Supporting Information

Synthesis of Functionalized, Unsymmetrical 1,3,4,6-Tetrasubstituted 2,5-Diketopiperazines

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General information:

^1H - and ^{13}C -NMR spectra were recorded in CDCl_3 if not otherwise noted at 400 and 100 MHz, respectively. The reactions were monitored by thin-layer chromatography (TLC), on silica plated aluminum sheets (Silica gel 60 F254), detecting spots by UV (254 nm). In case of flash chromatography, silica gel 60 (230-400 mesh) was used. Melting points are uncorrected. Microwave reactions were performed using a Biotage Initiator instrument. In the microwave experiments the temperature was measured using an IR-sensor.

Synthesis of *N*-substituted Amino Acid Methyl Esters***N*-Methyl-L-norleucine methyl ester (1)**

N-Methyl-L-NleOH (0.15 g; 1.1 mmol) was dissolved in HCl-saturated CH_3OH (3 mL) and refluxed overnight. The solvent was evaporated to give pure **1** as white crystals (0.16 g; 93%). Mp 92-94 °C. $[\alpha]_{\text{D}}^{25} +27$ (*c* 1, CH_3OH). ^1H NMR (CD_3OD) δ 4.06 (dd, $J = 6.6, 5.5$ Hz, 1H), 3.86 (s, 3H), 2.73 (s, 3H), 2.00-1.90 (m, 2H), 1.43-1.34 (m, 4H), 0.94 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR (CD_3OD) δ 169.0, 60.7, 52.4, 31.1, 28.6, 26.3, 21.9, 12.7. Anal. Calcd for $\text{C}_8\text{H}_{18}\text{ClNO}_2$: C, 49.10; H, 9.27; N, 7.16; Found C, 48.9; H, 9.2; N, 7.1.

***N*-Benzyl-L-norleucine methyl ester (3)**

L-NleOMe HCl (0.15 g; 1.1 mmol) and triethylamine (0.22 mL; 1.6 mmol) were dissolved in CH_2Cl_2 (20 mL) followed by addition of benzyl bromide (0.19 mL; 1.6 mmol) and heated to 40 °C overnight. The reaction mixture was concentrated *in vacuo* and the crude product was purified by flash chromatography using hexane: CH_3OH : CH_2Cl_2 (6 : 1 : 5) as eluent to give pure **3** as a colourless oil (0.21 g; 86%).

$[\alpha]_D -36.5$ (*c* 1, CH₂Cl₂). ¹H NMR (CD₃OD) δ 7.32-7.26 (m, 5H), 3.74 (d, *J* = 13.2 Hz, 1H), 3.68 (s, 3H), 3.59 (d, *J* = 13.2 Hz, 1H), 3.23 (t, *J* = 6.8 Hz, 1H), 1.61 (t, *J* = 7.0 Hz, 2H), 1.33-1.23 (m, 4H), 0.87 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CD₃OD) δ 175.6, 139.3, 128.3, 128.2, 127.0, 60.3, 51.6, 50.9, 32.7, 27.7, 22.3, 13.1. Anal. Calcd for C₁₄H₂₁NO₂: C, 71.46; H, 8.99; N, 5.95; Found C, 71.4; H, 9.1; N, 6.1.

***N*-Propargyl-L-norleucine methyl ester (4)**

L-NleOMe HCl (0.15 g; 1.1 mmol), triethylamine (0.22 mL; 1.6 mmol), and propargyl bromide (0.14 mL; 1.6 mmol) were reacted in CH₂Cl₂ (10 mL) as described for **3** to give pure **4** as a colourless oil (0.14 g; 74%).

$[\alpha]_D -27.5$ (*c* 1, CH₂Cl₂). ¹H NMR (CDCl₃) δ 3.58 (s, 3H), 3.33-3.23 (m, 3H), 2.09 (t, *J* = 2.6 Hz, 1H), 1.55-1.43 (m, 2H), 1.22-1.12 (m, 4H), 1.18 (t, *J* = 7.5 Hz, 3H). ¹³C NMR (CDCl₃) δ 175.3, 81.4, 71.6, 59.9, 51.6, 36.8, 33.0, 27.7, 22.4, 13.8. Anal. Calcd for C₁₀H₁₇NO₂: C, 65.54; H, 9.35; N, 7.64; Found C, 65.7; H, 9.3; N, 7.8.

***N*-Propargyl-L-serine-*O*-benzyl methyl ester (5)**

Compound **2** (0.20 g; 0.81 mmol) was dissolved in DMF (10 mL) and cooled to 0 °C followed by addition of triethylamine (0.17 mL; 1.2 mmol) and propargyl bromide (0.13 mL; 1.2 mmol) and the reaction mixture was run at rt for 24 h. The reaction mixture was diluted with water (10 mL) and extracted with ether (3×20 mL), the organic layers were dried (Na₂SO₄) and concentrated *in vacuo*. The crude product was purified by flash chromatography using EtOAc:hexane (3 : 2) as eluent to give pure **5** as a colourless oil (0.16 g; 78%).

$[\alpha]_D -16.5$ (*c* 2, CH₂Cl₂). ¹H NMR (CDCl₃) δ 7.34-7.24 (m, 5H), 4.54 (d, *J* = 12.1 Hz, 1H), 4.50 (d, *J* = 12.1 Hz, 1H), 3.70 (s, 3H), 3.69-3.66 (app s, 3H), 3.53 (dd, *J* = 16.9, 2.5 Hz, 1H), 3.43 (dd,

$J = 16.9, 2.5$ Hz, 1H), 2.20 (t, $J = 2.4$ Hz, 1H), 2.17 (s, 1H). ^{13}C NMR (CDCl_3) δ 171.3, 137.2, 128.5, 127.8, 127.8, 79.4, 73.3, 72.0, 70.7, 62.2, 51.8, 40.5. Anal. Calcd for $\text{C}_{14}\text{H}_{17}\text{NO}_3$: C, 68.00; H, 6.93; N, 5.66; Found C, 67.8; H, 6.9; N, 5.6.

Synthesis of Boc-Protected Dipeptide Methyl Esters

General procedure for peptide coupling reactions

The methyl ester of the C-terminal amino acid was dissolved in dry solvent (10 mL) followed by addition of NMM. The reaction mixture was stirred for 40 min at 0 °C whereupon either EDC, HOAt or HATU (if appropriate) and the Boc-protected N-terminal amino acid were added. The reaction was thereafter stirred for 3 h at 0 °C and then overnight at rt. The reaction mixture was diluted with CH_2Cl_2 and extracted with 10% aqueous citric acid. The organic layer was dried (MgSO_4), concentrated *in vacuo* and the crude product was purified by flash chromatography.

***N*-tert-Butoxycarbonyl-L-phenylalanyl-L-norleucine methyl ester (6)**

BocPheOH (0.20 g; 0.75 mmol), NleOMe (0.16 g; 0.90 mmol), NMM (99 μL ; 0.90 mmol), EDC (0.17 g; 0.90 mmol) were reacted in CH_2Cl_2 (15 mL). The crude product was purified using hexane: CH_3OH : CH_2Cl_2 (6 : 1 : 5) as eluent to give pure **6** as white crystals (0.27 g; 93%).

Mp 80-81 °C. $[\alpha]_{\text{D}} -3.0$ (c 1, CH_2Cl_2). ^1H NMR (CDCl_3) δ 7.29-7.18 (m, 5H), 6.42 (d, $J = 7.7$ Hz, 1H), 5.03 (d, $J = 7.2$ Hz, 1H), 4.53-4.48 (m, 1H), 4.39-4.31 (m, 1H), 3.81 (s, 3H), 3.08-3.00 (m, 2H), 1.80-1.71 (m, 1H), 1.63-1.54 (m, 1H), 1.39 (s, 9H), 1.29-1.13 (m, 4H), 0.84 (t, $J = 6.3$ Hz, 3H). ^{13}C NMR (CDCl_3) δ 172.5, 171.0, 155.4, 136.6, 129.44, 128.7, 127.0, 80.2, 55.71, 52.3, 52.3, 38.2, 32.2, 27.2, 22.3, 13.9. Anal. Calcd for $\text{C}_{21}\text{H}_{32}\text{N}_2\text{O}_5$: C, 64.26; H, 8.22; N, 7.14; Found C, 64.3; H, 8.3; N, 7.1.

***N*-tert-Butoxycarbonyl-L-phenylalaninyl-*N*-methyl-L-norleucine methyl ester (7)**

BocPheOH (0.15 g; 0.57 mmol), **1** (75 mg; 0.48 mmol), NMM (53 μ L; 0.48 mmol) and EDC (0.11 g; 0.57 mmol) were reacted in CH₂Cl₂ (5 mL). The crude product was purified using hexane:CH₃OH:CH₂Cl₂ (6 : 1 : 5) as eluent to give pure **7** as a colourless oil (0.17 g; 88%).

$[\alpha]_D$ -19.7 (*c* 1, CH₂Cl₂). ¹H NMR (CDCl₃) δ 7.18-7.10 (m, 5H), 5.42 (d, *J* = 9.1 Hz, 1H), 5.08 (dd, *J* = 10.8, 4.9 Hz, 1H), 4.76 (q, *J* = 7.3 Hz, 1H), 3.55 (s, 3H), 2.98 (dd, *J* = 13.5, 7.4 Hz, 1H), 2.81 (dd, *J* = 13.5, 6.2 Hz, 1H), 2.68 (s, 3H), 1.86-1.77 (m, 1H), 1.59-1.49 (m, 1H), 1.29 (s, 9H), 1.27-1.08 (m, 4H), 0.76 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CDCl₃) δ 172.8, 172.4, 155.2, 136.4, 126.70, 129.6, 128.3, 79.4, 56.2, 52.0, 51.7, 38.9, 31.1, 28.3, 27.9, 27.8, 22.2, 13.8. Anal. Calcd for C₂₂H₃₄N₂O₅: C, 65.00; H, 8.43; N, 6.89; Found C, 64.9; H, 8.3; N, 6.8.

***N*-tert-Butoxycarbonyl-L-phenylalaninyl-*N*-benzyl-L-norleucine methyl ester (8)**

BocPheOH (0.15 g; 0.57 mmol), **3** (0.26 g; 1.1 mmol), diisopropylethylamine (99 μ L; 0.57 mmol), EDC (0.11 g; 0.57 mmol) and HOAt (1.1 mL; 0.57 mmol) were reacted in CH₂Cl₂ (5 mL). The crude product was purified using hexane:CH₃OH:CH₂Cl₂ (6 : 1 : 5) as eluent to give pure **8** as a colourless oil (0.10 g; 54%).

$[\alpha]_D$ -25 (*c* 1, CHCl₃). ¹H NMR (CDCl₃) δ 7.30-7.03 (m, 10 H), 4.54-4.47 (m, 1H), 4.46-4.39 (m, 1H), 3.84 (d, *J* = 13.0 Hz, 1H), 3.57 (s, 3H), 3.57 (d, *J* = 13.0 Hz, 1H), 3.01-2.84 (m, 2H), 1.98-1.85 (m, 1H), 1.66-1.52 (m, 1H), 1.39 (s, 9H), 1.28-1.04 (m, 4H), 0.76 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CDCl₃) δ 173.2, 171.4, 155.0, 136.6, 136.5, 129.7, 128.7, 128.5, 127.7, 127.3, 126.8, 79.7, 58.5, 52.3, 51.9, 50.4, 39.7, 29.1, 28.6, 28.3, 22.4, 13.8. Anal. Calcd for C₂₈H₃₈N₂O₅: C, 69.68; H, 7.94; N, 5.80; Found C, 69.8; H, 8.1; N, 6.0.

***N*-tert-Butoxycarbonyl-L-phenylalaninyl-*N*-propargyl-L-norleucine methyl ester (9)**

BocPheOH (0.18 g; 0.66 mmol), **4** (0.10 g; 0.55 mmol) and EDC (0.13 g; 0.66 mmol) were reacted in CH₂Cl₂ (5 mL). The crude product was purified using hexane:CH₃OH:CH₂Cl₂ (6 : 1 : 5) as eluent to give pure **9** as a colourless oil (0.21 g; 87%).

[α]_D -22.1 (*c* 1, CH₂Cl₂). ¹H NMR (CDCl₃) δ 7.25-7.15 (m, 5H), 5.22 (d, *J* = 8.8 Hz, 1H), 5.03-4.96 (m, 1H), 4.94-4.86 (m, 1H), 4.28-4.21 (m, 1H), 3.86-3.77 (m, 1H), 3.64 (s, 3H), 3.12 (dd, *J* = 13.6, 7.0 Hz, 1H), 2.88 (dd, *J* = 13.6, 7.0 Hz, 1H), 2.26 (s, 1H), 1.96-1.84 (m, 1H), 1.75-1.64 (m, 1H), 1.34 (s, 9H), 1.36-1.21 (m, 4H), 0.83 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (CDCl₃) δ 173.1, 171.5, 155.2, 136.5, 129.6, 128.5, 126.9, 79.7, 78.8, 73.1, 56.9, 52.4, 52.1, 39.2, 34.8, 28.3, 28.4, 28.1, 22.3, 13.9. Anal. Calcd for C₂₄H₃₄N₂O₅: C, 66.95; H, 7.96; N, 6.51; Found C, 66.7; H, 7.9; N, 6.5.

***N*-tert-Butoxycarbonyl-L-4-bromophenylalaninyl-*N*-propargyl-*O*-benzyl-L-serine methyl ester (10)**

Boc-4-BrPheOH (0.20 g; 0.58 mmol), **5** (0.14 g; 0.58 mmol), HATU (0.11 g; 0.58 mmol), and DIPEA (0.20 mL; 1.2 mmol) were reacted in CH₂Cl₂ (10 mL) as described in the general procedure. The crude product was purified using EtOAc:hexane (3 : 2) as eluent to give pure **10** as a colourless, solid oil (0.24 g; 72%).

[α]_D -17.7 (*c* 1, CH₂Cl₂). ¹H NMR (CDCl₃) δ 7.34-7.24 (m, 8H), 7.07 (d, *J* = 7.3 Hz, 2H), 5.37 (d, *J* = 9.2 Hz, 1H), 5.05 (s, 1H), 4.95 (d, *J* = 7.3 Hz, 1H), 4.84 (app s, 1H), 4.54-4.31 (m, 2H), 3.92 (d, *J* = 4.4 Hz, 2H), 3.78-3.74 (m, 1H), 3.67 (s, 3H), 3.11-2.84 (m, 2H), 2.29 (s, 1H), 1.35 (s, 9H). ¹³C NMR (CDCl₃) δ 172.4, 169.5, 155.0, 137.7, 135.4, 131.5, 131.5, 128.5, 127.9, 127.8, 120.9, 79.8, 78.9, 73.4, 70.9, 68.3, 52.7, 52.6, 51.7, 38.6, 33.3, 28.4. Anal. Calcd for C₂₈H₃₃BrN₂O₆: C, 58.64; H, 5.80; N, 4.88; Found C, 58.6; H, 5.9; N, 4.6.

Synthesis of Dipeptide Methyl Ester Hydrochlorides

General procedure for Boc removal.

The Boc-protected dipeptide methyl ester was dissolved in HCl-saturated CH₃OH and stirred at rt for 3 h. The solvent was evaporated to give the dipeptide methyl ester as the corresponding hydrochloride salt.

L-Phenylalanyl-N-methyl-L-norleucine methyl ester hydrochloride (12)

Compound **7** (0.10 g; 0.25 mmol) and HCl-saturated CH₃OH (10 mL) gave pure **12** as sticky white crystals (75 mg; 89%).

$[\alpha]_D -12.2$ (*c* 1, CH₃OH). ¹H NMR (CD₃OD) δ 7.38-7.29 (m, 5H), 5.04-4.95 (m, 1H), 4.72 (t, *J* = 6.4 Hz, 1H), 3.71 (s, 3H), 3.26-3.12 (m, 2H), 2.83 (s, 3H), 2.01-1.90 (m, 1H), 1.79-1.68 (m, 1H), 1.41-1.23 (m, 4H), 0.91 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CD₃OD) δ 171.1, 169.2, 133.9, 129.8, 128.8, 127.6, 57.4, 57.1, 51.8, 36.7, 31.1, 27.9, 27.7, 22.1, 13.1. Anal. Calcd for C₁₇H₂₇ClN₂O₃: C, 59.55; H, 7.94; N, 8.17; Found C, 59.6; H, 7.9; N, 8.1.

L-Phenylalanyl-N-benzyl-L-norleucine methyl ester hydrochloride (13)

Compound **8** (0.10 g; 0.21 mmol) and HCl-saturated CH₃OH (5 mL) gave pure **13** as a colourless oil (78 mg; 90%).

$[\alpha]_D -32$ (*c* 1, CH₃OH). ¹H NMR (CD₃OD) δ 7.44-7.22 (m, 10H), 4.72 (app s, 2H), 4.51-4.43 (m, 1H), 4.36-4.29 (m, 1H), 3.69 (s, 3H), 3.27-3.11 (m, 2H), 2.02-1.91 (m, 1H), 1.61-1.54 (m, 1H), 1.21-1.10 (m, 4H), 0.82 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CD₃OD) δ 171.2, 169.3, 135.8, 133.8, 129.4, 129.0, 128.7, 128.0, 127.8, 127.2, 59.9, 52.2, 51.4, 50.8, 37.4, 28.9, 28.7, 22.0, 12.7. Anal. Calcd for C₂₃H₃₁ClN₂O₃: C, 65.94; H, 7.46; N, 6.69; Found C, 66.1; H, 7.4; N, 6.6.

L-Phenylalaninyl-N-propargyl-L-norleucine methyl ester hydrochloride (14)

Compound **9** (90 mg; 0.21 mmol) and HCl-saturated CH₃OH (5 mL) gave pure **14** as a colourless oil (68 mg; 89%).

$[\alpha]_D -60.0$ (*c* 1, CH₃OH). ¹H NMR (CD₃OD) δ 7.40-7.30 (m, 5H), 4.93-4.85 (m, 1H), 4.73 (t, *J* = 6.6 Hz, 1H), 4.22-4.14 (m, 1H), 4.95-4.87 (m, 1H), 3.72 (s, 3H), 3.36-3.26 (m, 1H), 3.25-3.13 (m, 1H), 3.00 (s, 1H), 2.05-1.96 (m, 1H), 1.89-1.77 (m, 1H), 1.40-1.30 (m, 4H), 0.91 (t, *J* = 6.4 Hz, 3H). ¹³C NMR (CD₃OD) δ 171.0, 169.2, 133.8, 129.5, 129.0, 127.8, 77.9, 72.0, 58.2, 52.3, 51.7, 37.0, 35.0, 28.3, 28.1, 22.1, 13.0. Anal. Calcd for C₁₉H₂₇ClN₂O₃: C, 62.20; H, 7.42; N, 7.64; Found C, 62.1; H, 7.4; N, 7.5.

Synthesis of N-Terminally Substituted Dipeptide Methyl Esters

General procedure for reductive amination reactions using sodium triacetoxyborohydride

Compound **11** and triethylamine were dissolved in 1,2-dichloroethane (15 mL) and stirred for 20 min. Thereafter the aldehyde was added and the reaction was stirred over night. Acetic acid was added and the mixture was stirred for 30 min whereupon sodium triacetoxyborohydride was added. The reaction was stirred for 2 h and diluted with 1,2-dichloroethane (20 mL) and extracted with brine (10 mL) and dried (Na₂SO₄), concentrated *in vacuo* and the crude product was purified by flash chromatography.

N-Benzyl-L-phenylalaninyl-L-norleucine methyl ester (15)

Compound **11** (0.41 g; 1.3 mmol), triethylamine (0.18 mL; 1.3 mmol), benzaldehyde (0.13 mL; 1.3 mmol), acetic acid (68 μ L; 1.3 mmol) and sodium triacetoxyborohydride (0.39 g; 1.8 mmol) were reacted as described in the general procedure. The crude product was purified using

hexane:CH₃OH:CH₂Cl₂ (6 : 1 : 5) as eluent. Pure **15** was isolated as a translucent oil (0.44 g; 88%).

[α]_D -10.2 (*c* 2, CH₃OH). ¹H NMR (CDCl₃) δ 7.88 (d, *J* = 8.4 Hz, 1H), 7.11-7.30 (m, 10 H), 4.65 (td, *J* = 8.2, 5.1 Hz, 1H), 3.81 (d, *J* = 12.8 Hz, 1H), 3.56 (d, *J* = 12.8 Hz, 1H), 3.42 (dd, *J* = 9.5, 4.0 Hz, 1H), 3.21 (dd, *J* = 13.9, 4.0 Hz, 1H), 2.75 (dd, *J* = 13.9, 9.5 Hz, 1H), 1.89-1.77 (m, 1H), 1.73-1.64 (m, 1H), 1.35-1.23 (m, 4H), 0.89 (t, *J* = 7.1 Hz, 1H). ¹³C NMR (CD₃OD) δ 173.4, 173.0, 139.2, 137.3, 129.2, 128.8, 128.6, 128.2, 127.3, 127.0, 63.2, 52.6, 52.3, 51.7, 39.9, 32.3, 27.5, 22.4, 14.0. Anal. Calcd for C₂₃H₃₀N₂O₃: C, 72.22; H, 7.91; N, 7.32; Found C, 71.9; H, 7.9; N, 7.4.

***N*-(*trans*-3-Phenyl-2-propenyl)-L-phenylalaninyl-L-norleucine methyl ester (16)**

Compound **11** (0.42 g; 1.3 mmol), triethylamine (0.18 mL; 1.3 mmol), cinnamic aldehyde (0.16 mL; 1.3 mmol), acetic acid (71 μ L; 1.3 mmol) and sodium triacetoxymethylborohydride (0.40 g; 1.9 mmol) were reacted as described in the general procedure. The crude product was purified using hexane:CH₃OH:CH₂Cl₂ (6 : 1 : 5) as eluent. Pure **16** was isolated as a yellowish oil (0.32 g; 60%).

[α]_D -19 (*c* 2, CH₃OH). ¹H NMR (CDCl₃) δ 7.81 (d, *J* = 8.4 Hz, 1H), 7.30-7.17 (m, 10H), 6.34 (d, *J* = 15.7 Hz, 1H), 6.02 (d, *J* = 15.7 Hz, 1H), 4.62 (dt, *J* = 8.2, 5.1 Hz, 1H), 3.68 (s, 3H), 3.45 (dd, *J* = 9.3, 3.8 Hz, 1H), 3.38 (dd, *J* = 4.4 Hz, 1H), 3.22 (dd, *J* = 9.3, 3.8 Hz, 1H), 3.21 (dd, *J* = 13.7, 9.3 Hz, 1H), 2.76 (dd, *J* = 13.7, 9.3 Hz, 1H), 1.87-1.77 (m, 1H), 1.74-1.62 (m, 1H), 1.33-1.22 (m, 4H), 0.88 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CDCl₃) δ 173.6, 173.0, 137.48, 136.89, 131.78, 129.26, 128.84, 128.55, 127.54, 127.36, 127.03, 126.39, 63.0, 52.25, 51.67, 50.36, 39.39, 32.25, 27.51, 22.39, 14.01. Anal. Calcd for C₂₅H₃₂N₂O₃: C, 73.50; H, 7.90; N, 6.86; Found C, 73.2; H, 8.0; N, 6.8.

***N*-(3-Cyclohexenylmethyl)-L-phenylalaninyl-L-norleucine methyl ester (17)**

Compound **11** (0.42 g; 1.3 mmol), triethylamine (0.18 mL; 1.3 mmol), 3-cyclohexene-1-carboxaldehyde (0.15 mL; 1.3 mmol), acetic acid (71 μ L; 1.3 mmol) and sodium triacetoxyborohydride (0.40 g; 1.9 mmol) were reacted as described in the general procedure. The crude product was purified using hexane:CH₃OH:CH₂Cl₂ (6 : 1 : 5) as eluent. Pure **17** was isolated as a yellowish oil (0.35 g; 70%).

$[\alpha]_D$ -35.2 (*c* 2, CH₃OH). ¹H NMR (CDCl₃) δ 7.74 (d, *J* = 8.1 Hz, 1H), 7.25-7.12 (m, 5H), 5.51 (m, 2H), 4.53 (dt, *J* = 8.2, 5.3 Hz, 1H), 3.63 (s, 3H), 1.92-1.83 (m, 2H), 1.81-1.72 (m, 1H), 1.63-1.56 (m, 1H), 1.56-1.46 (m, 3H), 1.29-1.14 (m, 5H), 1.10-1.02 (m, 1H), 0.82 (t, *J* = 7.1 Hz, 3H). ¹³C NMR (CDCl₃) δ 173.7, 172.8, 137.5, 129.1, 128.7, 127.1, 126.9, 126.0, 125.9, 64.1, 64.0, 54.7, 52.1, 39.3, 33.8, 32.2, 29.6, 27.4, 26.7, 24.9, 22.3, 13.9. Anal. Calcd for C₂₃H₃₄N₂O₃: C, 71.47; H, 8.87; N, 7.25; Found C, 71.6; H, 8.6; N, 7.4.

Synthesis of Trisubstituted 2,5-Diketopiperazines

General procedure for dipeptide cyclization.

The Boc-protected dipeptide methyl ester or the hydrochloride salt of the Boc-deprotected dipeptide methyl ester was added to water (4 mL). The Boc-deprotected dipeptide methyl ester was heated in the microwave at 200 °C for 10 min and the hydrochloride salt of the dipeptide methyl ester at 140 °C for 10 min in the presence of 2.0 eq of triethylamine. 50 mg of the starting material was used in each reaction. The reaction mixture was concentrated *in vacuo* and the crude product was purified by flash chromatography using EtOAc:hexane (3 : 2) as eluent. The isolated yields are shown in Table 2 in the main article.

c(L-phenylalanyl-N-methyl-L-norleuciny) (18)

Compound **12** (50 mg; 0.15 mmol) and triethylamine (42 μ L; 0.30 mmol) gave pure **18** as white crystals.

Mp 170-172 °C. $[\alpha]_D$ -18 (*c* 1, CH₃OH). ¹H NMR (CD₃OD) δ 7.31-7.16 (m, 5H), 6.22 (s, 1H), 4.32 (t, *J* = 4.6 Hz, 1H), 3.74-3.70 (m, 1H), 3.25 (dd, *J* = 13.7, 5.0 Hz, 1H), 2.97 (dd, *J* = 13.7, 5.0 Hz, 1H), 2.86 (s, 3H), 1.35-1.24 (m, 1H), 1.14-1.04 (m, 2H), 0.84-0.80 (m, 3H), 0.78 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD) δ 168.0, 166.1, 135.8, 130.2, 128.3, 127.1, 61.4, 56.3, 39.3, 31.8, 31.4, 26.7, 22.0, 12.8. Anal. Calcd for C₁₆H₂₂N₂O₂: C, 70.04; H, 8.08; N, 10.21; Found C, 70.0; H, 8.2; N, 10.25.

c(L-phenylalanyl-N-benzyl-L-norleuciny) (19)

Compound **13** (50 mg; 0.12 mmol) and triethylamine (34 μ L; 0.24 mmol) gave pure **19** as a colourless oil.

$[\alpha]_D$ -34.0 (*c* 1, CH₃OH). ¹H NMR (CD₃OD) δ 7.32-7.21 (m, 10 H), 4.97 (d, *J* = 15.0 Hz, 1H), 4.42 (t, *J* = 4.8 Hz, 1H), 4.22 (d, *J* = 15.0 Hz, 1H), 3.66-3.62 (m, 1H), 3.30 (dd, *J* = 13.7, 4.6 Hz, 1H), 3.05 (dd, *J* = 13.7, 4.6 Hz, 1H), 1.43-1.35 (m, 1H), 1.01-0.90 (m, 2H), 0.85-0.74 (m, 3H), 0.69 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD) δ 168.0, 166.6, 136.1, 135.8, 130.2, 128.5, 127.9, 127.5, 127.2, 59.2, 56.5, 47.6, 39.5, 31.4, 26.6, 21.9, 12.8. Anal. Calcd for C₂₂H₂₆N₂O₂: C, 75.40; H, 7.48; N, 7.99; Found C, 75.5; H, 7.5; N, 8.0.

c(4-Bromo-L-phenylalanyl-N-propargyl-O-benzyl-L-seriny) (21)

Compound **10** (50 mg; 87 μ mol) was suspended in H₂O (4 mL) and heated from 20 °C to 200 °C during 3 min and then at 200 °C for 10 min. The reaction mixture was concentrated *in vacuo* and

the crude product was purified by flash chromatography using CH₂Cl₂:CH₃OH (8 : 1) as eluent to give pure **21** as a colourless oil (30 mg; 78%).

[α]_D -208.8 (*c* 1, CH₂Cl₂). ¹H NMR (CDCl₃) δ 7.39 (d, *J* = 8.1 Hz, 2H), 7.39-7.25 (m, 5H), 6.88 (d, *J* = 8.4 Hz, 2H), 4.74 (dd, *J* = 17.6, 2.6 Hz, 1H), 4.54 (q, *J* = 11.4, 14.2 Hz, 2H), 4.32 (t, *J* = 2.4 Hz, 1H), 4.07 (dt, *J* = 11.0, 2.9 Hz, 1H), 3.83 (d, *J* = 2.6 Hz, 2H), 3.77 (dd, *J* = 17.6, 2.6 Hz, 1H), 3.21 (dd, *J* = 13.6, 3.3 Hz, 1H), 2.89 (dd, *J* = 13.6, 11.0 Hz, 1H), 2.29 (t, *J* = 2.4 Hz, 1H). ¹³C NMR (CDCl₃) δ 165.8, 165.7, 136.9, 135.3, 132.1, 131.3, 128.7, 128.4, 128.4, 121.2, 77.0, 73.9, 73.7, 67.7, 59.8, 57.0, 41.2, 33.6. Anal. Calcd for C₂₂H₂₁BrN₂O₃: C, 59.87; H, 4.80; N, 6.35; Found C, 59.8; H, 4.9; N, 6.4.

Synthesis of Tetrasubstituted 2,5-Diketopiperazines

N-Alkylation of trisubstituted 2,5-Diketopiperazines

c(*N*-propargyl-*L*-phenylalanyl-*L*-*N*-methyl-*L*-norleuciny) (22**)**

Compound **18** (50 mg; 0.18 mmol) was dissolved in CH₂Cl₂ (1 mL) and PS-BEMP (0.17g; 0.36 mmol) and propargyl bromide (23 μ L; 0.27 mmol) were added to the solution. After 24 h the polymer was filtered off and the reaction mixture was concentrated *in vacuo* and purified by flash chromatography using EtOAc:hexane (3 : 2) as eluent to give pure **22** as a colourless oil (43 mg; 77%).

[α]_D -11.4 (*c* 1, CHCl₃). ¹H NMR (CDCl₃) δ 7.29-7.19 (m, 3H), 7.12 (d, *J* = 6.6 Hz, 2H), 4.85 (dd, *J* = 17.6, 2.6 Hz, 1H), 4.59 (t, *J* = 4.8 Hz, 1H), 3.62-3.54 (m, 2H), 3.29 (dd, *J* = 14.1, 4.6 Hz, 1H), 3.19 (dd, *J* = 14.1, 4.6 Hz, 1H), 2.87 (s, 3H), 2.29 (t, *J* = 2.4 Hz, 1H), 1.22-0.99 (m, 5H), 0.79 (t, *J* = 7.0 Hz, 3H), 0.59-0.50 (m, 1H). ¹³C NMR (CDCl₃) δ 166.0, 165.1, 135.7, 130.1, 128.9, 127.7, 77.2, 73.8, 62.4, 60.5, 37.7, 33.5, 33.1, 33.0, 28.8, 22.3, 13.9. Anal. Calcd for C₁₉H₂₄N₂O₂: C, 73.05; H, 7.74; N, 8.97; Found C, 73.2; H, 7.7; N, 9.0.

c(*N*-allyl-*L*-phenylalanyl-*L*-*N*-methyl-norleuciny)l) (23)

Compound **18** (56 mg; 0.20 mmol) was dissolved in CH₂Cl₂ (1 mL) and PS-BEMP (0.19 g; 0.40 mmol) and allyl bromide (27 μ L; 0.30 mmol) were added to the solution. After 24 h the polymer was filtered off and the reaction mixture was concentrated *in vacuo* and purified by flash chromatography using EtOAc:hexane (3 : 2) as eluent to give pure **23** as sticky white crystals (46 mg; 72%).

$[\alpha]_D -60.7$ (*c* 1, CHCl₃). ¹H NMR (CDCl₃) δ 7.29-7.20 (m, 3H), 7.13-7.09 (m, 2H), 5.68 (dddd, *J* = 17.4, 16.1, 9.9, 7.9, 4.4 Hz, 1H), 5.20 (dd, *J* = 10.3, 1.1 Hz, 1H), 5.10 (dd, *J* = 17.2, 1.1 Hz, 1H), 4.70 (dddd, *J* = 15.0, 4.6, 1.4 Hz, 1H), 4.25 (t, *J* = 4.9 Hz, 1H), 3.59 (dd, *J* = 8.2, 4.9 Hz, 1H), 3.22-3.13 (m, 3H), 2.87 (s, 3H), 1.28-1.01 (m, 5H), 0.80 (t, *J* = 7.1 Hz, 3H), 0.61 (dddd, *J* = 18.7, 8.5, 5.2, 4.9 Hz, 1H). ¹³C NMR (CDCl₃) δ 166.2, 165.5, 136.0, 131.5, 130.0, 128.9, 127.6, 119.5, 62.6, 60.4, 46.7, 38.1, 33.1, 33.1, 29.0, 22.4, 13.9. Anal. Calcd for C₁₉H₂₆N₂O₂: C, 72.58; H, 8.33; N, 8.91; Found C, 72.5; H, 8.4; N, 8.9.

c(*N*-benzyl-*L*-phenylalanyl-*N*-methyl-*L*-norleuciny)l) (25)

Compound **19** (70 mg; 0.26 mmol) was dissolved in DMF (4 mL) and BEMP (0.15 mL; 0.52 mmol) and benzyl bromide (47 μ L; 0.39 mmol) were added to the solution and the mixture was heated in a microwave oven at 60 °C for 30 min. The reaction mixture was concentrated *in vacuo* and the crude product was purified by flash chromatography using EtOAc:hexane (3 : 2) as eluent to give pure **25** as off white crystals (69 mg; 74%).

Mp 105 °C. $[\alpha]_D -51.1$ (*c* 3, CH₂Cl₂). ¹H NMR (CDCl₃) δ 7.32-7.09 (m, 10H), 5.46 (d, *J* = 14.7 Hz, 1H), 4.15 (t, *J* = 4.9 Hz, 1H), 3.68 (d, *J* = 14.7 Hz, 1H), 3.66 (dd, *J* = 8.4, 4.8 Hz, 1H), 3.27-

3.17 (m, 2H), 2.86 (s, 3H), 1.30-1.06 (m, 5H), 0.83 (t, $J = 7.1$ Hz, 3H), 0.65 (m, 1H) ^{13}C NMR (CDCl_3) δ 166.5, 165.5, 136.1, 135.6, 130.0, 129.0, 128.9, 128.7, 128.1, 127.6, 62.5, 60.1, 46.9, 37.9, 33.2, 33.0, 29.0, 22.4, 13.9. Anal. Calcd for $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_2$: C, 75.79; H, 7.74; N, 7.69; Found C, 75.7; H, 7.7; N, 7.6.

c(*N*-benzyl-*L*-phenylalanyl-*N*-propargyl-*L*-norleuciny) (26)

Compound **20** (30 mg; 86 μmol) was dissolved in DMF (4 mL) and BEMP (49 μL ; 0.17 mmol) and benzyl bromide (15.4 μL ; 0.13 mmol) were added to the solution and the reaction mixture was heated in a microwave oven at 60 $^\circ\text{C}$ for 30 min. The reaction mixture was concentrated *in vacuo* and the crude product was purified by flash chromatography using hexane: CH_3OH : CH_2Cl_2 (6 : 1 : 5) as eluent to give pure **26** as a pale yellow oil (31 mg; 83%).

$[\alpha]_{\text{D}} -71.6$ (c 1, CH_2Cl_2). ^1H NMR (CDCl_3) δ 7.37 (d, $J = 9.2$ Hz, 2H), 7.35-7.15 (m, 11 H), 7.09 (dd, $J = 7.3, 2.2$ Hz, 2H), 5.40 (d, $J = 15.0$ Hz, 1H), 5.27 (d, $J = 15.0$ Hz, 1H), 4.24 (dd, $J = 6.0, 4.6$ Hz, 1H), 3.92 (d, $J = 15.0$ Hz, 1H), 3.73 (dd, $J = 9.0, 3.8$ Hz, 1H), 3.56 (d, $J = 15.0$ Hz, 1H), 3.34 (dd, $J = 11.7, 4.4$ Hz, 1H), 3.24 (dd, $J = 13.9, 6.2$ Hz, 1H), 1.40-1.10 (m, 5H), 0.93-0.90 (m, 1H), 0.85 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (CDCl_3) δ 166.9, 166.0, 136.4, 135.8, 135.7, 130.0, 129.2, 129.1, 129.0, 129.0, 128.9, 128.5, 128.3, 128.1, 128.1, 128.7, 60.7, 59.2, 47.7, 47.3, 38.7, 33.7, 33.3, 22.4, 14.0. Anal. Calcd for $\text{C}_{25}\text{H}_{28}\text{N}_2\text{O}_2$: C, 77.09; H, 7.25; N, 7.19; Found C, 77.4; H, 7.2; N, 7.1.

c(*N*-(2-phthalimidoethyl)-*L*-phenylalanyl-*N*-methyl-*L*-norleuciny) (27)

Compound **18** (60 mg; 0.22 mmol) was dissolved in DMF (4 mL) and BEMP (0.13 mL; 0.44 mmol), *N*-(2-bromoethyl)phthalimide (56 mg; 0.22 mmol) and tetrabutylammonium iodide (81 mg; 0.22 mmol) were added to the solution and the mixture was heated in a microwave to 60 $^\circ\text{C}$

for 3 h. The reaction mixture was concentrated *in vacuo* and the crude product was purified by flash chromatography using EtOAc:hexane (3 : 2) as eluent to give pure **27** as a colourless oil (51 mg; 52%).

$[\alpha]_D -26.0$ (*c* 1, CH₂Cl₂). ¹H NMR (CD₃OD) δ 7.73-7.66 (m, 2H), 7.63-7.55 (m, 2H), 7.31-7.16 (m, 5H), 6.22 (s, 1H), 4.32 (t, *J* = 4.6 Hz, 1H), 4.00-3.92 (m, 1H), 3.74-3.70 (m, 1H), 3.25 (dd, *J* = 13.7, 5.0 Hz, 1H), 2.97 (dd, *J* = 13.7, 5.0 Hz, 1H), 2.86 (s, 3H), 2.85-2.77 (m, 1H), 2.63-2.57 (m, 1H), 1.35-1.24 (m, 1H), 1.14-1.04 (m, 2H), 0.84-0.80 (m, 3H), 0.78 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (CD₃OD) δ 168.4, 168.0, 167.8, 166.1, 135.8, 134.2, 133.9, 132.1, 131.8, 130.2, 128.3, 127.1, 123.4, 123.1, 61.4, 56.3, 45.9, 39.3, 33.1, 31.8, 31.4, 26.7, 22.0, 12.8. Anal. Calcd for C₂₆H₂₉N₃O₄: C, 69.78; H, 6.53; N, 9.39; Found C, 69.7; H, 6.6; N, 9.5.

^1H - and ^{13}C -NMR spectra of compounds 28 – 31

^1H -NMR spectrum of **29** in CDCl_3

^{13}C -NMR spectrum of **29** in CDCl_3

^1H -NMR spectrum of **31** in CDCl_3

^{13}C -NMR spectrum of **31** in CDCl_3