Supporting Information

Dipeptide self-assembly into water-channels and gel biomaterial

Ottavia Bellotto, a Giovanni Pierri, b Petr Rozhin, a Maurizio Polentarutti, c Slavko Kralj, d,e Paola D’Andrea, f Consiglia Tedesco, b and Silvia Marchesan a

* ctedesco@unisa.it, smarchesan@units.it

a University of Trieste, Chem. Pharm. Sc. Dept., Via L. Giorgieri 1, 34127 Trieste, Italy.
b University of Salerno, Chemistry and Biology Dept. “A. Zambelli”, Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy.
c Elettra-Sincrotrone Trieste, S.S. 114 km 163.5, Basovizza, 34149 Trieste, Italy
d Jožef Stefan Institute, Materials Synthesis Dept., Jamova 39, 1000 Ljubljana, Slovenia
e University of Ljubljana, Pharmaceutical Technology Dept., Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia
f University of Trieste, Life Sciences Dept., Via L. Giorgieri 5, 34127 Trieste, Italy

Table of Contents

S1. D-Phe-L-Val spectroscopic data.......................................................... 2
S2. D-Val-L-Phe spectroscopic data......................................................... 5
S3. HPLC traces.......................................................................................... 8
S4. CD spectra........................................................................................... 8
S5. Thioflavin T fluorescence assay........................................................... 9
S6. Single-crystal XRD data......................................................................... 10
S7. Oscillatory rheometry data................................................................. 14
S8. Thermoreversibility test......................................................................... 14
S1. d-Phe-L-Val spectroscopic data

\[ \text{H NMR (400 MHz, DMSO-d}_6, \text{TMS)} \delta \text{ (ppm): 8.59 (d, } J = 8.6 \text{ Hz, 1H), 7.36 – 7.23 (m, 5H), 4.23 – 4.13 (m, 2H), 3.05 (dd, } J = 13.8, 6.7 \text{ Hz, 1H), 2.97 (dd, } J = 13.8, 7.9 \text{ Hz, 1H), 2.01 – 1.88 (m, 1H), 0.75 (d, } J = 6.8 \text{ Hz, 3H), 0.73 (d, } J = 6.8 \text{ Hz, 3H). C NMR (100 MHz, DMSO-d}_6, \text{TMS)} \delta \text{ (ppm): 172.4, 168.3 (2 x CO); 134.9, 129.5, 128.5, 127.1 (Ar); 57.3, 53.2 (2 x } \alpha \text{C); 37.6, 30.1 (2 x } \beta \text{C); 18.8, 17.7 (2 x } \gamma \text{C). MS (ESI): m/z 265.1 (M+H\textsuperscript{+}), 287.1 (M+Na\textsuperscript{+}), 263 (M-H\textsuperscript{-}).} \]

Fig. S1. \(^1\text{H-NMR spectrum of d-Phe-L-Val.}\)
Fig. S2. gCOSY 2D-NMR spectrum of d-Phe-L-Val.

Fig. S3. $^{13}$C-NMR spectrum of d-Phe-L-Val.
Fig. S4. ESI-MS spectrum of D-Phe-L-Val (positive ion mode).

Fig. S5. ESI-MS spectrum of D-Phe-L-Val (negative ion mode).
S2. d-Val-L-Phe spectroscopic data

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{O} \\
\text{H} & \quad \text{N} \\
\text{O} & \quad \text{COH}
\end{align*}
\]

\[\text{\textbf{H NMR}}\ (400\ MHz,\ \text{DMSO-d}_6,\ \text{TMS})\ \delta\ (ppm):\ 8.69\ (d,\ J = 8.4\ Hz,\ 1H),\ 7.31 - 7.15\ (m,\ 5H),\ 4.59\ (ddd,\ J = 10.3,\ 8.4,\ 4.4\ Hz,\ 1H),\ 3.57\ (d,\ J = 4.6\ Hz,\ 1H),\ 3.16\ (dd,\ J = 13.9,\ 4.4\ Hz,\ 1H),\ 2.83\ (dd,\ J = 13.9,\ 10.4\ Hz,\ 1H),\ 1.94 - 1.80\ (m,\ 1H),\ 0.72\ (d,\ J = 6.9\ Hz,\ 3H),\ 0.51\ (d,\ J = 7.0\ Hz,\ 3H).\ \text{\textbf{C NMR}}\ (100\ MHz,\ \text{DMSO-d}_6,\ \text{TMS})\ \delta\ (ppm):\ 172.6,\ 167.9\ (2\ \times\ CO);\ 137.3,\ 129.1,\ 128.2,\ 126.5\ (\text{Ar});\ 57.3,\ 53.5\ (2\ \times\ \alpha\text{C});\ 37.1,\ 29.7\ (2\ \times\ \beta\text{C});\ 18.3,\ 16.6\ (2\ \times\ \gamma\text{C}).\ \text{\textbf{MS (ESI)}}:\ m/z\ 265.1\ (\text{M+H})^+,\ 287.1\ (\text{M+Na})^+,\ 263\ (\text{M-H})^-.
\]

Fig. S6. \textbf{H}-NMR spectrum of d-Val-L-Phe.
Fig. S7. gCOSY 2D-NMR spectrum of d-Val-L-Phe.

Fig. S8. $^{13}$C-NMR spectrum of d-Val-L-Phe.
**Fig. S9.** ESI-MS spectrum of d-Val-L-Phe (positive ion mode).

**Fig. S10.** ESI-MS spectrum of d-Val-L-Phe (negative ion mode).
S3. HPLC traces

![HPLC traces](image)

**Fig. S11.** HPLC traces of D-Phe-L-Val (left) and D-Val-L-Phe (right) in water/MeCN gradient system from 5% of MeCN with 0.1% formic acid over 17 min (C-18 Luna Column, 5 µm, 100 Å, 150 x 2 mm, Phenomenex).

S4. CD spectra

![CD spectra](image)

**Fig. S12.** CD spectra (top) and corresponding HT (bottom) of D-Phe-L-Val (left) and D-Val-L-Phe (right) in solution at 1 mM.
S5. Thioflavin T fluorescence assay

**Fig. S13.** Thioflavin T fluorescence assay for D-Phe-L-Val reveals lack of fluorescence with a signal intensity (I) that is not significantly different relative to the control without peptide (I₀) across all concentrations tested, both in solution and gel phase (mgc = 40 mM).

**Fig. S14.** Thioflavin T fluorescence assay for D-Val-L-Phe reveals lack of fluorescence with a signal intensity (I) that is not significantly different relative to the control without peptide (I₀) across all concentrations tested.
S6. Single-crystal XRD data

D-Phe-L-Val (CCDC 2160978) and D-Val-L-Phe (CCDC 2160976).

Crystals of D-Phe-L-Val (CCDC 2160978) and D-Val-L-Phe (CCDC 2160976) were mounted on the diffractometer at the synchrotron Elettra, Trieste (Italy), beamline XRD1 and measured at 100 K. Data collection were performed using synchrotron radiation ($\lambda = 0.7000$ Å) with the rotating crystal method (0.5°/image) for a total of 720 images. Data indexing were performed using MOSFLM, while space groups were determined using POINTLESS. The software AIMLESS was used for scaling the data. The structures were solved using the software SHELXT and refined through full matrix least-squares based on $\mathbf{F}^2$ using the program SHELXL and OLEX2 as a GUI. For both crystal structures, non-hydrogen atoms were refined anisotropically, hydrogen atoms were positioned geometrically and included in structure factors calculations but not refined. In the case of D-Val-L-Phe crystal structure, it was possible to localise and refine the water molecules’ hydrogen atoms. On the contrary, for the crystal structure of D-Phe-L-Val, it was not possible to do that or add them automatically. ORTEP diagrams (Figure S15) were drawn using OLEX2. Crystallographic data and refinement details are reported in Table S1.
**Figure S15.** ORTEP diagrams of (a) D-Phe-L-Val (CCDC 2160978) and (b) D-Val-L-Phe (CCDC 2160976). Atom types: C grey, H white, O red, N blue. Ellipsoids are drawn at 20% probability level. Water molecules were omitted for clarity.

**Figure S16.** (a) The figure shows the water channels in the crystal structure of D-Phe-L-Val (shown in blue) surrounded by four dipeptides arranged head-to-tail. (b) Anphipathic layers in crystal structure of D-Val-L-Phe (hydrophilic regions with solvent molecules are shown in light blue and hydrophobic areas in pink). Atom types: C grey, H white, O red, N blue. Water molecules are depicted in ball-and-stick style.
**Figure S17.** Typical hydrogen bond pattern of Phe-Phe dipeptide group. Only the interacting water molecule O3W is reported in ball-and-stick style.

**Table S1.** Crystallographic data and refinement details for the crystal structures of D-Phe-L-Val (CCDC 2160978) and D-Val-L-Phe (CCDC 2160976).

<table>
<thead>
<tr>
<th></th>
<th>D-Phe-L-Val</th>
<th>D-Val-L-Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T (K)</strong></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Formula</strong></td>
<td>4(C_{14}H_{20}N_{2}O_{3}), 4(H_{2}O)</td>
<td>2(C_{14}H_{20}N_{2}O_{3}), 5(H_{2}O)</td>
</tr>
<tr>
<td><strong>Formula weight</strong></td>
<td>1129.34</td>
<td>618.72</td>
</tr>
<tr>
<td><strong>System</strong></td>
<td>tetragonal</td>
<td>monoclinic</td>
</tr>
<tr>
<td><strong>Space group</strong></td>
<td>4</td>
<td>C2</td>
</tr>
<tr>
<td><strong>a (Å)</strong></td>
<td>22.947(3)</td>
<td>19.615(4)</td>
</tr>
<tr>
<td><strong>b (Å)</strong></td>
<td>22.947(3)</td>
<td>6.7520(14)</td>
</tr>
<tr>
<td><strong>c (Å)</strong></td>
<td>5.6120(11)</td>
<td>13.402(3)</td>
</tr>
<tr>
<td><strong>α (°)</strong></td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td><strong>β (°)</strong></td>
<td>90</td>
<td>114.86(3)</td>
</tr>
<tr>
<td><strong>γ (°)</strong></td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td><strong>V (Å³)</strong></td>
<td>2955.1(10)</td>
<td>1610.5(7)</td>
</tr>
<tr>
<td><strong>Z</strong></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Dæ (g cm⁻³)</strong></td>
<td>1.260</td>
<td>1.276</td>
</tr>
<tr>
<td><strong>λ (Å)</strong></td>
<td>0.70000</td>
<td>0.70000</td>
</tr>
<tr>
<td><strong>μ (mm⁻¹)</strong></td>
<td>0.090</td>
<td>0.095</td>
</tr>
<tr>
<td><strong>F_{000}</strong></td>
<td>1200.0</td>
<td>668.0</td>
</tr>
<tr>
<td><strong>R1 (I &gt; 2σI)</strong></td>
<td>0.0661(2954)</td>
<td>0.0262(4354)</td>
</tr>
<tr>
<td><strong>wR2</strong></td>
<td>0.1921(4314)</td>
<td>0.0714(4372)</td>
</tr>
<tr>
<td><strong>N. of param.</strong></td>
<td>140</td>
<td>219</td>
</tr>
<tr>
<td><strong>GooF</strong></td>
<td>1.043</td>
<td>1.065</td>
</tr>
<tr>
<td><strong>ρ_{min}, ρ_{max} (eÅ⁻³)</strong></td>
<td>-0.37, 0.53</td>
<td>-0.27, 0.23</td>
</tr>
<tr>
<td><strong>Restraints</strong></td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>
S7. Oscillatory rheometry data

Fig. S18. Frequency sweep for D-Phe-L-Val at 40 mM.

Fig. S19. Time sweep for D-Val-L-Phe at 40 mM.

S8. Thermoreversibility test

<table>
<thead>
<tr>
<th>Sequence</th>
<th>MGC</th>
<th>RT</th>
<th>55-60°C</th>
<th>60°C</th>
<th>RT after 1h</th>
<th>RT after O/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Phe-L-Val</td>
<td>40 mM</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Fig. S20. Thermoreversibility test for D-Phe-L-Val hydrogel