Electronic Supplementary Information (ESI)

Sizes of amyloid-β oligomers predicted using atomic force microscopy and two-point crosslinked dimers as standards

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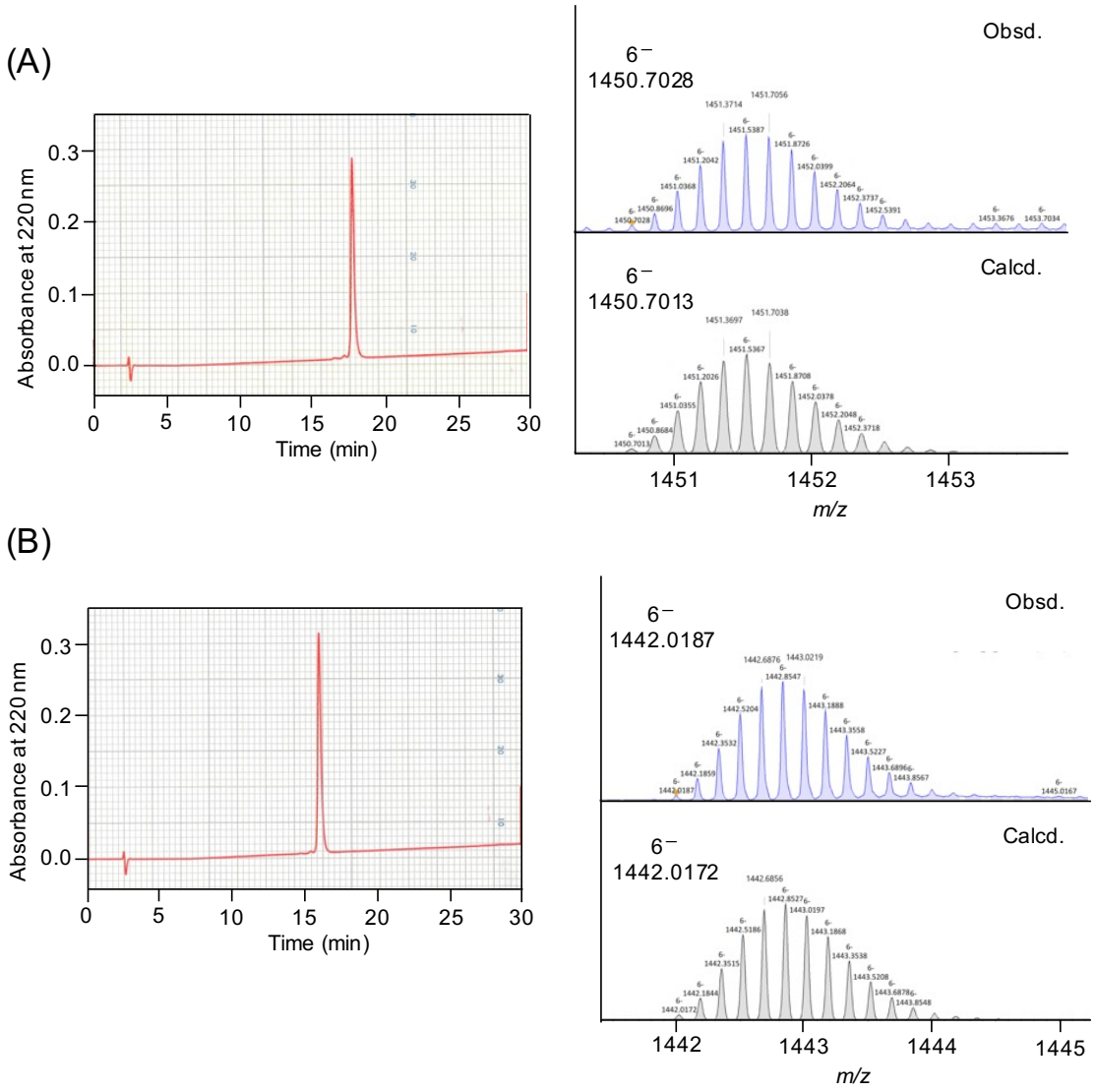


Figure S1-1 HPLC profiles and ESI-qTOF-MS data of **2** and **3**. HPLC conditions: Protein RP (100 × 6.0 mm I.D.), 1 mL/min, UV 220 nm, 20–60% acetonitrile containing 0.1% TFA (30 min linear gradient), 10 μg/10 μL (0.1% NH_4OH) injection. (A) **2**, *m/z* 1450.7028 (calcd for $C_{390}H_{584}N_{106}O_{114}S_4^{6-}$, 1450.7013). (B) **3**, *m/z* 1442.0187 (calcd for $C_{384}H_{572}N_{106}O_{116}S_4^{6-}$, 1442.0172).

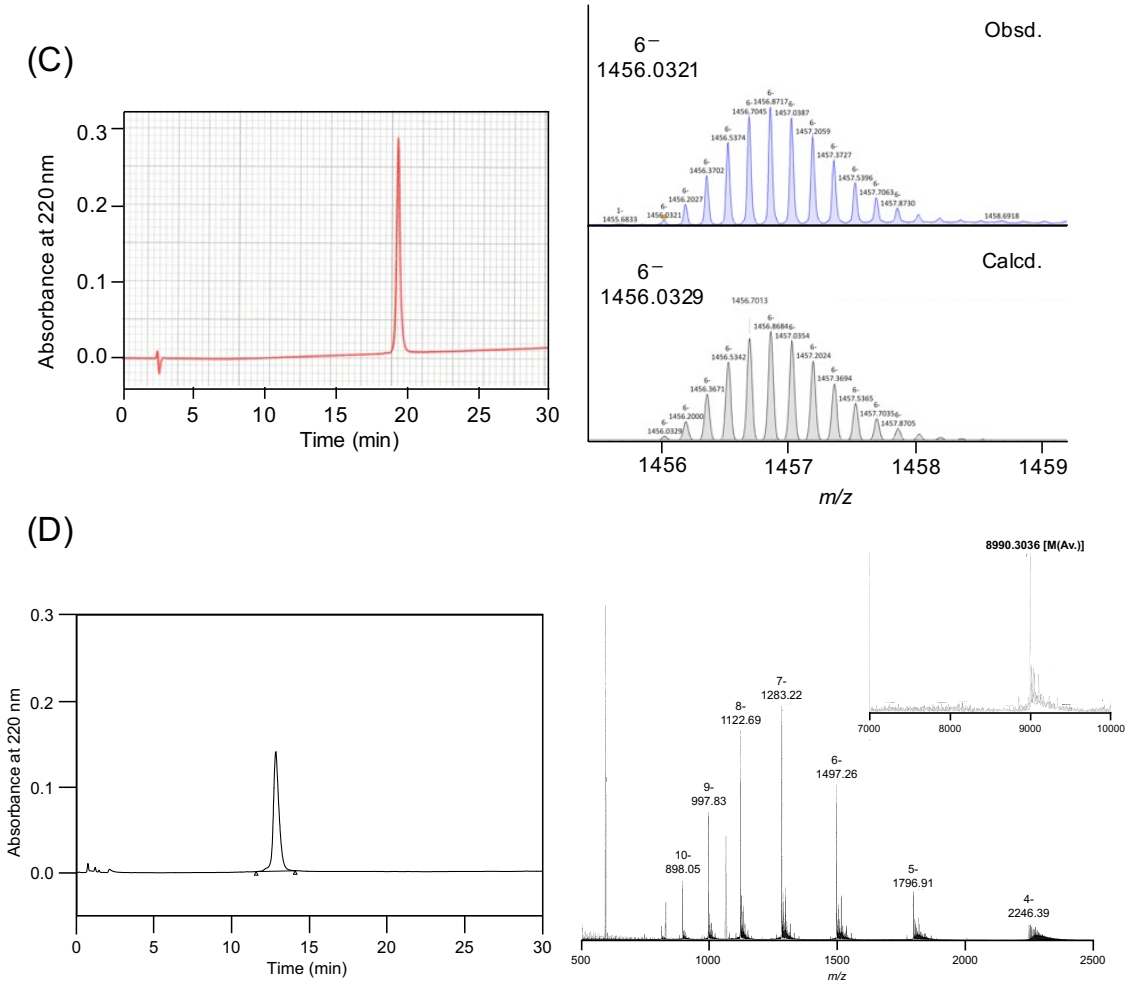


Figure S1-2 HPLC profiles and ESI-qTOF-MS data of **4** and **7**. (C) HPLC conditions: Protein RP (100 × 6.0 mm I.D.), 1 mL/min, UV 220 nm, 20–60% acetonitrile containing 0.1% TFA (30 min linear gradient), 10 μg/10 μL (0.1% NH_4OH) injection. **4**, *m/z* 1456.0321 (calcd for $C_{390}H_{584}N_{106}O_{116}S_4^{6-}$, 1456.0329). (D) HPLC conditions: X-Bridge (100 × 4.6 mm I.D.), 1 mL/min, UV 220 nm, 20–50% acetonitrile containing 0.1% NH_4OH (30 min linear gradient), 10 μg/10 μL (0.2% NH_4OH) injection. **7**, *m/z* 8991.08 (calcd for av. mass, 8690.30).

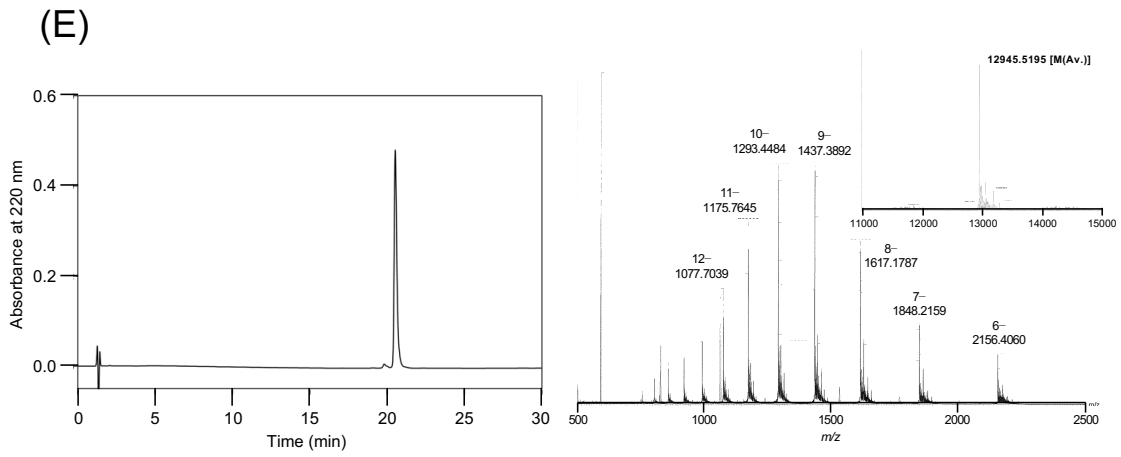


Figure S1-3 HPLC profiles and ESI-qTOF-MS data of **5**. (E) HPLC conditions: X-Bridge (100 × 4.6 mm I.D.), 1 mL/min, UV 220 nm, 20–50% acetonitrile containing 0.1% TFA (30 min linear gradient), 10 μg/10 μL (0.2% NH_4OH) injection. **5**, *m/z* 12945.52 (calcd for av. mass, 12945.49).

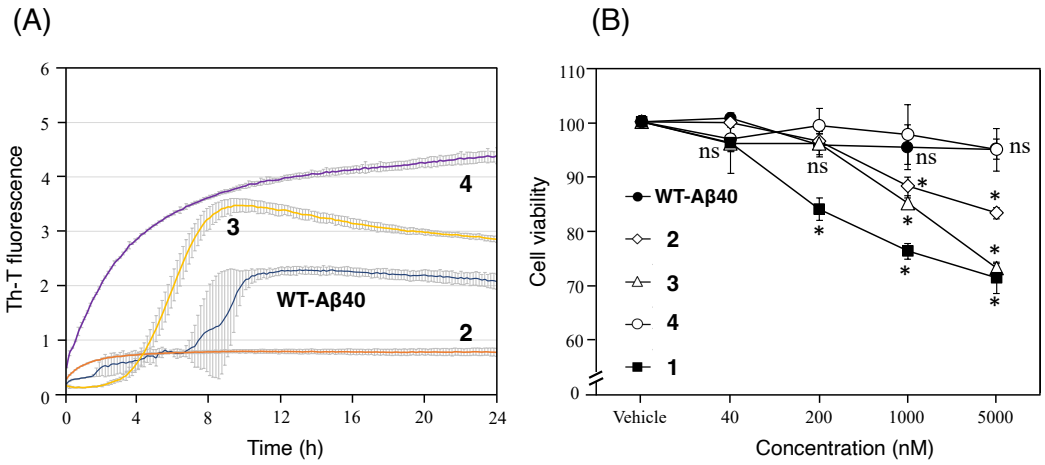


Figure S2 (A) Th-T fluorescence of new dimer models (**2**–**4**). Peptide concentration was 10 μM. Data are expressed as mean ± SD (n = 6). (B) MTT assay results for SH-SY5Y cells treated with the new dimer models (**2**–**4**) after 24 hr of incubation at 37 ℃ in 5% fetal bovine serum. Absorbance measured after adding the vehicle (0.1% NH_4OH) was set at 100%. Data are expressed as mean ± SD (n = 3). *p < 0.01 versus vehicle; ns: not significant. Peptides used in the cytotoxicity assay were pre-treated with 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) to improve reproducibility.

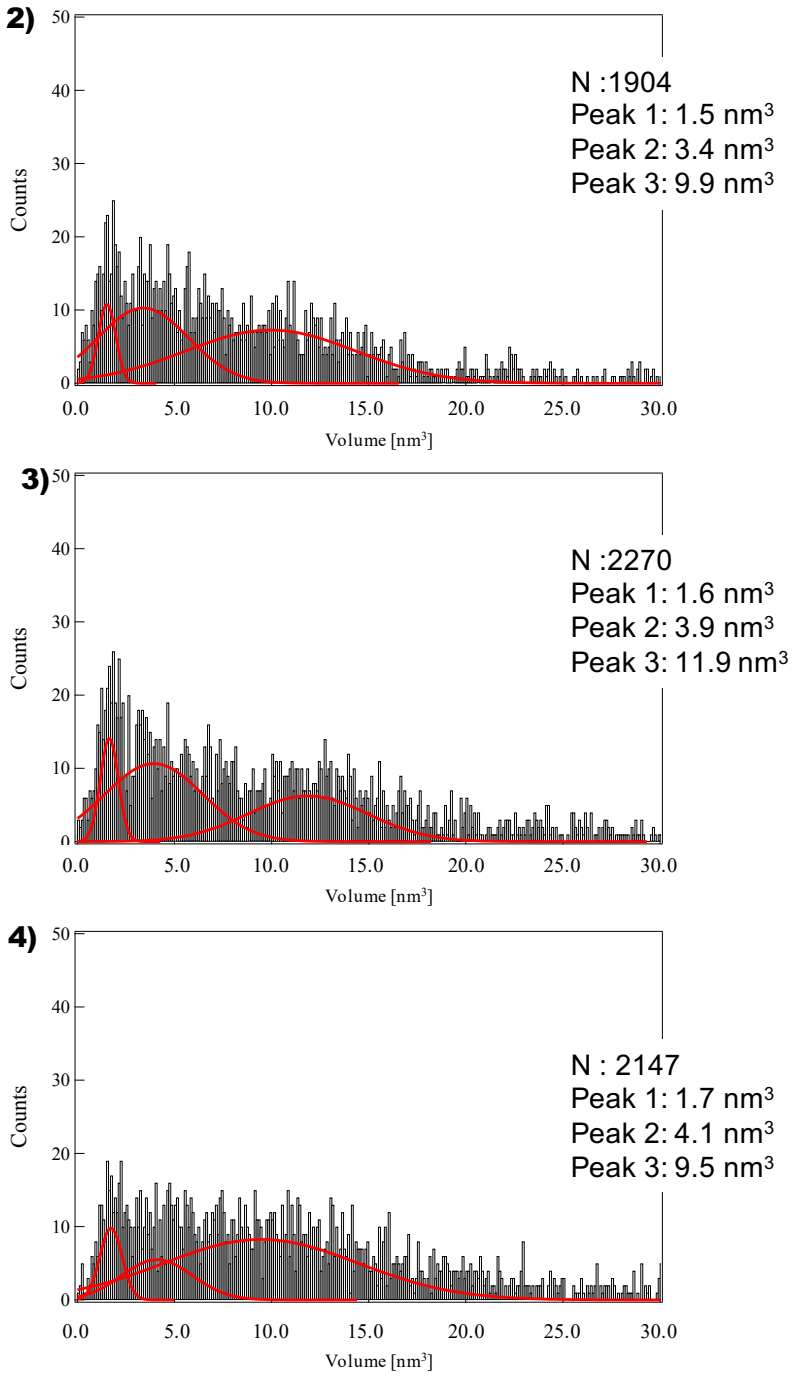
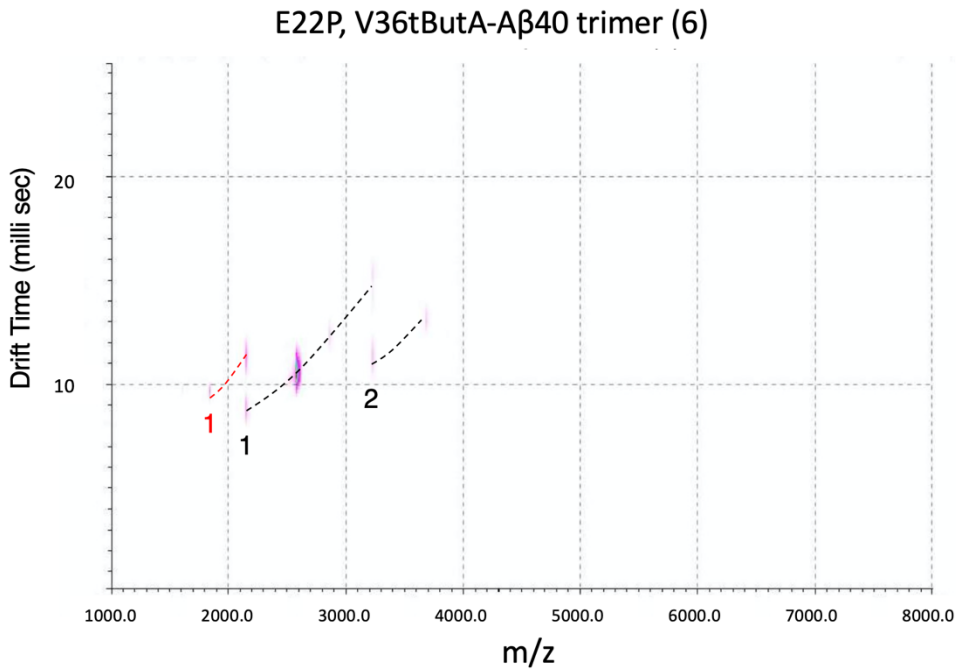


Figure S3 The volume histograms were created by calculating the ellipsoid volume of Aβ spherical particles (height multiplied by the square of the width).[1] Models **2**–**4** all converged to volumes of approximately 1.6 nm³, 3.9 nm³, and 10.0 nm³, respectively. As with the height histograms, Gaussian fitting was applied to characterize the general shape of the histograms, and all **2**–**4** showed three peaks in common, with similar volume values at all peak positions.

Despite the limited lateral resolution of AFM, the commonality of the volume histograms supports the consistency between the three dimer models.

(A)



(B)

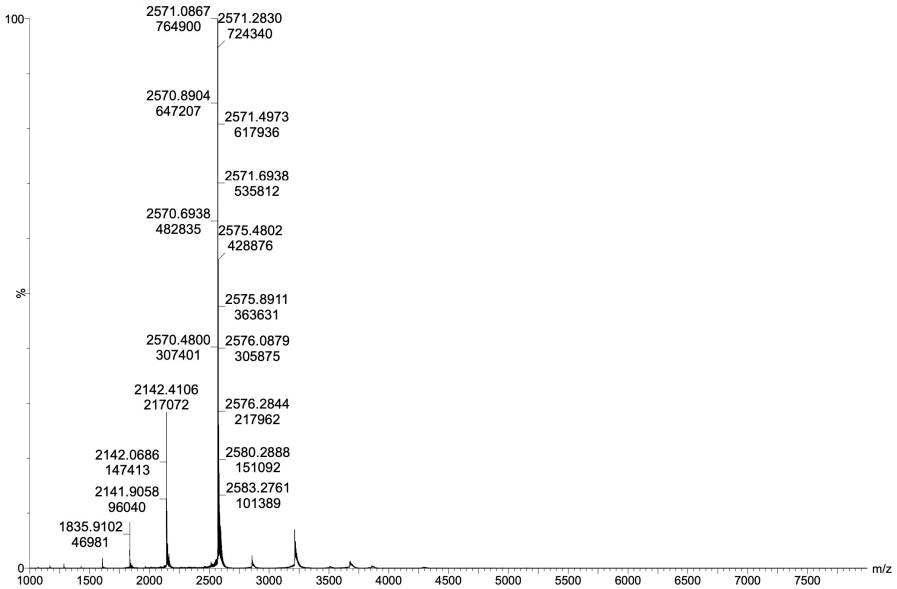


Figure S4 (A) IM-MS of trimer model **6**[3] (8 μM) immediately after dissolution in 25 mM ammonium acetate. "*n*" denotes an integer corresponding to the number of units coexisting in the solution [*n* = 1, 2, 3,... of a trimer model denotes trimer, hexamer, nonamer,..., respectively depending on their drift time. (B) The projection of 2D spectra of **6**.

Table S1 The calculated and observed masses of **6** in **Figure S4**.

E22P, V36tButA-Aβ40 trimer (**6**)

n		1	2	3	4	5	6	7	8	9	10	11	12
mass		12861.3	25722.7	38584.0	51445.3	64306.6	77168.0	90029.3	102890.6	115752.0	128613.3	141474.6	154335.9
z	1	12860.3	25721.6	38583.0	51444.3	64305.6	77167.0	90028.3	102889.6	115751.0	128612.3	141473.6	154334.9
	2	6429.7	12860.3	19291.0	25721.6	32152.3	38583.0	45013.6	51444.3	57875.0	64305.6	70736.3	77167.0
	3	4286.1	8573.2	12860.3	17147.4	21434.5	25721.6	30008.8	34295.9	38583.0	42870.1	47157.2	51444.3
	4	3214.3	6429.7	9645.0	12860.3	16075.7	19291.0	22506.3	25721.6	28937.0	32152.3	35367.6	38583.0
	5	2571.3	5143.5	7715.8	10288.1	12860.3	15432.6	18004.9	20577.1	23149.4	25721.6	28293.9	30866.2
	6	2142.5	4286.1	6429.7	8573.2	10716.8	12860.3	15003.9	17147.4	19291.0	21434.5	23578.1	25721.6
	7	1836.3	3673.7	5511.0	7348.3	9185.7	11023.0	12860.3	14697.7	16535.0	18372.3	20209.7	22047.0
	8	1606.7	3214.3	4822.0	6429.7	8037.3	9645.0	11252.7	12860.3	14468.0	16075.7	17683.3	19291.0
	9	1428.0	2857.1	4286.1	5715.1	7144.2	8573.2	10002.2	11431.3	12860.3	14289.4	15718.4	17147.4
	10	1285.1	2571.3	3857.4	5143.5	6429.7	7715.8	9001.9	10288.1	11574.2	12860.3	14146.5	15432.6
	11	1168.2	2337.4	3506.6	4675.8	5845.1	7014.3	8183.5	9352.7	10521.9	11691.1	12860.3	14029.5
	12	1070.8	2142.5	3214.3	4286.1	5357.9	6429.7	7501.4	8573.2	9645.0	10716.8	11788.5	12860.3
	13	988.3	1977.7	2967.0	3956.3	4945.7	5935.0	6924.3	7913.7	8903.0	9892.3	10881.7	11871.0
	14	917.7	1836.3	2755.0	3673.7	4592.3	5511.0	6429.7	7348.3	8267.0	9185.7	10104.3	11023.0
	15	856.4	1713.8	2571.3	3428.7	4286.1	5143.5	6000.9	6858.4	7715.8	8573.2	9430.6	10288.1
	16	802.8	1606.7	2410.5	3214.3	4018.2	4822.0	5625.8	6429.7	7233.5	8037.3	8841.2	9645.0
	17	755.5	1512.1	2268.6	3025.2	3781.7	4538.3	5294.8	6051.4	6807.9	7564.5	8321.0	9077.6
	18	713.5	1428.0	2142.5	2857.1	3571.6	4286.1	5000.6	5715.1	6429.7	7144.2	7858.7	8573.2
	19	675.9	1352.8	2029.7	2706.6	3383.6	4060.5	4737.4	5414.3	6091.2	6768.1	7445.0	8121.9
	20	642.1	1285.1	1928.2	2571.3	3214.3	3857.4	4500.5	5143.5	5786.6	6429.7	7072.7	7715.8
	21	611.4	1223.9	1836.3	2448.8	3061.2	3673.7	4286.1	4898.5	5511.0	6123.4	6735.9	7348.3
	22	583.6	1168.2	1752.8	2337.4	2922.0	3506.6	4091.2	4675.8	5260.4	5845.1	6429.7	7014.3
	23	558.2	1117.4	1676.6	2235.7	2794.9	3354.1	3913.3	4472.5	5031.7	5590.9	6150.1	6709.3
	24	534.9	1070.8	1606.7	2142.5	2678.4	3214.3	3750.2	4286.1	4822.0	5357.9	5893.8	6429.7
	25	513.4	1027.9	1542.4	2056.8	2571.3	3085.7	3600.2	4114.6	4629.1	5143.5	5658.0	6172.4
	26	493.7	988.3	1483.0	1977.7	2472.3	2967.0	3461.7	3956.3	4451.0	4945.7	5440.3	5935.0
	27	475.3	951.7	1428.0	1904.4	2380.7	2857.1	3333.4	3809.8	4286.1	4762.4	5238.8	5715.1
	28	458.3	917.7	1377.0	1836.3	2295.7	2755.0	3214.3	3673.7	4133.0	4592.3	5051.7	5511.0
	29	442.5	886.0	1329.5	1773.0	2216.5	2660.0	3103.5	3546.9	3990.4	4433.9	4877.4	5320.9
	30	427.7	856.4	1285.1	1713.8	2142.5	2571.3	3000.0	3428.7	3857.4	4286.1	4714.8	5143.5

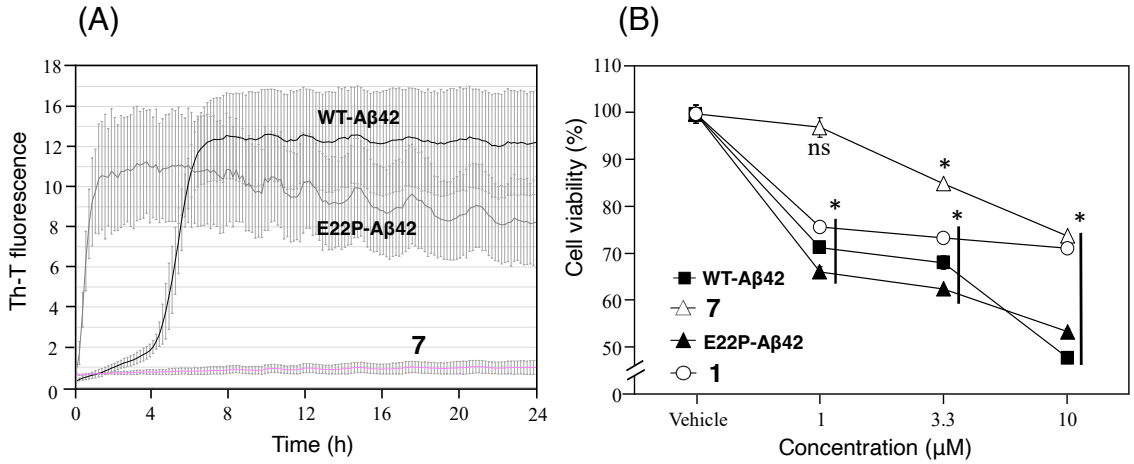


Figure S5 (A) Th-T fluorescence of the dimer model **7**. Concentration of **7** was 10 μM, and that of WT- and E22P-Aβ42 was 20 μM. Data are expressed as mean ± SD (n = 8). Th-T fluorescence of the dimer model **1** was reported previously; the Th-T fluorescence of **1** gradually increased after 12 hr to reach the intensity "2" after 24 hr.[3] (B) MTT assay using SH-SY5Y cells of the dimer models **1** and **7** after 24 hr incubation at 37 ℃ in 10% FBS. Absorbance obtained after adding vehicle (0.15% NH_4OH) was taken 100%. *p < 0.001 versus vehicle, ns: not significant. Data are expressed as mean ± SD (n = 3).

Synthesis of dimer and trimer models of Aβ

General remarks. Analytical thin-layer chromatography was performed with TLC Silica gel 60 F_{254} (Merck, Darmstadt, Germany). Silica gel column chromatography was performed with Wakogel C-200 (Fujifilm Wako Pure Chemical, Osaka, Japan) or Chromatorex BW-300 (Fuji Silica Chemical, Aichi, Japan). HPLC was performed with Model 600E with a Model 2487 UV detector (Waters, Milford, MA, USA). ^{1}H NMR spectra were recorded on an Ascend 400 (Bruker, Germany) at 400 MHz or an AVANCE III 500 (Bruker) at 500 MHz. Chemical shifts were reported relative to Me_4Si (δ 0.0) in $CDCl_3$, and residual solvent of CD_3OD (δ 3.31) and D_2O (δ 4.79). Multiplicity is indicated by one or more of the following: s (singlet); d (doublet); m (multiplet); br (broad). ^{13}C NMR spectra were recorded on an Ascend 400 (Bruker) at 101 MHz or an AVANCE III 500 (Bruker) at 126 MHz. Chemical shifts were reported relative to $CDCl_3$ (δ 77.0) and CD_3OD (δ 49.0). Infrared spectra were recorded on FT-IR-4X Fourier-transform infrared spectrometer (Jasco). Specific rotations were recorded on a P-2200 digital polarimeter (Jasco). Low- and high-resolution mass spectra of dimer and trimer linkers were recorded on JMS700 mass spectrometer (JEOL, Tokyo, Japan) for fast atom bombardment (FAB)-MS (matrix, *m*-nitrobenzyl alcohol), or timsTOF (Bruker) for electrospray ionization (ESI)-MS.

The Aβ dimer and trimer models were synthesized using an Initiator+ Alstra peptide synthesizer (Biotage, Uppsala, Sweden). Analytical HPLC for purity check of the peptides was performed with a Waters model 1525 binary HPLC pump with a model 2489 UV/Visible detector and EmpowerTM3 software (Waters). HR-ESI-qTOF-MS spectra were recorded on a Waters Xevo G2-S qTOF (Waters) and timsTOF (Bruker, Germany). Absorbance on microplates was recorded on microplate readers, Multiskan FC (Thermo Fisher Scientific, Waltham, MA, USA). Fluorescence on microplates was recorded on a Fluoroskan Ascent microplate reader (Thermo Fisher Scientific). HPLC was carried out on YMC PROTEIN-RP column (20 mm I.D. x 150 mm, 5 mm, YMC) and XBridge Peptide BEH C18 OBD^{TM} Prep Column (10 mm I.D. x 250 mm, 300 Å, 5 mm, Waters, Tokyo, Japan).

Materials for synthesis of the linker of the Aβ trimer model (**5**) were obtained from Tokyo Chemical Industry (Tokyo, Japan), Sigma-Aldrich (St. Louis, MO, USA), Fujifilm Wako Pure Chemical, Nacalai Tesque (Kyoto, Japan), and other commercial suppliers, and used without further purification. HATU, CH_2Cl_2, and NH_4OH (25%) were purchased from Fujifilm Wako Pure Chemical. *N*,*N*-Diisopropylethylamine (DIPEA) and Fmoc-L-Val-PEG-PS resin were purchased from Applied Biosystems (Foster City, CA, USA). DMF, trifluoroacetic acid (TFA), *m*-cresol, 1,2-ethanedithiol, thioanisole, and diethyl ether (peroxide free) were purchased from Nacalai Tesque. Piperidine was purchased from Sigma-Aldrich. Protected Fmoc amino acids were purchased from Applied Biosystems, Watanabe Chemical Industries (Hiroshima, Japan), Merck, or PerSeptive Biosystems (Framingham, MA, USA). Synthesis of Aβ peptides **1** and **6** was reported previously.[2,3]

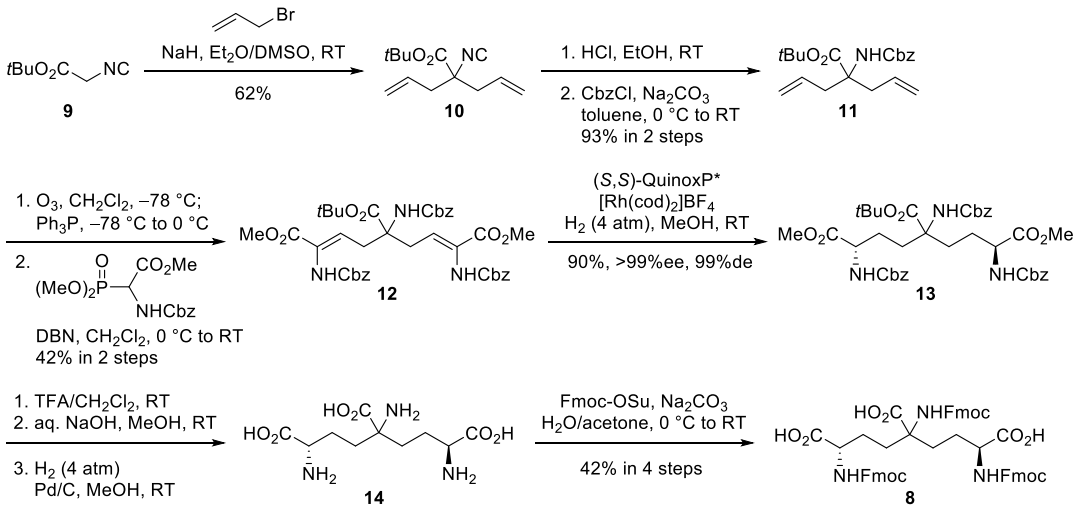
*Synthesis of dimer models **2**–**4**.* The Aβ40 dimer models (**2**–**4**) were synthesized in a stepwise fashion on 0.1 mmol of preloaded Fmoc L-Val TentaGel^{TM} PHB resin (loading: 0.20 mmol/g) supplied by Rapp Polymere (Tübingen, Germany) using the Fmoc method. One-half of a molar equivalent of di-Fmoc-L,L-diaminoadipic acid was used instead of Fmoc-L-Gly at position 38 in order to avoid the formation of the mono-coupled peptides.[2] The position at 17, 26, or 30 was replaced with a cysteine residue. Each coupling reaction was carried out using each Fmoc amino acid (0.4 mmol, 0.5 M in DMF), HATU (0.4 mmol, 0.5 M in DMF), and DIPEA (0.8 mmol, 1.0 M in DMF) for 5 min at 75 °C with microwave irradiation. The coupling reaction of Fmoc-L-His(Trt)-OH was carried out for 10 min at 50 °C to avoid racemization. The coupling reaction of di-Fmoc-L,L-diaminoadipic acid was conducted for 10 min at 75 °C. After each coupling reaction, the N-terminal Fmoc group was deblocked with 20% piperidine in DMF.

To improve the purity and yields of peptides, the pseudo-proline type dipeptide, which is an Fmoc-protected oxazolidine or thiazolidine consisting of two amino acid residues, was employed to disrupt β-sheets and prevent aggregation.[4] Pseudo-proline type dipeptides were employed instead of Fmoc-L-Asp and Fmoc-L-Ser at positions 7/8, and Fmoc-L-Gly and Fmoc-L-Ser at positions 25/26.

After the completion of chain elongation followed by Fmoc deblocking, the resultant peptide resin was washed with DMF and CH_2Cl_2, and shaken for 2 h at room temperature in a mixture containing TFA (16 mL), *m*-cresol (0.4 mL), 1,2-ethanedithiol (1.2 mL), and thioanisole (2.4 mL) for final deprotection and cleavage from the resin. Each crude peptide was precipitated by diethyl ether at 4 °C. A portion of the crude peptide was purified using HPLC (a Model 600E pump with a Model 2487 UV detector, Waters, Milford, MA, USA) on a YMC Pack PROTEIN-RP column (20 mm I.D. × 150 mm, 5 mm, YMC Co., Ltd., Kyoto, Japan) with elution at 8.0 mL/min by an 60-min linear gradient (curve 6) of 20–60% CH_3CN containing 0.1% TFA. Each peptide was oxidized for at least 2 hr in 5% DMSO/0.1% NH_4OH at 5 mg/mL under gentle stirring to form intramolecular disulfide bonds at positions 17, 26, or 30.[5] The reaction mixture was purified again using preparative HPLC described above. Lyophilization produced each peptide, the purity of which was checked by HPLC (Figure S1A–C). Molecular weights and formulae were confirmed by HR-ESI-qTOF-MS measurements (Figure S1A–C).

*Synthesis of dimer model **7**.* For the synthesis of E22P,G38DAA-Aβ42 dimer (**7**), Fmoc-L-Ala TentaGel R PHB resin (loading: 0.21 mmol/g) was employed. After the chain assembly and cleavage from the resin, followed by ether precipitation, a portion of the crude peptide was purified using HPLC on XBridge Peptide BEH C18 OBD^{TM} Prep Column (300 Å, 5 μm, 19 mm × 150 mm, Waters, Tokyo, Japan) with elution at 8.0 mL/min by a 60-min exponential gradient (curve 7) of 20–50% CH_3CN containing 0.1% NH_4OH. Lyophilization produced **7**, the purity of which was checked by HPLC (Figure S1D). Molecular weights and formulae were confirmed by HR-ESI-qTOF-MS measurements (Figure S1D). The yield of **7** was 6.6%.

*Synthesis of trimer model **5***. For the synthesis of E22P,G38di-hA-Gly-Aβ40 trimer (**5**), the one-third molar equivalent of the trimer linker **8** (0.033 mmol) was employed instead of Fmoc-L-Val at position 38 in order to avoid formation of the mono-coupled and/or di-coupled peptides.[3] The trimer linker **8** was synthesized by a similar method reported previously.[6]



Compound **10**: To a suspension of NaH (60%) (741 mg, 18.5 mmol) in dry Et_2O/dry DMSO (20 mL/1.2 mL) was added **9** (538 mg, 3.81 mmol) dropwise under an argon atmosphere at room temperature. The mixture was stirred for 30 min. Allyl bromide (1.20 g, 9.93 mmol) was added and the reaction mixture was stirred at room temperature for 1.5 hr. Five milliliters of H_2O was added dropwise to the reaction mixture at room temperature. The two layers were separated, and the aqueous layer was extracted with EtOAc three times. The organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane/Et_2O = 1/0 to 9/1) to afford **10** (523 mg, 62%) as a colorless oil. ^{1}H NMR: (500 MHz, $CDCl_3$): δ 5.85–5.77 (m, 2H), 5.26–5.20 (m, 4H), 2.62 (dd, *J* = 13.9, 7.5 Hz, 2H), 2.53–2.48 (m, 2H), 1.48 (s, 9H) ppm; ^{13}C NMR: (126 MHz, $CDCl_3$): δ 166.6, 159.0, 130.1 (2C), 120.8 (2C), 83.8, 67.6, 42.7 (2C), 27.8 (3C) ppm; IR (neat on KBr plate): ν_{max} 3084, 2982, 2933, 2139, 1748, 1644, 1371, 1254, 1235, 1148, 993, 927, 843 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{13}H_{19}NO_2Na$: 244.1308 $[M+Na]^+$; found: 244.1306.

Compound **11**: Isocyanide **10** (508 mg, 2.30 mmol) in EtOH (20 mL) was added concentrated HCl, and the resulting mixture was stirred at room temperature for 2 hr. The solvent was removed under reduced pressure, and the remaining oil was taken up in toluene (20 mL). The mixture was cooled to 0 °C, and CbzCl (1.20 mg, 7.03 mmol) and 1 M aqueous Na_2CO_3 solution (11 mL) were added. The reaction mixture was stirred at room temperature for 3.5 hr. The two layers were separated, and the aqueous layer was extracted with EtOAc three times. The organic layers were washed with 0.1

M aqueous HCl solution and brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane/EtOAc = 1/0 to 9/1) to afford **11** (738 mg, 93% in 2 steps) as a colorless oil. ^{1}H NMR: (400 MHz, $CDCl_3$): δ 7.40–7.28 (m, 5H), 5.81 (s, 1H), 5.67–5.56 (m, 2H), 5.08–5.06 (m, 4H), 5.05–5.03 (m, 2H), 3.08 (dd, *J* = 13.8, 7.1 Hz, 2H), 2.51–2.45 (m, 2H), 1.45 (s, 9H) ppm; ^{13}C NMR: (101 MHz, $CDCl_3$): δ 171.5, 154.1, 136.8 (2C), 132.2, 128.4 (2C), 127.9, 127.8 (2C), 118.8 (2C), 82.5, 66.1, 63.2, 39.7 (2C), 28.0 (3C) ppm; IR (neat on KBr plate): ν_{max} 3418, 3078, 2980, 2932, 1718, 1642, 1498, 1245, 1159, 1085, 921, 845, 698 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{20}H_{28}NO_4$: 346.2018 $[M+H]^+$; found: 346.2023.

Compound **12**: A solution of **11** (615 mg, 1.78 mmol) in CH_2Cl_2 (20 mL) was cooled to −78 °C. The solution was bubbled with O_3 until its color turned blue (8 min). Excess O_3 was removed from the reaction mixture by purging with O_2 for 3 min. To the mixture was added Ph_3P (1.39 g, 5.31 mmol) at −78 °C. The reaction mixture was stirred at −78 °C for 10 min and warmed to 0 °C with stirring for an additional 30 min. The reaction mixture was washed with water, and the aqueous layer was extracted with $CHCl_3$ three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane/EtOAc = 7/3 to 2/3) to afford a mixture containing dialdehyde as a colorless oil. To a solution of (*Z*)-α-phosphonoglycine trimethyl ester (1.26 g, 3.79 mmol) in CH_2Cl_2 (20 mL) was added DBN (468 mg, 3.77 mmol). The mixture was stirred at 0 °C for 10 min, to which a solution of the above-mentioned mixture containing dialdehyde in CH_2Cl_2 (20 mL) was then added slowly at 0 °C. The reaction mixture was gradually warmed to room temperature with stirring overnight and was washed with 1 M aqueous HCl solution. The aqueous layer was further extracted with EtOAc three times. The organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography carefully (hexane/EtOAc = 1/0 to 1/1, then toluene/EtOAc = 9/1 to 4/1) to afford **12** (571 mg, 42% in 2 steps) as a colorless oil. ^{1}H NMR: (500 MHz, $CDCl_3$): δ 7.39–7.27 (m, 15H), 6.46 (br s, 2H), 6.29–6.26 (m, 2H), 6.01 (s, 1H), 5.15 (s, 4H), 5.04 (s, 2H), 3.72 (s, 6H), 3.26 (dd, *J* = 15.1, 8.1 Hz, 2H), 2.72 (dd, *J* = 15.7, 6.6 Hz, 2H), 1.43 (s, 9H) ppm; ^{13}C NMR: (126 MHz, $CDCl_3$): δ 170.9, 164.5 (2C), 154.8, 153.9 (2C), 136.2 (3C), 135.9 (2C), 128.54 (4C), 128.52 (2C), 128.3 (2C), 128.23 (4C), 128.17 (2C), 128.1, 128.0 (2C), 83.9, 67.5 (2C), 66.7, 62.1, 52.5 (2C), 34.8 (2C), 27.7 (3C) ppm; IR (neat on KBr plate): ν_{max} 3323, 3033, 2953, 1714, 1661, 1505, 1228, 1045, 754, 698 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{40}H_{46}N_3O_{12}$: 760.3081 $[M+H]^+$; found: 760.3086.

Compound **13**: A solution of $[Rh(cod)_2]BF_4$ (2.1 mg, 5.2 μmol) and (*S*,*S*)-QuinoxP* (2.1 mg, 6.3 μmol) in degassed MeOH (0.5 mL) was stirred vigorously at room temperature under a nitrogen atmosphere. After 50 min, additional degassed MeOH (0.5 mL) was added to the mixture. Compound **12** (28.4 mg, 37.4 μmol) was charged in a hydrogenation bottle. After the bottle was evacuated and filled with hydrogen several times, the catalyst solution was added. The hydrogen pressure was

adjusted to 4 atm, and the mixture was stirred vigorously overnight at room temperature. The reaction mixture was concentrated, and the crude product was purified by silica gel column chromatography (hexane/EtOAc = 7/3 to 1/1) to afford **13** (25.8 mg, 90%) as a colorless oil; ee and de were determined by HPLC (Daicel CHIRAL CEL OD-RH, λ = 254 nm, CH_3CN/H_2O = 11/9, 0.5 mL/min, >99%ee, 99%de). $[\alpha]_D^{23}$ +20.9 (*c* = 0.91 in $CHCl_3$); ^{1}H NMR: (500 MHz, $CDCl_3$): δ 7.36–7.27 (m, 15H), 5.84 (s, 1H), 5.32–5.29 (m, 2H), 5.13–4.98 (m, 6H), 4.35–4.30 (m, 2H), 3.72 (s, 3H), 3.67 (s, 3H), 2.42–2.27 (m, 2H), 1.84–1.52 (m, 6H), 1.42 (s, 9H) ppm; ^{13}C NMR: (126 MHz, $CDCl_3$): δ 172.3, 172.2, 171.6, 155.73, 155.67, 153.7, 136.6, 136.2 (2C), 128.49 (4C), 128.48 (2C), 128.14 (2C), 128.07 (4C), 128.0, 127.8 (2C), 83.2, 66.9 (2C), 66.2, 63.0, 53.4, 53.3, 52.42, 52.41, 31.0, 30.8, 27.7 (3C), 27.1, 26.9 ppm; IR (neat on KBr plate): ν_{max} 3364, 3034, 2953, 1714, 1505, 1216, 1060, 698 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{40}H_{50}N_3O_{12}$: 764.3394 $[M+H]^+$; found: 764.3400.

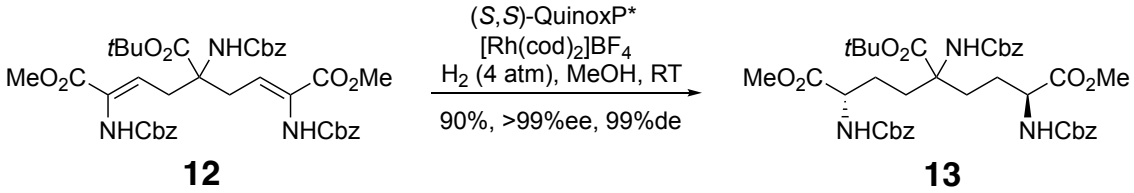
Compound **8**: To a solution of **13** (240 mg, 314 μmol) in CH_2Cl_2 (3 mL) was added TFA (3 mL) at room temperature. The mixture was stirred for 2 hr. The volatile component in the reaction mixture was removed under reduced pressure. The residue was reconstituted in Et_2O and washed with saturated aqueous NH_4Cl solution. The aqueous layer was further extracted twice with Et_2O. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to afford monocarboxylic acid as a pale-yellow oil. To a solution of the monocarboxylic acid in MeOH (10 mL) was added a 2 M aqueous NaOH solution (1.57 mL). The reaction mixture was stirred at room temperature for 1 hr. The volatile component in the reaction mixture was removed under reduced pressure. The residual water layer was adjusted to a pH of 1 using 1 M aqueous HCl solution and extracted with EtOAc three times. The organic layers were washed with brine and dried over Na_2SO_4. Filtration and concentration afforded a crude tricarboxylic acid as a white foam. To a solution of the tricarboxylic acid in MeOH (5 mL) in a hydrogenation bottle was added Pd/C (10 wt%, 77.7 mg). The bottle was filled with hydrogen and the hydrogen pressure was adjusted to 4 atm. The reaction mixture was stirred overnight under a hydrogen atmosphere at room temperature. The reaction mixture was filtered through celite and washed with H_2O to afford **14** as a white solid. ^{1}H NMR: (500 MHz, D_2O): δ 3.89–3.73 (m, 2H), 2.01–1.75 (m, 8H) ppm; ^{13}C NMR: (126 MHz, D_2O): δ 174.0, 173.6, 173.4, 63.6, 54.1, 53.8, 32.0, 31.1, 25.0, 24.6 ppm.

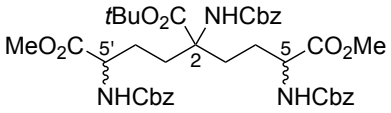
To a suspension of **14** in H_2O/acetone (5 mL/5 mL) were added Fmoc-OSu (289 mg, 859 μmol) and Na_2CO_3 (156 mg, 1.47 mmol) at 0 °C. The reaction mixture was gradually warmed to room temperature with stirring for 2 days. The reaction mixture was adjusted to a pH of 1 using 1 M aqueous HCl solution and extracted with EtOAc three times. The organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (hexane/EtOAc = 7/3 to 3/7, 1% acetic acid) and HPLC (YMC Pack ODS-A No.2015000608, λ = 254 nm, CH_3CN/H_2O = 7/3, 0.1% TFA, 8.0 mL/min, retention time 17.6 min) to afford **8** (126 mg, 42% in 4 steps) as a white solid. $[\alpha]_D^{24}$ +4.28 (*c* = 0.74 in MeOH); ^{1}H NMR: (500 MHz, CD_3OD): δ 7.76–7.55 (m, 12H), 7.36–7.25 (m, 12H), 4.35–4.12 (m, 11H), 2.29–1.43 (m, 8H)

ppm; ^{13}C NMR: (126 MHz, CD_3OD): δ 176.0, 175.4, 175.2, 158.5 (2C), 156.4, 145.32, 145.30, 145.28, 145.26, 145.2 (2C), 142.5 (6C), 128.7 (6C), 128.2 (6C), 126.32 (2C), 126.29 (2C), 126.2 (2C), 120.9 (6C), 68.1 (2C), 67.7, 63.5, 55.2, 55.1, 48.4, 48.3 (2C), 32.3, 32.2, 27.3 (2C) ppm; IR (neat on KBr plate): ν_{max} 3397, 3333, 3065, 2952, 2554, 1713, 1512, 1450, 1337, 1215, 740 cm^{-1}; HRMS (FAB): *m/z* calcd for $C_{55}H_{49}N_3O_{12}Na$: 966.3214 $[M+Na]^+$; found: 966.3233.

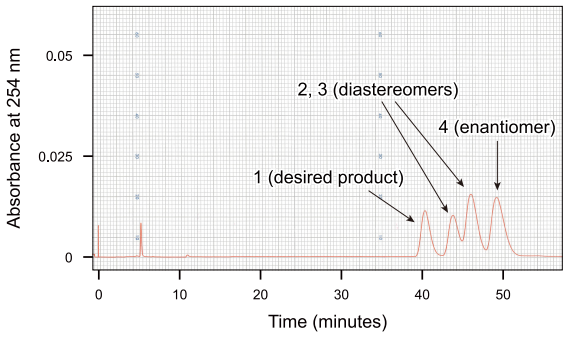
For the synthesis of E22P,G38di-hA-Gly-Aβ40 trimer (**5**), Fmoc-L-Val-PEG-PS resin (loading: 0.18 mmol/g) supplied by Applied Biosystems (Foster City, CA, USA) was employed. The one-third molar equivalent of the trimer linker (0.033 mmol) was employed instead of Fmoc-L-Gly at position 38 in order to avoid formation of the mono-coupled and/or di-coupled peptides. The coupling reaction of the trimer linker was carried out for 20 min at 75 °C.

The crude peptide was precipitated by diethyl ether at 4 ℃, followed by purification using HPLC on a YMC PROTEIN-RP column with elution at 8.0 mL/min by an 80-min linear gradient (curve 6) of 20–60% CH_3CN containing 0.1% TFA. Subsequent purification was carried out using XBridge Peptide BEH C18 OBD^{TM} Prep Column (300 Å, 5 μm, 10 mm × 250 mm, Waters, Tokyo, Japan) with elution at 5.5 mL/min by a 60-min exponential gradient (curve 7) of 30–50% CH_3CN containing 0.1% TFA. Lyophilization produced **5**, the purity of which was checked by HPLC (Figure S1E). Molecular weights and formulae were confirmed by HR-ESI-qTOF-MS measurements (Figure S1E). The yield of **5** was 0.72%.

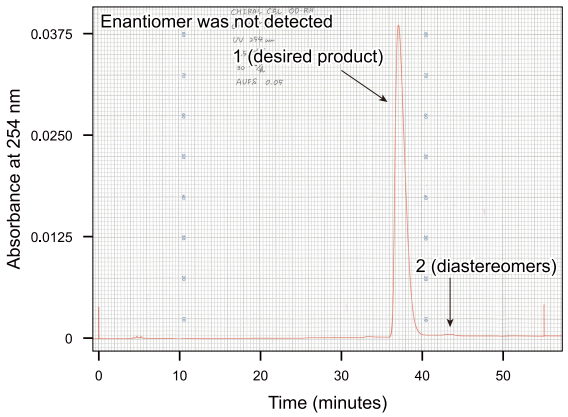


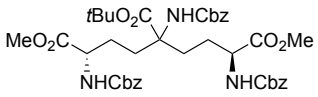


(5*S*,5'*S*)-, (2*S*,5*S*,5'*R*)-,
(2*R*,5*S*,5'*R*)-, (5*R*,5'*R*)-**13**



Peak No.	Ret. time (minutes)	Weight of paper (mg)	(%)
1	40.5	24.8	18.5
2, 3	43.8, 46.1	64.6	48.3
4	49.3	44.4	33.2





13

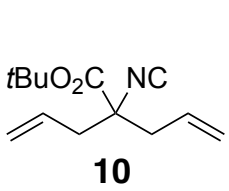
Peak No.	Ret. time (minutes)	Weight of paper (mg)	(%)
1	35.2	178.4	99.7
2	43.5	0.6	0.3

Enantiomer (4) was not detected.

Figure S6 HPLC profiles for ee and de determination of **13**; ee and de were calculated from the peak area of each stereoisomer in HPLC (Daicel CHIRAL CEL OD-RH, λ = 254 nm, CH_3CN/H_2O = 11/9, 0.5 mL/min) by weighing each peak.

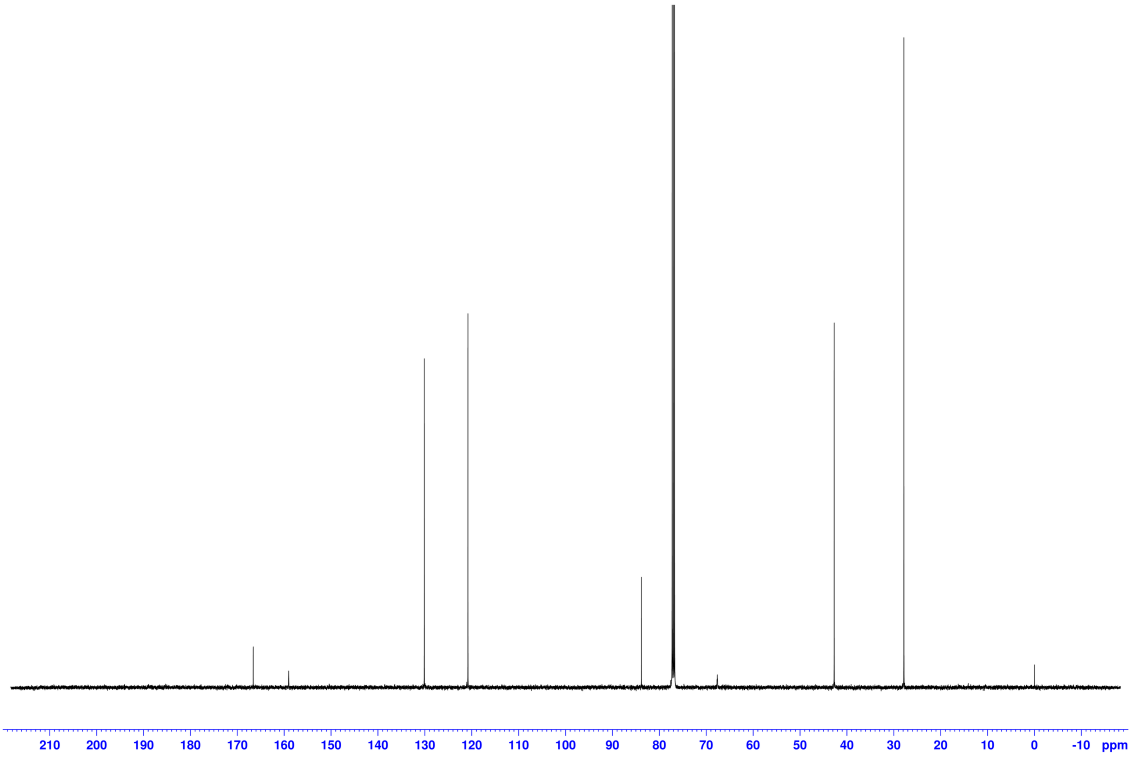
NMR spectra of 10, 11, 12, 13, 14 and 8

^{1}H NMR spectrum of **10** (500 MHz, $CDCl_3$, 297 K, 0.070 M)

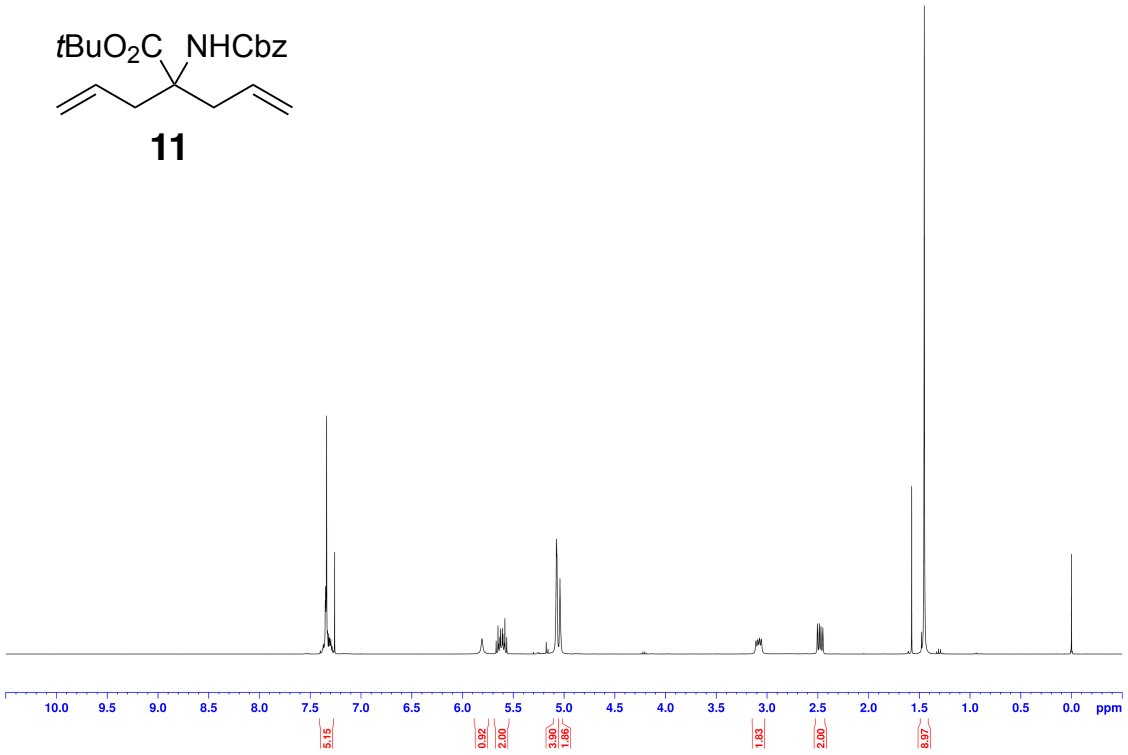


10

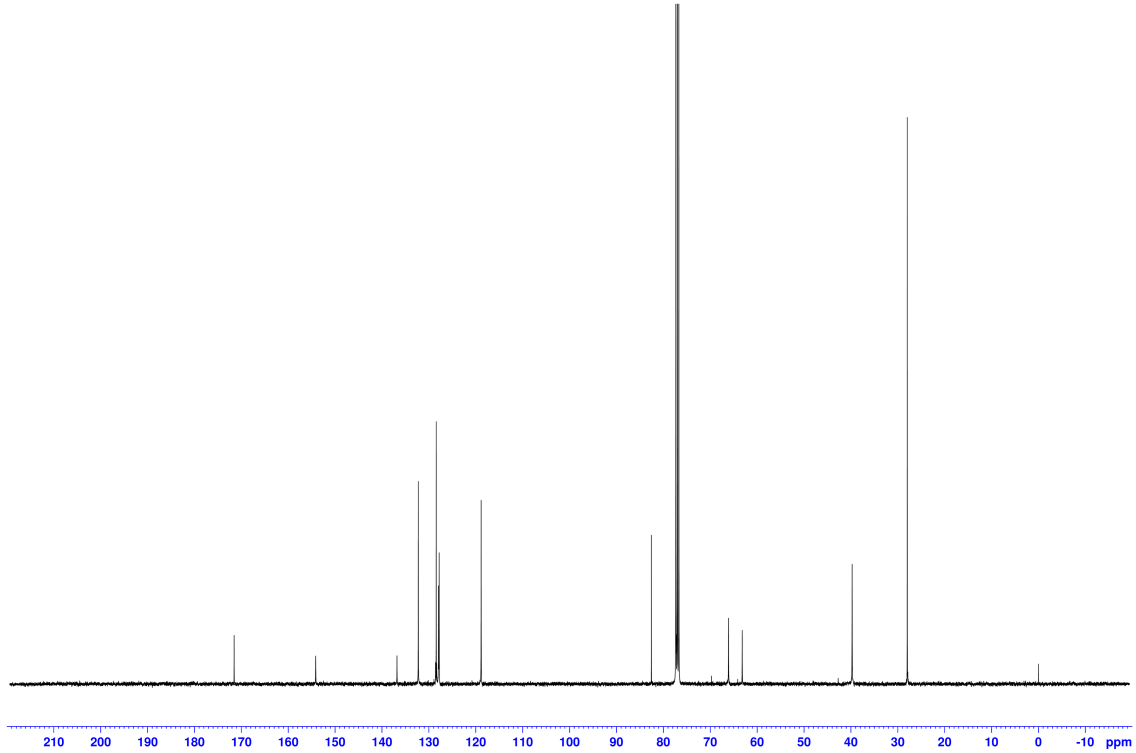
^{13}C NMR spectrum of **10** (126 MHz, $CDCl_3$, 298 K, 0.070 M)



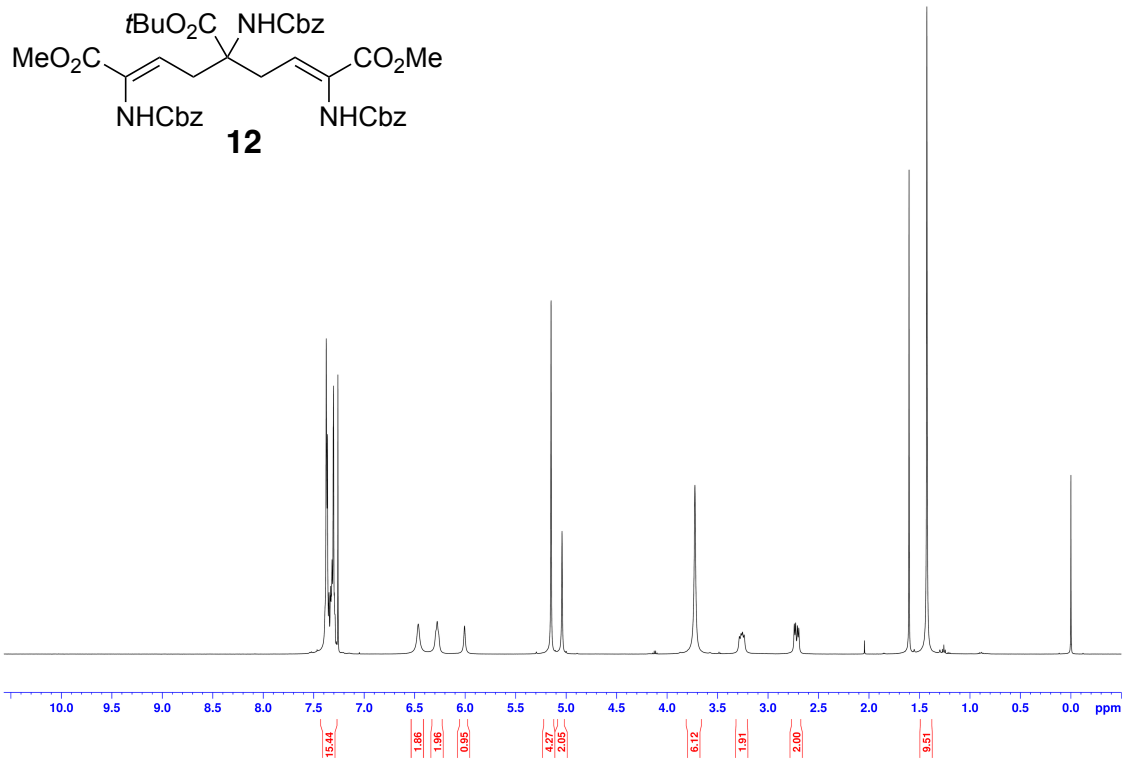
^{1}H NMR spectrum of **11** (400 MHz, $CDCl_3$, 298 K, 0.049 M)



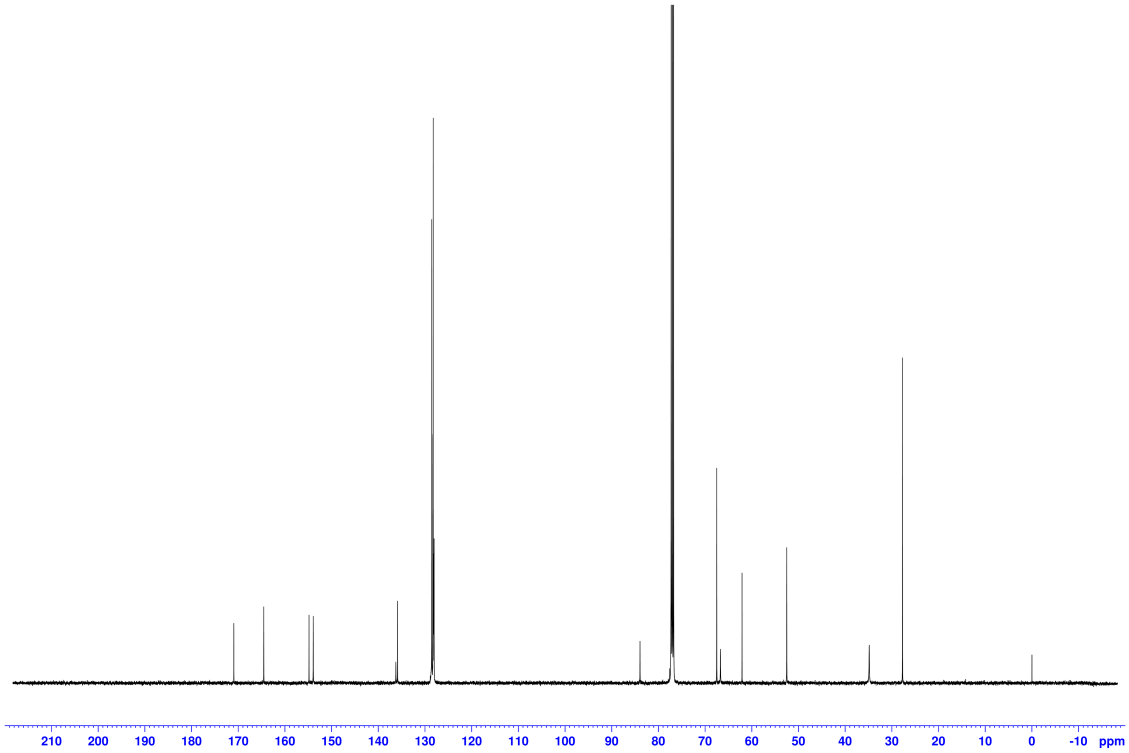
^{13}C NMR spectrum of **11** (101 MHz, $CDCl_3$, 299 K, 0.049 M)



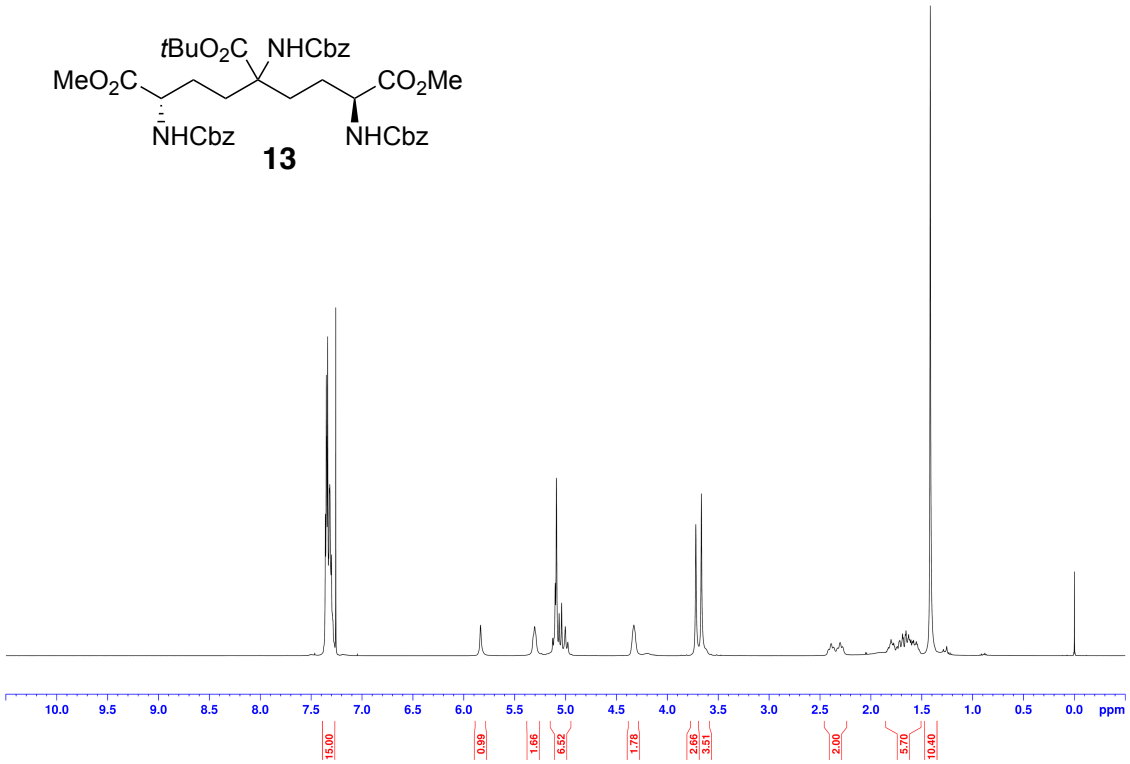
^{1}H NMR spectrum of **12** (500 MHz, $CDCl_3$, 297 K, 0.026 M)



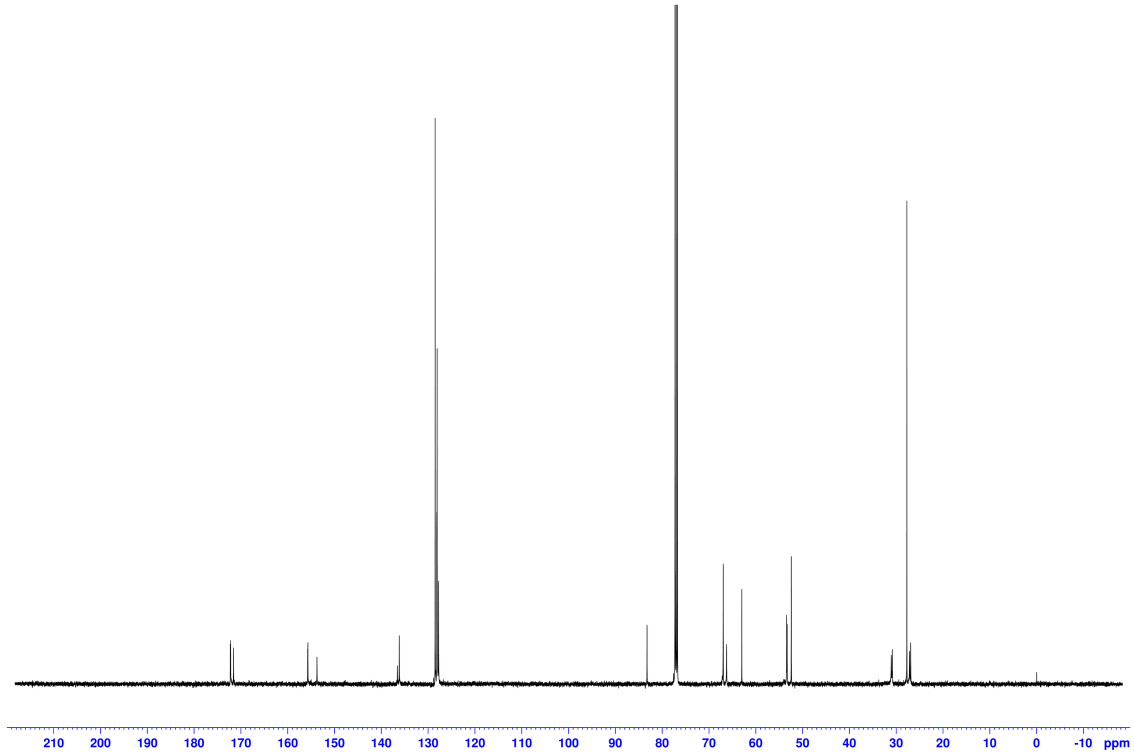
^{13}C NMR spectrum of **12** (126 MHz, $CDCl_3$, 298 K, 0.026 M)



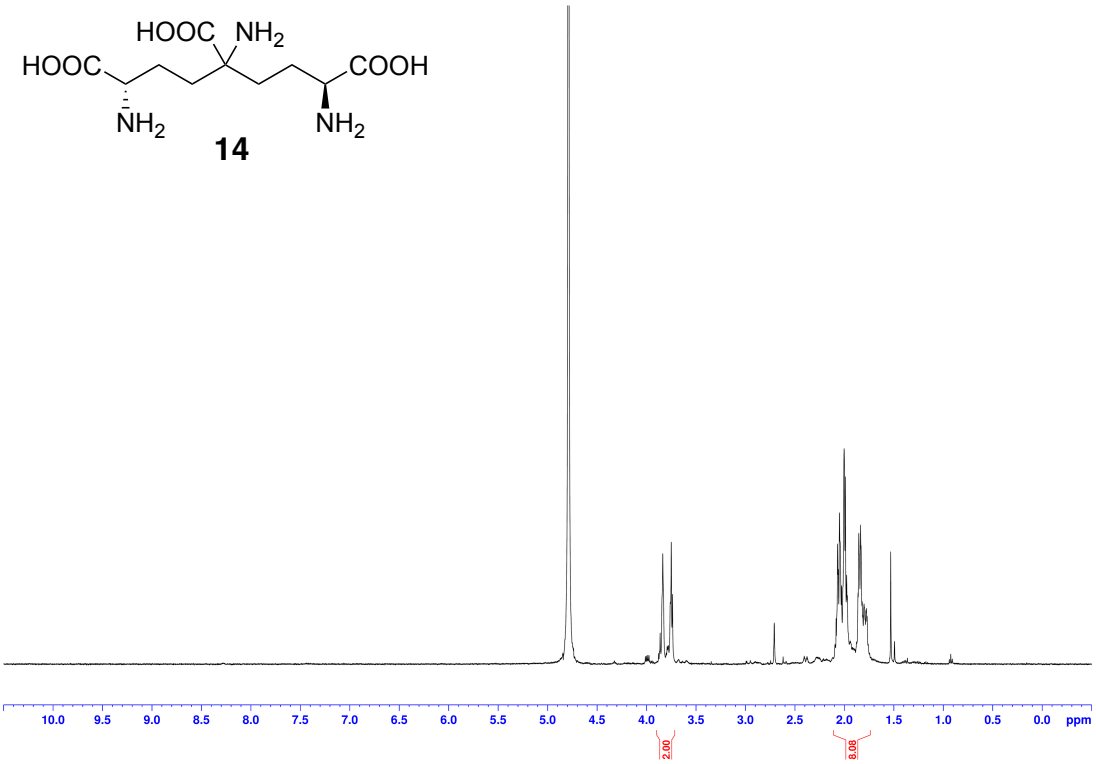
^{1}H NMR spectrum of **13** (500 MHz, $CDCl_3$, 300 K, 0.068 M)



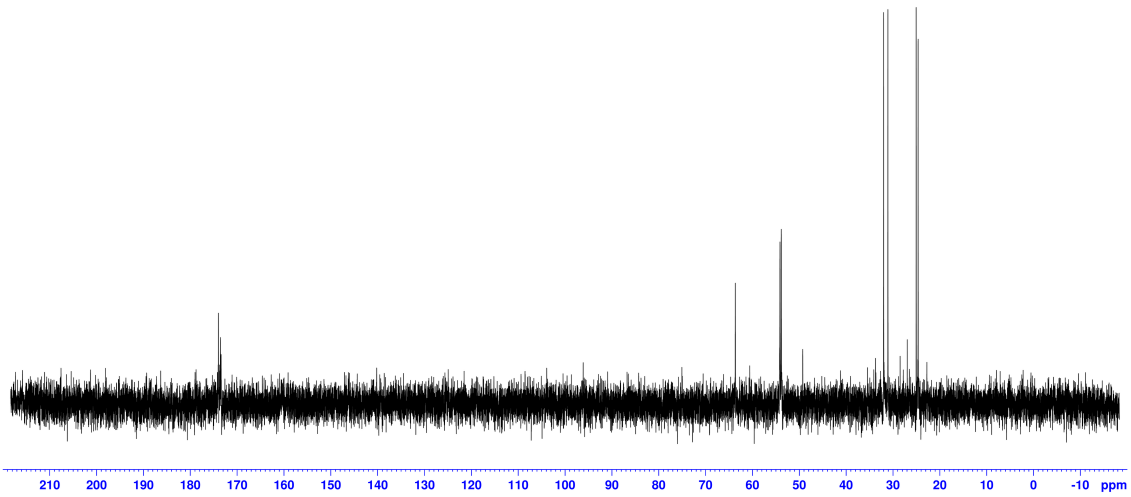
^{13}C NMR spectrum of **13** (126 MHz, $CDCl_3$, 300 K, 0.068 M)



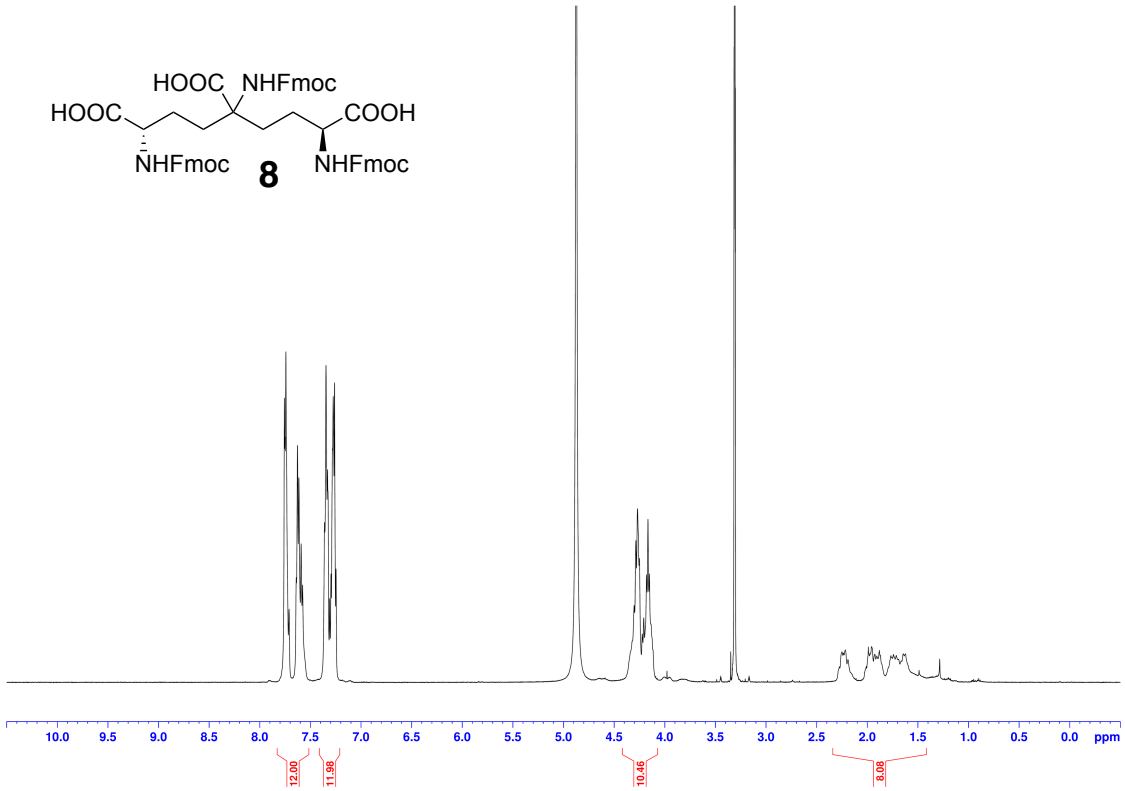
^{1}H NMR spectrum of **14** (500 MHz, D_2O, 297 K, 0.028 M)



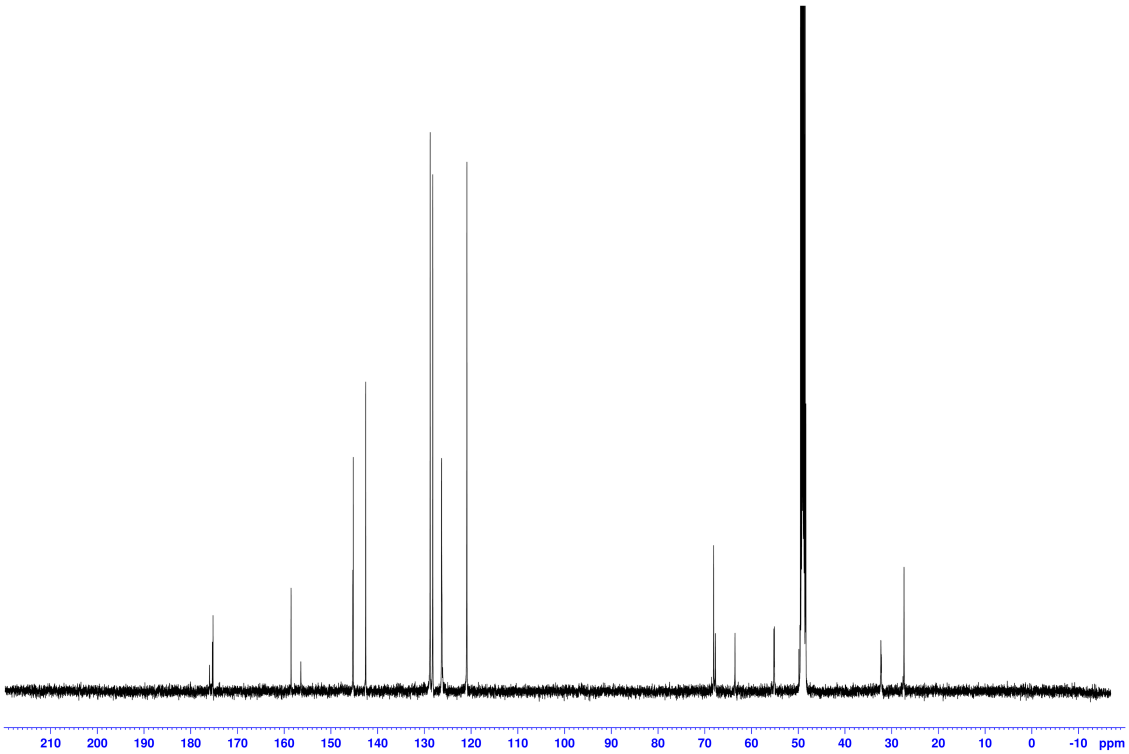
^{13}C NMR spectrum of **14** (126 MHz, D_2O, 298 K, 0.028 M)



^{1}H NMR spectrum of **8** (500 MHz, CD_3OD, 300 K, 0.016 M)



^{13}C NMR spectrum of **8** (126 MHz, CD_3OD, 300 K, 0.016 M)



MTT assay

MTT assay was carried out using the method reported previously.[2,3,5] A human neuroblastoma SH-SY5Y cell line (Figure S5: ATCC, Manassas, VA, USA; Figure S2: ECACC, UK) was maintained in a 1:1 mixture of Eagle's minimum essential medium and Ham's F12 medium (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) containing 10% fetal bovine serum (Biological Industries, Beit Heamek, Israel) and 1% MEM non-essential amino acid (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Each Aβ dimer was dissolved in 0.1% NH_4OH (Figure S2) or 0.15% NH_4OH (Figure S5) to give a 110 μM (Figure S5) or 55 μM (Figure S2) stock solution, which was diluted with 0.1% NH_4OH to appropriate concentrations (11 and 36 μM, or 0.44, 2.2, and 11 μM), and each 10 μL aliquot was subsequently added to 100 μL of the culture medium containing near-confluent cells (2×10^4 cells/well) to give final concentrations, 1, 3.3, and 10 μM (Figure S5) or 0.04, 0.2, 1, and 5 μM (Figure S2). After being treated at 37 °C for 24 hr in 10% FBS (Figure S5, Biological Industries, Beit Heamek, Israel) or 5% FBS (Figure S2, SERANA, Germany), 15 μL/well of Dye solution in CellTiter 96 Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA) was added to the cells, followed by a 4 hr incubation at 37 °C. The solubilization/stop solution (100 μL/well) was subsequently added to the cells. Next, the resultant cell lysate was incubated overnight in the dark at room temperature before performing measurements at 570 nm using a microplate reader (Multiskan FC, Thermo Fisher Scientific). Absorbance obtained by the addition of vehicle (0.1% NH_4OH) was taken as 100%.

Thioflavin-T (Th-T) assay

The aggregative ability of each Aβ was assessed by Th-T fluorescence assay. Th-T fluorescence was measured automatically based on the previously described procedure with slight modifications.[2,3,5] For instance, 390 μL of 50 mM sodium phosphate and 100 mM NaCl was aliquoted into a 1.5 mL tube, and 10 μL of 1 mM Th-T solution in distilled water was added. Each Aβ was dissolved with 0.1% NH_4OH (Figure S2) or 0.15% NH_4OH (Figure S5) and adjusted to the desired concentration. One hundred μL of each Aβ solution was added to the tube so as to become the final desired concentration (10 μM or 20 μM). Each solution (100 μL) was aliquoted into a 96-well black plate and fluorescence was measured automatically every 10 min at 430 nm excitation and 485 nm emission using a microplate reader (Fluoroskan Ascent, Thermo Fisher Scientific). For each assay, a vehicle control without Aβ solution was subtracted as blank to calculate relative fluorescence.

Ion mobility-mass spectrometry (IM-MS)

IM-MS was measured by the method reported previously.[2,3] Compound **6** was dissolved in 0.2% NH_4OH at 80 μM, respectively, followed by a 10-fold dilution with 25 mM ammonium acetate. The sample was centrifuged for 5 min at 18,000 *g* before infusion into the MS apparatus using a glass

capillary (Nanoflow Probe Tip, Waters, Milford, MA, USA). Mass spectra and ion mobility experiments were conducted on SYNAPT XS HDMS (Waters, Milford, MA, USA) using a nano electrospray as an ionization source. The instrument was operated in negative ion mode with a capillary voltage of 0.7–1.0 kV, a sample cone voltage of 150 V, and a source temperature of 50 °C. For the ion mobility measurement, nitrogen gas was used in an ion mobility cell, and the cell pressure was maintained at approximately 2.83 mbar with a wave velocity of 650 m/s and a wave height of 40 V. Data acquisition and processing were performed with the MassLynx (V4.2) and DriftScope (V3.0) software supplied with the instrument. The CsI cluster ions were used for *m/z* scale as a calibrator.

Statistical analysis

The data in Figures S2 and S5 were analyzed with Tukey's HSD test using the R (version 4.4.1) software.

Sample preparation

Sample preparation was conducted following a previously established protocol with minor modifications.[7,8] Synthesized Aβ solutions stored at –80 °C until required was diluted at a concentration of about 1–10 μM in cold (4 °C) 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) solvent and sonicated for 10 minutes. A volume of 2.5–10 μL of this solution was deposited onto freshly cleaved mica discs (V-1 grade, 9.5 nm × 0.15 nm, Alliance Biosystems Co., Ltd, Osaka, Japan) and rapidly dried under vacuum. HFIP, owing to its rapid drying characteristics and amphiphilic nature, serves as an ideal solvent to minimize artifacts on the mica surface. These artifacts often arise from varying hydration landscapes that promote the aggregation of Aβ and other crystals from the buffer.[12] The mica was affixed to magnetic stainless steel using carbon tape to enable mounting on the AFM observation stage. Mica samples were stored in a hinged case within a desiccator until needed to prevent moisture and static electricity.[9,10]

AFM observation

Topography images of the specimens were acquired using an AFM MultiMode 8 (Bruker, USA) operating in air at room temperature and humidity. The system was equipped with silicon cantilevers (RTESP-150, Bruker, USA) with a spring constant of 5 N/m (nominal value) and a tip radius of 8–12 nm. Measurements were performed in tapping mode with a resonance frequency of approximately 130 kHz and a scan rate of 0.7 Hz (1 line/second). Tapping mode is a Bruker method suitable for AFM observation of soft matter. AFM topography images (1 x 1 μm, 512 x 512 pixels) were acquired using NanoDrive software (v8.02, Bruker, USA).

Height histograms distribution & python program

Cross-sectional analysis of particles within AFM images was automatically performed by incorporating original Python code into the AFM image processing software Gwyddion (v.2.56, Czech Metrology Institute, Czech Republic). This code is based on the concept of separating the topography noise of the mica substrate (0.2 nm) from the particle height (>0.2 nm) before performing cross-sectional analysis, systematically explores cross-sections that intersect each particle at its highest point, ensuring no other particles are intersected, by varying the angle of the cross-section.[11] The AFM images automatically cross-sectionally analyzed by the Python program were later manually checked to confirm that the maximum height of the spherical particles was accurately selected. Igor Pro 9 (HULINKS Inc., Japan) was used for Gaussian fitting and histogram creation. Scan noise and tip curvature artifacts were removed by Gaussian fitting.[12,13] Height histograms obtained from AFM topography images were also analyzed by Gaussian fitting. The method and interpretation of Gaussian fitting to height histograms have been explained in our previous paper.[7,8] The median value of the Gaussian fitting curve fitted to the histogram represents the characteristic height value of particles in the Aβ solution. The height values of spherical Aβ42 appearing in the circular contrast of AFM data were measured from cross-sectional analysis, as this is more accurate than direct determination of width from AFM measurements, which may not be absolute due to convolution of the AFM tip and particle radius.

Acknowledgement

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