Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

α-Helical Peptide Vesicles with Chiral Membranes for Enantioselective Nanoreactors

Xi Chen, Yanqiu Wang, Huaxin Wang, Yongju Kim, and Myongsoo Lee*

State Key Lab of Supramolecular Structure and Materials

College of Chemistry, Jilin University, Changchun 130012, China

E-mail: mslee@jlu.edu.cn

1. General method

All starting materials were obtained from commercial suppliers (Aldrich, Lancaster, Anaspec, Novabiochem, and TCI, etc.) and were used without further purification. Dichloromethane (DCM), N, N–dimethylformamide (DMF), ethyl acetate (EA), methanol (MeOH) were distilled before use. Compound **6** and dendrimer were prepared according to the procedures described previously.[S1] Mass spectrometry was performed on a Bruker Microflex MALDI-TOF mass spectrometer using an α-cyano-4-hydroxy cinnamic acid (CHCA) as a matrix. HPLC analysis was performed with Prominence LC-20AP (SHIMADZU) equipped with Shim-pack PREP-ODS (H) KIT C18 reverse phase column (C18, 250 × 4.6 mm I.D.) for general separation and YMC CHIRALART Cellulose-C column (250 × 4.6 mm I.D. S-5 μm) for chiral separation. The fluorescence spectra were obtained from Hitachi F-7000 fluorescence spectrophotometer. Circular Dichroism (CD) spectra were measured using a JASCO J810 spectropolarimeter. Spectra were recorded from 280 nm to 190 nm using a 1 mm path length cell. Scans were repeated for 3 times and averaged. Samples have been kept at room temperature for 24 h before the measurement. Dynamic Light Scattering (DLS) experiments were performed by using ALV/CGS-3. The Scanning Electron Microscope (SEM) was performed at 3 kV using JEOL-JSM-6700F. ^{1}H NMR spectra were obtained on 500 MHz FT-NMR spectrometer. Compounds were synthesized according to the procedure described in **scheme 1** and then purified by silica gel column chromatography and preparatory HPLC.

TEM Experiments. A drop of the sample solution was placed on a carbon-coated copper grid (Carbon Type B (15–25 nm) on 200 mesh, with Formvar; Ted Pella, Inc.) at room temperature. These samples were stained by depositing a drop of uranyl acetate aqueous solution (1.0–0.2 wt %) onto the surface of the sample-loaded grid. The dried specimen was observed by a JEOL–JEM HR 2100 operated at 120 kV. The cryogenic transmission electron microscopy (cryo-TEM) experiments were performed with a thin film of a water solution of the peptide (5 μL) transferred to a lacey supported grid. The thin films were prepared at room temperature and humidity conditions (97–99 %) within a custom–built environmental

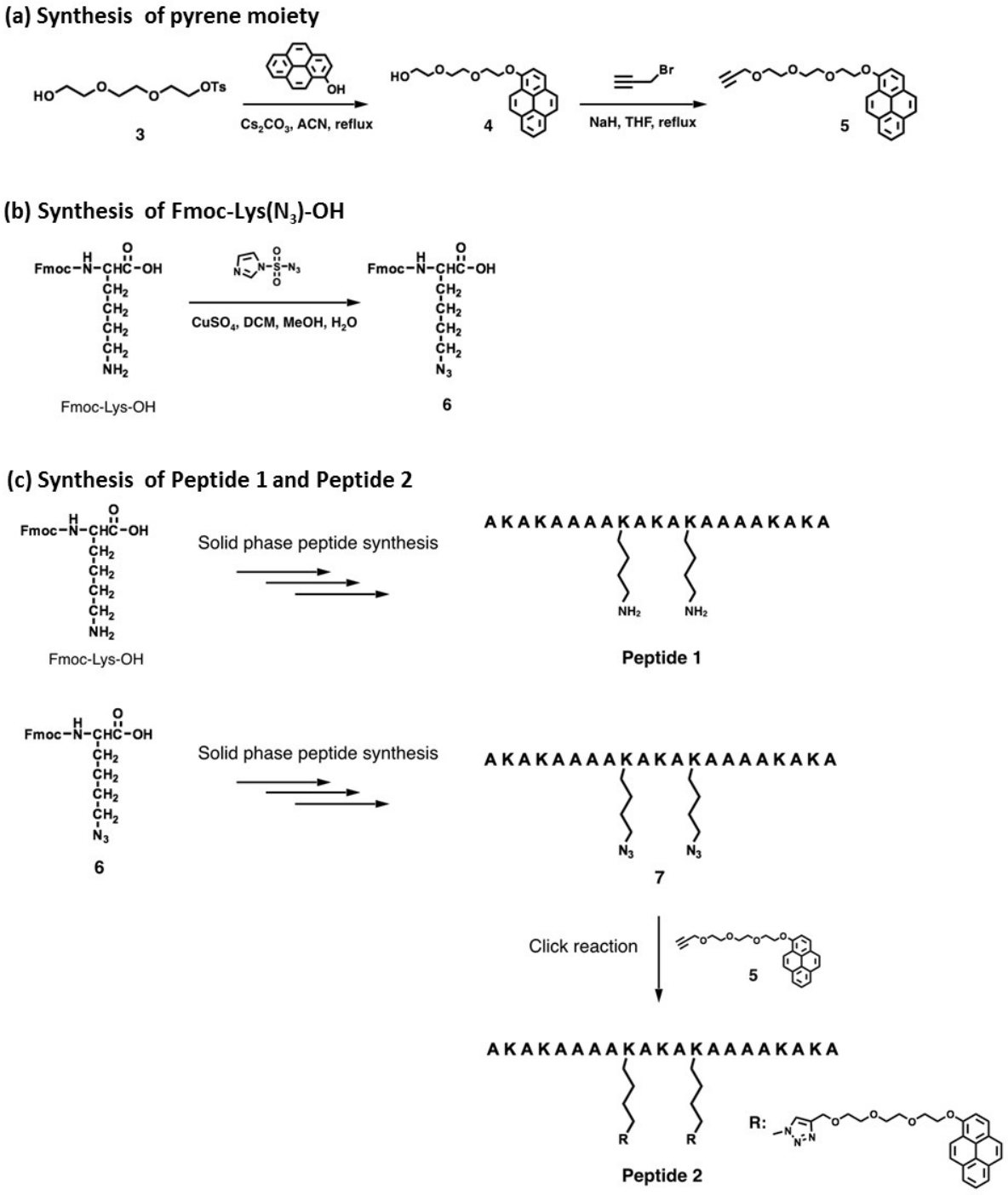
chamber in order to prevent evaporation of water from sample solution. The excess liquid was blotted with filter paper for 2 – 3 seconds and the thin water films were rapidly vitrified by plunging them into liquid ethane (cooled by liquid nitrogen) at its freezing point. The grid was transferred, on a Gatan 626 cryo-holder, using a cryo–transfer device and transferred to the JEOL–JEM HR 2100 TEM. Direct imaging was carried out at a temperature of approximately –175 ℃ and with a 120 kV accelerating voltage, using the images acquired with a Dual vision 300 W and SC 1000 CCD camera (Gatan, Inc.; Warrendale, PA). The data were analyzed using Digital Micrograph software.

Separation of racemic mixtures. A series of the racemic mixture was subjected to the evaluating the enantioselective permeability of the vesicular walls. Aqueous solution (2.0 mL) of peptide (0.1 wt%) which was stabilized for 1 hour at room temperature and was added to racemic mixtures to provide final concentration of 0.2 mM. Then 0.2 mL samples with different incubation time were subjected to Sephadex G-50 (800 mg) column to remove untrapped guest molecule. Three fractions of 3.0 mL eluate (each 1 mL) were collected and monitored for chiral molecules through HPLC analysis.

ITC Experiments. The microcalorimetric measurements for the binding study with enantiomers were performed on an isothermal titration calorimeter (MicroCal Auto-ITC200) from GE Healthcare. The ITC instrument was periodically calibrated using the internal electric heater. Each microcalorimetric titration experiment consisted of 30 successive injections of a constant volume (1 μL per injection) of 0.73 mM peptide **2** in the water, 0.073 mM D- and L-enantiomers in the water, respectively, into the microcalorimetric reaction cell. The final titration curves were obtained by subtracting the control enthalpies from the enthalpies measured in the titration experiments. Binding isotherms were obtained from the integration of raw data and fitted to a one-site model. The Origin program supplied by Auto-ITC200 was used to calculate the binding constant (KS), binding ratios (n) and molar enthalpy change (ΔH) from the titration curve

Molecular simulations. The packing arrangement of peptide **2** was simulated through the MacroModel module from Schrödinger Suites (Schrödinger K.K.) with the following parameters:[S2] force field, OPLS3; solvent, water; cutoff, Van der Waals (8.0)/electrostatic (20.0)/hydrogen bond (4.0); minimization method, PRCG (Polak–Ribiere conjugate gradient); maximum iterations, 2,500; converge on, gradient; convergence threshold, 0.05; dynamics method, stochastic dynamics; simulation temperature, 300.0 K; time step, 1.5 fs; equilibrium time, 1.0 ps; simulation time, 5,000 ps.

2. Synthetic Method



Scheme 1. Synthetic method for peptide **1** and **2**

Compound 3. Triethylene glycol (600 mg) and freshly distilled THF (750 μL) were placed in a round bottomed flask under N_2 atmosphere. Sodium hydroxide (240 mg) dissolved in H_2O (600 μL) was added to the solution, a solution of TsCl (635 mg) in THF (3.875 mL) was added to reaction mixture at 0 ℃ The mixture was stirred at room temperature for 12 h. The reaction was quenched with water and extracted with methylene chloride. The organic layer was then dried by anhydrous $MgSO_4$ and the solvent was then removed by rotary evaporator. The crude products were purified by silica gel flash column chromatography using ethyl acetate as eluent to yield 80 % (350 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 – 7.78 (m, 2H), 7.37 – 7.32 (m, 2H), 4.19 – 4.15 (m, 3H), 3.74 – 3.69 (m, 5H), 3.63 – 3.60 (m, 5H), 2.45 (s, 3H).

Compound 4. Compound **3** (200 mg), 1-hydroxypyrene (150 mg) and freshly distilled dry acetonitrile (6 mL) were placed in a dry round bottomed flask under N_2 atmosphere. Cesium carbonate (2.14 g) was added to this mixture dropwise at room temperature. The mixture was refluxed at 80 ℃ for 12 h. After cooling to room temperature, the reaction was quenched with water and extracted with methylene chloride. The organic layer was then dried by anhydrous $MgSO_4$ and the solvent was then removed by rotary evaporator. The crude products were purified by silica gel flash column chromatography using ethyl acetate as eluent to yield 56 % (129 mg).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (d, J = 9.1 Hz, 1H), 8.13 (t, J = 8.2 Hz, 3H), 8.07 (d, J = 9.2 Hz, 1H), 8.01 – 7.96 (m, 2H), 7.92 (d, J = 8.9 Hz, 1H), 7.58 (t, J = 7.0 Hz, 1H), 4.55 – 4.50 (m, 2H), 4.11 (dd, J = 9.4, 4.4 Hz, 2H), 3.88 (dd, J = 5.6, 3.9 Hz, 2H), 3.74 (tdd, J = 17.2, 7.5, 5.4 Hz, 6H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 152.75, 131.67, 131.62, 127.18, 126.41, 126.09, 125.81, 125.53, 125.41, 125.14, 124.87, 124.31, 124.20, 121.23, 120.60, 109.55, 72.47, 71.05, 70.50, 69.98, 68.70, 61.80.

Compound 5. Compound **4** (86 mg) and freshly distilled dry THF (5 mL) were placed in a dry round bottomed flask under N_2 atmosphere. NaH (15 mg) was added to this solution

dropwise at room temperature. The mixture was refluxed at 65 ℃ for 20 mins. Propargyl bromide (43.7 mg) was added to this mixture dropwise. The mixture was stirred at 65 ℃ for 12 hours. After cooling to room temperature, the reaction was quenched with water and extracted with methylene chloride. The organic layer was then dried by anhydrous $MgSO_4$ and the solvent was then removed by rotary evaporator. The crude products were purified by silica gel flash column chromatography using ethyl acetate as eluent to yield 33.4 % (32 mg).
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (d, *J* = 9.1 Hz, 1H), 8.10 (t, *J* = 8.2 Hz, 3H), 8.04 (d, *J* = 9.2 Hz, 1H), 7.98 – 7.93 (m, 2H), 7.89 (d, *J* = 8.9 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 1H), 4.53 – 4.46 (m, 2H), 4.19 (d, *J* = 2.3 Hz, 2H), 4.08 (dd, *J* = 9.4, 4.4 Hz, 2H), 3.85 (dd, *J* = 5.6, 3.9 Hz, 2H), 3.75 – 3.67 (m, 6H), 2.39 (t, *J* = 2.3 Hz, 1H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 152.87, 131.70, 131.66, 127.21, 126.39, 126.09, 125.83, 125.49, 125.43, 125.11, 124.90, 124.28, 124.19, 121.33, 120.63, 109.58, 79.64, 74.48, 71.06, 70.77, 70.50, 70.01, 69.13, 68.78, 58.40.

Peptide 1 was synthesized on Rink amide MBHA resin with standard Fmoc protection process using HCTU as coupling reagent. *tert*-Butyloxycarbonyl (Boc) protecting group was used for Lysine. The resin was swollen in DCM for 1h before the synthesis. Deprotection of Fmoc protecting group, a cocktail solution (20 % pyridine in DMF) was treated several times (2 × 2 min). The resin was washed with DCM and DMF several times after each step was finished. Resin was treated with a cleavage cocktail solution (TFA : anisole : Water : TIPS; 88 : 5 : 5 : 2) for 3 h after synthetic and the mixture solution was washed with ether. Peptide **1** was purified by reverse phase HPLC (water/acetonitrile with 0.1 % Formic Acid) using C18 prep scale column. Peptide **1** was confirmed by MALDI-TOF mass spectrometer. Yield of peptide **1** was calculated from the initial loading amount of the resin and after purification process, total reaction yield: 5.4 %.

Peptides 2. A solution of compound **7** (synthesized by the similar method with peptide **1**) and compound **5** were dissolved in H_2O/THF (1/1) with catalytic amounts of sodium ascorbate (5 mol%) and $CuSO_4$ (2 mol%) was stirred at 50 ℃ for 2 h. After completion of the reaction as

monitored by MALDI-TOF, the solvent was removed in a rotary evaporator. The crude product was purified by reverse phase HPLC (C18 column). Yields: 51 %.

Guest molecules G3 [Fmoc-Trp-Trp-OH] and G4 [Fmoc-Trp-Lys(N_3)-OH]. Trityl Resin (50 mg), DIPEA (20 μL) and Fmoc-Trp(Boc)-OH (80 mg for **G3**), Compound **6** (60 mg for **G4**) were placed in DMF (1 mL) and stirred for 2h. The resin was washed with DCM and DMF several times. A cocktail solution (20 % pyridine in DMF) was treated several times (2 × 2 min) to remove the Fmoc protecting group. A DMF solution of Fmoc-Trp(Boc)-OH (80 mg), HCTU (42 mg) and NMM (21 μL) was added to the resin. Resins were treated with a cleavage cocktail solution (TFA: anisole: Water: TIPS; 88: 5: 5: 2) for 1 h after synthetic and the mixture solution was washed with ether. **G3** and **G4** were purified by reverse phase HPLC using C4 preparatory column. **G3** and **G4** were confirmed by MALDI-TOF mass spectrometer. Yield: 55 % for **G3** and yield: 43 % for **G4**.

3. Supporting figures

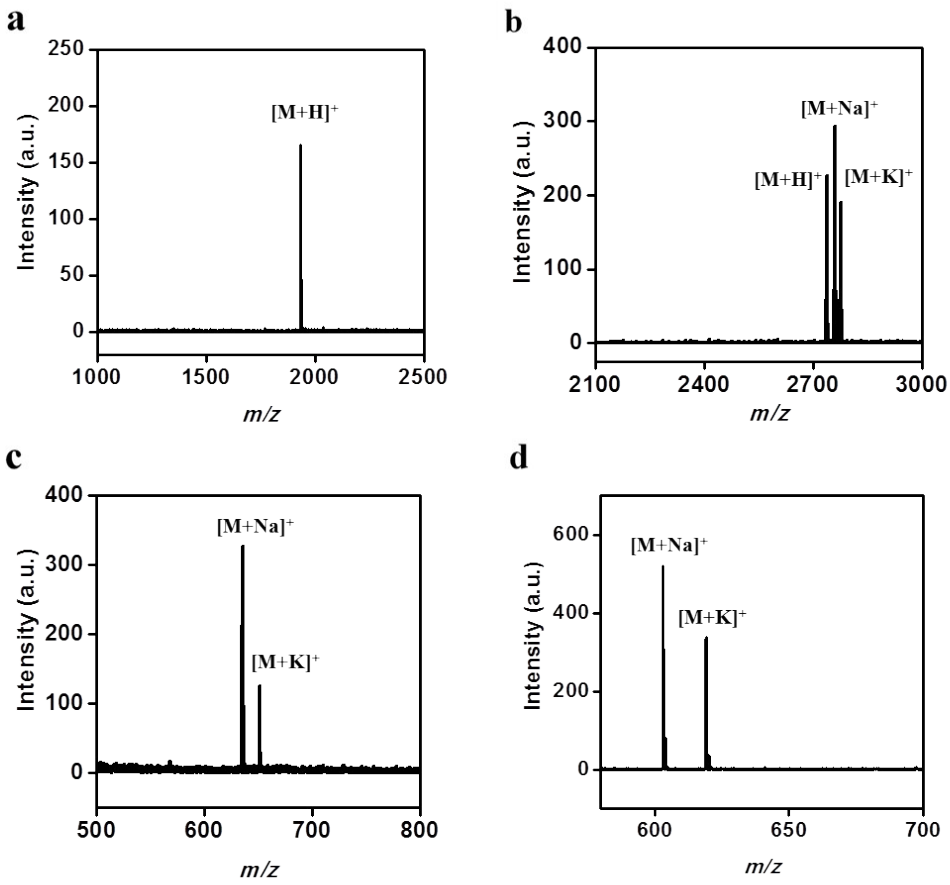


Figure S1. MALDI-TOF spectra of (a) peptide **1** calcd. for $C_{84}H_{156}N_{28}O_{22}$ $[M+H]^+$:1911.35; found: 1911.51; (b) peptide **2** calcd. for $C_{134}H_{201}N_{33}O_{29}$ $[M+H]^+$: 2737.53; found: 2737.99; (c) **G3** calcd for $C_{37}H_{32}N_4O_5$ $[M+Na]^+$: 635.68; found: 635.54; and (d) **G4** calcd for $C_{32}H_{32}N_6O_5$ $[M+Na]^+$: 603.63; found: 603.16.

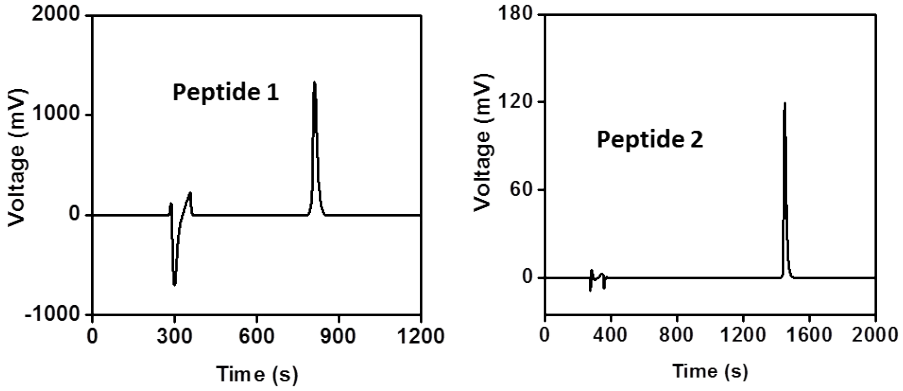


Figure S2. HPLC results of peptides **1** and **2**. Purified samples were analyzed with an analytical Shim-pack PREP-ODS (H) KIT column (C18, 250 ×4.6 mm I.D). Solvent was changed from 5 % CH_3CN in H_2O (0.1 % formic acid) to 100 % CH_3CN (0.1 % formic acid) for 50 min with 0.7 ml/min flow rate. Absorbance at 210 nm was detected.

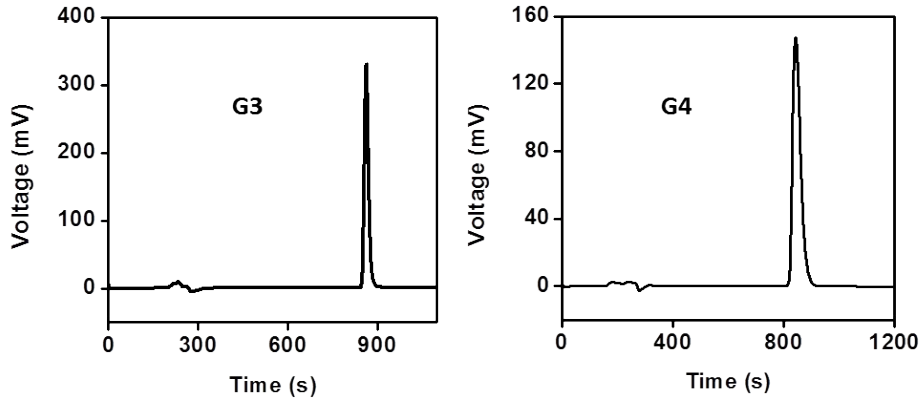


Figure S3. HPLC results of **G3** and G**4**. Purified samples were analyzed with an analytical Shim-pack PREP-ODS (H) KIT column (C4, 250 ×4.6 mm I.D). The solvent was 60 % CH_3CN and 40 % H_2O (0.1 % formic acid) with 0.7 ml/min flow rate. Absorbance at 254 nm was detected.

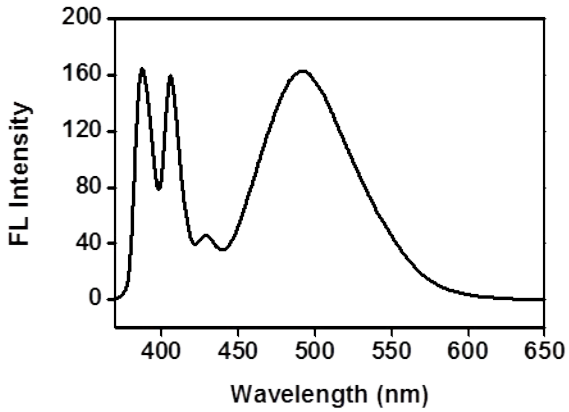


Figure S4. Fluorescence spectrum of peptide **2** (0.1 wt%) in aqueous solution. Excitation wavelength: 360 nm. Excimer wavelength: 494 nm.

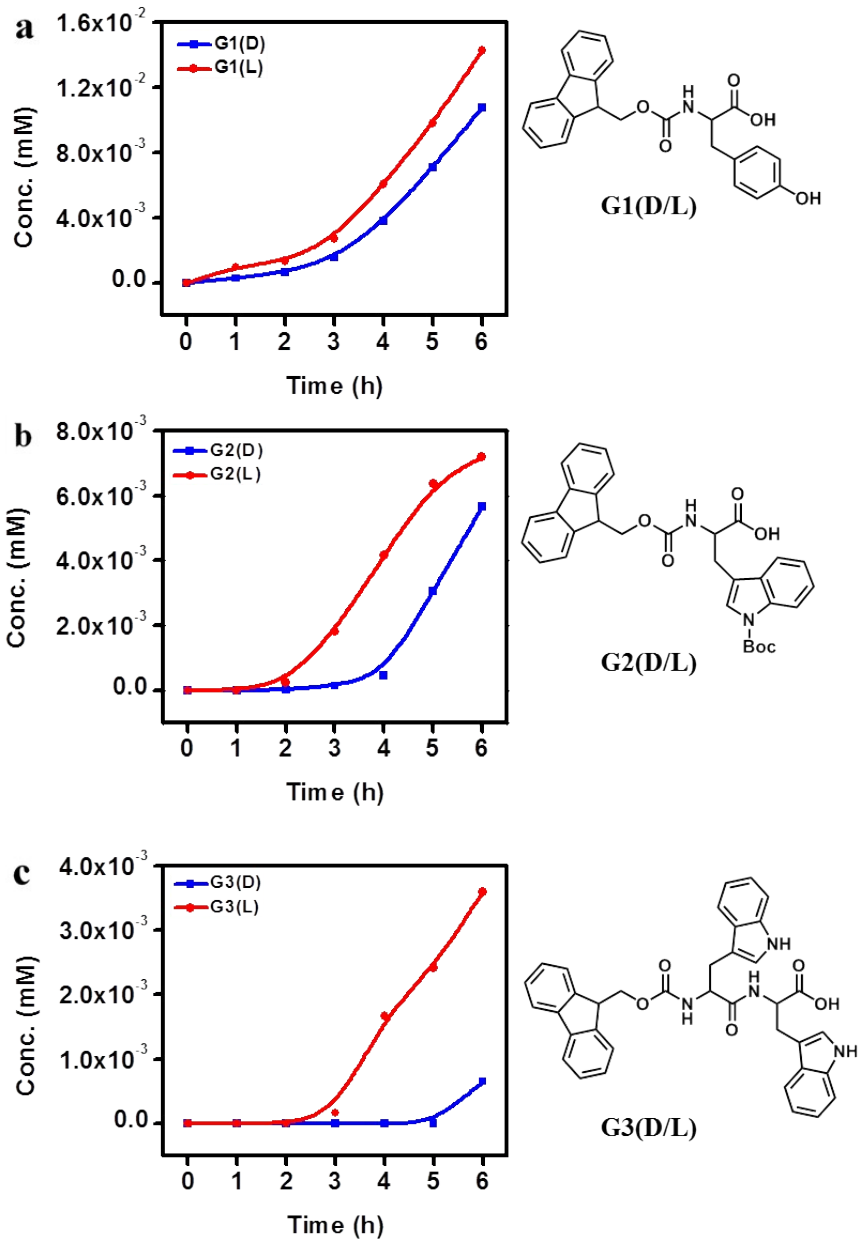


Figure S5. The separation experiment results of (a) **G1**; (b) **G2**; and (c) **G3**.

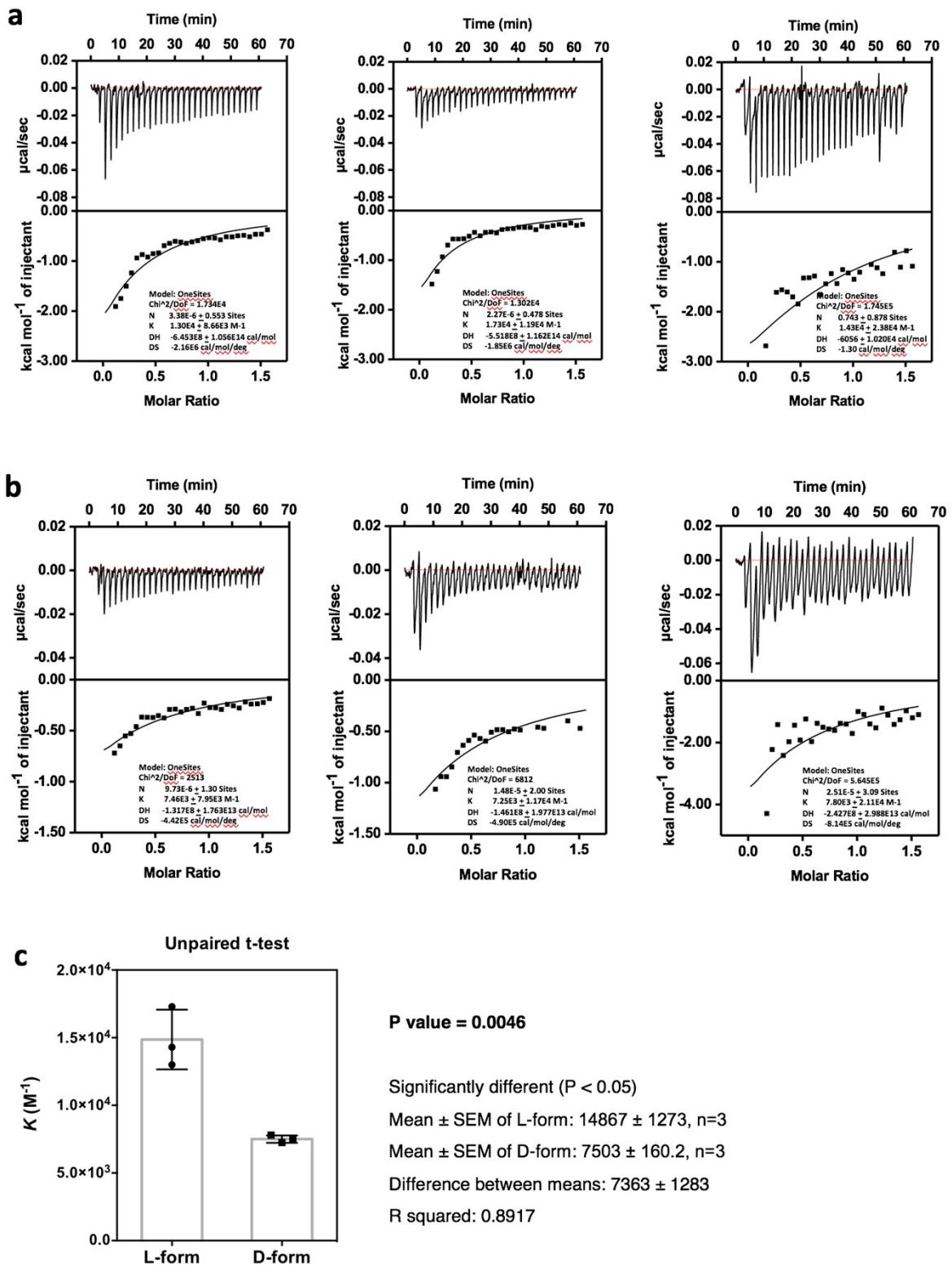


Figure S6. Isothermal titration calorimetry (ITC) measurements. ITC results showed the binding affinity with (a) **G2**(L) and (b) **G2**(D). Each microcalorimetric titration experiment consisted of 30 successive injections of a constant volume (1 µL/injection) of 0.73 mM peptide **2** aqueous solution into the microcalorimetric reaction cell, filled with guest solution

(73 μM) of (a) **G2**(L) and (b) **G2**(D) respectively. Binding isotherms were obtained from the integration of raw data and fitted to a one-sites model. The experiments were repeated three times independently. (c) Statistical binding affinity between peptide **2** and guest molecules. Data are the mean ± SD of n= 3 with different guest molecules. Statistical significance was analyzed using unpaired t-test with equal SD. The P values (0.0046) less than 0.05 were considered to provide statistically significant difference with different guest molecules. The binding affinity with the **G2**(L) (K_a= 14867±1273) is higher than **G2**(D) (K_a= 7503±160.2).

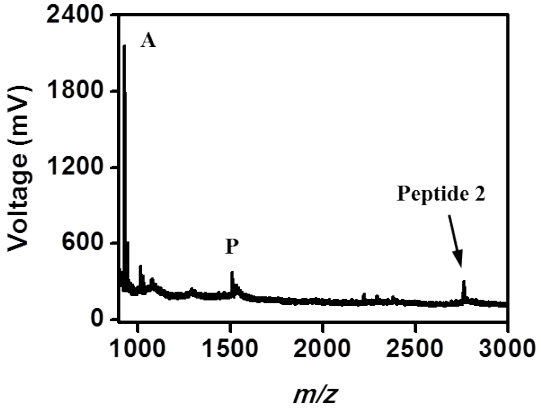
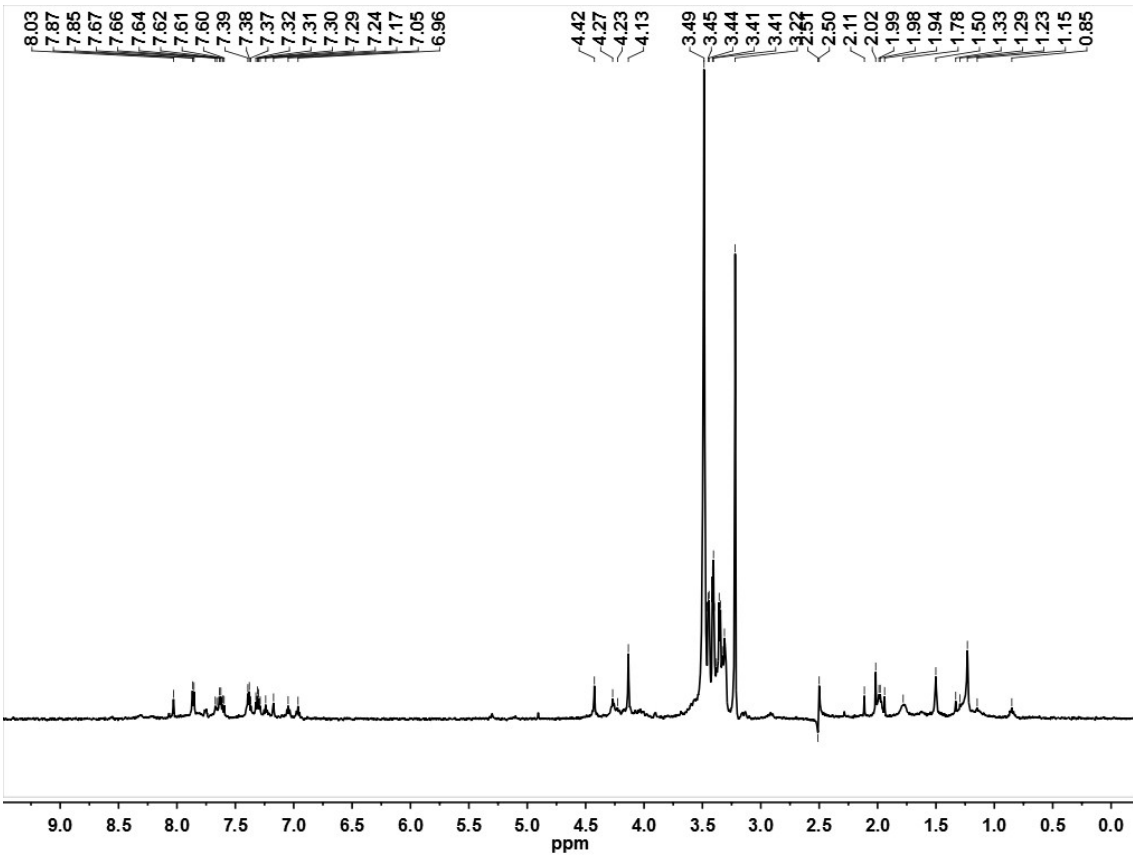


Figure S7. MALDI-TOF spectrum from reaction mixture after 1 hr. The product peak in enantioselective nanoreactors is as follows: calcd for $C_{75}H_{116}N_6NaO_{24}$ $[M+Na]^+$: 1508.76; found: 1509.65.



^{1}H NMR (600 MHz, DMSO-d_6) δ 8.03 (s, 1H), 7.86 (d, J = 8.0 Hz, 2H), 7.75 (s, 1H), 7.65 – 7.61 (m, 2H), 7.41 – 7.36 (m, 2H), 7.31 (dd, J = 14.4, 7.9 Hz, 2H), 7.24 (t, J = 7.3 Hz, 1H), 7.17 (s, 1H), 7.07 – 7.02 (m, 1H), 6.96 (t, J = 7.5 Hz, 1H), 4.42 (s, 2H), 4.25 (d, J = 25.3 Hz, 3H), 4.13 (s, 3H), 3.49 (s, 40H), 3.45 (d, J = 4.9 Hz, 8H), 3.43 – 3.39 (m, 11H), 3.35 (d, J = 6.1 Hz, 8H), 3.31 (s, 7H), 3.22 (s, 12H), 1.98 (d, J = 6.4 Hz, 2H), 1.78 (s, 2H), 1.50 (s, 3H), 1.23 (s, 6H), 0.84 (d, J = 8.5 Hz, 1H).

Figure S8. ^{1}H-NMR spectrum of reaction product.

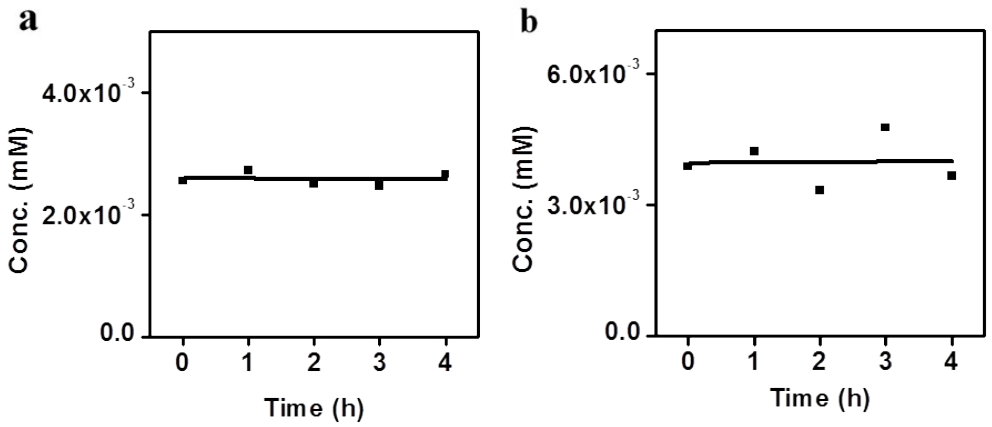


Figure S9. Release out experiments of (a) dendrimer and (b) reaction product with vesicle assembly of peptide **2**.

4. Reference

(1) Chen, X.; He, Y.; Kim, Y.; Lee, M. *J. Am. Chem. Soc.* **2016**, *138*, 5773-5776.

(2) Kim, Y.; Li, H.; He, Y.; Chen, X.; Ma, X.; Lee, M. *Nature Nanotech.* **2017,** *12,* 551-556.