Studies of Unusual Amino Acids and Their Peptides. VIII. The Syntheses of an Iminohexapeptide as a Model of Bottromycin and Its Related Iminopeptides[1]

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As a model of bottromycin, the structure of which had been proposed by Nakamura *et al.*, an iminohexapeptide, Z–Val–Val–ImPro–Gly–Phe–Phe–OMe, and its related iminopeptides were synthesized in good yields. These syntheses were achieved by the condensation of the imidates, Z–ImPro–OEt, Z–Val–ImPro–OEt·HCl, or Z–Val–Val–ImPro–OEt·HCl, with the peptide esters, Gly–Phe–OMe·HBr or Gly–Phe–Phe–OMe·HCl, in the presence of triethylamine. All of the resulting iminopeptides were isolated as the hydrobromide or the hydrochloride. The pK_a values of these iminopeptide salts were measured to be 9.75—9.3 in methanol–water; they showed that the pK_a rule of Nakamura *et al.* could not be applied in the cases of these iminopeptides.

In a previous paper,[2] we have reported on some properties and reactions of iminodipeptides and concluded that it is impossible to elongate the *C*-terminal of *N*-benzyloxycarbonyl(Z)-(iminoprolyl)glycine[3] because of the easy formation of an imidazolone derivative. This means that bottromycin (Fig. 1), the structure of which was proposed by Nakamura *et al.*,[1,4] could not be synthesized by the fragment condensation of the three already known dipeptide components; pivaloyl-*t*-leucylvaline,[5] (iminoprolyl)glycine,[2] and *β*-methylphenylalanyl-*β*-(2-thiazolyl)-*β*-alanine methyl ester.[6] Another possible route to build up bottromycin may be through the coupling of the two tripeptide components (pivaloyl-*t*-leucylvalyl(iminoproline) and glycyl-*β*-methylphenylalanyl-*β*-(2-thiazolyl)-*β*-alanine methyl ester) at the iminopeptide bond situated in the middle of this antibiotic molecule, because imidazolone formation could successfully be avoided by locating a carboxyl group (or an alkoxycarbonyl group) sufficiently apart from an imino group in the molecule—*e.g.*, the imidate (I) could be coupled with the dipeptide ester (II), affording the desired iminotripeptide (III)[2] (Scheme 1).

In order to ascertain the possibility of the latter method, as well as to reveal the properties of iminopeptides, we tried to synthesize an iminohexapeptide, Z–Val–Val–ImPro–Gly–Phe–Phe–OMe (XVII), as a model of bottromycin, and its related iminopeptides.

As the *N*-terminal fragments for this purpose, three sorts of imidates were prepared: ethyl *N*-Z-2-pyrrolidinecarboximidate (I),[2] ethyl *N*-(*N*-Z-valyl)-2-pyrrolidinecarboximidate hydrochloride (VII), and ethyl *N*-(*N*-Z-valylvalyl)-2-pyrrolidinecarboximidate hydrochloride (XI). The synthetic routes of these compounds are shown in Scheme 2. The imidates thus obtained were used for the next reaction without further purification.

As the *C*-terminal components, glycylphenylalanine methyl ester hydrobromide (II) and glycylphenylalanylphenylalanine methyl ester hydrochloride (XII) were used. Every coupling reaction was carried out by stirring a methanol solution of a free imidate, prepared from the hydrochloride just before the reaction, or an imidate hydrochloride itself, and a peptide ester salt in the presence of triethylamine at room temperature for 2 days. The products were isolated by chromatography on silica gel.

By the coupling of the imidate (I) with the dipeptide

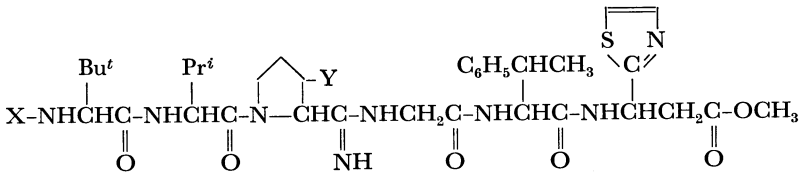
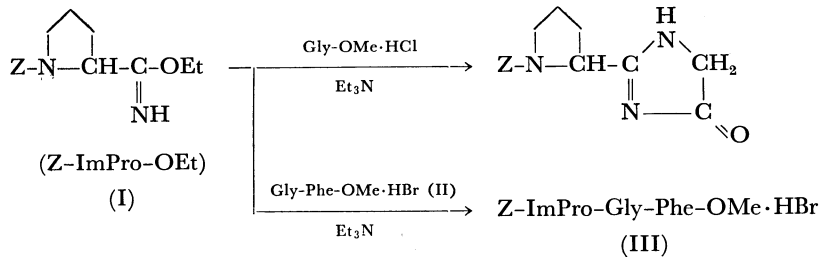


Fig. 1. The structure of bottromycin proposed by Nakamura *et al.*
X=pivaloyl or 4-methyl-2-pentenoyl; Y=H or CH_3;
Bu^t=*t*-butyl; Pr^i=isopropyl

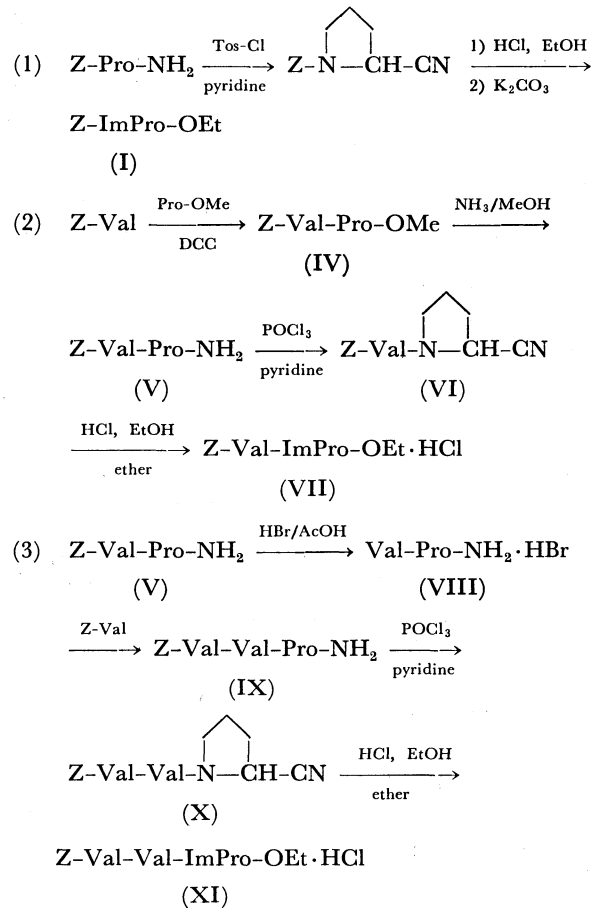


Scheme 1.

TABLE 1. SYNTHESES OF *N*-BENZYLOXYCARBONYL IMINOPEPTIDE HYDROHALIDES

Compd	Iminopeptide hydrohalides	Yield (%)	$[\alpha]_D^{20}$ (c 1, MeOH)	Molecular formula	Found (Calcd) (%) C	H	N
XIII	Z-ImPro-Gly-Phe-Phe-OMe·HCl	60	−17.0°	$C_{34}H_{39}N_5O_6 \cdot HCl \cdot H_2O$	61.26 (61.12)	6.45 (6.34)	10.11 (10.48)
XIV	Z-Val-ImPro-Gly-Phe-OMe·HBr	25[a)]	−40.9°	$C_{30}H_{39}N_5O_6 \cdot HBr \cdot 2H_2O$	52.22 (52.79)	6.58 (6.50)	10.13 (10.25)
XV	Z-Val-ImPro-Gly-Phe-OMe·HCl	36[a)]	−28.9°	$C_{30}H_{39}N_5O_6 \cdot HCl \cdot H_2O$	58.24 (58.10)	7.10 (6.83)	11.07 (11.29)
XVI	Z-Val-ImPro-Gly-Phe-Phe-OMe·HCl	59	−10.6°	$C_{39}H_{48}N_6O_7 \cdot HCl \cdot H_2O$	60.98 (61.05)	6.54 (6.70)	10.92 (10.95)
XVII	Z-Val-Val-ImPro-Gly-Phe-Phe-OMe·HCl	70	−35.7°	$C_{44}H_{57}N_7O_8 \cdot HCl \cdot H_2O$	61.30 (60.99)	7.35 (6.98)	10.80 (11.32)

a) Both compounds were isolated from the same reaction mixture. See Experimental part.



Scheme 2. Synthetic routes of the imidates of *N*-terminal fragments.

ester hydrobromide (II), the desired iminotripeptide derivative was obtained as a foamy solid; however, unexpectedly, it proved to be the hydrobromide instead of the free compound, as described previously.[2)] This was also the case for the coupling of the imidate (I) with the tripeptide ester hydrochloride (XII), which yielded the iminotetrapeptide hydrochloride (XIII). In the coupling of the imidate hydrochloride (VII) with the ester hydrobromide (II), the resulting iminotetrapeptide derivative was found to be a mixture of the hydrobromide (XIV) and the hydrochloride (XV), which could be separated chromatographically, though two equivalents of triethylamine were used. The coupling of the dipeptide imidate (VII) with the tripeptide ester (XII) was carried out by using both the hydrochloride in the presence of triethylamine, and the iminopentapeptide derivative was obtained as the hydrochloride (XVI).

Finally, the coupling of the tripeptide imidate hydrochloride (XI) with the tripeptide ester hydrochloride (XII) was attempted under the same conditions as above; the desired model compound (XVII) could be obtained in a 70% yield as the hydrochloride. The synthetic results of these iminopeptides are summarized in Table 1. Supported by this success, we decided to couple the two tripeptide fragments at the iminopeptide bond for the total synthesis of bottromycin. The results will be published later.[7)]

TABLE 2. pK_a VALUES OF IMINOPEPTIDES

	Iminopeptides	pK_a	(temp)
III	Z-ImPro-Gly-Phe-OMe·HBr	9.75[a)]	(22 °C)
XIII	Z-ImPro-Gly-Phe-Phe-OMe·HCl	9.65[b)]	(17 °C)
XIV	Z-Val-ImPro-Gly-Phe-OMe·HBr	9.4[a)]	(18 °C)
XVI	Z-Val-ImPro-Gly-Phe-Phe-OMe·HCl	9.45[a)]	(18 °C)
XVII	Z-Val-Val-ImPro-Gly-Phe-OMe·HCl	9.3[b)]	(18 °C)

a) MeOH-H_2O (1 : 1). b) MeOH-H_2O (3 : 2).

The iminopeptides seemed to be more basic than triethylamine, because all the iminopeptides were isolated as salts in spite of the presence of equimolecular triethylamine, as has been seen above. Therefore, we measured the pK_a values of these iminopeptides, thereby examining the validity of the pK_a rule used by Nakamura *et al.*[4b,8)] in order to elucidate the position of the imino group in the bottromycin molucule. The results are shown in Table 2. The basicities of these iminopeptides were unexpectedly found to be less than that of triethylamine (pK_a=10.4 in MeOH-H_2O (3 : 2)) but the differences were small, so that the unusual isolation of the iminopeptide salts could be explained by also considering the volatility of triethylamine. There also exsists a tendency, though not so marked, that the more inner the iminopeptide bond comes to, the smaller the pK_a value.

It should be pointed out that the pK_a rule of Nakamura *et al.* could not be applied in the cases of our iminopeptides, because the rule predicts that every pK_a value of them is 8.1—8.4. Furthermore, it is noteworthy thet the iminohexapeptide derivative (XVII), prepared here as a model of bottromycin, has a pK_a value of 9.3, in contrast with the 8.1—8.3 value[4b] of the antibiotic.

The antimicrobial activities of these iminopeptides against several microorganisms (gram-positive bacteria containing *Mycobacterium*, a negative one, and some fungi) were examined, but no activities were observed in any.

Experimental

All the melting points are uncorrected. The optical rotations were measured by means of a Yanagimoto polarimeter, OR-10. The pK_a values were measured by means of a Hitachi-Horiba pH meter, F-7. Thin-layer chromatography (TLC) was done on Merck's Kieselgel GF_{254} (Type 60), and circular paper chromatography, on Toyo Roshi No. 2.

Z-Val-Pro-OMe (IV). Into a cold mixture of Z-Val-OH (5.03 g, 20 mmol), H-Pro-OMe·HCl (3.31 g, 20 mmol), and Et_3N (2.02 g, 20 mmol) in CH_2Cl_2 (50 ml) and dioxane (20 ml), a solution of DCC (4.32 g, 21 mmol) in CH_2Cl_2 (10 ml) was stirred below −5 °C. The mixture was stirred at 0 °C for 3 h and then at room temperature overnight. The reaction mixture, treated as usual, gave a crude dipeptide as an oil, which was chromatographed on a silica gel column with benzene–AcOEt (4 : 1) to afford a colorless oil; yield, 5.81 g (80.1%); $[\alpha]_D^{20}$ −87.7° (*c*, 1 MeOH). Found: C, 62.77; H, 7.28; N, 7.64%. Calcd for $C_{19}H_{26}N_2O_5$: C, 62.96; H, 7.23; N, 7.73%.

Z-Val-Pro-NH$_2$ (V). A solution of IV (1.631 g, 4.5 mmol) in a saturated solution of NH_3 in MeOH (21.9%) (20 ml) was allowed to stand at room temperature for 5 days. The solution was then evaporated under reduced pressure to give a syrup, which afforded white crystals when treated with AcOEt; yield, 535 mg (34.2%); mp 131—132.5 °C, $[\alpha]_D^{20}$ −81.6° (*c* 1, MeOH).

When condensed, the filtrate gave a syrup (1.060 g) which was mainly composed of the starting material. The syrup was treated again with a saturated solution of NH_3 in MeOH (20 ml) at room temperature for 15 days, affording white crystals after the treatment described above; yield, 733 mg (46.9 %), mp 131—132 °C, $[\alpha]_D^{20}$ −81.3° (*c* 1, MeOH).

All the crystals obtained were combined and recrystallized from AcOEt–petroleum ether; mp 133—134 °C, $[\alpha]_D^{20}$ −84.3° (*c* 1, MeOH). Found: C, 62.02; H, 7.38; N, 11.92%. Calcd for $C_{18}H_{25}N_3O_4$: C, 62.23; H, 7.25; N, 12.01%.

N-(Z-Val)-2-cyanopyrrolidine (VI). To a stirred solution of V (1.390 g, 4 mmol) in dry pyridine (7 ml), $POCl_3$ (0.48 ml, 5.2 mmol) in CH_2Cl_2 (0.9 ml) was added, drop by drop, below −7 °C. The resulting solution was stirred at about −10 °C for 1 h, and then treated with ice (50 g) and extracted with AcOEt. The organic layer was successively washed with 1M-HCl, water, 1M-$NaHCO_3$ and water, and dried over $MgSO_4$; yield, 1.278 g (97%); slightly yellow oil, $[\alpha]_D^{20}$ −89.0° (*c* 1, MeOH). Found: C, 65.79; H, 7.23; N, 12.64%. Calcd for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76%.

Z-Val-ImPro-OEt·HCl (VII). This compound was prepared by bubbling dry HCl into a cold solution of VI (658 mg, 2 mmol) and absolute EtOH (120 mg, 2.6 mmol) in dry ether (10 ml) according to our previously described procedures;[2] yield, 820 mg (99.5%); a foamy solid. This imidate hydrochloride was used for the next reaction without purification.

H-Val-Pro-NH$_2$·HBr (VIII). This compound was prepared by the removal of the Z group from V (2.084 g, 6 mmol) with 25% HBr in AcOH (6 g) as usual. A crude product, a yellow syrup, was treated with MeOH–ether to give white crystals; yield, 1.632 g (92.5%); mp 214—217 °C (sublim.), $[\alpha]_D^{20}$ −45.6° (*c* 1, MeOH). Found: C, 40.71; H, 7.26, N, 14.01%. Calcd for $C_{10}H_{19}N_3O_2$·HBr: C, 40.83; H, 6.85; N, 14.28%.

Z-Val-Val-Pro-NH$_2$ (IX). Into a cold solution of Z-Val-OH (754 mg, 3 mmol) and *N*-methylmorpholine (304 mg, 3 mmol) in THF (10 ml), *i*Boc-Cl (410 mg, 3 mmol) was stirred, drop by drop, below −10 °C, after which the turbid mixture was stirred for 15 min at the same temperature. To the mixture we then added a mixture of VIII (883 mg, 3 mmol) and *N*-methylmorpholine (304 mg, 3 mmol) in DMF (10 ml) below −10 °C for 15 min, at about −5 °C for 1 h, and then at room temperature overnight. After the removal of a precipitate, the filtrate was concentrated under reduced pressure. The residual DMF solution was diluted with water (100 ml), extracted with AcOEt, washed with water, 1M-$NaHCO_3$, and water, and dried over $MgSO_4$. The solution was evaporated to a foamy solid; yield, 1.275 g (95.2%); $[\alpha]_D^{20}$ −94.5° (*c* 1, MeOH). Found: C, 61.61; H, 7.72; N, 12.45%. Calcd for $C_{23}H_{34}N_4O_5$: C, 61.86; H, 7.68; N, 12.55%.

N-(Z-Val-Val)-2-cyanopyrrolidine (X). This compound was prepared by the dehydration of IX (1.340 g, 3 mmol) with $POCl_3$ (0.36 ml, 3.9 mmol) in a mixture of dry pyridine (5 ml) and CH_2Cl_2 (0.7 ml) below −10 °C, as has been described above for the preparation of VI; yield, 1.131 g (88%); a foamy solid, $[\alpha]_D^{20}$ −103.0° (*c* 1, MeOH). Found: C, 64.26; H, 7.56; N, 13.19%. Calcd for $C_{23}H_{32}N_4O_4$: C, 64.46; H, 7.53; N, 13.08%.

Z-Val-Val-ImPro-OEt·HCl (XI). This compound was prepared by passing dry HCl into a mixture of X (900 mg, 2.1 mmol) and absolute EtOH (126 mg, 2.73 mmol) in dry ether as has been described above; yield, 1.002 g (93.4%); a foamy solid.

H-Gly-Phe-Phe-OMe·HCl (XII). This compound was prepared by the coupling of Z-Gly-ONp[9] with H-Phe-Phe-OMe in DMF according to the method of Katsoyannis *et al.*,[10] followed by the hydrogenation of the resulting Z-tripeptide ester (5.17 g, 10 mmol) over 5% palladium-carbon (1.5 g) in MeOH (200 ml) containing concentrated hydrochloric acid (0.9 ml); yield, 3.75 g (76.5% based on Z-Gly-ONp); mp 190—192.5 °C (dec), $[\alpha]_D^{20}$ +8.4° (*c* 1, MeOH). Found: C, 59.49; H, 6.28; N, 9.48%. Calcd for $C_{21}H_{25}N_3O_4$·HCl: C, 60.07; H, 6.24; N, 10.01%.

Iminopeptides. a) General Procedure: A solution of an imidate hydrochloride (1.2 mmol), a peptide ester hydrochloride (or hydrobromide) (1.0 mmol), and Et_3N (2.2 mmol) in dry MeOH (5 ml) was stirred at room temperature for 2 days. The solution was then evaporated under reduced pressure, leaving a syrup with some crystals. The syrup was taken up in AcOEt and freed from any insoluble materials by filtration. The filtrate was chromatographed on a silica gel column with MeOH–AcOEt (1 : 4), or on preparative layers of silica gel with MeOH–AcOEt (1 : 4) or $CHCl_3$–MeOH–AcOH (95 : 15 : 3), to give a foamy solid. The results are summarized in Table 1.

b) The Hydrobromide (XIV) and the Hydrochloride (XV) of Z-Val-ImPro-Gly-OMe: A solution of the imidate hydrochloride (VII) (820 mg, 2.0 mmol), the dipeptide ester hydrobromide (II)[2] (634 mg, 2.0 mmol), and Et_3N (404 mg, 4.0 mmol) in MeOH (10 ml) was stirred at room temperature

for 2 days. After the procedure described above, the resulting foamy solid was chromatographed on a silica gel column with MeOH–AcOEt (1 : 9) eluting one compound (A: 336 mg), and then with MeOH eluting the other compound (B: 430 mg); TLC: Compound A, R_f 0.37, Compound B, R_f 0.23 (MeOH–AcOEt (1 : 4)). Circular paper chromatography (1-BuOH–AcOH–H_2O (4 : 1 : 2, upper phase)) of the hydrolysates of these compounds showed both to contain 4 amino acids, and elemental analyses revealed that Compound A is iminotetrapeptide hydrobromide (XIV) and Compound B is the hydrochloride (XV).

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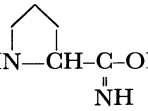
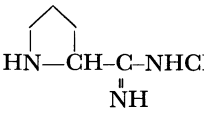
1) This work was presented at the 34th National Meeting of the Chemical Society of Japan, Hiratsuka, April, 1976, Preprint II, p. 699. In the course of the preparation of this paper, a revised study of bottromycin A_2 was published (Y. Takahashi, H. Naganawa, T. Takita, H. Umezawa, and S. Nakamura, *J. Antibiot.*, **29**, 1120 (1976)); it was reported 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 with our study.[7])

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

3) The following nomenclatures and abbreviations are used for iminopeptides and their derivatives. An amino acid containing =NH instead of =O in the carboxyl group is called an imino amino acid and abbreviated as ImAA (AA=amino acid); *e.g.*,  is called iminoproline (ImPro). Therefore,  is called (iminoprolyl)glycine (ImPro–Gly), and ethyl *N*-Z-2-pyrrolidinecarboximidate (I) may be abbreviated as Z–ImPro–OEt. Further, an iminopeptide bond means an amidino group (–C(=NH)–NH–) situated between amino acid residues. In addition, abbreviations according to the IUPAC-IUB Commission (*J. Biol. Chem.*, **247**, 977 (1972)) are used throughout. Additional abbreviations: DCC, dicyclohexylcarbodiimide; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; BuOH, 1-butanol; *i*Boc–Cl, isobutyloxycarbonyl chloride; Tos–Cl, *p*-toluenesulfonyl chloride. The amino acids and their derivatives used here are all of the L-configuration.

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7) Our synthetic study of bottromycins B_1 and B_2 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 Peptide Chemistry, Japan (1976), p. 113.

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