

## Supporting information

# Self-Assembly and Gelation Study of Dipeptide Isomers with Norvaline and Phenylalanine

Erica Scarel <sup>1</sup>, Giovanni Pierri <sup>2</sup>, Petr Rozhin <sup>1</sup>, Simone Adorinni <sup>1</sup>, Maurizio Polentarutti <sup>3</sup>, Consiglia Tedesco <sup>2,\*</sup> and Silvia Marchesan <sup>1,\*</sup>

1 Chemical and Pharmaceutical Sciences Department, University of Trieste,  
34127 Trieste, TRS, Italy

2 Department of Chemistry and Biology "A. Zambelli", University of Salerno,  
84084 Fisciano, SA, Italy

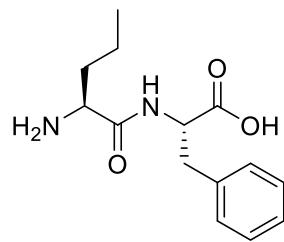
3 Elettra-Sincrotrone Trieste, S.S. 114 km 163.5, Basovizza (TS), 34149 Trieste, TRS, Italy

\* Correspondence: ctedesco@unisa.it (C.T.); smarchesan@units.it (S.M.)

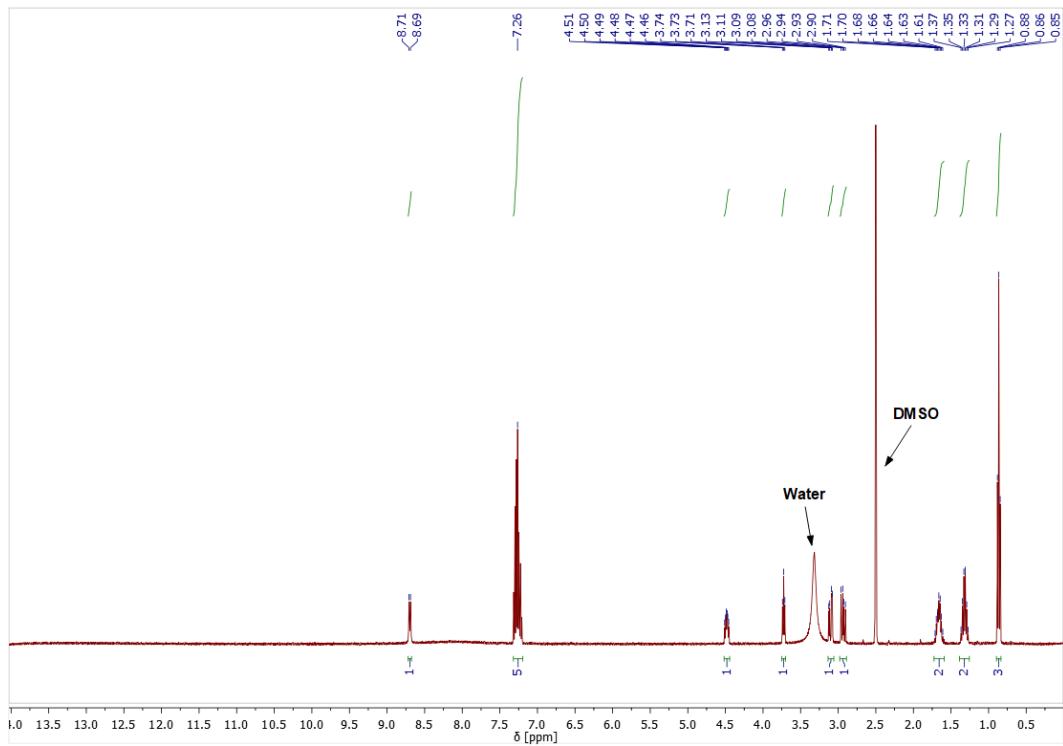
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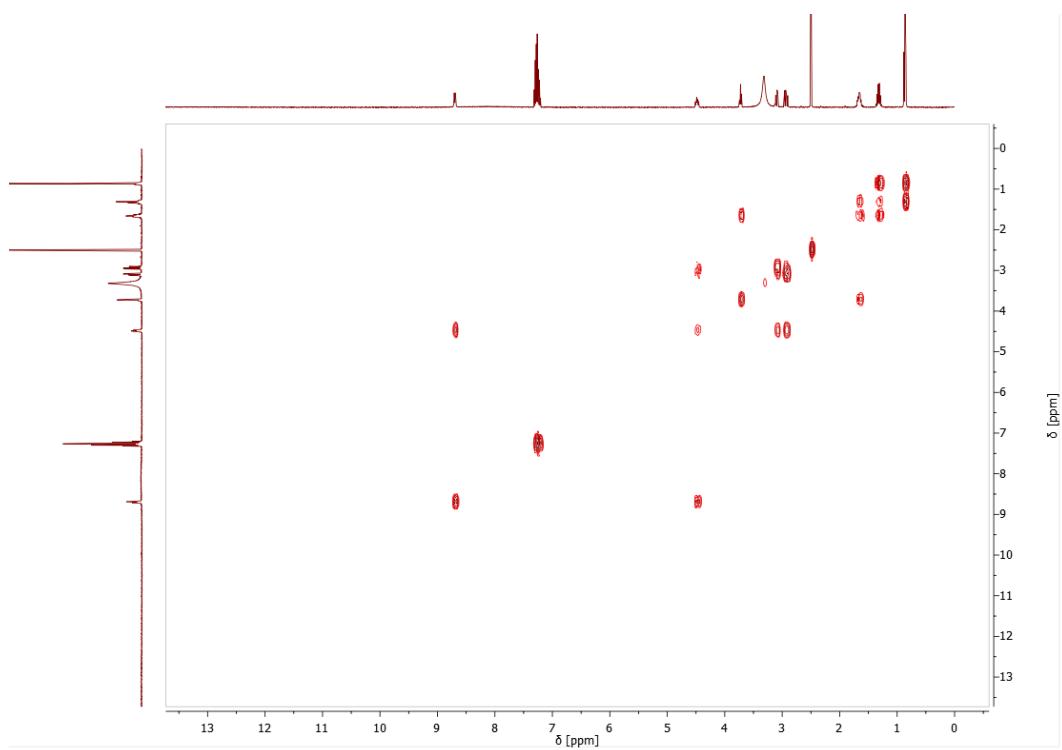
## 1. L-Nva-L-Phe spectroscopic data



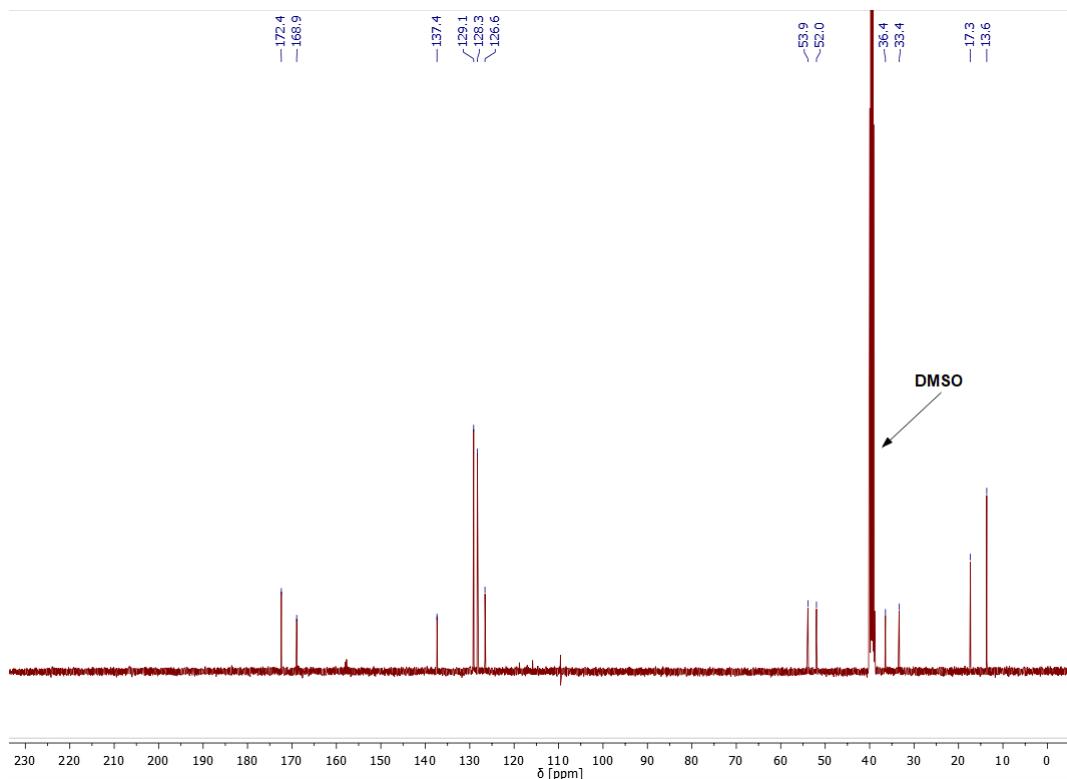
**<sup>1</sup>H NMR** (400 MHz, TMS, DMSO-*d*<sub>6</sub>) δ (ppm): 8.70 (1H, d, *J* = 8.4 Hz, NH), 7.26 (5H, m, Ar), 4.48 (1H, ddd, *J* = 4.8, 4.8 Hz, 8.8 Hz, CHα Phe), 3.72 (1H, dd, *J* = 6.4, 6.4 Hz, CHα Nva), 3.10 (1H, dd, *J* = 4.8, 14.0 Hz, CH<sub>2</sub>β Phe), 2.93 (1H, dd, *J* = 8.8, 14.0 Hz, CH<sub>2</sub>β Phe), 1.66 (2H, m, CH<sub>2</sub>β Nva), 1.32 (2H, m, CH<sub>2</sub>γ Nva), 0.87 (3H, dd, *J* = 7.2 Hz, CH<sub>3</sub>). **<sup>13</sup>C NMR** (100 MHz, TMS, DMSO-*d*<sub>6</sub>) δ (ppm): 172.4 (1C, COOH), 168.9 (1C, CONHR), 137.4 (1C, Ar), 129.1 (2C, Ar), 128.3 (2C, Ar), 126.6 (1C, Ar), 53.9 (1C, Cα), 52.0 (1C, Cα), 36.4 (1C, Cβ), 33.4 (1C, Cβ), 17.3 (1C, Cγ), 13.6 (1C, Cδ). **MS (ESI):** C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> Exact mass: 264.2 m/z. Found 265.0 [M+H]<sup>+</sup>, 287.0 [M+Na]<sup>+</sup>, 263.0 [M-H]<sup>-</sup>.



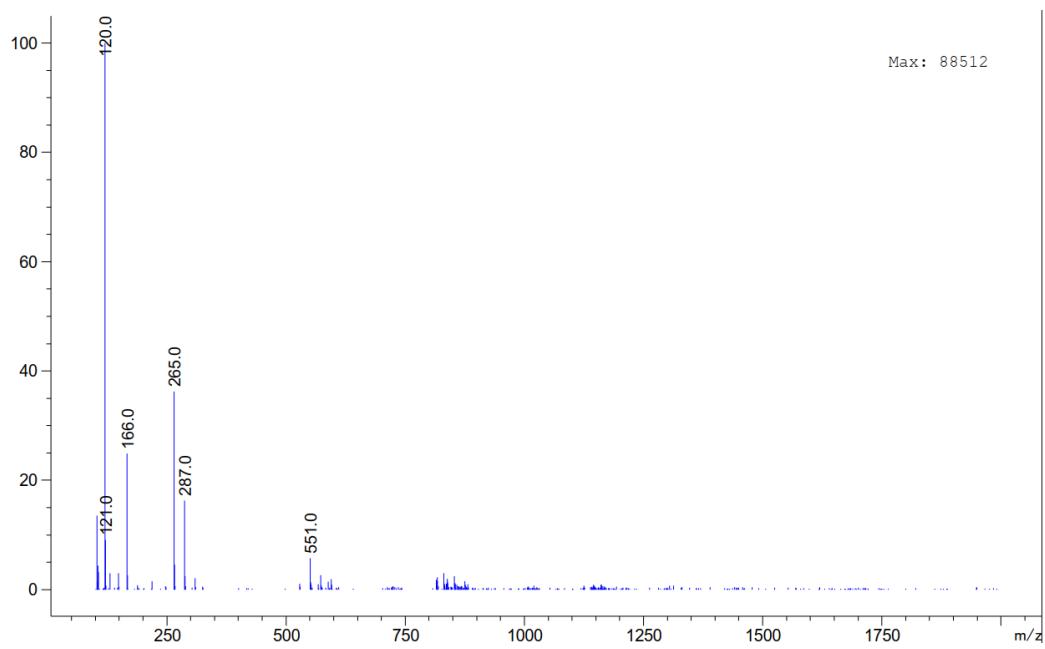
**Figure S1**  $^1\text{H}$  NMR spectrum of L-Nva-L-Phe.



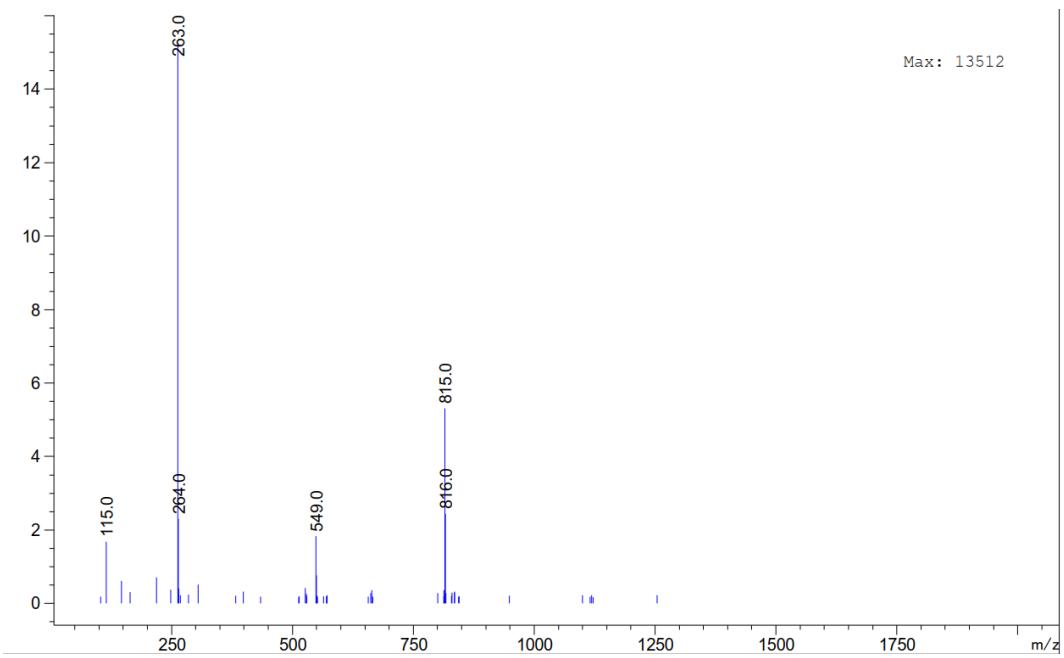
**Figure S2** gCOSY 2D NMR spectrum of L-Nva-L-Phe.



**Figure S3**  $^{13}\text{C}$  NMR spectrum of L-Nva-L-Phe.

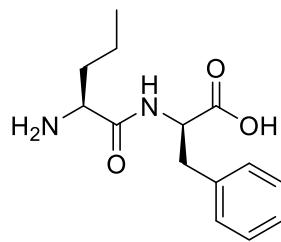


**Figure S4** ESI-MS spectrum of L-Nva-L-Phe (positive mode).

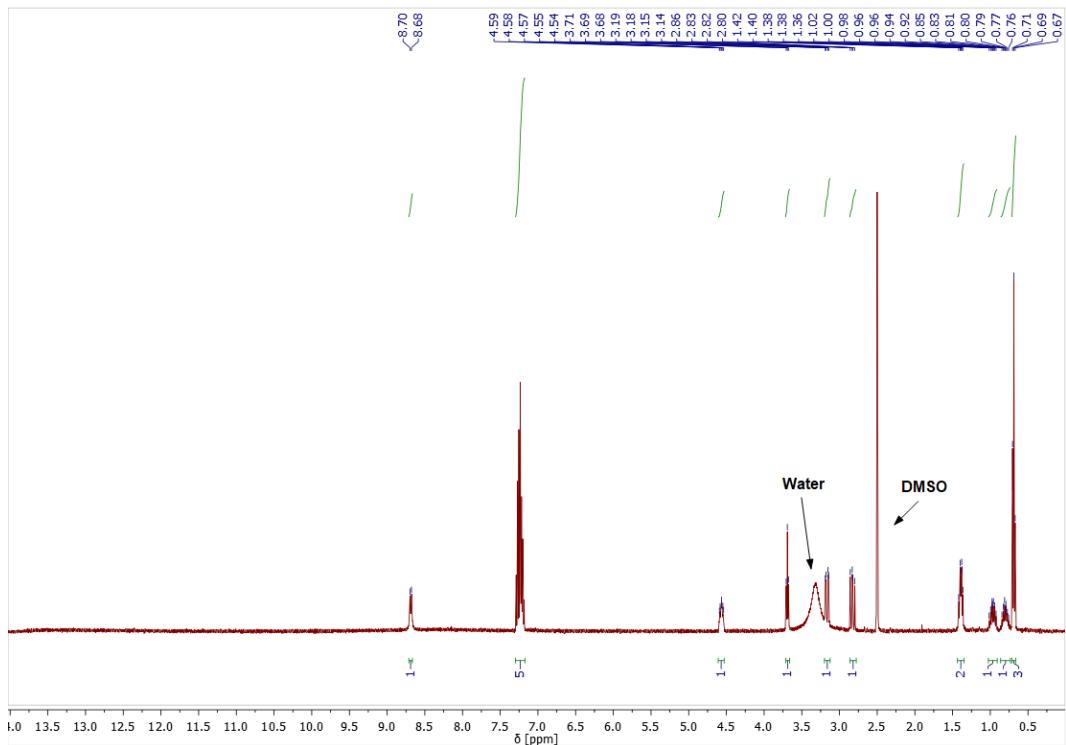


**Figure S5** ESI-MS spectrum of L-Nva-L-Phe (negative mode).

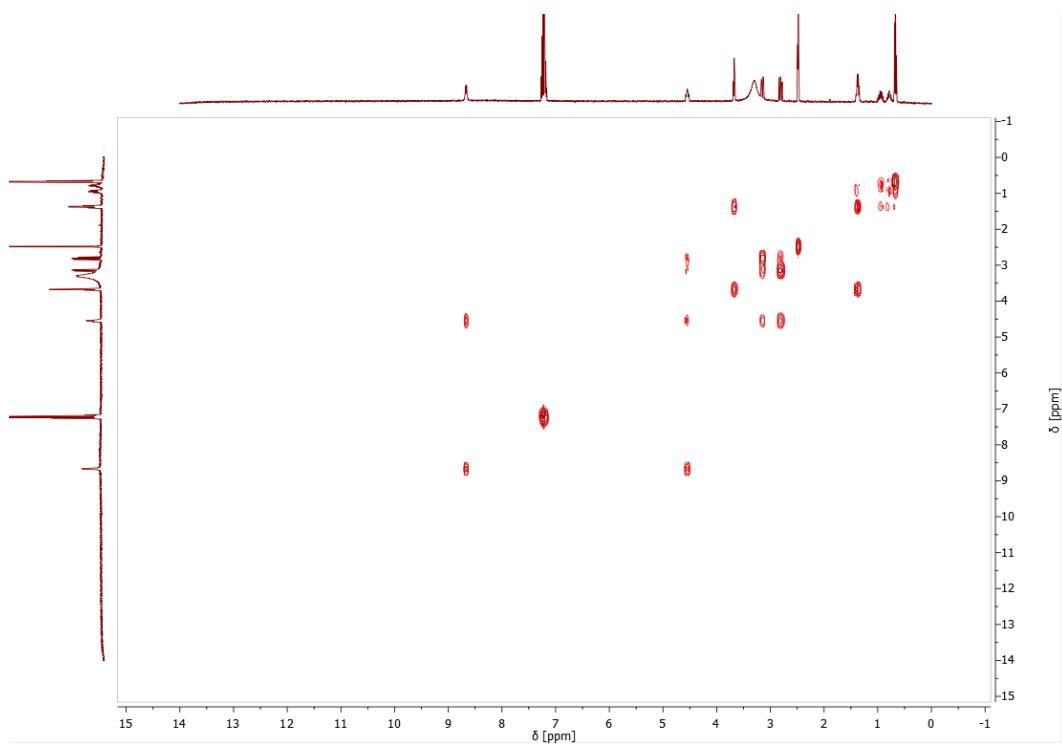
## 2. L-Nva-D-Phe spectroscopic data



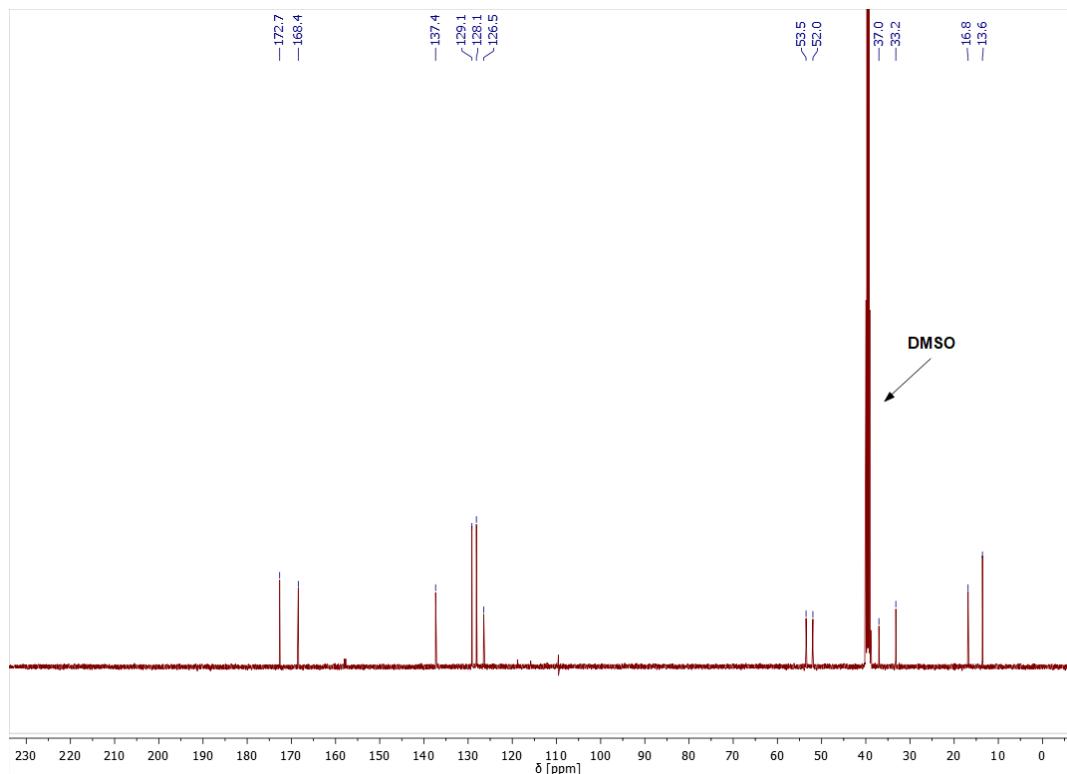
**<sup>1</sup>H NMR** (400 MHz, TMS, DMSO-*d*<sub>6</sub>) δ (ppm): 8.69 (1H, d, *J* = 8.0 Hz, NH), 7.24 (5H, m, Ar), 4.57 (1H, ddd, *J* = 4.4, 8.0, 10.4 Hz, CHα Phe), 3.69 (1H, dd, *J* = 6.0, 6.0 Hz, CHα Nva), 3.16 (1H, dd, *J* = 4.4, 14.0 Hz, CH<sub>2</sub>β Phe), 2.83 (1H, dd, *J* = 10.4, 14.0 Hz, CH<sub>2</sub>β Phe), 1.39 (2H, m, CH<sub>2</sub>β Nva), 0.96 (1H, m, CH<sub>2</sub>γ Nva), 0.81 (1H, m, CH<sub>2</sub>γ Nva), 0.69 (3H, dd, *J* = 7.2 Hz, CH<sub>3</sub>). **<sup>13</sup>C NMR** (100 MHz, TMS, DMSO-*d*<sub>6</sub>) δ (ppm): 172.7 (1C, COOH), 168.4 (1C, CONHR), 137.4 (1C, Ar), 129.1 (2C, Ar), 128.1 (2C, Ar), 126.5 (1C, Ar), 53.5 (1C, Cα), 52.0 (1C, Cα), 37.0 (1C, Cβ), 33.2 (1C, Cβ), 16.8 (1C, Cγ), 13.6 (1C, Cδ). **MS (ESI)**: C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> Exact mass: 264.2 m/z. Found 265.0 [M+H]<sup>+</sup>, 263.0 [M-H]<sup>-</sup>.



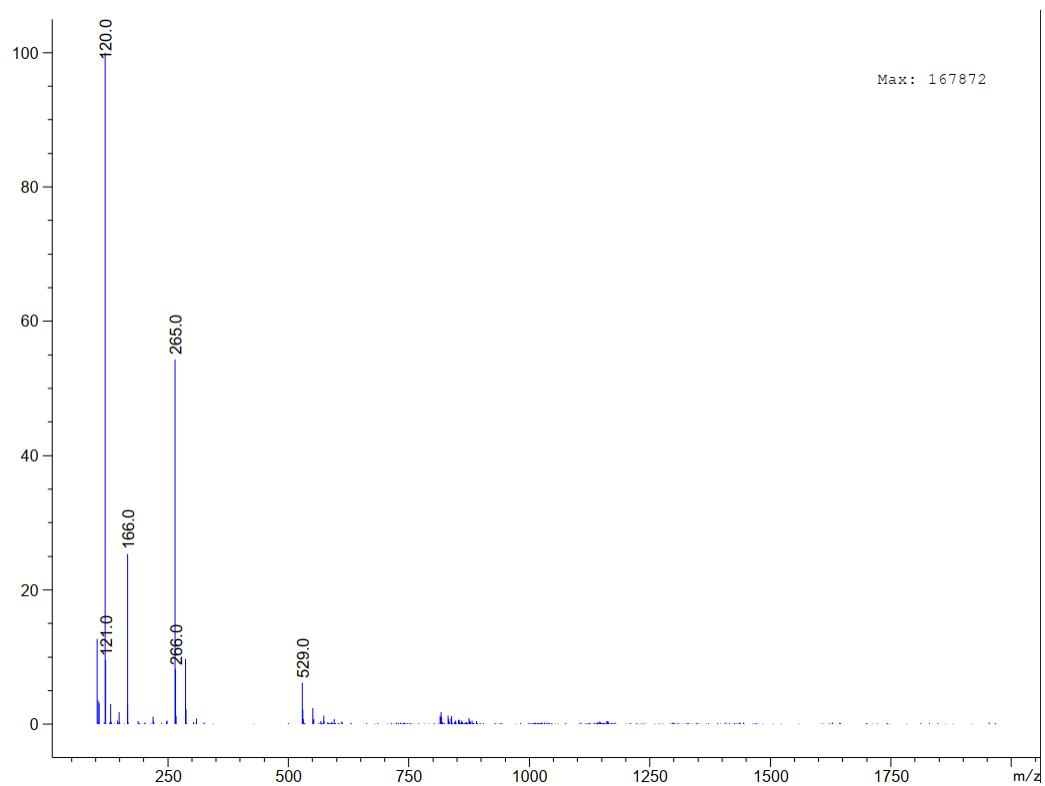
**Figure S6** <sup>1</sup>H NMR spectrum of L-Nva-D-Phe.



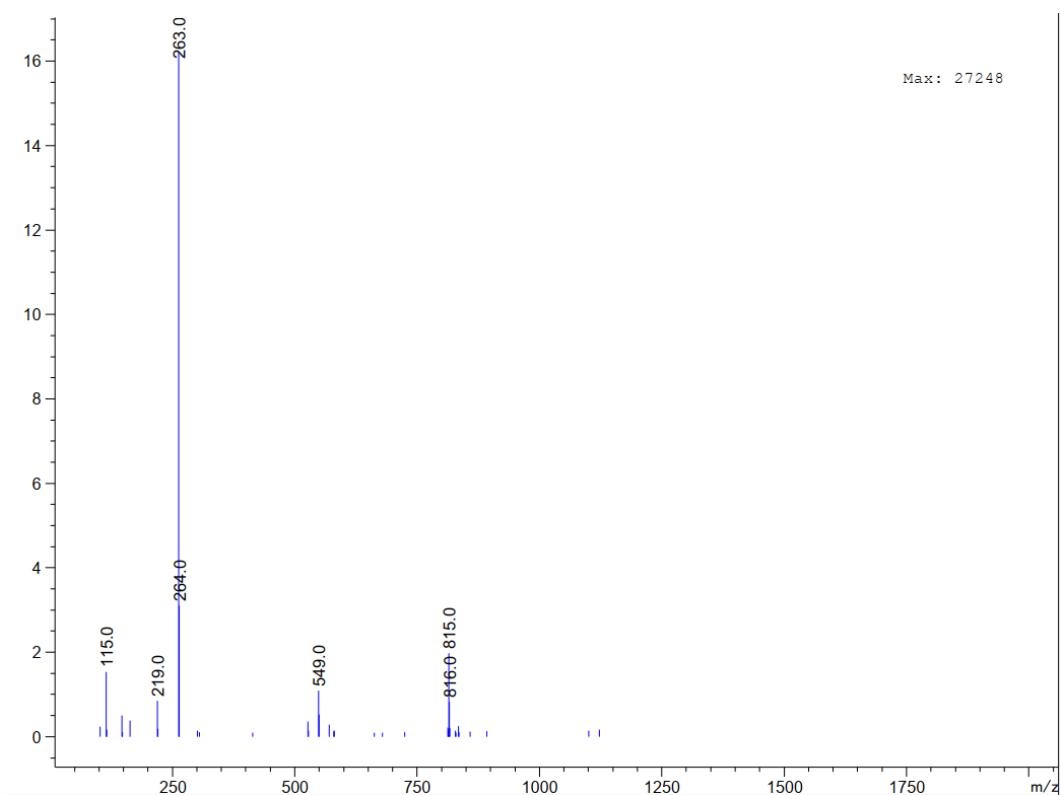
**Figure S7** gCOSY 2D NMR spectrum of L-Nva-D-Phe.



**Figure S8**  $^{13}\text{C}$  NMR spectrum of L-Nva-D-Phe.

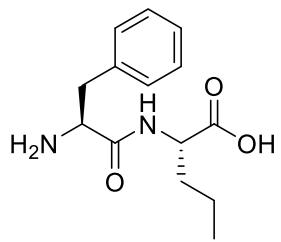


**Figure S9** ESI-MS spectrum of L-Nva-D-Phe (positive mode).

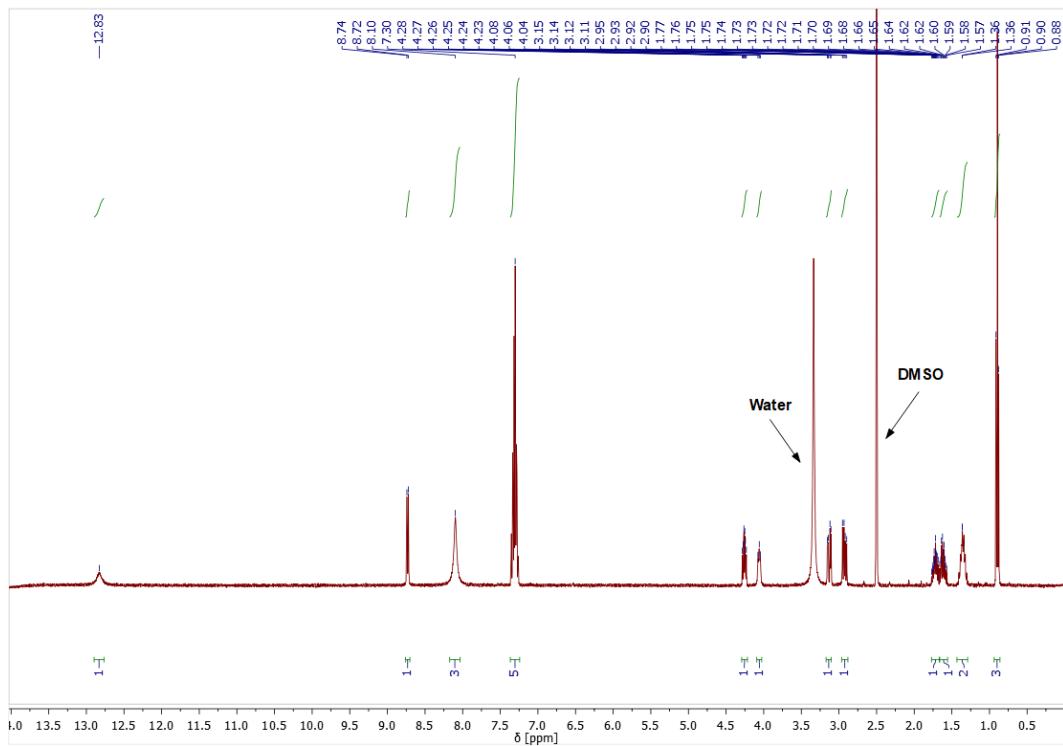


**Figure S10** ESI-MS spectrum of L-Nva-D-Phe (negative mode).

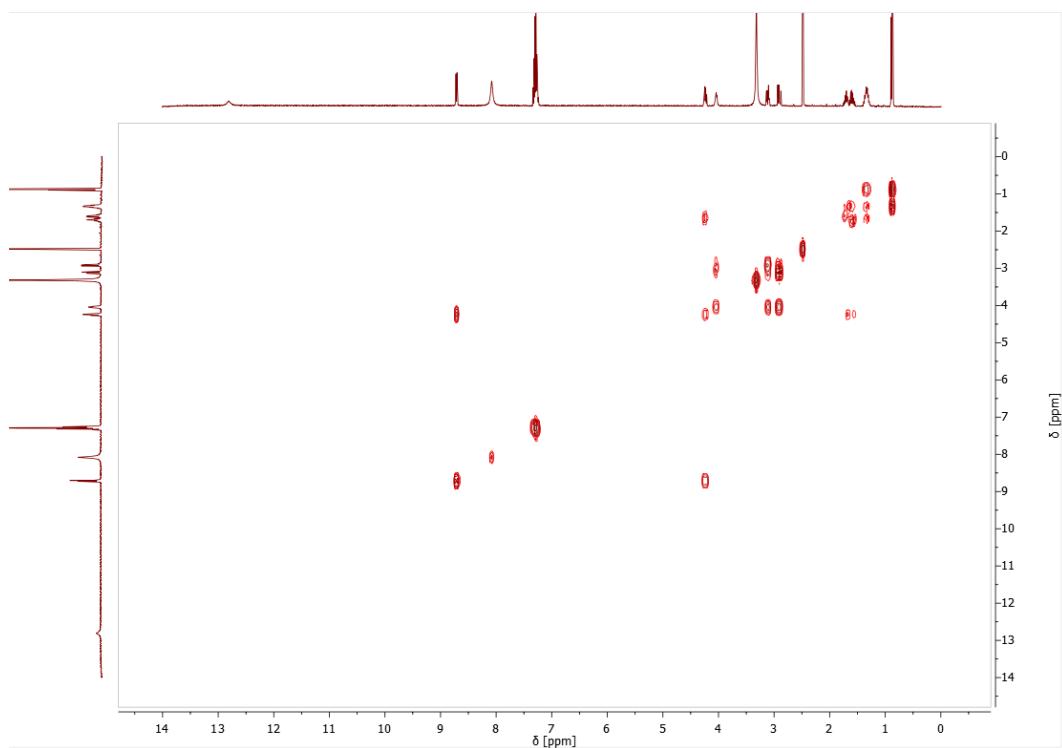
### 3. L-Phe-L-Nva spectroscopic data



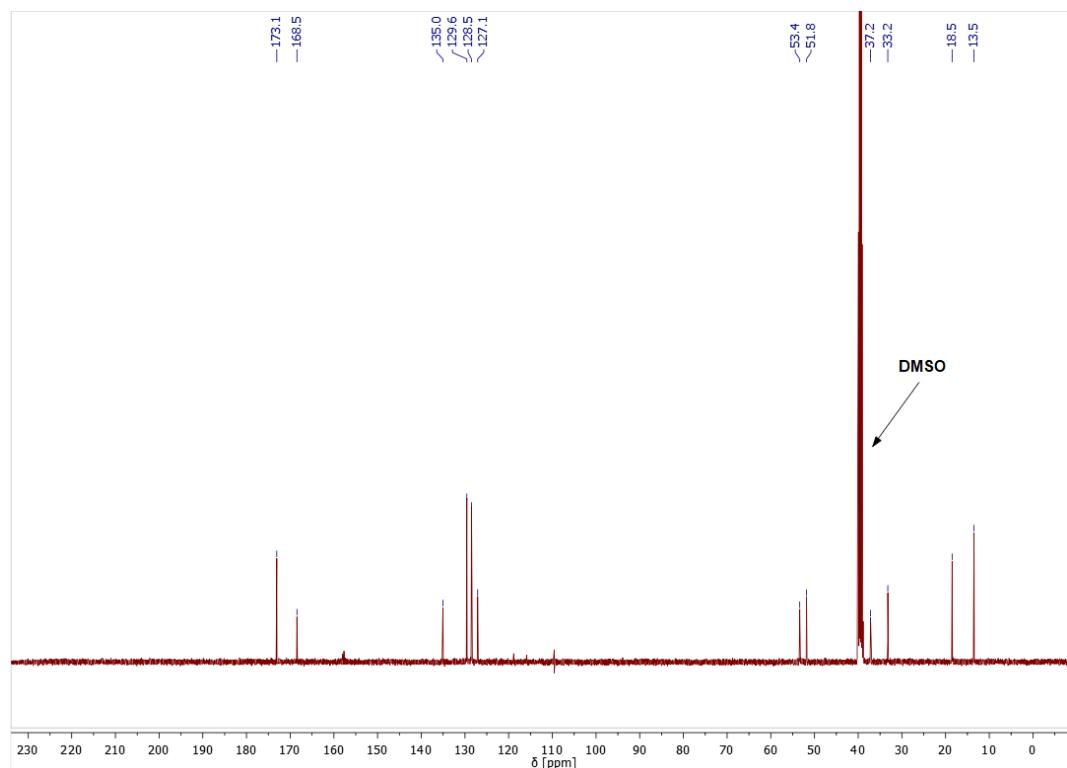
**<sup>1</sup>H NMR** (400 MHz, TMS, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 12.83 (1H, s, COOH), 8.73 (1H, d, *J* = 8.0 Hz, NH), 8.10 (3H, s, NH<sub>3</sub><sup>+</sup>), 7.30 (5H, m, Ar), 4.26 (1H, ddd, *J* = 8.0, 6.4, 4.8 Hz, CH $\alpha$  Nva), 4.06 (1H, d, *J* = 8.4, 5.2 Hz, CH $\alpha$  Phe), 3.13 (1H, dd, *J* = 5.2, 14.0 Hz, CH $\beta\beta$  Phe), 2.93 (1H, dd, *J* = 8.4, 14.0 Hz, CH $\beta\beta$  Phe), 1.72 (1H, m, CH $\beta\beta$  Nva), 1.62 (1H, m, CH $\beta\beta$  Nva), 1.35 (2H, m, CH $\gamma\gamma$ ), 0.90 (3H, dd, *J* = 7.2 Hz, CH<sub>3</sub>). **<sup>13</sup>C NMR** (100 MHz, TMS, DMSO-*d*<sub>6</sub>)  $\delta$  (ppm): 173.1 (1C, COOH), 168.5 (1C, CONHR), 135.0 (1C, Ar), 129.6 (2C, Ar), 128.5 (2C, Ar), 127.1 (1C, Ar), 53.4 (1C, C $\alpha$ ), 51.8 (1C, C $\alpha$ ), 37.2 (1C, C $\beta$ ), 33.2 (1C, C $\beta$ ), 18.5 (1C, C $\gamma$ ), 13.5 (1C, C $\delta$ ). **MS (ESI)**: C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> Exact mass: 264.2 m/z. Found 265.0 [M+H]<sup>+</sup>, 287.0 [M+Na]<sup>+</sup>, 263.0 [M-H]<sup>-</sup>.



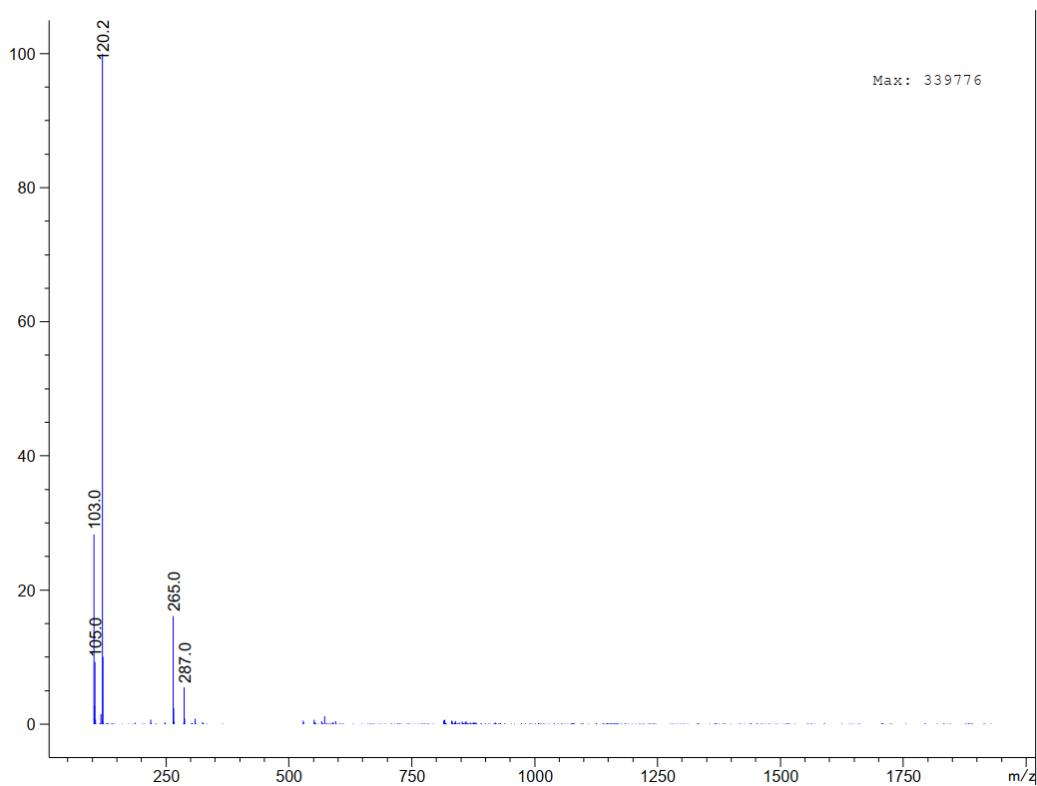
**Figure S11** <sup>1</sup>H NMR spectrum of L-Phe-L-Nva.



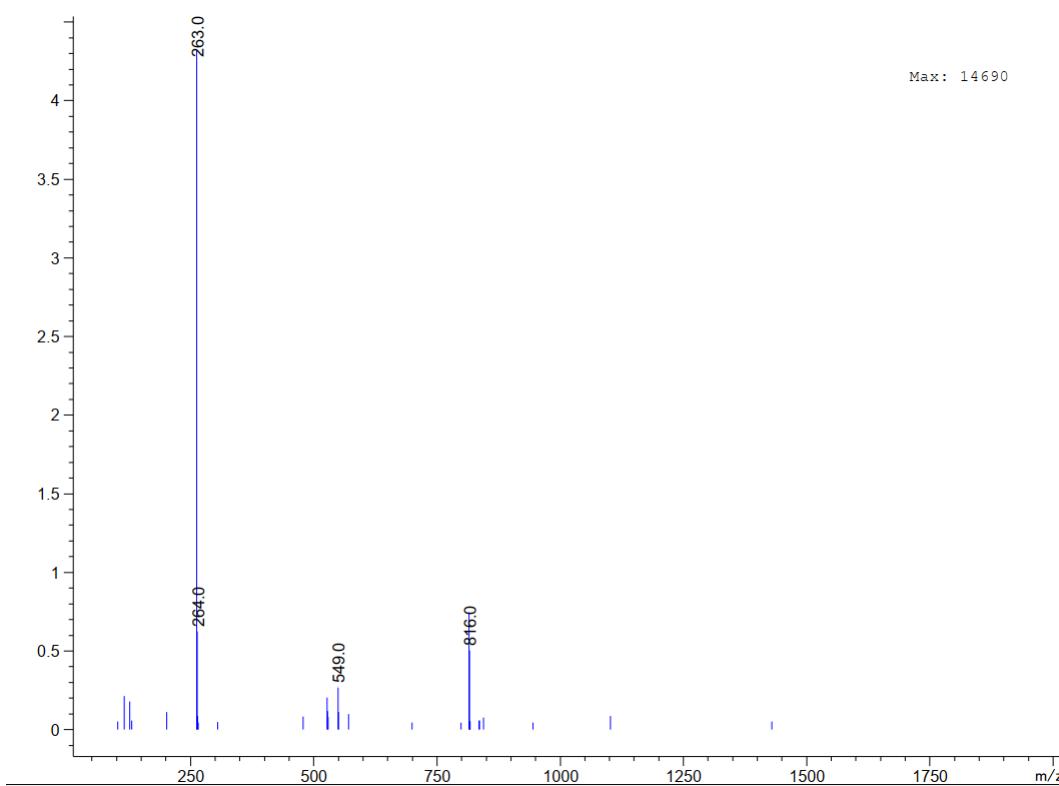
**Figure S12** gCOSY 2D NMR spectrum of L-Phe-L-Nva.



**Figure S13**  $^{13}\text{C}$  NMR spectrum of L-Phe-L-Nva.

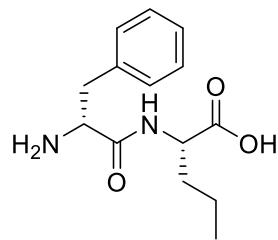


**Figure S14** ESI-MS spectrum of L-Phe-L-Nva (positive mode).



**Figure S15** ESI-MS spectrum of L-Phe-L-Nva (negative mode).

#### 4. D-Phe-L-Nva spectroscopic data



**<sup>1</sup>H NMR** (400 MHz, TMS, DMSO-*d*<sub>6</sub>) δ (ppm): 8.60 (1H, d, *J* = 8.0 Hz, NH), 7.29 (5H, m, Ar), 4.20 (1H, ddd, *J* = 8.4, 8.0, 7.2 Hz, CH<sub>α</sub> Nva), 4.07 (1H, dd, *J* = 8.0, 7.2 Hz, CH<sub>α</sub> Phe), 2.99 (2H, dd, *J* = 13.4, 8.0, 7.2 Hz, CH<sub>β</sub> Phe), 1.49 (2H, m, CH<sub>β</sub> Nva), 1.05 (2H, m, CH<sub>γ</sub> Nva), 0.78 (3H, dd, *J* = 7.2 Hz, CH<sub>3</sub>). **<sup>13</sup>C NMR** (100 MHz, TMS, DMSO-*d*<sub>6</sub>) δ (ppm): 173.0 (1C, COOH), 167.9 (1C, CONHR), 134.8 (1C, Ar), 129.5 (2C, Ar), 128.5 (2C, Ar), 127.1 (1C, Ar), 53.2 (1C, C<sub>α</sub>), 51.6 (1C, C<sub>α</sub>), 37.4 (1C, C<sub>β</sub>), 33.3 (1C, C<sub>β</sub>), 18.1 (1C, C<sub>γ</sub>), 13.5 (1C, C<sub>δ</sub>). **MS (ESI)**: C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> Exact mass: 264.2 m/z. Found 265.0 [M+H]<sup>+</sup>, 287.0 [M+Na]<sup>+</sup>, 263.0 [M-H]<sup>-</sup>.

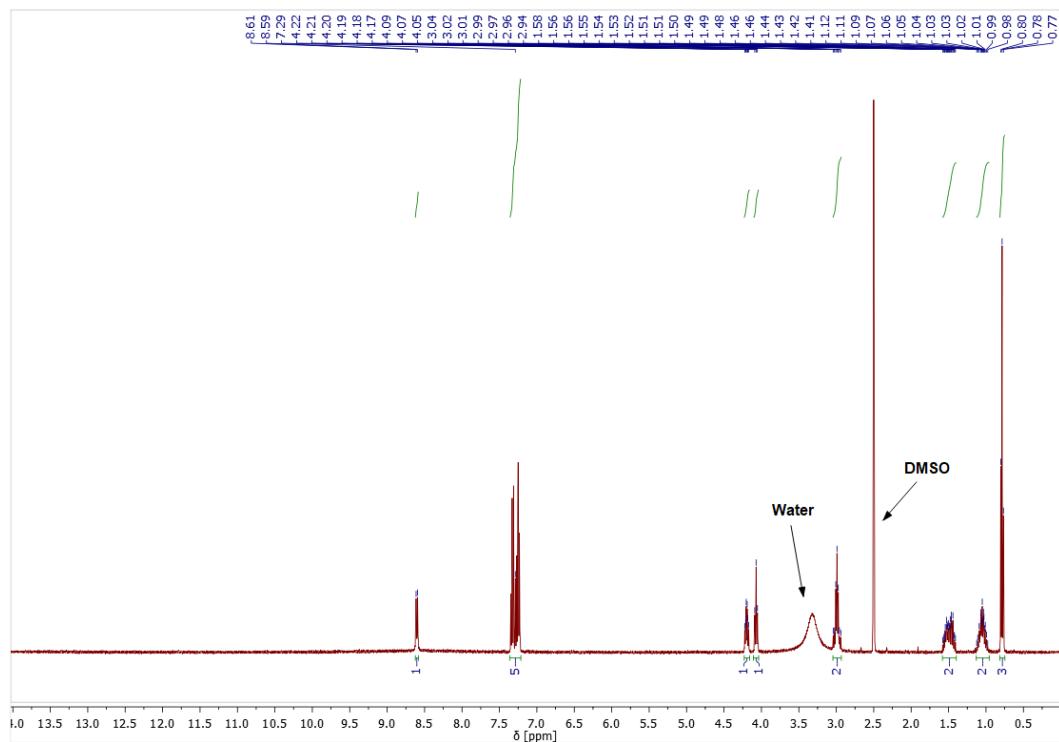
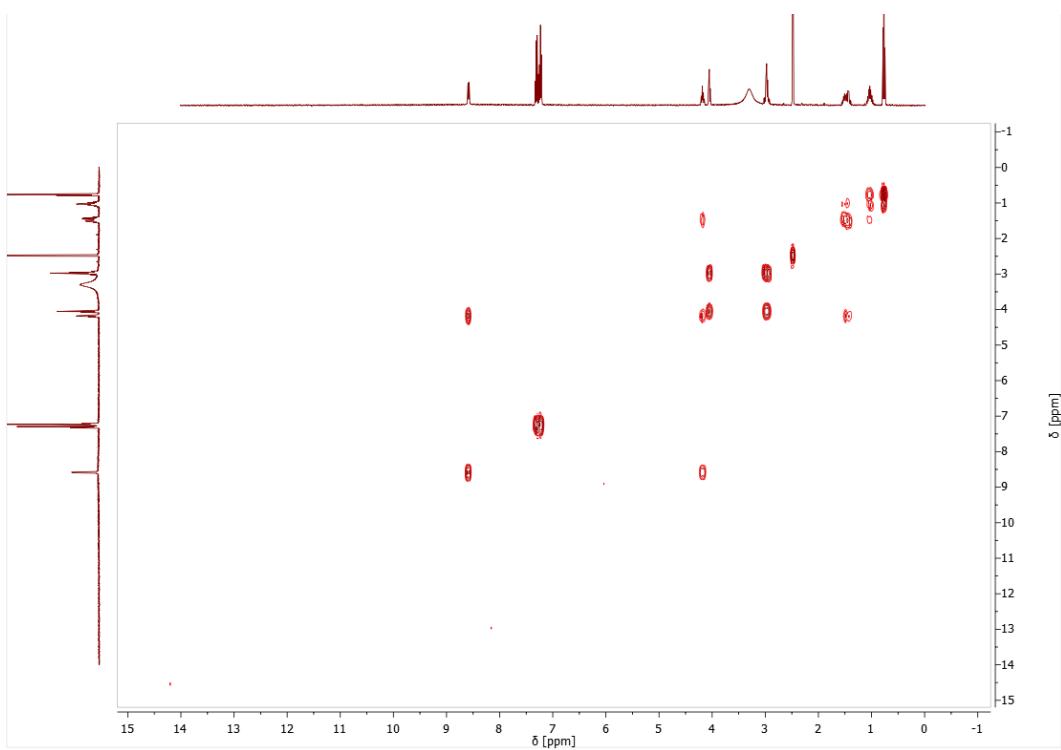
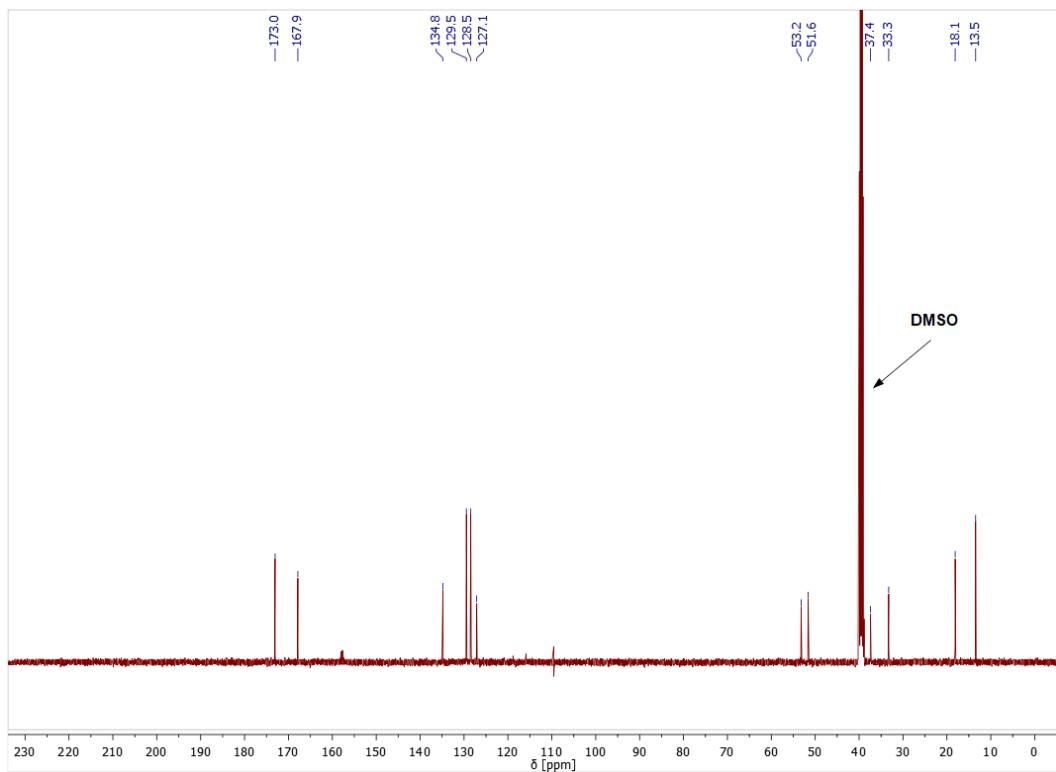


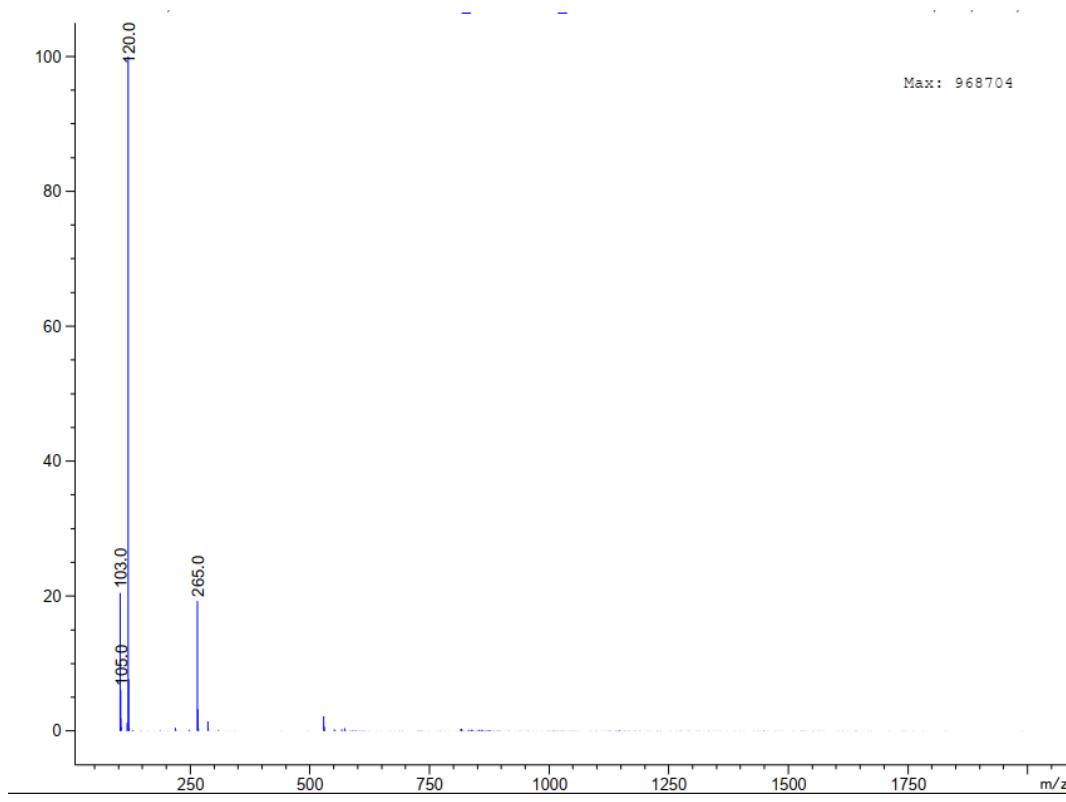
Figure S16 <sup>1</sup>H NMR spectrum of D-Phe-L-Nva.



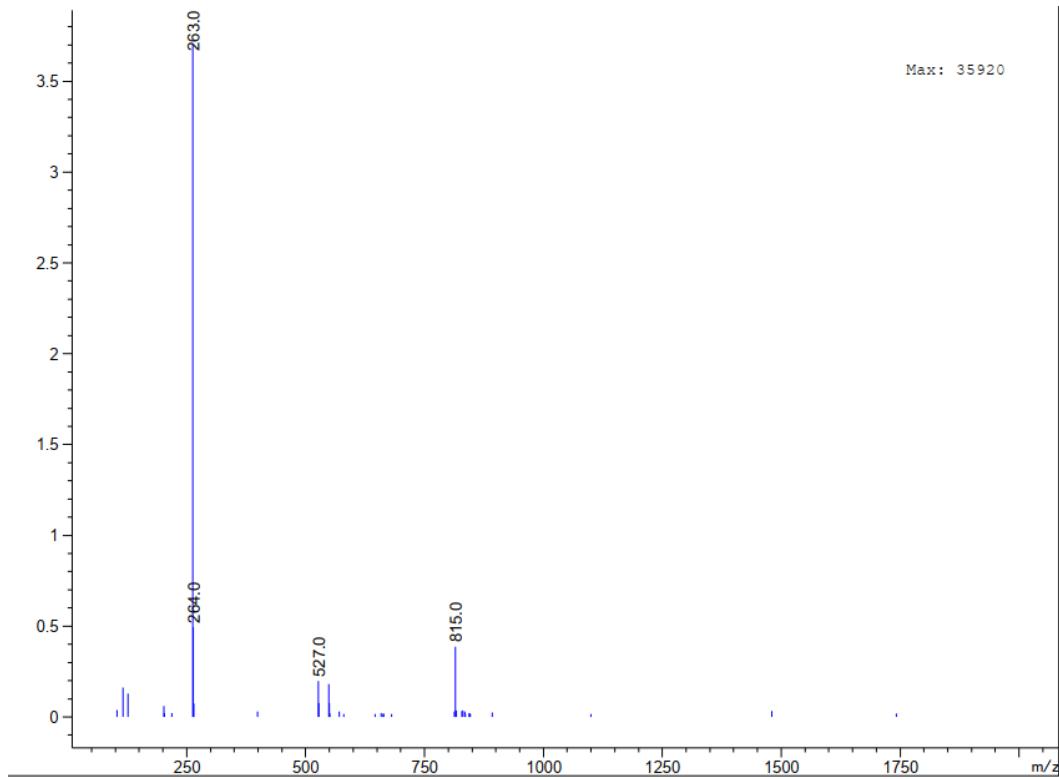
**Figure S17** gCOSY 2D NMR spectrum of D-Phe-L-Nva.



**Figure S18**  $^{13}\text{C}$  NMR spectrum of D-Phe-L-Nva.

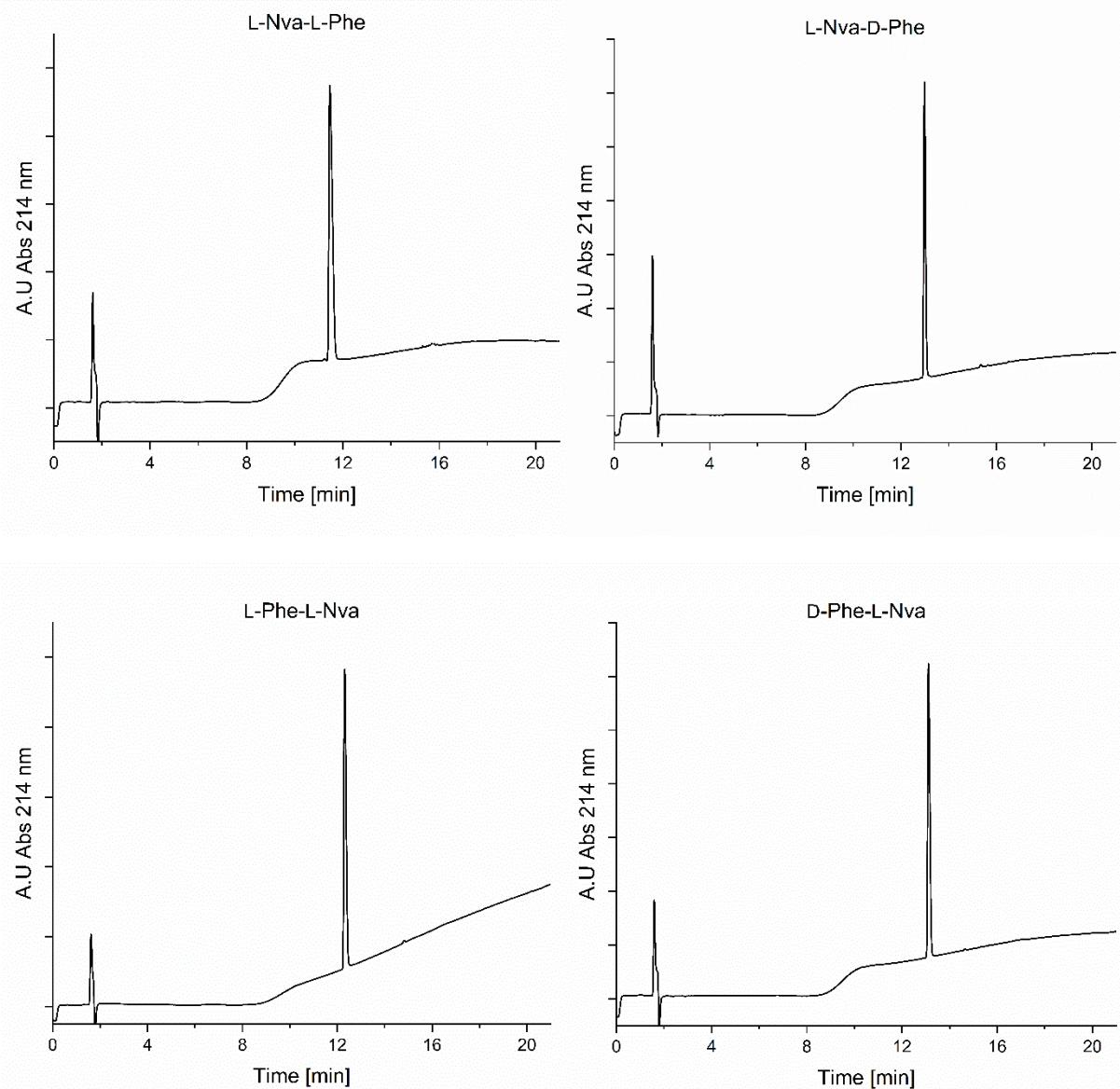


**Figure S19** ESI-MS spectrum of D-Phe-L-Nva (positive mode).



**Figure S20** ESI-MS spectrum of D-Phe-L-Nva (negative mode).

## 5. HPLC traces

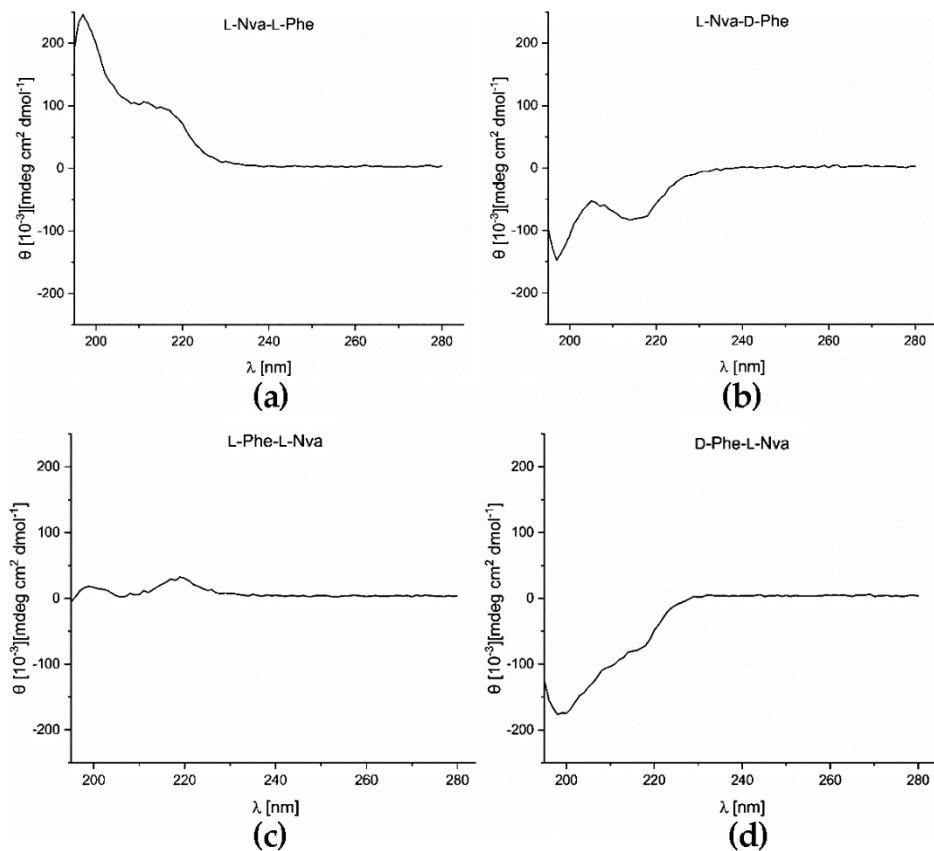


**Figure S21** LC traces of the four peptides. Retention times are reported in **table S1**.

	Retention time [min]
L-Nva-L-Phe	11.4
L-Nva-D-Phe	13.0
L-Phe-L-Nva	12.4
D-Phe-L-Nva	13.2

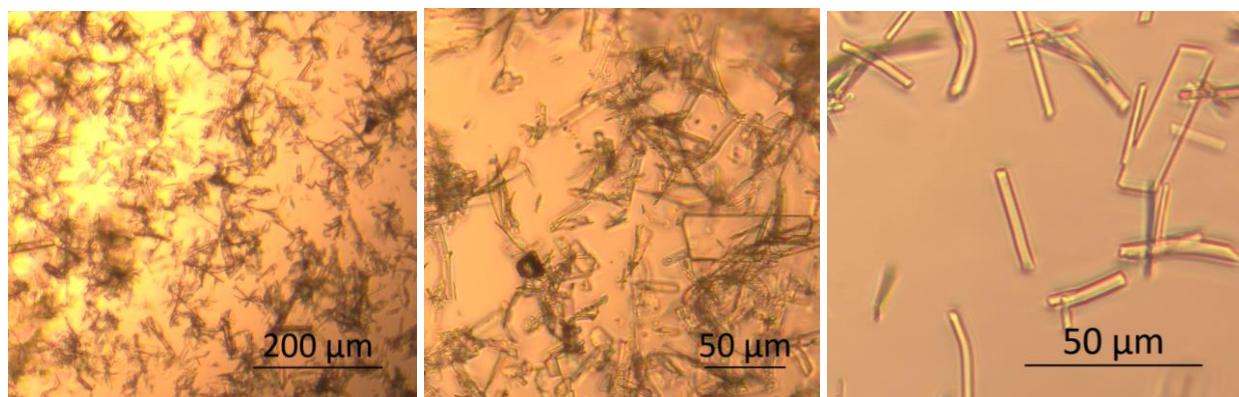
**Table S1.** Retention times of the four peptides. LC method: linear gradient of the mobile phase H<sub>2</sub>O/MeCN 0.1% FA from 5% MeCN to 95% in 20 min with 0.3 ml/min flow. Phenomenex LUNA column C18 (2), 5 µm, 100 Å, 150x2 mm.

## 6. CD spectra



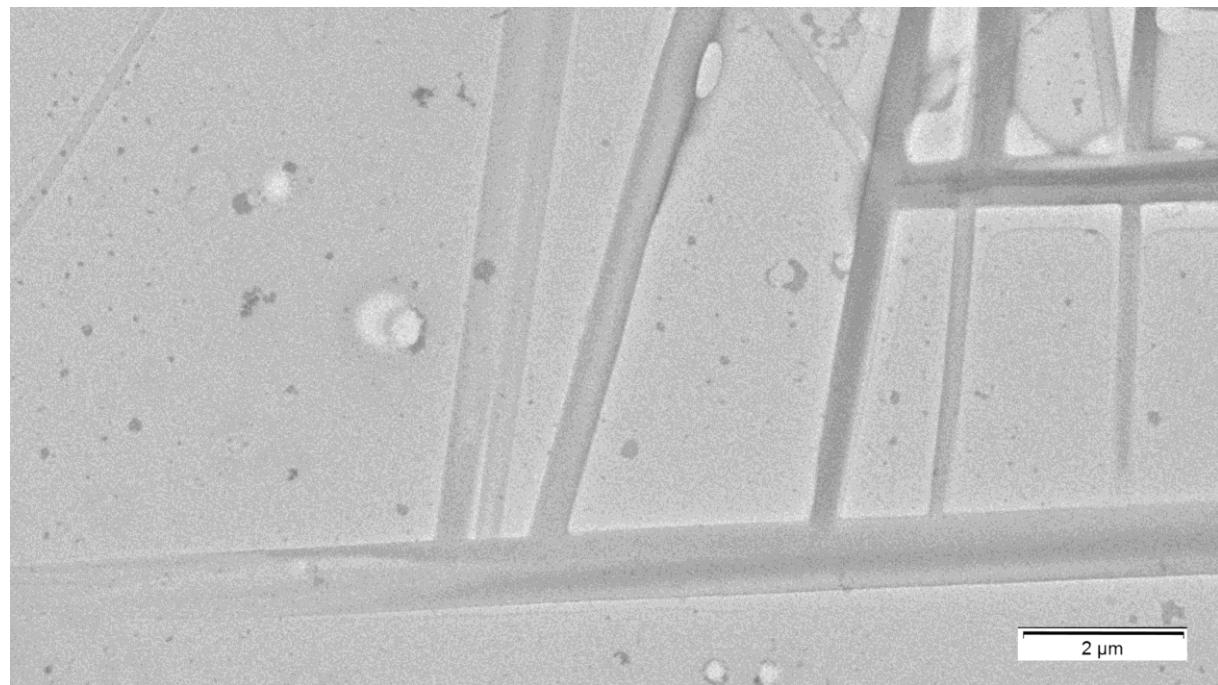
**Figure S22.** CD spectra of 5 mM aqueous solutions of the four compounds: (a) **1**, (b) **2**, (c) **3**, and (d) **4**.

## 7. Optical microscopy images



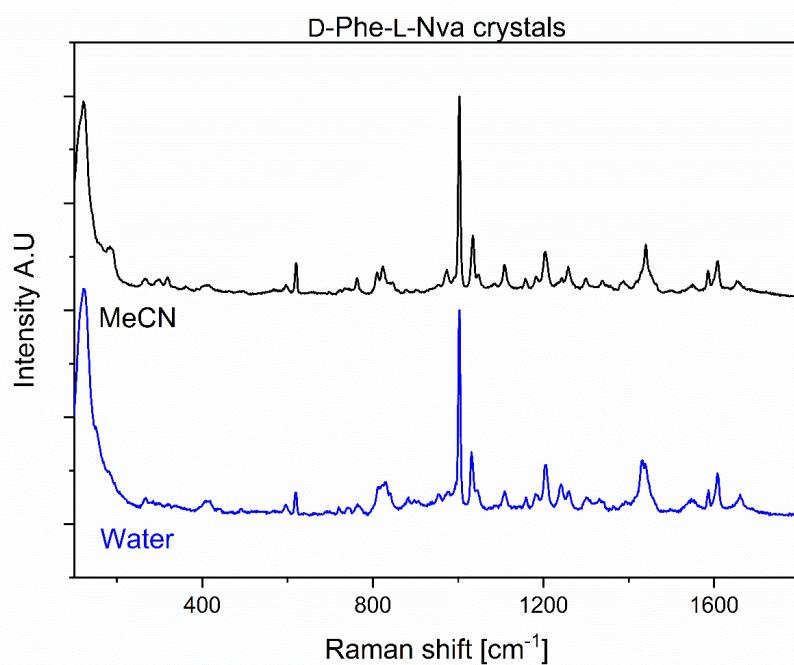
**Figure S23.** Sequence of optical microscopy images of dipeptide **4** in PBS (left) rapidly forming (center) large crystals (right).

## 8. TEM imaging



**Figure S24.** TEM micrograph of dipeptide **4** in MeCN.

## 9. Raman spectra

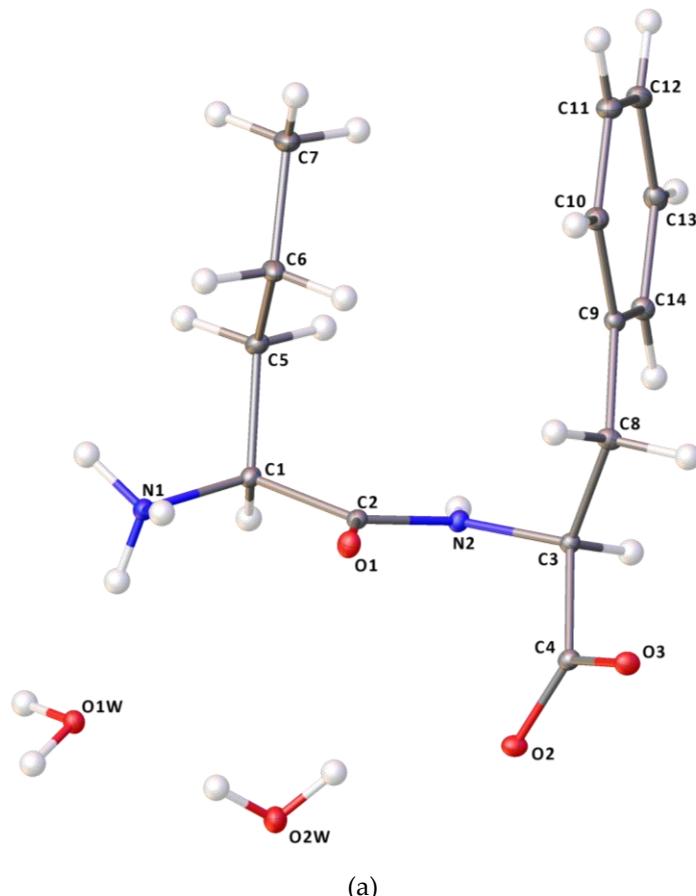


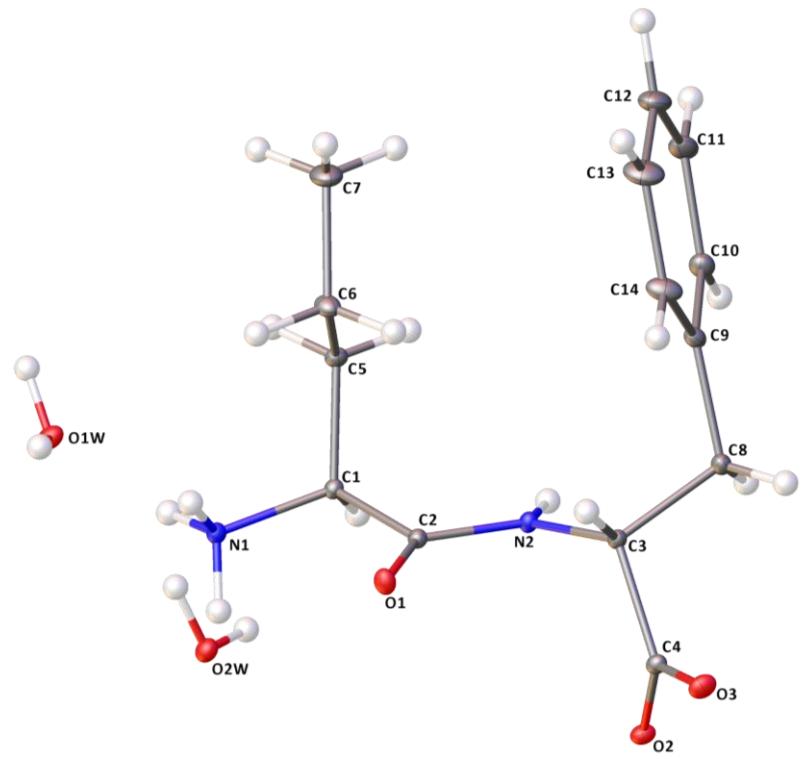
**Figure S25.** Raman spectra of D-Phe-L-Nva (**4**) after crystallisation in water and in acetonitrile.

## 10. Single-crystal XRD

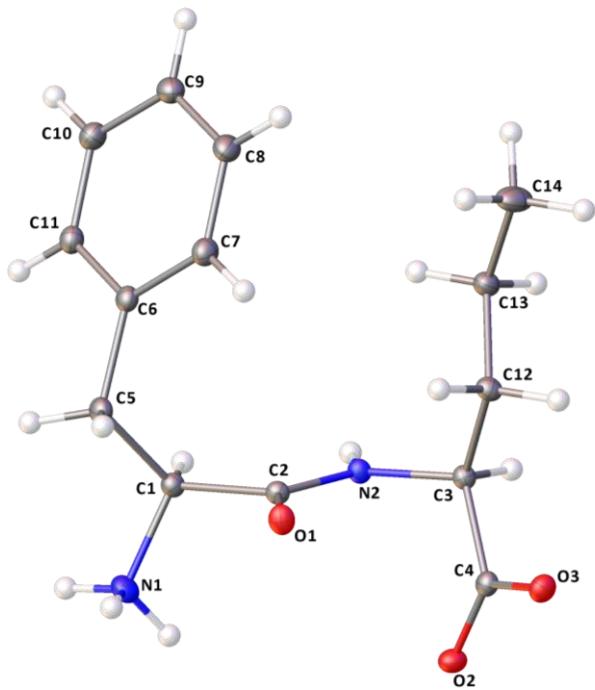
Crystals of **1**, **2**, **3** and **4** were mounted on the diffractometer at the synchrotron Elettra, Trieste (Italy), beamline XRD2 and measured at 100 K. Data collection were performed using synchrotron radiation ( $\lambda = 0.6200 \text{ \AA}$ ) with the rotating crystal method (0.5°/image) for a total of 720 images for each crystal. Data indexing were performed using MOSFLM,<sup>1</sup> while space groups were determined using POINTLESS.<sup>2</sup> The software AIMLESS<sup>3</sup> was used for scaling the data. The structures were solved using the software SHELXT<sup>4</sup> and refined through full matrix least-squares based on  $F^2$  using the programs SHELXL<sup>5</sup> and OLEX2<sup>6</sup> as a GUI. Non-hydrogen atoms were refined anisotropically, whereas hydrogen atoms were geometrically positioned and included in structure factor calculations but not refined.

Considering the highly disordered water molecules in crystal structure of **3** we decided to apply a solvent mask. In particular, a solvent mask was calculated and 118 electrons were found in a volume of  $252 \text{ \AA}^3$ . This is consistent with the presence of  $3(\text{H}_2\text{O})$  per asymmetric unit which account for 120 electrons per unit cell. ORTEP diagrams (Figure S26) were drawn using OLEX2. In Table S2 are reported relevant the crystallographic data.

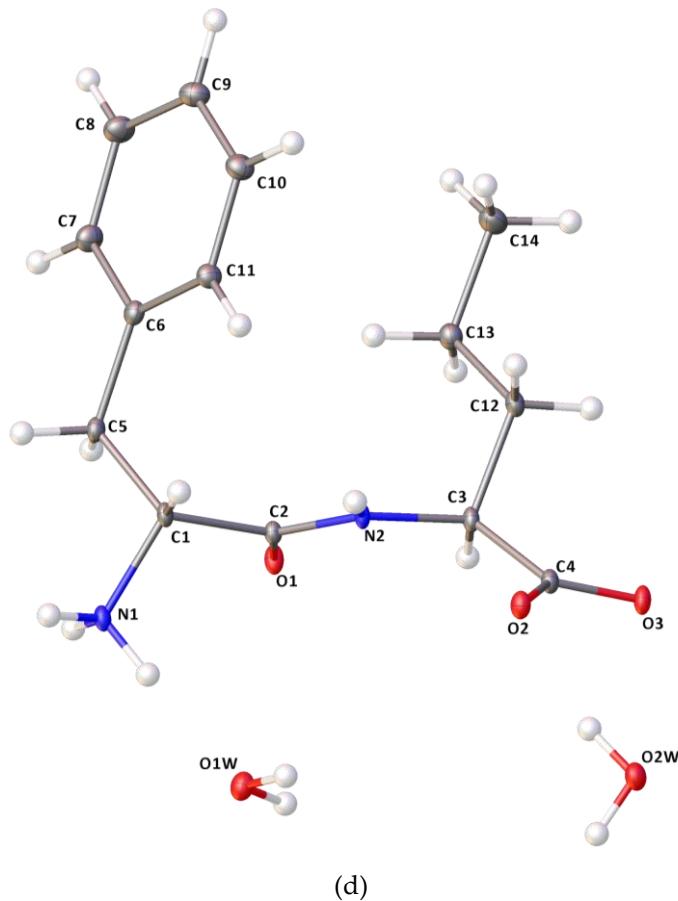




(b)

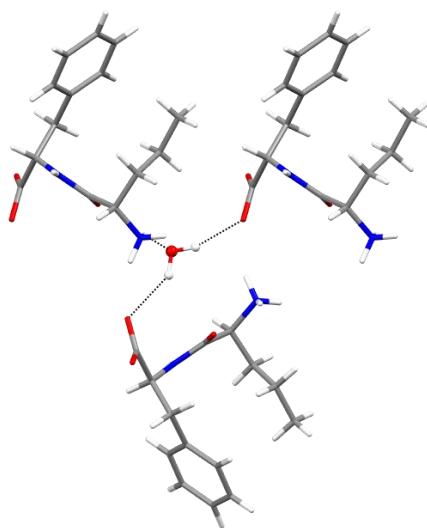


(c)



(d)

**Figure S26.** ORTEP diagrams of (a) **1** (CCDC 2210050), (b) **2** (CCDC 2210051), (c) **3** (CCDC 2210055) and (d) **4** (CCDC 2210056). Ellipsoids are drawn at 20% probability level. Atom types: C grey, H white, O red, N blue.



**Figure S27.** Host-guest interactions between O2W and three dipeptides in **1** crystal structure.

**Table S2.** Relevant crystallographic data for the crystal structures **1**, **2**, **3** and **4**.

	<b>1</b> CCDC 2210050	<b>2</b> CCDC 2210051	<b>3</b> CCDC 2210055	<b>4</b> CCDC 2210056
<b>T (K)</b>	100	100	100	100
<b>Formula</b>	C <sub>14</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> , 2(H <sub>2</sub> O)	C <sub>14</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> , 2(H <sub>2</sub> O)	C <sub>14</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> , 3(H <sub>2</sub> O)	C <sub>14</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> , 2(H <sub>2</sub> O)
<b>Formula weight</b>	300.35	300.35	318.37	300.35
<b>System</b>	P <sub>2</sub> 12 <sub>1</sub> 2 <sub>1</sub>	P2 <sub>1</sub>	P <sub>2</sub> 12 <sub>1</sub> 2 <sub>1</sub>	C2
<b>Space group</b>	orthorhombic	monoclinic	orthorhombic	monoclinic
<i>a</i> (Å)	5.6190(11)	6.0040(12)	4.780(1)	16.414(3)
<i>b</i> (Å)	8.2010(16)	8.2670(16)	8.9990(18)	4.792(1)
<i>c</i> (Å)	33.608(7)	16.108(3)	38.420(8)	19.859(4)
$\alpha$ (°)	90	90	90	90
$\beta$ (°)	90	91.19(3)	90	100.14(3)
$\gamma$ (°)	90	90	90	90
<i>V</i> (Å <sup>3</sup> )	1548.7(5)	799.3(3)	1652.6(6)	1537.6(5)
<b>Z</b>	4	2	4	4
<i>D<sub>x</sub></i> (g cm <sup>-3</sup> )	1.288	1.248	1.280	1.297
$\lambda$ (Å)	0.62000	0.62000	0.62000	0.62000
$\mu$ (mm <sup>-1</sup> )	0.072	0.070	0.074	0.073
<i>F</i> <sub>000</sub>	648.0	324.0	688.0	648.0
<b>R1 (I &gt; 2σI)</b>	0.0288(5355)	0.0268(5467)	0.0466(5589)	0.0500(4958)
<b>wR<sub>2</sub></b>	0.0836(5442)	0.0733(5521)	0.1385(5831)	0.1431(5297)
<b>N. of param.</b>	199	199	174	198
<b>GooF</b>	1.041	1.054	1.047	1.089
$\rho_{min}$ , $\rho_{max}$ (eÅ <sup>-3</sup> )	-0.19, 0.32	-0.18, 0.29	-0.39, 0.43	-0.28, 0.49
<b>Flack <i>x</i></b>	-0.1(2)	-0.18(16)	0.2(4)	0.4(5)
<b>Resolution (Å)</b>	0.67	0.67	0.67	0.67

## References

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- [4] Sheldrick, G. M., *Acta Crystallogr., Sect. A*, 2015, **71**, 3–8.
- [5] Sheldrick, G. M., *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.