

Supplementary Information

Synthetic procedures

***N*-tert-butoxycarbonyl L-prolyl L-proline methyl ester (1) METHOD 1** *N*-tert-butoxycarbonyl L-proline (99.69 g, 0.4631 mol) was dissolved in CH₂Cl₂ (800 mL). The solution was cooled to 0°C (icebath) and triethylamine (51.50 g, 0.5089 mol) added. Pivaloyl chloride (61.45 g, 0.5096 mol) was added dropwise, resulting in the precipitation of a white solid. The reaction mixture was stirred for 1 hour at 0°C before triethylamine (102.99 g, 1.018 mol) was added. Next, L-proline methyl ester hydrochloride (76.87 g, 0.4641 mol) was added in small portions. The icebath was removed and the reaction mixture stirred at room temperature for 17 hours. The volume was increased by addition of CH₂Cl₂ (2 L) and the solution washed with 30% (w/w) aqueous citric acid (3 × 1400 mL), saturated NaHCO₃ solution (3 × 1400 mL) and saturated brine (2 × 1400 mL). The solution was dried with anhydrous MgSO₄ and the solvent evaporated affording the title compound as a clear oil (129.14 g, 85%) with spectral characteristics in accordance with literature data.¹

METHOD 2 L-proline methyl ester (1.82 g, 14.1 mmol) was dissolved in CH₂Cl₂ (15 mL). *N*-tert-butoxycarbonyl L-proline (3.49 g, 16.2 mmol) dissolved in CH₂Cl₂ (10 mL) was added and the solution cooled to 0°C (icebath). HOBt hydrate (2.16 g, 14.1 mmol) and EDC hydrochloride (2.97 g, 15.5 mmol) were dissolved in CH₂Cl₂ (15 mL) and added dropwise. The reaction mixture was stirred for 16 hours at room temperature before being diluted with CH₂Cl₂ (60 mL) and washed with 2M aqueous H₂SO₄ (3 × 25 mL). The aqueous phase formed an emulsion which was extracted with CH₂Cl₂ (50 mL). The combined organic fractions were washed with 7.5% (w/w) aqueous K₂CO₃ (3 × 25 mL) and saturated brine (25

mL). The solution was dried with anhydrous MgSO_4 and the solvent evaporated affording the title compound as a very slightly yellow oil (3.79 g, 82%) with spectral characteristics in accordance with published data¹; δ_{H} (200 MHz; d_6 -DMSO) 4.56-4.31 (2H, m, $\text{C}^{\alpha}\text{H}(\text{Pro}_1)/\text{C}^{\alpha}\text{H}(\text{Pro}_2)$), 3.78-3.28 (4H, m, $\text{C}^{\delta}\text{H}_2(\text{Pro}_1)/\text{C}^{\delta}\text{H}_2(\text{Pro}_2)$), 3.67 (3H, s, OCH_3 , rotomer 1), 3.65 (3H, s, OCH_3 , rotomer 2), 2.26-1.65 (8H, m, CH_2), 1.40/1.34/1.22 (9H, s, $(\text{CH}_3)_3$, all rotomers); δ_{C} (75 MHz; CDCl_3) 172.8, 172.5, 171.5, 171.0, 154.4, 153.6, 79.3, 58.5, 57.6, 57.5, 53.3, 52.0, 51.9, 46.7, 46.5, 46.3, 29.8, 28.9, 28.7, 28.6, 28.3, 28.2, 24.9, 24.8, 23.9, 23.4

***N*-tert-butoxycarbonyl L-prolyl L-proline (2)** *N*-tert-butoxycarbonyl L-prolyl L-proline methyl ester **1** (4.90 g, 15.0 mmol) was dissolved in THF (100 mL) and the solution cooled to 0°C (icebath). LiOH monohydrate (0.692 g, 16.5 mmol) was dissolved in de-ionized H_2O (50 mL) and the solution cooled to 0°C before being added dropwise to the solution of **1** over 15 min. The reaction mixture was stirred for an additional 3 h 30 min before solid NaHCO_3 (2.52 g, 30.0 mmol) was added. Stirring was continued for 20 minutes and the THF evaporated. The solution was diluted with H_2O (50 mL), washed with Et_2O (2×50 mL) and acidified to pH 2 by addition of 2M aqueous H_2SO_4 . The resulting solution was extracted with CH_2Cl_2 (300 mL + 4×100 mL). The combined organic extracts were dried with anhydrous MgSO_4 and the solvent evaporated affording the title compound as a white solid (4.12 g, 88%) with spectral characteristics in accordance with literature data²; δ_{H} (300 MHz; d_6 -DMSO) 12.41 (1H, br s, COOH), 4.45-4.21 (2H, m, $\text{C}^{\alpha}\text{H}(\text{Pro}_1)/\text{C}^{\alpha}\text{H}(\text{Pro}_2)$), 3.73-3.13 (4H, m, $\text{C}^{\delta}\text{H}_2(\text{Pro}_1)/\text{C}^{\delta}\text{H}_2(\text{Pro}_2)$), 2.29-1.61 (8H, m, CH_2), 1.37/1.30/1.16 (9H, s, $(\text{CH}_3)_3$, all rotomers); δ_{C} (75 MHz; CDCl_3) 173.8, 173.5, 173.3, 173.0, 154.6, 153.6, 79.9, 59.6, 59.5, 57.7, 57.6, 47.1, 47.0, 46.9, 46.7, 30.0, 29.2, 28.4, 28.3, 27.7, 27.5, 25.0, 24.2, 23.6; m/z (ESI) 335.1595 ($[\text{M}+\text{Na}]^+$; $\text{C}_{15}\text{H}_{24}\text{N}_2\text{O}_5\text{Na}$ requires 335.1582)

N-tert-butoxycarbonyl O-allyl L-serine (3) *N-tert*-butoxycarbonyl L-serine (27.04 g, 0.1318 mol) was dissolved in DMF (220 mL). The solution was cooled to 0°C (icebath) before sodium hydride (60% dispersion in mineral oil, 11.59 g, 0.2898 mol) was added slowly under stirring. After gas evolution had ceased allyl bromide (17.54 g, 0.1450 mol) was added dropwise and the reaction mixture stirred for 18 hours at room temperature. The solvent was evaporated and the residue dissolved in H₂O (500 mL). The solution was washed with Et₂O (2 × 250 mL), cooled to 0°C (icebath) and acidified to pH 2.5 by addition of 2M aqueous H₂SO₄. The resulting solution/suspension was extracted with EtOAc (5 × 250 mL). The combined organic extracts were dried with anhydrous MgSO₄ and the solvent evaporated overnight affording the title compound as a slightly yellow-orange viscous liquid (26.86 g, 83%) with spectral characteristics in accordance with published data;^{3,4} δ_{H} (200 MHz; d₆-DMSO) 12.52 (1H, br s, COOH), 6.84 (1H, d, *J* 8, NH), 5.81 (1H, ddt, *J* 5, 10 and 17, CH=CH₂), 5.20 (1H, ddd, *J* 1, 3 and 17, CH=CHH), 5.09 (1H, ddd, *J* 1, 3 and 10, CH=CHH), 4.10 (1H, dt, *J* 5 and 8, C^{*α*}H), 3.96-3.92 (2H, dt, *J* 1 and 5, CH₂CH=CH₂), 3.56 (2H, d, *J* 5, CH₂), 1.34 (9H, s, (CH₃)₃); δ_{C} (75 MHz; d₆-DMSO) 171.9, 155.3, 134.8, 116.5, 78.1, 71.0, 69.1, 53.7, 28.1; *m/z* (ESI) 268.0 ([M+Na]⁺)

WARNING: As explosions are known to have occurred when using sodium hydride in DMF on pilot plant scale this is not an ideal method for synthesis of large amounts.^{5,6} However, mainly unreacted starting material was isolated using otherwise identical conditions in THF.

N-tert-butoxycarbonyl L-valyl L-valine methyl ester (4) *N-tert*-butoxycarbonyl L-valine (28.36 g, 0.1305 mol) was dissolved in DMF (120 mL) and the solution cooled to 0°C (icebath). L-valine methyl ester hydrochloride (21.88 g, 0.1305 mol) was suspended in DMF (60 mL) and *N,N*-diisopropylethyl amine (16.87 g, 0.1305 mol) added. The resulting suspension was added to the solution of *N-tert*-butoxycarbonyl L-valine in one portion. HOBt

hydrate (19.99 g, 0.1305 mol) and EDC hydrochloride (27.52 g, 0.1436 mol) were added together with additional DMF (80 mL). After 1 hour the icebath was removed and the reaction mixture stirred for 20 hours. The solvent was evaporated and the residue taken up in EtOAc (600 mL) and washed with 2 M aqueous H₂SO₄ (3 × 250 mL), 7.5% (w/w) aqueous K₂CO₃ (3 × 250 mL) and saturated brine (200 mL). The solution was dried with anhydrous MgSO₄ and the solvent evaporated affording the title compound as a white solid (37.50 g, 87%) with spectral characteristics in accordance with literature data²; δ_{H} (200 MHz; d₆-DMSO) 7.97 (1H, d, *J* 8, NH(Val₂)), 6.68 (1H, d, *J* 9, NH(Val₁)), 4.18 (1H, dd, *J* 6 and 9, C ^{α} H(Val₁)), 3.86 (1H, dd, *J* 8 and 8, C ^{α} H(Val₂)), 3.61 (3H, s, OCH₃), 2.12-1.83 (2H, m, CH(CH₃)₃), 1.37 (9H, s, (CH₃)₃), 0.91-0.80 (12H, m, CH₃); δ_{C} (50 MHz; d₆-DMSO) 171.7, 171.7, 155.3, 77.9, 59.4, 57.2, 51.5, 30.2, 29.8, 28.0, 19.0, 18.8, 18.1, 18.1; *m/z* (ESI) 353.2 ([M+Na]⁺)

***N*-tert-butoxycarbonyl α,α' -dimethylglycyl L-valine methyl ester (5)** *N*-tert-butoxycarbonyl α,α -dimethylglycine (25.10 g, 0.1235 mol) was dissolved in DMF (150 mL). L-valine methyl ester hydrochloride (20.70 g, 0.1235 mol) and *N,N*-diisopropylethyl amine (15.96 g, 0.1235 mol) were dissolved in DMF (30 mL) and added. HOBt hydrate (18.91 g, 0.1235 mol) and EDC hydrochloride (26.05 g, 0.1359 mol) were added together with additional DMF (70 mL). The reaction mixture was stirred for 47 hours before the solvent was evaporated and the residue taken up in EtOAc (600 mL). The solution was washed with 2 M aqueous H₂SO₄ (3 × 250 mL), 7.5 % (w/w) aqueous K₂CO₃ (3 × 250 mL) and saturated brine (250 mL). The solution was dried with anhydrous MgSO₄ and the solvent evaporated affording the title compound as a white solid (37.07 g, 95%) with spectral characteristics in accordance with literature data⁷; δ_{H} (200 MHz; d₆-DMSO) 7.38 (1H, d, *J* 8, NH(Val)), 6.93 (1H, br s, NH(Aib)), 4.21-4.14 (1H, m, C ^{α} H(Val)), 3.63 (3H, s, OCH₃), 2.13-1.97 (1H, m, CH(CH₃)₂), 1.37 (9H, s, (CH₃)₃), 1.32 (3H, s, CH₃(Aib)), 1.29 (3H, s, CH₃(Aib)), 0.85 (3H, d,

J 7, $\text{CH}_3(\text{Val})$), 0.84 (3H, d, J 7, $\text{CH}_3(\text{Val})$); δ_{C} (75 MHz; d_6 -DMSO) 174.4, 171.9, 154.1, 78.0, 57.1, 55.7, 51.6, 30.2, 28.0, 25.3, 24.6, 18.8, 17.9

***N*-tert-butoxycarbonyl glycyl glycine methyl ester (19)** *N*-tert-butoxycarbonyl glycine (10.57 g, 60.33 mmol) and glycine methyl ester hydrochloride (7.58 g, 60.4 mmol) were dissolved in DMF (60 mL). *N,N*-diisopropylethyl amine (7.80 g, 60.4 mmol) and HOBt hydrate (9.24 g, 60.3 mmol) dissolved in DMF (40 mL) were added. The solution was cooled to 0°C (icebath) and EDC hydrochloride (12.72 g, 66.35 mmol) added in small portions. The reaction mixture was stirred for 15 hours before the solvent was evaporated. The residue was taken up in EtOAc (300 mL) and washed with 2 M aqueous H_2SO_4 (3×50 mL), 7.5 % (w/w) aqueous K_2CO_3 (3×50 mL) and saturated brine (50 mL). The solution was dried with anhydrous MgSO_4 and the solvent evaporated affording the title compound as a clear liquid (10.34 g, 70%) with spectral characteristics in accordance with literature data⁸; δ_{H} (300 MHz; d_6 -DMSO) 8.17 (1H, t, J 6, $\text{NH}(\text{Gly}_2)$), 6.97 (1H, t, J 6, $\text{NH}(\text{Gly}_1)$), 3.85 (2H, d, J 6, $\text{C}^\alpha\text{H}_2(\text{Gly}_2)$), 3.62 (3H, s, OCH_3), 3.57 (2H, d, J 6, $\text{C}^\alpha\text{H}_2(\text{Gly}_1)$), 1.38 (9H, s, $(\text{CH}_3)_3$); δ_{C} (75 MHz; d_6 -DMSO) 170.2, 169.9, 155.7, 78.0, 51.6, 42.9, 40.4, 28.1; m/z (ESI) 269.1110 ($[\text{M}+\text{Na}]^+$; $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_5$ requires 269.1113)

Glycyl glycine methyl ester trifluoroacetate (21) *N*-tert-butoxycarbonyl glycyl glycine methyl ester **19** (9.20 g, 37.4 mmol) was dissolved in a 50% (v/v) solution of TFA in CH_2Cl_2 (120 mL) at room temperature. The reaction mixture was stirred for 1 h 30 min before the solvent and bulk of excess TFA were evaporated. The residue was washed with Et_2O (3×50 mL) and CH_2Cl_2 (50 mL) and dried affording the title compound as a slightly reddish viscous liquid in quantitative yield and with spectral characteristics in accordance with literature data.⁹; δ_{H} (300 MHz; d_6 -DMSO) 8.91 (1H, t, J 6, NH), 8.20 (3H, br s, NH_3^+), 3.96 (2H, d, J 6,

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$C^{\alpha}H_2(\text{Gly}_2)$), 3.67-3.59 (5H, m, $C^{\alpha}H_2(\text{Gly}_1)/OCH_3$); ^{13}C -NMR (75 MHz, d_6 -DMSO): δ_{C}
169.9, 166.7, 158.7 (q, J_{CF} 33), 116.4 (q, J_{CF} 294), 54.9, 51.8, 40.6; m/z (ESI) 147.0769 (M^+ ;
 $\text{C}_5\text{H}_{11}\text{N}_2\text{O}_3$ requires 147.0769)

ROESY spectra

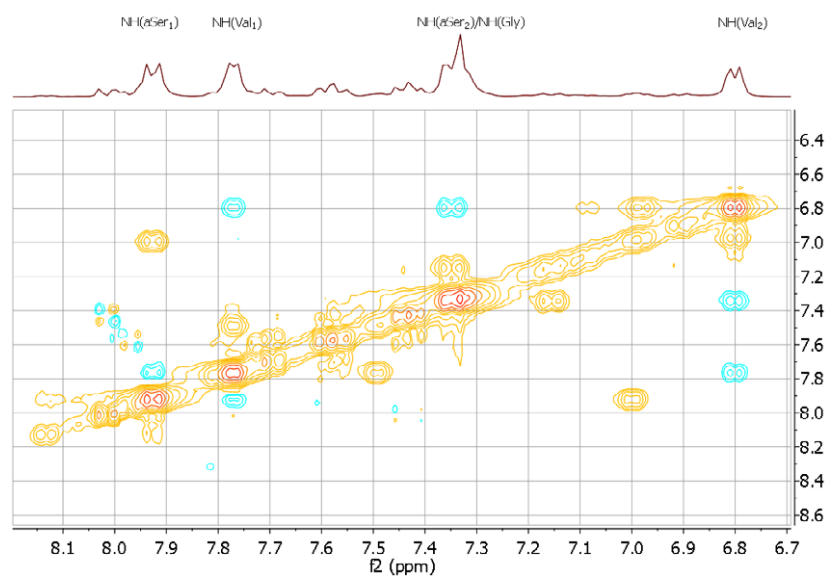


Fig. 1 Partial ROESY spectrum of **29** displaying NH(*i*)→NH(*i*+1) ROEs.

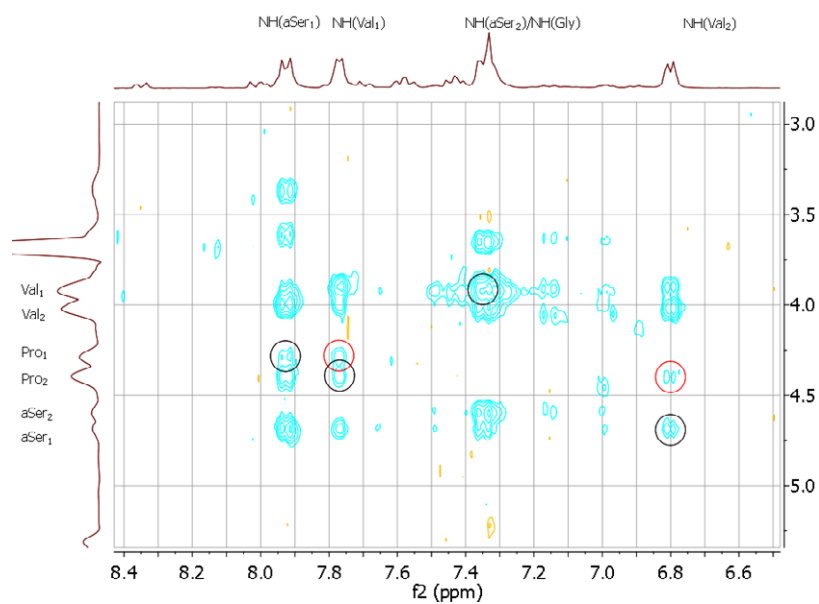


Fig. 2 Partial ROESY spectrum of **29** displaying C^αH(*i*)→NH(*i*+2) and C^αH(*i*)→NH(*i*+3) ROEs (black and red circles, respectively).

CD spectra

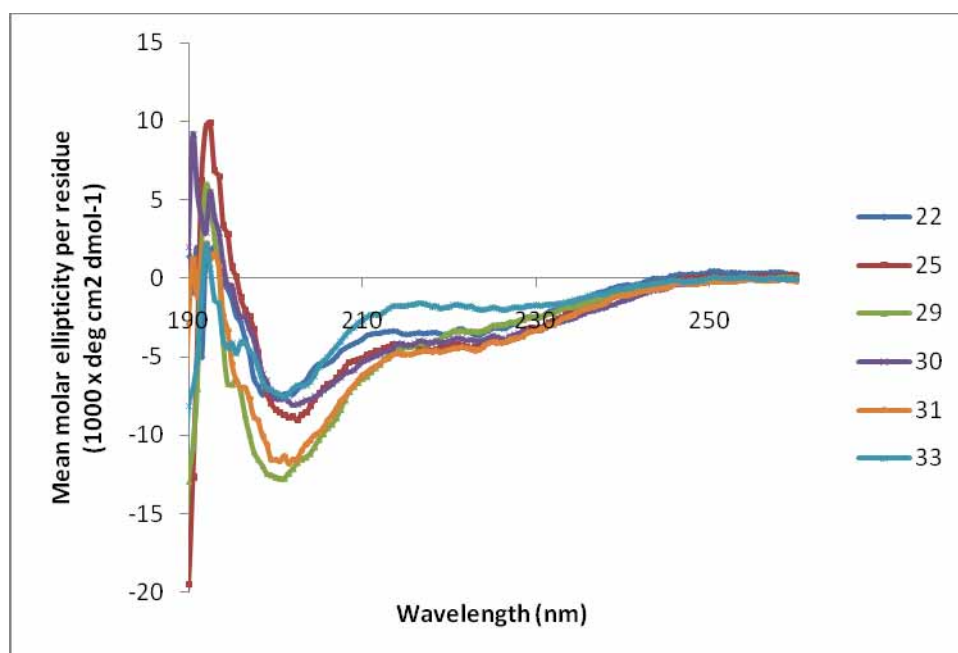


Fig. 3 CD spectra of selected penta- and heptapeptides in MeOH.

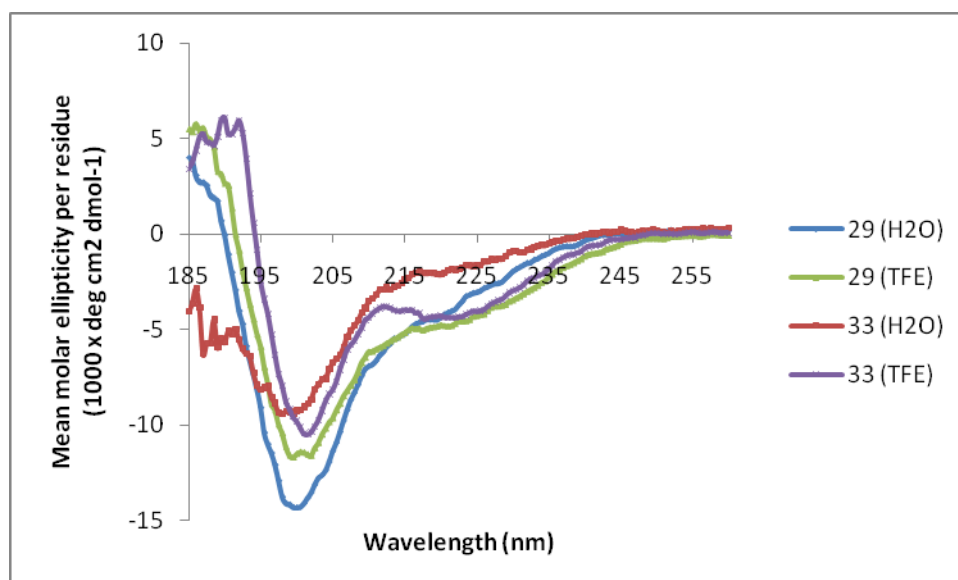


Fig. 4 Comparison of the CD spectra of the heptapeptides **29** and **33** in TFE and H₂O/MeOH (39:1).

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