Total Synthesis of Cryptophycin 3

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Keywords: Alkylation / Amino acids / Natural products / Peptidomimetics

The depsipeptide cryptophycin 3 (**5**) and the cryptophycin analogue **43** were prepared from the corresponding four subunits. The tripeptide analogue **34** was acquired from the starting amino ester **33**, which contains fragments D and C. After extension at the carboxyl function by esterification with the hydroxy ester **10**, the *seco* compounds **37** and **42** were obtained. Ring closure was achieved by macrolactamization in the presence of TBTU as condensing agent. This work features a streamlined synthesis of the hydroxy ester **10**, a short synthesis of the amino acid **14** by enantioselective alkylation of the glycinimine **21**, and the use of the Fmoc protecting group for the amino functions.

Introduction

Cyclodepsipeptides are cyclic peptides containing at least one hydroxy acid, resulting in the replacement of an amide by an ester bond. As well as the hydroxy acid(s), cyclodepsipeptides often also contain unusual amino acids, such as extended, *N*-methylated, hydroxylated, or halogenated ones. The hydroxy acids frequently derive from the polyketide pathway, so many cyclodepsipeptides are macrocyclic hybrid peptide/polyketide-like molecules. Compounds of this type have quite recently been accessed by a chemoenzymatic route.[1] Thanks to their modular natures and the presence of peptide subunits, cyclodepsipeptides are ideal lead compounds for the generation of libraries of natural product-like compounds, compound collections of interest in the context of chemical genetics and target discovery.[2] It might also be argued that an organism, by producing cyclodepsipeptides, would have a flexible tool available for responding to a possible hostile environment by simple combination of post-translationally modified amino acids and one larger building block from another biosynthetic pathway. A quite prominent family of cyclodepsipeptides are the cryptophycins. The first representative of these depsipeptides, cryptophycin 1 (**1**), was isolated in 1990 from a blue-green algae (Figure 1).[3,4] Some time later, the isolation and structure of cryptophycin 24 (**3**), also known as arenastatin A, was described.[5] Initially, cryptophycin 1 was classified as an antifungal agent, but it was later discovered that it also has powerful antitumor activity caused by disruption of microtubule assembly.[6] Of particular interest was the fact that cryptophycin showed activity against some multiple drug resistance (MDR) cell lines. Because of the interesting bio-

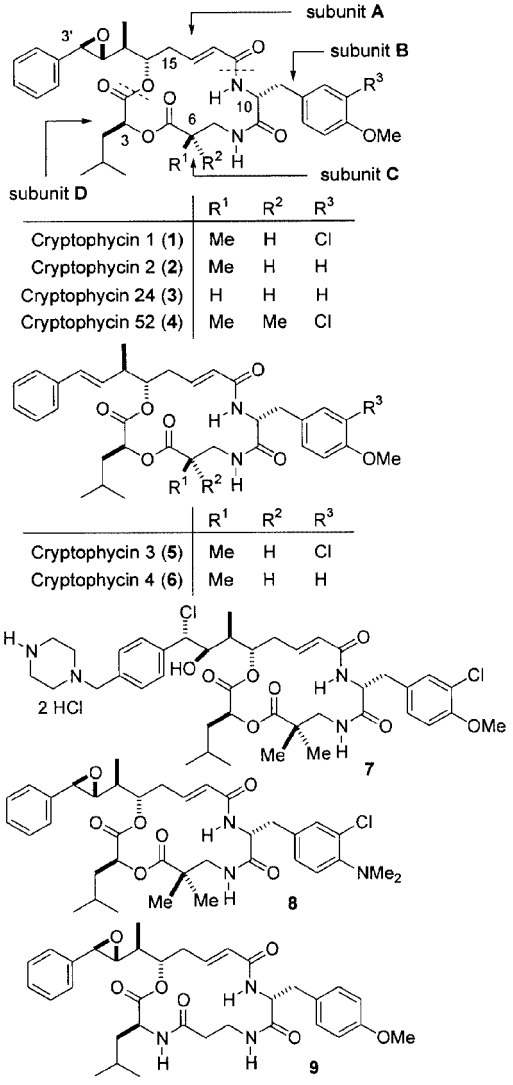

Figure 1. Structures of important cryptophycins

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syntheses of the natural products[7,8,9,10] and their analogues have been reported. Out of around 450 analogues, cryptophycin 52 (**4**) has emerged as a promising clinical candidate,[4] advancing even to Phase 2 clinical studies (Table 1).[11] Because of steric hindrance around the ester group, the analogue **4** is more stable towards hydrolysis, and structure/activity studies also showed that variations in the fragment A epoxide are tolerated well, even giving compounds that are active against murine Panc-03 tumors at much lower doses.[12] The chlorohydrin **7** is one example. Compounds such as **5** or **6**, lacking the epoxide ring, are still quite active, which indicates that there is no covalent bond-formation involved in binding to the tubulin. On the other hand, fragment B has turned out to be rather sensitive to modifications.[13] One of the most active compounds from this series turned out to be the analogue **8**.[14] The triamide **9** is characterized by poor solubility and low bioavailability but still shows reasonable activity.

While in vitro and in vivo studies with cryptophysin 52 were very promising, clinical studies revealed significant neurological toxicity and only weak or no therapeutic response. Nevertheless, from a chemical point of view, the ω-hydroxy acid of cryptophycin should be an interesting building block allowing restriction of the conformations of tri- and tetrapeptides inserted between the hydroxy and carboxyl functions.

Table 1. IC_{50} values for some representative cryptophycins

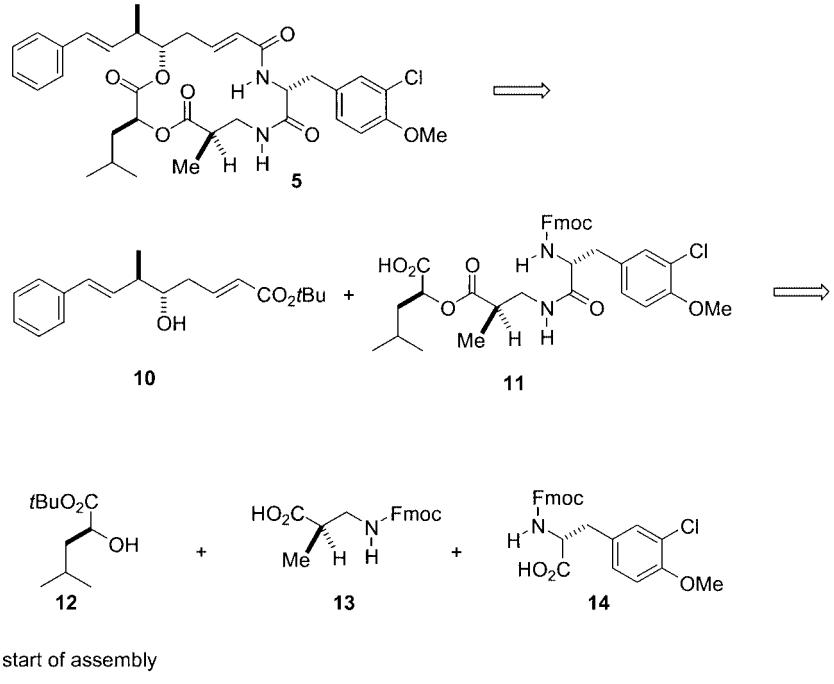
Compound	IC_{50} [nM][a]	Cell type
1	0.0092	KB
2	0.057	KB
3	0.198	KB
4	0.022	CCRF-CEM
5	3.23	KB
6	2.15	KB
7	0.021	CCRF-CEM
8	0.054	CCRF-CEM
9	6.0	KB

[a] Data from references[4f] (compounds **1**, **2**, **3**, **5**, **9**),[12] (compounds **4**, **7**),[3b] (compound **6**), and[14] (compound **8**); KB = human nasopharyngeal carcinoma cell line, CCRF-CEM = human leukemia cell line.

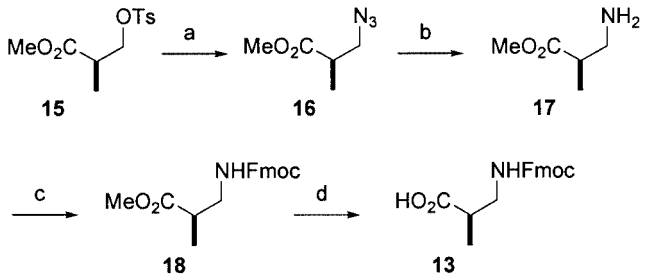
Results and Discussion

In order to make a larger number of such macrocycles, an efficient synthesis of fragment A is required. In addition, use of the Fmoc protecting group should in principle allow a solid-phase assembly of a suitable *seco* compound. In this paper we describe a formal total synthesis of cryptophycin 3 (**5**) in which all amino acids are Fmoc-protected and in which the amino acid B was prepared by an enantioselective alkylation. From a strategic point of view it seems advantageous to combine a tripeptide unit such as **11** with a ω-hydroxy ester **10** (Scheme 1).

The α-hydroxy ester **12** was prepared from isoleucine as in the literature.[15] The synthesis of the β-amino acid **13** started from the tosylate **15**, derived in turn from the Roche ester (Scheme 2).[16] Substitution of the tosylate with azide, followed by catalytic hydrogenation of the azide function, provided the amine **17**.[17] The amino group of **17** was then immediately protected under Schotten−Baumann conditions, resulting in the Fmoc-protected ester **18**. The hy-



Scheme 1. Retrosynthetic disconnection of cryptophycin 3 (**5**) to give the ω-hydroxy ester **10** and the building blocks **12**, **13**, and **14**

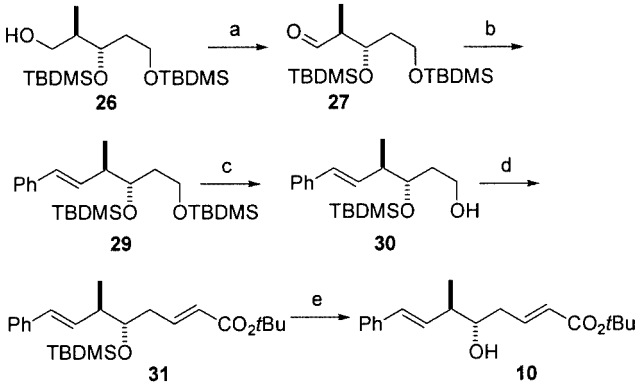


Scheme 2. Synthesis of the Fmoc-protected 3-amino-2-methylpropionic acid **13** from the tosylate **15**: a) NaN_3, DMSO, 80 °C, 3 h (80%); b) H_2, Pd/C, MeOH, 23 °C, 15 h (90%); c) FmocCl, Na_2CO_3 (10%), 23 °C, 14 h (90%); d) HCl, AcOH, 100 °C, 15 h (67%)

drolysis of the ester function to give the amino acid **13** was achieved under acidic conditions.[18]

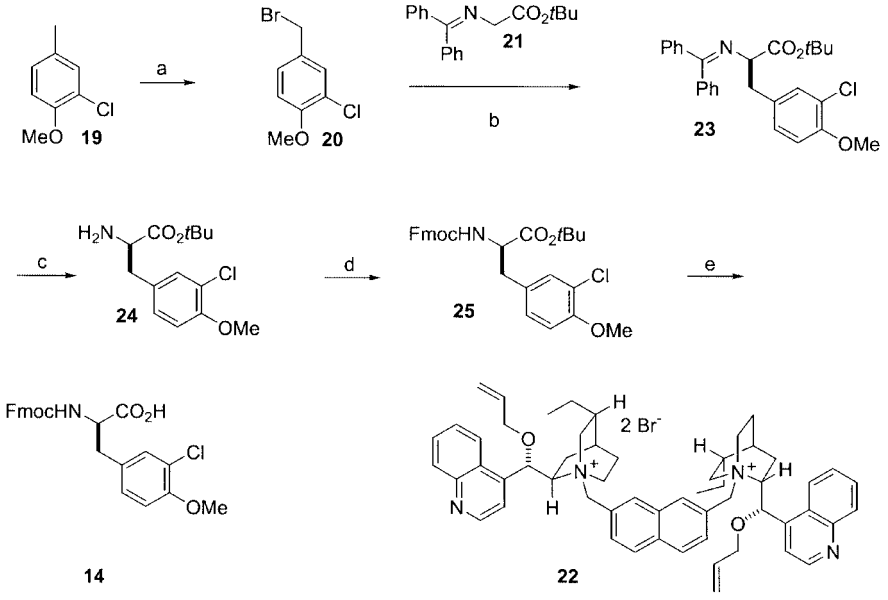
The phenylalanine derivative (cf. **14**), also called subunit B, is usually prepared from D-tyrosine through chlorination of the aromatic core. Since these conditions would probably not be compatible with the Fmoc protecting group, a new synthesis based on the enantioselective alkylation of the glycine derivative **21** was developed (Scheme 3). Accordingly, the methyl ether **19** was converted into the benzylic bromide **20** with *N*-bromosuccinimide. The enantioselective alkylation of the glycine imine[19] **21** with **20** was carried out under basic conditions with use of the chiral ligand **22**.[20–22] Hydrolysis of the imine **23** and protection of the amine with (fluorenylmethoxy)carbonyl chloride provided the fully protected amino acid **25**. Treatment of **25** with trifluoroacetic acid furnished the desired amino acid **14**.

Because of the use of the Fmoc strategy, the carboxyl group of the cyclization point has to be an ester that can be cleaved under non-basic conditions, so our original synthesis[23] of the subunit A was slightly modified (Scheme 4), with the alcohol **26**, obtained by the hydroboration pathway, being oxidized to the aldehyde **27**, which was then extended with $(EtO)_2P(O)CH_2Ph$ (**28**) under Wittig–Horner conditions to give the styrene derivative **29**. After selective cleavage of the primary silyl ether, resulting in alcohol **30**, one-pot oxidation and Wittig treatment[24] with $Ph_3P{=}CHCO_2tBu$[25] provided the unsaturated ester **31** in good yield. Finally, removal of the silicon protecting group furnished the key building block **10**.



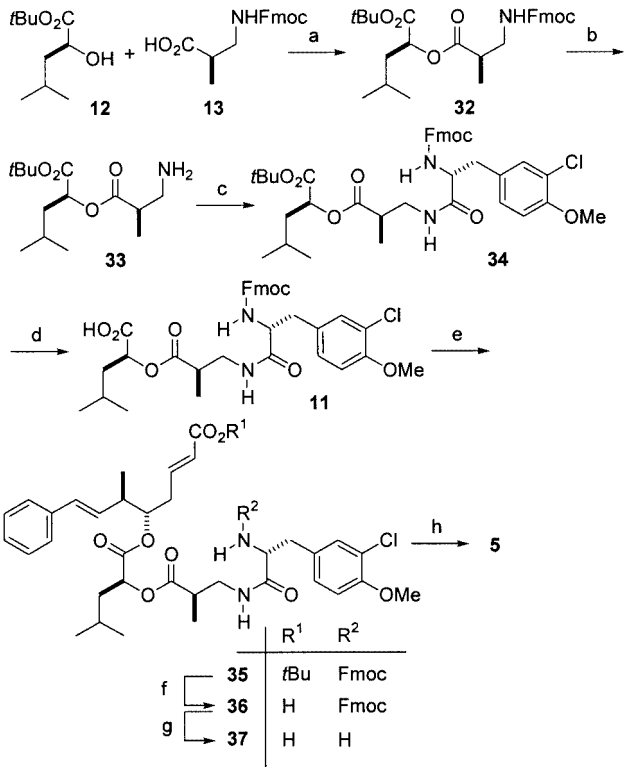
Scheme 4. Synthesis of the 5-hydroxy ester **10**: a) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C, 1 h, NEt_3, −70 to 0 °C, 3 h; b) $(EtO)_2P(O)CH_2Ph$ (**28**), *n*BuLi, THF, −78 °C, 1 h, add aldehyde, −78 to 23 °C, 7 h (58%, 2 steps); c) PPTS, MeOH, 50 °C, 4 h (85%); d) i) DMSO, $(COCl)_2$, CH_2Cl_2, −78 °C, 1 h, then Et_3N, −78 to 0 °C, 3 h; ii) add $Ph_3P{=}CHCO_2tBu$, 0 to 23 °C, 12 h (78%); e) TBAF, THF, 0 to 23 °C, 2 h (73%)

The assembly of the tripeptide **11** began with ester formation between the secondary alcohol of **12** and the amino acid **13** (Scheme 5). Use of DCC as a condensing agent provided an excellent yield of the depeptide analogue **32**, and treatment of compound **32** with diethylamine in THF caused cleavage of the Fmoc protecting group, resulting in



Scheme 3. Synthesis of the D-phenylalanine derivative **14** by enantioselective alkylation: a) NBS, AIBN (cat.), CCl_4, reflux, 16 h (68%); b) 50% KOH, toluene/$CHCl_3$ (7:3), ammonium salt **22** (0.01 equiv.), 0 °C, 20 h (87%); c) citric acid (15%), THF, 23 °C, 16 h; d) FmocCl, Na_2CO_3, 23 °C, 14 h (72%, 2 steps); e) TFA, CH_2Cl_2, 0 to 23 °C, 5 h (93%)

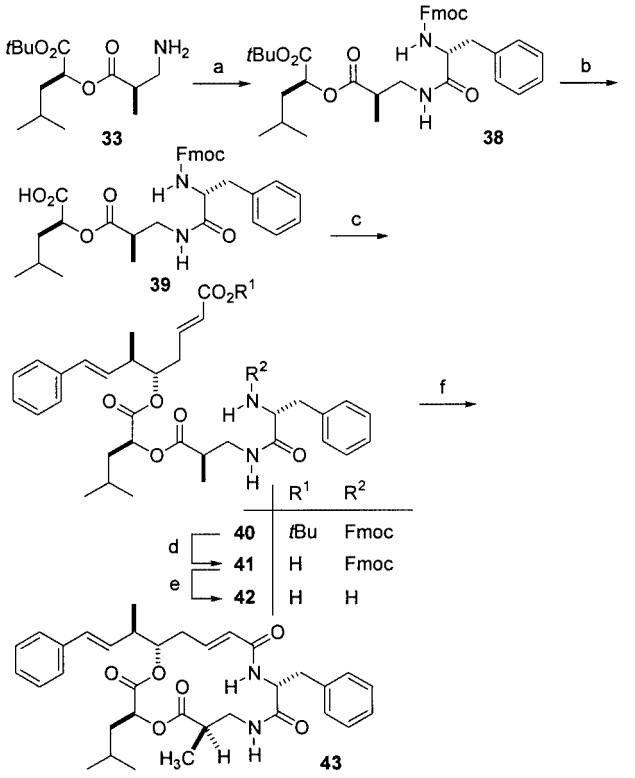
the primary amine **33**. This compound turned out to be stable towards intramolecular amide formation. Coupling of the amine **33** with the Fmoc-protected amino acid **14** in the presence of DCC/HOBt provided the D-C-B section, compound **34**, in good yield.



Scheme 5. Synthesis of desoxy cryptophycin **5** by the Fmoc strategy: a) DCC, DMAP (cat.), CH_2Cl_2, 0 to 23 °C, 5 h (80%); b) Et_2NH, THF, 0 to 23 °C, 12 h (67%); c) **14**, DCC, HOBt, 0 to 23 °C, 7 h (85%); d) TFA, CH_2Cl_2, 0 to 23 °C (79%); e) hydroxy ester **10**, 2,4,6-$Cl_3C_6H_2COCl$, iPr_2NEt, DMAP, THF, 23 °C, 2 h (73%, 2 steps); f) TFA, CH_2Cl_2, 0 to 23 °C, 2 h; g) Et_2NH, THF, 0 to 23 °C, 2 h; h) TBTU, HOBt, iPr_2NEt, DMF, 23 °C, 2 h (42%, 3 steps); TBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate

The next stage in the plan was to form the ester bond that connects A and D. Accordingly, treatment of the ester **34** with trifluoroacetic acid liberated the C-terminal carboxyl group, giving the acid **11**. While the esterification of **11** with the hydroxy ester **10** in the presence of DCC was low-yielding, esterification with the Yamaguchi reagent[26] took place in high yield. For the generation of the *seco* compound **37**, the *tert*-butyl ester was first cleaved, followed by removal of the Fmoc protecting group. Cyclization was achieved with the reagent TBTU giving the macrocycle **5** in good yield.

By replacement of the Fmoc-protected amino acid **14** with Fmoc-phenylalanine in the coupling with the amine **33**, fragment **38** was obtained (Scheme 6). The subsequent combination of the acid **39** with **10** provided the ester **40**. This compound could also be converted into the corresponding cryptophycin **43** in good overall yield by cleavage of the protecting groups and macrolactam formation.



Scheme 6. Synthesis of desoxy cryptophycin **43**: a) Fmoc-Phe-OH, DCC, HOBt, 0 to 23 °C, 7 h (85%); b) TFA, CH_2Cl_2, 0 to 23 °C, 3 h; c) hydroxy ester **10**, 2,4,6-$Cl_3C_6H_2COCl$, iPr_2NEt, DMAP, THF, 23 °C, 2 h (75%); d) TFA, CH_2Cl_2, 0 to 23 °C; e) Et_2NH, THF, 0 to 23 °C, 3 h; f) TBTU, HOBt, iPr_2NEt, DMF, 23 °C, 2 h (76%)

Conclusion

To summarize, we have been able to demonstrate that the Fmoc strategy is quite suitable for the production of the cryptophycins **5** and **43** in a concise fashion. In particular, this strategy is well suited with the enantioselective alkylation of the glycine derivative **21**. Further work to bridge the hydroxy acid **10** with tri- and tetrapeptides obtained by solid-phase synthesis is underway in our laboratory.

Experimental Section

General: ^1H and ^{13}C NMR: Bruker Avance 400, spectra were recorded at 295 K in $CDCl_3$; chemical shifts are calibrated to the residual proton and carbon resonance of the solvent: $CDCl_3$ (δ_H = 7.25, δ_C = 77.0 ppm), CD_3OD (δ_H = 4.78, 3.30, δ_C = 49.0 ppm). IR: Jasco FT/IR-430. Optical rotation: Jasco polarimeter P-1020, reported in degrees $[\alpha]_D$ {*c* [g/100 mL], solvent}; recorded at 298 K. MS: Finnigan Triple-Stage-Quadrupole TSQ-70 (ionizing voltage of 70 eV) or Intectra AMD 402 mass spectrometer. HRMS: Intectra AMD MAT-711A (EI) or Bruker Daltonic APEX 2 (ESI). Flash chromatography: J. T. Baker silica gel 43−60 μm. Thin-layer chromatography Macherey−Nagel Polygram Sil G/UV_{254}. All solvents used in the reactions were distilled before use. Dry tetrahydrofuran, and toluene were distilled from sodium and benzophenone,

whereas dry dichloromethane, dimethylformamide, and triethylamine were distilled from CaH_2. Petroleum ether with a boiling range of 40−60 °C was used. Reactions were generally run under argon. All commercially available compounds were used as received unless stated otherwise.

Cryptophycin 3 (5): Trifluoroacetic acid (5 mL) was added slowly at 0 °C to a solution of the protected *seco* acid **35** (80 mg, 0.085 mmol) in CH_2Cl_2 (2 mL) and the mixture was stirred for 2 hours at room temperature. The solvent was removed in vacuo, and toluene (5 mL) was added. After removal of the solvent, the residue (the acid **36**) was redissolved in THF (3 mL) and diethylamine (3 mL) was added dropwise at 0 °C. The reaction mixture was again stirred at room temperature for 2 hours, followed by the removal of the solvents in vacuo. The crude amino acid **37** was dissolved in dry DMF (15 mL), and TBTU (30 mg, 0.081 mmol), HOBT (2 mg), and DIEA (33 μL, 0.19 mmol) were then added successively at room temperature. The reaction mixture was stirred for 2 hours at room temperature, saturated $NaHCO_3$ solution (5 mL) was added, and stirring was continued for 1 hour. The mixture was extracted with CH_2Cl_2 (15 mL), and the organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (50% EtOAc in petroleum ether) to give compound **5** (22 mg, 42% from **35**). R_f = 0.31. $[\alpha]_D^{27}$ = +24.68 (c = 0.40, $CHCl_3$) {ref.[7e] $[\alpha]_D^{22}$ = +29.5 (c = 2.0, $CHCl_3$)}. IR (neat): $\tilde{\nu}$ = 3430, 3310, 2959, 1727, 1667, 1504, 1250, 1173 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.34−7.17 (m, 6 H), 7.04 (dd, J = 8.3, 1.8 Hz, 1 H), 6.96−6.91 (m, 1 H), 6.80 (d, J = 8.3 Hz, 1 H), 6.65 (ddd, J = 15.2, 9.8, 5.5 Hz, 1 H), 6.37 (d, J = 15.9 Hz, 1 H), 5.97 (dd, J = 15.8, 8.7 Hz, 1 H), 5.74 (d, J = 15.4 Hz, 1 H), 5.66 (d, J = 8.6 Hz, 1 H), 5.08−4.93 (m, 1 H), 4.87−4.72 (m, 2 H), 3.83 (s, 3 H), 3.51−3.41 (m, 1 H), 3.32-3-22 (m, 1 H), 3.10 (dd, J = 14.4, 5.3 Hz, 1 H), 3.00 (dd, J = 14.4, 7.0 Hz, 1 H), 2.71−2.62 (m, 1 H), 2.60−2.45 (m, 2 H), 2.41−2.28 (m, 1 H), 1.75−1.49 (m, 3 H), 1.38−1.26 (m, 1 H), 1.19 (d, J = 7.0 Hz, 3 H), 1.10 (d, J = 6.8 Hz, 3 H), 0.74 (d, J = 6.3 Hz, 3 H), 0.69 (d, J = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.6, 170.9, 170.2, 165.4, 153.9, 141.4, 136.6, 131.8, 131.0, 130.3, 130.0, 129.7, 128.6, 127.5, 126.1, 125.1, 122.3, 112.1, 71.5, 56.1, 53.5, 42.2, 41.1, 39.5, 38.2, 36.4, 35.0, 24.4, 22.7, 21.1, 17.3, 14.0 ppm. HRMS (FT-ICR): calcd. for $C_{35}H_{43}ClN_2O_7$ $[M + Na]^+$ 661.2651, found 661.2654.

***tert*-Butyl (2*E*,5*S*,6*R*,7*E*)-5-Hydroxy-6-methyl-8-phenylocta-2,7-dienoate (10):** Tetra-*n*-butylammonium fluoride (1 M in THF, 0.87 mL, 0.87 mmol) was added at 0 °C to a solution of enoate **31** (110 mg, 0.26 mmol) in dry THF (4 mL) and the reaction mixture was stirred at room temperature for 3 hours. The mixture was washed with brine, dried with $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (first 30% and then 50% EtOAc in petroleum ether) to give the hydroxy ester **10** (55 mg, 73%) as a colorless oil. R_f = 0.29 (30% EtOAc in petroleum ether). $[\alpha]_D^{25}$ = +40.0 (c = 1.42, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3444, 3025, 2976, 2931, 1712, 1652, 1494, 1361 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.70−7.22 (m, 5 H), 6.89−6.81 (m, 1 H), 6.41 (d, J = 15.9 Hz, 1 H), 6.06 (dd, J = 15.9, 8.5 Hz, 1 H), 5.77 (d, J = 15.6 Hz, 1 H), 3.61−3.56 (m, 1 H), 2.42−2.22 (m, 3 H), 1.41 (s, 9 H), 1.07 (d, J = 6.8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.7, 143.9, 137.0, 131.9, 130.9, 128.5, 127.4, 126.1, 125.9, 80.2, 73.8, 43.2, 37.1, 28.1, 16.7 ppm. HRMS (FT-ICR): calcd. for $C_{19}H_{26}O_3$ $[M + Na]^+$ 325.1774, found 325.1775.

(2*S*)-2-{[(2*R*)-3-({3-Chloro-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*O*-methyl-D-tyrosyl}amino)-2-methylpropanoyl]oxy}-4-methylpentanoic Acid (11): The *tert*-butyl ester **34** (100 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (5 mL), followed by the addition of trifluoroacetic acid (5 mL) at 0 °C. The reaction mixture was stirred for 3 hours at room temperature. Toluene (5 mL) was then added, and the mixture was concentrated in vacuo. This procedure was repeated twice to give the crude acid, which was purified by flash chromatography (5% MeOH in CH_2Cl_2 + drops of AcOH), resulting in acid **11** (82 mg, 79%) as a white solid. R_f = 0.21. $[\alpha]_D^{24}$ = −34.87 (c = 0.44, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3416, 3310, 2850, 2051, 1700, 1630, 1502, 1180 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ = 7.67−6.58 (m, 11 H), 4.86 (d, J = 9.8 Hz, 1 H), 4.24−4.00 (m, 4 H), 3.58 (s, 3 H), 3.45−3.30 (m, 1 H), 3.08−2.89 (m, 1 H), 2.72−2.43 (m, 2 H), 1.73−1.51 (m, 3 H), 1.25−1.18 (m, 1 H), 1.04 (d, J = 6.8 Hz, 3 H), 0.82 (d, J = 6.3 Hz, 6 H) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 174.0, 172.2, 156.6, 153.3, 142.5, 140.7, 131.3, 128.7, 128.1, 127.9, 126.2, 120.9, 114.8, 74.5, 63.1, 57.4, 55.5, 45.8, 44.7, 41.7, 38.2, 36.1, 23.7, 21.9, 16.8, 15.0 ppm. HRMS (FT-ICR): calcd. for $C_{35}H_{39}ClN_2O_8$ $[M + Na]^+$ 674.1350 found 674.1343.

(2*R*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-2-methylpropanoic Acid (13): A solution of the ester **18** (500 mg, 1.4 mmol) in acetic acid (50 mL) was treated with concentrated HCl (5 mL), and the mixture was then heated at 100 °C for 15 hours. After cooling it was poured into water (500 mL), and the colorless precipitate was collected by filtration. The colorless solid was purified by flash chromatography (5% MeOH in CH_2Cl_2) to provide the acid **13** (320 mg, 67%) as a colorless solid. R_f = 0.38 (5% MeOH in CH_2Cl_2 + 2 drops of AcOH). $[\alpha]_D^{25}$ = −10.85 (c = 0.273, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3344, 2360, 1715, 1520, 1450, 1247 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.71−7.19 (m, 8 H), 5.16 (s, br, 1 H), 4.46−4.14 (m, 3 H), 3.39−3.23 (m, 2 H), 2.72−2.69 (m, 1 H), 1.16 (d, J = 7.1 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.8, 157.2, 144.2, 141.0, 128.1, 127.4, 125.4, 120.3, 67.1, 47.6, 43.5, 40.1, 15.0 ppm. HRMS (FT-ICR): calcd. for $C_{19}H_{19}NO_4$ $[M + Na]^+$ 348.1206, found 348.1204.

3-Chloro-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*O*-methyl-D-tyrosine (14): The *tert*-butyl ester **25** (300 mg, 0.59 mmol) was dissolved in CH_2Cl_2 (5 mL), and trifluoroacetic acid (5 mL) was added dropwise at 0 °C. After stirring at room temperature for 5 hours, the reaction mixture was concentrated in vacuo. Toluene (5 mL) was added to the residue, and this mixture was again concentrated. The residue was purified by flash chromatography (5% MeOH in CH_2Cl_2) to yield the Fmoc-protected acid **14** (245 mg, 93%) as a white solid. R_f = 0.25. $[\alpha]_D^{25}$ = −20.71 (c = 0.38, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3410, 3316, 3065, 2953, 2930, 1722, 1716, 1606, 1504, 1450, 1280, 1259, 1065 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.76−7.18 (m, 9 H), 6.98 (d, J = 8.0 Hz, 1 H), 6.80 (d, J = 8.0 Hz, 1 H), 5.27 (d, J = 7.0 Hz, 1 H, NH), 4.67−4.56 (m, 1 H), 4.47−4.29 (m, 2 H), 4.24−4.11 (m, 1 H), 3.84 (s, 3 H), 3.16−2.96 (m, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.3, 156.2, 154.5, 144.0, 141.2, 131.4, 129.0, 128.1, 127.5, 125.4, 120.4, 112.5, 67.5, 56.5, 54.1, 47.4, 37.0 ppm. HRMS (FT-ICR): calcd. for $C_{25}H_{22}ClNO_5$ $[M + Na]^+$ 474.1079, found 474.1077.

Methyl (2*R*)-3-Azido-2-methylpropanoate (16): A mixture of tosylate **15** (2.0 g, 7.3 mmol) and sodium azide (1.0 g, 15.3 mmol) in DMSO (30 mL) was heated at 80 °C for 2−3 h. After the mixture had cooled to room temperature, water (30 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography with 20% EtOAc in petroleum ether to provide **16** (0.9 g, 85%) as a colorless oil. R_f = 0.47. $[\alpha]_D^{22}$ = −14.32 (c = 0.99, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 2982, 2103, 1732, 1463, 1381, 1199 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.68 (s, 3 H), 3.56−3.46 (m, 1 H), 3.35 (dd, J = 12.1,

5.8 Hz, 1 H), 2.73−2.60 (m, 1 H), 1.28 (d, J = 7.0 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.7, 54.1, 52.9, 40.0, 15.1 ppm. HRMS (FT-ICR): calcd. for $C_5H_9N_3O_2$ [M + Na]$^+$ 166.0587, found 166.0585.

Methyl (2*R*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-2-methylpropanoate (18): A mixture of azide **16** (0.28 g, 2.0 mmol) and Pd-C (10%, 140 mg) in MeOH (2 mL) was stirred under hydrogen atmosphere at room temperature for 15 hours. The reaction mixture was filtered through a pad of celite. Concentration of the filtrate gave the crude amine **17**, which was immediately protected without further purification. The crude amino ester **17** (300 mg, 2.5 mmol) was dissolved in THF (10 mL), and aqueous Na_2CO_3 (10%, 10 mL) was added, followed by FmocCl (1.0 g, 3.8 mmol). The reaction mixture was stirred at room temperature for 14 hours. The aqueous layer was extracted with EtOAc (2 × 30 mL), and the combined organic extracts were dried with $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (20% EtOAc in petroleum ether) to provide the protected amine **18** (0.61 g, 90%) as a colorless solid. R_f = 0.25. $[\alpha]_D^{22}$ = −13.2 (c = 0.962, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3310, 2250, 1760, 1520, 1350, 1250 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77−7.28 (m, 8 H), 5.43−5.33 (m, 1 H), 4.40 (d, J = 6.3 Hz, 2 H), 4.22 (t, J = 6.8 Hz, 1 H), 3.71 (s, 3 H), 3.46−3.29 (m, 2 H), 2.79−2.68 (m, 1 H), 1.20 (d, J = 7.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 176.1, 156.8, 144.3, 141.7, 128.0, 127.4, 125.4, 120.3, 67.0, 52.2, 47.6, 43.7, 40.2, 15.1 ppm. HRMS (FT-ICR): calcd. for $C_{20}H_{21}NO_4$ [M + Na]$^+$ 362.3850, found 362.3855.

4-(Bromomethyl)-2-chloro-1-methoxybenzene (20): A mixture of 2-chloro-1-methoxy-4-methylbenzene (**19**, 4.0 g, 25.5 mmol), NBS (5.0 g, 28.1 mmol), and AIBN (190 mg, 1.15 mmol) in dry CCl_4 (160 mL) was heated at reflux overnight. After being cooled to room temperature, the mixture was washed with NaOH solution (1.5 N, 75 mL) and water (75 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/diethyl ether, 3:1) to provide the benzyl bromide **20** (4.1 g, 68%) as a colorless oil. R_f = 0.52. IR (neat): $\tilde{\nu}$ = 2946, 1698, 1603, 1503, 1261, 1065 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40 (d, J = 2.0 Hz, 1 H), 7.22 (dd, J = 8.4, 2.1 Hz, 1 H), 6.85 (d, J = 8.5 Hz, 1 H), 4.43 (s, 2 H), 3.86 (s, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.7, 131.3, 129.0, 126.4, 122.8, 112.5, 56.6, 33.2 ppm. HRMS (FT-ICR): calcd. for C_8H_8BrClO [M + Na]$^+$ 258.5050, found 258.5034.

***tert*-Butyl *N*-(Diphenylmethylene)glycinate (21):** A solution of *tert*-butyl 2-bromoacetate (7.0 g, 35.9 mmol) in acetonitrile (40 mL) was treated with benzophenonimine (6.5 g, 35.8 mmol) and diisopropylethylamine (6.2 mL, 4.6 g, 35.6 mmol), and the mixture was then heated at reflux for 12 hours. After the system had cooled to room temperature, most of the acetonitrile was removed in vacuo. The residue was partitioned between water (40 mL) and diethyl ether (60 mL) and the phases were separated. The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo until the mixture became turbid. Cooling in an ice bath provided a first fraction of 4.1 g. Concentration of all the filtrates resulted in another crop; total yield 10.2 g (96%), slightly yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.71−7.20 (m, 10 H), 4.17 (s, 2 H), 1.44 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.3, 169.6, 139.2, 136.0, 132.2, 130.2, 129.9, 128.6, 128.4, 128.3, 128.1, 127.9, 127.5, 80.8, 56.2, 27.9 ppm.

***tert*-Butyl 3-Chloro-*N*-(diphenylmethylene)-*O*-methyl-D-tyrosinate (23):** The benzyl bromide **20** (500 mg, 2.1 mmol) was added to a stirred mixture of *N*-(diphenylmethylene)glycine *tert*-butyl ester **21** (500 mg, 1.7 mmol) and chiral catalyst[20a] **22** (17.0 mg, 0.017 mmol) in toluene/chloroform (volume ratio = 7:3, 10 mL). The reaction mixture was cooled to 0 °C and treated with aqueous KOH (50%, 2.5 mL). The mixture was stirred at room temperature for approximately 20 hours (TLC monitoring). The suspension was diluted with diethyl ether (100 mL) and washed with water (2 × 50 mL), and the phases were separated. The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/diethyl ether, 2:1) to give the alkylation product **23** (0.65 g, 87%) as a colorless oil. The *ee* was determined by HPLC on a chiral column (DAICEL Chiral OB-H, 250 × 2.6 mm; heptane/2-propanol, 98:02, flow 0.5 mL·min^{-1}, t_{minor} = 8.82 min, t_{major} = 9.79 min) to be 96%. R_f = 0.29. $[\alpha]_D^{23}$ = 155.41 (c = 0.62, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 2975, 2928, 1731, 1622, 1502, 1445, 1256, 1150 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (d, J = 7.0 Hz, 2 H), 7.30−7.16 (m, 6 H), 6.94−6.83 (m, 2 H), 6.70−6.58 (m, 3 H), 4.00 (dd, J = 8.9, 4.4 Hz, 1 H), 3.72 (s, 3 H), 3.08−2.93 (m, 2 H), 1.35 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.0, 153.8, 139.8, 136.7, 131.7, 130.6, 129.5, 129.1, 128.8, 128.6, 128.3, 128.0, 122.2, 112.0, 81.6, 68.0, 56.5, 38.8, 28.4 ppm. HRMS (FT-ICR): calcd. for $C_{27}H_{28}ClNO_3$ [M + Na]$^+$ 472.5035, found 472.5030.

***tert*-Butyl 3-Chloro-*O*-methyl-D-tyrosinate (24):** A solution of the alkylated imine **23** (500 mg, 1.1 mmol) in THF (10 mL) and aqueous citric acid (15%, 5 mL) was stirred at room temperature for 14 hours. The mixture was then diluted with Et_2O (10 mL) and extracted with HCl (1 M, 3 × 10 mL). The combined aqueous layers were washed with Et_2O (10 mL), basified with solid K_2CO_3, and then extracted with EtOAc (3 × 15 mL). The organic extracts were dried with $MgSO_4$, filtered, and concentrated in vacuo to provide the crude amino acid **24**, which was used in the next step without further purification.

***tert*-Butyl 3-Chloro-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*O*-methyl-D-tyrosinate (25):** The crude amino ester **24** (400 mg, 1.4 mmol) was dissolved in THF (10 mL), and aqueous Na_2CO_3 (10%, 10 mL) was added, followed by FmocCl (0.4 g, 1.5 mmol,). The reaction mixture was stirred for 14 hours at room temperature, and the aqueous layer was extracted with EtOAc (3 × 10 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (10% EtOAc in petroleum ether) to give **25** (510 mg, 72%) as a colorless oil. R_f = 0.30. $[\alpha]_D^{25}$ = −25.07 (c = 1.22, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3421, 3335, 3064, 2978, 2933, 1732, 1606, 1503, 1450, 1368, 1280 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, J = 7.3 Hz, 2 H), 7.61−7.17 (m, 7 H), 6.98 (d, J = 8.0 Hz, 1 H), 6.81 (d, J = 8.3 Hz, 1 H), 5.30 (d, J = 7.8 Hz, 1 H), 4.53−4.41 (m, 2 H), 4.36−4.28 (m, 1 H), 4.21 (t, J = 6.9 Hz, 1 H), 3.86 (s, 3 H), 3.05−2.96 (m, 2 H), 1.43 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.3, 155.4, 154.0, 143.7, 141.2, 131.2, 128.7, 127.7, 127.0, 125.0, 122.1, 119.9, 111.9, 82.7, 66.9, 56.1, 55.0, 47.1, 37.1, 27.9 ppm. HRMS (FT-ICR): calcd. for $C_{25}H_{22}ClNO_5$ [M + Na]$^+$ 474.1078, found 474.1077.

(2*S*,3*S*)-3,5-Bis{[*tert*-butyl(dimethyl)silyl]oxy}-2-methylpentanal (27): Dimethyl sulfoxide (0.22 mL, 3.12 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise at −78 °C to a solution of oxalyl chloride (0.139 mL, 1.6 mmol) in CH_2Cl_2 (10 mL). After 5 min, alcohol **26** (0.5 g, 1.3 mmol) in CH_2Cl_2 (10 mL) was added dropwise to the reaction mixture, and stirring was continued at −78 °C for 1 hour. Triethylamine (0.91 mL, 6.5 mmol) was then added dropwise, and the mixture was warmed to room temperature over 3 hours. For the workup the mixture was treated with water (15 mL) and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic layers were washed

with brine (30 mL), dried with $MgSO_4$, filtered, and concentrated in vacuo to give the crude aldehyde **27**, which was used for the next step without further purification.

[(1*E*,3*R*,4*S*)-4,6-Di{[*tert*-butyl(dimethyl)silyl]oxy}-3-methylhex-1-enyl]benzene (29): A solution of diethyl benzylphosphonate **28** (0.77 mL, 3.71 mmol) in THF (15 mL) was treated at −78 °C with *n*BuLi (2.5 M in hexane, 0.83 mL, 2.07 mmol). Stirring was continued at −78 °C for 1 hour, after which a solution of aldehyde **27** (from the previous step) in THF (7 mL) was added dropwise. After having been stirred at −78 °C for 1 hour, the reaction mixture was warmed gradually to room temperature over 6 hours. Aqueous NH_4Cl solution (25 mL) was added, and the mixture was extracted with Et_2O (3 × 40 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried with $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (5% EtOAc in petroleum ether) to give the styrene **29** (345 mg, 58%, 2 steps) as a colorless oil. $R_f = 0.55$. $[\alpha]_D^{25} = +17.8$ ($c = 1.00$, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 2955, 2928, 1471, 1463, 1256, 1067 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.39−7.10 (m, 5 H), 6.37 (d, J = 15.9 Hz, 1 H), 6.18 (dd, J = 15.6, 7.8 Hz, 1 H), 3.88−3.79 (m, 1 H), 3.74−3.59 (m, 2 H), 2.54−2.43 (m, 1 H), 1.65 (q, J = 6.5 Hz, 2 H), 1.11 (d, J = 7.0 Hz, 3 H), 0.95 (s, 9 H), 0.85 (s, 9 H), 0.11 (s, 6 H), 0.00 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 137.8, 132.8, 129.7, 128.4, 126.8, 126.0, 72.6, 60.1, 42.7, 36.7, 25.9, 18.1, 15.5, −4.5, −5.2 ppm. HRMS (FT-ICR): calcd. for $C_{25}H_{46}O_2Si_2$ $[M + Na]^+$ 457.7920, found 457.7925.

(3*S*,4*R*,5*E*)-3-{[*tert*-Butyl(dimethyl)silyl]oxy}-4-methyl-6-phenylhex-5-en-1-ol (30): A solution of disilyl ether **29** (0.36 g, 0.85 mmol) and pyridinium *para*-toluenesulfonate (70 mg, 0.27 mmol) in MeOH (20 mL) was stirred for 4 hours at 50 °C. Most of the MeOH was then removed under reduced pressure, and the mixture was partitioned between water and Et_2O. The aqueous layer was extracted with Et_2O, and the combined organic layers were washed with saturated $NaHCO_3$ solution and brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (15% EtOAc in petroleum ether) to give the primary alcohol **30** (225 mg, 85%) as a colorless oil. $R_f = 0.25$. $[\alpha]_D^{25} = +44.4$ ($c = 0.75$, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3352, 2955, 2930, 1465, 1378, 1255, 1093 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.26−7.06 (m, 5 H), 6.27 (d, J = 16.1 Hz, 1 H), 6.02 (dd, J = 15.9, 7.5 Hz, 1 H), 3.81−3.73 (m, 1 H), 3.68−3.57 (m, 2 H), 2.50−2.37 (m, 1 H), 1.84 (s, br, 1 H), 1.61 (q, J = 5.8 Hz, 2 H), 0.99 (d, J = 6.8 Hz, 3 H), 0.80 (s, 9 H), 0.00 (2 s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 137.5, 132.5, 130.0, 128.4, 127.0, 125.9, 74.5, 60.4, 42.6, 34.9, 25.8, 18.0, 14.7, −4.3, −4.5 ppm. HRMS (FT-ICR): calcd. for $C_{19}H_{32}O_2Si$ $[M + Na]^+$ 343.5310, found 343.5314.

***tert*-Butyl (2*E*,5*S*,6*R*,7*E*)-5-{[*tert*-Butyl(dimethyl)silyl]oxy}-6-methyl-8-phenylocta-2,7-dienoate (31):** A solution of dimethyl sulfoxide (0.086 mL, 1.2 mmol) in CH_2Cl_2 (5 mL) was added dropwise at −78 °C to a stirred solution of oxalyl chloride (0.050 mL, 0.6 mmol) in CH_2Cl_2 (8 mL). After 10 min, a solution of the alcohol **30** (0.160 g, 0.50 mmol) in CH_2Cl_2 (5 mL) was added. After having been stirred at −78 °C for 30 min, the reaction mixture was treated with Et_3N (0.346 mL, 2.50 mmol) and warmed to 0 °C. At this point *tert*-butyl (triphenylphosphoranylidene)acetate[25] (0.546 g, 0.150 mmol) in CH_2Cl_2 (6 mL) was added and the mixture was stirred at room temperature overnight. The reaction mixture was poured into half-saturated NH_4Cl solution (50 mL) and the layers were separated. The organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography (5% EtOAc in petroleum ether) gave the enoate **31** (180 mg, 78%) as a colorless oil. $R_f = 0.25$. $[\alpha]_D^{24} = +50.1$ ($c = 0.95$, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3350, 2950, 2856, 1715, 1471, 1366, 1256 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.48−7.11 (m, 5 H), 6.78 (dt, J = 15.0, 7.0 Hz, 1 H), 6.31 (d, J = 15.9 Hz, 1 H), 6.11 (dd, J = 15.9, 8.0 Hz, 1 H), 5.69 (d, J = 15.6 Hz, 1 H), 3.72−3.64 (m, 1 H), 2.46−2.34 (m, 1 H), 2.31−2.19 (m, 2 H), 1.41 (s, 9 H), 1.04 (d, J = 6.8 Hz, 3 H), 0.85 (s, 9 H), 0.0 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.7, 144.8, 137.6, 132.0, 130.3, 128.4, 127.0, 126.0, 125.0, 80.0, 75.1, 42.8, 37.2, 28.1, 25.8, 18.0, 16.0, −4.3 ppm. HRMS (FT-ICR): calcd. for $C_{25}H_{40}O_3Si$ $[M + Na]^+$ 439.2639, found 439.2638.

***tert*-Butyl (2*S*)-2-[((2*R*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-amino}-2-methylpropanoyl)oxy]-4-methylpentanoate (32):** A solution of DCC (293 mg, 1.42 mmol) in CH_2Cl_2 (3 mL) was added dropwise at 0 °C to a solution of hydroxy ester **12** (180 mg, 0.95 mmol), the amino acid **13** (373 mg, 1.14 mmol), and DMAP (50 mg, 0.40 mmol) in CH_2Cl_2 (3 mL). The clear solution was stirred for 30 min at 0 °C and then for 5 hours at room temperature. The white precipitate was filtered off, and the filtrate was concentrated. The residue was dissolved in Et_2O and washed with HCl (0.5 N, 10 mL), saturated $NaHCO_3$ solution (10 mL), and brine (10 mL). The ether layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (10% of EtOAc in petroleum ether) to give the ester **32** (378 mg, 80%) as a colorless oil. $R_f = 0.25$. $[\alpha]_D^{24} = -32.41$ ($c = 0.44$, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3366, 2958, 1732, 1522, 1450, 1369, 1249, 1159 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.77−7.22 (m, 8 H), 6.03−5.92 (m, 1 H), 4.92 (dd, J = 9.3, 4.5 Hz, 1 H), 4.38−4.26 (m, 2 H), 4.18 (t, J = 7.3 Hz, 1 H), 3.59−3.50 (m, 1 H), 3.35−3.25 (m, 1 H), 2.83−2.73 (m, 1 H), 1.83−1.57 (m, 3 H), 1.47 (s, 9 H), 1.21 (d, J = 7.0 Hz, 3 H), 0.94 (d, J = 6.3 Hz, 3 H), 0.91 (d, J = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.3, 170.3, 156.5, 144.0, 141.2, 127.5, 126.9, 125.2, 119.8, 82.5, 71.3, 66.7, 47.1, 43.6, 40.8, 39.5, 27.9, 24.7, 23.0, 21.5, 14.5 ppm. HRMS (FT-ICR): calcd. for $C_{29}H_{37}NO_6$ $[M + Na]^+$ 518.2513, found 518.2511.

***tert*-Butyl (2*S*)-2-{[(2*R*)-3-Amino-2-methylpropanoyl]oxy}-4-methylpentanoate (33):** A solution of compound **32** (300 mg, 0.60 mmol) in THF (85 mL) was treated at 0 °C with diethylamine (5 mL). The reaction mixture was stirred for 15 min at 0 °C and for 12 hours at room temperature. After evaporation of the solvent in vacuo, the residue was purified by flash chromatography (2% MeOH in CH_2Cl_2) to give the amine **33** (110 mg, 67%) as a colorless liquid. The amine **33** was immediately used for the next step. $R_f = 0.19$. IR (neat): $\tilde{\nu}$ = 3286, 2959, 2872, 2285, 1738, 1651, 1556, 1369, 1141 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.89 (dd, J = 9.3, 4.5 Hz, 1 H), 2.98−2.90 (m, 1 H), 2.82−2.75 (m, 1 H), 2.62−2.53 (m, 1 H), 1.80−1.69 (m, 2 H), 1.66−1.54 (m, 1 H), 1.43 (s, 9 H), 1.18 (d, J = 7.0 Hz, 3 H), 0.94 (d, J = 6.3 Hz, 3 H), 0.90 (d, J = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.0, 169.8, 81.9, 71.2, 45.5, 43.4, 39.6, 27.9, 24.7, 23.0, 21.5, 14.6 ppm. HRMS (FT-ICR): calcd. for $C_{14}H_{27}NO_4$ $[M + H]^+$ 274.2013, found 274. 2014.

***tert*-Butyl (2*S*)-2-{[(2*R*)-3-({3-Chloro-*N*-[(9*H*-fluoren-9-ylmethoxy)-carbonyl]-*O*-methyl-D-tyrosyl}amino)-2-methylpropanoyl]oxy}-4-methylpentanoate (34):** The amine **33** (150 mg, 0.54 mmol), the amino acid **14** (243 mg, 0.54 mmol), and HOBT (73 mg, 0.54 mmol) were dissolved in dry THF (3 mL), followed by addition of DCC (166 mg, 0.81 mmol), dissolved in THF (2 mL) at 0 °C. The mixture was stirred for 7 hours at room temperature, after which it was filtered and concentrated. The residue was diluted with Et_2O and washed with water. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (30% EtOAc in petroleum ether) to give the

tripeptide analogue **34** (325 mg, 84%) as a colorless oil. R_f = 0.32. $[\alpha]_D^{24}$ = −21.93 (*c* = 1.25, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3317, 2950, 2152, 1738, 1670, 1504, 1257 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.74 (d, *J* = 7.5 Hz, 2 H), 7.56−7.15 (m, 7 H), 7.03 (d, *J* = 8.0 Hz, 1 H), 6.79 (d, *J* = 8.3 Hz, 1 H), 5.61 (d, *J* = 8.3 Hz, 1 H), 5.00−4.95 (m, 1 H), 4.51−4.38 (m, 2 H), 4.22−4.07 (m, 2 H), 3.81 (s, 3 H), 3.78−3.70 (m, 1 H), 3.13 (ddd, *J* = 13.9, 10.1, 4.6 Hz, 1 H), 3.04 (dd, *J* = 13.9, 6.1, 1 H), 2.95 (dd, *J* = 13.9, 6.8 Hz, 1 H), 2.82−2.70 (m, 1 H), 1.78−1.53 (m, 3 H), 1.43 (s, 9 H), 1.17 (d, *J* = 6.8 Hz, 3 H), 0.93 (d, *J* = 6.3 Hz, 3 H), 0.90 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.6, 171.8, 155.6, 153.8, 143.7, 141.2, 131.0, 129.5, 128.4, 127.6, 127.0, 125.0, 119.9, 112.1, 83.1, 71.0, 67.0, 60.3, 56.0, 47.0, 41.8, 40.6, 39.4, 38.0, 27.9, 24.8, 22.9, 21.4, 14.7, 14.1 ppm. HRMS (FT-ICR): calcd. for $C_{39}H_{47}ClN_2O_8$ $[M + Na]^+$ 729.2913, found 729.2912.

***tert*-Butyl (2*E*,5*S*,6*R*,7*E*)-5-[(2*S*)-2-{[(2*R*)-3-({3-Chloro-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-*O*-methyl-D-tyrosyl}amino)-2-methylpropanoyl]oxy}-4-methylpentanoyl)oxy]-6-methyl-8-phenylocta-2,7-dienoate (35):** DIEA (35 μL, 0.201 mmol), 2,4,6-trichlorobenzoyl chloride (27 μL, 0.173 mmol), and DMAP (2 mg) were added at room temperature to a solution of the amino acid **11** (90 mg, 0.153 mmol) in THF (3 mL). After 30 min, the alcohol **10** (25 mg, 0.082 mmol), dissolved in THF (1 mL), was added slowly in dropwise fashion. After the system had been stirred for an additional 2 hours, saturated aqueous $NaHCO_3$ solution (5 mL) was added. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (30% EtOAc in petroleum ether) to give the *seco* compound **35** (94 mg, 73%) as a colorless oil. R_f = 0.23. $[\alpha]_D^{24}$ = 1.19 (*c* = 0.28, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3566, 2925, 2900, 1750, 1730, 1353, 1506, 1456, 1258 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.74 (d, *J* = 7.3 Hz, 2 H), 7.57−7.16 (m, 12 H), 7.11 (dd, *J* = 8.5, 2.0 Hz, 1 H), 7.08−7.02 (m, 1 H), 6.82−6.73 (m, 2 H), 6.14 (d, *J* = 8.6 Hz, 1 H), 6.00 (dd, *J* = 15.9, 8.5 Hz, 1 H), 5.81 (d, *J* = 15.9 Hz, 1 H), 5.12−4.88 (m, 2 H), 4.57−4.29 (m, 2 H), 4.21−3.97 (m, 2 H), 3.78 (s, 3 H), 3.74−3.61 (m, 1 H), 3.29−3.06 (m, 2 H), 3.01−2.85 (m, 1 H), 2.82−2.30 (m, 4 H), 1.80−1.52 (m, 3 H), 1.44 (s, 9 H), 1.15 (d, *J* = 7.1 Hz, 3 H), 1.10 (d, *J* = 7.1 Hz, 3 H), 0.80 (d, *J* = 6.3 Hz, 3 H), 0.74 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.6, 171.5, 171.1, 165.3, 153.8, 144.0, 144.2, 136.9, 129.9, 128.5, 127.6, 127.0, 126.0, 125.1, 122.1, 119.9, 112.0, 80.5, 70.7, 67.0, 55.9, 47.1, 44.3, 42.1, 41.0, 37.5, 33.8, 28.1, 24.6, 21.4, 17.5, 14.5 ppm. HRMS (FT-ICR): calcd. for $C_{54}H_{63}ClN_2O_{10}$ $[M + Na]^+$ 957.4063, found 957.4061.

***tert*-Butyl (2*S*)-2-{[(2*R*)-3-({*N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-D-phenylalanyl}amino)-2-methylpropanoyl]oxy}-4-methylpentanoate (38):** A mixture of the amine **33** (150 mg, 0.54 mmol), the Fmoc-protected D-phenylalanine (212 mg, 0.54 mmol), and HOBT (50 mg, 0.37 mmol) in dry THF (3 mL) was treated at 0 °C with DCC (166 mg, 0.81 mmol), dissolved in THF (2 mL). The reaction mixture was stirred for 7 h at room temperature, filtered, and concentrated. The residue was diluted with Et_2O, and the resulting mixture was washed with water. The organic layer was dried with $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (30% EtOAc in petroleum ether) to give the tripeptide analogue **38** (280 mg, 81%) as a colorless oil. R_f = 0.33. $[\alpha]_D^{24}$ = −23.63 (*c* = 1.25, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3064, 2957, 2341, 1732, 1665, 1539, 1450, 1246, 1106 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.65 (d, *J* = 7.3 Hz, 2 H), 7.51−6.95 (m, 11 H), 5.49 (d, *J* = 8.0 Hz, 1 H), 4.91−4.84 (m, 1 H), 4.45−4.27 (m, 2 H), 4.16−3.98 (m, 2 H), 3.69−3.55 (m, 1 H), 3.09−3.00 (m, 1 H), 2.95 (dd, *J* = 13.7, 7.2 Hz, 1 H), 2.85−2.75 (m, 1 H), 2.70−2.61 (m, 1 H), 1.69−1.40 (m, 3 H), 1.34 (s, 9 H), 1.06 (d, *J* = 7.0 Hz, 3 H), 0.84 (d, *J* = 6.3 Hz, 3 H), 0.81 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.7, 170.7, 155.6, 143.7, 141.2, 136.4, 129.2, 128.5, 127.6, 127.0, 125.0, 119.9, 82.9, 71.0, 66.9, 56.1, 47.0, 41.8, 40.5, 39.5, 39.1, 27.9, 24.7, 23.0, 21.5, 14.7 ppm. HRMS (FT-ICR): calcd. for $C_{38}H_{46}N_2O_7$ $[M + Na]^+$ 665.3197, found 665.3202.

***tert*-Butyl (2*E*,5*S*,6*R*,7*E*)-5-[(2*S*)-2-{[(2*R*)-3-({*N*-[(9*H*-Fluoren-9-ylmethoxy)carbonyl]-D-phenylalanyl}amino)-2-methylpropanoyl]oxy}-4-methylpentanoyl)oxy]-6-methyl-8-phenylocta-2,7-dienoate (40):** Trifluoroacetic acid (7 mL) was added at 0 °C to a solution of the fully protected amino acid **38** (230 mg, 0.35 mmol) in CH_2Cl_2 (7 mL), and the reaction mixture was then stirred for 3 hours at room temperature. Toluene (5 mL) was then added, and the mixture was concentrated. This was repeated twice more. The crude acid **39** was subjected to the next reaction without further purification.

DIEA (45 μL, 0.40 mmol), 2,4,6-trichlorobenzoyl chloride (91 μL, 0.374 mmol), and DMAP (2 mg) were added to a solution of the *N*-protected amino acid **39** (200 mg, 0.34 mmol) in THF (3 mL). After the system had been stirred for 30 min, the alcohol **10** (51 mg, 0.17 mmol), dissolved in THF (1 mL), was added slowly in a dropwise fashion. After 2 hours, saturated aqueous $NaHCO_3$ solution (5 mL) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography (30% EtOAc in petroleum ether) to give the protected *seco* compound **40** (231 mg, 75%). R_f = 0.21. $[\alpha]_D^{25}$ = 1.88 (*c* = 0.84, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3318, 3063, 2959, 1735, 1600, 1545, 1450, 1254, 1152, 1081 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.65 (d, *J* = 7.6 Hz, 2 H), 7.47−7.02 (m, 16 H), 6.97−6.90 (m, 1 H), 6.83−6.74 (m, 1 H), 6.23 (d, *J* = 15.6 Hz, 1 H), 6.03 (dd, *J* = 15.9, 8.4 Hz, 1 H), 5.81 (d, *J* = 8.4 Hz, 1 H), 5.76−5.69 (m, 1 H), 5.04−4.79 (m, 2 H), 4.52−4.26 (m, 2 H), 4.13−3.85 (m, 2 H), 3.69−3.57 (m, 1 H), 3.30−3.20 (m, 1 H), 3.13−2.93 (m, 2 H), 2.70−2.23 (m, 4 H), 1.76−1.43 (m, 3 H), 1.41 (s, 9 H), 1.06 (d, *J* = 7.0 Hz, 3 H), 0.72 (d, *J* = 6.3 Hz, 3 H), 0.67 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.5, 171.2, 171.0, 165.9, 156.0, 144.0, 141.1, 136.6, 131.7, 129.3, 128.5, 127.5, 127.0, 126.0, 125.0, 119.8, 80.5, 70.6, 67.0, 56.5, 47.1, 42.0, 39.4, 38.5, 34.8, 28.1, 24.6, 22.8, 21.2, 16.8, 14.5 ppm. HRMS (FT-ICR): calcd. for $C_{53}H_{62}N_2O_9$ $[M + Na]^+$ 893.4347, found 893.4344.

Cryptophycin (43): Trifluoroacetic acid (5 mL) was added slowly at 0 °C to a solution of **40** (100 mg, 0.11 mmol) in CH_2Cl_2 (3 mL), and the mixture was stirred at room temperature for 2 hours. The solvent was removed in vacuo, and toluene (5 mL) was added. After removal of the solvent in vacuo, the residue was dissolved in THF (3 mL), and diethylamine (3 mL) was added dropwise at 0 °C. The reaction mixture was stirred for 2 hours at room temperature followed by removal of the solvents in vacuo. The crude amino acid **37** was dissolved in dry DMF (15 mL) and the mixture was treated successively with TBTU (60 mg, 0.16 mmol), HOBT (2 mg), and DIEA (66 μL, 0.38 mmol) at room temperature. The reaction mixture was stirred for 2 hours at room temperature, after which saturated $NaHCO_3$ solution (10 mL) was added and stirring was continued for 1 hour. The mixture was extracted with CH_2Cl_2 (3 × 15 mL) and the combined organic layers were dried with $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (50% EtOAc in petroleum ether) to provide macrocycle

43 (47 mg, 74%) as a slightly yellow oil. R_f = 0.29. $[\alpha]_D^{24}$ = 7.04 (c = 0.32, CH_2Cl_2). IR (neat): $\tilde{\nu}$ = 3271, 2960, 1744, 1714, 1675, 1540, 1457, 1341, 1175 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.34−7.13 (m, 10 H), 7.06−6.99 (m, 1 H), 6.66 (ddd, J = 15.1, 10.0, 4.9 Hz, 1 H), 6.36 (d, J = 15.9 Hz, 1 H), 5.97 (dd, J = 15.9, 8.8 Hz, 1 H), 5.70 (d, J = 15.6 Hz, 1 H), 5.62 (d, J = 8.3 Hz, 1 H), 5.05−4.94 (m, 1 H), 4.86−4.74 (m, 2 H), 3.26−3.22 (m, 2 H), 3.18 (dd, J = 14.1, 5.3 Hz, 1 H), 3.08 (dd, J = 14.4, 7.3 Hz, 1 H), 2.70−2.60 (m, 1 H), 2.57−2.44 (m, 2 H), 2.39−2.26 (m, 1 H), 1.67−1.54 (m, 2 H), 1.43−1.36 (m, 1 H), 1.20 (d, J = 8.0 Hz, 3 H), 1.09 (d, J = 6.8 Hz, 3 H), 0.71 (d, J = 6.3 Hz, 3 H), 0.68 (d, J = 6.3 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.9, 171.1, 170.8, 165.3, 141.6, 136.7, 131.8, 130.1, 129.2, 128.6, 127.5, 126.9, 126.1, 125.0, 71.1, 53.7, 42.3, 40.8, 39.5, 38.1, 35.9, 24.4, 22.7, 21.2, 17.3, 14.2 ppm. HRMS (FT-ICR): calcd. for $C_{34}H_{42}N_2O_6$ $[M + Na]^+$ 597.2935, found 597.2934.

Acknowledgments

Financial support by the Deutsche Forschungsgemeinschaft (grant MA 1012/13−1) and the Fonds der Chemischen Industrie is gratefully acknowledged. P. Phukan thanks the Alexander von Humboldt Stiftung for a postdoctoral fellowship.

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Received August 5, 2004

