mmol) and the resulting mixture was stirred at 70°C for 2.5 hours. The resulting residue was diluted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude oil was purified by silica gel column chromatography (Hexanes : EtOAc=3 : 1) to give **16** (1.09 g, 70%) as an oil. IR (KBr) cm^{-1} 2950, 2786, 1747, 1689, 1450, 1270, 1183, 1138; ^1H NMR (400 MHz, $CDCl_3$) δ 1.95~2.06 (1H, m), 2.30 (3H, s), 2.71 (1H, dt, J=13.9, 8.8 Hz), 2.86 (1H, dd, J=10.3, 6.6 Hz), 3.06~3.13 (2H, m), 3.95~4.02 (1H, m), 4.65~4.69 (2H, m), 5.23~5.38 (2H, m), 5.78~5.99 (1H, m). MS (FAB) m/z 244 $(M+H)^+$.

(2*S*,4*S*)-4-Acetylthio-1-methyl-2-pyrrolidinecarboxylic acid (**17**)

To a solution of **16** (0.30 g, 1.23 mmol) in EtOAc (6 ml) were added PPh_3 (0.07 g, 0.27 mmol), dimedone (0.19 g, 1.70 mmol) and $Pd(PPh_3)_4$ (0.07 g, 0.06 mmol) and the mixture was stirred at room temperature for 1.5 hours. The precipitate was isolated by filtration to give **17** (0.20 g, 82%) as a powder. IR (KBr) cm^{-1} 1699, 1683, 1622, 1361, 1307, 1202, 1116; ^1H NMR (400 MHz, D_2O) δ 2.16~2.22 (1H, m), 2.38 (3H, s), 2.96~3.04 (1H, m), 2.99 (3H, s), 3.69~3.78 (2H, m), 4.06~4.10 (1H, m), 4.22~4.28 (1H, m). MS (FAB) m/z 204 $(M+H)^+$.

(2*S*,4*S*)-4-Acetylthio-1-methyl-2-[(*S*)-3-[2-[3-(4-nitrobenzyloxycarbonyl)guanidino]acetylamino]pyrrolidine-1-ylcarbonyl]pyrrolidine (**18**)

To a solution of **17** (0.47 g, 2.29 mmol) in CH_3CN (10 ml) were added diisopropylethylamine (0.63 g, 4.87 mmol) and pivaloyl chloride (0.29 g, 2.40 mmol) under ice cooling and the mixture was stirred at the same temperature for 1 hour. The solution of **13** (1.00 g, 2.28 mmol) and diisopropylethylamine (1.10 g, 9.12 mmol) in CH_3CN (15 ml) was added to the reaction mixture at −5°C. The resulting mixture was stirred at the same temperature for 2 hours and 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 residue was purified by silica gel column chromatography (EtOAc : MeOH=85 : 15) to give **18** (0.78 g, 62%) as an amorphous solid. IR (KBr) cm^{-1} 3295, 2950, 1685, 1639, 1549, 1522, 1448, 1347, 1290, 1110; ^1H NMR (400 MHz, CD_3OD) δ 1.70~1.78 (1H, m), 1.85~2.04 (1H, m), 2.11~2.26 (1H, m), 2.28, 2.29 (3H, s×2), 2.34, 2.36 (3H, s×2), 2.70~2.83 (1H, m), 2.89~2.97 (1H, m), 3.03~3.08 (1H, m), 3.20~3.43 (3H, m), 3.54~3.74 (3H, m), 3.84~4.12 (4H, m), 4.34~4.45 (1H, m), 7.60~7.63 (2H, m), 8.22~8.34 (2H, m). MS (FAB) m/z 550 $(M+H)^+$.

4-Nitrobenzyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-[(2*S*,4*S*)-2-[(*S*)-3-[2-[3-(4-nitrobenzyloxycarbonyl)-guanidino]acetylamino]pyrrolidine-1-ylcarbonyl]-1-methylpyrrolidine-4-ylthio]-1-carbapen-2-em-3-carboxylate (**19**)

To a solution of **18** (1.0 g, 1.82 mmol) in MeOH (20 ml) was added NaOMe (0.10 g, 1.82 mmol) under ice cooling and the resulting mixture was stirred for 1 hour at the same temperature. The mixture was neutralized with 4 M HCl in

EtOAc (0.46 ml, 1.82 mmol) and evaporated under reduced pressure. The residue was diluted with DMF (20 ml) and then 4-nitrobenzyl (1*R*,5*S*,6*S*)-2-diphenylphosphoryloxy-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapenem-3-carboxylate **1** (1.08 g, 1.82 mmol) and diisopropylethylamine (0.24 g, 1.82 mmol) were added to a solution under ice cooling. The resulting mixture was stirred for 12 hours at 0°C and 1% aqueous $NaHCO_3$ was added to the mixture. The precipitate was filtrated and washed with water. The crude powder was purified by silica gel column chromatography (EtOAc : MeOH=3 : 1~2 : 1) to give **19** (0.78 g, 62%) as a powder. IR (KBr) cm^{-1} 3384, 3113, 3080, 2970, 2875, 2789, 1770, 1643, 1609, 1522, 1450, 1379, 1346, 1322, 1287, 1209, 1181, 1136; ^1H NMR (400 MHz, D_2O) δ 1.08~2.22 (6H, m), 1.75~2.26 (6H, m), 2.44~2.76 (2H, m), 2.89~3.00 (1H, m), 3.03~3.15 (1H, m), 3.18~3.65 (6H, m), 3.68~3.90 (3H, m), 3.93~4.06 (1H, m), 4.13~4.35 (2H, m), 5.05~5.15 (2H, m), 5.37 (2H, d, J=14.1 Hz), 7.58 (2H, dd, J=8.8, 2.7 Hz), 7.74 (2H, d, J=8.7 Hz), 8.18~8.33 (4H, m). MS (FAB) *m/z* 852 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-2-[(*S*)-3-(2-Guanidinoacetylamino)pyrrolidine-1-ylcarbonyl]-1-methylpyrrolidine-4-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid (**8i**)-1/2 EtOH

To a solution of **19** (0.11 g, 0.13 mmol) in THF (2 ml) were added water (2 ml) and 7.5% Pd/C (0.11 g) and the resulting mixture was stirred for 2 hours at 35°C. The catalyst was filtered away, and the filtrate was washed with Et_2O. The aqueous layer was concentrated to 1 ml by evaporation, and then EtOH was added to the residue for crystallization under ice cooling. The obtained crystals were isolated by filtration and washed with a aqueous EtOH (EtOH : H_2O=2 : 1), EtOH and Et_2O, separately, and dried to give **8i** (0.05 g, 61%) as crystals containing a half molar equivalent of EtOH. mp 235~250°C (dec.); IR (KBr) cm^{-1} 3405, 3344, 3273, 3207, 2969, 2883, 2795, 1760, 1673, 1644, 1591, 1553, 1452, 1415, 1381, 1370, 1341, 1311, 1283, 1255; ^1H NMR (400 MHz, D_2O) δ 1.15~1.25 (4.5H, m), 1.30 (3H, d, J=6.4 Hz), 1.57~1.72 (1H, m), 1.93~2.13 (1H, m), 2.15~2.35 (1H, m), 2.27, 2.29 (3H, s×2), 2.68~2.88 (2H, m), 3.08 (1H, d, J=10.7 Hz), 3.29~3.73 (7H, m), 3.75~3.93 (2H, m), 4.01 (3H, s), 4.16~4.31 (2H, m), 4.37~4.49 (1H, m).

Anal Calcd for $C_{23}H_{35}N_7O_6S$ 1/2C_2H_6O:
C 51.41, H 6.83, N 17.49, S 5.72.
Found: C 51.13, H6.96, N 17.17, S 5.72.

Determination of Antibacterial Activity

MICs were determined on Nutrient agar (Eiken Chemical Co., Ltd.) by the two-fold dilution method except for those in Table 6. The inoculum size of the bacteria was one loopful of 10^7 cfu/ml.

Determination of Urinary Recovery in Mice

Carbapenem derivatives (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 of carbapenems was determined by bioassay using *Bacillus subtilis* ATCC 6633. Urinary recovery (%, 0~24 hours) was calculated based on the excretion and initial dose.

Hydrolysis Rates of Carbapenems by DHP-I in Human Renal Homogenate

The test compound solutions (0.3 ml) at a concentration of 2 mM in 50 mM MOPS (3-(*N*-morpholino)-propanesulfonic acid) buffer (pH 7.0) with or without cilastatin (20 mM) were added to an equal volume of human renal homogenate and incubated at 37°C to start the reaction. Aliquots of the reaction mixture were collected at 0, 5, 10, 15, 20, 40 and 80 minutes after starting the incubation and then mixed with three volumes of methanol using a vortex mixer. After centrifugation of the mixture at 4°C, the supernatant was injected into an HPLC system for the assay. The column-switching HPLC conditions are shown in Table 8.

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