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Synthesis of *N*-Acyl-γ-D-glutamyl Peptide Derivatives Containing a C-Terminal Small Fragment of Cholecystokinin and Their Effects on Gastric Secretion[1)]

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N-Acyl-γ-D-glutamyl peptide derivatives containing a C-terminal small fragment of cholecystokinin were prepared and their effects on gastric secretion were investigated. PhCO–D-Glu(Phe–NH_2)–NPr_2 and PhCO–D-Glu(Asp–Phe–NH_2)–NPr_2 inhibited gastric secretion, while PhCO–D-Glu(Met–Asp–Phe–NH_2)–NPr_2 and PhCO–D-Glu(Trp–Met–Asp–Phe–NH_2)–NPr_2 stimulated gastric secretion. The substitution of the acyl function at the N-terminal of PhCO–D-Glu(Phe–NH_2)–NPr_2 affected the activity. Z–D-Glu(Phe–NH_2)–NPr_2, 4-chlorobenzoyl-D-Glu-(Phe–NH_2)–NPr_2 and isonicotinoyl-D-Glu(Phe–NH_2)–NPr_2 were found to have more potent inhibitory activity against gastric secretion than proglumide (PhCO–DL-Glu–NPr_2).

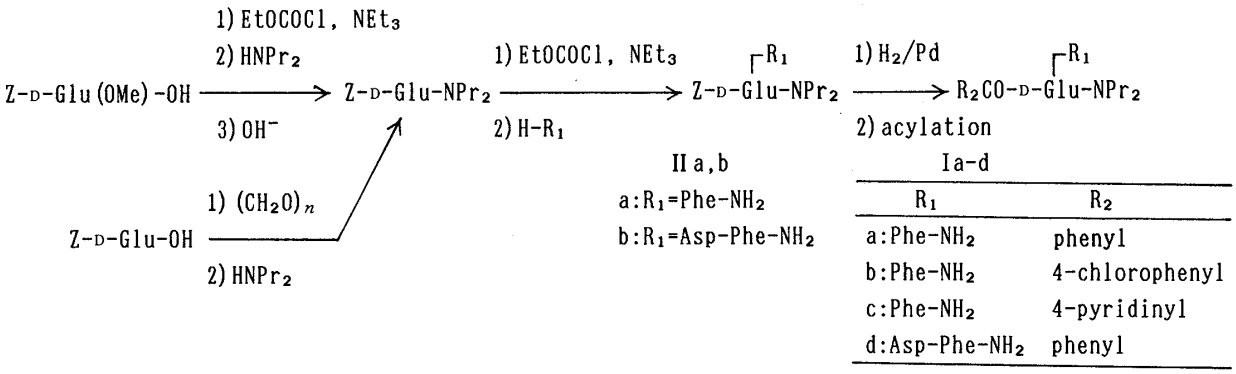
Keywords——proglumide derivative; *N*-acyl-γ-D-glutamyl peptide; CCK C-terminal small fragment; peptide synthesis; anti-gastric secretion

Proglumide (PhCO–DL-Glu–NPr_2) was found to inhibit gastric acid production,[2)] and this inhibitory activity was indicated by the results of binding assay to be due to interference of proglumide with the affinity of gastrin for gastric mucosal membrane receptors.[3)] It was also reported that proglumide functioned as an antagonist of cholecystokinin (CCK).[4)]

On the other hand, CCK behaved as a fairly specific inhibitor of gastrin-stimulated gastric acid secretion and this observation has been explained by considering CCK to act on the same receptor as that for gastrin[5)] or to act on a low-affinity gastrin-receptor which leads to inhibition of acid secretion.[6)] Not only the C-terminal tetrapeptide of gastrin but also the C-terminal tripeptide of gastrin stimulated gastric acid secretion in the dog[7)] and C-terminal fragments of CCK having fewer than four amino acids functioned as antagonists of CCK.[8)]

Gastrin and CCK have a common peptide sequence at their C-terminal (–Gly–Trp–Met–Asp–Phe–NH_2). The above reports suggested to us that C-terminal small fragments of gastrin, that is, C-terminal small fragments of CCK, would have affinity for gastrin-receptors as well as CCK-receptors.

Therefore, we considered that a compound constructed from a C-terminal small fragment of CCK and a proglumide moiety would also have affinity for the gastrin-receptor or the CCK-receptor, and that it may be possible to synthesize such peptide derivatives which would inhibit gastric acid secretion and CCK-like activity more strongly than proglumide. This consideration prompted us to investigate proglumide derivatives containing a C-terminal fragment of CCK. Our preliminary study on proglumide activity against gastric secretion showed that the D-isomer, PhCO–D-Glu–NPr_2 (D-proglumide), had more potent inhibitory activity than the L-isomer, PhCO–Glu–NPr_2 (L-proglumide). The present report describes the synthesis of *N*-acyl-γ-D-glutamyl peptide derivatives which contains a C-terminal small fragment of CCK. The CCK-antagonistic activities of some of these compounds have been reported elsewhere.[9)]



R_1	R_2
a:Phe-NH_2	phenyl
b:Phe-NH_2	4-chlorophenyl
c:Phe-NH_2	4-pyridinyl
d:Asp-Phe-NH_2	phenyl

Fig. 1

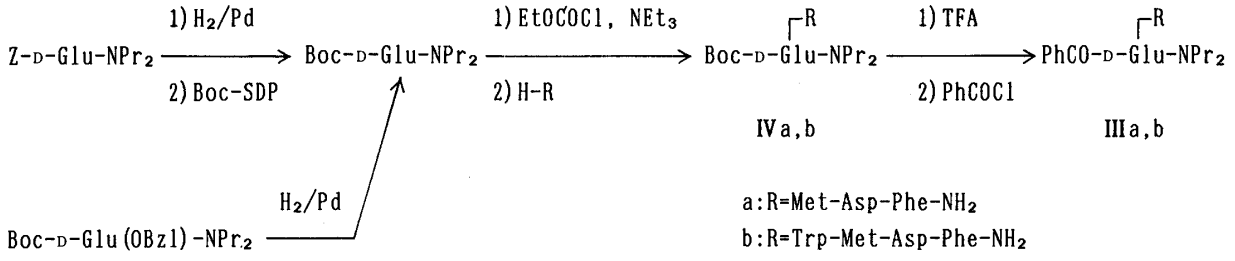


Fig. 2

N-Acyl-D-Glu(Phe–NH_2)–NPr_2 (Ia—c) and PhCO–D-Glu(Asp–Phe–NH_2)–NPr_2 (Id) were synthesized as shown in Fig. 1. The α-amino function was protected with the Z group. *N*-Acylation of peptides was performed in the final step in order to avoid racemization. Z–D-Glu–NPr_2 was prepared by the saponification of Z–D-Glu(OMe)–NPr_2 obtained from Z–D-Glu(OMe)–OH[10] and di-*n*-propylamine ($HNPr_2$), or was prepared from Z–D-Glu–OH[11] according to the procedure of Itoh.[12] Z–D-Glu–NPr_2 was coupled with H–Phe–NH_2[13] or H–Asp–Phe–NH_2[13] by the mixed acid anhydride method to give Z–γ-D-glutamyl peptide derivatives (IIa, b). Compounds IIa, b were deprotected by hydrogenolysis over palladium catalyst, then the deprotected products were acylated with aromatic acyl chloride in the presence of $NaHCO_3$ to give Ia, b and Id. The isonicotinoyl compound (Ic) was obtained by acylation with the mixed acid anhydride of isonicotinic acid.

PhCO–D-Glu(Met–Asp–Phe–NH_2)–NPr_2 (IIIa) and PhCO–D-Glu(Trp–Met–Asp–Phe–NH_2)–NPr_2 (IIIb) were synthesized as shown in Fig. 2. In order to avoid catalytic hydrogenolysis after introduction of the methionine residue, the intermediates were protected with the Boc group. Z–D-Glu–NPr_2 was deprotected by hydrogenolysis and reprotected with the Boc group using *tert*-butyl 4,6-dimethylpyrimidyl-2-thiol carbonate (Boc-SDP)[14] to give Boc–D-Glu–NPr_2. This compound could also be obtained in good yield by the hydrogenolysis of Boc–D-Glu(OBzl)–NPr_2 which was prepared from Boc–D-Glu(OBzl)–OH.[15] Boc–D-Glu–NPr_2 was coupled with H–Met–Asp–Phe–NH_2[13] or H–Trp–Met–Asp–Phe–NH_2[13] by the mixed acid anhydride method to give Boc–γ-D-glutamyl peptide derivatives (IVa, b). Compounds IVa, b were deprotected with TFA and acylated in the same manner as described for Ia to give IIIa, b.

Proglumide was extracted from the commercially available preparation of proglumide, Promid®. D-Proglumide was prepared from Z–D-Glu–NPr_2. Thus, Z–D-Glu–NPr_2 was deprotected by hydrogenolysis, then acylated in the same way as described for Ia to give D-proglumide. L-Proglumide was also prepared analogously using the corresponding starting materials.

The synthesized peptides were shown to be homogeneous by thin-layer chromatography

TABLE I. Effects of *N*-Acyl-glutamyl Compounds on Gastric Secretion

Compound	R_2CO–Glu(R_1)–NPr_2			Dose, i.p. mg/kg (mmol/kg)	Gastric juice (%, mean ± S.E.)[a]		
	R_1[e]	R_2[f]	Glu		Volume	Free acidity	Total acidity
Ia	CCK-1	Ph	D-Glu	300 (624)	48.9 ± 9.1[d]	78.9 ± 12.4	84.1 ± 7.4
Id	CCK-2	Ph	D-Glu	300 (504)	47.9 ± 6.2[c]	32.2 ± 7.7[b]	63.9 ± 1.8[d]
IIIa	CCK-3	Ph	D-Glu	300 (408)	131.8 ± 29.0[c]	143.3 ± 13.1[b]	111.0 ± 8.5
IIIb	CCK-4	Ph	D-Glu	300 (322)	194.5 ± 16.4[d]	211.1 ± 4.3[d]	141.6 ± 1.3[d]
Ib	CCK-1	4-Cl-Ph	D-Glu	300 (582)	26.7 ± 8.2[c]	35.3 ± 11.3[b]	68.5 ± 5.1[c]
Ic	CCK-1	4-Py	D-Glu	300 (622)	27.4 ± 4.8[c]	25.2 ± 8.3[c]	66.4 ± 4.6[d]
IIa	CCK-1	$PhCH_2O$	D-Glu	300 (587)	27.1 ± 2.1[d]	13.4 ± 8.5[c]	72.9 ± 7.8[b]
D-Proglumide	OH	Ph	D-Glu	300 (897)	35.6 ± 4.1[c]	47.4 ± 21.1[b]	73.0 ± 11.4[b]
L-Proglumide	OH	Ph	L-Glu	300 (897)	57.7 ± 12.4[b]	92.7 ± 5.8	98.0 ± 5.0
Proglumide	OH	Ph	DL-Glu	300 (897)	53.8 ± 7.6[b]	60.6 ± 10.8	85.4 ± 4.0
Proglumide	OH	Ph	DL-Glu	1000 (2990)	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*) $p<0.001$. *e*) CCK-1 = Phe–NH_2, CCK-2 = Asp–Phe–NH_2, CCK-3 = Met–Asp–Phe–NH_2, CCK-4 = Trp–Met–Asp–Phe–NH_2. *f*) 4-Cl-Ph = 4-chlorophenyl, 4-Py = 4-pyridinyl.

(TLC) on silica gel and gave the expected elemental analyses. Amino acid analyses of acid hydrolysates of these peptides were in good agreement with the theoretically expected values.

The effects of the synthesized peptides (Ia—d, IIa, IIIa, b), proglumide and its optical isomers on gastric secretion were examined in a manner analogous to that described by Watanabe *et al.* for the evaluation of proglumide.[2c] The test compound suspended in 1% gum arabic was injected intraperitoneally into rats. The control rats were injected with 1% gum arabic alone. The volume of gastric juice secreted during 4 h after injection of the test compound, and the free acidity and total acidity of the gastric juice were measured and expressed as the ratio (%) with respect to the control values (Table I).

Proglumide inhibited gastric secretion, in agreement with the earlier report.[2c] D-Proglumide was more active than L-proglumide and the activity of proglumide was intermediate. PhCO–D-Glu(Phe–NH_2)–NPr_2 (Ia) as well as PhCO–D-Glu(Asp–Phe–NH_2)–NPr_2 (Id) inhibited gastric secretion, while the compound (IIIa, b) having the C-terminal tri- or tetrapeptide of CCK stimulated gastric secretion. Compounds Ib, c and IIa inhibited gastric secretion more strongly than the others, and IIa showed the most potent inhibition against free acid.

The first entries of Table I indicate that the introduction of Phe–NH_2 or Asp–Phe–NH_2 into D-proglumide can be done with retention of the inhibitory effect, but the introduction of Met–Asp–Phe–NH_2 or Trp–Met–Asp–Phe–NH_2 into D-proglumide inverts the activity. This phenomenon may be explained by speculating that two types of gastrin-receptor exist, a low-affinity gastrin-receptor which leads to inhibition of secretion and a high-affinity gastrin-receptor which activates secretion.[6] That is, Ia and Id may bind to a low-affinity gastrin-receptor, while the introduction of Met–Asp–Phe–NH_2 into D-proglumide may result in greater affinity for the high-affinity gastrin-receptor than for the low-affinity gastrin-receptor, and Trp–Met–Asp–Phe–NH_2 shows the same effect to an even greater extent. Although the actual existence of the putative low-affinity gastrin-receptor has not been demonstrated,[6] two types of gastrin-receptor, a low-affinity gastrin-receptor whose physiological role has not been clarified and a high-affinity receptor which activates acid secretion, have already been found.[16]

The substituent at the N-terminal of Ia had an effect on the activity, and it was found that 4-chlorobenzoyl, isonicotinoyl and Z groups were advantageous for the inhibitory activity of

D-Glu(Phe–NH_2) derivatives. This may mean that these acyl groups are more suitable for binding to the low-affinity gastrin-receptor than the benzoyl group. The advantage of the Z group over the benzoyl group has also been observed for the CCK antagonistic action in hog duodenal circular muscle.[9]

In conclusion, we have found some compounds which are more potent than proglumide in terms of inhibition of gastric secretion.

Experimental

Every reaction which involves a methionine residue was performed under a nitrogen atmosphere, and peroxide-free THF and ether stored over ferrous sulfate were used in order to prevent oxidation of the methionine residue.[17] 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.*[18] Elementary analyses were carried out with a Yanagimoto MT-3 CHN Corder. 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, upper phase); Rf^5, AcOEt–pyridine–AcOH–H_2O (60:20:6:11); Rf^6, *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2); Rf^7, *n*-BuOH–AcOH–pyridine–H_2O (60:20:6:24).

Z-D-Glu–NPr_2——(a) A solution of $KHSO_4$ (2.6 g) in H_2O (30 ml) was added to a suspension of Z-D-Glu(OMe)–OH·DCHA[10] (8 g) in AcOEt (100 ml) and the mixture was stirred vigorously under cooling with ice for 30 min. The organic layer was washed with H_2O and brine, then dried over Na_2SO_4. The solvent was evaporated off *in vacuo* to give oily Z-D-Glu(OMe)–OH (4.7 g), which was dissolved in anhydrous THF (50 ml). To this solution, NEt_3 (1.75 g) and ethyl chloroformate (1.9 g) were added successively at −15—0 °C, followed by stirring for 10 min, then a solution of $HNPr_2$ in anhydrous THF (10 ml) was added at the same temperature. The whole was stirred at −10—0 °C for 6 h and the solvent was evaporated off *in vacuo*. The residue was dissolved in AcOEt, and the solution was washed successively with 5% HCl, 5% $NaHCO_3$, H_2O and brine, then dried over Na_2SO_4. Evaporation of the solvent *in vacuo* gave oily Z-D-Glu(OMe)–NPr_2 (5.2 g). This compound was dissolved in THF (20 ml) and 1 N NaOH (16.4 ml) was added. The mixture was stirred at room temperature for 2 h, then concentrated *in vacuo* to about 15 ml, and H_2O (30 ml) was added. The water layer was washed with AcOEt, acidified with 3 N HCl, then extracted with AcOEt (2 × 50 ml). The extract was washed with H_2O and brine, then dried over Na_2SO_4. The solvent was evaporated off *in vacuo* and the residue was crystallized from ether to give needles. Yield 3.5 g (70%), mp 83—85 °C, $[\alpha]_D^{20}$ +26.2° (c=5, MeOH).

(b) Z-D-Glu–OH[11] (78.4 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[12] to give benzyloxycarbonyl-5-oxo-4-oxazolizinepropionic acid as an oil (86 g). A solution of this oil and $HNPr_2$ (56.6 g) in THF (180 ml) was refluxed for 8 h. After evaporation of the solvent *in vacuo*, the residue was dissolved in AcOEt (750 ml). The solution was washed with 3% HCl and H_2O, then extracted with 1 N NaOH (600 ml). The aqueous solution was acidified 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 39.4 g (39%), mp 83—84 °C, $[\alpha]_D^{20}$ +26.4° (c=5, MeOH). *Anal.* Calcd for $C_{19}H_{28}N_2O_5$: C, 62.62; H, 7.74; N, 7.69. Found: C, 62.66; H, 7.95; N, 7.64.

Z-D-Glu(Phe–NH_2)–NPr_2 (IIa)——Ethyl chloroformate (5.71 ml) was added to a mixture of Z-D-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, then a mixture of NEt_3 (8.4 ml) and H–Phe–NH_2·HBr (14.7 g, obtained by deprotection of Z–Phe–NH_2[13] with HBr/AcOH) in anhydrous THF (225 ml) was added at −10——5 °C. 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), and the solution was washed successively with 3% $NaHCO_3$, 3% HCl and H_2O, and then dried over Na_2SO_4. The solvent was evaporated off *in vacuo* and the residue was recrystallized from AcOEt. Yield 22.4 g (73%), mp 159—160 °C, $[\alpha]_D^{20}$ +9.5° (c=1, MeOH), Rf^1 0.58, Rf^2 0.58. *Anal.* Calcd for $C_{28}H_{38}N_4O_5$: C, 65.86; H, 7.50; N, 10.97. Found: C, 65.80; H, 7.51; N, 10.96.

PhCO-D-Glu(Phe–NH_2) (Ia)——A solution of IIa (3.0 g) in MeOH (60 ml) containing 5.6 N HCl/dioxane (1.5 ml) was hydroganated over a palladium catalyst (10% Pd–C, 0.1 g) with bubbling of H_2 at room temperature for 2 h. After removal of the catalyst, the solvent was evaporated off *in vacuo* to give H-D-Glu(Phe–NH_2)·HCl, which was dissolved in H_2O (20 ml) containing $NaHCO_3$ (1.1 g). To this aqueous solution, benzoyl chloride (0.8 g) in ether (20 ml) was added under cooling with ice, and the mixture was stirred for 3 h. The resulting precipitate was collected by filtration, washed successively with 5% HCl, H_2O and ether, then recrystallized from iso-PrOH. Yield 2.1 g (75%), mp 185—187 °C, $[\alpha]_D^{20}$ +12.0° (c=1, MeOH), Rf^1 0.52, Rf^3 0.58. Amino acid ratio in an acid hydrolysate: D-Glu, 0.97; Phe, 1.03 (average recovery, 94%). *Anal.* Calcd for $C_{27}H_{36}N_4O_4$: C, 67.48; H, 7.55; N, 11.66. Found: C, 67.50; H, 7.42; N, 11.68.

4-Chlorobenzoyl-D-Glu(Phe–NH_2) (Ib)——This compound was obtained from IIa and 4-chlorobenzoyl chloride in the same manner as described above and recrystallized from iso-PrOH–petroleum ether. Yield 60%, mp 179—182 °C, $[\alpha]_D^{20}$ +2.0 (c=0.5, MeOH), Rf^1 0.59, Rf^2 0.67, Rf^3 0.37. Amino acid ratio in an acid hydrolysate: D-Glu, 1.04; Phe, 0.96 (average recovery, 92%). *Anal.* Calcd for $C_{27}H_{35}ClN_4O_4$: C, 62.96; H, 6.85; N, 10.88. Found: C, 62.76; H, 6.83; N, 10.88.

Isonicotinoyl-D-Glu(Phe–NH_2)–NPr_2 (Ic)——Compound IIa (4.3 g) was hydrogenated in the same manner as described above to give H-D-Glu(Phe–NH_2)–NPr_2·HCl (3.7 g). On the other hand, a mixed acid anhydride was prepared from NEt_3 (910 mg), ethyl chloroformate (0.86 ml) and isonicotinic acid (1.11 g) at −10—−9 °C in anhydrous THF (60 ml). To this mixed acid anhydride, a mixture of H-D-Glu(Phe–NH_2)–NPr_2·HCl (3.7 g) and NEt_3 (910 mg) in anhydrous THF (40 ml) was added at −10—0 °C, followed by stirring for 4.5 h. After evaporation of the solvent *in vacuo*, the residue was washed successively with H_2O, 2% $NaHCO_3$ and H_2O, dissolved in hot MeOH, treated with active charcoal and recrystallized from MeOH. Yield 2.1 g (48%), mp 207—209 °C, $[\alpha]_D^{20}$ −10.2° (c=0.5, DMF), Rf^1 0.12, Rf^2 0.08, Rf^3 0.03. Amino acid ratio in an acid hydrolysate: D-Glu, 0.96; Phe, 1.04 (average recovery, 92%). *Anal.* Calcd for $C_{26}H_{35}N_5O_4$: C, 64.84; H, 7.33; N, 14.54. Found: C, 64.80; H, 7.25; N, 14.66.

Z-D-Glu(Asp–Phe–NH_2)–NPr_2 (IIb)——A mixed acid anhydride was prepared from Z-D-Glu–NPr_2 (1.46 g), ethyl chloroformate (0.43 g) and NEt_3 (0.41 g) in anhydrous THF (25 ml) according to the procedure described for IIa. H–Asp–Phe–NH_2·H_2O[13] (1.19 g) was dissolved in cold H_2O (25 ml) containing NEt_3 (0.4 g) and the solution was added to the above mixed acid anhydride at −5—0 °C. The mixture was stirred for 5 h, concentrated *in vacuo* to about 20 ml and acidified with concentrated HCl. The precipitate was collected by filtration, washed with H_2O, then recrystallized from MeOH–H_2O. Yield 1.9 g (75%), mp 162—164 °C, $[\alpha]_D^{20}$ −25.0° (c=1, MeOH). *Anal.* Calcd for $C_{32}H_{43}N_5O_8 \cdot 1/2H_2O$: C, 60.55; H, 6.99; N, 11.03. Found: C, 60.64; H, 7.05; N, 11.08.

PhCO-D-Glu(Asp–Phe–NH_2)–NPr_2 (Id)——This compound was obtained in the same way as described for Ia. Recrystallization was performed from MeOH. Yield 65%, mp 197—198 °C, $[\alpha]_D^{20}$ −45.3° (c=1, DMF), Rf^1 0.48, Rf^4 0.76. Amino acid ratio in an acid hydrolysate: Asp, 0.99; D-Glu, 0.97; Phe, 1.04 (average recovery, 94%). *Anal.* Calcd for $C_{31}H_{41}N_5O_7$: C, 62.51; H, 6.94; N, 11.76. Found: C, 62.60; H, 6.92; N, 11.69.

Boc-D-Glu(OBzl)–NPr_2——A mixed acid anhydride was prepared from Boc-D-Glu(OBzl)–OH[15] (17.9 g), NEt_3 (7.9 ml) and ethyl chloroformate (5.4 ml), then reacted with $HNPr_2$ (5.7 g) in the same way as described for Z-D-Glu(OMe)–NPr_2 in the preparation of Z-D-Glu–NPr_2. NEt_3·HCl was removed by filtration, the solvent was evaporated off *in vacuo* and the residue was dissolved in AcOEt. The solution was washed successively with 5% citric acid, 3% $NaHCO_3$, H_2O and brine, then dried over Na_2SO_4. Evaporation of the solvent *in vacuo* and recrystallization of the residue from AcOEt–hexane gave needles. Yield 16 g (70%), mp 69—70 °C, $[\alpha]_D^{20}$ +26.8° (c=1, MeOH). *Anal.* Calcd for $C_{23}H_{36}N_2O_5$: C, 65.69; H, 8.63; N, 6.66. Found: C, 65.72; H, 8.70; N, 6.71.

Boc-D-Glu–NPr_2——(a) A solution of Boc-D-Glu(OBzl)–NPr_2 (15.8 g) was hydrogenated over palladium black (300 mg) at room temperature under the pressure of 2 kg/cm^2 for 1 h. After removal of the catalyst, the solvent was evaporated off *in vacuo* and the residue was recrystallized from AcOEt to give prisms. Yield 11.8 g (95%), mp 139.5—141 °C, $[\alpha]_D^{20}$ +32.2° (c=1, MeOH). *Anal.* Calcd for $C_{16}H_{30}N_2O_5$: C, 58.16; H, 9.15; N, 8.48. Found: C, 58.11; H, 9.11; N, 8.59.

(b) A solution of Z-D-Glu–NPr_2 (6.6 g) in MeOH (80 ml) was hydrogenated over palladium black (50 mg) with bubbling of H_2 at room temperature for 3 h. The catalyst and the solvent was removed, then the residue was triturated with ether to give H-D-Glu–NPr_2 (3.6 g, mp 93—94.5 °C). This compound (2.3 g) was reacted with Boc-SDP in the presence of NEt_3 (3.2 ml) in dioxane–H_2O (1 : 1, 15 ml) and the reaction mixture was worked up according to the procedure described for *tert*-butyloxycarbonization of amino acids.[14] The product was recrystallized from AcOEt–petroleum ether. Yield 2.7 g (71%), mp 140—141 °C, $[\alpha]_D^{20}$ +32.0° (c=1, MeOH).

Boc-D-Glu(Met–Asp–Phe–NH_2)–NPr_2 (IVa)——A mixed acid anhydride was prepared in anhydrous THF (15 ml) using Boc-D-Glu–NPr_2 (825 mg), ethyl chloroformate (270 mg) and NEt_3 (252 mg) in the same way as described for IIa. A cold mixture of H–Met–Asp–Phe–NH_2·TFA[13] (1.32 g) and NEt_3 (510 mg) in H_2O (13 ml) was added under ice cooling. The whole was stirred for 4 h, acidified with citric acid and concentrated to about 10 ml. The resulting precipitate was collected by filtration, washed with H_2O and AcOEt, then recrystallized from ether–petroleum ether. Yield 1.22 g (67%), mp 179—180 °C (dec.), $[\alpha]_D^{20}$ −24.1° (c=1, MeOH), Rf^5 0.64, Rf^6 0.66, Rf^7 0.68. *Anal.* Calcd for $C_{34}H_{54}N_6O_9S$: C, 56.49; H, 7.53; N, 11.63. Found: C, 56.45; H, 7.48; N, 11.72.

PhCO-D-Glu(Met–Asp–Phe–NH_2)–NPr_2 (IIIa)——A mixture of IVa (1.45 g), TFA (5.5 ml) and thioanisole (0.3 ml) was stirred under cooling in an ice bath for 1 h and poured into ether (100 ml). The resulting precipitate was collected by filtration, washed with ether and dried over KOH to give H-D-Glu(Met–Asp–Phe–NH_2)–NPr_2·TFA (1.47 g). This compound (1.4 g) was dissolved in THF–H_2O (1 : 1, 30 ml), benzoyl chloride (267 mg) in ether (10 ml) was added under cooling with ice, and the mixture was stirred for 2 h. After acidification of the reaction mixture with 4 N HCl, the reaction mixture was concentrated to about 15 ml. The precipitate was collected by filtration, washed with H_2O and recrystallized from MeOH–ether. Yield 910 mg (67%), mp 222—224 °C, $[\alpha]_D^{20}$ −27.8° (c=0.49, DMF), Rf^5 0.55, Rf^6 0.65, Rf^7 0.69. Amino acid ratio in an acid hydrolysate: Asp, 0.98; D-Glu, 0.98; Met, 1.02; Phe, 1.02. *Anal.* Calcd for $C_{36}H_{50}N_6O_8S$: C, 59.49; H, 6.93; N, 11.56. Found: C, 59.33; H, 7.01; N, 11.27.

PhCO-D-Glu(Trp–Met–Asp–Phe–NH_2)–NPr_2 (IIIb)——From Boc-D-Glu–NPr_2 (1.19 g) and H–Trp–Met–

Asp–Phe–NH_2·TFA[13] (2.56 g), Boc-D-Glu(Trp–Met–Asp–Phe–NH_2)–NPr_2 (IVb, 2.5 g, Rf^5 0.65, Rf^6 0.67, Rf^7 0.72) was obtained in the same manner as described for IVa. This compound contained a small amount of impurity with a low *Rf* value, but was used as such in the next reaction. Compound IVb (1.8 g) was deprotected with TFA and acylated with benzoyl chloride (274 mg) in the same way as described for IIIa, then recrystallized from EtOH–petroleum ether to give IIIb. Yield 948 mg (54%), mp 202—205 °C, $[\alpha]_D^{20}$ −21.6° (c=0.5, DMF), Rf^5 0.58, Rf^6 0.66, Rf^7 0.71. Amino acid ratio in an acid hydrolysate: Asp, 1.07; D-Glu, 0.97; Met, 0.99; Phe, 0.97 (average recovery, 93%). *Anal.* Calcd for $C_{47}H_{60}N_8O_9S \cdot H_2O$: C, 60.63; H, 6.71; N, 12.03. Found: C, 60.38; H, 6.61; N, 11.88.

PhCO–D-Glu–NPr_2 (D-Proglumide)——H–D-Glu–NPr_2 (1.15 g), obtained from Z–D-Glu–NPr_2 by hydrogenolysis, was acylated using benzoyl chloride (0.7 g) and $NaHCO_3$ (0.84 g) in H_2O (8 ml) according to the procedure described for Ia. The reaction mixture was acidified with concentrated HCl and the precipitate was recrystallized from 50% EtOH. Yield 1.0 g (69%), mp 132—134 °C, $[\alpha]_D^{20}$ +27.0° (c=5, MeOH). *Anal.* Calcd for $C_{18}H_{26}N_2O_4$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.56; H, 7.94; N, 8.46.

PhCO–Glu–NPr_2 (L-Proglumide)——This compound was prepared in the same way as described above and recrystallized from 50% EtOH. Yield 75%, mp 132—134 °C, $[\alpha]_D^{20}$ −26.2° (c=5, MeOH). *Anal.* Calcd for $C_{18}H_{26}N_2O_4$: C, 64.65; H, 7.84; N, 8.38. Found: C, 64.61; H, 7.84; N, 8.38.

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.[2c] 148—149 °C).

Determination of Gastric Acid Secretion——Male Sprague-Dawley rats (5—6 rats per group) were fasted for 48 h and then anesthetized with ether. Their pylorus was ligated by the method of Shay *et al.*,[19] and a test compound suspended in 1% gum arabic was injected intraperitoneally immediately after ligation. The volume of injected suspension was 5 ml/kg for each dosage. Then, the rats were anesthetized with ether and their stomachs were removed 4 h after the ligation according to Watanabe *et al.*[2c] The content of 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 using phenolphthalein 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; Boc-SDP, *tert*-butyl 4,6-dimethylpyrimidyl-2-thiol carbonate; 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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