Cliff McDonald for some of the ^1H NMR spectra, Peter Demou for the ^2H NMR spectra, recorded at the Northeast Regional NSF-NMR facility, Department of Chemistry, Yale University, supported in part by the National Science Foundation, Grant CHE-7916210, from the Chemistry Division, and Dr. Thomas Leipert, Department of Chemistry, University of Connecticut for the ^{13}C NMR spectra, recorded on an instrument purchased in part with Grant CHE 76-05739 from the National Science Foundation. We thank Dr. Catherine Costello for some of the mass spectra, provided by the facility at M.I.T., supported by National Institutes of Health Grant RR 00317 from the Biotechnology Resources Branch, Division of Research Resources. We thank Prof. H. A. Barker for some helpful information and a sample of β-lysine and (3S,5S)-diaminohexanoic acid. This work was supported by Grants GM 25919 (D.J.A.) and GM 25996 (S.J.G.) from the National Institutes of Health.

Registry No. **1b**·2HCl, 86146-77-2; **1b**·HCl, 86146-81-8; **1c**, 86146-83-0; **1d**·2HCl, 86195-10-0; **1e**·2HCl, 86195-11-1; **2c**, 86146-87-4; **4c**, 86146-85-2; **4d**, 86146-82-9; **5a**, 86195-07-5; **5f**, 86195-08-6; **5g**, 86146-84-1; **5i**, 86146-88-5; **6a**, 3153-36-4; **6b**, 79595-64-5; **7a**, 66502-79-2; **7b**, 86146-71-6; **7c**, 79595-65-6; **7d**, 79595-66-7; **7e**, 79595-67-8; **7f**, 79595-68-9; **8a**, 86146-72-7; **8b**, 86146-73-8; **8c**, 86146-74-9; **8d**, 86146-75-0; **8e**, 86162-04-1; **8f**, 86146-76-1; **10a**, 79595-56-5; **10b**, 79595-57-6; **11a**, 86146-68-1; **11b**, 79595-58-7; **11c**, 86146-69-2; **12a**, 86195-06-4; **12b**, 86146-70-5; **13a**, 86088-67-7; **13b**, 86146-80-7; **16a**, 86146-78-3; **16b**, 86146-79-4; lysine 2,3-aminomutase, 9075-20-1; 4-phthalimidobutanoyl chloride, 10314-06-4; (1*RS*)-4-chloro-1-butanol-*1*-d_1, 86195-09-7; ethyl 2-acetamido-6-chloro-2-cyanohexanoate-*3*-d_1, 79595-69-0; ethyl 2-acetamido-2-cyano-6-iodohexanoate-*3*-d_1, 79595-70-3; ethyl 2-acetamido-2-cyano-6-phthalimido hexanoate-*3*-d_1, 79791-12-1.

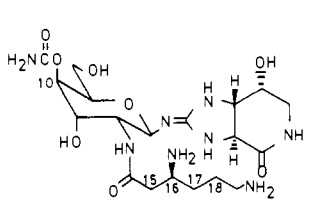
Biosynthesis of Streptothricin F. 5. Formation of β-Lysine by *Streptomyces* L-1689-23

T. K. Thiruvengadam,[†] Steven J. Gould,*[†1] D. John Aberhart,*[‡] and Horng-Jau Lin[‡]

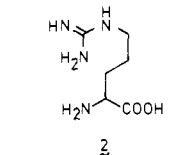
Contribution from the School of Pharmacy, Section of Medicinal Chemistry and Pharmacognosy, The University of Connecticut, Storrs, Connecticut 06268, and Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545. *Received December 2, 1982*

Abstract: The formation of the β-lysine moiety of streptothricin F has been studied by feeding to *Streptomyces* L-1689-23 α-[3-^{13}C,^{15}N]-, α-[(3*RS*)-$^2H_2$]-, α-[(3*R*)-^2H]-, and α-[(3*S*)-^2H]lysine and β-[(2*S*)-^2H]lysine. From the analysis of either the ^{13}C NMR or ^2H NMR spectrum of the derived antibiotics, it has been determined that the α-nitrogen migrates to C-3 with inversion of configuration by an intramolecular process, and the 3-*pro-R* hydrogen migrates to C-2 with inversion of configuration by a process that is substantially or completely intermolecular. The very high degree of incorporation of labeled β-lysine indicates it is probably an intermediate in the biosynthesis of streptothricin F.

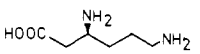
Streptothricin F (**1**),[2] representative of a large family of ubiquitous antibiotics, has been a major target in our studies on the biosynthesis of nitrogen-containing antibiotics.[3-6] Its structure was elucidated by chemical degradations[7] and, in part, by X-ray crystallography.[8] Recently, the location of the carbamate moiety was reassigned to C-10 on the basis of model studies,[9] and this was confirmed by the same authors by a total synthesis of **1**.[10] We have also assigned the carbamate to C-10 by a different approach, using deuterium-induced isotope shifts in ^{13}C NMR spectroscopy.[11] Thus, the antibiotic contains a gulosamine unit with three groups appended and is logically formed by a convergent biosynthetic pathway.



1



2



3

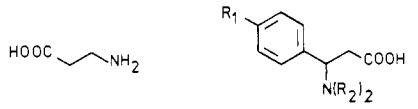
We have provided evidence for the specific incorporation of L-arginine (**2**) into the bicyclic moiety (streptolidine),[12] and through feedings of **2** labeled with ^{13}C and ^{15}N or with ^2H we have subsequently revealed the chemistry of this branch of the biosynthesis of **1**.[6] While all members of the streptothricin family contain D-gulosamine and the streptolidine moiety, the amino acid side chain at C-8 is variable.[13] However, L-β-lysine (**3**) is the most frequently encountered side chain, and all homologues up to seven β-lysine units (as a polyamide side chain) are known. The formation of β-lysine has been a particularly intriguing problem.

Naturally occurring β-amino acids are relatively rare and include β-lysine, β-alanine (**4**), β-tyrosine (**5**),[14] N^6-methyl-β-arginine (**6**),[15] β-leucine (**7**),[16] (dimethylamino)-β-phenylalanine (**8**),[17] and

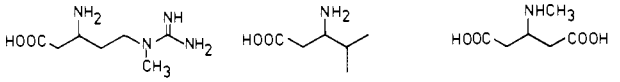
[†] University of Connecticut.
[‡] Worcester Foundation for Experimental Biology.

(1) Career Development Awardee of the National Cancer Institute (CA00627), 1979-1984. Present address: Department of Chemistry, Oregon State University, Corvallis, OR 97331.
(2) Waksman, S. A. *J. Bacteriol.* **1943**, *46*, 299-310.
(3) Gould, S. J.; Chang, C. C.; Darling, D. S.; Roberts J. D.; Squillacote, M. *J. Am. Chem. Soc.* **1980**, *102*, 1707-1712.
(4) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *J. Am. Chem. Soc.* **1981**, *103*, 4639-4640.
(5) Gould, S. J.; Thiruvengadam, T. K. *J. Am. Chem. Soc.* **1981**, *103*, 6752-6754.
(6) Martinkus, K. J.; Tann, C.-H.; Gould, S. J. *Tetrahedron* **1983**, in press.
(7) Van Tamelen, E. E.; Dyer, J. R.; Whaley, H. A.; Carter, H. E.; Whitfield, G. B., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 4295-4296.
(8) Bycroft, B. W.; King, T. J. *J. Chem. Soc., Chem. Commun.* **1972**, 652-653.
(9) Kusumoto, S.; Kambayashi, Y.; Imaoka, S.; Shima, K.; Shiba, T. *J. Antibiot.* **1982**, *35*, 925-927.
(10) Kusumoto, S.; Imaoka, S.; Kambayashi, Y.; Shiba, T. *Tetrahedron Lett.* **1982**, 2961-2964.
(11) Gould, S. J.; Martinkus, K. J., unpublished results.
(12) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *J. Am. Chem. Soc.* **1981**, *103*, 2871-2872.
(13) For a listing of all known members of the streptothricin family, see: Khokhlov, A. S. *J. Chromatogr. Libr.* **1978**, *15*, 617-713.
(14) Kurylo-Borowska, Z.; Abramsky, T. *Biochim. Biophys. Acta* **1972**, *264*, 1-10.

0002-7863/83/1505-5470$01.50/0



4 5, R_1=OH, R_2=H; 8, R_1=H, R_2=CH_3



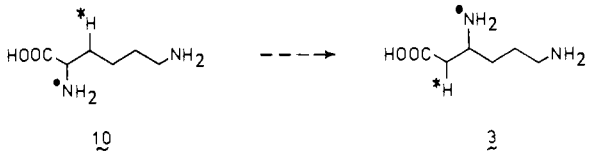
6 7 9

N-methyl-β-glutamic acid (**9**).[18] It would be reasonable to expect that each is derived from an α-amino acid. However, except for **4**, which is formed by the degradation of uracil[19] or by the decarboxylation of aspartic acid,[20] very little was known about the biosynthesis of any of these when we began our studies. Recently it has been shown that **5** is derived from tyrosine;[21] the pathway was shown to involve loss of the original α-nitrogen and loss of the 3-*pro*-*S* hydrogen. This suggested to the authors the intermediacy of an ammonia lyase like reaction, although certain differences with phenylalanine ammonia lyase were pointed out. Preliminary work[22] on β-leucine has so far indicated only that the (2*S*)-α- and (3*R*)-β-leucines are the metabolically active compounds; no data have been reported for the fate of isotope labels.

In addition to its occurrence in *Streptomyces* antibiotics, L-lysine is also the first isolable product from the breakdown of L-α-lysine (**10**) by *Clostridia* capable of using **10** as a carbon source. The enzyme, L-lysine 2,3-aminomutase, was isolated in 1966.[23] Data indicated that no hydrogen was exchanged with—or taken up from—the medium, and the presence of pyridoxal phosphate but not of coenzyme B_{12} was demonstrated spectroscopically.[24] Earlier workers have provided evidence for α-lysine as the precursor to the β-lysine moiety of several antibiotics.[25-27]

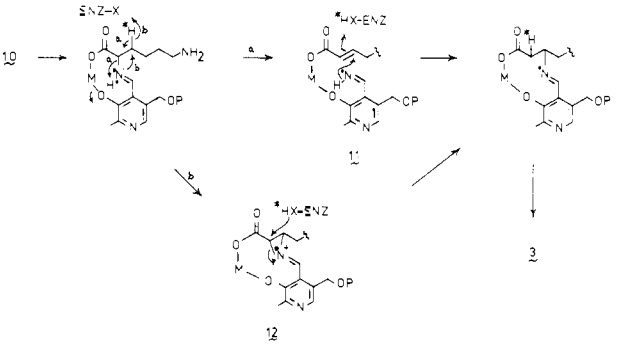
Although coenzyme B_{12} was apparently not involved in the catabolic reaction of *Clostridium* species, we felt a "B_{12}-like" reaction involving transposition of the α-nitrogen and a β-hydrogen (Scheme I) could account for the literature data. Two general mechanisms seemed plausible (Scheme II). One possibility (pathway a) would involve an ammonia lyase like reaction via an unsaturated acid **11** in which the originally eliminated hydrogen and nitrogen are returned in a conjugate addition.[28] The other possibility (pathway b) envisioned an aziridinium intermediate **12**—so that nitrogen always remained covalently bonded to the

Scheme I



10 3

Scheme II



carbon skeleton—with the original hydrogen returning to open the ring at the distal end. Pyridoxal phosphate presumably could react with the ω-amino group to provide a general anchor in the active site or with the α-amino group and participate directly in the reaction. With the extrapolation of this hypothesis to the anabolic reaction of aerobic *Streptomyces*, β-lysine became a major target of our studies of streptothricin biosynthesis.

Results and Discussion

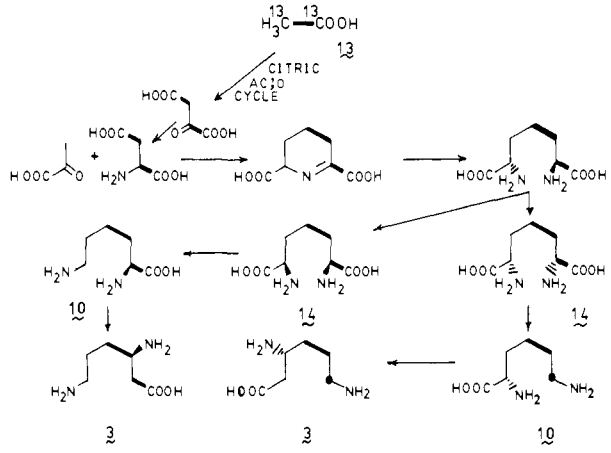
Having obtained *Streptomyces* L-1689-23, a streptothricin F producer, it was necessary to first develop a protocol for the isolation of **1** in high yield from the typical relatively small fermentations to be used in our labeling experiments. Since **1** is only sparingly soluble in any organic solvent, aqueous chromatography with a variety of supports was examined. Most supports proved to be unsuitable, including charcoal, DEAE-Sephadex, carboxymethylcellulose, Sephadex G-10, and Bio-Rad P-2. However, a relatively simple sequence involving Amberlite IRC-50 (K^+) followed by Sephadex LH-20 provided nearly pure **1** with ca. 60% recovery of the antibiotic.

A crucial requirement for any definitive biosynthetic study is a recrystallizable form of the metabolite in order to ensure radiochemical purity. While a few derivatives had been described,[29-31] none could be prepared in high yield and none were easily recrystallized. All known simple salts of **1** are amorphous, but a number of complex salts are crystalline.[32,33] We have found that only the salt formed with methyl orange (commonly referred to as a helianthate) proved easy to generate,[32] easy to recrystallize, and easy to convert to a simple inorganic salt for NMR spectroscopy.

[1,2-$^{13}C_2$]Acetate. Our first key experiment utilized sodium [1,2-$^{13}C_2$]acetate (**13**) as a general probe into the primary and secondary metabolism leading to **1**. Sawada et al.[34] had fed sodium [1-^{13}C]- and [2-^{13}C]acetates to a streptothricin-producing *Streptomyces* and detected a specific but uninterpretable labeling of only the streptolidine moiety. We recognized that acetate, as

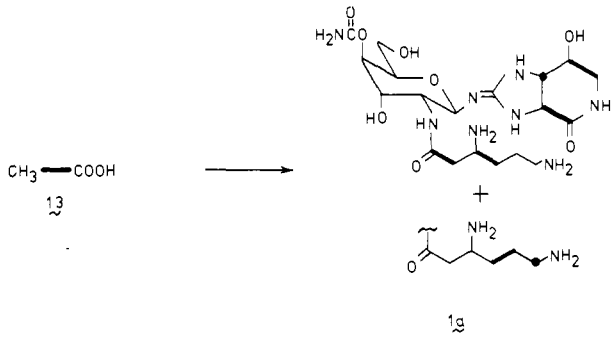
(15) Fox, J. J.; Watanabe, K. A. *Tetrahedron Lett.* **1966**, 897–904. Seto, H.; Otake, N.; Yonehara, H. *Agric. Biol. Chem.* **1968**, *32*, 1299–1305.
(16) Poston, J. M. *Phytochemistry* **1978**, *17*, 401–402, and references cited therein.
(17) Winterstein, E.; Iatrides, A. *Hoppe*-Seyler's *Z. Physiol. Chem.* **1921**, *117*, 240. Winterstein, E.; Guyer, A. *Ibid.* **1923**, *128*, 175. Graf, E.; Boedekker, H. *Arch. Pharm.* (*Weinheim, Ger.*) **1956**, *289*, 364.
(18) Summons, R. E. *Phytochemistry* **1981**, *20*, 1125–1127.
(19) "Principles of Biochemistry"; 5th ed.; White A.; Handler, P.; Smith, E. L.; Eds.; McGraw-Hill: New York, 1973; pp 723–724.
(20) "Enzymatic Reaction Mechanisms"; Walsh, C., Ed.; W. H. Freeman: San Francisco, 1979; p 801.
(21) Parry, R. J.; Kurylo-Borowska, Z. *J. Am. Chem. Soc.* **1980**, *102*, 836–837.
(22) Freer, I.; Pedrocchi-Fantoni, G.; Picken, D. J.; Overton, K. H. *J. Am. Chem. Soc., Chem. Commun.* **1981**, 80–82.
(23) Costilow, R. N.; Rochovansky, O. M.; Barker, H. A. *J. Biol. Chem.* **1966**, *241*, 1573–1580.
(24) Chirpich, T. P.; Zappia, V.; Costilow, R. N.; Barker, H. A. *J. Biol. Chem.* **1970**, *245*, 1778–1789.
(25) Sawada, Y.; Kubo, T.; Taniyama, H. *Chem. Pharm. Bull.* **1976**, *24*, 2163–2167.
(26) Carter, J. H., II; Du Bus, R. H.; Dyer, J. R..; Floyd, J. C.; Rice, K. C.; Shaw, P. D. *Biochemistry* **1974**, *13*, 1227–1233.
(27) (a) Voronina, O. I.; Tovarova, I. I.; Khokhlov, A. S. *Izv. Akad. Nauk SSSR, Ser. Biol.* **1970**, 435–440; *Chem. Abstr.* **1970**, *73*, 42518*m*. (b) Voronina, O. I.; Tovarova, I. I.; Khokhlov, A. S. *Izv. Akad. Nauk SSSR, Ser. Biol.* **1973**, 228–232; *Chem. Abstr.* **1973**, *79*, 16847*y*.
(28) Gould, S. J. *J. Nat. Prod.* **1982**, *45*, 38–49.
(29) Sawada, Y.; Taniyama, H. *Yakugaku Zasshi* **1974**, *4*, 264–266; *Chem. Abstr.* **1974**, *81*, 20772*d*.
(30) Taniyama, H.; Sawada, Y.; Tanaka, S. *Chem. Pharm. Bull.* **1974**, *22*, 337–341.
(31) Sawada, Y.; Sakamoto, H.; Taniyama, H. *Yakugaku Zasshi* **1974**, *94*, 176–180; *Chem. Abstr.* **1974**, *81*, 20771*c*.
(32) Peck, R. L.; Walti, A.; Grabner, R. P.; Flynn, E.; Hoffhine, C. E.; Jr.; Allfrey, V.; Folkers, K. *J. Am. Chem. Soc.* **1946**, *68*, 772–776.
(33) Kuehl, F. A.; Peck, R. L.; Walti, A.; Folkers, K. *Science* (*Washington, D.C.*) **1945**, *102*, 34–35.
(34) Sawada, Y.; Kawakami, S.; Taniyama, H.; Inamori, Y. *J. Antibiot.* **1977**, *30*, 632–640.

Scheme III



its coenzyme A thio ester, is the most central of all primary metabolites. Thus, it can, on the one hand, label a large array of intermediates interconnected by primary metabolic grids and, on the other hand, can fail to provide detectable levels of labeling due to extensive dilution by in vivo metabolite pools. By using double-labeled acetate, we could expect an increased sensitivity of detection by ^{13}C NMR spectroscopy. Furthermore, the analysis of ^{13}C–^{13}C spin couplings would be opaque to pathways generating specific labelings while transparent to those that would lead to random enrichments.

In the event, when feeding equal portions of **13** (mixed with



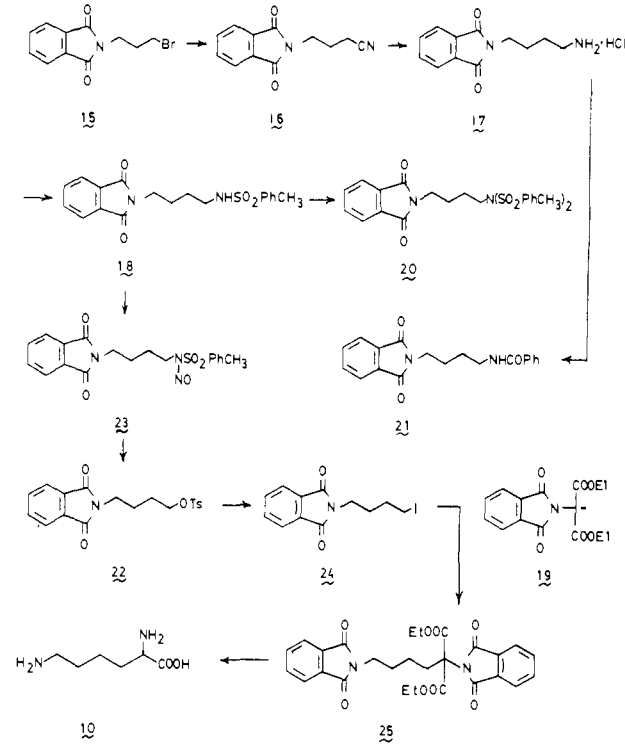
sodium [2-^{14}C]acetate) were fed at the start of the fermentation and 12 h later—at the onset of streptothricin F production—a 1.3% incorporation of ^{14}C was obtained. Analysis of the 67.88-MHz broad band decoupled ^{13}C NMR spectrum of the derived antibiotic **1a** provided the labeling pattern shown.[12] The labeling pattern observed for the streptolidine unit could be explained by incorporation via arginine derived from the citric acid cycle, while that of the β-lysine unit could be explained by incorporation via rearrangement of α-lysine produced by the diaminopimelic acid (DAP) pathway. As shown in Scheme III, the spin coupling of C-17 to both C-16 and C-18 resulted from decarboxylation of the unsymmetrically labeled, otherwise symmetrical *meso*-DAP (**14**). This experiment gave strong support for **10** as precursor to **3**, and defined which of the two known microbial pathways to **10**[35] was involved.

α-[^{13}C,2-^{15}N]Lysine. We next examined whether or not the original α-nitrogen of **10** was retained and, if so, whether the migration was of an intermolecular or intramolecular nature. Our goal was to synthesize α-[3-^{13}C,2-^{15}N]lysine (**10a**) and analyze the ^{13}C NMR spectrum of derived **1b** for the presence of ^{13}C–^{15}N spin coupling.[3]

A new α-lysine synthesis that would permit introduction of the ^{13}C label was required. This is shown in Scheme IV. (Bromopropyl)phthalimide (**15**) was converted to the nitrile **16** in 85% yield and then reduced catalytically in ethanolic HCl to afford the unstable amine salt **17**, which was converted to the sulfonamide

(35) Rodwell, V. W. *Metab. Pathways, 3rd Ed.* **1969**, *3*, 335–349.

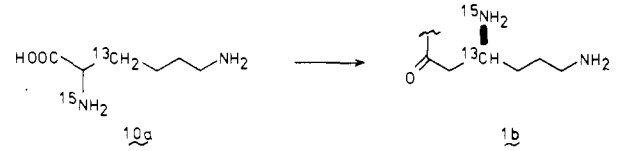
Scheme IV



18.[36] Our intention was to generate a facile leaving group that would allow coupling with the sodium salt of diethyl [^{15}N]-phthalimidomalonate (**19**).[6,37] The sulfonimide **20** could only be prepared with difficulty, and our best result was a 19% yield when prepared at −50 °C in dimethylformamide. In any event, treatment of **20** with either KI[36] or directly with **19** under a variety of conditions led only to recovery of **20**. Similarly, the benzamide **21** could not be converted to an alkyl halide by a von Braun type rearrangement;[38] when treated with either $SOCl_2$ or PBr_5, **21** was again recovered.

Our objective was achieved with the preparation of the tosylate **22** in 55–60% yield by thermal rearrangement[39] of the *N*-nitroso derivative **23**.[40] While **22** did not react with **19**, the iodide **24** coupled easily,[6] and hydrolysis in acid gave the hydrochloride of **10**. When this route was used, the ^{13}C label was introduced with sodium [^{13}C]cyanide and the ^{15}N label was introduced with potassium [^{15}N]phthalimide.[5]

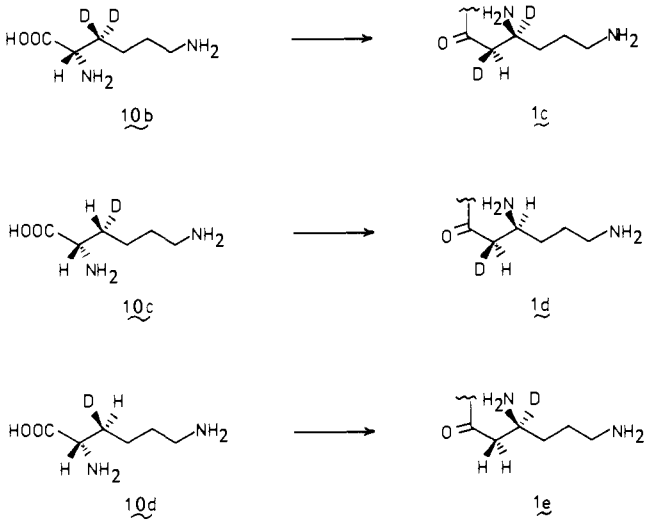
In designing the biosynthetic experiment, we were concerned with the possibility of losing the ^{15}N label by an untimely transamination unrelated to the streptothricin pathway. We, therefore, fed equal portions of **10a** (mixed with L-[U-^{14}C]lysine) at 12, 20,



and 30 h after inoculation of the production broths, hoping that at least one pulse would enter the streptothricin pathway rapidly enough for our needs. A 15% incorporation of radioactivity into **1b** was obtained, and this corresponded to a 4.3% enrichment in ^{13}C if only the L enantiomer were utilized.

(36) DeChristopher, P. J.; Adamek, J. P.; Lyon, G. D.; Galante, J. J.; Haffner, H. E.; Boggio, R. J.; Baumgarten, R. J. *J. Am. Chem. Soc.* **1969**, *91*, 2384–2385.
(37) Stetten, M. R. *J. Biol. Chem.* **1951**, *189*, 499–507.
(38) Vaughan, W. R.; Carlson, R. D. *J. Am. Chem. Soc.* **1962**, *84*, 769–774.
(39) White, E. H. *J. Am. Chem. Soc.* **1955**, *77*, 6008–6010.
(40) White, E. H. *J. Am. Chem. Soc.* **1955**, *77*, 6011–6014.

Scheme V



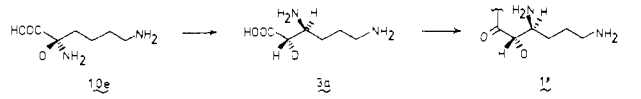
The ^{13}C NMR spectrum of **1b** revealed a doublet (J_{CN} = 3.4 Hz) at δ 45.9, sufficiently large to completely encompass the natural abundance singlet of C-16, revealing the formation of a new ^{13}C-^{15}N bond. A comparison of normalized integrals for the C-5 and C-8 singlets with the C-16 doublet indicated a 4.7% enrichment of ^{13}C. Apparently only the L enantiomer was utilized, and nonrelated transamination was not occurring at any time during the experiment. More importantly, the doublet clearly indicated that the α-nitrogen had been retained and had migrated to C-3 by an *intramolecular* process, since an intermolecular migration would have generated a doublet in only 0.3% of the **1b** molecules.[41]

α-[^{2}H]Lysines. We next investigated the fate of the C-3 hydrogens of **10**. α-[(3*RS*)-$^2H_2$]-, α-[(3*R*)-^{2}H]-, and α-[(3*S*)-^{2}H]lysines, **10b**, **10c**, and **10d**, respectively, had already been synthesized for a study of lysine 2,3-aminomutase in a *Clostridium*.[42] Quantities of each of these, mixed with L-[U-^{14}C]lysine, were fed in separate experiments and resulted in the following incorporations: 14.0% for **10b**, 9.1% for **10c**, and 11.5% for **10d**. Had the deuterium been retained in all three cases, enrichments of 3.43%, 0.96% and 1.97%, respectively, would have been expected.

The derived antibiotics were next analyzed by ^{2}H NMR. We had already assigned the ^{1}H NMR spectrum of **1**[43] and had found that the resonance for the C-16 hydrogen occurred at δ 3.70 (m), while those for the C-15 methylene appeared as AB quartets at δ 2.70 and 2.82.[44] Our samples were prepared in deuterium-depleted water. Since the chemical shift of the residual HDO would be dependent upon pH and temperature, each sample was spiked with *tert*-butanol to provide a chemical shift reference at δ 1.28.

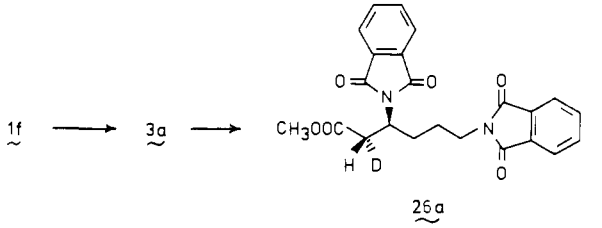
The spectrum of sample **1c** exhibited two deuterium resonances—at δ 2.63[44a] and 3.67[44b]—of roughly equal intensity, revealing that both of the original C-3 hydrogens of **10** had been retained and that one had migrated to C-2. The spectra of **1d** and **1e** exhibited resonances at δ 2.73 and 3.64, respectively, demonstrating that the 3-*pro-R* hydrogen had migrated to C-2 while the 3-*pro-S* hydrogen had remained in place (Scheme V). These results were consistent with those previously found for the *Clostridium* reaction, where the 3-*pro-R* hydrogen had been shown to migrate to the 2-*pro-R* position.[42,45] This information also revealed that the intramolecular migration of nitrogen had occurred with inversion of stereochemistry at C-16.

L-β-[(2*S*)-^{2}H]Lysine. The stereochemical course of the hydrogen migration was next defined by the incorporation of a chirally deuterated sample of L-β-lysine. α-[2-^{2}H,U-^{14}C]Lysine (**10e**) was prepared and treated with the partially purified



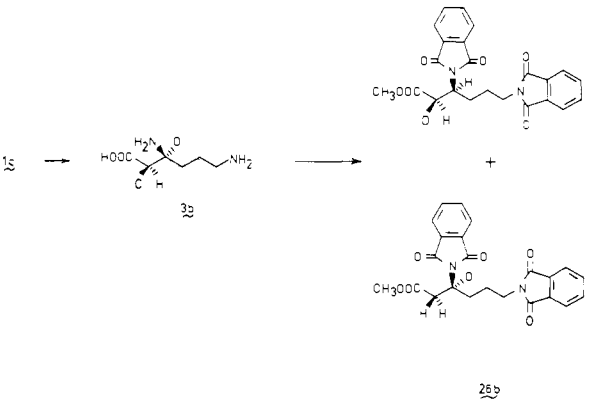
Clostridium enzyme to yield L-β-[(2*S*)-^{2}H,U-^{14}C]lysine (**3a**).[42] The deuterium enrichment of this material was 70%. A 29% incorporation of **3a** into streptothricin F was obtained, based on measured radioactivity, and the ^{2}H NMR spectrum of the antibiotic, **1f**, showed a resonance at δ 2.80. Thus, the methylene hydrogen labeled in **1f** was diastereotopic to that labeled in **1d**, implying the migrated hydrogen in **1a** occupied the 15-*pro-R* position.

The stereochemistry of the deuterium labels in **1d** and **1f** was



unequivocally defined by hydrolysis of **1f** with 1 N HCl at reflux for 6 h, followed by ion-exchange chromatography and derivatization of the β-lysine, **3a**, as the di-*N*-phthaloyl methyl ester **26a**.[42,45] Only one deuterium resonance, at δ 2.76, was observed for **26a**.[42,45] Thus, the 2*S* stereochemistry of the deuterium label of the **3a** initially fed had not been altered during incorporation into **1**. Clearly, no significant amount of exchange at C-2 occurred during the hydrolysis or the workup, since no deuterium resonance at δ 3.23 was observed. The δ 2.82 doublet of doublets in the ^{1}H NMR spectrum of **1** could now be assigned to the 15-*pro-S* hydrogen and the doublet of doublets of δ 2.70 could be assigned to the 15-*pro-R* hydrogen. The migration of the 3-*pro-R* hydrogen of **10** had, in fact, led to inversion of configuration at C-15 of **1**, as shown in Scheme V.

Hydrolysis of Streptothricin 1c. Results from the incorporation of **10a** had clearly demonstrated the intramolecularity of the nitrogen migration during the mutase reaction. It remained to determine whether the hydrogen migration was also intramolecular. To this end **1c** was hydrolyzed and the β-lysine **3b** de-



rivatized to **26b**. In a trial run, mass spectrometric analysis of **26** from hydrolysis of **1** in DCl had indicated the presence of only 9% d_1 and 4% d_2 material. Even in the absence of an isotope effect, only 12.5% [9/2 + (2 × 4)] of the labeled molecules would be

(41) This value was obtained by multiplying the effective ^{13}C concentration (5.1%) that accounts for both natural abundance and enrichment contributions.

(42) See preceding paper in this issue.

(43) Martinkus, K. J.; Gould, S. J.; Nugent, R.; Kuroda, Y., unpublished results.

(44) (a) These values are for pD 7.1. (b) The chemical shifts are pH dependent and complete pH profiles for both ^{1}H and ^{13}C NMR have been measured (ref 43).

(45) Aberhart, D. J.; Lin, H.-J.; Weiller, B. H. *J. Am. Chem. Soc.* **1981**, *103*, 6750–6752.

expected to lose deuterium from C-2 of **1c** (C-15 of streptothricin). Thus, an intramolecular migration would be observable by a 1.7% enrichment in the M + 2 peak of **26b**. The mass spectrum of **26b** showed no measurable enrichment in the M + 2 peak, but a 3.0% enrichment was observed in the M + 1 peak. This result is only possible if the hydrogen migration from C-3 to C-2 is substantially or completely *intermolecular*.

Conclusions

Through a series of feedings with specifically labeled substrates, α-lysine was first implicated and then confirmed as a primary precursor of streptothricin F (**1**). Furthermore, the very high incorporation of specifically labeled L-β-lysine indicated that it is an intermediate in the biosynthetic pathway. These feedings have clearly defined the mechanistic parameters for the *Streptomyces* α-lysine 2,3-aminomutase reaction: intramolecular migration of nitrogen from C-2 to C-3 and intermolecular migration of the *pro-R*-hydrogen from C-3 to C-2, with both steps resulting in inversion at the respective centers. These are identical with our findings with the catabolic *Clostridium* enzyme.[42] The parameters are distinctly different from those of the other known pathways converting α- to β-amino acids. Thus, what at first appeared to be a homogeneous group of metabolites actually is not, and the enzymes involved apparently did not all evolve from a single "grandparent".

Three other aminomutase enzymes that act on basic amino acids have been isolated from clostridia. These act on the ω-amino group of L-β-lysine,[46] D-α-lysine,[47] and D-α-ornithine[48] and transfer the nitrogen to the ω-1 carbon. Each enzyme requires coenzyme B_{12} and pyridoxal phosphate or pyruvate. In the case of the L-β-lysine 6,5-aminomutase, it has been demonstrated that the C-6 nitrogen is retained at C-5 (mechanism unknown),[49] while the C-5 hydrogen migrates intermolecularly to C-6 (stereochemistry unknown).[50] That these are very similar to our findings would not be surprising except that the L-α-lysine 2,3-aminomutase of *Clostridium*—and presumably of *Streptomyces*—requires pyridoxal phosphate but has no requirement for B_{12}. With these latter enzymes it is not clear what the hydrogen acceptor would be. Either mechanism in Scheme II is consistent with our data, although an aziridine intermediate seems more attractive in view of the intramolecularity of the nitrogen migration. Nonetheless, a clear definition of the mechanisms involved must await additional experimental data.

Experimental Section

General. ^1H NMR spectra of synthetic intermediates were taken on a Hitachi Perkin-Elmer R-24 spectrometer; ^{13}C NMR spectra were taken at 67.88 and 125 MHz on Bruker HX 270 and WM 500 spectrometers, respectively; ^2H NMR spectra were taken at 41.44 MHz on a Bruker HX 270 spectrometer. All ^{13}C NMR spectra were broad band decoupled, and ^2H NMR spectra were proton decoupled and run unlocked. Samples run on the HX 270 were contained in 10-mm tubes containing a cylindrical 0.50-mL capacity insert (Wilmad Glass Co.), and samples run on the WH 500 were contained in 5-mm tubes. Samples for ^{13}C NMR spectra were dissolved in 2% pyridine/D_2O with the middle pyridine resonance at 135.5 ppm used for reference, while those for ^2H NMR spectra were dissolved in deuterium-depleted water (Aldrich Chemical Co.) and spiked with *tert*-butanol to provide a chemical shift reference at 1.28 ppm. ^2H NMR spectra of streptothricin F were obtained at 10 °C, and the pH of these samples was between 6.5 and 7.0.

IR spectra were obtained with a Beckman Microlab MX 620 computing spectrometer, and mass spectra were measured on an AEI MS-902 with EI. All radioactivity measurements were carried out in Packard Tri-Carb 3375 or Beckman LS 8000 liquid scintillation counters. Samples were prepared by dissolution in 0.2 mL of methanol, addition of 0.2 mL of saturated aqueous dithionite to decolorize the orange solution, and then addition of 10 mL of Bray's scintillation cocktail (New England Nuclear). Microsamples were weighed on a Cahn Model 4400 electrobalance. All measurements were done in duplicate to ±1.5% standard deviation. Counting efficiencies were determined by spiking with *n*-[^{14}C]hexadecane standard purchased from Amersham/Searle. Melting points (uncorrected) were determined in a Hoover capillary melting point apparatus.

Linear thin-layer chromatography (TLC) was done by using Baker-flex silica gel 1B-F sheets and circular TLC was done by using 3.5 in. × 3.5 in. squares of Merck silica gel 60 F-254 aluminum-backed sheets. Bio-Rad IRC-50 and AG3-X4 resins wre purchased from Bio-Rad; Sephadex LH-20 was purchased from Sigma Chemical Co. Sodium [^{13}C]cyanide was purchased from Merck and Co., and potassium [^{15}N]phthalimide was provided by the Los Alamos Scientific Laboratory. L-[U-^{14}C]Lysine was purchased from ICN Radiochemicals. *Bacillus subtilis* ATCC 6633 spore suspension was purchased from Baltimore Biological Laboratories. *Streptomyces* L-1689-23 and quantities of streptothricin F were generous gifts from Dr. Donald Borders of Lederle Laboratories. All chemicals were of reagent grade and all solvents were distilled prior to use.

Culture Conditions. An improved strain of *Streptomyces* L-1689-23 (produced 200–400 mg/L antibiotic) was maintained at 5 °C on agar slants composed of 1% malt extract, 0.4% yeast extract, 0.4% dextrose, and 2% agar, adjusted to pH 7.3. Seed cultures were prepared by inoculating 50–250 mL of medium, containing 0.3% beef extract, 0.5% yeast extract, 0.5% tryptone, 0.1% dextrose, and 2.4% cornstarch with spores from an agar slant. The cultures, contained in Erlenmeyer flasks of 4–5 times the broth volume, were incubated at 29 °C on a New Brunswick G-25 gyrotory shaker at 200 rpm for 2.5 days. Production media, 250 mL in 1-L Erlenmeyer flasks, consisting of 3% cornstarch, 2% molasses, 1.5% soy flour, and 1% calcium carbonate, were inoculated with 20 mL of seed culture and incubated for 2–2.5 days. [3-^{13}C,2-^{15}N]Lysine was added in a sterile manner through disposable Millipore filters at 12, 20, and 30 h after inoculation of the production broth (four flasks). [^2H]Lysines and [^2H]-β-lysine were added in a sterile manner at 12 h after inoculation.

Isolation. The cultures were centrifuged at ca. 5000 *g* for 15 min in an IEC Model K centrifuge. The supernatant was loaded onto an IRC-50 (K^+ form) column (1.6 × 18 cm) and then washed with 300 mL of water. Antibiotic was eluted off the column with 0.3 N HCl, and the ninhydrin-positive fractions was neutralized to pH 6–7 with AG3-X4 (OH^- form) resin. After freeze-drying, the residue was extracted with methanol to remove salts, and the methanol solution was reduced to dryness, dissolved in 3 mL of water, and loaded onto a Sephadex LH-20 column (2.5 cm × 225 cm).

After elution with water, those ninhydrin-positive fractions not containing low R_f impurities (circular TLC with 1-propanol/pyridine/HOAc/water = 5/15/3/17) were combined, bioassayed, and lyophilized. The resulting powder was dissolved in ca. 5 mL of methanol and to this was added a 4-fold molar excess (based on antibiotic bioassay) of methyl orange in a minimal volume of hot water. The solution was stirred 1 h and taken to dryness by rotary evaporation and lyophilization, and the residue taken up in hot methanol and filtered to remove excess methyl orange. The helianthate salt of streptothricin F was then recrystallized to constant specific radioactivity from methanol–water.

The helianthate was converted to the hydrochloride for NMR studies. The salt was suspended in water, acidified with 1 N HCl to pH 1–2, and centrifuged to remove methyl orange. Residual traces of methyl orange were removed by passing the supernatant through a column of AG3-4X (OH^- form) eluted with water (pH 1); the ninhydrin-positive fractions were adjusted to pH 6–7 with HCl and then lyophilized.

Bioassay. A 0.2-mL aliquot of *B. subtilis* ATCC 6633 spore suspension was pipetted into 10.0 mL of sterile saline for a stock suspension. Brain heart infusion agar (Difco) Petri dishes were innoculated with 0.2 mL of the spore suspension, and these were spread evenly. Filter paper disks (Schleicher and Schuell 704-E, 0.5-in. diameter) were placed on the surface and each moistened with 50 μL of the test solution. The dishes were incubated at 37 °C for 18 h and antibiotic concentrations determined by comparison of the zone of inhibition with a standard curve.

***N*-(3-[^{13}C]Cyanopropyl)phthalimide (16a).** Sodium cyanide (2.4 g, 48 mmol) was added to a stirred solution of *N*-(3-bromopropyl)phthalimide (10.7 g, 40 mmol) in dry dimethyl sulfoxide (50 mL), and the mixture was stirred at 65 °C for 2 h. After being cooled, the mixture was poured into ice water (400 mL), and the precipitate was filtered, washed with water, and dried. Recrystallization from chloroform/petroleum ether yielded 7.5 g (88%) of crystals: mp 78–82 °C (lit.[51] mp 79–80 °C); IR ($CHCl_3$) 2250, 1762, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.9–2.6 (6 H, m), 3.8 (2 H, t, J = 6 Hz), 7.75 (4 H, m); ^{13}C NMR ($CDCl_3$) δ 15.1, 24.7,

(46) Dekker, E. E.; Barker, H. A. *J. Biol. Chem.* **1968**, *243*, 3232–3237.
(47) Morley, C. G. D.; Stadtman, T. C. *Biochemistry* **1971**, *10*, 2325–2329.
(48) Tsuda, Y.; Friedmann, H. C. *J. Biol. Chem.* **1970**, *245*, 5914–5926.
(49) Bray, R. C.; Stadtman, T. C. *J. Biol. Chem.* **1968**, *243*, 381–385.
(50) Rétey, J.; Kunz, F.; Stadtman, T. C.; Arigoni, D. *Experientia* **1969**, *25*, 801–802; *Helv. Chim. Acta* **1978**, *61*, 2989–2998.
(51) McKay, A. F.; Garmaise, D. L.; Gaudry, R.; Baker, H. A.; Paris, G. Y.; Kay, R. W.; Just, G. E.; Schwartz, R. *J. Am. Chem. Soc.* **1959**, *81*, 4328–4335.

36.7, 119.0, 123.4, 131.9, 134.2, 168.2; MS *m/z* 214.

When sodium [^{13}C]cyanide (2.64 g, 91.2 atom % ^{13}C) was used, 9.84 g (85.5%) of **16a** was obtained: mp 78–80 °C; ^{13}C NMR enhancement of the 119.0-ppm resonance; MS *m/z* 215.

***N*-(4-Amino-1-butyl-4-^{13}*C*)phthalimide Hydrochloride (17a).** A mixture of **16** (3 g, 14 mmol) and PtO_2 (100 mg) in absolute ethanol (200 mL) and concentrated HCl (3 mL) was shaken under an H_2 atmosphere at 45 psi in a Parr hydrogenator. After 24 h the catalyst was removed by filtration and the filtrate concentrated to dryness in vacuo. The residue was recrystallized from absolute ethanol to give 1.95 g (54.5%) of **17**: mp 197–199 °C (lit.[52] mp 196 °C); ^{1}H NMR (D_2O) δ 1.8 (4 H, m), 3.16 (2 H, br t), 3.65 (2 H, br t), 7.7 (4 H, m).

With portions of **16a** (2 g, 9.3 mmol), the hydrogenation was continued for 24–40 h. The crude solid containing **17a** was immediately converted to the *p*-toluenesulfonamide, **18a**.

***N*-[4-(*p*-Toluenesulfonamido)-1-butyl-4-^{13}*C*]phthalimide (18a).**[53] A solution of tosyl chloride (1.35 g, 7.1 mmol) in methylene chloride (10 mL) was slowly added to an ice-cold stirred suspension of **17** (1.8 g, 7.1 mmol) in methylene chloride (100 mL) containing triethylamine (1.42 g, 14.2 mmol). The mixture was stirred 4 h and then washed 3 times with water. The organic phase was dried ($MgSO_4$), filtered, and concentrated to yield a solid. Recrystallization from methylene chloride/petroleum ether gave **18** (2.31 g, 87.6%): mp 129–130 °C; IR ($CHCl_3$) 3300, 1760, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.6 (4 H, m), 2.37 (3 H, s), 2.95 (2 H, dt, *J* = 6 Hz), 3.62 (2 H, t, *J* = 6 Hz), 4.6 (1 H, t, *J* = 6 Hz), 7.20 (2 H, d, *J* = 8 Hz), 7.7 (6 H, m); ^{13}C NMR ($CDCl_3$) δ 21.4, 25.7, 26.9, 37.3, 42.6, 123.2, 127.1, 129.7, 132.1, 134.0, 143.2, 168.4; MS *m/z* 372.

Beginning with **17a** (6 g), the reaction was continued for 14 h. The mixture was washed with saturated NH_4Cl, water, and brine, then dried, filtered, and concentrated to yield 4.75 g of **18a**. The mother liquors were separated by flash chromatography on silica gel 60 (5 × 15 cm column) eluting with chloroform to give the starting material (0.95 g) and crude **18a** that was then recrystallized. The combined yield of **18a** was 5.27 g (60.7%): mp 124–126 °C; ^{13}C NMR enhancement of the 42.6-ppm resonance; MS *m/z* 373.

***N*-[4-(*N*-Nitroso-*p*-toluenesulfonamido)-1-butyl-4-^{13}C]phthalimide (23a).** An excess of sodium nitrite (6 g) was added in small portions over 4 h to a stirred suspension of **18** (1.49 g, 4 mmol) in acetic acid (10 mL) and acetic anhydride (20 mL) cooled at 0 °C. The mixture was maintained at 4 °C overnight, and then diluted with ice water (100 mL). After the mixture was stirred 5 min, the fine pale yellow crystals were removed by filtration, washed with water, dried, and then recrystallized from methylene chloride–petroleum ether to give **23** (1.55 g, 96%): mp 102–103 °C; IR ($CHCl_3$) 1760, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.5 (4 H, m), 2.4 (3 H, s), 3.65 (4 H, dt, *J* = 6, 4 Hz), 7.3 (2 H, d, *J* = 8 Hz), 7.75 (6 H, m); ^{13}C NMR ($CDCl_3$) δ 25.0, 25.8, 37.0, 42.4, 123.3, 128.1, 130.3, 132.0, 134.1, 133.4, 145.7, 168.2.

Beginning with 3.73 g of **18a**, 3.84 g (96%) of **23a** was obtained: mp 99–100 °C; ^{13}C NMR enhancement of the 42.4-ppm resonance.

***N*-(4-Hydroxy-1-butyl-4-^{13}*C*)phthalimide Tosylate (22a).** Nitrosotosylamide **23** (602 mg, 1.5 mmol) and anhydrous sodium carbonate (164 mg, 1.55 mmol) in carbon tetrachloride (300 mL) was heated at reflux for 40 h under an N_2 atmosphere. The solvent was then removed in vacuo and the residue triturated with methylene chloride. After filtration and concentration the crude material was dissolved in methylene chloride. Fractional crystallization by addition of petroleum ether yielded first toluenesulfonamide **18** (100 mg, 17.9%) and then the tosylate **22** (302 mg, 54%): mp 112–113 °C; IR ($CHCl_3$) 1765, 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.7 (4 H, m), 2.4 (3 H, s), 3.62 (3 H, t, *J* = 7 Hz), 4.05 (3 H, t, *J* = 7 Hz), 7.3 (2 H, d, *J* = 8 Hz), 7.7 (6 H, m); ^{13}C NMR ($CDCl_3$) δ 21.6, 24.7, 26.4, 37.0, 69.7, 123.2, 127.9, 129.9, 132.0, 133.0, 134.0, 146.8, 168.2.

Beginning with **23a** (1.20 g, 3 mmol), a small amount of the sulfonamide **18a** (120 mg, 10.8%) was obtained in addition to the tosylate **22a** (958 mg, 85.6%): mp 108–110 °C; ^{13}C NMR enhancement of the 69.7-ppm resonance.

***N*-(4-Iodo-1-butyl-4-^{13}*C*)phthalimide (24a).** A mixture of the tosylate **22** (1.20 g, 3.2 mmol) and anhydrous sodium iodide (2.41 g, 16 mmol) in acetone (50 mL) was heated at reflux for 2 h. After removal of the solvent in vacuo, water (50 mL) was added and the precipitate filtered and dried. Recrystallization from aqueous ethanol yielded 956 mg (90.4%) of **24**: mp 88–89 °C (lit.[54] mp 88–89 °C); IR ($CHCl_3$) 1765, 1703 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.85 (4 H, m), 3.20 (2 H, t, *J* = 6 Hz), 3.70 (2 H, t, *J* = 6 Hz), 7.75 (4 H, m); ^{13}C NMR ($CDCl_3$) δ 5.2, 29.5, 30.6, 36.7, 123.2, 132.1, 133.9, 168.2; MS *m/z* 329.

Beginning with 1.80 g of **22a**, 1.44 g (92%) of **24a** was obtained: mp 87–89 °C; ^{13}C NMR enhancement of the 5.2-ppm resonance.

α-[3-^{13}C,2-^{15}N]Lysine Dihydrochloride (10a). Diethyl phthalimidomalonate (303 mg, 1 mmol) was added to sodium hydride (50 mg, 50% suspension in Nujol) in absolute ethanol (50 mL) at 60 °C under an N_2 atmosphere. The solvent was removed under reduced pressure and replaced by dry toluene (50 mL). Lumps were broken up with a spatula. The mixture was then stirred at 80 °C while the solvent was removed at reduced pressure. *N*-(4-Iodobutyl)phthalimide (**24**) (329 mg, 1 mmol) in dry toluene (5 mL) was added and the mixture stirred for 5 min. The solvent was again removed and the residue was heated at 155–160 °C for 3 h. After being cooled, the mixture was triturated with chloroform, filtered through Celite, and concentrated in vacuo to yield the crude bis(phthalimide) **25** (537 mg), which was chromatographed on SiO_2 eluted with chloroform to afford 404 mg (93.5%) of **25**: IR ($CHCl_3$) 1765, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.2 (3 H, t, *J* = 7 Hz), 1.2–2.0 (5 H, m), 2.4–2.7 (1 H, m), 2.5–4.0 (3 H, m), 4.2 (2 H, q, *J* = 7 Hz), 7.7 (8 H, m). The bis(phthalimide) **25** (240 mg, 0.5 mmol) in concentrated HCl (4 mL) and acetic acid (2 mL) was heated at reflux for 20 h. The solvent was removed under reduced pressure and the residue triturated with water (10 mL). This mixture was filtered, and the filtrate was concentrated in vacuo. The product was recrystallized from ethanol/ether to yield 61 mg of **10** (50%): mp 185–191 °C dec; ^{13}C NMR (D_2O plus DSS) δ 19.2, 24.1, 27.6, 36.8, 52.2, 168.9.

When the procedure described above was used, diethyl [^{15}N]phthalimidomalonate (1.212 g, 4 mmol) and *N*-(4-iodobutyl-4-^{13}*C*)phthalimide (**24a**) (1.316 g, 4 mmol) were condensed to yield 2.25 g of crude **25a**. This was hydrolyzed with concentrated HCl (12 mL), glacial acetic acid (12 mL) and water (12 mL) heated at reflux for 5 h, whereupon a distillate boiling up to 108 °C was collected and the remainder heated at reflux for an additional 16 h. After the mixture was cooled and refrigerated, the phthalic acid was removed by filtration and washed with water, and the combined filtrates were concentrated to dryness. The residue was recrystallized to yield 425 mg (49%) of **10a**: mp 183–190 °C dec; ^{13}C NMR enhancement of the 27.6-ppm resonance.

***N*-[4-[*N*,*N*-Bis(*p*-toluenesulfonylamino)]butyl]phthalimide (20).** A solution of toluenesulfonamide **18** (186 mg, 0.5 mmol) in DMF (3 mL) was added to a suspension of sodium hydride (50 mg, 1 mmol, 50% suspension in Nujol) in DMF (1 mL) at −50 °C. After 30 min tosyl chloride (100 mg, 0.5 mmol) in DMF (1 mL) was added, and the mixture was stirred an additional 30 min at −50 °C. After the mixture was warmed to room temperature, the solvent was removed in vacuo and the residue in chloroform filtered through a small column of Florisil in the same solvent. The crystalline product **20** was thus obtained (50 mg, 19%): mp 142–144 °C; ^{1}H NMR ($CDCl_3$) δ 1.7 (4 H, m), 7.3 (4 H, d, *J* = 8 Hz), 7.8 (4 H, m), 7.9 (4 H, d, *J* = 8 Hz).

***N*-(4-Benzamido-1-butyl)phthalimide (21).** Benzoyl chloride (460 mg, 3.3 mmol) was added to a suspension of the amine hydrochloride **17** (763 mg, 3 mmol) in dry benzene (50 mL), and the mixture was then heated at reflux for 24 h. The resulting mixture was cooled and filtered, and the filtrate concentrated in vacuo. The residue was crystallized from benzene/petroleum ether to give 700 mg (72%) of **21**: mp 152–153 °C; ^{1}H NMR ($CDCl_3$) δ 1.7 (4 H, m), 3.5 (2 H, d, *J* = 7 Hz), 3.7 (2 H, t, *J* = 7 Hz), 6.4 (1 H, br s), 7.35 (3 H, m) 7.7 (6 H, m).

Di-*N*-phthaloyl-β-lysine Methyl Ester 26 from Hydrolysis of Streptothricin F. (a) Streptothricin F (100 mg, 0.16 mmol) in 1 N HCl (3 mL) was heated in a sealed tube for 6 h, and the mixture was then cooled and applied to a column of Dowex 50 W-X8 (H^+) (1.2 cm × 15 cm) in water. After being washed with water, the column was eluted with 2 N NH_4OH, collecting 3-mL fractions. Ninhydrin-positive fractions were pooled and lyophilized to give 68 mg of material that was dissolved in water (2 mL) containing sodium carbonate (22 mg, 0.2 mmol) and then treated with *N*-carbethoxyphthalimide (88 mg, 0.4 mmol). After the mixture was stirred 1 h, the turbid solution was filtered, acidified, and lyophilized. The residue was dissolved in ether/methanol and treated with an ether solution of diazomethane for 1 h. The solvent was removed, and the residue was chromatographed on a small silica gel column (1.2 × 10 cm, Woelm 70–150 mesh) eluted with ethyl acetate/hexane (1/1). This afforded 60 mg of crude product that was purified by HPLC (Partisil PXS 10/25 column, 25.0 cm × 4.1 cm, 70% isooctane/30% ethyl acetate, 4 mL/min, 1500 psi) to give 10 mg of **26**: mp 153–154 °C (lit.[42] mp 148–150 °C); identical by TLC and HPLC with authentic material. (b) Using this same procedure with 1 N DCl (3 mL), 51 mg of crude β-lysine was obtained and was derivatized after dissolving in D_2O (5 mL) containing sodium carbonate. After 45 min this solution was acidified with 1 N DCl, and the remainder of the procedure was carried out to yield **26**, 11 mg: mp 153–154 °C; MS, 86.8% d_0, 9.1% d_1, and 4.1% d_2. (c) Streptothricin F **1b** (90 mg, 0.14 mmol), derived from α[3,3-$^2H_2$]-lysine was hydrolyzed and the β-lysine derivatized to yield **26b** (11 mg):

(52) Wälchli-Schaer, E.; Eugster, C. H. *Helv. Chim. Acta* **1978**, *61*, 928–935.

(53) Maier, W.; Schuette, H. R. *Z. Chem.* **1967**, *7*, 155–156.

(54) Arnstein, H. R. V.; Hunter, G. D.; Muir, H. M.; Neuberger, A. *J. Chem. Soc.* **1952**, 1329–1334.

MS, 97.0% d_0 and 3.0% d_1. (d) Streptothricin F **1f** (92 mg, 0.15 mmol) yielded 8 mg of **26a**: MS, 95.6% d_0 and 6.4% d_1.

Acknowledgment. This work was supported by Publich Health Service Research Grants GM 25996 (S.J.G.) and GM 25919 (D.J.A.) from the National Institute of General Medical Sciences. The potassium [^{15}N]phthalimide was provided by the Stable Isotopes Resource at the Los Alamos Scientific Laboratory, jointly supported by the U.S. Department of Energy and the National Institutes of Health (RR-00962-02, Division of Research Resources). Dr. Chou-Hong Tann and Kazys Martinkus are warmly thanked for expert technical assistance. We are grateful for generous gifts of streptothricin F and *Streptomyces* L-1689-23. Peter Demou of the Chemistry Department, Yale University, is thanked for obtaining the ^{13}C NMR spectra. The Bruker HX-270 NMR instrument facility at Yale used in this work was supported by the National Science Foundation, Grant CHE-7916210, from the Chemistry Division.

Registry No. 1, 3808-42-2; **1**·*x*helianthate, 86118-03-8; **1**·*x*HCl, 18800-56-1; **2**, 74-79-3; **3**, 504-21-2; **10**, 6899-06-5; **10a**·2HCl, 86088-61-1; **13**, 64-19-7; **14**, 922-54-3; **15**, 5460-29-7; **16**, 3184-61-0; **16a**, 79634-10-9; **17**, 35517-18-1; **17a**, 86088-62-2; **18**, 15544-49-7; **18a**, 86101-32-8; **19**, 5680-61-5; **20**, 86088-63-3; **21**, 86088-64-4; **22**, 86088-65-5; **22a**, 86088-66-6; **23**, 86101-33-9; **23a**, 86101-34-0; **24**, 5457-30-7; **24a**, 79634-09-6; **25**, 86088-67-7; **25a**, 86088-68-8; **26**, 86088-69-9; α-lysine 2,3-aminomutase, 9075-20-1.

Communications to the Editor

Novel Alkyne Reactions with Binuclear Molybdenum-Sulfur Complexes: Structure of the Dimethyl Acetylenedicarboxylate Adduct of $[Mo_2O_2(\mu\text{-}S)_2(S_2)_2]^{2-}$

Thomas R. Halbert,* Wie-Hin Pan, and Edward I. Stiefel*

Exxon Research and Engineering Company
Corporate Research-Science Laboratories
Linden, New Jersey 07036
Received January 10, 1983

Recent years have seen dramatic expansion in synthetic and structural molybdenum–sulfur chemistry. Many new inorganic thioanions,[1] organic-ligand-bearing complexes,[2] and polymetallic heteronuclear clusters[3,4] have been prepared and their significance to biochemistry[5] and industry[1a,6,7] has been duly noted. Despite the plethora of new compounds, relatively few studies have been reported on reactivity toward organic reagents. As part of a systematic investigation of such reactivity we have begun to study the reactions of alkynes with Mo–S systems.

Known reactions of alkynes with S-ligand complexes are of two types. In the first or classical type the alkyne adds directly to the metal to form either π-bound[8a-c] or σ-bound[8d] complexes. In the second type the alkyne reacts with sulfide, disulfide, or polysulfide ligands to form a 1,2-dithiolene ligand.[7,8e,f] Here we report preliminary results on reactions of Mo–S complexes with alkynes, including a case in which addition of the alkyne follows neither of the above patterns.

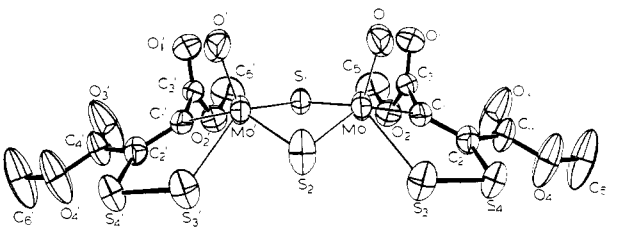


Figure 1. Perspective drawing adapted from ORTEP plot of the $Mo_2O_2S_2(S_2C_2(CO_2CH_3)_2)_2^{2-}$ dianion **4**. Bond lengths: Mo–S_1, 2.318 (2); Mo–S_2, 2.337 (2); Mo–O, 1.676 (6); Mo–S_3, 2.382 (3); Mo–Mo′, 2.882 (1); S_3–S_4, 2.061 (3); S_4–C_2, 1.731 (9); C_1–C_2, 1.36 (1); Mo–C_1, 2.215 (8) Å. Bond angles: Mo–C_1–C_2, 126.0 (6)°; C_1–C_2–S_4, 123.0 (6)°.

We chose the dianion $[Mo_2S_2(\mu\text{-}S)_2(SCH_2CH_2S)_2]^{2-}$ (**1**), whose structure contains a *syn*-$Mo_2S_4^{2+}$ core,[9] as a starting point for our studies. When a red-orange solution of the tetraethylammonium salt of **1** in CH_3CN at 0 °C is treated with two or more equivalents of dimethyl acetylenedicarboxylate (DMAC), the solution rapidly darkens and 2 equiv of ethylene are liberated (as quantitated by GC). Apparently, in the major reaction the activated acetylene attacks the 1,2-ethanedithiolate ligands to displace ethylene[10] rather than adding to the *syn*-$Mo_2S_4^{2+}$ core by a process analogous to that observed for the *anti*-$Mo_2S_4^{2+}$ core in $(Me_nCp)_2Mo_2S_2(\mu\text{-}S)_2$ (n = 1, 5) complexes.[7] Identification of pure Mo/S products from the reaction of **1** with DMAC has proved troublesome, perhaps due to some reactivity at the $Mo_2S_4^{2+}$ core in

(1) (a) Simhon, E. D.; Baenziger, N. C.; Kanatzidis, M.; Draganjac, M.; Coucouvanis, D. *J. Am. Chem. Soc.* **1981**, *103*, 1218. (b) Clegg, W.; Mohan, N.; Müller, A.; Neumann, A.; Rittner, W.; Sheldrick, G. M. *Inorg. Chem.* **1980**, *19*, 2066; **1979**, *18*, 530. (c) Clegg, W.; Christou, G.; Garner, C. D.; Sheldrick, G. M. *Ibid.* **1981**, *20*, 1562. (d) Müller, A.; Nolte, W. O.; Krebs, B. *Ibid.* **1980**, *19*, 2835 and references cited therein. (e) Pan, W. H.; Leonowicz, M. E.; Stiefel, E. I. *Ibid.* **1983**, *22*, 672. (f) Müller, A.; Bhattacharyya, R. G.; Pfefferkorn, B. *Chem. Ber.* **1979**, *112*, 778.

(2) (a) Miller, K. F.; Bruce, A. E.; Corbin, J. L.; Wherland, S.; Stiefel, E. I. *J. Am. Chem. Soc.* **1980**, *102*, 5104–5106 and references cited therein. (b) Rakowski DuBois, M.; DuBois, D. L.; Van Derveer, M. C.; Haltiwanger, R. C. *Inorg. Chem.* **1981**, *20*, 3064–3071. (c) Müller, A.; Reinsche, U. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 72–73. (d) Vergamini, P. J.; Vahrenkamp, H.; Dahl, L. F. *J. Am. Chem. Soc.* **1971**, *93*, 6327–6329. (e) Keck, H.; Kuchen, W.; Mathow, J.; Meyer, B.; Mootz, D.; Wunderlich, H. *Angew. Chem., Int. Ed. Engl.* **1980**, *20*, 975–976.

(3) Coucouvanis, D. *Acc. Chem. Res.* **1981**, *14*, 201–209 and references cited therein.

(4) Christou, G.; Mascharak, P. K.; Armstrong, W. H.; Papaefthymiou, G. C.; Frankel, R. B.; Holm, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 2820–2831 and references cited therein.

(5) (a) "Molybdenum and Molybdenum Containing Enzymes"; M. Coughlan, Ed.; Pergamon Press: New York, 1980. (b) Holm, R. H. *Chem. Soc. Rev.* **1981**, *10*, 455.

(6) (a) Chianelli, R. R. Advances in Catalytic Chem. II, Salt Lake City, Utah, 1982. (b) Stiefel, E. I.; Chianelli, R. R. In "Nitrogen Fixation: Chemistry, Biochemistry, Genetics Interface"; Müller, A., Diemann, E., Ed.; Plenum Press: New York, 1983; p 341.

(7) (a) Rakowski DuBois, M.; Haltiwanger, R. C.; Miller, D. J.; Glatzmaier, G. J. *J. Am. Chem. Soc.* **1979**, *101*, 5245. (b) Miller, D. J.; Rakowski DuBois, M. *Ibid.* **1980**, *102*, 4925. (c) DuBois, D. L.; Miller, W. K.; Rakowski DuBois, M. *Ibid.* **1981**, *103*, 3429.

(8) (a) Newton, W. E.; McDonald, J. W.; Corbin, J. L.; Ricard, L.; Weiss, R. *Inorg. Chem.* **1980**, *19*, 1997 and references cited therein. (b) Tanaka, K.; Miyaka, S.; Tanaka, T. *Chem. Lett.* **1981**, 189. (c) Kamata, M; Yoshida, T.; Otsuka, S.; Hirotsu, K.; Higuchi, T.; Kido, M.; Tatsumi, K.; Hoffman, R. *Organometallics* **1982**, *1*, 227. (d) Davidson, J. L.; Harrison, W.; Sharp, D. W. A.; Sim, G. A. *J. Organomet. Chem.* **1972**, *47*, C47. (e) Seyferth, D.; Henderson, R. S. *J. Organomet. Chem.* **1979**, *182*, C39–C42. (f) Bolinger, C. M.; Rauchfuss, T. B.; Wilson, S. R. *J. Am. Chem. Soc.* **1981**, *103*, 5620–5621.

(9) (a) Bunzey, G.; Enemark, J. H.; Howie, J. K.; Sawyer, D. T. *J. Am. Chem. Soc.* **1977**, *99*, 4168. (b) Bunzey, G.; Enemark, J. H. *Inorg. Chem.* **1978**, *17*, 682. A general high-yield synthetic route to **1** and related compounds is now available.[1e,2a]

(10) The reaction of acetylene with $CpMo(SCH_2CH_2S)_2MoCp$ has been reported to liberate $H_2C{=}CH_2$.[7a] This reaction involves a bridging ethanedithiolate, whereas the currently reported reaction starts with terminal ethanedithiolate ligands. In a related organic reaction DMAC displaces ethylene from ethylene trithiocarbonate (O'Conner, B. R.; Jones, F. N. *J. Org. Chem.* **1970**, *35*, 2002).

0002-7863/83/1505-5476$01.50/0