DOI: 10.1002/ejoc.200500856

Synthesis and Precursor-Directed Biosynthesis of New Hormaomycin Analogues

Boris D. Zlatopolskiy,[a] Markus Radzom,[a] Axel Zeeck,*[a] and Armin de Meijere*[a]

Keywords: Natural products / Amino acids / Synthetic methods / Peptides / Biosynthesis

Several new analogues of hormaomycin (**1**), a peptide lactone with interesting biological activities, were prepared by total synthesis or by precursor-directed biosynthesis. The new analogues **2a**–**c**, **3a**–**c**, *O*-MOM-**1** and *epi*-*O*-MOM-**1** as well as the model acyl tripeptides **20a**–**c** and **21a**–**e** were tested for their antibiotic activities to give new insights into structure–activity relationships of this class of compounds. In this context, an unexpected activity of **2c** against *C. albicans* was discovered. The precursors necessary for feeding experiments, the amino acids **14a**, **14b** and **17**, were prepared in 31, 48 and 55% yield over 4 and 3 steps, respectively. In addition, these studies provided some new information about the biosynthetic route to furnish compound **1**. They also support the notion that the combination of chemical and biological methods may provide a broad range of analogues of an interesting biologically active natural product.

Definitely the most commonly exploited way to compounds with interesting biological activities is the design of analogues of lead structures, which are frequently derived from naturally occurring compounds. This can be achieved either by conventional chemical synthesis or by precursor-directed biosynthesis. If successful, this approach eventually furnishes compounds with an enhanced biological activity or even a totally changed activity pattern.[1]

Some time ago, we embarked on a project to develop a total synthesis and to elucidate the biosynthesis of hormaomycin (**1**), a peptide lactone produced by *Streptomyces griseoflavus* (strain W-384).[2,3] Besides challenging structural features, **1** exhibits quite an interesting spectrum of biological activities, including a marked influence on the secondary metabolite production of other streptomycetes, an exceptionally selective inhibitory activity against coryneform bacteria[2] as well as an interesting antimalarial activity.[4] Once our total synthesis of hormaomycin (**1**) had been completed,[3b] we concentrated our efforts on the preparation of new hormaomycin analogues as well as stripped-down mimics, in order to determine its minimal structural unit, which would still be biologically active. These studies included precursor-directed biosynthetic approaches which, at the same time, provided some clues about the biosynthesis of **1** itself[5] (Figure 1).

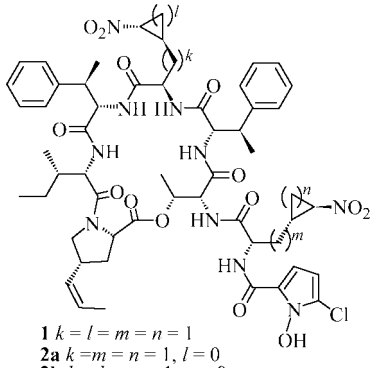


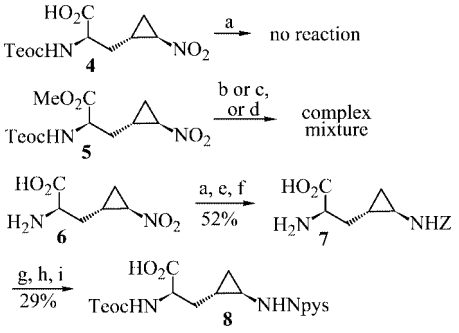
Figure 1. Structures of hormaomycin (**1**) and its analogues **2a**–**c** and **3a**–**c**.

As a first target, the total synthesis of an analogue containing an (2*S*,1′*R*,2′*R*)-3-(2′-aminocyclopropyl)alanine [(3-Acp)Ala] instead of the (2*S*,1′*R*,2′*R*)-3-(2′-nitrocyclopropyl)alanine [(3-Ncp)Ala] moiety in the side chain was addressed. Feeding experiments with H-(3-Acp)Ala-OH had shown that the latter was not incorporated, and thus such an analogue could not be prepared by precursor-directed biosynthesis.[5]

Because an initially attempted reduction of the *N*-Teoc-protected (3-Ncp)Ala-OH **4**[3b] or its methyl ester **5** failed, the free amino acid **6** was hydrogenated over 10% Pd/C in methanol according to the protocol of Larionov et al.[6] to give the desired diamino acid. Without any purification, it was acylated via the corresponding copper complex with Z-

[a] Institut für Organische und Biomolekulare Chemie der Georg-August-Universität Göttingen,
Tammannstrasse 2, 37077 Göttingen, Germany
Fax: +49-551-399475
E-mail: Armin.deMeijere@chemie.uni-goettingen.de
azeeck@gwdg.de
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

OSu to yield the N_ω-Z-protected H-(3-Acp)Ala-OH **7** (52% yield over two steps, Scheme 1).



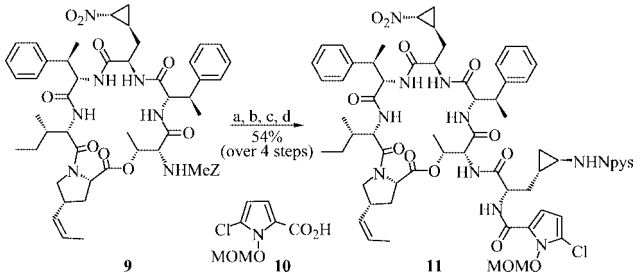
Scheme 1. Synthesis of the suitably protected diamino acid **8**. Reagents and conditions: a) H_2, 10% Pd/C, MeOH, 20 °C, 16 h. b) H_2, 10% Pd/C, EtOAc, 20 °C, 2 h. c) Zn/AcOH, 20 °C, 3 h. d) $NiCl_2$, $NaBH_4$, MeOH/THF, 20 °C, 2 h. e) $CuSO_4$, $NaHCO_3$, H_2O, 20 °C, 10 min, then Z-OSu, acetone, 20 °C, 1.5 h. f) disodium ethylenediaminetetraacetate (Trilon B), H_2O, reflux, 10 min. g) Teoc-OSu, $NaHCO_3$, acetone/H_2O (1:1), 20 °C, 16 h. h) H_2, 10% Pd/C, MeOH, 20 °C, 2.5 h. i) Npys-Cl, Et_3N, DMF, 0 → 20 °C, 15 h. Teoc-OSu = 2-(trimethylsilyl)ethyl *N*-hydroxysuccinimidyl carbonate, Z-OSu = benzyl *N*-hydroxysuccinimidyl carbonate, Npys-Cl = 3-nitro-2-pyridinesulfenyl chloride.

The latter was first N_α-protected with Teoc-OSu and then, after hydrogenolytic removal of the Z-group, treated with 3-nitro-2-pyridinesulfenyl chloride (NpysCl) to give the orthogonally bisprotected derivative **8** in 29% yield over three steps.

The Npys group, which was proposed for amino group protection by Matsueda et al.,[7] is stable towards trifluoroacetic acid, but easily removed with highly diluted (0.1–0.2 N) HCl in organic solvents. It has been reported that it can also be cleaved off under nearly neutral conditions with triphenylphosphane and a proton source, as well as under essentially neutral conditions with thiols such as 2-pyridinethiol *N*-oxide or 2-mercaptobenzothiazole. This therefore appeared to be the group of choice for protection of the ω-amino function of (3-Acp)Ala units taking into account the limitations imposed on the deprotection conditions by other sensitive moieties in the hormaomycin molecule (Scheme 2).[8]

After removal of the MeZ group from the lactone **9**,[3b] was acylated with the orthogonally bisprotected diamino acid **8** (Scheme 2). After removal of the Teoc group, the resulting intermediate product was in turn coupled with the *O*-MOM-protected Chpca **10**[9] to give the *O*-MOM-protected hormaomycin analogue **11** in 54% yield over four steps. The latter was treated first with $MgBr_2{\cdot}Et_2O$ and EtSH in CH_2Cl_2, and then with PPh_3 and Py·HCl.[10] Although the reaction mixture, according to TLC, contained two compounds sensitive to Ehrlich's reagent, attempted separation by exclusion chromatography did not provide any compound containing a pyrrole fragment.

Cyclopropylalanines with electron-withdrawing substituents (like NO_2, CO_2Me) in the cyclopropyl fragment have already been shown to be acceptable substrates for the hormaomycin synthetase.[3b] However, the question whether



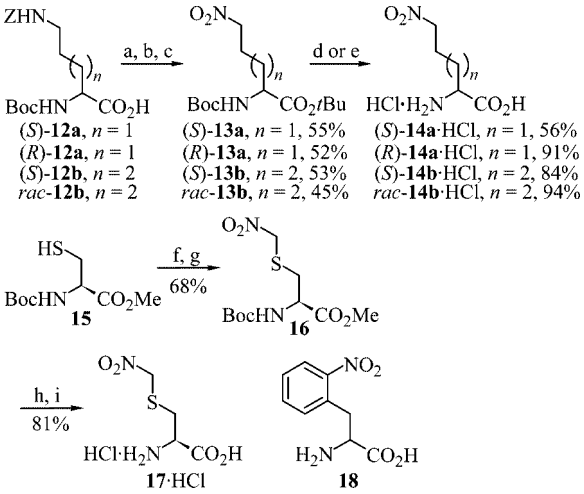
Scheme 2. Synthesis of the *O*-MOM-protected hormaomycin analogue **11**. Reagents and conditions: a) anisole, TFA, 20 °C, 2 h. b) Teoc-(2*S*,1′*R*,2′*R*)-(3-Acp)AlaOH (**8**), HATU, HOAt, DIEA, TMP, CH_2Cl_2, 20 °C, 15 h. c) TFA, 20 °C, 1 h. d) **10**, HATU, HOAt, DIEA, TMP, CH_2Cl_2, 20 °C, 4 h. HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOAt = 7-aza-1-hydroxybenzotriazole, TMP = 2,4,6-trimethylpyridine, DIEA = *N*,*N*-diisopropylethylamine, MOM = methoxymethyl.

the hormaomycin synthetase would accept amino acid substrates without a cyclopropyl group, and the importance of the two cyclopropyl fragments in hormaomycin (**1**) for its biological activities remained open. Therefore, feeding experiments with nitro-substituted amino acids devoid of a cyclopropyl moiety, e.g. **14a**, **14b**, **17** and **18**, were carried out.

These precursors were prepared as follows (Scheme 3). The N_α-Boc-N_ω-Z-protected ornithines **12a** and lysines **12b** were converted into the corresponding *tert*-butyl esters, Boc-Orn-O*t*Bu and Boc-Lys-O*t*Bu,[11] according to a protocol of Chevallet et al.[12] These diamino esters, after removal of the Z-group, were oxidized to the α-amino ω-nitro amino esters **13a** and **13b**, respectively, with *m*-chloroperbenzoic acid (MCPBA) in refluxing 1,2-dichloroethane following a protocol of Gilbert et al.[13] Final deprotection with TFA or HCl in EtOAc gave the desired 5-nitronorvalines, (*S*)-**14a** and (*R*)-**14a**, and 6-nitronorleucines, (*S*)-**14b** and *rac*-**14b**, as hydrochlorides in 31, 44, 48 and 42% yields, respectively, over four steps.[14,15]

5-Nitro-4-thianorvaline (**17**) was prepared in 55% yield over three steps, starting from the *N*,*O*-protected cysteine **15**. The latter was chlorinated at the sulfur atom with SO_2Cl_2 to give an unstable sulfenyl chloride, which was immediately treated with potassium methylnitronate to furnish the protected amino acid **16**. The ester was hydrolyzed under basic conditions with strict exclusion of oxygen, and subsequently the Boc group was removed by treatment with hydrochloric acid to furnish compound **17**. 2′-Nitrophenylalanine (**18**), a phenyl analogue of H-(3-Ncp)Ala-OH, was prepared according to a published procedure.[16]

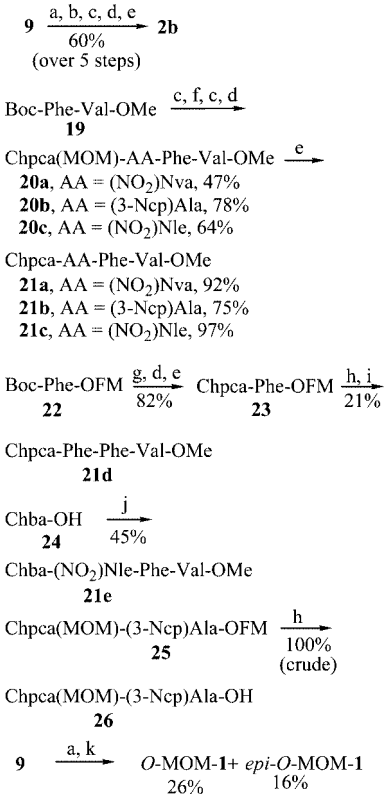
Feeding of 5-nitronorvaline (*S*)-**14a** to growing cultures of *Streptomyces griseoflavus* (strain W-384) was successful and, according to ESI-MS spectra of the isolated crude product, gave a mixture of hormaomycin (**1**) and the analogues **2a**–**c**. This indicates that the cyclopropyl ring in H-(3-Ncp)Ala-OH is not essential for the recognition of the amino acid by the hormaomycin synthetase.[17] The mixture was separated by HPLC to give the pure analogue **2c** with two, and one of the analogues with one nitronorvaline



Scheme 3. Syntheses of nitro amino acids **14a**,**b** and **17**. Reagents and conditions: a) *t*BuBr, K_2CO_3, TEBAC, DMA, 55 °C, 24 h. b) H_2, 10% Pd/C, EtOAc, 20 °C, 3 h. c) MCPBA, $ClCH_2CH_2Cl$, reflux, 20 min. d) TFA, 20 °C, 1 h, then 2 M HCl/EtOAc, 20 °C, 2 min. e) 4 M HCl/EtOAc, 0 → 20 °C, 2 h. f) SO_2Cl_2, CH_2Cl_2, –40 → –20 °C, 3 h·g) CH_3NO_2, *t*BuOK, THF, –78 → 0 °C, 2.5 h. h) 0.25 M NaOH, THF, 0 °C, 80 min. i) 4 M HCl/EtOAc, 20 °C, 20 min. TEBAC = triethylbenzylammonium chloride, MCPBA = 3-chloroperbenzoic acid.

[(NO_2)Nva] instead of a (3-Ncp)Ala residue. The latter, according to its ^{1}H-NMR spectrum and HPLC-MS analysis of its total hydrolysate applying the advanced Marfey method,[18] contained a (NO_2)Nva moiety in the peptide lactone ring of the molecule, corresponding to **2a**. The second analogue **2b** with only one (NO_2)Nva was also observed in tiny quantities, but could not be isolated in pure form. If an external H-(3-Ncp)Ala-OH epimerase really participates in the biosynthesis of hormaomycin (**1**) (see ref.[3a] for the relevant discussion), then an additional supply of (*R*)-H-(NO_2)Nva-OH should give a mixture of hormaomycin (**1**) and its analogues **2a**–**c**, possibly enriched with **2b**. Therefore, a feeding experiment with (*R*)-**14a** was carried out. However, only hormaomycin (**1**) itself was isolated in low yields. Apparently, (*R*)-**14a** is not accepted by the hormaomycin synthetase because of its inappropriate configuration, and the accepted (*S*)-**14a** is epimerized in the peptide lactone ring position to (*R*)-nitronorvaline [(*R*)-**14a**] during the biosynthesis of analogues **2a**,**c** by an epimerase in the appropriate module of the hormaomycin synthetase.[19] The different yields of **2a** and **2b** in the experiment with (*S*)-**14a** indicate different affinities of the binding sites for this amino acid in the multienzyme complex.

Although analogue **2b** could not be prepared in pure form by feeding experiments, it ought to be synthesized in order to estimate the importance of *each* nitrocyclopropyl moiety in **1** for its biological activity. To evaluate the applicability of 5-nitronorvaline in peptide syntheses, the acylated tripeptide **21a** was prepared (Scheme 4).[15,20] Towards that, the known dipeptide **19**[21] after removal of the Boc group, was coupled with Teoc-(NO_2)Nva-OH to give Teoc-(NO_2)Nva-Phe-Val-OMe in 59% yield. Subsequent deprotection of its *N*-terminus was followed by acylation with Chpca(MOM)-OH (**10**) to give, after removal of the MOM group, the tripeptide ester **21a** (73%).



Scheme 4. Syntheses of hormaomycin analogue **2b**, acyl tripeptides **21a**–**e** as well as *O*-MOM-protected hormaomycin *O*-MOM-**1** and its epimer *epi*-*O*-MOM-**1**. Reagents and conditions: a) anisole, TFA, 20 °C, 2 h. b) Teoc-(*S*)-(NO_2)Nva-OH, HATU, HOAt, DIEA, TMP, CH_2Cl_2, 20 °C, 15 h. c) TFA, 20 °C, 1 h. d) **10**, HATU, DIEA, TMP, CH_2Cl_2, 20 °C, 4–16 h or **10**, EDC, HOBt, DIEA, CH_2Cl_2, 0 → 20 °C, 4 h. e) $MgBr_2$·Et_2O, EtSH, CH_2Cl_2, 20 °C, 3–4 h. f) Teoc-AA-OH, EDC, HOAt, DIEA, TMP, CH_2Cl_2, 0 → 20 °C, 15 h·g) 5 M HCl in Et_2O, 20 °C, 1.5 h. h) 20% Et_2NH, MeCN, 40 min. i) HCl·H-Phe-Val-OMe, HATU, DIEA, CH_2Cl_2, 20 °C, 1.5 h. j) TFA·H-(NO_2)Nle-Phe-Val-OMe, HATU, DIEA, TMP, CH_2Cl_2, 20 °C, 6 h. k) **26**, PyAOP, HOAt, DIEA, TMP, CH_2Cl_2, 20 °C, 15 h. EDC = *N*-(3-dimethylamino)propyl-*N*′-ethylcarbodiimide hydrochloride, Chba-OH = 3-chloro-2-hydroxybenzoic acid, PyAOP = [(7-azabenzotriazol-1-yl)oxy]tris(pyrrolidino)-phosphonium hexafluorophosphate.

After removal of the MeZ group, the peptide lactone **9**[3b] was coupled with the *N*-Teoc-protected 5-nitronorvaline. After removal of the Teoc group, the resulting intermediate was acylated with Chpca(MOM)-OH (**10**). Finally, the desired hormaomycin analogue **2b** was obtained after removal of the MOM group in 60% overall yield over 5 steps. The HPLC retention time of this substance was the same as that of the presumed **2b**, observed in the feeding experiment with H-(NO_2)Nva-OH.

Feeding experiments with both enantiomerically pure (*S*)-**14b** and racemic 6-nitronorleucine *rac*-**14b** were successful in giving, according to HPLC and ESI-MS spectra of the crude extract, a mixture of hormaomycin (**1**) and the three analogues **3a**–**c** (Table 1). In this case, the isolation of the individual components was complicated because of the

very similar retention times. Repeated preparative HPLC eventually furnished the analogue **3a** in pure form, as well as a mixture of **3b** and **3c** enriched with the former (ca. 5:1). Furthermore, it was impossible to separate the analogue **3c** from hormaomycin (**1**) itself. On the one side, 6-nitronorleucine is accepted as a substrate that leads to the three possible hormaomycin analogues **3a**–**c**, yet on the other side compounds **3a**–**c** were never observed in the culture broth unless **14b** was supplied as a precursor – these facts demonstrate that this amino acid cannot be an intermediate along the biosynthetic route to H-(3-Ncp)Ala-OH.[22]

Table 1. Influence of the configuration and side-chain structure of selected amino acid precursors on the production of hormaomycin analogues by *Streptomyces griseoflavus* (strain W-384).

Substance fed	Incorporation	Analogues produced
(*S*)-**14a**	+	**2a**–**c**
(*R*)-**14a**	–	–
(*S*)-**14b**	+	**3a**–**c**
rac-**14b**	+	**3a**–**c**
(*S*)-**17**	–	–
rac-**18**	–	–

Feeding experiments with 5-nitro-3-thianorvaline (**17**) and (2′-nitrophenyl)alanine (**18**) failed to produce any new hormaomycin analogues.[23] Apparently, these amino acids are no suitable substrates for the hormaomycin synthetase.

Shortly after the first identification of hormaomycin (**1**), it had been shown that upon removal of both, the chlorine and the *N*-hydroxy group from the Chpca unit of **1**, at least the antibiotic and morphogenic activity was completely lost.[24] In order to find out, whether the free hydroxy function of Chpca of hormaomycin (**1**) is crucial for its activity, the *O*-MOM-protected peptolide *O*-MOM-**1** and its epimer at C-2 of (3-Ncp)Ala in the side chain *epi*-*O*-MOM-**1** were prepared and tested. Towards this, the cyclic precursor **9**[3b] was deprotected and then coupled with the *N*-acylamino acid **25**[9] applying the PyAOP reagent.[25] This coupling step was accompanied by significant racemization at C-2 of the (*S*)-(3-Ncp)Ala moiety, thus *O*-MOM-**1** (26%) and *epi*-*O*-MOM-**1** (16%) were obtained by separation of the obtained mixture of epimers by preparative TLC (Scheme 4).[26]

All of the newly obtained hormaomycin analogues and some model compounds were tested for their antibiotic activity against hormaomycin-hypersensitive *Arthrobacter* species (Table 2, Table 3) as well as against other bacteria and *Candida albicans*.[2c]

The hormaomycin analogues **2a**–**c** and **3a**–**c** containing (NO_2)Nva and (NO_2)Nle, respectively, exhibited high antibiotic activity against *Arthrobacter* species, yet none of the analogues was more active than hormaomycin (**1**) itself. This indicates that the cyclopropyl groups in **1** are not essential for its antibacterial activity. Alkylation of the *N*-hydroxy group of the Chpca moiety caused almost complete loss of the antibiotic activity (*O*-MOM-**1** and *epi*-*O*-MOM-**1** vs. **1**) pointing out that the free hydroxy group of Chpca is indeed very important. Surprisingly, the *O*-MOM-protected

Table 2. Relative antibacterial activities of hormaomycin (**1**), alkylated hormaomycins *O*-MOM-**1** and *epi*-O-MOM-**1** as well as analogue **2b** and model compounds **20a** and **21d** in serial dilution plate diffusion tests against *Arthrobacter crystallopoites* (strain 20117) (%) (estimated relative to the activity of hormaomycin at $5{\cdot}10^{-2}$ mg per 9×0.5 mm plate) at 28 °C.

Compound (mg per plate)	$5{\cdot}10^{-2}$	$5{\cdot}10^{-3}$	$5{\cdot}10^{-4}$	$5{\cdot}10^{-5}$
Hormaomycin (**1**)	100	94	71	39
O-MOM-**1**	19	0	–	–
epi-*O*-MOM-**1**	13	0	–	–
2b	90	84	58	26
20a	39	23	2	0
21d	0	–	–	–

Table 3. Relative antibacterial activities (%) of hormaomycin (**1**), hormaomycin analogues and model peptides compared to that of penicillin G in serial dilution plate diffusion tests against *Arthrobacter oxidans* (strain 20119) (estimated relative to the activity of hormaomycin at $1.5{\cdot}10^{-2}$ mg per 6×0.65 mm plate) at 28 °C. Mixtures of **3b/3c** and **3c/1** were tested.

Compound (mg per plate)	$1.5{\cdot}10^{-2}$	$1.5{\cdot}10^{-3}$	$1.5{\cdot}10^{-4}$
Hormaomycin (**1**)	100	72	44
Penicillin G	78	0	0
2a	57	53	33
2c	87	83	57
3a	97	–	–
3b/3c (ca. 5:1)	94	–	–
3c/1 (1:1)	97	–	–
20b	9	≈ 0	0
20c	15	0	–
21a	47	27	13
21b	0	0	–
21c	21	0	–
21e	0	0	–

model acyl tripeptide ester **20a** exhibited a relatively high activity, noticeably higher than those of *O*-MOM-**1** and *epi*-*O*-MOM-**1**. This unexpected result triggered the syntheses and tests of several similar compounds **20a**–**c** and **21a**–**e** (Scheme 4).

The *N*-acylated tripeptide esters **20b** and **20c** were prepared in a similar manner as **20a** starting from HCl·H-Phe-Val-OMe in 78 and 62% yield over three steps, respectively. The removal of the *O*-MOM protecting group with $MgBr_2{\cdot}Et_2O$ and EtSH gave the compounds **21b** (75%) and **21c** (97%).

Compound **21d** was prepared in 21% yield by coupling HCl·H-Phe-Val-OMe with Chpca-Phe-OH obtained after *O*-FM deprotection of **23**. The latter was synthesized starting from the known Boc-Phe-OFM[27] which, after removal of the Boc group from its *N*-terminus, was first acylated with Chpca(MOM)-OH (**10**) and then *O*-MOM-deprotected to furnish the acylamino acid **23** in 82% yield over three steps. Finally, the HATU-mediated acylation of H-(NO_2)Nle-Phe-Val-OMe with Chba-OH (3-chloro-2-hydroxybenzoic acid) yielded **21e** in 45% yield.[28]

The corresponding biological assays indicated that all of the compounds **20b**–**c** and **21b**–**e** at best had rather low hormaomycin-like antibiotic activity. Removal of the *O*-MOM

group from **20a** does not improve the antibiotic activity (**20a** vs. **21a**, compared to *O*-MOM-**1** vs. **1**). A possible reason for the unexpectedly high activity of the acyltripeptides **20a** and **21a**, especially compared to the very low activity of (3-Ncp)Ala containing compounds **20b** and **21b**, might be a different mode of action compared to that of hormaomycin (**1**). The simple tripeptides **21d**–**e**, which show no biological activity at all, demonstrate the importance not only of Chpca but also of its combination with the neighboring amino acids in the model peptides for activity against the tested strains.

As an unexpected result, the hormaomycin analogue **2c** showed a remarkable in vitro antibiotic activity against *C. albicans*, which, with inhibition zones of 21 and 13 mm ($1.5 \cdot 10^{-2}$ and $1.5 \cdot 10^{-3}$ mg per 6×0.65 mm plate, respectively) in the serial dilution plate diffusion assay, was in the same range as that for the antimycotic agent nystatine (20 and 14 mm). This is particularly noteworthy, as hormaomycin (**1**) as well as all of the hormaomycin analogues described here and before,[3c,5] were totally inactive against yeasts.

Conclusion

In summary, these studies show that a replacement of the cyclopropyl units in the (3-Ncp)Ala moiety by one or two methylene groups is tolerated by the respective binding domains of the hormaomycin synthetase. Furthermore, 6-nitronorleucine cannot be an intermediate along the biosynthesis route to H-(3-Ncp)Ala-OH. Hormaomycin analogues with nitronorvaline or nitronorleucine instead of (3-Ncp)Ala residues exhibit a similar biological activity as hormaomycin (**1**) itself. However, in the case of an *O*-alkyl group on the chloro(hydroxy)pyrrolcarboxylic acid residues of **1**, the antibiotic activity is almost completely lost. The side chain of hormaomycin (**1**) alone is not responsible for its biological activity.

Experimental Section

General: ^{1}H NMR spectra: Bruker AM 250 (250 MHz), Varian Unity 300 (300 MHz), Varian Inova 600 (600 MHz). ^{1}H chemical shifts are reported in ppm relative to residual peaks of deuterated solvents 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 = quadruplet, quin = quintuplet, 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 Unity 300 (75.5 MHz) or Varian Inova 600 (125.7 MHz) instruments. ^{13}C chemical shifts are reported relative to peaks of solvents or tetramethylsilane. The following abbreviations are used: DEPT: + = primary or tertiary C (positive signal in DEPT), – = secondary C (negative signal in DEPT), C_{quat} = quaternary C (no signal in DEPT); APT: + = primary or tertiary C (positive signal in APT), – = secondary or quaternary C (negative signal in APT); whenever it was necessary and possible, HMBC (Heteronuclear Multiple Bond Connectivity) and/or HMQC (Heteronuclear Multiple Quantum Coherence) spectra were also measured. The signals marked with an asterisk could not be assigned unambiguously. 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; pre-selected ion peak matching at $R >> 10000$ to be within ±2 ppm of the exact masses. 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), preparative: A – Kromasil C18 (7 μm, 20 mm × 250 mm), B – Knauer Nucleosil-100 C18 (5 μm, 8 mm × 250 mm), C – Kromasil C18 (7 μm, 8 mm × 250 mm). Optical rotations: Perkin–Elmer 241 digital polarimeter, 1-dm cell; optical rotation values are given in 10^{-1} deg cm^2 g^{-1}; concentrations (*c*) are given in g/100 mL. Circular dichroism: Jasco J 500A. Molar ellipticities (Θ) are given in degree 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 ninhydrine (0.2% in ethanol), or Ehrlich's reagent (freshly prepared solution of 1 g of 4-dimethylaminobenzaldehyde in 25 mL of 36% HCl and 75 mL of 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 procedures by distillation with drying agents and were stored under argon. All other solvents were distilled before use. All reactions were carried out with magnetic stirring if not stated otherwise and, if air or moisture sensitive, substrates and/or reagents were handled in flame-dried glassware under argon or nitrogen. Organic extracts were dried with anhydrous $MgSO_4$. (2*S*,1′*S*,2′*R*)-[*N*-(2-trimethylsilyl)ethyloxycarbonyl]-(2′-nitrocyclopropyl)alanine (**4**),[3b] 2-(trimethylsilyl)ethyl *N*-succinimidyl carbonate,[29] 3-nitro-2-pyridinesulfenyl chloride,[30] the cyclic peptide lactone **9**,[3b] HOAt,[31] Chpca(MOM)-OH (**10**),[9] racemic (2-nitrophenyl)alanine (**18**),[16] Boc-Phe-Val-OMe (**19**),[21] Boc-Phe-OFM (**22**),[27] Chpca(MOM)-(3-Ncp)Ala-OFM (**24**),[9] PyAOP[31] were prepared as described previously elsewhere. Feeding experiments and biological assays were carried out as described elsewhere.[3a,22,32]

***N*$_\omega$-Z-Protected (2*S*,1′*S*,2′*R*)-3-(2′-Aminocyclopropyl)alanine (7):** (2*S*,1′*S*,2′*R*)-3-(2′-Nitrocyclopropyl)alanine (**6**, 0.150 g, 0.86 mmol) in anhydrous methanol (20 mL) was hydrogenated at ambient pressure of hydrogen over 10% Pd on charcoal (0.150 g) for 15 h. The mixture was then filtered through a pad of Celite®, and the filtrate concentrated under reduced pressure to give the crude diamino acid as a yellow oil, which was used directly for the next step without any purification. This material was dissolved in water (4 mL) and $CuSO_4$ (72 mg, 0.45 mmol) was added. After 1 h $NaHCO_3$ (0.145 g, 1.73 mmol) and then a solution of ZOSu (0.28 g, 1.12 mmol) in acetone (2 mL) were added to the resulting deep violet solution, and stirring was continued for an additional 1.5 h. The formed precipitate was then filtered off, washed with water (100 mL), Et_2O (100 mL) and dried. The resulting copper complex (0.265 g) was dissolved in boiling water (5 mL), and Trilon B (0.509 g, 1.367 mmol) was added. The reaction mixture was vigorously stirred for 10 min and then cooled in an ice bath. After 30 min the precipitate was filtered off, washed with ice-cold water (50 mL),

Et_2O (100 mL) and dried to give the first crop of **7** (0.112 g) as a colorless solid. The mother liquor was concentrated under reduced pressure to ca. 5 mL and cooled in an ice bath. The precipitate was filtered off and crystallized from methanol to give the second crop of **7** (12 mg, 52% overall yield over 2 steps). R_f = 0.19 (MeCN/H_2O/AcOH, 10:1:1); m.p. 212–216 °C. $[\alpha]_D^{20}$ = –14.47 (*c* = 0.32, 0.1 N HCl). ^{1}H NMR (250 MHz, CD_3OD): δ = 0.56–0.68 (m, 1 H, 3′-H_a), 0.78–0.98 (m, 2 H, 3′-H_b, 3-H_a), 1.06 (ddd, *J* = 10.5, 10.5, 10.5 Hz, 1 H, 3-H_b), 2.34 (ddd, *J* = 7.0, 3.5, 3.5 Hz, 1 H, 1′-H), 2.60 (d, *J* = 14.3 Hz, 1 H, 2′-H), 3.74 (d, *J* = 10.8 Hz, 1 H, 2-H), 5.20 (s, 2 H, Bzl-H), 7.28–7.44 (m, 5 H, Ar-H) ppm. ^{13}C NMR (62.9 MHz, DCl in D_2O): δ = 11.5 (–, C-3′), 17.3 (+, C-1′), 29.1 (+, C-2′), 32.4 (–, C-3), 54.2 (+, C-2), 68.4 (–, Bzl-C), 128.3, 129.1, 129.4 (+, Ar-C), 136.8 (C_{quat}, Ar-C), 160.6 (C_{quat}, NCO_2), 172.2 (C_{quat}, C-1). IR (KBr): $\tilde{\nu}$ = 3730–2700 cm^{-1}, 3341, 3035, 2949, 1696, 1599, 1529, 1276 ppm. MS (ESI): positive, *m*/*z* = 323 (8) [M – H^+ + 2Na^+], 301 (80) [M + Na^+], 279 (3) [M + H^+]; negative, *m*/*z* = 277 (100) [M – H^+]. $C_{14}H_{18}N_2O_4$ (278.3): calcd. C 60.42, H 6.52, N 10.07; found C 60.26, H 6.32, N 9.89.

Deprotection of the *N*-MeZ-Protected Cyclic Peptide Lactone 9: The *N*-MeZ-protected cyclic peptide lactone (10.0 mg, 10.1 μmol) was deprotected by treatment with 10% anisole in TFA (1.1 mL) in the dark at ambient temperature for 2 h. All volatiles were removed under reduced pressure (0.05 Torr) at 20 °C. The residues were triturated with hexane (6 × 5 mL) and dried to give the deprotected material as a trifluoroacetate, which was directly used in the next condensation step.

Peptide Condensation Step for the Preparation of Hormaomycin Analogues 2b and 11. General Procedure (GP 1): Peptide lactone **9** (10 μmol) was deprotected as described above, taken up with anhydrous CH_2Cl_2 (5 mL), the respective protected amino acid (19 μmol), HATU (19 μmol) and HOAt (19 μmol) were added, and the reaction mixture was cooled to 4 °C. After this, a solution of DIEA (11 μmol) and TMP (57 μmol) in CH_2Cl_2 (2 mL) were 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. Then the reaction mixture was diluted with Et_2O (30 mL), and the mixture washed with 1 M $KHSO_4$ (3 × 5 mL), water (2 × 5 mL), saturated 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 preparative TLC and/or recrystallization to give the respective intermediates.

Deprotection of *O*-MOM-Protected Hormaomycin Analogues *O*-MOM-2b and 11 as Well as Model Acyl Tripeptides 21a–c and Acyl Amino Ester 23. General Procedure (GP 2): The respective *O*-MOM-protected compound (15 μmol) was deprotected by treatment with $MgBr_2$·Et_2O (0.30 mmol) and EtSH (0.10 mmol) in CH_2Cl_2 or Et_2O (10 mL) at ambient temperature for 3 h. The mixture was taken up with Et_2O (40 mL) and washed with 1 M $KHSO_4$ (3 × 10 mL), water (4 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residue was crystallized from CH_2Cl_2/pentane and, if necessary, further purified by column chromatography or preparative TLC.

Attempted Deprotection of 11: A solution of the protected depsipeptide (6.0 mg, 4.62 μmol) in CH_2Cl_2 (4 mL) was treated with $MgBr_2$·Et_2O (48 mg, 18.6 μmol) and EtSH (11.7 mg, 14 μL, 18.9 μmol) according to GP 2 for 4 h. The crude product after the usual aqueous work-up (GP, 2) was dried, dissolved in CH_2Cl_2 (0.5 mL) and treated with 0.1 M PPh_3 in CH_2Cl_2 (0.4 mL) and 0.1 M Py·HCl in CH_2Cl_2 (0.4 mL) for 10 min. The mixture, containing (according to TLC) two Ehrlich reagent active compounds, was then placed on the top of a column with Sephadex LH-20. Elution with CH_2Cl_2, then CH_2Cl_2/MeOH, 10:1, then CH_2Cl_2/MeOH, 3:1, CH_2Cl_2/MeOH, 1:1 and finally with pure MeOH did not give the desired product.

Preparation of *N*,*O*-Protected ω-Nitroamino Acids 13a,b. General Procedure (GP 3): To a solution of the N_α-Boc-N_ω-Z-protected diamino acid (4.09 mmol) in DMA (31 mL) were added *tert*-butyl bromide (197 mmol), K_2CO_3 (106.4 mmol) and benzyltriethylammonium chloride (4.09 mmol), and the mixture was vigorously stirred at 55–60 °C for 24 h. The reaction mixture was then poured into water (0.7 L), and the resulting emulsion was extracted with Et_2O (2 × 200 mL). The organic layer was washed with water (14 × 50 mL), brine (2 × 50 mL), dried, filtered and concentrated to give the respective *tert*-butyl ester as a colorless oil, which was directly used for the next step without any further characterization.

The respective *tert*-butyl ester (3.86 mmol) in EtOAc (15 mL) was hydrogenated over 10% Pd on charcoal (0.28 g) under ambient pressure of hydrogen for 2–3 h. The mixture was then filtered and concentrated under reduced pressure to give the crude monoprotected diamino ester which was immediately used without any purification for the next step. This material was dissolved in 1,2-dichloroethane (50 mL), and the solution added to a vigorously stirred solution of MCPBA (90% purity, 42.86 mmol) in 1,2-dichloroethane (200 mL) under intensive reflux for 2 min. Stirring under reflux was continued for an additional 20 min, and the reaction flask was then placed in an ice-water bath. After 15 min the reaction mixture was concentrated under reduced pressure to ca. 120 mL, the residue diluted with Et_2O (400 mL), the solution washed with saturated aqueous Na_2SO_3 (2 × 100 mL), saturated aqueous $NaHCO_3$ (3 × 100 mL), water (2 × 100 mL), brine (2 × 50 mL), dried, filtered and concentrated under reduced pressure. The residual brown oil was purified by column chromatography to give the desired products as pale yellow oils.

Boc-(*S*)-(NO_2)Nva-O*t*Bu [(*S*)-13a]: Boc-(*S*)-Orn(*Z*)-OH [(*S*)-**12a**, 1.50 g, 4.09 mmol] was esterified according to GP 3 to give Boc-(*S*)-Orn(*Z*)-O*t*Bu (1.63 g, 94%, R_f = 0.21, EtOAc/hexane, 1:4), which was further hydrogenated over 10% Pd on charcoal (0.28 g) according to GP 3 for 3 h to give the crude monoprotected diamino ester (R_f = 0.20 $CHCl_3$/MeOH, 10:1) as a colorless oil. The latter was oxidized according to GP3 using MCPBA (7.40 g, 90% purity, 38.6 mmol). The crude product was purified by column chromatography (EtOAc/hexane, 1:6) to give (*S*)-**13a** (0.73 g, 55% over three steps). R_f = 0.36, EtOAc/hexane, 1:6. $[\alpha]_D^{20}$ = 12.0 (*c* = 0.73, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.44 [s, 9 H, $C(CH_3)_3$], 1.46 [s, 9 H, $C(CH_3)_3$], 1.63–1.80 (m, 1 H, 4-H_a), 1.80–1.95 (m, 1 H, 4-H_b), 1.95–2.05 (m, 2 H, 3-H), 4.10–4.28 (m, 1 H, 2-H), 4.43 (t, *J* = 6.6 Hz, 2 H, 5-H), 5.12 (d, *J* = 7.5 Hz, 1 H, NH) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 23.0 (–, C-4), 27.8 [+, $C(CH_3)_3$], 28.1 [+, $C(CH_3)_3$], 29.6 (–, C-3), 52.9 (+, C-2), 74.8 (–, C-5), 79.8 [C_{quat}, *C* $(CH_3)_3$], 82.4 [C_{quat}, *C* $(CH_3)_3$], 155.3 (C_{quat}, NCO_2), 170.9 (C_{quat}, C-1) ppm. IR (film): $\tilde{\nu}$ = 3443 cm^{-1}, 3008, 2980, 2868, 1743, 1556, 1499, 1456, 1393, 1369, 1253, 1149, 1055. MS (ESI): positive mode, *m*/*z* = 341 (100) [M + Na^+]. $C_{14}H_{26}N_2O_6$ (318.4): calcd. C 52.82, H 8.23, N 8.80; found C 52.56, H 8.57, N 8.84.

Boc-(*R*)-(NO_2)Nva-O*t*Bu [(*R*)-13a]: Boc-(*R*)-Orn(*Z*)-OH [(*S*)-**12a**, 4.35 g, 11.87 mmol] was esterified according to GP 3 to give Boc-(*R*)-Orn(*Z*)-O*t*Bu (4.70 g, 94%, R_f = 0.35, EtOAc/hexane, 1:3), which was further hydrogenated in EtOAc (40 mL) over 10% Pd on charcoal (0.3 g) according to GP 3 for 3 h to give the crude monoprotected diamino ester as a faint yellow oil. The latter was oxidized according to GP 3 using MCPBA (21.30 g, 90% purity, 111.1 mmol). The crude product was purified by column

chromatography (EtOAc/hexane, 1:6) to give (*R*)-**13a** (1.95 g, 52% over three steps). $[\alpha]_D^{20}$ = –11.8 (*c* = 0.68, $CHCl_3$).

(*S*)-5-Nitronorvaline Hydrochloride [(*S*)-14a·HCl]: Compound (*S*)-**13a** (1.20 g, 3.77 mmol) was deprotected with TFA (5 mL) in the dark at ambient temperature for 1 h. All volatiles were then removed under reduced pressure, and the residue was taken up with toluene (2 × 20 mL) which was then distilled off to remove the last traces of TFA. To transform the resulting trifluoroacetate into hydrochloride it was taken up with 2 M HCl in EtOAc (3 × 20 mL) which was then distilled off. The resulting oil was triturated with acetone to give a light brown precipitate. Et_2O was then added to complete precipitation. The crude product was filtered off and washed with acetone/Et_2O, 1:1 (200 mL) to give (*S*)-**14a**·HCl (0.420 g, 56%) as an off-white solid. M.p. 132–133 °C (dec.). $[\alpha]_D^{20}$ = 16.8 (*c* = 0.22, 0.1 M HCl) [lit. (for free base):[14b] $[\alpha]_D^{20}$ = 22.8 (*c* = 0.56, diluted. HCl)]. ^{1}H NMR (300 MHz, D_2O): δ = 1.89–2.24 (m, 4 H, 3-H, 4-H), 4.06 (t, *J* = 5.0 Hz, 1 H, 2-H), 4.56 (t, *J* = 5.4 Hz, 2 H, 5-H) ppm. ^{13}C NMR (50.3 MHz, D_2O): δ = 22.4 (–, C-4), 26.8 (–, C-3), 52.5 (+, C-2), 74.5 (–, C-5), 171.7 (C_{quat}, C-1) ppm. IR (KBr): $\tilde{\nu}$ = 3750–1800 cm^{-1}, 2933, 1735, 1552, 1500, 1379, 1349, 1244. MS (ESI): positive mode, *m*/*z* = 229 (5) [M – 2H$^+$ + 3Na$^+$], 207 (8) [M – H$^+$ + 2Na$^+$], 185 (12) [M + Na$^+$]; negative mode, *m*/*z* = 345 (100) [2M – 2H$^+$ + Na$^+$]. $C_5H_{11}ClN_2O_4$ (198.6): calcd. C 30.24, H 5.58, N 14.10; found C 30.50, H 5.30, N 13.97.

(*R*)-5-Nitronorvaline Hydrochloride [(*R*)-14a·HCl]: Compound (*R*)-**13a** (1.95 g, 6.12 mmol) was dissolved in the ice-cold saturated solution (ca. 4–5 M) of HCl in EtOAc (50 mL). After 30 min the cooling bath was removed, and the reaction mixture was allowed to stand at ambient temperature for an additional 90 min. All volatiles were then removed under reduced pressure, and the residue was dissolved with MeOH (ca. 5 mL). EtOAc (ca. 60 mL) was then added and after standing in the refrigerator (4 °C) for 15 h the precipitate was filtered off to give (*R*)-**14a**·HCl (0.73 g, 60%) as a colorless solid. The mother liquor was concentrated, and the residue was recrystallized from MeOH/EtOAc to give a second crop of (*R*)-**14a**·HCl (0.38 g, 91% overall yield). $[\alpha]_D^{20}$ = –12.1 (*c* = 0.52, H_2O).

(*S*)-6-Nitronorleucine Hydrochloride [(*S*)-14b·HCl]: Compound (*S*)-**13b** (2.22 g, 6.68 mmol) was deprotected with TFA (11 mL) in the dark at ambient temperature for 1 h. All volatiles were then removed under reduced pressure, and the residue was taken up with toluene (2 × 15 mL) which was then distilled off to remove the last traces of TFA. To transform the resulting trifluoroacetate into a hydrochloride it was taken up with 1 M HCl (30 mL) which was then distilled off. The resulting oil was triturated with acetone to give a light brown precipitate. The residue was then dissolved in methanol (15 mL), the resulting solution was filtered through Celite®, and the filtrate concentrated to ca. 2–3 mL. EtOAc (50 mL) was added, the precipitate was filtered off and washed with EtOAc (100 mL) to give (*S*)-**14b**·HCl (1.19 g, 84%) as a colorless solid. R_f = 0.09, MeCN/H_2O/AcOH, 10:1:1; m.p. 180–181 °C (dec.). $[\alpha]_D^{20}$ = 10.3 (*c* = 0.95, H_2O). ^{1}H NMR (250 MHz, D_2O): δ = 1.40–1.61 (m, 2 H, 4-H), 1.94 (dd, *J* = 6.8, 6.8 Hz, 1 H, 3-H$_a$), 1.97–2.12 (m, 3 H, 3-H$_b$, 5-H), 4.06 (t, *J* = 6.1 Hz, 1 H, 2-H), 4.57 (t, *J* = 6.6 Hz, 2 H, 6-H) ppm. ^{13}C NMR (62.9 MHz, D_2O): δ = 21.8 (–, C-4), 26.7 (–, C-3), 29.7 (–, C-5), 53.4 (+, C-2), 75.7 (–, C-6), 172.8 (C_{quat}, C-1) ppm. IR (KBr): $\tilde{\nu}$ = 3432 cm^{-1}, 3250–2750, 1747, 1556, 1518, 1218, 1120. MS (ESI): positive mode, *m*/*z* = 221 (15) [M –H$^+$ + 2Na$^+$], 199 (52) [M + Na$^+$]. $C_6H_{13}ClN_2O_4$ (212.6): calcd. C 33.89, H 6.16, N 13.17; found C 34.15, H 5.94, N 13.18.

***rac*-6-Nitronorleucine Hydrochloride [*rac*-14b·HCl]:** Compound *rac*-**13b** (2.50 g, 7.52 mmol) was dissolved in an ice-cold saturated solution (ca. 4–5 M) of HCl in EtOAc (50 mL). After 30 min the cooling bath was removed, and the reaction mixture was allowed to stand at ambient temperature for an additional 90 min. All volatiles were then removed under reduced pressure, and the residue was recrystallized from MeOH/EtOAc to give *rac*-**14b**·HCl (1.50 g, 94%) as a colorless solid.

Methyl *N*-Boc-(*S*)-5-nitro-4-thianorleucinate (16): SO_2Cl_2 (0.70 mL, 1.17 g, 8.65 mmol) was added to a solution of Boc-Cys-OMe (**15**, 2.0 g, 8.50 mmol) in CH_2Cl_2 (80 mL) at –40 °C within 10 min and the reaction mixture was stirred at this temperature for an additional 3 h. Then the reaction flask was connected to a vacuum pump, the cooling bath was removed and volatiles were evaporated until the temperature of the reaction flask reached 0 °C (the ice on walls of the flask began to thaw) to leave the crude sulfenyl chloride. The reaction flask was immediately cooled to –78 °C, and the oily orange residue was dissolved in cold THF (60 mL). Then a cold (ca. –10 to –20 °C) jelly-like suspension of potassium methylnitronate [prepared from nitromethane (0.92 mL, 1.04 g, 17.04 mmol) and *t*BuOK (1.34 g, 11.94 mmol)] in THF (50 mL) was added through a Teflon tube. The transparent orange solution immediately became turbid. After 2 h the cooling bath was removed, and aqueous AcOH (4 mL in 20 mL H_2O) was added to the reaction mixture when its temperature reached ca. –10–0 °C. All volatiles were removed under reduced pressure, the residue was diluted with Et_2O (70 mL), the solution washed with H_2O (4 × 20 mL), 1 M $KHSO_4$ (3 × 20 mL), saturated aqueous $NaHCO_3$ (20 mL), H_2O (2 × 20 mL), brine (2 × 20 mL), dried, filtered and concentrated under reduced pressure. The residual orange oil was purified by column chromatography (EtOAc/hexane, 1:3) to give **16** (1.70 g, 68%) as a faint yellow oil with a distinct odor, which gradually solidified to give a colorless solid. R_f = 0.21, EtOAc/hexane, 1:3; m.p. 59–61 °C. $[\alpha]_D^{20}$ = –2.2 (*c* = 0.60, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.451, 1.454 [2 × s, 9 H, C(CH$_3$)$_3$], 3.15 (dd, *J* = 5.0, 5.0 Hz, 1 H, 3-H$_a$), 3.50 (dd, *J* = 5.0, 5.0 Hz, 1 H, 3-H$_b$), 3.789, 3.793 (2 × s, 3 H, OCH$_3$), 4.58–4.66 (m, 1 H, 2-H), 5.21 (d, *J* = 13.8 Hz, 1 H, 5-H$_a$), 5.25–5.40 (br., 1 H, NH), 5.33 (d, *J* = 13.8 Hz, 1 H, 5-H$_b$) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 28.1 [+, C(*C*H$_3$)$_3$], 35.3 (–, C-3), 52.8 (+, C-2), 52.9 (+, OCH$_3$), 76.2 (–, C-5), 80.5 [C_{quat}, *C*(CH$_3$)$_3$], 155.0 (C_{quat}, NCO$_2$), 170.7 (C_{quat}, C-1) ppm. IR (KBr): $\tilde{\nu}$ = 3750–2850 cm^{-1}, 2400–1850, 1734, 1629, 1558, 1487, 1404. MS (ESI): positive mode, *m*/*z* = 317 (30) [M + Na$^+$], 295 (100) [M + H$^+$]. $C_{10}H_{18}N_2O_6S$ (294.3): calcd. C 40.81, H 6.16, N 9.52; found C 40.79, H 5.93, N 9.65.

(*S*)-5-Nitro-4-thianorleucine Hydrochloride (17·HCl): Degassed NaOH (0.25 N, 43.4 mL, 10.85 mmol) was added to an ice-cold degassed solution of **16** (1.24 g, 4.21 mmol) in THF (10 mL) within 5 min (partial precipitation of the starting material was observed), and the mixture was stirred at the same temperature. More THF was then added (2, 3 and 5 mL after 5, 10 and 20 min, respectively). After 80 min Et_2O (50 mL), NaCl (4–5 g) and then dropwise 2 N H_2SO_4 (5.6 mL, 11.2 mmol) were added to the resulting thick colorless suspension. The organic layer was separated, washed with 1 N $KHSO_4$ (2 × 15 mL), brine (2 × 15 mL), dried, filtered and concentrated under reduced pressure. The residual yellow oil was immediately treated with 4 M HCl in EtOAc (25 mL) at ambient temperature. After 20 min all volatiles were removed under reduced pressure and the residual solid was triturated with EtOAc, and the mixture cooled to 4 °C. After 30 min the precipitate was filtered off and washed with EtOAc to give **17**·HCl (0.74 g, 81% over two steps) as a colorless solid. This substance has only limited stability in DMSO and water solution. Because of rapid H/D exchange it was impossible to observe the signal of C-5 in the ^{13}C NMR spectrum of **17**·HCl measured in D_2O. R_f = 0.85, MeCN/H_2O/AcOH, 10:1:1; m.p. 128–129 °C (dec.). $[\alpha]_D^{20}$ = 9.0 (*c* = 0.49, H_2O). ^{1}H

NMR (300 MHz, D_2O): δ = 3.38 (dd, J = 6.3, 6.3 Hz, 1 H, 3-H_a), 3.53 (dd, J = 3.8, 3.8 Hz, 1 H, 3-H_b), 4.33 (dd, J = 6.3, 3.8 Hz, 1 H, 2-H), 5.64 (s, 2 H, 5-H) ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 32.0 (–, C-3), 51.8 (+, C-2), 76.7 (–, C-5), 169.1 (C_{quat}, C-1) ppm. MS (ESI): positive mode, m/z = 203 (40) [M + Na$^+$], 181 (100) [M + H$^+$]; negative mode, m/z = 359 (100) [2M – H$^+$], 217/215 (23/68) [M + Cl$^-$], 179 (28) [M – H$^+$]. $C_4H_9ClN_2O_4S$ (216.6): calcd. C 22.18, H 4.19, N 12.93; found C 22.13, H 4.06, N 13.04.

Teoc-(*S*)-(NO$_2$)Nle-OH: A solution of TeocOSu (183 mg, 0.71 mmol) in acetone (7 mL) was added to a vigorously stirred solution of (*S*)-**14b**·HCl (150 mg, 0.71 mmol) and $NaHCO_3$ (0.218 g, 2.60 mmol) in water (7 mL) (if an emulsion formed acetone and/or water were added to obtain a homogeneous solution), and the mixture was stirred for an additional 15 h. Acetone was removed under reduced pressure, and the pH value of the residual water solution was adjusted to 2–3 with 1 M $KHSO_4$. The resulting emulsion was extracted with Et_2O (50 mL), and the ethereal layer was washed with 1 M $KHSO_4$ (3 × 10 mL), water (10 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography [EtOAc/hexane, 1:2 (2% AcOH)]. The resulting colorless oil was taken up with hexane (3 × 15 mL) which was then distilled off under reduced pressure to remove the last traces of AcOH. The partially solidified residue was triturated with hexane and filtered off to give the title compound (160 mg, 71%) as a colorless solid. R_f = 0.37 EtOAc/hexane, 1:2 (2% AcOH) (staining with ninhydrin); m.p. 71–72 °C. $[\alpha]_D^{20}$ = 23.7 (c = 0.675, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.04 [s, 9 H, Si(CH$_3$)$_3$], 1.00 (t, J = 7.5 Hz, 2 H, 2-H, *Teoc*), 1.43–1.61 (m, 2 H, 4-H), 1.68–1.85 (m, 1 H, 3-H_a), 1.85–2.18 (m, 3 H, 3-H_b, 5-H), 4.18 (t, J = 8.0 Hz, 2 H, 1-H, *Teoc*), 4.40 (t, J = 8.8 Hz, 2 H, 6-H), 6.68–6.82 (d, J = 7.8 Hz, br, 1 H, NH) 6.50–7.80 (br., 1 H, CO_2H) ppm; the signal of 1-H of *Teoc* and 6-H overlapped the signal of 2-H. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = –1.6 [+, Si(CH$_3$)$_3$], 17.6 (–, C-2, *Teoc*), 21.9 (–, C-4), 26.6 (–, C-3), 31.6 (–, C-5), 53.05, 53.7 (+, C-2), 63.8, 64.8 (–, C-1, *Teoc*), 75.1 (–, C-6), 156.5, 157.7 (C_{quat}, NCO_2), 175.8, 176.6 (C_{quat}, C-1) ppm. IR (KBr): $\tilde{\nu}$ = 3550–2800 ·cm^{-1}, 3427, 2959, 2908, 2880, 1750, 1726, 1664, 1553, 1410, 1252, 1202, 1068. MS (ESI): negative mode, m/z = 661 (100) [2M + Na$^+$ – 2H$^+$], 319 (10) [M – H$^+$]. $C_{12}H_{24}N_2O_6Si$ (320.4): calcd. C 44.98, H 7.55, N 8.74; found C 45.16, H 7.29, N 8.66.

***O*-MOM-Protected Hormaomycin *O*-MOM-1 and its Epimer *epi-O*-MOM-1:** The ester **25** (5.4 mg, 10.0 μmol) was deprotected with 20% Et_2N in MeCN (1 mL) for 40 min. The mixture was then concentrated under reduced pressure, the residue was washed with hexane, taken up with Et_2O (30 mL), washed with 1 M $NaHSO_4$ (3 × 5 mL), water (3 × 5 mL), brine (2 × 5 mL). The organic layer was dried, filtered and concentrated under reduced pressure. The resulting crude *N*-acyl amino acid **26** [R_f = 0.42, EtOAc/hexane, 1:2 (2% AcOH)] was dried at 0.01 Torr for 1 h and then introduced in the coupling reaction with the deprotected depsipeptide obtained after treatment of the *N*-MeZ-protected cyclodepsipeptide **9** (8.0 mg, 8.18 μmol) with 10% anisole in TFA (1 mL) as described before using PyAOP (8.4 mg, 16.1 μmol), HOAt (2.2 mg, 16.2 μmol), DIEA (1.3 mg, 10.1 μmol) and TMP (3.8 mg, 31.4 μmol) in CH_2Cl_2 (1 mL) according to GP 1 for 15 h. The mixture was then diluted with Et_2O and subjected to the usual aqueous work-up (GP, 1). TLC analysis showed the presence of the two main reaction products which were separated by preparative TLC (100 × 200 mm, acetone/hexane, 1:3, threefold development). Each fraction was additionally purified by preparative TLC (40 × 80 mm, acetone/hexane, 1:3, threefold development) to give *O*-MOM-**1** (2.5 mg, 26%) as a colorless glass and *epi-O*-MOM-**1** (1.5 mg, 16%) as a colorless glass.

***O*-MOM-1:** R_f = 0.11, acetone/hexane, 1:3. ^{1}H NMR (600 MHz, $CDCl_3$): δ = –0.26 [ddd, J = 6.0, 6.0, 6.0 Hz, 1 H, 3′-H_a, (*3-Ncp*)*Ala*], 0.07–0.17 [m, 1 H, 3-H_a, (*3-Ncp*)*Ala*], 0.52–0.61 [m, 1 H, 1′-H, (3-*Ncp*)*Ala*], 0.68–0.79 [m, 1 H, 3-H_b, (*3-Ncp*)*Ala*], 0.88 (t, J = 7.2 Hz, 3 H, 5-H, *Ile*), 1.02 (d, J = 6.6 Hz, 3 H, 1′-H, *Ile*), 1.05 [ddd, J = 6.0, 6.0, 6.0 Hz, 1 H, 3′-H_b, (*3-Ncp*)*Ala*], 1.10–1.14 [m, 1 H, 3′-H_a, (*3-Ncp*)*Ala*], 1.19–1.27 [m, 2 H, 4-H_a, *Ile*, 3′-H_b, (*3-Ncp*)*Ala*], 1.29 [d, J = 6.6 Hz, 3 H, 4-H, (*βMe*)*Phe*], 1.39 [d, J = 6.6 Hz, 3 H, 4-H, (*βMe*)*Phe*], 1.50–1.56 (m, 1 H, 4-H_b, *Ile*), 1.59 (d, J = 7.2 Hz, 3 H, 4-H, *a-Thr*), 1.64–1.70 [m, 1 H, 3-H_a, (*3-Ncp*)*Ala*], 1.69 [dd, J = 7.2, 1.8 Hz, 3 H, 3′-H, (*4-Pe*)-*Pro*], 1.75–1.85 [m, 2 H, 3-H, *Ile*, 3-H_a, (*4-Pe*)*Pro*], 1.90–1.95 [m, 1 H, 3-H_b, (*3-Ncp*)*Ala*], 1.95–2.00 [m, 1 H, 1′-H, (*3-Ncp*)*Ala*], 2.40 [ddd, J = 12.0, 6.0, 6.0 Hz, 1 H, 3-H_b, (*4-Pe*)-*Pro*], 3.05 [dq, J = 10.8, 6.6 Hz, 1 H, 3-H, (*βMe*)*Phe*], 3.11–3.17 [m, 1 H, 2′-H, (*3-Ncp*)*Ala*], 3.24–3.32 [m, 2 H, 4-H, 5-H_a, (*4-Pe*)-*Pro*], 3.71 [dq, J = 4.2, 7.2 Hz, 1 H, 3-H, (*βMe*)*Phe*], 3.75 (s, 3 H, OMe), 3.90 (dd, J = 6.6, 5.4 Hz, 1 H, 2-H), 3.93–3.98 [m, 1 H, 5-H_b, (*4-Pe*)-*Pro*], 4.09 [ddd, J = 6.6, 3.0, 3.0 Hz, 1 H, 2′-H, (*3-Ncp*)*Ala*], 4.26 (dd, J = 13.2, 13.2 Hz, 1 H, 2-H), 4.30 (dd, J = 11.4, 6.0 Hz, 1 H, 2-H), 4.61 (dd, J = 8.4, 4.8 Hz, 1 H, 2-H), 4.64–4.70 (m, 2 H, 2-H), 5.10–5.17 [m, 1 H, 2-H, (*3-Ncp*)*Ala*], 5.24–5.29 [m, 1 H, 1′-H, (*4-Pe*)-*Pro*], 5.39 (dq, J = 1.8, 7.2 Hz, 1 H, 3-H, *a-Thr*), 5.47 (d, J = 6.0 Hz, 1 H, H_a, OCH_2O), 5.50 (d, J = 6.0 Hz, 1 H, H_b, OCH_2O), 5.64 [dq, J = 10.5, 7.2 Hz, 1 H, 2′-H, (*4-Pe*)-*Pro*], 5.75–5.81 (br., 2 H, NH), 6.17 (d, J = 4.5 Hz, 1 H, 4-H, Chpca), 6.70 (d, J = 9.0 Hz, 1 H, NH), 6.84 (d, J = 4.5 Hz, 1 H, 3-H, Chpca), 7.06 (dd, J = 7.5, 7.5 Hz, 1 H, Ar-H), 7.09 (d, J = 7.2 Hz, 2 H, Ar-H), 7.15 (dd, J = 7.5, 7.5 Hz, 3 H, Ar-H), 7.16 (d, J = 7.2 H, 1 H, NH), 7.22–7.31 (m, 5 H, Ar-H, NH), 7.38 (d, J = 9.6 Hz, 1 H, NH), 7.75 (d, J = 9.0 Hz, 1 H, NH), 8.79 (d, J = 9.0 Hz, 1 H, NH) ppm. ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 10.8 (+, C-5, *Ile*), 13.2 [+, C-4, (*βMe*)*Phe*], 13.3 [+, C-3′, (*4-Pe*)-*Pro*], 15.5 (+, C-1′, *Ile*), 17.2 [–, C-3′, (*3-Ncp*)*Ala*], 17.3 [–, C-3′, (*3-Ncp*)*Ala*], 17.5 (+, C-4, *a-Thr*), 18.3 [+, C-4, (*βMe*)*Phe*], 20.7 [+, C-1′, (*3-Ncp*)*Ala*], 21.3 [+, C-1′, (*3-Ncp*)*Ala*], 24.9 (–, C-4, *Ile*), 29.7 [–, C-3, (*3-Ncp*)*Ala*], 34.0 [–, C-3, (*3-Ncp*)*Ala*], 35.4 [–, C-3, (*4-Pe*)-*Pro*], 36.7 [+, C-4, (*4-Pe*)-*Pro*], 38.6 (+, C-3, *Ile*), 39.4 [+, C-3, (*βMe*)*Phe*], 42.7 [+, C-3, (*βMe*)*Phe*], 50.2 [+, C-2′, (*3-Ncp*)*Ala*], 51.5 (+, C-2), 52.9 [–, C-5, (*4-Pe*)-*Pro*], 54.7 (+, C-2), 54.9 (+, C-2), 58.5 [+, C-2′, (*3-Ncp*)*Ala*], 59.4 (+, OMe, C-2), 59.6 (+, C-2), 60.2 (+, C-2), 61.5 (+, C-2), 69.9 (+, C-3, *a-Thr*), 104.5 (–, OCH_2O), 106.1 (+, C-4, *Chpca*), 111.5 (+, C-3, *Chpca*), 119.8 (C_{quat}, C-2, *Chpca*), 121.5 (C_{quat}, C-5, *Chpca*), 126.9, 127.1, 127.5, 127.6, 128.5, 128.6 (+, Ar-C), 127.4 [+, C-1′, (*4-Pe*)-*Pro*], 128.5 [+, C-2′, (*4-Pe*)-*Pro*], 142.3 (2 × C_{quat}, Ar-C), 158.6 (C_{quat}, C-1, *Chpca*), 168.8, 169.9, 170.1, 170.2, 171.1, 171.3, 171.4 (C_{quat}, C-1) ppm. UV (MeOH): neutral: λ_{max} (ε) = 268 (1.8·10^4), 201 (7.5·10^5) nm; basic: 341 (1.4·10^3), 268 (1.5·10^4), 205 (9.1·10^4) nm; acidic: 341 (2.7·10^3), 268 (1.4·10^4)nm. CD (MeOH): λ_{max} (Θ) = 268.2 (2.71·10^4), 259.3 (2.40·10^4), 222.1 (–41.7·10^4) nm (c = 8.18·10^{-6} M). MS (ESI): positive, m/z (%) = 1196 (100) [M + Na$^+$]; negative, m/z (%) = 1171 (100) [M – H$^+$].

***epi-O*-MOM-1:** R_f = 0.09, acetone/hexane, 1:3. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.68 [ddd, J = 6.6, 6.6, 6.6 Hz, 1 H, 3′-H_a, (*3-Ncp*)*Ala*], 0.79–0.92 [m, 2 H, 1′-H, 3-H_a, (*3-Ncp*)*Ala*], 0.98 (t, J = 7.2 Hz, 3 H, 5-H, *Ile*), 1.01 (d, J = 6.6 Hz, 3 H, 1′-H, *Ile*), 1.15 [ddd, J = 6.6, 6.6, 6.6 Hz, 1 H, 3′-H_b, (*3-Ncp*)*Ala*], 1.19–1.32 [m, 6 H, 3′-H, (*3-Ncp*)*Ala*, 4-H, (*βMe*)*Phe*, 4-H_a, *Ile*], 1.34 [d, J = 7.2 Hz, 3 H, 4-H, (*βMe*)*Phe*], 1.46–1.62 [m, 3 H, 4-H_b, *Ile*, 3-H, (*3-Ncp*)*Ala*], 1.64–1.70 [m, 1 H, 3-H_a, (*3-Ncp*)*Ala*], 1.69 [dd, J = 7.2, 1.8 Hz, 3 H, 3′-H, (*4-Pe*)-*Pro*], 1.76 [ddd, J = 11.4, 11.4, 11.4 Hz,

3-H_a, (*4-Pe*)*Pro*], 1.90–1.99 (m, 1 H, 3-H, *Ile*), 2.05–2.13 [m, 1 H, 1′-H, (*3-Ncp*)*Ala*], 2.40 [ddd, J = 11.4, 6.0, 6.0 Hz, 1 H, 3-H_b, (*4-Pe*)-*Pro*], 3.13–3.23 [m, 1 H, 3-H, (*βMe*)*Phe*], 3.23–3.32 [m, 2 H, 4-H, 5-H_a, (*4-Pe*)-*Pro*], 3.62 (s, 3 H, OMe), 3.67–3.75 [m, 1 H, 3-H, (*βMe*)*Phe*], 3.85 [ddd, J = 6.6, 3.0, 3.0 Hz, 1 H, 2′-H, (*3-Ncp*)*Ala*], 3.96–4.01 (m, 1 H, 2-H), 4.05–4.09 [m, 1 H, 5-H_b, (*4-Pe*)-*Pro*], 4.13 [ddd, J = 6.6, 3.0, 3.0 Hz, 1 H, 2′-H, (*3-Ncp*)*Ala*], 4.13–4.21 (m, 1 H, 2-H), 4.56–4.72 (m, 3 H, 2-H), 4.61 (dd, J = 9.0, 4.5 Hz, 1 H, 2-H), 4.84 (dd, J = 9.0, 2.4 Hz, 1 H, 2-H), 5.20–5.35 [m, 2 H, 1′-H, (*4-Pe*)*Pro*, 3-H, *a-Thr*], 5.22 (d, J = 6.0 Hz, 1 H, H_a, OCH_2O), 5.28 (d, J = 6.0 Hz, 1 H, H_b, OCH_2O), 5.61 [dq, J = 10.5, 7.2 Hz, 1 H, 2′-H, (*4-Pe*)-*Pro*], 6.03 (d, J = 4.8 Hz, 1 H, 4-H, Chpca), 6.33–6.46 (br., 1 H, NH), 6.57–6.69 (br., 1 H, NH), 6.70 (d, J = 4.8 Hz, 1 H, 3-H, Chpca), 7.13–7.34 (m, 14 H, Ar-H, NH), 7.57–7.71 (br., 1 H, NH) ppm; the absorption of water masked the signal of 4-H of *a-Thr* residue. ^{13}C NMR (150.8 MHz, $CDCl_3$): δ = 10.2 (+, C-5, *Ile*), 13.4 [+, C-4, (*βMe*)*Phe*, C-3′, (*4-Pe*)-*Pro*], 14.7 (+, C-1′, *Ile*), 17.4 [–, C-3′, (*3-Ncp*)*Ala*], 17.9 [–, C-3′, (*3-Ncp*)*Ala*], 18.6 (+, C-4, *a-Thr*), 21.5 [+, C-4, (*βMe*)*Phe*], 24.4 [+, C-1′, (*3-Ncp*)*Ala*], 24.9 (–, C-4, *Ile*), 29.3 [+, C-1′, (*3-Ncp*)*Ala*], 29.7 [–, C-3, (*3-Ncp*)*Ala*], 30.9 [–, C-3, (*3-Ncp*)*Ala*], 35.6 [–, C-3, (*4-Pe*)-*Pro*], 36.6 [+, C-4, (*4-Pe*)-*Pro*], 37.5 (+,C-3, *Ile*), 39.0 [+, C-3, (*βMe*)*Phe*], 40.2 [+, C-3, (*βMe*)*Phe*], 52.3 (+, C-2), 52.9 [–, C-5, (*4-Pe*)-*Pro*], 53.2 (+, C-2), 54.4 (+, C-2), 55.8 (+, C-2), 59.0 [+, C-2′, (*3-Ncp*)*Ala*], 59.3 [+, C-2′, (*3-Ncp*)*Ala*], 59.49 (+, OMe, C-2), 59.53 (+, C-2), 60.7 (+, C-2), 72.2 (+, C-3, *a-Thr*), 104.2 (–, OCH_2O), 104.9 (+, C-4, *Chpca*), 110.9 (+, C-3, *Chpca*), 118.7 (C_{quat}, C-2, *Chpca*), 122.1 (C_{quat}, C-5, *Chpca*), 126.8, 127.1, 127.3, 128.6, 128.7, 128.8 (+, Ar-C), 127.7 [+, C-1′, (*4-Pe*)-*Pro*], 128.1 [+, C-2′, (*4-Pe*)-*Pro*], 141.6 (2 × C_{quat}, Ar-C), 157.9 (C_{quat}, C-1, *Chpca*), 169.8, 170.0, 170.2, 170.9 (× 2), 171.2, 171.3 (C_{quat}, C-1) ppm. UV (MeOH): neutral: λ_{max} (ε) = 261 ($1.7 \cdot 10^4$), 201 ($6.6 \cdot 10^5$) nm; basic: 341 ($1.4 \cdot 10^3$), 267 ($1.4 \cdot 10^4$), 226 ($2.1 \cdot 10^4$), 204 ($7.6 \cdot 10^4$) nm; acidic: 341 ($2.7 \cdot 10^3$), 269 ($2.0 \cdot 10^4$) nm. CD (MeOH): λ_{max} (Θ) = 274 ($2.08 \cdot 10^4$), 221.6 ($-4.99 \cdot 10^4$) nm (c = $6.48 \cdot 10^{-6}$ M). MS (ESI): positive, m/z (%) = 1196 (100) [M + Na^+]; negative, m/z (%) = 1207 (40) [M + Cl^-], 1171 (100) [M – H^+].

Supporting Information (for details see the footnote on the first page of this article): Full experimental procedures and spectroscopic data for **2a**–**c**, **3a**–**c**, Teoc-(2*S*)-(3-Acp)Ala(Z)-OH, **8**, **11**, (*S*)-**13b**, *rac*-**13b**, Teoc-(*S*)-(NO_2)Nva-OH, **20a**–**c**, **21a**–**e**, Chpca-(MOM)-Phe-OFm, **23**, Chpca-Phe-OH, and description of the feeding experiments with **14a**,**b**.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 416, Projects A3 and B5) and the Fonds der Chemischen Industrie. The authors are indebted to Mrs. M. Klingebiel and Mr. H.-P. Kroll (Göttingen) for excellent technical assistance and to Dr. B. Knieriem (Göttingen) for his careful proofreading of the final manuscript.

[1] For the isolation of a cyclosporine analogue which lacks immunosuppressive, but has potential *anti*-HIV1-activity see: A. Billich, F. Hammerschmid, P. Reichl, R. Wender, G. Zenke, V. Quesniaux, B. Rosenwirth, *J. Virol.* **1995**, *69*, 2451–2461.

[2] a) N. Andres, H. Wolf, H. Zähner, E. Rössner, A. Zeeck, W. A. König, V. Sinnwell, *Helv. Chim. Acta* **1989**, *72*, 426–437; b) E. Rössner, A. Zeeck, W. A. König, *Angew. Chem.* **1990**, *102*, 84–85; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 64–65; c) N. Andres, H. Wolf, H. Zähner, *Z. Naturforsch., Teil C* **1990**, *45*, 851–855.

[3] For the final elucidation of the structure of hormaomycin (**1**) see: a) B. D. Zlatopolskiy, K. Loscha, P. Alvermann, S. I. Kozhushkov, S. V. Nikolaev, A. Zeeck, A. de Meijere, *Chem. Eur. J.* **2004**, *10*, 4708–4717; for the total synthesis of **1** see: b) B. D. Zlatopolskiy, A. de Meijere, *Chem. Eur. J.* **2004**, *10*, 4718–4727; c) For an investigation of its structure in solution and the synthesis of some new analogues see: U. M. Reinscheid, B. D. Zlatopolskiy, C. Griesinger, A. Zeeck, A. de Meijere, *Chem. Eur. J.* **2005**, *11*, 2929–2945.

[4] K. Otoguro, H. Ui, A. Ishiyama, N. Arai, M. Kobayashi, Y. Takahashi, R. Masuma, K. Shiomi, H. Yamada, S. Omura, *J. Antibiot.* **2003**, *56*, 322–324.

[5] S. I. Kozhushkov, B. D. Zlatopolskiy, M. Brandl, P. Alvermann, M. Radzom, B. Geers, A. de Meijere, A. Zeeck, *Eur. J. Org. Chem.* **2005**, 854–863.

[6] O. V. Larionov, S. I. Kozhushkov, M. Brandl, A. de Meijere, *Mendeleev Commun.* **2003**, 199–200.

[7] R. Matsueda, R. Walter, *Int. J. Peptide Protein Res.* **1980**, *16*, 392–401.

[8] a) See ref.[3b] for an appropriate discussion; b) Moreover, the side chain of hormaomycin (**1**) showed only limited stability to conditions [20% piperidine or diethylamine in MeCN, 10% tris(aminomethyl)amine in CH_2Cl_2, etc] routinely used for the Fmoc deprotection (cf. ref.[9]).

[9] B. D. Zlatopolskiy, H.-P. Kroll, E. Melotto, A. de Meijere, *Eur. J. Org. Chem.* **2004**, 4492–4502.

[10] These deprotection conditions worked quite satisfactorily for *Z*-Orn(Npys)-(*S*)-1-phenethylamide to completely cleave off the Npys group within 2 min and give the appropriate amino amide, in: 75% yield. For this model compound the Npys group was only partially cleaved off with $MgBr_2 \cdot Et_2O$/EtSH in CH_2Cl_2, and it was rather insensitive to 2-pyridinethiol *N*-oxide to give only traces of deprotected material after 5 h. Cf. B. D. Zlatopolskiy, *Dissertation*, University of Göttingen, **2003**.

[11] F. Yokokawa, H. Sugiyama, T. Shioiri, N. Katagiri, O. Oda, H. Ogawa, *Tetrahedron* **2001**, *57*, 4759–4766.

[12] P. Chevallet, P. Garrouste, B. Malawska, J. Martinez, *Tetrahedron Lett.* **1993**, *34*, 7409–7412.

[13] a) K. E. Gilbert, W. T. Borden, *J. Org. Chem.* **1979**, *44*, 659–661; b) P. Peterli-Roth, M. P. Maguire, E. Leon, H. Rapoport, *J. Org. Chem.* **1994**, *59*, 4186–4193.

[14] a) Previously, the amino acids **14a** and **14b** were obtained in enantiomerically pure form by separation of racemates. The nucleophilic substitution of iodine on a suitably protected 4-iodohomoserine with $AgNO_2$ in benzene gave the corresponding derivative of racemic 5-nitronorvaline in low yield, which could be deprotected.[14b] Racemic 6-nitronorleucine was obtained by hydrolysis of the corresponding *N*,*N*,*O*-tris(trimethylsilyl)derivative, which in turn was prepared by alkylation of trimethylsilyl *N*,*N*-bis(trimethylsilyl)glycinate with 1-nitro-4-bromobutane (41%);[14c] b) B. Mauer, W. Keller-Schierlein, *Helv. Chim. Acta* **1969**, *52*, 388–396; c) E. Bayer, K. Schmidt, *Tetrahedron Lett.* **1973**, *14*, 2051–2054.

[15] Deprotection with HCl always gave better yields of these amino acids, with TFA significant amounts of unidentified side products were formed.

[16] a) N. A. Meanwell, H. R. Roth, E. C. R. Smith, D. L. Wedding, J. J. K. Wright, J. S. Fleming, E. Gillespie, *J. Med. Chem.* **1991**, *34*, 2906–2916; b) Y. Endo, M. Ohno, M. Hirano, A. Itai, K. Shudo, *J. Am. Chem. Soc.* **1996**, *118*, 1841–1855.

[17] The same hormaomycin analogues, albeit only in trace quantities precluding their isolation and characterization, were observed after feeding of 2-(2′-nitrocyclopropyl)glycine (cf. ref.[5]).

[18] a) K. Fujii, Y. Ikai, T. Mayumi, H. Oka, M. Suzuki, K. Harada, *Anal. Chem.* **1997**, *69*, 3346–3352; b) Y. Suzuki, M. Ojika, Y. Sakagami, K. Kaida, R. Fudou, T. Kameyama, *J. Antibiot.* **2001**, *54*, 22–28; c) Unfortunately, (NO_2)Nva totally decomposed under the relatively harsh conditions of total hydrolysis (6 M HCl/AcOH = 1:1, 105 °C, 24 h). The fact that only the (*S*)-epimer of (3-Ncp)Ala was observed in this case indicates that analogue **2a** had been formed; d) See Supporting Infor-

mation (for details see the footnote on the first page of this article) to ref.[3a] for experimental details.
[19] J. Piel, University of Bonn, personal communication.
[20] The only described peptide coupling with (NO_2)Nva (via a mixed anhydride) gave the desired dipeptide Boc-(NO_2)Nva-(NO_2)Nva-OMe only in low (ca. 40%) yield (cf. B. Mauer, W. Keller-Schierlein, *Helv. Chim. Acta* **1969**, *52*, 603–610).
[21] T. Kitagawa, H. Kuroda, H. Sasaki, K. Kawasaki, *Chem. Pharm. Bull.* **1987**, *35*, 4294–4301.
[22] For an appropriate discussion on the biosynthetic pathway to the (3-Ncp)Ala units in hormaomycin (**1**) see: M. Brandl, S. I. Kozhushkov, B. D. Zlatopolskiy, P. Alvermann, B. Geers, A. Zeeck, A. de Meijere, *Eur. J. Org. Chem.* **2005**, 123–135.
[23] In the case of **18** this may be associated with its limited stability under the conditions of the feeding experiment.
[24] E. Rössner, *Dissertation*, University of Göttingen, **1989**.
[25] L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397–4398.
[26] More efficiently *O*-MOM-**1** was obtained by stepwise attachment of the side chain. In this case no epimerization at C-2 of (*S*)-(3-Ncp)Ala was observed.[3b].
[27] S. A. M. Merette, A. P. Burd, J. J. Deadman, *Tetrahedron Lett.* **1999**, *40*, 753–754.
[28] Feeding with Chba-OH did not give any hormaomycin analogue.
[29] R. E. Shute, D. H. Rich, *Synthesis* **1987**, 346–349.
[30] M. Ueki, M. Honda, Y. Kazama, T. Katoh, *Synthesis* **1994**, 21–22.
[31] L. A. Carpino, Patent US 5,580,981, **1996**; *Chem. Abstr.* **1996**, *126*, 104088.
[32] B. Geers, Dissertation, University of Göttingen, **1998**.

Received: November 2, 2005
Published Online: January 19, 2006

