Electronic Supplementary Material (ESI) for Soft Matter
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Electronic supplementary information (ESI)

of

Branched Peptide Fibers Self-assembled from Gemini-like Amphiphilic Peptide

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Experimental part

Materials

N-Fluorenyl-9-methoxycarbonyl (FMOC) protected L-amino acids (Fmoc-Orn (Boc)-OH, Fmoc-Cys (Trt)-OH) and 2-chloritrityl chloride resin (100-200 mesh, loading 1.32 mmol/g) were purchased from GL Biochem (i) Ltd. (China) and used as received. Piperdine, trifluoroacetic (TFA), o-benzotriazole-N,N, N′, N′-tetramethyluroniumhexafluorophosphate (HBTU), and N-hydroxybenzotriazole (HOBt) were provided by Shanghai Regent Chemical Co. (China) and used directly. N, N-dimethylformamide (DMF), and diisoproylethylamine (DIEA) were obtained from Shanghai Reagent Chemical Co. (China) and distilled prior to use. Decanoic acid, dodecanoic acid, myristicic acid and palmitic acid, were purchased from Shanghai Reagent Chemical Co. (China) and used after recrystallization from ethanol. Triisopropylsilane (TIS) and dimethylsulphoxide (DMSO) were purchased from ACROS (USA) and used without further purification. All other reagents and solvents were of analytical grade and were used directly.

Synthesis of peptide amphiphile by solid-phase peptide synthesis (SPPS)

Peptide amphiphiles were manually synthesized on the 2-chlorotrityl chloride resin employing a standard Fmoc solid phase peptide synthesis (SPPS) protocols. Before the synthesis, the resin was washed with dichloromethane (DCM) (three times) and DMF (three times) and then immersed in DMF for 30min. Thereafter, a DMF solution of FMOC protected amino acid (4 equiv relative to resin loading) and DIEA (6 equiv) was added to the resin and shaken for 2 h at room temperature. After the coupling, the FMOC group was removed by 20% (v/v) piperidine/DMF twice. After stirring for 30 min at room temperature, the reaction solution was drained off and the resin was washed with DMF (three times). The presence of free amino groups was indicated by

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a blue color in the Kaiser test. Then, a DMF solution of the mixture of FMOC protected amino acid (2 equiv), HBTU (3 equiv), HOBt (3 equiv) and DIEA (4 equiv) was added. After shaking for 1.5 h at room temperature, the reaction solution was drained off and the resin was washed with DMF (three times). The absence of free amino groups was indicated by a yellow color in the Kaiser test. After the repetition of deprotection and acylation reaction, fatty acid, i.e. dodecyl acid, myristicic acid and palmitic acid, was conjugated to the resin with HBTU and HOBt as activating agents finally. After completion of the synthesis, the resin was washed with DMF and DCM three times, respectively. The solvent of the resin was removed under vacuum for 24 h.

Synthesis of Gemini-like amphiphilic peptides (GAPs)

Cleavage of the expected peptide amphiphiles and removal of side chain protected groups from the dried resin were performed using a cleavage cocktail comprised of TFA, TIS and deionized water in the ratio of 95:4:1. After shaking at room temperature for 2 h, the cleavage mixture and subsequently TFA washing were collected. Then the total 22.5% (v/v) DMSO was added into the combined solution in twice at 4 h intervals, directly oxidizing -SH to form disulfide bond, which connected two single peptide amphiphiles to form GAPs.[10,11] After shaking for 24 h, the final mixture solution was concentrated to viscous solution by rotary evaporator. The remaining viscous GAPs solution was precipitated with cold ether. After washing with cold ether (five times), the resulting white product was collected and vacuum dried. Then, the product was dissolved in deionized water and was freeze-dried under vacuum for 3 days.

ESI-MS (LCQ Advantage, Finigan, USA, see Fig. S1): $(C_{10}\text{-}C\text{-}O_3)_2$: Calcd. 1232.8; Found 617.9 ($[M+2H]^{2+}$), 1233.8 ($[M+H]^{+}$) (Fig. S1A); $(C_{12}\text{-}C\text{-}O_3)_2$: Calcd. 1288.8;

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Found 645.8 ($[M+2H]^{2+}$), 1289.8 ($[M+H]^{+}$) (Fig. S1B); $(C_{14}\text{-}C\text{-}O_3)_2$: Calcd. 1344.9; Found 673.6 ($[M+2H]^{2+}$), 1345.9 ($[M+H]^{+}$) (Fig. S1C); $(C_{16}\text{-}C\text{-}O_3)_2$: Calcd. 1401; Found 701.8 ($[M+2H]^{2+}$), 1402 ($[M+H]^{+}$) (Fig. S1D).

Element analysis: $(C_{10}\text{-}C\text{-}O_3)_2$: Calcd. C 54.52, N 15.90, H 8.82; Found C 54.12, N 15.59, H 8.69; $(C_{12}\text{-}C\text{-}O_3)_2$: Calcd. C 55.87, N 15.20, H 9.07; Found C 55.46, N 14.91, H 8.94; $(C_{14}\text{-}C\text{-}O_3)_2$: Calcd. C 57.11, N 14.57, H 9.29; Found C 56.66, N 14.31, H 9.17; $(C_{16}\text{-}C\text{-}O_3)_2$: Calcd. C 58.25, N 13.99, H 9.49; Found C 57.86, N 13.66, H 9.38.

^{1}H-NMR (Fig S2, 300 MHz, D_2O, δ ppm): $(C_{10}\text{-}C\text{-}O_3)_2$ (Fig. S2A): 4.64-4.65 (2H, -CO-C***H***$(CH_2\text{-S-})$-NH-), 4.38-4.26 (6H, -CO-C***H***$((CH_2)_3\text{-}NH_2)$-NH-), 3.05-2.95 (12H, NH_2-C***H***$_2$-CH_2-), 2.77-2.75 (4H, -CH-C***H***$_2$-S-S-C***H***$_2$-CH-), 2.31-2.25 (4H, C_8H_{17}-C***H$_2$***-CO-NH-), 1.95-1.50 (28H, $NH_2\text{-}(CH_2)_2$-C***H$_2$***-CH-, $NH_2\text{-}CH_2$-C***H$_2$***-CH_2-CH-, C_7H_{15}-C***H***$_2$-CH_2-CO-NH-), 1.32-1.14 (24H, CH_3-(C***H***$_2)_6$-$(CH_2)_2$-CO-NH-), 0.88-0.79 (6H, C***H***$_3$-$(CH_2)_8$-CO-NH-). $(C_{12}\text{-}C\text{-}O_3)_2$: (Fig. S2B) 4.71-4.67 (2H, -CO-C***H***$(CH_2\text{-S-})$-NH-), 4.39-4.31 (6H, -CO-C***H***$((CH_2)_3\text{-}NH_2)$-NH-), 3.08-2.95 (12H, NH_2-C***H***$_2$-CH_2-), 2.78-2.74 (4H, -CH-C***H***$_2$-S-S-C***H***$_2$-CH-), 2.33-2.23 (4H, $C_{10}H_{21}$-C***H$_2$***-CO-NH-), 1.90-1.50 (28H, $NH_2\text{-}(CH_2)_2$-C***H$_2$***-CH-, $NH_2\text{-}CH_2$-C***H$_2$***-CH_2-CH-, C_9H_{19}-C***H***$_2$-CH_2-CO-NH-), 1.32-1.13 (32H, CH_3-(C***H***$_2)_8$-$(CH_2)_2$-CO-NH-), 0.89-0.81 (6H, C***H***$_3$-$(CH_2)_{10}$-CO-NH-). $(C_{14}\text{-}C\text{-}O_3)_2$: (Fig. S2C) 4.71-4.68 (2H, -CO-C***H***$(CH_2\text{-S-})$-NH-), 4.40-4.31 (6H, -CO-C***H***$((CH_2)_3\text{-}NH_2)$-NH-), 3.08-2.95 (12H, NH_2-C***H***$_2$-CH_2-), 2.77-2.68 (4H, -CH-C***H***$_2$-S-S-C***H***$_2$-CH-), 2.33-2.24 (4H, $C_{12}H_{25}$-C***H$_2$***-CO-NH-), 1.96-1.51 (28H, $NH_2\text{-}(CH_2)_2$-C***H$_2$***-CH-, $NH_2\text{-}CH_2$-C***H$_2$***-CH_2-CH-, $C_{11}H_{23}$-C***H***$_2$-CH_2-CO-NH-), 1.32-1.13 (40H, CH_3-(C***H***$_2)_{10}$-$(CH_2)_2$-CO-NH-), 0.89-0.81 (6H, C***H***$_3$-$(CH_2)_{12}$-CO-NH-). $(C_{16}\text{-}C\text{-}O_3)_2$: (Fig. S2D) 4.68-4.65 (2H, -CO-C***H***$(CH_2\text{-S-})$-NH-), 4.40-4.25 (6H,

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-CO-C***H***((CH_2)$_3$-NH_2)-NH-), 3.09-2.92 (12H, NH_2-C***H***$_2$-CH_2-), 2.78-2.69 (4H, -CH-C***H***$_2$-S-S-C***H***$_2$-CH-), 2.31-2.25 (4H, $C_{14}H_{29}$-C***H***$_2$-CO-NH-), 1.94-1.51 (28H, NH_2-(CH_2)$_2$-C***H***$_2$-CH-, NH_2-CH_2-C***H***$_2$-CH_2-CH-, $C_{13}H_{27}$-C***H***$_2$-CH_2-CO-NH-), 1.34-1.07 (48H, CH_3-(C***H***$_2$)$_{12}$-(CH_2)$_2$-CO-NH-), 0.90-0.81 (6H, C***H***$_3$-(CH_2)$_{14}$-CO-NH-).

^{13}C-NMR (Fig S2, 300 MHz, D_2O, δ ppm): (C_{10}-C-O_3)$_2$ (Fig. S3A): 176.61-173.10 (HO-***C***O-CH(-CH_2-)-NH-, C_9H_{19}-***C***O-NH-), 163.11-162.16 (-NH-***C***O-CH(-CH_2-CH_2-CH_2-NH_2)-NH-, -NH-***C***O-CH(-CH_2-S-)-NH-), 53.49-53.24 (HO-CO-***C***H(-CH_2-CH_2-CH_2-NH_2)-NH-, -CO-***C***H(-CH_2-CH_2-CH_2-NH_2))-NH-, -CO-***C***H(-CH_2-S-)-NH-CO-C_9H_{19}), 39.13 (NH_2-***C***H_2-CH_2-), 36.03 (C_8H_{19}-***C***H_2-CO-, -S-***C***H_2-CH(-CO)-NH-), 31.96 (CH_3-CH_2-***C***H_2-(CH_2)$_6$-CO-NH-), 30.56-28.03 (CH_3-(CH_2)$_2$-***C***H_2-***C***H_2-(CH_2)$_4$-CO-NH-, HO-CO-CH(-NH-CO-)-***C***H_2-(CH_2)$_2$-NH_2, CH_3-(CH_2)$_4$-***C***H_2-***C***H_2-(CH_2)$_2$-CO-NH-, NH_2-(CH_2)$_2$-***C***H_2-CH(-NH-)-CO-), 25.88 (CH_3-(CH_2)$_6$-***C***H_2-CH_2-CO-NH-), 23.54-22.67 (CH_3-***C***H_2-(CH_2)$_7$-CO-NH-, NH_2-CH_2-***C***H_2-CH_2-CH(-NH-)-CO-, NH2-CH2-***C***H2-CH2-CH(-NH-)-CO-OH), 14.00 (***C***H_3-(CH_2)$_8$-CO-NH-); (C_{12}-C-O_3)$_2$ (Fig. S3B): 176.55-172.79 (HO-***C***O-CH(-CH_2-)-NH-, $C_{11}H_{23}$-***C***O-NH-), 163.52-162.11 (-NH-***C***O-CH(-CH_2-CH_2-CH_2-NH_2)-NH-, -NH-***C***O-CH(-CH_2-S-)-NH-), 53.44-53.27 (HO-CO-***C***H(-CH_2-CH_2-CH_2-NH_2)-NH-, -CO-***C***H(-CH_2-CH_2-CH_2-NH_2))-NH-, -CO-***C***H(-CH_2-S-)-NH-CO-$C_{11}H_{23}$), 39.10 (NH_2-***C***H_2-CH_2-), 36.00 ($C_{10}H_{21}$-***C***H_2-CO-, -S-***C***H_2-CH(-CO)-NH-), 32.04 (CH_3-CH_2-***C***H_2-(CH_2)$_8$-CO-NH-), 30.54-27.69 (CH_3-(CH_2)$_2$-***C***H_2-***C***H_2-***C***H_2-***C***H_2-(CH_2)$_4$-CO-NH-, HO-CO-CH(-NH-CO-)-***C***H_2-(CH_2)$_2$-NH_2, CH_3-(CH_2)$_6$-***C***H_2-***C***H_2-(CH_2)$_2$-CO-NH-, NH_2-(CH_2)$_2$-***C***H_2-CH(-NH-)-CO-), 25.85 (CH_3-(CH_2)$_8$-***C***H_2-CH_2-CO-NH-), 24.01-22.39 (CH_3-***C***H_2-(CH_2)$_9$-CO-NH-, NH_2-CH_2-***C***H_2-CH_2-CH(-NH-)-CO-,

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NH2-CH2-***C***H2-CH2-CH(-NH-)-CO-OH), 14.00 (***C***H_3-$(CH_2)_{10}$-CO-NH-); (C_{14}-C-O_3)$_2$ (Fig. S3C): 176.44-172.71 (HO-***C***O-CH(-CH_2-)-NH-, $C_{13}H_{27}$-***C***O-NH-), 163.39-161.99 (-NH-***C***O-CH(-CH_2-CH_2-CH_2-NH_2)-NH-, -NH-***C***O-CH(-CH_2-S-)-NH-), 53.35-52.43 (HO-CO-***C***H(-CH_2-CH_2-CH_2-NH_2)-NH-, -CO-***C***H(-CH_2-CH_2-CH_2-NH_2))-NH-, -CO-***C***H(-CH_2-S-)-NH-CO-$C_{13}H_{27}$), 38.96 (NH_2-***C***H_2-CH_2-), 35.98 ($C_{12}H_{25}$-***C***H_2-CO-, -S-***C***H_2-CH(-CO)-NH-), 32.02 (CH_3-CH_2-***C***H_2-$(CH_2)_{10}$-CO-NH-), 30.48-27.58 (CH_3-$(CH_2)_2$-***C***H_2-***C***H_2-***C***H_2-***C***H_2-***C***H_2-***C***H_2-$(CH_2)_4$-CO-NH-, HO-CO-CH(-NH-CO-)-***C***H_2-$(CH_2)_2$-NH_2, CH_3-$(CH_2)_8$-***C***H_2-***C***H_2-$(CH_2)_2$-CO-NH-, NH_2-$(CH_2)_2$-***C***H_2-CH(-NH-)-CO-), 25.80 (CH_3-$(CH_2)_{10}$-***C***H_2-CH_2-CO-NH-), 24.01-22.39 (CH_3-***C***H_2-$(CH_2)_{11}$-CO-NH-, NH_2-CH_2-***C***H_2-CH_2-CH(-NH-)-CO-, NH2-CH2-***C***H2-CH2-CH(-NH-)-CO-OH), 14.00 (***C***H_3-$(CH_2)_{12}$-CO-NH-); (C_{16}-C-O_3)$_2$ (Fig. S3D): 176.39-172.73 (HO-***C***O-CH(-CH_2-)-NH-, $C_{15}H_{31}$-***C***O-NH-), 163.50-162.11 (-NH-***C***O-CH(-CH_2-CH_2-CH_2-NH_2)-NH-, -NH-***C***O-CH(-CH_2-S-)-NH-), 53.32-52.20 (HO-CO-***C***H(-CH_2-CH_2-CH_2-NH_2)-NH-, -CO-***C***H(-CH_2-CH_2-CH_2-NH_2))-NH-, -CO-***C***H(-CH_2-S-)-NH-CO-$C_{15}H_{31}$), 39.13 (NH_2-***C***H_2-CH_2-), 36.03 ($C_{14}H_{29}$-***C***H_2-CO-, -S-***C***H_2-CH(-CO)-NH-), 32.13 (CH_3-CH_2-***C***H_2-$(CH_2)_{12}$-CO-NH-), 30.59-28.00 (CH_3-$(CH_2)_2$-***C***H_2-***C***H_2-***C***H_2-***C***H_2-***C***H_2-***C***H_2-***C***H_2-***C***H_2-$(CH_2)_4$-CO-NH-, HO-CO-CH(-NH-CO-)-***C***H_2-$(CH_2)_2$-NH_2, CH_3-$(CH_2)_{10}$-***C***H_2-***C***H_2-$(CH_2)_2$-CO-NH-, NH_2-$(CH_2)_2$-***C***H_2-CH(-NH-)-CO-), 25.91 (CH_3-$(CH_2)_{12}$-***C***H_2-CH_2-CO-NH-), 24.01-22.81 (CH_3-***C***H_2-$(CH_2)_{13}$-CO-NH-, NH_2-CH_2-***C***H_2-CH_2-CH(-NH-)-CO-, NH2-CH2-***C***H2-CH2-CH(-NH-)-CO-OH), 14.02 (***C***H_3-$(CH_2)_{14}$-CO-NH-);

Self-assembly of GAPs

The GAPs were dissolved in buffer of acetic acid/sodium acetate at pH 4 at 10

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mg/ml for 12 hours to form stable assemblies for following characterization.

Transmission electron microscopy (TEM)

A 5~10μl aliquot of above GAPs stock solutions was placed on a copper grid. After several min, excess fluid was removed and then dried in air. Samples were observed by using a TEM (JEOL2010, Japan) with an acceleration voltage of 200 kV.

Circular dichroism spectrum (CD)

The solution of the self-assembled GAPs was fixed in a 0.5 mm quartz cell and CD was recorded on a Jasco J-810 spectropolarimeter (Jasco, Japan).

Fourier transform infrared spectroscopy (FT-IR)

The solution of the self-assembled GAPs was rapid-freezing by the liquid nitrogen and was freeze-dried in a Freeze Drier (Labconco, CA) under vacuum. Then the sample was pressed into potassium bromide (KBr) pellets and FT-IR measurement was performed on a FT-IR spectrophotometer (Perkin-Elmer Spectrum One).

Differential scanning calorimeter (DSC)

Before the measurement, the solution of self-assembled GAPs was rapid-freezing by the liquid nitrogen and was freeze-dried in a Freeze Drier (Labconco, CA) under vacuum. Then, the sample with accurate weight was placed inside a hermetic aluminum pan, which subsequently was sealed tightly by a hermitic aluminum lid. The thermograms were recorded on a DSC (Pyris 1, Perkin-Elmer, USA) under dry nitrogen and the scan speed for the heating cycle was 10 ^{o}C/min.

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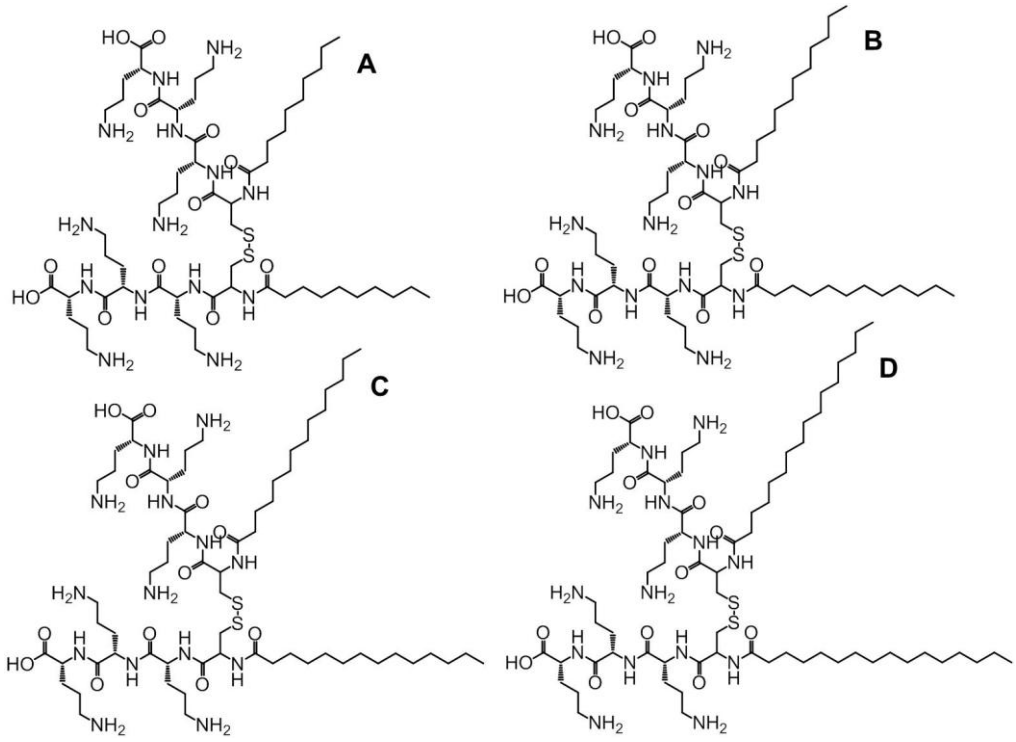

Scheme S1. The molecular structure of the Gemini-like amphiphilic peptides. A: $(C_{10}\text{-}C\text{-}O_3)_2$; B: $(C_{12}\text{-}C\text{-}O_3)_2$; C: $(C_{14}\text{-}C\text{-}O_3)_2$; D: $(C_{16}\text{-}C\text{-}O_3)_2$.

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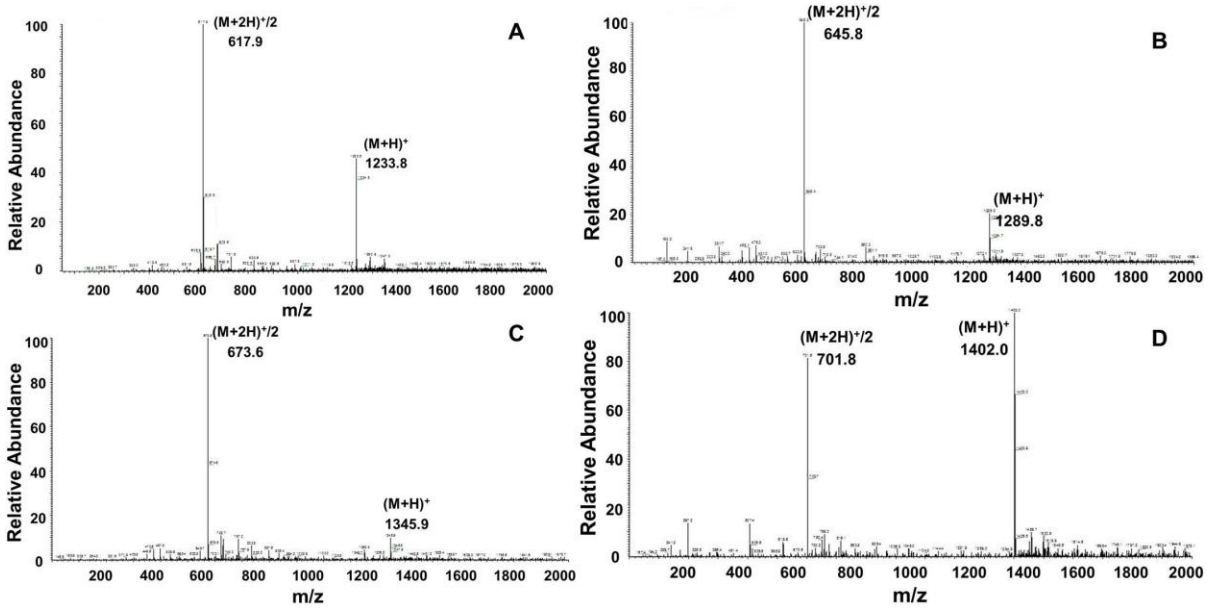

Figure S1. The ESI-MS profiles of the Gemini-like amphiphilic peptides. A: $(C_{10}\text{-}C\text{-}O_3)_2$; B: $(C_{12}\text{-}C\text{-}O_3)_2$; C: $(C_{14}\text{-}C\text{-}O_3)_2$; D: $(C_{16}\text{-}C\text{-}O_3)_2$.

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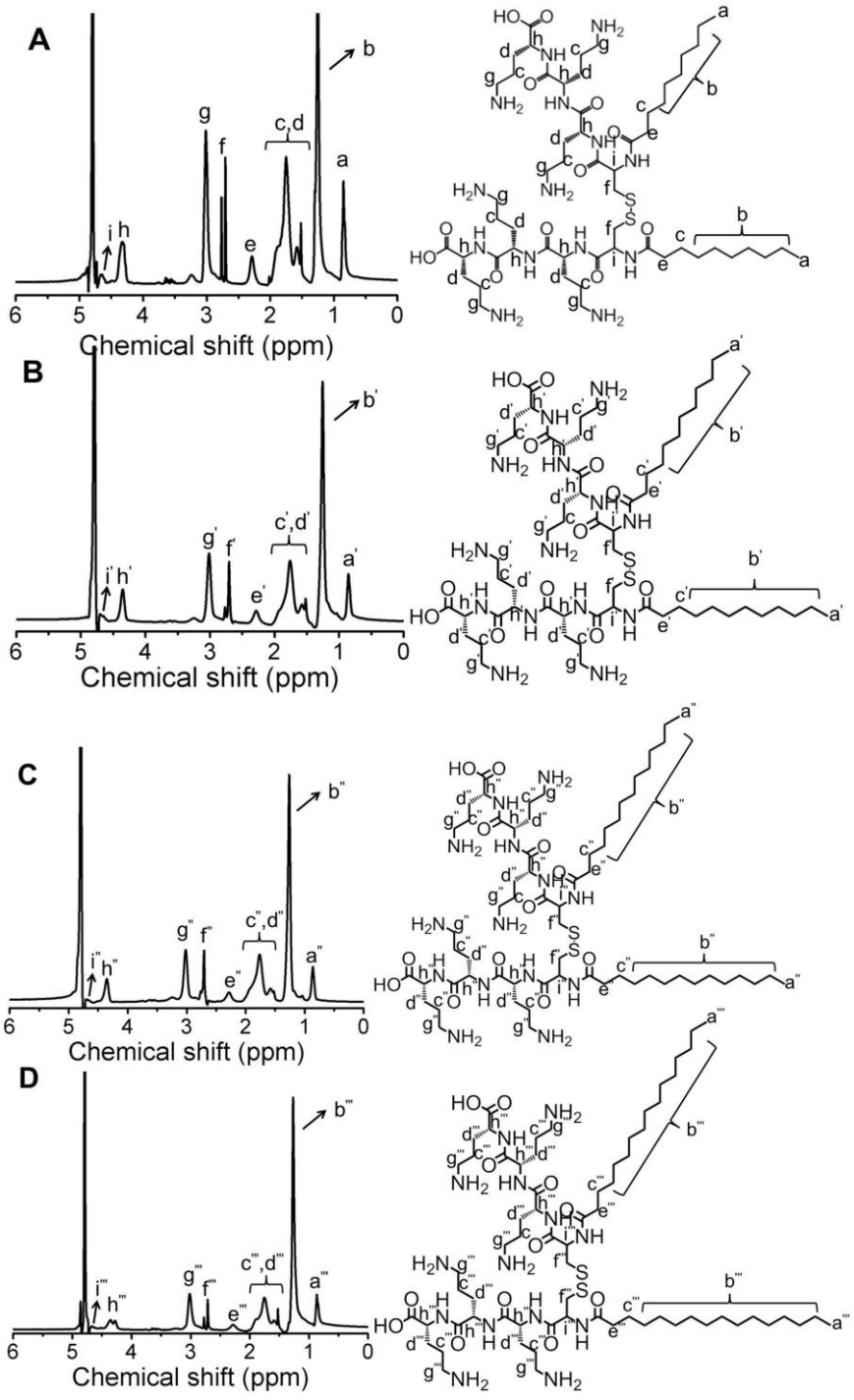

Figure S2. ^{1}H-NMR spectrum of the Gemini-like amphiphilic peptides. A: $(C_{10}\text{-}C\text{-}O_3)_2$; B: $(C_{12}\text{-}C\text{-}O_3)_2$; C: $(C_{14}\text{-}C\text{-}O_3)_2$; D: $(C_{16}\text{-}C\text{-}O_3)_2$.

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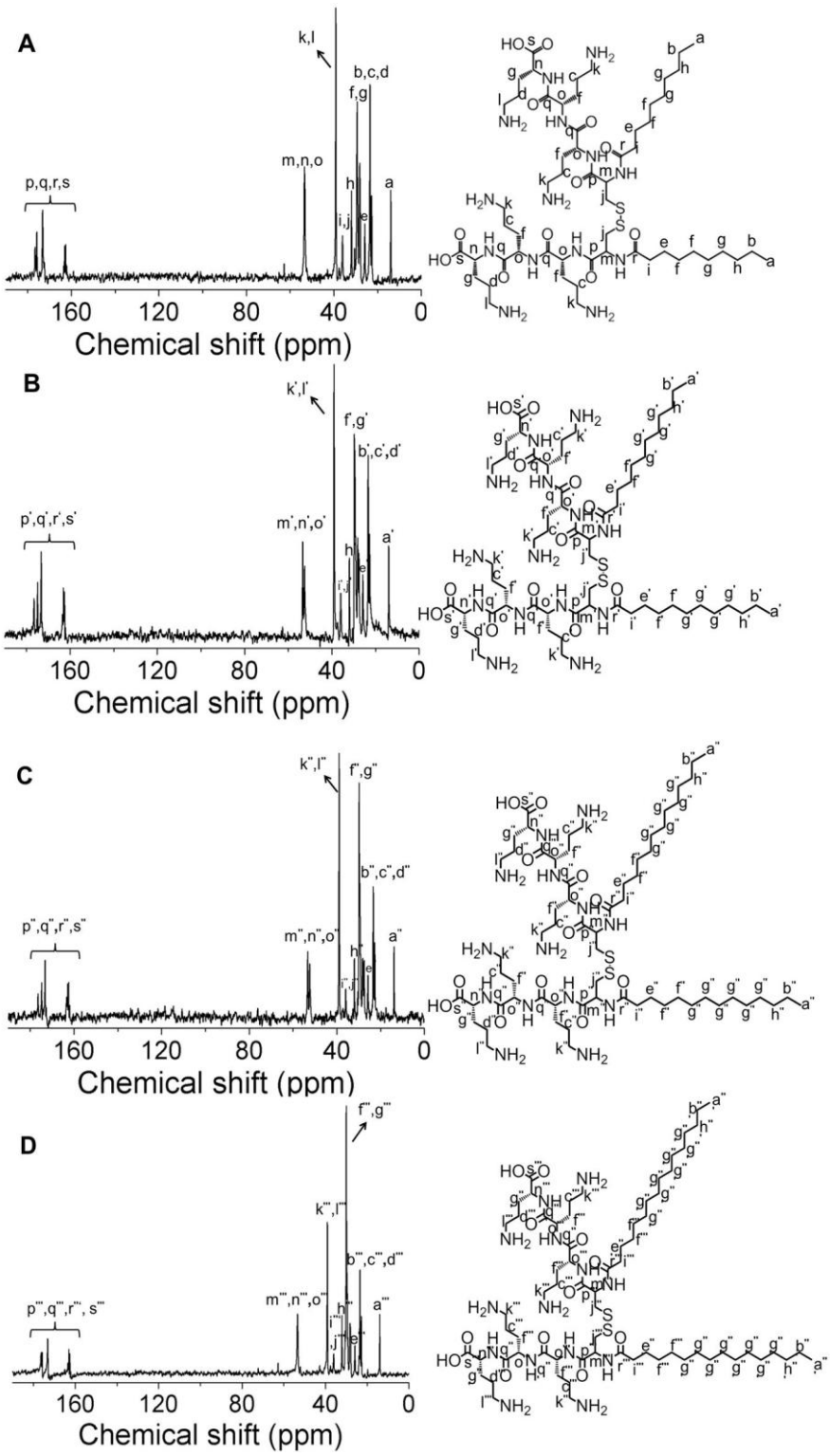

Figure S3. ^{13}C-NMR spectrum of the Gemini-like amphiphilic peptides. A: $(C_{10}\text{-}C\text{-}O_3)_2$; B: $(C_{12}\text{-}C\text{-}O_3)_2$; C: $(C_{14}\text{-}C\text{-}O_3)_2$; D: $(C_{16}\text{-}C\text{-}O_3)_2$.