VOL. 52 NO. 10, OCT. 1999 THE JOURNAL OF ANTIBIOTICS pp. 900 — 907

Synthesis and Biological Evaluation of New Oral Carbapenems with 1-Methyl-5-oxopyrrolidin-3-ylthio Moiety

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(Received for publication June 9, 1999)

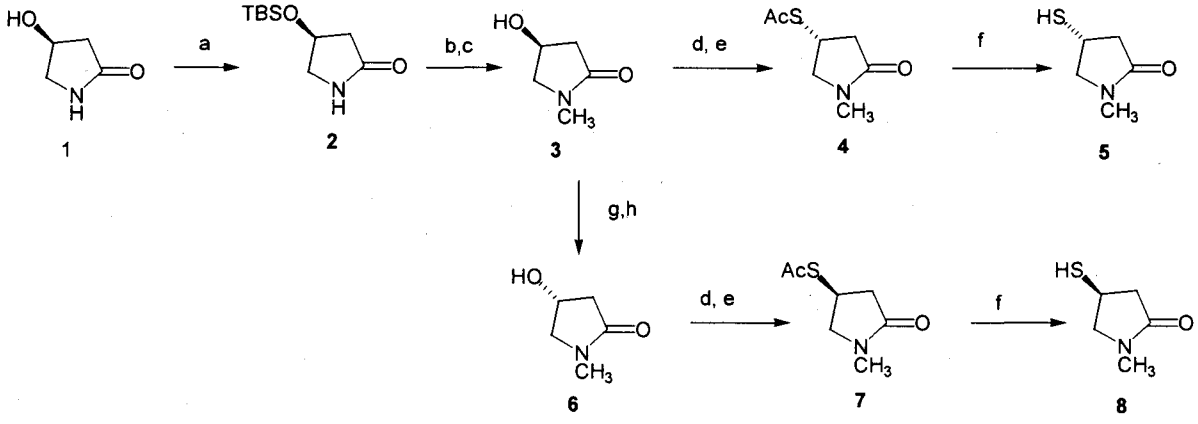
The synthesis and biological properties of 1β-methylcarbapenems with 1-methyl-5-oxopyrrolidin-3-ylthio group at the C-2 position were studied. The sodium (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[(*R*)-1-methyl-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate and its (*S*)-isomer at the 2-position show potent and well-balanced antibacterial activity. The pharmacokinetic parameters of the pivaloyloxymethyl esters of these two carbapenems were compared in mice. The *in vivo* potency of these carbapenems was compared with that of cefdinir. Good *in vivo* efficacy of these ester prodrugs reflected the high and prolonged blood levels in parent drugs achieved after oral administration to mice.

Carbapenems are the most potent β-lactam antibiotics, displaying broad antibacterial spectrum and have potent bactericidal activity against both Gram-positive and Gram-negative organisms.[1,2] Three parenteral carbapenems, imipenem/cilastatin,[3,4] panipenem/betamipron,[5~7] and meropenem,[8] have already been launched. However, oral carbapenems are not yet available although oral cephalosporins such as cefpodoxime proxetil and cefdinir have been used clinically.[9~11] In the course of structure-activity relationship studies of CS-834,[12,13] our attention was focused on the 1-methylated compound of the 5-oxopyrrolidin-3-ylthio group at the C-2 position. We were especially interested in increasing the activity and improving the pharmacokinetics of oral carbapenems when compared with oral cephalosporins. For this purpose, we synthesized sodium (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[(*R*)-1-methyl-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate and its (*S*)-isomer at the 2-position, and evaluated these derivatives for their antibacterial activity and pharmacokinetics after intravenous administration in mice. Also, the pivaloyloxymethyl esters of these two carbapenems were synthesized and the pharmacokinetics and *in vivo* efficacy were evaluated after oral administration in mice. Both carbapenem esters showed good oral absorption and the prolonged half-lives in mice. The half-lives ($T_{1/2}$) in both parent carboxylic acids were superior compared with that of cefdinir. The results showed that the favorable pharmacokinetics of these compounds have good *in vivo* efficacy against experimental infections in mice.

Chemistry

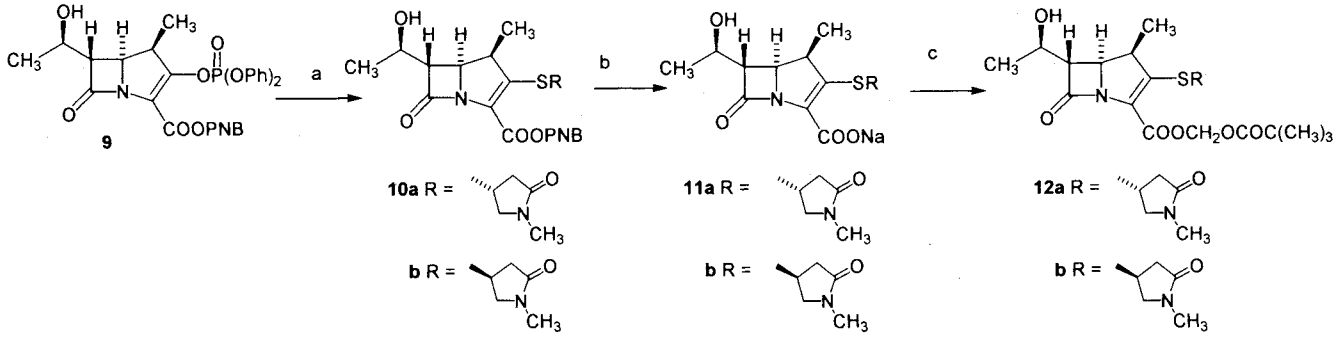
1β-Methyl carbapenems with (*R*)- and (*S*)-1-methyl-5-oxopyrrolidin-3-ylthio groups (**11a** and **11b**) were synthesized from (*S*)-4-hydroxy-2-oxopyrrolidine **1** *via* the routes as shown in Scheme 1 and 2, respecively. Optically active (*R*)-3-mercapto-1-methyl-5-oxopyrrolidine (**5**) was prepared from **1**. The hydroxy group of **1** was protected with *t*-butyldimethylsilyl chloride (TBSCl) and imidazole to give silyl compound **2** in quantitative yield. *N*-Methylation of **2** was carried out by using 60% NaH and methyl iodide to afford *N*-methylated compound in 57% yield. Deprotection of the *t*-butyldimethylsilyl group of the *N*-methyl compound with 1 N-HCl-MeOH afforded (*S*)-4-hydroxy-1-methyl-2-oxopyrrolidine (**3**) in 97% yield. Mesylation of **3** with methanesulfonyl chloride (MsCl) followed by thioacetylation with potassium thioacetate (AcSK) afforded thioacetate (**4**) in 67% yield. Hydrolysis of the thioacetyl group of **4** afforded mercaptan **5** in quantitative yield. Condensation of 1β-methylcarbapenem-2-yl diphenylphosphate (**9**) with **5** gave the carbapenem **10a**

Scheme 1.



Reagents: (a) TBSCl, imidazole; (b) NaH, then CH_3I; (c) 1 N-HCl; (d) MsCl, Et_3N; (e) AcSK; (f) 1 N-NaOMe; (g) Ph_3P, DEAD, 4-nitrobenzoic acid; (h) K_2CO_3.

Scheme 2.



Reagents: (a) **5** or **8**, $(iPr)_2NEt$; (b) H_2, 10% Pd-C; (c) $ICH_2OCOC(CH_3)_3$.

in 75% yield. Deprotection of the 4-nitrobenzyl (PNB) group of **10a** was carried out by hydrogenation in the presence of 10% Pd-C. Purification of the product by reversed phase column chromatography and lyophilization afforded the parent carbapenem **11a** in 80% yield. Esterification of **11a** with pivaloyloxymethyl iodide in *N*, *N*-dimethylformamide furnished carbapenem ester **12a** in 94% yield. Alternatively, (*R*)-4-hydroxy-1-methyl-2-oxopyrrolidine (**6**) was prepared in 59% yield from **3** by Mitsunobu reaction with triphenylphosphine, dietyl azodicarboxylate (DEAD) and 4-nitrobenzoic acid followed by ester hydrolysis. Optically active (*S*)-3-mercapto-1-methyl-5-oxopyrrolidine (**8**) was prepared *via* **7** from **6** in a similar manner to **5**. The carbapenem **11b** was synthesized by condensation of **9** with **8** followed by deprotection of the PNB group. Esterification of **11b** with pivaloyloxymethyl iodide afforded the carbapenem ester **12b** in the same way.

Biological Properties

The antimicrobial activities (MICs) of the parent carbapenems **11a** and **11b** are shown in Table 1. The activities of both carbapenems are compared with that of cefdinir which is a representative oral cephalosporins. The carbapenems **11a** and **11b** showed a particularly broad spectrum and more potent activity than cefdinir against

Table 1. Antibacterial activity (MIC, μg/ml)[a] of carbapenems **11a** and **11b**, and cefdinir.

Organism	**11a**	**11b**	**Cefdinir**
Staphylococcus aureus 209P	0.05	0.05	0.02
S. aureus 56R	0.05	0.05	0.05
S. aureus 535 (MRSA)	12.5	6.2	100
Enterococcus faecalis 681	1.5	1.5	3.1
Escherichia coli NIHJ	≤0.01	≤0.01	0.2
E. coli 609	0.05	0.05	0.4
Salmonella enteritidis	≤0.01	0.02	0.2
Klebsiella pneumoniae 806	≤0.01	≤0.01	0.1
K. pneumoniae 846	0.1	0.05	3.1
Enterobacter cloacae 903	0.8	0.8	3.1
Serratia marcescens 1184	0.02	0.02	0.2
Proteus vulgaris 1420	0.05	0.05	0.1
Morganella morganii 1510	0.2	0.2	12.5
Pseudomonas aeruginosa 1001	25	50	>200

[a] MIC was determined by the agar dilution method with an inoculum of 10^7 cfu/ml.

Table 2. Pharmacokinetic parameters of oral carbapenems **12a** and **12b** in mice.

	12a	**12b**
dose (administration route)	50 mg/kg (po)[a]	50 mg/kg (po)[a]
Cmax (μg/ml)[b]	24.60	33.5
Tmax (hour)[c]	0.50	0.50
$T_{1/2}$ (hour)[d]	1.08	1.01
AUC (μg·h/ml)[e]	23.37	31.26
Absolute bioavailability (%)[f]	44.95	55.34

[a] Esters **12a** and **12b** were orally administered at a dose of 50 mg/kg as **11a** and **11b**, respectively.

[b] Maximum plasma concentration.

[c] Time at which Cmax is achieved.

[d] Harmonic mean apparent terminal disposition half-life.

[e] The area under the concentration-time curve.

[f] Absolute oral bioavailability assuming linear pharmacokinetics = $[(AUC/dose)_{po} / (AUC/dose)_{iv}] \times 100$. The AUCs of **11a** and **11b** were 52.01 and 60.57 μg·h/ml, respectively, after intravenous administration (50 mg/kg).

Gram-positive and -negative bacteria. Both the carbapenems showed moderately potent activity against methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa*, which are resistant to cefdinir. The antibacterial activity of both the carbapenems against MRSA and *P. aeruginosa* was only slightly affected by the stereochemistry at the 3-position of the oxopyrrolidine side chain. The (*R*)-isomer **11a** showed slightly inferior activity against MRSA, but

Table 3. Protective effect of oral carbapenems **12a** and **12b**, and cefdinir against experimental infection in mice.

Organism	ED_{50} (mg/kg)[a]		
	12a	**12b**	**Cefdinir**
S. aureus Smith[b]	1.35	1.21	5.94
E. coli 704	0.70	0.46	14.1
K. pneumoniae 866[b]	1.06	2.01	9.46

[a] 50% effective po dose.
[b] Challenged with 5% mucin.

showed slightly more potent activity against *P. aeruginosa*. In order to estimate possible differences in oral absorption, the pharmacokinetics in mice were compared with each other (Table 2). The carbapenems **12a** and **12b** showed good oral bioavailability and prolonged half-lives in the parent carboxylic acids which were 1.08 and 1.01 hours, respectively. These values are longer than that of cefdinir; 0.39 hours in mice as previously reported.[14] The Cmax, AUC and absolute bioavailablity of **12b** were slightly better than those of **12a**. In order to clarify advantages in the *in vivo* actvity, the protective effects of **12a** and **12b** against experimental infections in mice were compared with those of cefdinir as shown in Table 3. Although a significant difference of *in vivo* activity between **12a** and **12b** was not observed, both the carbapenems showed good protection against *S. aureus* Smith, *E. coli* 704 and *K. pneumoniae* 866. These carbapenems exhibited approximately 4 to 30 times greater *in vivo* efficacy against these 3 organisms compared with cefdinir. Clearly, these protective effects reflect the pharmakokinetics of both carbapenems.

Conclusion

New oral active 1β-methylcarbapenems with (*R*)- and (*S*)-1-methyl-5-oxopyrrolidin-3-ylthio moieties at the 2-position were synthesized. As expected, the parent carbapenems **11a** and **11b** showed more potent *in vitro* activity against Gram-positive and Gram-negative bacteria when compared with cefdinir. The respective carbapenem esters **12a** and **12b** showed prolonged half-lives after oral administration in mice. Also, both the carbapenems had higher *in vivo* potency against *S. aureus* Smith, *E. coli* 704 and *K. pneumoniae* 866 compared with cefdinir. On the whole, both carbapenems are superior to cefdinir, but no major difference of *in vitro* and *in vivo* activities between **12a** and **12b** based on the stereochemistry of (*R*)- and (*S*)-1-methyl-5-oxopyrrolidin-3-ylthio group was observed. Consequently, the pharmacokinetic evaluation of both carbapenems using other experimental animals are of interest in future research.

Experimental

General Methods

IR spectra were recorded on a Nicolet NIC FT-IR (5SXC) spectrometer. NMR spectra were determined on a Jeol GX-270 (270 MHz) or GX-400 (400 MHz) spectrometer using tetramethylsilane (TMS) or sodium 3-(trimethylsilyl)-propionate-d_4 (TSP) as an internal standard. The mp was determined using a Yanagimoto micro-melting point apparatus and was not corrected. Optical rotations were obtained with a Jasco DIP-370 polarimeter. UV spectra were recorded on a Shimadzu UV-3100 spectrometer. Column chromatography was carried out on Silica gel 60 (230~400 mesh, Art. 9385, Merck), Cosmosil 75C_{18} PREP (75 μm, Nacalai Tesque, Inc.).

Preparation of (*R*)-3-Mercapto-1-methyl-5-oxopyrrolidine (**5**)

i) (*S*)-4-*t*-Buthyldimethylsilyloxy-2-oxopyrrolidine (**2**)

To a solution of (*S*)-4-hydroxy-2-oxopyrrolidine **1** (10.1 g, 0.1 mol) in DMF (150 ml) were added imidazole (10.2 g, 0.15 mol) and *t*-butyldimethylsilyl chloride (18.1 g, 0.12 mol) at room temperature, and then the mixture was stirred for 3 hours. The mixture was diluted with EtOAc and washed with water and brine. The EtOAc layer was dried (Na_2SO_4) and concentrated by evaporation under reduced pressure to give compound **2** as a colorless powder (22.28 g): ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 0.04 (3H, s), 0.08 (3H, s), 0.12 (9H, s), 2.20~2.36 (1H, m), 2.48~2.64 (1H, m), 3.18~3.32 (1H, m), 3.55~3.67 (1H, m), 4.51~4.65 (1H, m), 5.75 (1H, br s).

ii) (*S*)-4-Hydroxy-1-methyl-2-oxopyrrolidine (**3**)

To a solution of **2** (4.31 g, 20 mmol) in DMF (45 ml) was added sodium hydride (0.96 g, 24 mmol, 60% w/w dispersion in mineral oil) under ice-cooling, and then the mixture was stirred at the same temperature for 30 minutes. To this mixture was added methyl iodide (2.49 ml) under ice-cooling and then the mixture was stirred at room temperture for 4 hours. The mixture was poured into ice-water and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane - EtOAc, 1 : 4) to give (*S*)-4-*t*-buthyldimethylsilyloxy-1-methyl-2-oxopyrrolidine as a pale-brown powder (2.6 g, 57% from **1**): ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 0.07 (6H, s), 0.88 (9H, s), 2.33 (1H, dd, J=17.2, 4.0 Hz), 2.61 (1H, dd, J=17.2, 7.3 Hz), 2.85 (3H, s), 3.23 (1H, dd, J=10.5, 3.0 Hz), 3.58 (1H, dd, J=10.5, 6.6 Hz), 4.42~4.52 (1H, m).

To a solution of (*S*)-4-*t*-buthyldimethylsilyloxy-1-methyl-2-oxopyrrolidine (9.52 g, 41.5 mmol) in MeOH (55 ml) was added 1 N hydrochloric acid (33.2 ml, 33.2 mmol) under ice-cooling, and the mixture was stirred at room temperature for 15 minutes. To the mixture was added $NaHCO_3$ (3.53 g, 42 mmol) and concentrated by evaporation under reduced pressure and dried *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2 - EtOH, 9 : 1) to afford the title compound **3** as colorless crystals (4.64 g, 97.0%): mp <50°C ; $[\alpha]_D^{24}$=−34.0° (c=0.67, H_2O); IR (KBr) cm^{-1} 1681, 1494, 1471, 1460, 1441, 1417, 1407, 1382, 1361, 1319, 1298, 1269, 1253, 1219, 1171, 1112; ^{1}H NMR (270 MHz, D_2O, TSP) δ 2.32 (1H, dd, J=18.1 Hz), 2.82 (1H, dd, J=18.1, 6.6 Hz), 2.85 (3H, S), 3.36 (1H, dd, J=11.5, 5.3 Hz), 4.54 (1H, m).

iii) (*R*)-3-Acetylthio-1-methyl-5-oxopyrrolidine (**4**)

To a solution of **3** (4.6 g, 40 mmol) in CH_2Cl_2 (90 ml) were added methane sulfonyl chloride (3.71 ml, 48 mmol) and triethyl amine (6.65 ml, 48 mmol) under ice-cooling, and then the mixture was stirred at the same temperature for 40 minutes. After addition of saturated aqueous $NaHCO_3$ (2 ml), the mixture was concentrated by evaporation under reduced pressure. To the residue was added CH_2Cl_2 - AcOEt (1 : 1, 250 ml) which was then filtered. The filtrate was concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2 - EtOAc - MeOH, 47 : 47 : 6) to give (*S*)-4-methanesulfonyloxy-1-methyl-2-oxopyrrolidine as a colorless powder (7.45 g, 96.5%): IR (KBr) cm^{-1} 1702, 1683, 1498, 1460, 1439, 1404, 1380, 1337, 1306, 1263, 1214, 1182, 1173, 1161, 1104; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 2.65 (1H, dd, J=17.8, 2.6 Hz), 2.82 (1H, dd, J=17.8, 7.9 Hz), 2.90 (3H, s), 3.07 (3H, s), 3.64 (1H, dd, J=11.9, 2.0 Hz), 3.80 (1H, 11.9, 5.9 Hz), 5.30~5.37 (1H, m).

To a solution of (*S*)-4-methanesulfonyloxy-1-methyl-2-oxopyrrolidine (7.45 g, 39 mmol) in CH_3CN (260 ml) was added potassium thioacetate (6.16 g, 54 mmol) at room temperature, and the mixture was refluxed for 4.5 hours. The mixture was diluted with AcOEt (160 ml) and filtered. The filtrate was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc) to give the title compound **4** as a pale-brown oil (5.83 g, 87.3%): IR (liquid film) cm^{-1} 1683, 1560, 1499, 1440, 1404, 1301, 1264, 1199, 1110; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 2.35 (3H, s), 2.35 (1H, dd, J=17.2, 6.6 Hz), 2.85 (1H, dd, J=17.2, 5.9 Hz), 2.86 (3H, s), 3.29 (1H, dd, J=10.6, 5.3 Hz), 3.88 (1H, dd, J=10.6, 7.3 Hz), 4.02~4.13 (1H, m).

iv) (*R*)-3-Mercapto-1-methyl-5-oxopyrrolidine (**5**)

To a solution of **4** (5.80 g, 33.5 mmol) in MeOH (60 ml) was added 28% sodium methoxide in MeOH (6.8 ml, 33.5 mmol) for 5 minutes under ice-cooling, and the mixture was stirred at the same temperature for 10 minutes. To this solution was added 1 N hydrochloric acid (35.5 ml, 33.5 mmol) and the mixture was concentrated by evaporation under reduced pressure and dried *in vacuo*. To the residue was added AcOEt (200 ml) which was then filtered. The filtrate was concentrated by evaporation under reduced pressure and dried to give the title mercaptan **5** as a pale-brown oil (4.13 g, 94.1%): IR (liquid film) cm^{-1} 1690, 1499, 1435, 1403, 1357, 1302, 1264, 1127; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 1.93 (1H, d, J=6.6 Hz), 2.36 (1H, dd, J=17.2, 6.6 Hz), 2.75~2.29 (1H, m), 2.87 (3H, s),

3.29 (1H, dd, J=10.6, 5.3 Hz), 3.50~3.66 (1H, m), 3.77 (1H, dd, J=10.6, 7.3 Hz).

Preparation of (*S*)-3-Mercapto-1-methyl-5-oxopyrrolidine (**8**)

i) (*R*)-4-Hydroxy-1-methyl-2-oxopyrrolidine (**6**)

To a solution of tripenylphosphine (11.4 g, 43.4 mmol) in dry THF (120 ml) was added diethyl azodicarboxylate (6.83 ml) under ice-cooling and then nitrobenzoic acid was added. The mixture was stirred at the same temperature for 1 hour and was cooled to −25~−20°C. To this mixture was added **3** (2.5 g, 21.7 mmol) in THF (50 ml) at the same temperature, and then the mixture was warmed to room temperature and was allowed to stand overnight. The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc - MeOH, 9 : 1) to give (*R*)-4-(4-nitrobenzoyloxy)-1-methyl-2-oxopyrrolidine as a colorless powder (3.95 g, 68.9%): IR (KBr) cm^{-1} 1722, 1687, 1645, 1611, 1600, 1526, 1492, 1438, 1406, 1399, 1353, 1321, 1307, 1271, 1222, 1183, 1122, 1109; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 2.66 (1H, dd, J=17.8, 1.9 Hz), 2.92 (1H, dd, J=17.8, 6.3 Hz), 2.93 (3H, S), 3.54 (1H, dd, J=11.8, 1.9 Hz), 3.91 (1H, dd, J=11.8, 6.1 Hz), 5.57~5.66 (1H, m), 8.21 (2H, d, J=9.1 Hz), 8.31 (2H, d, J=9.1 Hz).

To a solution of (*R*)-4-(4-nitrobenzoyloxy)-1-methyl-2-oxopyrrolidine (5.1 g, 19.3 mmol) in MeOH (100 ml) was added K_2CO_3 (0.7 g, 5.1 mmol) under ice-cooling and the mixture was stirred at the same temperature for 20 minutes. To the mixture was added ammonium chloride (1.1 g, 20.5 mmol) and the mixture was stirred for 5 minutes and filtered. The filtrate was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc - MeOH, 9 : 1) to afford the title compound **6** as an oil (1.90 g): $[\alpha]_D^{24}$=+34.4° (c=1.23, H_2O), IR (liquid) cm^{-1} 1667, 1503, 1446, 1406, 1339, 1305, 1267, 1220, 1112; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 2.37 (1H, dd, J=17.2, 2.3 Hz), 2.69 (1H, dd, J=17.2, 6.6 Hz), 2.87 (3H, s), 3.03 (1H, br s), 3.32 (1H, dd, J=10.9, 2.0 Hz), 3.66 (1H, dd, J=10.9, 5.7 Hz), 4.48~4.57 (1H, m).

ii) (*S*)-3-Mercapto-1-methyl-5-oxopyrrolidine (**10**)

The title compound **8** was prepared as an oil from **6** by a similar manner as that described for the preparation of **5**.

Synthesis of Pivaloyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydoxyethyl]-1-methyl-2-[(*R*)-1-methyl-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (**12a**)

i) 4-Nitrobenzyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydoxyethyl]-1-methyl-2-[(*R*)-1-methyl-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (**10a**)

To a solution of 4-nitrobenzyl (1*R*,5*S*,6*S*)-2-diphenylphosphoryloxy-6-[(*R*)-1-hydoxyethyl]-1-methyl-1-carbapenam-3-carboxylate (18.58 g, 31.3 mmol)) in acetonitrile (260 ml) were added a solution of **3** in acetonitrile (20 ml) and *N*,*N*-diisopropylethylamine (5.46 ml) under ice-cooling and the mixture was stirred for 30 minutes and was allowed to stand in a refrigerator overnight. The mixture was concentrated by evaporation under reduced pressure and to the residue were added water (100 ml) and acetonitrile (150 ml). The precipitated crystals were collected by filtration, washed with acetonitrile - water (9 : 1), and dried to give the title compound **11a** as pale-brown crystals (12.8 g, 86.0%): mp 169°C; IR (KBr) cm^{-1} 1772, 1702, 1692, 1667, 1608, 1546, 1518, 1501, 1467, 1455, 1420, 1410, 1401, 1383, 1342, 1322, 1308, 1281, 1267, 1256, 1229, 1206, 1183, 1138, 1101; ^1H NMR (400 MHz, DMSO-d_6, TMS) δ 1.16 (3H, d, J=6.5 Hz), 1.17 (3H, d, J=7.5 Hz), 2.24 (1H, dd, J=17.1, 3.8 Hz), 2.74 (3H, s), 2.89 (1H, dd, J=17.1, 7.9 Hz), 3.22 (1H, dd, J=11.0, 3.7 Hz), 3.29 (1H, dd, J=6.3, 2.6 Hz), 3.42~3.50 (1H, m), 3.81 (1H, dd, J=11.0, 7.5 Hz), 3.94~4.05 (2H, m), 4.25 (1H, dd, J=9.5, 2.7 Hz), 5.09 (1H, d, J=5.1 Hz), 5.29, 5.45 (2H, AB, J=14.0 Hz), 7.71 (2H, d, J=8.7 Hz), 8.23 (2H, d, J=8.7 Hz).

ii) Sodium (1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydoxyethyl]-1-methyl-2-[(*R*)-1-methyl-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (**11a**)

To a solution of **10a** (6.18 g, 13 mmol) in THF (240 ml) and aqueous $NaHCO_3$ (1.09 g, 13 mmol; water 120 ml) was added 7.5% Pd-charcoal (10 g, wet, 52.4% water, Kawaken Fine Chemicals Co., Ltd.) and the mixture was stirred rigorously at 30°C in an atomosphere of hydrogen for 2 hours. The catalyst was removed by filtration from the reaction mixture, and the filtrate was washed twice with diethyl ether. The aqueous layer was then concentrated by evaporation under reduced pressure and the residue was purified by a reverse phase column chromatography (Nacalai Tesque, Inc., Cosmosil 75C_{18}-PREP, water). The desired fraction was concentrated and then lyophilized to give the title compound **11a** as a colorless powder (3.46 g, 73.4%):

Anal Calcd for $C_{15}H_{19}N_2O_5SNa \cdot 1/2H_2O$:
C 48.51, H 5.43, N 7.54, S 8.63, Na 6.19.
Found: C 48.25, H 5.68, N 7.43, S 8.62, Na 6.40.

UV (H_2O) λ_{max} nm 300; IR (KBr) cm^{-1} 1752, 1678, 1603, 1504, 1450, 1398, 1306, 1267, 1226, 1183, 1147, 1110; ^1H NMR (270 MHz, D_2O, TSP) δ 1.22 (3H, d, J=7.3 Hz), 1.30 (3H, d, J=6.6 Hz), 2.49 (1H, dd, J=17.8, 3.3 Hz), 2.86 (3H, s), 3.02 (1H, dd, J=17.8, 7.6 Hz), 3.28~3.47 (3H, m), 3.92~4.05 (2H, m), 4.21~4.30 (2H, m).

iii) Pivaloyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydoxyethyl]-1-methyl-2-[(*R*)-1-methyl-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (**12a**)

To a solution of **11a** (2.3 g, 6.34 mmol) in *N*,*N*-dimethylacetamide (25 ml) was added pivaloyloxymethyl iodide (1.14 ml, 7.61 mmol) under ice-cooling, and then the mixture was stirred for 1 hour. The mixture was diluted with AcOEt (200 ml) and was washed with 2% sodium thiosulfate, water and brine. The organic layer was dried ($MgSO_4$) and was concentrated by evaporation under reduced pressure. Crystals were formed during the evaporation. The resulting crystals were collected, filtered, and dried to give the title compound **12a** (2.70 g, 93.7% yield): mp 192°C;

Anal Calcd for $C_{21}H_{30}N_2O_7S$:
C 55.49, H 6.65, N 6.16, S 7.05.
Found: C 55.93, H 6.57, N 6.21, S 6.96.

$[\alpha]_D^{23}$=+24.6° (c=1.0, MeOH); UV (CH_3CN) λ_{max} nm 323; IR (KBr) cm^{-1} 1787, 1757, 1709, 1664; ^1H NMR (400 MHz, DMSO-d_6, TMS) δ 1.13~1.17 (15H, m), 2.22 (1H, dd, J=17.1, 3.9 Hz), 2.73 (3H, s), 2.88 (1H, dd, J=17.1, 7.9 Hz), 3.20 (1H, dd, J=11.0, 3.9 Hz), 3.26 (1H, dd, J=6.4, 2.7 Hz), 3.41~3.49 (1H, m), 3.81 (1H, dd, J=11.0, 7.8 Hz), 3.94~4.05 (2H, m), 4.23 (1H, dd, J=9.5, 2.7 Hz), 5.08 (1H, d, J=5.1 Hz), 5.71 (1H, d, J=5.9 Hz).

Synthesis of Pivaloyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydoxyethyl]-1-methyl-2-[(*S*)-1-methyl-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (**12b**)

i) 4-Nitrobenzyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydoxyethyl]-1-methyl-2-[(*S*)-1-methyl-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (**10b**)

The title compound **10b** was prepared in 85% yield as a powder from **8** by a similar manner as that described for the preparation of **10a**: ^1H NMR (400 MHz, DMSO-d_6, TMS) δ 1.16 (3H, d, J=6.4 Hz), 1.18 (3H, d, J=7.6 Hz), 2.14 (1H, dd, J=17.2, 4.7 Hz), 2.71 (3H, s), 3.28~3.31 (2H, m), 3.41~3.48 (1H, m), 3.85 (1H, dd, J=10.4, 6.7 Hz), 3.96~4.06 (2H, m), 4.24 (1H, dd, J=9.3, 2.9 Hz), 5.09 (1H, d, J=5.2 Hz), 5.29, 5.46 (2H, AB, J=14.1 Hz), 7.72 (2H, d, J=8.7 Hz), 8.23 (2H, d, J=8.7 Hz).

ii) Sodium (1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydoxyethyl]-1-methyl-2-[(*S*)-1-methyl-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (**11b**)

The title compound **11b** was prepared in 80% yield as a powder from **10b** by the similar manner as that described for the preparation of **11a**:

Anal Calcd for $C_{15}H_{19}N_2O_5SNa \cdot H_2O$:
C 47.36, H 5.57, N 7.36, S 8.43.
Found: C 46.84, H 5.71, N 7.24, S 8.45.

UV (H_2O) λ_{max} nm 300; IR (KBr) cm^{-1} 1744, 1673, 1640, 1597, 1553, 1504, 1451, 1403, 1309, 1266, 1230, 1183, 1165, 1149, 1111; ^1H NMR (270 MHz, D_2O, TPS) δ 1.23 (3H, d, J=7.3 Hz), 1.30 (3H, d, J=6.6 Hz), 2.37 (1H, dd, J=17.2, 3.3 Hz), 2.84 (3H, s), 2.99 (1H, dd, J=17.2, 8.3 Hz), 3.27~3.49 (3H, m), 3.92~4.03 (2H, m), 4.21~4.31 (m).

iii) Pivaloyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydoxyethyl]-1-methyl-2-[(*S*)-1-methyl-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (**12b**)

The title compound **12b** was prepared in 80% yield as a powder from **11b** by a similar manner as that described for the preparation of **12a**: mp 137°C;

Anal Calcd for $C_{21}H_{30}N_2O_7S$:
C 55.49, H 6.65, N 6.16, S 7.05.
Found: C 55.11, H 6.66, N 5.99, S 6.96.

$[\alpha]_D^{23}$=−3.4° (c=1.0, MeOH); UV (CH_3CN) λ_{max} nm 323; IR (KBr) cm^{-1} 1784, 1754, 1725, 1685, 1667; ^1H NMR (400 MHz, DMSO-d_6, TMS) δ 1.14~1.18 (15H, m), 2.12 (1H, dd, J=17.3, 4.7 Hz), 2.70 (3H, s), 2.81 (1H, dd, J=17.3, 8.8 Hz), 3.24~3.28 (2H, m), 3.41~3.48 (1H, m), 3.85 (1H, dd, J=10.6, 6.7 Hz), 3.94~4.02 (2H, m), 4.22 (1H, dd, J=9.5, 2.6 Hz), 5.08 (1H, d, J=5.2 Hz), 5.73 (1H, d, J=5.9 Hz), 5.88 (1H, d, J=5.9 Hz).

Measurement of Antibacterial Activity

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

Pharmacokinetics

Plasma levels of carbapenems **11a** and **11b** were determined by HPLC, after oral administration of **12a** and **12b** (50 mg/kg as **11a** and **11b**, respectively) as a 0.2 ml suspension of 0.5% tragacanth or intravenous administration of **11a** and **11b** (50 mg/kg, respectively) in 0.2 ml saline to SPF male *dd*Y mice (n=3).

Therapeutic Effect on Systemic Infection in Mice

Overnight cultures of organisms grown at 37°C in Trypto-soy broth (Eiken Chemical Co., Ltd.) were diluted according to their virulence. The diluted cultures, if necessary, were mixed with the same amount of gastric mucin (Tokyo Kasei Kogyo Co., Ltd.). Seven male SPF *dd*Y mice in each group were infected intraperitoneally with 0.2 ml portions of these bacterial mixtures. β-Lactam antibiotics (**12a**, **12b** and cefdinir) were administered orally as a 0.2 ml suspension of 0.5% sodium carboxymethyl cellose (Kanto Chemical Co., Inc.) immediately and 4 hours after infection. The ED_{50}s of the mice were calculated by the probit method from the survival rates on the 5th day after infection.

Acknowledgments

We wish to thank Mr. R. ENDO and Dr. A. NAKAGAWA for their help with the large scale preparation and pharmacokinetic studies.

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