Supporting Information

Supramolecular hydrogels from unprotected dipeptides: a comparative study on stereoisomers and structural isomers.

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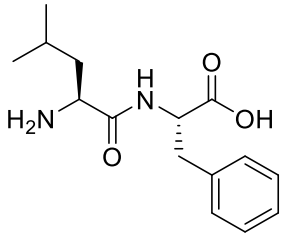
1. L-Leu-L-Phe spectroscopic data



^{1}H NMR (400 MHz, DMSO-d_6, TMS), δ (ppm): 12.89 (s, 1H, COOH), 8.80 (d, J = 7.7 Hz, 1H, NH), 8.09 (s, 2H, NH_2), 7.34 – 7.20 (m, 5H, Ar), 4.49 (m, 1H, αCH), 3.76 (t, 1H, αCH), 3.09 (dd, J = 14.1, 5.3 Hz, 1H, βCH_2), 2.96 (dd, J = 14.1, 8.8 Hz, 1H, βCH_2), 1.67 (m, 1H, ɣCH), 1.60 – 1.46 (m, 2H, βCH_2), 0.89 (d, J = 4.9 Hz, 3H, CH_3), 0.88 (d, J = 4.9 Hz, 3H, CH_3). **^{13}C NMR** (100 MHz, DMSO-d_6, TMS), δ (ppm): 172.3, 169.2 (2 x CO); 137.3, 129.1, 128.3, 126.6 (Ar); 53.8, 50.6 (2 x αC); 40.3, 36.4 (2 x βC); 23.4 (1 ɣC); 22.9, 21.6 (2 x δC). **MS (ESI):** m/z 279.1 $(M+H)^+$, 301.1 $(M+Na)^+$; 277.1 $(M-H)^-$.

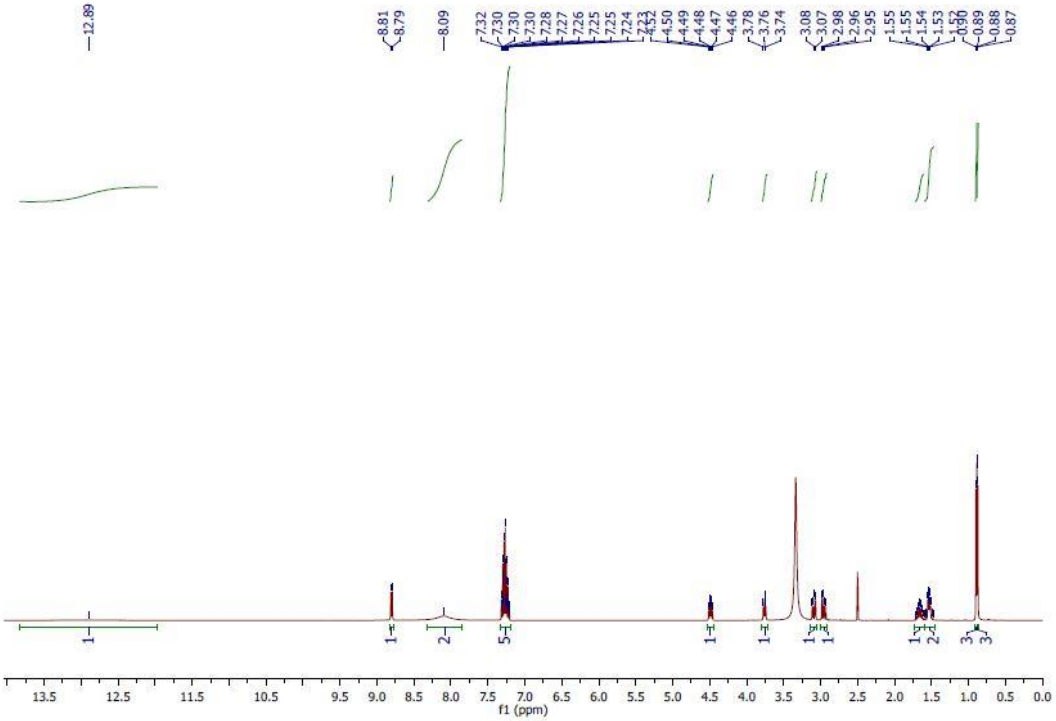


Fig. S1. ^{1}H-NMR spectrum of L-Leu-L-Phe.

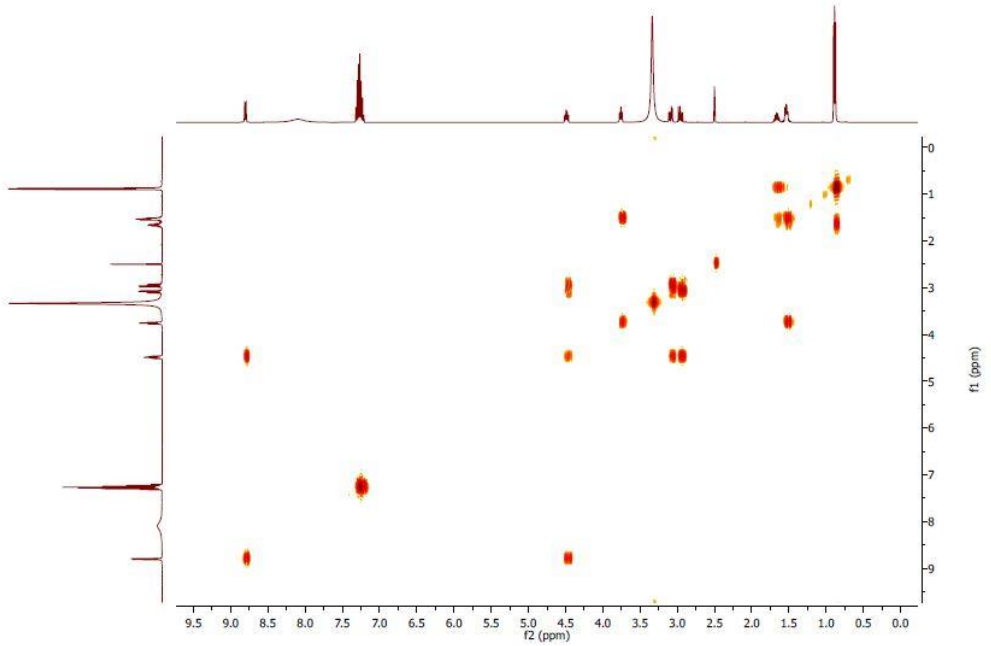


Fig. S2. gCOSY 2D-NMR spectrum of L-Leu-L-Phe.

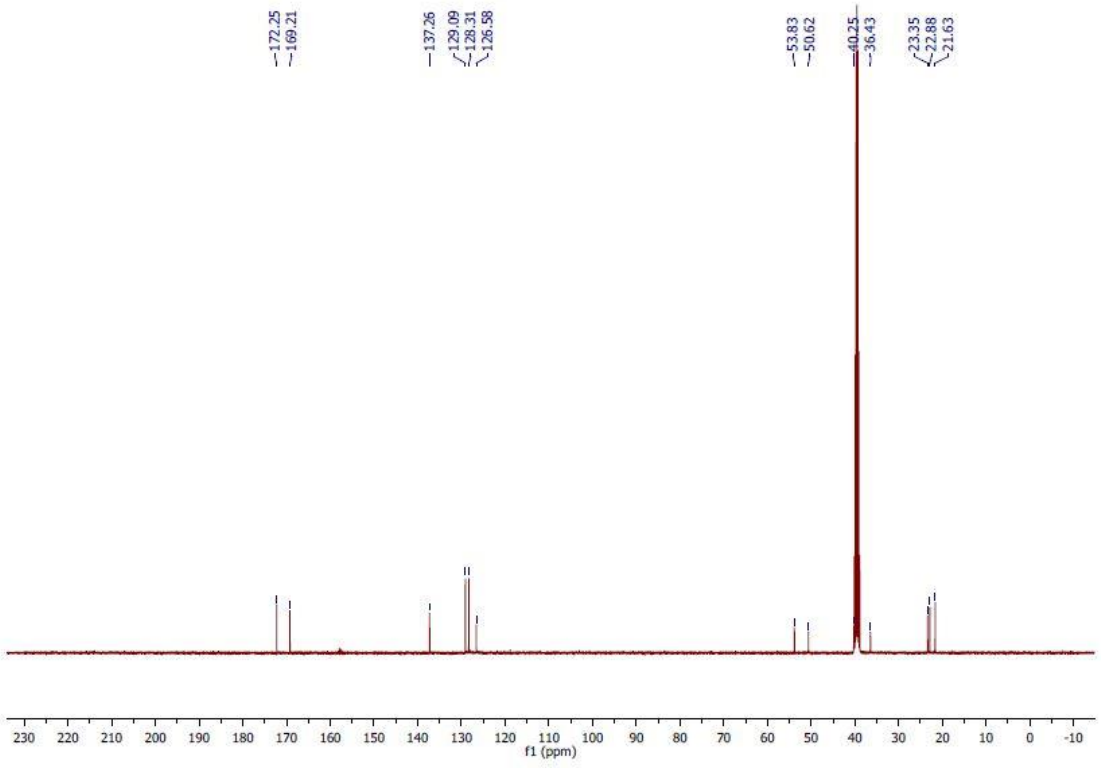


Fig. S3. ^{13}C-NMR spectrum of L-Leu-L-Phe.

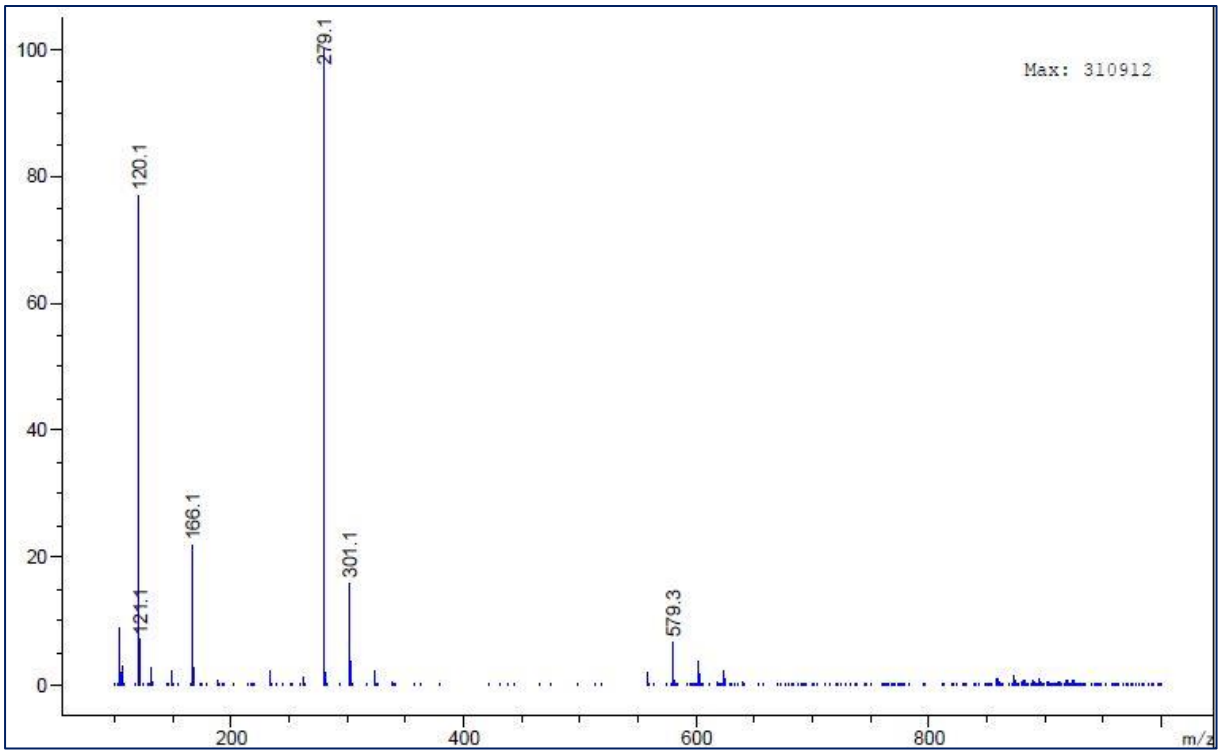


Fig. S4. ESI-MS spectrum of L-Leu-L-Phe (positive ion mode).

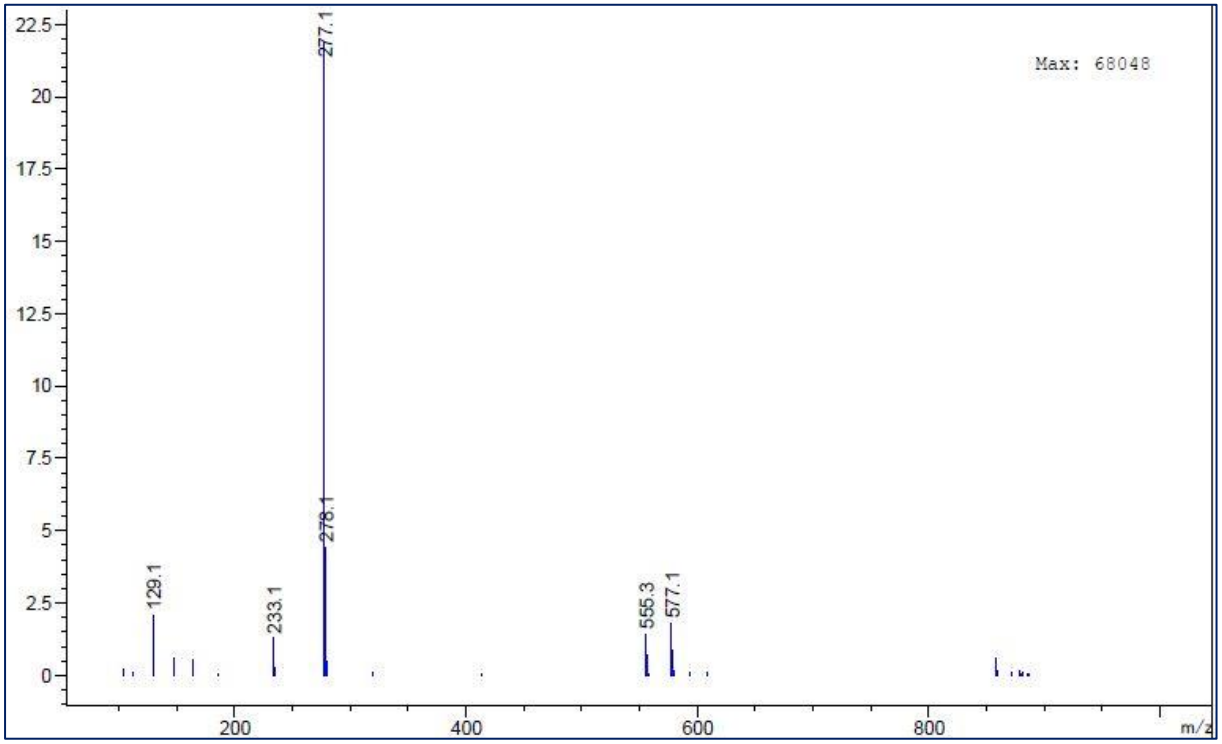
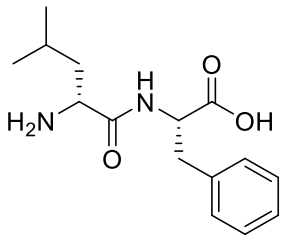


Fig. S5. ESI-MS spectrum of L-Leu-L-Phe (negative ion mode).

2. D-Leu-L-Phe spectroscopic data



^{1}H NMR (400 MHz, DMSO-d_6, TMS), δ (ppm): 8.83 (d, J = 8.5 Hz, 1H, NH), 8.10 (s, 2H, NH_2), 7.29 – 7.18 (m, 5H, Ar), 4.58 (m, 1H, αCH), 3.69 (m, 1H, αCH), 3.17 (dd, J = 13.8, 4.4 Hz, 1H, βCH_2), 2.82 (dd, J = 13.8, 10.6 Hz, 1H, βCH_2), 1.26 (m, 1H, ɣCH), 1.23 – 1.17 (m, 2H, βCH_2), 0.74 (d, J = 4.7 Hz, 3H, CH_3), 0.73 (d, J = 4.7 Hz, 3H, CH_3). **^{13} C NMR** (100 MHz, DMSO-d_6, TMS), δ (ppm): 172.5, 168.9 (2 x CO); 137.2, 129.2, 128.2, 126.5 (Ar); 53.4, 50.7 (2 x αC); 40.3, 37.0 (2 x βC); 23.2 (1 ɣC); 22.5, 21.8 (2 x δC). **MS (ESI):** m/z 279.1 $(M+H)^+$, 301.1 $(M+Na)^+$; 277.1 $(M-H)^-$.

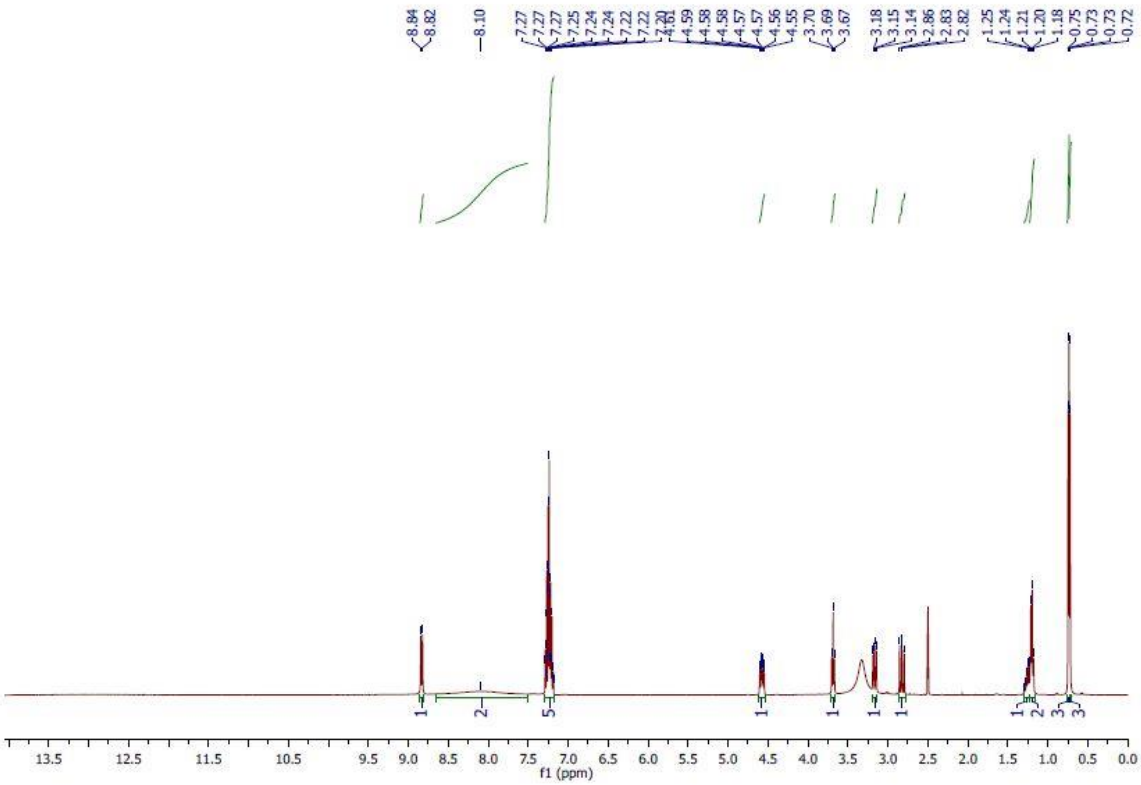


Fig. S6. ^{1}H-NMR spectrum of D-Leu-L-Phe.

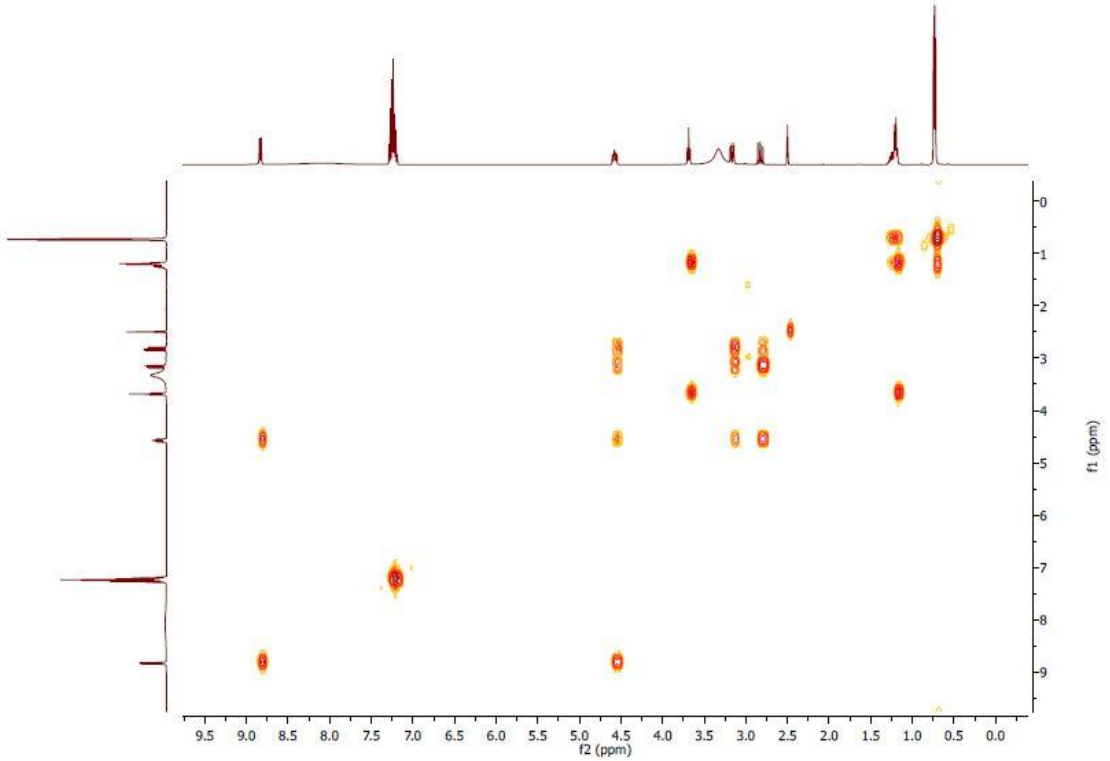


Fig. S7. gCOSY 2D-NMR spectrum of D-Leu-L-Phe.

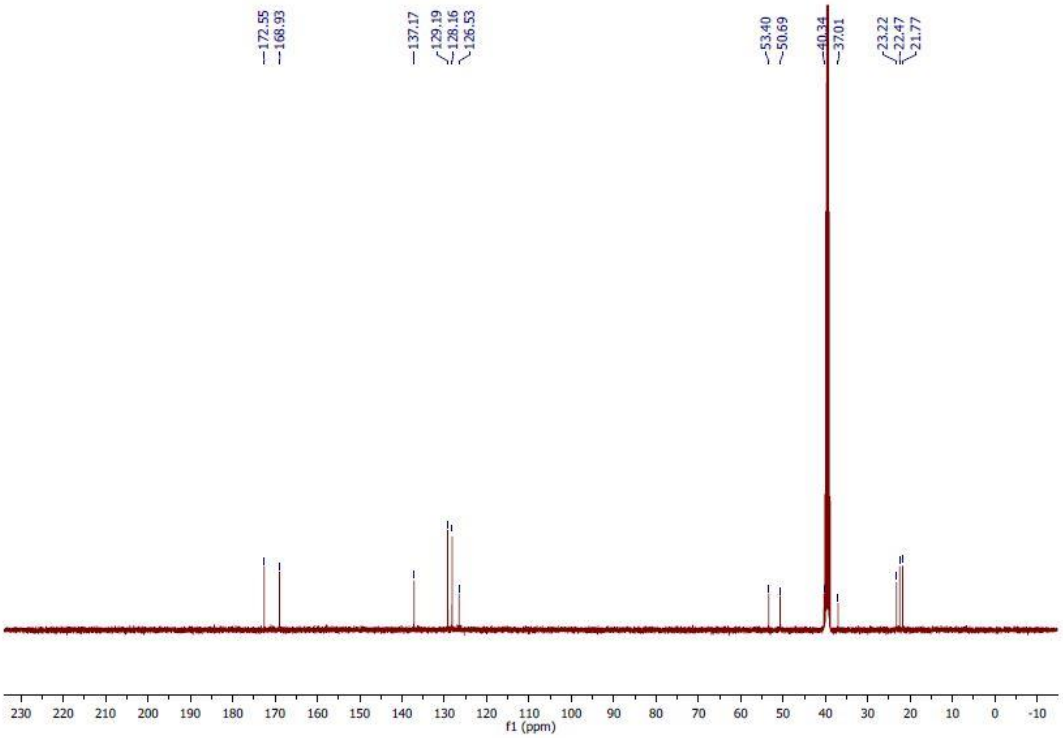


Fig. S8. ^{13}C-NMR spectrum of D-Leu-L-Phe.

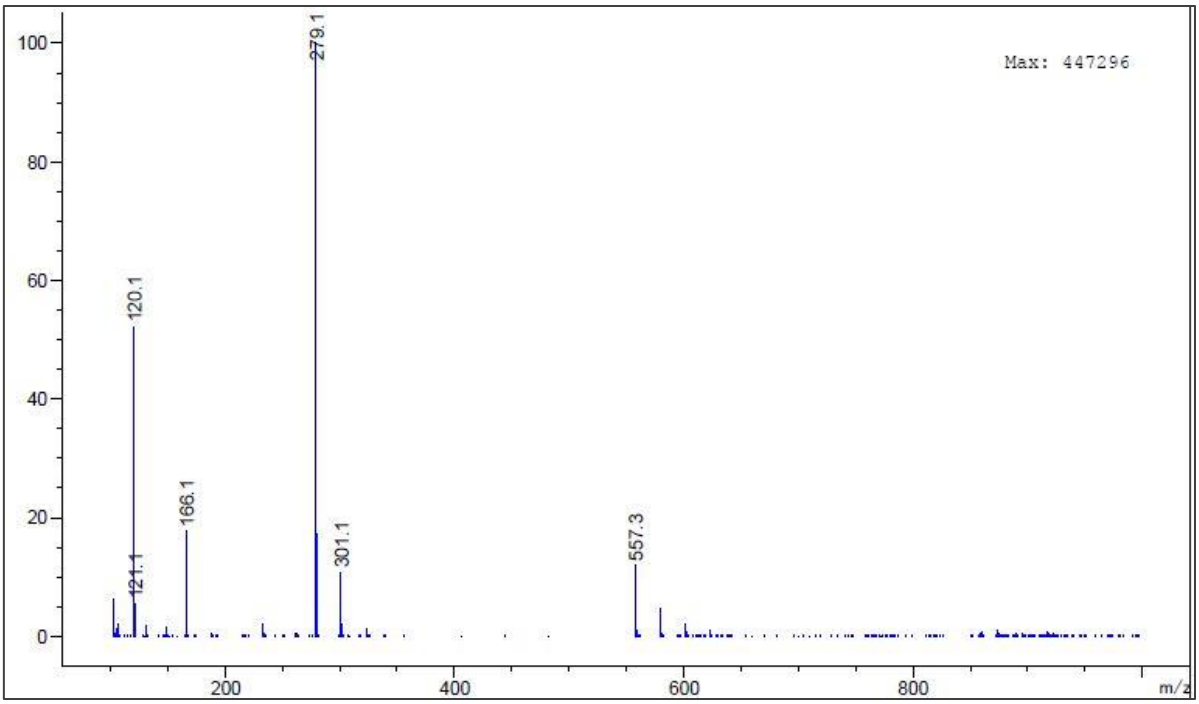


Fig. S9. ESI-MS spectrum of D-Leu-L-Phe (positive ion mode).

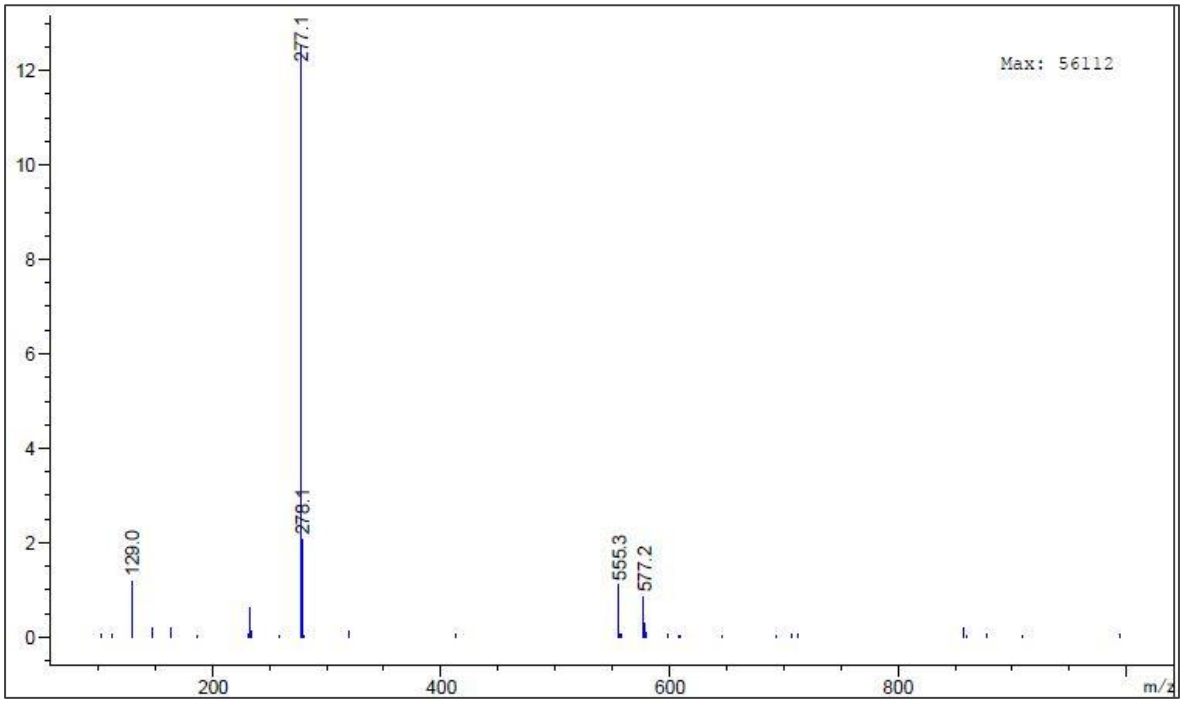
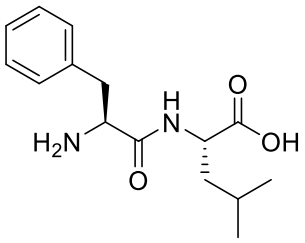


Fig. S10. ESI-MS spectrum of D-Leu-L-Phe (negative ion mode).

3. L-Phe-L-Leu spectroscopic data



^{1}H NMR (400 MHz, DMSO-d_6, TMS), δ (ppm): 12.83 (s, 1H, COOH), 8.76 (d, J = 7.9 Hz, 1H, NH), 8.12 (s, 2H, NH_2), 7.37 – 7.24 (m, 5H, Ar), 4.28 (m, 1H, αCH), 4.05 (m, 1H, αCH), 3.14 (dd, J = 14.2, 5.0 Hz, 1H, βCH_2), 2.98 – 2.88 (m, 1H, βCH_2), 1.75 – 1.61 (m, 1H, ɣCH), 1.60 – 1.49 (m, 2H, βCH_2), 0.92 (d, J = 6.6 Hz, 3H, CH_3), 0.88 (d, J = 6.5 Hz, 3H, CH_3). **^{13}C NMR** (100 MHz, DMSO-d_6, TMS), δ (ppm): 173.4, 168.1 (2 x CO); 134.7, 129.6, 128.5, 127.2 (Ar); 53.2, 50.5 (2 x αC); 40.0, 36.9 (2 x βC); 24.2 (1 ɣC); 22.8, 21.3 (2 x δC). **MS (ESI):** m/z 279.1 $(M+H)^+$, 301.1 $(M+Na)^+$; 277.1 $(M-H)^-$.

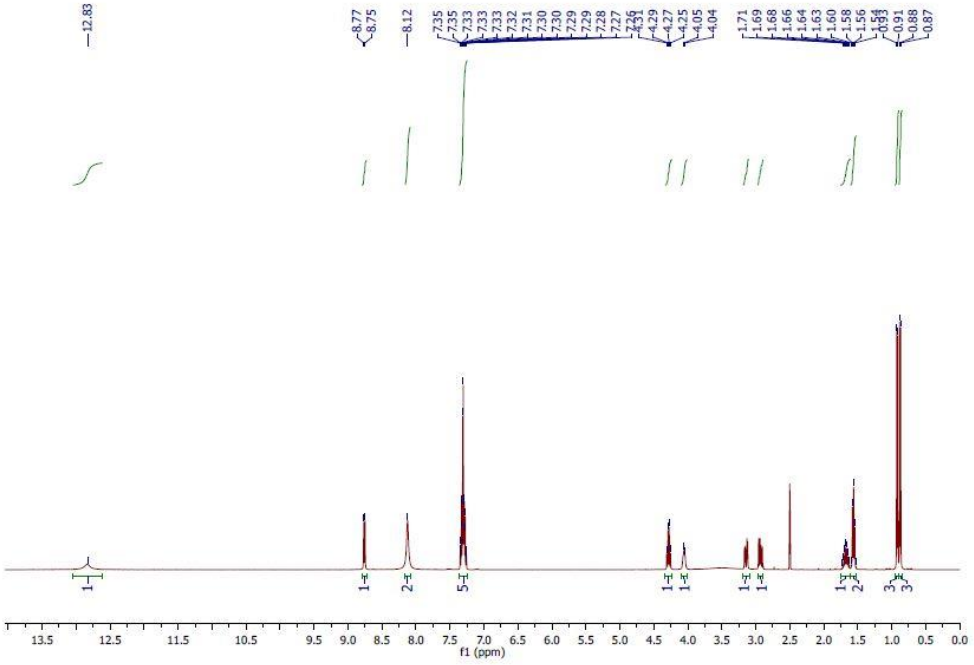


Fig. S11. ^{1}H-NMR spectrum of L-Phe-L-Leu.

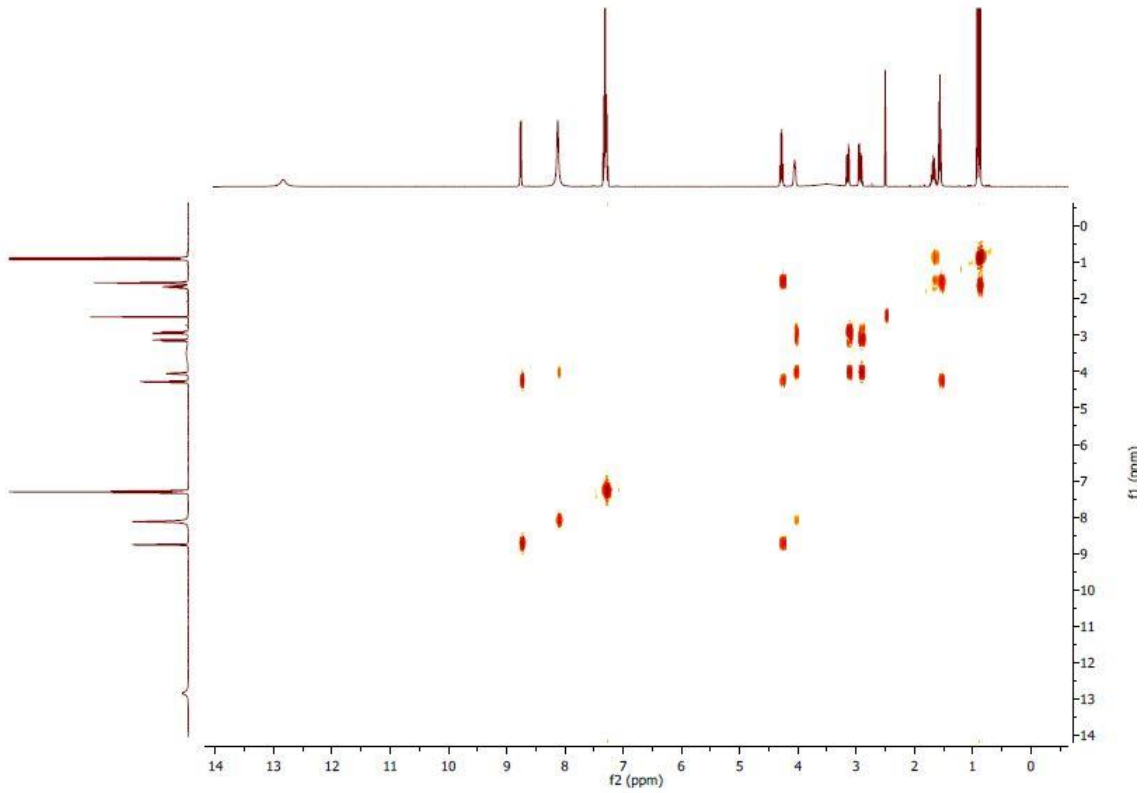


Fig. S12. gCOSY 2D-NMR spectrum of L-Phe-L-Leu.

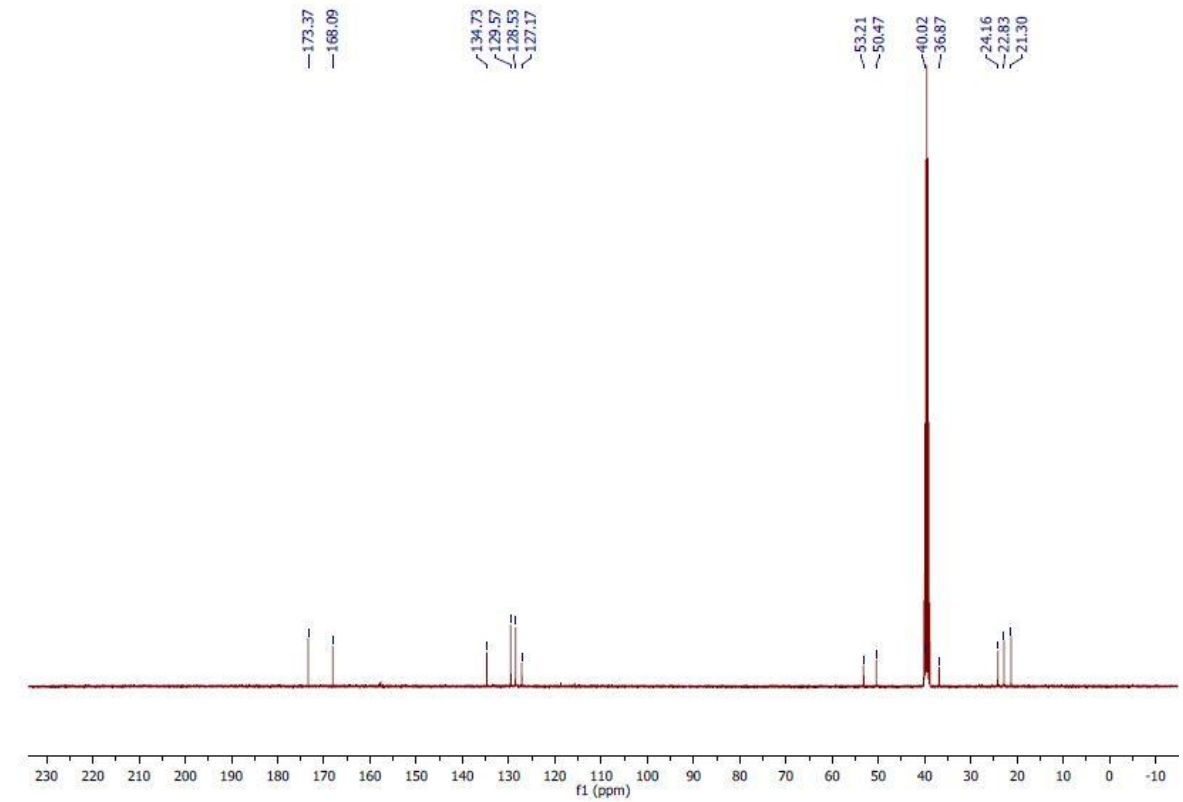


Fig. S13. ^{13}C-NMR spectrum of L-Phe-L-Leu.

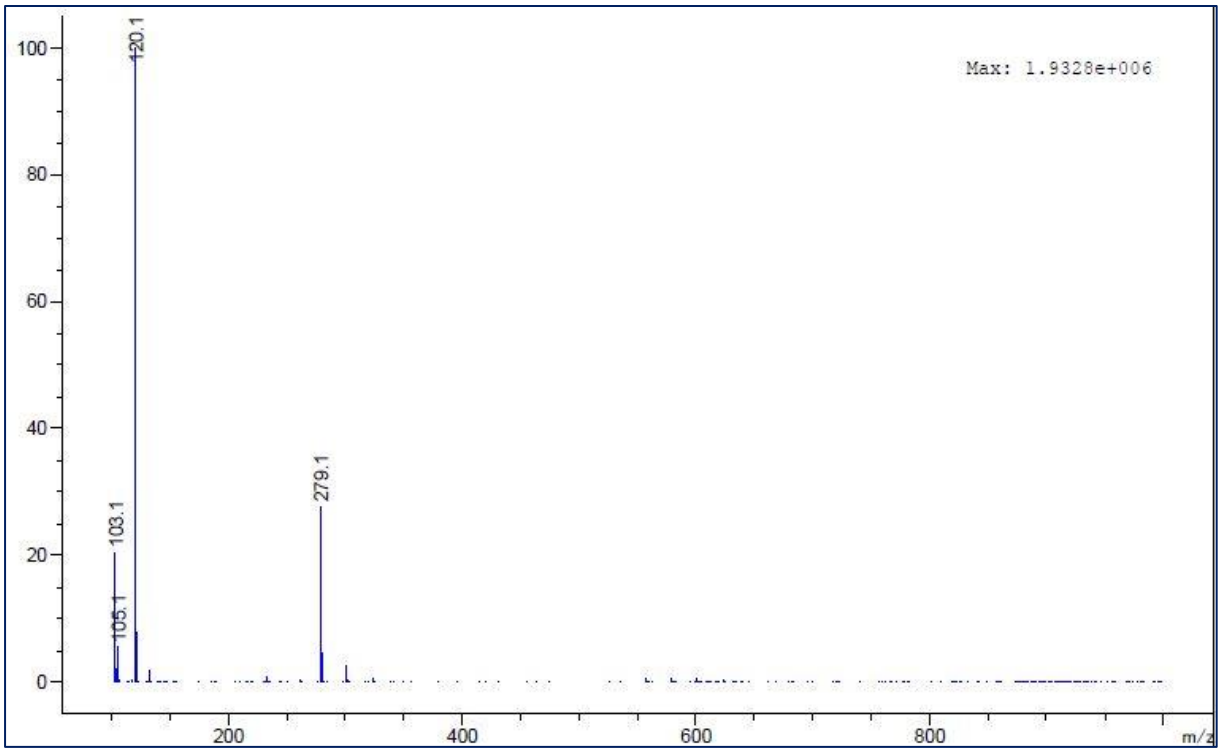


Fig. S14. ESI-MS spectrum of L-Phe-L-Leu (positive ion mode).

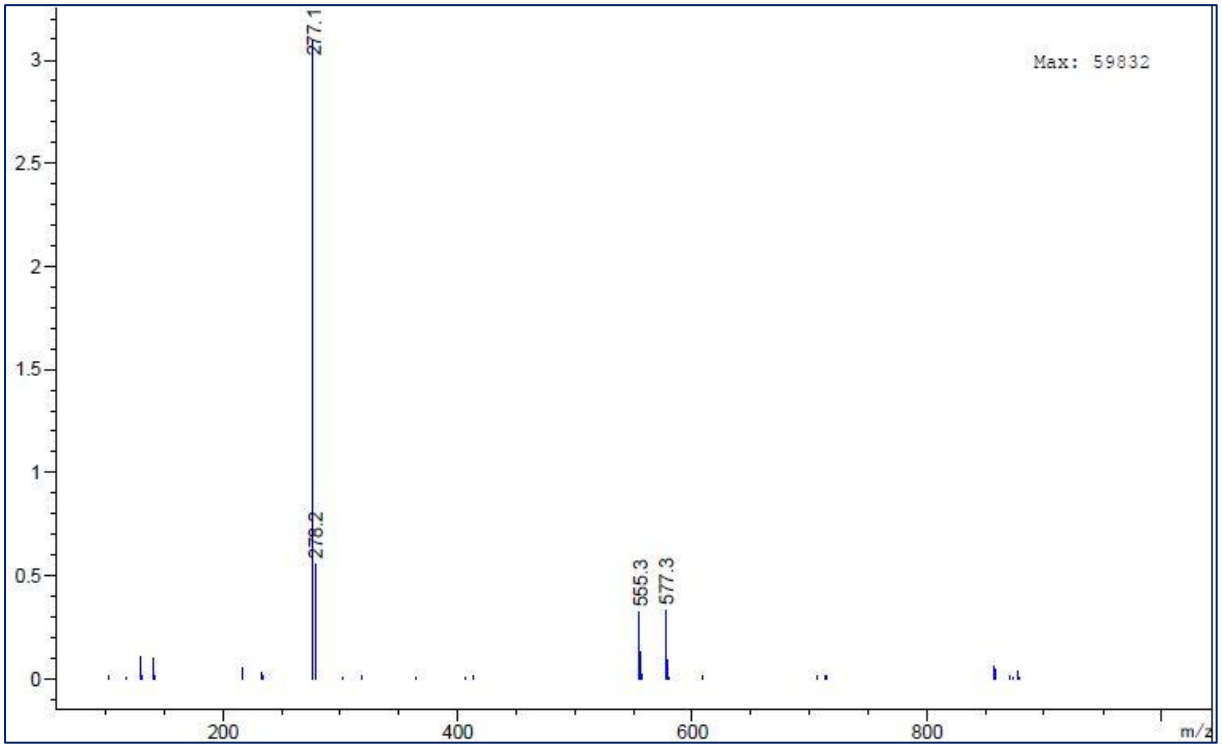
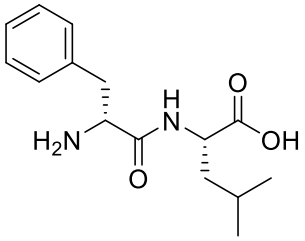


Fig. S15. ESI-MS spectrum of L-Phe-L-Leu (negative ion mode).

4. D-Phe-L-Leu spectroscopic data



^{1}H NMR (400 MHz, DMSO-d_6, TMS), δ (ppm): 8.62 (d, *J* = 8.1 Hz, 1H, NH), 7.36 – 7.21 (m, 5H, Ar), 4.19 (m, 1H, αCH), 4.05 (dd, *J* = 7.2 Hz, 7.2 Hz, 1H, αCH), 3.05 – 2.93 (m, 2H, βCH_2), 1.47 – 1.32 (m, 2H, βCH_2), 1.30 – 1.18 (m, 1H, ɣCH), 0.80 (d, *J* = 6.6 Hz, 3H, CH_3), 0.75 (d, *J* = 6.5 Hz, 3H, CH_3). **^{13}C NMR** (100 MHz, DMSO-d_6, TMS), δ (ppm): 173.5, 168.2 (2 x CO); 135.0, 129.4, 128.4, 127.1 (Ar); 53.4, 50.3 (2 x αC); 40.3, 37.6 (2 x βC); 23.9 (1 ɣC); 22.8, 21.2 (2 x δC). **MS (ESI):** m/z 279.1 $(M+H)^+$, 301.1 $(M+Na)^+$; 277.1 $(M-H)^-$.

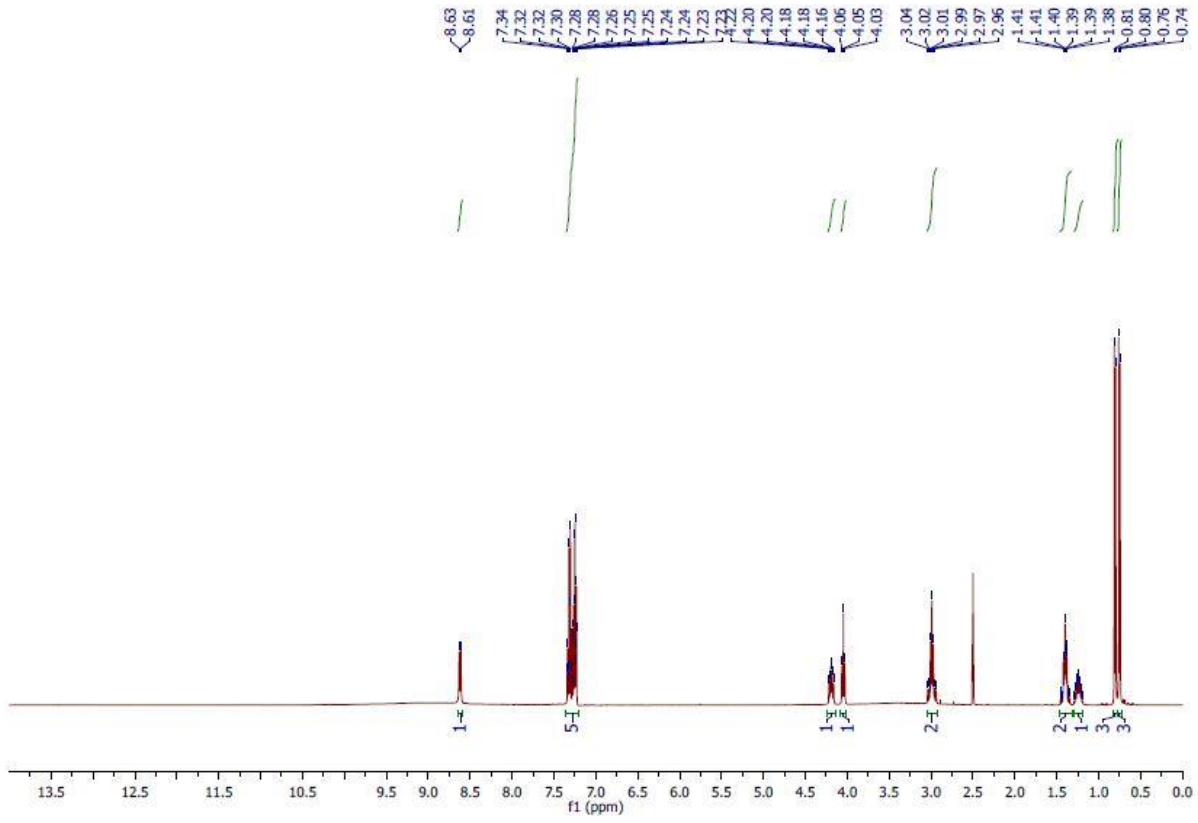


Fig. S16. ^{1}H-NMR spectrum of D-Phe-L-Leu.

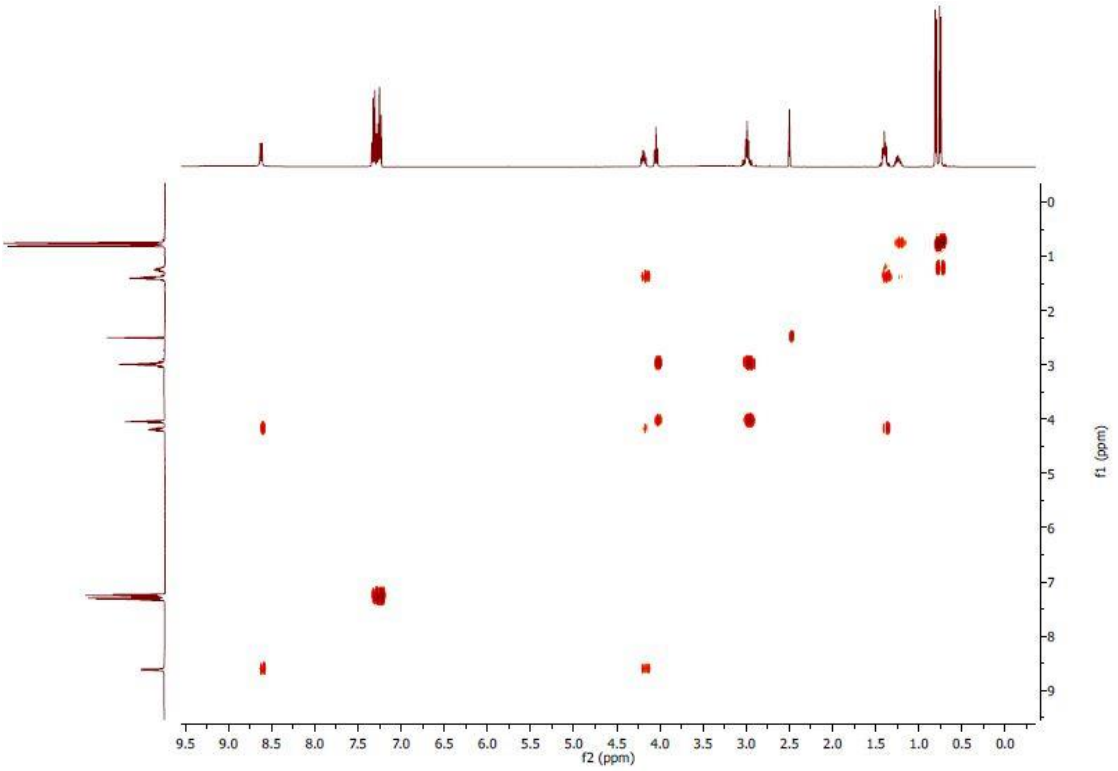


Fig. S17. gCOSY 2D-NMR spectrum of D-Phe-L-Leu.

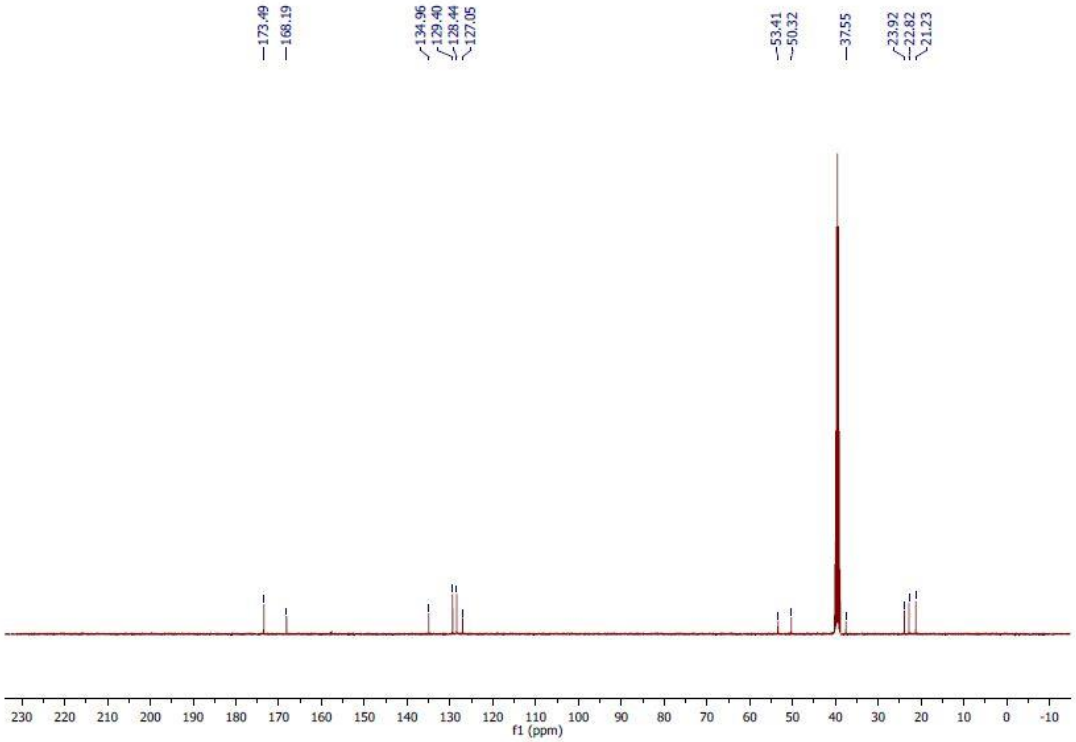


Fig. S18. ^{13}C-NMR spectrum of D-Phe-L-Leu.

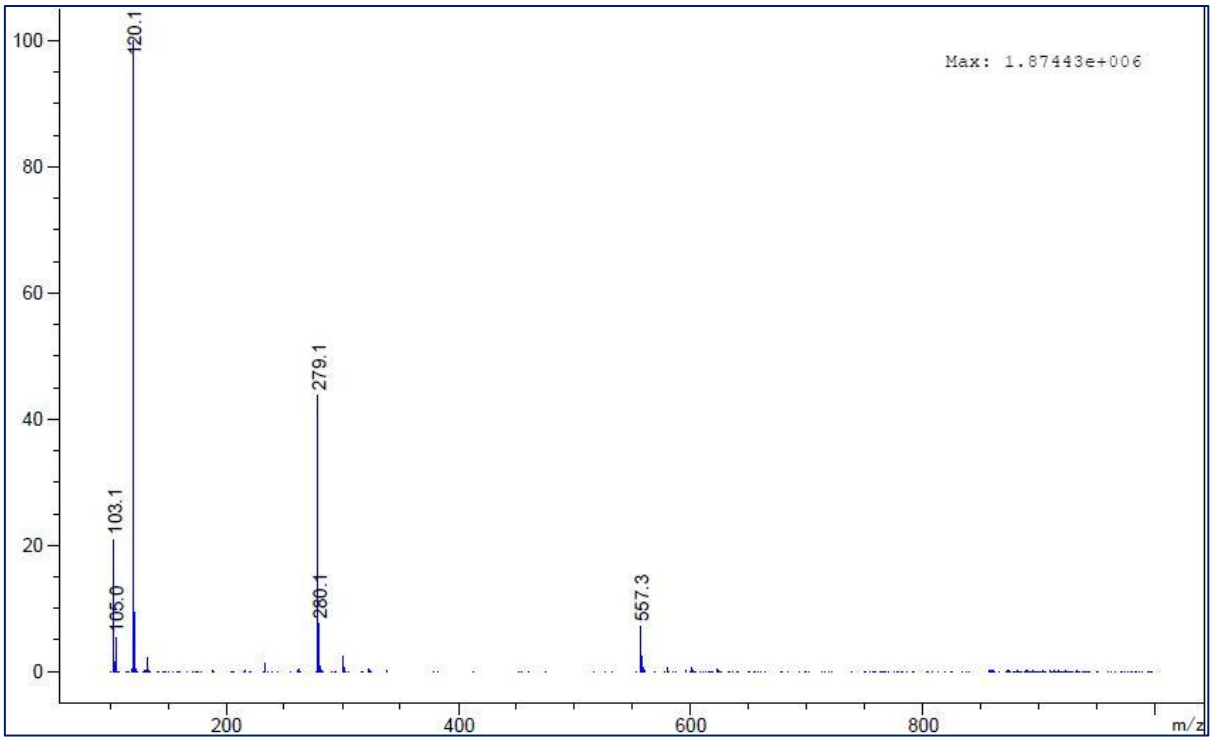


Fig. S19. ESI-MS spectrum of D-Phe-L-Leu (positive ion mode).

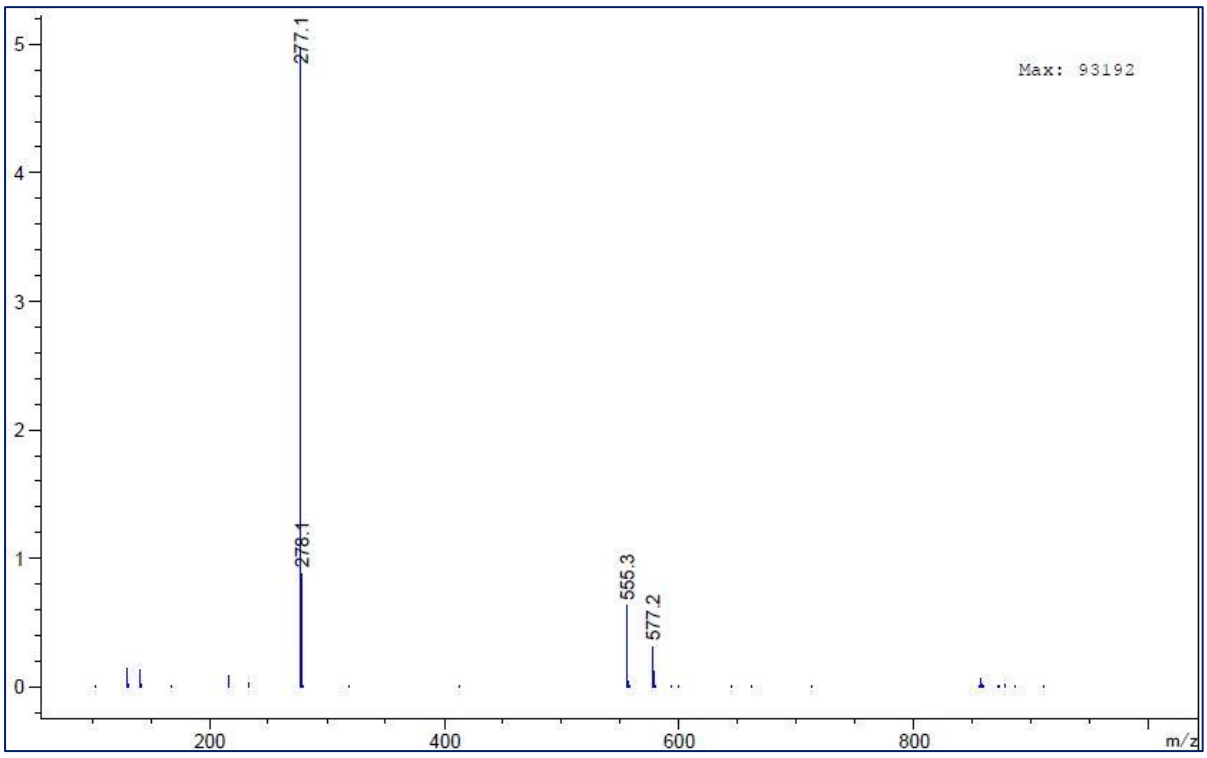


Fig. S20. ESI-MS spectrum of D-Phe-L-Leu (negative ion mode).

5. HPLC traces

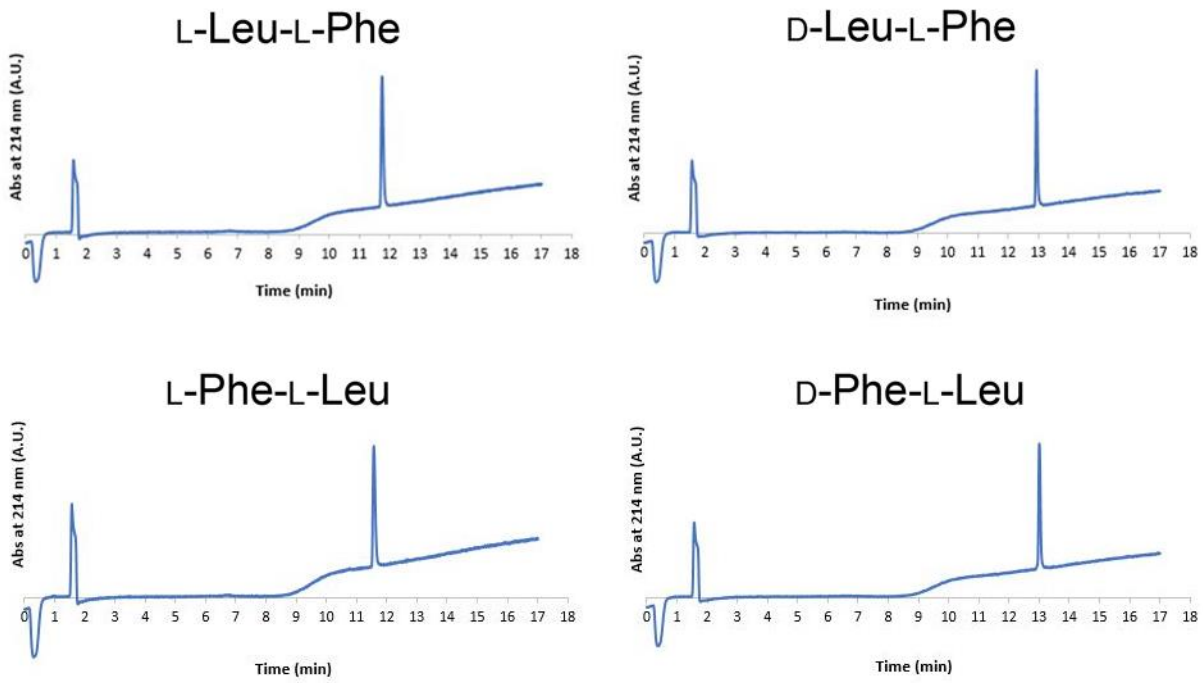


Fig. S21. HPLC traces for the four dipeptides. Retention times are reported in **Table 1** in the manuscript.

6. CD spectra for the hydrogels

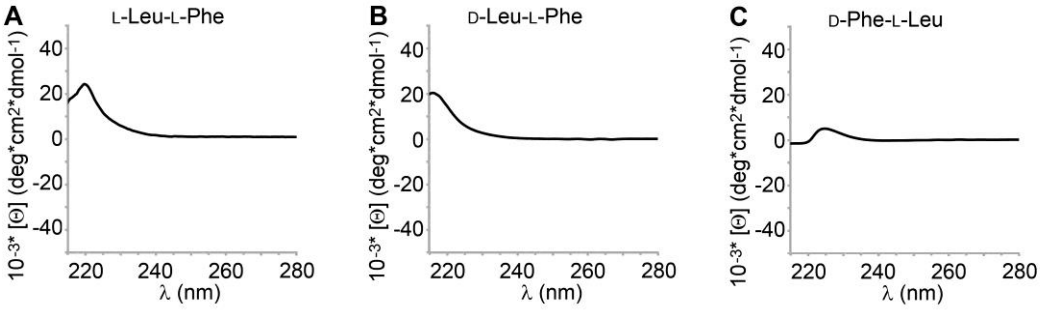


Fig. S22. CD spectra for the hydrogels at 40 mM, except for L-Leu-L-Phe that was at the mgc of 45 mM.

7. Rheology data

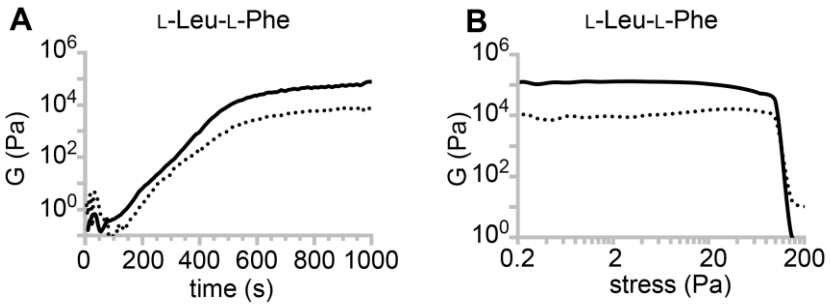


Fig. S23. Time sweep (A) and stress sweep (B) for L-Leu-L-Phe at 45 mM.

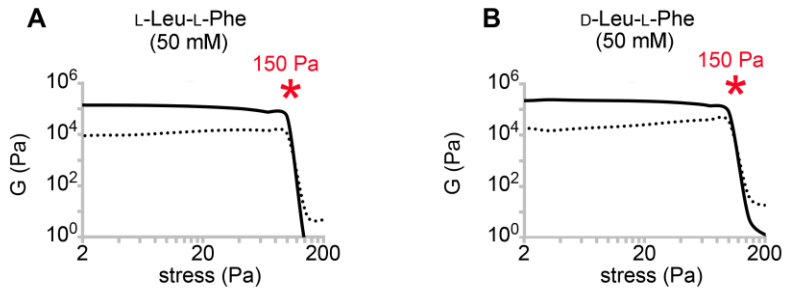


Fig. S24. Stress sweeps for (A) L-Leu-L-Phe, and (B) D-Leu-L-Phe at 50 mM.

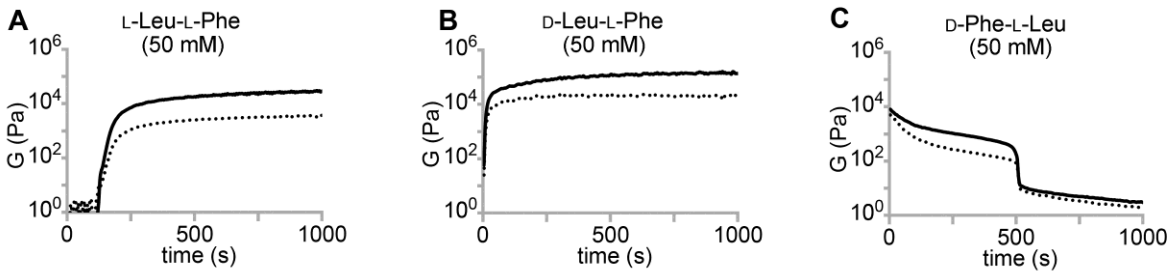


Fig. S25. Time sweeps for (A) L-Leu-L-Phe, (B) D-Leu-L-Phe, and (C) D-Phe-L-Leu at 50 mM.

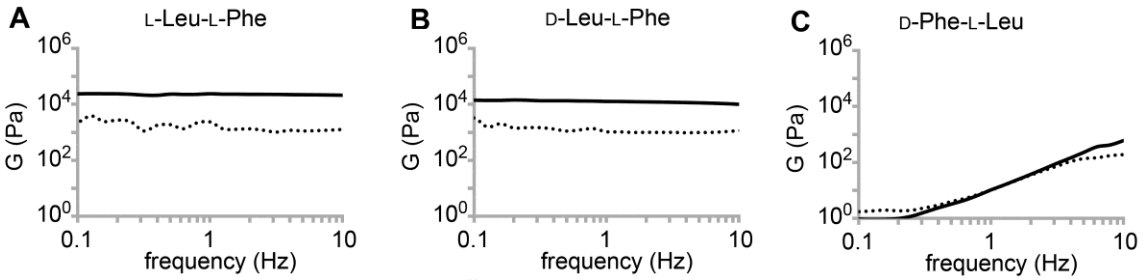


Fig. S26. Frequency sweeps for L-Leu-L-Phe (A), D-Leu-L-Phe (B), and D-Phe-L-Leu (C).

8. Single-crystal XRD data

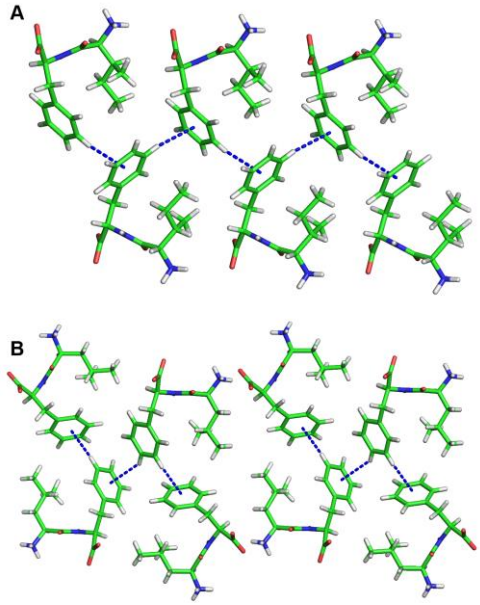


Fig. S27. Phe zipper for for L-Leu-L-Phe (A) and L-Ile-L-Phe (B), from C. H. Görbitz, *Chem. Eur. J.* **2001**, *7* (23), 5153 and C. H. Görbitz, *Acta Crystallogr. C*, **2004**, *60*, o371.

Structure determination for D-Phe-L-Leu

A rectangular-shaped single crystal of the peptide was collected with a loop, cryoprotected by dipping the crystal in glycerol, and stored frozen in liquid nitrogen. The crystal was mounted on the diffractometer at the synchrotron Elettra, Trieste (Italy), beamline XRD1, using the robot present at the facility. Temperature was kept at 100 K by a stream of nitrogen on the crystal. Diffraction data were collected by the rotating crystal method using synchrotron radiation, wavelength 0.70 Å, rotation interval 1°/image, crystal-to-detector distance of 85 mm. A total of 360 images were collected to increase redundancy. Reflections were indexed and integrated using the software MOSFLM [1], space group *C2* was determined using POINTLESS [2] and the resulting data set was scaled using AIMLESS [3]. Phase information were obtained by direct methods using the software SIR92 [4]. Refinements cycles were conducted with SHELXL-14 [5], operating through the WinGX GUI [6], by full-matrix least-squares methods on F^2. Analysis of the data using the software PLATON [7] revealed that the crystal had a non-merohedral twinning, with a contribution of the second twin component of about 30%. The correction for twinning was implemented in the following refinement. The asymmetric unit contains a molecule of the peptide, a molecule of water at full occupancy and 3.5 additional molecules of water in 7 positions, each at 50% occupancy. Hydrogen atoms of the peptide molecule and of the solvent molecules were added at geometrically calculated positions and refined isotropically. All the atoms, except the hydrogen atoms, within the asymmetric unit have

been refined with anisotropic thermal parameters. Unit cell parameters, scaling statistics, and refinement statistics are reported in Table S1.

Table S1: Crystallographic data.

	^{D}Phe-^{L}Leu
Formula	$C_{15}H_{22}N_2O_3 \cdot 4.5H_2O$
Temperature (K)	100
Wavelength (Å)	0.7
Crystal system	Monoclinic
Space group	*C 2*
a (Å)	16.870(4)
b (Å)	5.770(1)
c (Å)	19.890(3)
α (°)	90
β (°)	105.90(1)
γ (°)	90
V (Å^3)	1862.0(6)
Z, ρcalc (g/cm^3)	4, 1.282
μ (mm^{-1})	0.097
F (000)	780
Data collection θ range	1.048 - 28.521
Refl. Collected / unique	30445 / 2679
Rint	0.090
Completeness (%)	98.4
Data/Restraints/Parameters	2679 / 1 / 258
GooF	1.117
R1, wR2 [I>2σ(I)]	0.0772 / 0.2235
R1, wR2 all data	0.0797 / 0.2284

References:

[1] Battye T.G.G., Kontogiannis L., Johnson O., Powell H.R. and Leslie A.G.W. Acta Crystallogr., Sect. D. 2011, 67, 271-281.

[2] Evans, P. R. Acta Crystallogr., Sect. D. 2006, 62, 72−82.

[3] Evans, P. R., Murshudov, G. N. Acta Crystallogr., Sect. D. 2013, 69, 1204−14.

[4] Altomare, A., Cascarano, G., Giacovazzo, C., Guagliardi, A., Burla, M. C., Polidori, G. & Camalli, M. J. Appl. Cryst. 1994, 27, 435.

[5] Sheldrick, G. M. Acta Crystallogr., Sect. C. 2015, 71, 3–8.

[6] Farrugia, L.J. J. Appl. Cryst. 2012, 45, 849−854.

[7] Spek, A. L. Acta. Cryst., Sect. D. 2009, 65, 148-155.