

Supporting Information

The Efficient Synthesis and Purification of Amyloid- β (1-42) using an Oligoethylene Glycol-containing Photocleavable Lysine Tag

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1. General Experimental and Materials

Unless otherwise stated, all reagents were purchased from commercial sources and used without further purification. 2-Nitrobenzaldehyde (98%), Ethyl Magnesium Bromide (0.5M in THF) and Boc anhydride (99%) were sourced from Sigma Aldrich (Australia). Triphenylphosphine (99%) and Tosyl Chloride (98%) were purchased from Merck Millipore (Germany). Sodium azide (99%) was obtained from AJAX Scientific Ltd (Australia). 4-nitrophenylchloroformate was sourced from Matric Scientific (USA). 1,1,1,3,3,3-hexafluoroisopropanol (HFIP, 99%) was purchased from TCI Chemicals (Japan). Compounds **1**¹ and **2**² were prepared according to reported procedures. Tentagel R-PHB Fmoc-Ala (sub = 0.18 mmol/g) was sourced from Rapp Polymere (Germany). All Fmoc amino acids (98%) and oxyma pure (98%) were sourced from CEM Corporation (USA) except for Fmoc-Lys(Mmt)-OH (98%) which was purchased from Merck Millipore (Germany). O-(6-Chlorobenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HCTU, 98%) was obtained from GL Biochem (China). Diethyl ether, acetonitrile, acetic acid, 33% aqueous ammonium hydroxide, ammonium acetate, dimethylformamide (DMF), dichloromethane (DCM), ethyl acetate were sourced from Merck Millipore (Australia). TFA (99%), piperidine (99%), diisopropylethylamine (DIEA, 98%), triisopropylsilane (98%) and thioanisole (98%) were purchased from Sigma-Aldrich (Australia). Nitrogen gas was purchased from Coregas (Australia). Flash column chromatography was carried out using silica gel (40-63 microns) as the stationary phase. Analytical TLC was performed on pre-coated silica gel plates (0.25 mm thick, 60F254, Merck, Germany) and visualised under UV light. ¹H, ¹³C NMR spectra were recorded either on a 400 MHz Varian/Agilent 400-MR or 500 MHz AR spectrometer at 298 K. Chemical shifts are reported in parts per million (ppm) and referenced to residual solvent peak. Coupling constants (*J*) are reported in Hertz (Hz). Standard abbreviations indicating multiplicity were used as follows: m = multiplet, quint. = quintet, q = quartet, t = triplet, d = doublet, s = singlet, br = broad. ESI-MS spectra were recorded on an Agilent 6510 ESI-TOF LC/MS mass spectrometer or Thermo Fisher Orbitrap infusion mass spectrometer.

2. Synthesis of Photolabile OEGylated linker

Synthesis of 3

A mixture of alkyne **1**¹ (500 mg, 2.82 mmol), azide **2**² (900 mg, 2.82 mmol), CuSO₄·5H₂O (423 mg, 1.69 mmol) and sodium ascorbate (559 mg, 2.82 mmol) were combined in 4:1 DMF/water (10 mL) and stirred for 48 h at ambient temperature in the absence of light. EDTA/NH₄OH(aq) (0.1N, 30 mL) was added to the reaction mixture and stirred for 5 min then extracted with CH₂Cl₂ (3 × 50 mL).

Combined organic layers were washed with brine and dried over MgSO_4 . Solvent was removed under reduced pressure, and the product was purified by column chromatography (silica gel, $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$, 1:19) to give compound **3** (1.12 g, 72%) as a colorless oil. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 7.95 (d, $J = 1.1$ Hz, 1H), 7.93 (d, $J = 1.2$ Hz, 1H), 7.89 (dd, $J = 7.9, 1.2$ Hz, 1H), 7.86 (s, 1H), 7.78 – 7.73 (m, $J = 7.6, 1.2$ Hz, 1H), 7.58 – 7.52 (m, 1H), 6.73 (t, $J = 5.4$ Hz, 1H), 6.41 (d, $J = 5.3$ Hz, 1H), 6.31 (d, $J = 5.3$ Hz, 1H), 4.46 (t, $J = 5.3$ Hz, 2H), 3.77 (t, $J = 5.3$ Hz, 2H), 3.50 – 3.42 (m, 8H), 3.35 (t, $J = 6.1$ Hz, 2H), 3.04 (q, $J = 6.0$ Hz, 2H), 1.36 (s, 9H); ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 162.8, 156.0, 149.6, 148.1, 138.5, 133.7, 129.0, 128.9, 124.5, 123.4, 78.0, 70.1, 70.1, 70.0, 69.9, 69.6, 69.1, 63.7, 49.7, 28.7; ESI-MS: $m/z = [\text{M}+\text{H}]^+$ 496.2405 (experimental), 496.2402 (calculated for $[\text{C}_{22}\text{H}_{34}\text{N}_5\text{O}_8]^+$).

Synthesis of **4**

A solution of **3** (500 mg, 1.0 mmol), 4-nitrophenylchloroformate (244 mg, 1.2 mmol) and pyridine (200 μL) in dichloromethane (50 mL) was stirred at ambient temperature for 24 hrs under N_2 atmosphere. The mixture was concentrated by rotary evaporation and the residue was purified by flash column chromatography (eluted with 0-40% ethyl acetate in hexane). Evaporation of solvent gave **4** (305 mg, 45%) as a viscous yellow oil which was stored in freezer in the absence of light. ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.31 (d, $J = 9.3$ Hz, 2H), 8.24 (s, 1H), 8.12 (dd, $J = 8.2, 1.2$ Hz, 1H), 7.98 (dd, $J = 7.9, 1.4$ Hz, 1H), 7.91 (td, $J = 7.5, 1.1$ Hz, 1H), 7.74 – 7.67 (m, 1H), 7.55 (d, $J = 9.3$ Hz, 2H), 7.42 (s, 1H), 6.72 (t, $J = 5.4$ Hz, 1H), 4.56 – 4.51 (m, 2H), 3.81 (t, $J = 5.3$ Hz, 2H), 3.52 – 3.41 (m, 8H), 3.36 – 3.32 (m, $J = 11.7, 5.5$ Hz, 2H), 3.07 – 2.98 (m, $J = 12.0, 6.0$ Hz, 2H), 1.35 (s, 9H); ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 170.8, 156.0, 155.4, 151.6, 147.8, 145.8, 143.4, 134.9, 132.6, 130.4, 129.2, 125.9, 125.7, 125.2, 122.9, 78.0, 70.7, 70.1, 70.1, 70.0, 69.9, 69.6, 69.0, 60.2, 50.0, 28.7, 21.2, 14.5; ESI-MS: $m/z = [\text{M}+\text{H}]^+$ 661.2470 (experimental), 661.2464 (calculated for $[\text{C}_{29}\text{H}_{37}\text{N}_6\text{O}_{12}]^+$).

3. Solid Phase Peptide Synthesis of $\alpha\text{Nb-OEG}_3\text{-A}\beta_{42}$

Peptide assembly was performed on a Liberty microwave peptide synthesiser from CEM Corporation (USA). The $\text{A}\beta_{42}$ analogue was synthesised on a 0.1 mmol scale. For coupling, 5 equivalents of Fmoc amino acid and HCTU, plus 10 equivalents of DIEA were used. Residues 28-42 were coupled once at 75 $^\circ\text{C}$ for 5 minutes and residues 1-27 were double coupled under the same conditions except for Fmoc-His(Trt)-OH which was double coupled at 50 $^\circ\text{C}$. Fmoc cleavage was performed using a solution of 0.1 M oxyma pure in 20% piperidine/DMF at 90 $^\circ\text{C}$ for 1 minute, except for residues 23-27 which were deprotected for 10 minutes. Cleavage of the Mmt protecting group at Lys²⁸ was effected via

multiple treatments with 1% TFA in DCM. After neutralisation of the resin, 3 equivalents of the *o*Nb-OEG₃ tag and 6 equivalents of DIEA were added and coupled under microwave conditions for 1 hour at 75 °C. After synthesis, the resin-bound peptide was deprotected and cleaved with a cocktail of 2% water/2% thioanisole/1% triisopropylsilane in TFA for 3 hours. The resin was filtered and the volume of TFA reduced to ~2 mL via nitrogen aspiration, followed by a precipitation step and two further washes with diethyl ether. 300 mg of crude material was recovered. 50 mg was purified as described below and a final mass of 4.8 mg was isolated. The purity was found to be >95%. ESI-MS (Figure S1): $m/z = [M+4H]^+$ 1234.6174 (experimental), 1234.6163 (calculated for [C₂₂₁H₃₃₄N₆₀O₆₇S]⁺).

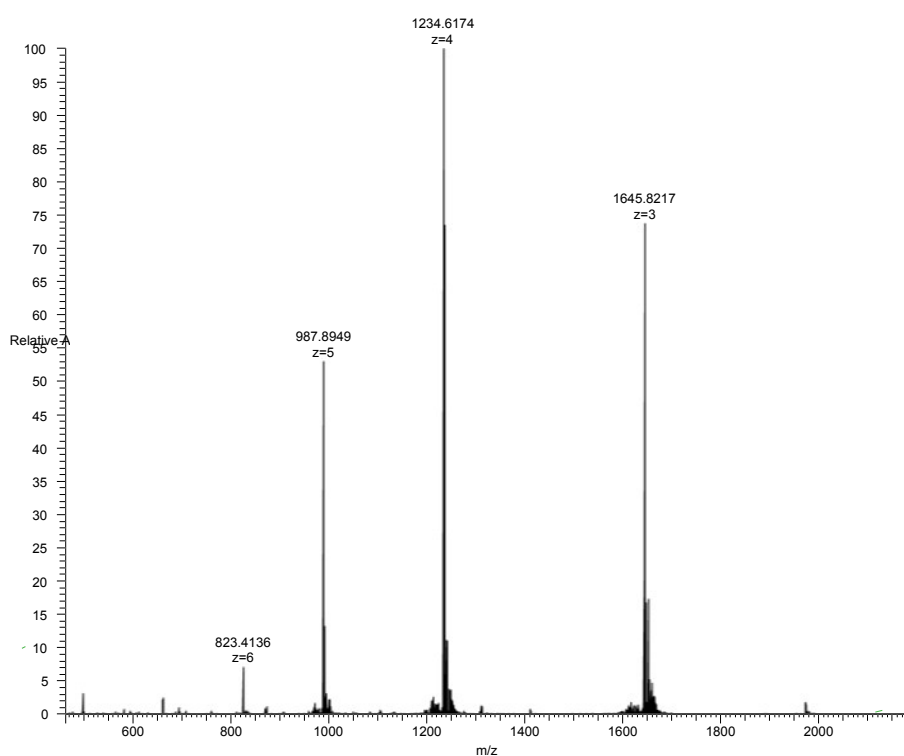


Figure S1: ESI-MS of purified *o*Nb-OEG₃-Aβ₄₂.

4. Reversed-phase HPLC Purification

All RP-HPLC was performed on an Agilent 1100 instrument, which was equipped with a degasser, auto-sampler, fraction collector and multichannel UV-Vis detector. Mobile phases: buffer A = 10 mM NH₄OAc in water, pH 9.2; buffer B = 10 mM NH₄OAc in 80% acetonitrile/20% water, pH 9.2. Purification of OEGylated Aβ₄₂ was carried out on a Phenomenex Kinetex 5μ XB-C18, 100 Å, 21.2 x 150 mm AXIA packed column at 60 °C. Gradient conditions: 20 – 60% buffer B over 40 minutes with a

5 mL/min flow rate and detection at 230 nm. Native A β ₄₂ was purified with a Phenomenex Jupiter 10 μ C4, 300 Å, 4.6 x 150 mm column. Gradient conditions: 20 – 60% buffer B over 40 minutes with a 5 mL/min flow rate and detection at 230 nm. Peptidic material was lyophilised on a Christ freeze dryer and the identity of the peptides were confirmed by mass spectrometry.

5. Photolysis of oNb-OEG₃-A β ₄₂

All photolysis reactions were performed in the 365 nm range with a RPR-100 Rayonet Photochemical Chamber Reactor. OEGylated A β ₄₂ (1.1 mg) was dissolved in 1 mL of 80% HFIP/20% H₂O. After irradiation for 20 minutes the solution was then injected directly onto the RP-HPLC column for purification affording peptide **8** (660 μ g, 60% yield). ESI-MS (Figure S2): $m/z = [M+4H]^+$ 1129.3268 (experimental), 1129.3263 (calculated for [C₂₀₃H₃₁₁N₅₅O₆₀S]⁺).

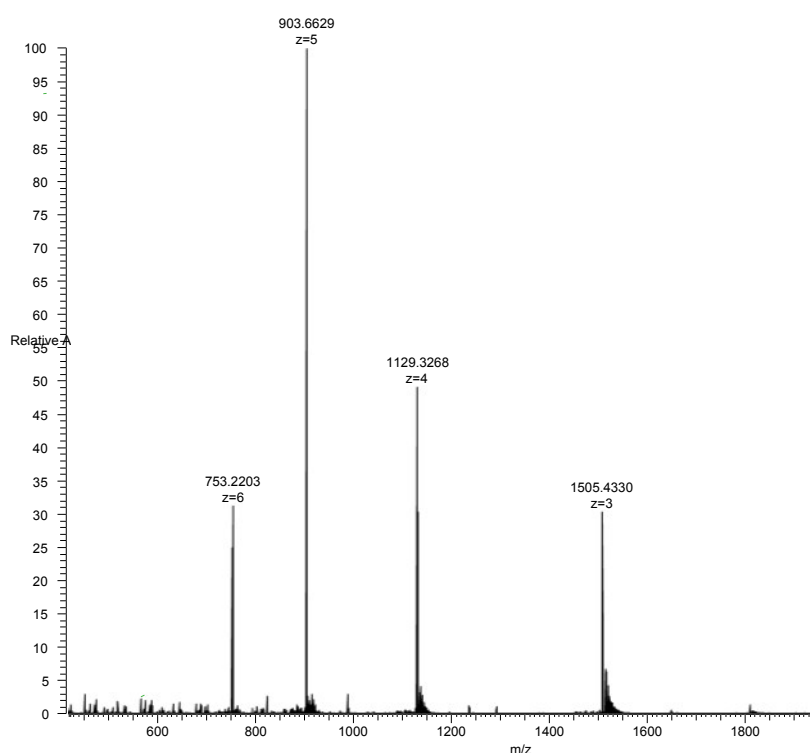


Figure S2: ESI-MS of purified A β ₄₂ after photolysis.

6. Aggregation Studies

Preparation of A β ₁₋₄₂ peptide stock solutions:

Synthetic peptide was dissolved in HFIP (1 mM), incubated for 1 hour on ice then allowed to evaporate overnight in fume hood. The residue was further dried under high vacuum to remove any residual HFIP and moisture and then stored at -80 °C. For a 1 mg/mL stock solution in PBS, the HFIP treated peptide was dissolved in 2 parts of 60 mM NaOH and incubated for 3 min at room temperature. To this solution 7 parts of de-ionised water was added and vortexed briefly followed by ultrasonication for 5 minutes on ice. Added 1 part of 10x PBS, vortexed briefly and centrifuged for 5 min. Supernatant was transferred to a fresh tube and kept on ice. The concentration of the stock solution was determined by measuring the absorbance at 214 nm ($\epsilon = 75\,887\text{ M}^{-1}\text{ cm}^{-1}$).

ThT fluorescence A β ₄₂ binding assays

Stock solution of A β ₄₂ (100 μM) was incubated in PBS at 37 °C and agitated at 300 rpm for 48 hours to promote the formation of fibrils. Stock solution of ThT (1 mM in PBS) was freshly prepared and final samples were composed of A β ₄₂ peptides (20 μM) and ThT (40 μM) at a final volume of 300 μL in PBS. The ThT fluorescence intensity of each sample was recorded by excitation at 444 nm.

TEM imaging

Stock solution of A β ₄₂ (100 μM) was prepared as detailed above and fibril formation confirmed using ThT binding assay. Stock solutions were diluted to 20 μM and then spotted onto a 300-mesh carbon-coated copper grid, allowed to incubate for 2 min, and then excess solution was blotted off. Samples were subsequently stained with uranyl acetate (0.5 % w/v, 2 min) and analysed on a FEI Tecnai F20 TEM transmission electron at a voltage of 200 kV.

7. References

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