Synthesis and Modification of a Novel 1β-Methyl Carbapenem Antibiotic, S-4661

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We describe an efficient method for introducing a sulfamoylamino group into the C-2′ position of pyrrolidine using the Mitsunobu reaction. S-4661, its *N*-methyl analogues and stereoisomers were synthesized using this method and their structure-activity relationships were investigated.

In the preceding paper[1], we reported the synthesisand structure-activity relationships of 2-(5-substituted aminomethylpyrrolidin-3-ylthio)-1β-methylcarbapenems and demonstrated that S-4661, (1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-sulfamoylaminomethyl-pyrrolidin-3-ylthio]-6-[(1*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic acid (**1a**), exhibited potent, broad and well-balanced antibacterial activity against both Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*. This finding led us to devise an efficient and industrially feasible synthetic route to this new antibiotic, S-4661, for further evaluation. Here we report an efficient method for preparing C-2 thiols (**7**), which involves the Mitsunobu reaction for introducing a sulfamoylamino group as a key step. Also described are the synthesis of *N*-methyl analogues (**1b, c**) and stereoisomers (**1d**～**f**) of S-4661 and their structure-activity relationships.

Chemistry

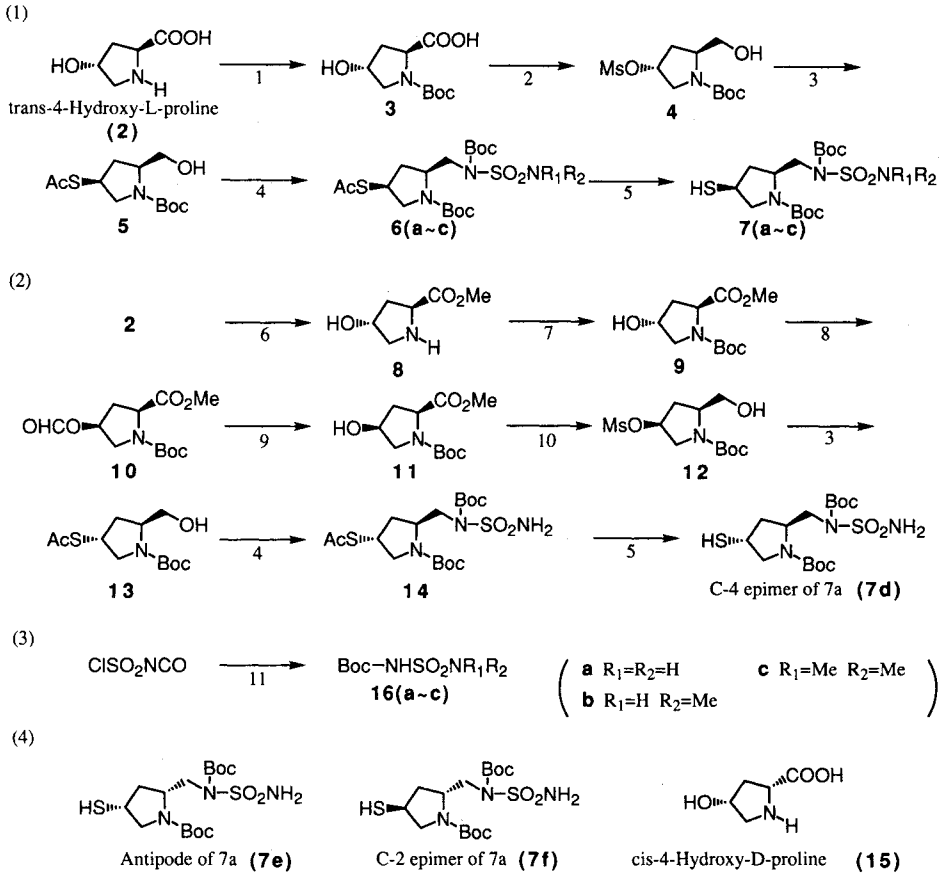
2-Substituted 4-mercaptopyrrolidine derivatives (**7**) were prepared by the sequence of reactions shown in Scheme 1. *trans*-4-Hydroxy-L-proline (**2**) was treated with di-*tert*-butyl dicarbonate ($(Boc)_2O$) to give *N*-protected 4-hydroxyproline (**3**), which was converted into mesylate (**4**) in one flask in 94% yield by a sequence of reactions, namely mixed anhydride formation with ethyl chloroformate and triethylamine, *O*-mesylation with methanesulfonyl chloride and triethylamine, and reduction of the mixed anhydride with aqueous sodium borohydride under phase transfer condition (n-Bu_4NBr catalyst)[2] in CH_2Cl_2. The mesylate (**4**) was treated with potassium thioacetate in DMF to give thioacetate (**5**) with inversion of the C-4 configuration. Conversion of the hydroxyl group of **5** into a sulfamoylamino group was successfully carried out by the Mitsunobu reaction. This reaction of **5** with **16(a**～**c)** using diethyl azodicarboxylate and triphenylphosphine in THF was done at room temperature for 4 hours and the *N*-protected sulfamoylaminomethyl derivatives at C-2 (**6(a**～**c)**) were isolated in good yield after removal of diethyl hydrazinedicarboxylate crystals by filtration. **16(a**～**c)** was prepared by treatment of chlorosulfonyl isocyanate with *tert*-butanol and then with ammonia or the corresponding amine. Finally the acetyl group of **6(a**～**c)** was removed with sodium methoxide to give the corresponding mercaptan (**7(a**～**c)**). The new synthetic route including Mitsunobu reaction for introduction of a sulfamoylamino group is superior to the previous one[1] in every respect such as shortness, high overall yield and good crystalline property of the intermediates, and is more suitable for large scale preparation.

C-2 and C-4 epimeric thiols (**7f, 7d**) of **7a** were prepared by a different synthetic route as shown in Scheme 1. Esterification and subsequent *N*-protection of **2** gave the *N*-protected methyl ester (**9**). **9** was subjected to the Mitsunobu reaction with formic acid and then hydrolysis to afford the epimer alcohol (**11**). **11** was then mesylated at C-4 and reduced with sodium borohydride to give **12**, which was followed by the method described above to furnish **7d**.

The antipode (**7e**) and C-2 epimer (**7f**) of **7a** were obtained by using *cis*-4-hydroxy-D-proline (**15**) as the starting material instead of **2** under similar reaction conditions to those described above.

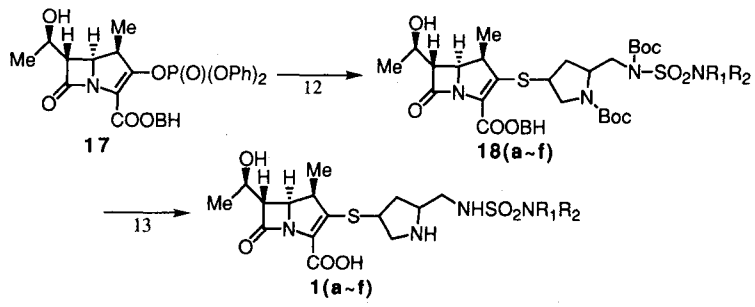
Protected 1β-methylcarbapenem (**18(a**～**f)**) was synthesized by treatment of enol phosphate (**17**)[3] with the freshly prepared mercaptan **7(a**～**f)** in the presence of diisopropylethylamine. The final deprotection step of **18(a**～**f)** was carried out by treatment with $AlCl_3$ in the presence of anisole[4] to provide the corresponding deprotected 1β-methylcarbapenem (**1(a**～**f)**), which was

Scheme 1.



1, $(Boc)_2O$, NaOH; 2,(i) ClCOOEt, NEt_3, (ii) MsCl, NEt_3, (iii) $NaBH_4$, n-Bu_4NBr; 3, AcSK; 4, **16(a~c)**, diethyl azodicarboxylate, PPh_3; 5, NaOMe; 6, MeOH, AcCl, $SOCl_2$; 7, $(Boc)_2O$; 8, HCOOH, diethyl azodicarboxylate, PPh_3; 9, NaOH; 10,(i) MsCl, NEt_3, (ii) $NaBH_4$; 11,(i) t-BuOH, (ii) HNR^1R^2

Scheme 2.



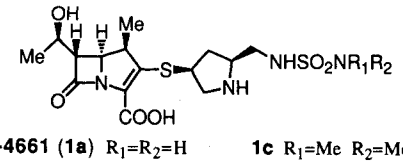
12, i-Pr_2EtN, **7(a~f)**; 13, $AlCl_3$, anisole

purified by Diaion HP-20AG column chromatography. (Scheme 2)

Biological Properties

The MIC values of the new carbapenems prepared as above (**1a**~**1f** in Fig. 1 and Fig. 2) against Gram-positive and Gram-negative bacteria are listed in Table 1, together with those of imipenem (IMP)[5~7] and meropenem (MEPM)[8].

Fig. 1. Prepared *N*-methyl analogues of S-4661.



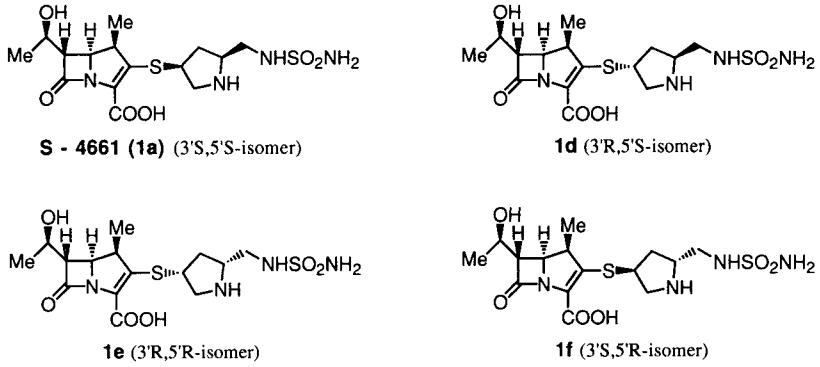
S-4661 (1a) $R_1=R_2=H$ **1c** $R_1=Me$ $R_2=Me$
1b $R_1=H$ $R_2=Me$

Table 1. Antibacterial activity (MIC, μg/ml) of carbapenem compounds.

Organism	MIC (μg/ml)							
	1a	**1b**	**1c**	**1d**	**1e**	**1f**	IPM	MEPM
S.a.	0.02	0.05	0.05	0.05	0.02	0.02	0.006	0.1
S.a.(R)	0.2	0.4	0.8	0.2	0.8	0.4	0.05	0.8
E.c.	0.02	0.02	0.05	0.02	0.02	0.02	0.1	0.02
K.p.	0.05	0.05	0.05	0.05	0.02	0.02	0.2	0.02
E.cl.	0.05	0.1	0.1	0.1	0.05	0.05	0.4	0.05
P.m.	0.1	0.1	0.1	0.4	0.1	0.1	0.4	0.05
P.v.	0.1	0.1	0.1	0.2	0.1	0.1	0.4	0.05
S.m.	0.1	0.1	0.1	0.2	0.2	0.1	0.4	0.05
*P.a.*1	0.4	0.8	3.1	3.1	6.3	0.4	1.6	0.8
*P.a.*2	0.1	0.2	0.4	1.6	3.1	0.4	1.6	0.1

S.a., *Staphylococcus aureus* FDA 209P JC-1; *S.a.*(R), *Staphylococcus aureus* SR3131; *E.c.*, *Escherichia coli* NIHJ JC-2; *K.p.*, *Klebsiella pneumoniae* SR1; *E.cl.*, *Enterobacter cloacae* SR233; *P.m.*, *Proteus mirabilis* PR-4; *P.v.*, *Proteus vulgaris* CN-329; *S.m.*, *Serratia marcescens* ATCC 13880; *P.a.*1, *Pseudomonas aeruginosa* SR1012; *P.a.*2, *Pseudomonas aeruginosa* SR24.

Fig. 2. Prepared stereoisomers of S-4661 (**1a**).



S - 4661 (1a) (3'S,5'S-isomer)

1d (3'R,5'S-isomer)

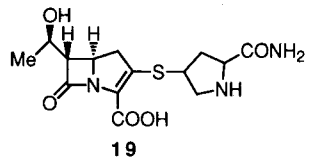
1e (3'R,5'R-isomer)

1f (3'S,5'R-isomer)

The effects of introduction of a methyl group at the end of the sulfamoylamine were first investigated. Comparison of **1a**, **1b** and **1c** in Table 1 shows that introduction of a methyl group does not contribute to enhancement of antibacterial activities and instead tends to cause a slight decrease.

The effects of the stereochemistry at the C-3 and C-5 positions on the pyrrolidine of the C-2 side chain moiety were investigated using each stereoisomer of **1a** (**1d**, **1e**, **1f**). As shown in Table 1, all four stereoisomers had relatively similar antibacterial activities against Gram-positive and Gram-negative bacteria, while obvious differences were observed against *Pseudomonas aeruginosa*. SUNAGAWA *et al.* also reported about the anti-pseudomonal activity of similar stereoisomers of **19** (Fig. 3) and concluded that *cis*-isomers ((3′*S*,5′*S*)- and (3′*R*,5′*R*)-isomers) were more active than *trans*-isomers ((3′*R*,5′*S*)- and (3′*S*,5′*R*)-isomers)[8]. However, in our series, 3′*S*-isomers (**1a**, **1f**) were more active than 3′*R*-isomers (**1d**, **1e**), and as a result, (3′*S*,5′*S*)-isomer (**1a** (S-4661)) showed the best antibacterial activity including that against *Pseudomonas aeruginosa*.

Fig. 3



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Experimental

Chemistry

MP was determined with a Yanagimoto micro melting point apparatus and is uncorrected. IR spectra were taken on a Jasco IR-700 spectrometer. ^{1}H NMR spectra were recorded at 200 MHz on a Varian VXR-200 NMR

spectrometer using TMS or sodium 2,2-dimethyl-2-silapentan-5-sulfonate (in D_2O) as an internal standard. All reactions under anhydrous conditions were carried out using anhydrous solvents dried over Molecular Sieves type 4A in a nitrogen atmosphere.

Measurement of *In Vitro* Antibacterial Activity

MICs were determined by agar dilution method using test agar. An overnight culture of bacteria in tryptosoy broth was diluted to about 10^6 cells/ml with the same broth and inoculated with an inoculating device onto agar containing serial twofold dilutions of the test compounds. The organisms were incubated at 37°C for 18~20 hours. The MIC of a compound was defined as the lowest concentration that visibly inhibited growth.

(2*S*,4*R*)-1-*tert*-Butoxycarbonyl-2-carboxy-4-hydroxypyrrolidine (**3**)

To a suspension of *trans*-4-hydroxy-L-proline (**2**) (50 g, 0.381 mol) in MeOH (250 ml), a solution of 4 N NaOH (95.4 ml, 0.381 mol) and di-*tert*-butyl dicarbonate (91.6 g, 0.42 mol) in MeOH (55 ml) was added at −20°C. After being stirred at 20°C for 3 hours, the reaction mixture was concentrated and then diluted with toluene (100 ml) and shaken. The aqueous layer was acidified with conc. HCl (36 ml) and extracted with EtOAc under salting-out condition, and the extract was washed with saturated brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was crystallized from a toluene-EtOAc mixture to give **3** (84.7 g, 96%): MP 126~128°C; IR ($CHCl_3$) cm^{-1} 3360, 1735, 1656; ^1H NMR ($CDCl_3$) δ 1.43, 1.46 (2×s, 9H), 1.95~2.36 (m, 2H), 3.36~3.6 (m, 2H), 4.23~4.44 (m, 2H).

(2*S*,4*R*)-1-*tert*-Butoxycarbonyl-2-hydroxymethyl-4-methanesulfonyloxypyrrolidine (**4**)

To a solution of **3** (84.5 g, 0.365 mol) and triethylamine (61.1 ml, 0.438 mol) in CH_2Cl_2 (1.27 liters) at −30 °C, ethyl chloroformate (38.4 ml, 0.402 mol) was added, and the mixture was stirred for 40 minutes. To this mixture cooled to −40°C, triethylamine (61.1 ml, 0.438 mol) and methanesulfonyl chloride (31.1 ml, 0.402 mol) were added, and the mixture was stirred for 40 minutes at the same temperature. To the resulting mixture, without any further operations, *tetra-n*-butylammonium bromide (11.8 g, 0.0365 mol) and a solution of sodium borohydride (52.5 g, 1.35 mol) in water (55 ml) were added, and the mixture was allowed to warm to −10°C and stirred for 1 hour. After the aqueous layer was acidified with dilute HCl to pH 3, the organic layer was taken, successively washed with aqueous $NaHCO_3$ and water, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was crystallized from a toluene-hexane mixture to give **4** (101.3 g, 94%): MP 95~96°C; IR ($CHCl_3$) cm^{-1} 3460, 1680; ^1H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.78~2.02 (m, 1H), 2.3~2.48 (m, 1H), 3.05 (s, 3H), 3.5~3.65 (m, 2H), 3.65~4.0 (m, 2H), 4.03~4.25 (m, 1H), 5.2 (s, 1H).

(2*S*,4*S*)-1-*tert*-Butoxycarbonyl-2-hydroxymethyl-4-acetylthiopyrrolidine (**5**)

After a solution of **4** (11.8 g, 40 mmol) and potassium thioacetate (5.94 g, 52 mmol) in DMF (120 ml) was stirred at 65°C for 3.75 hours, the reaction mixture was mixed with EtOAc (330 ml), ice water (100 ml), and 1 N HCl (20 ml) to adjust the aqueous layer at pH 4. The organic layer was taken, successively washed with water and saturated brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by silica gel chromatography (toluene-EtOAc=2:1) to give **5** as a pale-orange-colored oil (9.48 g, 86%): IR ($CHCl_3$) cm^{-1} 3380, 1690; ^1H NMR ($CDCl_3$) δ 1.47 (s, 9H), 2.34 (s, 3H), 2.4~3.2 (m, 2H), 3.58~4.1 (m, 6H).

(2*S*,4*S*)-1-*tert*-Butoxycarbonyl-2-(*N*-*tert*-butoxycarbonyl-*N*-sulfamoylaminomethyl)-4-mercaptopyrrolidine (**7a**)

To a solution of **5** (9.04 g, 32.8 mmol) in THF (95 ml), triphenylphosphine (10.16 g, 38.7 mmol), the corresponding *N*-*tert*-butoxycarbonylsulfamide (**16a**) (49.2 mmol), and diethyl azodicarboxylate (6.20 ml, 39.4 mmol) were successively added under ice cooling. After being stirred at room temperature for 4 hours, the reaction mixture was diluted with toluene (60 ml), and the resulting crystals were filtered off. After the filtrate was evaporated *in vacuo*, the residue containing the corresponding (2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-acetylthio-2-(*N*-*tert*-butoxycarbonyl-*N*-sulfamoylaminomethyl)-pyrrolidine (**6a**) was dissolved in toluene (95 ml), then 4.92 M sodium methoxide in MeOH (20 ml, 98.4 mmol) was added at −35°C, and the mixture was stirred for 30 minutes. After the reaction mixture was partitioned with water (100 ml), the aqueous layer was acidified with conc. HCl (10 ml) under ice cooling and extracted with EtOAc (300 ml). The extract was washed with water and brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by silica gel chromatography, and the colorless oil obtained was crystallized from toluene-hexane mixture to give **7a** (9.32 g, 69%): MP 92~93°C; IR ($CHCl_3$) cm^{-1} 3380, 3220, 1718, 1680; ^1H NMR ($CDCl_3$) δ 1.2~1.5 (m, 1H), 1.42 (s, 9H), 1.54 (s, 9H), 1.82 (d, J=6.2 Hz, 1H), 2.5~2.7 (m, 1H), 4.09, 3.05 (ABX, J_1=12.0 Hz, J_2=7.4 Hz, J_3=3.2 Hz, 2H), 4.06, 3.62 (ABX, J_1=15.0 Hz, J_2=10.8 Hz, J_3=3.2 Hz, 2H), 4.2~4.6 (m, 1H), 6.08 (s, 2H).

Anal Calcd for $C_{15}H_{29}N_3O_6S_2$:
C 43.78, H 7.10, N 10.21, S 15.58.
Found:
C 43.64, H 7.10, N 10.19, S 15.34.

The following compounds (**7b**~**7f**) were also prepared as described for **7a**.

7b: IR ($CHCl_3$) cm^{-1} 3352, 1711, 1686; ^1H NMR ($CDCl_3$) δ 1.46 (s, 9H), 1.55 (s, 9H), 1.70 (d, J=7.0 Hz, 1H), 2.45~2.60 (m, 1H), 2.78 (s, 3H), 2.98~3.27 (m, 2H), 3.71~3.80 (m, 1H), 3.90~4.31 (m, 4H), 5.68 (s, 1H).

7c: IR ($CHCl_3$) cm^{-1} 3355, 1715, 1683; ^1H NMR

($CDCl_3$) δ 1.47 (s, 9H), 1.54 (s, 9H), 1.67 (d, J=7.0 Hz, 1H), 2.43~2.58 (m, 1H), 2.91 (s, 6H), 2.97~3.25 (m, 2H), 3.75~3.88 (m, 1H), 4.00~4.28 (m, 4H).

7d: MP 90~91.5°C; IR (KBr) cm^{-1} 3220, 1698, 1683; ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.52 (s, 9H), 1.72 (d, J=7.0 Hz, 1H), 1.9~2.0 (m, 2H), 3.2~3.8 (m, 5H), 4.46~4.54 (m, 1H), 6.11 (s, 2H).

Anal Calcd for $C_{15}H_{29}N_3O_6S_2$:
C 43.78, H 7.10, N 10.21, S 15.58.
Found:
C 43.61, H 7.08, N 10.25, S 15.44.

7e: MP 92~93°C; IR ($CHCl_3$) cm^{-1} 3380, 3220, 1718, 1680; ^1H NMR ($CDCl_3$) δ 1.2~1.5 (m, 1H), 1.42 (s, 9H), 1.54 (s, 9H), 1.82 (d, J=6.2 Hz, 1H), 2.5~2.7 (m, 1H), 4.09, 3.05 (ABX, J_1=12.0 Hz, J_2=7.4 Hz, J_3=8.2 Hz, 2H), 4.06, 3.62 (ABX, J_1=15.0 Hz, J_2=10.8 Hz, J_3=3.2 Hz, 2H), 4.2~4.6 (m, 1H), 6.08 (s, 2H).

Anal Calcd for $C_{15}H_{29}N_3O_6S_2$:
C 43.78, H 7.10, N 10.21, S 15.58.
Found:
C 43.59, H 7.07, N 10.31, S 15.68.

7f: MP 90~91°C; IR (KBr) cm^{-1} 3220, 1698, 1683; ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.52 (s, 9H), 1.72 (d, J=7.0 Hz, 1H), 1.9~2.0 (m, 2H), 3.2~3.8 (m, 5H), 4.47~4.54 (m, 1H), 6.11 (s, 2H).

Anal Calcd for $C_{15}H_{29}N_3O_6S_2$:
C 43.78, H 7.10, N 10.21, S 15.58.
Found:
C 43.55, H 7.11, N 10.37, S 15.75.

(2*S*,4*R*)-2-Methoxycarbonyl-4-hydroxypyrrolidine Hydrochloride (**8**)

To a suspension of **2** (200 g, 1.525 mol) in MeOH (800 ml), acetyl chloride (163 ml, 2.288 mol) was added under ice cooling, and the mixture was warmed to room temperature. Next, thionyl chloride (55.7 ml, 0.763 mol) was added, and the mixture was stirred for 4 hours at 40 °C to give **8** after the usual work up as colorless crystals (244.27 g, 88%): IR (KBr) cm^{-1} 3380, 3330, 2960, 2695, 1742; ^1H NMR (D_2O) δ 1.8~2.0 (m, 1H), 2.0~2.2 (m, 1H), 2.9~3.1 (m, 1H), 3.17 (dd, J=12.6 Hz, J=3.6 Hz, 1H), 3.49 (s, 3H), 4.2~4.4 (m, 2H).

(2*S*,4*R*)-1-*tert*-Butoxycarbonyl-2-methoxycarbonyl-4-hydroxypyrrolidine (**9**)

To a suspension of **8** (12.71 g, 70 mmol) in CH_2Cl_2 (70 ml), triethylamine (10.7 ml, 77 mmol) was added under ice cooling, and the mixture was stirred for 5 minutes at room temperature. After a solution of di-*tert*-butyl dicarbonate (19.10 g, 87.5 mmol) in CH_2Cl_2 (72 ml) was added, the mixture was stirred for 45 minutes at room temperature to give **9** after the usual work up as a colorless oil (14.06 g, 82%): IR ($CHCl_3$) cm^{-1} 3460, 1730, 1680; ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H × 2/3), 1.46 (s, 9H × 1/3), 1.9~2.4 (m, 3H), 3.4~3.7 (m, 2H), 3.74 (s, 3H), 4.3~4.6 (m, 2H).

(2*S*,4*S*)-1-*tert*-Butoxycarbonyl-2-methoxycarbonyl-4-formyloxypyrrolidine (**10**)

To a solution of **9** (7.36 g, 30 mmol) in THF (30 ml), formic acid (1.36 ml, 36 mmol), triphenylphosphine (9.44 g, 36 mmol) and diethyl azodicarboxylate (5.67 ml, 36 mmol) were successively added under ice cooling. After being stirred for 40 minutes at the same temperature, the reaction mixture was diluted with toluene (60 ml) and the resulting crystals were filtered off. The filtrate was evaporated *in vacuo*. and the residue was purified by silica gel column chromatography (toluene-EtOAc=4:1) to give **10** as colorless crystals (5.38 g, 66%): IR (KBr) cm^{-1} 3420, 1748, 1712, 1681; ^1H NMR ($CDCl_3$) δ 1.43 (s, 9H × 2/3), 1.47 (s, 9H × 1/3), 2.2~2.4 (m, 1H), 2.4~2.7 (m, 1H), 3.5~3.9 (m, 2H), 3.75 (s, 3H), 4.3~4.6 (m, 1H), 5.3~5.5 (m, 1H), 7.98 (s, 1H).

(2*S*,4*S*)-1-*tert*-Butoxycarbonyl-2-methoxycarbonyl-4-hydroxypyrrolidine (**11**)

To a solution of **10** (5.12 g, 18.7 mmol) in MeOH (51.0 ml), aqueous 1 N NaOH (18.7 ml) was added under ice cooling and the mixture was stirred at the same temperature for 20 minutes. After concentration of the mixture, the concentrate was partitioned between EtOAc and water, and the organic layer was washed with water and evaporated *in vacuo* to give **11** as colorless crystals (4.09 g, 89%): IR (KBr) cm^{-1} 3460, 1728, 1677; ^1H NMR ($CDCl_3$) δ 1.42 (s, 9H × 2/3), 1.46 (s, 9H × 1/3), 2.0~2.2 (m, 1H), 2.2~2.5 (m, 1H), 3.2~3.8 (m, 3H), 3.79 (s, 3H), 4.2~4.4 (m, 2H).

(2*S*,4*S*)-1-*tert*-Butoxycarbonyl-2-hydroxymethyl-4-methanesulfonyloxypyrrolidine (**12**)

To a solution of **11** (9.81 g, 40 mmol) and triethylamine (6.67 ml, 48 mmol) in CH_2Cl_2 (49 ml) under ice cooling, methanesulfonyl chloride (3.70 ml, 48 mmol) was added, and the mixture was stirred at the same temperature for 20 minutes to give the mesylate after the usual work up as a crude oil (13.05 g, 101%). To a solution of this crude mesylate in THF (40 ml)/EtOH (60 ml), sodium borohydride (6.06 g, 160 mmol) was added, and the reaction mixture was stirred for 75 minutes at room temperature. After being acidified with dilute HCl, the mixture was extracted with EtOAc and the extract was washed with aqueous $NaHCO_3$ and water, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was crystallized from a toluene-hexane mixture to give **12** (9.85 g, 83%): MP 94~95°C; IR ($CHCl_3$) cm^{-1} 3490, 1688; ^1H NMR ($CDCl_3$) δ 1.48 (s, 9H), 1.9~2.2 (m, 1H), 2.3~2.5 (m, 1H), 3.06 (s, 3H), 3.65 (dd, J=11.2 Hz, J=4.0 Hz, 1H), 3.5~3.9 (m, 2H), 3.84 (dd, J=11.2 Hz, J=7.6 Hz, 1H), 4.02~4.18 (m, 1H), 5.2 (s, 1H).

General Procedure for Preparation of **16**

To a solution of *tert*-butanol (4.27 ml, 50 mmol) in EtOAc (100 ml), chlorosulfonyl isocyanate (4.35 ml, 50 mmol) was added at −40°C, and the mixture was stirred at −18°C for 20 minutes. After the corresponding

gaseous amine (2 mol) was bubbled into the reaction mixture cooled to −72°C with stirring, the mixture was stirred for 50 minutes at 10°C. The reaction mixture was acidified with 5 N HCl (30 ml), and the organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The crystalline residue was crystallized from hexane-EtOAc to give **16** as colorless crystals.

16a: 89%; MP 130~131°C; IR (Nujol) cm^{-1} 3360, 3270, 1718, 1548; ^1H NMR (d_6-DMSO) δ 1.43 (s, 9H), 7.27 (s, 2H).

Anal Calcd for $C_5H_{12}N_2O_4S$:
C 30.60, H 6.17, N 14.28, S 16.34.
Found:
C 30.39, H 6.11, N 14.30, S 16.30.

16b: 88%; IR ($CHCl_3$) cm^{-1} 3388, 1733, 1436; ^1H NMR ($CDCl_3$) δ 1.50 (s, 9H), 2.77 (d, J=5.5 Hz, 3H), 5.03 (s, 1H), 7.03 (s, 1H).

Anal Calcd for $C_6H_{14}N_2O_4S$:
C 34.28, H 6.71, N 13.32, S 15.25.
Found:
C 34.13, H 6.70, N 13.28, S 15.16.

16c: 87%; IR ($CHCl_3$) cm^{-1} 3390, 1741, 1423; ^1H NMR ($CDCl_3$) δ 1.50 (s, 9H), 2.97 (s, 6H), 6.92 (s, 1H).

Anal Calcd for $C_7H_{16}N_2O_4S$:
C 37.49, H 7.19, N 12.49, S 14.30.
Found:
C 37.21, H 7.11, N 12.60, S 14.21.

General Procedure for Preparation of **18**

To a solution of (1*R*,5*S*,6*S*)-2-diphenoxy-phosphonyloxy-6-[(1*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic acid diphenylmethyl ester (**17**) (6.88 g, 11 mmol) in MeCN (70 ml), the corresponding mercaptopyrrolidine (**7**) (13.2 mmol) and diisopropylethylamine (13.2 mmol) were added under ice cooling. After being stirred for 4.5 hours, the reaction mixture was partitioned between EtOAc and ice water. The organic layer was washed with water and saturated brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel chromatography to give **18**.

18a: 87%; MP 163~164°C; IR ($CHCl_3$) cm^{-1} 3385, 3230, 1778, 1715, 1685; ^1H NMR ($CDCl_3$) δ 1.27 (d, J=7.2 Hz, 3H), 1.37 (d, J=6.2 Hz, 3H), 1.39 (s, 9H), 1.42 (s, 9H), 1.78~1.87 (m, 1H), 2.45~2.65 (m, 1H), 3.1~3.35 (m, 2H), 3.28 (dd, J=7.2 Hz, J=2.6 Hz, 1H), 3.5~3.77 (m, 2H), 3.9~4.15 (m, 2H), 4.26 (dd, J=7.0 Hz, J=2.6 Hz, 1H), 4.2~4.37 (m, 1H), 4.45~4.66 (m, 1H), 6.07 (br, 2H), 6.95 (s, 1H), 7.2~7.6 (m, 10H).

Anal Calcd for $C_{38}H_{50}N_4O_{10}S_2$:
C 57.99, H 6.40, N 7.12, S 8.15.
Found:
C 57.87, H 6.46, N 6.99, S 7.93.

18b: 73%; IR ($CHCl_3$) cm^{-1} 3356, 1770, 1705; ^1H NMR ($CDCl_3$) δ 1.25 (d, J=7 Hz, 3H), 1.36 (d, J=6.2 Hz, 3H), 1.47 (s, 9H), 1.52 (s, 9H), 1.8~1.9 (m, 1H), 2.42~2.58 (m, 1H), 2.76 (s, 3H), 3.1~3.8 (m, 6H), 4.05~4.35 (m, 4H), 5.8 (br, 1H), 6,96 (s, 1H), 7.2~7.58 (m, 10H).

18c: 70%; IR ($CHCl_3$) cm^{-1} 1769, 1721, 1686; ^1H NMR ($CDCl_3$) δ 1.24 (d, J=7 Hz, 3H), 1.36 (d, J=6.2 Hz, 3H), 1.48 (s, 9H), 1.53 (s, 9H), 1.81~1.89 (m, 1H), 2.45~2.58 (m, 1H), 2.90 (s, 6H), 3.09~3.81 (m, 6H), 4.07~4.33 (m, 4H), 6.96 (s, 1H), 7.2~7.6 (m, 10H).

18d: 88%; IR (KBr) cm^{-1} 3420, 1770, 1710; ^1H NMR ($CDCl_3$) δ 1.26 (d, J=7.2 Hz, 3H), 1.36 (d, J=6.2 Hz, 3H), 1.42 (s, 9H), 1.52 (s, 9H), 1.8~2.1 (m, 2H), 3.2~3.9 (m, 7H), 4.1~4.4 (m, 2H), 4.4~4.6 (m, 1H), 6.04 (br, 2H), 6.94 (s, 1H), 7.1~7.6 (m, 10H).

18e: 86%; IR (KBr) cm^{-1} 3400, 3240, 1770, 1710, 1670; ^1H NMR ($CDCl_3$) δ 1.26 (d, J=7.2 Hz, 3H), 1.39 (d, J=6.2 Hz, 3H), 1.43 (s, 9H), 1.51 (s, 9H), 1.79~1.91 (m, 1H), 2.43~2.56 (m, 1H), 3.1~3.9 (m, 6H), 4.0~4.7 (m, 4H), 6.1 (br, 1H), 6.98 (s, 1H), 7.1~7.6 (m, 10H).

18f: 73%; IR (KBr) cm^{-1} 3400, 3240, 1772, 1708, 1682; ^1H NMR ($CDCl_3$) δ 1.28 (d, J=7.0 Hz, 3H), 1.36 (s, 9H), 1.40 (d, J=6.2 Hz, 3H), 1.52 (s, 9H), 1.8~1.9 (m, 1H), 2.01~2.12 (m, 1H), 3.2~3.9 (m, 7H), 4.2~4.4 (m, 2H), 4.4~4.6 (m, 1H), 6.01 (br, 2H), 6.94 (s, 1H), 7.1~7.6 (m, 10H).

General Procedure for Deprotection of **18**

To a solution of $AlCl_3$ (3.20 g, 24 mmol) in a mixture of anisole (24 ml) and CH_2Cl_2 (24 ml) at −40°C, **18** (3 mmol) in CH_2Cl_2 (12 ml) was gradually added dropwise. After being vigorously stirred at −30°C for 1.5 hours, the reaction mixture was partitioned between NaOAc (5.91 g, 72 mmol)/water (48 ml) and ether (48 ml). The aqueous layer was washed with ether, concentrated *in vacuo* to remove remaining organic solvent and subjected to Diaion HP-20AG column chromatography. The fraction eluting with MeOH-water was lyophilized to give 1 as a colorless foam.

1a: 72%; IR (KBr) cm^{-1} 3400, 1750; ^1H NMR (D_2O) δ 1.22 (d, J=7.2 Hz, 3H), 1.27 (d, J=6.3 Hz, 3H), 1.64~1.82 (m, 1H), 2.62~2.80 (m, 1H), 3.26~3.59 (m, 5H), 3.69 (dd, J=7.0 Hz, J=12.4 Hz, 1H), 3.84~4.10 (m, 2H), 4.16~4.29 (m, 2H).

Anal Calcd for $C_{15}H_{24}N_4O_6S_2 \cdot 1.0H_2O$:
C 41.08, H 5.98, N 12.78, S 14.62.
Found:
C 40.83, H 5.97, N 13.06, S 14.58.

1b: 69%; IR (KBr) cm^{-1} 3380, 1755; ^1H NMR (D_2O) δ 1.15 (d, J=7.0 Hz, 3H), 1.22 (d, J=6.6 Hz, 3H), 1.59~1.72 (m, 1H), 2.59 (s, 3H), 2.6~2.72 (m, 1H), 3.22~3.41 (m, 5H), 3.57~4.01 (m, 3H), 4.12~4.24 (m, 2H).

Anal Calcd for $C_{16}H_{26}N_4O_6S_2 \cdot 3.2H_2O$:
C 39.05, H 6.64, N 11.38, S 13.03.
Found:
C 38.73, H 6.32, N 11.52, S 13.23.

1c: 65%; IR (KBr) cm^{-1} 3400, 1750; ^1H NMR (D_2O)

δ 1.2 (d, J=7.4 Hz, 3H), 1.28 (d, J=6.4 Hz, 3H), 1.65~1.80 (m, 1H), 2.65~2.80 (m, 1H), 2.81 (s, 6H), 3.29~3.55 (m, 5H), 3.65~3.75 (m, 1H), 3.80~4.10 (m, 2H), 4.16~4.30 (m, 2H).

Anal Calcd for $C_{17}H_{28}N_4O_6S_2 \cdot 2.3H_2O$:
C 41.67, H 6.71, N 11.43, S 13.09.
Found:
C 41.70, H 6.58, N 11.31, S 13.04.

1d: 88%; IR (KBr) cm^{-1} 3400, 1750, 1585; ^1H NMR (D_2O) δ 0.86 (d, J=7.4 Hz, 3H), 0.93 (d, J=6.4 Hz, 3H), 1.90 (dd, J=9.0 Hz, J=4.4 Hz, 2H), 2.9~3.3 (m, 5H), 3.48 (dd, J=13.2 Hz, J=7.2 Hz, 1H), 3.7~3.8 (m, 2H), 3.8~4.0 (m, 2H).

Anal Calcd for $C_{15}H_{24}N_4O_6S_2 \cdot 3.3H_2O$:
C 37.54, H 6.43, N 11.67, S 13.36.
Found:
C 37.33, H 6.20, N 11.74, S 13.30.

1e: 86%; IR (KBr) cm^{-1} 3360, 1750; ^1H NMR (D_2O) δ 1.18 (d, J=7.2 Hz, 3H), 1.27 (d, J=6.2 Hz, 3H), 1.85~1.95 (m, 1H), 2.64~2.75 (m, 1H), 3.2~3.6 (m, 5H), 3.6~3.8 (m, 1H), 3.8~4.1 (m, 2H), 4.1~4.3 (m, 2H).

Anal Calcd for $C_{15}H_{24}N_4O_6S_2 \cdot 1.8H_2O$:
C 39.78, H 6.14, N 12.37, S 14.16.
Found:
C 39.84, H 6.04, N 12.31, S 14.02.

1f: 73%; IR (KBr) cm^{-1} 3340, 1765, 1740, 1620, 1575, 1548; ^1H NMR (d_6-DMSO) δ 1.09 (d, J=7.0 Hz, 3H), 1.14 (d, J=6.2 Hz, 3H), 1.7~2.0 (m, 1H), 1.9~2.2 (m, 1H), 2.9 (m, 1H), 3.0~3.3 (m, 4H), 3.3~3.6 (m, 1H), 3.6~3.8 (m, 2H), 3.86~3.94 (m, 1H), 4.05~4.14 (m, 1H).

SI-MS (m/z) 421 $(M+H)^+$.

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