Supporting Information

for

Studies on the synthesis of peptides containing dehydrovaline and dehydroisoleucine based on copper-mediated enamide formation

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Syntheses and analytical descriptions of reagents and peptides and copies of $^1$H and $^{13}$C NMR spectra

1. General information
2. Description of synthetic procedures
3. References

1. General information
All reactions were performed in oven-dried glassware under an atmosphere of nitrogen or argon, respectively. Tetrahydrofuran (THF) was distilled under nitrogen from sodium/benzophenone. Diethyl ether ($\text{Et}_2\text{O}$), methylene chloride ($\text{CH}_2\text{Cl}_2$) and $N,N$-dimethylformamide (DMF) were dried using a Braun Solvent Purification System (SPS). Commercially available reagents were used as supplied. Anhydrous magnesium sulfate and sodium sulfate were used as drying agents during work up. Unless otherwise noted, all
aqueous solutions used are saturated. If necessary, solvents were degassed prior to use by pump freeze-thaw technique.

Analytical thin-layer chromatography was performed using precoated silica gel ALUGRAM®Xtra SIL G/UV_254_ plates (Macherey-Nagel) and the spots were visualized with UV light at 254 nm or alternatively by staining with potassium permanganate or ninhydrin solutions.

Flash column chromatography was performed on Merck silica gel 60 (230–400 mesh). Eluents used for flash chromatography were distilled prior to use.

\(^1\)H NMR spectra were recorded at 400 MHz with a Bruker AVS-400 or Bruker DRX-400 as well as at 500 MHz with a Bruker DRX-500. Chemical shift values of \(^1\)H NMR spectra are commonly reported as values in ppm relative to residual solvent signal as internal standard\[^{S1}\]. The coupling constants are commonly reported in Hertz (Hz). Multiplicities are described using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sext = sextet, m = multiplet, b = broad.

\(^{13}\)C NMR spectra were recorded at 100 MHz with a Bruker AVS-400 or Bruker DRX-400 and at 125 MHz with a Bruker DRX-500. Chemical shift values of \(^{13}\)C NMR spectra are commonly reported as values in ppm relative to residual solvent signal as internal standard\[^{S1}\]. Multiplicities are reported using the following abbreviations: p = primary (methyl), s = secondary (methylene), t = tertiary (methine), q = quaternary (quaternary carbon). For full characterization \(^1\)H–\(^1\)H correlation spectra (COSY) and \(^1\)H–\(^{13}\)C correlation spectra (HMBC, HSQC) were recorded.

Mass spectra (EI) were obtained at 70 eV with a type VG Autospec spectrometer (Micromass), with a type LCT (ESI) (Micromass) or with a type Q-TOF (Micromass) spectrometer in combination with a Waters Aquisy Ultraperformance LC system.

Preparative high performance liquid chromatography was performed by using a Merck Hitachi LaChrom system [pump L- 7150, interface D-7000, diode array detector L-7450 (λ = 220–400 nm, preferred monitoring at λ = 230 nm)] with the following columns: Trentec Reprosil-Pur 120 C18 ISIS AQ 5 µm, 250 × 8 mm, with guard column Trentec Reprosil-Pur 120 C18 AQ 5 µm, 40 × 8 mm. Alternatively, a Varian HPLC system [pump Prepstar Model 218, variable wavelength detector Prostar (λ = 248 nm)] with parallel mass detection (Micromass typ ZMD ESI quad spectrometer) was used in combination with column: Trentec Reprosil-Pur 120 C18 AQ 5 µm, 250 mm × 25 mm, with guard column Trentec Reprosil-Pur 120 C18, AQ 10 µm, 30 mm × 20 mm. Membrane filtered, bidistilled water as well as commercial available HPLC quality solvents were used, which were degassed prior to use by using a ultrasonic bath.
Optical rotations $[\alpha]$ were measured on a Polarimeter 341 (Perkin Elmer) at a wavelength of 589 nm and are given in $10^{-1}$ deg cm² g⁻¹.
Melting points were measured using a SRS OptiMelt apparatus (Stanford research system).

2. Description of synthetic procedures

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Synthesis of 2-iodo-3-methylbut-2-enoic acid (10)

To a solution of CuI (2.46 g, 12.9 mmol, 1.50 equiv) in THF (42.5 mL) at −10 °C MeLi (1.6 M in Et₂O; 16.2 mL, 25.9 mmol, 3.00 equiv) was added. After 30 min, the reaction mixture was cooled to −78 °C and charged dropwise with ethyl 2-butyrate (8; 967 mg, 8.62 mmol, 1.00 equiv) in THF (5.00 mL). The reaction mixture was stirred for 2.5 h at −78 °C and subsequent a solution of iodine (6.57 g, 25.9 mmol, 3.00 equiv) in THF (5.00 mL) was added dropwise. The reaction mixture was stirred for additional 30 min at −78 °C and then terminated by addition of a saturated Na₂S₂O₃ solution. The layers were separated and the aqueous layer was extracted with Et₂O (4×). The combined organic layers were washed with a saturated NH₄Cl solution, followed by brine. The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was directly used in the next step without further purification.

The ethyl ester was dissolved in ethanol (2.00 mL) at rt. Then, LiOH (826 mg, 34.5 mmol, 4.00 equiv) in H₂O (15.0 mL) was added and the reaction mixture was heated to 60 °C. After 3.5 h, the reaction mixture was cooled to rt and EtOAc was added and the layers were separated. The aqueous layer was acidified with HCl (1.0 M) to pH ~ 1 and subsequently extracted with EtOAc (4×). The combined organic layers were washed with brine and then dried over MgSO₄, filtered and concentrated under reduced pressure. Acid 10 (1.68 g, 7.41 mmol, 86% over two steps) was obtained as a yellow solid. The analytical and physical data were in accordance with those reported in the literature[52].

mp = 74 – 78 °C (mp = 79 – 80 °C ref.[53]; ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 2.25 (3H, s, H-4), 2.17 (3H, s, H-4) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 170.5 (q, C-1), 157.1 (q, C-3), 84.5 (q, C-2), 33.5 (p, C-4), 23.5 (p, C-4) ppm; HRMS (ESI): m/z calculated for C₅H₆IO₂[M - H]: 224.9413 found 224.9416.

Synthesis of compound 11 through intermediate S1
Synthesis of ethyl (E)-2-iodo-3-methylpent-2-enoate (S1)

To a solution of CuI (2.86 g, 15.0 mmol, 1.50 equiv) in THF (42.5 mL) MeLi (1.6 M in Et₂O; 18.8 mL, 30.0 mmol, 3.00 equiv) was added at 0 °C. After 30 min, the reaction mixture was cooled to −78 °C and charged dropwise with ethyl pent-2-ynoate (9; 1.26 g, 10.0 mmol, 1.00 equiv) in THF (10 mL). The reaction mixture was stirred for 3 h at −78 °C and subsequently a solution of iodine (7.61 g, 30.0 mmol, 3.00 equiv) in THF (10 mL) was added dropwise. The reaction mixture was stirred for additional 15 min at −78 °C and then terminated by addition of a saturated Na₂S₂O₃ solution. The layers were separated and the aqueous layer was extracted with Et₂O (4×). The combined organic phases were washed with a saturated NH₄Cl solution, followed by brine. The combined organic phases were dried over MgSO₄, filtered and concentrated under reduced pressure. Product S1 was directly used without further purification.

¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 4.25 (2H, q, J = 7.2 Hz, H-7), 2.45 (2H, q, J = 7.5 Hz, H-5), 2.05 (3H, s, H-4), 1.32 (3H, t, J = 7.2 Hz, H-8), 1.08 (3H, t, J = 7.5 Hz, H-6) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 165.9 (q, C-1), 155.6 (q, C-3), 84.8 (q, C-2), 62.0 (s, C-7), 30.1 (p, C-4), 28.8 (s, C-5), 14.2 (p, C-8), 13.0 (p, C-6) ppm; HRMS (ESI): m/z calculated for C₈H₁₄I₂O₂ [M + H]⁺: 269.0039; found 269.0038.

nOe (400 MHz, CDCl₃):

<table>
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Synthesis of (E)-2-iodo-3-methylpent-2-enoic acid (11)
Ethyl ester S1 (348 mg, 1.30 mmol, 1.00 equiv) was dissolved in a mixture of ethanol/H\textsubscript{2}O (degassed; 1:7.5; 2.50 mL) at rt. Then, LiOH (125 mg, 5.21 mmol, 4.00 equiv) was added and the reaction mixture was heated to 60 °C. After 22.5 h, the reaction mixture was cooled to rt and EtOAc was added. The layers were separated. The aqueous layer was acidified with HCl (1.0 M) to pH ~ 1 and then extracted with EtOAc (4x). The combined organic layers were washed with brine and then dried over MgSO\textsubscript{4}, filtered and concentrated under reduced pressure. Acid 11 (268 mg, 1.12 mmol, 86% over two steps) was obtained as a yellow solid which was directly used without further purification.

**Synthesis of methyl (2-iodo-3-methylbut-2-enonyl)-L-alaninate (12)**

Acid 10 (600 mg, 2.65 mmol, 1.00 equiv) and L-alanine methylester hydrochloride (11; 482 mg, 3.45 mmol, 1.30 equiv) were dissolved in DMF (14.4 mL) at 0 °C. Then, HOAt (387 mg, 2.84 mmol, 1.07 equiv) and PyAOP (1.45 g, 2.79 mmol, 1.05 equiv) were added. Afterwards, DIPEA (1.57 g, 12.2 mmol, 4.60 equiv) was added dropwise. The reaction mixture was warmed up to rt. After 23 h, the reaction was stopped by adding saturated NH\textsubscript{4}Cl solution. The layers were separated and the aqueous layer was extracted with EtOAc (7×). The combined organic layers were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered and finally concentrated under reduced pressure. Purification by flash column chromatography on silica (petroleum ether/ethyl acetate 3:1) yielded the desired product 12 (710 mg, 2.28 mmol, 86%) as a colourless solid.

R\textsubscript{f} = 0.64 (CH\textsubscript{2}Cl\textsubscript{2}/CH\textsubscript{3}OH 98:2); mp = 74 – 76 °C; [\alpha]\textsubscript{D}\textsuperscript{20} = -10.8 (c 1.18, CH\textsubscript{2}Cl\textsubscript{2}); \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}, CDCl\textsubscript{3} = 7.26 ppm): δ = 6.26 (1H, brm, NH), 4.61 (1H, dq, J = 7.2 Hz, H-2), 3.77 (3H, s, H-8), 2.06 (3H, s, H-7), 2.01 (3H, s, H-7), 1.45 (3H, d, J = 7.2 Hz, H-3) ppm; \textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3}, CDCl\textsubscript{3} = 77.16 ppm): δ = 173.4 (q, C-1), 168.8 (q, C-4), 146.3 (q, C-6), 86.7 (q, C-5), 52.7 (p, C-8), 48.7 (t, C-2), 30.2 (p, C-7), 22.1 (p, C-7), 18.3 (p, C-3) ppm; HRMS (ESI): m/z calculated for C\textsubscript{9}H\textsubscript{15}INO\textsubscript{3} [M + H]\textsuperscript{+}: 312.0097 found 312.0096.

**Synthesis of 3-methylbutanamide (13)**

3-Methylbutanoic acid (6.00 g, 58.7 mmol, 1.00 equiv) and N-methylmorpholine (6.42 g, 63.4 mmol, 1.08 equiv) were dissolved in 1,2-dimethoxyethane (300 mL) at 0 °C. Then, isobutylchloroformiate (8.66 g, 63.4 mmol, 1.08 equiv) was added dropwise. The reaction mixture was stirred for 15 min at 0 °C. Afterwards, an aqueous ammonia solution (25%;
26.0 mL, 386 mmol, 6.57 equiv) was added dropwise. Now, the reaction mixture was stirred at 0 °C → rt. After 14 h, the reaction was terminated by adding HCl (1.0 M). The aqueous layer was extracted with EtOAc (4×). The combined organic layers were washed with HCl (0.1 M) and subsequently dried over MgSO₄, filtered and concentrated under reduced pressure. Amide 13 (4.14 g, 40.8 mmol, 70%) was isolated as a colourless solid.

mp = 123 – 126 °C; ⁱH-NMR (400 MHz, DMSO-d₆, DMSO-d₆ = 2.50 ppm): δ = 7.22 (1H, s, NH), 6.70 (1H, s, NH), 2.00-1.88 (3H, m, H-2, H-3), 0.96-0.89 (6H, m, H-4) ppm; ¹³C-NMR (100 MHz, DMSO-d₆, DMSO-d₆ = 39.52 ppm): δ = 173.8 (q, C-1), 44.4 (s, C-2), 25.3 (t, C-3), 22.3 (2C, p, C-4) ppm; HRMS (ESI): m/z calculated for C₅H₁₂ONa [M + H]⁺: 102.0919; found 102.0923.

**Synthesis of methyl [3-methyl-2-(3-methylbutanamido)but-2-enoyl]-L-alanine (14)**

![Diagram](attachment:image.png)

3-Methylbutanamide (13; 20.3 mg, 0.20 mmol, 1.00 equiv) and vinyl iodide 12 (125 mg, 0.40 mmol, 2.00 equiv) were dissolved in 1,4-dioxane (degassed; 0.25 mL) at rt. Then, K₂CO₃ (125 mg, 0.40 mmol, 2.00 equiv), Cul (23.0 mg, 0.12 mmol, 0.60 equiv) and trans-N,N-dimethyl-1,2-cyclohexanediamine (116 mg, 0.81 mmol, 4.05 equiv) were added. The reaction mixture was heated to 70 °C. After 20 h, the solvent was removed under reduced pressure. The resulting residue was diluted with a saturated NH₄Cl solution. Then, the aqueous layer was extracted with EtOAc (4×). The combined organic layers were washed with brine and subsequently dried over MgSO₄, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica (100% CH₂Cl₂ → CH₂Cl₂/CH₃OH 99:1 → CH₂Cl₂/CH₃OH 98:2) gave the desired α,β-dehydropeptide 14 (20.0 mg, 70.3 µmol, 35%) as a colourless solid.

Rᵣ = 0.37 (CH₂Cl₂/CH₃OH= 98:2); mp = 126 – 129 °C; [α]D₂⁰ = -2.1 (c 0.81, CH₂Cl₂); ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.15 (1H, s, NH-b), 6.97 (1H, d, J = 7.2 Hz, NH-a), 4.58 (1H, dq, J = 7.2 Hz, H-2), 3.73 (3H, s, H-12), 2.15 (3H, m, H-9, H-10), 1.98 (3H, s, H-7), 1.73 (3H, s, H-7), 1.42 (3H, d, J = 7.2 Hz, H-3), 0.99-0.97 (6H, d, J = 6.1 Hz, H-11) ppm; ¹³C-NMR (100 MHz, CDCl₃, CHCl₃ = 77.16 ppm): δ = 173.3 (q, C-1), 172.8 (q, C-8), 166.7 (q, C-4), 137.9 (q, C-5), 124.9 (q, C-6), 52.5 (p, C-12), 48.3 (t, C-2), 45.6 (s, C-9), 26.4 (t, C-10), 22.61 (p, C-11), 22.56 (p, C-11), 20.6 (p, C-7), 20.4 (p, C-7), 18.2 (p, C-3) ppm; HRMS (ESI): m/z calculated for C₁₅H₂₄N₂O₄Na [M + Na]⁺: 307.1634; found 307.1633.
Analytical data for the decomposition product 15:

$\text{H-NMR (400 MHz, CDCl}_3$, CDCl$_3$ = 7.26 ppm): $\delta$ = 7.44 (1H, m, NH), 4.55 (1H, dq, $J$ = 7.5 Hz, H-2), 3.76 (3H, s, H-8), 3.56 (1H, sept, $J$ = 6.8 Hz, H-6), 1.45 (3H, d, $J$ = 7.4 Hz, H-3), 1.12 (6H, d, $J$ = 6.8 Hz, H-7) ppm; $\text{C-NMR (100 MHz, CDCl}_3$, CDCl$_3$ = 77.16 ppm): $\delta$ = 201.7 (q, C-5), 172.5 (q, C-1), 159.4 (q, C-4), 52.7 (p, C-8), 48.1 (t, C-2), 34.2 (t, C-6), 18.2 (p, C-3 o. C-7), 17.82 (p, C-3 o. C-7), 17.80 (p, C-3 o. C-7) ppm; HRMS (ESI): m/z calculated for C$_9$H$_{15}$NO$_4$Na [M + Na]$^+$: 224.0899; found 224.0902.

Synthesis of compound 24 through intermediate S2

Synthesis of methyl [(R)-5-{{(benzyloxy)carbonyl}amino}-2-{{(tert-butoxyvarbonyl) aminopentanoyl]-D-valinate (S2)}

Boc-D-Orn(Cbz)-OH (200 mg, 0.55 mmol, 1.0 equiv) and D-valine methylester hydrochloride (101 mg, 0.60 mmol, 1.10 equiv) were dissolved in CH$_2$Cl$_2$ (64.0 mL) at rt. The reaction mixture was cooled to 0 °C and HOBT (111 mg, 0.82 mmol, 1.50 equiv) was added. Then, DIPEA (106 mg, 0.82 mmol, 1.50 equiv) was added dropwise followed by EDC·HCl (131 mg, 0.68 mmol, 1.25 equiv). The reaction mixture was stirred for 16 h at 0 °C → rt. The solvent
was removed under reduced pressure. Purification by flash chromatography on silica (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH = 99:1) gave dipeptide S2 (260 mg, 0.54 mmol, 99%) as colourless oil.

R<sub>f</sub> = 0.23 (CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH = 99:1); [α]<sup>20</sup> = -3.9 (c 1.30, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>, CHCl<sub>3</sub> = 7.26 ppm): δ = 7.35 (5H, m, ArH), 6.88 (1H, brd, J = 7.9 Hz, NH-a), 5.17 (1H, brd, J = 7.2 Hz, NH-c), 5.11 (2H, m, H-14), 4.96 (1H, brt, J = 4.1 Hz, NH-b), 4.52 (1H, dd, J = 4.1, 7.9 Hz, H-2), 4.34 (1H, m, H-6), 3.71 (3H, s, H-19), 3.45 (1H, brs, H-12), 3.16 (1H, brs, H-12'), 2.19 (1H, sept, J = 7.9 Hz, H-3), 1.88 (1H, brs, H-10), 1.74 (1H, brs, H-10'), 1.59 (2H, m, H-11), 1.44 (9H, s, H-9), 0.94 (6H, d, J = 7.9 Hz, H-4) ppm; <sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>, CDCl<sub>3</sub> = 77.16 ppm): δ = 172.4 (2C, q, C-1, C-5), 157.2 (q, C-13), 156.0 (q, C-7), 136.7 (q, C-15), 128.7 (2C, t, C-17), 128.3 (t, C-18), 128.3 (2C, t, C-16), 80.0 (q, C-8), 66.9 (s, C-14), 57.3 (t, C-2), 53.1 (t, C-6), 52.3 (p, C-19), 39.8 (s, C-12), 31.1 (t, C-3), 30.1 (s, C-10), 28.5 (3C, p, C-9), 26.4 (s, C-11), 19.2 (p, C-4), 17.8 (p, C-4) ppm; HRMS(ESI): m/z calculated for C<sub>24</sub>H<sub>37</sub>N<sub>3</sub>O<sub>7</sub>Na [M + Na]<sup>+</sup>: 502.2529; found 502.2524.


Boc-protected dipeptide S2 (118 mg, 0.25 mmol, 1.00 equiv) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3.60 mL) at 0 °C. Afterwards, TFA (1.41 g, 12.3 mmol, 50.0 equiv) was added dropwise. After 2.5 h the reaction was terminated by addition of a saturated sodium bicarbonate solution. The layers were separated and the aqueous layer was extracted with EtOAc (4×). The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The title compound 18 (93.6 mg, 0.25 mmol, quantitative) was obtained as a colourless oil. The product was used without further purification.

Acid 10 (50.7 mg, 0.22 mmol, 1.00 equiv) and dipeptide 18 (93.6 mg, 0.25 mmol, 1.10 equiv) were dissolved in DMF (1.20 mL) at 0 °C. Then, HOAt (32.7 mg, 0.24 mmol, 1.07 equiv) and PyAOP (123 mg, 0.24 mmol, 1.05 equiv) were added. Afterwards, DIPEA (133 mg, 1.03 mmol, 4.60 equiv) was added dropwise. The reaction mixture was warmed up to rt. After 22 h, the reaction was terminated by addition of a saturated NH₄Cl solution. The layers were separated and the aqueous layer was extracted with EtOAc (7×). The combined organic
layers were washed with brine and subsequently dried over Na$_2$SO$_4$, filtered as well as concentrated under reduced pressure. Purification by flash column chromatography over silica (petroleum ether/ethyl acetate = 3:1 → petroleum ether/ethyl acetate = 1:1) gave the desired vinyl iodide 24 (119 mg, 0.20 mmol, 90%) as a colourless solid.

$R_f = 0.70$ (CH$_2$Cl$_2$/CH$_3$OH 98:2); $mp = 143 – 146 ^\circ C$; $[\alpha]_D^{20} = +4.2$ ($c = 1.73$, CH$_2$Cl$_2$); $^1$H-NMR (400 MHz, CDCl$_3$, CHCl$_3$ = 7.26 ppm): $\delta = 7.37$-$7.28$ (5H, m, ArH), 7.05 (1H, d, $J = 8.9$ Hz, NH-a), 6.49 (1H, d, $J = 8.2$ Hz, NH-b), 5.12 (2H, t, $J = 6.1$ Hz, H-11), 5.03 (1H, m, NH-c), 4.80 (1H, m, H-6), 4.51 (1H, dd, $J = 4.9$, 8.8 Hz, H-2), 3.71 (3H, s, H-20), 3.57 (1H, m, H-9), 3.15 (1H, m, H-9'), 2.19 (1H, m, H-3), 2.02 (3H, s, H-19), 1.99 (3H, s, H-19), 1.95 (1H, m, H-7'), 1.69-1.53 (3H, m, H-7', H-8), 0.97-0.94 (6H, m, H-4) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$, CDCl$_3$ = 77.16 ppm): $\delta = 172.4$ (q, C-1), 171.8 (q, C-5), 167.5 (q, C-16), 157.5 (q, C-10), 145.8 (q, C-18), 136.6 (q, C-12), 128.7 (2C, t, C-14), 128.29 (t, C-15), 128.28 (2C, t, C-13), 86.8 (q, C-17), 67.0 (s, C-11), 57.5 (t, C-2), 52.3 (p, C-20), 51.9 (t, C-6), 39.5 (s, C-9), 30.9 (s, C-7), 30.4 (t, C-3), 30.1 (p, C-19), 26.6 (s, C-8), 22.1 (p, C-19), 19.2 (p, C-4), 17.9 (p, C-4) ppm; HRMS (ESI): $m/z$ calculated for C$_{24}$H$_{35}$N$_3$O$_6$ [M + H]$^+$: 588.1571; found 588.1579.

**Synthesis of compound 25 through intermediates S3-S5**

**Synthesis of N,N'-bis(allyloxycarbonyl)guanidine (S3)**
Guanidine hydrochloride (4.00 g, 41.9 mmol, 1.00 equiv) was dissolved in CH₂Cl₂ (80.0 mL) at rt. Then, NaOH (6.0 M; 28.0 mL) and BnEt₃NCl (191 mg, 0.84 mmol, 20 mol %) were added. The reaction mixture was cooled to 0 °C and stirred for additional 15 min. Afterwards, allylchloroformiate (20.2 g, 167.5 mmol, 4.00 equiv) was added at 0 °C. The reaction mixture was stirred for additional 6.5 h at 0 °C. The resulting colourless solid was filtered and the filtrate was diluted with H₂O. The layers were separated and the aqueous phase was extracted with CH₂Cl₂ (3×). The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. Purification by flash column chromatography on silica (CH₂Cl₂/Et₂O = 98:2 → CH₂Cl₂/Et₂O = 95:5 → CH₃OH/CH₂Cl₂ = 1:1) furnished guanidine S₃ (7.24 g, 31.8 mmol, 76%) as a colourless solid.

The spectroscopic and physical data were in accordance with those reported in the literature[S4].

Rᵣ = 0.32 (CH₂Cl₂/Et₂O= 9:1); mp = 109 – 114 °C; ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 8.32 (2H, brs, NH-a), 5.91 (2H, ddt, J = 5.1, 11.0, 17.1 Hz, H-4), 5.33 (2H, dd, J = 11.0, 17.1 Hz, H-5), 5.25 (2H, dd, J = 11.0, 17.1 Hz, H-5), 4.61 (4H, d, J = 5.1 Hz, H-3) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 158.9 (2C, q, C-2), 132.0 (2C, t, C-4), 118.7 (2C, s, C-5), 66.7 (2C, s, C-3) ppm; HRMS(ESI): m/z calculated for C₉H₁₄N₄O₄ [M + H]+: 228.0984; found 228.0983.
The quaternary carbon atom C-1 was not detectable in the ¹³C NMR spectra.

Synthesis of N,N’-bis(allyloxycarbonyl)-N’’-trifluoromethylsulfonylguanidine (S₄)

N,N’-Bis(allyloxycarbonyl)guanidine (S₃; 1.64 g, 7.23 mmol, 1.0 equiv) was dissolved in CH₂Cl₂ (34.0 mL) at rt. The reaction mixture was cooled to −78 °C and stirred for 10 min. Then, freshly distilled Et₃N (1.10 g, 10.8 mmol, 1.50 equiv) was added dropwise and the mixture was stirred for additional 10 min at −78 °C. Subsequently, trifluoromethanesulfonyl anhydride (3.05 g, 10.8 mmol, 1.50 equiv) was added dropwise while vigorously stirring over a period of 15 min at −78 °C. The reaction mixture was warmed up to rt and after 7 h the mixture was diluted with CH₂Cl₂ and washed with HCl (1.0 M; 3×). The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. Purification by flash column chromatography over silica (CH₂Cl₂ = 100%) furnished title compound S₄ (1.32 g, 3.69 mmol, 51%) as a colourless oil.

The spectroscopic and physical data were in accordance with those reported in the literature[S4].

Rᵣ = 0.31 (CH₂Cl₂= 100%); ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 10.30 (2H, brs, NH), 5.92 (2H, ddt, J = 6.0, 10.4, 17.1 Hz, H-4), 5.41 (2H, dd, J = 1.0, 1.4, 10.4 Hz, H-5),
5.36 (2H, dd, $J = 1.0$, 1.4, 10.4 Hz, H-5), 4.74 (4H, d, $J = 6.0$ Hz, H-3) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$, CDCl$_3$ = 77.16 ppm): $\delta$ = 151.0 (2C, q, C-2), 150.0 (q, C-1), 130.2 (2C, t, C-4), 120.9 (2C, s, C-5), 117.7 (q, SO$_2$CF$_3$), 68.7 (2C, s, C-3) ppm; HRMS (ESI): $m/z$ calculated for C$_{10}$H$_{12}$F$_3$N$_3$O$_6$Na [M + Na]$^+$: 382.0297; found 382.0298.

**Synthesis of methyl (E)-$N''$,N''-bis[(allyloxy)carbonyl]-$N^2$-(tert-butoxycarbonyl)-D-arginyl-D-valinate (S5)**

Dipeptide S2 (225 mg, 0.47 mmol, 1.0 equiv) was dissolved in CH$_3$OH (18.0 mL) at rt. Then, palladium on charcoal (10%; 27.5 mg, 0.55 equiv) was added. The reaction mixture was flushed with hydrogen gas (3x) and stirred under an H$_2$ atmosphere at rt. After 20 h the reaction mixture was filtered over a pad of Celite$^\text{TM}$ and washed with CH$_3$OH. The solvent was removed under reduced pressure. The resulting residue was dissolved in CH$_2$Cl$_2$ (7.60 mL) at rt. Freshly distilled Et$_3$N (47.5 mg, 0.47 mmol, 1.00 equiv) was added dropwise and followed by compound S4 (185 mg, 0.52 mmol, 1.10 equiv). After 21 h stirring at rt the solvent was removed under reduced pressure. Purification by flash chromatography (CH$_2$Cl$_2$/CH$_3$OH = 99:1) afforded title compound S5 (248 mg, 0.45 mmol, 95% over two steps) as a colourless oil. $R_f$ = 0.28 (CH$_2$Cl$_2$/CH$_3$OH = 99:1); $[\alpha]_{D}^{20}$ = +4.5 (c 1.21, CH$_2$Cl$_2$); $^1$H-NMR (400 MHz, CD$_3$OD, CD$_3$OH = 3.31 ppm): $\delta$ = 6.03-5.93 (2H, m, H-16, H-20), 5.40-5.19 (4H, m, H-17, H-21), 4.70 (2H, d, $J = 5.8$ Hz, H-19), 4.58 (2H, d, $J = 5.8$ Hz, H-15), 4.33 (1H, d, $J = 6.4$ Hz, H-2), 4.11 (1H, m, H-6), 3.71 (3H, s, H-22), 3.43 (2H, m, H-12), 2.15 (1H, sept, $J = 6.4$ Hz, H-3), 1.82-1.59 (4H, m, H-10, H-11), 1.44 (9H, s, H-9), 0.95 (6H, d, $J = 6.4$ Hz, H-4) ppm; $^{13}$C-NMR (100 MHz, CD$_3$OD, CD$_3$OD = 49.00 ppm): $\delta$ = 175.1 (q, C-5), 173.4 (q, C-1), 164.8 (q, C-13), 157.6 (2C, q, C-7, C-14), 154.7 (q, C-18), 134.4 (t, C-16), 132.8 (t, C-20), 119.5 (s, C-21), 117.9 (s, C-17), 80.6 (q, C-8), 68.1 (s, C-19), 67.3 (s, C-15), 59.0 (t, C-2), 55.5 (t, C-6), 52.5 (p, C-22), 41.6 (s, C-12), 31.9 (t, C-3), 30.4 (s, C-10), 28.7 (3C, p, C-9), 26.6 (s, C-11), 19.4 (p, C-4), 18.4 (p, C-4) ppm; HRMS(ESI): $m/z$ calculated for C$_{25}$H$_{41}$N$_5$O$_9$Na [M + Na]$^+$: 578.2802; found 578.2796.
Synthesis of methyl (\(E\))-\(N^\omega,N'^\omega\)-bis[(allyloxy)carbonyl]-\(N^\gamma\)-(2-iodo-3-methylbut-2-enoyl)-D-arginyl-D-valinate (25)

Boc-protected dipeptide S5 (611 mg, 1.10 mmol, 1.0 equiv) was dissolved in CH\(_2\)Cl\(_2\) (13.5 mL) at 0 °C. Afterwards, TFA (5.31 g, 46.6 mmol, 50.0 equiv) was added dropwise. After 2.5 h the reaction was terminated by addition of a saturated sodium bicarbonate solution. The layers were separated and the aqueous layer was extracted with EtOAc (5 ×). The combined organic layers were dried over MgSO\(_4\), filtered and concentrated under reduced pressure. The desired product 19 (501 mg, 1.10 mmol, quantitative) was obtained as a colourless foam.

\(^1\)H-NMR (200 MHz, CD\(_3\)OD, CD\(_3\)OH = 3.31 ppm): \(\delta = 5.99\) (2H, m, H-13, H-17), 5.43-5.18 (4H, m, H-14, H-18), 4.70 (2H, dt, \(J = 1.3, 5.8\) Hz, H-16), 4.59 (2H, dt, \(J = 1.4, 5.6\) Hz, H-12), 4.36 (1H, d, \(J = 5.7\) Hz, H-6), 3.79 (1H, t, \(J = 6.6\) Hz, H-2), 3.71 (3H, s, OC\(_2\)H\(_3\)), 3.43 (2H, m, H-9), 2.19 (1H, m, H-3), 1.85-1.69 (4H, m, H-7, H-8), 0.97 (6H, d, \(J = 6.6\) Hz, H-4) ppm; HRMS (ESI): \(m/z\) calculated for C\(_{20}\)H\(_{34}\)N\(_5\)O\(_7\) [M + H]\(^+\): 456.2458; found 456.2451.

Acid 10 (219 mg, 0.97 mmol, 1.0 equiv) and dipeptide 19 (486 mg, 1.07 mmol, 1.10 equiv) were dissolved in DMF (5.30 mL) at 0 °C. Then, HOAt (141 mg, 1.04 mmol, 1.07 equiv) and PyAOP (530 mg, 1.02 mmol, 1.05 equiv) were added. Afterwards, DIPEA (575 mg, 4.45 mmol, 4.60 equiv) was added dropwise. The reaction mixture was warmed up to rt. After 23 h, the reaction was terminated by addition of a saturated NH\(_4\)Cl solution. The layers were separated and the aqueous layer was extracted with EtOAc (7 ×). The combined organic layers were washed with brine and subsequently dried over Na\(_2\)SO\(_4\), filtered and concentrated under reduced pressure. Purification by flash column chromatography over silica (petroleum ether/ethyl acetate = 3:1 → petroleum ether/ethyl acetate = 1:1) gave the desired vinyl iodide 25 (576 mg, 0.87 mmol, 90%) as a colourless solid. 

\(R_f = 0.24\) (CH\(_2\)Cl\(_2\)/CH\(_3\)OH 98:2); mp = 127 – 132 °C; \(\alpha\)\(_D\)^{20} = +11.7 (c = 2.47, CH\(_2\)Cl\(_2\)); 

\(^1\)H-NMR (400 MHz, CDCl\(_3\), CHCl\(_3\) = 7.26 ppm): \(\delta = 11.71\) (1H, brs, NH-d), 8.38 (1H, t, \(J = 5.5\) Hz, NH-c), 6.62 (1H, d, \(J = 8.5\) Hz, NH-a), 6.56 (1H, d, \(J = 8.2\) Hz, NH-b), 6.01-5.85 (2H, m, H-13, H-17), 5.38-5.21 (4H, m, H-14, H-18), 4.64 (2H, m, H-16), 4.58 (2H, m, H-12), 4.55-4.48 (2H, m, H-2, H-6), 3.72 (3H, s, H-23), 3.49 (2H, m, H-9), 2.16 (1H, m, H-3), 2.01 (3H, s, H-22), 1.99 (3H, s, H-22), 1.95 (1H, m, H-7), 1.79-1.68 (3H, m, H-7', H-8), 0.93 (3H, d, \(J = \) ...
6.8 Hz, H-4), 0.90 (3H, d, J = 6.8 Hz, H-4) ppm; $^{13}\text{C}$-NMR (100 MHz, CDCl$_3$, CDCl$_3$ = 77.16 ppm): $\delta$ = 172.0 (q, C-1), 171.0 (q, C-5), 167.7 (q, C-19), 163.6 (q, C-10), 156.4 (q, C-11), 153.9 (q, C-15), 146.3 (q, C-21), 133.1 (t, C-13), 131.1 (t, C-17), 119.6 (s, C-18), 118.1 (s, C-14), 86.1 (q, C-20), 67.2 (s, C-16), 66.4 (s, C-12), 57.5 (t, C-2), 53.5 (t, C-6), 52.3 (p, C-23), 40.5 (s, C-9), 31.2 (t, C-3), 30.1 (p, C-22), 29.1 (s, C-7), 25.5 (s, C-8), 22.1 (p, C-22), 19.1 (p, C-4), 17.9 (p, C-4) ppm; HRMS (ESI): $m/z$ calculated for $\text{C}_{25}\text{H}_{39}\text{N}_{5}\text{O}_{8}$ [M + H]$^+$: 664.1843; found 664.1842.

**Synthesis of compound 20 through intermediates S6-S9**

\[
\begin{align*}
&\text{H}_2\text{N}\text{N} &\rightarrow &\text{BocH}\text{N}\text{NH}B\text{oc} \\
&\text{BocH}\text{N} &\rightarrow &\text{NTf} \\
&\text{FmocH}\text{N} &\rightarrow &\text{BocH}\text{N} \\
&\text{FmocH}\text{N} &\rightarrow &\text{S9} \\
&\text{BocH}\text{N} &\rightarrow &\text{20}
\end{align*}
\]

**Synthesis of N,N'-bis(tert-butoxycarbonyl)guanidine (S6)**

Guanidine hydrochloride (4.00 g, 41.9 mmol, 1.00 equiv) and NaOH (6.70 g, 167 mmol, 4.00 equiv) were dissolved in a mixture of 1,4-dioxane/H$_2$O (2:1; 126 mL) at 0 °C. Afterwards, (Boc)$_2$O (20.1 g, 92.1 mmol, 2.20 equiv) was added. The reaction mixture was warmed to rt and stirred for 21 h. Then, the solvent was removed under reduced pressure. The resulting residue was washed with H$_2$O and extracted with EtOAc (3×). The combined organic layers were washed with citric acid (10%) as well as H$_2$O and brine. Subsequently, the combined organic layers were dried over MgSO$_4$ and filtered. The solvent was removed under reduced pressure. Purification by flash column chromatography over silica (CH$_2$Cl$_2$/CH$_3$OH = 97:3) gave the desired product S6 (5.56 g, 21.4 mmol, 51%) as colourless solid. The spectroscopic and physical data were in accordance with those reported in the literature.$^{[56]}$
Synthesis of \( N,N'-\text{bis(tert-butoxycarbonyl)}-N'-'\text{trifluoromethansulfonyl} \text{guanidine} \) (S7)

\( N,N'-\text{Bis(tert-butoxycarbonyl)} \text{guanidine} \) (S6; 4.38 g, 16.9 mmol, 1.00 equiv) was dissolved in \( \text{CH}_2\text{Cl}_2 \) (84.4 mL) at rt. The reaction mixture was cooled to \(-78^\circ\text{C}\) and stirred for 10 min. Then, freshly distilled \( \text{Et}_3\text{N} \) (1.79 g, 17.7 mmol, 1.05 equiv) was added dropwise. Subsequently, trifluoromethanesulfonic anhydride (5.00 g, 17.7 mmol, 1.05 equiv) was added dropwise while vigorously stirring over a period of 15 min at \(-78^\circ\text{C}\). The reaction mixture was warmed to rt. After 6 h, the mixture was washed with a KHSO\(_4\) solution (1.0 M), followed by \( \text{H}_2\text{O} \). The combined organic layers were dried over \( \text{Na}_2\text{SO}_4\) and filtered. The solvent was removed under reduced pressure. Purification by flash column chromatography over silica (\( \text{CH}_2\text{Cl}_2 = 100\% \)) furnished the title compound S7 (5.51 g, 14.1 mmol, 83\%) as a colourless solid. The spectroscopic and physical data were in accordance with those reported in the literature\(^{[55]}\).

Synthesis of methyl \([([R]-2-\{[[9H-\text{fluorene-9-yl}]\text{methoxy}]}\text{carbonyl}]\text{amino})-5-\{[\text{tert-butoxycarbonyl}]}\text{amino}\}\text{pentanoyl]}\text{-D-valinate} \) (S8)

Fmoc-\text{D-Orn(Boc)}-\text{OH} (500 mg, 1.10 mmol, 1.0 equiv) and D-valine methylester hydrochloride (221 mg, 1.32 mmol, 1.20 equiv) were dissolved in \( \text{CH}_2\text{Cl}_2 \) (50.0 mL) at 0 \( ^\circ\text{C} \). Then, HOAt (225 mg, 1.65 mmol, 1.50 equiv), EDC-HCl (316 mg, 1.65 mmol, 1.50 equiv) and
NaHCO₃ (490 mg, 5.50 mmol, 5.0 equiv) were added. The reaction mixture was stirred at 0 °C → rt. After 22 h, the solvent was removed under reduced pressure. Purification by flash chromatography (CH₂Cl₂/CH₃OH 99:1 → CH₂Cl₂/CH₃OH 97:3) furnished the desired dipeptide S₈ (615 mg, 1.08 mmol, 98%) as colourless foam. Rf = 0.24 (CH₂Cl₂/CH₃OH 99:1); [α]D²⁰ = -1.8 (c = 1.44, CH₂Cl₂); ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.76 (2H, d, J = 7.5 Hz, H-21, H-27), 7.60 (2H, d, J = 7.5 Hz, H-18, H-24), 7.40 (2H, t, J = 7.5 Hz, H-20, H-26), 7.30 (2H, t, J = 7.5 Hz, H-19, H-25), 6.69 (1H, brs, NH-a), 5.62 (1H, brs, NH-c), 4.70 (1H, brs, NH-b), 4.51 (1H, dd, J = 6.5, 8.9 Hz, H-2), 4.41-4.35 (3H, m, H-6, H-9), 3.11 (1H, m, H-9'), 2.19 (1H, m, H-3), 1.90 (1H, m, H-7), 1.69-1.52 (3H, m, H-7', H-8), 1.44 (9H, s, H-12), 0.92 (6H, d, J = 6.5 Hz, H-4) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 172.3 (q, C-1), 172.0 (q, C-5), 156.7 (q, C-13), 156.4 (q, C-10), 144.0 (q, C-15 o. C-22), 143.9 (q, C-15 o. C-22), 141.5 (q, C-17 o. C-23), 141.4 (q, C-17 o. C-23), 127.9 (2C, t, C-19 o. C-20 o. C-25 o. C-26), 127.2 (2C, t, C-19 o. C-20 o. C-25 o. C-26), 125.3 (2C, t, C-18, C-24), 120.12 (t, C-21 o. C-27), 120.10 (t, C-21 o. C-27), 79.5 (q, C-11), 67.2 (s, C-14), 57.5 (t, C-2), 54.0 (t, C-6), 52.3 (p, C-28), 47.3 (t, C-15), 31.0 (t, C-3), 30.1 (s, C-7), 28.6 (3C, p, C-12), 26.5 (s, C-8), 19.2 (p, C-4), 18.0 (p, C-4) ppm; HRMS (ESI): m/z calculated for C₃₁H₄₁N₃O₇Na [M + Na]⁺: 590.2842; found 590.2841. The carbon atom C-9 could not be detected in the ¹³C NMR spectrum.

Synthesis of methyl (E)-N²-{[(9H-fluorene-9-yl)methoxy]carbonyl}-N°,N°'-bis(tert-butoxycarbonyl)-D-arginyl-D-valinate (S₉)

Boc-protected dipeptide S₈ (260 mg, 0.46 mmol, 1.00 equiv) was dissolved in CH₂Cl₂ (4.60 mL) at 0 °C. Afterwards, TFA (2.61 g, 22.9 mmol, 50.0 equiv) was added dropwise. After 2.5 h, the reaction was terminated by addition of a saturated sodium bicarbonate solution. The layers were separated and the aqueous layer was extracted with EtOAc (6×). The combined organic layers were washed with brine and then dried over MgSO₄ as well as filtered. The solvent was removed under reduced pressure. The product was used without further purification.

The crude product was dissolved in CH₂Cl₂ (4.60 mL) at rt. Then, NaHCO₃ (204 mg, 2.29 mmol, 5.0 equiv) and guanidine derivative S₇ (179 mg, 0.46 mmol, 1.00 equiv) were
added. The reaction mixture was stirred for 5 d at rt. Afterwards, the solvent was removed under reduced pressure. Purification by flash chromatography over silica (CH$_2$Cl$_2$/CH$_3$OH 99:1 → CH$_2$Cl$_2$/CH$_3$OH 98:2) gave the desired product S9 (175 mg, 0.25 mmol, 54% over two steps) as colourless foam.

R$_f$ = 0.65 (CH$_2$Cl$_2$/CH$_3$OH 95:5); [α]$_D^{20}$ = -2.2 (c = 0.87, CH$_2$Cl$_2$); $^1$H-NMR (400 MHz, CD$_3$OD, CD$_3$OH = 3.31 ppm): δ = 7.75 (2H, d, J = 7.5 Hz, H-25, H-31), 7.63 (2H, t, J = 7.5 Hz, H-22, H-28), 7.36 (2H, t, J = 7.5 Hz, H-24, H-30), 7.27 (2H, t, J = 7.5 Hz, H-23, H-29), 4.40-4.31 (3H, m, H-2, H-20), 4.24-4.17 (2H, m, H-6, H-19), 3.68 (3H, s, H-32), 3.37 (2H, m, H-9), 2.13 (1H, m, H-3), 1.80 (1H, m, H-7), 1.70-1.59 (3H, m, H-7', H-8), 1.49 (9H, s, H-13 o. H-16), 1.46 (9H, s, H-13 o. H-16), 0.93 (6H, d, J = 6.6 Hz, H-4) ppm; $^{13}$C-NMR (100 MHz, CD$_3$OD, CD$_3$OD = 49.00 ppm): δ = 174.8 (q, C-1), 173.3 (q, C-5), 164.4 (q, C-10), 158.3 (q, C-17), 157.6 (q, C-11 o. C-14), 154.1 (q, C-11 o. C-14), 145.3 (q, C-20 o. C-26), 145.1 (q, C-20 o. C-26), 142.6 (q, C-21 o. C-27), 142.5 (q, C-21 o. C-27), 128.7 (2C, t, C-23 o. C-24 o. C-29 o. C-30), 128.1 (2C, t, C-23 o. C-24 o. C-29 o. C-30), 126.2 (2C, t, C-22, C-28), 120.9 (2C, t, C-25, C-31), 84.4 (q, C-12 o. C-15), 80.4 (q, C-12 o. C-15), 67.8 (s, C-18), 59.1 (t, C-2), 55.8 (t, C-6), 52.5 (p, C-32), 48.4 (t, C-19), 41.3 (s, C-9), 31.7 (t, C-3), 30.5 (s, C-7), 28.6 (3C, p, C-13 o. C-16), 28.2 (3C, p, C-13 o. C-16), 26.7 (s, C-8), 19.5 (p, C-4), 18.5 (p, C-4) ppm; HRMS (ESI): m/z calculated for C$_{37}$H$_{62}$N$_8$O$_9$ [M + H]$^+$: 710.3765; found 710.3764.

**Synthesis of methyl (E)-N$^\alpha$-tert-butoxycarbonyl)-D-arginyl-D-valinate (20)**

Dipeptide S9 (106 mg, 0.15 mmol, 1.00 equiv) was dissolved in CH$_2$Cl$_2$ (11.0 mL) at 0 °C. Afterwards, tris(2-aminoethyl)amine (1.10 mL) was added dropwise. The reaction mixture was warmed to rt and stirred for additional 3 h. The reaction was terminated by addition of H$_2$O. The layers were separated and the organic layer was washed with H$_2$O again. The combined organic layers were washed with brine, dried over MgSO$_4$ and filtered. The solvent was removed under reduced pressure. Purification by flash chromatography over silica (CH$_2$Cl$_2$/CH$_3$OH 98:2 → CH$_2$Cl$_2$/CH$_3$OH 96:4 → CH$_2$Cl$_2$/CH$_3$OH 94:6) yielded amine 20 (65.5 mg, 0.13 mmol, 90%) as a colourless oil.

R$_f$ = 0.58 (CH$_2$Cl$_2$/CH$_3$OH 9:1); [α]$_D^{20}$ = -1.4 (c = 1.31, CH$_3$OH); $^1$H-NMR (400 MHz, CD$_3$OD, CD$_3$OH = 3.31 ppm): δ = 4.33 (1H, d, J = 6.6 Hz, H-2), 3.72 (3H, s, H-17), 3.45-3.37 (3H, m, H-6, H-9), 2.17 (1H, m, H-3), 1.76-1.59 (4H, m, H-7, H-8), 1.52 (9H, s, H-13 o. H-16), 1.47 (9H, s, H-13 o. H-16), 0.97 (6H, d, J = 6.6 Hz, H-4) ppm; $^{13}$C-NMR (100 MHz, CD$_3$OD,
CD$_3$OD = 49.00 ppm): δ = 177.6 (q, C-5), 173.4 (q, C-1), 164.6 (q, C-10), 157.6 (q, C-11 o. C-14), 154.1 (q, C-11 o. C-14), 84.4 (q, C-12 o. C-15), 80.3 (q, C-12 o. C-15), 59.1 (t, C-2), 55.4 (t, C-6), 52.5 (p, C-17), 41.4 (s, C-9), 33.7 (t, C-3), 31.7 (s, C-7), 28.6 (3C, p, C-13 o. C-16), 28.2 (3C, p, C-13 o. C-16), 26.3 (s, C-8), 19.5 (p, C-4), 18.5 (p, C-4) ppm; HRMS (ESI): m/z calculated for C$_{22}$H$_{42}$N$_5$O$_7$ [M + H]$^+$: 488.3084; found 488.3081.

Synthesis of methyl (E)-N$^\omega$-N$'^\omega$-bis(tert-butoxycarbonyl)-N$^2$-(2-iodo-3-methylbut-2-enoyl)-D-arginyl-D-valinate (26)

Acid 10 (64.2 mg, 0.28 mmol, 1.20 equiv) and amine 20 (100 mg, 0.26 mmol, 1.0 equiv) were dissolved in DMF (2.60 mL) at 0 °C. Then, HOAt (37.6 mg, 0.28 mmol, 1.07 equiv) and PyAOP (141 mg, 0.27 mmol, 1.05 equiv) were added. Afterwards, DIPEA (153 mg, 1.18 mmol, 4.60 equiv) was added dropwise. The reaction mixture was stirred at 0 °C → rt. After 22 h, the reaction was terminated by addition of a saturated NH$_4$Cl solution. The layers were separated and the aqueous layer was extracted with EtOAc (7×). The combined organic layers were washed with brine and subsequently dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography over silica (petroleum ether/ethyl acetate = 3:1 → petroleum ether/ethyl acetate = 1:1) gave the title compound 26 (108 mg, 0.15 mmol, 60%) as a colourless oil.

$^1$H-NMR (400 MHz, CDCl$_3$, CHCl$_3$ = 7.26 ppm): δ = 11.47 (1H, s, NH-c), 8.41 (1H, s, NH), 6.62 (1H, d, J = 8.5 Hz, NH), 6.43 (1H, d, J = 7.2 Hz, NH), 4.51 (2H, m, H-2, H-6), 3.74 (3H, s, H-21), 3.48 (2H, m, H-9), 2.18 (1H, m, H-3), 2.03 (3H, s, H-20), 2.00 (3H, s, H-20), 1.98 (1H, m, H-7), 1.77-1.59 (3H, m, H-7’, H-8), 1.49 (18H, s, H-13, H-16), 0.95-0.92 (6H, m, H-4) ppm; HRMS (ESI): m/z calculated for C$_{27}$H$_{47}$N$_5$O$_8$ [M + H]$^+$: 696.2469; found 696.2458.
Synthesis of compound 21 through intermediate S10

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\text{Fmoc-Arg(Pbf)-OH (2.00 g, 3.08 mmol, 1.00 equiv) and D-valine methylester hydrochloride (620 mg, 3.70 mmol, 1.20 equiv) were dissolved in CH}_2\text{Cl}_2 (140 mL) at 0 \, ^\circ\text{C. Then, HOAt (629 mg, 4.62 mmol, 1.50 equiv), EDC-HCl (886 mg, 4.62 mmol, 1.50 equiv) and NaHCO}_3 (1.37 g, 15.4 mmol, 5.00 equiv) were added. The reaction mixture was stirred at 0 \, ^\circ\text{C} \rightarrow \text{rt. After 22 h, the solvent was removed under reduced pressure. Purification by flash chromatography over silica (CH}_2\text{Cl}_2/\text{CH}_3\text{OH 99:1} \rightarrow \text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH 97:3} \rightarrow \text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH 96:4) gave the desired dipeptide S10 (2.53 g, with impurities of D-Val-OMe) as a colourless foam. The product was used without further purification.}
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\[\text{R}_f = 0.20 (\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH 98:2}); \text{^1H-NMR (400 MHz, CDCl}_3, \text{CHCl}_3 = 7.26 \text{ ppm}): \delta = 7.71 (2H, d, J = 7.3 \text{ Hz, H-31, H-37}), 7.54 (2H, d, J = 7.3 \text{ Hz, H-28, H-34}), 7.34 (3H, t, J = 7.3 \text{ Hz, H-30, H-36, NH-a}), 7.21 (2H, t, J = 7.3 \text{ Hz, H-29, H-35}), 6.40 (2H, s, NH), 6.24 (1H, d, J = 7.2 \text{ Hz, NH-e}), 4.40 (2H, m, H-2, H-6), 4.30 (2H, m, H-24), 4.12 (1H, m, H-25), 3.64 (3H, s, H-38), 3.24 (2H, m, H-9), 2.88 (2H, s, H-20), 2.58 (3H, s, H-17 o. H-19), 2.50 (3H, s, H-17 o. H-19), 2.11 (1H, m, H-3), 2.05 (3H, s, H-18), 1.90 (1H, m, H-7), 1.75 (1H, m, H-7'), 1.61 (2H, m, H-} \]
Synthesis of methyl Nω-[(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl]-D-arginyl-D-valinate (21)

Dipeptide S10 (800 mg, 1.05 mmol, 1.0 equiv) was dissolved in CH₂Cl₂ (70.0 mL) at 0 °C. Then, tris(2-aminoethyl)amine (7.00 mL) was added dropwise. The reaction mixture was warmed to rt and stirred for 2.5 h. The reaction was terminated by adding H₂O. The layers were separated and the organic layer was washed with H₂O (2×). Afterwards, the combined organic layers were washed with brine and then dried over MgSO₄ as well as filtered. The solvent was removed under reduced pressure. Purification by flash chromatography over silica (CH₂Cl₂/CH₃OH 98:2 → CH₂Cl₂/CH₃OH 96:4 → CH₂Cl₂/CH₃OH 94:6 → CH₂Cl₂/CH₃OH 92:8 → CH₂Cl₂/CH₃OH 9:1) gave the desired amine 21 (535 mg, 0.99 mmol, 94% over two steps) as a colourless foam.

R<sub>f</sub> = 0.33 (CH₂Cl₂/CH₃OH 9:1); [α]<sup>20</sup> = +1.4 (c = 1.03, CH₃OH); <sup>1</sup>H-NMR (400 MHz, CD₃OD, CD₃OH = 3.31 ppm): δ = 4.31 (1H, d, J = 6.6 Hz, H-2), 3.70 (3H, s, H-23), 3.40 (1H, m, H-6), 3.17 (2H, m, H-9), 2.99 (3H, s, H-20), 2.57 (3H, s, H-17 o. H-19), 2.51 (3H, s, H-17 o. H-19), 2.15 (1H, m, H-3), 2.08 (3H, s, H-18), 1.67 (1H, m, H-7), 1.54 (3H, m, H-7'; H-8), 1.45 (6H, s, H-22), 0.95 (6H, d, J = 6.6 Hz, H-4) ppm; <sup>13</sup>C-NMR (100 MHz, CD₃OD, CD₃OD = 49.0 ppm): δ = 177.5 (q, C-5), 173.5 (q, C-1), 159.8 (q, C-14), 158.0 (q, C-10), 139.4 (q, C-11), 134.4 (q, C-12 o. C-13 o. C-16), 133.5 (q, C-15), 126.0 (q, C-12 o. C-13 o. C-16), 118.4 (q, C-12 o. C-13 o. C-16), 87.6 (q, C-21), 59.1 (t, C-2), 55.3 (t, C-6), 52.5 (p, C-23), 43.9 (s, C-20), 41.7 (s, C-9), 33.5 (t, C-3), 31.7 (s, C-7), 28.7 (2C, p, C-22), 26.6 (s, C-8), 19.6 (p, C-4 o. C-17 o. C-19), 19.5 (p, C-4 o. C-17 o. C-19), 18.5 (p, C-4 o. C-17 o. C-19), 18.4 (p, C-4 o. C-17 o. C-19), 12.5 (p, C-18) ppm; HRMS (ESI): m/z calculated for C₃₂H₄₂N₅O₆S [M + H]<sup>+</sup>: 540.2856; found 540.2855.
Synthesis of methyl N₂(2-iodo-3-methylbut-2-enoyl)-N⁴-[2,2,4,6,7-pentamethyl-2,3-dihydrobenzofurane-5-yl)sulfonyl]-D-arginyl-D-valinate (27)

Acid 10 (6114 mg, 0.50 mmol, 1.20 equiv) and amine 21 (227 mg, 0.42 mmol, 1.0 equiv) were dissolved in DMF (4.20 mL) at 0 °C. Then, HOAt (61.2 mg, 0.45 mmol, 1.07 equiv) and PyAOP (230 mg, 0.44 mmol, 1.05 equiv) were added. Afterwards, DIPEA (249 mg, 1.93 mmol, 4.60 equiv) was added dropwise. The reaction mixture was stirred at 0 °C → rt. After 22 h, the reaction was terminated by addition of a saturated NH₄Cl solution. The layers were separated and the aqueous layer was extracted with EtOAc (6 × ). The combined organic layers were washed with brine and subsequently dried over MgSO₄, filtered as well as concentrated under reduced pressure. Purification by flash column chromatography over silica (petroleum ether/ethyl acetate = 2:1 → petroleum ether/ethyl acetate = 1:1 → 100% ethyl acetate → ethyl acetate/CH₃OH = 20:1) gave the title compound 27 (250 mg, 0.33 mmol, 80%) as a colourless oil.

Rᵣ = 0.11 (CH₂Cl₂/CH₃OH 98:2); [α]D²⁰ = +10.8 (c = 1.02, CH₂Cl₂); ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.37 (1H, d, J = 7.9 Hz, NH-a), 7.07 (1H, d, J = 7.9 Hz, NH-e), 6.34 (2H, s, NH-b, NH-d), 4.62 (1H, m, H-6), 4.38 (1H, dd, J = 5.5, 7.9 Hz, H-2), 3.69 (3H, s, H-27), 3.27 (2H, brm, H-9), 2.94 (2H, s, H-20), 2.56 (3H, s, H-17 o. H-19), 2.49 (3H, s, H-17 o. H-19), 2.14 (1H, m, H-3), 2.08 (3H, s, H-18), 1.99 (1H, m, H-7), 1.96 (3H, s, H-26), 1.91 (3H, s, H-26), 1.79 (1H, m, H-7'), 1.63 (2H, m, H-8), 1.45 (6H, s, H-22), 0.90 (6H, m, H-4) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 172.4 (q, C-1), 171.9 (q, C-5), 168.2 (q, C-23), 158.9 (q, C-14), 156.5 (q, C-10), 145.2 (q, C-25), 138.5 (q, C-11), 132.9 (q, C-12 o. C-13 o. C-15 o. C-16), 132.4 (q, C-12 o. C-13 o. C-15 o. C-16), 124.7 (q, C-12 o. C-13 o. C-16), 117.6 (q, C-12 o. C-13 o. C-16), 86.5 (q, C-21), 86.3 (q, C-24), 58.1 (t, C-2), 53.3 (t, C-6), 52.3 (p, C-27), 43.4 (s, C-20), 40.8 (s, C-9), 30.7 (t, C-3), 29.71 (s, C-7), 29.66 (p, C-26), 28.7 (2C, p, C-22), 25.5 (s, C-8), 22.0 (p, C-26), 19.5 (p, C-17 o. C-19), 19.2 (p, C-4), 18.3 (p, C-4), 18.1 (p, C-17 o. C-19), 12.6 (p, C-18) ppm; HRMS (ESI): m/z calculated for C₃₀H₄₆N₂O₇SNa [M + Na]⁺: 770.2060; found 770.2052.

Signals of NH⁺-proton could not be detected in the ¹H NMR spectrum.
Synthesis of compound 23 through intermediate S11

Fmoc-D-Orn(Boc)-OH (2.00 g, 5.50 mmol, 1.00 equiv) was dissolved in toluene (22.0 mL) and CH₃OH (8.80 mL) at 0 °C. Then, TMSCHN₂ (2.0 M in Et₂O; 3.00 mL, 7.15 mmol, 1.30 equiv) was added dropwise. Now, the reaction mixture was stirred at 0 °C → rt. After 22 h, the solvent was removed under reduced pressure. The resulting residue was diluted with CH₂Cl₂ and afterwards washed with a NaHCO₃ solution (5%). The layers were separated and the aqueous layer was washed with CH₂Cl₂ (4 ×). The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. Methyl ester S11 (2.58 g, 5.50 mmol, quantitative) was obtained as a colourless solid. 

Rᶠ = 0.60 (CH₂Cl₂/CH₃OH 98:2); mp = 123 – 124 °C; [α]D²⁰ = +5.8 (c = 1.04, DMF); ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.77 (2H, d, J = 7.5 Hz, H-17, H-23), 7.61 (2H, m, H-14, H-20), 7.40 (2H, m, H-16, H-22), 7.32 (2H, m, H-15, H-21), 5.47 (1H, brm, NH-a), 4.59 (1H, brm, NH-b), 4.40 (3H, m, H-2, H-10), 4.22 (1H, t, J = 7.0 Hz, H-11), 3.76 (3H, s, H-24), 3.15 (2H, brm, H-5), 1.88 (1H, m, H-3), 1.68 (1H, m, H-3'), 1.60-1.48 (2H, m, H-4), 1.45 (9H, s, H-8) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 172.9 (q, C-1), 156.11 (q, C-6), 156.05 (q, C-9), 144.0 (q, C-12 o. C-18), 143.9 (q, C-12 o. C-18), 141.5 (q, C-13 o. C-19), 141.4 (q, C-13 o. C-19), 129.2 (t, C-15 o. C-16 o. C-21 o. C-22), 128.4 (t, C-15 o. C-16 o. C-21 o. C-22), 127.8 (t, C-15 o. C-16 o. C-21 o. C-22), 127.2 (t, C-15 o. C-16 o. C-21 o. C-22), 125.4 (t, C-14 o. C-20), 125.2 (t, C-14 o. C-20), 120.11 (t, C-17 o. C-23), 79.4 (q, C-7), 67.1 (s, C-10), 53.7 (t, C-2), 52.6 (p, C-24), 47.3 (t, C-11), 40.1 (s, C-5), 30.0 (s, C-3), 28.5 (3C, p, C-8), 26.2 (s, C-4) ppm; HRMS (ESI): m/z calculated for C₂₆H₃₂N₂O₆Na [M + Na]⁺: 491.2158; found 491.2160.
Synthesis of methyl (R)-2-amino-5-[(tert-butoxycarbonyl)amino]pentanoate (23)

Fmoc-D-Orn(Boc)-OMe (S11; 1.00 g, 2.13 mmol, 1.00 equiv) was dissolved in CH$_2$Cl$_2$ (150 mL) at 0 °C. Then, tris(2-aminoethyl)amine (15.0 mL) was added dropwise. The reaction mixture was warmed to rt and stirred for 3 h. Afterwards, H$_2$O was added. The layers were separated and the organic layer was washed with H$_2$O (2×). The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography over silica (100% CH$_2$Cl$_2$ → CH$_2$Cl$_2$/CH$_3$OH = 99:1 → CH$_2$Cl$_2$/CH$_3$OH = 98:2 → CH$_2$Cl$_2$/CH$_3$OH = 96:4) gave the title compound 23 (484 mg, 1.96 mmol, 92%) as a colourless oil.

R$_f$ = 0.24 (CH$_2$Cl$_2$/CH$_3$OH 98:2); [α]$_D^{20}$ = -17.9 (c = 1.83, CH$_3$OH); $^1$H-NMR (400 MHz, CD$_3$OD, CD$_3$OH = 3.31 ppm): δ = 3.72 (3H, s, H-9), 3.45 (1H, t, J = 6.3 Hz, H-2), 3.05 (2H, t, J = 6.7 Hz, H-5), 1.72 (1H, m, H-3), 1.61 (1H, m, H-3'), 1.56-1.49 (2H, m, H-4), 1.43 (9H, s, H-8) ppm; $^{13}$C-NMR (100 MHz, CD$_3$OD, CD$_3$OD = 49.00 ppm): δ = 177.0 (q, C-1), 158.4 (q, C-6), 79.8 (q, C-7), 54.8 (t, C-2), 52.4 (p, C-9), 41.0 (s, C-5), 32.8 (s, C-3), 28.8 (3C, p, C-8), 27.0 (s, C-4) ppm; HRMS (ESI): m/z calculated for C$_{11}$H$_{22}$N$_2$O$_4$Na [M + Na]$^+$: 269.1477; found 269.1476.

Synthesis of methyl (R)-5-[(tert-butoxycarbonyl)amino]-2-(2-iodo-3-methylbut-2-enamido)pentanoate (29)

Acid 10 (371 mg, 1.64 mmol, 1.00 equiv) and amine 23 (445 mg, 1.81 mmol, 1.10 equiv) were dissolved in DMF (9.80 mL) at 0 °C. Then, HOAt (239 mg, 1.76 mmol, 1.07 equiv) and PyAOP (897 mg, 1.72 mmol, 1.05 equiv) were added. Afterwards, DIPEA (974 mg, 7.53 mmol, 4.60 equiv) was added dropwise. The reaction mixture was warmed to rt. After 22 h of stirring, the reaction was terminated by addition of a saturated NH$_4$Cl solution. The layers were separated and the aqueous layer was extracted with EtOAc (7×). The combined organic layers were washed with brine and subsequently dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography over
silica (petroleum ether/ ethyl acetate = 4:1 → petroleum ether/ ethyl acetate = 3:1 → petroleum ether/ ethyl acetate = 1:1) gave the title compound 29 (641 mg, 1.41 mmol, 86%) as a colourless solid. 

R_f = 0.83 (CH$_2$Cl$_2$/CH$_3$OH 98:2); mp = 112 – 113 °C; [α]$_D^{20}$ = +3.5 (c = 1.04, CH$_2$Cl$_2$); $^1$H-NMR (400 MHz, CDCl$_3$, CHCl$_3$ = 7.26 ppm): δ = 6.38 (1H, d, $J$ = 6.1 Hz, NH-a), 4.61 (2H, m, H-2, NH-b), 3.77 (3H, s, H-13), 3.15 (2H, m, H-5), 2.05 (3H, s, H-12), 2.01 (3H, s, H-12), 1.94 (1H, m, H-3), 1.73 (1H, m, H-3'), 1.57 (2H, m, H-4), 1.43 (9H, s, H-8) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$, CDCl$_3$ = 77.16 ppm): δ = 172.6 (q, C-1), 167.1 (q, C-9), 156.3 (q, C-6), 146.2 (q, C-11), 86.3 (q, C-10), 79.4 (q, C-7), 52.7 (t, C-2), 52.4 (p, C-13), 40.1 (s, C-5), 30.2 (p, C-12), 29.7 (s, C-3), 28.5 (3C, p, C-8), 26.3 (s, C-4), 22.1 (p, C-12) ppm; HRMS (ESI): m/z calculated for C$_{16}$H$_{27}$N$_2$O$_5$Na [M + Na]$^+$: 477.0862; found 477.0858.

**Synthesis of methyl [(R)-5-[(tert-butoxycarbonyl)amino]-2-(2-iodo-3-methylbut-2-enamido)pentanoyl]-D-valinate (35)**

Dipeptide 29 (262 mg, 0.58 mmol, 1.00 equiv) was dissolved in THF (3.20 mL) at 0 °C. Then, LiOH (1.0 M; 3.20 mL) was added. The reaction mixture was warmed up to rt and stirred for 16 h. The reaction mixture was acidified with HCl (1.0 M) to pH ~ 2 and extracted with EtOAc (6×). The combined organic layers were washed with brine and then dried over MgSO$_4$, filtered and concentrated under reduced pressure. The product was used without further purification.

The resulting crude product (254 mg, 0.58 mmol, 1.00 equiv) and D-valine methylester hydrochloride (126 mg, 0.75 mmol, 1.30 equiv) were dissolved in DMF (3.60 mL) at rt. The reaction mixture was cooled to 0 °C. Then, HOAt (84.1 mg, 0.62 mmol, 1.05 equiv) and PyAOP (316 mg, 0.61 mmol, 1.07 equiv) were added. Afterwards, DIPEA (343 mg, 2.65 mmol, 4.60 equiv) was added dropwise. The reaction mixture was stirred at 0 °C → rt. After 23 h, a saturated NH$_4$Cl solution was added. The layers were separated and the aqueous layer was extracted with EtOAc (8×). The combined organic layers were washed with brine and subsequently dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography over silica (petroleum ether/ethyl acetate = 3:1 → petroleum ether/ethyl acetate = 2:1) yielded the title compound 35 (279 mg, 0.50 mmol, 87% over two steps) as a colourless solid.
R_f = 0.67 (CH_2Cl_2/CH_3OH 98:2); mp = 123 – 126 °C; [α]_D^{20} = +7.2 (c = 1.39, CH_2Cl_2); ^1H-NMR (400 MHz, CDCl_3, CHCl_3 = 7.26 ppm): δ = 6.96 (1H, brm, NH-a), 6.46 (1H, dd, J = 5.3, 8.4 Hz, H-2), 3.70 (3H, s, H-17), 3.27 (1H, m, H-9), 3.09 (1H, m, H-9'), 2.16 (1H, m, H-3), 1.99 (3H, s, H-16), 1.96 (3H, s, H-16), 1.92 (1H, m, H-7), 1.67 (1H, m, H-7'), 1.56 (2H, m, H-8), 1.40 (9H, s, H-12), 0.93-0.90 (6H, m, H-4) ppm; ^13C-NMR (100 MHz, CDCl_3, CDCl_3 = 77.16 ppm): δ = 172.1 (q, C-1), 171.6 (q, C-5), 167.5 (q, C-13), 156.6 (q, C-10), 145.6 (q, C-15), 86.6 (q, C-14), 79.3 (q, C-11), 57.6 (t, C-2), 52.7 (t, C-6), 52.2 (p, C-17), 39.6 (s, C-9), 31.4 (t, C-3), 30.9 (p, C-16), 30.0 (s, C-7), 28.5 (3C, p, C-12), 26.4 (s, C-8), 22.0 (p, C-16), 19.2 (p, C-4), 18.0 (p, C-4) ppm; HRMS (ESI): m/z calculated for C_{21}H_{36}IN_3O_6Na [M + Na]^+: 576.1547; found 576.1548.

Synthesis of methyl (R)-5-[[[(benzylxy)carbonyl]amino]-2-(2-iodo-3-methylbut-2-enamido)pentanoate (28)

Boc-D-Orn(Cbz)-OH (400 mg, 1.09 mmol, 1.00 equiv) was dissolved in CH_3OH (2.30 mL) at 0 ºC. Then, freshly distilled thionylchloride (773 mg, 6.50 mmol, 5.95 equiv) was added dropwise. The reaction mixture was stirred at 0 ºC → rt. After 18 h, the solvent was removed under reduced pressure. The resulting residue was charged with Et_2O and the solvent was removed under reduced pressure. This procedure was repeated several times. The crude product 22 was used without further purification.

Acid 10 (138 mg, 0.61 mmol, 1.00 equiv) und amine 22 (222 mg, 0.79 mmol, 1.30 equiv) were dissolved in DMF (3.30 mL) at 0 ºC. Then, HOAt (88.7 mg, 0.65 mmol, 1.07 equiv) and PyAOP (333 mg, 0.64 mmol, 1.05 equiv) were added. Afterwards, DIPEA (361 mg, 2.79 mmol, 4.59 equiv) was added dropwise. The reaction mixture was stirred at 0 ºC → rt. After 22 h, the reaction was terminated by adding a saturated NH_4Cl solution. The layers were separated and the aqueous layer was extracted with EtOAc (7×). The combined organic layers were washed with brine and subsequently dried over MgSO_4 as well as filtered. The solvent was removed under reduced pressure. Purification by flash chromatography over silica (petroleum ether/ethyl acetate = 3:1 → petroleum ether/ethyl acetate = 1:1) yielded the desired vinyl iodide 28 (197 mg, 0.40 mmol, 66% over two steps) as a colourless solid.
R_f = 0.65 (CH_2Cl_2/CH_3OH 98:2); mp = 122 – 124 °C; [α]_D^{20} = +3.2 (c = 1.11, CH_2Cl_2); ^1H-NMR (400 MHz, CDCl_3, CHCl_3 = 7.26 ppm): δ = 7.37-7.28 (5H, m, ArH), 6.39 (1H, d, J = 7.5 Hz, NH-a), 5.07 (2H, s, H-7), 4.98 (1H, brm, NH-b), 4.60 (1H, m, H-2), 3.74 (3H, s, H-16), 3.22 (2H, q, J = 6.4 Hz, H-5), 2.02 (3H, s, H-15), 1.99 (3H, s, H-15), 1.94 (1H, m, H-3), 1.76 (1H, m, H-3'), 1.55 (2H, m, H-4) ppm; ^13C-NMR (100 MHz, CDCl_3, CHCl_3 = 77.16 ppm): δ = 172.4 (q, C-1), 167.1 (q, C-12), 156.6 (q, C-6), 146.2 (q, C-14), 136.6 (q, C-8), 128.6 (3C, t, C-10, C-11), 128.2 (2C, t, C-9), 86.4 (q, C-13), 66.8 (s, C-7), 52.7 (p, C-16), 52.3 (t, C-2), 40.5 (s, C-5), 30.1 (p, C-15), 29.6 (s, C-3), 26.1 (s, C-4), 22.1 (p, C-15) ppm; HRMS (ESI): m/z calculated for C_19H_25N_2O_5Na [M + Na]^+: 511.0706; found 511.0704.

Synthesis of tert-butyl (S)-1-(1-amino-3-methyl-1-oxobutan-2-yl)carbamate (30)

Boc-L-valine (4.00 g, 18.4 mmol, 1.00 equiv) and N-methylmorpholine (2.01 g, 19.9 mmol, 1.08 equiv) were dissolved in 1,2-dimethoxyethane (94.0 mL) at 0 °C. Then, isobutylchloroformiate (2.72 g, 19.9 mmol, 1.08 equiv) was added dropwise. The reaction mixture was stirred for 15 min at 0 °C. Afterwards, an aqueous ammonia solution (25%; 8.20 mL, 121 mmol, 6.57 equiv) was added dropwise. The temperature was raised to rt while stirring. After 22.5 h, the reaction was terminated by adding HCl (1.0 M). The aqueous layer was extracted with EtOAc (4 ×). The combined organic layers were washed with HCl (0.1 M) and subsequently dried over MgSO_4, filtered and concentrated under reduced pressure. Amide 30 (3.91 g, 18.1 mmol, 98%) was obtained as a colourless solid. The spectroscopic data matches those reported in the literature.[66]

R_f = 0.20 (CH_2Cl_2/CH_3OH = 98:2); mp = 155 – 157 °C (mp = 155 – 157 ºC, ref.[57]); [α]_D^{20} = -2.4 (c 1.16, CH_3OH) ([α]_D^{20} = -0.4 (c 1.46, CH_3OH) ref.[66]); ^1H-NMR (400 MHz, CDCl_3, CHCl_3 = 7.26 ppm): δ = 6.08 (1H, brs, NH-b), 5.67 (1H, brs, NH-b), 5.08 (1H, brm, NH-a), 3.97 (1H, t, J = 7.0 Hz, H-2), 2.14 (1H, m, H-3), 1.44 (9H, s, H-7), 0.99 (3H, d, J = 6.8 Hz, H-4), 0.94 (3H, d, J = 6.8 Hz, H-4) ppm; ^13C-NMR (100 MHz, CDCl_3, CDCl_3 = 77.16 ppm): δ = 174.2 (q, C-1), 156.1 (q, C-5), 80.2 (q, C-6), 59.6 (t, C-2), 30.8 (t, C-3), 28.5 (3C, p, C-7), 19.4 (2C, p, C-4) ppm; HRMS (ESI): m/z calculated for C_{10}H_{20}N_2O_5Na [M + Na]^+: 239.1372; found 239.1372.
Synthesis of methyl (E)-N\[^{\omega}\],N\[^{\omega}'\]-bis[(allyloxy)carbonyl]-N\[^{2}\]-2-[[(S)-2-[(tert-butoxy-carbonyl)amino]-3-methylbutanamido]-3-methylbut-2-enoxy]-D-arginyl-D-valinate (31)

Boc-L-Val-NH\(_2\) (30; 32.6 mg, 0.15 mmol, 1.00 equiv) and vinyl iodide 25 (205 mg, 0.31 mmol, 2.00 equiv) were dissolved in 1,4-dioxane (degassed; 0.15 mL) at rt. Then, K\(_2\)CO\(_3\) (41.7 mg, 0.31 mmol, 2.00 equiv), Cul (17.2 mg, 90.0 µmol, 0.60 equiv) and trans-N,N-dimethyl-1,2-cyclohexanediamine (86.8 mg, 0.61 mmol, 4.05 equiv) were added. The reaction mixture was heated to 70 °C. After 22 h, the solvent was removed under reduced pressure. The resulting residue was dissolved in CH\(_3\)OH (1.8 mL) and purified by preparative HPLC (C18) (gradient CH\(_3\)OH/H\(_2\)O = 20:80 (5 min) ➔ CH\(_3\)OH/H\(_2\)O = 50:50 (25 min) ➔ CH\(_3\)OH/H\(_2\)O = 50:50 (15 min) ➔ CH\(_3\)OH/H\(_2\)O = 100:0 (40 min) ➔ CH\(_3\)OH/H\(_2\)O = 100:0 (20 min) 15 mL/min.) (t\(_R\) = 69.5 min.). The desired tetrapeptide 31 (13.6 mg, 18.1 µmol, 13%) was obtained as a colourless oil.

\(^1\)H-NMR (400 MHz, CDCl\(_3\), CHCl\(_3\) = 7.26 ppm): \(\delta = 11.71\) (1H, brs, NH-c), \(8.34\) (1H, t, \(J = 5.1\) Hz, NH-b), \(7.34\) (1H, s, NH-e), \(7.05\) (1H, m, NH-a o. NH-d), \(6.97\) (1H, d, \(J = 8.5\) Hz, NH-a o. NH-d), \(5.94\) (2H, m, H-13, H-17), \(5.38-5.20\) (4H, m, H-14, H-18), \(5.02\) (1H, m, NH-f), \(4.64-4.58\) (4H, m, H-12, H-16), \(4.45\) (2H, m, H-2, H-6), \(3.87\) (1H, t, \(J = 5.6\) Hz, H-24), \(3.71\) (3H, s, H-30), \(3.47\) (2H, m, H-9), \(2.16\) (2H, m, H-3, H-25), \(2.09\) (3H, s, H-22), \(2.01\) (1H, m, H-7), \(1.78\) (3H, s, H-22), \(1.71\) (3H, m, H-7', H-8), \(1.43\) (9H, s, H-29), \(1.02\) (6H, d, \(J = 6.8\) Hz, H-4 o. H-26), \(0.98\) (3H, d, \(J = 6.8\) Hz, H-4 o. H-26), \(0.90\) (3H, d, \(J = 6.8\) Hz, H-4 o. H-26) ppm; HRMS (ESI): \(m/z\) calculated for C\(_{35}\)H\(_{57}\)N\(_7\)O\(_{11}\)Na [M + Na]\(^+\): 774.4014; found 774.4015.

Boc-L-Val-NH₂ (30; 14.5 mg, 67.0 µmol, 1.00 equiv) and vinyl iodide 24 (78.8 mg, 0.13 mmol, 2.00 equiv) were dissolved in 1,4-dioxane (degassed; 0.13 mL) at rt. Then, K₂CO₃ (18.5 mg, 0.13 mmol, 2.00 equiv), Cul (7.66 mg, 40.2 µmol, 0.60 equiv) and trans-N,N-dimethyl-1,2-cyclohexanediamine (38.6 mg, 0.27 mmol, 4.05 equiv) were added. The reaction mixture was heated to 70 °C. After 22 h, the solvent was removed under reduced pressure. The resulting residue was charged with a saturated NH₄Cl solution. Then, the aqueous layer was extracted with EtOAc (4×). The combined organic layers were washed with brine and subsequently dried over MgSO₄ as well as filtered. The solvent was removed under reduced pressure. Purification by flash chromatography on silica (100% CH₂Cl₂ → CH₂Cl₂/CH₃OH 99:1 → CH₂Cl₂/CH₃OH 98:2) yielded the desired product 32 (22.5 mg, 33.3 µmol, 50%) as a colourless solid.

R₇ = 0.21 (CH₂Cl₂/CH₃OH 98:2); [α]²⁰D = +21.3 (c = 0.97, CH₂Cl₂); ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.80 (1H, s, NH-d), 7.37-7.28 (5H, m, ArH), 7.17 (2H, m, NH-a, NH-b), 5.32 (1H, t, J = 4.1 Hz, NH-c), 5.20 (1H, d, J = 6.2 Hz, NH-e), 5.07 (2H, s, H-11), 4.60 (1H, m, H-6), 4.45 (1H, dd, J = 6.0, 8.0 Hz, H-2), 3.90 (1H, m, H-21), 3.70 (3H, s, H-27), 3.27 (1H, m, H-9), 3.15 (1H, m, H-9'), 2.14 (2H, m, H-3, H-22), 2.06 (3H, s, H-19), 1.93 (1H, m, H-7), 1.76 (3H, s, H-19), 1.70-1.53 (3H, m, H-7', H-8), 1.42 (9H, s, H-26), 0.98 (6H, d, J = 6.8 Hz, H-4), 0.94-0.90 (6H, m, H-23) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 172.3 (q, C-1), 172.0 (q, C-5 o. C-20), 171.6 (q, C-5 o. C-20), 166.4 (q, C-16), 157.1 (q, C-10), 156.6 (q, C-24), 140.7 (q, C-17), 136.7 (q, C-12), 128.6 (3C, t, C-14, C-15), 128.2 (2C, t, C-13), 123.5 (q, C-18), 80.7 (q, C-25), 66.8 (s, C-11), 60.8 (t, C-21), 57.5 (t, C-2), 52.5 (t, C-6), 52.2 (p, C-27), 40.2 (s, C-9), 31.0 (t, C-3 o. C-22), 30.3 (t, C-3 o. C-22), 28.6 (s, C-7), 28.4 (3C, p, C-26), 25.9 (s, C-8), 21.5 (p, C-19), 21.0 (p, C-19), 19.6 (p, C-4), 19.1 (p, C-4), 18.2 (p, C-23), 18.0 (p, C-23) ppm; HRMS (ESI): m/z calculated for C₃₂H₅₃N₅OₙNa [M + Na]⁺: 698.3741; found 698.3740.


![Chemical Structure](image)

Boc-L-Val-NH₂ (30; 11.3 mg, 52.2 µmol, 1.00 equiv) and vinyl iodide 28 (50.8 mg, 0.10 mmol, 2.00 equiv) were dissolved in 1,4-dioxane (degassed; 0.11 mL) at rt. Then, K₂CO₃ (14.4 mg, 0.10 mmol, 2.00 equiv), Cul (5.94 mg, 31.1 µmol, 0.60 equiv) and trans-N,N-dimethyl-1,2-
cyclohexanediamine (30.0 mg, 0.21 mmol, 4.05 equiv) were added. The reaction mixture was heated to 70 °C. After 22 h, the solvent was removed under reduced pressure. The resulting residue was charged with a saturated NH₄Cl solution. Then, the aqueous layer was extracted with EtOAc (4×). The combined organic layers were washed with brine and subsequently dried over MgSO₄ as well as filtered. The solvent was removed under reduced pressure. Purification by flash chromatography on silica (100% CH₂Cl₂ → CH₂Cl₂/CH₃OH 99:1 → CH₂Cl₂/CH₃OH 98:2) yielded the desired product 33 (15.1 mg, 26.2 µmol, 50%) as a colourless solid. 

Rᵣ = 0.21 (CH₂Cl₂/CH₃OH 98:2); ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.71 (1H, s, NH-c), 7.36-7.28 (5H, m, ArH), 7.04 (2H, d, J = 7.5 Hz, NH-b), 5.45 (1H, t, J = 5.0 Hz, NH-a), 5.17 (1H, m, NH-d), 5.08 (2H, s, H-7), 4.61 (1H, m, H-2), 3.89 (1H, t, J = 4.2 Hz, H-17), 3.70 (3H, s, H-23), 3.19 (2H, m, H-5), 2.11 (1H, m, H-18), 2.06 (3H, s, H-15), 1.96 (1H, m, H-3), 1.85 (1H, m, H-3'), 1.75 (3H, s, H-15), 1.68-1.53 (2H, m, H-4), 1.44 (9H, s, H-22), 0.95-0.93 (6H, m, H-19) ppm; HRMS (ESI): m/z calculated for C₂₉H₄₄N₄O₈Na [M + Na⁺]: 599.3057; found 599.3503.

Synthesis of compound 34 through intermediate S12

Synthesis of [(allyloxy)carbonyl]-L-valine (S12)

L-Valine (2.00 g, 17.1 mmol, 1.00 equiv) and K₂CO₃ (3.54 g, 25.6 mmol, 1.50 equiv) were dissolved in THF/H₂O (1:1; 80.0 mL) at 0 °C. Afterwards, allylchloroformiate (2.47 g, 20.5 mmol, 1.20 equiv) was added dropwise. The reaction mixture was stirred for 16 h at 0 °C → rt. Then, the solvent was removed under reduced pressure. The resulting residue was extracted with Et₂O (3x). The aqueous layer was acidified with HCl (conc.) towards pH ~ 2 and subsequently extracted with CH₂Cl₂ (5x). The combined organic layers were washed with brine and dried over MgSO₄. After filtration, the solvent was removed under reduced pressure. The desired product S12 (3.44 g, 17.1 mmol, quantitative) was obtained as a colourless oil.

The spectroscopic data matches those reported in the literature.⁵⁸
$[\alpha]_D^{20} = -2.9 \ (c = 2.48, \text{DMSO}) \ \{\text{ref.}^{[S9]}: \ [\alpha]_D^{20} = -10 \ (c = 1.00, \text{EtOH})\}; \ 1^H-\text{NMR} \ (400 \text{ MHz, DMSO-d}_6, \text{DMSO-d}_5 = 2.50 \text{ ppm}): \ \delta = 12.55 \ (1\text{H, brs, OH}), \ 7.41 \ (1\text{H, d, } J = 8.5 \text{ Hz, NH}), \ 5.90 \ (1\text{H, m, H-7}), \ 5.30 \ (1\text{H, dd, } J = 1.7, 17.4 \text{ Hz, H-8}), \ 5.17 \ (1\text{H, dd, } J = 1.7, 10.6 \text{ Hz, H-8}), \ 4.48 \ (2\text{H, dt, } J = 0.9, 5.1 \text{ Hz, H-6}), \ 3.85 \ (1\text{H, dd, } J = 6.4, 8.5 \text{ Hz, H-2}), \ 2.04 \ (1\text{H, m, H-3}), \ 0.88 \ (6\text{H}, \ t, J = 6.4 \text{ Hz, H-4}) \ \text{ppm}; \ ^{13}C-\text{NMR} \ (100 \text{ MHz, DMSO-d}_6, \text{DMSO-d}_5 = 39.52 \text{ ppm}): \ \delta = 173.3 \ (q, \text{ C-1}), \ 156.2 \ (q, \text{ C-5}), \ 133.6 \ (t, \text{ C-7}), \ 117.0 \ (s, \text{ C-8}), \ 64.5 \ (s, \text{ C-6}), \ 59.5 \ (t, \text{ C-2}), \ 29.5 \ (t, \text{ C-3}), \ 19.2 \ (p, \text{ C-4}), \ 18.0 \ (p, \text{ C-4}) \ \text{ppm}; \ \text{HRMS (ESI)}: \ m/z \ \text{calculated for C}_{9}H_{15}NO_{4}Na [M + Na]^+: 224.0899; \ \text{found 224.0900}.

**Synthesis of allyl (S)-1-(1-amino-3-methyl-1-oxobutan-2-yl)carbamate (34)**

Alloc-L-valine (S12; 3.44 g, 17.1 mmol, 1.00 equiv) and N-methylmorpholine (1.87 g, 18.4 mmol, 1.08 equiv) were dissolved in 1,2-dimethoxyethane (87.0 mL) at 0 °C. Then, isobutylchloroformiate (2.52 g, 18.4 mmol, 1.08 equiv) was added dropwise. The reaction mixture was stirred for 15 min at 0 °C. Afterwards, an aqueous ammonia solution (25%; 7.60 mL, 112 mmol, 6.57 equiv) was added dropwise. The temperature was raised to rt while stirring. After 23 h, the reaction was terminated by adding HCl (1.0 M). The aqueous layer was extracted with EtOAc (6×). The combined organic layers were washed with HCl (0.1 M) and subsequently dried over MgSO$_4$, filtered and the solvent was removed under reduced pressure. Amide 34 (3.00 g, 15.0 mmol, 88%) was obtained as a colourless solid. $R_f = 0.18 \ (\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH} \ 98:2); \ \text{mp} = 147 - 149 \degree \text{C}; \ [\alpha]_D^{20} = -5.9 \ (c = 1.39, \text{CH}_3\text{OH}); \ 1^H-\text{NMR} \ (400 \text{ MHz, DMSO-d}_6, \text{DMSO-d}_5 = 2.50 \text{ ppm}): \ \delta = 7.32 \ (1\text{H, brs, NH-a}), \ 7.01 \ (1\text{H, brs, NH-b}), \ 7.06 \ (1\text{H, d, } J = 9.0 \text{ Hz, NH-a}), \ 5.90 \ (1\text{H, m, H-7}), \ 5.29 \ (1\text{H, ddd, } J = 1.4, 3.1, 17.1 \text{ Hz, H-8}), \ 5.17 \ (1\text{H, ddd, } J = 1.4, 1.4, 10.6 \text{ Hz, H-8}), \ 4.47 \ (2\text{H, dt, } J = 1.4, 5.1 \text{ Hz, H-6}), \ 3.78 \ (1\text{H, dd, } J = 6.8, 9.0 \text{ Hz, H-2}), \ 1.94 \ (1\text{H, m, H-3}), \ 0.86 \ (3\text{H, d, } J = 6.8 \text{ Hz, H-4}), \ 0.83 \ (3\text{H, d, } J = 6.8 \text{ Hz, H-4}) \ \text{ppm}; \ ^{13}C-\text{NMR} \ (100 \text{ MHz, DMSO-d}_6, \text{DMSO-d}_5 = 39.52 \text{ ppm}): \ \delta = 173.2 \ (q, \text{ C-1}), \ 156.0 \ (q, \text{ C-5}), \ 133.7 \ (t, \text{ C-7}), \ 116.9 \ (s, \text{ C-8}), \ 64.4 \ (s, \text{ C-6}), \ 59.9 \ (t, \text{ C-2}), \ 30.1 \ (t, \text{ C-3}), \ 19.3 \ (p, \text{ C-4}), \ 18.0 \ (p, \text{ C-4}) \ \text{ppm}; \ \text{HRMS (ESI)}: \ m/z \ \text{calculated for C}_{9}H_{16}N_{2}O_{3}Na [M + Na]^+: 223.1059; \ \text{found 223.1059}.
Synthesis of methyl (2R,8S)-2-[3-[(tert-butoxycarbonyl)amino]propyl]-8-isopropyl-4,7,10-trioxo-5-(propan-2-ylidene)-11-oxa-3,6,9-triazatetradec-13-enoate (36)

Alloc-L-Val-NH₂ (34; 35.3 mg, 0.18 mmol, 1.00 equiv) and vinyl iodide 29 (160 mg, 0.35 mmol, 2.00 equiv) were dissolved in 1,4-dioxane (degassed; 0.18 mL) at rt. Then, K₂CO₃ (48.7 mg, 0.35 mmol, 2.00 equiv), CuI (20.1 mg, 0.11 mmol, 0.60 equiv) and trans-N,N-dimethyl-1,2-cyclohexanediame (101 mg, 0.71 mmol, 4.05 equiv) were added. The reaction mixture was heated to 70 °C. After 22 h, the reaction was terminated by adding a saturated NH₄Cl solution. Then, the aqueous layer was extracted with EtOAc (6×). The combined organic layers were washed with brine and subsequently dried over MgSO₄ as well as filtered. The solvent was removed under reduced pressure. The resulting residue was dissolved in CH₃OH (1.8 mL) and purified by preparative HPLC (C18) (gradient CH₃OH/H₂O 10:90 → CH₃OH/H₂O = 100:0 → CH₃OH/H₂O = 100:0 → CH₃OH/H₂O = 100:0, 15.0 mL/min) (tᵣ = 70.8 min). Dehydropeptide 36 (10.4 mg, 19.7 µmol, 11%) was obtained as a colourless oil.

¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.64 (1H, brs, NH-c), 7.01 (1H, brd, NH-a), 5.90 (1H, m, H-19), 5.43 (1H, brs, NH-d), 5.35-5.27 (2H, m, H-20), 4.88 (1H, brm, NH-b), 4.56 (2H, m, H-18), 4.46 (1H, m, H-2), 3.94 (1H, t, J = 6.8 Hz, H-14), 3.71 (3H, s, H-21), 3.12 (2H, m, H-5), 2.17 (1H, m, H-15), 2.10 (3H, s, H-12), 1.91 (1H, m, H-3), 1.81 (3H, s, H-12), 1.79 (1H, m, H-3'), 1.53 (2H, m, H-4), 1.42 (9H, s, H-8), 0.92 (6H, m, H-16) ppm; HRMS (ESI): m/z calculated for C₂₅H₄₂N₄O₈Na [M + Na]⁺: 549.2900; founded 549.2899.


Alloc-L-Val-NH₂ (34; 28.0 mg, 0.14 mmol, 1.00 equiv) and vinyl iodide 35 (155 mg, 0.28 mmol, 2.00 equiv) were dissolved in 1,4-dioxane (degassed; 0.15 mL) at rt. Then, K₂CO₃ (38.7 mg, 0.28 mmol, 2.00 equiv), CuI (16.0 mg, 84.1 µmol, 0.60 equiv) and trans-N,N-dimethyl-1,2-cyclohexanediame (80.6 mg, 0.57 mmol, 4.05 equiv) were added. The
reaction mixture was heated to 70 °C. After 22 h, the reaction was terminated by adding a saturated NH₄Cl solution. Then, the aqueous layer was extracted with EtOAc (6×). The combined organic layers were washed with brine and subsequently dried over MgSO₄ as well as filtered. The solvent was removed under reduced pressure. The resulting residue was dissolved in CH₂OH (1.8 mL) and purified by preparative HPLC (C18) (gradient CH₂OH/H₂O 10:90 {5 min} → CH₃OH/H₂O = 100:0 {85 min} → CH₃OH/H₂O = 100:0 {10 min}, 15.0 mL/min) (tᵣ = 73.8 min). Dehydropeptide 37 (11.5 mg, 18.4 µmol, 13%) was obtained as a colourless oil.

¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.64 (1H, brs, NH-e), 7.06 (1H, brd, NH-c), 7.01 (1H, brd, NH-a), 5.90 (1H, m, H-23), 5.43 (1H, brs, NH-d), 5.31-5.22 (2H, m, H-24), 4.88 (1H, brm, NH-b), 4.61-4.50 (2H, m, H-22), 4.44-4.39 (2H, m, H-2, H-6), 3.95 (1H, t, J = 6.8 Hz, H-18), 3.71 (3H, s, H-25), 3.12 (2H, m, H-9), 2.17 (2H, m, H-3, H-19), 2.10 (3H, s, H-16), 1.92 (1H, m, H-7), 1.81 (3H, s, H-16), 1.79 (1H, m, H-7'), 1.55-1.50 (2H, m, H-8), 1.42 (9H, s, H-12), 0.98-0.92 (12H, m, H-4, H-20) ppm; HRMS (ESI): m/z calculated for C₃₀H₅₁N₅O₉Na [M + Na]+: 648.3584; found 648.3588.

Synthesis of compound 38 through intermediates S13-S15

\[ \text{TMS} \overset{\text{OH}}{\longrightarrow} \text{TMS} \overset{\text{OCl}}{\longrightarrow} \text{TMS} \overset{\text{OSu}}{\longrightarrow} \]

\[ \text{H₂N} \overset{\text{OH}}{\longrightarrow} \text{TeocHN} \overset{\text{OH}}{\longrightarrow} \text{TeocHN} \overset{\text{NH₂}}{\longrightarrow} \]

Synthesis of (2-(trimethylsilyl)ethoxycarbonyloxy)chloride (S13)

\[ \overset{\text{Si}}{\overset{\text{3}}{\overset{\text{O}}{\overset{\text{Cl}}{\longrightarrow}}}} \]

2-Trimethylsilylethanol (4.13 g, 34.9 mmol, 1.00 equiv) and K₂CO₃ (4.15 g, 30.0 mmol, 0.86 equiv) were dissolved in toluene (20.0 mL) at −10 °C. Afterwards, phosgene (20% in toluene; 24.0 mL, 45.4 mmol, 1.30 equiv) was added dropwise. The reaction mixture was stirred for 30 min at −10 °C, then warmed to rt and stirred for additional 60 min. Excess of phosgene was removed by passing a stream of nitrogen gas through the flask. Then, the flask was neutralized by washing it with a saturated K₂CO₃ solution. Then, the solvent was removed under reduced pressure (waterbath temperature max. 30 °C; pressure max. 45 mbar). The resulting residue was filtered over MgSO₄ and washed with Et₂O. Again, the solvent was removed under reduced pressure (water bath temperature max. 30 °C; pressure
max. 45 mbar). The product S13 was used without further purifications. The spectroscopic data were in accordance with those reported in the literature.\[^{[510]}\]

$^1$H-NMR (400 MHz, CDCl$_3$, CHCl$_3$ = 7.26 ppm): $\delta$ = 4.41 (2H, m, H-2), 1.13 (2H, m, H-3), 0.06 (9H, s, H-4) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$, CDCl$_3$ = 77.16 ppm): $\delta$ = 156.7 (q, C-1), 71.5 (s, C-2), 17.6 (s, C-3), -1.5 (3C, p, C-4) ppm.

**Synthesis of 2,5-dioxopyrrolidin-1-yl [2(trimethylsilyl)ethyl] carbonate (S14)**

TeocCl reagent S13 was dissolved in CH$_3$CN (105 mL) at 0 °C. Then, NHS (5.22 g, 45.4 mmol, 1.30 equiv) and Et$_3$N (4.59 g, 45.4 mmol, 1.30 equiv; in 11.0 mL CH$_3$CN) were added. The reaction mixture was stirred at 0 °C $\rightarrow$ rt. After 16 h, the reaction mixture was poured into H$_2$O. The aqueous layer was extracted with Et$_2$O (6 ×). The combined organic layers were washed with H$_2$O (2 ×), HCl (1.0 M), followed by H$_2$O and subsequent dried over MgSO$_4$, filtered as well as concentrated under reduced pressure. The desired TeocOSu S14 (7.72 g, 29.8 mmol, 85% over two steps) was collected as colourless solid. The spectroscopic and physical data were in accordance with those reported in the literature.\[^{[510]}\] $R_f$ = 0.72 (petroleum ether/ethyl acetate = 1:1); mp = 92 – 97 °C (mp = 98 – 99 °C, ref.\[^{[510]}\]);

$^1$H-NMR (400 MHz, CDCl$_3$, CHCl$_3$ = 7.26 ppm): $\delta$ = 4.41 (2H, m, H-2), 2.83 (4H, s, H-6), 1.15 (2H, m, H-3), 0.07 (9H, s, H-4) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$, CDCl$_3$ = 77.16 ppm): $\delta$ = 168.9 (2C, q, C-5), 151.6 (q, C-1), 70.8 (s, C-2), 25.6 (2C, s, C-6), 17.7 (s, C-3), -1.5 (3C, p, C-4) ppm.

**Synthesis of [(2-(trimethylsilyl)ethoxy)carbonyl]-L-valine (S15)**

L-Valine (1.17 g, 10.0 mmol, 1.00 equiv) was dissolved in H$_2$O (10.0 mL) at rt. Then, Et$_3$N (1.52 g, 15.0 mmol, 1.50 equiv) in 1,4-dioxane (10.0 mL) was added, followed by TeocOSu S14 (2.85 g, 11.0 mmol, 1.10 equiv). The reaction mixture was stirred for 15 h at rt, then diluted with H$_2$O and acidified with saturated KH$_2$SO$_4$ solution. Afterwards, the reaction mixture was extracted with EtOAc (6 ×). The combined organic layers were washed with H$_2$O (4x) and subsequently dried over MgSO$_4$, filtered as well as concentrated under reduced pressure. The product was directly used in the next step without further purifications.

$^1$H-NMR (200 MHz, CD$_3$OD, CD$_3$OH = 3.31 ppm): $\delta$ = 4.15 (2H, m, H-6), 4.05 (1H, d, $J$ = 6.8 Hz, H-2), 2.15 (1H, m, H-3), 1.02 (2H, m, H-7), 0.98 (3H, d, $J$ = 6.8 Hz, H-4), 0.94 (3H, d, $J$ =
6.8 Hz, H-4), 0.05 (9H, s, H-8) ppm; HRMS (ESI): m/z calculated for C_{11}H_{23}NO_{2}SiNa [M + Na]^+: 284.1294; found 284.1295.

**Synthesis of 2-(trimethylsilyl)ethyl (S)-(1-amino-3-methyl-1-oxobutan-2-yl)carbamate (38)**

Teoc-L-valine S15 and N-methylmorpholine (1.09 g, 10.8 mmol, 1.08 equiv) were dissolved in 1,2-dimethoxyethane (51.0 mL) at 0 °C. Then, isobutylchloroformiate (1.48 g, 10.8 mmol, 1.08 equiv) was added dropwise. The reaction mixture was stirred for 20 min at 0 °C. Afterwards, an aqueous ammonia solution (25%; 5.00 mL, 65.7 mmol 6.57 equiv) was added dropwise. The reaction mixture was warmed up to rt. After 23 h, the reaction was terminated by addition of HCl (1.0 M). The aqueous layer was extracted with EtOAc (7 ×). The combined organic layers were washed with HCl (0.1 M) and subsequently dried over MgSO₄, filtered and concentrated under reduced pressure. Amide 38 (2.60 g, 10.0 mmol, quantitative over two steps) was obtained as a colourless solid. mp = 138 – 139 °C; [α]_D^{20} = -4.5 (c 1.21, CH₃OH); ¹H-NMR (400 MHz, DMSO-d₆, DMSO-d₅ = 2.50 ppm): δ = 7.28 (1H, s, NH-b), 7.00 (1H, s, NH-b), 6.80 (1H, d, J = 8.8 Hz, NH-a), 4.03 (2H, m, H-6), 3.77 (1H, dd, J = 6.8, 8.8 Hz, H-2), 1.92 (1H, m, H-3), 0.92 (2H, t, J = 8.4 Hz, H-7), 0.85 (3H, d, J = 6.8 Hz, H-4), 0.82 (3H, d, J = 6.8 Hz, H-4), 0.02 (9H, s, H-8) ppm; ¹³C-NMR (100 MHz, DMSO-d₆, DMSO-d₅ = 39.52 ppm): δ = 173.3 (q, C-1), 156.3 (q, C-5), 61.7 (s, C-6), 59.8 (t, C-2), 30.1 (t, C-3), 19.3 (s, C-7), 17.9 (p, C-4), 17.4 (p, C-4), -1.4 (3C, p, C-8) ppm; HRMS (ESI): m/z calculated for C_{11}H_{24}N_{2}O_{3}SiNa [M + Na]^+: 283.1454; found 283.1457.


Teoc-L-Val-NH₂ (38; 30.7 mg, 0.12 mmol, 1.00 equiv) and vinyl iodide 29 (107 mg, 0.24 mmol, 2.00 equiv) were dissolved in 1,4-dioxane (degassed; 0.24 mL) at rt. Then, K₂CO₃ (32.6 mg, 0.24 mmol, 2.00 equiv), Cul (13.5 mg, 70.9 µmol, 0.60 equiv) and trans-
N,N-dimethyl-1,2-cyclohexanediame (67.9 mg, 0.48 mmol, 4.05 equiv) were added. The reaction mixture was heated to 70 °C. After 18 h, the reaction mixture was terminated by adding a saturated NH₄Cl solution. Then, the aqueous layer was extracted with EtOAc (8 ×). The combined organic layers were washed with brine and dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting residue was dissolved in CH₃OH (1.8 mL) and purified by preparative HPLC (C18) (gradient CH₃OH/H₂O 10:90 [5 min] → CH₃OH/H₂O = 100:0 [85 min] → CH₃OH/H₂O = 100:0 [10 min], 15 mL/min) (tᵣ = 79.9 min). The desired tripeptide 39 (22.7 mg, 38.7 µmol, 33%) was obtained as a colourless foam. [α]D²⁰ = -11.5 (c = 1.39, CH₂Cl₂); ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.52 (1H, brs, NH-c), 7.01 (1H, brm, NH-a), 5.24 (1H, brm, NH-d), 4.91 (1H, brm, NH-b), 4.59 (1H, m, H-2), 4.17 (2H, m, H-18), 4.00 (2H, m, J = 5.5 Hz, H-19), 3.95 (1H, t, J = 6.8 Hz, H-14), 3.71 (3H, s, H-21), 3.12 (2H, m, H-5), 2.15 (1H, m, H-15), 2.09 (3H, s, H-12), 1.91 (1H, m, H-3), 1.76 (3H, s, H-12), 1.73 (1H, m, H-3′), 1.57 (2H, m, H-4), 1.42 (9H, s, H-8), 1.04-0.98 (8H, m, H-16, H-19), 0.03 (9H, s, H-20) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 172.8 (q, C-1), 171.2 (q, C-13), 165.8 (q, C-9), 157.5 (q, C-17), 156.3 (q, C-6), 141.5 (q, C-10), 123.4 (q, C-11), 79.2 (q, C-7), 64.2 (s, C-18), 61.3 (t, C-14), 52.4 (p, C-21), 52.1 (t, C-2), 40.2 (s, C-5), 30.2 (t, C-15), 29.2 (s, C-3), 28.6 (3C, p, C-8), 25.9 (s, C-4), 21.6 (p, C-12), 21.0 (p, C-12), 19.6 (s, C-19), 18.2 (p, C-16), 17.9 (p, C-16), -1.4 (3C, p, C-20) ppm; HRMS (ESI): m/z calculated for C₂₇H₅₅N₄O₈SiNa [M + Na]+: 609.3296; found 609.3298.


Teoc-L-Val-NH₂ (38; 31.6 mg, 0.12 mmol, 1.00 equiv) and vinyl iodide 35 (134 mg, 0.24 mmol, 2.00 equiv) were dissolved in 1,4-dioxane (degassed; 0.25 mL) at rt. Then, K₂CO₃ (33.5 mg, 0.24 mmol, 2.00 equiv), Cul (13.9 mg, 73.0 µmol, 0.60 equiv) and trans-N,N-dimethyl-1,2-cyclohexanediame (69.9 mg, 0.49 mmol, 4.05 equiv) were added. The reaction mixture was heated to 70 °C. After 22 h, the reaction mixture was terminated by adding a saturated NH₄Cl solution. Then, the aqueous layer was extracted with EtOAc (8 ×). The combined organic layers were washed with brine and subsequently dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting residue was dissolved in CH₃OH (1.8 mL) and purified by preparative HPLC (C18) (gradient CH₃OH/H₂O 10:90 [5...
min) → CH₂OH/H₂O = 100:0 {85 min} → CH₂OH/H₂O = 100:0 {10 min, 15 mL/min} (tᵣ = 81.9 min). The desired tetrapeptide 40 (38.7 mg, 56.4 µmol, 47%) was obtained as a colourless foam.

[α]D²₀ = +3.5 (c = 1.00, CH₂Cl₂); ¹H-NMR (500 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.57 (1H, s, NH-d), 7.06 (1H, d, J = 7.5 Hz, NH-c), 7.03 (1H, d, J = 8.6 Hz, NH-a), 5.28 (1H, d, J = 6.8 Hz, NH-e), 4.88 (1H, brm, NH-b), 4.44 (1H, dd, J = 5.3, 7.8 Hz, H-2), 4.15 (2H, m, H-22), 3.92 (1H, t, J = 6.8 Hz, H-18), 3.70 (3H, s, H-25), 3.13 (2H, m, H-9), 2.16 (2H, m, H-3, H-19), 2.10 (3H, s, H-16), 1.78 (3H, s, H-16), 1.74 (1H, m, H-7'). 1.59 (2H, m, H-8), 1.42 (9H, s, H-12), 1.03 (6H, d, J = 6.8 Hz, H-4), 0.98 (2H, d, J = 8.1 Hz, H-23), 0.91 (6H, d, J = 6.8 Hz, H-20). 0.03 (9H, s, H-24) ppm; ¹³C-NMR (125 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 172.3 (q, C-1), 171.8 (q, C-5), 171.3 (q, C-17), 166.1 (q, C-13), 157.5 (q, C-21), 156.5 (q, C-10), 141.2 (q, C-14), 123.5 (q, C-15), 79.2 (q, C-11), 64.2 (s, C-22), 61.2 (t, C-18), 57.6 (t, C-2), 52.9 (t, C-6), 52.2 (p, C-25), 40.0 (s, C-9), 31.0 (t, C-3 o. C-19), 30.3 (t, C-3 o. C-19), 28.6 (3C, p, C-12), 28.4 (s, C-7), 26.2 (s, C-8), 21.7 (p, C-16), 21.0 (p, C-16), 19.7 (s, C-23), 19.2 (p, C-4), 18.3 (p, C-4), 18.2 (p, C-20), 17.8 (p, C-20), -1.3 (3C, p, C-24) ppm; HRMS (ESI): m/z calculated for C₃₂H₅₈N₅O₅SiNa [M + Na⁺]: 708.3980; found 708.3982.


Teoc-L-Val-NH₂ (38; 43.5 mg, 0.17 mmol, 1.00 equiv) and vinyl iodide 27 (250 mg, 0.33 mmol, 2.00 equiv) were dissolved in 1,4-dioxane (degassed; 0.17 mL) at rt. Then, K₂CO₃ (46.2 mg, 0.33 mmol, 2.00 equiv), Cul (19.1 mg, 0.10 mmol, 0.60 equiv) and trans-N,N-dimethyl-1,2-cyclohexanediame (96.3 mg, 0.68 mmol, 4.05 equiv) were added. The reaction mixture was heated to 70 °C. After 22 h, the reaction mixture was terminated by adding a saturated NH₄Cl solution. Then, the aqueous layer was extracted with EtOAc (6×). The combined organic layers were washed with brine and subsequently dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting residue was dissolved in
CH₃OH (1.8 mL) and purified by preparative HPLC (C18) (Gradient CH₃OH/H₂O 10:90 (5 min) → CH₃OH/H₂O = 100:0 (85 min) → CH₃OH/H₂O = 100:0 (10 min), 15.0 mL/min) (tᵣ = 85.5 min). The desired dehydropeptide 41 (3.70 mg, 4.20 µmol, 2.5%) was obtained as a colourless foam.

¹H-NMR (500 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 8.16 (1H, s, NH-f), 7.22 (1H, m, NH-e), 7.13 (1H, m, NH-g), 6.34 (2H, brs, NH-b, NH-d), 5.32 (1H, m, NH-g), 4.50 (1H, m, H-6), 4.37 (1H, m, H-2), 4.17 (1H, m, H-32), 3.70 (3H, s, H-35), 3.21 (2H, m, H-9), 2.95 (2H, s, H-20), 2.57 (3H, s, H-17 o. H-19), 2.16 (2H, m, H-3, H-29), 2.09 (6H, s, H-18, H-26), 1.98 (1H, m, H-7), 1.80 (3H, s, H-26), 1.70-1.63 (3H, m, H-7', H-8), 1.46 (6H, s, H-22), 1.03-0.90 (14H, m, H-4, H-30, H-33), 0.02 (9H, s, H-34) ppm; HRMS (ESI): m/z calculated for C₄₁H₆₉N₇O₁₀SSiNa [M + Na]⁺: 902.4494; found 902.4488.

Signals of NH⁺-proton could not be detected in the ¹H NMR spectrum.

Synthesis of methyl (E)-(2-iodo-3-methylpent-2-enoyl)-D-alaninate (42)

Acid 11 (268 mg, 1.12 mmol, 1.00 equiv) and D-alanine methylester hydrochloride (202 mg, 1.45 mmol, 1.30 equiv) were dissolved in DMF (6.10 mL) at 0 °C. Then, HOAt (162 mg, 1.19 mmol, 1.07 equiv) and PyAOP (610 mg, 1.17 mmol, 1.05 equiv) were added. Afterwards, DIPEA (662 mg, 5.12 mmol, 4.60 equiv) was added dropwise. The reaction mixture was warmed up to rt. After 22 h, the reaction was terminated by addition of a saturated NH₄Cl solution. The layers were separated and the aqueous layer was extracted with EtOAc (7 ×). The combined, organic layers were washed with brine and subsequently dried over Na₂SO₄, filtered and concentrated under reduced pressure. Purification by flash column chromatography over silica (petroleum ether/ethyl acetate= 4:1) yielded the desired product 42 (210 mg, 0.65 mmol, 58%) as a colourless solid.

Rᵣ = 0.73 (CH₂Cl₂/CH₃OH 98:2); mp = 58 – 60 °C; [α]ᵣ²⁰ = +12.5 (c = 1.00, CH₂Cl₂); ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 6.26 (1H, brm, NH), 4.59 (1H, m, H-2), 3.75 (3H, s, H-10), 2.38 (2H, q, J = 7.4 Hz, H-8), 1.96 (3H, s, H-7), 1.42 (3H, d, J = 7.2 Hz, H-3), 1.05 (3H, t, J = 7.4 Hz, H-9) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 173.2 (q, C-1), 166.7 (q, C-4), 151.0 (q, C-6), 87.0 (q, C-5), 52.6 (p, C-10), 48.5 (t, C-2), 29.3 (p, C-7), 27.2 (s, C-8), 18.2 (p, C-3), 13.2 (p, C-9) ppm; HRMS (ESI): m/z calculated for C₁₀H₁₆INO₂Na [M + Na]⁺: 348.0073; found 348.0075.
Synthesis of tert-butyl (R)-1-(1-amino-3-methyl-1-oxobutan-2-yl)carbamate (43)

Boc-D-valine (2.00 g, 9.21 mmol, 1.00 equiv) and N-methylmorpholine (1.01 g, 9.94 mmol, 1.08 equiv) were dissolved in 1,2-dimethoxyethane (47.0 mL) at 0 °C. Then, isobutylchloroformiate (1.36 g, 9.94 mmol, 1.08 equiv) was added dropwise. The reaction mixture was stirred for 15 min at 0 °C. Afterwards, an aqueous ammonia solution (25%; 4.10 mL, 60.5 mmol, 6.57 equiv) was added dropwise. Now, the reaction mixture was stirred at 0 °C → rt. After 20 h, the reaction was terminated by addition of HCl (1.0 M). The aqueous layer was extracted with EtOAc (5 × ). The combined organic layers were washed with HCl (0.1 M) and dried over MgSO₄, filtered and concentrated under reduced pressure. Amide 43 (1.99 g, 9.21 mmol, quantitative) was obtained as a colourless solid. The analytical data were in accordance with those reported in the literature[S1].

R_f = 0.20 (CH₂Cl₂/CH₃OH 98:2); mp = 155 -157 °C (mp = 156 - 157 °C, ref.[S1]; [α]_D^20 = +2.5 (c 1.14, CH₂OH) ([α]_D^20 = +2.5 (c 1.05, CH₃OH) ref.[S12]; ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 6.22 (1H, brs, NH-b), 5.82 (1H, brs, NH-b), 5.14 (1H, d, J = 8.5 Hz, NH-a), 3.98 (1H, dd, J = 6.6, 8.5 Hz, H-2), 2.12 (1H, m, H-3), 1.44 (9H, s, H-7), 0.98 (3H, d, J = 6.6 Hz, H-4), 0.94 (3H, d, J = 6.6 Hz, H-4) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃= 77.16 ppm): δ = 174.4 (q, C-1), 156.1 (q, C-5), 80.1 (q, C-6), 59.6 (t, C-2), 30.8 (t, C-3), 28.5 (3C, p, C-7), 19.4 (2C, p, C-4) ppm; HRMS (ESI): m/z calculated for C₁₀H₂₀N₂O₃Na [M + Na]^+: 239.1372; found 239.1369.

Synthesis of methyl {{(E)-2-[(R)-2-[(tert-butoxycarbonyl]amino]-3-methylbutanamido]-3-methylpent-2-enoyl]-D-alaninate (44)

Boc-D-Val-NH₂ (43; 82.1 mg, 0.38 mmol, 1.00 equiv) and vinyl iodide 42 (245 mg, 0.76 mmol, 2.00 equiv) were dissolved in 1,4-dioxane (degassed; 0.38 mL) at rt. Then, K₂CO₃ (105 mg, 0.76 mmol, 2.00 equiv), Cul (43.4 mg, 0.23 mmol, 0.60 equiv) und trans-N,N-dimethyl-1,2-cyclohexanediambine (216 mg, 1.52 mmol, 4.05 equiv) were added. The reaction mixture was heated to 70 °C. After 20 h, the solvent was removed under reduced pressure. The resulting residue was diluted with a saturated NH₄Cl solution. Then, the aqueous layer was extracted with EtOAc (4×). The combined organic layers were washed with brine and dried over MgSO₄, filtered and concentrated under reduced pressure.
Purification by flash column chromatography over silica (100% CH₂Cl₂ → CH₂Cl₂/CH₃OH = 99:1 → CH₂Cl₂/CH₃OH = 98:2) gave the desired dehydropeptide **44** (75.4 mg, 0.18 mmol, 48%) as a colourless solid.

R_f = 0.27 (CH₂Cl₂/CH₃OH = 98:2); mp > 177 °C (decomposition); [α]_D^20 = +21.2 (c 1.82, CH₂Cl₂); ¹H-NMR (400 MHz, CDCl₃, CHCl₃ = 7.26 ppm): δ = 7.47 (1H, brs, NH-b), 7.02 (1H, d, J = 6.2 Hz, NH-a), 5.08 (1H, d, J = 7.2 Hz, NH-c), 4.58 (1H, m, H-2), 3.91 (1H, m, H-11), 3.72 (3H, s, H-17), 2.48-2.30 (2H, m, H-8), 2.16 (1H, m, H-12), 1.72 (3H, s, H-7), 1.42-1.40 (12H, m, H-3, H-16), 1.09 (3H, t, J = 7.5 Hz, H-9), 1.00 (3H, d, J = 6.8 Hz, H-13), 0.96 (3H, d, J = 6.8 Hz, H-13) ppm; ¹³C-NMR (100 MHz, CDCl₃, CDCl₃ = 77.16 ppm): δ = 173.4 (q, C-1), 171.5 (q, C-10), 165.9 (q, C-4), 156.2 (q, C-14), 144.5 (q, C-6), 123.8 (q, C-5), 80.4 (q, C-15), 60.6 (t, C-11), 52.5 (p, C-17), 48.4 (t, C-2), 30.5 (t, C-12), 28.4 (3C, p, C-16), 27.3 (s, C-8), 19.5 (2C, p, C-13), 18.2 (p, C-3), 18.0 (p, C-7), 12.9 (p, C-9) ppm; HRMS (ESI): m/z calculated for C₂₀H₃₅N₃O₆Na [M + Na]^+: 436.2424; found 436.2425.

### nOe (500 MHz, CDCl₃):

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**Figure S1:** Structure relevant nOe contacts of **44**; red: contacts of methyl group, blue: contacts of methylene group.
References supporting information

Copies of $^1$H and $^{13}$C-NMR spectra