Supporting Information

Dipeptide self-assembly into water-channels and gel biomaterial

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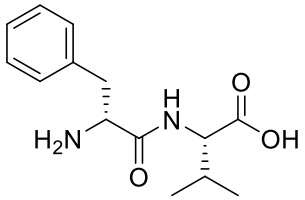
S1. D-Phe-L-Val spectroscopic data



^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ (ppm): 8.59 (d, *J* = 8.6 Hz, 1H), 7.36 – 7.23 (m, 5H), 4.23 – 4.13 (m, 2H), 3.05 (dd, *J* = 13.8, 6.7 Hz, 1H), 2.97 (dd, *J* = 13.8, 7.9 Hz, 1H), 2.01 – 1.88 (m, 1H), 0.75 (d, *J* = 6.8 Hz, 3H), 0.73 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (100 MHz, DMSO-d_6, TMS) δ (ppm): 172.4, 168.3 (2 x CO); 134.9, 129.5, 128.5, 127.1 (Ar); 57.3, 53.2 (2 x αC); 37.6, 30.1 (2 x βC); 18.8, 17.7 (2 x γC). **MS (ESI):** m/z 265.1 $(M+H)^+$, 287.1 $(M+Na)^+$, 263 $(M-H)^-$.

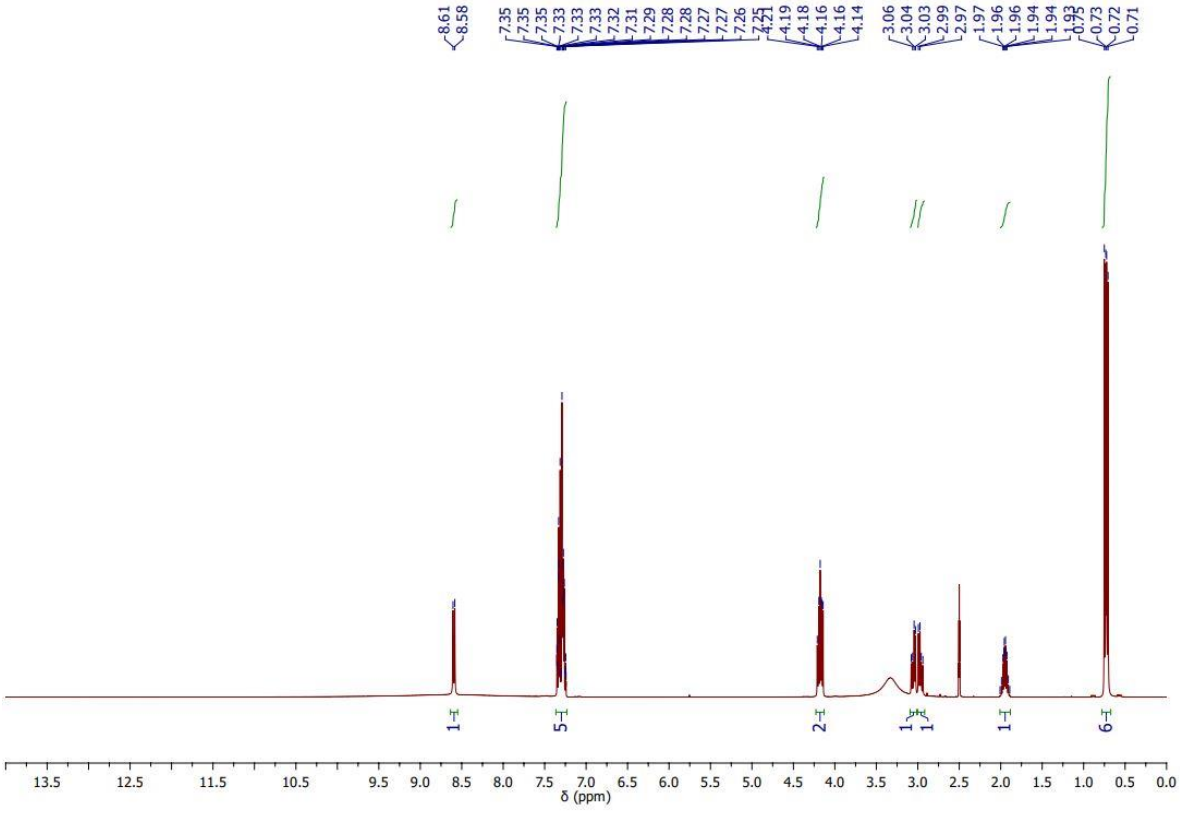


Fig. S1. ^{1}H-NMR spectrum of D-Phe-L-Val.

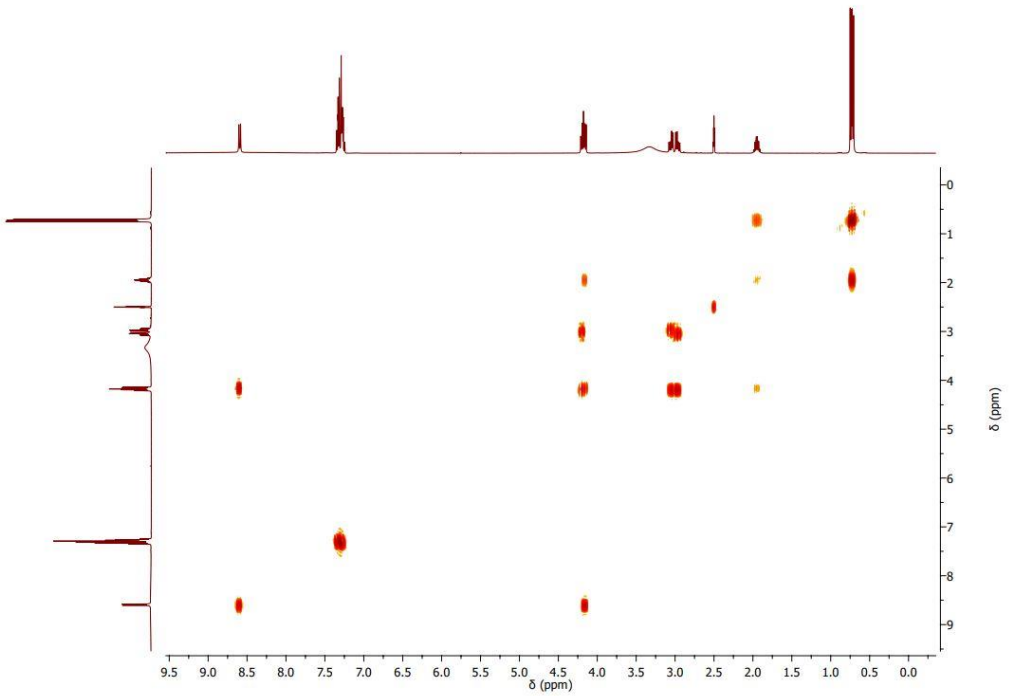


Fig. S2. gCOSY 2D-NMR spectrum of D-Phe-L-Val.

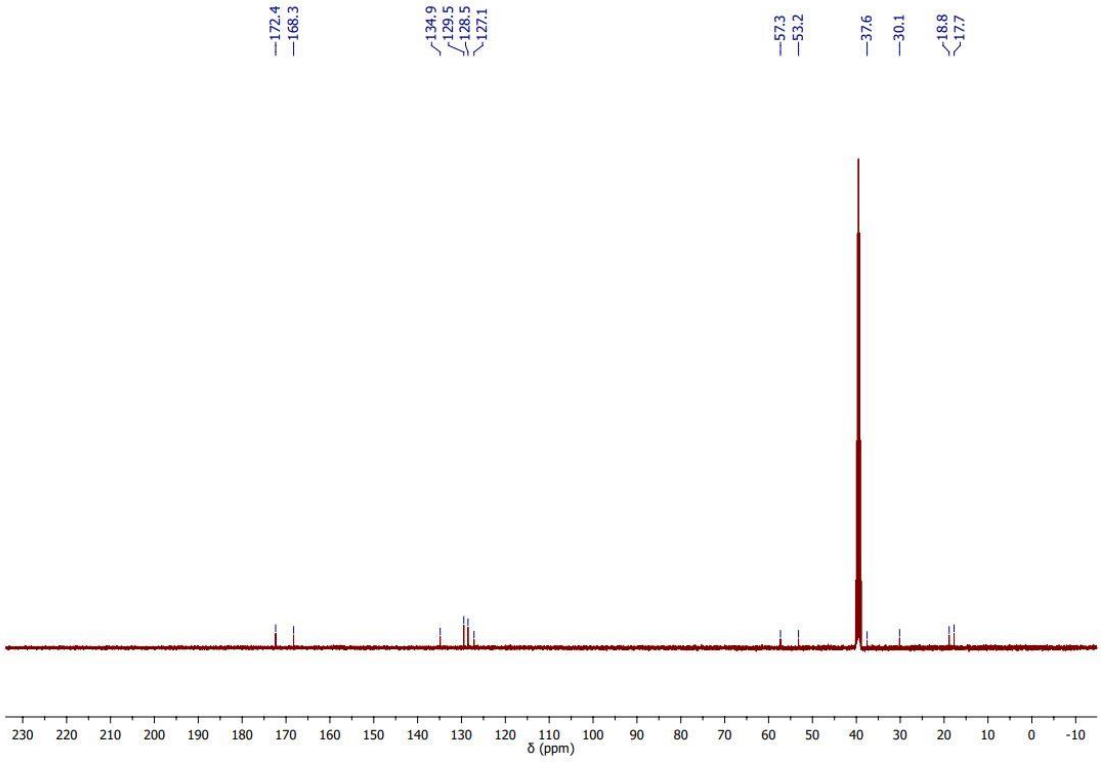


Fig. S3. ^{13}C-NMR spectrum of D-Phe-L-Val.

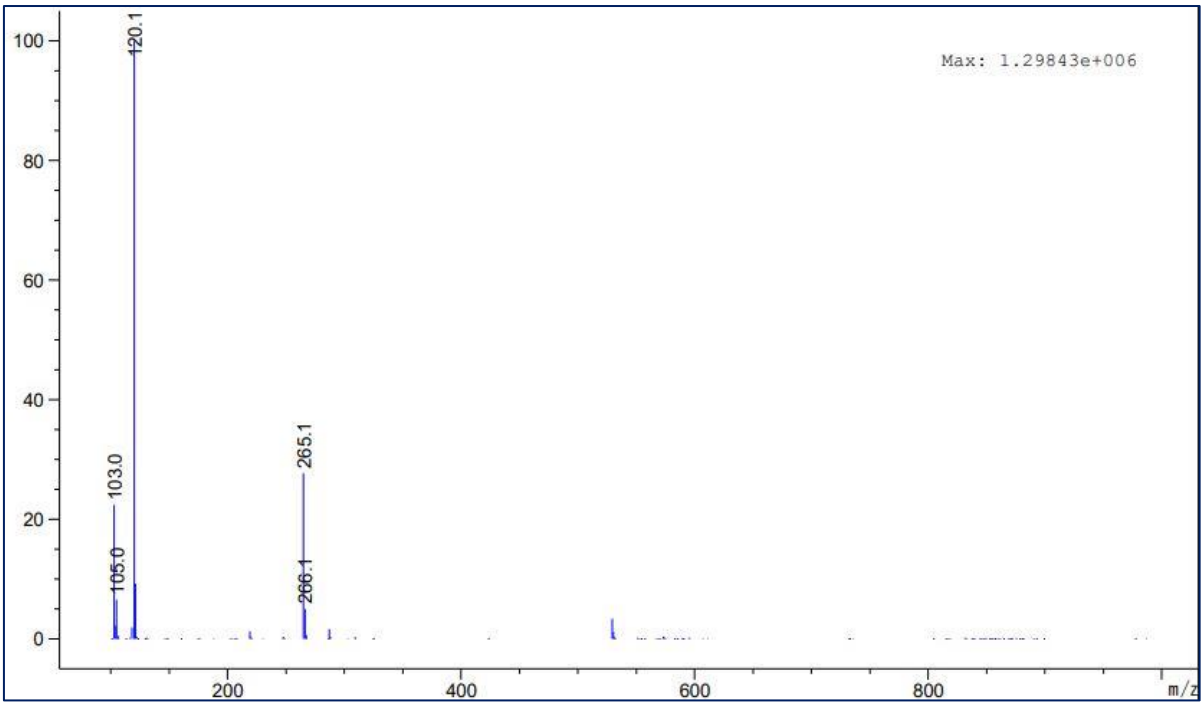


Fig. S4. ESI-MS spectrum of D-Phe-L-Val (positive ion mode).

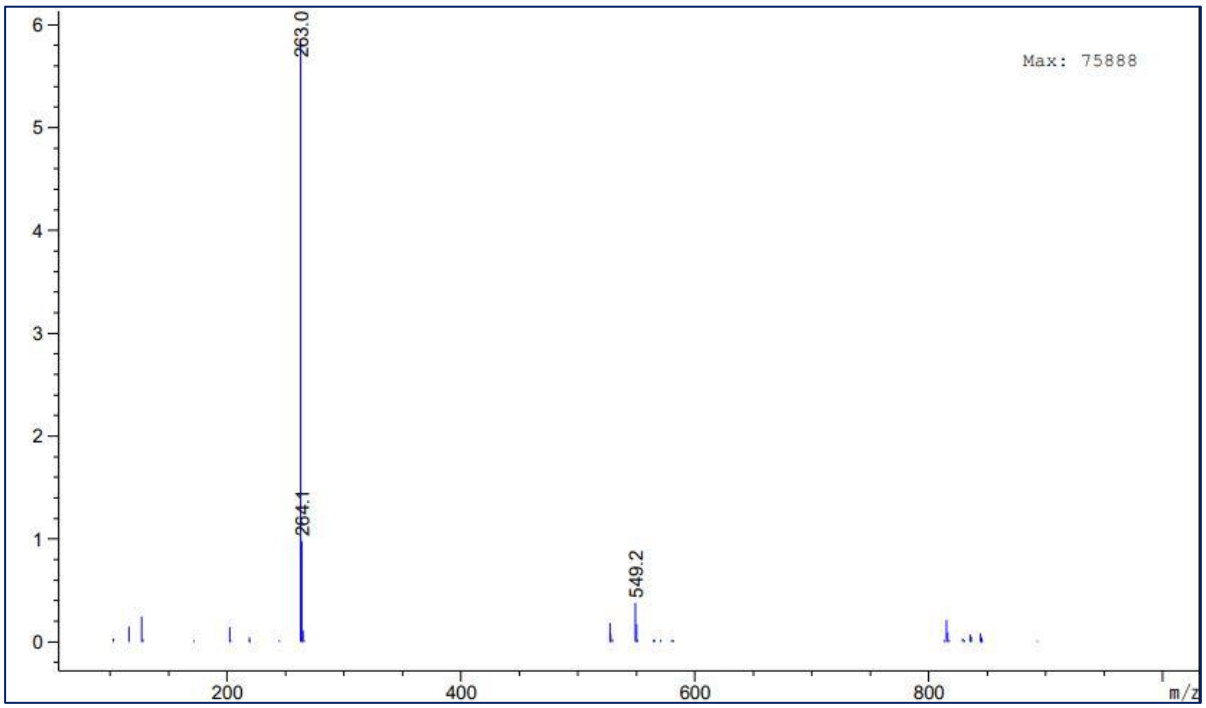
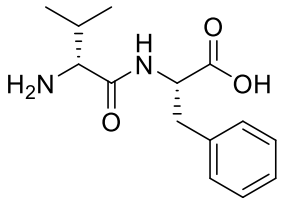


Fig. S5. ESI-MS spectrum of D-Phe-L-Val (negative ion mode).

S2. D-Val-L-Phe spectroscopic data



^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ (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). **^{13}C NMR** (100 MHz, DMSO-d_6, TMS) δ (ppm): 172.6, 167.9 (2 x CO); 137.3, 129.1, 128.2, 126.5 (Ar); 57.3, 53.5 (2 x αC); 37.1, 29.7 (2 x βC); 18.3, 16.6 (2 x γC). **MS (ESI):** m/z 265.1 $(M+H)^+$, 287.1 $(M+Na)^+$, 263 $(M-H)^-$.

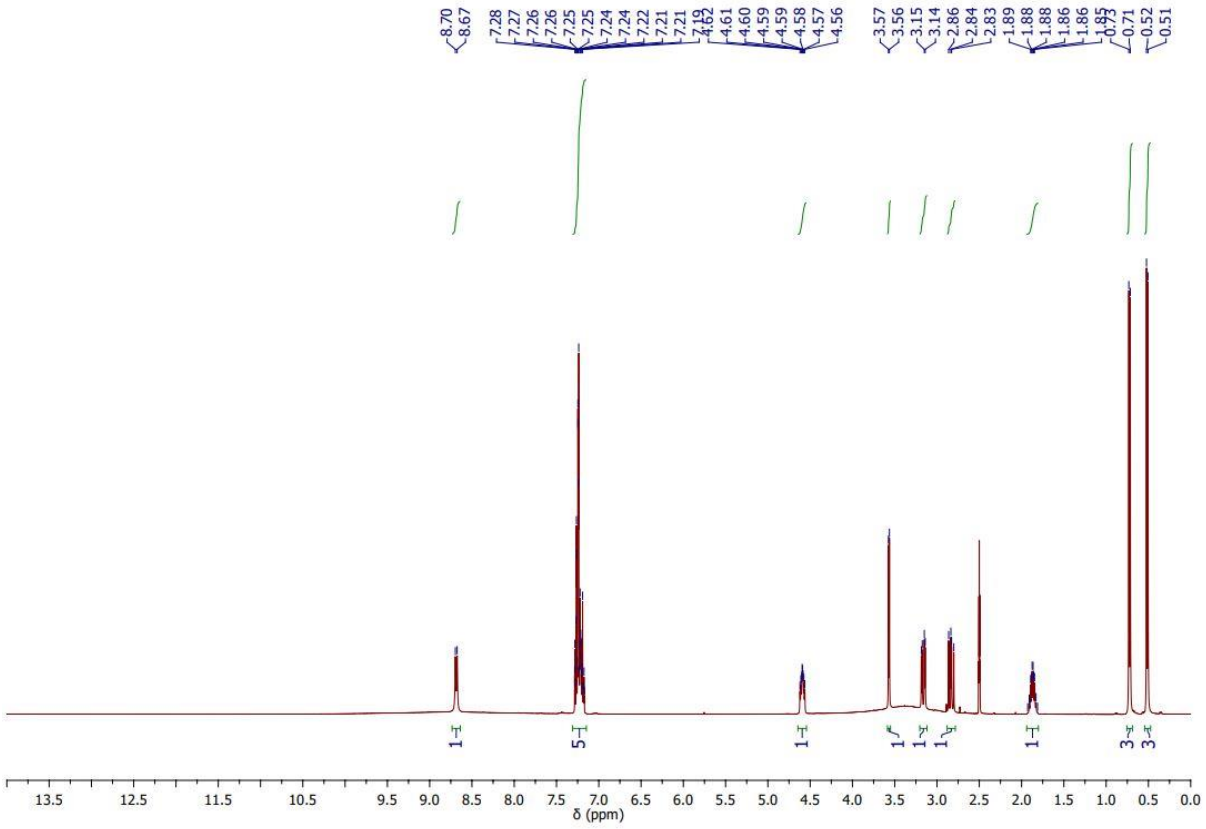


Fig. S6. ^{1}H-NMR spectrum of D-Val-L-Phe.

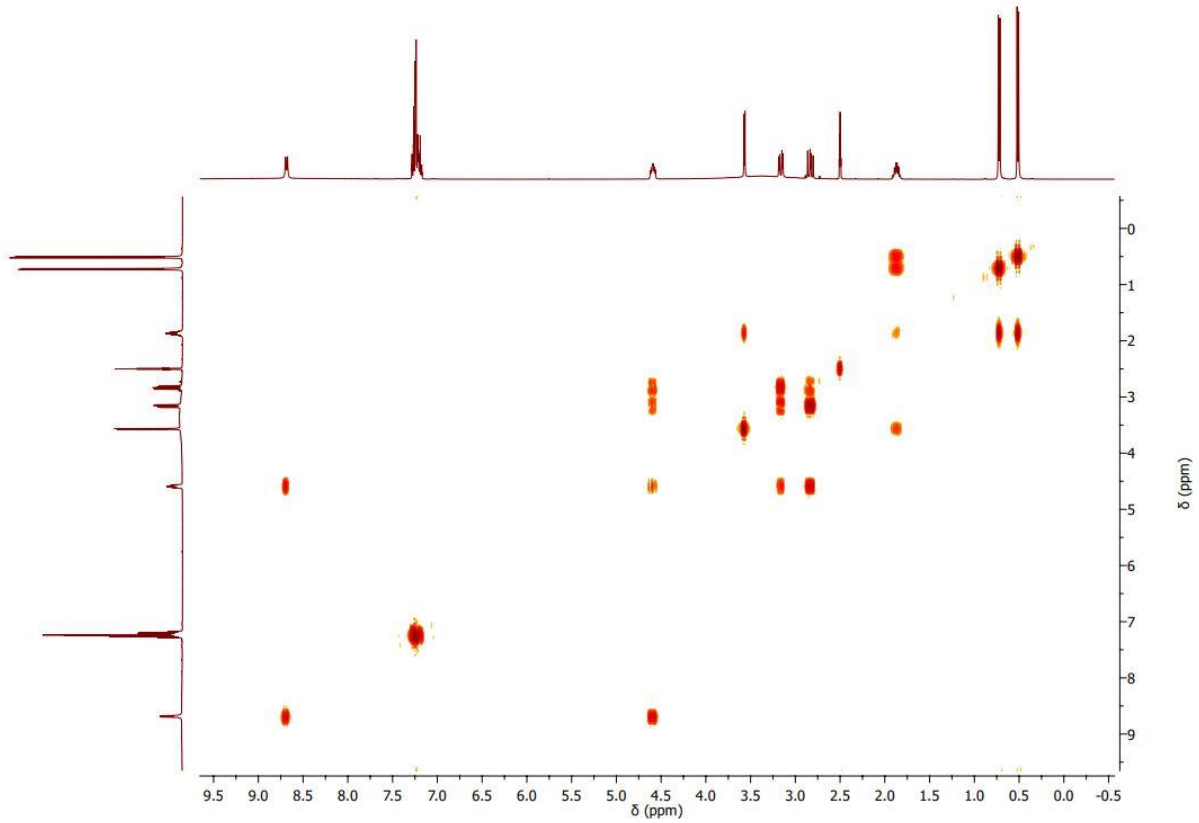


Fig. S7. gCOSY 2D-NMR spectrum of D-Val-L-Phe.

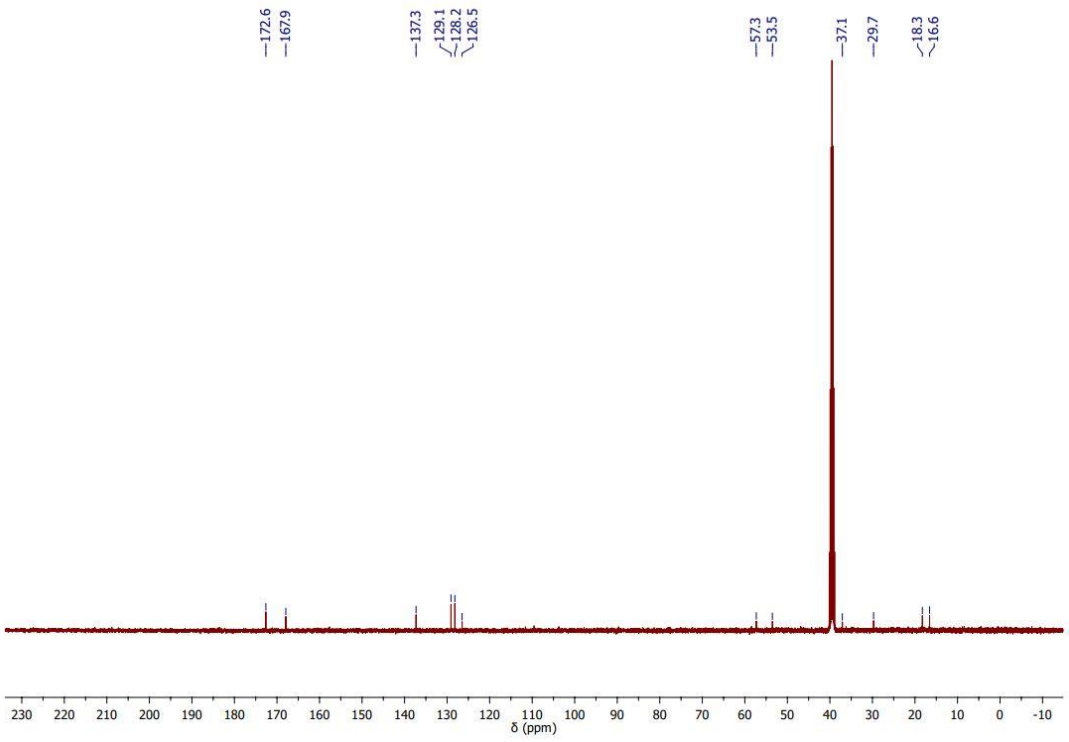


Fig. S8. ^{13}C-NMR spectrum of D-Val-L-Phe.

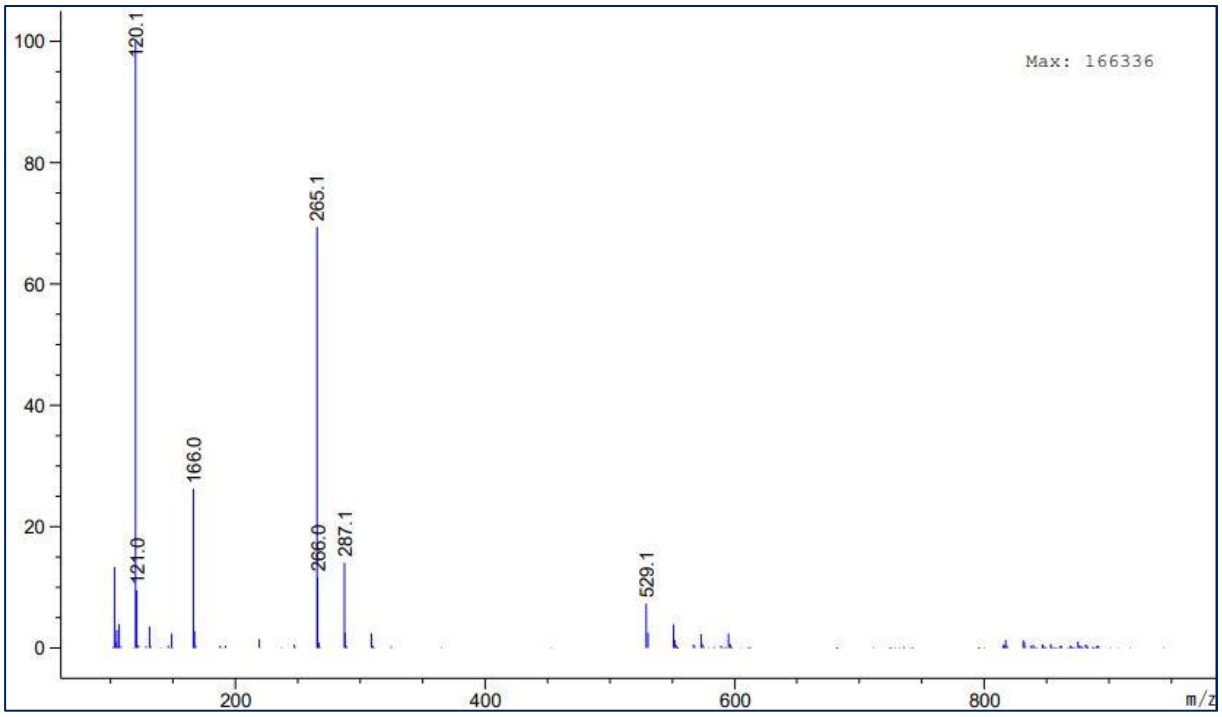


Fig. S9. ESI-MS spectrum of D-Val-L-Phe (positive ion mode).

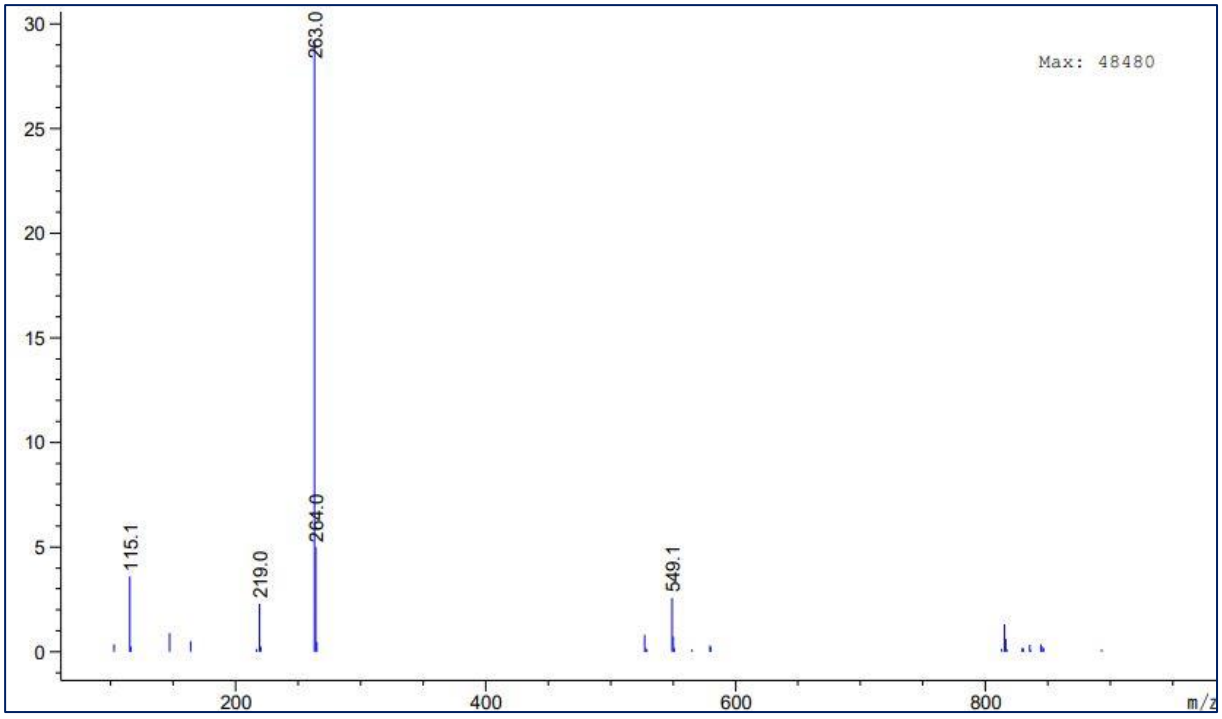


Fig. S10. ESI-MS spectrum of D-Val-L-Phe (negative ion mode).

S3. HPLC traces

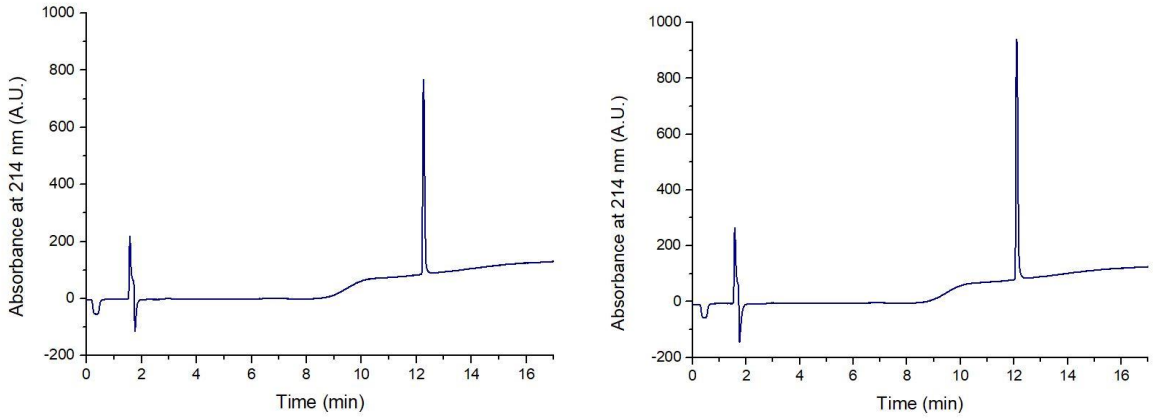


Fig. S11. HPLC traces of D-Phe-L-Val (left) and D-Val-L-Phe (right) in water/MeCN gradient system from 5% of MeCN with 0.1% formic acid over 17 min (C-18 Luna Column, 5 µm, 100 Å, 150 x 2 mm, Phenomenex).

S4. CD spectra

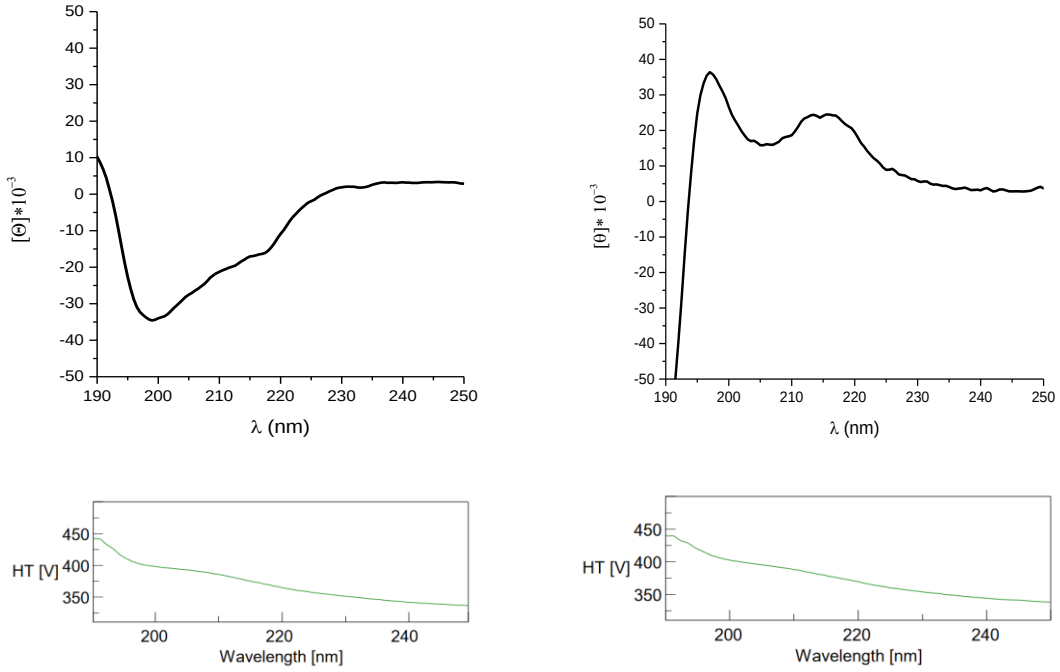


Fig. S12. CD spectra (top) and corresponding HT (bottom) of D-Phe-L-Val (left) and D-Val-L-Phe (right) in solution at 1 mM.

S5. Thioflavin T fluorescence assay

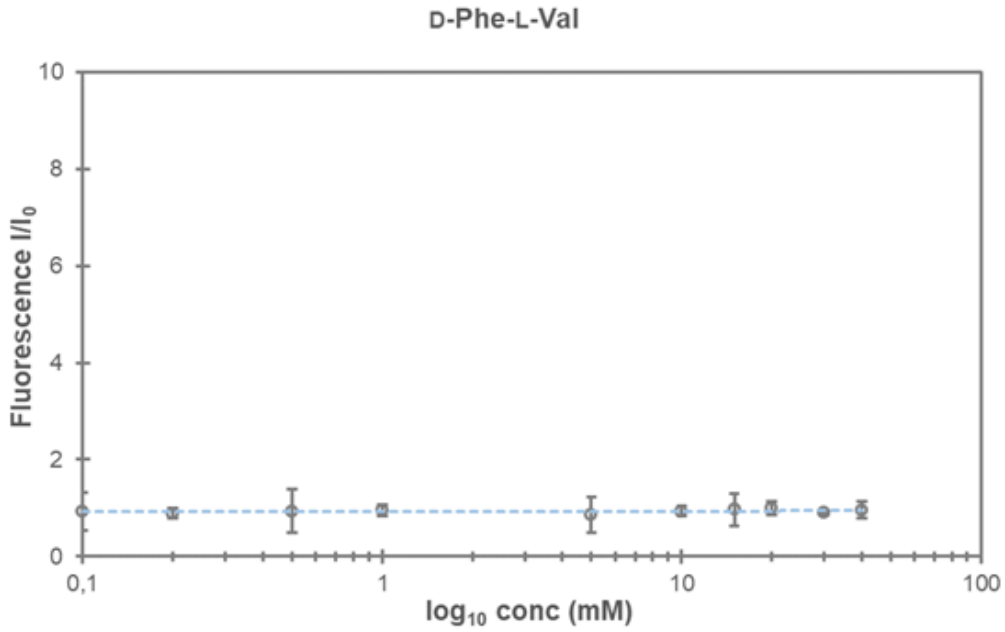


Fig. S13. Thioflavin T fluorescence assay for D-Phe-L-Val reveals lack of fluorescence with a signal intensity (I) that is not significant different relative to the control without peptide (I_0) across all concentrations tested, both in solution and gel phase (mgc = 40 mM).

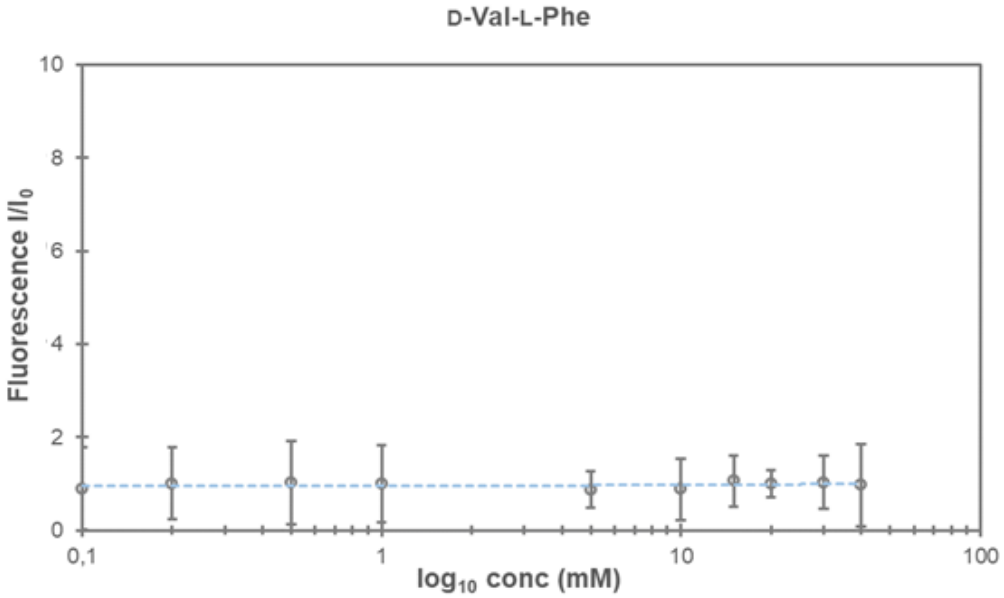


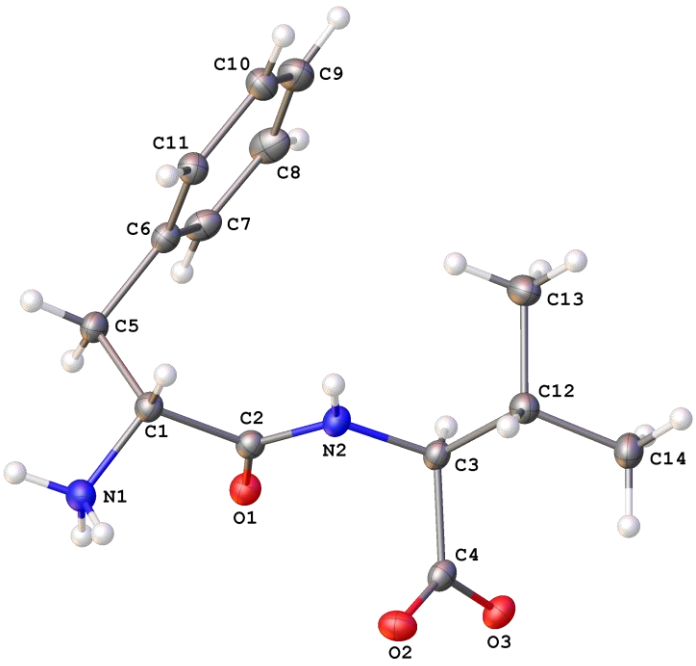
Fig. S14. Thioflavin T fluorescence assay for D-Val-L-Phe reveals lack of fluorescence with a signal intensity (I) that is not significant different relative to the control without peptide (I_0) across all concentrations tested.

S6. Single-crystal XRD data

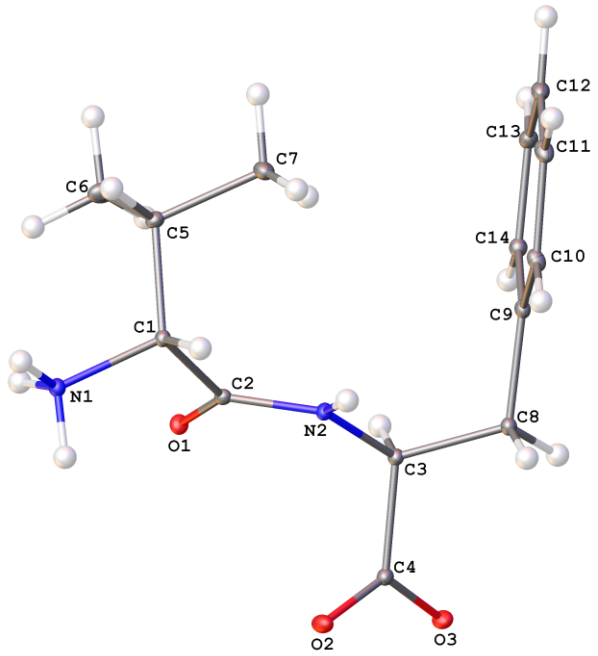
D-Phe-L-Val (CCDC 2160978) and D-Val-L-Phe (CCDC 2160976).

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 (λ = 0.7000 Å) with the rotating crystal method (0.5°/image) for a total of 720 images. Data indexing were performed using MOSFLM,[1] while space groups were determined using POINTLESS.[2] The software AIMLESS[3] was used for scaling the data. The structures were solved using the software SHELXT[4] and refined through full matrix least-squares based on F^2 using the program SHELXL[5] and OLEX2[6] 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**.

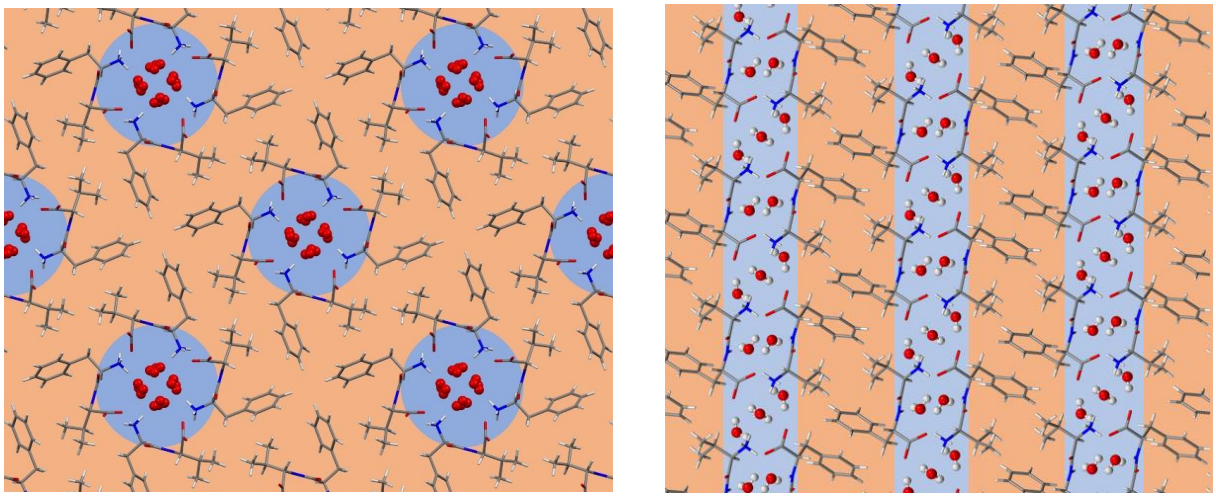


(a)



(b)

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.



(a) **(b)**

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.

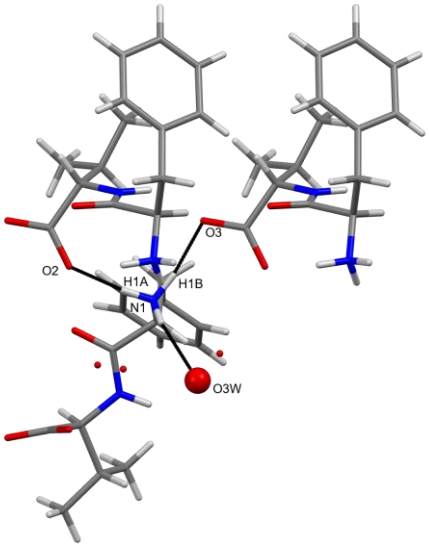


Figure S17. Typical hydrogen bond pattern of Phe-Phe dipeptide group. Only the interacting water molecule O3W is reported in ball-and-stick style.

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).

	D-Phe-L-Val (CCDC 2160978)	D-Val-L-Phe (CCDC 2160976)
T (K)	100	100
Formula	4($C_{14}H_{20}N_2O_3$), 4(H_2O)	2($C_{14}H_{20}N_2O_3$), 5(H_2O)
Formula weight	1129.34	618.72
System	tetragonal	monoclinic
Space group	*I*4	*C*2
***a* (Å)**	22.947(3)	19.615(4)
***b* (Å)**	22.947(3)	6.7520(14)
***c* (Å)**	5.6120(11)	13.402(3)
***α* (°)**	90	90
***β* (°)**	90	114.86(3)
***γ* (°)**	90	90
***V* (Å^3)**	2955.1(10)	1610.5(7)
Z	2	2
D_x (g cm^{-3})	1.260	1.276
λ (Å)	0.70000	0.70000
μ (mm^{-1})	0.090	0.095
F_{000}	1200.0	668.0
R1 (I > 2σI)	0.0661(2954)	0.0262(4354)
$_wR_2$	0.1921(4314)	0.0714(4372)
N. of param.	140	219
GooF	1.043	1.065
ρ_{min}, ρ_{max} (eÅ^{-3})	-0.37, 0.53	-0.27, 0.23
Restraints	/	/

[1] T. G. G. Battye, L. Kontogiannis, O. Johnson, H. R. Powell and A. G. W. Leslie, *Acta Crystallogr., Sect. D*, 2011, **67**, 271−281.
[2] P. R. Evans, *Acta Crystallogr., Sect. D*, 2006, **62**, 72−82.
[3] P. R. Evans and G. N. Murshudov, *Acta Crystallogr., Sect. D*, 2013, **69**, 1204−1214.
[4] G. M. Sheldrick, *Acta Crystallogr., Sect. A*, 2015, **71**, 3−8.
[5] G. M. Sheldrick, *Acta Crystallogr., Sect. C*, 2015, **71**, 3−8.
[6] O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, *J. Appl. Cryst.*, 2009, **42**, 339−341.

S7. Oscillatory rheometry data

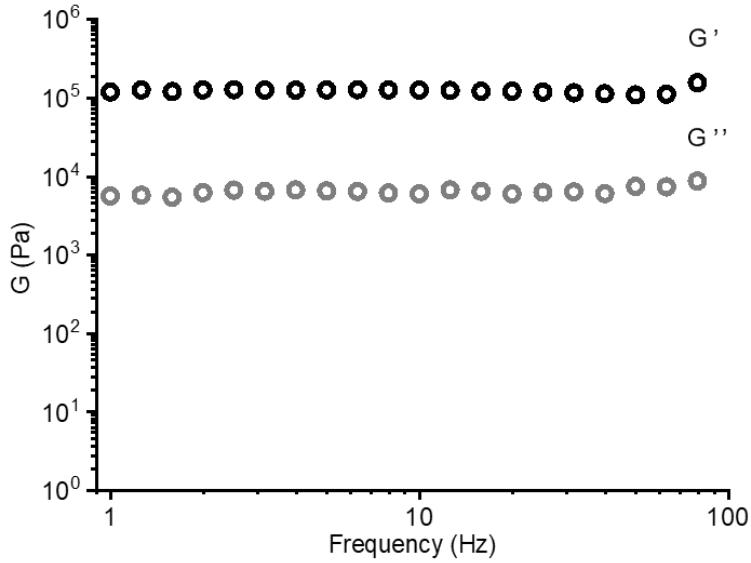


Fig. S18. Frequency sweep for D-Phe-L-Val at 40 mM.

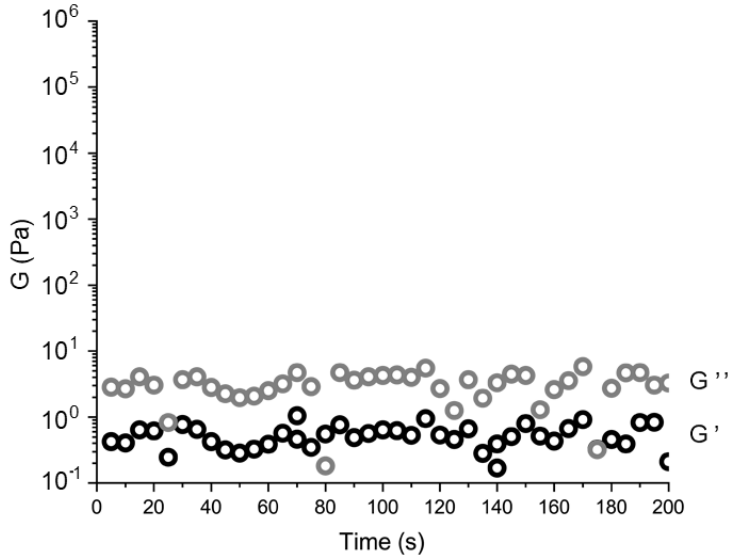


Fig. S19. Time sweep for D-Val-L-Phe at 40 mM.

S8. Thermoreversibility test

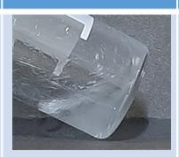
Sequence	MGC	RT	55-60°C	60°C	RT after 1h	RT after O/N
D-Phe-L-Val	40 mM					

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