Synthesis and Structure of Oxetane Containing Tripeptide Motifs

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Methyl [3-(nitromethyl)oxetan-3-yl]-D-valinate, (R)-4a

3-Oxetanone (130 µL, 2.03 mmol), nitromethane (154 µL, 2.85 mmol), and NEt₃ (57 µL, 0.41 mmol) were stirred at rt for 30 min then diluted with CH₂Cl₂ (10 mL) and cooled to −78 °C. To this solution was added NEt₃ (565 µL, 4.05 mmol), followed by MsCl (157 µL, 2.03 mmol) dropwise over 10 min. The reaction mixture was stirred at −78 °C for 40 min. Meanwhile, to a stirred solution of D-valine methyl ester hydrochloride (682 mg, 4.07 mmol) in CH₂Cl₂ (12.5 mL) was added NEt₃ (565 µL, 4.05 mmol). This mixture was stirred at rt for 10 min then added to the oxetane solution at −78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with sat. NH₄Cl (15 mL). The mixture was extracted with CH₂Cl₂ (5 x 30 mL) and the combined organics dried over MgSO₄, filtered and concentrated in vacuo. Purification by column chromatography (15 → 25% EtOAc in hexane) provided (R)-4a (337 mg, 68%) as a yellow oil. Rₚ = 0.23 (40% EtOAc in hexane); [α]₂⁵^D +16.6 (c 0.22, CHCl₃); IR (thin film) 3330, 2960, 2877, 1729, 1555, 1466, 1434, 1379, 1275, 1201 cm⁻¹; δ_H (400 MHz, CDCl₃) 4.85 (1H, dd, J = 13.1, 0.8 Hz, CHH), 4.76 (1H, d, J = 13.1 Hz, CHH), 4.60 (1H, d, J = 7.3 Hz, CHH), 4.59 (1H, d, J = 7.3 Hz, CHH), 4.49 (1H, d, J = 7.3 Hz, CHH), 4.45 (1H, d, J = 7.3 Hz, CHH), 3.71 (3H, s, CH₃), 3.21 (1H, dd, J = 8.5, 6.5 Hz, CH), 2.27 (1H, d, J = 8.5 Hz, NH), 1.97 – 1.85 (1H, m, CH), 0.91 (3H, d, J = 6.8 Hz, CH₃), 0.88 (3H, d, J = 6.8 Hz, CH₃); δ_C (100 MHz, CDCl₃) 175.9 (C=O), 79.0 (CH₂), 78.7 (CH₂), 78.5 (CH₂), 61.3 (CH), 59.5 (C), 52.3 (CH₃), 32.1 (CH), 19.4 (CH₃), 18.1 (CH₃); MS (ES⁺) m/z 247 [M+H]^+, 269 [M+Na]^+; HRMS (ES⁺) calcd. for C₁₀H₁₈N₂NaO₅ [M+Na]^⁺: 269.1108; found: 269.1101.

Methyl [3-(nitromethyl)oxetan-3-yl]-L-valinate, (S)-4a

3-Oxetanone (130 µL, 2.03 mmol), nitromethane (154 µL, 2.85 mmol), NEt₃ (57 µL, 0.41 mmol) were stirred at rt for 30 min then diluted with CH₂Cl₂ (10 mL) and cooled to −78 °C. To this solution was added NEt₃ (565 µL, 4.05 mmol), followed by MsCl (157 µL, 2.03 mmol) dropwise over 10 min. The reaction mixture was stirred at −78 °C for 40 min. Meanwhile, to a stirred solution of L-valine methyl ester hydrochloride (681 mg, 4.06 mmol) in CH₂Cl₂ (12.5 mL) was added NEt₃ (565 µL, 4.05 mmol). This mixture was stirred at rt for 10 min then added to the oxetane solution at −78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with sat. NH₄Cl (15 mL). The mixture was extracted with CH₂Cl₂ (5 x
30 mL) and the combined organics dried over MgSO₄, filtered and concentrated in vacuo. Purification by column chromatography (15 → 25% EtOAc in hexane) provided 4a (323 mg, 65%) as a yellow oil. Rf = 0.19 (100% CH₂Cl₂); [α]D²³³ −19.4 (c 0.24, CHCl₃); IR (thin film) 3333, 2962, 2878, 1730, 1555, 1467, 1435, 1379, 1276, 1202 cm⁻¹; δH (400 MHz, CDCl₃) 4.85 (1H, d, J = 13.1 Hz, CHH), 4.77 (1H, d, J = 13.1 Hz, CHH), 4.60 (2H, d, J = 7.0 Hz, CHH), 4.50 (1H, d, J = 7.0 Hz, CHH), 4.46 (1H, d, J = 7.0 Hz, CHH), 3.72 (3H, s, CH₃), 3.22 (1H, dd, J = 10.8, 6.0 Hz, CH), 2.27 (1H, d, J = 10.8 Hz, NH), 1.98 – 1.85 (1H, m, CH), 0.92 (3H, d, J = 6.8 Hz, CH₃), 0.89 (3H, d, J = 6.8 Hz, CH₃); δC (100 MHz, CDCl₃) 175.9 (C=O), 79.0 (CH₂), 78.7 (CH₂), 78.6 (CH₂), 61.4 (CH), 59.5 (C), 52.3 (CH₃), 32.2 (CH), 19.4 (CH₃), 18.1 (CH₃); MS (ES⁺) m/z 269 [M+Na⁺]; HRMS (ES⁺) calcd. for C₁₀H₁₄N₂O₃ [M+Na⁺]: 269.1108; found: 269.1105.

Benzy1 [3-(nitromethyl)oxetan-3-yl]-L-valinate, 4b

Nitromethane (177 µL, 3.27 mmol), 3-oxetanone (273 µL, 4.26 mmol) and NEt₃ (91 µL, 0.65 mmol) were stirred at rt for 30 min then diluted with CH₂Cl₂ (16 mL) and cooled to −78 °C. To this solution was added NEt₃ (0.91 mL, 6.53 mmol), followed by MsCl (255 µL, 3.29 mmol) dropwise over 10 min. This mixture was stirred at −78 °C for 40 min. Meanwhile, to a stirred solution of L-valine benzyl ester p-toluenesulfonate salt (2.49 g, 6.56 mmol) in CH₂Cl₂ (19 mL) was added NEt₃ (0.91 mL, 6.53 mmol). The reaction mixture was stirred at rt for 10 min before it was added to the oxetane solution at −78 °C. The reaction mixture was allowed to warm to rt and stirred for 21 h before being quenched with sat. NH₄Cl (30 mL). The mixture was extracted with CH₂Cl₂ (2 x 40 mL) and EtOAc (2 x 40 mL). The combined organics were dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by column chromatography (15 → 40% EtOAc in heptane) provided 4b (876 mg, 83%) as a pale pink oil. Rf = 0.33 (30% EtOAc in heptane); [α]D¹⁷ −4.5 (c 0.20, CHCl₃); IR (KBr) 3338, 2963, 2877, 1732, 1560, 1498, 1457, 1379, 1268, 1184, 983, 738 cm⁻¹; δH (400 MHz, CDCl₃) 7.40 – 7.31 (5H, m, ArH), 5.17 (1H, d, J = 12.0 Hz, CHH), 5.11 (1H, d, J = 12.0 Hz, CHH), 4.80 (1H, dd, J = 13.1, 0.9 Hz, CHH), 4.72 (1H, dd, J = 13.1, 0.9 Hz, CHH), 4.55 (1H, d, J = 7.1 Hz, CHH), 4.53 (1H, d, J = 7.1 Hz, CHH), 4.45 (1H, d, J = 7.1 Hz, CHH), 4.35 (1H, d, J = 7.1 Hz, CHH), 4.29 (1H, d, J = 10.9, 5.9 Hz, CH), 2.29 (1H, d, J = 10.9 Hz, NH), 1.98 – 1.88 (1H, m, CH), 0.91 (3H, d, J = 6.8 Hz, CH₃), 0.86 (3H, d, J = 6.8 Hz, CH₃); δC (175 MHz, CDCl₃) 175.3 (C=O), 135.4 (C), 128.9 (CH), 128.8 (CH), 128.8 (CH), 78.9 (CH₂), 78.7
(CH₂), 78.7 (CH₂), 67.3 (CH₂), 61.5 (CH), 59.6 (C), 32.2 (CH), 19.4 (CH₃), 18.0 (CH₃); MS (ES⁺) m/z 323 [M+H]⁺; HRMS (ES⁺) calcd. for C₁₆H₂₃N₂O₅ [M+H]⁺: 323.1602; found: 323.1606.

**Benzyl [3-(1-nitro-2-phenylethyl)oxetan-3-yl]glycinate, 4c**

![Chemical Structure](attachment:image.png)

(2-Nitroethyl)benzene (196 mg, 1.30 mmol), 3-oxetanone (108 µL, 1.68 mmol), and NEt₃ (36 µL, 0.26 mmol) were stirred at rt for 90 min then diluted with CH₂Cl₂ (6.4 mL) and cooled to −78 °C. To this mixture was added NEt₃ (358 µL, 2.57 mmol), followed by MsCl (100 µL, 1.29 mmol) dropwise. The reaction mixture was stirred at −78 °C for 90 min. Meanwhile, to glycine benzyl ester p-toluenesulfonate salt (965 mg, 2.86 mmol) in CH₂Cl₂ (9 mL) was added NEt₃ (360 µL, 2.58 mmol). This mixture was stirred at rt for 10 min before being added to the oxetane solution at −78 °C. The combined mixture was allowed to warm to rt overnight before being quenched with sat. NH₄Cl (15 mL). The mixture was extracted with CH₂Cl₂ (2 x 50 mL) and EtOAc (2 x 50 mL), and the combined organics dried over MgSO₄, filtered and concentrated in vacuo. Purification by repeat column chromatography (35 → 100% EtOAc in hexane; 20 → 30% EtOAc in hexane) provided 4c (280 mg, 58%) as a pale yellow solid. M.p. 53–54 °C; Rf = 0.34 (40% EtOAc in hexane); IR (thin film) 3342, 2958, 2883, 1736, 1604, 1551, 1496, 1455, 1367, 1189, 1083, 737 cm⁻¹; δH (400 MHz, CDCl₃) 7.39–7.19 (10H, m, ArH), 5.20 (2H, s, CH₂), 5.11 (1H, dd, J = 9.9, 4.4 Hz, CH), 4.61 (1H, d, J = 7.5 Hz, CHH), 4.59 (1H, d, J = 7.5 Hz, CHH), 4.47 (2H, s, CH₂), 3.75 (1H, d, J = 7.5 Hz, CHH), 3.65 (1H, d, J = 17.6 Hz, CHH), 3.51 (1H, dd, J = 14.7, 9.9 Hz, CHH), 3.27 (1H, dd, J = 14.7, 4.4 Hz, CHH), 2.50 (1H, br s, NH); δC (100 MHz, acetone-d₆) 172.6 (C=O), 137.2 (C), 137.1 (C), 129.9 (CH), 129.5 (CH), 129.3 (CH), 129.1 (CH), 129.0 (CH), 128.0 (CH), 92.8 (CH), 77.5 (CH₂), 77.1 (CH₂), 67.1 (CH₂), 63.1 (C), 45.2 (CH₂), 35.1 (CH₂); MS (ES⁺) m/z 371 [M+H]⁺, 393 [M+Na]⁺; HRMS (ES⁺) calcd. for C₂₀H₂₃N₂O₅ [M+H]⁺: 371.1601; found: 371.1602.

**Benzyl [3-(nitromethyl)oxetan-3-yl]glycinate, 4d**

![Chemical Structure](attachment:image.png)

Nitromethane (220 µL, 4.06 mmol), 3-oxetanone (340 µL, 5.30 mmol) and NEt₃ (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with CH₂Cl₂ (20 mL) and cooled to −78 °C. To this solution was added
NEt₃ (1.13 mL, 8.11 mmol), followed by MsCl (315 µL, 4.07 mmol) dropwise over 10 min. This mixture was stirred at −78 °C for 40 min. Meanwhile, to a stirred solution of glycine benzyl ester p-toluene sulfonate salt (2.75 g, 8.15 mmol) in CH₂Cl₂ (25 mL) was added NEt₃ (1.13 mL, 8.11 mmol). The reaction mixture was stirred at rt for 10 min before it was added to the oxetane solution at −78 °C. The reaction mixture was allowed to warm to rt and stirred for 21 h before being quenched with sat. NH₄Cl (30 mL). The mixture was extracted with CH₂Cl₂ (4 x 75 mL). The combined organics were dried over MgSO₄, filtered and concentrated in vacuo. Purification by column chromatography (60 → 80% Et₂O in hexane) gave a yellow solid. Recrystallisation (EtOH/hexane) provided 4d (728 mg, 64%) as off-white crystals. M.p. 77 – 79 °C (EtOH); Rf = 0.30 (50% EtOAc in petroleum ether); IR (thin film) 3342, 2962, 2885, 1736, 1550, 1498, 1456, 1380, 1190, 1102, 976, 737, 696 cm⁻¹; δ_H (400 MHz, CDCl₃) 7.41 – 7.32 (5H, m, Ar_H), 5.18 (2H, s, CH₂), 4.82 (2H, s, CH₂), 4.61 (2H, d, J = 7.3 Hz, CHH), 4.56 (2H, d, J = 7.3 Hz, CHH), 3.58 (2H, d, J = 5.3 Hz, CHH), 2.39 (1H, br t, J = 5.3 Hz, NH); δ_C (100 MHz, CDCl₃) 171.7 (C=O), 135.2 (C), 128.8 (CH), 128.7 (CH), 128.6 (CH), 78.5 (CH₂), 78.3 (CH₂), 67.3 (CH₂), 59.5 (C), 44.9 (CH₂); MS (ES⁺) m/z 281 [M+H⁺], 303 [M+Na⁺]; HRMS (ES⁺) calcd. for C₁₃H₁₆N₂O₅ [M+Na⁺]: 303.0951; found: 303.0957.

Benzyl [3-(nitromethyl)oxetan-3-yl]-L-threoninate, 4e

3-Oxanetone (260 µL, 4.05 mmol), nitromethane (307 µL, 5.67 mmol) and NEt₃ (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with CH₂Cl₂ (20 mL) and cooled to −78 °C. To this solution was added NEt₃ (1.13 mL, 8.11 mmol), followed by MsCl (314 µL, 4.06 mmol) dropwise over 10 min. The reaction mixture was stirred at −78 °C for 40 min. Meanwhile, to a stirred solution of L-threonine benzyl ester hydrochloride (1.99 g, 8.10 mmol) in CH₂Cl₂ (25 mL) was added NEt₃ (1.13 mL, 8.11 mmol). This mixture was stirred at rt for 10 min then added to the oxetane solution at −78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with sat. NH₄Cl (30 mL). The mixture was extracted with CH₂Cl₂ (5 x 50 mL) and the combined organics dried over MgSO₄, filtered and concentrated in vacuo. Purification by repeat column chromatography (30 → 50% EtOAc in hexane; 0 → 2% MeOH in CH₂Cl₂; 70 → 85% Et₂O in hexane) provided 4e (741 mg, 56%) as a pale yellow oil. Rf = 0.22 (50% EtOAc in hexane); [α]D²³ −7.7 (c 0.22, CHCl₃); IR (thin film) 3347, 2925, 1729, 1552, 1499, 1457, 1378, 1265, 1173, 974, 736, 697 cm⁻¹; δ_H (400 MHz,
Benzyl [3-(nitromethyl)oxetan-3-yl]-L-leucinate, 4f

3-Oxetanone (260 µL, 4.05 mmol), nitromethane (307 µL, 5.67 mmol) and NEt₃ (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with CH₂Cl₂ (20 mL) and cooled to −78 °C. To this solution was added NEt₃ (1.13 mL, 8.11 mmol), followed by MsCl (314 µL, 4.06 mmol) dropwise over 10 min. The reaction mixture was stirred at −78 °C for 40 min. Meanwhile, to a stirred solution of L-leucine benzyl ester p-toluenesulfonate salt (3.19 g, 8.11 mmol) in CH₂Cl₂ (25 mL) was added NEt₃ (1.13 mL, 8.11 mmol). This mixture was stirred at rt for 10 min then added to the oxetane solution at −78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with sat. NH₄Cl (30 mL). The mixture was extracted with CH₂Cl₂ (5 x 50 mL) and the combined organics dried over MgSO₄, filtered and concentrated in vacuo. Purification by column chromatography (15 → 30% EtOAc in hexane) provided 4f (798 mg, 59%) as a pale yellow oil. Rₐ = 0.31 (30% EtOAc in hexane); [α]²⁰D +5.0 (c 0.16, CHCl₃); IR (thin film) 3338, 2958, 2874, 1730, 1553, 1499, 1457, 1379, 1268, 1169, 976, 737, 697 cm⁻¹; δH (300 MHz, CDCl₃) 7.42 – 7.31 (5H, m, ArH), 5.17 (1H, d, J = 12.1 Hz, CHH), 5.08 (1H, d, J = 12.1 Hz, CHH), 4.83 (1H, dd, J = 12.8, 0.8 Hz, CHH), 4.75 (1H, d, J = 12.8 Hz, CHH), 4.58 (1H, d, J = 7.2 Hz, CHH), 4.53 (1H, d, J = 7.0 Hz, CHH), 4.45 (1H, d, J = 7.2 Hz, CHH), 4.26 (1H, d, J = 7.0 Hz, CHH), 3.51 (1H, s, CH), 2.22 (1H, br s, NH), 1.74 – 1.57 (1H, m, CH), 1.55 – 1.35 (2H, m, CH₂), 0.90 (3H, d, J = 6.8 Hz, CH₃), 0.88 (3H, d, J = 6.8 Hz, CH₃); δC (75 MHz, CDCl₃) 175.9 (C=O), 135.2 (C), 128.8 (CH), 128.8 (CH), 79.1 (CH₂), 78.9 (CH₂), 78.3 (CH₂), 67.3 (CH₂), 59.7 (C), 54.5 (CH), 43.5 (CH₂), 24.7 (CH), 22.9 (CH₃), 22.3 (CH₃); MS (ES⁻) m/z 337 [M+H]⁻, 359 [M+Na]⁺; HRMS (ES⁻) calcd. for C₁₇H₂₄N₂NaO₅ [M+Na]⁺: 359.1577; found: 359.1564.
Benzyl [3-(nitromethyl)oxetan-3-yl]-L-isoleucinate, 4g

3-Oxetanone (260 µL, 4.05 mmol), nitromethane (307 µL, 5.67 mmol) and NEt₃ (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with CH₂Cl₂ (20 mL) and cooled to −78 °C. To this solution was added NEt₃ (1.13 mL, 8.11 mmol), followed by MsCl (314 µL, 4.06 mmol) dropwise over 10 min. The reaction mixture was stirred at −78 °C for 40 min. Meanwhile, to a stirred solution of L-isoleucine benzyl ester p-toluenesulfonate salt (3.19 g, 8.11 mmol) in CH₂Cl₂ (25 mL) was added NEt₃ (1.13 mL, 8.11 mmol). This mixture was stirred at rt for 10 min then added to the oxetane solution at −78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with sat. NH₄Cl (30 mL). The mixture was extracted with CH₂Cl₂ (5 x 50 mL) and the combined organics dried over MgSO₄, filtered and concentrated in vacuo. Purification by column chromatography (10 → 25% EtOAc in hexane) provided 4g (704 mg, 52%) as a pale yellow oil. R_f = 0.23 (30% EtOAc in hexane); [α]ᵢ²³DCF −2.2 (c 0.24, CHCl₃); IR (thin film) 3339, 2965, 2879, 1728, 1555, 1499, 1457, 1379, 1177, 1147, 979, 751, 698 cm⁻¹; δH (400 MHz, CDCl₃) 7.41 – 7.32 (5H, m, ArH), 5.16 (1H, d, J = 12.1 Hz, CHH), 5.12 (1H, d, J = 12.1 Hz, CHH), 4.80 (1H, dd, J = 13.1, 0.8 Hz, CHH), 4.72 (1H, d, J = 13.1 Hz, CHH), 4.55 (1H, d, J = 7.3 Hz, CHH), 4.54 (1H, d, J = 7.3 Hz, CHH), 4.45 (1H, d, J = 7.3 Hz, CHH), 4.33 (1H, d, J = 7.3 Hz, CHH), 3.31 (1H, dd, J = 11.0, 6.2 Hz, CH), 2.30 (1H, d, J = 11.0 Hz, NH), 1.72 – 1.61 (1H, m, CH), 1.52 – 1.41 (1H, m, CHH), 1.13 – 1.01 (1H, m, CHH), 0.88 – 0.81 (6H, m, CH₃); δC (100 MHz, CDCl₃) 175.3 (C=O), 135.3 (C), 128.9 (CH), 128.8 (CH), 78.9 (CH₂), 78.7 (CH₂), 78.5 (CH₂), 67.2 (CH₂), 60.6 (CH), 59.6 (C), 39.0 (CH), 24.9 (CH₂), 15.7 (CH₃), 11.6 (CH₃); MS (ES⁺) m/z 337 [M+H]⁺, 359 [M+Na]⁺; HRMS (ES⁺) calcd. for C₁₇H₂₄N₂O₅ [M+Na]⁺: 359.1577; found: 359.1576.

Benzyl [3-(nitromethyl)oxetan-3-yl]-L-phenylalaninate, 4h

Nitromethane (220 µL, 4.06 mmol), 3-oxetanone (339 µL, 5.28 mmol) and NEt₃ (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with CH₂Cl₂ (20 mL) and cooled to −78 °C. To this solution was added NEt₃ (1.13 mL, 8.11 mmol), followed by MsCl (317 µL, 4.09 mmol) dropwise over 10 min. This mixture was stirred at −78 °C for 35 min. Meanwhile, to a stirred solution of L-phenylalanine benzyl ester hydrochloride (2.38 g, 8.16 mmol) in CH₂Cl₂ (25 mL) was added NEt₃ (1.13 mL, 8.11 mmol). The reaction mixture was stirred at rt for 10
min before it was added to the oxetane solution at −78 °C. The reaction mixture was allowed to warm to rt and stirred for 22 h before being quenched with sat. NH₄Cl (30 mL). The mixture was extracted with CH₂Cl₂ (1 x 30 mL) and EtOAc (3 x 40 mL). The combined organics were dried over Na₂SO₄, filtered and concentrated in vacuo. Purification by column chromatography (15 → 40% EtOAc in heptane) gave a yellow solid. Recrystallisation (MeOH) provided 4h (1.10 g, 73%) as pale yellow crystals. M.p. 92 – 93 °C (MeOH); Rᵣ = 0.25 (30% EtOAc in heptane); [α]¹⁷⁰⁰D = −9.6 (c 0.20, CHCl₃); IR (KBr) 3335, 2957, 2880, 1732, 1603, 1556, 1497, 1455, 1379, 1275, 1175, 982, 749 cm⁻¹; δH (400 MHz, CDCl₃) 7.37 – 7.33 (3H, m, ArH), 7.28 – 7.21 (5H, m, ArH), 7.13 – 7.09 (2H, m, ArH), 5.11 (1H, d, J = 12.0 Hz, CHH), 5.05 (1H, d, J = 12.0 Hz, CHH), 4.75 (1H, dd, J = 13.1, 0.8 Hz, CHH), 4.69 (1H, d, J = 13.1 Hz, CHH), 4.47 (1H, d, J = 7.2 Hz, CHH), 4.35 (1H, d, J = 7.3 Hz, CHH), 4.33 (1H, d, J = 7.2 Hz, CHH), 4.25 (1H, d, J = 7.3 Hz, CHH), 3.77 – 3.70 (1H, m, CH), 2.97 (1H, dd, J = 13.4, 6.3 Hz, CHH), 2.87 (1H, dd, J = 13.4, 7.1 Hz, CHH), 2.37 (1H, d, J = 9.6 Hz, NH); δc (175 MHz, CDCl₃) 174.5 (C=O), 136.6 (C), 135.2 (C), 129.6 (CH), 128.8 (CH), 128.6 (CH), 127.2 (CH), 78.8 (CH₂), 78.7 (CH₂), 78.6 (CH₂), 67.5 (CH₂), 59.6 (C), 57.7 (CH), 40.8 (CH₂); MS (ES⁺) m/z 371 [M+H⁺]; HRMS (ES⁺) calcd. for C₂₀H₂₃N₂O₅ [M+H⁺]: 371.1607; found: 371.1607.

**Methyl [3-(nitromethyl)oxetan-3-yl]-L-serinate, 4i**

Nitromethane (70 µL, 1.29 mmol), 3-oxatanone (107 µL, 1.67 mmol) and NEt₃ (36 µL, 0.26 mmol) were stirred at rt for 30 min then diluted with CH₂Cl₂ (6.3 mL) and cooled to −78 °C. To this solution was added NEt₃ (357 µL, 2.56 mmol), followed by MsCl (99 µL, 1.28 mmol) dropwise over 10 min. This mixture was stirred at −78 °C for 30 min. Meanwhile, to a stirred solution of L-serine methyl ester hydrochloride (401 mg, 2.58 mmol) in CH₂Cl₂ (10 mL) was added NEt₃ (357 µL, 2.56 mmol). The reaction mixture was stirred at rt for 10 min before it was added to the oxetane solution at −78 °C. The reaction mixture was allowed to warm to rt and stirred for 25 h before being quenched with sat. NH₄Cl (15 mL). The mixture was extracted with CH₂Cl₂ (2 x 30 mL) and EtOAc (2 x 30 mL). The combined organics were dried over MgSO₄, filtered and concentrated in vacuo. Purification by column chromatography (65 → 70% EtOAc in hexane) provided 4i (146 mg, 48%) as a colourless oil. Rᵣ = 0.28 (80% EtOAc in petroleum ether); [α]³⁰⁰⁰D = −23.3 (c 0.20, CHCl₃); IR (thin film) 3346, 2956, 2886, 1732, 1553, 1459, 1380, 1207, 1060 cm⁻¹; δH (400 MHz, CDCl₃) 4.85 (1H,
found: m/z 67.4 (CH, (C=O), 135.2 (C), 128.8 (CH), 128.7 (CH), 128.6 (CH), 79.5 (CH)

d, J = 13.2 Hz, CHH), 4.80 (1H, d, J = 13.2 Hz, CHH), 4.61 (2H, d, J = 7.3 Hz, CHH), 4.53 (2H, d, J = 7.3 Hz, CHH), 3.79 – 3.73 (4H, m, CH3, CHH), 3.65 – 3.59 (2H, m, CHH, CH), 2.70 (2H, br s, OH, NH); δC (100 MHz, CDCl3) 173.6 (C=O), 78.9 (CH2), 78.7 (CH2), 78.5 (CH2), 63.9 (CH2), 59.3 (C), 57.5 (CH), 52.9 (CH3); MS (ES+) m/z 257 [M+Na]+; HRMS (ES+) calcd. for C8H14N2NaO6 [M+Na]+: 257.0744; found: 257.0746.

**Benzyl [[3-(nitromethyl)oxetan-3-yl]-L-valyl]glycinate, 4j**

3-Oxetanone (260 µL, 4.05 mmol), nitromethane (307 µL, 5.67 mmol) and NEt3 (113 µL, 0.81 mmol) were stirred at rt for 30 min then diluted with CH2Cl2 (20 mL) and cooled to −78 °C. To this solution was added NEt3 (1.13 mL, 8.11 mmol), followed by MsCl (314 µL, 4.06 mmol) dropwise over 10 min. The reaction mixture was stirred at −78 °C for 40 min. Meanwhile, to a stirred solution of L-valylglycine benzyl ester hydrochloride (2.44 g, 8.11 mmol) in CH2Cl2 (25 mL) was added NEt3 (1.13 mL, 8.11 mmol). This mixture was stirred at rt for 10 min then added to the oxetane solution at −78 °C. The reaction mixture was allowed to warm to rt, stirred for 20 h, then quenched with sat. NH4Cl (30 mL). The mixture was extracted with CH2Cl2 (5 x 50 mL) and the combined organics dried over MgSO4, filtered and concentrated in vacuo. Purification by repeat column chromatography (40 → 50% EtOAc in hexane; 80 → 100% Et2O in hexane; 0 → 2% MeOH in CH2Cl2) provided 4j (949 mg, 62%) as a pale yellow oil. Rf = 0.31 (60% EtOAc in hexane); [α]D23 = 17.1 (c 0.22, CHCl3); IR (thin film) 3321, 2962, 2878, 1746, 1652, 1552, 1499, 1457, 1380, 1278, 1187, 977, 738, 698 cm⁻¹; δH (400 MHz, CDCl3) 7.40 – 7.30 (6H, m, ArH, NH), 5.20 – 5.13 (2H, m, CH2), 4.85 (1H, d, J = 13.8 Hz, CHH), 4.79 (1H, dd, J = 13.8, 0.5 Hz, CHH), 4.69 (1H, d, J = 7.3 Hz, CHH), 4.59 (1H, d, J = 7.5 Hz, CHH), 4.47 (1H, d, J = 7.3 Hz, CHH), 4.45 (1H, d, J = 7.5 Hz, CHH), 4.14 (1H, dd, J = 18.2, 6.3 Hz, CHH), 4.02 (1H, dd, J = 18.2, 5.5 Hz, CHH), 3.36 (1H, t, J = 4.0 Hz, CH), 2.26 – 2.14 (2H, m, CH, NH), 1.01 (3H, d, J = 6.8 Hz, CH3), 0.91 (3H, d, J = 7.0 Hz, CH3); δC (100 MHz, CDCl3) 174.3 (C=O), 169.7 (C=O), 135.2 (C), 128.8 (CH), 128.7 (CH), 128.6 (CH), 79.5 (CH2), 77.9 (CH2), 77.5 (CH2), 67.4 (CH2), 62.4 (CH), 59.3 (C), 41.0 (CH2), 31.7 (CH), 19.7 (CH3), 17.6 (CH3); MS (ES+) m/z 380 [M+H]+, 402 [M+Na]+; HRMS (ES+) calcd. for C18H28N3NaO6 [M+Na]+: 402.1636; found: 402.1631.
Benzyl [[3-(1-nitro-2-phenylethyl)oxetan-3-yl]glycyl]glycinate, 4k

(2-Nitroethyl)benzene (303 mg, 2.00 mmol), 3-oxetanone (167 µL, 2.60 mmol) and NEt₃ (56 µL, 0.40 mmol) were stirred at rt for 90 min then diluted with CH₂Cl₂ (12.5 mL) and cooled to −78 °C. To this mixture was added NEt₃ (559 µL, 4.01 mmol), followed by MsCl (156 µL, 2.01 mmol) dropwise. The reaction mixture was stirred at −78 °C for 90 min.

Meanwhile, to glycylglycine benzyl ester p-toluene sulfonate salt (1.74 g, 4.41 mmol) in CH₂Cl₂ (10 mL) was added NEt₃ (559 µL, 4.01 mmol). This mixture was stirred at rt for 10 min before being added to the oxetane solution at −78 °C. The combined mixture was allowed to warm to rt overnight before being quenched with sat. NH₄Cl (20 mL). The mixture was extracted with CH₂Cl₂ (3 x 50 mL) and EtOAc (1 x 50 mL), and the combined organics dried over MgSO₄, filtered and concentrated in vacuo. Purification by repeat column chromatography (65 → 100% EtOAc in heptane; 45 → 80% EtOAc in heptane) provided 4k (549 mg, 64%) as a sticky yellow solid. Rf = 0.23 (80% EtOAc in heptane); IR (KBr) 3379, 3342, 2956, 2887, 1744, 1668, 1605, 1549, 1497, 1455, 1189, 1030, 736, 698 cm⁻¹; δH (700 MHz, CDCl₃) 7.39 – 7.28 (9H, m, ArH, NH), 7.22 (2H, d, J = 7.3 Hz, ArH), 5.19 (2H, s, CH₂), 5.17 (1H, dd, J = 10.3, 3.8 Hz, CH), 4.66 (1H, d, J = 7.7 Hz, CHH), 4.61 (1H, d, J = 7.7 Hz, CHH), 4.59 (1H, d, J = 8.0 Hz, CHH), 4.53 (1H, d, J = 8.0 Hz, CHH), 4.20 (1H, dd, J = 18.4, 6.1 Hz, CHH), 4.06 (1H, dd, J = 18.4, 6.1 Hz, CHH), 3.66 (1H, d, J = 17.2 Hz, CHH), 3.60 (1H, d, J = 17.2 Hz, CHH), 3.56 (1H, dd, J = 14.8, 10.3 Hz, CHH), 3.28 (1H, dd, J = 14.8, 3.8 Hz, CHH), 2.51 (1H, br s, NH); δC (175 MHz, CDCl₃) 171.3 (C=O), 169.7 (C=O), 135.2 (C), 135.2 (C), 129.3 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 127.9 (CH), 93.3 (CH), 77.0 (CH₂), 76.6 (CH₂), 67.5 (CH₂), 62.4 (C), 46.1 (CH₂), 41.2 (CH₂), 34.8 (CH₂); MS (ES⁺) m/z 428 [M+H]⁺, 450 [M+Na]⁺; HRMS (ES⁺) calcd. for C₂₂H₂₈N₃O₆ [M+H]⁺: 428.1816; found: 428.1812.
Synthesis of the Tripeptides, 1a-f.

**General Method A: Nitro reduction**\(^1\) and amide coupling.\(^2\)

To a stirred suspension of the nitro compound (4) (1.0 molar equiv) in THF/H\(_2\)O (1:3, 1.0 mL) was added In metal (4.0 molar equiv), followed by 6 M HCl (6.0 molar equiv). The reaction mixture was stirred at rt for 90 min, then basified with sat. NaHCO\(_3\) (8 mL) and diluted with EtOAc (20 mL). The solution was filtered through Celite\(^\circledR\) and extracted with EtOAc (3 x 20 mL). The combined organics were dried over MgSO\(_4\), filtered and concentrated in vacuo to give the crude amine. To this amine (1.0 molar equiv) was added the Cbz protected amino acid (1.03 molar equiv), HOBt (0.15 molar equiv), 4-methylmorpholine (2.2 molar equiv) in EtOH. The reaction mixture was cooled in an ice-water bath before EDC-HCl (1.2 molar equiv) was added. The reaction mixture was stirred at rt for 18 h before water (18 mL) was added and the solution extracted with EtOAc (4 x 30 mL). The combined organics were dried over MgSO\(_4\), filtered and concentrated in vacuo. Purification by column chromatography provided the product.

**General Method B: Deprotection to tripeptides,**\(^3\) 1a-f.

A suspension of the protected oxetane tripeptide (9a-f) and 10% Pd/C (20% by wt) in MeOH was stirred under an atmosphere of hydrogen at rt for 18 h then filtered through Celite\(^\circledR\), washed with MeOH and concentrated in vacuo to give the product.

\[\text{[3-[(2-Aminoacetamido)methyl]oxetan-3-yl]-L-valine, 1a} \]

4b (97 mg, 0.30 mmol), In metal (142 mg, 1.24 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-Gly-OH (68 mg, 0.33 mmol), HOBt (8 mg, 0.05 mmol), NMM (75 µL, 0.68 mmol), EDC-HCl (70 mg, 0.37 mmol) in EtOH (3.1 mL). Work-up, followed by purification by column chromatography (60 → 80% EtOAc in hexane) provided 9a as a colourless oil (71 mg, 49%). \(R_t = 0.31\) (80% EtOAc in petroleum ether); \([\alpha]^{33}_D +12.3\) (c 0.22, CHCl\(_3\)); IR (thin film) 3316, 2960, 2875, 1722, 1666, 1520, 1456, 1241, 1152, 974, 737, 699 cm\(^{-1}\); \(\delta_H\) (400
MHZ, CDCl$_3$) 7.39 – 7.29 (10H, m, ArH), 6.50 (1H, br s, NH), 5.41 (1H, br s, NH), 5.16 – 5.08 (4H, m, CH$_2$), 4.37 (1H, d, $J = 6.4$ Hz, CHH), 4.30 (1H, d, $J = 6.5$ Hz, CHH), 4.23 (1H, d, $J = 6.5$ Hz, CHH), 4.18 (1H, d, $J = 6.4$ Hz, CHH), 3.86 (2H, d, $J = 5.5$ Hz, CH$_2$), 3.78 (1H, dd, $J = 14.0$, 5.8 Hz, CHH), 3.41 (1H, dd, $J = 5.3$ Hz, CHH), 2.01 – 1.65 (2H, m, CH, NH), 0.94 (3H, d, $J = 6.8$ Hz, CH$_3$), 0.87 (3H, d, $J = 6.8$ Hz, CH$_3$); $\delta$C (100 MHz, CDCl$_3$) 176.0 (C=O), 169.7 (C=O), 156.7 (C=O), 136.2 (C), 135.3 (C), 128.8 (CH), 128.7 (CH), 128.7 (CH), 128.6 (CH), 128.3 (CH), 128.2 (CH), 79.7 (CH$_2$), 79.4 (CH$_2$), 67.3 (CH$_2$), 67.2 (CH$_2$), 61.1 (CH), 59.4 (C), 44.7 (CH$_2$), 43.6 (CH$_2$), 32.0 (CH), 19.5 (CH$_3$), 17.9 (CH$_3$); MS (ES$^+$) m/z 484 [M+H]$^+$, 506 [M+Na]$^+$; HRMS (ES$^+$) calcd. for C$_{26}$H$_{34}$N$_3$O$_6$ [M+H]$^+$: 484.2442; found: 484.2444. 9a (73 mg, 0.15 mmol) and 10% Pd/C (15 mg) in MeOH (3 mL) were further reacted according to General Method B to give 1a (33 mg, 84%) as a cream oily solid. $[\alpha]_{D}^{25}$ +12.3 ($c$ 0.20, MeOH); IR (thin film) 3266, 3077, 2963, 2879, 1682, 1568, 1557, 1470, 1436, 1393 cm$^{-1}$; $\delta$H (500 MHz, D$_2$O) 4.68 (1H, d, $J = 7.3$ Hz, CHH), 4.62 (1H, d, $J = 7.3$ Hz, CHH), 4.50 (1H, d, $J = 7.3$ Hz, CHH), 4.43 (1H, d, $J = 7.3$ Hz, CHH), 3.86 (2H, s, CH$_2$), 3.79 (1H, d, $J = 14.3$ Hz, CHH), 3.65 (1H, d, $J = 14.3$ Hz, CHH), 3.13 (1H, d, $J = 5.2$ Hz, CH), 1.98 – 1.87 (1H, m, CH), 0.95 (3H, d, $J = 7.6$ Hz, CH$_3$), 0.94 (3H, d, $J = 7.6$ Hz, CH$_3$); $\delta$C (125 MHz, D$_2$O) 181.2 (C=O), 167.9 (C=O), 79.0 (CH$_2$), 78.8 (CH$_2$), 64.2 (CH), 59.9 (C), 43.3 (CH$_2$), 40.5 (CH$_2$), 31.3 (CH), 18.3 (CH$_3$), 18.2 (CH$_3$); MS (ES$^+$) m/z 260 [M+H]$^+$, 282 [M+Na]$^+$; HRMS (ES$^+$) calcd. for C$_{11}$H$_{22}$N$_3$O$_4$ [M+H]$^+$: 260.1605; found: 260.1605.

[3-[(S)-2-Aminopropanamido)methyl]oxetan-3-yl]-L-valine, 1b

4b (101 mg, 0.31 mmol), In metal (146 mg, 1.27 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Ala-OH (72 mg, 0.32 mmol), HOBt (8 mg, 0.05 mmol), NMM (75 µL, 0.68 mmol), EDC-HCl (71 mg, 0.37 mmol) in EtOH (3.1 mL). Work-up, followed by purification by column chromatography (50 → 70% EtOAc in hexane) provided 9b (69 mg, 44%) as a colourless oil. $R_f = 0.31$ (70% EtOAc in hexane); $[\alpha]_{D}^{20} +9.4$ ($c$ 0.16, CHCl$_3$); IR (thin film) 3327, 2962, 2876, 1721, 1663, 1521, 1499, 1456, 1239, 1178, 1153, 974, 737, 697 cm$^{-1}$; $\delta$H (400 MHz, CDCl$_3$) 7.39 – 7.28 (10H, m, ArH), 6.48 (1H, br s, NH), 5.31 (1H, br s, NH), 5.14 (1H, d, $J = 12.1$ Hz, CHH), 5.11 (1H, d, $J = 12.1$ Hz, CHH), 5.10 (2H, s, CH$_2$), 4.35 (1H, d, $J = 6.5$ Hz, CHH), 4.28 (1H, d, $J = 6.5$ Hz, CHH), 4.25 – 4.19 (2H, m, CHH, CH), 4.17 (1H, d,
$J = 6.5$ Hz, CHH), 3.75 (1H, dd, $J = 13.9, 5.8$ Hz, CHH), 3.39 (1H, dd, $J = 13.9, 4.9$ Hz, CHH), 3.13 (1H, d, $J = 5.5$ Hz, CH), 2.03 – 1.89 (2H, m, CH, NH), 1.38 (3H, d, $J = 7.0$ Hz, CHJ), 0.94 (3H, d, $J = 6.8$ Hz, CHJ), 0.87 (3H, d, $J = 6.8$ Hz, CHJ); δC (100 MHz, CDCl3) 176.1 (C=O), 173.0 (C=O), 156.0 (C=O), 136.3 (C), 135.3 (C), 128.8 (CH), 128.8 (CH), 128.7 (CH), 128.7 (CH), 128.4 (CH), 128.4 (CH), 79.7 (CH2), 79.4 (CH2), 67.2 (CH2), 67.1 (CH2), 61.1 (CH), 59.5 (C), 50.9 (CH3), 43.7 (CH2), 32.0 (CH), 19.6 (CH3), 18.8 (CH3), 18.0 (CH3); MS (ES+) m/z 520 [M+Na]+; HRMS (ES+) calcd. for C25H35N3O6 [M+Na]+: 520.2418; found: 520.2420. 9b (53 mg, 0.11 mmol) and 10% Pd/C (11 mg) in MeOH (2.13 mL) were further reacted according to General Method B to give 1b (29 mg, 100%) as a cream solid. M.p. 113–114 °C; [α]$_D^{25}$ +31.5 (c 0.16, MeOH); IR (thin film) 3245, 2960, 2876, 1672, 1558, 1539, 1468, 1393 cm$^{-1}$; δH (400 MHz, D$_2$O) 4.65 (1H, d, $J = 7.0$, CHH), 4.60 (1H, d, $J = 7.3$, CHH), 4.48 (1H, d, $J = 7.3$, CHH), 4.40 (1H, d, $J = 7.0$, CHH), 4.03 (1H, q, $J = 7.0$, CH), 3.75 (1H, d, $J = 14.3$, CHH), 3.59 (1H, d, $J = 14.3$, CHH), 3.07 (1H, d, $J = 5.3$, CH), 1.95 – 1.83 (1H, m, CH), 1.52 (3H, d, $J = 7.0$, CH), 0.93 (6H, d, $J = 6.8$, CH); δC (100 MHz, D$_2$O) 182.3 (C=O), 172.6 (C=O), 79.4 (CH2), 79.2 (CH2), 64.1 (CH), 59.7 (C), 49.4 (CH), 43.6 (CH2), 31.5 (CH), 18.5 (CH3), 18.2 (CH3), 17.0 (CH3); MS (ES+) m/z 274 [M+H]+, 296 [M+Na]+; HRMS (ES+) calcd. for C$_{12}$H$_{24}$N$_3$O$_4$ [M+H]+: 274.1761; found: 274.1769.

[3-[(S)-2-Amino-3-phenylpropanamido)methyl][oxetan-3-yl]-L-isoleucine, 1c

4g (100 mg, 0.30 mmol), In metal (137 mg, 1.19 mmol) and 6 M HCl (298 µL, 1.79 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Phe-OH (95 mg, 0.32 mmol), HOBt (7 mg, 0.05 mmol), NMM (72 µL, 0.65 mmol), EDC-HCl (70 mg, 0.37 mmol) in EtOH (3 mL). Work-up, followed by purification by column chromatography (30 → 50% EtOAc in hexane) provided 9c (102 mg, 58%) as a colourless oil. $R_f = 0.31$ (50% EtOAc in hexane); [α]$_D^{23}$ +16.3 (c 0.20, CHCl3); IR (thin film) 3313, 3033, 2963, 2876, 1724, 1663, 1523, 1498, 1456, 1255, 1174, 976, 746, 698 cm$^{-1}$; δH (400 MHz, CDCl3) 7.39 – 7.20 (13H, m, ArH), 7.18 (2H, d, $J = 7.3$ Hz, ArH), 6.17 – 6.12 (1H, m, NH), 5.41 (1H, d, $J = 7.5$ Hz, NH), 5.10 (1H, d, $J = 12.3$ Hz, CHH), 5.08 (2H, s, CH2), 5.07 (1H, d, $J = 12.3$ Hz, CHH), 4.43 – 4.36 (1H, m, CH), 4.28 (1H, d, $J = 6.5$ Hz, CHH), 4.14 (1H, d, $J = 6.5$ Hz, CHH), 4.06 (1H, d, $J = 6.5$ Hz, CHH), 4.01 (1H, d, $J = 6.5$ Hz, CHH), 3.64 (1H, dd, $J = 13.9, 5.4$ Hz, CHH), 3.32 (1H, dd, $J = 13.9, 4.9$ Hz, CHH), 3.16
(1H, dd, J = 13.7, 5.8 Hz, CHH), 3.05 (1H, d, J = 5.8 Hz, CH), 2.99 (1H, dd, J = 13.7, 7.8 Hz, CHH), 1.83 (1H, br s, NH), 1.64 – 1.54 (1H, m, CH), 1.39 – 1.28 (1H, m, CHH), 1.04 – 0.90 (1H, m, CHH), 0.83 – 0.78 (6H, m, CH2); δC (100 MHz, CDCl3) 176.0 (C=O), 171.3 (C=O), 155.9 (C=O), 136.6 (C), 136.3 (C), 135.2 (C), 129.4 (CH), 128.9 (CH), 128.8 (CH), 128.7 (CH), 128.7 (CH), 128.4 (CH), 127.2 (CH), 79.7 (CH2), 79.6 (CH2), 67.2 (2 x CH2), 60.2 (CH), 59.0 (C), 56.5 (CH), 43.7 (CH2), 38.9 (CH), 38.8 (CH), 24.9 (CH2), 15.9 (CH3), 11.6 (CH3); MS (ES+) m/z 588 [M+H]+, 610 [M+Na]+; HRMS (ES+) calcd. for C34H42N2O6 [M+H]+: 588.3050; found: 588.3068.

9c (91 mg, 0.15 mmol) and 10% Pd/C (18 mg) in MeOH (3.1 mL) were further reacted according to General Method B to give 1e (54 mg, 96%) as a pale orange solid. M.p. 162 – 164 °C (dec); [α]D26 +43.2 (c 0.18, MeOH); IR (thin film) 3313, 3030, 2974, 2874, 1670, 1560, 1555, 1497, 1453, 1437, 1387, 972, 742, 721, 396 cm⁻¹; δH (500 MHz, DMSO-d6) 8.20 (1H, br t, J = 5.4 Hz, NH), 7.31 – 7.26 (2H, m, ArH), 7.25 – 7.18 (3H, m, ArH), 4.34 (1H, d, J = 6.2 Hz, CHH), 4.31 (1H, d, J = 6.3 Hz, CHH), 4.18 (1H, d, J = 6.3 Hz, CHH), 4.13 (1H, d, J = 6.2 Hz, CHH), 3.61 (1H, dd, J = 7.8, 5.4 Hz, CH), 3.48 – 3.40 (2H, m, CH2), 3.10 (1H, d, J = 5.5 Hz, CH), 3.00 (1H, dd, J = 13.6, 5.4 Hz, CHH), 2.74 (1H, dd, J = 13.6, 7.8 Hz, CHH), 1.59 – 1.44 (2H, m, CH and CHH), 1.12 – 1.01 (1H, m, CHH), 0.87 – 0.79 (6H, m, CH3); δC (125 MHz, DMSO-d6) 177.2 (C=O), 173.2 (C=O), 137.6 (C), 129.3 (CH), 128.2 (CH), 126.4 (CH), 78.5 (CH2), 78.2 (CH2), 60.1 (CH), 59.8 (C), 55.4 (CH), 43.4 (CH2), 39.7 (CH2), 38.1 (CH), 24.5 (CH2), 15.7 (CH3), 11.6 (CH3); MS (ES+) m/z 364 [M+H]+, 386 [M+Na]+; HRMS (ES+) calcd. for C19H30N3O4 [M+H]+: 364.2231; found: 364.2228.

[3-[(S)-2-Amino-3-methylbutanamido)methyl]oxetan-3-yl]-L-valine, 1d

4b (102 mg, 0.32 mmol), In metal (144 mg, 1.25 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Val-OH (82 mg, 0.33 mmol), HOBt (8 mg, 0.05 mmol), NMM (75 µL, 0.68 mmol), EDC·HCl (73 mg, 0.38 mmol) in EtOH (3.1 mL). Work-up, followed by purification by column chromatography (35 → 50% EtOAc in hexane) provided 9d (78 mg, 47%) as a white oily solid. Rf = 0.24 (50% EtOAc in hexane); [α]D20 +20.5 (c 0.20, CHCl3); IR (thin film) 3311, 2963, 2876, 1723, 1659, 1521, 1498, 1456, 1233, 1177, 1152, 1027, 976, 737, 696 cm⁻¹; δH (400 MHz, CDCl3) 7.39 – 7.28 (10H, m, ArH), 6.46 – 6.41 (1H, m, NH), 5.39 (1H, d, J = 8.3 Hz, NH), 5.14 (1H, d, J = 12.1 Hz, CHH), 5.11 (1H, d, J = 12.1 Hz, CHH),
5.10 (2H, s, CH₃), 4.35 (1H, d, J = 6.5 Hz, CHH), 4.29 (1H, d, J = 6.5 Hz, CHH), 4.22 (1H, d, J = 6.5 Hz, CHH), 4.17 (1H, d, J = 6.5 Hz, CHH), 3.99 (1H, dd, J = 8.3, 6.0 Hz, CH), 3.74 (1H, dd, J = 13.8, 5.5 Hz, CHH), 3.42 (1H, dd, J = 13.8, 5.0 Hz, CHH), 3.13 (1H, d, J = 5.3 Hz, CH), 2.22 – 2.09 (1H, m, CH), 2.07 – 1.90 (2H, m, CH, NH), 0.98 – 0.93 (6H, m, CH₃), 0.91 (3H, d, J = 7.0 Hz, CH₃), 0.87 (3H, d, J = 6.8 Hz, CH₃); δ_C (100 MHz, CDCl₃) 176.1 (C=O), 171.8 (C=O), 156.5 (C=O), 156.4 (C), 135.3 (C), 128.8 (CH), 128.8 (CH), 128.7 (CH), 128.7 (CH), 128.3 (CH), 128.2 (CH), 79.7 (CH₂), 79.5 (CH₂), 67.2 (CH₂), 67.2 (CH₂), 61.1 (CH), 60.8 (CH), 59.4 (C), 43.7 (CH₂), 32.0 (CH), 31.0 (CH), 19.6 (CH₃), 19.3 (CH₃), 18.0 (CH₃), 17.9 (CH₃); MS (ES⁺) m/z 526 [M+H]⁺, 548 [M+Na]⁺; HRMS (ES⁺) calcd. for C₂₀H₂₆N₄O₆ [M+Na]⁺: 548.2731; found: 548.2724. 9d (61 mg, 0.12 mmol) and 10% Pd/C (12 mg) in MeOH (2.3 mL) were reacted according to General Method B to give 1d (33 mg, 94%) as a cream solid. M.p. 102 – 103 °C; [α]D₂⁵ +56.6 (c 0.16, MeOH); IR (thin film) 3244, 2960, 2875, 1680, 1555, 1540, 1467, 1393 cm⁻¹; δ_H (400 MHz, D₂O) 4.66 (1H, d, J = 7.0 Hz, CHH), 4.61 (1H, d, J = 7.3 Hz, CHH), 4.48 (1H, d, J = 7.3 Hz, CHH), 4.42 (1H, d, J = 7.0 Hz, CHH), 3.77 (1H, d, J = 14.3 Hz, CHH), 3.75 (1H, d, J = 6.0 Hz, CH), 3.61 (1H, d, J = 14.3 Hz, CHH), 3.09 (1H, d, J = 5.3 Hz, CH), 2.28 – 2.15 (1H, m, CH), 1.95 – 1.83 (1H, m, CH), 1.04 (3H, d, J = 7.0 Hz, CH₃), 1.03 (3H, d, J = 7.0 Hz, CH₃), 0.93 (6H, d, J = 6.8 Hz, CH₃); δ_C (100 MHz, D₂O) 182.3 (C=O), 171.1 (C=O), 79.3 (CH₂), 79.1 (CH₂), 64.1 (CH), 59.7 (C), 59.1 (CH), 43.8 (CH₂), 31.5 (CH), 30.0 (CH), 18.5 (CH₃), 18.2 (CH₃), 17.8 (CH₃), 16.9 (CH₃); MS (ES⁺) m/z 302 [M+H]⁺, 324 [M+Na]⁺; HRMS (ES⁺) calcd. for C₁₄H₂₈N₃O₄ [M+H]⁺: 302.2074; found: 302.2066.

[3-[(S)-2-Amino-4-methylpentanamido)methyl]oxetan-3-yl]-L-isoleucine, 1e

4g (104 mg, 0.31 mmol), In metal (145 mg, 1.26 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Leu-OH (98 mg, 0.33 mmol), HOBt (8 mg, 0.05 mmol), NMM (75 μL, 0.68 mmol), EDC-HCl (73 mg, 0.38 mmol) in EtOH (3.1 mL). Work-up, followed by purification by repeat column chromatography (30 → 45% EtOAc in hexane; 2 → 4% MeOH in CH₂Cl₂) provided 9e (92 mg, 54%) as a colourless oil. Rₚ = 0.37 (50% EtOAc in hexane); [α]D²³ +9.1 (c 0.20, CHCl₃); IR (thin film) 3314, 3034, 2960, 2875, 1724, 1718, 1660, 1529, 1456, 1237, 1172, 975, 737, 697 cm⁻¹; δ_H (400 MHz, CDCl₃) 7.39 – 7.28 (10H, m, ArH), 6.58 – 6.52 (1H, m, NH), 5.23 (1H, d, J = 7.8 Hz, NH), 5.12 (2H, s, CH₂), 5.09 (2H, s, CH₂), 4.35 (1H, d, J =
6.13 (1H, m, CHH), 4.29 (1H, d, J = 6.3 Hz, CHH), 4.23 – 4.14 (3H, m, CHH, CH), 3.74 (1H, dd, J = 14.0, 5.7 Hz, CHH), 3.39 (1H, dd, J = 14.0, 5.0 Hz, CHH), 3.20 (1H, d, J = 5.5 Hz, CH), 2.03 (1H, br s, NH), 1.75 – 1.60 (3H, m, CHH, CH), 1.55 – 1.37 (2H, m, CHH, CHH), 1.17 – 1.04 (1H, m, CHH), 0.97 – 0.81 (12H, m, CH₃); δ (100 MHz, CDCl₃) 176.1 (C=O), 172.9 (C=O), 156.2 (C=O), 136.3 (C), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.3 (CH), 128.2 (CH), 79.8 (CH₂), 79.4 (CH₂), 67.2 (2 x CH₂), 60.3 (CH), 59.5 (C), 53.8 (CH), 43.7 (CH₂), 41.7 (CH₂), 39.0 (CH), 24.9 (CH₂), 24.9 (CH), 23.0 (CH₃), 22.1 (CH₃), 16.0 (CH₃), 11.7 (CH₃); MS (ES⁺) m/z 554 [M+H]⁺, 576 [M+Na]⁺; HRMS (ES⁺) calcd. for C₃₁H₄₄N₅O₆ [M+H]⁺: 554.3225; found: 554.3210. 9e (90 mg, 0.16 mmol) and 10% Pd/C (18 mg) in MeOH (3.25 mL) were further reacted according to General Method B to give 1e (53 mg, 99%) as a pale cream/orange solid. M.p. 130 – 132 °C (dec); [α]D²⁵ +36.6 (c 0.18, MeOH); IR (thin film) 3200, 2960, 2875, 1673, 1557, 1550, 1466, 1391 cm⁻¹; δH (500 MHz, D₂O) 4.70 (1H, d, J = 7.4 Hz, CHH), 4.64 (1H, d, J = 7.6 Hz, CHH), 4.52 (1H, d, J = 7.6 Hz, CHH), 4.45 (1H, d, J = 7.4 Hz, CHH), 4.05 (1H, t, J = 7.3 Hz, CH), 3.77 (1H, d, J = 14.5 Hz, CHH), 3.66 (1H, d, J = 14.5 Hz, CHH), 3.32 (1H, d, J = 4.6 Hz, CH), 1.81 – 1.65 (4H, m, CH₂ and CH), 1.57 – 1.48 (1H, m, CHH), 1.25 – 1.15 (1H, m, CHH), 0.98 (3H, d, J = 6.5 Hz, CH₃), 0.96 (3H, d, J = 6.5 Hz, CH₃), 0.93 – 0.89 (6H, m, CH₃); δ (125 MHz, D₂O) 179.9 (C=O), 171.5 (C=O), 78.3 (CH₂), 78.0 (CH₂), 63.0 (CH), 60.4 (C), 52.1 (CH), 43.2 (CH₂), 39.8 (CH₂), 38.2 (CH), 25.5 (CH₂), 23.9 (CH), 21.7 (CH₃), 20.9 (CH₃), 14.8 (CH₃), 11.2 (CH₃); MS (ES⁺) m/z 330 [M+H]⁺, 352 [M+Na]⁺; HRMS (ES⁺) calcd. for C₁₆H₁₇N₄O₄ [M+Na]⁺: 352.2207; found: 352.2208.

[3-[(S)-2-Amino-3-phenylpropanamido)methyl]oxetan-3-yl]-L-valine, 1f

4b (101 mg, 0.31 mmol), In metal (145 mg, 1.26 mmol) and 6 M HCl (0.31 mL, 1.86 mmol) were reacted according to General Method A. The crude amine was further reacted with Z-L-Phe-OH (97 mg, 0.32 mmol), HOBT (9 mg, 0.06 mmol), NMM (75 µL, 0.68 mmol), EDC-HCl (73 mg, 0.38 mmol) and EtOH (3.1 mL). Work-up, followed by purification by column chromatography (40 → 50% EtOAc in hexane) provided 9f (107 mg, 60%) as a colourless oil. Rf = 0.26 (50% EtOAc in hexane); [α]D⁰²¹ +15.5 (c 0.20, CHCl₃); IR (thin film) 3320, 2962, 2875, 1724, 1663, 1525, 1498, 1456, 1254, 1179, 1152, 977, 747, 698 cm⁻¹; δH (400 MHz, CDCl₃) 7.39 – 7.21 (13H, m, ArH), 7.18 (2H, d, J = 7.5 Hz, ArH), 6.19 – 6.13 (1H, m, NH), 5.43 – 5.36 (1H, m, NH), 5.12 – 5.04 (4H, m, CH₂), 4.43 – 4.35 (1H, m,
CH), 4.28 (1H, d, J = 6.5 Hz, CHH), 4.14 (1H, d, J = 6.5 Hz, CHH), 4.06 (1H, d, J = 6.5 Hz, CHH), 4.02 (1H, d, J = 6.5 Hz, CHH), 3.64 (1H, dd, J = 13.9, 5.4 Hz, CHH), 3.33 (1H, dd, J = 13.9, 5.0 Hz, CHH), 3.15 (1H, dd, J = 13.8, 6.3 Hz, CHH), 3.04 – 2.97 (2H, m, CHH, CHH), 1.92 – 1.78 (2H, m, CH, NH), 0.86 (3H, d, J = 6.8 Hz, CH$_3$), 0.78 (3H, d, J = 6.8 Hz, CH$_3$); δc (100 MHz, CDCl$_3$) 176.0 (C=O), 171.3 (C=O), 155.9 (C=O), 136.6 (C), 136.3 (C), 135.3 (C), 129.4 (CH), 128.9 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.3 (CH), 128.2 (CH), 127.2 (CH), 79.6 (CH), 79.6 (CH), 67.2 (CH$_2$), 67.2 (CH$_2$), 61.0 (CH), 59.0 (C), 56.6 (CH), 43.7 (CH$_2$), 38.8 (CH$_2$), 32.0 (CH), 19.5 (CH$_3$), 18.0 (CH$_3$); MS (ES$^+$) m/z 596 [M+Na]$^+$; HRMS (ES$^+$) calcd. for C$_{13}$H$_{30}$N$_3$NaO$_6$ [M+Na]$^+$: 596.2731; found: 596.2722.

9f (89 mg, 0.16 mmol) and 10% Pd/C (18 mg) in MeOH (3.1 mL) were further reacted according to General Method B to give 1f (52 mg, 96%) as a pale yellow solid. M.p. 165 – 167 °C (dec); [α]$^2$$_D$ +41.3 (c 0.18, MeOH); IR (thin film) 3045, 2958, 2890, 1666, 1559, 1512, 1455, 1436, 1390, 974, 742, 697 cm$^{-1}$; δh (500 MHz, DMSO-$d_6$) 8.23 – 8.19 (1H, m, NH), 7.31 – 7.27 (2H, m, ArH), 7.25 – 7.20 (3H, m, ArH), 4.35 (1H, d, J = 6.2 Hz, CHH), 4.29 (1H, d, J = 6.3 Hz, CHH), 4.17 (1H, d, J = 6.3 Hz, CHH), 4.13 (1H, d, J = 6.2 Hz, CHH), 3.67 – 3.63 (1H, m, CH), 3.49 – 3.40 (2H, m, CH$_2$), 3.06 (1H, d, J = 5.7 Hz, CH), 3.02 (1H, dd, J = 13.6, 5.2 Hz, CHH), 2.75 (1H, dd, J = 13.6, 8.0 Hz, CHH), 1.87 – 1.79 (1H, m, CH), 0.88 (3H, d, J = 6.8 Hz, CH$_3$), 0.83 (3H, d, J = 6.8 Hz, CH$_3$); δc (125 MHz, DMSO-$d_6$) 177.1 (C=O), 172.8 (C=O), 137.4 (C), 129.4 (CH), 128.3 (CH), 126.5 (CH), 78.4 (CH$_2$), 78.2 (CH$_2$), 60.7 (CH), 59.7 (C), 55.3 (CH), 43.4 (CH$_2$), 38.9 (CH$_2$), 31.1 (CH), 19.4 (CH$_3$), 18.0 (CH$_3$); MS (ES$^+$) m/z 350 [M+H]$^+$, 372 [M+Na]$^+$; HRMS (ES$^+$) calcd. for C$_{18}$H$_{28}$N$_3$O$_4$ [M+H]$^+$: 350.2074; found: 350.2070.

(S)-6-Benzyl-2-oxa-5,8-diazaspiro[3.5]nonan-7-one, 7

To a stirred solution of 4h (98 mg, 0.26 mmol) in glacial AcOH (4.5 mL) was added Zn (628 mg, 9.60 mmol).[4] The reaction mixture was stirred vigorously at rt for 67 h then filtered through Celite® and concentrated in vacuo. The residue was diluted with sat. NaHCO$_3$ (15 mL) and extracted with EtOAc (3 x 20 mL). The combined organic extracts were dried over MgSO$_4$, filtered and concentrated in vacuo. Purification by column chromatography (0 → 10% MeOH in EtOAc with 0.5% NEt$_3$) provided 7 (42 mg, 68%) as a white solid. M.p. 156 – 158 °C; R$_f$ = 0.30 (10% MeOH in EtOAc); [α]$^2$$_D$ –98.9 (c 0.18, CHCl$_3$); IR (thin film) 3286, 3061, 2945, 2869, 1665, 1604, 1495, 1455, 1314, 975, 734, 700 cm$^{-1}$; δh (400 MHz, CDCl$_3$) 7.37 – 7.32 (2H, m,
ArH), 7.31 – 7.25 (3H, m, ArH), 6.30 (1H, br s, NH), 4.57 (1H, d, \( J = 6.5 \) Hz, CHH), 4.49 (1H, d, \( J = 6.5 \) Hz, CHH), 4.41 (1H, d, \( J = 7.0 \) Hz, CHH), 4.38 (1H, d, \( J = 7.0 \) Hz, CHH), 3.72 (1H, dd, \( J = 10.0, 3.4 \) Hz, CHH), 3.68 (1H, dd, \( J = 11.5, 4.0 \) Hz, CHH), 3.52 – 3.44 (2H, m, CHH), 2.80 (1H, dd, \( J = 13.7, 10.0 \) Hz, CHH), 1.86 (1H, br s, NH); \( \delta_c \) (100 MHz, CDCl\(_3\)) 170.8 (C=O), 137.8 (C), 129.4 (CH), 129.0 (CH), 127.0 (CH), 81.8 (CH\(_2\)), 79.9 (CH\(_2\)), 56.3 (CH), 55.2 (C), 49.4 (CH\(_2\)), 38.5 (CH\(_2\)); MS (ES\(^+\)) \( m/z \) 233 [M+H]\(^+\), 255 [M+Na]\(^+\); HRMS (ES\(^+\)) calcd. for C\(_{13}\)H\(_{16}\)N\(_2\)O\(_2\) [M+Na]\(^+\): 255.1104; found: 255.1106.
$^1$H NMR

Chemical Shift (ppm)

$^{13}$C NMR

Chemical Shift (ppm)
\[ ^1H \text{ NMR} \]

Compound (R)-4a

\[ ^{13}C \text{ NMR} \]
**$^1$H NMR**

Compound 4b

![NMR spectrum for Compound 4b](image)

**$^{13}$C NMR**

![C NMR spectrum for Compound 4b](image)
$^1$H NMR

Compound 4c

$^{13}$C NMR
\[^1\text{H NMR}\]

**Compound 4d**

\[\text{H}_2\text{N-CON_2Bn}\]

\[\begin{align*}
\text{Chemical Shift (ppm)} & \quad 4.61 & \quad 2.00 & \quad 2.03 & \quad 2.08 & \quad 2.05 & \quad 2.00 & \quad 0.89 \\
\end{align*}\]

\[\begin{align*}
\text{Chemical Shift (ppm)} & \quad 7.0 & \quad 6.5 & \quad 6.0 & \quad 5.5 & \quad 5.0 & \quad 4.5 & \quad 4.0 & \quad 3.5 & \quad 3.0 & \quad 2.5 & \quad 2.0 & \quad 1.5 & \quad 1.0 & \quad 0.5 & \quad 0 \\
\end{align*}\]

\[\begin{align*}
\text{Chemical Shift (ppm)} & \quad 171.70 & \quad 135.22 & \quad 128.80 & \quad 128.74 & \quad 128.63 & \quad 78.53 & \quad 78.32 & \quad 67.32 & \quad 59.52 & \quad 44.91 \\
\end{align*}\]

\[^{13}\text{C NMR}\]

\[\begin{align*}
\text{Chemical Shift (ppm)} & \quad 170 & \quad 160 & \quad 150 & \quad 140 & \quad 130 & \quad 120 & \quad 110 & \quad 100 & \quad 90 & \quad 80 & \quad 70 & \quad 60 & \quad 50 & \quad 40 & \quad 30 & \quad 20 & \quad 10 & \quad 0 \\
\end{align*}\]

\[\begin{align*}
\text{Chemical Shift (ppm)} & \quad 171.70 & \quad 135.22 & \quad 128.80 & \quad 128.74 & \quad 128.63 & \quad 78.53 & \quad 78.32 & \quad 67.32 & \quad 59.52 & \quad 44.91 \\
\end{align*}\]
$^1$H NMR

Compound 4e

$^{13}$C NMR
$^1$H NMR

Compound 4f

$^{13}$C NMR
$^1$H NMR

Compound 4g

$^{13}$C NMR
$^1$H NMR

**Compound 4h**

\[ \text{Compound 4h} \]

\[
\begin{align*}
\text{Chemical Shift (ppm)} & \\
1.00 & \quad 1.06 & \quad 1.07 & \quad 0.04 & \quad 1.06 & \quad 1.06 & \quad 1.65 & \quad 1.05 & \quad 1.04 & \quad 1.04 & \quad 1.05 & \quad 1.05 & \quad 1.06 & \quad 1.06 & \quad 1.04 & \quad 1.07 & \quad 1.06 & \quad 1.00
\end{align*}
\]

$^{13}$C NMR

\[
\begin{align*}
\text{Chemical Shift (ppm)} & \\
174.52 & \quad 173.60 & \quad 136.60 & \quad 123.55 & \quad 128.81 & \quad 127.15 & \quad 78.84 & \quad 78.73 & \quad 78.63 & \quad 77.16 & \quad 59.45 & \quad 59.34 & \quad 56.77 & \quad 50.77
\end{align*}
\]
$^1$H NMR

Compound 4i

13C NMR
$^1$H NMR

Compound 4j

$^{13}$C NMR
Compound 4k

$^1$H NMR

Chemical Shift (ppm)

$^{13}$C NMR

Chemical Shift (ppm)
$^1$H NMR

Compound 1a

![NMR Spectrum](image)

$^{13}$C NMR

![C NMR Spectrum](image)

Chemical Shift (ppm)

Chemical Shift (ppm)
$^1$H NMR

Compound 1b

\[
\begin{align*}
\text{Chemical Shift (ppm)} &= 0.5 \quad 1.0 \quad 1.5 \quad 2.0 \quad 2.5 \quad 3.0 \quad 3.5 \quad 4.0 \quad 4.5 \\
\end{align*}
\]

$^{13}$C NMR

\[
\begin{align*}
\text{Chemical Shift (ppm)} &= 0 \quad 10 \quad 20 \quad 30 \quad 40 \quad 50 \quad 60 \quad 70 \quad 80 \quad 90 \quad 100 \quad 110 \quad 120 \quad 130 \quad 140 \quad 150 \quad 160 \quad 170 \quad 180 \\
\end{align*}
\]
$^1$H NMR

Compound 1c

$^{13}$C NMR
$^1$H NMR

Compound 1d

$^{13}$C NMR
$^1$H NMR

Compound 1e

\[ \text{Chemical Shift (ppm)} \]

\[
\begin{align*}
\delta & = 1.07, 1.01, 0.97, 0.96, 0.97, 0.89, 4.15, 1.04, 1.05, 6.01, 6.00
\end{align*}
\]

$^{13}$C NMR

\[ \text{Chemical Shift (ppm)} \]

\[
\begin{align*}
\delta & = 179.90, 171.54, 78.31, 77.97, 63.04, 60.37, 52.09, 43.22, 39.79, 38.18, 25.48, 23.87, 21.73, 20.94, 14.77, 11.24
\end{align*}
\]
$^1$H NMR

Compound 1f

$^{13}$C NMR
$^1$H NMR

Compound 7

$^{13}$C NMR
Chiral HPLC analysis of 4a
(HPLC OD-H, 95: 5 hexane : ‘PrOH, flow rate = 0.30 µL/min, λ = 254 nm)

Mixture of (R)-4a and (S)-4a
(S)-4a

(R)-4a
X-Ray Crystallography

1c [CCDC 996044]:

Contents of the unit cell of 1c showing the two crystallographically independent molecules and a partially occupied molecule of methanol.

Part of the infinite hydrogen bonded anti-parallel sheet in the solid state of 1c that travels along the b axis of the cell (closest Miller plane 1 0 -2).
1e [CCDC 996043]:

Solid state structure of one of the crystallographically independent molecules in the unit cell of 1e
**Molecular Dynamics Simulations**

**Computational Methods**

*CHARMM-compatible forcefield parameters for the 3-amino oxetane residue*

The CHARMM forcefield is one of the most widely used forcefields for molecular dynamics (MD) simulations of peptides, proteins and other biomolecules;[5] however there are no parameters for oxetanes within the CHARMM forcefield. Therefore to enable MD simulations of oxetane isosteres it was necessary to develop a set of CHARMM-compatible forcefield parameters for this class of molecules. Following the general guidelines for the CHARMM forcefield we adopted a ‘divide-and-conquer’ approach by developing parameters for small molecular fragments (see Figure A) that capture the essential chemistry of the modified peptide bond (carbonyl to oxetane substitution and rotation about the $\psi$ and $\omega$ dihedral angles). Non-bonded Lennard-Jones parameters for the C, O and H atoms comprising the oxetane ring (hereafter denoted atom types COX, OOX and HOX respectively and depicted in Figure B) were taken from existing CHARMM parameters for tetrahydrofuran (THF).[6] For completeness, the Lennard-Jones parameters are reported in Table A.

![Figure A](image)

**Figure A** Molecular fragments used in the parameterisation of oxetane isosteres. (A) Oxetane molecule and oxetane molecules with (B) NH$_2$ group, (C) CH$_3$ group and (D) both NH$_2$ and CH$_3$ groups, used to develop bonded parameters.
Figure B (A) Atomic partial charges on oxetane atoms; (B) non-bonded atom types referred to in this work.

Table A Non-bonded Lennard-Jones parameters for the oxetane atoms.

<table>
<thead>
<tr>
<th>Atom type</th>
<th>$\sigma$/nm</th>
<th>$\epsilon$/kJ mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>OOX</td>
<td>0.2940</td>
<td>0.41840</td>
</tr>
<tr>
<td>COX</td>
<td>0.3599</td>
<td>0.25104</td>
</tr>
<tr>
<td>HOX</td>
<td>0.2316</td>
<td>0.14644</td>
</tr>
</tbody>
</table>

Atomic partial charges were determined using the Force Field Toolkit (ffTK) Plugin\cite{7} to the Visual Molecular Dynamics (VMD) software package.\cite{8} Quantum mechanical (QM) calculations were performed using the Gaussian 03 program.\cite{9} In brief, partial charges were chosen to reproduce the QM interaction energy with a TIP3P\cite{10} water molecule. First, an oxetane molecule was energy minimized at the MP2/6-31G* level of theory. Putative hydrogen bond acceptor or donor interaction sites were identified and a water molecule automatically constructed in an ideal orientation for hydrogen bonding with each interaction site. For each oxetane-water complex, separate calculations were carried out at the HF/6-31G* level of theory to optimize the distance of the water molecule to the target atom and the rotation angle of the water about the line connecting them. The interaction energy was determined from the difference between the energy of the complex and the independent energies of an oxetane and a water molecule. Consistent with the CHARMM procedure to better approximate the bulk-phase, QM-optimised distances were shifted by -0.2 Å and the interaction energies scaled by 1.16. The ffKT then iteratively fit atomic partial charges to the QM target data so that the molecular mechanics (MM) interaction energy reproduced the QM interaction energy, subject to the constraints that hydrogen atoms have a default charge of
+0.09 and the molecule is charge neutral. The final set of partial charges for an oxetane-substituted amino acid derivative is given in Figure B.

Target data for fitting bond stretching, angle bending and dihedral angle rotation parameters were obtained from QM calculations at the MP2/6-31G* level of theory. In each case the relevant molecular fragment (see Figure A and Tables B-D) was selected and a relaxed potential energy surface scan was carried out where the particular bond, angle or dihedral of interest was constrained in increments about the energy minimum (increments of 0.1 Å for bonds, 1° for angles and 5° for dihedrals involving rings) or about a 360° rotation (increments of 10° for all other dihedrals). Equilibrium values and force constants for bond stretching (see Table B for parameters) and angle bending (see Table C for parameters) were obtained by fitting a harmonic function to the resulting potential energy surface. For dihedral angle rotation, the Lennard-Jones and electrostatic contribution needed to be taken into account. For each QM structure the CHARMM MM energy (excluding the dihedral to parameterize) was calculated and the periodic function \( V_{\text{dih}} = k_{\phi} \left( 1 + \cos(n\phi - \gamma) \right) \) fitted to the difference between the QM and MM energies, where \( V_{\text{dih}} \) is the potential energy associated with rotation about the dihedral angle \( \phi \), \( k_{\phi} \) defines the barrier height, \( n \) is the multiplicity, and \( \gamma \) is the phase shift (see Table D for parameters). All other peptide parameters were taken from the standard CHARMM all atom forcefield.\([11,12]\)

Table B Bond stretching parameters for the bond energy between atoms \( i \) and \( j \), where \( b_{\text{eq}} \) is the equilibrium bond length and \( k_b \) is the force constant.

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Atom type ( i )</th>
<th>Atom type ( j )</th>
<th>( b_{\text{eq}}/\text{nm} )</th>
<th>( k_b/\text{kJ mol}^{-1}\text{nm}^2 )</th>
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</thead>
<tbody>
<tr>
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<td>COX</td>
<td>COX</td>
<td>0.1549</td>
<td>135381</td>
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<td>136963</td>
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<td>C</td>
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<td>0.1530</td>
<td>148818</td>
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<tr>
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<td>HOX</td>
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<tr>
<td>B</td>
<td>COX</td>
<td>NH1(^*)</td>
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<td>137639</td>
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\(^*\)CT2 and NH1 are the CHARMM atom types for the C\(_\alpha\) atom in Gly and the amide N in a peptide bond respectively.
Table C Angle bending parameters for the angle bending energy involving atoms $i, j$ and $k$, where $\theta_{eq}$ is the equilibrium bond angle and $k_\theta$ is the force constant.

<table>
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<tr>
<th>Fragment</th>
<th>Atom type $i$</th>
<th>Atom type $j$</th>
<th>Atom type $k$</th>
<th>$\theta_{eq}$/degrees</th>
<th>$k_\theta$/kJ mol$^{-1}$ rad$^{-2}$</th>
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<tr>
<td>D</td>
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<tr>
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<td>COX</td>
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<tr>
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<td>COX</td>
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<tr>
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<td>HOX</td>
<td>COX</td>
<td>OOX</td>
<td>COX</td>
<td>180.00</td>
</tr>
</tbody>
</table>
Initial configurations of the peptides

Starting coordinates of the peptides were generated using the protein builder tool in the Tinker software package,[13] assuming a linear conformation ($\phi$, $\psi$ and $\omega$ backbone dihedral angles were set to 180°) and zwitterionic ends. The C=O to oxetane substitution was made by replacing the O atom with energy minimized oxetane coordinates from the QM calculation described above. Input topology files for Gromacs[14] were generated using the Gromacs pdb2gmx tool after first modifying the residue database to include oxetane-substituted residues. Each structure was solvated with 2100 TIP3P[10] water molecules.

Simulation parameters

Energy minimization and MD simulations were carried out using the Gromacs simulation package version 4.5.5.[14] Each solvated peptide was subjected to 50000 steps of steepest descents energy minimization. This was followed by 50000 steps of simulation at 300 K in the NVT ensemble and 50000 steps of simulation at 300 K and 1 bar in the NPT ensemble to equilibrate the temperature and density of the system respectively. To overcome the issue of kinetic trapping in local minima and enhance the sampling of conformational space, each peptide was then simulated for 100 ns at 500 K in the NVT ensemble. Cluster analysis (see below) was performed on the resultant trajectory to group peptide conformations according to their structural similarity. The central structure of the top ten most populated clusters (which accounted for >99.9% of the total population) was then used as the starting configuration for ten independent 100 ns simulations of each peptide at 300K and 1 bar in the NPT ensemble. Accordingly, each peptide was simulated for a total simulation time of 1 µs.

In all MD simulations, all bonds were constrained using the LINCS algorithm[15] and a simulation timestep of 2 fs was used. Periodic boundary conditions were applied in all directions. Lennard-Jones interactions were cutoff at 1.0 nm. Electrostatic interactions were handled using the particle mesh Ewald approach with a real-space cutoff of 1.0 nm. The temperature was controlled using velocity rescaling with a stochastic term[16] with a time constant of 0.1 ps and the pressure was isotropically maintained at 1 bar using the Berenden barostat[17] with time constant 1.0 ps and compressibility 4.5 x 10^-5 bar^-1. Atomic coordinates were saved every 10 ps for analysis.
Assessment of convergence and sampling

Cluster analysis was used to identify distinct peptide conformations from the trajectories. We used the algorithm proposed by Daura et al.\textsuperscript{[18]} whereby the root mean square deviation (RMSD) of atom positions between all pairs of structures is determined. For each structure, the number of other structures for which the RMSD of the backbone atoms was $\leq 0.07$ nm (neighbour conformations) was calculated. The structure with the highest number of neighbours was taken as the centre of a cluster and together with its neighbours formed the first cluster. All these structures were eliminated from the pool of structures and the process repeated to find new clusters until the pool of structures was empty. In this way, each structure belonged to only one cluster. To assess whether the sampling was adequate in our simulations, we calculated the number of clusters as a function of the total simulation time (i.e. the number of clusters in the first independent 100 ns trajectory was determined, then the first and second trajectories were joined together and the number of clusters determined, then the first, second and third trajectories were joined together and so on until all ten trajectories were included). As shown in Figure C, no new structures for 8 are found after the first two 100 ns simulations, whereas eight 100 ns simulations are needed before no new structures are found for the oxetane-modified peptide, 1e. The relative population of each cluster with increasing simulation time is shown in Figure D. It can be seen that the cluster populations have converged with the last 200 ns of simulation essentially contributing no new structural information.

![Figure C](image)

**Figure C** Number of clusters as the 10 independent 100 ns simulations are added to the trajectory.
**Simulation Results**

Analysis of the MD simulations was carried out on the entire 1 µs trajectory. The distribution of the distance between the C-terminal C atom and the N-terminal N atom of each peptide is presented in Figure E where folded conformations are characterised by a C-N distance of between 3 and 5 Å. The distribution for 1e has two distinct peaks which indicates that 1e interchanges between folded and extended conformations. On the other hand, the distribution for 8 is dominated by a single broader peak centred around 8 Å which indicates that the peptide spends most of the time in an extended conformation with the folded conformation occurring only rarely. The distribution of the distance between the Leu and Ile Cα atoms is shown in Figure F. Hydrogen bonds were identified using geometric criteria whereby a hydrogen bond is said to exist if the donor-acceptor distance < 2.0 Å and the angle donor-hydrogen-acceptor ≤ 20°. The percentage occupancy of all intra-peptide hydrogen bonds is given in Table E. Hydrogen-bonding is negligible in the natural peptide, 8. In the oxetane-containing tripeptide 1e limited (~12-15% occupancy) hydrogen bonding is identified between the C and N termini (this may simply be a consequence of the electrostatic...
interaction between the termini) and the C terminus and the N atom of the modified Gly residue, which may further stabilise the folded conformation.

**Figure E** Normalised distribution of the distance between the C-terminal C atom and the N-terminal N atom obtained from MD simulations in solution.

**Figure F** Normalised distribution of the distance between the Leu and Ile Cα atoms obtained from MD simulations in solution.

**Table E** Occupancy of intra-peptide hydrogen-bonds for 8 and 1e in solution.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Donor Atom</th>
<th>Acceptor Atom</th>
<th>% Occupancy</th>
</tr>
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<tbody>
<tr>
<td>8</td>
<td>Leu N</td>
<td>Ile O</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>Leu N</td>
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<tr>
<td>1e</td>
<td>Gly N</td>
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<td>Ile N</td>
<td>Leu O</td>
<td>0.13</td>
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References