Synthesis and Biological Evaluation of Novel Tricyclic Carbapenems (Trinems)

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Recently, MRSA has been causing serious problems in hospitals.12 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 citextel (GV-118819, active form: sanfetrinem sodium, GV-104326) has been developed as an oral trinem by Glaxo SpA (Figure 1).78) 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 pyrrolidinylmethylthio or pyrrolidinylthiomethyl group at the C-4 position. Among them, (4R)-[(5)-pyrrolidin-3-ylthiomethyl]trinem (14a) exhibited potent activity against MRSA in vitro and in vivo.

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We synthesized two types of trinems with pyrrolidinylmethylthio or pyrrolidinylthiomethyl and pyrrolidinylmethylthiomethyl groups at the C-4 position. Among them, (4R)-[(5)-pyrrolidin-3-ylthiomethyl]trinem (14a) exhibited potent activity against MRSA in vitro and in vivo.

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-acetyltiethiomethyl-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-acetyltiethiomethyl-1-allyloxycarbonylpyrrolidine in the presence of ethylenediamine and (S)-1-allyloxycarbonyl-2-mercaptoethylpyrrolidine 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.
Scheme 1.

Reagents: (a) R^SAc, NH₂CH₂CH₂NH₂ (2a, b) or R^SH, Et₃N (2c); (b) Swern oxidation; (c) CI(CO)₂All, Et₃N; (d) EtP(OEt)₂; (e) TBAF or HF·NH₄F; (f) PdCl₂(Ph₃P)₂, Bu₃SnH.

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 Rᵢ group. Deprotection of both 4b and 4e in a similar manner afforded trinems 5b and 5e in 46% and 36% yield, respectively.

Alternatively, thiomethyltrinem derivatives were synthesized from 2-hydroxymethylcyclohexanone derivative 6, 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-allyloxy carbonyl-3-mercapto pyrrolidine and ((S)-1-allyloxy carbonyl-2-mercaptomethyl pyrrolidine 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 proceed 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. 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.
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 activity against P. aeruginosa 1001. The
Table 1. Antibacterial activity (MIC, μg/ml)\(^a\) of tricyclic carbapenems.

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

\(^a\) 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.

<table>
<thead>
<tr>
<th>Organism</th>
<th>14a</th>
<th>PAPM</th>
<th>MEPM</th>
<th>BIPM</th>
<th>VCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus 507(^b)</td>
<td>3.68</td>
<td>23.94</td>
<td>87.10</td>
<td>31.63</td>
<td>1.48</td>
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<tr>
<td>[MIC, μg/ml](^c)</td>
<td>[0.78]</td>
<td>[0.20]</td>
<td>[3.13]</td>
<td>[1.56]</td>
<td>[0.78]</td>
</tr>
</tbody>
</table>

\(^b\) 50% effective sc dose.
\(^c\) Challenged with 5% mucin.

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 ddY 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 (55XC) 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-d₄ (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 75C₁₈ PREP (75 μm, Nacalai Tesque, Inc.).

Synthesis of (4S,8S,9R,10S)-10-[(R)-1-Hydroxyethyl]-11-oxo-4-[(S)-pyrrolidin-3-ylmethylthio]-1-azatricyclo[7.2.0.0³⁷]undec-2-ene-2-carboxylic Acid (5a)

(1) (3S,4R)-4-[(2S,6R)-2-[(S)-1-Allyloxyacarboryl]pyrrolidin-3-ylmethylthio]cyclohexan-6-yl]-3-[(R)-1-(tert-butyldimethylsiloxy)ethyl]azetidin-2-one (2a)

A solution of (3S,4R)-4-[(1R,2S,3R)-2,3-epoxycyclohexyl]-3-[(R)-1-(tert-butyldimethylsiloxy)ethyl]azetidin-2-one (1, 892 mg, 2.74 mmol) and (S)-3-acetyltithio-1-allyloxyacarborylpyrrolidine (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 Na₂SO₄ and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane-EtOAc, 3:1) to give 2a (600 mg, 1.14 mmol) and triethylamine (384 μl, 2.74 mmol) in dichloromethane (7 ml) was added dropwise allyloxy acril 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 (428 mg, 60%) as an oil: IR (neat) cm⁻¹ 3277, 2935, 2858, 1760, 1704, 1412; ¹H NMR (270 MHz, CDCl₃, TMS) δ 0.07 (3H, s), 0.08 (3H, s), 0.08 (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)⁺.

To a solution of (3S,4R)-4-[(S)-1-Allyloxyacarboryl]pyrrolidin-3-ylmethylthio]cyclohexan-6-yl]-3-[(R)-1-(tert-butyldimethylsiloxy)ethyl]azetidin-2-one (2a) (600 mg, 1.14 mmol) and triethylamine (384 μl, 2.74 mmol) in dichloromethane (7 ml) was added dropwise allyloxy acril 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 3277, 2935, 2858, 1808, 1756, 1704, 1409, 1215; ¹H NMR (270 MHz, CDCl₃, TMS) δ 0.04 (3H, s), 0.07 (3H, s), 0.08 (9H, s), 1.20 (3H, d, J=6.3 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)⁺.


A solution of 3a (423 mg, 0.66 mmol) and diethyl ethylphosphonite (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 3275, 2934, 2858, 1760, 1704, 1410, 1106; ¹H NMR (270 MHz, CDCl₃, 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 = 5.5 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) (3S,4S,9R,10S)-10-[(R)-1-Hydroxyethyl]-11-oxo-4-[(S)-pyrrolidin-3-ylmethylthio]-1-azatricyclo[7.2.0.0³,8]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 atmosphere. 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 Na₂SO₄. The residue was purified by reversed phase chromatography to afford allyl (4S,8S,9R,10S)-4-[(S)-(1-allyloxy carbonyl)pyrrolidin-3-ylmethylthio]-11-oxo-4-[(R)-pyrrolidin-3-ylmethylthio]-1-azatricyclo[7.2.0.0³,8]undec-2-ene-2-carboxylic acid (5a).

Synthesis of (4S,8S,9R,10S)-10-[(R)-1-Hydroxyethyl]-11-oxo-4-[(S)-pyrrolidin-3-ylmethylthio]-1-azatricyclo[7.2.0.0³,8]undec-2-ene-2-carboxylic Acid (5b)

The title compound 5b (270 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, CDCl₃, 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) (3S,4R)-1-Allyloxyalyl-4-[(2S,6R)-2-[(R)-1-Allyloxycarbonyl]pyrrolidin-3-ylmethylthio]-cyclohexanox-6-yl]-2-oxocyclohexyl]-3-[(R)-1-(tert-butyl dimethylsililyloxy)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 3275, 2934, 2858, 1756, 1703, 1409, 1215; ¹H NMR (270 MHz, CDCl₃, 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)⁺.


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, CDCl₃, 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) (3S,4R)-1-allyloxycarbonyl-4-[(2S,6R)-2-[(S)-(1-allyloxycarbonyl)pyrrolidin-2-ylmethylthio]-1-azatricyclo[7.2.0.0³,⁸]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, D₂O, TSP) 8 1.84 (6H, s), 5.77–5.82 (1H, m), 5.93 (1H, br s), 5.17–5.82 (4H, m), 5.90–6.01 (2H, m). FAB-MS m/z 605 (M+H)+.


(1) (3S,4R)-4-[(2S,6R)-2-[(S)-(1-allyloxy carbonyl)pyrrolidin-2-ylmethylthio]-cyclohexan-6-yl]-3-[(R)-1-(tert-butyl dimethylsilyloxy)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-allyloxy carbonyl-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 Na₂SO₄. After concentration of the mixture under reduced pressure, the residue was purified by silica gel column chromatography (Hexane-EtOAc, 1:1) to give 3c (1.39 g, 2.18 mmol) by a similar manner as that described for the preparation of 3a: IR (KBr) cm⁻¹ 3382, 2929, 1808, 1756, 1703, 1401, 1214; ¹H NMR (270 MHz, CDCl₃, 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), 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), 5.18–5.48 (4H, m), 5.90–6.01 (2H, m). FAB-MS m/z 637 (M+H)+.

(2) (3S,4R)-1-allyloxy carbonyl-4-[(2S,6R)-2-[(S)-(1-allyloxy carbonyl)pyrrolidin-2-ylmethylthio]-cyclohexan-6-yl]-3-[(R)-1-(tert-butyl dimethylsilyloxy)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 3276, 2925, 2859, 1758, 1704, 1105; ¹H NMR (270 MHz, CDCl₃, 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, ddd, 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)+.

(3) Allyl (4S,8S,9R,10S)-4-[(S)-(1-allyloxy carbonyl)pyrrolidin-2-ylmethylthio]-10-[(R)-1-(tert-butyl dimethylsilyloxy)ethyl]cyclodecane-6-yl]-11-oxo-1-azatricyclo[7.2.0.0³,⁸]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 (KBr) cm⁻¹ 3291, 2857, 1781, 1706, 1403, 1284; ¹H NMR (270 MHz, CDCl₃, 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)+.

To a solution of 4c (806 mg, 1.33 mmol) in dimethylformamide (5 ml) and N-methylpyrrolidone (3.2 ml) was added ammonium hydrogen fluoride (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 (4S,8S,9R,10S)-4-[(S)-(1-allyloxy carbonyl)pyrrolidin-2-ylmethylthio]-10-[(R)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.0³,⁸]undec-2-ene-2-carboxylic acid (5c)

The title compound 5c (808 mg, 61%) was prepared as an oil from 4c (806 mg, 1.33 mmol) by a similar manner as that described for the preparation of 5c: IR (KBr) cm⁻¹ 3291, 2931, 2857, 1781, 1706, 1403, 1284; ¹H NMR (270 MHz, CDCl₃, TMS) δ 0.09 (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)+.

as an oil: IR (neat) cm⁻¹ 3439, 2936, 1777, 1705, 1285, 1195; ¹H NMR (270 MHz, CDCl₃, 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 (4S,8S,9R,10S)-4-[[S-(1-allyloxycarbonyl)pyrrolidin-2-yl]methylthio]-10-[(R)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.0³⁸]undec-2-ene-2-carboxylate (430 mg, 0.88 mmol) by a similar manner as that described for the preparation of 5a:

**Anal Caled for C₅₆H₇₇N₂O₅S: C 56.23, H 7.34, N 7.29, S 8.34.**

**Found:** C 56.19, H 7.06, N 7.28, S 8.34.

**Synthesis of (3S,4R)-4-[[S-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]cyclohexanon-6-yl]-11-oxo-1-azatricyclo[7.2.0.0³⁸]undec-2-ene-2-carboxylic Acid (12a)**

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

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 (302 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 8a (723 mg, 39%) as colorless oils.

8a: IR (CHCl₃) cm⁻¹ 3417, 2953, 2860, 1698, 1413; ¹H NMR (400 MHz, CDCl₃, TMS) δ 0.07 (3H, s), 0.08 (3H, s), 0.92 (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⁺H⁺).

9a: IR (CHCl₃) cm⁻¹ 3418, 2953, 2860, 1753, 1702, 1412; ¹H NMR (400 MHz, CDCl₃, 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, dd, J = 10.3 Hz), 5.31 (1H, dd, J = 19.0 Hz), 5.72 (1H, br s), 5.89–5.99 (1H, m). FAB-MS m/z 525 (M⁺H⁺).

(2) Allyl (4S,8S,9R,10S)-4-[[S-(1-allyloxycarbonyl)pyrrolidin-3-ylthiomethyl]-10-[(R)-1-(tert-butyldimethylsilyloxy)ethyl]-11-oxo-1-azatricyclo[7.2.0.0³⁸]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 (302 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 8a (723 mg, 39%) as colorless oils.

8a: IR (CHCl₃) cm⁻¹ 3418, 2953, 2860, 1698, 1413; ¹H NMR (400 MHz, CDCl₃, TMS) δ 0.07 (3H, s), 0.08 (3H, s), 0.92 (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⁺H⁺).
To a solution of 11a (670 mg, 1.11 mmol) in dimethylformamide (10 ml) and N-methylpyrrolidone (3.4 ml) was added ammonium hydrogen fluoride (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 NaHCO₃ and then the mixture was extracted with AcOEt (100 ml×3). The extract was washed with brine, dried over Na₂SO₄ and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane – AcOEt, 1:5) to give allyl (4S,8S,9R,10S)-4-[(5)-(1-allyloxy-carbonyl)pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo[7.2.0.0³⁸]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 hydrogen fluoride (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 NaHCO₃ and then the mixture was extracted with AcOEt (100 ml×3). The extract was washed with brine, dried over Na₂SO₄ and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane – AcOEt, 1:5) to give allyl (4S,8S,9R,10S)-4-[(5)-(1-allyloxy-carbonyl)pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo[7.2.0.0³⁸]undec-2-ene-2-carboxylic acid (12a).

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To a solution of 11a (670 mg, 1.11 mmol) in dimethylformamide (10 ml) and N-methylpyrrolidone (3.4 ml) was added ammonium hydrogen fluoride (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 NaHCO₃ and then the mixture was extracted with AcOEt (100 ml×3). The extract was washed with brine, dried over Na₂SO₄ and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane – AcOEt, 1:5) to give allyl (4S,8S,9R,10S)-4-[(5)-(1-allyloxy-carbonyl)pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo[7.2.0.0³⁸]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 hydrogen fluoride (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 NaHCO₃ and then the mixture was extracted with AcOEt (100 ml×3). The extract was washed with brine, dried over Na₂SO₄ and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane – AcOEt, 1:5) to give allyl (4S,8S,9R,10S)-4-[(5)-(1-allyloxy-carbonyl)pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo[7.2.0.0³⁸]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 hydrogen fluoride (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 NaHCO₃ and then the mixture was extracted with AcOEt (100 ml×3). The extract was washed with brine, dried over Na₂SO₄ and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane – AcOEt, 1:5) to give allyl (4S,8S,9R,10S)-4-[(5)-(1-allyloxy-carbonyl)pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo[7.2.0.0³⁸]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 hydrogen fluoride (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 NaHCO₃ and then the mixture was extracted with AcOEt (100 ml×3). The extract was washed with brine, dried over Na₂SO₄ and concentrated by evaporation under reduced pressure. The residue was purified by silica gel column chromatography (hexane – AcOEt, 1:5) to give allyl (4S,8S,9R,10S)-4-[(5)-(1-allyloxy-carbonyl)pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo[7.2.0.0³⁸]undec-2-ene-2-carboxylic acid (12a).
(1H, d, J=17.0 Hz), 5.89 (2H, m). FAB-MS m/z 605 (M+H)+.

(2) (4R,8S,9R,10S)-10-[(R)-1-Hydroxyethyl]-4-[(S)-pyrrolidin-3-ylthiomethyl]-11-oxo-1-azatricyclo[7.2.0.03'8]undec-2-ene-2-carboxylic acid (14a)


The title compound 14a (80 mg, 49%) was prepared as a powder from allyl (4R,8S,9R,10S)-4-[(S)-1-allyloxy carbonyl]pyrrolidin-3-ylthiomethyl]-10-[(R)-1-hydroxyethyl]-11-oxo-1-azatricyclo[7.2.0.03'8]undec-2-ene-2-carboxylate (13b)


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 11a: [α]D 4S = +37.2° (c=0.79, CHCl3); IR (neat) cm⁻¹ 2931, 2858, 1759, 1702, 1404; 1H NMR (400 MHz, CDCl3, TMS) δ 0.09 (6H, s), 1.78 (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 539 (M+H)+.
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: $[\alpha]_D^{25} = +54.5^\circ$ (c=0.82, CHCl$_3$); IR (neat) cm$^{-1}$ 2930, 2856, 1775, 1701, 1405; $^1$H NMR (270MHz, CDCl$_3$, TMS) $\delta$ 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) (4R,8S,10S)-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 (59mg, 40%) was prepared as a powder from 13b (250mg, 0.404mmol) by a similar manner as that described for the preparation of 12a: IR (KBr) cm$^{-1}$ 3363, 2927, 1758, 1583, 1390; $^1$H NMR (400 MHz, D$_2$O, TSP) $\delta$ 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$_2$O$_4$S $\cdot$ 2H$_2$O: 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$\times$10$^7$ CFU/mouse). The diluted cultures were mixed with the same amount of 5% gastric mucin (Tokyo Kasei Kogyo Co., Ltd.). Seven male SPF ddY mice in each group were infected intraperitoneally with 0.2ml portions of these bacterial cultures. $\beta$-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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References