Synthesis of Thyrotropin-Releasing Hormone Analogues. 1. Complete Dissociation of Central Nervous System Effects from Thyrotropin-Releasing Activity†[1]

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Twenty-four thyrotropin-releasing hormone (TRH) analogues containing mainly aliphatic amino acids in position 2 were synthesized and tested for central nervous system (CNS) and hormonal (TSH) activity. Application of the pentafluorophenyl ester method in the syntheses resulted in optimal yields and high purity of the products. The neutral tripeptides pGlu-Nva-Pro-NH_2 (**9**), pGlu-Nle-Pro-NH_2 (**10**), and pGlu-Leu-Pro-NH_2 (**3**) with a three- or four-membered straight or branched alkyl side chain in the position of the central amino acid had 2.5 to 10 times stronger anticataleptic effect than TRH, demonstrating that the presence of histidine is not essential for the CNS activity. Analogue **9** exhibited tenfold anticataleptic activity as compared to TRH, and it was found to be fully inactive in the release of TSH.

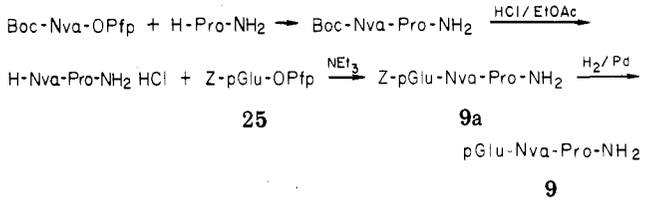
Since the discovery of the direct central nervous system (CNS) effects of thyrotropin-releasing hormone (TRH, L-pyroglutamyl-L-histidyl-L-prolinamide),[2] a relatively large number of TRH analogues have been synthesized[3-8] in order to separate these effects from the well-demonstrated hormonal (thyrotropin-releasing) activity of the parent peptide.[9] Synthetic analogues having elevated CNS activity and decreased or no hormonal potency were initially thought to be advantageously applicable in the treatment of human mental depression.[10]

Early structure–hormonal activity studies of TRH analogues synthesized so far seemed to have shown that pyroglutamic acid and prolinamide residues are fundamentally responsible for the full thyrotropin-releasing activity of the natural hormone. Actually, replacement of the pyrrolidone ring of pyroglutamic acid and the pyrrolidine ring of proline by other five- or six-membered heterocycles resulted in analogues with enhanced CNS effects and practically unaltered hormonal activity. In contrast, the presence of the specific basic, aromatic, and steric properties of the imidazole side chain of the central histidine is regarded as a decisive factor in the binding of TRH to appropriate adenohypophyseal receptors and, thus, for the full thyrotropin-releasing activity. This concept can be supported by the extremely high hormonal activity of two analogues, namely, [$N^{3\text{-im}}$-methyl]TRH[11] and [β-pyrazol-1-yl-Ala^2]TRH,[12] whose structural characteristics in position 2 are essentially similar to TRH. Disjunction of the above-mentioned triple unity of the central structural elements leads to considerable loss of hormonal activity.

Therefore, we have decided to synthesize analogues in which the central histidine is replaced by a natural or uncommon amino acid containing a neutral aromatic or aliphatic side chain in order to gain information about the role of the central histidine in CNS activity. The TSH-releasing activities of the analogues were measured according to the literature,[13] whereas the CNS activities were determined by their ability to inhibit the cataleptic effect of haloperidol, as a measure of dopaminergic stimulation.[14]

Chemistry. Peptide Synthesis. Benzyloxycarbonyl-protected tripeptides (**1a–24a**) were synthesized by the pentafluorophenyl ester method developed in our laboratory.[15] Scheme I shows the synthesis of pGlu-Nva-Pro-NH_2 (**9**) as an example. Overall yields and physical data of the protected tripeptides and analogues are listed in Tables I and II, respectively. Slight modifications of the synthesis are indicated as footnotes in the tables.

Scheme I



Results and Discussion

In the design of a simple and general method for the synthesis of neutral TRH analogues, there are two main

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Table I. Characterization Data of the Benzyloxycarbonyl-Protected Tripeptides

no.	compd[a]	overall yield,[b] %	mp, °C (recrystn solvent)	R_f (C)	$[\alpha]^{25}_D$, deg (c 1, AcOH)
1a	Z-pGlu-Phe-Pro-NH_2	71	141-143 (EtOAc)	0.65	-55.7
2a	Z-pGlu-Met-Pro-NH_2	72	157-159 (EtOH/EtOAc)	0.56	-74.9
3a	Z-pGlu-Leu-Pro-NH_2	67	103-105 (EtOH/Et_2O)	0.60	-93.3
4a	Z-pGlu-Gly-Pro-NH_2	64	191-194 (EtOH)	0.42	-95.0
5a	Z-pGlu-Ala-Pro-NH_2	65	189-191 (EtOH)	0.47	-120.5
6a	Z-pGlu-Val-Pro-NH_2	71	116-118 (EtOAc/Et_2O)	0.54	-100.5
7a	Z-pGlu-Ile-Pro-NH_2	71	103-105 (EtOAc/Et_2O)	0.57	-91.4
8a	Z-pGlu-Abu-Pro-NH_2	66	170-172 (EtOH)	0.48	-99.6
9a	Z-pGlu-Nva-Pro-NH_2	69	166-167 (EtOH/Et_2O)	0.58	-87.5
10a	Z-pGlu-Nle-Pro-NH_2	68	146-148 (EtOAc)	0.58	-79.8
11a	Z-pGlu-Ade-Pro-NH_2	77	103-105 (EtOAc)	0.68	-63.7
12a	Z-pGlu-Cha-Pro-NH_2	79	165-166 (EtOAc)	0.63	-67.3
13a	Z-pGlu-Pro-Pro-NH_2	60	247-248 (EtOH)	0.40	-82.8
14a	Z-pGlu-Ser(*t*-Bu)-Pro-NH_2[c]	75	226-229 (EtOH)	0.61	-71.6
15a	Z-pGlu-Thr(Bzl)-Pro-NH_2	71	A[f]	0.67	
16a	Z-D-pGlu-Leu-Pro-NH_2	75	192-194 (EtOAc)	0.66	-31.2
17a	Z-pGlu-D-Leu-Pro-NH_2	71	190-193 (EtOH)	0.70	-33.1
18a	Z-pGlu-Leu-D-Pro-NH_2	56	153-157 (EtOAc)	0.62	-24.5
19a	Z-pGlu-Leu-Pro-NH-Et	67	A	0.70	-89.8
20a	Z-pGlu-Leu-Pro-NH-Dec	63	108-109 (Et_2O/*n*-hexane)	0.60[g]	-74.5
21a	Z-pGlu-Leu-Pro-NH-DMAE	57	oil	0.23	
22a	Z-pGlu-Leu-Pro-OMe[d]	66	A	0.56[g]	-100.2
23a	Z-pGlu-Leu-Pro-OH[e]	57	98-100 (EtOAc)	0.48	-98.1
24a	Z-pGlu-Leu-pyrrolidide	52	109-110 (EtOH/Et_2O)	0.46[g]	-53.3

[a] Abbreviations used are those recommended by the IUPAC-IUB Comission on Biochemical Nomenclature [*J. Biol. Chem.* 1972, *247*, 977]. Other abbreviations: Abu, L-2-aminobutyric acid; Ade, L-2-aminodecanoic acid; Cha, L-cyclohexylalanine; Dec, *n*-decyl; DMAE, 2-(dimethylamino)ethyl. C, H and N elemental analyses were correct for each assigned structure. [b] Calculated for the amount of central amino acid derivative used in the dipeptide coupling reaction. [c] Through Z-Ser(*t*-Bu)-Pro-NH_2 deprotected by hydrogenolysis. [d] Through Boc-Leu-Pro-OMe coupled by DCC method. [e] Through H-Leu-Pro-OH obtained from Boc-Leu-Pro-OMe by saponification and subsequent acidolysis. [f] A = amorphous. [g] In solvent system B (for composition of the solvent systems; see Experimental Section).

Table II. Characterization Data of TRH Analogues

no.	compd	overall yield, %	mp, °C (recrystn solvent)	R_f (C)	R_f (E)	amino acid anal.[a] Glu	amino acid anal.[a] Pro	$[\alpha]^{25}_D$, deg (c 1, AcOH)
1	pGlu-Phe-Pro-NH_2[b]	64	155-159 (H_2O)	0.40	0.33	1.01	1.02	-46.6[c]
2	pGlu-Met-Pro-NH_2[d]	40[e]	A[f]	0.32	0.20			-68.8
3	pGlu-Leu-Pro-NH_2	62	A	0.37	0.29	1.08	1.00	-91.6[g]
4	pGlu-Gly-Pro-NH_2	58	213-216 (EtOH/Et_2O)	0.18[h]	0.20[i]	0.94	0.95	-121.3[j]
5	pGlu-Ala-Pro-NH_2	59	A	0.12	0.20	1.02	0.92	-113.9[k]
6	pGlu-Val-Pro-NH_2	60	A	0.33	0.28	1.03	0.94	-104.9
7	pGlu-Ile-Pro-NH_2	64	A	0.39	0.32	0.95	1.02	-100.7
8	pGlu-Abu-Pro-NH_2	60	A	0.27	0.23	1.01	0.97	-102.5
9	pGlu-Nva-Pro-NH_2	66	A	0.33	0.23	0.97	0.98	-87.0
10	pGlu-Nle-Pro-NH_2	66	A	0.40	0.24	0.96	1.00	-77.1
11	pGlu-Ade-Pro-NH_2	65	A	0.51	0.31	1.03	0.99	-66.2
12	pGlu-Cha-Pro-NH_2	75	A	0.45	0.27	1.00	0.97	-70.9
13	pGlu-Pro-Pro-NH_2	50	A	0.15[h]	0.15	1.04	1.96	-223.1
14	pGlu-Ser(*t*-Bu)-Pro-NH_2	64	186-187 (EtOH/Et_2O)	0.40	0.34	1.03	1.00	-60.8
15	pGlu-Thr-Pro-NH_2	42[e]	A	0.12	0.13	1.01	1.04	-90.0
16	D-pGlu-Leu-Pro-NH_2	68	A	0.33	0.20	0.96	0.96	-23.6
17	pGlu-D-Leu-Pro-NH_2	62	A	0.37	0.26	0.98	1.04	-83.3
18	pGlu-Leu-D-Pro-NH_2	45	A	0.39	0.30	1.03	0.99	+8.4
19	pGlu-Leu-Pro-NH-Et	51[e]	A	0.47	0.43	1.03	1.10	-94.5
20	pGlu-Leu-Pro-NH-Dec	50	140-141 (EtOAc)	0.74	0.70	0.97	1.01	-71.3
21	pGlu-Leu-Pro-NH-DMAE	43	A	0.11[h]	0.05[i]	1.04	0.94	-71.7
22	pGlu-Leu-Pro-OMe	46[e]	A	0.64	0.66	0.97	0.98	-122.2[l]
23	pGlu-Leu-Pro-OH	51	A	0.27	0.14[i]	0.98	1.05	-101.4
24	pGlu-Leu-pyrrolidide	47	102-103 (Et_2O)	0.57	0.63	1.00		-31.6

[a] Calculated for the amount of the central amino acid taken as 1.00. [b] Characterized as the monohydrate. [c] -41.4° (*c* 1.57, MeOH); lit.[29] $[\alpha]^{25}_D$ -49.3° (*c* 1, AcOH); lit.[30] $[\alpha]^{25}_D$ -44.0° (*c* 1.56, MeOH). [d] Deprotected in HBr/AcOH. [e] Purified by column chromatography. [f] A = amorphous. [g] -90.2° (*c* 1.11, MeOH); lit.[31a] $[\alpha]^{25}_D$ -81.8° (*c* 1.1, MeOH). [h] In solvent system D. [i] In solvent system F. [j] -72.4° (*c* 0.98, MeOH); lit.[29] $[\alpha]^{25}_D$ -110.0° (*c* 1, AcOH); lit.[31a] $[\alpha]^{25}_D$ -65.5° (*c* 0.99, MeOH). [k] lit.[29] $[\alpha]^{25}_D$ -90.0° (*c* 1, AcOH). [l] -94.1° (*c* 1.1, $CHCl_3$); lit.[31a] $[\alpha]^{25}_D$ -80.7° (*c* 1.1, $CHCl_3$).

difficulties that have to be overcome: the known tendency of proline dipeptides to form diketopiperazines[16] and the poor crystallization tendency of the products, similar to TRH, allowing purification only by laborious column chromatography. Therefore, the synthesis of benzyloxycarbonyl-protected tripeptides (**1a–24a**) as crystalline key intermediates by the pentafluorophenyl ester method was chosen to solve both problems simultaneously. In view of the high reactivity of pentafluorophenyl esters,[17] it was

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Table III. CNS and Hormonal Activities of TRH Analogues

no.	compd	anticataleptic effect: inhibition % (80 mg/kg iv)	anticataleptic effect: ED_{50}, mg/kg iv	TSH-releasing act., %: present report	TSH-releasing act., %: lit.
	pGlu-His-Pro-NH_2	40	113.0		100
1	pGlu-Phe-Pro-NH_2	40			10,[b] 40[c]
2	pGlu-Met-Pro-NH_2	40			1[d]
3	pGlu-Leu-Pro-NH_2		40.0	2.7	2[e]
4	pGlu-Gly-Pro-NH_2	10			0[e]
5	pGlu-Ala-Pro-NH_2	0			0[f]
6	pGlu-Val-Pro-NH_2		>80	3.1	
7	pGlu-Ile-Pro-NH_2	10		0.2	
8	pGlu-Abu-Pro-NH_2		⩾80	0	
9	pGlu-Nva-Pro-NH_2		11.4	0	
10	pGlu-Nle-Pro-NH_2		16.8	3.8	
11	pGlu-Ade-Pro-NH_2	0		np[a]	
12	pGlu-Cha-Pro-NH_2	20		45.6	
13	pGlu-Pro-Pro-NH_2		80.0	np	
14	pGlu-Ser(*t*-Bu)-Pro-NH_2	0		0.2	
15	pGlu-Thr-Pro-NH_2		80.0	25.7	
16	D-pGlu-Leu-Pro-NH_2		70.0	0	
17	pGlu-D-Leu-Pro-NH_2		>80	0	
18	pGlu-Leu-D-Pro-NH_2		>80	0	
19	pGlu-Leu-Pro-NH-Et		56.6	0	
20	pGlu-Leu-Pro-NH-Dec	10		0	
21	pGlu-Leu-Pro-NH-DMAE	10		0	
22	pGlu-Leu-Pro-OMe	0			0[e]
23	pGlu-Leu-Pro-OH	0		0.1	
24	pGlu-Leu-pyrrolidide	20		np	

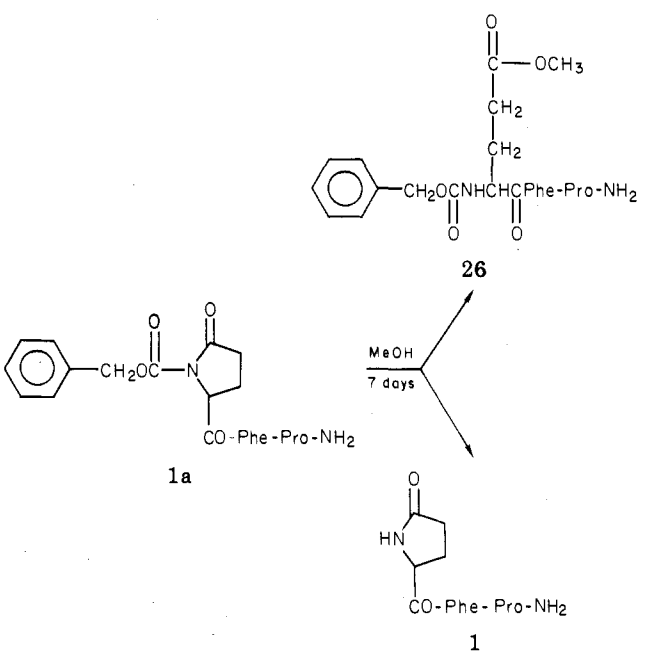
[a] np = significant but not parallel TSH response to the lower and upper doses used. (For details of the pharmacological experiments, see Experimental Section.) [b] Reference 30. [c] Reference 32. [d] Reference 33. [e] Reference 31. [f] Reference 29.

assumed that the reaction time of tripeptide coupling would be short enough to prevent diketopiperazine formation. Indeed, the coupling of dipeptides with Z-pGlu-OPfp (**25**) occurred within a few minutes and resulted in pure protected tripeptides, which could be readily isolated from the reaction mixture by simple washing techniques in good yields. Most of the protected tripeptides are crystalline substances, which can be purified by recrystallization (Table I).

Analogues **1–24** were obtained from the benzyloxycarbonyl-protected tripeptides by hydrogenolysis without any side reactions in about 90% yield and high purity. Except for a few cases, there was no need to purify the products by column chromatography, despite the fact that most compounds were amorphous substances. The purity of the analogues was confirmed by high-pressure liquid chromatography, too.

A possible, but in the present case unexpected, methanolysis of the (benzyloxycarbonyl)pyroglutamyl peptides was observed when they were recrystallized from methanol. Sensitivity of the protected pyroglutamyl derivatives to external bases is well documented.[18,19] A similar simultaneous nucleophilic attack of methanol on the carbonyl groups of both the pyroglutamyl ring and protecting group occurred when Z-pGlu-His-Pro-NH_2 or Z-pGlu-His-NH_2 was allowed to stand in methanol for 9 days, resulting in the appropriate Z-γ-Me-Glu and pGlu peptides. The presence of the imidazole group of histidine as an internal base had been assumed to be responsible for catalyzing this process.[20] However, the same type of methanolysis of our *neutral* benzyloxycarbonyl-protected tripeptides, for example, that of Z-pGlu-Phe-Pro-NH_2 (**1a**), which completely

Scheme II



occurred within 7 days to give a similar ratio of the products **26** and **1** (Scheme II), has demonstrated that the reaction is independent of the presence of either external or internal bases, taking place also under neutral conditions, providing further evidence for the sensitivity of protected pyroglutamyl derivatives to nucleophiles. The presence of a free carboxyl group can prevent methanolysis, as proven by the stability of the acidic peptide, Z-pGlu-Leu-Pro-OH (**23a**) in methanol. The rate of ethanolysis of the tripeptides is much slower; therefore, ethanol is a suitable solvent for the recrystallization of the protected tripeptides.

The biological results obtained (Table III) have supported the correctness of our assumption. Replacement

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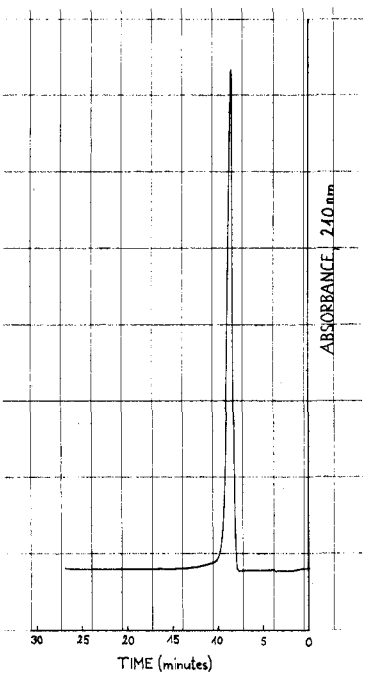

Figure 1. High-pressure liquid chromatogram of pGlu-Nva-Pro-NH_2 (**9**).

of the histidine in TRH by an amino acid containing aliphatic side chain resulted in neutral TRH analogues without any remarkable thyrotropin-releasing activity and with stronger CNS effects in the inhibition of haloperidol catalepsy as compared with TRH (compounds **8**, **9**, and **16–19**). Similarly, five other analogues (**3**, **6**, **10**, **13**, and **15**) had a powerful anticataleptic effect, yet they retained more or less of the hormonal activity of TRH. The thyrotropin-releasing activity of analogues **3**, **6**, and **10** is rather negligible, but that of [Thr^2]TRH (**15**) is surprisingly high. The remarkably high TSH-releasing effect of [Cha^2]TRH (**12**) seems to emphasize the important role of the steric properties of the central amino acid side chain in the hormonal activity of TRH. An interesting TSH response of analogues **11**, **13**, and **24** was observed when they were administered in two different doses. With the lower dose (1 μg), a small yet significant and definitely higher rise of the plasma TSH level was found than when applying the higher dose (10 μg), suggesting the possibility that these analogues might be inhibitors of TRH.

Our results demonstrate that the presence of histidine is not essential for the CNS activity, because analogues **3**, **9**, and **10** with a three- and four-membered straight or branched alkyl side chain in position 2 had 2.5 to 10 times greater CNS effects than TRH. From the practical point of view, pGlu-Nva-Pro-NH_2 ([Nva^2]TRH, **9**) can be regarded as a useful TRH analogue: it exhibits tenfold CNS activity in comparison to TRH and is fully inactive as a TSH releaser.

A wider pharmacological profile of the analogues will be published elsewhere.

Experimental Section

Melting points were determined with a Tottoli (Büchi) capillary apparatus and are uncorrected. IR spectra were taken with a Perkin-Elmer 700 spectrophotometer. ^1H NMR spectra were obtained on a Varian EM 360 instrument in $CDCl_3$ with Me_4Si as internal standard. HPLC was performed on a LABOR MIM apparatus coupled to an OE 308 UV detector set at 210 nm. The column (25 cm × 4.6 mm) was packed with Nucleosil 10 C_{18} (Chrompack), and MeOH/H_2O (25:75) was used as eluent. Chromatography was conducted at room temperature with a solvent flow rate of 1.0 mL/min. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. For TLC, precoated plates (silica gel 60, 0.25 mm, Merck) were used in the following solvent systems (all v/v). Solvent systems A–D were made by mixing EtOAc and a stock solution of pyridine/AcOH/H_2O (20:6:11) in the following proportions: (A) EtOAc/stock, 19:1; (B) EtOAc/stock, 4:1; (C) EtOAc/stock, 3:2; (D) EtOAc/stock, 1:1; (E) $CHCl_3$/MeOH, 4:1; (F) $CHCl_3$/MeOH, 2:1; (G) EtOAc/*n*-hexane, 4:1. Column chromatography was carried out on Fluka silica gel 60 (0.063–0.200 mm). All new crystalline compounds gave elemental analyses (C, H, and N) that agreed with the calculated values within 0.3%. For amino acid analysis, the peptides were hydrolyzed in 6 N HCl for 24 h at 110 °C, and the hydrolysates were analyzed on a Chinoin OE 975 amino acid analyzer.

Table IV. Physical Data of the New Pentafluorophenyl Esters

compd[a]	yield, %	mp, °C	R_f (G)	$[\alpha]^{25}_D$,[b] deg
Boc-Abu-OPfp	76	83–84	0.75	−32.8
Boc-Cha-OPfp	85	75–77	0.78	−23.9
Boc-Thr(Bzl)-OPfp	86	85–87	0.70	−24.1
Boc-D-Leu-OPfp	88	53–55	0.75	+31.7
Z-Ser(*t*-Bu)-OPfp	87	oil	0.76	−4.3
Z-pGlu-OPfp (**25**)	90	80–82	0.60	−40.4
Z-D-pGlu-OPfp	85	81–82	0.60	+40.1
Z-Glu(OMe)-OPfp (**26b**)	84	59–60	0.72	−17.9

[a] Structure of the compounds was confirmed by elemental analysis (C, H, N, and F) and IR and ^1H NMR spectroscopy. [b] *c* 1, EtOAc.

Pentafluorophenyl esters of the Boc-protected amino acids were prepared as described earlier.[21,22] The new pentafluorophenyl esters were synthesized in the same way. Physical data of these compounds are shown in Table IV. Protected amino acids Boc-Abu-OH,[24] Boc-Cha-OH,[25] Boc-Thr(Bzl)-OH,[26] Z-Ser(*t*-Bu)-OH,[27] Z-pGlu-OH,[18] and Z-Glu(OMe)-OH[28] were prepared according to the known methods. L-Prolinamide and D-prolinamide and substituted prolinamides (H-Pro-NH-Et, H-Pro-NH-Dec, H-Pro-NH-DMAE) were synthesized by the literature procedure.[23]

The anticataleptic effect of the new TRH analogues was tested in adult male Wistar rats. The test compounds were administered in doses of 5 to 80 mg/kg intravenously (iv) 2 h after an intraperitoneal (ip) injection of 4 mg/kg of haloperidol. The anticataleptic effect of the analogues that inhibited haloperidol catalepsy by less than 50% in a dose of 80 mg/kg is expressed in percentage of noncataleptic animals. ED_{50} values are given only for the more potent analogues.

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The TSH-releasing activity of the analogues was tested in adult male albino rats. TSH response was determined after a single ip injection of each sample in a two-point assay (1 and 10 μg, respectively). Blood samples were withdrawn 15 min after injection, and serum TSH concentration was measured by radioimmunoassay[13] (NIAMDD rat TSH-RIA system). Relative potencies of the analogues are expressed in percentage, the effect of TRH being taken as 100%. Synthetic TRH was used as reference standard in both biological tests.

***N*-(Benzyloxycarbonyl)-L-pyroglutamyl-L-norvalyl-L-prolinamide (Z-pGlu-Nva-Pro-NH_2, 9a).** A solution of Boc-Nva-OPfp[22] (3.83 g, 10 mmol) and H-Pro-NH_2 (1.37 g, 12 mmol) in DMFA (20 mL) was allowed to stand for 1 h at room temperature. The solvent was evaporated, the residue was dissolved in $CHCl_3$, and the solution was washed with 1 N HCl and then three times with 1 N $NaHCO_3$ and H_2O, dried over Na_2SO_4, and filtered. The filtrate was evaporated to give oily Boc-Nva-Pro-NH_2 [3.3 g; R_f (A) 0.29], which was dissolved in 2 N HCl/EtOAc (20 mL). After standing for 1 h at room temperature, the mixture was diluted with Et_2O, the precipitate was filtered off and washed subsequently with ice-cold Et_2O, EtOAc, and Et_2O, and dried in vacuo over NaOH pellets to yield 2.12 g of H-Nva-Pro-NH_2·HCl as a hygroscopic substance [R_f (C) 0.07].

An ice-cold suspension of H-Nva-Pro-NH_2·HCl (2.12 g, 8.5 mmol) in DMFA (20 mL) was mixed with NEt_3 (2.38 mL, 17 mmol) and Z-pGlu-OPfp (**25**, 3.86 g, 9.0 mmol). After the mixture was stirred for 10 min at 0–5 °C, the solvent was evaporated, the residue was dissolved in $CHCl_3$, and the solution was washed with 1 N HCl and then three times with 1 N $NaHCO_3$ and H_2O, dried over Na_2SO_4, and filtered. The filtrate was evaporated, and the residual oil was crystallized from Et_2O to give 4.24 g of the crude product; this was recrystallized from EtOH/Et_2O to yield 3.16 g (69%, calculated for Boc-Nva-OPfp) of **9a**: mp 166–167 °C; $[\alpha]^{25}_D$ −87.5° (*c* 1, AcOH); R_f (C) 0.58; IR (KBr) 3420, 3330, 3190 (NH, NH_2), 3050, 3020 (aromatic CH), 1778 (lactam ring CO), 1695, 1688, 1665, 1635 (carbonyls), 1520 (amide II), 738, 692 (Ar) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (t, 3, CH_3), 15 (br m, 4, 2 CH_2), 1.8–3.0 (br m, 8, 4 CH_2), 3.5 (br m, 2, NCH_2), 4.2–4.9 (br m, 3, 3 NCH), 5.2 (s, 2, benzyl CH_2), 6.2 and 6.65 (2 br s, 2, $CONH_2$, exchangeable with D_2O), 7.3 (s, 5, Ar H), 7.4 (d, 1, CONH, exchangeable with D_2O).

Physical data of compounds **1a–8a** and **10a–24a**, synthesized essentially in the same way, are listed in Table I.

L-Pyroglutamyl-L-norvalyl-L-prolinamide (pGlu-Nva-Pro-NH_2, 9). Compound **9a** (2.29 g, 5 mmol) was hydrogenated in EtOH (100 mL) in the presence of 10% Pd/charcoal for 1 h. The catalyst was removed by filtration, the filtrate was evaporated, and the residue was solidified on rubbing with Et_2O. The resulting crude product (1.60 g) was dissolved in H_2O (50 mL), the solution was clarified with charcoal and freeze-dried to give 1.54 g (95%) of **9** as an amorphous substance: $[\alpha]^{25}_D$ −87.0° (*c* 1, AcOH); R_f (C) 0.33; R_f (E) 0.23; ^1H NMR ($CDCl_3$) δ 0.91 (t, 3, CH_3), 1.1–1.8 (br m, 4, 2 CH_2), 2.06 (m, 4, 2 CH_2), 2.3 (m, 4, 2 CH_2), 3.7–4.03 (br m, 3, NCH_2 + NCH), 4.44–4.6 (m, 2, 2 NCH), 5.8 and 7.45 (2 br s, 2, $CONH_2$, exchangeable with D_2O), 7.9–8.0 (d, s, 2, 2 CONH, exchangeable with D_2O). Amino acid analysis: Glu, 0.97 (1.00); Nva, 1.00 (1.00); Pro, 0.98 (1.00).

Characterization data of compounds **1–8** and **10–24**, synthesized in the same way, are summarized in Table II.

***N*-(Benzyloxycarbonyl)-L-γ-methylglutamyl-L-phenylalanyl-L-prolinamide (26).** An ice-cold solution of H-Phe-Pro-NH_2·HCl (298 mg, 1 mmol) in DMFA (3 mL) was mixed with NEt_3 (0.28 mL, 2 mmol) and Z-Glu(OMe)-OPfp (**26b**; 484 mg, 1.1 mmol). After the mixture was stirred for 10 min, the solvent was evaporated, the residue was dissolved in $CHCl_3$, and the solution was washed with 1 N HCl then three times with 1 N $NaHCO_3$, and H_2O, dried over Na_2SO_4, and filtered. The solvent was evaporated, and the oily residue was crystallized from Et_2O to give 474 mg of **26**, which was recrystallized from EtOAc: yield 420 mg (78%); mp 137–139 °C; $[\alpha]^{25}_D$ −34.7° (*c* 1, AcOH); R_f (C) 0.84; IR (KBr) 3380, 3300 (NH, NH_2), 3055, 3025 (aromatic CH), 1723 (ester CO), 1665 (urethane), 1635 (carboxamide), 1545, 1532 (amide II), 755, 743, 693 (Ar) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5–2.5 (m, 8, 4 CH_2), 2.9 (m, 2, CH_2), 3.4 (br s, 2, NCH_2), 3.53 (s, 3, OCH_3), 4.4 (m, 2, 2 NCH), 4.9 (m, 1, NCH), 5.0 (s, 2, benzyl CH_2), 5.8 (d, 1, NH, exchangeable with D_2O), 5.7 and 6.55 (2 br s, 2, $CONH_2$, exchangeable with D_2O), 7.1 and 7.26 (2 s, 10, 2 Ar H), 8.02 (d, 1, CONH).

Methanolysis of Z-pGlu-Phe-Pro-NH_2 (1a). The title compound (**1a**; 4.05 g, 8.0 mmol) was dissolved in MeOH (405 mL), and the solution was allowed to stand at room temperature for 7 days. The solvent was evaporated, the residue was dissolved in $CHCl_3$, and the solution was extracted six times with H_2O. The organic phase was dried over Na_2SO_4 and filtered, then the solvent was evaporated. The residue was crystallized from Et_2O/*n*-hexane to give Z-Glu(OMe)-Phe-Pro-NH_2 (**26**) as compared with an authentic control substance by IR and ^1H NMR spectra: yield 3.60 g (6.7 mmol, 84%); mp 139–141 °C; $[\alpha]^{25}_D$ −34.0° (*c* 1, AcOH); R_f (C) 0.84.

Evaporation of the water extract gave a crystalline material, which was identical with pGlu-Phe-Pro-NH_2·H_2O (**1**): yield 0.43 g (1.1 mmol, 14%); mp 150–153 °C; $[\alpha]^{25}_D$ −46.3° (*c* 1, AcOH); R_f (C) 0.40; R_f (E) 0.33; IR (KBr) 3535 (H_2O), 3440, 3240 (broad, $CONH_2$, CONH), 2970, 2925, 2890 (CH_2), 1655 (broad, carbonyls), 1532 (amide), 740, 700 (Ar) cm^{-1}.

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Registry No. **1**, 34783-35-2; **1a**, 69461-91-2; **2**, 35259-10-0; **2a**, 88888-68-0; **3**, 49760-92-1; **3a**, 88888-69-1; **4**, 42294-01-9; **4a**, 88888-70-4; **5**, 42390-94-3; **5a**, 88888-71-5; **6**, 78058-07-8; **6a**, 78058-06-7; **7**, 78058-11-4; **7a**, 78058-10-3; **8**, 78058-13-6; **8a**, 78082-29-8; **9**, 78058-02-3; **9a**, 78058-01-2; **10**, 78057-99-5; **10a**, 78057-98-4; **11**, 78058-16-9; **11a**, 78058-15-8; **12**, 78058-20-5; **12a**, 78058-19-2; **13**, 78058-04-5; **13a**, 78058-03-4; **14**, 78082-31-2; **14a**, 78058-27-2; **15**, 78058-24-9; **15a**, 78058-23-8; **16**, 78664-76-3; **16a**, 78664-75-2; **17**, 78058-30-7; **17a**, 78058-29-4; **18**, 78664-40-1; **18a**, 78664-39-8; **19**, 78058-34-1; **19a**, 78058-33-0; **20**, 78058-37-4; **20a**, 78058-36-3; **21**, 78058-38-5; **21a**, 78082-35-6; **22**, 49760-98-7; **22a**, 88888-72-6; **23**, 85146-12-9; **23a**, 87171-07-1; **24**, 88888-73-7; **24a**, 88888-74-8; **25**, 78148-14-8; **26**, 88888-75-9; **26b**, 39993-97-0; Boc-Abu-OPfp, 78082-28-7; Boc-Cha-OPfp, 78058-17-0; Boc-Thr(Bzl)-OPfp, 70989-89-8; Boc-D-Leu-OPfp, 78082-32-3; Z-Ser(*t*-Bu)-OPfp, 78082-30-1; Z-D-pGlu-OPfp, 78685-16-2; Boc-Nva-OPfp, 67861-97-6; H-Pro-NH_2, 7531-52-4; H-Nva-Pro-NH_2·HCl, 78058-00-1; H-Phe-Pro-NH_2·HCl, 83871-05-0.