A new type of thienamycin derivatives (3a~3j, 4a, 4b), having a monothioacetal or a thioacetal side chain at the C-2 position was prepared, and the susceptibility to renal dehydropeptidase-1 (DHP-1) and the antimicrobial activity of these compounds were determined. The structure-activity relationships of these derivatives are also discussed.

There has been intense interest in the chemical modifications of thienamycin (THM) in the pursuit of higher physico-chemical stability and antimicrobial activity, and the first carbapenem antibiotic, imipenem (MK0787) has been developed recently. This antibiotic, however, is reported to be metabolized by dehydropeptidase-1 (DHP-1) and it is used clinically in combination with cilastatin, a DHP-1 inhibitor.

To date, only a few studies on the relationships between the structure and the DHP-1 susceptibility of carbapenem antibiotics have been reported in the literature, and it was of interest to investigate THM derivatization further for the purpose of improving the susceptibility to DHP-1. The present paper deals with the synthesis and structure-activity relationships of a series of THM derivatives (3a~3j, 4a, 4b) having a monothioacetal or a thioacetal moiety.

Chemistry

In previous papers, we have described a facile method using a masked thiol for the preparation of the S-a-methoxy THM derivative (3a), which, it was thought, might have high antimicrobial activity as well as improved stability to DHP-1. This method was considered to be very convenient for the synthesis of a series of THM derivatives (3b~3j, 4a, 4b) having an acetal moiety (see Fig. 2), and preparation of these compounds was carried out as shown in Schemes 1 and 2. Thus, masked thiols such as 6b~6i were prepared by the Pummerer reaction of 5 with N-chlorosuccinimide (NCS) followed by a replacement reaction using various nucleophiles in the presence of a catalyst. Although compound 6b was obtained in 60% yield by the use of Ag2O as a catalyst, none of the desired com-
Compound, 6c, was obtained under similar reaction conditions. After surveying a variety of conditions, this reaction was found to proceed smoothly by the use of Cu powder or Ag$_2$CO$_3$ as a catalyst instead of Ag$_2$O. Therefore Cu powder, which was more effective than Ag$_2$CO$_3$, was employed in the subsequent experiments. When the protected THM sulfoxide (8) was treated with the masked thiols (6b~6i) thus obtained in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), compounds 7b~7i were obtained in fairly good yields after purification by reverse-phase HPLC, respectively. Finally, catalytic hydrogenation of compounds 7b~7i in the presence of PtO$_2$ afforded the target compounds 3b~3i after purification by column chromatography on Diaion HP-20.

Compounds 4a and 4b were prepared similarly from the starting materials, 9a and 9b, as shown in Scheme 2, respectively.

Fig. 2.

![Scheme 1](image)

Compounds 4a and 4b were prepared similarly from the starting materials, 9a and 9b, as shown in Scheme 2, respectively.

![Scheme 2](image)

PNZ = p-Nitrobenzylxoycarbonyl
PNB = p-Nitrobenzyl
For the preparation of a sulfur analogue (3j) of 3a, the masked thiol method described above was considered to be unsuitable because of the volatility and foul smell of methylmercaptan, and S-α-methylthio THM (3j) was prepared by another method (Scheme 3). Thus, the Pummerer reaction of 13, prepared by protection of the amino group of 12 with a p-nitrobenzylxycarbonyl group (PNZ), with sulfuryl chloride followed by replacement reaction using thiobenzoic acid in the presence of DBU afforded compound 14 in 47% yield. Although the instability of thiohemiacetals such as 15 was anticipated, the desired compound (15) was isolated in 40% yield on treatment of 14 with 3,4-dimethoxyphenethylamine after silica gel column chromatography. The replacement reaction of the protected THM sulfoxide (8) with the thiohemiacetal (15) in the presence of N,N-diisopropylethylamine gave 16 in 74% yield after purification by reverse-phase HPLC. Finally, catalytic hydrogenation of 16 in the presence of PtO₂ as described above afforded the target compound 3j in 63% yield.

Scheme 2.

Scheme 3.
Biological Properties and Discussion

The susceptibility to DHP-1 and the MICs of the THM derivatives (3a~3j, 4a, 4b) against Gram-positive and Gram-negative bacteria are listed in Table 1 comparing with those of THM and MK0787. Christensen has reported\(^\text{22}\) that introduction of an alkyl group such as a methyl or ethyl group at the S-\(\alpha\)-position of the cysteamine side chain of THM increased the stability to hydrolysis by DHP-1 in the order of increasing bulkiness of the substituents. However, methoxy (3a) and methylthio compounds (3j) showed only a little improvement in stability to DHP-1 compared with THM, and no such increases as were seen with the introduction of an alkyl group were observed. As seen in compounds 3c, 3e and 3f, introduction of a more bulky substituent at the \(\alpha\)-position of the cysteamine side chain afforded moderate stability. These results indicate that acetal-type modification is not so effective for the stability to hydrolysis by DHP-1 as the introduction of alkyl group.\(^\text{22}\) Surprisingly, compound 4b, which has a heteroaromatic ring, was 10 times more susceptible to hydrolysis by DHP-1 than THM. As judged from the result of compounds 3d and 4b, the heteroaromatic ring seemed particularly to increase the susceptibility to hydrolysis by DHP-1 in spite of its bulkiness. On the other hand, introduction of basic substituents 3b and 3g~3i resulted in considerable DHP-1-resistance whose degree rose in the order of their increasing bulkiness. Of these substituents, compound 3i was 11 times more stable than THM. These results indicate that the basicity of the substituent at the S-\(\alpha\)-position of the cysteamine side chain of THM, as well as its bulkiness, also plays an important role in the stability to hydrolysis by DHP-1.

The antimicrobial activities of the THM derivatives (3a~3j, 4a, 4b) against Gram-positive bacteria were generally excellent as well as THM; potent activity of methylthio compound 3j against *Streptococcus faecalis* should be noted. On the other hand, as reported by Christensen,\(^\text{21}\) introduction of a substituent at the S-\(\alpha\)-position of the cysteamine side chain of THM resulted in lowering the activity against Gram-negative bacteria, particularly against *Pseudomonas aeruginosa*. Of this series of derivatives, only the activity of compound 3g was roughly comparable to that of THM. Introduction of a basic substituent retained the anti-pseudomonal activity of the parent compound compared with that of a neutral substituent although a tendency for the activity against Gram-negative bacteria to decrease was observed. For example, 3g was 8 times more active than 3e against *P. aeruginosa*, but only half as active against *Escherichia coli*. Compounds 3a and 3j showed excellent antimicrobial properties comparable to those of THM except against *P. aeruginosa*. In comparison with thioacetal and monothioacetal derivatives, 3j had slightly higher activity against Gram-positive and Gram-negative bacteria than 3a, whereas 3g was significantly more active than 3b, indicating that thioacetal derivatives should be superior to monothioacetal derivatives with regard to antimicrobial activity.

In summary, of a new type of THM derivatives (3a~3j, 4a, 4b) having a monothioacetal or a thioacetal side chain at the C-2 position, compound 3g was the most active against Gram-positive and Gram-negative bacteria including *P. aeruginosa*, which was close to that of THM. Moreover, it displayed fairly good improvement in stability to hydrolysis by DHP-1 compared with THM.

**Experimental**

**Antibiotics**

THM and MK0787 were gifts of Merck, Sharp & Dohme Research Laboratories. The substrate
Table 1. Antibacterial activity and DHP-1 stability of thienamycin and its derivatives.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>MK0787</th>
<th>THM</th>
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<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3f</th>
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<td>100</td>
<td>80</td>
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* Abbreviations: *E.*, *Escherichia*; *C.*, *Citrobacter*; *Pr.*, *Proteus*; *K.*, *Klebsiella*; *Ent.*, *Enterobacter*; *Ser.*, *Serratia*; *Ps.*, *Pseudomonas*; *S.*, *Staphylococcus*; *Str.*, *Streptococcus*.

b DHP-1 susceptibility is given relative to MK0787 = 100.
for DHP-1, glycyldehydrophenylalanine (Gly-dh-Phe), was prepared according to the directions of Campbell.  

Measurement of In Vitro Antibacterial Activity

Minimal inhibitory concentrations (MICs) were determined according to the 2-fold broth dilution method using Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) with the inoculum size of $10^5$ cfu/ml. The MIC was defined as the lowest concentration which prevented visible growth of bacteria after incubation at 37°C for 18 hours.

Partial Purification of DHP-1

The samples of DHP-1 were prepared from hog kidney cortex by a modification of the Campbell method with Gly-dh-Phe as an assay substrate.

Fresh hog kidney cortex (300 g) was cut into small pieces, and homogenized in a Waring blender with 2 volumes of cold deionized water. The homogenate was mixed with 1/5 volumes of BuOH, and the mixture was vigorously stirred at 4°C for 60 minutes. The mixture was then centrifuged at $9,000 \times g$ for 30 minutes at 4°C, and the aqueous phase of the supernatant was dialyzed overnight against running water. After removal of insoluble aggregations by centrifugation at $9,000 \times g$ for 10 minutes, ammonium sulfate was added to the supernatant to give a final concentration of 50% saturation (313 g/liter), and the solution was gently stirred for 6 hours at 4°C. The precipitates were collected by centrifugation at $9,000 \times g$ for 30 minutes, and discarded. To the supernatant containing solubilized protein, more ammonium sulfate was then added to give a final concentration of 75% saturation, and after standing overnight at 4°C, the suspension was centrifuged at $9,000 \times g$ for 30 minutes. The sediment was dissolved in 160 ml of 0.01 m Tris-HCl buffer (pH 8.0) containing 0.01 mM ZnCl$_2$, and dialyzed against the same buffer overnight with four changes of dialyzing buffer. The dialysate was then applied to Pharmacia DEAE-Sephadex A-50 (Lot No. 7930, capacity 3.5±0.5 mequiv/g, particle size 40~120 μ) column chromatography. The resin was equilibrated in the same buffer mentioned above, and the equilibrated gel was poured into a column of dimensions 1.6×55 cm. Elution was carried out with the initial buffer at a flow rate of 20 ml/hour, and then with salt gradient buffer at a final concentration of 0.4 m of NaCl. Fractions were collected as 4.5 ml of effluent per tube with continuous UV monitoring at 280 nm. The enzyme activity to Gly-dh-Phe was measured spectrophotometrically.

Stability Test of THM Derivatives against DHP-1

Prior to the test with enzyme, an examination was made of the stability of each THM derivative to non-enzymatic degradation in 0.025 m phosphate buffer adjusted to pH 6.0 and 7.0, in 0.025 m Tris-HCl buffer at pH 8.0 and 9.0, and in 0.05 m 3-(N-morpholino)propanesulfonic acid (MOPS) buffer at pH 7.1, respectively. The degradation rate was measured by spectrophotometric assay.

On the basis of the results of the above test, each THM derivative was dissolved in 3 ml of MOPS buffer (pH 7.1) at a concentration of 0.1 mM. Five minutes after preliminary incubation at 35°C, 0.1 ml of enzyme solution at a flow rate of 20 ml/hour, and then with salt gradient buffer at a final concentration of 0.4 m of NaCl. Fractions were collected as 4.5 ml of effluent per tube with continuous UV monitoring at 280 nm. The enzyme activity to Gly-dh-Phe was measured spectrophotometrically.

General Analytical Methods

Melting points were taken on a Yanagimoto melting point apparatus and were uncorrected. IR spectra were recorded on a Hitachi 260-30 IR spectrophotometer. $^1$H NMR spectra were obtained on a Hitachi R-40 (90 MHz) or a Varian XL-200 (200 MHz) spectrometer, in the designated solvent, using tetramethylsilane or residual HOD (δ 4.80) as an internal reference. Mass spectra were recorded on a Jeol JMS-D300 mass spectrometer. HPLC purifications were performed on a Waters ALC/GPC Model 201 using μBondapak C$_{18}$ column 7.8 mm x 30 cm. Preparative thin-layer chromatography (preparative TLC) was performed, using Merck Silica gel 60 F$_{254}$ plates.

2-Methyl-1-nitro-2-[1-(2-p-nitrobenzyloxy carbonylaminoethoxy)-2-p-nitrobenzyloxy carbonyl]-
aminoethylthio]propane (6b)

To a stirred solution of 5 (360 mg, 1.01 mmol) in CH₂Cl₂ (8 ml) was added NCS (155 mg, 1.16 mmol) in an ice-cooled bath, and stirring was continued for 30 minutes. After addition of p-nitrobenzoylcarbonylaminomethanol (250 mg, 1.04 mmol) and Ag₂O (235 mg, 1.10 mmol) to the reaction mixture, the mixture was heated under reflux for 6.5 hours. The reaction mixture was filtered and the filtrate was washed with water, dried over MgSO₄ and concentrated under reduced pressure to give an oily residue which was purified by preparative TLC using benzene - EtOAc (1:1) to afford 6b (361 mg, 60%) as a viscous oil: ¹H NMR (CDCl₃) δ 1.53 (6H, s, CH₃×2), 3.3-3.7 (4H, m, NCH₂×2), 3.7 (2H, s, CH₂NO₂), 4.56 (2H, s, CH₂NO₂), 4.85 (1H, t, J=6 Hz, SCHO), 5.23 (4H, s, NCO₂CH₂Ar×2), 5.2-5.5 (2H, m, NH×2), 7.53 (4H, d, J=9 Hz, ArH), 8.23 (4H, d, J=9 Hz, ArH).

2-[1-(2-Trifluoroethoxy)-2-[(7-nitrobenzyloxycarbonylaminoethylthio]-2-methyl-1-nitropropane (6c)

To a stirred solution of 5 (356 mg, 1 mmol) in CHCl₃ (5 ml) was added NCS (147 mg, 1.1 mmol) at room temperature under argon, and stirring was continued for 5 minutes. To the reaction mixture were added CF₃CH₂OH (300 mg, 3 mmol) and Cu powder (189 mg, 3 mmol) at room temperature, then the mixture was heated under reflux for 1 hour. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel using benzene - acetone (19:1) to afford 6c (211 mg, 46%) as a pale yellow viscous oil: ¹H NMR (CDCl₃) δ 1.53 (6H, s, CH₃×2), 3.52 (2H, t, J=6Hz, NCH₂), 4.00 (2H, q, J=9Hz, CH₂CF₃), 4.53 (2H, s, CH₂NO₂), 5.03 (1H, t, J=6 Hz, SCHO), 5.20 (2H, s, NCO₂CH₂Ar), 5.45 (1H, br s, NH), 7.50 (2H, d, J=9 Hz, ArH), 8.67 (2H, d, J=9 Hz, ArH); field desorption mass spectra (FD-MS) m/z 455 (M+H).

2-Methyl-1-nitro-2-[2-[(7-nitrobenzyloxycarbonylaminoethylthio)-1-(thiazol-2-ylmethyloxy)]propane (6d)

To a stirred solution of 5 (2.14 g, 6 mmol) in CHCl₃ (15 ml) was added NCS (0.88 g, 6.6 mmol) at room temperature, and stirring was continued for 5 minutes. To the reaction mixture were added a solution of 2-hydroxymethylthiazole (3.46 g, 30 mmol) in CHCl₃ (10 ml) and Cu powder (1.91 g, 30 mmol), and then the mixture was concentrated under reduced pressure to give a viscous residue, which was stirred at 60°C for 30 minutes. The reaction mixture was taken up in CHCl₃ and 5% NaHCO₃ and filtered. The separated organic layer was washed with water, dried over Na₂SO₄ and concentrated in vacuo. The residue was chromatographed on silica gel (30 g) using benzene, benzene - CHCl₃ and CHCl₃. The fraction eluted with CHCl₃ was concentrated to give a pale yellow oil, which was purified by preparative TLC using benzene - EtOAc (2:1) to afford 6d (0.25 g, 9%) as a viscous oil: ¹H NMR (CDCl₃) δ 1.55 (6H, s, CH₃×2), 3.6 (2H, m, NCH₂), 4.62 (2H, s, CH₂NO₂), 4.9 (1H, m, NH), 7.37 (1H, d, J=A Hz, thiazole 5-H), 7.52 (2H, d, J=9 Hz, ArH), 7.81 (1H, d, J=4 Hz, thiazole 4-H), 8.25 (2H, d, J=9 Hz, ArH); FD-MS m/z 471 (M+H).

The following compounds (6e-6i) were prepared from 5 as described for the preparation of 6d, in 80%, 29%, 40%, 30% and 27% yields, respectively.

6e: Pale yellow oil; ¹H NMR (CDCl₃) δ 1.53 (6H, s, CH₃×2), 2.87 (2H, t, J=6 Hz, SCHO), 3.51 (2H, t, J=6 Hz, NCH₂×2), 3.86 (2H, t, J=6 Hz, CH₂OH), 4.20 (1H, t, J=6 Hz, SCHO), 4.57 and 4.77 (2H, ABq, J=10Hz, CH₂NO₂), 5.23 (2H, s, NCO₂CH₂Ar), 5.67 (1H, m, NH), 7.51 (2H, d, J=9 Hz, ArH), 8.21 (2H, d, J=9 Hz, ArH).

6f: ¹H NMR (CDCl₃) δ 1.49 (6H, s, CH₃×2), 1.8-2.1 (4H, m, pyrrolidine 3-H₂, pyrrolidine 4-H₂), 3.06 (1H, s, SCH₂), 3.15 (1H, s, SCH₂), 3.4-3.7 (6H, m, NCH₂, pyrrolidine 2-H₂, pyrrolidine 5-H₂), 4.51 (2H, s, CH₂NO₂), 4.98 (0.5H, t, J=8 Hz, SCHO), 5.07 (0.5H, t, J=8 Hz, SCHO), 5.23 (2H, s, NCO₂CH₂Ar), 6.8 (1H, m, NH), 7.55 (2H, d, J=9 Hz, ArH), 8.22 (2H, d, J=9 Hz, ArH); FD-MS m/z 500 (M+H).

6g: IR (CHCl₃) cm⁻¹ 1720, 1600; ¹H NMR (CDCl₃) δ 1.50 (3H, s, CH₃), 1.52 (3H, s, CH₃), 2.85 (2H, t, J=6 Hz, SCHO), 3.50 (4H, q, J=6 Hz, NCH₂×2), 4.09 (1H, t, J=6 Hz, SCHO), 4.53 and 4.73 (2H, ABq, J=10 Hz, CH₂NO₂), 5.22 (4H, s, NCO₂CH₂Ar×2), 5.36 (1H, m, NH), 5.62 (1H, m, NH), 7.55 (4H, d, J=9 Hz, ArH), 8.21 (4H, d, J=9 Hz, ArH); FD-MS m/z 611 (M+H).

6h: Colorless oil; ¹H NMR (CDCl₃) δ 1.51 (6H, br s, CH₃×2), 2.88 (2H, t, J=6 Hz, SCHO),

6i:
3.00 (3H, s, NCH3), 3.35-3.70 (4H, m, NCH2x2), 3.96-4.20 (1H, m, SCHS), 4.54 and 4.71 (2H, ABq, J=15 Hz, CH2NO2), 5.23 (4H, br s, NCO2CH2Arx2), 7.50 (4H, d, J=9 Hz, ArH), 8.18 (4H, d, J=9 Hz, ArH).

6i: Caramel; 1H NMR (CDCl3) δ 1.52 (6H, s, CH3x2), 1.80-2.16 (1H, m, pyrrolidine 4-H), 2.16-2.50 (1H, m, pyrrolidine 4-H), 3.24-3.75 (6H, m, NCH2, pyrrolidine 2-H2, pyrrolidine 5-H2), 3.80-3.99 (1H, m, pyrrolidine 3-H), 4.06 (1H, t, J=7 Hz, SCHS), 4.57 and 4.67 (2H, ABq, J=13 Hz, CH2NO2), 5.26 (4H, s, NCO2CH2Arx2), 5.40-5.54 (1H, m, NH), 7.50 (4H, d, J=9 Hz, ArH), 8.25 (4H, d, J=9 Hz, ArH); FD-MS m/z 637 (M+).

jj-Nitrobenzyl (5R,6S)-(1R-Hydroxyethyl)-2-[1-(2-p-nitrobenzyloxycarbonylaminoethyloxy)-2-(2-p-nitrobenzyloxycarbonylaminoethylthio)carbapen-2-em-3-carboxylate (7b)

To a stirred solution of 8 (60 mg, 0.1 mmol) and 6b (70 mg, 0.12 mmol) in DMF (3 ml) was added DBU (18 mg, 0.12 mmol) at -50°C under argon. After stirring for 20 minutes at the same temperature, the reaction mixture was diluted with EtOAc, washed successively with 0.5 n HC1, water and brine and dried over MgSO4. Evaporation of the solvent in vacuo gave an oily residue which was purified by HPLC [acetonitrile - water (3:2)] to afford 7b (29 mg, 35%) as a colorless powder: IR (KBr) cm⁻¹ 1770, 1715; 1H NMR (CDCl3) δ 1.37 (3H, d, J=6 Hz, CH3), 3.0-3.3 (2H, m, 1-H2), 3.45 (4H, m, NCH2x2), 3.60 (3H, d, J=15 Hz, OCH2), 4.26 (2H, m, 5-H, 8-H), 4.96 (1H, t, J=6 Hz, SCHS), 5.22 (4H, s, NCO2CH2Arx2), 5.40-5.54 (1H, m, NH), 7.55 (4H, d, J=9 Hz, ArH), 8.25 (6H, d, J=9 Hz, ArH).

The following compounds (7c-7i) were prepared from 8 as described for the preparation of 7b, in 57%, 59%, 71%, 62%, 35%, 47% and 58% yields, respectively.

7c: Caramel; IR (KBr) cm⁻¹ 1770; 1H NMR (CDCl3) δ 1.34 (3H, m, J=6 Hz, CH3), 2.70-3.50 (3H, m, 1-H, 6-H), 3.60 (2H, t, J=6 Hz, NCH2), 3.80-4.50 (4H, m, CH2CF3, 5-H, 8-H), 4.95-5.70 (5H, m, NCO2CH2Ar, CO2CH2Ar, SCHO), 7.51 (2H, d, J=9 Hz, ArH), 7.65 (2H, d, J=9 Hz, ArH), 8.22 (4H, d, J=9 Hz, ArH); FD-MS m/z 684 (M+).

7d: Pale yellow powder; IR (KBr) cm⁻¹ 1770, 1700; 1H NMR (CDCl3) δ 1.37 (3H, d, J=6 Hz, CH3), 3.0-3.8 (5H, m, 1-H, 6-H, NCH2, OCH2), 4.2-4.4 (2H, m, 5-H, 8-H), 5.05 (2H, m, OCH2), 5.22 (3H, m, NCO2CH2Ar, SCHO), 5.27 and 5.53 (2H, ABq, J=14 Hz, CO2CH2Ar), 7.40 (1H, d, J=3 Hz, thiazole 5-H), 7.53 (2H, d, J=9 Hz, ArH), 7.70 (2H, d, J=9 Hz, ArH), 7.82 (1H, d, J=3 Hz, thiazole 4-H), 8.16 (4H, d, J=9 Hz, ArH).

7e: Caramel; IR (KBr) cm⁻¹ 1770; 1H NMR (CDCl3) δ 1.28 (3H, d, J=6 Hz, CH3), 2.8-3.9 (9H, m, SCH2, 1-H, 6-H, NCH2, OCH3), 4.10 (1H, m, 5-H), 4.30 (1H, m, 8-H), 4.70 (1H, t, J=6 Hz, SCHS), 5.31 (2H, s, NCO2CH2Ar), 5.34 and 5.59 (2H, ABq, J=14 Hz, CO2CH2Ar), 7.10 (1H, m, NH), 7.70 (2H, d, J=9 Hz, ArH), 7.86 (2H, d, J=9 Hz, ArH), 8.28 (4H, d, J=9 Hz, ArH); FD-MS m/z 622 (M+).

7f: Colorless powder; IR (KBr) cm⁻¹ 1775, 1720, 1620; 1H NMR (CDCl3) δ 1.37 (3H, d, J=6 Hz, CH3), 1.8-2.1 (4H, m, pyrrolidine 3-H2, pyrrolidine 4-H2), 3.2-3.8 (11H, m, 1-H, SCHs, NCH2, pyrrolidine 2-H2, pyrrolidine 5-H2, 6-H), 4.2-4.4 (2H, m, 5-H, 8-H), 4.66 (1H, m, SCHS), 5.25 (2H, s, NCO2CH2Ar), 5.42 (2H, ABq, J=14 Hz, CO2CH2Ar), 7.54 (2H, d, J=9 Hz, ArH), 7.69 (2H, d, J=9 Hz, ArH), 8.26 (2H, d, J=9 Hz, ArH); FD-MS m/z 729 (M+).

7g: Colorless powder; IR (KBr) cm⁻¹ 1775, 1720, 1620; 1H NMR (CDCl3) δ 1.37 (3H, d, J=6 Hz, CH3), 1.8-2.1 (4H, m, pyrrolidine 3-H2, pyrrolidine 4-H2), 3.2-3.8 (11H, m, 1-H, SCHs, NCH2, pyrrolidine 2-H2, pyrrolidine 5-H2, 6-H), 4.2-4.4 (2H, m, 5-H, 8-H), 4.66 (1H, m, SCHS), 5.25 (2H, s, NCO2CH2Ar), 5.42 (2H, ABq, J=14 Hz, CO2CH2Ar), 7.54 (2H, d, J=9 Hz, ArH), 7.69 (2H, d, J=9 Hz, ArH), 8.26 (2H, d, J=9 Hz, ArH); FD-MS m/z 729 (M+).

7h: Colorless powder; IR (KBr) cm⁻¹ 1775, 1720, 1620; 1H NMR (CDCl3) δ 1.37 (3H, d, J=6 Hz, CH3), 2.75-3.05 (2H, m, SCH2), 3.15-3.35 (2H, m, 1-H, 6-H), 3.35-3.80 (5H, m, NCH2x2, 6-H), 4.20-4.45 (3H, m, 5-H, 8-H, SCHS), 5.24 (4H, s, NCO2CH2Arx2), 5.28 and 5.54 (2H, ABq, J=14 Hz, CO2CH2Ar), 7.54 (4H, d, J=9 Hz, ArH), 7.69 (2H, d, J=9 Hz, ArH), 8.25 (6H, d, J=9 Hz, ArH).

7i: Colorless powder; IR (KBr) cm⁻¹ 1780; 1H NMR (CDCl3) δ 1.38 (3H, d, J=6 Hz, CH3), 1.8-2.1 (1H, m, pyrrolidine 4-H), 2.2-2.5 (1H, m, pyrrolidine 4-H), 3.10-3.34 (2H, m, 1-H, 6-H), 3.34-
3.76 (6H, m, NCH₂, pyrrolidine 2-H₂, pyrrolidine 5-H₂), 3.76~3.95 (2H, m, 6-H, pyrrolidine 3-H), 
4.10~4.24 (3H, m, 5-H, 8-H, SCH₂), 5.14~5.36 (4H, m, NCO₂CH₂Ar×2), 5.36~5.46 (1H, m, NH), 
5.29 and 5.55 (2H, ABq, J=15 Hz, CO₂CH₂Ar), 7.54 (4H, d, J=9 Hz, ArH), 7.68 (2H, d, J=9 Hz, 
ArH), 8.28 (6H, d, J=9 Hz, ArH).

(5R,6S)-2-[(1-(2-Aminooxy)ethoxy)-2-aminoethylthio]-6-[(R)-1-hydroxyethyl]carbapen-2-em-3-carboxylic Acid (3b)

A mixture of 7b (50 mg, 0.06 mmol) and PtO₂ (75 mg) in tetrahydrofuran (THF) (5 ml), EtOH 
(2 ml), deionized water (2 ml), and 0.1 m MOPS buffer (pH 7.1, 2 ml) was subjected to catalytic hy- 
drogenation under 4.5 atm for 2.5 hours at room temperature. The catalyst was filtered off and 
washed with water. The filtrate and washings were combined and washed with Et₂O. The separated 
aqueous layer was concentrated briefly under reduced pressure to remove any residual organic solvents 
and then applied to a Diaion HP-20 column (1.8 x 15 cm) which was eluted successively with water 
and water - THF (95 :5). The fractions having UV absorption at 286 nm were combined and lyo- 
philized to give 3b (5 mg, 27%) as a colorless powder: IR (KBr) cm⁻¹ 1760; ¹H NMR (D₂O) 3 1.35 
(3H, d, J=6Hz, CH₃), 3.2-3.6 (7H, m, 1-H₂, NCH₂, 6-H), 3.6-3.9 (2H, m, OCH₂), 4.1-4.4 
(2H, m, 5-H, 8-H), 4.80 (HOD); UV ƛmax (H₂O) 286 nm.

The following compounds (3c~3j) were prepared from 7c~7i and 16 as described for the prepa-
ration of 3b, in 31%, 33%, 27%, 18%, 59%, 23%, 18% and 63% yields, respectively.

3c: Pale yellow powder; IR (KBr) cm⁻¹ 1760; ¹H NMR (D₂O) 3 1.27 (3H, d, J=6 Hz, CH₃), 
2.8~3.2 (2H, m, SCH₂), 3.2~3.7 (5H, m, 1-H₂, NCH₂, 6-H), 3.86 (2H, m, OCH₂), 4.00~4.70 (3H, 
m, 5-H, 8-H, SCH₂), 4.80 (HOD); UV ƛmax (H₂O) 295 nm.

3d: Pale yellow powder; IR (KBr) cm⁻¹ 1760; ¹H NMR (D₂O) 3 1.27 (3H, d, J=6 Hz, CH₃), 
4.1~4.3 (2H, m, 5-H, 8-H), 4.80 (HOD), 5.0~5.4 (3H, m, SCH, OCH₃), 7.67 (1H, d, J=3 Hz, thiazole 5-H), 7.85 (1H, d, J=3 Hz, thiazole 4-H); UV ƛmax (H₂O) 
290 nm.

3e: Pale yellow powder; IR (KBr) cm⁻¹ 1750; ¹H NMR (D₂O) 3 1.30 (3H, d, J=6 Hz, CH₃), 
1.8~2.1 (4H, m, pyrrolidine 3-H₂, pyrrolidine 4-H₂), 3.2~3.8 (4H, m, 1-H₂, NCH₂, 6-H), 3.86 (2H, m, OCH₂), 4.20~4.33 (2H, m, 5-H, 8-H), 4.45~4.60 (1H, m, SCH₂), 4.80 (HOD); UV ƛmax (H₂O) 297 nm.

3f: Pale yellow powder; IR (KBr) cm⁻¹ 1750; ¹H NMR (D₂O) 3 1.28 (3H, d, J=6 Hz, CH₃), 
1.8~2.1 (4H, m, pyrrolidine 3-H₂, pyrrolidine 4-H₂), 3.2~3.9 (11H, m, 1-H₂, SCH₂, NCH₂, pyrrolidine 
2-H₂, pyrrolidine 5-H₂, 6-H), 4.1~4.3 (2H, m, 5-H, 8-H), 4.60 (1H, m, SCH₂), 4.80 (HOD); UV ƛmax 
(H₂O) 295 nm.

3g: Pale yellow powder; IR (KBr) cm⁻¹ 1755, 1580, 1380; ¹H NMR (D₂O) 3 1.27 (3H, d, J= 
6 Hz, CH₃), 3.10 (2H, m, SCH₂), 3.2~3.9 (7H, m, 1-H₂, NCH₂, 6-H), 4.25 (2H, m, 5-H, 8-H), 
4.45~4.60 (1H, m, SCH₂), 4.80 (HOD); UV ƛmax (H₂O) 293 nm.

3h: Pale yellow powder; IR (KBr) cm⁻¹ 1760, 1580; ¹H NMR (D₂O) 3 1.27 (3H, d, J=6 Hz, 
CH₃), 2.72 (1.5H, s, NCH₃), 2.73 (1.5H, s, NCH₃), 3.00~3.80 (9H, m, 1-H₂, SCH₂, NCH₂, pyrrolidine 
2-H₂, pyrrolidine 5-H₂, 6-H), 4.45~4.60 (1H, m, SCH₂), 4.80 (HOD); UV ƛmax (H₂O) 295 nm.

3i: Pale yellow powder; IR (KBr) cm⁻¹ 1730; ¹H NMR (D₂O) 3 1.26 (3H, d, J=6 Hz, CH₃), 
1.90~2.70 (2H, m, pyrrolidine 4-H₂), 3.10~4.00 (10H, m, 1-H₂, SCH₂, pyrrolidine 2-H₂, pyrrolidine 
5-H₂, 6-H, pyrrolidine 3-H), 4.80 (HOD); UV ƛmax (H₂O) 293 nm.

3j: Pale yellow powder; IR (KBr) cm⁻¹ 1760; ¹H NMR (D₂O) 3 1.28 (3H, d, J=6 Hz, CH₃), 
2.21 (1.5H, s, SCH₂), 2.28 (1.5H, s, SCH₂), 3.10~3.50 (5H, m, 1-H₂, NCH₂, 6-H), 4.18~4.50 (3H, 
m, 5-H, 8-H, SCH₂), 4.80 (HOD); UV ƛmax (H₂O) 298 nm.

2-Methyl-1-nitro-2-[2-(p-nitrobenzyloxy)carbonyloxy)ethylthio]propane (9a)

A solution of 2-methyl-1-nitropropene²⁵ (2.39 g, 23.7 mmol), 2-mercaptoethanol (1.85 g, 23.7 
mmol), and triethylamine (4 drops) in CHCl₃ (8 ml) was stirred for 24 hours at room temperature under 
argon. To the reaction mixture was added 2-methyl-1-nitropropene (1.20 g, 11.8 mmol) and stirring 
was continued for more 15 hours. The reaction mixture was concentrated under reduced pressure, 
and the residue was chromatographed on silica gel (40 g) using benzene - EtOAc (4 :1) to give 2-
methyl-1-nitro-2-(2-hydroxyethylthio)propane (1.63 g, 38%) as a colorless oil: ¹H NMR (CDCl₃)
δ 1.40 (6H, s, CH₃×2), 2.2 (1H, m, OH), 2.78 (2H, t, J=6 Hz, SCH₂), 3.75 (2H, t, J=6 Hz, CH₂OH), 4.50 (2H, s, CH₂NO₂); FD-MS m/z 179 (M⁺).

4-Nitrobenzyl chloroformate (999 mg, 4.7 mmol) was added to a solution of the above alcohol (550 mg, 3.1 mmol) and 4-dimethylaminopyridine (756 mg, 6.2 mmol) in CH₂Cl₂ (10 ml), and stirring was continued for 2 hours at room temperature. The reaction mixture was washed with water, dried over MgSO₄ and concentrated in vacuo. The residue was chromatographed on silica gel (40 g) using benzene - EtOAc (30 : 1) to give 9a (442 mg, 40%) as an oil, which crystallized after standing in a refrigerator, having an mp of 58~59°C: IR (KBr) cm⁻¹ 1740; ¹H NMR (CDCl₃) δ 1.50 (6H, s, CH₃×2), 2.86 (2H, t, J=6 Hz, SCH₂), 4.29 (2H, t, J=6 Hz, OCH₂), 4.48 (2H, s, CH₂NO₂), 5.24 (2H, s, NCO₂CH₂Ar), 7.53 (2H, d, J=9 Hz, ArH), 8.21 (2H, d, J=9 Hz, ArH).

Anal Calcd for C₁₄H₁₈N₂O₇S: C 46.92, H 5.06, N 7.82.

Found: C 46.77, H 5.02, N 7.73.

2-[2-[(2-Nitrobenzyl oxycarbonylamino)thiazol-4-yl]methylthio]-2-methyl-1-nitropropane (9b)

To a stirred solution of 2-amino-4-chloromethylthiazole hydrochloride²⁶) (3.69 g, 20 mmol), pyridine (7.00 g, 88 mmol) and 4-nitrobenzyl chloroformate (5.60 g, 26 mmol) in DMF (5 ml) and CHCl₃ (30 ml) was added 4-dimethylaminopyridine (6.34 g, 52 mmol) at room temperature. After stirring for 3 hours, thiobenzoic acid (4.14 g, 30 mmol) was added to the reaction mixture, and the resulting mixture was heated under reflux for 1 hour. The reaction mixture was diluted with EtOAc, then washed with 5% NaHCO₃, 5% HCI, water and brine, dried over MgSO₄ and concentrated in vacuo. The residue was chromatographed on silica gel using benzene - EtOAc (92 : 8) to give [2-(p-nitrobenzyloxycarbonylamino)thiazol-4-yl]methyl thiobenzoate (3.78 g, 44 %) as a pale yellow powder:

²H NMR (CDCl₃) δ 4.28 (2H, s, CH₂S), 5.40 (2H, s, NCO₂CH₂Ar), 6.90 (1H, s, thiazole 5-H), 7.25~7.75 (5H, m, ArH), 7.80~8.05 (2H, m, ArH), 7.87 (2H, d, J=9 Hz, ArH).

A mixture of the above thioester (1.51 g, 3.5 mmol), cone HCI (20 ml), water (20 ml) and MeOH (100 ml) was heated under reflux for 8 hours. The reaction mixture was concentrated under reduced pressure and the residue was suspended in CHCl₃ (15 ml) and MeOH (10 ml). To the resulting suspension were added 2-methyl-1-nitropropane (0.43 g, 4.2 mmol) and triethylamine (1 ml), and the mixture was stirred for 3 hours at room temperature. The reaction mixture was concentrated in vacuo and the resulting residue was chromatographed on silica gel using benzene - EtOAc (19 : 1) to give 9b (838mg, 56 %) as a pale yellow powder:

²H NMR (CDCl₃) δ 1.43 (6H, s, CH₃×2), 3.78 (2H, s, CH₂S), 4.40 (2H, s, CH₂NO₂), 5.37 (2H, s, NCO₂CH₂Ar), 6.80 (1H, s, thiazole 5-H), 7.55 (2H, d, J=9 Hz, ArH), 8.22 (2H, d, J=9 Hz, ArH).

Preparation of Masked Thiols (10a, 10b)

The masked thiols (10a, 10b) were prepared from 9a and 9b as described for the preparation of 6d, in 44 % and 39 % yields, respectively.

10a: Colorless oil; ¹H NMR (CDCl₃) δ 1.53 (6H, s, CH₃×2), 2.86 (2H, t, J=6 Hz, SCH₂), 3.43 (2H, q, J=6 Hz, NCH₂), 4.2~4.4 (3H, m, OCH₂, SCH₂), 4.66 (2H, s, CH₂NO₂), 5.16 (2H, s, CO₂CH₂Ar), 5.25 (2H, s, CO₂CH₂Ar), 5.2~5.5 (1H, m, NH), 7.46 (2H, d, J=9 Hz, ArH), 7.52 (2H, d, J=9 Hz, ArH), 8.13 (2H, d, J=9 Hz, ArH), 8.16 (2H, d, J=9 Hz, ArH); FD-MS m/z 612 (M⁺).

10b: Pale yellow caramel; ¹H NMR (CDCl₃) δ 1.45 (3H, s, CH₃), 1.50 (3H, s, CH₃), 2.65~3.05 (2H, m, SCH₂), 3.25~3.60 (2H, m, NCH₂), 4.46 and 4.55 (2H, ABq, J=11 Hz, CH₂NO₂), 5.20 (2H, s, NCO₂CH₂Ar), 5.36 (2H, s, NCO₂CH₂Ar), 5.20~5.60 (1H, m, NH), 6.96 (1H, s, thiazole 5-H), 7.30~7.70 (4H, m, ArH), 8.05~8.30 (4H, m, ArH).

Preparation of 11a and 11b

The protected thienamycin derivatives (11a, 11b) were prepared from 8 as described for the preparation of 7b, in 43 % and 44 % yields, respectively.

11a: Colorless powder; IR (KBr) cm⁻¹ 1750, 1700; ¹H NMR (CDCl₃) δ 1.37 (3H, d, J=6 Hz, CH₃), 2.8~3.8 (7H, m, SCH₂, 1-H₂, NCH₂, 6-H), 4.2~4.6 (5H, m, OCH₂, 5-H, 8-H, SCH₂), 5.22 (2H, s, CO₂CH₂Ar), 5.30 (2H, s, CO₂CH₂Ar), 5.27 and 5.54 (2H, ABq, J=14 Hz, CO₂CH₂Ar), 7.53 (2H, d, J=9 Hz, ArH), 7.57 (2H, d, J=9 Hz, ArH), 7.70 (2H, d, J=9 Hz, ArH), 8.27 (6H, d, J=9 Hz, ArH).
lib: Colorless powder; IR (KBr) cm⁻¹ 1780; ¹H NMR (CDCl₃) δ 1.36 (3H, d, J=6 Hz, CH₃), 2.70-3.10 (2H, m, SCH₂), 3.10-3.30 (2H, m, 1-H₂), 3.30-3.60 (3H, m, NCH₂, 6-H), 4.10-4.20 (2H, ABq, J=14 Hz, CO₂CH₂Ar), 5.00-5.70 (2H, m, NHx₂), 7.02 (1H, s, thiazole 5-H), 7.51 (2H, d, J=9 Hz, ArH), 7.60 (2H, d, J=9 Hz, ArH), 7.67 (2H, d, J=9 Hz, ArH), 8.24 (4H, d, J=9 Hz, ArH), 8.29 (2H, d, J=9 Hz, ArH).

Preparation of 4a and 4b

Compounds 4a and 4b were prepared from lib and lib as described for the preparation of 3b, in 44% and 35% yields, respectively.

4a: Colorless powder; IR (KBr) cm⁻¹ 1750; ¹H NMR (D₂O) δ 1.30 (3H, d, J=6 Hz, CH₃), 3.0-3.6 (7H, m, SCH₂, 1-H₂, NCH₂, 6-H), 3.8-4.0 (2H, m, OCH₂), 4.2-4.4 (2H, m, 5-H, 8-H), 4.4-4.5 (1H, m, SCHS), 4.80 (HOD); UV λmax (H₂O) 297 nm.

4b: Colorless powder; IR (KBr) cm⁻¹ 1755; ¹H NMR (D₂O) δ 1.24 (3H, d, J=6 Hz, CH₃), 2.8-3.1 (2H, m, SCH₂), 3.1-3.5 (5H, m, 1-H₂, NCH₂, 6-H), 4.0-4.3 (2H, m, 5-H, 8-H), 4.80 (HOD), 5.40 (0.5H, s, SCHS), 5.43 (0.5H, s, SCHS), 6.73 (0.5H, s, thiazole 5-H), 6.79 (0.5H, s, thiazole 5-H); UV λmax (H₂O) 299 nm.

Methyl 2-(α-Nitrobenzyloxycarbonylamino)ethyl Sulfide (13)

To a stirred solution of 12 (1.60 g, 17.6 mmol) and triethylamine (2.40 g, 23.8 mmol) in Et₂O (50 ml) was added 4-nitrobenzyl chloroformate (3.80 g, 17.6 mmol) in Et₂O (50 ml) in an ice-cooled bath over a period of 10 minutes. The reaction mixture was allowed to return to room temperature and was stirred for 3 hours. The reaction mixture was washed with 0.5 n HCl and brine, dried over MgSO₄ and concentrated under reduced pressure. The resulting crystals were recrystallized from Et₂O - pentane to give 13 (3.02 g, 64%) as pale yellow needles: MP 63-64°C; IR (KBr) cm⁻¹ 3325, 1685; ¹H NMR (CDCl₃) δ 2.13 (3H, s, CH₃S), 2.68 (2H, t, J=6Hz, SCH₂), 3.46 (2H, q, J=6Hz, NCH₂), 5.23 (2H, s, NCO₂CH₂Ar), 5.3 (1H, m, NH), 7.55 (2H, d, J=6 Hz, ArH), 8.25 (2H, d, J=9 Hz, ArH).

Found: C 48.82, H 4.99, N 10.37.

1-Benzoylthio-1-methylthio-2-(α-nitrobenzyloxycarbonylamino)ethane (14)

Sulfuryl chloride (86 mg, 0.64 mmol) was added to a stirred solution of 13 (154 mg, 0.57 mmol) in CH₂Cl₂ (5 ml) and stirring was continued for 1 hour at room temperature. Thiobenzoic acid (93 mg, 0.67 mmol) and DBU (8 drops) were added successively to the reaction mixture. After being stirred for 2 hours at room temperature, the reaction mixture was diluted with CH₂Cl₂, washed with water and brine, dried over MgSO₄ and concentrated in vacuo. The residue was purified by preparative TLC using CHCl₃ - EtOAc (15 : 1) to give 14 (110 mg, 47%) as a colorless oil: ¹H NMR (CDCl₃) δ 2.25 (3H, s, CH₃S), 3.72 (2H, t, J=7 Hz, NCH₂), 4.84 (1H, t, J=7 Hz, SCH₂), 5.22 (2H, s, NCO₂CH₂Ar), 5.3 (1H, m, NH), 7.4-8.3 (9H, m, ArH).

2-Mercapto-2-methylthio-N,N'-p-nitrobenzyloxycarbonylamineethane (15)

A mixture of 14 (407 mg, 1 mmol) and 3,4-dimethoxyphenethylamine (217 mg, 1.1 mmol) in benzene (0.5 ml) was stirred for 4 hours at room temperature. The reaction mixture was concentrated in vacuo and the residue was chromatographed on silica gel (15 g) using benzene - EtOAc (10 : 1) to give 15 (120 mg, 40%) as a colorless oil: ¹H NMR (CDCl₃) δ 2.25 (3H, s, CH₃S), 3.72 (2H, t, J=7 Hz, NCH₂), 4.84 (1H, t, J=7 Hz, SCH₂), 5.22 (2H, s, NCO₂CH₂Ar), 5.3 (1H, m, NH), 7.4-8.3 (9H, m, ArH).

2-Mercapto-2-methylthio-N,N'-p-nitrobenzyloxycarbonylamineethane (16)

A mixture of 15 (407 mg, 1 mmol) and 3,4-dimethoxyphenethylamine (217 mg, 1.1 mmol) in benzene (0.5 ml) was stirred for 4 hours at room temperature. The reaction mixture was concentrated in vacuo and the residue was chromatographed on silica gel (15 g) using benzene - EtOAc (10 : 1) to give 16 (120 mg, 40%) as a colorless oil: ¹H NMR (CDCl₃) δ 2.25 (3H, s, CH₃S), 3.72 (2H, t, J=7 Hz, NCH₂), 4.84 (1H, t, J=7 Hz, SCH₂), 5.22 (2H, s, NCO₂CH₂Ar), 5.3 (1H, m, NH), 7.4-8.3 (9H, m, ArH).

p-Nitrobenzyl (5R,6S)-6-[(R)-1-Hydroxyethyl]-2-(1-methylthio-2-p-nitrobenzyloxycarbonylaminomethylthio)carbapen-2-em-3-carboxylate (16)

To a stirred solution of 8 (120 mg, 0.2 mmol) and 15 (132 mg, 0.44 mmol) in DMF (2 ml) was added diisopropylethylamine (31 mg, 0.24 mmol) at -50°C under argon. After being stirred for 20 minutes at the same temperature, the reaction mixture was diluted with EtOAc, washed with 0.5 n HCl and brine, dried over MgSO₄ and concentrated in vacuo. The residue was chromatographed on silica gel (15 g) using benzene - EtOAc (10 : 1) to give 16 (120 mg, 40%) as a colorless oil: ¹H NMR (CDCl₃) δ 2.25 (3H, s, CH₃S), 3.72 (2H, t, J=7 Hz, NCH₂), 4.84 (1H, t, J=7 Hz, SCH₂), 5.22 (2H, s, NCO₂CH₂Ar), 5.3 (1H, m, NH), 7.4-8.3 (9H, m, ArH).
HCl and brine, dried over MgSO4 and concentrated in vacuo. The residue was purified by HPLC [acetonitrile - water (3 :2)] to give 16 (95 mg, 74%) as a colorless powder: IR (KBr) cm⁻¹ 1770, 1720; 
¹H NMR (CDCl3) δ 1.37 (3H, d, J=6 Hz, CH₃), 2.21 (1.5H, s, SCH₃), 2.23 (1.5H, s, SCH₃), 3.15~
3.75 (5H, m, 1-H₂, 6-H, NCH₂), 4.09~4.40 (3H, m, 5-H, 8-H, SCH₂), 5.20 (2H, s, NCO₂CH₂Ar),
5.21 and 5.52 (2H, ABq, J=14 Hz, CO₂CH₂Ar), 7.50 (2H, d, J=9 Hz, ArH), 7.65 (2H, d, J=9 Hz,
ArH), 8.20 (4H, d, J=9 Hz, ArH).

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