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Studies of Bitter Peptides from Casein Hydrolyzate. VII.[1] Bitterness of the retro-BPIa (Val-Ile-Phe-Pro-Pro-Gly-Arg) and Its Fragments[2]

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To explain the bitter taste exhibited by BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val) which was isolated from casein hydrolyzate, we have proposed the following requirement: its characteristic spatial structure: the basic moiety in the N-terminal and the hydrophobic moiety in the C-terminal were affected to each other by prolylprolyl residue, is necessary for the bitterness to be exhibited. As for BPIc (Val-Tyr-Pro-Phe-Pro-Pro-Gly-Ile-Asn-His), which is the other fraction, although it exhibited as strong and bitter a taste as BPIa, the basic moiety of BPIc is located in the C-terminal and its hydrophobic moiety is located in the N-terminal. The authors synthesized retro-BPIa with the reverse peptide sequence and its fragments. The retro-BPIa exhibited as strong and bitter a taste as BPIa. However, the bitterness of the fragments of retro-BPIa was far weaker than that of retro-BPIa.

In studies of the relationship between bitterness and chemical structure of BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val) which was isolated by Minamiura *et al.*,[3] the authors reported that the basic amino acid residue in the N-terminal, the hydrophobic amino acid residues in the C-terminal, and its characteristic spatial structure, which may derive from prolylprolyl residues at 3 and 4 positions of BPIa, are necessary for an intense bitter taste to be exhibited by BPIa.[4-7] In the previous paper,[1] we reported that a decapeptide BPIc (Val-Tyr-Pro-Phe-Pro-Pro-Gly-Ile-Asn-His) in which basic moiety is located in the C-terminal and hydrophoic moiety is located in the N-terminal exhibited an extremely bitter taste on the same level as that of BPIa. From those results, it was expected that the retro-BPIa (Val-Ile-Phe-Pro-Pro-Gly-Arg) possessing the reverse amino acid sequence of BPIa would exhibit as bitter a taste as BPIa. Thus the authors synthesized retro-BPIa and its fragments, as shown in the Table, and investigated their bitterness.

The synthetic route to compound **1** (retro-BPIa) is shown in Fig. 1. *N*-(Benzyloxycarbonyl)isoleucine and phenylalanine benzyl ester were coupled by the mixed anhydride method to yield *N*-(benzyloxycarbonyl)-isoleucylphenylalanine benzyl ester (**12**). The free dipeptide (**5**) was given by the catalytic hydrogenation of **12**. *N*-(Benzyloxycarbonyl)valine *N*-hydroxysuccinimide ester and **5** were coupled to yield *N*-(benzyloxycarbonyl)valylisoleucylphenylalanine (**13**). *N*-(*t*-Butoxycarbonyl)prolylproline and glycine benzyl ester were coupled by the mixed anhydride method to yield *N*-(*t*-butoxycarbonyl)prolylprolylglycine benzyl ester (**14**). After treatment of **14** with hydrogen chloride in dioxane, the tripeptide benzyl ester hydrochloride (**15**) and **13** were coupled by the DCC-HONSu method to yield *N*-(benzyloxycarbonyl)valylisoleucylphenylalanylprolylprolylglycine benzyl ester (**16**). *N*-(Benzyloxycarbonyl)valylisoleucylphenylalanylprolylprolylglycine (**17**) was obtained by the saponification of **16**. **17** and N^G-nitroarginine benzyl ester were coupled by the DCC-HOBt method to yield *N*-(benzyloxycarbonyl)valylisoleucylphenylalanylprolylprolylglycyl-N^G-nitroarginine benzyl ester (**18**). The protected heptapeptide was hydrogenated in the presence of palladium black to give the retro-BPIa (**1**).

The synthetic route to compounds **2** and **3** is shown in Fig. 2. These compounds were obtained by the catalytic hydrogenation of **16** and **13**, which are the intermediates in the synthesis of retro-BPIa. The details

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

	Compound	Taste	T.V./mM	$R_{caf.}$[a]
1.	Val-Ile-Phe-Pro-Pro-Gly-Arg	Bitter	0.08	12.50
2.	Val-Ile-Phe-Pro-Pro-Gly	Bitter	2.10	0.48
3.	Val-Ile-Phe	Bitter	1.30	0.77
4.	Val-Ile	Bitter	6.00	0.17
5.	Ile-Phe	Bitter	1.50	0.67
6.	Pro-Pro	Bitter	4.50	0.22
7.	Pro-Pro-Gly	Bitter	9.50	0.11
8.	Pro-Gly	Sweet	—	—
9.	Pro-Gly-Arg	Bitter	25.00	0.04
10.	Gly-Arg	Bitter	75.00	0.01
11.	Arg-Gly-Pro-Pro-Phe-Ile-Val	Bitter	0.05	20.00

a) Ratio of caffeine.

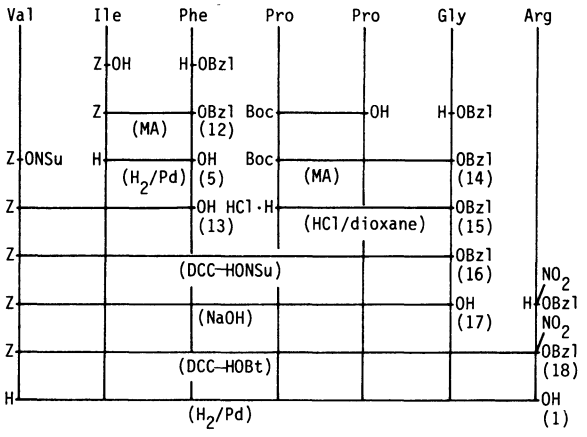

Fig. 1. Syntheses of compound **1** (retro-BPIa) and **5**.

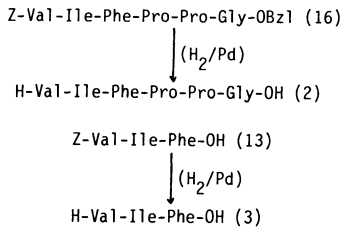

Fig. 2. Syntheses of compounds **2** and **3**.

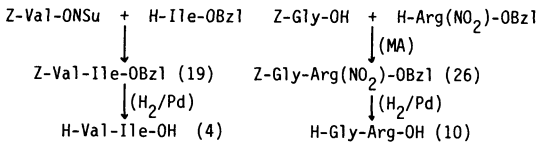

Fig. 3. Syntheses of compounds **4** and **10**.

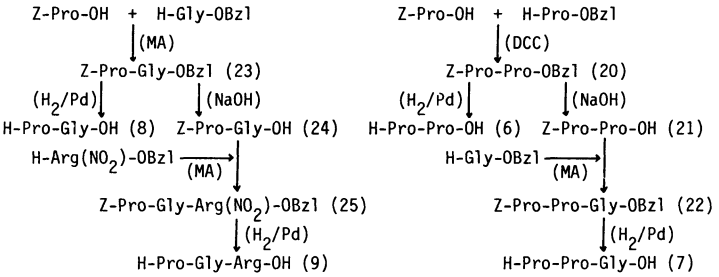

Fig. 4. Syntheses of compounds **6**, **7**, **8**, and **9**.

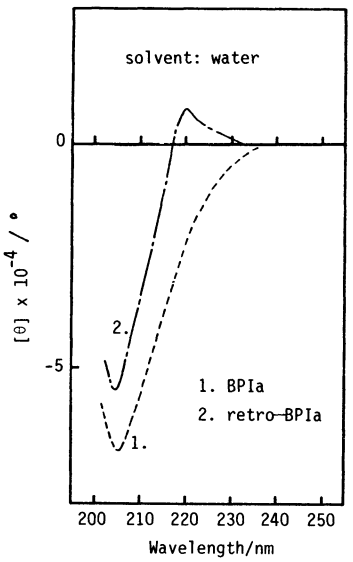

Fig. 5. CD curves of BPIa and retro-BPIa.

of the synthetic route to other peptides (compounds **4**—**10**) are shown in Figs. 3 and 4, and are described in the Experimental part. The purity of the synthetic peptides and their intermediates was confirmed by thin-layer chromatography in two solvent systems, elemental analyses, and amino acid analyses.

The bitterness of the synthetic peptides were organoleptically determined by panel evaluation of five people (see Table). The threshold value of retro-BPIa (compound **1**) was 0.08 mM (1 M=1 mol dm^{-3}). The value is about the same as that of BPIa. On the other hand, the bitterness of des-Arg^7-retro-BPIa (**2**) was far weaker than that of retro-BPIa. In the previous paper,[4] we reported that des-Arg^1-BPIa also exhibited a weakly bitter taste. The result indicated that the arginine residue plays an important role for the bitter taste exhibited by BPIa and retro-BPIa, as has been described in the previous papers.[4,7] As for the other fragments (**4**—**10**), they did not exhibit as strong and bitter a taste as BPIa.

We have reported that the strong bitter taste exhibited by BPIa may derive from its characteristic spatial structure.[4,5] In order to investigate that requirement, we measured the circular dichroism (CD) of the retro-BPIa, and compared its CD curves with that of BPIa, as shown in Fig. 5. The CD curves of BPIa and retro-BPIa were similar in shape.

The results described above suggest that the spatial structure of retro-BPIa possessing the reverse amino acid sequence may be similar to that of BPIa. Thus, in order for the bitter taste to be exhibited by BPIa, the spatial structure of the molecule is more important than the position of the basic and hydrophobic moieties.

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 hydrochloric acid and propionic acid (1:1) at 110 °C for 72 h.

Z-Ile-Phe-OBzl (12). Z-Ile-OH·DCHA (14.74 g, 33 mmol) was dissolved in ethyl acetate (100 ml), and 0.5 M sulfic acid (50 ml) was added to the mixture during stirring. The organic layer was washed with water and dried over anhydrous sodium sulfate. The solution was con-

centrated to dryness *in vacuo*, and the oily residue was dissolved in THF (50 ml) and NMM (3.3 ml, 30 mmol). ECF (3.0 ml, 30 mmol) was added to the mixture at −5 °C. After 10 min, a solution of H-Phe-OBzl·TsOH[8] (12.80 g, 30 mmol) in chloroform (50 ml) was added to the mixture. The reaction mixture was stirred in an ice bath for 1 h, then at room temperature overnight. The reaction mixture was evaporated *in vacuo*, and the oily residue was dissolved in ethyl acetate. The solution was washed with water, 0.5 M hydrochloric 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 11.88 g (71%); mp 158 °C; $[\alpha]_D^{15}$ −15.5° (*c* 1, *N,N*-dimethylformamide); R_f^1 0.99 and R_f^2 0.97.

Found: C, 71.63; H, 7.01; N, 5.50%. Calcd for $C_{30}H_{34}O_5N_2$: C, 71.69; H, 6.82; N, 5.57%.

H-Ile-Phe-OH (5). A solution of compound **12** (10.05 g, 20 mmol) in methanol (20 ml) and acetic acid (5 ml) was hydrogenated in the presence of palladium black at room temperature overnight. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was crystallized by the aid of acetone: yield 4.96 g (90%); mp 250 °C (decomp); $[\alpha]_D^{15}$ +68.0° (*c* 1, acetic acid); R_f^1 0.83 and R_f^2 0.06.

Found: C, 65.16; H, 7.87; N, 10.11%. Calcd for $C_{15}H_{22}O_3N_2$: C, 64.72; H, 7.97; N, 10.07%.

Z-Val-Ile-Phe-OH (13). To a solution of Z-Val-ONSu[9] (4.20 g, 12 mmol) in THF (20 ml), a solution of compound **5** (2.78 g, 10 mmol) and Et_3N (1.4 ml, 10 mmol) in water (20 ml) was added. The reaction mixture was allowed to stand at room temperature for 24 h, and then suspended by the aid of water (200 ml). The insoluble product was collected by filtration and recrystallized from hot ethanol and ether: yield 3.58 g (70%); mp 208—210 °C; $[\alpha]_D^{15}$ +7.5° (*c* 1, *N,N*-dimethylformamide); R_f^1 0.89 and R_f^2 0.74.

Found: C, 65.10; H, 7.63; N, 8.40%. Calcd for $C_{28}H_{37}O_6N_3 \cdot 1/3\ H_2O$: C, 64.97; H, 7.34; N, 8.12%.

Boc-Pro-Pro-Gly-OBzl (14). Boc-Pro-Pro-OH[10] (6.24 g, 20 mmol) and H-Gly-OBzl·TsOH[11,12] (6.74 g, 20 mmol) were coupled by the same method as described for the preparation of **12**: yield 6.95 g (76%); mp 108—109 °C; $[\alpha]_D^{15}$ −122.5° (*c* 1, methanol); R_f^1 0.85 and R_f^2 0.67.

Found: C, 62.71; H, 7.36; N, 9.04%. Calcd for $C_{24}H_{33}O_6N_3$: C, 62.72; H, 7.24; N, 9.14%.

H-Pro-Pro-Gly-OBzl·HCl (15). To a solution of compound **14** (4.60 g, 10 mmol) in dioxane (20 ml), 4 M HCl/dioxane (50 ml) was added. The solution was allowed to stand at room temperature. After 2 h, the solution was evaporated *in vacuo*. The residual oil was solidified with ether: yield 3.92 g (99%); mp 65—67 °C; $[\alpha]_D^{15}$ −115.0° (*c* 1, methanol); R_f^1 0.62 and R_f^2 0.13.

Found: C, 56.74; H, 6.83; N, 10.38%. Calcd for $C_{19}H_{25}O_4N_3 \cdot HCl \cdot 1/3\ H_2O$: C, 56.78; H, 6.68; N, 10.45%.

Z-Val-Ile-Phe-Pro-Pro-Gly-OBzl (16). To a solution of compound **13** (2.56 g, 5 mmol) and compound **15** (1.98 g, 5 mmol) and NMM (0.55 ml, 5 mmol) and DMF (20 ml), DCC (1.55 g, 7.5 mmol) and HONSu (0.86 g, 7.5 mmol) were added to the mixture. The reaction mixture was cooled at −10 °C for 1 h and allowed to stand at room temperature for 48 h. 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, 0.5 M 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 4.12 g (97%); mp 108—109 °C; $[\alpha]_D^{15}$ −54.5° (*c* 1, *N,N*-dimethylformamide); R_f^1 0.94 and R_f^2 0.60.

Found: C, 66.00; H, 7.33; N, 10.02%. Calcd for $C_{47}H_{60}O_9N_6$: C, 66.17; H, 7.09; N, 9.85%.

Z-Val-Ile-Phe-Pro-Pro-Gly-OH (17). To a solution of compound **16** (2.56 g, 3 mmol) in methanol (15 ml), 2 M sodium hydroxide (1.8 ml, 3.6 mmol) was added under stirring. The solution was allowed to stand at room temperature. After 2 h, the solution was evaporated *in vacuo*, and then diluted with water. The solution was washed with ethyl acetate. The aqueous layer was acidified to pH 4 with 2 M hydrochloric acid. The precipitate thus obtained was collected and washed with water. It was recrystallized from hot ethanol-ether: yield 2.16 g (92%); mp 130—134 °C; $[\alpha]_D^{15}$ −56.0° (*c* 1, *N,N*-dimethylformamide); R_f^1 0.78 and R_f^2 0.35.

Found: C, 62.01; H, 7.19; N, 10.84%. Calcd for $C_{40}H_{54}O_9N_6 \cdot 1/3\ H_2O$: C, 62.08; H, 7.12; N, 10.86%.

Z-Val-Ile-Phe-Pro-Pro-Gly-Arg(NO$_2$)-OBzl (18). To a solution of compound **17** (0.77 g, 1 mmol) and H-Arg(NO$_2$)-OBzl·2TsOH[13] (0.65 g, 1 mmol) in NMM (0.22 ml, 2 mmol) and DMF (10 ml), DCC (0.31 g, 1.5 mmol) and HOBt (0.20 g, 1.5 mmol) were added. The reaction mixture was cooled in an ice bath for an hour and allowed to stand at room temperature overnight. The DCUrea was removed by filtration, and the filtrate was evaporatd *in vacuo*. The oily residue was dissolved in ethyl acetate and the solution was washed with water, 0.5 M 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 oily residue was crystallized with ether-petroleum ether: yield 0.75 g (71%); mp 130—133 °C; $[\alpha]_D^{15}$ −39.5° (*c* 1, *N,N*-dimethylformamide); R_f^1 0.98 and R_f^2 0.57.

Found: C, 59.93; H, 7.00; N, 14.17%. Calcd for $C_{53}H_{71}O_{12}N_{11} \cdot 1/2\ H_2O$: C, 59.87; H, 6.82; N, 14.49%.

H-Val-Ile-Phe-Pro-Pro-Gly-Arg-OH (1). Compound **18** (0.53 g, 0.5 mmol) was treated as described for the preparation of **5**: yield 0.37 g (87%); mp 180 °C (decomp); $[\alpha]_D^{15}$ −124.5° (*c* 1, H_2O); R_f^1 0.47 and R_f^2 0.00.

Found: C, 56.32; H, 7.82; N, 16.15%. Calcd for $C_{38}H_{60}O_8N_{10} \cdot CH_3COOH \cdot 1/2\ H_2O$: C, 56.25; H, 7.75; N, 16.40%.

Amino acid ratios in acid hydrolyzate: Val 1.03, Ile 1.00, Phe 1.01, Pro 2.02, Gly 1.00, Arg 0.96.

H-Val-Ile-Phe-Pro-Pro-Gly-OH (2). Compound **16** (0.85 g, 1 mmol) was treated as described in the case of **5**: yield 0.48 g (77%); mp 162—164 °C; $[\alpha]_D^{15}$ −86.5° (*c* 1, acetic acid); R_f^1 0.62 and R_f^2 0.03.

Found: C, 57.30; H, 7.75; N, 12.39%. Calcd for $C_{32}H_{48}O_7N_6 \cdot 7/3\ H_2O$: C, 57.29; H, 7.91; N, 12.53%.

Amino acid ratios in acid hydrolyzate: Val 1.02, Ile 1.00, Phe 0.99, Pro 2.01, Gly 1.01.

H-Val-Ile-Phe-OH (3). Compound **13** (0.51 g, 1 mmol) was treated as described in the case of **5**: yield 0.30 g (80%); mp 233 °C (decomp); $[\alpha]_D^{15}$ +9.0° (*c* 1, acetic acid); R_f^1 0.80 and R_f^2 0.13.

Found: C, 62.53; H, 8.23; N, 10.88%. Calcd for $C_{20}H_{31}O_4N_3 \cdot 1/3\ H_2O$: C, 62.63; H, 8.32; N, 10.95%.

Amino acid ratios in acid hydrolyzate: Val 1.01, Ile 1.00, Phe 0.99.

Z-Val-Ile-OBzl (19). Z-Val-ONSu[9] (2.10 g, 6 mmol) and H-Ile-OBzl·TsOH[8] (1.97 g, 5 mmol) were coupled by the same method as described for the preparation of **13**: yield 1.38 g (61%); mp 108—109 °; $[\alpha]_D^{15}$ −14.0° (*c* 1, methanol); R_f^1 0.98 and R_f^2 0.95.

Found: C, 68.74; H, 7.59; N, 6.20%. Calcd for $C_{26}H_{34}$-

O_5N_2: C, 68.70; H, 7.63; N, 6.14%.

H-Val-Ile-OH (4). Compound **19** (0.91 g, 2 mmol) was treated as described in the case of **5**: yield 0.44 g (96%); melting point above 250 °C; $[\alpha]_D^{15}$ +17.0° (*c* 1, H_2O); R_f^1 0.71 and R_f^2 0.21.

Found: C, 57.28; H, 9.68; N, 12.14%. Calcd for $C_{11}H_{22}O_3N_2$: C, 57.36; H, 9.65; N, 12.21%.

Amino acid ratios in acid hydrolyzate: Val 1.02, Ile 1.00.

Z-Pro-Pro-OBzl (20). To a solution of Z-Pro-OH[14-16] (2.49 g, 10 mmol) in CH_3CN (10 ml), DCC (2.48 g, 12 mmol) was added to the mixture at 0 °C. After 20 min, a solution of H-Pro-OBzl·HCl[17,18] (2.42 g, 10 mmol) and NMM (1.1ml, 10 mmol) in CH_3CN (10 ml) was added to the mixture. The resulting 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, 0.5 M hydrochloric acid, 4% sodium hydrogencarbonate, and water successively. The solution was dried over anhydrous sodium sulfate. Sodium sulfate as removed by filtration, and the filtrate was evaporated *in vacuo*. This compound was obtained in an oily form, while Appel *et al.*[19] reported that they obtained this compound as a crystalline form: yield 3.93 g (90%); R_f^1 0.89 and R_f^2 0.94.

H-Pro-Pro-OH (6). Compound **20** (3.93 g, 9 mmol) was treated as described in the case of **5**: yield 1.70 g (89%); mp 130—132 °C (lit, 125—127 °C,[19] 144—145 °C,[20] and 220—222 °C[21]); $[\alpha]_D^{15}$ −167.5° (*c* 1, H_2O) (lit, −166° (H_2O),[19] −169° (H_2O),[20] and −87° (H_2O)[21]); R_f^1 0.33 and R_f^2 0.10.

Found: C, 56.63; H, 7.58; N, 13.15%. Calcd for $C_{10}H_{16}O_3N_2$: C, 56.59; H, 7.63; 13.23%.

Z-Pro-Pro-OH (21). Compound **20** (5.13 g, 11.75 mmol) was treated as described for the preparation of **17**: yield 3.30 g (81%); mp 186 °C (lit, 187—190 °C[22] and 191.5—193.5 °C[23]); $[\alpha]_D^{15}$ −84.5° (*c* 1, *N,N*-dimethylformamide) (lit, −83° (*N,N*-dimethylformamide[23])); R_f^1 0.69 and R_f^2 0.17.

Found: C, 59.47; H, 6.58; N, 7.77%. Calcd for $C_{18}H_{22}O_5N_2 \cdot H_2O$: C, 59.32; H, 6.65; N, 7.69%.

Z-Pro-Pro-Gly-OBzl (22). Compound **21** (1.73 g, 5 mmol) and H-Gly-OBzl·TsOH[11,12] (1.69 g, 5 mmol) were coupled by the same method as described for the preparation of **12**: yield 2.42 g (98%); mp 121—124 °C (lit, 120—122 °C[24]); $[\alpha]_D^{15}$ −113.0° (*c* 1, methanol) (lit, −115° (methanol)[24]); R_f^1 0.87 and R_f^2 0.71.

Found: C, 65.82; H, 6.31; N, 8.61%. Calcd for $C_{27}H_{31}O_6N_3$: C, 65.70; H, 6.34; N, 8.52%.

H-Pro-Pro-Gly-OH (7). Compound **22** (0.99 g, 2 mmol) was treated as described for the preparation of **5**: yield 0.50 g (93%); mp 108—111 °C (lit, 112—114 °C[24]); $[\alpha]_D^{15}$ −97.0° (*c* 1, H_2O); R_f^1 0.27 and R_f^2 0.07.

Found: C, 51.89; H, 7.13; N, 14.98%. Calcd for $C_{12}H_{19}O_4N_3 \cdot 1/2\ H_2O$: C, 51.79; H, 7.26; N, 15.10%.

Amino acid ratios in acid hydrolyzate: Pro 2.10, Gly 1.00.

Z-Pro-Gly-OBzl (23). Z-Pro-OH[14-16] (4.99 g, 20 mmol) H-Gly-OBzl·TsOH[11,12] (6.75 g, 20 mmol) were coupled by the same method as described for the preparation of **12**: yield 6.99 g (88%); mp 81—83 °C (lit, 88—89 °C[19]); $[\alpha]_D^{15}$ −65.0° (*c* 1, methanol); R_f^1 0.90 and R_f^2 0.74.

Found: C, 66.58; H, 6.02; N, 7.18%. Calcd for $C_{22}H_{24}O_5N_2$: C, 66.65; H, 6.11; N, 7.07%.

H-Pro-Gly-OH (8). Compound **23** (0.79 g, 2 mmol) was treated as described in the case of **5**: yield 0.30 g (87%); mp 225—228 °C (lit, 230—232 °C[25]); $[\alpha]_D^{15}$ −23.5° (*c* 1, H_2O) (lit, −23° (H_2O[26])); R_f^1 0.23 and R_f^2 0.07.

Found: C, 46.27; H, 7.21; N, 15.38%. Calcd for $C_7H_{12}O_3N_2 \cdot 1/2\ H_2O$: C, 46.39; H, 7.25; N, 15.46%.

Amino acid ratios in acid hydrolyzate: Pro 1.07, Gly 1.00.

Z-Pro-Gly-OH (24). Compound **23** (5.95 g, 15 mmol) was treated as described in the case of **17**. This compound was obtained in an oily form:[27] yield 2.48 g (54%); R_f^1 0.77 and R_f^2 0.34.

Z-Pro-Gly-Arg(NO$_2$)-OBzl (25). Compound **24** (2.48 g, 8.1 mmol) and H-Arg(NO_2)-OBzl·TsOH[13] (5.29 g, 8.1 mmol) were coupled by the same method as described for the preparation of **12**: yield 4.02 g (83%); mp 75—79 °C; $[\alpha]_D^{15}$ −30.5° (*c* 2, methanol); R_f^1 0.90 and R_f^2 0.50.

Found: C, 53.70; H, 6.08; N, 15.83%. Calcd for $C_{28}H_{35}O_8N_7 \cdot 3/2\ H_2O$: C, 53.84; H, 6.14; N, 15.70%.

H-Pro-Gly-Arg-OH (9). Compound **25** (1.20 g, 2 mmol) was treated as described for the preparation of **5**. This compound was obtained in a hygroscopic form: yield 0.58 g (89%); R_f^1 0.23 and R_f^2 0.00.

Z-Gly-Arg(NO$_2$)-OBzl (26). Z-Gly-OH[28-30] (1.15 g, 5.5 mmol) and H-Arg(NO_2)-OBzl·2TsOH[13] (3.60 g, 5.5 mmol) were coupled by the same method as described for the preparation of **12**: yield 1.84 g (67%); mp 117—119 °C; $[\alpha]_D^{15}$ −14.0° (*c* 1, methanol); R_f^1 0.95 and R_f^2 0.53.

Found: C, 55.11; H, 5.68; N, 16.74%. Calcd for $C_{23}H_{28}O_7N_6$: C, 55.19; H, 5.65; N, 16.80%.

H-Gly-Arg-OH (10). Compound **26** (1.00 g, 2 mmol) was treated as described in the case of **5**: yeld 0.47 g (80%); mp 68—72 °C; $[\alpha]_D^{15}$ +4.0° (*c* 1, H_2O); R_f^1 0.19 and R_f^2 0.00.

Found: C, 38.71; H, 8.07; N, 22.74%. Calcd for $C_8H_{19}O_3N_5 \cdot CH_3COOH \cdot H_2O$: C, 38.83; H, 8.16; N, 22.65%.

Amino acid ratios in acid hydrolyzate: Pro 1.05, Gly 1.00, Arg 0.97.

CD Measurement. The measurements were performed with 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 solvent of distilled water are shown in Fig. 5.

Sensory Test. The bitterness of the synthetic peptide was organoleptically determined *via* panel evaluation by five people. A series of solutions of decreasing concentration was prepared in which each solution was half as strong as its proceeding one. Before testing the sample, the panelist's mouth 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 seconds and then spit out. The threshold value and the $R_{caf.}$ (ratio of caffeine) value, which expresses the extent of the strength of bitterness of the synthetic peptides, are shown in the Table.

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2) The abbreviations recommended by the IUPAC-IUB commission of Biochemical Nomenclature (*J. Biol. Chem.*, **247**, 977 (1972)) have been used. Amino acid symbols except glycine denote the L-configuration. Additional abbreviations: DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DCUrea, *N,N'*-dicyclohexylurea; DMF, *N,N*-dimethylformamide; ECF, ethyl chloroformate; Et_3N, triethylamine; HOBt, 1-hydroxybenzotriazole; HONSu, *N*-hydroxysuccinimide; MA, mixed anhydride; NMM, *N*-meth-

ylmorpholine; TsOH, *p*-toluenesulfonic acid; THF, tetrahydrofuran.

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