Studies of Bitter Peptides from Casein Hydrolyzate. IV.[1] Relationship between Bitterness and Hydrophobic Amino Acids Moiety in the C-Terminal of BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val)[2]

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(Received June 15, 1983)

In the synthetic studies of bitter peptide BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val), we synthesized several BPIa analogs to elucidate the participation of the hydrophobic amino acids moiety in the C-terminal in the bitter taste exhibited by BPIa. The syntheses of the peptides were performed by the usual MA (mixed anhydride) and DCC (dicyclohexylcarbodiimide)-HOBt (1-hydroxybenzotriazole) method,[3] and the bitterness of the peptides was organoleptically determined. The BPIa analogs in which more than two hydrophobic amino acids were located in the C-terminal exhibited a bitter taste as strong as that of BPIa.

In 1972, Minamiura *et al.*[4] isolated a bitter heptapeptide named BPIa from casein hydrolyzate by alkaline proteinase of *Bacillus subtilis*. They decided its amino acid sequence as follows: Arg-Gly-Pro-Pro-Phe-Ile-Val. In our laboratory, the authors have investigated the relationship between the bitterness and the chemical structure of BPIa. In a previous paper,[5] we explained that at least two hydrophobic amino acid residues in the C-terminal group are necessary for the intense bitter taste exhibited by BPIa. However, we did not deal with the relationship between the bitterness and the position of the hydrophobic amino acids in the C-terminal group of BPIa in that paper. In order to elucidate this, we synthesized several C-terminal analogs of BPIa, as shown in the Table.

Figure 1 indicates the route for the synthesis of the compound **1** (Arg-Gly-Pro-Pro-Gly-Gly-Val). *N*-(*t*-Butoxycarbonyl)glycylglycylvaline benzyl ester (**12**) was prepared by the reaction of *N*-(*t*-butoxycarbonyl)glycylglycine with valine benzyl ester by means of a mixed anhydride method. The subsequent treatment of **12** with hydrogen chloride in dioxane gave the tripeptide benzyl ester hydrochloride (**13**). *N*-(*t*-Butoxycarbonyl)prolylproline and **13** were coupled by the mixed anhydride method to yield *N*-(*t*-butoxycarbonyl)prolylprolylglycylglycylvaline benzyl ester (**14**). After treatment of **14** with hydrogen chloride, the pentapeptide benzyl ester hydrochloride (**15**) and N^{α}-benzyloxycarbonyl-N^{G}-nitroarginylglycine were coupled by the mixed anhydride method to yield N^{α}-benzyloxycarbonyl-N^{G}-nitroarginylglycylprolylprolylglycylglycylvaline benzyl ester (**16**). The protected heptapeptide was hydrogenated in the presence of palladium black to give the desired heptapeptide (H-Arg-Gly-Pro-Pro-Gly-Gly-Val-OH) (**1**).

Figure 2 indicates the route for the synthesis of the compound **2** (Arg-Gly-Pro-Pro-Gly-Ile-Gly). *N*-(*t*-Butoxycarbonyl)isoleucine and glycine benzyl ester were coupled by the mixed anhydride method to

TABLE. THE THRESHOLD VALUE FOR BITTER TASTE OF THE SYNTHETIC PEPTIDES

Compound	T.V./mM	$R_{caf.}$[b]
1. Arg-Gly-Pro-Pro-Gly-Gly-Val	3.30	0.3
2. Arg-Gly-Pro-Pro-Gly-Ile-Gly	1.70	0.6
3. Arg-Gly-Pro-Pro-Phe-Gly-Gly	0.60	1.7
4. Arg-Gly-Pro-Pro-Gly-Phe-Gly	0.21	4.8
5. Arg-Gly-Pro-Pro-Gly-Gly-Phe	0.82	1.2
6. Arg-Gly-Pro-Pro-Phe-Phe	0.06	17.0
7. Arg-Gly-Pro-Pro-Gly-Phe-Phe	0.04	25.0
8. Arg-Gly-Pro-Pro-Gly-Gly-Phe-Phe	0.08	13.0
9. Arg-Gly-Pro-Pro-Gly-Gly-Gly-Phe-Phe	0.11	9.0
10. Arg-Gly-Pro-Pro-Phe-Phe-Phe	0.01	100.0
11. Arg-Gly-Pro-Pro-Phe-Ile-Val[a]	0.05	20.0

a) BPIa, isolated by Minamiura *et al.* b) Ratio of caffeine.

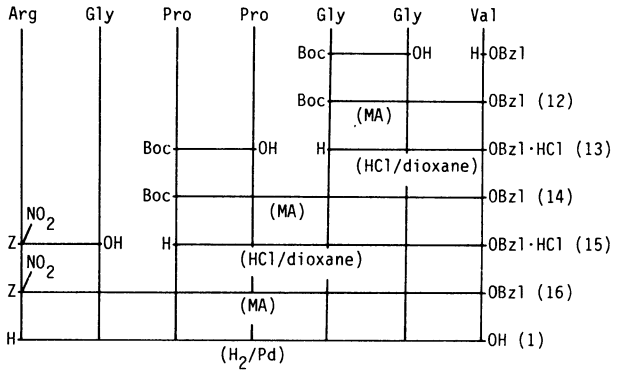

Fig. 1. Synthesis of compound **1**.

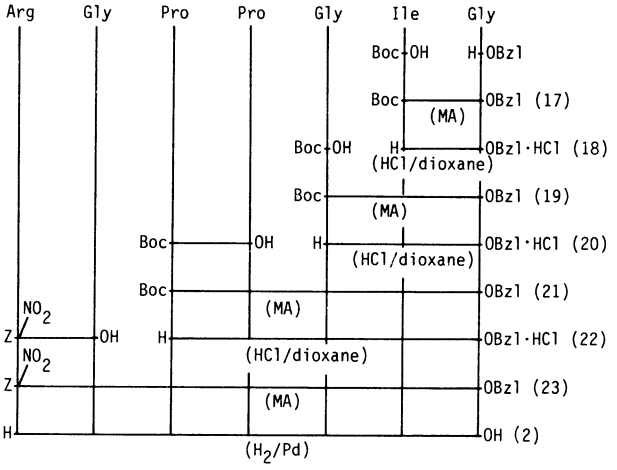

Fig. 2. Synthesis of compound **2**.

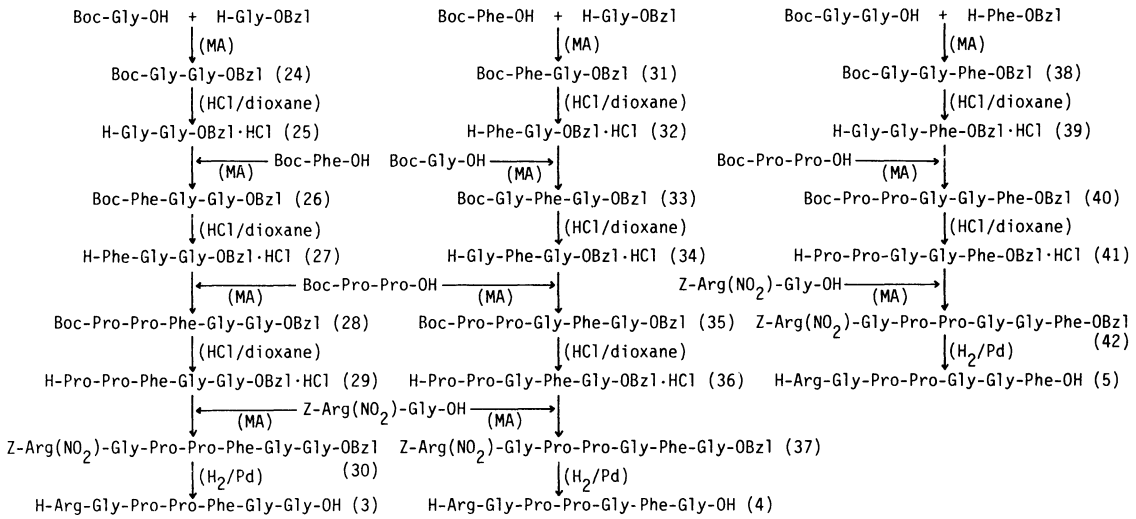

Fig. 3. Syntheses of compounds **3**, **4**, and **5**.

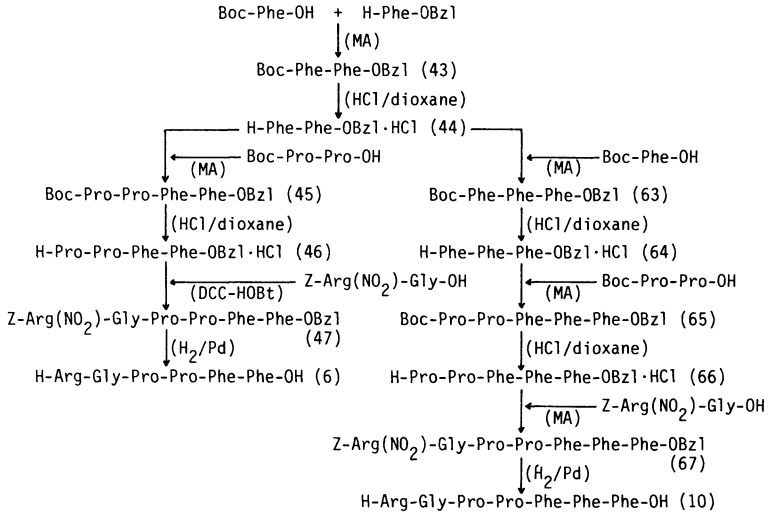

Fig. 4. Syntheses of compounds **6** and **10**.

yield *N*-(*t*-butoxycabonyl)isoleucylglycine benzyl ester (**17**). The subsequent treatment of **17** with hydrogen chloride in dioxane gave the dipeptide ester hydrochloride (**18**). The reaction of *N*-(*t*-butoxycarbonyl)glycine and **18** gave the tripeptide derivative (**19**). After treatment of **19** with hydrogen chloride in dioxane, the tripeptide benzyl ester hydrochloride (**20**) and *N*-(*t*-butoxycarbonyl)prolylproline were coupled by the mixed anhydride method to yield *N*-(*t*-butoxycarbonyl)prolylprolylglycylisoleucylglycine benzyl ester (**21**). The removal of the *t*-butoxycarbonyl group from **21** with hydrogen chloride in dioxane afforded the corresponding pentapeptide benzyl ester hydrochloride (**22**). N^{α}-Benzyloxycarbonyl-N^{G}-nitroarginylglycine and **22** were coupled by the mixed anhydride method to yield N^{α}-benzyloxycarbonyl-N^{G}-nitroarginylglycylprolylprolylglycylisoleucylglycine benzyl ester (**23**). The protected heptapeptide was hydrogenated in the presence of palladium black to give the desired compound (H-Arg-Gly-Pro-Pro-Gly-Ile-Gly-OH) (**2**).

The details of the synthetic routes to other peptides (compounds **3**—**10**) are shown in Figs. 3—5, and are described in the Experimental part. The purity of the synthetic peptides and their intermediates was confirmed by thin-layer examinations in two solvent systems, elemental analyses, and amino acid analyses.

The bitterness of the synthetic peptides was organoleptically determined by a panel evaluation of five people (see Table). Compounds **1**—**3** were synthesized to confirm the participation of the hydrophobic moiety in the bitter taste exhibition; bitterness of these compounds was weaker than that of BPIa. Compounds **3**, **4**, and **5** in which an L-phenylalanine residue is located at 5, 6, and 7 position[6] respectively, also exhibited a weak bitter taste. On the other hand, the bitterness of compounds **6**—**9**, in which two L-phenylalanine residues are located in the C-terminal, was on the same level as that of BPIa.

We treated the relationship between bitterness and the position of the hydrophobic amino acid residue. The bitterness of compound **4**, in which an L-phenylalanine residue locates at δ position, was stronger than that of compounds **3** and **5**, which contain the hydrophobic amino acid residue at 5 and 7 posi-

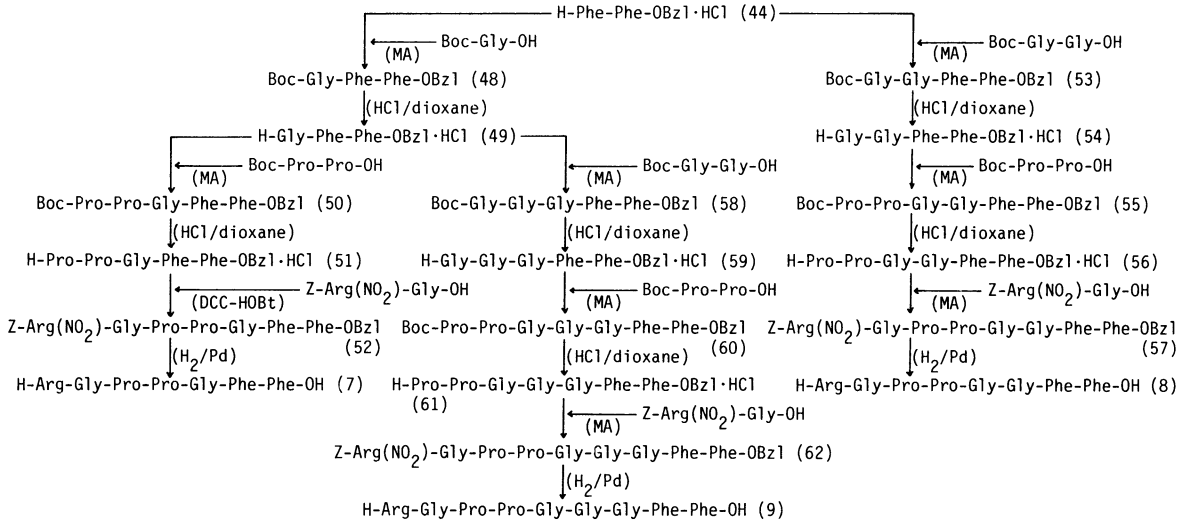

Fig. 5. Syntheses of compounds **7**, **8**, and **9**.

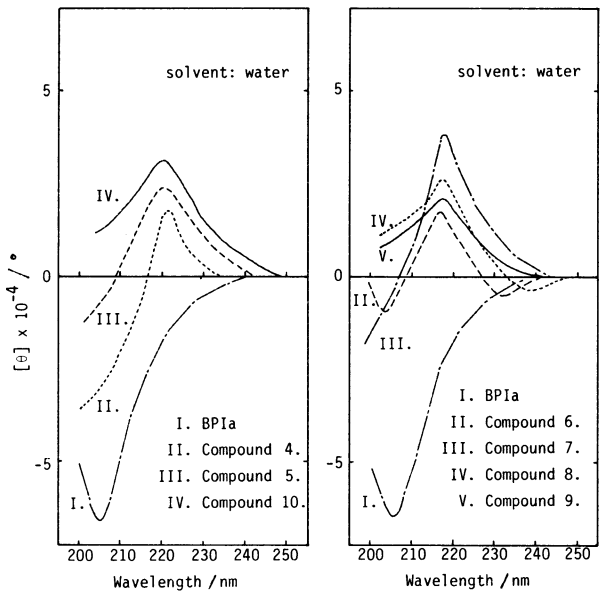

Fig. 6. CD curves of BPIa and its C-terminal analogs.

tion respectively. For compounds **6—9**, similar results obtained. In the previous paper,[5] we reported that the des-Gly[2]-BPIa, des-Pro[4]-BPIa, and des-Val[7]-BPIa, in which an L-hydrophobic amino acid residue is located at 6-position, exhibited a bitter taste as strong as that of BPIa.

The bitterness of compound **10**, in which three L-phenylalanine residues are located in the C-terminal, was five times stronger than that of BPIa. The result indicated that the strength of the bitterness increased with an increase of the hydrophobicity in the C-terminal. In conclusion, the presented data suggest that the hydrophobicity of the C-terminal moiety and the L-hydrophobic amino acid at 6-position were necessary for an intense bitter taste to be exhibited by BPIa.

The authors measured the circular dichroism (CD) of the synthetic peptides in this study, as shown in Fig. 6. In the previous papers,[1,5] the BPIa analogs that exhibited as strong and bitter a taste as BPIa have similarly shaped CD curves, with a negative trough at about 205 nm just as BPIa possesses. However, both the BPIa analogs in this study, the one which exhibited a strong bitter taste and the one with a weak bitter taste, have CD curves shaped oppositely to those of the BPIa analogs in the previous papers.[1,5] Further studies of this question are now in progress.

Experimental

All the melting points are uncorrected. Thin-layer chromatography (TLC) was carried out on Merck silica gel G with the following solvent systems: R_f^1, 1-butanol–acetic acid–pyridine–water (4:1:1:2, v/v); R_f^2, chloroform–methanol (5:1, v/v). Materials possessing a free amino group on a thin-layer plate were detected by spraying with ninhydrin. Compounds with blocked amino groups were detected by spraying with 25% hydrogen bromide in acetic acid and then with ninhydrin. The optical rotations were measured on a Union PM-101 polarimeter. Amino acid analyses were performed on a Hitachi amino acid analyzer KLA-5 type, after hydrolysis in a mixture of 6 M HCl (1 M=1 mol dm^{-3}) and propionic acid (1:1) at 110 °C for 72 h.

Boc-Gly-Gly-Val-OBzl (12). To a solution of Boc-Gly-Gly-OH[1] (2.32 g, 10 mmol) in DMF (20 ml) and NMM (1.1 ml, 10 mmol), ECF (1.0 ml, 10 mmol) was added at −5 °C. After 10 min, a solution of H-Val-OBzl·TsOH[7,8] (3.80 g, 10 mmol) and NMM (1.1 ml, 10 mmol) in chloroform (20 ml) was added. The reaction mixture was stirred in an ice bath for 1 h, then at room temperature overnight. The mixture was evaporated *in vacuo*, and the oily residue was dissolved in ethyl acetate. The solution was washed with water, 4% citric acid, 4% sodium hydrogencarbonate, and water successively, then dried over anhydrous sodium sulfate. Sodium sulfate was removed by filtration, and the filtrate was evaporated *in vacuo*. The oily residue was crystallized with ether–petroleum ether: yield 2.82 g (84%); mp 127—130 °C; $[\alpha]_D^{20}$ −21.0° (*c* 1, methanol); R_f^1 0.93 and R_f^2 0.67.

Found: C, 59.50; H, 7.43; N, 9.95%. Calcd for $C_{21}H_{31}O_6N_3$: C, 59.84; H, 7.41; N, 9.97%.

H-Gly-Gly-Val-OBzl·HCl (13). To a solution of compound **12** (2.40 g, 5.7 mmol) in dioxane (10 ml), 4 M HCl/dioxane (20 ml) was added. The reaction mixture was allowed to stand at room temperature. After 1.5 h, the solution was evaporated *in vacuo*. The residual oil was crystallized with ether: yield 1.82 g (89%); mp 205—206 °C; $[\alpha]_D^{20}$ −32.0° (*c* 1, methanol); R_f^1 0.80 and R_f^2 0.25.

Found: C, 53.16; H, 6.74; N, 11.63%. Calcd for $C_{16}H_{23}O_4N_3 \cdot HCl \cdot 1/4\ H_2O$: C, 53.03; H, 6.82; N, 11.60%.

Boc-Pro-Pro-Gly-Gly-Val-OBzl (14). Boc-Pro-Pro-OH[7,9] (1.25 g, 4 mmol) and compound **13** (1.43 g, 4 mmol) were coupled by the same method as described for the preparation of **12**: yield 2.12 g (86%); mp 73—76 °C; $[\alpha]_D^{20}$ −67.5° (*c* 1, methanol): R_f^1 0.82 and R_f^2 0.52.

Found: C, 59.16; H, 7.21; N, 10.72%. Calcd for $C_{31}H_{45}O_8N_5 \cdot H_2O$: C, 58.75; H, 7.47; N, 11.05%.

H-Pro-Pro-Gly-Gly-Val-OBzl·HCl (15). Compound **14** (2.11 g, 3.4 mmol) was treated as described in the case of **13**: yield 1.93 g (99%); mp 97—101 °C; $[\alpha]_D^{20}$ −50.0° (*c* 1, methanol); R_f^1 0.71 and R_f^2 0.55.

Found: C, 54.96; H, 6.87; N, 12.29%. Calcd for $C_{26}H_{37}O_6N_5 \cdot HCl \cdot H_2O$: C, 54.77; H, 7.07; N, 11.73%.

Z-Arg(NO$_2$)-Gly-Pro-Pro-Gly-Gly-Val-OBzl (16). Z-Arg(NO$_2$)-Gly-OH[7,10] (0.82 g 2 mmol) and compound **15** (1.10 g 2 mmol) were coupled by the same method as described for the preparation of **12**: yield 1.52 g (84%); mp 114—117 °C; $[\alpha]_D^{20}$ −69.0° (*c* 1, methanol); R_f^1 0.71 and R_f^2 0.55.

Found: C, 54.40; H, 6.30; N, 16.46%. Calcd for $C_{42}H_{57}O_{12}N_{11} \cdot H_2O$: C, 54.47; H, 6.42; N, 16.64%.

H-Arg-Gly-Pro-Pro-Gly-Gly-Val-OH (1). A solution of **16** (0.79 g, 1 mmol) in methanol (2 ml) and acetic acid (2 ml) was hydrogenated in the presence of palladium black at room temperature for 24 h. The catalyst was removed by filtration, then the filtrate was evaporated *in vacuo*. The residue was crystallized with acetone: yield 0.39 g (94%); mp 176—178 °C $[\alpha]_D^{20}$ −83.5° (*c* 1, H_2O); R_f^1 0.26 and R_f^2 0.00.

Found: C, 48.70; H, 7.20; N, 19.36%. Calcd for $C_{27}H_{46}O_8N_{10} \cdot H_2O \cdot CH_3COOH$: C, 48.59; H, 7.33; N, 19.55%.

Amino acid ratios in acid hydrolyzate: Arg 0.95, Gly 2.89, Pro 2.08, Val 1.00.

Boc-Ile-Gly-OBzl (17). Boc-Ile-OH·DCHA,[7,11] (13.62 g, 33 mmol) and H-Gly-OBzl·TsOH[12] (10.12 g, 30 mmol) were coupled by the same method as described for the preparation of **12**: yield 9.28 g (82%); mp 99—100 °C; $[\alpha]_D^{20}$ −22.5° (*c* 1, methanol); R_f^1 0.98 and R_f^2 0.71.

Found: C, 63.63; H, 8.09; N, 7.40%. Calcd for $C_{20}H_{30}O_5N_2$: C, 63.47; H, 7.99; N, 7.40%.

H-Ile-Gly-OBzl·HCl (18). Compound **17** (3.78 g, 10 mmol) was treated as described in the case of **13**: yield 2.96 g (94%); mp 52—56 °C; $[\alpha]_D^{20}$ +4.0° (*c* 1, methanol); R_f^1 0.78 and R_f^2 0.62.

Found: C, 56.48; H, 7.48; N, 8.79%. Calcd for $C_{15}H_{22}O_3N_2 \cdot HCl \cdot 1/4\ H_2O$: C, 56.42; H, 7.42; N, 8.77%.

Boc-Gly-Ile-Gly-OBzl (19). Boc-Gly-OH[13] (1.75 g, 10 mmol) and compound **18** (3.20 g, 10 mmol) were coupled by the same method as described for the preparation of **12**: yield 3.47 g (87%); mp 97—99 °C; $[\alpha]_D^{20}$ −28.5° (*c* 1, methanol); R_f^1 0.98 and R_f^2 0.43.

Found: C, 60.61; H, 7.69; N, 9.61%. Calcd for $C_{22}H_{33}O_6N_3$: C, 60.67; H, 7.64; N, 9.65%.

H-Gly-Ile-Gly-OBzl·HCl (20). Compound **19** (2.62 g, 6 mmol) was treated as described in the case of **13**: yield 2.21 g (99%); mp 185—187 °C; $[\alpha]_D^{20}$ −33.0° (*c* 1, methanol); R_f^1 0.77 and R_f^2 0.43.

Found: C, 53.25; H, 6.99; N, 11.19%. Calcd for $C_{17}H_{25}O_4N_3 \cdot HCl \cdot 2/3\ H_2O$: C, 53.18; H, 7.17; N, 10.95%.

Boc-Pro-Pro-Gly-Ile-Gly-OBzl (21). Boc-Pro-Pro-OH[7,9] (1.56 g, 5 mmol) and compound **20** (1.86 g, 5 mmol) were coupled by the same method as described for the preparation of **12**: yield 2.90 g (92%); 80—82 °C; $[\alpha]_D^{20}$ −63.0° (*c* 1, methanol); R_f^1 0.85 and R_f^2 0.50.

Found: C, 60.00; H, 7.52; 10.56%. Calcd for $C_{32}H_{47}O_8N_5 \cdot 3/4\ H_2O$: C, 59.75; H, 7.60; N, 10.89%.

H-Pro-Pro-Gly-Ile-Gly-OBzl·HCl (22). Compound **21** (2.79 g, 4.4 mmol) was treated as described in the case of **13**: yield 2.48 g (98%); mp 96—100 °C; $[\alpha]_D^{20}$ −62.0° (*c* 1, methanol); R_f^1 0.75 and R_f^2 0.40.

Found: C, 55.23; H, 6.97; N, 11.72%. Calcd for $C_{27}H_{39}O_6N_5 \cdot HCl \cdot H_2O$: C, 55.52; H, 7.08; N, 11.99%.

Z-Arg(NO$_2$)-Gly-Pro-Pro-Gly-Ile-Gly-OBzl (23). Z-Arg(NO$_2$)-Gly-OH[7,10] (0.82 g, 2 mmol) and compound **22** (1.14 g, 2 mmol) were coupled by the same method as described for the preparation of **12**: yield 0.78 g (85%); mp 114—117 °C; $[\alpha]_D^{20}$ −70.0° (*c* 1, methanol); R_f^1 0.85 and R_f^1 0.53.

Found: C, 55.20; H, 6.41; N, 16.34%. Calcd for $C_{43}H_{59}O_{12}N_{11} \cdot H_2O$: C, 54.94; H, 6.51 N, 16.39%.

H-Arg-Gly-Pro-Pro-Gly-Ile-Gly-OH (2). Compound **23** (0.92 g, 1 mmol) was treated as described in the case of **1**: yield 0.62 g (87%); mp 183—185 °C; $[\alpha]_D^{20}$ −116.0° (*c* 1, H_2O); R_f^1 0.20 and R_f^2 0.00.

Found: C, 50.47; H, 7.41; N, 19.80%. Calcd for $C_{28}H_{48}O_8N_{10} \cdot CH_3COOH$: C, 50.54; H, 7.35; N, 19.65%.

Amino acid ratios in acid hydrolyzate: Arg 0.93, Gly 3.01, Pro 1.97, Ile 1.00.

Boc-Gly-Gly-OBzl (24). Boc-Gly-OH[13] (5.26 g, 30 mmol) and H-Gly-OBzl·TsOH (10.12 g, 30 mmol) were coupled by the same method as described for the preparation of **12** : yield 8.89 g (92%); mp 84—85 °C; R_f^1 0.98 and R_f^2 0.76.

Found: C, 59.61; H, 6.91; N, 8.68%. Calcd for $C_{16}H_{22}O_5N_2$: C, 59.61; H, 6.88; N, 8.69%.

H-Gly-Gly-OBzl·HCl (25). Compound **24** (6.45 g, 20 mmol) was treated as described in the case of **13**: yield 4.93 g (95%); mp 158—160 °C (lit,[14] 160 °C) R_f^1 0.76 and R_f^2 0.26.

Found: C, 51.00; H, 5.87; N, 10.86%. Calcd for $C_{11}H_{14}O_3N_2 \cdot HCl$: C, 51.07; H, 5.84; N, 10.83.

Boc-Phe-Gly-Gly-OBzl (26). Boc-Phe-OH·DCHA[15] (4.91 g, 11 mmol) and compound **25** (2.59 g, 10 mmol) were coupled by the same method as described for the preparation of **13**: yield 4.01 g (85%); mp 100—102 °C; $[\alpha]_D^{20}$ +8.5° (*c* methanol); R_f^1 0.97 and R_f^2 0.75.

Found: C, 64.06; H, 6.71; N, 9.03%. Calcd for $C_{25}H_{31}O_6N_3$: C, 63.94; H, 6.66; N, 8.95%.

H-Phe-Gly-Gly-OBzl·HCl (27). Compound **26** (3.29 g, 7 mmol) was treated as described in the case of **13**: yield 2.69 g (92%); mp 60—63 °C; $[\alpha]_D^{20}$ −35.5° (*c* 1, methanol); R_f^1 0.80 and R_f^2 0.49.

Found: C, 55.42; H, 6.25; N, 9.98%. Calcd for $C_{20}H_{23}O_4N_3 \cdot HCl \cdot 3/2\ H_2O$: C, 55.48; H, 6.29; N, 9.71%.

Boc-Pro-Pro-Phe-Gly-Gly-OBzl (28). Boc-Pro-Pro-OH[7,9] (1.25 g, 4 mmol) and compound **27** (1.63 g, 4 mmol) were coupled by the same method as described for the preparation of **12**: yield 2.32 g (89%); mp 78—81 °C; $[\alpha]_D^{20}$ −73.0° (*c* 1, methanol); R_f^1 0.91 and R_f^2 0.50.

Found: C, 61.65; H, 6.86; N, 10.00%. Calcd for $C_{35}H_{45}O_8N_5 \cdot H_2O$: C, 61.66; H, 6.95; N, 10.27%.

H-Pro-Pro-Phe-Gly-Gly-OBzl·HCl (29). Compound **28** (2.77 g, 4.2 mmol) was treated as described in the case of **13**: yield 2.37 g (93%); mp 102—107 °C; $[\alpha]_D^{20}$ −68.5° (*c* 1, methanol); R_f^1 0.78 and R_f^2 0.42.

Found: C, 57.62; H, 6.33; N, 10.88%. Calcd for $C_{30}H_{37}O_6N_5 \cdot HCl \cdot 3/2\ H_2O$: C, 57.45; H, 6.59; N, 11.17%.

Z-Arg(NO$_2$)-Gly-Pro-Pro-Phe-Gly-Gly-OBzl (30). Z-Arg(NO$_2$)-Gly-OH[7,10] (0.82 g, 1 mmol) and compound

29 (1.20 g, 1 mmol) were coupled by the same method as described for the preparation of **12**: yield 0.70 g (72%); mp 114—117 °C; $[\alpha]_D^{20}$ −72.5° (*c* 1, methanol); R_f^1 0.86 and R_f^2 0.56.

Found: C, 56.88; H, 6.00; N, 15.79%. Calcd for $C_{46}H_{57}O_{12}N_{11}\cdot H_2O$: C, 56.71; H, 6.11; N, 15.81%.

H-Arg-Gly-Pro-Pro-Phe-Gly-Gly-OH (3). Compound **30** (0.96 g, 1 mmol) was treated as described in the case of **1**: yield 0.61 g (81%) mp 180 °C (decomp); $[\alpha]_D^{20}$ −106.0° (*c* 1, H_2O); R_f^1 0.21 and R_f 0.00.

Found: C, 51.98; H, 6.81; N, 17.95%. Calcd for $C_{31}H_{46}O_8N_{10}\cdot H_2O\cdot CH_3COOH$: C, 51.82; H, 6.87; N, 18.32%.

Amino acid ratios in acid hydrolyzate: Arg 0.92, Gly 3.00, Pro 1.99, Phe 1.00.

Boc-Phe-Gly-OBzl (31). Boc-Phe-OH·DCHA[15] (9.83 g, 22 mmol) and H-Gly-OBzl·TsOH[12] (6.75 g, 20 mmol) were coupled by the same method as described for the preparation of **12**: yield 7.76 g (94%); mp 134—135 °C; $[\alpha]_D^{20}$ −7.0° (*c* 1, methanol); R_f^1 0.94 and R_f^2 0.91.

Found: C, 66.99; H, 6.81; N, 6.84%. Calcd for $C_{23}H_{28}O_5N_2$: C, 66.96: H, 6.86; N, 6.79%.

H-Phe-Gly-OBzl·HCl (32). Compound **31** (6.18 g, 15 mmol) was treated as described in the case of **13**. This product was obtained in a hygroscopic form: yield 5.37 g (98%); R_f^1 0.72 and R_f^2 0.61.

Boc-Gly-Phe-Gly-OBzl (33). Boc-Gly-OH[13] (1.75 g, 10 mmol) and compound **32** (3.65 g, 10 mmol) were coupled by the same method as described for the preparation of **12**. This product was obtained in an oily form: yield 4.51 g (96%); R_f^1 0.96 and R_f^2 0.77.

H-Gly-Phe-Gly-OBzl·HCl (34). Compound **33** (4.51 g, 9.6 mmol) was treated as described in the case of **13**. This product was obtained in an oily form: yield 3.76 g (97%); R_f^1 0.70 and R_f^2 0.41.

Boc-Pro-Pro-Gly-Phe-Gly-OBzl (35). Boc-Pro-Pro-OH[7,9] (2.91 g, 9.33 mmol) and compound **34** (3.76 g, 9.33 mmol) were coupled by the same method as described for the preparation of **12**. This product was obtained in an oily form: yield 4.70 g (76%); R_f^1 0.85 and R_f^2 0.69.

H-Pro-Pro-Gly-Phe-Gly-OBzl·HCl (36). Compound **35** (4.71 g, 7.09 mmol) was treated as described in the case of **13**: yield 4.00 g (94%); mp 76—78 °C; $[\alpha]_D^{20}$ −36.5° (*c* 1, methanol); R_f^1 0.72 and R_f^2 0.33.

Found: C, 58.27; H, 6.60; N, 11.29%. Calcd for $C_{30}H_{37}O_6N_5\cdot HCl\cdot H_2O$: C, 58.28; H, 6.53; N, 11.33%.

Z-Arg(NO₂)-Gly-Pro-Pro-Gly-Phe-Gly-OBzl (37). Z-Arg(NO_2)-Gly-OH[7,10] (0.82 g, 2 mmol) and compound **36** (1.19 g, 2 mmol) were coupled by the same method as described for the preparation of **12**: yield 1.39 g (73%); mp 125—128 °C; $[\alpha]_D^{20}$ −77.0° (*c* 1, acetic acid); R_f^1 0.75 and R_f^2 0.53.

Found: C, 57.21; H, 6.11; N, 16.02%. Calcd for $C_{46}H_{57}O_{12}N_{11}\cdot 1/2\ H_2O$: C, 57.25; H, 6.07; N, 15.97%.

H-Arg-Gly-Pro-Pro-Gly-Phe-Gly-OH (4). Compound **37** (0.96 g, 1 mmol) was treated as described in the case of **1**: yield 0.59 g (79%); mp 87—89 °C; $[\alpha]_D^{20}$ −83.0° (*c* 1, H_2O); R_f^1 0.20 and R_f^2 0.00.

Found: C, 52.01; H, 6.89; N, 18.24%. Calcd for $C_{31}H_{46}O_8N_{10}\cdot H_2O\cdot CH_3COOH$: C, 51.82; H, 6.87; N, 18.32%.

Amino acid ratios in acid hydrolyzate: Arg 0.93, Gly 2.99, Pro 1.98, Phe 1.00.

Boc-Gly-Gly-Phe-OBzl (38). Boc-Gly-Gly-OH[1] (2.58 g, 8 mmol) and H-Phe-OBzl·TsOH[16,17] (3.42 g, 8 mmol) were coupled by the same method as described for the preparation of **12**. This product was obtained in an oily form: yield 2.59 g (69%); R_f^1 0.93 and R_f^2 0.69.

H-Gly-Gly-Phe-OBzl·HCl (39). Compound **38** (2.59 g, 5.49 mmol) was treated as described in the case of **13**: yield 2.15 g (97%); mp 122—123 °C; $[\alpha]_D^{20}$ −2.0° (*c* 1, methanol); R_f^1 0.67 and R_f^2 0.27.

Found: C, 56.61; H, 6.02; N, 9.87%. Calcd for $C_{20}H_{23}O_4N_3\cdot HCl\cdot H_2O$: C, 56.65; H, 5.96; N, 9.91%.

Boc-Pro-Pro-Gly-Gly-Phe-OBzl (40). Boc-Pro-Pro-OH[7,9] (1.56 g, 5 mmol) and compound **39** (2.02 g, 5 mmol) were coupled by the same method as described for the preparation of **12**. This product was obtained in a hygroscopic form: yield 2.13 g (64%); R_f^1 0.76 and R_f^2 0.65.

H-Pro-Pro-Gly-Gly-Phe-OBzl·HCl (41). Compound **40** (2.13 g, 3.2 mmol) was treated as described in the case of **13**: yield 1.80 g (94%); mp 94—97 °C; $[\alpha]_D^{20}$ −35.0° (*c* 1, methanol); R_f^1 0.68 and R_f^2 0.32.

Found: C, 59.08; H, 6.44; N, 11.59%. Calcd for $C_{30}H_{37}O_6N_5\cdot HCl\cdot 1/2\ H_2O$: C, 59.14; H, 6.47; N, 11.50%.

Z-Arg(NO₂)-Gly-Pro-Pro-Gly-Gly-Phe-OBzl (42). Z-Arg(NO_2)-Gly-OH[7,10] (0.82 g 2 mmol) and compound **41** (1.19 g, 2 mmol) were coupled by the same method as described for the preparation of **12**: yield 1.43 g (79%); mp 121—123 °C; $[\alpha]_D^{20}$ −63.0° (*c* 1, acetic acid); R_f^1 0.75 and R_f^2 0.53.

Found: C, 56.11; H, 6.13; N, 15.72%. Calcd for $C_{46}H_{57}O_{12}N_{11}\cdot 3/2\ H_2O$: C, 56.19; H, 6.16; N, 15.67%.

H-Arg-Gly-Pro-Pro-Gly-Gly-Phe-OH (5). Compound **42** (0.96 g, 1 mmol) was treated as described in the case of **1**: yield 0.63 g (84%); mp 124 °C; $[\alpha]_D^{20}$ −72.0° (*c* 1, H_2O); R_f^1 0.19 and R_f^2 0.00.

Found: C, 52.35; H, 6.85; N, 18.47%. Calcd for $C_{31}H_{46}O_8N_{10}\cdot 1/2\ H_2O\cdot CH_3COOH$: C, 52.43; H, 6.81; N, 18.53%.

Amino acid ratios in acid hydrolyzate: Arg 0.92, Gly 3.01, Pro 1.98, Phe 1.00.

Boc-Phe-Phe-OBzl (43). Boc-Phe-OH·DCHA[15] (14.74 g, 33 mmol) and H-Phe-OBzl·TsOH[16] (12.82 g, 30 mmol) were coupled by the same method as described for the preparation of **12**: yield 13.01 g (86%); mp 144—146 °C (lit,[17] 148—149 °C); $[\alpha]_D^{20}$ −6.0° (*c* 1, DMF); R_f^1 0.99 and R_f^2 0.95.

Found: C, 71.61; H, 6.87; N, 5.55%. Calcd for $C_{30}H_{34}O_5N_2$: C, 71.68; H, 6.83; N, 5.58%.

H-Phe-Phe-Bzl·HCl (44). Compound **43** (10.05 g, 20 mmol) was treated as described in the case of **13**: yield 7.89 g (83%); mp 155—157 °C; $[\alpha]_D^{20}$ +10° (*c* 1, DMF); R_f^1 0.87 and R_f^2 0.71.

Found: C, 63.15; H, 5.79; N, 6.01%. Calcd for $C_{25}H_{26}O_3N_2\cdot HCl$: C, 63.21; H, 5.74; N, 5.90%.

Boc-Pro-Pro-Phe-Phe-OBzl (45). Boc-Pro-Pro-OH[7,9] (1.56 g, 5 mmol) and compound **46** (2.37 g, 5 mmol) were coupled by the same method as described for the preparation of **12**: yield 3.03 g (87%); mp 130—131 °C; $[\alpha]_D^{20}$ −80.0° (*c* 1, methanol); R_f^1 0.98 and R_f^2 0.68.

Found: C, 69.20; H, 6.81; N, 7.99%. Calcd for $C_{40}H_{48}O_7N_4$: C, 68.93; H, 6.96; N, 8.04%.

H-Pro-Pro-Phe-Phe-OBzl·HCl (46). Compound **45** (2.09 g, 3 mmol) was treated as described in the case of **13**: yield 1.89 g (98%); mp 78—79 °C; $[\alpha]_D^{20}$ −79.0° (*c* 1, methanol); R_f^1 0.69 and R_f^2 0.35.

Found: C, 65.57; H, 6.71; N, 8.68%. Calcd for $C_{35}H_{40}O_5N_4\cdot HCl\cdot 1/2\ H_2O$: C, 65.46; H, 6.61; N, 8.73%.

Z-Arg(NO₂)-Gly-Pro-Pro-Phe-Phe-OBzl (47). To a solution of Z-Arg(NO_2)-Gly-OH[7,10] (0.82 g, 2 mmol) in DMF (10 ml), DCC (0.41 g, 2 mmol), and HOBt (0.27 g, 2 mmol) were added at 0 °C. After 20 min, a solution of compound **46** (1.27 g, 2 mmol) and NMM (0.22 ml, 2 mmol) in DMF (10 ml) was added to the mixture. The mixture was cooled at 0 °C for 1 h and allowed to stand overnight at room temperature. The DCUrea was removed by filtration, and the filtrate was evaporated *in vacuo*. The oily residue was dissolved in ethyl acetate and the solution was

washed with water, 2% hydrochloric acid, 4% sodium hydrogencarbonate, and water successively. The solution was dried over anhydrous sodium sulfate. Sodium sulfate was removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was crystallized with ether-petroleum ether: yield 1.07 g (54%); mp 115—117 °C; $[\alpha]_D^{20}$ −64.0° (*c* 1, acetic acid); R_f^1 0.69 and R_f^2 0.42.

Found: C, 60.35; H, 6.21; N, 13.68%. Calcd for $C_{51}H_{60}O_{11}N_{10}\cdot 3/2\ H_2O$: C, 60.27; H, 6.26; N, 13.79%.

H-Arg-Gly-Pro-Pro-Phe-Phe-OH (6). Compound **47** (0.79 g, 0.8 mmol) was treated as described in the case of **1**: yield 0.50 g (80%); mp 108—111 °C; $[\alpha]_D^{20}$ −73.0° (*c* 1, H_2O); R_f^1 0.25 and R_f^2 0.00.

Found: C, 54.91; H, 6.98; N, 14.21%. Calcd for $C_{36}H_{49}O_7N_9\cdot 2CH_3COOH\cdot 2H_2O$: C, 54.83; H, 7.03; N, 14.39%.

Amino acid ratios in acid hydrolyzate: Arg 0.93, Gly 1.01, Pro 1.98, Phe 2.00.

Boc-Gly-Phe-Phe-OBzl (48). Boc-Gly-OH[13] (1.75 g, 10 mmol) and compound **44** (4.75 g, 10 mmol) were coupled by the same method as described for the preparation of **12**: yield 5.26 g (94%); mp 129—130 °C; $[\alpha]_D^{20}$ −11.0° (*c* 1, methanol); R_f^1 0.89 and R_f^2 0.63.

Found: C, 68.74; H, 6.62; N, 7.55%. Calcd for $C_{32}H_{37}O_6N_3$: C, 68.67; H, 6.68; N, 7.51%.

H-Gly-Phe-Phe-OBzl·HCl (49). Compound **48** (4.48 g, 8 mmol) was treated as described in the case of **13**: yield 3.56 g (90%); mp 143—145 °C; $[\alpha]_D^{20}$ −2.0° (*c* 1, methanol); R_f^1 0.70 and R_f^2 0.63.

Found: C, 63.37; H, 6.25; N, 8.24%. Calcd for $C_{27}H_{29}O_4N_3\cdot HCl\cdot H_2O$: C, 63.44; H, 6.32; N, 8.22%.

Boc-Pro-Pro-Gly-Phe-Phe-OBzl (50). Boc-Pro-Pro-OH[7,9] (1.56 g, 5 mmol) and compound **49** (2.47 g, 5 mmol) were coupled by the same method as described for the preparation of **12**: yield 3.17 g (84%); mp 75—76 °C; $[\alpha]_D^{20}$ −54.0° (*c* 1, methanol); R_f^1 0.77 and R_f^2 0.46.

Found: C, 66.97; H, 6.75; N, 9.33%. Calcd for $C_{42}H_{51}O_8N_5$: C, 66.91; H, 6.83; N, 9.29%.

H-Pro-Pro-Gly-Phe-Phe-OBzl·HCl (51). Compound **50** (2.26 g, 3 mmol) was treated as described in the case of **13**: yield 1.90 g (92%); mp 95—97 °C; $[\alpha]_D^{20}$ −44.0° (*c* 1, methanol); R_f^1 0.46 and R_f^2 0.30.

Found: C, 62.83; H, 6.42; N, 9.77%. Calcd for $C_{37}H_{43}O_6N_5\cdot HCl\cdot H_2O$: C, 62.74; H, 6.49; N, 9.81%.

Z-Arg(NO$_2$)-Gly-Pro-Pro-Gly-Phe-Phe-OBzl (52). Z-Arg(NO_2)-Gly-OH[7,10] (0.82 g, 2 mmol) and compound **51** (1.38 g, 2 mmol) were coupled by the same method as described for the preparation of **47**: yield 1.68 g (80%); mp 93—96 °C; $[\alpha]_D^{20}$ −54.0° (*c* 1, acetic acid); R_f^1 0.94 and R_f^2 0.50.

Found: C, 60.35; H, 6.13; N, 14.54%. Calcd for $C_{53}H_{63}O_{12}N_{11}\cdot 1/2\ H_2O$: C, 60.32; H, 6.13; N, 14.60%.

H-Arg-Gly-Pro-Pro-Gly-Phe-Phe-OH (7). Compound **52** (1.05 g, 1 mmol) was treated as described in the case of **1**: yield 0.74 g (88%); mp 110 °C; $[\alpha]_D^{20}$ −71.0° (*c* 1, H_2O); R_f^1 0.22 and R_f^2 0.00.

Found: C, 57.36; H, 6.80; N, 16.77%. Calcd for $C_{38}H_{52}O_8N_{10}\cdot CH_3COOH$: C, 57.40; H, 6.76; N, 16.74%.

Amino acid ratios in acid hydrolyzate: Arg 0.92, Gly 2.00, Pro 1.98, Phe 2.00.

Boc-Gly-Gly-Phe-Phe-OBzl (53). Boc-Gly-Gly-OH[1] (2.32 g, 10 mmol) and compound **44** (4.74 g, 10 mmol) were coupled by the same method as descried for the preparation of **12**: yield 5.24 g (85%); mp 81—82 °C; $[\alpha]_D^{20}$ −6.0° (*c* 1, methanol); R_f^1 0.93 and R_f^2 0.55.

Found: C, 63.39; H, 6.71; N, 8.74%. Calcd for $C_{34}H_{40}O_7N_4\cdot 3/2\ H_2O$: C, 63.43; H, 6.75; N, 8.71%.

H-Gly-Gly-Phe-Phe-OBzl·HCl (54). Compound **53** (4.32 g, 7 mmol) was treated as described in the case of **13**: yield 3.76 g (97%); mp 159—161 °C; $[\alpha]_D^{20}$ −13.0° (*c* 1, methanol); R_f^1 0.75 and R_f 0.43.

Found: C, 61.05; H, 6.14; N, 9.80%. Calcd for $C_{29}H_{32}O_5N_4\cdot HCl\cdot H_2O$: C, 60.98; H, 6.19; N, 9.81%.

Boc-Pro-Pro-Gly-Gly-Phe-Phe-OBzl (55). Boc-Pro-Pro-OH[7,9] (1.56 g, 5 mmol) and compound **54** (2.77 g, 5 mmol) were coupled by the same method as described for the preparation of **12**: yield 3.51 g (87%); mp 88—90 °C; $[\alpha]_D^{20}$ −53.0° (*c* 1, methanol); R_f^1 0.74 and R_f^2 0.41.

Found: C, 63.70; H, 6.79; N, 10.17%. Calcd for $C_{44}H_{54}O_9N_6\cdot H_2O$: C, 63.75; H, 6.82; N, 10.14%.

H-Pro-Pro-Gly-Gly-Phe-Phe-OBzl·HCl (56). Compound **55** (2.43 g, 3 mmol) was treated as described in the case of **13**: yield 2.09 g (93%); mp 101—103 °C; $[\alpha]_D^{20}$ −38.0° (*c* 1, methanol); R_f^1 0.58 and R_f^2 0.39.

Found: C, 61.17; H, 6.52; N, 10.95%. Calcd for $C_{39}H_{46}O_7N_6\cdot HCl\cdot H_2O$: C, 61.21; H, 6.47; N, 10.98%.

Z-Arg(NO$_2$)-Gly-Pro-Pro-Gly-Gly-Phe-Phe-OBzl (57). Z-Arg(NO_2)-Gly-OH[7,10] (0.82 g, 2 mmol) and compound **56** (1.49 g, 2 mmol) were coupled by the same method as described for the preparation of **12**: yield 1.79 g (81%); mp 116—119 °C; $[\alpha]_D^{20}$ −48.0° (*c* 0.5, acetic acid); R_f^1 0.90 and R_f^2 0.32.

Found: C, 59.30; H, 6.02; N, 15.17%. Calcd for $C_{55}H_{66}O_{13}N_{12}\cdot 1/2\ H_2O$: C, 59.39; H, 6.08; N, 15.12%.

H-Arg-Gly-Pro-Pro-Gly-Gly-Phe-Phe-OH (8). Compound **57** (1.10 g, 1 mmol) was treated as described in the case of **1**: yield 0.72 g (81%); mp 128—130 °C; $[\alpha]_D^{20}$ −61.0° (*c* 1, H_2O); R_f^1 0.19 and R_f^2 0.00.

Found: C, 54.81; H, 6.85; N, 16.70%. Calcd for $C_{40}H_{55}O_9N_{11}\cdot 3/2\ H_2O\cdot CH_3COOH$: C, 54.76; H, 6.80; N, 16.73%.

Amino acid ratios in acid hydrolyzate: Arg 0.93, Gly 2.99, Pro 2.02, Phe 2.00.

Boc-Gly-Gly-Gly-Phe-Phe-OBzl (58). Boc-Gly-Gly-OH[1] (2.32 g, 10 mmol) and compound **49** (4.93 g, 10 mmol) were coupled by the same method as described for the preparation of **12**: yield 5.46 g (81%); mp 175—176 °C; $[\alpha]_D^{20}$ −11.0° (*c* 1, methanol); R_f^1 0.93 and R_f^2 0.58.

Found: C, 64.15; H, 6.49; N, 10.39%. Calcd for $C_{36}H_{43}O_8N_5$: C, 64.17; H, 6.45; N, 10.40%.

H-Gly-Gly-Gly-Phe-Phe-OBzl·HCl (59). Compound **58** (4.72 g, 7 mmol) was treated as described in the case of **13**: yield 4.03 g (94%); mp 170—173 °C; $[\alpha]_D^{20}$ −12.0° (*c* 1, methanol); R_f^1 0.63 and R_f^2 0.34.

Found: C, 60.97; H, 5.99; N, 11.53%. Calcd for $C_{31}H_{35}O_6N_5\cdot HCl$: C, 61.02; H, 5.96; N, 11.50%.

Boc-Pro-Pro-Gly-Gly-Gly-Phe-Phe-OBzl (60). Boc-Pro-Pro-OH[7,9] (1.56 g, 5 mmol) and compound **59** (3.05 g, 5 mmol) were coupled by the same method as described for the preparation of **12**: yield 3.63 g (84%); mp 130—131 °C; $[\alpha]_D^{20}$ −52.0° (*c* 1, methanol) R_f^1 0.73 and R_f^2 0.54.

Found: C, 63.60; H, 6.81; N, 11.33%. Calcd for $C_{46}H_{57}O_{10}N_7\cdot H_2O$: C, 63.64; H, 6.86; N, 11.30%.

H-Pro-Pro-Gly-Gly-Gly-Phe-Phe-OBzl·HCl (61). Compound **60** (2.60 g, 3 mmol) was treated as described in the case of **13**: yield 2.34 g (97%); mp 137—140 °C; $[\alpha]_D^{20}$ −29.0° (*c* 1, methanol); R_f^1 0.58 and R_f^2 0.30.

Found: C, 59.93; H, 6.31; N, 11.90%. Calcd for $C_{41}H_{49}O_8N_7\cdot HCl\cdot H_2O$: C, 59.88; H, 6.37; N, 11.93%.

Z-Arg(NO$_2$)-Gly-Pro-Pro-Gly-Gly-Gly-Phe-Phe-OBzl (62). Z-Arg(NO_2)-Gly-OH[7,10] (0.82 g, 2 mmol) and compound **61** (1.61 g, 2 mmol) were coupled by the same method as described for the preparation of **12**. This product was obtained in a hygroscopic form: yield 1.72 g (74%); R_f^1 0.85 and R_f^2 0.52.

H-Arg-Gly-Pro-Pro-Gly-Gly-Gly-Phe-Phe-OH (9). Compound **62** (1.15 g, 1 mmol) was treated as described in the case of **1**: yield 0.67 g (70%); mp 137—140 °C; $[\alpha]_D^{20}$

−50.0° (*c* 1, H_2O); R_f^1 0.21 and R_f^2 0.00.

Found: C, 54.55; H, 6.61; N, 17.33%. Calcd for $C_{42}H_{58}O_{10}N_{12}\cdot H_2O\cdot CH_3COOH$: C, 54.52; H, 6.67; N, 17.35%.

Amino acid ratios in acid hydrolyzate: Arg 0.93, Gly 3.98, Pro 1.99, Phe 2.00.

Boc-Phe-Phe-Phe-OBzl (63). Boc-Phe-OH·DCHA[15] (5.36 g, 12 mmol) and compound **49** (4.74 g, 10 mmol) were coupled by the same method as described for the preparation of **12**: yield 5.78 g (89%); mp 170—172 °C; $[\alpha]_D^{20}$ −27.5° (*c* 1, methanol); R_f^1 0.99 and R_f^2 0.89.

Found: C, 72.13; H, 6.76; N, 6.49%. Calcd for $C_{39}H_{43}O_6N_3$: C, 72.09; H, 6.77; N, 6.46%.

H-Phe-Phe-Phe-OBzl·HCl (64). Compound **63** (5.30 g, 8.15 mmol) was treated as described in the case of **13**: yield 4.55 g (95%); mp 186 °C (decomp); $[\alpha]_D^{20}$ −9.0° (*c* 1, methanol); R_f^1 0.91 and R_f^2 0.81.

Found: C, 69.70; H, 6.19; N, 7.15%. Calcd for $C_{34}H_{35}O_4N_3\cdot HCl$: C, 69.67; H, 6.19; N, 7.13%.

Boc-Pro-Pro-Phe-Phe-Phe-OBzl (65). Boc-Pro-Pro-OH[7,9] (1.56 g, 5 mmol) and compound **64** (2.93 g, 5 mmol) were coupled by the same method as described for the preparaton of **12**: yield 3.46 g (82%); mp 109—112 °C; $[\alpha]_D^{20}$ −66.5° (*c* 1, methanol); R_f^1 0.99 and R_f^2 0.84.

Found: C, 69.80; H, 6.83; N, 8.26%. Calcd for $C_{49}H_{57}O_8N_5$: C, 69.76; H, 6.81; N, 8.30%.

H-Pro-Pro-Phe-Phe-Phe-OBzl·HCl (66). Compound **65** (2.53 g, 3 mmol) was treated as described in the case of **13**: yield 2.22 g (95%); mp 100—103 °C; $[\alpha]_D^{20}$ −76.5° (*c* 1, methanol); R_f^1 0.82 and R_f^2 0.40.

Found: C, 66.92; H, 6.48; N, 8.88%. Calcd for $C_{44}H_{49}O_6N_5\cdot HCl\cdot 1/2\ H_2O$: C, 66.95; H, 6.51; N, 8.87%.

Z-Arg(NO$_2$)-Gly-Pro-Pro-Phe-Phe-Phe-OBzl (67). Z-Arg(NO_2)-Gly-OH[7,10] (0.82 g, 2 mmol) and compound **66** (1.56 g, 2 mmol) were coupled by the same method as described for the preparation of **12**: yield 1.75 g (77%); mp 114—117 °C; $[\alpha]_D^{20}$ −69.5° (*c* 1, methanol); R_f^1 0.84 and R_f 0.55.

Found: C, 62.47; H, 6.27; N, 13.32%. Calcd for $C_{60}H_{70}O_{12}N_{11}\cdot H_2O$: C, 62.43; H, 6.30; N, 13.35%.

H-Arg-Gly-Pro-Pro-Phe-Phe-Phe-OH (10). Compound **67** (1.14 g, 1 mmol) was treated as described in the case of **1**: yield 0.74 g (76%); mp 127—130 °C; $[\alpha]_D^{20}$ −64.0° (*c* 1, acetic acid); R_f^1 0.29 and R_f^2 0.00.

Found: C, 60.25; H, 6.79; N, 15.03%. Calcd for $C_{45}H_{58}O_8N_{10}\cdot 1/2\ H_2O\cdot CH_3COOH$: C, 60.29; H, 6.80; N, 14.96%.

Amino acid ratios in acid hydrolyzate: Arg 0.95, Gly 1.00, Pro 2.08, Phe 2.99.

CD Measurement. The measurements were performed with a JASCO J-20 Automatic Recording Spectropolarimeter over a wavelength range of 200 to 250 nm. A cell of path length 0.2 mm was used and the runs were made at ambient temperature. Patterns in a solvent of distilled water are shown in Fig. 6.

Sensory Test. The bitterness of the synthetic BPIa analogs was organoleptically determined *via* panel evaluation by five people. A series of solution of decreasing concentration was prepared in which each solution was half as strong as its proceeding one. Before testing the sample, the tester's month was thoroughly rinsed with distilled water. The sample size was usually 2—3 ml. The sample solution was held in the mouth for *ca.* 10 s and then spit out. The threshold values of synthetic BPIa analogs are shown in the Table.

The authors wish to express their thanks to Professor Sakuzo Fukui of Hiroshima University for his helpful discussion in the course of this study. Thanks are also due to Associate Professor Michio Kondo of Saga University for his help in the CD measurements, and to Dr. Shinji Okumura of Ajinomoto Co. Inc., for supplying some amino acids.

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