Supporting Information

Synthesis of natural and unnatural cyclooligomeric depsipeptides enabled by flow chemistry

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# Contents

1. General Methods ........................................................................................................... 3

2. Optimisation of flow conditions .................................................................................. 4

   2.1. Boc-Leu-Phe-OMe 16 ................................................................................................. 4

   2.1.1. Method A (Optimised Batch) ................................................................................. 4

   2.1.2. Method B (Optimised Flow) ................................................................................ 5

3. Flow general methods ...................................................................................................... 5

   3.1. Intermolecular coupling (General Method 1) .......................................................... 5

   3.1.1. Acid deprotection ................................................................................................ 5

   3.1.2. Amine deprotection ............................................................................................. 6

   3.1.3. Coupling .............................................................................................................. 6

   3.2. Macrocyclisation (General Method 2) ..................................................................... 6

   3.2.1. Acid deprotection ................................................................................................ 6

   3.2.2. Amine deprotection ............................................................................................. 6

   3.2.3. Coupling .............................................................................................................. 7

4. Synthesis of N-Me-Phe containing depsipeptides .......................................................... 7

   4.1. d-Hydroxyisovaleric acid 3 ....................................................................................... 7

   4.2. d-Hiv-Obn 4 ............................................................................................................. 8

   4.3. Boc-Me-Phe-d-Hiv-Obn 6a ......................................................................................... 8

   4.4. Boc-Me-Phe-d-Hiv-Me-Phe-d-Hiv-Obn 8a ................................................................. 9

   4.4.1. Method A (Batch) ............................................................................................... 9

   4.4.2. Method B (Flow) ............................................................................................... 10

   4.5. Boc-Me-Phe-d-Hiv-Me-Phe-d-Hiv-Obn 10a ............................................................... 10

   4.5.1. Method A (Batch) ............................................................................................. 10

   4.5.2. Method B (Flow) ............................................................................................. 11


   4.6.1. Method A (Batch) ............................................................................................. 12

   4.6.2. Method B (Flow) ............................................................................................. 12

   4.7. d-[Phe-d-Hiv-Me-Phe-d-Hiv] 9a ................................................................................ 13

   4.7.1. Method A (Batch) ............................................................................................. 13

   4.7.2. Method B (Flow) ............................................................................................. 14


   4.8.1. Method A (Batch) ............................................................................................. 14

   4.8.2. Method B (Flow) ............................................................................................. 15


   4.9.1. Method A (Batch) ............................................................................................. 15

   4.9.2. Method B (Flow) ............................................................................................. 16

5. Synthesis of N-Me-Leu containing depsipeptides .......................................................... 16

   5.1. Boc-Me-Leu-d-Hiv-Obn 6b ......................................................................................... 16

   5.2. Boc-Me-Leu-d-Hiv-Me-Leu-d-Hiv-Obn 8b ................................................................. 17

   5.2.1. Method A (Batch) ............................................................................................. 17

   5.2.2. Method B (Flow) ............................................................................................. 17


   5.3.1. Method A (Batch) ............................................................................................. 18

   5.3.2. Method B (Flow) ............................................................................................. 19


   5.4.1. Method A (Batch) ............................................................................................. 19

   5.4.2. Method B (Flow) ............................................................................................. 20

   5.5. d-[Me-Leu-d-Hiv-Me-Leu-d-Hiv] 9b ........................................................................... 20

   5.5.1. Method A (Batch) ............................................................................................. 20

   5.5.2. Method B (Flow) ............................................................................................. 21


   5.6.1. Method A (Batch) ............................................................................................. 21

   5.6.2. Method B (Flow) ............................................................................................. 22


   5.7.1. Method A (Batch) ............................................................................................. 22

   5.7.2. Method B (Flow) ............................................................................................. 23

6. References ....................................................................................................................... 23

7. NMR Spectra .................................................................................................................... 23
1 General Methods

All reactions were carried out under argon atmosphere using oven-dried glassware, and were monitored by TLC. Unless otherwise stated, reagents were obtained from commercial sources and used without further purification. Amino acids were all of the natural (L) enantiomeric form unless otherwise stated. Solvents were freshly distilled over calcium hydride and lithium aluminium hydride (tetrahydrofuran and diethyl ether) or calcium hydride (dichloromethane, methanol, toluene, ethyl acetate and 40-60 petroleum ether). Additional anhydrous solvents were obtained from commercial sources and used directly (N,N-dimethylformamide).

Flow reactions were carried out using Vapourtec R2+\textsuperscript{[1]} and Syrris Asia\textsuperscript{[2]} pumps and a Polar Bear Plus cooling unit\textsuperscript{[3]} (Cambridge Reactor Design).

Thin layer chromatography (TLC) was carried out using 0.25 mm thick glass backed Merck TLC Silica gel 60 F\textsubscript{254} plates which were visualised using ultraviolet radiation and aqueous acidic ammonium molybdate (VII) solution or potassium permanganate solution. Preparatory TLC was carried out using Analtech 20 x 20 cm UNIPLATE™ Silica gel GF (preparative layer with UV254) plates of either 500, 1000 or 2000 microns depth and were visualised using UV. Flash column chromatography was carried out using high-purity grade silica gel (Merck grade 9385) with a pore size 60 Å and 230–400 mesh particle size.

Infrared Spectroscopy was recorded using a PerkinElmer Spectrum One FT-IR spectrometer using Universal ATR sampling accessories. Absorbances were recorded in the range 4000-650 cm\textsuperscript{-1}.

Nuclear Magnetic Resonance Spectroscopy (NMR) spectra were recorded using either a 400 MHz DPX-400 Dual Spectrometer, a 500 MHz AV III HD Smart Probe Spectrometer or a 600 MHz Avance 600 BBI spectrometer as indicated. Unless otherwise stated, all samples were run at room temperature in deuterated solvent, with chemical shift (δ) reported to the nearest 0.01 (\textsuperscript{1}H)/0.1 (\textsuperscript{13}C) ppm, relative to the residual protic solvent; δ(DCl\textsubscript{3}) = 7.26 (\textsuperscript{1}H)/77.16 (\textsuperscript{13}C) ppm, δ(toluene-d\textsubscript{8}) = 2.09, 6.98, 7.00, 7.09 (\textsuperscript{1}H)/20.40, 125.49, 128.33, 129.24, 137.86 (\textsuperscript{13}C) or δ(d\textsubscript{6}-DMSO) 2.50 (\textsuperscript{1}H)/39.52 (\textsuperscript{13}C) ppm. All carbon NMRs were run with broadband proton decoupling. Multiplicity of a signal in \textsuperscript{1}H NMR is indicated by: s = singlet, d = doublet, t = triplet or, q = quartet, quint = quintet, m = multiplet, or a combination thereof. Multiplets are reported as the range of ppm values covered by the signals, otherwise the centre of the signal is given. Coupling constants, J, are quoted in Hz and recorded to the nearest 0.1 Hz. Assignments were confirmed using Distortionless Enhanced Polarisation Transfer NMR (DEPT 135) and two dimensional NMR (\textsuperscript{1}H–\textsuperscript{1}H Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC)) experiments gave information used to assign both the \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra. Copies of all spectra are included at the end of this supporting information.

High Resolution Mass Spectrometry (HRMS) was performed using either a Waters Micromass LCT Premier spectrometer or by using a Bruker Bioapex 47e FTICR spectrometer, using positive Electron Spray Ionisation (ESI+). Masses are given in m/z-units, and the reported values all lie within Δ5 ppm of calculated values.

Melting points (mp) were measured using a Stanford Research Systems OptiMelt automated melting point system using a gradient of 1 °C/min, and are uncorrected.
Specific Optical Rotation was recorded on a Perkin-Elmer Model 343 digital polarimeter, using a Na/Hal lamp set at 589 nm and with a path length of 100 mm. All \([\alpha]_D\) values were measured using spectroscopy grade solvent at the specified concentration (in g/cm\(^3\)) and temperature, with units of 10\(^{-1}\)cm\(^2\)g\(^{-1}\).

The compound numbering system used is based on IUPAC conventions. Each amino acid is numbered individually, starting from the carboxyl group and proceeding up the side chain. Position labels thus refer first to the individual amino acid, and then to the position on it, with the atom to be defined (C or H) underlined. Non-amino acid derived functionality is numbered based on the position from which it branches. The entire branch is thus given the label of the position from which it begins, appended with a prime, and each position within the chain is further denoted by a lowercase letter.

2 Optimisation of flow conditions

2.1 Boc-Leu-Phe-OMe 16

\[ \text{Leu} \quad \text{Phe} \]

2.1.1 Method A (Optimised Batch)

A solution of Ghosez reagent (0.013 mL, 0.1 mmol) in dichloromethane (5 mL) was added to a solution of Boc-leucine 14 (0.012g, 0.05 mmol) in dichloromethane (5 mL) at 0 °C and the resulting mixture was stirred for 5 min at this temperature. A solution of phenylalanine methyl ester HCl salt 15 (0.011 g, 0.05 mmol) and N,N-diisopropylethylamine (0.032 mL, 0.18 mmol) in dichloromethane (5 mL) was added and the reaction mixture was allowed to warm to room temperature and stirred for 6 min 40 s before cooling to 0 °C and addition of aqueous HCl (10 mL, 1M). The reaction mixture was extracted with dichloromethane (3 x 10 mL) and the combined organic layers were washed with water (10 mL), saturated sodium bicarbonate (NaHCO\(_3\)) solution (10 mL) and saturated sodium chloride (NaCl) solution (10 mL) before drying over magnesium sulfate (MgSO\(_4\)) and removing the solvent in vacuo. The crude product was purified via preparative TLC using 40-60 petroleum ether:ethyl acetate (1:1) as eluent to afford the title compound 16 (0.009 g, 0.023 mmol, 46%) as a colourless solid.

\( R_f = 0.66 \) [40-60 petroleum ether:diethyl ether = 1:1]; m.p. = 84 – 84.5 °C (literature\(^{[1]}\) = 83.5 – 84 °C);
\( \alpha_D^{27.7} = +18.5 \) (c 1.00, CHCl\(_3\)); \(^1\)H NMR (600 MHz, CDCl\(_3\)) \( \delta = 7.31 – 7.26 \) (m, 2H, 2 x Phe-C5H), 7.26 – 7.22 (m, 1H, Phe-C7H), 7.13 – 7.08 (m, 2H, 2 x Phe-C6H), 6.49 (d, J = 7.8 Hz, 1H, Phe-NH), 4.89 – 4.77 (m, 2H, Phe-C2H and Leu-NH), 4.14 – 4.04 (m, 1H, Leu-C2H), 3.71 (s, 3H, Phe-C1’aH\(_3\)), 3.19 – 3.06 (m, 2H, Phe-C3H\(_2\)), 1.68 – 1.57 (m, 2H, Leu-C3HH, Leu-C4H), 1.44 (br. s, 10H, Leu-C3HH and 3 x Leu-C2’CH\(_3\)), 0.92 (t, J = 6.6 Hz, 6H, 2 x Leu-C5H\(_3\)); \(^13\)C NMR (151 MHz, CDCl\(_3\)) \( \delta = 172.2 \) (quat., Phe-C1), 171.8 (quat. Leu-C1), 155.1 (quat., Leu-C2’a), 135.9 (quat., Phe-C4), 129.4 (CH, Phe-C6), 128.7 (CH, Phe-C5), 127.3 (CH, Phe-C7), 80.2 (quat., Leu-C2’b), 53.3 (2 x CH, Phe-C2, Leu-C2), 52.4 (CH\(_3\), Phe-C1’a), 41.4 (CH\(_2\), Leu-C3), 38.1 (CH\(_2\), Phe-C3), 28.4 (CH\(_3\), 3 x Leu-C2’c), 24.8 (CH, Leu-C4), 23.0 (CH\(_3\), Leu-C5), 22.1 (CH\(_3\), Leu-C5); IR (neat/cm\(^{-1}\)) : 3339, 3302, 2960, 1742, 1664, 1520, 1275, 1245, 1163, 1020, 697; HRMS (ESI): found 415.2224 (M+Na\(^+\)) \( C_{21}H_{32}N_{2}O_{5}Na \) requires 415.2209 \( \Delta = 3.6 \) ppm.
2.1.2 Method B (Optimised Flow)

**Plug flow:** Boc-leucine 14 (0.012 g, 0.05 mmol) was taken up in dichloromethane (5 mL) and filled into loop 1. Ghosez reagent (0.013 mL, 0.1 mmol) was taken up in dichloromethane (5 mL) and filled into loop 2. Phenylalanine methyl ester HCl salt 15 (0.011 g, 0.05 mmol) and diisopropylethylamine (0.032 mL, 0.18 mmol) were taken up in dichloromethane (4.968 mL) and filled into loop 3. Pumps 1 – 3 were run at 1 mL.min\(^{-1}\) with dichloromethane and injection loops 1 – 3 injected at the appropriate time to ensure coordinated meeting of streams at the T-pieces (determined by runs using Sudan red dye) – loop 1 at t=0, loop 2 at t=23 s and loop 3 at t=4 min 53 s. From t = 15 min to 1 = 26 min the reaction outflow was collected in stirring aqueous HCl (10 mL, 1M) at 0 °C. The reaction mixture was extracted with dichloromethane (3 x 20 mL) and the combined organic extracts were washed with water (10 mL), saturated NaHCO\(_3\) solution (10 mL) and saturated NaCl solution (10 mL) before drying over MgSO\(_4\) and removing the solvent in vacuo. The resultant crude product was then purified via preparative TLC using 40-60 petroleum ether:ethyl acetate (1:1) as eluent to afford the **title compound 16** (0.009 g, 0.023 mmol, 46%) as a colourless solid, which was spectroscopically identical to that reported using method A.

**Continuous flow:** The flow coupling was also run continuously with pump 1 pumping a solution of Boc-Leucine 14 (0.01 M) in dichloromethane, pump 2 pumping a solution of Ghosez reagent (0.02 M) in dichloromethane and pump 3 pumping a solution of phenylalanine methyl ester HCl salt 15 (0.01 M) and diisopropylethylamine (0.036 M) in dichloromethane. The reactor was allowed to reach steady state (30 min) and then the outflow was collected for 60 min into stirring aqueous HCl at 0 °C (150 mL, 1M). The reaction mixture was extracted with dichloromethane (3 x 150 mL) and the combined organic extracts were washed with water (150 mL), saturated NaHCO\(_3\) (150 mL) and saturated NaCl (150 mL). The combined organic extracts were dried over magnesium sulphate and the solvent removed in vacuo. The resultant crude product was purified using preparative TLC using 40-60 petroleum ether:ethyl acetate (1:1) as eluent to afford the **title compound 16** (0.110 g, 0.28 mmol, 47%) as a colourless solid, which was spectroscopically identical to that reported using method A.

3 Flow general methods

3.1 Intermolecular coupling (General Method 1)

3.1.1 Acid deprotection

**Coupling partner A** (0.05 mmol) was taken up in **solvent** and this solution was hydrogenated using an H-cube® Pro (ThalesNano) with a 10% Pd/C CatCart®. The pump was run at 1 mL.min\(^{-1}\) using...
solvent with the temperature set to 60 °C and the pressure to 6 bar. The solvent was then removed in vacuo and the crude acid used directly in the coupling.

3.1.2 Amine deprotection

**Coupling partner B** (0.05 mmol) was taken up in dioxane (1 mL) and anhydrous HCl (1 mL, 4M in dioxane) was added and the reaction mixture stirred at room temperature until the reaction was judged to be complete using TLC. The solvent was then removed in vacuo and the crude oil taken up in ethanol (2 x 2 mL) and methanol (2 mL) successively, removing the solvent in vacuo after each addition to afford the amine HCl salt which was used directly in the coupling.

3.1.3 Coupling

![Scheme 2. Flow equipment set up for General Method 1.](image)

**Acid** (0.05 mmol) was taken up in dichloromethane (5 mL) and filled into loop 1. Ghosez reagent (0.013 mL, 0.1 mmol) was taken up in dichloromethane (5 mL) and filled into loop 2. **Amine HCl** (0.05 mmol) and diisopropylethylamine (0.032 mL, 0.18 mmol) were taken up in dichloromethane (4.968 mL) and filled into loop 3. Pumps 1 – 3 were run a 1 mL min⁻¹ with dichloromethane and injection loops 1 – 3 injected at the appropriate time to ensure coordinated meeting of streams at the T-pieces (determined by runs using Sudan red dye) – loop 1 at t=0, loop 2 at t=23 s and loop 3 at t=4 min 53 s. From t = 15 min to t = 26 min the reaction outflow was collected in stirring aqueous HCl (10 mL, 1M) at 0 °C. The reaction mixture was extracted with dichloromethane (3 x 20 mL) and the combined organic extracts were washed with water (10 mL), saturated NaHCO₃ solution (10 mL) and saturated NaCl solution (10 mL) before drying over MgSO₄ and removing the solvent in vacuo. The resultant crude product was then purified via flash chromatography or preparative TLC.

3.2 Macrocyclisation (General Method 2)

3.2.1 Acid deprotection

**Protected precursor** (0.05 mmol) was taken up in solvent and this solution was hydrogenated using an H-cube® Pro (ThalesNano) with a 10% Pd/C CatCart®. The pump was run at 1 mL min⁻¹ using solvent with the temperature set to 60 °C and the pressure to 6 bar. The solvent was then removed in vacuo and the crude acid used directly in the amine deprotection.

3.2.2 Amine deprotection

**Crude acid** (0.05 mmol) was taken up in dioxane (1 mL) and anhydrous HCl (1 mL, 4M in dioxane) was added and the reaction mixture stirred at room temperature until the reaction was judged to be complete using TLC. The solvent was then removed in vacuo and the crude oil taken up in ethanol (2 x 2 mL) and methanol (2 mL) successively, removing the solvent in vacuo after each addition to afford the cyclisation precursor HCl salt which was used directly in the coupling.
3.2.3 Coupling

**Cyclisation precursor HCl** (0.05 mmol) was taken up in dichloromethane (5 mL) and filled into loop 1. Ghosez reagent (0.013 mL, 0.1 mmol) was taken up in dichloromethane (5 mL) and filled into loop 2. Pumps 1 and 2 were run at 1 mL.min\(^{-1}\) with dichloromethane and pump 3 at 1 mL.min\(^{-1}\) with a solution of diisopropylethylamine (0.036M) in dichloromethane. Injection loops 1 and 2 were injected at the appropriate time to ensure coordinated meeting of streams at the T-pieces (determined by runs using Sudan red dye) – loop 1 at t=0, loop 2 at t=23 s. From t = 15 min to t = 26 min the reaction outflow was collected in stirring aqueous HCl (20 mL, 1M) at 0 °C. The reaction mixture was extracted with dichloromethane (3 x 20 mL) and the combined organic extracts washed with water (10 mL), saturated NaHCO\(_3\) solution (10 mL) and saturated NaCl solution (10 mL) and was dried over MgSO\(_4\) and the solvent was removed in vacuo before purification via flash chromatography or preparative TLC.

4 Synthesis of N-Me-Phe containing depsipeptides

4.1 D-Hydroxyisovaleric acid 3

\[
\text{HO} \quad ^{\sigma} \quad \text{CH} \quad \text{CH}_2 \quad \text{OH}
\]

**Scheme 4.** Flow equipment set up for synthesis of 3

D-Hydroxyisovaleric acid 3 was synthesised using a modification of the procedure previously reported by the Ley group.\(^5\) A solution of d-valine (3.41 g, 29.1 mmol) in sulfuric acid (58.2 mL, 0.5 M in water) at a flow rate of 0.167 mL.min\(^{-1}\) was mixed with a solution of sodium nitrite (4.02 g, 58.2 mmol) in water (58.2 mL) at a flowrate of 0.167 mL.min\(^{-1}\) at a T-piece (0.333 mL.min\(^{-1}\) total). The reaction mixture was passed through a 20 mL reactor coil at 60 °C and a 100 psi back pressure regulator before the output was collected in stirring ethyl acetate (150 mL). The aqueous layer was extracted with ethyl acetate (4 x 100 mL) and the combined organic layers were washed with
saturated NaCl solution (250 mL) before drying over MgSO₄ and removing the solvent in vacuo. The crude title compound 3 (2.74 g, 23.2 mmol, 80%) was obtained as a yellow solid, which was used in the next step without further purification. The ¹H and ¹³C NMR data was in agreement with that reported in the literature.[⁶]

¹H NMR (400 MHz, d₆-DMSO) δ = 3.77 (d, J = 4.8 Hz, 1H, C₂H₆J), 1.95 (hept.d, J = 6.9, 4.8 Hz, 1H, C₃H₇J), 0.93 (d, J = 6.9 Hz, 3H, C₄H₉), 0.87 (d, J = 6.9 Hz, 3H, C₄H₉); ¹³C NMR (101 MHz, d₆-DMSO) δ = 175.3 (quat., C₁), 74.5 (CH, C₂), 31.4 (CH, C₃), 18.9 (CH₃, C₄), 16.8 (CH₃, C₄).

4.2 d-Hiv-OBn 4

Caesium carbonate (4.45 g, 13.7 mmol) was added to a solution of d-hydroxyisovaleric acid 3 (3.22 g, 27.3 mmol) in N,N-dimethylformamide (20 mL) at 0 °C and the resulting mixture was stirred for 40 min. Benzyl bromide (3.6 mL, 30.0 mmol) was added and the reaction mixture was stirred at room temperature for 15 h. The reaction mixture was filtered and the filtrate diluted with 40-60 petroleum ether:ethyl acetate (4:1, 120 mL). The organic layer was washed with saturated ammonium chloride solution (100 mL), saturated NaHCO₃ solution (100 mL) and saturated NaCl solution (100 mL) before drying over MgSO₄ and removing the solvent in vacuo. The crude product was purified via flash column chromatography using 40-60 petroleum ether:ethyl acetate (10:1) as eluent to afford the title compound 4 (4.56 g, 21.9 mmol, 80%) as a colourless oil. The ¹H and ¹³C data was in agreement with that reported in the literature[⁷].

Rₗ = 0.29 [40-60 petroleum ether:diethyl ether = 10:1]; αₖº²⁵.₈ = +17.8 (c 3.35 EtOH) literature[⁸] αₖº = +15.4 (c 2.1, EtOH); ¹H NMR (400 MHz, d₆-DMSO) δ = 7.49 – 7.26 (m, 5H, C₁′CH – C₁′eH), 5.32 (br s., 1H, C₂OH), 5.25 – 5.02 (m, 2H, C₁′aH₂), 3.86 (d, J = 5.0 Hz, 1H, C₂H), 2.04 – 1.81 (m, 1H, C₃H), 0.87 (d, J = 6.9 Hz, 3H, C₄H₉), 0.81 (d, J = 6.9 Hz, 3H, C₄H₉); ¹³C NMR (151 MHz, d₆-DMSO) δ = 172.5 (quat., C₁), 135.6 (quat., C₁′b), 127.6, 127.5, 127.2 (5 x CH, C₁′c – C₁′e), 74.7 (CH₂, C₁′a), 64.8 (CH, C₂), 31.1 (CH, C₃), 17.7 (CH₃, C₄), 16.3 (CH₃, C₄).

4.3 Boc-Me-Phe-d-Hiv-OBn 6a

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (1.72 g, 9.0 mmol) was added to a solution of d-Hiv-OBn 4 (1.30 g, 6.3 mmol), Boc-N-methyl-L-phenylalanine 5a (2.50 g, 8.95 mmol) and 4-dimethylaminopyridine (4-DMAP) (0.99 g, 8.1 mmol) in dichloromethane (25 mL) at 0 °C and the resulting mixture was stirred at room temperature for 14.5 h. The reaction was ended by the addition of aqueous HCl (25 mL, 1M) and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 30 mL) and the combined organic layers were washed with water (100 mL), saturated NaHCO₃ solution (100 mL) and saturated NaCl solution (100 mL) before drying over MgSO₄ and removing the solvent in vacuo. The crude product was purified via flash column chromatography using 40-60 petroleum ether:ethyl acetate (10:1) as eluent to afford the title compound 6a (2.69 g, 5.7 mmol, 92%) as a colourless oil.
\( R_f = 0.34 \) [40-60 petroleum ether:diethyl ether = 10:1]; \( \alpha_0^{23.7} = -30.9 \) (c 1.00, CHCl₃); \( ^{1}\text{H NMR} \) (400 MHz, d₆-DMSO, T = 403 °K) δ = 7.47 – 7.13 (m, 10H, d-Hiv-C¹·CH – d-Hiv-C¹′eH and Phe-C5H – Phe-C7H), 5.20 (s, 2H, d-Hiv-C¹′aH₂), 4.99 (dd, J = 10.4, 5.4 Hz, 1H, Phe-C2H₂), 4.92 (d, J = 4.6 Hz, 1H, d-Hiv-C2H), 3.23 (dd, J = 14.6, 5.4 Hz, 1H, Phe-C3H), 3.01 (dd, J = 14.6, 10.4 Hz, 1H, Phe-C3H), 2.70 (s, 3H, Phe-NCH₃), 2.28 – 2.13 (m, 1H, d-Hiv-C3H), 1.33 (s, 9H, Phe-C2′CH₃), 0.96 (d, J = 6.9 Hz, 3H, d-Hiv-C4H₉), 0.93 (d, J = 6.9 Hz, 3H, d-Hiv-C4H₉); \( ^{13}\text{C NMR} \) (101 MHz, d₆-DMSO, T = 403 °K) δ = 169.6 (quat., Phe-C¹), 167.8 (quat., P-Hiv-C¹), 155.5 (quat., Phe-C²′a), 136.8 (quat., Phe-C₄), 135.0 (quat., d-Hiv-C¹′b), 128.0, 127.7, 127.4, 127.3, 125.6 (10 x CH, d-Hiv-C¹′c – d-Hiv-C¹′e, Phe-C₅ – Phe-C⁷), 78.7 (quat., Phe-C²′b), 76.4 (CH, d-Hiv-C²), 65.7 (CH₂, d-Hiv-C¹′a), 58.8 (CH, Phe-C₂), 33.8 (CH₃, Phe-C₃), 30.2 (CH₃, Phe-NC), 29.1 (CH, d-Hiv-C₃), 27.3 (3 x CH₃, Phe-C²′c), 17.5 (CH₃, d-Hiv-C₄), 16.2 (CH₃, d-Hiv-C⁴); \( IR \) (neat/cm⁻¹): 2972, 2933, 2880, 1741, 1694, 1455, 1390, 1366, 1167, 1127, 1029, 750, 697; \( \text{HRMS (ESI)} \): found 470.2531 (M+H)⁺ C₂₂H₃₆NO₆ requires 470.2543 Δ = 2.4 ppm.

4.4 Boc-Me-Phe-d-Hiv-Me-Phe-d-Hiv-OBn 8a

4.4.1 Method A (Batch)
Anhydrous HCl (2.5 mL, 4M in dioxane) was added to a solution of Boc-Me-Phe-d-Hiv-OBn 6a (0.31 g, 0.66 mmol) in dioxane (2.5 mL) at room temperature and the resulting mixture was stirred for 3 h. The solvent was removed \textit{in vacuo} and the residue was taken up sequentially in ethanol (2 x 5 mL) and methanol (5 mL) with the solvent removed \textit{in vacuo} after each addition to afford the amine HCl salt which was used in the next step without further purification.

Hydrogen gas was bubbled through a solution of Boc-Me-Phe-d-Hiv-OBn 6a (0.31 g, 0.66 mmol) and palladium (70 mg, 10% on charcoal, 0.07 mmol) in tetrahydrofuran (5 mL) for 3 min at room temperature before the resulting mixture was stirred under a hydrogen atmosphere for 2.5 h. The reaction mixture was filtered through celite, eluting with ethyl acetate (20 mL). The solvent was removed \textit{in vacuo} from the filtrate to afford the acid which was used in the next step without further purification.

Ghosez reagent (0.096 mL, 0.72 mmol) was added to a solution of the crude acid in dichloromethane (2.5 mL) at 0 °C and the resulting mixture was stirred for 20 min at this temperature. A solution of the crude amine HCl salt and \( N,N \)-diisopropylethylamine (0.40 mL, 2.36 mmol) in dichloromethane (2.5 mL) was added and the reaction mixture was allowed to warm to room temperature and stirred for 17 h. Aqueous HCl (20 mL, 1M) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 20 mL) and the combined organic layers were washed with water (40 mL), saturated NaHCO₃ solution (40 mL) and saturated NaCl solution (40 mL) before drying over MgSO₄ and removing the solvent \textit{in vacuo}. The crude product was purified \textit{via} flash column chromatography using 40-60 petroleum ether:ethyl acetate (10:1) as eluent to afford the \textit{title compound} 8a (0.33 g, 0.45 mmol, 68%) as a colourless solid.

\( R_f = 0.06 \) [40-60 Petroleum ether:diethyl ether = 10:1]; \( mp = 120-122 \) °C; \( \alpha_0^{23.7} = -47.4 \) (c 1.00, CHCl₃); \( ^{1}\text{H NMR} \) (400 MHz, d₆-DMSO, T = 403 °K) δ = 7.51 – 7.31 (m, 5H, d-Hiv⁸-C¹·CH – d-Hiv⁸-C¹′eH), 7.31 –
7.08 (m, 10H, Phe<sup>A</sup>B-C5H – Phe<sup>A</sup>B-C7H), 5.39 (br. s, 1H, Phe<sup>B</sup>-C2H), 5.21 (d, J = 1.4 Hz, 2H, d-Hiv<sup>B</sup>-C1′aH3J, 5.11 (d, J = 4.8 Hz, 1H, d-Hiv<sup>B</sup>-C2H), 4.99 – 4.90 (m, 2H, Phe<sup>B</sup>-C2H and d-Hiv<sup>B</sup>-C2H), 3.32 (dd, J = 14.6, 5.4 Hz, 1H, Phe<sup>A</sup>-C3H-H), 3.21 (dd, J = 14.6, 5.2 Hz, 1H, Phe<sup>A</sup>-C3H-H), 3.07 – 2.96 (m, 2H Phe<sup>A</sup>-C3HH and Phe<sup>B</sup>-C3HH), 2.94 (s, 3H, Phe<sup>B</sup>-NCH<sub>3</sub>), 2.72 (s, 3H, Phe<sup>A</sup>-NCH<sub>3</sub>), 2.22 (pd, J = 6.9, 4.8 Hz, 1H, d-Hiv<sup>A</sup>-C3H), 1.81 (br. s, 1H, d-Hiv<sup>A</sup>-C3H), 1.31 (s, 9H, 3 x Phe<sup>A</sup>-C2′cH<sub>3</sub>), 0.95 (dd, J = 6.9, 4.3 Hz, 6H, 2 x d-Hiv<sup>B</sup>-C4H<sub>3</sub>), 0.78 (d, J = 6.8 Hz, 3H, d-Hiv<sup>B</sup>-C4H<sub>3</sub>), 0.71 – 0.55 (m, 3H, d-Hiv<sup>B</sup>-C4H<sub>3</sub>); <sup>13</sup>C NMR (101 MHz, <sup>1</sup>H-DMSO, T = 403 °K) δ = 169.3 (quat., Phe<sup>A</sup>-C1), 169.1 (quat., Phe<sup>B</sup>-C1), 167.8 (quat., d-Hiv<sup>A</sup>-C1), 154.1 (quat., Phe<sup>A</sup>-C2′a), 136.9 (quat., Phe<sup>A</sup>-C4), 136.4 (quat., Phe<sup>B</sup>-C4), 135.0 (quat., d-Hiv<sup>B</sup>-C1′b), 128.1, 128.0, 127.7, 127.5, 127.4, 127.4, 125.7, 125.6 (15 x CH, Phe<sup>A</sup>B-C5 – Phe<sup>A</sup>B-C7 and d-Hiv<sup>B</sup>-C1′c – d-Hiv<sup>B</sup>-C1′e), 78.5 (quat., Phe<sup>A</sup>-C2′b), 76.7 (CH, d-Hiv<sup>B</sup>-C2), 74.4 (CH, d-Hiv<sup>B</sup>-C2), 65.7 (CH<sub>2</sub>, d-Hiv<sup>B</sup>-C1′a), 58.7 (CH, Phe<sup>A</sup>-C2), 57.5 (CH, Phe<sup>A</sup>-C2), 33.9 (CH<sub>2</sub>, Phe<sup>B</sup>-C3), 33.6 (CH<sub>2</sub>, Phe<sup>B</sup>-C3), 31.0 (CH<sub>3</sub>, Phe<sup>B</sup>-NC), 30.0 (CH<sub>3</sub>, Phe<sup>A</sup>-NC), 29.0 (CH, d-Hiv<sup>B</sup>-C3), 28.6 (CH, d-Hiv<sup>B</sup>-C3), 27.3 (3 x CH<sub>3</sub>, Phe<sup>A</sup>-C2′c), 17.8 (CH<sub>3</sub>, d-Hiv<sup>A</sup>-C4), 17.4 (CH<sub>3</sub>, d-Hiv<sup>B</sup>-C4), 16.3 (CH<sub>3</sub>, d-Hiv<sup>B</sup>-C4), 15.7 (CH<sub>3</sub>, d-Hiv<sup>B</sup>-C4); IR (neat/cm<sup>-1</sup>): 2962, 2937, 1761, 1737, 1683, 1662, 1454, 1394, 1290, 1167, 1128, 1029, 745, 696; HRMS (ESI): found 731.3878 (M+H)<sup>+</sup> C<sub>42</sub>H<sub>55</sub>N<sub>2</sub>O<sub>9</sub> requires 731.3907 Δ = 3.9 ppm.

4.4.2 Method B (Flow)

According to General Method 1, a solution of coupling partner A 6a (0.024 g, 0.05 mmol) in dichloromethane (5 mL) was hydrogenated to give the acid and the nitrogen of coupling partner B 6a (0.024 g, 0.05 mmol) was deprotected using acid (reaction time = 3 h) and the resultant crude products were coupled in flow. The crude product was purified using preparative TLC using 40-60 petroleum ether:ethyl acetate (2:1) as eluent to afford the title compound 8a (0.031 g, 0.042 mmol, 82%) as a colourless solid, which was spectroscopically identical to that reported using method A.

4.5 Boc-Me-Phe-d-Hiv-Me-Phe-d-Hiv-OBn 10a

4.5.1 Method A (Batch)

Anhydrous HCl (2 mL, 4M in dioxane) was added to a solution of Boc-Me-Phe-d-Hiv-Me-Phe-d-Hiv-OBn 8a (0.17 g, 0.23 mmol) in dioxane (2 mL) at room temperature and the resulting mixture was stirred for 6 h. The solvent was removed in vacuo and the residue was taken up sequentially in ethanol (2 x 5 mL) and methanol (5 mL) with the solvent removed in vacuo after each addition to afford the crude amine HCl salt which was used in the next step without further purification.

Hydrogen gas was bubbled through a solution of Boc-Me-Phe-d-Hiv-OBn 6a (0.11 g, 0.23 mmol) and palladium (24 mg, 10% on charcoal, 0.02 mmol,) in tetrahydrofuran (5 mL) for 3 min at room temperature before the resulting mixture was stirred under hydrogen for 2.5 h. The reaction mixture was filtered through celite, which was washed with ethyl acetate (20 mL). The solvent was removed in vacuo from the filtrate to afford the crude acid which was used in the next step without further purification.
Ghosez reagent (31 µL, 0.23 mmol) was added to a solution of the crude acid in dichloromethane (3 mL) at 0 °C and the resulting mixture was stirred for 20 min. A solution of the crude amine and \(N,N\)-diisopropylethylamine (0.14 mL, 0.84 mmol) in dichloromethane (3 mL) was added and the reaction mixture was stirred at room temperature for 18 h. Aqueous HCl (5 mL, 1M) was added, the reaction mixture was diluted with diethyl ether (10 mL) and the phases were separated. The aqueous layer was extracted with diethyl ether (3 x 10 mL) and the combined organic layers were washed with water (30 mL), saturated NaHCO\(_3\) solution (30 mL) and saturated NaCl solution (30 mL) before drying over MgSO\(_4\) and removing the solvent in vacuo. The crude product was purified via flash column chromatography using 40-60 petroleum ether:ethyl acetate (10:1 → 5:1) as eluent to afford the title compound 10a (0.18 g, 0.18 mmol, 75%) as a colourless solid.

\[
R_f = 0.16 \text{ [40-60 petroleum ether:diethyl ether = 5:1]; } mp = 142-143; \ 
\alpha_0^{23.7} = -60.8 \text{ (c 1.00 CHCl}_3); \ 
^1H NMR (500 MHz, d\textsubscript{6}-DMSO, T = 393 °K) \delta = 7.45 - 7.33 \text{ (m, 5H, d-Hiv}^\text{C1}^1\text{CH} - d\text-Hiv}^\text{C1}^1\text{eH}), 7.33 - 7.12 \text{ (m, 15H, Phe}^\text{A}^\text{C5H} - Phe}^\text{A}^\text{C7H}), 5.37, 5.33 \text{ (2 overlaid br. s, 2H, Phe}^\text{B}^\text{C2H}), 5.21 \text{ (d, J = 2.9 Hz, 2H, d-Hiv}^\text{A}^\text{B}^\text{C2H}), 5.12 \text{ (d, J = 4.8 Hz, 2H, d-Hiv}^\text{A}^\text{B}^\text{C2H}), 4.96-4.92 \text{ (m, 2H, d-Hiv}^\text{C2H} \text{ and Phe}^\text{A}^\text{C2H}), 3.38 - 3.24 \text{ (m, 2H, Phe}^\text{B}^\text{C3H}^\text{HH}, 3.20 \text{ (dd, J = 14.6, 5.1 Hz, 1H, Phe}^\text{A}^\text{C3}^\text{HH}), 3.12 - 2.90 \text{ (m, 9H, Phe}^\text{A}^\text{C3H}^\text{HH}, Phe}^\text{B}^\text{C3}^\text{HH}), 2.70 \text{ (s, 3H, Phe}^\text{A}^\text{NCH}^\text{3}), 2.31 - 2.16 \text{ (1H, m, d-Hiv}^\text{C2}^\text{C3H}), 1.81 \text{ (br. s, 2H, d-Hiv}^\text{A}^\text{B}^\text{C2H}), 1.30 \text{ (s, 9H, Phe}^\text{A}^\text{C2}^\text{CH}^\text{3}), 0.95 \text{ (dd, J = 6.9, 5.2 Hz, 6H, d-Hiv}^\text{C4}^\text{H}^\text{4H}), 0.78 \text{ (d, J = 6.8 Hz, 6H, d-Hiv}^\text{A}^\text{C4}^\text{H}^\text{4H}, 0.66 \text{ (s, 6H, d-Hiv}^\text{A}^\text{B}^\text{C4}^\text{H}^\text{4H}); \ 
^13C NMR (126 MHz, d\textsubscript{6}-DMSO, T = 393 °K) \delta = \ 
170.4, 170.3, 170.0 \text{ (3 x quat., Phe}^\text{A}^\text{B}^\text{C1}^\text{C1}), 169.5, 169.3 \text{ (2 x quat., d-Hiv}^\text{A}^\text{B}^\text{C1}^\text{C1}), 168.9 \text{ (quat., d-Hiv}^\text{C}^\text{C1}), 155.3 \text{ (quat., Phe}^\text{A}^\text{C1}^\text{C2}^\text{a}), 138.1 \text{ (quat., Phe}^\text{A}^\text{C4}), 137.6, 137.5 \text{ (2 x quat., Phe}^\text{B}^\text{C4}), 136.1 \text{ (quat., d-Hiv}^\text{B}^\text{C1}^\text{b}), 129.3, 129.1, 128.8, 128.7, 128.6, 128.6, 128.6, 126.9, 126.9, 126.7 \text{ (20 x CH, Phe}^\text{A}^\text{B}^\text{C5} \text{ - Phe}^\text{A}^\text{B}^\text{C7} \text{ and d-Hiv}^\text{C1}^\text{C1}^\text{c} - d\text-Hiv}^\text{C1}^\text{e}^\text{e}), 79.6 \text{ (quat., Phe}^\text{A}^\text{B}^\text{C2b}), 77.9 \text{ (CH, d-Hiv}^\text{C}^\text{C2}), 76.0, 75.6 \text{ (2 x CH, d-Hiv}^\text{A}^\text{B}^\text{C2}), 66.9 \text{ (CH}^\text{2}, d\text-Hiv}^\text{C1}^\text{a}), 59.9 \text{ (CH, Phe}^\text{A}^\text{C2}), 58.6, 58.5 \text{ (2 x CH, Phe}^\text{B}^\text{C2}), 35.0 \text{ (CH}^\text{2}, \text{ Phe}^\text{C3}), 34.8, 34.7 \text{ (2 x CH}^\text{2}, \text{ Phe}^\text{C3}), 32.4, 32.2 \text{ (2 x CH}^\text{2}, \text{ Phe}^\text{C}^\text{N}^\text{C}), 31.2 \text{ (CH}^\text{3}, \text{ Phe}^\text{N}^\text{C}), 30.2 \text{ (CH, d-Hiv}^\text{C2}^\text{C3}), 29.7, 29.6 \text{ (2 x CH, d-Hiv}^\text{A}^\text{B}^\text{C3}), 28.4 \text{ (3 x CH}^\text{3}, \text{ Phe}^\text{C2}^\text{c}), 19.0, 18.9 \text{ (2 x CH}^\text{3}, \text{ d-Hiv}^\text{A}^\text{C}^\text{C4}), 18.6, 17.5 \text{ (2 x CH}^\text{3}, \text{ d-Hiv}^\text{C}^\text{C4}), 16.9, 16.8 \text{ (2 x CH}^\text{3}, \text{ d-Hiv}^\text{A}^\text{B}^\text{C4}); \text{ IR (neat/cm}^{-1}): \text{ 2968, 2936, 1733, 1694, 1653, 1455, 1391, 1367, 1264, 1169, 1130, 1025, 741, 696}; \text{ HRMS (ESI): found 992.5297 (M+H)^+ \text{ C}_{57}H_{74}N_3O_{12} requires 992.5273 } \Delta = 2.4 \text{ ppm.}

4.5.2 Method B (Flow)

According to General Method 1, a solution of coupling partner A 6a (0.024 g, 0.05 mmol) in dichloromethane (5 mL) was hydrogenated to give the acid and the nitrogen of coupling partner B 8a (0.037 g, 0.05 mmol) was deprotected using HCl (reaction time = 6 h) and the resultant crude products were coupled in flow. The crude product was purified using preparative TLC using 40-60 petroleum ether:ethyl acetate (2:1) as eluent to afford the title compound 10a (0.039 g, 0.039 mmol, 78%) as a colourless solid, which was spectroscopically identical to that reported using method A.

4.6 Boc-Me-Phe-d-Hiv-Me-Phe-d-Hiv -Me-Phe-d-Hiv-Me-Phe-d-Hiv-OBn 11a

![](image)
4.6.1 Method A (Batch)

Anhydrous HCl (3 mL, 4M in dioxane) was added to a solution of 8a (0.10 g, 0.14 mmol) in dioxane (3 mL) and the reaction mixture was stirred at room temperature for 6 h before the solvent was removed in vacuo. Ethanol (2 x 3 mL) and methanol (3 mL) were added successively, removing the solvent in vacuo after each addition to afford the crude amine HCl salt which was used in the next step without further purification.

Hydrogen gas was bubbled through a solution of 8a (0.10 g, 0.14 mmol) and palladium (0.015 g, 10% on charcoal, 0.014 mmol) in tetrahydrofuran (4 mL) for 5 min before stirring under a hydrogen atmosphere for 6 h. The reaction mixture was filtered through cotton wool and a syringe filter and the solvent was removed in vacuo to afford crude acid which was used in the next step without further purification.

Ghosez reagent (0.02 mL, 0.15 mmol) was added to a solution of the crude acid in dichloromethane (5 mL) at 0 °C and the reaction mixture was stirred at this temperature for 20 min before a solution of the crude amine HCl salt and disopropylethylamine (0.088 mL, 0.5 mmol) in dichloromethane (5 mL) was added and the reaction mixture was warmed to room temperature and stirred for 20.5 h. The solvent was removed in vacuo and the crude taken up in diethyl ether (5 mL) and aqueous HCl (5 mL, 1M). The reaction mixture was extracted with diethyl ether (3 x 5 mL) and the combined organic extracts were washed with water (5 mL), saturated NaHCO₃ (5 mL) and saturated NaCl (5 mL) and were dried over MgSO₄ before the solvents were removed in vacuo to afford a crude oil. This was purified using preparative TLC using 40-60 petroleum ether:ethyl acetate (2:1) as eluent to afford the title compound 11a (0.10 g, 0.08 mmol, 57%) as a colourless solid.

\[ R_f = 0.36 \text { [40-60 petroleum ether:diethyl ether = 2:1]; mp } = 168 - 169 ^\circ \text{C; } \alpha_0^{27.8} = -48.8 \ (c \ 1.00 \text{CHCl}_3); \]

\[ ^1H \text{ NMR (500 MHz, d}_2\text{-DMSO, } T = 393 \text{ K}) \delta = 7.47 - 7.31 \ (m, 5H, d-HivD-C1′C3H) - d-HivC-C1′eH), 7.30 - 7.10 \ (m, 20H, PheA-D-C5H - PheA-D-C7H), 5.58 - 5.25 \ (m, 3H, PheB-D-C2H), 5.25 - 5.15 \ (m, 2H, d-HivD-C1′aH), 5.12 \ (d, J = 6.1 Hz, 3H, d-HivA-C2H), 5.02 - 4.78 \ (m, 2H, PheA-D-C2H and d-HivD-C2H), 3.99 - 3.22 \ (m, 3H, PheB-D-C3H), 3.19 \ (dd, J = 14.6, 5.1 Hz, 1H, PheA-D-C3H), 3.11 - 2.81 \ (m, 13H, PheA-D-C3H, PheB-D-NCH₃), 2.69 \ (s, 3H, PheA-D-NCH₃), 2.32 - 2.06 \ (m, 1H, d-HivD-C3H), 1.77 \ (br. s, 3H, d-HivA-C3H), 1.29 \ (s, 9H, PheB-D-C2H), 0.94 \ (t, J = 6.2 Hz, 6H, d-HivA-C4H), 0.76 \ (s, 9H, d-HivA-C4H), 0.63 \ (s, 9H, d-HivA-C4H); \]

\[ ^{13C} \text{ NMR (126 MHz, d}_2\text{-DMSO, } T = 393 \text{ K}) \delta = 170.6 \text{(quat., PheA-C1), 170.2, 169.93, 169.86 (3 x quat., PheB-D-C1), 169.4, 169.3 (quat., HivA-C-C1), 168.9 (quat., HivA}-C-C1), 153.2 \text{(quat., PheB-D-C2’a), 138.0 (quat., PheB-C4), 137.6, 137.4, 136.0 (3 x quat., PheB-D-C4), 129.2 (quat., d-HivB-C1'b), 129.1, 128.8, 128.6, 128.5, 128.5, 126.8, 126.8, 126.6, 126.6 \ (25 x CH, PheB-D-C5 - PheB-D-C7 and d-HivD-C1’c – d-HivD-C1’e), 79.5 \text{(quat., PheB-D-C2’b), 77.8 (CH, d-HivD-C2), 75.9, 75.5 \ (3 x CH, d-HivA-C-C2), 66.8 (CH₂, d-HivD-C1’a), 59.8 (CH, PheA-D-C2), 58.5(3 x CH, PheB-D-C2), 34.9 \ (4 x CH₂, PheB-D-C3), 32.3 \ (3 x CH₃, PheB-D-NCH₃), 31.1 \ (CH₃, PheA-NCH₃), 30.1 \ (CH, d-HivD-C3), 29.6 \ (3 x CH, d-HivA-C-C3), 28.4 \ (3 x CH₃, PheB-C2’c), 18.94, 18.89, 18.85 \ (4 x CH₂, from d-HivA-C-C4), 18.5, 17.4 \ (2 x CH₃, d-HivD-C4), 16.8, 16.8 \ (2 x CH₃, from d-HivA-C-C4); \]

IR (neat/cm⁻¹): 2965, 2929, 1733, 1685, 1657, 1457, 1270, 1225, 1170, 1027, 739, 698; HRMS (ESI): found 1275.6443 (M+Na)⁺ C₇₂H₉₂N₄O₁₃Na requires 1275.6451, Δ = 0.6 ppm.

4.6.2 Method B (Flow)

According to General Method 1, a solution of coupling partner A 8a (0.037 g, 0.05 mmol) in dichloromethane (5 mL) was hydrogenated to give the acid and coupling partner B 8a (0.037 g,
0.05 mmol) was acid deprotected (reaction time = 6 h) and the resultant crude products were coupled in flow. The crude product was purified using preparative TLC using 40-60 petroleum ether:ethyl acetate (2:1) as eluent to afford the title compound 11a (0.042 g, 0.034 mmol, 67%) as a colourless solid, which was spectroscopically identical to that reported using method A.

### 4.7 –[Me-Phe-d-Hiv-Me-Phe-d-Hiv]- 9a

![Image of structure](image)

#### 4.7.1 Method A (Batch)

Palladium (0.02 g, 10% on charcoal, 0.01 mmol) was added to a solution of Boc-Me-Phe-d-Hiv-Me-Phe-d-Hiv-OBn 6a (0.10 g, 0.14 mmol) and hydrogen was bubbled through the reaction mixture for 5 min before stirring under a hydrogen atmosphere for 6 h. The reaction mixture was filtered through a plug of cotton wool and a syringe filter and the solvent was removed in vacuo from the filtrate to afford the crude acid. This was taken up in dioxane (3 mL) and anhydrous HCl (3 mL, 4M in dioxane) added. The reaction mixture was stirred at room temperature for 6 h before the solvent was removed in vacuo. Ethanol (2 x 3 mL) and methanol (3 mL) were added successively and the solvent removed in vacuo after each addition to afford the doubly deprotected depsipeptide HCl salt. This was taken up in dichloromethane (14 mL) and was cooled to 0 °C before Ghosez reagent (0.02 mL, 0.15 mmol) was added. The reaction mixture was stirred at 0 °C for 20 min before diisopropylethylamine (0.09 mL, 0.50 mmol) was added and the reaction mixture warmed to room temperature and stirred for 21 h. The solvent was removed in vacuo and the crude taken up in diethyl ether (5 mL) and aqueous HCl (5 mL, 1M). The reaction mixture was extracted with diethyl ether (3 x 5 mL) and the combined organic extracts were washed with water (5 mL), saturated NaHCO3 (5 mL) and saturated NaCl (5 mL). The combined aqueous extracts were further extracted with ethyl acetate (3 x 5 mL) and the combined organics were dried over MgSO4 before the solvent was removed in vacuo. The resulting crude oil was purified using preparative TLC using 40-60 petroleum ether:ethyl acetate (1:1) as eluent to afford the title compound 9a (0.012 g, 0.024 mmol, 17%) as a colourless solid and cyclic tetramer 2a (0.030 g, 0.029 mmol, 41%) as a colourless solid which was spectroscopically identical to that reported for this compound below.

**Characterisation of 9a:** Rf = 0.30 [toluene:ethyl acetate = 2:1]; mp = 54.5 – 55 °C, αD 27.8° = +16.7 (c 1.00, CHCl3); 1H NMR (600 MHz, CDCl3) δ = 7.31 (dd, J = 8.1, 6.6 Hz, 2H, 2 x Phe-C5H), 7.28 – 7.23 (m, 1H, Phe-C7H), 7.18 (dd, J = 6.9, 1.7 Hz, 2H, 2 x Phe-C6H), 4.88 (t, J = 7.8 Hz, 1H, Phe-C2H), 4.76 (d, J = 10.4 Hz, 1H, d-Hiv-C2'H), 3.14 (s, 4H, Phe-C3'H'H and Phe-NCH3), 3.03 (dd, J = 13.8, 8.2 Hz, 1H, Phe-C3'H'H), 1.69 (ddt, J = 16.3, 12.5, 6.3 Hz, 1H, d-Hiv-C3'H), 0.67 (dd, J = 13.3, 6.6 Hz, 6H, 2 x d-Hiv-C4'H'2); 13C NMR (151 MHz, CDCl3) δ = 169.4 (quat., Phe-Ć1), 168.5 (quat., d-Hiv-Ć1), 135.4 (quat., Phe-Ć4), 129.1 (2 x CH, Phe-Ć5), 129.0 (2 x CH, Phe-C6), 127.5 (CH, Phe-Ć7), 83.3 (CH, d-Hiv-Ć2), 59.9 (CH, Phe-Ć2), 36.8 (CH2, Phe-Ć3), 30.6 (CH, d-Hiv-Ć3), 30.3 (CH3, Phe-NĆ), 19.7 (CH3, d-Hiv-Ć4), 18.1 (CH3, d-Hiv-Ć4); IR (neat/cm⁻¹): 2966, 2929, 1749, 1652, 1435, 1404, 1366., 1285, 1007, 700, 668; HRMS (ESI): found 523.2800 (M+H)+ C30H38N2O6 requires 523.2803, Δ = 0.5 ppm.
4.7.2 Method B (Flow)
According to General Method 2, a solution of protected precursor 8a (0.037 g, 0.05 mmol) in dichloromethane (5 mL) was hydrogenated to give the acid and then the nitrogen acid deprotected (reaction time = 6 h) and the resultant crude product macrocyclised in flow. The crude product was purified using preparative TLC using toluene:ethyl acetate (2:1) as eluent to afford the title compound 9a (0.018 g, 0.034 mmol, 68%) as a colourless solid, which was spectroscopically identical to that reported using method A.

4.8  –[Me-Phe-d-Hiv-Me-Phe-d-Hiv-Me-Phe-d-Hiv]- 1a (beauvericin)

4.8.1 Method A (Batch)
Hydrogen gas was bubbled through a solution of Boc-Me-Phe-d-Hiv-Me-Phe-d-Hiv-Me-Phe-d-Hiv-OBn 10a (83 mg, 84 µmol) and palladium (9 mg, 10% on charcoal, 8.4 µmol) in tetrahydrofuran (2 mL) for 3 min at room temperature before the mixture was stirred under hydrogen for 6 h. The reaction mixture was filtered through celite, washing with ethyl acetate (30 mL). The solvent was removed in vacuo from the filtrate to afford the crude acid, which was then taken up in dioxane (2 mL). Anhydrous HCl (2 mL, 4M in dioxane) was added and the resulting mixture was stirred for 6 h. The solvent was removed in vacuo and the residue was taken up sequentially in ethanol (2 x 5 mL) and methanol (5 mL) removing the solvent in vacuo after each addition to afford the deprotected linear precursor. This was taken up in dichloromethane (20 mL) and cooled to 0 °C before Ghosez reagent (12 µL, 92 µmol) was added at and the resulting mixture was stirred for 30 min at this temperature. N,N-diisopropylethylamine (51 µL, 301 µmol) was added and the reaction mixture was warmed to room temperature and stirred for 18.5 h. The solvent was removed in vacuo and the residue was taken up in diethyl ether (60 mL) and aqueous HCl (60 mL, 1M). The aqueous layer was extracted with diethyl ether (3 x 30 mL) and the combined organic layers were washed with water (100 mL), saturated NaHCO₃ solution (100 mL) and saturated NaCl solution (100 mL) before drying over MgSO₄ and removing the solvent in vacuo. The crude product was purified via flash column chromatography using 40-60 petroleum ether:ethyl acetate (2:1 → 1:1) as eluent to afford the title compound 1a (46 mg, 59 µmol, 70%) as a colourless solid. The ¹H and ¹³C NMR spectrum were in agreement with that published in the literature.[⁹]

Rₚ = 0.15 [40-60 petroleum ether:diethyl ether = 1:1]; mp = 92 – 93 °C (literature = 92 – 93 °C⁹); αₒ = 24° = +75 (c 0.1 MeOH) literature¹⁰ [αₒ = +69 (c 0.1, MeOH)]; ¹H NMR (600 MHz, CDCl₃) δ = 7.41 – 7.07 (m, 5H, Phe-C₅H Fayette – Phe-C₇H Fayette), 5.46 (dd, J = 11.5, 4.9 Hz, 1H, Phe-C₂H Fayette), 4.92 (d, J = 8.6 Hz, 1H, d-Hiv-C₂H Fayette), 3.36 (dd, J = 14.6, 4.9 Hz, 1H, Phe-C₃H Fayette), 3.11 – 2.91 (m, 4H, Phe-C₃H Fayette and Phe-NCH₃ Fayette), 2.02 (hept., J = 6.7 Hz, 1H, d-Hiv-C₃H Fayette), 0.80 (d, J = 6.7 Hz, 3H, d-Hiv-C₄H Fayette), 0.43 (d, J = 6.7 Hz, 3H, d-Hiv-C₄H Fayette); ¹³C NMR (151 MHz, CDCl₃) δ = 170.0 (quat., Phe-C₁), 169.5 (quat., d-Hiv-C₁), 136.8 (quat., Phe-C₄), 129.0, 128.7, 126.9 (5 x CH, Phe-C₅ – Phe-C₇), 75.6 (CH, d-Hiv-C₂), 57.5 (CH, Phe-C₂), 34.9 (CH₂, Phe-C₃), 32.5 (CH₃, Phe-NC), 29.8 (CH, d-Hiv-C₃), 18.4, 17.6 (2 x CH₃, d-Hiv-C₄); IR (neat/cm⁻¹):
2964, 2933, 1743, 1659, 1456, 1411, 1370, 1263, 1178, 1106, 1015, 731, 697; HRMS (ESI): found 784.4183 (M+H)\(^{+}\) \(\text{C}_{45}\text{H}_{58}\text{N}_{9}\text{O}_{9}\) requires 784.4173 \(\Delta = 1.3\) ppm.

4.8.2 Method B (Flow)
According to General Method 2, a solution of protected precursor 10a (0.050 g, 0.05 mmol) in dichloromethane (5 mL) was hydrogenated to give the acid and then the nitrogen acid deprotected (reaction time = 6 h) and the resultant crude product macrocyclised in flow. The crude product was purified using preparative TLC using toluene:ethyl acetate (2:1) as eluent to afford the title compound 1a (0.030 g, 0.038 mmol, 76%) as a colourless solid, which was spectroscopically identical to that reported using method A.

4.9 –[Me-Phe-\text-d}-Hiv–Me-Phe-\text-d}-Hiv–Me-Phe-\text-d}-Hiv–Me-Phe-\text-d}-Hiv]– 2a

4.9.1 Method A (Batch)
Boc-Me-Phe-\text-d}-Hiv–Me-Phe-\text-d}-Hiv–Me-Phe-\text-d}-Hiv–Me-Phe-\text-d}-Hiv–OBn 11a (0.086 g, 0.068 mmol) was taken up in methanol (5 mL) and dichloromethane (2 mL) and this solution was hydrogenated using an H-cube\textsuperscript{®} Pro (ThalesNano) with a 10\% Pd/C CatCart\textsuperscript{®}. The pump was run at 1 mL min\(^{-1}\) using methanol with the temperature set to 60 °C and the pressure to 6 bar. The solvent was then removed \textit{in vacuo} and the crude acid was taken up in dioxane (1.5 mL) and anhydrous HCl (1.5 mL, 4 M in dioxane) was added. The reaction mixture was stirred at room temperature for 6 h 50 min before the solvent was removed \textit{in vacuo}. The resultant crude was taken up successively in ethanol (2 x 2 mL) and methanol (2 mL) removing the solvent \textit{in vacuo} after each addition. The resultant HCl salt was taken up in dichloromethane (10 mL) and was cooled to 0 °C before Ghosez reagent (0.01 mL, 0.075 mmol) was added and the reaction mixture was stirred at this temperature for 20 min. Disopropylethylamine (0.042 mL, 0.24 mmol) was added and the reaction mixture was warmed to room temperature and stirred for 19.5 h before the solvent was removed \textit{in vacuo}. The resultant oil was taken up in aqueous HCl (10 mL, 1M) and was extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were washed with water (10 mL), saturated NaHCO\textsubscript{3} (10 mL) and saturated NaCl (10 mL) before drying over MgSO\textsubscript{4} and removing the solvent \textit{in vacuo}. The resulting crude oil was purified using preparative TLC using toluene:ethyl acetate (1:1) as eluent to afford the title compound 2a (0.021 g, 0.020 mmol, 30%) as a colourless solid.

\(R_f = 0.35\) [toluene:diethyl ether = 1:1]; mp = 102 – 103 °C; \(\delta_0^{27,8} = -20.2\) (c 1.00, CHCl\textsubscript{3}); \(^1\)H NMR (500 MHz, \(\text{d}_{6}\)-toluene, \(T = 363°\text{K}\)) \(\delta = 7.34 – 6.88\) (m, 5H, Phe-\text{C}SH – Phe-\text{C}7H), 6.43 – 6.06 (m, 1H, Phe-\text{C}2H), 5.25 (d, \(J = 7.8\) Hz, 1H, d-Hiv-\text{C}2H)), 3.42 (dd, \(J = 14.8, 5.3\) Hz, 1H, Phe-\text{C}3H\textsubscript{2}H)), 3.04 – 2.67 (m, 4H, Phe-\text{C}3H\textsubscript{2}H and Phe-NCH\textsubscript{3})), 2.24 – 2.09 (m, 1H, d-Hiv-\text{C}3H)), 0.78 (d, \(J = 6.7\) Hz, 3H, d-Hiv-\text{C}4H\textsubscript{3})), 0.56 (d, \(J = 6.7\) Hz, 3H, d-Hiv-\text{C}4H\textsubscript{3})); \(^{13}\)C NMR (126 MHz, \(\text{d}_{6}\)-toluene, \(T = 363°\text{K}\)) \(\delta = 170.5\) (quat., Phe-\text{C}1), 169.2 (quat., d-Hiv-\text{C}1), 137.4 (quat., Phe-\text{C}4), 129.5, 128.7, 126.8 (5 x CH, Phe-\text{C}5 – Phe-\text{C}7), 75.2 (CH, d-Hiv-\text{C}2), 57.3 (CH, Phe-\text{C}2), 57.3 (CH, Phe-\text{C}2), 34.9 (CH\textsubscript{2}, Phe-\text{C}3), 31.2 (CH\textsubscript{3}, Phe-\text{N}C), 30.4 (CH, d-Hiv-\text{C}3), 18.3,
18.1 (2 x CH₃, d-Hiv-C4); IR (neat/cm⁻¹): 2963, 2929, 1742, 1663, 1456, 1413, 1261, 1194, 1091, 1018, 745, 699; HRMS (ESI): found 1045.5528 (M+H)⁺ C₆₀H₇₇NaO₁₂ requires 1045.5533 Δ = 0.5 ppm.

4.9.2 Method B (Flow)

According to General Method 2, a solution of protected precursor 11a (0.063 g, 0.05 mmol) in methanol (5 mL) and dichloromethane (2 mL) and was hydrogenated to give the acid and then the nitrogen acid deprotected (reaction time = 6 h 25 min) and the resultant crude product macrocylised in flow. The crude product was purified using preparative TLC using toluene:ethyl acetate (1:1) as eluent to afford the title compound 2a (0.041 g, 0.040 mmol, 80%) as a colourless solid, which was spectroscopically identical to that reported using method A.

5 Synthesis of N-Me-Leu containing depsipeptides

5.1 Boc-Me-Leu-d-Hiv-OBn 6b

![Diagrams](image)

EDCI (0.37 g, 2.0 mmol) was added to a solution of d-Hiv-OBn 4 (2.14 g 10.3 mmol), Boc-N-methyl-L-leucine 5b (3.78 g, 15.4 mmol) and 4-DMAP (1.63 g, 13.6 mmol) in dichloromethane (30 mL) at 0 °C and the resulting mixture was stirred at room temperature for 14 h. Aqueous HCl (30 mL, 1M) was added and the phases were separated. The aqueous layer was extracted with dichloromethane (3 x 30 mL) and the combined organic layers were washed with water (100 mL), saturated NaHCO₃ solution (100 mL) and saturated NaCl solution (100 mL) before drying over MgSO₄ and removing the solvent in vacuo. The crude product was purified via flash column chromatography using 40-60 petroleum ether:ethyl acetate (10:1) as eluent to afford the title compound 6b (4.13 g, 9.5 mmol, 92%) as a colourless oil.

R_f = 0.45 [40-60 petroleum ether:diethyl ether = 10:1]; αD = 32.2° (c 1.00, CHCl₃); ¹H NMR (500 MHz, d⁵-DMSO, T = 393 °K) δ = 7.60 – 7.09 (m, 5H, d-Hiv-C1’cH – d-Hiv-C1’eH), 5.18 (d, J = 1.5 Hz, 2H, d-Hiv-C1’aH), 4.90 (d, J = 4.6 Hz, 1H, d-Hiv-C2H), 4.75 (dd, J = 8.8, 6.2 Hz, 1H, Leu-C2H), 2.73 (s, 3H, Leu-NCH₃), 2.22 (pd, J = 6.8, 4.6 Hz, 1H, d-Hiv-C3H), 1.70 – 1.65 (m, 2H, Leu-C3H₂), 1.65 – 1.54 (m, 1H, Leu-C4H), 1.43 (s, 9H, Leu-C2’CH₃), 1.01 – 0.87 (m, 12H,d-Hiv-C4H₃ and Leu-C5H₃); ¹³C NMR (126 MHz, d⁵-DMSO, T = 393 °K) δ = 170.4 (quat., Leu-C1), 167.8 (quat., d-Hiv-C1), 154.4 (quat., Leu-C2’a), 134.9 (quat., d-Hiv-C1’b), 127.6, 127.4, 127.3 (5 x CH, d-Hiv-C1’c – d-Hiv-C1’e), 78.7 (quat., Leu-C2’b), 76.2 (CH, d-Hiv-C2), 65.7 (CH₂, d-Hiv-C1’a), 55.8 (CH, Leu-C2), 36.7 (CH, Leu-C3), 29.4 (CH₃, Leu-NC), 29.0 (CH, d-Hiv-C3), 27.4 (CH₃, Leu-C2’c), 23.9 (CH, Leu-C4), 21.8, 20.6 (CH₃, Leu-C5), 17.5, 16.1 (CH₃, d-Hiv-C4); IR (neat/cm⁻¹): 2966, 2875, 1741, 1694, 1456, 1390, 1366, 1318, 1188, 1149, 1126, 1026, 1004, 906, 751, 697; HRMS (ESI): found 436.2701 (M+H)⁺ C₂₆H₃₈NO₆ requires 436.2699 Δ = 0.4 ppm.

5.2 Boc-Me-Leu-d-Hiv-Me-Leu-d-Hiv-OBn 8b

![Diagrams](image)
5.2.1 Method A (Batch)

Anhydrous HCl (2.5 mL, 4M in dioxane) was added to a solution of Boc-Me-Leu-d-Hiv-OBn 6b (0.12 g, 0.26 mmol) in dioxane (2.5 mL) at room temperature and the resulting mixture was stirred for 3 h. The solvent was removed in vacuo and the residue was taken up sequentially in ethanol (2 x 5 mL) and methanol (5 mL) with the solvent removed in vacuo after each addition to afford the amine HCl salt which was used directly in the next step.

Hydrogen gas was bubbled through a solution of Boc-Me-Leu-d-Hiv-OBn 6b (0.12 g, 0.26 mmol) and palladium (26 mg, 10% on charcoal, 26.4 μmol) in tetrahydrofuran (5 mL) for 3 min at room temperature before the resulting mixture was stirred under a hydrogen atmosphere for 3 h. The reaction mixture was filtered through celite, eluting with ethyl acetate (20 mL). The solvent was removed in vacuo from the filtrate to afford the acid which was used directly in the next step.

Ghosez reagent (38 μL, 0.29 mmol) was added to a solution of the acid in dichloromethane (5 mL) at 0 °C and the resulting mixture was stirred for 25 min. A solution of amine HCl salt and N,N-diisopropylethylamine (0.17 mL, 0.95 mmol) in dichloromethane (5 mL) was added, the reaction mixture was allowed to warm to room temperature and stirred for 22 h. Aqueous HCl (20 mL, 1M) was added, the reaction mixture was diluted with diethyl ether (40 mL) and the phases were separated. The aqueous layer was extracted with diethyl ether (3 x 20 mL) and the combined organic layers were washed with water (100 mL), saturated NaHCO₃ solution (100 mL) and saturated NaCl solution (100 mL) before drying over MgSO₄ and removing the solvent in vacuo. The crude product was purified via flash column chromatography using 40-60 petroleum ether:ethyl acetate (10:1 → 5:1) as eluent to afford the title compound 8b (0.11 g, 0.17 mmol, 64%) as a colourless solid.

\[ R_f = 0.12 \] [40-60 petroleum ether:diethyl ether = 10:1]; mp = 80 – 82 °C; \[ \alpha_d^{23.8} = -33.0 \] (c 1.00, CHCl₃);

\[ ^1H \text{ NMR} \] (500 MHz, d₆-DMSO, T = 393 °K) δ = 7.42 – 7.30 (m, 5H, d-Hiv⁺⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-

\[ ^1C \text{ NMR} \] (126 MHz, d₆-DMSO, T = 393 °K) δ = 170.2 (quat., Leu⁺⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻~-~-

HRMS (ESI): found 663.4215 (M+H)⁺ C₃₆H₅₀N₄O₃ requires 663.4220 Δ = 0.8 ppm.

5.2.2 Method B (Flow)

According to General Method 1, a solution of coupling partner A 6b (0.022 g, 0.05 mmol) in methanol (5 mL) was hydrogenated to give the acid and coupling partner B 6b (0.022 g, 0.05 mmol) was deprotected using HCl (reaction time = 5 h) and the resultant crude products were coupled in flow. The product was purified using flash column chromatography using 40-60 petroleum ether:ethyl acetate (10:1) as eluent to afford the title compound 8b (0.030 g, 0.045 mmol, 90%) as a colourless solid, which was spectroscopically identical to that reported using method A.

5.3.1 Method A (Batch)
Anhydrous HCl (3 mL, 4M in dioxane) was added to a solution of Boc-Me-Leu-d-Hiv-OBn 6b (0.10 g, 0.15 mmol) in dioxane (3 mL) at room temperature and the resulting mixture was stirred for 6 h. The solvent was removed in vacuo and the residue was taken up sequentially in ethanol (2 x 4 mL) and methanol (4 mL) with the solvent removed in vacuo after each addition to afford the amine HCl salt which was used directly in the next step.

Hydrogen gas was bubbled through a solution of Boc-Me-Leu-d-Hiv-OBn 6b (0.07 g, 0.15 mmol) and palladium (16 mg, 10% on charcoal, 0.015 mmol) in tetrahydrofuran (4 mL) for 5 min at room temperature before the resulting mixture was stirred under a hydrogen atmosphere for 3 h 15 min. The reaction mixture was filtered through cotton wool and syringe filter (0.2 µm) and the solvent was removed in vacuo from the filtrate to afford the acid which was used directly in the next step.

Ghosez reagent (0.022 mL, 0.17 mmol) was added to a solution of the acid in dichloromethane (5 mL) at 0 °C and the resulting mixture was stirred for 20 min. A solution of amine hydrochloride salt and N,N-diisopropylethylamine (0.094 mL, 0.54 mmol) in dichloromethane (5 mL) was added, the reaction mixture was allowed to warm to room temperature and stirred for 18 h. The solvent was removed in vacuo and aqueous HCl (5 mL, 1M) was added before the reaction mixture was extracted with ethyl acetate (3 x 15 mL) and the combined organic layers were washed with water (5 mL), saturated NaHCO3 solution (5 mL) and saturated NaCl solution (5 mL) before drying over MgSO4 and removing the solvent in vacuo. The crude product was purified via preparative TLC using 40-60 petroleum ether:ethyl acetate (2:1) as eluent to afford the title compound 10b (0.10 g, 0.12 mmol, 78%) as a colourless foam.

Rf = 0.67 (40-60 petroleum ether:diethyl ether = 2:1), 0.14 (40-60 petroleum ether:diethyl ether = 5:1); 1H NMR (500 MHz, d6-DMSO, T = 393 °K) δ = 7.58 – 7.16 (m, 5H, d-Hiv5-C1′CH – d-Hiv6-C1′eH), 5.24 – 5.15 (m, 4H, d-Hiv6-C1′aH2 and d-HivA′B′C′-C2H), 5.06 (br. s, 2H, LeuB′C′-C2H), 4.88 (br. s, 1H, d-HivC′-C2H), 4.74 (t, J = 8.0 Hz, 1H, LeuA′-C2H), 3.03 (br. s, 3H, LeuB′ or C-NCH3), 2.96 (br. s, 3H, LeuB′ or C-NCH3), 2.77 (s, 3H, LeuA′-NCH3), 2.27 – 2.07 (m, 3H, d-HivA′B′C′-C3H), 1.86 – 1.65 (m, 6H, LeuA′B′C′-C3H), 1.62 – 1.51 (m, 3H, LeuA′B′C′-C4H), 1.43 (s, 9H, LeuA′-C2′cH3), 1.02 – 0.88 (m, 36H, d-HivA′B′C′-C4H and LeuA′B′C′-C5H3); 13C NMR (126 MHz, d6-DMSO, T = 393 °K) δ = 170.2 (quat., LeuA′-C1′), 169.9, 169.7 (quat., LeuB′C′-C1′), 168.4, 168.3 (quat., d-HivA′B′-C1′), 167.8 (quat., d-HivC′-C1′), 154.5 (quat., LeuA′-C2′a), 135.0 (quat., d-HivA′-C1′b), 127.7, 127.5, 127.4 (5 x CH, d-HivC′-C1′c – d-HivC′-C1′e), 78.6 (quat., LeuA′-C2′b), 76.6 (CH, d-HivC′-C2′), 74.9, 74.7 (2 x CH, d-HivA′B′-C2′), 65.7 (CH2, d-HivA′-C1′a), 55.8 (CH, LeuB′-C2′), 54.5 (2 x CH, LeuB′-C2′), 36.8 (CH2, LeuA′-C3), 30.6 (2 x CH3, LeuB′-NC), 29.4 (CH3, LeuA′-NC), 29.0 (CH, d-HivC′-C3), 28.7 28.6, (2 x CH, d-HivA′B′-C3), 27.4 (3 x CH3, LeuA′-C2′c), 23.9 (CH, LeuA′-C4′), 23.8 (2 x CH, LeuB′-C4′), 22.0, 21.9, 20.6, 18.0 (6 x CH3, LeuA′B′-C5′), 17.9, 17.4, 16.3, 16.0 (6 x CH3, d-HivA′B′C′-C4′); IR (neat/cm−1): 2960, 2873, 1737, 1695, 1662, 1470, 1390, 1368, 1262, 1203, 1150, 1125, 1020, 699; HRMS (ESI): found 890.5738, (M+H)+ C48H80N3O12 requires 890.5737 Δ = 0.1 ppm.
5.3.2 Method B (Flow)
According to General Method 1, a solution of coupling partner A 6b (0.022 g, 0.05 mmol) in methanol (5 mL) was hydrogenated to give the acid and coupling partner B 8b (0.033 g, 0.05 mmol) was deprotected using HCl (reaction time = 7 h 10 min) and the resultant crude products were coupled in flow. The crude product was purified using flash column chromatography using 40-60 petroleum ether:ethyl acetate (5:1) as eluent to afford the title compound 10b (0.038 g, 0.043 mmol, 86%) as a colourless foam, which was spectroscopically identical to that reported using method A.


5.4.1 Method A (Batch)
Anhydrous HCl (2 mL, 4M in dioxane) was added to a solution of Boc-Me-Leu-d-Hiv-Me-Leu-d-Hiv-OBn 8b (80 mg, 0.12 mmol) in dioxane (2 mL) at room temperature and the resulting mixture was stirred for 5 h. The solvent was removed in vacuo and the residue was taken up sequentially in ethanol (2 x 5 mL) and methanol (5 mL) with the solvent removed in vacuo after each addition to afford the HCl salt of the amine which was used directly in the next step.

Hydrogen gas was bubbled through a solution of Boc-Me-Leu-d-Hiv-Me-Leu-d-Hiv-OBn 8b (80 mg, 0.12 mmol) and palladium (12 mg, 10% on charcoal, 12 µmol) in tetrahydrofuran (2 mL) for 3 min at room temperature before the mixture was stirred under a hydrogen atmosphere for 5.5 h. The reaction mixture was filtered through celite, eluting with ethyl acetate (20 mL). The solvent was removed in vacuo from the filtrate to afford the acid which was used directly in the next step.

Ghosez reagent (18 µL, 0.13 mmol) was added to a solution of the crude acid in dichloromethane (2.5 mL) at 0 °C and the resulting mixture was stirred for 25 min before a solution of crude amine and N,N-diisopropylethylamine (0.07 mL, 0.43 mmol) in dichloromethane (2.5 mL) was added and the reaction mixture was allowed to warm to room temperature and stirred for 16 h. Aqueous HCl (10 mL, 1M) was added, the reaction mixture was diluted with diethyl ether (20 mL) and the phases were separated. The aqueous layer was extracted with diethyl ether (3 x 10 mL) and the combined organic layers were washed with water (50 mL), saturated NaHCO3 solution (50 mL) and saturated NaCl solution (50 mL) before drying over MgSO4 and removing the solvent in vacuo. The crude product was purified via flash column chromatography using 40-60 petroleum ether:ethyl acetate (5:1 → 3:1) as eluent to afford the title compound 11b (0.10 g, 91 µmol, 75%) as a colourless solid.

Rf = 0.12 [40-60 petroleum ether:ethyl acetate = 5:1]; mp = 124 – 125 °C; αD23.8 = -68.9 (c 1.00, CHCl3); 1H NMR (500 MHz, d6-DMF, T = 393 °K) δ = 7.42 – 7.30 (m, 5H, d-HivD-C1’CH – d-HivD-C1’eH), 5.27 – 5.15 (m, 5H, d-HivD-C1’aH2 and d-HivA-C2H2), 5.02 (br. s, 3H, LeuB-D-C2H2), 4.88 (d, J = 4.6 Hz, 1H, d-HivD-C2H2), 4.78 – 4.70 (m, 1H, LeuA-C2H2), 3.14 – 2.94 (m, 9H, LeuB-D-CNCH3), 2.77 (s, 3H, LeuA-NCH3), 2.26 – 2.09 (m, 4H, d-HivA-D-C3H), 1.76 – 1.62 (m, 8H, LeuA-D-C3H2), 1.63 – 1.52 (m, 4H, LeuA-D-C4H), 1.43 (s, 9H, LeuA-C2’CH3), 0.99 – 0.87 (m, 48H, d-HivA-B-D-C4H2 and LeuA-B-D-CNCH3), 13C NMR (126 MHz, d6-DMF, T = 393 °K) δ = 170.4, 170.3, 170.0, 169.9 (4 x quat., LeuA-D-C1), 168.9, 168.8, 168.7 (3 x quat., d-HivA-C1), 168.2 (quat., d-HivD-C1), 155.4 (quat., LeuA-C2’a), 135.1 (quat., d-HivD-C1), 115.6 (quat., d-HivA-C2), 111.9 (quat., d-HivA-C1), 83.1 (quat., d-HivD-C1), 56.3 (quat., d-HivD-C1), 31.0 (quat., d-HivD-C1).
was colourless CatCart®.

1263, 16.0 28.8, LeuB C2), (1.5 cooled with chromatography 0.014 0.013 22.0, 19%)

The product was removed and the acid was deprotected and the resultant crude products were coupled in flow. The crude product was purified using flash column chromatography using 40-60 petroleum ether:ethyl acetate (5:1) as eluent to afford the title compound 11b (0.042 g, 0.038 mmol, 76%) as a colourless solid, which was spectroscopically identical to that reported using method A.

5.5 -[Me-Leu-d-Hiv-Me-Leu-d-Hiv]- 9b

5.5.1 Method A (Batch)

Boc-Me-Leu-d-Hiv-Me-Leu-d-Hiv-OBn 8b (0.050 g, 0.075 mmol) was taken up in dichloromethane (1.5 ml) and this solution was hydrogenated using an H-cube® Pro (ThalesNano) with a 10% Pd/C CatCart®. The pump was run at 1 mL.min⁻¹ using dichloromethane with the temperature set to 60 °C and the pressure to 6 bar. The solvent was then removed in vacuo and the crude acid was taken up in dioxane (1 ml) and anhydrous HCl (1 ml, 4 M in dioxane) was added. The reaction mixture was stirred at room temperature for 5 h 25 min before the solvent was removed in vacuo. The resultant crude was taken up successively in ethanol (2 x 5 ml) and methanol (5 ml) removing the solvent in vacuo after each addition. The resultant HCl salt was taken up in dichloromethane (0.5 ml) and was cooled to 0 °C before Ghosez reagent (0.011 ml, 0.083 mmol) was added and the reaction mixture was stirred at this temperature for 15 min. Diisopropylethylamine (0.05 ml, 0.27 mmol) was added and the reaction mixture was warmed to room temperature and stirred for 20 h before the solvent was removed in vacuo. The resultant oil was taken up in aqueous HCl (6 ml, 1M) and was extracted with diethyl ether (3 x 6 ml). The combined organic extracts were washed with water (6 ml), saturated NaHCO₃ solution (6 ml) and saturated NaCl solution (6 ml) before drying over MgSO₄ and removing the solvent in vacuo. The resulting crude oil was purified using flash column chromatography using 2:1 toluene:ethyl acetate as eluent to afford the title compound 9b (0.0065 g, 0.014 mmol, 19%) as a colourless solid and cyclic tetramer 2b (0.015 g, 0.017 mmol, 45%) as a colourless solid which was spectroscopically identical to that reported for this compound below.

Rₚ = 0.36 [toluene:ethyl acetate= 2:1]; mp = 155.5 – 157 °C (literature = 156 – 1578 °C\textsuperscript{[11]}); \( \alpha_\text{D}^{27.8} = +34.4 \text{ (c } 0.90 \text{ CHCl}_3 \text{), literature}^{[11]} = \alpha_\text{D}^{20} = +35 \text{ (c } 1.0 \text{ CHCl}_3 \text{); } ^1\text{H NMR} \text{ (600 MHz, CDCl}_3 \text{) } \delta = 4.90 \text{ (d, J} = 20$
9.9 Hz, 1H, d-Hiv-C2H), 4.82 (dd, J = 8.9, 6.7 Hz, 1H, Leu-C2H), 3.02 (s, 3H, Leu-NCH₃), 2.09 (dt, J = 9.9, 6.7 Hz, 1H, d-Hiv-C3H), 1.92 (ddd, J = 13.9, 8.9, 5.3 Hz, 1H, Leu-C3H/H), 1.58 – 1.52 (m, 1H, Leu-C4H), 1.49 (ddd, J = 13.4, 8.2, 6.7 Hz, 1H, Leu-C3H/H). 1.11 – 0.98 (m, 12H, d-Hiv-C4H₃ and Leu-C5H₂H): ¹³C NMR (151 MHz, CDCl₃) δ = 170.3 (quat., Leu-C1), 168.5 (quat., d-Hiv-C1), 83.3 (CH, d-Hiv-C2), 56.3 (CH, Leu-C2), 39.7 (CH₂, Leu-C3), 30.5 (CH, d-Hiv-C3), 29.9 (CH₂, Leu-NC), 25.0 (CH, Leu-C4), 23.1, 22.1 (2 x CH₃, Leu-C5), 19.9, 18.6 (2 x CH₃, d-Hiv-C4); IR (neat/cm⁻¹): 2957, 2937, 2875, 1750, 1655, 1468, 1369, 1175, 1147, 1001; HRMS (ESI): found 455.3113 (M+H)+ C₂₈H₄₃N₂O₆ requires 455.3116 \( \Delta = 0.6 \) ppm.

5.5.2 Method B (Flow)

According to General Method 2, a solution of protected precursor 8b (0.033 g, 0.05 mmol) in methanol (5 mL) and was hydrogenated to give the acid and then the nitrogen acid deprotected (reaction time = 5 h 35 min) and the resultant crude product macrocyclised in flow. The crude product was purified using preparative TLC using toluene:ethyl acetate (1:1) as eluent to afford the title compound 9b (0.020 g, 0.043 mmol, 86%) as a colourless solid, which was spectroscopically identical to that reported using method A.

5.6 -[Me-Leu-d-Hiv-Me-Leu-d-Hiv-Me-Leu-d-Hiv]- 1b (enniatin C)

![Enniatin C structure]

5.6.1 Method A (Batch)

Boc-Me-Leu-d-Hiv-Me-Leu-d-Hiv-Me-Leu-d-Hiv-OBn 10b (0.23 g, 0.26 mmol) was taken up in methanol (30 mL) and this solution was hydrogenated using an H-cube® Pro (ThalesNano) with a 10% Pd/C CatCart®. The pump was run at 1 mL.min⁻¹ using dichloromethane with the temperature set to 60 °C and the pressure to 6 bar. The solvent was then removed in vacuo and the crude acid was taken up in dioxane (7 mL) and anhydrous HCl (7 mL, 4 M in dioxane) was added. The reaction mixture was stirred at room temperature for 6 h before the solvent was removed in vacuo. The crude was then taken up successively in ethanol (2 x 7 mL) and methanol (7 mL) removing the solvent in vacuo after each addition. The resultant HCl salt was taken up in dichloromethane (20 mL) and was cooled to 0 °C before Ghosez reagent (0.038 mL, 0.29 mmol) was added and the reaction mixture was stirred at this temperature for 30 min. Diisopropylethylamine (0.16 mL, 0.94 mmol) was added and the reaction mixture was warmed to room temperature and stirred for 18 h before the solvent was removed in vacuo. The resultant oil was taken up in aqueous HCl (20 mL, 1M) and was extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with water (20 mL), saturated NaHCO₃ solution (20 mL) and saturated NaCl solution (20 mL) before drying over MgSO₄ and removing the solvent in vacuo. The resulting crude oil was purified using flash column chromatography using 1:2 40-60 petroleum ether:ethyl acetate as eluent to afford the title compound 1b (0.075 g, 0.11 mmol, 42%) as a colourless solid.

\( R_f = 0.13 \) [40-60 petroleum ether:ethyl acetate= 1:2]; \( m_p = 166 – 167 ^\circ C \) (literature = 129 \(^\circ C\)\(^{[12]}\)); \( \alpha_o^{278} = -21.8 \) (c 1.00, CHCl₃) literature\(^{[12]}\) \( \alpha_o^{20.0} = -24 \) (c 0.1, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ = 5.25 (dd, J = 11.3, 4.9 Hz, 1H, Leu-C2H), 4.93 (d, J = 8.6 Hz, 1H, d-Hiv-C2H), 3.09 (s, 3H, Leu-NCH₃), 2.30 –
1.72 (ddd, J = 15.3, 11.3, 4.3 Hz, 1H, Leu-C3H), 1.65 (ddd, J = 14.3, 10.0, 4.9 Hz, 1H, Leu-C3H), 1.53 – 1.38 (m, 1H, Leu-C4H), 1.02 – 0.86 (m, 12H, d-Hiv-C4H3 and Leu-C5H3); 13C NMR (151 MHz, CDCl3) δ = 171.0 (quint., Leu-C1), 169.9 (quat., d-Hiv-C1), 75.6 (CH, d-Hiv-C2), 54.4 (CH, Leu-C2), 37.6 (CH2, Leu-C3), 31.8 (CH3, Leu-NC), 30.1 (CH, d-Hiv-C3), 25.3 (CH, Leu-C4), 23.5, 21.3 (2 x CH3, Leu-C5), 18.8, 18.2 (2 x CH3, d-Hiv-C4); IR (neat/cm⁻¹): 2965, 2929, 2869, 1746, 1662, 1470, 1413, 1370, 1266, 1178, 1129, 1010; HRMS (ESI): found 682.4635 (M+H)+ C36H64N3O9 requires 682.4637 Δ = 0.33 ppm.

5.6.2 Method B (Flow)
According to General Method 2, a solution of protected precursor 10b (0.044 g, 0.05 mmol) in methanol (5 mL) was hydrogenated to give the acid and then the nitrogen acid deprotected (reaction time = 6 h) and the resultant crude product macrocyclised in flow. The resulting crude oil was purified using flash column chromatography using 1:2 40-60 petroleum ether:ethyl acetate as eluent to afford the title compound 1b (0.029 g, 0.042 mmol, 84%) as a colourless solid, which was spectroscopically identical to that reported using method A.


5.7.1 Method A (Batch)
Hydrogen gas was bubbled through a solution of Boc-Me-Leu-d-Hiv-Me-Leu-d-Hiv-Me-Leu-d-Hiv-Me-Leu-d-Hiv-OBn 11b (102 mg, 91 µmol) and palladium (10 mg, 10% on charcoal, 9.1 µmol) in tetrahydrofuran (2 mL) for 3 min at room temperature before the mixture was stirred under a hydrogen atmosphere for 6 h. The reaction mixture was filtered through celite, eluting with ethyl acetate (30 mL). The solvent was removed in vacuo from the filtrate to afford crude acid as a colourless oil that was taken up in dioxane (2 mL).

HCl (2 mL, 4M in dioxane) was added and the resulting mixture was stirred for 5 h. The solvent was removed in vacuo and the residue was taken up sequentially in ethanol (2 x 5 mL) and methanol (5 mL) removing the solvent in vacuo after each addition to afford the deprotected linear precursor. This was taken up in dichloromethane (20 mL) and cooled to 0 °C before Ghosez reagent (13 µL, 100 µmol) was added. The resulting mixture was stirred for 30 min at 0 °C before N,N-diisopropylethylamine (55 µL, 326 µmol) was added and the reaction mixture was warmed to room temperature and stirred for 18 h. The solvent was removed in vacuo and the residue was taken up in diethyl ether (60 mL) and aqueous HCl (60 mL, 1M). The aqueous layer was extracted with diethyl ether (3 x 30 mL) and the combined organic layers were washed with water (100 mL), saturated NaHCO₃ solution (100 mL) and saturated NaCl solution (100 mL) before drying over MgSO₄ and removing the solvent in vacuo. The crude product was purified via flash column chromatography using 40-60 petroleum ether:ethyl acetate (3:1 → 2:1) as eluent followed by preparatory TLC using 40-60 petroleum ether:ethyl acetate (2:1) as eluent to afford the title compound 2b (56 mg, 61 µmol, 67%) as a colourless solid. The δRᶠ = 0.17 [40-60 petroleum ether:ethyl acetate= 2:1]; mp = 100 –
101 °C; $\alpha_0^{24.6} = -56.3$ (c 3.3 CHCl$_3$) literature$^{[14]}$ $\alpha_0^{22.0} = -69$ (c 3.3, CHCl$_3$); $^1$H NMR (500 MHz, $d_6$-toluene, T = 363 °K) $\delta$ = 5.84 (br. s, 1H, Leu-C2H), 5.37 (d, $J = 7.1$ Hz, 1H, d-Hiv-C2H), 2.83 (s, 3H, Leu-NCH$_3$), 2.35 (br. s, 1H, d-Hiv-C3H), 1.96 – 1.81 (m, 1H, Leu-C3H), 1.75 – 1.59 (m, 1H, Leu-C3H), 1.51 (s, 1H, Leu-C4H), 1.01 – 0.85 (m, 12H, d-Hiv-C4H$_3$ and Leu-C5H$_3$); $^{13}$C NMR (126 MHz, $d_6$-toluene, T = 363 °K) $\delta$ = 171.5 (quat., Leu-C1), 169.7 (quat., d-Hiv-C1), 75.5 (CH, d-Hiv-C2), 55.4 (CH, Leu-C2), 37.9 (CH$_2$, Leu-C3), 31.1 (CH$_3$, Leu-NCH$_3$), 30.9 (CH, d-Hiv-C3), 26.0 (CH, Leu-C4), 23.7, 22.1 (2 x CH$_3$, d-Hiv-C4), 19.1, 18.4 (2 x CH$_3$, Leu-C5); IR (neat/cm$^{-1}$): 2959, 2873, 1740, 1660, 1469, 1412, 1388, 1262, 1191, 1125, 1087, 1009, 758; HRMS (ESI): found 931.6010 (M+Na)$^+$ C$_{48}$H$_{63}$O$_{12}$N$_4$Na requires 931.5983 $\Delta = 2$ ppm.

5.7.2 Method B (Flow)

According to General Method 2, a solution of protected precursor 11b (0.056 g, 0.05 mmol) in methanol (5 mL) and was hydrogenated to give the acid and then the nitrogen acid deprotected (reaction time = 6 h 5 min) and the resultant crude product macrocycised in flow. The crude product was purified using flash column chromatography using toluene:ethyl acetate (2:1) as eluent to afford the title compound 2b (0.042 g, 0.046 mmol, 93%) as a colourless solid, which was spectroscopically identical to that reported using method A.

6 References


7 NMR Spectra
16
CDCl₃
DEPT 135
151 MHz

16
CDCl₃
COSY
600 MHz
$^{1}$H NMR
400 MHz
403 K

$^{13}$C NMR
101 MHz
403 K
10a
d$_2$-DMSO
$^1$H NMR
500 MHz
393 K

$^{13}$C NMR
126 MHz
393 K
11a
d_p-DMSO
HSQC
500/126 MHz
393 K

11a
d_p-DMSO
HMBC
500/126 MHz
393 K
beauvericin 1a
CDCl₃
DEPT 135
151 MHz
2a
d$_5$-Toluene
$^1$H NMR
500 MHz
363 K

2a
d$_5$-Toluene
$^{13}$C NMR
126 MHz
363 K
6b
d$_6$-DMSO
DEPT 135
126 MHz
393 K

6b
d$_6$-DMSO
COSY
500 MHz
393 K
$^1$H NMR
500 MHz
393 K

$^1$C NMR
126 MHz
393 K
10b
d-DMSO
DEPT 135
126 MHz
393 K

10b
d-DMSO
COSY
500 MHz
393 K