

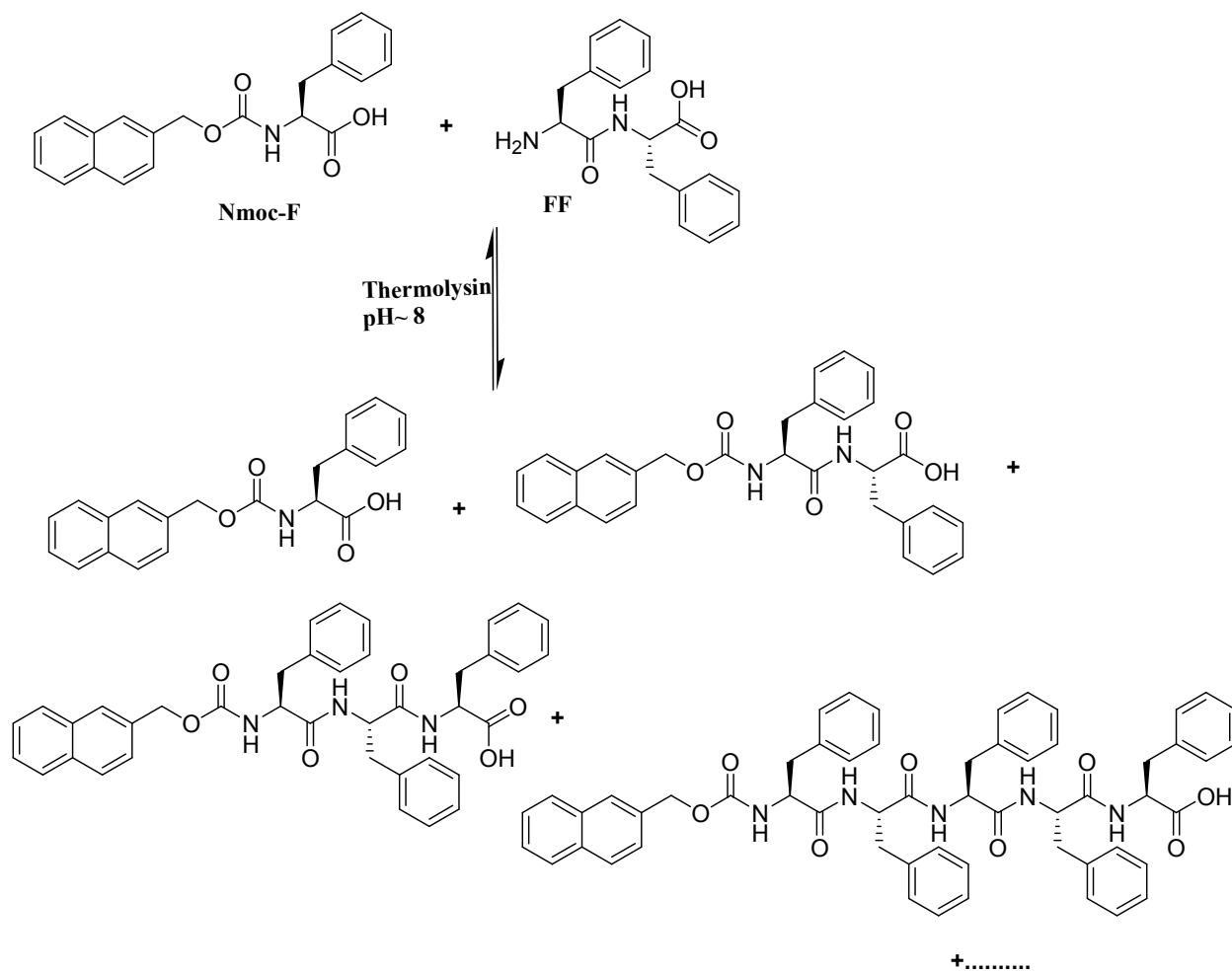
# Exploring Thermodynamically Downhill Nanostructured Peptide Libraries: From Structural to Morphological Insight

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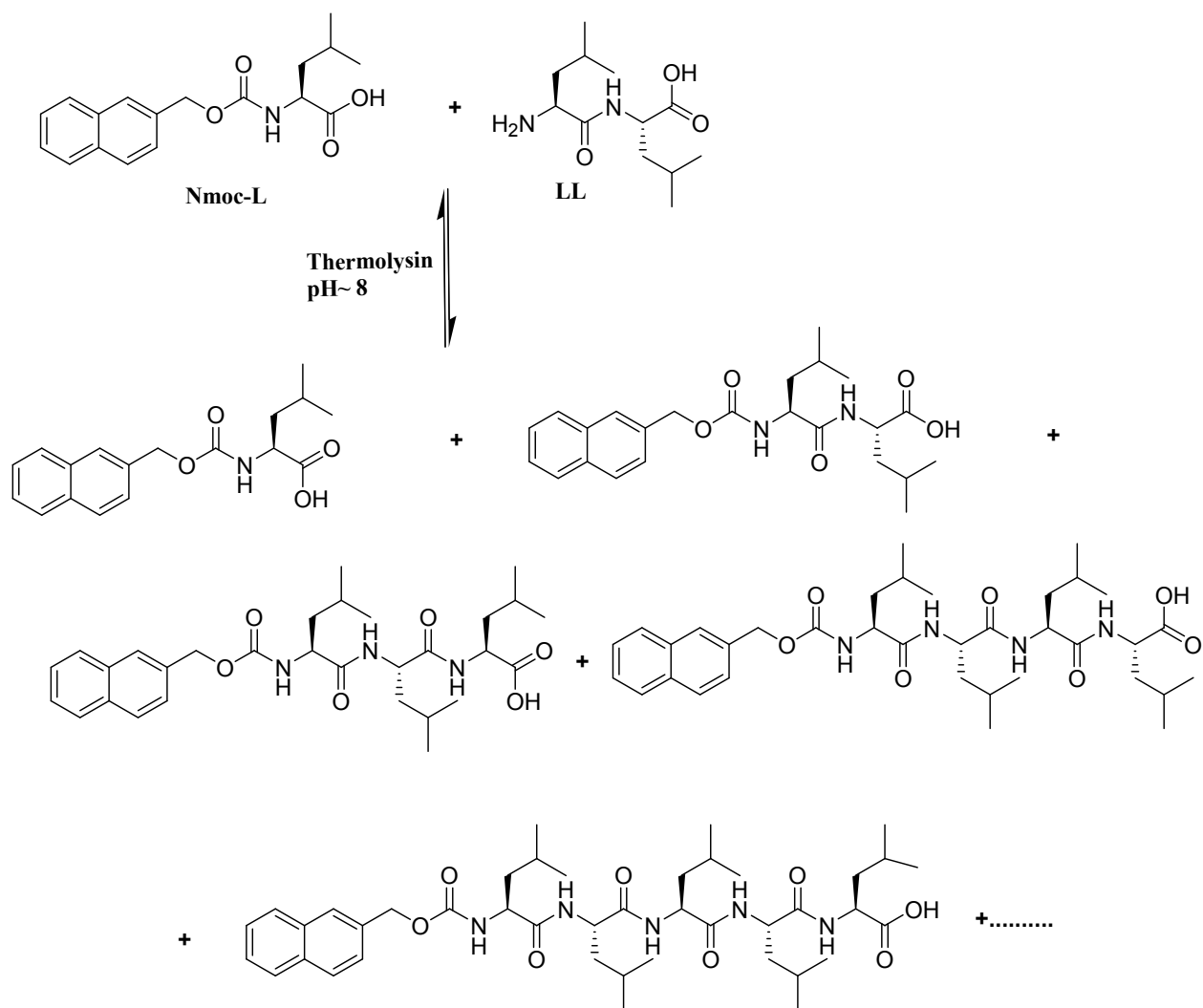
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**General Methods.**

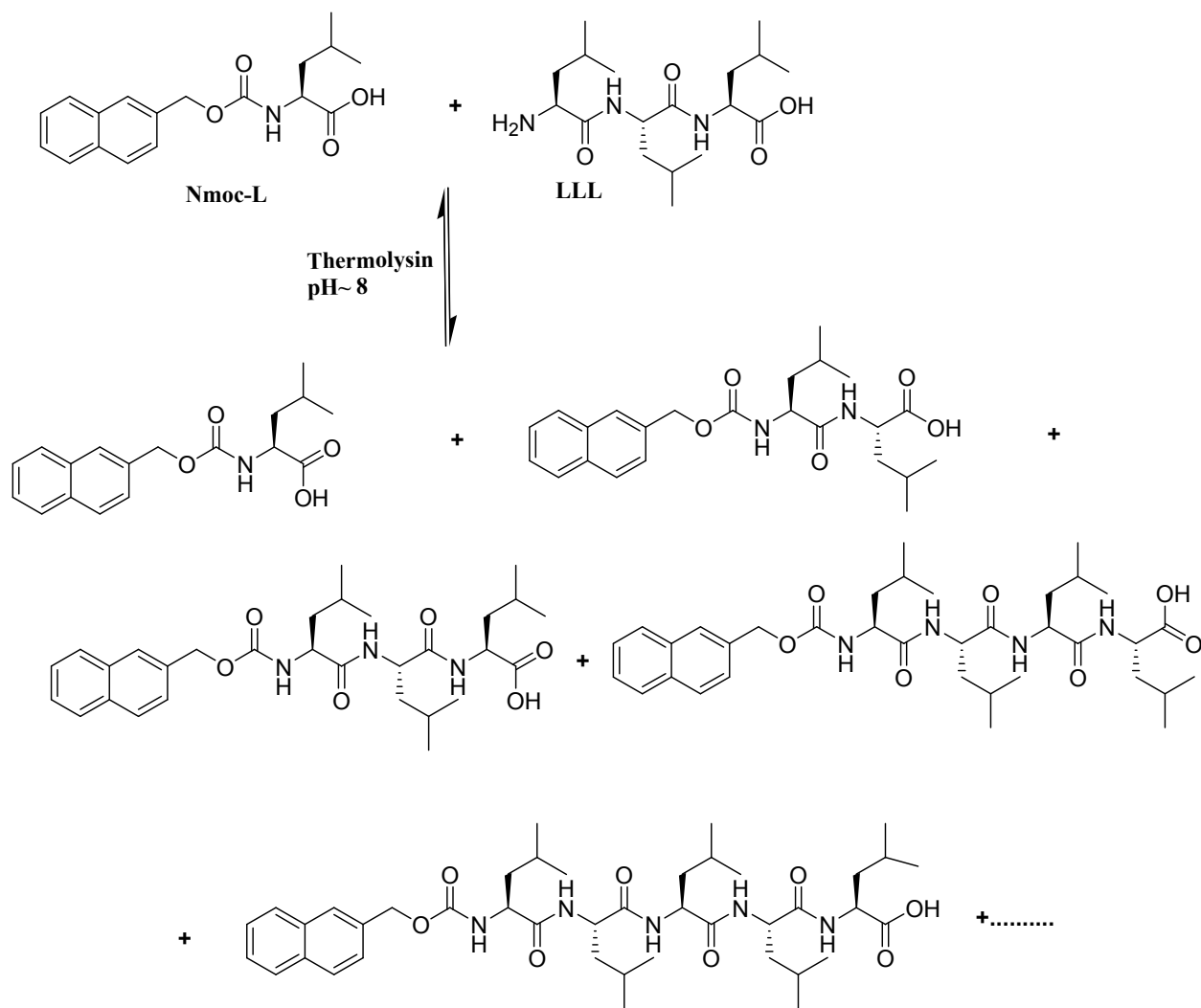
All the chemicals and reagents were obtained commercially. All NMR spectra were recorded at 400 MHz Bruker AV 400 NMR. Compounds concentrations were in the range of 1-10 mmol in  $(\text{CD}_3)_2\text{SO}$  and  $\text{CDCl}_3$ . Mass spectra were recorded on Bruker micrOTOF-Q II by positive and negative mode electrospray ionisations. All the reported FT-IR spectra were taken using Bruker (Tensor 27) FT-IR spectrophotometer. Specific rotations of the synthesized compounds were measured on an Autopol<sup>R</sup> V automatic polarimeter (Rudolph research analytical). The cell (length = 100 mm, capacity = 2 mL) was used for this study at 25 °C.



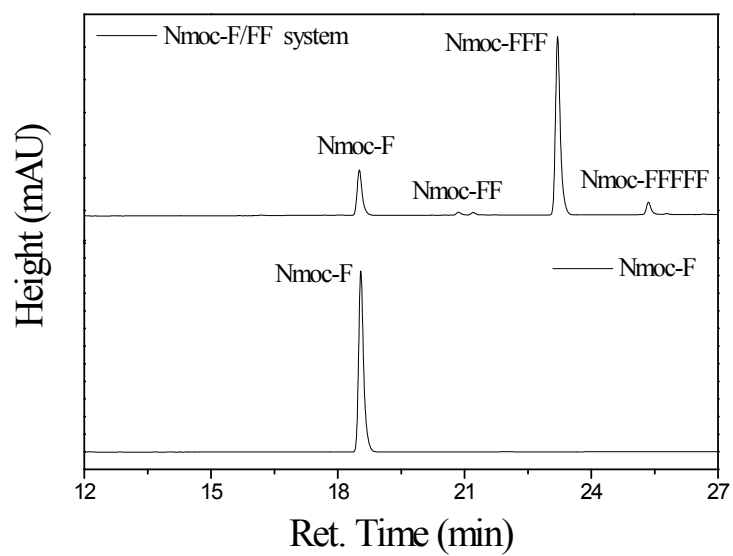
**Scheme S1:** Generation of dynamic library for Nmoc-F/FF system.



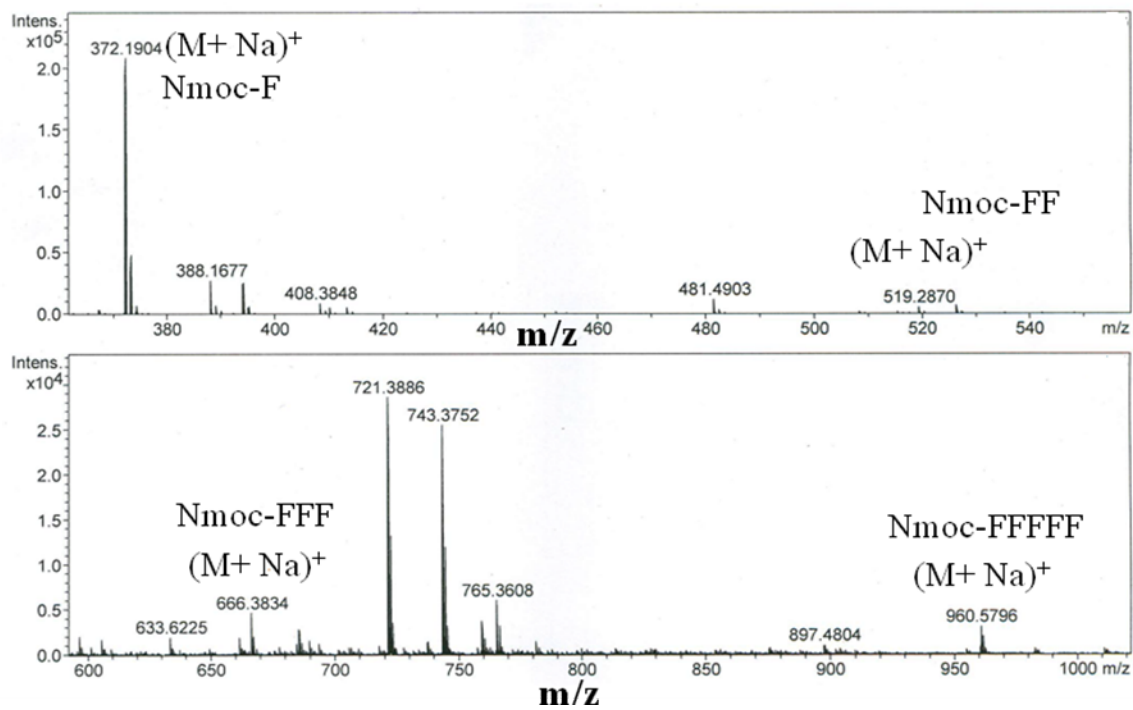
**Scheme S2:** Generation of dynamic library for Nmoc-L/LL system.



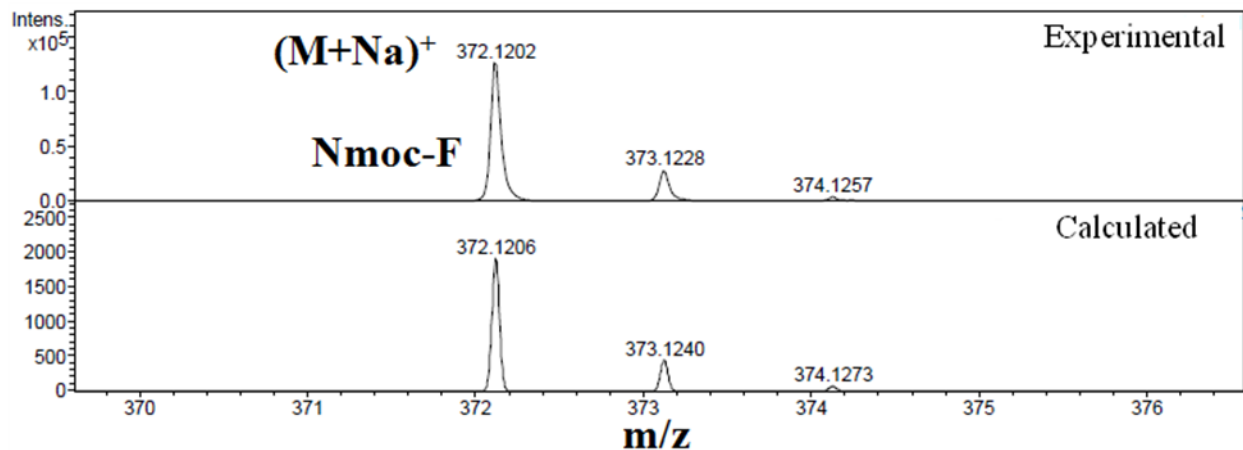
**Scheme S3:** Generation of dynamic library for Nmoc-L/LLL system.



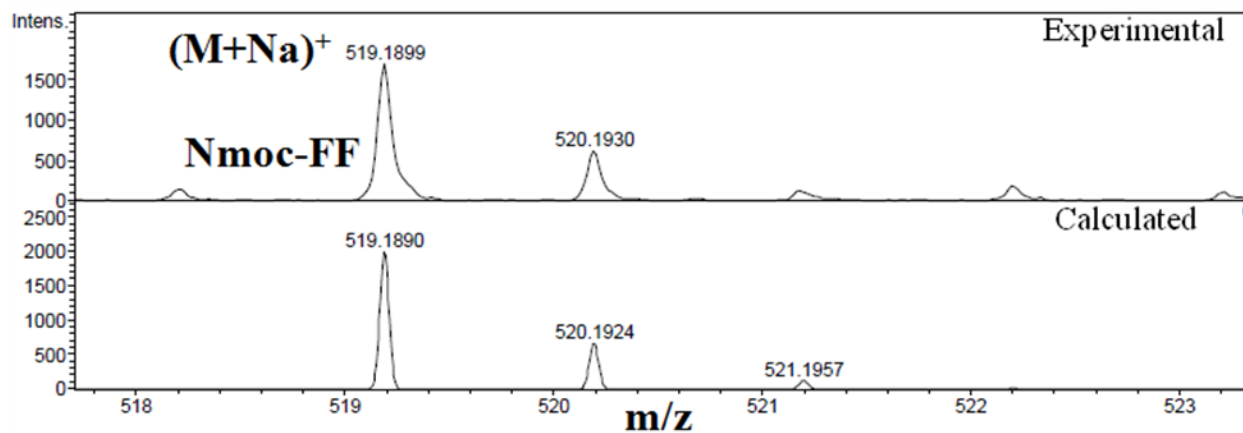
**Figure S1:** High performance liquid chromatography (HPLC) analysis for system Nmoc-F/FF which form dynamic library of peptides upon treatment with thermolysin after 24h.



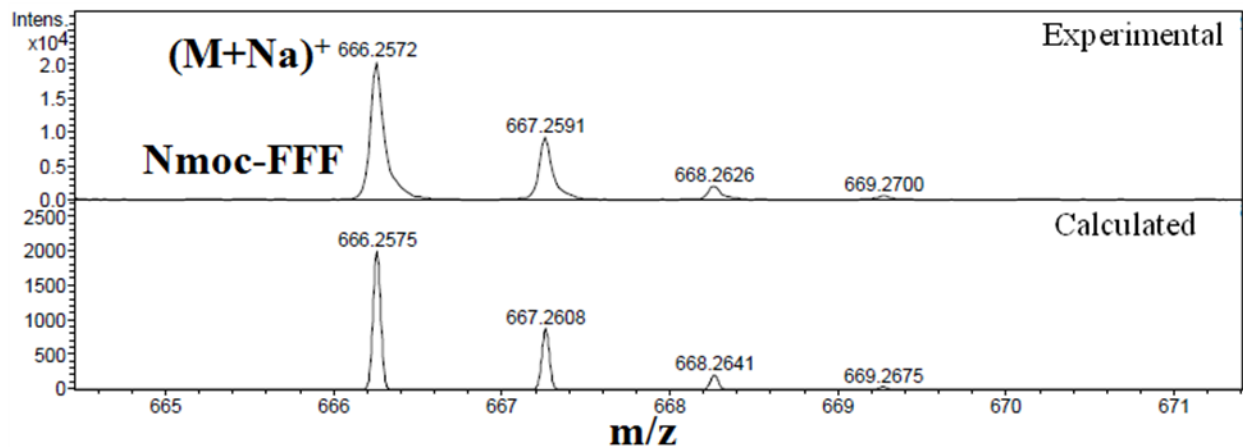
**Figure S2:** ESI-MS analysis for dynamic library formed from Nmoc-F/FF system.



**Figure S3:** HRMS (ESI) analysis for dynamic library component formed from Nmoc-F/FF system.

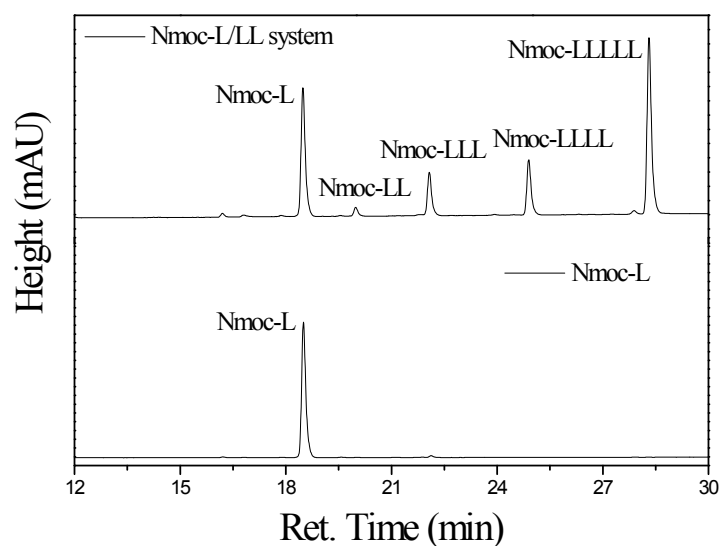


**Figure S4:** HRMS (ESI) analysis for dynamic library component formed from Nmoc-F/FF system.

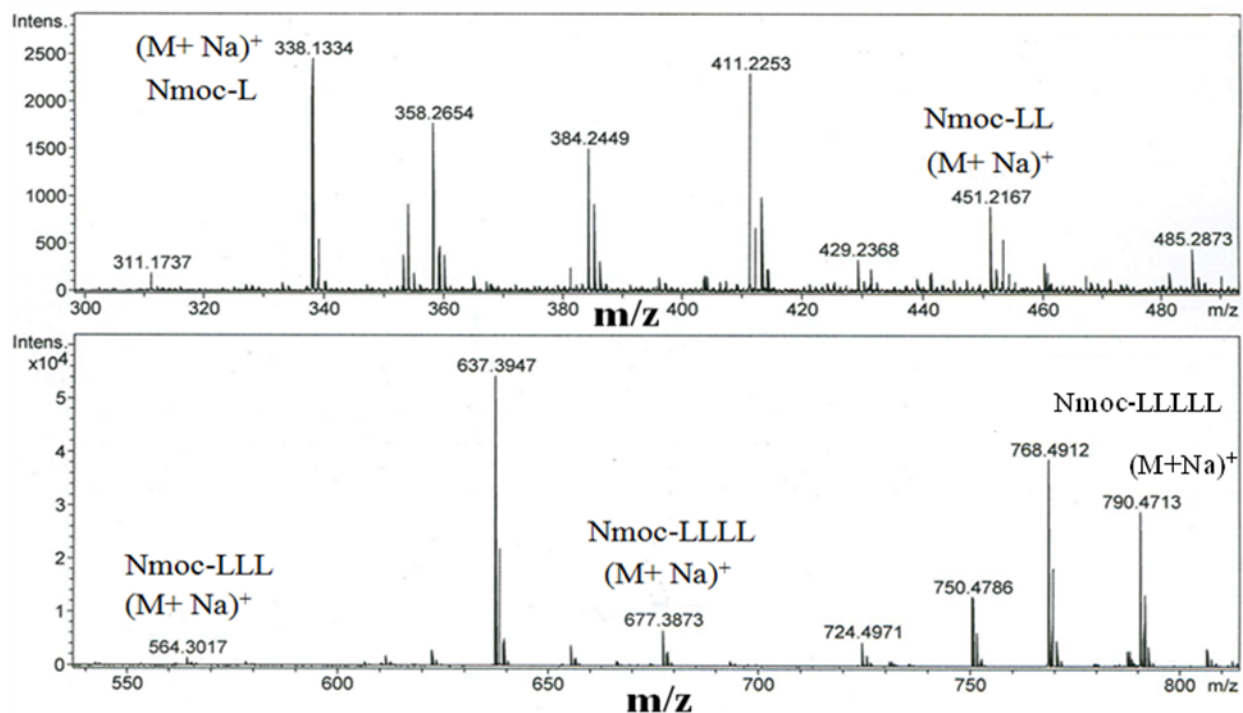


**Figure S5:** HRMS (ESI) analysis for dynamic library component formed from Nmoc-F/FF system.

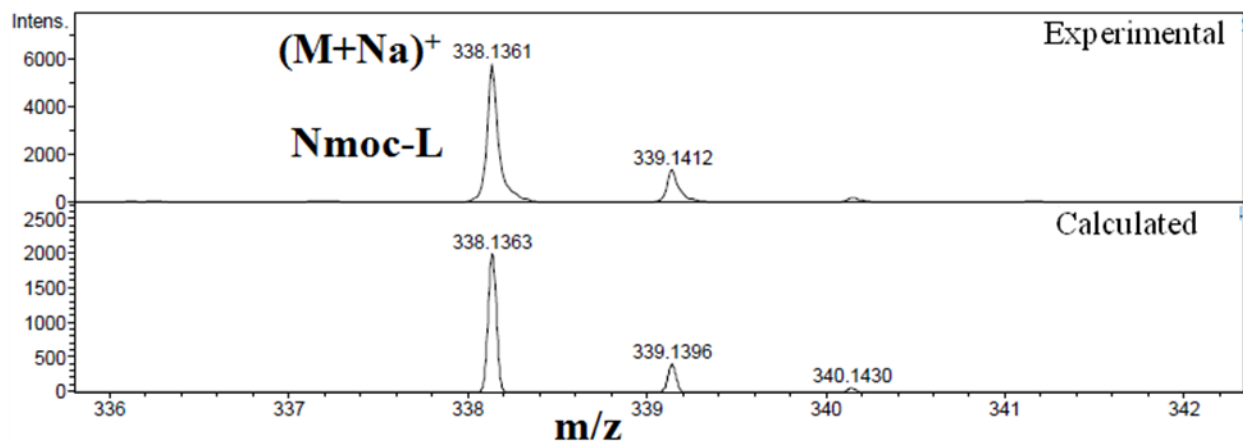




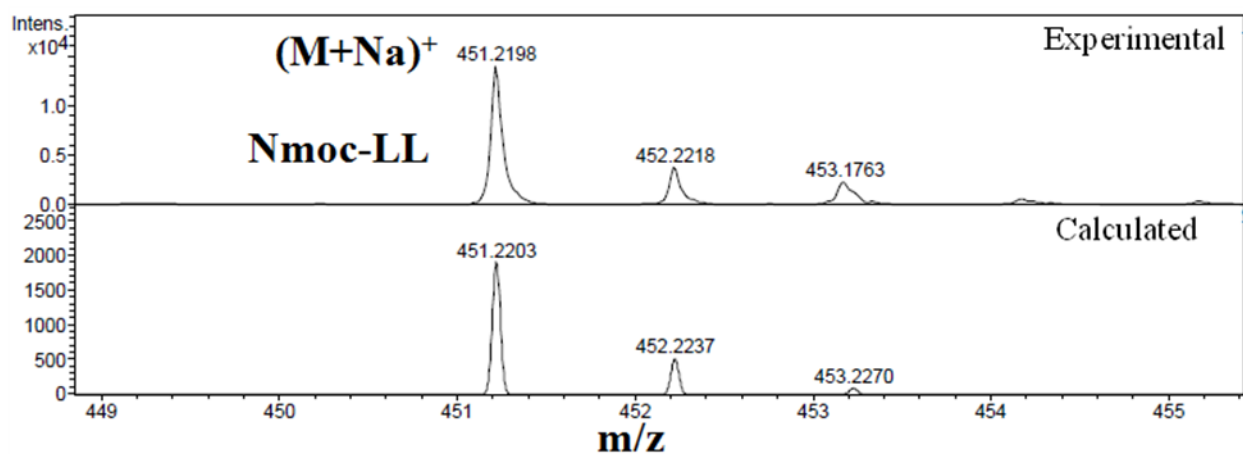
**Figure S6:** High performance liquid chromatography (HPLC) analysis for Nmoc-L/LL system, which form dynamic library of peptides upon treatment with thermolysin after 36h.



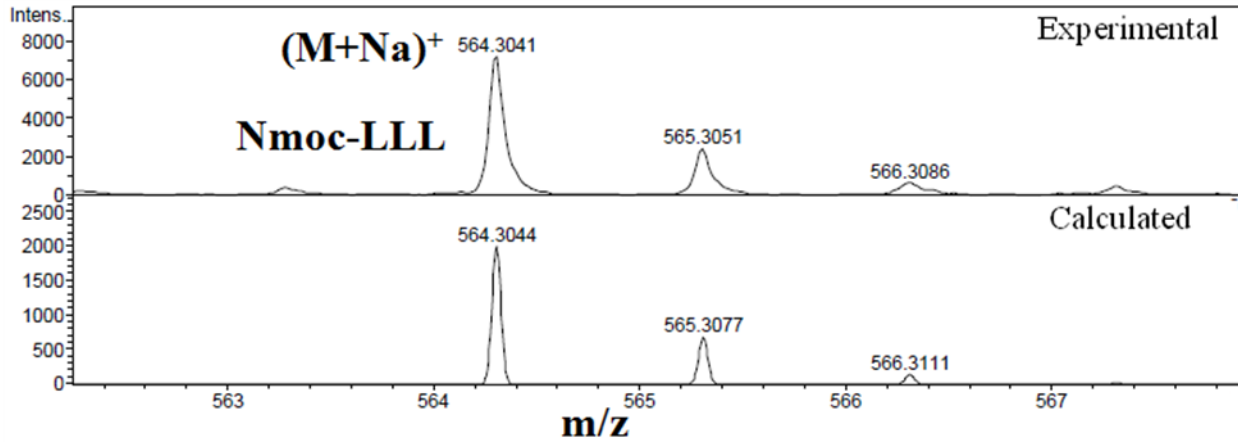
**Figure S7:** ESI-MS analysis for dynamic library formed from Nmoc-L/LL system.



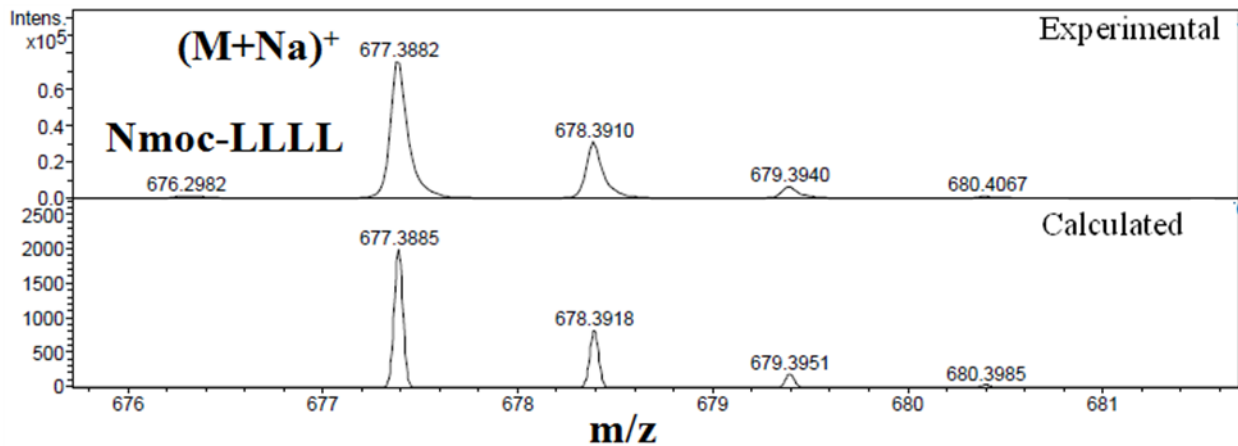
**Figure S8:** HRMS (ESI) analysis for dynamic library component formed from Nmoc-L/LL system.



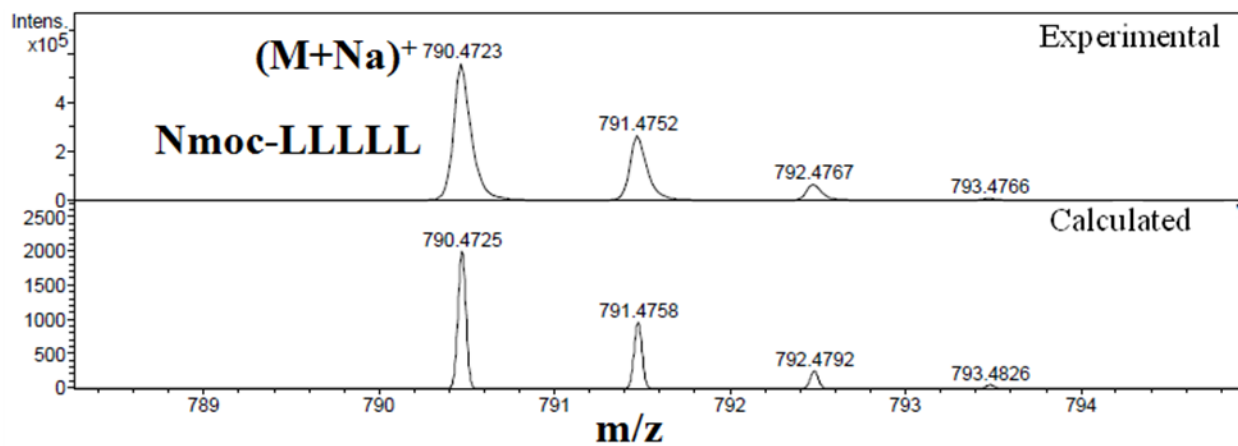
**Figure S9:** HRMS (ESI) analysis for dynamic library component formed from Nmoc-L/LL system.



**Figure S10:** HRMS (ESI) analysis for dynamic library component formed from Nmoc-L/LL system.



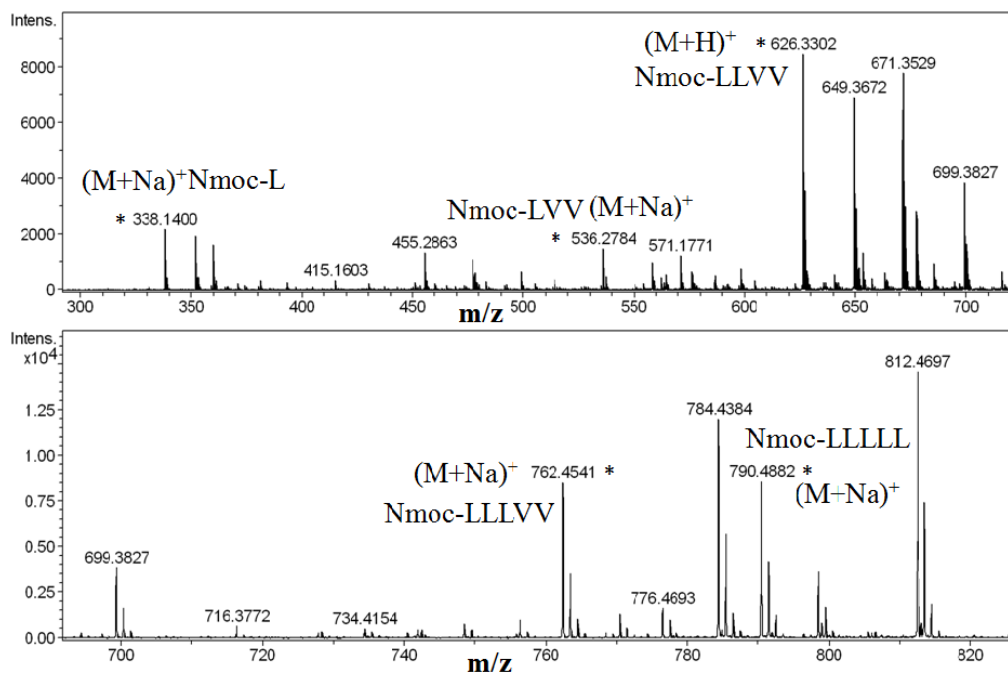
**Figure S11:** HRMS (ESI) analysis for dynamic library component formed from Nmoc-L/LL system.



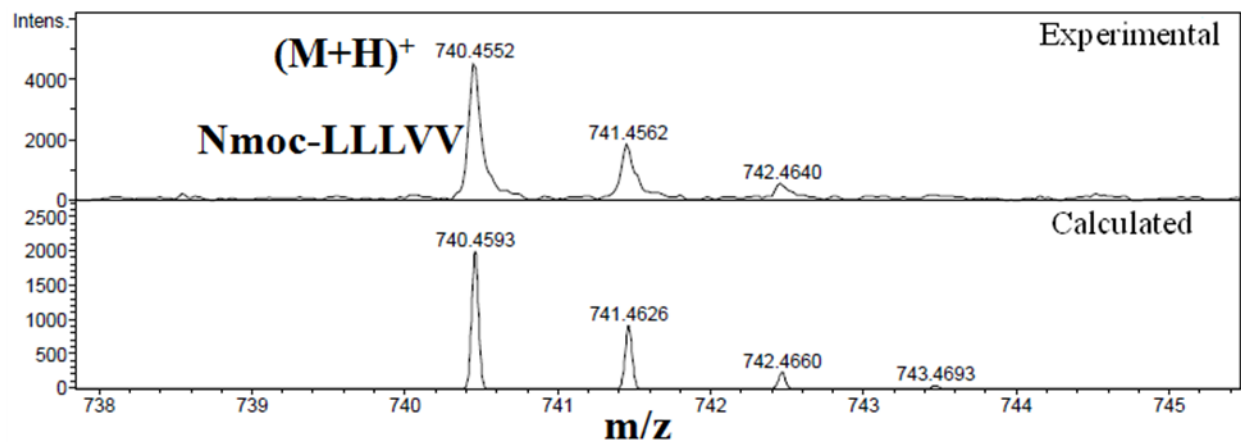
**Figure S12:** HRMS (ESI) analysis for dynamic library component formed from Nmoc-L/LL system.

### Competitive experiment:

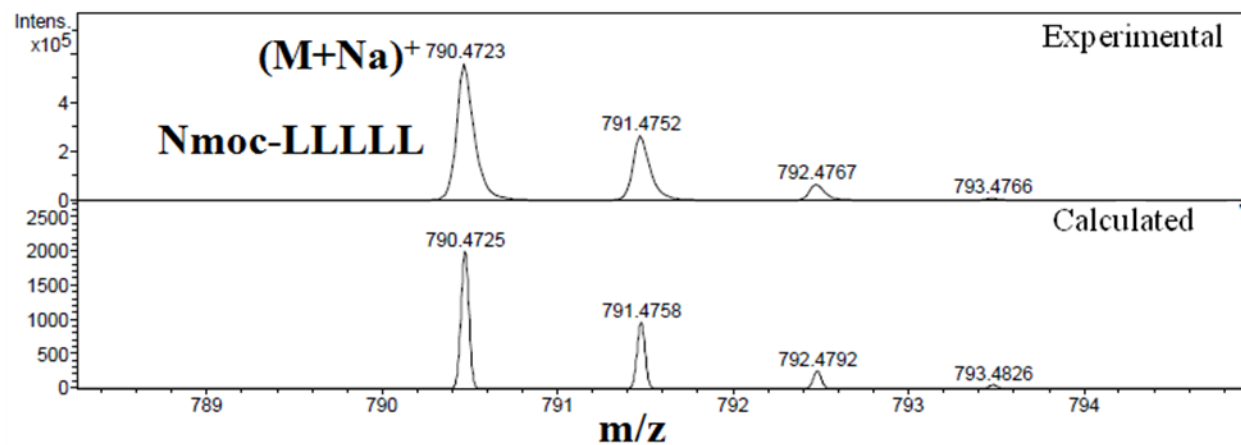
20 mmol of Nmoc-L and 60 mmol of corresponding dipeptides (LL, VV, AA and GG) were suspended in 2 mL of 0.1 M sodium phosphate buffer (pH 8) in a glass vial. 1 mg/mL lyophilised thermolysin powder (bacillus *Thermoproteolyticus rokko* from Sigma-Aldrich) was added to the reaction mixture. The mixture was vortex mixed for 15 s to ensure dissolution. A number of dynamic library components were observed after analysing by HPLC and ESI-MS. In the competitive experiment, the system Nmoc-L/LL/VV/AA/GG remained in solution phase. Nmoc-LLVV and Nmoc-L<sub>5</sub> formed as major components among the library members.



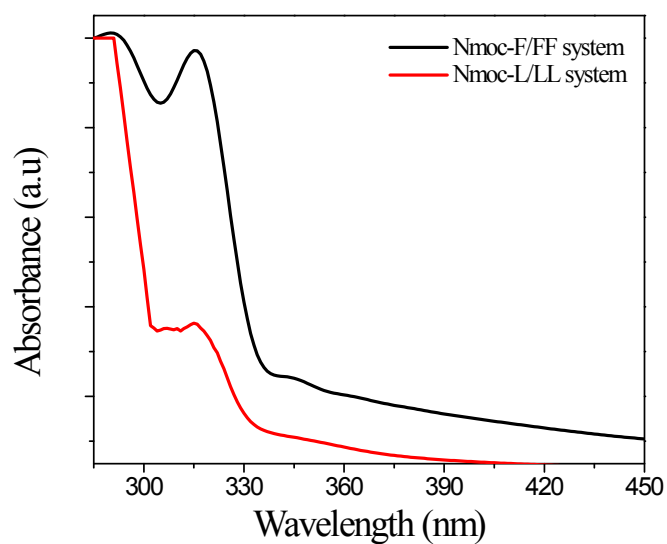
**Figure S13:** ESI-MS analysis for dynamic library formed from Nmoc-L/LL, VV, AA, GG system.



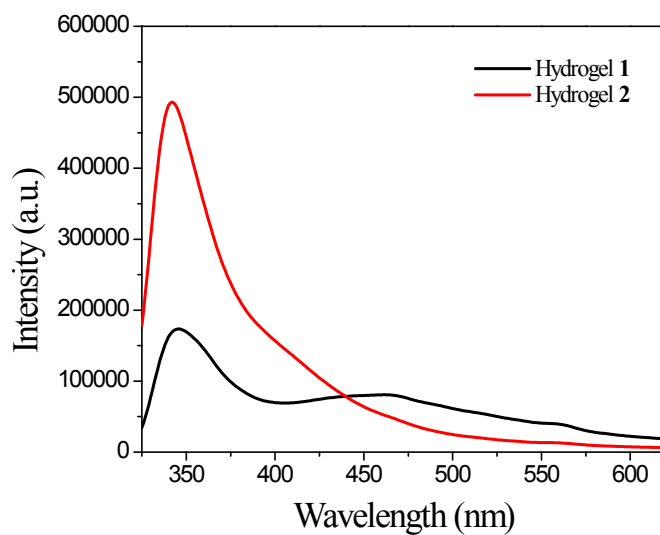
**Figure S14:** HRMS (ESI) analysis for dynamic library component formed from Nmoc-L/LL, VV, AA, GG system.



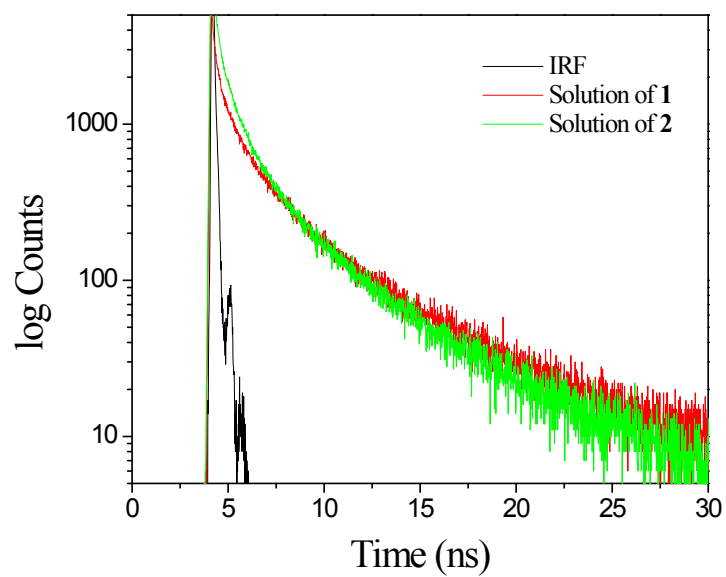
**Figure S15:** HRMS (ESI) analysis for dynamic library component formed from Nmoc-L/LL, VV, AA, GG system.



**Figure S16:** UV-Vis absorption spectra of hydrogel **1** and **2** at 20 mmol L<sup>-1</sup> concentration.

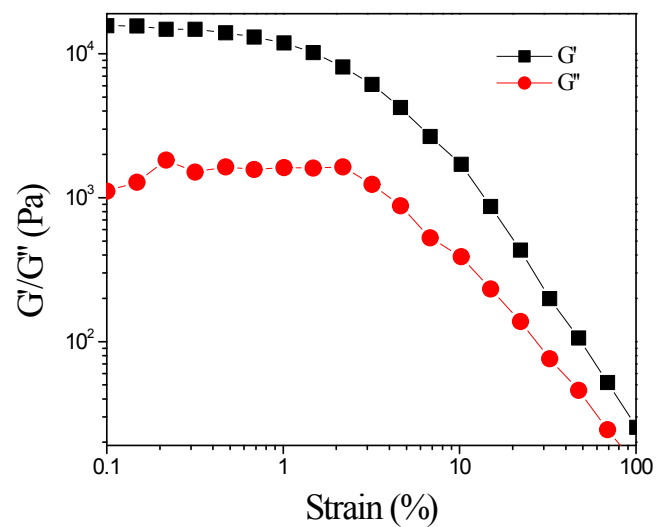


**Figure S17:** Fluorescence spectra taken during the self-assembly process of hydrogel **1** (Nmoc-F/FF system) and hydrogel **2** (Nmoc-L/LL system) at 20 mmol L<sup>-1</sup> concentration ( $\lambda_{\text{ex}} = 315$  nm).

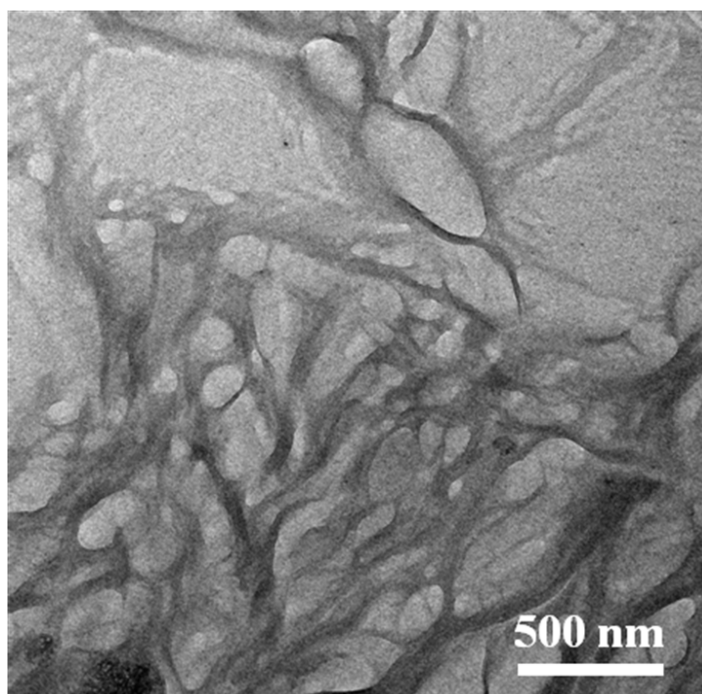


**Figure S18:** Emission decay curves of solution of **1** and **2** (concentration: 20 mmol L<sup>-1</sup>) monitored at 468 nm ( $\lambda_{\text{ex}} = 367$  nm) (IRF: instrument response function).





**Figure S19:** Rheological measurement of LVE at constant frequency 10 rad s<sup>-1</sup> for hydrogel **1** at 20 mmol/L concentration.



**Figure S20.** TEM image of hydrogel **2** (concentration = 5 mmol L<sup>-1</sup>) shows nanofibrillar morphology.

Table S1. Decay parameters for Nmoc-F/FF and Nmoc-L/LL systems.

Entry	$\alpha_1$	$\alpha_2$	$\alpha_3$	$\alpha_4$	$\tau_{1(\text{ns})}$	$\tau_{2(\text{ns})}$	$\tau_{3(\text{ns})}$	$\tau_{4(\text{ns})}$	$\tau^a(\text{ns})$	$\chi^2$
Solution of <b>1</b>	0.12	0.06	0.01	0.81	0.424	1.95	7.09	0.049	0.306	1.15
Hydrogel of <b>1</b>	0.24	0.13	0.04	0.60	0.793	3.19	10.07	0.137	1.07	1.21
Solution of <b>2</b>	0.13	0.05	0.01	0.81	0.382	1.52	5.55	0.049	0.214	1.73
Hydrogel of <b>2</b>	0.12	0.05	0.01	0.82	0.388	1.90	6.55	0.049	0.267	1.42

$\tau^a$  The amplitude weighted average lifetime, Normalized amplitude of each component is given by  $\alpha$

## Synthesis of precursors

Naphthalene-2-methyloxichloroformate **6**,<sup>1</sup> Nmoc-Phe-OH **7**,<sup>2</sup> Nmoc-Leu-OH **8**,<sup>2</sup> Nmoc-V-OH **9**,<sup>2</sup> Nmoc-Ala-OH **10**,<sup>3</sup> and H<sub>2</sub>N-Phe-Phe-OMe **13**<sup>4</sup> were prepared according to the literature. The general procedures used for peptide coupling are as follows: A solution of Boc-Leu-OH **15** (1.31 g, 5.6 mmol) and HOBT (5.6 mmol, 0.756 g) were stirred in 2 mL of DMF. A neutralized solution of leucine methyl ester was extracted from its corresponding hydrochloride salt and concentrated to add to the reaction mixture followed by DCC (5.7 mmol, 1.17 g) at 0 °C. The mixture was allowed to stir at room temperature for 12 h. The mixture was diluted with ethyl acetate and organic layer was washed with 1 M HCl (2 x 30 mL), brine solution, 1 M Na<sub>2</sub>CO<sub>3</sub> (3 x 30 mL) and brine solution. The ethyl acetate layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated under vacuum to yield white solid product **16**. Purification was done by silica gel column (100-200 mesh) using ethyl acetate-toluene (3:1) as eluent.

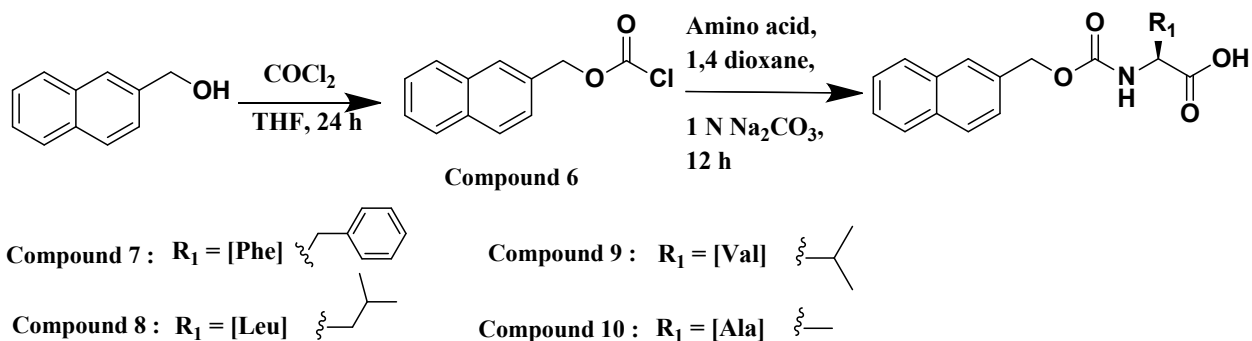
A solution of Boc-Leu-Leu-OCH<sub>3</sub> (1.6 g, 4.4 mmol) **16** in TFA was stirred for 12 h under argon at room temperature. The excess TFA was removed under vacuum. The oily residue was taken in 100 mL of water and washed with diethyl ether (2 x 20 mL). White product **17** was obtained after lyophilization.

H<sub>2</sub>N-Leu-Leu-OH **18** was synthesized according to the following procedure. A solution NH<sub>2</sub>-Leu-Leu-OMe (0.950 g, 3.6 mmol) in 100 mL of dry MeOH was allowed to react with a solution of 2 M NaOH. The progress of reaction was monitored by thin layer chromatography (TLC). The reaction mixture was stirred for 12 h. Then, the methanol was removed under vacuum. The residue was taken in 100 mL of water and washed with diethyl ether (2 x 20 mL). Then the pH of aqueous layer was adjusted to 2 using 2 M HCl and it was extracted with ethyl

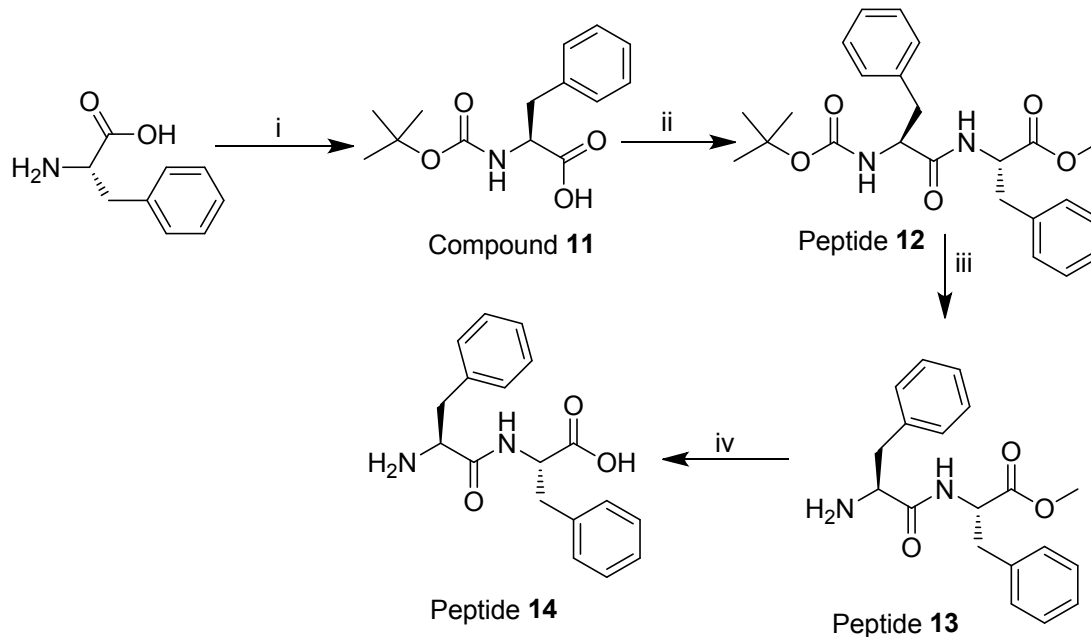
acetate (3 x 30 mL). The ethyl acetate layer was dried over anhydrous sodium sulfate and evaporated under vacuum to yield **18** as white solid and used further without purification.

Compounds **19-23** were synthesized as per above-mentioned procedures. Compound **24** was synthesized with valine benzyl ester instead of taking methyl ester. The general procedure was used for peptide coupling for the synthesis of compound **24**. Compounds **25-29** were also synthesized as the above-mentioned procedures.

### Synthesis of Nmoc-amino acids 7-10



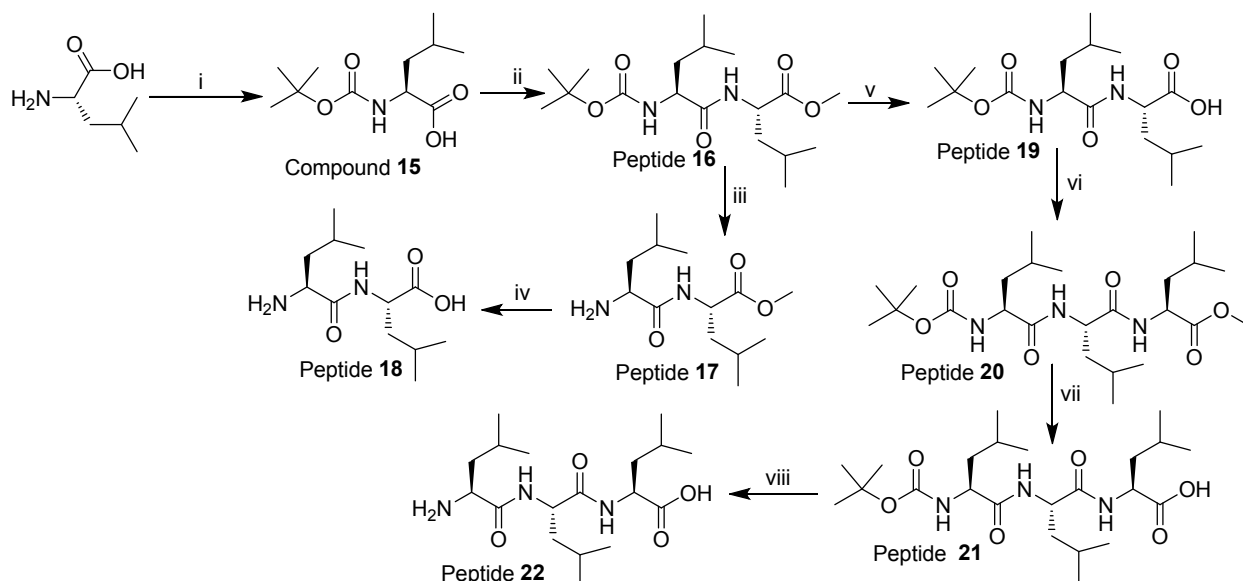
## Synthesis of Phe-Phe (14)



i) Boc-anhydride, 1 M NaOH, 1,4 Dioxane, ii) Phenylalanine methyl ester, DCC/HOBt, DMF, 12h, iii) TFA, 12 h, iv) 1M NaOH, MeOH, 12 h

**NH<sub>2</sub>-Phe<sup>2</sup>-Phe<sup>1</sup>-OH (14):** Yield = 0.490 g (1.5 mmol, 88.23%).  $[\alpha]_D^{25} = -20$  ( $c = 1$ , MeOH); FT-IR (KBr):  $\tilde{\nu} = 3309$  (s), 3096 (br), 2929 (br), 1732 (s), 1687 (m), 1566 (m), 1448 (m), 1332 (s), 1202(m)  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  ppm 8.86 (d, 1H, NH of Phe), 7.23-7.28 (m, 12H, aromatic Hs of Phe<sup>1</sup> and Phe<sup>2</sup> and NH<sub>2</sub>), 4.50 (m, 1H, C <sup>$\alpha$</sup> H of Phe<sup>2</sup>), 4.02 (m, 1H, C <sup>$\alpha$</sup> H of Phe<sup>1</sup>), 3.10 (m, 2H, C <sup>$\beta$</sup> Hs of Phe), 2.97 (m, 2H, C <sup>$\beta$</sup> Hs of Phe); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  172.1, 168.1, 137.1, 134.6, 129.5, 129.1, 128.4, 128.2, 127.1, 126.5, 53.79, 53.10, 36.89, 36.63; HRMS (ESI)  $m/z$  for C<sub>18</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> ( $M + H$ )<sup>+</sup> calcd.: 313.1547, found: 313.1546.

## Synthesis of Leu-Leu (18) and Leu-Leu-Leu (22)



Reagents: i) Boc-anhydride, 1 M NaOH, 1,4 Dioxane, ii) Leucine methyl ester, DCC/HOBt, DMF, 12 h, iii) TFA, 12 h, iv) 1M NaOH, MeOH, 12 h, v) Leucine methyl ester, DCC/HOBt, DMF, 12 h, vi) 1 M NaOH, MeOH, 12 h, viii) TFA, 12 h.

**Boc-Leu<sup>2</sup>-Leu<sup>1</sup>-OMe (16):** Yield = 1.8 g, (5 mmol, 89.28 %).  $[\alpha]_{\text{D}}^{25} = -56$  ( $c = 1$ , MeOH); FT-IR (KBr):  $\tilde{\nu} = 3353$  (s), 3274 (m), 3088 (m), 2960 (s), 1759 (s), 1688 (s), 1561 (m), 1520 (m), 1445 (s), 1365 (m)  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 6.47 (d, 1H,  $J = 7.52$  Hz, NH of Leu<sup>1</sup>), 4.89 (d, 1H, NH of Leu<sup>2</sup>), 4.60 (m, 1H, C <sup>$\alpha$</sup> H of Leu<sup>2</sup>), 4.2 (m, 1H, C <sup>$\alpha$</sup> H of Leu<sup>1</sup>), 3.69 (s, 3H, OCH<sub>3</sub>), 1.66 (m, 4H, C <sup>$\beta$</sup> Hs of Leu<sup>1</sup> and Leu<sup>2</sup>), 1.46 (m, 2H, C <sup>$\gamma$</sup> Hs of Leu<sup>1</sup> and Leu<sup>2</sup>), 1.44 (s, 9H, Boc), 0.91 (m, 12H, C <sup>$\delta$</sup> Hs of Leu<sup>1</sup> and Leu<sup>2</sup>). HRMS (ESI)  $m/z$  for  $\text{C}_{18}\text{H}_{34}\text{N}_2\text{O}_5$  ( $M + \text{Na}$ )<sup>+</sup> calcd.: 381.2360, found: 381.2366.

**NH<sub>2</sub>-Leu<sup>2</sup>-Leu<sup>1</sup>-OCH<sub>3</sub> (17):** Yield = 1.1 g (4.2 mmol, 95.45 %),  $[\alpha]_{\text{D}}^{25} = -6$  ( $c = 1$ , MeOH);  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-d}_6$ ):  $\delta = 8.83$  (d, 1H,  $J = 6.52$  Hz, NH of Leu<sup>1</sup>), 8.21 (d, 3H, NH<sub>3</sub> of Leu<sup>1</sup>), 4.32 (m, 1H, C <sup>$\alpha$</sup> H of Leu<sup>2</sup>), 3.79 (m, 1H, C <sup>$\alpha$</sup> H of Leu<sup>1</sup>), 3.61 (s, 3H, OCH<sub>3</sub>), 1.68 (m, 4H,

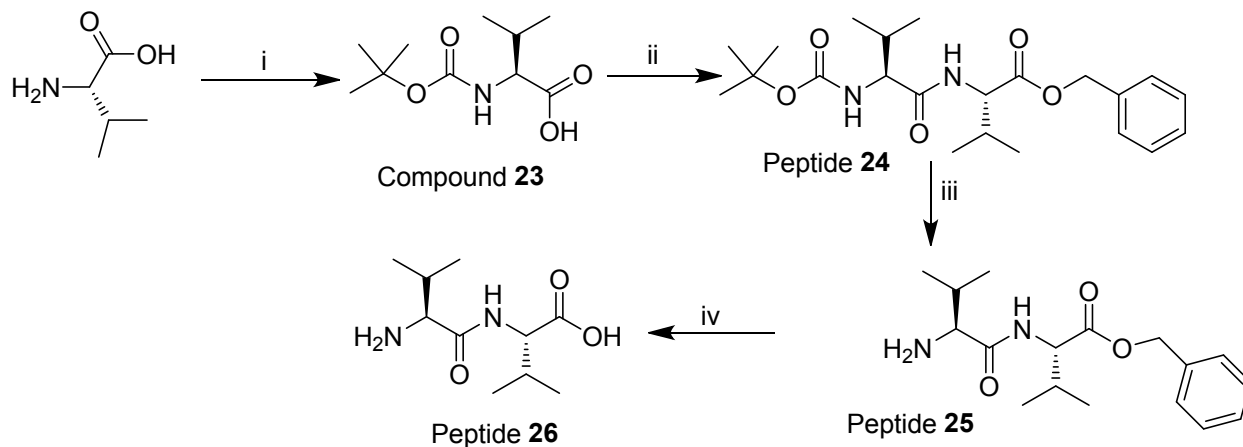
C<sup>β</sup>Hs of Leu<sup>1</sup> and Leu<sup>2</sup>), 1.55 (m, 2H, C<sup>γ</sup>Hs of Leu<sup>1</sup> and Leu<sup>2</sup>), 0.89 (m, 12H, C<sup>δ</sup>Hs of Leu<sup>1</sup> and Leu<sup>2</sup>); HRMS (ESI)  $m/z$  for C<sub>13</sub>H<sub>26</sub>N<sub>2</sub>O<sub>3</sub> ( $M + H$ )<sup>+</sup> calcd.: 259.2016, found: 259.2011.

**NH<sub>2</sub>-Leu<sup>2</sup>-Leu<sup>1</sup>-OH (18)**: Yield= 0.789 g (3.2 mmol, 88.88 %). [ $\alpha$ ]<sub>D</sub><sup>25</sup> = - 11 ( $c = 1$ , MeOH); FT-IR (KBr):  $\tilde{\nu} = 3435$  (br), 3234 (br), 3081 (br), 2966 (m), 1670 (br), 1560 (m), 1464 (m), 1438 (m), 1199 (s), cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  ppm 8.65 (d, 1H,  $J = 7.76$  Hz, NH of Leu<sup>1</sup>), 8.11 (d, 2H, NH<sub>2</sub>), 4.26 (m, 1H, C <sup>$\alpha$</sup> H of Leu<sup>2</sup>), 3.77 (m, 1H, C <sup>$\alpha$</sup> H of Leu<sup>1</sup>), 1.69 (m, 4H, C<sup>β</sup>Hs of Leu<sup>1</sup> and Leu<sup>2</sup>), 1.55 (m, 2H, C<sup>γ</sup>Hs of Leu<sup>1</sup> and Leu<sup>2</sup>), 0.89 (m, 12H, C<sup>δ</sup>Hs of Leu<sup>1</sup> and Leu<sup>2</sup>); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  ppm 173.77, 169.46, 51.08, 50.82, 24.50, 23.75, 22.8, 22.6, 21.7, 21.2; HRMS (ESI)  $m/z$  for C<sub>12</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> ( $M + Na$ )<sup>+</sup> calcd.: 267.1679, found: 267.1674.

**NH<sub>2</sub>-Leu<sup>1</sup>-Leu<sup>2</sup>-Leu<sup>3</sup>-OH (22)**: Yield= 0.180 g (0.5 mmol, 86.20 %). [ $\alpha$ ]<sub>D</sub><sup>25</sup> = - 13; FT-IR (KBr):  $\tilde{\nu} = 3297.09$  (br), 3082.83 (m), 1659.63 (s), 1551.95 (s), 1470.06 (m), 1390.02 (m), 1370.76 (m). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  8.43 (d, 1H,  $J = 8$  Hz, NH), 8.13 (d, 1H,  $J = 8$  Hz, NH), 7.96 (s, 2H, NH<sub>2</sub>), 4.28 (m, 1H, C <sup>$\alpha$</sup> H of Leu<sup>3</sup>), 4.1 (m, 1H, C <sup>$\alpha$</sup> H of Leu<sup>2</sup>), 3.64 (m, 1H, C <sup>$\alpha$</sup> H of Leu<sup>1</sup>), 1.48 (m, 3H, C<sup>γ</sup>Hs of Leu<sup>1,2,3</sup>), 1.35 (m, 6H, C<sup>β</sup>Hs of Leu<sup>1,2,3</sup>), 0.75 (m, 18H, C<sup>δ</sup>Hs of Leu<sup>1,2,3</sup>); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  173.75, 171.29, 168.49, 50.86, 49.94, 40.88, 40.26, 24.16, 23.84, 23.41, 22.91, 22.48, 22.04, 21.82, 20.98; HRMS (ESI)  $m/z$  for C<sub>18</sub>H<sub>35</sub>N<sub>3</sub>O<sub>4</sub> ( $M + H$ )<sup>+</sup> calcd.: 358.2706, found: 358.2703.



## Synthesis of Val-Val (26)



Reagents: i) Boc-anhydride, 1 M NaOH, 1,4 Dioxane, ii) Valine benzyl ester, DCC/HOBt, DMF, 12h, iii) TFA, 12h, iv) 1M NaOH, MeOH, 12 h.

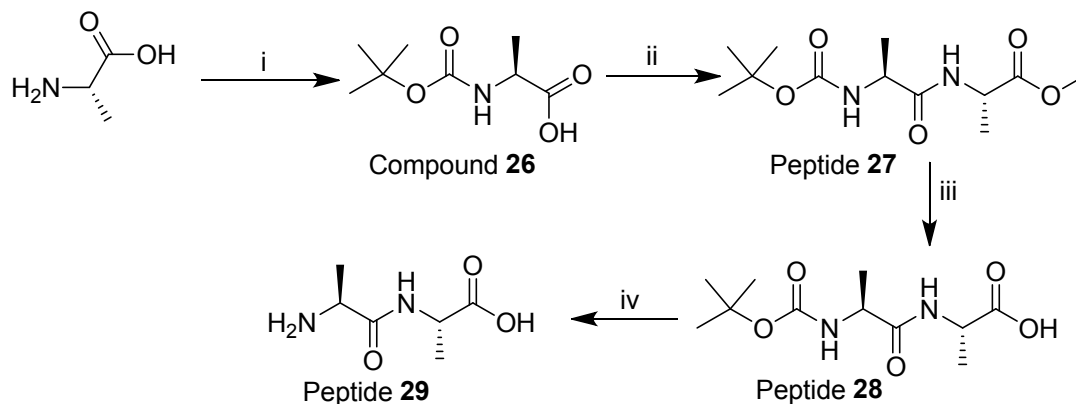
**Boc-Val<sup>2</sup>-Val<sup>1</sup>-OCH<sub>2</sub>Ph (24):** Yield= 2.8 g, (6.8 mmol, 85 %).  $[\alpha]_D^{25} = -47$  ( $c = 1$ , MeOH); FT-IR (KBr):  $\tilde{\nu} = 3315$  (s), 3066 (m), 2969 (s), 2879 (m), 1745 (s), 1683 (m), 1657 (s), 1535 (m), 1460 (m), 1373 (m), 1175 (s)  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  ppm 7.32 (m, 5H, aromatic Hs of Ph ring), 6.43 (s, 1H, benzyl CH<sub>2</sub>), 5.18 (d, 1H, NH of Val<sup>1</sup>), 5.12 (d, 1H, NH of Val<sup>2</sup>), 4.56 (m, 1H, C <sup>$\alpha$</sup> H of val<sup>1</sup>), 3.89 (m, 1H, C <sup>$\alpha$</sup> H of val<sup>2</sup>), 2.17 (m, 1H, C <sup>$\beta$</sup> H of Val<sup>1</sup>), 2.09 (m, 1H, C <sup>$\beta$</sup> H of Val<sup>2</sup>), 1.41 (s, 12H, Boc), 0.95 (s, 9H, Hs of Boc-), 0.93-0.84 (m, 12H, C <sup>$\gamma$</sup> Hs of Val<sup>1</sup> and Val<sup>2</sup>). HRMS (ESI)  $m/z$  for C<sub>22</sub>H<sub>34</sub>N<sub>2</sub>O<sub>5</sub> ( $M + \text{Na}$ )<sup>+</sup> calcd.: 429.2360, found: 429.2347.

**NH<sub>2</sub>-Val<sup>2</sup>-Val<sup>1</sup>-OCH<sub>2</sub>Ph (25):** Yield= 1.95 g (6.3 mmol, 98.4 %).  $[\alpha]_D^{25} = -11$  ( $c = 1$ , MeOH); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$  ppm 9.60 (d, 1H,  $J = 7.76$  Hz, NH of Val<sup>1</sup>), 8.61 (d, 2H,  $J = 7.52$  Hz, NH<sub>2</sub>), 7.44-7.34 (m, 5H, aromatic Hs of Ph ring), 5.12 (s, 2H, CH<sub>2</sub> of Ph), 4.25 (m, 1H, C <sup>$\alpha$</sup> H of Val<sup>2</sup>), 4.13 (m, 1H, C <sup>$\alpha$</sup> H of Val<sup>1</sup>), 2.09 (m, 2H, C <sup>$\beta$</sup> Hs of Val<sup>1</sup> and Val<sup>2</sup>), 0.89 (m, 12H,

C $\gamma$ Hs of Val<sup>1</sup> and Val<sup>2</sup>). HRMS (ESI) m/z for C<sub>17</sub>H<sub>26</sub>N<sub>2</sub>O<sub>3</sub> (*M* + H)<sup>+</sup> calcd.: 307.2016, found: 307.2012.

**NH<sub>2</sub>-Val<sup>2</sup>-Val<sup>1</sup>-OH (26)**: Yield = 0.651 g (3 mmol, 93.75 %). [ $\alpha$ ]<sub>D</sub><sup>25</sup> = 11 (*c* = 1, MeOH); FT-IR (KBr):  $\tilde{\nu}$  = 3308 (s), 3200 (br), 3074 (br), 2973 (m), 1670 (s), 1557 (m), 1518 (m), 1469 (m), 1309 (s), 1141 (m) cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  ppm 8.44 (d, 2H, *J* = 7.76 Hz, NH of Val<sup>1</sup>), 8.09 (d, 1H, NH<sub>2</sub>), 4.15 (m, 1H, C $\alpha$ H of Val<sup>2</sup>), 3.72 (m, 1H, C $\alpha$ H of Val<sup>1</sup>), 2.07 (m, 2H, C $\beta$ Hs of Val<sup>1</sup> and Val<sup>2</sup>), 0.92 (m, 12H, C $\gamma$ Hs of Val<sup>1</sup> and Val<sup>2</sup>); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  172.3, 168.1, 158.3, 157.9, 57.5, 56.9, 29.9, 29.6, 18.9, 18.2, 17.9, 17.4; HRMS (ESI) m/z for C<sub>10</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> (*M* + H)<sup>+</sup> calcd.: 217.1547, found: 217.1545.

### Synthesis of Ala-Ala (29)



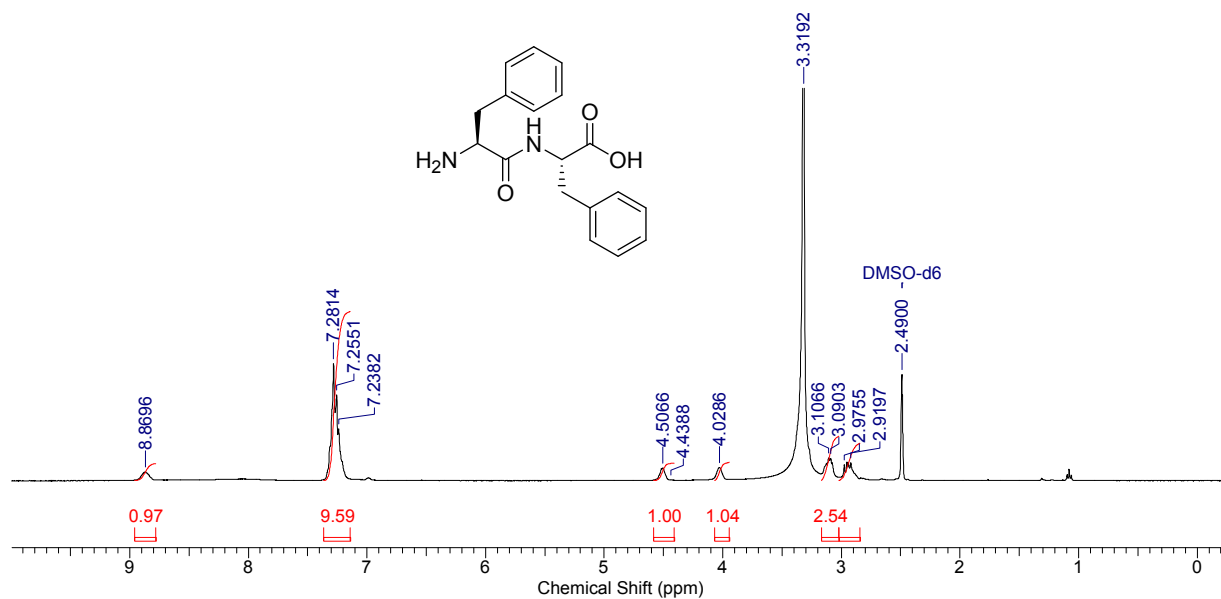
Reagents: i) Boc-anhydride, 1 M NaOH, 1,4 Dioxane, ii) Alanine methyl ester, DCC/HOBt, DMF, 12h, iii) 1 M NaOH, MeOH, 12 h, iv) TFA, 12 h.

**Boc-Ala<sup>2</sup>-Ala<sup>1</sup>-OMe (27)**: Yield = 1.85 g (6.7 mmol, 84.81 %). [ $\alpha$ ]<sub>D</sub><sup>25</sup> = -56 (*c* = 1, MeOH); FT-IR (KBr):  $\tilde{\nu}$  = 3319 (s), 3093 (m), 2984 (s), 2942 (m), 1746 (s), 1685 (s), 1656 (s), 1557 (s), 1524

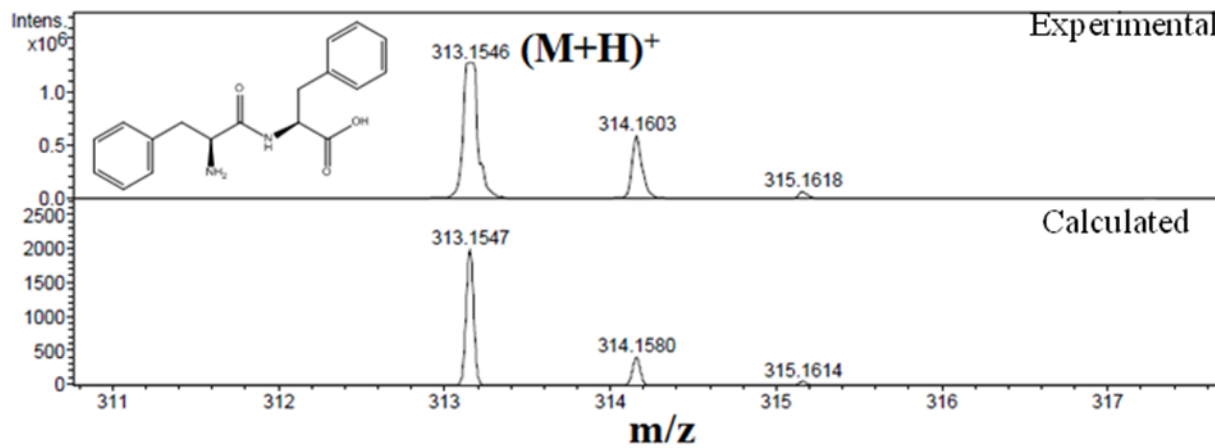
(m), 1452 (s), 1372 (m), 1261 (m)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 6.75 (d, 1H, NH of Ala<sup>1</sup>), 5.12 (d, 1H,  $J = 7.04$  Hz, NH of Ala<sup>2</sup>), 4.54 (m, 1H, C <sup>$\alpha$</sup> H of Ala<sup>1</sup>), 4.17 (m, 1H, C <sup>$\alpha$</sup> H of Ala<sup>2</sup>), 3.71 (s, 3H, OCH<sub>3</sub>), 1.41 (s, 9H, Boc), 1.38-1.32 (m, 6H, C <sup>$\beta$</sup> Hs of Ala<sup>1</sup> and Ala<sup>2</sup>). HRMS (ESI)  $m/z$  for  $\text{C}_{12}\text{H}_{22}\text{N}_2\text{O}_5$  ( $M + \text{Na}$ )<sup>+</sup> calcd.: 297.1421, found: 297.1423.

**Boc-Ala<sup>2</sup>-Ala<sup>1</sup>-OH (28)**: Yield= 1.37 g (5.2 mmol, 89.65 %).  $[\alpha]_{\text{D}}^{25} = -56$  ( $c = 1$ , MeOH);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  ppm 7.97 (d, 1H,  $J = 7.04$  Hz, NH of Ala<sup>1</sup>), 6.83 (d, 1H,  $J = 7.28$  Hz, NH of Ala<sup>2</sup>), 4.18 (m, 1H, C <sup>$\alpha$</sup> H of Ala<sup>1</sup>), 3.97 (m, 1H, C <sup>$\alpha$</sup> H of Ala<sup>2</sup>), 1.35 (s, 9H, Boc), 1.26-1.14 (m, 6H, C <sup>$\beta$</sup> Hs of Ala<sup>1</sup> and Ala<sup>2</sup>); HRMS (ESI)  $m/z$  for  $\text{C}_{11}\text{H}_{20}\text{N}_2\text{O}_5$  ( $M + \text{Na}$ )<sup>+</sup> calcd.: 283.1264, found: 283.1263.

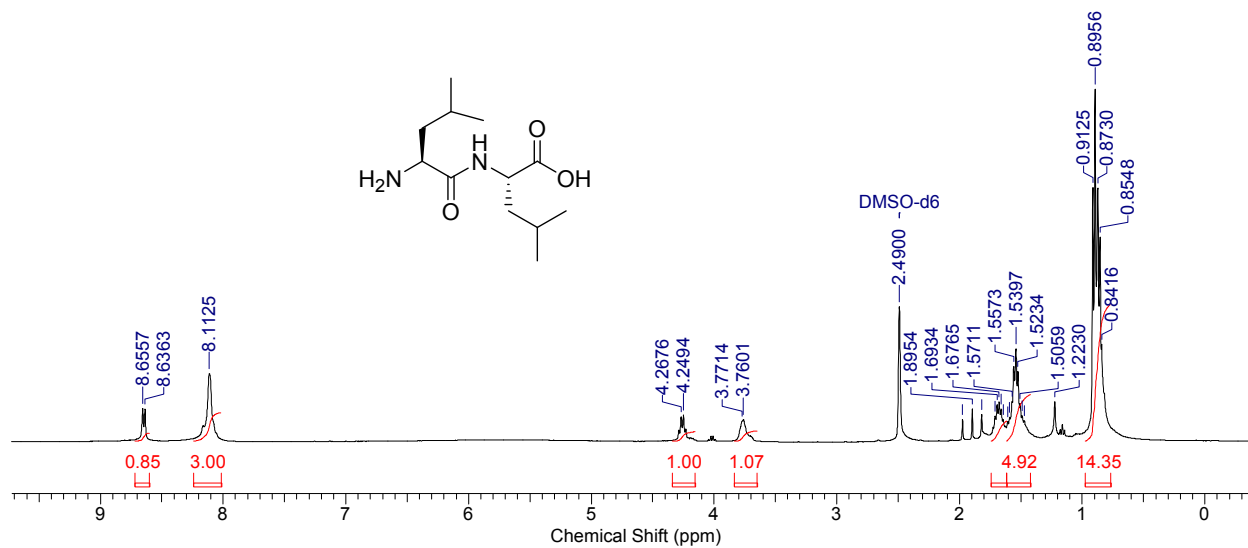
**NH<sub>2</sub>-Ala<sup>2</sup>-Ala<sup>1</sup>-OH (29)**: Yield= 0.651 g (4 mmol, 86.95 %).  $[\alpha]_{\text{D}}^{25} = -9$  ( $c = 1$ , MeOH);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  ppm 8.67 (d, 1H,  $J = 8.28$  Hz, NH of Ala<sup>1</sup>), 8.11 (d, 1H, NH<sub>2</sub> of Ala<sup>2</sup>), 4.27 (m, 1H, C <sup>$\alpha$</sup> H of Ala<sup>2</sup>), 3.84 (m, 1H, C <sup>$\alpha$</sup> H of Ala<sup>1</sup>), 1.38-1.28 (m, 6H, C <sup>$\beta$</sup> Hs of Ala<sup>1</sup> and Ala<sup>2</sup>);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  173.7, 169.4, 158.7, 158.3, 48.2, 47.8, 17.2; HRMS (ESI)  $m/z$  for  $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_3$  ( $M + \text{H}$ )<sup>+</sup> calcd.: 161.0921, found: 161.0926.



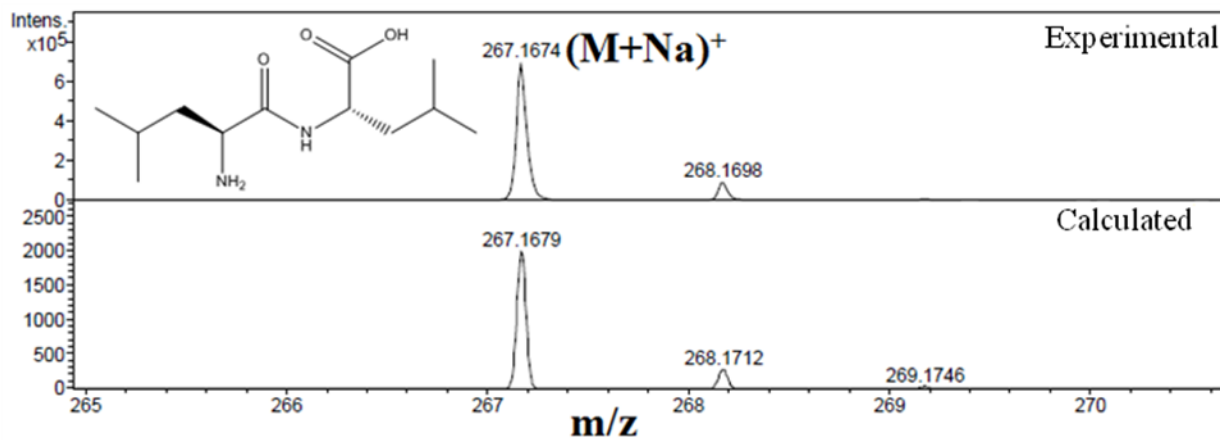
**Figure S21:** 400 MHz  $^1\text{H}$  NMR spectrum of **FF** in  $\text{DMSO-d}_6$ .



**Figure S22:** HRMS (ESI) spectrum of **FF** (**14**).



**Figure S23:** 400 MHz  $^1\text{H}$  NMR spectrum of LL (18) in DMSO- $d_6$ .



**Figure S24:** HRMS (ESI) spectrum of LL (18).

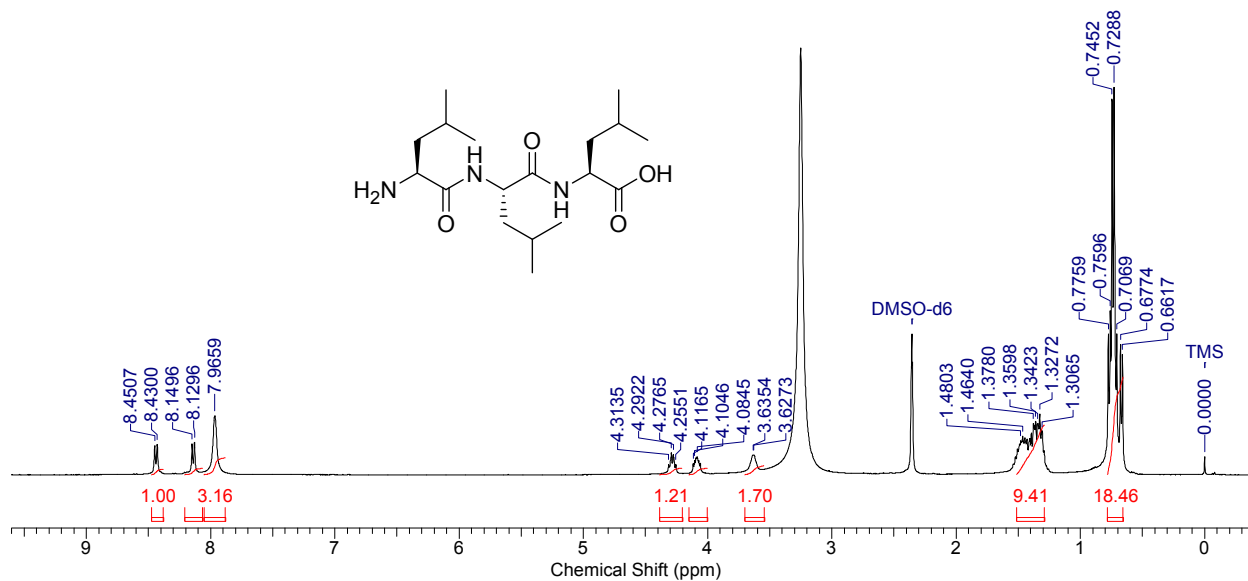


Figure S25: 400 MHz  $^1\text{H}$  NMR spectrum of LLL (22) in  $\text{DMSO-d}_6$ .

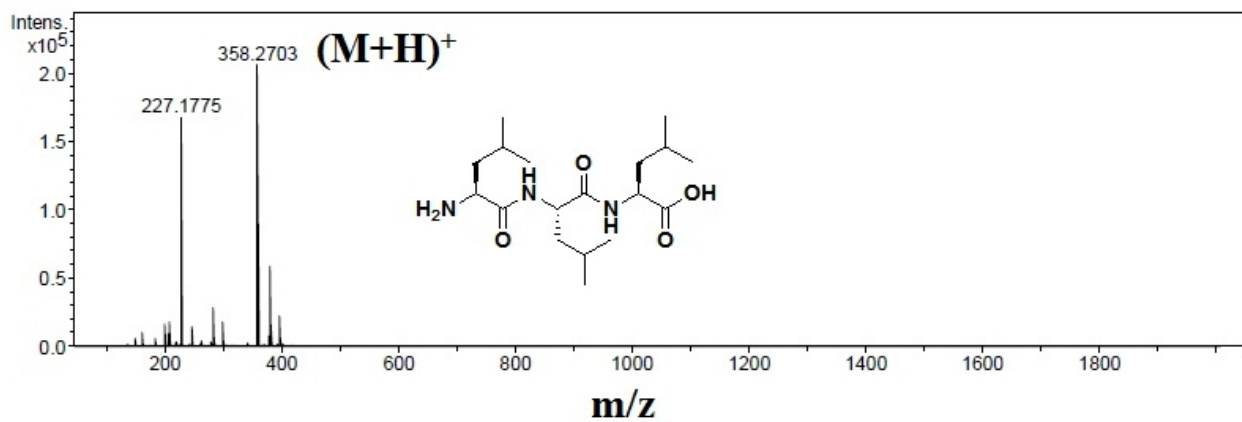


Figure S26: HRMS (ESI) spectrum of LLL (22).

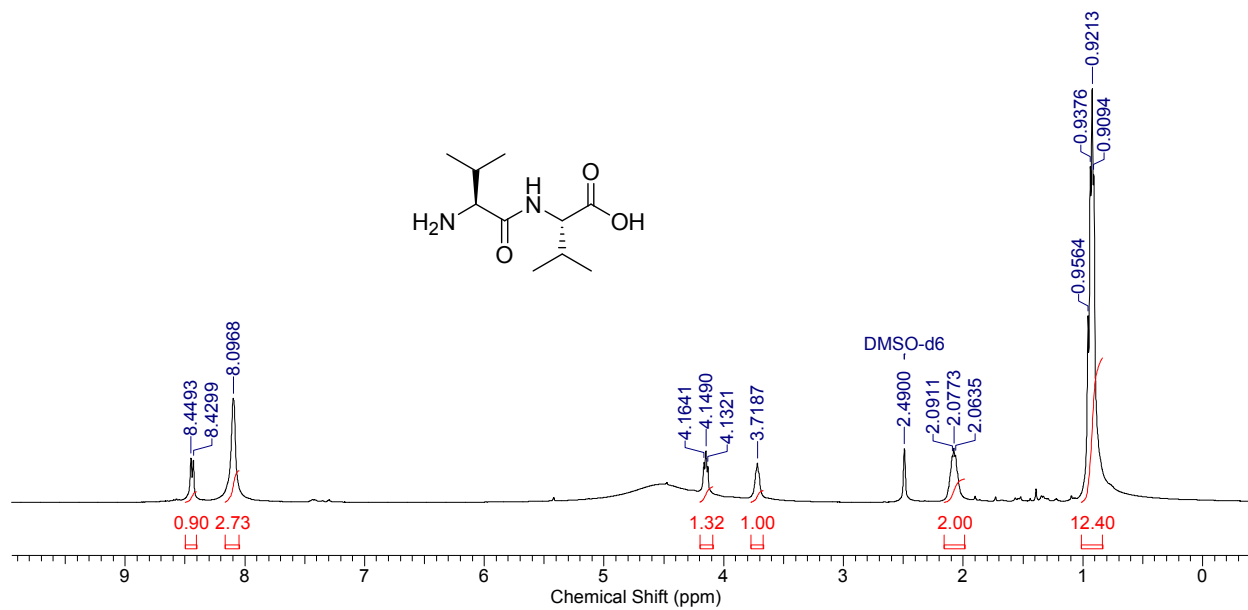


Figure S27: 400 MHz  $^1\text{H}$  NMR spectrum of VV (26) in DMSO- $d_6$ .

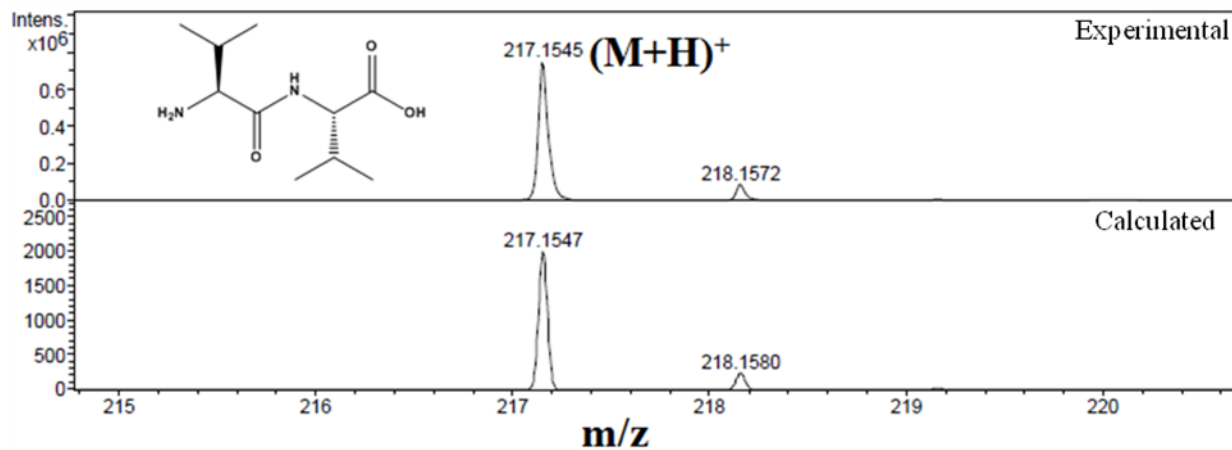
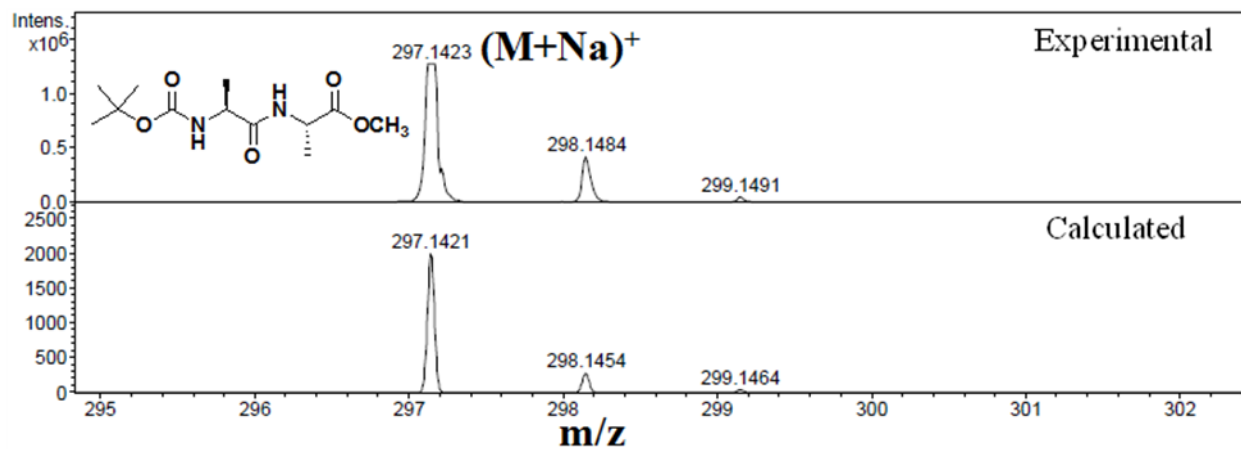
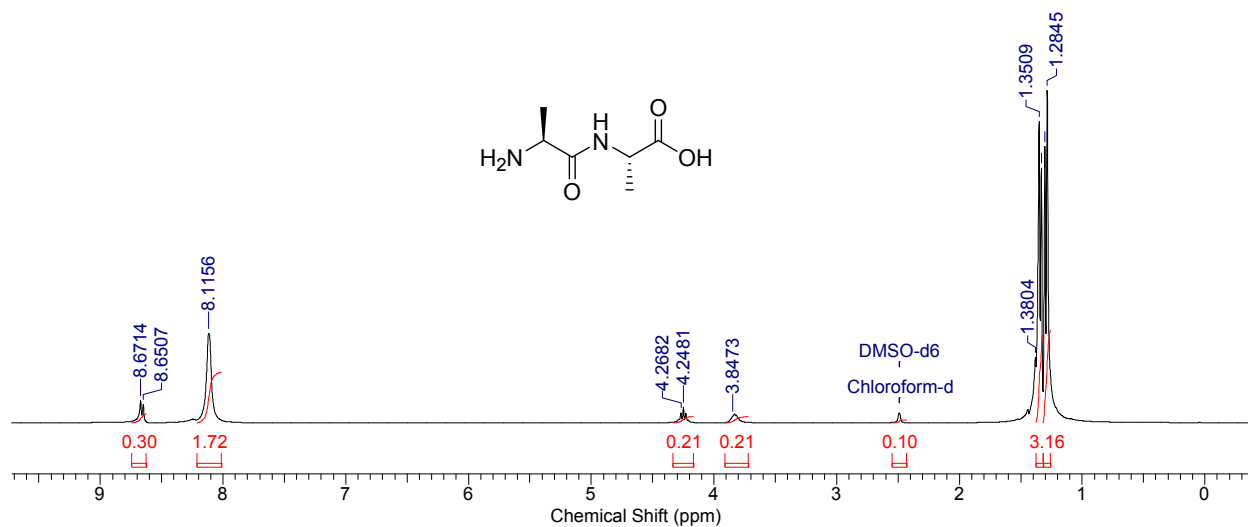


Figure S29: HRMS (ESI) spectrum of VV (26).

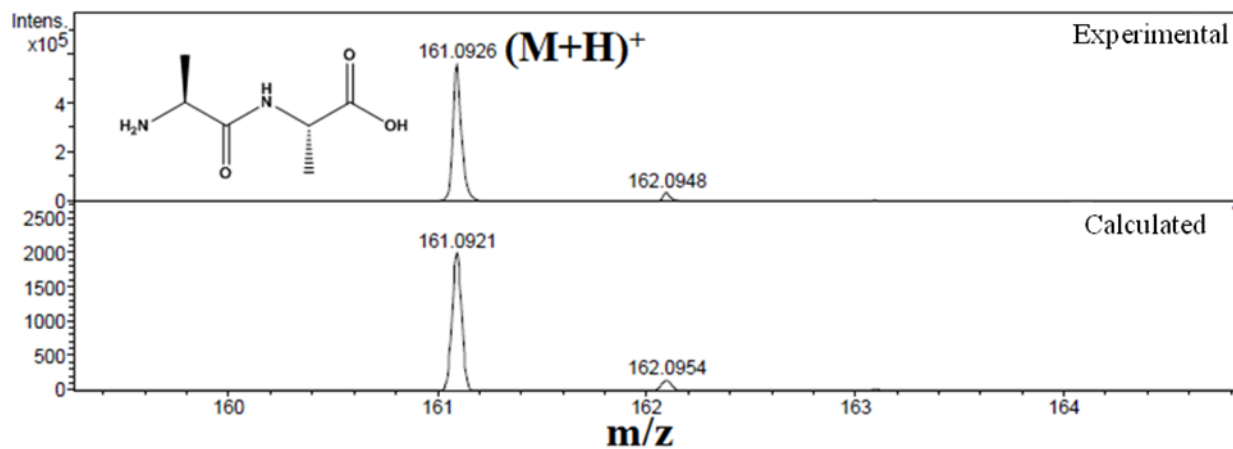


**Figure S30:** HRMS (ESI) spectrum of Boc-Ala-Ala-OMe (27).



**Figure S31:** 400 MHz  $^1\text{H}$  NMR spectrum of AA (29) in  $\text{DMSO-d}_6$ .





**Figure S32:** HRMS (ESI) spectrum of AA (29).

#### References:

- [1] D. B. Rasale, I. Maity, A. K. Das, *Chem. Commun.*, **2014**, *50*, 8685-8688.
- [2] D. B. Rasale, I. Maity, A. K. Das, *Chem. Commun.*, **2014**, *50*, 11397-11400.
- [3] D. B. Rasale, I. Maity, M. Konda, A. K. Das, *Chem. Commun.*, **2013**, *49*, 4815-4817.
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