MODIFICATION OF THE CYSTEAMINE SIDE CHAIN OF THIENAMYCIN. II

MAKOTO SATO, MAKOTO TAKEMURA, SHOHGO ATARASHI, KUNIO HIGASHI, HIROYUKI FUJIWARA, TAKAYASU NAGAHARA and MINORU FURUKAWA

Laboratory of Medicinal Chemistry,

TORU IKEUCHI, SEIICHI OZAWA, NOBUKO NISHIZAWA and YASUAKI OSADA

Laboratory of Microbiology & Pathology, Research Institute,
Daiichi Seiyaku Co., Ltd.,
1-16-13, Kitakasai, Edogawa-ku, Tokyo 134, Japan

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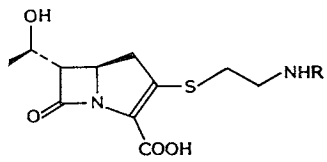
A new type of thienamycin derivatives (**3a**~**3j**, **4a**, **4b**), having a monothioacetal or a thioacetal side chain at the C-2 position was prepared, and the susceptibility to renal dehydropeptidase-1 (DHP-1) and the antimicrobial activity of these compounds were determined. The structure-activity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

There has been intense interest in the chemical modifications of thienamycin (THM)[1~3] in the pursuit of higher physico-chemical stability and antimicrobial activity,[4,5] and the first carbapenem antibiotic, imipenem (MK0787)[6,7] has been developed recently. This antibiotic, however, is reported to be metabolized by dehydropeptidase-1 (DHP-1),[8,9] and it is used clinically in combination with cilastatin,[7,10,11] 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,[8,12,13] 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.

Fig. 1.



Thienamycin (**1**) R = H
Imipenem (**2**) R = CH=NH

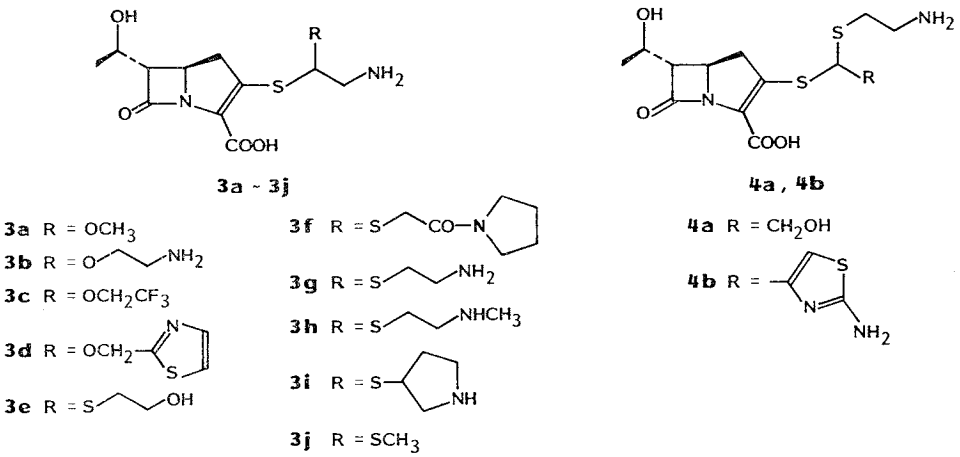
Chemistry

In previous papers,[14,15] we have described a facile method using a masked thiol for the preparation of the *S*-α-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**[15] 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 Ag_2O as a catalyst, none of the desired com-

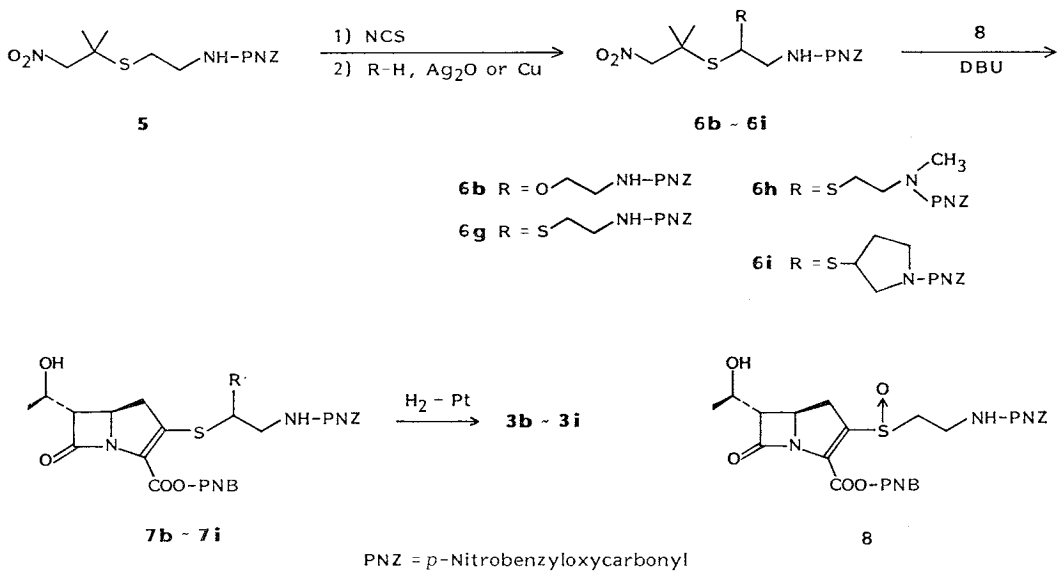
pound, **6c**, was obtained under similar reaction conditions. After surveying a variety of conditions, this reaction was found to proceed smoothly by the use of Cu powder or Ag_2CO_3 as a catalyst instead of Ag_2O. Therefore Cu powder, which was more effective than Ag_2CO_3, was employed in the subsequent experiments. When the protected THM sulfoxide (**8**)[16] 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[17] 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.

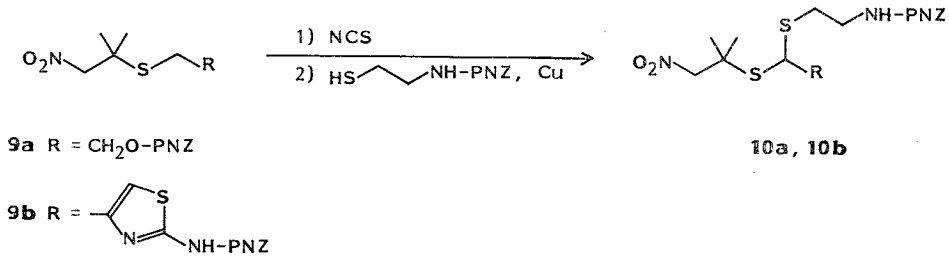


Scheme 1.

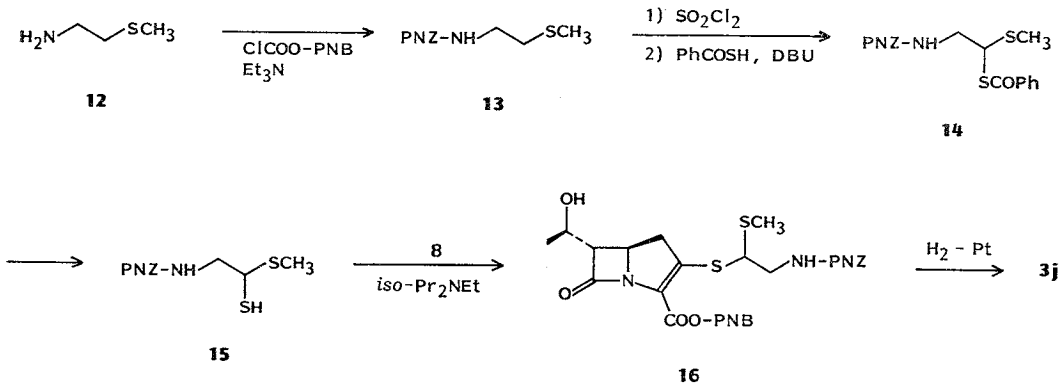


For the preparation of a sulfur analogue (**3j**) of **3a**, the masked thiol method described above was considered to be unsuitable because of the volatility and foul smell of methylmercaptan, and *S*-α-methylthio THM (**3j**) was prepared by another method (Scheme 3). Thus, the Pummerer reaction of **13**, prepared by protection of the amino group of **12**[18] with a *p*-nitrobenzyloxycarbonyl 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,[19,20] 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 PtO_2 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[22] that introduction of an alkyl group such as a methyl or ethyl group at the *S*-α-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 α-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.[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*-α-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,[22] introduction of a substituent at the *S*-α-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.

Organisms[a]	MIC (μg/ml)													
	MK0787	THM	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	4a	4b
E. coli NIHJ	0.20	0.20	0.20	1.56	0.39	0.20	0.39	0.39	0.78	1.56	0.78	0.20	0.39	0.78
C. freundii IID 976	0.10	0.20	0.39	3.13	1.56	0.39	0.39	0.39	0.78	1.56	3.13	0.39	0.39	0.78
Pr. vulgaris 3167	0.39	1.56	1.56	6.25	0.78	0.39	1.56	0.39	0.78	1.56	3.13	0.78	0.39	0.78
Pr. mirabilis IFO 3894	0.10	0.20	0.20	25	0.39	0.20	0.39	0.39	0.39	0.39	0.78	1.56	0.20	0.39
K. pneumoniae 501	0.10	0.20	0.20	1.56	0.39	0.20	0.39	0.39	0.39	0.39	0.78	0.20	0.39	0.39
Ent. cloacae 12005	0.78	1.56	3.13	>25	3.13	1.56	1.56	6.25	0.78	1.56	12.5	1.56	0.78	3.13
Ser. marcescens 13001	0.78	0.78	0.39	3.13	3.13	0.78	1.56	1.56	3.13	1.56	3.13	0.39	0.39	0.78
Ps. aeruginosa 2044	1.56	3.13	12.5	6.25	>50	50	25	>50	3.13	3.13	>50	12.5	12.5	100
S. aureus 209P	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.10	0.20	<0.10	0.10	0.39	<0.10	<0.10	0.10
S. epidermidis 7035	0.10	0.10	<0.10	0.20	0.20	0.10	0.20	0.39	0.10	0.20	0.78	0.10	0.20	0.10
Str. pyogenes G-36	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	0.10	<0.10	0.10	<0.10	<0.10	<0.10
Str. faecalis ATCC 19433	0.78	0.78	6.25	12.5	6.25	1.56	6.25	6.25	6.25	6.25	12.5	0.20	1.56	1.56
DHP-1 Susceptibility[b]	100	110	91	39	68	107	73	65	27	15	10	100	80	1,119

[a] 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.[21]

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[21] 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 $40 \sim 120$ 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 in MOPS buffer containing 9.2 units per ml was added to the above test tubes. Immediately after the addition of the enzyme, the residual amount of substrate was measured spectrophotometrically at 35°C for 5 minutes. The rate of the hydrolysis of each derivative by the enzyme was calculated from the reaction curve (decrease rate in absorbance) recorded on the chart.

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 $\times$ 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*-nitrobenzyloxycarbonylaminoethyloxy)-2-*p*-nitrobenzyloxycarbonyl-

aminoethylthio]propane (**6b**)

To a stirred solution of **5** (360 mg, 1.01 mmol) in CH_2Cl_2 (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*-nitrobenzyloxycarbonylaminoethanol[23] (250 mg, 1.04 mmol) and Ag_2O (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 $MgSO_4$ 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: ^{1}H NMR ($CDCl_3$) δ 1.53 (6H, s, $CH_3\times2$), 3.3~3.7 (4H, m, $NCH_2\times2$), 3.7 (2H, m, OCH_2), 4.56 (2H, s, CH_2NO_2), 4.85 (1H, t, J=6 Hz, SCHO), 5.23 (4H, s, $NCO_2CH_2Ar\times2$), 5.2~5.5 (2H, m, $NH\times2$), 7.53 (4H, d, J=9 Hz, ArH), 8.23 (4H, d, J=9 Hz, ArH).

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

To a stirred solution of **5** (356 mg, 1 mmol) in $CHCl_3$ (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 CF_3CH_2OH (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: ^{1}H NMR ($CDCl_3$) δ 1.53 (6H, s, $CH_3\times2$), 3.52 (2H, t, J=6 Hz, NCH_2), 4.00 (2H, q, J=9 Hz, CH_2CF_3), 4.53 (2H, s, CH_2NO_2), 5.03 (1H, t, J=6 Hz, SCHO), 5.20 (2H, s, NCO_2CH_2Ar), 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 spetra (FD-MS) m/z 455 (M+H).

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

To a stirred solution of **5** (2.14 g, 6 mmol) in $CHCl_3$ (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[24] (3.46 g, 30 mmol) in $CHCl_3$ (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 $CHCl_3$ and 5% $NaHCO_3$ and filtered. The separated organic layer was washed with water, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (30 g) using benzene, benzene - $CHCl_3$ and $CHCl_3$. The fraction eluted with $CHCl_3$ 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: ^{1}H NMR ($CDCl_3$) δ 1.55 (6H, s, $CH_3\times2$), 3.6 (2H, m, NCH_2), 4.62 (2H, s, CH_2NO_2), 4.9 (1H, m, SCHO), 5.00 (2H, s, OCH_2), 5.22 (2H, s, NCO_2CH_2Ar), 6.5 (1H, m, NH), 7.37 (1H, d, J=4 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; ^{1}H NMR ($CDCl_3$) δ 1.53 (6H, s, $CH_3\times2$), 2.87 (2H, t, J=6 Hz, SCH_2), 3.51 (2H, t, J=6 Hz, NCH_2), 3.86 (2H, t, J=6 Hz, CH_2OH), 4.20 (1H, t, J=6 Hz, SCHS), 4.57 and 4.77 (2H, ABq, J=11 Hz, CH_2NO_2), 5.23 (2H, s, NCO_2CH_2Ar), 5.67 (1H, m, NH), 7.51 (2H, d, J= 9 Hz, ArH), 8.21 (2H, d, J=9 Hz, ArH).

6f: ^{1}H NMR ($CDCl_3$) δ 1.49 (6H, s, $CH_3\times2$), 1.8~2.1 (4H, m, pyrrolidine 3-H_2, pyrrolidine 4-H_2), 3.06 (1H, s, SCH_2), 3.15 (1H, s, SCH_2), 3.4~3.7 (6H, m, NCH_2, pyrrolidine 2-H_2, pyrrolidine 5-H_2), 4.51 (2H, s, CH_2NO_2), 4.98 (0.5H, t, J=8 Hz, SCHS), 5.07 (0.5H, t, J=8 Hz, SCHS), 5.23 (2H, s, NCO_2CH_2Ar), 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^+).

6g: IR ($CHCl_3$) cm^{-1} 1720, 1600; ^{1}H NMR ($CDCl_3$) δ 1.50 (3H, s, CH_3), 1.52 (3H, s, CH_3), 2.85 (2H, t, J=6 Hz, SCH_2), 3.50 (4H, q, J=6 Hz, $NCH_2\times2$), 4.09 (1H, t, J=6 Hz, SCHS), 4.53 and 4.73 (2H, ABq, J=10 Hz, CH_2NO_2), 5.22 (4H, s, $NCO_2CH_2Ar\times2$), 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^+).

6h: Colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.51 (6H, br s, $CH_3\times2$), 2.88 (2H, t, J=6 Hz, SCH_2),

3.00 (3H, s, NCH_3), 3.35~3.70 (4H, m, $NCH_2\times2$), 3.96~4.20 (1H, m, SCHS), 4.54 and 4.71 (2H, ABq, J=15 Hz, CH_2NO_2), 5.23 (4H, br s, $NCO_2CH_2Ar\times2$), 7.50 (4H, d, J=9 Hz, ArH), 8.18 (4H, d, J=9 Hz, ArH).

6i: Caramel; ^1H NMR ($CDCl_3$) δ 1.52 (6H, s, $CH_3\times2$), 1.80~2.16 (1H, m, pyrrolidine 4-H), 2.16~2.50 (1H, m, pyrrolidine 4-H), 3.24~3.75 (6H, m, NCH_2, pyrrolidine 2-H_2, pyrrolidine 5-H_2), 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, CH_2NO_2), 5.26 (4H, s, $NCO_2CH_2Ar\times2$), 5.40~5.54 (1H, m, NH), 7.56 (4H, d, J=9 Hz, ArH), 8.25 (4H, d, J=9 Hz, ArH); FD-MS m/z 637 (M^+).

p-Nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-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 HCl, water and brine and dried over $MgSO_4$. 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 ($CDCl_3$) δ 1.37 (3H, d, J=6 Hz, CH_3), 3.0~3.3 (2H, m, 1-H_2), 3.45 (4H, m, $NCH_2\times2$), 3.6 (3H, m, 6-H, OCH_2), 4.26 (2H, m, 5-H, 8-H), 4.96 (1H, t, J=6 Hz, SCHO), 5.22 (4H, s, $NCO_2CH_2Ar\times2$), 5.28 and 5.54 (2H, ABq, J=14 Hz, CO_2CH_2Ar), 7.55 (4H, d, J=9 Hz, ArH), 7.69 (2H, 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 ($CDCl_3$) δ 1.34 (3H, m, J=6 Hz, CH_3), 2.70~3.50 (3H, m, 1-H_2, 6-H), 3.60 (2H, t, J=6 Hz, NCH_2), 3.80~4.50 (4H, m, CH_2CF_3, 5-H, 8-H), 4.95~5.70 (5H, m, NCO_2CH_2Ar, CO_2CH_2Ar, 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 ($CDCl_3$) δ 1.37 (3H, d, J=6 Hz, CH_3), 3.0~3.8 (5H, m, 1-H_2, NCH_2, 6-H), 4.2~4.4 (2H, m, 5-H, 8-H), 5.05 (2H, m, OCH_2), 5.22 (3H, m, NCO_2CH_2Ar, SCHO), 5.27 and 5.53 (2H, ABq, J=14 Hz, CO_2CH_2Ar), 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 ($CDCl_3$) δ 1.28 (3H, d, J=6 Hz, CH_3), 2.8~3.9 (9H, m, SCH_2, 1-H_2, 6-H, NCH_2, OCH_2), 4.10 (1H, m, 5-H), 4.30 (1H, m, 8-H), 4.70 (1H, t, J=6 Hz, SCHS), 5.31 (2H, s, NCO_2CH_2Ar), 5.34 and 5.59 (2H, ABq, J=14 Hz, CO_2CH_2Ar), 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 ($CDCl_3$) δ 1.37 (3H, d, J=6 Hz, CH_3), 1.8~2.1 (4H, m, pyrrolidine 3-H_2, pyrrolidine 4-H_2), 3.2~3.8 (11H, m, 1-H_2, SCH_2, NCH_2, pyrrolidine 2-H_2, pyrrolidine 5-H_2, 6-H), 4.2~4.4 (2H, m, 5-H, 8-H), 4.66 (1H, m, SCHS), 5.25 (2H, s, NCO_2CH_2Ar), 5.42 (2H, ABq, J=14 Hz, CO_2CH_2Ar), 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} 1770, 1720, 1700; ^1H NMR ($CDCl_3$) δ 1.38 (3H, d, J=6 Hz, CH_3), 2.75~3.05 (2H, m, SCH_2), 3.15~3.35 (2H, m, 1-H_2), 3.35~3.80 (5H, m, $NCH_2\times2$, 6-H), 4.20~4.45 (3H, m, 5-H, 8-H, SCHS), 5.24 (4H, s, $NCO_2CH_2Ar\times2$), 5.28 and 5.54 (2H, ABq, J=14 Hz, CO_2CH_2Ar), 7.54 (4H, d, J=9 Hz, ArH), 7.69 (2H, d, J=9 Hz, ArH), 8.25 (6H, d, J=9 Hz, ArH).

7h: Colorless powder; IR (KBr) cm^{-1} 1775, 1700, 1600; ^1H NMR ($CDCl_3$) δ 1.37 (3H, d, J=6 Hz, CH_3), 2.98 (3H, s, NCH_3), 4.00~4.52 (3H, m, 5-H, 8-H, SCHS), 5.23 (4H, s, $NCO_2CH_2Ar\times2$), 5.34 and 5.54 (2H, ABq, J=15 Hz, CO_2CH_2Ar), 7.52 (4H, d, J=9 Hz, ArH), 7.68 (2H, d, J=9 Hz, ArH), 8.24 (6H, d, J=9 Hz, ArH).

7i: Colorless powder; IR (KBr) cm^{-1} 1780; ^1H NMR ($CDCl_3$) δ 1.38 (3H, d, J=6 Hz, CH_3), 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_2), 3.34~

3.76 (6H, m, NCH_2, pyrrolidine 2-H_2, pyrrolidine 5-H_2), 3.76~3.95 (2H, m, 6-H, pyrrolidine 3-H), 4.10~4.24 (3H, m, 5-H, 8-H, SCHS), 5.14~5.36 (4H, m, $NCO_2CH_2Ar \times 2$), 5.36~5.46 (1H, m, NH), 5.29 and 5.55 (2H, ABq, J=15 Hz, CO_2CH_2Ar), 7.54 (4H, d, J=9 Hz, ArH), 7.68 (2H, d, J=9 Hz, ArH), 8.28 (6H, d, J=9 Hz, ArH).

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

A mixture of **7b** (50 mg, 0.06 mmol) and PtO_2 (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 hydrogenation 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 Et_2O. 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 (1.8×15 cm) which was eluted successively with water and water - THF (95 : 5). The fractions having UV absorption at 286 nm were combined and lyophilized to give **3b** (5 mg, 27%) as a colorless powder: IR (KBr) cm^{-1} 1760; ^1H NMR (D_2O) δ 1.35 (3H, d, J=6 Hz, CH_3), 3.2~3.6 (7H, m, 1-H_2, $NCH_2 \times 2$, 6-H), 3.6~3.9 (2H, m, OCH_2), 4.1~4.4 (2H, m, 5-H, 8-H), 4.80 (HOD); UV λ_{max} (H_2O) 286 nm.

The following compounds (**3c~3j**) were prepared from **7c~7i** and **16** as described for the preparation 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 (D_2O) δ 1.27 (3H, d, J=6 Hz, CH_3), 3.00~3.80 (5H, m, 1-H_2, NCH_2, 6-H), 3.80~4.80 (4H, m, CH_2CF_3, 5-H, 8-H), 4.80 (HOD), 5.20 (1H, t, J=6 Hz, OCHS); UV λ_{max} (H_2O) 288 nm.

3d: Pale yellow powder; IR (KBr) cm^{-1} 1760; ^1H NMR (D_2O) δ 1.27 (3H, d, J=6 Hz, CH_3), 3.0~3.9 (5H, m, 1-H_2, NCH_2, 6-H), 4.1~4.3 (2H, m, 5-H, 8-H), 4.80 (HOD), 5.0~5.4 (3H, m, SCHO, OCH_2), 7.67 (1H, d, J=3 Hz, thiazole 5-H), 7.85 (1H, d, J=3 Hz, thiazole 4-H); UV λ_{max} (H_2O) 290 nm.

3e: Pale yellow powder; IR (KBr) cm^{-1} 1750; ^1H NMR (D_2O) δ 1.30 (3H, d, J=6 Hz, CH_3), 2.8~3.2 (2H, m, SCH_2), 3.2~3.7 (5H, m, 1-H_2, NCH_2, 6-H), 3.86 (2H, m, OCH_2), 4.00~4.70 (3H, m, 5-H, 8-H, SCHS), 4.80 (HOD); UV λ_{max} (H_2O) 297 nm.

3f: Pale yellow powder; IR (KBr) cm^{-1} 1750; ^1H NMR (D_2O) δ 1.28 (3H, d, J=6 Hz, CH_3), 1.8~2.1 (4H, m, pyrrolidine 3-H_2, pyrrolidine 4-H_2), 3.2~3.9 (11H, m, 1-H_2, SCH_2, NCH_2, pyrrolidine 2-H_2, pyrrolidine 5-H_2, 6-H), 4.1~4.3 (2H, m, 5-H, 8-H), 4.60 (1H, m, SCHS), 4.80 (HOD); UV λ_{max} (H_2O) 295 nm.

3g: Pale yellow powder; IR (KBr) cm^{-1} 1755, 1580, 1380; ^1H NMR (D_2O) δ 1.27 (3H, d, J=6 Hz, CH_3), 3.10 (2H, m, SCH_2), 3.2~3.9 (7H, m, 1-H_2, $NCH_2 \times 2$, 6-H), 4.25 (2H, m, 5-H, 8-H), 4.45~4.60 (1H, m, SCHS), 4.80 (HOD); UV λ_{max} (H_2O) 293 nm.

3h: Pale yellow powder; IR (KBr) cm^{-1} 1760, 1580; ^1H NMR (D_2O) δ 1.27 (3H, d, J=6 Hz, CH_3), 2.72 (1.5H, s, NCH_3), 2.73 (1.5H, s, NCH_3), 3.00~3.80 (9H, m, 1-H_2, SCH_2, $NCH_2 \times 2$, 6-H), 4.20~4.33 (2H, m, 5-H, 8-H), 4.45~4.60 (1H, m, SCHS), 4.80 (HOD); UV λ_{max} (H_2O) 295 nm.

3i: Pale yellow powder; IR (KBr) cm^{-1} 1730; ^1H NMR (D_2O) δ 1.26 (3H, d, J=6 Hz, CH_3), 1.90~2.70 (2H, m, pyrrolidine 4-H_2), 3.10~4.00 (10H, m, 1-H_2, NCH_2, pyrrolidine 2-H_2, pyrrolidine 5-H_2, 6-H, pyrrolidine 3-H), 4.80 (HOD); UV λ_{max} (H_2O) 293 nm.

3j: Pale yellow powder; IR (KBr) cm^{-1} 1760; ^1H NMR (D_2O) δ 1.28 (3H, d, J=6 Hz, CH_3), 2.21 (1.5H, s, SCH_3), 2.28 (1.5H, s, SCH_3), 3.10~3.50 (5H, m, 1-H_2, NCH_2, 6-H), 4.18~4.50 (3H, m, 5-H, 8-H, SCHS), 4.80 (HOD); UV λ_{max} (H_2O) 298 nm.

2-Methyl-1-nitro-2-[2-(*p*-nitrobenzyloxycarbonyloxy)ethylthio]propane (**9a**)

A solution of 2-methyl-1-nitropropene[25] (2.39 g, 23.7 mmol), 2-mercaptoethanol (1.85 g, 23.7 mmol), and triethylamine (4 drops) in $CHCl_3$ (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 ($CDCl_3$)

δ 1.40 (6H, s, $CH_3\times2$), 2.2 (1H, m, OH), 2.78 (2H, t, J=6 Hz, SCH_2), 3.75 (2H, t, J=6 Hz, CH_2OH), 4.50 (2H, s, CH_2NO_2); 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 CH_2Cl_2 (10 ml), and stirring was continued for 2 hours at room temperature. The reaction mixture was washed with water, dried over $MgSO_4$ 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 ($CDCl_3$) δ 1.50 (6H, s, $CH_3\times2$), 2.86 (2H, t, J=6 Hz, SCH_2), 4.29 (2H, t, J=6 Hz, OCH_2), 4.48 (2H, s, CH_2NO_2), 5.24 (2H, s, NCO_2CH_2Ar), 7.53 (2H, d, J=9 Hz, ArH), 8.21 (2H, d, J=9 Hz, ArH).

Anal Calcd for $C_{14}H_{18}N_2O_7S$: C 46.92, H 5.06, N 7.82.
Found: C 46.77, H 5.02, N 7.73.

2-[2-(*p*-Nitrobenzyloxycarbonylamino)thiazol-4-yl]methylthio-2-methyl-1-nitropropane (**9b**)

To a stirred solution of 2-amino-4-chloromethylthiazole hydrochloride[26] (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 $CHCl_3$ (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% $NaHCO_3$, 5% HCl, water and brine, dried over $MgSO_4$ 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: ^1H NMR ($CDCl_3$) δ 4.28 (2H, s, CH_2S), 5.40 (2H, s, NCO_2CH_2Ar), 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), conc HCl (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 $CHCl_3$ (15 ml) and MeOH (10 ml). To the resulting suspension were added 2-methyl-1-nitropropene (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** (838 mg, 56%) as a pale yellow powder: ^1H NMR ($CDCl_3$) δ 1.43 (6H, s, $CH_3\times2$), 3.78 (2H, s, CH_2S), 4.40 (2H, s, CH_2NO_2), 5.37 (2H, s, NCO_2CH_2Ar), 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 ($CDCl_3$) δ 1.53 (6H, s, $CH_3\times2$), 2.86 (2H, t, J=6 Hz, SCH_2), 3.43 (2H, q, J=6 Hz, NCH_2), 4.2~4.4 (3H, m, OCH_2, SCHS), 4.66 (2H, s, CH_2NO_2), 5.16 (2H, s, CO_2CH_2Ar), 5.25 (2H, s, CO_2CH_2Ar), 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 ($CDCl_3$) δ 1.45 (3H, s, CH_3), 1.50 (3H, s, CH_3), 2.65~3.05 (2H, m, SCH_2), 3.25~3.60 (2H, m, NCH_2), 4.46 and 4.55 (2H, ABq, J=11 Hz, CH_2NO_2), 5.20 (2H, s, NCO_2CH_2Ar), 5.36 (2H, s, NCO_2CH_2Ar), 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 ($CDCl_3$) δ 1.37 (3H, d, J=6 Hz, CH_3), 2.8~3.8 (7H, m, SCH_2, 1-H_2, NCH_2, 6-H), 4.2~4.6 (5H, m, OCH_2, 5-H, 8-H, SCHS), 5.22 (2H, s, CO_2CH_2Ar), 5.30 (2H, s, CO_2CH_2Ar), 5.27 and 5.54 (2H, ABq, J=14 Hz, CO_2CH_2Ar), 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).

11b: Colorless powder; IR (KBr) cm^{-1} 1780; ^1H NMR ($CDCl_3$) δ 1.36 (3H, d, J=6 Hz, CH_3), 2.70~3.10 (2H, m, SCH_2), 3.10~3.30 (2H, m, 1-H_2), 3.30~3.60 (3H, m, NCH_2, 6-H), 4.10~4.20 (2H, m, 5-H, 8-H), 5.22 (2H, s, NCO_2CH_2Ar), 5.40 (3H, s, NCO_2CH_2Ar, SCHS), 5.26 and 5.54 (2H, ABq, J=14 Hz, CO_2CH_2Ar), 5.00~5.70 (2H, m, NH×2), 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 **11a** and **11b** as described for the preparation of **3b**, in 44% and 35% yields, respectively.

4a: Colorless powder; IR (KBr) cm^{-1} 1750; ^1H NMR (D_2O) δ 1.30 (3H, d, J=6 Hz, CH_3), 3.0~3.6 (7H, m, SCH_2, 1-H_2, NCH_2, 6-H), 3.8~4.0 (2H, m, OCH_2), 4.2~4.4 (2H, m, 5-H, 8-H), 4.4~4.5 (1H, m, SCHS), 4.80 (HOD); UV λ_{max} (H_2O) 297 nm.

4b: Colorless powder; IR (KBr) cm^{-1} 1755; ^1H NMR (D_2O) δ 1.24 (3H, d, J=6 Hz, CH_3), 2.8~3.1 (2H, m, SCH_2), 3.1~3.5 (5H, m, 1-H_2, NCH_2, 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 λ_{max} (H_2O) 299 nm.

Methyl 2-(*p*-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 Et_2O (50 ml) was added 4-nitrobenzyl chloroformate (3.80 g, 17.6 mmol) in Et_2O (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 $MgSO_4$ and concentrated under reduced pressure. The resulting crystals were recrystallized from Et_2O - pentane to give **13** (3.02 g, 64%) as pale yellow needles: MP 63~64°C; IR (KBr) cm^{-1} 3325, 1685; ^1H NMR ($CDCl_3$) δ 2.13 (3H, s, CH_3S), 2.68 (2H, t, J=6 Hz, SCH_2), 3.46 (2H, q, J=6 Hz, NCH_2), 5.23 (2H, s, NCO_2CH_2Ar), 5.3 (1H, m, NH), 7.55 (2H, d, J=9 Hz, ArH), 8.25 (2H, d, J=9 Hz, ArH).

Anal Calcd for $C_{12}H_{14}N_2O_4S$: C 48.88, H 5.22, N 10.36.
Found: C 48.82, H 4.99, N 10.37.

1-Benzoylthio-1-methylthio-2-(*p*-nitrobenzyloxycarbonylamino)ethane (**14**)

Sulfuryl chloride (86 mg, 0.64 mmol) was added to a stirred solution of **13** (154 mg, 0.57 mmol) in CH_2Cl_2 (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 CH_2Cl_2, washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by preparative TLC using $CHCl_3$ - EtOAc (15 : 1) to give **14** (110 mg, 47%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 2.25 (3H, s, CH_3S), 3.72 (2H, t, J=7 Hz, NCH_2), 4.84 (1H, t, J=7 Hz, SCHS), 5.22 (2H, s, NCO_2CH_2Ar), 5.3 (1H, m, NH), 7.4~8.3 (9H, m, ArH).

2-Mercapto-2-methylthio-*N*-*p*-nitrobenzyloxycarbonylaminoethane (**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 ($CDCl_3$) δ 2.04 (1H, d, J=8 Hz, SH), 2.24 (3H, s, CH_3S), 3.45~3.65 (2H, m, NCH_2), 3.85~4.06 (1H, m, SCHS), 5.23 (2H, s, NCO_2CH_2Ar), 7.53 (2H, d, J=9 Hz, ArH), 8.24 (2H, d, J=9 Hz, ArH).

p-Nitrobenzyl (5*R*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-(1-methylthio-2-*p*-nitrobenzyloxycarbonylaminoethylthio)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 $MgSO_4$ 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 ($CDCl_3$) δ 1.37 (3H, d, J=6 Hz, CH_3), 2.21 (1.5H, s, SCH_3), 2.23 (1.5H, s, SCH_3), 3.15~3.75 (5H, m, 1-H_2, 6-H, NCH_2), 4.09~4.40 (3H, m, 5-H, 8-H, SCHS), 5.20 (2H, s, NCO_2CH_2Ar), 5.21 and 5.52 (2H, ABq, J=14 Hz, CO_2CH_2Ar), 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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References

1) KAHAN, J. S.; F. M. KAHAN, R. GOEGELMAN, S. A. CURRIE, M. JACKSON, E. O. STAPLEY, T. W. MILLER, A. K. MILLER, D. HENDLIN, S. MOCHALES, S. HERNANDEZ, H. B. WOODRUFF & J. BIRNBAUM: Thienamycin, a new β-lactam antibiotic. I. Discovery, taxonomy, isolation and physical properties. J. Antibiotics 32: 1~12, 1979
2) ALBERS-SCHÖNBERG, G.; B. H. ARISON, O. D. HENSENS, J. HIRSHFIELD, K. HOOGSTEEN, E. A. KACZKA, R. E. RHODES, J. S. KAHAN, F. M. KAHAN, R. W. RATCLIFFE, E. WALTON, L. J. RUSWINKLE, R. B. MORIN & B. G. CHRISTENSEN: Structure and absolute configuration of thienamycin. J. Am. Chem. Soc. 100: 6491~6499, 1978
3) TALLY, F. P.; N. V. JACOBUS & S. L. GORBACH: In vitro activity of thienamycin. Antimicrob. Agents Chemother. 14: 436~438, 1978
4) LEANZA, W. J.; K. J. WILDONGER, T. W. MILLER & B. G. CHRISTENSEN: *N*-Acetimidoyl- and *N*-formimidoylthienamycin derivatives: Antipseudomonal β-lactam antibiotics. J. Med. Chem. 22: 1435~1436, 1979
5) MIYADERA, T.; Y. SUGIMURA, T. HASHIMOTO, T. TANAKA, K. IINO, T. SHIBATA & S. SUGAWARA: Synthesis and *in vitro* activity of a new carbapenem, RS-533. J. Antibiotics 36: 1034~1039, 1983
6) KROPP, H.; J. G. SUNDELOF, J. S. KAHAN, F. M. KAHAN & J. BIRNBAUM: MK0787 (*N*-Formimidoyl thienamycin): Evaluation of in vitro and in vivo activities. Antimicrob. Agents Chemother. 17: 993~1000, 1980
7) KAHAN, F. M.; H. KROPP, J. G. SUNDELOF & J. BIRNBAUM: Thienamycin: development of imipenem-cilastatin. J. Antimicrob. Chemother. 12 (Suppl. D): 1~35, 1983
8) KROPP, H.; J. G. SUNDELOF, R. HAJDU & F. M. KAHAN: Metabolism of thienamycin and related carbapenem antibiotics by the renal dipeptidase, dehydropeptidase-I. Antimicrob. Agents Chemother. 22: 62~70, 1982
9) NORRBY, S. R.; K. ALESTIG, F. FERBER, J. L. HUBER, K. H. JONES, F. M. KAHAN, M. A. P. MEISINGER & J. D. ROGERS: Parmacokinetics and tolerance of *N*-formimidoyl thienamycin (MK0787) in humans. Antimicrob. Agents Chemother. 23: 293~299, 1983
10) NORRBY, S. R.; K. ALESTIG, B. BJÖRNEGÅRD, L. Å. BURMAN, F. FERBER, J. L. HUBER, K. H. JONES, F. M. KAHAN, J. S. KAHAN, H. KROPP, M. A. P. MEISINGER & J. G. SUNDELOF: Urinary recovery of *N*-formimidoyl thienamycin (MK0787) as affected by coadministration of *N*-formimidoyl thienamycin dehydropeptidase inhibitors. Antimicrob. Agents Chemother. 23: 300~307, 1983
11) NORRBY, S. R.; J. D. ROGERS, F. FERBER, K. H. JONES, A. G. ZACCHEI, L. L. WEIDNER, J. L. DEMETRIADES, D. A. GRAVALLESE & J. Y.-K. HSIEH: Disposition of radiolabeled imipenem and cilastatin in normal human volunteers. Antimicrob. Agents Chemother. 26: 707~714, 1984
12) CAMA, L. D.; K. J. WILDONGER, R. GUTHIKONDA, R. W. RATCLIFFE & B. G. CHRISTENSEN: Total synthesis of thienamycin analogs-III. Syntheses of 2-aryl and 2-heteroaryl analogs of thienamycin. Tetrahedron 39: 2531~2549, 1983
13) ANDRUS, A.; F. BAKER, F. A. BOUFFARD, L. D. CAMA, B. G. CHRISTENSEN, R. N. GUTHIKONDA, J. V. HECK, D. B. R. JOHNSTON, W. J. LEANZA, R. W. RATCLIFFE, T. N. SALZMANN, S. M. SCHMITT, D. H. SHIH, N. V. SHAH, K. J. WILDONGER & R. R. WILKENING: Structure-activity relationships among some totally synthetic carbapenems. *In* Recent Advances in the Chemistry of β-Lactam Antibiotics. *Eds.*, A. G. BROWN & S. M. ROBERTS, pp. 86~99, Royal Soc. Chem., London, 1985
14) TAKEMURA, M.; K. HIGASHI, H. FUJIWARA, M. SATO & M. FURUKAWA: Pummerer reaction of thiena-

mycin-type cyclic vinylogous sulfide and sulfoxide. Chem. Pharm. Bull. 33: 5190~5196, 1985
15) TAKEMURA, M.; K. HIGASHI, H. FUJIWARA, M. SATO & M. FURUKAWA: Modification of the cysteamine side chain of thienamycin. 1. Chem. Pharm. Bull. 34: 1089~1093, 1986
16) CHRISTENSEN, B. G.; J. HANNAH, W. J. LEANZA, R. W. RATCLIFFE & D. H. SHIH (Merck): *O*- and *N*-Carboxyl derivatives of thienamycin sulfoxide and sulfone. Jpn. Kokai 52,092 ('79), Apr. 24, 1979 [Chem. Abstr. 92: 41785c, 1980]
17) SCHMITT, S. M.; D. B. R. JOHNSTON & B. G. CHRISTENSEN: Thienamycin total synthesis. 3. Total synthesis of (±)-thienamycin and (±)-8-epithienamycin. J. Org. Chem. 45: 1142~1148, 1980
18) WOODBURN, H. M. & B. G. PAUTLER: The reaction of cyanogen with organic compounds VIII. 2-Mercaptoethylamine and its alkyl derivatives. J. Org. Chem. 19: 863~867, 1954
19) ALTAMURA, M. R.; T. HASSELSTROM & L. LONG, Jr.: Synthesis of di(methylthiomethyl) polysulfides. J. Org. Chem. 28: 2438~2440, 1963
20) SCHUTTE, L.: One-step synthesis of dithiohemiacetals. A new class of compounds. Tetrahedron Lett. 1971: 2321~2322, 1971
21) CAMPBELL, B. J.: Renal dipeptidase. Methods Enzymol. 19: 722~729, 1970
22) CHRISTENSEN, B. G.: Carbapenems. Symposium on chemistry and structure-activity relationships of the new β-lactams. Program and Abstracts of the 22nd Intersci. Conf. on Antimicrob. Agents Chemother., Session 35, p. 20, Miami Beach, Oct. 4~6, 1982
23) GRAHAM, E. R. B. & A. NEUBERGER: The synthesis and some properties of 2-aminoethyl β-D-glucopyranoside. J. Chem. Soc. 1968: 1638~1641, 1968
24) BERLIN, A. Y. & V. P. BRONOVITSKAYA: *p*-Bis(2-chloroethyl)aminophenylalanine (sarcolysine) and its derivatives. VI. Amides of *N*-acetylsarcolysine and some amines of the thiazole series. Zh. Obshch. Khim. 31: 1356~1361, 1961 [Chem. Abstr. 55: 24719g, 1961]
25) SHECHTER, H. & J. W. SHEPHERD: Base-catalyzed isomerization and tautomeric equilibria of the system 2-methyl-3-nitropropene and 2-methyl-1-nitropropene. J. Am. Chem. Soc. 76: 3617~3621, 1954
26) SPRAGUE, J. M.; A. H. LAND & C. ZIEGLER: Derivatives of 2-amino-4-methylthiazole. J. Am. Chem. Soc. 68: 2155~2159, 1946