April, 1976] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 49(4), 1081—1084 (1976) 1081

# Studies of Unusual Amino Acids and Their Peptides. VI. The Syntheses and the Optical Resolutions of β-Methylphenylalanine and Its Dipeptide Present in Bottromycin

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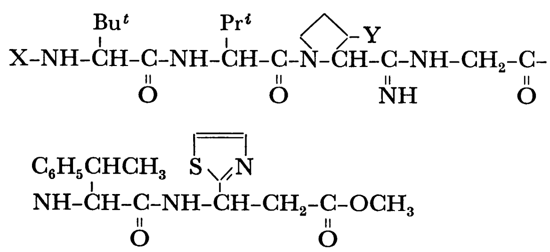
(Received July 25, 1975)

β-Methyl-phenylalanine was prepared by the condensation of α-methylbenzyl bromide with acetaminomalonate, followed by the hydrolysis and decarboxylation of the condensate. The decarboxylation occurred diastereoselectively to some extent. The *erythro* and the *threo* isomers could be separated by the fractional crystallization of their benzoyl derivatives, and differentiated by means of their NMR spectra. The optical resolution of the respective isomers was attained by treating the benzyloxycarbonyl derivatives with qunine or quinidine; thus, the four isomers could be obtained in a pure state. A dipeptide present in the C-terminal of bottromycin was synthesized by coupling N-protected *erythro*-L-β-methyl-phenylalanine with either L-β-(2-thiazolyl)-β-alanine ester or, more conveniently, with the DL-compound, and by separating thereafter.

Bottromycin, a peptidic antibiotic discovered in the fermentation broths of *Streptomyces bottropensis* by Waisvisz and his co-workers,[1)] contains many unusual amino acids. Structural studies of this antibiotic were carried out by Waisvisz[1,2)] and by Umezawa *et al.*;[3)] the formula shown in Fig. 1 has been proposed by the latter.

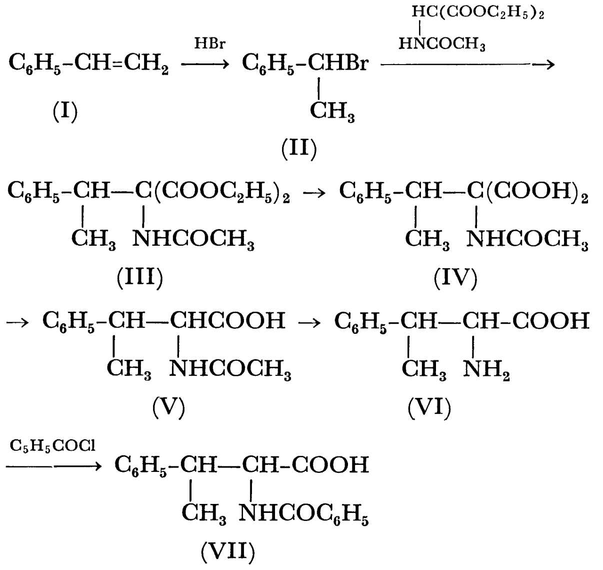


X = Pivaloyl or Δ¹-isocaproyl
Y = H or $CH_3$

Fig. 1. Structure of bottromycin.

In a previous paper[4)] we have made clear the absolute configuration of β-(2-thiazolyl)-β-alanine, situated at the carboxyl terminal in this peptide oligomer, by resolving the synthesized racemate into antipodes and by then investigating their ORD curves.

This paper will treat β-methyl-phenylalanine, the amino acid just neighboring β-(2-thiazolyl)-β-alanine. Umezawa and his co-workers[3)] attributed this amino acid to the L-series from the facts that its dithioethoxycarbonyl derivative shows a positive Cotton effect in methanol and that the amino acid is not decomposed by D-amino acid oxidase. Arold and his co-warkers[5)] have shown that the amino acid present in bottromycin belongs to the *erythro*-L series by preparing *ethythro*-β-methyl-phenylalanine and by then resolving it into antipodes. We, too, prepared this amino acid by another route and succeeded in obtaining the four possible isomers by resolving both *erythro*- and *threo*-compounds into antipodes; the scheme is illustrated in Schemes 1 and 2. α-Methylbenzyl bromide, prepared by the addition of hydrogen bromide to styrene according to Walling,[6)] was condensed with acetaminomalonate. The condensate, α-methylbenzylacetaminomalonate (III), was then hydrolyzed with aqueous sodium hydroxide and decarboxylated without isolation of the intermediate hydrolysate (IV). In order to separate the product mixture into *erythro*- and *threo*-compounds, it was first converted into the easily crystallizable benzoyl derivative, which was then separated, by crystallization from aqueous ethanol, into two : A high-melting substance (mp 188—190 °C)(VII-a) and a low-melting one (mp 130—132 °C) (VII-b).



Scheme 1. Synthetic route of β-Methyl-phenylananine.

It has been reported that *erythro* and *threo* isomers can easily be differentiated by means of a comparison of their NMR spectra; *i.e.*, in β-alkylsubstituted α-amino acids, the coupling constant ($J_{\alpha\beta}$) between $C_\alpha$–H and $C_\beta$–H is larger in the *erythro* form than in the *threo* form.[7)] In our case, the β-methyl-phenylalanines obtained from VII-a always gave larger values than those obtained from VII-b, as may be seen in Table 1. This suggests that the former belongs to an *erythro* type.

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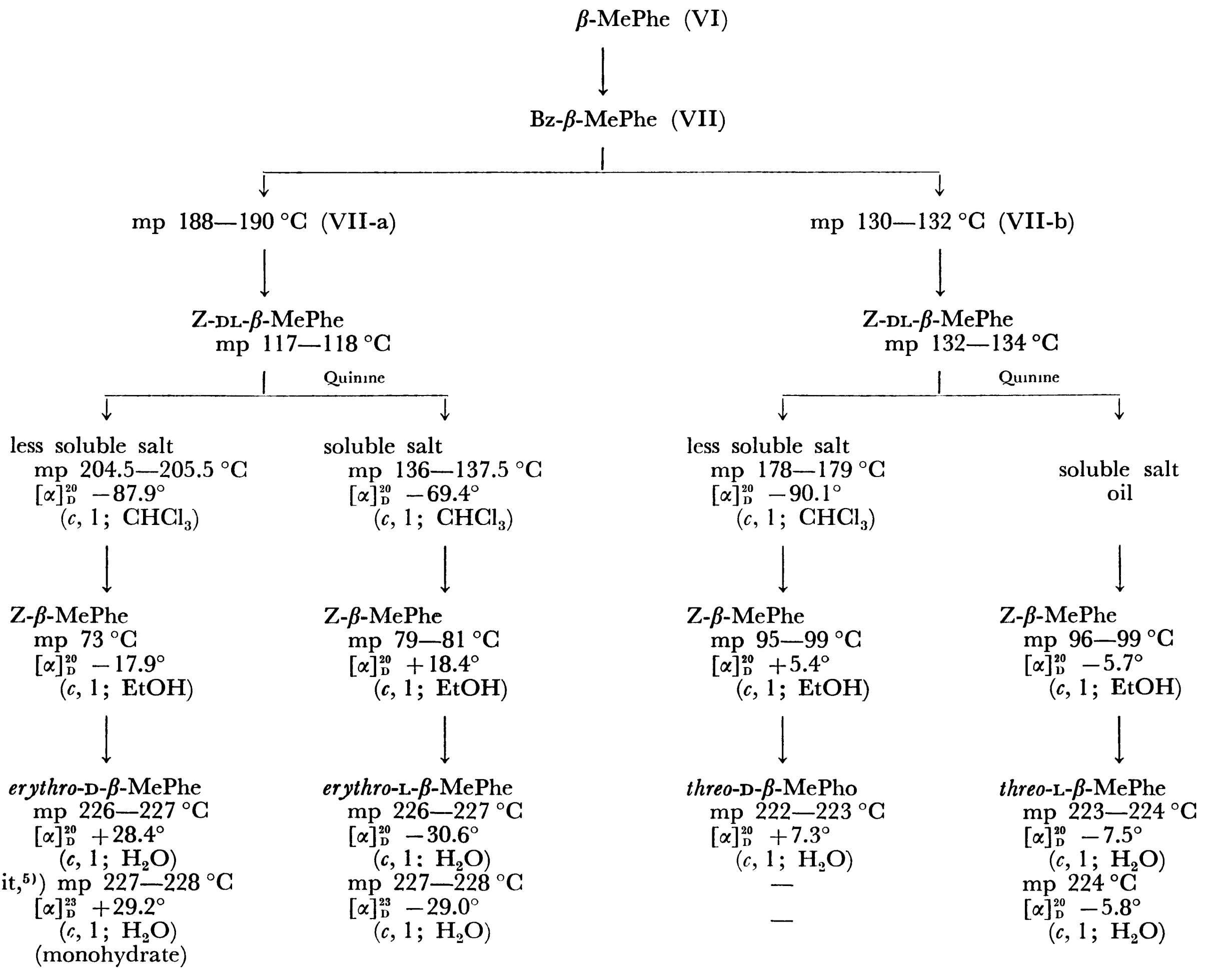
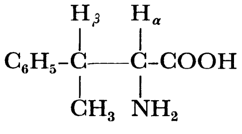


Scheme 2. Separation of β-methyl-phenylalanine (β-MePhe) into four isomers.

Table 1. NMR data of β-methyl-phenylalanine (60 MHz, DSS)



$$C_6H_5\text{-}\underset{CH_3}{\overset{H_\beta}{C}}\text{—}\underset{NH_2}{\overset{H_\alpha}{C}}\text{-}COOH$$

| | Compound | Medium D$_2$O | D$_2$O+DCl | D$_2$O+NaOD |
|---|---|---|---|---|
| $\delta_{H_\alpha}$ (ppm) | VI-a | 3.81 | 4.26 | 3.34 |
| | VI-b | 3.94 | 4.34 | 3.41 |
| $\delta_{H_\beta}$ (ppm) | VI-a | 3.31 | 3.49 | 2.98 |
| | VI-b | 3.55 | 3.59 | 3.19 |
| $J_{CH_3\text{-}H_\beta}$ (Hz) | VI-a | 6.9 | 7.4 | 6.8 |
| | VI-b | 7.2 | 7.5 | 7.2 |
| $J_{H_\alpha\text{-}H_\beta}$ (Hz) | VI-a | 7.5 | 7.2 | 7.5 |
| | VI-b | 4.8 | 6.0 | 5.2 |

It was noted that the difference in $J_{\alpha\beta}$ appeared more remarkable in a neutral medium than in an acidic or a basic condition.

In order to determine the *erythro*/*threo* ratio, the condensation product (III) was converted into a mixture of free amino acids (VI) by hydrolyzing it with hydrochloric acid. From the NMR spectra of the amino acids, the *erythro*/*threo* ratios were determined as (1.5—1.7) : 1. The benzyloxycarbonylation of the mixture of the free amino acids afforded the crystalline *threo* isomer and the oily, difficult-to-refine *erythro* derivative; the ratio of the amount of the *erythro* to that of the *threo* compounds was again found to be about 2 : 1. These results apparently indicate that the diastereoselective decarboxylation of the malonic acid derivative occurred quite similarly to Arold's observation.

To resolve each diastereomer, a diastereoselective reaction between *N*-acetyl-β-methyl-phenylalanine and hydrazine in the presence of papain was attempted, but no crystalline hydrazide was obtainable. The benzoyl derivative could not be used in this case because of its low solubility in a buffer solution of pH 5. By using 30% aqueous dioxane, it could be brought into solution, but no desired results were obtained. The resolution of the *N*-acyl derivatives by alkaloid bases was alternatively tried in various combinations; the results are shown in Table 2. The *N*-benzoyl derivative gave no definite alkaloid salt so far as we examined; with a quaternary ammonium hydroxide obtained from quinine by methylation, it gave crystalline salt, but the operations were too troublesome. As to the *erythro* derivative, the best result was obtained by treating the *N*-benzyloxycarbonyl-derivative with quinine in ethanol. *N*-Benzyloxycarbonyl-*erythro*-β-methyl-phenylalanine was mixed with equimolar quinine in ethanol, the less soluble salt was treated with hydrochloric acid to remove the quinine, and then

TABLE 2. SALT FORMATION OF ACYL-β-METHYL-PHENYLALANINE WITH ALKALOID BASES

| Solvent | Quinine | | | Chinchonine | | | Brucine | | |
|---|---|---|---|---|---|---|---|---|---|
| | Bz | Ac | Z | Bz | Ac | Z | Bz | Ac | Z |
| Acetone | ± | − | + | | − | − | − | − | − |
| Methanol | − | | | − | | | | | |
| Ethanol | | − | + | | − | − | − | − | − |
| 1-Propanol | − | − | ± | − | − | − | | − | − |
| Ethyl acetate | | − | | ± | | − | | | |
| Benzene | | | | | | − | | | |
| Chloroform | | | − | | | − | | − | − |

Bz=Benzoyl derivative; Ac=Acetyl derivative; Z=Benzyloxycarbonyl derivative. +, crystal; ±, trace; −, no precipitate.

with hydrogen bromide in acetic acid to remove the benzyloxycarbonyl group. We thus obtained a free amino acid of $[\alpha]_D+28.4°$. From the soluble quinine salt, its antipode of $[\alpha]_D-30.6°$ was isolated. The latter is identical with the β-methyl-phenylalanine ($[\alpha]_D-31°$) obtained from bottromycin by Umezawa and his co-workers[3] as well as with the one belonging to the *erythro*-L form reported by Arold.[5] Another way to isolate the *erythro*-L isomer was to treat the benzyloxy-carbonyl compound with quinidine and to regenerate the free amino acid from the less soluble alkaloid salt. In this case, it was not the D-isomer but the L-isomer that made the less soluble salt, so it was more convenient to obtain the L-isomer present in bottromycin, though the yield was somewhat inferior to that of the preceding method. In a similar way, the *threo*-compound was also resolved into its antipodes. Thus, all four theoretically possible isomers were successfully prepared.

Now both the optically-active amino acids present in the carboxyl terminal of bottromycin, *i.e.*, L-β-(2-thiazolyl)-β-alanine and L-β-methyl-phenylalanine, could be prepared, so we tried to build up the dipeptide which constitutes the most important part of bottromycin.[8] *N*-Benzyloxycarbonyl-*erythro*-L-β-methyl-phenylalanine was coupled with L-β-(2-thiazolyl)-β-alanine methyl ester by the carbodiimide method; the desired protected dipeptide was thus obtained in a 70% yield. As the standard sample was prepared in this way, we attempted a more convenient method of synthesizing this dipeptide by coupling the optically-active β-methyl-phenylalanine with the racemic β-(2-thiazolyl)-β-alanine, and by then separating the product mixture into its respective diastereomers. After coupling the components by the carbodiimide method, the product was fractionally recrystallized from ethanol; we thus obtained a diastereomer with a mp of 159—161 °C, and one with a mp of 182—182.5 °C in *ca.* a yield 60% of the theoretical amount respectively. The ORD curve and IR spectrum of the former were completely identical with those of the standard sample described above. The other compound must be a diasteromer composed of L-β-methyl-phenylalanine and D-β-(2-thiazolyl)-β-alanine, because it has a different melting point and a different value of $[\alpha]_{300}$ from those of the L-L-compound, though the IR spectra of the two compounds in chloroform coincided completely. In order to ascertain the configuration of the β-(2-thiazolyl)-β-alanine contained in this dipeptide, it was hydrolyzed by refluxing with 6 M hydrochloric acid for a short time (1.5 h); the desired amino acid was then isolated by silica-gel dry-column chromatography. Its ORD curve apparently showed that it belongs to the D-series, though the values of α are very small because of its easily racemizable property under acid-hydrolytic conditions.[4] The phenomenon that the curve shifts to the positive side when 2 drops of 6 M hydrochloric acid or a concentrated sodium hydroxide solution (more remarkably) are added supports this conclusion.[9]

## Experimental[10]

*α-Methylbenzyl Bromide (II).* This compound was prepared according to the method of Walling[6] by passing dry hydrogen bromide gas to freshly distilled styrene under ice-cooling; bp 79—80 °C/8 mmHg, $n_D^{22}$ 1.564. lit,[11]: bp 78—82 °C/10 mmHg, $n_D^{20}$ 1.5612.

*Diethyl α-Methylbenzylacetaminomalonate (III).* Diethyl acetaminomalonate (9.1 g) was dissolved in a sodium ethoxide solution in ethanol (sodium, 0.97 g; absolute ethanol, 50 ml); II (9.3 g) was then added, and the mixture was kept at 30 °C for 20 h. After the sodium bromide had then been filtered off and washed with hot ethanol, the combined ethanolic solution was concentrated *in vacuo*. The residual solid was recrystallized from 50% aqueous ethanol. The yield was 9.9 g (73%); mp 122—122.5 °C. Found: C, 63.31; H, 7.41; N, 4.40%. Calcd for $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.36%.

N-*Acetyl*-DL-*β-methyl-phenylalanine (V).* III (6.43 g) was hydrolyzed by refluxing with a 10% sodium hydroxide solution (30 ml) for 30 min; then to this boiling solution 3 M hydrochloric acid (*ca.* 23 ml) was carefully added, drop by drop, until no more evolution of carbon dioxide was observed. The resulting mixture was allowed to stand in a refrigerator overnight, after which the separated acetylamino acid (V) was collected. The yield was 3.91 g (88%). The crude product was used for the next reaction without further purification, though it showed a broad melting point.

*Diastereomeric Mixture of* erythro- *and* threo-DL-*β-Methyl-phenylalanine Hydrochloride (VI).* V was refluxed with 6 M hydrochloric acid for 8 h, after which the solution was concentrated to dryness *in vacuo*. The residue was diluted with some water and concentrated again; the repetition of this operation gave a crude diastereomeric mixture (VI) in an almost quantitative yield. TLC and a paper chromatogram showed only one spot. For analysis it was recrystallized from water; mp 181—190 °C. Found: C, 55.56; H, 6.48; N, 6.68%. Calcd for $C_{10}H_{13}NO_2\cdot HCl$; C, 55.69; H, 6.49; N, 6.49.%

N-*Benzoyl*-DL-*β-methyl-phenylalanine and Its Separation into* erythro- *and* threo-*Forms.* VI was benzoylated in a usual manner in a 93% yield. When the crude product was fractionally crystallized from aqueous ethanol, compounds melting at 188—190 °C (VII-a) and 130—132 °C (VII-b) were obtained.

*Determination of the Structures of the* erythro- *and the* threo-*Compounds (VII-a and VII-b) by Means of the NMR Data.* Both the *N*-benzoylated derivatives (VII-a and VII-b) were hydrolyzed with 6 M hydrochloric acid, and the resulting hydrochlorides were converted into free amino acids by treating them with an ion-exchange resin (Amberite IR 120). The

NMR data of both the amino acids are shown in Table 1. In order to estimate the *erthro/threo* ratio of the decarboxylation product (V), the crude sample of V was hydrolyzed to a free amino acid mixture and its NMR spectrum was measured. From the intensity of the peaks corresponding to the two $\alpha$-H signals (100 MHz), the *erythro/threo* ratio was calculated as 1.7 : 1. The free amino acids derived from the acetaminomalonate in one step by treating it with 6 M hydrochloric acid also gave a similar *erythro/threo* ratio (1.5 : 1), as estimated from their NMR spectra.

*N-Benzyloxycarbonyl* erythro- *and* threo-DL-*β-Methyl-phenylalanine (IX-a and IX-b).* VII-a and VII-b were hydrolyzed with 6 M hydrochloric acid and benzyloxycarbonylated by the usual method (yields, >90%).

VIII-a derived from VII-a (mp 117—118 °C). Found: C, 68.95; H, 6.20; N, 4.48%. Calcd for $C_{18}H_{19}NO_4$: C, 69.00; H, 6.11; N, 4.47%.

VIII-b derived from VII-b (mp 132—134 °C). Found: C, 69.09; H, 6.16; N, 4.63%.

*Separation of the* N-*Benzyloxycarbonylated β-Methyl-phenylalanine into the* erythro- *and the* threo-*Derivatives.* The amino acid mixture obtained by the hydrolysis of V was benzyloxycarbonylated as usual. Part of the crude reaction product crystallized easily and could be isolated by filtration, though the rest remained oily (the ratio of the oil to the crystal was about 2 : 1). The crystalline compound was found to be identical with the *threo*-compound (VIII-b) derived from VII-b, and the residual oil was concluded to be composed of the *erythro*-derivative contaminated with some *threo*-compound, judging from the NMR spectrum of the free amino acid reproduced from it.

NMR data of VIII-a and VIII-b (60 MHz, DMSO-$d_6$):

VIII-a (*erythro*): $\delta$ 1.27 (3H, $CH_3$, d, $J$=7.2 Hz), 3.15 (1H, $H_\beta$, d-q, $J$=9.1 Hz and 7.1 Hz), 4.29 (1H, $H_\alpha$, t, $J$=9.0 Hz), 4.99 (2H, benzyl-$CH_2$, s).

VIII-b (*threo*): $\delta$ 1.25 (3H, $CH_3$, d, $J$=7.5 Hz), 3.26 (1H, $H_\beta$, qi, $J$=7.0 Hz), 4.27 (1H, $H_\alpha$, d-d, $J$=6.9 Hz and 9.0 Hz), 5.01 (2H, benzyl-$CH_2$, s), 7.66 (1H, NH, d, $J$=9.0 Hz).

*Optical Resolutions of* N-*Benzyloxycarbonyl*-erythro- *and* threo-DL-*β-Methyl-phenylalanine.* Equimolar amounts of VIII-a and quinine were dissolved in hot ethanol, and the solution was allowed to stand overnight. A white crystalline salt which separated as needles was collected. From the salt, the quinine was removed by treatment with 2 M hydrochloric acid in ether; thus *N*-benzyloxycarbonyl-*erythro*-D-β-methyl-phenylalanine was obtained. From the mother liquor, the *erythro*-L-derivative was obtained by a similar treatment. By the same proceddre, the *threo*-D-compound was obtained from VIII-b through easily crytallizable quinine salt, and the *threo*-L-compound, from the mother liquor. The results are summarized in Scheme 2.

Similarly, VIII-a was treated with quinidine, and from the less soluble salt (mp 171—171.5 °C (from chloroform-ethanol)$[\alpha]^{20}_{D}$ +122.1° (*c* 1, $CHCl_3$)), the L-compound ($[\alpha]^{20}_{D}$ +17.6° (*c* 1, $C_2H_5OH$)) was obtained in a 62% yield.

*Four Isomers of Optically Active β-Methyl-phenylalanine.* Four isomers of the optically-active benzyloxycarbonyl derivative of the amino acid obtained above were debenzyloxycarbonylated by hydrogen bromide in acetic acid and converted into free amino acids by treatment with an ion-exchange resin (Amberlite IR 120). Their optical properties are shown in Scheme 2.

*Methyl β-(2-Thiazolyl)-β-alaninate Dihydrochloride.* Thionyl chloride (1.31 g) was gradually stirred into methanol (30 ml) at about −10 °C, and to the resulting solution DL-β-(2-thiazolyl)-β-alanine[4] (1.72 g) was added. After stirring for 1 h at that temperature and for 6 h at room temperature, the reaction mixture was concentrated to dryness *in vacuo*, and after which anhydrous ether was added. The colorless crystals (1.72 g; 73%) precipitated were collected and recrystallized from methanol-ether. The yield was 1.16 g (48%); mp 153—155 °C. Found: C, 32.29; H, 4.79; N, 10.76%. Calcd for $C_7H_{10}N_2O_2S \cdot 2HCl$: C, 32.44; H. 4.67; N, 10.81%.

Similarly, the L-compound was prepared; mp 152—153 °C, $[\alpha]^{19}_{270}$ −8.8° (*c* 1, $CH_3OH$).

N-*Benzyloxycarbonyl*-erythro-L-*β-methyl-phenylalanyl*-L- *and* DL-*β-(2-thiazolyl)-β-alanine Methyl Ester and Its Resolution into Diastereomers.* Into a solution of *N*-benzyloxycarbonyl-*erythro*-L-β-methyl-phenylalanine (175 mg) in dichloromethane (4 ml), dicyclohexylcarbodiimide (115 mg) in the same solvent (2 ml) was stirred under ice-cooling. After 5 min, a solution of methyl L-β-(2-thiazolyl)-β-alanine dihydrochloride (145 mg) and *N*-methylmorpholine (113 mg) in dichloromethane (4 ml) was added, and the mixture was stirred overnight at room temperature. After treatment as usual, the crude dipeptide was obtained; 256 mg (95%); mp 152—155 °C. Recrystallization from aqueous ethanol gave a sample with a mp of 159.5—162 °C, $[\alpha]^{19}_{300}$ +89.3° (*c* 0.268, $CHCl_3$). Found: C, 62.34; H, 5.70; N, 8.62%. Calcd for $C_{25}H_{27}N_3O_5S$: C, 62.35; H, 5.65; N, 8.73%.

The coupling of the *erythro*-L-acid (1.38 g) and the DL-ester (1.08 g) was quite similarly carried out by using triethylamine in place of *N*-methylmorpholine. The crude dipeptide (1.89 g; 89%) was fractionally crystallized from 70% ethanol and could be separated into two components:

L-D-Compound, mp 182—182.5 °C, $[\alpha]^{27}_{300}$ +103.7° (*c* 0.27, $CHCl_3$). Found: C, 62.65; H, 5.69; N, 8.60%.

L-L-Compound, mp 159—161 °C, $[\alpha]^{19}_{300}$ +90.0° (c, 0.242, $CHCl_3$). Found: C, 62.33; H, 5.68; N, 8.56%.

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10) All the melting points are uncorrected. The optical rotations were measured by means of a Yanagimoto Polarimeter OR-20. ORD-curves were recorded on a JASCO ORD/UV-5 spectropolarimeter. NMR spectra were recorded on JEOL PS-100 and Hitachi R-20A spectrometer, using DSS as the internal standard. Thin-layer chromatographies were performed on Merck silica gel G using 1-butanol-acetic acid-water (4 : 1 : 2 v/v, upper layer) as the developing solvent. Circular paper chromatographies were performed on Toyo Roshi No. 2.

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