

## Supplementary Information

# Reversible Transformation of Peptide Assembly Between Densified-Polysarcosine-Driven Kinetically and Helix-Orientation-Driven Thermodynamically Stable Morphologies

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## Materials and Methods

### Materials

All amino acids and condensation reagents were purchased from Watanabe Chemical Ind., Ltd., Hiroshima, Japan. The membrane-fluidity-sensitive dyes 1,6-Diphenyl-1,3,5-hexatriene (DPH, CAS:1720-32-7) and 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene *p*-toluene sulfonate (TMA-DPH, CAS: 115534-33-3) were purchased from Cayman Chemical Company, MI, USA. Saline (product ID: 991-4987035132509) was obtained from Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan.

### Synthesis of amphiphilic polypeptides $S_nL_{12}S_n$ and $S_nL_{12}$

The synthetic schemes for the bola-type amphiphilic polypeptides of  $PSar_n-b-(L-Leu-Aib)_6-b-PSar_n$  ( $S_nL_{12}S_n$ ) are shown in Scheme S1 (see Supporting Information), in which  $n$  = degree of polymerization of PSar. Briefly, the methoxy group of Boc-(L-Leu-Aib)<sub>6</sub>-OMe, which was synthesized using the established method<sup>1-4</sup>, was deprotected by alkaline hydrolysis using 1N NaOH. Then Boc-(L-Leu-Aib)<sub>6</sub>-OH and Boc-ethylenediamine (Boc-NH-CH<sub>2</sub>CH<sub>2</sub>-NH<sub>2</sub>) were condensed to obtain Boc-(L-Leu-Aib)<sub>6</sub>-NH-CH<sub>2</sub>CH<sub>2</sub>-NH-Boc. After the deprotection of both Boc groups by acid hydrolysis with 4N HCl/dioxane, the hydrophilic PSar chain was elongated from both terminal amino groups of H-(L-Leu-Aib)<sub>6</sub>-NH-CH<sub>2</sub>CH<sub>2</sub>-NH<sub>2</sub>, with sarcosine *N*-

carboxyanhydride (SarNCA) synthesized as previously reported<sup>1, 2, 5-7</sup>. After purification using Sephadex LH-20 columns, S<sub>n</sub>L<sub>12</sub>S<sub>n</sub> was obtained as white powder.

PSar<sub>n</sub>-*b*-(L-Leu-Aib)<sub>6</sub> (S<sub>n</sub>L<sub>12</sub>) was synthesized with the method previously reported<sup>1, 2, 5, 8, 9</sup>.

Synthesized compounds were confirmed by <sup>1</sup>H NMR spectroscopy and matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) (data summarized in Supporting Information).

### **Preparation of peptide assemblies**

50 mg/mL amphiphilic peptide (S<sub>n</sub>L<sub>12</sub>S<sub>n</sub> or S<sub>n</sub>L<sub>12</sub>) stock solutions were prepared by dissolving the synthesized polypeptides in super dehydrated ethanol (EtOH). Peptide assemblies were prepared using the EtOH injection method. 10 μL of the stock solutions were injected into 990 μL saline at room temperature (final concentration 0.5 mg/mL) and stirred for 10 min. The resulting dispersions were heated at varying temperatures (indicated in the text) for 1 h and naturally cooled to room temperature.

### **Transmission electron microscopy (TEM)**

The morphologies of the assemblies were observed using TEM. TEM images were obtained using the JEM-1230 TEM (JEOL, Tokyo, Japan) operated at 80 kV accelerating voltage. The

samples were prepared by mounting 5  $\mu\text{L}$  of the peptide assembly dispersion on a carbon-coated copper grid (Okensoji Co., Ltd., Tokyo, Japan), then negatively stained with 2% samarium acetate. Excess fluid was removed using filter paper.

### **Dynamic light scattering (DLS)**

The hydrodynamic diameter of assemblies in saline was measured with an ELSZ-2PL apparatus (Photal Otsuka Electronics Co. Ltd., Osaka, Japan) using a He-Ne laser (633 nm). All measurements were performed in triplicate at 25 °C.

### **Membrane fluidity evaluation with DPH and TMA-DPH**

The membrane fluidities of the peptide assemblies were ascertained at room temperature with a JASCO FP-6500 spectrofluorimeter (JASCO Corporation, Tokyo, Japan) using the dyes DPH and TMA-DPH<sup>10-12</sup>. In each trial, 4  $\mu\text{L}$  of a 250  $\mu\text{M}$  ethanol solution containing DPH or TMA-DPH were added to a 0.15 mM (0.5 mg/mL) assembly dispersion, after which each sample was incubated for 90 min in the dark. The excitation and emission wavelengths were 360 and 430 nm, respectively. The excitation was vertically polarized, while the emission was recorded in both parallel,  $I_{\parallel}$  ( $0^\circ, 0^\circ$ ), and perpendicular,  $I_{\perp}$  ( $0^\circ, 90^\circ$ ), modes. The polarization ( $P$ ) values of the DPH and TMA-DPH were calculated using the formula  $P = (I_{\parallel} - GI_{\perp}) / (I_{\parallel} + GI_{\perp})$ , where  $G$  is a correction factor equal to  $i_{\perp}/i_{\parallel}$ , and  $i_{\perp}$  and  $i_{\parallel}$  are the perpendicular ( $90^\circ, 0^\circ$ ) and

parallel (90°, 90°) emission intensities recorded using horizontally polarized light, respectively.

### **Circular dichroism (CD)**

CD measurements were carried out on a JASCO J-720 (JASCO Corporation, Tokyo, Japan) using a cell with an optical path length of 1 cm. Stock solutions (50 mg/mL) of amphiphilic polypeptides in super dehydrated EtOH were prepared. Before CD data collection, each peptide solution was diluted five hundred-fold in EtOH (final concentration: 0.1 mg/mL). Four accumulation cycles were used for each sample, and the data were recorded at 25 °C.

The helix content was calculated with the following formula;

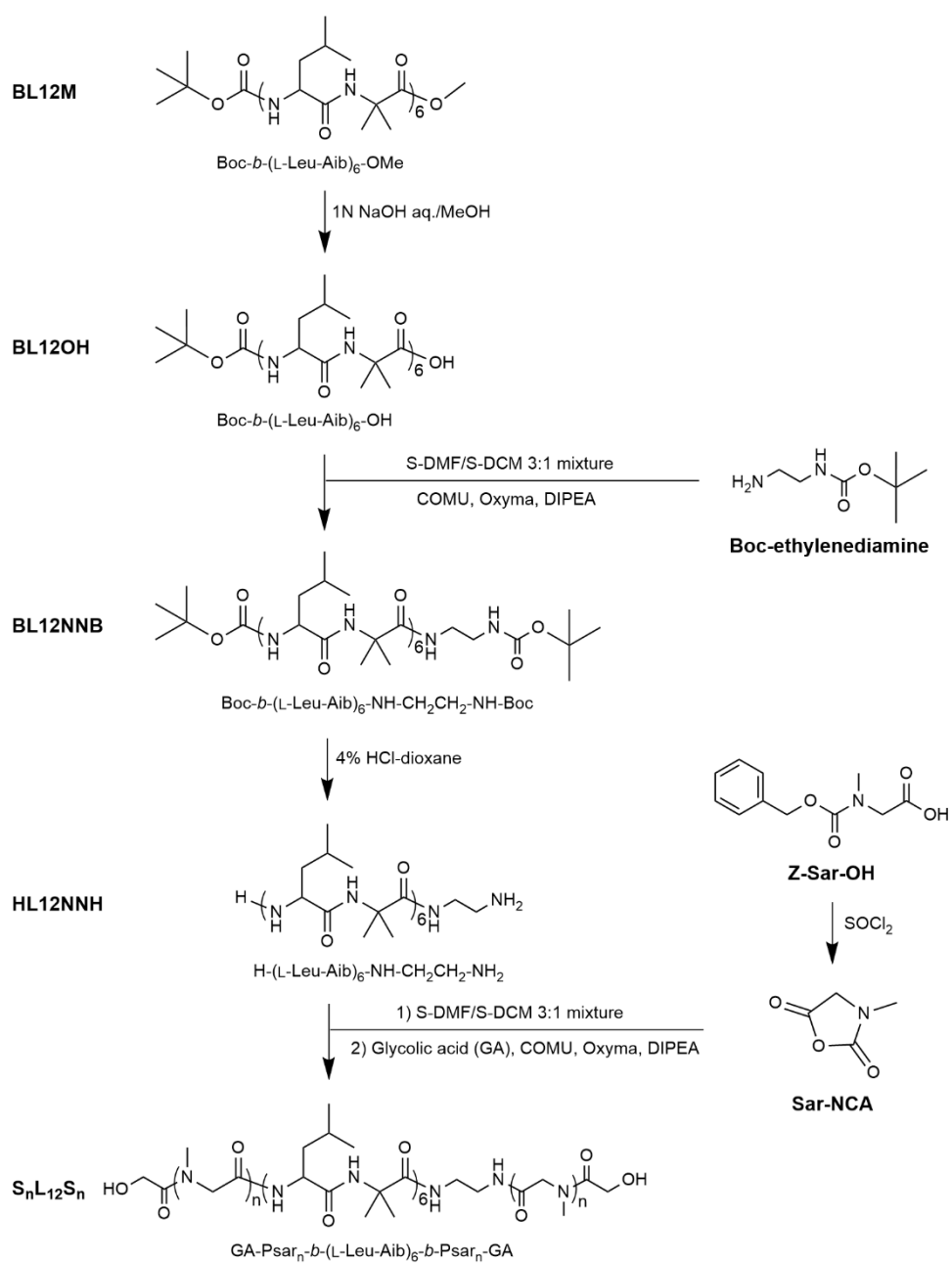
$$\text{Helix content (\%)} = -([\theta_{222}] - 2340) / 30300 \times 100^{13}$$

## SI-1. Synthesis of amphiphilic polypeptides $S_nL_{12}S_n$ (Scheme S1)

### Synthesis of $S_nL_{12}S_n$

$S_nL_{12}S_n$  was synthesized according to the following scheme S1.

#### Scheme S1. Synthesis of $S_nL_{12}S_n$ .



## BL12OH

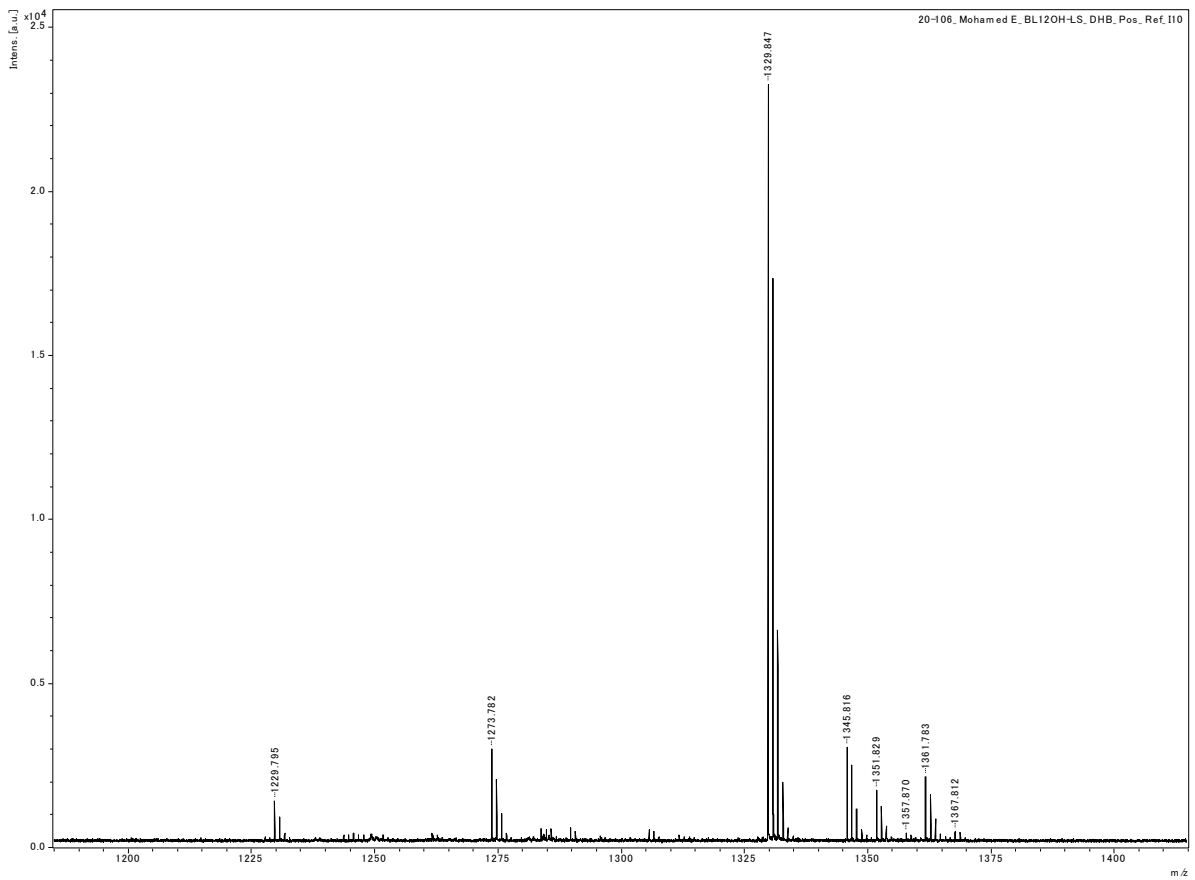
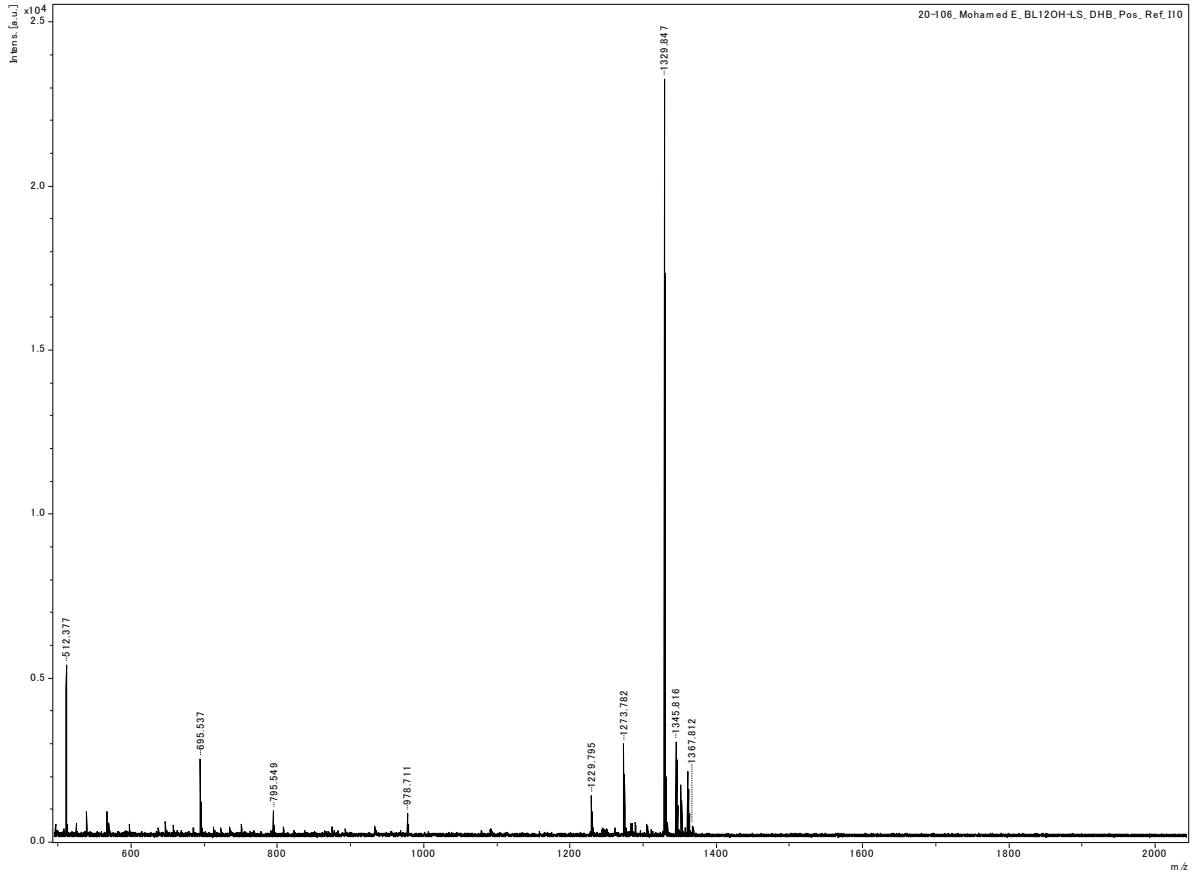
Methyl ester deprotection of BL12M was executed by solubilizing the BL12M (500 mg, 378.28  $\mu\text{mol}$ ) in MeOH. To BL12M solution, NaOH (1N) dissolved in 80% MeOH was added (mixture ratio; 7:3 v/v). The reaction was implemented at 38 °C under a condenser and monitored by thin layer chromatography (TLC) with the ninhydrin staining. To collect the pure BL12OH, firstly, the solution was neutralized with 1N HCl to pH 6 and evaporated under vacuum using a rotary evaporator. Secondly, the dried powder was dissolved in  $\text{CHCl}_3$  and subjected to wash by shaking with 4%  $\text{KHSO}_4$  aqueous solution. The  $\text{KHSO}_4$  was back-extracted with  $\text{CHCl}_3$ , then the  $\text{CHCl}_3$  part was collected and dried over  $\text{MgSO}_4$  powder. To collect the compound, the mixture was finally filtered through filter paper, evaporated, and dried under vacuum. The targeted compound obtained was a white powder (340 mg, 68.73 % yield).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 8.3–7.3 [m, 12H, amide], 4.6–3.8 [br, 6H, Leu $C^\alpha$ H], 2.0–1.3 [m, 63H, Leu $\text{CH}_2$ , Leu $C^\gamma$ H, Aib( $\text{CH}_3$ )<sub>2</sub>, Boc( $\text{CH}_3$ )<sub>3</sub>], 1.1–0.8 [m, 36H, Leu( $\text{CH}_3$ )<sub>2</sub>].

MALDI-TOF MS calculated for  $\text{C}_{65}\text{H}_{118}\text{N}_{12}\text{O}_{15}\text{Na}^+$  [M+Na]<sup>+</sup>  $m/z$  1329.88, found: 1329.847.

MALDI-TOF MS calculated for  $\text{C}_{65}\text{H}_{118}\text{N}_{12}\text{O}_{15}\text{K}^+$  [M+K]<sup>+</sup>  $m/z$  1345.88, found: 1345.816.



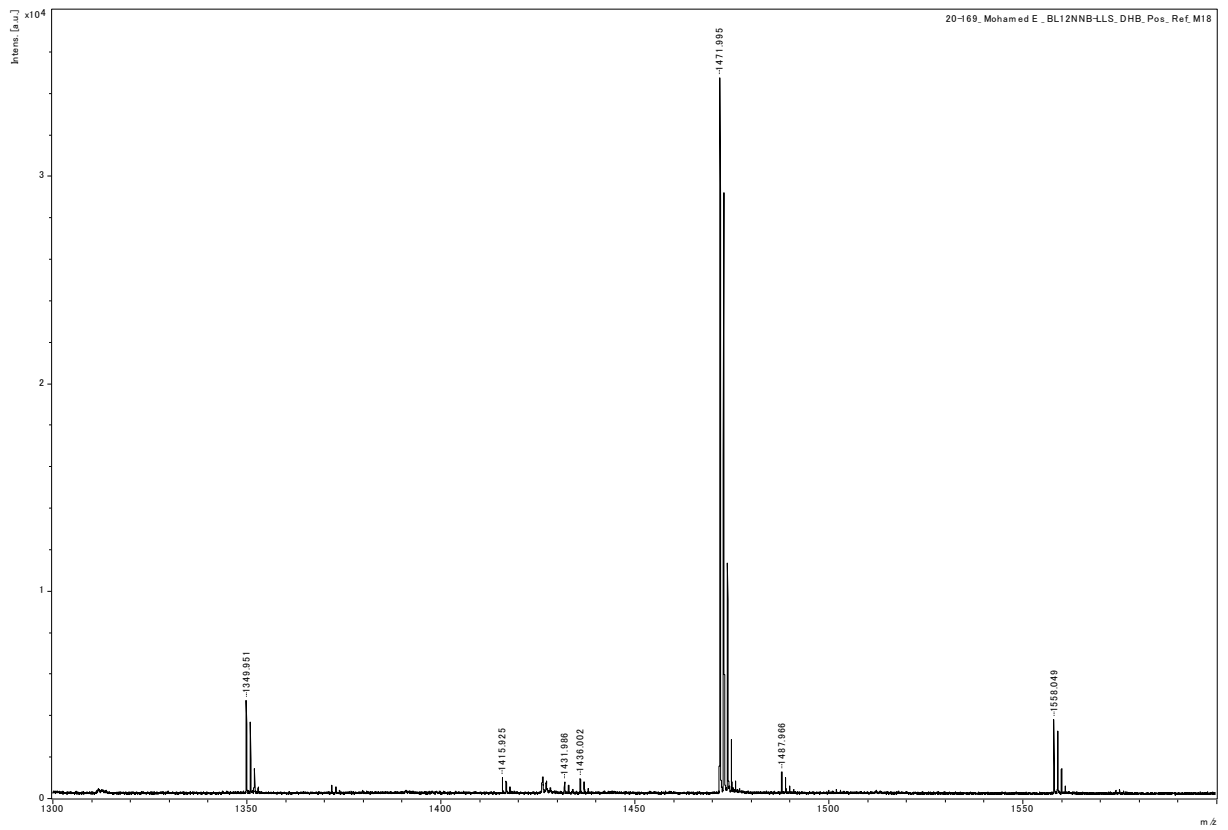
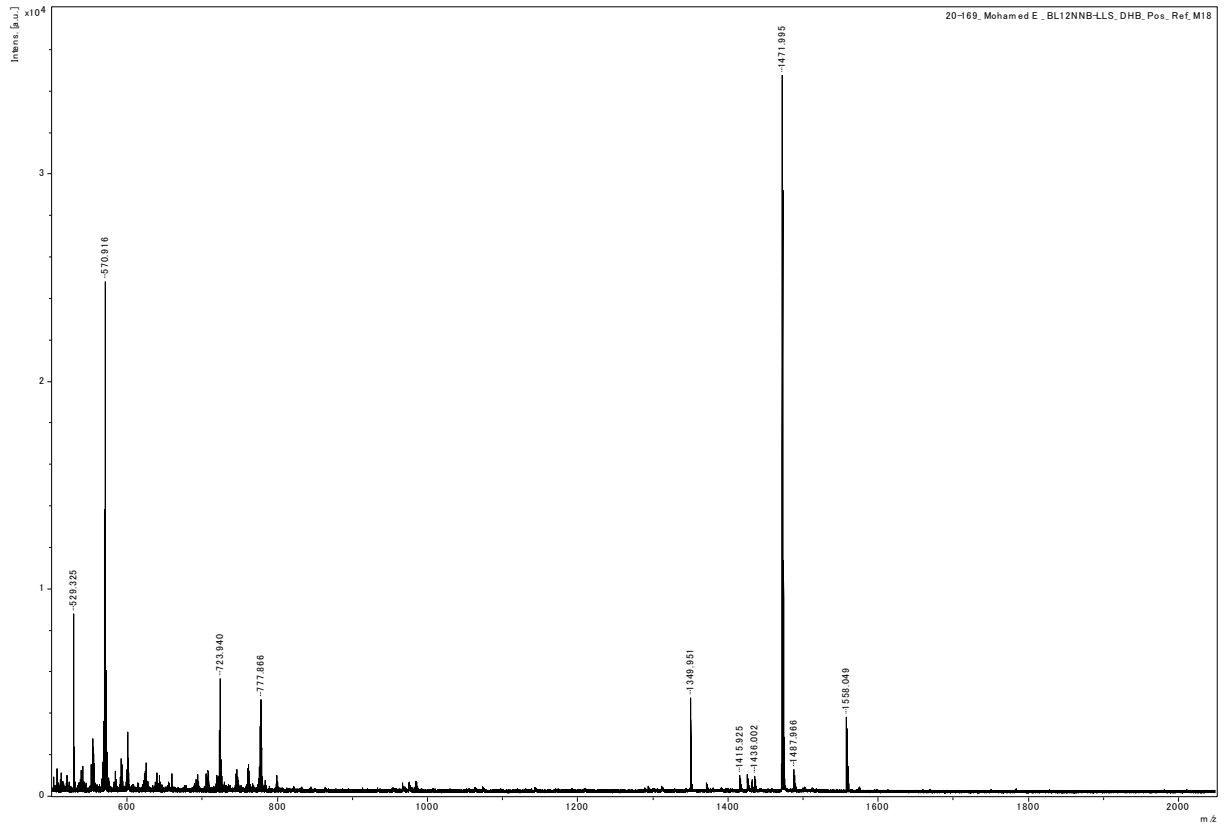


## BL12NNB

The BL12OH powder (340 mg, 259.99  $\mu\text{mol}$ ) was dissolved in a mixture of superdehydrated dichloromethane (DCM) and superdehydrated Dimethylformamide (DMF) with a mixing ratio of 1:3 (v/v). To this solution, *N*-Boc-ethylenediamine (1.5 eq.), (1-cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylaminomorpholino-carbenium hexafluorophosphate (COMU) (1.5 eq.), ethyl cyano(hydroxyimino)acetate (Oxyma Pure) (1.5 eq.), and *N,N*-diisopropylethylamine (DIPEA) (2.4 eq.) were added. The reaction was stirred overnight at 25 °C under argon atmosphere. To detect completion, the reaction was monitored by TLC using the ninhydrin stain. To gain the compound, the reaction mixture was firstly concentrated and evaporated by a rotary evaporator followed by drying under vacuum, then, the dried crude was dissolved in chloroform and purified through a Sephadex LH-20 column with MeOH elution. The fractions were identified and collected based on UV measurements at 260 nm and mass analysis. The purified compound was obtained as a white powder (307 mg, 81.44 % yield). This compound was identified by MALDI-TOF MS and moved to the next deprotection step.

MALDI-TOF MS calculated for  $\text{C}_{72}\text{H}_{132}\text{N}_{14}\text{O}_{16}\text{Na}^+$   $[\text{M}+\text{Na}]^+$   $m/z$  1471.994, found: 1471.995.

MALDI-TOF MS calculated for  $\text{C}_{72}\text{H}_{132}\text{N}_{14}\text{O}_{16}\text{K}^+$   $[\text{M}+\text{K}]^+$   $m/z$  1487.994, found: 1487.966.



## HL12NNH

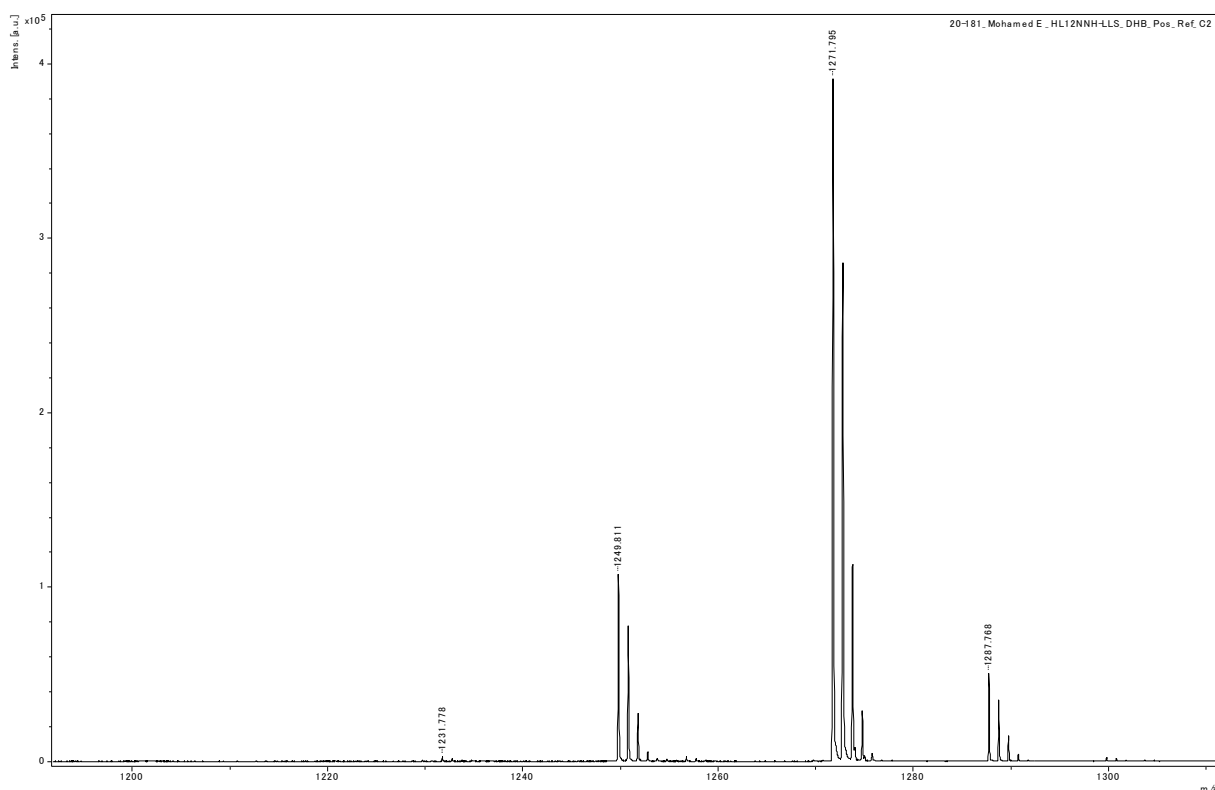
Deprotection of the two *tert*-butoxycarbonyl (Boc) groups of BL12NNB (307 mg, 211.73  $\mu\text{mol}$ ) was initiated by dissolving the compound in 4N HCl-dioxane (529.34 mL 2.12 mmol). The solution was stirred for 1 h at room temperature, and the reaction progress was monitored by TLC stained with ninhydrin. After concentration and dryness, washing of the crude powder was performed by saturated  $\text{NaHCO}_3$  aqueous solution, followed by extraction in organic  $\text{CHCl}_3$ . The collected  $\text{CHCl}_3$  phase was dried over  $\text{MgSO}_4$  powder and eventually filtered through filter paper. To get the HL12NNH in powder form, the filtered solution was evaporated and dried under vacuum. The gained powder was white in color (250 mg, 95.48 % yield).

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 8.5–7.6 [m, 14H, amide,  $\text{NHCH}_2\text{CH}_2\text{NH}_2$ ], 6.7 [s, 1H, urethane] 4.1–3.8 [br, 6H,  $\text{Leu}^{\alpha}\text{H}$ ], 3.8–3.5 [t, 2H,  $\text{NHCH}_2\text{CH}_2\text{NH}_2$ ], 3.4–3.1 [t, 2H,  $\text{NHCH}_2\text{CH}_2\text{NH}_2$ ], 2.0–1.3 [m, 54H,  $\text{LeuCH}_2$ ,  $\text{Leu}^{\gamma}\text{H}$ ,  $\text{Aib}(\text{CH}_3)_2$ ], 1.1–0.8 [m, 36H,  $\text{Leu}(\text{CH}_3)_2$ ].

MALDI-TOF MS calculated for  $\text{C}_{62}\text{H}_{116}\text{N}_{14}\text{O}_{12}\text{H}^+$   $[\text{M}+\text{H}]^+$   $m/z$  1249.89, found: 1249.811.

MALDI-TOF MS calculated for  $\text{C}_{62}\text{H}_{116}\text{N}_{14}\text{O}_{12}\text{Na}^+$   $[\text{M}+\text{Na}]^+$   $m/z$  1271.89, found: 1271.795.

MALDI-TOF MS calculated for  $\text{C}_{62}\text{H}_{116}\text{N}_{14}\text{O}_{12}\text{K}^+$   $[\text{M}+\text{K}]^+$   $m/z$  1287.89, found: 1287.768.



### $S_nL_{12}S_n$

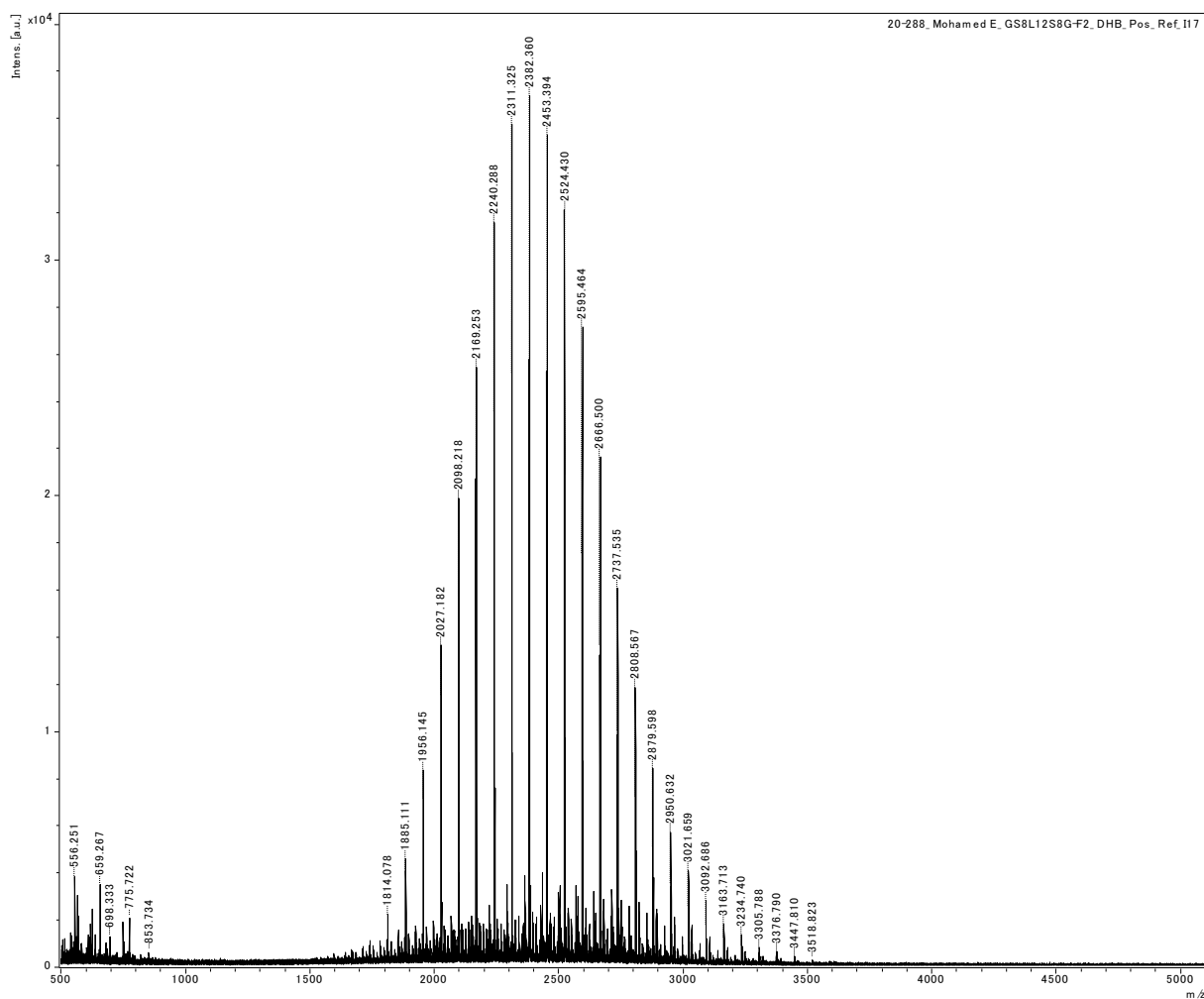
In order to control the sarcosine chain length, the polymerization conditions were kept strictly dry. For that, the freshly prepared SarNCA (25.79 mg, 224.05  $\mu$ mol, 14 eq.), (46.05 mg, 400.10  $\mu$ mol, 20 eq.), (107.75 mg, 936.23  $\mu$ mol, 26 eq.), (132.61 mg, 1.15 mmol, 32 eq.), and (223.79 mg, 1.94 mmol, 54 eq.), were dissolved in a minimum volume of super dehydrated DMF. To start polymerization, solutions of HL12NNH as an initiator (20 mg, 16  $\mu$ mol, 1 eq.), (25 mg, 20  $\mu$ mol, 1 eq.), (45 mg, 36.01  $\mu$ mol, 1 eq.), (45 mg, 36.01  $\mu$ mol, 1 eq.), and (45 mg, 36.01  $\mu$ mol, 1 eq.) in a mixture of super dehydrated DMF and super dehydrated  $CHCl_3$  with a mixing ratio of 3:1 (v/v) were added to SarNCA solutions to prepare  $S_nL_{12}S_n$  ( $n = 7, 10, 13, 16$  and  $27$ ), respectively. The mixtures were stirred for 6 h at room temperature under an atmosphere of

argon. For capping the end of polymerized sarcosine chains, glycolic acid (GA) (1.5 eq.), COMU (1.5 eq.), Oxyma Pure (1.5 eq.), and DIPEA (2.4 eq.) were added, and reaction mixtures were left to stir overnight under argon atmosphere at 25 °C. To obtain compounds, mixtures were concentrated by rotary evaporator, then, purified through Sephadex LH-20 columns using MeOH as eluent. The column fractions were identified and collected based on UV measurements at 260 nm and mass analysis. The purified compounds were obtained as pale yellow to off-white powders (S<sub>7</sub>L<sub>12</sub>S<sub>7</sub>; 32 mg, 84.5 % yield, S<sub>10</sub>L<sub>12</sub>S<sub>10</sub>; 49 mg, 87.0 % yield, S<sub>13</sub>L<sub>12</sub>S<sub>13</sub>; 97 mg, 83.5 % yield, S<sub>16</sub>L<sub>12</sub>S<sub>16</sub>; 114 mg, 86.9 % yield, and S<sub>27</sub>L<sub>12</sub>S<sub>27</sub>; 161 mg, 85.6 % yield).

### **S<sub>7</sub>L<sub>12</sub>S<sub>7</sub>**

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 8.1–7.3 [m, 13H, amide], 7.0 [s, 1H, urethane], 4.5–3.8 [br, 42H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>14</sub>, HOCH<sub>2</sub>CO, NH(CH<sub>2</sub>)<sub>2</sub>NH], 3.3–2.8 [m, 42H, (SarCH<sub>3</sub>)<sub>14</sub>], 1.9–1.3 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

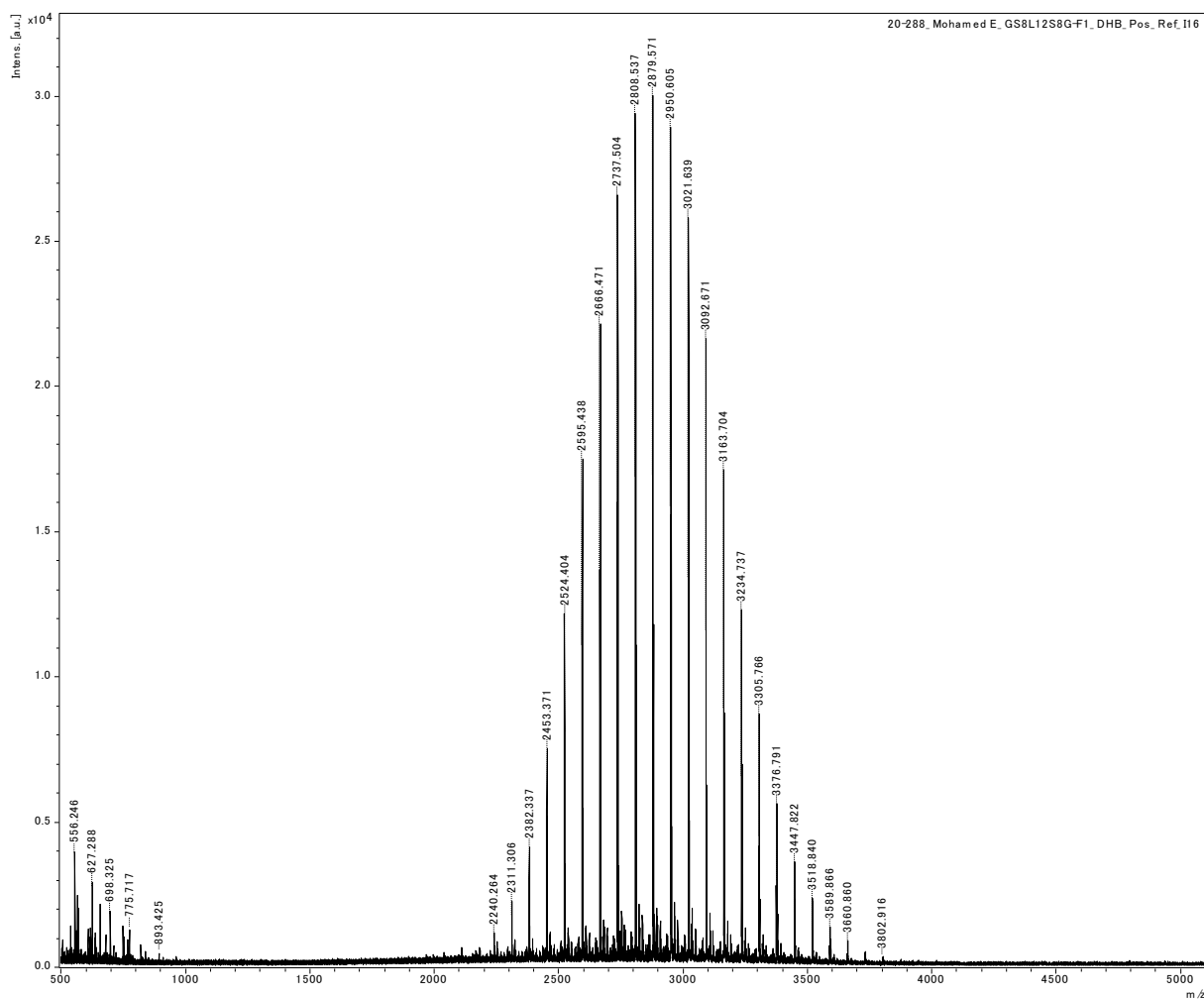
MALDI-TOF MS calculated for C<sub>108</sub>H<sub>190</sub>N<sub>28</sub>O<sub>30</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 2382.420, found: 2382.360.



## S<sub>10</sub>L<sub>12</sub>S<sub>10</sub>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 8.2–7.3 [m, 13H, amide], 7.0 [s, 1H, urethane], 4.6–3.8 [br, 54H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>20</sub>, HOCH<sub>2</sub>CO, NH(CH<sub>2</sub>)<sub>2</sub>NH], 3.3–2.8 [m, 60H, (SarCH<sub>3</sub>)<sub>20</sub>], 2.0–1.3 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

MALDI-TOF MS calculated for C<sub>126</sub>H<sub>220</sub>N<sub>34</sub>O<sub>36</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 2808.642, found: 2808.537.

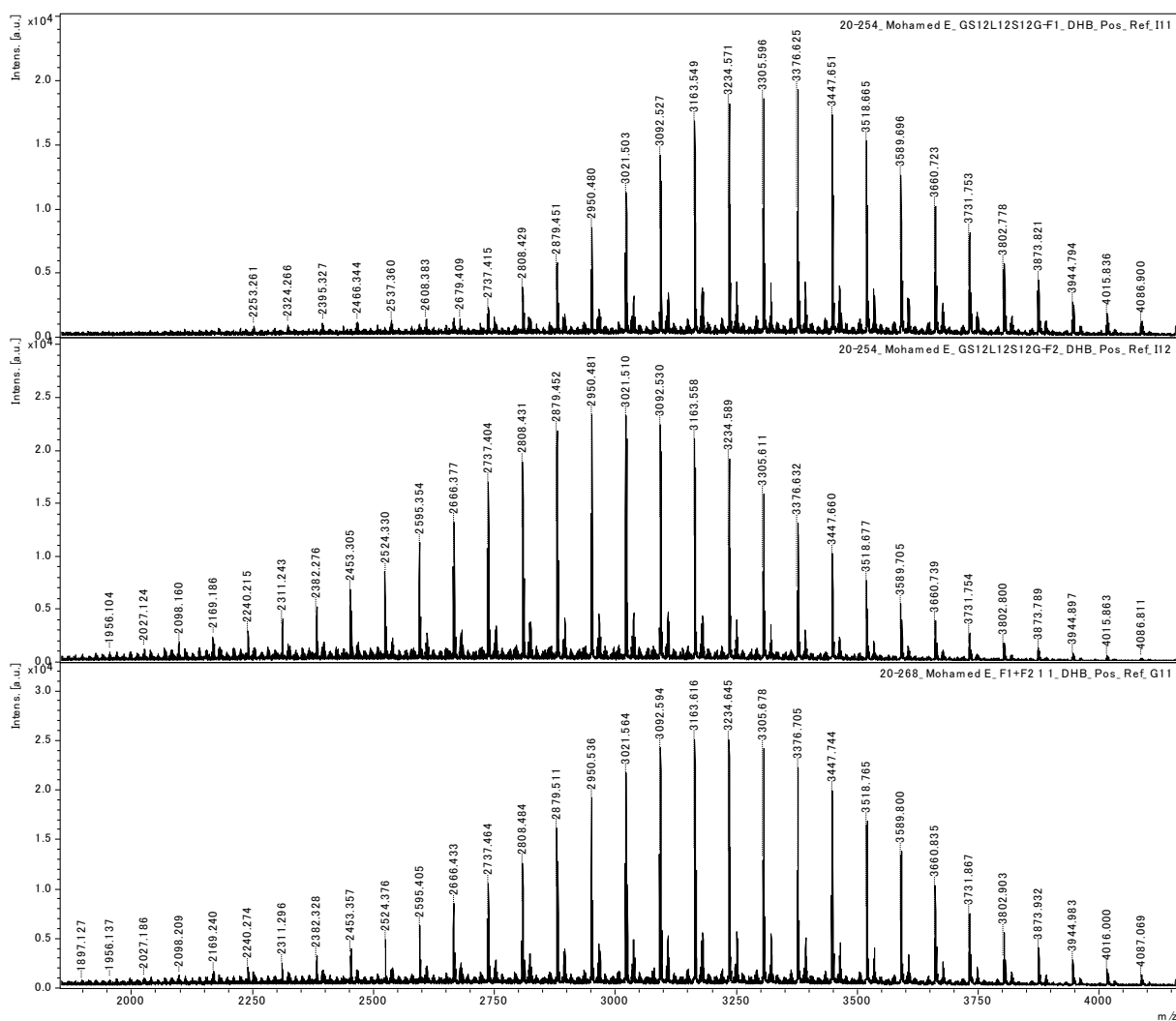


### S<sub>13</sub>L<sub>12</sub>S<sub>13</sub>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 8.2–7.3 [m, 13H, amide], 7.0 [s, 1H, urethane], 4.5–3.8 [br, 66H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>26</sub>, HOCH<sub>2</sub>CO, NH(CH<sub>2</sub>)<sub>2</sub>NH], 3.3–2.8 [m, 72H, Sar(CH<sub>3</sub>)<sub>26</sub>], 2.1–1.3 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

MALDI-TOF MS calculated for C<sub>144</sub>H<sub>250</sub>N<sub>40</sub>O<sub>42</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 3234.865, found: 3234.645.

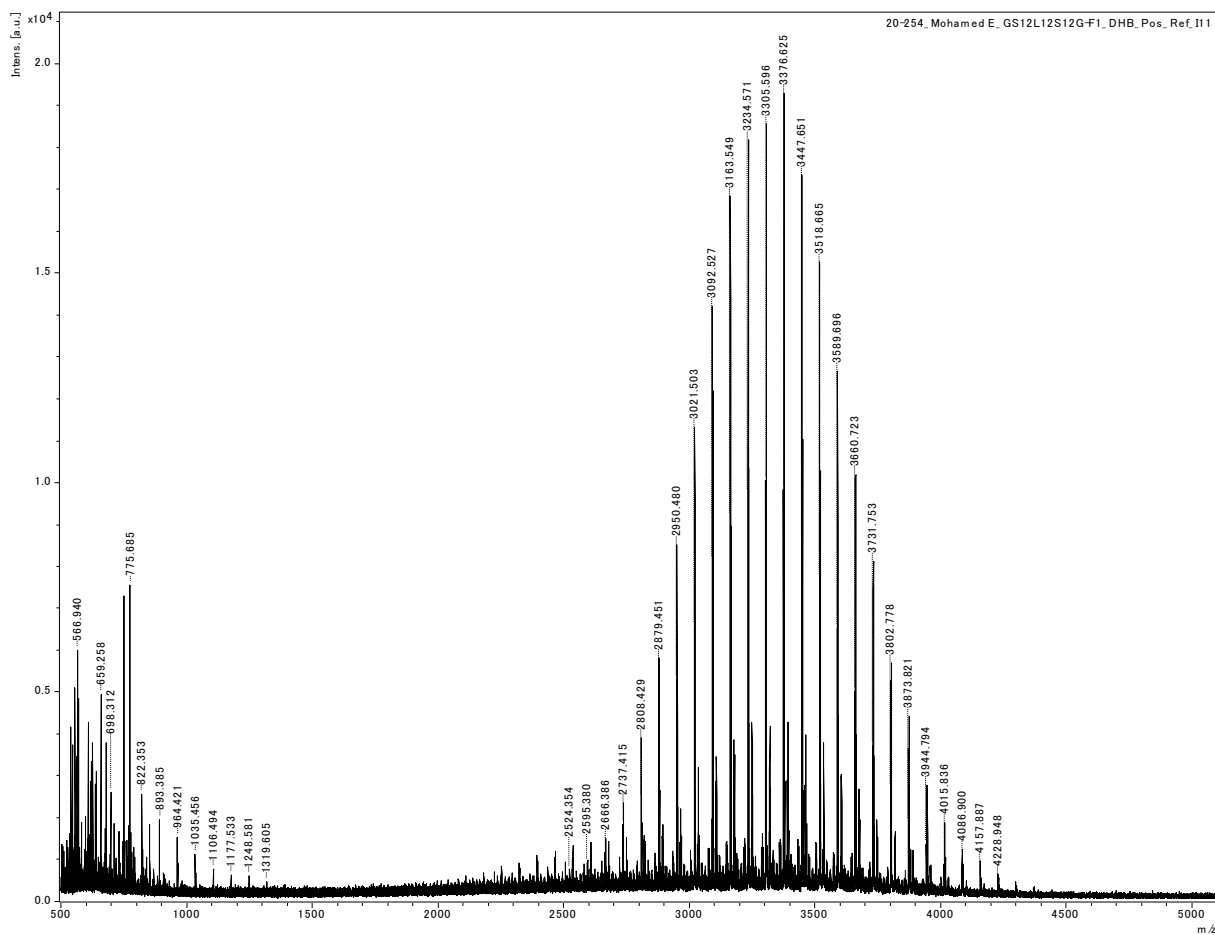




## S<sub>14</sub>L<sub>12</sub>S<sub>14</sub>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 8.3–7.2 [m, 13H, amide], 7.0 [s, 1H, urethane], 4.6–3.8 [br, 70H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>28</sub>, HOCH<sub>2</sub>CO, NH(CH<sub>2</sub>)<sub>2</sub>NH], 3.3–2.8 [m, 84H, Sar(CH<sub>3</sub>)<sub>28</sub>], 2.3–1.3 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

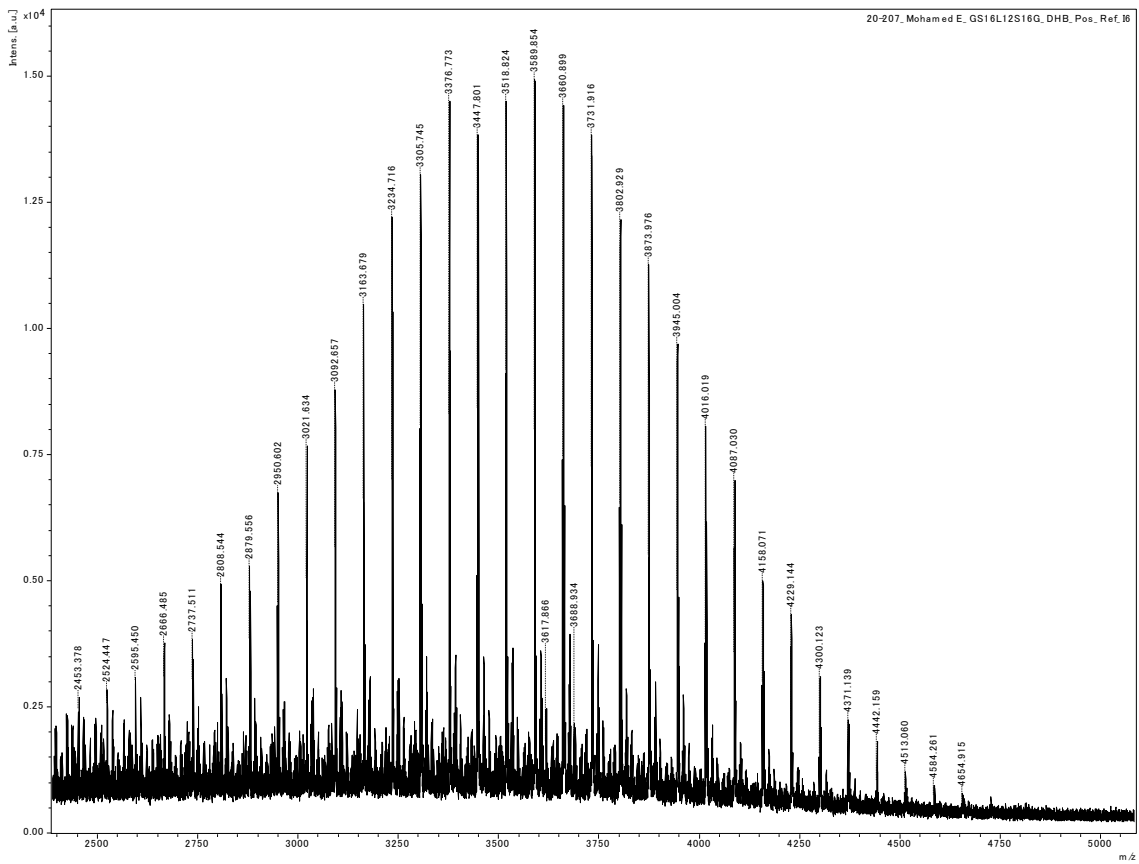
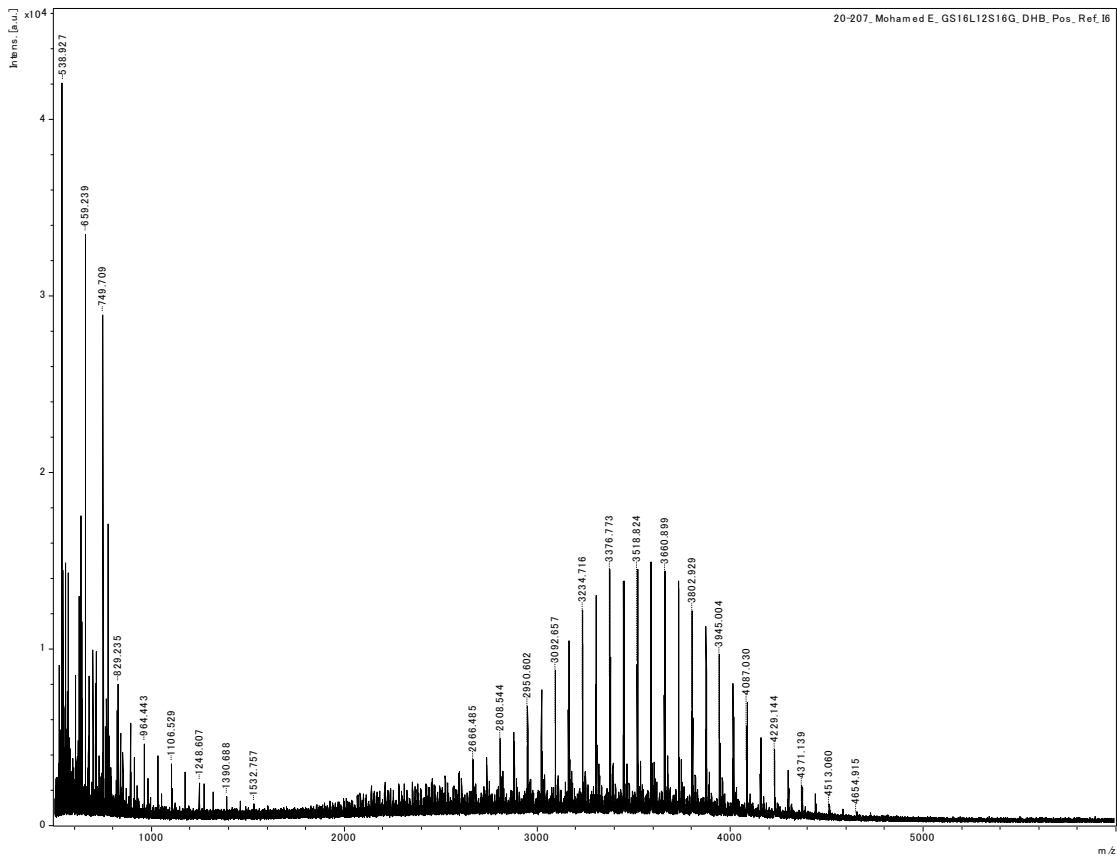
MALDI-TOF MS calculated for C<sub>150</sub>H<sub>260</sub>N<sub>42</sub>O<sub>44</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 3376.939, found: 3376.625.



### S<sub>16</sub>L<sub>12</sub>S<sub>16</sub>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 8.1–7.3 [m, 13H, amide], 7.0 [s, 1H, urethane], 4.5–3.8 [br, 78H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>32</sub>, HOCH<sub>2</sub>CO, NH(CH<sub>2</sub>)<sub>2</sub>NH], 3.3–2.8 [m, 96H, Sar(CH<sub>3</sub>)<sub>32</sub>], 2.0–1.3 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

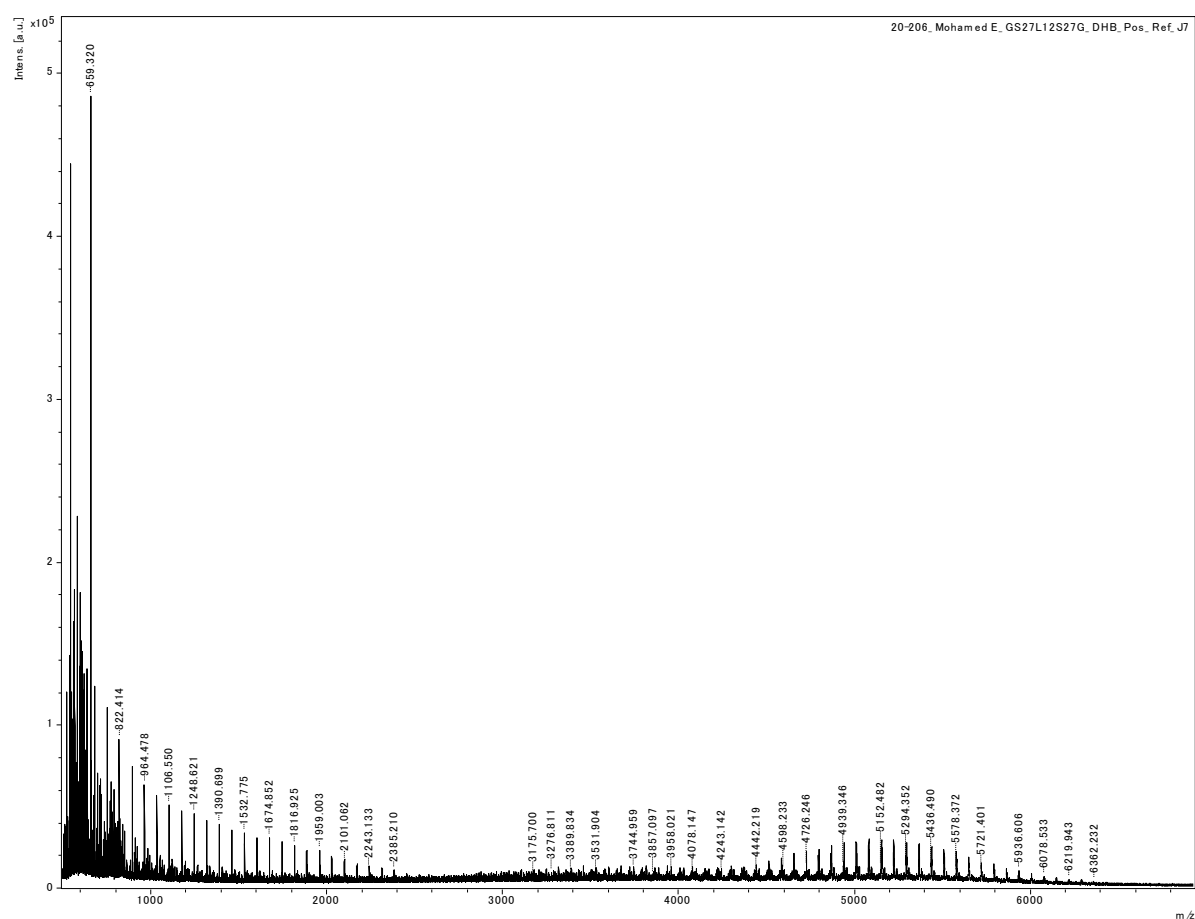
MALDI-TOF MS calculated for C<sub>162</sub>H<sub>280</sub>N<sub>46</sub>O<sub>48</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 3661.088, found: 3660.899.

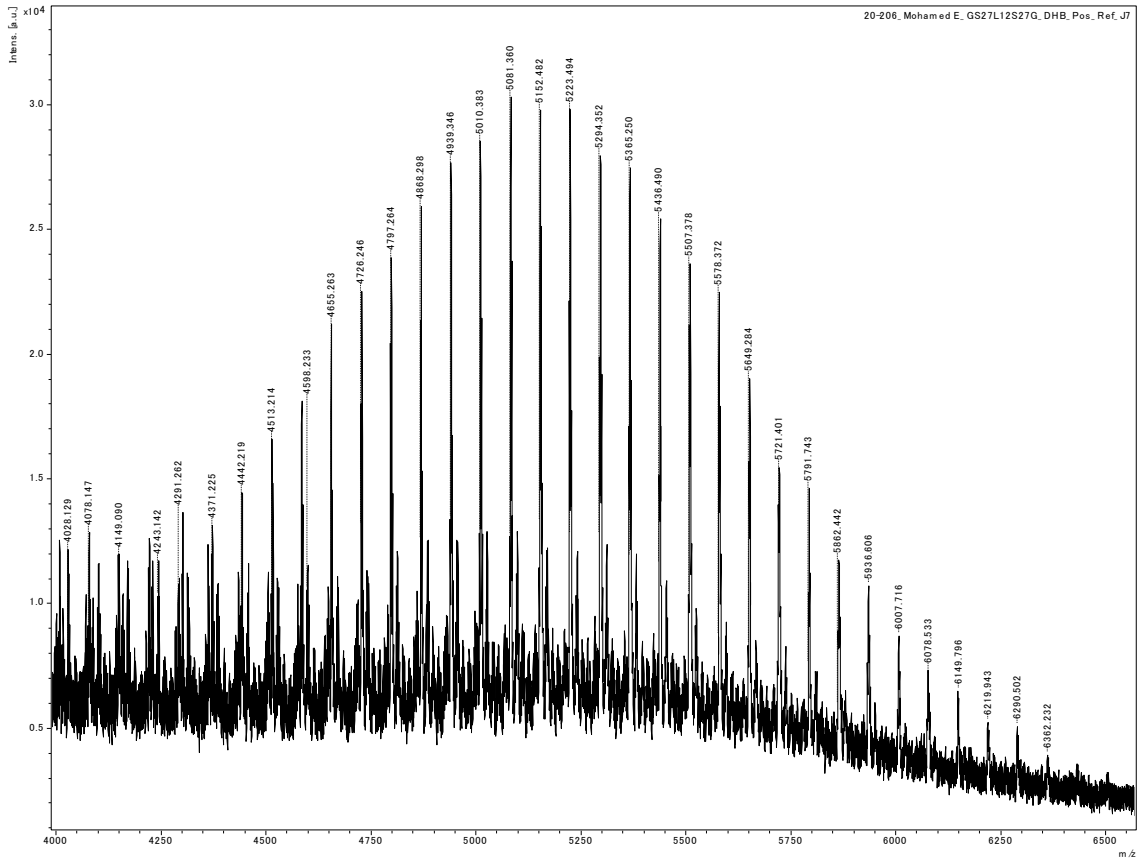


## S<sub>27</sub>L<sub>12</sub>S<sub>27</sub>

<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ (ppm) 8.1–7.4 [m, 14H, amide], 7.0 [s, 1H, urethane], 4.5–3.8 [br, 122H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>54</sub>, HOCH<sub>2</sub>CO, NH(CH<sub>2</sub>)<sub>2</sub>NH], 3.3–2.8 [m, 72H, Sar(CH<sub>3</sub>)<sub>54</sub>], 2.1–1.3 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

MALDI-TOF MS calculated for C<sub>228</sub>H<sub>390</sub>N<sub>68</sub>O<sub>70</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 5223.904, found: 5223.494.



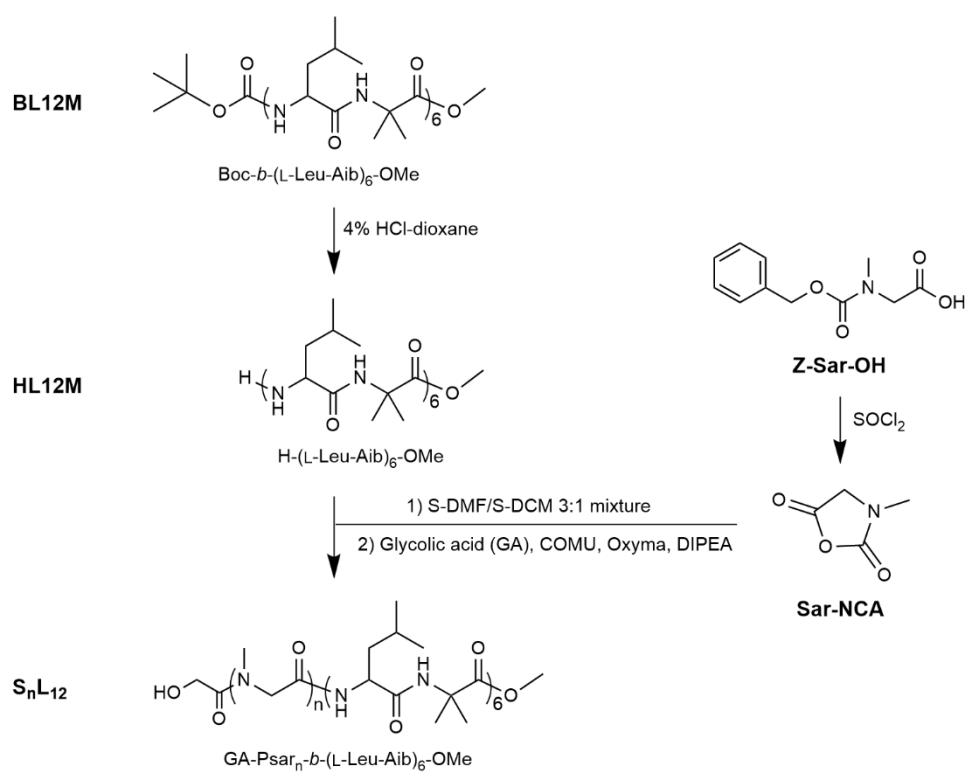


## SI-2. Synthesis of amphiphilic polypeptides $S_nL_{12}$ (Scheme S2)

### Synthesis of $S_nL_{12}$

$S_nL_{12}$  was synthesized according to the scheme S2.

### Scheme S2. Synthesis of $S_nL_{12}$ .



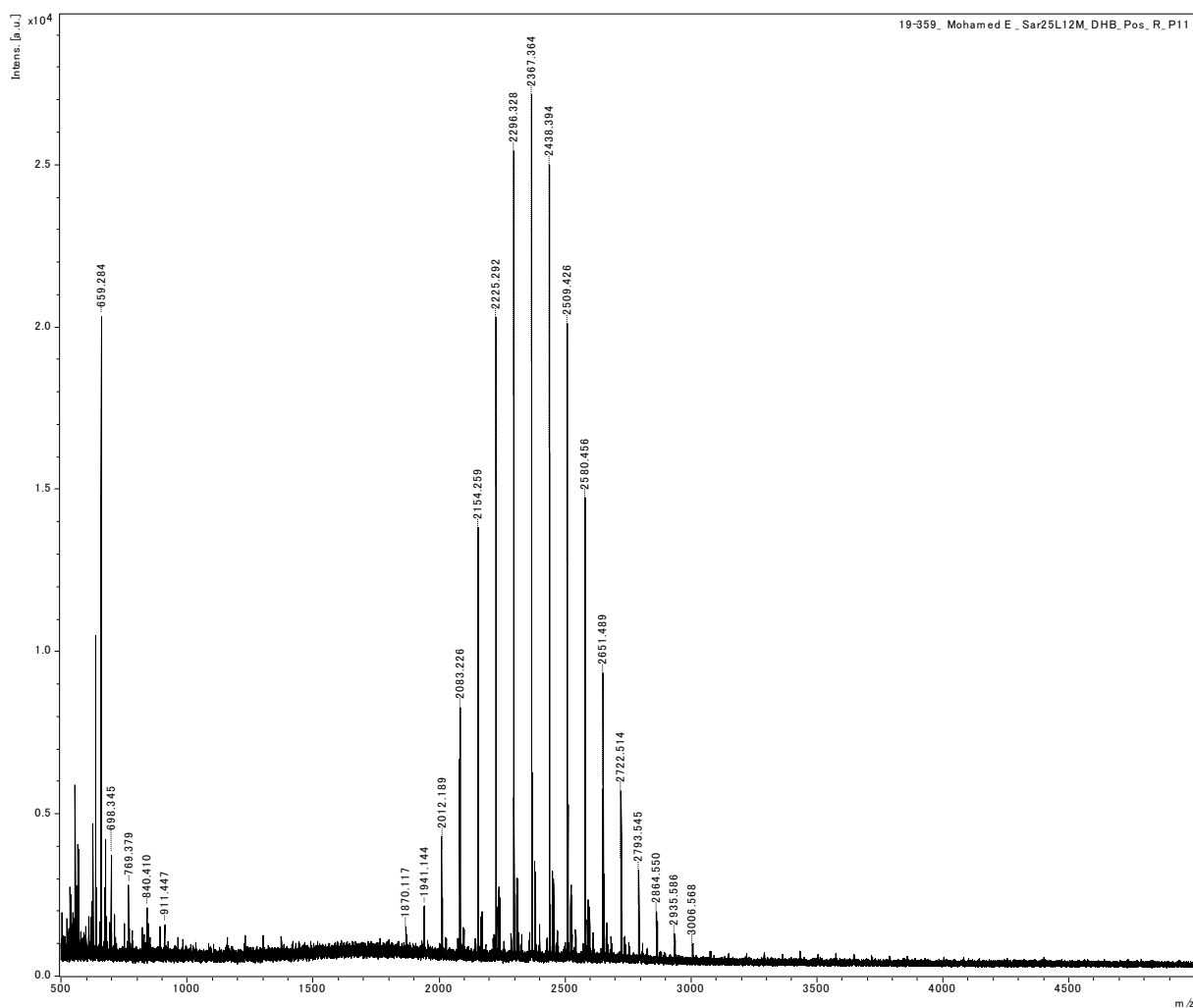
## **S<sub>n</sub>L<sub>12</sub>**

Amphiphilic Polypeptides PSar<sub>n</sub>-*b*-(L-Leu-Aib)<sub>6</sub> (S<sub>15</sub>L<sub>12</sub>, S<sub>21</sub>L<sub>12</sub>, S<sub>25</sub>L<sub>12</sub>, S<sub>28</sub>L<sub>12</sub>, S<sub>32</sub>L<sub>12</sub>, and S<sub>55</sub>L<sub>12</sub>) were synthesized as previously reported. Briefly, through consecutive steps of  $\alpha$ -aminoisobutyric acid (Aib) and leucine (Leu) amino acid condensation, Boc-(L-Leu-Aib)<sub>6</sub>-OMe which constitute the hydrophobic helical block was prepared. Then, the Boc group of Boc-(L-Leu-Aib)<sub>6</sub>-OMe was deprotected through acidic hydrolysis by 4N HCL in dioxane. Thereafter, hydrophilic polysarcosine chain elongation was performed on the N-terminal amine units of H-(L-Leu-Aib)<sub>6</sub>-OMe with SarNCA, followed by sarcosine end chain capping with GA (The detailed reaction condition of Boc deprotection, sarcosine elongation, and GA capping were previously mentioned in S<sub>n</sub>L<sub>12</sub>S<sub>n</sub> synthesis section). Finally, the formed compounds were purified through sephadex LH-20 column with MeOH elution. All synthesized compounds were confirmed by <sup>1</sup>H NMR spectroscopy and matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS).

## **S<sub>15</sub>L<sub>12</sub>**

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD):  $\delta$  (ppm) 7.9–7.3 [m, 11H, amide], 7.0 [s, 1H, urethane], 4.4–3.8 [br, 38H, LeuC <sup>$\alpha$</sup> H, Sar(CH<sub>2</sub>)<sub>15</sub>, HOCH<sub>2</sub>CO], 3.66 [s, 3H, OCH<sub>3</sub>], 3.3–2.8 [m, 45H, Sar(CH<sub>3</sub>)<sub>15</sub>], 1.9–1.4 [m, 54H, LeuCH<sub>2</sub>, LeuC <sup>$\gamma$</sup> H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

MALDI-TOF MS calculated for C<sub>108</sub>H<sub>189</sub>N<sub>27</sub>O<sub>30</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 2367.409, found: 2367.364.

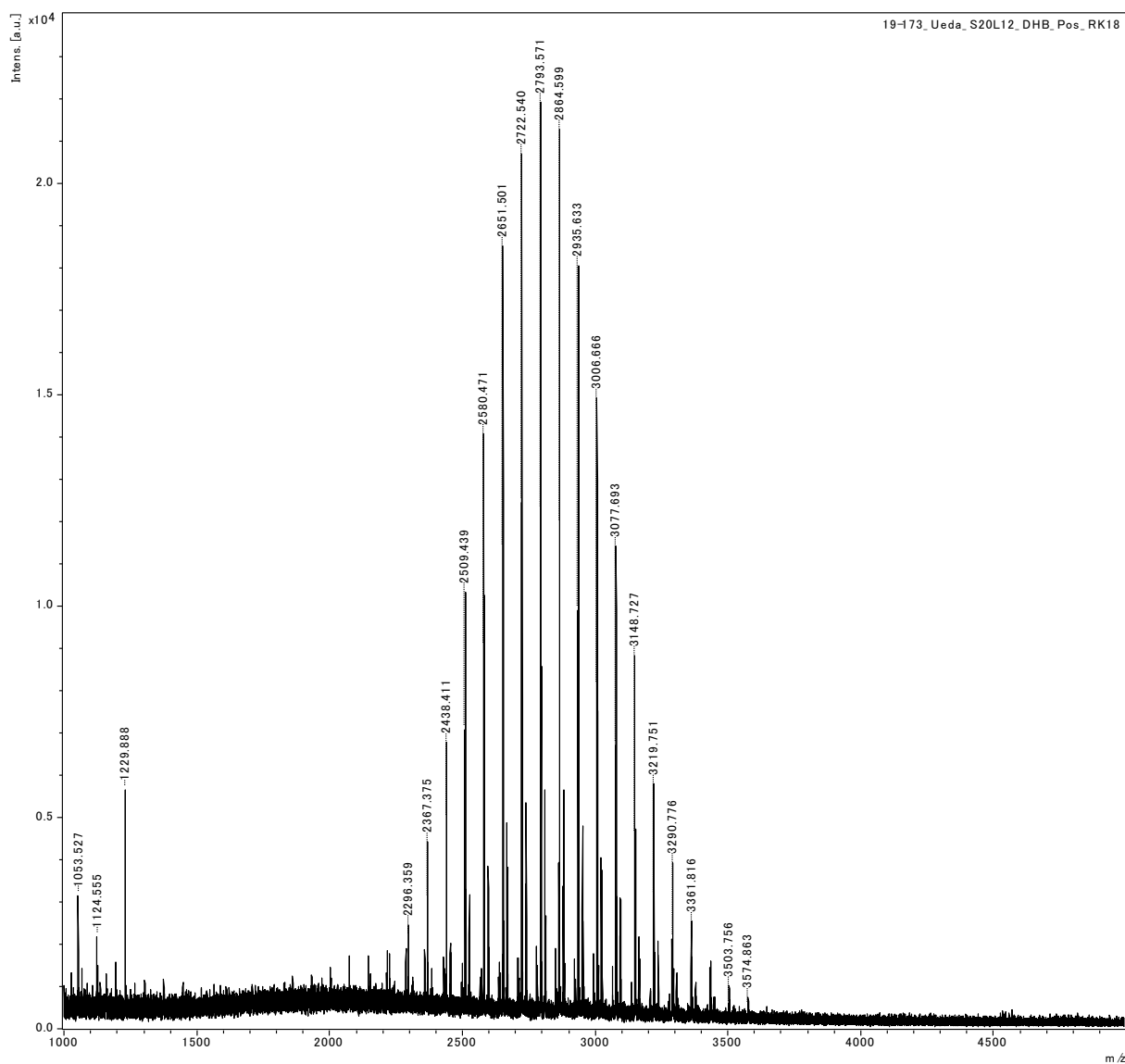


## S<sub>21</sub>L<sub>12</sub>

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ (ppm) 8.0–7.4 [m, 11H, amide], 7.0 [s, 1H, urethane], 4.4–3.9 [br, 52H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>21</sub>, HOCH<sub>2</sub>CO], 3.66 [s, 3H, OCH<sub>3</sub>], 3.3–2.8 [m, 63H, Sar(CH<sub>3</sub>)<sub>21</sub>], 2.0–1.4 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

MALDI-TOF MS calculated for C<sub>126</sub>H<sub>219</sub>N<sub>33</sub>O<sub>36</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 2793.632, found: 2793.571.

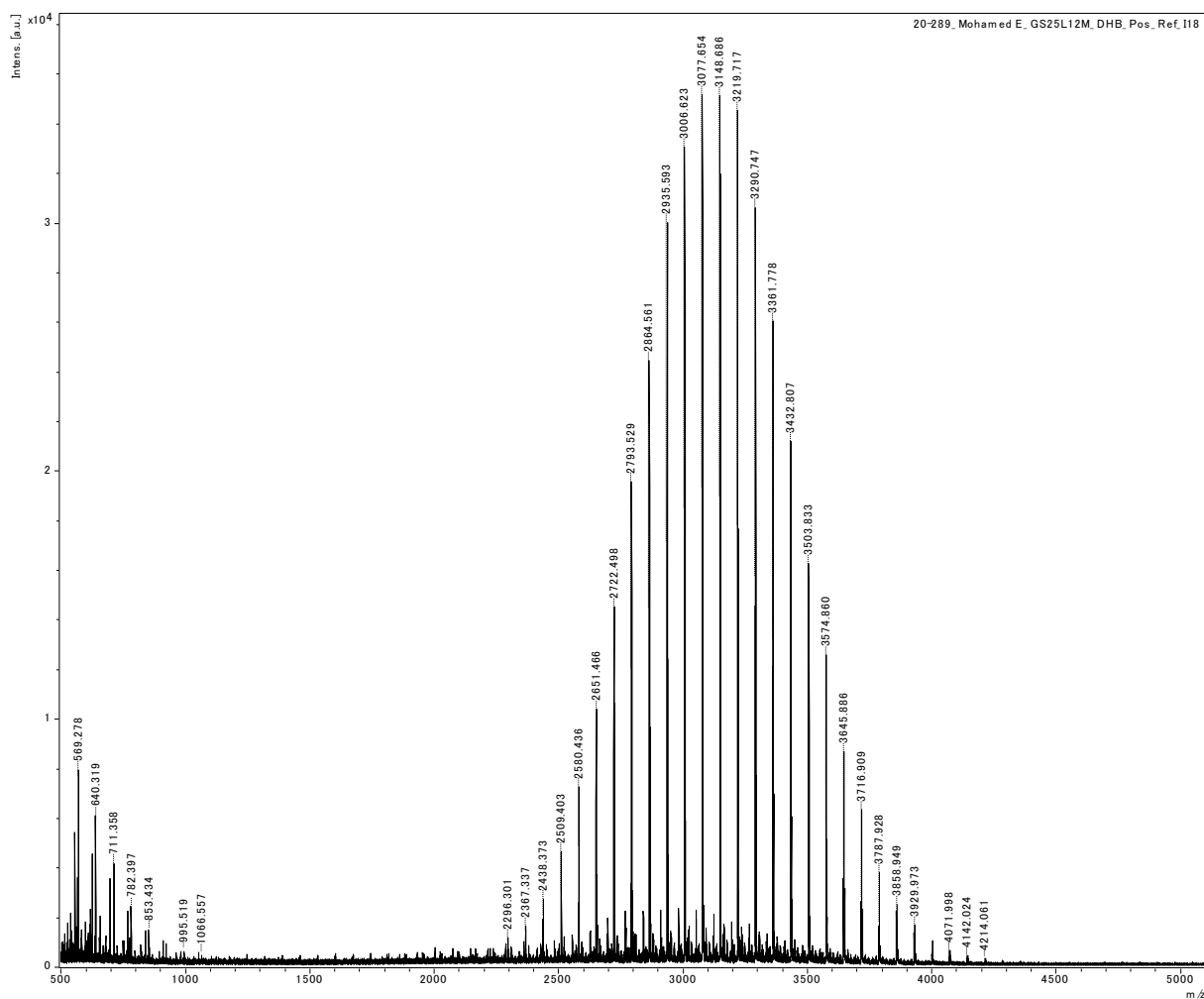




## S<sub>25</sub>L<sub>12</sub>

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ (ppm) 8.0–7.4 [m, 11H, amide], 7.0 [s, 1H, urethane], 4.4–3.8 [br, 60H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>25</sub>, HOCH<sub>2</sub>CO], 3.66 [s, 3H, OCH<sub>3</sub>], 3.3–2.8 [m, 75H, Sar(CH<sub>3</sub>)<sub>25</sub>], 2.1–1.3 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

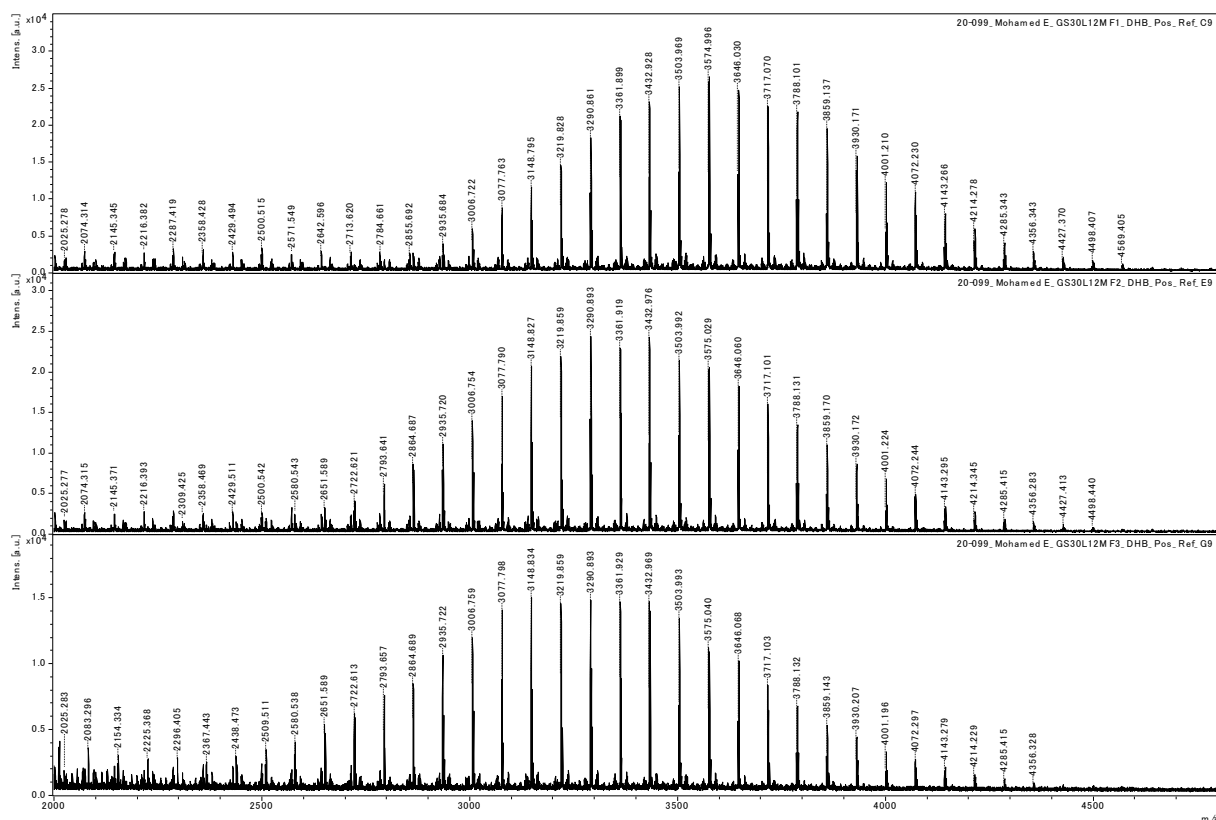
MALDI-TOF MS calculated for C<sub>138</sub>H<sub>239</sub>N<sub>37</sub>O<sub>40</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 3077.780, found: 3077.654.



## S<sub>28</sub>L<sub>12</sub>

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ (ppm) 8.1–7.4 [m, 11H, amide], 7.0 [s, 1H, urethane], 4.4–3.9 [br, 66H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>28</sub>, HOCH<sub>2</sub>CO], 3.66 [s, 3H, OCH<sub>3</sub>], 3.3–2.8 [m, 84H, Sar(CH<sub>3</sub>)<sub>28</sub>], 2.1–1.4 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

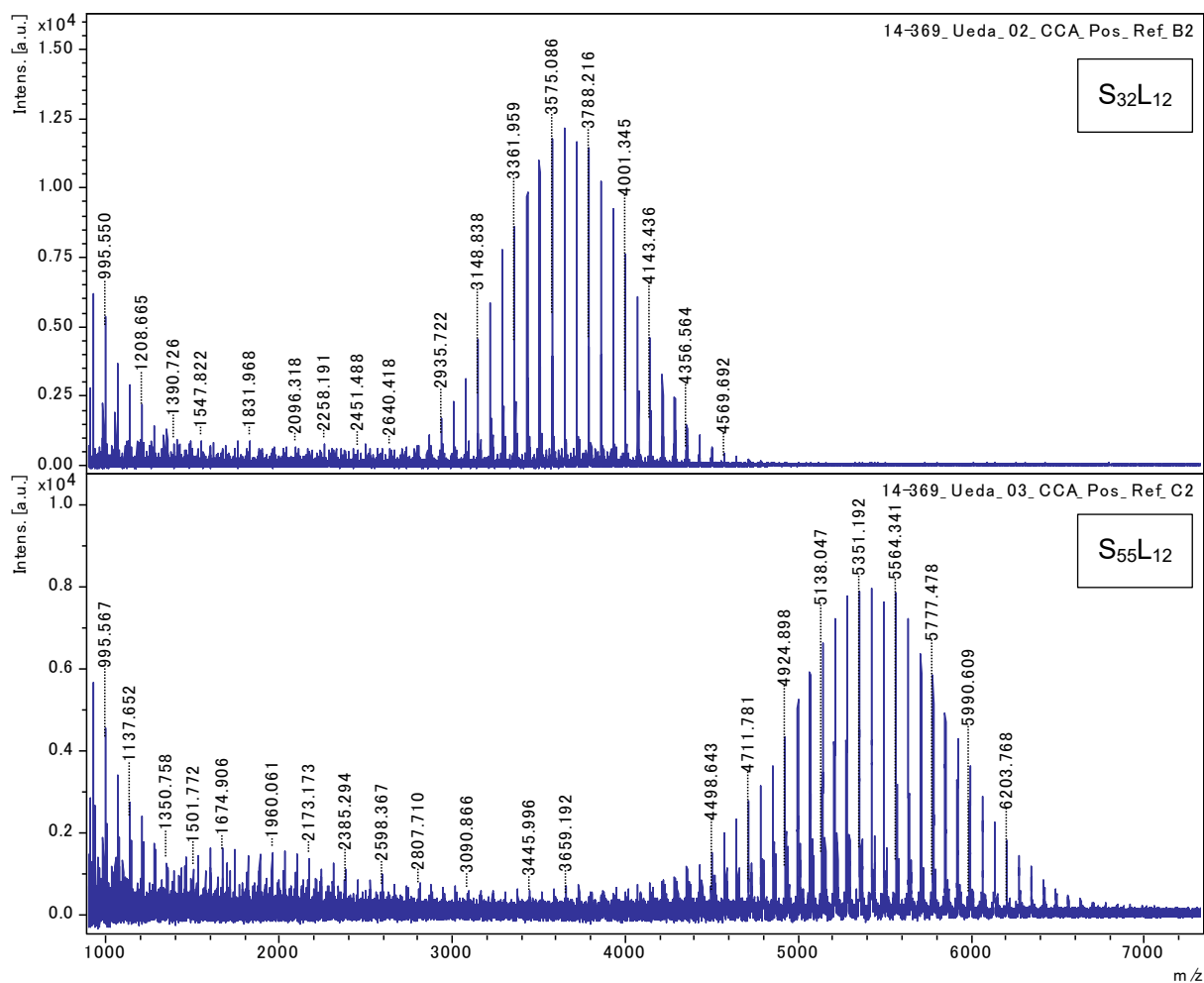
MALDI-TOF MS calculated for C<sub>147</sub>H<sub>254</sub>N<sub>40</sub>O<sub>43</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 3290.891, found: 3290.893.



## S<sub>32</sub>L<sub>12</sub>

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ (ppm) 8.0–7.3 [m, 11H, amide], 7.0 [s, 1H, urethane], 4.4–3.9 [br, 74H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>32</sub>, HOCH<sub>2</sub>CO], 3.66 [s, 3H, OCH<sub>3</sub>], 3.3–2.8 [m, 96H, Sar(CH<sub>3</sub>)<sub>32</sub>], 2.0–1.3 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

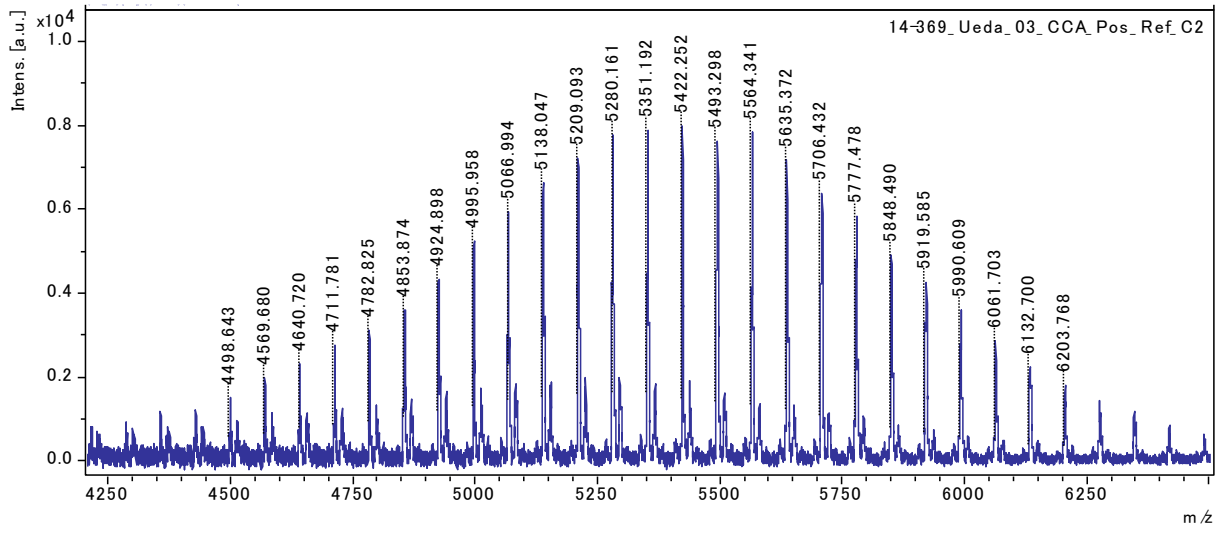
MALDI-TOF MS calculated for C<sub>159</sub>H<sub>274</sub>N<sub>44</sub>O<sub>47</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 3575.040, found: 3575.086.



## S<sub>55</sub>L<sub>12</sub>

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ (ppm) 8.1–7.4 [m, 12H, amide], 7.0 [s, 1H, urethane], 4.4–3.9 [br, 120H, LeuC<sup>α</sup>H, Sar(CH<sub>2</sub>)<sub>55</sub>, HOCH<sub>2</sub>CO], 3.66 [s, 3H, OCH<sub>3</sub>], 3.3–2.8 [m, 165H, Sar(CH<sub>3</sub>)<sub>55</sub>], 2.0–1.4 [m, 54H, LeuCH<sub>2</sub>, LeuC<sup>γ</sup>H, Aib(CH<sub>3</sub>)<sub>2</sub>], 1.1–0.8 [m, 36H, Leu(CH<sub>3</sub>)<sub>2</sub>].

MALDI-TOF MS calculated for C<sub>228</sub>H<sub>389</sub>N<sub>67</sub>O<sub>70</sub>Na<sup>+</sup> [M+Na]<sup>+</sup> *m/z* 5208.893, found: 5209.093.



SI-3. Low-magnification TEM images of assemblies prepared by  $S_nL_{12}S_n$  (Fig. S1)

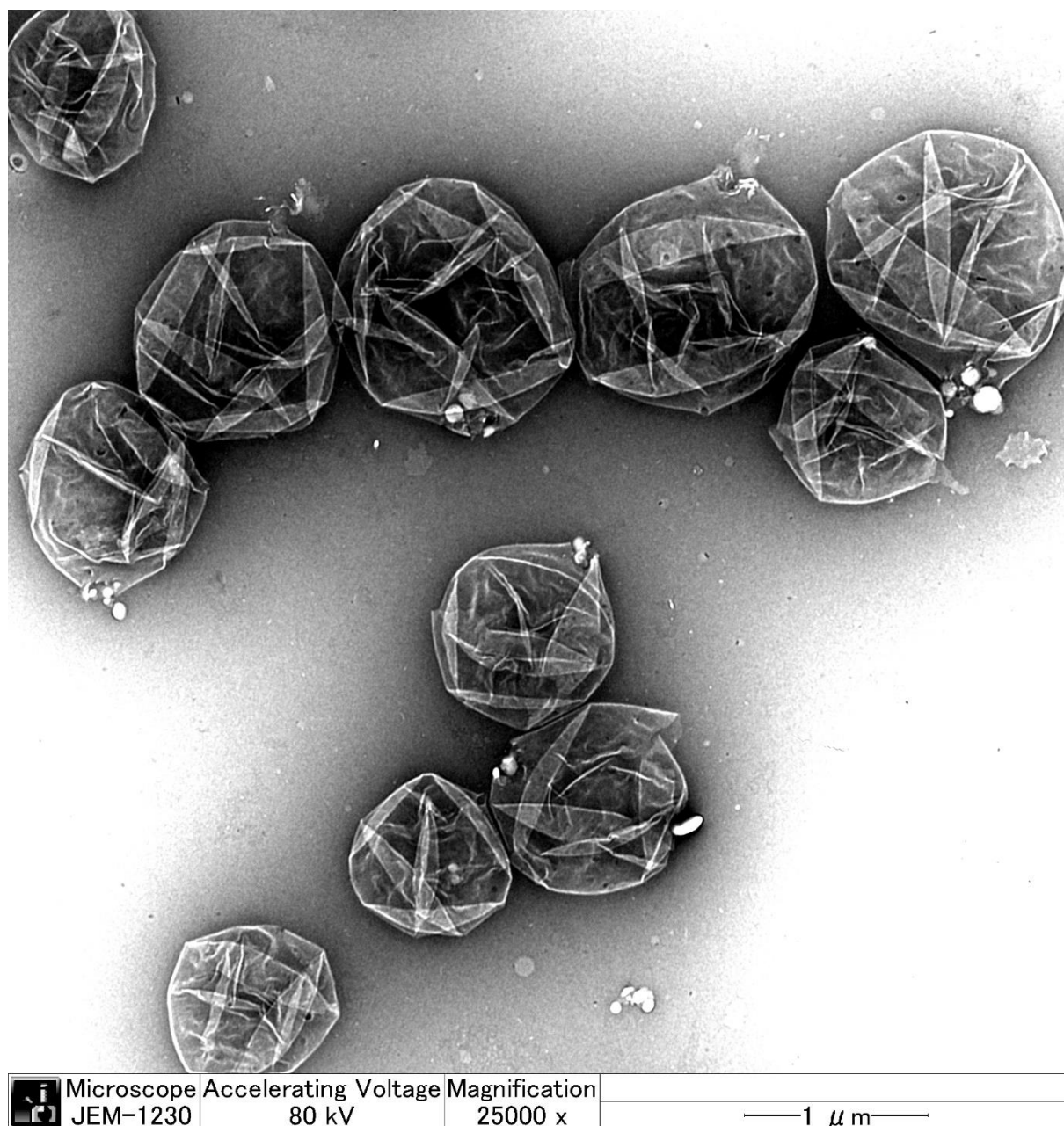


Fig. S1. TEM images of assembly of  $S_{14}L_{12}S_{14}$  after heat treatment at 90 °C for 1h.

#### SI-4. Calculation of PSar density on the assembly surface.

The theoretical densities of PSar chain on the surface of assemblies prepared from a mixture of  $S_{13}L_{12}S_{13}$  and  $S_{15}L_{12}$  with a mixing molar ratio of 1:0, 1:0.2, 1:0.3, 1:0.5, 1:1, and 0:1, were estimated with the following calculation. The calculation was performed based on two conditions; (1) the peptide membrane is a monolayer amphiphilic polypeptide packs interdigitately,<sup>14</sup> and (2) the occupied area of amphiphilic polypeptide is  $1.5 \text{ nm}^2/\text{chain}$ .<sup>15</sup>

In the mixing ratio of 1:0 ( $S_{13}L_{12}S_{13}:S_{15}L_{12}$ ), the assembly is composed only of  $S_{13}L_{12}S_{13}$  having PSar chains on its both termini. So, an inverse of the occupied area can be regarded as a PSar density on the assembly surface. As a result, the PSar density of  $S_{13}L_{12}S_{13}$  assembly is  $0.67 \text{ chains/nm}^2$ .

In the ratio of 0:1 ( $S_{13}L_{12}S_{13} : S_{15}L_{12}$ ), the assembly is composed only of  $S_{15}L_{12}$  having PSar chain only on its *N*-terminus. Thus, an inverse of [occupied area  $\times 2$ ] means a PSar density on the outer surface of assembly because the interdigitate packing of  $S_{15}L_{12}$  has just one PSar chain outside and inside per two molecules, respectively. The PSar density of  $S_{15}L_{12}$  assembly is  $0.33 \text{ chains/nm}^2$ .

In the mixture of  $S_{13}L_{12}S_{13}$  and  $S_{15}L_{12}$ , we estimated PSar chain density from averaged number of PSar chains. 1.2 amphiphiles of 1  $S_{13}L_{12}S_{13}$ s and 0.2  $S_{15}L_{12}$ s have total 2.2 PSar chains (inner and outer surface has 1.1 PSar chains, respectively) and have the surface area of  $1.5 \text{ nm}^2/\text{chain} \times 1.2 = 1.8 \text{ nm}^2$ . So, the PSar density of the assembly with a mixing ratio of 1:0.2 is  $11/18 =$

0.61 chains/nm<sup>2</sup>.

In the mixing ratio of 1:0.3, 1:0.5 and 1:1, 1.3, 1.5 and 2 amphiphiles of 1 S<sub>13</sub>L<sub>12</sub>S<sub>13</sub>S and 0.3, 0.5 and 1 S<sub>15</sub>L<sub>12</sub>S have total 2.3, 2.5 and 3 PSar chains (inner and outer surface has 1.15, 1.25 and 1.5 PSar chains, respectively) and have the surface are of 1.5 nm<sup>2</sup>/chain × 1.3, 1.5, and 2 = 1.95, 2.25, 3 nm<sup>2</sup>. As results, the PSar densities of the assembly with a mixing ratio of 1:0.3, 1:0.5, and 1:1 are 1.15/1.95 = 0.59, 1.25/2.25 = 0.56, and 1.5/3 = 0.5 chains/nm<sup>2</sup>, respectively.

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