1β-Methyl-2-(5-substituted pyrrolidin-3-ylthio)carbapenems; 2. Synthesis and Antibacterial Activity of 5-Aminopropyl and 5-Aminopropenyl Pyrrolidine Derivatives

NORIKAZU OHTAKE*, OSAMU OKAMOTO, SHINJI KATO, RYOSUKE USHIJIMA, HIROSHI FUKATSU and SUSUMU NAKAGAWA

Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Okubo 3, Tsukuba 300-26, Japan

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The synthesis and biological activity of (1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-substituted pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic acid, in which aminopropyl, aminopropenyl, and aminopropynyl groups were introduced as substituents, are described. Aminopropyl and aminopropenyl derivatives showed potent *in vitro* and *in vivo* antibacterial activity against Gram-positive and Gram-negative bacteria including *P. aeruginosa*.

In the preceding paper[1], we reported the synthesis and biological properties of the novel cationic carbapenems, bearing cyclic amine substituted pyrrolidinylthio moieties as the C-2 side chain. Among the series, BO-2502A (Fig. 1) was selected as a lead compound, due to its improved properties including antibacterial activity against *P. aeruginosa* and pharmacokinetic profiles compared to those of meropenem. We conceived that the introduction of an additional amine moiety on the pyrrolidinylthio side chain was responsible for the improvements.

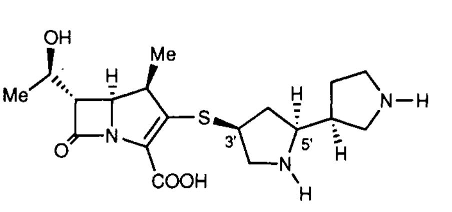
Taking these results into consideration, modification of the C-2 side chains on the cationic carbapenems was continued by replacing the C-5′ cyclic amine moiety with simple acyclic amines such as 3-aminopropyl, 3-aminopropenyl, and 3-aminopropynyl which could be prepared readily.

The resulting new carbapenems having aminopropyl and aminopropenyl moiety were found to show excellent antibacterial activity against Gram-positive and Gram-negative bacteria including *P. aeruginosa* and possess good stability to DHP-I. This paper describes the synthesis and structure activity relationships of a new series of cationic carbapenems.

Chemistry

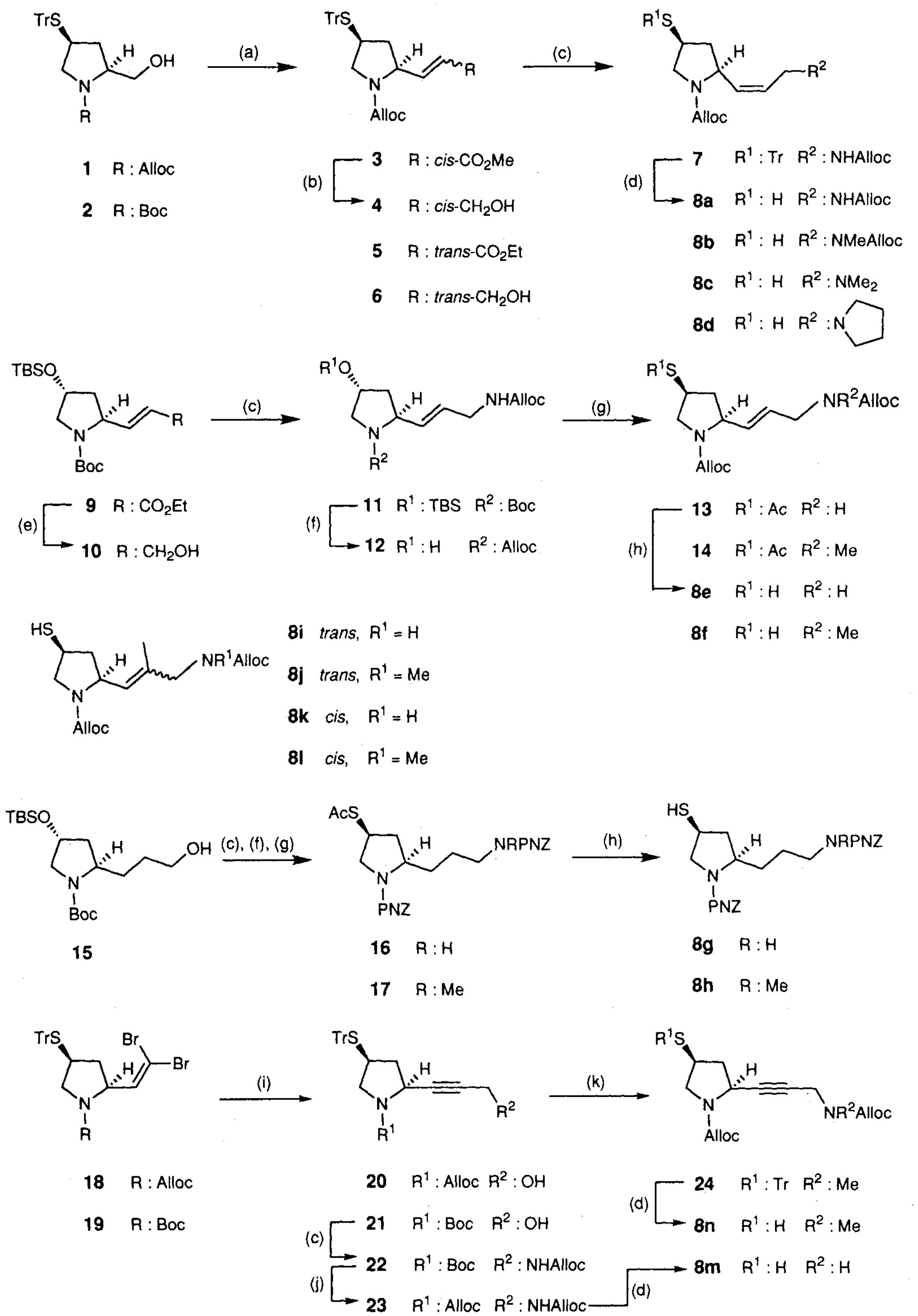
Preparation of some representative pyrrolidinethiols bearing aminopropyl, aminopropenyl and aminopropynyl moieties at the C-2 position were shown in Scheme 1. Preparation of aminopropenyl pyrrolidinethiols was initiated with commercially available (2*S*,4*R*)-L-hydroxyproline, which was converted to the alcohol (**1**) by a five-step process: (1) esterification with HCl-MeOH, (2) *N*-protection with allyl chloroformate, (3) mesylation of the secondary alcohol with MsCl (methanesulfonyl chloride) and triethylamine, (4) trytylthiolation of the mesylate with TrSH (triphenylmethanethiol) and sodium hydride in DMF, and (5) reduction of the ester moiety with sodium borohydride-lithium chloride. Swern oxidation of (**1**) followed by stereoselective Horner-Emmons reaction of the resulting aldehyde afforded *cis*[2] and *trans* α,β-unsaturated esters (**3** and **5**), respectively. 1, 2-Reduction of the α,β-unsaturated ester (**3**) was achieved by using DIBAL-H (diisobutylalminiumhydride) to give **4** in 51% yield. The DIBAL-H reduction of the *trans*-esters (**5**, **9**) resulted in poor yields of **6** and **10**[3], however **10** was obtained in good yield under *n*-butyllithium—DIBAL-H system.[4] The *cis*-propenol (**4**) was converted to the corresponding amine (**7**) in the following reaction sequence: (1) mesylation, (2) displacement of the mesylate with sodium azide, (3) reduction of the azide group with triphenylphosphine-H_2O, and (4) protection of the resulting amine by an allyloxycarbonyl group. Other *cis*-propenylamine derivatives such as **8b**, **8c**, and **8d** were prepared from the mesylate by displacement with the

Fig. 1.



BO-2502A

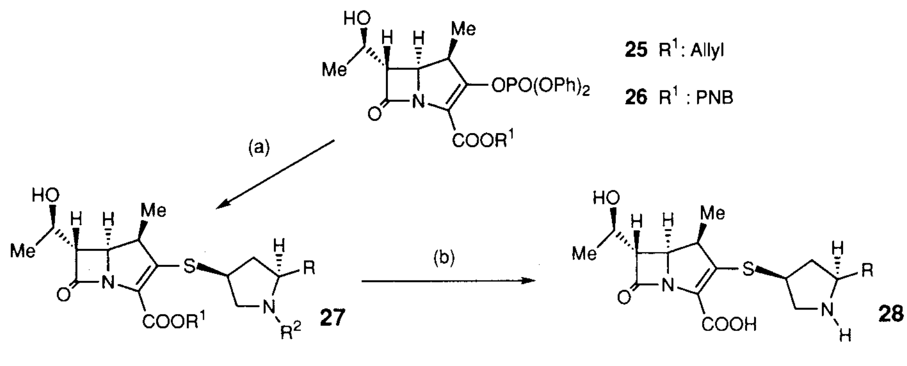
Scheme 1.



Alloc ; Allyloxycarbonyl. PNZ ; *p*-nitrobenzyloxycarbonyl

(a): 1) DMSO, $(COCl)_2$, NEt_3, CH_2Cl_2, -78 °C, 2) $(CF_3CH_2O)_3POCH_2CO_2Me$, $KN(TMS)_2$, 18-crown-6, THF, -78 °C, 2') 60% NaH, $(EtO)_3POCH_2CO_2Et$, THF, 4 °C, (b) DIBAL-H, toluene, -78 °C, (c): 1) MsCl, NEt_3, THF, 4 °C, 2) NaN_3, DMF, 50 °C, 3) PPh_3, H_2O, THF, r.t., 4) AllocCl or PNZCl, NEt_3, 4 °C, (d) Et_3SiH, TFA-CH_2Cl_2, 4 °C, (e) n-BuLi, DIBAL-H, toluene, -50 °C, (f): 1) HCl-MeOH, 2) AllocCl or PNZCl, $NaHCO_3$, dioxane-H_2O, (g): 1) MsCl, NEt_3, THF, 4 °C, 2) AcSK, DMF, 60 °C, (h) 1N-NaOH, MeOH, (i) n-BuLi, THF, -78 °C, then paraformaldehyde, -20 °C (j): 1) TFA, CH_2Cl_2, 4 °C, 2) AllocCl, NEt_3, CH_2Cl_2, 4 °C,(k) 60% NaH, MeI, DMF, r.t..

Scheme 2.



R^1=Allyl, R^2=Alloc:
(a); iPr$_2$NEt, **8a–8f**, **8i–8n**, CH_3CN, 0~4°C, (b); tributyltin hydride, $(PPh_3)_2PdCl_2$, CH_2Cl_2–H_2O, 4°C.

R^1=PNB, R^2=PNZ:
(a); iPr$_2$NEt, **8g**, **8h**, CH_3CN, 0–4 °C, (b); 10 % Pd–C, H_2, THF–MOPS buffer (pH = 7.0).

corresponding amines. Finally deprotection of the trityl group was accomplished by using triethylsilane—TFA[5] to afford the thiols (**8a**, **8b**, **8c**, and **8d**) in 80~90% yield.

The *trans*-propenylamine (**11**) derived from the *trans*-propenol (**10**) in a similar manner described above, was deprotected with HCl-MeOH and reprotected with allyl chloroformate to afford the alcohol (**12**), which was converted to the corresponding thioacetate (**13**) *via* the mesylate. The thiol (**8e**) was obtained from **13** by basic or acidic hydrolysis.

The 2-methyl-3-aminopropenylamine derivatives (**8i**, **8j**, **8k**, and **8l**) were prepared from **1** by a similar method mentioned above.

The 3-aminopropyl derivatives (**8g** and **8h**) were easily prepared from the propanol (**15**), which was obtained by catalytic hydrogenation of the α,β-unsaturated ester (**9**) followed by reduction of the ester group with sodium borohydride—lithium chloride.

Finally preparation of the aminopropynyl derivatives (**8m** and **8n**) began with the alcohol (**1**). Swern oxidation of **1** followed by treatment with triphenylphosphine and carbon tetrabromide afforded the dibromoolefin (**18**). Conversion of **18** to propynyl alcohol (**20**)[6] was not successful, probably due to the lability of the allyloxycarbonyl group under the reaction condition (2.2 eq. n-butyllithium at -78°C, then addition of paraformaldehyde at -20°C). The use of the dibromoolefin (**19**) protected with a Boc group which was derived from the alcohol (**2**), afforded the propynyl alcohol (**21**) in a moderate yield. The alcohol moiety of **21** was transformed to the corresponding allyloxycarbonylamine by a similar method described above to give **22**. The Boc group of **22** was replaced with an allyloxycarbonyl group affording **23**, which was treated with triethylsilane in TFA to give **8m**. N-Methylation of **23** was easily achieved by treatment with sodium hydride and iodomethane in DMF to give **24**, which was deprotected under the above condition, furnishing **8n** in a good yield.

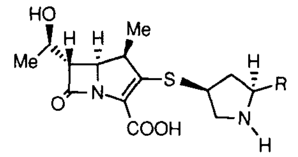
The thiols, obtained above, except for **8g** and **8h** were coupled with allyl (1R,5S,6S)-2-diphenylphosphoryloxy-6-[(R)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (**25**)[7,8] in the presence of iPr$_2$NEt in CH_3CN at 0~5°C to give the protected carbapenems (**27**) in moderate to good yields. Deprotection of **27** (**a**~**f**, **i**~**n**) were easily accomplished according to the method developed by GUIBE *et al.*,[9] and the resulting crude carbapenems were purified by reversed phase column chromatography, giving **28** (**a**~**f**, **i**~**n**) in 30~40% yield. The coupling reaction of the thiols (**8g** and **8h**) with the p-nitrobenzyl ester (**26**) followed by deprotection under catalytic hydrogenation and purification as above, afforded **28g** and **28h**, respectively.

Biological Properties

The MICs of the above prepared carbapenems against Gram-positive and Gram-negative bacteria, and the stability data to porcine DHP-I [relative hydrolysis rate to imipenem (=1)] are shown in Table 1, together with those of imipenem and meropenem.

All the aminopropyl, aminopropenyl, and aminopropynyl derivatives exhibited potent antibacterial activity against Gram-positive and Gram-negative bacteria and

Table 1. *In vitro* antibacterial activity (MIC, μg/ml) and DHP-I stability of carbapenem compounds.



Organism	**28a**	**28b**	**28c**	**28d**	**28e**	**28f**	**28g**	**28h**
R :	NH_2	NHMe	NMe_2	N	NH_2	NHMe	NH_2	NHMe
S. aureus 209P NIHJ JC1	0.012	0.012	0.012	<0.006	0.012	0.012	0.012	0.012
S. aureus BB5939*	3.13	3.13	3.13	3.13	3.13	1.56	3.13	3.13
S. aureus pMS520/Smith	6.25	6.25	6.25	6.25	6.25	3.13	6.25	6.25
S. faecalis MB4966	1.56	1.56	3.13	1.56	3.13	1.56	1.56	1.56
E. coli NIHJ JC2	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
S. marcescence NO.16-2*	0.39	0.39	0.39	0.39	0.78	0.78	0.78	0.39
C. freundii GN346*	0.05	0.05	0.10	0.05	0.10	0.10	0.10	0.10
M. morganii MB5168	0.39	0.39	0.39	0.39	0.78	0.39	0.78	0.39
P. aeruginosa MB5002	0.78	0.78	1.56	1.56	1.56	1.56	1.56	0.78
P. aeruginosa MB5178	3.13	3.13	6.25	6.25	6.25	6.25	3.13	6.25
P. aeruginosa AKR17*	3.13	3.13	3.13	3.13	3.13	3.13	1.56	3.13
DHP-I susceptibility**	<0.05	0.19	0.18	0.17	0.11	<0.05	<0.05	0.07

Organism	**28i**	**28j**	**28k**	**28l**	**28m**	**28n**		
R :	NH_2 Me	NHMe Me	NH_2 Me	NHMe Me	NH_2	NHMe	IPM	MEPM
S. aureus 209P NIHJ JC1	<0.006	0.012	<0.006	0.012	0.012	0.012	0.006	0.05
S. aureus BB5939*	3.13	3.13	3.13	3.13	6.25	6.25	6.25	12.5
S. aureus pMS520/Smith	3.13	6.25	3.13	6.25	6.25	6.25	25	25
S. faecalis MB4966	1.56	1.56	1.56	1.56	3.13	1.56	1.56	3.13
E. coli NIHJ JC2	0.05	0.05	0.05	0.05	0.05	0.05	0.10	0.012
S. marcescens No. 16-2*	0.78	0.78	0.39	0.39	0.78	0.78	1.56	0.20
C. freundii GN346*	0.05	0.05	0.05	0.10	0.10	0.10	0.20	0.025
M. morganii MB5168	0.39	0.78	0.39	0.39	0.39	0.39	1.56	0.10
P. aeruginosa MB5002	1.56	0.78	1.56	0.78	12.5	6.25	1.56	3.13
P. aeruginosa MB5178	3.13	6.25	3.13	3.13	12.5	12.5	12.5	6.25
P. aeruginosa AKR17*	3.13	3.13	3.13	1.56	12.5	12.5	3.13	3.13
DHP-I susceptibility**	0.05	0.07	<0.05	0.10	0.08	0.08	1.0	0.20

* ß-lactamase producing strain.
** Relative to imipenem, porcine renal dehydropeptidase-I.

Table 2. Therapeutic effect against experimental systemic infection in mice*.

Organisms (Infection dose; cfu/mouse)	Compounds	MIC (μg/ml)	ED_{50} (95% confidence limit)**
S. aureus 4970 (8.5x10^5)	**28f**	0.025	0.05 (0.02-0.08)
	28h	0.025	0.05 (0.02-0.09)
	28l	0.025	0.08 (0.04-0.13)
	MEPM	0.10	1.59 (0.84-2.77)
	IPM	0.025	0.13 (0.07-0.25)
S. aureus pMS520/Smith*** (2.9x10^7)	**28f**	3.13	2.11 (0.94-4.35)
	28h	1.56	2.23 (1.14-3.99)
	28l	3.13	1.77 (0.62-4.85)
	MEPM	12.50	43.8 (undefined)
	IPM	6.25	16.1 (undefined)
P. aeruginosa BB5746 (2.9x10^4)	**28f**	0.39	0.71 (0.17-1.82)
	28h	0.39	0.35 (0.14-0.66)
	28l	0.39	0.44 (undefined)
	MEPM	0.39	2.41 (0.76-6.72)
P. aeruginosa BB5935 (3.9x10^5)	**28f**	1.56	1.12 (0.27-3.23)
	28h	1.56	1.53 (0.51-3.13)
	28l	3.13	1.54 (0.23-7.34)
	MEPM	1.56	11.8 (4.35-34.5)

* DDY male mice.
** Antibiotics were administered at 1 hour after infection and ED values were calculated by probit method (n=7 or 8).
*** Methicillin-resistant strain.

Table 3. Pharmacokinetics of carbapenems after s.c. administration of a 20 mg/kg dose to mice (n=3).

Compounds	Pharmacokinetic parameters			Urinary recovery
	Cmax (μg/ml)	T1/2 (hr)	AUC (μg·hr/ml)	0-6 hr (%)
28f	26.6	0.13	12.5	60.7
28h	26.7	0.13	12.6	69.8
28l	25.1	0.11	10.1	40.2
MEPM	22.9	0.10	7.9	22.8
IPM	21.5	0.12	9.5	21.5

excellent DHP-I stability. In particular, they showed improved antibacterial activity against the *S. aureus* strains compared to meropenem. However their antipseudomonal activity was found to depend on the respective side chains. The aminopropyl and aminopropenyl derivatives were generally more potent than meropenem and imipenem against *P. aeruginosa*, however the aminopropynyl derivatives showed unexpectedly reduced activity compared to imipenem.

As to the geometry of the amino propenyl side chain, the *cis*-isomers (**28a**, **28b**, **28k**, and **28l**) were generally more potent than the corresponding *trans*-isomers (**28e**, **28f**, **28i**, and **28j**) against the *P. aeruginosa*.

The *N*-unsubstituted derivative (**28a**) and *N*-methyl derivative (**28b**) showed better antipseudomonal activity than the *N*,*N*-disubstituted one (**28c** and **28d**).

In summary, the aminopropyl and aminopropenyl carbapenems showed well balanced potent antibacterial activity against Gram-positive and Gram-negative bacteria including *P. aeruginosa*. The potent antistaphylococal and -pseudomonal activity superior to that of meropenem indicates that acyclic amines including aminopropyl and aminopropenyl moieties have the same enhancing effect on antibacterial activity as does the second pyrrolidine moiety of BO-2502A.

The three selected compounds (**28f**, **28h**, and **28l**) were evaluated for *in vivo* therapeutic efficacy in systemic infections caused by *S. aureus* and *P. aeruginosa* (2 strains each) and pharmacokinetics in mice. All of these compounds were as active as or more active than imipenem, and more active than meropenem in the infectious models. The favorable *in vivo* efficacy might be the reflection of the good *in vitro* activity and pharmacokinetics. In particular it is interesting to note that these compounds were more effective in the MRSA model (*S. aureus* pMS520/Smith) than imipenem and meropenem. In addition they showed much less epileptogenic potential than imipenem by the rat head assay (intracerebroventricular injection).

Experimental

MIC Determination

MICs were determined by an agar dilution method using Mueller-Hinton medium. The culture grown overnight at 37°C for 20 hours was diluted to 3×10^6 CFU/ml, and about 10^4 CFU/ml was spotted onto the agar plates containing serial two-fold dilutions of antibiotics with a replicating device (Microplanter; Sakuma Seisakusyo, Tokyo, Japan). The plates were incubated at 37°C for 20 hours. The MIC was defined as the lowest concentration of antibiotics, at which visible growth was inhibited.

DHP-I Stability

Susceptibility of carbapenems to hydrolysis by DHP-I was determined by using partially purified swine renal DHP-I (specific activity, 0.3 U/mg of protein). One unit of activity was defined as the amount of enzyme hydrolyzing 1 μmol of glycyldehydrophenylalanine per minute when the substrate and 0.04 U of DHP-I per ml was incubated at 35°C in 50 mM MOPS buffer (pH 7.0). Hydrolysis was monitored spectrophotometrically, and expressed as the relative hydrolysis rate, taking the hydrolysis rate of imipenem as 1.0.

Determination of Antibiotic Levels in Mouse Plasma and Urine

Groups of three mice each were injected subcutaneously with 20 mg of each carbapenem per kg of body weight. The levels of carbapenems were determined by biological assay with a paper disk method using *Bacillus subtilis* ATCC 12432 as the indicator organism. The inoculated agar plates (antibiotic medium No.1; Difco) were incubated at 37°C for 16 hours. The contents of the disk were caluculated from a standard curve.

Systemic Infection

DDY male mice, 4 weeks old, were intraperitonealy infected with Gram-positive and Gram-negative bacteria, which were suspended in 5% gastric mucin. Antibiotics were subcutaneously administered to the mice once at 1 hour after infection. The therapeutic efficacy (ED_{50}) was calculated by probit method from the survival rate on the day 4 after treatment.

General Methods

All reactions were carried out under a nitrogen atmosphere unless indicated otherwise. IR spectra were recorded on a Horiba FT-200 IR spectrometer. ^1H NMR spectra were taken with Varian XL-200 and GEM-300 FT spectrometer, in the designated solvent, using tetramethylsilane or residual DOH (δ 4.80) as an internal reference. Mass spectra were obtained on JEOL JMS-SX102A. Silica gel column chromatography was carried out on WAKO gel C-300. Reversed phase column chromatography was carried out on YMC-gel ODS-AQ 120-S50.

(2*S*,4*S*)-*N*-Allyloxycarbonyl-2-hydroxymethyl-4-tritylthiopyrrolidine (**1**)

1) To an ice-cooled solution of (2*S*,4*R*)-*N*-allyloxycarbonyl-4-hydroxyproline methylester (2.4 g, 10 mmol) in THF (30 ml) were added MsCl (0.96 ml, 12.4 mmol) and NEt_3 (1.74 ml, 12.5 mmol) dropwise and the mixture was stirred for 30 minutes at the same temperature. The mixture was poured into H_2O and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. To an ice-cooled solution of TrSH (3.1 g 11 mmol) in DMF (50 ml) was added 60% NaH (440 mg, 11 mmol) in portions. After being stirred for 30 minutes at the same temperature, the above residue in DMF (15 ml) was added, and the mixture was further stirred for 3 hours at room temperature. The mixture was quenched with aqueous NH_4Cl solution, poured into H_2O, and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give (3*S*,5*S*)-*N*-allyloxycarbonyl-5-

methoxycarbonyl-3-tritylthiopyrrolidine (3.5 g, 70%).

2) To a solution of the above compound (3.5 g, 7.2 mmol) in THF (30 ml) was added LiCl (610 mg, 14.4 mmol) and $NaBH_4$ (550 mg, 14.4 mmol). EtOH (30 ml) was added below 5°C, and the mixture was stirred for 17 hours. The mixture was cooled with ice-water, adjusted to pH 4 by adding AcOH, and concentrated *in vacuo*. The residue was poured into H_2O, and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give **1** (2.6 g, 77%): IR (KBr) 3442, 1691 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.35 (1H, m), 1.95 (1H, m), 2.65~3.10 (3H, m), 3.5~3.8 (3H, m), 4.45~4.7 (3H, m), 5.2~5.3 (2H, m), 5.90 (1H, m), 7.20~7.60 (15H, m); HRFAB-MS *m/z* Calcd for $C_{28}H_{30}NO_3S$ $(M+H)^+$ 460.1926; Found 460.1946.

(2*S*,4*S*)-*N*-*t*-Butoxycarbonyl-2-hydroxymethyl-4-tritylthiopyrrolidine (**2**)

2 was prepared from (2*S*,4*R*)-*N*-*t*-butoxycarbonyl-4-hydroxyproline methylester as described for the preparation of **1**.

2: IR (KBr) 3427, 1687 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.39, 1.42 (9H, each s), 1.74 (1H, m), 2.05 (1H, m), 2.65~2.87 (H, m), 2.98 (1H, m),; HRFAB-MS *m/z* Calcd for $C_{29}H_{34}NO_3S$ $(M+H)^+$ 476.2260; Found 476.2265.

(2*S*,4*S*)-*N*-Allyloxycarbonyl-2-[(*Z*)-2-methoxycarbonylvinyl]-4-tritythiopyrrolidine (**3**)

To a stirred solution of oxalyl chloride (1.36 ml, 15.6 mmol) in CH_2Cl_2 (60 ml) was added dropwise a solution of DMSO (1.65 ml, 23.2 mmol) in CH_2Cl_2 (10 ml) at −78°C and the mixture was stirred for 30 minutes. To the mixture was added **1** (5.1 g, 11 mmol) in CH_2Cl_2 (20 ml) dropwise, and the mixture was stirred for 20 minutes. After addition of NEt_3 (5.1 ml, 37 mmol) at −78°C, the mixture was further stirred for 30 minutes at that temperature and then the temperature was raised to 0°C. The reaction mixture was diluted with CH_2Cl_2 and washed successively with 10% aqueous citric acid solution, $NaHCO_3$ aqueous solution, and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give the crude aldehyde. To a stirred solution of bis(2,2,2-trifluoroethyl)(methoxycarbonylmethyl)-phosphonate (3.6 ml, 12.0 mmol) and 18-crown-6 (14.7 g, 55.4 mmol) in THF (100 ml) was added a 0.5 M solution of potassium hexamethyldisilazide in toluene (23.2 ml) dropwise at −78°C. After being stirred for 30 minutes at the same temperature, the above aldehyde in THF (25 ml) was added, and the mixture was further stirred for 1 hour. The reaction was quenched with NH_4Cl aqueous solution and the mixture was extracted with EtOAc. The organic layer was washed with H_2O (×3) and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The oily residue was purified by silica gel column chromatography to give **3** (4.3 g, 75%): IR (KBr) 1711, 1647, 1404 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.47 (1H, m), 2.10~2.54 (1H, m), 3.67 (3H, s), 4.30~4.54 (2H, m), 5.62~5.96 (2H, m), 6.12 (1H, m), 7.10~7.50 (15H, m); EI-MS *m/z* 513 (M^+).

(2*S*,4*S*)-*N*-Allyloxycarbonyl-2-[(*Z*)-3-hydroxy-1-propenyl]-4-tritylthiopyrrolidine (**4**)

To a stirred solution of **3** (3.73 g, 7.27 mmol) in toluene (80 ml) was added a 1.0 M solution of DIBAL-H in toluene (18.2 ml) dropwise over 30 minutes at −78°C. After being stirred for 1 hour at −78°C and then the temperature being raised to −10°C, the reaction was quenched by adding AcOH (3.5 ml, 61 mmol) and the mixture was further stirred for 30 minutes at 0°C. The resulting mixture was diluted with EtOAc and washed successively with 1 N aqueous NaOH solution, H_2O and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give the oily residue, which was purified by silica gel column chromatography affording **4** (1.74 g, 49%): IR (KBr) 3448, 1691, 1549 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.53 (1H, m), 2.17 (1H, m), 2.64~3.00 (3H, m), 3.68~3.98 (2H, m), 4.24~4.67 (4H, m), 5.11~5.40 (3H, m), 5.73~5.97 (2H, m), 7.16~7.54 (15H, m); HRFAB-MS *m/z* Calcd for $C_{30}H_{32}NO_3S$ $(M+H)^+$ 486.2113; Found 486.2076.

(2*S*,4*S*)-*N*-Allyloxycarbonyl-2-[(*Z*)-3-allyloxycarbonylamino-1-propenyl]-4-tritylthiopyrrolidine (**7**)

1) To an ice-cooled solution of **4** (843 mg, 1.74 mmol) in THF (15 ml) was added NEt_3 (0.39 ml, 0.28 mmol) and MsCl (0.175 ml, 2.26 mmol) and the mixture was stirred for 30 minutes at the same temperature. The mixture was poured into H_2O and extracted with EtOAc. The combined organic layers were washed brine, dried over $MgSO_4$, and concentrated *in vacuo*. To a solution of the residue in DMF (10 ml) was added NaN_3 (340 mg, 5.23 mmol) and the mixture was stirred for 1 hour at 50°C. The mixture was poured into H_2O and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the oily residue by silica gel column chromatography gave (3*S*,5*S*)-*N*-allyloxycarbonyl-5-

[(*Z*)-3-azido-1-propenyl]-3-tritylthiopyrrolidine (826 mg, 93%): IR (KBr) 2100, 1703, 1402 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56 (1H, m), 1.86~2.22 (1H, m), 5.12~5.70 (4H, m), 5.83 (1H, m), 7.15~7.55 (15H, m); HRFAB-MS *m/z* Calcd for $C_{30}H_{31}N_4O_2S$ $(M+H)^+$ 511.2168; Found 511.2138.

2) To a stirred solution of the above compound (570 mg, 1.1 mmol) in THF (10 ml) was added PPh_3 (440 mg, 1.7 mmol) and H_2O (0.1 ml) and the mixture was stirred overnight at room temperature. To the mixture was added NEt_3 (0.77 ml, 5.6 mmol) and AllocCl (0.18 ml, 1.7 mmol), and the mixture was further stirred for 2 hours. The mixture was poured into H_2O and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography gave **7** (460 mg, 73%): IR (KBr) 1695, 1571, 1403 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.54 (1H, m), 2.14 (1H, m), 2.64~3.02 (3H, m), 3.64~4.02 (3H, m), 4.22~4.63 (5H, m), 5.10~6.02 (8H, m), 7.16~7.56 (15H, m); HRFAB-MS *m/z* Calcd for $C_{34}H_{37}N_2O_4S$ $(M+H)^+$ 569.2474; Found 569.2465.

(2*S*,4*R*)-*N*-*t*-Butoxycarbonyl-4-*t*-butyldimethylsilyloxy-2-[(*E*)-3-hydroxy-1-propenyl]pyrrolidine (**10**)

To a stirred solution of DIBAL-H (26.7 mmol) in toluene (80 ml) was added a 1.6 M solution of *n*-BuLi in hexane (16.7 ml) dropwise at −50°C. After being stirred for 30 minutes at the same temperature, **9** (3.55 g, 10.2 mmol) in toluene (15 ml) was added dropwise and the mixture was further stirred for 1 hour at −50°C. After the reaction temperature was raised to −10°C over 30 minutes, the reaction was quenched with MeOH (5 ml) and the solution was diluted with EtOAc. The organic layer was washed with 1 N aqueous NaOH solution, H_2O, and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The oily residue was purified by silica gel column chromatography to afford **10** (2.3 g, 72%): IR (KBr) 1691, 1549, 1402 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.04 (6H, s), 0.86 (9H, s), 1.42 (9H, s), 1.78 (1H, m), 2.01 (1H, m), 3.40 (2H, m), 4.12 (2H, d, J=5.0 Hz), 4.34 (2H, m), 5.58 (1H, dd, J=5.0 and 16.0 Hz), 5.74 (1H, dt, J=5.0 and 16.0 Hz); HRFAB-MS *m/z* Calcd for $C_{18}H_{36}NO_4Si$ $(M+H)^+$ 358.2413; Found 358.2446.

(2*S*,4*R*)-2-[(*E*)-3-Allyloxycarbonylamino-1-propenyl]-*N*-*t*-butoxycarbonyl-4-*t*-butyldimethylsilyloxypyrro-lidine (**11**)

11 was prepared from **10** as described for the preparation of **7**: IR (KBr) 1693, 1531, 1402 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.01 (6H, s), 0.82 (9H, s), 1.40 (9H, s), 1.73 (1H, m), 1.98 (1H, m), 3.38 (2H, m), 3.78 (2H, m), 4.19 (2H, m), 4.54 (2H, m), 5.13~5.35 (2H, m), 5.52 (2H, m), 5.91 (1H, m); FAB-MS *m/z* 441 $(M+H)^+$.

(2*S*,4*R*)-2-[(*E*)-3-Allyloxycarbonylamino-1-propenyl]-*N*-allyloxycarbonyl-4-hydroxypyrrolidine (**12**)

To a solution of **11** (680 mg, 1.5 mmol) in MeOH (5 ml) was added a 4.5 N solution of hydrogen chloride in MeOH (5 ml). After being stirred overnight at room temperature, the mixture was concentrated *in vacuo*. To an ice-cooled solution of the residue in CH_2Cl_2 (10 ml) was added triethylamine (0.8 ml, 7 mmol) and AllocCl (0.56 ml, 3 mmol), and the mixture was stirred for 1 hour. The reaction mixture was concentrated *in vacuo*, poured into H_2O and extracted with EtOAc. The combined organic layer was washed with 10% aqueous citric acid solution, saturated $NaHCO_3$ aqueous solution, and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography gave **12** (440 mg, 91%): IR (KBr) 3392, 1728, 1666, 1545, 1248 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.84 (1H, m), 2.17 (1H, m), 3.55 (2H, m), 3.79 (2H, m), 4.32~4.46 (6H, m), 5.12~5.38 (4H, m), 5.57 (2H, m), 5.90~6.04 (2H, m); HRFAB-MS *m/z* Calcd for $C_{15}H_{23}N_2O_5$ $(M+H)^+$ 311.1607; Found 311.1595.

(2*S*,4*S*)-4-Acetylthio-*N*-allyloxycarbonyl-2-[(*E*)-3-allyloxycarbonylamino-1-propenyl]pyrrolidine (**13**)

To an ice-cooled solution of **12** (440 mg, 1.35 mmol) in THF (10 ml) was added NEt_3 (0.21 ml, 49 mmol) and MsCl (0.12 ml, 1.45 mmol) and the mixture was stirred for 1 hour. The reaction mixture was poured into H_2O and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. To a solution of the residue in DMF (20 ml) was added potassium thioacetate (900 mg, 3.9 mmol) and the mixture was stirred for 4 hours at 60~70°C. The reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by silica gel chromatography gave **13** (400 mg, 75%): IR (KBr) 1703, 1404, 1097 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.76 (1H, m), 2.35 (3H, s), 2.48 (1H, m), 3.26 (1H, m), 3.82 (2H, m), 3.88~4.14 (2H, m), 4.42 (1H, m), 4.66 (4H, m), 5.16~5.40 (4H, m), 5.63 (2H, m), 5.80~6.04 (2H, m); HRFAB-MS *m/z* Calcd for $C_{17}H_{25}N_2O_5S$ $(M+H)^+$ 369.1484; Found 369.1462.

(2*S*,4*S*)-*N*-*t*-Butoxycarbonyl-2-(3-hydroxy-1-propynyl)-4-tritylthiopyrrolidine (**21**)

To a stirred solution of **19** (3.1 g, 4.8 mmol) in THF (80 ml) was dropwise added a 1.6 M solution of *n*-butyllithium in hexane (6.56 ml) at −78°C. After being stirred for 2 hours at the same temperature, paraformaldehyde (1.5 g) was added, and the mixture was further stirred for 4 hours at −20°C. The reaction was quenched with aqueous NH_4Cl solution and extracted with EtOAc. The combined organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography gave **21** (1.21 g, 51%). IR (KBr) 3446, 1691, 1402 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (9H, br s), 1.88 (1H, m), 2.31 (1H, m), 2.74 (2H, m), 3.02 (0.5H, m), 3.38 (0.5H, m), 4.03~4.30 (3H, m), 7.16~7.57 (15H, m); FAB-MS *m*/*z* 522 $(M+Na)^+$.

(2*S*,4*S*)-2-(3-Allyloxycarbonylamino-1-propynyl)-*N*-*t*-butoxycarbonyl-4-tritylthiopyrrolidine (**22**)

22 was prepared from **21** as described for the preparation of **11**: IR (KBr) 1693, 1396, 1248 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39, 1.42 (9H, each s), 1.80 (1H, m), 2.72 (1H, m), 3.00 (1H, m), 3.86, 3.96 (2H, each d, *J*=5.1 Hz), 4.56 (2H, br d, *J*=4.0 Hz), 5.17~5.37 (2H, m), 5.90 (1H, m), 7.15~7.51 (15H, m); FAB-MS *m*/*z* 583 $(M+H)^+$.

(2*S*,4*S*)-*N*-Allyloxycarbonyl-2-(3-allyloxycarbonylamino-1-propynyl)-4-tritylthiopyrrolidine (**23**)

To an ice-cooled solution of **22** (440 mg, 0.75 mmol) in CH_2Cl_2 (5 ml) was added TFA (5 ml). After being stirred for 1 hour at the same temperature, the mixture was concentrated *in vacuo*. To an ice-cooled solution of the residue in CH_2Cl_2 (10 ml) was added NEt_3 (1.04 ml, 7.5 mmol) and AllocCl (0.12 ml, 1.13 mmol). After being stirred for 1 hour at the same temperature, the mixture was concentrated *in vacuo*. The residue was poured into H_2O and extracted with EtOAc. The combined organic layer was washed successively with 10 % aqueous citric acid solution, $NaHCO_3$ aqueous solution and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography gave **23** (370 mg, 88%): IR (KBr) 1707, 1408, 1248, 1095 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.84 (1H, m), 1.95~2.28 (1H, m), 2.72 (1H, quint, *J*=8.0 Hz), 2.97 (1H, m), 3.97 (2H, d, *J*=5.0 Hz), 4.22 (1H, m), 4.38~4.66 (4H, m), 5.12~5.40 (4H, m), 5.78~6.02 (2H, m), 7.16~7.56 (15H, m); FAB-MS *m*/*z* 567 $(M+H)^+$.

(2*S*,4*S*)-*N*-Allyloxycarbonyl-2-[3-(*N*-allyloxycarbonyl-*N*-methylamino)-1-propynyl]-4-tritylthiopyrrolidine (**24**)

To an ice-cooled solution of **23** (560 mg, 0.96 mmol) in DMF (15 ml) was added 60% NaH (58 mg, 1.4 mmol). After being stirred for 30 minutes at the same temperature, MeI (0.24 ml, 3.84 mmol) was added and the mixture was further stirred for 2 hours at room temperature. The reaction mixture was poured into H_2O and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give **24** (535 mg, 93%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.85 (1H, m), 1.97~2.32 (1H, m), 2.74 (1H, quint, *J*=8.0 Hz), 2.80~3.12 (4H, m), 4.10 (2H, br s), 4.25 (1H, m), 4.38~4.66 (4H, m), 5.08~5.40 (4H, m), 5.75 (2H, m), 7.15~7.55 (15H, m).

(2*S*,4*S*)-*N*-Allyloxycarbonyl-2-[(*Z*)-3-allyloxycarbonylamino-1-propenyl]-4-mercaptopyrrolidine (**8a**)

To an ice-cooled solution of **7** (902 mg, 1.59 mmol) in CH_2Cl_2 (2.5 ml) and TFA (2.5 ml) was added Et_3SiH (0.268 ml, 1.68 mmol). After being stirred for 1 hour at the same temperature, the reaction mixture was concentrated *in vacuo* and diluted with EtOAc. The organic layer was washed with 1.0 M phosphate buffer (p.H 5.5) and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give **8a** (460 mg, 89%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.50 (2H, m), 2.62 (1H, m), 3.10~3.42 (2H, m), 3.74~4.20 (4H, m), 4.58 (4H, m), 5.16~6.08 (8H, m).

The following compounds (**8b**, **8c**, **8i**~**8n**) were prepared from **1** as described for the preparation of **8a**.

8b (90%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.62 (1H, m), 2.60 (1H, m), 2.93 (3H, s), 3.13~3.38 (2H, m), 3.73~4.40 (4H, m), 4.54~4.76 (4H, m), 5.14~5.42 (4H, m), 5.56 (2H, m), 5.92 (2H, m).

8c (69%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.62 (1H, m), 2.62 (1H, m), 2.84 (6H, br s), 3.12~3.42 (3H, m), 3.74~4.22 (3H, m), 4.42~4.66 (2H, m), 5.16~5.40 (2H, m), 5.55~6.10 (3H, m).

8i (91%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.47~1.82 (4H, m), 2.57(1H, m), 3.10~3.36 (2H, m), 3.72 (2H, m), 4.02 (1H, m), 4.40~4.66 (4H, m), 4.68~5.00 (1H, m), 5.12~5.40 (4H, m), 5.78~6.04 (2H, m).

8j (81%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.46~1.75 (4H, m), 2.60 (1H, m), 2.82 (3H, s), 3.25 (2H, m), 3.86 (2H, m), 4.04 (1H, m), 4.46~4.68 (5H, m), 5.14~5.42 (5H, m), 5.92 (2H, m).

8k (86%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.60 (1H, m), 1.78 (3H, s), 2.58 (1H, m), 3.08~3.36 (2H, m), 3.88 (2H, m), 4.02 (1H, m), 4.44~4.82 (5H, m), 5.10~5.44 (5H, m), 5.80~6.16 (2H, m).

8l (85%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.48~1.75 (4H, m), 2.76 (1H, m), 2.88 (3H, br s), 3.22 (2H, m), 3.76~4.32 (4H, m), 4.44~4.70 (4H, m), 5.15~5.42 (5H, m), 5.92 (2H, m).

8m (95%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.80~2.18 (2H, m), 2.69 (1H, m), 3.14~3.42 (2H, m), 3.52~4.24 (5H, m), 4.60 (4H, m), 5.14~5.48 (4H, m), 5.80~6.08 (2H, m).

8n (92%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.02 (2H, m), 2.72 (1H, m), 2.96 (3H, s), 3.34 (1H, m), 3.50~4.22 (4H, m), 4.48~4.68 (4H, m), 5.14~5.42 (4H, m), 5.82~6.06 (2H, m).

(2*S*,4*S*)-*N*-Allyloxycarbonyl-2-[(*E*)-3-allyloxycarbonylamino-2-propenyl]-4-mercaptopyrolidine (**8e**)

To an ice-cooled solution of **13** (400 mg, 1.0 mmol) in MeOH (12 ml) was added 1 N aqueous NaOH solution (1.1 ml). After being stirred for 15 minutes at the same temperature, 1 N hydrochloric acid (1.1 ml) was added and the mixture was concentrated *in vacuo*. The residue was poured into H_2O, and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give **8e** (360 mg, 100%), which was used for the next reaction without further purification. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.56~1.78 (2H, m), 2.58 (1H, m), 3.23 (2H, m), 3.82 (2H, m), 4.06 (1H, m), 4.35 (1H, m), 4.60 (4H, m), 5.17~5.42 (4H, m), 5.66 (2H, m), 5.82~6.06 (2H, m).

8f was peprared as described for the preparation of **8e**.

8f (98%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.70 (1H, m), 2.58 (1H, m), 2.86 (3H, s), 3.10~3.38 (2H, m), 3.88 (2H, m), 4.06 (1H, m), 4.34 (1H, m), 4.45~4.66 (4H, m), 5.10~5.40 (4H, m), 5.60 (2H, m), 5.95 (2H, m).

Allyl (1*R*,5*S*,6*S*)-2-{(3*S*,5*S*)-*N*-Allyloxycarbonyl-5-[(*E*)-3-allyloxycarbonylamino-1-propenyl]pyrrolidin-3-ylthio}-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**27f**)

To a stirred solution of allyl (1*R*,5*S*,6*S*)-2-diphenylphosphoryloxy-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**25**, 1.50 g, 3.0 mmol) and **8f** (850 mg, 2.51 mmol) in CH_3CN (50 ml) was added *N*,*N*-diisopropylethylamine (0.19 ml, 1.09 mmol) dropwise at −10°C. After being stirred overnight at 4°C, the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give **27f** (630 mg, 35%). IR (KBr) 1770, 1700, 1400, 1200 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.26 (3H, d, J=8.0 Hz), 1.36 (3H, d, J=7.0 Hz), 1.76 (1H, m), 2.60 (1H, m), 2.86 (3H, s), 4.50~4.95 (6H, m), 5.15~5.55 (6H, m), 5.60 (2H, m), 5.95 (2H, m).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-{(3*S*,5*S*)-5-[(*E*)-3-methylamino-1-propenyl]pyrrolidin-3-ylthio}-1-methyl-1-carbapen-2-em-3-carboxylic acid·hydrochloride (**28f**)

To an ice-cooled solution of **27f** (592 mg, 1.0 mmol) in CH_2Cl_2 (22.5 ml) was succesively added H_2O (91 μl), bis(triphenylphosphine)palladiumdichloride (35.2 mg, 0.05 mmol) and tributyltin hydride (1.03 ml, 3.83 mmol). After being stirred for 20 minutes at the same temperature, the temperature was raised to room temperature and the mixture was further stirred for 20 minutes. The mixture was poured into H_2O. The aqeuous layer was washed with $CHCl_3$ (×2) and concentrated *in vacuo* to *ca*. 20 ml. After the insoluble was removed by filtration, the filtrate was subjected to reversed phase column chromatography, which was eluted with 20% MeOH-H_2O. The fractions detected by HPLC were combined, and the pH of the solution was adjusteded to 6.2 with 0.1 N HCl. The solution was concentrated *in vacuo* and lyophilized to give **28f** (190 mg, 45%) as an amorphous powder, which was crystallized from MeOH-EtOH (1:2).

28f: IR (KBr) 1750, 1700, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.22 (3H, d, J=8.0 Hz), 1.30 (3H, d, J=7.0 Hz), 1.89 (1H, m), 2.74 (3H, s), 2.82 (1H, m), 3.28~3.52 (3H, m), 3.64~3.80 (3H, m), 4.08 (1H, m), 4.18~4.44 (3H, m), 6.04 (1H, dt, J=6.0 and 15.0 Hz), 6.19 (1H, dd, J=8.0 and 15.0 Hz); FAB-MS m/z 382 $(M+H)^+$.

Anal Calcd for $C_{18}H_{27}N_3O_4S \cdot HCl \cdot 0.75H_2O$:
C 50.11, H 6.89, N 9.74, S 7.43.
Found:
C 50.31, H 6.85, N 9.78, S 7.40.

The following compounds (**28a**~**28e**, **28i**~**28n**) were prepared from **25** and the thiols (**8a**~**8e**, **8i**~**8n**) as described for the preparation of **28f**, respectively.

28a (13%): IR (KBr) 1750, 1580, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.21 (3H, d, J=8.0 Hz), 1.29 (3H, d, J=7.0 Hz), 1.44 (1H, m), 2.76 (1H, m), 3.04 (1H, dd, J=4.0 and 12.0 Hz), 3.20~3.48 (3H, m), 3.70 (2H, d, J=8.0 Hz), 4.23 (2H, m), 5.68 (1H, dt, J=8.0 and 10.0 Hz), 5.87 (1H, dd, J=8.0 and 10.0 Hz); FAB-MS m/z 368 $(M+H)^+$.

28b (17%): IR (KBr) 1750, 1590, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.18 (3H, d, J=8.0 Hz), 1.26 (3H, d, J=7.0 Hz), 1.44 (1H, m), 2.55 (1H, m), 2.67 (3H, s), 3.04 (1H, dd, J=4.0 and 12.0 Hz), 3.22~3.46 (3H, m), 3.73 (2H, d, J=8.0 Hz), 3.82 (1H, m), 4.05 (1H, q, J=8.0 Hz), 4.20 (2H, m), 5.67 (1H, m), 5.94 (1H, t, J=10.0 Hz); FAB-MS m/z 382 $(M+H)^+$.

28c (5%): IR (KBr) 1760, 1600, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.20 (3H, d, J=8.0 Hz), 1.28 (3H, d, J=7.0 Hz), 1.53 (1H, m), 2.60 (1H, m), 2.79 (6H, s), 3.10 (1H, dd, J=4.0 and 12.0 Hz), 3.28~3.48 (3H, m), 3.75 (2H, d, J=8.0 Hz), 3.87 (1H, m), 4.21 (3H, m), 5.94 (2H, m); FAB-MS m/z 396 $(M+H)^+$.

28d (7%): IR (KBr) 1760, 1600, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.18 (3H, d, J=8.0 Hz), 1.26 (3H, d, J=7.0 Hz), 1.42 (1H, m), 2.02 (4H, m), 2.54 (1H, m), 3.00 (1H, dd, J=4.0 and 12.0 Hz), 3.82 (3H, m), 4.00 (1H, q, J=8.0 Hz), 4.18 (2H, m), 5.68 (1H, m), 5.94 (1H, t, J=10.0 Hz); FAB-MS m/z 422 $(M+H)^+$.

28e (16%): IR (KBr) 1750, 1580, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.20 (3H, d, J=7.0 Hz), 1.29 (3H, d, J=6.0 Hz), 1.47 (1H, m), 2.54 (1H, m), 3.02 (1H, dd, J=3.0 and 12.0 Hz), 3.20~3.47 (3H, m), 3.59 (2H, d, J=6.0 Hz), 3.78 (2H, m), 4.22 (2H, m), 5.81 (1H, dt, J=6.0 and 16.0 Hz), 5.98 (1H, dd, J=7.0 and 16.0 Hz); FAB-MS m/z 368 $(M+H)^+$.

28i (11%): IR (KBr) 1750, 1600, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.24 (3H, d, J=8.0 Hz), 1.30 (3H, d, J=7.0 Hz), 1.70~1.93 (4H, m), 2.82 (1H, m), 3.30~3.54 (3H, m), 3.58~3.80 (3H, m), 4.06 (1H, m), 4.27 (2H, m), 5.70 (1H, d, J=8.0 Hz); FAB-MS m/z 382 $(M+H)^+$.

28j (18%): IR (KBr) 1750, 1590, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.25 (3H, d, J=8.0 Hz), 1.32 (3H, d, J=7.0 Hz), 1.74~1.96 (4H, m), 2.86 (1H, m), 3.34~3.46 (3H, m), 3.64~3.82 (3H, m), 4.10 (1H, m), 4.29 (2H, m), 4.64 (1H, m), 5.83 (1H, d, J=8.0 Hz); FAB-MS m/z 396 $(M+H)^+$.

28k (12%): IR (KBr) 1760, 1600, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.22 (3H, d, J=8.0 Hz), 1.29 (3H, d, J=7.0 Hz), 1.80 (1H, m), 1.92 (3H, s), 2.78 (1H, m), 3.27~3.52 (3H, m), 3.58~3.86 (3H, m), 4.25 (2H, m), 4.56 (1H, m), 5.76 (1H, d, J=8.0 Hz); FAB-MS m/z 382 $(M+H)^+$.

28l (12%): IR (KBr) 1750, 1590, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.22 (3H, d, J=8.0 Hz), 1.28 (3H, d, J=7.0 Hz), 1.78 (1H, m), 1.92 (3H, s), 2.68~2.85 (4H, m), 3.28~3.56 (3H, m), 3.66 (1H, m), 3.78 (2H, s), 4.04 (1H, m), 4.24 (2H, m), 4.52 (1H, q, J=8.0 Hz), 5.84 (1H, d, J=8.0 Hz); FAB-MS m/z 396 $(M+H)^+$.

28m (13%): IR (KBr) 1755, 1590, 1390 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.23 (3H, d, J=8.0 Hz), 1.31 (3H, d, J=7.0 Hz), 2.00 (1H, dt, J=6.0 and 15.0 Hz), 2.80 (1H, dt, J=8.0 and 15.0 Hz), 3.27 (1H, dd, J=4.0 and 12.0 Hz), 3.33~3.57 (3H, m), 3.85~4.02 (3H, m), 419~4.40 (3H, m); FAB-MS m/z 366 $(M+H)^+$.

28n (12%): IR (KBr) 1755, 1590, 1385 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.23 (3H, d, J=8.0 Hz), 1.32 (3H, d, J=7.0 Hz), 2.02 (1H, m), 2.68~2.88 (4H, m), 3.15 (1H, dd, J=4.0 and 12.0 Hz), 3.30~3.57 (3H, m), 3.87~4.03 (3H, m), 420~4.40 (3H, m); FAB-MS m/z 380 $(M+H)^+$.

27g was prepared from **26** and **8g** as described for the preparation of **27f**.

27g (57%): IR (KBr) 3420, 1770, 1705, 1345 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28 (3H, d, J=8.0 Hz), 1.38 (3H, d, J=7.0 Hz), 1.76 (1H, m), 2.56 (1H, m), 3.00~3.50 (5H, m), 3.60 (1H, m), 3.98 (2H, m), 4.25 (2H, m), 4.90~5.60 (6H, m), 7.51 (4H, d, J=8.0 Hz), 7.67 (2H, d, J=8.0 Hz), 8.24 (6H, m).

(1*R*,5*S*,6*S*)-2-[(3*S*,5*R*)-5-(3-Aminopropyl)pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylic Acid·hydrochloride (**28g**)

To a solution of **27g** (180 mg, 0.21 mmol) in THF (15 ml), EtOH (3 ml) and a 0.1 M solution of MOPS buffer (pH 7.0, 15 ml) was added 10% Pd-C (200 mg), and the mixture was stirred for 2 hours under a hydrogen atmosphere. The catalyst was filtered off and washed with THF and H_2O. The combined filtrate and washings were concentrated *in vacuo* to *ca.* 10 ml, which was filtered to remove the insoluble. The filtrate was subjected to reversed phase column chromatography, which was eluted with 20% MeOH-H_2O. The fractions detected by HPLC were combined, and the pH of the solution was adjusted to 6.2 with 0.1 N NaOH. The solution was concentrated *in vacuo* and lyophilized to give **28g** (42 mg, 54%).

28g: IR (KBr) 3430, 1745, 1600 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.20 (3H, d, J=7.0 Hz), 1.27 (3H, J=6.0 Hz), 1.50~1.90 (4H, m), 2.75 (1H, m), 2.85~3.10 (3H, m), 3.25~3.50 (3H, m), 3.53~3.75 (2H, m), 4.00 (1H, m), 4.21 (2H, m); FAB-MS m/z 370 $(M+H)^+$.

28h was prepared as described for the preparation of **28g**.

28h (55%): IR (KBr) 3420, 1760, 1590 cm^{-1}; ^{1}H NMR (200 MHz, D_2O) δ 1.22 (3H, d, J=7.0 Hz), 1.29 (3H, J=6.0 Hz), 1.63~2.03 (5H, m), 2.68~2.86 (4H, m), 3.10 (2H, br t, J=7.0 Hz), 3.30~3.45 (2H, m), 3.62~3.78 (2H, m), 4.03 (1H, m), 4.23 (2H, m); FAB-MS m/z 384 $(M+H)^+$; HRFAB-MS m/z Calcd for $C_{18}H_{30}N_3O_4S$ $(M+H)^+$ 384.1957; Found 384.1967.

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