Design of *N*-Acylprolyltyrosine "Tripeptoid" Analogues of Neurotensin as Potential Atypical Antipsychotic Agents

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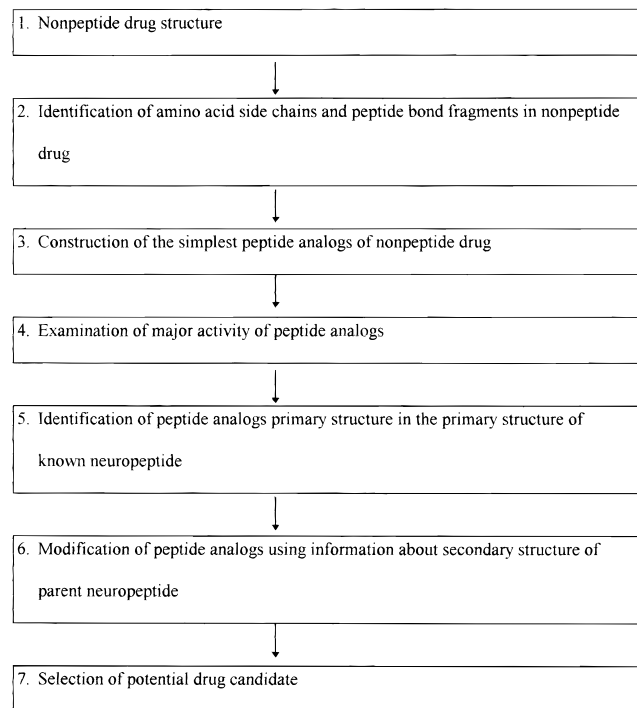
A series of *N*-acylprolyltyrosine amides was designed as tripeptoid analogues of neurotensin. The substituted dipeptides were tested in vivo for antidopamine activity by their ability to inhibit the apomorphine-induced climbing in mice and the dopamine-induced extrapolatory behavior impairment in rats. The *N*-acylprolyltyrosine amides structure−activity relationships have indicated the size of the *N*-acyl group and the configuration of amino acids that are important for the activity. We found that the bioactivity has been increased dramatically when the *n*-hydrocarbon chain on the *N*-acyl group was increased from four to five carbon atoms. The activity seems to reside exclusively in the L-Tyr diastereomers. All of the compounds tested were inactive in the cataleptogenic action and did not exhibit the acute toxicity even at doses 500−1000 times higher than ED_{50} in climbing test. On this basis, the *N*-acylprolyltyrosine amides could potentially be a novel class of atypical antipsychotic agents.

Introduction

Neurotensin (NT, pGlu[1]-Leu[2]-Tyr[3]-Glu[4]-Asn[5]-Lys[6]-Pro[7]-Arg[8]-Arg[9]-Pro[10]-Tyr[11]-Ile[12]-Leu[13]) is an endogenous tridecapeptide[1] that has a broad range of biological effects including hypotension, analgesia, and increase of vascular permeability. In addition, NT has been shown to possess pharmacological properties similar to those of dopamine antagonists that are effective antipsychotics.[2−6] Closer scrutiny of the behavioral data revealed even closer similarities of centrally administrated NT and atypical antipsychotic drugs. For example, direct injection of NT into the nucleus accumbens, a major terminal site of the mesolimbic DA system, attenuates D-amphetamine-induced hyperactivity, whereas direct injection of NT into the nucleus caudatus, a major terminal site of the nigroneostriatal DA system, does not attenuate D-amphetamine-induced stereotypic behavior. This finding suggests that NT might selectively modulate mesolimbicortical DA neurons while exerting few if any effects on the nigroneostriatal DA system—a profile desirable in drug development because of the presumed absence of both extrapyramidal side effect and tardive dyskinesia liability.[7] Thus, NT analogues could potentially be a novel class of neuroleptics (antipsychotics), which could replace current anti-dopamine therapies.

Small di-/tripeptides and their derivatives could be the most perspective drugs among these NT analogues. These peptides are more available and less polyfunctional in comparison with polypeptides. Besides, small peptides in some cases are more enzymatically stable and hence could be orally active.

Scheme 1. Stepwise Design of Therapeutically Useful "Peptoids" from Nonpeptide Drugs and Putative Endogenous Neuropeptides



We now report the design, synthesis, and pharmacological properties of the substituted prolyltyrosines, "tripeptoid" analogues of NT. The dipeptide lead was designed using a drug-based peptide design approach, which has as a ground the hypothesis that some psychotropic exogenous drugs are the ligands of specific neuropeptide receptors. Our approach to the design of peptide analogues of nonpeptide drugs is outlined in Scheme 1. Previously we have developed this approach for the design of dipeptide analogues of the classical nootropic piracetam, pyroglytamyl-containing dipep-

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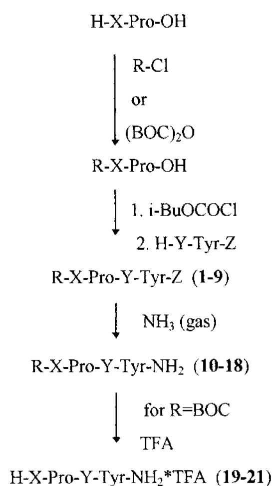
Scheme 2



1: R=$(CH_3)_3$CO C(O), Z=OC_2H_5, X=Y=L

2: R=$(CH_3)_3$CO C(O), Z=OCH_3, X=D, Y=L

3: R=$(CH_3)_3$CO C(O), Z=OCH_3, X= L, Y= D

4: R=$C_6H_5CH_2$C(O), Z=OCH_3, X=Y=L

5: R=$C_6H_5CH_2$C(O), Z= OC_2H_5, X=D, Y=L

6: R=$C_6H_5CH_2$C(O), Z= OC_2H_5, X= L, Y= D

7: R= $CH_3(CH_2)_3$C(O), X=Y=L, Z=OCH_3

8: R= $CH_3(CH_2)_4$C(O), X=Y=L, Z=OCH_3

9: R= $CH_3(CH_2)_7$C(O), X=Y=L, Z=OC_2H_5

10: R=$(CH_3)_3$CO C(O), X=Y=L

11: R=$(CH_3)_3$CO C(O), X=D, Y=L

12: R=$(CH_3)_3$COC(O), X=L, Y=D

13: R=$C_6H_5CH_2$C(O), X=Y=L

14: R=$C_6H_5CH_2$C(O), X=D, Y=L

15: R=$C_6H_5CH_2$C(O), X=L, Y=D

16: R= $CH_3(CH_2)_3$C(O), X=Y=L

17: R= $CH_3(CH_2)_4$C(O), X=Y=L

18: R= $CH_3(CH_2)_7$C(O), X=Y=L

19: X=Y=L

20: X=D, Y=L

21: X=L, Y=D

tides, bridging the gap between piracetam and the major metabolite of the memory peptide vasopressin.[8−10]

The structure of the atypical benzamide neuroleptic, sulpiride, was used as a starting point for the present drug-based peptide design. Sulpiride [5-(aminosulfonyl)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide] is a proven antipsychotic agent,[11] and its action is associated with a low incidence of extrapyramidal disturbances. In this respect sulpiride has a certain similarity with NT. We assumed that sulpiride acts not only on D-2 dopamine receptors but also on NT receptors, which are known as able to modulate dopaminergic system.[12]

Chemistry

The general synthetic route for prolyltyrosines is presented in Scheme 2. N-Acylprolines were prepared by acylation of proline with the appropriate acyl chloride according to Schotten-Baumann; BOC-prolines were synthesized using di-*tert*-butyl dicarbonate according to Pozdnev.[13] A coupling of *N*-acylproline with the corresponding ester of tyrosine was carried out by the mixed anhydride method using isobutyl chloroformate in the presence of *N*-ethylmorpholine according to Anderson.[14] Amides of dipeptides were synthesized by ammonolysis of the corresponding esters. Unsubstituted prolyltyrosines were obtained as its trifluoroacetic salts by treatment of corresponding BOC derivatives with trifluoroacetic acid. Diastereomeric purity of the prepared compounds was not below 98% according to ^{1}H-NMR spectroscopy.

Table 1. Antagonism of Apomorphine-Induced Mice Climbing of Prolyltyrosines

R-(X)-Pro-(Y)-Tyr-NH_2

compd	R	X	Y	ED_{50},[a] mg/kg ip
10	$(CH_3)_3COC(O)$	L	L	2.5 (2.0−3.1)
11	$(CH_3)_3COC(O)$	D	L	0.4 (0.3−0.5)
13	$C_6H_5CH_2C(O)$	L	L	2.2 (1.7−2.8)
				3.4 (po)
14	$C_6H_5CH_2C(O)$	D	L	4.9 (3.2−75)
15	$C_6H_5CH_2C(O)$	L	D	>8
16	$CH_3(CH_2)_3C(O)$	L	L	3.2 (2.1−5.1)
17	$CH_3(CH_2)_4C(O)$	L	L	0.5 (0.4−0.7)
18	$CH_3(CH_2)_7C(O)$	L	L	0.5 (0.4−0.7)
19	H	L	L	3.4 (2.2−5.3)
20	H	D	L	3.6 (2.7−4.7)
21	H	L	D	>16
(−)-sulpiride				17.5 (11.3−27.1)

[a] ED_{50} (doses required for 50% of maximal response) were calculated on percent antagonism; 95% confidence limits are included in parentheses.

Pharmacology

Pharmacological results are presented in Tables 1 and 2. As an indication of potential antipsychotic activity, the present compounds were evaluated in vivo for their ability to antagonize the apomorphine-induced climbing response in mice.[15] Most of the substances under study were able to antagonize this climbing. For some of the active compounds, an additional behavioral test associated with antipsychotic efficacy, dopamine dependent extrapolatory behavior paradigm,[16] was used. This test measures the ability of rats placed into a cylinder plunged in water to solve an "extrapolatory task". The rats treated with L-DOPA lost this ability. The typical and atypical neuroleptics restored the ability of L-DOPA-treated rats to escape from an acute stress situation. All of the compounds tested demonstrated antidopamine activity in this test. The tendency to induce catalepsy in rats was used as an indication of the propensity to cause extrapyramidal side effects.[17] All of the compounds tested were inactive in this cataleptogenic action even in doses 500−1000 times higher than the ED_{50} in the climbing test. The dipeptides studied did not exhibit the acute toxicity in doses up to 500−1000 mg/kg ip (experiments on albino male mice).

Results and Discussion

The structure of sulpiride contains a pyrrolidine ring and an electronegatively substituted phenyl group separated by an amide group. From this we have assumed that sulpiride is a structure analogue of the -Pro-Tyr- dipeptide fragment of some polypeptides. The structure of this dipeptide consists of the pyrrolidine-containing amino acid Pro and the aromatic amino acid

Table 2. In Vivo Characterization of Some Prolyltyrosines, Sulpiride, and Other Neuroleptics

compound	ED_{50} (ip), mg/kg			LD_{50} (ip), mg/kg
	apom[a]	dopa[b]	cata[c]	acute tox in mice
13	2.2 (1.7−2.8)	1.5 (1.2−2.0)	>1000	>1000
17	0.5 (0.4−0.7)	0.4 (0.3−0.6)	>500	>500
19	3.4 (2.2−5.3)	1.6 (1.2−2.1)	>500	>500
sulpiride	17.5 (11.3−27.1)	16.5 (9.11−24.3)	≥120	250
remoxipride	1.2 (0.5−2.4)	0.31 (0.29−0.35)	8.7[d]	302[d]
clozapine	26.2 (9.4−73.2)	20 (18−27)	>32[e]	
haloperidol	0.1 (0.05−0.16)	0.14 (0.10−0.20)	0.5 (0.4−0.6)[f]	35[g]

[a] Inhibition of apomorphine-induced climbing in mice. [b] Antagonism of extrapolatory behavior impairement induced by DOPA in rats. [c] Induction of catalepsy in rats. [d] Value taken from Florvall and Ogren.[23] [e] Value taken from Howard et al.[24] [f] Value taken from Henning et al.[25] [g] Value taken from de Paulis et al.[26]

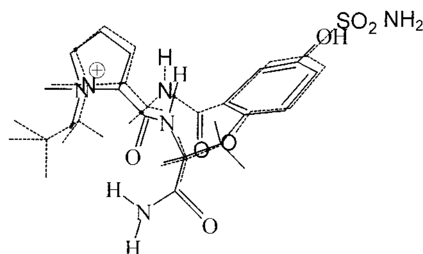
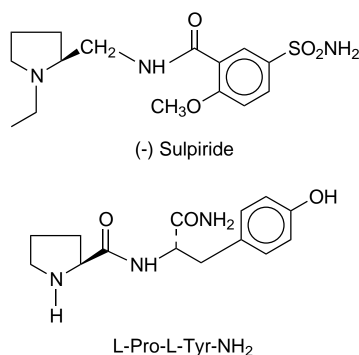


Figure 1. Superposition of the (*S*)-sulpiride (open lines) and L-Pro-L-Tyr-NH_2 (solid lines).

with the OH-substitution Tyr. The simplest model of such fragment is dipeptide prolyltyrosine amide (Pro-Tyr-NH_2).



Superimposition of L-Pro-L-Tyr-NH_2 and (*S*)-sulpiride (using of Dreiding molecular models) revealed the overlap of pyrrolidine cycles and phenyl rings of both molecules in unstrained conformations (see Figure 1). Dipeptide Pro-Tyr-NH_2 has been shown to inhibit apomorphine-induced climbing in mice without cataleptogenic activity (Table 2). This result supports our assumption about the peptidergic mechanism of sulpiride action. The sequence Pro-Tyr corresponds to the neurotensin fragment NT(10−11), which is present in the major biologically active metabolite of neurotensin, NT(8−13).

It is known that the replacement of the Pro^{10}-Tyr^{11} sequence in NT(8−13) with β-turn mimetics has not resulted in activity loss that has suggested the presence of a β-turn-like structure.[18−20] NT(8−13) structure−activity relationships have revealed the residues that are critical for receptor recognition. For example, an alanine scan of NT(8−13) suggested[21] the relative importance of the individual amino acid side chains to be as follows: $Leu^{13} > Tyr^{11} \gg Ile^{12} > Arg^9 > Pro^{10} > Arg^8$. In order to increase the Pro-Tyr-NH_2 dipeptide activity, we included into its structure an *N*-acyl group,

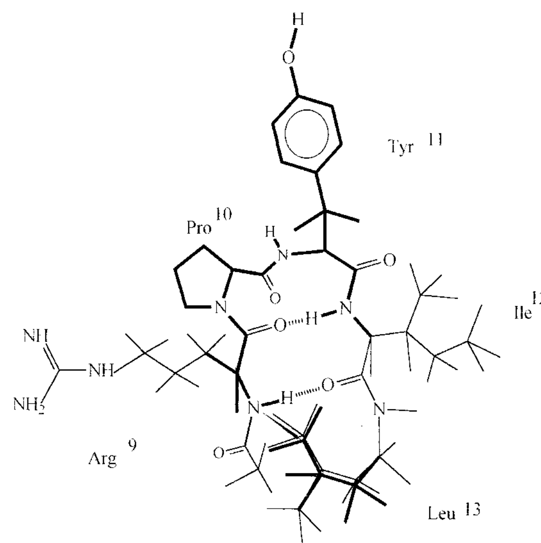


Figure 2. Superposition of the neurotensin (9−13) and its "tripeptoid" analogue *N*-caproyl-L-Pro-L-Tyr-NH_2 (bold lines). The type I β-turn conformation is depicted.

which could imitate the hydrophobic side chain of Leu^{13} in the hypothetical β-turn conformation of NT(8−13) (see Figure 2). In this case the type I β-turn conformation was used because the Pro-Tyr fragment has better overlay with sulpiride than that in the type II β-turn conformation. Thus *N*-acylprolyltyrosine amides may be considered as the "tripeptoid" analogues of NT.

In fact, introduction of the *N*-acyl moiety into dipeptide Pro-Tyr-NH_2 resulted in increased or at least conserved activity in the apomorphine climbing test in mice (cf. **10**, **13**, **16**−**18**, Table 1). The *N*-acylprolyltyrosine amides SAR have indicated the size of *N*-acyl group that is critical for the activity increase. The activity of *N*-valerylprolyltyrosine amide (cf. **16**) is the same as that of nonsubstituted H-Pro-Tyr-NH_2 (cf. **19**). The lengthening of *N*-valeryl moiety by one CH_2 group (*N*-caproyl-Pro-Tyr-NH_2, cf. **17**) results in the increased activity. Further augmentation of *N*-caproyl moiety by three CH_2 groups did not change the activity (cf. **18**). *N*-BOC-prolyltyrosine amide (cf. **10**) and *N*-phenylacetylprolyltyrosine amide (cf. **13**) with *N*-acyl moiety shorter than *N*-valeryl have demonstrated activity at the level of nonsubstituted Pro-Tyr-NH_2.

These findings are in a good agreement with our assumption that a sufficiently long *N*-acyl moiety is able to imitate the Leu^{13} side chain which is important to reveal the activity. The above results are also consistent the data reported elsewhere[20] that NT(8−13) is biologically active being in the β-turn conformation. The

results obtained suggest the distance between the nitrogen atom of Pro[10] and the side chain Leu[13] to be not less than six σ-bonds.

To evaluate the influence the amino acid residue configurations exerts over the activity of compounds studied, the diastereomers of both Pro-Tyr NH_2 and its *N*-acyl derivatives were synthesized. The replacement of L-Tyr by D-Tyr (cf. **15**, **21**) resulted in the loss of activity. In contrast, replacement of L-Pro by D-Pro (cf. **11**, **14**, **20**) resulted in activity conservation. This demonstrates that the effect of the dipeptides studied is stereoselective in respect to tyrosine, but not proline.

It is concluded that the *N*-acylprolyltyrosine amides which carry an *N*-acyl group longer than *N*-valeryl and have the L-configuration of Tyr and the L- or D-configuration of Pro constitute a new type of potent antipsychotic agents with the ability to inhibit the apomorphine-induced climbing without production catalepsy even at high doses.

Experimental Section

Molecular Modeling. The structures demonstrating the overlay of sulpiride with the prolyltyrosine peptide fragments were obtained by handling with Molecular Dreiding stereomodels (W. Buchi Scientific Apparatus Flawil, Switzerland).

The following sequence of operations was used. First, the hydrophobic regions of the molecules (the phenyl groups and the pyrrolidine rings) were superimposed. Second, the hydrophilic parts were fitted: the positive charged nitrogen atoms of the pyrrolidine rings were fitted precisely; the polar OH-group of the Tyr side chain and the sulpiride SO_2NH_2 group were superimposed; the sulpiride amide group and the peptide group were placed closely; the corresponding N−H and C═O bond vectors were oriented parallel. Then, the conformations have been selected that demonstrated the least strain of the valent angles and bonds. Finally, the conformations with the minimum unsuperimposed molecular volumes were chosen.

Chemistry. Melting points were determined on a capillary melting point apparatus in open capillary tubes and are uncorrected. The structures of the compounds were confirmed by elemental analysis and ^{1}H NMR spectroscopy. Microanalyses agree with calculated values within ±0.4%. The NMR spectra were obtained on a Bruker AC-250 spectrometer using tetramethylsilane as an internal standard. The NMR peaks were designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Specific optical rotations were recorded by automatic polarimeter Perkin-Elmer 241. The TLC was carried out on Merck silica gel 60 F 254 plates, and spots were developed in an iodine chamber or under UV light. For column chromatography, Merck silica gel 60, 230−400 mesh was used. If required, all solvents used in reaction mixtures were dried and purified by standard procedures.

General Synthetic Method for the Preparation of *N*-Acylprolyltyrosine Esters. To a well-stirred solution of *N*-acylproline (10.0 mmol)[13,22] in 100 mL of chloroform were added *N*-ethylmorpholine (10.0 mmol) and isobutyl chloroformate (10.0 mmol) dropwise at −10 °C. After 2 min a mixture of the hydrochloride methyl or ethyl ester of tyrosine (10.0 mmol) and *N*-ethylmorpholine (10.0 mmol) in dimethylformamide (20 mL) was added. Stirring continued for 30 min at −5 °C, and then the mixture was allowed to stand for 1 h. The precipitate was separated by filtration; the solvent was evaporated in vacuo; the residue was dissolved in chloroform, washed with 5% solution of $NaHCO_3$, water, 1 N HCl, and water, and dried with Na_2SO_4; and the solvent was evaporated to dryness. The residue was purified by column chromatography using chloroform as eluent or crystallization from suitable solvent.

***N*-(*tert*-Butoxycarbonyl)-L-prolyl-L-tyrosine ethyl ester (1):** yield 82% (after column chromatography); oil; $[\alpha]^{20}_D$ −48.2° (*c* 0.39, $CHCl_3$): R_f 0.75 ($CHCl_3/C_2H_5OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 1.08 (t, CH_3CH_2O, 3H, minor conformer), 1.11 (t, CH_3CH_2O, 3H, major conformer), 1.15−1.59 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 1.21 (s, $(CH_3)_3C$, 9H, major conformer), 1.37 (s, $(CH_3)_3C$, 9H, minor conformer), 2.76−3.0 (m, $C^\beta H_2$ Tyr, 2H), 3.15−3.35 (m, $C^\delta H_2$ Pro, 2H), 4.0 (q, CH_3CH_2O, 2H, minor conformer), 4.03 (q, CH_3CH_2O, 2H, major conformer), 4.11 (m, $C^\alpha H$ Pro, 1H), 4.31 (m, $C^\alpha H$ Tyr, 1H, minor conformer), 4.36 (m, $C^\alpha H$ Tyr, 1H, major conformer), 6.63, 7.0 (each m, AA′BB′ system, C_6H_4 Tyr, 4H), 8.10 (d, NH Tyr, 1H, minor conformer), 8.15 (d, NH Tyr, 1H, major conformer), 9.23 (s, OH, 1H, major conformer), 9.25 (s, OH, 1H, minor conformer). Anal. ($C_{21}H_{30}N_2O_6$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-D-prolyl-L-tyrosine methyl ester (2):** yield 86% (after column chromatography); oil; $[\alpha]^{20}_D$ +72.0° (*c* 0.23, $CHCl_3$); R_f 0.53 ($CHCl_3/C_2H_5OH$, 11:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 1.29 (s, $(CH_3)_3C$, 9H, major conformer), 1.39 (s, $(CH_3)_3C$, 9H, minor conformer), 1.41−2.09 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 2.67−2.99 (m, $C^\beta H_2$ Tyr, 2H), 3.11−3.35 (m, $C^\delta H_2$ Pro, 2H), 3.59 (s, CH_3O, 3H, major conformer), 3.62 (s, CH_3O, 3H, minor conformer), 4.05 (dd, $C^\alpha H$ Pro, 1H, major conformer), 4.09 (dd, $C^\alpha H$ Pro, 1H, minor conformer), 4.46 (m, $C^\alpha H$ Tyr, 1H), 6.64, 6.97 (each m, AA′BB′ system, C_6H_4 Tyr, 4H); 8.09 (d, J = 8.77 Hz, NH, 1H, minor conformer), 8.26 (d, J = 8.05 Hz, NH, 1H, major conformer), 9.22 (s, OH, 1H). Anal. ($C_{20}H_{28}N_2O_6$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-prolyl-D-tyrosine methyl ester (3):** yield 87% (after column chromatography); oil; $[\alpha]^{20}_D$ −72.0° (*c* 0.23, $CHCl_3$); R_f 0.60 ($CHCl_3/C_2H_5OH$, 11:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 1.29 (s, $(CH_3)_3C$, 9H, major conformer), 1.39 (s, $(CH_3)_3C$, 9H, minor conformer), 1.41−2.09 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 2.67−2.99 (m, $C^\beta H_2$ Tyr, 2H), 3.11−3.35 (m, $C^\delta H_2$ Pro, 2H), 3.59 (s, CH_3O, 3H, major conformer), 3.62 (s, CH_3O, 3H, minor conformer), 4.05 (dd, $C^\alpha H$ Pro, 1H, major conformer), 4.09 (dd, $C^\alpha H$ Pro, 1H, minor conformer), 4.46 (m, $C^\alpha H$ Tyr, 1H), 6.64, 6.97 (each m, AA′BB′ system, C_6H_4 Tyr, 4H), 8.09 (d, NH, 1H, minor conformer), 8.26 (d, NH, 1H, major conformer), 9.22 (s, OH, 1H). Anal. ($C_{20}H_{28}N_2O_6$) C, H, N.

***N*-(Phenylacetyl)-L-prolyl-L-tyrosine methyl ester (4):** yield 75% (after crystallization from methanol−ether, 1:2, v/v); mp 146−148 °C; $[\alpha]^{20}_D$ −48.0° (*c* 0.15, DMF); R_f 0.57 ($CHCl_3/CH_3OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 1.56−2.18 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 2.80−2.90 (m, $C^\beta H_2$ Tyr, 2H, major conformer), 2.82, 3.02 (each dd, $C^\beta H_2$ Tyr, 2H, minor conformer), 3.20, 3.35 (each d, J = 15.7 Hz, C_6H_5-CH_2, 2H, major conformer), 3.40−3.50 (m, $C^\delta H_2$ Pro, 2H), 3.55 (s, CH_3O, 3H, major conformer), 3.63 (s, CH_3O, 3H, minor conformer), 3.64 (s, $C_6H_5CH_2$, 2H, minor conformer), 4.35 (m, $C^\alpha H$ Pro, $C^\alpha H$ Tyr major conformer, 2H), 4.54 (m, $C^\alpha H$ Tyr, 1H, minor conformer), 6.61, 7.01 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 6.64, 6.96 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 7.0−7.35 (m, $C_6H_5CH_2$, 5H), 8.13 (d, J = 7.68 Hz, NH Tyr, 1H, major conformer), 8.54 (d, J = 8.20 Hz, NH Tyr, 1H, minor conformer), 9.17 (s, OH, 1H, minor conformer), 9.21 (s, OH, 1H, major conformer). Anal. ($C_{23}H_{26}N_2O_5$) C, H, N.

***N*-(Phenylacetyl)-D-prolyl-l-tyrosine ethyl ester (5):** yield 66% (after trituration with hexane); mp 186−188 °C; $[\alpha]^{20}_D$ +49.5° (*c* 0.3, CH_3OH); R_f 0.56 ($CHCl_3/CH_3OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 1.09 (t, J = 7.04 Hz, CH_3CH_2O, 3H, minor conformer), 1.13 (t, J = 7.04 Hz, CH_3CH_2O, 3H, major conformer), 1.48−2.2 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 2.7−3.05 (m, $C^\beta H_2$ Tyr, 2H), 3.18−3.4 (m, $C^\delta H_2$ Pro, 2H), 3.65 (s, $C_6H_5CH_2$, 2H), 4.04 (q, CH_3CH_2O, 2H), 4.27 (dd, $C^\alpha H$ Pro, 1H, minor conformer), 4.33 (dd, $C^\alpha H$ Pro, 1H, major conformer), 4.40 (m, $C^\alpha H$ Tyr, 1H, major conformer), 4.44 (m, $C^\alpha H$ Tyr, 1H, minor conformer), 6.62, 7.01 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 6.63, 6.95 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 7.1−7.34 (m, $C_6H_5CH_2$, 5H), 8.15 (d, J = 8.41 Hz, NH, 1H, major conformer), 8.59 (d, J = 8.02 Hz, NH, 1H, minor conformer), 9.18 (s, OH, 1H, major conformer), 9.19 (s, OH, 1H, minor conformer). Anal. ($C_{24}H_{28}N_2O_5$) C, H, N.

***N*-(Phenylacetyl)-L-prolyl-D-tyrosine ethyl ester (6):** yield 59% (after trituration with ether); mp 187−188 °C; $[\alpha]^{20}_D$ −58.5° (*c* 0.3, CH_3OH); R_f 0.5 ($CHCl_3/CH_3OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 1.10 (t, J = 7.04 Hz, CH_3CH_2O, 3H, minor conformer), 1.14 (t, J = 7.04 Hz, CH_3CH_2O, 3H, major conformer), 1.5−2.2 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 2.7−3.05 (m, $C^\beta H_2$ Tyr, 2H), 3.2−3.4 (m, $C^\gamma H_2$ Pro, 2H), 4.04 (q, CH_3CH_2O, 2H, minor conformer), 4.05 (q, CH_3CH_2O, 2H, major conformer), 4.31 (dd, $C^\alpha H$ Pro, 1H, minor conformer), 4.38 (dd, $C^\alpha H$ Pro, 1H, major conformer), 4.40 (m, $C^\alpha H$ Tyr, 1H, major conformer), 4.45 (m, $C^\alpha H$ Tyr, 1H, minor conformer), 6.45, 6.62 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 6.97, 7.01 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 7.09−7.35 (m, $C_6H_5CH_2$, 5H), 8.15 (d, J = 8.4 Hz, NH, 1H, major conformer), 8.59 (d, J = 8.02 Hz, NH, 1H, minor conformer), 9.25 (s, OH, 1H, major conformer), 9.26 (s, OH, 1H, minor conformer). Anal. ($C_{24}H_{28}N_2O_5$) C, H, N.

***N*-Valeryl-L-prolyl-L-tyrosine methyl ester (7):** yield 51% (after column chromatography); oil; $[\alpha]^{20}_D$ −58° (*c* 0.5, $CHCl_3$); R_f 0.77 (*n*-$C_4H_9OH/AcOH/H_2O$, 5:1:2). NMR (DMSO-d_6) two conformations evident in spectrum δ 0.82 (t, $CH_3(CH_2)_3$, 3H, minor conformer), 0.88 (t, $CH_3(CH_2)_3$, 3H, major conformer), 0.98−2.17 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 0.98−2.0 (m, $CH_3(CH_2)_2$, 4H), 2.23 (t, $C_3H_7CH_2C(O)$, 2H), 2.70−3.04 (dd, $C^\beta H_2$ Tyr, 2H), 3.2−3.5 (m, $C^\delta H_2$ Pro, 2H), 3.56 (s, CH_3O, 3H, major conformer), 3.63 (s, CH_3O, 3H, minor conformer), 4.25 (dd, $C^\alpha H$ Pro, 1H, minor conformer), 4.33 (dd, $C^\alpha H$ Pro, 1H, major conformer), 4.33 (m, $C^\alpha H$ Tyr, 1H, major conformer), 4.47 (m, $C^\alpha H$ Tyr, 1H, minor conformer), 6.64, 6.99 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 6.65, 6.98 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 8.07 (d, NH Tyr, 1H, major conformer), 8.4 (d, NH Tyr, 1H, minor conformer), 9.18 (s, OH Tyr, 1H, major conformer), 9.2 (s, OH Tyr, 1H, minor conformer). Anal. ($C_{20}H_{28}N_2O_5$) C, H, N.

***N*-Caproyl-L-prolyl-L-tyrosine methyl ester (8):** yield 54% (after crystallization from EtOAc); mp 115−116 °C; $[\alpha]^{20}_D$ −58° (*c* 0.4, $CHCl_3$); R_f 0.56 ($CHCl_3/C_2H_5OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 0.84 (t, $(CH_3(CH_2)_4$, 3H, minor conformer), 0.87 (t, $CH_3(CH_2)_4$, 3H, major conformer), 1.02−2.20 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, $CH_3(CH_2)_4$, 12H), 2.7−3.1 (m, $C^\beta H_2$ Tyr, 2H), 3.2−3.45 (m, $C^\delta H_2$ Pro, 2H), 3.56 (s, CH_3O, 3H, major conformer), 3.63 (s, CH_3O, 3H, minor conformer), 4.26 (m, $C^\alpha H$ Pro, 1H, minor conformer), 4.35 (m, $C^\alpha H$ Pro, 1H, major conformer), 4.35 (m, $C^\alpha H$ Tyr, 1H, major conformer), 4.48 (m, $C^\alpha H$ Tyr, 1H, minor conformer), 6.65, 7.00 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 6.66, 6.98 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 8.04 (d, NH Tyr, 1H, major conformer), 8.42 (d, NH Tyr, 1H, minor conformer), 9.23 (s, OH Tyr, 1H, major conformer), 9.26 (s, OH Tyr, 1H, minor conformer). Anal. ($C_{21}H_{30}N_2O_5$) C, H, N.

***N*-Nonanoyl-L-prolyl-L-tyrosine ethyl ester (9):** yield 60% (after trituration with ether); mp 115−117 °C; $[\alpha]^{20}_D$ −50.4° (*c* 0.4, $CHCl_3$); R_f 0.9 ($CHCl_3/CH_3OH$, 9:1); NMR ($CDCl_3$) two conformations evident in spectrum δ 0.87 (t, $CH_3(CH_2)_7$, 3H), 1.26 (m, $CH_3(CH_2)_5$, CH_3CH_2O, 13H), 1.5−1.7 (m, $C_6H_{13}CH_2CH_2$, 2H), 1.75−2.06 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 2.28 (t, $C_7H_{15}CH_2C(O)$, 2H), 2.90, 3.11 (each dd, $C^\beta H_2$ Tyr, 2H, minor conformer), 2.93, 3.09 (each dd, $C^\beta H_2$ Tyr, 2H, major conformer), 3.30−3.54 (m, $C^\delta H_2$ Pro, 2H), 4.16 (q, CH_3CH_2O, 2H), 4.55 (dd, $C^\alpha H$ Pro, 1H), 4.77 (m, $C^\alpha H$ Tyr, 1H), 6.28 (d, NH Tyr, 1H, minor conformer), 6.67, 6.97 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 6.76, 6.93 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 7.27 (s, OH Tyr, 1H); 7.28 (d, NH Tyr, 1H, major conformer). Anal. ($C_{25}H_{38}N_2O_5$) C, H, N.

General Synthetic Method for the Preparation of *N*-Acylprolyltyrosine Amides. A solution of *N*-acylprolyltyrosine methyl or ethyl ester (5 mmol) in 50 mL of methanol was cooled to 0 °C. NH_3 (dried through NaOH trap) was then bubbled through the solution for 30 min. The solution was maintained at room temperature for 48 h. Methanol was evaporated in vacuo, and the residue was purified by recrystallization or column chromatography using chloroform−ethanol (9:1, v/v) as eluent.

***N*-(*tert*-Butoxycarbonyl)-L-prolyl-L-tyrosine amide (10):** yield 60% (after column chromatography); mp 72−75 °C; $[\alpha]^{20}_D$ −49.7° (*c* 0.38, CH_3OH); R_f 0.33 ($CHCl_3/C_2H_5OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 1.22 (s, $(CH_3)_3C$, 9H, major conformer), 1.39 (s, $(CH_3)_3C$, 9H, minor conformer), 1.56−2.10 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 2.6−3.0 (m, $C^\beta H_2$ Tyr, 2H), 3.2−3.4 (m, $C^\delta H_2$ Pro, 2H), 4.05 (m, $C^\alpha H$ Pro, 1H), 4.28 (m, $C^\alpha H$ Tyr, 1H, minor conformer), 4.35 (m, $C^\alpha H$ Tyr, 1H, major conformer), 6.62, 6.98 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 6.62, 7.0 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 7.0, 7.30 (each s, NH_2, 2H, minor conformer), 7.08, 7.15 (each s, NH_2, 2H, major conformer), 7.70 (d, NH, 1H, major conformer), 7.75 (d, NH, 1H, minor conformer), 9.18 (s, OH, 1H, major conformer), 9.20 (s, OH, 1H, minor conformer). Anal. ($C_{19}H_{27}N_3O_5$) C, H, N.

***N*-(tert-Butoxycarbonyl)-D-prolyl-L-tyrosine amide (11):** yield 40% (after column chromatography); mp 76−78 °C; $[\alpha]^{20}_D$ +33.2° (*c* 0.3, $CHCl_3$), R_f 0.36 ($CHCl_3/C_2H_5OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 1.28 (s, $(CH_3)_3C$, 9H, major conformer), 1.38 (s, $(CH_3)_3C$, 9H, minor conformer), 1.55−2.08 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 2.64, 2.84 (each dd, $C^\beta H_2$ Tyr, 2H, major conformer), 2.64, 2.98 (each dd, $C^\beta H_2$ Tyr, 2H, minor conformer), 3.10−3.32 (m, $C^\delta H_2$ Pro, 2H), 4.05 (dd, $C^\alpha H$ Pro, 1H), 4.26 (m, $C^\alpha H$ Tyr, 1H, minor conformer), 4.46 (m, $C^\alpha H$ Tyr, 1H, major conformer), 6.62, 6.98 (each m, AA′BB′ system, C_6H_4 Tyr, 4H), 7.05, 7.37 (each s, NH_2, 2H, major conformer), 7.16, 7.28 (each s, NH_2, 2H, minor conformer), 7.88 (d, NH Tyr, J = 8.9 Hz, 1H, major conformer), 8.12 (d, NH Tyr, J = 8.6 Hz, 1H, minor conformer), 9.15 (c, OH Tyr, 1H). Anal. ($C_{19}H_{27}N_3O_5$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-prolyl-D-tyrosine amide (12):** yield 46% (after column chromatography); mp 79−80 °C; $[\alpha]^{20}_D$ −34.0° (*c* 0.3, $CHCl_3$); R_f 0.36 ($CHCl_3/C_2H_5OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 1.28 (s, $(CH_3)_3C$, 9H, major conformer), 1.38 (s, $(CH_3)_3C$, 9H, minor conformer), 1.55−2.08 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 2.64, 2.84 (each dd, $C^\beta H_2$ Tyr, 2H, major conformer), 2.64, 2.98 (each dd, $C^\beta H_2$ Tyr, 2H, minor conformer), 3.10−3.32 (m, $C^\delta H_2$ Pro, 2H), 4.05 (dd, $C^\alpha H$ Pro, 1H), 4.26 (m, $C^\alpha H$ Tyr, 1H, minor conformer), 4.46 (m, $C^\alpha H$ Tyr, 1H, major conformer), 6.62, 6.98 (each m, AA′BB′ system, C_6H_4 Tyr, 4H), 7.05, 7.37 (each s, NH_2, 2H, major conformer), 7.16, 7.28 (each s, NH_2, 2H, minor conformer), 7.88 (d, NH Tyr, J = 8.9 Hz, 1H, major conformer), 8.12 (d, NH Tyr, J = 8.6 Hz, 1H, minor conformer), 9.15 (c, OH Tyr, 1H). Anal. ($C_{19}H_{27}N_3O_5$) C, H, N.

***N*-(Phenylacetyl)-L-prolyl-L-tyrosine amide (13):** yield 75% (after crystallization from methanol−ether, 1:2, v/v); mp 201−203 °C; $[\alpha]^{20}_D$ −89.6° (*c* 0.3, DMF); R_f 0.75 ($CHCl_3/C_2H_5OH$, 9:3); NMR (DMSO-d_6) two conformations evident in spectrum δ 1.54−2.16 (m, $C^\alpha H_2$-$C^\gamma H_2$ Pro, 4H), 2.69, 2.92 (each dd, $C^\beta H_2$ Tyr, 2H, minor conformer), 2.74, 2.94 (each dd, $C^\beta H_2$ Tyr, 2H, major conformer), 3.40−3.65 (m, $C^\delta H_2$ Pro, 2H), 3.66, 3.71 (each d, AB system, $C_6H_5CH_2$, 2H), 4.21 (dd, $C^\alpha H$ Pro, 1H, major conformer), 4.38 (dd, $C^\alpha H$ Pro, 1H, minor conformer), 4.28 (m, $C^\alpha H$ Tyr, 1H, major conformer), 4.51 (m, $C^\alpha H$ Tyr, 1H, minor conformer), 6.57, 7.05 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 6.62, 6.95 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 7.0−7.5 (m, $C_6H_5CH_2$, NH_2, 7H), 7.71 (d, NH, 1H, major conformer), 8.20 (d, NH, 1H, minor conformer), 9.09 (s, OH, 1H, minor conformer); 9.14 (s, OH, 1H, major conformer). Anal. ($C_{22}H_{25}N_3O_4$) C, H, N.

***N*-(Phenylacetyl)-D-prolyl-L-tyrosine amide (14):** yield 54% (after crystallization from methanol−ether, 1:2, v/v); mp 195−197 °C; $[\alpha]^{20}_D$ +61.7° (*c* 0.4, CH_3OH); R_f 0.3 ($CHCl_3/CH_3OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum in ratio 4:1 δ 1.4−2.1 (m, $C^\beta H_2$-$C^\gamma H_2$ Pro, 4H), 2.61 (dd, J = 14.1 Hz, J = 10.8 Hz, $C^\beta H_2$ Tyr, 1H, major conformer), 2.68 (dd, J = 13.9 Hz, J = 10.8 Hz, $C^\beta H_2$ Tyr, 1H, minor conformer), 2.94 (dd, J = 13.9 Hz, J = 4.0 Hz, $C^\beta H_2$ Tyr, 1H, minor conformer), 3.03 (dd, J = 14.1 Hz, J = 3.7 Hz, $C^\beta H_2$ Tyr, 1H, major conformer), 3.3−3.5 (m, $C^\delta H_2$ Pro, 2H), 3.65

(s, $C_6H_5CH_2$, 2H), 4.2–4.3 (m, $C^{\alpha}H$ Pro, $C^{\alpha}H$ Tyr, 2H, major conformer), 4.4–4.5 (m, $C^{\alpha}H$ Pro, $C^{\alpha}H$ Tyr, 2H, minor conformer), 6.6, 7.0, 7.1 (each m, AA′BB′ system, C_6H_4 Tyr, 4H), 7.1–7.4 (m, $C_6H_5CH_2$, 5H), 8.3 (d, NH, 1H), 9.23 (s, OH, 1H, major conformer), 9.25 (s, OH, 1H, minor conformer). Anal. ($C_{22}H_{25}N_3O_4$) C, H, N.

***N*-(Phenylacetyl)-L-prolyl-D-tyrosine amide (15):** yield 64% (after column chromatography); mp 196–198 °C; $[\alpha]^{20}_D$ −65.8° (*c* 0.4, CH_3OH); R_f 0.39 ($CHCl_3/CH_3OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum in ratio of 3:1 δ 1.4–2.1 (m, $C^{\beta}H_2$-$C^{\gamma}H_2$ Pro, 4H), 2.61 (dd, J = 13.89 Hz, J = 10.8 Hz, $C^{\beta}H_2$ Tyr, 1H, major conformer), 2.68 (dd, J = 14.1 Hz, J = 10.8 Hz, $C^{\beta}H_2$ Tyr, 1H, minor conformer), 2.94 (dd, J = 13.9 Hz, J = 4.0 Hz, $C^{\beta}H_2$ Tyr, 1H, minor conformer), 3.03 (dd, J = 14.1 Hz, J = 3.65 Hz, $C^{\beta}H_2$ Tyr, 1H, major conformer), 3.3–3.45 (m, $C^{\delta}H_2$ Pro, 2H), 3.65 (s, $C_6H_5CH_2$, 2H), 4.17–4.31 (m, $C^{\alpha}H$ Pro, $C^{\alpha}H$ Tyr, 2H, major conformer), 4.4–4.54 (m, $C^{\alpha}H$ Pro, $C^{\alpha}H$ Tyr, 2H, minor conformer), 6.63, 6.99 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 6.63, 7.05 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 7.08–7.36 (m, $C_6H_5CH_2$, 5H), 8.25 (d, NH, 1H), 9.23 (s, OH, 1H, major conformer), 9.25 (s, OH, 1H, minor conformer). Anal. ($C_{22}H_{25}N_3O_4$) C, H, N.

***N*-Valeryl-L-prolyl-L-tyrosine amide (16):** yield 91% (after trituration with ether); mp 202–204 °C; $[\alpha]^{20}_D$ −60.7° (*c* 0.3, CH_3OH); R_f 0.48 ($CHCl_3/C_2H_5OH$, 4:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 0.79 (t, $CH_3(CH_2)_3$, 3H, minor conformer), 0.89 (t, $CH_3(CH_2)_3$, 3H, major conformer), 1.00–2.35 (m, $CH_3(CH_2)_3$, $C^{\beta}H_2$-$C^{\gamma}H_2$ Pro, 10H), 2.60–2.98 (m, $C^{\beta}H_2$ Tyr, 2H), 3.13–3.59 (m, $C^{\delta}H_2$ Pro, 2H), 4.16 (m, $C^{\alpha}H$ Pro, 1H, major conformer), 4.24 (m, $C^{\alpha}H$ Pro, 1H, minor conformer), 4.24 (m, $C^{\alpha}H$ Tyr, 1H, major conformer), 4.42 (m, $C^{\alpha}H$ Tyr, 1H, minor conformer), 6.63, 6.97 (each m, AA′BB′ system, C_6H_4 Tyr, 4H), 7.03, 7.49 (each s, NH_2, 2H, minor conformer), 7.09, 7.12 (each s, NH_2, 2H, major conformer), 7.67 (d, NH Tyr, 1H, major conformer), 8.12 (d, NH Tyr, 1H, minor conformer), 9.17 (s, OH Tyr, 1H, minor conformer), 9.18 (s, OH Tyr, 1H, major conformer). Anal. ($C_{19}H_{27}N_3O_4$) C, H, N.

***N*-Caproyl-L-prolyl-L-tyrosine amide (17):** yield 59% (after trituration from $CHCl_3$); mp 112–113 °C; $[\alpha]^{20}_D$ −66° (*c* 0.3, CH_3OH); R_f 0.4 ($CHCl_3/C_2H_5OH$, 9: 1); NMR (DMSO-d_6) two conformations evident in spectrum δ 0.83 (t, $CH_3(CH_2)_4$, 3H, minor conformer), 0.87 (t, $CH_3(CH_2)_4$, 3H, major conformer), 0.99–2.27 (m, $CH_3(CH_2)_4$, 8H), 1.20–2.36 (m, $C^{\beta}H_2$-$C^{\gamma}H_2$ Pro, 4H), 2.66, 2.88 (each m, $C^{\beta}H_2$ Tyr, 2H, minor conformer), 2.71, 2.99 (each m, $C^{\beta}H_2$ Tyr, 2H, major conformer), 3.26–3.59 (m, $C^{\delta}H_2$ Pro, 2H), 4.16 (dd, $C^{\alpha}H$ Pro, 1H, major conformer), 4.25 (m, $C^{\alpha}H$ Pro, 1H, minor conformer), 4.25 (m, $C^{\alpha}H$ Tyr, 1H, major conformer), 4.43 (m, $C^{\alpha}H$ Tyr, 1H, minor conformer), 6.63, 6.98 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 6.61, 6.99 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 7.09, 7.10 (each s, NH_2, 2H, major conformer), 7.10, 7.47 (each s, NH_2, 2H, minor conformer), 7.66 (d, NH Tyr, 1H, major conformer), 8.10 (d, NH Tyr, 1H, minor conformer), 9.18 (s, OH Tyr, 1H, minor conformer), 9.20 (s, OH Tyr, 1H, major conformer). Anal. ($C_{20}H_{29}N_3O_4$) C, H, N.

***N*-Nonanoyl-L-prolyl-L-tyrosine amide (18):** yield 62% (after column chromatography); mp 178–180 °C; $[\alpha]^{20}_D$ −57.6° (*c* 0.3, CH_3OH); R_f 0.7 ($CHCl_3/CH_3OH$, 9:1); NMR (DMSO-d_6) two conformations evident in spectrum δ 0.86 (t, $CH_3(CH_2)_7$, 3H), 1.10–2.27 (m, $CH_3(CH_2)_7$, 14H), 1.50–2.10 (m, $C^{\beta}H_2$-$C^{\gamma}H_2$ Pro, 4H), 2.6–3.0 (m, $C^{\beta}H_2$ Tyr, 2H), 3.20–3.50 (m, $C^{\delta}H_2$ Pro, 2H), 4.16 (m, $C^{\alpha}H$ Pro, 1H, major conformer), 4.26 (m, $C^{\alpha}H$ Pro, 1H, minor conformer), 4.26 (m, $C^{\alpha}H$ Tyr, 1H, major conformer), 4.42 (m, $C^{\alpha}H$ Tyr, 1H, minor conformer), 6.62, 6.97 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, major conformer), 6.62, 7.01 (each m, AA′BB′ system, C_6H_4 Tyr, 4H, minor conformer), 6.95–7.15 (m, NH_2, 2H), 7.67 (d, NH Tyr, 1H, major conformer), 8.10 (d, NH Tyr, 1H, minor conformer), 9.17 (s, OH Tyr, 1H, minor conformer), 9.18 (s, OH Tyr, 1H, major conformer). Anal. ($C_{23}H_{35}N_3O_4$) C, H, N.

General Strategy of the *N*-(*tert*-Butoxycarbonyl)-dipeptide Amide Deprotection. A solution of BOC-dipeptide amide (1 mmol) in 10 mL of CF_3COOH was stirred for 15 min at a room temperature, and the solvent was evaporated in vacuo. Ether was added to the residue, and the dipeptide amide crystals were separated as a trifluoroacetic salt.

L-Prolyl-L-tyrosine amide trifluoroacetate (19): yield 96%; mp 161–163 °C; $[\alpha]^{20}_D$ −13° (*c* 0.37, C_2H_5OH); R_f 0.28 (n-$C_4H_9OH/AcOH/H_2O$, 5:1:2); NMR (DMSO-d_6) δ 1.7–2.4 (m, $C^{\beta}H_2$-$C^{\gamma}H_2$ Pro, 4H), 2.68, 2.92 (each dd, J = 13.9 Hz, J = 9.96 Hz, J = 4.14 Hz, J = 13.71 Hz, $C^{\beta}H_2$ Tyr, 2H), 3.07–3.25 (m, $C^{\delta}H_2$ Pro, 2H), 4.11 (m, $C^{\alpha}H$ Pro, 1H), 4.41 (m, $C^{\alpha}H$ Tyr, 1H), 6.65, 7.05 (each m, AA′BB′ system, C_6H_4 Tyr, 4H), 7.14, 7.61 (each s, NH_2, 2H), 8.48 (s, NH Pro, 1H), 8.68 (d, J = 8.1 Hz, NH Tyr, 1H), 9.31 (s, OH Tyr, 1H). Anal. ($C_{14}H_{19}N_3O_3$·CF_3COOH) N.

D-Prolyl-L-tyrosine amide trifluoroacetate (20): yield 93%; mp 93–95 °C (hydroscopic); $[\alpha]^{20}_D$ +32° (*c* 0.16, $CHCl_3$); R_f 0.26 ($C_4H_9OH/CH_3COOH/H_2O$, 5:1:2). NMR (DMSO-d_6) δ 1.40–2.13 (m, $C^{\beta}H_2$-$C^{\gamma}H_2$ Pro, 4H), 2.60, 2.99 (each dd, $C^{\beta}H_2$ Tyr, 2H), 3.12 (m, $C^{\delta}H_2$ Pro, 2H), 4.12 (m, $C^{\alpha}H$ Pro, 1H), 4.47 (m, $C^{\alpha}H$ Tyr, 1H), 6.63, 7.01 (each m, AA′BB′ system, C_6H_4 Tyr, 4H), 7.20, 7.64 (each s, NH_2, 2H), 8.39 (s, NH Pro, 1H), 8.69 (d, NH Tyr, 1H), 9.17 (s, OH Tyr, 1H). Anal. ($C_{14}H_{19}N_3O_3$·CF_3COOH) N.

L-Prolyl-D-tyrosine amide trifluoroacetate (21): yield 94%; mp 94–95 °C (hydroscopic); $[\alpha]^{20}_D$ −32° (*c* 0.2, $CHCl_3$); R_f 0.26 ($C_4H_9OH/CH_3COOH/H_2O$, 5:1:2); NMR (DMSO-d_6) δ 1.41–2.13 (m, $C^{\beta}H_2$-$C^{\gamma}H_2$ Pro, 4H), 2.60, 2.99 (each dd, $C^{\beta}H_2$ Tyr, 2H), 3.11 (m, $C^{\delta}H_2$ Pro, 2H), 4.12 (m, $C^{\alpha}H$ Pro, 1H), 4.47 (m, $C^{\alpha}H$ Tyr, 1H), 6.62, 7.00 (each m, AA′BB′ system, C_6H_4 Tyr, 4H), 7.18, 7.64 (each s, NH_2, 2H), 8.39 (s, NH Pro, 1H), 8.69 (d, NH Tyr, 1H), 9.17 (s, OH Tyr, 1H). Anal. ($C_{14}H_{19}N_3O_3$·CF_3COOH) N.

Pharmacology. Inhibition of Apomorphine-Induced Climbing Behavior in Mice. Male mice (C57BL6) weighing 22–25 g were placed in wire mesh cages (12 cm diameter, 14 cm high) and were allowed 1 h for adaptation and exploration of the new environment. Apomorphine was dissolved ex tempora in saline containing 0.1% ascorbic acid and injected 5.0 mg/kg sc. This dose caused climbing in all animals for 15 min. The test compound dissolved in saline containing 3% Tween 80 was injected ip 15 min prior to the apomorphine. For evaluation of climbing 15 min after apomorphine, 30 readings were taken every 2 min (scoring scale: four paws on bottom (no climbing), 0; one paw on the wall, 1; two paws on the wall, 2; three paws on the wall, 3; four paws on the wall (full climbing), 4). Mice consistently climbing before apomorphine injection were discarded. The climbing scores were individually totaled (maximum score: 120 per mouse) and the total score of the control (vehicle ip − apomorphine sc) was set to 100%. ED_{50} values with 95% confidence limits were calculated by linear regression analysis (log dose transformation) using 8–16 mice per dose.

Inhibition of Extrapolatory Behavior Impairment Induced by DOPA in Rats. The experimental animals were outbread albino male rats (Moscow region, Stolbovaya) weighing 180–200 g. A rat put into a cylinder plunged in water with the temperature of 22 °C should solve an "extrapolatory task", i.e., to dive under the cylinder's edge, thus escaping the stress situation. The animal was taken out of water immediately after the solution of the task. The time during which the animals were in water did not exceed 2 min. From 80 to 90% of the control rats solved the task. The rats which did not escape in 2 min were discarded. One day later 0.9% NaCl solution (intact group) or the suspension in 3% Tween 80 of Madopar (100 mg/kg L-DOPA + 25 mg/kg benzerazide) (control group) were administrated intraperitonially 60 min prior the rat being put into a cylinder. The test compounds dissolved in saline + 3% Tween 80 were injected ip 10 min prior to Madopar (test group). The animals with successful escape response were registered. ED_{50} values with 95% confidence limits were calculated by linear regression analysis with eight rats per dose.

Catalepsy in Rats. Induction of catalepsy was tested in adult outbread male rats (200–250 g) 0.5, 1.0, 1.5, and 2 h

after ip administration of the test compound dissolved in saline containing 3% Tween 80 in the apparatus Morpurgo.

The rats were placed with the forepaws onto a bar situated 7 cm above the floor of a perspex jar (22 × 38 × 16 cm) and tested for catalepsy according to a rating score (time resting upon the bar: 10 s, 0; 10−20 s, 1; 20−30 s, 2; >30 s, 3). Testing was repeated twice for each animal at 2 min intervals. The cumulated maximal score for one animal during the 2 h observation period was 15. Scores are expressed as percent of the maximal score obtainable.

Acute Toxicity. Male outbred albino mice (Moscow region) weighing 20−25 g were used. The mice were housed five per cage and had free access to food and water. The mice were kept at a temperature of 21−22 °C and humidity of 40−50% and in a light-controlled room (12/12 h light/dark cycle). The number of surviving animals was recorded 2, 24, and 48 h after the injection.

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