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Biosynthesis of Streptothricin F

7. The Fate of the Arginine Hydrogens

STEVEN J. GOULD, JUNNING LEE, AND JOHN WITYAK

Department of Chemistry, Oregon State University, Corvallis, Oregon 97331-4003

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DL-[2,3,3,5,5-$^2H_5$]Arginine was synthesized, fed to *Streptomyces* L-1689-23, and incorporated into the antibiotic streptothricin F, **1.** Deuterium was retained at C-5, but completely lost from C-2 and C-3. *Erythro-* and *threo*-DL-[5,5-$^2H_2$]-β-hydroxyarginines were synthesized and fed to *S.* L-1689-23, but neither was incorporated into **1,** and isotope-trapping experiments failed to detect *de novo* biosynthesis of either β-hydroxyarginine. DL-[4,4-$^2H_2$]-, L[4,4-$^2H_2$]-, L-[4*R*-^{2}H]-, and L-[4*S*-^{2}H]arginines were synthesized and fed to *S.* L-1689-23, as well. The results of these latter feedings revealed that hydroxylation during the biosynthesis of **1** occurs with retention of configuration at C-4 of arginine.

Streptothricin F, **1,** one of the earliest detected antibiotics (*1*), is representative of a large group of ubiquitous natural products differing almost exclusively in the nature of the amide side-chain (*2–5*). We have studied the biosynthesis of **1** by *Streptomyces* L-1689-23 (*6*), including the origin of β-lysine, **2,** from L-α-lysine, **3** (*7, 8*), the gulosamine moiety from D-glucosamine, **4** (9), and the heterocyclic moiety (streptolidine) from L-α-arginine, **5** (*10, 11*) (Scheme 1).

Through the synthesis and incorporation of a set of arginines doubly labeled with ^{13}C and ^{15}N we established that formation of streptolidine (shown in its lactam form **6**), involves the creation of three new carbon–nitrogen bonds (*10, 11*). Bycroft had proposed (*12*) that **6** was formed *via* capreomycidine, **7** (*13*), which was consistent with these results (Scheme 2). However, it had further been proposed (*12*) that **7** was derived from α,β-dehydroarginine, **8.** This proved to be inconsistent with data we obtained from feedings of [2-^{2}H]-, [3,3-$^2H_2$]-, and [2,3,3-$^2H_3$]arginines (*11*). In all these latter feedings the derived **1** was devoid of deuterium enrichment. In this report we describe further experiments to delineate the metabolism from arginine to **1.**

RESULTS AND DISCUSSION

Although the incorporations of DL-[1-^{14}C]arginine cofed with the deuterated arginines were substantial (1.6–13.7%), the critical biosynthetic conclusions mentioned above were based on negative data since no deuterium was incorporated. We therefore prepared [2,3,3,5,5-$^2H_5$]arginine, **5a,** so that a positive intramolecu-

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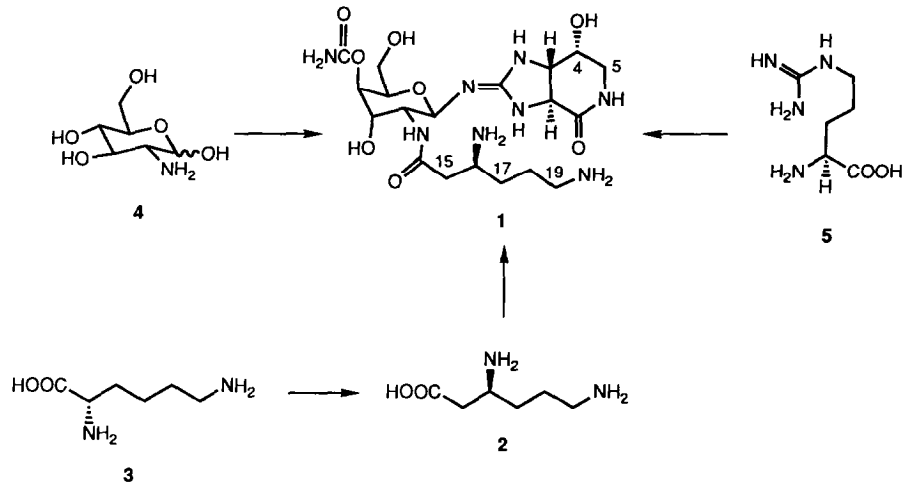
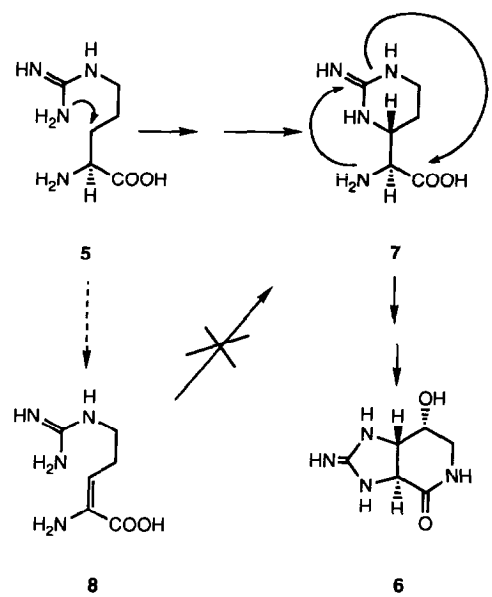
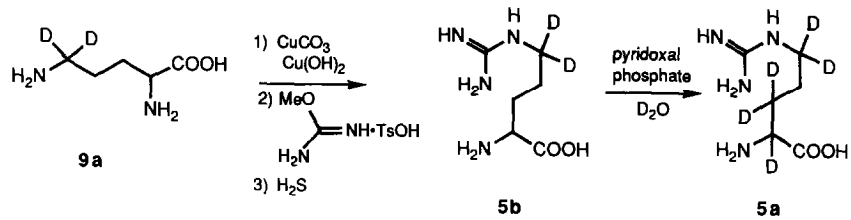


SCHEME 1

lar standard would be available. The synthesis (*14*) of **5a** is shown in Scheme 3. [5,5-$^2H_2$]Ornithine, **9a,** had been prepared in a 98% overall yield with a 96% enrichment in deuterium (*14*), and this was now converted to [5,5-$^2H_2$]arginine, **5b,** in 52% yield under standard conditions (*16*). Pyridoxal-catalyzed exchange (*17*) of the hydrogens at C-2 and C-3 then afforded a 95% enrichment at these positions and a 62% recovery of the desired **5a.**

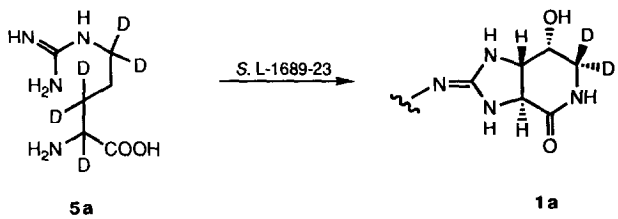


SCHEME 2



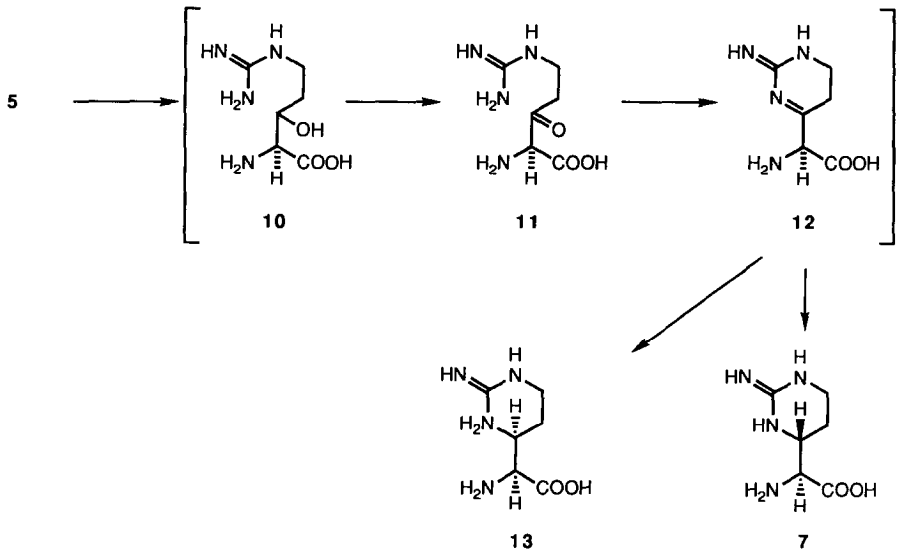
SCHEME 3

A sample of **5a** (200 mg) was fed to cultures of *S.* L-1689-23 (4 × 250 ml) and mixed with [1-^{14}C]arginine, and workup (*4*) after two days yielded 118 mg of streptothricin **1a,** as its helianthate salt. A second fermentation of the same size, fed 60 mg of **5a,** yielded 44 mg more of the helianthate. Incorporations of **5a** of 5.75 and 9.09%, respectively, were obtained. The combined salts were converted to the trihydrochloride (*11*) and the ^{2}H NMR spectrum was obtained at pH 6.8 in deuterium-depleted water with *t*-butanol present as the chemical shift reference and the deuterium quantitation standard. Enrichments of 4.6 and 5.0% were observed at the 5*R*- (δ 3.37) and 5*S*- (δ 3.83) positions, respectively, but no deuterium enrichment at H-2 or H-3 was observed. Some deuterium was also observed at H-15 (δ 2.73), H-17 (δ 1.85), and H-19 (δ 3.25) of the β-lysine moiety due to metabolism of the arginine fed.



This direct proof for the loss of the hydrogens from C-2 and C-3 of arginine supported the hypothesis (*11*) that the pathway involved hydroxylation to β-hydroxyarginine, **10,** oxidation to β-ketoarginine, **11,** and then cyclization to the dihydropyrimidine **12** (Scheme 4), providing a simple rationale for the formation of both **7** (2*S*, 3*S*) and its diastereomer **13** (2*S*, 3*R*), which occurs as part of elastatinal (*18*) and of chymostatin (*19*), *via* stereospecific enzymatic reductions (*11*). The loss of H-2 could then come either as a consequence of the mechanism for enzymatic hydroxylation at C-3 (*11*) or by chemical exchange of **11** since it would be adjacent to two carbonyls. This was tested next by examining the involvement of **10.**

Scheme 5 shows the route developed (*20*) for the synthesis of both the *erythro*- and the *threo*-isomers **14** and **15,** respectively (*21*), which allowed for convenient introduction of deuterium labels via the nitrone cycloaddition with formaldehyde-d_2. As shown, C-5 was again selected for deuterium labeling. Separation at the stage of the diastereomeric acids **16a** and **17a** proved most effective, and ultimately yielded [5,5-$^2H_2$]**14a** and [5,5-$^2H_2$]**15a**. Each of these was fed separately to



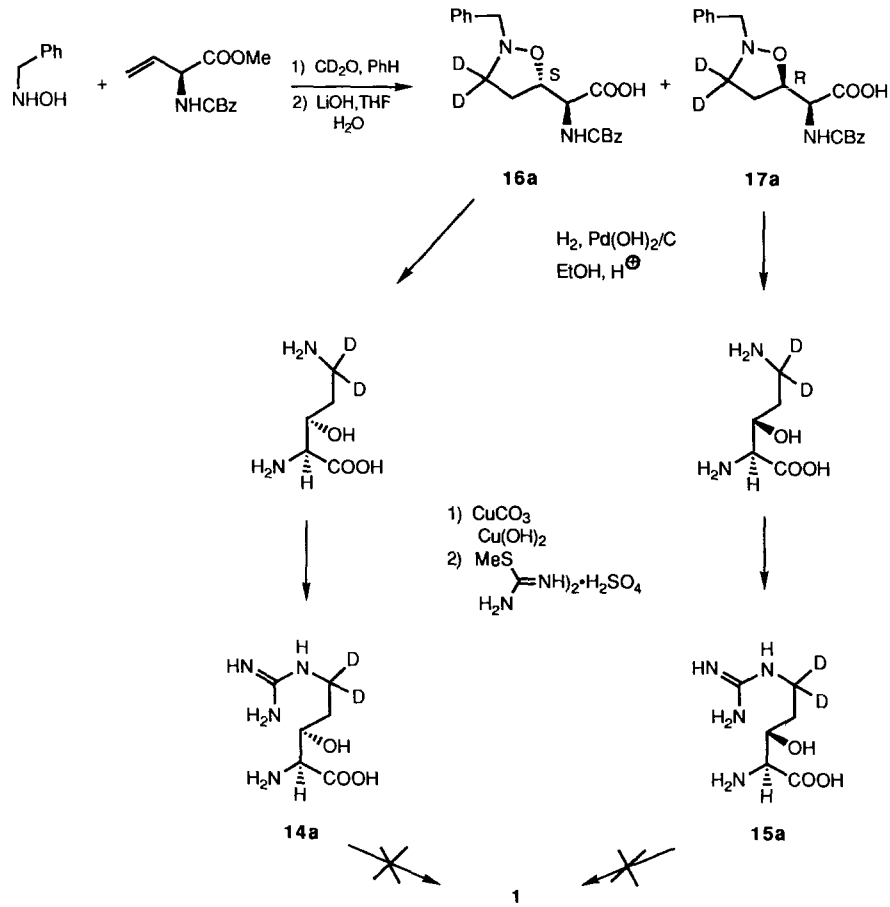
SCHEME 4

cultures of *S.* L-1689-23, but the samples of streptothricin F obtained were devoid of deuterium.

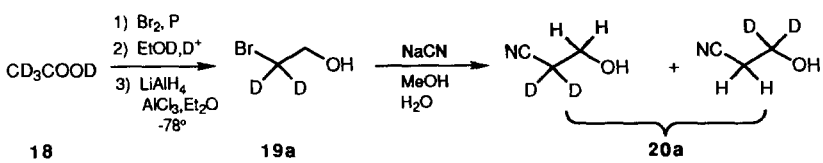
Not only were **14** and **15** (as **14a** and **15a**) not incorporated, but radioisotope trapping experiments failed to provide evidence for their formation by *S.* L-1689-23. Thus, portions of DL-α-[1-^{14}C]arginine were fed to 12, 20, and 32-h production broths, each incubated at 28–29°C. Three hours after each feeding the flask was removed, the contents sonicated and centrifuged, and the supernatant divided into two portions. A small amount of **14** or **15** (100 mg or 50 mg, respectively) was added to each portion. Each was then chromatographed on Amberlite IRC-50 with an ammonium hydroxide gradient. Ninhydrin-positive fractions were combined, ammonia was removed *in vacuo*, and the residue was lyophilized. Finally, each sample was recrystallized repeatedly and each was ultimately found to be devoid of radioactivity. Had any sample remained radioactive, it would have been *prima facie* evidence for *de novo* biosynthesis of that β-hydroxyarginine. However, in all cases the samples ended up at background levels of radioactivity.

To this point the fate of hydrogen from all positions of **5** had been determined except for those at C-4. This position ultimately is hydroxylated in all streptothricins except one recently reported example (*23*). The stereochemistry of hydroxylation in the formation of **1** was now established by the synthesis and feeding the 4-deuterio arginines, **5c, 5d, 5e,** and **5f.**

In order to synthesize DL-[4,4-$^{2}H_2$]arginine, **5c,** perdeuterio acetic acid, **18,** was converted to 2-bromo[2,2-$^{2}H_2$]ethanol, **19a** (*24*), and then treated with sodium cyanide to give **20a** (11% overall from **18**). However, examination of the ^{1}H NMR spectrum of **20a** revealed that deuterium was now present equally at C-2 and C-3, indicating the intermediacy of ethylene oxide during the cyanation reaction (Scheme 6) (*25*). Fortunately, in the course of this work it was found that β-

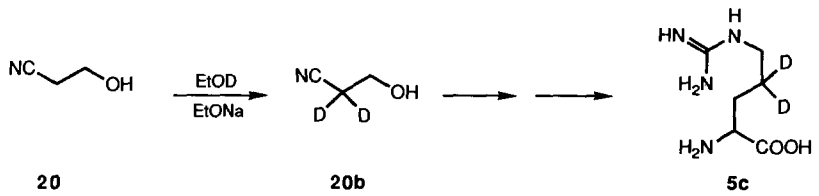


SCHEME 5



SCHEME 6

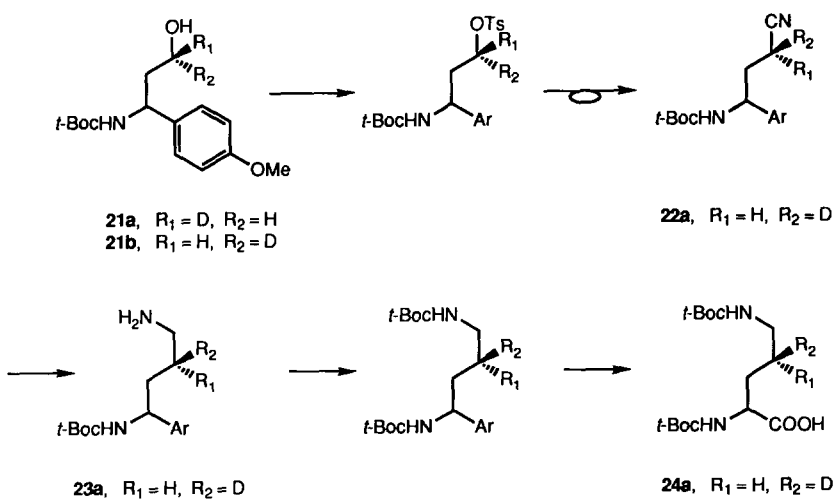
cyanoethanol, **20,** readily underwent exchange at C-2 in ethanol-d_1 with a catalytic amount of sodium ethoxide to give **20b.** The remainder of the synthesis of **5c** followed Scheme 3.



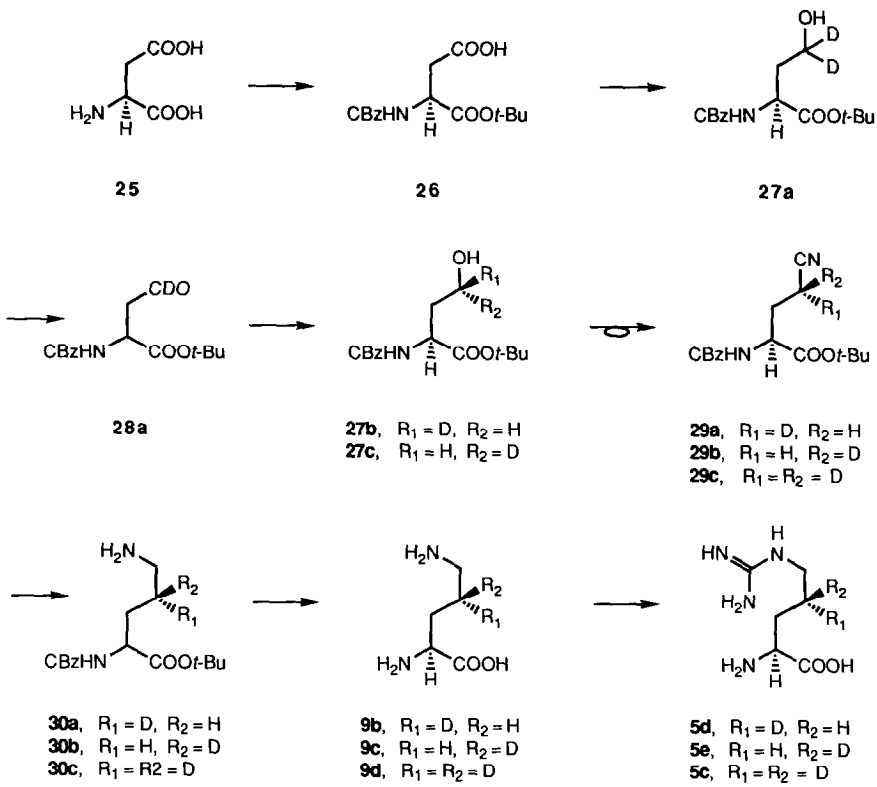
20 **20b** **5c**

In a first attempt to synthesize the chirally deuterated L-arginines [4*R*-^{2}H]**5d** and [4*S*-^{2}H]**5e**, the chirally deuterated alcohols **21a** and **21b** were prepared using the procedure reported by Woodard (*26*). Alcohol **21a** was next tosylated (*p*-TsCl/pyridine/0°C) and then treated with KCN in DMSO while the mixture was being sonicated, yielding 34% of **22a.** The nitrile was reduced with a variety of agents (B_2H_6 · $NiBr_2$, H_3B · $S(Me)_2$, $NaBH_3(OCOCF_3)$, H_2/PtO_2, H_2NNH_2/Ra · Ni, $LiAlH_4$), and lithium aluminum hydride gave the best result (53% yield of **23a**). Destructive oxidation of the aromatic ring of **23a** with RuO_4 afforded an unattractive mixture of products; protection of the primary amine with *t*-Boc prior to RuO_4 oxidation gave only a modest yield of **24a** (Scheme 7).

An alternative route to **5d** and **5e** from L-aspartic acid, **25,** was successful (Scheme 8) (*27*). The *N*-CBz-α-*t*-Boc derivative **26** was prepared in 40% overall yield (*28*). This was treated with ethylchloroformate followed by $NaBD_4$ reduction to give the dideuterated alcohol **27a,** which was oxidized under Swern conditions (*29*) to give **28a** in 50% yield for the two steps. Portions of **28a** were reduced with



SCHEME 7



SCHEME 8

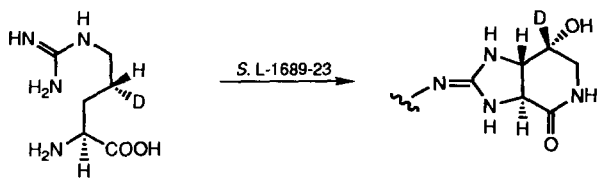
R-Alpine borane (*30*) and with *S*-Alpine borane to give **27b** and **27c**, respectively (*31*). Mesylation of **27b**, followed by cyanide displacement (KCN/DMSO/sonication) gave nitrile **29b** (70%), which was reduced to the protected L-ornithine, **30b**, with $NaBH_4/CoCl_6 \cdot 6H_2O$ in MeOH and then hydrolyzed (6 N HCl) to give L-ornithine, **9c** (53%). This was then converted to **5d** under standard conditions. In a similar manner **5e** was prepared. The route was then adapted to prepare L-[4,4-$^2H_2$]arginine, **5f**, by leaving out the Swern/Midland steps.

The enantiomeric excess at C-4 in each series was determined from the ^1H NMR spectra of the respective (−)-camphanates in the presence of $Eu(fod)_3$ at 15–20 mol% shift reagent. In the 4*R*-series the excess was 76% and in the 4*S*-series it was 80%. As had previously been observed with the phthalimidocamphanate of 3-aminopropanol (*32*), the resonance of the methine distal to the camphanate shifted downfield fastest *and* the relative positions of the resonances from the methine adjacent to the camphanate were, again, reversed from the Gerlach/Zagalak rule (*33*).

Portions of each compound, **5c–5f**, were mixed with DL-[1-^{14}C]arginine and fed to fermentations of *S.* L-1689-23 (3 × 250 ml) in three equal portions (12, 20, and 30 h after inoculation). After a total of 48 h each experiment was worked up to

yield between 112 mg and 150 mg of strepthothricin F (**1b–1e**) after Amberlite IRC-50 and Sephadex LH-20 chromatography. Each was converted to its helianthate salt, recrystallized, and then converted to its trihydrochloride for ^2H NMR analysis. The recrystallized helianthate was also used to determine the specific activity and, therefore, the percentage incorporation and expected enrichment in deuterium.

The ^2H NMR spectra (deuterium-depleted H_2O, *t*-butanol for reference) of **1b** and **1e** showed a broad resonance for deuterium at H-4 (δ 4.7 and 4.9, respectively, the chemical shift varying due to small changes in pD (*4*)), as expected. In the latter case this overlapped with the resonance from residual HOD. The spectrum of **1c** also contained the deuterium resonance at δ 4.75 for H-4, whereas the spectrum of **1d** only contained the narrow HOD resonance. Thus, it is the *pro*-4*R* hydrogen of arginine that is retained and the *pro*-4*S* hydrogen that is replaced by oxygen. This corresponds to net retention of configuration for the hydroxylation at this position.



5d **1c**

We have now unequivocally established that in the biosynthesis of **1** by *S.* L-1689-23 all three hydrogens from C-2 and C-3 of arginine are lost, while both hydrogens of arginine at C-5 and the *pro*-4*R* hydrogen are retained. Retention of configuration in the hydroxylation at what had been C-4 of arginine is consistent with the stereochemistry that has been observed in other biological hydroxylations at unactivated carbon centers (*34*). However, the combined loss of the C-2 and C-3 hydrogens and the noninvolvement of either *erythro-* or *threo*-β-hydroxyarginine are perplexing and leave the mechanism of formation of the apparent intermediate, capreomycidine, unresolved. Further experiments aimed at understanding how the capreomycidine ring is formed are in progress.

EXPERIMENTAL

General

^1H NMR spectra were taken on a Bruker AM 400 spectrometer at 400.13 MHz, on a Bruker AC 300 spectrometer at 300 MHz, or on a Varian FT-80A spectrometer at 80 MHz with TMS (0.00 ppm) or *t*-BuOH (1.28 ppm) as internal standard. ^{13}C NMR spectra were obtained with the Bruker instruments at 100.6 or 75 MHz, respectively. ^2H NMR spectra were obtained with the AM 400 spectrometer at 61.4 MHz in deuterium depleted water (Aldrich Chemical Co., Milwaukee, WI) with *t*-BuOH as internal standard (1.28 ppm). Infrared spectra were obtained with a Nicolet 5DXB FT-IR spectrometer. Low resolution mass spectra were mea-

sured on a Varian MAT CH-7 instrument and high resolution mass spectra were obtained on a Kratos MS 50 TC spectrometer. Optical rotations were determined on a Perkin–Elmer 243 polarimeter.

Flash chromatography was carried out on silica gel (EM Reagents, Keiselgel 60, 230–400 mesh). Analytical thin layer chromatography (TLC) was performed on a precoated Kieselgel 60 F_{254} plates (aluminum- or plastic-backed). Ion-exchange resins were purchased from either Bio-Rad Laboratories (Richmond, CA) or Sigma Chemical Co. (St. Louis, MO) and were converted to the necessary ionic form according to the manufacturer's directions. Melting points were determined on a Büchi melting point apparatus and are uncorrected. Sonications were done with a Branson Model 2000 water bath sonicator. Fermentations were carried out in a Lab-line or New Brunswick gyrotory incubator shaker. Microsamples were weighed on a Cahn Model 29 electrobalance. All radioactive measurements were carried out in a Beckman LS 7800 liquid scintillation counter; all measurements were done in duplicate to a ±3% standard deviation. Counting efficiencies were determined automatically by using the Beckman DPM program and sealed ^{14}C-quenched standards.

Paraformaldehyde-d_2 (99 atom% D) and sodium borodeuteride (99 atom% D) were purchased from Cambridge Isotope Laboratories (Woburn, MA); ethanol-d_1 (99.5 atom% D) from Aldrich Chemical Co., acetic acid-d_4 (99.5 atom% D) from ICN Biochemicals, Inc. (Cambridge, MA), and 38% DCl in D_2O (99 atom%) from Stohler/KOR. Eu(fod)$_3$ was purchased from Sigma, (-)-camphanyl chloride from Fluka Chemical Corp. (Ronkonkoma, NY), and (*R*)- and (*S*)-Alpine boranes (0.5 M in THF) from Aldrich. All other reagents and solvents were used without further purification unless noted, and were obtained from Aldrich, Sigma, Fluka, VWR Scientific (Seattle, WA), or American Scientific Products (Redmond, WA).

DL-[*5,5-*$^2H_2$]*Arginine*, **5b.** $CuCO_3 \cdot Cu(OH)_2$ (3.77 g, 17.0 mmol) was suspended in boiling water (100 ml) and DL-[5,5-$^2H_2$]ornithine (1.904 g, 11.16 mmol) was added. After 1 min, the excess salts were removed by filtration and washed with water. The combined filtrate was concentrated *in vacuo* to a volume of 10 ml, *O*-methyl isourea tosylate (*16*) (2.7 g, 11 mmol) was added, and the pH was adjusted to 11.0. Supplemental additions of the isourea (1.0 g, 4.0 mmol) were made after 6, 31, and 72 h. After 102 h, precipitation of the copper with H_2S and lyophilization gave a powder that was dissolved in water, adjusted to pH 4, and loaded onto a column of Dowex 50W-X8 (H^+ form, 100 mesh, 3 × 21 cm). Elution with 0.1 M NH_4OH, lyophilization of the arginine-containing fractions, and recrystallization from water/ethanol at pH 6.5 afforded 1.242 g (52%) of **5b**: mp 231°C dec (lit. (*11*) 223°C); ^1H NMR (D_2O, 80 MHz) δ 3.81 (t, J = 5.6 Hz, 1 H), 3.25 (m, 0.05 H), 1.83 (m, 4 H).

DL-[*2,3,3,5,5-*$^2H_5$]*Arginine*, **5a.** This was prepared using the procedure of LeMaster and Richards (*17*) except as follows. Arginine **5a** (1.18 g, 5.56 mmol), pyridoxal hydrochloride (120 mg, 0.589 mmol), and $Al_3(SO_4)_3 \cdot 16H_2O$ (94 mg, 0.15 mmol) were stirred in D_2O (6 ml) for 15 min followed by lyophilization. Collidine (0.25 ml, 3.0 mmol) and D_2O (6 ml) were added and the vessel was sealed under vacuum (5 Torr) after freezing. After reaction at 115–130°C for 2 days, the standard isolation procedure afforded 750 mg (62%) of the desired pentadeuterio

arginine: mp 228°C dec; ^{1}H NMR (D_2O, 80 MHz) δ 3.76 (m, 0.05 H), 3.2 (m, 0.05 H), 1.68 (m, 2.1 H).

2(S)-[2-Benzyl-3(S)- and 2(S)-[2-benzyl-3(R)-isoxazolidinyl][N-((benzyloxy) carbonyl)amino)][5,5-$^2H_2$]acetic acids, **16a** *and* **17a**. Methyl *N*-(benzyloxy)carbonyl-L-vinylglycinate (*22*) (8.00 g, 32.1 mmol), paraformaldehyde-d_2 (4.07 g, 128.4 mmol), hydroxylamine (4.36 g, 35.4 mmol), and 4-Å molecular sieves (10.0 g) were added to benzene (480 ml) and heated at a gentle relflux for 12 h. The sieves were removed by filtration and the filtrate concentrated *in vacuo* to a syrup. Purification by flash chromatography (95 : 4 : 1 benzene/EtOAc/MeOH, 4 × 34 cm column) afforded 10.28 g (83%) of the partially separated isoxazolidines in a ratio of 1.2 : 1.0 *threo/erythro*. The mixture of esters (9.44 g, 24.4 mmol) was dissolved in THF (300 ml) and cooled to 5°C in an ice-water bath. To this was added 0.56 N LiOH (10 ml). Purification by flash chromatography in 1-g batches (92 : 7 : 1 $CHCl_3$/MeOH/AcOH, 2.7 × 30 cm column) afforded 2.78 g of **16a** and 4.81 of **17a** (82% combined) (*20*). *erythro* (**16a**): mp 121–123°C; $[\alpha]_D^{25}$ + 16.47° (*c* 0.2, MeOH); ^{1}H NMR (MeOH-d_4, 400 MHz, 270 K) δ 7.3 (m, 10 H), 6.05 (br s, 1 H), 5.08 (s, 2 H), 4.65 (m, 1 H), 4.41 (d, J = 5.2 Hz, 1 H), 3.95 (d, J = 12.5 Hz, 1 H), 3.81 (d, J = 12.5 Hz, 1 H), 2.50 (m, 1 H), 2.28 (m, 1 H).

erythro-β-Hydroxy-L-[5,5-$^2H_2$]ornithine hydrochloride. Isoxazolidine **16a** (2.93 g, 7.7 mmol) and 20% $Pd(OH)_2$/C (2.1 g) in a mixture of ethanol (120 ml) and 4 N HCl (3.9 ml) were hydrogenated for 4 days. The catalyst was removed by filtration through celite and the filtrate concentrated *in vacuo*. The residue was dissolved in a small volume of water, adjusted to pH 6.5 with 2.8 M LiOH, and treated with ethanol to yield the crystalline hydrochloride (880 mg, 60%) of the deuterated β-hydroxyornithine: mp 232°C (dec); $[\alpha]_D^{25}$ +17.5° (*c* 3, H_2O); ^{1}H NMR (80 MHz, D_2O) δ 4.25 (m, 1H), 3.94 (d, J = 3.7 Hz, 1 H), 1.9 (m, 2H).

threo-β-Hydroxy-L-[5,5-$^2H_2$]ornithine hydrochloride. Using the same procedure, a mixture of **17a** (4.57 g, 12.3 mmol), 20% $Pd(OH)_2$/C (3.3 g), ethanol (190 ml), and 4 N HCl (6.1 ml) afforded 2.14 g (93%) of the hydrochloride salt: mp 120–121°C (dec); $[\alpha]_D^{25}$ +3.63° (c 2, H_2O); ^{1}H NMR (80 MHz, D_2O) δ 4.2 (m, 1H), 3.7 (d, J = 5.0 Hz, 1 H), 2.76 (m, 0.02 H), 1.95 (m, 2H).

erythro-β-Hydroxy-L-[5,5-$^2H_2$]arginine hydrochloride, **14a.** To the labeled *erythro*-β-hydroxyornithine (870 mg, 4.66 mmol) in water (30 ml) was added $CuCO_3 \cdot Cu(OH)_2$ (2.0 g, 9.0 mmol) and the resulting mixture was heated to near-boiling. After the excess salts were removed by filtration and washed with water, *S*-methyl isothiourea sulfate (1.44 g, 5.18 mmol) was added to the combined filtrate, and the pH was adjusted to 9.7 with 10% NaOH. After 2 h, most of the blue color had faded and was replaced by a yellow precipitate of copper methylthiolate. This precipitate was removed by filtration after 4 days and washed with water. The combined filtrate was adjusted to pH 5.5 with 10% HCl and lyophilized. The resulting lyophilizate was dissolved in water and adjusted to pH 2.5 followed by passage through a column of Dowex 50W-X8 (H^+ form, 100 mesh, 1.5 × 10 cm column, 120 ml H_2O : 6 N NH_4OH linear gradient). Combination and lyophilization of the appropriate fractions then afforded a powder, which was crystallized from ethanol/water at pH 6.6 to afford 805 mg (76%) of **14a:** mp 216–217°C (dec); $[\alpha]_D^{25}$

$-4.11°$ (c 2, H_2O); ^1H NMR (D_2O, 80 MHz) δ 4.2 (m, 1 H), 3.92 (d, J = 3.7 Hz, 1 H), 1.8 (m, 2 H).

threo-β-Hydroxy-L-[5,5-$^2H_2$]arginine hydrochloride, **15a.** This was prepared as above except for the following. To labeled *threo-β*-hydroxyornithine (889 mg, 4.76 mmol) in water (20 ml) was added $CuCO_3 \cdot Cu(OH)_2$ (1.4 g, 6.3 mmol) and the resulting mixture was heated to near-boiling. After the excess salts were removed *S*-methyl isothiourea sulfate (1.46 g, 5.24 mmol) was added, and the pH adjusted to 9.7. Isolation as above afforded 725 mg (67%) of **15a:** mp 204–206°C, $[\alpha]_D^{25}$ +14.58% (*c* 2, H_2O); ^1H NMR (D_2O, 80 MHz) δ 4.10 (m, 1 H), 3.68 (d, J = 5.2 Hz, 1 H), 1.9 (m, 2 H).

2-Cyano[2,2-$^2H_2$]ethanol, **20b.** To a solution of NaCN (0.32 g, 6.53 mmol) in D_2O (25 ml) and methanol-OD (15 ml) was added 5 g (4.8 ml, 70.34 mmol) of 3-hydroxypropionitrile (**20**), and the resulting solution was heated at reflux for 23 h. This solution was then concentrated to 10 ml, saturated with NaCl, and extracted with EtOAc. The EtOAc solution was dried over Na_2SO_4, filtered, and concentrated to yield 2.03 g (66.7%) of **20b.** Infrared (neat) 3414 (br), 2253, 1122 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.85 (s, 2H), 2.60 (m, 0.1 H).

(2S) N-Benzyloxycarbonyl[4,4-$^2H_2$]homoserine t-butylester, **27a.** Following literature procedures (*28*), to a solution of **26** (651 mg, 2.04 mmol) and triethylamine (230 mg, 2.25 mmol) in anhydrous THF (4 ml) was added dropwise ethyl chloroformate (232 mg, 2.15 mmol) in THF (3 ml) at −5°C. The reaction mixture was stirred at 0°C for 15 min and 10°C for 45 min. After removal of the white precipitate *via* filtration, the filtrate was again cooled to −5°C. A solution of $NaBD_4$ (170 mg, 4.5 mmol) in H_2O (5 ml) was added dropwise. Stirring was continued at RT for 5 h. Dilute HCl (0.3 M, 25 ml) was added and the resulting mixture was extracted with EtOAc (3 × 30 ml). The combined organic layer was washed with saturated NH_4Cl and saturated brine and then dried over $MgSO_4$. After removal of the solvent the crude product was chromatographed on silica gel eluting with hexane/EtOAc (2 : 1) to afford **27a** (338 mg, 54%) as a colorless oil: $[\alpha]_D^{25}$ −27.1° (c 2.6, EtOH), lit. for unlabeled −32.2° (c 2.6, EtOH); ir 3345, 2979, 1719, 1702, 1517, 1465, 1369, 1250, 1052, 751, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.34 (m, 5 H), 5.70 (d, J = 7.7 Hz, 1 H), 5.10 (ABq, J = 12.4, 2.8 Hz, 2 H), 4.40 (m, 1 H), 3.18 (br m, 1 H), 2.11 (dd, J = 14.0, 3.6 Hz, 1 H), 1.61 (dd, J = 14.0, 10.1 Hz, 1 H), 1.45 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.6, 156.8, 136.0, 128.4, 128.2, 128.0, 82.4, 67.1, 58.1, 57.9, 57.5, 57.3, 57.1, 51.4, 35.7, 27.8; FAB-MS (glycerol) *m/z* (relative intensity) 310.1 (M^+-1, 58%), 146.1 (100%), 128.0 (57%), 102.1 (49%).

(2S) 2-N-Benzyloxycarbonylamino-4-oxo-[4-^2H]butanoic acid t-butylester, **28a.** Oxalyl chloride (250 mg, 1.97 mmol) in dichloromethane (15 ml) was cooled to −65°C. DMSO (339 mg, 4.33 mmol) in dichloromethane (2 ml) was added dropwise. The resultant solution was stirred at −60°C for 15 min, alcohol **27a** (511 mg, 1.64 mmol) in dichloromethane (8 ml) was added, and the reaction was continued at −60°C for 15 min. Triethylamine (1.1 ml) was added and the reaction was warmed to room temperature. After the literature (*28*) workup, purification of the product by silica gel chromatography yielded **28a** (490 mg, 97%): $[\alpha]_D^{25}$ +22.3° (c 1.1, $CHCl_3$), lit. for unlabeled +17.0° (c 1.0, $CHCl_3$); ir 3356, 2977, 2937, 1717,

1702, 1517, 1508, 1368, 1221, 1059, 751, 697 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33 (m, 5 H), 5.79 (d, J = 7.9 Hz, 2 H), 5.10 (s, 2 H), 5.53 (ABX, J = 8.0, 5.1 Hz, 1 H), 2.97 (ABX, J = 13.4, 5.1 Hz, 2 H), 1.43 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 200.2, 199.8, 199.4, 169.4, 155.7, 136.0, 128.3, 128.0, 127.8, 82.6, 66.8, 49.4, 45.7, 27.7.

(*2S,4S*) *N-Benzyloxycarbonyl[4-^{2}H]homoserine t-butylester,* **27b.** To a solution of aldehyde **28a** (800 mg, 2.6 mmol) in anhydrous THF (18 ml) under argon was added dropwise (*R*)-Alpine borane (6.4 ml, 3.2 mmol), under argon. After stirring overnight at room temperature the mixture was heated at reflux for 2 h, and then cooled to room temperature. Acetaldehyde (2.8 ml) was added to the reaction mixture, and the solvent was then removed *in vacuo*. The resulting greenish oil was dissolved in anhydrous ether (50 ml) and cooled to −10°C. Ethanolamine (0.2 ml, 3.2 mmol) was added and the mixture was stirred for 20 min. The white precipitate that formed was removed by filtration through celite, and the filtrate was washed with water (2 × 20 ml), saturated brine, and dried over $MgSO_4$. After removal of solvent the residue was chromatographed on silica gel eluting with hexane/EtOAc (3 : 2) to give pure **27b** (660 mg, 81.7%) as a colorless oil: ir 3365, 2977, 2934, 1718, 1709, 1455, 1393, 1366, 1256, 1227, 1158, 1056, 745, 699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38 (m, 5 H), 5.62 (d, J = 7.3 Hz, 1 H), 5.12 (ABq, J = 14.1, 2.4 Hz, 2 H), 4.41 (m, 1 H), 3.68 (m, 1 H), 3.11 (d, J = 8.7 Hz, 1 H), 2.13 (m, 1 H), 1.60 (m, 1 H), 1.46 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.5, 156.9, 136.0, 128.5, 128.1, 82.4, 67.1, 58.1, 57.9, 57.6, 51.4, 35.9, 27.8; FAB-MS (glycerol) *m/z* (relative intensity) 311.1 (M^+ + 1, 90%), 255.1 (100%), 211.2 (98%), 165.2 (20%), 121.1 (23%).

(*2S,4R*) *N-Benzyloxycarbonyl[4-^{2}H]homoserine t-butylester,* **27c.** In the same fashion, **27c** was prepared in 85% yield from the aldehyde **28a** using (*S*)-Alpine borane: ir, ^{1}H NMR, ^{13}C NMR, and mass spectra were identical to those described above.

(*2S,4S*) *N-Benzyloxycarbonyl[4-^{2}H]homoserine-4-mesylate t-butylester.* To a solution of alcohol **27b** (602 mg, 1.94 mmol), triethylamine (344 mg, 3.0 mmol), and DMAP (24 mg, 0.194 mmol) in anhydrous CH_2Cl_2 (28 ml) was added mesyl chloride (245 mg, 2.14 mmol) in CH_2Cl_2 (5 ml) at 0°C. The reaction mixture was stirred at 0°C for 4 h and at room temperature overnight. Ice water (30 ml) was then added and the aqueous solution extracted with CH_2Cl_2 (2 × 20 ml). The combined organic layer was washed with water and saturated brine and dried over $MgSO_4$. After removal of solvent the residue was purified by chromatography on silica gel eluting with hexane/EtOAc (2 : 1) to afford the mesylate (740 mg, 98.4%): $[\alpha]_D^{25}$ +9.7° (c 2.2, $CHCl_3$); ir 3361, 2979, 2939, 1721, 1525, 1456, 1356, 1175, 1157, 975, 943, 843, 801, 699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.30–7.37 (m, 5 H), 5.45 (d, J = 7.43 Hz, 1 H), 5.11 (s, 2 H), 4.37 (ABX, J = 7.0, 5.0 Hz, 2 H), 3.14 (s, 3 H), 2.33 (ABX, J = 14.0, 5.0 Hz, 1 H), 2.09 (ABX, J = 14.0, 7.0 Hz, 1 H), 1.47 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.4, 155.8, 136.1, 128.5, 128.2, 128.1, 82.9, 67.0, 65.9, 65.6, 65.3, 51.3, 37.1, 31.9, 27.8.

(*2S,4S*) *N-Benzyloxycarbonyl[4-^{2}H]homoserine-4-mesylate t-butylester.* Using the same conditions, **27c** (708 mg, 2.28 mmol) was converted to the [4*R*-^{2}H]mesylate in 100% yield (887 mg): $[\alpha]_D^{25}$ +10.0° (c 1.0, $CHCl_3$); ir, ^{1}H NMR and ^{13}C NMR spectra were identical to those described above.

N-Benzyloxycarbonyl-4-cyanobutanoic acid t-butylester, **29.** Mesylate (237 mg, 0.512 mmol) and KCN (excess) were dissolved in anhydrous DMSO, and the resulting mixture was subjected to ultrasound irradiation at room temperature for 4 h. After removal of DMSO *in vacuo* the residue was chromatographed on silica gel, eluting with hexane/EtOAc (2:1) to afford the nitrile **29** (133 mg, 82%) as a colorless oil: ir 3342, 2980, 1721, 1527, 1455, 1359, 1252, 1227, 1154, 1050, 846, 743, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35 (m, 5 H), 5.43 (d, J = 6.5 Hz, 1 H), 5.12 (s, 2 H), 4.29 (m, 1 H), 2.41 (m, 2 H), 2.34 (m, 1 H), 2.03 (m, 1 H), 1.48 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.8, 156.0, 135.8, 128.6, 128.1, 128.0, 118.6, 83.5, 67.2, 53.3, 28.9, 27.8, 13.4; FAB-MS (glycerol) m/z (relative intensity) 319.0 (M^+ +1, 35%), 309.0 (54%), 263.0 (55%), 91.0 (100%), 57.1 (85%); HR FAB-MS (glycerol) exact mass calcd for $C_{17}H_{23}N_2O_4$: 319.1642 ($M+H^+$), found: 319.1658.

(2S,4R) N-Benzyloxycarbonyl-4-cyano[4-^2H]butanoic acid t-butylester, **29a.** [4*S*-^2H]Mesylate (270 mg, 0.696 mmol) and KCN (excess) were dissolved in anhydrous DMSO, and the resulting mixture was subjected to ultrasound at room temperature for 4 h. Removal of DMSO *in vacuo* and purification as above afforded nitrile **29** (218 mg, 97% yield): ir 3345, 2979, 1724, 1527, 1455, 1360, 1252, 1227, 1155, 1050, 846, 743, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35 (m, 5 H), 5.43 (d, J = 6.5 Hz, 1 H), 5.11 (s, 2 H), 4.31 (m, 1 H), 2.32 (m, 1 H), 2.27 (m, 1 H), 1.99 (m, 1 H), 1.47 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.8, 155.9, 135.9, 128.5, 128.1, 118.8, 83.3, 67.2, 53.3, 28.8, 27.8, 13.6, 13.4, 13.1; FAB-MS (glycerol) m/z (relative intensity) 320.1 (M^++1, 48%), 264.1 (96%), 220.1 (8%), 181.2 (5%), 154.1 (4%), 91.1 (100%), 57.1 (63%); HR FAB-MS (glycerol) exact mass calcd for $C_{17}H_{22}DN_2O_4$: 320.1726 ($M+H^+$), found: 320.1721.

(2S,4S) N-Benzyloxycarbonyl-4-cyano[4-^2H]butanoic acid t-butylester, **29b.** Nitrile **29b** was synthesized in 100% yield (460 mg) in the same fashion from the [4*R*-^2H]mesylate (560 mg, 1.44 mmol): ir, ^1H NMR, and ^{13}C NMR spectra were identical to those described above; FAB-MS (glycerol) m/z (relative intensity) 320.1 (M^++1, 45%), 264.1 (98%), 91.1 (100%), 57.1 (62%); HR FAB-MS (glycerol) exact mass calcd for $C_{17}H_{22}DN_2O_4$: 320.1726 ($M+H^+$), found: 320.1721.

(2S,4R) N-Benzyoxycarbonyl[4-^2H]ornithine t-butylester, **30a.** To a solution of nitrile **29a** (205 mg, 0.645 mmol) and $CoCl_2 \cdot 6H_2O$ (165 mg, 0.694 mmol) in methanol (3 ml) was added $NaBH_4$ (132 mg, 3.47 mmol) and the mixture was stirred 1 h at room temperature. Hydrochloric acid (3 M, 1 ml) was added and the methanol removed under high vacuum. Extraction with ether removed unreduced nitrile, and the aqueous residue was made basic with NH_4OH. This was extracted with EtOAc and the extracts were filtered through celite and then concentrated *in vacuo* to give **30a** in 66% yield (138 mg): $[\alpha]_D^{25}$ −5.5° (c 1.0, EtOH), lit(*35*) for unlabeled −6.5° (c 8.0, acetone); ir 3326, 2976, 2938, 1714, 1672, 1501, 1404, 1363, 1342, 1258, 1156, 1052, 745, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35, (m, 5 H), 5.65 (br s, 1 H), 5.09 (s, 2 H), 4.25 (m, 1 H), 2.70 (d, J = 6.8 Hz, 2 H), 1.83 (m, 1 H), 1.71 (m, 1 H), 1.45 (s, 9 H), 1.42 (m, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.5, 155.9, 136.3, 128.3, 128.0, 127.9, 81.8, 66.6, 54.0, 41.2, 29.9, 28.4, 28.2, 27.8; FAB-MS (glycerol) m/z (relative intensity) 324.1 (M^++1, 18%), 304.1 (28%), 268.1 (24%), 204.1 (45%), 91.1 (100%), 57.1 (30%); HR FAB-MS (glycerol) exact mass calcd for $C_{17}H_{26}DN_2O_4$: 324.2033 ($M+H^+$), found: 324.2033.

(2S,4S) N-Benzyloxycarbonyl[4-^2H]ornithine t-butylester, **30b.** In the same

manner **29b** (460 mg, 1.45 mmol) was reduced with $NaBH_4$ (435 mg, 11.45 mmol) in the presence of $CoCl_2 \cdot 6H_2O$ (690 mg, 2.90 mmol) to **30b** (430 mg, 92%): $[\alpha]_D^{25}$ −5.8° (c 0.95, EtOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.35 (m, 5 H), 5.69 (br s, 1 H), 5.12 (s, 2 H), 4.12 (m, 1 H), 3.34 (d, J = 7.0 Hz, 2 H), 1.82 (m, 1 H), 1.71 (m, 1 H), 1.45 (s, 9 H), 1.41 (m, 1 H); HR FAB-MS (glycerol) exact mass calcd for $C_{17}H_{26}DN_2O_4$: 324.2033 ($M+H^+$), found: 324.2033.

(2S,4R)-[4-^{2}H]-Ornithine, **9b.** A solution of **30a** (479 mg, 1.74 mmol) in 6 N HCl was heated at reflux for 5 h, cooled, and extracted with EtOAc to remove water insoluble impurities. The aqueous solution was then concentrated *in vacuo* to remove HCl, and the residue was dissolved in water (3 ml) and lyophilized. The crude ornithine was taken up in 95% EtOH and concentrated NH_4OH was added dropwise until cloudiness appeared. After standing at 4°C overnight the product was filtered and dried to afford **9b** as a white powder (169 mg, 73%): mp 139°C; $[\alpha]_D^{25}$ +7.1° (c 0.55, H_2O); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.83 (t, J = 6.3 Hz, 1 H), 3.07 (d, J = 7.5 Hz, 2 H), 1.97 (m, 2 H), 1.81 (m, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.0, 54.7, 41.0, 32.0, 25.0, 24.7, 24.5; FAB-MS (glycerol) m/z (relative intensity) 132.2 (M^+-1, 100%), 113.1 (22%), 91.1 (64%), 71.1 (24%), 59.1 (47%); HR FAB-MS (glycerol) exact mass calcd for $C_5H_{12}DN_2O_2$: 134.1055 ($M+H^+$), found: 134.1040.

(2S,4S)-[4-^{2}H]Ornithine, **9c.** In the same fashion **9c** (140 mg, 67%) was made from amine **30b** (400 mg, 1.24 mmol): mp 139°C; $[\alpha]_D^{25}$ +7.2° (c 0.55, H_2O); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.82 (t, J = 6.3 Hz, 1 H), 3.08 (d, J = 7.5 Hz, 2 H), 1.97 (m, 2 H), 1.79 (m, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 179.7, 55.6, 39.9, 30.5, 24.2, 23.9, 23.7; FAB-MS (glycerol) m/z (relative intensity) 132.2 (M^+-1, 100%), 113.1 (22%), 91.1 (64%), 71.1 (24%), 59.1 (47%); HR FAB-MS (glycerol) exact mass calcd for $C_5H_{12}DN_2O_2$: 134.1055 ($M+H^+$), found 134.1040.

(2S,4R)-[4-^{2}H]Arginine, **5d.** A solution of ornithine **9b** (267 mg, 1.59 mmol) and excess $CuCO_3 \cdot Cu(OH)_2$ (527 mg, 2.38 mmol) in water (3 ml) was boiled for 1 min. The undissolved copper carbonate was removed by filtration and washed with boiling water (2 × 1.5 ml) and the combined filtrates (about 10 ml) were cooled and concentrated to 2 ml. *S*-Methyl isothiouronium sulfate (662 mg, 2.38 mmol) was added to the solution, and, after adjustment to pH 10.0 with 10% NaOH, the mixture was stirred for 5 days. During this period several additional portions of *S*-methyl isothiouronium sulfate were added to the reaction. After the reaction was complete (by TLC) the precipitated yellow copper methylthioate was removed by filtration and the filtrate was concentrated by rotary evaporation, diluted with water, adjusted to pH 5, and purified by ion-exchange chromatography (Dowex 50W-X8, H^+, 100 mesh, 15 × 2.5 cm, eluted with a H_2O:6 N NH_4OH linear gradient). The appropriate fractions were combined and lyophilized to give 198 mg of crude arginine as a yellowish glassy solid which was further purified by recrystallization with H_2O–EtOH to afford pure **5d** (110 mg, 39%) as colorless crystals: mp 230°C (dec); $[\alpha]_D^{25}$ +7.6° (c 1, H_2O); ^1H NMR (D_2O, 300 MHz) δ 3.36 (t, J = 7.7 Hz, 1 H), 3.23 (d, J = 6.3 Hz, 2 H), 1.67 (m, 3 H); ^{13}C NMR (D_2O, 75 MHz) δ 184.5, 159.1, 57.8, 43.2, 33.4, 26.4, 26.3, 26.2; HR FAB-MS (glycerol) exact mass calcd for $C_6H_{14}DN_4O_2$ ($M+H^+$): 176.1276, found: 176.1258.

(2S,4S)-[4-^{2}H]Arginine, **5e.** In the same fashion, **9c** (265 mg, 1.58 mmol) was

converted into **5e** in 44% yield (124 mg): mp 231°C (dec);$[\alpha]_D^{25}$ +7.7° (c 1, H_2O); ^1H NMR (D_2O, 300 MHz) δ 3.37 (t, J = 7.2 Hz, 1 H), 3.25 (d, J = 6.3 Hz, 2 H), 1.67 (m, 3 H).

(*2S*) *N-Benzyloxycarbonyl[4,4-$^2H_2$]homoserine-4-mesylate t-butylester.* From **27a** (1.63 g, 5.24 mmol) and MsCl (0.66 g, 5.76 mmol) using the procedure previously described, [4,4-$^2H_2$]mesylate (1.70 g, 83%) was obtained as a colorless oil: ir 3369, 2980, 2939, 1726, 1703, 1526, 1455, 1365, 1228, 1181, 1152, 979, 844, 800, 749, 700 cm^{-1}; ^1H NMR (benzene-d_6, 300 MHz) δ 7.30 (m, 5 H), 5.6 (d, J = 7.9 Hz, 1 H), 5.06 (s, 2 H), 4.33 (ABX, J = 7.8, 4.8 Hz, 1 H), 2.91 (s, 3 H), 2.26 (ABX, J = 14.6, 4.8 Hz, 1 H), 2.01 (ABX, J = 14.6, 7.8 Hz, 1 H), 1.43 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.3, 155.7, 136.0, 128.3, 128.0, 127.9, 82.6, 66.7, 65.8, 65.6, 65.3, 65.0, 64.8, 51.1, 36.8, 31.4, 27.6.

(*2S*) *N-Benzyloxycarbonyl-4-cyano[4,4-$^2H_2$]butanoic acid t-butylester,* **29c.** Mesylate (1.68 g, 4.31 mmol) and excess KCN in anhydrous DMSO (35 ml) afforded nitrile **29c** (1.087 g, 78.8%) as a colorless oil: ir 3336, 2979, 2938, 1724, 1530, 1370, 1224, 845, 748, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.36 (m, 5 H), 5.44 (d, J = 6.6 Hz, 1 H), 5.11 (s, 2 H), 4.32 (td, J = 6.5, 4.1 Hz, 1 H), 2.27 (dd, J = 13.7, 4.1 Hz, 1 H), 1.98 (dd, J = 13.7, 6.5 Hz, 1 H), 1.47 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.7, 155.8, 135.9, 128.3, 128.1, 127.9, 118.7, 80.9, 66.9, 53.2, 28.4, 27.7, 13.5, 13.3, 13.0, 12.8, 12.6; FAB-MS (glycerol) *m/z* (relative intensity) 319.2 (M^+-1, 19%), 263.1 (17%), 229.2 (63%), 211.2 (24%), 155.1 (100%), 131.1 (25%), 99.0 (16%), 83.1 (15%), 57.1 (14%); HR FAB-MS (glycerol) exact mass calcd for $C_{17}H_{21}D_2N_2O_4$: 321.1814, found: 321.1783.

(*2S*) *N-Benzyloxycarbonyl[4,4-$^2H_2$]ornithine t-butylester,* **30c.** Nitrile **29c** (0.495 g, 1.55 mmol) was reduced with $NaBH_4$ (0.589 g, 15.5 mmol) to give **30c** (363 mg, 72%): $[\alpha]_D^{25}$ −6.7° (c 2.0, EtOH); ir 3321, 2978, 2936, 1718, 1702, 1528, 1455, 1368, 1342, 1259, 1227, 1156, 1052, 848, 740, 698 cm^{-1}; ^1H NMR (benzene-d_6, 300 MHz) δ 7.34 (m, 5 H), 5.86 (br s, 1 H), 5.09 (s, 2 H), 4.24 (br d, J = 5.4 Hz, 1 H), 2.68 (s, 2 H), 1.82 (dd, J = 5.4, 13.7 Hz, 1 H), 1.68 (dd, J = 7.2, 13.7 Hz, 1 H), 1.46 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.4, 155.8, 136.3, 128.3, 128.0, 127.9, 81.8, 66.6, 54.0, 41.3, 29.9, 27.8, 27.7, 27.6, 27.5; FAB-MS (glycerol) *m/z* (relative intensity) 325.1 (M^++1, 50%), 269.1 (58%), 206.1 (6%), 116.1 (6%), 91.1 (100%), 72.1 (10%), 57.1 (23%); HR FAB-MS (glycerol) exact mass calcd for $C_{17}H_{25}D_2N_2O_4$ ($M+H^+$): 325.2127, found: 325.2096.

(*2S*)-[*4,4-$^2H_2$]Ornithine,* **9d.** A solution of **30c** (324 mg, 1.0 mmol) in 6 N HCl (15 ml) was heated to reflux for 5 h, and workup gave the ornithine **9d** (99 mg, 58%): mp 138°C; $[\alpha]_D^{25}$ +8.8° (c 1, H_2O); ^1H NMR (D_2O, 300 MHz) δ 4.21 (t, J = 6.38 Hz, 1 H), 3.12 (s, 2 H), 2.08 (2 ABq, J = 14.3, 6.4, 2 H); ^{13}C NMR (D_2O, 75 MHz) δ 172.9, 53.8, 40.1, 28.1, 23.8, 23.5, 23.3, 23.0, 22.7; FAB-MS (glycerol) *m/z* (relative intensity) 170.1 (M^++1, 23%), 135.1 (100%), 118.1 (19%), 93.1 (18%), 72.1 (32%), 57.1 (21%).

(*2S*)-[*4,4-$^2H_2$]Arginine,* **5f.** Ornithine **9d** (160 mg, 0.9 mmol), in H_2O (5 ml), was chelated with excess $CuCO_3 \cdot Cu(OH)_2$ (331 mg, 1.5 mmol) and treated with *S*-methyl isothiourea sulfate to yield after workup **5f** (65 mg, 41%) as a white powder: mp 239°C (dec); $[\alpha]_D^{25}$ +9.6° (c 1, H_2O); ^1H NMR (D_2O, 300 MHz) δ 3.79 (t, J = 5.8 Hz, 1 H), 3.25 (br s, 2 H), 1.91 (br d, J = 5.8 Hz, 2 H).

Feeding Experiments

Culture conditions, bioassay protocols, and isolation of streptothricin F have been described (*11*). One-third of the precursor was added at 12, 20, and 22 h after inoculation. Fermentations were worked up after a total of 45 h.

a. DL-[*2,3,3,5,5-*$^2H_5$]*Arginine,* **5a.** To four 250-ml broths, **5a** (200 mg, 0.927 mmol) and 3.79×10^7 dpm of DL-[1-^{14}C]arginine were fed, and workup yielded 237 mg of **1a** by bioassay. Purification by chromatography on Amberlite IRC-50 and Sephadex LH-20 afforded 210 mg, which was converted to its helianthate and recrystallized (2.82×10^6 dpm/mmol, 5.75% incorporation). Regeneration of the trihydrochloride gave 101 mg (0.165 mmol), which was used for ^{2}H NMR analysis: 90° pulse angle, 4K data points zero-filled to 8K, 1.245-s acquisition time, 3-Hz line broadening, 15,302 scans.

b. DL-[*4,4-*$^2H_2$]*Arginine,* **5c.** Three 250-ml broths were fed **5c** (100 mg, 0.575 mmol) and 1.51×10^7 dpm of DL-[1-^{14}C]arginine. Workup yielded 530 mg of **1b** by bioassay, of which 144 mg remained after LH-20 chromatography. A portion (63 mg) was converted to the helianthate, recrystallized (1.54×10^6 dpm/mmol, 16.1% incorporation), converted to the trihydrochloride, and used for ^{2}H NMR analysis: 5798 scans.

c. L-[*4,4-*$^2H_2$]*Arginine,* **5f.** A mixture of **5f** (60 mg, 0.34 mmol) and 1.48×10^7 dpm of DL-[1-^{14}C]arginine was fed to three 250-ml broths and workup yielded 536 mg of **1e.** Purification yielded 148 mg (1.77×10^6 dpm/mmol, 25.0% incorporation), which was used for ^{2}H NMR analysis: 4712 scans.

d. L-[*4R-*^{2}H]*Arginine,* **5d.** A mixture of **5d** (103 mg, 0.575 mmol) and 1.49×10^7 dpm of DL-[1-^{14}C]arginine was fed to three 250-ml broths and workup yielded 530 mg of **1c.** Purification through LH-20 chromatography yielded 158 mg, of which 48 mg was converted to the helianthate and recrystallized (1.73×10^6 dpm/mmol). Total incorporation was 12.3%. After conversion to the trihydrochloride, the sample was analyzed by ^{2}H NMR analysis: 3356 scans.

e. L-[*4S-*^2H]*Arginine,* **5e.** A mixture of **5e** (102 mg, 0.575 mmol) and 1.29×10^7 dpm of DL-[1-^{14}C]arginine was fed to three 250-ml broths and workup yielded 180 mg of **1d.** Purification through LH-20 chromatography yielded 112 mg, of which 104 mg was converted to the helianthate and recrystallized (4.06×10^6 dpm/mmol, 22.6% incorporation). After conversion to the trihydrochloride, the sample was analyzed by ^{2}H NMR analysis: 8737 scans.

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