ELECTRONIC SUPPLEMENTARY INFORMATION (ESI) for

Efficient synthesis and characterisation of the amyloid beta peptide, $A\beta_{1-42}$, using a double linker system

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CONTENTS

I. Materials

All reagents were purchased as reagent grade and used without further purification. *N,N*-diisopropylethylamine (DIPEA), piperidine, *N*-methylmorpholine (NMM), triisopropylsilane (TIPS), *N,N'*-diisopropylcarbodiimide (DIC), and thioflavin T (ThT) were purchased from Sigma Aldrich (St. Louis, USA). Trifluoroacetic acid (TFA) was purchased from Scharlau (Barcelona, Spain) and 1,2-ethanedithiol (EDT) was supplied by Fisher Scientific (New Hampshire, USA). Fmoc-Asp(OtBu)-OH, Fmoc-Ala-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Phe-OH, Fmoc-Arg(Pbf)-OH, Fmoc-His(Trt)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Gly-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Val-OH, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ile-OH, and Fmoc-Met-OH were purchased from GL Biochem (Shanghai, China). Aminomethyl ChemMatrix resin was purchased from PCAS Biomatrix Inc. (Quebec, Canada), 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt), O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HATU), Fmoc-Asp(OtBu)-Ser($\psi^{Me,Me}$pro)-OH, and Fmoc-Gly-Ser($\psi^{Me,Me}$pro)-OH was purchased from Aapptec (Kentucky, USA), and acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany). 4-Hydroxymethylbenzoic acid (HMBA) linker was purchased from CS Bio (Shanghai, China). 4-(Hydroxymethyl)phenoxyacetic acid (HMP) linker was purchased from AK Scientific (San Francisco, USA). Rink amide linker was purchased from Chempep (Florida, USA).

LC-MS spectra were acquired on an Agilent Technologies (California, USA) 1260 Infinity LC equipped with an Agilent Technologies 6120 Quadrupole mass spectrometer. An analytical column (Zorbax 300SB-C3, 3.5 µm, 3.0 x 150 mm) was used at a flow rate of 0.3 mL/min. A linear gradient of 5% B to 95% B was employed over 30 minutes (*ca* 3% B/min), where solvent A was 0.1% formic acid in water and solvent B was 0.1% formic acid in acetonitrile.

II. Methods

Method 1. Attachment of HMP linker to aminomethyl ChemMatrix resin

To aminomethyl ChemMatrix resin (161.3 mg, 0.1 mmol, 0.62 mmol/g loading) pre-swollen in DMF (5 mL, 10 minutes), a mixture of HMP linker (55 mg, 0.3 mmol), DIC (62 µL, 0.4 mmol), and 6-Cl-HOBt (102 mg, 0.6 mmol) in DMF (2 mL) was added. The reaction mixture was gently agitated at room temperature for 1 hour, filtered, and the reaction repeated with fresh reagents for a further hour. The resin was then filtered and washed with DMF (3 x 3 mL) and CH_2Cl_2 (3 x 3 mL).

Method 2. Attachment of alanine to HMP-ChemMatrix resin

To HMP-ChemMatrix resin was added a solution of Fmoc-Ala-OH (62 mg, 0.2 mmol), DMAP (1.2 mg, 0.09 mmol), and DIC (31 µL, 0.2 mmol) in DMF (2 mL). The reaction mixture was then agitated at room temperature for 2 hours, and then filtered. This reaction was repeated once for a further 2 hours with fresh reagents. The resin was then filtered, and washed with DMF (3 x 3 mL), CH_2Cl_2 (3 x 3 mL), and dried *in vacuo* to afford Fmoc alanine-bound resin.

Method 3. Quantitative estimation of alanine attachment on resin

Three 10 mm-matched silica UV spectrophotometric cuvettes were used, and vacuum-dried resin weighed in two of them. 3 mL of 20% piperidine in DMF (*v/v*) was dispensed into all cuvettes; the two containing resin were agitated for 10 minutes using an automatic pipette. The reference cuvette, containing no resin, was used to zero the spectrophotometer at λ = 290 nm. The absorbance of the resin-containing cuvettes was then quantified, and the loading of alanine calculated using **Equation 1** below:

$$Loading = \frac{Absorbance\ of\ sample}{(Mass\ of\ resin\ sample\ \times 1.75)}$$

Equation 1. Estimation of level of first residue attachment. Loading is measured in mmol/g.[1]

Method 4. Assembly of the linear peptide using automated Fmoc-SPPS

Fmoc-deprotections: to the peptidyl resin was added a solution of 20% piperidine in DMF (*v/v*, 3 mL), and the reaction mixture agitated at room temperature for 5 minutes. This procedure was repeated once, after which the resin was filtered and washed with DMF (2 mL, 5 x 30 seconds).

Fmoc-amino acid couplings: to the peptidyl resin was added a mixture of Fmoc-AA-OH (0.50 mmol), HATU (174.8 mg, 0.46 mmol), and NMM (1.00 mmol) in DMF (2 mL). The reaction mixture was agitated at room temperature for 30 minutes, after which the resin was filtered and washed with DMF (2 mL, 5 x 30 seconds).

Capping: following every coupling of Fmoc-AA-OH, a solution of 20% acetic anhydride in DMF (*v/v*, 3 mL) was added, and the reaction mixture agitated at room temperature for 1 minute. The resin was then filtered and washed with DMF (2 mL, 5 x 30 seconds).

Method 5. Pseudoproline coupling

To the peptidyl resin was added a mixture of Fmoc-ψPro-OH (0.50 mmol), HATU (174.8 mg, 0.46 mmol), and NMM (1.00 mmol) in DMF (2 mL). The reaction mixture was agitated at room temperature for 30 minutes, after which the resin was filtered and washed with DMF (2 mL, 5 x 30 seconds).

Method 6. Full cleavage of the peptide from the resin

Following deprotection of the final Fmoc group on the peptide, the peptidyl resin was washed with DMF (3 x 3 mL), CH_2Cl_2 (3 x 3 mL), and dried *in vacuo*. A mixture of TFA/TIPS/H_2O/EDT (94:1:2.5:2.5 *v/v/v/v*, 5 mL) was then added to the dry resin-bound peptide, and the reaction mixture agitated at room temperature for 2 hours. The resulting mixture was filtered, and the resin bed washed with the cleavage cocktail (2 x 2 mL) to ensure that all cleaved peptide had been filtered off. The combined filtrates were then concentrated under a gentle flow of nitrogen to evaporate excess TFA. Afterwards, the crude peptide was precipitated with cold Et_2O (40 mL), and isolated by centrifugation. The ether was removed, fresh ether (40 mL) added, and the peptide recovered by centrifugation. The precipitate was then dissolved in equal volumes of 0.1% TFA in acetonitrile and 0.1% TFA in water, prior to lyophilisation.

Method 7. Purification of crude peptide using basic mobile phases

Semi-preparative reverse phase high-performance liquid chromatography (RP-HPLC) was undertaken on a Thermo Scientific Dionex Ultimate 3000 HPLC equipped with a four channel UV detector at 210, 225, 254, and 280 nm. A semi-preparative column (Zorbax 300SB-C3, 5 µM, 9.4 x 250 mm) was used at 70 °C, and at a flow rate of 4 mL per minute. A linear gradient of 1% B to 61% B was employed, at a rate of 1% B per minute; solvent A was 0.1 % NH_4OH in water, whereas solvent B was 0.1% NH_4OH in acetonitrile.

Method 8. Analysis of purified peptide fractions using basic mobile phases

All analyses of collected fractions were performed on a Thermo Scientific Dionex Ultimate 3000 HPLC, which is equipped with a four channel UV detector at wavelengths 210, 225, 254, and 280 nm. An analytical column (xTerra MS C18, 5 µM, 4.6 x 150 mm) was used at 50 °C, and at a flow rate of 1 mL per minute. A linear gradient of 1% B to 61% B was employed, at a rate of 3% B per minute; solvent A was 0.1% NH_4OH in water, whereas solvent B was 0.1% NH_4OH in acetonitrile.

Method 9. Attachment of Fmoc-Rink amide linker to aminomethyl ChemMatrix resin

To aminomethyl ChemMatrix resin (161.3 mg, 0.1 mmol, 0.62 mmol/g loading) pre-swollen in DMF (5 mL, 10 minutes), a mixture of Fmoc-Rink amide linker (215.6 mg, 0.4 mmol), DIC (62 µL, 0.4 mmol), and 6-Cl-HOBt (67.8 mg, 0.4 mmol) in DMF (2 mL) was added. The reaction mixture was gently agitated at room temperature for 1 hour, filtered, and the reaction repeated with fresh reagents for a further hour. The resin was then filtered and washed with DMF (3 x 3 mL) and CH_2Cl_2 (3 x 3 mL).

Method 10. Assembly of hexalysine tag on Rink amide linker

To resin-bound Fmoc Rink amide pre-swollen in DMF (5 mL, 10 minutes), a solution of 20% piperidine in DMF (*v/v*, 3 mL) was added, and the mixture agitated at room temperature for 2 x 5 minutes, after which the resin bed was filtered and washed with DMF (3 x 3 mL). A mixture of Fmoc-Lys(Boc)-OH (234.2 mg, 0.5 mmol), HATU (174.8 mg, 0.46 mmol), and DIPEA (174 µL, 1 mmol) in DMF (2 mL) was added. The reaction mixture was then agitated at room temperature for 45 minutes, and then filtered. A solution of 20% acetic anhydride in DMF (*v/v*, 3 mL) was then added to cap any free amine (NH_2) groups on resin, and the mixture agitated at room temperature for 5 minutes, filtered, and washed with DMF (3 x 3 mL). This protocol was repeated a further 5 times, such that 6 lysine residues were coupled to the resin in total.

Method 11. Attachment of HMBA linker to hexalysine tag

To the peptidyl resin was added a solution of 20% piperidine in DMF (*v/v*, 3 mL) to remove the N^{α}-Fmoc protecting group on the lysine residue. The reaction mixture was agitated at room temperature for 2 x 5 minutes, after which the resin bed was filtered, and washed. A solution of HMBA (60.9 mg, 0.4 mmol), 6-Cl-HOBt (67.8 mg, 0.4 mmol), and DIC (62 µL, 0.4 mmol) in DMF (2 mL) was then added onto the resin, and the mixture agitated at room temperature for 2 hours, after which the resin was filtered, and the reaction repeated for a further 2 hours with fresh reagents. The resin was then filtered, and washed with DMF (3 x 3 mL).

Method 12. Attachment of alanine to HMBA-hexalysine tag

To the peptidyl resin was added a solution of Fmoc-Ala-OH (62 mg, 0.2 mmol), DMAP (1.2 mg, 0.09 mmol), and DIC (31 µL, 0.2 mmol) in DMF (2 mL). The reaction mixture was agitated at room temperature for 2 hours, after which the resin was filtered, and the reaction repeated for a further 2 hours with fresh reagents. Following completion of reaction, the resin was washed with DMF (3 x 3 mL) and DCM (3 x 3 mL) and left to dry *in vacuo*.

Method 13. Purification of $A\beta_{42}$-HMBA-Lys_6-$CONH_2$ using acidic mobile phases

Semi-preparative reverse phase high-performance liquid chromatography (RP-HPLC) was undertaken on a Thermo Scientific Dionex Ultimate 3000 HPLC equipped with a four channel UV detector at 210, 225, 254, and 280 nm. A semi-preparative column (Zorbax 300SB-C3, 5 µM, 9.4 x 250 mm) was used at room temperature, and at a flow rate of 4 mL per minute. A linear gradient between 1% B to 61% B was employed, at a rate of 1% B per minute; solvent A was 0.1 % TFA in water, whereas solvent B was 0.1% TFA in acetonitrile.

Method 14. Analysis of purified $A\beta_{42}$-HMBA-Lys_6-$CONH_2$ fractions using acidic mobile phases

All analyses of collected fractions were performed on a Thermo Scientific Dionex Ultimate 3000 HPLC equipped with a four channel UV detector at wavelengths 210, 225, 254, and 280 nm. An analytical column (xTerra MS C18, 5 µM, 4.6 x 150 mm) was used at room temperature, and at a flow rate of 1 mL per minute. A linear gradient between 1% B to 61% B was employed, at a rate of 3% B per minute; solvent A was 0.1% TFA in water, whereas solvent B was 0.1% TFA in acetonitrile.

Method 15. Cleavage of HMBA-Lys_6 from $A\beta_{42}$-HMBA-Lys_6

Lyophilised, pure peptide-linker was treated with 1 M NaOH for 1 hour to remove the solubilising linker from the peptide. The reaction was then quenched by addition of neat TFA, and the mixture lyophilised. $A\beta_{42}$ peptide was then precipitated in cold Et_2O (40 mL), and isolated by centrifugation. This isolation protocol was repeated twice. The precipitate was treated with type 1 water (18.2 mΩ-cm, 5 mL) to completely dissolve the linker, centrifuged for 5 minutes, and water carefully removed using an automatic pipette. This procedure was repeated a further two times, after which the precipitate was reconstituted in equal volumes of 0.1% TFA in water and 0.1% TFA in acetonitrile, and lyophilised.

Method 16. Sample pre-treatment

Pure $A\beta_{42}$ was treated with 10% *w/v* NH_4OH (0.5 mg/mL) to remove preformed aggregate in LoBind Protein 1.5 mL microcentrifuge tube (Eppendorf, Germany) to remove preformed aggregates.[2] Following reconstitution, the peptide was left at room temperature (21 °C) for 10 minutes and sonicated for 5 minutes at a frequency band of 50-60 Hz. Sonicated samples were then centrifuged at 16,100 x *g* for 10 minutes at room temperature, and the supernatant carefully removed and dispensed as 100 µL aliquots (0.05 mg), which were lyophilised and stored at -20 °C.

Method 17. Spectrophotometric quantitation of peptide concentration

NH_4OH pre-treated lyophilised aliquot of $A\beta_{42}$ was reconstituted in 155 µL of 0.22 µm filtered 0.33 mM NaOH/1 x DPBS and centrifuged at 16,100 x *g* for 10 minutes at 21 °C. 150 µL of the supernatant was immediately analysed in a Cary 4000 UV spectrophotometer (Varian, USA) at 280 nm, using a 10 mm path-length quartz cuvette. The Beer-Lambert law (**Equation 2**) was used to quantify the concentration of the peptide sample, with reference to its absorbance value at 280 nm, and the molar extinction coefficient of a single tyrosine residue on the $A\beta_{42}$ peptide (1280 $M^{-1}\,cm^{-1}$). At least two absorbance readings were taken and used to calculate the average peptide concentration in solution.

$$A = \varepsilon c l$$

Equation 2. Beer-Lambert law, where A: absorbance, ε: molar extinction coefficient ($M^{-1}cm^{-1}$), c: concentration (M), l: path length (cm)

Method 18. Transmission electron microscopy (TEM) imaging

To facilitate fibril formation, NH_4OH pre-treated peptide was incubated in 10 mM HCl for up to 3 days at 37 °C.[3] HCl-incubated peptide was sampled every 24 hours for the duration of the experiment as specified above. 2 µL of peptide sample was adsorbed onto glow-discharged, carbon-coated 400-mesh copper grids (Gilder, UK) for 30 seconds, after which the grid was washed in type 1 water (18.2 mΩ-cm) for a further 30 seconds and stained with 2% *w/v* uranyl acetate for 60 seconds. Excess uranyl acetate was wicked on a filter paper, and the copper grid subsequently imaged in a CM12 transmission electron microscope (Philips, Netherlands) at an operating voltage of 120 kV, using a Bioscan 792 Camera (Gatan, USA) at a magnification of × 140,000. At least 5-6 fields of view were obtained for each sample for each day.

Method 19. Thioflavin T (ThT) assay

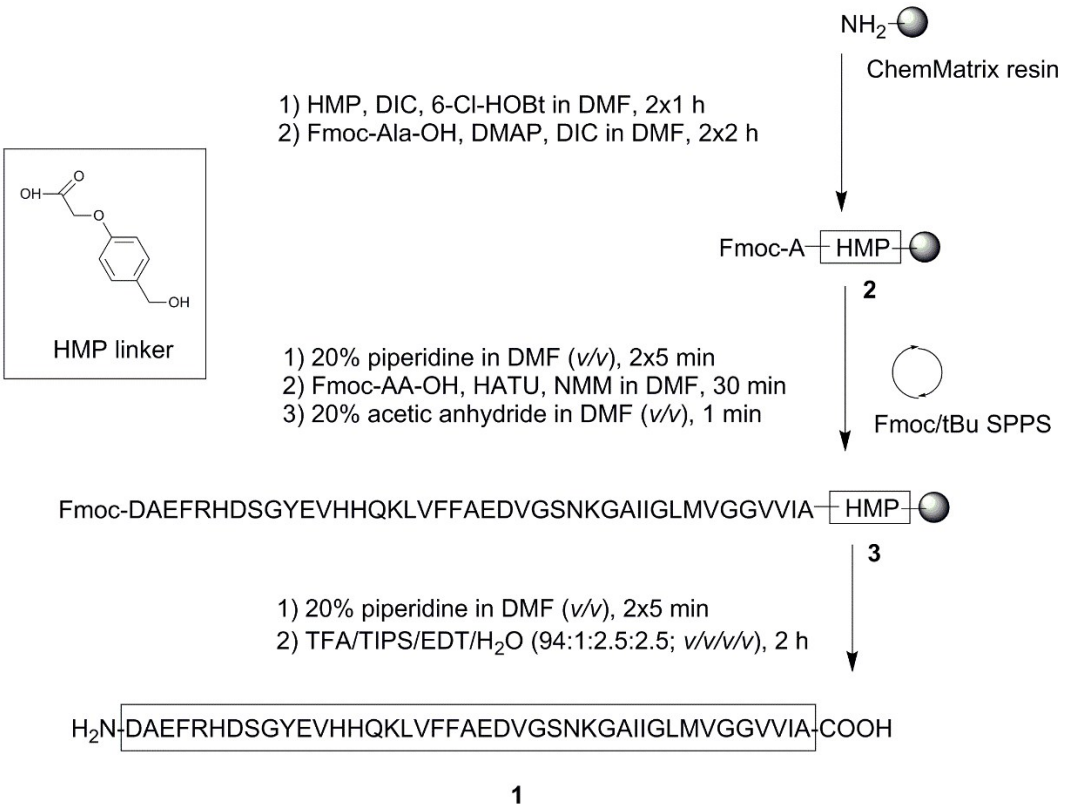
1 mM of thioflavin T (ThT) was freshly prepared prior to every experiment in type 1 water (18.2 mΩ-cm). This stock solution was then diluted in ice-cold 10 mM sodium phosphate buffer (pH 7.4) to a final concentration of 10 μM. Peptides were reconstituted in ice-cold 10 μM ThT in 10 mM sodium phosphate buffer (pH 7.4), and immediately diluted in a non-binding opaque black 96 well plate (GN655900, Greiner, USA) on ice. The plate was then sealed with a clear plastic cover, and placed in an EnVision plate reader (PerkinElmer, USA) at 30 °C. Fluorescence intensity was monitored at 440 nm (excitation) and 485 nm (emission) for 600 minutes with 2-minute intervals between each read. The number of flashes was set to 1000. ThT signal of the peptides was subtracted from the background signal (10 μM ThT in 10 mM sodium phosphate buffer (pH 7.4) with no peptide).

Method 20. Circular dichroism (CD) spectroscopy

Frozen, lyophilised peptide was left to warm to room temperature and then diluted into a 1 mm path-length quartz cuvette (Hellman Analytics, Germany) to 10 μM with 10 mM sodium phosphate buffer (pH 7.4). The secondary structure of the peptide was quantified in a Chirascan circular dichroism spectrophotometer (Applied Biophysics, UK) at chamber temperature of 30 °C after 27 minutes with a bandwidth of 2.5 nm and a read time of 2 seconds per 1 nm from 260 to 180 nm. Mdeg values were subtracted from the baseline (10 mM sodium phosphate buffer (pH 7.4) with no peptide). Spectral deconvolution and estimation of secondary structure was carried out using *BeStSel*.[4]

III. Synthesis of amyloid beta peptide, $A\beta_{1-42}$

1. Standard Fmoc/tBu SPPS



Scheme S1. Standard Fmoc/tBu SPPS of $A\beta_{42}$.

HMP linker was attached to ChemMatrix resin (**Method 1**), followed by coupling of alanine, the first residue of $A\beta_{42}$ (**Method 2**) to afford **2**. Alanine loading was spectrophotometrically quantitated to be 0.523 mmol/g (**Method 3**). Peptide was elongated on a Tribute Peptide Synthesizer (Protein Technologies Inc.) as according to **Method 4** to afford peptidyl resin **3**, after which it was cleaved (**Method 6**) to afford the crude peptide as a colourless solid (150 mg, 33% yield based on 0.1 mmol ChemMatrix resin loading, Fig. S1). Attempted purification of the crude peptide (5 mg) using **Method 7** unfortunately yielded impure fractions, likely due to peptide aggregation.

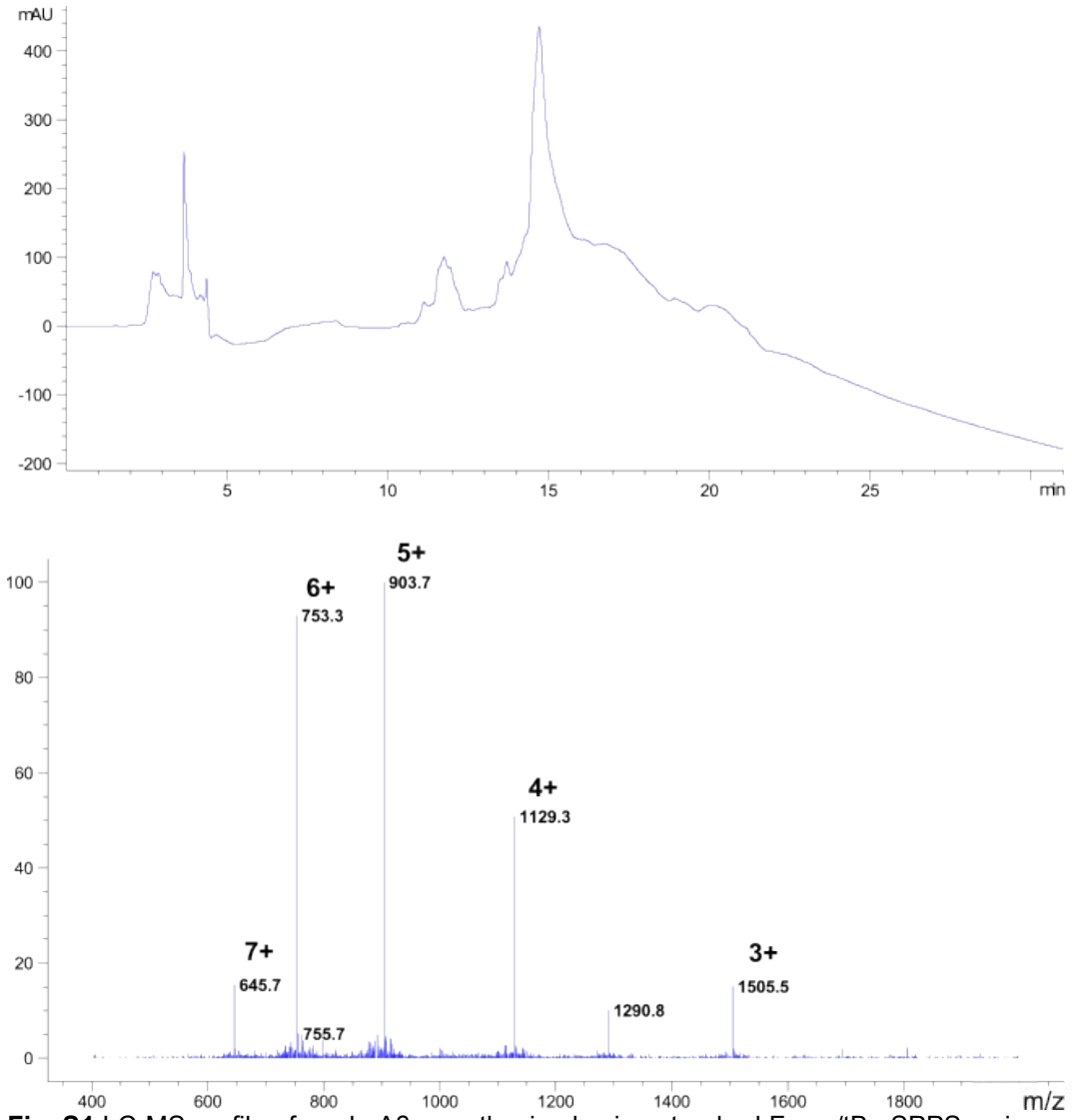
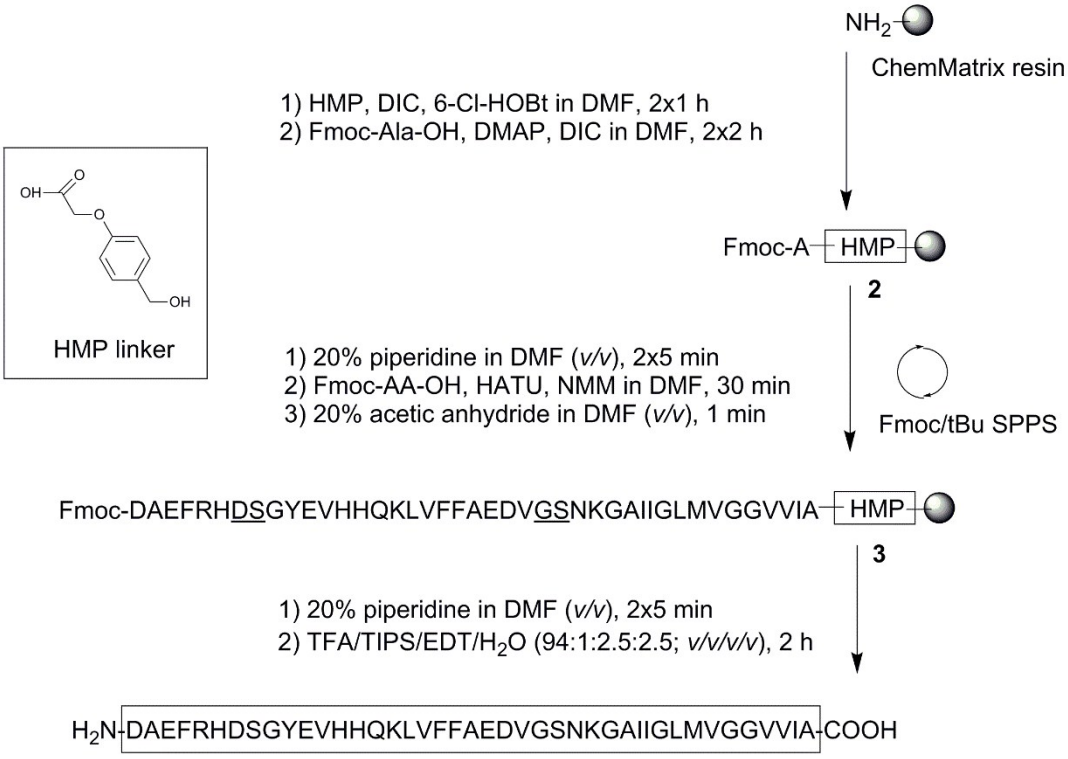


Fig. S1 LC-MS profile of crude $A\beta_{42}$ synthesised using standard Fmoc/tBu SPPS, using an analytical column (Zorbax 300SB-C3, 3.5 µm, 3.0 x 150 mm) at room temperature with a linear gradient of 5-95% buffer B over 30 minutes (buffer A = 0.1% formic acid in H_2O; buffer B = 0.1% formic acid in acetonitrile). Mass spectrum of the peak over the range of 400-1800 m/z showed the desired product (R_T *ca* 15-25 minutes due to aggregation; $[M+5H]^{5+}$ m/z calculated 903.8, observed 903.7; deconvoluted mass 4513.38 ± 0.34 Da, calculated mass 4514.08).

2. Pseudoproline incorporation



Scheme S2. Fmoc/tBu SPPS of $A\beta_{42}$ employing pseudoproline dipeptides (underlined residues) as aggregation disruptor.

HMP linker was attached to ChemMatrix resin (**Method 1**), followed by coupling of alanine, the first residue of $A\beta_{42}$ (**Method 2**) to afford **2**. Alanine loading was spectrophotometrically quantitated to be 0.523 mmol/g (**Method 3**). Peptide was elongated on a Tribute Peptide Synthesizer (Protein Technologies Inc.) as according to **Methods 4** and **5** to afford peptidyl resin **3**, after which it is cleaved (**Method 6**) to afford the crude peptide as a colourless solid (259 mg, 57% yield based on 0.1 mmol ChemMatrix resin loading, Fig. S2). Attempted purification of the crude peptide (5 mg) using **Method 7** unfortunately yielded impure fractions, likely due to peptide aggregation.

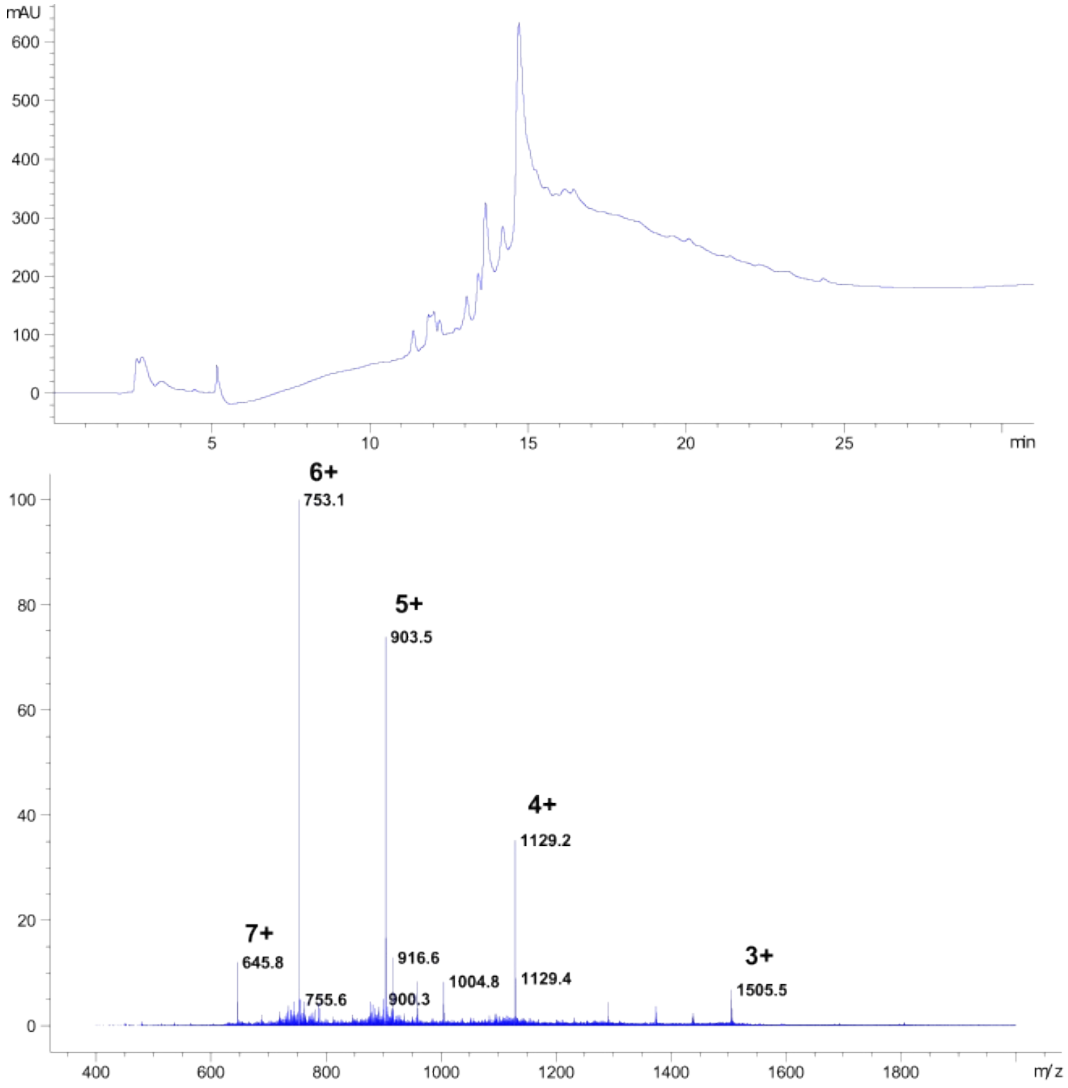
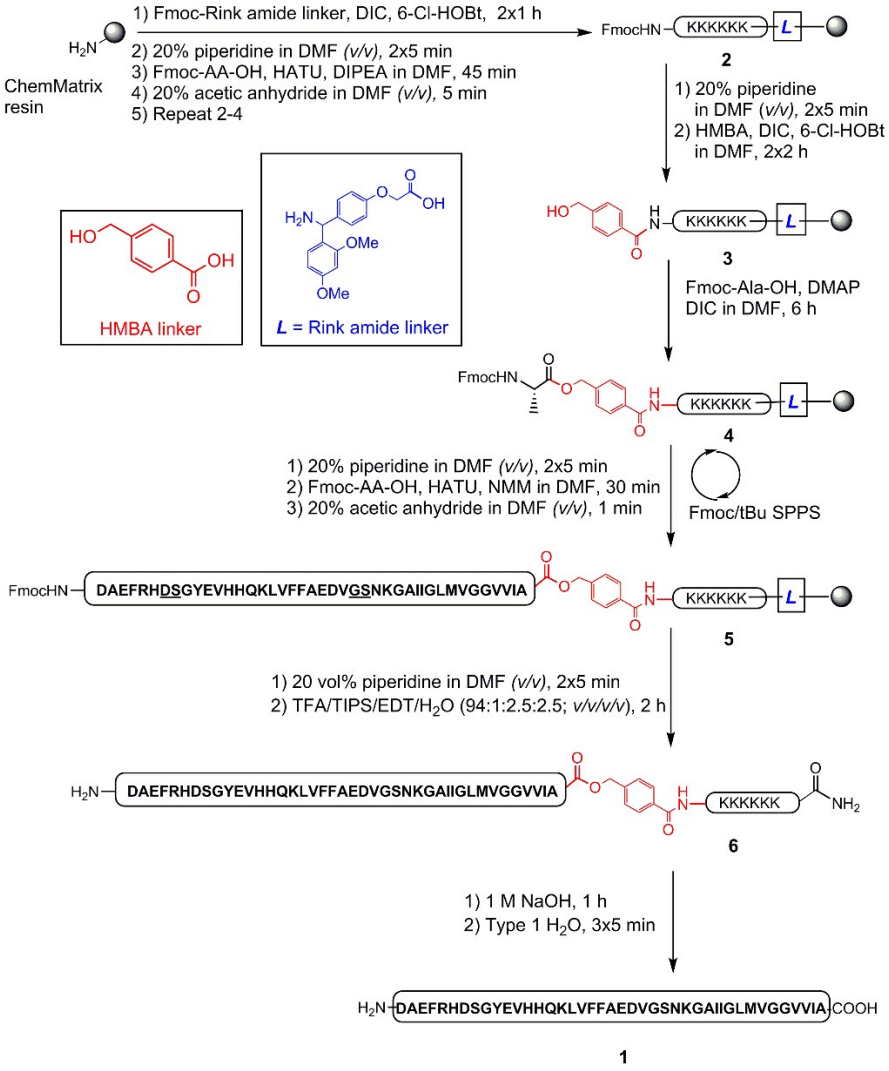


Fig. S2 LC-MS profile of crude $A\beta_{42}$ synthesised with pseudoproline dipeptides, using an analytical column (Zorbax 300SB-C3, 3.5 µm, 3.0 x 150 mm) at room temperature with a linear gradient of 5-95% buffer B over 30 minutes (buffer A = 0.1% formic acid in H_2O; buffer B = 0.1% formic acid in acetonitrile). Mass spectrum of the peak over the range of 400-1800 m/z showed the desired product (R_T *ca* 15-25 minutes due to aggregation; $[M+6H]^{6+}$ m/z calculated 753.3, observed 753.1; deconvoluted mass 4513.00 ± 0.51 Da, calculated mass 4514.08).

3. Double linker system and pseudoproline incorporation



Scheme S3. Fmoc/tBu SPPS of $A\beta_{42}$ using a double linker system, and employing pseudoproline dipeptides (underlined residues) as aggregation disruptor.

Fmoc Rink amide linker was firstly coupled on ChemMatrix resin (**Method 9**), and its loading spectrophotometrically quantitated to be 0.42 mmol/g (**Method 3**). This was followed by assembly of hexalysine tag (**Method 10**) to afford **2**, followed by HMBA linker coupling (**Method 11**) to afford **3**, and alanine attachment (**Method 12**) to afford **4**. Elongation of peptidyl resin **5** was performed on a Tribute Peptide Synthesizer (Protein Technologies Inc.), using the conditions described in **Methods 4** and **5**, after which it was cleaved off resin (**Method 6**) to afford crude $A\beta_{42}$-HMBA-Lys_6-$CONH_2$ as a colourless solid (303 mg, 56% yield based on 0.1 mmol ChemMatrix resin loading, Fig. S3). Crude $A\beta_{42}$-HMBA-Lys_6-$CONH_2$ (90 mg, 0.017 mmol) was purified by semi-preparative RP-HPLC using **Method 13** to afford pure $A\beta_{42}$-HMBA-Lys_6-$CONH_2$ (16.5 mg, 18.3% crude yield, 92% purity, Fig. S4), as determined by **Method 14**. HMBA-Lys_6-$CONH_2$ is then separated from $A\beta_{42}$ using **Method 15** to afford pure $A\beta_{42}$ **1** (13.8 mg, 92% purity, Fig. S5).

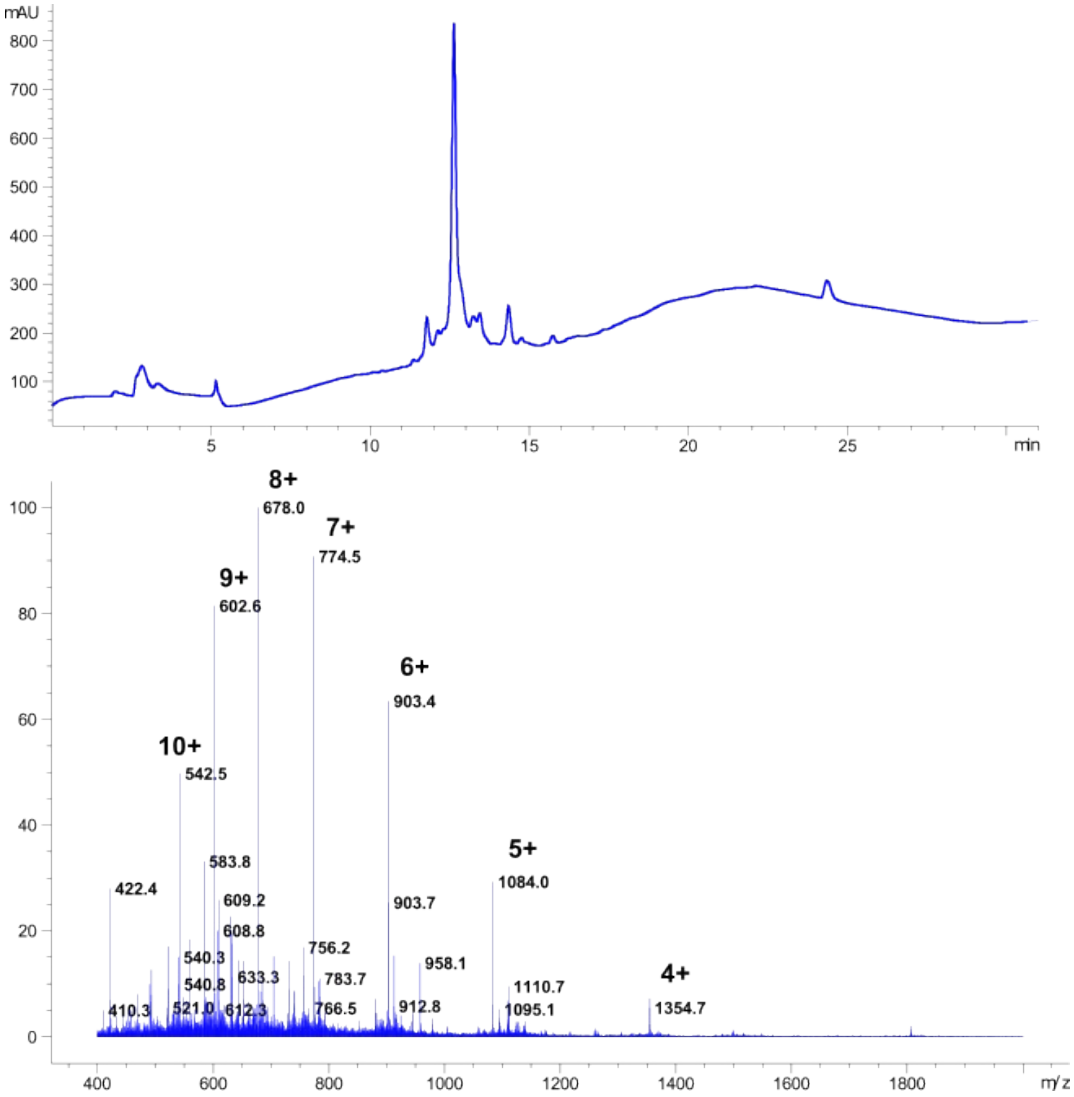


Fig. S3 LC-MS profile of crude $A\beta_{42}$ synthesised using double linker system, using an analytical column (Zorbax 300SB-C3, 3.5 µm, 3.0 x 150 mm) at room temperature with a linear gradient of 5-95% buffer B over 30 minutes (buffer A = 0.1% formic acid in H_2O; buffer B = 0.1% formic acid in acetonitrile). Mass spectrum of the peak over the range of 400-1800 m/z showed the desired product (R_T *ca* 12 minutes; $[M+8H]^{8+}$ m/z calculated 678.03, observed 678.0; deconvoluted mass 5414.94 ± 0.64 Da, calculated mass 5416.31).

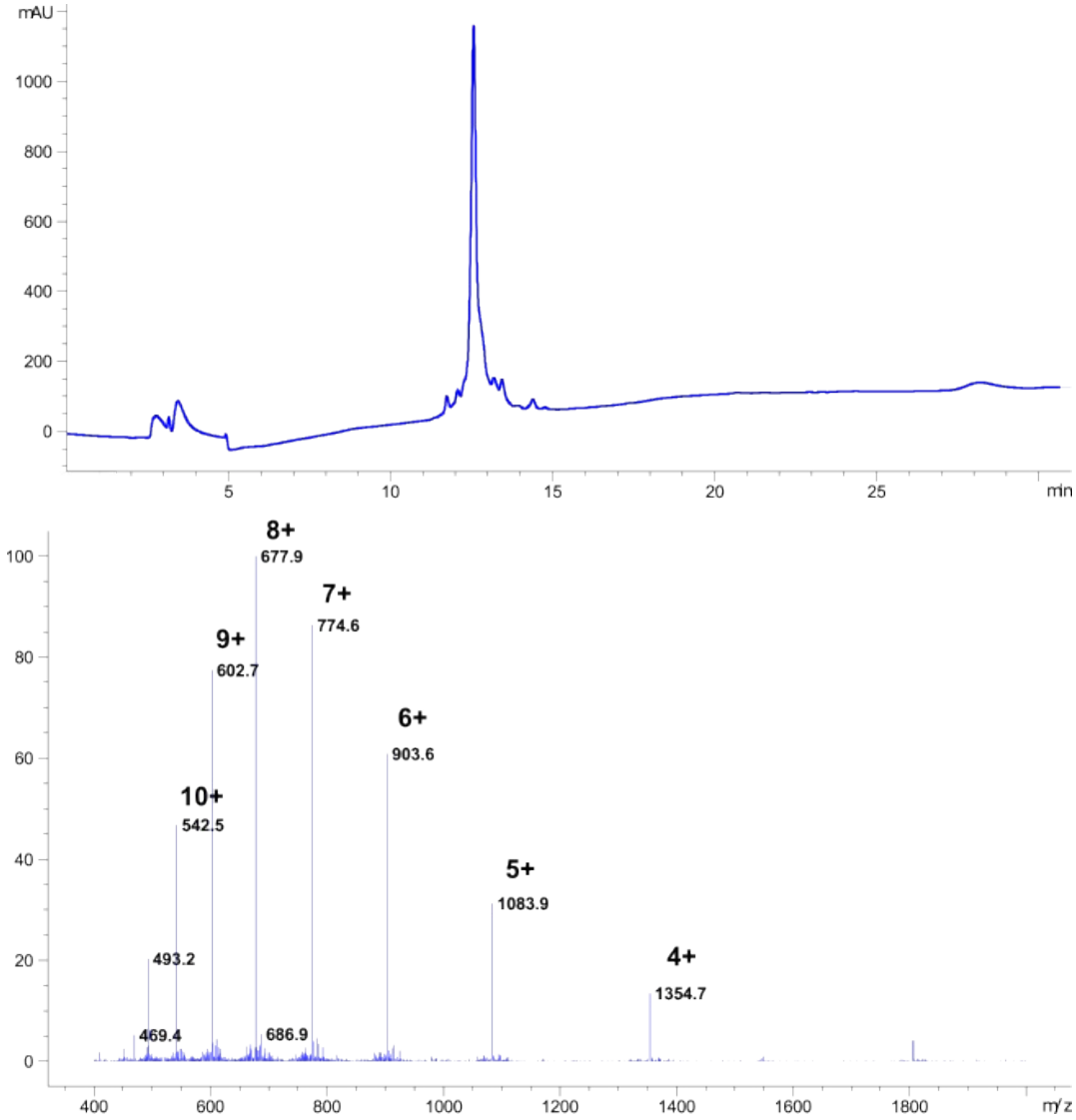


Fig. S4 LC-MS profile of pure $A\beta_{42}$-HMBA-Lys_6-$CONH_2$, using an analytical column (Zorbax 300SB-C3, 3.5 µm, 3.0 x 150 mm) at room temperature with a linear gradient of 5-95% buffer B over 30 minutes (buffer A = 0.1% formic acid in H_2O; buffer B = 0.1% formic acid in acetonitrile). Mass spectrum of the peak over the range of 400-1800 m/z showed the desired product (R_T 12.6 minutes; $[M+8H]^{8+}$ m/z calculated 678.03, observed 677.9; deconvoluted mass 5415.06 ± 0.42 Da, calculated mass 5416.31). The purity was determined to be 92%.

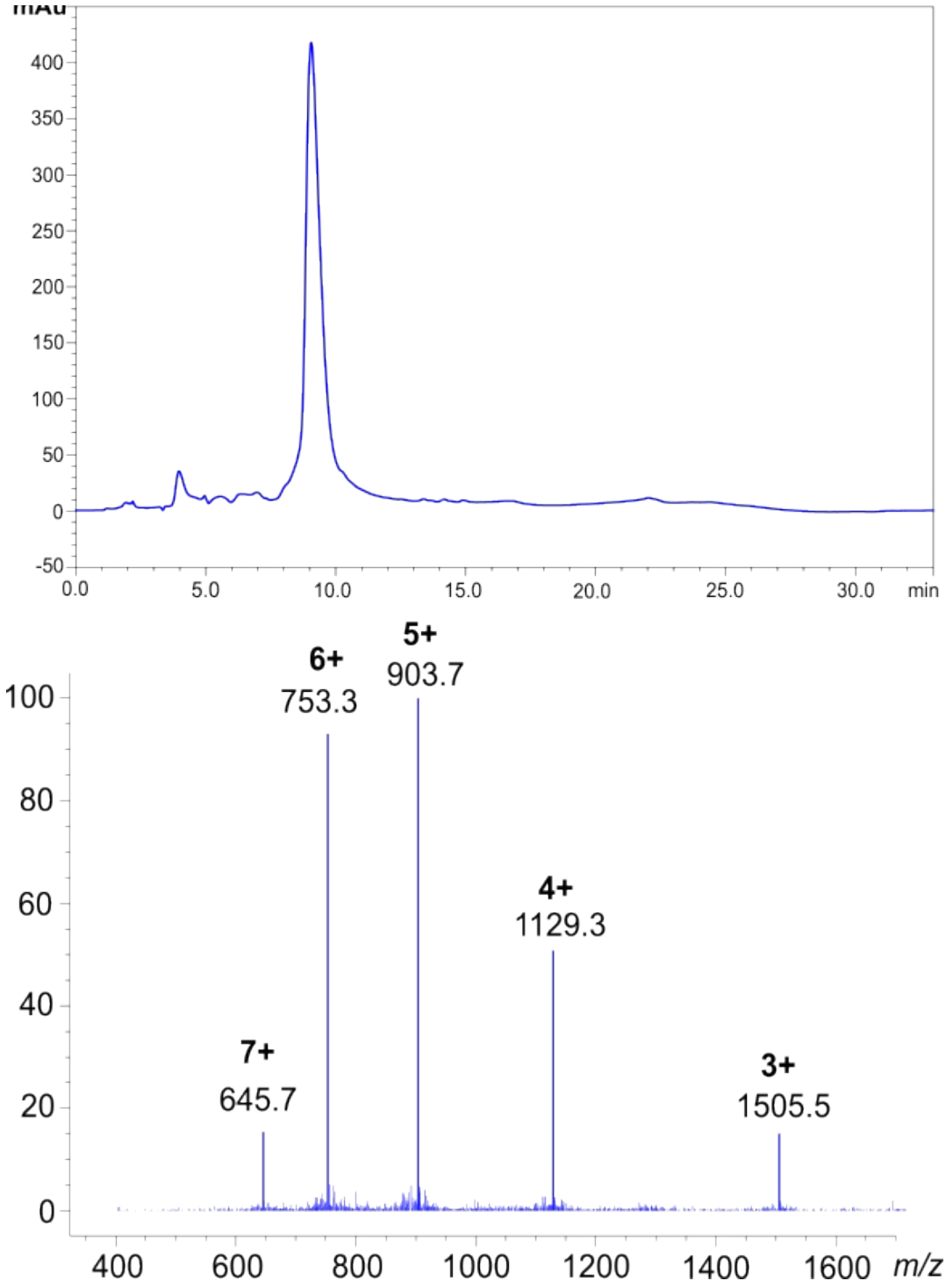


Fig. S5 Analytical RP-HPLC profile of pure $A\beta_{42}$ following removal of HMBA-Lys_6-$CONH_2$, using an analytical column (xTerra MS C18, 5 µM, 4.6 x 150 mm) at 50 °C with a linear gradient of 1-61% buffer B over 20 minutes (buffer A = 0.1% NH_4OH in H_2O; buffer B = 0.1% NH_4OH in acetonitrile). Mass spectrum of the peak over the range of 400-1800 m/z showed the desired product (R_T 9.8 minutes; $[M+5H]^{5+}$ m/z calculated 903.8, observed 903.7; deconvoluted mass 4513.38 ± 0.34 Da, calculated mass 4514.08). Buffer precipitation was observed beyond 61% B. The peptide purity was determined to be 92%.

IV. Biophysical characterisation

Lyophilised, pure peptide samples were pre-treated as according to **Method 16**, and their concentration quantitated as per **Method 17** prior to assessment of biophysical properties. TEM imaging was undertaken as described in **Method 18** (Fig. S5), followed by ThT assay (**Method 19**) and CD spectroscopy (**Method 20**).

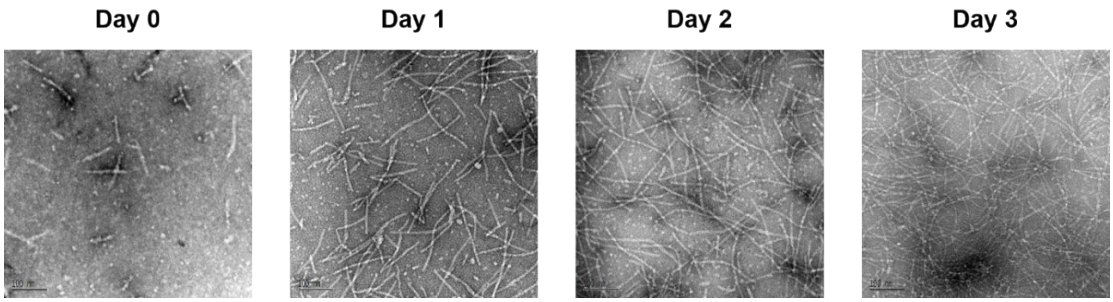


Fig. S6 TEM images of $A\beta_{42}$ in fibril forming solution (10 mM HCl), incubated at 37 °C over 3 days (Day 0-3) (Magnification: × 140 000; Scale bar: 100 nm).

V. References

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