A Unimolecular Channel Formed by Dual Helical Peptide Modified Pillar[5]arene: Correlating Transmembrane Transport Properties with Antimicrobial Activity and Haemolytic Toxicity.

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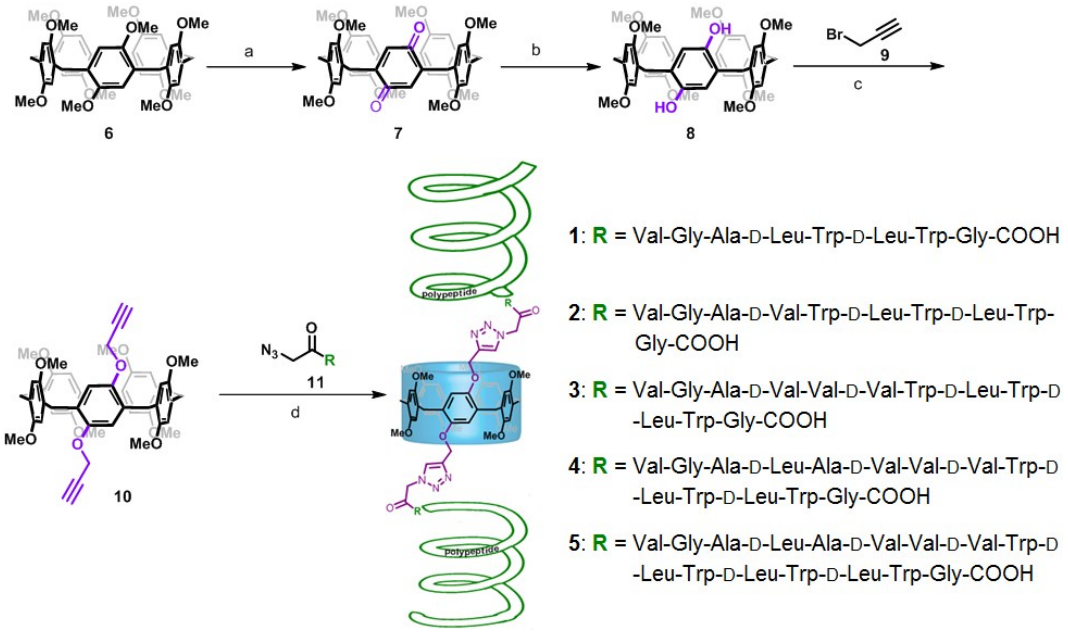
Contents

1. General:

Egg yolk L-α-phosphatidylcholine was obtained from Sigma-Aldrich as ethanol solution (100 mg/mL). 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (diPhyPC) was obtained from Avanti Polar Lipids as chloroform solution (10 mg/mL). The Fmoc-protected amino acids were obtained from GL Biochem (Shanghai) Ltd. The peptides were synthesized by PTI PS3 automated peptide synthesizer. The ^{1}H and ^{13}C NMR spectra were recorded on commercial instruments (600 MHz) at 298 K. Chemical shifts were referenced to solvent residue. Mass spectra were recorded with Bruker MicroTOF II spectrometer by using positive or negative mode. The fluorescent experiments on vesicles were performed on Varian Cary Eclipse fluorescence spectrophotometer. The conductance measurement on planar lipid bilayer was performed on Warner BC-535D Planar Lipid Bilayer Workstation. The CD measurements were performed with Chirascan spectropolarimeter.

2. Synthetic procedures and characterization data:



Scheme S1 Synthesis of compounds **1**-**5**: (a) $(NH_4)_2Ce(NO_3)_6$, DMA/H_2O, r.t.; (b) $NaBH_4$, THF/MeOH, r.t.; (c) K_2CO_3, CH_3CN, 60°C; (d) $CuSO_4 \cdot 5H_2O$, Sodium ascorbate, DMSO, r.t.

Compound 7. To a solution of **6**[1] (3.0 g, 4 mmol) in dichloromethane (DCM) (300 mL) was added $(NH_4)_2Ce(NO_3)_6$ (4.38 g, 8 mmol) and water (3 mL).[2] After addition, the mixture was stirred at room temperature for 10 minutes. Then the mixture was

washed with water and the organic solution was dried over anhydrous Na_2SO_4. After removing of the solvent, the crude product was purified by column chromatography on silica gel to yield **7** as red solid.

7: Yield: 35%. ^{1}H NMR ($CDCl_3$, 600MHz) δ: 6.84(s, 2H), 6.81(s, 2H), 6.79 (s, 2H), 6.67 (s, 2H), 6.66 (s, 2H), 3.79 (s, 6H), 3.74 (s, 6H), 3.71 (s, 12H), 3.63 (s, 6H), 3.59 (s, 4H). HRMS: Calcd for $C_{43}H_{44}NaO_{10}$ $[M+Na]^+$: 743.2832. Found: 743.2867.

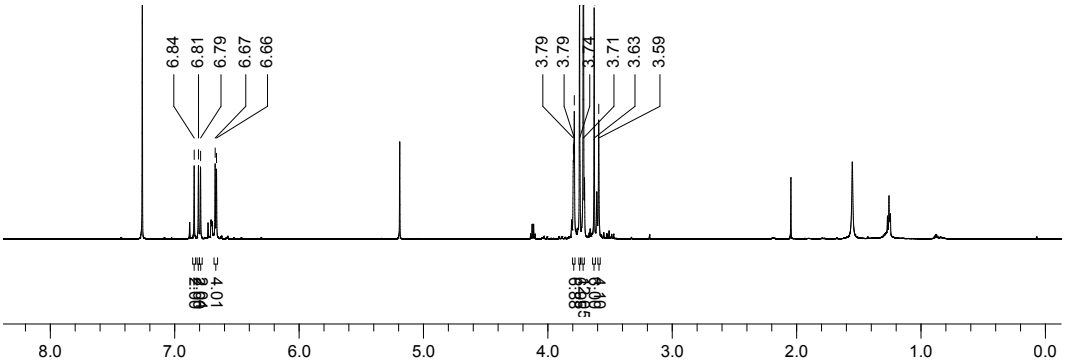


Fig. S1. ^{1}H NMR spectrum of **7** in $CDCl_3$.

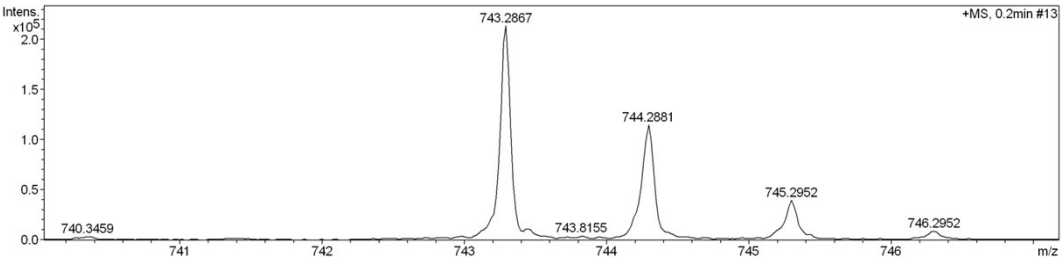


Fig. S2. HR-MS of **7**.

Compound 8. To a solution of **7** (0.5 g, 0.7 mmol) in THF (15 mL) and CH_3OH (5 mL) was added $NaBH_4$ (0.13 mg, 3.50 mmol).[3] The mixture was stirred at room temperature for 30 min. The reaction was quenched by pouring into 0.5 M HCl aqueous solution. Then ethyl acetate and water were added to the mixture. The organic layer was dried over anhydrous Na_2SO_4. After removing of the solvent, the crude product was purified by column chromatography on silica gel to yield **8** as white solid.

8: Yield: 57%. ^{1}H NMR (DMSO-*d6*, 600MHz) δ: 8.22 (s, 2H), 6.84(s, 2H), 6.83 (s, 2H), 6.79 (s, 2H), 6.78 (s, 2H), 6.54 (s, 2H), 3.71 (s, 6H), 3.68 (s, 6H), 3.65 (s, 12H), 3.63 (s, 6H), 3.55 (s, 4H). HRMS: Calcd for $C_{43}H_{46}NaO_{10}$ $[M+Na]^+$: 745.2989. Found: 745.2997.

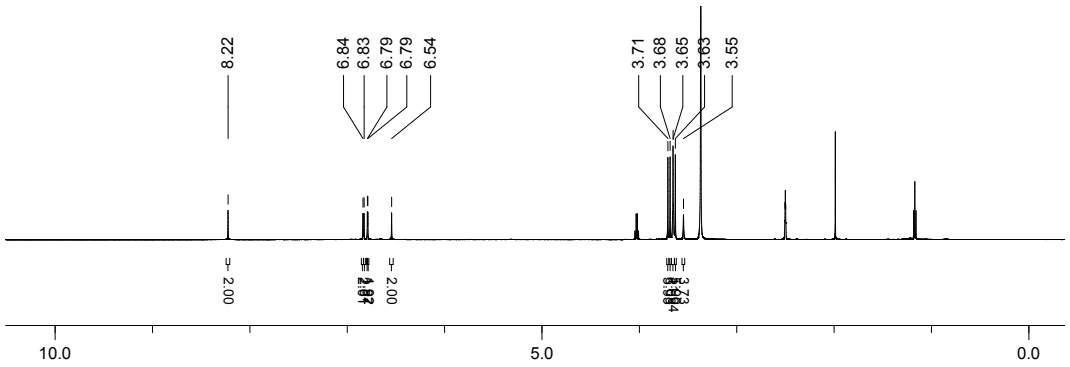


Fig. S3. ^{1}H NMR spectrum of **8** in $CDCl_3$.

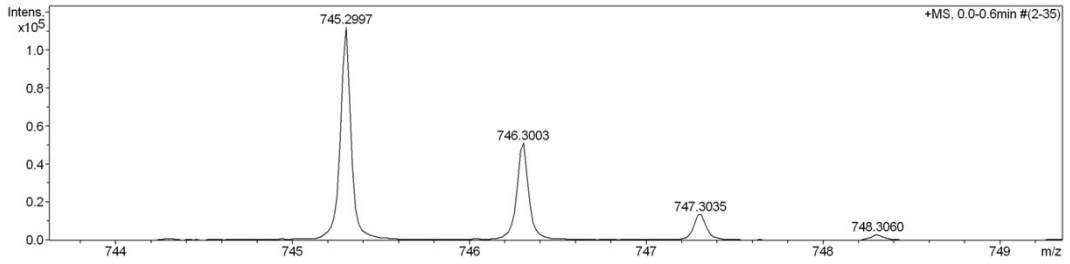


Fig. S4. HR-MS of **8**.

Compound 10. Under a nitrogen atmosphere, **8** (0.2 g, 0.28 mmol) was dissolved in CH_3CN (10 mL). K_2CO_3 (0.6 mg, 1.4 mmol) was added, and the reaction mixture was stirred at 60 °C for 2 h. Then propargyl bromide **9** (0.17 mL, 2.24 mmol) was added, and the reaction mixture was stirred at 60 °C for 24 h. After removal of the solvent, the resulting solid was dissolved in ethyl acetate and water. The organic layer was dried over anhydrous Na_2SO_4. After removing of the solvent, the crude product was purified by column chromatography on silica gel to yield **10** as white solid.

10: Yield: 52%. ^{1}H NMR ($CDCl_3$, 600MHz) δ: 6.79 (s, 2H), 6.78(s, 2H), 6.76 (s, 2H), 6.74 (s, 2H), 6.69 (s, 2H), 4.47 (d, 4H), 3.79-3.77 (br, 10H), 3.68 (s, 6H), 3.66 (s, 6H), 3.62 (d, 12H), 1.94 (br, 2H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 150.3, 150.3, 149.1, 128.4, 128.0, 127.8, 127.7, 127.4, 115.2, 113.7, 113.7, 113.5, 113.5, 80.2, 77.7, 60.2, 56.5, 55.8, 55.7, 55.7, 31.3, 29.5, 29.4, 29.3, 22.3, 21.2, 14.5. HRMS: calcd for $C_{49}H_{50}NaO_{10}$ $[M+Na]^+$ 821.3302, found 821.3261.

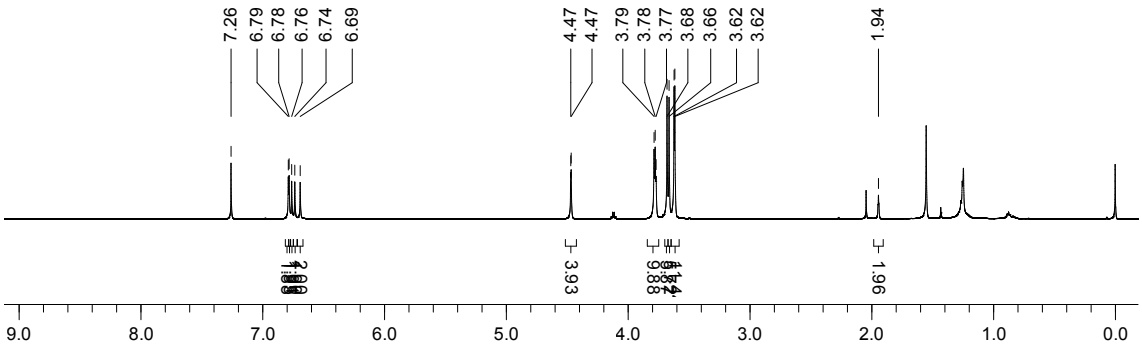


Fig. S5. ^{1}H NMR spectrum of **10** in $CDCl_3$.

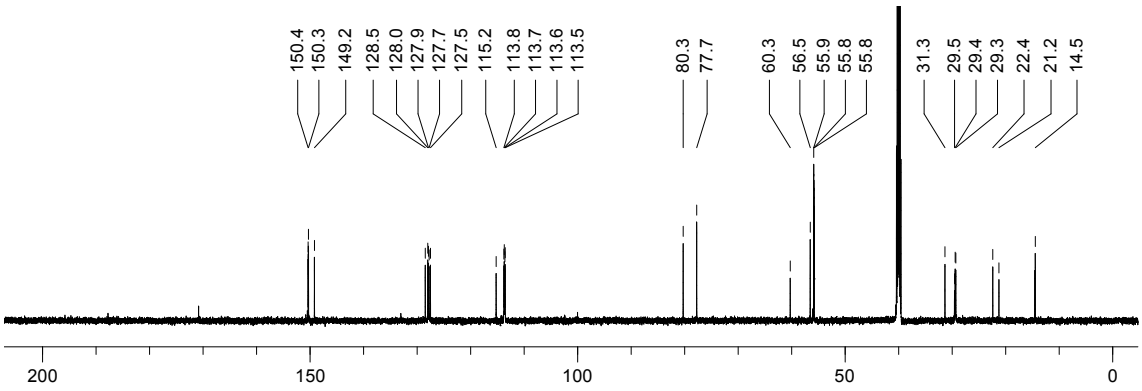


Fig. S6. ^{13}C NMR spectrum of **10** in DMSO-*d6*.

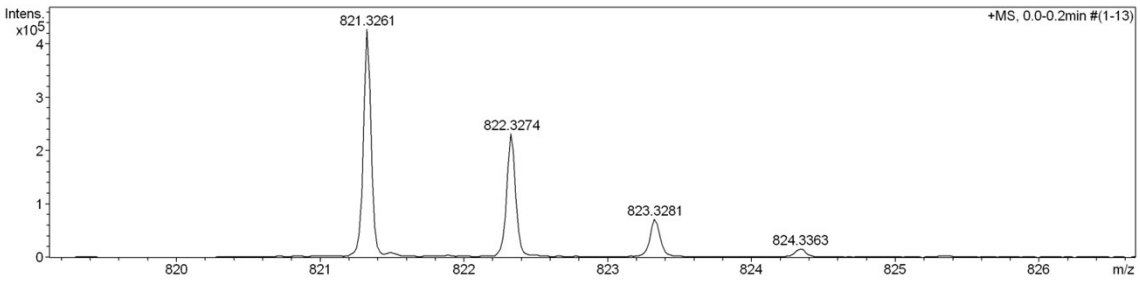


Fig. S7. HR-MS of **10**.

Compounds 11a-11e. The peptides **11a-11e** were synthesized from solid phase peptide synthesis (SPPS), which was performed by using an automated peptide synthesizer (PTI PS3). 2-chlorotrityl chloride resin and Fmoc-protected amino acids were used for the solid phase peptide synthesis. Fmoc-protected L- and D-amino acids were purchased from GL Biochem and used without further purification. The synthesized peptide was analysed by HPLC using SB-C18 column (5 μm, 4.6×250 mm) with *A* (100% acetonitrile containing 0.1% TFA) and *B* (100% water containing 0.1% TFA) as eluent and UV detection at 254 nm.

11a: N_3-CH_2-CO-Val-Gly-Ala-D-Leu-Trp-D-Leu-Trp-Gly-COOH

HRMS: calcd for $C_{48}H_{66}N_{13}O_{10}$ $[M+H]^+$ 984.5056, found 984.5060.

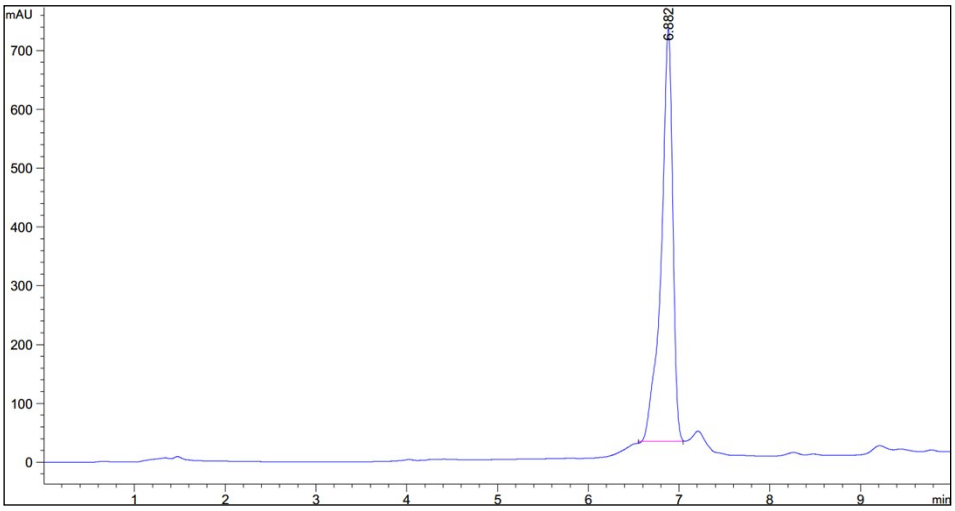


Fig. S8. HPLC analytic trace of **11a**

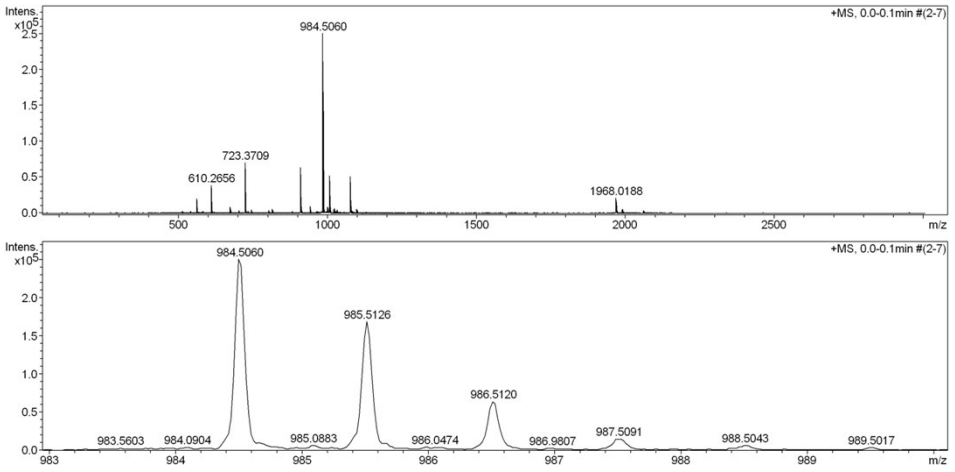


Fig. S9. HR-MS of **11a**.

11b: N_3-CH_2-CO-Val-Gly-Ala-D-Val-Trp-D-Leu-Trp-D-Leu-Trp-Gly-COOH
HRMS: calcd for $C_{64}H_{85}N_{16}O_{12}$ $[M+H]^+$ 1269.6533, found 1269.6525.

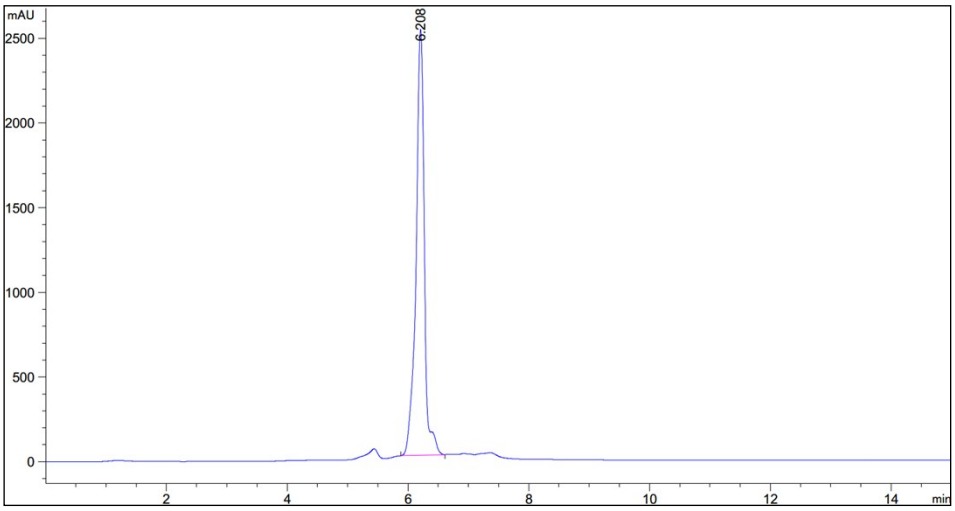


Fig. S10. HPLC analytic trace of **11b**

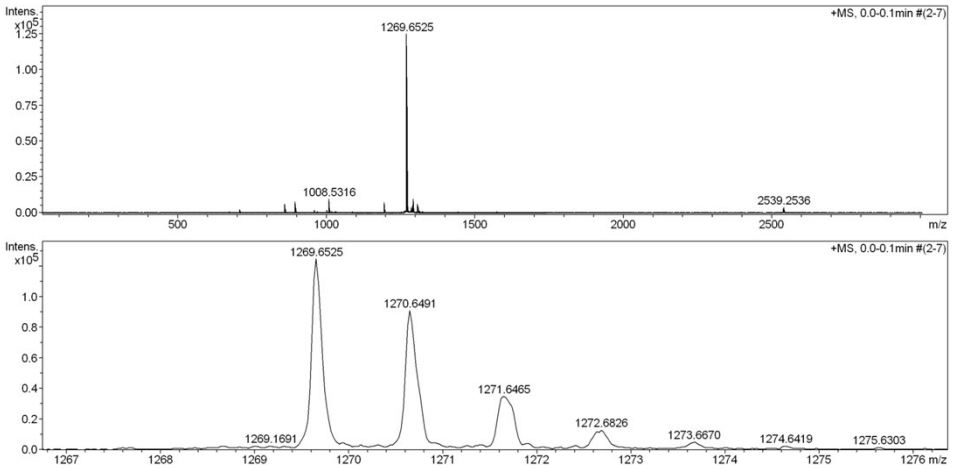


Fig. S11. HR-MS of **11b**.

11c:N_3-CH_2-CO-Val-Gly-Ala-D-Val-Val-D-Val-Trp-D-Leu-Trp-D-Leu-Trp-Gly-COOH

HRMS: calcd for $C_{74}H_{103}N_{18}O_{14}$ $[M+H]^+$ 1467.7901, found 1467.7898.

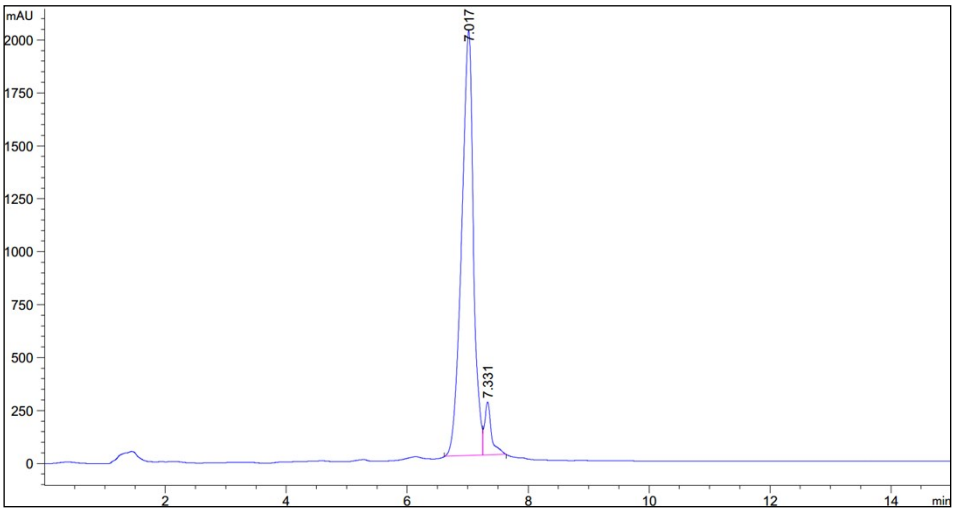


Fig. S12. HPLC analytic trace of **11c**

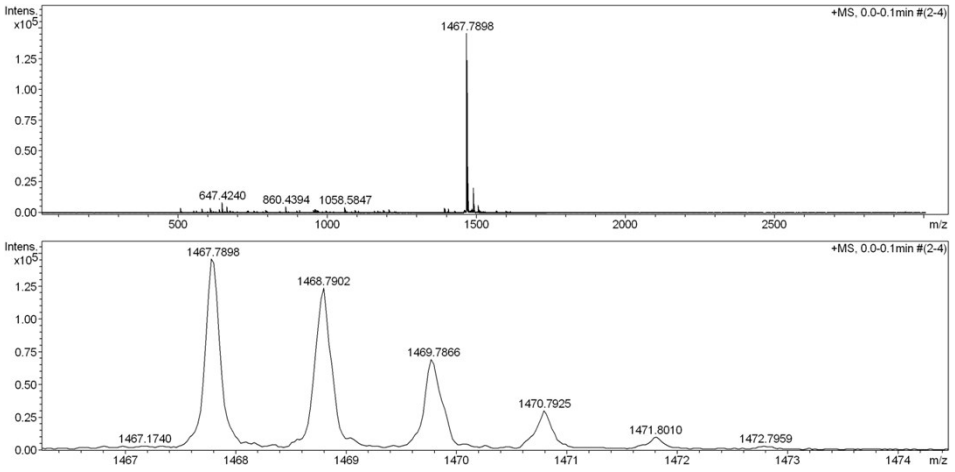


Fig. S13. HR-MS of **11c**.

11d: N_3-CH_2-CO-Val-Gly-Ala-D-Leu-Ala-D-Val-Val-D-Val-Trp-D-Leu-Trp-D-Leu-Trp-Gly-COOH

HRMS: calcd for $C_{83}H_{119}N_{20}O_{16}$ $[M+H]^+$ 1651.9113, found 1651.9179.

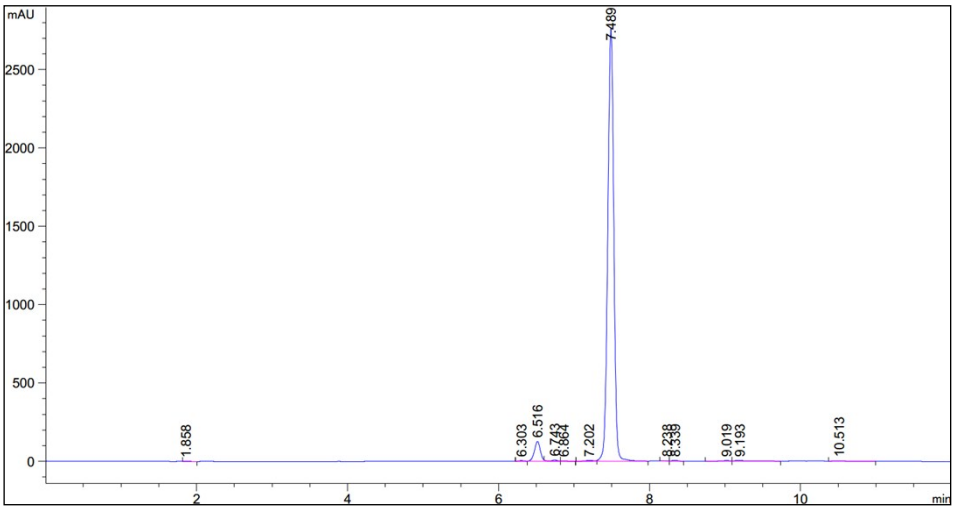


Fig. S14. HPLC analytic trace of **11d**

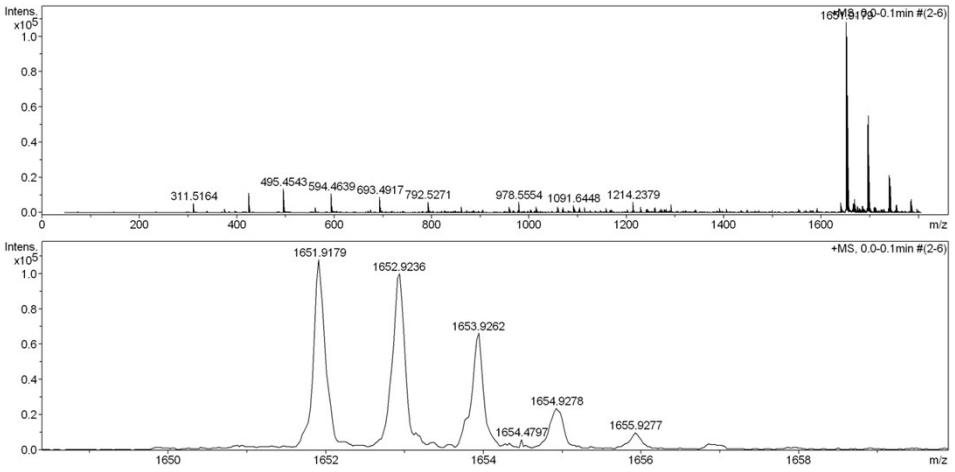


Fig. S15. HR-MS of **11d**.

11e: N_3-CH_2-CO-Val-Gly-Ala-D-Leu-Ala-D-Val-Val-D-Val-Trp-D-Leu-Trp-D-Leu-Trp-D-Leu-Trp-Gly-COOH

HRMS: calcd for $C_{100}H_{139}N_{23}O_{18}$ $[M+H]^+$ 1951.0702, found 1951.0801.

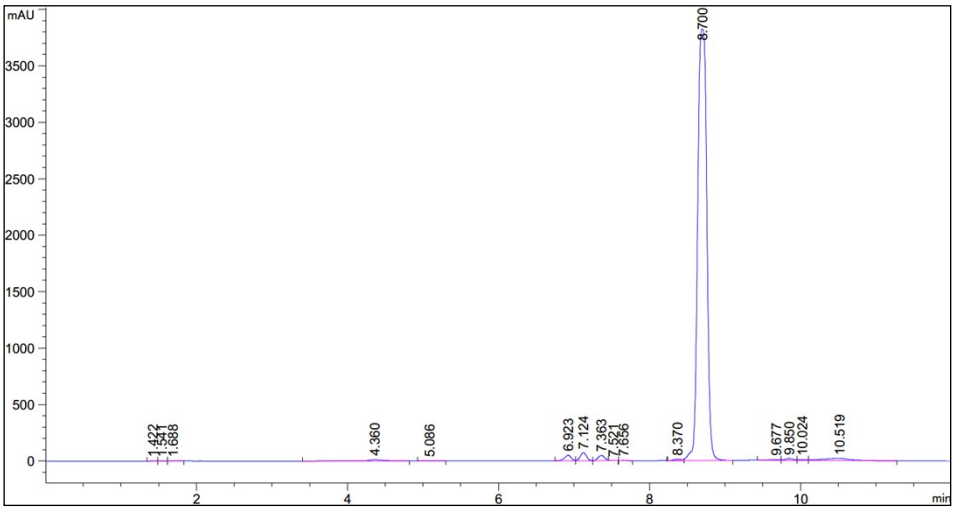


Fig. S16. HPLC analytic trace of **11e**

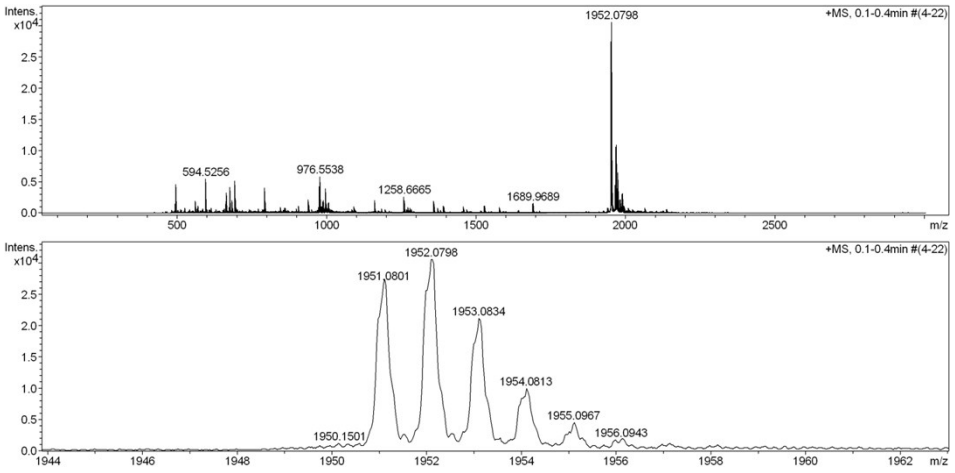


Fig. S17. HR-MS of **11e**.

Compound 1. Compound **11a** (74 mg, 0.075 mmol) was dissolved in DMSO (8 mL). Sodium ascorbate (20 mg, 0.1 mmol) and Compound **10** (20.5 mg, 0.025 mmol) was added, and the reaction mixture was stirred for 15 minutes. Then $CuSO_4 \cdot 5H_2O$ (6.3 mg, 0.025 mmol) was added, the reaction mixture was stirred at room temperature for 48 h. 10 portions of water was added to the reaction mixture, the precipitate were collected by centrifugation and washed twice with water. The crude product was purified by HPLC to yield **1** as white solid.

1: Yield: 67%. ^{1}H NMR (DMSO-*d6*, 600MHz) δ: 10.76 (s, 2H), 10.74 (s, 2H), 8.87 (d, 1H), 8.49 (d, 2H), 8.36-8.33 (m, 4H), 8.28 (d, 2H), 8.18 (d, 2H), 8.08 (d, 2H), 8.02 (d, 2H), 7.97-7.91 (m, 2H), 7.93-7.91 (m, 2H), 7.60 (t, 4H), 7.29 (t, 4H), 7.13 (d, 2H), 7.09 (d, 2H), 7.04-6.99 (m, 6H), 6.96-6.92 (m, 4H), 6.78 (d, 4H), 6.75 (d, 4H),

5.29-5.20 (m, 4H), 5.02-4.97 (m, 4H), 4.55-4.50 (m, 4H), 4.28-4.14 (m, 18H), 3.99-3.97 (m, 2H), 3.79 (d, 4H), 3.51 (d, 8H), 3.20-3.17 (m, 3H), 3.10-3.06 (m, 3H), 2.93-2.87 (m, 5H), 2.04-1.96 (m, 3H), 1.47-1.44 (m, 5H), 1.24-0.99 (m, 26H), 0.88 (d, 12H), 0.69-0.59 (m, 24H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 172.5, 172.4, 172.2, 171.8, 171.5, 171.4, 168.7, 166.1, 159.0, 158.8, 158.5, 158.3, 154.6, 150.3, 149.6, 144.1, 143.5, 136.5, 136.5, 130.1, 128.4, 128.0, 127.9, 127.8, 127.6, 127.6, 127.0, 126.0, 124.4, 121.1, 118.9, 118.8, 118.6, 118.5, 117.1, 115.1, 115.0, 113.8, 113.7, 111.6, 111.5, 110.5, 110.2, 80.4, 77.8, 73.9, 66.5, 62.2, 58.4, 57.2, 55.9, 55.9, 55.8, 54.1, 53.8, 51.9, 51.6, 51.5, 48.7, 46.2, 42.3, 41.2, 41.1, 40.8, 39.5, 31.7, 31.0, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 28.4, 28.2, 27.6, 27.0, 25.6, 24.4, 24.2, 23.2, 22.0, 21.8, 19.6, 18.9, 18.4, 14.4, 9.1. HRMS: calcd for $C_{145}H_{182}N_{26}O_{30}$ $[M+2H]^{2+}$ 1384.1775, found 1384.1839.

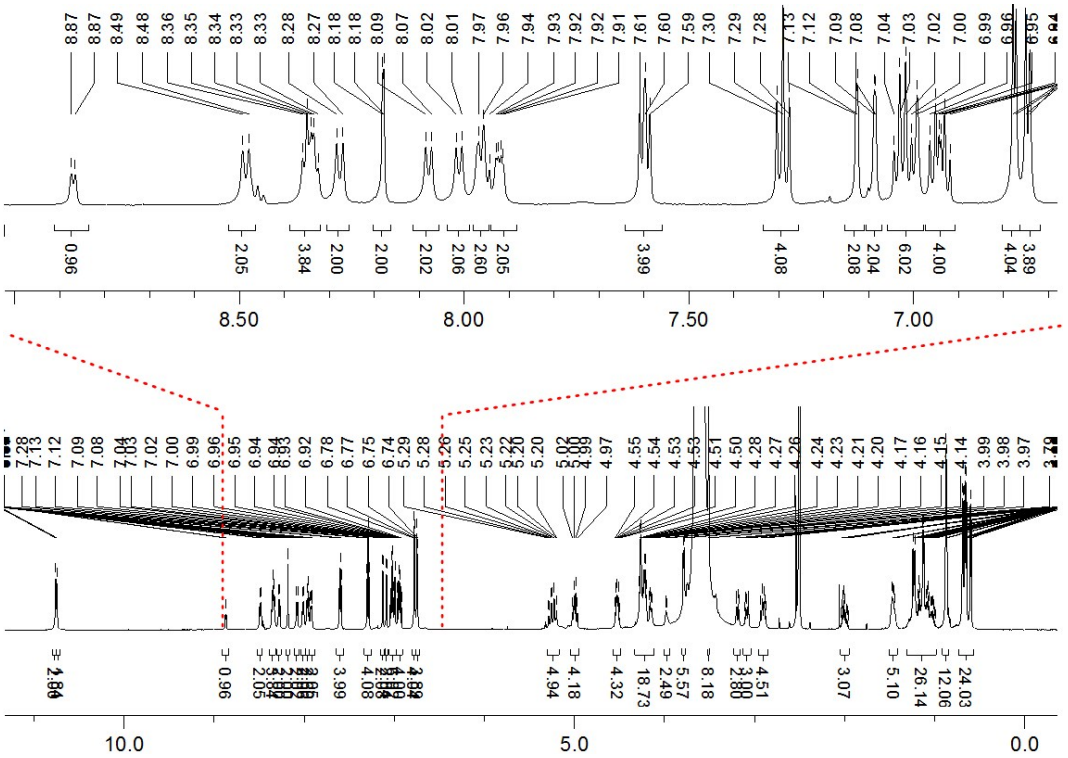


Fig. S18. ^{1}H NMR spectrum of **1** in DMSO-*d6*.

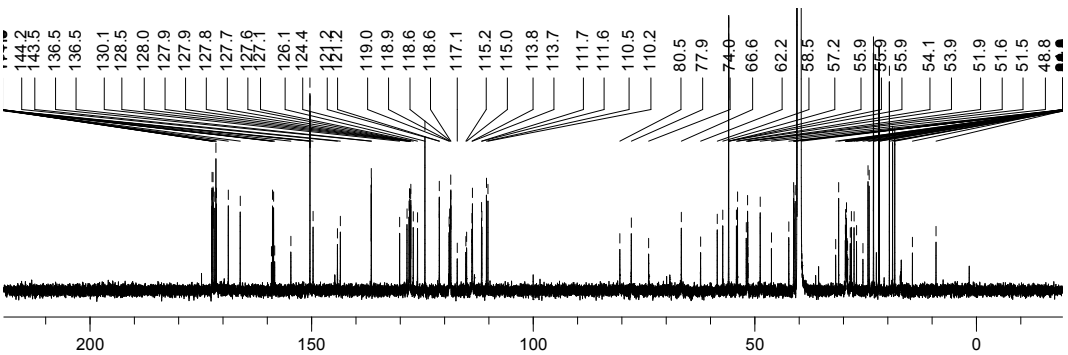


Fig. S19. ^{13}C NMR spectrum of **1** in DMSO-*d6*.

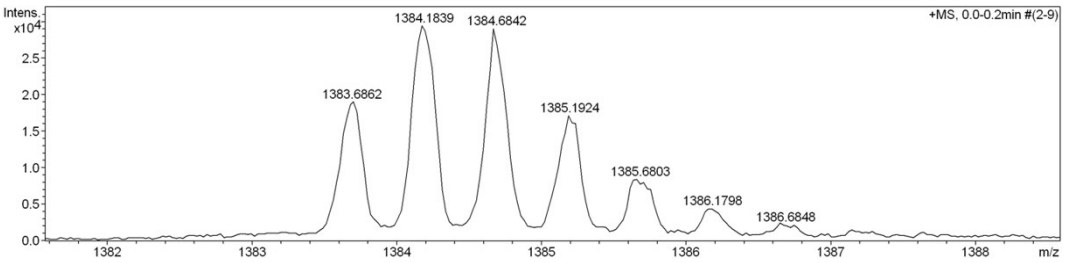


Fig. S20. HR-MS of **1**.

Compound 2. This compound was synthesized from **10** and **11b** according to the same procedure with compound **1**.

2: Yield: 56%. ^{1}H NMR (DMSO-*d6*, 600MHz) δ: 10.76 (s, 2H), 10.72 (s, 2H), 10.71 (s, 2H), 8.48 (d, 2H), 8.38 (t, 2H), 8.34-8.31 (m, 4H), 8.18 (s, 2H), 8.15 (d, 4H), 8.04 (d, 2H), 7.99 (d, 2H), 7.92 (d, 2H), 7.83 (d, 2H), 7.59 (q, 6H), 7.30-7.27 (m, 6H), 7.12 (s, 2H), 7.08 (s, 4H), 7.02-6.99 (m, 8H), 6.96-6.91 (m, 6H), 6.78 (d, 4H), 6.75 (d, 4H), 5.32 (t, 2H), 5.28-5.19 (m, 4H), 5.02-4.96 (m, 4H), 4.60-4.52 (m, 6H), 4.40-4.35 (m, 2H), 4.25-4.15 (m, 8H), 3.80 (d, 4H), 3.66-3.65 (m, 24H), 3.51 (d, 6H), 3.21-3.18 (m, 4H), 3.09-3.07 (m, 4H), 2.93-2.86 (m, 6H), 2.02-1.96 (m, 6H), 1.82-1.77 (m, 2H), 1.48-1.43 (m, 2H), 1.30-1.28 (m, 4H), 1.16 (d, 6H), 1.08-1.04 (m, 6H), 1.01-0.97 (m, 2H), 0.93-0.90 (m, 2H), 0.87 (d, 12H), 0.63 (d, 6H), 0.58-0.56 (m, 16H), 0.53 (d, 10H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 174.8, 172.6, 172.4, 172.3, 172.1, 171.8, 171.6, 171.5, 171.4, 171.2, 168.6, 166.0, 150.3, 150.3, 149.6, 143.5, 136.5, 136.5, 136.5, 130.1, 128.4, 128.0, 127.9, 127.7, 127.6, 126.6, 126.1, 118.9, 118.8, 118.6, 118.5, 113.8, 113.7, 111.6, 111.5, 110.5, 110.2, 110.0, 71.8, 65.4, 62.2, 58.4, 57.8, 55.9, 55.8, 54.1, 53.8, 51.9, 51.7, 48.7, 42.3, 41.2, 41.1, 35.6, 31.7, 31.1, 31.0, 29.5, 29.5, 29.4, 29.3, 29.3, 29.2, 29.1, 29.0, 28.5, 28.4, 28.2, 27.0, 25.6, 24.1, 23.1, 23.1, 22.5, 22.0, 21.9, 19.6, 19.4, 19.2, 18.4, 17.8, 16.9, 15.6, 14.4. HRMS: calcd for $C_{177}H_{220}N_{32}O_{34}$ $[M+2H]^{2+}$ 1669.3252, found 1669.3451.

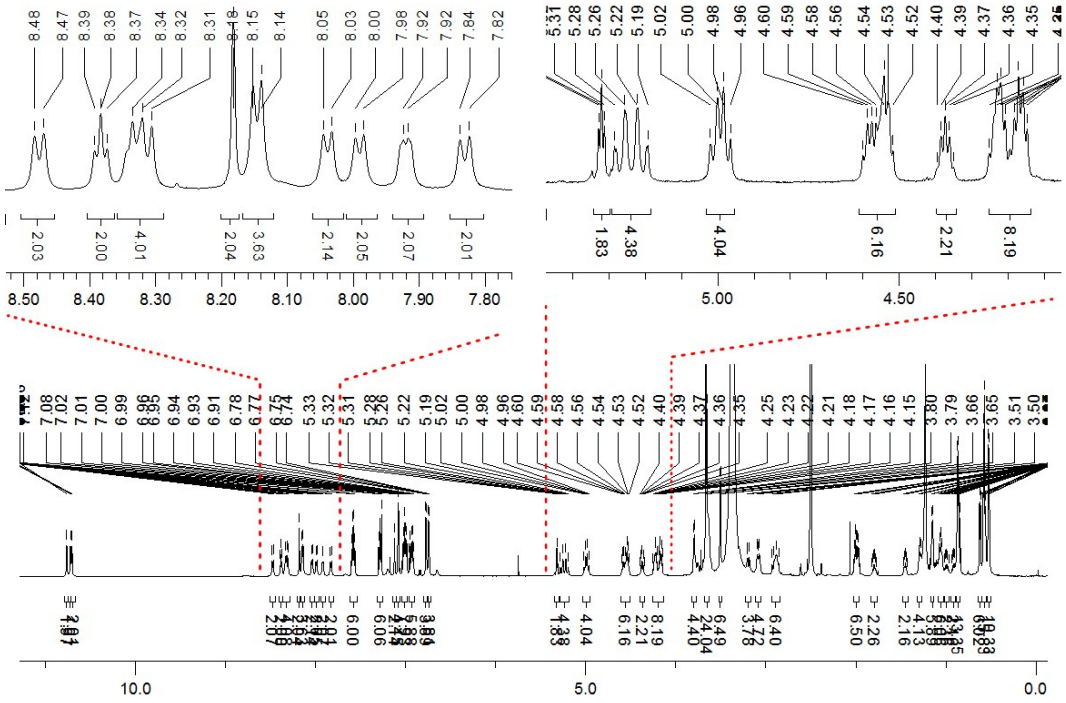


Fig. S21. ^{1}H NMR spectrum of **2** in DMSO-*d6*.

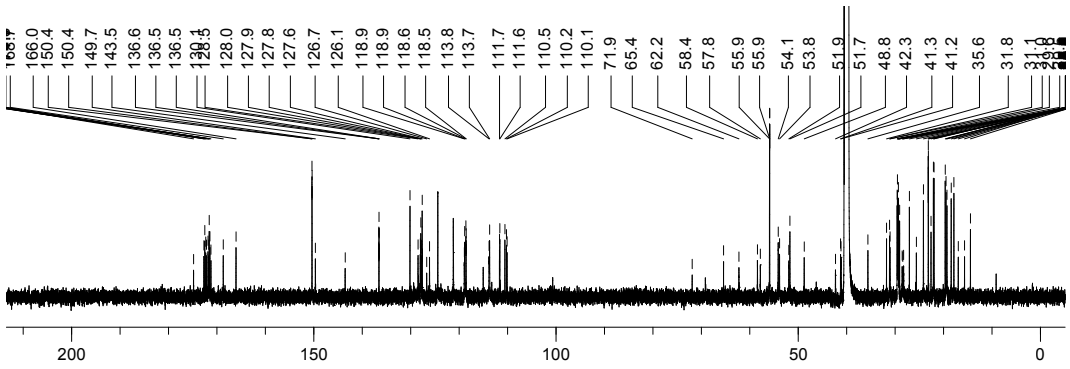


Fig. S22. ^{13}C NMR spectrum of **2** in DMSO-*d6*.

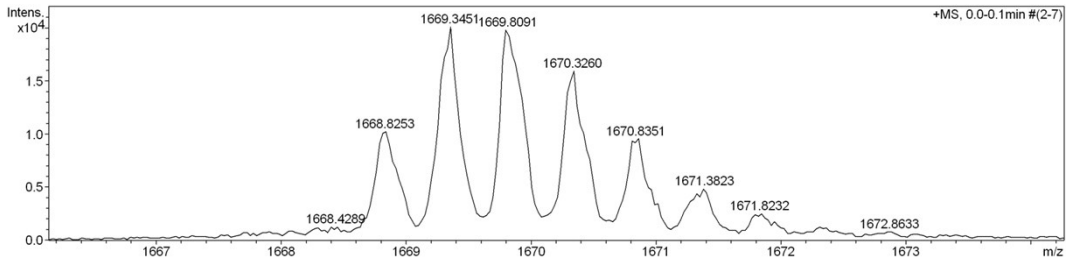


Fig. S23. HR-MS of **2**.

Compound 3. This compound was synthesized from **10** and **11c** according to the same procedure with compound **1**.

3: Yield: 48%. ^{1}H NMR (DMSO-*d6*, 600MHz) δ: 10.79 (s, 2H), 10.76 (s, 2H), 10.71 (s, 2H), 8.51 (d, 2H), 8.42 (t, 2H), 8.36-8.33 (m, 4H), 8.20 (br, 6H), 8.07-8.06 (m, 2H), 8.00-7.89 (m, 8H), 7.81 (d, 2H), 7.59 (d, 4H), 7.55 (d, 2H), 7.30-7.26 (m, 6H), 7.12 (s, 2H), 7.08 (s, 4H), 7.04-6.99 (m, 8H), 6.96-6.90 (m, 6H), 6.78 (d, 4H), 6.75 (d, 4H), 5.33-5.21 (m, 6H), 5.02-4.97 (m, 4H), 4.59-4.52 (m, 6H), 4.41-4.39 (m, 2H), 4.32-4.30 (m, 4H), 4.23 (br, 4H), 4.18-4.15 (m, 4H), 3.80 (d, 4H), 3.67-3.66 (m, 24H), 3.52 (d, 6H), 3.19 (d, 2H), 3.10-3.08 (m, 4H), 2.92-2.86 (m, 6H), 2.03-1.98 (m, 8H), 1.80-1.75 (m, 2H), 1.29-1.27 (m, 2H), 1.17 (d, 6H), 1.10-1.04 (m, 10H), 0.98-0.94 (m, 2H), 0.88 (br, 12H), 0.85-0.83 (m, 4H), 0.80-0.78 (m, 24H), 0.62 (d, 6H), 0.57-0.53 (m, 22H), 0.50 (d, 6H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 172.6, 172.4, 172.2, 172.1, 171.8, 171.5, 171.4, 171.3, 171.2, 168.5, 166.0, 159.2, 158.6, 158.4, 150.3, 150.3, 149.6, 143.5, 136.6, 136.5, 136.5, 130.1, 128.4, 128.0, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 126.0, 124.3, 121.1, 119.7, 118.9, 118.8, 118.5, 118.5, 118.5, 115.0, 113.8, 113.6, 111.6, 111.6, 111.5, 110.5, 110.2, 110.0, 62.2, 58.4, 58.1, 58.0, 57.9, 55.9, 55.8, 55.8, 54.1, 54.1, 53.8, 51.9, 51.7, 48.7, 42.3, 41.2, 41.1, 41.0, 40.9, 35.6, 31.7, 31.3, 31.1, 31.0, 30.8, 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 28.4, 28.3, 28.2, 27.0, 25.5, 24.1, 24.0, 23.0, 22.5, 22.1, 21.9, 19.8, 19.7, 19.6, 19.4, 19.3, 18.4, 18.1, 18.0, 14.4. HRMS: calcd for $C_{197}H_{256}N_{36}O_{38}$ $[M+2H]^{2+}$ 1867.9637, found 1867.9733.

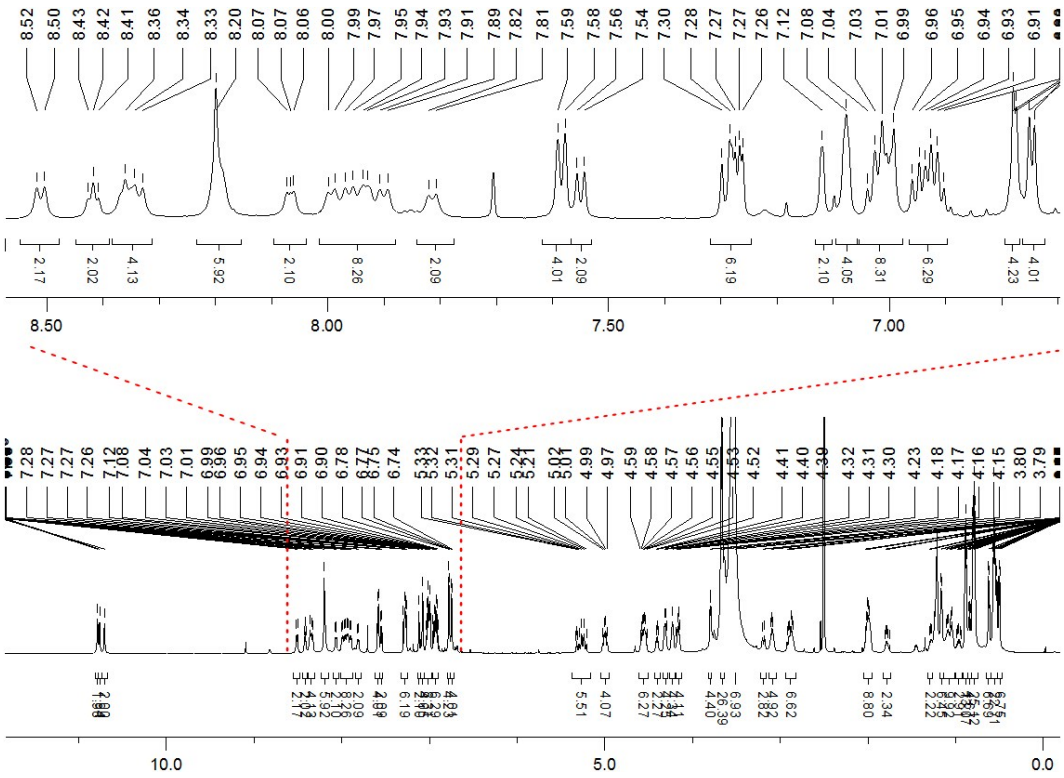


Fig. S24. ^{1}H NMR spectrum of **3** in DMSO-*d6*.

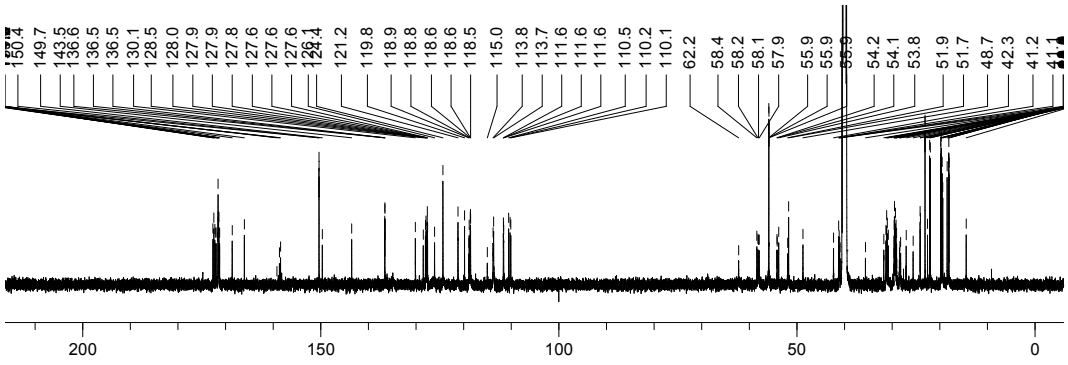


Fig. S25. ^{13}C NMR spectrum of **3** in DMSO-*d6*.

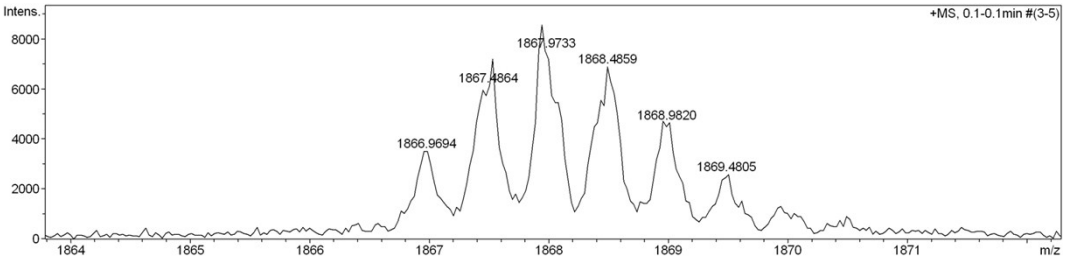


Fig. S26. HR-MS of **3**.

Compound 4. This compound was synthesized from **10** and **11d** according to the same procedure with compound **1**.

4: Yield: 74%. ^{1}H NMR (DMSO-*d6*, 600MHz) δ: 12.56 (br, 2H), 10.76 (br, 4H), 10.68 (s, 2H), 8.51 (d, 2H), 8.37 (br, 4H), 8.32 (br, 2H), 8.19 (s, 2H), 8.18-8.15 (m, 4H), 8.04 (br, 2H), 7.95-7.91 (m, 8H), 7.81 (br, 2H), 7.73 (br, 2H), 7.58 (d, 4H), 7.54 (d, 2H), 7.30-7.26 (m, 6H), 7.11 (s, 2H), 7.08 (s, 4H), 7.04-6.99 (m, 8H), 6.96-6.89 (m, 6H), 6.78 (d, 4H), 6.75 (d, 4H), 5.33-5.21 (m, 4H), 5.02-4.97 (m, 4H), 4.55-4.53 (m, 6H), 4.31-4.17 (m, 18H), 3.79-3.75 (m, 6H), 3.70-3.64 (m, 30H), 3.52 (d, 6H), 3.21-3.16 (m, 2H), 3.09 (br, 4H), 2.90-2.88 (m, 6H), 2.03-1.94 (m, 8H), 1.81 (br, 2H), 1.57-1.52 (m, 2H), 1.46-1.44 (m, 4H), 1.22 (s, 4H), 1.20 (d, 6H), 1.16 (d, 6H), 1.09 (br, 8H), 0.96 (br, 2H), 0.89 (d, 12H), 0.84 (d, 8H), 0.81-0.77 (m, 28H), 0.63 (d, 6H), 0.57-0.52 (m, 28H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 172.7, 172.6, 172.2, 172.1, 171.4, 171.4, 171.2, 168.8, 166.1, 150.3, 150.3, 149.6, 136.5, 136.5, 130.1, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 126.1, 124.3, 121.1, 121.1, 118.9, 118.8, 118.5, 118.5, 113.8, 113.6, 111.6, 111.5, 62.2, 58.5, 58.2, 57.9, 55.9, 55.8, 54.1, 51.9, 51.7, 51.6, 49.0, 48.9, 42.2, 41.0, 31.2, 31.0, 29.5, 29.5, 29.3, 29.1, 29.0, 27.0, 24.7, 24.1, 23.4, 23.0, 22.1, 22.0, 21.7, 19.8, 19.7, 19.6, 19.5, 18.8, 18.6, 18.4, 18.1, 18.0, 14.4. HRMS: calcd for $C_{215}H_{288}N_{40}O_{42}$ $[M+2H]^{2+}$ 2052.0849, found 2052.1042.

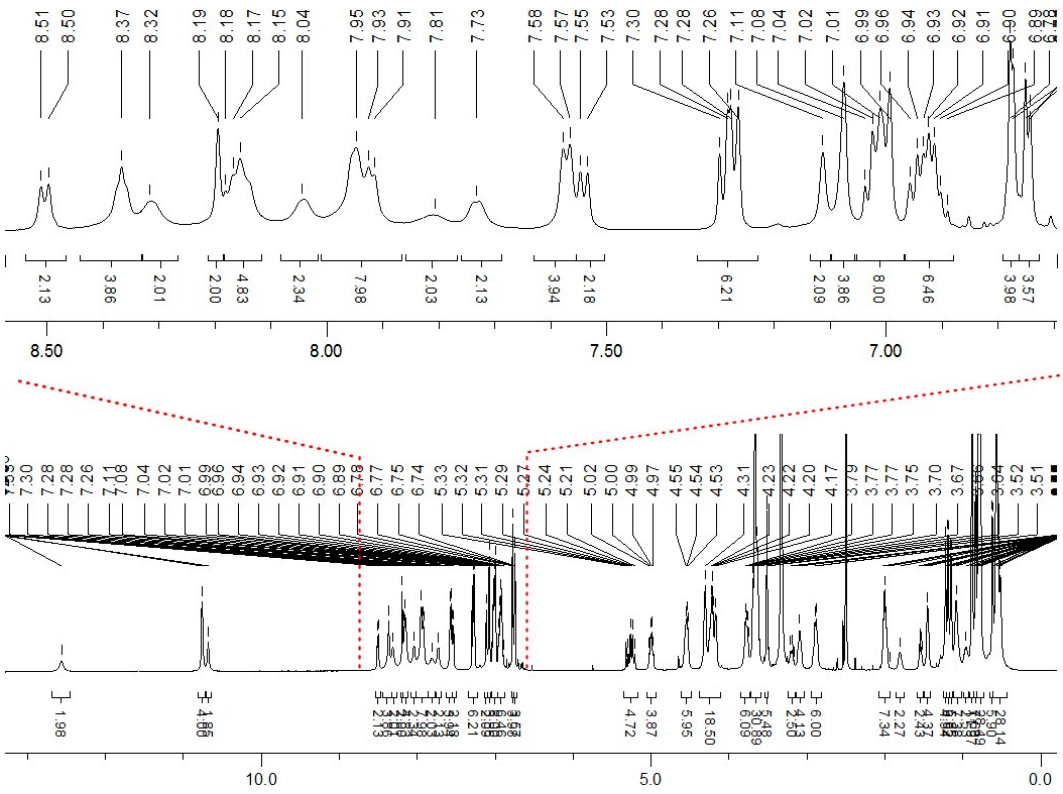


Fig. S27. ^{1}H NMR spectrum of **4** in DMSO-*d6*.

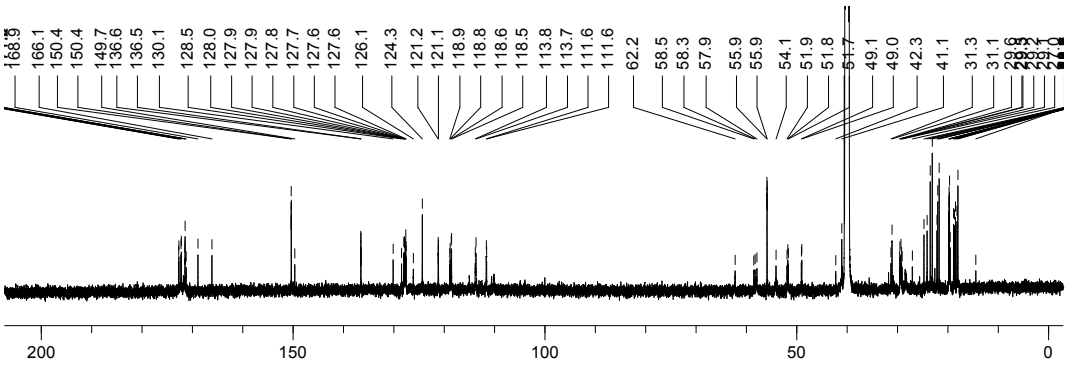


Fig. S28. ^{13}C NMR spectrum of **4** in DMSO-*d6*.

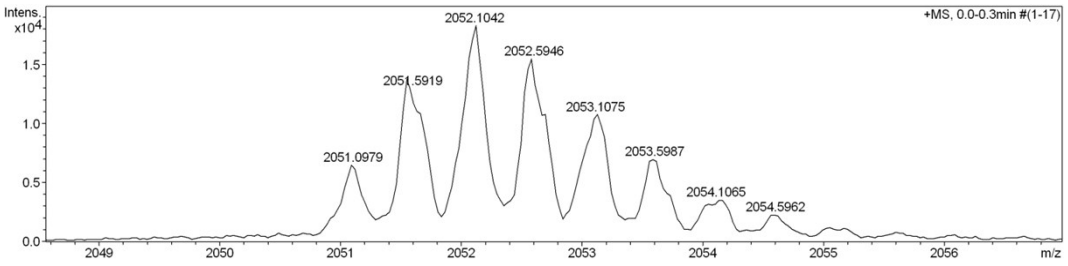


Fig. S29. HR-MS of **4**.

Compound 5. This compound was synthesized from **10** and **11e** according to the same procedure with compound **1**.

5: Yield: 61%. ^{1}H NMR (DMSO-*d6*, 600MHz) δ: 12.55 (br, 2H), 10.76 (br, 6H), 10.69 (s, 2H), 8.51 (d, 2H), 8.37 (br, 6H), 8.19-8.14 (m, 10H), 7.99-7.93 (m, 12H), 7.77-7.74 (m, 4H), 7.58-7.52 (m, 8H), 7.29-7.27 (m, 8H), 7.11 (s, 2H), 7.08 (s, 6H), 7.03-7.00 (m, 10H), 6.95-6.89 (m, 8H), 6.78 (br, 4H), 6.75 (d, 4H), 5.33-5.21 (m, 4H), 5.03-4.97 (m, 4H), 4.2 (br, 8H), 4.30-4.15 (m, 20H), 3.79-3.75 (m, 6H), 3.70-3.64 (m, 30H), 3.52 (d, 6H), 3.25-3.21 (m, 2H), 3.17-3.12 (m, 6H), 2.90 (br, 8H), 2.03-1.97 (m, 6H), 1.81-1.77 (m, 2H), 1.56-1.52 (m, 2H), 1.46 (br, 4H), 1.22-1.12 (m, 28H), 0.89-0.77 (m, 52H), 0.63-0.51 (m, 46H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ 172.7, 172.6, 172.3, 172.2, 171.8, 171.6, 171.5, 171.4, 171.2, 168.8, 166.1, 150.3, 149.6, 136.5, 136.5, 130.1, 128.4, 128.0, 127.9, 127.7, 127.6, 127.5, 124.3, 124.3, 124.2, 121.1, 118.7, 118.5, 113.7, 113.6, 111.6, 111.5, 110.6, 110.2, 110.0, 62.2, 58.5, 58.3, 58.1, 57.9, 55.9, 55.8, 54.2, 51.9, 51.6, 49.1, 49.0, 42.2, 41.0, 40.9, 31.2, 31.0, 30.9, 29.5, 29.4, 29.3, 29.1, 28.3, 28.0, 27.0, 24.7, 24.1, 23.4, 23.0, 22.5, 22.2, 22.1, 21.9, 21.7, 19.7, 19.7, 19.6, 19.5, 18.8, 18.6, 18.4, 18.2, 18.0. HRMS: calcd for $C_{249}H_{329}KN_{46}O_{46}$ $[M+H+K]^{2+}$ 2370.2262, found 2370.2216.

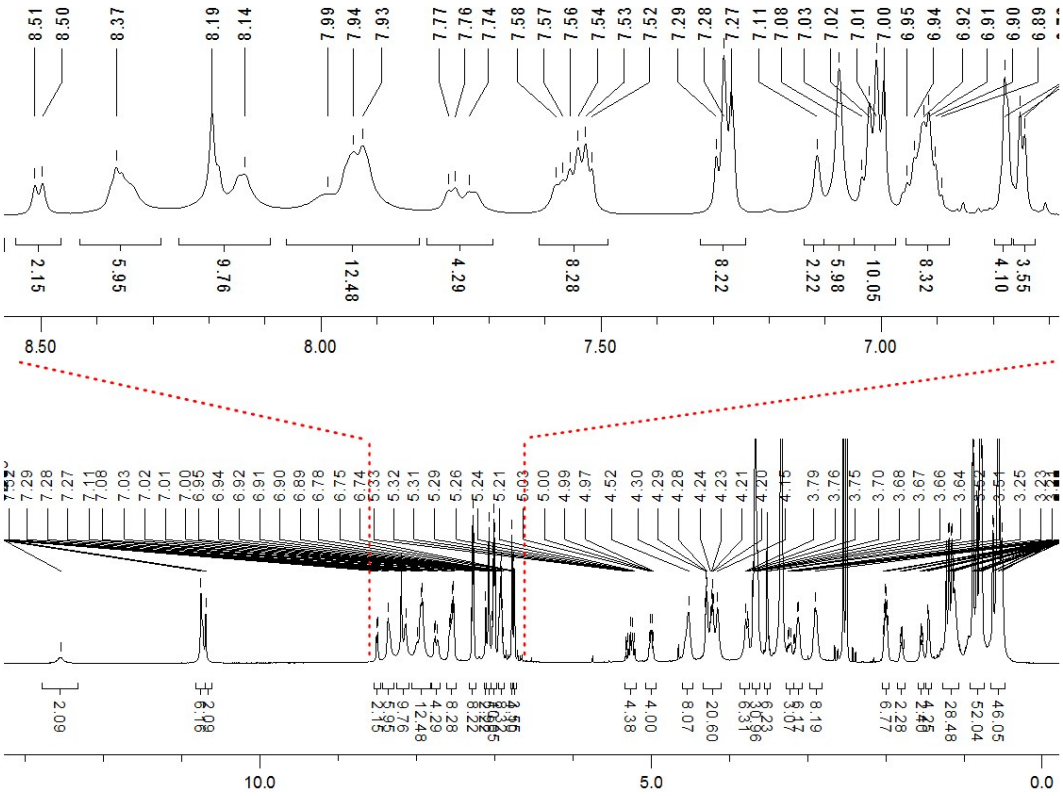


Fig. S30. ^{1}H NMR spectrum of **5** in DMSO-*d6*.

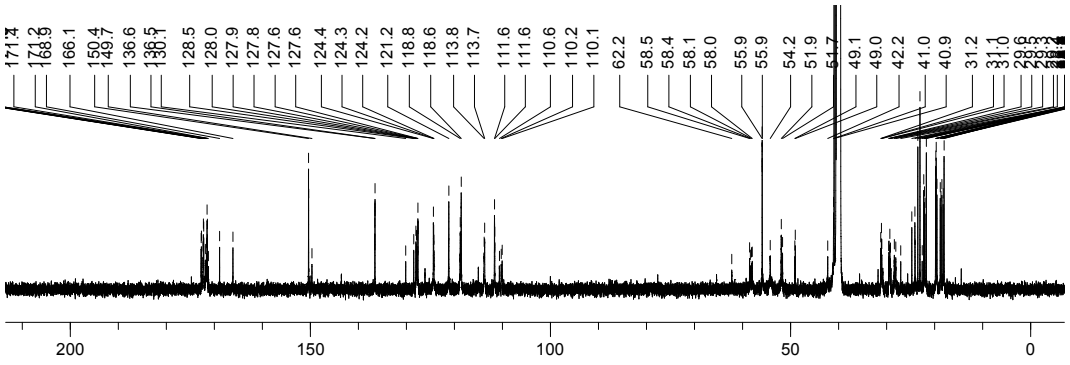


Fig. S31. ^{13}C NMR spectrum of **5** in DMSO-*d6*.

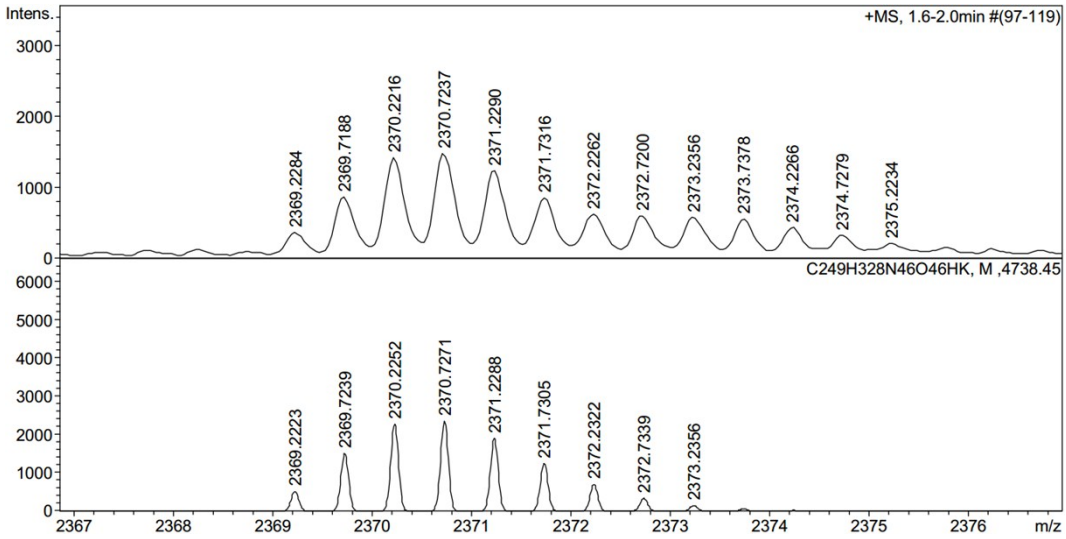


Fig. S32. HR-MS of **5**.

3. Procedures for proton and chloride transport experiments:[4]

Preparation of HPTS containing large unilamellar vesicles (LUVs): EYPC (15 mg, 20 μmol) in EtOH (0.15 mL) was diluted with EtOH (5.0 mL), the solution was evaporated under reduced pressure, and the resulting thin film was dried under high vacuum for 3 h. The lipid film was hydrated with HEPES buffer solution (1.5 mL, HEPES (10 mM), KCl (100 mM), pH = 7.2) containing HPTS (0.1 mM) at 40 °C for 2 h to give a milky suspension. The resulting suspension was subjected to ten freeze-thaw cycles by using liquid N_2 to freeze and warm water bath to thaw. The suspension was dialyzed with membrane tube (MWCO = 14000) against the same HEPES buffer solution (200 mL, without HPTS) for six times to remove un-entrapped HPTS and produce vesicle suspension ([lipid] = 13.3 mM).

Fluorescent experiments: HEPES buffer solution (2.0 mL, HEPES (10 mM), KCl (100 mM), pH = 6.0) and the prepared vesicle suspension (13.3 mM, 100 μL) were placed in a fluorimetric cuvette. To the cuvette, the solution of compound **1-5** in DMSO (5 μL) was added to reach a required channel concentration (molar ratio

relative to lipid, represented by x) with gentle stirring. Fluorescent intensity (I_t) was continuously monitored at 510 nm (excitation at 460 nm) in 10 min. Then, Triton aqueous solution (50%, 10 μL) was added with gentle stirring. The intensity was monitored until the fluorescent intensity (I_∞) did not change. The collected data were then normalized into the fractional change in fluorescence given by $(I_t\text{-}I_0)/(I_\infty\text{-}I_0)$, where I_0 is the initial intensity.

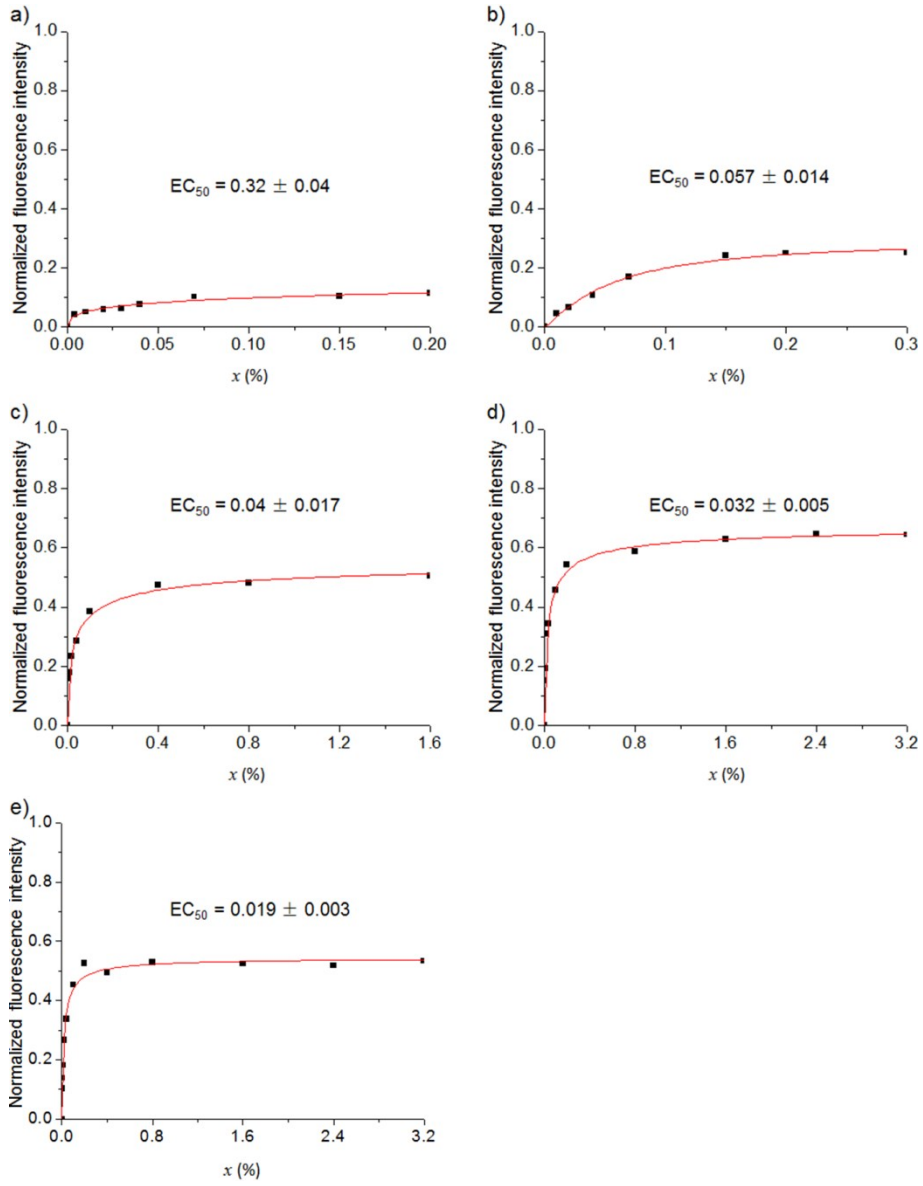


Fig. S33. Changes in normalized fluorescent intensity of HPTS (λ_{ex} = 460 nm, λ_{em} = 510 nm) in vesicles with the concentration of **1**-**5** (molar ratio relative to lipid, represented by x). By fitting the plot with Hill equation, the effective concentration needed for 50% activity (EC_{50}) for **1**-**5** was determined to be 0.32% (**1**), 0.057% (**2**), 0.04% (**3**), 0.032% (**4**) and 0.019% (**5**), respectively.

Preparation of LG containing large unilamellar vesicles (LUVs): EYPC (15 mg,

20 μmol) in $CHCl_3$ (0.15 mL) was diluted with $CHCl_3$ (5.0 mL), the solution was evaporated under reduced pressure, and the resulting thin film was dried under high vacuum for 3 h. The lipid film was hydrated with Mes buffer solution (1.5 mL, Mes (10 mM), K_2SO_4 (71 mM), KOH, pH = 6.2) containing lucigenin (LG, 2 mM) at 40 ^oC for 2 h to give a milky suspension. The resulting suspension was subjected to ten freeze-thaw cycles by using liquid N_2 to freeze and warm water bath to thaw. The suspension was dialyzed with membrane tube (MWCO = 14000) against the same Mes buffer solution (200 mL, without LG) for six times to remove un-entrapped LG and produce vesicle suspension ([lipid] = 13.3 mM).

Fluorescent experiments: Mes buffer solution (2.0 mL, Mes (10 mM), K_2SO_4 (71 mM), KOH, pH = 6.2), the prepared vesicle suspension (13.3 mM, 100 μL) and KCl solution (60 μL, 3.2M) were placed in a fluorimetric cuvette. To the cuvette, the solution of compound **1-5** in DMF (5 μL) was added to reach a required channel concentration (molar ratio relative to lipid, represented by x) with gentle stirring. Fluorescent intensity (I_t) was continuously monitored at 503 nm (excitation at 372 nm) in 10 min. Then, Triton aqueous solution (50%, 10 μL) was added with gentle stirring. The intensity was monitored until the fluorescent intensity (I_∞) did not change. The collected data were then normalized into the fractional change in fluorescence given by $(I_t\text{-}I_0)/(I_\infty\text{-}I_0)$, where I_0 is the initial intensity.

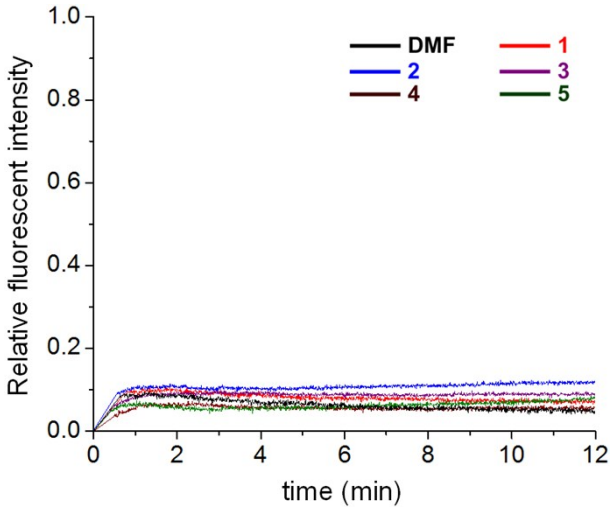


Fig. S34. Changes in the fluorescence intensity of LG (λ_{ex} = 372 nm, λ_{em} = 503 nm) in vesicles with time after the addition of **1-5** (x = 0.4%).

4. Procedures for planer lipid bilayer conductance experiments:[5]

The solution of diPhyPC in chloroform (10 mg/ml, 20 μL) was evaporated with nitrogen gas to form a thin film and re-dissolved in *n*-decane (5 μL). The lipid solution (0.5 μL) was injected on to the aperture (diameter = 200 μm) of the Delrin®

cup (Warner Instruments, Hamden, CT) and then evaporated with nitrogen gas. In a typical experiment for measurement of the channel conductance for an ion, the chamber (*cis* side) and the Delrin cup (*trans* side) were filled with aqueous MCl solution (1.0 M, 1.0 mL, M = Cs^+, Rb^+, K^+ or Na^+). Ag-AgCl electrodes were applied directly to the two solutions and the *cis* one was grounded. Planar lipid bilayer was formed by painting the lipids solution (1.0 μL) around the pretreated aperture and by judgment of capacitance (80-120 pF). Membrane currents were measured using a Warner BC-535D bilayer clamp amplifier and were collected by PatchMaster (HEKA) with sample interval at 5 kHz and then filtered with a 8-pole Bessel filter at 1 kHz (HEKA). The data were analyzed by FitMaster (HEKA) with a digital filter at 100 Hz.

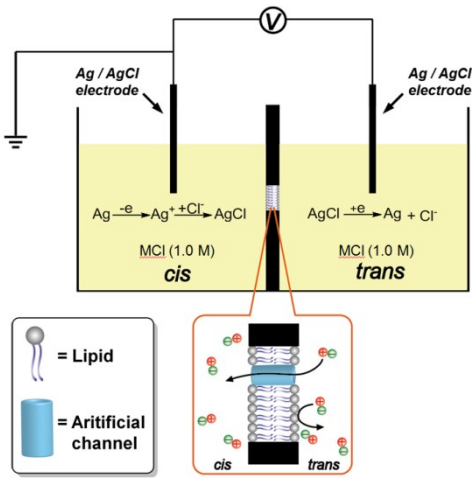


Fig. S35. Schematic representation for the patch clamp experiments with planar lipid bilayer. The redox reactions on both Ag/AgCl electrodes are inserted to illustrate the nature of charge balance during M^+ transmembrane transport.

For the single-channel conductance measurement, two chambers were charged with KCl (1 M, 1 mL). And the solution of compound **1-5** in DMSO (1 mM, 0.2 μL) was added to the *cis* compartment and the solution was stirred for 5 min.

For the measurement of the transport selectivity of K^+ over Cl^-, the KCl solutions (0.2M and 1M) were added to the both side of the bilayer (diPhyPC), *trans* chamber: KCl (1.0 M), *cis* chamber: KCl (0.2 M). The solution of compound **1-5** in DMSO (1 mM, 0.2 μL) was added to the *cis* compartment and the solution was stirred for 5 min.

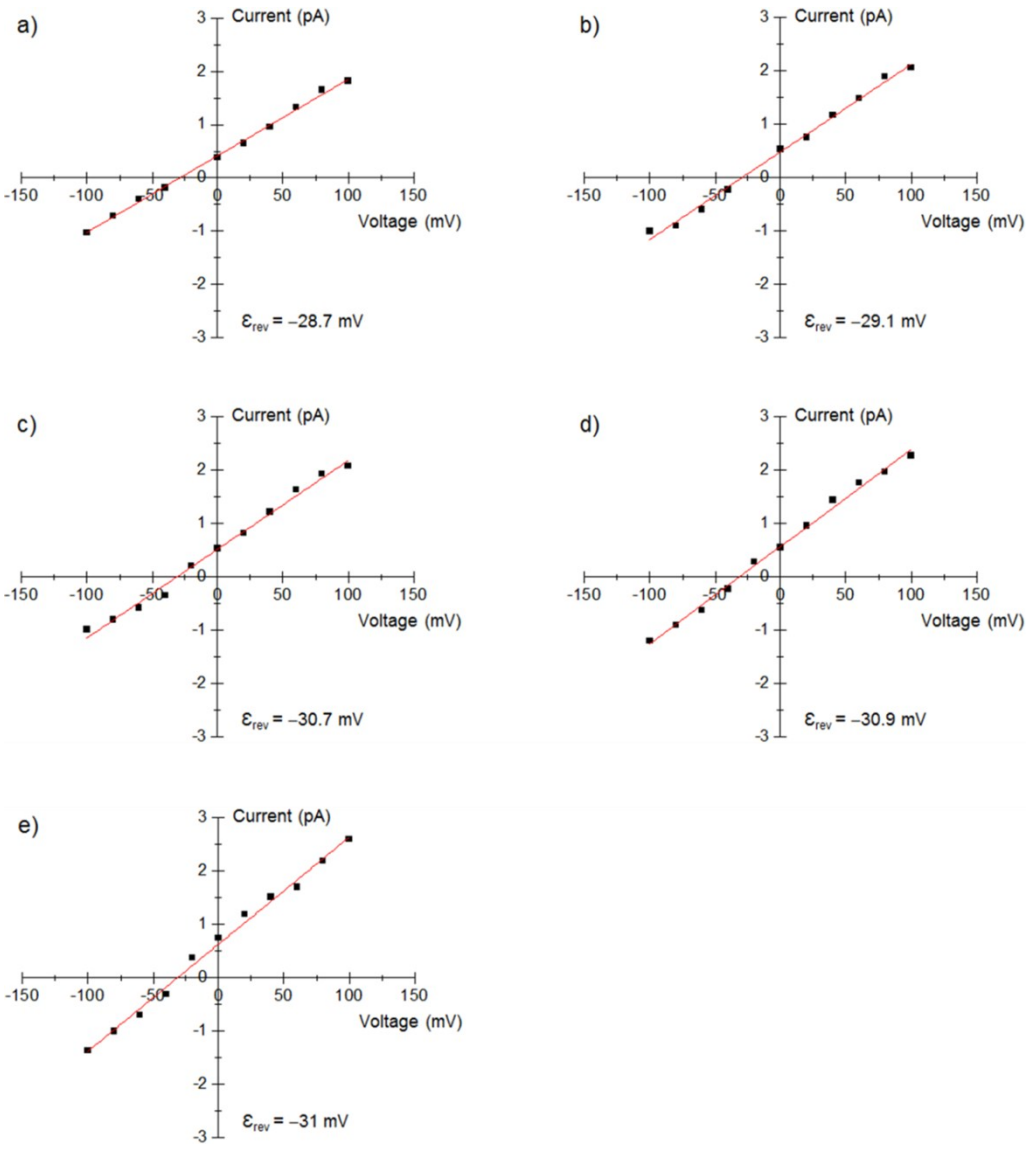


Fig. S36. *I–V* plots of **1-5** by using unsymmetrical solution at both side of the bilayer. *trans* chamber: KCl (1.0 M), *cis* chamber: KCl (0.2 M). (a) **1**, (b) **2**, (c) **3**, (d) **4** and (e) **5**.

5. Molecular modelling studies:

The initial conformation of the helical dimer of gramicidin A and 1,4-dimethoxypillar[5]arene were taken from the PDB database (PDB ID: 1MAG)[6] and experimental X-ray crystallographic data,[7] respectively. A pre-equilibrated DMPC bilayer with 128 lipids was downloaded from CHARMM-GUI.[8] The molecular dynamics simulation was performed in NAMD 2.9 using the CHARMM 36 force field.[9] The cgenff.paramchem.org server was used to construct the topology and RESP charges of 1,4-dimethoxypillar[5]arene. The van der Waals cut-off distance was 12 Å with a switching distance of 10 Å. The particle mesh Ewald was used for computing the Coulomb interactions under the periodic boundary condition.

The 1,4-dimethoxypillar[5]arene and gA-analogous peptides were connected by linkers and then minimized to obtain a reasonable model for the channel **5 (**Fig. S36**)**. Then, the optimized tubular model was inserted into a pre-equilibrated DMPC bilayer with 128 lipids, and the lipids overlapping **5** were removed. After solvation, the system was neutralized and set a KCl concentration of 0.15 M. The final system includes the **5** tubular model, 112 DMPC lipids, 4457 TIP3 water molecules, 15 K^+ and 13 Cl^-. The initial configuration was energy-minimized at two stages: with all atoms fixed except the alkyl tails of lipids; with heavy atoms of peptide side chains and phenyl groups in pillar[5]arene restrained. Then, a 300-ps equilibration was performed with the backbone of peptide and heavy atoms in pillar[5]arene restrained, which allowed the lipids and the water molecules to relax around tubular model after its insertion. The simulation was continued for 10 ns with only heavy atoms in pillar[5]arene restrained at constant pressure (1 bar) and constant temperature (310 K), at a 2-fs time-step. The tubular model was very stable during the 10-ns simulation, and the two peptides adopted helical conformations from beginning to end.

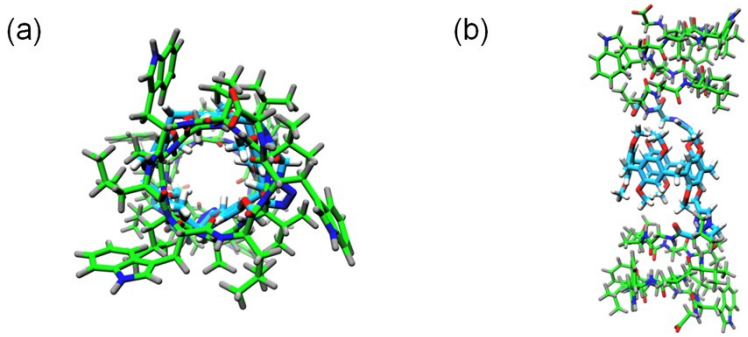


Fig. S37. (a) Top view and (b) side view of the optimized structures of channel **5**.

6. Procedures for CD experiments:[10]

All experiments were done using large unilamellar vesicles (LUVs) of EYPC containing 6% (mol/mol) channels. In general, 854 nmol of EYPC in ethanol was mixed with 51.2 nmol of channel **1** in methanol. The mixture was evaporated under reduced pressure, and the resulting thin film was dried under high vacuum for 3 h. Then, the lipid film was hydrated with buffer solution (1.5 mL, Sodium phosphate (10 mM), NaCl (150 mM), pH = 7.2) at 40 ^oC for 2 h to give a milky suspension. The suspension was subjected to ten freeze-thaw cycles by using liquid N_2 to freeze and warm water bath to thaw. The resulting samples were incubated at 65 ^oC for 12 h with continuous shaking followed by centrifugation. The collected vesicles were re-suspended in the buffer (1.5 mL) and incubate in dark at room temperature for 1 h. CD measurements were performed at room temperature with a Chirascan spectropolarimeter by using 1-cm-pathlength quartz cuvettes. All spectra were recorded in the range of 202-280 nm with 1 nm wavelength increments.

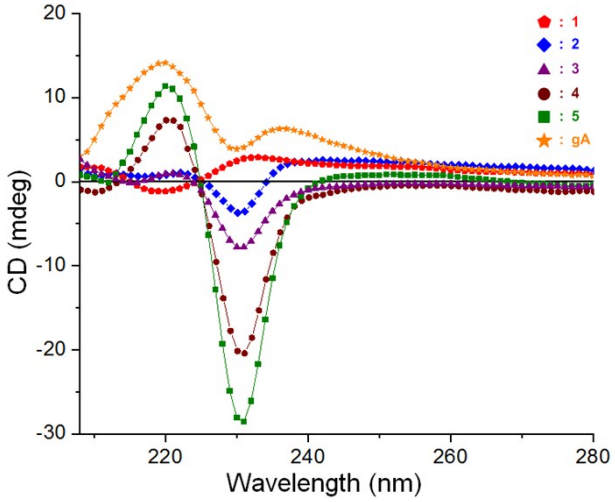


Fig. S38. CD spectra of **1-5** and gA in the lipid bilayer of vesicles.

The CD spectrum of gA displayed two characteristic peaks of positive ellipticity at 220 and 238 nm, and a minimum at 230 nm, which are consistent with the reported single-stranded β-helical conformation of gA dimer (Fig. S36).[11] Under the same conditions, **2-5** showed positive ellipticity at 220 nm, corresponding to the β-helical conformation of the amide backbone of the peptide chains.[12] The weak CD spectral features of **1** may be attributed to the 8-residue side chains, which merely form 1 turn helical structure. There may be a dynamic shift between the left-handed and right-handed helical structures of peptide side chains, which resulted in a weak CD spectral feature of **1**.

7. Procedures for the measurement of antimicrobial activity:[13]

B.subtilis (ATCC6633), *S. Epidermidis* (ATCC 12228), *S. aureus* (ATCC 25923) and *E. coli* (BL21) from a single colony were grown overnight in LB broth at 37 ºC with agitation. An aliquot was taken and diluted in fresh broth and cultured until the bacteria reach mid-logarithmic phase (OD595 ≈ 0.5). The bacteria were diluted to a concentration of 5×10^5 CFU/ml. Then, 200 μL bacterial suspension was added to each well of a sterile 96-well plate. 2.5 μL DMSO solutions of serial dilution of the channels were added to the wells in triplicates. The plates were shaken for 30 seconds and then incubated at 37 ºC overnight before the absorbance at 595 nm was monitored using a microtiter plate reader. The antimicrobial activities of the channels were normalized as bacteria survival (%) = $(OD_{sample+bac} - OD_{broth\ only}) / (OD_{DMSO+bac} - OD_{broth\ only}) \times 100\%$. The channel concentration required to inhibit 50% bacteria growth (IC_{50}) was read out directly from the graph.

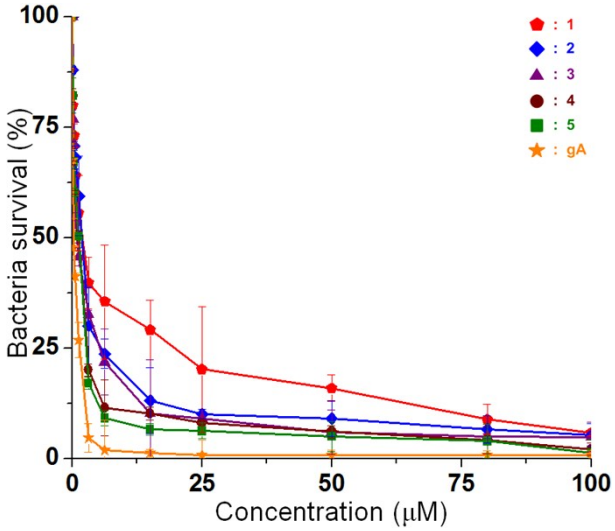


Fig. S39. Antimicrobial activity of **1-5** and gA against Gram-positive bacteria *B.subtilis*.

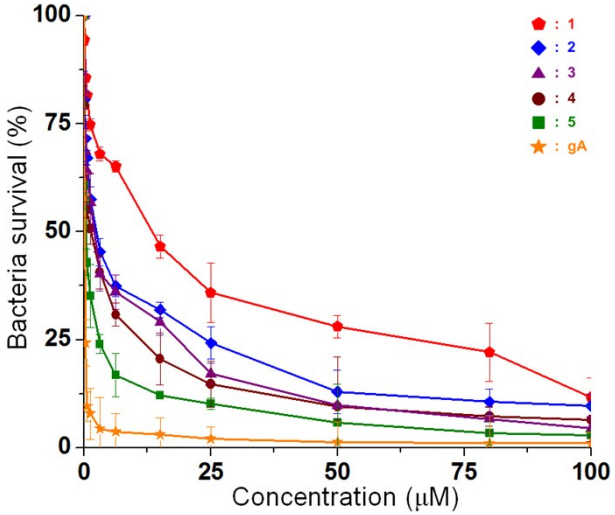


Fig. S40. Antimicrobial activity of **1-5** and gA against Gram-positive bacteria *S. Epidermidis*.

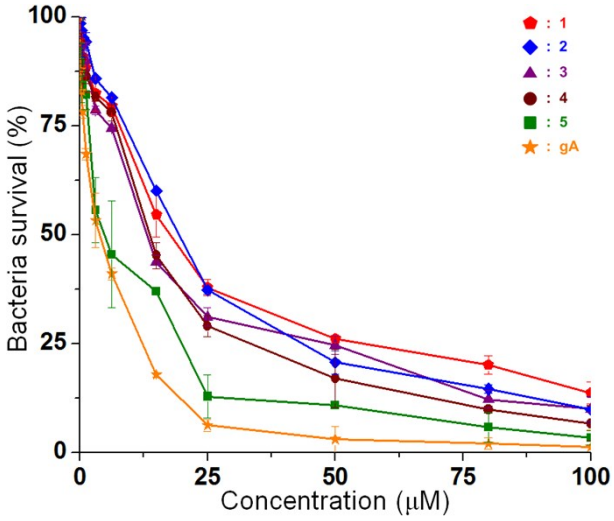


Fig. S41. Antimicrobial activity of **1-5** and gA against Gram-positive bacteria *S.aureus*.

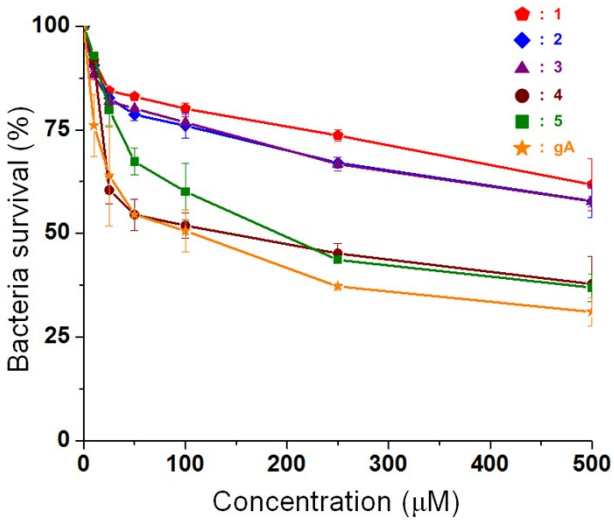


Fig. S42. Antimicrobial activity of **1-5** and gA against Gram-negative bacteria *E. coli*.

8. Procedures for the measurement of haemolytic toxicity:[13]

The haemolytic activity of the channels was determined using rat red blood cells (rRBCs). The rRBCs were isolated from fresh Sprague Dawley rat blood by centrifugation at 3500 rpm for 5 min and then washed with PBS buffer until the supernatant was clear. The rRBCs were then resuspended and diluted to a final concentration of 1% (v/v) in PBS and used immediately. To each well of sterile 96-well plate, 200 μL of the erythrocytes suspension was added. And then 2.5 μL of serial dilution of channels in DMSO or DMSO alone were added to the wells in triplicates to reach the required concentration. The plate was gently shaken and then incubated at 37 ºC for 30 min, followed by centrifugation at 3500 rpm for 10 min. Aliquots (50 μL) of the supernatant were transferred into a new sterile 96-well plate

containing 50 μL of PBS buffer in each well, and the absorbance was measured at 562 nm by a microtiter plate reader. The Percentage haemolysis was calculated as follows: $(A_{sample} - A_{DMSO}) / (A_{triton\ X\text{-}100} - A_{DMSO}) \times 100\%$. The complete haemolysis was achieved by mixing the erythrocytes with 1% Triton X-100. The channel concentration required to cause 50% haemolysis (HC_{50}) was read out directly from the graph.

9. Procedures for the stability assays:[14]

The trypsin stability of the channels was evaluated using viability assays. In brief, *S. Epidermidis* (ATCC 12228) was cultured to mid-logarithmic phase (OD595 ≈ 0.5) and diluted to 5×10^5 CFU/ml in the LB broth. Then, 200 μL bacterial suspension was added to each well of a sterile 96-well plate. Channels **1-5** (2 mM) were diluted in phosphate-buffered saline (PBS) and incubated with trypsin (2 mg/mL) for 1 or 6 h at 37 °C; subsequently, the enzymatic activity was terminated at 60 °C for 20 min. Finally, trypsin-treated samples were mixed with 5-μL DMSO and then incubated with *S. Epidermidis* at the final concentration of 25 μM in the 96-well plate overnight at 37 °C. The growth inhibitory effect was determined at 595 nm by measuring the absorbance. The bacteria survival (%) was calculated as the following: $(A_{sample+bac} - A_{broth\ only}) / (A_{DMSO+bac} - A_{broth\ only}) \times 100\%$. Data are the average of three independent experiments.

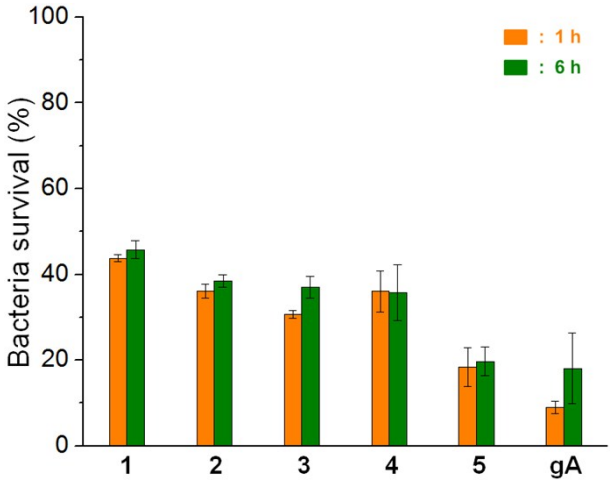


Fig. S43. The viability of bacteria incubated with trypsin-treated **1-5** and gA. Each bar indicates the mean ± SD of triplicate assays.

As shown in Fig. S43, the antimicrobial activity of **1-5** still remained after incubation with trypsin even at concentration 2 mg/mL for 6 h, while gA lost part of antimicrobial activity. From above results, we could cautiously conclude that the channels **1-5** might resistant to degradation by trypsin. Considering that the D-amino acid substitution is a potential strategy to improve the stability of peptides,[15] the

stability of **1**-**5** to trypsin might be due to their 'D-rich' peptide side chains.

10. References:

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