Studies on the Formation and Incorporation of Streptolidine in the Biosynthesis of the Peptidyl Nucleoside Antibiotic Streptothricin F

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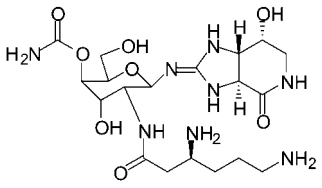
Streptothricin F (STF, **1**) is a peptidyl nucleoside antibiotic produced by *Streptomyces lavendulae*. Studies were conducted to address the formation and timing of incorporation of the arginine-derived base streptolidine (**4**) during the biosynthesis of **1**. [*guanidino*-^{13}C]Streptolidine (**10**) was prepared by modification of an established method and used in whole-cell incorporation experiments. Analysis of the purified STF by ^{13}C NMR revealed a 1.9% enrichment of the guanidino carbon, confirming **4** as an advanced precursor to **1** and supporting proposals that **1** is assembled via a convergent biosynthetic pathway. To identify advanced intermediates in the conversion of L-arginine to **4**, (2*S*,3*R*)-[*guanidino*-^{13}C]capreomycidine (**32**) was prepared from oxazolidine aldehyde (**18**) via 1,1-dimethylethyl (4*R*,1′*S*)-4-(1′,3′-diaminopropyl)-2,2-dimethyl-3-oxazolidinecarboxylate (**30**). Treatment of **30** with Br^{13}CN yielded the corresponding diprotected amino alcohol, which was readily converted to **32**. The STF isolated from whole-cell incorporation experiments with **32** showed no significant ^{13}C enrichment at the guanidino carbon. These results suggest that **32** may be an enzyme-bound intermediate, unable to enter the cell, or is not a precursor to STF.

Introduction

Streptothricin F (STF, **1**) is a broad-spectrum peptidyl nucleoside antibiotic first isolated from *Streptomyces lavendulae* in 1943 by Waksman and Woodruff.[1] STF consists of a carbamoylated D-gulosamine (**2**) to which is attached β-lysine (**3**) and the unusual amino acid streptolidine (**4**) in the amide form. It is one example of the streptothricin class of antibiotics, the members of which only differ in the number of β-lysine residues present.



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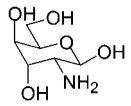

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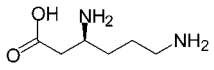

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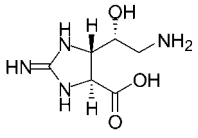

4

In the mid 1970s, two groups reported biosynthetic studies on **1**, but the use of universally labeled precursor compounds made it difficult to determine the specificity of incorporation as well as the pathway involved in the biosynthesis of streptolidine (**4**).[2,3] Sawada and co-workers concluded that two different pathways leading to the formation of **4** were present in *Streptomyces* species, one from arginine and the other from acetate. Later studies showed [1-^{13}C]- and [2-^{13}C]acetates were specifically incorporated, but a pathway was not proposed to explain the observed labeling pattern in **1**.[4] Using [1,2-^{13}C]-acetate, Gould and co-workers later determined that **1** was specifically, albeit indirectly, labeled by α-ketoglutarate generated by the TCA cycle and therefore also by arginine. Hence, there was no need to propose two separate pathways.[5]

In their early studies on **1**, Bycroft and King proposed the intermediacy of dehydroarginine (**5**) and the amino acid capreomycidine (**6**) in the biosynthesis of **4** (Scheme 1).[6] The incorporation studies using [1,2-^{13}C]acetate supported the direct biosynthesis of capreomycidine from arginine (**7**).[5] Added support for the proposal in Scheme 1 was obtained through studies using ^{13}C and ^{15}N dual-labeled arginine to identify the C−N bonds that are retained and cleaved during the formation of streptolidine.[7,8] Similarly, extensive studies with multiple forms of deuterated arginine established which hydrogens in **7** were retained and lost in the conversion to **4**.[8,9]

Convergent biosynthetic pathways can be thought of as processes where individual structural components of

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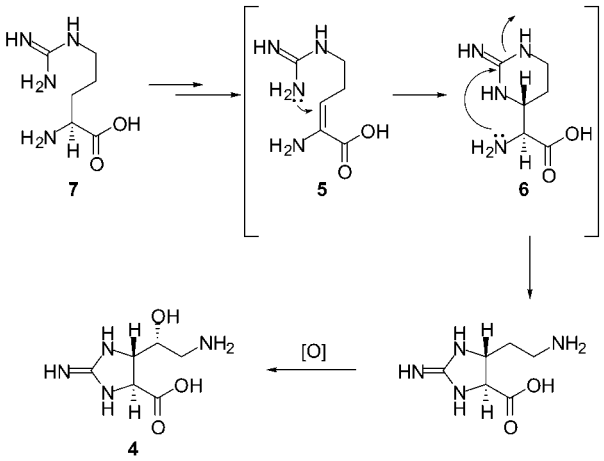
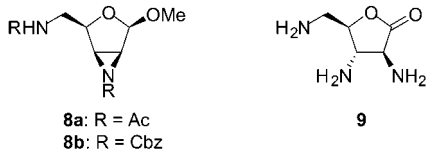
Scheme 1. Original Proposal for the Rearrangement of Arginine to Streptolidine[6]



the molecule are made independently and then assembled to form the core structure of the natural product. Postassembly modifications such as group transfer and redox reactions often occur before the molecule is presented in its biologically active form. Such pathways where individual components can be manipulated independently of one another represent excellent systems for combinatorial biosynthesis experiments. On the basis of the observed incorporation of β-lysine (**3**)[10] and D-glucosamine[11] into **1**, we sought to determine if streptolidine (**4**) was also a late-stage intermediate, supporting the proposal that STF is formed through a convergent process. Because earlier incorporation studies with labeled arginines supported the intermediacy of capreomycidine (**6**) and streptolidine (**4**), we launched efforts to prepare ^{13}C-labeled **4** and **6** for biosynthesis incorporation studies. Herein we report a new synthesis of [*guanidino*-^{13}C]capreomycidine, the preparation of [*guanidino*-^{13}C]streptolidine, and the results of whole-cell incorporation experiments in *S. lavendulae* var. L-1689-23.

Results and Discussion

Synthesis of [*guanidino*-^{13}C]Streptolidine and Subsequent Incorporation Studies. Two groups have reported syntheses of streptolidine. Independently, Goto and Kusumoto et al. reported the synthesis of **4** from ribose derivatives bearing stereochemical resemblance to streptolidine.[12−14] Important intermediates utilized by both groups were the protected aziridine acetals **8a** and **8b**,[15] and the triamine lactone **9**. The synthesis of **4** was completed in modest yield with the addition of cyanogen bromide to **9**.

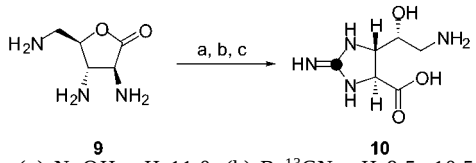


8a: R = Ac
8b: R = Cbz

9

Our preparation of [*guanidino*-^{13}C]streptolidine largely followed the route reported by Kusumoto et al. because unreacted starting material could easily be isolated for recycling and the Cbz protecting group provided a UV-active tag.[13] Some effort was devoted to improving the yield of the addition of the expensive ^{13}C-cyanogen bromide to **9**. It was noted that the pH of the reaction solution gradually dropped from pH 11 to 6.5 upon addition of cyanogen bromide and correlated with very poor yields of streptolidine. When the pH of the reaction mixture was maintained between 9.5 and 11, higher yields of [*guanidino*-^{13}C]streptolidine (**10**) were obtained with greater than 98% ^{13}C enrichment in the guanidino carbon (Scheme 2).

Scheme 2. Preparation of [^{13}C]Streptolidine from the Triamine 9[a]



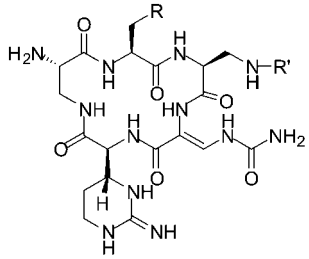
9 **10**

[a] Key: (a) NaOH, pH 11.0; (b) Br^{13}CN, pH 9.5−10.5; (c) 6 N HCl, 34−85% overall.

The [*guanidino*-^{13}C]streptolidine (**10**) thus synthesized was introduced to production flasks of *S.* L-1689-23 in one pulse, 12 h after inoculation. The labeled STF produced was isolated (18 mg) following the reported procedure.[5] Analysis of the ^{13}C NMR spectra of both labeled and control STF revealed a 1.9% enrichment of the guanidino carbon (C-6; 160 ppm), calculated by normalizing the resonance at 160 ppm to that of the lactam carbonyl at 168 ppm (Figure 1). Similar results were obtained when the experiment was repeated, serving to confirm **4** as a free intermediate in the formation of **1**.

It remains to be determined when the introduction of the hydroxyl in **4** occurs. One possibility is direct hydroxylation of deshydroxystreptolidine (**11**) (Scheme 3). An alternative is the oxidation of capreomycidine (**6**) to form **13**, which then undergoes rearrangement to **4**. It is also conceivable that 4-hydroxyarginine (**12**) is formed and cyclized to **13**.

Synthesis of L-[*guanidino*-^{13}C]Capreomycidine and Incorporation Studies. Capreomycidine (**6**) is an arginine-derived amino acid common among members of the tuberactinomycin class of peptide antibiotics, such as the capreomycins (**14**−**17**). Because capreomycidine is a likely intermediate in the biosynthesis of **4**,[6] we sought to test this hypothesis by preparing ^{13}C-labeled **6** for use in whole-cell incorporation studies.



Capreomycins

IA (**14**): R = OH, R' = β-Lysine
IB (**15**): R = H, R' = β-Lysine
IIA (**16**): R = OH, R' = H
IIB (**17**): R = H, R' = H

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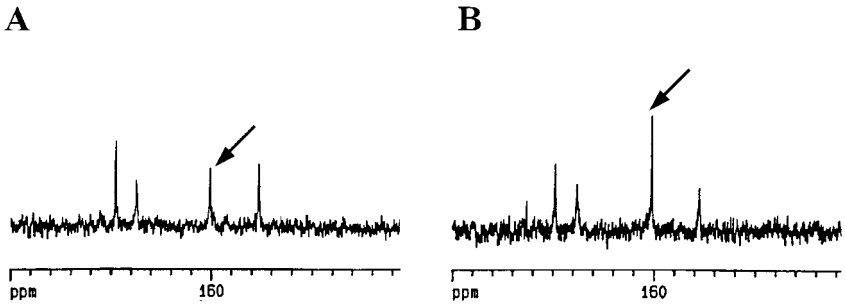
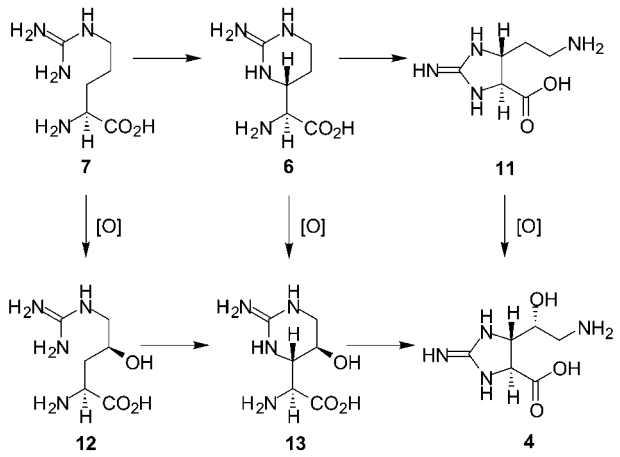
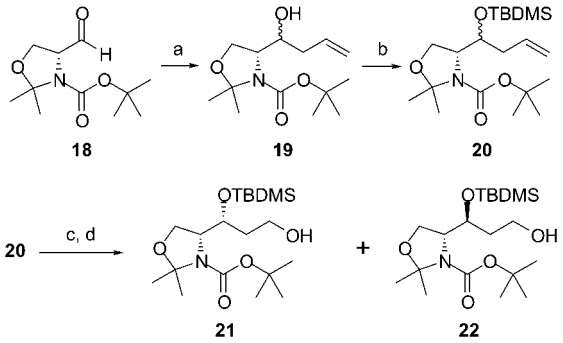


Figure 1. Partial ^{13}C NMR spectra of (A) streptothricin F (**1**) control and (B) [*guanidino*-^{13}C]-**1** isolated from incorporation studies.

Scheme 3. Timing of the Hydroxyl Introduction in Streptolidine



Scheme 4. Synthesis of TBDMS-Protected Alcohols 21 and 22[a]



[a] Key: (a) allylmagnesium chloride, THF, 86%; (b) TBDMSCl, DMF, imidazole, 98%; (c) O_3, MeOH, −78 °C; (d) $NaBH_4$, MeOH; 92%.

There are three reported procedures for the synthesis of **6**. The first synthesis of DL-**6** involved the sequential hydrogenation and hydrolysis of 2-amino-4-(α-methoxycarbonyl-α-hydroxyimino)methylpyrimidine.[16] Wakamiya et al. developed a synthesis of DL-**6** wherein the key intermediate was *β*-hydroxyornithine.[17] This method was later modified to include the separation of the *erythro* and *threo* diastereomers of *β*-hydroxyornithine by fractional crystallization followed by enzymatic resolution of the chloroacetamides. The (2*S*,3*R*)-*β*-hydroxyornithine thus prepared served as starting material for the preparation of (2*S*,3*R*)-capreomycidine.[18]

Our studies on STF (**1**) and other pathways in which capreomycidine may be involved required a stereospecific synthesis of labeled **6**. Because the isotopic label should be located at a site retained during biochemical transformations, we chose to introduce a ^{13}C label at the guanidine carbon, similar to our preparation of streptolidine. Our route to (2*S*,3*R*)-[^{13}C]capreomycidine began with Garner's D-serine-derived oxazolidine aldehyde **18** (Scheme 4).[19]

The reaction of aldehyde **18** and allylmagnesium chloride cleanly produced a diastereomeric mixture of the homoallylic alcohol **19**.[20] Protection of the hydroxyl with *tert*-butyldimethylsilyl chloride (TBDMS−Cl) afforded the TBDMS-protected homoallylic alcohol (**20**) in near quantitative yield.[21] Oxidative cleavage of the terminal double bond in **20** with ozone and in situ reduction with sodium borohydride afforded primary alcohols **21** and **22** in a 2:1 ratio, respectively.[22] Although the diastereomers of **20** can be separated using flash chromatography, the separation of alcohols **21** and **22** was achieved with greater efficiency.

To determine the relative stereochemical outcome of the Grignard reaction and establish the configuration of **21** and **22**, the TBDMS-protected alcohols were carefully separated by silica gel flash chromatography. The major diastereomer (**20a**) was deprotected to yield **19a**, which was converted to the α-methoxy-α-(trifluoromethyl)phenylacetate (MTPA) esters (Scheme 5).[23,24] Analysis of the proton NMR spectra of both diastereomers indicated that the configuration of the MTPA-bearing carbon in **19a** was *R*.[25] Further support for this assignment was obtained by comparing the spectral data of **19a** to those reported for its enantiomer.[26] Hence, the major product **20a** has the *R* configuration resulting from anti addition predicted by the Felkin−Ahn model.

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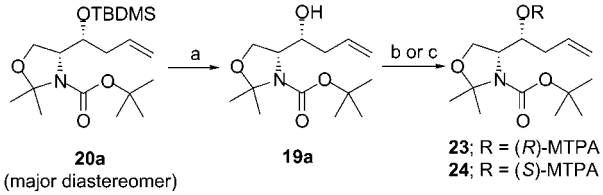
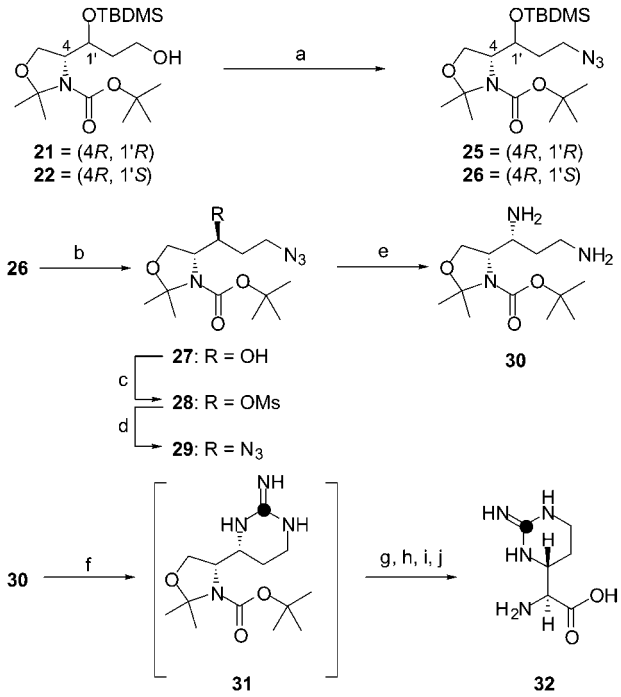
Scheme 5. Preparation of MTPA Esters for Configuration Analysis[a]



[a] Key: (a) TBAF, THF, 93%; (b) (*R*)-MTPA, DCC, DMAP, CH_2Cl_2, 60%; (c) (*S*)-MTPA, DCC, DMAP, CH_2Cl_2, 84%.

Scheme 6. Synthesis of [^{13}C]Capreomycidine[a]



[a] Key: (a) DPPA, $P(Ph)_3$, DEAD, THF, 0 °C, 86%; (b) TBAF, THF, 94%;(c) MsCl, Et_3N, 98%; (d) LiN_3, DMF, 90 °C, 38%; (e) $P(Ph)_3$, THF, H_2O, reflux, 100%; (f) Br^{13}CN, pH 11, NaOH; (g) 6 N HCl; (h) Boc_2O, NaOH; (i) $KMnO_4$; (j) 6 N HCl, 10% (from **31**).

Azide **25** was first prepared in high yield via nucleophilic displacement of the corresponding tosylate; however, formation of the tosylate only occurred in low yield. As an alternate route to **25**, we tried modified Mitsunobu conditions that have been used successfully in the synthesis of various secondary azides.[27−30] When alcohol **21** was exposed to the modified Mitsunobu conditions, the corresponding primary azide was obtained in high yield (Scheme 6).[31] Treatment of diastereomer **22** in the same manner generated the primary azide **26**, the required stereoisomer to take on for the synthesis of (2*S*,3*R*)-capreomycidine.

Compound **26** was deprotected (Scheme 6) with tetra-*n*-butylammonium fluoride (TBAF) to yield alcohol **27**, which was subjected to the same Mitsunobu conditions used in the conversion of the primary alcohol to the primary azides. Unfortunately, no diazide was detected and only starting material was recovered. There are reported cases in which the Mitsunobu reaction was unsuccessful in inverting the stereochemistry of secondary alcohols[32,33] as well as converting a secondary alcohol to the corresponding azide.[34] Diazide **29** was eventually obtained by converting **27** to the corresponding methanesulfonate ester **28**, followed by displacement with lithium azide.[35] None of our attempts to increase the yield of **29** by employing other sulfonate ester derivatives of **27** resulted in any improvement over methanesulfonate. Subsequent reduction of **29** with triphenylphosphine in wet THF cleanly afforded diamine **30**.[36] Condensation of **30** with Br^{13}CN produced the guanidino derivative **31** in moderate yield.[37] Deprotection of the oxazolidine in 6 N HCl, reprotection of the amines with di-*tert*-butyl dicarbonate (Boc_2O), and oxidation of the alcohol with $KMnO_4$ followed by acidic workup afforded L-[*guanidino*-^{13}C]-capreomycidine (**32**).

Incorporation studies were performed by adding 18 mg of **32** to each of two 100 mL production flasks containing *S. lavendulae* L-1689-23. STF (**1**) was isolated 24 h after initial inoculation and analyzed by ^{13}C NMR spectroscopy. There was no evidence for appreciable incorporation of **32** into the guanidino carbon of STF. Repeating the experiment yielded similar results. These findings suggest that capreomycidine is either not able to enter the cell, not a precursor to STF, or not involved as a free intermediate. To conclusively demonstrate that **6** does enter the cell, it would need to be prepared in radiolabeled form. However, the demonstrated incorporation of exogenous arginine (**7**) and streptolidine (**4**) into STF strongly supports a species such as **6** or 4-hydroxycapreomycidine (**13**, Scheme 3) as an intermediate between these two precursors. An equally plausible explanation for these results is that capreomycidine is not released as a free intermediate in the conversion of **7** to **4**.

Resolution of the steps leading from arginine to streptolidine and insight into the involvement of enzyme-bound intermediates will be facilitated by the reported cloning of the streptothricin biosynthesis gene cluster from *Streptomyces rochei*.[38] Of particular relevance to this work is the *sttL* gene, which exhibits high similarity to the gene encoding clavaminate synthase, a non-heme iron, α-ketoglutarate-dependent oxygenase that catalyzes three oxidative reactions—a hydroxylation, an oxidative cyclization, and a dehydrogenation—in the conversion of deoxyguanidinoproclavaminic acid to clavaminic acid.[39,40] It is possible that SttL is involved in one or more steps

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in the incorporation of arginine into **1** without the formation of **6** as a free intermediate.

Precedence for the oxidation of an amino acyl−enzyme intermediate comes from recent studies on novobiocin biosynthesis demonstrating that two enzymes, NovH and NovI, are responsible for the formation of a β-hydroxytyrosine intermediate.[41] NovH is a dual-action protein that activates L-tyrosine as an acyladenylate to facilitate transfer to the thiol of a 4′-phosphopantetheine cofactor attached to a peptidyl carrier protein domain in a process analogous to nonribosomal peptide activation and loading. NovI is a cytochrome P450-type monooxygenase that catalyzes the hydroxylation of the β-carbon of the covalent L-tyrosyl-*S*-NovH intermediate. Importantly, Walsh et al. identified similar adenylation/peptidyl carrier protein (A•PCP)/redox enzymes pairs in other biosynthetic gene clusters where β-hydroxylation of an amino acid is known or implied and suggested that this may be evidence of a general method for directing a portion of the amino acid pool toward secondary metabolism.[41]

A number of routes can be proposed for the conversion of arginine (**7**) to capreomycidine (**6**) or 4-hydroxycapreomycidine (**13**) that are consistent with chemistry known to be catalyzed by non-heme iron, α-ketoglutarate-dependent oxygenases. Previous incorporation studies with ^{2}H-labeled arginine isotopomers help exclude several pathways but do not completely support a single route. For example, only the *pro*-4*R* and both C-5 hydrogens from arginine are retained in STF (**1**).[9] The observed loss of the C-2 and C-3 hydrogens is partially consistent with the intermediacy of species such as 2,3-dehydro- or 3-ketoarginine. Formation of 2,3-dehydroarginine requires loss of the C-2 ^{2}H and one, but not both, C-3 ^{2}H. Involvement of 3-ketoarginine is supported by the loss of both C-3 ^{2}H but not loss of the C-2 ^{2}H, although the increased acidity of the proton at C-2 of an enzyme-bound β-keto thioester could lead to wash out of the label. Interestingly, neither *erythro*- nor *threo*-[5,5-$^2H_2$]-β-hydroxyarginine, logical precursors to both 2,3-dehydro- and 3-ketoarginine, were incorporated into **1**.[9] As discussed above for capreomycidine, β-hydroxyarginine may simply not be a precursor to **1** or may not be transported into the cell, or this could be further evidence for an enzyme-bound intermediate. Ultimately, the identity of intermediates in the pathway and substrate(s) for SttL will be revealed from studies with purified recombinant enzyme.

Conclusions

The findings disclosed here confirm that the streptolidine base of STF (**4**) is derived from arginine prior to condensation with the amino sugar to form the nucleoside. Together with earlier studies demonstrating that β-lysine and D-glucosamine are also free intermediates, it is clear that STF is assembled in a process culminating in the assembly of three individual components derived from L-α-arginine, L-α-lysine, and D-glucose. Discrete intermediates in the transformation of arginine to streptolidine remain to be identified. While previous studies with labeled arginine support the intermediacy of capreomycidine (**6**), we were unable to secure definitive proof through direct incorporation of labeled **6**. Further studies on the biosynthesis of **1**, focused on the transformation of arginine to streptolidine, are underway.

(41) Chen, H.; Walsh, C. T. *Chem. Biol.* **2001**, *8*, 301−312.

Experimental Section

General Methods. All chemicals were purchased through Aldrich Chemical Co. or Arcos Chemical Co. and used without further purification. Sodium cyanide (99.99% ^{13}C enriched) was purchased from Cambridge Isotope Laboratories (Cambridge, MA). Anhydrous THF, benzene, and toluene were obtained by refluxing over sodium in the presence of benzophenone. Anhydrous methanol was obtained by refluxing over magnesium methoxide. Anhydrous DMF was obtained by first letting it stand over 4 Å molecular sieves for 24 h and then distilling under argon into a receiving vessel containing 4 Å molecular sieves. NMR spectra were obtained on either a Bruker AC 300 or AM 400 spectrometer. *t*-BuOH or pyridine was added as an internal chemical shift reference when spectra were recorded in D_2O. Mass spectra were obtained on a Kratos MS 50 TL spectrometer. Optical rotations were obtained using a Perkin-Elmer model 141 polarimeter. IR spectra were obtained using a Nicolet Model 510 FT-IR spectrometer. Ozone was generated using an OREC ozone generator.

[*guanidino*-^{13}C]Streptolidine (10). The hydrobromide salt of the unprotected triamine **9** (60 mg, 0.15 mmol prepared according to the method of Kusumoto et al.[13]) was dissolved in 6 mL of water, the pH was adjusted to 11.0 with 1 M NaOH, and the reacton mixture was then left to stand at room temperature for 2 h. A solution of Br^{13}CN (85 mg, 0.8 mmol) in 3 mL of water was added dropwise over 2.5 h while a pH of 9.5−11.0 was maintained. The mixture was stirred for an additional 30 min and then evaporated to dryness. The residue was dissolved in 15 mL of 6 M HCl and refluxed for 2 h. Removal of the solvent afforded a dark brown residue that was dissolved in a small amount of water and loaded onto an AG 50W-X8 (NH_4^+ form, 100−200 mesh, 1.5 × 20 cm) cation exchange column. The column was washed with water (400 mL) and then eluted with 0.2 M NH_4OH. Fractions (10 mL) were collected, those that contained streptolidine were pooled, and the solvent was removed to afford 6 mg of **10**: $[\alpha]^{25}_D$ +53° (*c* 0.21, H_2O); IR (KBr) 1709, 1685 cm^{-1}; ^{1}H NMR (400 MHz, D_2O, 25 °C) δ (ppm) 4.45 (t, J = 4.3 Hz, 1H), 4.23 (m, 2H), 3.38 (dd, J = 3.8 and 9.1 Hz, 1 H), 3.26 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ (ppm) 178.3, 160.3, 69.8, 63.7, 60.0, 42.3; MS (FAB) *m*/*z* 190.1 (M + 1); HRMS (FAB) calcd for $C_5{}^{13}CH_{13}N_4O_3$ 190.1022, obsd 190.1011.

In Vivo Incorporation of [*guanidino*-^{13}C]Streptolidine (10). Culture conditions, bioassay protocols, and isolation of STF have been described.[8,11] To each of two flasks containing 100 mL of modified O'Brien's medium was added 7 mg of sterile [*guanidino*-^{13}C]streptolidine (**19**) in 2 mL of water. The addition of material was performed 12 h after inoculation. The production flasks were incubated for an additional 32 h with shaking at 29 °C and 225 rpm. After a total of 48 h from initial inoculation, the antibiotic was isolated as described.[8]

The white powder resulting from freeze-drying (approximately 0.6 g) was transferred to a 40 mL centrifuge tube and extracted with 10 mL portions of MeOH until the extracts were ninhydrin-negative. After evaporation of the MeOH, the material was dissolved in a small amount of water and loaded onto a column of LH-20 gel filtration resin (1 × 30 cm column) at a flow rate of 1.0 mL/min. The column was washed with water, collecting 3 mL fractions. Ninhydrin-positive fractions were analyzed by TLC (pyridine/H_2O/*n*-PrOH/AcOH, 15:12:5:3; detection, 0.2% ninhydrin). Fractions containing only STF (R_f 0.46) were pooled, and the pH was adjusted to 6.8 using 2% KOH prior to lyophilization. The STF obtained (18 mg) was analyzed by ^{13}C NMR, showing a 1.9% enrichment of the guanidino carbon.

In a second incorporation study using 22 mg of **10**, 55 mg of STF was obtained. The antibiotic was analyzed by ^{13}C NMR spectroscopy and a 2.2% enrichment of the guanidino carbon of **1** was observed.

1,1-Dimethylethyl (4*R*)-4-Formyl-2,2-dimethyl-3-oxazolidinecarboxylate (18). Compound **18** was prepared from D-serine methyl ester as described by Garner and Park.[20] The product exhibited physical properties fully in agreement with the published data.

1,1-Dimethylethyl (4*R*)-(1′-hydroxy-3-butenyl)-2,2-dimethyl-3-oxazolidinecarboxylate (19). To a solution of the aldehyde **18** (14.8 g, 6.45 mmol) in dry THF (350 mL) at −78 °C was slowly added 3.3 equiv of allylmagnesium chloride (0.213 mol, 107 mL of a 2 M solution in THF) with stirring and then the mixture was slowly warmed to room temperature. Analysis by TLC (hexanes/EtOAc, 3:1; detection, PMA) showed complete conversion of starting material to a product at R_f 0.38. Saturated NH_4Cl (100 mL) was added and the mixture extracted with ether (3 × 300 mL). The combined organic layers were washed with saturated $NaHCO_4$ (2 × 300 mL) and brine (1 × 300 mL) and dried over $MgSO_4$. The crude product was purified by flash chromatography (hexanes/EtOAc, 3:1; 7 × 20 cm column; detection, PMA) to afford 15.0 g of the desired homoallylic alcohol (**19**) as a mixture of diastereomers: $[\alpha]^{25}_D$ +28.8° (*c* 3.90, $CHCl_3$); IR (neat) 3465 (br), 1696 cm^{-1}; ^1H NMR (300 MHz, C_6D_6, 70 °C) δ (ppm) 1.35 (s, 9H), 1.44 (s, 3H), 1.61 (s, 3H), 3.52 (t, J = 8.2 Hz, 1H), 3.65 (d, J = 6.9 Hz, 1H), 3.78 (m, 1H), 9.33 (s, 1H); ^{13}C NMR (75 MHz, C_6D_6, 70 °C) d (ppm) 24.5, 26.5, 28.6, 64.0, 65.5, 80.9, 95.5, 152.0, 198.5; MS (FAB) *m*/*z* 230.131 (M + 1); HRMS (FAB) calcd for $C_{11}H_{20}NO_4$ 230.1392, obsd 230.1393.

1,1-Dimethylethyl (4*R*,1′*R*)-(1′-(*tert*-Butyldimethysilyloxy)-3′-butenyl)-2,2-dimethyl-3-oxazolidinecarboxylate (21) and 1,1-Dimethylethyl (4*R*,1′*S*)-(1′-(*tert*-Butyldimethylsilyloxy)-3′-butenyl)-2,2-dimethyl-3-oxazolidinecarboxylate (22). To 11.6 g (4.26 mmol) of **19** in dry DMF (23.1 mL) was added imidazole (8.69 g, 0.128 mol) followed by *tert*-butyldimethysilyl chloride (8.98 g, 0.06 mol), and the mixture was stirred at room temperature for 24 h. TLC analysis (hexanes/EtOAc, 4:1; detection, 2% PMA) showed complete conversion of starting material (R_f 0.28) to product (R_f 0.81). Water (100 mL) was added and the solution extracted with Et_2O (3 × 500 mL). The combined organic layers were washed with saturated $NaHCO_3$ (3 × 500 mL) and brine (1 × 500 mL) and dried over Na_2SO_4. Filtration and removal of the solvent afforded a yellow oil (16.4 g, 100%). The diastereomers were separated by flash chromatography (pentane/Et_2O, 9:1; 7 × 21 cm column; detection, 2% PMA) to afford 60% of the major diastereomer and 35% of the minor diastereomer. **21**, major diastereomer: $[\alpha]^{25}_D$ +27.1° (*c* 3.84, $CHCl_3$); IR (neat) 1702, 1384 cm^{-1}; ^1H NMR (300 MHz, C_6D_6, 70 °C) δ (ppm) 0.11 (s, 3H), 0.12 (s, 3H), 0.84 (s, 9H), 1.42 (s, 9H), 1.46 (s, 3H), 1.70 (s, 3H), 2.31 (m, 1H), 2.49 (m, 1H), 3.80 (dd, J = 7.2 and 9.3 Hz, 1H), 4.01 (bs, 1H), 4.22 (dd, J = 2.2 and 9.4 Hz, 1H) 4.01 (bs, 1H), 5.07 (m, 2H), 5.91 (m, 1H); ^{13}C NMR (75 MHz, C_6D_6, 70 °C) δ (ppm) 18.6, 23.8, 26.5, 27.0, 29.0, 36.6, 61.7, 63.8, 72.2, 80.1, 95.3, 117.0, 137.3, 153.0; MS (FAB) *m*/*z* 386.2 (M + 1); HRMS (FAB) calcd for $C_{20}H_{40}NO_4Si$ 386.2727, obsd 386.2721. **22**, minor diastereomer: $[\alpha]^{25}_D$ +50.7° (*c* 3.10, $CHCl_3$); IR (neat) 1695, 1363, 1254 cm^{-1}; ^1H NMR (300 MHz, C_6D_6, 70 °C) δ (ppm) 0.10 (s, 3H), 0.14 (s, 3H), 0.97 (s, 9H), 1.41 (s, 9H), 1.65 (s, 3H), 1.66 (m, 3H), 3.62 (bs, 2H), 3.70 (dd, J = 7.1 and 8.1 Hz, 1H), 3.98 (bs, 1H), 4.13 (dd, J = 3.3 and 8.4 Hz, 1H), 4.36 (bd, J = 6.2 Hz, 1H);^{13}C NMR (75 MHz, C_6D_6, 70 °C) δ (ppm) −3.7, −3.7, 18.7, 25.2, 26.7, 27.5, 28.9, 38.9, 59.4, 62.0, 64.0, 70.0, 80.4, 94.9, 153.7; MS (FAB) *m*/*z* 386.2 (M + 1); HRMS (FAB) calcd for $C_{20}H_{40}NO_4Si$ 386.2727, obsd 386.2728.

1,1-Dimethylethyl (4*R*,1′*R*)-(1′-Hydroxy-3′-butenyl)-2,2-dimethyl-3-oxazolidinecarboxylate (19a). To a solution of **20a** (0.519 g, 1.34 mmol) in dry THF (16 mL) at room temperature was added a solution of tetra-*n*-butylammonium fluoride in THF (3.36 mmol, 3.40 mL) with stirring. Upon completion of the addition, stirring was continued for 2 h at room temperature. Analysis by TLC (hexanes/EtOAc, 3:1; detection, I_2) showed the desired product (R_f 0.19) at the expense of starting material. The reaction mixture was partitioned between water (100 mL) and Et_2O (150 mL). The organic layer was washed with saturated $NaHCO_3$ (3 × 100 mL) and brine (1 × 100 mL) and dried over $MgSO_4$. Filtration and removal of the solvent afforded crude product (372 mg). Purification by flash chromatography (hexanes/EtOAc, 2:1; detection, I_2) yielded 363 mg (99%) of the desired product (**19a**) as an oil: $[\alpha]^{25}_D$ +19.2° (*c* 0.66, $CHCl_3$); IR (neat) 3472 (br), 3081, 2978, 2931, 1690 cm^{-1}; ^1H NMR (400 MHz, C_6D_6, 67 °C) δ (ppm) 1.38 (s, 9H), 1.44 (s, 3H), 1.62 (s, 3H), 2.09 (m, 1H), 2.30 (m, 1H), 3.62 (dd, J = 1.5 and 8.9 Hz, 1H), 3.70 (bd, J = 9.2 Hz, 1H), 3.91 (m, 1H), 5.00 (t, J = 1.3 Hz, 1H), 5.04 (m, 1H), 5.92 (m, 1H);^{13}C NMR (100 MHz, C_6D_6, 67 °C) δ (ppm) 24.6, 27.5, 28.8, 39.1, 62.4, 65.2, 73.2, 80.8, 94.8, 117.5, 135.8, 154.5; MS (CI) *m*/*z* 272.1 (M + 1); HRMS (CI) calcd for $C_{14}H_{26}NO_4$ 272.1862, obsd 272.1863.

(*R*)-(+)-MTPA Ester of 1,1-Dimethylethyl (4*R*,1′*R*)-(1′-Hydroxy-3′-butenyl)-2,2-dimethyl-3-oxazolidinecarboxylate (23). To a solution of **19a** (0.093 g, 0.34 mmol) in dry CH_2Cl_2 (2 mL) was added a catalytic amount of 4-dimethylaminopyridine (0.040 mmol, 5.0 mg), (*R*)-(+)-MTPA (0.391 mmol, 91 mg), and dicyclohexylcarbodiimide (0.37 mmol, 0.37 mL of a 1 M solution in CH_2Cl_2), and the mixture was stirred under argon for 2 h. TLC analysis of the reaction mixture (hexanes/EtOAc, 3:1; detection, UV, I_2) showed conversion of the starting material to a UV-active compound (R_f 0.56). The organic layer was washed with saturated NH_4OH (1 × 2 mL), saturated $NaHCO_3$ (2 × 2 mL), and brine (1 × 2 mL) and dried over Na_2SO_4. Filtration and removal of the solvent first afforded a dark yellow oil (143 mg), which was further purified by flash chromatography (hexanes/EtOAc, 3:1; 1 × 15 cm column; detection, UV) to give 100 mg (60%) of the desired MTPA ester (**23**): $[\alpha]^{25}_D$ +29.2° (*c* 0.25, $CHCl_3$); IR (neat) 2363, 2342, 1749, 1705 cm^{-1}; ^1H NMR (300 MHz, C_6D_6, 70 °C) δ (ppm) 1.43 (s, 3H), 1.46 (s, 9H), 1.66 (s, 3H), 2.31 (m, 1H), 2.54 (m, 1H), 3.37 (s, 3H), 3.63 (dd, J = 6.8 and 9.7 Hz, 1H), 3.92 (dd, J = 1.7 and 9.9 Hz, 1H), 4.12 (bs, 1H), 4.89 (d, J = 10 Hz, 1H), 4.95 (dd, J = 1.3 and 17 Hz, 1H), 5.66 (m, 1H), 5.80 (m, 1H), 7.10 (m, 3H), 7.64 (d, J = 7.2 Hz, 2H);^{13}C NMR (75 MHz, C_6D_6, 70 °C) δ (ppm) 23.5, 26.9, 28.8, 28.9, 33.7, 55.7, 58.8, 62.4, 64.1, 75.8, 81.0, 85.9, 95.4, 118.4, 122.8, 126.6, 130.2, 133.4, 133.8, 134.3, 152.8, 166.6; MS (FAB) *m*/*z* 488.2 (M + 1); HRMS (FAB) calcd for $C_{24}H_{33}F_3NO_6$ 488.2260, obsd 488.2268.

(*S*)-(−)-MTPA Ester of 1,1-Dimethylethyl (4*R*,1′*R*)-(1′-Hydroxy-3′-butenyl)-2,2-dimethyl-3-oxazolidinecarboxylate (24). Mosher ester **24** was prepared from the corresponding homoallylic alcohol (**19a**) and (*S*)-(−)-MTPA using the same procedure outlined for the synthesis of **23**. Starting with 99 mg (0.36 mmol) of **19a**, 210 mg of **24** (84%) was obtained: $[\alpha]^{25}_D$ −28.9° (*c* 0.55, $CHCl_3$); IR (neat) 3074, 2984, 2939, 1752, 1711, 1376 cm^{-1}; ^1H NMR (300 MHz, C_6D_6, 70 °C) δ (ppm) 1.39 (s, 3H), 1.48 (s, 9H), 1.64 (s, 3H), 2.35 (m, 1H), 2.58 (m, 1H), 3.44 (s, 3H), 3.53 (dd, J = 6.8 and 9.8 Hz), 3.80 (dd, J = 2.1 and 9.9 Hz), 4.08 (bs, 1H), 4.97 (d, J = 11 Hz), 5.02 (m, 2H), 5.74 (m, 1H), 5.84 (m, 1H), 7.08 (m, 3H), 7.64 (d, J = 7.9 Hz);^{13}C NMR (75 MHz, C6D6, 70 °C) (δ ppm) 23.4, 26.8, 28.8, 33.3, 55.9, 58.4, 63.9, 75.6, 80.9, 85.8, 95.4, 118.3, 122.7, 126.6, 130.1, 133.5, 134.8, 152.7, 166.4; MS (FAB) *m*/*z* 488.2 (M + 1); HRMS (FAB) calcd for $C_{24}H_{33}F_3NO_6$ 488.2260, obsd 488.2269.

1,1-Dimethylethyl (4*R*,1′*R*)-4-(3′-Azido-(1′-tert-butyldimethylsilyloxy)-1′-hydroxypropyl)-2,2-dimethyl-3-oxazolidinecarboxylate (25). To a solution of alcohol **21** (5.24 g, 0.0134 mol) in dry THF (200 mL) under argon atmosphere at 0 °C was added triphenylphosphine (TPP) (4.23 g, 1.61 mmol), followed by diethylazodicarboxylate (DEAD) (2.5 mL, 1.61 mmol), and the mixture stirred at 0 °C in the dark for 30 min. Diphenylphosphoryl azide (DPPA, 3.5 mL, 1.61 mmol) was then slowly added and the reaction mixture gradually warmed to room temperature, and then stirred in the dark overnight. TLC analysis (hexanes/EtOAc, 3:1; detection, 2% PMA) showed complete conversion of starting material to product (R_f 0.82). The solvent was removed and the resulting oil was purified by flash chromatography (hexanes/EtOAc, 4:1; 7 × 15 cm column; detection, 2% PMA) to afford 5.03 g (90%) of the desired azide (**25**). All physical data were identical to the primary azide prepared via nucleophilic substitution of the tosylate with sodium azide: $[\alpha]^{25}_D$ +47.9° (*c* 2.6, $CHCl_3$); IR (neat) 2097, 1691 cm^{-1}; ^1H NMR (300 MHz, C_6D_6, 70 °C) δ (ppm) 0.10 (s, 6H), 0.90 (s, 9H), 1.44 (s, 12H), 1.68 (s, 3H), 1.86 (m, 1H), 3.12 (m, 2H), 3.75 (t, J = 8.0 Hz, 1H), 3.98 (bs, 1H), 4.10 (d, J = 9.4 Hz, 1H), 4.44 (bs, 1H);^{13}C NMR (75 MHz, C_6D_6, 70 °C) δ (ppm) −4.32, −3.72, 18.5, 23.7, 26.4, 26.9, 28.9, 30.8, 48.9, 61.3, 63.6, 68.8, 80.4, 95.3, 152.9; MS (FAB) *m*/*z*

415.1 (M + 1); HRMS (FAB) calcd for $C_{19}H_{39}N_4O_4Si$ 415.2741, obsd 415.2737.

1,1-Dimethylethyl (4*R*,1′*S*)-4-(3′-Azido-(1′-tert-butyldimethylsilyloxy)-1′-hydroxypropyl)-2,2-dimethyl-3-oxazolidinecarboxylate (26). Azide **26** was synthesized from **22** (1.89 g, 4.85 mmol) using the procedure outlined for the preparation of **25**. Compound **26** (R_f 0.75) was obtained in 90% yield (1.80 g): $[\alpha]^{25}_D$ +26.3° (*c* 2.48, $CHCl_3$); IR (neat) 2092, 1697 cm^{-1}; ^1H NMR (300 MHz, C_6D_6, 70 °C) δ (ppm) 0.06 (s, 3H), 0.10 (s, 3H), 0.93 (s, 9H), 1.43 (s, 9H), 1.49 (s, 3H), 1.68 (s, 2H), 3.11 (m, 1H), 3.26 (bs, 1H), 3.61 (dd, J = 6.7 and 8.2 Hz), 3.76 (bs, 1H), 4.00 (dd, J = 2.5 and 8.5 Hz), 4.17 (q, J = 4.7 Hz);^{13}C NMR (75 MHz, C_6D_6, 70 °C) δ (ppm) −4.35, −4.25, 18.1, 24.4, 26.0, 27.3, 28.4, 34.2, 47.7, 61.2, 63.8, 69.8, 79.8, 94.4, 153.0; MS (CI) *m*/*z* 415.1 (M + 1); HRMS (CI) calcd for $C_{19}H_{39}N_4O_4Si$ 415.2736, obsd 415.2741.

1,1-Dimethylethyl (4*R*,1′*S*)-4-(3′-Azido-1′-hydroxypropyl)-2,2-dimethyl-3-oxazolidinecarboxylate (27). To a solution of the TBDMS-protected alcohol **26** (6.39 g, 1.54 mmol) in dry THF (129 mL) at room temperature was added TBAF (1 M solution in THF, 38.5 mL, 3.85 mmol) and the mixture stirred at room temperature for 2.5 h. Analysis by TLC (hexanes/EtOAc, 4:1; detection, 2% PMA) showed complete conversion of starting material to product (R_f 0.18). The mixture was partitioned between water (200 mL) and Et_2O (500 mL). The organic layer was washed with saturated $NaHCO_3$ (2 × 200 mL) and brine (1 × 200 mL) and dried over $MgSO_4$. Filtration and removal of the solvent afforded a light yellow oil (7.63 g), which was purified by flash chromatography (hexanes/EtOAc, 4:1; 7 × 15 cm column; detection, 2% PMA). A total of 4.33 g (94%) of the desired product (**27**) was obtained: $[\alpha]^{25}_D$ +24.3° (*c* 1.21, $CHCl_3$); IR (neat) 3451, 2099, 1695 cm^{-1}; ^1H NMR (400 MHz, C_6D_6, 70 °C) δ (ppm) 1.33 (s, 9H), 1.36 (s, 3H), 1.41 (m, 1H), 1.48 (m, 1H), 1.52 (s, 3H), 3.15 (m, 2H), 3.54 (bd, J = 5.6 Hz), 3.69 (m, 1H), 3.77 (bs, 1H);^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ (ppm) 24.4, 27.2, 28.7, 33.2, 49.2, 62.9, 65.2, 70.9, 81.0, 95.0, 154.3; MS (CI) *m*/*z* 301.1 (M + 1); HRMS (CI) calcd for $C_{13}H_{25}N_4O_4$ 301.1876, obsd 301.1878.

1,1-Dimethyl (4R,1′S)-4-(1′-(Methanesulfonyloxy)-3′-azidopropyl)-2,2-dimethyl-3-oxazolidinecarboxylate (28). Alcohol **27** was dissolved in dry CH_2Cl_2 (6.0 mL) followed by the addition of triethylamine (1.22 mL, 8.74 mmol). The solution was cooled to −48 °C and then methanesulfonyl chloride (0.260 mL, 3.30 mmol) was added dropwise with stirring over 15 min. The reaction was slowly allowed to warm to 0 °C over the course of 2 h. Analysis by TLC (hexanes/EtOAc, 3:1; detection, 2% PMA) showed complete conversion of starting material (R_f 0.33) to a lower R_f compound (R_f 0.28). The reaction was partitioned between water (35 mL) and CH_2Cl_2 (75 mL), and then the organic layer was washed again with water (35 mL) and dried over anhydrous $MgSO_4$. Filtration and removal of the solvent yielded a dark yellow oil (942 mg), which was purified by flash chromatography (hexanes/EtOAc, 2:1; 15 × 3 cm column; detection, PMA) to afford 615 mg of the desired mesylate (**28**): $[\alpha]^{25}_D$ +23.8° (*c* 1.34, $CHCl_3$); IR (neat) 2107, 1692 cm^{-1}; ^1H NMR (400 MHz, C_6D_6, 70 °C) δ (ppm) 1.41(s, 9 H), 1.43 (s, 3H), 1.62 (s, 3H), 2.41(s, 3H), 3.08 (m, 2H), 3.56 (dd, J = 6.8 and 9.0 Hz, 1H), 3.75 (bs, 1H), 3.79 (dd, J = 3.8 and 9.1 Hz, 1H), 5.17 (m, 1H);^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ (ppm) 24.6, 27.1, 28.7, 32.4, 38.6, 48.1, 60.2, 63.7, 78.2, 81.0, 95.3, 153.0; MS (CI) *m*/*z* 379 (M + 1); HRMS (CI) calcd for $C_{14}H_{27}N_4O_6S$ 379.1651, obsd 379.1656.

1,1-Dimethylethyl (4R,1′S)-4-(1′,3′-Diazidopropyl)-2,2-dimethyl-3-oxazolidinecarboxylate (29). Mesylate **28** (3.04 g, 8.03 mmol) was dissolved in dry DMF (80 mL) followed by the addition of lithium azide (1.04 g, 21.3 mmol). The mixture was heated at 90 °C for 24 h and monitored by TLC (hexanes/EtOAc, 3:1; detection, 2% PMA), which showed the conversion of starting material to a major component at R_f 0.78. The reaction mixture was partitioned between water (200 mL) and Et_2O (500 mL), and the organic layer was washed with water (2 × 200 mL) and brine (1 × 200 mL) and dried over Na_2SO_4. Filtration and removal of the solvent afforded a dark yellow oil (1.41 g), which was purified by flash chromatography (hexanes/EtOAc, 7:1; 7 × 18 cm column; detection, 2% PMA) to yield 0.92 g (35%) of diazide **29**: $[\alpha]^{25}_D$ +37.2° (*c* 1.15, $CHCl_3$); IR (neat) 2146, 2098, 1697 cm^{-1}; ^1H NMR (400 MHz, C_6D_6, 70 °C) δ (ppm) 1.18 (m, 2H), 1.40 (s, 9H), 1.47 (s, 3H), 1.68 (s, 3H), 2.84 (m, 2H), 3.50 (m, 1H), 3.68 (dd, J = 3.2 and 9.4 Hz, 1H), 4.00 (bs, 1H);^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ (ppm) 23.3, 26.8, 28.4, 28.9, 48.7, 59.7, 61.2, 64.0, 80.5, 94.9, 152.4; MS (CI) *m*/*z* 326.2 (M + 1); HRMS (CI) calcd for $C_{13}H_{24}N_7O_3$ 326.1941, obsd 326.1940.

1,1-Dimethylethyl (4*R*,1′*S*)-4-(1′,3′-Diaminopropyl)-2,2-dimethyl-3-oxazolidinecarboxylate (30). To a solution of the diazide **29** (0.92 g, 2.82 mmol) in THF (25 mL) was added triphenylphosphine (2.01 g, 6.18 mmol) and water (0.11 g, 6.18 mmol) and the mixture refluxed for 16 h. Analysis of the reaction mixture by TLC (CH_2Cl_2/MeOH/NH_4OH, 8:1:1; detection, 0.2% ethanolic ninhydrin) showed complete conversion of starting material to a product at R_f 0.24. The solvent was removed, and the resulting yellow oil (3.15 g) was purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH, 8:1:1; 4 × 15 cm column; detection, 0.2% ethanolic ninhydrin) to afford diamine **30** (0.70 g, 100%) as a yellow oil: $[\alpha]^{25}_D$ +26.2° (*c* 2.10, $CHCl_3$); IR (neat) 3405 (br), 1693 cm^{-1}; ^1H NMR (300 MHz, C_6D_6, 70 °C) δ (ppm) 0.71 (bs, 5H), 1.14 (m, 1H), 1.29 (m, 1H), 1.42 (s, 9H), 1.52 (s, 3H), 1.67 (s, 3H), 2.64 (bs, 1H), 3.09 (bs, 1H), 3.28 (bs, 1H), 3.62 (t, J = 8.4 Hz, 1H), 3.69 (bs, 1H), 3.83 (dd, J = 1.9 and 8.5 Hz, 1H);^{13}C NMR (75 MHz, C_6D_6, 70 °C) δ (ppm) 24.4, 27.5, 28.9, 38.8, 40.5, 51.3, 63.2, 64.2, 80.0, 94.5, 153.3; MS (FAB) *m*/*z* 274.1 (M + 1); HRMS (FAB) calcd for $C_{13}H_{28}N_3O_3$ 274.2131, obsd 274.2133.

L-[*guanidino*-^{13}C]Capreomycidine (32). A ^{13}C-cyanogen bromide solution (2 mmol in 4 mL water) was slowly added to diamine **30** (0.200 g, 0.730 mmol) in 21 mL of water at pH 11.5 over 2.5 h. During the addition, the pH was kept at 11−11.5 with 1 N NaOH. Upon completion of the addition, the mixture was stirred at room temperature for 1 h and then transferred to a separatory funnel. The solution was extracted with Et_2O (3 × 100 mL), the combined organic layers were washed with water (100 mL), and the aqueous layers were combined. The solvent from the aqueous layers was removed to afford a white, oily residue (1.05 g). The residue was washed with 100% ethanol (3 × 25 mL) and the solvent removed in vacuo to afford a light tan residue (0.69 g). This was dissolved in water (8 mL) and 2 mL of the solution was loaded onto a Varian Mega Bond Elute C_{18} column (500 mg). The column was washed with water (3 × 3 mL), followed by elution with acetonitrile. The fractions were analyzed by TLC (*n*-BuOH/H_2O/AcOH, 4:2:1; detection, 0.2% ethanolic ninhydrin), those fractions containing the product (R_f 0.79) were pooled, and the solvent was removed to give 150 mg of **31** as the HBr salt.

Crude **31** was dissolved in 6 N HCl (10 mL) and allowed to stand for 2 h at room temperature. The solvent was removed and the resulting residue was dissolved in 1 M NaOH (5 mL) and cooled to 4 °C. To this was added BOC_2O (2.19 mmol, 480 mg) in dioxane (5 mL). The reaction was stirred at room temperature for 4 h, then $KMnO_4$ (1.40 mmol, 64 mg) was added, and the mixture was stirred for an additional 6 h. The MnO_2 was filtered off and then the filtrate was acidified with 6 N HCl to pH 1. After standing at room temperature for 2 h, the solvent was removed to afford a white residue (140 mg). The residue was dissolved in water (25 mL) and loaded onto an AG50W-X8 cation exchange column (H^+ form, 13 × 2.5 cm) and the column was washed with water (300 mL). The column was eluted with a pH gradient (pH 7−9) of 0.14 M NH_4OAc, fractions that were ninhydrin-positive were pooled, and the solvent removed to afford 15 mg of **32**: $[\alpha]^{25}_D$ +6.1° (*c* 0.11, H_2O); IR (KBr) 1702, 1653 cm^{-1}; ^1H NMR (300 MHz, D_2O, 25 °C) δ (ppm) 2.05 (m, 1H), 2.21 (m, 1H), 3.49 (m, 2H), 3.90 (d, J = 5.3 Hz, 1H), 4.12 (m, 1H);^{13}C NMR (75 MHz, D_2O, 25 °C) δ (ppm) 23.4, 37.9, 50.1, 58.2, 156.0, 172.4; MS (FAB) *m*/*z* 174.1 (M + 1); HRMS (FAB) calcd for $C_5{}^{13}CH_{13}N_4O_2$ 174.1072, obsd 174.1073.

Incorporation Study Using [*guanidino*-^{13}C]Capreomycidine (32). Two 100 mL production flasks were prepared and inoculated with 8% seed medium, which had been

prepared 64 h earlier. Production flasks were incubated at 29 °C and 225 rpm for 12 h, at which time 18 mg of **32** in 2 mL of water was divided between the two flasks and added aseptically through a sterile filter. The production flasks were incubated at 29 °C and 225 rpm for an additional 36 h and worked up as previously described. A total of 13 mg of STF (**1**) was obtained and the antibiotic was analyzed by ^{13}C NMR spectroscopy.

A second incorporation study was performed using 18 mg of [*guanidino*-^{13}C]capreomycidine, as described above. From the 200 mL of production medium, 8 mg of STF was isolated and analyzed by ^{13}C NMR spectroscopy to determine the extent of incorporation.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for new compounds and intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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