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Electronic Supporting Information

for

Solid Phase Total Synthesis of the 3-Amino-6-hydroxy-2-piperidone (Ahp) cyclodepsipeptide and protease inhibitor Symplocamide A

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General methods and instruments

Reagents

Reagents were purchased from Acros, Senn Chemicals, Fluka, J.T. Baker, Merck, Novabiochem, Riedel de Haen, Iris, Roth or Sigma-Aldrich and were used without further purification. Anhydrous solvents were purchased from commercial suppliers.

Thin layer chromatography (TLC)

TLC was carried out on Merck aluminium precoated silica gel plates (20 × 20 cm, $60F_{254}$) using ultraviolet light irradiation at 254 nm or the following developing reagents. Eluents and R_f values are given with the respective experiment.

Reagent A: 20 g of phosphomolybdic acid hydrate in 80 mL ethanol

Reagent B: 1.5 g $KMnO_4$, 10 g K_2CO_3 and 1.25 mL of 10% aq. NaOH in 200 mL water.

Silica gel flash liquid chromatography

Column chromatography purifications were performed with silica gel from Acros (particle size 35-70 µm).

Reversed-phase liquid chromatography - electrospray ionization mass spectrometry (LC-MS)

LC-MS analyses were performed on an HPLC system from Agilent (1200 series) with an Eclipse XDB-C18, 5 µm column from Agilent (peak detection at 210 nm) and a Thermo Finnigan LCQ Advantage Max ESI-Spectrometer. A linear gradient of solvent B (0.1% formic acid in acetonitrile) in solvent A (0.1% formic acid in water) was used at 1 mL/min flow rate.

Gradient C_{18}: 0 min / 10% B → 1 min / 10% B → 10 min / 100% B → 12 min / 100% B → 15 min / 10% B.

Alternatively, a system consisting of an Agilent (1100 series) HPLC from Hewlett-Packard (peak detection at 210 nm) equipped with a CC 250/4 Nucleosil 120-5 C4 column from Macherey-Nagel, connected to a Thermo Finnigan LCQ Advantage Max ESI-Spectrometer was used. A linear gradient of solvent B (0.1% formic acid in acetonitrile) in solvent A (0.1% formic acid in water) was employed at 1mL/min flow rate.

Gradient C_4: 0 min / 10% B → 1 min / 10% B → 10 min / 100% B → 12 min / 100% B → 15 min / 10% B.

Preparative reversed-phase high performance liquid chromatography (prep HPLC)

Purification of the compounds was performed on a Varian HPLC system (Pro Star 215) with a VP 250/21 Nucleosil C18PPN-column from Macherey-Nagel and detection at 210 nm. Linear gradients of solvent B (0.1% TFA in acetonitrile) in solvent A (0.1% TFA in water) were applied at a 25 mL/min flow rate.

Nuclear magnetic resonance spectroscopy (NMR)

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury 400 system (400 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR), a Bruker Avance DRX 500 system (500 MHz for ^{1}H- and 126 MHz for ^{13}C-NMR) or a Varian Unity Inova 600 system (600 MHz for ^{1}H- and 151 MHz for ^{13}C-NMR). ^{1}H NMR spectra are reported in the following manner: chemical shifts (δ) in ppm calculated with reference to the residual signals of undeuterated solvent, multiplicity (s, singlet; d, doublet; t, triplet; dd, doublet of doublet; dt, doublet of triplet; td, m, multiplet; b, broad signal), coupling constants (J) in Hertz (Hz), and number of protons (H).

High resolution mass spectrometry (HRMS)

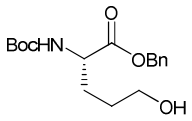
HRMS measurements were performed on a TRACE GC Ultra DFS (GC/EI) instrument from Thermo Scientific. Alternatively, an Accela/LTQ Orbitrap (LC/ESI) system from Thermo Scientific was used.

Optical rotation

Optical rotations were measured with a Polartronic HH8 polarimeter from Schmidt + Haensch. Concentrations *c* are given in g/100 mL, the solvent used is given with the respective data.

Solution synthesis of the Ahp precursor 8

(*S*)-Benzyl-2-(*tert*-butoxycarbonylamino)-5-hydroxypentanoate (1)



Boc-(*S*)-glutamic acid benzyl ester (5.00 g, 14.8 mmol) was dissolved in anhydrous THF (60 mL) under an argon atmosphere and cooled to -15 ℃. Successive addition of triethylamine (6.2 mL, 44.5 mmol, 3 eq.) and ethyl chloroformate (4.2 mL, 44.5 mmol, 3 eq.) resulted in the formation of a white precipitate. The reaction mixture was stirred for 1 h at -15 ℃. Sodium borohydride (2.24 g, 59.3 mmol, 4 eq.) dissolved in water (60 mL) was added slowly at -15 ℃, after 1 h the reaction mixture was allowed to warm to RT. A TLC check after 5 h indicated the completition of the reaction.

The reaction mixture was quenched with 1 M aq. $KHSO_4$ solution (100 mL) and the aqueous phase was extracted with ethyl acetate (3x 100 mL). The combined organic extracts were washed with brine (150 mL) and dried over Na_2SO_4. After evaporation to dryness, the crude product was purified by flash column chromatography (50% ethyl acetate in cyclohexane), affording (*S*)-benzyl-2-(*tert*-butoxycarbonylamino)-5-hydroxypentanoate (**1**).

Yield: 4.03 g (12.5 mmol, 84%) as a pale yellow oil.

TLC (50% ethyl acetate in cyclohexane): R_f = 0.36.

$[\alpha]_D^{20}$ -2.3 (*c* 0.98 in $CHCl_3$) (lit, [1].$[\alpha]_D^{24}$ -3.9 (*c* 1.0 in $CHCl_3$))

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.34 (m, 5 H), 5.10-5.19 (m, 2H), 4.29-4.34 (m, 1H), 3.58 (t, J = 6.20 Hz, 2H), 1.93-2.00 (m, 1H), 1.84-1.91 (m, 1H), 1.66-1.76 (m, 1H), 1.53-1.60 (m, 2H), 1.41 (s, 9H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6, 141.0, 135.3, 128.5, 128.4, 128.2, 79.8, 65.0, 61.8, 53.2, 29.2, 28.2, 25.4.

LC-MS: t_R = 8.90 min (C_{18}), *m/z* = 346($M^+ + Na^+$, 30%), 224(M^+ - Boc, 100).

HRMS (EI)*: m/z* calcd for $C_{17}H_{25}O_5N^+$ $[M + H]^+$ 324.1805, found 324.1810.

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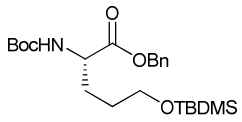
[1] F. Yokokawa, A. Inaizumi and T. Shioiri, *Tetrahedron*, 2005, **61**, 1459-1480.

(*S*)-Benzyl-2-(*tert*-butoxycarbonylamino)-5-(*tert*-butyldimethylsilyloxy)pentanoate (2)



(*S*)-Benzyl-2-(*tert*-butoxycarbonylamino)-5-hydroxypentanoate (**1**) (3.92 g, 12.1 mmol) was dissolved in DCM (80 mL) and cooled to 0 °C. After the addition of imidazole (2.00 g, 30.3 mmol, 1.2 eq.) and *tert*-butyldimethylsilyl chloride (2.19 g, 14.5 mmol , 2.5 eq.) the reaction mixture was stirred at 0 °C for 1 h and allowed to warm to RT over night. The reaction was quenched by the addition of water (80 mL) and the phases were separated. The aqueous phase was extracted with ethyl acetate (3x 80 mL), the combined extracts were washed with 1M aq. $KHSO_4$ solution (150 mL) and aq. saturated $NaHCO_3$ solution (150 mL) and dried over Na_2SO_4. After evaporation to dryness the crude product was purified by flash column chromatography (10% ethyl acetate in cyclohexane) affording (*S*)-Benzyl-2-(*tert*-butoxycarbonylamino)-5-(*tert*-butyldimethyl-silyloxy)pentanoate (**2**).

Yield: 5.05 g (11.5 mmol, 95%) as a yellow oil.

TLC (10% ethyl acetate in cyclohexane): R_f = 0.35.

$[\alpha]_D^{20}$ -5.8 (*c* 1.13 in $CHCl_3$).

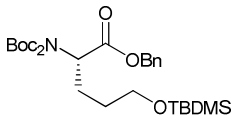
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.35 (m, 5H), 5.13-5.19 (m, 2H), 4.31-4,36 (m, 1H), 3.58-3.61 (t, J = 6.05, 2H), 1.94-1.95 (m, 1H), 1.76-1.85 (m, 1H), 1.51-1.56 (m, 2H), 1.43 (s, 9H), 0.88 (s, 9H), 0.03 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.6, 155.4, 135.5, 128.5, 128.3, 128,1, 79.6, 66.8, 62.2, 53.3, 29.0, 28.3, 25.9, 18.3, -5.4 .

LC-MS: t_R = 13.31 min (C_{18}), *m/z* = 460($M^+ + Na^+$, 40%), 338(M^+ - Boc, 100).

HRMS (EI)*: m/z* calcd for $C_{23}H_{40}O_5NSi^+$ $[M + H]^+$ 438.2670, found 438.2670.

(*S*)-Benzyl-2-(bis(tert-butoxycarbonyl)amino)-5-(*tert*-butyldimethylsilyloxy)-pentanoate (3)



(*S*)-Benzyl-2-(*tert*-butoxycarbonylamino)-5-(*tert*-butyldimethylsilyloxy)pentanoate (**2**) (4.94 g, 11.3 mmol) was dissolved in acetonitrile (80 mL). 4-(Dimethylamino)pyridine (2.07 g, 16.9 mmol, 1.5 eq.) was added at RT and the mixture was stirred for 10 min. Di-*tert*-butyl dicarbonate (13.0 mL, 56.5 mmol, 5 eq.) was added and the reaction mixture was stirred over night at RT, thereby turning from a pale yellow color to bright red. The reaction was quenched by the addition of water and the aqueous phase was extracted with ethyl acetate (3x 80 mL). The combined organic extracts were washed with 1M aq. HCl (150 mL) and brine (4x 150 mL). After evaporation to dryness the crude mixture of product and unreacted starting material was purified by flash column chromatography (5% ethyl acetate in cyclohexane), eluting (*S*)-Benzyl-2-(bis(tert-butoxycarbonyl)amino)-5-(*tert*-butyldimethylsilyloxy)pentanoate (**3**) first and then the starting material **2**.

Yield: 3.88 g (7.2 mmol, 64%, 81% b.r.s.m.) as a colorless oil.

TLC (5% ethyl acetate in cyclohexane): R_f = 0.26.

$[\alpha]_D^{20}$ -20.2 (*c* 1.26 in $CHCl_3$).

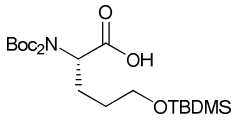
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31-7.33 (m, 5H), 5.11-5.18 (m, 2H), 4.88-4.92 (m, 1H), 3.61-3.65 (dt, J = 6.40, 1.38 Hz, 2H), 2.12-2.14 (m, 1H), 1.91-2.01 (m, 1H), 1.54-1.61 (m, 2H), 1.44 (s, 18H), 0.88 (s, 9H), 0.03 (s, 6H) .

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 152.2, 135.7, 128.4, 128.0, 127.9, 82.9, 66.7, 62.6, 58.1, 29.6, 27.9, 26.0, 25.9, 18.3, -5.3.

LC-MS: t_R = 11.29 min (C_4), *m/z* = 1097($2M^+ + Na^+$, 100%), 438(M^+ - Boc, 35).

HRMS (EI)*: m/z* calcd for $C_{56}H_{94}O_{14}N_2NaSi_2^+$ $[2M + Na]^+$ 1097.6136, found 1097.6141.

(*S*)-2-(bis(*tert*-Butoxycarbonyl)amino)-5-(*tert*-butyldimethylsilyloxy)pentanoic acid (4)



(*S*)-Benzyl-2-(bis(*tert*-butoxycarbonyl)amino)-5-(*tert*-butyldimethylsilyloxy)pentanoate (**3**) (5.85 g, 10.9 mmol) was dissolved in ethyl acetate (80 mL) and transferred to a 2-neck round bottom flask equipped with a rubber septum and a stop-cock under an argon atmosphere. Palladium on activated charcoal (10%) (2.93 g, 5 %wt) was added at RT in small portions through a funnel, after each portion the funnel was flushed with ethyl acetate (70 mL in total). After complete addition the flask was equipped with a hydrogenation balloon and carefully flushed by opening the stop-cock from time to time. The hydrogenation was carried out for 2.5 h, then the reaction mixture was filtered through a pad of Celite®, which was rinsed thoroughly with ethyl acetate (4x 100 mL). Evaporation of the solvent afforded (*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-5-(*tert*-butyldimethylsilyloxy)pentanoic acid (**4**).

Yield: 4.84 g (10.8 mmol, 99%) as a white solid.

$[\alpha]_D^{20}$ -19.8 (*c* 0.91 in $CHCl_3$).

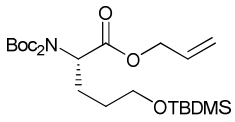
^{1}H NMR (400 MHz, $CDCl_3$): δ 4.91-4.95 (m, 1H), 3.62-3.65 (dt, J = 6.30, 1.74 Hz, 2H), 2.11-2.20 (m, 1H), 1.91-2.00 (m, 1H), 1.54-1.59 (m, 2H), 1.50 (s, 18H), 0.89 (s, 9H), 0.04 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 177.1, 152.0, 83.2, 62.5, 57.8, 29.5, 27.9, 26.1, 25.9, 18.3, -5.3.

LC-MS: t_R = 11.31 min (C_{18}), *m/z* = 917($2M^+ + Na^+$, 100%), 347(M^+ - Boc, 20).

HRMS (EI)*: m/z* calcd for $C_{42}H_{82}O_{14}N_2NaSi_2^+$ $[2M + Na]^+$ 917.5197, found 917.5191.

(*S*)-Allyl-2-(bis(*tert*-butoxycarbonyl)amino)-5-(*tert*-butyldimethylsilyloxy)-pentanoate (5)



(*S*)-2-(bis(*tert*-butoxycarbonyl)amino)-5-(*tert*-butyldimethylsilyloxy)pentanoic acid (**4**) (4.61 g, 10.3 mmol) and cesium carbonate (3.36 g, 10.3 mmol, 1 eq.) were dissolved in anhydrous methanol (40 mL). The solvent was evaporated and the resulting cesium salt of **4** was co-evaporated twice with toluene (60 mL) to dryness. The resulting white solid was suspended in DMF (80 mL) and allyl bromide (17.4 mL, 206 mmol, 20 eq.) was added slowly at RT. The reaction mixture was stirred over night at RT, the solvent was evaporated and the residue was co-evaporated twice with toluene to remove remaining DMF. Purification by flash chromatography (10% ethyl acetate in cyclohexane) afforded (*S*)-allyl-2-(bis(*tert*-butoxycarbonyl)amino)-5-(*tert*-butyldimethylsilyloxy)pentanoate (**5**).

Yield: 4.75 g (9.7 mmol, 95%) as a pale yellow oil.

TLC (5% ethyl acetate in cyclohexane): R_f = 0.13.

$[\alpha]_D^{20}$ -20.1 (*c* 1.21 in $CHCl_3$).

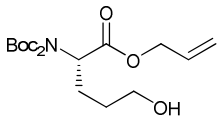
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.81-5.93 (m, 1H), 5.28-5.32 (dd, J = 17.20, 1.55 Hz, 1H), 5.18-5.21 (dd, J = 10.50, 1.45 Hz, 1H), 4.86-4.88 (m, 1H), 4.58-4.60 (m, 2H), 3.60-3.64 (dt, J = 6.40, 1.80 Hz, 2H), 2.12-2.21 (m, 1H), 1.89-1.96 (m, 1H), 1.54-1.60 (m, 2H), 1.48 (s, 9H), 0.87 (s, 9 xH), 0.03 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 152.1, 131.9, 117.9, 82.9, 65.5, 62.6, 58.1, 29.6, 27.9, 26.2, 25.9, 18.3, -5.3.

LC-MS: t_R = 14.37 min, *m/z* = 510(M^+ + Na^+, 100%), 410(M^+ - Boc + Na^+, 80).

HRMS (EI)*: m/z* calcd for $C_{24}H_{45}O_7NNaSi^+$ $[M + Na]^+$ 510.2858, found 510.2860.

(*S*)-Allyl-2-(bis(*tert*-butoxycarbonyl)amino)-5-hydroxypentanoate (6)



(*S*)-Allyl-2-(bis(*tert*-butoxycarbonyl)amino)-5-(*tert*-butyldimethylsilyloxy)pentanoate (**5**) (4.63 g, 9.5 mmol) was dissolved in a solution of 5% water in acetone (100 mL) at RT. Copper(II) chloride dihydrate (81 mg, 0.5 mmol, 0.05 eq.) was added and the reaction was heated to reflux. After refluxing for 1h a TLC check indicated the complete conversion of the starting material. The solvent was evaporated to dryness, the crude product was purified by flash column chromatography (33% ethyl acetate in cyclohexane) affording (*S*)-Allyl-2-(bis(*tert*-butoxycarbonyl)amino)-5-hydroxypentanoate (**6**).

Yield: 3.27 g (8.7 mmol, 92%) as a colorless oil.

TLC (33% ethyl acetate in cyclohexane): R_f = 0.26.

$[\alpha]_D^{20}$ -30.2 (*c* 1.01 in $CHCl_3$).

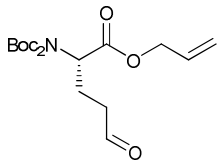
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.82-5.91 (m, 1H), 5.26-5.31 (dd, J = 17.20, 1.50 Hz, 1H), 5.17-5.20 (dd, J = 10.50, 1.40 Hz, 1H), 4.85-4.89 (m, 1H), 4.57-4.59 (m, 2H), 3.62-3.65 (t, J = 6.40, 2H), 2.17-2.26 (m, 1H), 1.87-1.97 (m, 1H), 1.57-1.64 (m, 2H), 1.46 (s, 18H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 152.1, 131.7, 118.0, 83.1, 65.6, 62.1, 57.9, 29.3, 27.9, 26.1.

LC-MS: t_R = 9.90 min (C_{18}), *m/z* = 396(M^+ + Na^+, 100%), 295(M^+ - Boc + Na^+, 80), 173(M^+ - 2Boc, 70).

HRMS (EI)*: m/z* calcd for $C_{18}H_{31}O_7NNa^+$ $[M + Na]^+$ 396.1990, found 396.1993.

(*S*)-Allyl-2-(bis(*tert*-butoxycarbonyl)amino)-5-oxopentanoate (7)



(*S*)-Allyl-2-(bis(*tert*-butoxycarbonyl)amino)-5-hydroxypentanoate (**6**) (1.60 g, 4.3 mmol) was dissolved in anhydrous DCM under an argon atmosphere. Dess-Martin´s periodinane (1.91 g, 4.5 mmol, 1.05 eq.) was added at RT. The reaction was stirred at RT for 1.5h, when a TLC check indicated the complete conversion. The reaction mixture was quenched with aq. saturated K_2CO_3 solution (60 mL), the phases were allowed to separate and the aqueous layer was extracted with ethyl acetate (3x 60 mL). The combined extracts were washed with brine (150 mL) and dried over Na_2SO_4. After evaporation to dryness the crude product was purified by flash column chromatography (17% ethyl acetate in cyclohexane) affording (*S*)-allyl-2-(bis(*tert*-butoxycarbonyl)amino)-5-oxopentanoate (**7**) which was directly used for the next step.

Yield: 1.34 g (3.6 mmol, 84%) as a colorless oil.

TLC (17% ethyl acetate in cyclohexane): R_f = 0.23.

$[\alpha]_D^{20}$ -26.2 (*c* 1.26 in $CHCl_3$).

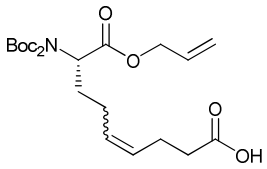
^{1}H NMR (400 MHz, $CDCl_3$): δ 9.75 (s, 1H), 5.83-5.92 (m, 1H), 5.27-5.32 (dd, J = 17.20, 1.50 Hz, 1H), 5.18-5.21 (dd, J = 10.50, 1.40 Hz, 1H), 4.86-4.90 (m, 1H), 4.58-4.60 (m, 2H), 2.48-2.59 (m, 3H), 2.13-2.20 (m, 1H), 1.47 (s, 18H).

^{13}C NMR (100 MHz, $CDCl_3$): δ 200.8, 169.9, 152.0, 131.7, 118.2, 83.4, 65.7, 57.4, 40.4, 27.9, 22.3.

LC-MS: t_R = 11.11 min (C_{18}), *m/z* = 394($M^+ + Na^+$, 80%), 294(M^+ - Boc + Na^+, 90).

HRMS (EI)*: m/z* calcd for $C_{18}H_{30}O_7N^+$ $[M + H]^+$ 372.2017, found 372.2018.

(*S*)-9-(Allyloxy)-8-(bis(*tert*-butoxycarbonyl)amino)-9-oxonon-4-enoic acid (8)



3-(Carboxypropyl)triphenylphosphonium bromide (1.92 g, 4.5 mmol, 1.5 eq.) was suspended in anhydrous THF (20 mL) under an argon atmosphere. 1 M Lithium bis(trimethylsilyl) amide solution in THF (9.7 mL, 9.7 mmol, 3.25 eq.) was added within 40 min to the suspension at RT and stirring was continued at RT for further 15 min. The resulting dark red solution was cooled to 0 ℃ and (*S*)-allyl-2-(bis(*tert*-butoxycarbonyl)-amino)-5-oxopentanoate (**7**) (1.11 g, 3.0 mmol) dissolved in THF (20 mL) was added within 40 min, after the addition the reaction mixture was allowed to warm to room temperature. A TLC check after 4h indicated the completition of the reaction.

2 M HCl was added to the reaction mixture until an acidic pH was obtained. The aqueous phase was extracted with ethyl acetate (3x 40mL) and the combined extracts were dried over Na_2SO_4. After evaporation to dryness the crude mixture was purified by flash column chromatography (50% ethyl acetate in cyclohexane) affording (*S*)-9-(Allyloxy)-8-(bis(*tert*-butoxycarbonyl)amino)-9-oxonon-4-enoic acid (**8**) in a 9:1 ratio (*Z*/*E*).

Yield: 824 mg (1.9 mmol, 64%) as a yellow oil.

TLC (50% ethyl acetate in cyclohexane): R_f = 0.35.

^{1}H NMR (500 MHz, $CDCl_3$): δ) 5.83-5.91 (m, 1H), 5.43-5.44 (t, J = 5.20 Hz 1H), 5.37-5.42 (dt, J = 11.10 Hz, 5.90 Hz, 1H), 5.27-5.31 (d, J = 16.20 Hz, 1H), 5.17-5.19 (d, J = 10.50 Hz, 1H), 4.83-4.86 (m, 1H), 4.57-4.58 (d, J = 5.35 Hz, 2H), 2.28-2.40 (m, 4H), 2.11-2.19 (m, 2H), 2.02-2.06 (m, 1H), 1.89-1.95 (m, 1H), 1.47 (s, 18H).

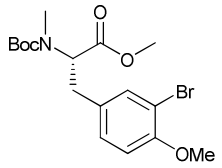
^{13}C NMR (126 MHz, $CDCl_3$): δ 178.9, 170.5, 152.1, 131.8, 130.3, 130.0, 128.9, 128.4, 117.9, 83.0, 65.6, 57.8, 33.9, 29.7, 29.5, 29.1, 27.9.

LC-MS: t_R = 10.73 min (C_{18}), *m/z* = 486(M^+ + 2Na^+, 45%), 464(M^+ + Na^+, 45), 242(M^+ - 2Boc, 100).

HRMS (EI)*: m/z* calcd for $C_{22}H_{36}O_8N^+$ $[M + H]^+$ 442.2435, found 442.2436.

Synthesis of the N,O-dimethyl3-bromotyrosine building block 16

(*S*)-Methyl-3-(3-bromo-4-methoxyphenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)-propanoate (15)



The starting material (*S*)-Methyl-3-(3-bromo-4-methoxyphenyl)-2-(*tert*-butoxycarbonylamino)propanoate (**14**) was prepared in 3 steps following a literature procedure[2].

To achieve the methylation of the amine, sodium hydride (60% suspension in mineral oil, 187 mg, 4.7 mmol, 1.2 eq.) was suspended in DMF (5 mL). (*S*)-Methyl-3-(3-bromo-4-methoxyphenyl)-2-(*tert*-butoxy-carbonylamino)propanoate (**14**) (1.51 mg, 3.9 mmol) and methyl iodide (927 µL, 15.6 mmol, 4 eq.) were dissolved in DMF (5 mL) and added to the sodium hydride suspension.

After stirring for 1h, the reaction was slowly quenched by the addition of aq. saturated NH_4Cl solution (10 mL). The aqueous phase was extracted with ether (3x 20 mL) and the combined organic extracts were washed with brine (2x 20 mL) and dried over Na_2SO_4. After evaporation to dryness the crude product was purified by flash column chromatography (33% ethyl acetate in cyclohexane) affording (*S*)-methyl-3-(3-bromo-4-methoxyphenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)-propanoate (**15**).

Yield: 1.41 g (3.5 mmol, 90%) as a colorless oil.

TLC (33% ethyl acetate in cyclohexane): $R_f = 0.54$.

$[\alpha]_D^{20}$ – 57.1 (*c* 0.93 in $CHCl_3$).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35 (s, 1H) 7.01-7.10 (m, 1H), 6.78-6.80 (d, J = 8.40 Hz, 1H), 4.80 (m, 0.5H), 4.50-4.51 (m, 0.5H) (Boc rotamers), 3.83 (s, 3H), 3.72 (s, 3H), 3.17-3.19 (m, 1H), 2.87-2.96 (m, 1H), 2.70 (s, 3H), 1.33-1.37 (d, J = 20.20 Hz, 9H) .

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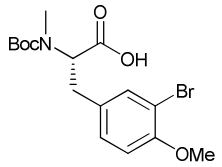
[2] M. Prieto, S. Mayor, K. Rodriguez, P. Lloyd-Williams and E. Giralt, *J. Org. Chem.*, 2007, **72**, 1047-1050.

^{13}C NMR (126 MHz, $CDCl_3$): δ (some peaks appear double due to Boc rotamers, for this case the second peak is given in parentheses) 171.5 (171.2), 155.7, 154.8 (154.6), 133.6 (133.5), 131.2 (131.0), 129.0 (128.0), 111.9, 111,5 (111.3), 80.3 (80.0), 61.2, 56.2, 52.1, 34.2 (33.7), 32.2 (32.0), 28.1.

LC-MS: t_R = 10.93 min (C_{18}), *m/z* = 302(M^+ - Boc, 100%).

HRMS (EI)*: m/z* calcd for $C_{17}H_{25}O_5NBr^+$ $[M + H]^+$ 402.0911, found 402.0910, *m/z* calcd for $C_{17}H_{25}O_5N^{18}Br^+$ $[M + H]^+$ 404.0890, found 404.0889.

(*S*)-3-(3-Bromo-4-methoxyphenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)propanoic acid (16)



(*S*)-Methyl-3-(3-bromo-4-methoxyphenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)-propanoate (**15**) (1.30 g, 3.2 mmol) was dissolved in a mixture of THF (10 mL) and methanol (6 mL). Lithium hydroxide (155 mg, 6.5 mmol, 2 eq.) dissolved in water (4 mL) was added to the solution at RT. The reaction mixture was stirred for 5h at RT.

1 M HCl was added to the reaction mixture until a pH of ~4 was reached. The solution was then diluted with brine (20 mL) and DCM (20 mL). The phases were separated and the aqueous phase was extracted with DCM (20 mL). The combined extracts were dried over Na_2SO_4. After evaporation to dryness the crude product was purified by flash column chromatography (10% methanol in chloroform) to afford (*S*)-3-(3-Bromo-4-methoxyphenyl)-2-(*tert*-butoxycarbonyl(methyl)amino)propanoic acid (**16**).

Yield: 985 mg (2.5 mmol, 78%) as white solid.

TLC (10% methanol in chloroform): R_f= 0.41.

$[\alpha]_D^{20}$ -44.3 (*c* 1.15 in $CHCl_3$).

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37-7.39 (d, J =10.50 Hz, 1H), 7.04-7.13 (m, 1H), 6.81-6.83 (d, J = 8.40 Hz, 1H), 4.74-4.75 (m, 0.5H), 4.61-4.62 (m, 0.5H) (Boc rotamers), 3.86 (s, 3H), 3.21-3.24 (m, 1H), 2.91-3.05 (m, 1H), 2.69-2.76 (d, J = 32.20 Hz, 3H), 1.35-1.41 (d, J = 27.15 Hz, 9H).

^{13}C NMR (126 MHz, $CDCl_3$): δ (some peaks appear double due to Boc rotamers, for this case the second peak is given in parentheses) 176.0 (175.8), 156.2, 154.8 (154.7), 133.7 (133.5), 131.0 (130.8), 129.0 (128.9), 112.0, 111.7 (117.2), 80.8, 61.1 (60.4), 56.2, 34.0 (33.5), 33.1 (32.3), 28.2 (28.1).

LC-MS: t_R = 9.45 min (C_{18}), *m/z* = 288(M^+ - Boc, 90%).

HRMS (EI)*: m/z* calcd for $C_{16}H_{23}O_5NBr^+$ $[M + H]^+$ 388.0754, found 388.0756, *m/z* calcd for $C_{16}H_{23}O_5N^{18}Br^+$ $[M + H]^+$ 390.0734, found 390.0735.

Solid phase synthesis of Symplocamide A

General methods

Method A: *Boc deprotection and neutralization*

The dry resin was transferred into a syringe reactor and swelled in an appropriate amount of DCM for 10 min. The solvent was removed, a freshly prepared solution of 50% trifluoracetic acid in DCM was added and the resulting suspension was shaken for 5 min. The solution was removed, fresh TFA/DCM solution was added and the resulting suspension was shaken for 1h. The resin was filtered off and washed with DCM (2x 1 min) and 2-propanol (2x 1 min).
For neutralization, a 10% solution of triethyl amine in DCM was added to the resin and the resulting suspension was shaken for 10 min. This procedure was repeated once more. The resin was washed alternatingly with DCM and 2-propanol (3x 1 min) and finally with DMF (2x 1 min).

Method B: *Fmoc deprotection*

A solution of 20% piperidine in DMF was added to resin and the resulting suspension was shaken for 15 min. The solution was removed from the resin and the resin was washed with DMF (5x 1 min). Fresh piperidine/DMF solution was added to resin and the resulting suspension was again shaken for 15 min. The solution was removed, the resin was washed alternatingly with methanol and DCM (3x 1 min) and finally with DMF (5x 1 min).

Method C: *amino acid coupling*

4 eq. of amino acid were dissolved in an appropriate amount of DMF. Subsequently 4 eq. HOBt, 3.5 eq. HBTU and 3 eq. DIPEA were added to the amino acid solution which was shaken gently for 1 min. The solution was added to the deprotected resin, which was shaken for an appropriate time (2 to 5h, respective values given in the experiment description). After this time, the solution was removed and the resin was washed with

DMF (2x 1 min), alternatingly with methanol and DCM (3x 1 min) and then again with DMF (5x 1 min).

Method D: *Fmoc determination*

A small resin aliquot (~ 5 mg) was transferred into a 25 mL pear shaped flask and a freshly prepared 20% piperidine in DMF solution (5 mL) was added to the resin (in total 10 mL of the piperidine/DMF solution were prepared for latter dilution and to use this solution as a blank for the UV measurement). The solution was shaken for 20 min. The resin was allowed to settle and the UV absorption of 1 mL of the cleavage solution or a dilution (e.g 33%) with piperidine/DMF solution was measured, using a Cary instrument type 100 Bio. The silica glass cuvettes had a thickness of 10 mm. The measurement was repeated at least 3 times.
According to the formula

$$c = \frac{A \cdot V}{\varepsilon \cdot d \cdot m \cdot F}\left[mmol / g\right]$$

in which *A* is the absorption at 300.7 nm, *V* the total volume (here: 5 [mL]), ε the extinction coefficient (here: 780 [ml/mmol · mm]), *d* the thickness of the cuvette (here: 10 [mm]), *m* the mass of resin in [g] and *F* the dilution factor (1, 1/3, 1/6, …) the loading of the resin was determined from the measurement of the absorption.

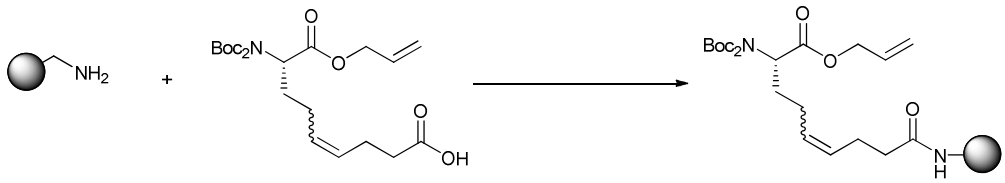
Method E: *Kaiser test*

A few beads were sampled into a small vial. 3 drops of a 5% solution of ninhydrine in ethanol, 2 drops of a 20% solution of phenole in ethanol and 3 drops of a 2% solution of aq. potassium cyanide (0.001M) in pyridine were added and the resulting suspension was heated to 100 ℃ for 5 min. A blue solution and/or blue beads indicated the presence of primary amines.

Method F: *Chloranil test*

A few beads were sampled into a small vial. 2 drops of a 2% solution of acetaldehyde in DMF and 2 drops of a 2% solution of chloranil in DMF were added to the resin. The resulting suspension was allowed to stand for 5 min at RT. Blue- to green-stained beads indicated the presence of secondary amines.

Coupling of (*S*)-9-(Allyloxy)-8-(bis(*tert*-butoxycarbonyl)amino)-9-oxonon-4-enoic acid (8) to NovaPEG amino resin



8 **9**

NovaPEG amino resin (0.66 mmol/g, 1.00 g, 0.66 mmol, 1 eq.) was transferred to a solid phase reactor under an argon atmosphere and swelled in anhydrous DCM (20 mL) for 1h. (*S*)-9-(Allyloxy)-8-(bis(*tert*-butoxycarbonyl)amino)-9-oxonon-4-enoic acid (**8**) (729 mg, 1.65 mmol, 2.5 eq.), HOBt (357 mg, 2.64 mmol, 4 eq.), HBTU (877 mg, 2.31 mmol, 3.5 eq.) and anhydrous DIPEA (327 µL, 1.98 mmol, 3 eq.) were dissolved in a solution of 10% anhydrous DMF in anhydrous DCM (15 mL) and preactivated for 30 min. The solvent was removed from the resin, which was resupended in a DMF/DCM solution (15 mL) and gently shaken for 10 min. The amino acid solution was added to the resin and the resulting suspension was shaken for further 24h.

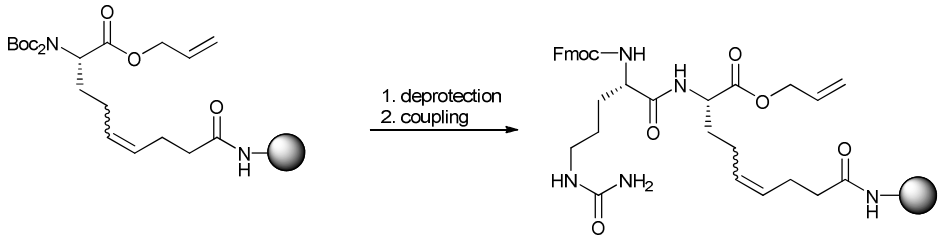
The reaction solution was removed and the resin was washed with DMF (2x 1 min), methanol (2x 1 min) and DCM (2x 1 min).

A capping step was subsequently performed to eliminate unreacted amines. To this end, a solution of DCM, DIPEA and acetic anhydride (3:1:1, (v/v/v)) was added to the resin and the resulting suspension was shaken for 2h. This procedure was repeated once more. The resin was washed with DMF (5x 1 min), alternatingly with methanol and DCM (3x 1 min) and finally with DMF (2x 1 min). A subsequent Kaiser test performed

according to *method E* was negative, thereby indicating complete capping of all unreacted amines.

The resin loaded with the Ahp precursor will be called NovaPEG-Ahp5COAll (**9**) in the following descriptions.

Coupling of Fmoc-Cit-OH to NovaPEG-Ahp5COAll (9)



9 **17**

The resin **9** (0.66 mmol) was deprotected according to *method A*. The coupling of Fmoc-Cit-OH to the resin was performed according to *method C*. The amounts of reagent were:

Fmoc-Cit-OH: 1.05 g, 2.64 mmol

HOBt: 357 mg, 2.64 mmol

HBTU: 877 mg, 2.31 mmol

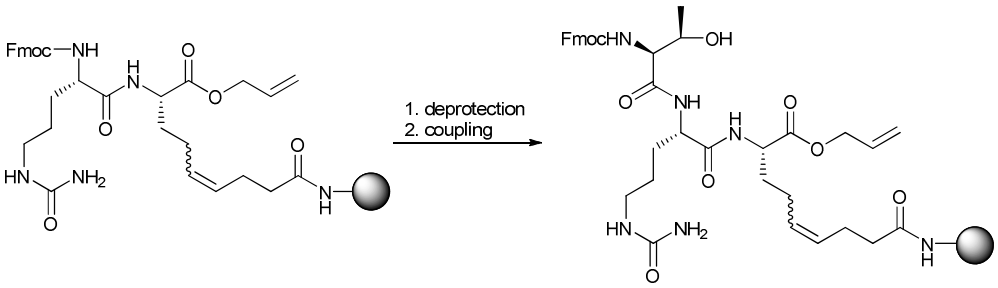
DIPEA: 327 µL, 1.98 mmol.

The coupling reaction was performed for 4h. After the regular washing, an additional washing procedure consisting of washing alternatingly with anhydrous methanol and DCM (3x 1 min) and finally anhydrous ether (3x 1 min) was performed. The resin was then dried under high vacuum over night.

A Kaiser test according to *method E* remained negative, thereby indicating quantitative coupling.

The loading of NovaPEG-Ahp5COAll-Cit4-Fmoc (**17**) was measured as described in *method D* using 5.8 mg of resin and resulted in loading determination of 0.24 mmol/g (36% loading yield).

Coupling of Fmoc-Thr-OH to NovaPEG-Ahp5COAll-Cit4-Fmoc (17)



NovaPEG-Ahp5COAll-Cit4-Fmoc (**17**) (0.24 mmol/g, 1.12 g, 0.27 mmol) was deprotected according to *method B*. The coupling of Fmoc-Thr-OH to the resin was performed using *method C.* The amounts of reagent were:

Fmoc-Thr-OH·H_2O: 388 mg, 1.08 mmol

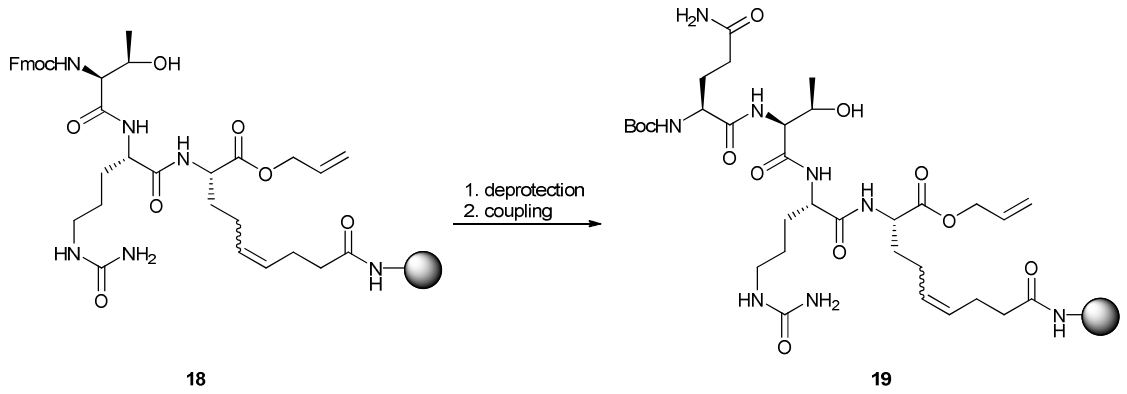
HOBt: 146 mg, 1.08 mmol

HBTU: 359 mg, 0.95 mmol

DIPEA: 134 µL, 0.81 mmol.

The coupling was performed for 5h. The Kaiser test performed according to *method E* remained negative, indicating a complete conversion of **17** to NovaPEG-Ahp5COAll-Cit4-Thr3-Fmoc (**18**).

Coupling of Boc-Gln-OH to NovaPEG-Ahp5COAll-Cit4-Thr3-Fmoc (18)



18 **19**

NovaPEG-Ahp5COAll-Cit4-Thr3-Fmoc (**18**) (0.27 mmol) was deprotected according to *method B*. The coupling of Boc-Gln-OH to the resin was performed using *method C.* The amounts of reagent were:

Boc-Gln-OH: 266 mg, 1.08 mmol

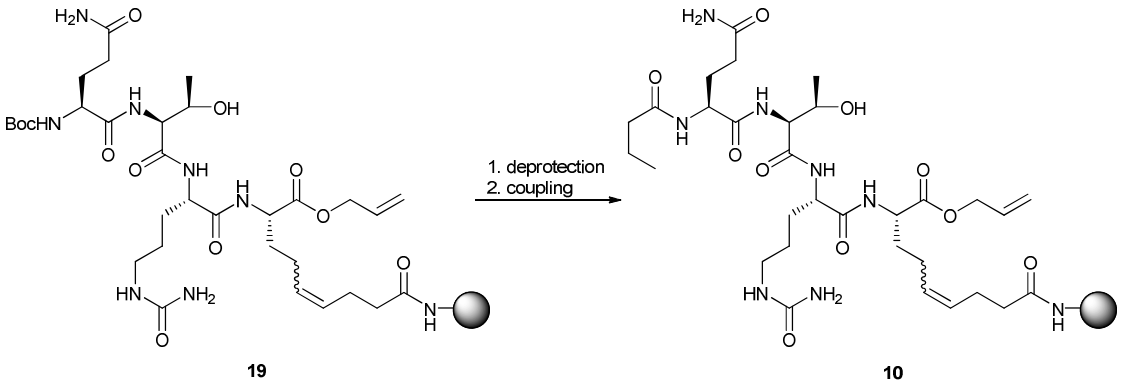
HOBt: 146 mg, 1.08 mmol

HBTU: 359 mg, 0.95 mmol

DIPEA: 134 µL, 0.81 mmol.

The coupling was performed for 4h. The Kaiser test performed according to *method E* remained negative, indicating a complete conversion of **18** to NovaPEG-Ahp5COAll-Cit4-Thr3-Gln2-Boc (**19**).

Coupling of butyric acid (But-OH) to NovaPEG-Ahp5COAll-Cit4-Thr3-Gln2-Boc (19)



19 **10**

NovaPEG-Ahp5COAll-Cit4-Thr3-Gln2-Boc (**19**) (0.27 mmol) was deprotected according to *method A*. The coupling of But-OH to the resin was performed using *method C.* The amounts of reagent were:

But-OH: 99 µL, 1.08 mmol

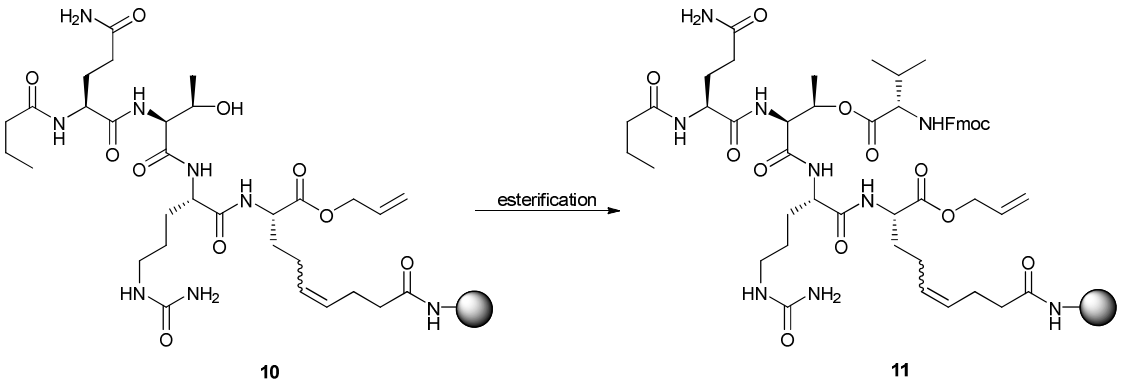
HOBt: 146 mg, 1.08 mmol

HBTU: 359 mg, 0.95 mmol

DIPEA: 134 µL, 0.81 mmol.

The coupling was performed for 4h. The Kaiser test performed according to *method E* remained negative, indicating a complete conversion of **19** to NovaPEG-Ahp5COAll-Cit4-Thr3-Gln2-But1 (**10**).

Esterification of NovaPEG-Ahp5COAll-Cit4-Thr3-Gln2-But1 (10) with Fmoc-Val-OH



10 **11**

For the esterification, the following amounts of reagents were dissolved in a 10% solution of DMF in DCM and added directly to resin **10**.

Fmoc-Val-OH: 916 mg, 2.70 mmol, 10 eq.

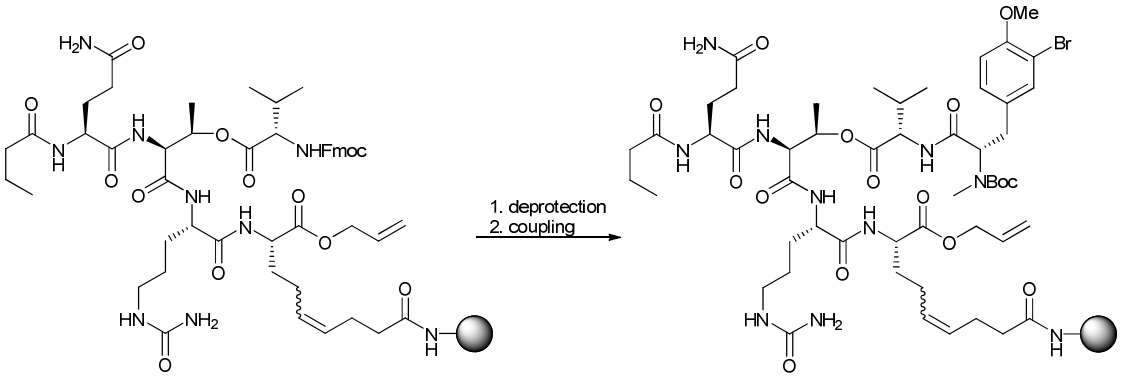
4-(dimethylamino)pyridine: 33 mg, 0.27 mmol, 1 eq.

N,N'-Di*iso*propylcarbodiimide: 421 µL, 2.70, 10 eq.

The reaction was performed for 2h. After the coupling, the resin was washed with DMF (2x 1 min), alternatingly with methanol and DCM (3x 1 min) and DMF (5x 1 min). The coupling was performed four times. After the final coupling an additional washing step consisting of washing alternatingly with anhydrous methanol and DCM (3x 1 min) and anhydrous ether (3x 1 min) was performed. The resin was then dried under high vacuum over night.

The loading of NovaPEG-Ahp5COAll-Cit4-Thr3(O-CO-Val8-Fmoc)-Gln2-But1 (**11**) was measured as described in *method D* using 6.8 mg of resin and resulting in a loading determination of 0.22 mmol/g (92%).

Coupling of *N*-Boc-*N*-Methyl-3-bromomethyltyrosine 16 (Boc-mTyr(3-Br, 4-OMe)-OH) to NovaPEG-Ahp5-COAll-Cit4-Thr3(O-CO-Val8-Fmoc)-Gln2-But1 (11)



11 **20**

NovaPEG-Ahp5COAll-Cit4-Thr3(O-CO-Val8-Fmoc)-Gln2-But1 (**11**) (0.22 mmol/g, 1.19 g, 0.26 mmol) was deprotected according to *method B*. The coupling of Boc-mTyr(3-Br, 4-OMe)-OH **13** to the resin was performed using *method C.* The amounts of reagent were:

Boc-mTyr(3-Br, 4-OMe)-OH **16**: 396 mg, 1.02 mmol

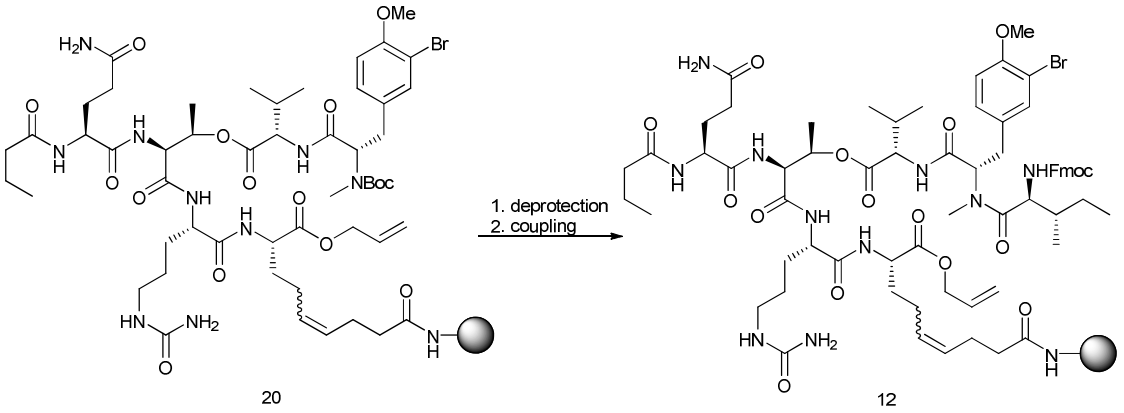
HOBt: 138 mg, 1.02 mmol

HBTU: 339 mg, 0.89 mmol

DIPEA: 126 µL, 0.77 mmol.

The coupling was performed for 4.5h. The Kaiser test performed according to *method E* remained negative, indicating a complete conversion of **11** to NovaPEG-Ahp5-COAll-Cit4-Thr3(O-CO-Val8-mTyr7(3-Br, 4-OMe)-Boc)-Gln2-But1 (**20**).

Coupling of Fmoc-Ile-OH to NovaPEG-Ahp5-COAll-Cit4-Thr3(O-CO-Val8-mTyr7(3-Br, 4-OMe)-Boc)-Gln2-But1 (20)



20 12

NovaPEG-Ahp5-COAll-Cit4-Thr3(O-CO-Val8-mTyr7(3-Br, 4-OMe)-Boc)-Gln2-But1 (**20**) (0.26 mmol) was deprotected according to *method A*. The coupling of Fmoc-Ile-OH to the resin was performed using PyBrOP as the coupling reagent. The following reagents were dissolved in DMF, preactivated for 1 min and then added to the resin.

Fmoc-Ile-OH: 451 mg, 1.28 mmol, 5 eq.

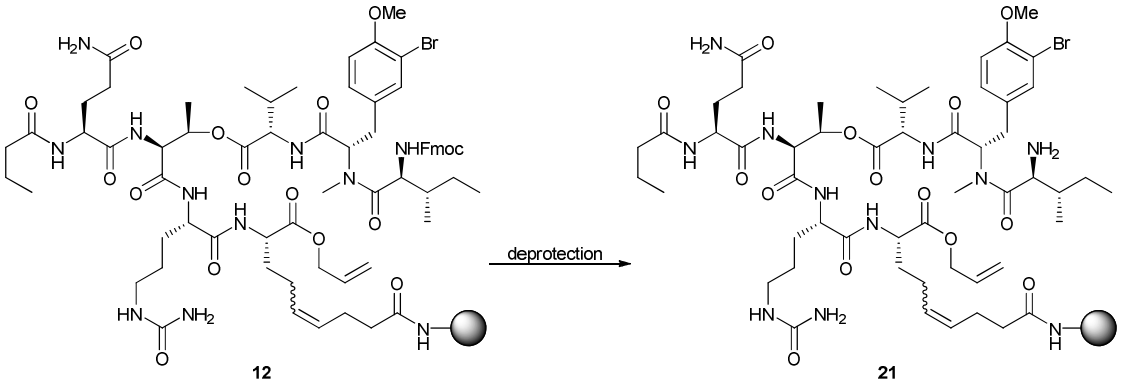
PyBrOP: 583 mg, 1.25 mmol, 4.9 eq.

DIPEA: 421 µL, 2.55 mmol, 10 eq.

The coupling was performed for 24h. After the coupling, the resin was washed with DMF (2x 1 min), alternatingly with methanol and DCM (3x 1 min), DMF (5x 1 min), alternatingly with anhydrous methanol and DCM (3x 1 min) and finally with anhydrous ether (3x 1 min). The resin was then dried under high vacuum over night. The chloranil test performed following *method F* remained negative, indicating a complete conversion of **20** to NovaPEG-Ahp5-COAll-Cit4-Thr3(O-CO-Val8-mTyr7(3-Br, 4-OMe)-Ile6-Fmoc)-Gln2-But1 (**12**)

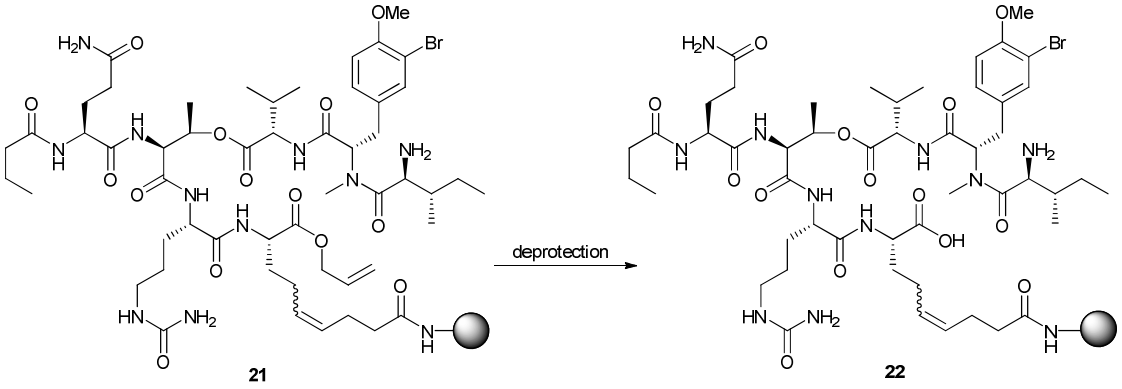
The loading of NovaPEG-Ahp5-COAll-Cit4-Thr3(O-CO-Val8-mTyr7-(3-Br, 4-OMe)-Ile6-Fmoc)-Gln2-But1 (**12**) was measured as described in *method D* using 5.0 mg of resin and resulted in a loading determination of 0.16 mmol/g (73%).

Final Fmoc deprotection of 12



The final Fmoc deprotection of **12** (0.16 mmol/g, 1.27 g, 0.20 mmol) was performed according to *method B*. After the second washing procedure, an additional washing step was performed, consisting of washing with methanol (2x 1 min), alternatingly with methanol and DCM (2x 1 min), DMF (5x 1 min), NMP (5x 1 min), DCM (2x 1 min) and finally diethyl ether (2x 1 min). The NovaPEG-Ahp5-COAll-Cit4-Thr3(O-CO-Val8-mTyr7-(3-Br, 4-OMe)-Ile6-OH)-Gln2-But1 (**21**) was then dried under high vacuum over night.

Removal of the allyl protecting group from NovaPEG-Ahp5-COAll-Cit4-Thr3(O-CO-Val8-mTyr7-(3-Br, 4-OMe)-Ile6-OH)-Gln2-But1 (21)



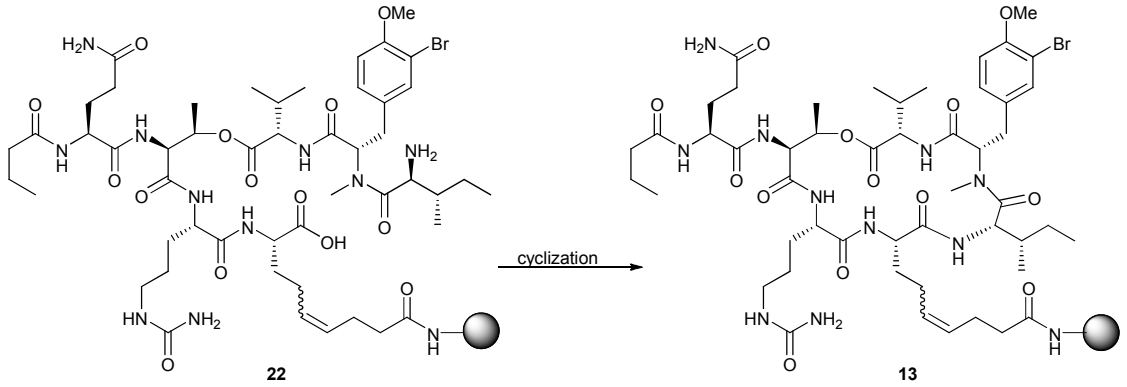
21 22

The resin **21** (0.20 mmol) was transferred into a pear shaped flask under an argon atmosphere and was degassed under high vacuum for 15 min using an ultrasonication bath. It was then washed with degassed DCM (3x 2 min) and during this washing step, argon was furthermore bubbled through the suspension. After the last washing, DCM was removed and a solution of degassed morpholine (420 µL, 4.80 mmol, 24 eq.) in degassed DCM (1 mL) was added. Again, argon was bubbled through the suspension for 2 min. $Pd(PPh_3)_4$ (58 mg, 0.05 mmol, 0.25 mmol) dissolved in degassed DCM was added to the suspension and argon was again bubbled through the suspension for 2 min. Finally, the flask containing the suspension was placed on an orbital shaker and shaken gently for 30 min.

The resin was then removed carefully using a glass pipette and transferred to a syringe reactor to simplify the washing. The resin was washed with DCM (3x 1 min), NMP (3x 1 min) and DCM (4x 1 min). This washing procedure was repeated once more. The washing procedure was then continued with DCM (3x 1min), NMP (3x 1 min), a 0.02 M solution of Et_2NCS_2Na in NMP (3x 5 min), NMP (5x 1 min) and DMF (2x 1 min).

This procedure then afforded the NovaPEG-Ahp5-COOH-Cit4-Thr3(O-CO-Val8-mTyr7-(3-Br, 4-OMe)-Ile6-OH)-Gln2-But1 (**22**) intermediate.

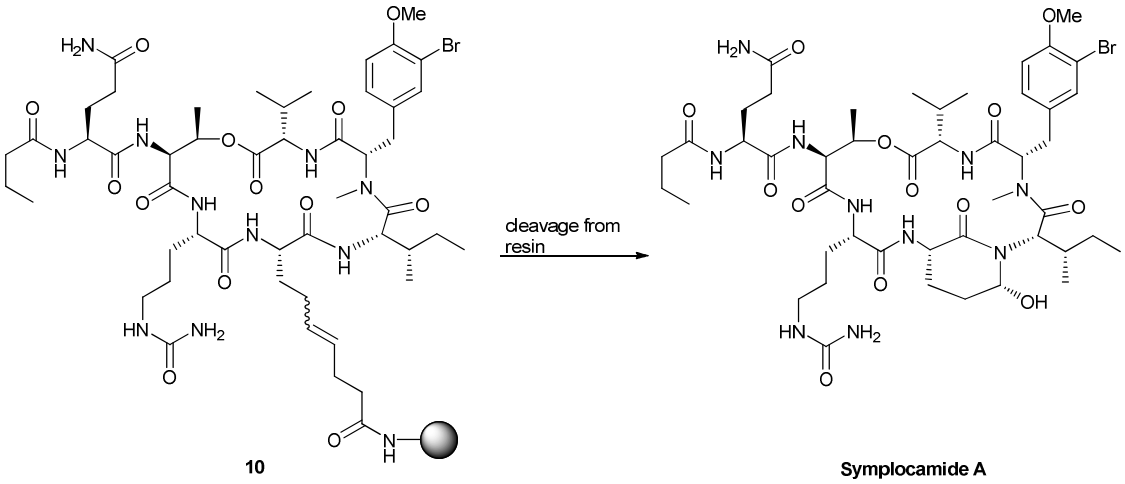
On resin cyclization of NovaPEG-Ahp5-COOH-Cit4-Thr3(O-CO-Val8-mTyr7-(3-Br, 4-OMe)-Ile6-OH)-Gln2-But1 (22) to macrolactam 13



To the fully deprotected resin (**22**) (0.20 mmol) was added a solution of HOBt (216 mg, 1.60 mmol, 8 eq.) and DIPEA (397 µL, 2.40 mmol, 12 eq.) in DMF. The resulting suspension was shaken 1 min for preactivation, then a solution of PyBOP (357 mg, 0.80 mmol, 4 eq.) in DMF was added and the resulting reaction mixture was shaken for further 24h.

The resin was washed with DMF (2x 1 min), alternatingly with methanol and DCM (3x 1 min), DMF (5x 1 min), DCM (2x 1 min) and finally diethyl ether (2x 1 min). It was then dried under high vacuum for 1h.

Cleavage of 13 to Symplocamide A



Sodium periodate (428 mg, 2.00 mol, 10 eq.) and DABCO (112 mg, 1.00 mmol, 5 eq.) were suspended in water (8 mL), sonificated briefly and added to resin **13** (0.20 mmol). The resin was allowed to stand in this mixture for 10 min, after which THF (8 mL) was added and the resulting suspension was shaken for 2 min. A 0.1 M solution of osmium tetroxide in *tert*-butanol (123 µL, 0.01 mmol, 0.05 eq.) was added to the suspension, which was then shaken for further 20h.

The cleavage solution was collected and quenched with an aq. saturated sodium metabisulfite solution. The resin was washed with a 1:1 (v/v) solution of THF/water (5x 20 mL), the washing phases were added to the sodium metabisulfite solution. The phases in this solution were allowed to separate and the aqueous phase was extracted with ethyl acetate (5x 80 mL). The combined extracts were dried over Na_2SO_4 and the solvent was evaporated. The resulting crude mixture was redissolved in ethyl acetate (1 mL) and a few drops of trifluoracetic acid were added dropwise, followed by transfer of the whole reaction mixture to cold ether, thereby causing the crude product to precipitate as a white solid. The precipitate was centrifuged; the supernatant removed and fresh ether was added. The suspension was sonificated briefly and centrifuged again, then the supernatant was removed and the precipitate was allowed to dry.

The crude product (65 mg) was purified by preparative HPLC (0 min / 10% B → 3 min / 10% B → 15 min / 32% B → 40 min / 45% B → 41 min / 100% B → 51 min / 100% B) to afford Symplocamide A.

Yield: 8.4 mg (8 µmol, 3%) as a light yellow glass.

$[\alpha]_D^{20}$ -21.7 (*c* 0.06 in MeOH) (lit., [3] $[\alpha]_D^{23}$ -43.2 (*c* 0.06 in MeOH)).

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.49 (d, J = 8.65 Hz, 1H), 8.05 (d, J = 7.30 Hz, 1H), 7.82 (d, J = 8.95 Hz, 1H), 7.66 (d, J = 9.00 Hz, 1H), 7.34 (d, J = 9.25 Hz, 1H), 7.31 (bs, 1H), 7.20 (d, J = 7.15 Hz, 1H), 7.03 (d, J = 8.40 Hz, 1H), 6.74 (bs, 1H), 6.13 (bs, 1H), 5.97 (bs, 1H), 5.51 (m, 1H), 5.37 (bs, 2H), 5.06 (d, J = 11.80 Hz, 1H), 4.93 (bs, 1H), 4.65 (m, 1H), 4.48-4.49 (m, 1H), 4.44 (m, 1H), 4.37-4.39 (m, 2H), 4.25 (m, 1H), 3.77 (s, 1H), 3.22-3.24 (m, 1H), 2.94 (m, 1H), 2.77-2.80 (m, 1H), 2.74 (s, 3H), 2.57-2.61 (m, 1H), 2.16-2.17 (m, 2H), 2.12 (t, J = 6.75 Hz, 2H), 2.04-2.06 (m, 1H), 1.98-2.01 (m, 2H), 1.78-1.80 (m, 1H), 1.73-1.75 (m, 4H), 1.53 (m, 2H), 1.46 (m, 1H), 1.24 (m, 2H), 1.21 (d, J = 5.50 Hz, 2H), 1.10 (m, 2H), 0.87 (m, 6H), 0.76 (d, J = 6.20 Hz, 3H), 0.63 (m, 3H), -0.14 (d, J = 5.50 Hz, 3H).

^{13}C NMR (151 MHz, DMSO-d_6): δ 174.4, 174.0, 172.5, 172.4, 170.3, 169.8, 169.5, 169.3, 169.3, 158.1, 154.6, 129.8, 128.1, 126.5, 116.5, 111.2, 74.1, 72.05, 63.0, 60.6, 56.4, 54.9, 54.3, 52.5, 52.2, 49.0, 37.2, 36.6, 35.2, 33.1, 32.9, 31.7, 31.4, 30.3, 28.9, 28.8, 28.7, 26.7, 25.2, 22.2, 19.4, 18.8, 17.8, 17.8, 13.9, 13.7, 10.4.

LC-MS: t_R = 7.35 min, *m/z* = 1053($M^+ + H^+$,75%), 1035 ($M^+ - H_2O$, 100).

HRMS (ESI)*: m/z* calcd for $C_{46}H_{72}O_{13}N_{10}Br^+$ $[M + H]^+$ 1051.4458, found 1051.4463, *m/z* calcd for $C_{46}H_{72}O_{13}N_{10}{}^{81}Br^+$ $[M + H]^+$ 1053.4438, found 1053.4446.

[3] R. G. Linington, D. J. Edwards, C. F. Shuman, K. L. McPhail, T. Matainaho and W. H. Gerwick, *J. Nat. Prod.*, 2008, **71**, 22-27.

Biochemical activity assay

The chymotrypsin inhibition potency of Symplocamide A has been determined as previously described,[4] using commercial bovine chymotrypsin from Sigma.

[4] P. Hauske, M. Meltzer, C. Ottmann, T. Krojer, T. Clausen, M. Ehrmann and M. Kaiser, *Bioorg. Med. Chem.*, 2009, **17**, 2920-2924.