Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Solid-phase Synthesis of Homodimeric Peptides: Preparation of Covalently-linked Dimers of Amyloid-beta Peptide

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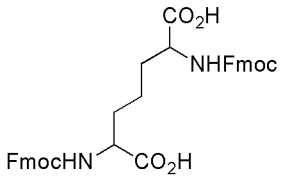
Experimental procedures

General Methods. Melting points were determined on an automated melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 499.69 MHz. Chemical shifts (δ) are reported in parts per million relative to the residual solvent peak of chloroform (δ 7.26). ^{13}C NMR spectra were recorded at 125.78 MHz. Chemical shifts (δ) are reported in parts per million relative to the residual solvent peak of chloroform (δ 77.0). RP-HPLC was performed on a C18 column (50 × 21.2 mm) with a linear gradient of 0–75% buffer B/buffer A [buffer A: 0.1% TFA in water (v/v); buffer B: 0.1% TFA in acetonitrile (v/v)] over 70 min, with a flow rate of 5 mL/min. Size exclusion chromatography was performed on a G3000SW 10μm 7.5 × 500 mm column, eluting with 1:1 buffer A:buffer B [buffer A: 0.1% TFA in water (v/v); buffer B: 0.1% TFA in acetonitrile (v/v)] over 60 min, with a flow rate of 0.5 mL/min. Mass spectra were recroded on a Q-TOF LC/MS mass spectrometer. All data were acquired and reference-mass-corrected via a dual-spray electrospray ionisation (ESI) source.

2,6-Di([9*H*-fluoren-9-yl]methoxycarbonylamino)pimelic acid (Fmoc$_2$DAP) 4



2,6-Diaminopimelic acid (0.10 g, 0.53 mmol), Fmoc-OSu (0.35 g, 1.05 mmol) and Na_2CO_3 (0.22 g, 2.1 mmol) were added to acetone/water (1:1, 4 mL) and stirred at room temperature overnight. The mixture was acidified to pH 2 with concentrated HCl and concentrated *in vacuo*. The mixture was then extracted with dichloromethane (2 × 6 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give a white solid. The solid was chromatographed on silica eluting with 0–1% formic acid/ethyl acetate to give the product **4** as a white solid (0.26 g, 80%), mp 104–106 °C; ^{1}H NMR (DMSO, 500 MHz) δ 7.88 (4H, d, J = 7.4 Hz), 7.72 (4H, d, J = 7.4 Hz), 7.65 (2H, m), 7.41 (4H, t, J = 7.4 Hz), 7.33 (4H, t, J = 7.4 Hz), 4.27 (4H, d, J = 6.4 Hz), 4.21 (2H, t, J = 6.4 Hz), 1.72–1.60 (4H, m), 1.45–1.41 (2H, m); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.84/173.79, 156.1, 143.8, 140.69/140.68, 127.6, 127.1, 125.28/125.25, 120.1, 65.6, 53.73/53.70, 46.6, 30.37/30.34, 22.56/22.33. MS (ESI+) *m*/*z* 635.239 (M+H$^+$); calc. for $C_{37}H_{34}N_2O_8$, 635.232.

Synthesis of peptide dimers. Standard Fmoc solid-phase peptide synthesis techniques were employed to prepare resin-bound linear peptides, either manually or in batch mode on an

automated microwave peptide synthesiser, using Fmoc-protected-L-α-amino acids with standard TFA-labile side-chain protecting groups.

General amino acid coupling protocol

Fmoc-L-α-amino acids were activated with 2-(1-H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HBTU) and *N*-ethyl-diisopropylethylamine (DIPEA) in DMF (5 mL) for 30 min. The solution was added to the resin, and the coupling reaction allowed to proceed for 1 hour.

Coupling of $Fmoc_2DAP$ to peptide

$Fmoc_2DAP$ **4** (0.5 to 2.0 equiv) and 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium (HATU) (1.0 equiv relative to $Fmoc_2DAP$) were dissolved in minimal volume of DMF. DIPEA (2.0 equiv relative to $Fmoc_2DAP$) was added and the mixture was added to the resin and allowed to react overnight. The coupling reaction was monitored for completion with the qualitative TNBSA test (see below).

Optimization of $Fmoc_2DAP$ coupling

Following $Fmoc_2DAP$ coupling, the resin-bound peptide was treated with 5% Ac_2O / 5% DIPEA in DMF for 30 min. The resin was washed (DCM, DMF), Fmoc-deprotected, then Fmoc-Gly was coupled following the general coupling protocol. The peptide was Fmoc-deprotected, then cleaved from the resin and analysed by C18 RP-HPLC.

General deprotection protocol

The resin-bound peptide was treated with a solution of 20% piperidine in DMF (containing 0.1M HOBt) for 20 minutes to remove the Fmoc group.

Analysis of coupling/deprotection reactions

A qualitative confirmation test was carried out after each coupling or deprotection reaction using the 2,4,6-trinitrobenzenesulphonic acid (TNBSA) test for free amino groups.[1] Approximately twenty resin beads were placed in a 1:1 mixture of 5% DIPEA in DMF, and 1% TNBSA in DMF. Clear beads indicated successful coupling, whilst red coloration confirmed the successful deprotection with the presence of free amino group.

Cleavage of peptides

The peptides were cleaved in 2.5% triisopropylsilane (TIPS), 2.5% water, 95% TFA solution at room temperature for 3 h. The volatiles were removed under a stream of nitrogen gas, and peptides precipitated with diethyl ether, redissolved in 30% acetonitrile in water (v/v), and lyophilised.

Purification of peptide dimers

Aβ(9–16) dimer 8a

Purification of the dimer was performed using the general RP HPLC method.

MS (ESI+) *m/z* 1822.89 ($M+H^+$) (calc. 1822.82).

Aβ(1–16) dimer 8b

Purification of the dimer was performed using the general RP HPLC method.

MS (ESI+) *m/z* 3737.76 (calc. 3737.74).

Aβ(1–28) dimer 8c

Purification of the dimer was performed using the general RP HPLC method, followed by the general size exclusion chromatographic method.

MS (ESI+) *m/z* 6352.88 (calc. 6352.72).

Aβ(1–40) dimer 8d

Purification of the dimer was performed using the general size-exclusion chromatographic method, followed by C4-RP HPLC (5μ, 300Å pore size, 4.6 × 240 mm) heated in a water bath to 60 °C, eluting with a linear gradient of 10–90% buffer A/buffer C [buffer A: 0.1% TFA in water (v/v); buffer B: 0.1% TFA in 80% acetonitrile and 20% isopropanol (v/v)] over 45 min, with a flow rate of 1 mL/min.

MS (ESI+) *m/z* 8487.56 (calc. 8487.44).

Dynamic Light Scattering

Lyophilised peptides were dissolved on ice to ~0.5 mg/mL under a range of buffer conditions including water alone, 20 mM HEPES pH 7.0, and 1 x PBS pH 7.4. Samples were subject to centrifugation (15,000g, 10 minutes, 4 °C) to remove particulates immediately prior to analysis. DLS measurements were made with a Malvern Instruments Zetasizer Nano ZS instrument. Size distribution profiles were measured repeatedly over the first 30 minutes following dissolution. All samples demonstrated some degree of polydispersity and no discernible difference was observed between buffer conditions. Peptide concentrations: Aβ(1–16) monomer, 0.26 mM; Aβ(1–16) dimer **8b**, 0.13 mM; Aβ(1–28) monomer, 0.15 mM; Aβ(1–28) dimer **8c**, 0.079 mM; Aβ(1–40) monomer, 0.11 mM; Aβ(1–40) dimer **8d**, 0.059 mM.

Circular dichroism spectroscopy

Dry peptide was weighed and dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at a concentration of 1 mg/mL to ensure solutions were free of aggregated peptide, then aliquotted and dried by speed vacuuming and stored at –80 °C. Peptide concentrations were determined using

absorbance at 214 nm and extinction coefficients of 48525 for Aβ(1–16), 90292 for Aβ(1–16) dimer **8b**, 61517 for Aβ(1–28), 137969 for Aβ(1–28) dimer **8c**, 55771 for Aβ(1–40), and 239706 for Aβ(1–40) dimer **8d**, as determined by amino acid analysis. Aliquots of HFIP-treated, dried peptide were dissolved in 20mM NaOH then diluted in deionised water and phosphate buffer (100mM potassium phosphate, pH 7.4) at a v/v/v ratio of 2:7:1. All solutions were sonicated at 0 °C for 10 min and filtered (20 μm) to ensure pre-formed aggregates were removed. Samples were run in a 0.1 cm quartz cuvette at 37 °C. For aging experiments, the peptide was incubated at 37 °C for up to 7 days with agitation. CD spectra were background subtracted, adjusted for concentration, and smoothed using the algorithm provided with the system. Peptide concentrations: Aβ(1–16) monomer, 76 μM; Aβ(1–16) dimer **8b**, 27 μM; Aβ(1–28) monomer, 61 μM; Aβ(1–28) dimer **8c**, 15 μM; Aβ(1–40) monomer, 37 μM; Aβ(1–40) dimer **8d**, 6 μM.

ThT assay for fibril formation

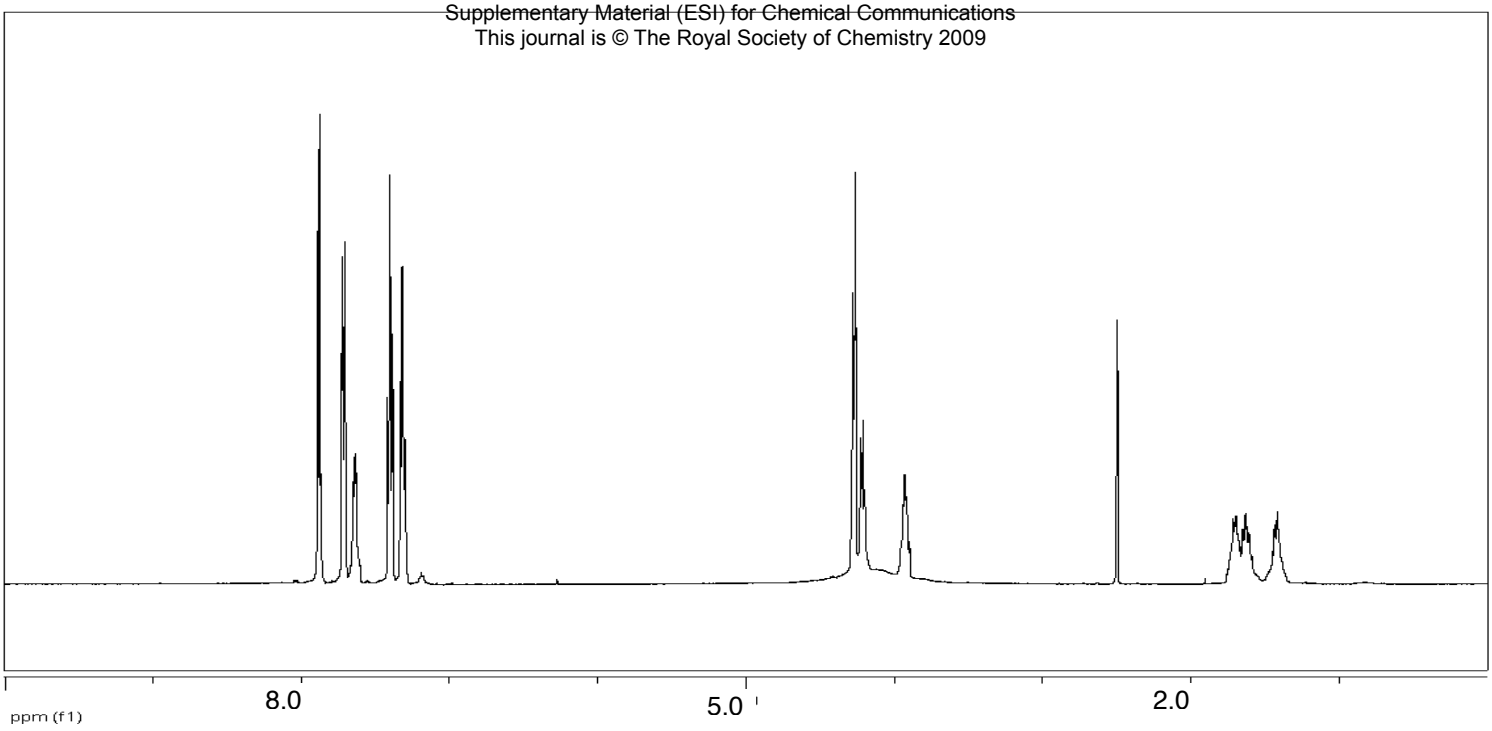
Peptide solutions were prepared as above for CD spectroscopy, with the Aβ(1–40) dimer **8d** at a final concentration of 7 μM and the Aβ(1–40) monomer at 14 μM, and Thioflavin-T (ThT) at 28 μM in 1X PBS buffer (10mM sodium phosphate, 137mM NaCl, 2.7mM KCl at pH 7.4) to a final volume of 600 μL. Each sample was incubated at 37 °C in a stirred cuvette. Excitation was at 444 nm with fluorescence emission measured at 480 nm. Readings were taken every 60 s for the first 15 min, then every 15 min for the next 885 min. Slit widths were 5 nm for both excitation and emission.

Electron Microscopy

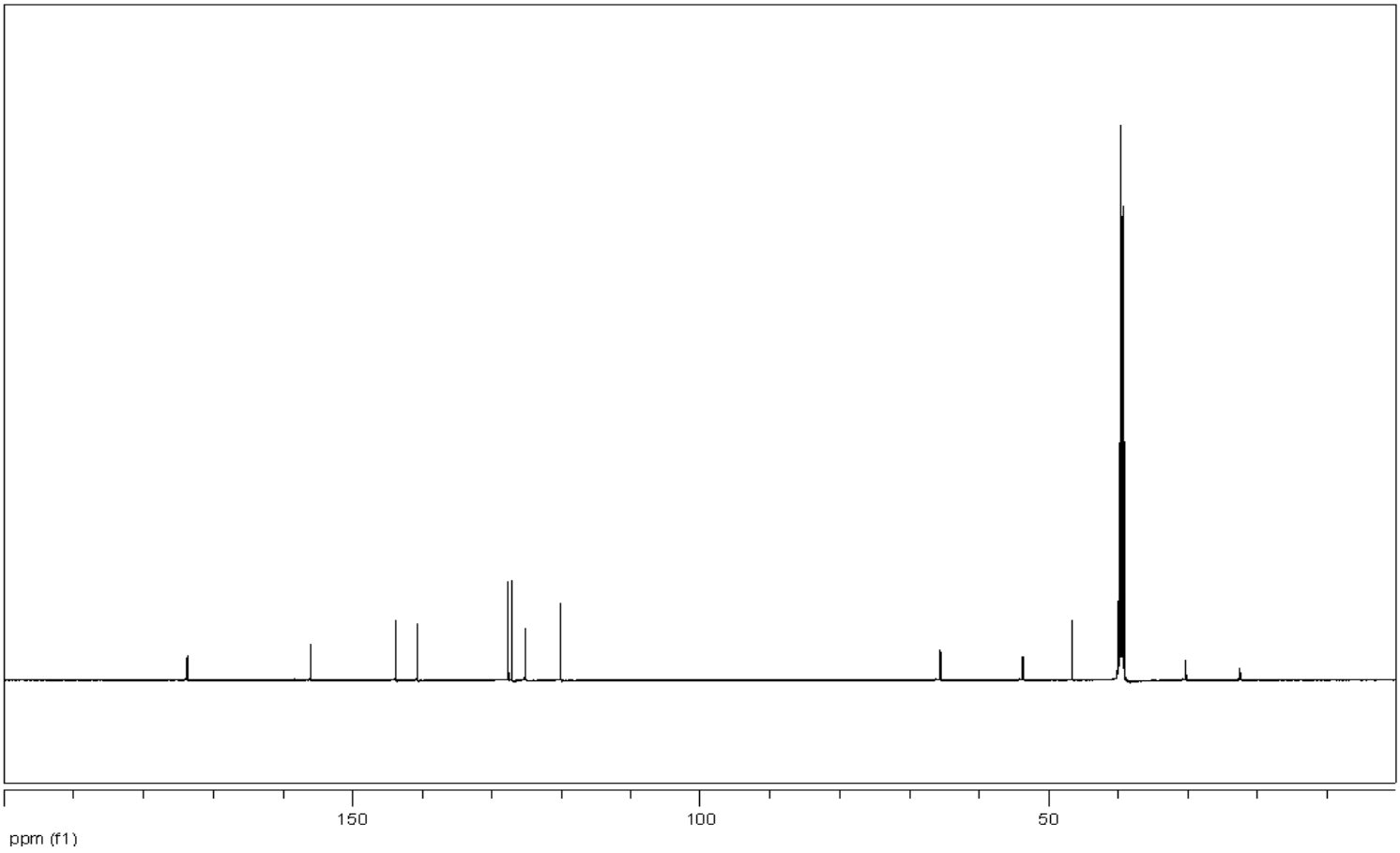
Transmission electron microscope (TEM) samples were prepared by absorbing a 3.5 μL aliquot of the sample solution used for ThT assay onto a carbon-coating Formwar film mounted on 300 mesh copper grids. Prior to adsorption, the grids were rendered hydrophilic by glow discharge in a reduced atmosphere of air for 10 s. After 30 s adsorption, samples were blotted and negatively stained with 1.5% aqueous uranyl acetate. The transmission electron microscope was operated at 200 kV, with images acquired digitally.

Reference

[1] Hancock, W. S.; Battersby, J. E. *Analytical Biochemistry* **1976**, *71*, 260.

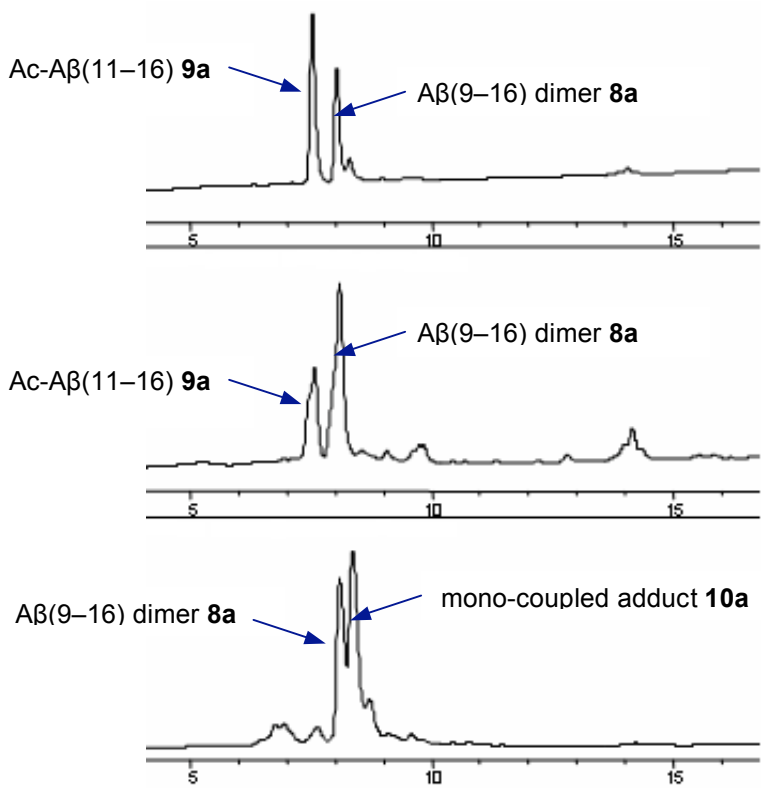


^{1}H NMR spectrum of Fmoc$_2$DAP **4** (in d_6-DMSO)



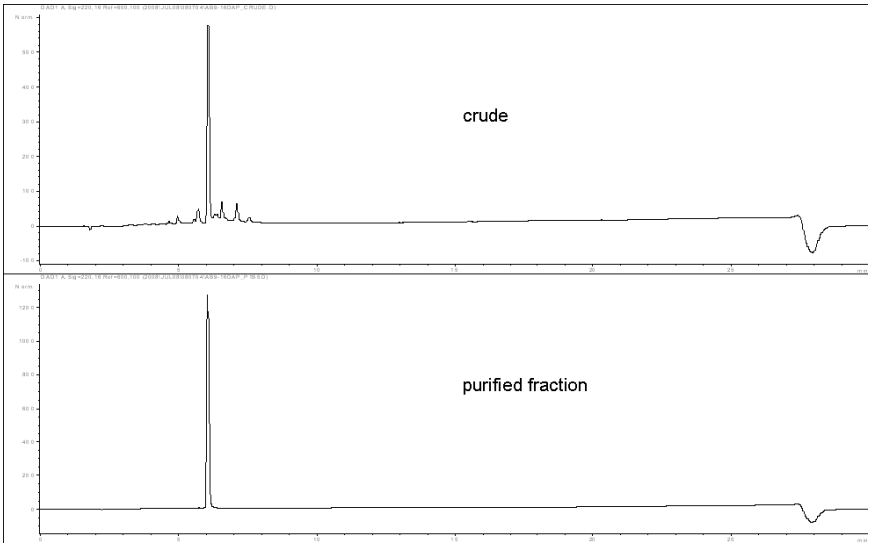
^{13}C NMR spectrum of Fmoc$_2$DAP **4** (in d_6-DMSO)

Optimization of $Fmoc_2DAP$ coupling

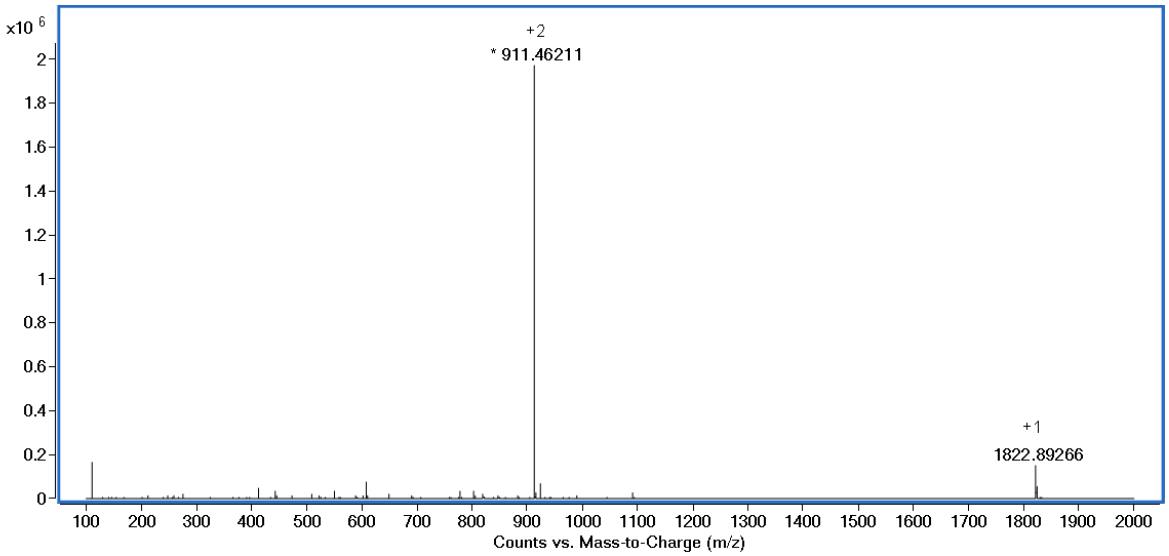


Optimisation of $Fmoc_2DAP$ coupling to resin-bound Aβ(11–16) peptide: 0.5 equiv. $Fmoc_2DAP$ **4** (top); 0.75 equiv. $Fmoc_2DAP$ **4** (middle); 2.0 equiv. $Fmoc_2DAP$ **4** (bottom) (relative to peptide loading).

Aβ(9–16) dimer 8a

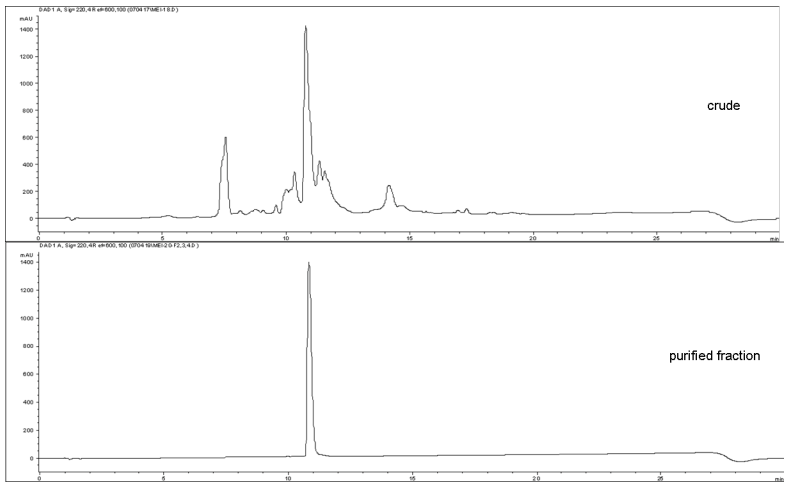


HPLC traces of crude Aβ(9–16) dimer cleaved from resin (top), and purified Aβ(9–16) dimer **8a** following preparative HPLC (bottom).

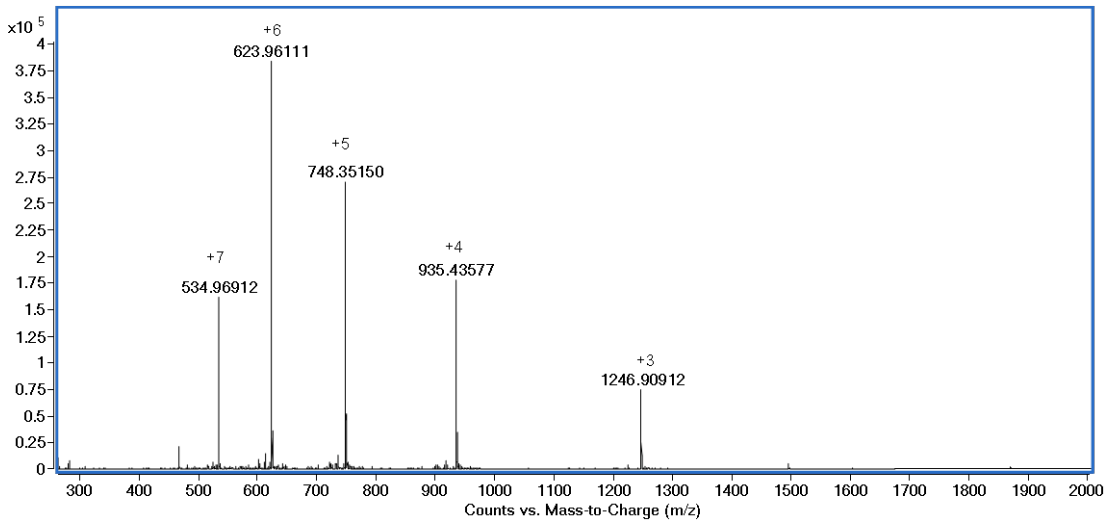


ESI mass spectrum of Aβ(9–16) dimer **8a**

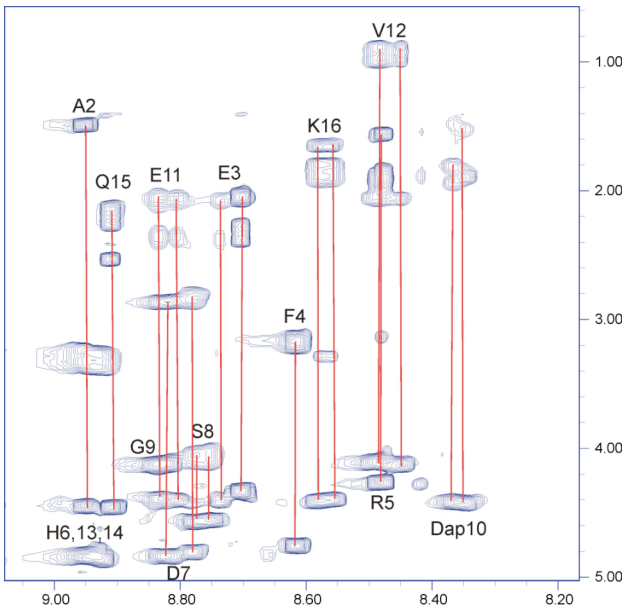
Aβ(1–16) dimer 8b



HPLC traces of crude Aβ(1–16) dimer cleaved from resin (top), and purified Aβ(1–16) dimer **8b** following preparative HPLC (bottom).

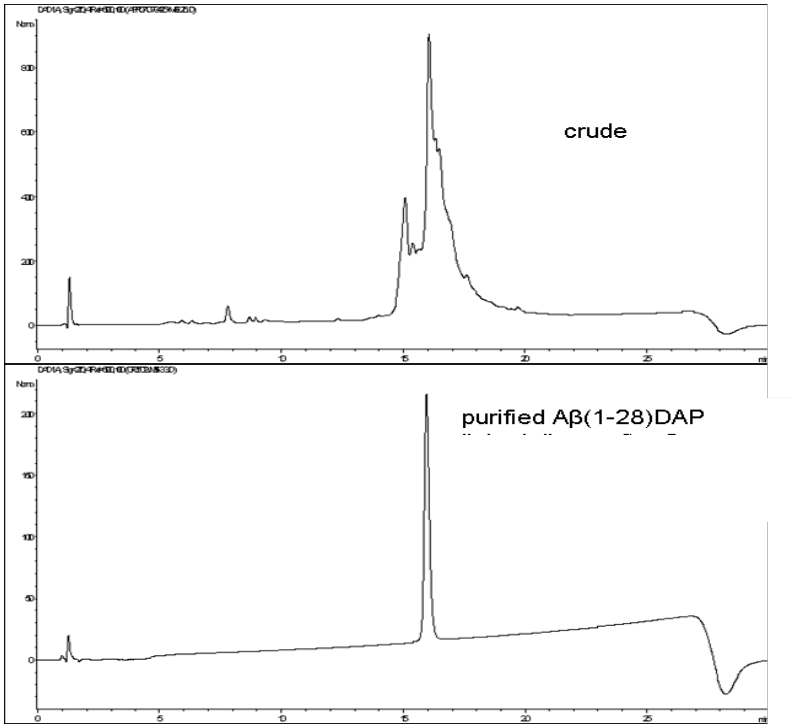


ESI mass spectrum of Aβ(1–16) dimer **8b**

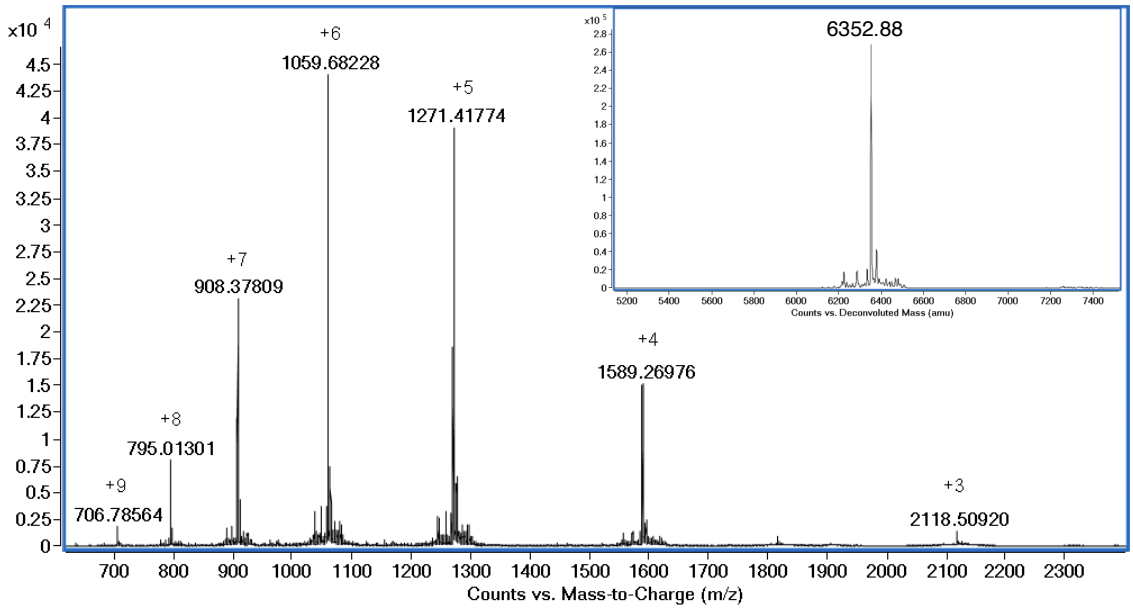


TOCSY NMR spectrum of Aβ(1–16) dimer **8b**

Aβ(1–28) dimer 8c

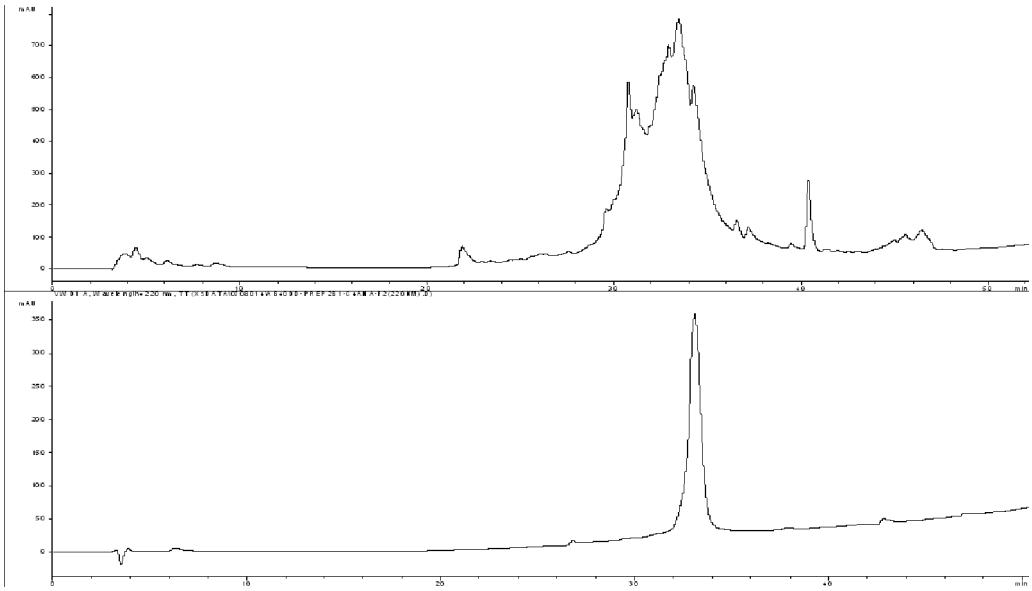


HPLC traces of crude Aβ(1–28) dimer cleaved from resin (top), and purified Aβ(1–28) dimer **8c** following preparative HPLC and size-exclusion chromatography (bottom).

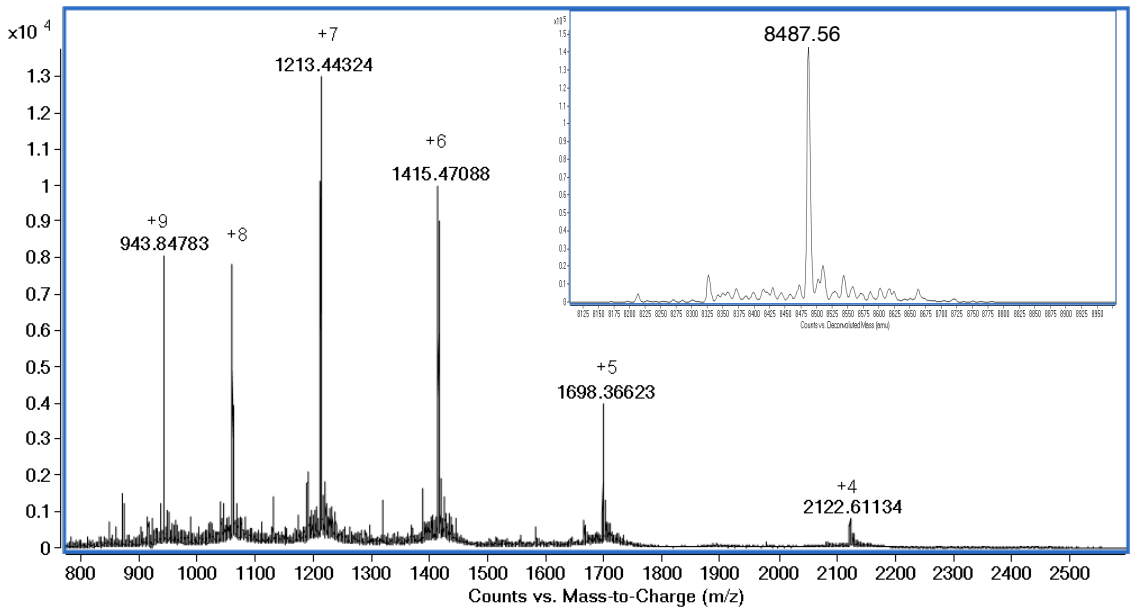


ESI mass spectrum of Aβ(1–28) dimer **8c**, and deconvoluted spectrum (inset).

Aβ(1–40) dimer 8d



Purification of Aβ(1–40) dimer: RP-HPLC following size exclusion chromatography (top); and subsequent C4 RP HPLC (bottom).



ESI mass spectrum of Aβ(1–40) dimer **8d**, and deconvoluted spectrum (inset).