Supplementary Information

Replacement of proline by $\Delta^3$-dehydroproline 1 in peptides and proteins has given analogs with similar and improved biological activity. For example, a $\Delta^3$-dehydroproline$^3$-bradikinin analog exhibited nearly the same pharmacological activity as the parent peptide$^1$ and was a competitive inhibitor for prolyl hydroxylase.$^2$ $\Delta^3$-Dehydroproline$^7$-oxytocin exhibited a two-fold increase in uterotonic and antidiuretic potency relative to the native peptide.$^3$ Moreover, in the protein adenylate kinase, mutation of the highly conserved Pro$^{17}$ residue with $\Delta^3$-dehydroproline gave a 7-fold increase in the $K_m$,$\text{MgATP}$ kinetic parameter.$^4$

Among the rare examples of a proline possessing a heterocycle fused to its 3- and 4-positions, regioisomeric 3-carboxy- and 3-hydroxyisoxazolinyl prolines were synthesized by dipolar cycloadditions on a protected $\Delta^3$-dehydroproline and exhibited neuroexcitatory activity and affinity at the AMPA and KAIN ionotropic receptors for glutamate.$^5$

![Scheme 1. Synthesis of Protected Pyrrolo-Proline 3 for Peptide Synthesis$^6$](image)

$^a$ (1) $n$-BuLi, THF, -78°C, (2) Boc-alaninal, THF, -78°C, (3) cat. HCl$\text{conc}$, CH$_2$Cl$_2$. $^b$ $\Delta$ 180°C, neat, 30 min. $^c$ (1) H$_2$, Pd(OH)$_2$, THF, MeOH, (2) FmocOSu, Na$_2$CO$_3$, dioxane, water.

**Synthesis.** $N^{\alpha}$-Fmoc-Pyrrolo-proline 3 was synthesized as reported by a sequence featuring the aldol condensation of Boc-alaninal onto the lithium enolate of $N$-PhF-4-oxo-proline 6 (PhF = 9-(9-phenylfluorenyl)),$^7$ acid catalyzed cyclisation, thermolytic removal of the Boc group, hydrogenolytic cleavage of the PhF-amine and benzyl ester groups, and acylation with FmocOSu (Scheme 1).$^6$ Prolyl peptides 4a and 5a were prepared by a $N^{\alpha}$-
Boc-protection strategy in solution using TBTU, DIEA in CH₂Cl₂⁸ for peptide couplings and HCl in dioxane for deprotections as described in detail in the experimental section.⁹

Pyrrolo-prolyl peptides 4b and 5b were synthesized by a Nα-Fmoc-protection strategy in solution (Scheme 2) because Boc-protected pyrrolo-prolines were found to decompose on prolonged exposure to acid, such as TFA in dichloromethane and HCl in dioxane. In solution, cleavage of the Fmoc group using the common piperidine/DMF method was inconvenient because of difficulties removing the poorly volatile solvent and dibenzofulvene adduct. We attempted different conditions such as piperazino-fuctionalized silica gel¹⁰ in chloroform,¹¹ that gave no reaction, and tris(2-aminoethyl)amine (TAEA) in chloroform,¹² which gave low yield, presumably because of the phosphate buffer extractions that were necessary for removing TAEA from the reaction mixture. The best conditions found for Fmoc group cleavage employed a catalytic amount of DBU in THF in the presence of octanethiol as a scavenger and provided the free amine of pyrrolo-proline peptides 10 and 12 in 85 and 89 % yields after purification by chromatography on silica gel.¹³ The free amines were then directly coupled to the acetylated amino acid or dipeptide using TBTU to provide the final peptides after preparative HPLC. Some epimer (less the 5 %) due to coupling of acetyl amino acid or dipeptide, could be detected by LCMS and was removed during purification.

**Scheme 2.** Synthesis of Model Peptides 4b and 5b from FmocPyPro 3.

\[
\begin{align*}
\text{FmocPyPro-Gly-D-Leu-NMe₂} & \quad \text{FmocPyPro-Val-NMe₂} \\
\text{PyPro-Gly-D-Leu-NMe₂} & \quad \text{PyPro-Val-NMe₂} \\
\text{Ac-D-Val-PyPro-Gly-D-Leu-NMe₂} & \quad \text{Ac-Leu-D-Phe-PyPro-Val-NMe₂}
\end{align*}
\]

\(a\) HCl-Gly-D-Leu-NMe₂, TBTU, DIEA, CH₂Cl₂. \(b\) HCl-Val-NMe₂, TBTU, DIEA, CH₂Cl₂. \(c\) HS(CH₂)₇CH₃, DBU, THF. \(d\) Ac-D-Val, TBTU, DIEA, CH₂Cl₂. \(e\) Ac-Leu-D-Phe, TBTU, DIEA, CH₂Cl₂.
Conformational analysis. No aggregation of the enantiomer of 4a was found previously between 0.01 and 50 mM. The variation of the chemical shift of the amide protons of peptide 5a was plotted as a function of the logarithm of the peptide concentration in CD$_2$Cl$_2$ (Figure 4). No significant variation of the chemical shift was observed at $\leq$10 mM, indicative of a monomeric state below this concentration range.

![Figure 4](image.png)

Figure 4. Amide proton NMR chemical shifts in CD$_2$Cl$_2$ at room temperature as a function of the logarithm of peptide concentration for 5a

Similar chemical shift and coupling constant data were observed for Pro and PyPro peptides 4 in CD$_2$Cl$_2$ and 2:1 CD$_2$Cl$_2$:DMSO (Table 2). In CD$_2$Cl$_2$, D-Val and D-Leu amide proton were downfield of the Gly-NH, indicative of an intramolecular hydrogen bond for these residues. In DMSO, the Gly amide proton appeared further downfield relative to the amide protons of D-Val and D-Leu probably due to hydrogen bonding with DMSO. In both solvents, larger coupling constants $^3$J$_{\phi}$ were measured for Val and Leu relative to Gly, indicative of $\phi$ dihedral angles in extended versus bent geometry.
Table 2. Chemical shifts and coupling constants for peptides 4a and 4b in CD$_2$Cl$_2$ and 2:1 CD$_2$Cl$_2$:DMSO-$d_6$$^b$

<table>
<thead>
<tr>
<th>Peptide$^a$</th>
<th>Solvent</th>
<th>D-Val-NH $\delta$ ($^3$J$_{\alpha H-NH}$)</th>
<th>Gly-NH $\delta$ ($^3$J$_{\alpha H-NH}$)</th>
<th>D-Leu-NH $\delta$ ($^3$J$_{\alpha H-NH}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro 4a</td>
<td>CD$_2$Cl$_2$</td>
<td>7.84 (7.8)</td>
<td>6.92 (5.7)</td>
<td>7.55 (8.3)</td>
</tr>
<tr>
<td>PyPro 4b</td>
<td></td>
<td>8.31 (8.1)</td>
<td>6.49 (5.4)</td>
<td>7.61 (7.9)</td>
</tr>
<tr>
<td>Pro 4a</td>
<td>2:1 CD$_2$Cl$_2$:DMSO-$d_6$</td>
<td>7.83 (8.0)</td>
<td>7.95 (5.8)</td>
<td>7.54 (8.4)</td>
</tr>
<tr>
<td>PyPro 4b</td>
<td></td>
<td>7.97 (8.5)</td>
<td>7.83 (5.8)</td>
<td>7.47 (8.3)</td>
</tr>
</tbody>
</table>

$^a$ conc. $\cong$ 10 mM.  $^b$ major isomer.

In the case of peptide 5 (Table 3), the chemical shift order in CD$_2$Cl$_2$ was Leu-NH downfield from Phe-NH downfield from Val-NH, indicative of a hydrogen-bound Leu amide proton and a Phe amide proton experiencing the influence of aromatic ring current. Similarly, downfield shifted Leu and Phe amide proton signals have been observed in the related cyclic peptide gramicidin S (GS) in DMSO-$d_6$ (Table 3).$^{14}$ In this case, as in the case of 5 in 2:1 CD$_2$Cl$_2$:DMSO-$d_6$, Phe-NH comes furthest downfield, followed by Leu-NH and Val-NH.

The $^3$J$_{\alpha H-NH}$ coupling constant pattern for 5 follows the same trend as for GS, with the amide protons for Leu and Val exhibiting larger values than for Phe, which in GS is due to the syn-periplanar geometry of the Phe $\alpha$H and NH protons. The larger $^3$J$_{\alpha H-NH}$ value for the Phe amide protons in 5a and 5b relative to GS is likely due to greater conformational flexibility of the linear peptide analogues.

Table 3. Comparative chemical shifts and coupling constants for the peptides 5a and 5b,$^b$ and for Gramcidin S$^c$

<table>
<thead>
<tr>
<th>Peptide$^a$</th>
<th>Solvent</th>
<th>Leu-NH $\delta$ ($^3$J$_{\alpha H-NH}$)</th>
<th>Phe-NH $\delta$ ($^3$J$_{\alpha H-NH}$)</th>
<th>Val-NH $\delta$ ($^3$J$_{\alpha H-NH}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro 5a</td>
<td>CD$_2$Cl$_2$</td>
<td>7.78 (7.8)</td>
<td>7.12 (5.9)</td>
<td>6.86 (7.1)</td>
</tr>
<tr>
<td>PyPro 5b</td>
<td></td>
<td>7.63 (8.5)</td>
<td>7.40 (7.2)</td>
<td>6.64 (8.9)</td>
</tr>
<tr>
<td>Pro 5a</td>
<td>2:1 CD$_2$Cl$_2$:DMSO-$d_6$</td>
<td>7.64 (7.9)</td>
<td>8.18 (6.2)</td>
<td>7.32 (9.0)</td>
</tr>
<tr>
<td>PyPro 5b</td>
<td></td>
<td>7.60 (7.8)</td>
<td>8.00 (7.4)</td>
<td>7.27 (8.4)</td>
</tr>
<tr>
<td>Gramicidin S$^c$</td>
<td>DMSO-$d_6$</td>
<td>8.32 (9.2)</td>
<td>9.05 (2.6)</td>
<td>7.21 (9.7)</td>
</tr>
</tbody>
</table>

$^a$ conc. $\cong$10 mM.  $^b$ of the major isomer.  $^c$ values from ref 12
Different Phe side-chain orientations in 5a and 5b are suggested by the chemical shift differences (Δδ) for their δ protons, which were respectively 1.05 ppm and 0.66 ppm. The phenylalanine aromatic ring current influences the upfield δ-proton greater in the Pro analog. Similar aromatic ring current effects have been suggested to upfield shift one of the proline δ-protons in a GS analogue. Further support for the greater proximity of the aromatic ring to the δ-protons in Pro peptide 5a was the apparent NOE between the aromatic protons and the upfield shifted δ-proton in 5a, which was not observed with 5b (Figure 3 and 5).

**Figure 5**: Selected 1H-1H NOESY cross peaks in CD$_2$Cl$_2$ as well as potential hydrogen bonds from DMSO titration curves for peptides 4a and 5a.
Figure 6: Expanded NOE spectrum of peptide 5a in CD$_2$Cl$_2$

Figure 7: Expanded NOE spectrum for peptide 5b in CD$_2$Cl$_2$
The NH-stretch region of the IR spectra of peptides 4 and 5 was analysed in CH$_2$Cl$_2$ at [1 mM]. As reported previously for the enantiomer of 4a, a characteristic hairpin IR profile was observed exhibiting a minor band at around 3410-3440 cm$^{-1}$ corresponding to the exposed NH-amide and a stronger band near 3320 cm$^{-1}$ due to the two hydrogen-bonded NHs. Similar characteristic bands were observed in the IR spectra of the other three peptides (Figure 8). PyPro peptides 4b and 5b exhibited an additional band near 3460 cm$^{-1}$ due to the pyrrole NH-stretch that shouldered the minor amide-NH. Comparisons of the IR data for the Pro and PyPro peptides demonstrate as well a conservation of conformation.

**Figure 8:** NH-stretch regions from IR spectra of 4 and 5 in CH$_2$Cl$_2$ at [1 mM]
Materials and Methods

**General.** Unless otherwise noted, all reactions were run under an argon atmosphere and distilled solvents were transferred by syringe. Dry dichloromethane and THF were taken from a Solvent Dispensing System (GlassContour™); DIEA was double distilled from ninhydrin and CaH₂. Final reaction mixture solutions were dried over Na₂SO₄. Chromatography was on 230-400 mesh silica gel; TLC on aluminum-backed silica plates. Mass spectral data, HRMS (EI & FAB), were obtained by the Regional Center for Mass Spectrometry at the Université de Montréal. Chemical shifts for NMR data are given in ppm (δ units) and coupling constants are given in hertz. Final peptides were characterized by HPLC using the following conditions: 40-80% CH₃CN/H₂O 0.1% TFA, over 20 min with a flow rate of 0.5 ml/min on a Prevail C₁₈ column (250x4.6mm).

**IR Spectroscopy.** Spectra were obtained on a Perkin Elmer FT-IR spectrometer. Samples were prepared in dried CH₂Cl₂, the spectrum of the pure solvent was subtracted prior to analysis. Peaks in the amide NH stretch region were baseline corrected and analysed without further manipulation.

**NMR Spectroscopy.** 1D and 2D spectra for aggregation studies and conformational analyses were performed on a Varian 600 MHz spectrometer and the data were processed with SpinWorks 2.3. Samples were prepared by serial dilution of a 50 mM stock solution of peptide in CD₂Cl₂ (dried over 3Å molecular sieves). Samples for NOESY (1 mM) were degassed using the Freeze-Pump-Thaw technique. NOESY spectra were recorded with a time domain size of 2048 by 512 points and 64 scan with a mixing time of 900 ms.

**Synthesis of the Proline Containing Peptides.** Prolyl peptides 4a and 5a were prepared by a solution phase strategy using \( O-(\text{benzotriazol-1-yl})-N,N,N',N'-\text{tetramethyluronium tetrafluoroborate (TBTU)}^\text{8} \) as coupling reagent with Boc-amino acids. Representative procedures are given below.

**General Procedure for Boc deprotections, HCl-Val-NMe₂.** A solution of Boc-Val-NMe₂ (500 mg, 2.05 mmol) in dioxane (10 ml) was treated for 2 h with HCl gas bubbles that were generated by slow addition of H₂SO₄(conc) onto NaCl. The mixture was evaporated and triturated with Et₂O (3 x 10 ml) to give HCl-Val-NMe₂ (300 mg, 1.66 mmol, 81%) as a hygroscopic white solid.
General Procedure for coupling reactions, Boc-Pro-Val-NMe₂. A solution of Boc-Pro-OH (100 mg, 0.46 mmol) in 6 mL of CH₂Cl₂ was treated with TBTU (177 mg, 0.55 mmol) and DIEA (82 µL, 0.47 mmol), stirred for 15 min, cooled to 0°C, treated with a solution of HCl-Val-NMe₂ (92 mg, 0.51 mmol) in CH₂Cl₂ (3 mL), followed by DIEA (160 µL, 0.92 mmol). After stirring for 10-12 h at room temperature, the reaction mixture was washed with water (5 mL), 0.1 N HCl (3 x 5 mL), saturated NaHCO₃ (3 x 5 mL) and brine, dried and concentrated to a residue, that was chromatographed eluting with 0-5 % MeOH in chloroform to give 145 mg (0.42 mmol, 92%) of the desired dipeptide as a white solid.

General Procedure for final peptide acetylation, Ac-Leu-D-Phe-Pro-Val-NMe₂ (5a). A solution of the tetrapeptide Boc-Leu-D-Phe-Pro-Val-NMe₂ (60 mg, 0.1 mmol) was deprotected with HCl bubbles as described above. The resulting HCl salt was dissolved in CH₂Cl₂ (4 mL), cooled to 0°C, treated with DIEA (52 µL, 0.3 mmol) and Ac₂O (15 µL, 0.16 mM) and stirred at room temperature for 3 h, then washed with 0.1 N HCl (2 x 4 mL), saturated NaHCO₃ (2 x 4 mL) and brine, dried and concentrated to a residue, that was chromatographed eluting with 0-5 % MeOH in chloroform to give 42 mg (0.073 mmol, 73%) of the desired tetrapeptide as a white solid.

Ac-D-Val-Pro-Gly-D-Leu-NMe₂ (4a): ¹H NMR (400 MHz, CD₂Cl₂, 10 mM) 0.90 (d, 3 H, J = 3.2), 0.92 (d, 3 H, J = 6.7), 0.99 (d, 3 H, J = 6.7), 1.41 (ddd, 1 H, J = 4.5, 8.9, 13.6), 1.64 (m, 1 H), 1.76 (m, 1 H), 1.94 (s, 3 H), 1.95-2.14 (m, 4 H), 2.20 (m, 1 H), 2.90 (s, 3 H), 3.14 (s, 3 H), 3.56 (dd, 1 H, J = 5.9, 17.3), 3.62 (m, 1 H), 3.96 (dd, 1 H, J = 7.2, 17.3), 4.1 (m, 1 H), 4.35 (m, 2 H), 5.32 (ddd, 1 H, J = 4.5, 8.6, 13.0), 6.92 (t, 1 H, J = 5.7), 7.55 (d, 1 H, J = 8.3), 7.84 (d, 1 H, J = 7.8); ¹³C NMR (100 MHz, CD₂Cl₂) δ 19.0, 19.3, 21.9, 22.7, 23.4, 24.9, 25.3, 8.3, 30.9, 36.0, 37.5, 41.1, 42.9, 47.4, 48.1, 57.6, 61.8, 169.0, 172.1, 172.5, 172.6, 173.2; HPLC RT: 12.26 min; MS: 454.2 (MH⁺).

Ac-Leu-D-Phe-Pro-Val-NMe₂ (5a): ¹H NMR (400 MHz, CD₂Cl₂, 10 mM) δ 0.88 (m, 12 H), 1.49 (m, 1 H), 1.49-1.65 (m, 5 H), 2.00 (s, 3 H), 2.04 (m, 1 H), 2.13 (m, 1 H), 2.67 (m, 1 H), 2.90 (dd, 1 H, J = 6.2, 12.8), 2.93 (s, 3 H), 2.97 (dd, 1 H, J = 9.7, 12.8), 3.18 (s, 3 H), 3.72 (m, 1 H), 4.33 (d, 1 H, J = 6.4), 4.51 (q, 1 H, J = 6.8), 4.56-4.62 (m, 2 H), 6.86 (d, 1 H, J = 7.1), 7.12 (d, 1 H, J = 5.9), 7.19-7.30 (m, 5 H), 7.78 (d, 1 H, J = 7.8); ¹³C NMR (100 MHz, CD₂Cl₂) δ 18.6, 19.2, 22.4, 22.9, 23.1, 24.3, 25.0, 29.7, 31.0, 36.0, 37.5, 37.9, 39.5, 47.0,
50.6, 53.8, 54.3, 60.9, 127.3, 128.8, 129.7, 136.8, 171.1, 171.3, 171.7, 172.3, 173.8. HPLC RT: 19.17 min; MS: 544.2 (MH+).

**Fmoc-PyPro-Gly-d-Leu-NMe₂ (9).** Fmoc-PyPro-OH (3, prepared as described in Ref 6, 87 mg, 0.22 mmol) was coupled to HCl-Gly-d-Leu-NMe₂ (68 mg, 0.27 mmol) using the general coupling procedure described above to give 86 mg (0.147 mmol, 67%) of the desired tripeptide.

**Fmoc-PyPro-Val-NMe₂ (11).** Fmoc-PyPro-OH (3, 80 mg, 0.20 mmol) was coupled to HCl-Val-NMe₂ (40 mg, 0.22 mmol) using the general coupling procedure described above to give 72 mg (0.14 mmol, 70%) of the desired dipeptide.

**General Procedure for the Fmoc Deprotection. PyPro-Gly-d-Leu-NMe₂ (10).** At room temperature, a stirred solution of Fmoc-PyPro-Gly-d-Leu-NMe₂ (9, 200 mg, 0.34 mmol) and octanethiol (590 µL, 3.4 mmol, 1000 mol%) in THF (4 mL) was treated with a 1% (v/v) solution of DBU in THF (225 µL, 5 mol%), stirred for 2 h and evaporated to a residue, that was triturated with Et₂O (3 x 10 mL) and chromatographed eluting with 0-5% MeOH in chloroform containing 1% of Et₃N to give PyPro-Gly-d-Leu-NMe₂ (10, 105 mg, 85%): ¹H NMR (400 MHz, pyridine-d⁵) δ 0.79 (d, 3 H, J = 6.4), 0.94 (d, 3 H, J = 6.4), 1.53 (m, 1 H), 1.65 (m, 1 H), 1.76 (m, 1 H), 2.27 (s, 3 H), 2.89 (s, 3 H), 2.97 (s, 3 H), 4.20 (s, 2 H), 4.35 (dd, 1 H, J = 16.9, 4.5), 5.53 (dd, 1 H, J = 16.9, 5.4), 5.17 (s, 1 H), 5.31 (m, 1 H), 6.29 (s, 1 H), 9.08 (d, 1 H, J = 8.3), 9.19 (s, 1 H), 11.2 (s, 1 H); ¹³C NMR (100 MHz, pyridine-d⁵) δ 14.3, 22.4, 23.8, 25.4, 35.9, 37.1, 42.4, 43.4, 46.7, 48.1, 63.1, 101.7, 125.5, 132.5, 133.5, 169.9, 172.8, 175.1; HRMS calcd for C₁₈H₂₉N₅O₃ (M⁺) 363.2270, found 363.2255.

**PyPro-Val-NMe₂ (12).** Fmoc-PyPro-Val-NMe₂ (11, 79 mg, 0.15 mmol) was deprotected using the general Fmoc deprotection procedure described above to give PyPro-Val-NMe₂ (12, 39 mg, 89%): ¹H NMR (300 MHz, CDCl₃) δ 0.80 (d, 3 H, J = 6.7), 0.88 (d, 3 H, J = 6.7), 1.99 (m, 1 H), 2.23 (s, 3 H), 2.96, (s, 3 H), 3.13 (s, 3 H), 4.13 (s, 2 H), 4.71 (1 H, dd, J = 6.9, 9.2), 4.81 (m, 1 H), 5.84 (s, 1 H), 7.99 (d, 1 H, J = 9.2), 8.16 (s, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 13.8, 17.8, 19.6, 31.7, 35.8, 37.6, 46.0, 53.4, 61.9, 100.8, 124.0, 131.1, 132.8, 172.0, 172.7; HRMS calcd for C₁₅H₂₄N₄O₂ (M⁺) 292.1899, found 292.1900.

**Ac-d-Val-PyPro-Gly-d-Leu-NMe₂ (4b):** At 0°C, a solution of PyPro-Gly-d-Leu-NMe₂ (10, 100 mg, 0.275 mmol) and Ac-d-Val-OH (50 mg, 0.3 mmol) in 10 mL of CH₂Cl₂ was treated with TBTU (106 mg, 0.33
mmol) and DIEA (105 µL, 0.6 mmol). After stirring for 12 h, the mixture was washed with water (8 mL), 0.1 N HCl (3 x 8 mL), saturated NaHCO₃ (3 x 8 mL) and brine, dried and concentrated to a residue that was purified by HPLC (20-80 % CH₃CN in water, 0.01 % TFA, C₁₈ column) to provide Ac-D-Val-PyPro-Gly-D-Leu-NMe₂ (5a, 81 mg, 54 %): ¹H NMR (400 MHz, CD₂Cl₂, ≅10 mM) δ 0.92 (d, 3 H, J = 6.8), 0.94 (d, 3 H, J = 6.8), 0.98 (d, 3 H, J = 6.7), 1.02 (d, 3 H, J = 6.7), 1.43 (ddd, 1 H, J = 4.3, 9.0, 13.5), 1.67 (m, 1 H), 1.84 (ddd, 1 H, J = 4.5, 10.5, 14.2), 1.98 (s, 3 H), 2.15 (m, 1 H), 2.25 (s, 3 H), 2.92 (s, 3 H), 3.16 (s, 3 H), 3.47 (dd, 1 H, J = 5.5, 17.4), 4.11 (dd, 1 H, J = 7.4, 17.4), 4.43 (t, 1 H, J = 9.1), 4.77 (d, 1 H, J = 11.6), 4.95 (m, 1 H), 5.25 (dd, 1 H, J = 2.6, 11.6), 5.31 (mask by CD₂Cl₂, 1H), 5.76 (s, 1 H), 6.49 (t, 1 H, J = 5.4), 7.61 (d, 1 H, J = 7.9), 8.27 (s, 1 H), 8.31 (d, 1 H, J = 8.1); ¹³C NMR (100 MHz, CD₂Cl₂) δ 13.6, 18.8, 19.3, 21.7, 22.4, 23.3, 24.9, 30.7, 36.0, 37.5, 40.8, 42.8, 47.6, 48.2, 57.5, 63.9, 100.2, 119.9, 127.7, 133.4, 169.4, 171.2, 172.6, 173.22, 173.27. HPLC RT: 12.66 min; MS: 505.2 (MH⁺).

Ac-Leu-d-Phe-PyPro-Val-NMe₂ (5b): At 0°C, a solution of PyPro-Val-NMe₂ (12, 49 mg, 0.168 mmol) and Ac-Leu-d-Phe-OH (54 mg, 0.168 mmol) in 4.2 mL of CH₂Cl₂ was treated with TBTU (59 mg, 0.18 mmol) and DIEA (88 µL, 0.5 mmol). After stirring for 12 h, the mixture was washed with water (4 mL), 0.1 N HCl (3 x 4 mL), saturated NaHCO₃ (3 x 4 mL) and brine, dried and concentrated to a residue that was purified by HPLC (20-80 % CH₃CN in water, 0.01 % TFA, C₁₈ column) to provide Ac-Leu-d-Phe-PyPro-Val-NMe₂ (5b, 42 mg, 42 %): ¹H NMR (400 MHz, CD₂Cl₂, ≅10 mM) δ 0.67 (d, 3 H, J = 6.7), 0.77 (d, 3 H, J = 6.7), 0.85 (d, 3 H, J = 6.1), 0.88 (d, 3 H, J = 6.1), 1.50 (m, 1 H), 1.57 (m, 2 H), 1.98 (m, 1 H), 2.00 (s, 3 H), 2.21 (s, 3 H), 2.91 (dd, 1 H, J = 6.5, 13.4), 2.93 (s, 3 H), 3.10 (ddd, 1 H, J = 8.7, 13.4), 3.17 (s, 3 H), 4.19 (dd, 1 H, J = 11.7), 4.53 (m, 2 H), 4.74 (q, 1 H, J = 7.6), 4.84 (ddd, 1 H, J = 3.1, 11.7), 5.14 (dd, 1 H, J = 3.1), 5.72 (s, 1 H), 6.64 (d, 1 H, J = 8.9), 7.19-7.25 (m, 5 H), 7.40 (d, 1 H, J = 7.2), 7.63 (d, 1 H, J = 8.5), 7.93 (s, 1 H); ¹³C NMR (100 MHz, CD₂Cl₂) δ 13.6, 18.0, 19.1, 22.2, 22.99, 23.02, 25.0, 31.2, 36.1, 37.5, 37.9, 40.1, 47.5, 51.3, 53.2, 54.0, 63.2, 100.5, 121.2, 126.9, 127.2, 128.8, 129.7, 132.7, 137.0, 170.76, 170.83, 171.8, 172.5, 173.6. HRMS calcd for C₃₂H₄₆N₆O₅ (MH⁺) 595.3608, found 595.3602. HPLC RT: 15.54 min.
References:


(10) We thank Silicycle Inc. for providing a sample of piperazine functionalized silica gel.


