Synthesis and Antibacterial Activity of 2-(Isoxazolidinio-5-yl)carbapenem Derivatives

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The synthesis and antibacterial activity of the title compounds having an isoxazolidine ring at the C-2 position are described. These derivatives were synthesized by the 1,3-dipolar cycloaddition reaction of nitrone with 2-vinyl carbapenems. This 1,3-dipolar cycloaddition reaction proceeded regioselectively to give diastereomeric isomers of 2-(isoxazolidin-5-yl)carbapenems. It was ascertained that the antibacterial activity of 1β-methylcarbapenem derivatives was superior to that of the corresponding 1H-carbapenem derivatives, and between the 2-(isoxazolidin-5-yl)-1β-methylcarbapenems the antibacterial activity of the 5'*R*-isomer was slightly better than that of the 5'*S*-isomer.

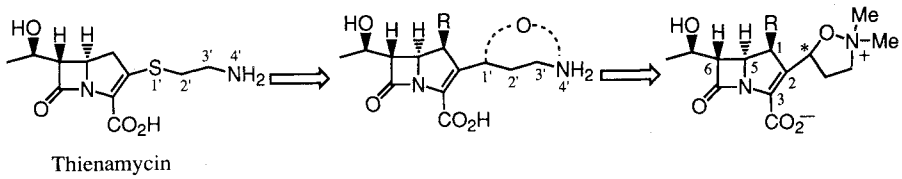
The discovery of thienamycin[1] in 1976 opened up a new antibiotic field, and numerous chemical modifications[2~5] were introduced for exploiting this novel antibiotic as a clinical candidate. However, these efforts have been mostly limited to the natural-type alkylthio side chain at the C-2 position because of the instability of the carbapenem nucleus. In our laboratories, we have been interested in carbapenems having unnatural-type side chains, which directly connect a carbon unit in place of sulfur at the C-2 position, and have found that 2-(N-methyl-4-pyridinio)thiomethyl derivatives show excellent activities against both Gram-positive and Gram-negative bacteria.[6,7]

We planned to prepare unnatural-type carbapenems which were based on the side chain at the C-2 position of thienamycin. We first intended to replace $S^{1'}$-$C^{2'}$-$C^{3'}$-$N^{4'}$ of thienamycin side chain with unnatural-type $C^{1'}$-$C^{2'}$-$C^{3'}$-$N^{4'}$, forming a rigid 5-membered ring by combining $C^{1'}$ and $N^{4'}$ through a spacer of one atom to fix its conformation. We anticipated that the adoption of an electron-withdrawing oxygen atom as a spacer should enhance the chemical reactivity of the β-lactam ring, and would improve biological activity.[8] Moreover we chose to quaternarize the nitrogen atom $N^{4'}$ because a cation charge on the side chain had tended to improve activity. Finally, we decided on a isoxazolidinio substituent as a side chain at the C-2 position. An isoxazolidine ring could be constructed by 1,3-dipolar cycloaddition reaction. Herein, we describe the synthesis of 2-(isoxazolidinio-5-yl)carbapenem derivatives *via* the 1,3-dipolar cycloaddition reaction of 2-vinyl carbapenems with nitrone and their potent antibacterial activities.

Chemistry

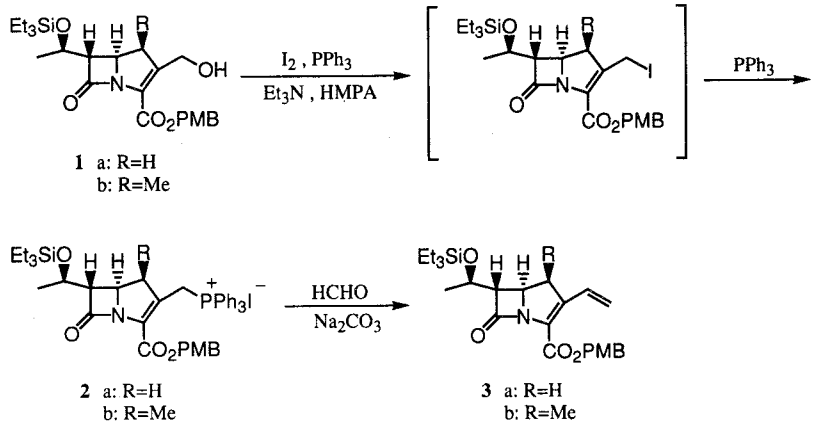
Requisite material for 1,3-dipolar cycloaddition reaction, 2-vinylcarbapenems **3**, were synthesized from 2-hydroxymethylcarbapenems **1**[6] (Scheme 1). 2-Hydroxymethylcarbapenems **1** were transformed to 2-iodomethylcarbapenems by the direct iodination method ($PPh_3/I_2/Et_3N$/HMPA) *in situ*, and treatment of 2-iodomethyl carbapenems with triphenylphosphine resulted in the formation of somewhat stable phosphonium derivatives **2**.[9] Wittig reaction of phosphonium salts **2** with aqueous formaldehyde in the presence of sodium carbonate as a base gave 2-vinylcarbapenems **3**. We stored **3b** as a solution of toluene since pure neat compound **3b** polymerized even stored in a freezer. The isoxazolidine ring was constructed by the 1,3-dipolar cycloaddition reaction of 2-vinyl-1β-methylcarbapenem

Fig. 1. Design of novel unnatural-type carbapenems.

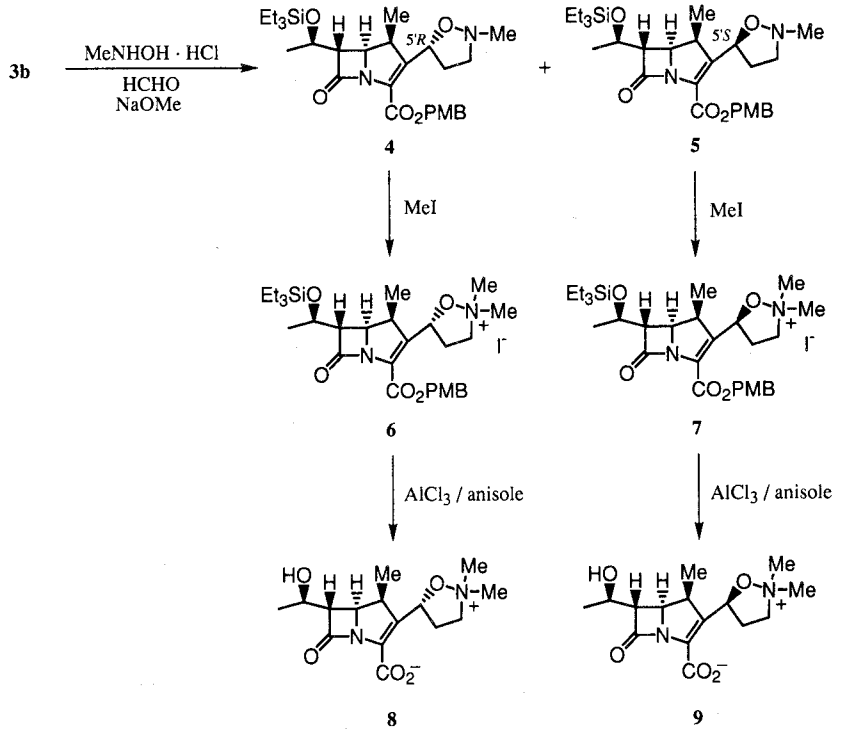


3b with nitrone (Scheme 2), which was generated *in situ* from N-methylhydroxylamine hydrochloride and formaldehyde in the presence of sodium methoxide. This cycloaddition reaction gave two diastereomeric isomers (**4**, **5**) of 2-(2-methylisoxazolidin-5-yl)-1β-methylcarbapenem in 22% and 45% yield, respectively. The corresponding regioisomers, 2-(2-methylisoxazolidin-4-yl)-1β-methylcarbapenems, were not detected in this re-

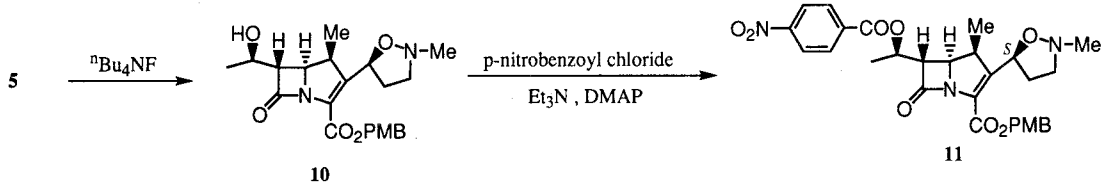
Scheme 1. Synthesis of 2-vinylcarbapenems **3**.



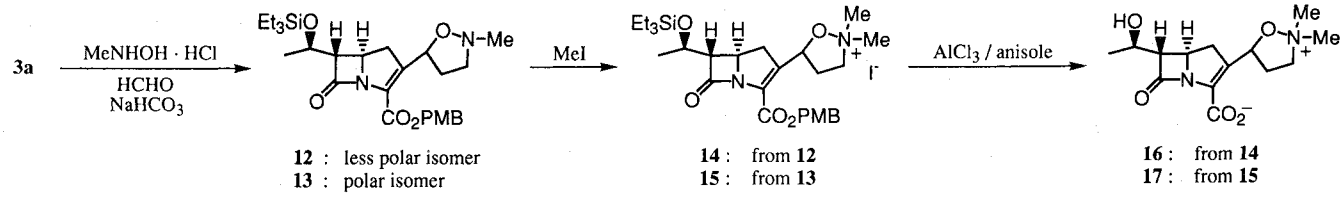
Scheme 2. Synthesis of 2-(isoxazolidinio-5-yl)-1β-methylcarbapenems **8** and **9**.



Scheme 3. Synthesis of compound **11**.



Scheme 4. Synthesis of 2-(isoxazolidinio-5-yl)carbapenems **16** and **17**.



12 : less polar isomer
13 : polar isomer

14 : from **12**
15 : from **13**

16 : from **14**
17 : from **15**

Table 1. *In vitro* antibacterial activities (MIC; μg/ml) of carbapenem derivatives.

Organism	**8**	**9**	**16**	**17**	Imipenem
Staphylococcus aureus FDA 209 JC-1	0.01	0.01	0.02	0.02	0.01
Staphylococcus aureus Smith	0.01	0.01	0.05	0.02	0.01
Staphylococcus aureus SR 3131	0.1	0.1	0.2	0.2	0.05
Streptococcus pyogenes C-203	<0.003	<0.003	0.006	0.01	<0.003
Streptococcus pneumoniae Type 1	<0.003	<0.003	0.006	0.006	<0.003
Enterococcus faecalis SR 1004	0.8	0.8	1.6	1.6	1.6
Escherichia coli NIHJ JC-2	0.05	0.05	0.1	0.2	0.1
Escherichia coli EC-14	0.05	0.05	0.1	0.2	0.1
Klebsiella pneumoniae SR 1	0.1	0.2	0.2	0.4	0.2
Proteus mirabilis PR-4	0.4	0.4	12.5	12.5	0.4
Proteus vulgaris CN-329	1.6	1.6	1.6	3.1	0.4
Enterobacter cloacae SR 233	0.1	0.1	0.4	0.4	1.6
Serratia marcescens ATCC 13880	3.1	3.1	1.6	1.6	0.4
Pseudomonas aeruginosa ATCC 25619	0.8	0.8	3.1	6.3	0.8
Pseudomonas aeruginosa SR 24	0.8	1.6	6.3	6.3	1.6

action. These two diastereomeric isomers **4** and **5** could be separated by silica gel column chromatography. After quaternarization of compounds **4** and **5** with methyl iodide, conventional deprotection by $AlCl_3$/anisole method[10] easily gave **8** and **9**, respectively. With respect to stereochemistry at the 5′-position of isoxazolidine ring, we determined that compound **5** has *S*-configuration at the 5′-position by X-ray crystallographic analysis of compound **11** which was prepared according to Scheme 3. Therefore, it was ascertained that configurations at the 5′-position of compounds **8** and **9** are 5′*R* and 5′*S*, respectively. The corresponding 1H carbapenem derivatives (**16**, **17**) were synthesized from compound **3a** by the similar method (Scheme 4), and two diastereomeric isomers **12** and **13** could be separated by silica gel column chromatography. Stereochemistry at the 5′-position of isoxazolidine ring in diastereomeric isomers **16** and **17** was not determined.

Antibacterial Activity

The *in vitro* antibacterial activity of the new carbapenems **8**, **9**, **16** and **17** against selected Gram-positive and Gram-negative bacteria were summarized in Table 1. For comparison, the MIC values of imipenem are also listed.

As shown in Table 1, all compounds prepared in this study and imipenem had a comparable antibacterial activity against both Gram-positive and Gram-negative bacteria including *P. aeruginosa*. Stereochemistry at the 5′-position of isoxazolidine ring was found to have some effect on their antibacterial activity. 5′*R*-isomer **8** and 5′*S*-isomer **9** are almost equivalent against Gram-positive bacteria, while 5′*R*-isomer **8** possessed a slightly better activity than 5′*S*-isomer against some Gram-negative bacteria. In general, 1β-methylcarbapenem derivatives, **8** and **9**, were superior to the corresponding 1H analogues, **16** and **17**.

In vivo activities (ED_{50}) of 1β-methyl carbapenem

Table 2. *In vivo* activities (EC_{50}; mg/kg[a]) against infections in mice.

	S. aureus Smith	*E. faecalis* SR 1004	*E. coli* EC-14	*P. aeruginosa* SR 24
8	0.02(0.01)[b]	1.8(0.8)	0.06(0.05)	0.8(0.8)
9	0.04(0.01)	N.T.[c]	N.T.	1.5(1.6)
Imipenem	0.02(0.01)	3.5(1.6)	0.3(0.1)	0.6(1.6)

[a] Challenge dose: 2×10^6 CFU/mouse for *S.a.*
3×10^6 CFU/mouse for *E.f.*
2×10^6 CFU/mouse for *E.c.*
3×10^4 CFU/mouse for *P.a.*
[b] MIC values.
[c] Not tested.

derivatives **8** and **9** together with the data of imipenem as reference were shown in Table 2. Therapeutic efficacy of 1β-methyl compound **8** was excellent and better than that of imipenem against *E. coli* infection.

In conclusion, we found that 2-(isoxazolidinio-5-yl)carbapenem derivatives, especially 2-(5*R*-isoxazolidinio-5-yl)-1β-methylcarbapenem, had an excellent broad spectrum antibacterial activity.

Experimental

Chemistry

Reagents were used as supplied unless otherwise noted. Reactions were carried out under nitrogen using dry solvents. Silica gel (E. Merck, 230~400 mesh) and high porous polymer CHP 20P (Mitsubishi Kasei, 75~150 μ) were used for column chromatography. Melting points were determined on a Yanagimoto apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 260-10 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Varian VXR-200 (200 MHz) spectrometer. Chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal standard in $CDCl_3$, and determined by using DOH (δ 4.80) as an internal standard in D_2O. Mass spectra (MS) were obtained on a Hitachi M-90 (SIMS) mass spectrometer. Most of the compounds reported here are either non-crystalline solid or viscous oil or unstable. Hence, their analytical data could not be obtained.

Determination of Antibacterial Activity

The *in vitro* antibacterial activity is given as minimum inhibitory concentration (MIC) in μg/ml as determined by the serial agar dilution method (sensitivity test agar) after incubation at 37°C for 18~20 hours with an inoculum size of one loopful of 10^6 CFU/ml.

In vivo Antibacterial Activity Test

ICR female mice (age: 5 weeks) were infected intraperitoneally with the bacterial suspension in 5% mucin and compounds were administered subcutaneously at 1 and 5 hours after infection. ED_{50} values were calculated from survival ratio of mice on 7th day after infection by the probit method.

p-Methoxybenzyl (5*R*,6*S*)-6-[(1*R*)-1-(Triethylsilyloxy)-ethyl]-2-(triphenylphosphonio)-methylcarbapen-2-em-3-carboxylate Iodide (**2a**)

To a solution of **1a** (6.73 g, 14.6 mmol) in CH_2Cl_2 (70 ml) were added triethylamine (2.24 ml, 16.0 mmol), iodine (4.07 g, 16.0 mmol), triphenylphosphine (5.74 g, 21.9 mmol) at −50°C. After being stirred at the same temperature for 1 hour, hexamethylphosphoramide (5.07 ml, 29.2 mmol) was added. After being stirred for more 30 minutes, a solution of 2-iodomethylcarbapenem derivative was obtained and then treated with triphenylphosphine (3.82 g, 14.6 mmol). The reaction mixture was left at −20°C overnight then diluted with water and ethyl acetate, and extracted with ethyl acetate. The extracts were washed with water, dried over magnesium sulfate, and evaporated *in vacuo*. After being washed the residue with ether, a crude phosphonium salt **2a** (11.85 g, 97%) was obtained as a yellow foam: IR ($CHCl_3$) 1779, 1709 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.55 (q, J=8.0 Hz, 6H), 0.90 (t, J=8.0 Hz, 9H), 1.18 (d, J=6.2 Hz, 3H), 2.64~2.82 (m, 1H), 3.01 (dd, J=5.2, 3.0 Hz, 1H), 3.28~3.55 (m, 1H), 3.83 (s, 3H), 4.02~4.25 (m, 2H), 4.80 and 4.85 (ABq, J=12.1 Hz, 2H), 5.29 (br-dd, J=17.7, 14.4 Hz, 1H), 5.75 (br-t, J=15.5 Hz, 1H), 6.86 (d, J=8.8 Hz, 2H), 7.19 (d, J=8.8 Hz, 2H), 7.40~7.90 (m, 15H); HR-MS Calcd for $C_{42}H_{49}NO_5PSi$ $(M-I)^+$ 706.3115; Found 706.3118.

p-Methoxybenzyl (1*S*,5*R*,6*S*)-1-Methyl-6-[(1*R*)-1-(triethylsilyloxy)ethyl]-2-(triphenyl-phosphonio)methyl-carbapen-2-em-3-carboxylate Iodide (**2b**)

This compound **2b** (87.57 g, 91% purity as 100% yield) was prepared from **1b** (43.68 g, 92 mmol) by a similar procedure used for **2a** as a yellow foam: IR ($CHCl_3$) 1778, 1709 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.55 (q, J=7.8 Hz, 6H), 0.90 (t, J=7.8 Hz, 9H), 1.17 (d, J=6.2 Hz, 3H), 1.35 (d, J=7.2 Hz, 3H), 2.65~2.90 (m, 1H), 3.23 (dd, J=4.6, 3.0 Hz, 1H), 3.82 (s, 3H), 4.06 (dd, J=10.2, 3.0 Hz, 1H), 4.22 (dq, J=6.2, 4.6 Hz, 1H), 4.77 (s, 2H), 5.19 (br-t, J=15.6 Hz, 1H), 5.45 (br-t, J=15.6 Hz, 1H), 6.86 (d, J=8.8 Hz, 2H), 7.18 (d, J=8.8 Hz, 2H), 7.41~7.87 (m, 15H); HR-MS Calcd for $C_{43}H_{51}NO_5PSi$ $(M-I)^+$ 720.3272; Found 720.3286.

p-Methoxybenzyl (5*R*,6*S*)-6-[(1*R*)-1-(Triethylsilyloxy)-ethyl]-2-vinylcarbapen-2-em-3-carboxylate (**3a**)

To an ice-cooled solution of crude **2a** (1.00 g, 1.2 mmol) in a mixed solvent of toluene (6 ml), CH_2Cl_2 (0.5 ml) and water (1 ml) were added 37% aqueous formaldehyde (1.95 ml, 24 mmol) and sodium bicarbonate (191 mg, 1.8 mmol), and the mixture was stirred for 1 hour. The reaction mixture was poured into water

and ethyl acetate, and extracted with ethyl acetate. The combined extracts were washed with water and brine, dried over magnesium sulfate, and evaporated under reduced pressure. Purification of the residue by silica gel column chromatography gave 2-vinylcarbapenem **3** (73 mg, 13%) as a viscous colorless oil: IR ($CHCl_3$) 1778, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.60 (q, J=8.0 Hz, 6H), 0.95 (t, J=8.0 Hz, 9H), 1.28 (d, J=6.0 Hz, 3H), 2.85~3.15 (m, 2H), 3.12 (dd, J=6.6, 2.8 Hz, 1H), 3.80 (s, 3H), 4.12 (dt, J=9.6, 2.8 Hz, 1H), 4.21 (quint, J=6.4 Hz, 1H), 5.21 and 5.25 (ABq, J=12.2 Hz, 2H), 5.35 (dd, J=16.0, 1.2 Hz, 1H), 5.42 (dd, J=9.4, 1.2 Hz, 1H), 6.88 (d, J=8.8 Hz, 2H), 7.39 (d, J=8.8 Hz, 2H), 7.39 (dd, J=16.0, 9.4 Hz, 2H); HR-MS Calcd for $C_{25}H_{36}NO_5Si$ $(M+H)^+$ 458.2360; Found 458.2358.

p-Methoxybenzyl (1*S*,5*R*,6*S*)-1-Methyl-6-[(1*R*)-1-(triethylsilyloxy)ethyl]-2-vinyl-carbapen-2-em-3-carboxylate (**3b**)

This compound **3b** (12.0 g) was synthesized from **2b** (37.23 g of 91% purity, 40 mmol) by a similar procedure used for **3a**: 64% yield as a viscous colorless oil. This compound was stored as a solution of toluene since pure neat compound polymerized under storage in freezer; IR ($CHCl_3$) 1760, 1708 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.59 (q, J=8.0 Hz, 6H), 0.94 (t, J=8.0 Hz, 9H), 1.20 (d, J=7.6Hz, 3H), 1.29 (d, J=6.0 Hz, 3H), 3.19 (dd, J=6.7, 2.6 Hz, 1H), 3.35 (quint, J=7.6 Hz, 1H), 3.80 (s, 3H), 4.12 (dd, J=9.4, 2.6 Hz, 1H), 4.22 (quint, J=6.4 Hz, 1H), 5.23 (s, 2H), 5.43 (dd, J=11.2, 0.9 Hz, 1H), 5.50 (dd, J=18.0, 0.9 Hz, 1H), 6.88 (d, J=8.7 Hz, 2H), 7.34 (dd, J=18.0, 11.2 Hz, 1H), 7.39 (d, J=8.7 Hz, 2H); HR-MS Calcd for $C_{26}H_{38}NO_5Si$ $(M+H)^+$ 472.2518; Found 472.2525.

p-Methoxybenzyl (1*S*,5*R*,6*S*)-1-Methyl-2-[(5*R*)-2-methylisoxazolidin-5-yl]-6-[(1R)-1-(triethylsilyloxy)-ethyl]carbapen-2-em-3-carboxylate (**4**) and *p*-Methoxybenzyl (1*S*,5*R*,6*S*)-1-Methyl-2-[(5*S*)-2-methylisoxazolidin-5-yl]-6-[(1*R*)-1-(triethylsilyloxy)-ethyl]carbapen-2-em-3-carboxylate (**5**)

N-methylhydroxylamine hydrochloride (3.32 g, 39.8 mmol) and 37% aqueous formaldehyde (24 ml, 0.296 mol) were added to a solution of 1 M sodium methoxide (in MeOH, 40 ml, 40 mmol) at room temperature. This mixture was added to a solution of **3b** (6.24 g, 13.2 mmol) in toluene (100 ml) at the same temperature, and heated to 110°C for 4 hours. After cooling, the reaction mixture was poured into water and ether, and extracted with ether. The combined organic layer was washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography, and gave 5′*R*-isomer **4** (962 mg, 22%), less polar isomer, and 5′*S*-isomer **5** (1.97 g, 45%), polar isomer, as viscous colorless oils, respectively:

5′*R*-isomer **4**; IR ($CHCl_3$) 1763, 1709 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.58 (q, J=7.8 Hz, 6H), 0.94 (t, J=7.8 Hz, 9H), 1.23(d, J=7.0 Hz, 3H), 1.26 (d, J=6.0 Hz, 3H), 1.88~2.14 (m, 1H), 2.39~2.58 (m, 1H), 2.60~2.86 (m, 1H), 2.67 (s, 3H), 3.10~3.45 (m, 2H), 3.18 (dd, J=6.6, 2.4 Hz, 1H), 3.80 (s, 3H), 4.06 (dd, J=9.3, 2.4 Hz, 1H), 4.20 (quint, J=6.3 Hz, 1H), 5.15 and 5.23 (ABq, J=12.3 Hz, 2H), 5.43~5.62 (m, 1H), 6.88 (d, J=8.8 Hz, 2H), 7.38 (d, J=8.8 Hz, 2H); HR-MS Calcd for $C_{28}H_{43}N_2O_6Si$ $(M+H)^+$ 531.2888; Found 531.2894.

5′*S*-isomer **5**; IR ($CHCl_3$) 1764, 1709 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.58 (q, J=7.8 Hz, 6H), 0.94 (t, J=7.8 Hz, 9H), 1.23 (d, J=7.4 Hz, 3H), 1.27 (d, J=6.0 Hz, 3H), 1.27~2.09 (m, 1H), 2.22~2.48 (m, 1H), 2.56~2.89 (m, 1H), 2.66 (s, 3H), 3.15~3.58 (m, 2H), 3.20 (dd, J=7.0, 2.8 Hz, 1H), 3.80 (s, 3H), 4.09 (dd, J=9.9, 2.8 Hz, 1H), 4.19 (quint, J=6.4 Hz, 1H), 5.19 (s, 2H), 5.34~5.55 (m, 1H), 6.88 (d, J=8.8 Hz, 2H), 7.37 (d, J=8.8 Hz, 2H); HR-MS Calcd for $C_{28}H_{43}N_2O_6Si$ $(M+H)^+$ 531.2888; Found 531.2890.

p-Methoxybenzyl (1*S*,5*R*,6*S*)-2-[(5*R*)-2,2-Dimethyl-isoxazolidinio-5-yl]-1-methyl-6-[(1*R*)-1-(triethylsilyl-oxy)ethyl]carbapen-2-em-3-carboxylate Iodide (**6**)

To a solution of **4** (839 mg, 1.58 mmol) in CH_3CN (10 ml) was added iodomethane (10 ml) at room temperature, and the mixture was stirred at the same temperature for 10 minutes. Concentration of the reaction mixture under reduced pressure gave a crude quaternarized salt **6** as a pale yellow foam: IR ($CHCl_3$) 1776, 1717 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.58 (q, J=8.0 Hz, 6H), 0.93 (t, J=8.0 Hz, 9H), 1.20 (d, J=7.2 Hz, 3H), 1.23 (d, J=6.2 Hz, 3H), 2.86~3.06 (m, 2H), 3.26 (dd, J=5.4, 3.0 Hz, 1H), 3.36 (dd, J=9.8, 7.2 Hz, 1H), 3.80 (s, 3H), 3.82 (s, 3H), 3.87 (s, 3H), 4.24 (quint, J=6.2 Hz, 1H), 4.28 (dd, J=9.8, 3.0 Hz, 1H), 4.47~4.66 (m, 1H), 4.76~4.93 (m, 1H), 5.21 (s, 2H), 6.05 (dd, J=9.1, 7.1 Hz, 1H), 6.89 (d, J=8.8 Hz, 2H), 7.38 (d, J=8.8 Hz, 2H); HR-MS Calcd for $C_{29}H_{45}N_2O_6Si$ $(M+H)^+$ 545.3044; Found 545.3039.

p-Methoxybenzyl (1*S*,5*R*,6*S*)-2-[(5*S*)-2,2-Dimethyl-isoxazolidinio-5-yl]-1-methyl-6-[(1*R*)-1-(triethylsilyl-oxy)ethyl]carbapen-2-em-3-carboxylate Iodide (**7**)

Quaternarization of **5** (5.0 g, 9.42 mmol) gave **7** by the same way used for **6**: a pale yellow foam; IR ($CHCl_3$) 1778, 1714 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.58 (q, J=8.2 Hz, 6H), 0.93 (t, J=8.2 Hz, 9H), 1.24 (d, J=6.2 Hz, 3H), 1.27 (d, J=6.4 Hz, 3H), 2.57~2.84 (m, 1H), 3.00~3.19 (m, 1H), 3.19~3.38 (m, 2H), 3.81 (s, 3H), 3.91 (s, 3H), 3.94 (s, 3H), 4.22 (dd, J=10.2, 3.0 Hz, 1H), 4.24 (quint, J=6.2 Hz, 1H), 4.50~4.83 (m, 2H), 5.23 (s, 2H), 5.91 (dd, J=10.0, 6.2 Hz, 1H), 6.89 (d, J=8.7 Hz, 2H), 7.38 (d, J=8.7 Hz, 2H); HR-MS Calcd for $C_{29}H_{45}N_2O_6Si$ $(M+H)^+$ 545.3044; Found 545.3036.

(1*S*,5*R*,6*S*)-2-[(5*R*)-2,2-Dimethylisoxazolidinio-5-yl]-6-[(1*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (**8**)

To a solution of crude **6** in CH_2Cl_2 (6 ml) and nitromethane (3 ml) were added at −60°C a solution of

aluminum chloride (843 mg, 6.32 mmol) and anisole (3.5 ml). The mixture was allowed to warm to −15°C over 1.5 hours, and was stirred at the same temperature for 30 minutes. The reaction mixture was diluted with CH_2Cl_2, a aqueous solution (10 ml) of sodium bicarbonate (707 mg, 8.41 mmol) was added, and the mixture was stirred under ice cooling for 10 minutes. After filtration of the mixture, aqueous filtrate was separated, washed with CH_2Cl_2, and purified by CHP-20P column chromatography. The fractions containing the product were concentrated and freeze-dried to give **8** (301 mg, 61% from **4**) as a colorless powder: IR (KBr) 3400, 1750, 1598 cm^{-1}; ^1H NMR (D_2) δ 1.15 (d, J=7.2 Hz, 3H), 1.27 (d, J=6.2 Hz, 3H), 2.61~2.93 (m, 2H), 3.35 (dq, J=9.4, 7.2 Hz, 1H), 3.47 (dd, J=6.2, 2.6 Hz, 1H), 3.60 (s, 6H), 5.98 (dd, J=9.7, 6.3 Hz, 1H); UV $\lambda_{max}^{H_2O}$ 274 nm (ε=6800); HR-MS Calcd for $C_{15}H_{23}N_2O_5$ $(M+H)^+$ 311.1606; Found 311.1610.

(1*S*,5*R*,6*S*)-2-[(5*S*)-2,2-Dimethylisoxazolidinio-5-yl]-6-[(1*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (**9**)

Deprotection of **7** gave **9** (1.375 g) by a similar procedure used for **8**: 47% from **5** as a colorless powder; IR (KBr) 3500, 1750, 1595 cm^{-1}; ^1H NMR (D_2) δ 1.23 (d, J=7.2 Hz, 3H), 1.30 (d, J=6.4 Hz, 3H), 2.59~3.13 (m, 2H), 3.29~3.47 (m, 1H), 3.50 (dd, J=5.1, 2.9 Hz, 1H), 3.63 (s, 6H), 4.08~4.37 (m, 4H), 5.91(dd, J=9.9, 6.3 Hz, 1H); UV $\lambda_{max}^{H_2O}$ 274 (ε=5000), 225 (ε=5500) nm; HR-MS Calcd for $C_{15}H_{23}N_2O_5$ $(M+H)^+$ 311.1606; Found 311.1600.

p-Methoxybenzyl (1*S*,5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-1-methyl-2-[(5*S*)-2-methyl-isoxazolidin-5-yl]-carbapen-2-em-3-carboxylate (**10**)

To an ice-cooled solution of **5** (116 mg, 0.22 mmol) in THF (1 ml) were added acetic acid (38 μl, 0.66 mmol) and tetrabutylammonium fluoride (1 M solution in THF, 0.66 ml, 0.66 mmol). After being stirred for 30 minutes, the reaction mixture was poured into water and ethyl acetate, and extracted with ethyl acetate. The extracts were washed with saturated aqueous sodium bicarbonate and brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was chromatographied on silica gel column to afford **10** (70 mg, 77%) as a colorless foam: IR ($CHCl_3$) 3424, 1767, 1712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.24 (d, J=7.4 Hz, 3H), 1.33 (d, J=6.2 Hz, 3H), 1.70~2.12 (m, 1H), 2.28~2.52 (m, 1H), 2.56~2.86 (m, 1H), 2.66 (s, 3H), 3.19~3.60 (m, 2H), 3.25 (dd, J=6.6, 2.8 Hz, 1H), 3.80 (s, 3H), 4.15 (dd, J=9.8, 2.8 Hz, 1H), 4.23 (quint, J=6.4 Hz, 1H), 5.16 and 5.24 (ABq, J=12.1 Hz, 2H), 5.36~5.55 (m, 1H), 6.89 (d, J=8.7 Hz, 2H), 7.37 (d, J=8.7 Hz, 2H); HR-MS Calcd for $C_{22}H_{29}N_2O_6$ $(M+H)^+$ 417.2024; Found 417.2031.

p-Methoxybenzyl (1*S*,5*R*,6*S*)-1-Methyl-2-[(5*S*)-2-methylisoxazolidin-5-yl]-6-[(1*R*)-1-(*p*-nitrobenzoyloxy)-ethyl]carbapen-2-em-3-carboxylate (**11**)

To a solution of **10** (188 mg, 0.45 mmol) in CH_2Cl_2 (4 ml) were added triethylamine (94 ml, 0.68 mmol), 4-nitrobenzoyl chloride (126 mg, 0.68 mmol) and 4-dimethylaminopyridine (28 mg, 0.23 mmol) at −40°C, and the mixture was stirred for 2 hours. The reaction mixture was diluted with water and CH_2Cl_2, extracted with CH_2Cl_2, and dried over magnesium sulfate. After evaporation, purification of the residue by silica gel column chromatography gave **11** (227 mg, 89%) as a colorless crystal. This compound was recrystalized from ether and hexane. X-ray crystallographic analysis determined that the configuration at the 5'-position of isoxazolidine ring is the *S*-configuration: mp 131~132°C; IR ($CHCl_3$) 1774, 1720, 1528, 1341 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (d, J=7.4 Hz, 3H), 1.53 (d, J=6.2 Hz, 3H), 1.78~2.14 (m, 1H), 2.28~2.52 (m, 1H), 2.58~2.90 (m, 1H), 2.67 (s, 3H), 3.16~3.63 (m, 2H), 3.55 (dd, J=6.4, 2.8 Hz, 1H), 3.78 (s, 3H), 4.22 (dd, J=9.7, 2.8 Hz, 1H), 5.20 (s, 2H), 5.35~5.60 (m, 1H), 5.54 (quint, J=6.4 Hz, 1H), 6.85 (d, J=8.6 Hz, 2H), 7.34 (d, J=8.6 Hz, 2H), 8.15 (dd, J=6.6, 2.6 Hz, 2H), 8.22 (dd, J=6.6, 2.6 Hz, 2H).

Anal Calcd for $C_{29}H_{31}N_3O_9$: C 61.59, H 5.52, N 7.43.
Found: C 61.46, H 5.64, N 7.44.

p-Methoxybenzyl (5*R*,6*S*)-2-(2-Methylisoxazolidin-5-yl)-6-[(1*R*)-1-(triethylsilyloxy)-ethyl]carbapen-2-em-3-carboxylate (**12**, **13**)

1,3-Dipolar cycloaddition reaction of **3a** (696 mg, 1.52 mmol) gave less polar isomer **12** (90 mg) and polar isomer **13** (131 mg) by a similar procedure used for **4** and **5**: **12** (11%) as a viscous oil; IR ($CHCl_3$) 1775, 1713 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.59 (q, J=8.0 Hz, 6H), 0.94 (t, J=8.0 Hz, 9H), 1.26 (d, J=6.2 Hz, 3H), 1.88~2.14 (m, 1H), 2.24~3.38 (m, 5H), 2.66 (s, 3H), 3.11 (dd, J=6.4, 2.8 Hz, 1H), 3.80 (s, 3H), 4.09 (dt, J=9.2, 2.8 Hz, 1H), 4.19 (quint, J=6.2 Hz, 1H), 5.16 and 5.23 (ABq, J=12.0 Hz, 2H), 5.39~5.64 (m, 1H), 6.88 (d, J=8.8 Hz, 2H), 7.37 (d, J=8.8 Hz, 2H); HR-MS Calcd for $C_{27}H_{41}N_2O_6Si$ $(M+H)^+$ 517.2732; Found 517.2738. **13** (17%) as a viscous oil; IR ($CHCl_3$) 1773, 1712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.59 (q, J=8.0 Hz, 6H), 0.94 (t, J=8.0 Hz, 9H), 1.27 (d, J=6.0 Hz, 3H), 1.84~2.10 (m, 1H), 2.34~3.36 (m, 5H), 2.67 (s, 3H), 3.08 (dd, J=6.8, 2.8 Hz, 1H), 3.80 (s, 3H), 4.02~4.27 (m, 2H), 5.19 (s, 2H), 5.35~5.61 (m, 1H), 6.88 (d, J=8.6 Hz, 2H), 7.36 (d, J=8.6 Hz, 2H); HR-MS Calcd for $C_{27}H_{41}N_2O_6Si$ $(M+H)^+$ 517.2732; Found 517.2738.

p-Methoxybenzyl (5*R*,6*S*)-2-(2,2-Dimethylisoxazolidinio-5-yl)-6-[(1*R*)-1-(triethyl-silyloxy)ethyl]carbapen-2-em-3-carboxylate Iodide (**14**)

Quaternization of **12** (161 mg, 0.31 mmol) gave **14** (178 mg) by the same procedure used for **6**: 87% yield as a pale yellow foam; IR ($CHCl_3$) 1783, 1713 cm^{-1}; ^1H

NMR ($CDCl_3$) δ 0.59 (q, J=8.0 Hz, 6H), 0.94 (t, J=8.0 Hz, 9H), 1.24 (d, J=6.2 Hz, 3H), 2.84~3.06 (m, 3H), 3.12~3.35 (m, 2H), 3.80 (s, 6H), 3.95 (s, 3H), 4.13~4.57 (m, 3H), 4.85~5.03 (m, 1H), 5.22 (s, 2H), 6.20 (t, J=7.8 Hz, 1H), 6.89 (d, J=8.6 Hz, 2H), 7.38 (d, J=8.6 Hz, 2H); HR-MS Calcd for $C_{28}H_{43}N_2O_6Si$ $(M-I)^+$ 531.2888; Found 531.2892.

p-Methoxybenzyl (5*R*,6*S*)-2-(2,2-Dimethylisoxazolidinio-5-yl)-6-[(1*R*)-1-(triethylsilyl-oxy)ethyl]carbapen-2-em-3-carboxylate Iodide (**15**)

Quaternization of **13** (102 mg, 0.20 mmol) gave **15** (114 mg) by the same manner used for **6**: 87% yield as a pale yellow foam; IR ($CHCl_3$) 1784, 1712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.59 (q, J=8.0 Hz, 6H), 0.93 (t, J=8.0 Hz, 9H), 1.24 (d, J=6.2 Hz, 3H), 2.85~3.14 (m, 3H), 3.26~3.50 (m, 2H), 3.80 (s, 6H), 3.96 (s, 3H), 4.13~4.50 (m, 3H), 4.83~5.03 (m, 1H), 5.22 (s, 2H), 6.17 (t, J=7.8 Hz, 1H), 6.88 (d, J=8.6 Hz, 2H), 7.38 (d, J=8.6 Hz, 2H); HR-MS Calcd for $C_{28}H_{43}N_2O_6Si$ $(M-I)^+$ 531.2888; Found 531.2891.

(5*R*,6*S*)-2-(2,2-Dimethylisoxazolidinio-5-yl)-6-[(1*R*)-1-hydroxyethyl]carbapen-2-em-3-carboxylate (**16**)

Deprotection of **14** (156 mg, 0.24 mmol) gave **16** (39 mg) by a similar procedure used for **8**: 56% yield as a colorless powder; IR (KBr) 3420, 1760, 1600 cm^{-1}; ^1H NMR (D_2O) δ 1.26 (d, J=6.2 Hz, 3H), 2.53~3.17 (m, 4H), 3.44 (dd, J=5.8, 2.8 Hz, 1H), 3.55 (s, 3H), 3.59 (s, 3H), 4.06~4.31 (m, 4H), 6.13 (dd, J=9.4, 6.2 Hz, 1H); UV $\lambda_{max}^{H_2O}$ 224 (ε=4700), 273 (ε=3900) nm; HR-MS Calcd for $C_{14}H_{21}N_2O_5$ $(M+H)^+$ 297.1450; Found 297.1451.

(5*R*,6*S*)-2-(2,2-Dimethylisoxazolidinio-5-yl)-6-[(1*R*)-1-hydroxyethyl]carbapen-2-em-3-carboxylate (**17**)

Deprotection of **15** (217 mg, 0.33 mmol) gave **17** (46 mg) by a similar procedure used for **8**: 47% yield as a colorless powder; IR (KBr) 3400, 1760, 1600 cm^{-1}; ^1H NMR (D_2O) δ 1.25 (d, J=6.6 Hz, 3H), 2.53~3.10 (m, 4H), 3.44 (dd, J=5.8, 3.0 Hz, 1H), 3.56 (s, 3H), 3.58 (s, 3H), 4.09~4.30 (m, 4H), 6.15 (dd, J=9.4, 6.4 Hz, 1H); UV $\lambda_{max}^{H_2O}$ 223 (ε=5000), 272 (ε=4300) nm; HR-MS Calcd for $C_{14}H_{21}N_2O_5$ $(M+H)^+$ 297.1450; Found 297.1460.

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References

1) KAHAN, J. S.; F. M. KAHAN, R. GOEGELMAN, S. A. CURRIE, M. JACKSON, E. O. STAPLEY, T. W. MILLER, D. HENDLIN, S. MOCHALES, S. HERNADEZ, H. B. WOODRUFF & J. BIRNBAUM: Thienamycin, a new β-lactam antibiotic I. Discovery, taxonomy, isolation and physical properties. J. Antibiotics 32: 1~12, 1979

2) LEANZA, W. J.; K. J. WILDONGER, T. W. MILLER & B. G. CHRISTENSEN: N-Acetylimidoyl- and N-formimidoyl-thienamycin derivatives: Antipseudomonal β-lactam antibiotics. J. Med. Chem. 22: 1435~1436, 1979

3) MIYADERA, T.; Y. SUGIMURA, T. HASHIMOTO, T. TANAKA, K. IINO, T, SHIBATA & S. SUGAWARA: Synthesis and *in vitro* activity of a new carbapenem, RS-533. J. Antibiotics 36: 1034~1039, 1983

4) SUNAGAWA, M.; H. MATSUMURA, T. INOUE, M. FUKASAWA & M. KATO: A novel carbapenem antibiotic, SM-7338 structure-activity relationships. J. Antibiotics 43: 519~532, 1990

5) NAGAO, Y.; Y. NAGASE, T. KUMAGAI, H. MATSUNAGA, T. ABE, O. SHIMADA, T. HAYASHI & Y. INOUE: β-Lactams 3. Asymmetric total synthesis of new non-natural 1β-methylcarbapenems exhibiting strong antimicrobial activities and stability against human renal dehydropeptidase-I. J. Org. Chem 57: 4243~4249, 1992

6) IMUTA, M.; H. ITANI, H. ONA, Y. HAMADA, S. UYEO & T. YOSHIDA: Carbapenem and penem VI. Synthesis and antibacterial activity of 2-heteroaromatic-thiomethyl and 2-carbamoyloxymethyl 1-methylcarbapenems. Chem. Pharm. Bull. 39: 663~671, 1991

7) IMUTA, M.; H. ITANI, H. ONA, T. KONOIKE, S. UYEO, Y. KIMURA, H. MIWA, S. MATSUURA & T. YOSHIDA: Carbapenem and penem VII. Synthesis and antibacterial activity of 1β-methyl-2-(quaternary heteroaromatic-thiomethyl)carbapenems. Chem. Pharm. Bull. 39: 672~678, 1991

8) NISHIKAWA, J.; F. WATANABE, M. SHUDOU, Y. TERUI & M. NARISADA: ^1H NMR study of degradation mechanisms of oxacephem derivatives with various 3′-substituents in alkaline solution. J. Med. Chem. 30: 523~527, 1987

9) IMUTA, M.; H. ITANI, K. NISHI, H. ONA, S. UYEO & Y. KIMURA: Synthesis and antibacterial activity of 2-functionalized-vinyl 1β-methylcarbapenems and related compounds. Bioorg. & Med. Chem. Lett. 3: 2199~2204, 1993

10) OHTANI, M.; F. WATANABE & M. NARISADA: Mild deprotection of carbapenem esters with aluminum trichloride. J. Org. Chem. 49: 5271~5272, 1984