First Total Synthesis of Hormaomycin, a Naturally Occurring Depsipeptide with Interesting Biological Activities

Boris D. Zlatopolskiy and Armin de Meijere*[a]

Dedicated to Professor Axel Zeeck on the occasion of his 65th birthday

Abstract: Some unique features were disclosed during the structure elucidation of the cyclic depsipeptide hormaomycin (**1**), first isolated in 1989 from a *Streptomyces griseoflavus* strain and found to have quite an interesting spectrum of biological activities. Besides one residue of the proteinogenic amino acid isoleucine [(*S*)-Ile], it contains two units of 3-methylphenylalanine [(βMe)Phe], one of (2*R*)-*allo*-threonine [*a*-Thr] as well as two moieties of 3-(*trans*-2-nitrocyclopropyl)alanine [(3-Ncp)Ala] and one of 4-(*Z*)-propenylproline [(4-PE)Pro]. The latter two have never been found in any natural product before. The side chain of **1** is terminated with the residue of 5-chloro-1-hydroxypyrrole-2-carboxylic acid [Chpca]. This first synthetic access to hormaomycin **1** will make it possible to prepare structural analogues of this interesting natural depsipeptide in order to elucidate structure–activity relationships and the biologically active minimal unit.

Keywords: natural products · peptides · protective groups · synthetic methods · total synthesis

Introduction

The structure elucidation of the cyclic depsipeptide hormaomycin (**1**), first isolated in 1989 from a *Streptomyces griseoflavus* strain[1a] and found to have quite an interesting spectrum of biological activities,[1b,c] disclosed some unique features.[1a,d] Besides one residue of the proteinogenic amino acid isoleucine [(*S*)-Ile], it contains two units of 3-methylphenylalanine [(βMe)Phe], one of (2*R*)-*allo*-threonine [*a*-Thr] as well as two moieties of 3-(*trans*-2-nitrocyclopropyl)alanine [(3-Ncp)Ala] and one of 4-(*Z*)-propenylproline [(4-PE)Pro]. The latter two have never been found in any natural product before. The side chain of **1** is terminated with the residue of 5-chloro-1-hydroxypyrrole-2-carboxylic acid [Chpca]. These challenging structural features and the interesting biological properties prompted us to embark on a total synthesis of **1** (Figure 1).

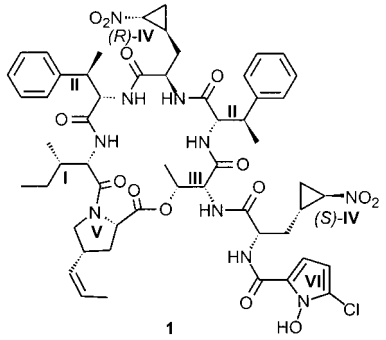

Figure 1. Structure and absolute configuration of hormaomycin (**1**). **I** (*S*)-Ile; **II** (2*S*,3*R*)-(βMe)Phe; **III** (*R*)-*a*-Thr; **IV** (1′*R*,2′*R*)-(3-Ncp)Ala; **V** (2*S*,4*R*)-4-(*Z*)-(4-PE)Pro; **VI** Chpca.

Results and Discussion

Once the absolute configuration of hormaomycin **1** had been established[2] and productive syntheses of (2*S*,1′*S*,2′*R*)- and (2*R*,1′*S*,2′*R*)-3-(*trans*-2-nitrocyclopropyl)alanine[3] as well as *N*-Boc-protected (2*S*,4*R*)-4-(*Z*)-propenylproline[4] had been developed, the assembly of the hexapeptolide segment of **1** was initiated.

All four peptide couplings towards the acyclic precursor **16a** were performed with the combination of *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC)

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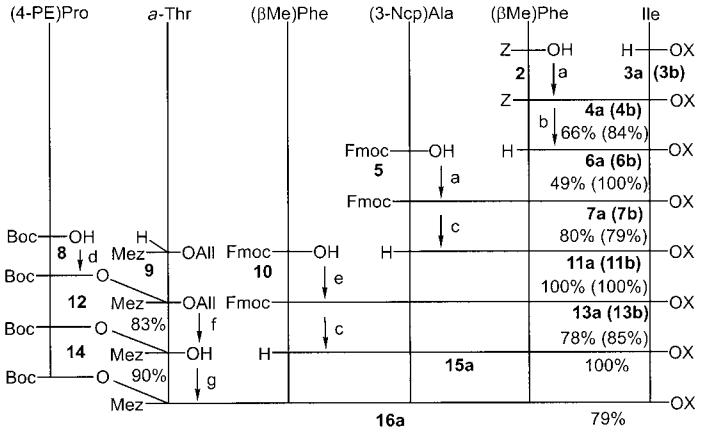
and 7-aza-1-hydroxybenzotriazole (HOAt).[5] Having in mind to ring-close an acyclic precursor already containing the ester bond between the (4-PE)Pro and the *a*-Thr residues by forming the peptide bond between the Ile and (4-PE)Pro moieties, the methoxymethyl or dicyclopropylmethyl ester[6] of Ile, **3a** or **3b**, were condensed with *N*-Z-protected (βMe)Phe-OH **2** (see Scheme 1). The latter was obtained



Scheme 1. Synthesis of the acyclic precursor **16a** to the hexapeptolide fragment of hormaomycin **1**. a) EDC, HOAt, DIEA, 2,4,6-collidine, CH_2Cl_2, 0→20 °C, 14 h; b) H_2, Pd/C, EtOAc, 20 °C, 40 min; c) 50 % Et_2NH/MeCN, 20 °C, 1 h; d) EDC, 4-pyrrolidinopyridine, CH_2Cl_2, 0→20 °C, 24 h; e) EDC, HOAt, 2,4,6-collidine, DMF, 0→20 °C, 14 h; f) $[Pd(PPh_3)_4]$, *N*-methylaniline, DME, 20 °C, 1 h; g) HATU, HOAt, DIEA, 2,4,6-collidine, CH_2Cl_2, 0→20 °C, 24 h. DCPM = dicyclopropylmethyl, All = allyl, Fmoc = 9-fluorenylmethyloxycarbonyl, DIEA = *N*,*N*-diisopropylethylamine, EDC = *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride, HATU = *O*-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOAt = 7-aza-1-hydroxybenzotriazole, Me*Z* = *p*-methylbenzyloxycarbonyl, MOM = methoxymethyl; X = MOM (or DCPM).

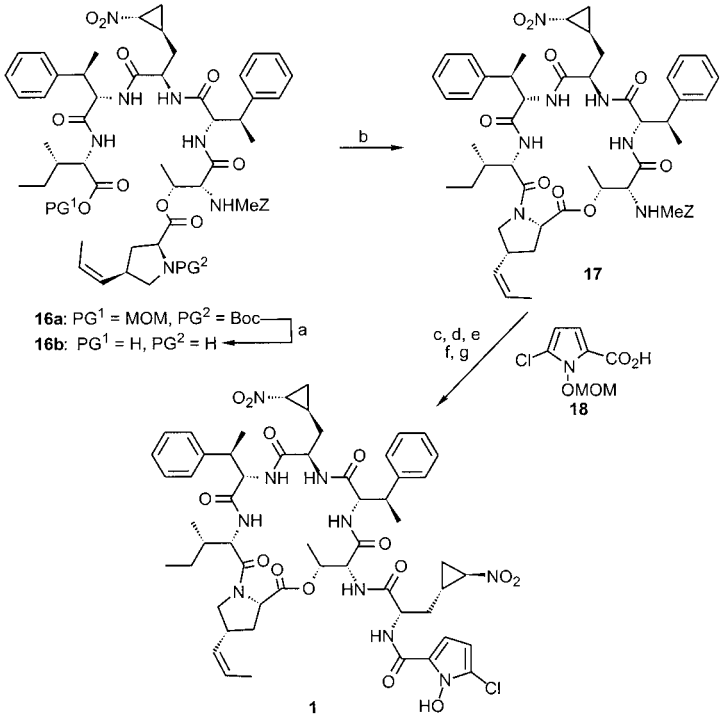
by acylation of the corresponding amino acid with benzyl *N*-succinimidyl carbonate (ZOSu) in 85 % yield. The alkylation of Z-Ile-OH with MOM iodide (generated in situ) gave the *N*-protected MOM ester of Ile-OH in 76 % yield, which, after removal of the Z group (100 %), was immediately used in the coupling step. The DCPM ester **3b** was obtained just before the next step by removal of the Fmoc group from the *N*-terminus of Fmoc-Ile-ODCPM (100 %), which was in turn prepared by esterification of the intermediate acid chloride Fmoc-Ile-Cl with dicyclopropylmethanol in the presence of pyridine in 57 % yield.

After removal of the Z group from the *N*-terminus of the resulting dipeptides, **4a** (66 %) and **4b** (84 %), by catalytic hydrogenation to give **6a** (54 %)[7] and **6b** (100 %), the latter were coupled with *N*-Fmoc-protected (2*R*,1′*S*,2′*R*)-(3-Ncp)Ala-OH **5** to yield tripeptides **7a** (80 %) and **7b** (79 %). The latter, after removal of the Fmoc-group (100 % yield), were coupled with *N*-Fmoc-protected (βMe)Phe-OH **10** to give tetrapeptides **13a** (78 %) and **13b** (85 %).[8]

The 4-pyrrolidinopyridine-catalyzed condensation of the *N*-Boc-protected (4-PE)Pro-OH **8** and *N*,*C*-protected *a*-Thr **9**, prepared by alkylation of *N*-MeZ protected D-*allo*-threonine, which in turn was obtained by reaction between H-*a*-Thr-OH and 4-methylbenzyl *N*-succinimidyl carbonate (MeZOSu), with allyl bromide in the presence of K_2CO_3 in MeCN (76 % over two steps), gave the ester **12** (83 %). The latter, after palladium-promoted removal of the allyl group, was coupled with the tetrapeptide **11a** using the *O*-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU)[9] to give the hexadepsipeptide **16a** in 79 % yield.

4-Methylbenzyloxycarbonyl *N*-protecting group (MeZ)[10] had to be applied in the course of this synthesis, since all other convenient *N*-protective groups orthogonal to the Boc group turned out to be incompatible with the restrictions imposed on the deprotection conditions due to the nitro group on the (3-Ncp)Ala moiety, the double bond in the (4-PE)Pro residue as well as the base-sensitive ester bond between the (4-PE)Pro and *a*-Thr fragments.[11a,b] The MeZ group was found to be stable towards 2 N HCl/EtOAc at ambient temperature for at least 90 min,[11c] whereas under these conditions the Boc group was cleaved off within 2 min.[11d] On the other hand, the MeZ group can be removed smoothly by treatment with 10 % anisole in trifluoroacetic acid for 1 h.[11e,f]

The MOM and Boc groups were removed from the termini of the depsihexapeptide **16a** (the ESI-mass spectrum showed that the MeZ group stayed intact), and the cyclizing peptide condensation succeeded under high dilution conditions,[12] using the HATU reagent. The cyclodepsipeptide **17** was obtained after HPLC purification in 53 % yield over two steps (Scheme 2).



Scheme 2. The final stages in the preparation of hormaomycin **1**. a) 2 N HCl/EtOAc, 20 °C, 45 min; b) HATU, DIEA, 2,4,6-collidine, CH_2Cl_2, 0.1 mM, 0→20 °C, 16 h, 53 % after HPLC purification (2 steps); c) anisole, TFA, 20 °C, 2 h; d) Teoc-(2*S*,1′*R*,2′*R*)-(3-Ncp)AlaOH, HATU, HOAt, DIEA, 2,4,6-collidine, CH_2Cl_2, 20 °C, 6 h; e) TFA, 20 °C, 1 h; f) **18**, HATU, HOAt, DIEA, 2,4,6-collidine, CH_2Cl_2, 20 °C, 4 h; g) $MgBr_2 \cdot Et_2O$, EtSH, CH_2Cl_2, 20 °C, 3.5 h, 67 % (5 steps). Teoc = (2-trimethylsilylethyl)-oxycarbonyl.

To complete the assembly of **1**, the *N*-MeZ-protected cyclic intermediate was deprotected and first coupled with *N*-Teoc-protected (2*S*,1′*R*,2′*R*)-(3-Ncp)Ala-OH. After removal of the Teoc group, the intermediate in turn was coupled with the *O*-MOM-protected 5-chloro-1-hydroxypyrrole-2-carboxylic acid **18**.[4,13]

Finally, the MOM group was removed by treatment with $MgBr_2 \cdot Et_2O$ and EtSH in dichloromethane[14] to give a compound which was expected to be identical with the native hormaomycin **1**. It did indeed disclose the expected [$M-H^+ +2Na^+$], [$M+Na^+$] and [$M-H^+$] peaks in its positive- and negative-mode ESI-mass spectra, had the same optical rotation, identical CD trace, at least in the wavelength region above 210 nm where no impurity interference is expected,[15a] identical UV spectrum, a very similar ^{13}C NMR spectrum and identical HPLC retention time as an authentic sample of the natural material. The synthetic product also showed the same capability to inhibit the growth of coryneform bacteria as native hormaomycin (**1**).[15b] However, there were some differences in their ^1H NMR spectra in $CDCl_3$ (Figure 2). These discrepancies were initially interpreted to indicate that a diastereomer of the natural product had been synthesized. Yet, this possibility was excluded by the results of LC/MS experiments according to the advanced Marfey method,[16] which rigorously proved that the MePhe, Ile, Ncpa and (*R*)-*a*-Thr residues were not epimerized in any of the peptide coupling and deprotection steps.[17] Because of this inconsistency, the last steps of the synthesis of hormaomycin (**1**), starting from the cyclodepsipeptide **17**, were repeated on a slightly larger scale. This made it possible to use simple recrystallizations instead of preparative TLC separations for the purification of intermediates. The thus obtained second sample of synthetic hormaomycin had a ^1H NMR spectrum which did not differ any more from that of an authentic sample of natural hormaomycin than two spectra taken of different samples of the natural material (see Figure 2).

This first synthetic access to hormaomycin (**1**) will make it possible to prepare structural analogues of this interesting natural depsipeptide in order to elucidate structure–activity relationships and the biologically active minimal unit.

Experimental Section

General aspects: ^1H NMR spectra: Bruker AM 250 (250 MHz), Varian Inova 600 (600 MHz). ^1H chemical shifts are reported in ppm relative to residual peaks of deuterated solvent or tetramethylsilane. Higher order NMR spectra were approximately interpreted as first-order spectra, if possible. The observed signal multiplicities are characterized as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, as well as br = broad, Ar-H = aryl-H. ^{13}C NMR spectra [additional DEPT (Distortionless Enhancement by Polarization Transfer) or APT (Attached Proton Test)]: Bruker AM 250 (62.9 MHz), Varian Inova 600 (125.7 MHz) instruments. ^{13}C chemical shifts are reported relative to peak of solvent or tetramethylsilane. The following abbreviations were applied: DEPT: + = primary or tertiary (positive signal in DEPT), − = secondary (negative signal in DEPT), C_{quat} = quaternary (no signal in DEPT); APT: + = primary or tertiary (positive signal in APT), − = secondary or quaternary (negative signal in APT); whenever it was necessary and possible HMBS (Heteronuclear Multiple Bond Connectivity) and/or HMQS (Heteronuclear Multiple Quantum Coherence) spectra were also measured. IR spectra: Bruker IFS 66 (FT-IR) spectrometer, samples measured as KBr pellets or oils between KBr plates. MS: EI-MS: Finnigan MAT 95, 70 eV, high resolution EI-MS spectra with perfluorokerosene as reference substance; ESI-MS: Finnigan LCQ. HPLC: pump: Kontron 322 system, detector: Kontron DAD 440, mixer: Kontron HPLC 360, data system: Kontron Kromasystem 200, columns: Knauer Nucleosil-100 C18 (analytical, 5 μm, 3 mm × 250 mm), Knauer Nucleosil-100 C18 (preparative, 5 μm, 8 mm × 250 mm). Optical rotations: Perkin–Elmer 241

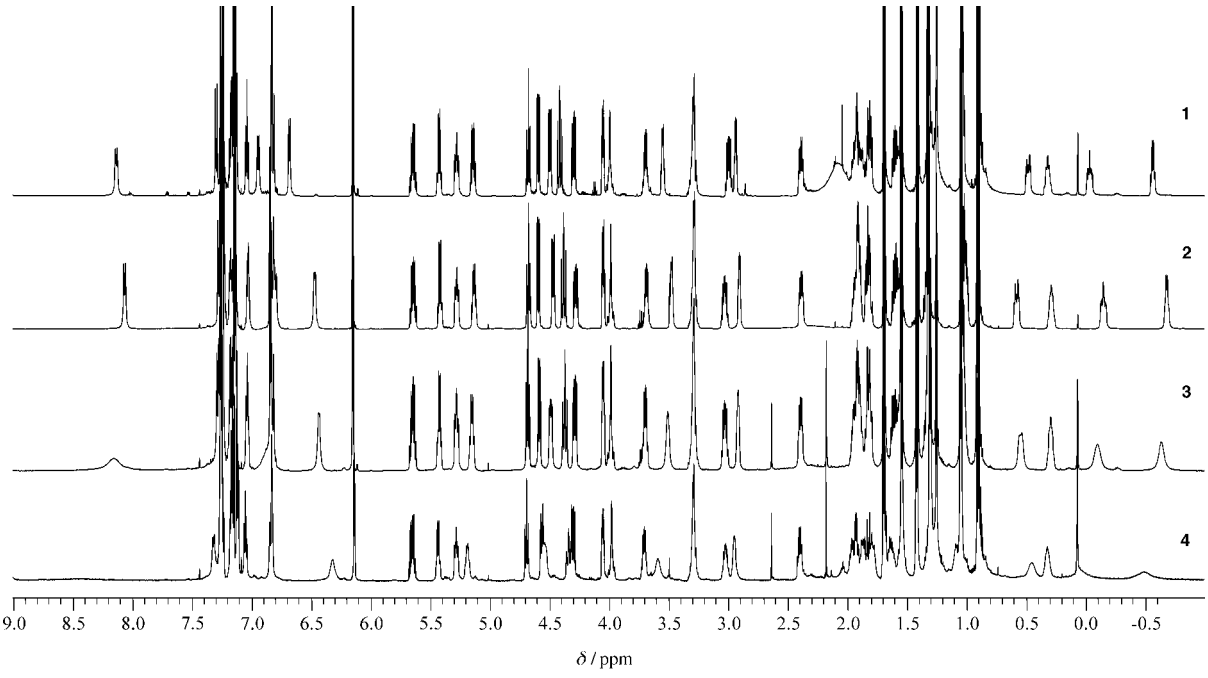


Figure 2. Comparison of ^1H NMR spectra (600 MHz, $CDCl_3$) of different samples of synthetic [traces **2** (second run) and **4** (first run)] and natural (traces **1** and **3**) hormaomycin **1**. All samples were purified by HPLC before measurements. All measurements were carried out at ambient temperature and with the same spectrometer.

digital polarimeter, 1-dm cell; optical rotation values are given in 10^{-1} deg cm^2 g^{-1}; concentrations (c) are given in g per 100 mL. Circular dichroism: Jasco J 500A. Molar ellipticities (Θ) are given in grad cm^2 10^{-1} mol^{-1}. M.p.: Büchi 510 capillary melting point apparatus, uncorrected values. TLC: Macherey-Nagel precoated sheets, 0.25 mm Sil G/UV$_{254}$. The chromatograms were viewed under UV light and/or by treatment with phosphomolybdic acid (10% in ethanol), or ninhydrin (0.2% in ethanol), or Ehrlich's reagent (freshly prepared solution of 1 g of 4-dimethylamino-benzaldehyde in 25 mL of 36% HCl and 75 mL methanol). Column chromatography: Merck silica gel, grade 60, 230–400 mesh and Baker silica gel, 40–140 mesh. Preparative TLC: Macherey-Nagel, silica gel SIL G/UV$_{254}$, layer thickness 0.25 mm (100×200 mm or 200×200 mm). Elemental analyses: Mikroanalytisches Laboratorium des Instituts für Organische und Biomolekulare Chemie der Universität Göttingen. Starting materials: Anhydrous solvents were prepared according to standard methods by distillation over drying agents and were stored under argon. All other solvents were distilled before use. All reactions were carried out with magnetic stirring and, if air or moisture sensitive, in flame-dried glassware under argon or nitrogen. Organic extracts were dried with anhydrous $MgSO_4$. (*R*)-*allo*-Threonine,[18] 1-hydroxy-7-azabenzotriazol,[19] 2-(trimethylsilyl)ethyl *N*-succinimidyl carbonate,[20] (2*S*,3*R*)-β-methylphenylalanine,[21] (2*R*,1′*R*,2′*R*)-3-(2′-nitrocyclopropyl)alanine,[3a] (2*S*,4*R*)-(*N*-*tert*-butyloxycarbonyl)-4-(*Z*)-propenylproline (**8**),[4] (2*S*,3*R*)-(*N*-9-fluorenylmethyloxycarbonyl)-β-methylphenylalanine (**10**),[22] 5-chloro-1-methoxymethoxypyrrole-2-carboxylic acid (**18**),[4] were prepared as described elsewhere. (2*S*,1′*R*,2′*R*)-3-(2′-Nitrocyclopropyl)alanine was kindly provided by Oleg V. Larionov (Göttingen).

Deprotection of *N*-Fmoc-protected peptides 7a, 7b, 13a, 13b—General procedure (GP 1): The protected peptides (1 mmol) were taken up with acetonitrile or THF (2 mL); diethylamine (2 mL) was added, and the resulting mixture left at ambient temperature for 30 min. All volatiles were evaporated under reduced pressure, the residue was taken up with toluene (2×5 mL), which was evaporated under reduced pressure to remove the last traces of diethylamine. The obtained crude *N*-deprotected peptides were directly used in the next condensation step.

Peptide condensation step for the preparation of peptides 4a, 4b, 7a, 7b, 13a, 13b—General procedure (GP 2): EDC (1.03 mmol) and HOAt (1.05 mmol) were added to a cooled (4°C) solution of the respective *N*-protected amino acid (1 mmol) in anhydrous CH_2Cl_2 (3 mL). After 10 min, the solution of the appropriate crude *N*-deprotected peptide (0.97 mmol) and TMP (3 mmol) in anhydrous CH_2Cl_2 (1 mL) was added at the same temperature. The temperature was allowed to reach 20°C, and stirring was continued for 15 h. Then the reaction mixture was diluted with Et_2O or EtOAc (30 mL) and washed with 1 M $KHSO_4$ (3×5 mL), water (2×5 mL), 3% aq. solution of $NaHCO_3$ (3×5 mL), water (3×5 mL), brine (2×5 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography or recrystallization.

***N*-Z-3-(2*S*,3*R*)-β-Methylphenylalanine (2)**: A solution of ZOSu (0.396 g, 1.59 mmol) in acetone (5 mL) was added to a vigorously stirred solution of 3-(2*S*,3*R*)-methylphenylalanine hydrochloride (0.35 g, 1.62 mmol) and $NaHCO_3$ (0.409 g, 4.87 mmol) in water (5 mL); stirring was continued for 2 h (if an emulsion formed, acetone and/or water were added to obtain a homogeneous solution). Acetone was then removed under reduced pressure, the residual fraction was diluted with water (25 mL) and washed with Et_2O (3×10 mL). The organic fraction was back-extracted with 5% aqueous $NaHCO_3$ (3×10 mL), the pH of the combined water fractions was adjusted to 1 with 1 M HCl and the resulting emulsion was extracted with Et_2O (2×50 mL). The organic layer was washed with 1 M HCl (2×10 mL), water (10×10 mL), brine (2×10 mL), dried, filtered and concentrated under reduced pressure. The residual oil was dissolved in Et_2O (3 mL) and dicyclohexylamine (0.277 g, 1.53 mmol) was added followed by hexane (20 mL) and the resulting precipitate was filtered and crystallized twice from EtOAc/hexane to give the dicyclohexylammonium salt of **2** (0.67 g, 85%) as a white solid. To obtain an analytical sample of **2**, a small quantity of the dicyclohexylammonium salt dissolved in EtOAc and washed twice with 1 M HCl, three times with water, twice with brine to give, after prolonged drying at 0.02 Torr and 60°C, **2** as a colorless solid. M.p. 76.5–79°C; $[\alpha]_D^{20}=17.3$ ($c=0.76$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=1.31$, 1.36 (2×d, $J=7.0$ Hz, 3H, 4-H), 3.33 (dq, $J=7.0$, 7.0 Hz, 1H, 3-H), 4.43–4.55 (m, 0.25H, 2-H), 4.60 (dd, $J=5.5$ Hz, 9 Hz, 0.75H, 2-H), 4.78 (d, $J=12.5$ Hz, 0.25H, Bzl-H), 4.96–5.20 (m, 1.75H, Bzl-H), 5.30 (d, $J=9$ Hz, 0.75H, NH), 6.25 (d, $J=8.8$ Hz, 0.25H, NH), 7.03–7.35 (m, 10H, Ar-H), 7.30–7.90 (br, 1H, CO_2H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta=14.3$, 15.8 (+, C-4), 41.5, 41.8 (+, C-3), 59.1, 59.8 (+, C-2), 67.0, 67.4 (−, Bzl-C), 127.0 (+, Ar-C), 127.5 (+, Ar-C), 127.6 (+, Ar-C), 127.9 (+, Ar-C), 128.0 (+, Ar-C), 128.3, 128.3 (+, Ar-C), 135.2, 135.9 (C_{quat}, Ar-C), 140.9, 141.4 (C_{quat}, Ar-C), 156.0, 157.1 (C_{quat}, NCO_2), 175.1, 175.4 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}=3750$–2250, 1718, 1518, 1497, 1454, 1416, 1347, 1217 cm^{-1}; MS (EI, 70 eV): m/z (%): 313 (1) [M^+], 209 (4) [$M^+-C_8H_8$], 162 (10), 105 (100) [$C_8H_9^+$], 91 (48) [$C_7H_7^+$], 65 (7) [$C_5H_5^+$]; HRMS (EI): calcd for $C_{18}H_{19}NO_4$: 313.1314; found 313.1314; elemental analysis calcd (%) for $C_{18}H_{19}NO_4$ (313.4): C 69.00, H 6.11, N 4.47; found C 69.16, H 6.00, N 4.45.

***N*-Z-Isoleucine methoxymethyl ester**: LiI (0.783 g, 5.85 mmol) was added to an ice-cold solution of MOM-Cl (0.43 mL, 5.65 mmol) in acetonitrile (7 mL). The mixture was stirred at the same temperature for 20 min, and a solution of Z-Ile-OH (0.75 g, 2.83 mmol), and DIEA (0.731 g, 5.65 mmol) in acetonitrile (5 mL) was then added dropwise within 5 min. The reaction mixture was allowed to warm to 20°C, stirred for an additional 2 h and then diluted with Et_2O (50 mL). After the usual aqueous work-up (GP 2), the organic layer was dried, and concentrated under reduced pressure to give an oily residue which was purified by column chromatography (2×; $R_f=0.21$, EtOAc/hexane 1:6) to give the title compound (0.665 g, 76%) as a colorless oil. $[\alpha]_D^{20}=3.9$ ($c=1.90$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=0.93$ (t, $J=6.9$ Hz, 3H, 5-H), 0.97 (d, $J=6.8$ Hz, 3H, 1′-H), 1.06–1.32 (m, 1H, 4-H$_a$), 1.35–1.54 (m, 1H, 4-H$_b$), 1.81–2.03 (m, 1H, 3-H), 3.47 (s, 3H, OMe), 4.38 (dd, $J=9$, 4.5 Hz, 1H, 2-H), 5.11 (s, 2H, Bzl-H), 5.22 (d, $J=5.8$ Hz, 1H, H$_a$, OCH_2O), 5.28 (d, $J=6.0$ Hz, 1H, NH), 5.35 (d, $J=5.8$ Hz, 1H, H$_b$, OCH_2O), 7.28–7.42 (m, 5H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta=11.2$ (+, C-5), 15.1 (+, C-1′), 24.5 (−, C-4), 37.4 (+, C-3), 57.3 (+, OMe), 58.1 (+, C-2), 66.5 (−, Bzl-C), 90.6 (−, OCH_2O), 127.7, 127.7, 128.1 (+, Ar-C), 136.0 (C_{quat}, Ar-C), 155.9 (C_{quat}, NCO_2), 171.4 (C_{quat}, C-1); IR (film): $\tilde{\nu}=3390$, 3066, 3034, 2965, 2937, 2878, 2832, 1724, 1525, 1455, 1412, 1337, 1220, 1087, 1041 cm^{-1}.

***N*-Fmoc-Isoleucine dicyclopropylmethyl ester**: To a stirred ice-cold solution of *N*-Fmoc-protected isoleucine (1.0 g, 2.83 mmol) in anhydrous CH_2Cl_2 (10 mL) were added oxalyl chloride (0.898 g, 7.07 mmol) and then DMF (5 drops), and stirring was continued at the same temperature for 2 h. The mixture was then allowed to warm to 20°C and stirred for an additional 1 h. The solvent was blown out with a nitrogen stream and the crude acyl chloride was dried at 0.01 Torr for 2 h and used without further purification. It was dissolved in anhydrous CH_2Cl_2 (10 mL) and a mixture of pyridine/dicyclopropylmethanol 1:1 (1.5 mL) was added. After 40 min, DMAP (0.02 g) was added to the mixture and stirring was continued for an additional 3 h. The reaction mixture was then diluted with Et_2O (50 mL), washed (according to GP 2), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography [$R_f=0.24$, EtOAc/hexane 1:10 (0.5% Et_3N)]. The appropriate fractions were combined, concentrated under reduced pressure, taken up with Et_2O/hexane 1:1 (100 mL), washed with water (3×20 mL), 3% aqueous $NaHCO_3$ (3×20 mL), water (3×20 mL), brine (2×10 mL), dried, filtered and concentrated under reduced pressure to give the title compound (0.72 g, 57%) as a turbid oil. $[\alpha]_D^{20}=-3.8$ ($c=0.26$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=0.16$–0.38 (m, 4H, 2′-H$_a$, *DCPM*), 0.38–0.51 (m, 2H, 2′-H$_b$, *DCPM*), 0.51–0.64 (m, 2H, 2′-H$_c$, *DCPM*), 0.94 (t, $J=7.5$ Hz, 3H, 5-H, *Ile*), 0.96 (d, $J=7.5$ Hz, 3H, 1′-H, *Ile*), 1.02–1.16 (m, 2H, 1′-H, *DCPM*), 1.17–1.34 (m, 1H, 4-H$_a$, *Ile*), 1.39–1.47 (m, 1H, 4-H$_b$, *Ile*), 1.86–2.09 (m, 1H, 3-H, *Ile*), 3.90 (t, $J=8.8$ Hz, 1H, 1-H, *DCPM*), 4.23 (dd, $J=7.3$, 7.3 Hz, 1H, 2-H, *Ile*), 4.34–4.44 (m, 3H, 1-H, 9′-H, *Fmoc*), 5.36 (d, $J=9.8$ Hz, 1H, NH), 7.23–7.46 (m, 4H, Ar-H), 7.61 (d, $J=7.5$ Hz, 2H, Ar-H), 7.76 (d, $J=8.3$ Hz, 2H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta=2.4$, 2.6, 2.9 (−, C-2′, *DCPM*), 11.6 (+, C-5, *Ile*), 14.5, 15.3 (+, C-1′, *DCPM*), 14.6 (+, C-1′, *Ile*), 24.9 (−, C-4, *Ile*), 38.1 (+, C-3, *Ile*), 47.1 (+, C-9′, *Fmoc*), 58.3 (+, C-2, *Ile*), 66.8 (−, C-1, *Fmoc*), 83.4 (+, C-1, *DCPM*), 119.8, 125.0, 126.9, 127.5 (+, Ar-C), 141.1 (C_{quat}, Ar-C), 143.7, 143.8 (C_{quat}, Ar-C), 156.0 (C_{quat}, NCO_2), 171.5 (C_{quat}, C-1, *Ile*); IR (film): $\tilde{\nu}=3400$, 3068, 3009, 2964, 2935, 2876, 2832, 1805, 1717, 1508, 1451, 1331, 1209, 1220, 1091, 1030 cm^{-1}.

Z-(βMe)Phe-Ile-OMOM (4a): Z-Ile-OMOM (0.415 g, 1.34 mmol) was hydrogenated over 10% Pd on charcoal (0.150 g) under hydrogen at ambient pressure in EtOAc (10 mL) with AcOH (0.080 g, 1.34 mmol) for

1 h. The mixture was then filtered, concentrated under reduced pressure, taken up with Et_2O (40 mL), washed with saturated aqueous $NaHCO_3$ (3×10 mL), brine (2×10 mL), dried, filtered and concentrated under reduced pressure to give the deprotected ester of isoleucine **3a** as a free base which was directly used for the condensation with *N*-Z-protected β-methylphenylalanine (**2**; 0.400 g, 1.28 mmol) employing EDC (0.257 g, 1.34 mmol), HOAt (0.173 g, 1.28 mmol) and TMP (0.500 mL, 3.78 mmol) in CH_2Cl_2 (5 mL) according to GP 2. After 2 h, the reaction mixture was subjected to the usual aqueous work-up and the resulting crude product was purified by crystallization from EtOAc/hexane to give the dipeptide **4a** (0.40 g, 66%) as a colorless solid. R_f=0.17 (EtOAc/hexane 1:6); R_f=0.35 (EtOAc/hexane 1:4); m.p. 111–112 °C; $[\alpha]_D^{20}=-8.5$ (c=0.30, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.81 (d, J=6.8 Hz, 3H, 1′-H, *Ile*), 0.87 (t, J=6.8 Hz, 3H, 5-H, *Ile*), 0.94–1.17 (m, 1H, 4-H_a, *Ile*), 1.24–1.45 (m, 1H, 4-H_b, *Ile*), 1.35 [d, J=7.3 Hz, 3H, 4-H, (β*Me*)*Phe*], 1.68–1.87 (m, 1H, 3-H, *Ile*), 3.17 [dq, J=7.3 Hz, 1H, 3-H, (β*Me*)*Phe*], 3.42 (s, 3H, OMe), 4.27–4.41 (m, 2H, 2×2-H), 5.09 (s, 2H, Bzl-H), 5.13 (d, J=6.0 Hz, 1H, H_a, OCH_2O), 5.21 (d, J=5.8 Hz, 1H, H_b, OCH_2O), 5.43 (d, J=9 Hz, 1H, NH), 5.98 (d, J=7.8 Hz, 1H, NH), 7.15–7.30 (m, 6H, Ar-H), 7.30–7.42 (m, 4H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=11.2 (+, C-5, *Ile*), 14.9 (+, C-1′, *Ile*), 16.9 [+, C-4, (β*Me*)*Phe*], 24.8 (−, C-4, *Ile*), 37.5 (+, C-3, *Ile*), 42.2 [+, C-3, (β*Me*)*Phe*], 56.1 (+, C-2, *Ile*), 57.5 (+, OMe), 60.2 [+, C-2, (β*Me*)*Phe*], 66.5 (−, Bzl-C), 90.6 (−, OCH_2O), 126.6, 127.5, 127.5, 127.7, 128.17 (×2) (+, Ar-C), 136.2, 141.9 (C_{quat}, Ar-C), 156.2 (C_{quat}, NCO_2), 170.4, 170.6 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}$=3330, 3275, 3065, 3033, 2965, 2928, 2874, 2832, 1741, 1689, 1654, 1536, 1293, 1275, 1253, 1242, 1155, 1071, 1012 cm^{-1}; MS (EI, 70 eV): *m*/*z* (%): 470 (4) [M^+], 366 (11) [$M^+-C_8H_8$], 319 (18) [$M^+-C_8H_9NO_2$], 289 (15), 268 (8), 224 (20), 199 (6), 105 (10) [$C_8H_9^+$], 91 (100) [$C_7H_7^+$], 86 (10), 45 (12) [CHO_2^+]; HRMS (EI): calcd for $C_{26}H_{34}N_2O_6$: 470.2417; found 470.2417; elemental analysis calcd (%) for $C_{26}H_{34}N_2O_6$ (470.6): C 66.36, H 7.28, N 5.95; found C 66.58, H 7.19, N 6.10.

Z-(βMe)Phe-Ile-ODCPM (4b): Fmoc-Ile-ODCPM (0.540 g, 1.21 mmol) was *N*-deprotected according to GP 1, and the resulting amino ester **3a** was coupled with *N*-Z-protected (β-methylphenylalanine) **2** (0.36 g, 1.15 mmol) employing EDC (0.227 g, 1.18 mmol), HOAt (0.160 g, 1.18 mmol) and TMP (0.418 g, 3.45 mmol) in CH_2Cl_2 (5 mL) according to GP 2. After 6 h, the reaction mixture was subjected to the usual aqueous work-up, and the resulting crude product was triturated with pentane and then purified by crystallization from hexane to give the dipeptide **4b** (0.50 g, 84%) as a colorless solid. R_f=0.17 [EtOAc/hexane 1:6 (0.5% Et_3N)]; m.p 105–106 °C; $[\alpha]_D^{20}=9.0$ (c=0.31, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.23–0.41 (m, 4H, 2′-H_a, *DCPM*), 0.41–0.53 (m, 2H, 2′-H_b, *DCPM*), 0.53–0.64 (m, 2H, 2′-H_c, *DCPM*), 0.81 (d, J=6.8 Hz, 3H, 1′-H, *Ile*), 0.89 (t, J=7.3 Hz, 3H, 5-H, *Ile*), 0.95–1.21 (m, 3H, 4-H_a, *Ile*, 1′-H, *DCPM*), 1.35 [d, J=7.3 Hz, 3H, (β*Me*)*Phe*], 1.37–1.48 (m, 1H, 4-H_b, *Ile*), 1.71–1.90 (m, 1H, 3-H, *Ile*), 3.19 [dq, J=7.3 Hz, 1H, (β*Me*)*Phe*], 3.90 (t, J=8.5 Hz, 1H, 1-H, *DCPM*), 4.26–4.42 (m, 2H, 2×2-H), 5.09 (s, 2H, Bzl-H), 5.42 (d, J=9.0 Hz, 1H, NH), 6.06 (d, J=7.5 Hz, 1H, NH), 7.15–7.30 (m, 6H, Ar-H), 7.30–7.40 (m, 4H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=2.4, 2.7 (−, C-2′, *DCPM*), 11.4 (+, C-5, *Ile*), 14.3, 14.5 (+, C-1′, *DCPM*), 14.8 (+, C-1′, *Ile*), 16.7 [+, C-4, (β*Me*)*Phe*], 24.9 (−, C-4, *Ile*), 37.9 (+, C-3, *Ile*), 42.2 [+, C-3, (β*Me*)*Phe*], 56.1 (+, C-2, *Ile*), 60.1 [+, C-2, (β*Me*)*Phe*], 66.5 (−, Bzl-C), 82.9 (+, C-1, *DCPM*), 126.5, 127.4, 127.5, 127.6, 128.1 (×2) (+, Ar-C), 136.2, 141.9 (C_{quat}, Ar-C), 156.1 (C_{quat}, NCO_2), 170.2, 170.5 (C_{quat}, C-1, *Ile*); IR (KBr): $\tilde{\nu}$=3300, 3270, 3010, 2965, 2875, 1732, 1690, 1655, 1535, 1454, 1379, 1334, 1292, 1275, 1255, 1240, 1196, 1137, 1013 cm^{-1}; MS (EI, 70 eV): *m*/*z* (%): 520 (8) [M^+], 416 (7) [$M^+-C_8H_8$], 381 (8) [$M^+-C_7H_9NO_2$], 296 (20), 268 (11), 224 (20), 176 (6), 105 (7) [$C_8H_9^+$], 95 (100), 86 (17), 67 (10); HRMS (EI): calcd for $C_{31}H_{40}N_2O_5$: 520.2937; found 520.2937; elemental analysis calcd (%) for $C_{31}H_{40}N_2O_5$ (520.7): C 71.51, H 7.74, N 5.38; found C 71.51, H 7.42, N 5.40.

***N*-Fmoc-3-(2*R*,1′*R*,2′*R*)-(*trans*-2′-Nitrocyclopropyl)alanine (5)**: A solution of Fmoc-OSu (0.416 g, 1.36 mmol) in acetone (7 mL) was added to a vigorously stirred solution of 3-(2*R*,1′*R*,2′*R*)-(*trans*-2′-nitrocyclopropyl)-alanine (0.20 g, 1.15 mmol) and $NaHCO_3$ (0.202 g, 2.40 mmol) in water (5 mL) (if a precipitate formed, acetone and/or water was added to obtain a homogeneous solution) and stirring continued for an additional 3 h. Acetone was then removed under reduced pressure, and the pH of the residual water solution was adjusted to 1 with 3 M HCl. The resulting emulsion was extracted with Et_2O (30 mL) and the ethereal layer was back-extracted with 3% aqueous $NaHCO_3$ (5×10 mL, TLC control for the completeness of extraction was necessary). The combined aqueous fractions were washed with Et_2O (2×10 mL), acidified to pH 2 with 3 M HCl, and the resulting emulsion was extracted with Et_2O (40 mL). The organic phase was washed with 3 M HCl (2×10 mL), water (3×10 mL), brine (2×5 mL), dried, filtered and concentrated under reduced pressure. The residue was triturated with cold pentane and filtered. The resulting semisolid was dried at 0.02 Torr for prolonged time to give **5** (0.423 g, 93%) as a colorless foam. R_f=0.08 (EtOAc/hexane 1:1); m.p. (softening) 50–57 °C; $[\alpha]_D^{20}=-56.7$ (c=0.36, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.71–0.82 (m, 0.4H, 3′-H_a), 1.11 (ddd J=6.2, 6.2, 6.2 Hz, 0.6H, 3′-H_a), 1.17–1.51 (m, 1H, 3′-H_b), 1.75–2.13 (m, 2H, 3-H), 3.61–3.76, 3.76–3.89 (2×m, 1H, 1′-H), 3.99–4.12 (m, 0.5H, 2-H), 4.12–4.27 (m, 1.5H, 9‴-H, 2-H), 4.27–4.56 (m, 2H, 1″-H), 4.56–4.69, 4.71–4.87 (2×m, 1H, 2′-H), 5.48 (d, J=7.0 Hz, 0.6H, NH), 7.01–7.13 (m, 0.4H, NH), 7.23–7.42 (m, 4H, Ar-H), 7.42–7.61 (m, 2H, Ar-H), 7.75 (d, J=7.5 Hz, 2H, Ar-H), 7.90–10.50 (br, 1H, CO_2H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=17.3, 17.6 (−, C-3′), 21.4, 22.0 (+, C-1′), 32.8, 33.2 (−, C-3), 46.8 (+, C-9‴), 53.0 (+, C-2), 59.0 (+, C-2′), 66.7, 67.0 (−, C-1″), 119.8 (+, Ar-C), 124.2, 124.4 (+, Ar-C), 124.8 (+, Ar-C), 126.9, 127.6 (+, Ar-C), 141.1 (C_{quat}, Ar-C), 143.0, 143.3 (C_{quat}, Ar-C), 143.3, 143.5 (C_{quat}, Ar-C), 155.9, 156.8 (C_{quat}, NCO_2), 173.8, 174.6 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}$=3331, 2965, 2934, 1736, 1689, 1653, 1525, 1455, 1391, 1367, 1251, 1176 cm^{-1}; MS (EI, 70 eV): *m*/*z* (%): 396 (1) [M^+], 196 (36) [$C_{14}H_{12}O^+$], 178 (51) [$C_{14}H_{10}^+$], 165 (100), 139 (6); HRMS (EI): calcd for $C_{21}H_{20}N_2O_6$: 396.1321; found 396.1321; elemental analysis calcd (%) for $C_{21}H_{20}N_2O_6$ (396.4): C 63.63, H 5.09, N 7.07; found C 63.56, H 5.21, N 7.00.

Fmoc-(2*R*)-(3-Ncp)Ala-(βMe)Phe-Ile-OMOM (7a): The compound **4a** (0.370 g, 0.786 mmol) was hydrogenated over 10% Pd on charcoal (0.100 g) under hydrogen at ambient pressure in EtOAc (10 mL) with AcOH (0.048 g, 0.800 mmol) for 1 h. The mixture was then filtered, concentrated under reduced pressure, taken up with Et_2O (40 mL), washed with saturated aqueous $NaHCO_3$ (3×10 mL), brine (2×10 mL), dried, filtered and concentrated under reduced pressure to give the deprotected dipeptide **6a** as a free base together with cyclo-[(βMe)Phe-Ile]. This mixture was directly used for the condensation with the *N*-Fmoc-protected amino acid **5** (0.320 g, 0.807 mmol) employing EDC (0.160 g, 0.835 mmol), HOAt (0.111 g, 0.816 mmol) and TMP (0.430 mL, 3.254 mmol) in CH_2Cl_2 (5 mL) according to GP 2. After 15 h, the reaction mixture was subjected to the usual aqueous work-up, and the resulting crude product was first crystallized from CH_2Cl_2/hexane and then purified by column chromatography [R_f=0.28, $CHCl_3$/MeOH 60:1 (1% EtOH)] to give tripeptide **7a** (0.220 g, 39% over two steps) as a colorless solid. $[\alpha]_D^{20}=-15.3$ (c=0.40, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.82 (q, J=7.3 Hz, 6H, 5-H, 1′-H, *Ile*), 0.93–1.17 [m, 2H, 4-H_a, *Ile*, 3′-H_a, (*3-Ncp*)*Ala*], 1.26–1.43 (m, 1H, 4-H_b, *Ile*), 1.32 [d, J=6.5 Hz, 3H, 4-H, (β*Me*)*Phe*], 1.48–1.70 [m, 1H, 3′-H_b, (*3-Ncp*)*Ala*], 1.70–1.84 [m, 2H, 3-H, *Ile*, 1′-H, (*3-Ncp*)*Ala*], 1.84–1.99 [m, 2H, 3-H, (*3-Ncp*)*Ala*], 3.22 [dq, J=7.4 Hz, 1H, 3-H, (β*Me*)*Phe*], 3.40 (s, 3H, OMe), 4.00–4.10 (m, 1H, 2-H), 4.20 (dd, J=6.5, 6.5 Hz, 1H, 2-H), 4.26–4.44 [m, 3H, 1-H_a, 9′-H_a, *Fmoc*, 2′-H, (*3-Ncp*)*Ala*], 4.49 (dd, J=10.6, 6.9 Hz, 1H, 1-H_b, *Fmoc*), 4.62 (dd, J=8.5, 8.5 Hz, 1H, 2-H), 5.09 (d, J=5.9 Hz, 1H, H_a, OCH_2O), 5.20 (d, J=5.9 Hz, 1H, H_b, OCH_2O), 5.63 (d, J=8.0 Hz, 1H, NH), 6.17 (d, J=8.0 Hz, 1H, NH), 6.95 (d, J=8.5 Hz, 1H, NH), 7.14–7.31 (m, 7H, Ar-H), 7.40 (dd, J=7.5, 7.5 Hz, 2H, Ar-H), 7.58 (dd, J=7.3, 5.0 Hz, 2H, Ar-H), 7.77 (d, J=7 Hz, 2H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=11.4 (+, C-5, *Ile*), 15.0 (+, C-1′, *Ile*), 17.1 [+, C-4, (β*Me*)*Phe*], 17.9 [−, C-3′, (*3-Ncp*)*Ala*], 22.1 [+, C-1′, (*3-Ncp*)*Ala*], 24.9 (−, C-4, *Ile*), 34.0 [−, C-3, (*3-Ncp*)*Ala*], 37.7 (+, C-3, *Ile*), 41.9 [+, C-3, (β*Me*)*Phe*], 46.9 (+, C-9′, *Fmoc*), 54.0 [+, C-2, (β*Me*)*Phe*], 56.3 [+, C-2, *Ile*], 57.7 (+, OMe), 58.7 [+, C-2, (*3-Ncp*)*Ala*], 59.0 [+, C-2′, (*3-Ncp*)*Ala*], 67.1 (−, C-1, *Fmoc*), 90.9 (−, OCH_2O), 119.9, 124.9, 126.9, 127.0, 127.6, 127.7, 128.4 (+, Ar-C), 141.1, 141.2 (C_{quat}, Ar-C, *Fmoc*), 141.6 [C_{quat}, Ar-C, (β*Me*)*Phe*], 143.5, 143.7 (C_{quat}, Ar-C, *Fmoc*), 156.0 (C_{quat}, NCO_2), 170.2, 170.6, 171.2 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}$=3330, 3275, 3067, 2966, 1718, 1643, 1542, 1451, 1368, 1227, 1163, 1139, 1091 cm^{-1}; MS (EI, 70 eV): *m*/*z* (%): 715 (0.04) [M^+], 518 (3), 429 (13), 321 (5), 196 (20), 178 (100), 165 (54), 134 (16), 86 (23), 45 (20) [CHO_2^+]; MS (ESI): positive mode: *m*/*z* (%): 737 (30) [M+Na^+]; negative mode: *m*/*z* (%): 749 (40) [M+Cl^-].

Fmoc-(2*R*)-(3-Ncp)Ala-(βMe)Phe-Ile-ODCPM (7b): The dipeptide **4b** (0.35 g, 0.67 mmol) was taken up with EtOAc (10 mL) and hydrogenated over 10% Pd/C (0.15 g) under hydrogen at ambient pressure for 2 h. The reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure to give the deprotected dipeptide **6b**, which was directly used for the coupling with **5** (274 mg, 0.69 mmol), using EDC (137 mg, 0.72 mmol), HOAt (96 mg, 0.71 mmol) and TMP (0.25 mL, 2.02 mmol) according to GP 2 to give compound **7b** (405 mg, 79%) as a colorless solid after two recrystallizations from THF/hexane 1:1. $R_f = 0.52$ (EtOAc/hexane 2:3); m.p 151–155 °C; $[\alpha]_D^{20} = -3.8$ ($c = 0.26$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.20$–0.38 (m, 4H, 2′-H, *DCPM*), 0.37–0.49 (m, 2H, 2′-H, *DCPM*), 0.49–0.62 (m, 2H, 2′-H, *DCPM*), 0.80 (d, $J = 7.0$ Hz, 3H, 1′-H, *Ile*), 0.86 (t, $J = 7.5$ Hz, 3H, 5-H, *Ile*), 0.94–1.20 [m, 4H, 4-H_a, *Ile*, 3′-H_a, *3-(Ncp)Ala*, 1′-H, *DCPM*], 1.33–1.49 [m, 1H, 3′-H_b, *3-(Ncp)Ala*], 1.34 [d, $J = 7.0$ Hz, 3H, 4-H, (β-*Me*)*Phe*], 1.50–1.68 (m, 1H, 4-H_b, *Ile*), 1.71–1.85 [m, 2H, 3-H, *Ile*, 1′-H, *3-(Ncp)Ala*], 1.85–2.05 [m, 2H, 3-H, *3-(Ncp)Ala*], 3.22 [dq, $J = 7.0$ Hz, 1H, 3-H, (β-*Me*)*Phe*], 3.79 (t, $J = 8.6$ Hz, 1H, 1-H, *DCPM*), 4.01–4.09 (m, 1H, 2-H), 4.21 (dd, $J = 6.5$ Hz, 1H, 2-H), 4.26–4.43 [m, 3H, 2′-H, *3-(Ncp)Ala*, 1-H_a, 9′-H_a, *Fmoc*], 4.49 (dd, $J = 10.3$, 7.0 Hz, 1H, 1-H_b, *Fmoc*), 4.61 (dd, $J = 5.5$ Hz, 1H, 2-H), 5.58 (d, $J = 8.3$ Hz, 1H, NH), 6.14 (d, $J = 7.8$ Hz, 1H, NH), 6.84 (d, $J = 8.0$ Hz, 1H, NH), 7.12–7.31 (m, 5H, Ar-H), 7.33 (d, $J = 7.8$ Hz, 2H, Ar-H), 7.40 (dd, $J = 7.3$, 7.3 Hz, 2H, Ar-H), 7.58 (d, $J = 7.0$ Hz, 2H, Ar-H), 7.77 (d, $J = 7.3$ Hz, 2H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 2.4$, 2.7 (−, C-2′, *DCPM*), 11.4 (+, C-5, *Ile*), 14.1, 14.4 (+, C-1′, *DCPM*), 14.8 (+, C-1′, *Ile*), 16.7 [+, C-4, (β-*Me*)*Phe*], 17.7 [−, C-3′, *3-(Ncp)Ala*], 21.97 [+, C-1′, *3-(Ncp)Ala*], 24.9 (−, C-4, *Ile*), 33.9 [−, C-3, *3-(Ncp)Ala*], 38.1 (+, C-3, *Ile*), 41.7 [+, C-3, (β-*Me*)*Phe*], 46.8 (+, C-9′, *Fmoc*), 53.8 (+, C-2), 56.2 (+, C-2), 58.5 (+, C-2), 58.8 [+, C-2′, *3-(Ncp)Ala*], 67.0 (−, C-1, *Fmoc*), 83.1 (+, C-1, *DCPM*), 119.7, 124.8, 126.6, 127.4 (× 2) (+, Ar-C), 128.2 (× 2) (+, Ar-C), 140.96, 141.49 (C_{quat}, Ar-C, *Fmoc*), 141.5 [C_{quat}, Ar-C, (β-*Me*)*Phe*], 143.5, 143.6 (C_{quat}, Ar-C, *Fmoc*), 155.9 (C_{quat}, NCO_2), 169.8, 170.5, 171.0 (C_{quat}, C-1); IR (KBr): $\tilde{\nu} = 3490$, 2966, 1717, 1645, 1543, 1451, 1368, 1147 cm^{-1}; MS (ESI): positive mode: *m/z* (%): 787 (70) [*M*+Na^+]; negative mode: *m/z* (%): 799 (38) [*M*+Cl^-]; elemental analysis calcd (%) for $C_{44}H_{52}N_4O_8$ (764.9): C 69.09, H 6.85, N 7.32; found C 69.06, H 6.79, N 7.19.

MeZ-*a*-Thr-OH: $NaHCO_3$ (0.360 g, 4.29 mmol) and then a solution of MeZOSu (0.486 g, 1.85 mmol) in dioxane (7 mL) were added to a vigorously stirred solution of *allo*-D-threonine (0.200 g, 1.68 mmol) in water (7 mL), and stirring was continued for 3 h (if a precipitate formed, dioxane and/or water was added to obtain a homogeneous solution). The mixture was then concentrated under reduced pressure, diluted with water (40 mL) and washed with CH_2Cl_2 (4 × 10 mL). The pH of the water fraction was adjusted to ca. 1–2 with solid $NaHSO_4$, and the resulting emulsion was extracted with EtOAc (2 × 40 mL). The organic layer was washed with water (4 × 20 mL), brine 2 × 10 mL), dried and concentrated under reduced pressure. The residue was recrystallized from Et_2O/hexane and then from CH_2Cl_2/hexane to give the title compound (0.175 g, 39%) as a colorless solid. The mother liquor from the second crystallization was concentrated, and the residue was recrystallized again from Et_2O/hexane to give a second crop of the title compound (0.23 g, 90% overall yield). $R_f = 0.13$ (EtOAc/hexane 1:3 (2% AcOH); threefold development was applied); m.p. 78–80 °C; $[\alpha]_D^{20} = -24.6$ ($c = 0.32$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.27$ (d, $J = 6.5$ Hz, 3H, 4-H), 2.34 (s, 3H, 1′-H), 3.70–4.40 (br, 2H, OH, CO_2H), 4.05–4.27 (m, 1H, 3-H), 4.33–4.44 (m, 1H, 2-H), 5.07 (s, 2H, Bzl-H), 5.77 (d, $J = 7.5$ Hz, 1H, NH), 7.15 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.24 (d, $J = 8.0$ Hz, 2H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 18.7$ (+, C-4), 21.1 (+, C-1′), 59.3 (+, C-2), 67.4 (−, Bzl-C), 69.0 (+, C-3), 128.3, 129.2 (+, Ar-C), 132.7, 138.1 (C_{quat}, Ar-C), 156.9 (C_{quat}, NCO_2), 173.3 (C_{quat}, C-1); IR (KBr): $\tilde{\nu} = 3400$, 3213, 2988, 2931, 2889, 1723, 1678, 1537, 1422, 1275, 1225, 1210 cm^{-1}; MS (EI, 70 eV): *m/z* (%): 267 (3) [M^+], 223 (5) [$M^+ - CO_2$], 162 (8) [$M^+ - C_8H_9$], 105 (100) [$C_8H_9^+$], 77 (6) [$C_6H_5^+$], 45 (5) [CHO_2^+]; HRMS (EI): calcd for $C_{13}H_{17}NO_5$: 267.1107; found 267.1107; elemental analysis calcd (%) for $C_{13}H_{17}NO_5$ (267.3): calcd. C 58.42, H 6.41, N 5.24; found C 58.59, H 6.13, N 5.08.

MeZ-*a*-Thr-OAll (9): A suspension of dried K_2CO_3 (0.034 g, 0.247 mmol) in a solution of the *N*-MeZ-protected *allo*-D-threonine (0.12 g, 0.449 mmol) and allyl bromide (0.08 mL, 0.946 mmol) in anhydrous MeCN (4 mL) was vigorously stirred in a sealed tube at 85 °C for 2 h. The mixture was then allowed to cool to 60 °C, and stirring was continued for an additional 16 h. The reaction mixture was cooled to 20 °C, Et_2O (50 mL) and water (20 mL) were then added. The organic layer was washed with water (4 × 10 mL), saturated aqueous $NaHCO_3$ (2 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residual oil was triturated with Et_2O/pentane 1:2 to give a colorless solid. Then more pentane was added to complete the precipitation, **9** was filtered off and dried under reduced pressure (0.116 g, 84%). $R_f = 0.16$ (EtOAc/hexane 1:3); m.p. 47–48 °C; $[\alpha]_D^{20} = -20.4$ ($c = 0.30$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.20$ (d, $J = 6.5$ Hz, 3H, 4-H), 2.35 (s, 3H, 1′-H), 2.74 (d, $J = 6.3$ Hz, 1H, OH), 4.09–4.26 (m, 1H, 3-H), 4.47 (dd, $J = 7.5$, 3.8 Hz, 1H, 2-H), 4.67 (d, $J = 5.3$ Hz, 2H, 1-H, *All*), 5.08 (s, 2H, Bzl-H), 5.27 (ddt, $J = 10.3$, 1.0, 1.0 Hz, 1H, *trans*-3-H, *All*), 5.34 (ddt, $J = 17.3$, 1.0, 1.0 Hz, 1H, *cis*-3-H, *All*), 5.66 (d, $J = 7.3$ Hz, 1H, NH), 5.90 (ddt, $J = 17.3$, 10.3, 5.8 Hz, 1H, 2-H, *All*), 7.17 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.26 (d, $J = 8.0$ Hz, 2H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 18.7$ (+, C-4), 20.9 (+, C-1′), 59.3 (+, C-2), 65.9 (−, C-1, *All*), 67.4 (−, Bzl-C), 68.4 (+, C-3), 118.7 (−, C-3, *All*), 128.0, 128.9 (+, Ar-C), 132.8 (+, C-2, *All*), 132.8, 137.7 (C_{quat}, Ar-C), 156.4 (C_{quat}, NCO_2), 169.9 (C_{quat}, C-1); IR (KBr): $\tilde{\nu} = 3409$, 3327, 3056, 2972, 2941, 2889, 1744, 1690, 1536, 1463, 1382, 1338, 1284, 1233, 1183 cm^{-1}; MS (EI, 70 eV): *m/z* (%): 307 (2) [M^+], 263 (4) [$M^+ - C_2H_4O$], 202 (8) [$M^+ - C_8H_9$], 105 (100) [$C_8H_9^+$], 77 (5) [$C_6H_5^+$], 41 (5) [$C_3H_5^+$]; HRMS (EI): calcd for $C_{16}H_{21}NO_5$: 307.1420; found 307.1420; elemental analysis calcd (%) for $C_{16}H_{21}NO_5$ (307.5): C 62.53, H 6.89, N 4.56; found C 62.80, H 6.84, N 4.41.

MeZ-*a*-Thr[(4-PE)Pro]-OAll (12): EDC (0.324 g, 1.69 mmol) was added to a cooled (4 °C) solution of the *N*-Boc-protected (2*S*,4*R*)-4-(*Z*)-propenylproline (**8**; 0.340 g, 1.33 mmol), the *N*,*C*-protected amino acid **9** (0.400 g, 1.30 mmol) and 4-pyrrolidinopyridine (0.250 g, 1.69 mmol) in anhydrous CH_2Cl_2 (3 mL). The temperature was allowed to reach 20 °C, and stirring was continued for 15 h. Then the reaction mixture was diluted with Et_2O (30 mL) and washed with 1 M $KHSO_4$ (3 × 5 mL), water (2 × 5 mL), 3% aqueous solution of $NaHCO_3$ (3 × 5 mL), water (3 × 5 mL), brine (2 × 5 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc/hexane 1:6) to give **12** (0.588 g, 83%) as a turbid oil. $R_f = 0.43$ (EtOAc/hexane 1:3); $[\alpha]_D^{20} = -35.4$ ($c = 0.28$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.32$, 1.34 (2 × d, $J = 6.5$ Hz, 3H, 4-H, *a-Thr*), 1.36, 1.39 [2 × s, 9H, $C(CH_3)_3$], 1.64, 1.65 [2 × dd, $J = 1.5$, 7.0 Hz, 3H, 3′-H, (*4-PE*)*Pro*], 1.63–1.81 [m, 1H, 3-H_a, (*4-PE*)*Pro*], 2.21–2.46 [m, 1H, 3-H_b, (*4-PE*)*Pro*], 2.33, 2.35 (2 × s, 3H, 1′-H, *MeZ*), 2.93–3.19 [m, 2H, 4-H, 5-H_a, (*4-PE*)*Pro*], 3.51–3.67, 3.69–3.83 [2 × m, 1H, 5-H_b, (*4-PE*)*Pro*], 4.10–4.27 [m, 1H, 2-H, (*4-PE*)*Pro*], 4.48–4.65 (m, 1H, 2-H, *a-Thr*), 4.68 (d, $J = 5.2$ Hz, 2H, 1-H, *All*), 4.97–5.13 (m, 2H, Bzl-H, *MeZ*), 5.17–5.43 [m, 4H, 3-H, *All*, 3-H, *a-Thr*, 1′-H, (*4-PE*)*Pro*], 5.54 [dq, $J = 10.2$, 7.0 Hz, 1H, 2′-H, (*4-PE*)*Pro*], 5.80–6.01 (m, 1H, 2-H, *All*), 6.42 (d, $J = 9.2$ Hz, 1H, NH), 7.13, 7.17 (2 × d, $J = 7.9$ Hz, 2H, Ar-H), 7.23, 7.25 (d, $J = 7.9$ Hz, 2H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 12.8$ [+, C-3′, (*4-PE*)*Pro*], 15.8, 16.3 (+, C-4, *a-Thr*), 20.8 (+, C-1′, *MeZ*), 27.9 [+, $C(CH_3)_3$], 35.3, 36.1 [+, C-4, (*4-PE*)*Pro*], 35.8, 36.9 [−, C-3, (*4-PE*)*Pro*], 51.1, 51.3 [−, C-5, (*4-PE*)*Pro*], 57.0, 57.4 [+, C-2, (*4-PE*)*Pro*], 59.0, 59.1 (+, C-2, *a-Thr*), 65.5, 66.0 (−, C-1, *All*), 66.4, 67.0 (−, Bzl-C), 70.8, 70.8 (+, C-3), 79.6, 79.7 [C_{quat}, $C(CH_3)_3$], 118.2, 118.9 (−, C-3, *All*), 126.3, 126.3 (+, Ar-C), 127.9 (+, Ar-C), 128.7, 128.8 [+, C-2′, (*4-PE*)*Pro*], 129.0, 129.1 [+, C-1′, (*4-PE*)*Pro*], 130.9, 131.2 (+, C-2, *All*), 132.7, 133.1 (C_{quat}, Ar-C), 137.2, 137.6 (C_{quat}, Ar-C), 153.0, 153.5 (C_{quat}, NCO_2, *Boc*), 155.4, 155.9 (C_{quat}, NCO_2, *MeZ*), 168.4, 168.4 (C_{quat}, C-1, *a-Thr*), 171.6, 171.9 [C_{quat}, C-1, (*4-PE*)*Pro*]; IR (KBr): $\tilde{\nu} = 3330$, 3009, 2977, 2932, 2871, 1747, 1703, 1520, 1478, 1453, 1399, 1367, 1253, 1161 cm^{-1}; MS (EI, 70 eV): *m/z* (%): 544 (2) [M^+], 488 (6) [$M^+ - C_4H_8$], 444 (5) [$M^+ - C_5H_8O_2$], 339 (26), 210 (11), 154 (100) [$C_8H_{12}NO_2^+$], 110 (83) [$C_7H_{12}N^+$], 105 (100) [$C_8H_9^+$], 57 (49) [$C_4H_9^+$], 41 (15) [$C_3H_5^+$]; HRMS (EI): calcd for $C_{29}H_{40}N_2O_8$: 544.2785; found 544.2785; elemental analysis calcd (%) for $C_{29}H_{40}N_2O_8$ (544.7): C 63.95, H 7.40, N 5.14; found C 63.99, H 7.32, N 4.99.

Fmoc-(βMe)Phe-(2*R*)-(3-Ncp)Ala-(βMe)Phe-Ile-OMOM (13a): The tripeptide **7a** (205 mg, 0.29 mmol) was deprotected according to GP 1 and then directly coupled with the *N*-Fmoc-protected β-methylphenylalanine (**10**)[21] (126 mg, 0.31 mmol) according to GP 2 using EDC (60 mg, 0.31 mmol), HOAt (42 mg, 0.31 mmol) and TMP (106 mg, 0.88 mmol) in CH_2Cl_2/MeCN 2:1 (3 mL) to give **13a** (195 mg, 78%) as a colorless solid after recrystallization from $CHCl_3$/hexane. $R_f = 0.27$ [$CHCl_3$/MeOH 70:1

(1% EtOH)]; m.p. 209–212°C (decomp.); $[\alpha]_D^{20} = -40.2$ ($c = 0.3$, THF); ^{1}H NMR (250 MHz, [D$_8$]THF): $\delta = 0.78$ (d, $J = 7.0$ Hz, 3H, 1′-H, *Ile*), 0.80 (t, $J = 7.0$ Hz, 3H, 5-H, *Ile*), 0.94–1.17 [m, 2H, 4-H$_a$, *Ile*, 3′-H$_a$, (*3-Ncp*)*Ala*], 1.20 [d, $J = 6.8$ Hz, 3H, 4-H, (β-*Me*)*Phe*], 1.33 [d, $J = 7.0$ Hz, 3H, 4-H, (β-*Me*)*Phe*], 1.34–1.54 [m, 4H, 3-H, 4-H$_b$, *Ile*, 1′-H, 3′-H$_b$, (*3-Ncp*)*Ala*], 1.59–1.87 [m, 2H, 3-H, (*3-Ncp*)*Ala*], 3.10 [dq, $J = 10.3$, 7.1 Hz, 1H, 3-H, (β-*Me*)*Phe*], 3.21 [dq, $J = 7.5$, 7.5 Hz, 1H, 3-H, (β-*Me*)*Phe*], 3.31 (s, 3H, OMe), 3.95 [ddd, $J = 6.8$, 3.3, 3.3 Hz, 1H, 2′-H, (*3-Ncp*)*Ala*], 4.13–4.40 (m, 5H, 2×2-H, 1-H, 9′-H, *Fmoc*), 4.46 [ddd, $J = 3.3$, 3.3, 3.3 Hz, 1H, 2-H, (*3-Ncp*)*Ala*], 4.55 (dd, $J = 8.6$, 8.6 Hz, 1H, 2-H), 5.03 (d, $J = 5.6$ Hz, 1H, H$_a$, OCH$_2$O), 5.11 (d, $J = 5.6$ Hz, 1H, H$_b$, OCH$_2$O), 6.97–7.46 (m, 16H, 4×NH, 12×Ar-H), 7.54 (dd, $J = 10.9$, 8.9 Hz, 2H, Ar-H), 7.66 (dd, $J = 9.9$, 7.6 Hz, 2H, Ar-H), 7.77 (d, $J = 7.5$ Hz, 2H, Ar-H); ^{13}C NMR (62.9 MHz, [D$_8$]THF): $\delta = 11.9$ (+, C-5, *Ile*), 15.9 (+, C-1′, *Ile*), 17.9 [+, C-4, (β-*Me*)*Phe*], 18.6 [+, C-4, (β-*Me*)*Phe*], 18.8 [−, C-3′, (*3-Ncp*)*Ala*], 23.1 [+, C-1′, (*3-Ncp*)*Ala*], 26.0 (−, C-4, *Ile*), 34.7 [−, C-3, (*3-Ncp*)*Ala*], 38.6 (+, C-3, *Ile*), 42.7 [+, C-3, (β-*Me*)*Phe*], 44.0 [+, C-3, (β-*Me*)*Phe*], 48.4 (+, C-9′, *Fmoc*), 52.7 (+, C-2), 57.3 (+, C-2), 57.9 (+, OMe), 59.6 (+, C-2), 60.1 [+, C-2′, (*3-Ncp*)*Ala*], 62.1 (+, C-2), 67.6 (−, C-1, *Fmoc*), 91.4 (−, OCH$_2$O), 126.3, 126.3, 127.4, 127.7, 128.0, 128.5, 128.9, 129.1, 129.2, 129.3 (+, Ar-C), 142.4, 142.4 (C$_{quat}$, Ar-C, *Fmoc*), 143.9 [C$_{quat}$, Ar-C, (β-*Me*)*Phe*], 144.2 [C$_{quat}$, Ar-C, (β-*Me*)*Phe*], 145.3, 145.5 (C$_{quat}$, Ar-C, *Fmoc*), 157.7 (C$_{quat}$, NCO$_2$), 171.3, 171.4, 171.6, 172.1 (C$_{quat}$, C-1); IR (KBr): $\tilde{\nu} = 3300$, 2967, 1708, 1668, 1637, 1543, 1370, 1247 cm^{-1}; MS (ESI): positive mode: *m*/*z* (%): 898 (100) [*M*+Na$^+$]; elemental analysis calcd (%) for $C_{49}H_{57}N_5O_{10}$ (876.0): C 67.18, H 6.56, N 7.99; found C 67.15, H 6.46, N 8.06.

Fmoc-(βMe)Phe-(2*R*)-(3-Ncp)Ala-(βMe)Phe-Ile-ODCPM (13b): The tripeptide **7b** (0.420 g, 0.549 mmol) was deprotected according to GP 1, and the resulting product **11b** was then directly coupled with **10** (0.242 g, 0.603 mmol) according to GP 2 using EDC (0.114 g, 0.595 mmol), HOAt (0.080 g, 0.588 mmol) and TMP (0.200 g, 1.650 mmol) in CH_2Cl_2 (3 mL). After 1 h, a precipitate formed in the reaction mixture, and anhydrous DMF (2 mL) was added to obtain a homogeneous solution. After 15 h, the reaction mixture was concentrated under reduced pressure. The resulting solid was washed with water (100 mL), 3% aqueous $NaHCO_3$ (100 mL), water (100 mL), Et_2O (100 mL), pentane (50 mL), dried at 0.5 Torr and then crystallized twice from THF/hexane to give **13b** (0.430 g, 85%) as an off-white solid. $R_f = 0.29$ ($CHCl_3$/MeOH 70:1); m.p 210–215°C (decomp.); $[\alpha]_D^{20} = -26.3$ ($c = 0.32$, THF); ^{1}H NMR (250 MHz, [D$_8$]THF): $\delta = 0.22$–0.57 (m, 7H, 2′-H, *DCPM*), 0.67–0.90 (m, 2H, 1′-H, 2′-H, *DCPM*), 0.78–0.86 (m, 1H, 1′-H, *DCPM*), 0.81 (d, $J = 6.5$ Hz, 3H, 1′-H, *Ile*), 0.83 (t, $J = 7.3$ Hz, 3H, 5-H, *Ile*), 0.92–1.19 [m, 4H, 4-H, *Ile*, 3′-H, (*3-Ncp*)*Ala*], 1.20 [d, $J = 7.3$ Hz, 3H, 4-H, (β*Me*)*Phe*], 1.32 [d, $J = 7.0$ Hz, 3H, 4-H, (β*Me*)*Phe*], 1.30–1.50 [m, 4H, 3-H, *Ile*, 1′-H, 3-H, (*3-Ncp*)*Ala*], 3.11 [dq, $J = 9.6$, 7.2 Hz, 1H, 3-H, (β*Me*)*Phe*], 3.24 [dq, $J = 7.4$, 7.4 Hz, 1H, 3-H, (β*Me*)*Phe*], 3.81 (t, $J = 8.5$ Hz, 1H, 1-H, *DCPM*), 3.94 [ddd, $J = 6.8$, 3.3, 3.3 Hz, 1H, 2′-H, (*3-Ncp*)*Ala*], 4.13–4.41 (m, 5H, 2×2-H, 1-H, 9′-H, *Fmoc*), 4.41–4.51 (m, 1H, 2-H), 4.61 (dd, $J = 8.5$, 7.8 Hz, 1H, 2-H), 6.97–7.40 (m, 16H, 2×NH, 14×Ar-H), 7.48 (d, $J = 8.5$ Hz, 1H, NH), 7.58 (d, $J = 8.3$ Hz, 1H, NH), 7.66 (dd, $J = 8.1$ Hz, 2H, Ar-H), 7.77 (d, $J = 7.8$ Hz, 2H, Ar-H); ^{13}C NMR (62.9 MHz, [D$_8$]THF): $\delta = 3.3$, 3.5, 3.6, 3.7 (−, C-2′, *DCPM*), 12.2 (+, C-5, *Ile*), 15.6, 15.9 (+, C-1′, *DCPM*), 16.1 (+, C-1′, *Ile*), 17.7 [+, C-4, (β*Me*)*Phe*], 18.7 [+, C-4, (β*Me*)*Phe*], 18.9 [−, C-3′, (*3-Ncp*)*Ala*], 23.2 [+, C-1′, (*3-Ncp*)*Ala*], 26.2 (−, C-4, *Ile*), 35.0 [−, C-3, (*3-Ncp*)*Ala*], 39.1 (+, C-3, *Ile*), 42.8 [+, C-3, (β*Me*)*Phe*], 44.1 [+, C-3, (β*Me*)*Phe*], 48.5 (+, C-9′, *Fmoc*), 52.8 (+, C-2), 57.4 (+, C-2), 59.3 (+, C-2), 60.2 [+, C-2′, (*3-Ncp*)*Ala*], 62.1 (+, C-2), 67.8 (−, C-1, *Fmoc*), 83.4 (+, C-1, *DCPM*), 126.4 (× 2), 127.5, 127.7, 128.1, 128.6, 129.1, 129.1, 129.3, 129.4 (+, Ar-C), 142.5 (C$_{quat}$, Ar-C, *Fmoc*), 144.1 [C$_{quat}$, Ar-C, (β*Me*)*Phe*], 144.4 [C$_{quat}$, Ar-C, (β*Me*)*Phe*], 145.4, 145.6 (C$_{quat}$, Ar-C, *Fmoc*), 157.8 (C$_{quat}$, NCO$_2$), 171.1, 171.5, 171.9, 172.1 (C$_{quat}$, C-1); IR (KBr): $\tilde{\nu} = 3250$, 3065, 2967, 2933, 1709, 1665, 1636, 1542, 1451, 1369, 1246 cm^{-1}; MS (ESI): positive mode: *m*/*z* (%): 948 (100) [*M*+Na$^+$]; elemental analysis calcd (%) for $C_{54}H_{63}N_5O_9$ (926.1): C 70.03, H 6.86, N 7.56; found C 69.94, H 6.68, N 7.32.

MeZ-*a*-Thr[(4-PE)Pro]-OH (14): [Pd(PPh$_3$)$_4$] (0.034 g, 29.4 μmol) was added to a vigorously stirred solution of the ester **12** (0.145 g, 0.266 mmol) and *N*-methylaniline (0.1 mL, 0.923 mmol) in DME (4.0 mL) and the resulting suspension was carefully heated with a heat-gun to obtain a homogeneous solution. The mixture was left to stir at 20°C for 45 min, was then again carefully heated with heat-gun until the catalyst had dissolved and stirred for another 15 min. The reaction mixture was then diluted with Et_2O (40 mL), washed with 1M $NaHSO_4$ (3× 10 mL), water (10×10 mL), brine (2×10 mL), dried, filtered and concentrated under reduced pressure. The residue was taken up with Et_2O/hexane 1:2, filtered, concentrated and purified by column chromatography [$R_f = 0.34$ (EtOAc/hexane 1:2 (1.5% AcOH)] to give **14** (0.121 g, 90%) as a yellow oil. $[\alpha]_D^{20} = -71.7$ ($c = 0.32$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.35$, 1.40 [2×s, 9H, C(CH$_3$)$_3$], 1.41, 1.43 (2×d, $J = 5.7$ Hz, 3H, 4-H, *a-Thr*), 1.63, 1.65 [2×dd, $J = 1.5$, 6.7 Hz, 3H, 3′-H, (*4-PE*)*Pro*], 1.67–1.86 [m, 1H, 3-H$_a$, (*4-PE*)*Pro*], 2.24–2.49 [m, 1H, 3-H$_b$, (*4-PE*)*Pro*], 2.33, 2.34 (2×s, 3H, 1′-H, *MeZ*), 2.91–3.20 [m, 2H, 4-H, 5-H$_a$, (*4-PE*)*Pro*], 3.40–4.30 (br, 1H, CO$_2$H), 3.61, 3.73 [2×ddd, $J = 2.7$ Hz, 1H, 5-H$_b$, (*4-PE*)*Pro*], 4.11–4.29 [m, 1H, 2-H, (*4-PE*)*Pro*], 4.51 (dd, $J = 8.6$, 3.5 Hz, 1H, 2-H, *a-Thr*), 5.05 (s, 2H, Bzl-H, *MeZ*), 5.15–5.31 [m, 1H, 1′-H, (*4-PE*)*Pro*], 5.31–5.44 (m, 1H, 3-H, *a-Thr*), 5.45–5.60 [m, 1H, 2′-H, (*4-PE*)*Pro*], 5.63 (d, $J = 7.6$ Hz, 0.4H, NH), 6.46 (d, $J = 9.2$ Hz, 0.6H, NH), 7.13, 7.17 (2×d, $J = 7.9$ Hz, 2H, Ar-H), 7.21, 7.22 (d, $J = 7.9$ Hz, 2H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 13.0$ [+, C-3′, (*4-PE*)*Pro*], 15.8, 16.3 (+, C-4, *a-Thr*), 21.0 (+, C-1′, *MeZ*), 28.1 [+, C(*C*H$_3$)$_3$], 35.5, 36.2 [+, C-4, (*4-PE*)*Pro*], 36.0, 37.0 [−, C-3, (*4-PE*)*Pro*], 51.3, 51.6 [−, C-5, (*4-PE*)*Pro*], 57.0, 57.5 [+, C-2, (*4-PE*)*Pro*], 59.1, 59.3 (+, C-2, *a-Thr*), 66.8, 67.0 (−, Bzl-C), 71.2, 71.4 (+, C-3, *a*-Thr), 80.5, 80.6 [C$_{quat}$, *C*(CH$_3$)$_3$], 126.5, 126.6 (+, Ar-C), 128.1, 128.2 [+, C-2′, (*4-PE*)*Pro*], 128.9, 129.0 [+, C-1′, (*4-PE*)*Pro*], 129.10, 129.13 (+, Ar-C), 132.8, 133.0 (C$_{quat}$, Ar-C), 137.6, 137.9 (C$_{quat}$, Ar-C), 153.7, 154.2 (C$_{quat}$, NCO$_2$, *Boc*), 155.8, 156.4 (C$_{quat}$, NCO$_2$, *MeZ*), 171.2, 171.7 (C$_{quat}$, C-1), 172.0, 172.2 (C$_{quat}$, C-1); IR (KBr): $\tilde{\nu} = 3700$–2750, 2979, 1729, 1520, 1404, 1369, 1257, 1162, 1061 cm^{-1}; MS (EI, 70 eV): *m*/*z* (%): 504 (2) [M^+], 448 (7) [$M^+ - C_4H_8$], 404 (27) [$M^+ - C_5H_8O_2$], 299 (21), 210 (14), 154 (100) [$C_8H_{12}NO_2^+$], 110 (92) [$C_7H_{12}N^+$], 105 (100) [$C_8H_9^+$], 57 (50) [$C_4H_9^+$], 41 (16) [$C_3H_5^+$]; HRMS (EI): calcd for $C_{26}H_{36}N_2O_8$: 504.2472; found 504.2472.

MeZ-*a*-Thr[Boc-(4-PE)Pro]-(βMe)Phe-(2*R*)-(3-Ncp)Ala-(βMe)Phe-Ile-OMOM (16a): The tetrapeptide **13a** (180 mg, 0.21 mmol) was deprotected according to GP 1 in MeCN (2 mL), taken up with anhydrous CH_2Cl_2 (5 mL), the dipeptide acid **14** (0.114 g, 0.23 mmol), HATU (96 mg, 0.25 mmol) and HOAt (31 mg, 0.23 mmol) were added, and the reaction mixture was cooled to 4°C. After this, a solution of DIEA (29 mg, 0.22 mmol) and TMP (75 mg, 0.62 mmol) in CH_2Cl_2 (2 mL) was added at the same temperature within 5 min. The temperature was allowed to reach 20°C, and stirring was continued for an additional 15 h. After aqueous work-up according to GP 2 and two recrystallizations from EtOAc/hexane 1:2, the depsipeptide **16a** (185 mg, 79%) was obtained as a colorless powder. $R_f = 0.46$ (EtOAc/hexane 1:1); m.p. 125–127°C; $[\alpha]_D^{20} = 0$ ($c = 0.21$, $CHCl_3$); $[\alpha]_D^{20} = -29.0$ ($c = 0.2$, THF); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 0.76$ (d, $J = 6.8$ Hz, 3H, 1′-H, *Ile*), 0.89 (t, $J = 7.3$ Hz, 3H, 5-H, *Ile*), 0.95–1.11 [m, 2H, 4-H$_a$, *Ile*, 3′-H$_a$, (*3-Ncp*)*Ala*], 1.11–1.60 [m, 5H, 3-H, 4-H$_b$, *Ile*, 3-H$_a$, 1′-H, 3′-H$_b$, (*3-Ncp*)*Ala*], 1.24 [d, $J = 7.5$ Hz, 3H, 4-H, (β-*Me*)*Phe*], 1.27 [d, $J = 7.5$ Hz, 3H, 4-H, (β-*Me*)*Phe*], 1.36 [s, 9H, C(CH$_3$)$_3$], 1.43 (d, $J = 6.5$ Hz, 3H, 4-H, *a-Thr*), 1.68 [dd, $J = 6.6$, 1.6 Hz, 3H, 3′-H, (*4-PE*)*Pro*], 1.76 [dd, $J = 11.6$, 11.6 Hz, 1H, 3-H$_a$, (*4-PE*)*Pro*], 1.87–2.02 [m, 1H, 3-H$_b$, (*3-Ncp*)*Ala*], 2.32 (s, 3H, 1′-H, MeZ), 2.43 [ddd, $J = 12.3$, 6.5, 6.5 Hz, 1H, 3-H$_b$, (*4-PE*)*Pro*], 2.99–3.20 [m, 1H, 4-H, (*4-PE*)*Pro*], 3.11 [dd, $J = 9.8$, 9.8 Hz, 1H, 5-H$_a$, (*4-PE*)*Pro*], 3.21–3.38 [m, 2H, 3-H, (β-*Me*)*Phe*, 5-H$_b$, (*4-PE*)*Pro*], 3.49 (s, 3H, OMe), 3.70 (dd, $J = 9.8$, 7.0 Hz, 1H, 2-H), 3.86 [dq, $J = 3.9$, 6.5 Hz, 1H, 3-H, (β-*Me*)*Phe*], 4.14 (dd, $J = 11.1$, 6.3 Hz, 1H, 2-H), 4.34–4.55 [m, 3H, 2-H, 2′-H, (*3-Ncp*)*Ala*], 4.59 (dd, $J = 9.9$, 5.1 Hz, 1H, 2-H), 4.67 (dd, $J = 10.4$, 10.4 Hz, 1H, 2-H), 5.03 (dd, $J = 2.9$, 2.9 Hz, 2H, Bzl-H), 5.27 [ddq, $J = 10.5$, 10.5, 1.6 Hz, 1H, 1′-H, (*4-PE*)*Pro*], 5.46–5.60 [m, 2H, 3-H, *a-Thr*, 2′-H, (*4-PE*)*Pro*], 5.61 (d, $J = 6.3$ Hz, 1H, H$_b$, OCH$_2$O), 6.57 (d, $J = 8.3$ Hz, 1H, NH), 6.99 (d, $J = 6.0$ Hz, 1H, NH), 7.02 (d, $J = 6.0$ Hz, 1H, NH), 7.07–7.38 (m, 14H, Ar-H), 7.57 (d, $J = 10.0$ Hz, 1H, NH), 7.95 (d, $J = 5.8$ Hz, 1H, NH); The peak of Bzl-H masked absorption for H$_a$ of OCH$_2$O; ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 11.5$ (+, C-5, *Ile*), 13.0 [+, C-3′, (*4-PE*)*Pro*], 15.6 (+, C-1′, *Ile*), 17.5 [+, C-4, (β-*Me*)*Phe*], 18.2 [−, C-3′, (*3-Ncp*)*Ala*], 18.4 [+, C-4, (β-*Me*)*Phe*], 19.2 (+, C-4, *a-Thr*), 20.9 (+, C-1′, *MeZ*), 21.7 [+, C-1′, (*3-Ncp*)*Ala*], 24.9 (−, C-4, *Ile*), 28.1 [+, C(*C*H$_3$)$_3$], 31.5 [−, C-3, (*3-Ncp*)*Ala*], 36.1 [−, C-3, (*4-PE*)*Pro*], 36.1 [+, C-4, (*4-PE*)*Pro*], 36.9 (+, C-3, *Ile*), 40.4 [+, C-3, (β-*Me*)*Phe*], 41.8 [+,

C-3, (β-*Me*)*Phe*], 50.8 (+, C-2), 51.9 [−, C-5, (*4-PE*)*Pro*], 56.1 (+, C-2), 57.5 (+, OMe), 59.1 (+, C-2), 59.3 (+, C-2), 60.7 (+, C-2), 61.2 (+, C-2), 61.6 [+, C-2′, (*3-Ncp*)*Ala*], 66.7 (−, Bzl-C), 70.4 (+, C-3, *a-Thr*), 80.7 [C_{quat}, $C(CH_3)_3$], 90.9 (−, OCH_2O), 126.3, 126.7, 127.5, 127.7, 128.2, 128.3, 128.4, 128.5, 128.7, 128.8 [+, Ar-C and C-1′, C-2′, (*4-PE*)*Pro*], 133.0, 137.6, 141.6, 141.8 (C_{quat}, Ar-C), 154.5, 154.6 (C_{quat}, NCO_2), 170.31, 170.33, 170.8, 171.1, 173.0, 173.8 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}$ = 3308, 3062, 2972, 2934, 1729, 1648, 1543, 1454, 1368, 1254, 1162, 1091 cm^{-1}; MS (ESI): positive mode: *m*/*z* (%): 1163 (100) [*M*+Na^+]; negative mode: *m*/*z* (%): 1138 (50) [*M*−H^+], 1175 (50) [*M*+Cl^-]; elemental analysis calcd (%) for $C_{60}H_{81}N_7O_{15}$ (1140.4): C 63.20, H 7.16, N 8.60; found C 63.09, H 7.27, N 8.46.

***N*-MeZ-protected cyclohexadepsipeptide (17)**: 2 M HCl in EtOAc (2 mL) was added to the hexadepsipeptide **16a** (0.188 g, 0.165 mmol). The reaction mixture was stirred at 20 °C for 45 min in a dark place and was then concentrated under reduced pressure at 20 °C. The residue was triturated with anhydrous Et_2O to give the hydrochloride of the deprotected material as a colorless solid (0.160 g, 0.157 mmol). MS (ESI): positive mode: *m*/*z* (%): 1019 (5) [*M*+Na^+], 997 (100) [*M*+H^+]; negative mode: *m*/*z* (%): 995 (50) [*M*−H^+]. It was taken up with anhydrous CH_2Cl_2 (1.5 L). The solution was cooled to 4 °C (internal temperature), HATU (73 mg, 0.192 mmol) and HOAt (22 mg, 0.163 mmol) were added, and then a solution of DIEA (62 mg, 0.480 mmol) in CH_2Cl_2 (50 mL) was added over 30 min. The cooling bath was removed and stirring continued for an additional 2 h at ambient temperature. Then the reaction mixture was cooled again to 4 °C, and a second portion of each, HATU (73 mg, 0.192 mmol) and HOAt (22 mg, 0.163 mmol), was added, and then a solution of DIEA (62 mg, 0.480 mmol) in CH_2Cl_2 (50 mL) was added within 30 min. The temperature was allowed to reach 20 °C and stirring was continued for 15 h. After this, the solvent was removed under reduced pressure, the residue was taken up with Et_2O, and after the usual aqueous work-up (GP 2) and concentration under reduced pressure, the crude product was purified first by column chromatography (R_f = 0.17, acetone/hexane 1:3) and then by recrystallization (Et_2O/pentane) to give a crude product (120 mg), which was finally purified by preparative HPLC to give cyclodepsipeptide **17** (86 mg, 53% over two steps) as a colorless solid. Preparative HPLC: isocratic, 75% MeCN in H_2O (0.1% TFA); analytical HPLC: isocratic, 50% MeCN in H_2O (0.1% TFA) for 20 min, then gradient 50% → 99% MeCN (0.1% TFA) for 15 min, then isocratic, 99% MeCN in H_2O (0.1% TFA) for 10 min, flow rate = 0.5 mL min^{-1}, t_R = 34.93 min, purity >98%; $[\alpha]_D^{20} = -15.5$ (*c* = 0.20, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.64 [ddd, *J* = 14.4, 7.2, 7.2 Hz, 1H, 3-H_a, (3-*Ncp*)*Ala*], 0.72 (d, *J* = 6.6 Hz, 3H, 1′-H, *Ile*), 0.74 [ddd, *J* = 6.6, 6.6, 6.6 Hz, 1H, 3′-H_a, (*3-Ncp*)*Ala*], 0.79 (t, *J* = 7.2 Hz, 3H, 5-H, *Ile*), 1.04–1.12 (m, 1H, 4-H_a, *Ile*), 1.23 [d, *J* = 6.6 Hz, 3H, 4-H, (β-*Me*)*Phe*], 1.27–1.34 [m, 1H, 1′-H, (3-*Ncp*)*Ala*], 1.37 [d, *J* = 6.6 Hz, 3H, 4-H, (β-*Me*)*Phe*], 1.37–1.43 [m, 1H, 3-H_b, (3-*Ncp*)*Ala*], 1.45–1.54 (m, 1H, 4-H_b, *Ile*), 1.54–1.57 [m, 1H, 3′-H_b, (*3-Ncp*)*Ala*], 1.57 (d, *J* = 6.6 Hz, 3H, 4-H, *a-Thr*), 1.65 [dd, *J* = 6.6, 1.5 Hz, 3H, 3′-H, (*4-PE*)-*Pro*], 1.66–1.76 [m, 2H, 3-H, *Ile*, 3-H_a, (4-*PE*)*Pro*], 2.23 [ddd, *J* = 12.0, 5.4, 5.4 Hz, 1H, 3-H_b, (4-*PE*)-*Pro*], 2.35 (s, 3H, 1′-H, *MeZ*), 3.05 [dq, *J* = 6.6, 6.6 Hz, 1H, 3-H, (β-*Me*)*Phe*], 3.15–3.28 [m, 2H, 4-H, 5-H_a, (*4-PE*)-*Pro*], 3.54 [dq, *J* = 7.2, 6.6 Hz, 1H, 3-H, (β-*Me*)*Phe*], 3.71 (dd, *J* = 6.0, 5.4 Hz, 1H, 2-H), 3.76 [ddd, *J* = 6.6, 3.3, 3.3 Hz, 1H, 2′-H, (*3-Ncp*)*Ala*], 3.98 (dd, *J* = 10.5, 6.3 Hz, 1H, 2-H), 4.01–4.08 [m, 1H, 5-H_b, (*4-PE*)-*Pro*], 4.46–4.54 (m, 2H, 2-H), 4.54 (dd, *J* = 9.6, 9.6 Hz, 1H, 2-H), 4.69 (dd, *J* = 8.4 Hz, 1H, 2-H), 5.03 (d, *J* = 12.0 Hz, 1H, Bzl-H_a), 5.15 (d, *J* = 12.0 Hz, 1H, Bzl-H_b), 5.19–5.25 [m, 1H, 1′-H, (*4-PE*)-*Pro*], 5.39 (dq, *J* = 1.8, 6.6 Hz, 1H, 3-H, *a-Thr*), 5.56 [dq, *J* = 10.8, 6.6 Hz, 1H, 2′-H, (*4-PE*)-*Pro*], 6.21–6.37 (br, 2H, NH), 6.44 (d, *J* = 8.4 Hz, 1H, NH), 6.48 (d, *J* = 9.6 Hz, 1H, NH), 7.06–7.18 (m, 4H, Ar-H), 7.19–7.27 (m, 8H, Ar-H), 7.26–7.34 (m, 2H, Ar-H), 7.48 (d, *J* = 8.4 Hz, 1H, NH); ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 10.3 (+, C-5, *Ile*), 13.3 [+, C-3′, (*4-PE*)-*Pro*], 14.6 [+, C-1′, *Ile*, C-4, (β-*Me*)*Phe*], 17.3 [−, C-3′, (*3-Ncp*)*Ala*], 17.7 [+, C-4, (β-*Me*)*Phe*], 18.4 (+, C-4, *a-Thr*), 21.2 (+, C-1′, *MeZ*), 21.3 [+, C-1′, (*3-Ncp*)*Ala*], 24.7 (−, C-4, *Ile*), 32.0 [−, C-3, (*3-Ncp*)*Ala*], 35.4 [−, C-3, (*4-PE*)-*Pro*], 36.6 [+, C-3, *Ile*, C-4, (*4-PE*)-*Pro*], 39.4 [+, C-3, (β-*Me*)*Phe*], 44.5 [+, C-3, (β-*Me*)*Phe*], 52.5 [−, C-5, (*4-PE*)-*Pro*], 53.3 (+, C-2), 54.6 (+, C-2), 58.6 (+, C-2), 59.0 [+, C-2′, (*3-Ncp*)*Ala*], 59.4 (+, C-2), 60.1 (+, C-2), 60.7 (+, C-2), 67.2 (−, Bzl-C), 72.6 (+, C-3, *a-Thr*), 127.1, 127.2, 127.5, 127.6, 128.3, 128.6, 128.8, 129.2 (+, Ar-C), 127.8 [+, C-1′, (*4-PE*)-*Pro*], 128.0 [+, C-2′, (*4-PE*)-*Pro*], 133.2, 137.9, 140.9, 142.6 (C_{quat}, Ar-C), 156.3 (C_{quat}, NCO_2), 169.0, 170.3, 170.6, 171.1, 171.4, 173.1 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}$ = 3340, 2969, 2936, 2878, 1730, 1637, 1541, 1515, 1452, 1370, 1179 cm^{-1}; MS (ESI): positive mode: *m*/*z* (%): 1001 (100) [*M*+Na^+]; negative mode: *m*/*z* (%): 977 (100) [*M*−H^+]; HRMS (ESI): calcd for [$C_{53}H_{67}N_7O_{11}Na^+$]: 1000.4792; found 1000.4792.

***N*-Teoc-3-(2*S*,1′*S*,2′*R*)-(*trans*-2′-Nitrocyclopropyl)alanine**: A solution of TeocOSu (0.358 g, 1.38 mmol) in acetone (5 mL) was added to a vigorously stirred solution of 3-(2*S*,1′*R*,2′*R*)-(*trans*-2′-nitrocyclopropyl)alanine (0.200 g, 1.15 mmol) and $NaHCO_3$ (0.202 g, 2.40 mmol) in water (7 mL) (if an emulsion formed, acetone and/or water was added to obtain a homogeneous solution), and stirring was continued for another 2 h. *N*,*N*-Dimethylaminopropylamine (0.055 mL, 0.44 mmol) was then added. After an additional 10 min acetone was removed under reduced pressure and the pH of the residual water solution was adjusted to 2–3 with 1 M $NaHSO_4$. The resulting emulsion was extracted with Et_2O (50 mL), and the ethereal layer was washed with 1 M $NaHSO_4$ (2 × 10 mL), water (10 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residual oil (0.300 g) was dissolved in Et_2O (5 mL), and cyclohexylamine (0.094 g, 0.95 mmol) was added. The mixture was concentrated under reduced pressure and treated with boiling hexane. The resulting precipitate was filtered off and washed with Et_2O/pentane 1:4 to give the cyclohexylammonium salt of the title compound (0.386 g, 81%) as a colorless solid. R_f = 0.24 [EtOAc/hexane 1:3 (2% AcOH)]; $[\alpha]_D^{20}$ = 22.80 (*c* = 0.46, $CHCl_3$) for CHA salt; ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.04 [s, 9H, $Si(CH_3)_3$], 1.00 (dd, *J* = 9.5, 7.3 Hz, 2H, 2-H, *Teoc*), 1.14 (ddd, *J* = 6.0, 6.0, 6.0 Hz, 1H, 3′-H_a), 1.60–1.95 (m, 2H, 1′-H, 3′-H_b), 1.98–2.19 (m, 2H, 3-H), 4.18 (dd, *J* = 8.4, 8.4 Hz, 2H, 1-H, *Teoc*), 4.33–4.46, 4.46–4.59 (2 × m, 1H, 2-H), 5.33–5.46 (m, 1H, NH), 7.08–7.25 (br, 1H, CO_2H); the signal of 2′-H is masked by the absorption of 1-H, *Teoc*; ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = −1.9 [+, $Si(CH_3)_3$], 17.3 (−, C-3′ and C-2, *Teoc*), 22.0 (+, C-1′), 33.1, 33.3 (−, C-3), 52.7, 53.2 (+, C-2), 59.0 (+, C-2′), 63.7, 64.8 (−, C-1, *Teoc*), 156.4, 157.4 (C_{quat}, NCO_2), 174.5, 174.8 (C_{quat}, C-1); IR (KBr; for CHA salt): $\tilde{\nu}$ = 3419, 3250–2600, 2952, 2863, 1722, 1632, 1541, 1492, 1406, 1368, 1248, 1206, 1163, 1053 cm^{-1}; MS (ESI): positive mode: *m*/*z* (%): 363 (28) [*M*−H^++2Na^+], 341 (26) [*M*+Na^+]; negative mode (%): *m*/*z*: 657 (100) [2*M*−2H^++Na^+], 317 (28) [*M*−H^+].

Hormaomycin 1: An ethereal solution (50 mL) of the CHA salt of *N*-Teoc-protected (2*S*,1′*R*,2′*R*)-(3-nitrocyclopropyl)alanine (23.2 mg, 55.61 μmol) was washed with 1 M H_2SO_4 (3 × 5 mL), 1 M $KHSO_4$ (2 × 5 mL), water (3 × 5 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The resulting *N*-protected amino acid was dried at 0.02 Torr for 2 h and then coupled with the depsipeptide, obtained after deprotection of the *N*-MeZ-protected cyclodepsipeptide **17** (17.0 mg, 17.4 μmol) with 10% anisole in TFA (1.1 mL) in the dark at ambient temperature for 2 h, using HATU (19.8 mg, 52.1 μmol), HOAt (7.1 mg, 52.2 μmol), DIEA (2.3 mg, 17.8 μmol) and TMP (19.0 mg, 156.8 μmol) in CH_2Cl_2 (1.5 mL) at ambient temperature for 15 h. The reaction mixture was then diluted with Et_2O (30 mL) and the crude product obtained after the usual aqueous work-up (GP 2) was purified by crystallization from CH_2Cl_2/pentane to give Teoc-(*S*)-(3-Ncp)Ala-cyclohexadepsipeptide (19.7 mg, 100%; R_f = 0.43, acetone/hexane 1:2) as a colorless solid, which was used for the next step without any characterization. The Teoc group was cleaved off from this substance (19.7 mg, max. 17.4 μmol) with TFA (1.0 mL) for 1 h. The mixture was concentrated under reduced pressure at 20 °C and then taken up with toluene (3 × 15 mL) which was distilled off to remove the last traces of TFA. The resulting deprotected depsipeptide was coupled with **18** (10.0 mg, 48.7 μmol) using HATU (17.8 mg, 46.7 μmol), DIEA (2.6 mg, 20.1 μmol) and TMP (17.7 mg, 146.1 μmol) in CH_2Cl_2 (1.5 mL) at ambient temperature for 2.5 h. The mixture was then taken up with Et_2O (30 mL), and the crude product obtained after the usual aqueous work-up (GP 2) was purified by recrystallization from CH_2Cl_2/pentane to give the *O*-MOM protected **1** (20.5 mg, 100%; R_f = 0.36 acetone/hexane 1:2) as a colorless solid which was used for the next step without any characterization. The *O*-MOM protected hormaomycin (20.5 mg, max. 17.4 μmol) was deprotected using $MgBr_2 \cdot Et_2O$ (120 mg, 0.464 mmol) and EtSH (30 μL, 0.405 mmol) in CH_2Cl_2 (10 mL) at ambient temperature for 3 h. The mixture was taken up with Et_2O (40 mL) and washed with 1 N $KHSO_4$ (3 × 10 mL), water (4 × 10 mL), brine (2 × 5 mL), dried, filtered and concen-

trated under reduced pressure. The residue was crystallized from CH_2Cl_2/pentane to give the crude hormaomycin **1** (17.5 mg, 90% purity according to HPLC), which was further purified by preparative HPLC. The fraction containing the desired product was collected, and its pH value was carefully adjusted to 6.9 (pH meter) with diluted aqueous ammonia, and then it was lyophilized. The residue was dissolved in EtOAc and filtered through cotton wool to give, after removal of the solvent under reduced pressure, the pure synthetic hormaomycin **1** (13.2 mg, 67% over five steps from **17**) as a colorless glass. $R_f = 0.24$ (acetone/hexane 3:7); preparative HPLC: column: JASCO Nucleosil C18 (250×8 mm); isocratic, 62% MeCN in 0.07% aqueous TFA; flow rate = 2.5 mL min^{-1}; analytical HPLC: the same column; the same conditions; the same flow rate; $t_R = 14.54$ min, purity >99%; $[\alpha]_D^{20} = 20.0$ ($c = 0.1$, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$): $\delta = -0.68$ [ddd, $J = 6.7, 6.7, 6.7$ Hz, 1H, 3′-H$_a$, (*3-Ncp*)*Ala*], −0.15 [dddd, $J = 14.2, 9.8, 3.5, 3.5$ Hz, 1H, 3-H$_a$, (*3-Ncp*)*Ala*], 0.23–0.33 [m, 1H, 1′-H, (*3-Ncp*)*Ala*], 0.58 [ddd, $J = 14.2, 4.3, 4.3$ Hz, 1H, 3-H$_b$, (*3-Ncp*)*Ala*], 0.90 (t, $J = 7.2$ Hz, 3H, 5-H, *Ile*), 0.98–1.04 [m, 1H, 3′-H$_b$, (*3-Ncp*)*Ala*], 1.02 [ddd, $J = 6.4, 6.4, 6.4$ Hz, 1H, 3′-H$_a$, (*3-Ncp*)*Ala*], 1.04 (d, $J = 6.8$ Hz, 3H, 1′-H, *Ile*), 1.27–1.35 (m, 1H, 4-H$_a$, *Ile*), 1.33 [d, $J = 7.2$ Hz, 3H, 4-H, (β-*Me*)*Phe*], 1.41 [d, $J = 7.2$ Hz, 3H, 4-H, (β-*Me*)*Phe*], 1.54 (d, $J = 6.7$ Hz, 3H, 4-H, *a-Thr*), 1.54–1.63 [m, 2H, 3-H$_a$, (*3-Ncp*)*Ala*, 4-H$_b$, *Ile*], 1.69 [dd, $J = 6.6, 1.8$ Hz, 3H, 3′-H, (*4-PE*)*Pro*], 1.75–1.99 [m, 4H, 3-H$_b$, 1′-H, 3′-H$_b$, (*3-Ncp*)*Ala*, 3-H, *Ile*], 1.82 [ddd, $J = 11.4, 11.4, 11.4$ Hz, 1H, 3-H$_a$, (*4-PE*)*Pro*], 2.39 [ddd, $J = 11.4, 6.0, 5.4$ Hz, 1H, 3-H$_b$, (*4-PE*)*Pro*], 2.90 [ddd, $J = 6.7, 3.5$ Hz, 1H, 2′-H, (*3-Ncp*)*Ala*], 3.03 [dq, $J = 11.1, 7.2$ Hz, 1H, 3-H, (β-*Me*)*Phe*], 3.22–3.34 [m, 2H, 4-H, 5-H$_a$, (*4-PE*)*Pro*], 3.43–3.52 [m, 1H, 2-H, (*3-Ncp*)*Ala*], 3.68 [dq, $J = 4.5, 7.2$ Hz, 1H, 3-H, (β-*Me*)*Phe*], 3.94–4.02 [m, 1H, 5-H$_b$, (*4-PE*)*Pro*], 4.05 [ddd, $J = 6.5, 3.8, 3.8$ Hz, 1H, 2′-H, (*3-Ncp*)*Ala*], 4.28 [dd, $J = 11.8, 6.2$ Hz, 1H, 2-H, (*4-PE*)*Pro*], 4.38 [dd, $J = 10.2, 10.2$ Hz, 1H, 2-H, (β-*Me*)*Phe*], 4.47 [dd, $J = 9.5, 4.5$ Hz, 1H, 2-H, (β-*Me*)*Phe*], 4.59 (dd, $J = 9.8, 2.5$ Hz, 1H, 2-H, *a-Thr*), 4.67 (dd, $J = 9.0, 9.0$ Hz, 1H, 2-H, *Ile*), 5.13 [ddd, $J = 7.5, 7.5, 7.5$ Hz, 1H, 2-H, (*3-Ncp*)*Ala*], 5.24–5.31 [m, 1H, 1′-H, (*4-PE*)*Pro*], 5.41 (dq, $J = 6.6, 2.5$ Hz, 1H, 3-H, *a-Thr*), 5.64 [dq, $J = 10.2, 6.6$ Hz, 1H, 2′-H, (*4-PE*)*Pro*], 6.15 (d, $J = 4.9$ Hz, 1H, 4-H, *Chpca*), 6.47 (d, $J = 6.0$ Hz, 1H, NH), 6.80 (d, $J = 8.4$ Hz, 1H, NH), 6.82 (d, $J = 9.6$ Hz, 1H, NH), 6.84 (d, $J = 4.9$ Hz, 1H, 3-H, *Chpca*), 7.00–7.06 (m, 1H, Ar-H), 7.11–7.16 (m, 4H, Ar-H), 7.15–7.19 (m, 1H, Ar-H), 7.21–7.25 (m, 4H, Ar-H), 7.27 (d, $J = 9.5$ Hz, 1H, NH), 8.06 (d, $J = 9.4$ Hz, 1H, NH), 9.08 (d, $J = 9.8$ Hz, 1H, NH), 10.60–11.25 (br, 1H, OH, *Chpca*); ^{13}C NMR (150.8 MHz, $CDCl_3$): $\delta = 10.6$ (+, C-5, *Ile*), 13.3 [+, C-3′, (*4-PE*)*Pro*], 13.3 [+, C-4, (β-*Me*)*Phe*], 15.0 (+, C-1′, *Ile*), 17.0 (+, C-4, *a-Thr*), 17.4 [−, 2×C-3′, (*3-Ncp*)*Ala*], 18.0 [+, C-4, (β-*Me*)*Phe*], 20.5 [+, C-1′, (*3-Ncp*)*Ala*], 21.8 [+, C-1′, (*3-Ncp*)*Ala*], 24.9 (−, C-4, *Ile*), 33.4 [−, C-3, (*3-Ncp*)*Ala*], 35.3 [−, C-3, (*3-Ncp*)*Ala*], 35.6 [−, C-3, (*4-PE*)*Pro*], 36.7 [+, C-4, (*4-PE*)*Pro*], 38.1 (+, C-3, *Ile*), 39.3 [+, C-3, (β-*Me*)*Phe*], 41.9 [+, C-3, (β-*Me*)*Phe*], 50.7 [+, C-2, (*3-Ncp*)*Ala*], 52.0 [+, C-2, (*3-Ncp*)*Ala*], 52.8 [−, C-5, (*4-PE*)*Pro*], 54.7 (+, C-2, *Ile*), 55.2 (+, C-2, *a-Thr*), 58.2 [+, C-2′, (*3-Ncp*)*Ala*], 59.3 [+, C-2′, (*3-Ncp*)*Ala*], 59.9 [+, C-2, (β-*Me*)*Phe*], 60.2 [+, C-2, (β-*Me*)*Phe*], 61.4 [+, C-2, (*4-PE*)*Pro*], 69.2 (+, C-3, *a-Thr*), 103.2 (+, C-4, *Chpca*), 109.3 (+, C-3, *Chpca*), 119.5 (C$_{quat}$, C-2, *Chpca*), 121.4 (C$_{quat}$, C-5, *Chpca*), 126.9, 127.2, 127.5, 127.6, 128.5, 128.7 (+, Ar-C), 127.5 [+, C-1′, (*4-PE*)*Pro*], 128.4 [+, C-2′, (*4-PE*)*Pro*], 141.6, 142.2 (C$_{quat}$, Ar-C), 159.6 (C$_{quat}$, C-1, *Chpca*), 168.7, 168.9, 169.8, 170.9, 171.3, 171.5, 172.1 (C$_{quat}$, C-1); UV (MeOH): λ_{max} (ε) = 204 (5×10^4), 275 nm (9.6×10^3); CD (MeOH): λ_{max} [Θ] = 279 (6.01×10^4), 224.2 nm (-6.500×10^3) ($c = 1.754\times10^{-4}$ M); MS (ESI): positive mode: *m*/*z* (%): 1174 (100) [$M-H^+ + 2Na^+$], 1151 (80) [$M+Na^+$]; negative mode: *m*/*z* (%): 1127 (100) [$M-H^+$].

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[2] The previously unknown absolute configurations of the two nitrocyclopropylalanine residues as well as the 4-propenylproline moiety in **1** were established by a series of feeding experiments with the appropriate enantiomerically pure deuterium-labeled amino acids. It was additionally confirmed (in the case of 4-propenylproline) by a series of HPLC and HPLC/MS experiments.

[3] For the synthesis of all four stereoisomers of 3-(*trans*-2-nitrocyclopropyl)alanine see: a) O. V. Larionov, T. F. Savelieva, K. A. Kochetkov, N. S. Ikonnikov, S. I. Kozhushkov, D. S. Yufit, J. A. K. Howard, V. N. Khrustalev, Yu. N. Belokon, A. de Meijere, *Eur. J. Org. Chem.* **2003**, 869–877; for a partial elucidation of the relative and absolute configurations of the two 3-(*trans*-2-nitrocyclopropyl)alanine moieties in hormaomycin **1**, and the synthesis of enantiomerically pure mixtures of epimers of 3-(*trans*-2-nitrocyclopropyl)alanine, see also: b) J. Zindel, A. de Meijere, *J. Org. Chem.* **1995**, *60*, 2968–2973; c) J. Zindel, A. Zeeck, W. A. König, A. de Meijere, *Tetrahedron Lett.* **1993**, *34*, 1917–1920.

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[7] The relatively low yield must be attributed to the noticeable susceptibility of the MOM ester to nucleophilic attack by the free amino group, especially when formation of a diketopiperazine ring is possible.

[8] The tetrapeptide **13b** can also be used towards the synthesis of various analogues of **1**.

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[10] Although several *N*-MeZ-acylated glycines were prepared as early as in 1951 (cf.: D. M. Channing, P. B. Turner, G. T. Young, *Nature* **1951**, *167*, 487–488), the authors considered that "these compounds appear to offer little advantage over the corresponding carbobenzyloxy compounds"; to the best of our knowledge, the application of *p*-methylbenzyloxycarbonyl group for peptide synthesis has never been considered again.

[11] a) The *N*-Alloc-protected cyclic fragment of **1** was successfully prepared, but all attempts to obtain the appropriate deprotected cyclic hexadepsipeptide failed, presumably due to an *O*→*N* acyl shift; an attempted transprotection with Boc_2O (cf.: E. C. Roos, P. Bernabe, H. Hiemstra, W. N. Speckamp, B. Kaptein, W. H. J. Bösten, *J. Org. Chem.* **1995**, *60*, 1733–1740) gave an inseparable mixture of unreacted and transprotected peptides in 34% yield, although with model peptides this technique worked pretty well (90–100% yield of the desirable product). b) The ester bond between the (4-PE)Pro and *a*-Thr fragments may also be cleaved under strongly acidic (TFMSA in TFA, TMSOTf) conditions. c) For the model dipeptide MeZ-Phe-Val-OMe. d) From the secondary amino group of the (4-PE)Pro residue of the C-TMSE-protected acyclic precursor of the cyclic part of **1**. e) The rate of cleavage of the conventional Z-protective group was unacceptably slow even in the case of the model ester Z-Thr(OFmocPro)-OMe (incomplete deprotection with 20% thioanisole in TFA in 12 h at ambient temperature). f) The 3-methylbenzyloxycarbonyl and 3,5-dimethylbenzyloxycarbonyl groups were also tested, but turned out to be somewhat less suitable for application in this synthesis.

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[13] a) Chpca-(3-Ncp)Ala-OH was also synthesized, but turned out to be too unstable to be used for condensation with the deprotected **17**. b) The condensation between Chpca(MOM)-(3-Ncp)Ala-OH and **17** (after its deprotection) using the PyAOP[9] reagent unexpectedly caused significant epimerization at the α-carbon of the (3-Ncp)Ala residue in the side chain and gave a separable mixture of epimers (MOM-**1** and *epi*-MOM-**1**) in moderate yield.
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[15] a) The CD spectra were measured in methanol. b) It is noteworthy that both MOM-**1** and *epi*-MOM-**1** were equally active in this test and their activity was about 3.5 times lower than the activity of **1**.
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[17] We are grateful to Prof. W. A. König (Hamburg) for repeating some GC separations[1d] to determine the absolute configuration of the 3-methylalanine residue in hormaomycin **1**, using pure (2*S*,3*R*)-3-methylphenylalanine, provided by us, as one of the reference compounds (previously[1d] a mixture of all stereoisomers of 3-methylphenylalanine had been used as a reference). These latest results were in accordance with those obtained before.
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