Negishi Cross-Coupling Provides Alkylated Tryptophans and Tryptophan Regioisomers

Steffen Dachwitz, Bjarne Scharkowski, and Norbert Sewald®
Instruments

Analytical HPLC

Analytical HPLC was performed on a Shimadzu NexeraXR 20A System with autosampler, degasser, column oven, diode array detector and a Phenomenex Luna C18 column (2.9 µm, 50 × 2.1 mm) with a gradient (in 5.5 min from 5 % B to 95 % B, 0.5 min 95 % B and back to 5 % B in 3 min, total run time 9 min) at a flow rate of 650 µL/min and column oven temperature of 40 °C. HPLC solvent A consists of 99.9% water with 0.1 % TFA, solvent B of 99.9 % acetonitrile with 0.1 % TFA.

Analytical LC-MS

Analytical LC-MS was performed on an Agilent 6220 TOF-MS with a Dual ESI-source, 1200 HPLC system with autosampler, degasser, binary pump, column oven, diode array detector and a Hypersil Gold C18 column (1.9 µm, 50 × 2.1 mm) with a gradient (in 11 min from 0 % B to 98 % B, back to 0 % B in 0.5 min, total run time 15 min) at a flow rate of 300 µL/min and column oven temperature of 40°C. HPLC solvent A consists of 94.9 % water, 5 % acetonitrile and 0.1 % formic acid, solvent B of 5 % water, 94.9 % acetonitrile with 0.1 % formic acid. ESI mass spectra were recorded after sample injection via 1200 HPLC system in extended dynamic range mode equipped with a Dual-ESI source, operating with a spray voltage of 2.5 kV.

NMR

NMR spectra were recorded on a Bruker Avance III 500 HD (¹H: 500 MHz, ¹³C: 126 MHz) or Avance 600 (¹H: 600 MHz, ¹³C: 151 MHz). Chemical shifts δ [ppm] are reported relative to residual solvent signal (CDCl₃, ¹H: 7.26 ppm, ¹³C: 77.1 ppm; DMSO-d₆, ¹H: 2.50 ppm, ¹³C: 39.5 ppm; Methanol-d₄, ¹H: 3.31 ppm ¹³C: 49.0 ppm). 2D spectra (COSY, HMQC, HMBC) were used for signal assignment.

General Procedures

GP1: Ni-catalysed reductive cross-coupling

Arylbromide (125 µmol), NiI₂ (4.7 mg, 13 µmol, 10 mol%) and 4,4’-Di-tert-butyl-2,2’dipyridine (3.8 mg, 13 µmol, 10 mol%) were placed in a glass vial under argon atmosphere. The mixture was suspended in DMPU (0.5 mL) and purged with argon. Alkyliodide (250 µmol, 2.0 eq.), pyridine (1.0 µL, 13 µmol, 10 mol%) and manganese (500 µmol, 4.0 eq.) were added. The green reaction mixture was heated to 60 °C and stirred for 21 The mixture was purified directly by column chromatography (Petrolether/EtOAc; 3:1).

GP2: Pd-catalysed Negishi cross-coupling (analytical scale)

Arylbromide (250 µmol, 1.0 eq.), zinc dust (1.5-4.0 eq.), Alkyliodide (1.5-2.0 eq.) and Pd(amphos)₂Cl₂ (5 mol%) were placed in a glass vial followed by DMF (0.5 mL) The suspension was purged with argon and stirred at room temperature for 24 h. The crude
mixture was diluted with water (30 mL) and extracted with EtOAc (3 x 30 mL) the organic phase was dried over MgSO4 and the solvent removed in vacuum. The crude product was purified by automated column chromatography using a Büchi Reveleris X2 (Petrolether:EtOAc).

**GP2b: Pd-catalysed Negishi cross-coupling (preparative scale)**

Arylbromide (5.00 mmol, 1.0 eq.), zinc dust (1.5 eq.), Alkyliodide (1.5 eq.) and Pd(amphos)2Cl2 (5 mol%) were placed in a glass vial followed by DMF (1-10 mL). The suspension was purged with argon and stirred at 37°C for 24 h. The crude mixture was filtered over a short plug of silica and washed with Petrolether/EtOAc; 4:1, the solvent was evaporated in vacuum, giving a dark red oil. The crude product was purified by column chromatography (DCM/MeOH; 99:1).

**GP3: Marfey’s Test**

The enantiopurities were determined using the chiral reagent FDAA (Marfey’s reagent, 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide). For this purpose, a 3.33 mM amino acid stock solution in a 1:2 mixture of aqueous 0.1 M NaHCO3 and acetone (300 μL, 1.0 eq.) and a 15 mM FDAA stock solution in acetone (100 μL, 1.5 eq.) were mixed and incubated for 1 h at 40 °C. The resulting suspension was neutralized with aqueous 0.1 M HCl solution (150 μL) and diluted 1:1 with of acetonitrile. The obtained solution was analysed by LC-MS.

Analytical LC-MS was performed on an Agilent 6220 TOF-MS with a Dual ESI-source, 1200 HPLC system with autosampler, degasser, binary pump, column oven, diode array detector and a Hypersil Gold C18 column (1.9 μm, 50 × 2.1 mm) with a gradient (in 30 min from 5 % B to 55 % B, 3 min at 55 % B, back to 5 % B in 3 min, total run time 40 min) at a flow rate of 300 μL/min and column oven temperature of 40°C. HPLC solvent A consists of 94.9 % water, 5 % acetonitrile and 0.1 % formic acid, solvent B of 5 % water, 94.9 % acetonitrile and 0.1 % formic acid. UV detection took place at 340 nm. ESI mass spectra were recorded after sample injection via 1200 HPLC system in extended dynamic range mode equipped with a Dual-ESI source, operating with a spray voltage of 2.5 kV. Nitrogen served both as the nebulizer gas and the dry gas. Nitrogen was generated by a nitrogen generator NGM 11.

**GP4: Acid free deprotection of regioisomeric tryptophan surrogates**

The protected tryptophan regioisomer (1.0 mmol) was suspended in Na2HPO4-Buffer (100 mM, pH = 7.4, 15 mL). The mixture was heated to reflux for 9 hours. The aqueous phase was washed with EtOAc (2x15 mL). The solvent of the aqueous phase was reduced in vacuum and the crude product was desalted using an acid-free reversed phase C18-column chromatography, washing with pure MPW (100 mL) and eluting the product with MeOH (100 mL). The enantiomeric excess was determined using Marfey’s Test (see GP3).

**GP5: Acidic deprotection of alkylated tryptophan derivatives**

The protected alkylated tryptophan (25 μmol) was suspended in aqueous HCl (5 M, 2 mL) and heated to 90 °C for 2 hours. The crude reaction mixture was purified by reversed phase HPLC. The enantiomeric excess was determined using Marfey’s Test (see GP3).
Figure S1: a) Comparison of $^1$H NMR of the 3-(5-, 6- and 7-indolyl)alanine 11b-d; b) Comparison of $^{13}$C NMR of the 3-(5-, 6- and 7-indolyl)alanine 11b-d.
Compounds

L-7-Bromotryptophan x TFA

L-7-bromotryptophan (2) was synthesized according to our previously reported procedure using RebH-PrnF-RR-ADH combiCLEAs.[3] The biocatalyst was produced using lyzed E. coli cells containing overexpressed tryptophan-7-halogenase RebH resulting from 1.5 L expression culture. The reaction buffer contained 1 mM L-tryptophan, 15 mM Na₂HPO₄, 30 mM NaBr, 0.1 mM NAD⁺, 1 µM FAD and 0.5 % (v/v) 2-propanol at pH = 7.4 in a total reaction volume of 1.250 L. Full conversion was usually observed after 2-4 days. The suspension was filtered and desalted. Therefore, the crude filtrate was concentrated up to a volume of about 100 mL and loaded on a 12 g RP-C₁₈-column and purified using an automated column chromatography using a Büchi Reveleris X2 with a binary pump and ELSD Detector. The gradient (4 min at 5% B, up to 25% B in 14 min, in 1 min up to 100 % B for 2 min and flushing with 80% B for 5 min, total run time 27 min) was used at a flow rate of 30 mL/min. Solvent A consisted of 99.9 % water and 0.1% TFA, solvent B of 99.9 % acetonitrile and 0.1 % TFA. Freeze drying gave L-7-bromotryptophan x TFA as a colorless to yellow solid.

Anal. RP-HPLC: tᵣ = 3.3 min;

LC-MS: tᵣ = 5.2 min;

¹H NMR (500 MHz, DMSO-d₆) δ [ppm] = 13.96 (br s, 1H, COOH), 11.29 (d, ³J = 2.7 Hz, 1H, indole-NH), 8.19 (brs, 3H, NH₃), 7.58 (d, ³J = 7.9 Hz, 1H, C4-H), 7.33 (d, ³J = 7.5 Hz, 1H, C6-H), 7.30 (d, ³J = 2.7 Hz, 1H, C2-H), 6.98 (dd, ³J = 7.8 Hz, ³J = 7.8 Hz, 1H, C5-H), 4.18 (dd, ³J = 7.1 Hz, ³J = 6.2 Hz, 1H, Cα-H), 3.27 (dd, ²J = 15.0 Hz, ³J = 5.7 Hz, 1H, Cβ-H), 3.23 (dd, ²J = 14.8 Hz, ³J = 6.9 Hz, 1H, Cβ-H);

MS (ESI): found [m/z] = 265.98 [M(⁷⁹Br)-NH₂]⁺, 267.98 [M(⁸¹Br)-NH₂]⁺, 283.01 [M(⁷⁹Br)+H]⁺, 285.01 [M(⁸¹Br)+H]⁺; calcd. [m/z] = 265.98 [M(⁷⁹Br)-NH₂]⁺, 267.98 [M(⁸¹Br)-NH₂]⁺, 283.01 [M(⁷⁹Br)+H]⁺, 285.01 [M(⁸¹Br)+H]⁺.
L-7-bromotryptophan x TFA (2, 159.7 mg, 402 µmol) was dissolved in 1,4-dioxane (5 mL) followed by addition of di-tert-butyl dicarbonate (105.8 mg, 484 µmol, 1.2 eq.) and aqueous NaOH (1.0 M, 800 µL, 800 µmol, 2.0 eq.). The reaction progress was monitored by analytical HPLC. After completion, the solvent was removed in vacuum and the crude residue was dissolved in water (25 mL) and adjusted to pH = 3 by addition of aqueous HCl (1.0 M). The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organic layers were dried over MgSO4. The organic solvent was removed in vacuum and the crude product was purified by RP-HPLC providing Nα-Boc-L-7-bromotryptophan (19) as a colorless solid (106.2 mg, 277 µmol, 69%).

**Anal. RP-HPLC:** $t_R = 5.0$ min;

**LC-MS:** $t_R = 8.7$ min;

$^1$H NMR (500 MHz, DMSO- $d_6$) δ [ppm] = 12.58 (br s, 1H, COOH), 11.07 (d, $^3J = 2.6$ Hz, indole-NH), 7.54 (d, $^3J = 7.8$ Hz, 1H, C4-H), 7.29 (d, $^3J = 7.5$ Hz, 1H, C6-H), 7.22 (d, $^3J = 2.6$ Hz, 1H, C2-H), 7.02 (d, $^3J = 7.9$ Hz, 1H, OCONH), 6.95 (dd, $^3J = 7.7$ Hz, $^3J = 7.7$ Hz, 1H, C5-H), 4.13 (ddd, $^3J = 9.3$ Hz, $^3J = 8.0$ Hz, $^3J = 4.7$ Hz, 1H, Cα-H), 3.12 (dd, $^2J = 14.7$, $^3J = 4.7$ Hz, 1H, Cβ-H), 2.97 (dd, $^2J = 15.0$ Hz, $^3J = 9.6$ Hz, 1H, Cβ-H), 1.32 (s, 9H, C(CH$_3$)$_3$; cis/trans ratio 5:1);

**MS (ESI):** found [m/z] = 381.05 [M($^{79}$Br)-H]; 383.05 [M($^{81}$Br)-H]; calcd. [m/z] = 381.05 [M($^{79}$Br)-H]; 383.05 [M($^{81}$Br)-H].
L-7-Bromotryptophan x TFA (17) (272.5 mg, 963 µmol) was dissolved in methanol (5 mL) and cooled to 0 °C followed by dropwise addition of thionyl chloride (150 µL, 2.0 mmol, 2.0 eq.). The mixture was heated to reflux and the reaction progress was monitored by analytical HPLC. After completion (1 h), the solvent was removed in vacuum and the crude residue was dissolved in ACN (10 mL) and adjusted to pH = 9 by addition of aqueous NaOH (1.0 M) followed by addition of di tert-butyl-dicarbonate (355.2 mg, 1.6 mmol, 1.7 eq.). The reaction progress was monitored by analytical HPLC and after completion (20 h), the solvent was removed in vacuum and the crude residue was suspended in water (50 mL) and adjusted to pH = 4 by addition of aqueous HCl (1.0 M). The aqueous layer was extracted with EtOAc (3 x 70 mL) and the combined organic layers were dried over MgSO₄. The organic solvent was removed in vacuum and the crude product was purified by column chromatography (Petrolether/EtOAc; 4:1) providing Nα-Boc-L-7-bromotryptophan methyl ester (17) as a colorless solid (280.5 mg, 706 µmol, 73 %).

**LC-MS:** \( t_R = 9.7 \text{ min} \);

**1H NMR** (500 MHz, DMSO-\(d_6\)) \( \delta \text{ [ppm]} = 11.09 \) (s, 1H, Indole-NH), 7.51 (d, \( 3J = 7.9 \text{ Hz} \), 1H, C4-H), 7.29 (d, \( 3J = 7.5 \text{ Hz} \), 1H, C6-H), 7.26 (d, \( 3J = 7.9 \text{ Hz} \), 1H, OCONH), 7.24 (d, \( 3J = 2.6 \text{ Hz} \), 1H, C2-H), 6.95 (dd, \( 3J = 7.8 \text{ Hz} \), 1H, C5-H), 4.20 (ddd, \( 3J = 9.4 \text{ Hz} \), \( 3J = 7.9 \text{ Hz} \), \( 3J = 5.0 \text{ Hz} \), 1H, Cα-H), 3.60 (s, 3H, C \( \alpha \)-H), 3.11 (ddd, \( 3J = 14.8 \text{ Hz} \), \( 3J = 14.6 \text{ Hz} \), \( 3J = 9.5 \text{ Hz} \), 1H, Cβ-H), 2.99 (dd, \( 3J = 14.6 \text{ Hz} \), \( 3J = 9.5 \text{ Hz} \), 1H, Cβ-H), 1.32 (s, 9H, C(C\( \alpha \)H\( \alpha \))\( \alpha \)); cis/trans ratio 5:1).

**13C NMR** (126 MHz, DMSO-\(d_6\)) \( \delta \text{ [ppm]} = 172.8 \) (COO(CH\( \alpha \)), 155.4 (NHC=O), 134.3 (C7a), 128.8 (C4a), 125.1 (C2), 123.5 (C6), 119.9 (C5), 117.7 (C4), 111.3 (C3), 104.2 (C7), 78.2 (C(CH\( \alpha \)\( \alpha \))\( \alpha \)), 54.5 (C\( \alpha \)), 51.8 (OCH\( \alpha \)), 28.1(C(CH\( \alpha \)\( \alpha \))\( \alpha \)), 26.7 (C\( \beta \)).

**MS (ESI):** found \([m/z] = 419.06 \text{ [M}^{79}\text{Br)+Na}^+] , 421.05 \text{ [M}^{81}\text{Br)+Na}^+] , 341.01 \text{ [M}^{79}\text{Br)-(tertButyl)+H}^+] , 343.01 \text{ [M}^{81}\text{Br)-(tertButyl)+H}^+] ; 299.02 \text{ [M}^{79}\text{Br)-Boc+H}^+] , 297.02 \text{ [M}^{81}\text{Br)-Boc+H}^+] , 279.99 \text{ [M}^{79}\text{Br)-Boc-NH}_2^+] , 281.99 \text{ [M}^{81}\text{Br)-Boc-NH}_2^+] , \text{ calcd. \([m/z] = 419.06 \text{ [M}^{79}\text{Br)+Na}^+] , 421.05 \text{ [M}^{81}\text{Br)+Na}^+] , 341.01 \text{ [M}^{79}\text{Br)-(tertButyl)+H}^+] , 343.01 \text{ [M}^{81}\text{Br)-(tertButyl)+H}^+] ; 299.02 \text{ [M}^{79}\text{Br)-Boc+H}^+] , 297.02 \text{ [M}^{81}\text{Br)-Boc+H}^+] , 279.99 \text{ [M}^{79}\text{Br)-Boc-NH}_2^+] , 281.99 \text{ [M}^{81}\text{Br)-Boc-NH}_2^+] .
5-Benzylindole (10) was synthesized according to GP2. 5-Bromoindole (1b, 50.5 mg, 258 µmol), zinc dust (69.9 mg, 1069 µmol, 4 eq.) and Pd(amphos)2Cl2 (8.5 mg, 12.5 µmol, 5 mol%) were placed in a glass vial followed by DMF (0.5 mL). The suspension was degassed by sparkling argon through it. Freshly distilled benzyl iodide (9, 103.5 µL, 523 µmol, 2 eq.) was added and the mixture was stirred at room temperature for 4 h. The solvent was evaporated in vacuum and the crude product was purified by column chromatography (Petrolether/EtOAc; 4:1) providing 10 as a pale brown solid (42.5 mg, 205 µmol, 79%).

Anal. RP-HPLC: \( t_R = 5.6 \) min;

LC-MS: \( t_R = 9.7 \) min;

\( ^1H \) NMR (500 MHz, Chloroform-\( d \)) \( \delta \) [ppm] = 8.05 (br s, 1H, Indole-NH), 7.52 (s, 1H, C4-H), 7.38 – 7.31 (m, 3H, C2'-H/C6'-H/C7-H), 7.30 (m, 2H, C3'-H/C5'-H ), 7.22 (m, 1H, C4'-H), 7.20 (dd, \( ^3J = 3.5 \) Hz, \( ^3J = 1.1 \) Hz, 1H, C2-H), 7.10 (dd, \( ^3J = 8.4 \) Hz, \( ^4J = 1.8 \) Hz, 1H, C6-H), 6.55 (dd, \( ^3J = 3.5 \) Hz, \( ^4J = 2.1 \) Hz, 1H, C3-H), 4.15 (s, 2H, CH2).

MS (ESI): found \([m/z] = 208.11 [M+H]^+\), calcd. \([m/z] = 208.11 [M+H]^+\).
Nα-Boc-L-3-(1H-4-Indolyl)alanine methyl ester (11a)

Analytical scale:
Nα-Boc-L-3-(1H-4-Indolyl)alanine methyl ester (11a) was synthesized according to GP2. Therefore, 4-Bromoindole (1a, 32 μL, 255 μmol), zinc dust (24.9 mg, 382 μmol, 1.5 eq.), Nα-Boc-L-3-iodoalanine methyl ester (164.4 mg, 399 μmol, 1.5 eq.) and Pd(amphos)2Cl2 (9.2 mg, 13 μmol, 5 mol%) were placed in a glass vial followed by DMF (0.5 mL) The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was diluted with water (30 mL) and extracted with EtOAc (3 x 30 mL) the organic phase was dried over MgSO4 and the solvent removed in vacuum. The crude product was purified by automated column chromatography using a Büchi Revelerie s X2 (Petrolether:EtOAc) giving 11a as a colorless solid (47.7 mg, 150 μmol, 59 %).

Upscaling:
Nα-Boc-L-3-(1H-5-Indolyl)alanine methyl ester (11a) was synthesized according to GP2b. Therefore, 4-Bromoindole (1a, 0.627 mL, 5.00 mmol), zinc dust (0.496 g, 7.59 mmol, 1.5 eq.), Nα-Boc-L-3-iodoalanine methyl ester (8, 2.456 g, 7.46 mmol, 1.5 eq.) and Pd(amphos)2Cl2 (176 mg, 250 µmol, 5 mol%) were placed in a glass vial followed by DMF (1 mL) The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was filtered over a short plug of silica and washed with Petrolether/EtOAc; 4:1, the solvent was evaporated in vacuum, giving a dark red oil. The crude product was purified by column chromatography (DCM/MeOH; 99:1) providing 11a as a colorless solid (487.2 mg, 1.53 mmol, 31 %).

LC-MS: tR = 8.7 min;

$^1$H NMR (500 MHz, CDCl3) δ [ppm] = 8.24 (s, 1H, Indole-NH), 7.30 (d, $^3$J = 8.1 Hz, 1H, C7-H), 7.21 (dd, $^3$J = 4.8 Hz, $^3$J = 2.9 Hz, 1H, C2-H), 7.12 (dd, $^3$J = 7.6 Hz, $^3$J = 4.6 Hz, 1H, C5-H), 5.03 (d, $^3$J = 6.3 Hz, 1H, OCONH), 4.71 (ddd, $^3$J = 6.6 Hz $^3$J = 6.3 Hz $^3$J = 6.1 Hz, 1H, Cα-H), 3.66 (s, 3H, CH3), 3.41 (dd, $^3$J = 13.9 Hz, $^3$J = 6.0 Hz, 1H, Cβ-H), 3.34 (dd, $^3$J = 14.1 Hz, $^3$J = 6.6 Hz, 1H, Cβ-H), 1.41 (s, 9H, C(CH3)3).

$^{13}$C NMR (126 MHz, CDCl3) δ [ppm] = 172.9 (COOME), 155.3 (NHCOO), 135.9 (C7a), 128.2 (C4a), 128.1 (C4), 124.2 (C2), 122.2 (C6), 120.5 (C5), 110.2 (C7), 101.1 (C3), 79.9 (C(CH3)3), 54.4 (Cα), 52.3 (COO(CH3)), 36.1 (Cβ), 28.5 (C(CH3)3).

MS (ESI): found [m/z] = 319.16 [M+H]+, 263.10 [M-(tertButyl)+H]+, 219.12[M-Boc+H]+, 202.09 [M-Boc-NH2]+, calcd. [m/z] = 319.17 [M+H]+, 263.10 [M-(tertButyl)+H]+, 219.11 [M-Boc+H]+, 202.09 [M-Boc-NH2]+.
Nα-Boc-L-3-(1H-5-Indolyl)alanine methyl ester (11b)

Analytical scale:

Nα-Boc-L-3-(1H-5-Indolyl)alanine methyl ester (11b) was synthesized according to GP2. Therefore, 5-Bromoindole (1b, 50.07 mg, 255 μmol), zinc dust (66.07 mg, 1.01 mmol, 4.0 eq.), Nα-Boc-L-3-iodoalanine methyl ester (8, 165.66 mg, 503 μmol, 2.0 eq.) and Pd(amphos)2Cl2 (9.1 mg, 13 µmol, 5 mol%) were placed in a glass vial followed by DMF (0.5 mL). The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was purified by column chromatography (PE/EtOAc; 5:1) providing 11b as a colorless solid (44.2 mg, 138.8 μmol, 54%).

Upscaling:

Nα-Boc-L-3-(1H-5-Indolyl)alanine methyl ester (11b) was synthesized according to GP2b. Therefore, 5-Bromoindole (1b, 0.981 g, 5.00 mmol), zinc dust (0.497 g, 7.60 mmol, 1.5 eq.), Nα-Boc-L-3-iodoalanine methyl ester (8, 1.816 g, 5.52 mmol, 1.1 eq.) and Pd(amphos)2Cl2 (179 mg, 250 µmol, 5 mol%) were placed in a glass vial followed by DMF (1 mL). The suspension was purged with argon and stirred at 37 °C for 24 h. The crude mixture was filtered over a short plug of silica and washed with Petroleum/EtOAc; 4:1, the solvent was evaporated in vacuum, giving a dark red oil. The crude product was purified by column chromatography (DCM/MeOH; 99:1) providing 11b as a colourless solid (809.2 mg, 2.542 mmol, 51%).

LC-MS: \( t_R = 8.9 \) min;

1H NMR (500 MHz, Chloroform-d) \( \delta \) [ppm] = 8.16 (s, 1H, Indole-NH), 7.39 (d, \( 4J = 1.8 \) Hz, 1H, C4-H), 7.32 (d, \( 3J = 8.4 \) Hz, 1H, C7-H), 7.20 (dd, \( 3J = 2.8 \) Hz, \( 3J = 2.7 \) Hz, 1H, C2-H), 6.95 (dd, \( 3J = 8.4 \), \( 4J = 1.7 \) Hz, 1H, C6-H), 6.50 (dd, \( 3J = 2.7 \) Hz, \( 4J = 1.6 \) Hz, 1H, C3-H), 4.96 (d, \( 3J = 7.6 \) Hz, 1H, OCON-H), 4.59 (ddd, \( 3J = 7.3 \) Hz, \( 3J = 6.6 \) Hz, \( 3J = 6.6 \) Hz, 1H, Cα-H), 3.72 (s, 3H, COOC3), 3.19 (dd, \( 3J = 11.5 \), \( 3J = 6.8 \) Hz, 1H, Cβ-H), 3.16 (dd, \( 3J = 12.0 \), \( 3J = 6.2 \) Hz, 1H, Cβ-H), 1.41 (s, 9H, C(CH3)3).

13C NMR (126 MHz, Chloroform-d) \( \delta \) [ppm] = 172.8 (COOME), 155.4 (OCNH), 135.1 (C7a), 128.3 (C3a), 127.2 (C5), 124.7 (C2), 123.4 (C6), 121.4 (C4), 111.3 (C7), 102.6 (C3), 79.9 (C(CH3)3), 55.0 (Cα), 52.3 (OCH3), 38.4 (Cβ), 28.5 (C(CH3)3).

MS (ESI): found [\( m/z \)] = 319.16 [M+H]+, 263.10 [M-(tert-Butyl)+H]+, 219.12 [M-Boc+H]+, 202.09 [M-Boc-NH2]+; calcd. [\( m/z \)] = 319.17 [M+H]+, 263.10 [M-(tert-Butyl)+H]+, 219.11 [M-Boc+H]+, 202.09 [M-Boc-NH2]+.
Analytical scale:

N\textsuperscript{α}-Boc-L-3-(1H-6-Indolyl)alanine methyl ester (11c) was synthesized according to GP2. Therefore, 6-Bromoindole (1c, 51.0 mg, 260 \( \mu \)mol), zinc dust (65.02 mg, 995 \( \mu \)mol, 3.8 eq.), N\textsuperscript{α}-Boc-L-3-iodoalanine methyl ester (8, 170.38 mg, 517 \( \mu \)mol, 2.0 eq.) and Pd(amphos)\textsubscript{2}Cl\textsubscript{2} (9.4 mg, 13 \( \mu \)mol, 5 mol%) were placed in a glass vial followed by DMF (0.5 mL). The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was diluted with water (30 mL) and extracted with EtOAc (3 x 30 mL). The organic phase was dried over MgSO\textsubscript{4} and the solvent removed in vacuum. The crude product was purified by automated column chromatography using a Büchi Reveleris X2 (Petrolether:EtOAc) giving 11c as a colourless solid (42.1 mg, 132 \( \mu \)mol, 51%).

Upscaling:

N\textsuperscript{α}-Boc-L-3-(1H-6-Indolyl)alanine methyl ester (11c) was synthesized according to GP2b. Therefore, 6-Bromoindole (1c, 980 mg, 5.00 mmol), zinc dust (490 mg, 7.50 mmol, 1.5 eq.), N\textsuperscript{α}-Boc-L-3-iodoalanine methyl ester (8, 1.833 g, 5.57 mmol, 1.1 eq.) and Pd(amphos)\textsubscript{2}Cl\textsubscript{2} (178 mg, 249 \( \mu \)mol, 5 mol%) were placed in a glass vial followed by DMF (10 mL). The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was filtered over a short plug of silica and washed with Petrolether/EtOAc (4:1), the solvent was evaporated in vacuum, giving a dark red oil. The crude product was purified by column chromatography (DCM/MeOH; 99:1) providing 11c as a colourless solid (482 mg, 1.51 mol, 30%).

LC-MS: \( t_R = 8.6 \) min;

\( \textsuperscript{1}H \) NMR (500 MHz, Chloroform-\textit{d}) \( \delta [ppm] = 8.14 \) (s, 1H, Indole-NH), 7.56 (d, \( ^3J = 8.4 \) Hz 1H, C4-H), 7.18 (m, 2H, C2-H/ C7-H), 6.88 (d, \( ^3J = 8.1 \) Hz, 1H, C5-H), 6.52 (d, \( ^3J = 2.0 \) Hz 1H, C3-H), 4.96 (d, \( ^3J = 8.5 \) Hz, 1H, OCON-H), 4.61 (dd, \( ^3J = 9.2 \) Hz, \( ^3J = 6.8 \) Hz, 1H, C\textalpha-H), 3.71 (s, 3H, COOC\textsubscript{3}), 3.19 (dd, \( ^3J = 13.2 \) Hz, \( ^3J = 6.8 \) Hz, 1H, C\textbeta-H), 3.16 (dd, \( ^3J = 13.0 \) Hz, \( ^3J = 6.8 \) Hz, 1H, C\textbeta-H), 1.41 (s, 9H, C(CH\textsubscript{3})\textsubscript{3}).

\( \textsuperscript{13}C \) NMR (126 MHz, Chloroform-\textit{d}) \( \delta [ppm] = 172.8 \) (COOME), 155.4 (OCONH), 136.2 (C7a), 129.8 (C3a), 127.1 (C6), 124.4 (C2), 121.5 (C4), 120.9 (C5), 111.6 (C7), 102.7 (C3), 80.0 (C(CH\textsubscript{3})\textsubscript{3}), 54.9 (C\textalpha), 52.3 (OCH\textsubscript{3}), 38.6 (C\textbeta), 28.5 (C(CH\textsubscript{3})\textsubscript{3}).

MS (ESI): found [m/z] = 319.16 [M+H]\textsuperscript{+}, 263.10 [M-\textsubscript{tert}Butyl]+H\textsuperscript{+}, 219.12[M-Boc+H]\textsuperscript{+}, 202.09 [M-Boc-NH\textsubscript{2}]+, calcd. [m/z] = 319.17 [M+H]\textsuperscript{+}, 263.10 [M-\textsubscript{tert}Butyl]+H\textsuperscript{+}, 219.11 [M-Boc+H]\textsuperscript{+}, 202.09 [M-Boc-NH\textsubscript{2}]+.
Analytical scale:

\( N^\alpha\)-Boc-L-3-(1H-7-Indolyl)alanine methyl ester (11d) was synthesized according to GP2. Therefore, 7-Bromoindole (1d, 50.1 mg, 255 \( \mu \)mol), zinc dust (65.1 mg, 995 \( \mu \)mol, 3.9 eq.), \( N^\alpha\)-Boc-L-3-iodoalanine methyl ester (8, 164.1 mg, 499 \( \mu \)mol, 2.0 eq.) and Pd(amphos)\(_2\)Cl\(_2\) (9.1 mg, 13 \( \mu \)mol, 5 mol%) were placed in a glass vial followed by DMF (0.5 mL). The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was diluted with water (30 mL) and extracted with EtOAc (3 x 30 mL) the organic phase was dried over MgSO\(_4\) and the solvent removed in vacuum. The crude product was purified by automated column chromatography using a Büchi Reveleris X2 (Petrolether:EtOAc) giving 11d as a colourless solid (46.5 mg, 146 \( \mu \)mol, 58%).

Upscaling:

\( N^\alpha\)-Boc-L-3-(1H-7-Indolyl)alanine methyl ester (11d) was synthesized according to GP2b. Therefore, 7-Bromoindole (1d, 980 mg, 5.00 mmol), zinc dust (490 mg, 7.50 mmol, 1.5 eq.), \( N^\alpha\)-Boc-L-3-iodoalanine methyl ester (8, 2.501 g, 7.60 mmol, 1.5 eq.) and Pd(amphos)\(_2\)Cl\(_2\) (178 mg, 249 \( \mu \)mol, 5 mol%) were placed in a glass vial followed by DMF (10 mL). The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was filtered over a short plug of silica and washed with Petrolether/EtOAc (4:1), the solvent was evaporated in vacuum, giving a dark red oil. The crude product was purified by column chromatography (DCM/MeOH; 99:1) providing 11d as a colourless solid (586 mg, 1.84 mol, 37%).

LC-MS: \( t_R = 9.4 \) min;

\( ^1H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \) [ppm] = 9.80 (s, 1H, Indole-NH), 7.56 (d, \( ^3J = 7.9 \) Hz, 1H, C4-H), 7.27 (dd, \( ^3J = 3.8 \) Hz, \( ^3J = 2.9 \) Hz, 1H, C2-H), 7.02 (dd, \( ^3J = 7.7 \) Hz, \( ^3J = 7.7 \) Hz, 1H, C5-H), 6.88 (d, \( ^3J = 7.2 \) Hz, 1H, C6-H), 6.55 (dd, \( ^3J = 2.8 \), \( ^3J = 2.8 \) Hz, 1H, C3-H), 5.48 (d, \( ^3J = 6.4 \) Hz, 1H, OCONH), 4.58 (ddd, \( ^3J = 9.0 \) Hz, \( ^3J = 6.8 \) Hz, \( ^3J = 6.7 \) Hz, 1H, Cα-H), 3.66 (s, 3H, CH\(_3\)), 3.49 (dd, \( ^3J = 13.0 \) Hz, \( ^3J = 6.5 \) Hz, 1H, Cβ-H), 3.34 (dd, \( ^3J = 14.9 \) Hz, \( ^3J = 9.0 \) Hz, 1H, Cβ-H), 1.41 (s, 9H, C(CH\(_3\))\(_3\)).

\( ^{13}C \) NMR (126 MHz, CDCl\(_3\)) \( \delta \) [ppm] = 173.0 (COOMe), 156.3 (NHCOO), 135.8 (C7a), 128.4 (C4a), 124.9 (C7), 123.2 (C2), 120.2 (C4), 120.1 (C5), 120.0 (C6), 102.9 (C3), 80.9 (C(CH\(_3\))\(_3\)), 54.3 (Cα), 52.8 (COO(CH\(_3\))\(_3\)), 36.9 (Cβ), 28.8 (C(CH\(_3\))\(_3\)).

MS (ESI): found [m/z] = 319.08 [M+H]\(^+\), 263.02 [M-(\text{tert}Butyl)+H]\(^+\), 219.04 [M-Boc+H]\(^+\), 202.02 [M-Boc-NH\(_2\)]\(^+\), calcd. [m/z] = 319.17 [M+H]\(^+\), 263.10 [M-(\text{tert}Butyl)+H]\(^+\), 219.11 [M-Boc+H]\(^+\), 202.09 [M-Boc-NH\(_2\)]\(^+\).
Nα-Boc-L-4-(1H-4-Indolyl)homoalanine benzyl ester (13a)

Nα-Boc-L-4-(1H-4-Indolyl)homoalanine benzyl ester (13a) was synthesized according to GP2. Therefore, 4-Bromoindole (1a, 32 µL, 255 µmol), zinc dust (65.9 mg, 1008 µmol, 4.0 eq.), Nα-Boc-L-4-iodo homoalanine benzyl ester (12, 223.4 mg, 523 µmol, 2.0 eq.) and Pd(ampmos)2Cl2 (8.9 mg, 13 µmol, 5 mol%) were placed in a glass vial followed by DMF (0.5 mL). The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was diluted with water (30 mL) and extracted with EtOAc (3 x 30 mL) the organic phase was dried over MgSO4 and the solvent removed in vacuum. The crude product was purified by automated column chromatography using a Büchi Reveleris X2 (Petrolether:EtOAc) giving 13a as a colourless solid (72.7 mg, 177 µmol, 70%).

LC-MS: \( t_R = 10.4 \text{ min} \);

\(^1\)H NMR (500 MHz, CDCl3) \( \delta \) [ppm] = 8.18 (s, 1H, Indole-NH), 7.39 – 7.30 (m, 5H, CPhenyl-H), 7.25 (d, \(^3\)J = 7.9 Hz, 1H, C7-H), 7.18 (dd, \(^3\)J = 2.9 Hz, \(^2\)J = 2.9 Hz, 1H, C2-H), 7.10 (dd, \(^3\)J = 7.6 Hz, \(^2\)J = 7.6 Hz, 1H, C6-H), 6.86 (d, \(^3\)J = 7.2 Hz, 1H, C5-H), 6.47 (dd, \(^4\)J = 3.0 Hz, \(^3\)J = 3.0 Hz, 1H, C3-H), 5.25 – 5.07 (m, 3H, OCONH/CH2Benzyl), 4.48 (ddd, \(^3\)J = 8.7 Hz, \(^3\)J = 6.6 Hz, \(^2\)J = 5.5 Hz, 1H, Cβ-H), 2.99 - 2.82 (m, 2H, Cμ-H), 2.30 (dd, \(^3\)J = 12.7 Hz, \(^3\)J = 5.5 Hz, 1H, Cβ-H), 2.09 (dd, \(^3\)J = 13.0 Hz, \(^3\)J = 6.6 Hz, 1H, Cβ-H), 1.45 (s, 9H, C(CH3)3).

\(^13\)C NMR (126 MHz, CDCl3) \( \delta \) [ppm] = 172.7 (COOBn), 155.5 (NHCOO), 135.8 (C7a), 135.5 (C\text{Benzyl}), 133.1 (C4a), 128.8 (C\text{Benzyl}), 128.6 (C\text{Benzyl}), 126.6 (C\text{Benzyl}), 127.2 (C4), 123.9 (C2), 122.3 (C5), 119.3 (C6), 109.4 (C7), 100.9 (C3), 80.0 (C(CH3)3), 67.2 ((C\text{H2})\text{Benzyl}), 53.8 (Cα), 33.4 (Cβ), 29.3 (Cμ), 28.5 (C(CH3)3).

MS (ESI): found [m/z] = 409.20 [M+H]+, 353.14 [M-(tertButyl)+H]+, 309.16 [M-Boc+H]+, 292.13 [M-Boc-NH2]+, calcld. [m/z] = 409.21 [M+H]+, 353.15 [M-(tertButyl)+H]+, 309.16 [M-Boc+H]+, 292.13 [M-Boc-NH2]+.
\[N^\alpha-\text{Boc-L-4-(1H-5-Indolyl)homoalanine benzyl ester (13b)}\]

\[N^\alpha-\text{Boc-L-4-(1H-5-Indolyl)homoalanine benzyl ester (13b)}\] was synthesized according to GP2. Therefore, 5-Bromoindole (1b, 49.0 mg, 250 \(\mu\)mol), zinc dust (68.9 mg, 1.053 mmol, 4.0 eq.), \(N^\alpha\)-Boc-\(\mu\)-iodo homoalanine benzyl ester (12, 213.1 mg, 518 \(\mu\)mol, 2.0 eq.) and \(\text{Pd(amporphos)}_2\text{Cl}_2\) (9.0 mg, 13 \(\mu\)mol, 5 mol\%) were placed in a glass vial followed by DMF (0.5 mL). The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was diluted with water (30 mL) and extracted with EtOAc (3 x 30 mL). The organic phase was dried over MgSO\(_4\) and the solvent removed in vacuum. The crude product was purified by automated column chromatography using a Büchi Revealeris X2 (Petrolether:EtOAc) giving \(N^\alpha\)-Boc-L-4-(1H-5-indolyl)homoalanine benzyl ester (13b) as a colourless solid (67.4 mg, 165 \(\mu\)mol, 66 %).

**LC-MS:** \(t_R = 10.3\) min;

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) [ppm] = 8.09 (s, 1H, Indole-NH), 7.41-7.32 (m, 6H, CPhenyl-H/C4-H), 7.29 (d, \(^3J = 8.2\) Hz, 1H, C7-H), 7.18 (dd, \(^3J = 2.9\) Hz, \(^3J = 2.9\) Hz, 1H, C2-H), 6.95 (dd, \(^3J = 8.3\) Hz, \(^4J = 1.8\) Hz, 1H, C6-H), 6.47 (d, \(^3J = 3.1\) Hz, 1H, C3-H), 5.18 (d, \(^2J = 12.5\) Hz, 1H, C\(\text{Benzyl}\)-H), 5.11 (d, \(^2J = 13.0\) Hz, 1H, C\(\text{Benzyl}\)-H), 5.07 (d, \(^3J = 6.9\) Hz, 1H, OCONH), 4.42 (ddd, \(^3J = 8.4\) Hz, \(^3J = 7.9\) Hz, \(^3J = 4.7\) Hz, 1H, C\(\alpha\)-H), 2.80-2.61 (m, 2H, C\(\mu\)-H), 2.18 (m, 1H, C\(\beta\)-H), 2.00 (m, 1H, C\(\beta\)-H), 1.45 (s, 9H, C(CH\(_3\))\(_3\)).

\(^13\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) [ppm] = 173.0 (COOBn), 155.8 (NHCOCO), 136.5 (C\(\text{7a}\)), 135.8 (C\(\text{Benzyl}\)), 135.1 (C\(\text{4a}\)), 129.0 (C\(\text{Benzyl}\)), 128.7 (C\(\text{Benzyl}\)), 128.6 (C\(\text{5}\)), 126.6 (C\(\text{Benzyl}\)), 124.3 (C\(\text{2}\)), 121.2 (C\(\text{6}\)), 121.0 (C\(\text{4}\)), 111.0 (C\(\text{7}\)), 102.8 (C\(\text{3}\)), 80.3 (C(CH\(_3\))\(_3\)), 67.4 ((CH\(_2\))\(_\text{Benzyl}\)), 53.8 (C\(\alpha\)), 35.4 (C\(\beta\)), 32.3 (C\(\mu\)) 28.7 (C(CH\(_3\))\(_3\)).

**MS (ESI):** found \([m/z] = 409.20\ [M+H]^+, 353.14\ [M-(\text{tert} Butyl)+H]^+, 309.16\ [M-Boc+H]^+, 292.13\ [M-Boc-NH\(_2\)]^+,\) calcd. \([m/z] = 409.21\ [M+H]^+, 353.15\ [M-(\text{tert} Butyl)+H]^+, 309.16\ [M-Boc+H]^+, 292.13\ [M-Boc-NH\(_2\)]^+.\)
$\text{N}^\alpha\text{-Boc-L-4-(1H-6-Indolyl)homoalanine benzyl ester (13c)}$

$\text{N}^\alpha\text{-Boc-L-4-(1H-6-Indolyl)homoalanine benzyl ester (13c)}$ was synthesized according to GP2. Therefore, 6-Bromoindole (1c, 50.1, 255 µmol), zinc dust (66.54 mg, 1.018 mmol, 4.0 eq.), $\text{N}^\alpha\text{-Boc-L-\mu-iodo homoalanine benzyl ester (12, 213.5 mg, 509 µmol, 2.0 eq.)}$ and $\text{Pd(ampphos)}_2\text{Cl}_2$ (8.9 mg, 13 µmol, 5 mol%) were placed in a glass vial followed by DMF (0.5 mL). The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was diluted with water (30 mL) and extracted with EtOAc (3 x 30 mL). The organic phase was dried over MgSO$_4$ and the solvent removed in vacuum. The crude product was purified by automated column chromatography using a Büchi Reveleris X2 (Petrolether:EtOAc) giving $\text{13c}$ as a colourless solid (60.5 mg, 148 µmol, 58%).

**LC-MS:** $t_R = 10.5 \text{ min}$;

$^{1}\text{H NMR}$ (500 MHz, CDCl$_3$) δ [ppm] = 8.06 (s, 1H, Indole-NH), 7.55 (d, $^3J = 8.1 \text{ Hz}$, 1H, C4-H), 7.41-7.32 (m, 5H, CPhenyl-H), 7.18 (dd, $^3J = 3.0 \text{ Hz}$, $^3J = 3.0 \text{ Hz}$, 1H, C2-H), 7.16 (s, 1H, C7-H), 6.91 (dd, $^3J = 8.1 \text{ Hz}$, $^4J = 1.6 \text{ Hz}$, 1H, C5-H), 6.52 (ddd, $^3J = 4.2 \text{ Hz}$, $^3J = 3.1 \text{ Hz}$, $^3J = 2.9 \text{ Hz}$, 1H, C3-H), 5.19 (d, $^2J = 12.4 \text{ Hz}$, 1H, C$_{$Benzyl$}$-H), 5.13 (d, $^2J = 12.4 \text{ Hz}$, 1H, C$_{$Benzyl$}$-H), 5.09 (d, $^3J = 8.3 \text{ Hz}$, 1H, OCONH), 4.44 (ddd, $^2J = 8.4 \text{ Hz}$, $^3J = 7.9 \text{ Hz}$, $^3J = 6.7 \text{ Hz}$, 1H, C$_{$a-H}$), 2.84-2.64 (m, 2H, C$_{$μ-H}$), 2.21 (m, 1H, C$_{$β-H}$), 2.03 (m, 1H, C$_{$β-H}$), 1.47 (s, 9H, C(C$_{H_3}$)$_3$).

$^{13}\text{C NMR}$ (126 MHz, CDCl$_3$) δ [ppm] = 172.8 (COOBn), 155.5 (NHCOO), 136.2 (C7a), 135.6 (C$_{Benzyl}$), 134.9 (C4a), 128.8 (C$_{Benzyl}$), 128.6 (C$_{Benzyl}$), 128.5 (C$_{Benzyl}$), 126.4 (C6), 124.0 (C2), 120.9 (C5), 120.8 (C4), 110.7 (C7), 102.6 (C3), 80.1 (C(CH$_3$)$_3$), 67.2 ((CH$_2$)$_{Benzyl}$), 53.6 (C$_a$), 35.1 (C$_{β}$), 32.0 (C$_μ$), 28.5 (C(CH$_3$)$_3$).

**MS (ESI):** found [m/z] = 409.21 [M+H]$^+$, 353.15 [M-(tertButyl)+H]$^+$, 309.16 [M-Boc+H]$^+$, 292.15 [M-Boc-NH$_2$]$^+$, calcd. [m/z] = 409.21 [M+H]$^+$, 353.15 [M-(tertButyl)+H]$^+$, 309.16 [M-Boc+H]$^+$, 292.13 [M-Boc-NH$_2$]$^+$. 
Nα-Boc-L-4-(1H-7-Indolyl)homoalanine benzyl ester (13d)

Nα-Boc-L-4-(1H-7-Indolyl)homoalanine benzyl ester (13d) was synthesized according to GP2. Therefore, 7-Bromoindole (1d, 51.1 mg, 260 µmol), zinc dust (31.0 mg, 497 µmol, 1.9 eq.), Nα-Boc-L-4-iodo homoalanine benzyl ester (12, 200.4 mg, 595 µmol, 2.2 eq.) and Pd(amphos)2Cl2 (9.1 mg, 13 µmol, 5 mol%) were placed in a glass vial followed by DMF (0.5 mL). The suspension was purged with argon and stirred at room temperature for 24 h. The crude mixture was diluted with water (30 mL) and extracted with EtOAc (3 x 30 mL) the organic phase was dried over MgSO4 and the solvent removed in vacuum. The crude product was purified by column chromatography using (Petrolether:EtOAc (5:1)) giving 13d as a colourless solid (75.6 mg, 185 µmol, 71 %).

**LC-MS**: $t_R = 10.9$ min;  

1H NMR (500 MHz, CDCl3) δ [ppm] = 8.81 (s, 1H, Indole-NH), 7.51 (d, $^3J = 7.8$ Hz, 1H, C4-H), 7.41 (m, 5H, CPhenyl-H), 7.14 (dd, $^3J = 2.7$ Hz, $^3J = 2.7$ Hz, 1H, C2-H), 7.03 (dd, $^3J = 7.6$ Hz, $^3J = 7.6$ Hz, 1H, C5-H), 6.93 (d, $^3J = 7.2$ Hz, 1H, C6-H), 6.53 (dd, $^4J = 2.0$, $^3J = 3.2$ Hz, 1H, C3-H), 5.26 (d, $^3J = 6.6$ Hz, 1H, OCONH), 5.24 (d, $^2J = 12.2$ Hz, 1H, CBenzy-H), 5.14 (d, $^2J = 12.2$ Hz, 1H, Cα-H), 2.89 (ddd, $^2J = 13.0$ Hz, $^3J = 9.6$ Hz, $^3J = 9.6$ Hz 1H, Cβ-H), 3.20 (ddd, $^2J = 13.1$ Hz, $^3J = 9.6$ Hz, $^3J = 6.1$ Hz 1H, Cµ-H), 2.30 (m, 1H, Cβ-H), 2.15 (m, 1H, Cµ-H), 1.46 (s, 9H, C(CH3)3).

13C NMR (126 MHz, CDCl3) δ [ppm] = 172.7 (COOBn), 155.7 (NHCOC), 135.4 (C7α), 135.1 (CβBenzy), 135.0 (C4α), 128.9 (CβBenzy), 128.8 (CβBenzy), 128.7 (CβBenzy), 128.1 (C7), 124.4 (C2), 121.7 (C6), 120.0 (C5), 119.1 (C4), 102.8 (C3), 80.5 (C(CH3)3), 67.5 ((CH2)Benzy), 53.9 (Cα), 33.3 (Cβ), 28.5 (C(CH3)3), 27.4 (Cµ).

**MS** (ESI): found $[m/z] = 409.21$ [M+H]+, 353.15 [M-(tertButyl)+H]+, 309.16 [M-Boc+H]+, 292.15 [M-Boc-NH2]+, calcd. $[m/z] = 409.21$ [M+H]+, 353.15 [M-(tertButyl)+H]+, 309.16 [M-Boc+H]+, 292.13 [M-Boc-NH2]+.
L-3-(1H-4-Indolyl)alanine (22a)

Nα-Boc-L-3-(1H-4-Indolyl)alanine methyl ester (11a) (348.7 mg, 1.095 mmol) was suspended in Na₂HPO₄-Buffer (100 mM, pH = 7.4, 15 mL). The mixture was heated to reflux for 9 hours. The aqueous phase was washed with EtOAc (2x15 mL). The solvent of the aqueous phase was reduced in vacuum and the crude product was desalted using an acid-free reversed phase C18-column chromatography, washing with pure MPW (100 mL) and eluting the product with MeOH (100 mL). The solvent was removed in vacuum, giving L-3-(1H-4-indolyl)alanine (22a) as a slightly yellow solid (122.9 mg, 0.602 mmol, 55 %). The enantiomeric excess was determined using Marfey’s Test (see GP3) giving an ee of 95 %.

**LC-MS:** $t_R = 2.8$ min;

$^1$H NMR (500 MHz, MeOD) $\delta$ [ppm] = 7.35 (d, $^3J = 8.2$ Hz, 1H, C7-H), 7.28 (d, $^3J = 3.1$ Hz, 1H, C2-H), 7.09 (dd, $^3J = 8.2$ Hz, $^3J = 7.1$ Hz, 1H, C6-H), 6.95 (d, $^3J = 7.2$ Hz, 1H, C5-H), 6.72 (d, $^3J = 3.1$ Hz, 1H, C3-H), 3.94 (dd, $^3J = 10.9$ Hz $^3J = 3.9$ Hz, 1H, Cα-H), 3.74 (dd, $^3J = 14.3$ Hz, $^3J = 3.9$ Hz, 1H, Cβ-H), 3.08 (dd, $^3J = 14.4$ Hz, $^3J = 10.8$ Hz, 1H, Cβ-H).

$^{13}$C NMR (126 MHz, CDCl₃) $\delta$ [ppm] = 174.8 (COOH), 137.9 (C7a), 128.8 (C3a), 128.7 (C4), 125.8 (C2), 122.5 (C6), 120.9 (C5), 111.8 (C7), 100.4 (C3), 57.1 (Cα), 37.0 (Cβ).

**MS (ESI):** found $m/z = 205.03$ [M+H]$^+$, calcd. $m/z = 205.10$ [M+H]$^+$.

**Marfey’s Derivatization:** $t_R$ (L-deriv.) = 6.3 min (97.5 %); $t_R$ (D-deriv.) = 6.9 min (2.5 %) found $m/z = 457.03$ [M+H]$^+$, calcd. $m/z = 457.15$ [M+H]$^+$. 

![Marfey's reagent](image)
L-3-(1H-5-Indolyl)alanine (22b)

\[ \text{N}^\text{a}-\text{Boc-L-3-(1H-5-Indolyl)alanine methyl ester (11b)} \]

(300.0 mg, 0.942 mmol) was suspended in Na$_2$HPO$_4$-Buffer (100 mM, pH = 7.4, 15 mL). The mixture was heated to reflux for 9 hours. The aqueous phase was washed with EtOAc (2x15 mL). The solvent of the aqueous phase was reduced in vacuum and the crude product was desalted using an acid-free reversed phase C18-column chromatography, washing with pure MPW (100 mL) and eluting the product with MeOH (100 mL). The solvent was removed in vacuum giving L-3-(1H-5-indolyl)alanine (22b) as a colourless solid (100.3 mg, 0.491 mmol, 52 %). The enantiomeric excess was determined using Marfey’s Test (see GP3) giving an ee of 94 %.

**LC-MS:** $t_R$ = 2.7 min;

\[ ^1\text{H} \text{NMR (500 MHz, DMSO-d$_6$) } \delta [\text{ppm}] = 11.03 \text{ (s, 1H, Indole-NH), 7.41 \text{ (s, 1H, C4-H), 7.30 \text{ (d, } ^3J = 8.3 \text{ Hz, 1H, C7-H), 7.29 \text{ (s, 1H, C2-H), 6.99 \text{ (d, } ^3J = 8.4 \text{ Hz, 1H, C6-H), 6.35 \text{ (m, 1H, C3-H), 3.40 \text{ (dd, } ^3J = 8.7 \text{ Hz, } ^3J = 4.3 \text{ Hz, 1H, Cα-H), 3.22 \text{ (dd, } ^3J = 14.3 \text{ Hz, } ^3J = 4.3 \text{ Hz, 1H, Cβ-H).}} } \]

\[ ^{13}\text{C NMR (126 MHz, DMSO-d$_6$) } \delta [\text{ppm}] = 169.9, 134.9 \text{ (C7a), 127.8 \text{ (C3a), 127.5 \text{ (C5), 125.3 \text{ (C2), 122.5 \text{ (C6), 120.5 \text{ (C4), 111.1 \text{ (C7), 100.8 \text{ (C3), 56.3 \text{ (Cα), 37.2 \text{ (Cβ).}}}} } \]

**MS (ESI):** found [m/z] = 205.04 [M+H]$^+$, calcd. [m/z] = 205.10 [M+H]$^+$.

Marfey’s Derivatization: $t_R$ (L-deriv.) = 6.6 min (97 %); $t_R$ (D-deriv.) = 7.3 min (3 %)

found [m/z] = 457.15 [M+H]$^+$, calcd. [m/z] = 457.15 [M+H]$^+$.
L-3-(1H-6-Indolyl)alanine (22c)

$N^\alpha$-Boc-L-3-(1H-6-Indolyl)alanine methyl ester (11c) (279.2 mg, 0.877 mmol) was suspended in Na$_2$HPO$_4$-Buffer (100 mM, pH = 7.4, 15 mL). The mixture was heated to reflux for 9 hours. The aqueous phase was washed with EtOAc (2x15 mL). The solvent of the aqueous phase was reduced in vacuum and the crude product was desalted using an acid-free reversed phase C18-column chromatography, washing with pure MPW (100 mL) and eluting the product with MeOH (100 mL). The solvent was removed in vacuum giving L-3-(1H-6-indolyl)alanine (22c) as a colourless solid (176.6 mg, 0.864 mmol, 98%). The enantiomeric excess was determined using Marfey’s Test (see GP3) giving an ee of 94%.

LC-MS: $t_R = 3.6$ min;

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ [ppm] = 11.10 (s, 1H, Indole-NH), 7.44 (d, $^3J = 8.1$ Hz 1H, C4-H), 7.29 (s, 1H, C7-H), 7.26 (dd, $^3J = 2.8$ Hz, $^3J = 2.8$ Hz, 1H, C2-H), 6.90 (d, $^3J = 8.1$ Hz, 1H, C5-H), 6.36 (dd, $^3J = 2.8$ Hz, 4J = 2.1 Hz 1H, C3-H), 3.38 (dd, $^3J = 8.5$ Hz $^3J = 4.0$ Hz, 1H, Cα-H), 3.22 (dd, $^3J = 14.2$ Hz, $^3J = 4.0$ Hz, 1H, Cβ-H), 2.89 (dd, $^3J = 14.2$ Hz, $^3J = 8.5$ Hz, 1H, Cβ-H).

$^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ = 169.8 (COOH), 136.2 (C7a), 130.2 (C3a), 126.3 (C6), 124.9 (C2), 120.6 (C4), 119.8 (C5), 112.1 (C7), 100.7 (C3), 56.3 (Cα), 37.5 (Cβ)

MS (ESI): found [m/z] = 205.03 [M+H]$^+$, calcd. [m/z] = 205.10 [M+H]$^+$.

Marfey’s Derivatization: $t_R$ (L-deriv.) = 6.4 min (97 %); $t_R$ (D-deriv.) = 7.2 min (3 %) found [m/z] = 457.15 [M+H]$^+$, calcd. [m/z] = 457.15 [M+H]$^+$. 
L-3-(1H-7-Indolyl)alanine (22d)

$N^\alpha$-Boc-L-3-(1H-7-Indolyl)alanine methyl ester (11d) (253.4 mg, 0.796 mmol) was suspended in Na$_2$HPO$_4$-Buffer (100 mM, pH = 7.4, 15 mL). The mixture was heated to reflux for 9 hours. The aqueous phase was washed with EtOAc (2x15 mL). The solvent of the aqueous phase was reduced in vacuum and the crude product was desalted using an acid-free reversed phase C18-column chromatography, washing with pure MPW (100 mL) and eluting the product with MeOH (100 mL). The solvent was removed in vacuum giving L-3-(1H-7-indolyl)alanine (22d) as a colourless solid (160.5 mg, 0.786 mmol, 99 %). The enantiomeric excess was determined using Marfey’s Test (see GP3) giving an ee of 38 %.

LC-MS: $t_R$ = 4.1 min;

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ [ppm] = 12.18 (br s, 1H, Indole-NH), 7.40 (d, $^3$J = 7.8 Hz, 1H, C4-H), 7.30 (d, $^3$J = 3.2 Hz, 1H, C2-H), 6.95-6.86 (m, 2H, C5-H/C6-H), 6.40 (d, $^3$J = 3.1 Hz, 1H, C3-H), 3.44 (m, 1H, C$\alpha$-H), 3.36 (dd, $^3$J = 14.3 Hz, $^3$J = 4.2 Hz, 1H, C$\beta$-H), 3.07 (dd, $^3$J = 14.2 Hz, $^3$J = 6.3 Hz, 1H, C$\beta$-H).

$^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ = 173.2 (COOH), 135.6 (C7a), 127.7 (C3a), 125.1 (C7), 122.4 (C2), 121.7 (C4), 118.7 (C6), 118.3 (C5), 101.2 (C3), 55.2 (Cα), 35.6 (Cβ).

MS (ESI): found [m/z] = 205.03 [M+H]+, calcd. [m/z] = 205.10 [M+H]+.

Marfey’s Derivatization: $t_R$ (L-deriv.) = 7.7 min (69 %); $t_R$ (D-deriv.) = 9.1 min (31 %) found [m/z] = 457.04 [M+H]+, calcd. [m/z] = 457.15 [M+H]+.
Nα-Boc-L-7-Butyltryptophan methyl ester (18a)

Method A: Nickel-catalysed reductive cross-coupling

Nα-Boc-L-7-Butyltryptophan methyl ester (18a) was synthesized according to GP1. Nα-Boc-L-7-bromotryptophan methyl ester (17) (49.2 mg, 124 μmol), NiI₂ (4.7 mg, 13 μmol, 10 mol%) and 4,4'-Di-tert-butyl-2,2'dipyridine (3.8 mg, 13 μmol, 10 mol%) were placed in a glass vial under argon atmosphere. Everything was suspended in DMPU (0.5 mL) and purged with argon. 1-iodobutane (3, 31 μL, 252 μmol, 2.2 eq.), pyridine (1.0 μL, 13 μmol, 10 mol%) and manganese (27.4 mg, 499 μmol, 4.0 eq.) were added. The green reaction mixture was heated to 60 °C and stirred for 21 h. The mixture was purified directly by column chromatography (Petrolether/EtOAc; 3:1) providing 18a as a colorless solid (32.2 mg, 86 μmol, 70%).

Method B: Negishi cross-coupling

Nα-Boc-L-7-butyl tryptophan methyl ester (18a) was synthesized according to GP2. Therefore, Nα-Boc-L-7-bromotryptophan methyl ester (17, 39.1 mg, 98 μmol), zinc dust (13.4 mg, 207 μmol, 2.1 eq.), 1-iodobutane (3, 22.7 μL, 200 μmol, 2.0 eq.) and Pd(amphos)Cl₂ (3.6 mg, 0.5 μmol, 5 mol%) were placed in a glass vial followed by DMF (0.2 mL). The suspension was degassed by sparking it with argon and stirred at 37°C for 24 h. The mixture was purified by column chromatography (Petrolether/EtOAc; 4:1) providing 18a as a colorless solid (30.5 mg, 81 μmol, 83%).

LC-MS: tᵣ = 11.0 min;

1H NMR (500 MHz, CDCl₃) δ [ppm] = 8.01 (s, Indole-NH), 7.40 (d, 3J = 7.9 Hz, 1H, C4-H), 7.06 (dd, 3J = 7.5 Hz, 3J = 7.5 Hz, 1H, C5-H), 7.03-6.99 (m, 2H, C2-H/C6-H), 5.07 (d, 3J = 7.9 Hz, 1H, OCONH), 4.65 (dd, 3J = 8.0 Hz, 3J = 5.2 Hz, 3J = 5.2 Hz, 1H, Cα-H), 3.69 (s, 3H, OC₃H₃), 3.31 (dd, 2J = 14.4 Hz, 3J = 5.2 Hz, 1H, Cβ-H), 2.81 (t, 3J = 7.7 Hz, 2H, C1'-H), 1.72 (tt, 3J = 7.7 Hz, 3J = 7.5 Hz, 2H, C2'-H), 1.50-1.25 (m, 11H, C(C₃H₃)₃/C₃'-H), 0.96 (t, 3J = 7.3 Hz, 3H, C4'-H).

13C NMR (126 MHz, CDCl₃) δ [ppm] = 172.9 (COOME), 155.4 (NHOCO), 135.3 (C7a), 127.7 (C4a), 125.4 (C2), 122.4 (C6), 121.8 (C7), 120.0 (C5), 116.6 (C4), 110.9 (C3), 79.9 (C(CH₃)₃), 54.3 (Cα), 52.4 (COO(CH₃)), 31.9 (C2'), 31.0 (C1'), 28.5 (C(CH₃)₃), 28.2 (Cβ), 22.9 (C3') 14.1 (C4').

MS (ESI): found [m/z] = 375.23 [M+H]⁺, 319.17 [M-(tertButyl)+H]⁺, 275.18 [M-Boc+H]⁺, 258.15 [M-Boc-NH₂]⁺, calcd. [m/z] = 375.23 [M+H]⁺, 319.17 [M-(tertButyl)+H]⁺, 275.18 [M-Boc+H]⁺, 258.15 [M-Boc-NH₂]⁺.
\(N^\alpha\)-Boc-L-7-Heptyltryptophan methyl ester (7)

\(N^\alpha\)-Boc-L-7-Heptyltryptophan methyl ester (7) was synthesized according to GPkll. \(N^\alpha\)-Boc-L-7-bromo-tryptophan methyl ester (17) (20.0 mg, 50 \(\mu\)mol), NiI\(_2\) (2.0 mg, 5 \(\mu\)mol, 10 mol\%) and 4,4''-Di-tert-butyl-2,2'-dipyridine (1.4 mg, 5 \(\mu\)mol, 10 mol\%) were placed in a glass vial under argon atmosphere. Everything was suspended in DMPU (0.2 mL) and purged with argon. 1-iodoheptane (4, 18 \(\mu\)L, 110 \(\mu\)mol, 2.2 eq.), pyridine (0.5 \(\mu\)L, 6 \(\mu\)mol, 12 mol\%) and manganese (11.1 mg, 203 \(\mu\)mol, 4.0 eq.) were added. The green reaction mixture was heated to 60 °C and stirred for 21 The mixture was purified directly by column chromatography (Petrolether/EtOAc; 3:1) providing 7 as a colorless solid (17.2 mg, 41 \(\mu\)mol, 82 %).

LC-MS: \(t_R = 11.7\) min;

\(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) [ppm] = 10.79 (s, Indole-NH), 7.30 (d, \(^3\)J = 7.8 Hz, 1H, C4-H), 7.18 (d, \(^3\)J = 7.8 Hz, 1H, OCONH), 7.12 (d, \(^3\)J = 2.1 Hz, 1H, C2-H), 6.91 (dd, \(^3\)J = 7.5 Hz, \(^3\)J = 7.5 Hz, 1H, C5-H), 6.86 (d, \(^3\)J = 7.0 Hz, 1H, C6-H), 4.20 (ddd, \(^3\)J = 9.3 Hz, \(^3\)J = 8.0 Hz, \(^3\)J = 4.7 Hz, 1H, Cα-H), 3.09 (dd, \(^3\)J = 14.4, \(^3\)J = 4.8 Hz, 1H, Cβ-H), 2.97 (dd, \(^3\)J = 14.5 Hz, \(^3\)J = 9.4 Hz, 1H, Cβ-H), 2.79 (t, \(^3\)J = 7.6 Hz, 2H, C1'-H), 1.63 (tt, \(^3\)J = 7.5 Hz, \(^3\)J = 7.5 Hz, 2H, C2'-H), 1.32 (s, 9H, C(CH\(_3\))\(_3\)), 1.29 – 1.15 (m, 8H, C3'-H/C4'-H/C5'-H/C6'-H), 0.85 (t, \(^3\)J = 6.9 Hz, 3H, C7'-H).

\(^13\)C NMR (126 MHz, DMSO-\(d_6\)) \(\delta\) [ppm] = 173.4 (COOMe), 155.8 (NHCOO), 135.4 (C7a), 127.4 (C3a), 125.9 (C7), 123.8 (C2), 120.9 (C6), 119.0 (C5), 116.0 (C4), 110.5 (C3), 78.7 (C(CH\(_3\))\(_3\)), 55.1 (Ca), 52.2 (OCH\(_3\)), 31.8 (C1'), 31.1 (C2'), 30.1 (C3'), 29.5 (C4'), 29.1 (C5'), 28.6 (C6'), 27.4 (C(CH\(_3\))\(_3\)), 22.6 (Cβ), 14.4 (C7').

MS (ESI): found [m/z] = 417.28 [M+H]\(^+\), 361.21 [M-(tertButyl)+H]\(^+\), 317.27 [M-Boc+H]\(^+\), 300.20 [M-Boc-NH\(_2\)]\(^+\), calcd. [m/z] = 417.27 [M+H]\(^+\), 361.21 [M-(tertButyl)+H]\(^+\), 317.27 [M-Boc+H]\(^+\), 300.20 [M-Boc-NH\(_2\)]\(^+\).
Nα-Boc-L-7-Cyclohexyltryptophan methyl ester (18d)

Nα-Boc-L-7-Cyclohexyltryptophan methyl ester (18c) was synthesized according to GP2. Nα-Boc-L-7-bromo-tryptophan methyl ester (XX) (40.0 mg, 100 μmol), zinc dust (13.2 mg, 202 μmol, 2.0 eq.), cyclohexyl iodide (15, 26 μL, 201 μmol, 2.0 eq.) and Pd(amphos)Cl2 (3.5 mg, 0.5 µmol, 5 mol%) were placed in a glass vial followed by DMF (0.2 ml). The suspension was degassed by sparkling it with argon and stirred at 37°C for 24 h. The mixture was purified by column chromatography (Petrolether/EtOAc; 4:1) providing 18d as a colorless solid (29.4 mg, 73 µmol, 73 %).

LC-MS: tR = 11.2 min;

1H NMR (600 MHz, DMSO-d6) δ [ppm] = 10.80 (d, 3J = 2.1 Hz, indole-NH), 7.29 (dd, 3J = 7.4 Hz, 3J = 1.8 Hz, 1H, C4-H), 7.18 (d, 3J = 7.8 Hz, 0.8H, OCONH), 7.12 (d, 3J = 2.6 Hz, 1H, C2-H), 6.96-6.93 (m, 2H, C6-H/C5-H), 6.81 (d, 3J = 6.5 Hz, 0.2H, OCONH), 4.20 (dd, 3J = 9.3 Hz, 3J = 8.0 Hz, 3J = 5.1 Hz, 1H, Cα-H), 3.62 (s, 3H, OCH3), 3.09 (dd, 3J = 14.6, 3J = 5.2 Hz, 1H, Cβ-H), 3.03-2.90 (m, 2H, Cβ-H/C1’-H), 1.90-1.71 (m, 4H, C2’-H1.38-1.20 (m, 10H, C(CH3)3; cis/trans ratio 4:1/C4’-).

13C NMR (151 MHz, DMSO-d6) δ [ppm] = 172.9 (COOMe), 155.4 (NHCOO), 135.3 (C7a), 130.8 (C3a), 127.0 (C7), 123.3 (C2), 118.8 (C6), 117.2 (C5), 115.5 (C4), 110.1 (C3), 78.2 (C(CH3)3, 54.6 (Cα), 51.7 (OCH3), 38.3 (C1’), 32.9 (C2’), 32.8 (C6’), 28.1 (C(CH3)3), 27.7 (Cβ), 26.8 (C3’), 26.5 (C5’), 25.8 (C4’).

MS (ESI): found [m/z] = 401.25 [M+H]+, 345.19 [M-(tert-Butyl)+H]+, 301.19 [M-Boc+H]+, 284.17 [M-Boc-NH2]+; calcd. [m/z] = 401.24 [M+H]+, 345.18 [M-(tert-Butyl)+H]+, 301.19 [M-Boc+H]+, 284.16 [M-Boc-NH2]+.
**Nα-Boc-L-7-benzyltryptophan methyl ester (18c)**

**Nα-Boc-L-7-Benzyltryptophan methyl ester (18c)** was synthesized according to GP2. Therefore, **Nα-Boc-L-7-bromotryptophan methyl ester (17, 41.1 mg, 100 µmol)**, zinc dust (13.1 mg, 200 µmol, 2.0 eq.), freshly distilled benzyl iodide (9, 39.5 µL, 200 µmol, 2.0 eq.) and Pd(amphos)Cl2 (3.5 mg, 0.5 µmol, 5 mol%) were placed in a glass vial followed by DMF (0.2 mL). The suspension was degassed by sparkling it with argon and stirred at 37°C for 24 h. The mixture was purified by column chromatography (Petrolether/EtOAc; 4:1) providing **18c** as a colorless solid (33.0 mg, 81 µmol, 81 %).

**LC-MS:** $t_R = 10.0$ min;

$^1$H NMR (500 MHz, DMSO-$d_6$) δ [ppm] = 10.92 (s, Indole-NH), 7.34 (d, $^3J = 7.9$ Hz, 1H, C4-H), 7.29 (m, 2H, C2'-H), 7.25 (m, 2H, C3'-H), 7.20 (d, $^3J = 7.8$ Hz, 1H, OCONH), 7.18-7.15 (m, 2H, C4'-H/C2-H), 6.93 (dd, $^3J = 7.8$ Hz, $^3J = 7.0$ Hz, 1H, C5-H), 6.85 (d, $^3J = 7.0$ Hz, 1H, C6-H), 4.20 (ddd, $^3J = 9.2$ Hz, $^3J = 8.0$ Hz, $^3J = 5.3$ Hz, 1H, Cα-H), 4.15 (s, 2H, CH2), 3.60 (s, 3H, OCH3), 3.10 (dd, $^3J = 14.4$, $^3J = 5.3$ Hz, 1H, Cβ-H), 2.99 (dd, $^3J = 14.6$ Hz, $^3J = 9.2$ Hz, 1H, Cβ-H), 1.33 (s, 9H, C(CH3)3; cis/trans ratio 5:1);

$^{13}$C NMR (151 MHz, DMSO-$d_6$) δ [ppm] = 172.9 (COOMe), 155.4 (NHCOO), 140.6 (C1'), 134.9 (C7a), 128.7 (C2'), 128.2 (C3'), 127.2 (C3a), 125.9 (C4'), 124.3 (C7), 123.7 (C2), 121.2 (C4), 118.8 (C6), 116.1 (C5), 110.3 (C3), 78.2 (C(CH3)3), 54.6 (Cα), 51.8 (OCH3), 36.3 (CH2), 28.2 (C(CH3)3), 26.9 (Cβ).

MS (ESI): found [m/z] = 409.20 [M+H]+, 353.15 [M-(tertButyl)+H]+, 309.16 [M-Boc+H]+, 292.13 [M-Boc-NH2]+; calcd. [m/z] = 409.21 [M+H]+, 353.15 [M-(tertButyl)+H]+, 309.16 [M-Boc+H]+, 292.13 [M-Boc-NH2]+.
Dimethyl (2S,2'S)-3,3’-(1H-indole-3,7-diyl)bis(2-((tert-butoxycarbonyl)amino)propanoate) (18b)

Dimethyl (2S,2'S)-3,3’-(1H-indole-3,7-diyl)bis(2-((tert-butoxycarbonyl)amino)propanoate) (18b) was synthesized according to GP3. Nα-Boc-L-7-Bromotryptophan methyl ester (17, 39.1 mg, 98 µmol), zinc dust (13.0 mg, 199 µmol, 2.0 eq.), Nα-Boc-L-3-iodoalanine methyl ester (8, 65.9 mg, 200 µmol, 2.0 eq.) and Pd(amphos)Cl2 (3.6 mg, 0.5 µmol, 5 mol%) were placed in a glass vial followed by DMF (0.2 mL). The suspension was degassed by sparkling it with argon and stirred at 37°C for 24 h. The mixture was purified by column chromatography (Petrolether/EtOAc; 2:1) providing 18b as a colorless solid (27.9 mg, 54 µmol, 55 %).

LC-MS: $t_R = 9.6$ min;

$^1$H NMR (500 MHz, DMSO-$_d_6$) $\delta$ [ppm] = 10.86 (s, Indole-NH, cis/trans ratio 4:1), 7.36 (d, $^3J = 7.8$ Hz, 1H, C4-H), 7.27 (d, $^3J = 7.5$ Hz, 1H, OCONH$_{Ala}$), 7.20 (d, $^3J = 7.5$ Hz, 1H, OCONH$_{Trp}$), 7.15 (d, $^3J = 2.6$ Hz, 1H, C2-H), 6.92 (dd, $^3J = 7.7$ Hz, $^3J = 7.7$ Hz, 1H, C5-H), 6.86 (d, $^3J = 7.5$ Hz, 1H, C6-H), 4.31 (dd, $^3J = 7.9$ Hz, $^3J = 7.9$ Hz, 1H, Cα$_{Ala}$), 4.19 (ddd, $^3J = 9.2$ Hz, $^3J = 7.5$ Hz, $^3J = 5.3$ Hz 1H, Cα$_{Trp}$), 3.60 (s, 3H, OCH$_3$), 3.56 (s, 3H, OCH$_3$), 3.29 (d, $^3J = 7.9$ Hz, 1H, C2-H), 2.99 (dd, $^2J = 14.9$ Hz, $^3J = 9.2$ Hz, 1H, Cβ-H), 1.34 (s, 9H, C(CH$_3$)$_3$$_{Ala}$; cis/trans ratio 4:1), 1.32 (s, 9H, C(CH$_3$)$_3$$_{Trp}$; cis/trans ratio 4:1);

$^{13}$C NMR (126 MHz, DMSO-$_d_6$) $\delta$ [ppm] = 172.9 (COOMe), 172.8 (COOMe), 155.4 (NHCOO), 155.3 (NHCOO), 135.2 (C7a), 127.1 (C3a), 123.7 (C2), 121.4 (C4), 120.5 (C7), 118.5 (C6), 116.5 (C5), 110.2 (C3), 78.2 (C(CH$_3$)$_3$), 54.6 (Cα$_{Trp}$), 53.8 (Cα$_{Ala}$), 51.7 (OCH$_3$), 51.6 (OCH$_3$), 32.2 (C$_{(CH_3)}$), 28.2 (C$_{(CH_3)}$), 28.1 (C$_{(CH_3)}$), 26.8 (Cβ$_{Trp}$);

MS (ESI): found [m/z] = 520.26 [M+H]$^+$, 464.21 [M-($^{tert}$Butyl)+H]$^+$, 420.21 [M-Boc+H]$^+$, 403.13 [M-Boc-NH$_2$]$^+$, 364.15 [M-Boc-($^{tert}$Butyl)+H]$^+$, 347.12 [M-Boc-($^{tert}$Butyl)-NH$_2$]$^+$, 320.16 [M-2Boc+H]$^+$, 303.13 [M-2Boc-NH$_2$]$^+$; calcld. [m/z] = 520.27 [M+H]$^+$, 464.20 [M-($^{tert}$Butyl)+H]$^+$, 420.21 [M-Boc+H]$^+$, 403.18 [M-Boc-NH$_2$]$^+$, 364.15 [M-Boc-($^{tert}$Butyl)+H]$^+$, 347.12 [M-Boc-($^{tert}$Butyl)-NH$_2$]$^+$, 320.16 [M-2Boc+H]$^+$, 303.13 [M-2Boc-NH$_2$]$^+$.
**Nα-Boc-L-7-Butyl tryptophan (20a)**

*Nα*-Boc-L-7-butyl tryptophan (20a) was synthesized according to GP2. Therefore, *Nα*-Boc-L-7-bromotryptophan (19, 38.3 mg, 97 μmol), zinc dust (13.2 mg, 202 μmol, 2.1 eq.), 1-iodobutane (3, 22.7 μL, 200 μmol, 2.0 eq.) and Pd(amphos)2Cl2 (3.5 mg, 0.5 μmol, 5 mol%) were placed in a glass vial followed by DMF (0.2 mL). The suspension was degassed by sparkling it with argon and stirred at 37°C for 24 h. The mixture was purified by preparative RP-HPLC providing 20a as a colorless solid (16.0 mg, 44 μmol, 46%).

**LC-MS:** *t*<sub>R</sub> = 9.6 min;

**1H NMR** (500 MHz, DMSO-<sup>d6</sup>) δ [ppm] = 12.58 (br s, 1H, COO), 10.76 (s, 1H, Indole-NH), 7.33 (d, 3<sub>J</sub> = 7.8 Hz, 1H, C4-H), 7.11 (d, 3<sub>J</sub> = 2.4 Hz, 1H, C2-H), 6.90 (dd, 3<sub>J</sub> = 7.4 Hz, 3<sub>J</sub> = 7.4 Hz, 1H, C5-H), 6.86 (d, 3<sub>J</sub> = 7.0 Hz, 1H, C6-H), 4.13 (ddd, 3<sub>J</sub> = 9.3 Hz, 3<sub>J</sub> = 7.0 Hz, 3<sub>J</sub> = 4.7 Hz, 1H, Co-H), 3.11 (dd, 3<sub>J</sub> = 14.5 Hz, 3<sub>J</sub> = 4.7 Hz, 1H, Cβ-H), 2.95 (dd, 2<sub>J</sub> = 14.6 Hz, 3<sub>J</sub> = 9.3 Hz, 1H, Cβ-H), 2.79 (t, 3<sub>J</sub> = 7.7 Hz, 2H, C1′-H), 1.62 (tt, 3<sub>J</sub> = 8.1 Hz, 3<sub>J</sub> = 7.6 Hz, 2H, C2′-H), 1.39-1.34 (m, 2H, C3′-H) 1.32 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub> (cis/trans ratio 5:1)), 0.91 (t, 3<sub>J</sub> = 7.3 Hz, 3H, C4′-H).

**13C NMR** (126 MHz, DMSO-<sup>d6</sup>) δ [ppm] = 173.9 (COOH), 155.4 (NHCOO), 135.0 (C7a), 127.2 (C4a), 125.3 (C2), 123.3 (C6), 120.4 (C7), 118.5 (C5), 115.8 (C4), 110.5 (C3), 77.9 (C(CH<sub>3</sub>)<sub>3</sub>), 54.5 (Cα), 31.8 (C2′), 30.4 (C1′), 28.2 (C(CH<sub>3</sub>)<sub>3</sub>), 27.8 (Cβ), 22.1 (C3′) 13.9 (C4′).

**MS (ESI):** found [**m/z**] = 361.20 [M+H]<sup>+</sup>, 305.14 [M-({tert-Butyl})+H]<sup>+</sup>, 261.15 [M-Boc+H]<sup>+</sup>, 244.13 [M-Boc-NH2]<sup>+</sup>, calcd. [**m/z**] = 361.21 [M+H]<sup>+</sup>, 305.15 [M-({tert-Butyl})+H]<sup>+</sup>, 261.16 [M-Boc+H]<sup>+</sup>, 244.13 [M-Boc-NH2]<sup>+</sup>. 

L-7-Butyl tryptophan (21a)

L-7-butyl tryptophan (21a) was synthesized according to GP5. Therefore, Nα-Boc-L-7-butyl tryptophan methyl ester (18a) (9.5 mg, 25 µmol) was suspended in aqueous HCl (5 M, 2 mL) and heated to 90 °C for 2 hours. The crude reaction mixture was purified by reversed phase HPLC, providing 21a as a colorless solid (8.0 mg, 21 µmol, 84 %). The enantiomeric excess was determined using Marfey’s Test (see GP3) giving an ee higher 99 %.

**LC-MS:** $t_R = 6.0$ min;

$^1$H NMR (500 MHz, DMSO-d$_6$) δ [ppm] = 10.83 (d, $^3$J = 2.6 Hz, 1H, Indole-NH), 7.52 (br s, 3H, NH$_3^+$), 7.37 (d, $^3$J = 6.7 Hz, 1H, C4-H), 7.17 (d, $^3$J = 2.6 Hz, 1H, C2-H), 6.91 (dd, $^3$J = 6.7 Hz, $^3$J = 6.0 Hz, 1H, C5-H), 6.87 (d, $^3$J = 6.0 Hz, 1H, C6-H), 3.52 (dd, $^3$J = 8.8 Hz $^3$J = 4.2 Hz, 1H, Cα-H), 3.29 (dd, $^3$J = 15.2 Hz, 1H, Cβ-H), 2.97 (dd, $^3$J = 15.2 Hz, $^3$J = 8.8 Hz, 1H, Cβ-H), 2.80 (t, $^3$J = 7.6 Hz, 2H, C1'-H), 1.62 (tt, $^3$J = 7.6 Hz, $^3$J = 6.5 Hz, 2H, C2'-H), 1.36 (t, $^3$J = 7.3 Hz, 2H, C3'-H) 0.91 (t, $^3$J = 7.4 Hz, 3H, C4'-H).

$^{13}$C NMR (126 MHz, DMSO-d$_6$) δ [ppm] = 169.9 (COOH), 135.2 (C7a), 127.1 (C3a), 125.5 (C7), 123.7 (C2), 120.5 (C6), 118.5 (C5), 115.9 (C4), 109.6 (C3), 54.4 (Cα), 31.8 (C2'), 30.4 (C1'), 27.1 (Cβ), 22.1 (C3') 13.9 (C4').

MS (ESI): found [m/z] = 261.09 [M+H]$^+$, 244.06 [M-NH$_2$]$^+$, calcd. [m/z] = 261.16 [M+H]$^+$, 244.13 [M-NH$_2$]$^+$.

Marfey’s Derivatization: $t_R$ (L-deriv.) = 12.7 min (>99 %)

found [m/z] = 513.09 [M+H]$^+$, calcd. [m/z] = 513.13 [M+H]$^+$.
L-7-Cyclohexyl tryptophan (21c)

L-7-butyl tryptophan (21c) was synthesized according to GP5. Therefore, Nα-Boc-L-7-cyclohexyl tryptophan methyl ester (18d, 10.0 mg, 25 μmol) was suspended in aqueous HCl (5 M, 2 mL) and heated to 90 °C for 2 hours. The crude reaction mixture was purified by reversed phase HPLC, providing 21c as a colorless solid (5.2 mg, 18 μmol, 73%). The enantiomeric excess was determined using Marfey’s Test (see GP3) giving an ee higher 99 %.

LC-MS: tR = 6.3 min;

1H NMR (500 MHz, DMSO-d6) δ [ppm] = 10.95 (d, 3J = 2.3 Hz, 1H, Indole-NH), 7.95 (br s, 3H, NH3+), 7.38 (d, 3J = 6.8 Hz, 1H, C4-H), 7.19 (d, 3J = 2.7 Hz, 1H, C2-H), 6.96 (dd, 3J = 6.9 Hz, 3J = 6.8 Hz, 1H, C5-H), 6.95 (d, 3J = 6.9 Hz, 1H, C6-H), 3.95 (dd, 3J = 7.4 Hz, 3J = 5.1 Hz, 1H, Cα-H), 3.27 (dd, 2J = 15.2 Hz, 3J = 5.1 Hz, 1H, Cβ-H), 3.14 (dd, 2J = 15.0 Hz, 3J = 7.4 Hz, 1H, Cβ-H), 2.98 (m, 1H, C1'-H), 1.83 (m, 4H, C2'-H), 1.79 (m, 1H, C4'-H), 1.49 (m, 4H, C3'-H) 1.31 (m, 1H, C4'-H).

13C NMR (126 MHz, DMSO-d6) δ [ppm] = 170.7 (COOH), 134.5 (C7a), 130.8 (C3a), 127.0 (C7), 124.2 (C2), 118.9 (C6), 117.4 (C5), 115.7 (C4), 107.8 (C3), 53.2 (Cα), 38.3 (C1'), 32.9 (C2'), 26.5 (C3'/C4'), 25.8 (Cβ).

MS (ESI): found [m/z] = 287.19 [M+H]+, 270.15 [M-NH2]+, calcd. [m/z] = 287.18 [M+H]+, 270.15 [M-NH2]+.

Marfey’s Derivatization: tR (L-deriv.) = 14.7 min (>99 %)

found [m/z] = 539.20 [M+H]+, calcd. [m/z] = 539.22 [M+H]+.
