Synthesis of Thyrotropin-Releasing Hormone Analogues. 2. Tripeptides Structurally Greatly Differing from TRH with High Central Nervous System Activity†

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A new series of thyrotropin-releasing hormone (TRH) analogues, obtained by further modifications of our most potent central nervous system (CNS) stimulating *neutral* tripeptides at both termini, were synthesized by the pentafluorophenyl ester method and tested for CNS and thyrotropin (TSH) releasing activity. Replacement of pyroglutamic acid by pyro-2-aminoadipic acid, 2-oxoimidazolidine-4-carboxylic acid or γ-butyrolactone-γ-carboxylic acid and that of proline by pipecolic acid, thiazolidine-4-carboxylic acid, or homoproline in [Leu^2]- and [Nva^2]TRH led to tripeptides structurally widely different from TRH. In spite of this fact, 7 of the 17 analogues (1, 2, 8-10, 16, and 17) have stronger anticataleptic effect than TRH, with negligible or no hormonal potency. The highest CNS activity was achieved when pyroglutamic acid was replaced by pyro-2-aminoadipic acid at the N-terminus [pAad-Leu-Pro-NH_2, 1 (RGH 2202), and pAad-Nva-Pro-NH_2, 2]. A novel synthesis of L-2-aminoadipic acid suitable for large-scale preparation is also described.

As a result of the first part of our research for dissociation of the CNS effects from the hormonal activity of thyrotropin-releasing hormone (TRH), we have reported the synthesis and biological properties of a series of TRH analogues containing aliphatic amino acids in the central position.[2]

Earlier studies also revealed that replacement of the terminal amino acids in TRH, for example that of pyroglutamic acid by pyro-2-aminoadipic acid,[3] γ-butyrolactone-γ-carboxylic acid,[4] or orotic acid[5] and that of proline by pipecolic acid or thiazolidine-4-carboxylic acid[3] resulted in remarkable enhancement of the CNS activity.

Based on these findings it was decided to synthesize a new series of mainly neutral tripeptide amides in which the central histidine was replaced by leucine or norvaline, while the terminal amino acids, pyroglutamic acid and proline, were exchanged for other acids, containing five- or six-membered heterocycles. In some cases, aliphatic amino acids were incorporated in the C-terminus, too.

The analogues were tested for hormonal (TSH-releasing) and CNS-stimulating (anticataleptic and barbiturate antagonistic) activity.

Results and Discussion

Chemistry. Peptide Synthesis. For the synthesis of most analogues (3, 5-15) the pentafluorophenyl ester method[6] and the previously well proved strategy[2] were employed. These analogues were obtained from benzyloxycarbonyl- (**3a**, **6a**, **8a-14a**) or *tert*-butyloxycarbonyl-protected (**5a**, **15a**) tripeptides by hydrogenolysis or acidolysis, respectively. Analogues containing pyro-2-aminoadipic acid (**1**, **2**, **16**, and **17**) or γ-butyrolactone-γ-carboxylic acid (**4**) at the N-terminus were synthesized by the same method but without the use of any protecting group in the last coupling step. Overall yields and physical data of the protected tripeptides and analogues are shown in Tables I and II, respectively.

Synthesis and Cyclization of L-2-Aminoadipic Acid. L-2-Aminoadipic acid (Aad) was synthesized in two different routes as can be seen in Scheme I. Route 1 shows a laboratory-scale preparation of Aad. L-2-Amino-6-hydroxycaproic acid (**18**), prepared from 2,3-dihydropyran involving a resolution step as described earlier,[7] was converted to the *N*-benzyloxycarbonyl derivative (**19**). This compound was oxidized with $KMnO_4$ by the same method as described for the *N*-benzoyl derivative[8] to yield Z-Aad-OH (**20**), from which Aad (**21**) was obtained by catalytic hydrogenolysis.

Scheme I

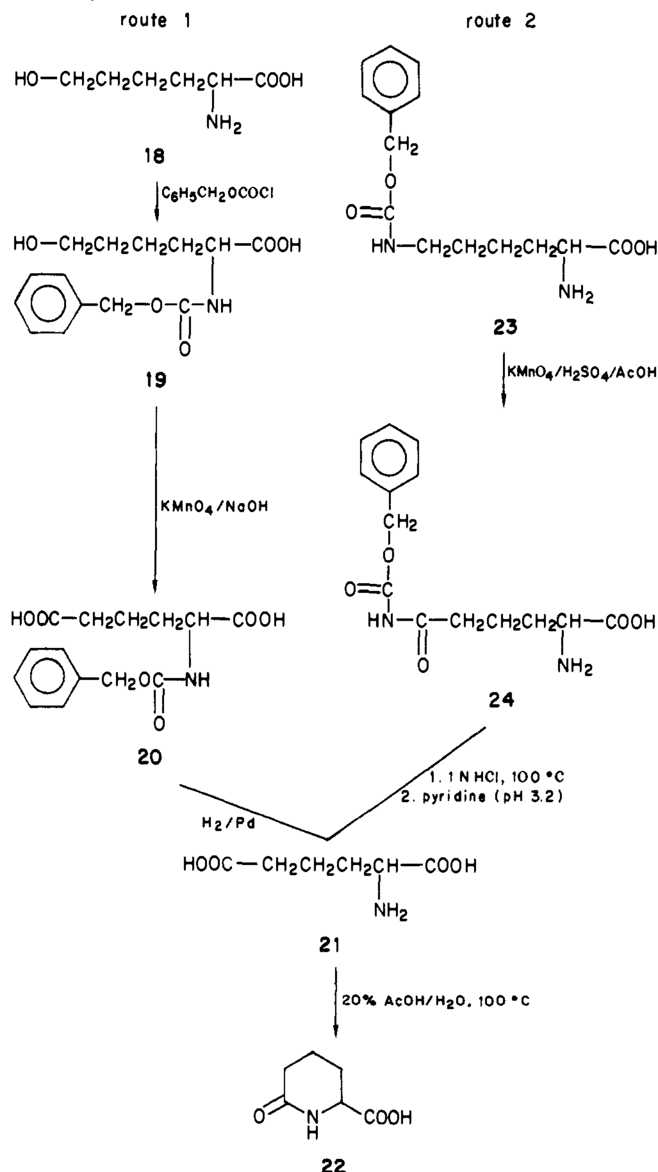

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Table I. Characterization of the Protected Tripeptides

no.	formula[a]	overall yield,[b] %	mp, °C (recrystn solvent)	R_f (A)	$[\alpha]^{25}_D$, deg (*c* 1, AcOH)	anal.
3a	Z-Ica-Leu-Pro-NH_2	61	172–174 (EtOAc)	0.35[c]	−102.6	C, H, N
5a	Boc-Tca-Leu-Pro-NH_2	76	A[d]	0.56	−148.4	C, H, N, S
6a	Z-Pro-Leu-Pro-NH_2	75	A	0.40	−144.2	C, H, N
8a	Z-pGlu-Leu-Pip-NH_2	65	143–144 (EtOAc/Et_2O)	0.39	−97.2	C, H, N
9a	Z-pGlu-Leu-Tca-NH_2	64	108–110 (EtOAc)	0.35	−133.8	C, H, N, S
10a	Z-pGlu-Nva-Tca-NH_2	46	116–118 (EtOAc/Et_2O)	0.37	−129.0	C, H, N, S
11a	Z-pGlu-Leu-piperidide	71	113–115 (EtOAc/Et_2O)	0.61	−42.5	C, H, N
12a	Z-pGlu-Leu-Gly-NH_2	76	206–209 (EtOH)	0.27	−57.4[e]	C, H, N
13a	Z-pGlu-Nva-Leu-NH_2[f]	62	241–242 (EtOH/Et_2O)	0.48	−62.6	C, H, N
14a	Z-pGlu-Nva-Ile-NH_2	69	252–253 (DMFA/Et_2O)	0.49	−57.5	C, H, N
15a	Boc-Gln-Nva-Met-NH_2[f]	49	237–238 (EtOH)	0.38	−37.1	C, H, N, S

[a] Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977). Other abbreviations: Ica, L-2-oxoimidazolidine-4-carboxylic acid; Tca, L-thiazolidine-4-carboxylic acid; Pip, L-pipecolic acid. [b] Calculated for the amount of central amino acid derivative used in the dipeptide coupling reaction. [c] In solvent system B (for composition of the solvent systems, see the Experimental Section). [d] A, amorphous. [e] −27.9° (*c* 0.8, DMFA); lit.[16] mp 217–218 °C; $[\alpha]^{20}_D$ −28.2° (*c* 0.8, DMFA). [f] Through Boc-protected dipeptide methyl ester amidated in NH_3/MeOH.

Table II. Characterization of TRH Analogues

no.	formula[a]	overall yield, %	mp, °C (recrystn solvent)	R_f B	R_f E	amino acid anal.[b] 1	amino acid anal.[b] 3	$[\alpha]^{25}_D$, deg (*c* 1, AcOH)	anal.
1	pAad-Leu-Pro-NH_2 (RGH-2202)	72	214–216 (EtOH/Et_2O)	0.39	0.43	0.99	0.98	−80.2	C, H, N
2	pAad-Nva-Pro-NH_2	68	192–193 (EtOH/Et_2O)	0.33	0.40	1.02	0.97	−83.2	C, H, N
3	Ica-Leu-Pro-NH_2	56	A[c]	0.33	0.25		1.04	−90.4	C, H, N
4	Blc-Leu-Pro-NH_2	40[d]	A	0.55[e]	0.55		0.98	−59.4	C, H, N
5	H-Tca-Leu-Pro-NH_2[f]	63	A	0.63	0.64		1.08	−162.9	C, H, N, S
6	H-Pro-Leu-Pro-NH_2	60	189–192 (EtOH/Et_2O)	0.45[g]	0.17	2.10		−126.0[h]	C, H, N
7	Ac-Pro-Leu-Pro-NH_2[i]	50	A	0.35	0.56	2.17		−129.6	C, H, N
8	pGlu-Leu-Pip-NH_2	57	A	0.48	0.60	1.06	1.05	−86.4	C, H, N
9	pGlu-Leu-Tca-NH_2	34[d]	A	0.46	0.57	1.11		−145.0	C, H, N, S
10	pGlu-Nva-Tca-NH_2	34[d]	A	0.41	0.52	0.99		−145.6	C, H, N, S
11	pGlu-Leu-piperidide	57	99–100 (Et_2O)	0.68	0.78	0.95		−30.4	C, H, N
12	pGlu-Leu-Gly-NH_2	65	175–177 (EtOAc)	0.33	0.39	1.00	1.02	−45.8[j]	C, H, N
13	pGlu-Nva-Leu-NH_2	60	239–240 (AcOH/Et_2O)	0.54	0.58	1.01	0.94	−55.8	C, H, N
14	pGlu-Nva-Ile-NH_2	67	267–270 (AcOH/Et_2O)	0.56	0.59	1.03	1.00	−50.2	C, H, N
15	pGlu-Nva-Met-NH_2[k]	48	249–250 (AcOH/Et_2O)	0.50	0.57	0.97	0.96	−48.4	C, H, N, S
16	pAad-Nva-Tca-NH_2	58	183–185 (EtOH/Et_2O)	0.46	0.47	1.05		−136.0	C, H, N, S
17	pAad-Nva-HPro-NH_2	46[d]	A	0.35	0.44	0.96		−44.6	C, H, N

[a] Abbreviations not indicated in Table I: pAad, L-pyro-2-aminoadipic acid; Blc, (*S*)-γ-butyrolactone-γ-carboxylic acid; HPro, L-homoproline (L-2-pyrrolidineacetic acid). [b] Calculated for the amount of the central amino acid taken as 1.00; 1 and 3 mean the N- and C-terminal amino acid, respectively. [c] A, amorphous. [d] Purified by column chromatography. [e] Contaminated with some percents of (*S*)-2-hydroxy-α-glutaryl-Leu-Pro-NH_2. [f] Obtained from **5a** by deprotection in HCl/EtOAc. [g] In solvent system C. [h] −120.0° (*c* 0.1, MeOH); lit.[13] mp 192–193.5 °C; $[\alpha]^{21}_D$ −120° (*c* 0.1, MeOH). [i] Obtained from **6** by acetylation. [j] −22.7° (*c* 1, MeOH); lit.[14] mp 175–176 °C; $[\alpha]^{20}_D$ −17.0° (*c* 1, MeOH); lit.[16] mp 174–175 °C; $[\alpha]^{20}_D$ −17.7° (*c* 0.9, MeOH). [k] Obtained from **15a** by deprotection in formic acid and subsequent ring closure in hot AcOH.

The second synthesis enabled us to prepare Aad on a large scale. We have modified and improved the synthesis of Liberek and Jablonska[9] starting from L-lysine (Scheme I, route 2). The key step of this synthesis is the oxidation of H-Lys(Z)-OH (**23**) with $KMnO_4$ in acidic medium to L-2-amino-5-[(benzyloxycarbonyl)carbamoyl]pentanoic acid [H-Aad(NH-Z)-OH, **24**] as described for the oxidation of several N-protected amino acids.[11] This reaction can be carried out in 40% yield without size limitation. In accord with our observation about the sensitivity of the *N*-benzyloxycarbonyl-protected δ-carboxamide group of **24** to acids, Aad (**21**) was readily obtained by a simple treatment of **24** with hot hydrochloric acid instead of the proposed but dangerous use of Na_2O_2.[9,12]

2-Aminoadipic acid is more prone to intramolecular cyclization on heating than glutamic acid: when a 2% water solution was refluxed for 2 h, an equilibrium value of 73% lactam (pyro-2-aminoadipic acid, **22**) and 27% starting amino acid was established by ninhydrin analysis.[10] This very low concentration of Aad is not practical for a large-scale preparation of **22**, but an increase of the

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Table III. CNS and Hormonal Activities of TRH Analogues

		anticataleptic effect[a]		effect on hexobarbital sleeping time[b]		
no.	formula	inhibn % (80 mg/kg iv)	ED_{50}, mg/kg iv (95% FL)	min ± SE	Δ, %	TSH-releasing act.,[c] %
	saline	0		38.4 ± 1.36		
	pGlu-His-Pro-NH_2 (TRH)	40	113.0 (–)	24.8 ± 3.44*	–35	100
1	pAad-Leu-Pro-NH_2 (RGH 2202)		23.5 (8.6–33.5)	16.4 ± 2.06**	–57	2.7
2	pAad-Nva-Pro-NH_2		10.6 (6.7–16.3)	19.7 ± 1.49**	–49	0
3	Ica-Leu-Pro-NH_2	40		25.8 ± 4.81	–33	0
4	Blc-Leu-Pro-NH_2	40		36.8 ± 1.51	–4	
5	H-Tca-Leu-Pro-NH_2	0		35.0 ± 1.90	–9	0
6	H-Pro-Leu-Pro-NH_2	0		65.6 ± 6.20**	+71	np[d]
7	Ac-Pro-Leu-Pro-NH_2	0		37.6 ± 0.11	–2	
8	pGlu-Leu-Pip-NH_2		38.5 (21.1–54.1)	16.6 ± 6.36**	–57	7.2
9	pGlu-Leu-Tca-NH_2		31.4 (19.6–53.4)	17.2 ± 2.47**	–55	0
10	pGlu-Nva-Tca-NH_2		56.0 (–)	26.4 ± 1.69**	–31	0
11	pGlu-Leu-piperidide	20		33.3 ± 1.55	–13	np
12	pGlu-Leu-Gly-NH_2	0		35.8 ± 3.08	–7	
13	pGlu-Nva-Leu-NH_2	20		43.0 ± 1.89	+12	0
14	pGlu-Nva-Ile-NH_2	pot[e]		$47.7 \pm 3.10^{+}$	+24	0
15	pGlu-Nva-Met-NH_2	20		46.1 ± 3.69	+20	0
16	pAad-Nva-Tca-NH_2		60.3 (45.4–84.0)	23.6 ± 1.81**	–39	np
17	pAad-Nva-HPro-NH_2		35.3 (30.1–41.2)	29.1 ± 1.79**	–24	np

[a] Based on measurement of the ability of varying doses of the analogue to inhibit the cataleptic effect of haloperidol in rat. [b] Based on measurement of the ability of the analogue to shorten the hexobarbital sleeping time in mice. Key (in relation to the control group): $^{+}$, $p < 0.02$; *, $p < 0.01$; **, $p < 0.001$. [c] Based on measurement of the TSH response to the analogue by radioimmunoassay in a two-point assay (1 and 10 μg, respectively) in the rat. [The compounds were dissolved in physiological saline before injection (in 5 mL/kg for rat and 10 mL/kg for mice). The control groups received the same volume of physiological saline alone. Groups of 10 animals were used for each treatment in all three pharmacological tests. For details of the pharmacological experiments, see the Experimental Section.] [d] np, Slightly significant but not parallel TSH response to the lower and upper doses used. [e] Potentiating effect.

concentration resulted in a lower equilibrium value of **22** (Szirtes, unpublished observation). Finally, an acceptable degree of the ring closure (about 80%) at a reasonable concentration of Aad (10%) was achieved by refluxing **21** in dilute acetic acid for 3 h.

Biology. The pharmacological data in Table III show that further modifications of our neutral TRH analogues[2] have led to newer tripeptides having stronger anticataleptic effect than TRH without any notable TSH-releasing potency (compounds **1**, **2**, **8**, **9**, **10**, **16**, and **17**). No or weak anticataleptic activity was only found in the case of peptides that had no N-terminal lactam ring (**3–7**) or when no C-terminal heterocyclic amino acid was present (**11–15**). The reported oxotremorine antagonists, H-Pro-Leu-Pro-NH_2[13] (**6**) and pGlu-Leu-Gly-NH_2[14,15] (**12**), were inactive in our test.

As also can be seen from the data of Table III, the barbiturate sleeping time was shortened most significantly by the analogues having strong anticataleptic effect, too (compounds **1**, **2**, **8**, and **9**). In the dose used (10 mg/kg) they produced approximately a 50% decrease in sleeping time, an effect greater than that of TRH and similar to those obtained for the known potent CNS-stimulating TRH analogues MK 771,[17] CG 3509,[5b] DN 1417,[4] and RX 77368[18] in this test. Drastic structural modifications in the analogues such as replacement of pyroglutamic acid by other than a lactam-type amino acid and that of proline by aliphatic amino acids have led not only to inactive tripeptides in the reversal of barbiturate sleeping time too (compounds **4**, **5**, **7**, and **12**) but have resulted in analogues having the opposite effect in this experiment (compounds **6**, **13**, **14**, and **15**). H-Pro-Leu-Pro-NH_2 (**6**) extended the barbiturate sleeping time most significantly while pGlu-Nva-Ile-NH_2 (**14**) showed perfectly inverse effects to TRH in both CNS tests.

In conclusion, simultaneous replacement of both the central histidine in TRH by leucine or norvaline and the terminal amino acids by other heterocyclic amino acids has led to a new series of neutral analogues structurally widely different from the parent peptide. It is quite surprising that some tripeptides show a qualitative similar CNS stimulating profile to TRH in the selected tests all the same. (A more detailed pharmacological profile of the analogues will be published separately.) The highest CNS activity was achieved when pyroglutamic acid was replaced by pyro-2-aminoadipic acid at the N-terminus (pAad-Leu-Pro-NH_2, **1** (RGH 2202), and pAad-Nva-Pro-NH_2, **2**). In view of its prominent CNS stimulating activity but negligible hormonal potency and, last but not least, convenient producibility and favorable physical properties, pAad-Leu-Pro-NH_2 (**1**, RGH 2202) has been selected for development.

The mechanism of the CNS action of the analogues is yet unknown, but further pharmacological, endocrinological, in addition, neurochemical, toxicological, receptor binding, and degradation studies with RGH 2202 to answer this question will be completed and published in the near furture.

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 Varian EM 360 and Bruker WM 250 instruments in $CDCl_3$, $CDCl_3/Me_2SO$-d_6 (4:1), or 2 N DCl/D_2O using Me_4Si and DSS as internal standards in the organic solvents and DCl/D_2O, respectively. 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. Solvent systems A–C were made by mixing EtOAc and a stock solution of pyridine/AcOH/H_2O (20:6:11) in the following proportions: (A) EtOAc/stock, 4:1; (B) EtOAc/stock, 3:2; (C) Et-

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Table IV. Physical Data of the New Pentafluorophenyl Esters

compd	yield, %	mp, °C (recrystn solvent)	R_f (F)	$[\alpha]^{25}_D$, deg (c 1, EtOAc)	^{1}H NMR, δ ($CDCl_3$)	anal.
pAad-OPfP (**25**)	92	96–99 (Et_2O/*n*-hexane)	0.37	+30.0	1.6–2.3 (m), 2.4 (t), 4.52 (m), 7.35 (br)	C, H, N, F
Z-Ica-OPfp	75	148–150 (EtOAc)	0.13	−39.7	3.32–4.1 (m), 5.1 (dd), 5.26 (s), 7.36 (s), 7.4 (br)	C, H, N, F
Blc-OPfp	92	45–47 (Et_2O/*n*-hexane)	0.58	+16.5	2.7 (m), 5.32 (m)	C, H, F
Boc-Tca-OPfp	92	55–56 (*n*-hexane)	0.73	−84.5	1.51 (s), 3.45 (m), 4.62 (ABq), 5.12 (m)	C, H, N, F, S

OAc/stock, 2:3; (D) 1-butanol/AcOH/H_2O, 4:1:1; (E) $CHCl_3$/MeOH, 2:1; (F) $CHCl_3$/*n*-hexane/AcOH, 8:1:1. Column chromatography was carried out on Fluka silica gel 60 (0.063–0.200 mm). All new compounds gave elemental analyses (C, H, and N) consistent 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.

Pentafluorophenyl esters of Boc-Gln-OH, Boc-Leu-OH,[19] Boc-Nva-OH,[20] and Z-Pro-OH[21] were prepared as described earlier. The new pentafluorophenyl esters were synthesized in the same way. Physical data of these new compounds are shown in Table IV. Z-Pip-OH and Boc-HPro-OH were kindly supplied by Dr. L. Balâspiri (Department of Medical Chemistry, University Medical School, Szeged). Amino acid amides were prepared by the literature procedure.[22]

The anticataleptic and TSH-releasing activity of the analogues were determined in rat according to the methods described earlier.[2]

The effect of the analogues on barbiturate sleeping time was tested in male LATI:CFLP mice weighing 18–22 g. All mice received a 10 mg/kg intraperitoneal (ip) dose of the test compounds dissolved in physiological saline 10 min after an intravenous (iv) injection of 60 mg/kg hexobarbital sodium. Groups of 10 mice were used for each treatment, and the control group received saline alone 10-min predose with 60 mg/kg of hexobarbital sodium. The sleeping time was recorded as the period elapsed between the loss and recovery of the righting reflex.

Synthesis of L-2-Aminoadipic Acid. Route 1. *N*-(Benzyloxycarbonyl)-L-2-amino-6-hydroxycaproic Acid (19). To an ice-cold solution of L-2-amino-6-hydroxycaproic acid[7] (**18**; 4.27 g, 29 mmol) in 2 N NaOH (14.5 mL) were simultaneously added 2 N Na_2CO_3 (22 mL) and benzyl chloroformate (6 g, 35 mmol) by drops, with stirring at 0–5 °C. The mixture was stirred for an additional 3 h at the same temperature; then the solution was extracted with Et_2O, acidified with concentrated HCl, and extracted again with EtOAc. The organic solution was dried over Na_2SO_4 and filtered. The filtrate was evaporated and the oily residue solidified on rubbing with *n*-hexane. The resulting crude product (6.93 g) was recrystallized from EtOAc/*n*-hexane to give 6.82 g (84%) of **19**: mp 101–103 °C; $[\alpha]^{25}_D$ −9.8° (*c* 1, MeOH); R_f (A) 0.42; IR (KBr) 3350, 3300, 1675 (br), 1245, 760, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$/Me_2SO-d_6, 4:1) δ 1.10–2.05 (m, 6, 3 CH_2), 3.54 (t, 2, OCH_2), 4.20 (m, 1, CH), 5.08 (s, 2, benzyl CH_2), 6.06 (d, 1, NH), 7.17 (br, 2, OH and COOH), 7.30 (s, 5, Ar H).

***N*-(Benzyloxycarbonyl)-L-2-aminoadipic Acid (Z-Aad-OH, 20).** To a cooled solution of **19** (6.46 g, 23 mmol) in 1 N NaOH (46 mL), $KMnO_4$ (4.84 g, 30.6 mmol) was added portionwise, with stirring at 5–10 °C. After the mixture was stirred for an additional 2 h at the same temperature, MnO_2 was filtered off and the filtrate acidified to pH 1 with concentrated HCl (5.5 mL). The precipitate was filtered off and the resulting crude material (5.90 g) recrystallized from H_2O to yield 5.51 g (82%) of Z-Aad-OH (**20**): mp 133–135 °C; $[\alpha]^{25}_D$ −10.2° (*c* 1, MeOH); R_f (A) 0.44; IR (KBr) 3300, 1680 (br), 1520, 1275, 755, 693 cm^{-1}; ^{1}H NMR ($CDCl_3$/Me_2SO-d_6, 4:1) δ 1.30–2.20 (m, 4, 2 CH_2), 2.27 (t, 2, $COCH_2$), 4.25 (m, 1, CH), 5.06 (s, 2, benzyl CH_2), 5.78 (d, 1, NH), 7.30 (s, 5, Ar H), 10.18 (br, 2, 2 COOH); lit[23] mp 136–137 °C.

L-2-Aminoadipic Acid (Aad, 21). Z-Aad-OH (**20**; 5.31 g, 18 mmol) was hydrogenated in 60% aqueous AcOH (250 mL) in the presence of 10% Pd/charcoal for 2 h. The catalyst was removed by filtration and washed with 1 N HCl (20 mL) and H_2O. The filtrate was evaporated, the residue dissolved in H_2O (25 mL), and the solution adjusted to pH 3.2 by the addition of pyridine. The resulting suspension was allowed to stand overnight at 5 °C, and the precipitate was filtered off and washed with cold H_2O and EtOH to give 2.40 g (83%) of Aad (**21**): mp 220–222 °C; $[\alpha]^{25}_D$ +23.8° (*c* 2, 5 N HCl); R_f (D) 0.23; lit[10] $[\alpha]^{25}_D$ +25.0 (*c* 2, 5 N HCl).

Route 2. L-2-Amino-5-[(benzyloxycarbonyl)carbamoyl]pentanoic Acid [H-Aad(NH-Z)-OH, 24]. To a cooled solution of H-Lys(Z)-OH (**23**; 327 g, 1.17 mol) in 1 M H_2SO_4/50% aqueous AcOH (14.8 L), $KMnO_4$ (740 g, 4.68 mol) was added portionwise, with stirring at 10–15 °C, during 20 min. The mixture was stirred for an additional 15 min; then saturated aqueous solution of Na_2SO_3 was added until the brown suspension turned into a clear solution. The solution was diluted with H_2O (5 L) and ajusted to pH 5 by the addition of 25% NH_4OH. The resulting white suspension was allowed to stand overnight at 5 °C; the precipitate was filtered off and washed with H_2O to yield 139 g (40.4%) of **24**. A sample recrystallized from H_2O had the following physical data: mp 176–179 °C dec; $[\alpha]^{25}_D$ +11.7° (*c* 1, 2 N HCl); R_f (D) 0.36; IR (KBr) 3350, 3190, 1745, 1678, 773, 733, 692 cm^{-1}; ^{1}H NMR (2 N DCl/D_2O) δ 1.97 (m, 4, 2 CH_2), 2.54 (t, 2, $COCH_2$), 4.20 (t, 1, CH), 5.16 (s, 2, benzyl CH_2), 7.41 (s, 5, Ar H).

L-2-Aminoadipic Acid (21). H-Aad(NH-Z)-OH (**24**; 133 g, 0.452 mol) was refluxed in 1 N HCl (1125 mL) for 3 h. The benzyl carbamate that precipitated on cooling to room temperature was extracted with Et_2O, and the water solution was adjusted to pH 3.2 by the addition of pyridine. The resulting white suspension was allowed to stand overnight at 5 °C; then the amino acid was filtered off and washed with ice-cold H_2O to give 59.28 g (81.5%) of Aad (**21**): mp 220–222 °C; $[\alpha]^{25}_D$ +24.0° (*c* 2, 5 N HCl); R_f (D) 0.23.

L-Pyro-2-aminoadipic Acid Monohydrate (pAad-OH·H_2O, 22). Aad (**21**; 20 g, 0.124 mol) was refluxed in 20% aqueous AcOH (200 mL) for 3 h. The solvent was evaporated, and the solid residue was taken up in EtOH (200 mL). The unchanged starting amino acid was filtered off (3.8 g) and the filtrate evaporated. The crude lactam obtained (14.2 g, 80%) was dissolved in hot H_2O (100 mL). From the water solution, the monohydrate crystallized in large prisms on cooling. It was filtered off to yield 13.6 g (68%) of pAad-OH·H_2O (**22**): mp 101–104 °C; $[\alpha]^{25}_D$ +13.7° (*c* 2, H_2O); R_f (D) 0.42; lit[10] $[\alpha]^{25}_D$ −16.5° (*c* 2, H_2O) for the anhydrous D isomer.

L-Pyro-2-aminoadipic Acid Pentafluorophenyl Ester (pAad-OPfp, 25). To an ice-cold suspension of pAad-OH·H_2O (**22**; 16.1 g, 0.1 mol) and PfpOH (20.2 g, 0.11 mol) in EtOAc (200 mL) was added DCC (22.6 g, 0.11 mol). The mixture was stirred for 3 h at 0–5 °C, DCU filtered off, and the solvent evaporated. The residue was dissolved in a mixture of Et_2O (30 mL) and *n*-hexane (90 mL) from which the product crystallized on cooling to yield 28.5 g (92%) of pAad-OPfp (**25**): mp 96–99 °C; $[\alpha]^{25}_D$ +30.0° (*c* 1, EtOAc); R_f (F) 0.37; IR (KBr) 3270, 1780, 1655, 1613, 1510, 1105, 990 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.6–2.3 (m, 4, 2 CH_2), 2.4 (t, 2, $COCH_2$), 4.52 (m, 1, CH), 7.35 (br, 1, NH).

L-Pyro-2-aminoadipyl-L-leucyl-L-prolinamide (pAad-Leu-Pro-NH_2, 1). To an ice-cold suspension of H-Leu-Pro-NH_2·HCl[2] (106.7 g, 0.403 mol) and pAad-OPfp (**25**; 130 g, 0.42 mol) in DMFA (1 L) was added NEt_3 (56.5 mL, 0.403 mol) dropwise, with stirring at 0–5 °C. The mixture was stirred for 2 h at the same temperature; the precipitate was filtered off, and the solvent was evaporated and the residue solidified by rubbing with EtOAc. The

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resulting crude product (148 g) was dissolved in boiling EtOH (800 mL); the solution was clarified with charcoal and allowed to stand overnight at 5 °C. The resulting white suspension was diluted with Et_2O (400 mL) and left to stand overnight at 5 °C. The product was filtered off and washed with an ice-cold mixture of EtOH/Et_2O (2:1) to give 1 as a white, crystalline substance: yield, 122.2 g (86%); mp 214-216 °C; $[\alpha]^{25}_D$ -80.2° (c 1, AcOH); R_f (B) 0.39; R_f (E) 0.43; ^{1}H NMR ($CDCl_3$) δ 0.92, 0.94 (dd, 6, 2 CH_3), 2.35 (m, 2, $COCH_2$), 3.65 (m, 1, Leu NCH), 3.88 (m, 2, NCH_2), 4.52 (m, 1, Pro NCH), 4.75 (m, 1, pAad NCH), 5.35, 7.67 (2 s, 2, NH_2), 8.00 (s, 1, Leu NH), 8.40 (d, 1, pAad NH). Amino acid analysis: Aad, 0.99 (1); Leu, 1.00 (1); Pro, 0.98 (1); NH_3, 1.02 (1).

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Topological Similarities between a Cyclic Enkephalin Analogue and a Potent Opiate Alkaloid: A Computer-Modeling Approach

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The cyclic enkephalin analogue H-Tyr-*cyclo*[-D-N^{δ}-Orn-Gly-Phe-Leu-] (**1-c**) and the rigid narcotic alkaloid 7α-[(1*R*)-1-hydroxy-1-methyl-3-phenylpropyl]-6,14-*endo*-ethenotetrahydrooripavine (PEO) (**2**) were studied by using computer graphics methods to investigate potential geometrical congruencies of their respective pharmacophoric elements. Particular emphasis was placed on the relative spatial disposition of the tyramine moiety and the additional aromatic ring that occurs in both molecules. A three-dimensional vector map was generated defining the locus of the C_{21} aromatic ring for all those conformers of PEO having up to 10 kcal above the minimum energy conformer. A systematic conformational search on the cyclic peptide afforded four allowable sets of conformers whose side chain of the phenylalanine residue coincided with the vector map of PEO. Local energy minima for the peptide within the revised mutual vector space were found and subjected to bimolecular energy refinement with correspondingly local energy minima for the opiate alkaloid. Several low-energy conformers of the cyclic peptide were identified that permitted a good fit with the alkaloid provided that the tyramine moiety of the respective molecules does not coincide. In the designated conformations the basic nitrogen of the former occupies a distinct geometrical locus, and the side chain of the leucine residue has no structural correlate in the alkaloid.

Intensive studies with synthetic analogues have established the relative importance and contribution to opiate activity of the structural elements in the peptide backbone and side chains of enkephalins.[1] The molecular conformation of these opioid peptides have also been investigated extensively[2] and several theories relating to the native and receptor-bound[3,4] conformation have been proposed. Furthermore, since enkephalins and rigid and semirigid opiate alkaloids bind, albeit with different degree of affinity and selectivity, to the same receptors, several authors have alluded to the possibility of chemical and spatial equivalence of key functional groups which could serve homologous roles at the receptor level. Among the plethora of structural analogies drawn, the *p*-hydroxyphenethylamine (tyramine) moiety common to opioid peptides and narcotics of the morphine, morphinan, and oripavine class has been proposed to serve a similar pharmacophoric role in receptor recognition and activation.[5-8,11,12] However, the spatial organization of the atoms comprising the tyramine pharmacophore may be different at the receptor level for these two classes of opioids[7,10] and this may be related to differences in regional flexibility and respective chirality of the α-carbon in the tyrosyl residue in the former and corresponding C_9 in the latter category. Several authors have also suggested that the secondary structure of enkephalins permits the additional aromatic ring present in the side chain of the phenylalanine residue to serve a functional role equivalent to the C_{21} ring in the potent

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