Solid Phase Total Synthesis and Structure Proof of Callipeltin B

Supporting Information

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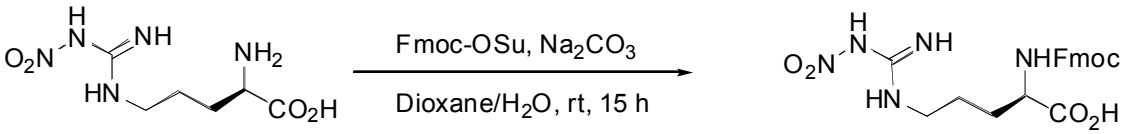
General Experimental

NMR spectra were recorded at 300 MHz and 75 MHz for proton and carbon nuclei respectively. ^{1}H NMR spectra of 1a-c were recorded at 500 MHz. Solvents were purified by passage through an activated column prior to use.[1]

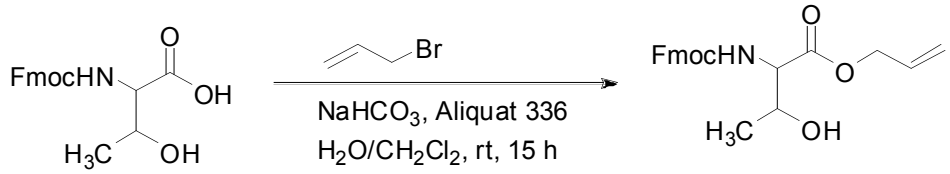
Fmoc-D-Arg(NO_2)



A suspension of D-Arg(NO_2) (340 mg, 1.55 mmol), Fmoc-OSu (784 mg, 2.32 mmol) and a 10% aqueous solution of Na_2CO_3 (6 mL) in Dioxane (3 mL) was stirred at room temperature for 15 h. The reaction mixture was washed with diethyl ether (2x10 mL). The aqueous layer was acidified to pH 1 with 5% citric acid and extracted with ethyl acetate (3x 50 mL). The combined organic layers were washed with brine solution and dried over Na_2SO_4. The solvent was evaporated in vacuo to afford Fmoc-D-Arg(NO_2) (502 mg, 73% yield) which was used without further purification.

mp 148 °C. ^{1}H NMR (300 MHz, CD_3OD) _ 7.81 (d, J = 7.0 Hz, 2H), 7.66 (dd, J = 6.5, 7.0 Hz, 2H), 7.40 (m, 4H), 4.41 (d, J = 7.0 Hz, 2H), 4.23 (t, J = 7.0, 6.4 Hz, 2H), 3.28 (t, J = 7.0, 5.9 Hz, 2H), 1.97 (m, 2H), 1.73 (m , 2H); ^{13}C NMR (75 MHz, CD_3OD) _ 174.2, 159.6, 157.4, 143.9, 143.7*, 141.2, 127.4, 126.8, 124.8, 119.5, 66.5, 53.5, 47.1, 40.3, 28.5, 24.8 (* denotes signals arising from a minor rotamer); IR (KBr) λ_{max} 3311, 2950, 1707, 1633, 1532, 1450, 1263, 1106 cm^{-1}; HRMS (ESI) calculated for $C_{21}H_{23}N_5O_6$ (M + Na)$^+$ 464.1546, found 464.1551.

Synthesis of Fmoc-Thr-OAllyl – General Procedure



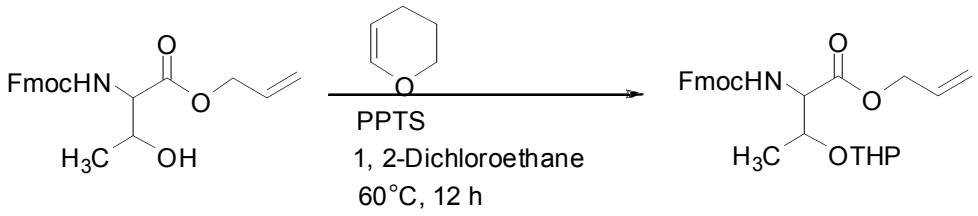
[1] Solvent columns are composed of activated alumina and supported copper redox catalyst reactant. See: *Organometallics* **1996**, 15, 1518-1520.

A mixture of aliquat 336 (500 mg) and allylbromide (5.3 eq) in CH_2Cl_2 (3 mL) was added to a mixture of Fmoc-Thr (1 eq) and $NaHCO_3$ (1 eq) in H_2O (3 mL). The resulting suspension was stirred vigorously at room temperature for 15 h. The reaction mixture was diluted with water (15 mL) and extracted with CH_2Cl_2 (3x 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concenterated in vacuo to afford the crude product. Flash column chromatography of the crude product (30% ethylacetate/hexanes) afforded Fmoc-Thr-OAllyl ester.

Fmoc-D-*a*Thr-OAllyl: Fmoc-D-*a*Thr (250 mg, 0.73 mmol), $NaHCO_3$ (61.5 mg, 0.732 mmol), allylbromide (0.34 mL, 3.9 mmol). Colorless solid, 271 mg, 97% yield, mp 108 °C. ^{1}H (300 MHz, $CDCl_3$) _ 7.74 (d, J = 7.6 Hz, 2H), 7.58 (d, J = 7.0 Hz, 2H), 7.37 (m, 4H), 5.88 (m, 1H), 5.32 (m, 2H), 4.66 (d, J = 5.3 Hz, 2H), 4.47 (m, 1H), 4.40 (d, J = 7.0 Hz, 2H), 4.21 (t, J = 7.0, 7.0 Hz, 2H), 2.98 (br s., 1H), 1.22 (d, J = 5.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) _ 170.2, 156.7, 143.8*, 143.7, 141.3, 131.3, 127.8, 127.2, 125.2, 120.1, 119.3*, 68.9, 67.4, 66.3, 59.6, 47.2, 19.0 (* denotes signals arising from a minor rotamer); IR (neat) λ_{max} 3383, 1718, 1520, 1450, 1255, 1196, 1035 cm^{-1}; HRMS (ESI) calculated for $C_{22}H_{23}NO_5$ $(M + Na)^+$ 404.1474, found 404.1470.

Fmoc-L-Thr-OAllyl: Fmoc-L-Thr (500 mg, 1.46 mmol), $NaHCO_3$ (122.6 mg, 0.732 mmol), allylbromide (0.67 mL, 7.74 mmol). Colorless solid, 527 mg, 94% yield, mp 109 °C. ^{1}H (300 MHz, $CDCl_3$) _ 7.75 (d, J = 7.4 Hz, 2H), 7.60 (d, J = 5.3 Hz, 2H), 7.38 (m, 4H), 5.90 (m, 1H), 5.72 (d, J = 8.2 Hz, 1H), 5.37 (m, 2H), 4.67 (d, J = 5.3 Hz, 2H), 4.41 (d, J = 7.6 Hz, 2H), 4.39 (d, J = 7.6 Hz, 1H), 4.24 (t, J = 7.0, 7.0 Hz, 1H), 2.34 (br s., 1H), 1.82 (br s., 1H), 1.25 (d, J = 5.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) _ 171.2, 157.1, 144.1*, 143.9, 141.6, 131.7, 128.0, 127.4, 125.4, 120.3, 119.3*, 68.3, 67.5, 66.6, 59.5, 47.4, 20.2 (* denotes signals arising from a minor rotamer); IR (neat) λ_{max} 3408, 1721, 1520, 1450, 1205, 1070 cm^{-1}; HRMS (EI) calculated for $C_{22}H_{23}NO_5$ M^+ 381.1576, found 381.1582

Synthesis of Fmoc-Thr(THP)-OAllyl – General Procedure



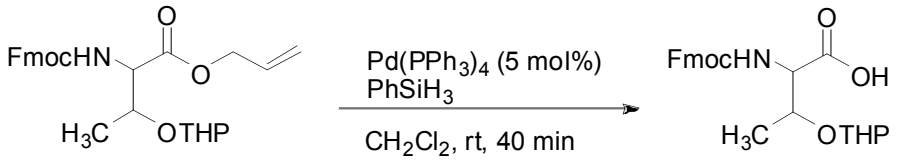
To a solution of Fmoc-Thr-OAllyl (1 eq) in 1,2- dichloroethane (5 mL) was added 3, 4-dihydro-2H-pyran (5 eq) followed by PPTS (2.5 mol%). The reaction was stirred at 60 °C for 12 h, whereupon the reaction mixture was diluted with water (15 mL) and extracted with CH_2Cl_2 (3x 20 mL). The combined organic layers were washed with brine solution, dried over Na_2SO_4 and concentrated in vacuo to afford the crude product. Flash column chromatography of the crude product (20% ethylacetate/hexanes) afforded Fmoc-Thr(THP)-OAllyl as a colorless, viscous oil in quantitative yield.

Fmoc-D-aThr(THP)-OAllyl: Fmoc-D-*a*Thr-OAllyl (162 mg, 0.43 mmol), 3, 4-dihydro-2H-pyran (160 _L, 1.75 mmol), PPTS (2 mg, 2.5 mol %). Colorless viscous oil, quantitative yield (200 mg). ^{1}H (300 MHz, $CDCl_3$) _ 7.75 (d, J = 7.5 Hz, 2H), 7.65* (d, J = 7.8 Hz, 2H), 7.6 (d, J = 7.8 Hz, 2H), 7.38 (m, 4H), 6.30 (d, J = 8.6 Hz, 1H), 5.92 (m, 1H), 5.55* (d, J = 8.6 Hz, 1H), 5.28 (m, 2H), 5.22* (m, 2H), 4.76* (br s., 1H), 4.68 (d, J = 5.6 Hz, 2H), 4.64* (d, J = 7.6 Hz, 2H), 4.61 (br s., 1H), 4.38 (d, J = 5.1 Hz, 2H), 4.32* (d, J = 4.5 Hz, 2H), 4.25 (t, J = 7.3, 9.1 Hz, 1H), 4.21* (t, J = 4.5, 6.4 Hz, 1H), 4.06 (q, J = 3.1, 3.2, 3.1 Hz, 1H), 3.94* (q, J = 5.7, 5.7, 5.3 Hz, 1H), 3.88* (m, 2H), 3.53 (t, J = 5.4, 5.7 Hz, 2H), 1.76 (m, 2H), 1.69* (m, 2H), 1.33 (d, J = 6.6 Hz, 3H), 1.27* (d, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) _ 170.1*, 169.6, 156.1, 144.1*, 143.9*, 143.8, 141.3, 131.8, 131.5*, 127.8, 127.7* 127.1, 127.0*, 125.3, 125.2*, 120.0, 119.9*, 119.1*, 118.4, 98.4*, 98.0*, 74.5, 72.9, 67.1, 67.0*, 66.1*, 65.8, 63.4, 62.7*, 58.6, 57.2*, 47.2, 31.1*, 30.6, 25.4*, 25.2, 20.0, 19.5*, 17.5 (* denotes minor rotamer); IR (neat) $\_{max}$ 3337, 2943, 1726, 1522, 1450, 1386, 1200, 1122, 1076, 1030 cm^{-1}; HRMS (ESI) calculated for $C_{27}H_{31}NO_6$ $(M + Na)^+$ 488.2049, found 488.2050.

Fmoc-L-Thr(THP)-OAllyl: Fmoc-L-Thr-OAllyl (483 mg, 1.27 mmol), 3, 4-dihydro-2H-pyran (580 _L, 6.35 mmol), PPTS (8 mg, 2.5 mol %). Colorless viscous oil, quantitative

yield (592 mg). ^{1}H (300 MHz, $CDCl_3$) _ 7.75 (d, J = 7.6 Hz, 2H), 7.67* (d, J = 6.5 Hz, 2H), 7.64 (d, J = 6.4 Hz, 2H), 7.38 (m, 4H), 5.94 (m, 1H), 5.76 (d, J = 8.8 Hz, 1H), 5.61* (d, J = 9.4 Hz, 1H), 5.34 (d, J = 15.8 Hz, 1H), 5.27 (m, 1H), 4.67 (t, J = 5.3, 5.9 Hz, 2H), 4.6* (d, J = 5.9 Hz, 1H), 4.56 (d, J = 3.5 Hz, 1H), 4.45 (m, 2H), 4.40* (m, 2H), 4.31 (t, J = 7.0, 7.6 Hz, 1H), 3.90 (q, J = 6.5, 3.5, 5.3 Hz, 1H), 3.73 (m, 1H), 3.47 (m, 1H), 1.74 (m, 2H), 1.65* (m, 2H), 1.53 (m, 4H), 1.34* (d, J = 6.5 Hz, 3H), 1.24 (d, J = 5.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) _ 170.8*, 170.7, 157.0, 156.9*, 144.2, 144.0*, 141.5, 131.9, 131.7*, 127.9, 127.2, 125.4, 125.1*, 120.2, 119.4*, 118.9, 100.6*, 96.1, 75.2*, 71.3, 67.4, 66.2*, 63.2*, 62.7, 59.2, 47.4, 31.0*, 30.9, 25.4, 20.0*, 19.7, 19.2*, 16.8 (* denotes minor rotamer); IR (neat) λ_{max} 3436, 3338, 2943, 1727, 1511, 1450, 1307, 1201, 1074, 1033 cm^{-1}; HRMS (EI) calculated for $C_{27}H_{31}NO_6$ M^+ 465.2151, found 465.2149.

Fmoc-Thr(THP) – General Procedure



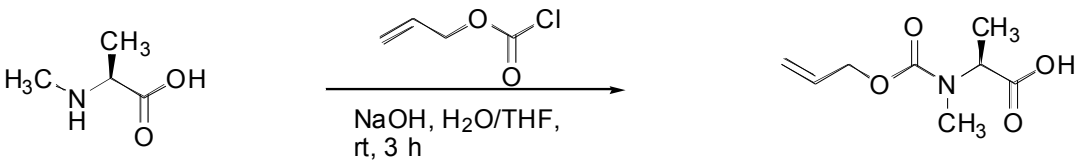
To a mixture of Fmoc-D-aThr(THP)-OAllyl (1 eq) and $PhSiH_3$ (2 eq) in CH_2Cl_2 (2 mL) was added $Pd(PPh_3)_4$[2] (5 mol%) and stirred at room temperature for 45 min. The reaction mixture was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (3x10 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was used immediately in the solid phase synthesis to avoid autocatalytic decomposition.

Fmoc-D-*a*Thr(THP): Fmoc-D-*a*Thr(THP)-OAllyl (29 mg, 0.063 mmol), $PhSiH_3$ (17.7 µL, 0.144 mmol), $Pd(PPh_3)_4$ (3.6 mg, 5 mol%). Crude product was obtained as a gummy brown solid.

Fmoc-L-Thr(THP): Fmoc-L-Thr(THP)-OAllyl (33.5 mg, 0.072 mmol), $PhSiH_3$ (23 µL, 0.19 mmol), $Pd(PPh_3)_4$ (4.2 mg, 5 mol%). Crude product was obtained as a gummy brown solid.

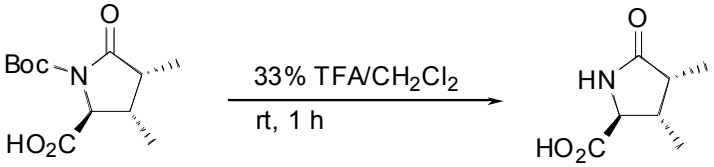
[2] Prior to use $Pd(PPh_3)_4$ was washed with DMSO (3x), EtOH (3x) and Et_2O (3x).

Alloc-MeAla



A mixture of *N*-methyl-L-alanine (600 mg, 5.8 mmol), allylchloroformate (3.1 mL, 5.8 mmol) and 2 M NaOH (5.8 mL) in 1:2 THF-H_2O (9 mL) was stirred at room temperature for 3 h. After completion the reaction mixture was concentrated in vacuo to reduce the volume, acidified to pH 2.0 with 2 M HCl and extracted with EtOAc (3x 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated in vacuo to afford the crude product. Flash column chromatography of the crude product (5% MeOH/ CH_2Cl_2) afforded Alloc-MeAla (952 mg, 87% yield) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) _ 10.1 (br s., 1H), 5.93* (m, 1H), 5.85 (m, 1H), 5.26* (m, 2H), 5.19 (m, 2H), 4.85 (q, J = 7.6, 7.0, 7.6 Hz, 1H), 4.70* (q, J = 7.0, 7.6, 7.0 Hz, 1H), 4.56 (d, J = 5.3 Hz, 2H), 2.86 (s, 3H), 1.39 (d, J = 7.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) _ 176.9, 156.7, 156.1*, 132.6, 117.5, 66.6, 54.1, 30.9*, 30.3, 15.1*, 14.6 (* denotes minor rotamer); IR (neat) λ_{max} 2947, 1746, 1706, 1674, 1456, 1402, 1320, 1164, 1096 cm^{-1}; HRMS (ESI) calculated for $C_8H_{13}NO_4$ $(M + Na)^+$ 210.0742, found 210.0745.

(3*S*, 4*R*)-Dimethyl-L-pyroglutamic acid



N-Boc-(3*S*, 4*R*)-Dimethyl-L-pyroglutamic acid[3] (17 mg, 0.065 mmol) was dissolved in 33% TFA/CH_2Cl_2 (2 mL) and stirred at room temperature for 1 h. After completion of the reaction, the solvent was evaporated under reduced pressure, redissolved in toluene and reevaporated to afford the crude product (10.2 mg), which was used without further purification in the synthesis.

[3] Acevedo, C. M.; Kogut , E. F.; Lipton, M. A. *Tetrahedron* **2001**, *57*, 6353.

Solid Phase Synthesis - Method A:

Novasyn Tentagel Sieber resin **2** (200 mg, 0.12 mmol/g) was swelled in DMF (2 mL) and treated with a 25% piperidine/DMF solution (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by filtration and the reaction was repeated. The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). The cyclic anhydride **3** (26.3 mg, 0.072 mmol) was added to the resin, followed by DMF (600 µL), and the resin was mixed by bubbling a slow stream of N_2 through the suspension for 1 h. The solution was removed from the reaction vessel by filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x). This reaction, and all subsequent reactions, was analyzed by HPLC and MALDI-MS by removing an aliquot of beads (2 mg).

The resin was swelled in 9:1 CH_2Cl_2/DMF (2 mL) and the coupling reagent HATU (21.7 mg, 0.057 mmol) was added to the resin, followed by a solution of (2*R*, 3*R*)-_-OMeTyr(Bn)-OAllyl·HCl **4** (19.5 mg, 0.057 mmol) in 9:1 CH_2Cl_2/DMF (600 µL), DIEA (20 µL, 0.114 mmol). The resin was mixed by bubbling a slow stream of N_2 through the suspension for 2 h. The solvent volume was maintained by adding CH_2Cl_2 as needed. The solution was removed by vacuum filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x).

The resin was swelled in DMF (2 mL) and treated with a 25% piperidine/DMF (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by vacuum filtration and the deprotection step was repeated. The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). The coupling reagents Fmoc-Leu (25.4 mg, 0.072 mmol), HATU (27.4 mg, 0.072 mmol) and HOAt (9.8 mg, 0.072 mmol) were added to the resin, followed by DMF (600 µL) and DIEA (25 µL, 0.144 mmol). The resin was mixed by bubbling a slow stream of N_2 through the suspension for 2 h. The solution was removed by vacuum filtration and the resin was washed with DMF (4x), CH_2Cl_2 (2x).

The resin was swelled in DMF (2 mL) and treated with a 25% piperidine/DMF solution (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by vacuum filtration and the deprotection step was repeated. The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). The coupling

reagents Fmoc-D-Arg(Z_2) (40 mg, 0.06 mmol), PyBOP (31.2 mg, 0.06 mmol) and HOBt·H_2O (9.2 mg, 0.060 mmol) were placed in a vial, followed by addition of DMF (600 μL) and DIEA (21 μL, 0.12 mmol). The solution was allowed to react for 2 min and then added to the resin suspension. The resin was mixed by bubbling a slow stream of N_2 through the suspension for 2.5 h. The solution was removed by vacuum filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x).

The resin was swelled in DMF (2 mL) and treated with a 2% DBU/DMF (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by vacuum filtration and the deprotection step was repeated. The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). The coupling reagents PyBOP (37.4 mg, 0.072 mmol) and HOBt·H_2O (11 mg, 0.072 mmol) were added to the flask containing Fmoc-L-Thr(THP) (0.072 mmol), used as a crude solution obtained from the deprotection of its allyl ester (*vide supra*), and the mixture was dissolved in DMF (600 μL) and DIEA (25 _L, 0.144 mmol). The solution was allowed to react for 2 min and then added to the resin suspension. The resin was mixed by bubbling a slow stream of N_2 through the suspension for 2.5 h. The solution was removed by vacuum filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x).

The resin was swelled in DMF (2 mL) and treated with a 2% DBU/DMF solution (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by vacuum filtration and the deprotection step was repeated (2x). The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). The coupling reagents Fmoc-Thr (for **1a**) or Fmoc-D-*a*Thr (for **1b**) (24.6 mg, 0.072 mmol), HATU (27.4 mg, 0.072 mmol) and HOAt (9.8 mg, 0.072 mmol) were placed in a vial and dissolved in DMF (600 _L) and DIEA (25 _L, 0.144 mmol). The mixture was allowed to react for 2 min and then added to the resin suspension. The resin was mixed by bubbling a slow stream of N_2 through the suspension for 2.5 h. The solution was removed by vacuum filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x).

The resin was swelled in DMF (2 mL) and treated with a 2% DBU/DMF solution (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by vacuum filtration and the deprotection step was repeated (2x). The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). HOSu (8.2 mg,

0.071 mmol) was added to a flask containing crude (3*S*, 4*R*)-Dimethyl-L-pyroglutamic acid (11.1 mg) followed by DMF (600 μL), DIC (11 μL, 0.071 mmol) and DIEA (12.4 μL, 0.071 mmol). The mixture was allowed to react for 2 min and then added to the resin suspension. The resin was mixed by bubbling a slow stream of N_2 through the suspension for 2 h. The solution was removed by vacuum filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x).

MSNT (85 mg, 0.288 mmol) and Alloc-MeAla (54 mg, 0.288 mmol) were placed separately in flame dried vials which were purged with N_2 for 2 min. To the vial containing the Alloc-MeAla, dry CH_2Cl_2 (400 _L) was added followed by *N*-methylimidazole (46 μL, 0.576 mmol). The solution was mixed for 1 min, added to the vial containing MSNT under a N_2 atmosphere and allowed to react for 5 min. The resin was swelled in dry CH_2Cl_2 (2 mL) and the preactivated amino acid solution was added to the resin. The resin was mixed by bubbling a slow stream of N_2 through the suspension for 2.5 h. The solvent volume was maintained by adding CH_2Cl_2 as needed. The solution was removed by vacuum filtration and the resin was washed with CH_2Cl_2 (2x), DMF (4x) and CH_2Cl_2 (2x).

CH_2Cl_2 (600 μL) was added to the resin, followed by $PhSiH_3$ (120 μL, 0.96 mmol) and the resin was mixed by bubbling N_2 through the suspension. After 5 min, $Pd(PPh_3)_4$[4] (19.4 mg, 16.8 μmol) was added to the resin and mixed for 45 min. The solvent volume was maintained by adding dry CH_2Cl_2 as needed. The solution was removed by vacuum filtration and the resin was washed with CH_2Cl_2 (2x), 19:1 CH_2Cl_2/DIEA (2x), DMF (2x) and CH_2Cl_2 (2x).

The resin was swelled in 9:1 CH_2Cl_2/DMF (2 mL). PyAOP (31.3 mg, 0.06 mmol) was added to the resin followed by 9:1 CH_2Cl_2/DMF (600 _L) and 2,4,6-collidine (15.8 μL, 0.12 mmol). The resin was mixed by bubbling a slow stream of N_2 through the suspension for 45 min. The solvent volume was maintained by adding CH_2Cl_2 as needed. The solution was removed by vacuum filtration and the resin was washed with Et_2O, CH_2Cl_2, DMF (4x) and CH_2Cl_2 (2x).

The resin was transferred to a vial and treated with 2% TFA/CH_2Cl_2 (2 mL) for 5 minutes. The solution was removed by pipette and filtered through a plug of cotton. The

[4] see footnote 2

remaining resin was sequentially treated with 2% TFA/ CH_2Cl_2 five times (for 5 min each). The combined 2% TFA/CH_2Cl_2 solution was evaporated in vacuo to get crude protected callipeltin B isomers.

The crude protected macrocycle was dissolved in 5% HCO_2H/MeOH (5 mL) and 20% $Pd(OH)_2$-C (6 mg, 2 μmol) was added. The reaction flask was evacuated, flushed with argon, evacuated again and filled with H_2. The reaction mixture was stirred under balloon pressure of H_2 for 6 h (H_2 balloon was refilled every 3 h). After completion of the reaction, the catalyst was removed by filtration through a cotton plug and the filtrate was evaporated in vacuo to afford the crude callipeltin B isomer. The crude product was purified by reverse phase HPLC (Vydac C8 column, 5-40% CH_3CN-H_2O gradient over 25 min).

Method B: Synthesis of 1c

Novasyn Tentagel Sieber resin **2** (100 mg, 0.12 mmol/g) was swelled in DMF (2 mL) and treated with a 25% piperidine/DMF solution (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by filtration and the reaction was repeated. The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). The cyclic anhydride **3** (22 mg, 0.060 mmol) was added to the resin, followed by DMF (400 μL), and the resin was mixed by bubbling a slow stream of N_2 through the suspension for 1 h. The solution was removed from the reaction vessel by filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x). This reaction, and all subsequent reactions, was analyzed by HPLC and MALDI-MS by removing an aliquot of beads (2 mg).

The resin was swelled in 9:1 CH_2Cl_2/DMF (2 mL) and the coupling reagent HATU (24 mg, 0.063 mmol) was added to the resin, followed by a solution of (2*R*, 3*R*)-_-OMeTyr(Bn)-OAllyl·HCl **4** (24 mg, 0.063 mmol) in 9:1 CH_2Cl_2/DMF (450 μL), DIEA (22 μL, 0.13 mmol). The resin was mixed by bubbling a slow stream of N_2 through the suspension for 1 h. The solvent volume was maintained by adding CH_2Cl_2 as needed. The solution was removed by vacuum filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x).

The resin was swelled in DMF (2 mL) and treated with a 25% piperidine/DMF (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by vacuum filtration and the deprotection step was repeated. The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). The coupling reagents Fmoc-Leu (20 mg, 0.56 mmol), HATU (21 mg, 0.056 mmol) and HOAt (7.6 mg, 0.056 mmol) were added to the resin, followed by DMF (400 μL) and DIEA (19.5 μL, 0.12 mmol). The resin was mixed by bubbling a slow stream of N_2 through the suspension for 1 h. The solution was removed by vacuum filtration and the resin was washed with DMF (4x), CH_2Cl_2 (2x).

The resin was swelled in DMF (2 mL) and treated with a 25% piperidine/DMF solution (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by vacuum filtration and the deprotection step was repeated (2x). The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). The coupling reagents Fmoc-D-Arg(NO_2) (28 mg, 0.063 mmol), PyBOP (33 mg, 0.63 mmol) and HOBt·H_2O (9.6 mg, 0.063 mmol) were placed in a vial, followed by addition of DMF (450 μL) and DIEA (21.9 μL). The solution was allowed to react for 2 min and then added to the resin suspension. The resin was mixed by bubbling a slow stream of N_2 through the suspension for 1.5 h. The solution was removed by vacuum filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x).

The resin was swelled in DMF (2 mL) and treated with a 25% piperidine/DMF (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by vacuum filtration and the deprotection step was repeated (2x). The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). The coupling reagents PyBOP (33 mg, 0.063 mmol) and HOBt·H_2O (9.6 mg, 0.063 mmol) were added to the flask containing Fmoc-D-*a*Thr(THP) (0.063 mmol), used as a crude solution obtained from the deprotection of its allyl ester (*vide supra*), and the mixture was dissolved in DMF (450 μL) and DIEA (22 _L). The solution was allowed to react for 2 min and then added to the resin suspension. The resin was mixed by bubbling a slow stream of N_2 through the suspension for 1.5 h. The solution was removed by vacuum filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x).

The resin was swelled in DMF (2 mL) and treated with a 25% piperidine/DMF solution (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by vacuum filtration and the deprotection step was repeated (2x). The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). The coupling reagents Fmoc-D-*a*Thr (22 mg, 0.063 mmol), HATU (24 mg, 0.063 mmol) and HOAt (8.6 mg, 0.063 mmol) were placed in a vial and dissolved in DMF (450 _L) and DIEA (22 _L, 0.13 mmol). The mixture was allowed to react for 2 min and then added to the resin suspension. The resin was mixed by bubbling a slow stream of N_2 through the suspension for 1.5 h. The solution was removed by vacuum filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x).

The resin was swelled in DMF (2 mL) and treated with a 25% piperidine/DMF solution (2 mL) by bubbling a slow stream of N_2 through the suspension for 15 min. The solution was removed by vacuum filtration and the deprotection step was repeated (3x). The resin was sequentially washed with Et_2O (2x), CH_2Cl_2 (2x) and DMF (4x). HOSu (7.5 mg, 0.065 mmol) was added to a flask containing crude (3*S*, 4*R*)-Dimethyl-L-pyroglutamic acid (10.2 mg) followed by DMF (465 µL), DIC (10 µL, 0.065 mmol) and DIEA (11 µL, 0.065 mmol). The mixture was allowed to react for 2 min and then added to the resin suspension. The resin was mixed by bubbling a slow stream of N_2 through the suspension for 2 h. The solution was removed by vacuum filtration and the resin was washed with DMF (4x) and CH_2Cl_2 (2x).

MSNT (57 mg, 0.19 mmol) and Alloc-MeAla (36 mg, 0.19 mmol) were placed separately in flame dried vials which were purged with N_2 for 2 min. To the vial containing the Alloc-MeAla, dry CH_2Cl_2 (400 _L) was added followed by *N*-methylimidazole (31 µL, 0.38 mmol). The solution was mixed for 1 min, added to the vial containing MSNT under a N_2 atmosphere and allowed to react for 5 min. The resin was swelled in dry CH_2Cl_2 (2 mL) and the preactivated amino acid solution was added to the resin. The resin was mixed by bubbling a slow stream of N_2 through the suspension for 1.5 h. The solvent volume was maintained by adding CH_2Cl_2 as needed. The solution was removed by vacuum filtration and the resin was washed with CH_2Cl_2 (2x), DMF (4x) and CH_2Cl_2 (2x).

CH_2Cl_2 (400 μL) was added to the resin, followed by $PhSiH_3$ (59 μL, 0.58 mmol) and the resin was mixed by bubbling N_2 through the suspension. After 5 min, $Pd(PPh_3)_4$[5] (9.7 mg, 8.4 μmol) was added to the resin and mixed for 40 min. The solvent volume was maintained by adding dry CH_2Cl_2 as needed. The solution was removed by vacuum filtration and the resin was washed with CH_2Cl_2 (2x), 19:1 CH_2Cl_2/DIEA (2x), DMF (2x) and CH_2Cl_2 (2x).

The resin was swelled in 9:1 CH_2Cl_2/DMF (2 mL). PyAOP (16 mg, 0.030 mmol) was added to the resin followed by 9:1 CH_2Cl_2/DMF (450 _L) and 2,4,6-collidine (8 μL, 0.06 mmol). The resin was mixed by bubbling a slow stream of N_2 through the suspension for 45 min. The solvent volume was maintained by adding CH_2Cl_2 as needed. The solution was removed by vacuum filtration and the resin was washed with Et_2O, CH_2Cl_2, DMF (4x) and CH_2Cl_2 (2x).

The resin was transferred to a vial and treated with 2% TFA/CH_2Cl_2 (2 mL) for 5 minutes. The solution was removed by pipette and filtered through a plug of cotton. The remaining resin was sequentially treated with 2% TFA/CH_2Cl_2 five times (for 5 min each). The combined 2% TFA/CH_2Cl_2 solution was evaporated in vacuo to get the crude protected callipeltin B isomer.

The crude protected macrocycle was dissolved in 5% HCO_2H/MeOH and 20% $Pd(OH)_2$-C (3 mg, 1 μmol) was added. The reaction flask was evacuated, flushed with argon, evacuated again and filled with H_2. The reaction mixture was stirred under balloon pressure of H_2 for 9 h (H_2 balloon was refilled every 1.5 h). After completion of the reaction, the catalyst was removed by filtration through a cotton plug and the filtrate was evaporated in vacuo to afford the crude callipeltin B isomer. The crude product was purified by reverse phase HPLC (Vydac C8 column, 5-40% CH_3CN-H_2O gradient over 25 min).

1a: Synthesized by Method A. Crude, protected macrocycle obtained as a yellow oil (35 mg). HPLC retention time: 13.2 min. **1a** was obtained as a colorless foam (3.6 mg, 15% overall yield). ^{1}H NMR (500 MHz, CD_3OD) _ 8.5 (m, 1H), 8.36* (d, J = 8.9 Hz, 1H), 8.10* (d, J = 8.4 Hz, 1H), 7.98 (br s., 1H), 7.89* (d, J = 8.5 Hz, 1H), 7.82 (d, J = 9.3 Hz,

[5] see footnote 2

1H), 7.77 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.17* (m, 1H), 7.05* (d, J = 6.0 Hz, 1H), 6.81 (d, J = 8.6 Hz, 2H), 6.79* (d, J = 8.5 Hz, 2H), 6.73* (d, J = 7.0 Hz, 1H), 5.54 (m, 1H), 5.21 (q, J = 7.3, 2.6, 8.8 Hz, 1H), 5.08 (t, J = 9.9, 9.9 Hz, 1H), 4.81 (m, 1H), 4.63 (d, J = 9.6 Hz, 1H), 4.56* (d, J = 6.7 Hz, 1H), 4.48 (m, 1H), 4.35 (d, J = 9.2 Hz, 1H), 4.08 (m, 1H), 3.99 (d, J = 3.3 Hz, 1H), 3.97* (d, J = 3.3 Hz, 1H), 3.48 (t, J = 1.5, 1.6 Hz, 1H), 3.22 (m, 3H), 3.19* (s, 1H), 3.16 (d, J = 8.0 Hz, 2H), 3.03 (s, 1H), 2.97 (m, 1H), 2.92* (s, 1H), 2.90 (s, 2H), 2.86* (bs, 1H), 2.80 (m, 1H), 2.73* (d, J = 7.8 Hz, 1H), 2.67 (s, 2H), 2.05 (m, 1H), 1.9 (m, 1H), 1.7 (m, 4H), 1.6* (m, 1H), 1.49 (t, J = 3.3, 4.5 Hz, 2H), 1.36 (t, J = 7.2, 7.4 Hz, 2H), 1.32 (s, 1H), 1.30* (s, 1H), 1.26 (t, J = 6.6, 6.1 Hz, 2H), 1.19 (m, 3H), 1.11 (d, J = 7.0 Hz, 3H), 0.99 (m, 6H); HRMS-MALDI calculated for $C_{47}H_{74}N_{12}O_{14}$ $(M + H)^+$ 1031.5526, found 1031.5492.

1b: Synthesized by Method A. Crude, protected macrocycle obtained as a yellow oil (35 mg). HPLC retention time: 12.9 min. **1b** was obtained as colorless foam (3.8 mg, 14% yield). ^{1}H NMR (500 MHz, CD_3OD) _ 8.81 (d, J = 9.7 Hz, 1H), 8.62 (d, J = 9.0 Hz, 1H), 8.36 (d, J = 9.3 Hz, 1H), 8.3* (m, 1H), 8.17 (m, 1H), 8.07* (d, J = 8.3 Hz, 1H), 7.80*(d, J = 7.7 Hz, 1H), 7.72 (d, J = 9.1 Hz, 1H), 7.60* (d, J = 7.5 Hz, 1H), 7.40* (m, 1H), 7.31 (d, J = 8.3 Hz, 2H), 7.29* (d, J = 7.4 Hz, 2H), 7.18 (d, J = 8.5 Hz, 2H), 7.10* (d, J = 8.4 Hz, 1H), 6.99* (d, J = 8.3 Hz, 1H), 6.76 (m, 2H), 6.68* (d, J = 8.1 Hz, 1H), 5.64 (q, J = 5.0, 7.2, 9.0 Hz, 1H), 5.40 (m, 1H), 5.25 (t, J = 9.8, 9.7 Hz, 1H), 5.05 (m, 1H), 4.67 (m, 1H), 4.54 (m, 1H), 4.48 (m, 1H), 4.39 (m, 1H), 4.23 (m, 1H), 4.08 (m, 1H), 3.86 (m, 1H), 3.77 (t, J = 4.1, 4.3 Hz, 1H), 3.65* (m, 1H), 3.43 (m, 1H), 3.19 (m, 3H), 3.12 (t, J = 8.2, 8.2 Hz, 1H), 2.94 (s, 1H), 2.85 (m, 2H), 2.74 (d, J = 10.2 Hz, 2H), 2.70* (s, 1H), 2.62 (m, 1H), 2.55 (s, 1H), 1.91 (m, 2H), 1.65* (m, 3H), 1.54 (m, 3H), 1.37 (m, 3H), 1.32* (m, 2H), 1.23 (d, J = 7.1 Hz, 2H), 1.12 (d, J = 6.8 Hz, 2H), 1.07 (m, 4H), 0.94 (d, J = 6.4 Hz, 2H), 0.93 (d, J = 6.6 Hz, 3H); HRMS-MALDI calculated for $C_{47}H_{74}N_{12}O_{14}$ $(M + H)^+$ 1031.5526, found 1031.5535.

1c: Synthesized using Method B. Crude, protected **1c** was obtained as a pale yellow, flocculent solid (12 mg). HPLC retention time: 15.5 min. **1c** was obtained as a colorless foam (1.8 mg, 15% overall yield). ^{1}H (500 MHz, CD_3OD) _ 8.40 (d, J = 9.9 Hz, 1H),

7.43 (d, J = 8.2 Hz, 1H), 7.24 (d, J = 8.5 Hz, 2H), 6.76 (d, J = 8.5 Hz, 2H), 5.48 (m, 1H), 5.47 (d overlapped, J = 5.1 Hz, 1H), 5.33 (q, J = 7.1 Hz, 1H), 4.91 (t, J = 9.9 Hz, 1H), 4.75 (m, 1H), 4.50 (d, J = 9.4 Hz, 1H), 4.14 (ddd, J = 5.3, 9.1, 9.4 Hz, 1H), 4.09 (d overlapped, J = 5.8 Hz, 1H), 4.08 (m overlapped, 1H), 3.93 (d, J = 3.1 Hz, 1H), 3.20 (m, 1H), 3.19 (m, 2H), 3.17 (s, 3H), 2.98 (s, 3H), 2.76 (m, 1H), 2.74 (s, 3H), 1.93 (m, 2H), 1.92 (m, 2H), 1.77 (m, 2H), 1.76 (m, 2H), 1.73 (m, 1H), 1.66 (m, 2H), 1.65 (m, 2H), 1.58 (m, 2H), 1.57 (m, 2H), 1.50 (m, 2H), 1.34 (m, 2H), 1.29 (d, J = 7.2 Hz, 3H), 1.28 (d, J = 7.2 Hz, 3H), 1.27 (d, J = 6.3 Hz, 3H), 1.21 (d, J = 7.2 Hz, 3H), 1.18 (d, J = 7.5 Hz, 3H), 0.94 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.5 Hz, 3H); HRMS (ESI) calculated for $C_{47}H_{74}N_{12}O_{14}$ $(M + H)^+$ 1031.5526, found 1031.5534.

Table 1. Comparsion of ^{1}H NMR spectra of natural callipeltin B and **1c**

aa	Natural callipeltin B _H	**1c** _H
MeAla		
_	5.34 q (7.1)	5.33 q (7.1)
_	1.30 d (7.1)	1.29 d (7.2)
NCH_3	2.74 s	2.74 s
_-OMeTyr		
_	4.90 t (9.5)	4.91 t (9.9)
_	4.52 d (9.5)	4.50 d (9.4)
C5/C9	7.26 d (8.1)	7.24 d (8.5)
C6/C8	6.78 d (8.1)	6.76 d (8.5)
OCH_3	3.18 s	3.17 s
CONH	8.44 d (9.5)	8.40 d (9.9)
MeGln		
_	4.94 overlapped	4.94 overlapped
_	1.59, 1.50 m	1.57, 1.50 m
_	1.94, 1.78 m	1.92, 1.79 m
NCH_3	3.01 s	3.00 s
Leu		
_	4.77 m	4.75 m
_	1.65, 1.38 m	1.65, 1.34 m
_	1.70 m	1.73 m
_	0.96 d (6.7)	0.94 d (6.7)
$_1$	0.93 d (6.7)	0.92 d (6.5)
CONH	7.44 d (8.1)	7.43 d (8.2)
Arg		
_	4.16 ddd (9.5, 8.1, 5.1)	4.14 ddd (9.4, 9.1, 5.3)
_	1.94, 1.78 m	1.93, 1.77 m
_	1.65, 1.59 m	1.66, 1.58 m

_	3.19 m	3.19 m
CONH	8.26 d (8.1)	-
Thr-1		
_	4.12 overlapped	4.09 overlapped
_	4.12 overlapped	4.08 overlapped
_	1.28 d (7.1)	1.28 d (7.2)
CONH	7.79 d (6.4)	-
Thr-2		
_	5.49 overlapped	5.47 overlapped
_	5.50 overlapped	5.48 overlapped
_	1.28 d (6.8)	1.27 d (6.3)
CONH	8.70 d (9.1)	8.63 d (9.5) (~ 90% exchanged)
DiMepyroGlu		
_	3.97 d (3.0)	3.93 d (3.1)
_	2.77 m	2.76 m
_Me	1.24 d (7.1)	1.21 d (7.2)
_	3.19 m	3.20 m
_Me	1.21 (6.5)	1.18 d (7.5)