Electronic Supplementary Information

Nanotubular Self-Organization of Amide Dendrons with Focal β-Sheet Forming Peptide Units

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Experimental

Materials

Trifluoroacetic acid (TFA), 4-dimethylaminopyridine (DMAP), triethylamine (TEA), piperidine, acetic anhydride, 3,6-dioxa-1,8-octandithiol, thioanisol, and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) obtained from Aldrich were used as received. N,N'-diisopropylcarbodiimide (DIPC) obtained from TCI was used as received. Rink amide MBHA resin and 1-hydroxybenzotriazole (HOBt) were obtained from BeadTech. Fmoc protected amino acids were obtained from NovaBiochem. The second generation amide dendron (2G) was synthesized as described elsewhere.[1]

Synthesis of peptides

All peptides and dendron-peptide conjugates were synthesized using Fmoc-chemistry by solid phase peptide synthesis. Fmoc protected amino acid was assembled on Rink Amide MBHA resin. The coupling reaction of each amino acid was carried out using 1 equivalent corresponding the Fmoc-protected amino acid and coupling reagents in DMF. DIC (10.85 mg, 0.086 mmol), HOBt (11.62 mg, 0.086 mmol), and Fmoc protected amino acid (0.086 mmol) in DMF (3 ml) were added to the rink amide resin (200 mg, 0.086 mmol). The resulting solution containing the resin was stirred for 4 hr at room temperatures. After filtration, the resin was washed 3 times with DMF (4 ml) and methanol (4 ml), sequentially. The amine group on the resin was then acetylated by a 20 molar excess of acetic anhydride (175.59 mg, 1.72 mmol) for 30 min and washed using above procedure. The coupling reaction was repeated until no colour change was observed in the ninhydrin test. After peptide synthesis, the purity of the resin-bound peptides was confirmed by HPLC and LCMS after the cleavage of peptides using TFA/H_2O/3,6-dioxa-1,8-oxtanedithiol/thioanisole (87.5 : 5 : 5 : 2.5) as shown in Figure S1-S4. VVLL $[M+H]^+$ calcd. 442.3, obs. 442.2 $[2M+H]^+$ calcd. 883.7, obs. 883.1; AAVV $[M+H]^+$ calcd. 358.2, obs. 358.1; $[2M+H]^+$ calcd. 715.5, obs. 715.2.

Synthesis of dendron-peptide conjugates

The coupling of the dendron (2G) with the peptides was performed according to the following procedure. To the resin-bound peptides (VVLL or AAVV, 200 mg 0.086 mmol), the solution of DIC (21.71 mg, 0.172 mmol), HOBt (23.24 mg, 0.172 mmol) and dendron (238.57 mg, 0.172 mmol) in DMF was added. The resulting solution containing resin was stirred under temperature-controlled

microwave irradiation (90 ℃, 150 W) for 5 min twice. After filtration, the resin was washed 3 times with DMF (4 ml), DMF/methanol (9:1 v/v, 4 ml), chloroform/methanol (9:1 v/v 4 ml), and methanol (4 ml), sequentially. The coupling reaction was repeated until no colour change was observed in the ninhydrin test. Cleavage was achieved by a treatment with a mixture of TFA/H_2O/3,6-dioxa-1,8-oxtanedithiol/thioanisole (87.5:5:5:2.5) at room temperature for 4 hrs. After cleavage of the product from the resin, the dendron-peptide conjugates were obtained by precipitation with acetonitrile at -20 ℃. The successful synthesis and purity of the dendron-peptide conjugates were confirmed by LCMS, as shown in Figures S3 and S4. 2G-VVLL $[M+H]^+$ calcd. 1811.5, obs. 1811.1 $[M+2H]^{2+}$ calcd. 906.2, obs. 906.1; 2G-AAVV $[M+2H]^{2+}$ calcd. 863.7, obs. 864.2, $[M+H]^+$ calcd. 1726.4, obs. 1727.1 $[M+Na]^+$ calcd. 1748.3, obs. 1748.5.

Sample preparation in an aqueous phase

In a typical procedure, 0.5 mg of the dendron-peptide conjugates were placed into a 20 ml vial and dissolved in a mixture of THF and methanol (3-5 ml). Subsequently, distilled water (10 ml) was added to the vial. After vigorous stirring for 60 sec, the solution was sonicated for 90 sec. The THF and methanol was evaporated under reduced pressure.

High performance liquid chromatography

Chromatograms of the samples were obtained using a Younglin YL9100 and SunFire Prep C18 (10 × 150 mm, 10 μm) analytic column. Stock solutions (200 μL, 1 mg/ml in H_2O/ACN 1:1 v/v mixed solvent) of each peptide were injected into a high performance liquid chromatography (HPLC) system. A HPLC grade acetonitrile and distilled water containing 0.1 % TFA were used as the mobile phases. The flow rate was 2 ml/min. The eluent was changed from 100 % distilled water to 100 % acetonitrile at a rate of 2 %/min. The chromatograms were acquired using a UV detector at 254 nm for 55 mins.

Transmission electron microscopy

Transmission electron microscopy (TEM) was performed using a Philips CM 200 operated at an acceleration voltage of 80 kV. For preparation of the TEM sample, a drop of sample solution was placed onto a 200-mesh carbon-coated copper grid. Approximately 40 min after air-drying, the grid was

stained using phosphotungstic acid or uranyl acetate (2 wt. %) for 10 min. After washing out the excess staining agent with distilled water, the grid was dried in vacuo.

Scanning transmission electron microscopy and energy-dispersive X-ray spectroscopy

Scanning transmission electron microscopy (STEM) images and energy-dispersive X-ray spectroscopy (EDX) spectra were obtained using a JEOL JEM-2100F operated at an acceleration voltage of 200kV. The samples were prepared using aforementioned way.

Field emission scanning electron microscopy

Field emission scanning electron microscopy (FE-SEM) was carried out on a Hitachi S-4300SE FE-SEM instrument with a field emission gun (acceleration voltage = 0.5-30kV). The FE-SEM samples were prepared by transferring a drop of the sample solution onto a silicon wafer. After transfer, the excess water was evaporated in a vacuum oven.

Circular dichroism spectrometry

All circular dichroism (CD) spectra were recorded on a JASCO-810 polarimeter by continuously purging dry N_2 gas during data acquisition. Each spectrum was collected from 250 nm to 190 nm in a quartz cuvette with a 1 cm path length at a response time of 16 s, a scan speed of 10 nm min^{-1} and a band width of 1 nm and averaged over three scans. Subsequently, the intensity of the CD spectra was converted to the molar ellipticity.

References

1 C. Kim, K. T. Kim, Y. Chang, H. H. Song, T.-Y. Cho and H.-J. Jeon, *J. Am. Chem. Soc.*, 2001, **123**, 5586-5587.

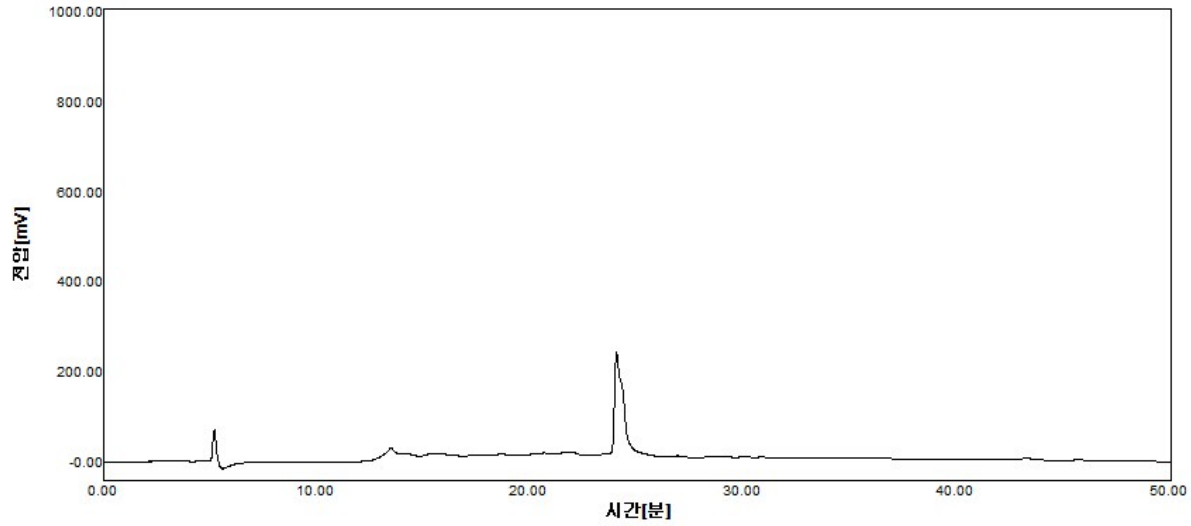

Figure S1. HPLC chromatogram of VVLL.

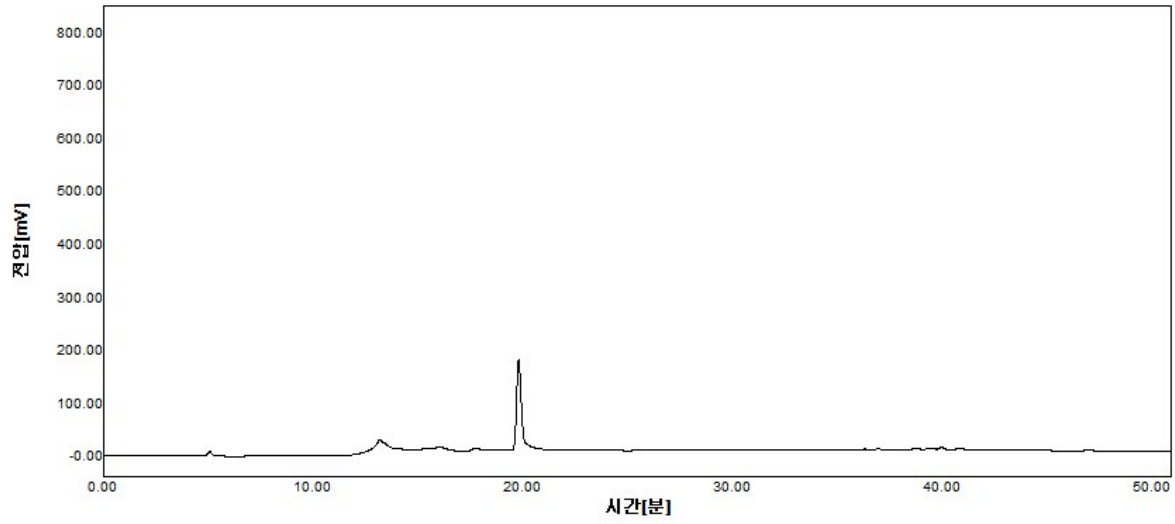

Figure S2. HPLC chromatogram of AAVV.

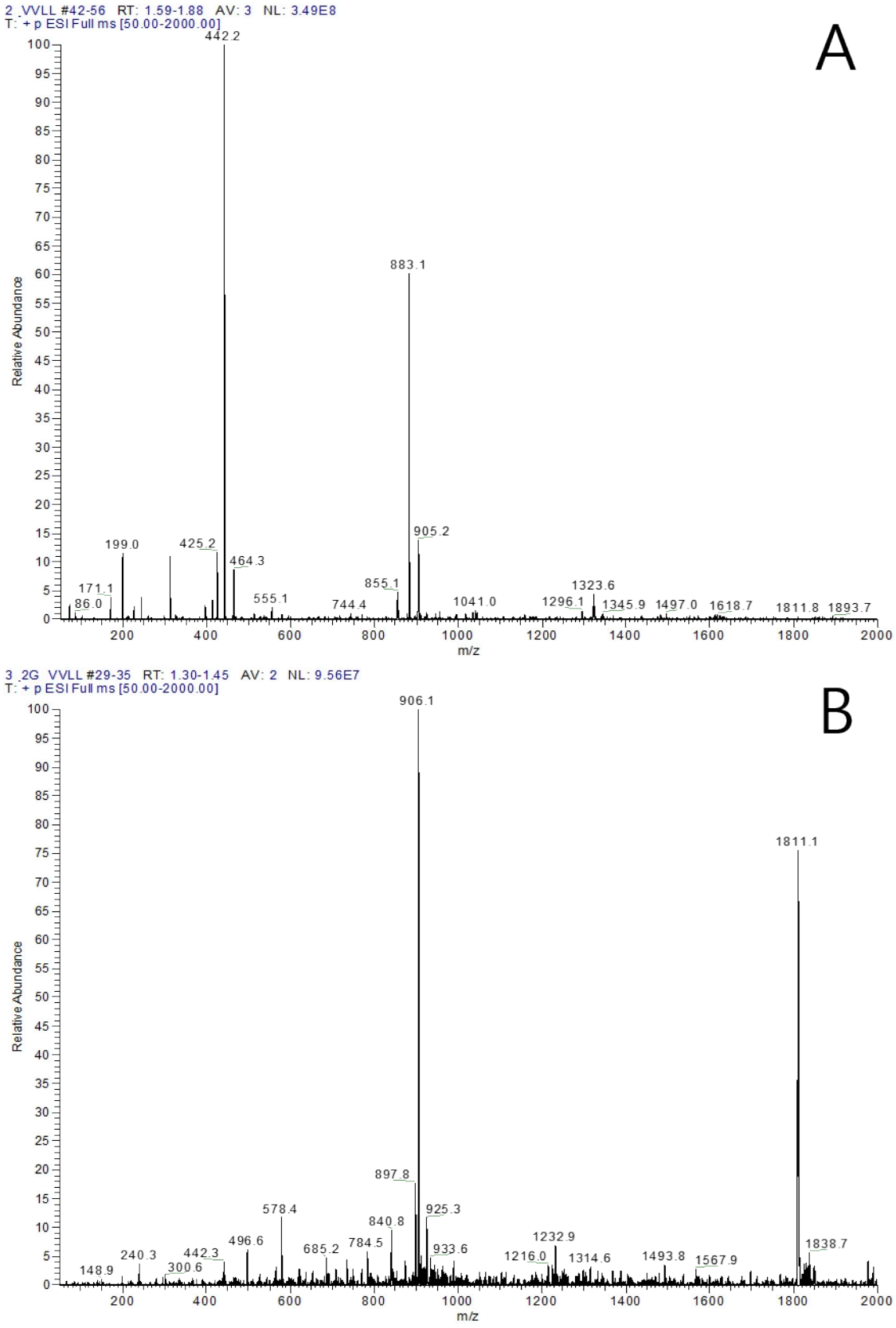

Figure S3. LCMS spectra of VVLL (a) and 2G-VVLL (b).

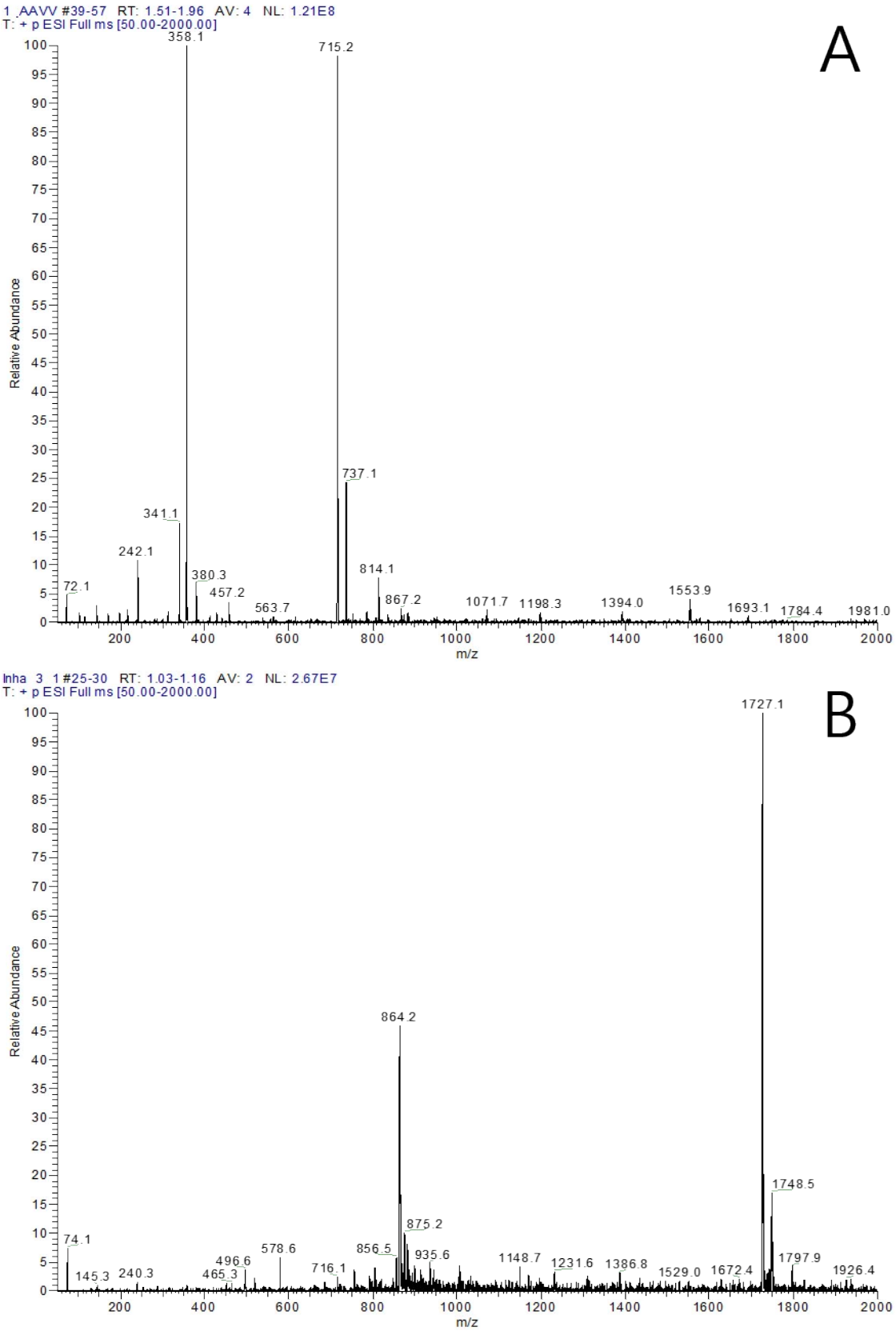

Figure S4. LCMS spectra of AAVV (a) and 2G-AAVV (b).