Supporting information for

Dissociation of Haemolytic and Oligomer-Preventing Activities of Gramicidin S Derivatives Targeting Amyloid-β N-Terminus
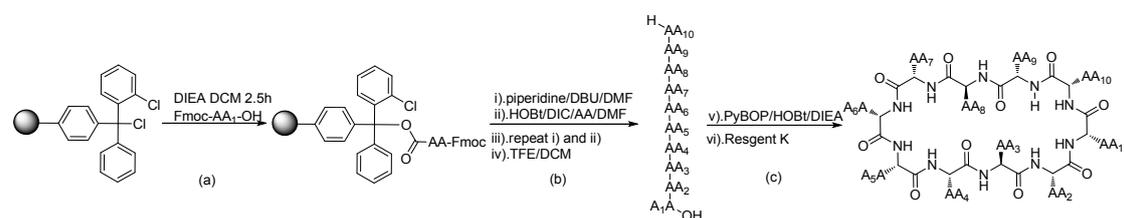
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1. Supplementary Results

Supplementary Scheme S1: General synthetic approach for the linear peptide and the cyclization of Gramicidin S derivatives. Reagents and conditions: *a*, Fmoc-AA-OH, DIEA/CH_2Cl_2, 2.5 h; *b*, Standard Fmoc/tBu SPPS. i) Deprotection: 5% piperidine, 2% DBU in DMF, 30 min; ii) coupling: Fmoc-amino acid, DIC, HOBt, 2.5h; iv) Cleavage from resin: 20% TFE in DCM; *c*, v) Cyclization: PyBOP/HOBt/DIEA (3:3:5 equiv); vi) Deprotection: CF_3COOH:phenol:i-Pr_3SiH:H2O = 88:5:5:2, 2 h.
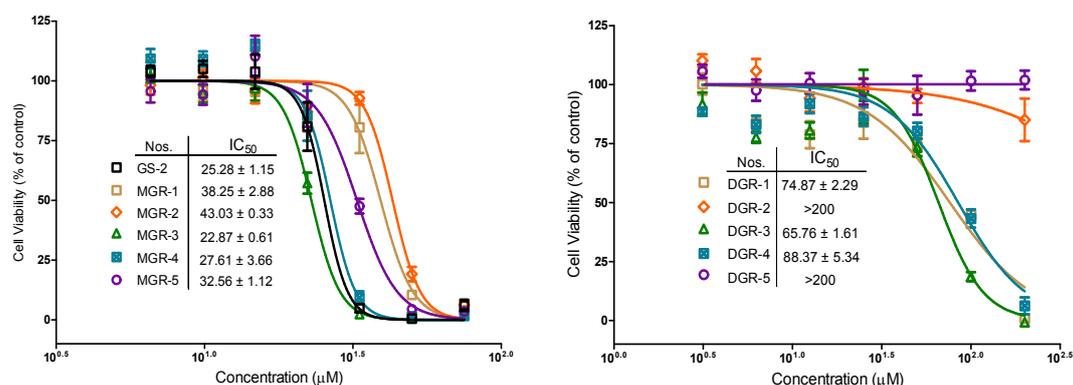

Figure S1: MTT toxicity assay of GS-2 and its analogues towards PC12 cells. Results are expressed as a percentage of control (untreated) cells with mean ± SD (n = 4).
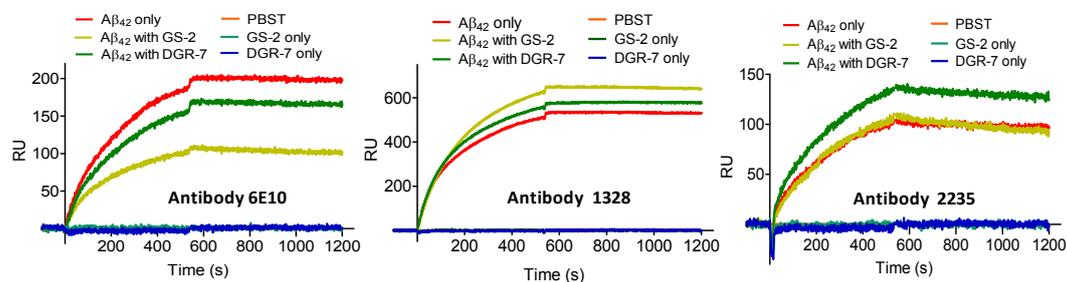

Figure S2: SPR analysis of binding selectivity of DGR-7 and GS-2 towards equivalent $A\beta_{42}$ using immobilized antibody 6E10, 1328, and 2235 respectively.
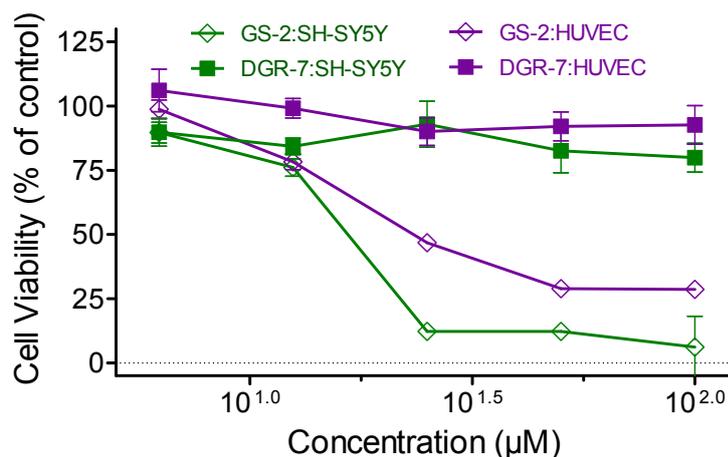
Figure S3: CCK-8 toxicity assay of DGR-7 and GS-2 towards SH-SY5Y and HUVEC cells. Results are expressed as a percentage of control (untreated) cells with mean ± SD (n = 5).

2. Supplementary Methods

2.1 General

All commercial reagents and solvents were purchased from vendors and used without further purification or distillation. Resins, protected amino acids, and coupling regents were purchased from GL Biochem(shanghai)Ltd. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Burker BioSpin Ultrashield 600 NMR system. The purity of peptides used for biological evaluation (>95%) were determined on a DIONEX Ultimate 3000 HPLC system (Chromeleon SR9 Build 2673): column, SHISEIDO C18,5 μm,4.6 × 250 mm. Separation conditions were: 1.0 mL min^{-1} flow rate, a linear gradient of 20% to 80% MeCN with 0.1% TFA in 25 min, 80% to 100% in another 10 min, washed with 100% for 5 min, and then calibrated at 20% for 10 min. All the cyclopeptides(20 mM) were dissolved in DMSO and stored at -20°C. Aβ$_{42}$ was purchased from Millipore (cat. No. AG970). Thioflavin-T, ammonium persulfate and Tris(2,2'-bipridyl)dichlororuthenium (II) were purchased from sigma. Antibody 6E10, 1328 (monoclonal anti-β-Amyloid 13-28 antibody) and 2235 (polyclonal anti-β-Amyloid 22-35 antibody) were obtained from Covance (clone 6E10, cat. No. SIG-39320), sigma (clone BAM90.1, cat. No. A8978), and sigma (clone polyclonal, cat. No. A3356) respectively. ECL reagent kit was purchased from CWBIO (China). All animal experiments were

conducted in compliance with the protocols approved by the IACUC of Peking University Shenzhen hospital.

2.2 Solid-phase synthesis of Gramicidin S analogues[1, 2]

General Procedure for Peptide Synthesis.

All the linear precursor of the Gramicidin S analogues were synthesized using standard Fmoc solid phase synthesis (SPSS) upon 2-Cl-Trityl resin, DPhe, Pro, Val, Arg, Leu, Ala, DAla, Thr, and Hyp were used with following protection form respectively: Fmoc-DPhe-OH, Fmoc-Pro-OH, Fmoc-Val-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Leu-OH, Fmoc-Ala-OH, Fmoc-DAla-OH, Fmoc-Thr(tBu)-OH, Fmoc-Hyp(Bzl)-OH. N,N'-Diisopropylcarbodiimide (DIC), 1-Hydroxybenzotriazole (HOBt), 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), Benzotriazol-1-yloxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP), N,N-Diisopropylethylamine (DIEA), 2,2,2-Trifluoroethanol(TFE) and Trifluoroacetic Acid(TFA) were used in the elongation and cyclization of linear precursor decapeptides to obtain the cyclopeptides.

Stepwise Elongation:

Prior to the first coupling step, 2-Chlorotrityl chloride resin (250 mg, loading 0.95 mmol/g, 1.0 *eq.*) was swollen in CH_2Cl_2 (DCM) for 30 min. The resin was added to a 25 mL round-bottle flask with a solution of Fmoc-protected amino acid (3.0 *eq.*), DIEA (10 *eq.*) and DCM (5 mL), the mixture was drained and washed with DCM (5×5 mL) after gently agitated for 2.5 h. The unreacted site on the resin were capped by a mixture of DCM /MeOH/DIEA (16:3:1, 5 mL) for 30 min. The resin was then washed with DMF (5×5 mL), followed by Fmoc deprotection using 5% piperidine and 2% DBU in DMF for 30 min. Chain elongation was performed with the solution of the Fmoc-protected amino acid (3.0 *eq.*), DIC (3.0 *eq.*) and HOBt (3.0 *eq.*) for 2.5 h. Each deprotection and coupling step was followed by washing the resin with DMF (5×5 mL). The completeness of each step was checked with Kaiser test. Finally, the *N*-terminal amine was liberated by Fmoc deprotection followed by washing 3 times with DMF, MeOH, DCM (5 mL, 3 min) respectively.

(b) Cleavage from the Resin: Resin-bound liner decapeptide was repeatedly treated with 20% TFE/DCM (10 mL) for 45min. The filtrate was combined and dried in vacuo to obtain crude linear product. The crude decapeptide was then precipitated by cold ether and used without further

purification in the cyclization step if the purity of crude peptide > 90%.

(c) Cyclization and Deprotection: The linear decapeptide was dissolved in cold DCM (400 mL) to a final concentration of 0.5 mg/mL and stirred for 3 h in the presence of (PyBOP)/HOBt/DIEA (3:3:5 equiv). After solvent removal in vacuo, the cyclized peptide was treated with 5 mL of Reagent B (TFA : phenol : i-Pr$_3$SiH : H$_2$O = 88 : 5 : 5 : 2) at 37°C for 2 h. The solvent was evaporated under reduced pressure, and the residue was then precipitated by cold ether and purified by reverse-phase HPLC and lyophilized to give white powders with purity >95%

Structureal data of **GS-2**, **MGR-4** (also namely **GS-3**), and **DGR-4** (also namely **GS-4**) have been shown in our previous report (*Chem. Commun.* **2017**, *53*, 7673).

MGR-1, cyclo (-**DAla**-Pro-Val-Arg-Leu-DPhe-Pro-Val-Arg-Leu)**:** 18.4mg, 15.2% yield as a white powder. HPLC purity 97.14%, t$_R$ = 19.50 min. ^{1}H NMR (600 MHz, MeOD): δ 8.69-7.71 (signal of amide protons) , 7.37 – 7.32 (m, 2H), 7.32 – 7.29 (m, 1H), 7.28 – 7.25 (m, 2H), 5.03 – 4.96 (m, 2H), 4.66 – 4.61 (m, 1H), 4.59 (m, 2H), 4.53 (m, 1H), 4.47 (m, 1H), 4.43 – 4.39 (m, 1H), 4.22 (t, *J* = 8.8 Hz, 1H), 4.19 (t, *J* = 8.8 Hz, 1H), 4.14 (m, 1H), 3.84 – 3.74 (m, 1H), 3.70 (td, *J* = 9.9, 7.4 Hz, 1H), 3.24 (t, *J* = 6.8 Hz, 2H), 3.22 – 3.14 (m, 1H,2H), 3.09 (dd, *J* = 12.7, 5.1 Hz, 1H), 2.96 (dd, *J* = 12.6, 11.1 Hz, 1H), 2.55 (q, *J* = 9.2 Hz, 1H), 2.38 – 2.31 (m, 1H), 2.31 – 2.25 (m, 1H), 2.25 – 2.19 (m, 2H), 2.05 – 1.98 (m, 2H), 1.90 – 1.84 (m, 2H), 1.83 – 1.78 (m, 1H), 1.73 (td, *J* = 8.4, 2.7 Hz, 2H), 1.68 – 1.50 (m, 11H), 1.45 (m, 2H), 1.34 (d, *J* = 6.9 Hz, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.93 (m, 18H). ^{13}C NMR (151 MHz, MeOD) δ 173.19, 172.52, 172.25, 172.17, 172.06, 171.55, 171.45, 171.34, 157.18, 157.04, 135.67, 129.03, 128.34, 127.09, 60.90, 60.54, 59.13, 59.09, 58.98, 58.93, 54.49, 51.86, 51.79, 50.48, 50.43, 46.90, 46.52, 40.92, 40.84, 40.73, 40.68, 35.94, 30.68, 30.62, 29.66, 29.36, 24.82, 24.73, 24.45, 24.42, 23.47, 23.11, 21.79, 21.77, 18.39, 18.35, 18.10, 17.99, 13.58. HRMS: calcd for C$_{56}$H$_{92}$N$_{16}$O$_{10}$ [M+2H]$^{2+}$ m/z 575.3664, found,575.3657.

MGR-2, cyclo (-DPhe-**Ala**-Val-Arg-Leu-DPhe-Pro-Val-Arg-Leu)**:** 18.6 mg, 17.4% yield as a white powder. HPLC purity 100%, t$_R$ = 19.91 min. ^{1}H NMR (600 MHz, MeOD) δ 8.95 – 7.69(signal of amide protons) , 7.36 – 7.30 (m, 5H), 7.29 – 7.24 (m, 5H), 5.04 – 4.95 (m, 2H), 4.67 – 4.56 (m, 2H), 4.53 (m, 1H), 4.44 – 4.40 (m, 1H), 4.39 – 4.33 (m, 1H), 4.21 (m, 3H), 3.78 (td, *J* = 8.2, 4.3 Hz, 1H),

3.25 (dt, *J* = 13.2, 6.7 Hz, 3H), 3.18 (m, 1H), 3.09 (dd, *J* = 12.6, 5.1 Hz, 1H), 3.04 (dd, *J* = 13.2, 6.4 Hz, 1H), 3.02 – 2.95 (m, 2H), 2.56 (q, *J* = 9.2 Hz, 1H), 2.36 – 2.30 (m, 1H), 2.30 – 2.24 (m, 1H), 2.06 – 1.99 (m, 1H), 1.91 – 1.77 (m, 2H), 1.72 (dt, *J* = 8.1, 5.9 Hz, 2H), 1.70 – 1.60 (m, 5H), 1.60 – 1.49 (m, 7H), 1.48 – 1.43 (m, 2H), 1.16 (d, *J* = 7.4 Hz, 3H), 1.02 (d, *J* = 6.7 Hz, 3H), 0.97 (d, *J* = 6.7 Hz, 3H), 0.91 (m, *J* = 13.3, 6.6 Hz, 18H). ^{13}C NMR (151 MHz, MeOD) δ 173.38, 172.85, 172.27, 172.23, 172.17, 172.08, 171.62, 171.54, 171.46, 171.41, 157.18, 136.14, 135.68, 129.03, 128.80, 128.35, 128.16, 127.09, 126.66, 60.54, 58.87, 58.83, 56.87, 54.47, 51.97, 51.87, 51.78, 50.83, 50.45, 48.90, 46.51, 41.06, 40.81, 40.77, 40.67, 35.94, 35.72, 30.79, 29.73, 29.54, 29.38, 24.87, 24.84, 24.51, 24.44, 23.11, 21.79, 21.77, 21.75, 18.37, 18.29, 17.91, 17.79, 16.23. HRMS: calcd for $C_{60}H_{94}N_{16}O_{10}$ [M+2H]$^{2+}$ m/z 600.3742, found,600.3738

MGR-3, cyclo (-DPhe-Pro-**Ala**-Arg-Leu-DPhe-Pro-Val-Arg-Leu): 27.3 mg, 21.6% yield as a white powder. HPLC purity 100%, t_R = 20.02 min. ^{1}H NMR (600 MHz, MeOD) δ 8.95-7.71 (signal of amide protons) 7.34 (m, 4H), 7.31 (t, *J* = 7.0 Hz, 2H), 7.27 (m, 4H), 5.01 (dt, *J* = 9.1, 6.8 Hz, 2H), 4.62 (qd, *J* = 7.7, 3.4 Hz, 2H), 4.57 – 4.50 (m, 3H), 4.42 (d, *J* = 7.6 Hz, 1H), 4.41 – 4.36 (m, 1H), 3.76 (m, 2H), 3.26 (dt, *J* = 9.1, 6.6 Hz, 4H), 3.09 (m, 2H), 3.03 – 2.92 (m, 2H), 2.60 – 2.48 (m, 2H), 2.06 – 1.96 (m, 2H), 1.86 (m, 2H), 1.80 – 1.49 (m, 17H), 1.50 – 1.37 (m, 2H,3H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.94 – 0.90 (m, 12H). ^{13}C NMR (151 MHz, MeOD) δ 172.97, 172.24, 172.12, 172.08, 172.02, 171.87, 171.71, 171.65, 171.57, 171.50, 157.23, 157.18, 135.69, 135.67, 129.03, 128.35, 127.09, 60.51, 59.00, 54.50, 51.99, 51.79, 50.66, 50.60, 48.31, 46.55, 46.47, 40.97, 40.71, 40.68, 40.48, 35.96, 35.91, 30.65, 30.06, 29.36, 29.32, 29.28, 24.76, 24.72, 24.68, 24.49, 23.08, 21.99, 21.82, 21.71, 21.51, 18.38, 18.05, 17.83, 17.78. HRMS: calcd for $C_{60}H_{92}N_{16}O_{10}$ [M+2H]$^{2+}$ m/z 599.3664, found,599.3654.

MGR-5, cyclo (-DPhe-Pro-Val-Arg-**Ala**-DPhe-Pro-Val-Arg-Leu)**:** 19.4 mg, 24.1% yield as a white powder. HPLC purity 98.63%, t_R = 19.01 min. ^{1}H NMR (600 MHz, MeOD) δ 8.67-7.66 (signal of amide protons), 7.37 – 7.33 (m, 4H), 7.31 (t, *J* = 7.1 Hz, 2H), 7.29 – 7.25 (m, 4H), 4.42 (d, *J* = 7.0 Hz, 2H), 3.79 (m, 2H), 3.25 (t, *J* = 6.7 Hz, 4H), 3.18 (td, *J* = 6.7, 3.6 Hz, 4H), 3.09 (d, *J* = 9.0 Hz, 2H), 2.97 (t, *J* = 11.8 Hz, 2H), 2.55 (m, 2H), 2.30 (m, 2H), 2.03 (m, 2H), 1.87 (q, *J* = 3.4 Hz, 3H), 1.86 – 1.80 (m, 2H), 1.77 – 1.60 (m, 12H), 1.55 (dq, *J* = 13.1, 6.6 Hz, 2H), 1.23 (d, *J* = 6.6 Hz, 3H), 1.00 – 0.95 (m,

6H), 0.95 – 0.90 (m, 12H). ^{13}C NMR (151 MHz, MeOD) δ 172.47, 172.33, 172.19, 172.18, 172.13, 172.08, 171.64, 171.56, 171.49, 171.37, 157.25, 157.20, 135.65, 129.03, 128.36, 127.12, 60.55, 59.07, 58.95, 54.50, 54.49, 51.84, 51.77, 50.46, 46.53, 45.99, 45.96, 40.87, 40.74, 40.70, 35.99, 35.93, 30.67, 30.52, 29.47, 29.36, 25.97, 25.92, 24.79, 24.76, 24.37, 23.12, 21.73, 18.40, 18.30, 18.01, 17.95, 16.43. HRMS: calcd for $C_{59}H_{90}N_{16}O_{10}$ [M+2H]$^{2+}$ m/z 592.3586, found, 592.3578.

DGR-1, cyclo (-**DAla**-Pro-Val-Arg-Leu-**DAla**-Pro-Val-Arg-Leu): 32.2 mg, 26.4% yield as a white powder. HPLC purity 95.15%, t_R = 17.57 min. ^{1}H NMR (600 MHz, MeOD) δ 8.75-7.84 (signal of amide protons), 5.02 – 4.96 (m, 2H), 4.63 – 4.54 (m, 4H), 4.47 (m, 2H), 4.21 (t, *J* = 8.9 Hz, 2H), 4.13 (m, 2H), 3.70 (td, *J* = 9.8, 7.3 Hz, 2H), 2.33 (m, 2H), 2.26 – 2.18 (m, 4H), 2.18 – 2.10 (m, 2H), 2.05 – 1.96 (m, 2H), 1.90 – 1.85 (m, 8H), 1.81 (m, 2H), 1.67 – 1.52 (m, 10H), 1.45 (m, 2H), 1.34 (d, *J* = 6.9 Hz, 6H), 1.03 (d, *J* = 6.7 Hz, 6H), 0.95 – 0.91 (m, 18H). ^{13}C NMR (151 MHz, MeOD) δ 173.16, 172.47, 172.18, 171.50, 171.47, 157.04, 60.90, 59.13, 51.83, 50.48, 46.91, 45.99, 45.97, 40.90, 40.69, 30.61, 29.65, 25.98, 25.92, 24.74, 24.45, 23.48, 21.81, 21.79, 18.38, 18.15, 13.60. HRMS: calcd for $C_{50}H_{88}N_{16}O_{10}$ [M+2H]$^{2+}$ m/z 537.3507, found, 537.3518.

DGR-2, cyclo (-DPhe-**Ala**-Val-Arg-Leu-DPhe-**Ala**-Val-Arg-Leu)**:** 8.7 mg, 6.8% yield as a white powder. HPLC purity 96.28%, t_R = 18.42 min. ^{1}H NMR (600 MHz, MeOD) δ 8.89-7.97 (signal of amide protons), 7.34 – 7.30 (m, 4H), 7.26 (m, 6H), 5.02 – 4.95 (m, 2H), 4.58 (dt, *J* = 8.9, 7.3 Hz, 2H), 4.36 (m, 2H), 4.22 (m, 4H), 3.25 (m, 2H), 3.20 – 3.15 (m, 4H),3.06 – 2.96 (m, 4H), 2.36 – 2.26 (m, 2H), 2.02 – 1.97 (m, 1H), 1.89 – 1.85 (m, 4H),1.83 – 1.76 (m, 2H), 1.63 – 1.48 (m, 10H), 1.48 – 1.43 (m, 2H), 1.15 (d, *J* = 7.4 Hz, 6H), 1.01 (d, *J* = 6.7 Hz, 6H), 0.94 – 0.88 (m, 18H).^{13}C NMR (151 MHz, MeOD) δ 173.40, 172.95, 172.34, 171.54, 157.16, 136.16, 128.80, 128.15, 126.65, 58.79, 57.01, 51.92, 50.88, 48.98, 45.99, 45.96, 41.03, 40.75, 35.78, 30.85, 29.73, 25.97, 25.92, 24.86, 24.52, 21.74, 18.28, 17.72, 16.30.HRMS: calcd for $C_{58}H_{92}N_{16}O_{10}$ [M+2H]$^{2+}$ m/z 587.3664, found, 587.3667.

DGR-3, cyclo (-DPhe-Pro-**Ala**-Arg-Leu–DPhe-Pro-**Ala**-Arg-Leu): 17.0 mg, 20.4% yield as a white powder. HPLC purity 97.23%, t_R = 18.59 min. ^{1}H NMR (600 MHz, MeOD) δ 8.45-7.81(signal of amide protons) , 7.37 – 7.33 (m, 4H), 7.32 – 7.29 (m, 2H), 7.29 – 7.26 (m, 4H), 4.98 (dt, *J* = 9.0, 6.8

Hz, 2H), 4.61 (dt, *J* = 8.6, 6.4 Hz, 2H), 4.58 − 4.50 (m, 4H), 4.39 (m, 2H), 3.76 (m, 2H), 3.26 (t, *J* = 6.7 Hz, 4H), 3.18 (m, *J* = 6.6, 3.7 Hz, 5H), 3.10 (dd, *J* = 12.7, 5.2 Hz, 2H), 2.98 (m, 2H), 2.52 (q, *J* = 8.9 Hz, 2H), 1.89 − 1.86 (m, 6H), 1.78 − 1.62 (m, 12H), 1.61 − 1.50 (m, 4H), 1.44 (d, *J* = 7.2 Hz, 6H), 0.95 (d, *J* = 6.6 Hz, 6H), 0.92 (d, *J* = 6.6 Hz, 6H).^{13}C NMR (151 MHz, MeOD) δ 173.27, 172.18, 172.08, 171.68, 157.16, 135.67, 129.03, 128.36, 127.12, 60.50, 54.55, 51.88, 50.88, 48.56, 46.54, 45.96, 40.84, 40.49, 35.93, 29.88, 29.32, 25.97, 25.92, 24.77, 24.59, 23.08, 22.05, 21.38, 17.64.

  HRMS: calcd for $C_{58}H_{88}N_{16}O_{10}$ [M+2H]$^{2+}$ m/z 585.3507, found, 585.3527.

DGR-5, cyclo (-DPhe-Pro-Val-Arg-**Ala**-DPhe-Pro-Val-Arg-**Ala**): 18.2 mg, 23.7% yield as a white powder. HPLC purity 96.28%, t_R = 16.17 min. ^{1}H NMR (600 MHz, MeOD) δ 8.66-7.72 (signal of amide protons), 7.37 − 7.33 (m, 4H), 7.32 − 7.29 (m, 2H), 7.29 − 7.26 (m, 4H), 4.99 (dt, *J* = 9.2, 7.2 Hz, 2H), 4.62 (dq, *J* = 8.5, 6.6 Hz, 2H), 4.54 (m, 2H), 4.47 − 4.39 (m, 2H), 4.15 (t, *J* = 8.9 Hz, 2H), 3.78 (m, 2H), 3.25 (td, *J* = 7.0, 2.0 Hz, 4H), 3.09 (dd, *J* = 12.6, 5.0 Hz, 2H), 2.97 (m, 2H), 2.54 (q, *J* = 9.4 Hz, 2H), 2.29 (m, 2H), 2.08 − 1.99 (m, 2H), 1.82 (m, 2H), 1.77 − 1.70 (m, 6H), 1.69 − 1.60 (m, 6H), 1.23 (d, *J* = 6.6 Hz, 6H), 0.97 (d, *J* = 6.7 Hz, 6H), 0.91 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (151 MHz, MeOD) δ 172.48, 172.23, 172.13, 171.69, 171.31, 157.20, 135.62, 129.03, 128.36, 127.13, 60.55, 59.26, 54.50, 51.77, 46.54, 40.72, 35.98, 30.40, 29.56, 29.32, 24.70, 23.12, 18.33, 18.09, 16.51. HRMS: calcd for $C_{56}H_{84}N_{16}O_{10}$ [M+2H]$^{2+}$ m/z 571.3351, found, 571.3368.

MGR-6, cyclo (-DPhe-**HyP**-Val-Arg-Leu-DPhe-Pro-Val-Arg-Leu): 27.8 mg, 34.2% yield as a yellow powder. HPLC purity 98.77%, t_R = 19.11 min. ^{1}H NMR (600 MHz, MeOD) δ 8.69-7.65 (signal of amide protons), 7.38 − 7.33 (m, 4H), 7.32 − 7.30 (m, 2H), 7.28 (m, 4H), 5.02 − 4.93 (m, 2H), 4.67 − 4.58 (m, 2H), 4.54 (m, 2H), 4.47 (dd, *J* = 8.8, 3.4 Hz, 1H), 4.45 − 4.39 (m, 1H), 4.32 (dq, *J* = 9.3, 6.2 Hz, 1H), 4.19 (td, *J* = 8.8, 2.8 Hz, 2H), 4.00 (m, 1H), 3.78 (m, 1H), 3.25 (m, 4H), 3.18 (td, *J* = 6.7, 3.7 Hz, 2H), 3.12 − 3.03 (m, 2H), 2.98 (m, 2H), 2.62 (m, 1H), 2.55 (q, *J* = 9.2 Hz, 1H), 2.28 (m, 2H), 1.91 − 1.81 (m, 5H), 1.73 (m, 2H), 1.71 − 1.63 (m, 6H), 1.62 − 1.57 (m, 2H), 1.57 − 1.53 (m, 2H), 1.54 − 1.49 (m, 1H), 1.48 − 1.41 (m, 2H), 0.98 (m, 6H), 0.94 − 0.88 (m, 18H). ^{13}C NMR (151 MHz, MeOD) δ 172.29, 172.26, 172.23, 172.21, 172.15, 172.07, 171.56, 171.51, 171.41, 171.37, 157.20, 157.15, 135.67, 135.65, 129.04, 128.84, 128.43, 128.35, 127.14, 127.10, 67.83, 60.54, 59.29, 58.92, 58.84, 54.49, 54.42, 53.45, 51.95, 51.85, 50.44, 50.36, 46.51, 45.99, 45.96, 40.85, 40.73, 40.71, 40.64,

37.45, 35.94, 35.68, 30.79, 30.70, 29.54, 29.49, 29.37, 25.98, 24.84, 24.40, 23.11, 21.81, 21.71, 18.37, 18.00. HRMS: calcd for $C_{62}H_{96}N_{16}O_{11}$ [M+2H]$^{2+}$ m/z 621.3795, found, 621.3822.

DGR-6, cyclo (-DPhe-**HyP**-Val-Arg-Leu-DPhe-**HyP**-Val-Arg-Leu): 26.9 mg, 24.9% yield as a white powder. HPLC purity 98.86%, t_R = 16.52 min. ^{1}H NMR (600 MHz, MeOD) δ 8.70-7.65 (signal of amide protons), 7.38 – 7.33 (m, 4H), 7.33 – 7.30 (m, 2H), 7.30 – 7.27 (m, 4H), 4.99 – 4.93 (m, 2H), 4.62 (dt, J = 9.1, 7.4 Hz, 2H), 4.53 (m, 2H), 4.47 (dd, J = 8.8, 3.4 Hz, 2H), 4.32 (dq, J = 7.9, 6.1 Hz, 2H), 4.18 (t, J = 8.7 Hz, 2H), 4.00 (dd, J = 10.3, 6.5 Hz, 2H), 3.23 (t, J = 6.8 Hz, 4H), 3.18 (td, J = 6.6, 3.7 Hz, 4H), 3.06 (dd, J = 13.1, 6.2 Hz, 2H), 3.00 (m, 2H), 2.62 (dd, J = 10.3, 6.1 Hz, 2H), 2.28 (m, 2H), 2.18 (m, 2H), 1.89 – 1.82 (m, 8H), 1.66 (m, 6H), 1.55 (m, 4H), 0.99 (d, J = 6.7 Hz, 6H), 0.94 – 0.88 (m, 18H). ^{13}C NMR (151 MHz, MeOD) δ 172.28, 172.23, 172.21, 171.55, 171.37, 157.15, 135.64, 128.83, 128.43, 127.14, 67.82, 59.29, 58.86, 54.42, 53.45, 51.87, 50.36, 45.96, 40.74, 40.65, 37.45, 35.67, 30.77, 29.48, 25.98, 25.92, 24.83, 24.40, 21.79, 21.71, 18.36, 18.02.HRMS: calcd for $C_{62}H_{96}N_{16}O_{12}$ [M+2H]$^{2+}$ m/z 629.3770, found, 629.3731.

MGR-7, cyclo (-DPhe-Pro-**Thr**-Arg-Leu-DPhe-Pro-Val-Arg-Leu): 17.6mg, 16.8% yield as a white powder. HPLC purity 95.28%, t_R = 19.31 min. ^{1}H NMR (600 MHz, MeOD) δ 8.72-7.78 (signal of amide protons), 7.35 (m, 4H), 7.33 – 7.30 (m, 2H), 7.28 (m, 4H), 4.86 (d, J = 7.4 Hz, 1H), 4.63 (q, J = 8.3 Hz, 2H), 4.56 – 4.51 (m, 3H), 4.48 (d, J = 8.1 Hz, 1H), 4.44 (d, J = 7.5 Hz, 1H), 4.18 (t, J = 8.9 Hz, 1H), 4.09 (m, 1H), 3.77 (m, 2H), 3.30 – 3.23 (m, 4H), 3.18 (td, J = 6.7, 3.6 Hz, 3H), 3.11 (m, 2H), 2.96 (m, 2H), 2.53 – 2.42 (m, 2H), 2.33 – 2.25 (m, 1H),2.06 (m, 2H), 1.93 – 1.84 (m, 4H), 1.74 – 1.47 (m, 17H), 1.43 (m, 2H), 1.19 (d, J = 6.4 Hz, 3H), 0.97 (t, J = 7.0 Hz, 6H), 0.95 – 0.91 (m, 12H). HRMS: calcd for $C_{61}H_{94}N_{16}O_{11}$ [M+2H]$^{2+}$ m/z 614.3714, found, 614.3717.

DGR-7, cyclo (-DPhe-Pro-**Thr**-Arg-Leu-DPhe-Pro-**Thr**-Arg-Leu): 66 mg, 25.3% yield as a white powder. HPLC purity 98.12%, t_R = 15.58 min. ^{1}H NMR (600 MHz, MeOD) δ 9.10-7.45（signal of amide protons）, 7.37 – 7.32 (m, 4 H), 7.30 (t, J = 6.0 Hz, 2 H), 7.27 (d, J = 7.1 Hz, 4 H), 4.68 – 4.58 (m, 2 H), 4.59 – 4.51 (m, 2 H), 4.50 – 4.42 (m, 4 H), 4.14 – 4.04 (m, 2 H), 3.79 (t, J = 8.5 Hz, 2 H), 3.77 – 3.64 (m, 1 H), 3.63 – 3.51 (m, 1 H), 3.30 – 3.19 (m, 4 H), 3.10 (dd, J = 12.6, 5.1 Hz, 2 H), 2.98 (t, J = 11.7 Hz, 2 H), 2.52 (s, 2 H，OH), 2.05 (dd, J = 12.0, 6.0 Hz, 2 H), 1.91 – 1.75 (m, 4 H),

1.75 – 1.62 (m, 8 H), 1.62 – 1.49 (m, 6 H), 1.45 – 1.36 (m, 8 H), 1.19 (d, J = 6.3 Hz, 6 H), 0.94 (d, J = 6.5 Hz, 6 H), 0.91 (d, J = 6.4 Hz, 6 H). ^{13}C NMR (151 MHz, MeOD) δ 173.76, 173.67, 173.46, 172.72, 171.74, 158.62, 137.05, 130.47, 129.76, 128.52, 69.22, 64.41, 61.93, 60.00, 55.87, 55.84, 53.68, 52.21, 47.97, 43.78, 42.40, 41.96, 37.40, 31.07, 30.67, 26.02, 25.97, 24.31, 23.26, 22.95, 20.01, 18.70, 17.27, 13.12. HRMS: calcd for $C_{60}H_{92}N_{16}O_{12}$ [M+2H]$^{2+}$ m/z 615.3613, found, 615.3592.

2.3 ThioflavinT (ThT) Assay[3]

Monomeric Aβ$_{42}$ preparation: Monomeric form of Aβ$_{42}$ was prepared by dissolving Aβ$_{42}$ (Millipore, AG970) in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) (1 mg/mL) and incubating overnight. The solution was aliquoted, lyophilized and the resulting monomeric Aβ$_{42}$ was stored at -80°C. Before using, the monomeric Aβ$_{42}$ was dissolved in 1% $NH_3 \bullet H_2O$, sonicated for 30 s to 1 min after it has gone into solution and then diluted into the appropriate concentration.

Disaggregation: Aβ$_{42}$ (40 μM) was aged in phosphate buffer saline (PBS, 50 mM, PH 7.4) for 4 days to afford the stable maximal ThT fluorescence. The aged Aβ$_{42}$ was incubated with or without a series concentration of the tested peptides for a further 72 h. The extent of disaggregation was determined by the fluorescence of amyloid-bound ThT assay, relative to Aβ$_{42}$ incubated without the peptides, The ThT fluorescence values of aged (96+72h) Aβ$_{42}$ were referred to 100% ThT fluorescence.

2.4 ThioflavinT (ThT) fluorescence kinetics assay

Aβ$_{42}$ and the tested peptide (GS-2 and DGR-7) were diluted with phosphate buffer saline (PBS, 10 mM, PH 7.4) containing ThT (5 μM). Aβ$_{42}$ (20 μL, 10 μM, final concentration) was incubated for 192 h at 15°C in the present or absent of the tested peptide (20 μL). All the samples (40 μL) were prepared over ice and added into a 384 well plate. The samples were prepared in quadruplicate. Fluorescence measurements were recorded with an Infinite M1000 EVO758 plus 1 microplate reader every 6 h, by using excitation and emission wavelengths of 444 and 484 nm, respectively. The fluorescence intensities were calculated by subtracting the background.

2.5 Photo-Induced Cross-Linking of Unmodified Proteins (PICUP) [4-6]

Samples prepared for PICUP were similar to that of ThT assay.

For Aβ$_{42}$ aggregation inhibition: Aβ$_{42}$ (9 μL, 25 μM, final concentration, (PBS, 50 mM, PH 7.4)) was incubated for different time at 37°C in the present or absent of the tested peptide (9 μL, various concentration).

For Aβ$_{42}$ fibrils disaggregation: Aβ$_{42}$ was aged in phosphate buffer saline (PBS, 50 mM, PH 7.4) for 4 days. The aged Aβ$_{42}$ (9 μL, 25 μM, final concentration) was incubated with or without the equivoluminal tested peptide for a further 72 h.

After each time interval, ammonium persulfate (APS, 1.0 μL, 40 mM), Tris (2,2'-bipridyl)dichlororuthenium (II) (1.0 μL, 2 mM) were added to the above prepared sample (18 μL) in a clear PCR tube. The mixture was then irradiated for 1 s using a 200 W lamp position 10 cm from the bottom of the PCR tube. The cross-linking reaction is quenched immediately by adding 5μL 5×loading buffer containing 5% β-mercaptoethanol. Samples were analyzed using Tricine-SDS-PAGE gels and visualized by western blot using monoclonal anti-β-Amyloid (13-28) antibody (1:1000 dilution ratio) as primary antibody.

2.6 Dot Blot Assay[7]

Binding selectivity: Monomeric Aβ$_{42}$ (5 μM) was incubated with or without different concentration of tested peptides in phosphate buffer saline (PBS, 50 mM, PH 7.4) at 37°C for 3 h, then the samples were diluted 10-fold with PBS, 80 μL of the diluted samples were spotted onto nitrocellulose membranes and dried at room temperature. The membranes were then blocked for 2 h with 5% nonfat milk in TBST (10 mM Tris buffered saline and 0.01% Tween 20). After washing, membranes were incubated at 4°C overnight with 6E10 (Covance, 1:1000 dilution ratio), monoclonal anti-β-Amyloid (1328) antibody (1:1000 dilution ratio) or polyclonal anti-β-Amyloid (2235) antibody (1:1000 dilution ratio) in primary antibody dilution buffer (Beyotime). The membranes were washed with TBST and then incubated for 2 h with appropriate horseradish peroxidase (HRP) conjugated secondary antibodies. After washed with TBST, the blots were developed using ECL reagent kit.

Fiber disassemble analysis: Samples prepared were same as ThT disaggregation assay. After

incubating, the samples were diluted with PBS to a final concentration containing 0.5 μM of $A\beta_{42}$ before spotted on the nitrocellulose membranes, the following experimental protocol was same as that of the binding selectivity dot-blot assay except the antibody used were anti-β-Amyloid fiber (OC) antibody (1:1000 dilution ratio) and anti-β-Amyloid oligomer (A11) antibody (1:500 dilution ratio)

2.7 Surface Plasmon Resonance (SPR)[8]

For SPR experiments NeutrAvidin-coated GLH sensor chips in combination with a ProteOn XPR36 protein interaction system (Bio-Rad Laboratories, Hercules, CA) were used.

Binding selectivity towards $A\beta_{42}$: In a typical experiment, sequence-specific antibodies (6E10, 1328 and 2235 were immobilized (~12000 RU) by EDC/NHS according to the protocol in flow cells, with one flow cell used as a blank. Prior to the experiment, $A\beta_{42}$ (10 μM) was incubated at 37°C for 3 h in the absence or presence of the equivalent tested peptide. The sample was diluted 5-fold with running buffer, and injected over immobilized antibody at a flow rate of 30 μL/min for 9 min of associating, followed by 11 min of disassociation at 25°C. The GLH chip was regenerated with a short injection of 0.85% H_3PO_4 between consecutive measurements. The final graphs were obtained by subtracting blank sensorgrams. The observed signal enhancements in the case of antibodies 1328 and 2235 are attributed to the increased molecular weight of the formed test peptide/Ab42 complex.

2.8 Cell Culture[9, 10]

PC-12, SH-SY5Y, and HUVEC cells were used to evaluate the cytotoxic effect of the tested compounds or aged $A\beta_{42}$ by MTT or CCK-8 assays. These cell lines have not been tested for mycoplasma contamination, but treated with MycoplasmaOUT™ (Genloci Biotechnologies Inc, China, cat. No. PT141212-1) to prevent mycoplasma contamination prior to the experiments. PC-12 cells were maintained in DMEM supplemented with 10% heat-inactivated horse serum and 5% fetal bovine serum. SH-SY5Y cells were grown in DMEM medium supplemented with 10% fetal bovine serum. HUVEC cells were cultured in Endothelial cell medium (ECM) supplemented with 5% fetal bovine serum. All cells were maintained at 37°C in 5% CO_2.

2.9 Toxicity of the tested peptides towards PC-12, SH-SY5Y, and HUVEC cells

PC-12, SH-SY5Y, and HUVEC cells were seeded on 96-well plates (5×10^3 cells/well). The cells were cultured for 24 h before the tested peptides containing medium (the total volume is 180 μL) were added. Control wells received medium without the tested peptides. After that, the live cell count was used MTT (Fig.S1) or CCK-8 (Fig.S3) assay. 20 μL of MTT or CCK-8 solution was added to each well. After the samples were incubated for another 4 h, the absorbance was measured at 490 nm (MTT) or 450 nm (CCK-8). Each of the samples was repeated with 4-6 replicates in at least two independent experiments. The results were normalized by setting the cell survival of the 1xPBS controls as 100%.

2.10 Detoxification of Aβ$_{42}$ by the tested peptide

Aβ$_{42}$ monomers (5 μM) were aged for 16 h at 37 °C in the absence or presence of DGR-7 in 1xPBS. After Aβ$_{42}$ were aged, the samples were diluted by a factor of 10 in the medium prior to add to the cells. After PC-12 cells were seeded on 96-well plates (4×10^3 cells/well) for 24 h, the samples were added to the cells for 24 h. Control wells received medium containing 10% PBS. All the wells contained an equal amount of DMSO. After that, the live cell count was used CCK-8 assay. 20 μL of CCK-8 solution was added to each well. After the samples were incubated for another 4 h, the absorbance was measured at 450 nm. Each of the samples was repeated with 5 replicates in three independent experiments. The results were normalized by setting the cell survival of the PBS controls as 100%.

2.11 Haemolysis assay[11, 12]

The experiments with blood samples (Human erythrocytes concentrates in 10% citrate-phosphate-dextrose medium) were conducted in accordance with our institutional guidelines, approved and obtained from Sun Yat-sen University hospital. The cells were washed five times in 0.9% saline solution by centrifugation at 3000 rpm, 4°C. The final pellet was diluted 50-fold in 0.9% saline solution and incubated for 5 min at 37°C just prior to the assay. The cyclic peptides were dissolved in dimethyl sulfoxide (DMSO) to produce 20 mM cyclic peptide stock solution and

stored at -20°C. Then, each concentration of cyclic peptides were prepared by a two-fold dilution series of the stock solution in 0.9% saline solution. To each micro tube, 65 μL of erythrocytes (2 % hematocrit in saline solution) and equivoluminal of each concentration of cyclic peptide was added. The micro tubes were incubated at 37°C for 2.5 h and centrifuged for 3 min at 3,000 rpm at 4°C. The supernatant of each micro tube was transferred to a 96-well plates, and the absorbance was measured at 540 nm by a microtiter plate reader. The absorption of the supernatant at 540 nm gives the extent of hemolysis, with 0% taken from the peptide-free control and 100% after treatment with 0.1% Triton X-100. To make sure that this step did not interfere with the analysis, the samples were back-converted before absorption measurements.

2.12 In vivo imaging of amyloid plagues[13]

The experiments with animals were conducted in accordance with national institutional guidelines and approved by IACUC of Peking University Shenzhen hospital. APPPS1/BL6 mice of 7-8 month of age were used for imaging studies before or after the treatment of DGR-7 (n = 6). A thin-skulled method was used to visualize amyloid plaque in vivo over a period of time. Methoxy-X04 (final concentration of 2 mg/mL) was injected i.v 24 h prior to imaging sessions. A region of cortex (posterior to Bregma, ~ 150 - 200 μm in depth) was identified which contained numerous plaques of different sizes and shapes. A z-stack of images of plaques was collected using two-photon imaging. After the first imaging session to determine the baseline plaque size, GS-5 was delivered into the brain. To do so, a glass pipette (2~3 μm in diameter) was filled with the peptide (1 mM), positioned at a 20° angle to the horizontal plane, until it was above the imaged cortex area (about 200 μm below the pial surface). GS-5 was pressured ejected with a volume about 2 μL. Imaging was performed again in the previous imaged brain regions 72 h after peptide injection. Two-photon imaging was performed on an Olympus BX 51 upright microscope equipped with a two-photon laser (Spectrum physics). Scanning was done in the XYZ directions (508 x 508 μm, 2 μm z-steps, 800 x 800 pixels). Image J was used to calculate the volume of plaques. To do so, plaques being repetitively imaged were selected, with a threshold set for the total area for a given plane was calculated. Then the sum of all the areas within the imaged planes for a given plaque was calculated to arrive at the volume for that plaque.

2.13 Statistical Analysis.

All values were expressed as mean ± SD. Two-tailed Student's t tests and graphs were performed using GraphPad Prism v5.0c software. Significance is represented by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control.

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