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Structure–Activity Relationships of 1β-Methyl-2-(5-phenylpyrrolidin-3-ylthio)carbapenems

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Abstract—Structure–activity relationship studies of 1β-methyl-2-[(3*S*,5*R*)-5-(4-aminomethylphenyl)pyrrolidin-3-ylthio]carbapenems, especially those pertaining to the relationship between antibacterial activity and side-chain structure were conducted. These studies suggested that the *trans*-(3*S*,5*R*)-5-phenylpyrrolidin-3-ylthio side-chain and the aminomethyl group at the 4-position of the phenyl ring play a key role in enhancing the antibacterial activity against the MRSA and *Pseudomonas aeruginosa* strains. In particular, the basicity of a substituent at the 4-position of the phenyl ring were shown to greatly contribute to the antibacterial activity against MRSA and methicillin-resistant *Staphyloccocus epidermidis* strains. In contrast, the amidine group was shown to lead to potent antibacterial activity against *P. aeruginosa* strains comparable to that of imipenem, however, a good correlation between the basicity of the 4-substituent and antipseudomonal activity was not observed. In conclusion, the 4-aminomethyl or methylaminomethyl group on the phenyl ring was the best substituent for antipseudomonal activity.

Introduction

The emergence of multidrug-resistant bacteria, especially methicillin-resistant *Staphyloccocus aureus* (MRSA), has caused serious concern in clinics worldwide. Due to the lack of better therapeutic agents, vancomycin has been used for the treatment of infections caused by MRSA despite severe side effects. Recently, the emergence of vancomycin-resistant MRSA has raised an alarm about the overuse of vancomycin.[1] Therefore, the development of new therapeutic agents with potent anti-MRSA activity, as well as good safety profiles, has been urgently demanded. Carbapenem antibiotics such as imipemem[2] and meropenem[3] possess antibacterial activity against a broad range of pathogens including *S. aureus* and *Pseudomonas aeruginosa* and have good safety profiles, however, their antibacterial activity against MRSA is still insufficient for clinical use.

Previously, we reported that in the course of developing a novel carbapenem with unique biological profiles, we identified 1β-methyl-2-[(3*S*,5*R*)-5-(4-aminomethylphenyl)pyrrolidin-3-ylthio]carbapenem **1a**, which is exceptionally broad spectrum antibiotic having anti-MRSA activity as well as activity against *P. aeruginosa* (Fig. 1).[4] We assumed that its ambidextrous antibacterial activity would be due to the basicity of an 4-aminoalkyl substituent on the phenyl ring and a 5-phenylpyrrolidinylthio group with a *trans*-(3*S*,5*R*)-configuration. To investigate the above assumption, further modifications of the pyrrolidinyl side chain were performed. In this paper, we describe the synthesis of 1β-methyl-2-[5-(substituted - phenyl)pyrrolidin - 3 - ylthio]carbapenems and related compounds, and the detailed structure–activity relationships (SARs) between the side-chain structures and the ambidextrous antibacterial activity against MRSA and *P. aeruginosa*.

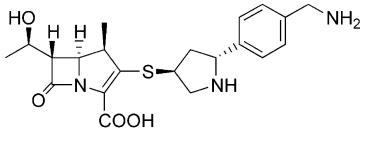

Figure 1. Chemical structure of **1a**.

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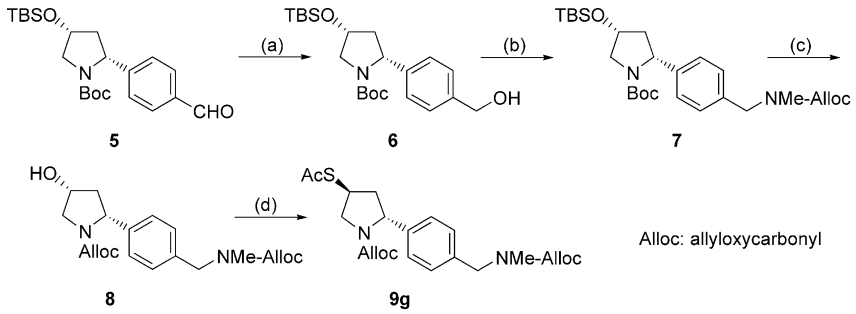
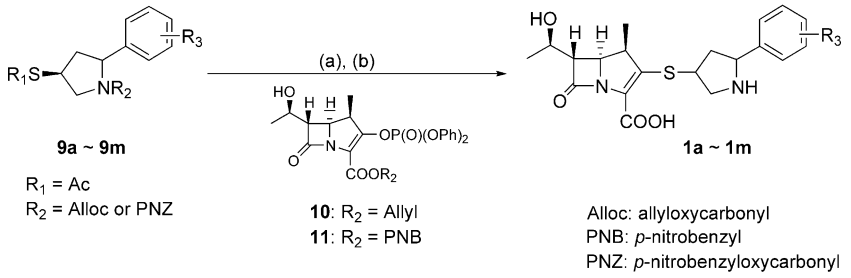


Scheme 1. Synthesis of **9g**. Reagents: (a) $NaBH_4$, MeOH, 0 °C; (b) (i) MsCI, Et_3N, CH_2CI_2, −30 °C; (ii) $MeNH_2$/MeOH, rt; (iii) Alloc–Cl, 0 °C; (c) (i) HCl–MeOH, reflux; (ii) Alloc–Cl, 0 °C; (d) (i) MsCI, Et_3N, CH_2CI_2, −40 °C; (ii) AcSK, DMF, 60 °C.



Scheme 2. Synthesis of **1a**–**1m**. Reagents: (a) (for **1a, 1b, 1c, 1d, 1e, 1f, 1g, 1h, 1i, lj, 1k** and **1m**) (i) NaOH, MeOH, 0 °C; (ii) **10**, *i*-Pr_2NEt, CH_3CN; (iii) $(PPh_3)_2PdCl_2$, *n*-Bu_3SnH, H_2O, CH_2CI_2, 0 °C, (b) (for **1l**) (i) NaOH, MeOH, 0 °C; (ii) **11**, *i*-Pr_2NEt, CH_3CN; (iii) H_2,Pd/C, rt.

Results and discussion

Chemistry

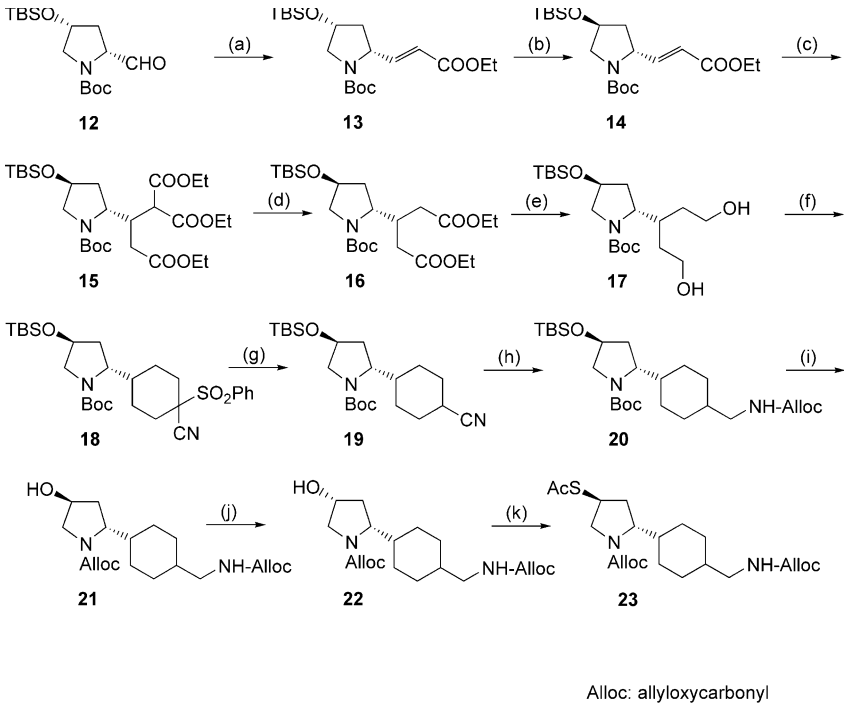
The synthesis of **1g** is highlighted in Schemes 1 and 2 as a representative example of an improved synthetic method of producing 1β-methyl-2-(5-phenylpyrrolidin-3-ylthio)carbapenems. The pyrrolidine aldehyde **5** which is easily derived from commercially available (*R*)-4-hydroxy-2-pyrrolidone, was used as the starting material.[5] Reduction of **5** with $NaBH_4$ in MeOH at 0 °C afforded the alcohol **6** in 90% yield. Mesylate, which was derived from **6**, was substituted with methylamine (40% $MeNH_2$/MeOH); subsequently, the obtained amine was protected as an allyloxycarbamate to provide **7** in 68% yield. Deprotection of **7** under acidic conditions (HCl/MeOH, reflux) and subsequent protection of the pyrollidine nitrogen with allyl chloroformate gave the alcohol, **8**, in quantitative yield, which was converted to the thioacetate, **9g**, in 88% yield by the treatment of the corresponding mesylate with potassium thioacetate. The thiol obtained by the hydrolysis of **9g** (NaOH/MeOH, 0 °C) was coupled with the carbapenem enolphosphate, **10**, in the presence of *N*,*N*-diisopropylethylamine in CH_3CN to produce an adduct in 53% yield.

Finally, deprotection of the adduct using the method reported by Guibe et al.[6] and subsequent purification by reversed-phase column chromatography furnished compound **1g** as an amorphous power after lyophilization in 41% yield. Protected thiols (**9a**–**9f** and **9h**–**9m**) were prepared according to our previous method starting from commercially available D-malic acid.[4e]

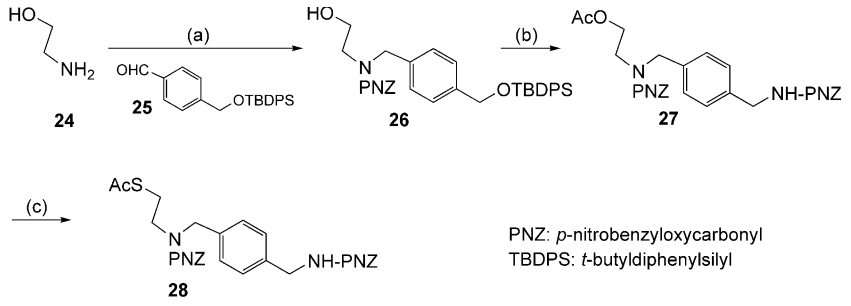
Similarly, the protected thiols, **9a**–**9f** and **9h**–**9m**, were converted to the carbapenems, **1a**–**1f** and **1h**–**1m**, respectively (Scheme 2).[7]

The synthesis of **2** is outlined in Scheme 3. The aldehyde, **12**,[8] was converted to the α,β-unsaturated ester, **13**, in 94% yield using Horner–Emmons reaction. Michael addition of a diethyl malonate anion to **13** did not proceed, probably due to the steric hindrance around the β-carbon of the conjugated ester caused by the bulky *cis*-configurated TBSO group. When the TBSO group of **13** was inverted to a *trans*-configuration, Michael addition of this compound to a diethyl malonate anion proceeded smoothly to give triester **15**, which was converted to the diol, **17**, via deethoxycarbonylation[9] and subsequent reduction of the resulting diester **16**. Cyclization of the diol **17** to cyclohexane **18** was achieved in 75% yield by a modified Mitsunobu reaction using phenylsulfonylacetonitrile and 1.1′-(azodicarbonyl)dipiperidine.[10] Removal of the phenylsulfonyl group of **18** by the treatment with $HgCl_2$–Mg gave **19**; the cyano moiety of **19** was hydrogenated with Raney Ni and subsequently protected with allyl chloroformate to produce **20**.

Transformation of **20** to **21** was carried out in a similar manner as that described above (80% yield). The secondary alcohol, **21**, was converted to **22** by inversion using Mitsunobu reaction[11] and subsequent basic hydrolysis. The alcohol **22** was then converted to thioacetate **23** by Mitunobu reaction using thioacetic acid. Finally, conversion of **23** to compound **2** was achieved by a method similar to that described for the preparation of **1g**.



Scheme 3. Synthesis of **23**. Reagents: (a) triethyl phosphonoacetate, NaH, THF, 0 °C; (b) (i) tetra-*n*-butylammonium fluoride, THF, 0 °C; (ii) PPh_3, DIAD, AcOH, THF; 0 °C; (iii) NaOH, MeOH, rt; (iv) TBS–Cl, imidazole, DMF, rt; (c) (i) diethyl malonate, tetra-*n*-butylammonium bromide, NaH, THF, 50 °C; (d) NaCI, DMSO, 160 °C; (e) $LiAlH_4$, THF, −20 °C; (f) PPh_3, $PhSO_2CH_2CN$, 1,1′-(azodicarbonyl)dipiperidine, THF, 50 °C; (g) $HgCl_2$, Mg, THF, MeOH, 18 °C; (h) (i) Raney Ni, rt; (ii) Alloc–CI, *i*-Pr_2NEt, 0 °C; (i) (i) HCl/MeOH, rt; (ii) Alloc–CI, NaOH, 1,4-dioxane, H_2O; (j) (i) PPh_3, DIAD, AcOH, THF, 0 °C; (ii) NaOH, MeOH, rt; (k) PPh_3, DIAD, thioacetic acid, THF, 0 °C.



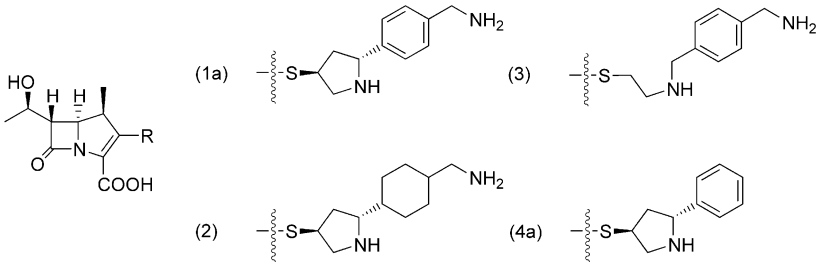
Scheme 4. Synthesis of **28**. Reagents: (a) (i) **25**, $Na(OAc)_3BH$, AcOH, THF, rt; (ii) PNZ–Cl, NaOH, 1,4-dioxane, H_2O; (b) (i) Ac_2O, pyndine, DMAP, THF, rt; (ii) tetra-*n*-butylammonium fluoride, THF, rt; (iii) MsCl, *i*-Pr_2NEt, THF, 0 °C; (iv) NaN_3, DMF, rt; (v) PPh_3, H_2O, THF, rt; (vi) PNZ–Cl, NaOH, 1,4-dioxane, H_2O; (c) (i) NaOH, MeOH, rt; (ii) PPh_3, DIAD, thioacetic acid, THF, 0 °C.

The synthesis of **3** is outlined in Scheme 4. Reductive amination of the 2-aminoethanol **24** with substituted benzaldehyde **25**, PNZ protection of resultant amine afforded alcohol **26**. The primary hydroxyl group of **26** was acetylated, and subsequent desilylation, azidation of resultant benzilic hydroxyl group with NaN_3 to afford azide. The azide was reduced with triphenylphosphine and H_2O to provide an amino group which was protected with an PNZ group to furnish **27**. The secondary alcohol, after deacetylation of **27**, was converted to thioacetate **28** by inversion using Mitsunobu reaction[11] Thioacetate **28** was hydrolized to the corresponding thiol which was in turn coupled with carbapenem diphenylphosphate **11** in the presence of *N*,*N*-diisopropylethylamine in CH_3CN. Deprotection of the coupling adduct was accomplished by hydrogenation with H_2 and Pd/C, and the resulting crude carbapenem was purified by reversed-phase column chromatography to furnish the final compound **3** as an amorphous powder after lyophilization.

Biological properties

The carbapenems obtained above were evaluated for in vitro antibacterial activity against *S. aureus*, including an MRSA (pMS520/Smith), a methicillin-resistant *Staphylococcus epidermidis* (MRSE, MB5181), *Escherichia coli* (NIHJ JC2), and *P. aeruginosa* including a ceftazidime-resistant *P. aeruginosa* (AKR17), and were evaluated for porcine renal dehydropeptidase-I (DHP-I) susceptibility (Tables 1–4). Standard serial dilution techniques were employed for the MIC determinations. Both imipenem and vancomycin were used as reference drugs.

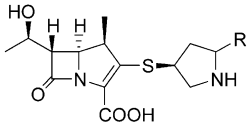
Table 1. In vitro activity[a] and biological properties of **1a** and related compounds

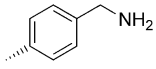
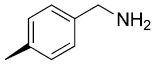
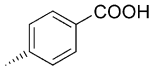
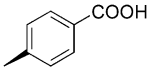


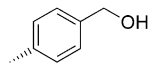
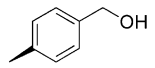
Organism	Compounds					
	(**1a**)	(**2**)	(**3**)	(**4a**)	VCM	IPM
S. *aureus* 209P NIHJ JC1	≤0.006	≤0.006	≤0.006	≤0.006	0.39	≤0.006
S. *aureus* pMS52O/Smith[b]	0.78	6.25	12.5	3.13	0.78	50
S. epidermidis MB5l8lb	1.56	6.25	6.25	6.25	1.56	50
E. coli NIHJ JC2	0.025	0.05	0.20	0.025	>100	0.1
P. aeruginosa AK109	0.39	0.78	3.13	6.25	>100	1.56
P. aeruginosa AKR17[c]	3.13	12.5	>25	6.25	>100	3.13
DHP-I susceptibility[d]	0.29	0.11	0.05	0.05	—	1.0

[a]MlC(μg/mL) determined by agar dilution method.
[b]Methicillin-resistant.
[c]Ceftazidime-resistant.
[d]Relative rate of hydrolysis to imipenem, porcine renal DHP-I.

Table 2. In vitro activity[a] and biological properties of la and related compounds



Organism	R = 			
	(**1a**)	(**1b**)	(**4b**)	(**4c**)
S. *aureus* 209P NIHJ JC1	≤0.006	≤0.006	≤0.006	≤0.006
S. *aureus* pMS520/Smith[b]	0.78	1.56	25	25
S. epidermidis MB5181[b]	1.56	3.13	>25	>25
E. coli NIHJ JC2	0.025	0.025	0.025	0.025
P. aeruginosa AK109	0.39	0.78	6.25	3.13
P. aeruginosa AKR1	3.13	6.25	25	6.25
DHP-I susceptibility[d]	0.29	0.12	<0.05	<0.05

Organism	R = 		VCM	IPM
	(**4d**)	(**4e**)		
S. aureus 209P NIHJ JC1	≤0.006	≤0.006	0.39	≤0.006
S. aureus pMS52O/Smith[b]	3.13	3.13	0.78	50
S. epidermidis MB5181[b]	12.5	12.5	1.56	50
E. coli NIHJ JC2	0.025	0.10	>100	0.1
P. aeruginosa AK109	3.13	6.25	>100	1.56
P. aeruginosa AKR17[c]	3.13	6.25	>100	3.13
DHP-l susceptibility[d]	—	—	—	1.0

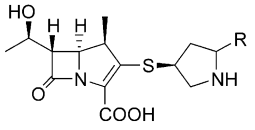
[a]MIC(μg/mL) determined by agar dilution method.
[b]Methicillin-resistant.
[c]Ceftazidime-resistant.
[d]Relative rate of hydrolysis to imipenem, porcine renal DHP-l.

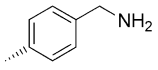
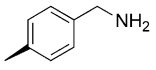
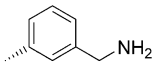
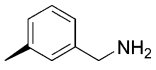
To examine which part(s) of the C-2 side chain in **1a** is/are essential for broad spectrum antibacterial activity, the following three compounds were prepared and their antibacterial activities were compared with that of **1a** (Table 1): compound **2**; the phenyl group of **1a** being replaced with a cyclohexyl group, compound **3**; the pyrrolidine ring being replaced with an aminoethyl group, and compound **4a**; the 4-aminomethyl group on the phenyl ring being removed. The cyclohexyl analogue, **2**, was 8-fold and 4-fold less active against MRSA (pMS520/Smith) and ceftazidime resistant *P. aeruginosa* strains, respectively, than **1a**, suggesting that the phenyl ring of **1a** plays a key role in enhancing the ambidextrous antibacterial activity. Comparison of the antibacterial activity of **3** and **1a** showed that the pyrrolidine ring was also essential to the broad spectrum antibacterial activity of **1a**. Moreover, the des-aminomethyl analogue **4a** exhibited 4-fold and 16-fold less antibacterial activity against the MRSA (pMS520/Smith) and *P. aeruginosa* (AKR17) strains, respectively, compared with **1a**. The above results suggest that both the aminomethylphenyl group and the (3*S*,5*R*)-configurated pyrrolidine ring contributed greatly to the ambidextrous antibacterial activity.

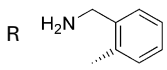
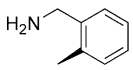
Subsequently, the effects of the aminomethyl group of the phenyl ring on the antibacterial activity was investigated further by comparing the antibacterial activity of compounds **4b**–**4e** with that of a non-basic functional group, such as a carboxylic acid or hydroxyl group in place of the aminomethyl group (Table 2). Replacement with a carboxyl group (**4b**) resulted in a 32-fold or more decrease in the antibacterial activity against the MRSA, MRSE, and *P. aeruginosa* strains. It is interesting to note that the *cis*-carboxyl analogue, **4c**, was more active against the *P. aeruginosa* strains than was the *trans*-analogue, **4b**. Replacement with a hydroxymethyl group (**4d**) resulted in an 8-fold reduction in the antibacterial activity against the MRSE and *P. aeruginosa* (AKR109) strains. These results suggest that a basic functional group on the phenyl ring of **1a** is necessary for the antibacterial activity against the gram-positive bacteria.

Table 3 shows the antibacterial activity of the regio-isomers (**1c** and **1e**) of **1a** with respect to the aminomethyl part, and shows the antibacterial activity of the corresponding *cis*-isomers (**1d** and **1f**). It is evident that the 4-aminomethyl analogue, **1a**, showed the best antibacterial activity against the MRSA, MRSE and *P. aeruginosa* strains among the three regio-isomers (**1a**, **1c** and **1e**). As expected, the *trans*-isomers (**1a**, **1c**, **1e**) showed better antibacterial activity against the MRSA and *P. aeruginosa* strains than the corresponding *cis*-isomers (**1b**, **1d**, **1f**). Only the *para*-isomer, **1a**, showed

Table 3. In vitro activity[a] and biological properties of **1a** and related compounds



Organism	R  (**1a**)	 (**1b**)	 (**1c**)	 (**1d**)
S. *aureus* 209P NIHJ JC1	≤0.006	≤0.006	≤0.006	≤0.006
S. *aureus* pMS520/Smith[b]	0.78	1.56	1.56	3.13
S. epidermidis MB5181[b]	1.56	3.13	3.13	3.13
E. coli NIHJ JC2	0.025	0.025	0.10	0.05
P. aeruginosa AK109	0.39	0.78	0.78	0.78
P. aeruginosa AKR1	3.13	6.25	6.25	12.5
DHP-I susceptibility[d]	0.29	0.12	0.16	<0.05

Organism	R  (**1e**)	 (**1f**)	VCM	IPM
S. *aureus* 209P NIHJ JC1	≤0.006	≤0.006	0.39	≤0.006
S. aureus pMS520/Smith[b]	6.25	6.25	0.78	50
S. epidermidis MB5181[b]	3.13	6.25	1.56	50
E. coIi NlHJ JC2	0.10	0.10	>100	0.1
P. aeruginosa AK1 09	3.13	6.25	>100	1.56
P. aeruginosa AKR17[c]	12.5	25	>100	3.13
DHP-I susceptibility[d]	<0.05	<0.05	—	1.0

[a]MlC(μg/mL) determined by agar dilution method.
[b]Methicillin-resistant.
[c]Ceftazidime-resistant.
[d]Relative rate of hydrolysis to imipenem, porcine renal DHP-l.

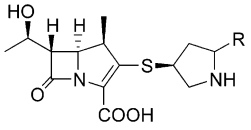
antibacterial activity against MRSA and MRSE strains comparable to that of vancomycin and more potent activity against *P. aeruginosa* strains than imipenem.

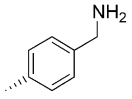
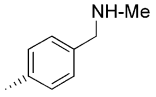
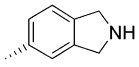
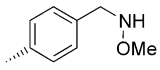
Finally, we investigated the relationship between the antibacterial activity and the basicity of a substituent installed on the 4-position of the phenyl ring in this class of compounds (Table 4).

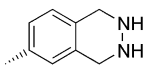
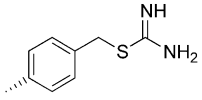
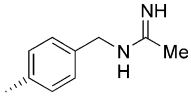
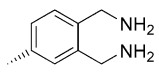
Previously reported, *N*-methylation (**1g**) of the primary amine of **1a** maintained broad spectrum antibacterial activity, while installment of other *N*-substituents brought about a large reduction in the antibacterial activity against the *P. aeruginosa* strain (AKR17). The cyclic analogue, **1h**, with a pyrroline group almost maintained the broad antibacterial activity. When the primary amine in **1a** was replaced with the weaker basic group, *O*-methylhydroxylamine, the resulting compound, **1i**, showed significantly decreased activity against the MRSA, MRSE and *P. aeruginosa* strains. Similarly, replacement of the pyrroline ring in **1h** with a weaker basic tetrahydropyridazine ring (**1j**) resulted in a large reduction in the antibacterial activity against the MRSA, MRSE and *P. aeruginosa* strains. In contrast, **1k**, which has basic thioamidine moiety, showed antibacterial activity against the MRSA and MRSE strains comparable to that of **1a**, while showing an 8-fold decrease in activity against the *P. aeruginosa* strain (AKR17). We considered that installment of a strong basic group into the phenyl ring would be necessary for enhancing the antibacterial activity against the *P. aeruginosa* strain (AKR17). The amidine analogue, **1l**, which has a more basic group than the primary amine, showed a 4-fold reduction in the antibacterial activity against the *P. aeruginosa* strain (AKR17) compared with **1a**. The diamine analogue, **1m**, was 2-fold less active than **1l** against the *P. aeruginosa* strain (AK109). These results suggest that the basicity of the substituent on the phenyl ring is necessary for the antipseudomonal activity but dose not correlated well with the antibacterial activity against *P. aeruginosa* strain (AKR17). In contrast, the antibacterial activity of the compounds with a basic group on the phenyl ring against the MRSA and MRSE strains was comparable to that of **1a**, suggesting that a substituent with some degree of basicity on the phenyl ring is likely to have good antibacterial activity against these gram-positive bacteria.

In summary, considering the structure of the C-2 side chain and antibacterial activity we have derivatized a

Table 4. In vitro activity[a] of *trans*-3,5-disubstituted pyrrolidinylthio 1β-methylcarbapenems



Organism	R  (**1a**)	 (**1g**)	 (**1 h**)	 (**1i**)
S. aureus 209P NIHJ JC1	≤0.006	≤0.006	≤0.006	≤0.006
S. aureus pMS520/Smith[b]	0.78	0.78	1.56	12.5
S. epidermidis MB5181[b]	1.56	1.56	1.56	12.5
E. coIi NIHJ JC2	0.025	0.025	0.025	0.10
P. aeruginosa AK109	0.39	0.39	0.39	25
P. aeruginosa AKR17[c]	1.56	1.56	3.13	>25
DHP-l susceptibility[d]	0.29	0.25	0.36	0.16

Organism	R  (**1j**)	 (**1k**)	 (**1l**)	 (**1m**)
S. aureus 209P NIHJ JC1	≤0.006	≤0.006	≤0.006	≤0.006
S. aureus pMS520/Smith[b]	12.5	1.56	1.56	0.78
S. *epidermidis* MB5181[b]	12.5	1.56	0.78	1.56
E. coli NIHJ JC2	0.10	0.05	0.05	0.05
P. aeruginosa AK109	12.5	1.56	0.78	1.56
P. aeruginosa AKR17[c]	25	12.5	6.25	6.25
DHP-I susceptibility[d]	0.14	0.30	0.25	0.26

[a]MIC(μg/mL) determined by agar dilution method.
[b]Methicillin-resistant.
[c]Ceftazidime-resistant.
[d]Relative rate of hydrolysis to imipenem, porcine renal DHP-I.

novel class of 1β-methyl-2-(5-phenylpyrrolidin-3-ylthio)-carbapenems. Our SAR studies suggest that a*trans*-(3*S*,5*R*)-disubstituted pyrrolidine skeleton with a 4-aminomethylphenyl moiety as the C-2 side chain is essential to the ambidextrous antibacterial activity against MRSA as well as *P. aeruginosa*. Various basic substituents on the phenyl ring showed potent antibacterial activity against the MRSA and MRSE strains comparable to that of vancomycin, while the neutral or acidic substituents were ineffective in the MRSE strain. In contrast, the 4-aminomethyl, 4-*N*-methylaminomethyl and amidine groups on the phenyl ring led to potent antipseudomonal activity comparable or superior to that of imipenem, however the antipseudomonal activity of the analogues was not correlated with the basicity of their 4-subsituent.

Experimental

Antibiotics and strains

Imipenem is a product of Banyu Pharmaceutical Co., Ltd., Japan. Vancomycin was purchased from Sigma Chemical Co., St. Louis, MO, USA. The antibiotics were dissolved in 10 mM 3-morpholino-propanesulfonate (MOPS) buffer, pH 7.0 on the day of use. The strains used in the study were our collections. Methicillin-resistant *S. aureus* pMS520/Smith was a generous gift from M. Inoue, Kitasato University, School of Medicine, Japan.

Determination of MIC

MICs were determined by the 2-fold serial agar dilution method with Mueller-Hinton medium (Difco Laboratories, Detroit, MI, USA). An overnight culture was diluted in the corresponding broth with phosphate-buffered saline supplemented with 0.01% gelatin to give a final concentration of approximately 10^6 CFU/mL. A portion of the dilution was delivered onto a drug-containing agar surface with an inoculum apparatus (Microplanter: Sakuma Seisakusho, Co., Ltd., Tokyo, Japan). The final inoculum size was approximately 10^4 CFU per spot. The plates were incubated at 37 °C for 20 h. The MIC was defined as the lowest concentration of antibiotics at which visible growth was inhibited.

Determination of susceptibility to renal dehydropeptidase-1 (DHP-I)

The relative hydrolysis rate of carbapenems was determined by porcine renal DHP-I, in which the initial hydrolysis rate of imipenem was considered to be 1.0. Partially purified porcine DHP-I (final concentration, 0.3 U/mL) was incubated with 50 μM carbapenem at 35 °C in 50 mM 3-morpholinopropanesulfonate (MOPS) buffer, pH 7.0. The initial hydrolysis rate was monitored by the spectrophotometric method. One unit of activity was defined as the amount of enzyme hydrolyzing 1 μM of glycyldehydrophenylalanine per min when the substrate (50 μM) was incubated at 35 °C in 50 mM MOPS buffer, pH 7.0.

General methods

Melting points were measured on a Yanaco MP micro-melting point apparatus and were not corrected. ^{1}H NMR spectra were recorded on a Varian VXR-300 spectrometer, a JEOL JNM-EX270 spectrometer, and a JEOL JNM-A500 spectrometer with tetramethylsilane (TMS) as an internal standard. ^{13}C NMR spectra were recorded on a JEOL JNM-A500 spectrometer and a JEOL JNM-EX270 spectrometer. IR absorption spectra were recorded with a Horiba FT-200 spectrometer. Specific rotations were measured with a Jasco DIP-370 polarimeter. UV spectra were taken on a Shimazu SPD-10A spectrometer in 0.1 M 3-morpholinopropanesulfonate (MOPS) buffer (pH 7.0). Mass spectra (MS) were measured on a JEOL JMS-SX102A spectrometer. TLC was performed with Merck Kieselgel F_{254} precoated plates. The silica gel used for column chromatography was Wako gel C-300. Reversed phase column chromatography was carried out on YMC gel ODS-AQ 120-S50. All reactions involving air-sensitive reagents were performed under nitrogen atmosphere using syringe-septum cap techniques.

General procedure for the preparation of 5-aryl pyrrolidinylthio-1β-methylcarbapenems

(2*R*,4*R*)-1-*tert*-Butoxycarbonyl-4-*tert*-butyldimethylsiloxy-2-[4-(hydroxymethyl)phenyl]pyrrolidine 6. To a solution of **5** (143 g, 35.5 mmol) in MeOH (3000 mL) was added $NaBH_4$ (14.8 g, 390 mmol) at 0 °C, and the mixture was stirred for 30 min at 0 °C. The reaction mixture was quenched by adding 10% aqueous NH_4Cl and was poured into H_2O. The whole was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5/1 ~ 4/1) to give 6 (130.1 g, 90.0%) as a colorless oil. $[\alpha]_D^{20}$ +34.6 (*c* 1.0, $CHCl_3$); IR ν_{max} (KBr) 3404, 1700,1365, 1253, 1159, 1091 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.08 (6H, s), 0.80 (9H, s), 1.16 (6H, s), 1.44 (3H, s), 1.87 (1H, m), 2.50 (1H, m), 3.40 (1H, m), 3.86 (1H, m), 4.37 (1H, m), 4.66 (2H, s), [4.72 (0.7H, m), 4.87 (0.3H, m), each rotamer], 7.26 (4H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$, major signals) δ −5.0, −4.9, 17.8, 25.6, 28.0, 44.6, 54.6, 60.0, 64.6, 69.8, 79.4, 125.9, 126.5, 139.3, 143.8, 154.4; FAB-HRMS calcd for $C_{22}H_{38}NO_4Si$ $(M+H)^+$: 408.2570, found 408.2572.

(2*R*,4*R*)-2-[4-(*N*-Allyloxycarbonyl-*N*-methylaminomethyl)-phenyl]-1-*tert*-butoxycarbonyl-4-*tert*-butyldimethylsiloxy-pyrrolidine 7. To a solution of **6** (130.1 g, 320 mmol) in CH_2Cl_2 (2600 mL) were added triethylamine (64.8 g, 640 mmol) and methanesulfonyl chloride (27.2 mL, 352 mmol) at −30 °C. The reaction mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, and dried over $MgSO_4$. After the removal of $MgSO_4$ by filtration, 40% methylamine/MeOH (1488 mL) was added to the filtrate at 0 °C and the mixture was stirred for 30 min at the same temperature. The mixture was evaporated under reduced pressure, and the residue was dissolved in 1,4-dioxane (3000 mL) and H_2O (500 mL). To this

solution was added allyl chloroformate (46.3 g, 384 mmol); the pH was maintained at 9.0 with 5 M aqueous NaOH at 5 C. The reaction mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5/1) to give **7** (110.5 g, 68.5%) as a colorless oil. $[\alpha]_D^{20}$ +30.4 (*c* 1.0, $CHCl_3$); IR ν_{max} (Nujol) 1704, 1394, 1253, 1139, 1091, 837, 775 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.02 (3H, s), 0.06 (3H, s), 0.78 (9H, s), 1.16 (6H, s), 1.42 (3H, s), 1.87 (1H, m), 2.49 (1H, m), [2.82 (1.4H, s), 2.83 (1.6H, s) each rotamer], 3.40 (1H, m), 3.83 (1H, m), 4.36 (1H, m), 4.42 (2H, s), 4.62 (2H, m), 4.70 (0.7H, m), 4.89 (0.3H, m), 5.27 (2H, m), 5.95 (1H, m), 7.15 (2H, d, *J* = 8.2 Hz), 7.20 (2H, d, *J* = 8.2 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$, major signals) δ −4.6, −4.5, 18.1, 25.9, 28.4, 34.3, 44.9, 52.3, 55.1, 60.3, 66.3, 70.3, 79.7, 117.4, 126.5, 127.4, 133.4, 135.7, 144.5, 154.7, 156.8; FAB-HRMS *m*/*z* calcd for $C_{27}H_{44}N_2O_5SiNa$ $(M+Na)^+$: 527.2917, found 527.2918.

(2*R*,4*R*)-2-[4-(*N*-Allyloxycarbonyl-*N*-methylaminomethyl)phenyl]-1-allyloxycarbonyl-4-hydroxypyrrolidine 8. To a solution of **7** (110.5 g, 219 mmol) in MeOH (1200 mL) was added concd HCl (83 mL), and the mixture was stirred for 30 min at reflux temperature. The mixture was evaporated under reduced pressure, and the residue was dissolved in 1,4-dioxane (1000 mL) and H_2O (200 mL). To this solution was added allyl chloroformate (46.3 mL, 384 mmol); the pH was maintained at 9.0 with 5 M aqueous NaOH at 5 °C. The reaction mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/2 ~ 1/4) to give **8** (82.0 g, 100%) as a colorless oil. $[\alpha]_D^{20}$ +49.2 (*c* 1.0, $CHCl_3$); IR ν_{max} (Nujol) 3440, 1693, 1286, 1143, 1081, 769 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.01 (1H, m), 2.59 (1H, m), 2.84 (3H, br s), 3.62 (1H, dd, *J* = 11.8, 3.6 Hz), 3.91 (1H, m), 4.48 (5H, m), 4.62 (2H, d, *J* = 5.5 Hz), 4.95 (2H, m), 5.27 (3H, m), 5.90 (2H, m), 7.20 (2H, d, *J* = 9.3 Hz), 7.25 (2H, d, *J* = 9.3 Hz); ^{13}C NMR (67.5 MHz, $CDCl_3$, major signals) δ 34.0, 42.9, 52.0, 54.9, 59.8, 65.4, 65.9, 69.4, 116.3, 117.0, 125.8, 127.5, 132.7, 135.5, 142.5, 143.0, 154.7, 156.3; FAB-HRMS *m*/*z* calcd for $C_{20}H_{27}N_2O_5$ $(M+H)^+$: 375.1920, found 375.1929.

(2*R*,4*S*)-4-Acetylthio-2-[4-(*N*-allyloxycarbonyl-*N*-methylaminomethyl)phenyl]-1-allyloxycarbonylpyrrolidine 9g. To a solution of **8** (81.9 g, 219 mmol) in CH_2Cl_2 (1600 mL) were added triethylamine (61.0 g, 438 mmol) and methanesulfonyl chloride (18.6 mL, 241 mmol) at −40 °C. The reaction mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Subsequently the mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3/2) to give (2*R*,4*R*)-2-[4-(*N*-allyloxycarbonyl-*N*-methylaminomethyl)phenyl]-1-allyloxycarbonyl-4-mesyloxypyrrolidine as a colorless oil (91.1 g, 92.0%). ^1H NMR (300 MHz, $CDCl_3$) δ 2.22 (1H, m), 2.63 (1H, m), 2.80 (3H, s), 2.91 (3H, s), 3.71 (1H, m), 3.86 (1H, m), 4.50 (5H, m), 4.61 (2H, d, *J* = 5.8 Hz), 4.94 (2H, m), 5.29 (3H, m), 5.83 (2H, m), 7.20 (2H, d, *J* = 8.8 Hz), 7.24 (2H, d, *J* = 8.8 Hz). To the above solution of the above (91.1 g, 201 mmol) in DMF (3000 mL) was added potassium thioacetate (46.1 g, 403 mmol) at room temperature; this solution was stirred for 3 h at 60 °C. The reaction mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2/1) to give **9g** as a colorless oil (83.9 g, 96.3%). $[\alpha]_D^{25}$ +50.6 (*c* 1.0, $CHCl_3$); IR ν_{max} (Nujol) 1700, 1658, 1147, 760 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.26 (1H, m), 2.32 (3H, s), 2.36 (1H, m), [2.86 (1.5H, s), 2.87 (1.5H, s) each rotamer], 3.62 (1H, m), 4.09 (2H, m), 4.46 (2H, s), 4.56 (1H, m), 4.64 (2H, d, *J* = 5.5 Hz), 4.94 (2H, m), 5.36 (4H, m), 5.78 (2H, m), 7.21 (4H, s); ^{13}C NMR (67.5 MHz, $CDCl_3$, major signals) δ 30.2, 34.0, 38.7, 39.8, 51.6, 52.5, 59.9, 65.3, 65.7, 77.4, 116.2, 116.9, 125.4, 127.2, 132.8, 136.1, 141.1, 141.7, 154.0, 156.1, 194.5; FAB-HRMS *m*/*z* calcd for $C_{22}H_{29}N_2O_5S$ $(M+H)^+$: 433.1797, found 433.1776.

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(3*S*,5*R*)-5-[4-(*N*-methylaminomethyl)phenyl]pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic acid dihydrochloride 1g. To an ice-cooled solution of **9g** (83.9 g, 194 mmol) in MeOH (850 mL) was added 1 M aqueous NaOH (214 mL). After being stirred for 30 min at the same temperature, the reaction mixture was adjusted to pH 7.0 with 1 M aqueous HCl and was concentrated under reduced pressure. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. To a stirred solution of the residue and allyl (1*R*,5*S*,6*S*)-2-diphenylphosphoryloxy-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate **10** (123 g, 247 mmol) in CH_3CN (1000 mL) was added *N*,*N*-diisopropylethylamine (42.5 mL, 252 mmol) in dropwise fashion at 0 °C. After being stirred overnight at 6 °C, the mixture was poured into H_2O and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/4) to give the adduct, allyl (1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-1-allyloxycarbonyl-5-[4-(*N*-allyloxycarbonyl-*N*-methylaminomethyl)phenyl]pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (66.8 g, 53.7%) as a foam. $[\alpha]_D^{25}$ +82.8 (*c* 1.0, $CHCl_3$); IR ν_{max} (KBr) 3367, 2937, 2875, 1754, 1700, 1403, 769 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.22 (3H, d, *J* = 7.2 Hz), 1.36 (3H, d, *J* = 6.3 Hz), 2.26 (1H, m), 2.40 (1H, m), 2.87 (3H, s), 3.23 (1H, dd, *J* = 7.1, 2.6 Hz), 3.30 (1H, m), 3.73 (2H, m), 4.03 (1H, m), 4.24 (2H, m), 4.46 (2H, s), 4.58 (1H, m), 4.64 (2H, m), 4.69 (1H, m), 4.83 (1H, dd, *J* = 13.5, 5.5 Hz), 4.93 (1H, m), 5.12 (1H, m), 5.34 (6H, m), 5.93 (3H, m), 7.14 (4H, m); ^{13}C NMR (67.5 MHz, $CDCl_3$, major signals) δ 16.1, 21.1, 32.9, 39.5, 40.6, 43.1, 51.2, 51.4, 54.2, 55.7, 59.0, 59.6, 65.0, 65.2, 65.4, 65.4, 115.8, 116.5, 177.7,

124.8, 127.3, 130.8, 132.2, 135.7, 140.7, 141.3, 147.3, 148.5, 153.5, 155.4, 159.6, 172.0; FAB-HRMS *m/z* calcd for $C_{33}H_{42}N_3O_8S$ $(M+H)^+$: 640.2693, found 640.2691.

To an ice-cooled solution of the adduct (66.3 g, 103.6 mmol) in CH_2Cl_2 (2072 mL) were successively added H_2O (9.32 mL), bis(triphenylphosphine)palladium(II) dichloride (3.61 g, 5.18 mmol), and tributyltin hydride (100.4 mL, 373 mmol). After being stirred for 30 min at the same temperature, the reaction mixture was concentrated under reduced pressure to ca. 300 mL and poured into H_2O. The separated aqueous layer was concentrated under reduced pressure to ca. 500 mL. After removal of the insoluble matter by filtration, the concentrated aqueous layer was subjected to reversed-phase column chromatography. The eluent was monitored by HPLC, and the fractions eluted with 15% CH_3CN/H_2O were combined and adjusted to pH 6.0 with 1 M aqueous HCl. The combined fractions were concentrated under reduced pressure and lyophilized to give **1g** as an amorphous powder (20.2 g, 41.7%). The amorphous powder was crystallized from 85% EtOH/H_2O to yield **1g** as dihydrochloride salt. $[\alpha]_D^{20}$ +9.0 (*c* 1.0, H_2O); IR (KBr) ν_{max} 3373, 1751, 1587, 1392, 1086 cm^{-1}; ^1H NMR (500 MHz, D_2O, as monohydrochloride) δ 1.02 (3H, d, *J* = 7.3 Hz), 1.08 (3H, d, *J* = 6.4 Hz), 2.33 (1H, dd, *J* = 14.0, 6.7 Hz), 2.52 (3H, s), 2.57 (1H, m), 3.17 (1H, dq, *J* = 9.1, 7.3 Hz), 3.27 (2H, m), 3.70 (1H, dd, *J* = 12.8, 5.8 Hz), 4.04 (5H, m), 4.88 (1H, dd, *J* = 11.0, 6.7 Hz), 7.35 (4H, m); ^{13}C NMR (125 MHz, D_2O, as monohydrochloride) δ 15.4, 19.7, 31.9, 35.9, 40.7, 42.1, 51.4, 51.9, 55.6, 58.5, 61.2, 64.7, 128.1, 130.2, 131.8, 134.2, 135.2, 136.7, 167.3, 176.3; FAB-HRMS *m/z* calcd for $C_{22}H_{30}N_3O_4S$ $(M+H)^+$: 432.1957, found 432.1950; Anal. calcd for $C_{22}H_{29}N_3O_4S{\cdot}2HCl{\cdot}H_2O$: C, 50.57; H, 6.37; N, 8.04; S, 6.14; found: C, 50.87; H, 6.45; N, 7.83; S, 6.02.

(1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-5-(4-Aminomethylphenyl)pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1a. To an ice-cooled solution of (2*R*,4*S*)-4-acetylthio-2-[4-(*N*-allyloxycarbonylaminomethyl)phenyl]-1-allyloxycarbonylpyrrolidine **9a** (227 mg, 0.54 mmol) in MeOH (8 mL) was added 1 M aqueous NaOH (540 μL) under a nitrogen atmosphere. After being stirred for 20 min at 0 °C, the reaction mixture was adjusted to pH 7.0 with 1 M aqueous HCl and concentrated under reduced pressure. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. To a stirred solution of the residue and 10 (271 mg, 0.54 mmol) in CH_3CN (10 mL) was added *N*,*N*-diisopropylethylamine (153 μL, 0.87 mmol) in a dropwise fashion at 0 °C. After being stirred overnight at 4 °C, the mixture was poured into H_2O and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2/3) to give the adduct, allyl (1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-1-allyloxycarbonyl-5-[4-(*N*-allyloxycarbonylaminomethyl)phenyl]pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate as a foam (222 mg, 65.4%). IR ν_{max} (KBr) 3373, 2966, 1751, 1587, 1392, 1086 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.22 (3H, d, *J* = 7.0 Hz), 1.36 (3H, d, *J* = 6.2 Hz), 2.24 (1H, m), 2.41 (1H, m), 3.21 (1H, dd, *J* = 7.2, 2.4 Hz), 3.30 (1H, m), 3.78 (2H, m), 4.03 (1H, m), 4.24 (2H, m), 4.49 (5H, m), 4.63 (2H, m), 4.64 (1H, m), 4.75 (1H, m), 5.04 (2H, m), 5.26 (4H, m), 5.43 (1H, m), 5.94 (2H, m), 7.20 (4H, m); FAB-HRMS *m/z* calcd for $C_{32}H_{40}N_3O_8S$ $(M+H)^+$: 626.2536, found: 626.2534.

To the ice-cooled solution obtained above (210 mg, 0.34 mmol) in CH_2Cl_2 (10 mL) were successively added H_2O (30 μL), bis(triphenylphosphine)palladium(II) dichloride (11.8 mg, 0.017 mmol), and tributyltin hydride (329 μL, 1.22 mmol) under a nitrogen atmosphere. After being stirred for 20 min at the same temperature, the reaction mixture was poured into H_2O. The separated aqueous layer was washed with CH_2Cl_2 and concentrated under reduced pressure to ca. 3 mL. After removal of the insoluble matter by filtration, the concentrated aqueous layer was subjected to reversed-phase column chromatography. The eluent was monitored by HPLC, and the fractions eluted with 15% CH_3CN/H_2O were combined and adjusted to pH 6.4 with 0.05 M aqueous HCl. The combined fractions were concentrated under reduced pressure and lyophilized to give **1a** as an amorphous powder (110 mg, 72.9%). IR ν_{max} (KBr) 3421, 1749, 1646, 1558 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.22 (3H, d, *J* = 7.0 Hz), 1.27 (3H, d, *J* = 6.5 Hz), 2.51 (1H, m), 2.73 (1H, m), 3.40 (3H, m), 3.86 (1H, dd, *J* = 12.5, 6.0 Hz), 4.25 (5H, m), 5.03 (1H, dd, *J* = 10.5, 7.0 Hz), 7.20 (4H, m); FAB-HRMS *m/z* calcd for $C_{21}H_{28}N_3O_4S$ $(M+H)^+$: 418.1801, found: 418.1800; UV λ_{max}298 (ε 9520).

(1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-(4-Aminomethylphenyl)pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1b. IR ν_{max} (KBr) 3421, 1749, 1652, 1558 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.22 (3H, d, *J* = 7.0 Hz), 1.27 (3H, d, *J* = 6.0 Hz), 2.14 (1H, m), 3.00 (1H, m), 3.37 (1H, m), 3.46 (2H, m), 3.80 (1H, m), 4.14 (1H, m), 4.20 (2H, s), 4.24 (1H, m), 7.52 (2H, d, *J* = 8.0 Hz), 7.55 (2H, d, *J* = 8.0 Hz); FAB-HRMS *m/z* calcd for $C_{21}H_{28}N_3O_4S$ $(M+H)^+$: 418.1801, found: 418.1793; UV λ_{max} 298 (ε 9910).

(1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-5-(3-Aminomethylphenyl)pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1c. IR ν_{max} (KBr) 1749, 1648, 1558 1540 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.21 (3H, d, *J* = 7.3 Hz), 1.25 (3H, d, *J* = 6.4 Hz), 2.51 (1H, m), 2.74 (1H, m), 3.41 (3H, m), 3.84 (1H, m), 4.22 (5H, s), 5.03 (1H, m), 7.52 (4H, m); FAB-HRMS *m/z* calcd for $C_{21}H_{28}N_3O_4S$ $(M+H)^+$: 418.1801, found: 418.1818; UV λ_{max} 299 (ε 10,700).

(1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-(3-Aminomethylphenyl)pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1d. IR ν_{max} (KBr) 1747, 1650, 1558, 1540 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.21 (3H, d, *J* = 7.3 Hz), 1.26 (3H, d, *J* = 6.3 Hz), 2.16 (1H, m), 3.02 (1H, m), 3.35 (1H, m), 3.43 (2H, m), 3.78

(1H, m), 4.20 (5H, m), 7.55 (4H, m); FAB-HRMS *m/z* calcd for $C_{21}H_{28}N_3O_4S$ $(M+H)^+$: 418.1801, found: 418.1794; UV λ_{max} 299 (ε 9920).

(1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-5-(2-Aminomethylphenyl)pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1e. IR ν_{max} (KBr) 1753, 1588, 1395 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.20 (3H, d, *J* = 7.3 Hz), 1.25 (3H, d, *J* = 6.3 Hz), 2.16 (1H, m), 2.44 (1H, m), 3.27 (3H, m), 3.52 (1H, dd, *J* = 12.3, 6.8 Hz), 4.15 (4H, m), 4.57 (1H, m), 7.55 (4H, m); FAB-HRMS *m/z* calcd for $C_{21}H_{28}N_3O_4S$ $(M+H)^+$: 418.1801, found: 418.1812.

(1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-(2-Aminomethylphenyl)pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1f. IR ν_{max} (KBr) 1751, 1576, 1394, 1286 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.20 (3H, d, *J* = 7.3 Hz), 1.25 (3H, d, *J* = 6.3 Hz), 1.99 (1H, m), 2.83 (1H, m), 3.29 (3H, m), 3.56 (1H, dd, *J* = 12.3, 6.8 Hz), 4.15 (4H, m), 4.63 (1H, m), 7.55 (4H, m); FAB-HRMS *m/z* calcd for $C_{21}H_{28}N_3O_4S$ $(M+H)^+$: 418.1801, found: 418.1796; UV λ_{max} 301 (ε 9480).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(3*S*,5*R*)-5-[4-(5-isoindoliniy)pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1 h. To an ice-cooled solution of (4*S*)-4-acetylthio-4-[5-(*N*-allyloxycarbonyl)isoindolinyl]-1-allyloxycarbonylpyrrolidine **9h** (435 mg, 1.09 mmol) in MeOH (10 mL) was added 1 M aqueous NaOH (1.09 mL) under a nitrogen atmosphere. After being stirred for 10 min at 0 °C, the reaction mixture was adjusted to pH 7.0 with 1 M aqueous HCl and concentrated under reduced pressure. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. To a stirred solution of the residue and **10** (600 mg, 1.20 mmol) in CH_3CN (10 mL) was added *N*,*N*-diisopropylethylamine (284 μL, 1.63 mmol) in a dropwise fashion at 0 °C. After being stirred overnight at 4 °C, the mixture was poured into H_2O and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/4 ~ EtOAc) to give both allyl (1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-5-[5-(*N*-allyloxycarbonyl)isoindolinyl]-1-allyloxycarbonylpyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**diastereomer I**, 238.7 mg, 38.0%) and allyl (1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-[5-(*N*-allyloxycarbonyl)isoindolinyl]-1-allyloxycarbonylpyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**diastereomer II**, 219.8 mg, 35.0%) as foams. **diastereomer I**: IR ν_{max} (KBr) 3378, 2988, 1759, 1086, 776 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.26 (3H, d, *J* = 6.9 Hz), 1.36 (3H, d, *J* = 6.2 Hz), 2.23 (1H, m), 2.42 (1H, m), 3.24 (1H, dd, *J* = 7.1, 2.6 Hz), 3.30 (1H, m), 3.69 (1H, m), 3.81 (1H, m), 4.04 (1H, m), 4.26 (2H, m), 4.50 (2H, m), 4.74 (6H, m), 4.77 (4H, m), 5.30 (6H, m), 5.96 (3H, m), 7.20 (3H, m); FAB-HRMS *m/z* calcd for $C_{39}H_{51}N_4O_{10}S$ $(M+H)^+$: 767.3326, found: 767.3326.

To an ice-cooled solution of **diastereomer I** (219 mg, 0.38 mmol) in CH_2Cl_2 (7.6 mL) were successively added H_2O (34 μL), bis(triphenylphosphine)palladium(II) dichloride (13.4 mg, 0.019 mmol), and tributyltin hydride (370 μL, 1.36 mmol) under a nitrogen atmosphere. After being stirred for 30 min at the same temperature, the reaction mixture was poured into H_2O. The separated aqueous layer was washed with CH_2Cl_2 and concentrated under reduced pressure. After removal of the insoluble matter by filtration, the concentrated aqueous layer was subjected to reversed-phase column chromatography. The eluent was monitored by HPLC, and the fractions eluted with 15% CH_3CN/H_2O were combined and adjusted to pH 6.0 with 0.05 M aqueous HCl. The combined fractions were concentrated under reduced pressure and lyophilized to give **1 h** as an amorphous powder (29.4 mg, 16.6%). IR ν_{max} (KBr) 1737, 1650, 1558 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.25 (3H, d, *J* = 7.3 Hz), 1.29 (3H, d, *J* = 6.3 Hz), 2.54 (1H, m), 2.78 (1H, m), 3.41 (3H, m), 3.89 (1H, dd, *J* = 12.6, 5.6 Hz), 4.26 (3H, m), 5.09 (1H, dd, *J* = 11.1, 7.0 Hz), 7.50 (3H, s); FAB-MS *m/z* 430 $(M+H)^+$; UV λ_{max} 298 (ε 9160).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(3*S*,5*R*)-5-[4-[*N*-(methoxy)aminomethyl]phenyl]pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1i. IR ν_{max} (KBr) 1747, 1650, 1558, 1540 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.23 (3H, d, *J* = 7.1 Hz), 1.27 (3H, d, *J* = 6.3 Hz), 2.52 (1H, m), 2.78 (1H, m), 3.44 (6H, m), 3.88 (1H, m), 4.05 (2H, s), 4.25 (3H, m), 5.08 (1H, m), 7.49 (4H, m); FAB-MS *m/z* 448 $(M+H)^+$; UV λ_{max} 297 (ε 10,100).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(3*S*,5*R*)-5-(1,2,3,4-tetrahydrophthalazin-6-yl)pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1j. IR ν_{max} (KBr) 3390, 1749, 1591, 1392 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.24 (3H, d, *J* = 7.3 Hz), 1.29 (3H, d, *J* = 6.4 Hz), 2.51 (1H, m), 2.77 (1H, m), 3.41 (3H, m), 3.88 (1H, dd, *J* = 12.5, 6.9 Hz), 4.06 (4H, s), 4.24 (3H, m), 5.03 (1H, dd, *J* = 11.2, 6.9 Hz), 7.27 (3H, m); FAB-HRMS *m/z* calcd for $C_{22}H_{29}N_4O_4S$ $(M+H)^+$: 445.1910, found: 445.1902; UV λ_{max} 298 (ε 11,100).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(3*S*,5*R*)-5-[4-(isothioureidomethyl)phenyl]pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1k. To an ice-cooled solution of allyl (1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-1-allyloxycarbonyl-5-[4-(iodomethyl)phenyl]pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (3.8 g, 5.82 mmol) in CH_3CN (100 mL) was added thiourea (443 mg, 5.82 mmol). After being stirred overnight at 4 °C, the mixture was poured into H_2O and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/acetone = 4/1 ~ 1/1) to give allyl (1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-1-allyloxycarbonyl-5-[4-(isothioureidomethyl)-phenyl]pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate hydroiodide salt as a foam (2.35 g, 55.4%). IR ν_{max} (KBr) 1754, 1682,

808, 777 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.22 (3H. d, $J=7.3$ Hz), 1.28 (3H, d, $J=6.4$ Hz), 2.28 (1H, m), 2.46 (1H, m), 3.52 (1H, m), 3.76 (1H, m), 4.06 (2H, m), 4.24 (1H, d, $J=10.8$, 2.8 Hz), 4.42 (4H, br s), 4.51 (1H, m), 4.70 (1H, m), 5.18 (4H, m), 5.40 (1H, m), 5.93 (2H, m), 7.29 (2H, d, $J=8.4$ Hz), 7.40 (2H, d, $J=8.4$ Hz); FAB-MS m/z 601 $(M+H)^+$.

To the ice-cooled solution of obtained above (1.65 g, 2.27 mmol) in CH_2Cl_2 (100 mL) were successively added H_2O (203 μL), bis(triphenylphosphine)palladium(II) dichloride (80.0 mg, 0.11 mmol), and tributylthin hydride (2.01 g, 7.49 mmol). After being stirred for 20 min at the same temperature, the reaction mixture was concentrated under reduced pressure and poured into H_2O (20 mL). The aqueous layer was washed with $CHCl_3$. After removal of the insoluble matter by filtration, the concentrated aqueous layer (100 mL) was subjected to reversed-phase column chromatography. The eluent was monitored by HPLC, and the fractions eluted with 30% MeOH/H_2O were combined and adjusted to pH 6.0 with 1 M aqueous HCl. The combined fractions were concentrated under reduced pressure and lyophilized to give **1k** as an amorphous powder (71.3 mg, 4.7%). IR ν_{max} (KBr) 3415, 2971, 1751, 1650, 1583, 1396, 773, 707 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.22 (3H, d, $J=7.7$ Hz), 1.27 (3H, d, $J=6.6$ Hz), 2.51 (1H, m), 2.76 (1H, m), 3.41 (4H, m), 3.88 (1H, m), 4.23 (2H, m), 4.42 (2H, s), 5.06 (1H, m), 7.48 (2H, d, $J=8.0$ Hz), 7.53 (2H, d, $J=8.0$ Hz); FAB-MS m/z 499 $(M+H)^+$; FAB-HRMS m/z 499 $(M+Na)^+$; FAB-HRMS m/z calcd for $C_{22}H_{29}N_4O_4S_2$ $(M+H)^+$: 477.1630, found: 477.1642; UV λ_{max} 298 (ε 5490).

(1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-5-[4-[*N*-(Acetimidoyl)aminomethyl]phenyl]pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 1l. To an ice-cooled solution of (2*R*,4*S*)-4-acetylthio-2-[4-[*N*-(*p*-nitrobenzyloxycarbonylacetimidoyl)aminomethyl]phenyl]-1-*p*-nitrobenzyloxycarbonylpyrrolidine **9l** (3.45 g, 5.32 mmol) in MeOH (70 mL) and THF (30 mL) was added 1 M aqueous NaOH (5.3 mL). After being stirred for 10 min at the same temperature, the reaction mixture was adjusted to pH 7.0 with 1 M aqueous HCl and concentrated under reduced pressure. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. To a stirred solution of the residue and *p*-nitrobenzyl (1*R*,5*S*,6*S*)-2-diphenylphosphoryloxy-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate **11** (3.16 g, 5.32 mmol) in CH_3CN (140 mL) was added *N*,*N*-diisopropylethylamine (1.29 mL, 7.44 mmol) in a dropwise fashion at 0 C. After being stirred for 17 h at 6 °C, the mixture was poured into H_2O and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/acetone = 4/1) to give *p*-nitrobenzyl (1*R*,5*S*,6*S*)-2-[5-[4-[*N*-(*p*-nitrobenzyloxycarbonylacetimidoyl)aminomethyl]-phenyl]-1-*p*-nitrobenzyloxycarbonylpyrrolidine-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (2.896 g, 57.2%) as a yellow foam. IR ν_{max} (KBr) 3338, 1760, 1680, 1560, 1390, 1266, 1080 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.27 (3H, m), 1.38 (3H, d, $J=6.2$ Hz), 2.21 (2H, m), 2.27 (2H, m), 2.44 (1H, m), 3.31 (2H, m), 3.80 (2H, m), 4.04 (1H, m), 4.28 (2H, m), 4.53 (2H, m), 5.15 (5H, m), 5.52 (1H, m), 6.98 (1H, m), 7.21 (5H, m), 7.55 (2H, d, $J=8.5$ Hz), 7.63 (2H, d, $J=8.5$ Hz), 8.02 (1H, m), 8.23 (4H, m); FAB-MS m/z 974 $(M+Na)^+$; FAB-HRMS m/z calcd for $C_{46}H_{46}N_7O_{14}S$ $(M+H)^+$: 952.2823, found: 952.2803.

The mixture obtained above (1.22 g, 1.28 mmol) and 10% Pd/C (1.22 g) in THF (86 mL), EtOH (11 mL), and 0.2 M sodium 3-morpholinopropanesulfonate buffer (MOPS buffer, pH 6.5, 64 mL) was stirred for 15 h at room temperature under a hydrogen atmosphere. The catalyst was removed by filtration and washed with H_2O. The combined filtrate and washings were concentrated under reduced pressure to ca. 20 mL. After the insoluble portion of the aqueous layer was removed by filtration, the filtrate was subjected to reversed-phase column chromatography. The eluent was monitored by HPLC, and the fractions eluted with 30% MeOH/H_2O were combined and adjusted to pH 6.0 with 0.1 M aqueous HCl. The combined fractions were concentrated under reduced pressure and lyophilized to give **1l** as an amorphous powder (282.6 mg, 44.6%). IR ν_{max} (KBr) 3338, 1749, 1681, 1648, 1560, 1394, 1263, 1180, 1147, 1072, 1054, 808, 773, 723 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.21 (3H, d, $J=7.1$ Hz), 1.26 (3H, d, $J=6.3$ Hz), 2.26 (3H, s), 2.48 (1H, m), 2.78 (1H, m), 3.36 (1H, m), 3.48 (2H, m), 3.87 (1H, dd, $J=12.5$, 5.9 Hz), 4.21 (3H, m), 4.51 (2H, s), 5.05 (1H, m), 7.42 (2H, d, $J=8.3$ Hz), 7.49 (2H, d, $J=8.3$ Hz); FAB-HRMS m/z calcd for $C_{23}H_{31}N_4O_4S$ $(M+H)^+$: 459.2066, found: 459.2094; UV λ_{max} 298 (ε 7590).

(1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-5-[3,4-Bis(aminomethyl)phenyl]-pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid dihydrochloride 1m. IR ν_{max} (KBr) 1737, 1650, 1558, 1540, 1070 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.23 (3H, d, $J=7.3$ Hz), 1.28 (3H, d, $J=6.3$ Hz), 2.48 (1H, m), 2.63 (1H, m), 3.39 (3H, m), 3.81 (1H, m), 4.20 (7H, m), 7.54 (3H, m); FAB-HRMS m/z calcd for $C_{22}H_{31}N_4O_4S$ $(M+H)^+$: 447.2066, found: 447.2037; UV λ_{max} 298 (ε 9640).

(2*R*,4*R*)-1-*tert*-Butoxycarbonyl-4-*tert*-butyldimethylsiloxy-2-[(*E*)-2-(ethoxycarbonyl)vinyl] pyrrolidine 13. To a mixture of 60% NaH (6.52 g, 163 mmol) in THF (500 mL) was added triethyl phosphonoacetate (36.6 g, 163 mmol) in a dropwise manner at 0 °C and the mixture was stirred for 1 h at the same temperature. A solution of **12** (53.5 g, 163 mmol) in THF (100 mL) was added in a dropwise manner at a temperature below 5 °C, and the mixture was stirred for 10 min at the same temperature. The mixture was poured into saturated aqueous NH_4Cl, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 9/1 ~ 6/1) to give **13** (61.5 g, 94.8%) as a colorless oil. IR (Nujol) ν_{max} 1698 cm^{-1}; ^1H NMR

(300 MHz, $CDCl_3$) δ 0.08 (6H, s), 0.89 (9H, s), 1.24 (9H, s), 1.24 (3H, t, $J=7.0$ Hz), 1.39 (6H, br s), 1.43 (3H, br s), 1.79 (1H, m), 2.18 (1H, m), 3.30 (1H, m), 3.54 (1H, m), 4.14 (2H, q, $J=7.0$ Hz), 4.38 (2H, m), 5.78 (1H, m), 6.96 (1H, dd, $J=15.4$, 7.5 Hz); FAB-MS m/z 422 $(M+Na)^+$.

(2*R*,4*S*)-1-*tert*-Butoxycarbonyl-4-*tert*-butyldimethylsiloxy-2 - [(*E*) - 2 - (ethoxycarbonyl)vinyl] pyrrolidine 14. To a solution of **13** (55.8 g, 140 mmol) in THF (500 mL) was added tetra-*n*-butylammonium fluoride (1 M in THF, 140 mL, 140 mmol) in a dropwise manner at 0 °C, and the mixture was stirred for 30 min at the same temperature. The mixture was poured into 0. 4 M sodium phosphate buffer (pH 6.5), and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/2) to give (2*R*,4*R*)-1-*tert*-butoxycarbonyl-2-[(*E*)-2-(ethoxycarbonyl)vinyl]-4-hydroxypyrrolidine (39.1 g, 98.1%) as a yellow oil. IR (Nujol) ν_{max} 1698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.24 (3H, t, $J=7.2$ Hz), 1.43 (9H, s), 1.90 (1H, m), 2.32 (1H, m), 3.44 (1H, m), 3.62 (1H, m), 4.18 (2H, q, $J=7.0$ Hz), 4.43 (2H, m), 5.87 (1H, m), 7.01 (1H, dd, $J=15.3$, 8.3 Hz); FAB-MS m/z 308 $(M+Na)^+$. To the ice-cooling solution obtained above (37.9 g, 133 mmol) in THF (1000 mL) were successively added triphenylphosphine (105 g, 399 mmol), diisopropyl azodicarboxylate (80.7 g, 399 mmol), and acetic acid (24 g, 399 mmol) in a dropwise fashion and the mixture was stirred for 30 min at the same temperature. The mixture was then poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. To a solution of the residue in EtOH (600 mL) was added 5 M aqueous NaOH (28 mL) at room temperature, and the mixture was stirred for 10 min at same temperature. After neutralization with 5 M aqueous HCl, the mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/2) to give (2*R*,4*S*)-1-*tert*-butoxycarbonyl - 2 - [(*E*) - 2 - (ethoxycarbonyl)vinyl]-4-hydroxypyrrolidine (31.1 g, 82.2%) as a yellow oil. IR (Nujol) ν_{max} 3400, 1698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.24 (3H, t, $J=7.2$ Hz), 1.42 (9H, s), 1.83 (1H, m), 2.16 (1H, m), 3.54 (2H, m), 4.18 (2H, q, $J=7.0$ Hz), 4.46 (2H, m), 5.85 (1H, d, $J=15.$ 4 Hz), 6.83 (1H, m); FAB-MS m/z 308 $(M+Na)^+$. To the solution obtained above (31.1 g, 109 mmol) in DMF (500 mL) were added imidazole (14.8 g, 218 mmol) and *tert*-butyldimethylchlorosilane (18.1 g, 120 mmol) at room temperature and the mixture was stirred for 3 h at same temperature. The mixture was poured into H_2O and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10/1) to give **14** (34.42 g, 78.9%) as a yellow oil. IR (Nujol) ν_{max} 1698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.04 (6H, s), 0.82 (9H, s), 1.22 (3H, t, $J=7.1$ Hz), 1.38 (9H, br s), 1.73 (1H, m), 2.01 (1H, m), 3.38 (2H, m), 4.12 (2H, m), 4.24 (1H, m), 4.43 (1H, m), 5.78 (1H, m), 6.74 (1H, m); FAB-MS m/z 422 $(M+Na)^+$.

(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-*tert*-butyldimethylsiloxy-2-(1,5-pentanediol-3-yl)pyrrolidine 17. To an ice-cooling solution of 60% NaH (4.12 g, 103 mmol) in THF (600 mL) was added diethyl malonate (15.6 mL, 103 mmol), and the mixture was stirred for 30 min at the same temperature. To the mixture were added tetra-*n*-butylammonium bromide (3.3 g, 10.3 mmol) and a solution of **14** (34.1 g, 85.5 mol) in THF (80 mL). The reaction mixture was stirred for 24 h at 50 °C. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was passed through silica gel (500 g, *n*-hexane/acetone = 20/1 ~ 10/1) to give crude **15** as a yellow oil. The residue was used for the next reaction without further purification. FAB-MS m/z 560 $(M+H)^+$. To a solution of crude 15 in DMSO (300 mL) were added NaCl (5.0 g, 85.4 mmol) and H_2O (3.1 mL), and the mixture was stirred for 3 h at 160 °C. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure to give crude 16 (27.94 g) as a brown oil. The residue was used for next reaction without further purification. FAB-MS m/z 510 $(M+Na)^+$. To a solution of crude 16 (27.9 g) in THF (500 mL) was added $LiAlH_4$ (5.4 g, 143 mmol) in a dropwise fashion at −20 °C, and the mixture was stirred for 30 min at the same temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl. Subsequently, the mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/3 ~ EtOAc) to give 17 (9.09 g, 26.4%, three steps) as a yellow oil. IR (Nujol) ν_{max} 3402, 1690 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.03 (6H, s), 0.83 (9H, s), 1.42 (9H, s), 1.55 (5H, m), 1.83 (1H, m), 2.39 (1H, m), 3.23 (1H, dd, $J=11.6$, 3.7 Hz), 3.63 (2H, t, $J=6.7$ Hz), 3.70 (2H, m), 4.06 (1H, m), 4.27 (1H, m); FAB-HRMS m/z calcd for $C_{20}H_{41}NO_5SiNa$ $(M+Na)^+$: 426.2652, found: 426.2645.

(2*S*,4*S*)-1-*tert*-Butoxycarbonyl-4-*tert*-butyldimethylsiloxy-2 - (4 - cyano - 4 - phenylsulfonylcyclohexyl)pyrrolidine 18. To a solution of 17 (7.09 g, 17.6 mmol) in THF (300 mL) were successively added 1,1′-(azodicarbonyl)dipiperidine (20 g, 79.3 mmol), phenylsulfonylacetonitrile (4.08 g, 22.5 mmol), and trimethylphosphine (1 M in THF, 70.4 mL, 70.4 mmol), and the mixture was stirred for 13 h at 50 °C. The resulting reaction mixture was evaporated under reduced pressure. The reaction mixture was quenched with saturated aqueous NH_4Cl. Subsequently, the mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5/1 ~ 4/1) to give **18** (7.22 g, 74.9%) as a solid.

IR (Nujol) ν_{max} 2235, 1692 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.03 (6H, s), 0.83 (9H, s), 1.43 (9H, s), 1.73 (7H, m), 1.96 (2H, m), 2.11 (2H, m), 3.11 (1H, dd, $J = 11.3$, 3.8 Hz), 3.50 (1H, m), 3.88 (1H, m), 4.22 (1H, m), 7.60 (2H, m), 7.72 (1H, m), 7.94 (2H, d, $J = 8.3$ Hz); FAB-HRMS m/z calcd for $C_{28}H_{45}N_2O_5SSi$ $(M+H)^+$: 549.2818, found: 549.2798.

(2*S*,4*S*)-1-*tert*-Butoxycarbonyl-4-*tert*-butyldimethylsiloxy-2-(4-cyanocyclohexyl)pyrrolidine 19. To an ice-cooling solution of 18 (6.72 g, 12.3 mmol) in THF (40 mL) and MeOH (40 mL) were successively added $HgCl_2$ (33 mg, 0.12 mmol) and Mg (1.5 g, 61.3 mmol), and the mixture was stirred for 1 h at 18 °C. The reaction mixture was quenched with saturated aqueous NH_4Cl. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4/1) to give **19** (4.88 g, 97.5%) as a colorless oil. IR (Nujol) ν_{max} 2230, 1694 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.03 (3H, s), 0.05 (3H, s), 0.84 (9H, s), 1.44 (9H, s), 1.68 (8H, m), 2.01 (1H, m), 2.12 (1H, m), 2.31 (1H, m), 2.94 (1H, br s), 3.18 (1H, m), 3.52 (1H, m), 3.89 (1H, m), 4.27 (1H, m); FAB-HRMS m/z calcd for $C_{22}H_{40}N_2O_3SiNa$ $(M+Na)^+$: 431.2706, found: 431.2697.

(2*S*,4*S*)-2-[4-(*N*-Allyloxycarbonylaminomethyl)cyclohexyl]-1-*tert*-butoxycarbonyl-4-*tert*-butyldimethylsiloxypyrrolidine 20. To a solution of **19** (4.88 g, 12.0 mmol) in MeOH (150 mL) was added Raney Ni (NDT-65, kawaken, 4.88 g), and the mixture was stirred for 22 h under 3.5 hydrogen atmosphere at room temperature. The catalyst was filtered off and washed with MeOH. The filtrate and washings were combined and evaporated under reduced pressure. To an ice-cooling solution of the residue in THF (90 mL) and H_2O (10 mL) were added *N*,*N*-diisopropylethylamine (10.5 mL, 60.6 mmol) and allyl chloroformate (1.9 mL, 18.0 mmol). The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4/1) to give **20** (5.74 g, 96.8%) as a colorless oil. IR (thin film) ν_{max} 1688 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.03 (6H, s), 0.80 (9H, s), 1.40 (9H, s), 1.48 (6H, m), 1.72 (4H, m), 2.98 (1H, m), 3.14 (1H, m), 3.42 (1H, m), 3.84 (1H, m), 4.23 (1H, m), 4.67 (1H, m), 5.20 (2H, m), 5.86 (1H, m); FAB-MS m/z 497 $(M+H)^+$.

(2*S*,4*S*)-2-[4-(*N*-Allyloxycarbonylaminomethyl)cyclohexyl]-1-allyloxycarbonyl-4-hydroxypyrrolidine 21. To a solution of **20** (5.74 g, 11.6 mmol) in MeOH (10 mL) was added 10% HCl/MeOH (100 mL), and the mixture was stirred for 20 h at room temperature. The resulting mixture was evaporated under reduced pressure. To a solution of the residue in 1,4-dioxane (90 mL) and H_2O (9 mL) was added allyl chloroformate (1.84 mL); the pH was maintained at 8.5 with 1 M aqueous NaOH at room temperature. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$, and the mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/2) to give **21** (3.41 g, 80.7%) as a colorless oil. IR ν_{max} (KBr) 3411, 1732, 1695 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (2H, m), 1.21 (1H, m), 1.53 (4H, m), 1.85 (5H, m), 2.36 (1H, m), 3.01 (1H, m), 3.19 (1H, m), 3.32 (1H, m), 3.68 (1H, m), 4.08 (1H, m), 4.39 (1H, br s), 4.57 (4H, m), 4.74 (1H, m), 5.24 (4H, m), 5.93 (2H, m); FAB-HRMS m/z calcd for $C_{19}H_{31}N_2O_5$ $(M+H)^+$: 367.2233, found: 367.2269.

(2*S*,4*R*)-2-[4-(*N*-Allyloxycarbonylaminomethyl)cyclohexyl]-1-allyloxycarbonyl-4-hydroxypyrrolidine 22. To an ice-cooled solution of **21** (3.41 g, 9.34 mmol) in THF (100 mL) were successively added triphenylphosphine (7.34 g, 28.0 mmol), diisopropyl azodicarboxylate (5.51 mL, 28.0 mmol), and acetic acid (1.6 mL, 28.0 mmol), and the mixture was stirred for 30 min at same temperature. The resulting mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2/1) to give (2*R*,4*R*)-4-acetoxy-2-[4-(*N*-allyloxycarbonylaminomethyl)cyclohexyl]-1-allyloxycarbonylpyrrolidine (3.56 g, 93.7%) as a colorless oil. IR ν_{max} (KBr) 1730, 1693 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (2H, m), 1.33 (1H, m), 1.42 (3H, m), 1.75 (5H, m), 2.05 (3H, s), 2.18 (1H, m), 3.02 (1H, m), 3.19 (2H, m), 3.81 (1H, m), 4.03 (1H, m), 4.57 (4H, m), 4.74 (1H, m), 5.24 (4H, m), 5.90 (2H, m); FAB-MS m/z 409 $(M+H)^+$. To the solution obtained above (3.57 g, 8.75 mmol) in MeOH (50 mL) was added 1 M aqueous NaOH (9 mL) at room temperature, and the mixture was stirred for 30 min at same temperature. After neutralization by 1 M aqueous HCl, the mixture was poured into H_2O and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/1) to give 22 (3.2 g, 100%) as a colorless oil. IR (thin film) ν_{max} 3433, 1730, 1696 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.00 (2H, m), 1.41 (4H, m), 1.76 (4H, m), 1.91 (1H, m), 2.11 (1H, m), 3.11 (3H, m), 3.88 (2H, m), 4.38 (1H, m), 4.58 (4H, m), 4.77 (1H, m), 5.26 (4H, m), 5.91 (2H, m); FAB-MS m/z 367 $(M+H)^+$.

(2*S*,4*S*)-4-Acetylthio-2-[4-(*N*-allyloxycarbonylaminomethyl)cyclohexyl]-1-allyloxycarbonylpyrrolidine 23. To an ice-cooled solution of **22** (3.16 g, 8.75 mmol) in THF (80 mL) were successively added triphenylphosphine (5.74 g, 21.9 mmol), diisopropyl azodicarboxylate (4.30 mL, 21.9 mmol), and thioacetic acid (1.57 mL, 21.9 mmol), and the mixture was stirred for 30 min at the same temperature. The resulting mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3/1) to give a mixture of **23** and triphenylphosphine oxide (5.15 g) as a colorless solid. ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (2H, m), 1.50 (7H, m), 1.80 (2H, m), 2.18 (1H, s), 2.32 (3H, s), 3.03 (1H, m), 3.17 (1H, m), 3.49 (1H, m), 3.69 (1H, m), 3.91 (2H, m), 4.58 (4H, m), 5.26 (4H, m), 5.92 (2H, m), 6.29 (1H, br s); FAB-MS m/z 425 $(M+H)^+$.

(1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-[4-(Aminomethyl)cyclohexyl]-pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 2. To an ice-cooled solution of crude **23** (5.15 g) in MeOH (60 mL) and THF (30 mL) was added 1 M aqueous NaOH (1.0 mL). After being stirred for 20 min at the same temperature, the reaction mixture was adjusted to pH 7.0 with 1 M aqueous HCl and concentrated under reduced pressure. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. To an ice-cooled solution of the residue and **10** (4.99 g, 10.0 mmol) in CH_3CN (90 mL) was added *N*,*N*-diisopropylethylamine (1.98 mL, 11.4 mmol) in a dropwise fashion at 0 °C. After being stirred overnight at 4 °C, the mixture was poured into H_2O and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/4) to give allyl (1*R*,5*S*,6*S*) - 2 - [(3*S*,5*R*) - 5 - [4 - (*N* - allyloxycarbonylaminomethyl)cyclohexyl]-1-allyloxycarbonylpyrrolidin-3-ylthio] - 6 - [(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (2.37 g, 42.9%) as a yellow foam. IR ν_{max} (KBr) 1749, 1688, 1554, 1396 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (2H, m), 1.26 (3H, m), 1.37 (3H, d, $J = 6.7$ Hz), 1.66 (8H, m), 1.90 (1H, m), 2.18 (1H, m), 3.08 (2H, m), 3.27 (2H, m), 3.66 (3H, m), 3.99 (1H, m), 4.24 (2H, m), 4.57 (4H, m), 4.73 (3H, m), 5.30 (6H, m), 5.92 (3H. m); FAB-HRMS *m*/*z* calcd for $C_{32}H_{46}N_3O_8S$ $(M+H)^+$: 632.3006, found: 632.2987.

To the ice-cooled solution obtained above (631 mg, 1.00 mmol) in CH_2Cl_2 (20 mL) were successively added H_2O (90 μL), bis(triphenylphosphine)palladium(II) dichloride (35 mg, 0.05 mmol) and tributyltin hydride (968 μL, 3.6 mmol). After being stirred for 30 min at the same temperature, the reaction mixture was concentrated under reduced pressure and poured into H_2O. The separated aqueous layer was concentrated under reduced pressure to ca. 10 mL. After removal of the insoluble matter by filtration, the concentrated aqueous layer was subjected to reversed (phase column chromatography. The eluent was monitored by HPLC, and the fractions eluted with 30% MeOH/H_2O were combined and adjusted to pH 6.4 with 1 M aqueous HCl. The combined fractions were concentrated under reduced pressure and lyophilized to give 2 (269 mg, 58.6%) as an amorphous powder. IR ν_{max} (Nujol) 1749, 1581 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.04 (1H, m), 1.20 (3H, d, $J = 7.2$ Hz), 1.25 (3H, d, $J = 7.2$ Hz), 1.46 (2H, m), 1.61 (3H, m), 1.82 (3H, m), 2.22 (2H, m), 2.83 (1H, d, $J = 7.1$ Hz), 2.95 (1H, d, $J = 7.1$ Hz), 3.32 (2H, m), 3.43 (1H, m), 3.68 (2H, m), 3.89 (1H, m), 4.03 (1H, m), 4.21 (2H, m); FAB-HRMS *m*/*z* calcd for $C_{22}H_{36}N_3O_4S$ $(M+H)^+$: 424.2270, found: 424.2312; UV λ_{max} 299 (ε 9760).

2-[*N*-(4-*tert*-Butyldiphenylsiloxymethylbenzyl)-*N*-*p*-nitrobenzyloxycarbonylamino]ethanol 26. To a solution of **24** (716 mg, 11.7 mmol) in THF (100 mL) were successively added **25** (4.0 g, 10.7 mmol), sodium triacetoxyborohydride (3.41 g, 16.1 mmol), and acetic acid (612 μL, 10.7 mmol), and the mixture was stirred for 20 h at room temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl. Subsequently, the mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. To an ice-cooled solution of the residue in 1,4-dioxane (90 mL) and H_2O (10 mL) was added *p*-nitrobenzyl chloroformate (2.52 g, 11.7 mmol); the pH was maintained at 8∼10 with 1 M aqueous NaOH. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$, and the mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3/2) to give **26** (2.08 g, 32.6%) as a brown oil. IR ν_{max} (Nujol) 1745 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (9H, s), 3.42 (2H, t, $J = 6.7$ Hz), 4.42 (2H, t, $J = 6.7$ Hz), 4.44 (4H, m), 5.26 (4H, m), 5.29 (1H, br s), 7.63 (18H, m), 8.22 (4H, m); FAB-HRMS *m*/*z* calcd for $C_{34}H_{39}N_2O_6Si$ $(M+H)^+$: 599.2577, found: 599.2589.

2-Acetoxy-[*N*-(4-*p*-nitrobenzyloxycarbonyl)aminomethylbenzyl-*N*-*p*-nitrobenzyloxycarbonyl]ethylamine 27. To a solution of the residue in pyridine (25 mL) were added acetic anhydride (10 mL) and catalytic amount of 4-dimethylaminopyridine, and the mixture was stirred for 18 h at room temperature. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$, and the mixture was evaporated under reduced pressure. To a solution of the residue in THF (30 mL) was added tetra-*n*-butylammonium fluoride (1 M in THF, 5.24 mL, 5.24 mmol), and the mixture was stirred for 1 h at room temperature. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$, and the mixture was evaporated under reduced pressure. To an ice-cooled solution of the residue in THF (30 mL) were added *N*,*N*-diisopropylethylamine (1.22 mL, 7.0 mmol) and methanesulfonyl chloride (351 μL, 4.54 mL), and the mixture was stirred for 30 min at the same temperature. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$, and the mixture was evaporated under reduced pressure. To a solution of the residue in DMF (80 mL) was added sodium azide (340 mg, 5.24 mmol), and the mixture was stirred for 1 h at room temperature. The organic layer was washed with brine and dried over $MgSO_4$, and the mixture was evaporated under reduced pressure. To a solution of the residue in THF (60 mL) were added triphenylphosphine (1.37 g, 5.24 mmol) and H_2O (10 mL). After being stirred for 14 h at room temperature, the reaction mixture was concentrated under reduced pressure. To an ice-cooled solution of the residue in 1,4-dioxane (80 mL) and H_2O (20 mL) was added *p*-nitrobenzyl chloroformate (980 mg, 4.54 mmol); the pH was maintained at 8.5 with 1 M aqueous NaOH. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$, and the mixture was evaporated under reduced pressure. The resi-

due was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3/2) to give **27** (990 mg, 48.9%) as a brown oil. IR ν_{max} (Nujol) 1745, 1735, 757 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.99 (3H, s), 3.54 (2H, m), 4.18 (2H,m), 4.39 (2H, d, $J = 6.4$ Hz), 4.57 (2H, s), 5.26 (4H, m), 5.29 (1H, br s), 7.54 (8H, m), 8.22 (4H, m); FAB-HRMS *m*/*z* calcd for $C_{28}H_{29}N_4O_{10}$ $(M+H)^+$: 581.1884, found: 581.1868.

2-Acetylthio-[*N*-(4-*p*-nitrobenzyloxycarbonyl)aminomethylbenzyl-*N*-*p*-nitrobenzyloxycarbonyl]ethylamine 28. To a solution of **27** (990 mg, 1.71 mmol) in MeOH (20 mL) was added 1 M aqueous NaOH (2 mL) at room temperature, and the mixture was stirred for 30 min at same temperature. After neutralization by 1 M aqueous HCl, the mixture was poured into H_2O and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/2) to give an alcohol (675 mg, 73.5%) as a colorless oil. To an ice-cooled solution of the alcohol (675 mg, 1.25 mmol) in THF (15 mL) were successively added triphenylphosphine (658 mg, 2.51 mmol), diisopropyl azodicarboxylate (494 μL, 2.51 mmol), and thioacetic acid (180 μL, 2.51 mmol), and the mixture was stirred for 30 min at same temperature. The resulting mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1/1) to give **28** (252 mg, 33.7%) as a colorless oil. IR ν_{max} (KBr) 1730, 1693, 747 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.31 (3H, s), 3.00 (2H, m), 3.42 (2H, m), 4.39 (2H, d, $J = 6.4$ Hz), 4.57 (2H, m), 5.28 (4H, m), 5.29 (1H, m), 7.22 (4H, m), 7.49 (4H, m), 8.22 (4H, m); FAB-HRMS *m*/*z* calcd for $C_{28}H_{29}N_4O_9S$ $(M+H)^+$: 597.1655, found: 597.1698.

(1*R*,5*S*,6*S*)-2-[4-(Aminomethyl)benzyl]aminoethylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid hydrochloride 3. To an ice-cooled solution of **28** (252 mg, 0.423 mmol) in MeOH (20 mL) and THF (10 mL) was added 1 M aqueous NaOH (486 μL). After being stirred for 20 min at the same temperature, the reaction mixture was adjusted to pH 7.0 with 1 M aqueous HCl and concentrated under reduced pressure. The mixture was poured into H_2O, and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. To a stirred solution of the residue and 11 (289 mg, 0.468 mmol) in CH_3CN (10 mL) was added *N*,*N*-diisopropylethylamine (96 μL, 0.55 mmol) in a dropwise manner at 0 °C. After being stirred overnight at 6 °C, the mixture was poured into H_2O and the whole was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc) to give *p*-nitrobenzyl (1*R*,5*S*,6*S*)-2-[*N*-[4-(*N*-*p*-nitrobenzyloxycarbonylaminomethyl)benzyl] - *N* - *p* - nitrobenzyloxycarbonylaminoethylthio] - 6 - [(*R*) - 1 - hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (267 mg, 70.3%) as a yellow foam. IR ν_{max} (KBr) 3373, 1751, 1587, 1086 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (1.2H, d, $J = 7.3$ Hz), 1.12 (1.8H, d, $J = 7.3$ Hz), 1.37 (3H, d, $J = 6.3$ Hz), 2.74 (3H, m), 3.20 (2H, m), 3.42 (1H, m), 4.22 (2H, m), 4.55 (4H, m), 5.37 (7H, m), 7.19 (4H, m), 7.50 (6H, m), 8.20 (6H, m); FAB-HRMS *m*/*z* calcd for $C_{43}H_{43}N_6O_{14}S$ $(M+H)^+$: 899.2558, found: 899.2529.

The mixture obtained above (267 mg, 0.297 mmol) and 10% Pd/C (100 mg) in THF (16 mL), EtOH (8 mL), and 0.2 M sodium 3-morpholinopropanesulfonate buffer (MOPS buffer, pH 6.5, 16 mL) was stirred for 15 h at room temperature under a hydrogen atmosphere. The catalyst was removed by filtration and washed with H_2O. The combined filtrate and washings were concentrated under reduced pressure to ca. 40 mL. After the insoluble portion of the aqueous layer was removed by filtration, the filtrate was subjected to reversed-phase column chromatography. The eluent was monitored by HPLC, and the fractions eluted with 10∼20% MeOH/H_2O were combined and adjusted to pH 6.0 with 0.05 M aqueous HCl. The combined fractions were concentrated under reduced pressure and lyophilized to give **3** as an amorphous powder (28.1 mg, 24.4%). IR ν_{max} (Nujol) 1754, 1575 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.20 (3H, d, $J = 7.6$ Hz), 1.21 (3H, d, $J = 5.7$ Hz), 2.85 (2H, m), 3.19 (2H, m), 3.34 (2H, m), 3.91 (1H, m), 4.22 (5H, m), 7.46 (2H, d, $J = 8.5$ Hz), 7.51 (2H, d, $J = 8.5$ Hz); FAB-HRMS *m*/*z* calcd for $C_{20}H_{28}N_3O_4S$ $(M+H)^+$: 406.1801, found: 406.1834; UV λ_{max} 299 (ε 7610).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(3*S*,5*R*)-5-phenylpyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic acid 4a. IR ν_{max} (KBr) 1753, 1589, 1390, 1082 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.22 (3H, d, $J = 7.3$ Hz), 1.26 (3H, d, $J = 6.3$ Hz), 2.54 (1H, m), 2.79 (1H, m), 3.42 (3H, m), 3.87 (1H, m), 4.22 (3H, m), 5.07 (1H, m), 7.47 (4H, s); FAB-HRMS *m*/*z* calcd for $C_{20}H_{25}N_2O_4S$ $(M+H)^+$: 389.1535, found: 389.1539; UV λ_{max} 298 (ε 9120).

Sodium (1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-5-(4-carboxyphenyl)pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate 4b. IR ν_{max} (KBr) 1755, 1641, 1552, 1402, 1107 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.23 (3H, d, $J = 7.4$ Hz), 1.28 (3H, d, $J = 6.5$ Hz), 2.47 (1H, m), 2.68 (1H, m), 3.37 (2H, m), 3.46 (1H, dd, $J = 6.2$, 2.4 Hz), 3.80 (1H, dd, $J = 12.7$, 5.8 Hz), 4.15 (1H, m), 4.23 (2H, m), 4.93 (1H, m), 7.49 (2H, d, $J = 7.9$ Hz), 7.88 (2H, d, $J = 7.9$ Hz); FAB-MS *m*/*z* 454 $(M+Na)^+$; UV λ_{max} 297 (ε 8790).

Sodium (1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-(4-carboxyphenyl)pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate 4c. IR ν_{max} (KBr) 3388, 1749, 1653, 1558, 1394, 1090 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.23 (3H, d, $J = 7.3$ Hz), 1.28 (3H, d, $J = 6.7$ Hz), 2.08 (1H, m), 2.94 (1H, m), 3.37 (2H, m), 3.43 (1H, m), 3.69 (1H, dd, $J = 12.2$, 7.6 Hz), 4.06 (1H, m), 4.24 (2H, m), 7.52 (2H, d, $J = 7.9$ Hz), 7.88 (2H, d, $J = 7.9$ Hz); FAB-MS *m*/*z* 454 $(M+Na)^+$; UV λ_{max} 298 (ε 6807).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(3*S*,5*R*)-5-(4-hydroxymethylphenyl)pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic acid 4d. IR ν_{max} (KBr) 3404,

1755, 1583, 1385, 1082, 609 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.26 (3H, d, $J=7.3$ Hz), 1.30 (3H, d, $J=6.2$ Hz), 2.55 (1H, dd, $J=10.9, 7.0$ Hz), 2.81 (1H, m), 3.40 (1H, m), 3.49 (2H, m), 3.90 (1H, dd, $J=12.5, 6.0$ Hz), 4.27 (3H, m), 4.66 (2H, m), 5.11 (1H, dd, $J=10.6, 6.6$ Hz), 7.49 (4H, s); FAB-MS m/z 419 $(M+H)^+$; UV λ_{max} 298 (ε 9962).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(3*S*,5*S*)-5-(4-hydroxymethylphenyl)pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic acid 4e. IR ν_{max} (KBr) 3394 1755, 1402, 1082, 609cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.24 (3H, d, $J=7.2$ Hz), 1.29 (3H, d, $J=6.6$ Hz), 2.29 (1H, m), 2.98 (1H, m), 3.42 (3H, m), 3.80 (1H, dd, $J=10.8$, 8.2 Hz), 4.13 (1H, m), 4.23 (3H, m), 4.66 (2H, m), 7.45 (2H, d, $J=8.4$ Hz), 7.49 (2H, d, $J=8.4$ Hz); FAB-MS m/z 419 $(M+H)^+$; UV λ_{max} 299 (ε 9627).

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