

Studies of Unusual Amino Acids and Their Peptides. IX. The Synthetic Study of Bottromycins B₁ and B₂¹⁾

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Bottromycins B₁ and B₂, peptide antibiotics, were synthesized according to the structures proposed by Nakamura *et al.* B₁ was obtained by the condensation of the imide, Piv-Tle-Val-ImPro-OEt·HCl with the ester, H-Gly-Phe(βMe)-β-Tha-OH in the presence of Et₃N, and B₂ by the replacement of the Z group of Z-Tle-Val-ImPro-Gly-Phe(βMe)-β-Tha-OMe by the *trans*-4-methyl-2-pentenoyl group. The structures of the synthetic B₁ and B₂ were confirmed by elemental analyses, amino acid analyses, and CI-mass spectra. However, the synthetic bottromycins differed from the natural ones in many respects, *e.g.*, IR spectra, behavior in TLC, and pK_a values. Most significantly, no antimicrobial activities were detected in the synthetic B₁. On the basis of these results, it is concluded that the structures proposed by Nakamura *et al.* for bottromycins are erroneous and should be revised.

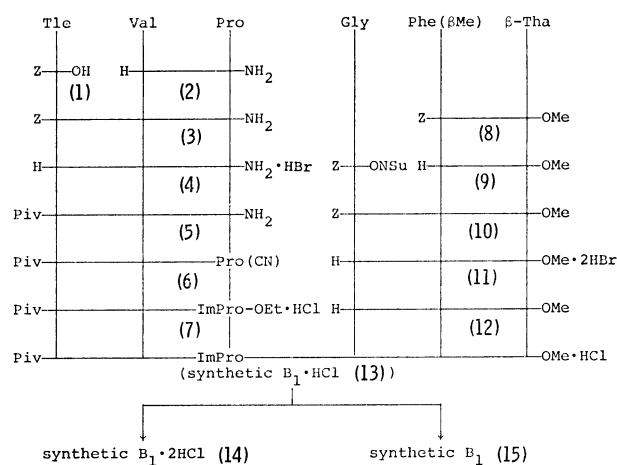
Bottromycin is a peptide antibiotic which was first isolated from the culture broth of *Streptomyces bottropensis* by Waisvisz *et al.*²⁾ They determined the C-terminal dipeptide to be β-methylphenylalanyl-β-(2-thiazolyl)-β-alanine methyl ester, though they gave no information about the configurations of the two amino acids. Later, Nakamura *et al.*,³⁾ who studied this antibiotic in detail, were able to separate it into 5 species and gave these components the structures shown in Fig. 1. Wolf and his co-workers⁴⁾ also isolated bottromycin and separated it into 5 species, 3 species of which, however, could not be identified. The configurations of the two unusual amino acids, β-methylphenylalanine and β-(2-thiazolyl)-β-alanine, were determined to be *erythro*-L by Arold *et al.*⁵⁾ and L by our group,⁶⁾ respectively.

Compared with other peptide antibiotics, the structure proposed for bottromycin is very unique, because it consists of a linear acyl peptide ester containing an iminopeptide bond^{7a)} in the middle of the molecule, and contains many unusual amino acids, but no D-amino acid. These structural features had strongly aroused our interest in the relationship between the structures and the biological activities, and prompted us to synthesize bottromycin.

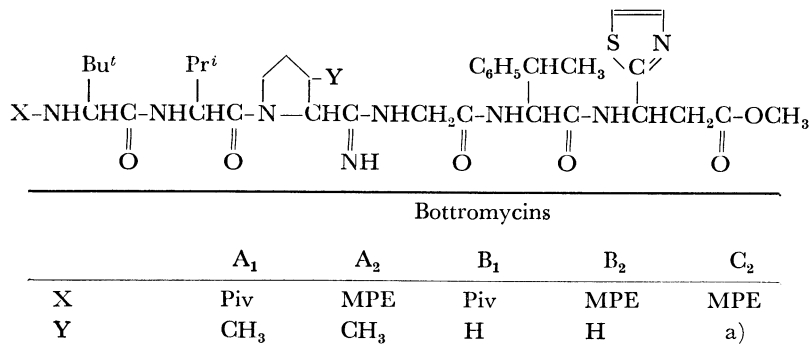
A preliminary trial⁸⁾ to build up bottromycin by coupling the three known dipeptide fragments, *i.e.*, pivaloyl-*t*-leucylvaline,⁹⁾ (iminopropyl)glycine,⁸⁾ and β-methylphenylalanyl-β-(2-thiazolyl)-β-alanine methyl ester,¹⁰⁾ failed due to the easy cyclization of the (imi-

nopropyl)glycine fragment into an imidazolone when the carboxyl group was activated. The imidazolone formation could successfully be avoided by locating a carboxyl (or alkoxycarbonyl) group sufficiently apart from the imino group in the molecule, as seen in the syntheses of model iminopeptides.¹¹⁾ On the basis of these previous results, we adopted the synthetic routes through the condensation of the tripeptide imide with the tripeptide ester to form an iminopeptide bond, as is shown in Schemes 1 and 2.

For the preparation of the N-terminal fragment of B₁, Z-Tle-OH^{7b)}(1) was coupled with H-Val-Pro-NH₂

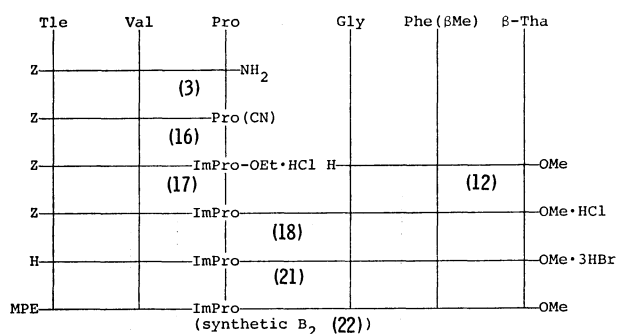


Scheme 1. Synthesis of bottromycin B₁.



a) Dimethyl- or ethyl-proline in the proline moiety.

Fig. 1. The structure of bottromycin group proposed by Nakamura *et al.*³⁾

Scheme 2. Synthesis of bottromycin B₂.

(2) by the mixed anhydride method, and the protected tripeptide amide (3) was obtained in 88% yield. The Z group of the amide (3) was replaced by the pivaloyl group through the debenzoyloxycarbonylation of 3 with hydrogen bromide in acetic acid and the successive acylation with pivaloyl chloride in the presence of *N,N*-diisopropylethylamine, to give *N*-pivaloyl tripeptide amide (5). The amide (5) was dehydrated to the nitrile (6) with phosphoryl chloride in pyridine in 87% yield, and then converted into the imidate hydrochloride (7) by the method of Pinner^{8,11,12} in 98% yield.

The C-terminal fragment was prepared by the coupling of Z-Gly-ONSu with H-Phe(βMe)-β-Tha-OMe (9), followed by the debenzoyloxycarbonylation of the resulting protected tripeptide (10) with hydrogen bromide in acetic acid. The tripeptide ester (11) was obtained as the dihydrobromide in 79% yield, based on the protected dipeptide ester¹⁰ (8).

The tripeptide imidate hydrochloride (7) was condensed with the tripeptide ester (12) derived from the dihydrobromide (11) in methanol in the presence of triethylamine and afforded bottromycin B₁ as the monohydrochloride (13) in 50% yield. The free B₁ (15) was obtained by the treatment of the hydrochloride (13) with an ion exchange resin, while the treatment of the compound (13) with 4.7% hydrogen chloride in methanol gave the B₁ dihydrochloride (14).

Bottromycin B₂ was synthesized as follows: The protected tripeptide amide (3) was dehydrated with phosphoryl chloride in pyridine in 97% yield, and the

resulting nitrile (16) was converted into the tripeptide imidate hydrochloride (17) by the method of Pinner. From the hydrochloride (17) and the tripeptide ester (12), the *N*-Z-iminohexapeptide ester (18) was obtained in 37% yield in the same manner as was used for the preparation of the B₁ hydrochloride (13). The compound (18) was debenzoyloxycarbonylated with hydrogen bromide in acetic acid, acylated with *trans*-4-methyl-2-pentenoyl chloride (MPE-Cl) in pyridine, and treated with Amberlyst A-29; thus the desired bottromycin B₂ was obtained in 43% yield. Although no information about the geometry of the MPE group in bottromycins A₂ and B₂ had been given, we used the *trans*-MPE-Cl for the preparation of B₂ after assuming that the MPE group of the natural compounds would be in the more stable *trans* form.

Near the end of this synthetic work, we received some information^{1b)} about a revised study of bottromycin A₂, which was published later.^{1c)} The revised structure was quite different from the old one.³⁾ Though nothing was mentioned about bottromycins A₁ and B₁, these structures also became doubtful. Therefore, we carefully investigated the properties of the compounds synthesized here according to the structures proposed by Nakamura *et al.*, by comparing with the structure of the natural A₂.¹³⁾

The coincidence of the structures of the synthetic bottromycins B₁ and B₂ with the proposed structures was confirmed by elemental analyses, amino acid analyses (Table 1), and the chemical ionization (CI) mass spectra (Fig. 2).

However, these synthetic bottromycins differed from the natural ones¹³⁾ in many respects: the IR spectra of the natural B₁ and the synthetic B₁ did not coincide with each other, though those of the synthetic B₁ and B₂ very much resemble each other and those of the natural B₁ and A₂ also resemble each other. It should be pointed out that the synthetic B₂ had an absorption band assigned to C-H bending deformation of *trans* disubstituted C=C at 980 cm⁻¹, while the natural A₂ had not absorption band in the same region. (Fig. 3) The *R_f* values (TLC, MeOH:AcOEt = 1:3) of the synthetic B₁ and B₂ were 0.37 and 0.33, respectively, while that of the natural A₂ containing some B₂¹³⁾ was

TABLE 1. ANALYTICAL DATA OF SYNTHETIC BOTTROMYCINS

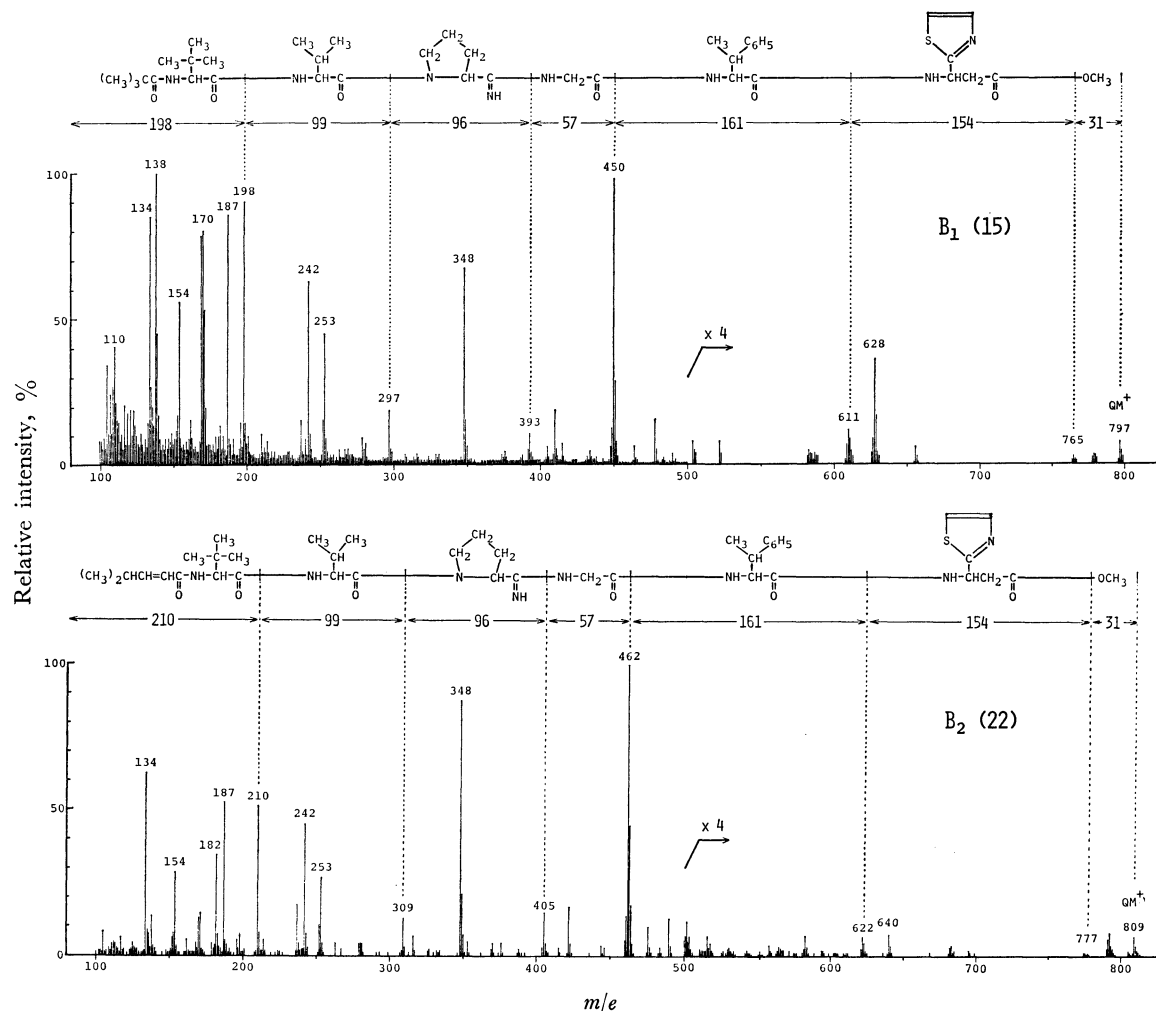
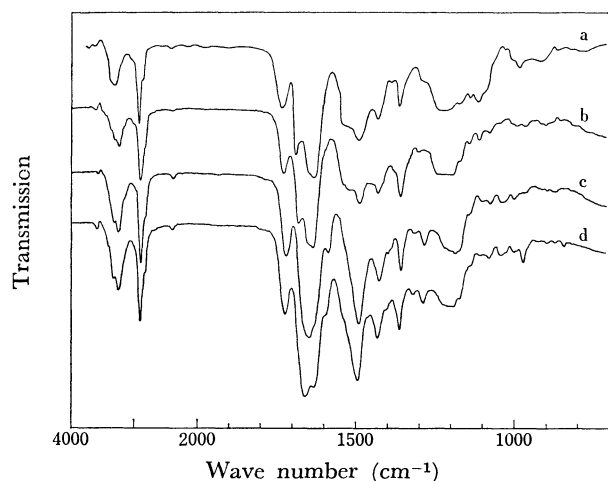
Bottromycin	[α] _D (MeOH)	Molecular formula	Found (Calcd) (%)		
			C	H	N
Synthetic B ₁ (15)	−54.1 ^{°a)}	C ₄₀ H ₆₀ N ₈ O ₇ S·H ₂ O	58.80 (58.95)	7.61 (7.67)	12.87 (13.75)
Synthetic B ₁ ·HCl (13)	−34.6 ^{°b)}	C ₄₀ H ₆₀ N ₈ O ₇ S·HCl·2H ₂ O	55.41 (55.25)	7.41 (7.53)	12.18 (12.89)
Synthetic B ₁ ·2HCl (14)	−34.2 ^{°c)}	C ₄₀ H ₆₀ N ₈ O ₇ S·2HCl·H ₂ O	54.32 (54.11)	7.34 (7.26)	11.96 (12.62)
Synthetic B ₂ (22)	−55.5 ^{°d)}	C ₄₁ H ₆₀ N ₈ O ₇ S·2H ₂ O	58.68 (58.27)	7.45 (7.63)	12.38 (13.25)

Amino acid analyses: 6 M HCl, 110°C, 5 days in sealed tube.

Synthetic B₁ (15): Tle 1.06, Val 1.09, Pro 0.98, Gly 1, Phe(βMe) 1.04, β-Tha 0.77^{e)}

Synthetic B₂ (22): Tle 1.01, Val 1.11, Pro 1.06, Gly 1, Phe(βMe) 1.02, β-Tha 0.74^{e)}

a) c 1 at 25°C. b) c 1 at 20°C. c) c 0.5 at 20°C. d) c 0.988 at 25°C. e) Amino acid produced from β-Tha in hydrolysis: Asp 0.05 (for B₁), 0.06 (for B₂).

Fig. 2. $\text{CI}(\text{CH}_4)$ -mass spectra of synthetic bottromycins B_1 (15) and B_2 (22).Fig. 3. IR spectra of natural and synthetic bottromycins in CHCl_3 . a: Natural B_1 (This spectrum is reproduced from Fig. 3 in Ref. 19), b: natural A_2 ,¹³⁾ c: synthetic B_1 (15), d: synthetic B_2 (22).

0.78,¹⁴⁾ a quite different value. Further, the behavior of the synthetic B_2 and the natural A_2 against KMnO_4 solution was striking: when a dilute KMnO_4 solution was sprayed on the thin-layer chromatogram, the syn-

thetic B_2 immediately decolorized KMnO_4 , but, unexpectedly, the natural A_2 remained unchanged, though it has been proposed that it contains an ethylene linkage. Furthermore, the pK_a values were also different: the pK_a value (in $\text{MeOH}:\text{H}_2\text{O} = 3:2$) of the synthetic B_1 was 9.1, similar to those of its related iminopeptides,¹¹⁾ while that of the natural A_2 was 8.3 (lit,^{3c)} 8.1–8.3). The most conclusive results were obtained from the comparison of the antimicrobial activities: no activities were detected in the synthetic B_1 so far examined; this can be seen in Table 2.

From all of the above results, we conclude that the structures of bottromycins proposed by Nakamura *et al.*, not only for A_2 and B_2 , but also for B_1 and probably A_1 , are erroneous and should be revised.

Experimental

All the melting points are uncorrected. The optical rotations were measured by means of a Yanagimoto polarimeter, OR-10, or a Perkin-Elmer 141 polarimeter. The NMR spectra were recorded on a JEOL PS-100 spectrometer or a Hitachi R-24A spectrometer. The IR spectra were recorded on a Hitachi EPI-S2 spectrophotometer. The pK_a values were measured by means of a Hitachi-Horiba pH meter, F-7. GLC analyses were carried out on a Hitachi 063 Gas

TABLE 2. ANTIMICROBIAL ACTIVITIES OF SYNTHETIC BOTTRMYCIN B₁ AND NATURAL A₂

Microorganism	MIC (μg/ml)	
	Synthetic B ₁	Natural A ₂
<i>Staphylococcus aureus</i> 209-p	>50	0.1
Terashima	>50	0.19
Smith	>50	0.19
252 R	>50	0.19
199 R	>50	0.19
664 R	>50	0.19
<i>Staphylococcus epidermidis</i> 10131 R	>50	≤0.005
Kawamura	>50	0.1
<i>Streptococcus faecalis</i>	>50	0.19
Urayama R	>50	0.19
<i>Sarcina lutea</i> PCI-1001	>50	0.1
<i>Bacillus subtilis</i> PCI-219	>50	≤0.05
<i>Escherichia coli</i> NIHJ	>50	50
K-12	>50	12.5
6065 R	>100	25
<i>Salmonella typhi</i> T-58	>100	>100
<i>Salmonella enteritidis</i>	>100	25
<i>Shigella flexneri</i> 2a	>100	25
<i>Shigella sonnei</i>	>100	25
<i>Citrobacter freundii</i> TU-786	>100	50
<i>Enterobacter cloacae</i> TL-14	>100	>100
<i>Serratia marcescens</i> OU-29	>100	>100
<i>Klebsiella pneumoniae</i> PCL-602	> 50	50
<i>Proteus mirabilis</i> 1698	> 50	>50
<i>Proteus vulgaris</i> IID-874	>100	>100
<i>Pseudomonas aeruginosa</i> PI-67	> 50	>50
No. 12	>100	50
No. 45	>100	>100
150	>100	50

Agar dilution method: Inoculum size: 10⁶ cells/ml. Medium: Heat infusion agar (pH 7.2). Cultivation: 18 h, 37 °C.

Chromatograph. TLC was done on Merck's Kieselgel GF₂₅₄ (Type 60) or precoated Kieselgel 60 F₂₅₄. For column chromatography, Merck's Kieselgel 60 (0.063–0.200 mm) was employed.

L-*t*-Leucine (Tle).²⁰ This compound was prepared from DL-*t*-leucine according to the method of Weygand,⁹ and was proved to be optically pure by GLC of Piv-Tle-Val-OMe¹⁵ derived from it without any purification; mp 250.5–251 °C (sublim.), [α]_D²⁵ –10.8° (c 1, H₂O). Lit, [α]_D²⁵ –9.4° (c 1, H₂O);¹⁶ [α]_D²⁰ –10.9° (c 2, H₂O);⁹ [α]_D –8.3° (c 0.53, H₂O).¹⁷

Z-Tle-OH (1). Into a cold solution of *t*-leucine (2.5 g, 19.1 mmol) in 1M NaOH (19.1 ml), *Z*-Cl (3.9 g, 22.9 mmol) and 1M NaOH (22.9 ml) were simultaneously added with vigorous stirring at 0–2 °C, and the mixture was stirred at about 0 °C for 1 h and then at room temperature for 1 h. After the usual treatment,⁹ the desired compound was obtained as an oil: Yield, 4.9 g (97%); [α]_D²⁰ –5.9° (c 1, MeOH); [α]_D²⁰ +4.0° (c 1, DMF). Lit,¹⁷ [α]_D +3.8° (c 0.39, DMF) *Dicyclohexylammonium Salt*: Mp 165.5–166.5 °C, [α]_D²⁰ –8.3° (c 1, MeOH). Lit,¹⁷ mp 165–168 °C, [α]_D –8.4° (c 0.59, MeOH).

Z-Tle-Val-Pro-NH₂ (3). Into a cold solution of

Z-Tle-OH (1) (1.857 g, 7 mmol) and *N*-methylmorpholine (708 mg, 7 mmol) in THF (15 ml), isobutyl chloroformate (956 mg, 7 mmol) was added, drop by drop, with vigorous stirring below –10 °C, and the mixture was stirred at the same temperature for 15 min. To the mixture was added H-Val-Pro-NH₂·HBr⁹ (2·HBr) (2.059 g, 7 mmol) and *N*-methylmorpholine (708 mg, 7 mmol) in DMF (15 ml) at about –10 °C. The mixture was stirred at about –10 °C for 20 min, at –5 °C for 1 h, at 0 °C for 1 h, and at room temperature overnight. After the removal of the precipitate, the yellow filtrate was evaporated under reduced pressure and the residual DMF solution was diluted with water (200 ml), extracted with AcOEt, washed with water, 1M NaHCO₃, and water again, and dried over MgSO₄. The solution was concentrated to give a colorless foamy solid: Yield, 2.845 g (88.2%). This product was used for the next reaction without further purification. The analytical sample was obtained by chromatography on a silica gel column with AcOEt-MeOH (15 : 1); [α]_D²⁰ –88.7° (c 1, MeOH). Found: C, 61.97; H, 8.07; N, 12.18%. Calcd for C₂₄H₃₆N₄O₅: C, 62.59; H, 7.88; N, 12.16%.

H-Tle-Val-Pro-NH₂·HBr (4). This compound was prepared by the removal of the *Z* group from 3 (2.073 g, 4.5 mmol) with 25% HBr in AcOH (4.5 g) as usual. The crude product was recrystallized from MeOH-ether to colorless crystals: Yield, 1.570 g (85.6%); mp 193–196 °C. Found: C, 45.22; H, 8.19; N, 13.19%. Calcd for C₁₆H₃₀N₄O₃·HBr·H₂O: C, 45.18; H, 7.82; N, 13.17%.

Piv-Tle-Val-Pro-NH₂ (5). Into a cold solution of 4 (522 mg, 1.28 mmol) and *N,N*-diisopropylethylamine (381 mg, 2.95 mmol) in CH₂Cl₂ (4 ml), pivaloyl chloride (201 mg, 1.67 mmol) in CH₂Cl₂ (2 ml) was stirred, drop by drop, at 2–3 °C, and stirring was continued at room temperature for 1 h. To the resulting clear solution, 1-(2-aminoethyl)pipe-razine (99 mg, 0.77 mmol) in CH₂Cl₂ (1 ml) was added.

After the mixture was stirred at room temperature for 10 min, the precipitate was filtered off. The filtrate was evaporated under reduced pressure and the residue was treated with AcOEt to remove any insoluble materials. The AcOEt solution was washed with water, 0.5 M citric acid, 1M NaHCO₃, and water again, and dried over MgSO₄. After the removal of the solvent, a foamy solid was obtained: Yield, 368 mg (69.9%). The analytical sample was obtained by chromatography on a silica gel column with AcOEt-MeOH (1 : 9); [α]_D²⁰ –109.8° (c 1, MeOH). Found: C, 61.15; H, 9.45; N, 13.02%. Calcd for C₂₁H₃₈N₄O₄: C, 61.43; H, 9.33; N, 13.65%.

N-(*Piv*-Tle-Val)-2-cyanopyrrolidine (6). To a stirred solution of 5 (493 mg, 1.2 mmol) in dry pyridine (2.5 ml), POCl₃ (0.14 ml, 1.44 mmol) in CH₂Cl₂ (0.4 ml) was added, drop by drop, below –10 °C. The solution was stirred at the same temperature for 1 h, treated with ice (10 g), and then extracted with AcOEt. The organic layer was washed with 1M HCl, 1M NaHCO₃, and water, and dried over MgSO₄: Yield, 408 mg (86.6%); a foamy solid, [α]_D²⁰ –118.2° (c 1, MeOH). Found: C, 63.80; H, 9.29; N, 13.94%. Calcd for C₂₁H₃₆N₄O₃: C, 64.25; H, 9.24; N, 14.27%.

Piv-Tle-Val-ImPro-OEt·HCl (7). To a cold solution of 6 (385 mg, 0.98 mmol) and absolute EtOH (60 mg, 1.3 mmol) in dry ether (10 ml), dry HCl was passed with stirring at about –15 °C until saturation, and then at 0 °C for 30 min and at room temperature for 30 min. The reaction mixture was evaporated under reduced pressure to afford a colorless foamy solid: Yield, 457 mg (98.2%). This imidate hydrochloride was immediately used for the next reaction without any purification.

Z-Gly-Phe(βMe)-β-Tha-OMe (10). Into a solution

of the crude product of H-Phe(β Me)- β -Tha-OMe-2HBr (**9**·2HBr) (prepared by the removal of the Z group from Z-Phe(β Me)- β -Tha-OMe¹⁰) (**8**) (960 mg, 1.99 mmol) with 25% HBr in AcOH as usual) and Et₃N (450 mg, 4.46 mmol) in CH₂Cl₂ (40 ml), Z-Gly-ONSu (660 mg, 2.15 mmol) was added with stirring and the mixture was allowed to stand at room temperature overnight. After the removal of the solvent, the residue was dissolved in AcOEt, washed with water, 1M HCl, water, 1M NaHCO₃, and water again, and dried over Na₂SO₄. The solution was evaporated under reduced pressure to leave a solid, which was recrystallized from MeOH: Yield, 852 mg (79.3%); mp 159.5–160 °C, $[\alpha]_D^{20} +18.4^\circ$ (*c* 1, MeOH). Found: C, 60.32; H, 5.59; N, 10.25%. Calcd for C₂₇H₃₀N₄O₆S: C, 60.21; H, 5.61; N, 10.40%.

H-Gly-Phe(β Me)- β -Tha-OMe-2HBr (**11**). This compound was prepared by the treatment of **10** (539 mg, 1 mmol) with 25% HBr in AcOH (1.5 g) as usual. The crude product was purified by the reprecipitation from MeOH-ether: Yield, 581 mg (99.5%); mp 149–152 °C. Found: C, 38.96; H, 4.86; N, 9.42%. Calcd for C₁₉H₂₄N₄O₄S·2HBr·H₂O: C, 39.06; H, 4.83; N, 9.59%.

The free ester, H-Gly-Phe(β Me)- β -Tha-OMe (**12**), was prepared by the treatment of **11** with Et₃N (in double the mole concentration of **11**) in THF, followed by the filtration of a precipitate and the removal of the solvent. The residual oil, the tripeptide ester (**12**), was used for the next reaction without any purification.

Piv-Tle-Val-ImPro-Gly-Phe(β Me)- β -Tha-OMe (Synthetic *Bottromycin B*₁).

a) *HCl Salt* (**13**): A solution of the imidate hydrochloride (**7**) (457 mg, 0.96 mmol), the tripeptide ester (**12**) prepared from **11** (453 mg, 0.8 mmol), and Et₃N (97 mg, 0.96 mmol) in dry MeOH (5 ml) was stirred at room temperature for 3 days. The solution was then evaporated under reduced pressure, leaving a foamy solid. The solid was taken up in AcOEt and freed from any insoluble materials by filtration. The filtrate was evaporated under reduced pressure, and the residue was chromatographed on preparative layers of silica gel with CHCl₃-MeOH-AcOH (95 : 15 : 3) to give a foamy solid: Yield, 344 mg (49.5%).

b) *2HCl Salt* (**14**). The hydrochloride (**13**) (100 mg) was treated with 4.7% HCl in MeOH (10 ml), and evaporated under reduced pressure, leaving a colorless solid: Yield, 98 mg; mp 156–160 °C (dec). *pK*_a (amidine group): 9.1 (MeOH-H₂O (3 : 2), 20 °C).

c) *Free Compound* (**15**): This compound was obtained by passing the HCl salt (**13**) (120 mg) through a Dowex 1×8 column (25% MeOH) or an Amberlyst A-29 column (90% MeOH): Yield, 112 mg, a foamy solid.

Analytical results of the compounds (**13**, **14**, and **15**) are summarized in Table 1.

N-(Z-Tle-Val)-2-cyanopyrrolidine (**16**). This compound was prepared by the dehydration of the tripeptide amide (**3**) (553 mg, 1.2 mmol) with POCl₃ (0.14 ml, 1.44 mmol) in a mixture of dry pyridine (2.5 ml) and CH₂Cl₂ (0.4 ml) at about -10 °C, as has been described above for the preparation of **6**: Yield, 455 mg (85.7%); a foamy solid, $[\alpha]_D^{20} -98.3^\circ$ (*c* 1, MeOH). Found: C, 64.78; H, 7.97; N, 12.73%. Calcd for C₂₄H₃₄N₄O₄: C, 65.14; H, 7.74; N, 12.66%.

Z-Tle-Val-ImPro-OEt·HCl (**17**). This compound was prepared by passing dry HCl into a mixture of **16** (398 mg, 0.9 mmol) and absolute EtOH (55 mg, 1.2 mmol) in dry ether, as has been described above for the preparation of **7**: Yield, 457 mg (96.7%); a foamy solid. This product was immediately used for the next reaction.

Z-Tle-Val-ImPro-Gly-Phe(β Me)- β -Tha-OMe·HCl (**18**).

A solution of the imidate hydrochloride (**17**) (457 mg, 0.87 mmol), the tripeptide ester (**12**) prepared from **11** (396 mg, 0.7 mmol), and Et₃N (88 mg, 0.87 mmol) in dry MeOH (5 ml) was stirred at room temperature for 3 days. After being treated as described above for **13**, the resulting foamy solid was chromatographed on preparative layers of silica gel with CHCl₃-MeOH-AcOH (95 : 15 : 3) to give a foamy solid: Yield, 229 mg (36.7%); $[\alpha]_D^{20} -21.2^\circ$ (*c* 0.5, MeOH). Found: C, 58.10; H, 6.85; N, 12.19%. Calcd for C₄₃H₅₈N₈O₈S·HCl·1/2H₂O: C, 57.87; H, 6.78; N, 12.55%.

trans-4-Methyl-2-pentenoic Acid (**19**). This compound was prepared from malonic acid and isobutyraldehyde in pyridine containing a small amount of piperidine.¹⁸ This acid obtained here was proved to be the *trans* isomer by the NMR and IR spectra, as shown below: Yield, 65.1%; bp 107–109 °C/14 Torr, *n*_D²⁰ 1.4469 (lit.¹⁸) bp 115–116 °C/20 Torr, *n*_D²⁰⁻⁶ 1.4489; IR(neat): 985 cm⁻¹ (σ C-H of *trans*-disubstituted olefin); NMR(CDCl₃): δ =1.09 (6H, d, *J*=7 Hz, (CH₃)₂CH-), 2.52 (1H, o, *J*=7 Hz, (CH₃)₂CHCH=), 5.82 (1H, dd, *J*=16 and *ca.* 1 Hz, -CH=CH-COOH), 7.11 (1H, dd, *J*=16 and 6.5 Hz, >CH-CH=CH-), 12.26 (1H, s, -COOH).

trans-4-Methyl-2-pentenoyl Chloride (**20**). This compound was prepared from **19** and SOCl₂ as usual:¹⁸ Yield, 73%; bp 65–66 °C/25 Torr (lit.¹⁸) bp 58–59 °C/18 Torr; NMR(CDCl₃): δ =1.12 (6H, d, *J*=7 Hz, (CH₃)₂CH-), 2.58 (1H, o, *J*=7 Hz, (CH₃)₂CHCH=), 6.04 (1H, dd, *J*=16 and *ca.* 1 Hz, -CH=CH-COCl), 7.19 (1H, dd, *J*=16 and 6.5 Hz, >CH-CH=CH-).

MPE-Tle-Val-ImPro-Gly-Phe(β Me)- β -Tha-OMe (Synthetic *Bottromycin B*₂) (**22**).

The compound (**18**) (121 mg, 0.136 mmol) was treated with 25% HBr in AcOH (2 g) at room temperature. The reaction mixture was diluted with dry ether, and the separated gummy material was triturated and thoroughly washed with dry ether. It afforded the iminohexapeptide methyl ester trihydrobromide(**21**) as a brownish amorphous powder (133 mg). Into a solution of the crude trihydrobromide (131 mg) in pyridine (2.5 ml), was stirred *trans*-MPE-Cl (**20**) (55 mg, 0.415 mmol) in benzene (1.5 ml) under ice-cooling, and the mixture was stirred at room temperature overnight. The solvent was thoroughly removed by repeated concentrations under reduced pressure after each addition of benzene. The residual syrup was chromatographed on preparative layers of silica gel with CHCl₃-MeOH-AcOH (96 : 15 : 3) to give a foamy solid, which was then passed through an Amberlyst A-29 column with 90% MeOH as a solvent. The eluent was evaporated under reduced pressure to afford a foamy solid: yield, 52 mg (43.3% based on **18**). Analytical results are summarized in Table 1.

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 NH

situated between amino acid residues. The nomenclatures and abbreviations for iminopeptides and their derivatives were described previously.^{8,11)} b) Abbreviations according to the IUPAC-IUB Commission (*J. Biol. Chem.*, **247**, 977 (1972)) are used throughout. Additional abbreviations: Tle, *t*-leucine; Phe(β Me), *erythro*- β -methylphenylalanine; β -Tha, β -(2-thiazolyl)- β -alanine; Pro(CN), 2-cyanopyrrolidine; ImPro, iminoproline;^{7a)} Bu^t, *t*-butyl; Pr^t, isopropyl; Z, benzoyloxycarbonyl; Piv, pivaloyl; MPE, 4-methyl-2-pentenoyl;

ONSu, succinimidooxy; DCC, dicyclohexylcarbodiimide; THF, tetrahydrofuran; DMF, *N,N*-dimethylformamide; BuOH, 1-butanol. The amino acids and their derivatives used here are all of the L-configuration.

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14) Although this sample of the natural A₂ contained some B₂,¹³⁾ TLC showed only one spot. This is understandable because bottromycin B₂ has been reported to behave like A₂ in TLC: e.g., *R_f* values of A₂ and B₂ were 0.81 and 0.80 (BuOH : AcOH : H₂O = 100 : 12 : 100), respectively.^{3d)}

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