# MODIFICATION OF THE CYSTEAMINE SIDE CHAIN OF THIENAMYCIN. III

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Thienamycin derivatives (**4**) having a cyclic amidine moiety at the C-2 position were prepared. The susceptibility to renal dehydropeptidase-1 and the antimicrobial activity of these compounds were determined. Their structure-activity relationships are also discussed.

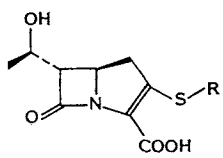
Carbapenem antibiotics, represented by thienamycin (THM),[1~3] have been reported to be hydrolyzed by renal dipeptidase, dehydropeptidase-1 (DHP-1).[4] To date, chemical modifications[5,6] of THM have been extensively undertaken to obtain DHP-1-resistant THM derivatives having potent antimicrobial activities. Recently, Merck chemists have found[7] that synthetic THM derivatives (**3**) having a 2-amidinoalkyl side chain at the C-2 position of carbapenem nucleus showed antimicrobial activities almost equal to, and resistance to DHP-1 greater than, that of *N*-formimidoyl THM (MK0787).[8~10]

In our previous papers,[11,12] derivatization of the *S*-$\alpha$-position of the cysteamine side chain of THM has been reported. As a part of the derivatization of this series, we have prepared THM derivatives (**4**) having a cyclic amidine moiety, in which the nitrogen atom and the *S*-$\alpha$-position of 2-amidinoalkyl moiety are cyclized as in Fig. 1. The present paper deals with the synthesis of these derivatives and the biological data in this series.

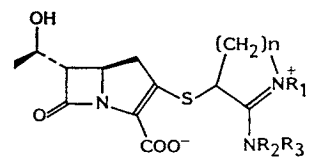
## Chemistry

In a previous paper,[13] we have described a facile method for the preparation of cyclic amidines,

Fig. 1.

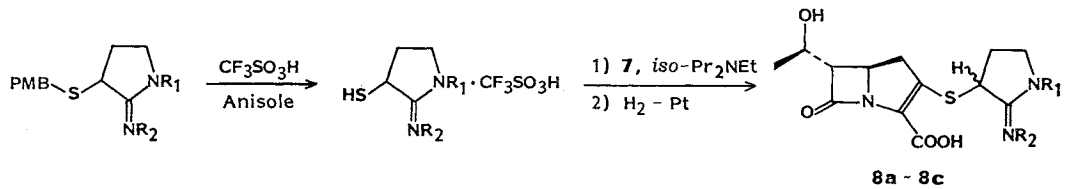



Thienamycin (**1**) R = $-CH_2CH_2NH_2$
MK0787 (**2**) R = $-CH_2CH_2NHCH{=}NH$
**3** R = $-CH_2CH_2C(=NR_1)NR_2R_3$



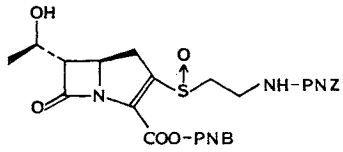

**4**
(n = 2, 3)

Scheme 1.

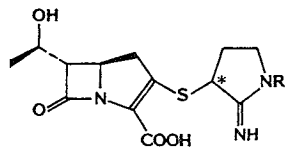



**5a ~ 5c** **6a ~ 6c** **8a ~ 8c**

**a** $R_1 = H$ $R_2 = H$
**b** $R_1 = H$ $R_2 = CH_3$
**c** $R_1 = CH_3$ $R_2 = H$




**7**




**9A** R = H (isomer A)
**9B** R = H (isomer B)
**10A** R = $CH_3$ (isomer A)
**10B** R = $CH_3$ (isomer B)

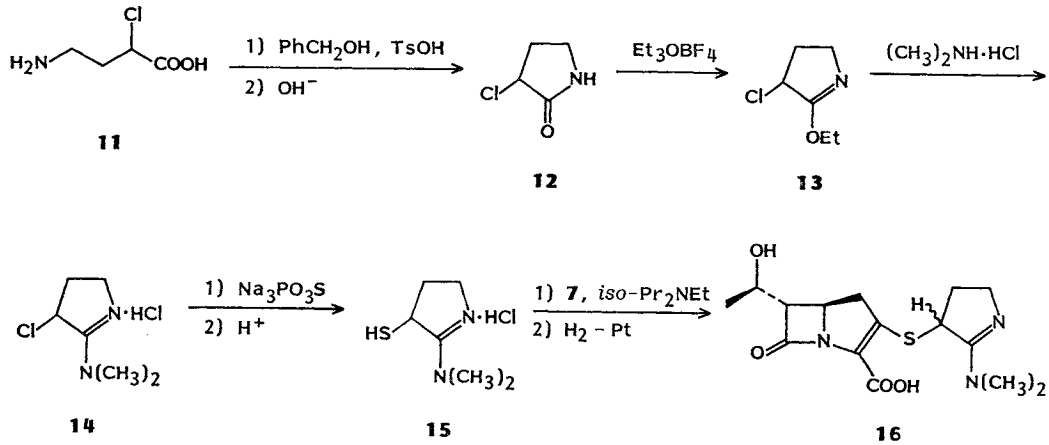
PNZ = *p*-Nitrobenzyloxycarbonyl
PNB = *p*-Nitrobenzyl
PMB = *p*-Methoxybenzyl

key intermediates for the preparation of the target compounds (**8a**~**8c**). Compounds **8a**~**8c** were prepared starting from **5a**~**5c**, as shown in Scheme 1. Thus, treatment of the cyclic amidines (**5a**~**5c**) with trifluoromethanesulfonic acid[14] and anisole in trifluoroacetic acid afforded the thiols (**6a**~**6c**) in good yields. Replacement[15] of the protected THM sulfoxide (**7**)[16] with the thiols (**6a**~**6c**) using diisopropylethylamine as a base, followed by catalytic hydrogenation[17] of the resulting reaction products in the presence of $PtO_2$ afforded target compounds **8a**~**8c** after purification by column chromatography on a Diaion HP-20 and by reverse-phase HPLC. An approximately 4 : 5 mixture of the two epimers **8a** was separated by HPLC to give **9A** and **9B**. Since the absolute configurations of **9A** and **9B** have not been confirmed, for convenience, we define the epimer having the shorter retention time in HPLC as isomer A, and the other as isomer B. Similarly, **8c** was separated into two epimers **10A** and **10B**. However, **8b** could not be separated by HPLC.

The dimethyl amidine derivative **16** was prepared as shown in Scheme 2. Esterification of amino acid **11**[18] with benzyl alcohol and *p*-toluenesulfonic acid, followed by cyclization of the resulting amino ester afforded lactam **12** in 53% yield. Treatment of lactam **12** with triethyloxonium fluoroborate (Meerwein reagent) gave the imido ester (**13**), which was converted into cyclic amidine **14** by treatment with dimethylamine hydrochloride in methanol. Then, displacement of the halogen with phosphorothioate anion[19] and subsequent acid hydrolysis produced the thiol (**15**). The target compound (**16**) was prepared by the conventional method outlined in Scheme 1 as a mixture of two diastereoisomers.

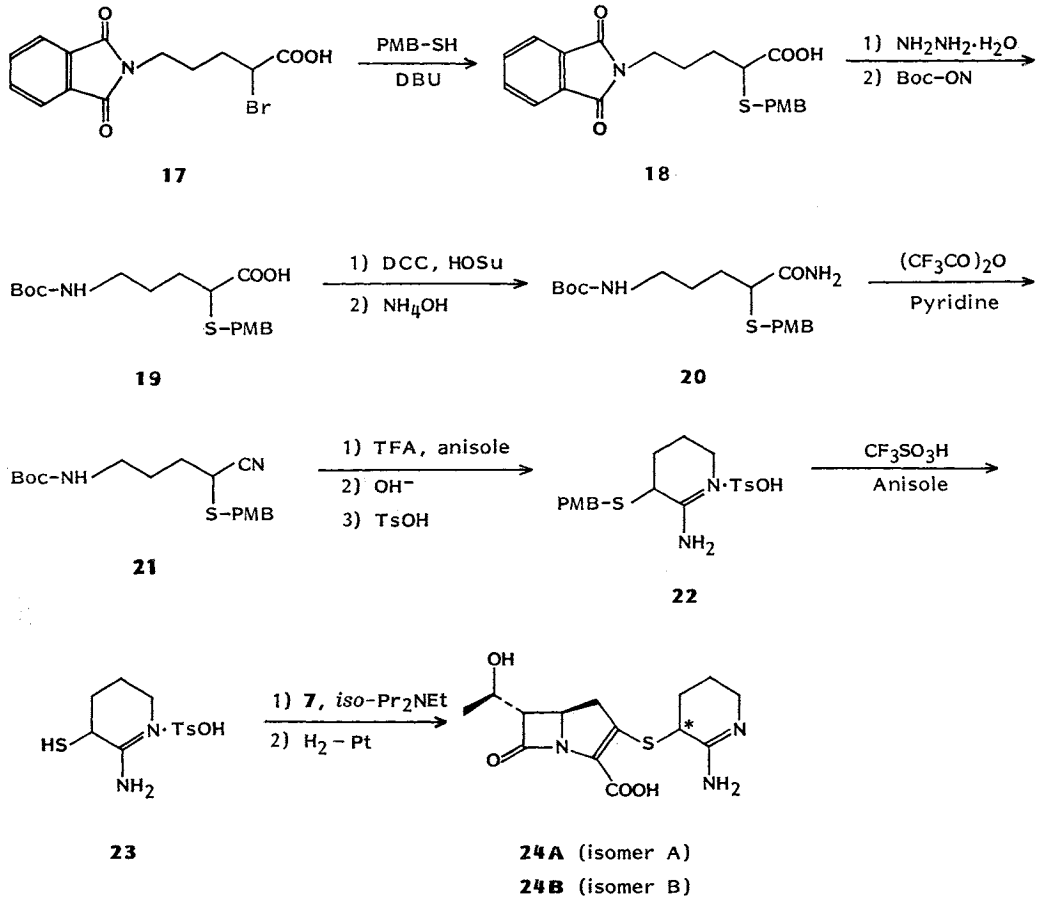
The six-membered amidine derivatives **24A** and **24B** were expected to be less susceptible to DHP-1 than the five-membered one (**9A** and **9B**) as a result of the steric hindrance[20] owing to the difference of

Scheme 2.




11 12 13 14 15 16

Scheme 3.




17 18 19 20 21 22 23 **24A** (isomer A) **24B** (isomer B)

ring size. Compounds **24A** and **24B** were prepared starting from **17** as shown in Scheme 3. Replacement of the bromo group of **17**[21] with *p*-methoxybenzylmercaptan using 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) as a base afforded **18** in 97% yield. Treatment of **18** with hydrazine monohy-

drate followed by protection of the resulting amino group with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) gave **19** in 80% yield. Activation of carboxylic acid **19** with *N*,*N*'-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (HOSu) and subsequent ammonolysis of the resulting active ester afforded amide **20** in 88% yield. Dehydration of **20** with trifluoroacetic anhydride[22] and pyridine gave a cyano compound (**21**) in 97% yield. After deprotection of the amino moiety, the cyclic amidine (**22**) was obtained by treatment of the resulting amino nitrile with *p*-toluenesulfonic acid[23] in xylene in 51% yield. The target compounds **24A** and **24B** were prepared as described for the preparation of **9A** and **9B**. Subsequent investigations revealed that **24B** crystallized as fine needles.

During the course of this work, we found that these cyclic amidine derivatives epimerized to about a 1 : 1 mixture of diastereoisomer in aqueous solution. For example, **9A**, which was completely separated by HPLC, was found to epimerize to **9B** in aqueous solution (2 mg/100 ml) after 72 hours at 4°C to give a *ca.* 12 : 1 mixture of **9A** and **9B**. The ratio was estimated by means of HPLC analysis. After being kept at room temperature for 5, 24 and 72 hours, the ratio of **9A** and **9B** became *ca.* 13 : 3, 12 : 7 and 4 : 5, respectively. On the other hand, an aqueous solution of **9B** attained equilibrium to give a *ca.* 4 : 5 mixture of **9A** and **9B** after 96 hours at room temperature. Similarly, **24A** epimerized to give a *ca.* 1 : 1 equilibrium mixture of **24A** and **24B** after being kept at room temperature for 96 hours.

## Biological Properties and Discussion

The susceptibility of the THM derivatives to DHP-1 and their minimal inhibitory concentrations (MICs) for Gram-positive and Gram-negative bacteria are listed in Table 1 in comparison with those of THM and MK0787.

Merck chemists have reported[7] that introduction of an alkyl group at the amidino function of **3** (see Table 1), reduced the susceptibility to hydrolysis by DHP-1 in proportion to the increase in bulkiness of the substituents. Similar results were observed in our series of derivatives (**4**). Thus, introduction of a methyl group into compound **8a** improved the DHP-1 stability as shown in compounds **8b**, **10A** and **10B**. Dimethyl derivative **16** was hardly hydrolyzed by DHP-1 in this reaction condition. As we expected, the six-membered cyclic amidine derivatives (**24A** and **24B**) showed considerably higher stability to DHP-1 than the five-membered ones (**9A** and **9B**) did. The stability of compound **9A** was roughly twice that of compound **9B**, indicating that a difference in the absolute configuration affects the stability. However, no significant difference was observed between **10A** and **10B**, or between **24A** and **24B**.

We have found that the antimicrobial activities of the THM derivatives against Gram-positive bacteria were generally excellent, as was that of MK0787. Of this series of THM derivatives tested, compound **8a** displayed the highest antimicrobial activity. Its activity was roughly twice that of MK0787 against Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*. In contrast to the results of 2-amidinoalkyl derivatives (**3**), introduction of a methyl group into the cyclic amidine moiety decreased antimicrobial activity, especially against *P. aeruginosa*. The activities of these mono- and dimethyl derivatives (**8b**, **10A**, **10B** and **16**) against Gram-negative bacteria were close to that of MK0787 except for the activity against *P. aeruginosa*. As for the activity against *P. aeruginosa*, the five-membered cyclic amidine derivatives (**9A** and **9B**) were twice as active than six-membered ones (**24A** and **24B**), although similar activities against other Gram-negative bacteria were observed.

Table 1. Antibacterial activity and DHP-1 stability of thienamycin and its derivatives.

| Organisms | MIC ($\mu$g/ml) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | MK0787 | THM | **8a** | **9A** | **9B** | **8b** | **10A** | **10B** | **16** | **24A** | **24B** |
| *Escherichia coli* NIHJ | 0.20 | 0.20 | <0.10 | 0.10 | 0.10 | <0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| *Citrobacter freundii* IID 976 | 0.10 | 0.20 | <0.10 | 0.10 | 0.10 | <0.10 | 0.20 | 0.39 | 0.10 | 0.20 | 0.20 |
| *Proteus vulgaris* 08601 | 0.39 | 1.56 | 0.20 | 0.20 | 0.20 | 0.20 | 0.39 | 0.78 | 0.78 | 0.39 | 0.39 |
| *P. mirabilis* IFO 3894 | 0.10 | 0.20 | 0.20 | 0.39 | 0.20 | 0.10 | 0.20 | 0.39 | 0.20 | 0.20 | 0.20 |
| *Klebsiella pneumoniae* type 1 | 0.10 | 0.20 | <0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.20 | 0.10 |
| *Enterobacter cloacae* 03402 | 0.78 | 1.56 | 0.10 | 0.10 | 0.10 | 0.20 | 0.39 | 0.39 | 0.20 | 0.20 | 0.20 |
| *Serratia marcescens* 10100 | 0.78 | 0.78 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.39 | 0.20 | 0.20 | 0.20 |
| *Pseudomonas aeruginosa* 32233 | 1.56 | 3.13 | 0.78 | 1.56 | 1.56 | 3.13 | 3.13 | 1.56 | 6.25 | 3.13 | 3.13 |
| *Staphylococcus aureus* 209P | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| *S. epidermidis* 56500 | 0.10 | 0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 | <0.10 |
| *Streptococcus 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 |
| *S. faecalis* ATCC 19433 | 0.78 | 0.78 | 0.78 | 1.56 | 1.56 | 1.56 | 0.78 | 1.56 | 0.78 | 0.78 | 0.78 |
| DHP-1 susceptibility[a] | 100 | 110 | 46 | 27 | 48 | 15 | 24 | 29 | <1 | 10 | 7 |

[a] DHP-1 susceptibility is given relative to MK0787=100.

In summary, of the new types of THM derivative with a cyclic amidine side chain at the C-2 position, **24B** was the most promising being a crystalline compound and having potent antimicrobial activity, comparable to that of MK0787. It was more resistant to hydrolysis by DHP-1 than MK0787; the relative stability was 7.

## Experimental

### Antibiotics

THM and MK0787 were provided by Merck, Sharp & Dohme Research Laboratories.

### Measurement of *In Vitro* Antibacterial Activity

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

### Test of Stability of THM Derivatives against Hydrolysis by DHP-1

The rate of the hydrolysis of each derivative by the enzyme was determined using the same method as described in the preceding paper.[12] The stability of compounds to hydrolysis by DHP-1 was represented in terms of the hydrolysis rate relative to that of the control compound, MK0787, represented as 100. The sample of DHP-1 used here was the same as that used in the preceding report.[12]

### 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 or 270-30 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 TMS or residual HOD ($\delta$ 4.80) as an internal reference. Mass spectra were recorded on a Jeol JMS-D300 mass spectrometer. UV spectra were measured on a Hitachi 323 spectrophotometer. HPLC purifications were performed on a Waters ALC/GPC Model 201 using a $\mu$Bondapak $C_{18}$ column (7.8 mm × 30 cm). Column chromatography was performed with Merck Silica gel 60 (70~230 mesh).

### 2-Imino-3-*p*-methoxybenzylthiopyrrolidine (**5a**)

DBU (786 mg, 5.16 mmol) was added to a stirred solution of **13** (760 mg, 5.15 mmol) and *p*-methoxybenzylmercaptan (797 mg, 5.17 mmol) in benzene (20 ml), and the reaction mixture was stirred for 0.5 hour at 50°C. The resulting precipitate was filtered and discarded. The filtrate was concentrated under reduced pressure and chromatographed on silica gel (15 g) with benzene - EtOAc (1 : 1) to give 2-ethoxy-3-*p*-methoxybenzylthio-1-pyrroline (937 mg, 68%) as a colorless oil.

A solution of the above imido ester (310 mg, 1.17 mmol) and ammonium chloride (69 mg, 1.29 mmol) in MeOH (20 ml) was heated under reflux for 15 hours. After concentration of the reaction mixture, the resulting residue was partitioned between water and EtOAc. The aqueous layer was separated, made basic with 1 N NaOH and extracted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting crystals were washed with isopropyl ether to give **5a** (160 mg, 58%): MP 89~90°C; IR (KBr) 1665 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.7~2.2 (1H, m, 4-H), 2.2~2.6 (1H, m, 4-H), 3.2~3.7 (3H, m, 3-H, 5-$H_2$), 3.68 (2H, s, $CH_2$Ar), 3.76 (3H, s, $OCH_3$), 4.87 (2H, br s, NH × 2), 6.80 (2H, d, $J$=9 Hz, ArH), 7.20 (2H, d, $J$=9 Hz, ArH).

*Anal* Calcd for $C_{12}H_{16}N_2OS$: C 61.07, H 6.82, N 11.86.
Found: C 60.87, H 6.71, N 11.74.

### 2-Imino-3-mercaptopyrrolidine Trifluoromethanesulfonate (**6a**)

A solution of **5a** (236 mg, 1 mmol), anisole (540 mg, 5 mmol) and trifluoromethanesulfonic acid (20 drops) in TFA (1.5 ml) was stirred for 1 hour at room temp. After concentration of the reaction mixture, the residue was washed successively with petroleum ether and isopropyl ether to give **6a** (260 mg, 98%) as a pale brown powder: $^1$H NMR ($D_2O$) $\delta$ 2.0~2.4 (1H, m, 4-H), 2.6~3.0 (1H, m,

4-H), 3.5~4.0 (2H, m, 5-$H_2$), 4.28 (1H, dd, $J$=7 and 9 Hz, 3-H), 4.80 (HOD).

The following compounds (**6b**, **6c** and **23**) were prepared from **5b**, **5c** and **22** as described for the preparation of **6a**, in 90%, 86% and 93% yields, respectively.

**6b**: A pale brown oil; $^1$H NMR ($D_2O$) $\delta$ 2.0~2.4 (1H, m, 4-H), 2.5~3.0 (1H, m, 4-H), 3.00 (3H, s, $CH_3$), 3.5~3.9 (2H, m, 5-$H_2$), 4.20 (1H, t, $J$=7 Hz, 3-H), 4.80 (HOD).

**6c**: A pale brown oil; $^1$H NMR ($D_2O$) $\delta$ 1.9~2.4 (1H, m, 4-H), 2.6~3.0 (1H, m, 4-H), 3.27 (3H, s, $CH_3$), 3.6~4.0 (2H, m, 5-$H_2$), 4.26 (1H, t, $J$=8 Hz, 3-H), 4.80 (HOD).

**23**: A pale brown oil; $^1$H NMR ($D_2O$) $\delta$ 1.70~2.20 (4H, m, 4-$H_2$, 5-$H_2$), 2.20 (3H, s, $CH_3$), 3.30~3.60 (2H, m, 6-$H_2$), 3.83~4.03 (1H, m, 3-H), 4.80 (HOD), 7.36 (2H, d, $J$=9 Hz, ArH), 7.70 (2H, d, $J$=9 Hz, ArH).

(5*R*,6*S*)-2-(2-Iminopyrrolidin-3-yl)thio-[(*R*)-1-hydroxyethyl]carbapen-2-em-3-carboxylic Acid (**8a**)

To a stirred solution of **7** (240 mg, 0.4 mmol) and **6a** (213 mg, 0.8 mmol) in DMF (3 ml) was added a solution of diisopropylethylamine (103 mg, 0.8 mmol) in DMF (1 ml) at −50°C under argon. After stirring for 0.5 hour at the same temp, the reaction mixture was poured into chilled $Et_2O$ (40 ml) and centrifuged. The separated gummy precipitate was dissolved in a mixture of THF (12 ml), water (4 ml) and 1/15 M phosphate buffer (pH 7.0, 14 ml) and the solution was subjected to catalytic hydrogenation under 4 atm for 1 hour at room temp in the presence of $PtO_2$ (170 mg). The catalyst was removed by filtration and washed with water. The combined filtrate and washing were concentrated under reduced pressure to remove organic solvents. The resultant aqueous solution was washed with $CHCl_3$ and concentrated to *ca.* 30 ml. The solution was applied to a Diaion HP-20 column (1.8×20 cm) which was eluted successively with water and water - THF (95 : 5). The fractions having UV absorption at 297 nm were combined, concentrated *in vacuo*, and then purified by HPLC (acetonitrile - water, 1 : 20) to give **8a** (8 mg, 6%) as a colorless powder after lyophilization.

An additional experiment was carried out using the sulfoxide (**7**, 300 mg, 0.5 mmol) and the thiol (**6a**, 266 mg, 1.0 mmol) as starting materials as described above, and the two diastereoisomers were separated by HPLC (acetonitrile - water, 1 : 50) to give **9A** (8 mg, 5%) and **9B** (11 mg, 7%) as a colorless powder, respectively.

The compounds **8b**, **10A**, **10B**, **16**, **24A** and **24B** were prepared from **6b**, **6c**, **15** and **23** as described for the preparation of **8a**, in 5%, 2%, 2%, 5%, 4% and 4% yields, respectively. Spectroscopic data of these compounds are listed in Table 2. In the additional experiment, **24B** was obtained as needles.

Compound **24B**: $[\alpha]_D^{25}$ +36.0° (*c* 0.21, $H_2O$).

*Anal* Calcd for $C_{14}H_{19}N_3O_4S \cdot 2H_2O$: C 46.53, H 6.41, N 11.63.
Found: C 46.88, H 5.88, N 11.09.

3-Chloro-2-pyrrolidone (**12**)

A mixture of (±)-4-amino-2-chlorobutyric acid (**11**, 4.11 g, 30 mmol), *p*-toluenesulfonic acid (6.19 g, 36 mmol) and benzyl alcohol (20 ml) in benzene (100 ml) was heated under reflux for 3 hours, the water liberated being removed by use of a Dean Stark apparatus. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between $CHCl_3$ and 5% $NaHCO_3$. The separated organic layer was extracted with 0.5 N HCl and the aqueous layer was neutralized with $NaHCO_3$ and then extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and heated under reflux for 0.5 hour. The reaction mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel (40 g) using EtOAc to give **12** (1.90 g, 53%) as prisms: MP 84~85°C; IR (KBr) 1700 $cm^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 2.0~2.9 (2H, m, 4-$H_2$), 3.2~3.7 (2H, m, 5-$H_2$), 4.43 (1H, dd, $J$=5 and 6 Hz, 3-H), 7.65 (1H, br s, NH).

*Anal* Calcd for $C_4H_6ClNO$: C 40.18, H 5.06, N 11.72.
Found: C 40.05, H 5.19, N 11.67.

3-Chloro-2-ethoxy-1-pyrroline (**13**)

A solution of **12** (2.10 g, 18 mmol) and triethyloxonium fluoroborate, prepared from boron trifluoride etherate (8.46 g, 60 mmol) and epichlorohydrin (4.16 g, 45 mmol), in $CH_2Cl_2$ (50 ml) was stirred for 1 hour at room temp. A solution of $K_2CO_3$ (8 g) in water (40 ml) was added to the reaction mixture cooled with ice, and the resulting precipitate was filtered off. The separated organic

Table 2. Spectroscopic data of thienamycin derivatives.

| Compound | IR (KBr) $cm^{-1}$ | | $^1H$ NMR ($D_2O$, 200 MHz) $\delta$ | UV $\lambda_{max}^{H_2O}$ nm |
|---|---|---|---|---|
| | Lactam | C=N | | |
| **8a** | 1760 | 1695 | 1.30 (3H, d), 2.2~2.4 (1H, m), 2.7~2.9 (1H, m), 3.1~3.3 (2H, m), 3.5 (1H, m), 3.6~3.9 (2H, m), 4.2~4.4 (2H, m), 4.6 (1H, m) | 297 |
| **9A** | 1765 | 1700 | 1.29 (3H, d), 2.2~2.5 (1H, m), 2.6~2.9 (1H, m), 3.22 (2H, d), 3.49 (1H, dd), 3.6~3.9 (2H, m), 4.2~4.4 (2H, m), 4.6~4.7 (1H, m) | 297 |
| **9B** | 1760 | 1700 | 1.29 (3H, d), 2.2~2.5 (1H, m), 2.6~2.9 (1H, m), 3.18 (1H, d), 3.21 (1H, d), 3.50 (1H, dd), 3.6~3.9 (2H, m), 4.2~4.4 (2H, m), 4.6~4.7 (1H, m) | 296 |
| **8b** | 1760 | 1695 | 1.29 (3H, d), 2.2~2.4 (1H, m), 2.7~2.9 (1H, m), 3.00 (3H, s), 3.1~3.3 (2H, m), 3.48 (1H, m), 3.6~3.9 (2H, m), 4.2~4.4 (2H, m), 4.5 (1H, m) | 296 |
| **10A** | 1760 | 1700 | 1.30 (3H, d), 2.2~2.4 (1H, m), 2.7~2.9 (1H, m), 3.14 (3H, s), 3.1~3.3 (2H, m), 3.50 (1H, m), 3.6~4.0 (2H, m), 4.2~4.4 (3H, m) | 298 |
| **10B** | 1760 | 1700 | 1.30 (3H, d), 2.2~2.4 (1H, m), 2.7~2.9 (1H, m), 3.14 (3H, s), 3.1~3.3 (2H, m), 3.50 (1H, m), 3.6~4.0 (2H, m), 4.2~4.4 (3H, m) | 296 |
| **16** | 1760 | 1690 | 1.27 (3H, d), 2.2~2.5 (1H, m), 2.6~2.9 (1H, m), 3.12 (3H, s), 3.1~3.3 (2H, m), 3.31 (1H, s), 3.34 (2H, s), 3.50 (1H, m), 3.6~4.0 (2H, m), 4.2~4.4 (2H, m), 4.6~4.7 (1H, m) | 298 |
| **24A** | 1760 | 1680 | 1.28 (3H, d), 1.70~2.32 (4H, m), 3.19~3.30 (2H, m), 3.40~3.60 (3H, m), 4.20~4.36 (3H, m) | 297 |
| **24B** | 1760 | 1680 | 1.27 (3H, d), 1.80~2.16 (3H, m), 2.20~2.36 (1H, m), 3.12~3.28 (2H, m), 3.40~3.60 (3H, m), 4.10~4.32 (3H, m) | 297 |

layer was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (20 g) using EtOAc to give **13** (2.23 g, 86%) as a colorless oil; IR (neat) 1645 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.35 (3H, t, $J$=7 Hz, $CH_3$), 2.1~2.8 (2H, m, 4-$H_2$), 3.5~4.0 (2H, m, 5-$H_2$), 4.26 (2H, q, $J$=7 Hz, $CH_2$), 4.55 (1H, dd, $J$=4 and 6 Hz, 3-H).

3-Chloro-2-dimethylamino-1-pyrroline Hydrochloride (**14**)

A solution of **13** (1.00 g, 6.8 mmol) and dimethylamine hydrochloride (0.50 g, 6.1 mmol) in MeOH (20 ml) was heated under reflux for 18 hours. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between $CHCl_3$ and water. The separated aqueous layer was concentrated under reduced pressure to give a mixture (1.00 g) of **14** and dimethylamine hydrochloride in the ratio of *ca.* 3:2. The oily product was used for the next reaction without further purification. IR (neat) 1690 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 2.1~2.6 (1H, m, 4-H), 2.6~3.2 (1H, m, 4-H), 3.36 (3H, s, $NCH_3$), 3.41 (3H, s, $NCH_3$), 3.8~4.1 (2H, m, 5-$H_2$), 5.30 (1H, m, 3-H).

2-Dimethylamino-3-mercapto-1-pyrroline Hydrochloride (**15**)

A solution of the above-mentioned chloride (1.00 g) and $Na_3PO_3S$ (1.00 g, 5.6 mmol) in water (10 ml) was heated under reflux for 2 hours under argon. After cooling, conc HCl (2 ml) was added to the reaction mixture and stirring was continued for another 15 minutes at 50~60°C under argon. The reaction mixture was concentrated *in vacuo*, and isopropyl alcohol (IPA) was added to the residue. The insoluble material was removed by filtration and the filtrate was concentrated *in vacuo*. A mixture of IPA (4 ml) and $Et_2O$ (20 ml) was added to the residue and the supernatant solution was decanted. The same treatment was repeated several times to remove phosphoric acid. The oily product was concentrated *in vacuo* to give a *ca.* 3:2 mixture (1.04 g) of **15** and dimethylamine hydrochloride.

This product was used for the next reaction without further purification. $^1$H NMR ($D_2O$) $\delta$ 2.0~2.5 (1H, m, 4-H), 2.5~3.0 (1H, m, 4-H), 3.06 (3H, s, $NCH_3$), 3.23 (3H, s, $NCH_3$), 3.6~4.0 (2H, m, 5-$H_2$), 4.26 (1H, m, 3-H), 4.80 (HOD).

2-*p*-Methoxybenzylthio-5-phthalimidoylpentanoic Acid (**18**)

To a solution of **17** (10.5 g, 32.2 mmol) and *p*-methoxybenzylmercaptan (PMB-SH, 5.8 g, 37.6 mmol) in DMF (35 ml) was added DBU (10.7 g, 70.3 mmol), and the reaction mixture was stirred for 20 minutes at room temp. The mixture was poured into cold 10% HCl and extracted with EtOAc. The extract was washed with water and dried over $MgSO_4$. Removal of the solvent *in vacuo* gave a residue which was chromatographed on silica gel (100 g) using $CHCl_3$ - MeOH (50 : 1) to give **18** (12.5 g, 97%) as a colorless, viscous oil, which crystallized after being kept at room temp: MP 85°C; IR (KBr) 1770, 1740, 1725, 1692 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.5~2.0 (4H, m, 3-$H_2$, 4-$H_2$), 3.0~3.3 (1H, m, 2-H), 3.4~3.8 (2H, m, 5-$H_2$), 3.77 (3H, s, $OCH_3$), 3.80 (2H, s, $CH_2Ar$), 6.80 (2H, d, $J$=9 Hz, ArH), 7.21 (2H, d, $J$=9 Hz, ArH), 7.6~7.9 (4H, m, ArH), 8.3~8.9 (1H, m, COOH).

*Anal* Calcd for $C_{21}H_{21}NO_5S$: C 63.14, H 5.30, N 3.51.
Found: C 63.16, H 5.45, N 3.55.

5-*tert*-Butoxycarbonylamino-2-*p*-methoxybenzylthiopentanoic Acid (**19**)

A solution of **18** (4.6 g, 11.5 mmol) and hydrazine monohydrate (3 ml) in EtOH (50 ml) was stirred for 17 hours at room temp. After concentration of the reaction mixture under reduced pressure, 5% HCl (100 ml) was added to the residue and then the mixture was centrifuged. The supernatant was concentrated under reduced pressure and the residue was dissolved in a mixture of dioxane (50 ml) and water (50 ml). Boc-ON (8.5 g, 34.5 mmol) and triethylamine (4.6 g, 45.5 mmol) were added to the solution and stirring was continued for 3 hours at room temp. The reaction mixture was diluted with water and washed with $Et_2O$. The separated aqueous layer was acidified with 0.5 N HCl and extracted with EtOAc. The extract was washed with water, dried over $Na_2SO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (75 g) using $CHCl_3$ - MeOH (99 : 1) to give **19** (3.4 g, 80%) as a pale yellow viscous oil: IR (neat) 1700 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.44 (9H, s, $CH_3\times3$), 1.40~1.95 (4H, m, 3-$H_2$, 4-$H_2$), 2.90~3.30 (3H, m, 2-H, 5-$H_2$), 3.78 (5H, s, $OCH_3$, $CH_2Ar$), 6.81 (2H, d, $J$=9 Hz, ArH), 7.24 (2H, d, $J$=9 Hz, ArH), 9.33 (1H, br s, COOH); field desorption mass spectra (FD-MS) $m/z$ 369 ($M^+$).

5-*tert*-Butoxycarbonylamino-2-*p*-methoxybenzylthiopentamide (**20**)

To a stirred solution of **19** (3.4 g, 9.2 mmol) in THF (30 ml) were added HOSu (1.1 g, 9.5 mmol) and DCC (1.9 g, 9.2 mmol) and the mixture was stirred for 2 hours at room temp. $NH_4OH$ (28%, 24 ml) was added to the reaction mixture and stirring was continued for another 2 hours. The reaction mixture was filtered off and the filtrate was concentrated *in vacuo*. The residue was partitioned between EtOAc and water and the separated organic layer was dried over $Na_2SO_4$ and then concentrated *in vacuo*. The residue was chromatographed on silica gel (50 g) using benzene - EtOAc (1 : 1) to give **20** (3.0 g, 88%) as a colorless powder: MP 130~132°C; IR (KBr) 1686, 1653 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.43 (9H, s, $CH_3\times3$), 1.40~2.00 (4H, m, 3-$H_2$, 4-$H_2$), 2.90~3.30 (3H, m, 2-H, 5-$H_2$), 3.70 (2H, s, $CH_2Ar$), 3.76 (3H, s, $OCH_3$), 4.79 (1H, m, NH), 6.23 (1H, br s, NH), 6.51 (1H, br s, NH), 6.81 (2H, d, $J$=9 Hz, ArH), 7.18 (2H, d, $J$=9 Hz, ArH).

*Anal* Calcd for $C_{18}H_{28}N_2O_4S$: C 58.67, H 7.66, N 7.60.
Found: C 58.70, H 7.63, N 7.64.

5-*tert*-Butoxycarbonylamino-2-*p*-methoxybenzylthiopentanonitrile (**21**)

To a stirred, ice-cooled solution of **20** (1.89 g, 5.13 mmol) and pyridine (0.81 g, 10.2 mmol) in dioxane (14 ml) was added dropwise $(CF_3CO)_2O$ (1.08 g, 5.14 mmol). After stirring for 1 hour at room temp the reaction mixture was diluted with EtOAc, washed with water, dried over $Na_2SO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (35 g) using benzene - EtOAc (4 : 1) to give **21** (1.75 g, 97%) as a colorless oil: IR (neat) 2260, 1705 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.42 (9H, s, $CH_3\times3$), 1.50~1.90 (4H, m, 3-$H_2$, 4-$H_2$), 2.95~3.20 (2H, m, 5-$H_2$), 3.23~3.45 (1H, m, 2-H), 3.76 (3H, s, $OCH_3$), 3.87 (2H, s, $CH_2Ar$), 4.65~4.86 (1H, m, NH), 6.81 (2H, d, $J$=9 Hz, ArH), 7.21 (2H, d, $J$=9 Hz, ArH); FD-MS $m/z$ 350 ($M^+$).

2-Imino-3-*p*-methoxybenzylthiopiperidine *p*-Toluenesulfonate (**22**)

A solution of **21** (700 mg, 2 mmol) and anisole (1.08 g, 10 mmol) in TFA (8 ml) was stirred for 1 hour with ice cooling. After concentration of the reaction mixture *in vacuo* the residue was partitioned between water and benzene. The separated aqueous layer was made basic with 1 N NaOH and extracted with $CHCl_3$. The extract was washed with water, dried over $Na_2SO_4$ and concentrated *in vacuo* to give 5-amino-2-*p*-methoxybenzylthiopentanonitrile (480 mg) as a colorless oil.

A mixture of the above amine (480 mg, 1.92 mmol) and *p*-toluenesulfonic acid monohydrate (380 mg, 2 mmol) in xylene (10 ml) was heated under reflux for 48 hours. After removal of the solvent, the residue was chromatographed on silica gel (10 g) using $CHCl_3$ - MeOH (20 : 1) to give **22** (430 mg, 53%), which was triturated with $Et_2O$ to give a yellowish powder: MP 96~103°C; IR (KBr) 1683 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.60~2.10 (4H, m, 4-$H_2$, 5-$H_2$), 2.34 (3H, s, $CH_3$), 3.25~3.46 (2H, m, 6-$H_2$), 3.6~3.8 (1H, m, 3-H), 3.76 (3H, s, $OCH_3$), 3.83 and 4.00 (2H, ABq, $J$=14 Hz, $CH_2Ar$), 6.76 (2H, d, $J$=9 Hz, ArH), 7.09 (2H, d, $J$=9 Hz, ArH), 7.16 (2H, d, $J$=9 Hz, ArH), 7.75 (2H, d, $J$=9 Hz, ArH), 7.90 (1H, br s), 9.10 (1H, br s), 9.85 (1H, br s).

*Anal* Calcd for $C_{20}H_{26}N_2O_4S_2$: C 56.84, H 6.20, N 6.63.
Found: C 56.81, H 6.12, N 7.01.

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