Studies of Unusual Amino Acids and Their Peptides. IX. The Synthetic Study of Bottromycins B_1 and B_2[1)]

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(Received September 16, 1977)

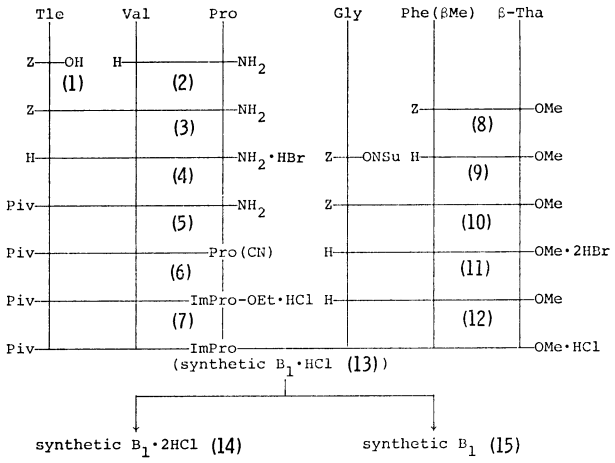
Bottromycins B_1 and B_2, peptide antibiotics, were synthesized according to the structures proposed by Nakamura *et al.* B_1 was obtained by the condensation of the imidate, Piv–Tle–Val–ImPro–OEt·HCl with the ester, H–Gly–Phe(βMe)–β-Tha–OH in the presence of Et_3N, and B_2 by the replacement of the 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 B_1 and B_2 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 B_1. 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-workers[4)] 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.

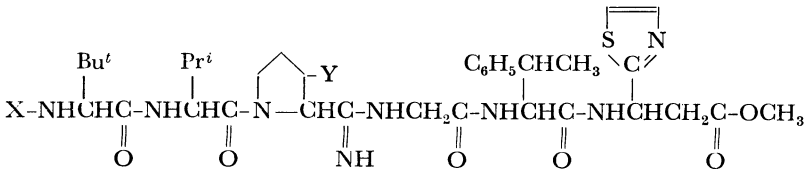
Compared with other peptide antibiotics, the structure proposed for bottromycin is very unique, because it consists of a linear acyl peptide ester containing an iminopeptide bond[7a)] in the middle of the molecule, and contains many unusual amino acids, but no D-amino acid. These structural features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

A preliminary trial[8)] to build up bottromycin by coupling the three known dipeptide fragments, *i.e.*, pivaloyl-*t*-leucylvaline,[9)] (iminoproly)glycine,[8)] and β-methylphenylalanyl-β-(2-thiazolyl)-β-alanine methyl ester,[10)] failed due to the easy cyclization of the (iminoprolyl)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 imidate 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 B_1, Z–Tle–OH[7b)] (**1**) was coupled with H–Val–Pro–NH_2



Scheme 1. Synthesis of bottromycin B_1.

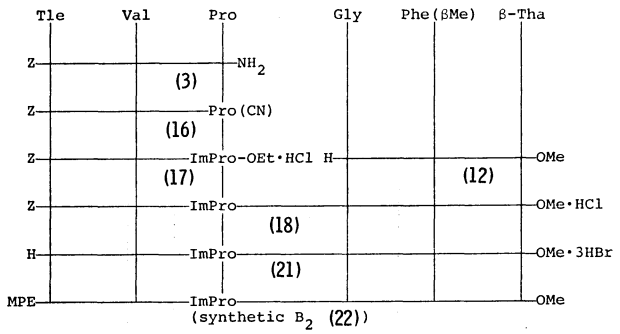


Bottromycins

	A_1	A_2	B_1	B_2	C_2
X	Piv	MPE	Piv	MPE	MPE
Y	CH_3	CH_3	H	H	a)

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 B_2.

(**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 debenzyloxycarbonylation 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(*β*Me)–*β*-Tha–OMe (**9**), followed by the debenzyloxycarbonylation 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 ester[10] (**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 B_1 as the monohydrochloride (**13**) in 50% yield. The free B_1 (**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 B_1 dihydrochloride (**14**).

Bottromycin B_2 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 B_1 hydrochloride (**13**). The compound (**18**) was debenzyloxycarbonylated 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 B_2 was obtained in 43% yield. Although no information about the geometry of the MPE group in bottromycins A_2 and B_2 had been given, we used the *trans*-MPE-Cl for the preparation of B_2 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 A_2, which was published later.[1c] The revised structure was quite different from the old one.[3] Though nothing was mentioned about bottromycins A_1 and B_1, 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 A_2.[13]

The coincidence of the structures of the synthetic bottromycins B_1 and B_2 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 ones[13] in many respects: the IR spectra of the natural B_1 and the synthetic B_1 did not coincide with each other, though those of the synthetic B_1 and B_2 very much resemble each other and those of the natural B_1 and A_2 also resemble each other. It should be pointed out that the synthetic B_2 had an absorption band assigned to C–H bending deformation of *trans* disubstituted C=C at 980 cm^{-1}, while the natural A_2 had not absorption band in the same region. (Fig. 3) The R_f values (TLC, MeOH:AcOEt = 1:3) of the synthetic B_1 and B_2 were 0.37 and 0.33, respectively, while that of the natural A_2 containing some B_2[13] was

Table 1. Analytical data of synthetic bottromycins

Bottromycin	$[\alpha]_D$ (MeOH)	Molecular formula	Found (Calcd) (%) C	H	N
Synthetic B_1 (**15**)	−54.1°[a]	$C_{40}H_{60}N_8O_7S\cdot H_2O$	58.80 (58.95)	7.61 (7.67)	12.87 (13.75)
Synthetic B_1·HCl (**13**)	−34.6°[b]	$C_{40}H_{60}N_8O_7S\cdot HCl\cdot 2H_2O$	55.41 (55.25)	7.41 (7.53)	12.18 (12.89)
Synthetic B_1·2HCl (**14**)	−34.2°[c]	$C_{40}H_{60}N_8O_7S\cdot 2HCl\cdot H_2O$	54.32 (54.11)	7.34 (7.26)	11.96 (12.62)
Synthetic B_2 (**22**)	−55.5°[d]	$C_{41}H_{60}N_8O_7S\cdot 2H_2O$	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 B_1 (**15**): Tle 1.06, Val 1.09, Pro 0.98, Gly 1, Phe(*β*Me) 1.04, *β*-Tha 0.77[e]
Synthetic B_2 (**22**): Tle 1.01, Val 1.11, Pro 1.06, Gly 1, Phe(*β*Me) 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 B_1), 0.06 (for B_2).

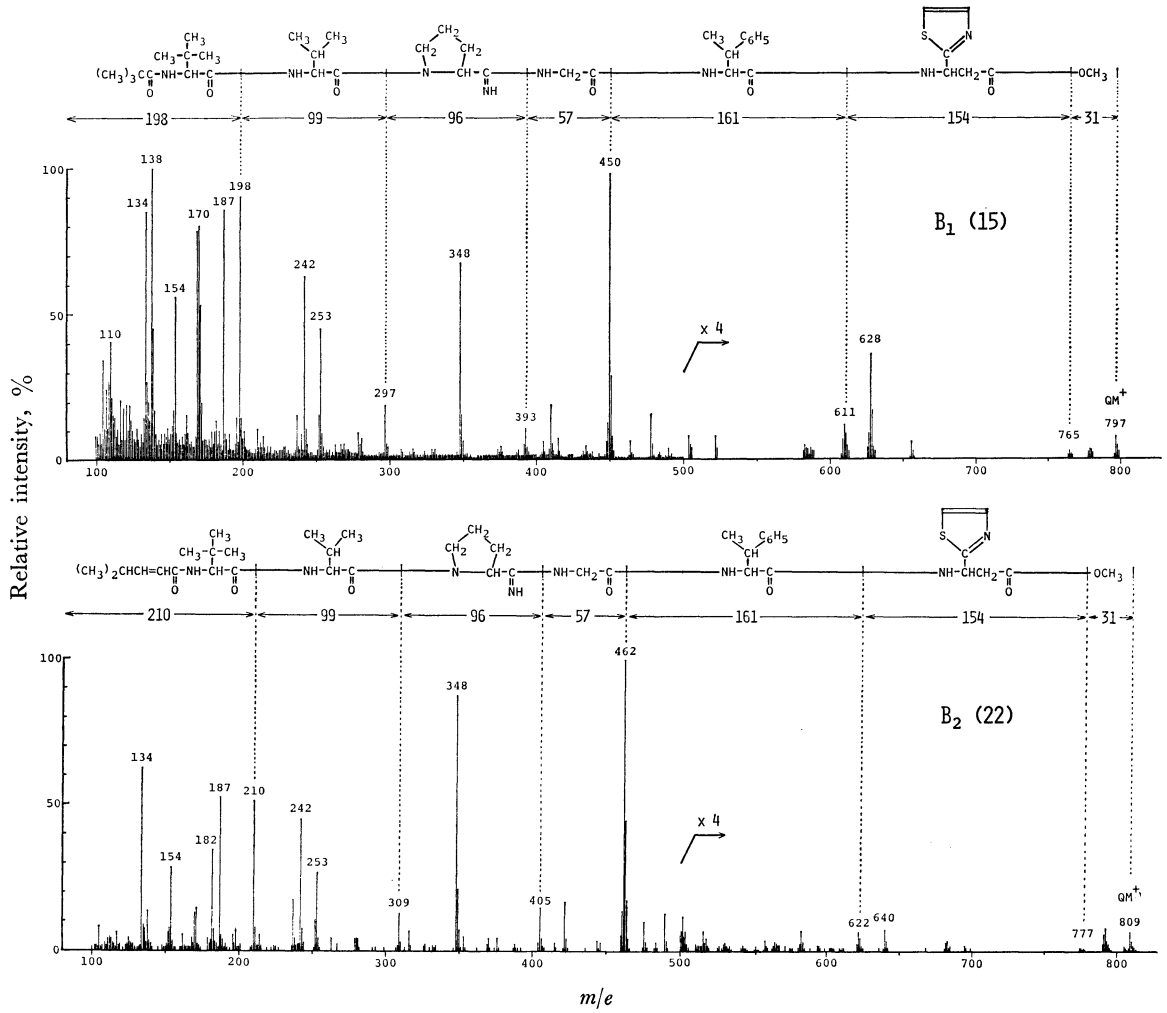


Fig. 2. CI(CH_4)-mass spectra of synthetic bottromycins B_1 (**15**) and B_2 (**22**).

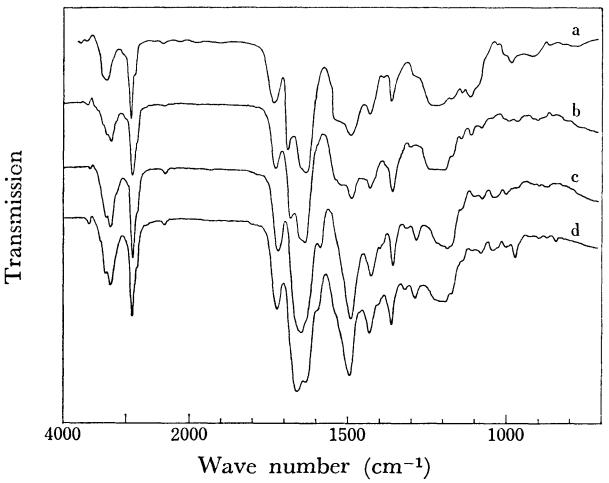


Fig. 3. IR spectra of natural and synthetic bottromycins in $CHCl_3$. a: Natural B_1 (This spectrum is reproduced from Fig. 3 in Ref. 19), b: natural A_2,[13] c: synthetic B_1 (**15**), d: synthetic B_2 (**22**).

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 synthetic 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 MeOH:H_2O = 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 bottromycin B_1 and natural A_2

Microorganism	MIC (μg/ml) Synthetic B_1	Natural A_2
Staphylococcus aureus 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
Staphylococcus epidermidis 10131 R	>50	≦0.005
Kawamura	>50	0.1
Streptococcus faecalis	>50	0.19
Urayama R	>50	0.19
Sarcina lutea PCI-1001	>50	0.1
Bacillus subtilis PCI-219	>50	≦0.05
Escherichia coli NIHJ	>50	50
K-12	>50	12.5
6065 R	>100	25
Salmonella typhi T-58	>100	>100
Salmonella enteritidis	>100	25
Shigella flexneri 2a	>100	25
Shigella sonnei	>100	25
Citrobacter freundii TU-786	>100	50
Enterobacter cloacae TL-14	>100	>100
Serratia marcescens OU-29	>100	>100
Klebsiella pneumoniae PCL-602	> 50	50
Proteus mirabilis 1698	> 50	>50
Proteus vulgaris IID-874	>100	>100
Pseudomonas aeruginosa PI-67	> 50	>50
No. 12	>100	50
No. 45	>100	>100
150	>100	50

Agar dilution method: Inoculumn size: 10^6 cells/ml. Medium: Heat infusion agar (pH 7.2). Cultivation: 18 h, 37 °C.

Chromatograph. TLC was done on Merck's Kieselgel GF_{254} (Type 60) or precoated Kieselgel 60 F_{254}. 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-OMe[15] derived from it without any purification; mp 250.5—251 °C (sublim.), $[\alpha]_D^{21}$ −10.8° (*c* 1, H_2O). Lit, $[\alpha]_D^{21}$ −9.4° (*c* 1, H_2O);[16] $[\alpha]_{546}^{20}$ −10.9° (*c* 2, H_2O);[9] $[\alpha]_D$ −8.3° (*c* 0.53, H_2O).[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%); $[\alpha]_D^{20}$ −5.9° (*c* 1, MeOH); $[\alpha]_D^{20}$ +4.0° (*c* 1, DMF). Lit,[17] $[\alpha]_D$ +3.8° (*c* 0.39, DMF) *Dicyclohexylammonium Salt*: Mp 165.5—166.5 °C, $[\alpha]_D^{20}$ −8.3° (*c* 1, MeOH). Lit,[17] mp 165—168 °C, $[\alpha]_D$ −8.4° (*c* 0.59, MeOH).

Z-Tle-Val-Pro-NH_2 (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-NH_2·HBr[9] (**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 $NaHCO_3$, and water again, and dried over $MgSO_4$. 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); $[\alpha]_D^{20}$ −88.7° (*c* 1, MeOH). Found: C, 61.97; H, 8.07; N, 12.18%. Calcd for $C_{24}H_{36}N_4O_5$: C, 62.59; H, 7.88; N, 12.16%.

H-Tle-Val-Pro-NH_2·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 $C_{16}H_{30}N_4O_3$·HBr·H_2O: C, 45.18; H, 7.82; N, 13.17%.

Piv-Tle-Val-Pro-NH_2 (5). Into a cold solution of **4** (522 mg, 1.28 mmol) and *N,N*-diisopropylethylamine (381 mg, 2.95 mmol) in CH_2Cl_2 (4 ml), pivaloyl chloride (201 mg, 1.67 mmol) in CH_2Cl_2 (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)piperazine (99 mg, 0.77 mmol) in CH_2Cl_2 (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 $NaHCO_3$, and water again, and dried over $MgSO_4$. 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); $[\alpha]_D^{20}$ −109.8° (*c* 1, MeOH). Found: C, 61.15; H, 9.45; N, 13.02%. Calcd for $C_{21}H_{38}N_4O_4$: 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), $POCl_3$ (0.14 ml, 1.44 mmol) in CH_2Cl_2 (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 $NaHCO_3$, and water, and dried over $MgSO_4$: Yield, 408 mg (86.6%); a foamy solid, $[\alpha]_D^{20}$ −118.2° (*c* 1, MeOH). Found: C, 63.80; H, 9.29; N, 13.94%. Calcd for $C_{21}H_{36}N_4O_3$: 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(βMe)–β-Tha–OMe·2HBr (**9**·2HBr) (prepared by the removal of the Z group from Z–Phe(βMe)–β-Tha–OMe[10] (**8**) (960 mg, 1.99 mmol) with 25% HBr in AcOH as usual) and Et_3N (450 mg, 4.46 mmol) in CH_2Cl_2 (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 $NaHCO_3$, and water again, and dried over Na_2SO_4. 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° (*c* 1, MeOH). Found: C, 60.32; H, 5.59; N, 10.25%. Calcd for $C_{27}H_{30}N_4O_6S$: C, 60.21; H, 5.61; N, 10.40%.

*H–Gly–Phe(βMe)–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 $C_{19}H_{24}N_4O_4S\cdot 2HBr\cdot H_2O$: C, 39.06; H, 4.83; N, 9.59%.

The free ester, H–Gly–Phe(βMe)–β-Tha–OMe (**12**), was prepared by the treatment of **11** with Et_3N (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(βMe)–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 Et_3N (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 $CHCl_3$–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–H_2O (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 $POCl_3$ (0.14 ml, 1.44 mmol) in a mixture of dry pyridine (2.5 ml) and CH_2Cl_2 (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° (*c* 1, MeOH). Found: C, 64.78; H, 7.97; N, 12.73%. Calcd for $C_{24}H_{34}N_4O_4$: 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(βMe)–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 Et_3N (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 $CHCl_3$–MeOH–AcOH (95 : 15 : 3) to give a foamy solid: Yield, 229 mg (36.7%); $[\alpha]_D^{20}$ −21.2° (*c* 0.5, MeOH). Found: C, 58.10; H, 6.85; N, 12.19%. Calcd for $C_{43}H_{58}N_8O_8S\cdot HCl\cdot 1/2H_2O$: 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_D^{19} 1.4469 (lit,[18] bp 115—116 °C/20 Torr, $n_D^{20.6}$ 1.4489); IR(neat): 985 cm^{-1} (σ C–H of *trans*-disubstituted olefin); NMR($CDCl_3$): δ=1.09 (6H, d, *J*=7 Hz, $(CH_3)_2CH$–), 2.52 (1H, o, *J*=7 Hz, $(CH_3)_2CHCH$=), 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 $SOCl_2$ as usual:[18] Yield, 73%; bp 65—66 °C/25 Torr (lit,[18] bp 58—59 °C/18 Torr); NMR($CDCl_3$): δ=1.12 (6H, d, *J*=7 Hz, $(CH_3)_2CH$–), 2.58 (1H, o, *J*=7 Hz, $(CH_3)_2CHCH$=), 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(βMe)–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 trituated and throughly 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 throughly removed by repeated concentrations under reduced pressure after each addition of benzene. The residual syrup was chromatographed on preparative layers of silica gel with $CHCl_3$–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.

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education (14703, 1976). We thank Drs. Hamao Umezawa, Tomohisa Takita, and Hiroshi Naganawa of the Institute of Microbial Chemistry, for measuring the mass spectra and for supplying bottromycin A_2, and Dr. Ichiro Chibata and his co-workers at Tanabe Seiyaku Co., Ltd., for the amino acid analyses and the microbiological assays.

References

1) a) This work was presented at the 14th Symposium on Peptide Chemistry (Japan), Hiroshima, November, 1976; T. Yamada, T. Miyazawa, S. Kuwata, and H. Watanabe, Proceedings of the 14th Symposium on Petide Chemistry, Japan (1976), p. 113; b) T. Takita, private communication

(August, 1976); c) Y. Takahashi, H. Naganawa, T. Takita, H. Umezawa, and S. Nakamura, *J. Antibiot.*, **29**, 1120 (1976); this was also presented at the 14th Symposium on Peptide Chemistry (Japan), Hiroshima, November, 1976 (Y. Takahashi, H. Naganawa, T. Takita, H. Umezawa, and S. Nakamura, Proceedings of the 14th Symposium on Peptide Chemistry, Japan (1976), p. 117), at the same time as our study.

2) J. M. Waisvisz, M. G. van der Hoeven, J. van Peppen, and W. C. M. Zwennis, *J. Am. Chem. Soc.*, **79**, 4520 (1975); J. M. Waisvisz, M. G. van der Hoeven, J. F. Hölscher, and B. te Nijenhuis, *ibid.*, **79**, 4522 (1957); J. M. Waisvisz, M. G. van der Hoeven, and B. te Nijenhuis, *ibid.*, **79**, 4524 (1957); J. M. Waisvisz and M. G. van der Hoeven, *ibid.*, **80**, 383 (1958).

3) a) S. Nakamura, T. Chikaike, K. Karasawa, N. Tanaka, H. Yonehara, and H. Umezawa, *J. Antibiot.*, **18A**, 47 (1965); b) S. Nakamura, T. Chikaike, H. Yonehara, and H. Umezawa, *ibid.*, **18A**, 60 (1965); S. Nakamura, T. Chikaike, H. Yonehara, and H. Umezawa, *Chem. Pharm. Bull.*, **13**, 599 (1965); c) S. Nakamura, N. Tanaka, and H. Umezawa, *J. Antibiot.*, **19A**, 10 (1966); S. Nakamura and H. Umezawa, *Chem. Pharm. Bull.*, **14**, 981 (1966); d) S. Nakamura, T. Yajima, Y. C. Lin, and H. Umezawa, *J. Antibiot.*, **20A**, 1 (1967).

4) W. J. Miller, L. Chaiet, G. Rasmussen, B. Christensen, J. Hannah, A. K. Miller, and F. J. Wolf, *J. Med. Chem.*, **11**, 746 (1968).

5) H. Arold, M. Eule, and S. Reissmann, *Z. Chem.*, **9**, 447 (1969).

6) Y. Seto, K. Torii, K. Bori, K. Inabata, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Jpn.*, **47**, 151 (1974).

7) a) An iminopeptide bond means a $-\overset{\overset{\displaystyle NH}{\|}}{C}-NH-$ group 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(βMe), *erythro*-β-methylphenylalanine; β-Tha, β-(2-thiazolyl)-β-alanine; Pro(CN), 2-cyanopyrrolidine; ImPro, iminoproline;[7a] Bu^t, *t*-butyl; Pr^i, isopropyl; Z, benzyloxycarbonyl; 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.

8) T. Yamada, K. Suegane, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Jpn.*, **50**, 1088 (1977).

9) W. Steglich, E. Frauendorfer, and F. Weygand, *Chem. Ber.*, **104**, 687 (1971).

10) Y. Kataoka, Y. Seto, M. Yamamoto, T. Yamada, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Jpn.*, **49**, 1081 (1976).

11) T. Yamada, T. Miyazawa, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Jpn.*, **50**, 1827 (1977).

12) R. Roger and D. G. Neilson, *Chem. Rev.*, **61**, 193 (1961); S. Patai, Ed., "The Chemistry of Amidines and Imidates," John Wiley & Sons, London (1975), p. 389.

13) Throughout this investigation, we were forced to use only bottromycin A_2 containing 5% of B_2 provided by Dr. T. Takita, as a natural standard sample, because neither Umezawa's group nor Wolf's group has any A_1 and B_1 (private communications from Dr. T. Takita (September, 1976) and from Dr. F. J. Wolf (September, 1976)), and they are the only two groups who have reported that they could isolate bottromycins.

14) Although this sample of the natural A_2 contained some B_2,[13] TLC showed only one spot. This is understand able because bottromycin B_2 has been reported to behave like A_2 in TLC: *e.g.*, R_f values of A_2 and B_2 were 0.81 and 0.80 (BuOH : AcOH : H_2O = 100 : 12 : 100), respectively.[3d]

15) E. Frauendorfer, W. Steglich, and F. Weygand, *Chem. Ber.*, **106**, 1019 (1973).

16) H. Pracejus and S. Winter, *Chem. Ber.*, **97**, 3173 (1964).

17) J. Pospíšec and K. Bláha, *Collect. Czech. Chem. Commun.*, **42**, 1069 (1977).

18) R. P. Linstead, *J. Chem. Soc.*, **1929**, 2498.

19) Y. Sumiki, "Koseibusshitsu," Supplement I, Tokyo Daigaku Shuppankai, Tokyo (1970), p. 325. See also Ref. 3c.

20) The configuration of *t*-leucine isolated from bottromycin has been assigned to the L-series by Nakamura *et al.*,[3b] although the compound showed the positive specific rotation, $[\alpha]_D^{22}$ +4° (*c* 1, H_2O), contrary to L-*t*-leucine prepared by us or all others.