Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

for

Distinct impact of glycation towards the aggregation and toxicity of murine and human amyloid-β

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Materials and Methods. All reagents were purchased from commercial suppliers and used as received unless otherwise stated. Murine $A\beta_{40}$ (DAEFGHDSGFEVRHQKLVFFAEDVGSN-KGAIIGLMVGGVV) and human $A\beta_{40}$ (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVG-GVV) were purchased from AnaSpec (Fremont, CA, USA). DAE was obtained from Anygen (Gwangju, Republic of Korea). Methylglyoxal (**MG**) and lysine (Lys) were acquired from Sigma-Aldrich (St Louis, MO, USA). HEPES [2-(4-(2-hydroxyethyl)piperazin-1-yl)ethanesulfonic acid] was obtained from Sigma-Aldrich. Double-distilled water (ddH_2O) used for all experiments was obtained from a Milli-Q Direct 16 system (18.2 MΩ·cm; Merck KGaA, Darmstadt, Germany). Trace metal contamination was removed from all solutions used for Aβ experiments by treating with Chelex (Sigma-Aldrich) overnight. The concentrations of peptides were determined by a Shimadzu 1900i UV−visible (UV−Vis) spectrophotometer (Shimadzu, Kyoto, Japan). ESI–MS experiments were performed by an Agilent 6530 Accurate Mass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer with an ESI source (Agilent, Santa Clara, CA, USA). The values of absorbance and fluorescence for biological assays were acquired by a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA). The membranes obtained by gel/Western blot were visualized by a ChemiDoc MP Imaging System (Bio-Rad, Hercules, CA, USA). Morphologies of peptide aggregates were recorded on a Tecnai F20 TEM [FEI Company, Eindhoven, Netherlands; KAIST Analysis Center for Research Advancement (KARA), Daejeon, Republic of Korea]. The secondary structure of peptides was analyzed by a JASCO-815 150-L CD spectropolarimeter (Jasco Inc., Tokyo, Japan; KARA). MALDI–MS studies were carried out by a Bruker Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA; KARA).

Preparation of Aβ Peptides. Murine $A\beta_{40}$ ($mA\beta_{40}$) and human $A\beta_{40}$ ($hA\beta_{40}$) were dissolved in ammonium hydroxide (NH_4OH; 1% w/w, aq). The resulting solutions were aliquoted, lyophilized overnight, and stored at –80 °C. A stock solution of $mA\beta_{40}$ and $hA\beta_{40}$ was then prepared by dissolving the lyophilized peptide with NH_4OH (1% w/w, aq; 10 μL) and diluting with ddH_2O, as previously reported.[1] The concentrations of $mA\beta_{40}$ and $hA\beta_{40}$ were determined by measuring the absorbance of the solution at 205 nm (for $mA\beta_{40}$, ε = 157,200 $M^{-1}cm^{-1}$; for $hA\beta_{40}$, ε = 159,880 $M^{-1}cm^{-1}$).[2]

ThT Fluorescence Assay. The aggregation kinetics of Aβ was analyzed by the ThT fluorescence assay. Samples containing $mA\beta_{40}$ or $hA\beta_{40}$ (20 μM) in the absence and presence of **MG** (200 μM)

were incubated for 0, 6, 12, 15, 18, 21, 24, 30, 36, 48, 60, and 72 h (for mAβ_{40}) or 0, 0.25, 0.4, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24 h (for hAβ_{40}) in 20 mM HEPES, pH 7.4, 150 mM NaCl at 37 °C with constant agitation (250 rpm). Upon addition of ThT (final concentration of 20 μM), the fluorescence intensity was measured with excitation at 440 nm and emission at 490 nm by a microplate reader. Data are presented as mean ± s.e.m. (standard error of the mean) of three independent experiments. The normalized fluorescence values were fitted by the equation below:

$$F(t) = F_0 + \frac{A}{1 + e^{-k(t-t_{1/2})}}$$

F_0 and A are the initial fluorescence intensity and the amplitude, respectively.[3] $t_{1/2}$ indicates the half time when fluorescence reaches 50% of its maximum intensity. *k* is the rate constant of the elongation phase.[3]

Gel/Western Blot. Molecular weight distributions of mAβ_{40} or hAβ_{40} upon aggregation were analyzed by gel/Western blot using an anti-Aβ antibody (Ab62658; 1:2,000, Abcam, Cambridge, UK). Samples containing mAβ_{40} or hAβ_{40} (20 μM) with or without **MG** (200 μM) were incubated for 0, 24, 36, and 72 h (for mAβ_{40}) or 0, 1, 6, and 24 h (for hAβ_{40}) in 20 mM HEPES, pH 7.4, 150 mM NaCl at 37 °C with constant agitation (250 rpm). The samples (10 μL) were separated on a 10–20% Tris-tricine gel (Invitrogen, Grand Island, NY, USA). Following separation, the proteins were transferred onto nitrocellulose membranes and blocked with bovine serum albumin (BSA, 3% w/v, Biosesang, Seongnam, Republic of Korea) in Tris-buffered saline (TBS) containing 0.1 % v/v Tween-20 (TBS-T) for 3 h at room temperature. After washing with TBS-T three times (10 min each), the horse radish peroxidase-conjugated goat anti-rabbit antibody (1:5,000; Promega, Madison, WI, USA) in 2% w/v BSA (in TBS-T) was added for 1 h at room temperature. A homemade ECL kit was used to visualize the gel/Western data on an imaging system.[4] The measurements were conducted in triplicate.

TEM. Samples for TEM measurements were prepared based on previously reported methods using Glow-discharged grids (Formvar/Carbon 300-mesh, Electron Microscopy Sciences, Hatfield, PA, USA).[1] The resultant samples were treated onto the grids for 2 min at room temperature. After removing the excess sample with a filter paper, each grid was washed three times with ddH_2O and incubated with uranyl acetate (1% w/v ddH_2O; 5 μL) for 1 min. Excess uranyl acetate was

removed, and the grids were dried for at least 4 h at room temperature. Images of the grids were taken at 200 kV with a magnification of 29000x. For the TEM analysis, we randomly selected locations of samples on the grids for imaging and collected at least 20 images from each grid.

CD Spectroscopy. mAβ_{40} or hAβ_{40} (50 μM) in the absence and presence of **MG** (500 μM) was incubated for 0, 24, 48, and 72 h (for mAβ_{40}) or 0, 1, 6, and 24 h (for hAβ_{40}) in 20 mM HEPES, pH 7.4, 150 mM NaF at 37 °C with constant agitation (250 rpm). CD spectra were collected in the range from 200 nm to 250 nm. The digital integration time, the bandwidth, and the scanning speed were 4 s, 2 nm, and 20 nm/min, respectively. Each spectrum was smoothed by Fourier transforms. The secondary structure of mAβ_{40} or hAβ_{40} was analyzed following the previously reported BeStSel method.[5] Data are presented as mean ± s.e.m. (standard error of the mean) of three independent experiments.

ESI–MS. mAβ_{40} or hAβ_{40} (100 μM) was incubated with and without **MG** (1 mM) in 100 mM ammonium acetate at 37 °C for 6 and 24 h. In addition, **MG** (1 mM) was treated with DAE (100 μM) in the presence of Lys (2 mM) in H_2O at 37 °C for 6 h. All samples were prepared without agitation. Before injection into the mass spectrometer, samples were diluted by 10 fold with H_2O. The capillary voltage, the drying gas flow, and the gas temperature were set to 5.8 kV, 12 L/min, and 300 °C, respectively. The fragment voltage was set to 170 V. The parameters of ESI–MS and ESI–MS^2 were the same as above. The collision-induced dissociation (CID) was conducted by applying the collision energy in the trap at 40 eV. More than 200 spectra were obtained for each sample and averaged for the analysis. The measurements were conducted in triplicate.

MALDI–MS. mAβ_{40} or hAβ_{40} (100 μM) was treated with or without **MG** (1 mM) in 20 mM HEPES, pH 7.4, 150 mM NaCl at 37 °C with constant agitation (250 rpm). The samples were incubated for 24 h. The resultant Aβ samples were mixed with sinapic acid (5 mg/mL) that were dissolved in 40% v/v CH_3CN and 2% v/v CF_3COOH in a 1:1 ratio and loaded onto the MALDI–MS target plate. Spectra were obtained by a MALDI-TOF mass spectrometer. The measurements were conducted in triplicate.

Cell Viability Studies. The human neuroblastoma SH-SY5Y cell line was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cell line was maintained in media containing 45% minimum essential medium (MEM), 45% Nutrient mixture F12, 10% fetal bovine serum (FBS, GIBCO, Grand Island, NY, USA), and 100 U/mL penicillin-streptomycin

(GIBCO). Cells were grown and maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cells used for our studies did not indicate mycoplasma contamination. Cell viability was determined by the MTT assay [MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. m$A\beta_{40}$ or h$A\beta_{40}$ (100 μM) with and without **MG** (1 mM) were pre-incubated for 0 h, 24 h, and 72 h (for m$A\beta_{40}$) or 0 h, 1 h, and 24 h (for h$A\beta_{40}$) in 20 mM HEPES, pH 7.4, 150 mM NaCl at 37 °C with constant agitation. Cells were seeded in a 96-well plate (12,000 cells in 100 μL per well) and treated with the resultant Aβ samples [final concentration: 20 μM (for Aβ) and 200 μM (for **MG**)]. After 24 h incubation, MTT [5 mg/mL in PBS (pH 7.4, GIBCO); 25 μL] was added to each well and the plate was incubated for 4 h at 37 °C. Formazan produced by cells was solubilized using an acidic solution of *N*,*N*-dimethylformamide (DMF; pH 4.5, 50% v/v, aq) and sodium dodecyl sulfate (SDS; 20% w/v) overnight at room temperature in the dark. The absorbance was measured at 600 nm by a microplate reader. The viability of cells was calculated, relative to that of cells containing an equivalent amount of the buffer solution (20 mM HEPES, pH 7.4, 150 mM NaCl). Data are presented as mean ± s.e.m. (standard error of the mean) of three independent experiments.

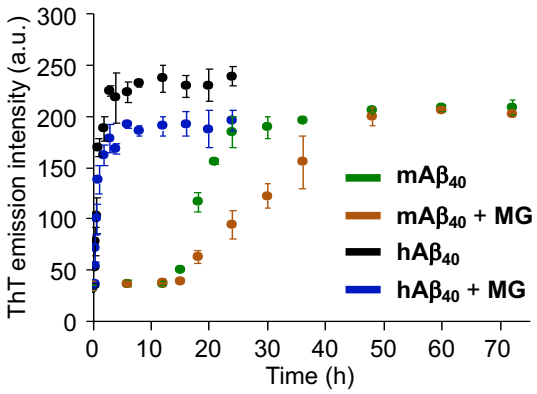
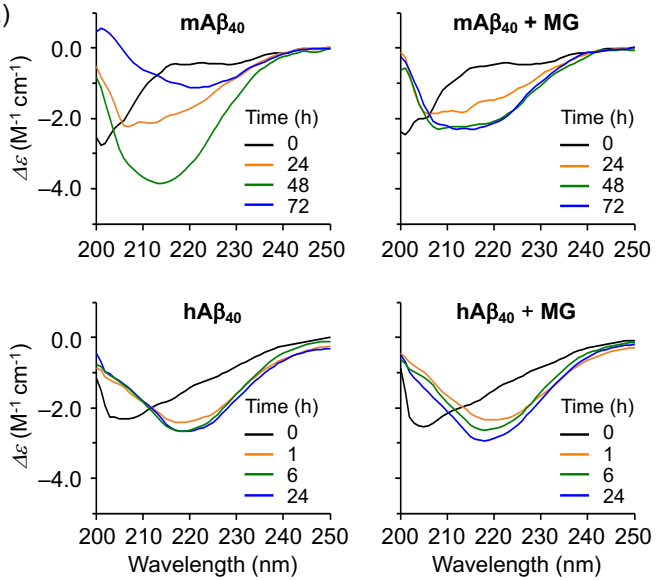


Fig. S1 ThT emission intensity of mAβ_{40} and hAβ_{40} in the absence and presence of **MG**. The normalized fluorescence values fitted by a modified Boltzmann sigmoidal equation are presented in Fig. 2b.[3]

(a)



(b)

	mAβ40				mAβ40 + MG			
Time (h)	0	24	48	72	0	24	48	72
α-Helix (%)	3.1 (± 0.2)	13.2 (± 3.6)	20.4 (± 6.0)	4.3 (± 0.2)	3.4 (± 0.3)	7.9 (± 1.1)	12.2 (± 0.2)	17.6 (± 4.8)
β-Sheet (%)	25.7 (± 3.3)	26.3 (± 3.2)	29.1 (± 2.2)	37.6 (± 2.5)	28.2 (± 1.3)	29.8 (± 4.8)	28.0 (± 5.4)	31.1 (± 1.9)
Turn (%)	19.0 (± 0.8)	16.3 (± 2.0)	12.5 (± 0.9)	12.1 (± 2.7)	18.6 (± 0.3)	16.0 (± 1.4)	15.0 (± 0.1)	12.6 (± 0.9)
Others (%)	52.3 (± 2.3)	44.3 (± 4.8)	38.1 (± 2.9)	46.1 (± 0.0)	49.8 (± 1.4)	46.3 (± 2.3)	44.9 (± 4.7)	38.8 (± 2.0)

	hAβ40				hAβ40 + MG			
Time (h)	0	1	6	24	0	1	6	24
α-Helix (%)	7.8 (± 1.6)	4.2 (± 1.7)	6.6 (±0.4)	6.0 (± 1.0)	4.8 (± 0.6)	4.3 (± 2.4)	6.1 (± 0.1)	6.4 (± 1.9)
β-Sheet (%)	26.1 (± 3.9)	26.8 (± 8.1)	31.0 (± 1.8)	35.6 (± 0.2)	30.5 (± 1.0)	24.3 (± 5.5)	32.4 (± 2.1)	33.6 (± 3.1)
Turn (%)	15.9 (± 2.0)	15.0 (± 1.7)	14.3 (± 0.8)	12.5 (± 0.9)	14.7 (± 1.0)	16.0 (± 0.4)	14.0 (± 0.2)	13.7 (± 0.1)
Others (%)	50.2 (± 0.4)	54.1 (± 8.1)	48.2 (± 1.4)	46.1 (± 1.7)	50.1 (± 2.5)	55.5 (± 7.4)	47.7 (± 2.8)	46.4 (± 1.2)

Fig. S2 Change in the secondary structures of mAβ40 and hAβ40 by treatment of **MG**. (a) CD spectra of mAβ40 and Aβ40 with or without **MG**. (b) Structural composition of mAβ40 and hAβ40 in the absence and presence of **MG** analyzed by BeStSel.[5] Data are presented as mean ± s.e.m. (standard error of the mean) of three independent experiments. Conditions: [mAβ40 or hAβ40] = 50 μM; [**MG**] = 500 μM; 20 mM HEPES, pH 7.4, 150 mM NaF; 37 °C; constant agitation (250 rpm).

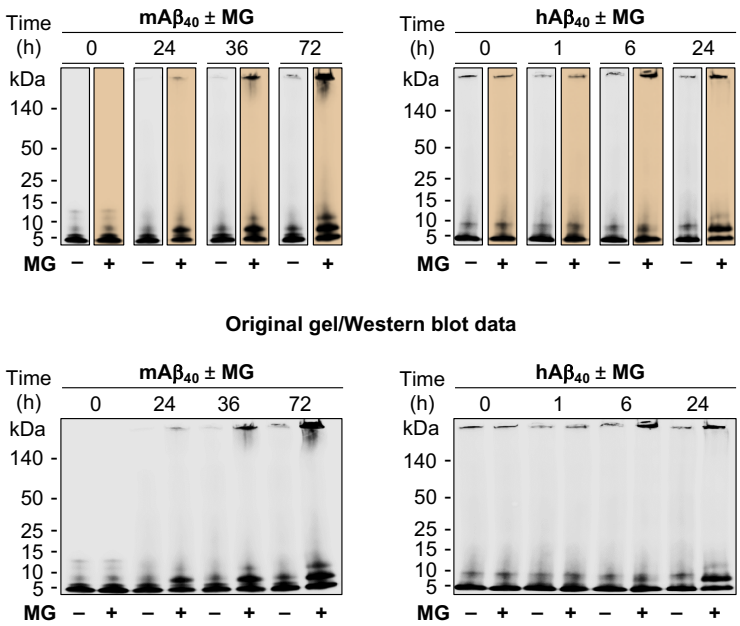


Fig. S3 Size distributions of mAβ_{40} and hAβ_{40} in the absence and presence of **MG**. Conditions: [mAβ_{40} or hAβ_{40}] = 20 μM; [**MG**] = 200 μM; 20 mM HEPES, pH 7.4, 150 mM NaCl; 37 ^{o}C; constant agitation (250 rpm).

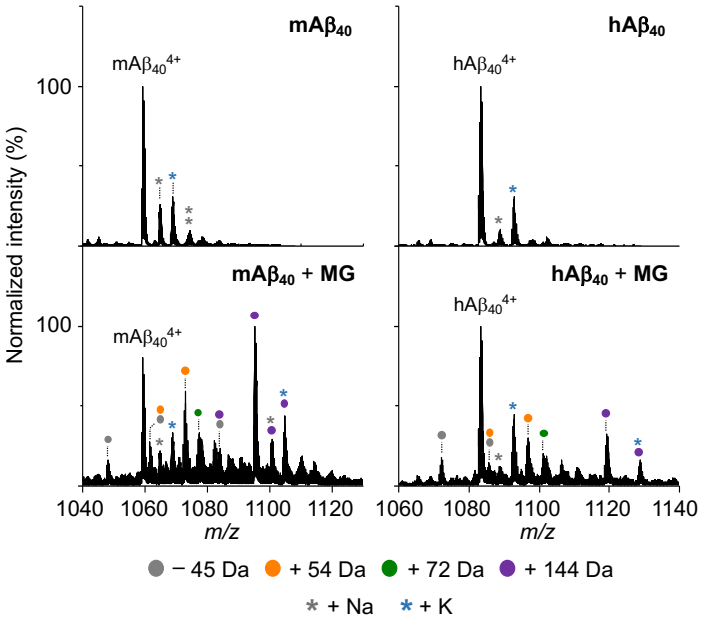


Fig. S4 ESI–MS spectra of mAβ_{40} and hAβ_{40} incubated with **MG** for 24 h. Conditions: [mAβ or hAβ_{40}] = 100 μM; [**MG**] = 1 mM; 100 mM ammonium acetate; 37 ^{o}C; 24 h incubation. The samples were diluted with H_2O by 10 fold before injection to the mass spectrometer.

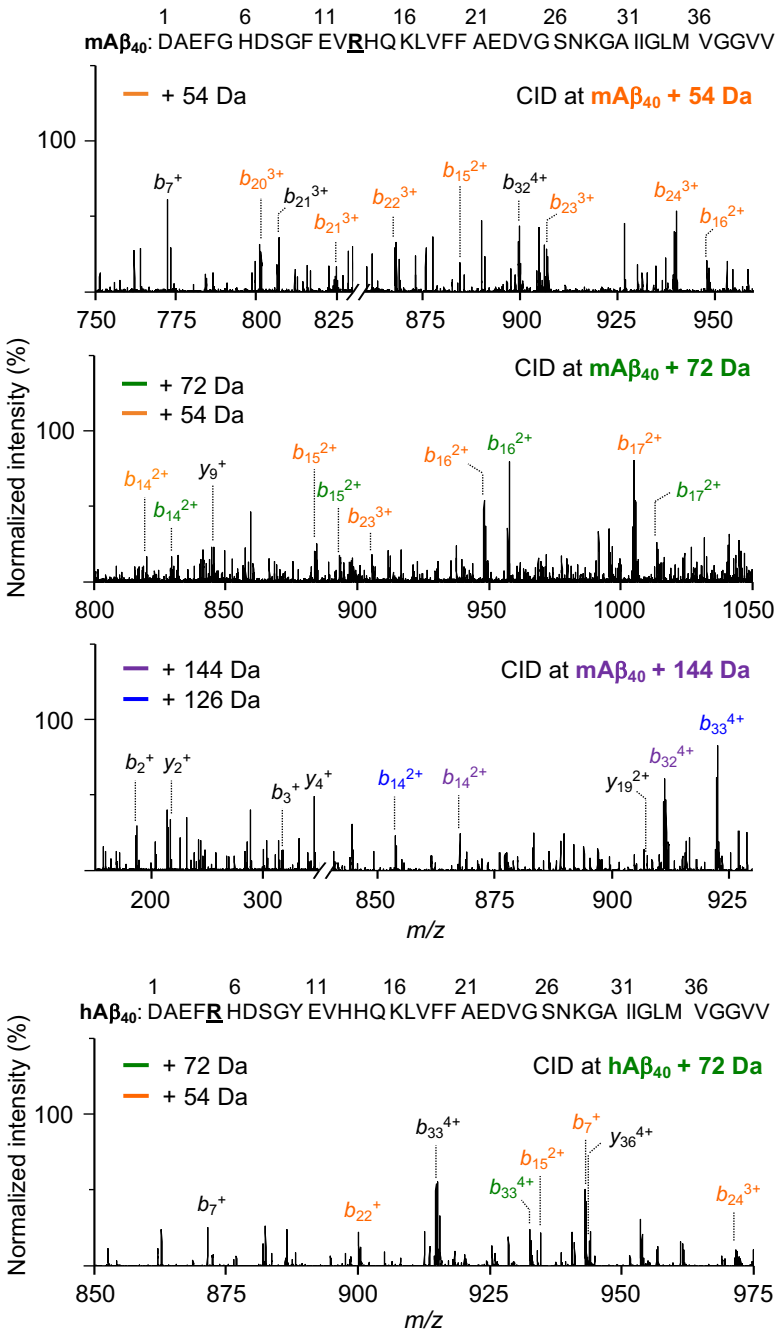


Fig. S5 Analysis of the amino acid residues in mAβ_{40} and hAβ_{40} that were modified upon treatment with **MG** by ESI–MS^2. Conditions: [mAβ_{40} or hAβ_{40}] = 100 μM; [**MG**] = 1 mM; 100 mM ammonium acetate; 37 °C; incubation for 6 h. The samples were diluted with H_2O by 10 fold prior to injection to the mass spectrometer.

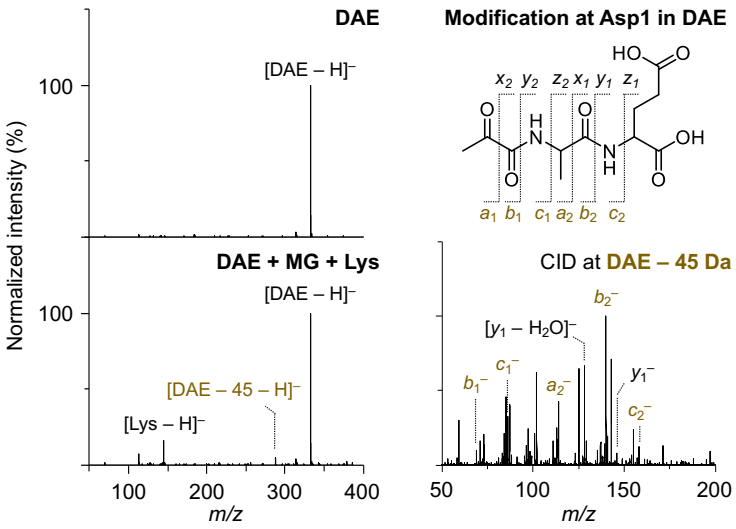


Fig. S6 Modification of DAE by **MG** in the presence of Lys. Conditions: [DAE] = 100 μM; [**MG**] = 1 mM; [Lys] = 2 mM; H_2O; 37 °C; 6 h incubation. The samples were diluted with H_2O by 10 fold before injection to the mass spectrometer.

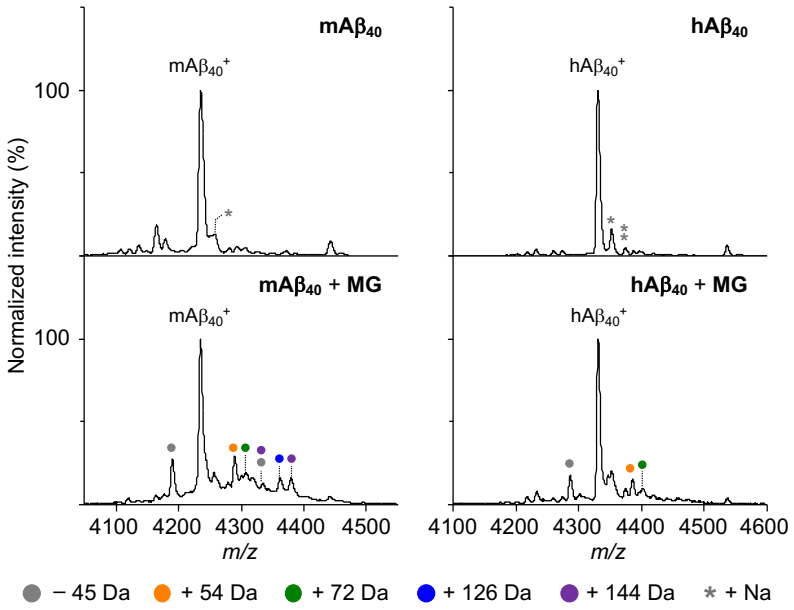


Fig. S7 MALDI–MS spectra of mAβ_{40} and hAβ_{40} in the absence and presence of **MG**. Conditions: [mAβ_{40} or hAβ_{40}] = 100 μM; [**MG**] = 1 mM; 20 mM HEPES, pH 7.4, 150 mM NaCl; 37 °C; incubation for 24 h; constant agitation (250 rpm).

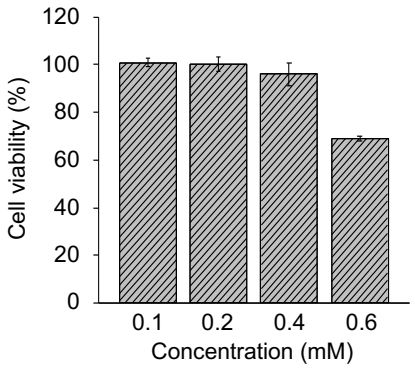


Fig. S8 Cytotoxicity of **MG**. The viability of cells, determined by the MTT assay, was calculated in comparison to that of the cells treated with an equivalent amount of the buffered solution. Data are presented as mean ± s.e.m. (standard error of the mean) of three independent experiments.

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