Supporting Information

Aziridine electrophiles in the functionalisation of peptide chains with amine nucleophiles

Anatol P. Spork and Timothy J. Donohoe*

Chemistry Research Laboratory, University of Oxford, 12 Mansfield Road, Oxford, OX1 3TA, United Kingdom
corresponding author* e-mail address: timothy.donohoe@chem.ox.ac.uk

Table of contents:

1. General Methods ................................................................. S2

2. Preparation of unknown compounds and key building block S3

3. Mechanistic control experiment ........................................... S39

4. NMR spectra of unknown compounds and key building block S40

5. References .................................................................................. S89
1. General Methods

Compounds $[S1]$, $[S2]$, and $[S3]$ as well as benzyl azide$[S4]$, 3-azido-7-hydroxycoumarine$[S5]$ and L-alanine dibenzylamide$[S6]$ are literature known (see references). Compound 13 and L-alanine dibenzylamide are also commercially available from Aurora Building Blocks (US) and Aldlab Chemicals Building Blocks (US). Benzyl azide is also commercially available but potentially explosive and was therefore only prepared in very small quantities.$[S4]$ All other chemicals were purchased from standard suppliers. All solvents and reagents were used as commercially supplied without further purification unless otherwise stated. In all aziridine ring-opening reactions only free amines have been used. In those cases in which only the corresponding hydrochlorides were commercially available, the desired free amines were obtained by dissolving the corresponding hydrochlorides in AcOEt, DCM or CHCl$_3$ and washing with sat. aqueous NaHCO$_3$ solution. The organic layer was dried over Na$_2$SO$_4$, filtered and the solvent of the filtrate evaporated in vacuo. All anhydrous reactions were carried out in flame-dried glassware and under an inert atmosphere of argon. Anhydrous solvents were dried by filtration through an activated alumina purification column or directly purchased as anhydrous solvents in sealed bottles. Petrol (PE) refers to petroleum ether in the boiling range 30-40 °C.

Flash column chromatography (FCC) was performed using Merck Kieselgel 60 (40-63 μm). Thin layer chromatography (TLC) analyses were performed on aluminum plates precoated with 0.25 mm silica gel 60 F$_{254}$ (VWR). Visualization of the spots was carried out using UV light (254 nm) and/or staining under heating (Vanillin-H$_2$SO$_4$ staining solution: 4 g vanillin, 25 mL conc. H$_2$SO$_4$, 80 mL AcOH and 680 mL MeOH).

Melting points $T_{mp}$ were obtained using a Leica VMTG heated-stage microscope and are uncorrected. Specific optical rotation values [$\alpha$]$_D^{20}$ are quoted in °cm$^2$g$^{-1}$dm$^{-1}$ and were recorded on a Perkin-Elmer polarimeter with a Na source using a 10 cm cell [concentrations c are quoted in g(100 mL)$^{-1}$]. $^1$H nuclear magnetic resonance spectra (NMR) were recorded on a Bruker AV400 (400 MHz) or Bruker AVII500 (500 MHz). $^{13}$C NMR spectra were recorded on a Bruker AV-400 (101 MHz) or AVII500 (126 MHz) as stated. Chemical shifts are reported relative to residual solvent peaks. Coupling constants $J$ are quoted to the nearest 0.1 Hz for $^1$H NMR. Chemical shifts $\delta$ are quoted in ppm (parts per million) to the nearest 0.01 ppm ($^1$H NMR) or 0.1 ppm ($^{13}$C NMR) with signal splittings recorded as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m). All NMR spectra were recorded at room temperature. Assignments were based upon DEPT, COSY, HSQC and HMBC experiments. Atoms have been numbered according to a self-consistent system used for clarity of assignment of the NMR data which does not reflect the IUPAC rules in naming compounds. Fourier transform infrared spectra (FTIR) were recorded neat on a Bruker Tensor 27 FT-IR spectrometer equipped with Attenuated Total Reflectance (ATR) sampling accessories. The nine most intense absorption maxima are quoted in wavenumbers $\nu$ [cm$^{-1}$]. Mass spectra (MS) under the conditions of electrospray ionization (ESI) were recorded on a Fisons Platform II and on a Bruker MicroTof (resolution = 10000 FWHM). Calibration was via the lock-mass of tetraoctyl ammonium bromide for positive ions and sodium dodecyl sulfate for negative ions.
2. Preparation of unknown compounds and key building block 9

Preparation of aziridine building block 9

The existing protocols for the preparation of 9 from 8(S1) were significantly improved by employing (MeSO₂)₂O instead of MeSO₂Cl or SO₂Cl₂ and thereby avoiding the formation of N-Trityl-β-chloro-L-alanine benzyl ester as side-product: Serine derivative 8(S1) (14.0 g, 32.0 mmol) was dissolved in abs. THF (100 mL) and was cooled to 0 °C. NEt₃ (13.4 mL, 96.0 mmol) and a solution of Ms₂O (8.36 g, 48.0 mmol) in abs. THF (25 mL) were added at 0 °C. The reaction mixture was stirred for 20 min at 0 °C, for 30 min at rt and for 60 h at 60 °C. The solvent was evaporated in vacuo. The residue was dissolved in Et₂O (300 mL) and washed with water (200 mL), 10 wt% aqueous citric acid solution (2 x 150 mL) and sat. aqueous NaHCO₃ solution (2 x 150 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (250 g, 5.0 x 21 cm, PE:DCM, 50:50 → 40:60) to give 12.7 g (30.3 mmol, 95%) of the title compound as a colourless solid.

**TLC:** R₇ = 0.39 (PE:DCM, 30:70).

**Melting point:** Tₚₚ = 109 °C [Lit. S1: Tₚₚ = 106-118 °C].

**Specific rotation:** [α]D²⁰ = -98.9 (c = 1.08, THF) [Lit. S1: [α]D²⁰ = -95.5(-98.3) (c = 0.92-1.0, THF)].

**¹H NMR** (400 MHz, CDCl₃): δ = 7.51-7.19 (m, 20 H, 20 x Hₐₐ), 5.26 (d, J = 12.2 Hz, 1 H, Bn-CH₃Hₐ), 5.21 (d, J = 12.2 Hz, 1 H, Bn-CH₃H₉), 2.30 (dd, J = 2.8, 1.7 Hz, 1 H, 3-Hₐ), 1.95 (dd, J = 6.2, 2.8 Hz, 1 H, 2-Hₐ), 1.43 (dd, J = 6.2, 1.7 Hz, 1 H, 3-H₉).

**¹³C NMR** (101 MHz, CDCl₃): δ = 171.6 (C-1), 143.7, 136.0 (4 x Cₐₐ), 129.5, 128.7, 128.5, 128.5, 127.2, 127.1 (20 x Hₐₐ), 74.5 (P₃C), 66.8 (Bn-CH₂), 31.9 (C-2), 29.0 (C-3).

**IR** (ATR): ν = 1729, 1447, 1364, 1234, 1171, 1015, 908, 746, 699, 631.

**MS** (ESI⁺): m/z = 861.4 [2M+Na]⁺, calculated: 442.1778 [M+Na]⁺, found: 442.1774 [-0.7 ppm] (ESI⁺-HRMS).

Preparation of dipeptide 11

To aziridine building block 9 (4.00 g, 9.53 mmol) in degassed EtOH (50 mL) degassed NEt₃ (3.99 mL, 28.6 mmol) and 10 wt% palladium on charcoal (250 mg, 0.235 mmol) were added. The resulting suspension was stirred under a hydrogen atmosphere (1 bar, balloon) for 1.5 h and then filtered through a syringe filter. The syringe filter was washed with EtOH (3 x 5 mL) and the solvent of the combined filtrates evaporated in vacuo. After co-evaporation with toluene:THF 1:1 (2 x 30 mL) the resulting colourless solid was dried in vacuo. With respect to its poor stability the unprotected carboxylate was always prepared freshly and used instantly in the subsequent transformation without further purification.

HOBt (709 mg, 5.25 mmol) was added to 1/2 of the crude product (only 1/2 of the initially prepared benzyl-deprotected aziridine building block, vide supra, was used in the 2nd step,
calculated maximal amount of substance: 4.77 mmol) in abs. DMF (18 mL). EDAC (1.01 g, 5.25 mmol) was added after cooling the solution to 0 °C. After stirring for 10 min at 0 °C NEt₃ (0.74 mL, 5.3 mmol) was added. Glycine derivative 1₃² (1.34 g, 5.25 mmol) in abs. DCM (4 mL) was added after additional 15 min of stirring at 0 °C. After stirring for 12 h and concomitantly slowly warming to rt the reaction mixture was diluted with Et₂O (300 mL) and washed with 10 wt% aqueous citric acid solution (2 x 200 mL) and sat. aqueous NaHCO₃ solution (2 x 200 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (150 g, 5.0 x 15 cm, PE:AcOEt, 80:20 → 70:30) to give 2.46 g (4.35 mmol, 91%) of the title compound as a colourless solid.

**TLC:** Rₜ = 0.25 (PE:AcOEt, 70:30).

**Melting point:** Tₘₚ = 78 °C.

**Specific rotation:** [α]D²⁰ = -73.2 (c = 0.98, CHCl₃)

**¹H NMR** (400 MHz, CDCl₃): δ = 7.79 (dd, J = 4.5, 4.4 Hz, 1 H, 2’-NH), 7.54-7.20 (m, 25 H, 25 x HC₅), 4.78 (d, J = 14.8 Hz, 1 H, Bn-CH₂H₂b), 4.64 (d, J = 14.8 Hz, 1 H, Bn-CH₂H₂b), 4.51 (d, J = 17.4 Hz, 1 H, Bn-CH₂H₃b), 4.46 (d, J = 17.4 Hz, 1 H, Bn-CH₂H₃b), 4.35 (dd, J = 17.4, 4.4 Hz, 1 H, 2’-Hₐ), 4.26 (dd, J = 17.4, 4.5 Hz, 1 H, 2’-Hₐ), 2.16 (dd, J = 2.7, 0.7 Hz, 1 H, 3-Hₐ), 2.05 (dd, J = 6.6, 2.7 Hz, 1 H, 2-H), 1.52 (dd, J = 6.6, 0.7 Hz, 1 H, 3-Hₐ).

**¹³C NMR** (101 MHz, CDCl₃): δ = 171.3, 168.8 (C-1, C-1’), 143.4, 136.6, 135.5 (5 x C₆), 129.6, 129.3, 128.9, 128.4, 128.1, 127.9, 127.2, 126.6 (25 x HC₅), 74.8 (Ph₃C), 49.1, 48.7 (2 x Bn-CH₂), 41.0 (C-2’), 34.2 (C-2’), 29.9 (C-3).

**IR** (ATR): ν = 1648, 1494, 1448, 1221, 1010, 909, 731, 699, 632.

**MS** (ESI⁺): m/z = 566.3 [M+H]^⁺, calculated: 588.2621 [M+Na]^⁺, C₃₈H₃₅N₅O₂ (565.70 g/mol⁻¹), found: 588.2593 [+4.9 ppm] (ESI⁺-HRMS).

**Preparation of dipeptide 12**

To aziridine building block 9 (4.00 g, 9.53 mmol) in degassed EtOH (50 mL) degassed NEt₃ (3.99 mL, 28.6 mmol) and 10 wt% palladium on charcoal (250 mg, 0.235 mmol) were added. The resulting suspension was stirred under a hydrogen atmosphere (1 bar, balloon) for 1.5 h and then filtered through a syringe filter. The syringe filter was washed with EtOH (3 x 5 mL) and the solvent of the combined filtrates evaporated in vacuo. After co-evaporation with toluene:THF 1:1 (2 x 30 mL) the resulting colourless solid was dried in vacuo. With respect to its poor stability the unprotected carboxylate was always prepared freshly and used instantly in the subsequent transformation without further purification.

HOBt (709 mg, 5.25 mmol) was added to 1/2 of the crude product (only 1/2 of the initially prepared benzyl-deprotected aziridine building block, vide supra, was used in the 2nd step, calculated maximal amount of substance: 4.77 mmol) in abs. DMF (18 mL). EDAC (1.01 g, 5.25 mmol) was added after cooling the solution to 0 °C. After stirring for 10 min at 0 °C NEt₃ (0.74 mL, 5.3 mmol) was added. Valine derivative 1₄³ (1.56 g, 5.25 mmol) in abs. DCM (4 mL) was added after additional 15 min of stirring at 0 °C. After stirring for 12 h and
concomitantly slowly warming to rt the reaction mixture was diluted with Et₂O (300 mL) and washed with 10 wt% aqueous citric acid solution (2 x 200 mL) and sat. aqueous NaHCO₃ solution (2 x 200 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (100 g, 4.5 x 14 cm, DCM:MeOH, 96:4 [R<sub>t</sub> = 0.21 (DCM:MeOH, 95:5)]). With respect to its poor stability the trityl-deprotected dipeptide was always prepared freshly and used in the subsequent transformation without any delay.

HOBT (92 mg, 0.68 mmol) was added to N-Carbobenzoxy-L-valine (172 mg, 0.684 mmol) in abs. DMF (2.5 mL). EDAC (131 mg, 0.684 mmol) was added after cooling the solution to 0 °C. After stirring for 10 min at 0 °C NEt₃ (95 µL, 0.68 mmol) was added. 1/6 of the crude product (only 1/6 of the initially prepared trityl-deprotected dipeptide, vide supra, was used in the 2nd step, calculated maximal amount of substance: 0.630 mmol) in abs. DMF (1.5 mL) was added after additional 15 min of stirring at 0 °C. After stirring for 16 h and concomitantly slowly warming to rt the reaction mixture was diluted with AcOEt (150 mL) and washed with water (100 mL), 10 wt% aqueous citric acid solution (100 mL) and sat. aqueous NaHCO₃ solution (200 mL) and sat. aqueous NaHCO₃ solution (200 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (150 g, 5.0 x 15 cm, PE:AcOEt, 85:15 → 80:20) to give 2.61 g (4.29 mmol, 90%) of the title compound as a colourless solid.

**TLC:** R<sub>t</sub> = 0.29 (PE:AcOEt, 80:20).

**Melting point:** T<sub>mp</sub> = 74 °C.

**Specific rotation:** [α]<sup>20</sup> = -109.3 (c = 1.03, CHCl₃)

**1H NMR** (400 MHz, CDCl₃): δ = 7.62 (d, *J* = 9.2 Hz, 1 H, 2′-NH), 7.53-7.21 (m, 25 H, 25 x H<sub>Ar</sub>), 7.04 (dd, *J* = 9.2, 6.2 Hz, 1 H, 2′-H), 4.98 (d, *J* = 14.6 Hz, 1 H, Bn-CH₂H₂), 4.73 (d, *J* = 16.5 Hz, 1 H, Bn-CH₂H₂), 4.49 (d, *J* = 16.5 Hz, 1 H, Bn-CH₂H₂), 4.34 (d, *J* = 14.6 Hz, 1 H, Bn-CH₂H₂), 2.20 (dqq, *J* = 6.7, 6.7, 6.2 Hz, 1 H, 3′-H), 2.10 (d, *J* = 2.4 Hz, 1 H, 3-H), 2.04 (dd, *J* = 6.5, 2.4 Hz, 1 H, 2-H), 1.50 (d, *J* = 6.5 Hz, 1 H, 3-H), 1.02 (d, *J* = 6.7 Hz, 3 H, 4′-H), 0.97 (d, *J* = 6.7 Hz, 3 H, 4′-H).  

**13C NMR** (101 MHz, CDCl₃): δ = 172.2, 170.8 (C-1, C-1′), 143.5, 137.1, 136 (5 x C<sub>Ar</sub>), 129.6, 129.1, 128.9, 128.5, 128.0, 127.9, 127.7, 127.3, 127.2 (25 x H<sub>Ar</sub>), 74.8 (PhC), 53.2 (C-2′), 50.0, 47.9 (2 x Bn-CH₂), 33.9 (C-2), 32.2 (C-3′), 30.3 (C-3), 19.9, 17.6 (2 x C-4′).  

**IR** (ATR): v = 1641, 1495, 1447, 1215, 1011, 909, 732, 705, 633.  

**MS** (ESI<sup>+</sup>): m/z = 608.3 [M+H]<sup>+</sup>, calculated: 630.3091 [M+Na]<sup>+</sup>, found: 630.3068 [+3.7 ppm] (ESI<sup>+</sup>-HRMS).

**Preparation of tripeptide 4**

![Tripeptide 4](image)

To a solution of dipeptide 12 (2.30 g, 3.78 mmol) in abs. MeOH (10 mL) and abs. CHCl₃ (10 mL) precooled to 0 °C, TFA (2.0 mL) was added dropwise. The solution was stirred for 3.5 h at 0 °C, diluted with AcOEt (150 mL) at 0 °C and washed with a preformed mixture of 1 M aqueous NaOH solution (100 mL) and sat. aqueous NaCl solution (100 mL), which was also cooled to 0 °C beforehand. The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (100 g, 4.5 x 14 cm, DCM:MeOH, 96:4 [R<sub>t</sub> = 0.21 (DCM:MeOH, 95:5)]).
solution (100 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (50 g, 4.5 x 7.0 cm, PE:AcOEt, 75:25 → 65:35) to give 256 mg (0.428 mmol, 68%) of the title compound as a colourless solid.

**TLC:** Rᵣ = 0.24 (PE:AcOEt, 60:40).

**Melting point:** T_mₚ = 47 °C.

**Specific rotation:** [α]_D^20 = -99.0 (c = 1.23, CHCl₃).

1H NMR (400 MHz, D₆-DMSO): δ = 8.88 (d, J = 8.8 Hz, 1 H, 2″-NH), 7.45 (d, J = 8.5 Hz, 1 H, 2-NH), 7.37-7.16 (m, 15 H, 15 x H₂C₆), 5.02 (d, J = 16.9 Hz, 1 H, Bn-CH₂H₃), 4.99 (d, J = 16.9 Hz, 1 H, Bn-CH₂H₃), 4.69-4.55 (m, 4 H, 2″-H, Bn-CH₂H₃, Bn-CH₂), 4.33 (d, J = 15.0 Hz, 1 H, Bn-CH₂H₃), 4.02 (dd, J = 8.5, 5.3 Hz, 1 H, 2-H), 3.34-3.30 (m, 1 H, 2″-H), 2.55 (dd, J = 5.4, 2.2 Hz, 1 H, 3″-Hₐ), 2.28 (dd, J = 2.6, 2.2 Hz, 1 H, 3″-Hₐ), 2.18-2.04 (m, 2 H, 3-H, 3″-Hₐ), 0.90-0.88 (m, 9 H, 4-H, 4″-Hₐ), 0.76 (d, J = 6.7 Hz, 3 H, 4″-Hₐ).

13C NMR (101 MHz, D₆-DMSO): δ = 181.7 (C-1), 171.4 (C-1′), 166.3 (C-1′), 156.2 (NC(CH₂)=O), 137.3, 137.1, 137.0 (3 x CH₃), 128.6, 128.5, 128.3, 127.8, 127.6, 127.5, 127.4, 127.1 (15 x CH₃), 65.4 (Bn-CH₂), 60.9 (C-2), 54.2 (C-2″), 49.9, 48.0 (2 x Bn-CH₂), 35.1 (C-2′), 30.1 (C-3, C-3′), 29.6 (C-3′), 19.3, 19.3, 17.9, 17.6 (2 x C-4, 2 x C-4′).

**IR (ATR):** ν = 1698, 1629, 1497, 1451, 1419, 1220, 1027, 909, 729, 698.

**MS (ESI):** m/z = 599.3 [M+H]^+, calculated: 621.3047 [M+Na]^+, found: 621.3056 [-1.3 ppm] (ESI^+ - HRMS).

Preparation of tripeptide 5

To a solution of dipeptide 12 (2.30 g, 3.78 mmol) in abs. MeOH (10 mL) and abs. CHCl₃ (10 mL) precooled to 0 °C, TFA (2.0 mL) was added dropwise. The solution was stirred for 3.5 h at 0 °C, diluted with AcOEt (150 mL) at 0 °C and washed with a preformed mixture of 1 M aqueous NaOH solution (100 mL) and sat. aqueous NaCl solution (100 mL), which was also cooled to 0 °C beforehand. The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (100 g, 4.5 x 14 cm, DCM:MeOH, 96:4 [Rᵣ = 0.21 (DCM:MeOH, 95:5)]). With respect to its poor stability the trityl-deprotected dipeptide was always prepared freshly and used in the subsequent transformation without any delay.

HOBt (92 mg, 0.68 mmol) was added to N-Carbobenzyoxy-glycine (143 mg, 0.684 mmol) in abs. DMF (2.5 mL). EDAC (131 mg, 0.684 mmol) was added after cooling the solution to 0 °C. After stirring for 10 min at 0 °C NEt₃ (95 μL, 0.68 mmol) was added. 1/6 of the crude product (only 1/6 of the initially prepared trityl-deprotected dipeptide, vide supra, was used in the 2nd step, calculated maximal amount of substance: 0.630 mmol) in abs. DMF (1.5 mL) was added after additional 15 min of stirring at 0 °C. After stirring for 16 h and concomitantly slowly warming to rt the reaction mixture was diluted with AcOEt (150 mL) and washed with water (100 mL), 10 wt% aqueous citric acid solution (100 mL) and sat. aqueous NaHCO₃ solution (100 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the
filtrate evaporated in vacuo. The resultant crude product was purified by FCC (50 g, 4.5 x 7.0 cm, PE:AcOEt, 45:55 → 40:60) to give 223 mg (0.401 mmol, 64%) of the title compound as a colourless solid.

**TLC:** \( R_t = 0.26 \) (PE:AcOEt, 40:60).

**Melting point:** \( T_{mp} = 48 \, ^\circ C \).

**Specific rotation:** \( \{\alpha\}^{20}_D = -95.7 \) (c = 1.09, CHCl₃)

**\(^1\)H NMR** (400 MHz, D₆-DMSO): \( \delta = 8.96 \) (d, \( J = 9.0 \, Hz \), 1 H, 2''-NH), 7.57 (dd, \( J = 6.1, 6.0 \, Hz, 1 \, H, 2''-NH \)), 7.38-7.17 (m, 15 H, 15 x HC₆H₄), 5.03 (s, 2 H, Bn-CH₂), 4.70-4.62 (m, 3 H, 2''-H, 2 x Bn-CH₃H₅), 4.55 (d, \( J = 16.8 \, Hz, 1 \, H, Bn-CH₃H₅ \)), 4.33 (d, \( J = 15.0 \, Hz, 1 \, H, Bn-CH₃H₅ \)), 3.84 (dd, \( J = 17.4, 6.1 \, Hz, 1 \, H, 2-H_{a} \)), 3.70 (dd, \( J = 17.4, 6.0 \, Hz, 1 \, H, 2-H_{b} \)), 3.35-3.33 (m, 1 H, 2'-H), 2.44 (dd, \( J = 5.3, 1.9 \, Hz, 1 \, H, 3''-H_{a} \)), 2.23 (dd, \( J = 2.8, 1.9 \, Hz, 1 \, H, 3''-H_{b} \)), 2.09 (dqq, \( J = 7.5, 6.8, 6.7 \, Hz, 1 \, H, 3''-H \)), 0.87 (d, \( J = 6.8 \, Hz, 3 \, H, 4''-H_{a} \)), 0.76 (d, \( J = 6.7 \, Hz, 3 \, H, 4''-H_{b} \)).

**\(^{13}\)C NMR** (101 MHz, D₆-DMSO): \( \delta = 179.8 \) (C-1), 171.3 (C-1''), 166.3 (C-1'), 156.4 (NC(=O)O), 137.3, 137.1, 137.0 (3 x C₆H₄), 128.6, 128.5, 128.4, 127.8, 127.7, 127.5, 127.4, 127.2, 127.0 (15 x HC₆H₄), 65.5 (Bn-CH₂), 54.2 (C-2''), 49.9, 48.0 (2 x Bn-CH₂), 44.5 (C-2'), 34.8 (C-2''), 30.2 (C-3'), 28.9 (C-3''), 19.3, 17.9 (2 x C-4'').

**IR (ATR):** \( \nu = 1707, 1632, 1524, 1451, 1249, 1167, 1049, 732, 699 \).

**MS (ESI)**: \( m/z = 579.3 \) [M+Na]⁺, calculated: 579.2578 [M+Na]⁺, found: 579.2583 [-0.8 ppm] (ESI⁺-HRMS).

### Preparation of tripeptide 6

![Tripeptide 6](image)

To a solution of dipeptide 11 (2.25 g, 3.98 mmol) in abs. MeOH (10 mL) and abs. CHCl₃ (10 mL) precooled to 0 °C, TFA (2.0 mL) was added dropwise. The solution was stirred for 3.5 h at 0 °C, diluted with AcOEt (200 mL) at 0 °C and washed with a preformed mixture of 1 M aqueous NaOH solution (100 mL) and sat. aqueous NaCl solution (100 mL), which was also cooled to 0 °C beforehand. The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (100 g, 5.0 x 9.0 cm, DCM:MeOH, 95:5 \( [R_t = 0.14 \) (DCM:MeOH, 94:6)]). With respect to its poor stability the trityl-deprotected dipeptide was always prepared freshly and used in the subsequent transformation without any delay.

HOBt (104 mg, 0.773 mmol) was added to N-Carbobenzyoxyl-L-valine (194 mg, 0.773 mmol) in abs. DMF (3 mL). EDAC (148 mg, 0.773 mmol) was added after cooling the solution to 0 °C. After stirring for 10 min at 0 °C NEt₃ (0.11 mL, 0.77 mmol) was added. 1/6 of the crude product (only 1/6 of the initially prepared trityl-deprotected dipeptide, vide supra, was used in the 2nd step, calculated maximal amount of substance: 0.663 mmol) in abs. DMF (1.5 mL) was added after additional 15 min of stirring at 0 °C. After stirring for 16 h and concomitantly slowly warming to rt the reaction mixture was diluted with AcOEt (150 mL) and washed with water (100 mL), 10 wt% aqueous citric acid solution (100 mL) and sat. aqueous NaHCO₃ solution (100 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the
The resultant crude product was purified by FCC (50 g, 4.5 x 7.0 cm, PE:AcOEt, 50:50) to give 261 mg (0.469 mmol, 71%) of the title compound as a colourless solid.

**TLC:** $R_t = 0.33$ (PE:AcOEt, 40:60).

**Melting point:** $T_{mp} = 51 \degree C$.

**Specific rotation:** $[\alpha]_D^{20} = -62.1$ (c = 1.01, CHCl$_3$)

**$^1$H NMR** (400 MHz, D$_6$-DMSO): $\delta = 8.55$ (t, $J = 5.5$ Hz, 1 H, 2''-NH), 7.54 (d, $J = 8.3$ Hz, 1 H, 2-NH), 7.39-7.21 (m, 15 H, 15 x HC$_{Ar}$), 5.02 (d, $J = 12.6$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.97 (d, $J = 12.6$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.54-4.45 (s, 4 H, 2 x Bn-CH$_2$), 4.09 (d, $J = 5.5$ Hz, 2 H, 2''-H), 4.03 (dd, $J = 8.3$, 5.9 Hz, 1 H, 2'-H), 3.23 (dd, $J = 5.7$, 3.1 Hz, 1 H, 2'-H), 2.62 (dd, $J = 5.7$, 2.0 Hz, 1 H, 3'-H$_a$), 2.38 (dd, $J = 3.1$, 2.0 Hz, 1 H, 3'-H$_b$), 2.15 (dq, $J = 6.8$, 6.8, 5.9 Hz, 1 H, 3-H), 0.92 (d, $J = 6.8$ Hz, 3 H, 4-H$_a$), 0.89 (d, $J = 6.8$ Hz, 3 H, 4-H$_b$).

**$^{13}$C NMR** (101 MHz, D$_6$-DMSO): $\delta = 182.5$ (C-1), 168.6 (C-1''), 166.8 (C-1''), 156.4 (NC(=O)O), 137.3, 137.0, 136.7 (3 x $C_{Ar}$), 128.8, 128.4, 128.3, 127.8, 127.7, 127.4, 127.1, 126.6 (15 x HC$_{Ar}$), 65.5 (Bn-CH$_2$), 61.0 (C-2'), 49.2, 48.5 (2 x Bn-CH$_2$), 40.7 (C-2''), 35.3 (C-2''), 29.9 (C-3, C-3''), 19.4, 17.8 (2 x C-4).

**IR** (ATR): $\nu = 1700, 1647, 1525, 1453, 1224, 1081, 1028, 732, 699.$


**Preparation of tripeptide 7**

To a solution of dipeptide 11 (2.25 g, 3.98 mmol) in abs. MeOH (10 mL) and abs. CHCl$_3$ (10 mL) precooled to 0 $\degree C$, TFA (2.0 mL) was added dropwise. The solution was stirred for 3.5 h at 0 $\degree C$, diluted with AcOEt (200 mL) at 0 $\degree C$ and washed with a preformed mixture of 1 M aqueous NaOH solution (100 mL) and sat. aqueous NaCl solution (100 mL), which was also cooled to 0 $\degree C$ beforehand. The organic layer was dried over Na$_2$SO$_4$, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (100 g, 5.0 x 9.0 cm, DCM:MeOH, 95:5 [$R_t = 0.14$ (DCM:MeOH, 94:6)]). With respect to its poor stability the trityl-deprotected dipeptide was always prepared freshly and used in the subsequent transformation without any delay.

HOBt (104 mg, 0.773 mmol) was added to N-Carbobenzoxy-glycine (162 mg, 0.773 mmol) in abs. DMF (3 mL). EDAC (148 mg, 0.773 mmol) was added after cooling the solution to 0 $\degree C$. After stirring for 10 min at 0 $\degree C$ NEt$_3$ (0.11 mL, 0.77 mmol) was added. 1/6 of the crude product (only 1/6 of the initially prepared trityl-deprotected dipeptide, *vide supra*, was used in the 2nd step, calculated maximal amount of substance: 0.663 mmol) in abs. DMF (1.5 mL) was added after additional 15 min of stirring at 0 $\degree C$. After stirring for 16 h and concomitantly slowly warming to rt the reaction mixture was diluted with AcOEt (150 mL) and washed with water (100 mL), 10 wt% aqueous citric acid solution (100 mL) and sat. aqueous NaHCO$_3$ solution (100 mL). The organic layer was dried over Na$_2$SO$_4$, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (50 g,
4.5 x 7.0 cm, PE:AcOEt, 40:60 → 25:75) to give 217 mg (0.422 mmol, 63%) of the title compound as a colourless solid.

**TLC:** $R_f = 0.13$ (PE:AcOEt, 30:70).

**Melting point:** $T_{\text{mp}} = 62 ^\circ \text{C}$.

**Specific rotation:** $[\alpha]_{D}^{20} = -56.9$ (c = 0.81, CHCl$_3$)

$^1$H NMR (400 MHz, D$_6$-DMSO): $\delta = 8.72$ (t, $J =$ 5.5 Hz, 1 H, 2''-NH), 7.61 (t, $J =$ 6.1 Hz, 1 H, 2-NH), 7.39-7.22 (m, 15 H, 15 x H$_{\text{Ar}}$), 5.03 (s, 2 H, Bn-CH$_2$), 4.53 (s, 2 H, Bn-CH$_2$), 4.50 (s, 2 H, Bn-CH$_2$), 4.10 (d, $J =$ 5.5 Hz, 2 H, 2''-H), 3.78 (d, $J =$ 6.1 Hz, 2 H, 2-H), 3.31 (dd, $J =$ 5.5, 3.0 Hz, 1 H, 2'-H), 2.47 (dd, $J =$ 5.5, 1.8 Hz, 1 H, 3'-H$_{\text{a}}$), 2.30 (dd, $J =$ 3.0, 1.8 Hz, 1 H, 3'-H$_{\text{b}}$).

$^{13}$C NMR (101 MHz, D$_6$-DMSO): $\delta = 180.4$ (C-1), 168.7 (C-1''), 166.9 (C-1'), 156.4 (NC(=O)O), 137.3, 137.0, 136.7 (3 x C$_{\text{Ar}}$), 128.8, 128.4, 128.3, 127.8, 127.7, 127.4, 127.1, 126.6 (15 x HC$_{\text{Ar}}$), 65.5, 49.2, 48.6 (3 x Bn-CH$_2$), 44.7 (C-2), 40.8 (C-2''), 35.2 (C-2'), 28.9 (C-3').

**IR** (ATR): $\nu =$ 3317, 1647, 1527, 1452, 1251, 1168, 1048, 733, 698.


### Preparation of tripeptide 15

To a solution of tripeptide 4 (15 mg, 0.025 mmol) in CHCl$_3$ (1.5 mL), diethylamine (16 $\mu$L, 0.15 mmol) was added. After stirring for 20 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt:MeOH, 60:40:0 → 98:0:2) to give 16 mg (0.024 mmol, 96%) of the title compound as a colourless solid.

**TLC:** $R_f = 0.14$ (DCM:MeOH, 96:4).

**Melting point:** $T_{\text{mp}} = 106 ^\circ \text{C}$.

**Specific rotation:** $[\alpha]_{D}^{20} = -3.5$ (c = 1.34, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): $\delta =$ 9.17 (d, $J =$ 8.7 Hz, 1 H, 2''-NH), 7.31-7.09 (m, 15 H, 15 x HC$_{\text{Ar}}$), 6.77 (d, $J =$ 4.9 Hz, 1 H, 2'-NH), 5.34 (d, $J =$ 8.6 Hz, 1 H, 2-NH), 5.06 (d, $J =$ 12.3 Hz, 1 H, Bn-CH$_2$H$_{\text{b}}$), 5.02 (d, $J =$ 12.3 Hz, 1 H, Bn-CH$_2$H$_{\text{b}}$), 4.55 (d, $J =$ 16.5 Hz, 1 H, Bn-CH$_2$H$_{\text{b}}$), 4.42 (d, $J =$ 16.5 Hz, 1 H, Bn-CH$_2$H$_{\text{b}}$), 4.33-4.28 (m, 1 H, 2'-H), 4.22 (d, $J =$ 14.8 Hz, 1 H, Bn-CH$_2$H$_{\text{b}}$), 4.02 (dd, $J =$ 8.6, 5.8 Hz, 1 H, 2-H), 2.81-2.74 (m, 3 H, 3'-H$_{\text{a}}$, 2 x CH$_2$H$_{\text{b}}$CH$_3$), 2.65-2.58 (m, 2 H, 2 x CH$_2$H$_{\text{b}}$CH$_3$), 2.46 (dd, $J =$ 11.7, 11.7 Hz, 1 H, 3'-H$_{\text{b}}$), 2.09-1.98 (m, 2 H, 3-H, 3'-H), 1.00 (dd, $J =$ 7.2, 7.1 Hz, 6 H, 2 x CH$_2$CH$_3$), 0.90 (d, $J =$ 6.8 Hz, 3 H), 0.89 (d, $J =$ 6.9 Hz, 3 H), 0.86 (d, $J =$ 6.8 Hz, 3 H), 0.83 (d, $J =$ 6.8 Hz, 3 H) (4-H$_{\text{a}}, 4$-H$_{\text{b}}, 4''$-H$_{\text{a}}, 4''$-H$_{\text{b}}$).

$^{13}$C NMR (126 MHz, CDCl$_3$): $\delta =$ 172.0, 171.3, 171.2 (C-1, C-1', C-1''), 156.4 (NC(=O)O), 137.2, 136.5, 136.4 (3 x C$_{\text{Ar}}$), 129.0, 128.7, 128.7, 128.4, 128.3, 128.2, 128.0, 127.6, 127.4 (15 x HC$_{\text{Ar}}$), 67.1 (Bn-CH$_2$), 60.3 (C-2), 55.5 (C-3'), 54.7 (C-2''), 50.1 (Bn-CH$_2$), 49.7 (C-2'),
Preparation of tripeptide 16

To a solution of tripeptide 5 (15 mg, 0.027 mmol) in CHCl₃ (1.5 mL), diethylamine (17 μL, 0.16 mmol) was added. After stirring for 20 h at rt and for 24 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:MeOH, 93:7) to give 16 mg (0.025 mmol, 94%) of the title compound as a colourless solid.

**TLC:** Rᵣ = 0.17 (DCM:MeOH, 95:5).

**Melting point:** Tₘᵣᵢₙ = 159 °C.

**Specific rotation:** [α]D° = -6.8 (c = 1.21, CHCl₃)

**1H NMR** (500 MHz, CDCl₃): δ = 9.17 (s, 1 H, 2''-NH), 7.31-7.09 (m, 15 H, 15 x HC₆H₅), 6.83 (s, 1 H, 2'-NH), 5.38 (s, 1 H, 2-NH), 5.08 (d, J = 12.5 Hz, 1 H, Bn-CH₂H₅), 4.75 (dd, J = 12.5 Hz, 1 H, Bn-CH₂H₅), 4.56 (d, J = 16.5 Hz, 1 H, Bn-CH₂H₅), 4.40-4.32 (m, 2 H, 2''-H, Bn-CH₂H₅), 4.18 (dd, J = 14.8 Hz, 1 H, Bn-CH₂H₅), 4.10 (dd, J = 8.9, 5.8 Hz, 1 H, 2''-H), 4.56 (d, J = 16.5 Hz, 1 H, Bn-CH₂H₅), 4.40-4.32 (m, 2 H, 2''-H, Bn-CH₂H₅), 2.08 (dq, J = 6.7, 6.7, 5.8 Hz, 1 H, 3''-H), 1.01 (dd, J = 7.0, 7.0 Hz, 6 H, 2 x CH₂CH₃), 0.89 (d, J = 6.7 Hz, 3 H, 4''-Hₐ), 0.83 (d, J = 6.7 Hz, 3 H, 4''-Hₐ).

**13C NMR** (126 MHz, CDCl₃): δ = 172.0, 171.3, 168.9 (C-1, C-1', C-1''), 156.5 (NC(=O)O), 137.1, 136.4, 136.4 (3 x C₆H₅), 129.0, 128.8, 128.7, 128.4, 128.3, 128.2, 128.0, 127.6, 127.4 (15 x HC₆H₅), 67.3 (Bn-CH₂), 55.4 (C-3'), 54.8 (C-2''), 50.1 (Bn-CH₂), 49.7 (C-2'), 48.0 (Bn-CH₂), 46.1 (2 x CH₂CH₃), 44.5 (C-2), 31.1 (C-3''), 20.1, 17.5 (2 x C-4''), 11.1 (2 x CH₂CH₃).

**IR** (ATR): ν = 3277, 1714, 1637, 1539, 1446, 1246, 1044, 738, 696.

**MS** (ESI⁺): m/z = 630.4 [M+H]⁺, calculated: 630.3650 [M+H]⁺, found: 630.3628 [-3.5 ppm] (ESI⁺-HRMS).

Preparation of tripeptide 17

To a solution of tripeptide 6 (15 mg, 0.027 mmol) in CHCl₃ (1.5 mL), diethylamine (17 μL, 0.16 mmol) was added. After stirring for 20 h at rt and for 24 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:MeOH, 97:3) to give 16 mg (0.025 mmol, 94%) of the title compound as a colourless solid.
TLC: \( R_f = 0.25 \) (DCM:MeOH, 95:5).
Melting point: \( T_{mp} = 105 \) °C.
Specific rotation: \( [\alpha]_D^{20} = +19.4 \) (c = 1.27, CHCl₃)

\(^1\)H NMR (500 MHz, CDCl₃): \( \delta = 9.07 \) (s, 1 H, 2'-NH), 7.40-7.14 (m, 15 H, 15 x HC₅H), 7.01 (s, 1 H, 2'-NH), 5.46 (d, \( J = 8.1 \) Hz, 1 H, 2-NH), 5.13 (d, \( J = 12.3 \) Hz, 1 H, Bn-CH₃Hb), 5.09 (d, \( J = 12.3 \) Hz, 1 H, Bn-CH₃Hb), 4.69 (d, \( J = 14.8 \) Hz, 1 H, Bn-CH₃Hb), 4.62 (d, \( J = 14.8 \) Hz, 1 H, Bn-CH₃Hb), 4.46-4.38 (m, 3 H, 2'-H, Bn-CH₂), 4.28-4.19 (m, 2 H, 2''-H), 4.14 (dd, \( J = 8.1, 6.2 \) Hz, 1 H, 2-H), 2.85-2.81 (m, 3 H, 3'-Hₐ, 2 x CH₃HbCH₃), 2.65-2.56 (m, 3 H, 3'-Hₐ, 2 x CH₃HbCH₃), 2.46 (dqq, \( J = 6.7, 6.6, 6.2 \) Hz, 1 H, 3-H), 1.12 (dd, \( J = 7.1, 7.0 \) Hz, 6 H, 2 x CH₂CH₃), 0.99 (d, \( J = 6.6 \) Hz, 3 H, 4-H₂ₐ), 0.95 (d, \( J = 6.7 \) Hz, 3 H, 4-Hₐ).

\(^13\)C NMR (126 MHz, CDCl₃): \( \delta = 171.2, 168.4 \), (C-1, C-1', C-1''), 156.4 (NC(=O)O), 136.7, 136.5, 135.6 (3 x C₆H), 129.2, 128.8, 128.6, 128.4, 128.2, 128.1, 128.0, 127.8, 126.5 (15 x HC₅H), 67.0 (Bn-CH₂), 60.2 (C-2), 55.2 (C-3'), 50.3 (C-2'), 49.1, 48.5 (2 x Bn-CH₂), 46.9 (2 x CH₂CH₃), 41.8 (C-2''), 31.7 (C-3), 19.3, 17.8 (2 x C-4), 11.7 (2 x CH₂CH₃).

IR (ATR): \( \nu = 3287, 1719, 1630, 1522, 1322, 1028, 733, 697, 646 \).

MS (ESI⁺): m/z = 630.4 [M+H]⁺, calculated: 630.3650 [M+H]⁺,
C₃₆H₄₇N₅O₅ (629.80 g/mol⁻¹), found: 630.3630 [-3.2 ppm] (ESI⁺-HRMS).

Preparation of tripeptide 18

To a solution of tripeptide 7 (14 mg, 0.027 mmol) in CHCl₃ (1.5 mL), diethylamine (17 µL, 0.16 mmol) was added. After stirring for 18 h at rt and for 20 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:MeOH, 96:4) to give 14 mg (0.026 mmol, 96%) of the title compound as a colourless solid.

TLC: \( R_f = 0.18 \) (DCM:MeOH, 95:5).
Melting point: \( T_{mp} = 78 \) °C.
Specific rotation: \( [\alpha]_D^{20} = +16.6 \) (c = 1.18, CHCl₃)

\(^1\)H NMR (500 MHz, CDCl₃): \( \delta = 9.00 \) (s, 1 H, 2'-NH), 7.40-7.07 (m, 16 H, 2'-NH, 15 x HC₅H), 5.59 (s, 1 H, 2-NH), 5.15 (d, \( J = 12.5 \) Hz, 1 H, Bn-CH₃Hb), 5.12 (d, \( J = 12.5 \) Hz, 1 H, Bn-CH₃Hb), 4.66 (d, \( J = 15.2 \) Hz, 1 H, Bn-CH₃Hb), 4.63 (d, \( J = 15.2 \) Hz, 1 H, Bn-CH₃Hb), 4.47-4.39 (m, 3 H, 2'-H, Bn-CH₂), 4.25-4.16 (m, 2 H, 2''-H), 3.99 (dd, \( J = 16.9, 5.4 \) Hz, 1 H, 2-H), 3.91 (dd, \( J = 16.9, 5.4 \) Hz, 1 H, 2-H), 2.88-2.78 (m, 3 H, 3'-Hₐ, 2 x CH₃HbCH₃), 2.67-2.58 (m, 3 H, 3'-Hₐ, 2 x CH₃HbCH₃), 1.11 (dd, \( J = 7.1, 7.1 \) Hz, 6 H, 2 x CH₂CH₃).

\(^13\)C NMR (126 MHz, CDCl₃): \( \delta = 171.2, 169.0, 168.4 \), (C-1, C-1', C-1''), 156.6 (NC(=O)O), 136.6, 136.4, 135.6 (3 x C₆H), 129.2, 128.8, 128.6, 128.4, 128.2, 128.2, 128.0, 127.8, 126.5 (15 x HC₅H), 67.2 (Bn-CH₂), 55.0 (C-3'), 50.2 (C-2'), 49.1, 48.5 (2 x Bn-CH₂), 46.9 (2 x CH₂CH₃), 44.5 (C-2), 41.7 (C-2''), 11.5 (2 x CH₂CH₃).

IR (ATR): \( \nu = 3287, 1717, 1633, 1523, 1452, 1223, 1046, 733, 697 \).

MS (ESI⁺): m/z = 588.3 [M+H]⁺, calculated: 588.3181 [M+H]⁺,
C₃₃H₄₁N₅O₅ (587.72 g/mol⁻¹), found: 588.3167 [-2.3 ppm] (ESI⁺-HRMS).
Preparation of tripeptide 19

To a solution of tripeptide 4 (72 mg, 0.12 mmol) in CHCl₃ (2.5 mL), N-methylpropargylamine (61 μL, 0.72 mmol) was added. After stirring for 8 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt, 80:20 → 70:30) to give 74 mg (0.11 mmol, 92%) of the title compound as a colourless solid.

TLC: Rₜ = 0.15 (DCM:AcOEt, 80:20).

Melting point: Tₘₚ = 123 °C.

Specific rotation: [α]D²⁰ = -13.8 (c = 1.84, CHCl₃)

¹H NMR (500 MHz, CDCl₃): δ = 8.52 (d, J = 8.9 Hz, 1 H, 2''-NH), 7.32-7.08 (m, 15 H, 15 x HC₆H₅), 6.80 (d, J = 5.3 Hz, 1 H, 1''-NH), 5.39 (d, J = 8.7 Hz, 1 H, 2-NH), 5.05 (d, J = 12.2 Hz, 1 H, Bn-CH₂H₃), 5.01 (d, J = 12.2 Hz, 1 H, Bn-CH₂H₃), 4.83-4.80 (m, 2 H, 2''-H, Bn-CH₂H₃), 4.56 (d, J = 16.4 Hz, 1 H, Bn-CH₂H₃), 4.35-4.30 (m, 2 H, 2''-H, Bn-CH₂H₃), 4.14 (d, J = 14.8 Hz, 1 H, Bn-CH₂H₃), 4.04 (dd, J = 8.7, 5.8 Hz, 1 H, 2-H), 3.52 (dd, J = 17.2, 2.3 Hz, 1 H, 4''-H₃), 3.37 (dd, J = 17.2, 2.3 Hz, 1 H, 4''-H₃), 2.76 (dd, J = 12.2, 4.1 Hz, 1 H, 3''-H₃), 2.50 (dd, J = 12.2, 10.9 Hz, 1 H, 3'-H₂), 2.44 (s, 3 H, NCH₃), 2.16 (dd, J = 2.3, 2.3 Hz, 1 H, 6'-H), 2.08-1.98 (m, 2 H, 3-H, 3''-H), [0.90 (d, J = 6.9 Hz, 3 H), 0.88 (d, J = 6.8 Hz, 3 H), 0.85 (d, J = 6.8 Hz, 3 H), 0.84 (d, J = 6.8 Hz, 3 H) (4-H₄, 4-H₄, 4''-H₄, 4''-H₄)].

¹³C NMR (126 MHz, CDCl₃): δ = 172.1, 171.3, 171.1 (C-1, C-1', C-1''), 156.4 (NC(=O)O), 137.1, 136.5, 136.2 (3 x C₆H₅), 129.1, 128.8, 128.6, 128.3, 128.2, 128.1, 128.0, 127.6, 127.4 (15 x HC₆H₅), 78.3 (C-5'), 73.7 (C-6'), 67.1 (Bn-CH₂), 60.2 (C-2), 57.3 (C-3'), 54.7 (C-2'), 50.0 (Bn-CH₂), 49.9 (C-2'), 47.9 (Bn-CH₂), 46.3 (C-4'), 41.0 (NCH₃), 31.7, 31.1 (C-3, C-3''), 20.1, 19.3, 17.8, 17.3 (2 x C-4, 2 x C-4'').

IR (ATR): ν = 3289, 2962, 1630, 1531, 1449, 1232, 1028, 733, 697.

MS (ESI⁺): m/z = 668.4 [M+H]⁺, calculated: 668.3807 [M+H]⁺, C₃₀H₄₉N₅O₅ (667.85 g/mol⁻¹), found: 668.3788 [-2.8 ppm] (ESI⁺-HRMS).

Preparation of tripeptide 20

To a solution of tripeptide 5 (25 mg, 0.045 mmol) in CHCl₃ (1.5 mL), N-methylpropargylamine (23 μL, 0.27 mmol) was added. After stirring for 20 h at rt and 20 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt, 50:50 → 40:60) to give 22 mg (0.035 mmol, 78%) of the title compound as a colourless solid.

TLC: Rₜ = 0.17 (DCM:AcOEt, 40:60).
Melting point: $T_{\text{mp}} = 118 \, ^{\circ}\text{C}$.
Specific rotation: $[\alpha]_D^{20} = -14.1 \, (c = 1.53, \text{CHCl}_3)$

$^1$H NMR (500 MHz, CDC$_3$): $\delta = 8.57$ (d, $J = 8.9 \, \text{Hz}$, 1 H, 2''-NH), 7.32-7.08 (m, 15 H, 15 x HC$_{\text{Ar}}$), 6.84 (d, $J = 5.3 \, \text{Hz}$, 1 H, 2''-NH), 5.42 (dd, $J = 5.3, 5.2 \, \text{Hz}$, 1 H, 2-NH), 5.07 (d, $J = 12.5 \, \text{Hz}$, 1 H, Bn-CH$_2$H$_{\text{b}}$), 5.04 (d, $J = 12.5 \, \text{Hz}$, 1 H, Bn-CH$_2$H$_{\text{b}}$), 4.84 (d, $J = 14.8 \, \text{Hz}$, 1 H, Bn-CH$_2$H$_{\text{b}}$), 4.80 (dd, $J = 8.9, 5.3 \, \text{Hz}$, 1 H, 2''-H), 4.57 (d, $J = 16.4 \, \text{Hz}$, 1 H, Bn-CH$_2$H$_{\text{b}}$), 4.36-4.29 (m, 2 H, 2''-H, Bn-CH$_2$H$_{\text{b}}$), 4.10 (d, $J = 14.8 \, \text{Hz}$, 1 H, Bn-CH$_2$H$_{\text{b}}$), 3.89 (dd, $J = 16.9, 5.3 \, \text{Hz}$, 1 H, 2-H$_{\text{b}}$), 3.81 (dd, $J = 16.9, 5.9 \, \text{Hz}$, 1 H, 2-H$_{\text{b}}$), 3.50 (dd, $J = 17.2, 2.2 \, \text{Hz}$, 1 H, 4''-H$_{\text{b}}$), 3.38 (dd, $J = 17.2, 2.2 \, \text{Hz}$, 1 H, 4''-H$_{\text{b}}$), 2.76 (dd, $J = 11.9, 3.4 \, \text{Hz}$, 1 H, 3''-H$_{\text{b}}$), 2.50 (dd, $J = 11.9, 11.4 \, \text{Hz}$, 1 H, 3''-H$_{\text{b}}$), 2.44 (s, 3 H, NCH$_3$), 2.40 (s, 3 H, 3''-H), 2.01 (dq, $J = 6.8, 6.3, 5.3 \, \text{Hz}$, 1 H, 3''-H), 0.87 (d, $J = 6.3 \, \text{Hz}$, 3 H, 4''-H$_{\text{b}}$), 0.84 (d, $J = 6.8 \, \text{Hz}$, 3 H, 4''-H$_{\text{b}}$).

$^{13}$C NMR (126 MHz, CDC$_3$): $\delta = 172.0, 171.2, 168.9$ (C-1, C-1', C-1''), 156.5 (NC(=O)O), 137.0, 136.4, 136.1 (3 x C$_{\text{Ar}}$), 129.1, 128.8, 128.7, 128.3, 128.2, 128.1, 127.7, 127.4 (15 x HC$_{\text{Ar}}$), 78.2 (C-5'), 73.7 (C-6'), 67.2 (Bn-CH$_2$), 57.4 (C-3'), 54.7 (C-2'), 50.0 (Bn-CH$_2$), 49.8 (C-2'), 47.9 (Bn-CH$_2$), 46.3 (C-4'), 44.4 (C-2), 41.1 (NCH$_3$), 31.0 (C-3''), 20.1, 17.2 (2 x C-4').

IR (ATR): $\nu = 3275, 1714, 1631, 1514, 1445, 1219, 1043, 730, 697$.

MS (ESI): $m/z = 626.4$ [M+H]$^+$, calculated: 626.3337 [M+H]$^+$, found: 626.3331 [-0.9 ppm] (ESI$^+$-HRMS).

Preparation of tripeptide 21
To a solution of tripeptide 6 (25 mg, 0.045 mmol) in CHCl$_3$ (1.5 mL), N-methylpropargylamine (23 $\mu$L, 0.27 mmol) was added. After stirring for 20 h at rt and 20 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt, 80:20 → 60:40) to give 26 mg (0.042 mmol, 93%) of the title compound as a colourless solid.

TLC: $R_f = 0.20$ (DCM:AcOEt, 60:40).

Melting point: $T_{\text{mp}} = 87 \, ^{\circ}\text{C}$.
Specific rotation: $[\alpha]_D^{20} = +16.1 \, (c = 1.87, \text{CHCl}_3)$
The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt, 40:60 → 20:80) to give 19 mg (0.033 mmol, 73%) of the title compound as a colourless solid.

**TLC:** Rf = 0.3 (DCM:AcOEt, 20:80).

**Melting point:** T_{mp} = 83 °C.

### Preparation of tripeptide 22

To a solution of tripeptide 7 (23 mg, 0.045 mmol) in CHCl₃ (1.5 mL), N-methylpropargylamine (23 μL, 0.27 mmol) was added. After stirring for 20 h at rt and 20 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt, 40:60 → 20:80) to give 19 mg (0.033 mmol, 73%) of the title compound as a colourless solid.

**TLC:** Rf = 0.3 (DCM:AcOEt, 20:80).

### 13C NMR (126 MHz, CDCl₃): δ = 171.4, 170.9, 168.5 (C-1, C-1‘, C-1“), 156.4 (NC(=O)O), 136.6, 136.4, 135.5 (3 x C₆H₅), 129.2, 128.9, 128.6, 128.4, 128.2, 128.1, 127.8, 126.6 (15 x HC₅N), 78.3 (C-5‘), 73.7 (C-6‘), 67.1 (Bn-CH₂), 60.2 (C-2), 57.1 (C-3‘), 50.3 (C-2‘), 49.1, 48.6 (2 x Bn-CH₂), 46.5 (C-4), 41.7 (C-2‘), 41.3 (NCH₃), 31.7 (C-3), 19.3, 17.8 (2 x C-4).

**IR (ATR):** ν = 3289, 1632, 1522, 1452, 1236, 1029, 735, 697, 632.

### MS (ESI⁺)

- m/z = 626.3 [M+H]⁺, calculated: 626.3337 [M+H]⁺,
- C₃₆H₄₃N₅O₅ (625.77 g/mol⁻¹), found: 626.3333 [-0.7 ppm] (ESI⁺-HRMS).

![Chemical structure](image)

**Preparation of tripeptide 23**

To a solution of tripeptide 4 (30 mg, 0.050 mmol) in CHCl₃ (2.0 mL), tert-butyl amine (32 μL, 0.30 mmol) was added. After stirring for 22 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt:MeOH, 60:40:0 → 96:0:4) to give 31 mg (0.046 mmol, 92%) of the title compound as a colourless solid.

**TLC:** Rf = 0.18 (DCM:MeOH, 95:5).

![Chemical structure](image)
Melting point: \( T_{\text{mp}} = 117 \, ^\circ\text{C} \).

Specific rotation: \([\alpha]_D^{20} = +20.3 \) (c = 2.56, CHCl\(_3\))

\(^1\text{H}\) NMR (500 MHz, CDCl\(_3\)):
\( \delta = 8.67 \) (d, \( J = 6.2 \, \text{Hz} \), 1 H, 2"-NH), 7.31-7.03 (m, 16 H, 2'-NH, 15 x HC\(_{\text{Ar}}\)), 5.41 (d, \( J = 8.5 \, \text{Hz} \), 1 H, 2'-NH), 5.05 (d, \( J = 12.3 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 5.01 (d, \( J = 12.3 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 4.83 (d, \( J = 14.8 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 4.80 (dd, \( J = 8.0, 6.2 \, \text{Hz} \), 1 H, 2"-H), 4.58 (d, \( J = 16.4 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 4.50-4.22 (m, 2 H, 2'-H, Bn-CH\(_2\)H\(_b\)), 4.11 (d, \( J = 14.8 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 4.03 (dd, \( J = 8.5, 5.9 \, \text{Hz} \), 1 H, 2'-H), 3.16-3.10 (m, 1 H, 3'-H\(_a\)), 2.60-2.52 (m, 1 H, 3'-H\(_b\)), 2.09-2.00 (m, 2 H, 3'-H, 3'-H), 1.11 (s, 9 H, C(CH\(_3\))\(_3\)), 0.93-0.85 (m, 12 H, 4'-H, 4''-H). The signal attributed to the secondary amine NH proton was not observed in the \(^1\text{H}\) NMR.

\(^{13}\text{C}\) NMR (126 MHz, CDCl\(_3\)):
\( \delta = 172.2, 171.4, 171.0 \) (C-1, C-1', C-1''), 156.5 (NC(=O)O), 137.0, 136.4, 136.1 (3 x C\(_{\text{Ar}}\)), 129.0, 128.8, 128.6, 128.3, 128.2, 128.0, 127.6, 127.4 (15 x HC\(_{\text{Ar}}\)), 67.1 (Bn-CH\(_2\)), 60.3 (C-2), 55.2 (C-2"), 52.4 (C-2), 50.1, 47.9 (2 x Bn-CH\(_2\)), 43.8 (C-3'), 31.6, 30.9 (C-3, C-3''), 28.6 (C(CH\(_3\))\(_3\)), 20.0, 19.4, 17.8, 17.5 (2 x C-4, 2 x C-4').

Despite several attempts, the \(^{13}\text{C}\) NMR signal attributed to quaternary carbon atom C(CH\(_3\))\(_3\) was not observed, probably due to very pronounced line-broadening. However, the presence of the Bn' group was unambiguously confirmed by appearance of signals for the corresponding three CH\(_3\) groups both in the \(^1\text{H}\) NMR and \(^{13}\text{C}\) NMR spectra, \textit{vide supra}.

IR (ATR): \( \nu = 3287, 2962, 1630, 1529, 1448, 1217, 1028, 733, 697 \).

MS (ESI\(^+$\)): m/z = 672.4 [M+H]\(^+$\), calculated: 672.4120 [M+H]\(^+$\),
\( \text{C}_{39}\text{H}_{53}\text{N}_{2}\text{O}_{5} \) (671.40 g/mol)\(^-1\),
found: 672.4095 [-3.6 ppm] (ESI\(^+$\)-HRMS).

Preparation of tripeptide 24

To a solution of tripeptide 5 (15 mg, 0.027 mmol) in CHCl\(_3\) (1.5 mL), \textit{tert}-butyl amine (17 \( \mu \text{L}, 0.16 \) mmol) was added. After stirring for 20 h at rt and for 24 h at 40 °C the solvent was evaporated \textit{in vacuo}. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:MeOH, 96:4) to give 16 mg (0.025 mmol, 94\%) of the title compound as a colourless solid.

TLC: \( R_f = 0.11 \) (DCM:MeOH, 95:5).

Melting point: \( T_{\text{mp}} = 96 \, ^\circ\text{C} \).

Specific rotation: \([\alpha]_D^{20} = -36.3 \) (c = 0.84, CHCl\(_3\))

\(^1\text{H}\) NMR (500 MHz, CDCl\(_3\)):
\( \delta = 8.69 \) (d, \( J = 6.2 \, \text{Hz} \), 1 H, 2"-NH), 7.32-7.08 (m, 16 H, 2'-NH, 15 x HC\(_{\text{Ar}}\)), 5.51 (s, 1 H, 2'-NH), 5.08 (d, \( J = 12.3 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 5.04 (d, \( J = 12.3 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 4.86 (d, \( J = 14.8 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 4.77 (dd, \( J = 8.0, 6.2 \, \text{Hz} \), 1 H, 2"-H), 4.58 (d, \( J = 16.4 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 4.51-4.42 (m, 1 H, 2'-H), 4.32 (d, \( J = 16.4 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 4.08 (d, \( J = 14.8 \, \text{Hz} \), 1 H, Bn-CH\(_2\)H\(_b\)), 3.89 (dd, \( J = 16.9, 5.5 \, \text{Hz} \), 1 H, 2'-H\(_a\)), 3.83 (dd, \( J = 16.9, 5.5 \, \text{Hz} \), 1 H, 2'-H\(_b\)), 3.16 (dd, \( J = 11.1, 2.4 \, \text{Hz} \), 1 H, 3'-H\(_a\)), 2.59 (dd, \( J = 11.1, 9.7 \, \text{Hz} \), 1 H, 3'-H\(_b\)), 2.05 (dq, \( J = 6.7, 6.7, 5.9 \, \text{Hz} \), 1 H, 3"-H), 1.13 (s, 9 H, C(CH\(_3\))\(_3\)), 0.92 (d, \( J = 6.7 \, \text{Hz} \), 3 H, 4'-H\(_a\)), 0.86 (d, \( J = 6.7 \, \text{Hz} \), 3 H, 4'-H\(_b\)). The signal attributed to the secondary amine NH proton was not observed in the \(^1\text{H}\) NMR.
\(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \(\delta = 172.2, 170.9, 169.1\) (C-1, C-1', C-1''), 156.6 (NC(=O)O), 137.0, 136.3, 136.0 (3 x C\(_4\)), 129.1, 128.8, 128.7, 128.4, 128.3, 128.2, 128.1, 127.7, 127.4 (15 x HC\(_{10}\)), 67.3 (Bn-CH\(_2\)), 55.3 (C-2''), 52.1 (C-2'), 50.1, 48.0 (2 x Bn-CH\(_2\)), 44.6 (C-2), 43.7 (C-3'), 30.9 (C-3''), 28.4 (C(CH\(_3\))\(_3\)), 20.0, 17.5 (2 x C-4'). Despite several attempts, the \(^{13}\)C NMR signal attributed to quaternary carbon atom C(CH\(_3\))\(_3\) was not observed, probably due to very pronounced line-broadening. However, the presence of the Bu' group was unambiguously confirmed by appearance of signals for the corresponding three CH\(_3\) groups both in the \(^1\)H NMR and \(^{13}\)C NMR spectra, video supra.

IR (ATR): \(\nu = 3286, 2963, 1630, 1526, 1449, 1216, 1046, 734, 697\).

MS (ESI\(^+\)): m/z = 630.4 [M+H]+, calculated: 630.3650 [M+H]+,
C\(_{36}\)H\(_{47}\)N\(_5\)O\(_5\) (629.80 g mol\(^{-1}\)), found: 630.3631 [-3.0 ppm] (ESI\(^+\)-HRMS).

Preparation of tripeptide 25
To a solution of tripeptide 6 (15 mg, 0.027 mmol) in CHCl\(_3\) (1.5 mL), tert-butyl amine (17 \(\mu\)L, 0.16 mmol) was added. After stirring for 20 h at rt and for 2 d at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:MeOH, 95:5) to give 16 mg (0.025 mmol, 94%) of the title compound as a colourless solid.

TLC: \(R_t = 0.11\) (DCM:MeOH, 95:5).

Melting point: \(T_{mp} = 81\) °C.

Specific rotation: \([\alpha]_D^{20} = +4.6\) (c = 1.21, CHCl\(_3\))

\(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta = 8.59\) (s, 1 H, 2'-NH, 15 x HC\(_{10}\)), 5.49 (s, 1 H, 2'-NH), 5.05 (d, \(J = 12.5\) Hz, 1 H, Bn-CH\(_2\)H\(_b\)), 5.02 (d, \(J = 12.5\) Hz, 1 H, Bn-CH\(_2\)H\(_b\)), 4.66-4.46 (m, 3 H, 2'-H, Bn-CH\(_2\)), 4.34 (d, \(J = 17.4\) Hz, 1 H, Bn-CH\(_2\)H\(_b\)), 4.30 (d, \(J = 17.4\) Hz, 1 H, Bn-CH\(_2\)H\(_b\)), 4.16-3.99 (m, 3 H, 2'H, 2''-H), 3.33-3.24 (m, 1 H, 3'H), 2.84-2.74 (m, 1 H, 3'H), 2.10 (dqq, \(J = 6.8, 6.7, 6.2, 6.2\) Hz, 1 H, 3'H), 1.20 (s, 9 H, C(CH\(_3\))\(_3\)), 0.92 (d, \(J = 6.7\) Hz, 3 H, 4-H\(_a\)), 0.87 (d, \(J = 6.8\) Hz, 3 H, 4-H\(_b\)). The signal attributed to the secondary amine NH proton was not observed in the \(^1\)H NMR.

\(^{13}\)C NMR (126 MHz, CDCl\(_3\)): \(\delta = 171.9, 170.6, 169.1\) (C-1, C-1', C-1''), 156.9 (NC(=O)O), 136.4, 135.2 (3 x C\(_4\)), 129.3, 128.9, 128.7, 128.4, 128.2, 128.2, 127.9, 127.6 (15 x HC\(_{10}\)), 67.2 (Bn-CH\(_2\)), 60.9 (C-2), 52.0 (C-2'), 49.4, 49.0 (2 x Bn-CH\(_2\)), 43.8 (C-3'), 41.9 (C-2''), 31.2 (C-3), 27.5 (C(CH\(_3\))\(_3\)), 19.4, 17.9 (2 x C-4'). Despite several attempts, the \(^{13}\)C NMR signal attributed to quaternary carbon atom C(CH\(_3\))\(_3\) was not observed, probably due to very pronounced line-broadening. However, the presence of the Bu' group was unambiguously confirmed by appearance of signals for the corresponding three CH\(_3\) groups both in the \(^1\)H NMR and \(^{13}\)C NMR spectra, vide supra.

IR (ATR): \(\nu = 2962, 1639, 1496, 1452, 1219, 1080, 1027, 735, 698\).

MS (ESI\(^+\)): m/z = 630.4 [M+H]+, calculated: 630.3650 [M+H]+,
C\(_{36}\)H\(_{47}\)N\(_5\)O\(_5\) (629.80 g mol\(^{-1}\)), found: 630.3633 [-2.7 ppm] (ESI\(^+\)-HRMS).
Preparation of tripeptide 26

To a solution of tripeptide 7 (23 mg, 0.045 mmol) in CHCl₃ (2.0 mL), tert-butyl amine (28 μL, 0.27 mmol) was added. After stirring for 20 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt:MeOH, 30:70:0 → 92:0:8) to give 24 mg (0.041 mmol, 91%) of the title compound as a colourless solid.

TLC: Rₜ = 0.15 (DCM:MeOH, 93:7).

Melting point: Tₘₚ = 63 °C.

Specific rotation: [α]₀D²⁰ = -5.1 (c = 1.83, CHCl₃)

¹H NMR (500 MHz, CDCl₃): δ = 8.47 (s, 1 H, 2'-NH), 7.77 (s, 1 H, 2'-NH), 7.31-7.07 (m, 15 H, 15 x HC₆H₅), 5.66 (s, 1 H, 2-NH), 5.05 (d, J = 12.6 Hz, 1 H, Bn-CH₂H₂), 5.02 (d, J = 12.6 Hz, 1 H, Bn-CH₂H₂), 4.55-4.47 (m, 3 H, 2'-H, Bn-CH₂), 4.31 (s, 2 H, Bn-CH₂), 4.13 (d, J = 17.0 Hz, 1 H, 2''-H₃), 4.01 (d, J = 17.0 Hz, 1 H, 2''-H₃), 3.91 (dd, J = 16.9, 5.7 Hz, 1 H, 2-H₃), 3.82 (dd, J = 16.9, 5.6 Hz, 1 H, 2-H₃), 3.32 (dd, J = 11.5, 2.9 Hz, 1 H, 3'-H₂), 2.72 (dd, J = 11.5, 6.9 Hz, 1 H, 3'-H₂), 1.13 (s, 9 H, C(CH₃)₃). The signal attributed to the secondary amine NH proton was not observed in the ¹H NMR.

¹³C NMR (126 MHz, CDCl₃): δ = 170.8, 169.7, 169.0 (C-1, C-1', C-1''), 156.9 (NC(=O)O), 136.5, 136.3, 135.3 (3 x C₆H₅), 129.3, 128.9, 128.6, 128.4, 128.3, 128.2, 128.1, 127.9, 127.6 (15 x HC₆H₅), 67.3 (Bn-CH₂), 53.0 (C(CH₃)₃), 52.3 (C-2'), 49.3, 48.9 (2 x Bn-CH₂), 44.7 (C-2), 43.6 (C-3'), 41.8 (C-2''), 28.0 (C(CH₃)₃).

IR (ATR): ν = 2962, 1647, 1496, 1451, 1218, 1047, 735, 698, 613.


Preparation of tripeptide 27

To a solution of tripeptide 4 (30 mg, 0.050 mmol) in CHCl₃ (2.0 mL), n-butyl amine (30 μL, 0.30 mmol) was added. After stirring for 22 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt:MeOH, 60:40:0 → 96:0:4) to give 33 mg (0.049 mmol, 98%) of the title compound as a colourless solid.

TLC: Rₜ = 0.18 (DCM:MeOH, 95:5).

Melting point: Tₘₚ = 115 °C.

Specific rotation: [α]₀D²⁰ = -23.6 (c = 2.77, CHCl₃)
Preparation of tripeptide 28

To a solution of tripeptide 6 (25 mg, 0.045 mmol) in CHCl₃ (2.0 mL), n-butyl amine (27 μL, 0.27 mmol) was added. After stirring for 20 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt:MeOH, 50:50:0 → 96:0:4) to give 24 mg (0.038 mmol, 85%) of the title compound as a colourless solid.

**TLC:** Rₛ = 0.05 (DCM:MeOH, 96:4).

**Melting point:** Tₘᵡ = 121 °C.

**Specific rotation:** [α]D²⁰ = +3.6 (c = 2.03, CHCl₃)

**1H NMR** (500 MHz, CDCl₃): δ = 8.42 (s, 1 H, 2''-NH), 7.37-7.07 (m, 16 H, 2'-NH, 15 x HC₆H₅), 5.45 (d, J = 7.7 Hz, 1 H, 2-NH), 5.05 (d, J = 12.2 Hz, 1 H, Bn-CH₂H₅), 5.01 (d, J = 12.2 Hz, 1 H, Bn-CH₂H₅), 4.55-4.45 (m, 3 H, 2'-H, Bn-CH₂), 4.32 (s, 2 H, Bn-CH₂), 4.11 (s, 2 H, 2''-H), 4.02 (dd, J = 7.7, 6.1 Hz, 1 H, 2-H), 3.18 (dd, J = 8.7, 3.3 Hz, 1 H, 3''-H), 2.73-2.59 (m, 3 H, 3''-H, CH₂CH₂CH₂CH₃), 2.09 (dq, J = 6.7, 6.6, 6.1 Hz, 1 H, 3-H), 1.50-1.44 (m, 2 H, CH₂CH₂CH₂CH₃), 1.32-1.25 (m, 2 H, CH₂CH₂CH₂CH₃), 0.92-0.82 (m, 9 H, 4-H, CH₃CH₂CH₂CH₃). The signal attributed to the secondary amine NH proton was not observed in the 1H NMR.

**13C NMR** (126 MHz, CDCl₃): δ = 171.6, 171.0, 168.8 (C-1, C-1', C-1''), 156.7 (NC(=O)O), 136.5, 136.4, 135.4 (3 x C₆H₅), 129.3, 128.8, 128.6, 128.4, 128.3, 128.2, 128.1, 127.8, 126.6 (15 x HC₆H₅), 67.2 (Bn-CH₂), 60.6 (C-2), 51.9 (C-2'), 50.5 (C-3'), 49.3 (CH₂CH₂CH₂CH₃), 49.2, 48.8 (2 x Bn-CH₂), 41.7 (C-2''), 31.4 (CH₂CH₂CH₂CH₃), 31.3 (C-3), 20.4 (CH₂CH₂CH₂CH₃), 19.4, 17.8 (2 x C-4), 14.0 (CH₂CH₂CH₂CH₃).

**IR (ATR):** ν = 3287, 2960, 1630, 1523, 1452, 1219, 1028, 736, 697.

**MS (ESI⁺):** m/z = 630.4 [M+H]⁺, calculated: 630.3650 [M+H]⁺, found: 630.3634 [-2.5 ppm] (ESI⁺-HRMS).
Preparation of lysine tripeptide conjugate 29

To lysine derivative S1 (100 mg, 0.179 mmol) in degassed EtOH (4 mL), 10 wt% palladium on charcoal (10 mg, 9.4 μmol) was added. The resulting suspension was stirred under a hydrogen atmosphere (1 bar, balloon) for 3 h and then filtered through a syringe filter. The syringe filter was washed with EtOH (3 x 3 mL) and the solvent of the combined filtrates evaporated in vacuo. The resulting colourless solid was dried in vacuo. The resulting free amine was always prepared freshly and used instantly in the subsequent transformation without further purification.

To a solution of tripeptide 4 (24 mg, 0.040 mmol) in CHCl₃ (1.5 mL), 1/2 of the crude product (only 1/2 of the initially prepared Cbz-deprotected lysine derivative, vide supra, was used in the 2nd step, calculated maximal amount of substance: 0.080 mmol) was added. After stirring for 24 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt:MeOH, 50:50:0 → 95:0:5) to give 35 mg (0.034 mmol, 85%) of the title compound as a colourless solid.

**TLC**: Rᵣ = 0.36 (DCM:MeOH, 93:7).

**Melting point**: Tᵣₚ = 142 °C.

**Specific rotation**: [α]₀²⁰ = −30.9 (c = 2.79, CHCl₃)

**¹H NMR** (500 MHz, CDCl₃): δ = 8.54 (d, J = 7.6 Hz, 1 H, 2''-NH), 7.39-7.16 (m, 25 H, 25 x HCₐₚ), 7.03 (s, 1 H, 2'-'NH), 5.59 (d, J = 7.7 Hz, 1 H, 2-NH), 5.46 (d, J = 8.4 Hz, 1 H, 2''-NH), 5.10 (s, 2 H, Bn-CH₂), 4.90-4.85 (m, 2 H, 2''-H, Bn-CH₂Hₐ), 4.73-4.52 (m, 6 H, 2''-H, Bn-CH₂Hₐ, b x Bn-CH₂), 4.43 (d, J = 16.5 Hz, 1 H, Bn-CH₂Hₐ), 4.36 (s, 1 H, 2'-H), 4.24 (d, J = 14.8 Hz, 1 H, Bn-CH₂Hₐ), 4.11 (dd, J = 7.7, 6.3 Hz, 1 H, 2-H), 3.10 (dd, J = 12.1, 3.6 Hz, 1 H, 3''-H₀), 2.67-2.53 (m, 3 H, 3''-H₀, 6''-H), 2.17-2.08 (m, 2 H, 3-H, 3''-H), 1.64-1.57 (m, 2 H, 3''-H), 1.44-1.27 (m, 13 H, 4''-H, 5''-H, C(CH₃)₃), 0.99-0.90 (m, 12 H, 4-H, 4''-H).

The signal attributed to the secondary amine NH proton was not observed in the ¹H NMR.

**¹³C NMR** (126 MHz, CDCl₃): δ = 173.5, 172.0, 171.4, 171.2 (C-1, C-1', C-1'', C-1''''), 156.5, 155.7 (2 x NC(=O)O), 137.1, 136.9, 136.5, 136.4, 136.2 (5 x Cₐ), 129.0, 128.8, 128.8, 128.6, 128.3, 128.2, 128.0, 127.9, 127.6, 127.3 127.0 (25 x HCₐ), 79.7 (C(CH₃)₃), 67.1 (Bn-CH₂), 60.4 (C-2), 54.7 (C-2''), 51.7 (C-2'), 50.5 (C-3'), 50.5 (C-2'''), 50.1, 50.0 (2 x Bn-CH₂), 49.2 (C-6''), 48.5, 47.9 (2 x Bn-CH₂), 33.3 (C-3'''), 31.4 (C-3'), 31.0 (C-3''), 29.7 (C-5''), 28.5 (C(CH₃)₃), 23.1 (C-4''), 20.0, 19.4, 17.8, 17.5 (2 x C-4, 2 x C-4 '').

**IR** (ATR): ν = 3286, 1678, 1637, 1527, 1450, 1218, 1167, 1028, 695.

**MS (ESI⁺)**: m/z = 1024.5 [M+H]⁺; calculated: 1024.5906 [M+H]⁺,

C₆₀H₇⁷N₃O₈ (1024.32 g/mol⁻¹), found: 1024.5907 [+0.1 ppm] (ESI⁺-HRMS).
Preparation of lysine tripeptide conjugate 30

To lysine derivative S1 (100 mg, 0.179 mmol) in degassed EtOH (4 mL), 10 wt% palladium on charcoal (10 mg, 9.4 µmol) was added. The resulting suspension was stirred under a hydrogen atmosphere (1 bar, balloon) for 3 h and then filtered through a syringe filter. The syringe filter was washed with EtOH (3 x 3 mL) and the solvent of the combined filtrates evaporated in vacuo. The resulting colourless solid was dried in vacuo. The resulting free amine was always prepared freshly and used instantly in the subsequent transformation without further purification.

To a solution of tripeptide 6 (22 mg, 0.040 mmol) in CHCl₃ (1.5 mL), 1/2 of the crude product (only 1/2 of the initially prepared Cbz-deprotected lysine derivative, vide supra, was used in the 2nd step, calculated maximal amount of substance: 0.080 mmol) was added. After stirring for 24 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt:MeOH, 30:70:0 → 95:0:5) to give 37 mg (0.038 mmol, 95%) of the title compound as a colourless solid.

**TLC:** $R_f = 0.25$ (DCM:MeOH, 93:7).

**Melting point:** $T_{mp} = 83 ^\circ$C.

**Specific rotation:** $[\alpha]_D^{20} = -11.9$ (c = 2.78, CHCl₃)

**¹H NMR** (500 MHz, CDCl₃): $\delta = 8.34$ (s, 1 H, 2''-NH), 7.38-7.14 (m, 26 H, 2'-NH, 25 x HCA₆), 5.73 (d, $J = 7.2$ Hz, 1 H, 2-NH), 5.55 (d, $J = 8.2$ Hz, 1 H, 2''-NH), 5.13 (s, 2 H, Bn-CH₂), 4.70-4.39 (m, 10 H, 2''-H, 2''-H, 4 x Bn-CH₂), 4.18 (s, 2 H, 2''-H), 4.09 (dd, $J = 7.2$, 6.4 Hz, 1 H, 2-H), 3.19 (dd, $J = 12.1$, 3.5 Hz, 1 H, 3''-Hₐ), 2.71-2.51 (m, 3 H, 3''-Hₐ, 6''-Hₐ), 2.17 (dqq, $J = 6.6$, 6.6, 6.4 Hz, 1 H, 3-H), 1.69-1.56 (m, 2 H, 3''-Hₐ), 1.47-1.27 (m, 13 H, 4''-H, 5''-H, C(CH₃)₃), 0.99 (d, $J = 6.6$ Hz, 3 H, 4-Hₐ), 0.93 (d, $J = 6.6$ Hz, 3 H, 4-Hₐ). The signal attributed to the secondary amine NH proton was not observed in the ¹H NMR.

**¹³C NMR** (126 MHz, CDCl₃): $\delta = 173.5$, 171.5, 171.1, 168.7 (C-1, C-1', C-1'', C-1''), 156.7, 155.7 (2 x NC(=O)O), 136.9, 136.6, 136.5, 136.4, 135.5 (5 x CA₆), 129.2, 129.0, 128.8, 128.8, 128.6, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.1, 126.6 (25 x HC₆), 79.7 (C(CH₃)₃), 67.1 (Bn-CH₂), 60.7 (C-2), 52.0 (C-2'), 50.5 (C-3'), 50.5 (C-2''), 50.1 (Bn-CH₂), 49.3 (C-6''), 49.1, 48.7, 48.5 (3 x Bn-CH₂), 41.7 (C-2''), 33.1 (C-3''), 31.2 (C-3), 29.3 (C-5''), 28.4 (C(CH₃)₃), 23.0 (C-4''), 19.4, 17.9 (2 x C-4).

**IR** (ATR): v = 3312, 1640, 1524, 1451, 1220, 1166, 1028, 732, 697.

**MS** (ESI⁺): m/z = 1004.5 [M+Na]⁺, calculated: 982.5437 [M+H]⁺, found: 982.5429 [-0.8 ppm] (ESI⁺-HRMS).
Preparation of 1,2,3-triazole 31
To a thoroughly degassed solution of alkyne 19 (24 mg, 0.036 mmol) in abs. CHCl₃ (1.5 mL), benzyl azide[54] (29 mg, 0.22 mmol), CuI (10 mg, 0.054 mmol) and NEt₃ (60 μL, 0.43 mmol) were added. After stirring for 3 h the reaction mixture was diluted with DCM (50 mL) and washed with water (50 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt, 60:40 → 40:60) to give 19 g (0.024 mmol, 67%) of the title compound as a pale yellow solid.

**TLC:** Rf = 0.13 (DCM:AcOEt, 40:60).
**Melting point:** Tmp = 74 ºC.
**Specific rotation:** [α]D²⁰ = +2.3 (c = 1.12, CHCl₃)

**¹H NMR** (500 MHz, CDCl₃): δ = 8.75 (d, J = 8.7 Hz, 1 H, 2'-NH), 7.77 (s, 1 H, 6'-H), 7.31-7.06 (m, 20 H, 20 x HC₆H₅), 6.85 (d, J = 4.2 Hz, 1 H, 2'-NH), 5.44 (d, J = 14.9 Hz, 1 H, Bn-CH₃H₂), 5.39 (d, J = 14.9 Hz, 1 H, Bn-CH₃H₂), 5.35 (d, J = 8.4 Hz, 1 H, 2'-NH), 5.06 (d, J = 12.2 Hz, 1 H, Bn-CH₃H₂), 5.02 (d, J = 12.2 Hz, 1 H, Bn-CH₃H₂), 4.80 (dd, J = 8.7, 6.0 Hz, 1 H, 2''-H), 4.76 (d, J = 14.9 Hz, 1 H, Bn-CH₃H₂), 4.56 (d, J = 16.5 Hz, 1 H, Bn-CH₃H₂), 4.39 (d, J = 16.5 Hz, 1 H, Bn-CH₃H₂), 4.37-4.33 (m, 1 H, 2'-H), 4.16 (d, J = 14.9 Hz, 1 H, Bn-CH₃H₂), 4.02 (dd, J = 8.4, 6.1 Hz, 1 H, 2'-H), 3.85 (d, J = 13.6 Hz, 1 H, 4'-H₂), 3.71 (d, J = 13.6 Hz, 1 H, 4'-H₂), 2.71 (dd, J = 11.9, 3.0 Hz, 1 H, 3'-H₃), 2.53 (dd, J = 11.9, 11.0 Hz, 1 H, 3'-H₃), 2.32 (s, 3 H, NCH₃), 2.05 (dqq, J = 6.7, 6.6, 6.1 Hz, 1 H, 3'-H₃), 1.96 (dqq, J = 6.7, 6.7, 6.0 Hz, 1 H, 3''-H₃), [0.90 (d, J = 6.6 Hz, 3 H), 0.84 (d, J = 6.7 Hz, 3 H), 0.80 (d, J = 6.7 Hz, 3 H), 0.78 (d, J = 6.7 Hz, 3 H)] (4-H₄, 4'-H₄, 4''-H₄, 4'''-H₄).

**¹³C NMR** (126 MHz, CDCl₃): δ = 172.0, 171.3, 170.8 (C-1, C-1', C-1''), 156.3 (NC(O)=O), 144.3 (C-5), 137.0, 136.4, 136.1, 134.9 (4 x C₆H₅), 129.0, 129.0, 128.7, 128.6, 128.6, 128.2, 128.1, 128.1, 127.9, 127.5, 127.2 (20 x HC₆H₅), 123.5 (C-6'), 67.0 (Bn-CH₂), 60.1 (C-2), 57.9 (C-3'), 54.5 (C-2''), 54.1 (Bn-CH₂), 51.7 (C-4''), 50.0 (Bn-CH₂), 49.6 (C-2'), 47.9 (Bn-CH₂), 41.6 (NCH₃), 31.5, 31.1 (C-3, C-3''), 19.9, 19.2, 17.7, 17.3 (2 x C-4, 2 x C-4'').

**IR (ATR):** ν = 3288, 2961, 1632, 1531, 1496, 1452, 1219, 1029, 697.

**MS (ESI⁺):** m/z = 801.4 [M+H]⁺, calculated: 801.4446 [M+H]⁺,
C₄₆H₅₆N₈O₅ (801.01 g/mol⁻¹),
found: 801.4423 [-2.9 ppm] (ESI⁺-HRMS).
Preparation of 1,2,3-triazole 32
To a thoroughly degassed solution of alkyne 19 (24 mg, 0.036 mmol) in abs. CHCl₃ (1.0 mL) and abs. EtOH (1.0 mL), 3-azido-7-hydroxycoumarine [55] (15 mg, 0.072 mmol), CuI (3.4 mg, 0.018 mmol) and NEt₃ (15 μL, 0.11 mmol) were added. After stirring for 3 h the reaction mixture was diluted with DCM (30 mL) and washed with water (30 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt, 50:50 → 30:70) to give 16 mg (0.018 mmol, 51%) of the title compound as a yellow solid.

**TLC:** Rᵣ = 0.18 (DCM:AcOEt, 40:60).

**Melting point:** Tᵣmp = 108 °C.

**Specific rotation:** \([\alpha]_D^{20} = -10.7 \ (c = 0.70, \ CHCl₃)\)

**¹H NMR** (500 MHz, CDCl₃): δ = 8.75 (d, J = 8.5 Hz, 1H, 2''-NH), 8.59 (s, 1H, 6'-H), 8.20 (s, 1H, 8'-H), 7.42-7.18 (m, 16H, 10'-H, 15 x HCAr), 7.13 (d, J = 4.9 Hz, 1H, 2'-NH), 6.76-6.72 (m, 2H, 11'-H, 13'-H), 5.53 (d, J = 8.0 Hz, 1H, 2-NH), 5.15 (d, J = 12.3 Hz, 1H, Bn-CH₂H₃b), 5.11 (d, J = 12.3 Hz, 1H, Bn-CH₂H₃b), 4.93 (dd, J = 8.5, 6.7 Hz, 1H, 2''-H), 4.83 (d, J = 14.9 Hz, 1H, Bn-CH₂H₃b), 4.70 (d, J = 16.5 Hz, 1H, Bn-CH₂H₃b), 4.63-4.57 (m, 2H, 2'-H, Bn-CH₂H₃b), 4.39 (d, J = 14.9 Hz, 1H, Bn-CH₂H₃b), 4.16 (dd, J = 8.0, 6.6 Hz, 1H, 2'-H), 4.06 (d, J = 14.2 Hz, 1H, 4'-H₃b), 3.85 (d, J = 14.2 Hz, 1H, 4'-H₃b), 2.89 (dd, J = 9.9, 4.0 Hz, 1H, 3'-H₃b), 2.72 (dd, J = 10.7, 9.9 Hz, 1H, 3'-H₃b), 2.47 (s, 3H, NCH₃), 2.25-2.17 (m, 2H, 3-H, 3''-H₃), [1.04 (d, J = 6.6 Hz, 3H), 1.03 (d, J = 6.5 Hz, 3H), 0.98 (d, J = 5.7 Hz, 3H), 0.97 (d, J = 6.5 Hz, 3H) (4-H₃b, 4'-H₃b, 4''-H₃b, 4''''-H₃b)]. The signal attributed to the hydroxy OH proton was not observed in the ¹H NMR.

**¹³C NMR** (126 MHz, CDCl₃): δ = 172.7, 171.9, 171.0 (C-1, C-1', C-1''), 162.0, 156.6, 156.1, 154.6 (C-12', C-14', C-15''), NC(=O)O), 143.8 (C-5'), 136.7, 136.4, 136.0 (3 x C₆Ar), 133.5 (C-8'), 130.1 (C-10'), 129.2, 128.9, 128.7, 128.3, 128.2, 128.1, 128.1, 127.8, 127.3 (15 x HCAr), 124.1 (C-6'), 119.4, 114.7, 110.7, 103.1 (C-7', C-9', C-11', C-13'), 67.2 (Bn-CH₂), 60.5 (C-2), 57.7 (C-3'), 55.0 (C-2''), 51.6 (C-4'), 50.5 (Bn-CH₂), 50.3 (C-2'), 48.5 (Bn-CH₂), 42.2 (NCH₃), 31.5, 31.3 (C-3, C-3''), 20.0, 19.4, 17.9, 17.8 (2 x C-4, 2 x C-4').

**IR** (ATR): ν = 2961, 1609, 1515, 1452, 1231, 1118, 1029, 734, 697.

**MS** (ESI⁺): m/z = 871.4 [M+H]⁺, calculated: 871.4137 [M+H]⁺,

C₄₈H₅₄N₆O₅ (871.01 g/mol)⁻¹,
found: 871.4113 [-2.8 ppm] (ESI⁺-HRMS).
Preparation of proline tripeptide conjugate 33
To a solution of tripeptide 4 (24 mg, 0.040 mmol) in CHCl₃ (1.5 mL), L-proline tert-butyl ester (14 mg, 0.080 mmol) was added. After stirring for 24 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt, 90:10 → 80:20) to give 30 mg (0.039 mmol, 98%) of the title compound as a colourless solid.

**TLC:** Rᵣ = 0.21 (DCM:AcOEt, 80:20).

**Melting point:** Tₘₚ = 81 °C.

**Specific rotation:** [α]D<sup>20</sup> = -44.4 (c = 2.83, CHCl₃)

**¹H NMR** (500 MHz, CDCl₃): δ = 8.30 (d, J = 8.5 Hz, 1 H, 2''-NH), 7.48 (d, J = 4.1 Hz, 1 H, 2'-NH), 7.38-7.18 (m, 15 H, 15 x HC₆H₅), 5.59 (d, J = 8.8 Hz, 1 H, 2-NH), 5.14 (d, J = 12.2 Hz, 1 H, Bn-CH₂H₂), 5.09 (d, J = 12.2 Hz, 1 H, Bn-CH₂H₂), 4.86-4.83 (m, 2 H, 2''-H, Bn-CH₂H₂), 4.62 (d, J = 16.6 Hz, 1 H, Bn-CH₂H₂), 4.52 (d, J = 16.6 Hz, 1 H, Bn-CH₂H₂), 4.32-4.22 (m, 3 H, 2-H, 2'-H, Bn-CH₂H₂), 3.40 (dd, J = 9.0, 4.4 Hz, 1 H, 2''-H), 3.23 (m, 1 H, 5''-H), 2.99 (dd, J = 12.7, 7.5 Hz, 1 H, 3'-H), 2.78 (dd, J = 12.7, 8.1 Hz, 1 H, 3'-H), 2.56-2.51 (m, 1 H, 5''-H), 2.23-2.12 (m, 3 H, 3-H, 3''-H, 3'''-H), 1.94-1.78 (m, 3 H, 3''-H, 4''-H), 1.48 (s, 9 H, C(CH₃)₃), 1.00-0.90 (m, 12 H, 4-H, 4''-H).

**¹³C NMR** (126 MHz, CDCl₃): δ = 175.1, 172.1, 171.8, 170.6 (C-1, C-1', C-1'', C-1'''), 156.4 (NC(=O)O), 137.2, 136.6, 136.5 (3 x CAr), 129.0, 128.7, 128.6, 128.4, 128.1, 127.9, 127.5, 127.4 (15 x HC₆H₅), 81.4 (C(CH₃)₃), 67.0 (Bn-CH₂), 66.2 (C-2''), 60.1 (C-2), 55.9 (C-3'), 54.7 (C-2''), 53.9 (C-5''), 51.9 (C-2'), 50.0, 48.0 (2 x Bn-CH₂), 31.8 (C-3), 31.2 (C-3'), 30.4 (C-3''), 28.2 (C(CH₃)₃), 24.2 (C-4''), 19.9, 19.3, 17.4, 17.4 (2 x C-4, 2 x C-4').

**IR** (ATR): ν = 1720, 1632, 1532, 1449, 1367, 1218, 1149, 1028, 697.

colourless solid.

**TLC:** $R_f = 0.11$ (DCM:AcOEt, 70:30).

**Melting point:** $T_{mp} = 128 \, ^{\circ}C$.

**Specific rotation:** $[\alpha]_D^{20} = -38.4$ (c = 2.58, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 8.45$ (d, $J = 8.5$ Hz, 1 H, 2''-NH), 7.39-7.18 (m, 16 H, 2'-NH, 15 x H$_2$A), 5.54 (dd, $J = 5.0$, 5.0 Hz, 1 H, 2'-NH), 5.13 (s, 2 H, Bn-CH$_2$), 4.85-4.82 (m, 2 H, 2''-H, Bn-CH$_2$H$_b$), 4.62 (d, $J = 16.5$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.51 (d, $J = 16.5$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.32-4.22 (m, 2 H, 2''-H, Bn-CH$_2$H$_b$), 3.97 (d, $J = 5.0$ Hz, 2 H, 2''-H), 3.41 (dd, $J = 9.1$, 4.2 Hz, 1 H, 2''-H), 3.25-3.20 (m, 1 H, 5''-H$_a$), 2.97 (dd, $J = 12.4$, 6.5 Hz, 1 H, 3''-H$_b$), 2.78 (dd, $J = 12.4$, 9.1 Hz, 1 H, 3''-H$_b$), 2.59-2.54 (m, 1 H, 5''-H$_b$), 2.21-2.11 (m, 2 H, 3''-H, 3''-H$_b$), 1.95-1.76 (m, 3 H, 3''-H$_b$, 4''-H), 1.46 (s, 9 H, C(CH$_3$)$_3$), 0.99 (d, J = 6.8 Hz, 3 H, 4''-H$_b$), 0.93 (d, J = 6.8 Hz, 3 H, 4''-H$_b$).

$^{13}$C NMR (126 MHz, CDCl$_3$): $\delta = 175.0$, 172.1, 170.6, 169.0 (C-1, C-1', C-1'', C-1'''), 155.5 (NC(=O)O), 137.2, 136.5, 136.4 (3 x C$_A$), 129.0, 128.7, 128.6, 128.3, 128.2, 127.9, 127.5, 127.4 (15 x C$_A$), 81.3 (C(CH$_3$)$_3$), 67.1 (Bn-CH$_2$), 66.4 (C-2''), 56.4 (C-3'), 54.9 (C-2''), 54.2 (C-5''), 51.7 (C-2'), 50.1, 48.0 (2 x Bn-CH$_2$), 44.4 (C-2), 31.1 (C-3''), 30.5 (C-3''), 28.2 (C(CH$_3$)$_3$), 24.2 (C-4''), 19.9, 17.3 (2 x C-4').

**IR (ATR):** $\nu = 1711, 1629, 1519, 1445, 1366, 1218, 1149, 1041, 698$.

**MS (ESI):** $m/z = 728.3$ [M+H]$^+$, calculated: 728.4018 [M+H]$^+$, found: 728.3986 [-4.3 ppm] (ESI$^+$-HRMS).

Preparation of proline tripeptide conjugate 35

To a solution of tripeptide 6 (22 mg, 0.040 mmol) in CHCl$_3$ (1.5 mL), l-proline tert-butyl ester (14 mg, 0.080 mmol) was added. After stirring for 24 h at 65 $^\circ$C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt, 80:20 $\rightarrow$ 75:25) to give 27 mg (0.037 mmol, 93%) of the title compound as a colourless solid.

**TLC:** $R_f = 0.19$ (DCM:AcOEt, 75:25).

**Melting point:** $T_{mp} = 75 \, ^{\circ}C$.

**Specific rotation:** $[\alpha]_D^{20} = -17.5$ (c = 1.78, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.90$ (dd, $J = 4.2$, 4.0 Hz, 1 H, 2''-NH), 7.62 (d, $J = 4.4$ Hz, 1 H, 2'-NH), 7.30-7.06 (m, 15 H, 15 x H$_2$A), 5.58 (d, $J = 8.9$ Hz, 1 H, 2'-NH), 5.08 (d, $J = 12.3$ Hz, 1 H, Bn-CH$_2$H$_b$), 5.08 (d, $J = 12.3$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.57 (d, $J = 14.8$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.50 (d, $J = 14.8$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.32 (s, 2 H, Bn-CH$_2$), 4.28-4.15 (m, 3 H, 2''-H), 4.03 (dd, $J = 17.4$, 4.0 Hz, 1 H, 2''-H), 3.27 (dd, $J = 8.9$, 5.0 Hz, 1 H, 2''-H), 3.11-3.06 (m, 1 H, 5''-H$_a$), 2.90 (dd, $J = 13.0$, 9.2 Hz, 1 H, 3''-H$_b$), 2.83 (dd, $J = 13.0$, 6.5 Hz, 1 H, 3''-H$_b$), 2.48-2.43 (m, 1 H, 5''-H$_b$), 2.23-2.05 (m, 2 H, 3'-H, 3''-H$_b$), 1.82-1.73 (m, 3 H, 3''-H$_b$, 4''-H), 1.35 (s, 9 H, C(CH$_3$)$_3$), 0.93 (d, $J = 6.8$ Hz, 3 H, 4''-H$_b$), 0.84 (d, $J = 6.9$ Hz, 3 H, 4''-H$_b$).
Preparation of proline tripeptide conjugate 36

To a solution of tripeptide 7 (21 mg, 0.040 mmol) in CHCl₃ (1.5 mL), 1-proline tert-butyl ester (14 mg, 0.080 mmol) was added. After stirring for 24 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt, 40:60 → 30:70) to give 26 mg (0.038 mmol, 95%) of the title compound as a colourless solid.

TLC: Rᵣ = 0.14 (DCM:AcOEt, 40:60).

Melting point: Tᵣ mp = 42 °C.

Specific rotation: [α]₀₂⁰ = -19.3 (c = 2.42, CHCl₃)

[^1]H NMR (500 MHz, CDCl₃): δ = 8.06 (dd, J = 4.5, 3.7 Hz, 1 H, 2''-NH), 7.64 (d, J = 4.5 Hz, 1 H, 2''-NH), 7.38-7.14 (m, 15 H, 15 x HC₆H₅), 5.61 (s, 1 H, 2-NH), 5.13 (s, 2 H, Bn-CH₂), 4.63 (d, J = 14.9 Hz, 1 H, Bn-CH₂H₅), 4.59 (d, J = 14.9 Hz, 1 H, Bn-CH₂H₆), 4.40-4.35 (m, 3 H, 2'-H, Bn-CH₂), 4.25 (dd, J = 17.1, 4.5 Hz, 1 H, 2'''-H₀), 4.12 (dd, J = 17.1, 3.7 Hz, 1 H, 2'''-H₀), 4.04-3.97 (m, 2 H, 2'-H), 3.35 (dd, J = 8.5, 4.4 Hz, 1 H, 2'''-H₀), 3.20-3.15 (m, 1 H, 3'''-H₁), 2.99 (dd, J = 12.4, 8.4 Hz, 1 H, 3'''-H₂), 2.89 (dd, J = 12.4, 6.6 Hz, 1 H, 3'''-H₂), 2.59-2.51 (m, 1 H, 5'''-H₁), 2.21-2.13 (m, 1 H, 3'''-H₆), 1.91-1.80 (m, 3 H, 3'''-H₆, 4'''-H), 1.45 (s, 9 H, C(CH₃)_₃).

[^13]C NMR (126 MHz, CDCl₃): δ = 174.8, 170.8, 169.8, 168.5 (C-1, C-1', C-1'', C-1''''), 156.5 (NC(=O)O), 136.7, 136.5, 135.5 (3 x C₆H₅), 129.2, 128.8, 128.6, 128.4, 128.2, 128.0, 127.8, 126.7 (15 x HC₆H₅), 81.5 (C(CH₃)_₃), 67.1 (Bn-CH₂), 66.3 (C-2''), 55.6 (C-3'), 53.6 (C-5'''), 52.6 (C-2'), 49.1, 48.6 (2 x Bn-CH₂), 44.4 (C-2), 41.6 (C-2''), 30.1 (C-3'''), 28.2 (C(CH₃)_₃), 23.9 (C-4''').

IR (ATR): ν = 1721, 1645, 1496, 1452, 1367, 1218, 1151, 732, 697.


Preparation of sarcosine tripeptide conjugate 37

To a solution of tripeptide 4 (24 mg, 0.040 mmol) in CHCl₃ (1.5 mL), sarcosine ethyl ester (9.4 mg, 0.080 mmol) was added. After stirring for 40 h at 65 °C the solvent was evaporated in vacuo. The resultant
crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt:MeOH, 90:10:0 → 99:0:1) to give 28 mg (0.039 mmol, 98%) of the title compound as a colourless solid.

**TLC:** Rf = 0.25 (DCM:AcOEt, 80:20).

**Melting point:** Tmp = 104 °C.

**Specific rotation:** [α]D<sup>20</sup> = -63.3 (c = 2.46, CHCl<sub>3</sub>)

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>): δ = 8.32 (d, J = 8.8 Hz, 1 H, 2''-NH), 7.31-7.08 (m, 16 H, 2'-NH, 15 x HC<sub>Ar</sub>), 5.40 (d, J = 8.8 Hz, 1 H, 2'-NH), 5.06 (d, J = 12.3 Hz, 1 H, Bn-CH<sub>2</sub>H<sub>5</sub>), 5.02 (d, J = 12.3 Hz, 1 H, Bn-CH<sub>2</sub>H<sub>5</sub>), 4.81-4.76 (m, 2 H, 2''-H, Bn-CH<sub>2</sub>H<sub>5</sub>), 4.56 (d, J = 16.4 Hz, 1 H, Bn-CH<sub>2</sub>H<sub>5</sub>), 4.38 (d, J = 16.4 Hz, 1 H, Bn-CH<sub>2</sub>H<sub>5</sub>), 4.27-4.23 (m, 1 H, 2'-H), 4.17 (d, J = 14.9 Hz, 1 H, Bn-CH<sub>2</sub>H<sub>5</sub>), 4.13-4.06 (m, 3 H, 2-H, Et-CH<sub>2</sub>), 3.50 (d, J = 17.3 Hz, 1 H, 2''-H<sub>a</sub>), 3.25 (d, J = 17.3 Hz, 1 H, 2''-H<sub>b</sub>), 2.88 (dd, J = 12.6, 4.5 Hz, 1 H, 3'-H<sub>a</sub>), 2.56 (dd, J = 12.6, 9.3 Hz, 1 H, 3'-H<sub>b</sub>), 2.43 (s, 3 H, NCH<sub>3</sub>), 2.12-2.02 (m, 2 H, 3-H, 3''-H), 1.19 (dd, J = 7.2, 7.1 Hz, 3 H, Et-CH<sub>2</sub>), 0.92-0.84 (m, 12 H, 4-H, 4''-H).

**<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>): δ = 172.1, 171.5, 171.3, 171.0 (C-1, C-1', C-1'', C-1'''), 156.4 (NC(=O)O), 137.2, 136.5, 136.3 (3 x C<sub>Ar</sub>), 129.0, 128.7, 128.6, 128.3, 128.2, 128.2, 128.0, 127.6, 127.4 (15 x HC<sub>Ar</sub>), 67.1 (Bn-CH<sub>2</sub>), 60.8 (Et-CH<sub>2</sub>), 60.2 (C-2), 58.3, 58.2 (C-2'', C-3'), 54.9 (C-2'''), 50.6 (C-2'), 50.0, 47.9 (2 x Bn-CH<sub>2</sub>), 42.7 (NCH<sub>3</sub>), 31.7, 30.9 (C-3, C-3''), 20.0, 19.3, 17.7, 17.3 (2 x C-4, 2 x C-4''), 14.4 (Et-CH<sub>3</sub>).

**IR** (ATR): ν = 2983, 2963, 1720, 1630, 1532, 1448, 1235, 1027, 697.

**MS** (ESI<sup>+</sup>): m/z = 716.4 [M+H]<sup>+</sup>, calculated: 716.4018 [M+H]<sup>+</sup>,

C<sub>40</sub>H<sub>53</sub>N<sub>5</sub>O<sub>7</sub> (715.89 g/mol)<sup>1</sup>, found: 716.3996 [-3.1 ppm] (ESI<sup>+</sup>-HRMS).

Preparation of sarcosine tripeptide conjugate 38
To a solution of tripeptide 5 (22 mg, 0.040 mmol) in CHCl<sub>3</sub> (1.5 mL), sarcosine ethyl ester (9.4 mg, 0.080 mmol) was added. After stirring for 40 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt:MeOH, 70:30:0 → 97:0:3) to give 25 mg (0.037 mmol, 93%) of the title compound as a colourless solid.

**TLC:** Rf = 0.31 (DCM:AcOEt, 50:50).

**Melting point:** Tmp = 106 °C.

**Specific rotation:** [α]D<sup>20</sup> = -17.2 (c = 2.19, CHCl<sub>3</sub>)

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>): δ = 8.30 (d, J = 8.8 Hz, 1 H, 2''-NH), 7.31-7.08 (m, 16 H, 2'-NH, 15 x HC<sub>Ar</sub>), 5.45 (s, 1 H, 2'-NH), 5.05 (s, 2 H, Bn-CH<sub>2</sub>), 4.81 (d, J = 15.5 Hz, 1 H, Bn-CH<sub>2</sub>H<sub>5</sub>), 4.78 (dd, J = 8.8, 5.9 Hz, 1 H, 2''-H), 4.56 (d, J = 16.4 Hz, 1 H, Bn-CH<sub>2</sub>H<sub>5</sub>), 4.35 (d, J = 16.4 Hz, 1 H, Bn-CH<sub>2</sub>H<sub>5</sub>), 4.28 (dd, J = 9.3, 5.0, 4.8 Hz, 1 H, 2'-H), 4.14 (d, J = 15.5 Hz, 1 H, Bn-CH<sub>2</sub>H<sub>5</sub>), 4.12-4.07 (m, 2 H, Et-CH<sub>2</sub>), 3.92-3.82 (m, 2 H, 2-H), 3.49 (d, J = 17.3 Hz, 1 H, 2''-H<sub>a</sub>), 3.25 (d, J = 17.3 Hz, 1 H, 2''-H<sub>b</sub>), 2.88 (dd, J = 12.6, 4.8 Hz, 1 H, 3'-H<sub>a</sub>), 2.57 (dd, J = 12.6, 9.3 Hz, 1 H, 3'-H<sub>b</sub>), 2.43 (s, 3 H, NCH<sub>3</sub>), 2.05 (dq, J = 6.7, 6.7,
5.9 Hz, 1 H, 3”-H), 1.19 (dd, J = 7.2, 7.2 Hz, 3 H, Et-CH₃), 0.90 (d, J = 6.7 Hz, 3 H, 4”-H₆), 0.84 (d, J = 6.7 Hz, 3 H, 4”-H₅).

**1³C NMR** (126 MHz, CDCl₃): δ = 172.0, 171.3, 171.1, 169.1 (C-1, C-1’, C-1”, C-1’’), 156.5 (NC(=O)=O), 137.1, 136.4, 136.3 (3 x C₅R), 129.0, 128.8, 128.6, 128.3, 128.2, 128.0, 127.6, 127.4 (15 x HC₅R), 67.2 (Bn-CH₂), 60.9 (Et-CH₂), 58.4, 58.2 (C-2”, C-3’), 54.9 (C-2’), 50.7 (C-2’’), 50.0, 47.9 (2 x Bn-CH₂), 44.4 (C-2), 42.7 (NCH₃), 30.9 (C-3’’), 20.0, 17.2 (2 x C-4’), 14.4 (Et-CH₃).

**IR** (ATR): ν = 3270, 1720, 1631, 1507, 1443, 1220, 1040, 733, 701.


C₁₉H₂₁N₂O₇ (673.81 g/mol⁻¹), found: 674.3524 [-3.6 ppm] (ESI⁺-HRMS).

Preparation of sarcosine tripeptide conjugate 39

To a solution of tripeptide 6 (22 mg, 0.040 mmol) in CHCl₃ (1.5 mL), sarcosine ethyl ester (9.4 mg, 0.080 mmol) was added. After stirring for 40 h at 65°C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt:MeOH, 80:20:0 → 98:0:2) to give 26 mg (0.039 mmol, 98%) of the title compound as a colourless solid.

**TLC**: Rᵣ = 0.37 (DCM:AcOEt, 70:30).

**Melting point**: Tₘₚ = 59°C.

**Specific rotation**: [α]D20 = +13.6 (c = 2.09, CHCl₃)

**¹H NMR** (500 MHz, CDCl₃): δ = 8.30 (dd, J = 4.7, 4.4 Hz, 1 H, 2”-NH), 7.30-7.07 (m, 16 H, 2’-NH, 15 x HC₅R), 5.41 (d, J = 8.7 Hz, 1 H, 2-NH), 5.05 (d, J = 12.3 Hz, 1 H, Bn-CH₆), 5.02 (d, J = 12.3 Hz, 1 H, Bn-CH₆), 4.54 (d, J = 14.9 Hz, 1 H, Bn-CH₆), 4.50 (d, J = 14.9 Hz, 1 H, Bn-CH₆), 4.32-4.21 (m, 4 H, 2’-H, 2”-H₆, Bn-CH₂), 4.12-4.06 (m, 3 H, 2-H, Et-CH₂), 4.02 (dd, J = 17.2, 4.4 Hz, 1 H, 2”-H₆), 3.36 (d, J = 17.3 Hz, 1 H, 2”-H₆), 3.31 (d, J = 17.3 Hz, 1 H, 2”-H₆), 2.89 (dd, J = 12.7, 5.3 Hz, 1 H, 3’-H₆), 2.65 (dd, J = 12.7, 8.6 Hz, 1 H, 3’-H₆), 2.41 (s, 3 H, NCH₃), 2.12 (dqq, J = 6.7, 6.6, 5.9 Hz, 1 H, 3-H), 1.18 (dd, J = 7.2, 7.2 Hz, 3 H, Et-CH₃), 0.92 (d, J = 6.6 Hz, 3 H, 4-H₆), 0.85 (d, J = 6.7 Hz, 3 H, 4-H₆).

**¹³C NMR** (126 MHz, CDCl₃): δ = 171.7, 171.7, 171.1, 168.6 (C-1, C-1’, C-1”, C-1’’), 156.5 (NC(=O)=O), 136.7, 136.5, 135.6 (3 x C₅R), 129.0, 128.8, 128.6, 128.4, 128.2, 128.2, 128.0, 127.7, 126.6 (15 x HC₅R), 67.1 (Bn-CH₂), 60.9 (Et-CH₂), 60.2 (C-2), 58.9 (C-2”), 58.3 (C-3’), 51.1 (C-2’), 49.0, 48.5 (2 x Bn-CH₂), 42.5 (NCH₃), 41.7 (C-2’’), 31.6 (C-3’), 19.3, 17.6 (2 x C-4’), 14.3 (Et-CH₃).

**IR** (ATR): ν = 3289, 1719, 1631, 1520, 1452, 1222, 1027, 734, 697.


C₁₉H₂₁N₂O₇ (673.81 g/mol⁻¹), found: 674.3524 [-3.6 ppm] (ESI⁺-HRMS).
Preparation of sarcosine tripeptide conjugate 40

To a solution of tripeptide 7 (21 mg, 0.040 mmol) in CHCl₃ (1.5 mL), sarcosine ethyl ester (9.4 mg, 0.080 mmol) was added. After stirring for 40 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt:MeOH, 70:30:0 → 97:0:3) to give 24 mg (0.038 mmol, 95%) of the title compound as a colourless solid.

**TLC:** R<sub>f</sub> = 0.12 (DCM:AcOEt, 50:50).

**Melting point:** T<sub>mp</sub> = 62 °C.

**Specific rotation:** [α]D<sub>20</sub> = +5.7 (c = 1.86, CHCl₃)

**¹H NMR** (500 MHz, CDCl₃): δ = 8.24 (dd, J = 5.0, 4.3 Hz, 1 H, 2''-NH), 7.35 (d, J = 5.2 Hz, 1 H, 2'-NH), 7.31-7.07 (m, 15 H, 15 x HC₆H₅), 5.49 (dd, J = 5.7, 5.2 Hz, 1 H, 2'-NH), 5.05 (s, 2 H, Bn-CH₂), 4.54 (d, J = 14.9 Hz, 1 H, Bn-CH₂H₅b), 4.50 (d, J = 14.9 Hz, 1 H, Bn-CH₂H₅b), 4.34-4.28 (m, 3 H, 2'-H, Bn-CH₂), 4.20 (dd, J = 17.2, 5.0 Hz, 1 H, 2''-Hₕ), 4.10-4.05 (m, 2 H, Et-CH₂), 4.02 (dd, J = 17.2, 4.3 Hz, 1 H, 2''-Hₕ), 3.91-3.87 (m, 2 H, 2'-H), 3.34 (d, J = 17.4 Hz, 1 H, 2''-Hₕ), 3.30 (d, J = 17.4 Hz, 1 H, 2''-Hₕ), 2.89 (dd, J = 12.7, 5.9 Hz, 1 H, 3''-Hₕ), 2.66 (dd, J = 12.7, 8.4 Hz, 1 H, 3''-Hₕ), 2.39 (s, 3 H, NCH₃), 1.18 (dd, J = 7.2, 7.2 Hz, 3 H, Et-CH₃).

**¹³C NMR** (126 MHz, CDCl₃): δ = 171.8, 171.1, 169.4, 168.6 (C-1, C-1', C-1'', C-1'''), 156.6 (NC(=O)O), 136.6, 136.4, 135.5 (3 x C₆H₅), 129.2, 128.8, 128.6, 128.4, 128.2, 128.0, 127.8, 126.6 (15 x HC₆H₅), 67.2 (Bn-CH₂), 61.0 (Et-CH₂), 58.9 (C-2'''), 58.3 (C-3'), 51.2 (C-2'), 49.1, 48.6 (2 x Bn-CH₂), 44.5 (C-2), 42.6 (NCH₃), 41.6 (C-2''), 14.3 (Et-CH₃).

**IR (ATR):** ν = 3290, 1717, 1630, 1521, 1452, 1217, 1028, 735, 696.

**MS (ESI⁺):** m/z = 632.3 [M+H]⁺, calculated: 632.3079 [M+H]⁺, found: 632.3058 [-3.3 ppm] (ESI⁺-HRMS).

Preparation of tert-leucine tripeptide conjugate 41

To a solution of tripeptide 4 (24 mg, 0.040 mmol) in CHCl₃ (1.5 mL), L-tert-leucine methyl ester (12 mg, 0.080 mmol) was added. After stirring for 20 h at 40 °C and for 70 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt, 80:20) to give 23 mg (0.031 mmol, 78%) of the title compound as a colourless solid.

**TLC:** R<sub>f</sub> = 0.32 (DCM:AcOEt, 80:20).

**Melting point:** T<sub>mp</sub> = 118 °C.

**Specific rotation:** [α]D<sub>20</sub> = -37.0 (c = 1.82, CHCl₃)

S28
$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.56$ (d, $J = 9.0$ Hz, 1 H, 2'-NH), 7.31-7.08 (m, 15 H, 15 x H$^1$C$_{ch}$), 6.90 (d, $J = 6.6$ Hz, 1 H, 2'-NH), 5.42 (d, $J = 7.4$ Hz, 1 H, 2-NH), 5.05 (d, $J = 12.2$ Hz, 1 H, Bn-CH$_2$H$_3$), 5.00 (d, $J = 12.2$ Hz, 1 H, Bn-CH$_2$H$_3$), 4.81 (dd, $J = 9.0$, 6.6 Hz, 1 H, 2'-H), 4.75 (d, $J = 14.7$ Hz, 1 H, Bn-CH$_2$H$_3$), 4.53 (d, $J = 16.5$ Hz, 1 H, Bn-CH$_2$H$_3$), 4.38 (d, $J = 16.5$ Hz, 1 H, Bn-CH$_2$H$_3$), 4.36-4.30 (m, 1 H, 2'-H), 4.20 (d, $J = 14.7$ Hz, 1 H, Bn-CH$_2$H$_3$), 4.00 (dd, $J = 7.4$, 6.2 Hz, 1 H, 2-H), 3.63 (s, 3 H, OCH$_3$), 2.98 (dd, $J = 11.9$, 2.7 Hz, 1 H, 3'-H), 2.86 (s, 1 H, 2''-H), 2.45 (dd, $J = 11.9$, 7.0 Hz, 1 H, 3''-H), 2.13-2.01 (m, 2 H, 3-H, 3''-H), 0.91-0.81 (m, 21 H, 4-H, 4''-H, 4'''-H). The signal attributed to the secondary amine NH proton was not observed in the $^1$H NMR.

$^{13}$C NMR (101 MHz, CDCl$_3$): $\delta = 175.2$, 171.9, 171.2, 170.5 (C-1, C-1', C-1'', C-1'''), 156.5 (NC(=O)O), 137.2, 136.4, 136.3 (3 x C$_{ar}$), 129.0, 128.8, 128.6, 128.4, 128.3, 128.2, 127.9, 127.6, 127.3 (15 x H$^1$C$_{ch}$), 71.2 (C-2''), 67.2 (Bn-CH$_2$), 60.5 (C-2), 54.6 (C-2''), 52.7 (C-2''), 51.5 (OCH$_3$), 50.2 (C-3''), 50.0, 48.0 (2 x Bn-CH$_2$), 34.3 (C-3''), 31.4 (C-3, C-3''), 27.0 (3 x C-4''), 19.9, 19.3, 17.8, 17.7 (2 x C-4, 2 x C-4'').

IR (ATR): $\nu = 2962$, 1731, 1630, 1528, 1448, 1217, 1155, 1028, 697.


Preparation of tert-leucine tripeptide conjugate 42

To a solution of tripeptide 6 (22 mg, 0.040 mmol) in CHCl$_3$ (1.5 mL), 1-tert-leucine methyl ester (12 mg, 0.080 mmol) was added. After stirring for 20 h at 40 °C and for 70 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt, 70:30) to give 21 mg (0.030 mmol, 75%) of the title compound as a colourless solid.

TLC: $Rf = 0.31$ (DCM:AcOEt, 70:30).

Melting point: $T_{mp} = 156$ °C.

Specific rotation: $[\alpha]_D^{20} = -5.8$ (c = 1.65, CHCl$_3$)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.71$ (dd, $J = 4.4$, 4.3 Hz, 1 H, 2''-NH), 7.30-7.05 (m, 15 H, 15 x H$^1$C$_{ch}$), 6.98 (d, $J = 6.7$ Hz, 1 H, 2'-NH), 5.36 (d, $J = 7.8$ Hz, 1 H, 2-NH), 5.05 (d, $J = 12.2$ Hz, 1 H, Bn-CH$_2$H$_3$), 5.01 (d, $J = 12.2$ Hz, 1 H, Bn-CH$_2$H$_3$), 4.52 (s, 2 H, Bn-CH$_2$), 4.41-4.35 (m, 1 H, 2'-H), 4.31 (s, 2 H, Bn-CH$_2$), 4.11-4.00 (m, 3 H, 2-H, 2''-H), 3.63 (s, 3 H, OCH$_3$), 3.05 (dd, $J = 11.8$, 3.2 Hz, 1 H, 3'-H), 2.84 (s, 1 H, 2''-H), 2.48 (dd, $J = 11.8$, 6.9 Hz, 1 H, 3''-H), 2.11 (dq, $J = 6.8$, 6.7, 5.8 Hz, 1 H, 3-H), 0.92-0.85 (m, 15 H, 4-H, 4''-H). The signal attributed to the secondary amine NH proton was not observed in the $^1$H NMR.

$^{13}$C NMR (101 MHz, CDCl$_3$): $\delta = 174.9$, 171.3, 170.8, 168.4 (C-1, C-1', C-1'', C-1'''), 156.6 (NC(=O)O), 136.7, 136.4, 135.6 (3 x C$_{ar}$), 129.2, 128.8, 128.7, 128.5, 128.3, 128.2, 128.0, 127.8, 126.6 (15 x H$^1$C$_{ch}$), 71.3 (C-2''), 67.2 (Bn-CH$_2$), 60.5 (C-2), 52.6 (C-2''), 51.5 (OCH$_3$), 50.1 (C-3), 49.1, 48.7 (2 x Bn-CH$_2$), 41.6 (C-2''), 34.3 (C-3''), 31.3 (C-3), 26.9 (3 x C-4''), 19.3, 17.8 (2 x C-4).
 Prepare the alanine tripeptide conjugate 43

To a solution of tripeptide 4 (24 mg, 0.040 mmol) in CHCl₃ (1.5 mL), alanine tert-butyl ester (12 mg, 0.080 mmol) was added. After stirring for 48 h at 40 °C and 20 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt, 70:30 → 60:40) to give 28 mg (0.038 mmol, 95%) of the title compound as a colourless solid.

**TLC:** \( R_f = 0.20 \) (DCM:AcOEt, 70:30).

**Melting point:** \( T_{mp} = 66 ^\circ C \).

**Specific rotation:** \( [\alpha]_D^{20} = -29.6 \) (c = 2.33, CHCl₃)

**1H NMR** (500 MHz, CDCl₃): \( \delta = 8.28 \) (d, \( J = 8.9 \) Hz, 1 H, 2''-NH), 7.31-7.08 (m, 15 H, 15 x HCAr), 6.96 (d, \( J = 6.2 \) Hz, 1 H, 2'-NH), 5.40 (d, \( J = 8.6 \) Hz, 1 H, 2-NH), 5.05 (d, \( J = 12.2 \) Hz, 1 H, Bn-CH₂H₂), 5.01 (d, \( J = 12.2 \) Hz, 1 H, Bn-CH₂H₂), 4.83-4.78 (m, 2 H, 2''-H, Bn-CH₂H₂), 4.55 (d, \( J = 16.5 \) Hz, 1 H, Bn-CH₂H₂), 4.43 (d, \( J = 16.5 \) Hz, 1 H, Bn-CH₂H₂), 4.30-4.25 (m, 1 H, 2'-H), 4.17 (d, \( J = 14.8 \) Hz, 1 H, Bn-CH₂H₂), 4.04 (dd, \( J = 8.6 \), 5.5 Hz, 1 H, 2-H), 3.20 (q, \( J = 7.0 \) Hz, 1 H, 2''-H), 3.11 (dd, \( J = 12.0 \), 3.6 Hz, 1 H, 3''-H), 2.48 (dd, \( J = 12.0 \), 9.0 Hz, 1 H, 3''-H), 1.39 (s, 9 H, C(CH₃)₃), 1.24 (d, \( J = 7.0 \) Hz, 3 H, 3''-H), 0.91-0.83 (m, 12 H, 4-H, 4''-H). The signal attributed to the secondary amine NH proton was not observed in the 1H NMR.

**13C NMR** (126 MHz, CDCl₃): \( \delta = 175.2, 172.1, 171.2, 170.9 \) (C-1, C-1', C-1'', C-1''''), 156.5 (NC(=O)=O), 137.1, 136.5, 136.2 (3 x CAr), 129.0, 128.8, 128.6, 128.4, 128.2, 128.0, 127.6, 127.3 (15 x HCAr), 81.4 (C(CH₃)₃), 67.1 (Bn-CH₂), 60.3 (C-2), 57.7 (C-2'''), 54.7 (C-2''), 52.3 (C-2'), 50.0 (Bn-CH₂), 49.5 (C-3'), 47.9 (Bn-CH₂), 31.6, 31.2 (C-3, C-3'''), 28.2 (C(CH₃)₃), 20.0, 19.4, 19.4, 17.7, 17.4 (C-3''', 2 x C-4, 2 x C-4'').

**IR** (ATR): \( \nu = 3291, 1726, 1632, 1530, 1449, 1216, 1149, 1028, 698 \).

**MS** (ESI⁺): \( m/z = 744.4 \) [M+H]⁺, calculated: 744.4331 [M+Na]⁺, found: 744.4324 [-0.9 ppm] (ESI⁺-HRMS).

Prepare the alanine tripeptide conjugate 44

To a solution of tripeptide 6 (22 mg, 0.040 mmol) in CHCl₃ (1.5 mL), alanine tert-butyl ester (12 mg, 0.080 mmol) was added. After stirring for 48 h at 40 °C and 20 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt, 70:30 → 60:40) to give 21 mg (0.030 mmol, 75%) of the title compound as a
colourless solid.

**TLC:** $R_t = 0.18$ (DCM:AcOEt, 60:40).

**Melting point:** $T_{mp} = 57 ^\circ C$.

**Specific rotation:** $[\alpha]_{D}^{20} = -3.8$ (c = 1.58, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 8.07$ (s, 1 H, 2"-NH), 7.31-7.09 (m, 16 H, 2'-NH, 15 x HC$_{Ar}$), 5.40 (d, $J = 8.5$ Hz, 1 H, 2'-NH), 5.05 (d, $J = 12.2$ Hz, 1 H, BN-CH$_2$H$_b$), 5.01 (d, $J = 12.2$ Hz, 1 H, BN-CH$_2$H$_b$), 4.56 (d, $J = 14.8$ Hz, 1 H, BN-CH$_2$H$_b$), 4.51 (d, $J = 14.8$ Hz, 1 H, BN-CH$_2$H$_b$), 4.36-4.30 (m, 3 H, 2'-H, BN-CH$_2$H$_b$), 4.16 (dd, $J = 17.4$, 4.7 Hz, 1 H, 2''-H$_a$), 4.09-4.04 (m, 2 H, 2'-H, 2''-H$_b$), 3.19-3.14 (m, 2 H, 2''-H, 3'-H$_a$), 2.53 (dd, $J = 12.0$, 7.4 Hz, 1 H, 3'-H$_b$), 2.11 (dq, $J = 6.7$, 6.7, 6.1 Hz, 1 H, 3'-H$_b$), 1.38 (s, 9 H, C(CH$_3$)$_3$), 1.24 (d, $J = 7.0$ Hz, 3 H, 3''-H$_b$), 0.92 (d, $J = 6.7$ Hz, 3 H, 4-H$_a$), 0.86 (d, $J = 6.7$ Hz, 3 H, 4-H$_b$). The signal attributed to the secondary amine NH proton was not observed in the $^1$H NMR.

$^{13}$C NMR (126 MHz, CDCl$_3$): $\delta = 174.9$, 171.4, 170.8, 168.4 (C-1, C-1', C-1'', C-1''''), 156.6 (NC(=O)O), 136.7, 136.4, 135.5 (3 x C$_{Ar}$), 129.2, 128.8, 128.6, 128.5, 128.3, 128.2, 128.1, 127.8, 126.6 (15 x HC$_{Ar}$), 81.4 (C(CH$_3$)$_3$), 67.2 (BN-CH$_2$), 60.4 (C-2), 57.7 (C-2''), 52.6 (C-2'), 49.1 (BN-CH$_2$), 49.0 (C-3'), 48.6 (BN-CH$_2$), 41.7 (C-2''), 31.5 (C-3), 28.2 (C(CH$_3$)$_3$), 19.4, 19.2 (C-3'', C$_{e-4}$), 17.7 (C$_{e-4}$).

**IR** (ATR): $\nu = 3317$, 1638, 1519, 1453, 1240, 1151, 1043, 744, 697.

**MS** (ESI$^+$): m/z = 702.4 [M+H]$^+$, calculated: 702.3861 [M+H]$^+$, found: 702.3855 [-0.9 ppm] (ESI$^+$-HRMS).

Preparation of glycine tripeptide conjugate 45

To a solution of tripeptide 4 (24 mg, 0.040 mmol) in CHCl$_3$ (1.5 mL), glycine tert-butyl ester (10 mg, 0.080 mmol) was added. After stirring for 40 h at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 6.0 cm, DCM:AcOEt, 80:20 → 50:50) to give 26 mg (0.036 mmol, 90%) of the title compound as a

colourless solid.

**TLC:** $R_t = 0.27$ (DCM:AcOEt, 60:40).

**Melting point:** $T_{mp} = 84 ^\circ C$.

**Specific rotation:** $[\alpha]_{D}^{20} = -37.2$ (c = 1.89, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 8.14$ (d, $J = 9.0$ Hz, 1 H, 2''-NH), 7.31-7.09 (m, 16 H, 2'-NH, 15 x HC$_{Ar}$), 5.46 (d, $J = 8.8$ Hz, 1 H, 2'-NH), 5.05 (d, $J = 12.4$ Hz, 1 H, BN-CH$_2$H$_b$), 5.02 (d, $J = 12.4$ Hz, 1 H, BN-CH$_2$H$_b$), 4.84-4.71 (m, 2 H, 2''-H, BN-CH$_2$H$_b$), 4.55 (d, $J = 17.5$ Hz, 1 H, BN-CH$_2$H$_b$), 4.34-4.28 (m, 2 H, 2'-H, BN-CH$_2$H$_b$), 4.13 (d, $J = 14.9$ Hz, 1 H, BN-CH$_2$H$_b$), 4.10 (dd, $J = 9.0$, 5.7 Hz, 1 H, 2'H), 3.37 (d, $J = 17.7$ Hz, 1 H, 2''-H$_a$), 3.27 (d, $J = 17.7$ Hz, 1 H, 2''-H$_b$), 3.13 (dd, $J = 12.5$, 3.3 Hz, 1 H, 3'-H$_a$), 2.55 (dd, $J = 12.5$, 7.9 Hz, 1 H, 3'-H$_b$), 2.14-2.01 (m, 2 H, 3-H, 3''-H$_b$), 1.39 (s, 9 H, C(CH$_3$)$_3$), 0.92 (d, $J = 6.8$ Hz, 3 H), 0.87 (d, $J = 6.8$ Hz, 3 H), 0.85 (d, $J = 6.8$ Hz, 3 H), 0.84 (d, $J = 6.7$ Hz, 3 H) (4-H$_a$, 4-H$_b$, 4-H$_c$, 4-H$_d$).
Preparation of dipeptide 47

To a solution of N-(tert-Butoxycarbonyl)-L-leucine (1.29 g, 5.58 mmol) in abs. DMF (10 mL), HOBt (754 mg, 5.58 mmol) was
added. EDAC (1.07 g, 5.58 mmol) was added after cooling the solution to 0 °C. After stirring for 5 min at 0 °C, NEt₃ (0.78 mL, 5.6 mmol) was added. L-Alanine dibenzylamide[86] (1.50 g, 5.58 mmol) in abs. DMF (5 mL) was added after additional 15 min of stirring at 0 °C. After stirring for 15 h and concomitantly slowly warming to rt the reaction mixture was diluted with AcOEt (200 mL) and washed with water (2 x 200 mL), 10 wt% aqueous citric acid solution (200 mL) and sat. aqueous NaHCO₃ solution (200 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resulting dipeptide was used in the subsequent transformation without further purification.

The crude product (calculated maximal amount of substance: 5.58 mmol) was dissolved in AcOEt (30 mL) and cooled to 0 °C. MeOH (4.53 mL, 112 mmol) and AcCl (3.98 mL, 55.8 mmol) were added at 0 °C. After stirring for 1 h at 0 °C and 4 h at rt the reaction mixture was diluted with AcOEt (150 mL) and washed with 1 M aqueous NaOH solution (150 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (150 g, 4.5 x 18 cm, DCM:MeOH, 95:5) to give 2.08 g (5.45 mmol, 98%) of the title compound as a colourless oil.

TLC: Rᵣ = 0.22 (DCM:MeOH, 93:7).

Specific rotation: [α]D²⁰ = -74.0 (c = 2.51, CHCl₃)

¹H NMR (400 MHz, CDCl₃): δ = 8.02 (d, J = 8.0 Hz, 1 H, 2-NH²), 7.41-7.19 (m, 10 H, 10 x CHAr), 5.04 (dq, J = 8.0, 6.8 Hz, 1 H, 2-H), 4.87 (d, J = 14.8 Hz, 1 H, Bn-CH₂Bn), 4.61 (d, J = 16.7 Hz, 1 H, Bn-CH₂Bn), 4.50 (d, J = 16.7 Hz, 1 H, Bn-CH₂Bn), 4.34 (d, J = 14.8 Hz, 1 H, Bn-CH₂Bn), 3.36 (dd, J = 9.8, 4.0 Hz, 1 H, 2'-H), 1.79-1.66 (m, 2 H, 3'-H₂, 4'-H), 1.43-1.36 (m, 4 H, 3-H, 3'-H₂), 0.99 (dd, J = 6.4 Hz, 3 H, 5'-H₂), 0.95 (d, J = 6.3 Hz, 3 H, 5'-H₂). The signal attributed to the primary amine NH₂ protons was not observed in the ¹H NMR.

¹³C NMR (101 MHz, CDCl₃): δ = 175.2, 173.4 (C-1, C-1'), 136.9, 136.0 (2 x CAr), 129.1, 128.8, 128.2, 127.9, 127.6, 127.0 (10 x CHAr), 53.6 (C-2), 49.8, 48.1 (2 x Bn-CH₂), 45.1 (C-2), 44.1 (C-3'), 24.9 (C-4'), 23.6, 21.5 (2 x C-5'), 19.2 (C-3).

IR (ATR): ν = 2954, 1637, 1495, 1451, 1365, 1220, 1079, 732, 698.

MS (ESI⁺): m/z = 382.3 [M+H]⁺, calculated: 382.2489 [M+H]⁺,
C₂₃H₃₃N₅O₂ (381.52 g/mol)¹, found: 382.2489 [-0.1 ppm] (ESI⁺-HRMS).

Preparation of dipeptide tripeptide conjugate 48

To a solution of tripeptide 5 (22 mg, 0.040 mmol) in CHCl₃ (1.5 mL), dipeptide 47 (23 mg, 0.060 mmol) was added. After stirring for 4 d at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt, 70:30 → 50:50) to give 28 mg (0.030 mmol, 75%) of the title compound as a colourless solid.

TLC: Rᵣ = 0.20 (DCM:AcOEt, 50:50).

Melting point: Tₘₚ = 65 °C.
Specific rotation: $[\alpha]_D^{20} = -38.3$ (c = 2.10, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 8.09$ (d, $J = 8.6$ Hz, 1 H, 2"-NH), 7.98 (d, $J = 7.4$ Hz, 1 H, 2'-NH), 7.49 (dd, $J = 4.3$, 4.0 Hz, 1 H, 2"-NH), 7.34-7.02 (m, 25 H, 25 x HC$_{Ar}$), 5.80 (d, $J = 9.0$ Hz, 1 H, 2'-NH), 5.06 (dd, $J = 8.6$, 6.9 Hz, 1 H, 2"'-H), 5.03 (d, $J = 12.3$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.96 (d, $J = 12.3$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.78 (d, $J = 14.9$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.58 (dd, $J = 10.2$, 7.4, 3.4 Hz, 1 H, 2'-H), 4.54 (d, $J = 16.5$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.52 (d, $J = 14.7$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.44 (d, $J = 14.7$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.38 (d, $J = 16.5$ Hz, 1 H, Bn-CH$_2$H$_b$), 4.28 (s, 2 H, Bn-CH$_2$).

To a solution of dipeptide (256 mg, 1.18 mmol) in abs. DMF (3 mL) was added. Dipeptide was not observed in the subsequent transformation without further purification. Preparation of tripeptide 49

To a solution of N-((tert-Butoxycarbonyl)-t-valine (256 mg, 1.18 mmol) in abs. DMF (5 mL), HOBr (159 mg, 1.18 mmol) was added. EDAC (226 mg, 1.18 mmol) was added after cooling the solution to 0°C. After stirring for 5 min at 0°C NEt$_3$ (0.17 mL, 1.2 mmol) was added. Dipeptide 47 (450 mg, 1.18 mmol) in abs. DMF (3 mL) was added after additional 15 min of stirring at 0°C. After stirring for 15 h and concomitantly slowly warming to rt the reaction mixture was diluted with AcOEt (150 mL) and washed with water (2 x 100 mL), 10 wt% aqueous citric acid solution (200 mL) and sat. aqueous NaHCO$_3$ solution (200 mL). The organic layer was dried over Na$_2$SO$_4$, filtered and the solvent of the filtrate evaporated in vacuo. The resulting tripeptide was used in the subsequent transformation without further purification.

AcOEt (6 mL) was added to the crude product (calculated maximal amount of substance: 1.18 mmol) and the resulting suspension was cooled to 0°C. MeOH (0.955 mL, 23.6 mmol) and AcCl (0.842 mL, 11.8 mmol) were added at 0°C. After stirring for 1 h at 0°C 3 h at rt the reaction mixture was diluted with AcOEt (150 mL) and washed with 1 M aqueous
NaOH solution (100 mL). The organic layer was dried over Na₂SO₄, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (100 g, 4.5 x 12 cm, DCM:MeOH, 95:5 → 90:10) to give 360 mg (0.749 mmol, 63%) of the title compound as a colourless solid.

**TLC:** Rᵣ = 0.22 (DCM:MeOH, 93:7).

**Melting point:** T_{mp} = 157 °C.

**Specific rotation:** [α]ᵣ²⁰ = -83.3 (c = 3.12, CHCl₃)

**¹H NMR** (400 MHz, CDCl₃): δ = 7.65 (d, J = 8.5 Hz, 1 H, 2'-NH), 7.30-7.06 (m, 11 H, 2-NH, 10 x HC₅Ar), 4.89 (dq, J = 7.2, 6.9 Hz, 1 H, 2-H), 4.68 (d, J = 14.9 Hz, 1 H, Bn-CH₂H₅), 4.47-4.38 (m, 3 H, 2'-H, Bn-CH₂), 4.31 (d, J = 14.9 Hz, 1 H, Bn-CH₂H₅), 3.21 (d, J = 3.8 Hz, 1 H, 2''-H), 2.27-2.20 (m, 1 H, 3''-H), 1.28-1.26 (m, 3 H, 3'-H, 4'-H), 1.27 (d, J = 6.9 Hz, 3 H, 3-H), 0.91 (d, J = 7.0 Hz, 3 H, 4''-H₅), 0.88 (d, J = 6.1 Hz, 3 H, 5''-H), 0.85 (d, J = 6.1 Hz, 3 H, 5''-H₅), 0.75 (d, J = 6.9 Hz, 3 H, 4''-H₅). The signal attributed to the primary amine NH₂ protons was not observed in the ¹H NMR.

**¹³C NMR** (101 MHz, CDCl₃): δ = 174.6, 173.0, 171.5 (C-1, C-1', C-1''), 136.7, 135.9 (2 x C₅), 129.1, 128.8, 128.1, 128.0, 127.6, 126.9 (10 x HC₅Ar), 60.2 (C-2''), 51.4 (C-2'), 49.7, 48.1 (2 x Bn-CH₂), 45.6 (C-2), 41.3 (C-3'), 30.8 (C-3''), 24.9 (C-4'), 23.2 (C₉-5'), 21.9 (C₉-5''), 19.8 (C₉-4''), 19.1 (C-3), 16.2 (C₉-4'').

**IR** (ATR): ν = 3267, 1637, 1540, 1428, 1221, 1078, 753, 719, 695.

**MS (ESI⁺):** m/z = 481.3 [M+H]⁺; calculated: 481.3173 [M+H]⁺, found: 481.3171 [-0.5 ppm] (ESI⁺-HRMS).

**Preparation of tripeptide tripeptide conjugate 50**

To a solution of tripeptide 5 (33 mg, 0.060 mmol) in CHCl₃ (2.0 mL), tripeptide 49 (43 mg, 0.090 mmol) was added. After stirring for 4 d at 65 °C, the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt, 50:50 → 30:70) to give 51 mg (0.049 mmol, 82%) of the title compound as a colourless solid.

**TLC:** Rᵣ = 0.20 (DCM:AcOEt, 30:70).

**Melting point:** T_{mp} = 163 °C.

**Specific rotation:** [α]ᵣ²⁰ = -29.4 (c = 2.52, CHCl₃)

**¹H NMR** (500 MHz, CDCl₃): δ = 7.84-7.81 (m, 2 H, 2''-NH, 2''-NH), 7.75 (d, J = 9.5 Hz, 1 H, 2''''-NH), 7.23-7.01 (m, 26 H, 2''''-NH, 25 x HC₅Ar), 5.55 (d, J = 8.7 Hz, 1 H, 2-NH), 5.02-5.00 (m, 2 H, 2''''-H, Bn-CH₂H₅), 4.95 (d, J = 12.3 Hz, 1 H, Bn-CH₂H₅), 4.70-4.59 (m, 3 H, 2''-H, 2''''-H, Bn-CH₂H₅), 4.48-4.35 (m, 4 H, 2 x Bn-CH₂), 4.31-4.23 (m, 4 H, 2''-H₅, Bn-CH₂H₅, Bn-CH₂), 4.07 (dd, J = 8.7, 6.6 Hz, 1 H, 2-H), 3.75 (dd, J = 17.2, 4.0 Hz, 1 H, 2''-H), 2.98 (dd, J = 11.8, 3.4 Hz, 1 H, 3''-H₅), 2.82 (d, J = 6.3 Hz, 1 H, 2''''-H), 2.72 (dd, J = 11.8, 8.3 Hz, 1 H, 3''-H₅), 2.02 (dqq, J = 6.7, 6.7, 6.6 Hz, 1 H, 3-H), 1.83 (dqq, J = 6.7, 6.7, 6.3 Hz, 1 H,
3''-H), 1.67-1.44 (m, 3 H, 3'''-H, 4'''-H), 1.25 (d, J = 6.7 Hz, 3 H, 3'''-H), 0.88-0.76 (m, 18 H, 4-H, 4'''-H, 5'''-H). The signal attributed to the secondary amine NH proton was not observed in the 1H NMR.

13C NMR (126 MHz, CDCl3): δ = 173.9, 173.3, 173.0, 172.3 170.5, 168.9 (C-1, C-1', C-1'', C-1''''), 156.5 (NC(=O)O), 136.5, 136.4, 136.3, 135.7, 135.4 (5 x CAr), 129.2, 129.1, 128.9, 128.8, 128.6, 128.2, 128.1, 128.0, 127.8, 127.7, 127.0, 126.6 (25 x HCAr), 70.2 (C-2''), 67.0 (Bn-CH2), 60.7 (C-2), 53.5 (C-2''''), 51.5 (C-2'''''), 50.4 (C-3''), 50.0, 49.1, 48.5, 48.2 (4 x Bn-CH2), 45.6 (C-2''''), 41.6 (C-3'''''), 41.4 (C-2'''), 31.7 (C-3''''), 31.2 (C-3), 25.0 (C-4'''''), 23.2, 21.5, 19.6, 19.4, 19.2, 18.0, 18.3 (C-3'''', 2 x C-4, 2 x C-4'''', 2 x C-5'''').

IR (ATR): ν = 3271, 2958, 1636, 1537, 1452, 1225, 1133, 1037.6 (m, 1037.5860 [M+H]+, calculated: 1037.5859 [M+H]+, found: 1037.5860 [+0.1 ppm] (ESI+HRMS).

Preparation of tripeptide 51

To a solution of tripeptide 6 (22 mg, 0.040 mmol) in CHCl3 (1.5 mL), MeNH2 (2 m in THF, 0.12 mL, 0.24 mmol) was added. After stirring for 20 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:MeOH, 96:4 → 94:6) to give 21 mg (0.036 mmol, 90%) of the title compound as a colourless solid.

TLC: Rf = 0.14 (DCM:MeOH, 93:7).

Melting point: Tmp = 98 °C.

Specific rotation: [α]D20 = +7.3 (c = 1.25, CHCl3)

1H NMR (400 MHz, CDCl3): δ = 8.37 (s, 1 H, 2''-NH), 7.30-7.05 (m, 16 H, 2''-NH, 15 x HCAr), 5.52 (d, J = 8.4 Hz, 1 H, 2'-NH), 5.03 (d, J = 12.3 Hz, 1 H, Bn-CH2Hb), 4.99 (d, J = 12.3 Hz, 1 H, Bn-CH2Hb), 4.57-4.46 (m, 3 H, 2'-H, Bn-CH2), 4.31 (s, 2 H, Bn-CH2), 4.17-4.03 (m, 3 H, 2'-H, 2''-H), 3.07 (dd, J = 11.9, 3.6 Hz, 1 H, 3''Hb), 2.67 (dd, J = 11.9, 7.8 Hz, 1 H, 3''Hb), 2.41 (s, 3 H, NCH3), 2.06 (dqq, J = 6.7, 6.7, 6.2 Hz, 1 H, 3-H), 0.90 (d, J = 6.7 Hz, 3 H, 4-Ha), 0.85 (d, J = 6.7 Hz, 3 H, 4-Hb). The signal attributed to the secondary amine NH proton was not observed in the 1H NMR.

13C NMR (101 MHz, CDCl3): δ = 171.6, 171.0, 168.7 (C-1, C-1', C-1''), 156.7 (NC(=O)O), 136.5, 136.4, 135.4 (3 x CAr), 129.2, 128.8, 128.6, 128.4, 128.2, 128.1, 127.8, 126.6 (15 x HCAr), 67.1 (Bn-CH2), 60.5 (C-2'), 52.7 (C-3'), 51.8 (C-2''), 49.1, 48.7 (2 x Bn-CH2), 41.7 (C-2'''), 36.1 (NCH3), 31.4 (C-3), 19.4, 17.8 (2 x C-4).

IR (ATR): ν = 3284, 1710, 1629, 1526, 1244, 1096, 1026, 739, 697.

MS (ESI+): m/z = 588.3 [M+H]+, calculated: 588.3181 [M+H]+, found: 588.3181 [+0.1 ppm] (ESI+HRMS).

Preparation of tripeptide tripeptide conjugate 52

From isolated and purified tripeptide 51: To a solution of tripeptide 5 (19 mg, 0.035 mmol) in CHCl3...
(1.5 mL), tripeptide 51 (31 mg, 0.053 mmol) was added. After stirring for 2 d at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt 40:60 → 20:80) to give 38 mg (0.033 mmol, 95%) of the title compound as a colourless solid.

**One-pot protocol without isolation and purification of tripeptide 51**: To a solution of tripeptide 6 (22 mg, 0.040 mmol) in CHCl₃ (1.5 mL), MeNH₂ (2 m in THF, 0.12 mL, 0.24 mmol) was added. After stirring for 20 h at 40 °C the solvent and any excess of MeNH₂ were evaporated in vacuo. The remaining colourless solid was dissolved in CHCl₃ (1.5 mL) and tripeptide 5 (15 mg, 0.027 mmol) was added. After stirring for 2 d at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (4 g, 1.0 x 8.0 cm, DCM:AcOEt, 40:60 → 20:80) to give 28 mg (0.024 mmol, 91%) of the title compound as a colourless solid.

**TLC**: Rₜ = 0.09 (DCM:AcOEt, 40:60).

**Melting point**: T_mₚ = 77 °C.

**Specific rotation**: [α]D[^2] = -11.4 (c = 2.59, CHCl₃)

**¹H NMR** (500 MHz, CDCl₃): δ = 8.03-7.89 (m, 3 H, 2''-NH, 2'''-NH, 2''''-NH), 7.40-7.10 (m, 31 H, 2''-NH, 30 x HCA₀), 5.78 (s, 1 H, 2''-NH), 5.65 (d, J = 9.0 Hz, 1 H, 2''-NH), 5.15-5.05 (m, 4 H, 2 x Bn-CH₂), 4.97 (dd, J = 8.7, 7.0 Hz, 1 H, 2'''-H), 4.83 (d, J = 14.8 Hz, 1 H, Bn-CH₂), 4.70-4.51 (m, 6 H, 2''-H, 2'''-H, 2 x Bn-CH₂), 4.40-4.33 (m, 3 H, Bn-CH₂), 4.28-4.25 (m, 2 H, 2''-H), 4.01 (dd, J = 17.5, 2.3 Hz, 1 H, 2''-H), 3.95-3.87 (m, 2 H, 2'''-H), 2.82-2.75 (m, 3 H, 3''-H, 3'''-H), 2.60 (dd, J = 12.3, 8.1 Hz, 1 H, 3''-H), 2.32 (s, 3 H, NCH₃), 2.20-2.04 (m, 2 H, 3-H, 3'''-H), 1.04-0.88 (m, 12 H, 4-H, 4'''-H).

**¹³C NMR** (126 MHz, CDCl₃): δ = 172.7, 172.5, 170.7, 169.3, 168.5 (C-1, C-1', C-1'', C-1'''', C-1'''''), 156.6, 156.5 (2 x NC(=O)O), 136.8, 136.5, 136.5, 136.5, 136.0, 135.3 (6 x C₅₀), 129.2, 129.1, 128.9, 128.8, 128.6, 128.4, 128.3, 128.2, 128.1, 128.1, 128.1, 127.8, 127.7, 127.3, 127.3, 126.5 (30 x HCA₀), 67.1, 67.0 (2 x Bn-CH₂), 60.6 (C-3'''), 60.3 (C-2'), 59.1 (C-3'''), 54.5 (C-2'''), 51.1, 51.0 (C-2', C-2''''), 50.3, 49.1, 48.6, 48.3 (4 x Bn-CH₂), 44.4 (C-2'''), 41.6 (C-2''), 41.1 (NCH₃), 31.8, 31.5 (C-3, C-3'''), 19.7, 19.5, 18.1, 17.9 (2 x C-4, 2 x C-4''').

**IR** (ATR): ν = 3296, 2961, 1635, 1496, 1452, 1207, 1028, 734, 697.


### Preparation of tripeptide tripeptide conjugate 53

![Diagram of tripeptide tripeptide conjugate 53](image)

To a solution of tripeptide 6 (42 mg, 0.075 mmol) in CHCl₃ (1.5 mL), MeNH₂ (2 m in THF, 0.13 mL, 0.25 mmol) was added. After stirring for 24 h at 40 °C and 2 d at 65 °C the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (3 g, 1.0 x 8.0 cm, DCM:AcOEt, 70:30 → 40:60) to give 26 mg (0.023 mmol, 92%) of the title compound as a colourless solid.
**TLC**: $R_f = 0.14$ (DCM:AcOEt, 50:50).

**Melting point**: $T_{\text{mp}} = 86$ °C.

**Specific rotation**: $[\alpha]_D^{20} = +6.0$ (c = 1.85, CHCl$_3$)

$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.79$ (s, 2 H, 2 x 2'-NH), 7.61 (d, $J = 6.0$ Hz, 2 H, 2 x 2'-NH), 7.28-7.02 (m, 30 H, 30 x H$_{\text{Ar}}$), 5.59 (d, $J = 8.9$ Hz, 2 H, 2 x 2'-NH), 5.01 (d, $J = 12.3$ Hz, 2 H, 2 x Bn-CH$_2$H$_2$), 4.95 (d, $J = 12.3$ Hz, 2 H, 2 x Bn-CH$_2$H$_2$), 4.62 (d, $J = 14.8$ Hz, 2 H, 2 x Bn-CH$_2$H$_2$), 4.55 (ddd, $J = 7.4$, 6.8, 6.0 Hz, 2 H, 2 x 2'-H), 4.38 (d, $J = 14.8$ Hz, 2 H, 2 x Bn-CH$_2$H$_2$), 4.35-4.24 (m, 6 H, 2 x 2'-H$_a$, 2 x Bn-CH$_2$), 4.04-3.98 (m, 4 H, 2 x 2'-H, 2 x 2'-H$_b$), 2.75 (ddd, $J = 12.6$, 7.4 Hz, 2 H, 2 x 3'-H$_a$), 2.64 (ddd, $J = 12.6$, 6.8 Hz, 2 H, 2 x 3'-H$_b$), 2.15 (s, 3 H, NCH$_3$), 2.03 (dqq, $J = 6.8$, 6.7, 6.7 Hz, 2 H, 2 x 3'-H), 0.90 (d, $J = 6.7$ Hz, 6 H, 2 x 4'-H$_a$), 0.86 (d, $J = 6.7$ Hz, 6 H, 2 x 4'-H$_b$).

$^{13}$C NMR (126 MHz, CDCl$_3$): $\delta = 172.3$, 170.8, 168.9 (2 x C-1, 2 x C-1', 2 x C-1''), 156.6 (2 x NC(=O)O), 136.5, 136.5, 135.3 (6 x C$_{\text{Ar}}$), 129.2, 128.8, 128.6, 128.4, 128.2, 128.1, 128.1, 127.8, 126.7 (30 x H$_{\text{Ar}}$), 67.1 (2 x Bn-CH$_2$), 60.5 (2 x C-2), 59.5 (2 x C-3), 51.1 (2 x C-2'), 49.1, 48.5 (4 x Bn-CH$_2$), 41.6 (2 x C-2'), 41.0 (NCH$_3$), 31.2 (2 x C-3), 19.5, 18.3 (4 x C-4).

**IR** (ATR): $\nu = 3288, 2961, 1636, 1528, 1452, 1218, 1026, 734, 697$.

**MS** (ESI$^+$/MS): $m/z = 1166.5$ [M+Na]$^+$, calculated: 1144.5866 [M+H]$^+$, found: 1144.5863 [-0.3 ppm] (ESI$^+$-HRMS).

**Preparation of lysine derivative S1**

To a solution of $N^\alpha$-(tert-Butoxycarbonyl)$-N^\varepsilon$-carbobenzoxy-L-lysine (500 mg, 1.31 mmol) in abs. DMF (12 mL), HOBt (177 mg, 1.31 mmol) was added. EDAC (251 mg, 1.31 mmol) was added after cooling the solution to 0 °C. After stirring for 5 min at 0 °C NEt$_3$ (0.36 mL, 2.6 mmol) was added. Dibenzylamine (0.25 mL, 1.3 mmol) was added after additional 15 min of stirring at 0 °C. After stirring for 15 h and concomitantly slowly warming to rt the reaction mixture was diluted with AcOEt (200 mL) and washed with water (200 mL), 10 wt% aqueous citric acid solution (200 mL) and sat. aqueous NaHCO$_3$ solution (200 mL). The organic layer was dried over Na$_2$SO$_4$, filtered and the solvent of the filtrate evaporated in vacuo. The resultant crude product was purified by FCC (50 g, 4.5 x 7.0 cm, PE:AcOEt, 85:15 → 60:40) to give 682 mg (1.22 mmol, 94%) of the title compound as a colourless solid.

**TLC**: $R_f = 0.29$ (PE:AcOEt, 60:40).

**Melting point**: $T_{\text{mp}} = 68$ °C.

**Specific rotation**: $[\alpha]_D^{20} = -24.5$ (c = 2.28, CHCl$_3$)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.28-7.09$ (m, 15 H, 15 x H$_{\text{Ar}}$), 5.32 (d, $J = 8.7$ Hz, 1 H, 2-NH), 5.00 (s, 2 H, Bn-CH$_2$), 4.74 (s, 1 H, 6-NH), 4.63-4.56 (m, 2 H, 2-H, Bn-CH$_2$H$_2$), 4.51-4.43 (m, 3 H, Bn-CH$_2$H$_2$, Bn-CH$_2$), 3.02 (ddd, $J = 6.2$, 6.1, 5.6 Hz, 2 H, 6-H), 1.57-1.48 (m, 2 H, 3-H), 1.35 (s, 9 H, C(CH$_3$)$_3$), 1.31-1.17 (m, 4 H, 4-H, 5-H).

$^{13}$C NMR (101 MHz, CDCl$_3$): $\delta = 173.4$ (C-1), 156.5, 155.8 (2 x NC(=O)O), 136.9, 136.8, 136.3 (3 x C$_{\text{Ar}}$), 129.1, 128.8, 128.6, 128.3, 128.2, 128.0, 127.7, 127.0 (15 x H$_{\text{Ar}}$), 79.9
Preparation of tripeptide S2

To a solution of tripeptide 4 (180 mg, 0.30 mmol) in abs. CHCl₃ (4 mL), TMEDA (90 µL, 0.60 mmol) was added. After stirring for 40 h the solvent was evaporated in vacuo. The resultant crude product was purified by FCC (18 g, 2.5 x 7.0 cm, PE:AcOEt, 75:25) to give 162 mg (0.271 mmol, 90%) of the title compound as a colourless solid.

**TLC**: R_f = 0.42 (PE:AcOEt, 60:40).

**Melting point**: T_{mp} = 62 °C.

**Specific rotation**: [α]_D^{20} = -23.2 (c = 2.03, CHCl₃)

**1H NMR** (400 MHz, CDCl₃): δ = 8.31 (s, 1 H, 2'-NH), 7.29-7.08 (m, 16 H, 2''-NH, 15 x HCAr), 6.44 (d, J = 1.8 Hz, 1 H, 3'-H_a), 5.39-5.37 (m, 2 H, 2-NH, 3'-H_b), 5.06 (d, J = 12.2 Hz, 1 H, Bn-CH_aH_b), 5.01 (d, J = 12.2 Hz, 1 H, Bn-CH_bH_a), 4.95 (dd, J = 8.6, 5.5 Hz, 1 H, 3''-H), 4.90 (d, J = 14.6 Hz, 1 H, Bn-CH_aH_b), 4.60 (d, J = 16.5 Hz, 1 H, Bn-CH_bH_a), 4.30 (d, J = 16.5 Hz, 1 H, Bn-CH_bH_a), 4.09-4.06 (m, 2 H, 2-H, Bn-CH_aH_b), 2.12-2.03 (m, 2 H, 3-H, 3''-H), 0.91-0.84 (m, 12 H, 4-H, 4''-H).

**13C NMR** (101 MHz, CDCl₃): δ = 171.8 (C-1''), 170.5 (C-1), 163.5 (C-1'), 156.5 (NC(=O)O), 136.7, 136.4, 135.7 (3 x CAr), 133.8 (C-2'), 129.1, 128.8, 128.6, 128.4, 128.2, 128.1, 127.7, 127.1 (15 x HCAr), 102.8 (C-3'), 67.1 (Bn-CH₂), 60.9 (C-2), 54.5 (C-2''), 50.0, 48.0 (2 x Bn-CH₂), 32.0, 31.5 (C-3, C-3''), 19.8, 19.3, 17.7, 17.3 (2 x C-4, 2 x C-4 '').

**IR (ATR)**: ν = 3301, 1720, 1503, 1448, 1215, 1032, 749, 699.

**MS** (ESI⁺): m/z = 621.3 [M+Na]^+, calculated: 621.3047 [M+Na]^+, found: 621.3053 [-0.9 ppm] (ESI⁺-HRMS).
3. Mechanistic control experiment

In agreement with the preparation of tripeptide 15 from aziridine containing tripeptide 4, didehydroalanine derivative S2 was subjected to identical reaction conditions:

Attempted preparation of tripeptide 15 from didehydroalanine derivative S2
To a solution of didehydroalanine containing tripeptide S2 (15 mg, 0.025 mmol) in CHCl$_3$ (1.5 mL), diethylamine (16 μL, 0.15 mmol) was added. After stirring for 20 h at 40 °C the solvent was evaporated in vacuo. The resultant crude product was filtered through silica (2 g, 1.0 x 4.0 cm, DCM:MeOH, 95:5) and the solvent of the filtrate evaporated in vacuo to give 13 mg of a colourless solid.

Based on rigorous TLC, MS and NMR analysis of the isolated material it was unambiguously proven that all starting material S2 was consumed while no indication for any formation of the expected product 15 was found. Furthermore, the NMR and MS analysis strongly suggested that the isolated material consisted of a mixture of compounds S3 and S4. Since it was not possible to fully purify and to separate S3 and S4 the identity of S3 and S4 was not unambiguously proven. Nevertheless, it was shown, that the reaction of S2 with diethylamine does not yield the ring-opening product 15 or any isomer, under the aziridine ring opening conditions.
4. NMR spectra of unknown compounds and key building block 9

\[ \text{H NMR spectrum of 9 (400 MHz, CDCl}_3\text{)} \]

\[ \text{C NMR spectrum of 9 (101 MHz, CDCl}_3\text{)} \]

\[ \text{\textsuperscript{13}C NMR spectrum of 9 (101 MHz, CDCl}_3\text{)} \]
$^1$H NMR spectrum of 11 (400 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 11 (101 MHz, CDCl$_3$)
$^1$H NMR spectrum of 12 (400 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 12 (101 MHz, CDCl$_3$)
$^1$H NMR spectrum of 4 (400 MHz, D$_6$-DMSO)

$^{13}$C NMR spectrum of 4 (101 MHz, D$_6$-DMSO)
$^1$H NMR spectrum of 5 (400 MHz, D$_6$-DMSO)

$^{13}$C NMR spectrum of 5 (101 MHz, D$_6$-DMSO)
$^1$H NMR spectrum of 6 (400 MHz, D$_6$-DMSO)

$^{13}$C NMR spectrum of 6 (101 MHz, D$_6$-DMSO)
$^1$H NMR spectrum of 7 (400 MHz, D$_6$-DMSO)

$^{13}$C NMR spectrum of 7 (101 MHz, D$_6$-DMSO)
$^1$H NMR spectrum of 15 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 15 (126 MHz, CDCl$_3$)
\(^1\)H NMR spectrum of 16 (500 MHz, CDCl\(_3\))

\(^{13}\)C NMR spectrum of 16 (126 MHz, CDCl\(_3\))
$^1$H NMR spectrum of 17 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 17 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 18 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 18 (126 MHz, CDCl$_3$)
1H NMR spectrum of 19 (500 MHz, CDCl₃)

13C NMR spectrum of 19 (126 MHz, CDCl₃)
$^1$H NMR spectrum of 20 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 20 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 21 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 21 (126 MHz, CDCl$_3$)

S54
$^1$H NMR spectrum of 22 (400 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 22 (101 MHz, CDCl$_3$)
$^1$H NMR spectrum of 23 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 23 (126 MHz, CDCl$_3$)
^1^H NMR spectrum of 24 (500 MHz, CDCl$_3$)

^13^C NMR spectrum of 24 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 25 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 25 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 26 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 26 (126 MHz, CDCl$_3$)

S59
$^1$H NMR spectrum of 27 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 27 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 28 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 28 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 29 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 29 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 30 (500 MHz, CDCl₃)

$^{13}$C NMR spectrum of 30 (126 MHz, CDCl₃)
$^1$H NMR spectrum of 31 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 31 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 32 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 32 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 33 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 33 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 34 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 34 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 35 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 35 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 36 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 36 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 37 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 37 (126 MHz, CDCl$_3$)
\[ ^1H \text{NMR spectrum of } 38 \text{ (500 MHz, CDCl}_3 \text{)} \]

\[ ^{13}C \text{NMR spectrum of } 38 \text{ (126 MHz, CDCl}_3 \text{)} \]
$^1$H NMR spectrum of 39 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 39 (126 MHz, CDCl$_3$)
$^{1}$H NMR spectrum of 40 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 40 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 41 (400 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 41 (101 MHz, CDCl$_3$)
$^1$H NMR spectrum of 42 (400 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 42 (101 MHz, CDCl$_3$)
$^{1} \text{H NMR spectrum of 43 (500 MHz, CDCl}_{3}$)

$^{13} \text{C NMR spectrum of 43 (126 MHz, CDCl}_{3}$)
\[ ^1H \text{NMR spectrum of } 44 \text{ (500 MHz, CDCl}_3) \]

\[ ^{13}C \text{NMR spectrum of } 44 \text{ (126 MHz, CDCl}_3) \]


\[ \text{H NMR spectrum of 45 (500 MHz, CDCl}_3\text{)} \]

\[ \text{\^{13}C NMR spectrum of 45 (126 MHz, CDCl}_3\text{)} \]

S78
$^1$H NMR spectrum of 46 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 46 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 47 (400 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 47 (101 MHz, CDCl$_3$)
$^1$H NMR spectrum of 48 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 48 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 49 (400 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 49 (101 MHz, CDCl$_3$)
$^1$H NMR spectrum of 50 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 50 (126 MHz, CDCl$_3$)
$^1$H NMR spectrum of 51 (400 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 51 (101 MHz, CDCl$_3$)
$^1$H NMR spectrum of 52 (500 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of 52 (126 MHz, CDCl$_3$)
\(^1\)H NMR spectrum of 53 (500 MHz, CDCl\(_3\))

\(^{13}\)C NMR spectrum of 53 (126 MHz, CDCl\(_3\))
$^1$H NMR spectrum of S1 (400 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of S1 (101 MHz, CDCl$_3$)
$^1$H NMR spectrum of S2 (400 MHz, CDCl$_3$)

$^{13}$C NMR spectrum of S2 (101 MHz, CDCl$_3$)
5. References


