Supporting information

γ-AApeptide-based small-molecule ligands that inhibit Aβ aggregation

Haifan Wu,[a] Yaqiong Li,[a] Ge Bai,[a] Youhong Niu,[a] Qiao Qiao,[a] Jeremiah D. Tipton,[b] Chuanhai Cao,*[,c] and Jianfeng Cai*[,a]

[a]Department of Chemistry, University of South Florida, 4202 E. Fowler Ave, Tampa, FL 33620

[b]College of Pharmacy, University of South Florida, 4202 E. Fowler Ave, Tampa, FL 33620

[c]Proteomics and Mass Spectrometry, USF CDDI, 3720 Spectrum Blvd., Tampa, FL 33612

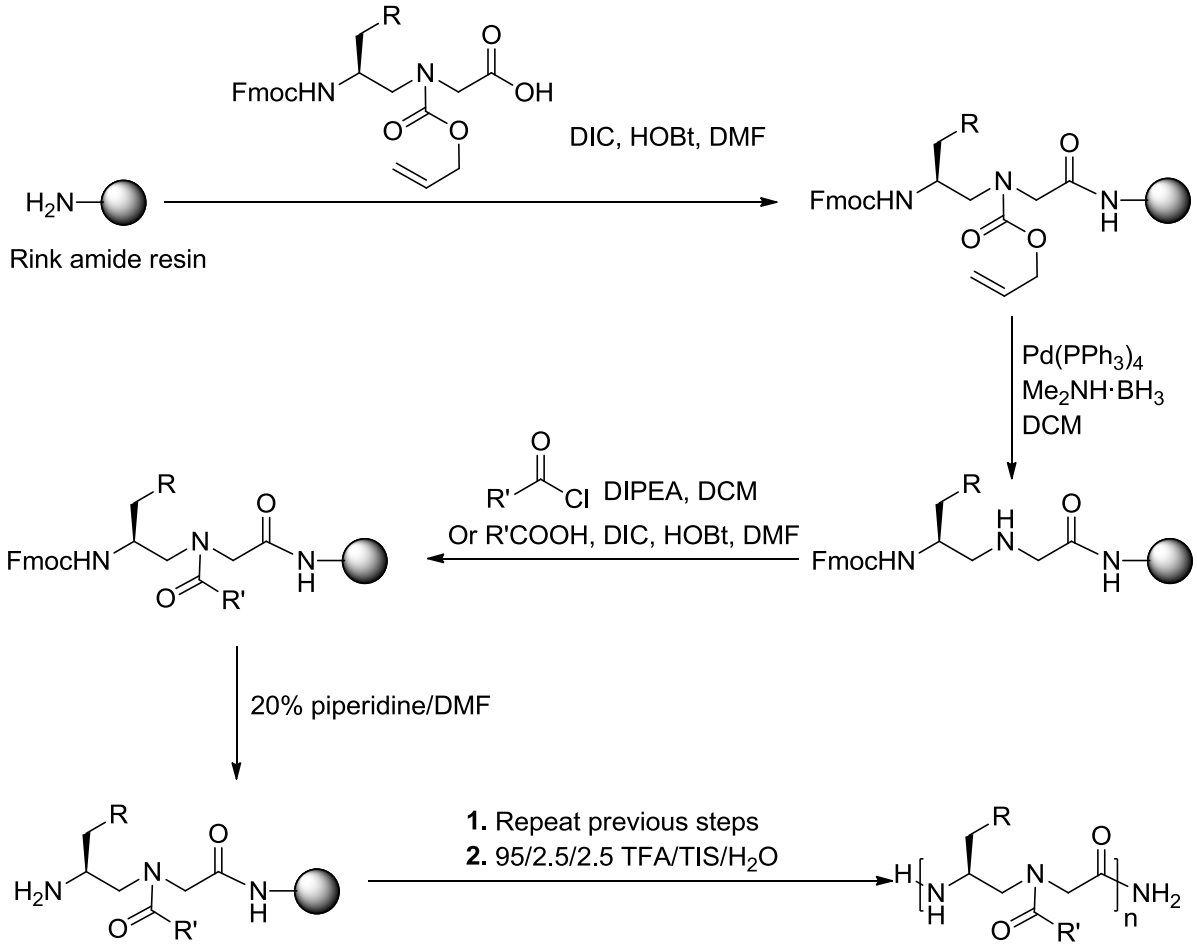
jianfengcai@usf.edu and ccao@health.usf.edu

Library synthesis

1. General information

All Fmoc protected α-amino acids and Rink amide resin (0.7 mmol/g, 200-400 mesh) were purchased from Chem-Impex International, Inc. TentaGel MB NH_2 resin (0.3 mmol/g, 140-170 μm) was purchased from RaPP Polymere GmbH. All the other solvents and reagents were purchased from either Sigma-Aldrich or Fisher Scientific. NMR data for building blocks was obtained on a Varian UnityInova400 spectrometer. High resolution masses of building blocks were determined on an Agilent 6540 Liquid Chromatography/Quadrupole Time-of Flight mass spectrometer. Masses of γ-AApeptides were obtained on an Applied Biosystems 4700 Proteomics Analyzer. MS/MS analysis was carried out with a Thermo LTQ Orbitrap XL. Solid phase synthesis was conducted in peptide synthesis vessels on a Burrell Wrist-Action shaker. γ-AApeptides were analyzed and purified on a Waters Breeze 2 HPLC system, and then lyophilized on a Labcono lyophilizer.

2. Solid phase synthesis of γ-AApeptides



Scheme S1. Solid phase γ-AApeptide synthesis.

Solid phase synthesis was conducted on Rink amide resin (0.7 mmol/g) in peptide synthesis vessels on a Burrell Wrist-Action shaker (Scheme S1). 100 mg resin (0.07 mmol) was treated with 3 mL 20% Piperidine/DMF solution for 15 min (× 2) to remove Fmoc protecting group. The solution was drained

and beads were washed with DCM (3 × 3 mL) and DMF (3 × 3mL). A solution of γ-AApeptide building block (2 equiv.),[1-5] HOBt (38 mg, 0.28 mmol), and DIC (44 μL, 0.28 mmol) in 3 mL DMF was shaken for 5 min, and then added to the resin in a peptide synthesis vessel. The mixture was allowed to react at room temperature for 6 h and drained. The beads were washed with DCM (3 × 3 mL) and DMF (3 × 3 mL), followed by a capping reaction with 500 μL acetic anhydride in 3 mL Pyridine. After washing with DMF (3 × 3 mL) and DCM (3 × 3 mL), to the beads were added $Pd(PPh_3)_4$ (8 mg, 0.007 mmol) and $Me_2NH\ BH_3$ (25 mg, 0.42 mmol) in 3 mL DCM. [6] The alloc deprotection reaction was shaken for 10 min and repeated one more time. The beads were washed with DCM and DMF, followed by the reaction with acid chloride (4 equiv.) and DIPEA (6 equiv.) in 3 mL DCM for 30 min (× 2) or with carboxylic acid (4 equiv.), HOBt (8 equiv.), and DIC (8 equiv.) for 4 h (× 2).

The previous steps were repeated until the desired sequences were obtained. After that, the resin were washed with DCM and dried in vacuo. Peptide cleavage was done in a 4 mL vial by treating resin with $TFA/H_2O/TIS$ (95/2.5/2.5) for 2 h. The solvent was evaporated and the crude was analyzed and purified on an analytical (1 mL/min) and a preparative (20 mL/min) Waters HPLC systems, respectively. 5% to 100% linear gradient of solvent B (0.1% TFA in acetonitrile) in A (0.1% TFA in water) over 40 min was used. The HPLC traces were detected at 215 nm. The products were confirmed on an Applied Biosystems 4700 Proteomics Analyzer. Then, the desired fractions were collected and lyophilized.

3. MS/MS analysis

The fragmentation pattern of a known γ-AApeptide was analyzed on a Thermo Scientific LTQ Orbitrap XL mass spectrometer (Figure S1). Higher Energy Collision Dissociation (HCD) was performed at collision energy of 35.

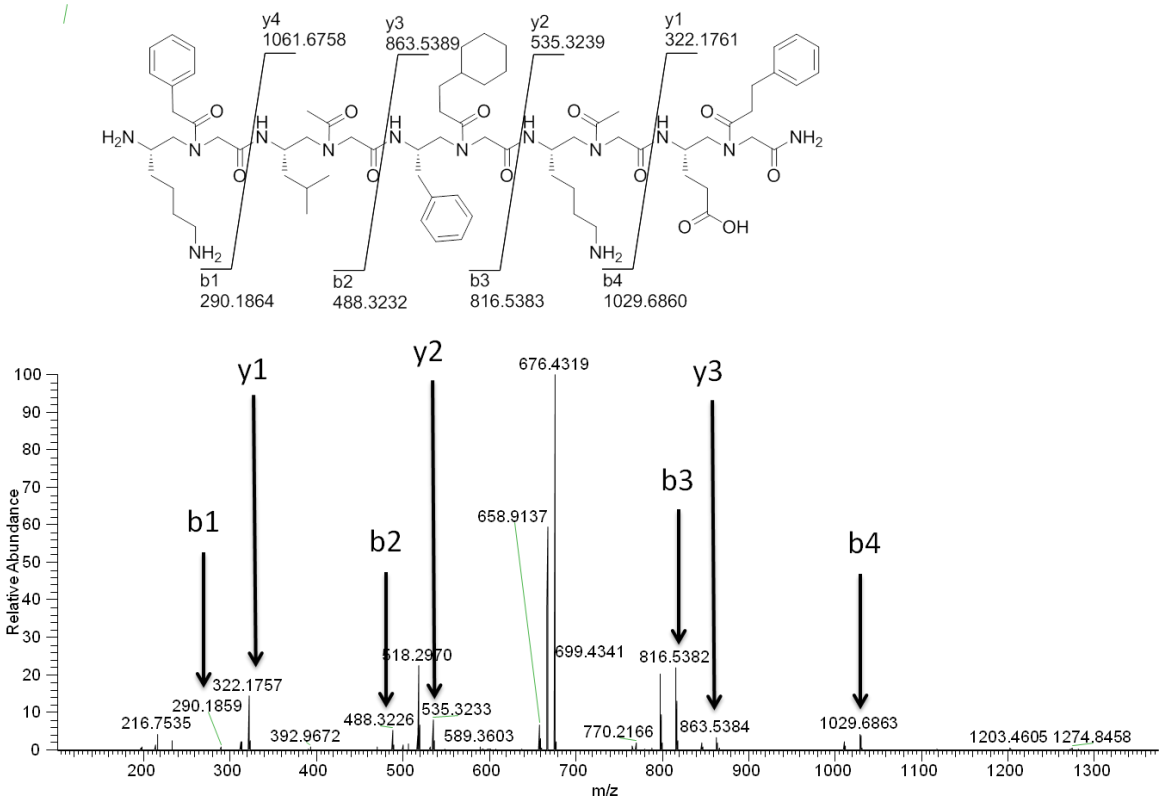
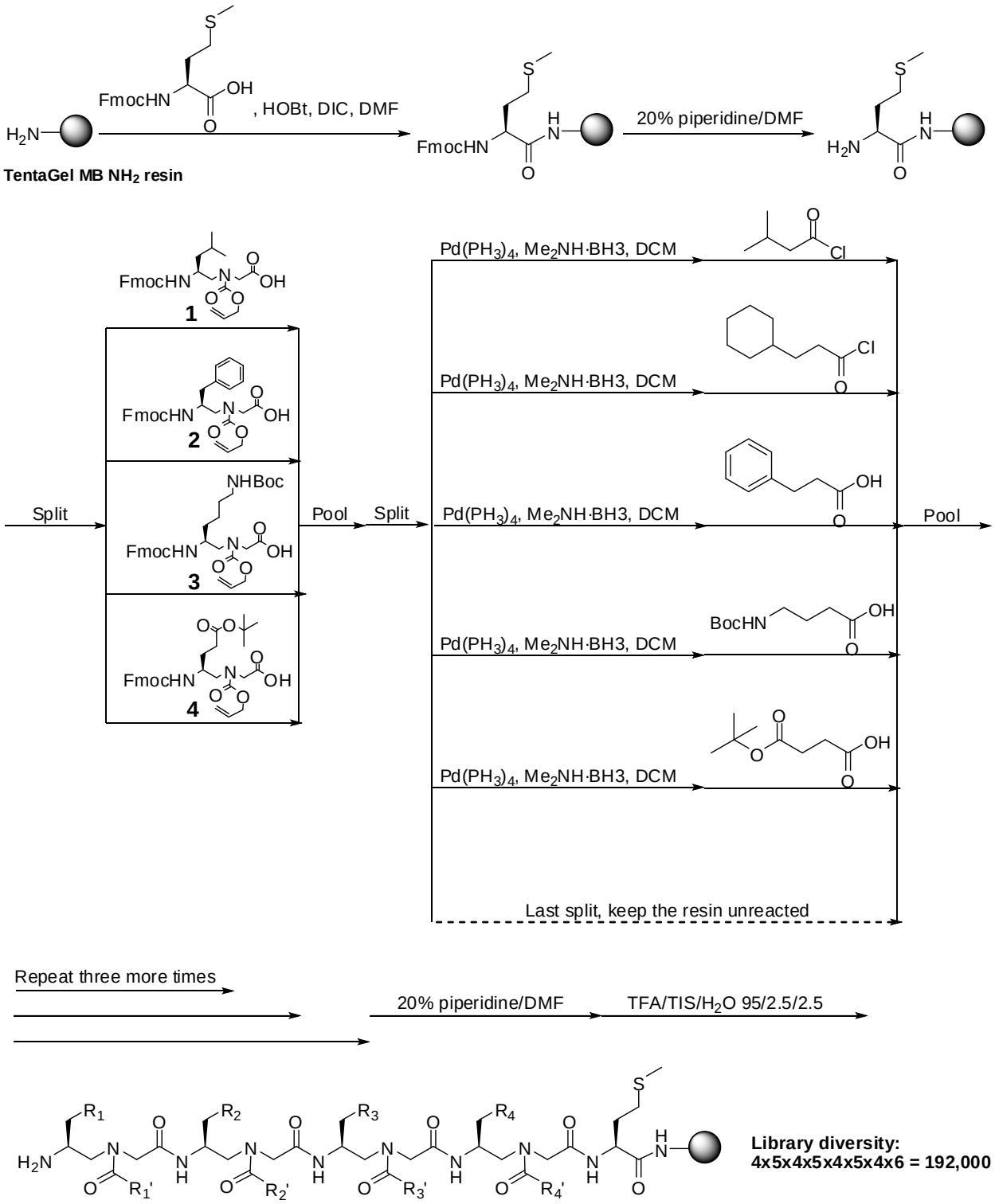


Figure S1. MS/MS analysis of a known γ-AApeptide. HCD fragmentation of a double charged precursor ion was performed at collision energy of 35.

4. Synthesis of the OBOC γ-AApeptide library

The TentaGel NH_2 resin (1.6 g, 0.48 mmol, 832,000 beads) was swelled in DMF for 1h, followed by the treatment with Fmoc-Met-OH (3 equiv.), HOBt (6 equiv.), and DIC (6 equiv.) in DMF. [7] The beads were shaken at room temperature in a peptide synthesis vessel for 4 h and repeated. The beads were washed with DCM (× 3) and DMF (× 3). Fmoc protecting group was removed with 20% piperidine in DMF for 20 min (× 2). The beads were washed and equally distributed into four peptide synthesis vessels. Each building block (2 equiv.) together with HOBt (4 equiv.) and DIC (4 equiv.) were dissolved in DMF and added to each vessel. The coupling reaction was performed at room temperature for 6 h and repeated. The beads in each vessel were then washed and mixed thoroughly by severe shaking for 1 h. The beads were equally split into five vessels. The Alloc protecting group was removed by treating beads with $Pd(PPh_3)_4$ (0.1 equiv.) and $Me_2NH{\cdot}BH_3$ (6 equiv.) in DCM for 10 min (× 2). After washing, each portion was reacted with either acid chloride or carboxylic acid. The reaction with acid chloride (5 equiv.) was carried out in the presence of DIPEA (5 equiv.) and DCM for 30 min (× 2). The carboxylic acids (3 equiv.) were pre-activated with DIC (6 equiv.) and HOBt (6 equiv.) in DMF, then added to beads. The reaction was carried out by shaking the vessel for 6 hours and repeated. After that, all the beads were pooled and mixed thoroughly. The previous split-and-pool process was repeated three times. The last time, after attachment of building blocks, beads were equally distributed into six portions, five of which were treated with alloc deprotection reagents then with acid chlorides and carboxylic acids as shown previously. The sixth portion was kept unreacted. At last, all beads were combined in one peptide synthesis vessel and washed thoroughly with DMF and DCM. Beads were treated with 20% piperidine in DMF for 20 min (× 2) and then with TFA/TIS/H_2O (95:2.5:2.5) for 2 h to remove all the protecting groups. The beads were washed with DCM thoroughly and dried in vacuo.



Scheme S2. Schematic representation of synthesizing OBOC γ-AApeptide library.

Combinatorial Library Screening

1. General information

The amyloid beta peptide $A\beta_{40}$ was used as a target for the combinatorial library screen because insoluble Aβ plaque was thought to be a pathological marker in Alzheimer's disease (AD). [8] The synthesized library was stored in a glass peptide synthesis vessel, and later washed and incubated in the same container. The antibodies were purchased from Fisher Scientific, and all the other chemicals were provided by Sigma-Aldrich. The beads were screened and picked up under Zeiss inverted fluorescence microscope 10x43HE filter.

2. Beads screening

The library synthesized on TentaGel beads was swelled in dimethylformamide (DMF) for 1 hour, washed with 1×TBST for five times and then equilibrated in 1×TBST overnight at room temperature. [9] The beads were blocked in 1% BSA in TBST for 1 hour, washed and equilibrated in 1×PBST before prescreening and screening.

Prescreening: In order to avoid any possible nonspecific binding, both the Aβ and antibodies solution were made in 1% BSA/TBST blocking buffer. The library was first incubated with mouse 6e10 primary antibody (200 ng/mL) which recognizes the first 16 amino acids of Aβ1-40, followed by five times PBST wash and incubation with goat anti-mouse IgG conjugated with dylight 549 (200 ng/ml). The beads were washed with PBST completely and transferred into a 6-well plate to be observed under Zeiss inverted fluorescence microscope 10×43HE filter, and the orange bright beads were picked up for they had suspicious nonspecific binding. These bright beads were excluded for further screening.

The rest of the beads were pooled together, washed with PBST, and then treated with 1% SDS at 90 ^{o}C for ten minutes to remove any bound proteins. We used both water and TBST to wash away the SDS and then the beads were washed and swelled in DMF for 1 hour. After washing and equilibrating in TBST overnight, the beads were ready for actual Aβ screening.

Screening: The prescreened beads were equilibrated in 1% BSA/PBST for 1 hour at room temperature. Aβ solution was made by dissolving $A\beta_{40}$ powder in the buffer right before the screening. After washing with PBST for three times, the beads were incubated with $A\beta_{40}$ peptide at a concentration of 20 μg/mL for 4 h at room temperature. Since $A\beta_{40}$ aggregates quite slowly and the screening was done in such as short time, the percentage of Aβ40 aggregation in the solution was very minimal. After thorough washing with PBST, the library beads were incubated in 5 mL of 1% BSA/PBST containing 1:5000 diluted mouse 6e10 antibodies for 2 hours at room temperature. The beads were gently washed with PBST and incubated with 1:500 diluted goat anti-mouse IgG-dylight 549 for 1 hour at room temperature. The beads were washed with PBST and transferred into the 6-well plate to be observed under Zeiss inverted fluorescence microscope 10×43HE filter. Again the bright orange ones were picked up as candidates for further study.

3. Sequence decoding

The beads were collected and washed with 1×PBST three times. The bound fluorescent dyes, proteins, and antibodies were removed by treating beads with 1% SDS solution at 90 ℃ for 10 min. After being washed with water, DMSO, and acetonitrile, beads were then cleaved and analyzed using previous procedure. The structure of one hit was determined (Figure S6) and designated as **HW-155-1**.

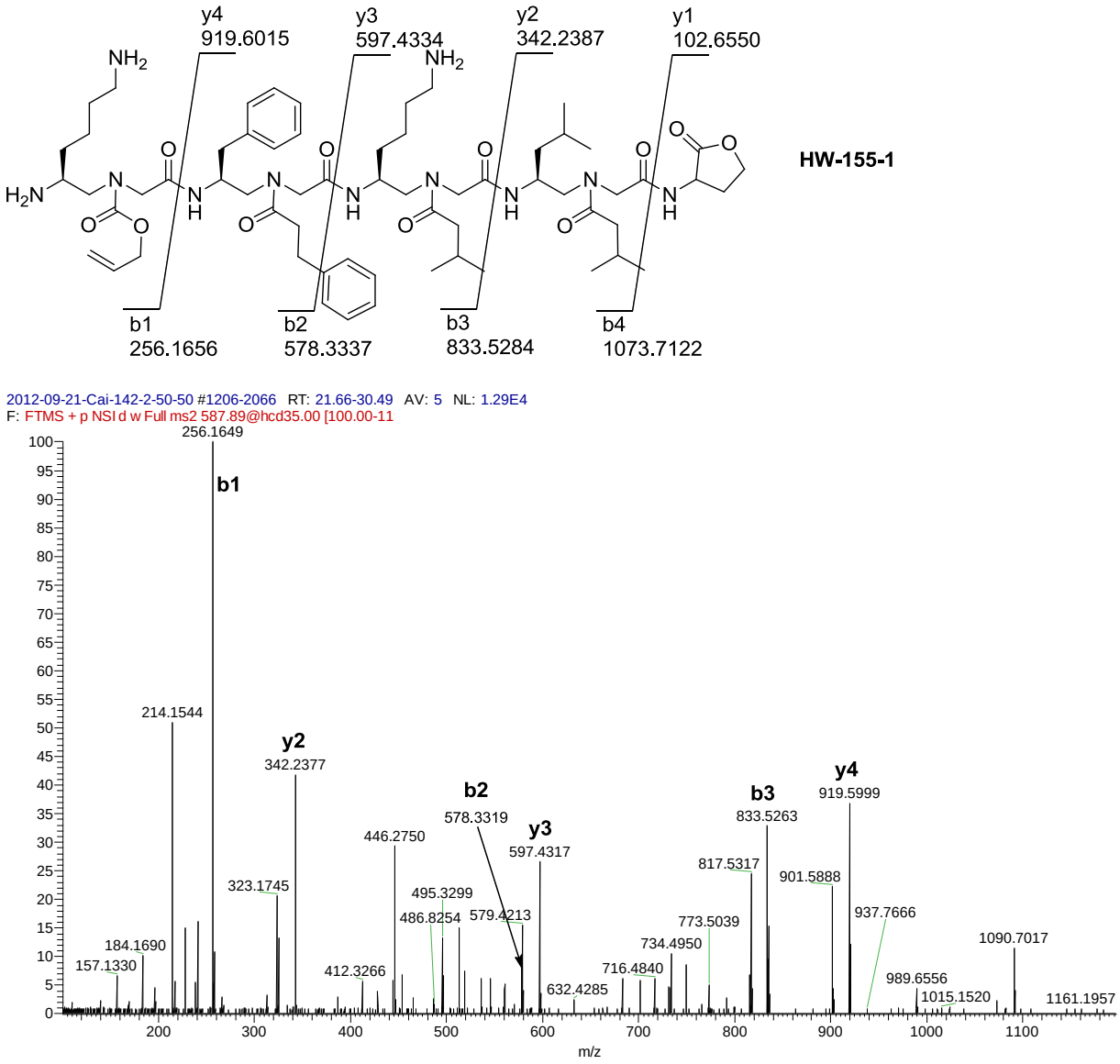


Figure S2. Structural identification of one hit by MS/MS analysis. HCD fragmentation was performed on a double charged precursor ion (587.8827) and the collision energy was set at 35.

4. Solid phase synthesis of HW-155-1 and KLVFF peptide

HW-155-1 was resynthesized on rink amide resin following previous procedure. (Scheme S3) The mass was determined to be 1091.1375 $(M+H)^+$ on an Applied Biosystems 4700 Proteomics Analyzer. The purity was analyzed on an analytical Waters HPLC system with flow rate of 0.8 mL/min and linear gradient from 5% to 100% (CH_3CN in water) in 40 min (Figure S7a). As a positive control, **KLVFF** sequence was synthesized manually using regular solid phase peptide synthesis method. Amino acids were assembled on rink amide resin individually using HOBt/DIC as coupling reagents. After cleavage with TFA/TIS/H_2O (95:2.5:2.5) for 3 h, TFA was removed under reduced pressure. The peptide was purified and analyzed on a preparative and analytical Waters HPLC system, respectively. (Figure S7b)

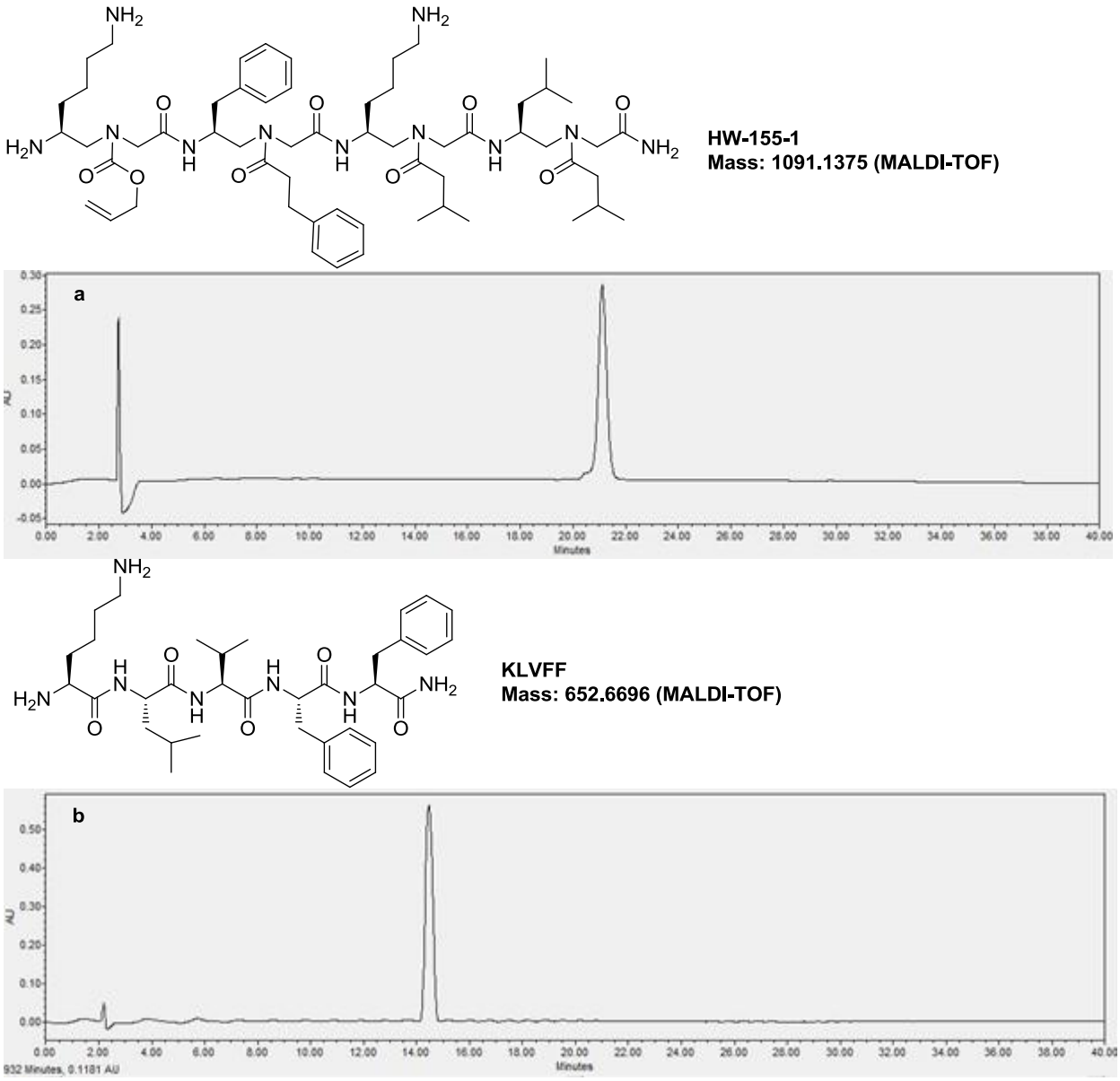


Figure S3. Pure HPLC trace of compound HW-155-1 (a) and KLVFF peptide (b).

Function bioassays

1. Thioflavin T assay

Compounds in different concentrations in Tris buffer Saline (TBS, pH 7.5) containing 10 μM ThT were added into a black 96 well plate corning@3721. $A\beta_{40}$ monomer was freshly thaw and used to make a stock solution in TBS with a concentration of 5 μM. Equal volume of Aβ solution was added into the 96 well plate (Final Aβ conc. = 2.5 μM). Time-dependant fluorescence change was monitored by a Synergy 2 plate reader at an excitation wavelength of 440 nm and emission at 482 nm. After 24 h, the fluorescence change was recorded. 100% aggregation is the fluorescence change of 2.5 μM $A\beta_{40}$ in TBS buffer containing 5 μM ThT.

2. Transmission electron microscopy (TEM)

$A\beta_{40}$ preparations were adsorbed onto 200-mesh copper grids for 1 hour (until it is dry), and then stained with 1% uranyl acetate for 20 sec. The excess fluid was removed and the grids were analyzed with FEI Morgagni 268D TEM operated at 60 kV.

3. MTT toxicity assay

In siliconized tubes, $A\beta_{42}$ peptide of 10 μM (in F-12 medium) was pre-incubated with 0, 0.5 and 1 equiv. **HW-155-1** γ-AApeptide, respectively. These solutions were incubated on rotating shaker (Barnstead 400100) at 8 rpm in 37 ^{o}C for 24 h. Meanwhile, N2a cells were plated in 96-well plates (10000 cells/well) with three replicates for 24 h at 37 ^{o}C. Then pre-aggregated mixtures were added into each well to make the final Aβ concentration 1 μM. The plate was incubated for another 24 h at 37 ^{o}C. Next, 10 μL MTT reagent was added to the cells. The plate was incubated for 4 h at 37 ^{o}C. After the addition of 100 μL solublization solution (10% Triton-X 100 in acidic Isopropanol (0.1N HCl)) and incubation overnight, OD values were read at 575 nm. The final cell viability was calculated as:

Cell viability % = $(OD_{575}-OD_{blank})/(OD_{ctrl} - OD_{blank})$ x 100%. OD_{ctrl} is the OD of the well containing cells only. OD_{blank} is the OD of the blank well.

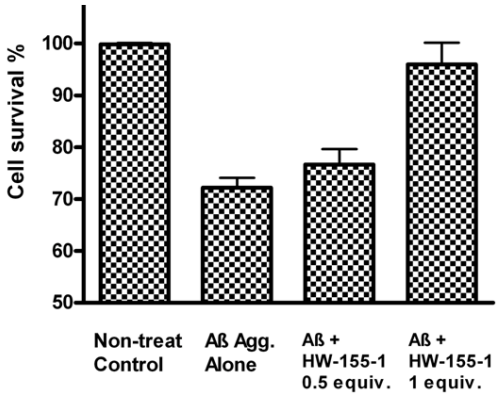


Figure S4. Detoxification of $A\beta_{42}$ aggregates by **HW-155-1**. N2a cells were cultured with 1 μM of pre-aggregated $A\beta_{42}$ in the absence/presence of **HW-155-1** for 24 h.

References

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