Synthesis of Analogs of the Thyrotropin-Releasing Hormone and Structure–Activity Relationships†

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Twenty-two analogs and derivatives of the thyrotropin-releasing hormone (TRH), which is pGlu-His-Pro-NH_2, have been synthesized by a generally useful procedure involving the amino acids with appropriate protective groups. Fourteen were analogs having the pGlu-His moiety of TRH but the Pro-NH_2 moiety was replaced by other amino acid amides or esters. Eight compounds possessed the benzyl group on the histidine moiety and included pGlu-His(Bzl)-Pro-NH_2. The structural analogs pGlu-His-Pro having $NHCH_3$, OCH_3, and His(Bzl) showed the hormonal activity of TRH at 10, 10, and 1000 times, respectively, the dose level of TRH. Replacement of Pro-NH_2 of TRH by Ala-NH_2, Abu-NH_2, Val-NH_2, and Leu-NH_2 greatly decreased the hormonal activity of TRH, but dosage at 500 to 1000 times the level of TRH released TSH *in vivo*. All other compounds were inactive.

The biological assays and the structure–activity relationships of 16 analogs and derivatives of the thyrotropin-releasing hormone (TRH) and citations to pertinent publications of other investigators on this aspect of TRH have been reported.[1] Subsequently, Hofmann and Bowers[2] have reported the synthesis of a new analog which has a pyrazole nucleus in place of the imidazole nucleus; it showed about 5% of the activity of TRH. We now describe the synthesis of these 16 compounds and their characterization as well as this information on 6 newer compounds; the biological assay data on these latter compounds are included.

L-Pyroglutamyl-L-histidyl-L-prolinamide[3] (TRH) (**1**) was synthesized by a procedure involving protecting groups. This procedure was particularly useful as a general procedure for the synthesis of many new analogs of TRH to study structure–activity relationships. This synthesis is based upon the coupling of *N*-carbobenzoxypyroglutamic acid[4] and N^{im}-benzylhistidine benzyl ester dibenzenesulfonate[5] by the mixed anhydride procedure (ethyl chloroformate). The resulting dipeptide *N*-carbobenzoxypyroglutamyl-N^{im}-benzylhistidine benzyl ester (**23**) is converted into pyroglutamyl-N^{im}-benzylhistidine (**24**) by selective hydrogenolysis. Reaction of the latter dipeptide (**24**) with prolinamide[6] mediated by *N*,*N*′-dicyclohexylcarbodiimide (DCI), afforded pyroglutamyl-N^{im}-benzylhistidylprolinamide (**15**). Hydrogenation of this N^{im}-benzyl derivative (**15**) yielded pGlu-His-Pro-NH_2 (TRH) (**1**). The basic procedure of this synthesis was modified to obtain analogs and derivatives of TRH as follows.

The protected dipeptide, pyroglutamyl-N^{im}-benzylhistidine (**24**) was coupled with the appropriate derivative of an amino acid by DCI to obtain the corresponding tripeptide. The pyroglutamyl-N^{im}-benzylhistidine (**24**) was the preferred intermediate in these syntheses mainly because the subsequent N^{im}-benzyl tripeptides were comparatively easy to purify and characterize. The purification was usually effected by repeated recrystallization, but in some cases (**15**, **16**, **17**, and **18**) column chromatography on silica gel with MeOH–$CHCl_3$ mixtures (5–20% MeOH) was used. In many cases, the N^{im}-benzyl tripeptides were also desired for biological assay to assess the significance of free histidine moieties (method A.)

Hydrogenation over Pd/C at atmospheric pressure removed the N^{im}-benzyl group from the tripeptides in yields of 75–96% and usually without side products. Generally, recrystallization or precipitation of the hydrogenation product was all that was required to obtain a satisfactory new tripeptide for chemical analysis and biological assay (method B).

Pyroglutamylhistidine (**25**) was obtained from pyroglutamyl-N^{im}-benzylhistidine (**24**) by hydrogenation and could be used in coupling reactions to give the final tripeptides in one step. However, this coupling reaction resulted in several side products and the desired tripeptide was then obtained by preparative tlc on silica gel G (method C).

The products from these procedures were then examined for purity and found to be satisfactory by tlc in 3 different solvent systems: acidic, neutral, and basic.

Chemical information on the 22 analogs and derivatives of the thyrotropin-releasing hormone (TRH) are summarized in Table I and other characterization data on these compounds are in Table II.

The information on the hormonal activity of some of these analogs and derivatives is summarized in Table III. The activities are expressed in dose levels in nanograms relative to TRH for hormonal activity in mice. The effective dose was determined as that level in nanograms which releases essentially the same level of TSH as 9 ng of synthetic TRH in the T_3-TRH method.[7–9]

† Hypothalamic Hormones. 17.

(1) C. Y. Bowers, A. Weil, J.-K. Chang, H. Sievertsson, F. Enzmann, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **40**, 683 (1970).

(2) K. Hofmann and C. Y. Bowers, *J. Med. Chem.*, **13**, 1099 (1970).

(3) F. Enzmann, J. Bøler, K. Folkers, C. Y. Bowers, and A. V. Schally, *J. Med. Chem.*, **14**, 469 (1971).

(4) H. Gibian and E. Klieger, *Justus Liebigs Ann. Chem.*, **640**, 145 (1961).

(5) D. Theodoropoulos and G. Fölsch, *Acta Chem. Scand.*, **12**, 1955 (1958).

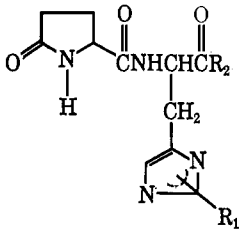
(6) R. W. Chambers and F. H. Carpenter, *J. Amer. Chem. Soc.*, **77**, 1522 (1955).

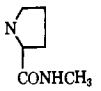
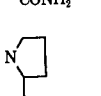
(7) C. Y. Bowers, A. V. Schally, G. A. Reynolds, and W. D. Hawley, *Endocrinology*, **81**, 741 (1967).

(8) C. Y. Bowers and A. V. Schally, "Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry," J. Meites, Ed., Williams and Wilkins Co., Baltimore, 1970, p 74.

(9) C. Y. Bowers, A. V. Schally, F. Enzmann, J. Bøler, and K. Folkers, *Endocrinology*, **86**, 1143 (1970).

TABLE I: ANALOGS AND DERIVATIVES OF THE THYROTROPIN-RELEASING HORMONE (TRH)



No.	Compd	R_1	R_2	$[\alpha]^{22}D$,	R_f value[g]			Mp, °C[g]	Recrystn solvent	Method[g]	Yield %
					R_f^1	R_f^2	R_f^3				
1	pGlu-His-Pro-NH_2[a]	H		−42.4[b] (c 1.00)	0.23	0.64	0.41	h	MeOH–Et_2O	B	75
2	pGlu-His-Pro-$NHCH_3$[a]	H		−45.1[b] (c 1.00)	0.23	0.72	0.44	h	MeOH–Et_2O	B	76
3	pGlu-His-Pro-OCH_3[a]	H		−19.8[b] (c 0.97)	0.40	0.84	0.55	h		B	96
4	pGlu-His-Gly-NH_2[a]	H	$NHCH_2CONH_2$	+0.6[b] (c 0.96)	0.23	0.42	0.38	h		B	82
5	pGlu-His-Ala-NH_2[a]	H	$NHC(CONH_2)HCH_3$	−9.9[b] (c 1.02)	0.26	0.48	0.43	240–243 dec	MeOH	B C	80 25
6	pGlu-His-Abu-NH_2	H	$NHC(CONH_2)HCH_2CH_3$	−28.7[c] (c 1.00)	0.33	0.61	0.46	h		C	35
7	pGlu-His-Val-NH_2[a]	H	$NHC(CONH_2)HCH(CH_3)_2$	−16.7[d] (c 1.00)	0.42	0.66	0.54	268–270 dec	MeOH–Et_2O	B	77
8	pGlu-His-Leu-NH_2[a]	H	$NHC(CONH_2)HCH_2CH(CH_3)_2$	−18.9[b] (c 1.00)	0.47	0.70	0.57	169–173 dec	EtOH–Et_2O	B	88
9	pGlu-His-Ileu-OCH_3	H	$NHC(CO_2CH_3)HCH(CH_3)CH_2CH_3$	−12.1[b] (c 1.06)	0.58	0.83	0.56	235–238 dec	MeOH–Et_2O	C	59
10	pGlu-His-Ileu-NH_2	H	$NHC(CONH_2)HCH(CH_3)CH_2CH_3$	−8.5[b] (c 0.80)	0.49	0.73	0.51	h	MeOH–Et_2O	D	80
11	pGly-His-Thr-NH_2	H	$NHC(CONH_2)HCH(OH)CH_3$	−4.1[b] (c 1.11)	0.25	0.47	0.43	h	EtOH–Et_2O	C	47
12	pGlu-His-Met-NH_2	H	$NHC(CONH_2)HCH_2CH_2SCH_3$	−37.4[c] (c 1.18)	0.45	0.66	0.50	218–220 dec	MeOH–Et_2O	C	47
13	pGlu-His-Phe-NH_2[a]	H	$NHC(CONH_2)HCH_2C_6H_5$	−3.6[b] (c 1.00)	0.53	0.63	0.54	250–255 dec	MeOH	B C	61 12
14	pGlu-His-Trp-NH_2[i]	H		−11.2[b] (c 1.00)	0.57	0.83	0.68	220–230 dec	MeOH–Et_2O	C	28
15	pGlu-His(Bzl)-Pro-NH_2	$CH_2C_6H_5$		−19.6[b] (c 1.50)	0.48		0.57	h		A	46
16	pGlu-His(Bzl)-Pro-$NHCH_3$	$CH_2C_6H_5$		−16.1[b] (c 1.47)	0.48	0.93	0.57	h		A	44
17	pGlu-His(Bzl)-Pro-OCH_3	$CH_2C_6H_5$		−60.8[b] (c 1.00)	0.54		0.63	h		A	31
18	pGlu-His(Bzl)-Gly-NH_2	$CH_2C_6H_5$	$NHCH_2CONH_2$	+6.8[b] (c 1.00)	0.49	0.74	0.56	h		A	34
19	pGlu-His(Bzl)-Ala-NH_2	$CH_2C_6H_5$	$NHC(CONH_2)HCH_3$	+7.6[e] (c 0.94)	0.53		0.58	218–220 dec	EtOH	A	40
20	pGlu-His(Bzl)-Val-NH_2	$CH_2C_6H_5$	$NHC(CONH_2)HCH(CH_3)_2$	−22.3[f] (c 0.91)	0.56		0.63	285–288 dec	MeOH	A	50
21	pGlu-His(Bzl)-Leu-NH_2	$CH_2C_6H_5$	$NHC(CONH_2)HCH_2CH(CH_3)_2$	−14.5[f] (c 0.95)	0.58		0.65	258–260 dec	MeOH	A	45
22	pGlu-His(Bzl)-Phe-NH_2	$CH_2C_6H_5$	$NHC(CONH_2)HCH_2C_6H_5$	−27.1[f] (c 0.99)	0.63		0.63	260–263 dec	MeOH	A	60

[a] Hydrolysis of the peptide was carried out in a sealed ampoule in 6 *N* HCl at 110° for 24 hr and the theoretical amino acids were identified. [b] MeOH. [c] H_2O. [d] 95% EtOH. [e] DMF. [f] AcOH. [g] See Experimental Section for details. [h] No sharp mp was obtained. [i] Pauly-, Cl-tolidine-, and Ehrlich-positive spot. [j] 80% MeOH.

TABLE II

CHARACTERIZATION DATA ON ANALOGS AND DERIVATIVES OF TRH

No.	Formula	Analyses[a]	Nmr[b] Imidazole protons	Nmr[b] Benzylic protons	Nmr[b] Methyl group
1	$C_{16}H_{22}N_6O_4 \cdot HCl$	*m*	*c*		
2	$C_{17}H_{24}N_6O_4 \cdot 1.5H_2O$	C, H, N[d]	2.32, s, 1 H[h] 3.02, s, 1 H		7.32, s, 3 H
3	$C_{17}H_{23}N_5O_5 \cdot HCl$	C, H, N	2.29, s, 1 H[i] 3.33, s, 1 H		6.63, s, 3 H
4	$C_{13}H_{18}N_6O_4$		2.28, s, 1 H[h] 2.98, s, 1 H		6.08, s, 2 H[l]
5	$C_{14}H_{20}N_6O_4$	C, H	2.30, s, 1 H[h] 3.02, s, 1 H		8.65, d, 3 H
6	$C_{13}H_{22}N_6O_4 \cdot H_2O$	C, H, N	2.10, s, 1 H[h] 2.98, s, 1 H		9.05, t, 3 H
7	$C_{16}H_{24}N_6O_4$	C, H, N	2.26, s, 1 H[h] 3.02, s, 1 H		9.10, d, 6 H
8	$C_{17}H_{26}N_6O_4 \cdot 2.5H_2O$	C, H, N[e]	2.22, s, 1 H[i] 3.18, s, 1 H		9.32, m, 6 H
9	$C_{18}H_{27}N_5O_5$	C, H, N			
10	$C_{17}H_{26}N_6O_4$	C, H, N[f]			
11	$C_{15}H_{22}N_6O_5 \cdot EtOH$	C, H, N			
12	$C_{16}H_{24}N_6O_4S$	C, H, N, S	2.28, s, 1 H[h] 3.05, s, 1 H		7.98, s, 3 H
13	$C_{20}H_{24}N_6O_4 \cdot 0.5H_2O$	C, H, N			
14	$C_{22}H_{25}N_7O_4 \cdot 2H_2O$	C, H, N			
15	$C_{23}H_{28}N_6O_4 \cdot 2H_2O$	C, H, N		2.68, s, 5 H[j] 4.94, s, 2 H	
16	$C_{24}H_{30}N_6O_4 \cdot 0.5H_2O$	C, H, N[g]		2.72, s, 5 H[j] 4.92, s, 2 H	7.26, d, 3 H
17	$C_{24}H_{29}N_5O_5 \cdot 0.5H_2O$	C, H, N		2.69, s, 5 H[j] 4.95, s, 2 H	6.36, s, 3 H
18	$C_{20}H_{24}N_6O_4$			2.70, s, 5 H[i] 4.85, s, 2 H	
19	$C_{21}H_{26}N_6O_4$	C, H, N			
20	$C_{23}H_{30}N_6O_4$	C, H, N		2.56, s, 5 H[k] 4.56, s, 2 H	9.02, d, 6 H
21	$C_{24}H_{32}N_6O_4$	C, H, N		2.62, s, 5 H[i] 4.82, s, 2 H	9.08, m, 6 H
22	$C_{27}H_{30}N_6O_4$	C, H, N			

[a] Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. [b] See Experimental Section for details. [c] J. Bøler, F. Enzmann, K. Folkers, C. Y. Bowers, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **37**, 705 (1969). [d] C: calcd, 50.55; found, 49.98. [e] H: calcd, 7.37; found, 6.82. [f] C: calcd, 53.95; found, 53.39. [g] N: calcd, 17.67; found, 17.21. [h] D_2O. [i] CD_3OD. [j] $CDCl_3$. [k] AcOH-d_4. [l] Methylene protons of glycine moiety. [m] J. Bøler, J. K. Chang, F. Enzmann, and K. Folkers, *J. Med. Chem.*, **14**, 475 (1971).

The presence of a Me group on the Pro-NH_2 moiety of TRH as in pGlu-His-Pro-$NHCH_3$ (**2**) decreases activity, and a dosage of 100 ng is necessary to achieve about the same release of TSH as does 9 ng of synthetic TRH.

Replacement of the Pro-NH_2 group of TRH with Pro-OCH_3 (**3**) also decreases activity with a relative dose level of 100 ng for the analog in comparison with 9 ng of TRH.

The presence of the N^{im}-benzyl group on the His moiety of TRH greatly decreases activity, but this substance released TSH at a dose level of 10,000 ng relative to 9 ng of TRH.

The replacement of the Pro-NH_2 moiety by Ala-NH_2, Abu-NH_2 Val-NH_2, and Leu-NH_2 to give the 4 corresponding tripeptides (**5**, **6**, **7**, **8**) greatly reduced activity. pGlu-His-Val-NH_2 (**7**) was the most active of these 4 analogs and showed release of TSH at a relative dose level of about 5000 ng/9 ng of TRH. The other 3 tripeptides released TSH at relative dose levels of approximately 10,000 ng/9 ng of TRH. One may consider that the Ala, Abu, Val, and Leu tripeptides are analogs of TRH which have an "open proline ring" and have 3–6 C atoms in this amino acid moiety in comparison with the 5 C atoms of the proline moiety. It is evident that the cyclic proline moiety of TRH is important for the great potency of TRH.

The replacement of the Pro-NH_2 moiety of TRH with Met-NH_2, Phe-NH_2, or Trp-NH_2 resulted in complete deactivation even at high dose levels.

All tripeptides possessing the His(Bzl) group except for the N^{im}-benzyl derivative ot TRH (**15**) were inactive even at high dose levels.

It is notable that a dose level of 10 μg per analog which fully releases TSH in the assay with mice is actually a comparatively low dose level. However 10 μg is about 1000 times the dose of TRH that releases the same level of TSH. Interpretations of the significance of such unique structure–activity relationships where one is comparing relative dosage of 100 to 10,000 for an analog in respect to that of the hormone have been reviewed.[1]

Experimental Section

Melting points were performed on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalyses

TABLE III

ACTIVITY OF ANALOGS AND DERIVATIVES OF TRH

No.	Compd	Dose level in ng relative to TRH for hormonal activity[a]
2	pGlu-His-Pro-NHCH$_3$	100
3	pGlu-His-Pro-OCH$_3$	100
4	pGlu-His-Gly-NH$_2$	Inactive[b]
5	pGlu-His-Ala-NH$_2$	$\leq$10,000
6	pGlu-His-Abu-NH$_2$	10,000
7	pGlu-His-Val-NH$_2$	5,000
8	pGlu-His-Leu-NH$_2$	10,000
9	pGlu-His-Ileu-OCH$_3$	Inactive[c]
10	pGlu-His-Ileu-NH$_2$	Inactive[b]
11	pGlu-His-Thr-NH$_2$	Inactive[c]
12	pGlu-His-Met-NH$_2$	Inactive[b]
13	pGlu-His-Phe-NH$_2$	Inactive[b]
14	pGlu-His-Trp-NH$_2$	Inactive[b]
15	pGlu-His-Pro-NH$_2$ (Bzl)	10,000
16–22	His-peptides (Bzl)	Inactive[b]

[a] Dose in ng which releases essentially the same level of TSH as 9 ng of synthetic TRH in the T^3-TRH method.[7-9] [b] Up to 10,000 ng. [c] Up to 50,000 ng.

were performed by the Mikroanalytisches Laboratorium, Bonn, West Germany. On tlc (silica gel G), R_f^1, R_f^2, and R_f^3 values refer to the systems of *n*-BuOH-glacial HOAc-EtOAc-H_2O (1:1:1:1); $CHCl_3$-MeOH-NH_4OH (60:45:20); and EtOH-H_2O (7:3), resp. The nmr spectra were measured at 60 Hz on a Varian Associates A-60 spectrometer (Me_4Si or sodium 2,2-dimethyl-2-silapentane-5-sulfonate) and the chemical shifts are expressed in τ values. Satisfactory nmr data were obtained from **23**, **24**, **26**, **28**, and **29**. The optical rotations were measured on a Perkin-Elmer Model 141 digital readout polarimeter. All amino acids used as starting material were purchased as the pure L isomers.

***N*-Carbobenzoxypyroglutamyl-*N*^{im}-benzylhistidine Benzyl Ester (23).**—To a soln of *N*-carbobenzoxy-L-pyroglutamic acid[4] 3.1 g) in dry THF (50 ml), which was magnetically stirred at 0°, Et_3N (2.01 ml) and ethyl chloroformate (1.27 ml) were successively added. After 1 hr, *N*^{im}-benzyl-L-histidine benzyl ester dibenzenesulfonate[5] (8.0 g) and Et_3N (3.38 ml) in dry THF (10 ml) were added. After 2 more hr at below 20°, the THF was evaporated *in vacuo*. The reaction mixt was dild with H_2O (50 ml) and extd with $CHCl_3$ (3 × 50 ml). The ext was dried ($MgSO_4$), filtered, and evapd. The residue was recrystd twice from Me_2CO-Et_2O affording pure **23** (4.62 g, yield 66%): mp 164–166°; $[\alpha]^{22}D$ −48.2° (*c* 0.99, $CHCl_3$); R_f^1 0.78 and R_f^3 0.76; single spot with Cl-tolidine reagent. *Anal.* ($C_{38}H_{32}N_4O_6$) C, H, N.

Pyroglutamyl-*N*^{im}-benzylhistidine (24).—Compd **23** (4.62 g) in 90% EtOH (200 ml) with 5% Pd/C as catalyst was hydrogenated during 2 hr at room temp (1 atm). The reaction mixt was filtered and evapd to give the dipeptide which was purified by recrystn from EtOH to afford pure **24**, (2.5 g, yield 90%): mp 236–239° dec; $[\alpha]^{22}D$ +0.4° (*c* 1.00, H_2O); R_f^1 0.46, R_f^2 0.61, and R_f^3 0.68; single spot with Cl-tolidine reagent. *Anal.* ($C_{18}H_{20}N_4O_4$) C, H, N.

Pyroglutamylhistidine (25).—Pyroglutamyl-*N*^{im}-benzylhistidine (**24**) (3 g) in 80% EtOH (300 ml) with 5% Pd/C (2 g) as catalyst was hydrogenated during 24 hr at room temp (1 atm). The reaction mixt was filtered and evapd *in vacuo*. The residue was recrystd twice from MeOH-EtOH to afford pure **25** (1.9 g, yield 85%): mp 217–219° dec; $[\alpha]^{22}D$ +3.8° (*c* 1.00, H_2O); R_f^1 0.24 and R_f^2 0.47; single spot with the Pauly and Cl-tolidine reagents. *Anal.* ($C_{11}H_{14}N_4O_4$) C, H, N.

***N*-Methylprolinamide·HCl (26).**—A soln of proline Me ester·HCl (5.48 g) in abs MeOH (20 ml) was treated with MeOH which was satd with $MeNH_2$ (80 ml) at 0°. After 48 hr at room temp, the reaction mixt was evapd *in vacuo* to dryness. The residue was dissolved in H_2O (20 ml) and was then extd with $CHCl_3$ (3 × 60 ml). The ext was dried ($MgSO_4$), filtered, and evapd to give *N*-methyl-L-prolinamide (4 g, yield 94%): R_f^1 0.36; single spot ninhydrin reagent; hydrochloride, mp 165–166°; $[\alpha]^{22}D$ −54.4° (*c* 1.00, MeOH). *Anal.* ($C_6H_{12}N_2O$·HCl) C, H, Cl, N.

L-Alaninamide·HCl (27).—L-Alanine Me ester (1 g) was converted as described[6] into **27**; (0.2 g, yield 22%); mp 225° dec; $[\alpha]^{22}D$ +11.0° (*c* 1.24, MeOH). *Anal.* ($C_3H_8N_2O$·HCl C, H, Cl, N.

L-α-Aminobutyramide·HCl (28).—L-α-Aminobutyric acid (1 g) was converted as described[6] into the Me ester and then **28** (0.67 g, yield 50%): mp 267–270° dec; $[\alpha]^{22}D$ +24.4° (*c* 1.65, MeOH). *Anal.* ($C_4H_{10}N_2O$·HCl) C, H, Cl, N.

L-Threoninamide·HCl (29).—L-Threonine Me ester (1.7 g) was converted as described[6] into **29** (1.2 g, yield 61%): mp 230–233° dec; $[\alpha]^{22}D$ +3.1° (*c* 1.53, MeOH). *Anal.* ($C_4H_{10}N_2O_2$·HCl) C, H, Cl.

Pyroglutamyl-*N*^{im}-benzylhistidylvalinamide (20). Method A.—Pyroglutamyl-*N*^{im}-benzylhistidine (**24**) (356 mg, 1 mmole), L-valinamide·HCl (153 mg, 1 mmole), and Et_3N (0.15 ml, 1 mmole) in dry DMF (30 ml) were treated with DCI (210 mg, 1.02 mmoles) at 0°. After being stirred at room temp during 24 hr, the reaction mixt was evapd *in vacuo*. The residue was recrystd from MeOH 3 times to afford pure **20** (228 mg, yield 50%).

Pyroglutamylhistidylvalinamide (7). Method B.—Compd **20** (96 mg) in EtOH (50 ml) with 10% Pd/C as catalyst (200 mg) was hydrogenated during 12 hr at room temp (1 atm). The reaction mixt was filtered and evapd. The residue of **7** was purified by recrystn twice from MeOH-Et_2O to afford the pure tripeptide (60 mg, yield 77%).

Pyroglutamylhistidylmethioninamide (12). Method C.—Pyroglutamylhistidine (**25**) (266 mg, 1 mmole), L-methioninamide·HCl (185 mg, 1 mmole), and Et_3N (0.15 ml, 1 mmole) in dry DMF (25 ml) were treated with DCI (210 mg, 1.02 mmoles) at 0°. After being stirred at room temp during 24 hr, the reaction mixt was evapd *in vacuo* to dryness. The residue was purified by prep chromatography on silica gel plates with elution by $CHCl_3$-MeOH-NH_4OH (60:30:5) to afford pure **12** (187 mg, yield 47%).

Pyroglutamylhistidylisoleucinamide (10). Method D.—A soln of L-pyroglutamylhistidylisoleucine Me ester (20 mg) (**9**) in dry MeOH satd with NH_3 (50 ml) was left at room temp during 6 days. The reaction mixt was evapd, and the residue was recrystd from MeOH-Et_2O to afford pure **10** (15 mg, yield 80%).

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