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# Supporting Information



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# Enzymatic Release and Macrolactonization of Cryptophycins from Safety-Catch Solid Support

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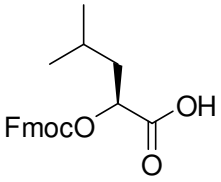
**Reagents and general procedures:** All reactions were performed under nitrogen atmosphere. Fmoc-protected amino and hydroxy acids were either purchased from Bachem and Novabiochem (Fmoc-Leu-OH, Fmoc-*β*-Ala-OH, Fmoc-D-Phe-OH, Fmoc-D-Tyr(Me)-OH) or synthesized in a few steps starting from previously described compounds (see below). 4-Sulfamylbutyryl AM PEGA resin was obtained from Novabiochem. Solvents were purchased from Fisher Scientific and freshly distilled before use ($CH_2Cl_2$, $CHCl_3$, THF, EtOAc, hexane, methanol, $Et_2O$) or obtained in Peptide Synthesis Grade (DMF, NMP). PyBOP (Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate), TBTU (2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate), HOBt (1-Hydroxybenzo-triazole), MSNT (1-(Mesitylene-2-sulfonyl)-3-nitro-1H-1,2,4-triazole), DIPEA (Diisopropyl-ethylamine), NMI (*N*-Methylimidazole), DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene) and all other chemicals were obtained from Aldrich or Novabiochem and used directly.

$^{1}H$ and $^{13}C$ NMR spectra were recorded on a Varian Inova 400 MHz spectrometer. Proton chemical shifts are reported in ppm from an internal standard of residual chloroform (7.26 ppm), carbon chemical shifts are reported in ppm using an internal standard of residual chloroform (77.16 ppm). Proton chemical data are described as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (in Hz) and integration. Mass spectra were recorded on a Micromass LCT time-of-flight mass spectrometer with electrospray ionization (ESI) mode. UV-VIS measurements were performed on a ABI SpectraMax M5 spectrophotometer. Analytical thin-layer chromatography (TLC) was performed on silica gel TLC aluminum sheets with a fluorescence indicator from EMD Chemicals. Visualization was accomplished with UV light (254 nm) and by dipping in a 20% solution of Phosphomolybdic acid (PMA) in ethanol or in a

$KMnO_4$ solution (3 g of $KMnO_4$, 20 g of $K_2CO_3$ and 0.25 g of NaOH in 300 mL of water) followed by heating.

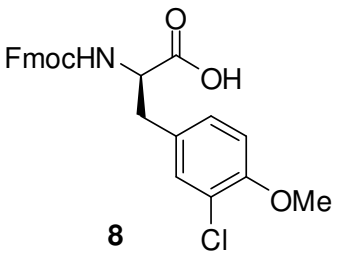
Expression and purification of cryptophycin thioesterase (CrpTE) was performed as previously reported.[1]

## Synthesis of Fmoc-leucic acid (7) and Fmoc-3-chloro-*O*-methyl-D-tyrosine (8)




**7**

**(*S*)-2-(9´-Fluorenylmethoxycarbonyloxy)-4-methylpentanoic acid (7):** To a stirring solution of (*S*)-leucic acid benzyl ester (6.00 g, 27.0 mmol)[2] in pyridine (150 mL) was added 9-fluorenylmethyl-chloroformate (8.38 g, 32.4 mmol). The reaction was stirred at 23 °C for 90 min then concentrated *in-vacuo*. The residue was partitioned between $CH_2Cl_2$ and water, the organic layer was dried with $MgSO_4$, filtered and concentrated. Purification by flash chromatography (10% EtOAc/hexane) yielded a colorless oil which was dissolved in EtOAc (50 mL) and 10% Pd/C (50 mg) was added. The reaction mixture was stirred under $H_2$ for 40 h and subsequently filtered through a pad of celite. Evaporation of the solvent and chromatographic purification (20% EtOAc/hexane) afforded the title compound **7** (7.12 g, 74%) as colorless oil which solidified upon standing. TLC $R_f$ = 0.05 (20% EtOAc/hexanes, PMA stain); $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.77 (d, *J* = 7.5, 2H), 7.64 (t, *J* = 7.5, 2H), 7.41 (t, *J* = 7.5, 2H), 7.33 (d, *J* = 7.5, 2H), 5.03 (dd, *J* = 9.8, 3.9, 1H), 4.54 (dd, *J* = 10.2, 7.0, 1H), 4.36 (dd, *J* = 10.2, 7.8, 1H), 4.29–4.32 (m, 1H), 1.70–1.95 (m, 3H), 1.01 (d, *J* = 6.5, 3H), 0.99 (d, *J* = 6.5, 3H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 176.3, 154.9, 143.5, 143.2, 141.4, 141.4, 128.1, 128.0, 127.4, 127.3, 125.4, 125.3, 120.2, 73.8, 70.5, 46.8, 39.7, 24.7, 23.1, 21.5; MS (ESI+) *m*/*z* 377.1 $[M + Na]^+$ ($C_{21}H_{22}NaO_5$ requires 377.1).



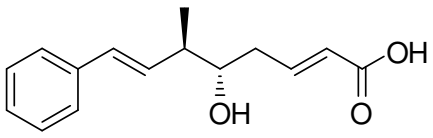

**8**

**Fmoc-3-Cl-D-Tyr(Me)-OH (8):**[3] Boc-3-Cl-D-Tyr(Me)-OH (9.60 g, 29.1 mmol)[4] was treated with 4 M HCl in dioxane (25 mL) at 23 °C for 1 h then the reaction mixture was concentrated *in-vacuo*. The residual white solid was dissolved in 10% aqueous $Na_2CO_3$ (60 mL), dioxane (60 mL) and *N*-(9-Fluorenylmethoxy-carbonyloxy)succinimide (9.82 g, 29.1 mmol) were added. The reaction mixture was stirred at 23 °C for 18 h then diluted with water (100 mL). The aqueous phase was extracted with $Et_2O$ (2 x 100 mL), acidified to pH 2 (conc. HCl) and extracted with EtOAc (3 x 100 mL). The combined EtOAc phases were washed with saturated aqueous

NaCl, dried with $MgSO_4$, filtered and concentrated. Without further purification the title compound **8** (12.65 g, 96%) was obtained as white foam. TLC $R_f$ = 0.26 (EtOAc, PMA stain); $^1$H NMR ($CDCl_3$, 400 MHz) δ 9.82 (br s, 1H), 7.76 (d, *J* = 7.6, 2H), 7.56 (t, *J* = 7.6, 2H), 7.39 (t, *J* = 7.6, 2H), 7.31 (d, *J* = 7.6, 2H), 7.19 (d, *J* = 1.6, 1H), 6.99 (dd, *J* = 8.3, 1.6, 1H), 6.80 (d, *J* = 8.3, 1H), 5.44 (d, *J* = 7.8, 1H), 4.64–4.69 (m, 1H), 4.46 (dd, *J* = 10.5, 7.0, 1H), 4.35 (dd, *J* = 10.5, 7.0, 1H), 4.21 (t, *J* = 7.0, 1H), 3.83 (s, 3H), 3.14 (dd, *J* = 14.1, 5.3, 1H), 3.02 (dd, *J* = 14.1, 6.3, 1H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 175.0, 155.9, 154.2, 143.7, 141.4, 131.1, 128.8, 128.8, 127.8, 127.2, 125.2, 125.1, 120.1, 112.2, 67.2, 56.1, 54.6, 47.2, 36.8; MS (ESI+) *m*/*z* 474.1 $[M + Na]^+$ ($C_{25}H_{22}ClNNaO_5$ requires 474.1).

## Synthesis of cryptophycin unit A (9)

Cryptophycin unit A, hydroxy group protected as TBS ether and carboxylic acid as *tert*-butyl ester, was synthesized according to the publication by Georg *et al.*[5] Removal of both protecting groups in one step was accomplished as described below.[6]




**9**

**(2*E*, 5*S*, 6*R*, 7*E*)-5-Hydroxy-6-methyl-8-phenylocta-2,7-dienoic acid (9):** *tert*-Butyl (2*E*, 5*S*, 6*R*, 7*E*)-5-[(*tert*-butyl-dimethylsilyl)-oxy]-6-methyl-8-phenyl-octa-2,7-dienoate (725 mg, 1.74 mmol)[5] was dissolved in $CH_2Cl_2$ (8 mL) and trifluoroacetic acid (2 mL). The solution was stirred at 23 °C for 4 h then concentrated *in-vacuo*. Residual trifluoroacetic acid was removed by coevaporation with toluene. Flash chromatography (2% MeOH/$CH_2Cl_2$ + 1% AcOH) afforded the title compound **9** (210 mg, 49%) as pale yellow oil. TLC $R_f$ = 0.24 (2% MeOH/$CH_2Cl_2$ + 1% AcOH, $KMnO_4$ stain); $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.23–7.39 (m, 5H), 7.16 (dt, *J* = 15.6, 7.4, 1H), 6.49 (d, *J* = 15.8, 1H), 6.13 (dd, *J* = 15.8, 8.7, 1H), 5.94 (dt, *J* = 15.6, 1.4, 1H), 3.68 (ddd, *J* = 7.9, 6.0, 3.9, 1H), 2.50–2.56 (m, 1H), 2.36–2.44 (m, 2H), 1.16 (d, *J* = 6.8, 3H); $^{13}$C NMR ($CDCl_3$, 101 MHz) δ 171.0, 148.5, 137.0, 132.4, 130.9, 128.8, 127.7, 126.4, 122.9, 73.9, 43.6, 37.5, 17.0; MS (ESI+) *m*/*z* 269.1 $[M + Na]^+$ ($C_{15}H_{18}NaO_3$ requires 269.1).

## Synthesis of linear cryptophycin thioesterase substrates 3a–c on solid support

**Attachment of first amino or hydroxy acid:** In a typical experiment, 4-sulfamylbutyryl AM PEGA resin (1 g, 0.28 mmol) was washed with $CH_2Cl_2$ and cooled to -20 °C. Fmoc-leucic acid **7** (397 mg, 1.12 mmol) or Fmoc-Leu-OH (396 mg, 1.12 mmol) and PyBOP (583 mg, 1.12 mmol), dissolved in $CH_2Cl_2$ (5 mL), and DIPEA (0.39 mL, 2.24 mmol) were added. The reaction mixture was left to stand at -20 °C for 8 h with occasional shaking and subsequently filtered and washed with $CH_2Cl_2$. The coupling procedure was repeated once to obtain resin with a loading value of 0.20 – 0.25 mmol/g.[7]

**General methods for solid-phase synthesis:** The Fmoc deprotection and the formation of peptide bonds were performed as follows: to the above prepared resin (0.25 mmol) was added a solution of 2% piperidine and 2% DBU in DMF (5 mL), shaked for 10 min, filtered and washed with DMF. The deprotection procedure was repeated twice. After thoroughly washing with DMF the resin was agitated with a solution of the Fmoc-protected amino acid (0.75 mmol), TBTU (0.75 mmol) and HOBt (0.75 mmol) in DMF (5 mL) and with DIPEA (1.50 mmol) for 30 min. The resin was filtered, washed with DMF and the coupling was repeated once. With a negative Kaiser test, the synthesis proceeded to the next round of deprotection and coupling. At last, cryptophycin unit A **9** (2 x 0.30 mmol) was coupled in the same way as the Fmoc-protected amino acids.

**Ester bond formation on solid support:** To form the ester bond between *β*-alanine and leucic acid on solid support, Fmoc-leucic acid loaded resin (0.25 mmol) was washed with DMF and the alcohol was deprotected with a solution of 2% piperidine and 2% DBU in DMF (5 mL) for 10 min. The deprotection procedure was repeated twice and the resin was subsequently washed with DMF and $CH_2Cl_2$. Fmoc-*β*-Ala-OH (233 mg, 0.75 mmol), dissolved in THF (3 mL), $CH_2Cl_2$ (3 mL), MSNT (222 mg, 0.75 mmol) and NMI (44 µL, 0.56 mmol) were added and the reaction mixture was agitated for 1h, then washed with THF and $CH_2Cl_2$. The esterification was repeated twice to obtain a loading value of 0.18 mmol/g.[7]

## Purification and characterization of cryptophycins 5a–c and *seco*-cryptophycins 6a–c

The cleaved cryptophycins **5a**–**c** and *seco*-cryptophycins **6a**–**c** from solid support were either separated by flash chromatography (5% MeOH/$CH_2Cl_2$) or by using reverse phase HPLC with a 10 to 100% gradient of acetonitrile in 0.1% TFA/water over the course of 40 min on an Alltech Econosil 10 µm C18 column (250 mm x 4.6 mm). The products were analyzed by $^1$H NMR spectroscopy and/or by (HR)-ESI-TOF mass spectrometry in the positive ion mode.

**Desepoxyarenastatin (5a):** Yield after purification (FC): 5 mg (8.5 µmol) of a white solid; $^1$H NMR ($CDCl_3$/$d^4$-MeOH , 400 MHz) δ 7.04–7.22 (m, 5H), 7.00 (d, *J* = 8.6, 2H), 6.67 (d, *J* = 8.6, 2H), 6.54 (ddd, *J* = 16.4, 10.8, 4.6, 1H), 6.28 (d, *J* = 15.8, 1H), 5.88 (dd, *J* = 15.8, 8.9, 1H), 5.66 (d, *J* = 16.4, 1H), 4.87–4.92 (m, 1H), 4.80 (dd, *J* = 9.8, 3.5, 1H), 4.46–4.51 (m, 1H), 3.64 (s, 3H), 3.30–3.34 (m, 1H), 3.20–3.24 (m, 1H), 3.03 (dd, *J* = 14.5, 5.7, 1H), 2.72 (dd, *J* = 14.5, 8.8, 1H), 2.41–2.44 (m, 3H), 2.16–2.25 (m, 2H), 1.43–1.65 (m, 3H), 1.01 (d, *J* = 6.8, 3H), 0.60 (d, *J* = 6.2, 3H), 0.56 (d, *J* = 6.4, 3H); MS (ESI+) *m*/*z* 591.3 $[M + H]^+$ ($C_{34}H_{43}N_2O_7$ requires 591.3).

**Cryptophycin-29 (5b):** Yield after purification (HPLC): 6 mg (9.6 µmol) of a white solid; $^1$H NMR ($CDCl_3$, 400 MHz) δ 7.18–7.33 (m, 6H), 7.08 (br t, 1H), 7.06 (dd, *J* = 8.4, 2.0, 1H), 6.81 (d, *J* = 8.4, 1H), 6.66 (ddd, *J* = 15.2, 10.4, 5.2, 1H), 6.47 (d, *J* = 8.4, 1H), 6.39 (d, *J* = 15.8, 1H), 5.98 (dd, *J* = 15.8, 8.8, 1H), 5.74 (d, *J* = 15.2, 1H), 4.98–5.02 (m, 1H), 4.89 (dd, *J* = 10.0, 3.3, 1H), 4.64–4.69 (m, 1H), 3.83 (s, 3H), 3.55–3.63 (m, 1H), 3.28–3.35 (m, 1H), 3.13 (dd, *J* = 14.3, 6.2, 1H), 2.87 (dd, *J* = 14.3, 8.0, 1H), 2.50–2.58 (m, 3H), 2.26–2.34 (m, 2H), 1.55–1.63 (m, 3H), 1.11 (d, *J* = 6.8, 3H), 0.72 (d, *J* = 6.3, 3H), 0.68 (d, *J* = 6.3, 3H); MS (ESI+) *m*/*z* 625.3 $[M + H]^+$ ($C_{34}H_{42}ClN_2O_7$ requires 625.3).

**Amide analog (5c):** Yield after purification (HPLC): 12 mg (21 µmol) of a white solid; $^1$H NMR ($CDCl_3$/$d^4$-MeOH , 400 MHz) δ 7.10–7.24 (m, 10H), 6.56 (ddd, *J* = 15.2, 11.2, 4.1, 1H), 6.32 (d, *J* = 15.8, 1H), 5.93 (dd, *J* = 15.8, 8.8, 1H), 5.69 (dd, *J* = 15.2, 1.5, 1H), 4.96–5.01 (m, 1H), 4.51–4.55 (m, 1H), 4.35 (dd, *J* = 8.8, 6.2, 1H), 4.54–3.60 (m, 1H), 3.14–3.23 (m, 2H), 2.76 (dd, *J* = 14.4, 10.1, 1H), 2.44–2.48 (m, 1H), 2.22–2.30 (m, 4H), 1.24–1.52 (m, 3H), 1.05 (d, *J* = 6.8, 3H), 0.65 (d, *J* = 6.4, 3H), 0.64 (d, *J* = 6.5, 3H); $^{13}$C NMR data could not been obtained due to very low solubility in standard NMR solvents; HRMS (ESI+) *m*/*z* 582.2949 $[M + Na]^+$ ($C_{33}H_{41}N_3NaO_5$ requires 582.2944).

***seco*-Desepoxyarenastatin (6a):** MS (ESI+) *m*/*z* 609.4 $[M + H]^+$ ($C_{34}H_{45}N_2O_8$ requires 609.3).

***seco*-Cryptophycin-29 (6b):** MS (ESI+) *m*/*z* 643.3 $[M + H]^+$ ($C_{34}H_{44}ClN_2O_8$ requires 643.3).

***seco*-Amide analog (6c):** MS (ESI+) *m*/*z* 578.4 $[M + H]^+$ ($C_{33}H_{44}N_3O_6$ requires 578.3).


[1] Z. Q. Beck, C. C. Aldrich, N. A. Magarvey, G. I. Georg, D. H. Sherman, *Biochemistry* **2005**, *44*, 13457-13466.

[2] A. K. Ghosh, A. Bischoff, *Eur. J. Org. Chem.* **2004**, 2131-2141.

[3] A different synthetic approach for Fmoc-3-Cl-D-Tyr(Me)-OH has recently been reported: P. Danner, M. Bauer, P. Phukan, M. E. Maier, *Eur. J. Org. Chem.* **2005**, 317-325.

[4] R. A. Barrow, T. Hemscheidt, J. Liang, S. Paik, R. E. Moore, M. A. Tius, *J. Am. Chem. Soc.* **1995**, *117*, 2479-2490.

[5] M. Eggen, C. J. Mossman, S. B. Buck, S. K. Nair, L. Bhat, S. M. Ali, E. A. Reiff, T. C. Boge, G. I. Georg, *J. Org. Chem.* **2000**, *65*, 7792-7799.

[6] An alternative one-step cleavage of both protecting groups has been described: J. D. White, H. Smits, E. Hamel, *Org. Lett.* **2006**, *8*, 3947-3950.

[7] Fmoc loading on solid support was determined as described in the Novabiochem catalog **2003**.