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Synthesis and Effect on Gastric Secretion of Several Di- or Tripeptides Related to Proglumide[1)]

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Several *N*-acyl di- or tripeptides related to proglumide (PhCO–DL-Glu–NPr_2) were prepared and their effects on gastric secretion were examined by intraperitoneal injection in rats. PhCO–Glu(Phe–NH_2)–NPr_2, Z–Glu(Phe–NH_2)–NPr_2, PhCO–Glu(NPr_2)–Phe–NH_2 and PhCO–Asp–(Phe–NH_2)–NPr_2 inhibited gastric secretion, while PhCO–Glu(Asp–Phe–NH_2)–NPr_2 stimulated gastric secretion. Of these peptides, PhCO–Glu(Phe–NH_2)–NPr_2 showed the most potent inhibitory activity against gastric secretion, and was more potent than proglumide.

Keywords——proglumide; *N*-acyl-γ-L-glutamyl peptide; γ-glutamylphenylalanine amide; peptide synthesis; anti-gastric secretion

In the previous study, we investigated the effects of *N*-acyl-γ-D-glutamyl peptides containing a C-terminal small fragment of cholecystokinin (CCK) on gastric secretion.[2)] It was found that PhCO–D-Glu(Phe–NH_2)–NPr_2 and PhCO–D-Glu(Asp–Phe–NH_2)–NPr_2 inhibited gastric secretion and that Z–D-Glu(Phe–NH_2)–NPr_2 was the most potent of the peptides investigated by us.

The present investigation on *N*-acyl-γ-L-glutamyl derivatives showed that the structure–activity relationship of these compounds was not parallel with that of *N*-acyl-γ-D-glutamyl compounds. Thus, PhCO–Glu(Phe–NH_2)–NPr_2 (Ia) inhibited gastric secretion more strongly than Z–Glu(Phe–NH_2)–NH_2 (IIa), while PhCO–Glu(Asp–Phe–NH_2)–NPr_2 (Ib) stimulated gastric secretion, in contrast to the inhibitory activity of PhCO–D-Glu(Asp–Phe–NH_2)–NPr_2. This report describes the syntheses of Ia, b and some analogs of Ia, as well as their effect on gastric secretion.

Compounds Ia, b were synthesized as shown in Fig. 1. Z–Glu–OH[3)] was reacted with paraformaldehyde to prepare the oxazoline compound according to the procedure of Itoh,[4)] followed by reaction with di-*n*-propylamine ($HNPr_2$) to give Z–Glu–NPr_2. Z–Glu–NPr_2 was coupled with H–Phe–NH_2[5)] or H–Asp–Phe–NH_2[5)] by the mixed acid anhydride method to produce the dipeptide derivative (IIa) and tripeptide derivative (IIb). Compounds IIa, b were deprotected by hydrogenolysis over Pd catalyst, then acylated with benzoyl chloride in the presence of $NaHCO_3$ to give Ia, b.

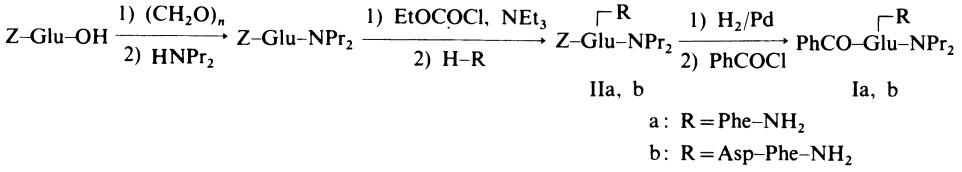

Fig. 1

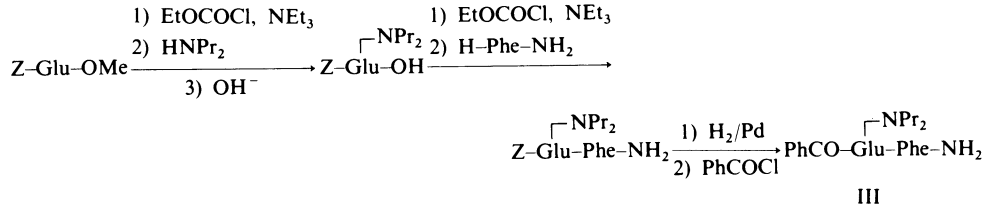

Fig. 2

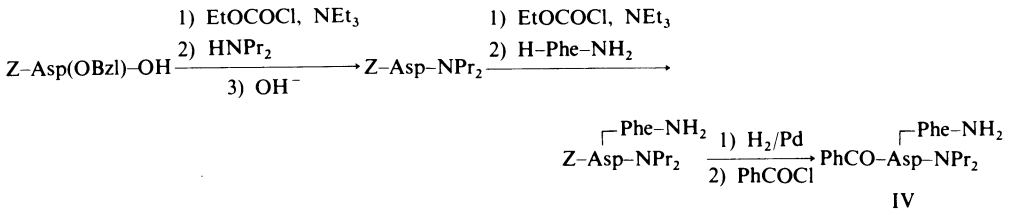

Fig. 3

In order to investigate analogs of Ia, PhCO–Glu(NPr_2)–Phe–NH_2 (III) and PhCO–Asp(Phe–NH_2)–NPr_2 (IV) were prepared. The synthetic scheme for III is shown in Fig. 2. Z–Glu–OMe[6] was condensed with $HNPr_2$ by the mixed acid anhydride method then saponified to afford Z–Glu(NPr_2)–OH, which was coupled with H–Phe–NH_2[5] by the mixed acid anhydride method to produce Z–Glu(NPr_2)–Phe–NH_2. This compound was deprotected and acylated to give III in the same way as described for Ia.

The synthetic method for PhCO–Asp(Phe–NH_2)–NPr_2 (IV) is illustrated in Fig. 3. Z–Asp(OBzl)–OH[7] was coupled with $HNPr_2$ by the mixed acid anhydride method then saponified to give Z–Asp–NPr_2. Z–Asp–NPr_2 was linked with H–Phe–NH_2, then the resulting Z–Asp(Phe–NH_2)–NPr_2 was deprotected and acylated to give IV according to the procedure described for Ia.

The reference compound, proglumide (PhCO–DL-Glu–NPr_2) was extracted from a commercial preparation of proglumide (PROMID®). Another reference compound, L-proglumide (L form in proglumide) was prepared from Z–Glu–NPr_2. Thus, Z–Glu–NPr_2 was deprotected by hydrogenolysis, then acylated with benzoyl chloride to give L-proglumide in the same way as described for Ia.

The synthesized peptides were shown to be homogeneous by thin-layer chromatography (TLC) on silica gel and gave the expected elemental analyses. Amino acid analyses of acid hydrolysates of these peptides gave results in good agreement with the theoretically expected values.

The effects of the synthesized peptides (Ia, Ib, IIa, III, IV), proglumide and L-proglumide on gastric secretion in rats were examined in the same manner as described by Watanabe *et al.* for the evaluation of proglumide.[8] The test compounds suspended in 1% gum arabic solution were injected intraperitoneally into rats. The control rats were injected with 1% gum arabic solution. The volume of gastric juice secreted during 4 h after injection of the test compound, free acidity and total acidity of the gastric juice were measured and expressed as the ratio (%) with respect to the control value (Table I).

Compound Ia having a Phe–NH_2 residue inhibited gastric secretion more strongly than proglumide or L-proglumide. On the other hand, compound Ib having a Asp–Phe–NH_2 residue stimulated gastric secretion but appeared to induce autoinhibition at a high dose, as pentagastrin and MBOC–Met–Asn–Phe–OH do.[9] These results may be explained in terms of two types of gastrin receptor, a low-affinity gastrin receptor which leads to inhibition of

TABLE I. Effects of Di- or Tripeptides Related to L-Proglumide on Gastric Secretion in Rats

Compound	Dose, i.p. mg/kg (mmol/kg)	Number of rats	Gastric juice (%, mean ± S.E.)[a]		
			Volume	Free acidity	Total acidity
Ia	100 (208)	3	66.7 ± 17.6	61.2 ± 28.5	89.0 ± 11.3
	300 (624)	3	37.0 ± 4.8[c]	30.1 ± 9.0[d]	51.4 ± 5.9[d]
Ib	100 (168)	3	195.8 ± 13.9[c]	151.6 ± 18.3[b]	123.6 ± 10.5[b]
	300 (504)	3	118.2 ± 7.2	162.7 ± 11.0[c]	137.7 ± 5.2[d]
	1000 (1679)	3	88.5 ± 26.7	93.8 ± 38.4	98.8 ± 25.4
IIa	300 (587)	5	55.7 ± 11.4[b]	75.0 ± 23.6	85.0 ± 7.8
III	300 (624)	5	45.5 ± 14.2[b]	64.4 ± 18.9	87.4 ± 6.6
IV	300 (643)	6	35.2 ± 6.3[d]	62.5 ± 18.5	99.1 ± 5.2
L-Proglumide	100 (299)	3	79.4 ± 22.0	98.6 ± 6.7	97.8 ± 1.5
	300 (897)	3	57.7 ± 12.4[b]	92.7 ± 5.8	98.0 ± 5.0
	1000 (2990)	3	25.8 ± 7.9[c]	38.8 ± 6.2[c]	70.2 ± 1.5[d]
Proglumide	100 (299)	6	63.0 ± 6.7	70.8 ± 7.3	98.0 ± 3.9
	300 (897)	6	53.8 ± 7.6[b]	60.6 ± 10.8	85.4 ± 4.0
	1000 (2990)	6	24.4 ± 1.7[c]	20.6 ± 6.9[c]	54.2 ± 5.5[c]

a) Data are expressed as the ratio (%) with respect to the control value; *b*) $p<0.05$; *c*) $p<0.01$; *d*) $p<0.001$.

secretion and a high-affinity gastrin receptor which activates secretion.[9a] Thus, L-proglumide would have specific affinity for the low-affinity gastrin receptor, and the introduction of a Phe–NH_2 residue into L-proglumide increased the affinity. On the other hand, the introduction of an Asp–Phe–NH_2 residue into L-proglumide resulted in higher affinity for the high-affinity gastrin receptor than the low-affinity gastrin receptor, consequently stimulating gastric secretion. When a high dose of Ib was given, some Ib would bind to the low-affinity gastrin receptor and would induce autoinhibition.

The stimulative activity of Ib forms a contrast with the inhibitory activity of the epimer [PhCO–D-Glu(Asp–Phe–NH_2)–NPr_2] described in the previous paper.[2] The present result for Ib and the previous result for the epimer of Ib show that the configuration of the glutamyl moiety of these tripeptide is very important for binding to the gastrin receptor.

The *N*-benzyloxycarbonyl compound (IIa) inhibited gastric secretion but appeared to be less potent than the *N*-benzoyl compound (Ia). In the previous study on D-glutamyl peptides, Z–D-Glu(Phe–NH_2)–NPr_2 was more potent than PhCO–D-Glu(Phe–NH_2)–NPr_2.[2] Not only the result for Ib but also the result for IIa show that the structure–activity relationship for gastric secretion of *N*-acyl–γ-L-glutamyl peptides is not parallel with that of *N*-acyl–γ-D-glutamyl peptides.

In order to investigate further the structural requirements for the glutamyl moiety, the α-glutamyl dipeptide (III) and β-aspartyl dispeptide (IV) were investigated. These compounds inhibited gastric secretion, but their inhibitory activities in terms of the acidity of gastric juice were less than that of Ia. This means that the Glu(Phe–NH_2) moiety is preferable to the Glu–Phe–NH_2 moiety or Asp(Phe–NH_2) moiety.

In conclusion, Ia has a more potent inhibitory activity against gastric secretion than proglumide, and the structure–activity relationship for gastric secretion of *N*-acyl–γ-L-glutamyl peptides is not parallel with that of *N*-acyl–γ-D-glutamyl peptides.

Experimental

The melting points are uncorrected. Optical rotations were measured with a DIP-181 polarimeter (Japan Spectroscopic Co.). Amino acid analyses of acid hydrolysates were performed according to the procedure of Lee *et*

al.[10] Elementary analyses were carried out with a Yanagimoto MT-3 CHN Corder. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNK PS-100 high-resolution NMR spectrometer; chemical shifts are given in δ (ppm) with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Ascending TLC was performed on silica gel TLC plates (Kieselgel 60 F_{254}, Merck) using the following solvent systems: Rf^1, benzene–AcOH (3 : 1); Rf^2, $CHCl_3$–acetone (1 : 1); Rf^3, $CHCl_3$–acetone (3 : 1); Rf^4, *n*-BuOH–AcOH–H_2O (4 : 1 : 5).

Z–Glu–NPr$_2$——Z–Glu–OH[3] (80 g) was reacted with paraformaldehyde (14 g) in the presence of *p*-toluenesulfonic acid hydrate (2.8 g) in benzene (2 l) according to the procedure of Itoh[4] to give benzyloxycarbonyl-5-oxo-4-oxazolizinepropionic acid as an oil (89.9 g). This oil (89.9 g) and $HNPr_2$ (62 g) were dissolved in THF (180 ml) and the mixture was refluxed for 8 h. After evaporation of the solvent *in vacuo*, the residue was dissolved in AcOEt, then washed with 3% HCl and H_2O. The solution was extracted with 1 N NaOH (600 ml). The water layer was acidified with concentrated HCl under cooling with ice and extracted with AcOEt (700 ml). The extract was washed with H_2O and brine, then dried over anhydrous $MgSO_4$. The solvent was evaporated off *in vacuo* and the residue was crystallized from ether to give needles. Yield 46 g (44%), mp 82—85 °C, $[\alpha]_D^{20}$ −25.8° (c=5, MeOH). *Anal.* Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; , 7.69. Found: C, 62.56; H, 7.77; N, 7.78.

Z–Glu(Phe–NH$_2$)–NPr$_2$ (IIa)——Ethyl chloroformate (5.71 ml) was added to a mixture of Z–Glu–NPr_2 (21.9 g) and NEt_3 (8.4 ml) in anhydrous THF (350 ml) at −15—−10 °C, followed by stirring for 10 min. A mixture of H–Phe–NH_2·HBr (14.7 g, obtained by deprotection of Z–Phe–NH_2[5] with HBr/AcOH) and NEt_3 (8.4 ml) in anhydrous THF (225 ml) was added at −10—−5 °C and the whole was stirred at −10—0 °C for 5 h. Insoluble material was removed by filtration and the solvent was evaporated off *in vacuo*. The residue was dissolved in AcOEt (1.2 l), washed successively with 3% $NaHCO_3$, 3% HCl and H_2O, then dried over Na_2SO_4. The solution was concentrated to about 200 ml *in vacuo* and cooled to about 15 °C. The resulting needles were collected by filtration. Yield 24.6 g (80.3%), mp 150—153 °C. For analysis and biological assay, the above product was recrystallized from AcOEt, mp 152—153 °C, $[\alpha]_D^{20}$ −9.9° (c=1, MeOH), Rf^1 0.43, Rf^2 0.28. *Anal.* Calcd for $C_{28}H_{38}N_4O_5$: C, 65.86; H, 7.50; N, 10.97. Found: C, 65.92; H, 7.64; N, 11.01.

PhCO–Glu(Phe–NH$_2$) (Ia)——A solution of IIa (3.2 g) in MeOH (60 ml) containing 5.6 N HCl/dioxane (2 ml) was hydrogenated over a palladium catalyst (10% Pd–C, 0.1 g) with bubbling of hydrogen at room temperature for 2 h. After removal of the catalyst, the solvent was evaporated off *in vacuo* to give H–Glu(Phe–NH_2)·HCl, which was dissolved in H_2O (50 ml) containing $NaHCO_3$ (1.1 g). To this aqueous solution, benzoyl chloride (0.89 g) in ether (20 ml) was added under cooling with ice, and the mixture was stirred for 3 h. The reaction mixture was extracted with AcOEt (200 ml), and the extract was washed with H_2O and dried over anhydrous $MgSO_4$. AcOEt was evaporated off *in vacuo* and the residue was triturated with ether to afford a crude product, which was recrystallized from AcOEt–hexane. Yield 2.2 g (68%), mp 149—151 °C, $[\alpha]_D^{20}$ −1.8° (c=1, DMF), $[\alpha]_{365}^{20}$ +16.1° (c=1, DMF), Rf^1 0.42, Rf^2 0.27. Amino acid ratio in an acid hydrolysate: Glu, 0.96; Phe, 1.04 (average recovery, 94%). *Anal.* Calcd for $C_{27}H_{36}N_4O_4$: C, 67.48; H, 7.55; N, 11.66. Found: C, 67.27; H, 7.56; N, 11.56.

Z–Glu(Asp–Phe–NH$_2$)–NPr$_2$ (IIb)——Boc–Asp–Phe–NH_2[11] (3.8 g) was added to TFA (15 ml) under cooling with ice and the mixture was stirred for 1 h. Ether (50 ml) was added to give a precipitate, which was washed with ether to give H–Asp–Phe–NH_2·TFA. This material was dissolved in H_2O (50 ml) containing NEt_3 (2.8 ml) under cooling with ice. To this aqueous solution was added a mixed acid anhydride prepared from Z–Glu–NPr_2 (3.2 g), ethyl chloroformate (0.96 g) and NEt_3 (1.3 ml) in anhydrous THF (50 ml) at 0—5 °C. After the mixture had been stirred at 7—8 °C overnight, the solvent was evaporated off *in vacuo*. The residue was triturated with 2% HCl to give a crude product, which was recrystallized from MeOH. Yield 3.9 g (70%), mp 196.0—197.5 °C, $[\alpha]_D^{20}$ −37.8° (c=1, DMF), Rf^1 0.25, Rf^4 0.69. *Anal.* Calcd for $C_{32}H_{43}N_5O_8 \cdot 1/4H_2O$: C, 60.98; H, 6.95; N, 11.11. Found: C, 60.95; H, 7.03; N, 11.18.

PhCO–Glu(Asp–Phe–NH$_2$)–NPr$_2$ (Ib)——Compound IIb (1.6 g) was deprotected and acylated with benzoyl chloride (0.37 g) in the same way as described for Ia. The reaction mixture was acidified with concentrated HCl. The resulting precipitate was collected by filtration, washed with ether and H_2O, then recrystallized from iso-PrOH. Yield 1.1 g (71%), mp 206—208 °C, $[\alpha]_D^{20}$ −48.4° (c=1, DMF), Rf^1 0.24, Rf^4 0.69. Amino acid ratio in an acid hydrolysate: Asp, 1.04; Glu, 0.99; Phe, 0.97 (average recovery, 98%). *Anal.* Calcd for $C_{31}H_{41}N_5O_7$: C, 62.51; H, 6.94; N, 11.76. Found: C, 62.27; H, 6.89; N, 11.76.

Z–Glu(NPr$_2$)–OH——A mixture of Z–Glu–OMe·DCHA[6] (17 g) and 5% $KHSO_4$ aqueous solution (150 ml) was stirred vigorously at room temperature for 1 h and the resulting oily product was extracted with AcOEt (150 ml). The extract was dried over anhydrous $MgSO_4$ and the solvent was evaporated *in vacuo* to give Z–Glu–OMe as an oil, which was dissolved in anhydrous THF (150 ml). NEt_3 (5 ml) and ethyl chloroformate (3.4 ml) were added at −10—−5 °C and the mixture was stirred for 15 min. HNPr (3.6 g) was added and the whole was stirred under cooling with ice for 4 h. Resulting NEt_3·HCl was removed by filtration, the solvent was evaporated off *in vacuo*, and the residue was dissolved in AcOEt (200 ml). The solution was washed successively with 2% HCl, 3% $NaHCO_3$ and H_2O, then dried over anhydrous $MgSO_4$. The solvent was evaporated off *in vacuo* to give Z–Glu(NPr_2)–OMe (14.2 g) as an oil. This oil was dissolved in MeOH (30 ml), then 1 N NaOH (45 ml) was added. The mixture was stirred at room temperature for 3 h and concentrated *in vacuo* to about 45 ml. The concentrate was washed with AcOEt, acidified with

concentrated HCl and extracted with AcOEt (100 ml). The extract was washed with H_2O and dried over anhydrous $MgSO_4$. The solvent was evaporated off *in vacuo* and the residue was crystallized from AcOEt–hexane. Yield 8.3 g (63%), mp 101—102 °C, $[\alpha]_D^{20}$ −11.4° (*c*=1, MeOH), Rf^1 0.54, Rf^4 0.66. *Anal.* Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.43; H, 7.69; N, 7.77.

Z–Glu(NPr$_2$)–Phe–NH$_2$——Z–Glu(NPr$_2$)–OH was coupled with H–Phe–NH$_2$ by the mixed acid anhydride method in the same manner as described for IIa. The product was recrystallized from AcOEt–hexane. Yield 82%, mp 128—130 C, $[\alpha]_D^{20}$ −21.0° (*c*=1, MeOH), Rf^1 0.62, Rf^2 0.63. *Anal.* Calcd for $C_{28}H_{38}N_4O_5$: C, 65.86; H, 7.50; N, 10.97. Found: C, 65.75; H, 7.50; N, 10.81.

PhCO–Glu(NPr$_2$)–Phe–NH$_2$ (III)——Z–Glu(NPr$_2$)–Phe–NH$_2$ (1.4 g) was deprotected and acylated with benzoyl chloride according to the procedure described for Ia. The reaction mixture was acidified with HCl and extracted with $CHCl_3$ (100 ml). The extract was washed with 2% $NaHCO_3$ and water, then dried over anhydrous $MgSO_4$. The solvent was evaporated off *in vacuo* and the residue was recrystallized from AcOEt. Yield 0.7 g (60%), mp 184—185.5 C, Rf^1 0.44, Rf^2 0.45, Rf^3 0.15, $[\alpha]_D^{20}$ −32.0° (*c*=1, MeOH). Amino acid ratio in an acid hydrolysate: Glu, 0.97; Phe, 1.03 (average recovery, 95%). *Anal.* Calcd for $C_{27}H_{36}N_4O_4$: C, 67.48; H, 7.55; N, 11.66. Found: C, 67.58; H, 7.55; N, 11.44.

Z–Asp–NPr$_2$——Z–Asp(OBzl)–OH[7] (10 g) was coupled with HNPr$_2$ (3.0 g) by the mixed acid anhydride method in the same way as described for IIa to give oily Z–Asp(OBzl)–NPr$_2$ (9.6 g). This compound was dissolved in MeOH (60 ml), then 1 N NaOH (26 ml) was added. The mixture was stirred at room temperature for 3 h and concentrated *in vacuo* to about 25 ml. After addition of H_2O (80 ml), the aqueous solution was washed with AcOEt, acidified with 4 N HCl and extracted with AcOEt (2 × 200 ml). The extract was washed successively with H_2O and brine, then dried over Na_2SO_4. The solvent was evaporated off *in vacuo* to give an oil. Yield 6 g (61%). ^{1}H-NMR (in $CDCl_3$): 0.72—1.04 (6H, m), 1.38—1.84 (4H, m), 2.54—2.96 (2H, m), 3.02—3.52 (4H, m), 4.86—5.20 (1H, m), 5.10 (2H, s), 6.02 (1H, d, *J*=10 Hz), 6.8—7.2 (1H, br), 7.52 (5H, s).

Z–Asp(Phe–NH$_2$)–NPr$_2$——This compound was obtained from Z–Asp–NPr$_2$ (6.0 g) and H–Phe–NH$_2$·HBr (4.19 g) according to the procedure described for IIa. The crude product was recrystallized from AcOEt–MeOH. Yield 5.6 g (66%), mp 161—163 °C, $[\alpha]_D^{20}$ −17.8° (*c*=0.51, MeOH). *Anal.* Calcd for $C_{27}H_{36}N_4O_5$: C, 65.30; H, 7.31; N, 11.28. Found: C, 65.32; H, 7.15; N, 11.12.

PhCO–Asp(Phe–NH$_2$)–NPr$_2$ (IV)——Z–Asp(Phe–NH$_2$)–NPr$_2$ (1.8 g) was deprotected and the deprotected compound was acylated with benzoyl chloride in the same way as described for Ia. The acylation produced a white precipitate. After acidification of the reaction mixture with 4 N HCl, the precipitate was collected by filtration, washed with H_2O and ether, then recrystallized from iso-PrOH. Yield 1.2 g (55%), mp 188—189 °C, $[\alpha]_D^{20}$ −42.9° (*c*=1, MeOH), Rf^1 0.52, Rf^2 0.44. Rf^3 0.12. Amino acid ratio in an acid hydrolysate: Asp, 1.02; Phe, 0.98 (average recovery, 94%). *Anal.* Calcd for $C_{26}H_{34}N_4O_4$: C, 66.93; H, 7.34; N, 12.00. Found: C, 66.99; H, 7.29; N, 11.89.

PhCO–Glu–NPr$_2$ (L-Proglumide)——Z–Glu–NPr$_2$ (5 g) in MeOH (100 ml) was hydrogenated over 10% Pd–C (1 g) at room temperature for 3 h by bubbling H_2 gas through the solution catalyst was removed by filtration and the solvent was evaporated off *in vacuo* to give H–Glu–NPr$_2$ (3.2 g). This compound was acylated with benzoyl chloride (2 g) in the same way as described for Ia. The reaction mixture was acidified with concentrated HCl to give a precipitate, which was collected by filtration, washed with H_2O and recrystallized from 50% EtOH. Yield 3.7 g (79%), mp 132—133 °C, $[\alpha]_D^{20}$ −25.9° (*c*=5, MeOH). *Anal.* Calcd for $C_{18}H_{26}N_2O_4$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.60; H, 7.78; N, 8.42.

PhCO–DL-Glu–NPr$_2$ (Proglumide)——This compound was extracted with AcOEt from PROMID® (Kaken Seiyaku) and recrystallized from 50% EtOH, mp 148.5—149.5 °C (lit.[8] 148—149 °C).

Determination of Gastric Acid Secretion——Male SD rats (150—200 g) were fasted for 48 h and then anesthetized with ether. Their pylorus was ligated by the method of Shay *et al.*,[12] and a test compound suspension in 1% gum arabic solution was injected intraperitoneally immediately after ligation. The volume of injected suspension was 5 ml/kg for each test compound. Then, 4 h after the ligation, the rats were anesthetized with ether and their stomachs were removed according to Watanabe *et al.*[8] The content in the stomach was centrifuged to obtain a supernatant. The gastric juice volume was measured by measuring the supernatant. An aliquot of the supernatant was titrated with 0.02 N NaOH by using phenolphtalain and methyl yellow as indicators to determine the acidity of free acid (meq/l) and the acidity of total acid (meq/l), respectively.

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References and Notes

1) The customary L indication for amino acid residues is omitted. Standard abbreviations for amino acids and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [*Biochemistry*, **5**, 2485 (1966); **6**, 362 (1967); **11**, 1726 (1972)]. Other abbreviations used are: Boc, *tert*-

butyloxycarbonyl; Z, benzyloxycarbonyl; PhCO, benzoyl; OMe, methyl ester; OBzl, benzyl ester; NPr_2, di-*n*-propylamino; DCHA, dicyclohexylamine; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; TFA, trifluoroacetic acid; AcOEt, ethyl acetate; AcOH, acetic acid; iso-PrOH, 2-propanol; *n*-BuOH, 1-butanol.

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