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Supporting Material

Peptide Adsorption to Lipid Bilayers: Slow Processes Revealed by Linear Dichroism Spectroscopy

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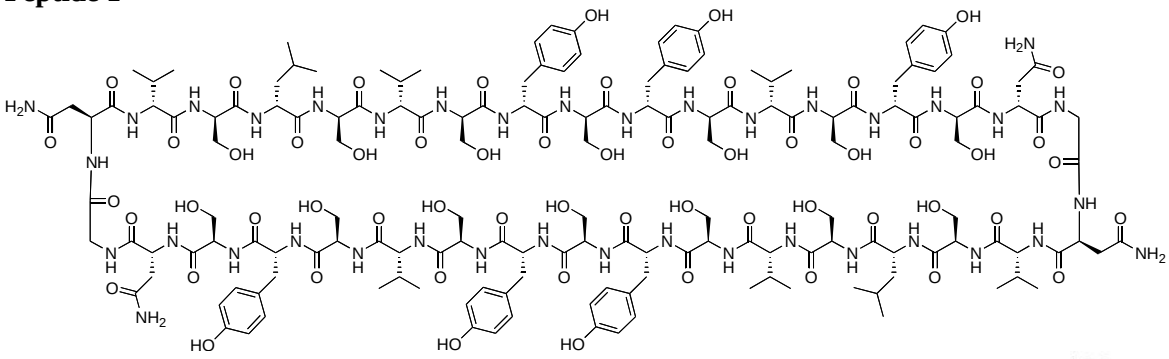
HPLC Data

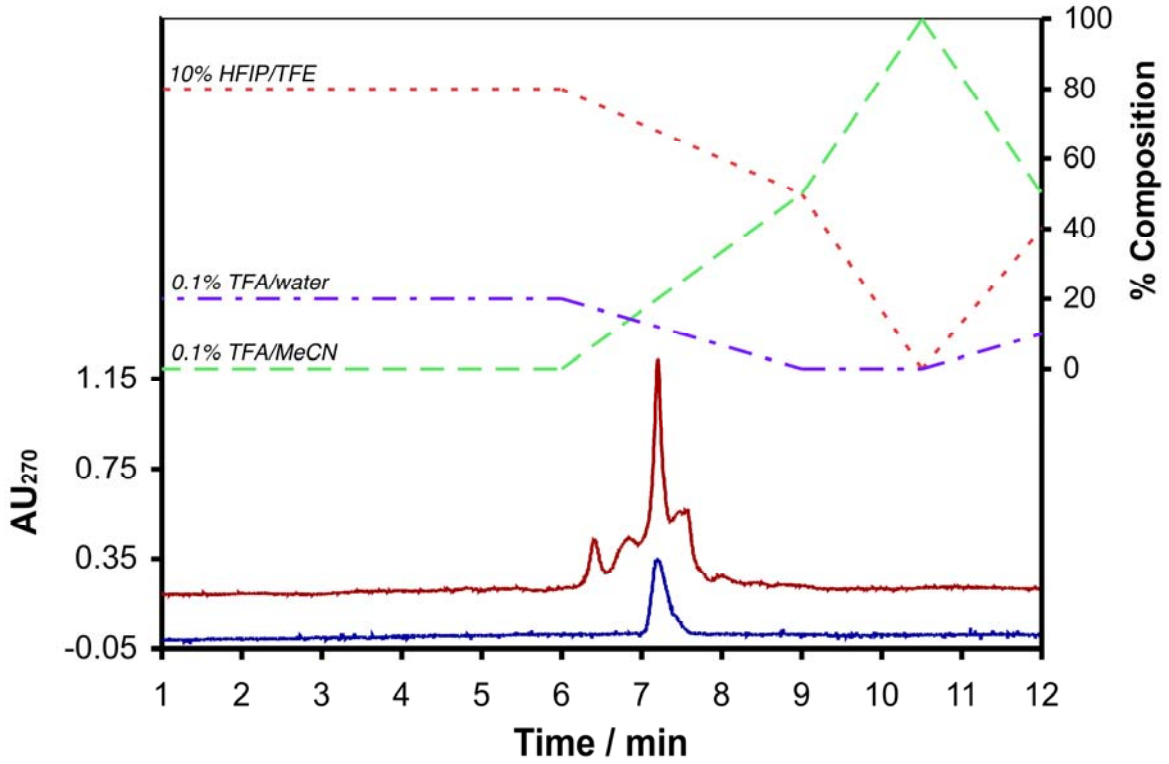
Column: Supelcosil LC-8 (C_8), 25 x 1 cm, 5 μm particle size, 100-300 Å pore size (Supelco).
Eluants: A, 10% HFIP in TFE; B, 0.1% TFA in MeCN; C, 0.1% TFA in water. Temperature: 30 °C. Detector wavelength: 220 nm or 270 nm.[1]
Equipment: Perkin-Elmer Series 200 lc pump, Gilson 231XL sample injector with 401C dilutor, Shimadzu CTO-6A column oven, Kontron DEG-104 in-line degasser, Waters 486 detector linked to a data-logger constructed in house using a National Instruments DAQ card and LabView 6i software running on Windows 2000.

Gradient:

t (min)	%A	%B	%C	Flow (ml/min)
0	80	0	20	2
6	80	0	20	2
9	50	50	0	2
10.5	0	100	0	2
13.5	80	0	20	2

Peptide 1

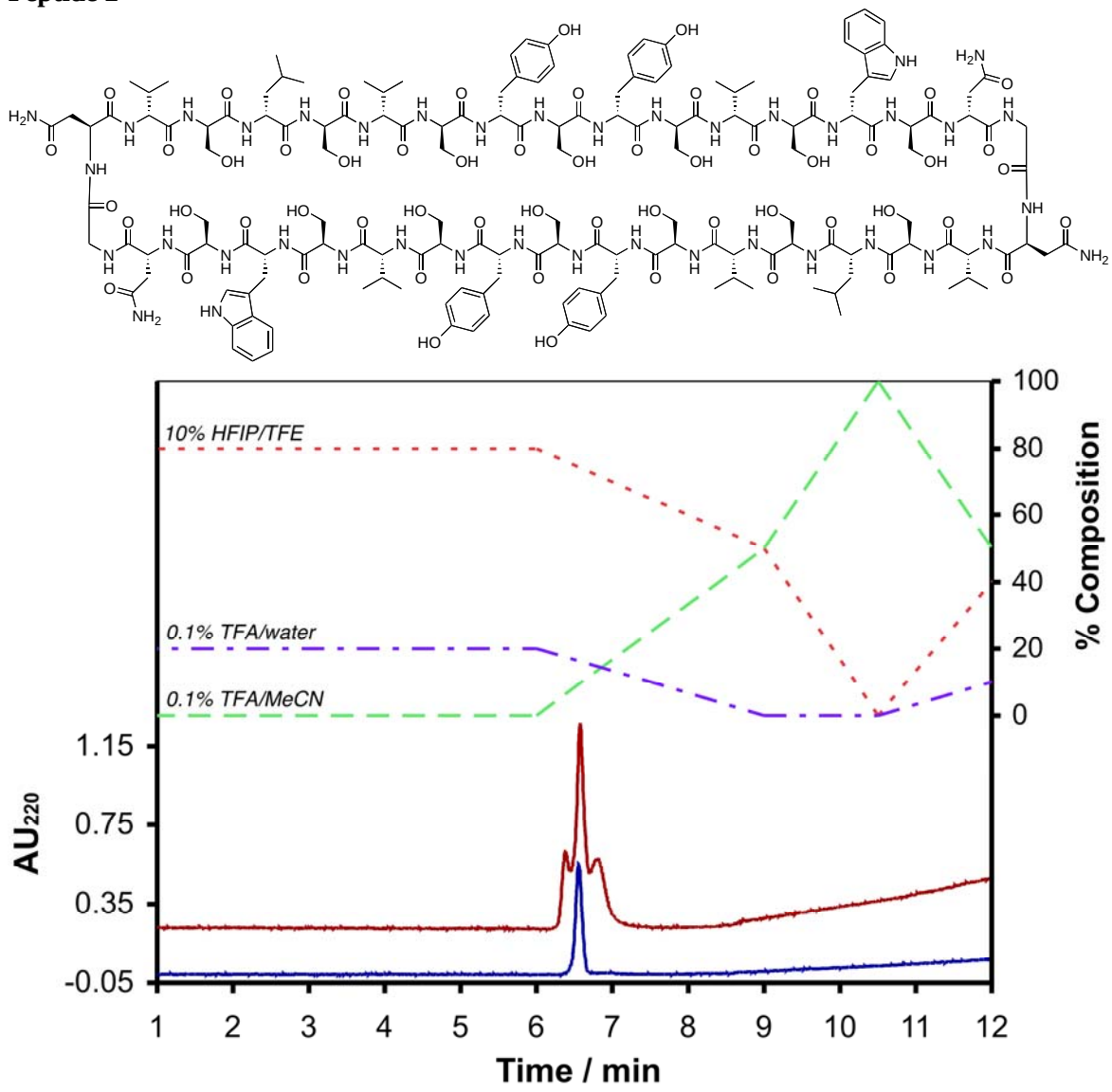




Key: brown (-), crude product; blue (-), product following chromatography. The profile of the crude product has been offset on the *y*-axis and the profile of the chromatographed product scaled by 2.5 for clarity.

[1] Abbreviations: HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; TFA, 2,2,2-trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol.

Peptide 2

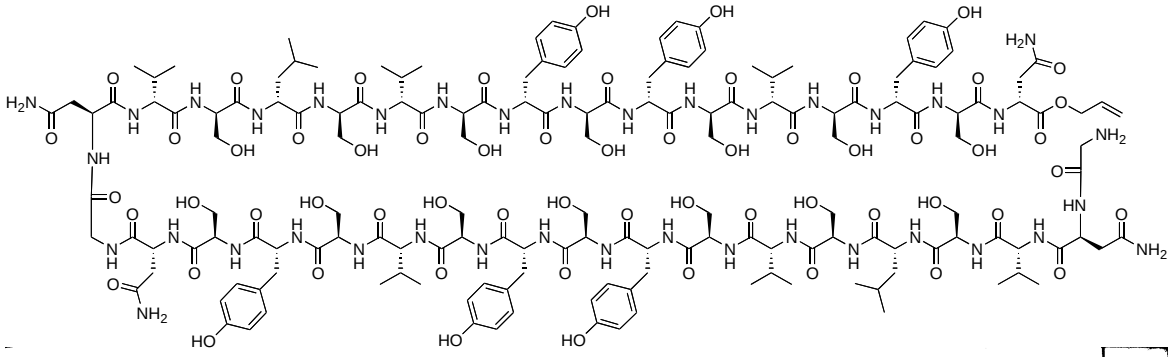


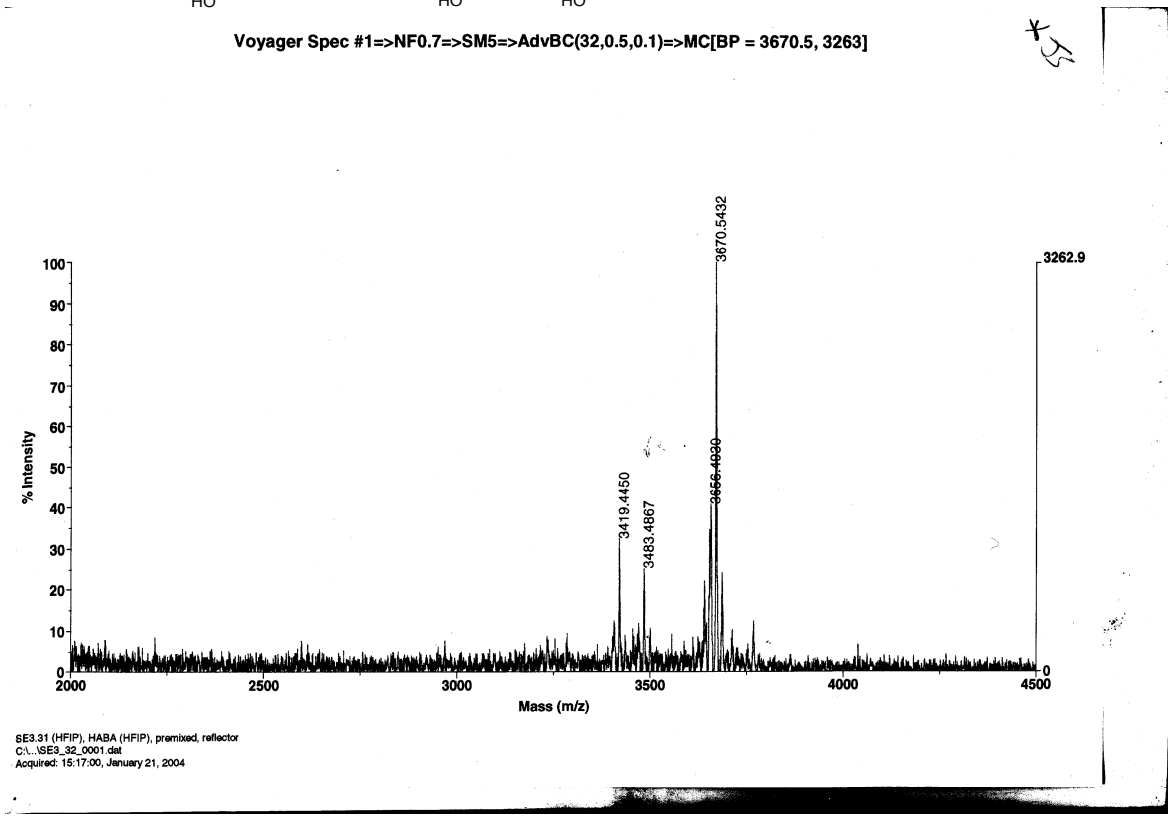
Key: brown (-), crude product; blue (-), product following chromatography. The profile of the crude product has been offset on the *y*-axis and the profile of the chromatographed product scaled by 0.3 for clarity.

Mass Spectra

Conditions: Matrix: HABA, 10 mg/ml in HFIP containing 0.1% TFA; peptide, ~1 mg/ml in HFIP;[2] target; stainless steel. The two were mixed at molar matrix to peptide ratios of 1:1400 to 1:3000 and 1 µl spotted on to the MALDI target.
Equipment: Applied Biosystems Voyager-DE STR; operating mode: reflector.

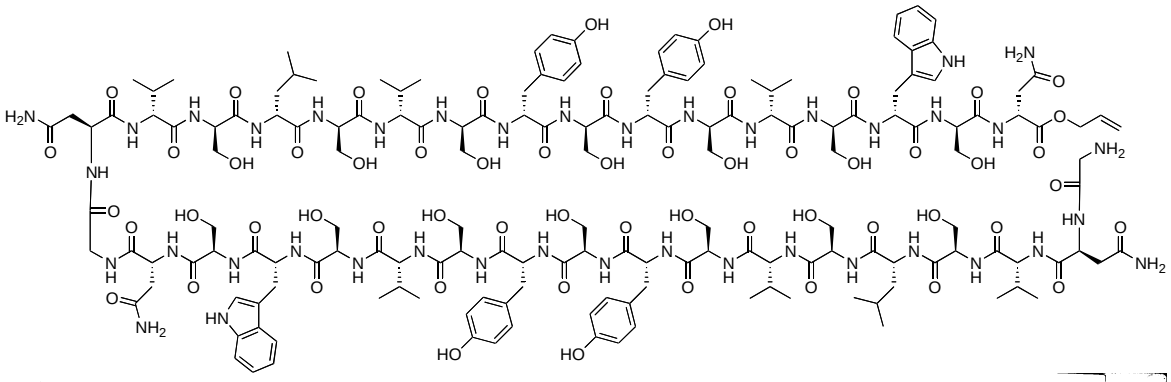
Linear Peptides (allyl esters, both crude products from test cleavages of small quantities of resin prior to the deprotection and cyclization steps)

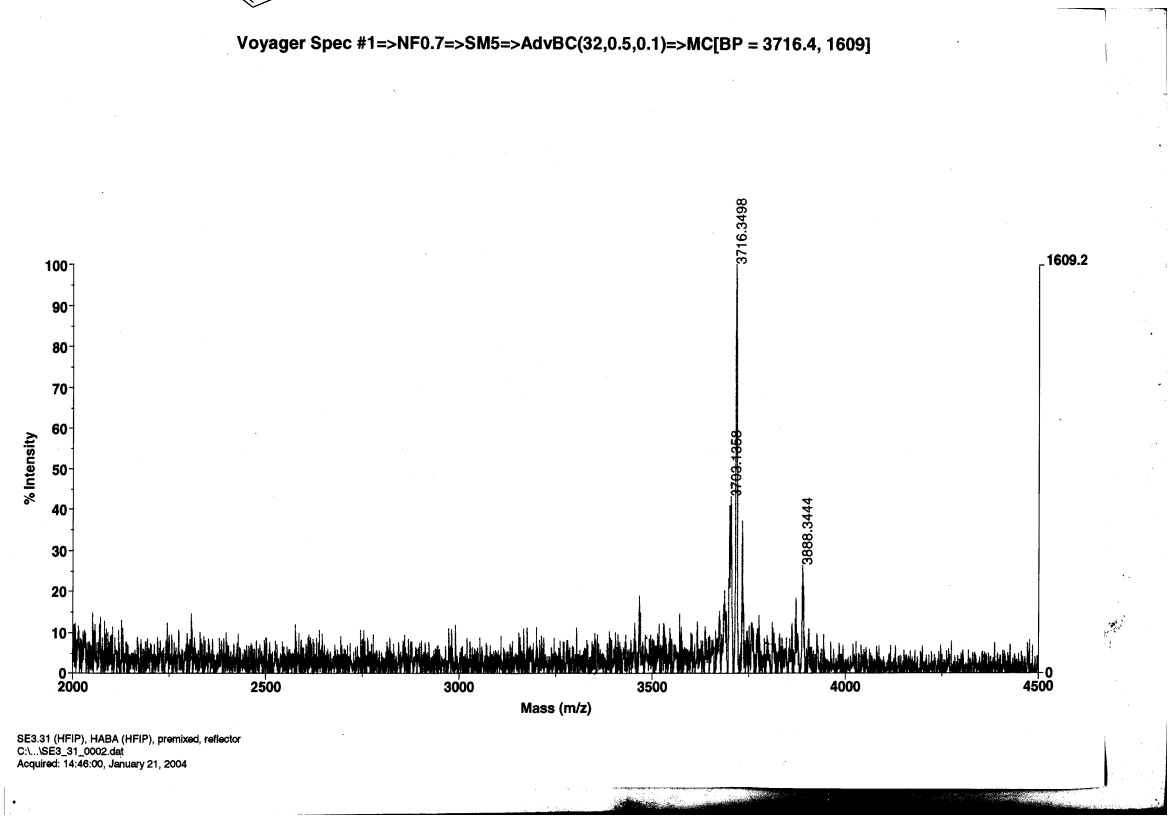




Calculated for $[C_{161}H_{236}N_{38}O_{59} + Na]^+ = 3670.81$ {$[M + Na]^+$}

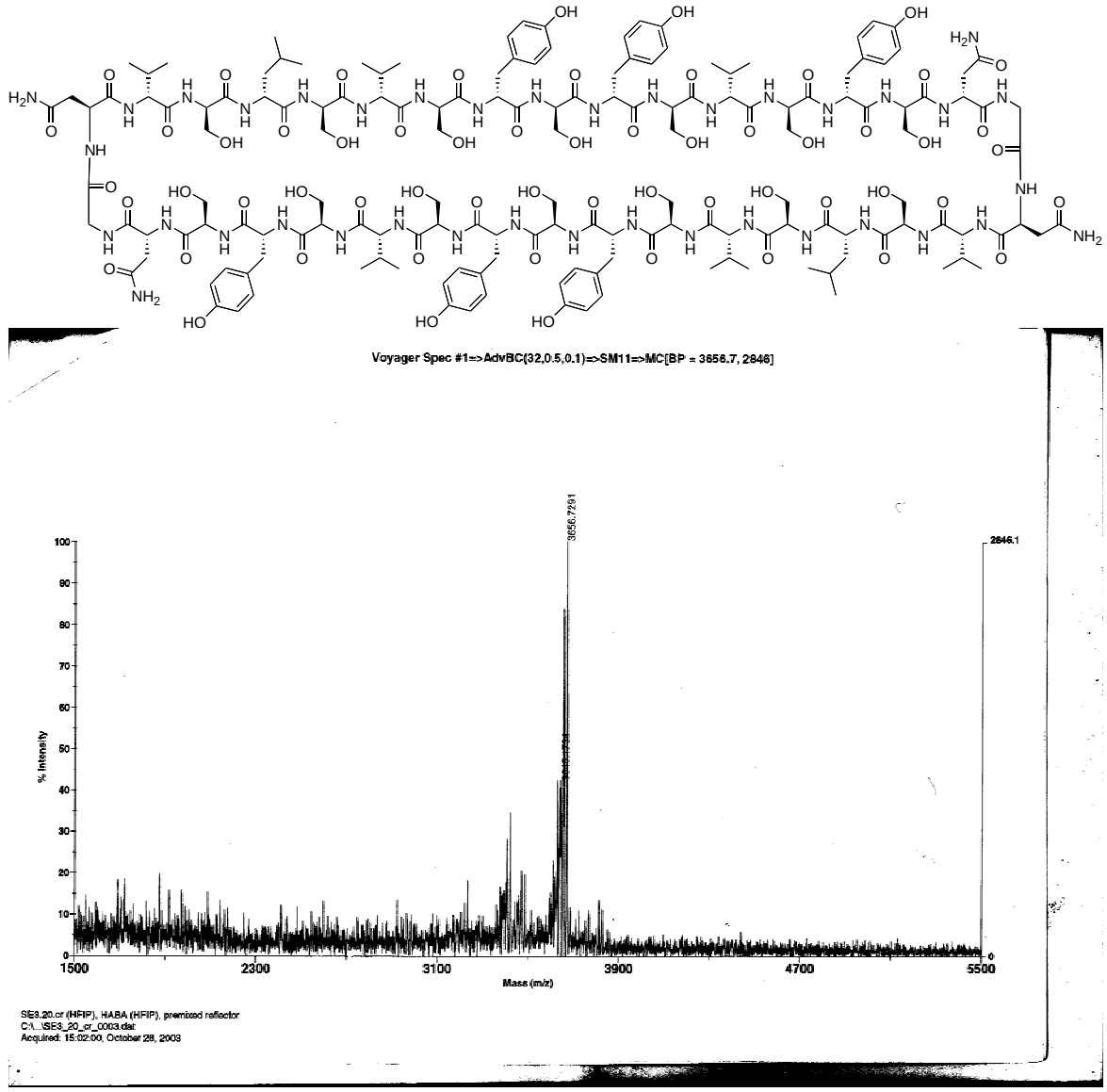
[2] Abbreviations: HABA, 2-(4-hydroxyphenylazo)benzoic acid; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; MALDI, matrix-assisted laser desorption/ionisation; TFA, 2,2,2-trifluoroacetic acid





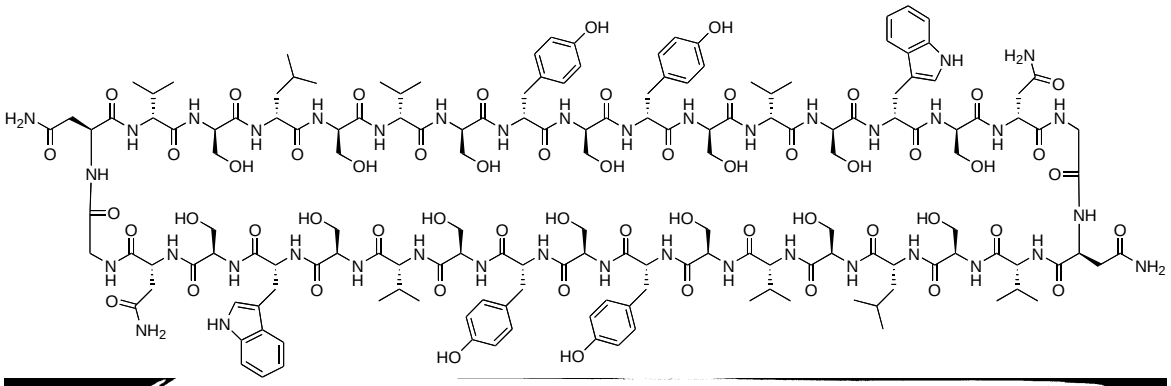
Calculated for $[C_{165}H_{238}N_{40}O_{57} + Na]^+ = 3716.88$ {$[M + Na]^+$}

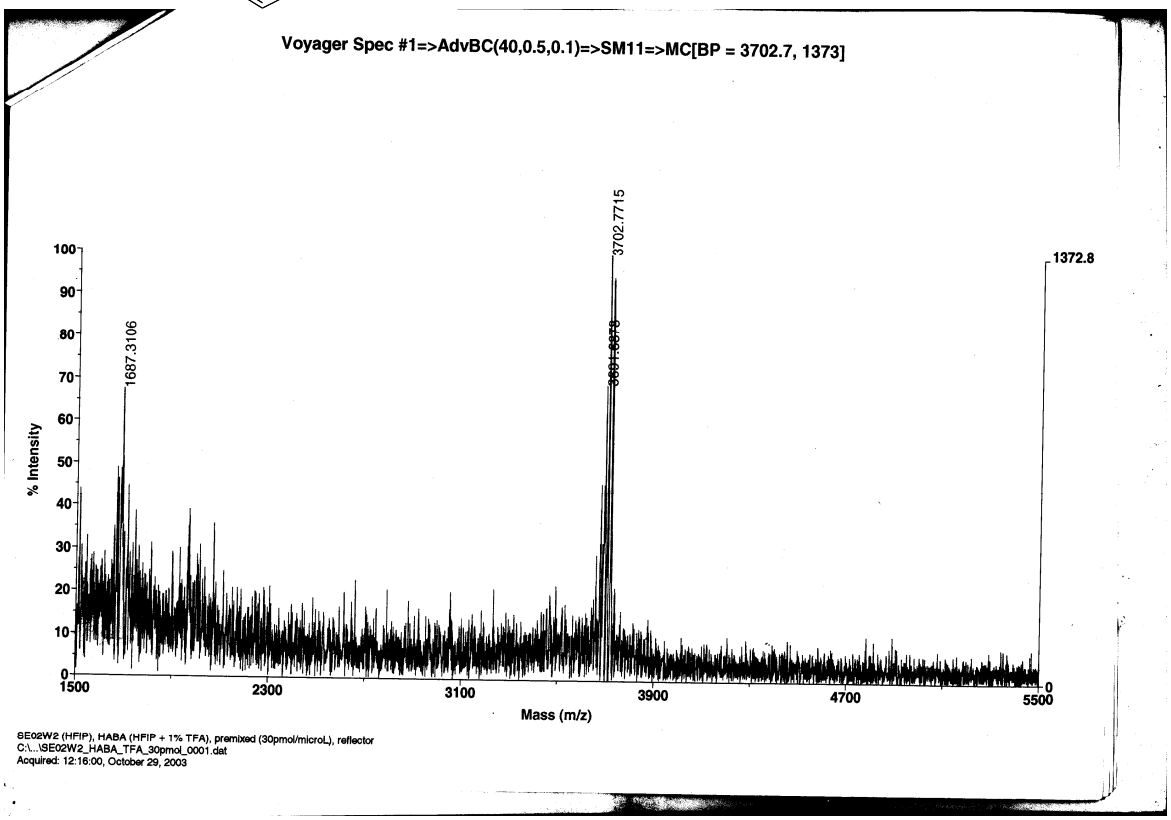
Cyclic Peptides (following deprotection, cyclization and HPLC purification steps)



Calculated for $[C_{158}H_{230}N_{38}O_{58} + 3Na - 2H]^+ = 3656.69$ $\{[M + 3Na - 2H]^+\}$[3]

[3] Other studies on cyclic peptides have documented triply sodiated species as a predominant form (Ngoka, L. C. M. and Michael L. Gross. 1999. Novel Sodium Binding Properties of Some Cyclopentapeptide Endothelin A Selective Receptor Antagonists: Electrospray and Fast-Atom-Bombardment Mass Spectrometric Studies. *Biochem. Biophys. Res. Comm.* 254:713–719).





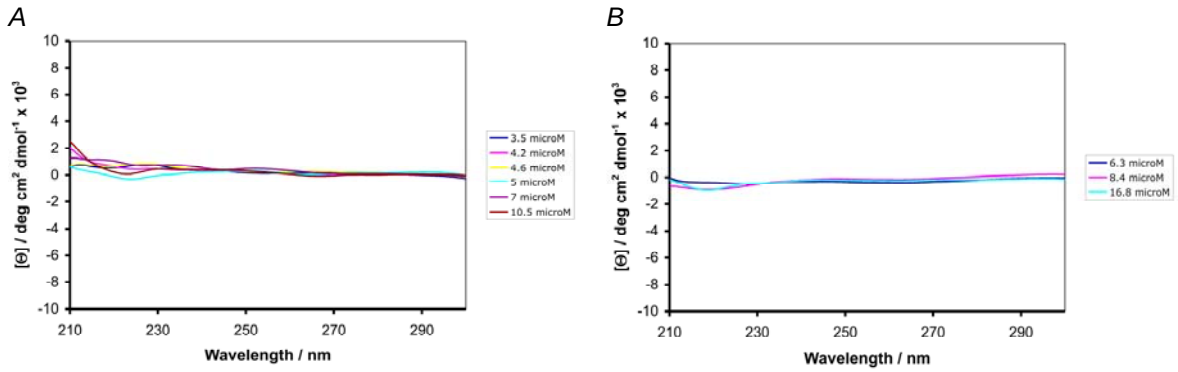
Calculated for $[C_{162}H_{232}N_{40}O_{56} + 3Na - 2H]^+ = 3702.76$ $\{[M + 3Na - 2H]^+\}$[4]

[4] See comments above in Footnote 2.

CD Data

CD spectra in the absence of lipids

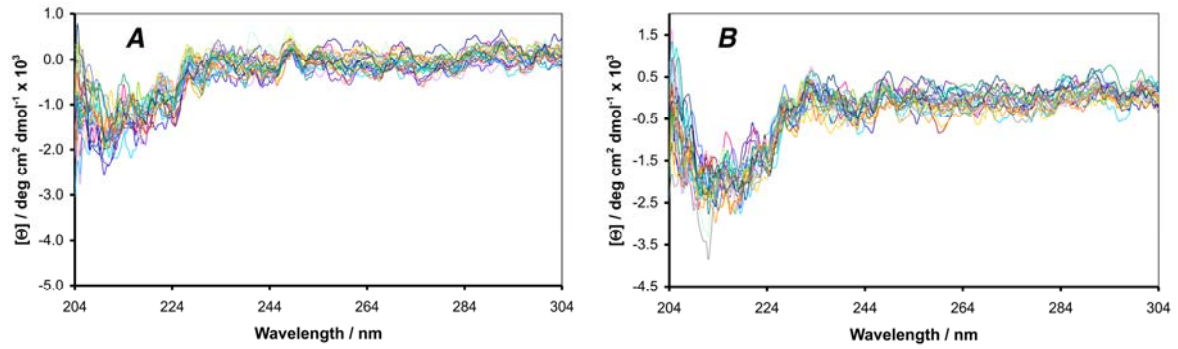
CD spectra of peptides **1** and **2** in 10 mM tris buffer at pH 7.4 containing 150 mM NaCl and 15% TFE v/v. Spectra were recorded at a data pitch of 0.5 nm and processed using the Jasco Spectra Analysis program. Background spectra for 15% TFE in buffer have been subtracted and the resulting spectra subjected to 15 point binary smoothing.



A: peptide **1**; B: peptide **2**.

Repeat binding experiment in a shorter path length cell

Time series spectra acquired in a quartz cell of path length 2 mm at 20 °C over a 4 h period following the addition of peptides to 100 nm EPC liposomes in 10 mM tris/150 mM NaCl at pH 7.4. CD spectra for **1** (A) and **2** (B) at respective peptide concentrations of 5.12 μM and 3.96 μM and an EPC concentration of 0.27 mg/ml (0.3 mM).



Miscellaneous Methods

Preparation of Tetrakis(triphenylphosphine) Palladium(0)

Palladium dichloride (0.44 g, 2.5 mmol) and triphenylphosphine (3.27 g, 12 mmol) were added to dimethyl sulfoxide (30 ml) and the mixture stirred at a temperature >140 °C under nitrogen until a clear solution formed. The solution was then stirred for a further 15 min without heating. Hydrazine hydrate (0.5 g, 10 mmol) was added rapidly by syringe and the resulting dark mixture was left to cool to room temperature under nitrogen. The reaction mixture was filtered and the filtrate washed with ethanol (2 x 2 ml) and diethyl ether (2 ml) before being dried under nitrogen to give the title compound as a yellow crystalline solid (2.57 g, 68%). The product was stored at -20 °C under argon.

Calculated elemental composition for $C_{72}H_{60}PdP_4$: C: 75.88; H: 5.25; P: 10.75; Found: C: 75.62; H: 5.54; P: 10.68.