The Biosynthesis of 3-(*trans*-2-Nitrocyclopropyl)alanine, a Constituent of the Signal Metabolite Hormaomycin

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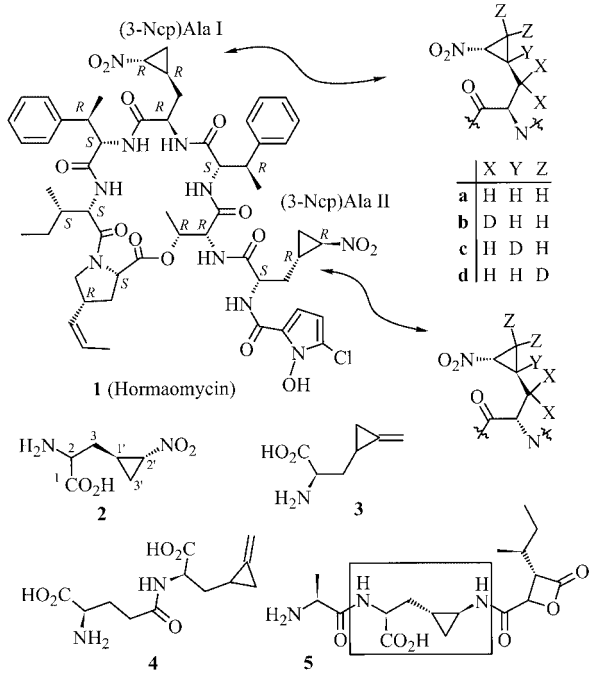
Feeding experiments with *Streptomyces griseoflavus* using deuterium-labeled racemic 3,3-[D_2]- (**6b**), 4,4-[D_2]- (**6c**), 5,5-[D_2]- (**6d**), and 6,6-[D_2]-lysine (**6e**), and 3-amino-5-(2-amino-1,1-dideuterioethyl)-4,5-dihydrofuran-2-one dihydrochloride (**34**·2HCl) were carried out in order to obtain detailed information about the hitherto unknown biosynthetic pathway from lysine to the unusual amino acid 3-(*trans*-2′-nitrocyclopropyl)alanine [(3-Ncp)Ala] (**2**), which is a building block of hormaomycin **1a**. The corresponding lysine dihydrochlorides were prepared in 33, 24, 19, and 30% overall yield, respectively, along a new efficient general synthetic route applying an alkylation of the lithium enolate of O′Donnel's glycine equivalent **7** as a key step. In the attempted preparation of 5,5-[D_2]-4-hydroxylysine (**29**), the respective γ-lactone (**34**·2 HCl) was obtained in five steps with 10% overall yield. The distribution of isotope labels in hormaomycins **1b–d** led to the formulation of a reasonable cyclization mechanism of 2-amino-4-hydroxy-6-(hydroxyimino)hexanoic acid, an ω-oxime analogue of 4-hydroxylysine.

Introduction

Among the natural products containing a cyclopropyl group, the peptide lactone hormaomycin **1a**, isolated from *Streptomyces griseoflavus* (strain *W 384*), which shows some selective antibacterial activity and was identified as a signal metabolite of streptomycetes,[1,2] is especially intriguing. The molecule of **1a** contains two moieties of the previously unknown 3-(*trans*-2′-nitrocyclopropyl)alanine [(3-Ncp)Ala] (**2**). The synthesis of the two diastereomeric building blocks of **1**[3] and an improved approach to their synthetic precursors[4] have previously been reported. The amino acid **2**, along with hypoglycines A **3** and B **4**,[5] as well as 3-(*trans*-2′-aminocyclopropyl)alanine [(3-Acp)Ala] in belactosine A **5**,[6] belongs to the group of very rare 4,5-methanoamino acids, the biosynthesis of which has not yet attracted much attention.[7]



Results and Discussion

First Feeding Experiments with Labeled Methionine and Lysines

In the first feeding experiment with (*S*)-[^{13}C-methyl]-methionine performed to elucidate the biosynthesis of Hormaomycin **1a**, no ^{13}C enrichment in any position of the cyclopropyl rings of the isolated metabolite was observed.[8] This excluded the first conceived possibility that the cyclopropyl ring in (3-Ncp)Ala would be formed by cyclopropanation of a nitroethenyl derivative by (*S*)-adenosylmethionine, which in some cases acts as a methylene donor in the biosynthesis of cyclopropane derivatives.[7] Based on a

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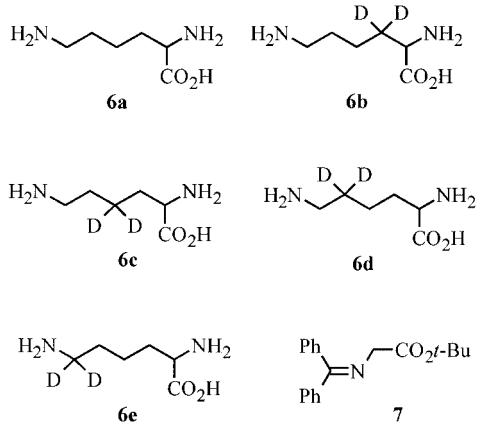
simple count of its C atoms, the proteinogenic diamino acid lysine **6a** might be a reasonable biosynthetic precursor to 3-(nitrocyclopropyl)alanine (**2**), in which case the amino group at C-6 would have to be converted into the nitro group at C-2′ in **2**. Indeed, a successful feeding experiment[8] with commercially available racemic [1-^{13}C]-lysine to give hormaomycin with a ^{13}C label in both (3-Ncp)Ala moieties rigorously proved this hypothesis. It is noteworthy that the ^{13}C enrichment at C-1, 10.4% in (3-Ncp)Ala I and 45.7% in the (3-Ncp)Ala II, was not the same. This experiment, however, could not prove whether the nitro group of (3-Ncp)Ala originates from the ω-amino group of lysine or not. This information was obtained from a feeding experiment with [6-^{15}N]-lysine: in this case the isolated hormaomycin showed in its FAB and MALDI-TOF mass spectra a significant increase in intensity of the [M^+ +2] peak relative to those of unlabeled hormaomycin **1a**. The cleanly observable splitting of the respective C-2′ signals in its ^{13}C NMR spectrum due to ^{15}N−^{13}C coupling (^1J = 14 Hz) also corroborates that the ^{15}N enrichment had taken place at both nitro groups of **1a**. Thus, the ω-amino group of lysine is retained in an oxidized form.

In view of these results we decided to synthesize selectively deuterium-labeled racemic lysines in order to obtain more detailed information about the intermediates along the biosynthetic pathway to the (3-Ncp)Ala residues in **1**. Feeding experiments with appropriately deuterium-labeled racemic 3,3-[D_2]- (**6b**), 4,4-[D_2]- (**6c**), 5,5-[D_2]- (**6d**), and 6,6-[D_2]-lysine (**6e**) were carried out, and the loss or the preservation of deuterium in the different positions of the (3-Ncp)Ala moieties in the isolated hormaomycins were used to propose a cyclization mechanism, which is compatible with all of the experimental facts.

Preparation of Various Deuterium-Labeled Lysines

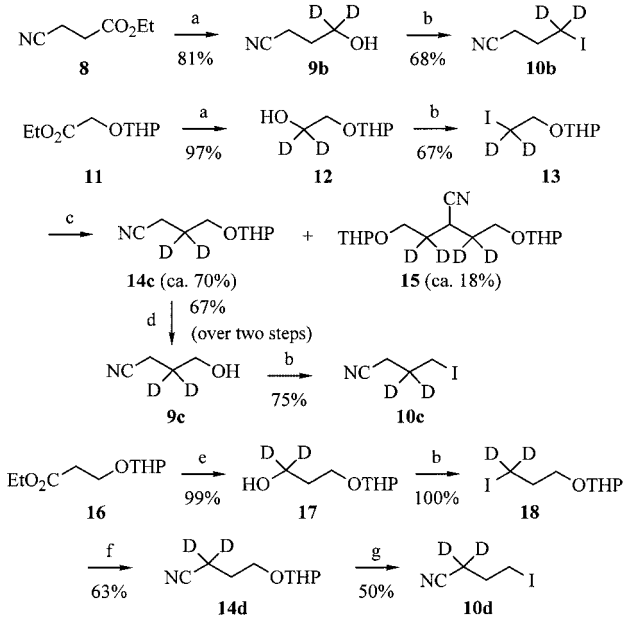
Although the deuterium-labeled racemic 3,3-[D_2]- (**6b**),[9,10] 4,4-[D_2]- (**6c**),[10] 5,5-[D_2]- (**6d**),[11a] and 6,6-[D_2]-lysine (**6e**)[10,11] had previously been prepared, more efficient and general approaches to all four compounds were elaborated. Firstly, in all four cases the incorporation of deuterium should be achieved by reduction of an ester functionality with lithium aluminum deuteride, which is the least expensive source of deuterium. Secondly, one of the most versatile methods to introduce an amino acid fragment into a molecule, namely by nucleophilic substitution of an appropriate leaving group such as a halide, with the lithium enolate of **7** (O′Donnel's glycine equivalent[12]), appeared to be most attractive. Thirdly, catalytic hydrogenation of a cyano group ought to be applicable to install a 6-amino substituent in **6b**−**d**.

The appropriate starting materials, the deuterated 4-iodobutyronitriles **10b**−**d** were each synthesized in a few steps from commercially available ethyl 3-cyanopropanoate **8**, THP-protected ethyl glycolate **11** (easily prepared from inexpensive glycolic acid in two steps[13]) and THP-protected ethyl 3-hydroxypropanoate **16** (obtained from freshly prepared ethyl 3-hydroxypropanoate[14] using a common

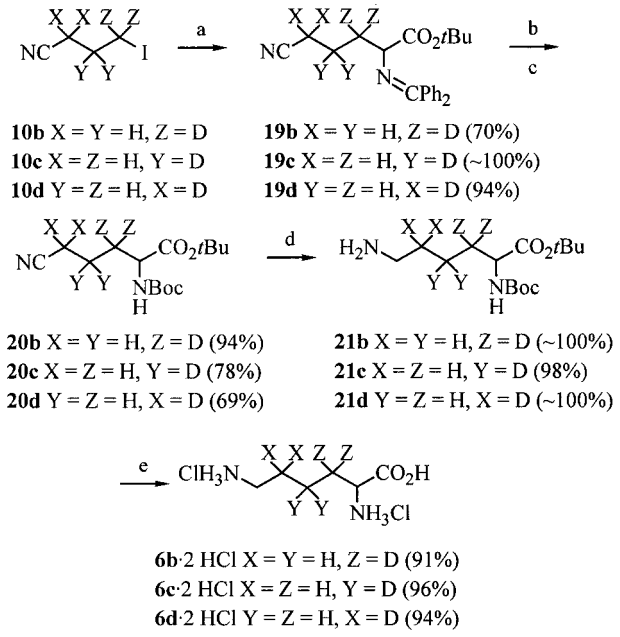


method, see Exp. Sect.), respectively, by applying essentially the same set of standard procedures.

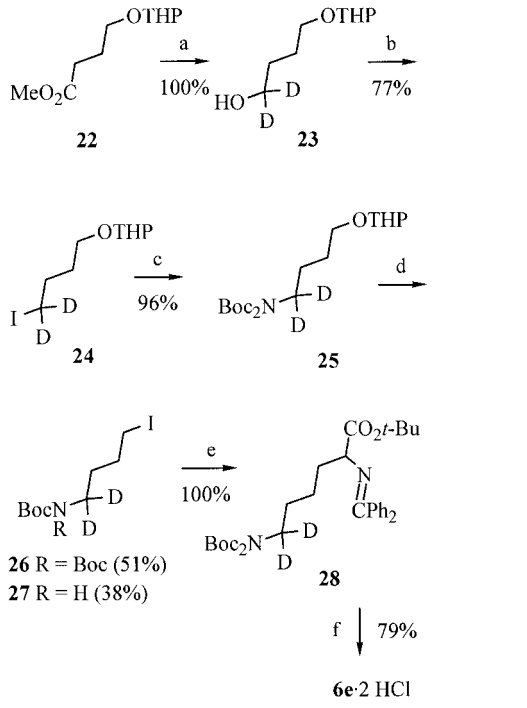
By adding a solution of lithium aluminumdeuteride to the cyano ester **8**, selective reduction of the ester function could be achieved. Two different methods, treatment with either iodine in the presence of triphenylphosphane and imidazole according to Corey et al.[15] or with the iodine-bis(diphenylphosphanyl)ethane reagent,[16] could be employed to transform the hydroxy to an iodo substituent in the presence of a tetrahydropyranyloxy group or convert the latter directly into an iodo substituent. The alkylation of lithiated acetonitrile with the iodide **13** under modified conditions[17] gave the desired product **14c**, along with about 18% of the bis-alkylated nitrile **15**, which could be separated by chromatography from **14c** only after deprotection. Because of this, the transformation of **14c** into **10c** was done in two steps (Scheme 1). The overall yields of the



Scheme 1. Preparation of dideuterium-labeled 4-iodobutyronitriles **10b**−**d**: a) $LiAlD_4$ (inverse addition of a solution in THF over 1 h, Et_2O, 20 °C, 2 h; b) Ph_3P, imidazole, I_2, Et_2O/MeCN, 0 °C, 2 h; c) $LiCH_2CN$, THF, 0 °C, 40 min; d) PPTS (pyridinium *p*-toluenesulfonate), MeOH, 50 °C, 1.5 h; e) $LiAlD_4$, Et_2O, 34 °C, 2 h; f) NaCN, DMSO, 40 °C, 3 h; g) I_2, dppe [1,2-bis(diphenylphosphanyl)-ethane], CH_2Cl_2, 0 → 20 °C, 2 h



Scheme 2. Preparation of the dideuterium-labeled lysines **6b**–**d**: a) **7**-Li, THF, −78→20 °C, 56 h; b) Et_2O/1 N aqueous HCl, 20 °C, 1–3 h; c) Boc_2O, MeOH, 20 °C, 12 h; d) Raney Ni, H_2 (4 bar), NH_3/MeOH, 20 °C, 15 h–3 d; e) 1 N aqueous HCl, 20 °C, 2 d



Scheme 3. Preparation of 6,6-dideuteriolysine **6e**: a) $LiAlD_4$, Et_2O, 34 °C, 2 h; b) Ph_3P, imidazole, I_2, Et_2O/MeCN, 0 °C, 2 h; c) $KNBoc_2$, DMF, 20 °C, 4 h; d) I_2, dppe, CH_2Cl_2, 0→20 °C, 2 h; e) **7**-Li, THF, −78→20 °C, 56 h; f) 1 N aqueous HCl, Et_2O, 20 °C, 2 d

prepared iodonitriles **10b**–**d** were 55, 33 and 31%, respectively.

The nucleophilic substitution on all the iodonitriles **10b**–**d** with the lithium enolate of O′Donnel's glycine equivalent **7** gave high yields (70, approximately 100 and 94%) of the protected amino acids **19b**–**d** (Scheme 2). An optimized sequence of all the substituent transformations was developed for the nonlabeled iodonitrile **10a**, prepared in an analogous manner to compound **10b**.

Surprisingly, deprotection of the nonlabeled **19a** (X = Y = Z = H) by treatment with 1 N aqueous HCl solution followed by Pd/C-catalyzed hydrogenation under acidic conditions yielded pipecolinic acid hydrochloride[18] as the sole product, and no reaction was observed with the protected nonlabeled **19a** upon attempted hydrogenation under the same conditions. Yet, when the diphenylmethyleneamino group was first deprotected and reprotected with a *tert*-butoxycarbonyl group,[19] the cyano group in the resulting nonlabeled amino acid derivative **20a** (X = Y = Z = H) was smoothly transformed into an aminomethyl moiety in very high yield upon Raney Ni-catalyzed hydrogenation under basic conditions (methanolic ammonia).[20] These conditions were also used for the dideuterionitriles **19b**–**d** and, after the usual one-pot deprotection, the dideuteriolysines **6b**–**d** were obtained as dihydrochlorides in 33, 24, and 19% overall yield, respectively.

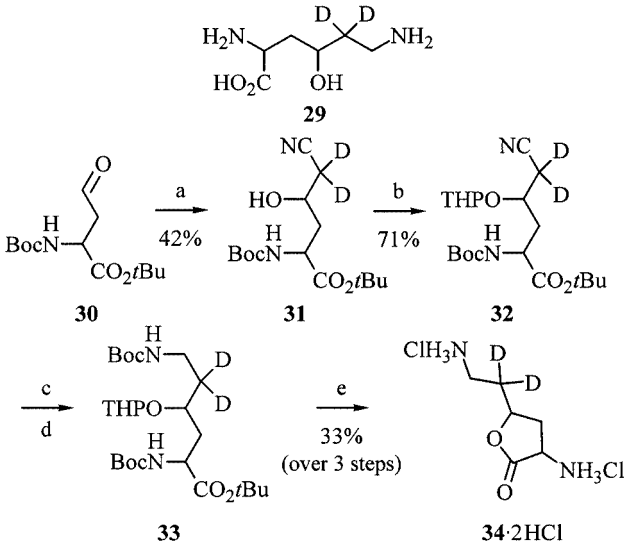
Because the very same sequence could not be used for the synthesis of 6,6-dideuteriolysine **6e**, it was prepared along a similar route in six steps from the known THP-protected methyl γ-hydroxybutyrate **22**[21] (Scheme 3).

The 6-amino substituent en route to lysine had previously been introduced by nucleophilic substitution of iodide in a protected 4-iodobutanol like **24** with potassium bis(*tert*-butoxycarbonyl)amide.[22] This method turned out to be rather efficient when applied to **24**. In spite of the fact that the resulting **25** was partially deprotected upon transformation to the iodide **26** with the I_2·dppe reagent (Scheme 3),[23] the overall yield of **6e** as the dihydrochloride after further conversion via **28** was 30%.

These new general synthetic approaches to isotopomers **6b**–**e** appear to be more efficient than those previously reported,[9–11] in which the lysines **6b**,[10] **6c**,[10] and **6e**[11b] were prepared over 7, 7, and 8 steps, respectively, in overall yields of 2.5, 2.3 and 17%, respectively.[11c]

The previously reported preparation of racemic nonlabeled 4-hydroxylysine by photochlorination of lysine in hydrochloric acid solution[24] could not be reproduced.[25a] Therefore, an alternative six-step preparation of **29** starting from the known *tert*-butyl 2-(*tert*-butoxycarbonylamino)-4-oxobutyrate (**30**)[26] was developed (Scheme 4).

Because of its reversibility, the aldol reaction of the aldehyde **30** with lithiated trideuterioacetonitrile turned out to at best furnish a mixture of **30** and **31** in 48 and 42% yield, respectively, even when the transformation was performed in the presence of anhydrous cerium(III) chloride.[27] However, as the starting material **30** can easily be recycled from the reaction mixture, the overall yield of **30** can be increased by several repetitions of the aldol reaction. After THP-protection of the hydroxy group in **31** and Raney Ni catalyzed



Scheme 4. Attempted preparation of racemic 5,5-dideuterio-4-hydroxylysine (**29**): a) $LiCD_2CN$, $CeCl_3$, THF, −78 °C, 20 min; b) DHP (3,4-dihydro-2*H*-pyran), PPTS (pyridinium *p*-toluenesulfonate), CH_2Cl_2, 20 °C, 24 h; c) Raney Ni, H_2 (4 bar), NH_3/MeOH, 20 °C, 3 h; d) Boc_2O, MeOH, 20 °C, 9 h; e) 1 N aqueous HCl, MeOH, 20 °C, 2 d

hydrogenation of the cyano group in **32** under basic conditions, the second amino group was also protected with a *tert*-butyloxycarbonyl (Boc) group to give the completely protected hydroxydiamino acid **33**. With this exhaustive protection, the 4-hydroxylysine derivative **33** could more easily be purified by chromatography, and, in addition, the spontaneous cyclization to the γ-lactone by way of a free hydroxy function under the basic conditions of hydrogenation that was observed in a similar preparation,[25a] could be prevented. However, upon complete deprotection of **33** under acidic conditions in a one-pot operation, the lactone 3-amino-5-(2-amino-1,1-dideuterioethyl)-4,5-dihydrofuran-2-one dihydrochloride (**34**·2HCl) was obtained as the sole product.[28]

Feeding Experiments with Deuterium-labeled Lysines

The first feeding experiments were carried out with the 3,3-[D_2]-lysine **6b** to rigorously exclude the possibility that the biosynthesis of (3-Ncp)Ala might proceed via an intermediate with a double bond between C-3 and C-4, which could serve as a precursor to the nitrocyclopropyl ring in close analogy to the postulated biosynthetic pathway to hypoglycine A **3**.[7] The loss of even one deuterium label would confirm this hypothesis. However, the deuterium-labeled hormaomycin **1b** which was obtained from this feeding experiment, according to its ESI mass spectrum, had a molecular mass which was 4 units higher than that of **1a**. The ^{2}H-, ^{13}C- and ^{1}H NMR spectra confirmed that both deuterium labels had been introduced in both (3-Ncp)Ala moieties to the same extent. This proved that no hydrogen is lost from C-3 of lysine during the conversion into (3-Ncp)Ala. Thus, the C-3 position is not involved in the construction of the nitrocyclopropyl ring.

Further information was obtained from a feeding experiment with 4,4-[D_2]-lysine **6c**. The ESI mass spectrum of the isolated hormaomycin shows, besides the [M + Na^+] peak of the unlabeled product, a significant increase in the [M + Na^+ +2] peak, which corroborates the introduction of only two deuterium labels. The ^{1}H NMR spectrum of **1c** clearly shows a decrease in the intensity of the cleanly visible signal for 1′-H of (3-Ncp)Ala I (δ = 0.33 ppm), while the signal of 1′-H of (3-Ncp)Ala II overlaps strongly with other signals. Also, the signals of C-1′ of (3-Ncp)Ala I and (3-Ncp)Ala II had lower intensities relative to the corresponding signals in the ^{13}C NMR spectrum of undeuterated **1a**. This experiment proved that during the biosynthesis of (3-Ncp)Ala only one hydrogen from C-4 of lysine is abstracted.

In order to see whether C-5 of lysine is involved in the formation of the cyclopropyl group of (3-Ncp)Ala, a feeding experiment with 5,5-[D_2]-lysine **6d** was carried out. In this case, the spectroscopic data indicates that a total of four deuterium labels [two for each (3-Ncp)Ala moiety] were retained in the isolated hormaomycin **1d** to a significant degree (about 30−35% according to the ESI mass spectrum). In the ^{1}H NMR spectrum of **1d**, the signal intensities for all four 3′-protons are significantly decreased, and in the ^{13}C NMR spectrum a significant decrease in signal intensity for C-3′ of both (3-Ncp)Ala residues is observed as well as an upfield shift for the respective signals of C-1′ and C-2′. This experiment shows that none of the hydrogens at C-5 of lysine is lost during the biosynthesis of (3-Ncp)Ala. In addition, the result strictly excludes the formation of a double bond between C-4/C-5 or C-5/C-6 at an intermediate stage.

In contrast to the preceding feeding experiments, all of the labels of 6,6-[D_2]-lysine **6e** had been lost in the hormaomycin **1** isolated from an analogous feeding experiment. This means that during the biosynthesis of (3-Ncp)Ala **2** either both hydrogens from C-6 are lost, or an exhaustive exchange of D with H takes place.

In order to test whether 4-hydroxylysine is a biosynthetic intermediate en route from lysine **6a** to (3-Ncp)Ala, a feeding experiment with the racemic 5,5-[D_2]-4-hydroxylysine (**29**) or the synthetically more easily accessible lactone **34** was of prime interest.[29] The feeding of **34** indeed led to deuterium-labeled hormaomycin **1d** as proved by ESI mass and D NMR spectra of the isolated product. The fact that the lactone **34** was accepted as a substrate in the biosynthesis of hormaomycin as well as the loss of one deuterium label from C-4 of 4,4-[D_2]-lysine makes 4-hydroxylysine (and/or its lactone) a highly probable intermediate.

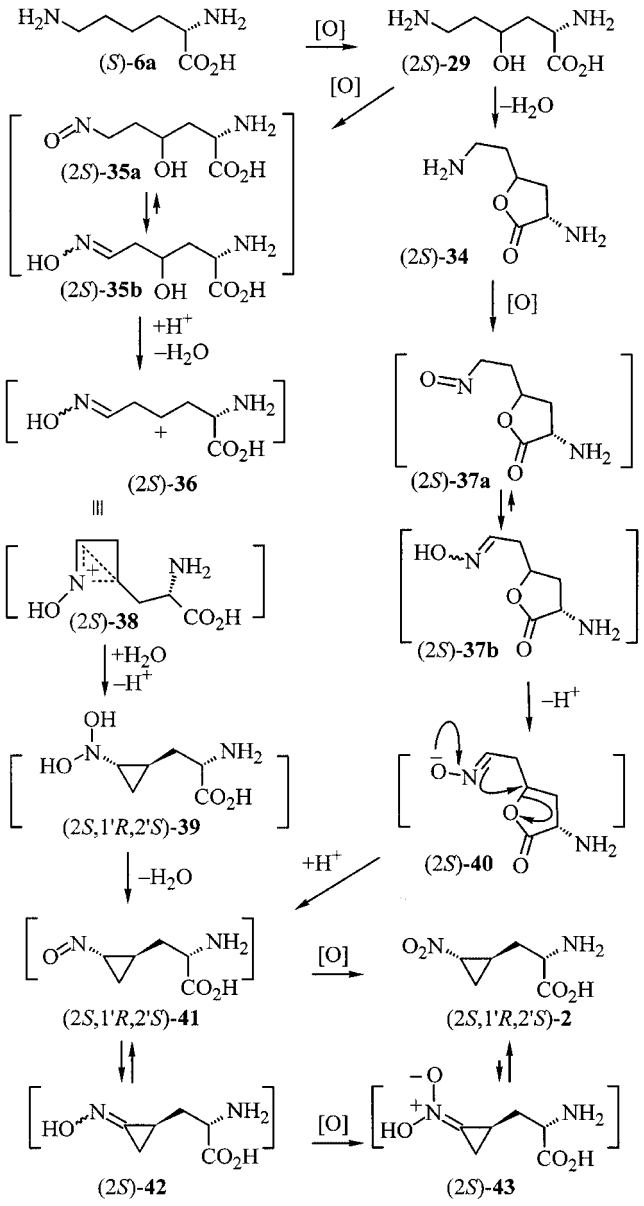
Conclusion

According to the results of the feeding experiments described, one may conclude that (i) lysine is definitely the precursor of both (3-Ncp)Ala moieties, (ii) both hydrogens at the C-3 and C-5 of lysine are retained all along the way to the nitrocyclopropyl ring, (iii) complete loss or exchange

of both hydrogens at C-6 of lysine as well as loss of *one* hydrogen at C-4 of lysine occurs, and (iv) the lactone **34** of 4-hydroxylysine (**29**) is an acceptable substrate, and thus **29** is the most probable intermediate in the biosynthesis of (3-Ncp)Ala from lysine.

Hormaomycin **1a** most probably is biosynthesized by a non-ribosomal peptide synthetase (NRPS, hormaomycin synthetase). The non-proteinogenic amino acids usually are substrates of a multienzyme complex and must be completely biosynthesized before they are assembled to a peptide. This had been proved in the case of 3-(2′-nitrocyclopropyl)alanine (**2**) by feeding a deuterium-labeled racemic sample.[2b,30] Thus, there must be a continuous biosynthetic route from lysine to (3-Ncp)Ala.

Taking into account also that (i) racemic 2-(3′-aminocyclopropyl)alanine is not a substrate for the hormaomycin synthetase and does not affect the biosynthesis of hormaomycin **1a**,[30] and (ii) 6-nitronorleucine [(*S*)-lysine with the oxidized ω-amino group] is accepted by *Streptomyces griseoflavus* strain W 384 as a substrate, but led to novel hormaomycin analogues with intact nitronorleucine (consequently nitronorleucine-containing intermediates have never been observed in the culture broth at any time without addition of this amino acid),[31] both precursors cannot be an intermediate along the route. On the basis of all this, a reasonable biosynthetic pathway for (3-Ncp)Ala that includes a cyclization mechanism can now be proposed (Scheme 5).



Scheme 5. Proposed biosynthetic pathway to 3-(2′-nitrocyclopropyl)-alanine (2*S*,1′*R*,2′*S*)-**2**

In the first step, (*S*)-lysine (*S*)-**6a** must undergo hydroxylation at C-4 to give 4-hydroxylysine (2*S*)-**29** which, in turn, would probably be oxidized at its ω-amino group to give the nitroso compound (2*S*)-**35a**,[32] or cyclized to the lactone **34** followed by oxidation to give the nitrosolactone **37a** as an intermediate. One conceivable route would be via the cationic intermediate (2*S*)-**36** or the azabicyclobutonium relative (2*S*)-**38** of the azahomoallyl cation (2*S*)-**36** formed by oxime tautomer (2*S*)-**35b** of (2*S*)-2-amino-4-hydroxy-6-nitrosohexanoic acid [(2*S*)-**35a**]. Homoallyl cations are capable of cyclizing to cyclopropylmethyl cations;[33] however, in the case of (2*S*)-**36**, such a cyclization would lead to a thermodynamically highly unfavorable intermediate with a positive charge on nitrogen. However, such a ring closure might be facilitated by nucleophilic assistance through attack of water on the azabicyclobutonium cation (2*S*)-**38**[34] followed by dehydration of the formed intermediate **39** to give 3-(2′-nitrosocyclopropyl)alanine (**41**).

A more likely alternative pathway to **41** would be via the oxime anion (2*S*)-**40** formed upon deprotonation of the oxime tautomer (2*S*)-**37b** of the nitrosolactone (2*S*)-**37a** which, by nucleophilic attack, would open the ring of the lactone moiety in **40** with simultaneous formation of the cyclopropyl ring (see ref.[35]) and subsequent reprotonation. Final oxidation of the nitroso group in **41** to the nitro group would then afford (*S*)-(3-Ncp)Ala **2**. The latter transformation could also occur as an NADP-dependent oxidation[36a] of the oxime (2*S*)-**42**, tautomeric to **41**,[36b] to afford the amino acid **2** in its *aci*-form (2*S*)-**43**. This would explain the loss of the deuterium label in the feeding experiment with 6,6-[D_2]-lysine (**6e**). In both tentative pathways the total exchange of deuterium of 6,6-[D_2]-lysine **6e** can also be accounted for by equilibration between the tautomeric nitroso and oxime intermediates **35a**/**35b** and **37a**/**37b**.[37] Epimerization of (*S*)-(3-Ncp)Ala to yield the (*R*)-(3-Ncp)Ala residue in **1a** needs an external epimerase before the assembly or more likely an internal epimerase as part of the hormaomycin synthetase.[2b]

As a result of these efforts a new biosynthetic pathway has been established by which lysine is converted into the 3-(2′-nitrocyclopropyl)alanine (**2**). Whether this pathway can be a model for a biomimetic synthesis of (3-Ncp)Ala, and the characterization of the enzyme for these steps, should be attractive projects for the future.

Experimental Section

General: ^{1}H and ^{13}C NMR spectra were recorded at 250/500/600 (^{1}H) and 62.9/125.7/150.8 MHz [^{13}C, additional DEPT (Distortionless Enhancement by Polarization Transfer)] with a Bruker AM 250, a Varian VXR 500, and a Varian Inova 600 instrument, respectively, in $CDCl_3$ solution, $CHCl_3/CDCl_3$ as internal reference (if not otherwise specified); δ in ppm, J in Hz. MS (EI): Finnigan MAT 95 spectrometer (70 eV); DCI-MS: Finnigan MAT 95, 200 eV, reactant gas NH_3. ESI-MS: Finnigan LCQ. IR: Bruker IFS 66 (FT-IR) spectrometer, measured as KBr pellets, oils between KBr plates. 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. Starting materials: Anhydrous diethyl ether and THF were obtained by distillation from sodium benzophenone ketyl, MeOH from magnesium methoxide, DMF and DMSO from CaH_2, MeCN and C_2H_2 from P_4O_{10}. *tert*-Butyl *N*-(diphenylmethylene)glycinate (**7**),[12a] ethyl (tetrahydropyran-2-yloxy)acetate (**11**),[13] methyl 4-(tetrahydropyran-2-yloxy)butyrate (**22**),[21] and *tert*-butyl 2-(*tert*-butoxycarbonylamino)-4-oxobutyrate (**30**)[26] were prepared according to published procedures. All other chemicals were used as available commercially (Merck, Acrõs, BASF, Bayer, Hoechst, Degussa AG, and Hüls AG). Organic extracts were dried with anhydrous $MgSO_4$. Microbiology: Shaking incubator: Braun BS4. Fermenter, Braun Biostat M. Media (per liter of demineralized 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), trace elements solution: $CaCl_2 \cdot 2\ H_2O$ (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), vitamins solution: 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). 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. After autoclaving, the vitamins solution (1 mL/L) was added to M10 after sterile filtration. All ingredients were purchased from Merck, Gibco, Difco, Sigma, Riedel de Haen and Henselwerk GmbH. (*RS*)-[1-^{13}C]-Lysine (99%) and (*RS*)-[6-^{15}N]-lysine (99%) were purchased from CamproScientific.

Ethyl 3-(Tetrahydropyran-2-yloxy)propanoate (16): To a solution of freshly prepared ethyl 3-hydroxypropanoate[14] (54.34 g, 0.46 mol) in anhydrous CH_2Cl_2 (180 mL) was added 3,4-dihydro-2*H*-pyran (DHP, 53.0 g, 0.63 mol) and then, under vigorous stirring and intensive cooling, two drops of concentrated hydrochloric acid was added. After the strongly exothermal reaction was completed, the reaction mixture was stirred at ambient temperature for an additional 1 h, then the reaction was quenched with $NaHCO_3$ (15.0 g, 0.18 mol), and the mixture stirred for 30 min. The mixture was filtered, dried, concentrated under reduced pressure and distilled in vacuo to give **16** (73.2 g, 79%) as a colorless liquid, b.p. 94−96 °C (0.6 mbar). ^{1}H NMR: δ = 1.20 (t, J = 7.1 Hz, 3 H, CH_3), 1.45−1.57 (m, 4 H, 4′-H, 5′-H), 1.59−1.76 (m, 2 H, 3′-H), 2.53 (t, J = 6.0 Hz, 2 H, 2-H), 3.41−3.57 (m, 1 H, 6′-H), 3.61−3.67 (m, 1 H, 6′-H), 3.75−3.82 (m, 1 H, 3-H), 3.84−3.98 (m, 1 H, 3-H), 4.11 (q, J = 7.1 Hz, 2 H, OCH_2), 4.55−4.58 (m, 1 H, 2′-H) ppm. ^{13}C NMR: δ = 14.1 (+, CH_3), 19.1 (−, C-4′), 25.3 (−, C-5′), 30.3 (−, C-3′), 35.0 (−, C-2), 60.3 (−, O*C*H_2CH$_3$), 61.9 (−, C-3), 62.8 (−, C-6′), 98.6 (+, C-2′), 171.5 (C_{quat}, C=O) ppm.

Reduction of Esters 8, 11, 16 and 22 with $LiAlD_4$. General Procedure (GP) 1: GP1a: To a stirred solution of the corresponding ester (50 mmol) in anhydrous diethyl ether (150 mL) was added $LiAlD_4$ (25 mmol) as a solution in THF at ambient temperature over a period of 1 h under Ar. After this, the reaction mixture was stirred at the same temperature for an additional 2 h, cooled to 10 °C, the reaction was quenched with saturated Na_2SO_4 solution, and the organic solution was dried and concentrated under reduced pressure. The resulting alcohols were purified by column chromatography or used without further purification. **GP1b:** To a vigorously stirred suspension of $LiAlD_4$ (25 mmol) in anhydrous diethyl ether (200 mL) was added dropwise a solution of the corresponding ester (47.5 mmol) in Et_2O (50 mL) under Ar. The resulting mixture was stirred under reflux for 2 h and then worked up according to **GP1a**.

4,4-Dideuterio-4-hydroxybutyronitrile (9b): Alcohol **9b**[38] (1.87 g, 81%) was obtained from the cyano ester **8** (3.35 g, 26.3 mmol) and $LiAlD_4$ (14.8 mmol, 16.1 mL of a 0.92 N solution in THF) according to GP1b, after column chromatography (30 g of silica gel, 3 × 15 cm column, Et_2O, R_f = 0.34) as a colorless oil. ^{1}H NMR: δ = 1.67 (t, J = 7.0 Hz, 2 H, 3-H), 2.32 (t, J = 7.0 Hz, 2 H, 2-H), 3.56 (s, 1 H, OH) ppm. ^{13}C NMR: δ = 13.0 (−, C-2), 27.2 (−, C-3), 58.6 (quint, J_{CD} = 22.0 Hz, C-4), 119.5 (C_{quat}, CN) ppm.

1,1-Dideuterio-2-(tetrahydropyran-2-yloxy)ethanol (12): Alcohol **12**[39] (28.71 g, 97%) was obtained from the ester **11** (37.64 g, 0.2 mol) and $LiAlD_4$ (0.1 mol, 91.7 mL of a 1.09 N solution in THF) according to GP1b as a viscous liquid which was used without further purification. ^{1}H NMR: δ = 1.46−1.54 (m, 4 H, 4′-H, 5′-H), 1.61−1.78 (m, 2 H, 3′-H), 3.25 (s, 1 H, OH), 3.41−3.50 (m, 1 H, 6′-H), 3.59 (d, J = 11.3 Hz, 1 H, 2-H), 3.70 (d, J = 11.3 Hz, 1 H, 2-H), 3.80−3.88 (m, 1 H, 6′-H), 4.51 (t, J = 2.6 Hz, 1 H, 2′-H) ppm. ^{13}C NMR: δ = 19.7 (−, C-4′), 25.0 (−, C-5′), 30.5 (−, C-3′), 61.1 (quint, J_{CD} = 19.9 Hz, C-1), 62.9 (−, C-6′), 70.1 (−, C-2), 99.8 (+, C-2′) ppm.

1,1-Dideuterio-3-(tetrahydropyran-2-yloxy)propanol (17): Alcohol **17** (12.15 g, 99%) was obtained from the ester **16** (15.2 g, 75.1 mmol) and $LiAlD_4$ (1.66 g, 39.5 mmol) according to GP1b as a viscous liquid. ^{1}H NMR: δ = 1.42−1.52 (m, 4 H, 4′-H, 5′-H,), 1.59−1.70 (m, 4 H, 2-H, 3′-H), 3.36−3.47 (m, 3 H, 3-H, OH), 3.71−3.82 (m, 2 H, 6′-H), 4.40−4.51 (m, 1 H, 2′-H) ppm. ^{13}C NMR: δ = 19.4 (−, C-4′), 25.0 (−, C-5′), 30.4 (−, C-3′), 31.7 (−, C-2), 59.4 (quint, J_{CD} = 26.0 Hz, C-1), 62.3 (−, C-3), 65.2 (−, C-6′), 98.9 (+, C-2′) ppm. MS (CI): *m*/*z* (%) = 180 (95) [M + NH_4^+], 163 (5) [M + H^+].

1,1-Dideuterio-4-(tetrahydropyran-2-yloxy)butanol (23): Alcohol **23** (12.15 g, 99%) was obtained from methyl 4-(tetrahydropyran-2-yloxy)butyrate (**22**) (10.1 g, 50.0 mmol) and $LiAlD_4$ (2.10 g, 50.0 mmol) according to GP1b as a viscous liquid. IR: $\tilde{\nu}$ = 3429 cm^{-1}, 2941, 2869, 1352, 1121, 1076, 1033. ^{1}H NMR: δ = 1.51−1.59 (m, 4 H, 2-H, 3-H), 1.62−1.81 (m, 6 H, 3′-H, 4′-H, 5′-H), 2.15 (br. s, 1 H, OH), 3.37−3.53 (m, 2 H, 4-H), 3.74−3.89 (m, 2 H, 6′-H), 4.56 (t, J = 3.3 Hz, 1 H, 2′-H) ppm. ^{13}C NMR: δ = 19.5 (−, C-4′), 25.3 (−, C-5′), 26.5 (−, C-3), 29.8 (−, C-2), 30.6 (−, C-3′), 62.0 (quint, J_{CD} = 20.0 Hz, C-1), 62.3 (−, C-4), 67.5 (−, C-6′), 98.8 (+, C-2′) ppm. MS (CI): *m*/*z* (%) = 370 (3) [2M + NH_4^+], 194 (100) [M + NH_4^+], 177 (3) [M + H^+]. For the NMR spectra of the non-deuterated compound see ref.[40]

Preparation of Iodides 10b−d, 13, 18, 24 and 26. General Procedure (GP) 2: GP2a: Iodine (5.07 g, 20 mmol) was added in small por-

tions over a period of 30 min to an efficiently cooled (0 °C) solution of the respective alcohol (10.1 mmol), Ph_3P (4.71 g, 18 mmol) and imidazole (1.29 g, 18.9 mmol) in a mixture of anhydrous MeCN (20 mL) and Et_2O (30 mL) under Ar. Stirring was continued for 2 h at 0 °C, the reaction mixture was diluted with Et_2O (150 mL), washed with 20% $Na_2S_2O_3$ aqueous solution (2 × 100 mL) and brine (100 mL), dried, and concentrated under reduced pressure. The residue was thoroughly extracted with hexane (100 mL) by vigorous stirring for 1 h in the dark. The solution was filtered and concentrated under reduced pressure. The residue was purified by column chromatography or used without further purification. **GP2b:** To a solution of 1,2-bis(diphenylphosphanyl)ethane (dppe) (12.0 mmol) in anhydrous CH_2Cl_2 (30 mL) was added a solution of iodine (12.0 mmol) in CH_2Cl_2 (60 mL) at −10 °C over a period of 30 min under Ar. After additional stirring for 30 min, a solution of the corresponding THP-protected alcohol (10.0 mmol) in CH_2Cl_2 (15 mL) was added dropwise at 0 °C, and the resulting mixture was warmed to ambient temperature over a period of 2 h. The reaction mixture was poured into hexane/Et_2O (2:1) mixture (250 mL), filtered through a pad of Celite (3 cm), and concentrated under reduced pressure. The product was purified by column chromatography.

4,4-Dideuterio-4-iodobutyronitrile (10b): Iodide **10b**[41] (9.65 g, 68%) was obtained from the alcohol **9b** (6.24 g, 71.6 mmol), PPh_3 (19.7 g, 75.1 mmol), imidazole (5.12 g, 75.2 mmol) and I_2 (19.1 g, 75.3 mmol) according to GP2a as a slightly yellow oil after column chromatography (150 g of silica gel, 6 × 15 cm column, hexane/Et_2O, 1:1, R_f = 0.35). IR: $\tilde{\nu}$ = 2942 cm^{-1}, 2246, 1438, 1421, 1192, 1108, 1046, 1016, 993. ^1H NMR: δ = 2.04 (t, J = 6.6 Hz, 2 H, 3-H), 2.44 (t, J = 6.6 Hz, 2 H, 2-H) ppm. ^{13}C NMR: δ = 3.1 (quint, J_{CD} = 23.2 Hz, C-4), 17.9 (−, C-2), 28.1 (−, C-3), 118.0 (C_{quat}, CN) ppm. MS (CI): m/z (%) = 232 (100) [M + NH_4^+ + NH_3], 215 (63) [M + NH_4^+].

3,3-Dideuterio-4-iodobutyronitrile (10c): Iodide **10c** (7.08 g, 75%) was obtained from the alcohol **9c** (4.18 g, 48.0 mmol), PPh_3 (13.11 g, 50.0 mmol), imidazole (3.47 g, 51.0 mmol) and I_2 (12.73 g, 50.2 mmol) according to GP2a as a slightly yellow oil after column chromatography (120 g of silica gel, 4.5 × 20 cm column, hexane/Et_2O, 1:1, R_f = 0.35). ^1H NMR: δ = 2.51 (s, 2 H, 2-H), 3.27 (s, 2 H, 4-H) ppm. ^{13}C NMR: δ = 2.83 (−, C-4), 18.2 (−, C-2), 28.4 (quint, J_{CD} = 20.3 Hz, C-3), 118.3 (C_{quat}, CN) ppm. MS (CI): m/z (%) = 232 (100) [M + NH_4^+ + NH_3], 215 (57) [M + NH_4^+].

2,2-Dideuterio-4-iodobutyronitrile (10d): Iodide **10d**[42] (7.05 g, 50%) was obtained from the protected alcohol **14d** (12.15 g, 71.0 mmol), dppe (23.9 g, 60.0 mmol) and I_2 (15.0 g, 59.1 mmol) according to GP2b after column chromatography (120 g of silica gel, 4 × 15 cm column, hexane/Et_2O, 1:1, R_f = 0.28) as a colorless oil. ^1H NMR: δ = 2.07 (t, J = 6.8 Hz, 2 H, 3-H), 3.23 (t, J = 6.8 Hz, 2 H, 4-H) ppm. ^{13}C NMR: δ = 3.2 (−, C-4), 17.6 (quint, J_{CD} = 20.8 Hz, C-2), 28.2 (−, C-3), 118.1 (C_{quat}, CN) ppm.

2-(2,2-Dideuterio-2-iodoethoxy)tetrahydropyran (13): Iodide **13** (33.53 g, 67%) was obtained from the alcohol **12** (28.70 g, 193.7 mmol), PPh_3 (50.80 g, 193.7 mmol), imidazole (13.21 g, 194 mmol) and I_2 (49.16 g, 193.7 mmol) according to GP2a after column chromatography (120 g of silica gel, 4 × 15 cm column, hexane/Et_2O, 10:1, R_f = 0.32) as a colorless oil. ^1H NMR: δ = 1.40−1.63 (m, 4 H, 5-H, 4-H), 1.64−1.84 (m, 2 H, 3-H), 3.43−3.52 (m, 1 H, 6-H), 3.66 (d, J = 10.8 Hz, 1 H, 1′-H), 3.77−3.81 (m, 1 H, 6-H), 3.89 (d, J = 10.8 Hz, 1 H, 1′-H), 4.63 (t, J = 3.3 Hz, 1 H, 2-H) ppm. ^{13}C NMR: δ = 3.20 (quint, J_{CD} = 20.7 Hz, C-2′), 19.1 (−, C-4), 25.2 (−, C-5), 30.4 (−, C-3), 62.1 (−, C-1′), 67.9 (−, C-6), 98.5 (+, C-2) ppm. For the NMR spectra of a non-deuterated compound see ref.[43]

2-(3,3-Dideuterio-3-iodopropoxy)tetrahydropyran (18): Iodide **18** (20.38 g, 100%) was obtained from the alcohol **17** (12.15 g, 74.9 mmol), PPh_3 (19.6 g, 74.7 mmol), imidazole (5.10 g, 74.9 mmol) and I_2 (19.0 g, 74.9 mmol) according to GP2a as a slightly yellow oil and used without further purification. ^1H NMR: δ = 1.40−1.52 (m, 4 H, 5-H, 4-H), 1.60−1.78 (m, 2 H, 3-H), 1.98 (t, J = 5.8 Hz, 2 H, 2′-H), 3.31−3.47 (m, 2 H, 6-H), 3.67−3.82 (m, 2 H, 1′-H), 4.51 (t, J = 3.3 Hz, 1 H, 2-H) ppm. ^{13}C NMR: δ = 3.11 (quint, J_{CD} = 24.9 Hz, C-3′), 19.2 (−, C-4), 25.2 (−, C-5), 30.3 (−, C-3), 33.0 (−, C-2′), 61.9 (−, C-1′), 66.5 (−, C-6), 98.5 (+, C-2) ppm. For the NMR spectra of a non-deuterated compound see ref.[44]

2-(4,4-Dideuterio-4-iodobutoxy)tetrahydropyran (24): Iodide **24** (10.97 g, 77%) was obtained from the alcohol **23** (8.81 g, 50.0 mmol), PPh_3 (13.1 g, 50.0 mmol), imidazole (3.40 g, 50.0 mmol) and I_2 (12.7 g, 50.0 mmol) according to GP2a as a colorless oil after column chromatography [50 g of silica gel, 2 × 10 cm column, hexane/Et_2O, 2:1, R_f = 0.35 (10:1)]. ^1H NMR: δ = 1.47−1.66 (m, 4 H, 4-H, 5-H), 1.68−1.82 (m, 4 H, 3-H, 2′-H), 1.91 (t, J = 7.3 Hz, 2 H, 3′-H), 3.35−3.53 (m, 2 H, 1′-H), 3.70−3.88 (m, 2 H, 6-H), 4.56 (t, J = 3.2 Hz, 1 H, 2-H) ppm. ^{13}C NMR: δ = 19.6 (−, C-4), 25.4 (−, C-5), 30.4 (−, C-2′), 30.5 (−, C-3′), 30.7 (−, C-3), 62.3 (−, C-1′), 66.2 (−, C-6), 98.8 (+, C-2) ppm. The intensity of the CD_2 carbon signals was too low. For the NMR spectra of the non-deuterated compound see ref.[45].

3,3-Dideuterio-4-(tetrahydropyran-2-yloxy)butyronitrile (14c): Under Ar, a solution of acetonitrile (6.13 g, 7.8 mL, 149.3 mmol) in anhydrous THF (100 mL) was added dropwise to a solution of *n*BuLi (149.3 mmol, 60.7 mL of a 2.46 N solution in hexane) in THF (60 mL) at −78 °C over a period of 1 h. After an additional stirring for 1 h at the same temperature, the resulting fine suspension was cannulated to a stirred solution of the iodide **13** (33.53 g, 129.9 mmol) in anhydrous THF (100 mL) over a period of 20 min, while maintaining the temperature of the reaction mixture at 0 °C. The mixture was stirred at the same temperature for an additional 15 min, and the reaction was quenched with water (20 mL) and partitioned between diethyl ether and brine (200 mL of each). The inorganic phase was extracted with diethyl ether (3 × 80 mL), the combined organic layers were washed with brine (100 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (250 g of silica gel, 7 × 20 cm column, hexane/Et_2O, 1:1.5, R_f = 0.40) to give a nonseparable mixture (22.2 g) which consisted, according to ^1H and ^{13}C NMR spectroscopy, of **14c** (estimated yield ca. 70%) and 3,3-dideuterio-2-[1,1-dideuterio-2-(tetrahydropyran-2-yloxy)ethyl]-4-(tetrahydropyran-2-yloxy)butyronitrile (**15**) (yield approximately 18%). **14c and 15:** ^1H NMR: δ = 1.49−1.59 (m, 4 H, tetrahydropyranyl-H), 1.61−1.77 (m, 2 H, tetrahydropyranyl-H), 3.41−3.51 (m, 2 H, CH_2O), 3.74−3.89 (m, 2 H, CH_2O), 4.53−4.58 (m, 1 H, CHO) (common signals); 2.43 (s, 2 H, 2-H, **14c**), 2.99 (s, 1 H, 2-H, **15**). **14c:** ^{13}C NMR: δ = 13.9 (−, C-2), 19.3 (−, C-4′), 25.2 (−, C-5′), 25.4 (quint, J_{CD} = 14.8 Hz, C-3), 30.34 (−, C-3′), 62.2 (−, C-4), 64.6 (−, C-6′), 98.9 (+, C-2′), 119.4 (C_{quat}, CN) ppm. For the properties of the non-deuterated compound **14b** see ref.[46]. **15** (a mixture of two diastereomers): ^{13}C NMR: δ = 19.1 (−, 2 CH_2), 25.1 (−, 2 CH_2), 25.17 (+, 2 CH), 30.30 (−, 2 CH_2), 61.8, 62.3, 63.7, 63.8 (−, CH_2O), 98.3, 99.1 (+, CHO), 121.6 (C_{quat}, 2 CN) ppm. The signals of CD_2 carbon could not be detected because of their low intensity.

3,3-Dideuterio-4-hydroxybutyronitrile (9c): The mixture of **14c** and **15** (22.17 g) obtained as indicated above was stirred in MeOH

(150 mL) with PPTS (2.0 g, 7.96 mmol) at 50 °C for 1.5 h. After cooling, the mixture was concentrated under reduced pressure, the residue was taken up with CH_2Cl_2 (5 mL) and separated by column chromatography (200 g of silica gel, 4.5 × 30 cm column, Et_2O, R_f = 0.33) to give **9c** (7.58 g, 67% over two steps) as a colorless oil. ^{1}H NMR: δ = 2.39 (s, 2 H, 2-H), 3.23 (s, 1 H, OH), 3.60 (s, 2 H, 4-H) ppm. ^{13}C NMR: δ = 13.2 (−, C-2), 27.0 (quint, J_{CD} = 19.8 Hz, C-3), 59.5 (−, C-4), 119.6 (C_{quat}, CN) ppm.

2,2-Dideuterio-4-(tetrahydropyran-2-yloxy)butyronitrile (14d): To a suspension of NaCN (5.60 g, 114 mmol) in anhydrous DMSO (180 mL) was added the iodide **18** (20.4 g, 75.0 mmol) at ambient temperature over a period of 20 min under Ar. After an additional stirring at 40 °C for 3 h, the reaction mixture was poured into ice-cold water (200 mL), and the resulting mixture extracted with Et_2O (3 × 100 mL). The combined ethereal phases were washed with H_2O (3 × 100 mL) and brine (100 mL), and dried and concentrated under reduced pressure. Column chromatography of the residue (100 g of silica gel, 4 × 15 cm column, hexane/Et_2O, 1:1, R_f = 0.27) gave **14d** (8.06 g, 63%) as a colorless oil. ^{1}H NMR: δ = 1.41−1.51 (m, 4 H, 5′-H, 4′-H), 1.59−1.73 (m, 2 H, 3′-H), 1.83 (t, J = 6.0 Hz, 2 H, 3-H), 3.34−3.46 (m, 2 H, 6′-H), 3.69−3.79 (m, 2 H, 4-H), 4.49 (t, J = 3.3 Hz, 1 H, 2′-H) ppm. ^{13}C NMR: δ = 13.5 (quint, J_{CD} = 21.0 Hz, C-2), 19.1 (−, C-4′), 25.0 (−, C-5′), 25.3 (−, C-3), 30.2 (−, C-3′), 62.0 (−, C-4), 64.5 (−, C-6′), 98.6 (+, C-2′), 119.3 (C_{quat}, CN) ppm. MS (CI): m/z (%) = 189 (38) [M + NH_4^+], 172 (100) [M + H^+]. For the properties of the non-deuterated compound see ref.[46].

Coupling of Iodides 10b−d and 26 with *tert*-Butyl *N*-(Diphenylmethylene)glycinate (7). General Procedure (GP) 3: To a stirred solution of protected glycine **7** (40 mmol) in anhydrous THF (300 mL) was added dropwise *n*BuLi (40 mmol as a solution in hexane) at −78 °C over a period of 1 h under Ar. After stirring for an additional 1 h at this temperature, a solution of iodide (40 mmol) in THF (50 mL) was added to the suspension of the lithio compound at the same temperature over 15 min. The mixture was stirred for 20 h at −78 °C, then warmed to 20 °C over a period of 24 h and stirred for 12 h at ambient temp. After this, the mixture was poured into ice-cold water (100 mL), extracted with diethyl ether (3 × 60 mL), and the combined organic layers were washed with H_2O (80 mL), brine (80 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel deactivated with triethylamine.

***tert*-Butyl 5-Cyano-3,3-dideuterio-2-(diphenylmethylenimino)pentanoate (19b):** Compound **19b** (12.43 g, 70%) was obtained from the iodide **10b** (9.62 g, 48.8 mmol), *tert*-butyl *N*-(diphenylmethylene)glycinate (**7**) (14.4 g, 48.8 mmol) and *n*BuLi (49.0 mmol, 29.7 mL of a 1.65 N solution in hexane) according to GP3 as a colorless oil after column chromatography (200 g of silica gel, 6 × 15 cm column, hexane/Et_2O, 1:1, R_f = 0.37). ^{1}H NMR: δ = 1.45 [s, 9 H, $C(CH_3)_3$], 1.64−1.68 (t, J = 7.1 Hz, 2 H, 4-H), 2.30 (t, J = 7.1 Hz, 2 H, 5-H), 3.95 (s, 1 H, 2-H), 7.15−7.19 (m, 2 H, Ph-H), 7.29−7.38 (m, 3 H, Ph-H), 7.44−7.46 (m, 3 H, Ph-H), 7.63−7.67 (m, 2 H, Ph-H) ppm. ^{13}C NMR: δ = 14.1 (−, C-5), 22.0 (−, C-4), 28.0 [+, $C(CH_3)_3$], 31.6 (quint, J_{CD} = 22.0 Hz, C-3), 64.8 (+, C-2), 81.3 [C_{quat}, $C(CH_3)_3$], 119.5 (C_{quat}, CN), 127.7, 128.1, 128.6, 128.7, 130.4 (+, Ph-C), 136.4, 139.3 (C_{quat}, Ph-C), 170.6 (C_{quat}, C=N), 170.7 (C_{quat}, C=O) ppm. MS (CI): m/z (%) = 729 (23) [2M + H^+], 365 (100) [M + H^+].

***tert*-Butyl 5-Cyano-4,4-dideuterio-2-(diphenylmethylenamino)pentanoate (19c):** Compound **19c** (8.83 g, 100%) was obtained from the iodide **10c** (4.93 g, 25.0 mmol), *tert*-butyl *N*-(diphenylmethylene)glycinate (**7**) (7.16 g 24.2 mmol) and *n*BuLi (24.2 mmol, 9.84 mL of a 2.46 N solution in hexane) according to GP3 as a colorless oil after column chromatography (200 g of silica gel, 6 × 20 cm column, hexane/Et_2O, 1:1, R_f = 0.33). ^{1}H NMR: δ = 1.45 [s, 9 H, $C(CH_3)_3$], 1.96−2.07 (m, 2 H, 3-H), 2.30 (s, 2 H, 5-H). 3.96 (dd, J = 5.0, 7.5 Hz, 1 H, 2-H), 7.15−7.19 (m, 2 H, Ph-H), 7.30−7.39 (m, 3 H, Ph-H), 7.45−7.47 (m, 3 H, Ph-H), 7.62−7.66 (m, 2 H, Ph-H) ppm. ^{13}C NMR: δ = 16.9 (−, C-5), 21.9 (quint, J_{CD} = 30.3 Hz, C-4), 27.9 [+, $C(CH_3)_3$], 32.1 (−, C-3), 64.7 (+, C-2), 81.2 [C_{quat}, $C(CH_3)_3$], 119.4 (C_{quat}, CN), 127.5, 127.9, 128.1, 128.6, 130.3 (+, Ph-C), 136.2, 139.1 (C_{quat}, Ph-C), 170.49 (C_{quat}, C=N), 170.52 (C_{quat}, C=O) ppm. MS (CI): m/z (%) = 729 (19) [2M + H^+], 365 (100) [M + H^+].

***tert*-Butyl 5-Cyano-5,5-dideuterio-2-(diphenylmethylenamino)pentanoate (19d):** Compound **19d** (12.26 g, 94%) was obtained from the iodide **10d** (7.05 g, 35.8 mmol), *tert*-butyl *N*-(diphenylmethylene)glycinate (**7**) (11.64 g, 39.4 mmol) and *n*BuLi (39.4 mmol, 24.0 mL of a 1.64 N solution in hexane) according to GP3 as a colorless oil after column chromatography (120 g of silica gel, 4 × 15 cm column, hexane/Et_2O, 1:1, R_f = 0.33). ^{1}H NMR: δ = 1.44 [s, 9 H, $C(CH_3)_3$], 1.65−1.69 (m, 2 H, 4-H), 1.96−2.08 (m, 2 H, 3-H), 3.94−3.99 (m, 1 H, 2-H), 7.15−7.19 (m, 2 H, Ph-H), 7.28−7.38 (m, 3 H, Ph-H), 7.43−7.48 (m, 3 H, Ph-H), 7.63−7.66 (m, 2 H, Ph-H) ppm. ^{13}C NMR: δ = 16.4 (quint, J_{CD} = 22.0 Hz, C-5), 21.7 (−, C-4), 27.8 [+, $C(CH_3)_3$], 32.1 (−, C-3), 64.7 (+, C-2), 81.0 [C_{quat}, $C(CH_3)_3$], 119.3 (C_{quat}, CN), 127.4, 127.8, 128.4, 128.5, 130.2 (+, Ph-C), 136.1, 139.0 (C_{quat}, Ph-C), 170.0 (C_{quat}, C=N), 170.4 (C_{quat}, C=O) ppm. MS (CI): m/z (%) = 729 (23) [2M + H^+], 365 (100) [M + H^+].

Change of a Protective Group in Imines 19b−d. General Procedure (GP) 4: To a solution of the respective imine (18.9 mmol) in Et_2O (200 mL) was added 1 N aqueous HCl solution (100 mL), and the resulting mixture was vigorously stirred with TLC-monitoring. The aqueous phase was washed with Et_2O (2 × 50 mL), brought to a pH of about 10 with concentrated ammonia solution, and the solvents were evaporated under reduced pressure. The residue was taken up with EtOAc (200 mL), and the solution was washed with aqueous 10% ammonia solution (2 × 50 mL), brine (50 mL), and dried and concentrated under reduced pressure. The crude amine was dissolved in anhydrous MeOH (150 mL), and to this solution was added in one portion di-*tert*-butyl pyrocarbonate (Boc_2O) (28.0 mmol). The resulting solution was stirred at ambient temperature for 12 h and concentrated under reduced pressure. The residue was taken up with diethyl ether (180 mL), washed with H_2O (3 × 50 mL), dried and concentrated under reduced pressure. The product was purified by column chromatography.

***tert*-Butyl 2-(*tert*-Butoxycarbonylamino)-5-cyano-3,3-dideuteriopentanoate (20b):** Compound **20b** (13.27 g, 94%) was obtained from **19b** (17.1 g, 46.9 mmol) according to GP4 after chromatographic purification (200 g of silica gel, 6 × 20 cm column, hexane/Et_2O, 2:1, R_f = 0.31) as a colorless solid, m.p. 72−74 °C (hexane). ^{1}H NMR: δ = 1.42, 1.45 [s, 9 H, $C(CH_3)_3$], 1.71 (t, J = 6.9 Hz, 2 H, 4-H), 2.38 (t, J = 6.9 Hz, 2 H, 5-H), 4.16 (d, J = 8.3 Hz, 1 H, 2-H), 5.11 (d, J = 8.3 Hz, 1 H, NH) ppm. ^{13}C NMR: δ = 16.7 (−, C-5), 21.2 (−, C-4), 27.9, 28.2 [+, $C(CH_3)_3$], 31.3 (quint, J_{CD} = 21.1 Hz, C-3), 52.8 (+, C-2), 79.8, 82.4 [C_{quat}, $C(CH_3)_3$], 119.1 (C_{quat}, CN), 155.3 (C_{quat}, NC=O), 171.1 (C_{quat}, C=O) ppm. MS (CI): m/z (%) = 618 (20) [2M + NH_4^+], 318 (100) [M + NH_4^+], 301 (5) [M + H^+]. $C_{15}H_{24}D_2N_2O_4$ (300.4): calcd. C 59.98, H(D) 9.39, N 9.32; found C 60.29, H(D) 9.19, N 9.15.

***tert*-Butyl 2-(*tert*-Butoxycarbonylamino)-5-cyano-4,4-dideuteriopentanoate (20c):** Compound **20c** (5.68 g, 78%) was obtained from **19c**

(8.83 g, 24.2 mmol) according to GP4 after chromatographic purification (200 g of silica gel, 6 × 20 cm column, hexane/Et_2O, 2:1, R_f = 0.27) as a colorless solid, m.p. 77−79 °C (hexane). ^1H NMR: δ = 1.41, 1.45 [s, 9 H, $C(CH_3)_3$], 1.65 (dd, J = 7.2, 13.8 Hz, 1 H, 3-H), 1.91 (dd, J = 5.2, 13.8 Hz, 1 H, 3-H), 2.37 (s, 2 H, 5-H) 4.15 (ddd, J = 5.0, 5.2, 7.2 Hz, 1 H, 2-H), 5.11 (d, J = 5.0 Hz, 1 H, NH) ppm. ^{13}C NMR: δ = 16.5 (−, C-5), 21.8 (quint, J_{CD} = 22.0 Hz, C-4), 27.9, 28.2 [+, $C(CH_3)_3$], 31.8 (−, C-3), 52.9 (+, C-2), 79.8, 82.4 [C_{quat}, $C(CH_3)_3$], 119.1 (C_{quat}, CN), 155.3 (C_{quat}, NC=O), 171.1 (C_{quat}, C=O) ppm. $C_{15}H_{24}D_2N_2O_4$ (300.4): calcd. C 59.98, H(D) 9.39, N 9.32; found C 60.10, H(D) 8.97 N 9.16.

***tert*-Butyl 2-(*tert*-Butoxycarbonylamino)-5-cyano-5,5-dideuteriopentanoate (20d):** Compound **20d** (3.94 g, 69%) was obtained from **19d** (6.90 g, 18.9 mmol) according to GP4 after chromatographic purification (100 g of silica gel, 4.5 × 15 cm column, hexane/Et_2O, 2:1, R_f = 0.27) as a colorless solid, m.p. 70−72 °C. IR (KBr): $\tilde{\nu}$ = 3273 cm^{-1}, 2978, 1735, 1703, 1397, 1366, 1155. ^1H NMR: δ = 1.39, 1.42 [s, 9 H, $C(CH_3)_3$], 1.60−1.78 (m, 2 H, 4-H), 1.81−1.98 (m, 2 H, 3-H), 4.08−4.21 (m, 1 H, 2-H), 5.15 (br. s, 1 H, NH) ppm. ^{13}C NMR: δ = 16.4 (quint, J_{CD} = 22.5 Hz, C-5), 21.4 (−, C-4), 27.9, 28.2 [+, $C(CH_3)_3$], 32.0 (−, C-3), 52.9 (+, C-2), 79.8, 82.3 [C_{quat}, $C(CH_3)_3$], 119.1 (C_{quat}, CN), 155.3 (C_{quat}, NC=O), 171.1 (C_{quat}, C=O) ppm. MS (CI): m/z (%) = 618 (25) [2M + NH_4^+], 318 (100) [M + NH_4^+]. $C_{15}H_{24}D_2N_2O_4$ (300.4): calcd. C 59.98, H(D) 9.39, N 9.32; found C 60.21, H(D) 9.35, N 9.26.

Raney Ni-Catalyzed Hydrogenation of Nitriles 20b−d and 32. General Procedure (GP) 5: To a solution of the respective nitrile (10 mmol) in MeOH (150 mL) was added a 3.5 N ammonia solution in MeOH (50 mL) and freshly prepared Raney Ni (3.0 g), and the resulting mixture was hydrogenated at ambient temperature in a Parr apparatus under a pressure of 4 bar of hydrogen with TLC-monitoring for the indicated time. The mixture was then filtered through a pad of Celite (3 cm) and concentrated under reduced pressure. The residue was taken up with EtOAc (150 mL), and the solution was washed with aqueous 10% ammonia solution (2 × 50 mL), dried and concentrated under reduced pressure again.

***tert*-Butyl 6-Amino-2-(*tert*-butoxycarbonylamino)-3,3-dideuteriohexanoate (21b):** Compound **21b** (3.08 g, 100%) was obtained from **20b** (3.05 g, 10.2 mmol) according to GP5 (reaction time 3 d) as a slightly yellow oil. ^1H NMR: δ = 1.27−1.40 (m, 4 H, 4-H, 5-H), 1.41, 1.43 [s, 9 H, $C(CH_3)_3$], 2.65 (t, J = 6.6 Hz, 2 H, 6-H), 4.10−4.15 (m, 1 H, 2-H), 5.03 (br. s, 1 H, NH), 5.25 (br. s, 2 H, NH_2) ppm. ^{13}C NMR: δ = 22.5 (−, C-4), 28.2, 28.6 [+, $C(CH_3)_3$], 30.2 (quint, J_{CD} = 22.0 Hz, C-3), 33.6 (−, C-5), 41.7 (−, C-6), 54.0 (+, C-2), 79.7, 81.9 [C_{quat}, $C(CH_3)_3$], 155.6 (C_{quat}, NC=O), 172.3 (C_{quat}, C=O) ppm.

***tert*-Butyl 6-Amino-2-(*tert*-butoxycarbonylamino)-4,4-dideuteriohexanoate (21c):** Compound **21c** (5.29 g, 98%) was obtained from **20c** (5.33 g, 17.73 mmol) according to GP5 (reaction time 2 d) as a slightly yellow oil. ^1H NMR: δ = 1.14 (t, J = 6.8 Hz, 2 H, 5-H), 1.33, 1.35 [s, 9 H, $C(CH_3)_3$], 1.48 (dd, J = 7.3, 13.8 Hz, 1 H, 3-H), 1.65 (dd, J = 6.5, 13.8 Hz, 1 H, 3-H), 2.58 (t, J = 6.8 Hz, 2 H, 6-H), 3.99 (dd, J = 6.5, 7.3 Hz, 1 H, 2-H), 5.15 (br. s, 1 H, NH), 5.30 (br. s, 2 H, NH_2) ppm. ^{13}C NMR: δ = 21.6 (quint, J_{CD} = 20.8 Hz, C-4), 27.8, 28.1 [+, $C(CH_3)_3$], 32.3 (−, C-3), 33.1 (−, C-5), 41.7 (−, C-6), 53.7 (+, C-2), 79.2, 81.3 [C_{quat}, $C(CH_3)_3$], 155.2 (C_{quat}, NC=O), 171.8 (C_{quat}, C=O) ppm.

***tert*-Butyl 6-Amino-2-(*tert*-butoxycarbonylamino)-5,5-dideuteriohexanoate (21d):** Compound **21d** (2.89 g, 100%) was obtained from **20d** (2.86 g, 9.52 mmol) according to GP5 (reaction time 15 h) as a slightly yellow oil. ^1H NMR: δ = 1.23−1.38 (m, 2 H, 4-H), 1.40, 1.42 [s, 9 H, $C(CH_3)_3$], 1.44−1.61 (m, 1 H, 3-H), 1.62−1.80 (m, 1 H, 3-H), 2.63 (s, 2 H, 6-H), 4.19−4.23 (m, 1 H, 2-H), 5.18 (br. s, 1 H, NH), 5.22 (br. s, 2 H, NH_2) ppm. ^{13}C NMR: δ = 22.2 (−, C-4), 27.9, 28.2 [+, $C(CH_3)_3$], 30.3 (−, C-3), 33.0 (quint, J_{CD} = 22.0 Hz, C-5), 41.7 (−, C-6), 53.8 (+, C-2), 79.4, 81.6 [C_{quat}, $C(CH_3)_3$], 155.3 (C_{quat}, NC=O), 171.9 (C_{quat}, C=O) ppm.

Deprotection of Protected Amino Acids 21b−d. General Procedure (GP) 6: The protected amino acids **21b−d** were taken up with 1 N aqueous HCl solution (250 mL), and the mixture vigorously stirred at ambient temperature for two days. After evaporation of the solvent, the residue was dried in a desiccator over P_2O_5 under reduced pressure to give amino acid dihydrochlorides in pure form.

***rac*-2,6-Diamino-3,3-dideuteriohexanoic Acid (*rac*-3,3-Dideuteriolysine) Dihydrochloride (6b·2 HCl):** Compound **6b**·2 HCl (2.04 g, 91%) was obtained from **21b** (3.08 g, 10.1 mmol) according to GP6 as a colorless powder, m.p. 168−170 °C. ^1H NMR (D_2O): δ = 1.28−1.35 (t, J = 7.6 Hz, 2 H, 4-H), 1.55 (tt, J = 7.6, 7.6 Hz, 2 H, 5-H), 2.84 (t, J = 7.6 Hz, 2 H, 6-H), 3.91 (br. s, 1 H, 2-H) ppm. ^{13}C NMR (D_2O): δ = 23.7 (−, C-4), 28.7 (−, C-5), 31.0 (quint, J_{CD} = 21.10 Hz, C-3), 41.5 (−, C-6), 55.0 (+, C-2), 174.5 (C_{quat}, C=O) ppm. MS (CI): m/z (%) = 166 (35) [M − 2HCl + NH_4^+], 149 (100) [M^+ − 2HCl + H].

***rac*-2,6-Diamino-4,4-dideuteriohexanoic Acid (*rac*-4,4-Dideuteriolysine) Dihydrochloride (6c·2 HCl):** Compound **6c**·2 HCl (3.69 g, 96%) was obtained from **21c** (5.29 g, 17.4 mmol) according to GP6 as a colorless powder, m.p. 178−180 °C. ^1H NMR (D_2O): δ = 1.50 (t, J = 7.4 Hz, 2 H, 5-H), 1.72 (dd, J = 6.1, 16.4 Hz, 1 H, 3-H), 1.82 (dd, J = 6.1, 16.4 Hz, 1 H, 3-H), 2.80 (t, J = 7.4 Hz, 2 H, 6-H), 3.88 (dd, J = 6.1, 6.1 Hz, 1 H, 2-H) ppm. ^{13}C NMR (D_2O): δ = 23.1 (quint, J_{CD} = 20.5 Hz, C-4), 28.5 (−, C-5), 31.5 (−, C-3), 41.2 (−, C-6), 55.0 (+, C-2), 174.4 (C_{quat}, C=O) ppm.

***rac*-2,6-Diamino-5,5-dideuteriohexanoic Acid (*rac*-5,5-Dideuteriolysine) Dihydrochloride (6d·2 HCl):** Compound **6d**·2 HCl (1.98 g, 94%) was obtained from **21d** (2.90 g, 9.53 mmol) according to GP6 as a colorless powder, m.p. 173−175 °C. ^1H NMR (D_2O): δ = 1.32−1.35 (m, 2 H, 4-H), 1.79−1.81 (m, 2 H, 3-H), 2.83 (s, 2 H, 6-H), 3.90 (dd, J = 5.6, 5.6 Hz, 1 H, 2-H) ppm. ^{13}C NMR (D_2O): δ = 26.7 (−, C-4), 31.0 (quint, J_{CD} = 20.9 Hz, C-5), 34.7 (−, C-3), 44.5 (−, C-6), 58.8 (+, C-2), 177.5 (C_{quat}, C=O) ppm. MS (CI): m/z (%) = 149 (100) [M^+ − 2HCl + H], 147 (90) [M^+ − 2HCl − H], 130 (80) [M^+ − 2HCl − H_2O].

***N*,*N*-Bis(*tert*-butoxycarbonyl)-1,1-dideuterio-4-(tetrahydropyran-2-yloxy)butylamine (25):** To a suspension of potassium bis(*tert*-butoxycarbonyl)amide (4.72 g, 18.5 mmol) in anhydrous DMF (40 mL) was added iodide **24** (5.15 g, 18.0 mmol) in one portion under Ar. The resulting mixture was stirred at ambient temperature for 4 h, poured into a mixture of ice-cold water (150 mL) and diethyl ether (50 mL). The aqueous layer was extracted with Et_2O (50 mL), and the combined organic phases were washed with H_2O (2 × 50 mL) and brine (50 mL), and dried and concentrated under reduced pressure. The product was purified by column chromatography (170 g of silica gel, 4.5 × 20 cm column, hexane/Et_2O, 2:1, R_f = 0.44) to give **25** (6.49 g, 96%) as a colorless oil. IR: $\tilde{\nu}$ = 2941 cm^{-1}, 2671, 1734, 1694, 1367, 1123, 733. ^1H NMR: δ = 1.48 [s, 18 H, 2 $C(CH_3)_3$], 1.56−1.77 (m, 10 H, 4′-H, 5′-H, 3′-H, 2-H, 3-H), 3.39−3.49 (m, 2 H, 4-H), 3.70−3.84 (m, 2 H, 6′-H), 4.55 (br. s, 1 H, 2′-H) ppm. ^{13}C NMR: δ = 19.5 (−, C-4′), 25.4 (−, C-5′), 27.0 (−, C-3), 28.0 [+, 2 $C(CH_3)_3$], 30.5 (−, C-2), 30.7 (−, C-3′), 62.2 (−, C-4), 67.1 (−, C-6′), 82.2 [C_{quat}, 2 $C(CH_3)_3$], 98.7 (+, C-2′), 152.6 (C_{quat}, 2 NC=O) ppm. The intensity of the CD_2 carbon signal was too low. MS (CI): m/z (%) = 768 (85) [2M + NH_4^+], 712

(40) [2M − C_4H_8 + NH_4^+], 393 (100) [M + NH_4^+], 337 (100) [M − C_4H_8 + NH_4^+], 253 (50).

***N*,*N*-Bis(*tert*-butoxycarbonyl)-1,1-dideuterio-4-iodobutylamine (26):** Column chromatography (200 g of silica gel, 5 × 30 cm column, hexane/Et_2O, 5:1) of a reaction mixture obtained from the protected amino alcohol **25** (6.67 g, 17.8 mmol), dppe (8.53 g, 21.4 mmol) and I_2 (5.45 g, 21.5 mmol) according to GP2b gave the diprotected aminoiodide **26** (3.65 g, 51%) and *N*-*tert*-butoxycarbonyl-1,1-dideuterio-4-iodobutylamine (**27**) (2.01 g, 38%). **26:** R_f = 0.34. ^{1}H NMR: δ = 1.45 [s, 18 H, 2 C(CH_3)$_3$], 1.61 (t, J = 8.2 Hz, 2 H, 2-H), 1.75−1.81 (m, 2 H, 3-H), 3.14 (t, J = 6.8 Hz, 2 H, 4-H) ppm. ^{13}C NMR: δ = 6.0 (−, C-4), 27.9 [+, 2 C(CH_3)$_3$], 29.6 (−, C-3), 30.6 (−, C-2), 44.4 (quint, J = 19.0 Hz, C-1), 82.1 [C_{quat}, 2 C(CH_3)$_3$], 152.4 (C_{quat}, 2 NC=O) ppm. **27:** R_f = 0.18.

***tert*-Butyl 6-[*N*,*N*-Bis(*tert*-butoxycarbonyl)]-6,6-dideuterio-2-(*N*-diphenylmethyleneamino)hexanoate (28):** Compound **28** (5.16 g, 100%) was obtained from the iodide **26** (3.65 g, 9.1 mmol), *tert*-butyl *N*-(diphenylmethylene)glycinate (**7**) (2.96 g, 10.0 mmol) and *n*BuLi (10.1 mmol, 6.1 mL of a 1.65 N solution in hexane) according to GP3 as a colorless oil after column chromatography (150 g of silica gel, 4.5 × 25 cm column, hexane/Et_2O, 3:1, R_f = 0.36). ^{1}H NMR: δ = 1.21−1.36 (m, 4 H, 3-H, 4-H), 1.42, 1.44, 1.45 [s, 9 H, C(CH_3)$_3$], 1.82−1.96 (m, 2 H, 5-H), 3.83 (t, J = 7.4 Hz, 1 H, 2-H), 7.15−7.19 (m, 2 H, Ph-H), 7.29−7.38 (m, 3 H, Ph-H), 7.44−7.46 (m, 3 H, Ph-H), 7.63−7.67 (m, 2 H, Ph-H) ppm. ^{13}C NMR: δ = 23.4 (−, C-4), 28.0 [+, 3 C(CH_3)$_3$], 28.7 (−, C-5), 33.4 (−, C-3), 66.0 (+, C-2), 80.8 [C_{quat}, C(CH_3)$_3$], 81.9 [C_{quat}, 2 C(CH_3)$_3$], 127.8, 127.9, 128.4, 128.7, 130.1 (+, Ph-C), 136.7, 139.6 (C_{quat}, Ph-C), 152.6 (C_{quat}, 2 NC=O), 170.0 (C_{quat}, C=N), 170.4 (C_{quat}, C=O) ppm. The intensity of CD_2 carbon signals was too low. MS (CI): *m*/*z* (%) = 569 (23) [M + H^+], 297 (72), 296 (100).

***rac*-2,6-Diamino-6,6-dideuteriohexanoic Acid (*rac*-6,6-Dideuteriolysine) Dihydrochloride (6e·2 HCl):** The protected amino acid **28** (5.80 g, 10.2 mmol) was taken up with diethyl ether (100 mL), 1 N aqueous HCl solution (150 mL) was added, and the resulting mixture was vigorously stirred at ambient temperature for two days. The aqueous phase was washed with Et_2O (2 × 80 mL), and the solvents evaporated in vacuo. The residue was washed with Et_2O (30 mL) and dried in a desiccator over P_2O_5 under reduced pressure to give amino acid dihydrochloride **6e**·2 HCl (1.79 g, 79%) as a colorless powder, m.p. 168−170 °C. ^{1}H NMR (D_2O): δ = 1.34 (tt, J = 6.8, 9.0 Hz, 2 H, 4-H), 1.54 (t, J = 7.1 Hz, 2 H, 5-H), 1.78−1.85 (m, 2 H, 3-H), 3.92 (dd, J = 6.1, 6.1 Hz, 1 H, 2-H) ppm. ^{13}C NMR (D_2O): δ = 23.8 (−, C-4), 28.5 (−, C-3), 31.7 (−, C-5), 41.3 (quint, J_{CD} = 32.7 Hz, C-6), 55.1 (+, C-2), 174.5 (C_{quat}, C=O) ppm. MS (CI): *m*/*z* (%) = 166 (32) [M − 2HCl + NH_4^+], 149 (100) [M^+ − 2HCl + H].

***tert*-Butyl 2-(*tert*-Butoxycarbonylamino)-5-cyano-5,5-dideuterio-4-hydroxypentanoate (31):** A mixture of anhydrous $CeCl_3$ (6.36 g, 25.8 mmol, dried at 140 °C/0.01 mbar for 2 h) and anhydrous THF (200 mL) was vigorously stirred under Ar at ambient temperature for 2 h. A solution of *tert*-butyl 2-(*tert*-butoxycarbonylamino)-4-oxobutyrate (**30**) (7.05 g, 25.8 mmol) in anhydrous THF (30 mL) was then added in one portion, the stirring was continued for an additional 1 h, and the reaction mixture was then cooled to −78 °C. After this, a cold suspension of $LiCD_2CN$ − prepared from [D_3]acetonitrile (CD_3CN) (2.27 g, 2.70 mL, 51.5 mmol) and *n*BuLi (51.5 mmol, 20.6 mL of a 2.5 N solution in hexane) in anhydrous THF (100 mL) at −78 °C as described above for the preparation of compound **14c** − was cannulated to the reaction mixture. After additional stirring at −78 °C for 20 min, the mixture was poured into ice-cold 10% aqueous HCl solution (200 mL), the aqueous phase was extracted with Et_2O (2 × 80 mL), and the combined organic layers were washed with H_2O (200 mL), and dried and concentrated under reduced pressure. Column chromatography (200 g of silica gel, 6 × 20 cm column, hexane/Et_2O, 1:1) gave the starting material **30** (3.42 g, 48%, R_f = 0.42) and the product **31** (3.46 g, 42%, R_f = 0.16) as a 2:1 mixture of diastereomers **31a** and **31b**. ^{1}H NMR (**31a**,**b**): δ = 1.45, 1.48 [s, 9 H, C(CH_3)$_3$, **31a**,**b**] 1.65−1.89 (m, 1 H, 3-H, **31a**,**b**), 1.98 (ddd, J = 3.0, 11.1, 12.5 Hz, 1 H, 3-H, **31a**), 2.12 (ddd, J = 3.0, 10.0, 13.5 Hz, 1 H, 3-H, **31b**), 3.49 (br. s, 1 H, OH, **31a**,**b**), 3.95 (dd, J = 4.0, 10.1 Hz, 1 H, 4-H, **31a**), 4.11 (dd, J = 3.0, 10.0 Hz, 1 H, 4-H, **31b**), 4.18 (ddd, J = 4.0, 7.4, 7.5 Hz, 1 H, 2-H, **31b**), 4.37 (ddd, J = 4.0, 6.7, 11.1 Hz, 1 H, 2-H, **31a**), 4.85 (d, J = 4.0 Hz, 1 H, NH, **31b**), 5.42 (d, J = 6.7 Hz, 1 H, NH, **31a**) ppm. ^{13}C NMR (**31a**): δ = 27.8, 28.1 [+, C(CH_3)$_3$], 40.8 (−, C-3), 50.7 (+, C-2), 63.2 (+, C-4), 80.8, 82.9 [C_{quat}, C(CH_3)$_3$], 117.4 (C_{quat}, CN), 156.4 (C_{quat}, NC=O), 171.1 (C_{quat}, C=O) ppm. ^{13}C NMR (**31b**): δ = 27.7, 28.0 [+, C(CH_3)$_3$], 39.3 (−, C-3), 51.6 (+, C-2), 64.93 (+, C-4), 80.2, 82.6 [C_{quat}, C(CH_3)$_3$], 117.35 (C_{quat}, CN), 155.6 (C_{quat}, NC=O), 170.9 (C_{quat}, C=O) ppm. The intensity of CD_2 carbon signals was too low.

***tert*-Butyl 2-(*tert*-Butoxycarbonylamino)-5-cyano-5,5-dideuterio-4-(tetrahydropyran-2-yloxy)pentanoate (32):** A solution of alcohol **31** (1.48 g, 4.68 mmol) and 3,4-dihydro-2*H*-pyran (DHP) (789 mg, 0.85 mL, 9.38 mmol) in anhydrous CH_2Cl_2 (50 mL) was stirred with PPTS (118 mg, 0.47 mmol) at ambient temperature for 24 h. After this, the reaction mixture was diluted with Et_2O (50 mL), washed with half-saturated sodium chloride solution (50 mL), and dried and concentrated under reduced pressure. Column chromatography of the residue (60 g of silica gel, 4 × 15 cm column, hexane/Et_2O, 1:1, R_f = 0.38) gave the cyano ester **32** (1.34 g, 71%) as a slightly yellow oil. ^{1}H NMR: δ = 1.37, 1.40 [s, 9 H, C(CH_3)$_3$], 1.48−1.65 (m, 4 H, 4′-H, 5′-H), 1.66−1.76 (m, 2 H, 3′-H), 1.90 (ddd, J = 3.5, 8.0, 14.2 Hz, 1 H, 3-H), 2.10 (ddd, J = 4.2, 8.8, 14.2 Hz, 1 H, 3-H), 3.39−3.46 (m, 1 H, 6′-H), 3.78−3.82 (m, 1 H, 6′-H), 4.04 (dd, J = 3.5, 8.8 Hz, 1 H, 4-H), 4.10−4.23 (m, 1 H, 2-H), 4.68 (br. s, 1 H, 2′-H), 5.53 (d, J = 8.5 Hz, 1 H, NH) ppm. ^{13}C NMR: δ = 19.5 (−, C-4′), 24.9 (−, C-5′), 27.8, 28.1 [+, C(CH_3)$_3$], 30.6 (−, C-3′), 36.5 (−, C-3), 51.4 (+, C-4), 63.2 (−, C-6′), 68.2 (+, C-2), 79.3, 81.1 [C_{quat}, C(CH_3)$_3$], 97.3 (+, C-2′), 116.8 (C_{quat}, CN), 155.3 (C_{quat}, NC=O), 171.0 (C_{quat}, C=O) ppm. The intensity of CD_2 carbon signals was too low. MS (CI): *m*/*z* (%) = 818 (5) [2M + NH_4^+], 418 (100) [M + NH_4^+], 334 (60).

***tert*-Butyl 2-(*tert*-Butoxycarbonylamino)-6-(*tert*-butoxycarbonylamino)-5,5-dideuterio-4-(tetrahydropyran-2-yloxy)hexanoate (33):** The residue obtained from the nitrile **32** (1.10 g, 2.75 mmol) after hydrogenation under the conditions described in GP5 [MeOH (20 mL), a 3.5 N ammonia solution in MeOH (5 mL), Raney Ni (1.0 g), 3 h] was stirred in MeOH (50 mL) with Boc_2O (830 mg, 3.8 mmol) at ambient temperature for 9 h. Workup according to GP4 and column chromatography (50 g of silica gel, 3 × 15 cm column, hexane/Et_2O, 1:1, R_f = 0.24) gave **33** as a colorless oil which was immediately deprotected. MS (CI): *m*/*z* (%) = 522 (100) [M + NH_4^+], 505 (8) [M + H^+].

3-Amino-5-(2-amino-1,1-dideuterioethyl)-4,5-dihydrofuran-2-one Dihydrochloride (34·2 HCl): The residue from the previous preparation was taken up with MeOH (40 mL), and the solution vigorously stirred with 1 N aqueous HCl solution (40 mL) at ambient temperature for two days. Methanol was evaporated under reduced pressure, the residual aqueous solution was washed with Et_2O (2 × 30 mL) and concentrated in vacuo to give 357 mg of a yellow oil, crystallization of which from aqueous EtOH gave lactone **34**·2HCl

(196 mg, 33% over three steps, a 1.1:1 mixture of diastereomers **34a** and **34b**) as a colorless powder, m.p. 176−181 °C. ^{1}H NMR (D_2O, **34a**,**b**): δ = 2.43−2.50 (m, 2 H, 4-H), 2.98−3.10 (m, 2 H, 2′-H), 4.35−4.49 (m, 1 H, 3-H) 4.81−4.90 (m, 1 H, 5-H) ppm. ^{13}C NMR (D_2O, **34a**): δ = 32.9 (−, C-4), 33.5 (quint, J = 20.2 Hz, C-1′), 38.8 (−, C-2′), 51.8 (+, C-3), 79.9 (+, C-5), 175.8 (C_{quat}, C=O) ppm. ^{13}C NMR (D_2O, **34b**): δ = 34.8 (−, C-4), 35.3 (quint, J = 20.2 Hz, C-1′), 38.9 (−, C-2′), 51.5 (+, C-3), 79.8 (+, C-5), 175.8 (C_{quat}, C=O) ppm. MS (CI): *m*/*z* (%) = 164 (100) [M − 2HCl + NH_4^+], 147 (100) [M − 2HCl + H^+].

Feeding Experiments. General Procedure (GP) 7: *Streptomyces griseoflavus* (strain W-384) was obtained from Prof. H. Wolf (Stuttgart). An M2Ca agar in a culture tube was inoculated with the spores of the strain W-384 and the tube was incubated at 28 °C until the sporulation was complete (grey colored aerial mycelia, orange colored agar, incubation up to 3 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 900 mL of medium M10 in a 1 L fermenter. Just before inoculation, 1 mL of the vitamin solution and 5 drops of olive oil (to prevent foaming) were added.

The production culture was incubated at 27 °C, with a stirring rate of 700 rpm and an air flow rate of 1.6 vvm over 20 h. The pH was maintained at 6.5 ± 1.0 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 50 mL of distilled, sterilized water (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 labeled hormaomycins had purities of about 90% or higher, as determined by HPLC analysis. t_R = 14.25 min [column: Nucleosil®-100C18, 5 μm, 250 × 3.0 mm; eluent: 0.1% TFA in water (A), 0.1% TFA in acetonitrile (B); gradient elution: 40 → 100% B for 15 min, flow rate −0.5 mL/min; detection: 220 or 270 nm].

Feeding Experiment with (*RS*)-[1−^{13}C]-Lysine: This was carried out according to GP 7 with (*RS*)-[1-^{13}C]-lysine dihydrochloride (560 mg, 2.54 mmol) to give the ^{13}C-labeled hormaomycin (10.2 mg). The isotopic enrichment was 10.4% for C-1 (δ = 168.4 ppm) of (3-Ncp)Ala I and 45.7% for C-1 of (3-Ncp)Ala II (δ = 171.).[8,47,48]

Feeding Experiment with (*RS*)-[6−^{15}N]-Lysine: This was carried out according to GP 7 with (*R*,*S*)-[6-^{15}N]-lysine dihydrochloride (500 mg, 2.27 mmol) to give the ^{15}N-labeled hormaomycin (9.3 mg). ^{13}C NMR: duplicated: δ = 58.0 ([^{15}N-^{13}C]-^1J = 14.1 Hz) and 58.2 [C-2′, (3-Ncp)Ala I], 59.1 ([^{15}N-^{13}C]-^1J = 14.1 Hz) and 59.2 [C-2′, (3-Ncp)Ala II] ppm. MS (FAB): 1131/1130/1129 [M + H^+].

Feeding Experiment with 6b·2 HCl: This was carried out according to GP 7 with **6b**·2 HCl (460 mg, 2.08 mmol) to give hormaomycin **1b** (4.6 mg). ^{1}H NMR: decreased: δ = −0.07 ppm [3-H_a, (3-Ncp)Ala I], 0.58 [3-H_b, (3-Ncp)Ala I], 1.60 [3-H_a, (3-Ncp)Ala II]; 1.81 [3-H_b, (3-Ncp)Ala II]. D NMR: δ = 0.00, 0.50, 1.60 (1:1:2) ppm. ^{13}C NMR: decreased: δ = 33.0 [C-3, (3-Ncp)Ala I], 35.1 [C-3, (3-Ncp)Ala II]; duplicated (β-shift): δ = 20.3 and 20.2 ppm[C-1′, (3-Ncp)Ala I], 21.9 and 21.6 [C-1′, (3-Ncp)Ala II], 51.1 and 51.0 [C-2, (3-Ncp)Ala II], 52.1 and 51.8 [C-2, (3-Ncp)Ala I] ppm. MS (ESI), positive *m*/*z* (%) = 1155/1153/1151 [M + Na^+].

Feeding Experiment with 6c·2 HCl: This was carried out according to GP 7 with **6c**·2 HCl (460 mg, 2.08 mmol) to give hormaomycin **1c** (7.6 mg). ^{1}H NMR: decreased: δ = 0.33 [1′-H, (3-Ncp)Ala I] ppm. ^{13}C NMR: decreased: δ = 20.0 [C-1′, (3-Ncp)Ala I], 21.6 [C-1′, (3-Ncp)Ala II] ppm. MS (ESI), positive *m*/*z* (%) = 1153/1152/1151 [M + Na^+], 1131/1130/1129 [M + H^+].

Feeding Experiment with 6d·2 HCl: This was carried out according to GP 7 with **6d**·2 HCl (460 mg, 2.08 mmol) to give hormaomycin **1d** (5.8 mg). ^{1}H NMR: decreased: δ = −0.60 ppm [3′-H_a, (3-Ncp)Ala I], 1.01 [3′-H_b, (3-Ncp)Ala I, 3′-H_a, (3-Ncp)Ala II], 1.90 [3′-H_b, (3-Ncp)Ala II]. D NMR: δ = −0.06, 1.00, 1.92 ppm. ^{13}C NMR: decreased: δ = 17.4 [C-3′, (3-Ncp)Ala I and C-3′, (3-Ncp)Ala II]; duplicated (β-shift): δ = 20.0 [C-1′, (3-Ncp)Ala I], 21.6 [C-1′, (3-Ncp)Ala II], 58.1 [C-2′, (3-Ncp)Ala I], 59.1 [C-2′, (3-Ncp)Ala II] ppm. MS (ESI), positive *m*/*z* (%) = 1155/1153/1151 [M + Na^+].

Feeding Experiment with 6e·2 HCl: This was carried out according to GP 7 with **6e**·2 HCl (460 mg, 2.08 mmol) to give the unlabeled hormaomycin **1a** (18.0 mg).

Feeding Experiment with 34·2 HCl: This was carried out according to GP 7 with **34**·2 HCl (100 mg, 0.49 mmol) to give hormaomycin **1d** (1.1 mg).

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