1β-Methyl-2-(5-substituted pyrrolidin-3-ylthio)carbapenems; 3. Synthesis and Antibacterial Activity of BO-2727 and Its Related Compounds†

NORIKAZU OHTAKE*, OSAMU OKAMOTO, RYUJI MITOMO, YOSHIAKI KATO, KATSUMI YAMAMOTO, YUJI HAGA, HIROSHI FUKATSU and SUSUMU NAKAGAWA

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

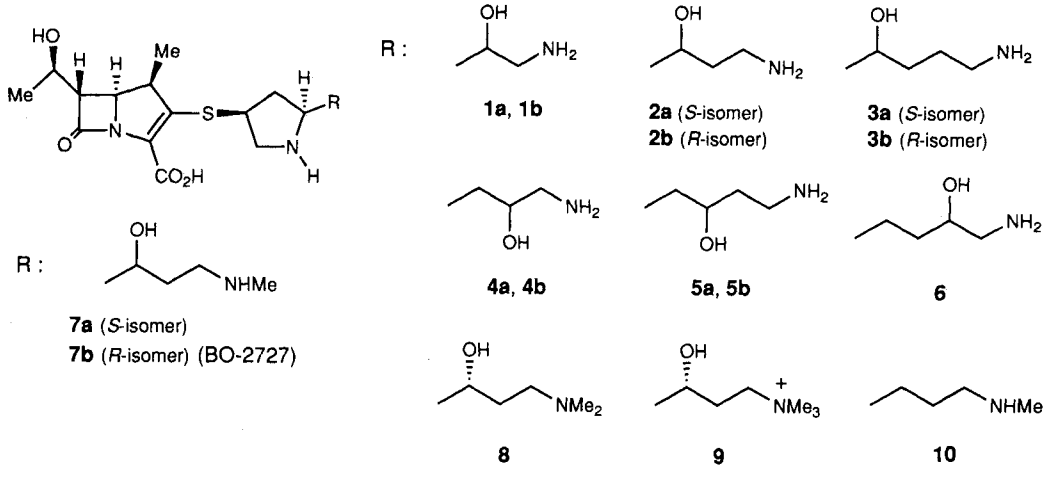
(Received for publication March 24, 1997)

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 hydroxy-substituted aminoethyl, aminopropyl, and aminobutyl groups were introduced as substituents, are described. These derivatives showed potent antibacterial activity against Gram-positive and Gram-negative bacteria including *P. aeruginosa*. Among them, lenapenem (BO-2727, **7b**), carrying an (*R*)-1-hydroxy-3-(*N*-methylamino)propyl group, was selected as a development candidate.

The discovery of the 1β-methyl carbapenem[2] by Merck group was a milestone in that it suggested the possibility of its clinical use as a single agent without dehydropeptidase-I (DHP-I) inhibitor, due to the improved chemical and metabolic stability. During the course of our derivatization of 1β-methyl-2-(5-substituted pyrrolidin-3-ylthio)carbapenems, the derivatives having either an aminopropyl or aminopropenyl substituent were previously demonstrated to show not only the improved antipseudomonal activity but also the higher stability to DHP-I compared to those of imipenem and meropenem.[3]

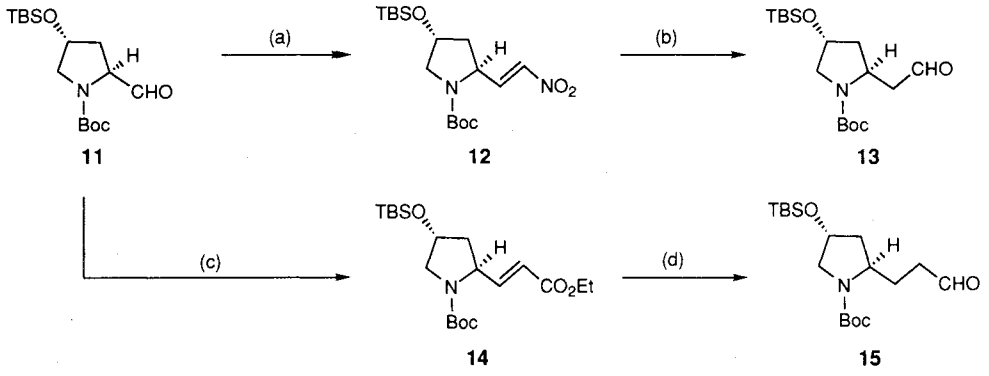
In our further effort to optimize the C-5′ substituents of the pyrrolidinylthio moiety, hydroxy-substituted aminoalkyl derivatives were found to bring about improved antipseudomonal activity compared to those of the aminoalkyl derivatives. In this paper we describe the synthesis and antibacterial activity of 1β-methyl-2-(pyrrolidin-3-ylthio)carbapenems, carrying a hydroxy-substituted aminoalkyl moiety at the C-5′ position of the pyrrolidine ring (Fig. 1). In addition, the physicochemical and biological properties of BO-2727 (**7b**), which was chosen as a development candidate, are described.

Fig. 1.



† See ref. 1.

Scheme 1.



(a);1) CH_3NO_2, cat.Et_3N, 2) $SOCl_2$, Et_3N / CH_2Cl_2, (b);1) $NaBH_4$, SiO_2 // $CHCl_3$-^{i}PrOH, 2) $KMnO_4$, 60% NaH / ^{t}BuOH-heptane-H_2O, (C) 60% NaH, $(EtO)_2POCH_2CO_2Et$ / THF, (d);1) H_2, 10% Pd-C / EtOH, 2) DIBAL-H / CH_2Cl_2.

Chemistry

Our general synthetic methods of 2-(hydroxy-substituted alkylamino)pyrrolidine thiols include the addition of appropriate nucleophiles to the pyrrolidine aldehydes (**11**[4], **13**, and **15**), which were prepared as shown in Scheme 1, and subsequent introduction of an amino function into the C-2 side chain.

Preparation of the 1-hydroxy-substituted aminoalkyl derivatives (**18**, **24**, and **27**) were conducted as shown in Scheme 2, using **11** as a starting material. Addition of trimethylsilyl cyanide to **11** followed by reduction of the nitrile moiety with LAH (lithium alminium hydride) and protection of the resulting primary amine with PNZCl (*p*-nitrobenzyl chloroformate) gave **16**, as a mixture of diastereomers (*syn* : *anti* = 1 : 1), which was separated by silica gel column chromatography to afford **16a** (less polar) and **16b**, respectively. The stereochemistries of the hydroxyls in **16a** and **16b** were not determined. Deprotection and reprotection of **16a** and **16b** furnished the diols (**17a** and **17b**), which were converted to the corresponding thioacetates (**18a** and **18b**) *via* preferential mesylation of the secondary alcohol on the pyrrolidine ring over that of the side chain.

Preparation of the 1-hydroxy-3-aminopropyl derivatives (**24a** and **24b**) was started from the aldol reaction of lithium enolate of ethyl acetate[5] with the aldehyde (**11**). The resulting diastereomeric mixture of the aldols was easily separated by silica gel column chromatography to give **20a** and **20b**, respectively, in a ratio of 3 : 1. The stereochemistry of the hydroxyls in **20a** and **20b** were determined as *S* and *R* configuration, respectively, by the X-ray analysis of **21b**.[1] Reduction of the ester moiety of **20a** and **20b** with $NaBH_4$-MeOH[6] followed by selective tosylation of the resulting primary alcohols gave the tosylates (**22a** and **22b**) in good yields. Transformations of **22a** and **22b** to **23a** and **23b** were carried out by the following four-step sequence: (1) substitution of the tosylates with sodium azide, (2) catalytic hydrogenation of the azide groups, (3) deprotection under acidic conditions, and (4) reprotection of the amino groups with PNZCl. The thioacetates (**24a** and **24b**) were obtained from **23a** and **23b** by a similar procedure for the preparation of **18** from **17**.

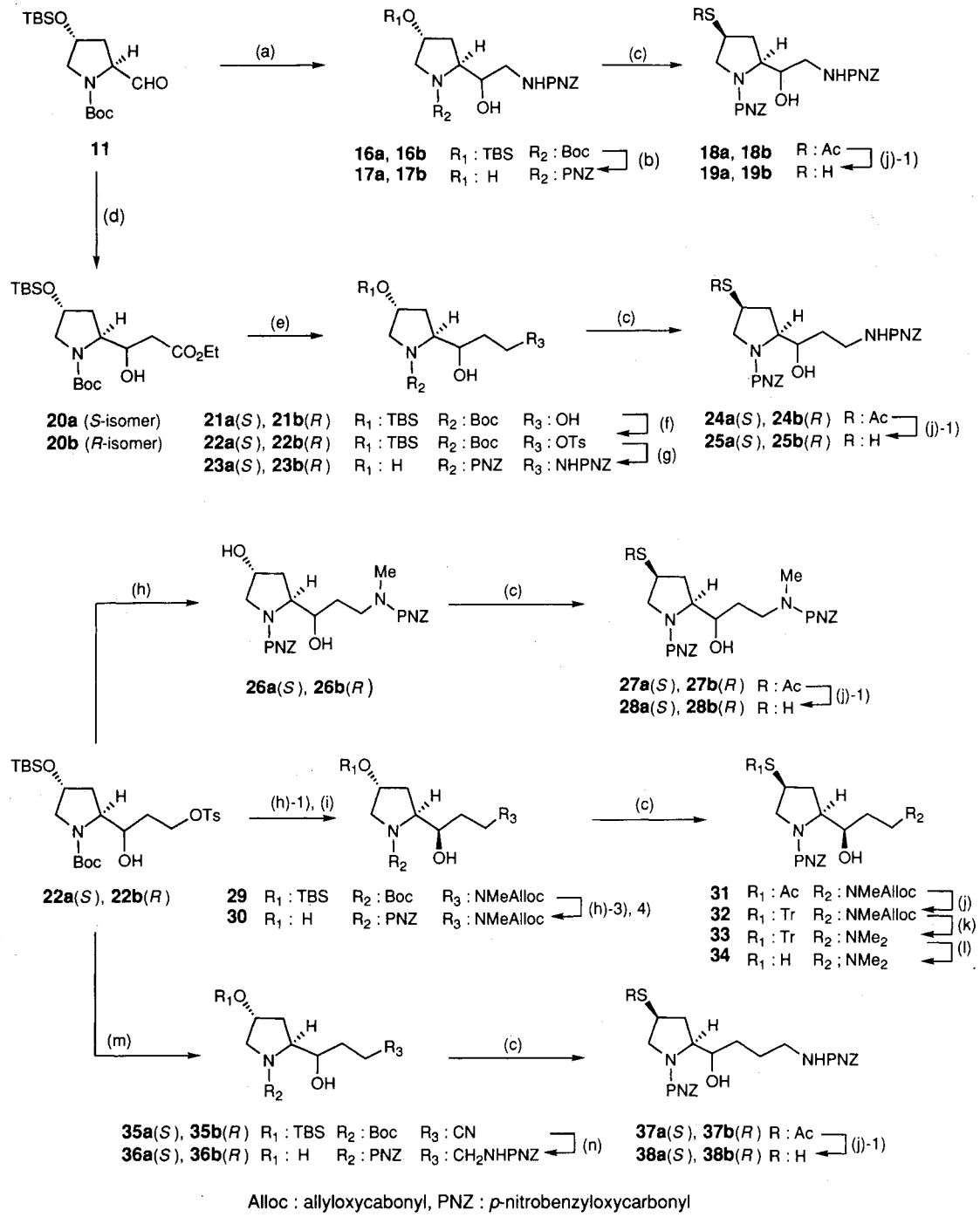
N-Methyl-1-hydroxypropyl derivatives (**27a** and **27b**) were prepared *via* substitution of the tosylates (**22a** and **22b**) with *N*-methylamine. The *N*,*N*-dimethylamino derivative (**34**) was derived from **32** by selective deprotection of allyloxycarbonyl group and subsequent reductive methylation of the secondary amine.

The 1-hydroxy-4-aminobutyl derivatives (**37a** and **37b**) were prepared from the tosylates (**22a** and **22b**) *via* substitution with NaCN and the subsequent reduction of the nitrile moiety with LAH.

The 2-hydroxy aminoalkyl derivatives (**43**, **51**, and **57**) were prepared from the aldehyde (**13**) as shown in Scheme 3. Addition of nitromethane to **13** followed by separation by column chromatography, afforded the nitroalcohols (**39a** and **39b**) in a ratio of 1 : 1. Reduction of **39a** and **39b** with Raney nickel, followed by protection of the resulting amine with di-*t*-butyl dicarbonate gave **40a** and **40b**, which were converted to the thioacetates (**43a** and **43b**), respectively, *via* the mesylates (**42a** and **42b**) by six steps including protection of the secondary alcohol of the C-2 side chains.

Preparation of the thioacetates (**51a** and **51b**) were

Scheme 2.



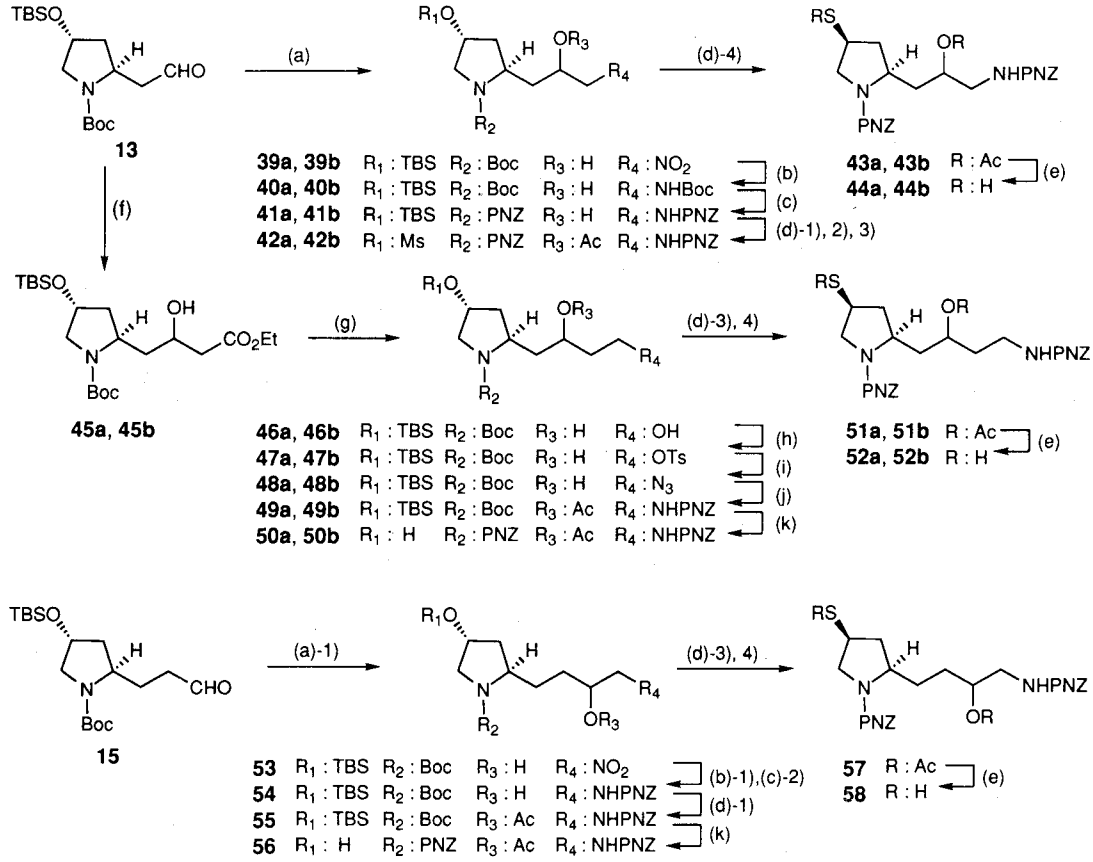
Alloc : allyloxycabonyl, PNZ : *p*-nitrobenzyloxycarbonyl

(a);1) TMSCN / benzene, 2) LAH / ether, 3) PNZCl, Et_3N / ether, 4) Separation, (b);1) TBAF / THF, 2) TFA / CH_2Cl_2, 3) PNZCl , Et_3N / CH_2Cl_2, (c);1) MsCl, Et_3N, 2) AcSK / DMF, (d);1) $(TMS)_2NLi$, EtOAc / THF, 2) Separation, (e) $NaBH_4$ / THF-MeOH, (f) TsCl, Et_3N, cat. DMAP / CH_2Cl_2, (g);1) NaN_3 / DMSO, 2) H_2,10% Pd-C/ MeOH, 3) HCl / MeOH, 4) PNZCl, Et_3N / CH_2Cl_2, (h);1) $MeNH_2$ / MeOH, 2) PNZCl, Et_3N / MeOH, 3) HCl / MeOH, 4) PNZCl, Et_3N / MeOH, (i) AllocCl, Et_3N / CH_2Cl_2, (j);1) aq. NaOH / MeOH, 2) Ph_3CCl / DMF, (k);1) cat. $(Ph_3P)_2PdCl_2$, nBu_3SnH, H_2O-CH_2Cl_2, 2) aq. CH_2O, AcOH, $NaBH(OAc)_3$ / THF, (l) TFA-Et_3SiH / CH_2Cl_2, (m) NaCN / DMSO, (n);1) LAH / ether, 2) PNZCl, Et_3N, 3) HCl / MeOH, 4) PNZCl, Et_3N.

carried out by a similar method for the preparation of **24** except for the protection of the alcohol on the C-2 side chain.

The 4-amino-3-hydroxybutyl derivative (**57**), which was a mixture of diastereomers, was prepared from the aldehyde (**15**) *via* the aldol reaction of nitromethane with

Scheme 3.



(a);1) CH_3NO_2, cat. Et_3N, 2) Separation, (b);1) H_2, Raney Ni / EtOH, 2) Boc_2O / CH_2Cl_2, (c);1) TFA / CH_2Cl_2, 2) PNZCl, Et_3N, (d);1) Ac_2O-Py, 2) TBAF / THF, 3) MsCl, Et_3N, 4) AcSK / DMF, (e) HCl / MeOH, (f);1) $(TMS)_2NLi$, EtOAc / THF, 2) Separation, (g) $NaBH_4$ / THF-MeOH, (h) TsCl, Et_3N, cat. DMAP / CH_2Cl_2, (i) NaN_3 / DMSO, (j);1) H_2, 10% Pd-C, 2) PNZCl, Et_3N, 3) Ac_2O-Py, (k);1) TBAF / THF, 2) TFA / CH_2Cl_2, 3) PNZCl, Et_3N.

15, followed by reduction of the nitro group with Raney nickel under a hydrogen atmosphere.

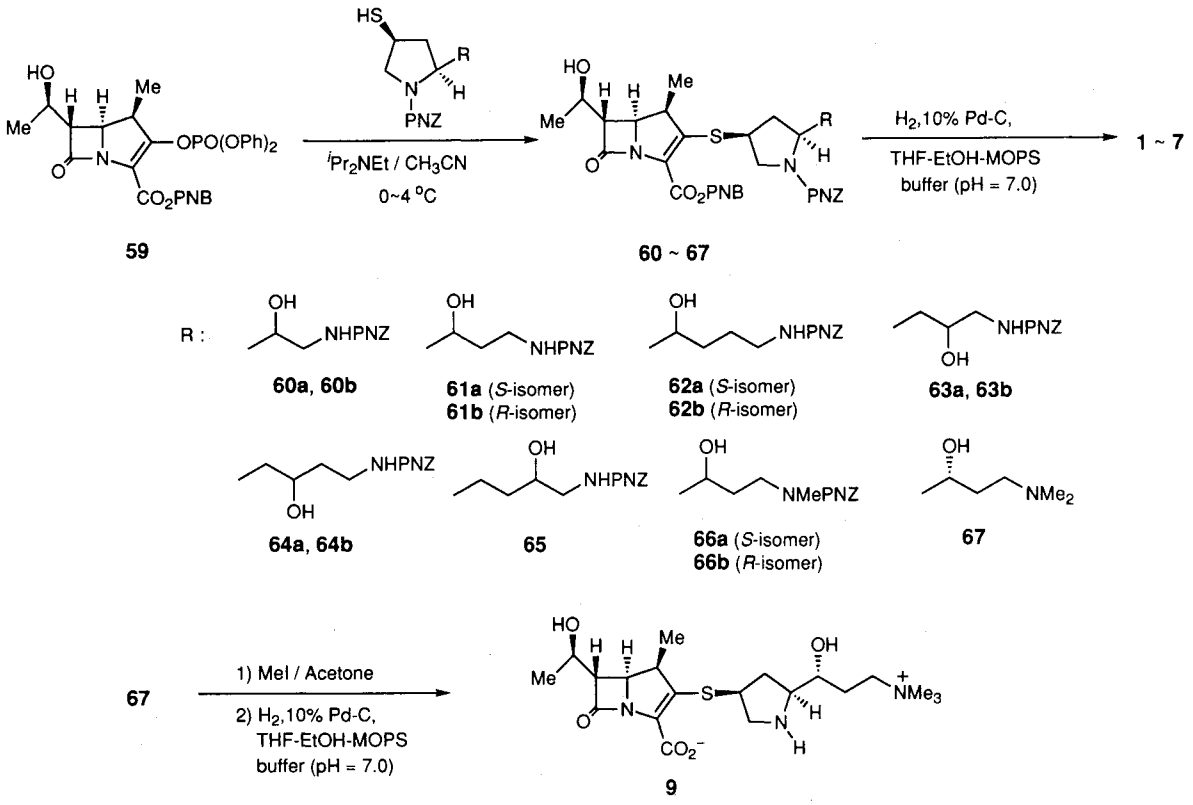
The thioacetates thus prepared were converted to the corresponding thiols by deacetylation under acidic or basic conditions. The thiols (**19**, **25**, **28**, **34**, **38**, **44**, **52**, and **58**) were coupled with the carbapenem enol phosphate (**59**)[3] in the presence of *N*,*N*-diisopropylethylamine to provide the protected 1β-methylcarbapenems (**60**$\sim$**67**) as shown in Scheme 4. Deprotection of these compounds by catalytic hydrogenation furnished the crude carbapenems, which were purified by reversed phase column chromatography as reported previously[7] to give the carbapenems (**1**$\sim$**8**) in moderate yields. The trimethylammonium carbapenem (**9**) was obtained by treatment of **67** with iodomethane followed by deprotection by catalytic hydrogenation.

Biological Properties

The MICs of the novel carbapenems prepared above against Gram-positive and Gram-negative bacteria, and the stability data to porcine renal DHP-I are shown in Table 1, together with those of imipenem and meropenem as reference compounds.

The 1β-methyl-2-[5-(hydroxy-substituted aminoalkyl)pyrrolidinylthio]carbapenems showed potent antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*, and higher stability to porcine renal DHP-I than meropenem did. In particular, their antipseudomonal activity was superior to those of imipenem and meropenem. The 3-amino-1-hydroxypropyl derivatives (**2a** and **2b**) were more active than the 2-amino-1-hydroxyethyl derivatives (**1a** and **1b**) and the 4-amino-1-hydroxybutyl derivatives (**3a** and **3b**) against the *P. aeruginosa*, although **3a** and **3b** were more active than **2a** and **2b** against the *S. aureus* including the methicillin-resistant strain BB5939 and pMS520/Smith. **2a** and **2b** were more active than **1a** and **1b** against the two MRSA strains. In view of the well balanced anibacterial activity

Scheme 4.



against the *S. aureus* and *P. aeruginosa* and synthetic feasibility, the derivatives carrying a 3-amino-1-hydroxypropyl side chain (**2a** and **2b**) were selected for further consideration.

The effect of the hydroxy group introduced at the 3-aminopropyl side chain was evident from the improved antipseudomonal activity, especially against the imipenem-resistant strain MB5178 compared to that of the 3-aminopropyl derivative (**10**) (Table 1). *N*-Monomethylation of the primary amino groups of **2** did not compromise the antipseudomonal activity, which was markedly reduced by *N,N*-dimethyl, and *N,N,N*-trimethylammonium substitution (**8** and **9**).

One of the concerns of this type of carbapenems was instability owing to intra- and intermolecular attacks of the side chain amino group on the β-lactam. A stability study revealed that *N*-monomethylation of **2a** considerably improved the solution stability at a high concentration (10% in water, pH 7.0, 20°C). The $T_{1/2}$ of **2a**, **2b**, **7a**, and **7b** were 5, 6, 8, and 12 hours, respectively. It is interesting to note that the stereochemistry of the hydroxy group affected both the solution stability and acute toxicity, although the antibacterial activity differed. The LD_{50}s, i.v. in mice, of **7a** and **7b** were 2.0 g/kg and 2.7 g/kg, respectively. In addition **7b** had no epileptogenic potential at 400 μg/head in rat head assay (intracerebroventricular injection), and was clean at 225 mg/kg, i.v., in rabbit nephrotoxicity study. The detailed *in vitro* and *in vivo* study[8,9] of **7b** demonstrated the superiority of its antibacterial activity against *S. aureus* including MRSA and *P. aeruginosa* to those of meropenem and imipenem.

Taking these results into consideration, coupled with good crystallinity of **7b** and some of its intermediates, **7b** (BO-2727) was selected as a development candidate from the series of carbapenems. An improved method for the preparation of BO-2727, including the coupling reaction of the unprotected thiol (**69**), derived from **22b** *via* the diol (**68**), with the enolphosphate (**59**) was developed for large scale preparation as shown in Scheme 5. The coupling product (**70**), obtained as a white solid, was deprotected by the catalytic hydrogenation to give the crude BO-2727, which was purified by the treatment with ion-exchange resin SA-10A (Cl^- type) and reversed phase column chromatography to give crystalline hydrochloride salt in 71% yield from **59**. The stereochemistry of BO-2727 was unambiguously determined by X-ray crystallographic analysis as shown in Fig. 2.

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

	Compounds									
Organism	**1a**	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	**5a**	**5b**
S.aureus 209P NIHJ JC1	0.012	0.025	0.012	0.012	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
S.aureus BB5939*	6.25	6.25	3.13	3.13	1.56	1.56	1.56	3.13	1.56	1.56
S.aureus pMS520/Smith	12.5	12.5	6.25	6.25	3.13	6.25	6.25	6.25	3.13	3.13
E.coli NIHJ JC2	0.05	0.025	0.05	0.05	0.05	0.05	0.10	0.05	0.05	0.05
P.aeruginosa MB5002	0.78	1.56	0.78	0.78	0.78	1.56	0.78	0.78	0.78	0.78
P.aeruginosa MB5178	3.13	3.13	3.13	3.13	3.13	6.25	6.25	6.25	6.25	6.25
P.aeruginosa IFO3445	0.39	0.39	0.2	0.2	0.78	0.39	0.39	0.39	0.39	0.39
DHP-I susceptibility**	<0.05	<0.05	0.18	0.05	0.11	<0.05	<0.05	0.07	0.07	0.07

	Compounds							
Organism	**6**	**7a**	**7b**	**8**	**9**	**10**	IPM	MEPM
S.aureus 209P NIHJ JC1	<0.006	0.012	0.012	0.012	0.025	0.012	0.006	0.05
S.aureus BB5939*	1.56	6.25	6.25	3.13	3.13	3.13	6.25	12.5
S.aureus pMS520/Smith	6.25	6.25	12.5	6.25	12.5	6.25	25	25
E.coli NIHJ JC2	0.05	0.05	0.05	0.1	0.1	0.10	0.1	0.012
P.aeruginosa MB5002	0.78	0.78	0.78	1.56	0.78	1.56	1.56	3.13
P.aeruginosa MB5178	3.13	3.13	3.13	6.25	12.5	3.13	12.5	6.25
P.aeruginosa IFO3445	0.39	0.2	0.39	0.39	0.39	0.39	3.13	3.13
DHP-I susceptibility**	0.07	0.07	0.11	0.10	0.08	<0.05	1.0	0.20

* β-lactamase producing strain.
** Relative to imipenem (=1), porcine renal dehydropeptidase-I.

Scheme 5.

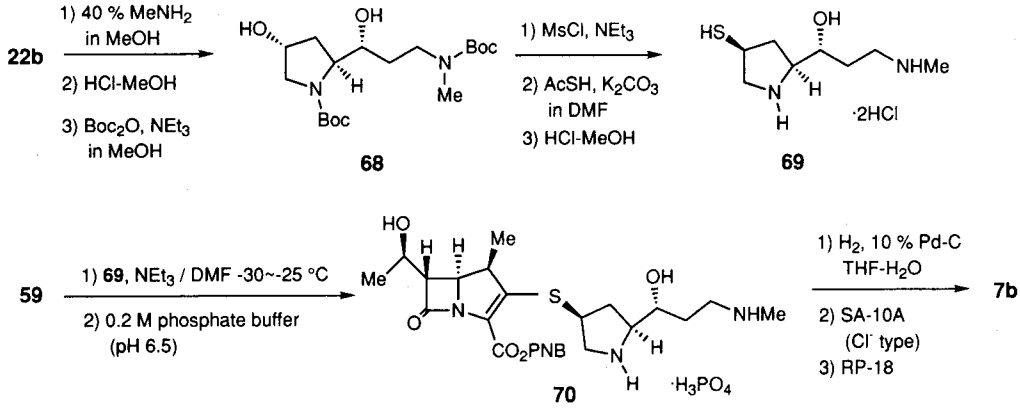
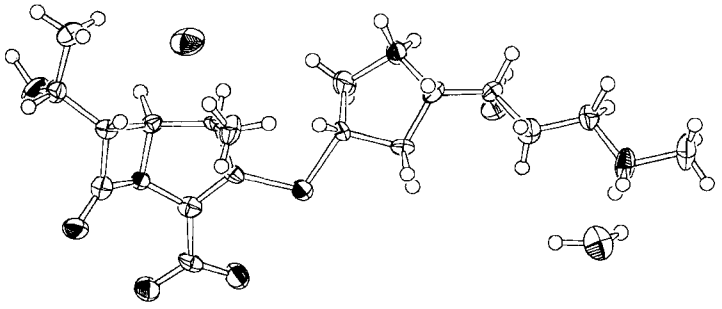


Fig. 2. X-ray structure of BO-2727·HCl·H_2O.



Experimental

Determination of MIC

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.

Determination of Susceptibility to Renal Dehydropeptidase-I (DHP-I)

Relative hydrolysis rate of carbapenems by porcine renal DHP-I was determined, taking the initial hydrolysis rate of imipenem as 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 spectrophotometric method. One unit of activity was defined as the amount of enzyme hydrolyzing 1 μM of glycyldehydrophenylalanine per minute when the substrate, 50 μM, was incubated at 35°C in 50 mM MOPS buffer, pH 7.0.

General Methods

All reactions involving air-sensitive reactions or compounds were carried out under nitrogen unless otherwise indicated. Melting points were taken on a Yanaco MP capillary melting point apparatus and were uncorrected. IR spectra were recorded on a Horiba FT-200 IR spectrometer. ^{1}H 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. UV spectra were taken in 0.1 M MOPS buffer (pH 7.0). Mass spectra were obtained on JEOL JMS-SX102A. TLC was done with Merck Kieselgel F_{254} precoated plates. 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*R*)-*N*-*t*-Butoxycarbonyl-4-*t*-butyldimethylsilyloxy-2-(2-nitrovinyl)pyrrolidine (**12**)

To a stirred solution of **11** (300 g, 0.91 mol) in nitromethane (1.5 liters) was dropwise added NEt_3 (76 ml) below 4°C. After being stirred for 12 hours at room temperature, the mixture was concentrated *in vacuo* to give the aldol. To a stirred solution of the aldol, prepared above, in CH_2Cl_2 (2 liters) was added thionyl chloride (87 ml, 1.2 mmol) dropwise over 30 minutes at −70°C. After being stirred for 30 minutes at the same temperature, NEt_3 (510 ml) was added to the mixture dropwise. The reaction was quenched by adding MeOH (500 ml), H_2O (1.5 liters), and a saturated aqueous $NaHCO_3$ solution. The mixture was stirred for 1 hour at 0°C, and then concentrated *in vacuo* to remove the organic solvent. The resulting mixture was 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 column chromatography afforded **12** (326 g, 96%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.03 (6H, s), 0.80 (9H, s), 1.35, 1.38 (total 9H, each s), 1.78 (1H, m), 2.09 (1H, m), 3.30~3.55 (2H, m), 4.28 (1H, m), 4.50 (1H, m), 6.96 (1H, d, J=13.2 Hz), 7.08 (1H, dd, J=6.6 and 13.2 Hz).

(2*S*,4*R*)-*N*-*t*-Butoxycarbonyl-4-*t*-butyldimethylsilyloxy-2-formylmethylpyrrolidine (**13**)

To a stirred solution of **12** (326 g, 0.876 mol) in a mixture of $CHCl_3$ (1.5 liters) and isopropanol (470 ml)

were added silica gel (470 g) and $NaBH_4$ (103 g) at 0°C. After being stirred for 1 hour at the same temperature, the reaction was quenched with H_2O (*ca.* 500 ml), and a saturated NH_4Cl aqueous solution (*ca.* 400 ml). The resulting mixture was filtered to remove the silica gel, and washed with CH_2Cl_2 (1 liter). The organic layer was concentrated *in vacuo* and the residue was diluted with EtOAc. The organic phase was washed with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. To a suspension of 60% NaH (67.5 g) in *t*-butanol (2.4 liters) was added the residue in *t*-butanol (850 ml) at 30°C. After being stirred for 30 minutes, the mixture was diluted with heptane (32 liters). To the resulting mixture were successively added crushed ice (20 kg), potassium permanganate (133 g) in H_2O (5 liters), and boric acid (103 g) in H_2O (5 liters). The reaction mixture was quenched by adding a sodium sulfite aqueous solution (2 liters), and 1 N sulfuric acid (2 liters). The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give an oily residue, which was purified by silica gel column chromatography affording **13** (190 g, 63%): ^1H NMR (200 MHz, $CDCl_3$) δ 0.05 (6H, s), 0.86 (9H, s), 1.44 (9H, s), 1.73 (1H, m), 2.15 (1H, m), 2.54 (1H, dd, J=7.2 and 16.4 Hz), 2.90 (1H, m), 3.28~3.55 (2H, m), 4.20~4.40 (2H, m), 9.76 (1H, s).

(2*S*,4*R*)-*N*-*t*-Butoxycarbonyl-4-*t*-butyldimethylsiloxy-2-(2-formylethyl)pyrrolidine (**15**)

A mixture of **14**[7] (200 mg, 0.5 mmol) and 10% Pd-C (20 mg) in EtOH (2 ml) was stirred for 8 hours under a hydrogen atmosphere. The mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. To a solution of the residue in CH_2Cl_2 (10 ml) was added a 1.0 M solution of DIBAL-H in toluene (0.50 ml) dropwise at −70°C. After being stirred for 1 hour at the same temperature, the reaction was quenched with MeOH (2.5 ml), and the resulting mixture was further stirred for 30 minutes at room temperature. The mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo* to give the residue, which was purified by silica gel column chromatography affording **15** (120 mg, 69%): IR (KBr) 1726, 1695, 1394, 1365, 1160, 1115 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.04 (6H, s), 0.85 (9H, s), 1.45 (9H, s), 1.58~2.10 (4H, m), 2.43 (2H, t, J=6.4 Hz), 3.27 (1H, dd, J=4.8 and 11.3 Hz), 3.40 (1H, m), 3.95 (1H, m), 4.32 (1H, m), 9.74 (1H, s).

(2*S*,4*R*)-*N*-*t*-Butoxycarbonyl-4-*t*-butyldimethylsilyloxy-2-[1-hydroxy-2-(*p*-nitrobenzyloxycarbonylamino)ethyl]pyrrolidine (**16a** and **16b**)

To a stirred solution of **11** (20 g, 61 mmol) in benzene (300 ml) was added trimethylsilyl cyanide (9.6 ml, 72 mmol), and the mixture was stirred for 10 hours at room temperature. The reaction was quenched by adding MeOH (100 ml), and the mixture was further stirred for 3 hours at room temperature and concentrated *in vacuo* to give the crude cyanohydrin. To a stirred suspension of LAH (4.60 g, 121 mmol) in diethyl ether (360 ml) was added the crude cyanohydrin in diethyl ether (120 ml) dropwise over 30 minutes at −5°C. After being stirred for 1 hour at 4°C, the reaction mixture was diluted with diethyl ether (500 ml) and quenched by carefully adding $Na_2SO_4 \cdot 10H_2O$ (55 g) below 0°C. After being stirred vigorously for 30 minutes at 0°C, anhydrous Na_2SO_4 (55 g) was added, and the mixture was further stirred for 10 hours at room temperature. The mixture was filtered, and the filtrate was concentrated *in vacuo* to *ca.* 300 ml. To this solution were added NEt_3 (12.7 ml, 91.0 mmol) and PNZCl (13.1 g, 60.7 mmol) in $CHCl_3$ (50 ml) at −20°C. After being stirred for 30 minutes at −10°C, the mixture was concentrated *in vacuo* and the residue was diluted with EtOAc. The organic phase was washed with 10% aqueous citric acid solution and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give **16a** [14.1 g, 43%, Rf=0.49 (TLC, 50% EtOAc in heptane)] and **16b** [10.8 g, 33%, Rf=0.38 (TLC, 50% EtOAc in heptane)].

16a: ^1H NMR (200 MHz, $CDCl_3$) δ 0.04 (6H, s), 0.85 (9H, s), 1.47 (9H, s), 1.65 (1H, m), 2.05 (1H, m), 3.14~3.32 (3H, m), 3.48~3.65 (2H, m), 4.03 (1H, m), 4.27 (1H, m), 5.18 (2H, s), 7.51 (2H, d, J=8.3 Hz), 8.22 (2H, d, J=8.3 Hz).

16b: ^1H NMR (200 MHz, $CDCl_3$) δ 0.05 (6H, s), 0.86 (9H, s), 1.44 (9H, s), 1.80~2.08 (2H, m), 3.16~3.72 (5H, m), 4.16 (1H, m), 4.30 (1H, m), 5.20 (2H, s), 7.50 (2H, d, J=8.3 Hz), 8.22 (2H, d, J=8.3 Hz).

(2*S*,4*R*)-4-Hydroxy-2-[1-hydroxy-2-(*p*-nitrobenzyloxycarbonylamino)ethyl]-*N*-(*p*-nitrobenzyloxycarbonyl)-pyrrolidine (**17a** and **17b**)

To a solution of **16a** (7.1 g, 13 mmol) in MeOH (13 ml) was added a 5.5 N solution of hydrogen chloride in MeOH (13 ml). After being stirred for 10 hours at room temperature, the mixture was concentrated *in vacuo*. To the residue in MeOH (13 ml) were added NEt_3 (7.3 ml, 52.5 mmol) and PNZCl (2.83 g, 13.1 mmol) in CH_2Cl_2

(5 ml) dropwise at −10°C. After being stirred for 30 minutes at the same temperature, the mixture was concentrated *in vacuo*. The residue was partitioned between EtOAc and H_2O. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined extract was washed with 10% aqueous citric acid solution and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give **17a** (5.2 g, 78%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.78 (1H, m), 2.07 (1H, m), 2.85~3.05 (2H, m), 3.30 (1H, m), 3.46 (1H, m), 3.90~4.20 (2H, m), 4.90 (1H, m), 5.10~5.30 (4H, m), 7.50~7.70 (4H, m), 8.08~8.30 (4H, m).

17b was prepared in 80% yield from **16b** by the same method described above.

17b: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.80~2.04 (2H, m), 2.80~3.20 (2H, m), 3.35 (1H, m), 3.48 (1H, m), 4.08 (1H, m), 4.28 (1H, m), 4.88 (1H, m), 5.13~5.30 (4H, m), 7.53~7.68 (4H, m), 8.08~8.24 (4H, m).

(2*S*,4*S*)-4-Acetylthio-2-[1-hydroxy-2-(*p*-nitrobenzyloxycarbonylamino)ethyl]-*N*-(*p*-nitrobenzyloxycarbonyl)pyrrolidine (**18a** and **18b**)

To a solution of **17a** (3.9 g, 7.7 mmol) in CH_2Cl_2 (40 ml) were added NEt_3 (1.4 ml, 10 mmol) and MsCl (0.66 ml, 8.5 mmol) dropwise at 0°C. After being stirred for 30 minutes at 0°C, the mixture was diluted with CH_2Cl_2, and washed with 10% aqueous citric acid solution and brine. The organic layer was dried over $MgSO_4$, and concentrated *in vacuo*. To the residue in DMF (40 ml) was added potassium thioacetate (1.8 g, 15 mmol), and the mixture was stirred for 3 hours at 70°C. The reaction mixture was cooled to room temperature, poured into H_2O, and extracted with EtOAc. The combined organic extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography afforded **18a** (2.4 g, 55%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.73 (1H, m), 2.33 (3H, s), 2.55 (1H, m), 3.18 (1H, m), 3.29 (1H, m), 3.62 (1H, m), 3.68~3.85 (2H, m), 4.00 (1H, m), 4.22 (1H, m), 5.10~5.37 (4H, m), 7.48~7.60 (4H, m), 8.17~8.30 (4H, m).

18b was prepared in 47% yield from **17b** by the same method described above.

18b: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.03 (1H, m), 2.34 (3H, s), 2.40 (1H, m), 2.95 (1H, m), 3.15 (1H, m), 3.59 (1H, m), 3.70~4.30 (4H, m), 4.00 (1H, m), 5.15~5.30 (4H, m), 5.75 (1H, m, NH), 7.45~7.60 (4H, m), 8.15~8.30 (4H, m).

(2*S*,4*R*)-*N*-*t*-Butoxycarbonyl-4-*t*-butyldimethylsilyloxy-2-(2-ethoxycarbonyl-1-hydroxyethyl)pyrrolidine (**20a** and **20b**)

To a stirred solution of hexamethyldisilazane (1.44 ml, 6.8 mmol) in THF (20 ml) was added a 1.6 M solution of *n*-BuLi in hexane (4.3 ml, 6.8 mmol) dropwise at 0°C, and the mixture was stirred for 15 minutes. To the mixture was added EtOAc (0.67 ml, 6.8 mmol) dropwise at −70°C, and further stirred for 15 minutes. To the resulting mixture was added **11** (1.5 g, 4.6 mmol) in THF (5 ml) at the same temperature. After being stirred for 30 minutes at that temperature, the reaction was quenched by adding a 0.2 M sodium phosphate buffer (50 ml, pH 6.5). The mixture was extracted with EtOAc, and the combined organic extract was washed with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography afforded **20a** [400 mg, 21%, Rf=0.27 (TLC, 25% EtOAc in heptane)], and **20b** [1.17 g, 62%, Rf=0.20 (TLC, 25% EtOAc in heptane)].

20a: IR (KBr) 1738, 1697, 1398, 1255, 1165 cm^{-1}, ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.06 (6H, s), 0.87 (9H, s), 1.27 (3H, t, J=7.1 Hz), 1.46 (9H, s), 1.69 (1H, m), 1.96 (1H, m), 2.35~2.52 (2H, m), 3.27 (1H, dd, J=3.8 and 11.4 Hz), 3.52 (1H, m), 3.90~4.15 (2H, m), 4.17 (2H, q, J=7.1 Hz), 4.33 (1H, m), 5.09 (1H, br s). HRFAB-MS *m/z* Calcd for $C_{20}H_{40}NO_6Si$ $(M+H)^+$ 418.2625, Found 418.2624;

Anal Calcd for $C_{20}H_{39}NO_6Si$: C 57.52, H 9.41, N 3.35.
Found: C 57.70, H 9.77, N 3.24.

20b: IR (KBr) 1740, 1697, 1406, 1255 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.06 (6H, s), 0.86 (9H, s), 1.27 (3H, t, J=7.3 Hz), 1.47 (9H, s), 1.80~1.98 (2H, m), 2.30~2.43 (2H, m), 3.30 (1H, dd, J=4.3 and 11.5 Hz), 3.53 (1H, m), 3.82~4.48 (3H, m), 4.19 (2H, q, J=7.1 Hz), 4.33 (1H, m); HRFAB-MS *m/z* Calcd for $C_{20}H_{40}NO_6Si$ $(M+H)^+$ 418.2625, Found 418.2633;

Anal Calcd for $C_{20}H_{39}NO_6Si$: C 57.52, H 9.41, N 3.35.
Found: C 57.76, H 9.70, N 3.30.

(2*S*,4*R*)-*N*-*t*-Butoxycarbonyl-4-*t*-butyldimethylsilyloxy-2-[1-hydroxy-3-(*p*-toluenesulfonyloxy)propyl]-pyrrolidine (**22a** and **22b**)

1) To a mixture of **20a** (20.9 g, 50 mmol) and $NaBH_4$ (2.84 g, 75 mmol) in THF (100 ml) was slowly added MeOH (25 ml) over 30 minutes at 65°C. The reaction was quenched by adding 10% citric acid aqueous solution (150 ml) and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give the crude diol **21a** (18.8 g,

100%), which was used in the next reaction without further purification.

2) To a mixture of **21a** (18.8 g, 50 mmol), dimethylaminopyridine (610 mg, 5 mmol) and NEt_3 (21 ml, 150 mmol) in CH_2Cl_2 (175 ml) was added *p*-toluenesulfonylchloride (11.4 g, 60 mmol) in portions at room temperature. After being stirred for 1 hour, the mixture was poured into a saturated $NaHCO_3$ aqueous solution, and extracted with CH_2Cl_2. The combined extract was washed successively with 10% aqueous citric acid solution, H_2O and brine, dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography afforded **22a** (21.4 g, 81%): IR (KBr) 1690, 1660, 1600, 1460, 1400, 1360 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.05 (6H, s), 0.85 (9H, s), 1.44 (9H, s), 1.50~1.70 (2H, m), 1.75~2.00 (2H, m), 2.43 (3H, s), 3.20 (1H, dd, J=3.5 and 11.7 Hz), 3.40~3.70 (2H, m), 3.92 (1H, dd, J=8.1 and 16.0 Hz), 4.10~4.40 (3H, m), 7.33 (2H, d, J=8.3 Hz), 7.78 (2H, d, J=8.3 Hz).

22b was prepared in 74% yield from **20b** by the same method described above.

22b: IR (KBr) 1690, 1670, 1600, 1460, 1400, 1360 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.04 (6H, s), 0.84 (9H, s), 1.43 (9H, s), 1.40~2.00 (4H, m), 2.43 (3H, s), 3.20 (1H, dd, J=3.6 and 11.5 Hz), 3.48 (1H, m), 3.74 (1H, m), 4.18~4.23 (4H, m), 7.32 (2H, d, J=7.3 Hz), 7.77 (2H, d, J=7.3 Hz).

(2*S*,4*R*)-4-Hydroxy-2-[1-hydroxy-3-(*p*-nitrobenzyloxycarbonylamino)propyl]-*N*-(*p*-nitrobenzyloxycarbonyl)pyrrolidine (**23a** and **23b**)

To a solution of **22a** (12.4 g, 23.4 mmol) in DMSO (24 ml) was added NaN_3 (4.6 g, 70.0 mmol), and the mixture was stirred for 1 hour at 70°C. The reaction mixture was poured into H_2O (200 ml), and extracted with EtOAc. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. A mixture of the residue and 10% Pd-C (0.90 g) in MeOH (50 ml) was stirred for 8 hours under a hydrogen atmosphere. The mixture was filtered through a pad of celite and the filtrate was concentrated *in vacuo* to give the crude amine, from which **23a** was prepared in 42% overall yield as described for the preparation of **17a**.

23a: IR (KBr) 1700, 1600, 1520, 1350 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.40~2.15 (4H, m), 3.15~3.41 (3H, m), 3.41~3.85 (2H, m), 4.12 (1H, m), 4.25 (1H, m), 5.08~5.29 (4H, m), 7.29 (2H, d, J=7.0 Hz), 7.30 (2H, d, J=7.0 Hz), 8.22 (2H, d, J=7.0 Hz), 8.23 (2H, d, J=7.0 Hz).

23b was prepared in 56% yield from **22b** by the above procedure.

23b: IR (KBr) 1700, 1620, 1520, 1340 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.29~2.05 (4H, m), 3.31 (1H, m), 3.37~3.62 (2H, m), 3.70 (1H, m), 4.00~4.22 (2H, m), 4.49 (1H, m), 5.10~5.35 (4H, m), 7.48 (2H, d, J=7.0 Hz), 7.53 (2H, d, J=7.0 Hz), 8.22 (2H, d, J=7.0 Hz), 8.23 (2H, d, J=7.0 Hz).

(2*S*,4*S*)-4-Acetylthio-2-[1-hydroxy-3-(*p*-nitrobenzyloxycarbonylamino)propyl]-*N*-(*p*-nitrobenzyloxycarbonyl)pyrrolidine (**24a** and **24b**)

24a and **24b** were prepared from **23a** and **23b**, respectively, as described for the preparation of **18a**.

24a (52%): IR (KBr) 1700, 1600, 1520, 1340 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.32~2.10 (4H, m), 2.34 (3H, s), 3.06~3.58 (3H, m), 3.66~4.28 (4H, m), 5.06~5.31 (4H, m), 7.49 (2H, d, J=6.0 Hz), 7.53 (2H, d, J= 6.0 Hz), 8.19 (2H, d, J=6.0 Hz), 8.20 (2H, d, J=6.0 Hz).

24b (57%): IR (KBr) 1700, 1610, 1520, 1340 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.42~1.86 (4H, m), 2.36 (3H, s), 3.16 (1H, m), 3.32 (1H, m), 3.48 (1H, m), 3.68~3.88 (2H, m), 4.00 (1H, m), 4.22 (1H, m), 5.10~5.30 (4H, m), 7.53 (2H, d, J=6.0 Hz), 7.54 (2H, d, J=6.0 Hz), 8.23 (2H, d, J=6.0 Hz), 8.24 (2H, d, J=6.0 Hz).

(2*S*,4*R*)-4-Hydroxy-2-{1-hydroxy-3-[*N*-(*p*-nitrobenzyloxycarbonyl)-*N*-methylamino]propyl}-*N*-(*p*-nitrobenzyloxycarbonyl)pyrrolidine (**26a** and **26b**)

To a stirred solution of **22a** (2.56 g, 4.8 mmol) in MeOH (10 ml) was added a 40% solution of $MeNH_2$ in MeOH (41 ml). After being stirred for 8 hours, the mixture was concentrated *in vacuo*. To the residue in MeOH (40 ml) was added NEt_3 (2.7 ml, 19.4 mmol) and PNZCl (1.03 g, 4.8 mmol) at −10°C, and the mixture was stirred for 1 hour at that temperature. The mixture was concentrated *in vacuo* to give the residue, which was poured into H_2O, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to give a crude oil including (2*S*,4*R*)-*N*-*t*-butoxycarbonyl-4-*t*-butyldimethylsilyloxy-2-{1-hydroxy-3-[*N*-(*p*-nitrobenzyloxycarbonyl)-*N*-methylamino]propyl}pyrrolidine. Conversion of this compound to **26a** was carried out according to the method for the preparation of **17a**.

26a (75%): IR (KBr) 1680, 1600, 1520, 1400, 1340 cm^{-1}; ^1H NMR (200MHz, $CDCl_3$) δ 1.50~2.20 (4H, m), 2.95 (3H, s), 3.30~3.90 (5H, m), 4.00~4.30 (2H, m), 5.17~5.32 (4H, m), 7.50~7.65 (4H, m), 8.20~8.25 (4H, m).

26b was prepared in 78% yield from **22b** as described

for the preparation of **26a**.

26b: IR (KBr) 1670, 1610, 1520, 1440, 1400, 1340 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.40 (1H, m), 1.55 (1H, m), 1.72 (1H, m), 2.05 (1H, m), 3.32 (3H, s), 3.70~4.05 (2H, m), 4.15 (1H, m), 5.10~5.35 (4H, m), 7.50~7.70 (4H, m), 8.10~8.30 (4H, m).

(2*S*,4*S*)-4-Acetylthio-2-{1-hydroxy-3-[*N*-(*p*-nitrobenzyloxycarbonyl)-*N*-methylamino]propyl}-*N*-(*p*-nitrobenzyloxycarbonyl)pyrrolidine (**27a** and **27b**)

27a and **27b** were prepared from **26a** and **26b**, respectively, as described for the preparation of **18a**.

27a (83%): IR (KBr) 1700, 1610, 1520, 1430, 1400, 1340 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.40~1.80 (3H, m), 2.34 (3H, s), 2.50 (1H, m), 2.96 (3H, s), 3.10~4.30 (7H, m), 5.10~5.30 (4H, m), 7.43~7.60 (4H, m), 8.18~8.30 (4H, m).

27b (82%): IR (KBr) 1700, 1610, 1520, 1430, 1400, 1350 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.30~2.10 (4H, m), 2.34 (3H, s), 2.96 (3H, s), 3.00~3.60 (3H, m), 3.60~4.30 (4H, m), 5.10~5.36 (4H, m), 7.40~7.60 (4H, m), 8.18~8.30 (4H, m).

(2*S*,4*R*)-4-Hydroxy-2-{(*R*)-1-hydroxy-3-[*N*-allyloxycarbonyl-*N*-methylamino]propyl}-*N*-(*p*-nitrobenzyloxycarbonyl)pyrrolidine (**30**)

30 was prepared from **22b** as described for the preparation of **26a**; **26b**.

30 (73%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.30~1.70 (3H, m), 2.03 (1H, m), 2.75 (1H, m), 2.88 (3H, s), 3.10~3.40 (3H, m), 3.55~3.85 (3H, m), 4.56 (2H, m), 5.02~5.34 (4H, m), 5.92 (1H, m), 7.15~7.50 (17H, m), 8.20~8.30 (2H, m).

(2*S*,4*S*)-4-Acetylthio-2-[1-hydroxy-3-(*N*-allyloxycarbonyl-*N*-methylamino)propyl]-*N*-(*p*-nitrobenzyloxycarbonyl)pyrrolidine (**31**)

31 was prepared in 74% yield from **30** by a similar method for the preparation of **18b**.

31: IR (KBr) 1690, 1520, 1420, 1215 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.30~1.75 (3H, m), 2.04 (1H, m), 2.34 (3H, s), 2.91 (3H, s), 3.14 (1H, m), 3.70~3.90 (2H, m), 3.90~4.04 (2H, m), 4.18 (1H, m), 4.56 (1H, m), 4.58 (2H, d, J=5.1 Hz), 5.20 (1H, m), 5.22 (2H, s), 5.29 (1H, dd, J=1.4 and 17.4 Hz), 5.92 (1H, m), 7.52 (2H, d, J=8.7 Hz), 8.23 (2H, d, J=8.7 Hz).

(2*S*,4*S*)-2-[1-Hydroxy-3-(*N*-allyloxycarbonyl-*N*-methylamino)propyl]-*N*-(*p*-nitrobenzyloxycarbonyl)-4-trityl thiopyrrolidine (**32**)

To a solution of **31** (9.80 g, 19.6 mmol) in MeOH (98 ml) was added a 1 N aqueous NaOH solution (19.6 ml) at room temperature, and the mixture was stirred for 30 minutes. To this solution was added 1 N hydrochloric acid (22 ml), and the mixture was concentrated *in vacuo* to give the residue, which was diluted with EtOAc. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. To the residue in DMF (65 ml) was added chlorotriphenylmethane (645 mg, 2.3 mmol), and the mixture was stirred for 10 hours at 50°C. The mixture was poured into H_2O (100 ml) and extracted with EtOAc. The organic layer was washed with a saturated aqueous $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give **32** (10.4 g, 73%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.30~1.70 (3H, m), 2.03 (1H, m), 2.75 (1H, m), 2.88 (3H, s), 3.10~3.40 (3H, m), 3.55~3.85 (3H, m), 4.56 (2H, m), 5.02~5.34 (4H, m), 5.92 (1H, m), 7.15~7.50 (17H, m), 8.20~8.30 (2H, m).

(2*S*,4*S*)-2-[3-(*N*,*N*-Dimethylamino)-1-hydroxypropyl]-*N*-(*p*-nitrobenzyloxycarbonyl)-4-tritylthiopyrrolidine (**33**)

To a solution of **32** (10.4 g, 14.9 mmol) in CH_2Cl_2 (50 ml) were added H_2O (0.7 ml, 37 mmol), bis(triphenylphosphine)palladium(II) chloride (209 mg, 0.3 mmol) and tributyltin hydride (12 ml, 45 mmol) under cooling with ice. After being stirred for 30 minutes at room temperature. The mixture was poured into a saturated $NaHCO_3$ aqueous solution and extracted with CH_2Cl_2. The combined extracts were dried over $MgSO_4$, and concentrated *in vacuo*. To the residue in THF (70 ml) were added 37% aqueous formalin (1.13 ml, 14.9 mmol), acetic acid (0.85 ml, 14.9 mmol) and $NaBH(OAc)_3$ (61.3 g, 22.4 mmol) at 0~4°C, and the mixture was stirred for 6 hours at room temperature. The mixture was concentrated *in vacuo*, diluted with CH_2Cl_2 and washed with a saturated $NaHCO_3$ aqueous solution and brine. Evaporation of the solvent *in vacuo* gave the residue, which was purified by silica gel column chromatography to afford **32** (6.98 g, 72%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.30~1.70 (3H, m), 2.05 (1H, m), 2.60~2.85 (4H, m), 2.88 (3H, s), 2.96 (3H, s), 3.55~3.65 (2H, m), 4.18 (1H, m), 5.00~5.18 (2H, m), 7.12~7.60 (17H, m), 8.23 (2H, d, J=8.4 Hz).

(2*S*,4*S*)-2-[3-(*N*,*N*-Dimethylamino)-1-hydroxypropyl]-4-mercapto-*N*-(*p*-nitrobenzyloxycarbonyl)pyrrolidine (**34**)

To a solution of **33** (3.65 g, 5.61 mmol) in CH_2Cl_2 (4 ml) were added TFA (4 ml) and Et_3SiH (0.94 ml, 5.89 mmol) at 0~4°C. After being stirred for 30 minutes at room temperature, the reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography to afford **33** (1.90 g, 83%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40~1.80 (3H, m), 1.92 (1H, m), 2.45 (1H, m), 2.82 (3H, s), 2.87 (3H, s), 3.10~3.28 (4H, m), 3.96 (1H, m), 4.00~4.20 (2H, m), 5.21 (2H, s), 7.53 (2H, d, J=8.9 Hz), 8.23 (2H, d, J=8.9 Hz).

(2*S*,4*R*)-*N*-*t*-Butoxycarbonyl-4-*t*-butyldimethylsilyloxy-2-(3-cyano-1-hydroxypropyl)pyrrolidine (**35a** and **35b**)

To a solution of **22a** (541 mg, 1.02 mmol) in DMSO (3 ml) was added NaCN (98 mg, 2.0 mmol), and the mixture was stirred for 1 hour at 70°C. The reaction mixture was poured into H_2O (30 ml), and extracted with EtOAc. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography afforded **35a** (234 mg, 60%): IR (KBr) 2247, 1693, 1649, 1467, 1416, 1365 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.03 (6H, s), 0.83 (9H, s), 1.43 (9H, s), 1.35~1.85 (3H, m), 1.92 (1H, m), 2.54 (2H, t, J=8.4 Hz), 3.20 (1H, dd, J=4.7 and 12.6 Hz), 3.44~3.68 (2H, m), 3.93 (1H, q, J=9.5 Hz), 4.24 (1H, br s), 5.76 (1H, br s, –OH).

35b was prepared in 52% yield from **22b** by the same procedure described above.

35b: IR (KBr) 2249, 1691, 1674, 1471, 1410, 1365 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.04 (6H, s), 0.86 (9H, s), 1.46 (9H, s), 1.15~1.80 (3H, m), 1.96 (1H, m), 2.38~2.73 (2H, m), 3.23 (1H, dd, J=3.4 and 11.7 Hz), 3.54 (1H, m), 3.71 (1H, m), 4.13~4.35 (2H, m), 4.74 (1H, m, –OH).

(2*S*,4*R*)-4-Hydroxy-2-[1-hydroxy-4-(*p*-nitrobenzyloxycarbonylamino)butyl]-*N*-(*p*-nitrobenzyloxycarbonyl)-pyrrolidine (**36a** and **36b**)

36a and **36b** were prepared from **35a** and **35b**, respectively, as described for the preparation of **17a**.

36a (65%): IR (KBr) 1705, 1695, 1522, 1348 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.23~1.85 (5H, m), 2.06 (1H, m), 3.24 (2H, q, J=6.4 Hz), 3.44 (1H, m), 3.59 (1H, m), 3.79 (1H, m), 4.10 (1H, m), 4.44 (1H, m), 5.18 (2H, s), 5.25 (2H, s), 7.51 (4H, d, J=8.6 Hz), 8.22 (4H, dd, J=2.2 and 8.6 Hz).

36b (59%): IR (KBr) 1691, 1670, 1520, 1348 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.10~1.73 (5H, m), 2.01 (1H, m), 2.96 (2H, m), 3.14~3.50 (2H, m), 3.70~4.00 (2H, m), 4.24 (1H, m), 5.10~5.24 (4H, m), 7.53~7.68 (4H, m), 8.16~8.28 (4H, m).

(2*S*,4*S*)-4-Acetylthio-2-[1-hydroxy-4-(*p*-nitrobenzyloxycarbonylamino)butyl]-*N*-(*p*-nitrobenzyloxycarbonyl)pyrrolidine (**37a** and **37b**)

37a and **37b** were prepared, respectively, from **36a** and **36b** as described for the preparation of **18a**.

37a (65%): IR (KBr) 1699, 1522, 1348 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30~1.80 (5H, m), 2.35 (3H, s), 2.43 (1H, m), 3.10~3.30 (3H, m), 3.64 (1H, m), 3.78 (1H, m), 3.94 (1H, m), 4.19 (1H, m), 5.19 (2H, s), 5.24 (2H, s), 7.51 (4H, dd, J=2.1 and 9.0 Hz), 8.15~8.26 (4H, m).

37b (36%): IR (KBr) 1697, 1520, 1348 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.20~1.80 (6H, m), 2.34 (3H, s), 3.00~3.30 (3H, m), 3.78 (1H, m), 3.90~4.10 (2H, m), 4.19 (1H, m), 5.19 (2H, s), 5.22 (2H, s), 7.51 (4H, dd, J=2.0 and 8.8 Hz), 8.16~8.27 (4H, m).

(2*S*,4*R*)-*N*-*t*-Butoxycarbonyl-4-*t*-butyldimethylsilyloxy-2-(2-hydroxy-3-nitropropyl)pyrrolidine (**39a** and **39b**)

To a stirred solution of **13** (34.4 g, 0.10 mol) in nitromethane (170 ml) was added NEt_3 (8.6 ml, 0.062 mol) dropwise at 0~4°C, and the mixture was stirred for 12 hours at room temperature. Evaporation of the mixture gave the residue, which was purified by silica gel column chromatography to afford **39a** [17.7 g, 44%, Rf=0.49 (TLC, 50% EtOAc in heptane)] and **39b** [20.9 g, 52%, Rf=0.38 (TLC, 50% EtOAc in heptane)].

39a: IR (KBr) 1670, 1558, 1404, 1367 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.06 (6H, s), 0.87 (9H, s), 1.47 (9H, s), 1.30~1.64 (3H, m), 1.74 (1H, m), 2.10 (1H, m), 3.28~3.48 (2H, m), 4.20~4.52 (4H, m).

39b: IR (KBr) 1668, 1558, 1410, 1367 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.06 (6H, s), 0.88 (9H, s), 1.46 (9H, s), 1.40~1.92 (4H, m), 2.12 (1H, m), 3.32 (1H, dd, J=4.5 and 11.4 Hz), 3.48 (1H, m), 4.11 (1H, m), 4.32 (1H, m), 4.43 (2H, s).

(2*S*,4*R*)-*N*-*t*-Butoxycarbonyl-2-(3-*t*-butoxycarbonylamino-2-hydroxypropyl)-4-*t*-butyldimethylsilyloxy pyrrolidine (**40a** and **40b**)

A mixture of **39a** (4.04 g, 9.99 mmol) and Raney Ni (W-II, 5.0 g) in ethanol (50 ml) was stirred under a hydrogen pressure (3 kg/cm^2) for 8 hours. The mixture

was filtered through a pad of celite and the filtrate was concentrated *in vacuo*. To the residue in CH_2Cl_2 (30 ml) was added di-*t*-butyl dicarbonate (1.86 g, 10.0 mmol) and the mixture was stirred for 2 hours. The mixture was concetrated *in vacuo* and the residue was purifed by silica gel column chromatography to give **40a** (2.67 g, 56%). IR (KBr) 1715, 1700, 1675, 1400, 1365 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 0.06 (6H, s), 0.87 (9H, s), 1.43 (9H, s), 1.45 (9H, s), 1.20~1.56 (2H, m), 1.72 (1H, m), 2.05 (1H, m), 2.93 (1H, m), 3.30~3.42 (3H, m), 3.62 (1H, m), 4.24 (1H, m), 4.38 (1H, m), 5.09 (1H, br s, –OH).

40b was prepared in 55% yield from **39b** by the same procedure described above.

40b: IR (KBr) 1693, 1679, 1408, 1365 cm^{-1}. ^1H NMR (200MHz, $CDCl_3$) δ 0.06 (6H, s), 0.86 (9H, s), 1.44 (9H, s), 1.46 (9H, s), 1.25~1.60 (2H, m), 1.75 (1H, m), 2.10 (1H, m), 2.98 (1H, m), 3.20~3.45 (3H, m), 3.76 (1H, m), 4.06 (1H, t, J=6.8 Hz), 4.32 (1H, t, J=4.6 Hz), 5.05 (1H, br s, –OH).

The following thiols (**44a**, **44b**, **52a**, **52b**, and **58**) were prepared, and their spectral data were shown below.

44a: IR (KBr) 1732, 1700, 1520, 1348, 1238 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.70~1.90 (2H, m), 2.03 (3H, s), 2.26 (1H, m), 2.34 (3H, s), 2.58 (1H, m), 3.20 (1H, m), 3.33 (1H, m), 3.56 (1H, m), 3.80~4.00 (2H, m), 4.08 (1H, dd, J=7.4 and 11.1 Hz), 4.94 (1H, m), 5.20 (4H, s), 7.51 (4H, d, J=8.3 Hz), 8.18~8.30 (4H, m).

44b: IR (KBr) 1730, 1701, 1524, 1348, 1236 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.60~1.80 (2H, m), 2.05 (3H, s), 2.35 (3H, s), 2.40 1H, m), 2.59 (1H, m), 3.20 (1H, dd, J=7.6 and 11.1 Hz), 3.30~3.50 (2H, m), 3.80~4.03 (2H, m), 4.12 (1H, dd, J=8.1 and 11.1 Hz), 5.01 (1H, m), 5.16~5.32 (4H, m), 7.45~7.60 (4H, m), 8.18~8.30 (4H, m), 8.22 (4H, d, J=9.6 Hz).

52a: IR (KBr) 1697, 1522, 1348 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.50~1.80 (5H, m), 2.66 (1H, m), 2.95~3.35 (4H, m), 3.49 (1H, m), 3.70 (1H, m), 4.07~4.30 (2H, m), 5.21 (4H, d, J=10.8 Hz), 7.51 (4H, d, J=8.4 Hz), 8.18~8.28 (4H, m).

52b: IR (KBr) 1699, 1522, 1348 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.50~1.80 (4H, m), 2.10 (1H, m), 2.63 (1H, m), 3.10~3.35 (3H, m), 3.40~3.80 (2H, m), 4.00~4.20 (2H, m), 5.20 (4H, br s), 5.50 (1H, m, –NH), 7.51 (4H, br d, J=8.4 Hz), 8.22 (4H, br d, J=8.4 Hz).

58: IR (KBr) 1699, 1522, 1346 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.35~1.80 (4H, m), 2.10 (1H, m), 2.55 (1H, m), 3.00~3.50 (4H, m), 3.60~4.20 (3H, m), 5.20 (4H, s), 7.51 (4H, d, J=8.9 Hz), 8.16~8.28 (4H, m).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(3*S*,5*S*)-5-(2-amino-1-hydroxyethyl)pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic acid·hydrochloride (**1a**)

1) To a solution of **18a** (2.5 g, 4.5 mmol) in MeOH (35 ml) was added a 1 N aqueous NaOH solution (4.68 ml) under cooling with ice. After being stirred for 10 minutes, the reaction mixture was quenched by adding 1 N hydrochloric acid (4.8 ml), and concentrated *in vacuo*. The residue was partitioned between EtOAc and H_2O, and extracted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to give the crude thiol.

2) To a stirred solution of the crude thiol (2.3 g, 4.5 mmol) in CH_3CN (5 ml) were added **59** (2.7 g, 4.5 mmol) in CH_3CN (60 ml) and *N*,*N*-diisopropylethylamine (0.93 ml, 5.4 mmol) at 0~5°C. After being stirred for 4 hours, the reaction mixture was concentrated *in vacuo* to *ca.* 5 ml, which was poured into H_2O, and extracted with EtOAc (100 ml). The 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 **60a** (1.6 g, 42%): ^1H NMR (200 MHz, $CDCl_3$) δ 1.27 (3H, d, J=6.9 Hz), 1.38 (3H, d, J=6.3 Hz), 1.90 (1H, m), 2.60 (1H, m), 3.20~3.60 (7H, m), 3.90~4.30 (4H, m), 5.10~5.35 (5H, m), 5.51 (1H, d, J=13.5 Hz), 7.50~7.60 (4H, m), 7.65 (2H, d, J=8.9 Hz), 8.15~8.30 (6H, m).

3) A mixture of **60a** (1.62 g, 1.87 mmol) and 10% Pd-C (1.13 g) in THF (19 ml), EtOH (6 ml) and 0.5 M MOPS buffer (pH 7.0, 19 ml)) was stirred under a hydrogen pressure (3.5 kg/cm^2) for 2.5 hours at room temperature. The catalyst was filtered off and washed successively with THF, EtOH, and H_2O. The combined filtrate and washing were concentrated *in vacuo* to *ca.* 5 ml, and the insoluble was removed by filtration. The filtrate was subjected to reversed phase column chromatography, which was eluted first with H_2O and with 20% MeOH-H_2O. The fractions containing the desired compound were combined, and the pH of the solution was adjusted to 5.9 with 0.1 N hydrochloric acid, concentrated *in vacuo* and lyophilized to give **1a** (208 mg, 27%).

1a: IR (KBr) 1745, 1600, 1390 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 1.29 (3H, d, J=7.3 Hz), 1.36 (3H, d, J=6.6 Hz), 1.74 (1H, m), 2.79 (1H, m), 2.98 (1H, m), 3.15~3.47 (4H, m), 3.59~3.80 (2H, m), 3.59~3.80 (2H, m), 3.95~4.27 (4H, m); HRFAB-MS Calcd for $C_{16}H_{26}N_3O_5S$ $(M+H)^+$ 372.1593; Found 372.1608; UV λ_{max} 298 (ε=8,810) nm.

The following compounds (**1b**, **2a**, **2b**, **3a**, **3b**, **4a**, **4b**,

5a, **5b**, **6**, **7a**, **7b**, **8**) were prepared from the thiols (**19b**, **25a**, **25b**, **28a**, **28b**, **34a**, **34b**, **38**, **44a**, **44b**, **52a**, **52b**, **58**) and the enolphosphate **59**, respectively, as described for the preparation of **1a**.

1b: IR (KBr) 1745, 1600, 1390 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 1.19 (3H, d, J=7.6 Hz), 1.25 (3H, d, J=6.6 Hz), 1.86 (1H, m), 2.58 (1H, m), 2.92 (1H, m), 3.15~3.46 (4H, m), 3.52~3.80 (2H, m), 3.95 (1H, m), 4.11~4.27 (3H, m); HRFAB-MS m/z Calcd for $C_{16}H_{26}N_3O_5S$ $(M+H)^+$ 372.1593; Found 372.1595; UV λ_{max} 300 (ε=9,300) nm.

2a; IR (KBr) 1760, 1730, 1600, 1400 cm^{-1}. ^1H NMR (200 MHz, D_2O) δ 1.18 (3H, d, J=7.7 Hz), 1.25 (3H, d, J=7.5 Hz), 1.60~2.04 (3H, m), 2.67 (1H, m), 3.08~3.20 (2H, m), 3.23~3.45 (3H, m), 3.58~3.76 (2H, m), 3.88~4.10 (2H, m), 4.12~4.28 (2H, m); HRFAB-MS m/z Calcd for $C_{17}H_{28}N_3O_5S$ $(M+H)^+$ 386.1750; Found 386.1770; UV λ_{max} 298 (ε=9,750) nm.

2b: IR (KBr) 1760, 1730, 1600, 1400 cm^{-1}. ^1H NMR (200 MHz, D_2O) δ 1.18 (3H, d, J=7.7 Hz), 1.25 (3H, d, J=7.5 Hz), 1.63~2.00 (3H, m), 2.61 (1H, m), 3.03~3.20 (2H, m), 3.23~3.38 (2H, m), 3.43 (1H, dd, J=3.1 and 6.7 Hz), 3.66 (1H, m), 3.78 (1H, m), 3.90~4.24 (4H, m). HRFAB-MS m/z Cald for $C_{17}H_{28}N_3O_5S$ $(M+H)^+$ 386.1750; Found 386.1761; UV λ_{max} 298 (ε=9,720) nm.

3a: IR (KBr) 1755, 1583, 1394 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 1.19 (3H, d, J=7.1 Hz), 1.26 (3H, d, J=6.4 Hz), 1.40~1.95 (5H, m), 2.65 (1H, m), 3.0 (2H, t, J=7.3 Hz), 3.25~3.39 (2H, m), 3.42 (1H, m), 3.55~3.75 (2H, m), 3.72 (1H, m), 4.03 (1H, m), 4.13~4.27 (2H, m); HRFAB-MS m/z Calcd for $C_{18}H_{30}N_3O_5S$ $(M+H)^+$ 400.1906; Found 400.1894; UV λ_{max} 298 (ε=8,480) nm.

3b: IR (KBr) 1755, 1583, 1394 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 1.19 (3H, d, J=7.0 Hz), 1.26 (3H, d, J=6.4 Hz), 1.40~2.00 (5H, m), 2.60 (1H, m), 3.02 (2H, t, J=7.2 Hz), 3.25~3.40 (2H, m), 3.45 (1H, m), 3.58~3.85 (2H, m), 3.90~4.05 (2H, m), 4.15~4.30 (2H, m); UV λ_{max} 298 (ε=9,180) nm.

4a: IR (KBr) 1757, 1633, 1574, 1392 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 1.27 (3H, d, J=7.3 Hz), 1.34 (3H, d, J=6.4 Hz), 1.84 (1H, m), 2.06 (1H, m), 2.19 (1H, m), 2.87 (1H, m), 3.04 (1H, dd, J=9.4 and 13.1 Hz), 3.23 (1H, dd, J=3.1 and 13.1 Hz), 3.41 (1H, m), 3.46 (1H, m), 3.52 (1H, dd, J=2.7 and 6.1 Hz), 3.71 (1H, dd, J=7.0 and 12.5 Hz), 3.97 (1H, m), 4.06~4.13 (2H, m), 4.27~4.32 (2H, m); HRFAB-MS m/z Calcd for $C_{17}H_{28}N_3O_5S$ $(M+H)^+$ 386.1750; Found 386.1749; UV λ_{max} 298 (ε=8,930) nm.

4b: IR (KBr) 1749, 1589, 1396 cm^{-1}. ^1H NMR (200 MHz, D_2O) δ 1.22 (3H, d, J=7.3 Hz), 1.29 (3H, d, J=6.4 Hz), 1.75 (1H, m), 1.98~2.09 (2H, m), 2.83 (1H, m), 3.02 (1H, dd, J=4.5 and 8.6 Hz), 3.19 (1H, dd, J=3.2 and 13.3 Hz), 3.30~3.45 (2H, m), 3.48 (1H, dd, J=2.7 and 6.1 Hz), 3.70 (1H, dd, J=7.2 and 12.4 Hz), 3.90 (1H, m), 3.95~4.14 (2H, m), 4.15~4.30 (2H, m). HRFAB-MS m/z Calcd for $C_{17}H_{28}N_3O_5S$ $(M+H)^+$ 386.1750; Found 386.1778; UV λ_{max} 298 (ε=7,400) nm.

5a: IR (KBr) 1751, 1720, 1591, 1456, 1394 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 1.16 (3H, d, J=7.0 Hz), 1.23 (3H, d, J=6.4 Hz), 1.60~2.10 (5H, m), 2.73 (1H, m), 3.00~3.18 (2H, m), 3.22~3.45 (3H, m), 3.58 (1H, m), 3.75~4.06 (3H, m), 4.10~4.27 (2H, m); HRFAB-MS m/z Calcd for $C_{18}H_{30}N_3O_5S$ $(M+H)^+$ 400.1906; Found 400.1893; UV λ_{max} 297 (ε=8,790) nm.

5b: IR (KBr) 1751, 1587, 1542, 1456, 1394 cm^{-1}. ^1H NMR (200 MHz, D_2O) δ 1.17 (3H, d, J=7.2 Hz), 1.24 (3H, d, J=6.4 Hz), 1.55~2.05 (5H, m), 2.74 (1H, m), 3.08 (2H, m), 3.21~3.46 (3H, m), 3.55~4.05 (4H, m), 4.12~4.27 (2H, m); HRFAB-MS m/z Calcd for $C_{18}H_{30}N_3O_5S$ $(M+H)^+$ 400.1906; Found 400.1928; UV λ_{max} 297 (ε=8,960) nm.

6: IR (KBr) 1749, 1585, 1456, 1394, 1288 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 1.17 (3H, d, J=7.3 Hz), 1.24 (3H, d, J=6.4 Hz), 1.40~1.75 (3H, m), 1.75~2.10 (2H, m), 2.72 (1H, m), 2.87 (1H, dd, J=9.8 and 13.1 Hz), 3.12 (1H, dd, J=2.7 and 13.1 Hz), 3.22~3.36 (2H, m), 3.42 (1H, dd, J=2.6 and 6.1 Hz), 3.56~3.74 (2H, m), 3.84 (1H, m), 3.98 (1H, m), 4.13~4.25 (2H, m); HRFAB-MS m/z Calcd for $C_{18}H_{30}N_3O_5S$ $(M+H)^+$ 400.1906; Found 400.1890; UV λ_{max} 298 (ε=8,280) nm.

7a: IR (KBr) 1760, 1590, 1390 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 1.37 (3H, d, J=7.3 Hz), 1.45 (3H, d, J=6.6 Hz), 1.85~2.22 (3H, m), 2.84 (1H, m), 2.90 (3H, s), 3.30~3.68 (5H, m), 3.80~4.00 (2H, m), 4.08~4.30 (2H, m), 4.30~4.45 (2H, m); HRFAB-MS m/z Calcd for $C_{18}H_{30}N_3O_5S$ $(M+H)^+$ 400.1906; Found 400.1895; UV λ_{max} 298 (ε=9,270) nm.

7b: IR (KBr) 1760, 1620, 1400 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 1.30 (3H, d, J=6.9 Hz), 1.37 (3H, d, J=6.3 Hz), 1.80~2.15 (3H, m), 2.70 (1H, m), 2.82 (3H, s), 3.20~3.60 (5H, m), 3.70~3.90 (2H, m), 3.95~4.20 (2H, m), 4.20~4.40 (2H, m); HRFAB-MS m/z Calcd for $C_{18}H_{30}N_3O_5S$ $(M+H)^+$ 400.1906; Found 400.1899; UV λ_{max} 298 (ε=9,030) nm.

8: IR (KBr) 1780, 1590, 1490, 1245, 1090 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.33 (3H, d, J=7.4 Hz), 1.41 (3H, d, J=6.4 Hz), 1.85~2.20 (3H, m), 2.70 (1H, m), 3.02 (6H, s), 3.26~3.60 (5H, m), 3.65~3.90 (2H, m), 4.00~4.20 (2H, m), 4.27~4.44 (2H, m); HRFAB-MS

m/z Calcd for $C_{19}H_{32}N_3O_5S$ $(M+H)^+$ 414.2063; Found 414.2088; UV λ_{max} 298 ($\varepsilon=5{,}340$) nm.

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-[(3*S*,5*S*)-5-(3-trimethylammonio-1-hydroxypropyl)-pyrrolidin -3-ylthio]-1-carbapen-2-em-3-carboxylate (**9**)

To a solution of **67** (1.00 g, 1.38 mmol), in acetone (31 ml) was added MeI (31 ml). After being stirred for 18 hours at room temperature, the mixture was concentrated *in vacuo*. A mixture of the residue and 10% Pd-C (1.0 g) in THF (48 ml), EtOH (9 ml) and 0.2 M MOPS buffer (pH 7.0, 48 ml) was stirred for 3.5 hours at room temperature under a hydrogen pressure (3.5 kg/cm^2). The catalyst was removed by filtration and washed with THF and H_2O. The filtrate was concentrated *in vacuo* to remove the organic solvent. The aqueous layer was subjected to reversed phase column chromatography, which was eluted first with H_2O and with 15% MeOH-H_2O. The fractions containing the desired compound were concentrated *in vacuo* and lyophilized to give **9** (66 mg, 11%): IR (KBr) 1750, 1590, 1390 cm^{-1}; ^{1}H NMR (300 MHz, D_2O) δ 1.27 (3H, d, $J=7.3$ Hz), 1.34 (3H, d, $J=6.4$ Hz), 1.80~2.30 (3H, m), 2.70 (1H, m), 3.20 (9H, s), 3.30~4.00 (7H, m), 4.00~4.20 (2H, m), 4.20~4.40 (2H, m); HRFAB-MS *m/z* Calcd for $C_{20}H_{34}N_3O_5S$ $(M)^+$ 428.2219; Found 428.2247; UV λ_{max} 299 ($\varepsilon=8{,}130$) nm.

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[(3*S*,5*S*)-5-(1-hydroxy-3-methylaminopropyl)pyrrolidin-3-ylthio]-1-methyl-1-carbapen-2-em-3-carboxylic Acid·hydrochloride (**7b**)

1) To a mixture of **59** (100 g, 168 mmol) and the thiol **69** (50 g, 190 mmol) in DMF (1 liter) was added NEt_3 (58 ml, 420 mmol) at -30°C, and the mixture was stirred for 3.5 hours at -30~-25°C. The reaction mixture was poured into 0.2 M phosphate buffer (9.5 liters, pH 6.5) at 0~4°C. After being stirred for 1 hour at room temperature, the mixture was cooled to 5°C, and further stirred for 16 hours. The resulting precipitates were collected by filtration, washed with H_2O and acetone, and dried to give **70** (95.4 g, 83%): IR (KBr) 3420, 1770, 1700, 1520, 1340 cm^{-1}; ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.17 (6H, m), 1.47 (2H, m), 1.76 (1H, m), 2.25 (1H, m), 2.40 (1H, m), 2.75 (1H, m), 2.81 (1H, m), 3.01 (1H, m), 3.25 (2H, m), 3.50 (2H, m), 3.59 (1H, m), 3.66 (1H, m), 3.97 (2H, m), 4.23 (2H, m), 5.28, 5.45 (2H, AB q, $J=14.2$ Hz), 7.72 (2H, d, $J=8.6$ Hz), 8.23 (2H, d, $J=8.6$ Hz); FAB-MS *m/z* 535 $(M+H)^+$;

Anal Calcd for $C_{25}H_{34}N_4O_7S\cdot H_3PO_4\cdot 3H_2O$:
C, 43.73, H, 6.31 N, 7.90.
Found:
C, 43.41, H, 6.73 N, 8.16.

2) A mixture of **70** (200 g, 250 mmol) and 10% Pd-C (40 g) in THF (12.5 liters) and H_2O (12.5 liters) was stirred for 1.5 hours under a hydrogen pressure (3.0 kg/cm^2) at room temperature. The catalyst was filtered off and washed with THF-H_2O (1:1). The combined filtrate and washings were washed with CH_2Cl_2 (12 liters) and concentrated *in vacuo* to *ca.* 12 liters. The aqueous layer was subjected to ion-exchange resin SA-10A (Cl-type, 7.5 liters), which was eluted with H_2O. The fractions containing the desired compound (16 liters) were combined, and the pH of the solution was adjusted to 5.4 with a 2 N aqueous NaOH solution and concentrated *in vacuo* to *ca.* 1.7 liters. The aqueous layer was subjected to reversed phase column chromatography, which was eluted first with H_2O and with 10% MeOH-H_2O. The fractions containing the desired compound were combined, and concentrated *in vacuo* to *ca.* 200 ml, to which was added EtOH and seeded at room temperature, and the mixture was stirred for 17 hours at 5°C. The resulting crystalline solid was collected by filtration, washed with 85% EtOH and acetone, and dried to give BO-2727·HCl·H_2O (**7b**, 98.1 g, 86%).

References

1) Okamoto, O.; H. Fukatsu, S. Nakagawa & N. Taknaka: BO-2727 and its related compounds, new β-methylcarbapenems. Program and Abstracts of 33rd Intersci. Conf. on Antimicrob. Agents Chemother., No. 898, New Orleans, 1993
2) Shih, D. H.; F. Baker, L. Cama & B. G. Christensen: Synthetic carbapenem antibiotics I, 1β-methylcarbapenem. Heterocycles 21: 29~40, 1984
3) Ohtake, N.; O. Okamoto, S. Kato, R. Ushijima, H. Fukatsu & S. Nakagawa: 1β-methyl-2-(5-substituted pyrrolidin-3-ylthio)carbapenems; 2. Synthesis and antibacterial activity of 5-aminopropyl and 5-aminopropenyl derivatives. J. Antibiotics 50: 586~597, 1997
4) Mori, S.; T. Ohno, H. Harada, T. Aoyama & T. Shioiri: New methods and reagents in organic synthesis. 92. A stereoselective synthesis of tilivalline and its analogs. Terahedron 47: 5051~5079, 1991
5) Hanson, G. J.; J. S. Baran & T. Lindberg: Stereoselective addition of lithioethyl acetate to Boc-L-prolinal. A conveinient chiral synthetic building block for the pyrrolizidine alkaloid ring system.Terahedron Lett. 27: 3577~3580, 1986
6) Soai, K.; H. Oyamada & M. Takase: The preparation of *N*-protected amino alcohols and *N*-protected peptide alcohol by reduction of the corresponding esters with sodium borohydride. An improved procedure involving a slow addition of a small amount of methanol. Bull. Chem. Soc. Jpn. 57: 2327~2328, 1984

7) OHTAKE, N.; K. YAMADA, O. OKAMOTO, E. MANO, R. USHIJIMA & S. NAKAGAWA: 1β-methyl-2-(5-substituted pyrrolidin-3-ylthio)carbapenems; 1. Synthesis and antibacterial activity of BO-2502A and its related compounds. J. Antibiotics 50: 567～585, 1997

8) NAKAGAWA, S.; T. HASHIZUME, K. MATSUDA, M. SANADA, O. OKAMOTO, H. FUKATSU & N. TANAKA: *In vitro* activity of a new carbapenem antibiotic, BO-2727, with potent antipseudomonal activity. Antimicrob. Agents Chemother. 37: 2756～2759, 1993

9) ASAHI, Y.; S. MIYAZAKI & K. YMAGUCHI: *In vitro* and *in vivo* antibacterial activities of BO-2727, a new carbapenem. Antimicrob. Agents Chemother. 39: 1030～1037, 1995