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Triproline Analogues of Pro-Leu-Gly-NH_2 with Pro/Leu and Pro/Phe Chimeric Amino Acids in Position 2

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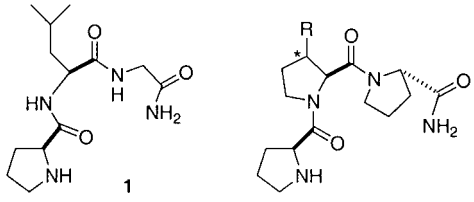
Abstract—Synthesis of the *cis*- and *trans*-isomers of the proline-leucine (3-*i*-Pr-Pro) and proline-phenylalanine (3-Ph-Pro) chimeric amino acids was accomplished by intramolecular reductive-cycloalkylation of the appropriate azido-olefin. These chimeric amino acids were incorporated into the triprolyl analogues of Pro-Leu-Gly-NH_2: Pro-*cis*-3-*i*-Pr-Pro-Pro-NH_2 (**3**), Pro-*trans*-3-*i*-Pr-Pro-Pro-NH_2 (**4**), Pro-*cis*-3-Ph-Pro-Pro-NH_2 (**5**), Pro-*trans*-3-Ph-Pro-Pro-NH_2 (**6**).

Introduction

Pro-Leu-Gly-NH_2 (**1**, PLG) modulates dopamine D_2 receptors within the central nervous system. One manifestation of this modulation in vivo is the ability of PLG to potentiate the contralateral rotational behavior induced by apomorphine in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway.[1–4] PLG brings about this modulation by increasing the percentage of D_2 receptors that exist in the high-affinity state, as well as by increasing the affinity of the high-affinity state for dopamine receptor agonists.[5–8]

Several attempts have been made to define the bioactive conformation of PLG through the design and synthesis of conformationally constrained analogues of this tripeptide.[8–11] In one early study, replacement of the glycinamide residue with either L- or D-prolinamide yielded active analogues of PLG.[12] More recently we found that simultaneously replacing both the leucyl and glycinamide residues of PLG with prolyl residues gave triprolyl analogues with biological activity. In particular, the triprolyl analogue Pro-Pro-Pro-NH_2 (**2**) possessed good dopamine receptor modulating activity both in vitro and in vivo.[3,13]

In the present study, the synthesis of triprolyl PLG analogues **3** and **4** in which the second prolyl residue is substituted at the 3-position with an isopropyl group to mimic the leucyl side chain has been carried out. Also, since conformationally constrained PLG analogues with isobutyl and benzyl groups have shown different selectivities with regard to modulation of the different dopamine receptor subtypes,[14] triprolyl PLG analogues **5** and **6** in which the 3-position of the second prolyl residue is substituted with a phenyl group were also made.



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2: R = H
3: R = *i*-Pr; * = *cis*-isomer
4: R = *i*-Pr; * = *trans*-isomer
5: R = Ph; * = *cis*-isomer
6: R = Ph; * = *trans*-isomer

Synthesis

3-Aryl-prolines, including 3-phenyl-proline, and 3-alkyl-prolines have been synthesized previously.[15–18] Typically, the approach used has been one involving the initial condensation of diethyl acetamidomalonate with an α,β-unsaturated aldehyde to give the substituted pyrrolidine ring system. Subsequent 5-deoxygenation followed by saponification and decarboxylation provides the 3-substituted proline. Although this approach is quite versatile it suffers from the fact that there is no control over stereochemistry and thus separation of diastereoisomers and resolution of enantiomers is necessary.

For the synthesis of the 3-isopropyl-prolines (3-*i*-Pr-Pro, **15a** and **15b**) and 3-phenyl-prolines (3-Ph-Pro, **16a** and **16b**) required in this work, we utilized an intramolecular reductive-cycloalkylation of azido-olefins procedure reported by Waid et al.[19] This route allowed us to control the stereochemistry of the 2-position in the synthesis. The

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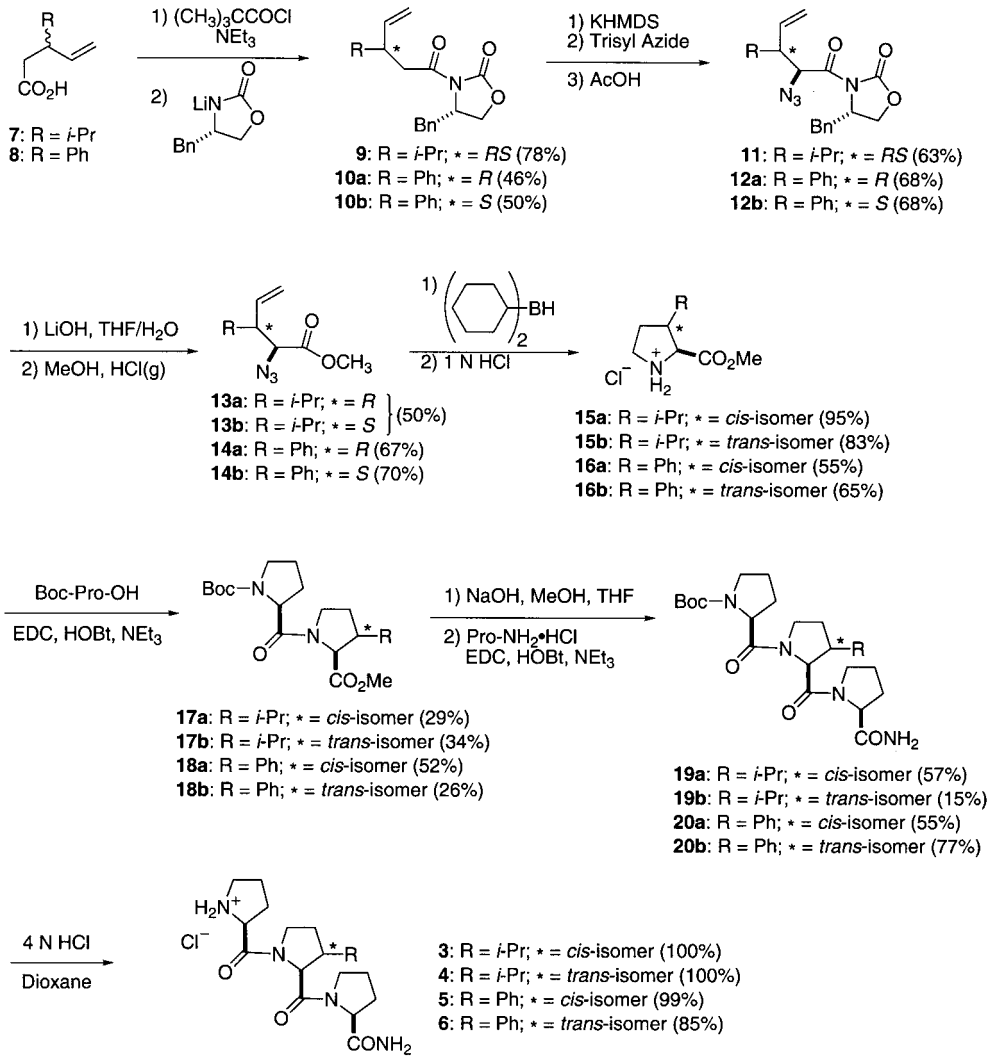

Scheme 1.

synthesis of these chimeric amino acids and their incorporation into the triprolyl peptides is outlined in Scheme 1.

The 3-substitited 4-pentenoic acids **7** and **8**[20] were converted to their corresponding mixed anhydrides with pivaloyl chloride. Reaction of the mixed anhydride in each case with lithiated *S*-4-benzyl-2-oxazolidinone[21] afforded a 1:1 mixture of diastereomeric *N*-acyloxazolidinones. In the case of *N*-acyloxazolidinones **9**, the diastereoisomers were not separable at this stage. In contrast, *N*-acyloxazolidinones **10** were chromatographically separable giving **10a** and **10b**. The chiral imide potassium enolates of **9**, **10a** and **10b** were formed and quenched by electrophilic azide transfer[22] using 2,4,6-triisopropylbenzenesulfonyl azide[23,24] to afford α-azido carboximides **11** as a mixture of diastereoisomers and **12a** and **12b** each as single diastereoisomers. The chiral auxiliary of **11** was removed with LiOH and the α-azido acid which was formed was treated with MeOH and HCl gas to afford an HPLC separable 1:1 mixture of diastereomeric methyl esters **13a** and **13b**. Likewise, the chiral auxiliaries of **12a** and **12b** were removed to give **14a** and **14b**, respectively. Hydroboration–cycloalkylation[25] of the azido-olefins **13a**, **13b**, **14a**, and **14b** with dicyclohexylborane[26] furnished the corresponding 3-substituted prolines **15a**, **15b**, **16a**, and **16b** in 95, 83, 55, and 65% yields, respectively.

The relative stereochemistries for **15a** and **15b** were assigned based on ^{1}H NMR GOESY (gradient NOE) data, which are summarized in Fig. 1. Irradiation of the α-CH

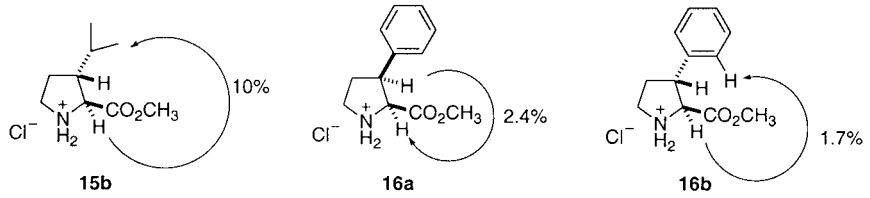

Figure 1. ^{1}H NMR GOESY data for chimeric amino acids **15b**, **16a**, and **16b**.

(δ 4.00) in **15a** showed no detectable NOE enhancement, while irradiation of the α-CH (δ 4.36) in **15b** showed a large NOE enhancement (10%) of the $CH(CH_3)_2$ protons (δ 0.87). Also, an NOE enhancement of 2.5% was seen for the α-CH of **15b** when the $CH(CH_3)_2$ protons were irradiated. These observed NOEs suggest that in **15b** the relationship between the ester and isopropyl group is *trans*, while for **15a** the relationship between these two groups is *cis*. Similar GOESY experiments were carried out on **16a** and **16b** (Fig. 1). Thus for **16a**, irradiation of the α-CH (δ 4.63) resulted in a 2% enhancement of the β-CH (δ 3.91), while a 2.4% enhancement was observed for the α-CH (δ 4.63) when the 3-Ph-Pro β-CH was irradiated (δ 3.91). In contrast, irradiation of the α-CH (δ 4.32) of **16b** showed a 1.7% enhancement of the *ortho*-aromatic protons (δ 7.24). These observations indicated a *cis* relationship between the ester and phenyl groups in **16a** and a *trans* relationship between these groups in **16b**.

The 3-substituted proline chimeras **15a**, **15b**, **16a**, and **16b** were coupled to Boc-Pro-OH with EDC·HCl to afford diprolyl peptides **17a**, **17b**, **18a**, and **18b**, respectively. These methyl esters were saponified with LiOH and the resulting acids were coupled to prolinamide to afford the respective protected triprolyl peptides **19a**, **19b**, **20a**, and **20b**. The step to afford tripeptide **19b**, which contains the *trans* proline-leucine chimera, was accomplished in a rather poor yield (15%) compared to those of the other three compounds (55–77%). In contrast, the yield observed for the *trans* proline-phenylalanine chimera case in which a flat phenyl ring is present instead of the rather bulky isopropyl substituent was quite good. This suggests that steric hindrance is playing a key role in the *trans* proline-leucine chimera case. The *tert*-butoxycarbonyl groups of **19a**, **19b**, **20a**, and **20b** were removed by HCl/dioxane to give the final triprolyl peptides **3**–**6**, respectively.

Discussion

The triprolyl compounds **3**–**6** represent novel conformationally constrained analogues of PLG. The pyrrolidine ring of each prolyl residue results in the restriction of each ϕ torsional angle in the peptide to a value around −60°. For the chimeric amino acid residues in the second position, the χ_1 torsional angle also is constrained. Preliminary MM2 molecular modeling of **3**–**6** using *Chem 3D* gave the χ_1 values shown in Fig. 2. In each isomeric case, a *trans* conformation for χ_1 was observed, although with opposite signs. The values were similar to those seen by Mosberg et al.[27] with their chimeric proline-tyrosine amino acids. On the basis of their work, a *gauche*$^+$ conformation also is possible for the *trans* isomers **4** and **6**, while a *gauche*$^-$ conformation is possible for the *cis* isomers **3** and **5**.

The triprolyl PLG analogues **3** and **4** with the proline-leucine chimeric amino acid in the second position have been designed such that the isopropyl group at position 3 could potentially mimic the leucyl side chain of PLG. Since previous structure-activity relationship studies on PLG have shown that the presence of the leucyl side chain results in enhanced binding of PLG analogues, it is expected that either **3** or **4** should show enhanced activity if the triproline peptides are interacting with the PLG binding site in the same manner as PLG. Also, since conformationally constrained PLG analogues with isobutyl and benzyl groups have shown different selectivities with regard to modulation of the different dopamine receptor subtypes,[14] a comparison of the dopamine receptor subtype selectivity of **3**–**6** should help shed light on the structural basis behind such selectivities.

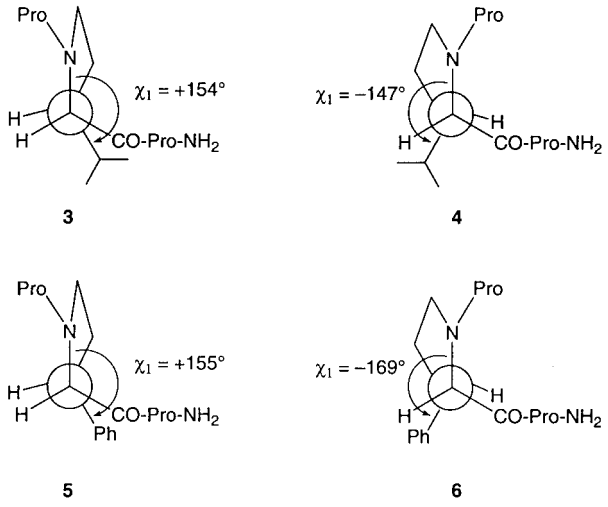

Figure 2. χ_1-Torsional angles of the chimeric amino acid residue in triprolines **3**–**6**.

Experimental

3-(3(*R*,*S*)-Isopropyl-4-ene-1-oxopentyl)-4(*S*)-(benzyl)-2-oxazolidinone (9). 3(*R*,*S*)-Isopropyl-pent-4-enoic acid (**7**, 285 mg, 2.0 mmol) was dissolved in THF and this solution was cooled to −78°C. Triethylamine (0.31 mL, 2.2 mmol) was added via syringe followed by trimethylacetyl chloride (0.27 mL, 2.2 mmol). The resulting white suspension was stirred at −78°C for 15 min, then at 0°C for 1 h, and finally at −78°C for 15 min. A slurry of lithiated 4(*S*)-benzyl-2-oxazolidinone at −78°C (prepared 10 min in advance at −78°C by the addition of 1.6 M *n*-butylithium in hexanes (1.4 mL, 2.2 mmol) into the solution of 4(*S*)-benzyl-2-oxazolidinone (394 mg, 2.2 mmol) in THF at −78°C) was added via a cannula to the suspension of the mixed anhydride. The resulting slurry was stirred at −78°C for 15 min, then for 4 h at room temperature. The reaction was quenched with saturated ammonium chloride (3 mL) and the volatiles were removed by rotary evaporation. Water was added to the mixture, which was then extracted with CH_2Cl_2 (3×). The combined organic extracts were dried over $MgSO_4$. Evaporation of the filtrate gave a yellow oil which was purified by silica gel flash chromatography (hexanes/EtOAc, 10:1→7.5:1→5:1) to yield 473 mg (78%) of an inseparable mixture of diastereoisomers: mp 51–54°C. ^{1}H NMR ($CDCl_3$) δ 7.21–7.37 (m, 5H), 5.64–5.78 (m, 1H), 5.01–5.10 (m, 2H), 4.63–4.69 (m, 1H), 4.13–4.20 (m, 2H), 2.91–3.34 (m, 3H), 2.49–2.75 (m, 2H), 1.70–1.77 (m, 1H), 0.89–0.97 (m, 6H) ^{13}C NMR ($CDCl_3$) δ 19.6, 21.0, 32.3, 32.4, 38.4, 38.6, 38.7, 38.8, 47.2, 47.4, 55.9, 56.0, 66.6, 66.8, 116.9, 117.0, 128.0, 129.6, 130.1, 136.1, 139.6, 139.7, 154.1, 154.2, 173.2, 173.3. FAB MS *m*/*z*=302

(60%) $[M+H]^+$. Anal. Calcd for $C_{18}H_{23}NO_3$: C, 71.73; H, 7.69; N, 4.65. Found: C, 72.00; H, 7.49; N, 4.68.

3-(3(*R*)-Phenyl-4-ene-1-oxopentyl)-4(*S*)-(benzyl)-2-oxazolidinone (10a) and 3-(3(*S*)-phenyl-4-ene-1-oxopentyl)-4(*S*)-(benzyl)-2-oxazolidinone (10b). The same conditions described for the synthesis of **9** were used to convert 3(*R*,*S*)-phenyl-pent-4-enoic acid (**8**, 1.63 g, 9.25 mmol) to **10a** and **10b**. Separation of these diastereoisomers was accomplished by two silica gel flash chromatography runs (hexanes/EtOAc, 10:1→7.5:1).

10a: Yield 713 mg (46%); mp 102–105°C; $[\alpha]_D$=+89.3 (*c* 1.0, CH_2Cl_2). TLC R_f=0.47 (hexanes/EtOAc, 5:2); ^{1}H NMR ($CDCl_3$) δ 7.20–7.38 (m, 10H), 6.10 (ddd, *J*=17.4, 10.2, 7.2 Hz, 1H), 5.17 (dd, *J*=10.2, 1.2 Hz, 2H), 4.54–4.61 (m, 1H), 4.02–4.14 (m, 3H), 3.44, 3.51 (2dd, *J*=16.2, 7.5 Hz, 2H), 3.27 (dd, *J*=9.9, 3.3 Hz, 1H), 2.72 (dd, *J*=13.2, 9.9 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 38.5, 41.2, 46.0, 55.9, 66.8, 115.6, 127.4, 128.0, 128.5, 129.3, 129.6, 130.1, 136.0, 141.1, 143.2, 154.1, 172.1; FAB MS *m*/*z* 336 (75%) $[M+H]^+$. Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18. Found: C, 75.30; H, 6.33; N, 4.16.

10b: Yield 775 mg (50%); mp 70–73°C; $[\alpha]_D$=+65.5 (*c* 1.0, CH_2Cl_2); TLC R_f=0.40 (hexanes/EtOAc, 5:2); ^{1}H NMR ($CDCl_3$) δ 7.22–7.43 (m, 8H), 7.09–7.14 (m, 2H), 6.09 (ddd, *J*=17.4, 10.2, 7.5 Hz, 1H), 5.14 (dd, *J*=10.2 Hz, 2H), 4.62–4.70 (m, 1H), 4.03–4.20 (m, 3H), 3.58 (dd, *J*=8.4, 7.5 Hz, 1H), 3.35 (dd, *J*=8.4, 7.5 Hz, 1H), 3.12 (dd, *J*=13.5, 3.3 Hz, 1H), 2.63 (dd, *J*=13.5, 9.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 38.3, 41.4, 46.0, 55.7, 66.7, 115.7, 127.4, 128.0, 128.5, 129.3, 129.6, 130.1, 135.8, 141.1, 143.2, 154.1, 172.1. FAB MS *m*/*z* 336 (20%) $[M+H]^+$. Anal. Calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18. Found: C, 75.41; H, 6.48; N, 4.26.

3-(2(*S*)-Azido-3(*R*,*S*)-isopropyl-4-ene-1-oxopentyl)-4(*S*)-(benzyl)-2-oxazolidinone (11). To 3 mL of dry THF at −78°C under N_2 was added 0.5 M potassium hexamethyldisilazide in toluene (3.07 mL, 1.53 mmol). To this solution was added via cannula a precooled (−78°C) solution of **9** (420 mg, 1.39 mmol) in 3 mL of dry THF. The reaction was stirred at −78°C for 30 min. To this solution of potassium enolate was added via cannula a precooled (−78°C) solution of trisyl azide (567 mg, 1.74 mmol) in 3 mL of THF. The reaction was quenched with acetic acid (0.37 mL, 6.4 mmol) after 1–2 min. The mixture was warmed immediately to 30°C and maintained at this temperature for 1.5 h. The solution was partitioned between CH_2Cl_2 and dilute brine. The aqueous phase was washed with CH_2Cl_2 (3×). The organic phases were combined and washed with 1 M $NaHCO_3$, dried over $MgSO_4$, filtered and then concentrated to a yellow oil. The crude product was purified by silica gel flash chromatography (hexanes/EtOAc, 5:1) to yield 299 mg (63%). An analytical sample of the inseparable mixture of diastereoisomers was obtained by HPLC with a Waters Spherisorb® 4.6×150 mm column (hexanes/EtOAc, 5:1; t_R=2.1 min). ^{1}H NMR ($CDCl_3$) δ 7.22–7.39 (m, 5H), 5.60–5.82 (m, 1H), 5.00–5.34 (m, 3H), 4.58–4.66 (m, 1H), 4.21–4.28 (m, 2H), 3.30–3.41 (m, 1H), 2.80–2.89 (m, 1H), 2.53–2.61 (m, 0.5H), 2.31–2.39 (m, 0.5H), 2.16–2.22 (m, 0.5H), 1.75–1.82 (m, 0.5H), 0.88–1.05 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 16.8, 20.6, 21.1, 21.8, 28.1, 29.5, 38.2, 38.5, 53.0, 53.9, 55.9, 56.7, 60.2, 63.5, 67.0, 67.4, 119.5, 120.9, 128.2, 129.7, 129.7, 130.1, 133.4, 135.5, 136.1, 153.5, 170.6, 172.0. FAB HRMS (NBAL matrix) *m*/*z*= 349.1867 ($C_{18}H_{22}N_4O_3$+Li^+ requires 349.1852).

3-(2(*S*)-Azido-3(*R*)-phenyl-4-ene-1-oxopentyl)-4(*S*)-(benzyl)-2-oxazolidinone (12a). Compound **10a** (282 mg, 0.84 mmol) was converted to **12a** by the same procedure described above for **11**. The material was purified by silica gel flash chromatography (hexanes/EtOAc, 5:1) to give 215 mg (68%). An analytical sample was obtained by HPLC with a Waters Spherisorb® 4.6×150 mm column (hexanes/EtOAc, 5:1; t_R=2.5 min): $[\alpha]_D$=+161.3 (*c* 0.6, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.17–7.36 (m, 10H), 6.23 (ddd, *J*= 16.8, 8.4 Hz, 1H), 5.51 (d, *J*=9.9 Hz, 1H), 5.38 (d, J_{trans}= 16.8 Hz, 1H), 5.33 (d, J_{cis}=8.1 Hz, 1H), 4.13–4.19 (m, 1H), 3.89–3.99 (m, 2H), 3.55 (dd, *J*=8.1 Hz, 1H), 3.22 (dd, *J*=13.5, 3.0 Hz, 1H), 2.72 (dd, *J*=13.5, 9.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 38.3, 53.2, 56.2, 62.4, 66.9, 119.3, 128.1, 128.3, 129.0, 129.4, 129.7, 130.1, 135.5, 136.6, 138.9, 153.2, 170.7; FAB HRMS (NBAL matrix) *m*/*z*=383.1689 ($C_{21}H_{20}N_4O_3$+Li^+ requires 383.1695).

3-(2(*S*)-Azido-3(*S*)-phenyl-4-ene-1-oxopentyl)-4(*S*)-(benzyl)-2-oxazolidinone (12b). Compound **10b** (250 mg, 0.75 mmol) was converted to **12b** by the same procedure described above for **11**. The material was purified by silica gel flash chromatography (hexanes/EtOAc, 5:1) to give 191 mg (68%). An analytical sample was obtained by HPLC with a Waters Spherisorb® 4.6×150 mm column (hexanes/EtOAc, 5:1; t_R=2.7 min): $[\alpha]_D$=+88.4 (*c* 0.56, MeOH). ^{1}H NMR ($CDCl_3$) δ 7.10–7.44 (m, 10H), 6.03 (ddd, *J*=17.1, 8.1 Hz, 1H), 5.60 (d, *J*=10.5 Hz, 1H), 5.21 (d, J_{trans}=17.1 Hz, 1H), 5.12 (d, J_{cis}=8.7 Hz, 1H), 4.66–4.72 (m, 1H), 4.18–4.26 (m, 2H), 3.91 (dd, *J*=9.6 Hz, 1H), 3.34 (dd, *J*=13.5, 3.3 Hz, 1H), 2.88 (dd, *J*=13.5, 9.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 38.4, 53.8, 55.9, 63.0, 67.2, 118.8, 128.2, 128.4, 128.7, 129.8, 130.2, 135.4, 137.2, 139.6, 153.9, 170.8; FAB HRMS (NBAL matrix) *m*/*z*=383.1692 ($C_{21}H_{20}N_4O_3$ + Li^+ requires 383.1695).

Methyl 2(*S*)-azido-3(*R*)-isopropyl-4-pentenoate (13a) and methyl 2(*S*)-azido-3(*S*)-isopropyl-4-pentenoate (13b). Compound **11** (1.9 g, 5.55 mmol) was dissolved in THF/H_2O (3:1, 25 mL) and this solution was placed in an ice bath. Solid LiOH (266 mg, 11.1 mmol) was added and the solution was allowed to stir at 0°C for 0.5 h. The solution was diluted with 1 M $NaHCO_3$ and then the THF was removed under aspirator pressure. The solution was washed with EtOAc (3×) and acidified to pH=3 with solid citric acid. The aqueous solution was extracted with EtOAc (3×) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated to afford the diastereoisomeric mixture of free acids. The mixture was dissolved in MeOH and an excess of anhydrous HCl gas was bubbled into the solution, which was then capped and stirred overnight at room temperature. Excess HCl and the MeOH were removed under aspirator pressure by forming an azeotrope with CH_2Cl_2 (3×). A yield of 543 mg (50%) of the mixture of diastereoisomers was obtained which were separated on a Waters PrepLC™ 25×100 mm silica PrepPak cartridge (hexanes/EtOAc, 60:1).

13a: Yield=202 mg; $[\alpha]_D=-6.4$ (*c* 1.07, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 5.61 (ddd, *J*=16.8, 9.9, 9.9 Hz, 1H), 5.10 (dd, J_{cis}=9.0, 1.8 Hz, 1H), 5.05 (dd, J_{trans}=15.6, 1.8 Hz, 1H), 4.08 (d, *J*=5.7 Hz, 1H), 3.73 (s, 3H), 2.26 (ddd, *J*=9.9, 7.8, 5.4 Hz, 1H), 1.64–1.73 (m, 1H), 0.95 (d, *J*=6.3 Hz, 3H), 0.85 (d, *J*=6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 20.4, 21.2, 29.0, 53.0, 53.9, 64.7, 119.7, 135.7, 171.2; EI HRMS (4% NH_3 in CH_4) *m/z*=215.1506 ($C_9H_{15}N_3O_2+NH_4^+$ requires 215.1550).

13b: Yield=157 mg; $[\alpha]_D=+71.1$ (*c* 1.22, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 5.57 (ddd, *J*=16.8, 10.2, 10.2 Hz, 1H), 5.12 (dd, J_{cis}=10.2, 1.8 Hz, 1H), 5.05 (dd, J_{trans}=16.8, 1.8 Hz, 1H), 3.71 (s, 3H), 3.70 (d, *J*=9.6 Hz, 1H), 2.38 (ddd, *J*=9.6, 4.5 Hz, 1H), 1.97–2.04 (m, 1H), 0.91 (d, *J*=6.6 Hz, 3H), 0.85 (d, *J*=6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 17.6, 21.7, 28.0, 52.5, 52.7, 64.2, 120.4, 133.8, 171.2; EI HRMS (4% NH_3 in CH_4) *m/z*=215.1510 ($C_9H_{15}N_3O_2$ + NH_4^+ requires 215.1550).

Methyl 2(*S*)-azido-3(*R*)-phenyl-4-pentenoate (14a). Compound **12a** (1.2 g, 3.18 mmol) was converted to **14a** (yield=495 mg (67%)) by the same method as that described above for the formation of **13a**: $[\alpha]_D=-6.1$ (*c* 0.59, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.26–7.38 (m, 5H), 6.17 (ddd, *J*=17.4, 9.0, 9.0 Hz, 1H), 5.28 (d, J_{trans}=15.9 Hz, 1H), 5.27 (d, J_{cis}=11.4 Hz, 2H), 4.13 (d, *J*=7.2 Hz, 1H), 3.93 (dd, *J*=8.1, 7.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 52.3, 53.1, 66.9, 119.2, 128.2, 128.8, 129.4, 136.3, 139.6, 170.4; FAB MS *m/z*=232 (30%) $[M+H]^+$. Anal. Calcd for $C_{16}H_{27}N_3O_4$: C, 62.33; H, 5.67; N, 18.17. Found: C, 62.42; H, 6.00; N, 18.02.

Methyl 2(*S*)-azido-3(*S*)-phenyl-4-pentenoate (14b). Compound **12b** (855 mg, 2.27 mmol) was converted to **14b** (yield=365 mg (70%)) by the same method as that described above for the formation of **13a**: $[\alpha]_D=+25.0$ (*c* 0.86, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.27–7.41 (m, 5H), 6.06 (ddd, *J*=18.0, 9.9, 8.4 Hz, 1H), 5.21 (d, J_{trans}=18.3 Hz, 1H), 5.20 (d, J_{cis}=9.9 Hz, 1H), 4.16 (d, *J*=9.0 Hz, 1H), 3.90 (dd, *J*=8.4, 8.4 Hz, 1H), 3.76 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 52.6, 53.0, 66.6, 118.5, 128.3, 128.9, 129.5, 137.1, 139.3, 170.5; FAB MS *m/z*=232 (5%) $[M+H]^+$. Anal. Calcd for $C_{16}H_{27}N_3O_4$: C, 62.33; H, 5.67; N, 18.17. Found: C, 62.13; H, 5.90; N, 17.97.

***cis*-3-Isopropyl-L-proline methyl ester hydrochloride (15a).** In a dry flask under Ar was placed cyclohexene (0.59 mL, 5.8 mmol) and anhydrous Et_2O (5 mL). The solution was cooled to 0°C. Borane dimethylsulfide complex (0.5 M in Et_2O, 0.58 mL, 2.9 mmol) was added and the reaction was allowed to stir at 0°C for 3 h. The Et_2O was blown off with a stream of Ar and the resulting dicyclohexylborane was dried under vacuum for 10 min and then suspended in freshly distilled CH_2Cl_2. Azide **13a** (191 mg, 0.97 mmol), which had been dissolved in CH_2Cl_2, was transferred via a cannula at 0°C into the white suspension. The reaction mixture was stirred for 2 d at room temperature. The CH_2Cl_2 was extracted with 1 M HCl and then the aqueous phase was evaporated to afford a 191 mg (95%) of a white solid. HPLC analysis of this material on a Waters Spherisorb® 4.6×150 mm column ($CHCl_3$/MeOH, 5:1) gave a single peak (t_R=1.4 min): mp 74–78°C; $[\alpha]_D=+17.0$ (*c* 0.17, H_2O); ^1H NMR (D_2O) δ 4.19 (d, *J*=7.2 Hz, 1H), 3.81 (s, 3H), 3.28–3.47 (m, 2H), 2.33–2.41 (m, 1H), 2.09–2.20 (m, 1H), 1.76–1.89 (m, 2H), 0.96 (d, *J*=6.6 Hz, 3H), 0.90 (d, *J*=6.9 Hz, 3H); ^{13}C NMR (D_2O) δ 18.3, 20.0, 27.1, 30.0, 46.7, 49.4, 54.4, 62.2, 171.5; GOESY NMR (D_2O, 500 MHz) irradiation at δ 4.00 (3-*i*-Pr-Pro α-CH) showed no NOE enhancement; FAB HRMS (MNBA matrix) *m/z*=172.1344 ($C_9H_{17}NO_2+H^+$ requires 172.1337).

***trans*-3-Isopropyl-L-proline methyl ester hydrochloride (15b).** This material was obtained from azide **13b** (142 mg, 0.72 mmol) through use of the same procedures as those described above for **15a**. A yield of 124 mg (83%) was obtained and HPLC analysis on a Waters Spherisorb® 4.6×150 mm column ($CHCl_3$/MeOH, 5:1) gave a single peak (t_R=1.0 min): mp 98–102°C; $[\alpha]_D=+27.5$ (*c* 0.16, H_2O); ^1H NMR (D_2O) δ 4.53 (d, *J*=7.2 Hz, 1H), 3.81 (s, 3H), 3.58–3.66 (m, 1H), 3.29–3.39 (m, 1H), 2.15–2.27 (m, 2H), 1.72–1.80 (m, 1H), 1.44–1.51 (m, 1H), 1.02 (d, *J*=6.6 Hz, 3H), 0.90 (d, *J*=6.6 Hz, 3H); ^{13}C NMR (D_2O) δ 21.2, 22.0, 27.3, 28.9, 46.0, 51.0, 53.9, 62.4, 170.5; GOESY NMR (D_2O, 500 MHz) irradiation at δ 4.36 (3-*i*-Pr-Pro α-CH) showed an NOE enhancement of 10% at δ 0.87 ($CH(CH_3)_3$) while irradiation at δ 0.87 ($CH(CH_3)_3$) showed an NOE enhancement of 2.5% at δ 4.36 (3-*i*-Pr-Pro α-CH); FAB HRMS (MNBA matrix) *m/z*=172.1346 ($C_9H_{17}NO_2+H^+$ requires 172.1337).

***cis*-3-Phenyl-L-proline methyl ester hydrochloride (16a).** This material was obtained from azide **14b** (340 mg, 1.47 mmol) through use of the same procedures as those described above for **15a**. A yield of 197 mg (55%) was obtained and HPLC analysis on a Waters Spherisorb® 4.6×150 mm column ($CHCl_3$/MeOH, 5:1) gave a single peak (t_R=1.5 min): mp 59–64°C; $[\alpha]_D=+61.4$ (*c* 0.5, MeOH); ^1H NMR (D_2O) δ 7.21–7.40 (m, 5H), 4.72 (d, *J*=9.0 Hz, 1H), 3.96–4.04 (m, 1H), 3.74–3.82 (m, 1H), 3.42–3.52 (m, 1H), 3.33 (s, 3H), 2.35–2.48 (m, 2H). ^{13}C NMR (D_2O) δ 29.6, 46.5, 46.9, 53.8, 64.3, 128.6, 129.0, 129.6, 136.6, 169.7; GOESY NMR (D_2O, 500 MHz) irradiation at δ 4.63 (3-Ph-Pro α-CH) showed an NOE enhancement (2.0%) at δ 3.91 (PhCH) while irradiation at δ 3.91 (PhCH) showed NOE enhancement (2.4%) at δ 4.63 (3-Ph-Pro α-CH); FAB HRMS (MNBA matrix) *m/z*=206.1181 ($C_{12}H_{16}NO_2+H^+$ requires 206.1181).

***trans*-3-Phenyl-L-proline methyl ester hydrochloride (16b).** This material was obtained from azide **14a** (482 mg, 2.08 mmol) through use of the same procedures as those described above for **15a**. A yield of 326 mg (65%) was obtained and HPLC analysis on a Waters Spherisorb® 4.6×150 mm column ($CHCl_3$/MeOH, 5:1) gave a single peak (t_R=1.7 min): mp 65–69°C; $[\alpha]_D=+31.5$ (*c* 0.71, MeOH); ^1H NMR (D_2O) δ 7.32–7.44 (m, 5H), 4.48 (d, *J*=9.9 Hz, 1H), 3.71 (s, 3H), 3.45–3.70 (m, 3H), 2.49–2.66 (m, 1H), 2.17–2.26 (m, 1H); ^{13}C NMR (D_2O) δ 33.7, 46.6, 48.4, 54.4, 65.3, 128.2, 128.8, 129.9, 138.6, 170.2; GOESY NMR (D_2O, 500 MHz) irradiation at δ 4.32 (3-Ph-Pro α-CH) showed an NOE enhancement (1.7%) at δ 7.24 (Ar-H); FAB HRMS (MNBA matrix) *m/z*=206.1185 ($C_{12}H_{16}NO_2+H^+$ requires 206.1181).

***N-tert*-Butoxycarbonyl-L-prolyl-*cis*-3-isopropyl-L-proline methyl ester (17a).** Boc-L-Pro-OH (118 mg, 0.55 mmol),

15a (100 mg, 0.48 mmol) and HOBt·H_2O (75 mg, 0.55 mmol) were dissolved in CH_2Cl_2 (10 mL) under N_2. The mixture was cooled to −78°C after which was added NEt_3 (0.13 mL, 0.97 mmol), followed by EDC·HCl (106 mg, 0.55 mmol). The mixture was stirred at a low temperature for 30 min and then warmed to room temperature where it was kept for two days. The organic layer was extracted with 1 M $NaHCO_3$, 10% citric acid, water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under aspirator pressure. The crude product was purified by silica gel flash chromatography (EtOAc/hexanes, 2:1) to give 52 mg (29%) of product: $[\alpha]_D$=−50.4 (*c* 0.91, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, rotomers observed in a ratio of 4:3) δ 4.49 (dd, *J*=8.4, 2.7 Hz, 0.57H), 4.37 (dd, *J*=8.1, 3.6 Hz, 0.43H), 4.25 (dd, *J*=6.0, 2.7 Hz, 1H), 3.82–3.86 (m, 1H), 3.67, 3.68 (2s, 3H), 3.33–3.58 (m, 3H), 1.66–2.16 (m, 8H), 1.36, 1.41 (s, 9H), 0.97 (dd, *J*=6.6 Hz), 0.91 (dd, *J*=6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, rotomers observed) δ 20.2, 21.6, 21.8, 24.1, 24.7, 29.0, 29.1, 29.3, 29.4, 30.5, 30.8, 30.9, 46.8, 47.0, 47.3, 47.4, 49.7, 50.1, 52.7, 52.8, 58.1, 58.3, 63.0, 63.2, 80.0, 154.4, 155.1, 171.7, 172.1, 174.0, 174.3; FAB MS (MNBA matrix) *m*/*z*=369 (40%) $[M+H]^+$. Anal. Calcd for $C_{19}H_{32}N_2O_5$: C, 61.93; H, 8.75; N, 7.60. Found: C, 61.87; H, 8.50; N, 7.39.

***N-tert*-Butoxycarbonyl-L-prolyl-*trans*-3-isopropyl-L-proline methyl ester (17b).** Boc-L-Pro-OH (72 mg, 0.33 mmol) and **15b** (60 mg, 0.29 mmol) were coupled together by the same method as that described above for **17a** to give 36 mg (34%) of product after silica gel flash chromatography (EtOAc/hexanes, 2:1): $[\alpha]_D$=−23.2 (*c* 1.08, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, rotomers observed in a ratio of 3:2) δ 4.69 (d, *J*=7.5 Hz, 0.6H), 4.64 (d, *J*=7.2 Hz, 0.4H), 4.43 (dd, *J*=8.1, 3.3 Hz, 0.6H), 4.32 (dd, *J*=9, 4.2 Hz, 0.4H), 3.69, 3.67 (2s, 3H), 3.35–3.65 (m, 4H), 1.78–2.17 (m, 7H), 1.41, 1.43 (2s, 9H), 1.06 (apparent dd, *J*=6.0, 10.8 Hz, 3H), 0.92 (apparent t, *J*=6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, rotomers observed) δ 22.2, 22.8, 22.9, 24.2, 24.7, 29.0, 29.1, 29.5, 29.6, 29.7, 30.4, 46.7, 46.8, 47.3, 47.6, 49.9, 50.2, 52.2, 52.4, 57.9, 58.1, 62.0, 80.0, 80.1, 155.2, 171.8, 172.3, 172.7, 173.0; LCQ MS (Direct Infusion Electrospray) *m*/*z*=391.1 (100%) $[M+Na]^+$. Anal. Calcd for $C_{19}H_{32}N_2O_5$: C, 61.93; H, 8.75; N, 7.60. Found: C, 62.15; H, 8.60; N, 7.39.

***N-tert*-Butoxycarbonyl-L-prolyl-*cis*-3-phenyl-L-proline methyl ester (18a).** Boc-L-Pro-OH (102 mg, 0.47 mmol) and **16a** (100 mg, 0.41 mmol) were coupled together by the same method as that described above for **17a** to give 85.5 mg (52%) of product after silica gel flash chromatography (EtOAc/hexanes, 2:1): mp 149–151°C; $[\alpha]_D$=−8.0 (*c* 0.57, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, rotomers observed in a 9:5 ratio) δ 7.19–7.34 (m, 5H), 4.82 (d, *J*=8.7 Hz, 0.65H), 4.77 (d, *J*=9.0 Hz, 0.35H), 4.51 (dd, *J*=8.4, 3.0 Hz, 0.65H), 4.41 (dd, *J*=8.4, 3.7 Hz, 0.35H), 3.83–3.93 (m, 1H), 3.30–3.71 (m, 4H), 3.22, 3.20 (2s, 3H), 2.62–2.70 (m, 1H), 1.81–2.27 (m, 5H), 1.45, 1.43 (2s, 9H); ^{13}C NMR ($CDCl_3$, rotomers observed) δ 24.7, 24.8, 29.1, 29.2, 29.4, 29.5, 29.7, 30.5, 46.5, 46.7, 46.8, 46.8, 47.3, 47.6, 52.0, 52.1, 58.0, 58.2, 64.3, 80.1, 80.2, 128.2, 128.3, 128.4, 128.5, 129.0, 129.1, 136.7, 137.1, 154.4, 155.2, 171.7, 171.9, 172.0, 172.4; LCQ MS (Direct Infusion Electrospray) *m*/*z*=425.1 (100%) $[M+Na]^+$. Anal. Calcd for $C_{22}H_{30}N_2O_5$: C, 65.65; H, 7.51; N, 6.96. Found: C, 65.64; H, 7.47; N, 7.03.

***N-tert*-Butoxycarbonyl-L-prolyl-*trans*-3-phenyl-L-proline methyl ester (18b).** Boc-L-Pro-OH (102 mg, 0.47 mmol) and **16b** (100 mg, 0.41 mmol) were coupled together by the same method as that described above for **17a** to give 44 mg (26%) of product after silica gel flash chromatography (EtOAc/hexanes, 2:1). Analytical HPLC analysis on a Waters Spherisorb® 4.6×150 mm column ($CHCl_3$/MeOH, 5:1) gave a single peak (t_R=1.25 min): $[\alpha]_D$=+10.9 (*c* 0.54, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, rotomers observed) δ 7.22–7.36 (m, 5H), 4.57–4.62 (m, 1.5H), 4.46 (dd, *J*=8.1 and 3.6 Hz, 0.5H), 4.05–4.13, 3.80–3.90 (m, 1H), 3.67, 3.70 (2s, 3H), 3.35–3.75 (m, 4H), 1.83–2.50 (m, 6H), 1.40, 1.46 (2s, 9H); ^{13}C NMR ($CDCl_3$, rotomers observed) δ 24.1, 24.8, 29.1, 29.2, 29.8, 30.8, 33.9, 34.7, 46.6, 47.1, 47.4, 47.4, 47.9, 48.8, 52.8, 53.0, 58.1, 58.4, 65.8, 65.9, 80.1, 80.2, 127.5, 127.8, 127.9, 128.0, 129.4, 129.5, 140.8, 141.0, 154.4, 155.2, 172.0, 172.2, 173.0, 173.2; FAB HRMS (MNBA matrix) *m*/*z*=403.2241 ($C_{22}H_{30}N_2O_5$ + H^+ requires 403.2233).

***N-tert*-Butoxycarbonyl-L-prolyl-*cis*-3-isopropyl-L-prolyl-L-prolinamide (19a).** Dipeptide **17a** (41 mg, 0.11 mmol) was reacted with 3N NaOH (0.06 mL, 0.17 mmol)/MeOH/THF (1:1:5) for 2 d at room temperature. The MeOH and THF were removed under aspirator pressure and the remaining aqueous residue was diluted with distilled water. The solution was washed with EtOAc (3×) and acidified to pH=3 with solid citric acid. The aqueous solution was extracted with EtOAc (3×) and the combined organic layers were washed with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated under aspirator pressure to afford the dipeptide free acid (20 mg, 0.13 mmol). This material was dissolved in CH_2Cl_2 (10 mL) along with L-prolinamide·HCl (40 mg, 0.11 mmol) and HOBt·H_2O (18 mg, 0.13 mmol). The solution under nitrogen was cooled to −78°C and then NEt_3 (0.03 mL, 0.23 mmol) and EDC·HCl (25 mg, 0.13 mmol) were added. The mixture was stirred at a low temperature for 30 min and then warmed to room temperature where the reaction was allowed to proceed for two days. The organic layer was extracted with 1 M $NaHCO_3$, 10% citric acid, water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under aspirator pressure and the crude product obtained was purified by silica gel flash chromatography (CH_2Cl_2/MeOH, 30:1→20:1) to give 29 mg (57%) of material, which yielded a single peak (t_R=1.3 min) by HPLC analysis on a Waters Spherisorb® 4.6×150 mm column ($CHCl_3$/MeOH, 20:1): $[\alpha]_D$=−84.3 (*c* 0.19, CH_2Cl_2); FAB HRMS (MNBA matrix) *m*/*z*=451.2926 ($C_{22}H_{30}N_2O_5$ + H^+ requires 451.2922).

***N-tert*-Butoxycarbonyl-L-prolyl-*trans*-3-isopropyl-L-prolyl-L-prolinamide (19b).** Dipeptide **17b** (224 mg, 0.61 mmol) was converted to **19b** by the same procedures as those described above for the synthesis of **19a**. A yield of 35 mg (15%) of material was obtained, which yielded a single peak (t_R=1.2 min) by HPLC analysis on a Waters Spherisorb® 4.6×150 mm column ($CHCl_3$/MeOH, 5:1): $[\alpha]_D$=−50 (*c* 0.15, CH_2Cl_2); FAB HRMS (MNBA matrix) *m*/*z*=451.2943 ($C_{23}H_{38}N_4O_5$ + H^+ requires 451.2922).

***N-tert*-Butoxycarbonyl-L-prolyl-*cis*-3-phenyl-L-prolyl-L-prolinamide (20a).** Dipeptide **18a** (70 mg, 0.16 mmol) was converted to **20a** by the same procedures as those described above for the synthesis of **19a**. A yield of 46 mg (55%) of material was obtained, which yielded a single peak (t_R=1.1 min) by HPLC analysis on a Waters Spherisorb® 4.6×150 mm column ($CHCl_3$/MeOH, 20:1): $[\alpha]_D$=−54.3 (*c* 0.96, CH_2Cl_2); FAB HRMS (MNBA matrix) *m*/*z*=485.2767 ($C_{22}H_{30}N_2O_5$ + H^+ requires 485.2764).

***N-tert*-Butoxycarbonyl-L-prolyl-*trans*-3-phenyl-L-prolyl-L-prolinamide (20b).** Dipeptide **18b** (52 mg, 0.13 mmol) was converted to **20b** by the same procedures as those described above for the synthesis of **19a**. A yield of 48 mg (77%) of material was obtained, which yielded a single peak (t_R=1.1 min) by HPLC analysis on a Waters Spherisorb® 4.6×150 mm column ($CHCl_3$/MeOH, 20:1): $[\alpha]_D$=−54.2 (*c* 1.1, CH_2Cl_2); FAB HRMS (MNBA matrix) *m*/*z*=485.2759 ($C_{22}H_{30}N_2O_5$ + H^+ requires 485.2764).

L-Prolyl-*cis*-3-isopropyl-L-prolyl-L-prolinamide hydrochloride (3). Triprolyl peptide **19a** (20 mg, 0.04 mmol) was dissolved in 4N HCl/dioxane (5 mL) and the resulting solution was stirred overnight at room temperature. Excess HCl and the dioxane were removed under aspirator pressure by forming an azeotrope with CH_2Cl_2 (3×). Lyophilization of the residue gave 17 mg (100%) of a fluffy white solid, which gave a single peak (t_R=1.2 min) by HPLC analysis on a Waters Spherisorb® 4.6×150 mm column eluting with 5:1 $CHCl_3$/MeOH: $[\alpha]_D$=−96.6 (*c* 0.8, MeOH); ^{1}H NMR (D_2O, rotomers observed) δ 4.53–4.60 (m, 1H), 4.31–4.36 (m, 1H), 3.87–3.94 (m, 1H), 3.49–3.80 (m, 4H), 3.29–3.41 (m, 2H), 2.48–2.55 (m, 2H), 2.16–2.32 (m, 3H), 1.78–2.07 (m, 7H), 0.96 (s, *J*=6.6 Hz, 3H), 0.90 (s, *J*=6.6 Hz, 3H); ^{13}C NMR (D_2O, rotomers observed) δ 18.3, 21.3, 24.5, 25.5, 26.4, 28.8, 29.1, 30.1, 43.9, 47.3, 47.6, 48.9, 49.4, 59.6, 60.9, 61.3, 62.9, 67.3, 70.2, 71.4, 72.2, 168.4, 172.7, 177.3; FAB HRMS (MNBA matrix) *m*/*z*=351.2400 ($C_{22}H_{30}N_2O_5$ + H^+ requires 351.2398).

L-Prolyl-*trans*-3-isopropyl-L-prolyl-L-prolinamide hydrochloride (4). Triprolyl peptide **19b** (34 mg, 0.08 mmol) was treated in the same manner as that described above for **3**. Lyophilization of the residue gave 29 mg (100%) of a fluffy white solid, which gave a single peak (t_R=1 min) by HPLC analysis on a Waters Spherisorb® 4.6×150 mm column eluting with 5:1 $CHCl_3$/MeOH: $[\alpha]_D$=−50.5 (*c* 0.39, MeOH); ^{1}H NMR (D_2O) δ 4.48–4.53 (m, 1H), 4.28–4.33 (m, 1H), 4.08–4.16 (m, 1H), 3.60–3.73 (m, 4H), 3.32–3.42 (m, 2H), 2.44–2.55 (m, 1H), 2.21–2.29 (m, 1H), 1.81–2.18 (m, 7H), 1.48–1.57 (m, 1H), 0.96 (d, *J*=6.6 Hz, 3H), 0.85 (d, *J*=6.6 Hz, 3H). ^{13}C NMR (D_2O, rotomers observed) δ 21.3, 23.2, 24.6, 25.6, 28.1, 29.1, 29.3, 30.2, 43.9, 47.5, 47.6, 49.5, 50.5, 59.2, 60.9, 60.9, 61.0, 70.6, 71.4, 72.3, 168.6, 171.8, 177.3; FAB HRMS (MNBA matrix) *m*/*z*=351.2376 ($C_{22}H_{30}N_2O_5$ + H^+ requires 351.2398).

L-Prolyl-*cis*-3-phenyl-L-prolyl-L-prolinamide hydrochloride (5). Triprolyl peptide **20a** (28 mg, 0.06 mmol) was treated in the same manner as that described above for **3**. Lyophilization of the residue gave 24 mg (99%) of a fluffy white solid, which gave a single peak (t_R=1.2 min) by HPLC analysis on a Waters Spherisorb® 4.6×150 mm column eluting with 5:1 $CHCl_3$/MeOH: $[\alpha]_D$=−27.4 (*c* 0.31, MeOH); ^{1}H NMR (D_2O) δ 7.32–7.44 (m, 5H), 4.58–4.71 (m, 1H), 4.07–4.17 (m, 1H), 3.85–3.97 (m, 1H), 3.59–3.82 (m, 3H), 3.24–3.49 (m, 3H), 3.02–3.08 (m, 1H), 1.46–2.68 (m, 10H); ^{13}C NMR (D_2O) δ 21.6, 24.5, 24.6, 24.9, 29.1, 29.2, 29.3, 29.4, 31.0, 31.1, 46.3, 47.1, 47.2, 47.3, 47.5, 48.8, 59.4, 59.4, 60.8, 61.3, 62.4, 63.4, 128.0, 128.9, 129.0, 129.1, 129.2, 129.3, 129.8, 129.9, 136.6, 136.8, 168.6, 168.8, 170.8, 171.3, 176.5, 176.7; FAB HRMS (MNBA matrix) *m*/*z*=385.2241 ($C_{22}H_{30}N_2O_5$ + H^+ requires 385.2240).

L-Prolyl-*trans*-3-phenyl-L-prolyl-L-prolinamide hydrochloride (6). Triprolyl peptide **20b** (13 mg, 0.03 mmol) was treated in the same manner as that described above for **3**. Lyophilization of the residue gave 10 mg (85%) of a fluffy white solid, which gave a single peak (t_R=1.2 min) by HPLC analysis on a Waters Spherisorb® 4.6×150 mm column eluting with 5:1 $CHCl_3$/MeOH: $[\alpha]_D$=−14.6 (*c* 0.21, MeOH); ^{1}H NMR (D_2O) δ 7.33–7.46 (m, 5H), 4.67–4.74 (m, 1H), 4.29–4.34 (m, 1H), 3.92–3.96 (m, 1H), 3.61–3.80 (m, 5H), 3.36–3.52 (m, 2H), 2.53–2.58 (m, 2H), 2.29–2.39 (m, 2H), 2.00–2.14 (m, 4H), 1.69–1.77 (m, 2H); ^{13}C NMR (D_2O) δ 24.5, 25.2, 28.7, 29.9, 34.1, 43.8, 43.9, 47.3, 48.4, 48.6, 49.3, 59.7, 61.0, 66.1, 67.3, 70.2, 71.4, 71.5, 72.3, 128.5, 128.7, 129.7, 129.8, 139.6, 168.2, 171.6, 177.0; FAB HRMS (MNBA matrix) *m*/*z*=385.2257 ($C_{22}H_{30}N_2O_5$+H^+ requires 385.2241).

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