Final Elucidation of the Absolute Configuration of the Signal Metabolite Hormaomycin

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Abstract: The complete absolute configuration of hormaomycin **1a** has been established by HPLC and HPLC/MS experiments with appropriately derivatized 4-propylprolines, (2*S*,4*S*)-**6** and (2*R*,4*R*)-**6**, as well as 4-(*Z*)-propenylprolines, *cis*-**5** and *trans*-**5**, and also feeding experiments with enantiomerically pure samples of the deuterium-labeled 3-(2′-nitrocyclopropyl)alanine, (2*S*)-3,3-[D_2]**15** and (2*S*)-2,2′-[D_2]**15**, and 4-(*Z*)-propenylproline 2′,4-[D_2]-(2*S*,4*R*)-**5**. The latter five amino acids were prepared for the first time and allowed one to unequivocally assign the hitherto unknown absolute configurations of the last four stereocenters in hormaomycin **1a**. As a bonus, some new information about the biosynthesis of this molecule has also been gathered.

Keywords: isotopic labeling · natural products · peptide lactone · structure elucidation

Introduction

Hormaomycin (**1a**), an unusual peptide lactone, which was first isolated in 1989 from *Streptomyces griseoflavus*[1] and found to have an interesting and surprisingly broad spectrum of biological activities,[2] shows structural features remarkable even for the wide range of structurally flexible secondary metabolites from microorganisms. The initial structure investigation[1] disclosed that besides one residue of the proteinogenic (*S*)-isoleucine [Ile], **1a** contains two units of 3-(2*S*,3*R*)-methylphenylalanine [(β-Me)Phe], one of (*R*)-*allo*-threonine [*a*-Thr] as well as two moieties of 3-(1′*R*,2′*R*)-(*trans*-2′-nitrocyclopropyl)alanine [(3-Ncp)Ala] and one of 4-(*Z*)-propenylproline [(4-Pe)Pro]. The side chain of **1a** is terminated with the residue of 5-chloro-1-hydroxypyrrole-2-carboxylic acid [Chpca]. The latter three acid residues have never been found in any natural product before. The absolute configuration of the first four abovementioned structural elements of **1a** and later, partially, the configurations of the (3-Ncp)Ala residues were clarified.[1,3] However, the absolute configuration of the two stereocenters in the (4-Pe)Pro and at the α-carbons of both (3-Ncp)Ala residues remained undetermined. Before the total synthesis of **1a** could be initiated,[4] a full elucidation of the structure of **1a** (Figure 1) had to be performed and this is presented here.

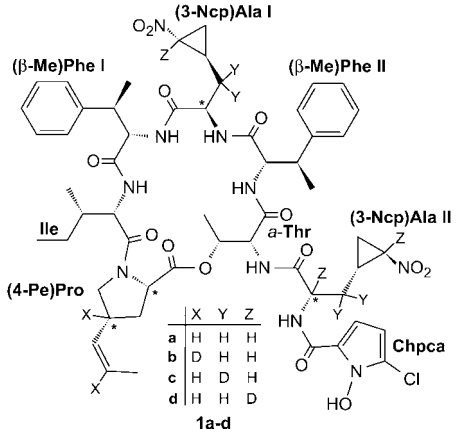

Figure 1. The absolute configuration of hormaomycin **1a**. Asterisks (*) mark the stereocenters for which the absolute configuration was unknown before.

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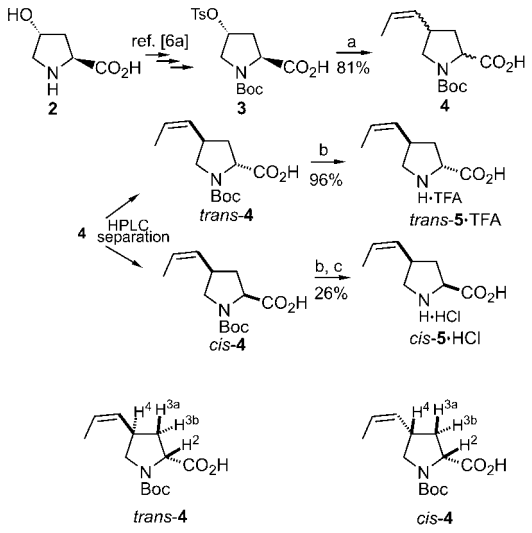
Results and Discussion

To begin with the determination of the configuration of the (4-Pe)Pro moiety in hormaomycin **1a**, a fast and simple access to 4-propenylproline [H-(4-Pe)Pro-OH], even as a mixture of stereoisomers, was desirable. In view of the lack of any reasonably effective preparative routes to H-(4-Pe)Pro-OH,[5] the procedure of Moniot et al.[6] was chosen as a starting point. Tosylate **3**, prepared from 4-hydroxyproline (**2**) according to the published procedure,[6] was successfully alkylated with an excess of lithium di-(*Z*)-propenylcuprate[7] in THF/Et_2O (1:2) to give a mixture of *N*-Boc-protected *cis*- (*cis*-**4**) and *trans*- (*trans*-**4**) 4-propenylprolines (ratio 1:2.2 according to the ^{13}C NMR spectrum), which was inseparable by conventional methods, in 81% yield. The isomerization of the double bond was insignificant and did not exceed 4–10% according to the ^{13}C NMR spectrum (determined by comparison of the signal intensities of the methyl groups: $\delta = 13.1$ ppm for the (*Z*)-isomer and 17.8 ppm for the (*E*)-isomer). A portion of this mixture was separated by preparative HPLC to give the pure individual diastereomers.[8]

The relative configuration of each stereoisomer was unambiguously established by NOESY experiments (Scheme 1). Although the ^1H NMR spectra of these substances showed the presence of two rotamers, the signals of 2-H, 3-H_a, 3-H_b and 4-H were cleanly separated from each other. For the *trans*-isomer, strong correlations between 2-H ($\delta =$ 4.40 ppm) and 3-H_a ($\delta = 1.82$, 2.01 ppm), as well as between 4-H ($\delta = 3.18$–3.33 ppm) and 3-H_b ($\delta = 2.18$, 2.41 ppm), while no correlations between either 2-H and 3-H_b, or 4-H and 3-H_a, or between 2-H and 4-H, were observed. On the contrary, for the *cis*-isomer the direct correlation between 2-H ($\delta = 4.22$, 4.28 ppm) and 4-H ($\delta = 2.99$–3.16 ppm) as well as strong correlations between 2-H and 3-H_a ($\delta = 2.38$, 2.44 ppm), and 4-H and 3-H_a could be seen, while no correlation between 2-H and 3-H_b ($\delta = 1.75$, 1.88 ppm) and only a weak correlation between 4-H and 3-H_b were observed. Both isomers were deprotected with trifluoroacetic acid, and the appropriate amino acids—*cis*-**5** and *trans*-**5**—were obtained. Whereas *trans*-**4** was deprotected smoothly to give the corresponding hydrotrifluoroacetate in virtually quantitative yield, the desired amino acid *cis*-**5**·HCl was obtained after several recrystallizations of the hydrochloride in only 26% yield.

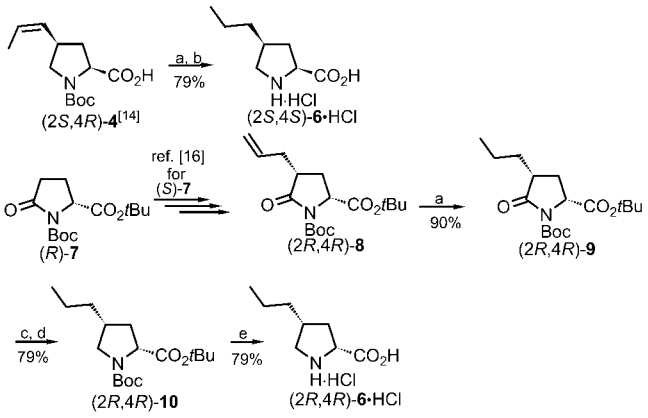
One sample of each amino acid was derivatized with the (*S*)-FDVA [N_α-2,4-dinitro-5-fluorophenyl-(*S*)-valine amide] reagent[9] and the enantiomeric purity of each isomer was determined according to the method of Marfey.[10] This experiment showed *ee* 69% for the *trans*-isomer, and almost full racemization for the *cis*-isomer. These data were in good agreement with the data on the enantiomeric purities of the isomeric 4-phenylprolines prepared by Moniot et al. according to this procedure.[6] A second sample of each stereoisomer of 4-(*Z*)-propenylproline was transformed into a dabsyl (4-dimethylaminoazobenzene-4′-sulfonyl) derivative,[11] and these were compared by HPLC with the sample of DABS-(4-Pe)Pro-OH obtained after the HPLC separation from the DABS-derivatized total hydrolysate of natural



Scheme 1. Synthesis of *cis*- (*cis*-**5**) and *trans*- (*trans*-**5**) 4-(*Z*)-propenylprolines and NOE effects for *cis*-**4** and *trans*-**4**. a) lithium di-(*Z*)-propenylcuprate, Et_2O/THF 2:1, $-40 \rightarrow 20$ °C, 16 h; b) TFA, 20 °C, 20 min; c) 2 M HCl/EtOAc, then four crystallizations from CH_2Cl_2/Et_2O.

hormaomycin **1a**. An MS/MS technique was used to determine the appropriate fraction. These experiments showed that **1a** definitely contains a *cis*-(4-Pe)Pro moiety.[12]

Once the relative configuration of the (4-Pe)Pro residue in hormaomycin **1a** was established, the absolute configuration of this fragment had to be determined. Several experiments failed, because it was impossible to isolate this amino acid (or its derivatives) from the total hydrolysate of the natural depsipeptide due to the instability of 4-(*Z*)-propenylproline in the presence of strong acids routinely applied for the total hydrolysis of peptides. At the same time, it had been known for some time that the catalytic hydrogenation of **1a** with palladium or platinum catalysts transforms the (4-Pe)Pro moiety into the 4-propylproline residue (and also reduces other functionalities of the molecule).[13] Therefore, both enantiomers of *cis*-4-propylproline, which were necessary for the determination of the absolute configuration, were prepared (Scheme 2).

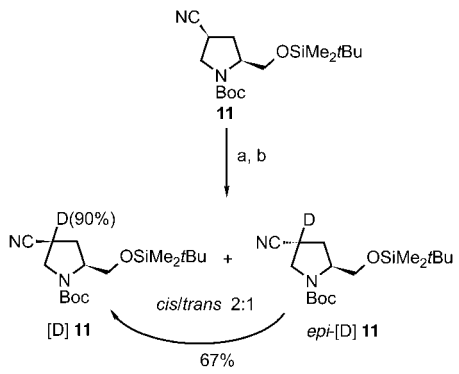


Scheme 2. Synthesis of both enantiomers of *cis*-4-propylproline (**6**). a) H_2, Pd/C, EtOAc, 20 °C, 15 h; b) 2 M HCl/EtOAc, 20 °C, 1 h; c) $LiBEt_3H$, THF, −78 °C, 1 h; d) Et_3SiH, $BF_3 \cdot Et_2O$, CH_2Cl_2, −78 °C, 2.5 h; e) 6 M HCl, 85 °C, 2 h.

Towards that end, the double bond of the enantiomerically pure *N*-Boc protected 4-(*Z*)-propenylproline (2*S*,4*R*)-**4**[14] was hydrogenated over 10% Pd/C in ethyl acetate, and then the *N*-Boc protection was removed with 2 M HCl in EtOAc to give the known (2*S*,4*S*)-**6**[15] as the hydrochloride in 79% yield. The enantiomer (2*R*,4*R*)-**6** was obtained after the deprotection of (2*R*,4*R*)-**10**. The latter was prepared from the *cis*-allyl derivative (2*R*,4*R*)-**8** which in turn was obtained by alkylation with allyl bromide of the enolate of the *N*-Boc-protected *tert*-butyl (*R*)-pyroglutamate (*R*)-**7**[16] in the same way as it was described for the (2*S*,4*S*)-enantiomer.[17] Hydrogenation of the double bond in (2*R*,4*R*)-**8** over 10% Pd/C, followed by two-step reduction of the lactam moiety of the intermediate *N*,*O*-protected 4-propylpyroglutamic acid (2*R*,4*R*)-**9** according to the protocol of Ezquerra et al. completed this synthesis.[18] Both enantiomers of *cis*-4-propylproline were transformed to (*S*)-FDVA derivatives, which were compared with the correspondingly derivatized hydrolysate of hydrogenated hormaomycin **1a** using an HPLC/MS technique according to the advanced Marfey method.[19] These experiments showed that hormaomycin **1a** contains a (2*S*,4*R*)-(4-Pe)Pro residue.

This conclusion was in accord with the results of the feeding experiments with doubly deuterated (2*S*,4*R*)-2′,4-[D_2]**5**. The positions for the deuterium labeling were chosen to establish the possible biotransformation of the deuterated amino acid into its stereoisomers: (*R*)-*cis* or (*S*)-*trans*. If epimerization at the chiral centers of this amino acid in the course of the biosynthesis of hormaomycin **1a** had taken place, it would have caused the loss of the deuterium label at C-4, and the peptide lactone labeled only at C-2′ of the (4-Pe)Pro fragment would have been obtained in the feeding experiments. In addition, the second deuterium label at C-2′ led to a visible change in the ^{1}H NMR spectrum of **1a** in a relatively peak-free region (between 5.3 and 6.0 ppm) as well as avoided any scrambling of the deuterium label during the construction of the double bond.

The nitrile **11**[14] was treated with a small excess of LDA in THF at −78 °C, and the intermediate enolate was quenched with the saturated solution of Na_2SO_4 in D_2O at the same temperature (to obtain more of the desired *cis*-isomer according to Takano et al.).[20] After this the separable mixture of [D]**11** and its epimer *epi*-[D]**11** was obtained in 2:1 ratio (Scheme 3).

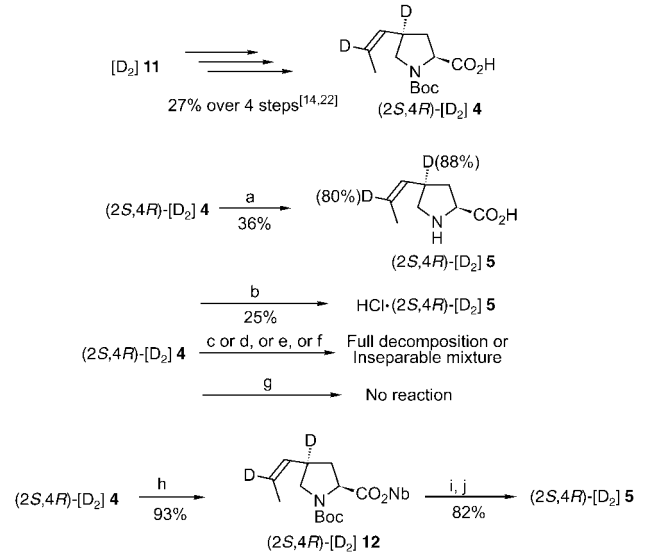


Scheme 3. Introduction of the deuterium label at C-4 of the nitrile **11**. a) LiHMDS, THF, −78 °C, 90 min; b) Na_2SO_4/D_2O.

If D_2O was used for the quenching of the enolate, a reversed selectivity was observed. The mixture of epimers was separated, the *trans*-isomer *epi*-[D]**11** was deprotonated again, and the same mixture of epimers was obtained after quenching of the enolate. The required isomer was separated and combined with the first portion to give the deuterated product [D]**11** (90% deuterium content according to ^{1}H NMR spectrum) in 67% overall yield.[21] This intermediate was transformed into the *N*-Boc protected doubly deuterium-labeled (2*S*,4*R*)-4-(*Z*)-propenylproline as it was previously described for the nondeuterated compound (Scheme 4).[14,22] In this case an ylide, obtained from (1,1-dideuterio)ethyltriphenylphosphonium bromide was used on the Wittig olefination step.[23] The cleavage of Boc group with TFA gave the necessary product only in 36% yield. Therefore, several other conditions for the Boc-deprotection were tried (Scheme 4).[24] To increase the yield, the *p*-nitrobenzyl ester [D_2]**12** (which was prepared by esterification of (2*S*,4*R*)-[D_2]**4**[25] with *p*-nitrobenzyl bromide and K_2CO_3 in MeCN in 93% yield) was *N*-Boc deprotected with 5 M HCl in Et_2O to give the corresponding amino ester as a hydrochloride in 82% yield. The latter was hydrolyzed with equimolar quantity of 1 M NaOH to give, after careful acidification, the dideuterated amino acid (2*S*,4*R*)-[D_2]**5** in almost quantitative yield as a mixture with NaCl. This mixture was directly used for the feeding experiment.

The feeding of (2*S*,4*R*)-2′,4-[D_2]**5** led to the correspondingly doubly deuterated **1b** without any loss of deuterium label. These experiments also cleanly demonstrated that the biosynthesis of H-(4-Pe)Pro-OH in cell takes place before the assembly of the peptide chain of **1a**.

In the course of previous investigations to elucidate the configurations of the (3-Ncp)Ala moieties in **1a** it became

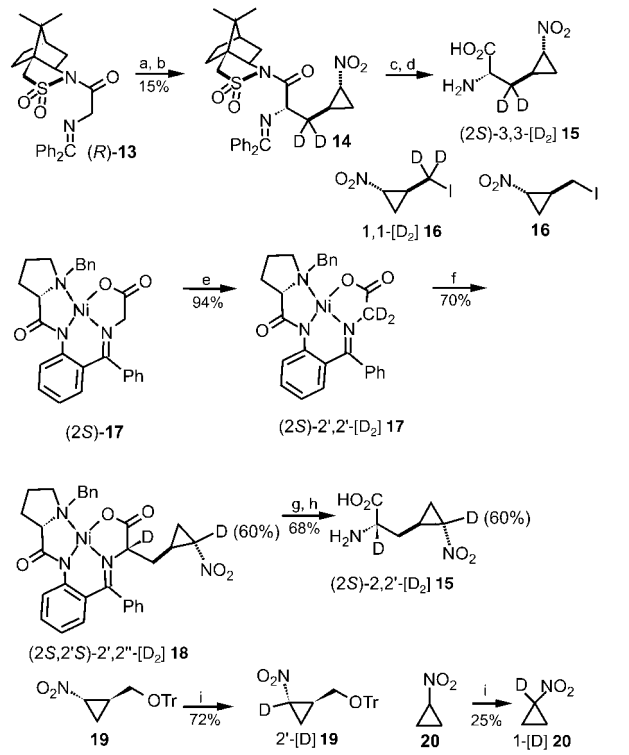


Scheme 4. The final stages in the preparation of the doubly deuterium-labeled amino acid (2*S*,4*R*)-[D_2]**5**. a) TFA (neat), 20 °C, 20 min, then 15 lyophilisations from water; b) 5 N HCl/Et_2O, 20 °C, 4 h; c) BF_3·Et_2O, CH_2Cl_2, 20 °C, 1 h; d) TMSI, CH_2Cl_2, 0 °C, 30 min; e) TFA/Me_2S/CH_2Cl_2, 0 °C, 1 h; f) $SnCl_4$, CH_2Cl_2, 20 °C, 2 h; g) 180 °C, 30 min; h) *p*-O_2N-PhCH_2Br, K_2CO_3, MeCN, 70 °C, 3 h; i) 5 N HCl/Et_2O, 20 °C, 3 h; j) 1 N NaOH, 0 °C, 30 min, then 1 N HCl.

known that both (3-Ncp)Ala residues have the same (1′*R*,2′*R*) configuration in the nitrocyclopropyl ring and are epimers with respect to the configuration at the α-carbons,[3] yet it remained unclear which epimer was incorporated in the ring and which was attached in the side chain. To clarify this situation, feeding experiments with enantiomerically pure deuterium-labeled H-(3-Ncp)Ala-OH had to be carried out. The enantioselective alkylation of the Oppolzer sultam-derived glycine equivalent **13**[26] with the known 1,1-dideuterio-(2′-nitrocyclopropyl)methyl iodide 1,1-[D_2]**16**[27] gave a mixture of enantiomerically pure diastereomers **14** in 15% yield (Scheme 5). The subsequent two-step hydrolysis of the latter under acidic and then under basic conditions gave the corresponding mixture of diastereomers of the 3,3-dideuterio-(2′-nitrocyclopropyl)alanine (2*S*)-3,3-[D_2]**15**.[28] The correspondingly deuterated hormaomycin **1c** was indeed obtained in the feeding experiments with (2*S*)-3,3-[D_2]**15**. According to its ESI-MS spectrum, the molecular mass of this product was four units higher than that of **1a**. The ^{2}H, ^{13}C and ^{1}H NMR spectra confirmed that the 3-[D_2]-(3-Ncp)Ala moiety had been incorporated both in the ring and in the side chain of **1a** with the same probability. This indicates firstly that H-(3-Ncp)Ala-OH is completely biosynthesized before the assembly of the amino acids takes place and secondly that the cells of the hormaomycin-producing strain comprise an epimerase which can convert a solely available (2*S*)-isomer of H-(3-Ncp)Ala-OH to the (2*R*)-isomer necessary to complete the biosynthesis of **1a**. In order to prove this implication, feeding experiments with one of the enantiomerically pure H-(3-Ncp)Ala-OH, labeled at C-2, had to be carried out.

Recently, the diastereoselective alkylation of the Belokon-type complex (2*S*)-**17**[29] with racemic *trans*-(2′-nitrocyclopropyl)methyl iodide **16** was applied to prepare all four possible isomers of H-(3-Ncp)Ala-OH in good yield.[30] Following this protocol, the deuterated compound (2*S*)-[D_2]**17**, which was prepared by a hydrogen/deuterium isotope exchange in excellent yield (94%) and with a very high degree of deuteration (> 97% of the incorporation of two deuterium atoms per molecule),[31] was deprotonated with sodium hydride. The enolate thus generated in turn was alkylated with the racemic iodide **16** in a mixture of [D_3]acetonitrile[32] and dimethylformamide to give a 2-deuterated (2*S*)-(2′-nitrocyclopropyl)alanine precursor in 70% yield. The MS and NMR spectra of this product showed that a hydrogen/deuterium exchange had also taken place in the 2″-position of the cyclopropyl ring, in other words, the product was the precursor to (2*S*)-2,2′-dideuterio-2-(2′-nitrocyclopropyl)alanine (2*S*)-2,2′-[D_2]**15**. This amino acid was obtained with 88% deuterium label in the 2- and 60% in the 2′-position by hydrolysis of (2*S*)-[D_2]**18** and ion exchange chromatography in 68% yield.

This observation appeared to markedly contradict earlier results by Seebach et al. on the possibility of deprotonating and alkylating of nitrocyclopropanes.[33] Therefore, the undeuterated Ni complex (2*S*,2′*S*,1″*R*,2″*R*)-**18**[24] in a CD_3CN/DMF mixture was treated with sodium hydride to give, after quenching with dilute D_2SO_4 in D_2O, 55 and 65%, respectively, incorporation of deuterium in the 2′- and 2″-positions



Scheme 5. Synthesis of deuterated (2*S*,1′*RS*,2′*RS*)-3-(*trans*-2′-nitrocyclopropyl)alanines 3,3-[D_2]**15** and 2,2′-[D_2]**15** as well as related deuterium exchange experiments. Reagents and conditions: a) *n*BuLi, THF, −70°C, 1 h; b) 1,1-[D_2]**16**, HMPT, THF, −78→20°C, 48 h; c) 0.5 M HCl, THF, 20°C, 48 h; d) LiOH, THF/H_2O, 20°C, 48 h; e) Na_2CO_3 (cat.), MeOD/$CDCl_3$/D_2O, 50°C, 24 h (twice); f) **16**, NaH, CD_3CN/DMF, −70→20°C, 50 min; g) 6 M HCl, MeOH, 65°C, 10 min; h) Amberlite IRA-120 in H^+ form; i) NaH, CD_3CN/DMF, −25→20°C, 50 min.

of the (3-Ncp)Ala moiety of **18**.[34] Under the same conditions, nitrocyclopropane (**20**) and the *O*-trityl protected (1′*S*,2′*S*)-(2′-nitrocyclopropyl)methanol **19**[3] gave deuterated analogues in 25 and 72% yield. In the case of **19**, the ^{1}H NMR spectrum of the crude product showed that partial (about 5–7%) epimerization had taken place.

The successful feeding experiment with (2*S*)-2,2′-[D_2]**15** gave the deuterium labeled hormaomycin **1d** which, according to its ^{1}H NMR data (see Figure 2), was enriched with deuterium at three positions, namely at the 2′-position of the (3-Ncp)Ala I moiety in the lactone ring of **1a** as well as at the C-2 and C-2′ positions of the (3-Ncp)Ala II residue in the side chain of **1a**.[35] This observation rigorously proves that the (2*R*)-(3-Ncp)Ala moiety is placed in the ring and the (2*S*)-residue is positioned in the side chain of **1a**.

It is also noteworthy that the degree of deuteration at C-2 position of (3-Ncp)Ala II (40%) was significantly lower than the 88% in the fed sample of (2*S*)-2,2′-[D_2]**15** as measured by the respective decrease of the intensity of the 2-H signal of (3-Ncp)Ala II moiety in the ^{1}H NMR spectrum of **1d** compared with the appropriate signal in the ^{1}H NMR spectrum of the nondeuterated hormaomycin **1a**. By contrast, the degree of deuteration at C-2′ of both (3-Ncp)Ala moieties of **1d** (60%) was as high as for (2*S*)-2,2′-[D_2]**15**. This corroborates that the deuterium at C-2 of (2*S*)-2,2′-[D_2]**15** is partly exchanged before the hormaomycin mole-

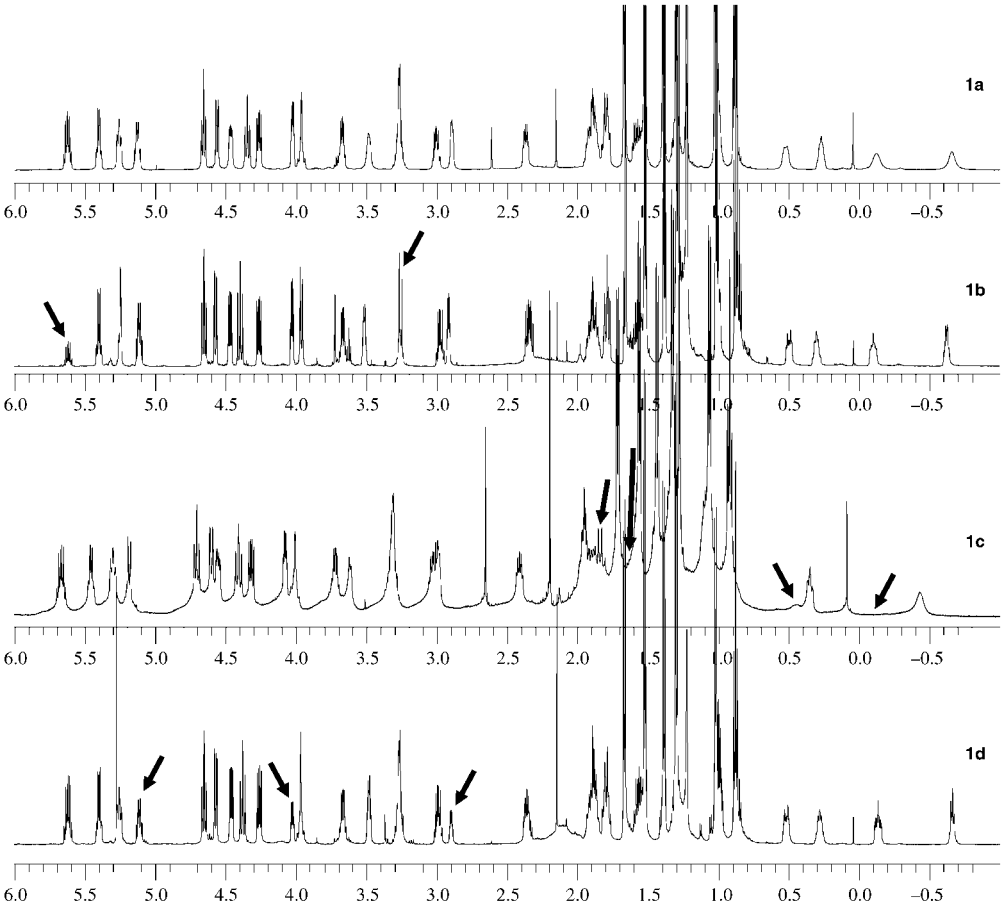


Figure 2. Comparison of ^{1}H NMR (600 MHz, $CDCl_3$) spectra of hormaomycin **1a** and deuterium-labeled hormaomycins **1b**, **1c**, **1d**. The arrows indicate signals, the intensity of which has decreased due to deuteration.

cule is assembled at the respective synthetase or synthetases. Normally, an epimerase is part of the corresponding module of the multienzyme complex. In the case of this amino acid two differently located epimerases possibly compete.

In summary, the hitherto unknown absolute configurations of four stereocenters in hormaomycin **1a** have been established by appropriate HPLC and HPLC/MS as well as feeding experiments with several enantiomerically pure deuterium-labeled 3-(2′-nitrocyclopropyl)alanine and *cis*-4-(*Z*)-propenylproline specimen, all prepared for the first time. As a benefit, some new information about the biosynthesis of this molecule has also been gathered.

Experimental Section

General remarks: Chemistry: ^{1}H NMR spectra: Bruker AM 250 (250 MHz), AMX 300 (300 MHz) or Varian Unity 300 (300 MHz), Inova 500 (500 MHz), Inova 600 (600 MHz). Proton chemical shifts are reported in ppm relative to residual peaks of deuterated solvents. Higher order NMR spectra were approximately interpreted as first-order spectra, if possible. ^{13}C NMR spectra [additional DEPT (Distortionless Enhancement by Polarization transfer) or APT (Attached Proton Test)]: Bruker AM 250 (62.9 MHz), AMX 300 (75.5 MHz) or Varian Unity 300 (75.5 MHz), Inova 500 (125.7 MHz), Inova 600 (150.9 MHz) instruments. ^{13}C chemical shifts are reported relative to peak of solvent or to dioxane in D_2O ($\delta = 67.19$ ppm). IR spectra: Bruker IFS 66 (FT-IR) spectrometer 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; DCI-MS: Finnigan MAT 95, 200 eV, reactant gas NH_3; ESI-MS: Finnigan LCQ. HPLC-MS: pump: Flux Instruments Rheos 4000; degasser: Flux Instruments ERC 3415α; detector: Linear UVIS-205; data system: Flux Instruments Janeiro; ESI: Finnigan LCQ, positive and negative mode; data system: Finnigan LCQ Xcalibur; column: Crom Superspher 100 RP-18 endcapped (4 μm, 2×100 mm); HPLC conditions: eluent A: H_2O (0.05% HCOOH), eluent B: 90% MeCN (0.05% HCOOH), 30→70% B for 30 min, flow rate: 300 μL min^{-1}. 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). HPLC conditions: A, isocratic, 55% MeCN in H_2O (0.1% TFA); B, isocratic 55% MeOH in H_2O for 20 min, then 55→75% MeOH for 8 min, then 75→80% MeOH for 10 min; C, isocratic, 78% acetonitrile in H_2O (0.05% HCOOH). Optical rotations: Perkin-Elmer 241 digital polarimeter, 1 dm^3 cell. M.p.: Büchi 510 capillary melting point apparatus, uncorrected values. TLC: Macherey-Nagel precoated sheets, 0.25 mm Sil G/UV_{254}. Column chromatography: Merck silica gel, grade 60, 230–400 mesh and Baker silica gel, 40–140 mesh. Starting materials: Anhydrous diethyl ether and THF were obtained by distillation from sodium/benzophenone, CH_2Cl_2 and DMF from molecular sieves 4 Å. Compounds **3**,[6] (2*S*,4*R*)-**4**,[14] (*R*)-**13**,[26] **16**,[27,30] 1,1-[D_2]**16**,[27] **17**,[29] **19**[3] were prepared as described elsewhere, and (2*R*,4*R*)-**8** as it was previously described for the (2*S*,4*S*)-isomer.[15b] All other chemicals were used as commercially available (Merck, Acros, BASF, Bayer, Aldrich, Fluka, Hoechst, Degussa AG). Organic extracts were dried over anhydrous $MgSO_4$. Microbiology: Shaking incubator: Braun

BS4. Fermenter: Braun Biostat M. media (per liter of distilled water): M2Ca: malt extract (10 g), glucose (4 g), yeast extract (4 g), agar-agar (20 g), $CaCO_3$ (0.5 g), pH 7.0; M6: D-mannitol (20 g), soybean flour (20 g), meat extract (20 g), NaCl (2 g), L-valine (0.3 g), $ZnSO_4 \cdot 6\,H_2O$ (0.5 g), pH 7.3; M10: D-mannitol (50 g), L-asparagine (3 g), K_2HPO_4 (1 g), NaCl (25 g), $MgSO_4 \cdot 7\,H_2O$ (50 mg), $CaCl_2 \cdot 2\,H_2O$ (50 mg), CH_3COONa (420 mg), *meso*-inositol (100 mg), vitamins solution (1 mL): thiamine hydrochloride (1 g), calcium D-pantothenate (1.2 g), flavine mononucleotide (1 g), nicotinic acid (2.3 g), pyridoxine hydrochloride (12 g), *p*-aminobenzoic acid (200 mg), vitamin B_{12} (100 mg), folic acid (10 mg), biotin (6 mg), trace elements solution (10 mL): $CaCl_2 \cdot 2\,H_2O$ (8 g), $MnCl_2 \cdot 2\,H_2O$ (5 g), $ZnCl_2$ (50 mg), $CuCl_2 \cdot 2\,H_2O$ (50 mg), $FeCl_2 \cdot 6\,H_2O$ (50 mg). In each case the quantities of compounds for the preparation of 1 L of media are given. For the dilution demineralized water was used. After adjusting the pH with 0.5 M NaOH or 0.5 M HCl, all media were sterilized at 121 °C for 30 min. The vitamins solution was added to M10 after sterile filtration and autoclavation. All ingredients were purchased from Merck, Gibco, Difco, Sigma, Riedel de Haen and Henselwerk GmbH.

Total hydrolysis of hormaomycin 1a and hydrogenated hormaomycin: The respective peptide was hydrolyzed with 6 M HCl/AcOH (1:1, 0.5 mL per 1 mg of peptide) in sealed tubes at 105 °C within 24 h. The solvent was then removed under reduced pressure, and the residue was used for further derivatization.

Derivatization of the amino acids 5 or the hydrolysate of 1a with DABS-Cl (4-dimethylaminoazobenzene-4′-sulfonyl chloride): 20 drops of a freshly prepared solution of DABS-Cl (4 nmol mL^{-1}) was added to the sample (ca. 0.1 mg) of *cis*-**5** or *trans*-**5**, or the hydrolysate of **1a** dissolved in five drops of 50 mM $NaHCO_3$ (pH 8.1), and it was heated at 70 °C for 10 min. After this, the reaction mixture was diluted with 50 mM phosphate buffer (1 mL; pH 7.0)/ethanol (1:1) and was directly used for the HPLC experiments.

Derivatization of the amino acids 6 or the hydrolysate of hydrogenated 1a with (*S*)-FDVA: The pH of a solution of 0.4 mg of the respective sample in H_2O (0.5 mL) was adjusted to 9 with 1 M $NaHCO_3$ and a 1% solution of (*S*)-FDVA in acetone (0.1 mL) was added. The reaction mixture was stirred at 40 °C for 1 h, and then the pH was adjusted to 6–7 with 0.1 M HCl. After this, the reaction mixture was diluted with MeCN (1 mL) and was directly used for HPLC/MS experiments.

(2*S*,4*R*)-4-Tosyloxy-*N*-Boc-proline (3):[6] M.p. 138–139 °C; $R_f = 0.19$ [hexane/EtOAc 1:2 (1.5% AcOH)]; $[\alpha] = -62.7$ ($c = 0.33$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.39$, 1.44 [2×s, 9H, $C(CH_3)_3$], 2.14–2.67 (m, 2H, 3-H_A, 5-H_A), 2.46 (s, 3H, CH_3), 3.45–3.81 (m, 2H, 3-H_B, 5-H_B), 4.39, 4.42 (2×t, $J = 8$ Hz, 1H, 2-H), 5.02 (m, 1H, 4-H), 7.37 (d, $J = 8.25$ Hz, 2H, 3′-H), 7.79 (d, $J = 8.25$ Hz, 2H, 2′-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 21.6$ (+, CH_3), 28.0, 28.2 [+, $C(CH_3)_3$], 35.3, 37.0 (−, C-3), 51.8, 52.3 (−, C-5), 51.2 (+, C-2), 78.2, 78.6 [C_{quat}, $C(CH_3)_3$], 81.2, 81.8 (+, C-4), 127.7 (+, C-3′), 130.1 (+, C-2′), 133.1, 133.2 (C_{quat}, C-4′), 145.3 (C_{quat}, C-1′), 153.2, 155.0 (C_{quat}, NCO_2), 175.2, 177.5 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}$ = 3062, 2985, 2956, 1754, 1635, 1433, 1171 cm^{-1}; elemental analysis calcd (%) for $C_{17}H_{23}NO_7S$ (385.4): C 52.98, H 6.01, N 3.63; found C 52.67, H 6.19, N 3.55.

***cis*- and *trans*-*N*-Boc-4-(*Z*)-Propenylprolines (*cis*-4 and *trans*-4)**: A solution of *cis*-1-bromopropene (0.3 mL, 5.01 mmol) in diethyl ether (3 mL) was added within 5 min with stirring at −10 °C under argon (Ar) to a Li chip (0.087 g, 12.53 mmol) in anhydrous diethyl ether (7 mL). The mixture was stirred at the same temperature for an additional 1 h and was then added to a suspension of CuBr·Me_2S (0.529 g, 2.57 mmol) in diethyl ether (15 mL) at −45 to −40 °C (internal temperature) within 10 min. After 1 h, a solution of **3** (0.29 g, 0.75 mmol) in THF (10.5 mL) was added within 15 min at the same temperature to the resulting almost clear yellowish-green solution of lithium di-(*Z*)-propenylcuprate, and the reaction mixture was allowed to warm up to 20 °C over a period of 16 h. The dark-colored reaction mixture was cooled in an ice-water bath and treated with saturated NH_4Cl (5–10 mL). Sodium hydroxide (10%) was added to adjust the pH to 10, and the organic layer was separated. The aqueous phase was washed with Et_2O (3×10 mL), acidified with 4 N H_2SO_4 to pH 2–3 and then extracted with ethyl acetate (2×20 mL). The combined organic layers were washed with water (2×10 mL), then with brine (2×5 mL), dried and concentrated under reduced pressure. The residue was filtered through a pad of silica gel (6 cm) with MeOH/$CHCl_3$ 1:10 to give, after evaporation of solvents, 4-(*Z*)-propenylproline (155 mg, 81%) as an inseparable mixture of diastereomers with a ratio of 2.2:1 (according to the ^{13}C NMR spectrum). An aliquot (25 mg) of this mixture was separated by HPLC (conditions A, flow rate 3 mL min^{-1}) to give the *trans*-**4** isomer (13.8 mg, $t_R = 6.84$ min) and the *cis*-**4** isomer (5.4 mg, $t_R = 6.35$ min) in pure form.

trans-**4**: M.p. 41–45 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.42$, 1.48 [2×s, 9H, $C(CH_3)_3$], 1.66 (d, $J = 6.0$ Hz, 3H; 3′-H), 1.82 (ddd, $J = 11.9$, 11.9, 9.1 Hz, 0.7H, 3-H_a), 2.01 (ddd, $J = 11.9$, 11.9, 10.3 Hz, 0.3H, 3-H_a), 2.11–2.24 (m, 0.3H; 3-H_b), 2.41 (dd, $J = 12.6$, 6.2 Hz, 0.7H; 3-H_b), 2.97 (dd, $J = 10.0$ Hz, 0.7H, 5-H_a), 3.05 (dd, $J = 9.5$ Hz, 0.3H, 5-H_a), 3.18–3.33 (m, 1H, 4-H), 3.58 (dd, $J = 8.5$, 8.5 Hz, 0.7H, 5-H_b), 3.76 (dd, $J = 9.3$, 9.3 Hz, 0.3H, 5-H_b), 4.31 (d, $J = 9.5$ Hz, 0.3H, 2-H), 4.40 (d, $J = 8.5$ Hz, 0.7H, 2-H), 5.25 (dd, $J = 9.8$, 9.8 Hz, 1H, 1′-H), 5.52 (dq, $J = 9.8$, 7.0 Hz 1H, 2′-H), 6.40–7.50 (br, 1H, CO_2H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 13.2$ (+, CH_3), 28.2, 28.3 [+, $C(CH_3)_3$], 34.2, 35.2 (+, C-4), 35.1, 37.0 (−, C-3), 51.4, 51.9 (−, C-5), 58.9, 59.2 (+, C-2), 80.3, 81.3 [C_{quat}, $C(CH_3)_3$], 126.7, 126.9 (+, C-2′), 129.2, 129.6 (+, C-1′), 153.8, 156.0 (C_{quat}, NCO_2), 175.4, 178.4 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}$ = 3023, 2981, 2880, 1722, 1650, 1404, 1227, 1159 cm^{-1}; MS (EI, 70 eV): *m*/*z* (%): 255 (1) [M^+], 210 (8) [$M^+ - CHO_2$], 182 (2) [$M^+ - C_4H_9O$], 154 (100) [$M^+ - C_5H_9O_2$], 110 (70) [$C_7H_{12}N^+$], 87 (8), 57 (80) [$C_4H_9^+$]; HRMS (EI): calcd for $C_{13}H_{21}NO_4$: 255.1471; found 255.1471.

cis-**4**: M.p. 65–68 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.41$, 1.45 [2×s, 9H, $C(CH_3)_3$], 1.65 (d, $J = 6.5$ Hz, 3H; 3′-H), 1.75 (ddd, $J = 12.5$, 10.3, 10.3 Hz, 0.5H, 3-H_b), 1.88 (ddd, $J = 12.0$, 10.0, 10.0 Hz, 0.5H, 3-H_b), 2.38 (dddd, $J = 13.0$, 7.0, 7.0, 7.0 Hz, 0.5H; 3-H_a), 2.44 (dddd, $J = 12.5$, 6.5, 6.5, 6.5 Hz, 0.5H; 3-H_a), 2.99–3.16 (m, 2H, 4-H, 5-H_a), 3.67 (dd, $J = 9.3$, 7.3 Hz, 0.5H, 5-H_b), 3.69–3.78 (m, 0.5H, 5-H_b), 4.22 (dd, $J = 8.3$, 8.3 Hz, 0.5H, 2-H), 4.28 (dd, $J = 8.3$, 8.3 Hz, 0.5H, 2-H), 5.23 (ddq, $J = 10.8$, 10.8, 1.8 Hz, 1H, 1′-H), 5.52 (dq, $J = 10.8$, 6.5 Hz, 1H, 2′-H), 6.82–7.57 (br, 1H, CO_2H); ^{13}C NMR (125.7 MHz, $CDCl_3$): $\delta = 13.2$ (+, CH_3), 28.2, 28.4 [+, $C(CH_3)_3$], 35.8, 36.0 (+, C-4), 35.9, 37.4 (−, C-3), 51.5, 52.1 (−, C-5), 59.3, 59.6 (+, C-2), 80.4, 81.0 [C_{quat}, $C(CH_3)_3$], 126.6, 126.9 (+, C-2′), 129.2, 129.3 (+, C-1′), 153.6, 155.6 (C_{quat}, NCO_2), 176.3, 178.6 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}$=3020, 2975, 2943, 1736, 1633, 1441, 1252, 1168 cm^{-1}; MS (EI, 70 eV): *m*/*z* (%): 255 (1) [M^+], 210 (8) [$M^+ - CHO_2$], 182 (2) [$M^+ - C_4H_9O$], 154 (100) [$M^+ - C_5H_9O_2$], 110 (70) [$C_7H_{12}N^+$], 87 (8), 57 (80) [$C_4H_9^+$]; HRMS (EI): calcd for $C_{13}H_{21}NO_4$: 255.1471; found 255.1471.

***trans*-4-(*Z*)-Propenylproline hydrotrifluoroacetate (*trans*-5·TFA)**: Compound *trans*-**4** (13.8 mg, 0.054 mmol) was deprotected with TFA (1 mL) at 20 °C for 20 min. The resulting solution was concentrated under reduced pressure to give the hydrotrifluoroacetate *trans*-**5**·TFA (13.9 mg, 96%) as a hydroscopic light pink solid. ^{1}H NMR (250 MHz, CD_3OD): $\delta = 1.72$ (dd, $J = 7.0$, 1.8 Hz, 3H, 3′-H), 2.11–2.29 (m, 1H, 3-H_a), 2.35–2.53 (m, 1H, 4-H), 3.02 (dd $J = 9.5$, 11 Hz, 1H, 3-H_b), 3.60 (dd, $J = 7.5$, 11.1 Hz, 1H, 5-H_b), 4.55 (dd, $J = 4.7$, 9.6 Hz, 1H, 2-H), 5.35 (ddq, $J = 9.8$, 9.8, 1.8 Hz, 1H, 1′-H), 5.74 (ddq, $J = 9.8$, 1.0, 7.0 Hz, 1H, 2′-H); the residual peak of CD_2HOD superimposed the signal of 5-H_a; ^{13}C NMR (62.9 MHz, CD_3OD): $\delta = 13.5$ (+, C-3′), 36.2 (−, C-3), 36.2 (+, C-4), 51.9 (−, C-5), 60.7 (+, C-2), 117.9 (q, $J = 289$ Hz, CF_3), 128.9 (+, C-2′), 129.7 (+, C-1′), 162.1 (q, $J = 37.4$ Hz, CF_3CO), 171.9 (C_{quat}, C-1); IR (film): $\tilde{\nu}$= 3250–1950, 1679, 1631, 1410, 1203, 1137 cm^{-1}; MS (EI, 70 eV): *m*/*z* (%): 155 (3) [M^+], 110 (100) [$M^+ - CHO_2$], 87 (16), 69 (43) [CF_3^+], 51 (16), 45 (45) [CHO_2^+], 41 (15) [$C_3H_5^+$]; HRMS (EI): calcd for $C_8H_{13}NO_2$: 155.0946·113.9928; found 155.0946.

***cis*-4-(*Z*)-Propenylproline hydrochloride (*cis*-5·HCl)**: Compound *cis*-**4** (10.2 mg, 0.040 mmol) was deprotected with TFA (1 mL) at 20 °C for 20 min. The resulting solution was concentrated under reduced pressure. The residue was taken up with 2 M HCl/EtOAc (3×2 mL), and the solution then was concentrated under reduced pressure. The residue was recrystallized four times from CH_2Cl_2/Et_2O to give, after prolonged drying at 0.01 Torr, hydrochloride *cis*-**5**·HCl (2.0 mg, 26%) as a hydroscopic brownish semisolid. ^{1}H NMR (250 MHz, D_2O): $\delta = 1.47$ (dd, $J = 6.9$, 1.9 Hz, 3H, 3′-H), 1.61–1.81 (m, 1H, 3-H_b), 2.45 (ddd, $J = 13.0$, 7.1, 7.1 Hz, 1H, 3-H_a), 2.90 (dd $J = 10.1$, 10.1 Hz, 1H, 5-H_a), 3.24 (ddddd, $J = 9.0$, 9.0, 9.0, 9.0, 9.0 Hz, 1H, 4-H), 3.31–3.42 (m, 1-H, 5-H_b), 4.25 (dd, $J = 8.9$ Hz, 1H, 2-H), 5.11 (ddq, $J = 8.8$, 8.8, 1.9 Hz, 1H, 1′-H), 5.44–5.58 (m, 1H, 2′-H); ^{13}C NMR (62.9 MHz, D_2O): $\delta = 13.1$ (+, C-3′), 35.6 (−, C-3), 36.5 (+, C-4), 50.8 (−, C-5), 60.5 (+, C-2), 127.7 (+, C-2′), 129.6 (+, C-

1′), 172.7 (C_{quat}, C-1); MS (ESI): positive mode: *m/z* (%): 178 (6) [M^++Na], 156 (100) [M^++H].

Determination of the relative configuration of the (4-Pe)Pro residue in 1: The total hydrolysate of **1**, derivatized with DABS-Cl, was separated by HPLC (conditions B, flow rate 2.5 mL min^{-1}), and then ESI/MS spectra were measured for the obtained fractions. The peak with t_R = 32.8 min corresponded, according to its ESI/MS spectrum, to the (4-Pe)Pro derivate. It was compared by HPLC with the derivates of *cis*- and *trans*-propenylproline. Conditions B, flow rate 0.8 mL min^{-1}: *cis*-isomer: t_R = 32.57 min, mixed sample: 1 peak; *trans*-isomer: t_R = 33.37 min, mixed sample: 2 peaks; conditions C, flow rate 0.8 mL min^{-1}: *cis*-isomer: t_R = 9.71 min, mixed sample: 1 peak, *trans*-isomer: t_R = 10.04 min, mixed sample: 2 peaks.

(2*S*,4*S*)-Propylproline hydrochloride [(2*S*,4*S*)-(6)·HCl]: (2*S*,4*R*)-**4** (20 mg, 0.078 mmol) was hydrogenated over 10% Pd/C (5 mg) in EtOAC (2 mL) under ambient pressure of hydrogen at 20 °C for 15 h. The reaction mixture was filtered, the solvent was removed under reduced pressure, and the residue was treated with 2 N HCl in EtOAc at 20 °C for 1 h. The solvent was removed and (2*S*,4*S*)-**6**·HCl was obtained as an extremely hygroscopic colorless solid (12 mg, 79%). $[\alpha]_D^{20} = -7.8$ (c = 1.0, $CHCl_3$).

***tert*-Butyl (2*R*,4*R*)-*N*-Boc-4-propylpyroglutaminate [(2*R*,4*R*)-9]**: Compound (2*R*,4*R*)-**8** (220 mg, 0.68 mmol) was hydrogenated over 10% Pd/C (90 mg) in EtOAc (10 mL) under ambient pressure of hydrogen at 20 °C for 15 h. The reaction mixture was filtered, the solvent was removed under reduced pressure, and the crude product was purified by column chromatography (R_f = 0.46, hexane/EtOAc 4:1) to give (2*R*,4*R*)-**9** as a colorless oil (198 mg, 90%). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.89 (t, J = 7.1 Hz, 3H, 3′-H), 1.28–1.50 (m, 3H, 1′-H_a, 2′-H), 1.46 [s, 9H, $C(CH_3)_3$], 1.48 [s, 9H, $C(CH_3)_3$], 1.52–1.67 (m, 1H, 3-H_a), 1.72–1.91 (m, 1H, 1′-H_b), 2.42–2.61 (m, 2H, 3-H_b, 4-H), 4.36 (dd, J = 8.8, 6.3 Hz, 1H, 2-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 13.6 (+, C-3′), 20.1 (−, C-2′), 27.7 (−, C-1′), 27.7 [+, 2×C(CH_3)$_3$], 33.0 (−, C-3), 42.2 (+, C-4), 57.9 (+, C-2), 81.8 [C_{quat}, $C(CH_3)_3$], 83.0 [C_{quat}, $C(CH_3)_3$], 149.3 (C_{quat}, NCO_2), 170.4 (C_{quat}, C-1), 175.4 (C_{quat}, C-5); IR (film): $\tilde{\nu}$ = 2978, 2934, 2874, 1792, 1744, 1719, 1316, 1158 cm^{-1}; MS (CI): *m/z* (%): 362 (8) [M^++2NH_4−H], 345 (88) [M^++NH_4], 328 (6) [M^++H].

***tert*-Butyl (2*R*,4*R*)-*N*-Boc-4-propylprolinate [(2*R*,4*R*)-10]**: A 1 M solution of lithium triethylborohydride in THF (0.8 mL) was added under an atmosphere of nitrogen to a solution of (2*R*,4*R*)-**9** (198 mg, 0.61 mmol) in THF (5 mL) at −78 °C. After 1 h the reaction was quenched with saturated aqueous $NaHCO_3$ (2 mL), and the mixture warmed to 0 °C. Three drops of 30% H_2O_2 were added, and the mixture was stirred at 0 °C. After 20 min, the organic solvent was removed under reduced pressure, and the aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried, filtered and concentrated. The residue was dried at 0.01 Torr for 3 h, then dissolved in anhydrous CH_2Cl_2 (5 mL) containing triethylsilane (0.1 mL, 0.62 mmol), and the mixture was cooled to −78 °C under an atmosphere of nitrogen. Boron trifluoride etherate (0.1 g, 0.71 mmol) was then added to the reaction mixture for 10 min. After 30 min, triethylsilane (0.1 mL, 0.62 mmol) and boron trifluoride etherate (100 mg, 0.71 mmol) were added, and the reaction mixture was stirred for 2 h at the same temperature. Saturated aqueous $NaHCO_3$ (2 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (3×10 mL), the extracts were dried, concentrated under reduced pressure, and the crude product was purified by column chromatography (R_f = 0.56, EtOAc/hexane 1:1) to give (2*R*,4*R*)-**10** (150 mg, 79%) as a colorless oil. $[\alpha]_D^{20} = 75.4$ (c = 0.98, $CHCl_3$) [lit. for the (2*S*,4*S*)-isomer: [15b]; $[\alpha]_D^{20} = -52$ (c = 1.1, $CHCl_3$)]; ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.89 (t, J = 6.9 Hz, 3H, 3′-H), 1.22–1.38 (m, 5H, 3-H_a, 1′-H, 2′-H), 1.42 [s, 9H, $C(CH_3)_3$], 1.42, 1.43 [2×s, 9H, $C(CH_3)_3$], 1.97–2.21 (m, 1H, 4-H), 2.37 (ddd, J = 8.1, 8.1, 8.1 Hz, 0.5H, 3-H_b), 2.42 (ddd, J = 7.8, 7.8, 7.8 Hz, 0.5H, 3-H_b), 2.94 (dd, J = 10.1, 10.1 Hz, 1H, 5-H_a), 3.62 (dd, J = 10.1, 7.3 Hz, 0.3H, 5-H_b), 3.74 (dd, J = 10.1, 7.3 Hz, 0.7H, 5-H_b), 4.06 (dd, J = 8.1, 8.1 Hz, 0.7H, 2-H), 4.11 (dd, J = 8.1, 8.1 Hz, 0.3H, 2-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 14.1 (+, C-3′), 21.3 (−, C-2′), 27.9, 28.0 [+, C(CH_3)$_3$], 28.3, 28.4 [+, C(CH_3)$_3$], 35.0, 36.2 (−, C-1′), 37.3 (−, C-3), 37.6, 38.3 (+, C-4), 52.1, 52.4 (−, C-5), 59.8 (+, C-2), 79.4, 79.6 [C_{quat}, $C(CH_3)_3$], 80.7 [C_{quat}, $C(CH_3)_3$], 153.8 (C_{quat}, NCO_2), 172.4 (C_{quat}, C-1); IR (film): $\tilde{\nu}$ = 2980, 2931, 2871, 1717, 1703, 1398, 1367, 1174, 1152 cm^{-1}; MS (CI): *m/z* (%): 331 (12) [M^++NH_4], 314 (100) [M^++H].

(2*R*,4*R*)-4-Propylproline hydrochloride [(2*R*,4*R*)-6·HCl]: Compound (2*R*,4*R*)-**10** (70 mg, 0.22 mmol) was heated with 6 M HCl (2 mL) at 85 °C for 2 h. The reaction mixture was concentrated under reduced pressure. The traces of water were removed by codistillation with toluene (2× 10 mL), and the residue was recrystallized three times from CH_2Cl_2/Et_2O to give, after prolonged drying at 0.01 Torr, (2*R*,4*R*)−**6**·HCl (34 mg, 79%) as an extremely hygroscopic colorless solid. $[\alpha]_D^{20} = 7.7$ (c = 0.88, $CHCl_3$); ^{1}H NMR (250 MHz, D_2O): δ = 0.83 (m, 3H, 3′-H), 1.19–1.54 (m, 4H, 1′-H, 2′-H), 1.70 (dddd, J = 12.8, 12.8, 10.0, 10.0 Hz, 1H; 4-H), 2.26–2.49 (m, 1H, 3-H_a), 2.50–2.68 (m, 1H, 3-H_b), 2.95 (dd, J = 10.9, 10.9 Hz, 1H, 5-H_a), 3.50 (dd, J = 10.9, 8.3 Hz, 1H, 5-H_b), 4.35 (dd, J = 8.9, 8.9 Hz, 1H, 2-H); ^{1}H NMR (250 MHz, CD_3OD): δ = 0.99 (t, J = 7 Hz, 3H, 3′-H), 1.30–1.62 (m, 4H, 1′-H, 2′-H), 1.74 (dddd, J = 11.7, 11.7, 10.2, 10.2 Hz, 1H; 4-H), 2.33–2.68 (m, 1H, 3-H_a), 2.65 (ddd, J = 12.2, 6.7, 6.7 Hz, 1H, 3-H_b), 2.98 (dd, J = 10.2, 10.2 Hz, 1H, 5-H_a), 3.53 (dd, J = 11.7, 7.8 Hz, 1H, 5-H_b), 4.37 (dd, J = 8.6, 8.6 Hz, 1H, 2-H); ^{13}C NMR (62.9 MHz, D_2O): δ = 14.1 (+, C-3′), 21.5 (−, C-2′), 34.6 (−, C-1′), 35.3 (−, C-3), 38.8 (+, C-4), 51.6 (−, C-5), 60.6 (+, C-2), 173.0 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}$ = 3432, 2960, 2931, 2873, 1736, 1389, 1252 cm^{-1}; MS (EI, 70 eV): *m/z* (%): 157 (1) [M^+], 112 (100) [M^+−HCO_2], 87 (4), 70 (5) [$C_4H_8N^+$], 69 (11), 43 (15) [$C_3H_7^+$], 41 (18) [$C_3H_5^+$]; HRMS (EI): calcd for $C_8H_{15}NO_2$: 157.1103; found 157.1103.

Determination of the absolute configuration of the (4-Pe)Pro residue in 1a: Hormaomycin **1a** (5 mg) was hydrogenated over 10% Pd on charcoal (30 mg) in methanol (3 mL) under ambient pressure of hydrogen for 30 min. The reaction mixture was filtered and concentrated under reduced pressure to give the crude material, an aliquot of which (ca. 0.5 mg) was further hydrolyzed, and after the derivatization with (*S*)-FDVA was used for HPLC/MS experiments. Detection: UV: 340 nm; ESI-MS (positive) *m/z*: 437.7–438.7; ESI-MS (negative) *m/z*: 435.7–436.7; (*S*)-FDVA-(2*S*,4*S*)-**6**: t_R = 14.77 min, mixed sample: 1 peak; (*S*)-FDVA-(2*R*,4*R*)-**6**: t_R = 17.98 min, mixed sample: 2 peaks.

(2*S*,4*S*)- and (2*S*,4*R*)-*N*-Boc-*O*-TBDMS-4-deuterio-4-cyanoprolinol, [D]11 and *epi*-[D]11: An ice-cold solution of LDA, prepared from *n*BuLi (15 mL of 2.44 M solution in hexane, 36.60 mmol) and diisopropylamine (5.9 mL, 41.85 mmol) in THF (30 mL), for 40 min was added dropwise to a solution of the nitrile **11** (9.5 g, 27.90 mmol) in THF (50 mL) at −78 °C. Stirring was continued for an additional 90 min, and then the reaction was quenched by addition of a saturated solution of Na_2SO_4 in D_2O (10 mL) under vigorous stirring at the same temperature. The mixture was allowed to warm to 20 °C, and it was then concentrated under reduced pressure, the residue was taken up with Et_2O (70 mL), filtered through Celite®, concentrated again and separated by column chromatography (EtOAc/hexane 1:3.7, *cis*-isomer: R_f = 0.38, *trans*-isomer: R_f = 0.54) to give [D]**11** (4.21 g) and a mixed fraction enriched with *epi*-[D]**11** (3.62 g, 10.63 mmol), which was dissolved in THF (20 mL), again treated with a solution of LDA, prepared from *n*BuLi (5.7 mL, 13.91 mmol) and diisopropylamine (2.23 mL, 15.91 mmol) in THF (15 mL), and quenched with a saturated solution of Na_2SO_4 in D_2O (3 mL) as it was described above. Separation of the mixture by column chromatography gave a second crop of the labeled *cis*-nitrile [D]**11** (2.40 g). It was combined with the first fraction and finally purified by column chromatography as described above to give [D]**11** (6.39 g, 67%, 90% D according to its ^{1}H NMR spectrum) as a colorless oil, which gradually solidified to a colorless solid.

[D]**11**: m.p. 53–55 °C; $[\alpha]_D^{20} = -25.9$ (c = 1.1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.01 [s, 6H, $Si(CH_3)_2$], 0.88 [s, 9H, $SiC(CH_3)_3$], 1.41 [s, 9H, $C(CH_3)_3$], 2.27–2.45 (m, 2H, 3-H), 2.92 (dd, J = 8.3, 8.3 Hz, 0.1H, 4-H), 3.35 (d, J = 11.3 Hz, 1H, 5-H_a), 3.53–4.05 (m, 4H, 1-H, 2-H, 5-H_b); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = −5.6 [+, $Si(CH_3)_2$], 18.0 (C_{quat}, SiC), 25.7 [+, $SiC(CH_3)_3$], 26.2 (t, J = 21.7 Hz, C-4), 28.2 [+,$C(CH_3)_3$], 30.9, 32.1 (−, C-3), 49.5, 49.9 (−, C-5), 57.7 (+, C-2), 62.1, 63.3 (−, C-1), 80.0 [C_{quat}, $C(CH_3)_3$], 119.9 (C_{quat}, CN), 153.3 (C_{quat}, NCO_2); IR (film): $\tilde{\nu}$ = 2952, 2895, 2858, 2241, 1700, 1471, 1405, 1258, 1177, 1101 cm^{-1}; MS (CI): *m/z* (%): 359 (1) [M^++NH_4], 342 (100) [M^++H]; elemental analysis (%) for $C_{17}H_{31}DN_2O_3Si$ (341.5): C 59.78, H 9.74, N 8.20; found C 59.54, H 9.60, N 8.03.

The mixed fractions enriched with the *trans*-epimer were purified by column chromatography as described before to give *epi*-[D]**11** (0.56 g, 5.9%, 82% D according to ESI-MS spectrum) as a colorless oil. $[\alpha]_D^{20} = -60.2$ (c = 0.48, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.01 [s, 6H,

$Si(CH_3)_2$], 0.85 [s, 9H, $SiC(CH_3)_3$], 1.44 [s, 9H, $C(CH_3)_3$], 2.15–2.46 (m, 2H, 3-H), 3.24–3.47 (m, 0.2H, 4-H), 3.41–3.80 (m, 3.5H, 0.5×1-H, 2-H, 5-H), 3.87–4.07 (m, 1.5H, 1.5×1-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = −5.7 [+, $Si(CH_3)_2$], 18.0 (C_{quat}, SiC), 25.7 [+, $SiC(CH_3)_3$], 26.5 (t, J = 15.7 Hz, C-4), 28.2 [+, $C(CH_3)_3$], 32.4, 33.2 (−, C-3), 49.4, 49.5 (−, C-5), 57.5 (+, C-2), 63.5, 64.0 (−, C-1), 80.0 [C_{quat}, $C(CH_3)_3$], 120.0 (C_{quat}, CN), 153.2 (C_{quat}, NCO_2); IR (film): $\tilde{\nu}$ = 2955, 2930, 2885, 2858, 2245, 1702, 1473, 1393, 1257, 1177, 1121 cm^{-1}; MS (ESI): positive mode: m/z (%): 364 (48) [M^++Na].

(2*S*,4*R*)-4,2′-Dideuterio-4-(*Z*)-propenylproline, (2*S*,4*R*)-[D$_2$]5: The *N*-protected acid (2*S*,4*R*)-[D$_2$]**4** (0.155 g, 0.602 mmol) was dissolved in trifluoroacetic acid (2 mL) at 20 °C. After 20 min, all volatiles were removed under reduced pressure, and the yellow oily residue was taken up with bidistilled water (5 mL). The solution was then concentrated under reduced pressure (this operation was repeated 15 times). The residue was recrystallized three times from MeOH/Et_2O to give (2*S*,4*R*)-[D$_2$]**5** (34 mg, 36%) as a colorless solid. $[\alpha]_D^{20}$ = −4.2 (c = 0.31, MeOH); ^{1}H NMR (250 MHz, D_2O): mixture of isotopomers: δ = 1.63 (s, 3H, 3′-H), 1.75 (dd, J = 12.5, 8.3 Hz, 1H, 3-H$_a$), 2.54 (dd, J = 8.3, 12.5 Hz, 1H, 3-H$_b$), 3.03 (d, J = 11.5 Hz, 1H, 5-H$_a$), 3.36 (m, 0.15H, 4-H), 3.48 (d, J = 11.5 Hz, 1H, 5-H$_b$), 4.16 (dd, J = 8.3, 8.3 Hz, 1H, 2-H), 5.27 (m, 1H, 1′-H), 5.52 (m, 0.15H, 2′-H); ^{13}C NMR (62.9 MHz, D_2O): δ = 12.3, 12.4 (+, CH_3), 35.5 (−, C-3), 35.8, (t, J = 21.6 Hz, C-4), 36.1 (+, C-4), 50.0 (−, C-5), 61.2 (+, C-2), 127.3, 127.4 (+, C-1′), 127.8 (t, J = 23.0 Hz, C-2′), 128.5 (+, C-2′), 174.2 (C_{quat}, C-1); IR (KBr): $\tilde{\nu}$ = 3101, 3008, 2969, 2915, 2856, 2361, 1626, 1388, 1315 cm^{-1}; MS (EI, 70 eV): m/z (%): 157 (1) [M^+], 112 (100) [M^+−CHO_2], 87 (16), 54 (7), 41 (36) [$C_3H_5^+$]; MS (ESI): positive mode: m/z (%) = 202 (16) [M^+−H+2Na], 180 (6) [M^++Na]; negative mode: m/z: 156 (100) [M^-−H]; HRMS (EI): calcd for $[C_8H_{11}D_2NO_2]$: 157.1072; found 157.1072.

***p*-Nitrobenzyl (2*S*,4*R*)-*N*-Boc-4,2′-dideuterio-4-(*Z*)-propenylprolinate, [D$_2$]12**: A suspension of K_2CO_3 (0.467 g, 3.379 mmol) in a solution of (2*S*,4*R*)-[D$_2$]**4** (0.87 g, 3.381 mmol) and *p*-nitrobenzyl bromide (0.767 g, 3.550 mmol) in MeCN (5 mL) was stirred in a sealed tube at 70 °C for 3 h. The mixture was then cooled, diluted with Et_2O (10 mL), filtered, concentrated under reduced pressure, and the residue was purified by column chromatography (R_f = 0.43, EtOAc/hexane 1:3) to give [D$_2$]**12** (1.23 g, 93%) as a colorless glass. $[\alpha]_D^{20}$ = −37.0 (c = 0.86, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): mixture of isotopomers: δ = 1.34, 1.44 [2 s, 9H, $C(CH_3)_3$], 1.63 (s, 3H, 3′-H), 1.63–1.80 (m, 1H, 3-H$_a$), 2.37–2.51 (m, 1H, 3-H$_b$), 3.06 (d, J = 10.5 Hz, 1H, 5-H$_a$), 3.67, 3.78 (2 d, J = 10.5 Hz, 1H, 5-H$_b$), 4.30, 4.37 (2 dd, J = 8.8, 8.8 Hz, 1H, 2-H), 5.19 (d, J = 13.8 Hz, 1H, Bzl-H$_a$), 5.20–5.26 (m, 1H, 1′-H), 5.35 (d, J = 13.8 Hz, 1H, Bzl-H$_b$), 5.49–5.59 (m, 0.2H, 2′-H), 7.52 (d, J = 8.5 Hz, 2H, Ar-H), 8.20 (dd, J = 8.5, 8.5 Hz, 2H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 12.9, 13.0 (+, CH_3), 28.0, 28.2 [+, $C(CH_3)_3$], 35.1 (t, J = 23.3 Hz, C-4), 36.3 (+, C-4), 36.4, 37.3 (−, C-3), 51.3, 51.7 (−, C-5), 58.9, 59.0 (+, C-2), 64.9 (−, C-Bzl), 79.9 [C_{quat}, $C(CH_3)_3$], 123.5, 123.6 (+, C-Ar), 126.7, 126.8 (+, C-2′), 128.0, 128.3 (+, C-Ar), 128.9, 129.0 (+, C-1′), 142.7, 143.1 (C_{quat}, C-Ar), 147.4, 147.6 (C_{quat}, C-Ar), 153.2, 154.0 (C_{quat}, NCO_2), 172.3, 172.5 (C_{quat}, C-1); MS (EI, 70 eV): m/z (%): 392/391 (1/0.4) [M^+], 291/290 (12/5) [M^+−$C_5H_9O_2$], 247/246 (4/2), 212/211 (50/20) [M^+−$C_8H_6NO_4$], 156 (100) [$C_8H_{10}D_2NO_2^+$], 155 (35) [$C_8H_{11}DNO_2^+$], 112 (80) [$C_7H_{10}D_2N^+$], 111 (28) [$C_7H_{11}DN^+$], 57 (92) [$C_4H_9^+$]; HRMS (EI): calcd for $C_{20}H_{24}D_2N_2O_6$: 392.1916; found 392.1916; elemental analysis (%) for $C_{20}H_{25}DN_2O_6$ (391.4): calcd for C 61.37, H 6.95, N 7.16; found C 61.41, H 6.66, N 7.08.

(2*S*,4*R*)-4,2′-Dideuterio-4-(*Z*)-propenylproline, (2*S*,4*R*)-[D$_2$]5: A 5 M HCl solution in Et_2O (20 mL) was added to a solution of the *N*-protected amino ester [D$_2$]**12** (1.108 g, 2.823 mmol) in Et_2O (5 mL) and stirring was continued in the dark for 2 h. The mixture was then filtered, and a fresh 5 M HCl solution in Et_2O (10 mL) was added. After 1 h, the mixture was diluted with hexane (10 mL), and the formed precipitate was separated, washed with Et_2O and dried to give *p*-nitrobenzyl (2*S*,4*R*)-4,2′-dideuterio-4-(*Z*)-propenylprolinate hydrochloride (0.775 g, 83%). MS (ESI): positive mode: m/z (%): 293/292 (100/35) [M^++H].

A 1 M NaOH solution (4.89 mL) was added dropwise to an ice-cold solution of *p*-nitrobenzyl (2*S*,4*R*)-4,2′-dideuterio-4-(*Z*)-propenylprolinate hydrochloride (0.775 g, 2.357 mmol) in MeOH (5 mL) within 10 min. Stirring was continued at the same temperature for an additional 30 min, after that the mixture was diluted with water (20 mL), methanol was removed under reduced pressure, and the reaction mixture was extracted with Et_2O (10×10 mL). The pH of the water fraction was carefully adjusted to 6.5–7.5 with 1 M HCl (ca. 2.40 mL), and water was removed under reduced pressure to give, after prolonged drying at 0.01 Torr, a mixture of (2*S*,4*R*)-[D$_2$]**5** and NaCl (0.655 g, 0.292 g of NaCl, 98%) as a colorless solid. This product had the same spectral characteristics as the one described above, and it was used for the feeding experiment without any further manipulations.

Alkylation of (*R*)-13 with *rac-trans*-1-(iododideuteriomethyl)-2-nitrocyclopropane, (1,1-[D$_2$]16)—Preparation of 14: *n*-BuLi (4.6 mL of 2.58 N solution in hexane, 11.90 mmol) within 30 min was added at −70 °C to a solution of (*R*)-**13** (2.43 g, 10.61 mmol) in anhydrous THF (600 mL). The reaction mixture was stirred at the same temperature for 30 min, and a solution of the iodide 1,1-[D$_2$]**16** (2.43 g, 10.63 mmol) in a mixture of HMPT (15 mL) and THF (40 mL) was then added within 45 min. Stirring was continued at the same temperature for an additional 24 h, and then the mixture was allowed to warm to 20 °C over a period of 48 h, the reaction was quenched with half-saturated sodium chloride (1 L) and the mixture was extracted with CH_2Cl_2 (3×200 mL). The combined organic layers were washed with water (2×100 mL), dried and concentrated under reduced pressure. The residue was recrystallized from Et_2O/light petroleum to give **14** (850 mg, 15%) as a colorless solid. M.p. 204 °C; ^{1}H NMR (250 MHz, $CDCl_3$): mixture of diastereomers: δ = 0.92 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 0.97–1.15 (m, 1H, 3″-H), 1.26–1.46 (m, 2H), 1.63, 1.73 (ddd, J = 5.7, 3.5, 9.4 Hz, 1H; 3″-H), 1.85–2.15 (m, 6H), 3.36 (s, 2H, 6-H), 3.88 (dd, J = 5.9, 6.4 Hz, 1H, 3-H), 3.97, 4.13 (ddd, J = 3.3, 3.5, 6.8 Hz, 1H, 2″-H), 4.76, 4.78 (s, 1H, 2′-H), 7.04–7.07, 7.12–7.16, 7.25–7.50 and 7.65–7.69 (m, 10H, Ph-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 17.7, 18.8 (−, C-3″), 19.8 (+, CH_3), 20.6 (+, CH_3), 23.0 (+, C-1″), 26.4 (−, C-9), 32.6 (−, C-8), 38.3 (−, C-2), 44.3 (+, C-1), 47.7 (C_{quat}, C-10*), 48.5 (C_{quat}, C-7*), 52.9 (−, C-6), 58.7, 59.9 (+, C-2″), 64.7, 64.9 (+, C-2′*), 65.1, 65.2 (+, C-3*), 127.4, 127.7, 128.0, 128.6, 128.8, 128.9 and 130.60 (+, Ph-C), 139.0 (C_{quat}, Ph-C), 171.4 (C_{quat}, C=N and C=O); the signal of the CD_2 carbon could not be detected because of its low intensity; IR (KBr): $\tilde{\nu}$ = 3084, 3061, 2983, 2960, 2914, 1689, 1628, 1577, 1539, 1445, 1364, 1333, 1166 cm^{-1}; MS (EI, 70 eV): m/z (%): 537 (7) [M^+], 491 (9) [M^+−NO_2], 295 (100) [$C_{18}H_{15}D_2N_2O_2^+$]; HRMS (EI): calcd for $C_{29}H_{31}D_2N_3O_5S$: 537.2266; found 537.2266.

(2*S*,1′*RS*,2′*RS*)-3,3-Dideuterio-3-(*trans*-2′-nitrocyclopropyl)alanine hydrochloride, (2*S*)-3,3-[D$_2$]15]: 0.5 M HCl (21 mL) was added with stirring to a solution of compound **14** (850 mg, 1.58 mmol) in THF (21 mL), and stirring was continued for 48 h. Then the reaction mixture was taken up with Et_2O (40 mL), the layers were separated and the aqueous phase was additionally extracted with Et_2O (3×20 mL). The organic phases were discarded, and the aqueous fraction was concentrated under reduced pressure. The residue was dissolved in THF/H_2O (1:1, 42 mL), and LiOH·H_2O (265 mg, 6.32 mmol) was added. After 48 h, H_2O (32 mL) was added, and the mixture was extracted with CH_2Cl_2 (3×20 mL). The pH of the aqueous fraction was adjusted to 5 with 1 M HCl, and it was concentrated to give (2*S*)-3,3-[D$_2$]**15** as a mixture with LiCl (540 mg, containing max. 280 mg of (2*S*)-3,3-[D$_2$]**15**), which was directly used for the feeding experiment. ^{1}H NMR (250 MHz, D_2O): δ = 1.13–1.28 (m, 1H, 3′-H), 1.71–1.84 (m, 1H, 3′-H), 1.92–2.06 (m, 1H, 1′-H), 3.71 (s, 1H, 2-H), 4.18–4.26 (m, 1H, 2′-H); ^{13}C NMR (62.9 MHz, D_2O): δ = 19.1 (−, C-3′), 24.0 (+, C-1′), 33.0 (quint, J = 25 Hz, C-3), 54.2 (+, C-2), 61.6 (+, C-2′), 173.0 (C_{quat}, C-1); IR (film): $\tilde{\nu}$ = 3200–2700, 2530, 1992, 1729, 1544, 1484, 1371, 1210 cm^{-1}.

2-Dideuterated nickel complex, (2*S*)-2′,2′-[D$_2$]17: Unlabelled (*S*)-**17** (6.00 g, 12.043 mmol) was taken up with a mixture of CH_3OD (10 mL), D_2O (7 mL) and $CDCl_3$ (5 mL), and Na_2CO_3 (200 mg, 2.410 mmol) was added. The mixture was stirred at 50 °C for 24 h in a sealed flask, concentrated under reduced pressure, CH_3OD (10 mL), D_2O (7 mL) and $CDCl_3$ (5 mL) were added again, and the resulting biphasic reaction mixture was stirred at 50 °C for an additional 24 h. The reaction mixture was then cooled, diluted with $CHCl_3$ (30 mL), the organic phase was separated, washed with H_2O (2×30 mL), dried and concentrated to give after recrystallization from $CHCl_3$/Et_2O/hexane (2*S*)-2′,2′-[D$_2$]**17** (5.66 g, 94%; D_2 and D contents > 97% and < 3% respectively, as determined by MS) as a dark red solid. M.p. 210–213 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.97–2.19 (m, 2H, 4-H), 2.28–2.46 (m, 1H, 3-H$_a$), 2.46–2.63 (m, 1H, 3-H$_b$), 3.21–3.41 (m, 1H, 5-H$_a$), 3.45 (dd, J = 10.9, 5.6 Hz, 1H, 5-H$_b$), 3.59–3.72 (m, 1H, 2-H), 3.68 (d, J = 25.0 Hz, 1H, Bzl-H$_a$), 4.46 (d, J = 25 Hz,

1H, Bzl-H_b), 6.63–6.72 (m, 1H, Ar-H), 6.74–6.81 (m, 1H, Ar-H), 6.88–7.01 (m, 1H, Ar-H), 7.03–7.12 (m, 1H, Ar-H), 7.14–7.24 (m, 1H, Ar-H), 7.24–7.33 (m, 1H, Ar-H), 7.35–7.46 (m, 2H, Ar-H), 7.44–7.58 (m, 3H, Ar-H), 8.04 (d, $J=8$ Hz, 2H, Ar-H), 8.26 (d, $J=8.5$ Hz, 1H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta=23.4$ (−, C-4), 30.3 (−, C-3), 57.2 (−, C-5), 62.8 (−, C-Bzl), 69.5 (+, C-2), 120.4, 123.9, 125.3, 125.9, 128.5, 128.7, 129.0, 129.2, 129.4, 131.4, 131.8 and 132.8 (+, Ar-C), 124.8, 133.0, 134.2, 142.2 (C_{quat}, Ar-C), 171.3 (C_{quat}, C=N), 176.9 (C_{quat}, C=O), 181.0 (C_{quat}, CO−N); the signal of the CD_2 carbon could not be detected because of its low intensity; IR (KBr): $\tilde{\nu}=2973$, 2869, 1673, 1642, 1589, 1442, 1336, 1264, 1176 cm^{-1}; MS (EI, 70 eV): *m/z* (%): 499 (40) [M^+], 455 (100) [M^+-CO_2], 363 (8), 267 (10), 265 (17), 217 (41), 160 (41), 91 (78) [$C_7H_7^+$]; HRMS (EI): calcd for $C_{27}H_{23}D_2N_3NiO_3$: 499.1375; found 499.1375; elemental analysis calcd (%) for $C_{27}H_{23}D_2N_3NiO_3$ (500.2): C 64.83, H 5.44, N 8.40; found C 65.03, H 5.77, N 8.32.

Alkylation of 2-dideuterated nickel complex, (2*S*)-2′,2′-[D_2]17 with *rac-trans*-1-(iodomethyl)-2-nitrocyclopropane 16—Preparation of (2*S*,2′*S*)-2′,2″-[D_2]18: A suspension of (*S*)-2′,2′-[D_2]**17** (4.60 g, 9.20 mmol) in a mixture of DMF (4.0 mL) and CD_3CN (8 mL) was degassed with three freeze-pump-thaw cycles at −70 °C. NaH (504 mg, 60% in oil, 12.60 mmol) and iodide **16** (2.12 g, 9.34 mmol) were then added. The cooling bath was removed, and the reaction mixture was vigorously stirred for 40 min. The reaction flask was then immersed in an ice/water bath and first D_2O (2 mL), then D_2SO_4 (1.26 g, 12.60 mmol) in D_2O (5 mL) were carefully added. After this, water (70 mL) was added, and the separated dark-red oil was triturated to give a red-brown solid (5.70 g) which was further purified by column chromatography ($R_f=0.37$, $CHCl_3$/acetone 6:1) and then by crystallization from $CHCl_3$/MeOH to give (2*S*,2′*S*)-2′,2″-[D_2]**18** (3.87 g, 70%) as a dark-red solid. According to its ^{1}H NMR spectrum, the deuterium content was 85% at C-2′ and 60% at C-2″, respectively. M.p. > 260 °C; $[\alpha]_D^{20}=1708$ ($c=0.05$, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$): mixture of diastereo- and isotopomers: $\delta=$ 0.46–0.58 (m, 0.5H, 3″-H_a), 0.85–0.96 (m, 0.5H, 3″-H_a), 1.05–1.28 (m, 0.5H, 3′-H_a), 1.45 (dd, $J=8.4$, 14.4 Hz, 0.5H, 3′-H_a), 1.73–1.84 (m, 0.5H, 3″-H_b), 1.84–1.96 (m, 0.5H, 3″-H_b), 1.97–2.32 (m, 2.5H, 4-H, 0.5×3′-H_b), 2.38–2.58 (m, 2H, 3-H_a, 1″-H), 2.58–2.83 (m, 1.5H, 3-H_a, 0.5×3′-H_b), 3.33–3.41 (m, 0.15H, 2′-H), 3.40–3.73 (m, 3H, 2-H, 5-H), 3.56 (dd, $J=2.3$, 12.8 Hz, 1H, Bzl-H_a), 3.92 (ddd, $J=3.4$, 3.4, 7.0 Hz, 0.30H, 2″-H, 2″-H), 4.00 (dd, $J=14.6$, 3.9 Hz, 0.13H, 2″-H), 4.44 (d, $J=12.8$ Hz, 1H, Bzl-H_b), 6.56–6.73 (m, 2H, Ar−H), 6.88 (t, $J=8$ Hz, 1H, Ar−H), 7.08–7.24 (m, 2H, Ar−H), 7.29–7.59 (m, 5H, Ar−H), 7.59–7.65 (m, 1H, Ar−H), 8.07 (dt, $J=7.8$, 1.5 Hz, 2H, Ar−H), 8.13 (dd, $J=3.3$, 8.5 Hz, 1H, Ar−H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta=17.7$, 17.8, 18.2, 18.4 (−, C-3″), 21.5, 21.57, 21.61, 21.7 (+, C-1″), 23.8 (−, C-4), 30.6 (−, C-3), 36.1, 36.4 (−, C-3′), 57.2 (−, C-5), 58.5 (+, C-2), 63.1, 63.2 (−, C-Bzl), 68.3 (t, $J=19.3$ Hz, C-2′), 69.97, 70.03 (+, C-2″), 120.6, 123.5, 123.6, 127.08, 127.10, 127.15, 127.19, 128.70, 128.72, 128.8, 129.0, 129.3, 129.8, 130.0, 131.3, 132.3 and 133.0 (+, Ar−C), 125.9, 133.2, 133.4, 133.4, 142.3 (C_{quat}, Ar−C), 170.9, 171.0 (C_{quat}, C=N), 178.1, 178.2 (C_{quat}, C=O), 180.4 (C_{quat}, CO−N); IR (KBr): $\tilde{\nu}=3071$, 2972, 2922, 2866, 1667, 1624, 1589, 1537, 1436, 1365, 1339, 1257, 1166 cm^{-1}; MS (EI, 70 eV): *m/z* (%): 598 (7) [M^+], 553 (15) [M^+-CO_2], 439 (9), 348 (7), 217 (22), 160 (100), 91 (23) [$C_7H_7^+$]; HRMS (EI): calcd for $C_{31}H_{29}DN_4NiO_5$: 597.1632; found 597.1632; elemental analysis calcd (%) for $C_{31}H_{29}DN_4NiO_5$ (598.3): C 62.23, H 5.22, N 9.36; found C 61.94, H 5.12, N 9.22.

Deuterium exchange experiments—Deuteration of (2*S*,2′*S*,1″*R*,2″*R*)-18, 19 and 20 (GP 1): NaH (0.6 mmol, 60% suspension in mineral oil) was added at −25 °C to a stirred solution or suspension of the respective nitro compound (0.3 mmol) in anhydrous CD_3CN/DMF 2:1 (1.5 mL). The mixture was allowed to warm to 20 °C, and stirring was continued for an additional 50 min. The reaction was quenched with 0.5 M D_2SO_4 in D_2O (1.2 mL), and the mixture was diluted with water (15 mL). The crude products were separated by filtration or extraction with Et_2O and purified by recrystallization or distillation.

Deuteration of (2*S*,2′*S*,1″*R*,2″*R*)-18: The nickel complex (2*S*,2′*S*,1″*R*,2″*R*)-**18** (250 mg, 0.42 mmol) was treated with NaH (38 mg, 60% suspension in mineral oil, 0.95 mmol) in CD_3CN/DMF (2:1, 1.5 mL) according to GP 1, and the crude product was crystallized from $CHCl_3$/Et_2O to give the deuterated compound (181 mg, 71%; 55% and 65% incorporation of the deuterium label at the 2′- and 2″- positions of the (3-Ncp)Ala fragment, respectively, according to the ^{1}H NMR spectrum).

Deuteration of 19: Compound **19** (300 mg, 0.84 mmol) was treated with NaH (40 mg, 60% suspension in mineral oil, 1.0 mmol) in CD_3CN/DMF (2:1, 1.5 mL) according to GP 1, and the crude product was purified by column chromatography ($R_f=0.41$, EtOAc/hexane 1:14) to give the deuterated product 2′-[D]**19** (215 mg, 72%; D content >99%, as determined by MS) as a colorless solid. $[\alpha]_D^{20}=63.4$ ($c=0.61$, $CHCl_3$) [lit. for the non-deuterated **19**:[3] $[\alpha]_D^{20}=66.2$ ($c=1.00$, $CHCl_3$)]; ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=1.31$ (dd, $J=6.0$, 7.5 Hz, 1H, 3′-H_a), 1.84 (dd, $J=5.8$, 10.5 Hz, 1H, 3′-H_b), 2.17–2.31 (m, 1H, 1′-H), 3.08 (dd, $J=5.6$, 10.1 Hz, 1H, 1-H), 3.30 (dd, $J=4.5$, 10.3 Hz, 1H), 7.22–7.43 (m, 15H, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta=15.3$ (−, C-3′), 25.2 (+, C-1′), 57.3 (t, $J=27.7$ Hz, C-2′), 61.6 (−, C-1), 86.7 (C_{quat}, CPh_3), 127.1, 127.9, 128.4 (+, Ar-C), 143.4 (C_{quat}, Ar−C); IR (KBr): $\tilde{\nu}=3111$, 3089, 3062, 3029, 2969, 2942, 2893, 2871, 1524, 1355, 1218, 1115, 1033 cm^{-1}; MS (EI, 70 eV): *m/z* (%): 360 (26) [M^+], 283 (12) [$M^+-C_6H_5$], 243 (100) [CPh_3^+], 165 (44) [CPh_2^+]; HRMS (EI): calcd for $C_{23}H_{20}DNO_3$: 360.1584; found 360.1584; elemental analysis calcd (%) for $C_{23}H_{20}DNO_3$ (360.4): C 76.65, H 6.15, N 3.89; found C 76.47, H 6.06, N 3.72.

Deuteration of 20: Nitrocyclopropane (**20**) (255 mg, 2.93 mmol) was treated with NaH (152 mg, 60% suspension in mineral oil, 3.80 mmol) in CD_3CN/DMF (2:1, 2.4 mL) according to GP 1. The reaction was quenched with 5% acetic acid in D_2O (3 mL), and the mixture was extracted with Et_2O (3×10 mL). The combined organic layers were washed with water (3×10 mL), brine (2×10 mL), dried, filtrated through a short (5 cm) column with alumina and concentrated under atmospheric pressure at a bath temperature <45 °C. The residue was subjected to bulb-to-bulb distillation under reduced pressure (125 Torr) and the fraction collected at a bath temperature >50 °C was 1-[D]**20** (72 mg, 90% purity, 25%; D content >97%, as determined by ^{1}H NMR) as a colorless liquid. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta=1.12$–1.22 (m, 2H), 1.62 (dd, $J=$ 5.3, 5.3 Hz, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta=11.4$ (−), 53.8 (t, $J=$ 28.3 Hz); MS (EI, 70 eV): *m/z* (%): 46 (38) [NO_2^+], 42 (100) [$C_3H_4D^+$].

(2*S*,1′*RS*,2′*RS*)-2,2′-dideuterio-3-(2′-nitrocyclopropyl)alanine, (2*S*)-2,2′-[D_2]15: 6 M HCl (32 mL) was added to a suspension of finely ground (2*S*,2′*S*)-2′,2″-[D_2]**18** (3.87 g, 6.47 mmol) in refluxing methanol (16 mL). The mixture was gently heated under reflux for 10 min. The resulting green syrup was then concentrated, the residue was taken up with ice-cold water (50 mL), the precipitate was filtered off, and washed with cold water (20 mL). The filtrate was combined with the washings, its pH was carefully adjusted to 6 with aqueous ammonia, and then it was extracted with $CHCl_3$ (3×50 mL). Preswollen Amberlite IRA-120 in the H^+ form (90 mL) was added to the water fraction, and the mixture was stirred for 15 h. The ion-exchange resin was filtered off, washed with water until the eluent reached pH 5 and treated with 10% aqueous NH_3 (2×150 mL) for 2 h. The combined filtrates were concentrated under reduced pressure to give a colorless solid, which was dissolved in hot water (10 mL). The solution was filtered and diluted with EtOH (20 mL). The formed precipitate was filtered off to give (2*S*)-2,2′-[D_2]**15** (770 mg, 68%; 60% of (2*S*,1′*S*,2′*R*)-isomer according to its ^{13}C NMR spectrum) as a colorless solid. The ^{1}H-spectrum showed 88% of deuterium at C-2 and 58% at C-2′, respectively. According to the ESI-MS spectrum, the D_2 and D contents were 52 and 42% respectively. M.p. 193–195 °C (decomp.); $[\alpha]_D^{20}=$ 3.3 ($c=0.3$, H_2O); ^{1}H NMR (250 MHz, D_2O): mixture of diastereo- and isotopomers: $\delta=1.24$–1.39 (m, 1H, 3′-H), 1.73–2.25 (m, 4H, 3-H, 1′-H, 3′-H), 3.80 (dd, $J=5.9$, 5.9 Hz, 1H, 2-H), 4.18–4.26 (m, 1H, 2′-H); ^{13}C NMR (62.9 MHz, D_2O): $\delta=20.4$, 20.5, 20.7, 20.9 (−, C-3′), 24.3, 24.4, 24.6, 24.7 (+, C-1′), 33.7, 33.8 (−, C-3), 55.9 (t, $J=23.4$ Hz, C-2), 61.6, 61.7 (+, C-2′), 176.0, 176.1 (C_{quat}, C-1); IR (film): $\tilde{\nu}=3418$, 3034, 2102, 1598, 1530, 1440, 1394, 1365 cm^{-1}; MS (ESI): positive mode: *m/z* (%): 221/220 (40/30) [M^++2Na−H]; negative mode: *m/z* (%): 175/174 (3/3) [$M-H^+$].

Feeding experiments—General procedure (GP 2): The *Streptomyces griseoflavus* strain W-384 was obtained from Prof. H. Wolf (Stuttgart). The M2Ca agar in a culture tube was inoculated with the spores of the strain W-384 and the tube incubated at 28 °C until the sporulation was completed (grey colored aerial mycelia, orange colored agar, incubation up to three weeks). The tubes were stored at 4 °C.

Aliquots of this material (2×2 mL, not older than 2 months) were used to inoculate medium M6 (2×50 mL) in two 250 mL flasks, and the flasks were incubated in a shaking incubator at 27 °C and 120 rpm for 31 h. The seed culture was transferred into medium M10 [900 mL; just before inoc-

ulation the vitamins solution (1 mL) and five drops of olive oil (to prevent foaming) were added] in a 1 L fermenter.

The production culture was incubated at 27 °C, with a stirring rate of 700 rpm and an air flow at a rate of 1.6 vvm over 20 h. pH 7 was maintained automatically by addition of 2 M citric acid. The temperature was then decreased to 20 °C. After 24 h, the solution of the respective synthetic precursor substance in distilled, sterilized water (50 mL; the pH of this solution was adjusted to 7.0 with 0.5 M NaOH or 0.5 M HCl) was added over a period of 10 h. After 66 h, the production culture was harvested by filtration. The separated mycelium was lyophilized, homogenized and extracted with ethyl acetate (2×250 mL) by applying sonification for 15 min. The collected organic extracts were filtered and concentrated under reduced pressure. The resulting crude material was purified twice by column chromatography (acetone/hexane 2:3, $R_f = 0.33$ and $CHCl_3/CH_3OH$ 9:1, $R_f = 0.63$). The thus prepared deuterated hormaomycins had purities of about 90% or higher, as determined by HPLC analyses.

Feeding experiment with (2*S*,4*R*)-2′,4-[D_2]5: It was carried out according to GP 2 with the mixture of (2*S*,4*R*)-2′,4-[D_2]**5** and NaCl (430 mg, max. 240 mg of (2*S*,4*R*)-2′,4-[D_2]**5**, 1.53 mmol) to give the deuterated hormaomycin **1b** (10.5 mg). ^{1}H NMR (600 MHz, $CDCl_3$) (see Figure 2): decreased: 5.65 [0.25 H, 2′-H, (4-Pe)Pro], 3.29 [0.15 H, 4-H, (4-Pe)Pro]; MS (ESI): positive mode: *m*/*z* (%): 1154 [M^++Na]; negative mode: *m*/*z* (%): 1130 [$M-H^+$] (undeuterated hormaomycin **1a**: MS (ESI): positive mode: *m*/*z* (%): 1152 [M^++Na]; negative mode: *m*/*z* (%): 1128 [$M-H^+$]).

Feeding experiment with (2*S*)-3,3-[D_2]15: It was carried out according to GP 2 with a mixture of (2*S*)-3,3-[D_2]**15** and LiCl (396 mg, max. 197 mg of (2*S*)-3,3-[D_2]**15**, 1.11 mmol) to give the deuterated hormaomycin **1c** (8.1 mg). ^{1}H NMR (500 MHz, $CDCl_3$) (see Figure 2): decreased: −0.07, 0.48 [2 H, 3-H, (3-Ncp)Ala I], 1.60, 1.81 [2 H, 3-H, (3-Ncp)Ala II]; D NMR (76.7 MHz, $CDCl_3$): 0.34, 1.69 (1:1); MS (ESI): positive mode: *m*/*z* (%): 1156 [M^++Na]; negative mode: *m*/*z* (%): 1132 [M^+-H].

Feeding experiment with (2*S*)-2,2′-[D_2]15: It was carried out according to GP 2 with (2*S*)-2,2′-[D_2]**15** (425 mg, 2.41 mmol) to give the deuterated hormaomycin **1d** (10.0 mg). ^{1}H NMR (600 MHz, $CDCl_3$) (see Figure 2): decreased: 5.14 [0.5 H, 2-H, (3-Ncp)Ala II], 4.05 [0.5 H, 2′-H, (3-Ncp)Ala II], 2.94 [0.4 H, 2′-H, (3-Ncp)Ala I]; MS (ESI): positive mode: *m*/*z* (%): 1155/1154/1153 [M^++Na]; negative mode: *m*/*z* (%): 1131/1130/1129 [M^+-H].

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