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SYNTHESIS AND DOPAMINE RECEPTOR MODULATING ACTIVITY OF UNSUBSTITUTED AND SUBSTITUTED TRIPROLINE ANALOGUES OF L-PROLYL-L-LEUCYL-GLYCINAMIDE (PLG)

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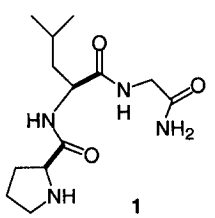
Abstract: Triprolines Pro-Pro-Pro-NH_2 (**4**), Pro-Pro-D-Pro-NH_2 (**5**), Pro-Pro(*trans*-3-Me)-D-Pro-NH_2 (**6**), and Pro-Pro(*cis*-3-Me)-D-Pro-NH_2 (**7**) were made as conformationally constrained analogues of Pro-Leu-Gly-NH_2. Triprolines **4–6** produced significant increases in the high- and low-affinity state ratio (R_H/R_L) of the dopamine receptor, but only **4** was found to increase apomorphine induced rotations in 6-hydroxydopamine-lesioned rats.

The ability of Pro-Leu-Gly-NH_2 (PLG, **1**) to modulate the dopamine D_2 receptor has been well documented both in vitro and in vivo. Examples of this modulation include the ability of PLG to increase the affinity of the high affinity state of the D_2 receptor for agonists[1–4] and to potentiate the contralateral rotational behavior induced by apomorphine in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway.[5–8] This type of pharmacological profile suggests that PLG and its analogues might have potential use in the treatment of dopamine receptor related disorders such as Parkinson's disease and tardive dyskinesia.[9–12]

Several conformationally constrained analogues of PLG have been designed and synthesized in order to elucidate the bioactive conformation of PLG.[13–18] In this paper, we wish to report on triprolines as unique conformationally constrained analogues of PLG. The design of these analogues, whereby prolyl residues replace both the leucyl and glycinamide residues of PLG, was prompted by two observations. Firstly, Pro-Leu-Pro-NH_2 (**2**) and Pro-Leu-D-Pro-NH_2 (**3**), previously, had been found to possess dopamine receptor modulating activity similar to that of PLG.[19] Secondly, since several recent reports[20–22] demonstrated the ability of unsubstituted and substituted homochiral and heterochiral diproline peptides to bias peptide conformations toward β-turns, it was postulated that such substitutions could potentially mimic the postulated bioactive β-turn conformation of PLG. Thus, triproline analogues **4–7** were made. In the case of **6** and **7**, a β-methyl group was placed on the second prolyl residue to possibly access the hydrophobic pocket with which the leucyl side chain is thought to interact.



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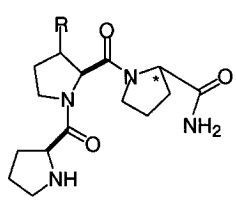

4: R = H; * = L-configuration
5: R = H; * = D-configuration
6: R = *trans*-CH_3; * = D-configuration
7: R = *cis*-CH_3; * = D-configuration

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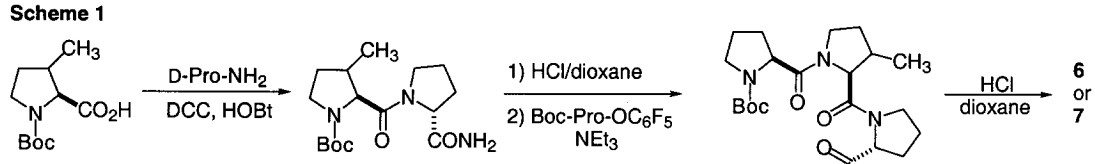
Triprolines **4** and **5** were made by standard peptide coupling methods, while **6** and **7** were made as shown in Scheme 1.[23] *tert*-Butoxycarbonyl-*trans*-3-methyl-L-proline (**8**) and *tert*-butoxycarbonyl-*cis*-3-methyl-L-proline (**9**), which served as the starting materials for **6** and **7**, respectively, were made as described by Herdeis, et al.[24]

Scheme 1



8: *trans*-3-CH_3
9: *cis*-3-CH_3
10: *trans*-3-CH_3
11: *cis*-3-CH_3
12: *trans*-3-CH_3
13: *cis*-3-CH_3

An X-ray crystal structure of **12** was obtained that confirmed the *trans* relationship of the 3-methyl substituent and provided information on the conformation of this triproline.[25] There were two unique molecules in the asymmetric unit, **12A** and **12B**. The molecular structure of **12A** is shown in Figure 1 and Table 1 lists the observed values for the backbone torsion angles of the two molecular structures. The major difference between the molecular conformations of **12A** and **12B** is the ω_1 torsion angle. In **12A** it is *cis* with a value of 1.0°, while for **12B** ω_1 has a value of 168.2°. Both molecular forms of **12** adopt a type II β-turn about the *trans*-3-methyl-L-prolyl-D-prolinamide part of the molecule, a result consistent with previous work.[22]

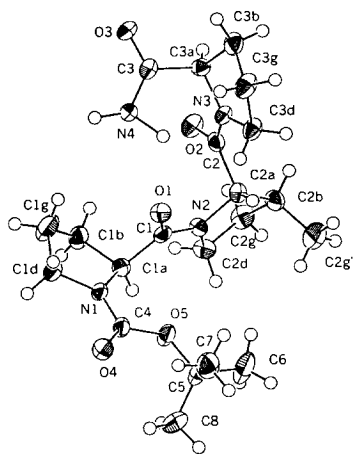
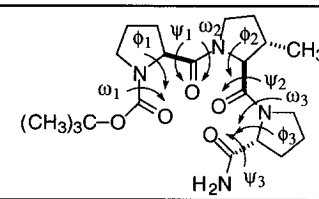


Figure 1. ORTEP diagram of **12A** with the atomic numbering scheme. The ellipsoids are drawn at the 50% probability level and the hydrogens are drawn as spheres of arbitrary size.

Table 1. Peptide Backbone Torsion Angles (°) of **12A** and **12B**.



angle	**12A**	**12B**	ideal type II β-turn
ω_1	1.0	168.2	
ϕ_1	-71.4	-59.7	
ψ_1	150.6	159.3	
ω_2	176.0	171.2	
ϕ_2	-66.2	-57.8	-60
ψ_2	124.6	127.7	120
ω_3	-175.8	179.0	180
ϕ_3	82.8	72.6	80
ψ_3	0.0	11.6	0

PLG analogues **2–7** were evaluated as modulators of apomorphine-induced rotational behavior in the 6-hydroxydopamine-lesioned rat model of hemi-parkinsonism.[7] Only **2–4** showed significant activity. Compound **2** produced a maximal increase in rotations of 30 ± 5% at a dose of 0.1 μg/kg, ip, while **3** produced a maximal increase in rotations of 26 ± 10% at a dose of 1 μg/kg, ip. In comparison, **4** produced its maximum effect of 47 ± 16% at a dose of 10 μg/kg, ip, while PLG produced its maximum effect of 30 ± 7% at a dose of 1 mg/kg, ip.[7]

The triprolines also were evaluated in the [^{3}H]spiroperidol/*N*-propylnorapomorphine (NPA) dopamine D_2 receptor competition binding assay.[4,17,18] The assay was carried out either in the presence or absence of 5'-guanylylimidodiphosphate (Gpp(NH)p), a non-hydrolyzable analogue of GTP. The kinetic binding data obtained are summarized in Table 2. At a concentration of 100 nM, triprolines **4–6** decreased, to varying degrees, the dissociation constant of the high affinity state of the dopamine receptor for the agonist NPA. This effect was seen when either Gpp(NH)p was absent or present. They also increased the percentage of D_2 receptors that existed in the high-affinity state. In the case of **4**, a significant increase in the high- and low-affinity state ratio (R_H/R_L) of the dopamine receptors was seen when either Gpp(NH)p was absent or present. Likewise, **5** produced significant increases in R_H/R_L, but this compound was less active than **4** in attenuating the Gpp(NH)p-induced shift to the low affinity state. Triproline **6** did not produce a significant change in R_H/R_L in the absence of Gpp(NH)p. It was, however, able to attenuate the Gpp(NH)p-induced shift to the low affinity state, but not to the extent that **4** did.

The above results show that replacing the leucyl and glycinamide residues of PLG with prolyl residues leads to analogues with greater potency than PLG in modulating dopamine receptors. Replacement of the glycinamide residue with L-prolinamide proved more effective than replacement with a D-prolinamide residue. The results also show that a β-methyl group, which was incorporated into the second prolyl residue in an attempt to access the hydrophobic pocket with which the leucyl side chain is believed to interact, yielded triproline analogues with diminished activity. The lower activity of the β-methyl triprolines **6** and **7** may result because the β-methyl group projects off the pyrrolidine ring in each case in such a manner that instead of accessing the hydrophobic pocket it actually protrudes into an area of the binding site that results in adverse steric interactions.

Table 2. Modulation of [^{3}H]Spiroperidol/*N*-Propylnorapomorphine Binding Competition by **4–6**.[a]

	binding parameters				
experiment	K_H (nM)	K_L (nM)	R_H (%)	R_L (%)	R_H/R_L
control for **4**					
–Gpp(NH)p	0.080 ± 0.011	120 ± 5	56 ± 3.5	44 ± 3.5	1.27 ± 0.18
+Gpp(NH)p	0.120 ± 0.009	172 ± 70	19 ± 3	81 ± 3	0.23 ± 0.02
pretreatment with **4** (100 nM)					
–Gpp(NH)p	0.086 ± 0.006	153 ± 16	71 ± 1[c]	29 ± 1	2.45 ± 0.16[c]
+Gpp(NH)p	0.054 ± 0.022[b]	140 ± 21	67 ± 1[d]	33 ± 1	2.03 ± 0.13[c]
control for **5** and **6**					
–Gpp(NH)p	0.098 ± 0.009	171 ± 18	49 ± 2	51 ± 2	0.96 ± 0.08
+Gpp(NH)p	0.146 ± 0.064	183 ± 7	18 ± 2	82 ± 2	0.2 ± 0.03
pretreatment with **5** (100 nM)					
–Gpp(NH)p	0.050 ± 0.010[c]	110 ± 5	73 ± 2[c]	27 ± 2	2.70 ± 0.29[c]
+Gpp(NH)p	0.089 ± 0.012	88 ± 7	54 ± 2[d]	46 ± 2	1.17 ± 0.12[c]
pretreatment with **6** (100 nM)					
–Gpp(NH)p	0.052 ± 0.012[b]	98 ± 7	52 ± 4	48 ± 4	1.08 ± 0.18
+Gpp(NH)p	0.087 ± 0.008	106 ± 18	54 ± 4[c]	46 ± 4	1.17 ± 0.22[c]

[a] Competition data were obtained as described in ref 18. K_H and K_L represent the inhibitor constant (K_i) of agonist calculated for the high- and low-affinity components of the [^{3}H]spiroperidol binding, respectively. R_H and R_L are percentage of receptors in the high- and low-affinity form for the agonist, respectively. Values for each preparation are the mean ± SEM of 3–4 separate experiments with each experiment carried out in duplicate or triplicate. Concentration of Gpp(NH)p was 100 μM. Statistical difference from the respective control group indicated as follows: [b] $p < 0.05$, [c] $p < 0.01$, [d] $p < 0.001$.

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23. **Pro-Pro-Pro-NH_2•HCl (4)**: $[\alpha]_D$ -130 (*c* 2.0, MeOH); ^{1}H NMR (MeOH-d_4) δ 4.73–4.77 (m, 1 H), 4.56–4.60 (m, 1 H), 4.36–4.41 (m, 1 H), 3.51–3.84 (m, 4 H), 3.28–3.39 (m, 2 H), 3.26–3.41 (m, 1 H), 2.43–2.55 (m, 1 H), 2.28–2.38 (m, 1 H), 2.19–2.27 (m, 1 H), 1.85–2.16 (m, 9 H); ^{13}C NMR ($CDCl_3$) δ 171.5, 171.4, 167.3, 59.8, 59.7, 59.3, 47.9, 47.8, 47.0, 30.1, 28.7, 28.7, 25.3, 24.5, 24.4; FABMS *m/z* 309 $[M+H]^+$. Anal. ($C_{15}H_{24}N_4O_3$•HCl) C, H, N.
Pro-Pro-D-Pro-NH_2•HCl (5): $[\alpha]_D$ -21.5 (*c* 2.0, MeOH); ^{1}H NMR (MeOH-d_4) δ 4.64–4.93 (m, 2 H), 4.36–4.46 (m, 1 H), 3.70–4.00 (m, 2 H), 3.46–3.67 (m, 2 H), 3.29–3.42 (m, 2 H), 2.44–2.60 (m, 1 H), 2.29–2.39 (m, 1 H), 1.91–2.25 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 176.6, 176.6, 176.5, 174.6, 174.5, 172.6, 172.5, 172.0, 171.4, 171.1, 171.0, 168.1, 167.9, 167.8, 167.3, 61.4, 61.2, 60.3, 60.2, 60.0, 59.9, 59.8, 59.7, 59.4, 59.3, 48.1, 47.9, 47.0, 46.9, 32.8, 31.6, 31.1, 31.0, 30.3, 30.0, 29.7, 29.6, 29.5, 29.4, 29.3, 28.7, 28.6, 28.5, 25.7, 25.6, 25.3, 25.2, 25.0, 24.6, 24.4, 22.9, 22.8; FABMS *m/z* 416 (M + thioglycerol matrix). Anal. ($C_{15}H_{24}N_4O_3$•HCl) C, H, N.
Pro-Pro(*trans*-3-Me)-D-Pro-NH_2•HCl (6): $[\alpha]_D$ +12.4 (*c* 0.50, MeOH); ^{1}H NMR (CD_2Cl_2) δ 11.02 (d, *J* = 4.8 Hz, 1 H), 8.18 (d, *J* = 4.8 Hz, 1 H), 6.86 (s, 1 H), 6.54 (s, 1 H), 4.52–4.54 (m, 1 H), 4.37–4.41 (m, 1 H), 4.22 (d, *J* = 5.4 Hz, 1 H), 3.86–3.93 (m, 1 H), 3.76–3.82 (m, 1 H), 3.47–3.60 (m, 3 H), 3.31–3.41 (m, 1 H), 2.36–2.46 (m, 2 H), 2.13–2.22 (m, 1 H), 1.97–2.10 (m, 7 H), 1.63–1.76 (m, 1 H), 1.16 (d, *J* = 6.0 Hz, 3 H); ^{13}C NMR (CD_2Cl_2) δ 174.9, 170.8, 167.9, 66.1, 61.3, 59.6, 48.1, 46.6, 44.0, 38.2, 34.0, 30.2, 28.7, 24.8, 24.7, 17.7; FABMS *m/z* 323 $[M+H]^+$. Anal. ($C_{16}H_{26}N_4O_3$•HCl•H_2O) C, H, N, Cl.
Pro-Pro(*cis*-3-Me)-D-Pro-NH_2•HCl (7): $[\alpha]_D$ +3.3 (*c* 0.24, MeOH); ^{1}H NMR (CD_2Cl_2) δ 11.50 (bs, 1 H), 7.79 (bs, 1 H), 6.63 (s, 1 H), 6.16 (s, 1 H), 4.73 (d, *J* = 8.4 Hz, 1 H), 4.43–4.56 (m, 2 H), 3.76–4.10 (m, 1 H), 3.41–3.74 (m, 5 H), 2.52–2.62 (m, 1 H), 2.39–2.48 (m, 1 H), 1.89–2.20 (m, 9 H), 1.03 (d, *J* = 6.0 Hz, 3 H); ^{13}C NMR (CD_2Cl_2) δ 176.1, 170.5, 169.8, 62.4, 60.2, 58.7, 48.1, 47.2, 47.1, 34.9, 33.2, 30.1, 29.7, 28.9, 24.8, 13.7; FABHRMS *m/z* 323.2067 ($C_{16}H_{26}N_4O_3$ + H^+ requires 323.2083).
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25. Colorless plate-shaped crystals of **12** were grown from EtOAc/hexane. All measurements were made on a Rigaku diffractometer with graphite monochromated Cu Kα radiation (λ = 1.54178 Å) using the ω-2θ scan mode at 173(1) K. The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The structure has been given the deposition number CCDC 128894. The coordinates can be obtained, upon request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK or via the Internet at http://www.ccdc.cam.ac.uk.