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Synthesis and Biological Evaluation of Novel Tricyclic Carbapenems (Trinems)

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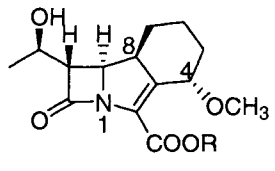
Synthesis of new tricyclic carbapenems (trinems) with a pyrrolidinyl moiety at the C-4 position of the tricyclic ring and their antimicrobial activities were studied. These trinems showed potent activities against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA). Among them, (4*R*)-[(*S*)-pyrrolidin-3-ylthiomethyl]trinem (**14a**) exhibited good activity against MRSA *in vitro* and *in vivo*.

Recently, MRSA has been causing serious problems in hospitals.[1,2] At present, worldwide, vancomycin is clinically the most popular treatment against MRSA. However, with the recent increased use of vancomycin, multiple-resistant *Enterococcus faecium* has emerged. In the field of carbapenem antibiotics, research to discover a new anti-MRSA agent to replace vancomycin continues today and these carbapenems are mostly 1β-methyl-carbapenems.[3~6] Recently, a novel class of tricyclic carbapenem (trinem) has been identified: sanfetrinem cilexetil (GV-118819, active form: sanfetrinem sodium, GV-104326) has been developed as an oral trinem by Glaxo SpA (Figure 1).[7,8] However, no trinems showing anti-MRSA activity have been reported previously. Our attention was focused on the synthesis of novel trinems by introduction of a pyrrolidinyl moiety at the C-4 position. We synthesized two types of trinems with pyrrolidinyl-methylthio or pyrrolidinylthiomethyl and pyrrolidinyl-methylthiomethyl groups at the C-4 position. Among them, (4*R*)-[(*S*)-pyrrolidin-3-ylthiomethyl]trinem (**14a**) exhibited potent anti-MRSA activity and good *in vivo* efficacy against experimental infection in mice compared with panipenem (PAPM), meropenem (MEPM), biapenem (BIPM) and vancomycin (VCM).

Chemistry

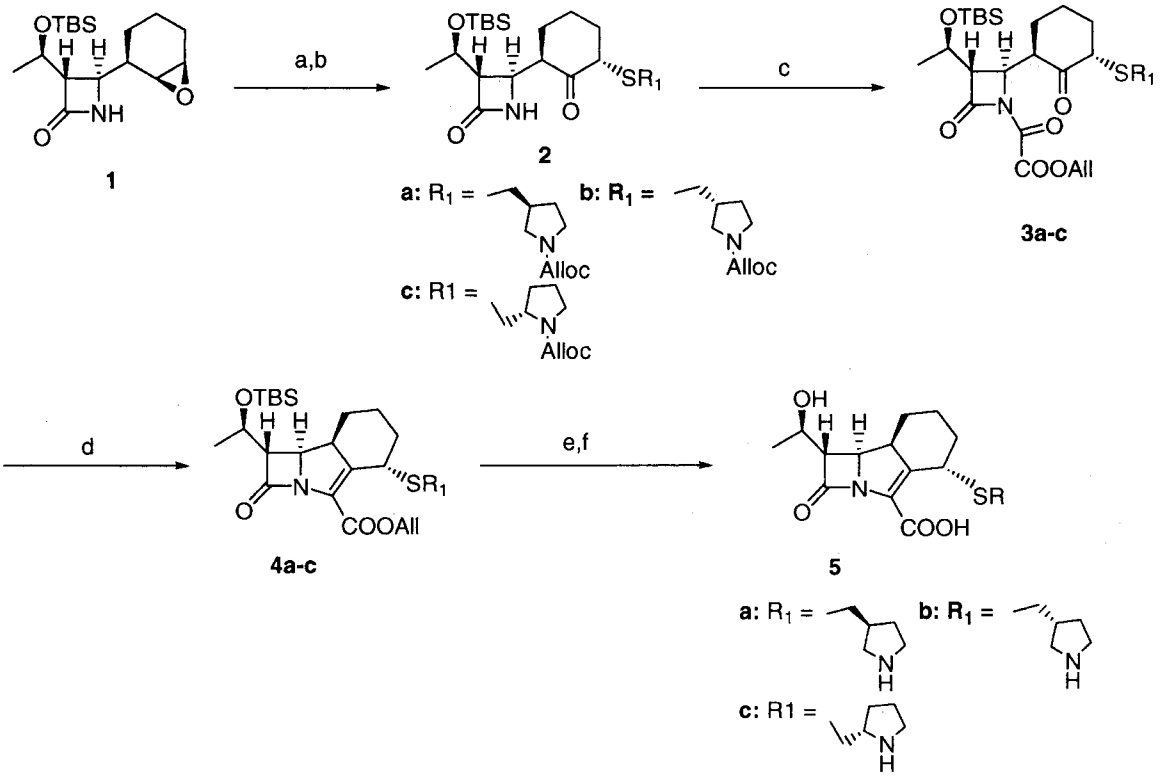
The two types of novel trinems were synthesized from Glaxo's epoxide intermediate (**1**)[9,10] and 2-hydroxymethylcyclohexanone intermediate (**6**)[11] as shown in Schemes 1 and 2. The ring cleavage of **1** with (*S*)-3-acetylthiomethyl-1-allyloxycarbonylpyrrolidine in the presence of ethylenediamine followed by Swern oxidation afforded cyclohexanone **2a** in 75% yield. Acylation of **2a** with allyl oxalyl chloride and triethylamine afforded oxalamide **3a** in 60% yield. The intramolecular Wittig type cyclization of **3a** with diethyl ethylphosphonite in refluxing toluene for 4 hours gave protected trinem **4a** in 36% yield. Deprotection of the *t*-butyldimethylsilyl (TBS) group of **4a** with tetrabutylammonium fluoride (TBAF) followed by bis(triphenylphosphine)palladium dichloride and tributyltin hydride afforded trinem **5a** in 58% yield (2 steps). Analogous reactions of **1** with (*R*)-3-acetylthiomethyl-1-allyloxycarbonylpyrrolidine in the presence of ethylenediamine and (*S*)-1-allyloxycarbonyl-2-mercaptomethylpyrrolidine afforded the corresponding ketones **2b** and **2c** in 68% and 70% yield, respectively. These ketones were acylated to give oxalamides **3b** and **3c** in 51% and 80% yield, respectively. The cyclization of

Fig. 1. Structure of sanfetrinem derivatives.



GV-104326 (sanfetrinem sodium) R = Na
GV-118819 (sanfetrinem cilexetil) R = —CHOCOO— (CH3)

Scheme 1.

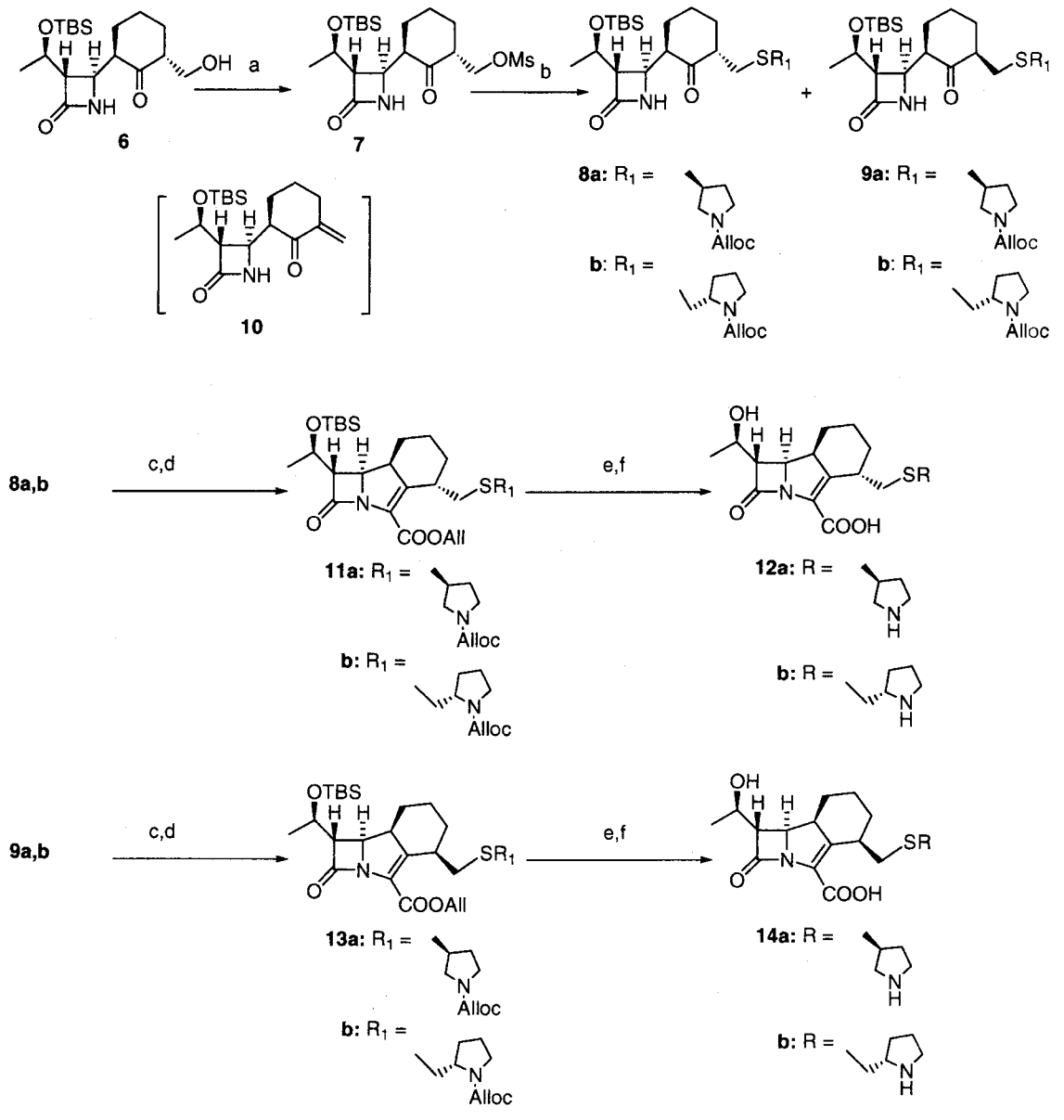


Reagents: (a) R_1SAc, $NH_2CH_2CH_2NH_2$ (**2a, b**) or R_1SH, Et_3N (**2c**); (b) Swern oxidation; (c) ClCOCOOAll, Et_3N; (d) $EtP(OEt)_2$; (e) TBAF or $HF \cdot NH_4F$; (f) $PdCl_2(Ph_3P)_2$, Bu_3SnH.

3b with diethyl ethylphosphonite in refluxing toluene for 9 hours resulted in 16% yield, but that of **3c** in refluxing toluene for 4 hours proceeded with 61% yield. These results in the cyclization reaction seem to reflect the steric hindrance of the R_1 group. Deprotection of both **4b** and **4c** in a similar manner afforded trinems **5b** and **5c** in 46% and 36% yield, respectively.

Alternatively, thiomethyltrinem derivatives were synthesized from 2-hydroxymethylcyclohexanone derivative **6**,[12] which was a versatile intermediate for the trinem synthesis. The mesylation of **6** with methanesulfonyl chloride and triethylamine afforded mesylate **7** in quantitative yield. Reaction of **7** with (*S*)-1-allyloxycarbonyl-3-mercaptopyrrolidine and (*S*)-1-allyloxycarbonyl-2-mercaptomethylpyrrolidine furnished *trans*- and *cis*-cyclohexanones **8a** and **8b**, and **9a** and **9b**, respectively. The ratios of **8a** to **9a** and **8b** to **9b** were both about 2 : 3. These reactions seem to proceeed *via* Michael addition to exo-methylene intermediate **10**, because the conversion of **8a** to **9a** hardly occurred under the same conditions without thiol. Acylation of **8a** followed by cyclization with diethyl ethylphosphonite in refluxing toluene afforded protected trinem **11a** in 81% yield. The stereochemistry at the C-4 position of **11a** was confirmed by the observation of NOE between methylene protons of the side chain next to the C-4 position and a proton at the C-8 position.[12] Deprotection of the TBS group of **11a** followed by deallylation with bis(triphenylphosphine)palladium dichloride and tributyltin hydride afforded trinem **12a** in 35% yield (2 steps). On the other hand, acylation of **9a** proceeded quantitatively, but cyclization of the oxalamide hardly occurred by refluxing in toluene due to the steric hindrance of the *cis* substituent groups of the cyclohexanone moiety. The cyclization occurred by refluxing in mesitylene for 2 hours to give trinem **13a** in 66% yield. Deprotection of the TBS and allyl groups of **13a** afforded trinem **14a** in 36% yield (2 steps). Analogous acylations of **8b** and **9b** followed by cyclizations in refluxing toluene furnished protected trinems **11b** and **13b** in 53% and 46%, respectively (2 steps). This suggests that lower yields of **13a** and **13b** than those of **11a** and **11b**

Scheme 2.



Reagents: (a) MsCl, Et_3N; (b) R_1SH, iPr_2NEt; (c) ClCOCOOAll, Et_3N; (d) $EtP(OEt)_2$; (e) $HF \cdot NH_4F$ or TBAF; (f) $PdCl_2(Ph_3P)_2$, Bu_3SnH.

were caused by steric bulkiness of the equatorial side chain. Deprotection of **11b** and **13b** afforded trinems **12b** and **14b** in 61% and 40%, respectively (2 steps).

Biological Properties

The antibacterial activity (MICs) of trinems is shown in Table 1. The trinems **5a~c**, **12a**, **12b**, **14a** and **14b** showed potent activity against Gram-positive bacteria such as *S. aureus* 209P, but weak or moderate activity against Gram-negative bacteria such as *E. coli* NIHJ, *K. pneumoniae* 806, and *S. marcescens* 1184. On the other hand, GV-104326 showed moderate activity against Gram-positive and Gram-negative bacteria, but weak activity against *S. aureus* 535 (MRSA). In spite of possessing an basic pyrrolidinyl moiety, these trinems which we synthesized rarely showed anti-pseudomonal activity with the exception of **5a**, which showed very weak activty against *P. aeruginosa* 1001. The

Table 1. Antibacterial activity (MIC, μg/ml)[a] of tricyclic carbapenems.

Organism	**5a**	**5b**	**5c**	**12a**	**12b**	**14a**	**14b**	GV-104326
Staphylococcus aureus 209P	≤0.01	≤0.01	≤0.01	0.02	0.05	0.02	0.02	0.02
Staphylococcus aureus 56R	0.05	0.05	0.05	0.1	0.1	0.05	0.1	0.05
Staphylococcus aureus 535 (MRSA)	3.1	6.2	3.1	12.5	12.5	1.5	6.2	12.5
Enterococcus faecalis 681	3.1	3.1	1.5	1.5	1.5	3.1	6.2	0.8
Escherichia coli NIHJ	6.2	12.5	6.2	0.8	3.1	1.5	12.5	0.2
Klebsiella pneumoniae 806	1.5	3.1	1.5	0.2	0.8	0.8	3.1	0.4
Serratia marcescens 1184	6.2	12.5	3.1	0.4	3.1	3.1	12.5	0.8
Pseudomonas aeruginosa 1001	50	>50	>50	>50	>50	>50	>50	100

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

Table 2. Protective effect of a tricyclic carbapenem **14a** compared with those of PAPM, MEPM, BIPM and VCM against experimental infection in mice.

Organism	ED_{50} (mg/kg)[a]				
	14a	PAPM	MEPM	BIPM	VCM
S. aureus 507[b]	3.68	23.94	87.10	31.63	1.48
[MIC, μg/ml][c]	[0.78]	[0.20]	[3.13]	[1.56]	[0.78]

[a] 50% effective sc dose.
[b] Challenged with 5% mucin.
[c] Mueller-Hinton II agar was used as a medium.

activity of 4-thiotrinems **5a**~**c** against *S. aureus* 535 (MRSA) was compared with that of 4-thiomethyltrinems **12a**, **12b**, **14a** and **14b**. Among these trinems, **14a** showed the most potent anti-MRSA activity and moderate activity against Gram-negative bacteria. The urinary recoveries of several trinems were measured after sc administration (50 mg/kg) in mice (n=5, male, SPF *dd*Y strain). The urinary recoveries of **5a**, **12a** and **14a** were 83%, 53% and 83%, respectively. In order to clarify *in vivo* anti-MRSA activity, the protective effect of **14a** was compared to those of PAPM, MEPM, BIPM and VCM. The trinem **14a** exhibited comparable *in vivo* efficacy to vancomycin against *S. aureus* 507 (MRSA, MIC against oxacillin: 32 μg/ml). *In vivo* anti-MRSA efficacy of **14a** was 6~23 times higher than those of PAPM, MEPM and BIPM. These results indicate a new possibility of trinem derivatives as potential anti-MRSA agents.

Conclusion

The structure-activity relationships of two types of tricyclic carbapenems (trinems) with a pyrrolidinyl moiety at the C-4 position were clarified. The trinem **14a** showed potent antimicrobial activity against Gram-positive bacteria including *S. aureus* 535 (MRSA) and higher *in vivo* efficacy against *S. aureus* 507 compared with those of panipenem, meropenem and biapenem. *In vivo* efficacy of **14a** was comparable to that of vancomycin. Trinem **14a** is of interest in the synthesis of potential anti-MRSA agents.

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 the internal standard. Mass spectra were recorded on JEOL HX-100, SX-102A or AX-505H mass spectrometer. The melting point (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 a Silica gel 60 (230~400 mesh, Art.9385, Merck) or a Cosmosil 75C_{18} PREP (75 μm, Nacalai Tesque, Inc.).

Synthesis of (4*S*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-11-oxo-4-[(*S*)-pyrrolidin-3-ylmethylthio]-1-azatricyclo-[7.2.0.0^{3,8}]undec-2-ene-2-carboxylic Acid (**5a**)

(1) (3*S*,4*R*)-4-[(2*S*,6*R*)-2-[(*S*)-(1-Allyloxycarbonyl) pyrrolidin-3-ylmethylthio]cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (**2a**)

A solution of (3*S*,4*R*)-4-[(1*R*,2*S*,3*R*)-2,3-epoxycyclohexyl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (**1**, 892 mg, 2.74 mmol) and (*S*)-3-acetylthiomethyl-1-allyl-oxycarbonylpyrrolidine (1.0 g, 4.11 mmol) in ethylene-diamine (2.74 ml, 4.11 mmol) was stirred at 60°C for 2.5 hours. Ethyl acetate (100 ml) was added to the reaction mixture and the mixture was washed with water, brine, dried over Na_2SO_4 and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane - EtOAc, 1 : 1) to give (3*S*,4*R*)-4-[(1*S*,2*S*,6*R*)-2-[(*S*)-(1-allyloxycarbonyl)-pyrrolidin-3-ylmethylthio]-1-hydroxycyclohexan-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (1.07 g, 75%) as an oil: IR (KBr) cm^{-1} 3424, 3285, 2931, 2857, 1740, 1706, 1685, 1412; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 0.10 (6H, s), 0.89 (9H, s), 1.28 (3H, d, J=6.1 Hz), 1.42~1.73 (6H, m), 1.95~2.13 (2H, m), 2.34~2.43 (1H, m), 2.52~2.65 (2H, m), 2.87~2.95 (2H, m), 3.07~3.20 (1H, m), 3.34~3.48 (1H, m), 3.48~3.58 (1H, m), 3.60~3.69 (2H, m), 3.92 (1H, br s), 4.10~4.20 (1H, m), 4.59 (2H, d, J=5.7 Hz), 5.21 (1H, d, J=10.1 Hz), 5.31 (1H, dd, J=17.2, 1.7 Hz), 5.88~6.02 (2H, m). FAB-MS *m/z* 527 $(M+H)^+$.

To a solution of oxalyl chloride (0.44 ml, 5.03 mmol) in CH_2Cl_2 (10 ml) was added dimethylsulfoxide (0.71 ml, 10.1 mmol) dropwise at −78°C under a dry nitrogen atomosphere. After stirring at −78°C for 10 minutes, a solution of (3*S*,4*R*)-4-[(1*S*,2*S*,6*R*)-2-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylmethylthio]-1-hydroxycyclohexan-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (1.06 g, 2.01 mmol) in CH_2Cl_2 (10 ml) was added to the mixture and the mixture was stirred at the same temperature for 10 minutes. Then, triethylamine (2.8 ml, 20 mmol) was added to the mixture followed by stirring at the same temperature for 30 minutes. The mixture was warmed to room temperature and EtOAc (100 ml) was added thereto. The mixture was washed with water, brine, dried over Na_2SO_4 and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane - EtOAc, 1 : 1) to give **2a** (1.02 g, 97%) as an oil: IR (neat) cm^{-1} 3277, 2935, 2858, 1760, 1704, 1413, 1106; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 0.07 (3H, s), 0.08 (3H, s), 0.88 (9H, s), 1.26 (3H, d, J=6.3 Hz), 1.70~2.21 (8H, m), 2.35~2.56 (3H, m), 2.90 (1H, dd, J=5.9, 2.2 Hz), 3.05~3.09 (1H, m), 3.33~3.68 (5H, m), 3.48~3.58 (1H, m), 4.08~4.23 (2H, m), 5.21 (1H, d, J=10.4 Hz), 5.30 (1H, dd, J=17.2, 1.3 Hz), 5.70 (1H, br d, J=7.8 Hz), 5.89~6.00 (1H, m). FAB-MS *m/z* 525 $(M+H)^+$.

(2) (3*S*,4*R*)-1-Allyloxalyl-4-[(2*S*,6*R*)-2-[(*S*)-(1-allyloxy-carbonyl)pyrrolidin-3-ylmethylthio]-cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (**3a**)

To a solution of **2a** (600 mg, 1.14 mmol) and triethyl-amine (384 μl, 2.74 mmol) in dichloromethane (7 ml) was added dropwise allyloxalyl chloride (374 mg, 2.52 mmol) in dichloromethane (3 ml) under ice-cooling and the mixture was stirred for 30 minutes. 2-Propanol (383 μl, 5.00 mmol) was added to the mixture and the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane - EtOAc, 3 : 1) to give **3a** (438 mg, 60%) as an oil: IR (neat) cm^{-1} 2933, 2859, 1808, 1756, 1704, 1409, 1215; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 0.04 (3H, s), 0.07 (3H, s), 0.85 (9H, s), 1.20 (3H, d, J=6.4 Hz), 1.44~1.80 (8H, m), 2.33~2.37 (3H, m), 3.02~3.09 (1H, m), 3.28~3.64 (5H, m), 4.23~4.33 (3H, m), 4.58 (2H, d, J=5.4 Hz), 4.79 (2H, d, J=6.0 Hz), 5.18~5.43 (4H, m), 5.89~6.01 (2H, m). FAB-MS *m/z* 637 $(M+H)^+$.

(3) Allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylmethylthio]-10-[(*R*)-1-(*tert*-butyldimethylsilyl-oxy)ethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]undec-2-ene-2-carboxylate (**4a**)

A solution of **3a** (423 mg, 0.66 mmol) and diethyl ethyl-phosphonite (299 mg, 1.99 mmol) in dry toluene (8 ml) was stirred under reflux for 4 hours. The mixture was concentrated under reduced pressure and the residue was

purified by silica gel column chromatography (hexane-EtOAc, 4:1) to give **4a** (144 mg, 36%) as an oil: IR (neat) cm^{-1} 2932, 2858, 1781, 1709, 1283, 1196; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 0.06 (6H, s), 0.89 (9H, s), 1.23 (3H, d, J=6.1 Hz), 1.31~2.07 (8H, m), 2.37~2.56 (3H, m), 3.03~3.12 (1H, m), 3.20 (1H, d, J=6.2, 3.4 Hz), 3.32~3.70 (4H, m), 4.11~4.25 (2H, m), 4.59 (2H, d, J=5 Hz), 4.62~4.80 (2H, m), 4.85 (1H, br s), 5.18~5.47 (4H, m), 5.88~6.04 (2H, m). FAB-MS m/z 605 $(M+H)^+$.

(4) (4*S*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-11-oxo-4-[(*S*)-pyrrolidin-3-ylmethylthio]-1-azatricyclo[7.2.0.$0^{3,8}$]-undec-2-ene-2-carboxylic acid (**5a**)

To a solution of **4a** (140 mg, 0.23 mmol) in tetrahydrofuran (3 ml) were added 1 M tetrabutylammonium fluoride in tetrahydrofuran (1.16 ml, 1.16 mmol) and acetic acid (80 μl, 1.39 mmol) under nitrogen atomosphere. The mixture was stirred at 0°C for 1 hour and left to stand in a refrigerator for 3 days. Ethyl acetate (100 ml) was added to the mixture and the mixture was washed with brine, aqueous sodium hydrogencarbonate and dried over Na_2SO_4. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to afford allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylmethylthio]-10-[(*R*)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.$0^{3,8}$]undec-2-ene-2-carboxylate (84 mg, 74%) as an oil: IR (neat) cm^{-1} 3436, 2936, 1779, 1707, 1445, 11985, 1135; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 1.32 (3H, d, J=6.2 Hz), 1.27~2.05 (8H, m), 2.34~2.56 (3H, m), 3.02~3.09 (1H, m), 3.25 (1H, dd, J=6.5, 3.5 Hz), 3.28~3.67 (4H, m), 4.21~4.32 (2H, m), 4.59 (2H, d, J=5.3 Hz), 4.62~4.86 (3H, m), 5.18~5.47 (4H, m), 5.88~6.04 (2H, m). FAB-MS m/z 491 $(M+H)^+$.

To a solution of allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylmethylthio]-10-[(*R*)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.$0^{3,8}$]undec-2-ene-2-carboxylate (81 mg, 0.165 mmol) in dichloromethane (1.6 ml) were added water (16.4 μl, 0.91 mmol), bis(triphenylphosphine)palladium dichloride (3.2 mg, 0.0046 mmol) and tributyltin hydride (222 μl, 0.83 mmol) at 0~5°C under nitrogen atomosphere. The mixture was stirred at room temperature for 30 minutes. Dichloromethane (20 ml) was added to the mixture and the mixture was extracted with water (10 ml×3). The aqueous layer was washed with dichloromethane and concentrated to 5 ml under reduced pressure. The residue was purified by reversed phase column chromatography (Cosmosil 75C_{18} PREP, eluted with 3~9% acetonitrile-water). The desired fraction was concentrated under reduced pressure followed by lyophilization to give **5a** (47 mg, 78%) as a powder: IR (KBr) cm^{-1} 3379, 2930, 1763, 1587, 1389; ^1H NMR (270 MHz, D_2O, TSP) δ 1.27 (3H, d, J=6.4 Hz), 1.36~1.45 (1H, m), 1.67~1.85 (3H, m), 1.85~1.93 (3H, m), 2.16~2.24 (1H, m), 2.50~2.63 (3H, m), 2.99 (1H, dd, J=11.8, 7.9 Hz), 3.27~3.55 (6H, m), 4.21 (1H, dd, J=10.6, 3.3 Hz), 4.24 (1H, q, J=6.1 Hz). FAB-MS m/z 367 $(M+H)^+$.

Anal Calcd for $C_{18}H_{26}N_2O_4S \cdot 1.5H_2O$:
C 54.94, H 7.43, N 7.12, S 8.15.
Found C 54.66, H 7.19, N 6.98, S 8.42.

Synthesis of (4*S*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-11-oxo-4-[(*R*)-pyrrolidin-3-ylmethylthio]-1-azatricyclo-[7.2.0.$0^{3,8}$]undec-2-ene-2-carboxylic Acid (**5b**)

(1) (3*S*,4*R*)-4-[(2*S*,6*R*)-2-[(*R*)-(1-Allyloxycarbonyl)pyrrolidin-3-ylmethylthio]-cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (**2b**)

The title compound **2b** (470 mg, 47%) was prepared as an oil from **1** (624 mg, 1.92 mmol) by a similar manner as that described for the preparation of **2a**: IR (neat) cm^{-1} 3275, 2934, 2858, 1760, 1704, 1410, 1106; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 0.07 (3H, s), 0.08 (3H, s), 0.88 (9H, s), 1.26 (3H, d, J=6.2 Hz), 1.54~2.19 (8H, m), 2.34~2.52 (3H, m), 2.90 (1H, dd, J=5.8, 2.4 Hz), 3.05~3.10 (1H, m), 3.32~3.62 (5H, m), 4.08~4.21 (2H, m), 4.59 (2H, d, J=5.4 Hz), 5.23 (1H, dd, J=11.1, 1.8 Hz), 5.31 (1H, dd, J=3.2, 1.3 Hz), 5.69 (1H, br s), 5.89~6.00 (1H, m). FAB-MS m/z 525 $(M+H)^+$.

(2) (3*S*,4*R*)-1-Allyloxalyl-4-[(2*S*,6*R*)-2-[(*R*)-(1-allyloxycarbonyl)pyrrolidin-3-ylmethylthio]-cyclohexanon-6-yl]-2-oxocyclohexyl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)-ethyl]azetidin-2-one (**3b**)

The title compound **3b** (287 mg, 51%) was prepared as an oil from **2b** (460 mg, 0.877 mmol) by a similar manner as that described for the preparation of **3a**: IR (neat) cm^{-1} 2934, 2859, 1809, 1756, 1703, 1409, 1215; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 0.04 (3H, s), 0.07 (3H, s), 0.85 (9H, s), 1.20 (3H, d, J=6.4 Hz), 1.44~1.80 (4H, m), 1.97~2.17 (5H, m), 2.30~2.48 (2H, m), 3.00~3.08 (1H, m), 3.30~3.39 (3H, m), 4.58 (2H, d, J=5.4 Hz), 4.76~4.80 (2H, m), 5.13~5.46 (4H, m), 5.87~6.03 (2H, m). FAB-MS m/z 637 $(M+H)^+$.

(3) Allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*R*)-(1-allyloxycarbonyl)pyrrolidin-3-ylmethylthio]-10-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]-11-oxo-1-azatricyclo[7.2.0.$0^{3,8}$]undec-2-ene-2-carboxylate (**4b**)

The title compound **4b** (42 mg, 16%) was prepared as an oil from **3b** (280 mg, 0.44 mmol) by a similar manner as that described for the preparation of **4a**. IR (neat) cm^{-1} 2932, 2858, 1781, 1709, 1283, 1196; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 0.08 (6H, s), 0.89 (9H, s), 1.25 (3H, d, J=

6.1 Hz), 1.30~2.07 (8H, m), 2.37~2.56 (3H, m), 3.03~3.12 (1H, m), 3.22 (1H, d, J=6.3, 3.4 Hz), 3.32~3.70 (4H, m), 4.13~4.26 (2H, m), 4.59 (2H, d, J=5.5 Hz), 4.60~4.87 (3H, m), 5.18~5.47 (4H, m), 5.88~6.04 (2H, m). FAB-MS m/z 605 $(M+H)^+$.

(4) (4*S*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-11-oxo-4-[(*R*)-pyrrolidin-3-ylmethylthio]-1-azatricyclo[7.2.0.0^{3,8}]-undec-2-ene-2-carboxylic Acid (**5b**)

The title compound **5b** (10 mg, 46%) was prepared as a powder from **4b** (40 mg, 0.081 mmol) by a similar manner as that described for the preparation of **5a**: IR (KBr) cm^{-1} 3382, 2929, 1759, 1600, 1389; ^1H NMR (270 MHz, D_2O, TSP) δ 1.27 (3H, d, J=6.4 Hz), 1.31~1.40 (1H, m), 1.67~1.97 (6H, m), 2.23~2.27 (1H, m), 2.54~2.60 (3H, m), 2.93~3.01 (3H, m), 3.25~3.49 (5H, m), 4.21 (1H, dd, J=9.5, 3.2 Hz), 4.25 (1H, q, J=6.3 Hz). FAB-MS m/z 367 $(M+H)^+$.

Synthesis of (4*S*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-11-oxo-4-[(*S*)-pyrrolidin-2-ylmethylthio]-1-azatricyclo-[7.2.0.0^{3,8}]undec-2-ene-2-carboxylic Acid (**5c**)

(1) (3*S*,4*R*)-4-[(2*S*,6*R*)-2-[(*S*)-(1-Allyloxycarbonyl)pyrrolidin-2-ylmethylthio]-cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (**2c**)

To a solution of **1** (1.46 g, 4.5 mmol) in methanol (30 ml) were added dropwise triethylamine (1.25 ml, 8.92 mmol) and (*S*)-1-allyloxycarbonyl-2-mercaptomethylpyrrolidine (1.36 g, 6.7 mmol) in methanol under ice-cooling and the mixture was stirred at room temperature for 2 days. Ethyl acetate (150 ml) was added to the mixture and the mixture was washed with water, brine and dried over Na_2SO_4. After concentration of the mixture under reduced pressure, the residue was purified by silica gel column chromatography (Hexane-EtOAc, 1:1) to give (3*S*,4*R*)-4-[(1*S*,2*S*,6*R*)-2-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-2-ylmethylthio]-1-hydroxycyclohexan-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (1.65 g, 70%) as an oil: IR (KBr) cm^{-1} 3426, 3285, 2931, 2857, 1740, 1705, 1680, 1412; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 0.09 (6H, s), 0.89 (9H, s), 1.27 (3H, d, J=5.6 Hz), 1.39~2.17 (10H, m), 2.30~2.50 (2H, m), 2.94 (1H, dd, J=6.0, 1.2 Hz), 2.79~3.14 (2H, m), 3.41~3.45 (2H, m), 3.65~3.69 (1H, m), 3.94~4.08 (2H, m), 4.08~4.24 (1H, m), 4.58 (2H, d, J=5.1 Hz), 5.22 (1H, dd, J=10.1, 1.8 Hz), 5.31 (1H, d, J=18.6 Hz), 5.93 (1H, br s), 5.24~5.89 (1H, m). FAB-MS m/z 527 $(M+H)^+$.

The title compound **2c** (1.46 g, 89%) was prepared as an oil from (3*S*,4*R*)-4-[(1*S*,2*S*,6*R*)-2-[(*S*)-(1-allyloxycarbonyl) pyrrolidin-2-ylmethylthio]-1-hydroxycyclohexan-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (1.64 g, 3.11 mmol) by a similar manner as that described for the preparation of **2a**: IR (KBr) cm^{-1} 3276, 2952, 2858, 1758, 1704, 1406, 1105; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 0.07 (3H, s), 0.08 (3H, s), 0.88 (9H, s), 1.26 (3H, d, J=6.2 Hz), 1.44~2.21 (10H, m), 2.32~2.48 (1H, m), 2.76~2.85 (1H, m), 2.91 (1H, dd, J=5.3, 3.6 Hz), 3.40~3.47 (4H, m), 3.94~3.99 (1H, m), 4.09 (1H, br s), 4.10~4.23 (1H, m), 4.58~4.64 (2H, m), 5.20~5.34 (2H, m), 5.69 (1H, br s), 5.89~6.02 (1H, m). FAB-MS m/z 525 $(M+H)^+$.

(2) (3*S*,4*R*)-1-Allyloxalyl-4-[(2*S*,6*R*)-2-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-2-ylmethylthio]-cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (**3c**)

The title compound **3c** (1.39 g, 80%) was prepared as an oil from **2c** (1.45 g, 2.76 mmol) by a similar manner as that described for the preparation of **3a**: IR (neat) cm^{-1} 2952, 2859, 1808, 1756, 1703, 1401, 1214; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 0.03 (3H, s), 0.07 (3H, s), 0.85 (9H, s), 1.22 (3H, d, J=7.3 Hz), 1.41~2.20 (10H, m), 2.24~2.41 (1H, m), 2.71~2.91 (1H, m), 3.29 (1H, br s), 3.35~3.44 (3H, m), 3.88~4.11 (1H, m), 4.12~4.21 (1H, m), 4.26~4.35 (2H, m), 4.57~4.61 (2H, m), 4.78 (2H, d, J=6.0 Hz), 5.18~5.88 (4H, m), 5.90~6.01 (2H, m). FAB-MS m/z 637 $(M+H)^+$.

(3) Allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-2-ylmethylthio]-10-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]undec-2-ene-2- carboxylate (**4c**)

The title compound **4c** (808 mg, 61%) was prepared as an oil from **3c** (1.39 g, 2.18 mmol) by a similar manner as that described for the preparation of 4a: IR (neat) cm^{-1} 2931, 2857, 1781, 1706, 1403, 1284; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 0.08 (6H, s), 0.89 (9H, s), 1.23 (3H, d, J=6.1 Hz), 1.29~1.42 (1H, m), 1.68~2.05 (9H, m), 2.39~2.60 (1H, m), 2.89~2.95 (1H, m), 3.17 (1H, dd, J=6.1, 3.1 Hz), 3.34~3.51 (3H, m), 3.94~4.04 (1H, m), 4.08~4.25 (2H, m), 4.57~4.83 (4H, m), 4.90 (1H, br s), 5.17~5.47 (4H, m), 5.88~6.01 (2H, m). FAB-MS m/z 605 $(M+H)^+$.

(4) (4*S*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-11-oxo-4-[(*S*)-pyrrolidin-2-ylmethylthio]-1-azatricyclo[7.2.0.0^{3,8}]-undec-2-ene-2-carboxylic acid (**5c**)

To a solution of **4c** (806 mg, 1.33 mmol) in dimethylformamide (5 ml) and *N*-methylpyrrolidone (3.2 ml) was added ammonium hydrogenfluoride (305 mg, 5.3 mmol) at room temperature and the mixture was stirred at room temperature for 3 days. The mixture was treated and purified in the same manner as that described for the deprotection of the TBS group of **4a** to give allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-2-ylmethylthio]-10-[(*R*)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]undec-2-ene-2-carboxylate (447 mg, 68%)

as an oil: IR (neat) cm^{-1} 3439, 2936, 1777, 1705, 1285, 1195; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 1.33 (3H, d, J= 6.1 Hz), 1.38~1.44 (1H, m), 1.68~2.04 (9H, m), 2.35~ 2.59 (1H, m), 2.90~3.01 (1H, m), 3.22 (1H, dd, J=6.4, 3.1 Hz), 3.35~3.52 (3H, m), 3.96~4.19 (1H, m), 4.20 (1H, dd, J=10.4, 3.1 Hz), 4.24 (1H, q, J=6.2 Hz), 4.51~4.86 (4H, m), 4.89~4.91 (1H, m), 5.18~5.47 (4H, m), 5.87~6.03 (2H, m). FAB-MS *m/z* 491 $(M+H)^+$.

The title compound **5c** (170 mg, 53%) was prepared as a powder from allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-2-ylmethylthio]-10-[(*R*)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.$0^{3,8}$]undec-2-ene-2-carboxylate (430 mg, 0.88 mmol) by a similar manner as that described for the preparation of **5a**: IR (KBr) cm^{-1} 3402, 2930, 1762, 1587, 1348, 1215; ^1H NMR (270 MHz, D_2O, TSP) δ 1.29 (3H, d, J=6.6 Hz), 1.30~1.42 (1H, m), 1.76~2.14 (10H, m), 2.20~2.34 (1H, m), 2.70~2.90 (1H, m), 3.39 (1H, dd, J=11.8, 6.6 Hz), 3.34~3.48 (4H, m), 3.73~3.78 (1H, m), 4.21~4.29 (2H, m), 4.90 (1H, br s). FAB-MS *m/z* 367 $(M+H)^+$.

Anal Calcd for $C_{18}H_{26}N_2O_4S \cdot H_2O$:
C 56.23, H 7.34, N 7.29, S 8.34.
Found: C 56.19, H 7.06, N 7.28, S 8.21.

Synthesis of (4*S*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-4-[(*S*)-pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo-[7.2.0.$0^{3,8}$]undec-2-ene-2-carboxylic Acid (**12a**)

(1) (3*S*,4*R*)-4-[(2*S*,6*R*)-2-[(*S*)-(1-Allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (**8a**) and (3*S*,4*R*)-4-[(2*R*,6*R*)-2-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (**9a**)

To a solution of (3*S*,4*R*)-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]-4-[(2*R*,6*R*)-2-(hydroxymethyl)cyclohexanon-2-yl]azetidin-2-one (**6**, 1.26 g, 3.54 mmol) in tetrahydrofuran (15 ml) was added triethylamine (596 μl, 4.25 mmol) and methanesulfonyl chloride (302 μl, 3.90 mmol) under ice-cooling. The mixture was stirred for 2 hours and the mixture was filtered. The filtrate was concentrated by evaporation under reduced pressure to give a mesylate. The mesylate was dissolved in dimethylformamide (15 ml) and (*S*)-1-allyloxycarbonyl-3-mercaptopyrrolidine (1.33 g, 7.08 mmol) in dimethylformamide (5 ml) and triethylamine (596 μl, 4.25 mmol) was added to the mesylate solution. The mixture was stirred at room temperature for 2 hours and 40°C for 3 hours. The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (Hexane : AcOEt, 1 : 3) followed by preparative HPLC (cosmosil $5C_{18}$AR 28×250 mm) to give **8a** (723 mg, 39%) and **9a** (1.03 g, 55%) as colorless oils.

8a: IR ($CHCl_3$) cm^{-1} 3417, 2953, 2860, 1698, 1413; ^1H NMR (400 MHz, $CDCl_3$, TMS) δ 0.06 (3H, s), 0.07 (3H, s), 0.87 (9H, s), 1.78 (3H, d, J=6.3 Hz), 1.58~2.26 (8H, m), 2.61~2.73 (3H, m), 2.85~2.92 (2H, m), 3.24~3.80 (5H, m), 4.04 (1H, dd, J=5.7, 1.7 Hz), 4.19 (1H, qd, J=6.3, 5.1 Hz), 4.59 (2H, d, J=5.5 Hz), 5.21 (1H, dd, 10.3, 1.3 Hz), 5.31 (1H, dd, J=17.5, 1.3 Hz), 5.85 (1H, br d, J=6.0 Hz), 5.89~5.99 (1H, m). FAB-MS *m/z* 525 $(M+H)^+$.

9a: IR ($CHCl_3$) cm^{-1} 3418, 2953, 2860, 1753, 1702, 1412; ^1H NMR (400 MHz, $CDCl_3$, TMS) δ 0.06 (3H, s), 0.07 (3H, s), 0.87 (9H, s), 1.23 (3H, d, J=6.3 Hz), 1.33~ 2.62 (11H, m), 2.86 (1H, dd, J=4.8, 2.4 Hz), 2.98 (1H, dd, J=13.9, 5.8 Hz), 3.26~3.81 (5H, m), 4.09~4.12 (1H, m), 4.20 (1H, qd, J=6.3, 4.8 Hz), 4.59 (2H, d, J=5.9 Hz), 5.21 (1H, d, J=10.3 Hz), 5.31 (1H, d, J=19.0 Hz), 5.72 (1H, br s), 5.89~5.99 (1H, m). FAB-MS *m/z* 525 $(M+H)^+$.

(2) Allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]-10-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]-11-oxo-1-azatricyclo[7.2.0.$0^{3,8}$]undec-2-ene-2-carboxylate (**11a**)

To a solution of **8a** (720 mg, 1.37 mmol) in dichloromethane (10 ml) were added triethylamine (384 μl, 2.74 mmol) and allyloxalyl chloride (305 mg, 2.06 mmol) under ice-cooling and the mixture was stirred for 1 hour. To the mixture was added 2-propanol (52 μl, 0.69 mmol) and the mixture was stirred for 15 minutes. The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (hexane - AcOEt, 1 : 1) to give (3*S*,4*R*)-1-allyloxalyl-4-[(2*S*, 6*R*)-2-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]-cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (873 mg, 100%). To a solution of (3*S*,4*R*)-1-allyloxalyl-4-[(2*S*, 6*R*)-2-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]-cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (873 mg, 1.37 mmol) in toluene (2 ml) was added diethyl ethylphosphonite (617 mg, 4.11 mmol) and the mixture was stirred at 60°C for 1.5 hours. The mixture was concentrated by evaporation under reduced pressure and mesitylene (50 ml) was added to the residue. The mixture was heated at 140°C for 1 hour and 120°C for 1.5 hours. The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (hexane - AcOEt, 3 : 2 to1 : 1) to give **11a** (672 mg, 81%) as an oil: IR ($CHCl_3$) cm^{-1} 2933, 2860, 1773, 1693, 1413; ^1H NMR (400 MHz, $CDCl_3$, TMS) δ 0.07 (3H, s), 0.08 (3H, s), 0.88 (9H, s), 1.23 (3H, d, J= 6.4 Hz), 1.2~2.33 (8H, m), 2.68~2.80 (2H, m), 2.94 (1H,

m), 3.17 (1H, dd, J=6.4, 3.1 Hz), 3.24~3.62 (4H, m), 3.75~3.80 (1H, m), 3.88 (1H, br s), 4.11 (1H, dd, J=10.3, 3.1 Hz), 4.20 (1H, q, J=6.4 Hz), 4.59 (2H, d, J=5.6 Hz), 4.65~4.80 (2H, m), 5.21 (1H, d, J=13.2 Hz), 5.24 (1H, d, J=11.1 Hz), 5.31 (1H, d, J=17.5 Hz), 5.44 (1H, d, J=17.5 Hz), 5.89~6.00 (2H, m). FAB-MS m/z 605 $(M+H)^+$.

(3) (4*S*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-4-[(*S*)-pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo-[7.2.0.0^{3,8}]undec-2-ene-2-carboxylic acid (**12a**)

To a solution of **11a** (670 mg, 1.11 mmol) in dimethylformamide (10 ml) and *N*-methylpyrrolidone (3.4 ml) was added ammonium hydrogenfluoride (316 mg, 5.54 mmol) at room temperature and the mixture was stirred at room temperature for 2 days. To the mixture was added 5% aqueous $NaHCO_3$ and then the mixture was extracted with AcOEt (100 ml×3). The extract was washed with brine, dried over Na_2SO_4 and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane - AcOEt, 1 : 5) to give allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]-10-[(*R*)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]undec-2-ene-2-carboxylate (253 mg, 47%): IR ($CHCl_3$) cm^{-1} 3610, 2940, 1771, 1693, 1413; ^1H NMR (400 MHz, $CDCl_3$, TMS) δ 1.33 (3H, d, J=6.2 Hz), 1.57~2.23 (9H, m), 2.69~2.81 (2H, m), 2.96~3.06 (1H, m), 3.22 (1H, d, J=6.6, 3.1 Hz), 3.20~3.62 (4H, m), 3.77 (1H, dd, J=10.6, 6.0 Hz), 3.90 (1H, m), 4.17 (1H, dd, J=10.3, 3.1 Hz), 4.21~4.28 (1H, m), 4.56 (2H, d, J=5.7 Hz), 4.66~4.84 (2H, m), 5.21 (1H, d, J=10.1 Hz), 5.26 (1H, d, J=10.1 Hz), 5.31 (1H, d, J=17.4 Hz), 5.44 (1H, d, J=17.4 Hz), 5.89~6.02 (1H, m). FAB-MS m/z 491 $(M+H)^+$.

To a solution of allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]-10-[(*R*)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]undec-2-ene-2-carboxylate (250 mg, 0.52 mmol) in dichloromethane (5.0 ml) were added water (51 μl, 2.84 mmol), bis-(triphenylphosphine)palladium dichloride (18 mg, 0.026 mmol) and tributyltin hydride (1.05 g, 3.61 mmol) at 0~5°C under nitrogen atomosphere. The mixture was stirred at room temperature for 30 minutes. Dichloromethane (30 ml) was added to the mixture and the mixture was extracted with water (30 ml×3). The aqueous layer was washed with dichloromethane and concentrated to 5 ml under reduced pressure. The residue was purified by reversed phase column chromatography (Cosmosil 75C_{18} PREP, eluted with 3~16% acetonitrile-water). The desired fraction was concentrated under reduced pressure followed by lyophilization to give **12a** (127 mg, 67%) as a colorless powder: IR (KBr) cm^{-1} 3408, 2929, 1758, 1586, 1392, 1252; ^1H NMR (270 MHz, D_2O, TSP) δ 1.27 (3H, d, J=6.2 Hz), 1.26~1.38 (1H, m), 1.60~1.71 (3H, m), 1.82~2.00 (3H, m), 2.34~2.44 (1H, m), 2.88 (1H, d, J=13.5, 6.1 Hz), 2.94 (1H, d, J=13.5, 10.3 Hz), 2.97~3.06 (1H, m), 3.24 (1H, q, J=6.8 Hz), 3.34~3.41 (2H, m), 3.49 (1H, dt, J=11.8, 7.5 Hz), 3.59~3.71 (3H, m), 4.15 (1H, dd, J=10.7, 3.0 Hz), 4.23 (1H, q, J=6.2 Hz). FAB-MS m/z 367 $(M+H)^+$.

Anal Calcd for $C_{18}H_{26}N_2O_4S \cdot 1.5H_2O$:
C 54.94, H 7.43, N 7.12, S 8.15.
Found: C 55.10, H 7.23, N 7.17, S 8.28.

Synthesis of (4*R*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-4-[(*S*)-pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo-[7.2.0.0^{3,8}]undec-2-ene-2-carboxylic Acid (**14a**)

(1) Allyl (4*R*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]-10-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]undec-2-ene-2-carboxylate (**13a**)

To a solution of **9a** (460 mg, 0.88 mmol) in dichloromethane (5 ml) were added triethylamine (245 μl, 4.69 mmol) and allyloxalyl chloride (195 mg, 1.31 mmol) under ice-cooling and the mixture was stirred for 1.5 hours. To the mixture was added 2-propanol (33 μl, 0.44 mmol) and the mixture was stirred for 10 minutes. The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (hexane - AcOEt, 1 : 1) to give (3*S*,4*R*)-1-allyloxalyl-4-[(2*S*, 6*R*)-2-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (556 mg, 99%). To a solution of (3*S*,4*R*)-1-allyloxalyl-4-[(2*S*, 6*R*)-2-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]-cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (556 mg, 0.87 mmol) in toluene (1 ml) was added diethyl ethylphosphonite (655 mg, 4.37 mmol) and the mixture was stirred at 60°C for 1.5 hours. The mixture was concentrated by evaporation under reduced pressure and mesitylene (50 ml) was added to the residue. The mixture was heated at 130°C for 2.5 hours and refluxed for 2 hours. The mixture was concentrated by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (hexane - AcOEt, 3 : 1 to1 : 1) to give **13a** (348 mg, 66%) as an oil: IR ($CHCl_3$) cm^{-1} 2933, 1769, 1691, 1413; ^1H NMR (400 MHz, $CDCl_3$, TMS) δ 0.07 (6H, s), 0.88 (9H, s), 1.22 (3H, d, J=6.2 Hz), 1.17~1.61 (3H, m), 1.79~2.32 (5H, m), 2.53~2.82 (3H, m), 3.13~3.79 (7H, m), 4.09~4.22 (2H, m), 4.59 (2H, d, J=5.4 Hz), 4.62~4.80 (2H, m), 5.21 (1H, d, J=11.3 Hz), 5.25 (1H, d, J=11.3 Hz), 5.30 (1H, d, J=17.0 Hz), 5.42

(1H, d, J=17.0 Hz), 5.89 (2H, m). FAB-MS m/z 605 $(M+H)^+$.

(2) (4*R*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-4-[(*S*)-pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo-[7.2.0.0^{3,8}]undec-2-ene-2- carboxylic acid (**14a**)

Allyl (4*R*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]-10-[(*R*)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]undec-2-ene-2-carboxylate (217 mg, 73%) was prepared as an oil from **13a** (365 mg, 0.60 mmol) by a similar manner as that described for the desilylation of **11a**: ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 1.33 (3H, d, J=6.3 Hz), 3.15~3.78 (7H, m), 4.11~4.24 (2H, m), 4.59 (2H, d, J=5.4 Hz), 4.67~4.81 (2H, m), 5.19~5.49 (4H, m), 5.87~6.06 (2H, m). FAB-MS m/z 491 $(M+H)^+$.

The title compound **14a** (80 mg, 49%) was prepared as a powder from allyl (4*R*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]-10-[(*R*)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]undec-2-ene-2-carboxylate (216 mg, 0.441 mmol) by a similar manner as that described for the preparation of **12a**: ^{1}H NMR (400 MHz, D_2O, TSP) δ 1.08 (3H, d, J=6.4 Hz), 0.97~1.39 (3H, m), 1.65~1.88 (4H, m), 2.15~2.24 (1H, m), 2.32~2.40 (1H, m), 2.58 (1H, dd, J=12.7, 7.3 Hz), 2.62~2.68 (1H, m), 3.00 (1H, dd, J=12.7, 7.8 Hz), 3.05 (1H, dd, J=13.7, 4.4 Hz), 3.14~3.22 (2H, m), 3.32 (1H, td, J=11.7, 7.8 Hz), 3.38~3.48 (2H, m), 3.96 (1H, dd, J=9.8, 2.9 Hz), 4.03 (q, J=6.4 Hz). FAB-MS m/z 367 $(M+H)^+$.

Anal Calcd for $C_{18}H_{26}N_2O_4S \cdot H_2O$:
C 56.23, H 7.34, N 7.29, S 8.34.
Found: C 56.46, H 7.42, N 7.09, S 8.59.

Synthesis of (4*S*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-4-[(*S*)-pyrrolidin-2-ylmethylthiomethyl]-11-oxo-1-azatricyclo-[7.2.0.0^{3,8}]undec-2-ene-2-carboxylic Acid (**12b**)

(1) (3*S*,4*R*)-4-[(2*S*,6*R*)-2-[(*S*)-(1-Allyloxycarbonyl)pyrrolidin-2-ylmethylthiomethyl]cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (**8b**) and (3*S*,4*R*)-4-[(2*R*,6*R*)-2-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-2-ylmethylthiomethyl]cyclohexanon-6-yl]-3-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]azetidin-2-one (**9b**)

The title compounds **8b** (383 mg, 26%) and **9b** (543 mg, 37%) were prepared as oils from **7** (980 mg, 2.76 mmol) by a similar manner as that described for the preparation of **8a** and **9a**.

8b: $[\alpha]_D^{25}$=+13.4° (c=0.70, $CHCl_3$); IR (neat) cm^{-1} 3276, 2932, 2858, 1761, 1705, 1406; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 0.06 (3H, s), 0.07 (3H, s), 0.87 (9H, s), 1.20 (3H, d, J=6.2 Hz), 1.64~2.16 (10H, m), 2.40~3.44 (7H, m), 3.38~3.44 (2H, m), 3.88~3.97 (1H, m), 4.02 (1H, m), 4.14~4.24 (1H, m), 4.57~4.62 (2H, m), 5.22 (2H, d, J=10.5 Hz), 5.31 (2H, d, J=17.2 Hz), 5.87~6.02 (1H, m), 6.23 (1H, br s). FAB-MS m/z 539 $(M+H)^+$.

9b: $[\alpha]_D^{25}$=−4.4° (c=0.85, $CHCl_3$); IR (neat) cm^{-1} 3277, 2931, 2858, 1760, 1705, 1405; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 0.06 (3H, s), 0.07 (3H, s), 0.87 (9H, s), 1.23 (3H, d, J=6.0 Hz), 2.38~2.66 (4H, m), 2.82~3.04 (2H, m), 2.87 (1H, dd, J=4.8, 2.4 Hz), 3.40~3.47 (2H, m), 3.92~3.98 (1H, m), 4.08~4.22 (2H, m), 4.59 (2H, m), 5.21 (1H, d, J=8.8 Hz), 5.31 (1H, d, J=17.2 Hz), 5.75 (1H, br s), 5.87~6.01 (1H, m). FAB-MS m/z 539 $(M+H)^+$.

(2) Allyl (4*S*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl)pyrrolidin-2-ylmetylthiomethyl]-10-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]undec-2-ene-2-carboxylate (**11b**)

The title compound **11b** (200 mg, 53%) was prepared as an oil from **8b** (330 mg, 0.612 mmol) by a similar manner as that described for the preparation of **11a**: $[\alpha]_D^{25}$=+37.2° (c=0.79, $CHCl_3$); IR (neat) cm^{-1} 2931, 2858, 1779, 1702, 1404; ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 0.08 (6H, s), 0.88 (9H, s), 1.11~2.05 (10H, m), 1.23 (3H, d, J=6.3 Hz), 2.37~2.54 (1H, m), 2.72~2.98 (4H, m), 3.16 (1H, dd, J=6.4, 3.1 Hz), 3.42~3.45 (2H, m), 3.84~4.00 (2H, m), 4.10 (1H, dd, J=10.4, 3.1 Hz), 4.20 (1H, q, J=6.3 Hz), 4.57~4.81 (4H, m), 5.18~5.46 (4H, m), 5.88~6.01 (1H, m). FAB-MS m/z 619 $(M+H)^+$.

(3) (4*S*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-4-[(*S*)-pyrrolidin-2-ylmethylthiomethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]-undec-2-ene-2- carboxylic acid (**12b**)

The title compound **12b** (71 mg, 61%) was prepared as a powder from **11b** (190 mg, 0.31 mmol) by a similar manner as that described for the preparation of **12a**: IR (KBr) cm^{-1} 3372, 2928, 1758, 1582, 1390; ^{1}H NMR (400 MHz, D_2O, TSP) δ 1.27 (3H, d, J=6.4 Hz), 1.28~1.38 (1H, m), 1.58~1.93 (6H, m), 1.99~2.16 (2H, m), 2.13~2.32 (1H, m), 1.99~2.16 (2H, m), 2.13~2.32 (1H, m), 2.78~2.85 (2H, m), 2.94~3.01 (2H, m), 3.03~3.11 (1H, m), 3.29~3.38 (2H, m), 3.40 (1H, dd, J=6.1, 3.1 Hz), 3.66~3.72 (1H, m), 3.74~3.83 (1H, m), 4.15 (1H, dd, J=10.2, 3.1 Hz), 4.23 (1H, q, J=6.4 Hz). FAB-MS m/z 381 $(M+H)^+$.

Anal Calcd for $C_{19}H_{28}N_2O_4S \cdot 2H_2O$:
C 54.79, H 7.74, N 6.73, S 7.70.
Found: C 55.81, H 7.65, N 6.72, S 7.52.

Synthesis of (4*R*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-4-[(*S*)-pyrrolidin-2-ylmethylthiomethyl]-11-oxo-1-azatricyclo-[7.2.0.0^{3,8}]undec-2-ene-2-carboxylic Acid (**14b**)

(1) Allyl (4*R*,8*S*,9*R*,10*S*)-4-[(*S*)-(1-allyloxycarbonyl) pyrrolidin-2-ylmethylthiomethyl]-10-[(*R*)-1-(*tert*-butyldimethylsilyloxy)ethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]-undec-2-ene-2-carboxylate (**13b**)

The title compound **13b** (265 mg, 46%) was prepared as an oil from **9b** (500 mg, 0.93 mmol) by a similar manner as that described for the preparation of **13a**: $[\alpha]_D^{25}=+54.5°$ (c=0.82, $CHCl_3$); IR (neat) cm^{-1} 2930, 2856, 1775, 1701,1405; ^1H NMR (270 MHz, $CDCl_3$, TMS) δ 0.07 (6H, s), 0.88 (9H, s), 1.11~2.96 (10H, m), 1.22 (3H, d, J=6.2 Hz), 3.07~3.14 (2H, m), 3.42~3.43 (2H, m), 3.89~3.98 (1H, m), 4.08~4.21 (2H, m), 4.57~4.81 (4H, m), 5.19~5.44 (4H, m), 5.87~6.04 (2H, m). FAB-MS *m/z* 619 $(M+H)^+$.

(2) (4*R*,8*S*,9*R*,10*S*)-10-[(*R*)-1-Hydroxyethyl]-4-[(*S*)-pyrrolidin-2-ylmethylthiomethyl]-11-oxo-1-azatricyclo[7.2.0.0^{3,8}]-undec-2-ene-2-carboxylic acid (**14b**)

The title compound **14b** (59 mg, 40%) was prepared as a powder from **13b** (250 mg, 0.404 mmol) by a similar manner as that described for the preparation of **12a**: IR (KBr) cm^{-1} 3363, 2927, 1758, 1583, 1390; ^1H NMR (400 MHz, D_2O, TSP) δ 1.23~1.37 (1H, m), 1.27 (3H, d, J=6.2 Hz), 1.49~1.63 (1H, m), 1.68~1.78 (1H, m), 1.84~1.98 (3H, m), 2.04~2.15 (2H, m), 2.22~2.30 (1H, m), 2.69 (1H, dd, J=11.8, 5.7 Hz), 2.79~2.87 (1H, m), 2.76 (1H, dd, J=14.6, 10.0 Hz), 3.01 (1H, dd, J=14.6, 4.5 Hz), 3.28 (1H, dd, J=11.8, 9.5 Hz), 3.34~3.42 (3H, m), 3.79~3.87 (1H, m), 4.15 (1H, dd, J=9.7, 2.9 Hz), 4.22 (1H, q, J=6.2 Hz). FAB-MS *m/z* 381 $(M+H)^+$.

Anal Calcd for $C_{19}H_{28}N_2O_4S\cdot 2H_2O$:
C 54.79, H 7.74, N 6.73, S 7.70.
Found: C 55.43, H 7.53, N 6.86, S 7.63.

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.

Therapeutic Effect on Systemic Infection in Mice

Overnight cultures of *S. aureus* 507 grown at 37°C in Trypto-soy broth (Eiken Chemical Co., Ltd.) were diluted according to their virulence (2.1×10^7 CFU/mouse). The diluted cultures were mixed with the same amount of 5% 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 cultures. β-Lactam antibiotics (**14a**, PAPM, MEPM, BIPM) and vancomycin were administered subcutaneously at 0 and 4 hours after infection. The ED_{50} values of the mice were calculated by the probit method from the survival rates on the 5th day after infection.

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