is three times larger than that of ^{13}C with a chemical shift anisotropy $\Delta\sigma$ = 673 ppm and an asymmetry parameter η = -0.47 ppm for ^{15}N in pyridine. Since the CSA contribution to relaxation increases with the square of the magnetic field strength, it may dominate relaxation for nonprotonated nitrogens in the 7.05T field used to obtain the spectra of Figure 1. Using the above values for the CSA parameters and assuming a single isotropic correlation time τ_{eff}, CSA contributions to the line width of 17 and 150 Hz may be estimated for τ_{eff} = 10 and 100 ns, respectively. Although chemical shift heterogeneity obfuscates line width measurements, it is evident in Figure 1 that line widths for the protonated and nonprotonated nitrogen resonances are similar. Although signal-to-noise ratio was poor, the ^{15}N spectrum of DNA at 9.115 MHz (not shown) was also observed. Line widths for protonated nitrogen resonances were comparable at both field strengths, but nonprotonated nitrogen resonances were roughly three times narrower at the low field strength. Chemical shift heterogeneity may be a factor, but different CSA contributions to line width undoubtedly occur at the two field strengths. The differential behavior of protonated and nonprotonated nitrogen resonances at the two field strengths was consistent with the peak assignments in Figure 1.

Acknowledgment. This work was supported in part by the National Institutes of Health via Grant GM25018. T.L.J. gratefully acknowledges seminal discussions with Dr. Philip H. Bolton. Thanks are also due to the Biological Service Unit of the Weizmann Institute for aid in growing the bacteria and the Chemistry Department, Hebrew University, for use of their NMR spectrometer. We are grateful to the Isotope Separation Plant of the Weizmann Institute for [^{15}N]nitric acid and Professor Ada Zamir for sizing our DNA.

Stereochemistry of Lysine 2,3-Aminomutase

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Lysine 2,3-aminomutase catalyzes the reversible interconversion of L-α-lysine (**1a**) and L-β-lysine (**2**).[1] The air-sensitive enzyme purified to near homogeneity from *Clostridium SB4* (*C. subterminale* strain SB4, ATCC 29748) has an equilibrium constant K = (L-β-Lys)/(L-α-Lys) = 6.7 at 37 °C and shows stimulation by *S*-adenosylmethionine, ferrous ion, and pyridoxal phosphate.[2] However, in contrast to most [1,3-8] other known aminomutases, the enzyme neither contains nor is stimulated by coenzyme B_{12}. The interconversion of L-α-lysine and L-β-lysine takes place without exchange of nitrogen or hydrogens with the medium[2,9] and thus, presumably, proceeds with intramolecular (or intermolecular) hydrogen and amino group transfer.[10,11]

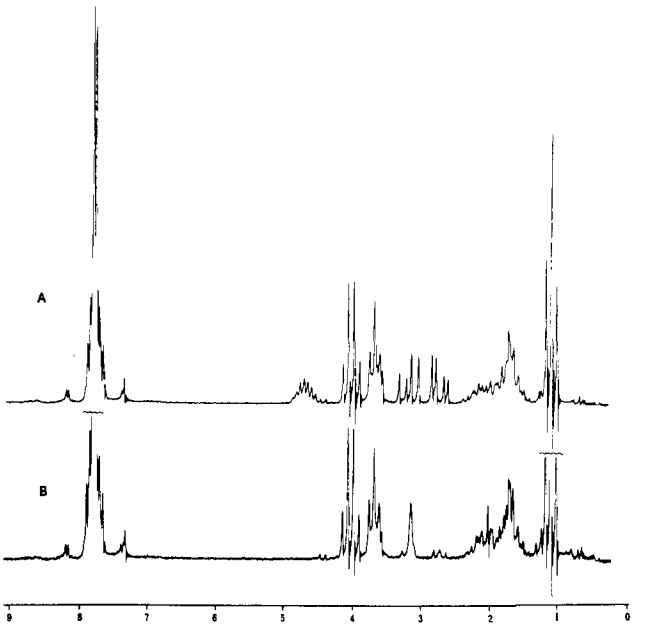

Figure 1. 90-MHz ^1H NMR spectra of **3a** and **3e**: (A) **3a**; (B) **3e** ($CDCl_3$ solutions).

Although the absolute configurations of the substrates of lysine 2,3-aminomutase have long been known, the mechanism of the reaction is still obscure. In an effort to shed some light on this problem, we investigated the cryptic stereochemistry[12,13] of the process and now report evidence which establishes that, in *Clostridium SB4*, the hydrogen and amino group transfers take place with inversion of configuration at both migration termini.[14]

In preliminary experiments, we found that upon incubation of L-lysine-^{14}C with a cell-free extract of *C. SB4* grown on a lysine-rich medium,[15] a reasonably high recovery (ca. 25%) of β-lysine could be obtained after conversion of the crude reaction product into the di-*N*-phthaloyl ethyl ester derivative (**3a**) by heating with phthalic anhydride followed by treatment with diazoethane.[16,17] It was therefore unnecessary to purify the enzyme, and all of our studies were conducted with this cell-free extract. Our ability to isolate β-lysine as the pure derivative **3** in milligram quantities was of extreme importance to our subsequent work. The ^1H NMR spectrum of this compound (Figure 1A) showed an excellent resolution of the signals for the C-2 and C-3 protons.[18]

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(4) Overton et al. recently reported[5] that L-leucine 2,3-aminomutase in tissue cultures of *Andrographis paniculata* did not show a coenzyme B_{12} dependence. This result contrasts with the results of Poston,[3] who has identified a coenzyme-B_{12}-dependent leucine 2,3-aminomutase in a wide variety of species.
(5) Freer, I.; Pedrocchi-Fantoni, G.; Picken, D. J.; Overton, K. H. *J. Chem. Soc., Chem. Commun. 1981*, 80-82.
(6) Tyrosine α,β-mutase has no requirement for coenzyme B_{12} but also has no requirement for pyridoxal phosphate.[7] The reaction proceeds with exchange of one C-3 hydrogen (3-pro-*S*) of L-tyrosine and both C-2 hydrogens of β-tyrosine with the medium. Also the amino group undergoes exchange with ammonium ions in the medium. The reaction thus differs in a fundamental way from lysine 2,3-aminomutase.[8]
(7) Kurylo-Borowska, Z.; Abramsky, T. *Biochim. Biophys. Acta* **1972**, *264*, 1-10.
(8) Parry, R. J.; Kurylo-Borowska, Z. *J. Am. Chem. Soc.* **1980**, *102*, 836-837.
(9) Crude cell-free extracts of *Clostridia* contain an α-lysine racemase which presumably exchanges the α-hydrogens of α-lysine. However, the pure enzyme has no racemase activity.
(10) Experiments designed to directly examine the question of intra- vs. intermolecular transfer of hydrogen and/or amino group in this reaction are in progress and will be reported elsewhere. For an experiment showing *intramolecular* transfer of the amino group in lysine 2,3-aminomutase in a *Streptomyces*, see the accompanying paper by Gould and Thiruvengadam.[11]
(11) Gould, S. J.; Thiruvengadam, T. K. *J. Am. Chem. Soc.*, following paper in this issue.
(12) Cryptic stereochemistry refers to the stereochemical course of a reaction which cannot be deduced simply by examining the absolute configurations of starting material and product.[13] Such sterochemistry can only be elucidated by an isotopic labeling experiment.
(13) Hanson, K. R.; Rose, I. A. *Acc. Chem. Res.* **1975**, *8*, 1-10.
(14) L-β-Lysine is also produced by certain *Streptomyces*, where it accumulates as a component of a wide variety of antibiotics: (a) Sawada, Y.; Nakashima, S.; Taniyama, H. *Chem. Pharm. Bull.* **1977**, *25*, 3210-3217. (b) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *J. Am. Chem. Soc.* **1981**, *103*, 2871-2872. (c) McGahren, W. J.; Hardy, B. A.; Morton, G. O.; Lovell, F. M.; Perkinson, N. A.; Hargreaves, R. T.; Borders, D. B.; Ellestad, G. A. *J. Org. Chem.* **1981**, *46*, 792-799.
(15) Clostridium SB4 (ATCC 29748) was grown on the medium of Barker et al: (a) Costilow, R. N.; Rochovansky, O. M.; Barker, H. A. *J. Biol. Chem.* **1966**, *241*, 1573-1580. (b) Chirpich, T. P.; Herbst, M. M.; Edmunds, H. N.; Baltimore, B. G.; Costilow, R. N.; Barker, H. A. *Prep. Biochem.* **1973**, *3*, 47-52.
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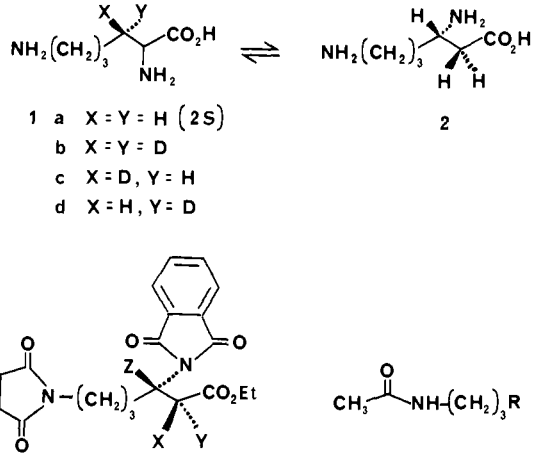
(17) Satisfactory analytical data have been obtained for all new compounds reported in this paper.

0002-7863/81/1503-6750$01.25/0

We first reexamined the question of whether a transfer of hydrogen (or hydrogens) from C-3 of α-lysine to C-2 of β-lysine accompanies the amino group transfer from C-2 to C-3. For this purpose, (2*RS*)-lysine-*3,3*-d_2 (**1b**) was synthesized. Methyl 4-acetamidobutyrate (**4a**) was reduced with $NaBD_4$ to 4-acetamido-1-butanol-*1,1*-d_2 (**4b**) which was converted with PBr_3 to 4-acetamido-1-bromobutane-*1,1*-d_2 (**4c**). Treatment of this with the sodium salt of ethyl acetamidocyanoacetate gave **4d**, which upon acidic hydrolysis yielded **1b**. Incubation of **1b** with the cell-free extract of *Clostridium SB4* gave, after workup, **3b**. The ^1H NMR of the product showed, in place of the well-defined ABX pattern for the C-2 and C-3 protons of **3a**, mainly a broadened singlet at δ 2.75. However, substantial absorption still remained at δ 3.23 and 4.70, as in Figure 1A, due to the presence of endogenous unlabeled material which made it difficult to evaluate the stereospecificity of the process. However, the deuterium NMR spectrum (Figure 2B) clearly shows the presence of equal amounts of deuterium at δ 4.69 (C-3^{2}H) and δ 3.18 (one of the C-2^{2}H).[19] This spectrum shows that deuterium transfer takes place from C-3 of α-lysine to C-2 of β-lysine and that this transfer occurs with a high degree of stereospecificity. The ^2H NMR (Figure 2A) of synthetic[20,21] (2*RS*)-β-lysine-d_1 di-*N*-phthaloyl ethyl ester (**3c + 3d**) confirms the expectation that the resonances of a 2-pro-*R* and 2-pro-*S* deuterium atom in **3** can readily be distinguished.



1 a X = Y = H (2S)
b X = Y = D
c X = D, Y = H
d X = H, Y = D

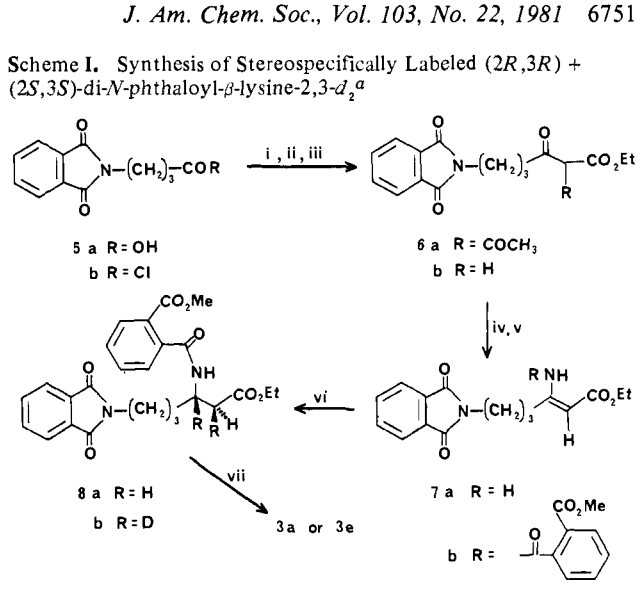
2

3 a X = Y = Z = H
b X = H, Y = Z = D
c X = H, Y = D, Z = H
d X = D, Y = Z = H
e X = D, Y = H, Z = D
f X = Y = H, Z = D

4 a R = CO_2Et
b R = CD_2OH
c R = CD_2Br
d R = CD_2 (CN, CO_2Et, $NHCOCH_3$)

For assignment of the C-2 stereochemistry of **3** obtained from **1b**, it was necessary to assign the C-2 proton (deuteron) resonances of **3**. For this purpose, a stereospecifically labeled reference compound **3e** was synthesized (Scheme I). 4-Phthalimidobutyric acid (**5a**)[22] was converted with $SOCl_2$ into the acid chloride **5b**.[22] Treatment of **5b** with the magnesium salt of ethyl acetoacetate gave **6a**, mp 73–75 °C, which upon treatment with dilute NH_4OH was converted to **6b**, mp 54–55 °C. Upon treatment with dry ammonia in ethanol at 25 °C, **6b** was converted into **7a**, mp 99–101 °C, which was acylated with *o*-(carbomethoxy)benzoyl chloride[23] in pyridine/$CHCl_3$ at 60 °C to give the (*Z*)-*o*-(carboxymethoxy)benzamide[24] (**7b**), mp 141–142 °C. Reduction of **7b** with hydrogen or deuterium (Wilkinson's catalyst,[25] 420 psi, 36 h)[26] gave racemic **8a** or **8b**, mp 150–152 °C, respectively. Upon heating at 200 °C (neat), **8a** or **8b** was converted to the desired, racemic **3a** or **3e**, mp 95–96 °C, respectively. The latter showed in the ^1H NMR spectrum (Figure 1B) a single broadened singlet at δ 3.16, with only traces of absorption in the δ 2.8 region.

Scheme I. Synthesis of Stereospecifically Labeled (2*R*,3*R*) + (2*S*,3*S*)-di-*N*-phthaloyl-β-lysine-2,3-d_2[a]



5 a R = OH
b R = Cl

6 a R = $COCH_3$
b R = H

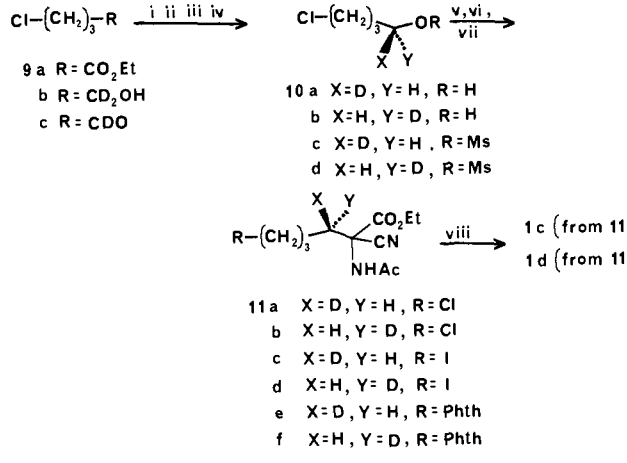
7 a R = H
b R = (o-carbomethoxybenzoyl)

8 a R = H
b R = D

3a or 3e

[a] Reagents: (i) $SOCl_2$; (ii) ethyl acetoacetate/$Mg(OEt)_2$/ether; (iii) 10% NH_4OH/ether, 10 °C; (iv) NH_3, EtOH, 25 °C, 15 h; (v) *o*-(carbomethoxy)benzoyl chloride, pyridine/$CHCl_3$ (1:2), 60 °C, 12 h; (vi) H_2 or D_2, $(Ph_3P)_3RhCl$, 420 psi, 36 h, 25 °C; (vii) heat (neat) at 200 °C (0.3 atm), 3 h.

Scheme II. Synthesis of Stereospecifically Labeled (2*RS*,3*R*)-Lysine-d_1 and (2*RS*,3*S*)-Lysine-d_1[a]



9 a R = CO_2Et
b R = CD_2OH
c R = CDO

10 a X = D, Y = H, R = H
b X = H, Y = D, R = H
c X = D, Y = H, R = Ms
d X = H, Y = D, R = Ms

11 a X = D, Y = H, R = Cl
b X = H, Y = D, R = Cl
c X = D, Y = H, R = I
d X = H, Y = D, R = I
e X = D, Y = H, R = Phth
f X = H, Y = D, R = Phth

1c (from 11e)
1d (from 11f)

[a] Reagents: (i) $LiAlD_4$, ether, −10 °C; (ii) pyridinium chlorochromate, CH_2Cl_2; (iii) (+)- or (−)-pinanyl-9-BBN; (iv) mesyl chloride, pyridine; (v) sodium salt of ethyl acetamidocyanoacetate, benzene, reflux; (vi) NaI, acetone, reflux 16 h; (vii) potassium phthalimide, neat, 140 °C 3.5 h; (viii) 6 N HCl, reflux, 15 h.

(18) Chemical shifts and couplings for **3a**: C-2H, δ_1 2.75, δ_2 3.23; C-3H, δ_3 4.70; J_{12} = 16 Hz, J_{13} = 6 Hz, J_{23} = 9 Hz.

(19) A very minor amount of absorption at δ 2.75 probably results from a little racemization in the rather vigorous phthalimido derivatization step; also a minor unidentified impurity appears at δ 4.03; this impurity did not appear in a second run.

(20) D,L-β-Lysine·HCl was synthesized by the method of Pelizzoni and Jommi.[21] A portion of the product was refluxed for 44 h with concentrated HCl (4 mL) + D_2O (1 mL). After isolation of the β-lysine by cation exchange, it was converted to **3** in the usual manner.

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(22) K. Kalenko, S. S.; Gracheva, N. A.; Chilikin, L. G. *Zh. Org. Khim.* **1973**, *9*, 1401–1404.

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(24) Product **7b** has a vinyl proton singlet at δ 5.10. Formulation of this product as the *Z* stereoisomer is based on extensive modeling studies in our laboratory, reported elsewhere: Aberhart, D. J.; Lin, H.-J. *J. Org. Chem.* **1981**, *46*, 3749–3751.

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(26) For an analogous reduction of a β-acylaminoacrylic ester see: Achiwa, K.; Soga, T. *Tetrahedron Lett.* **1978**, 1119–1120.

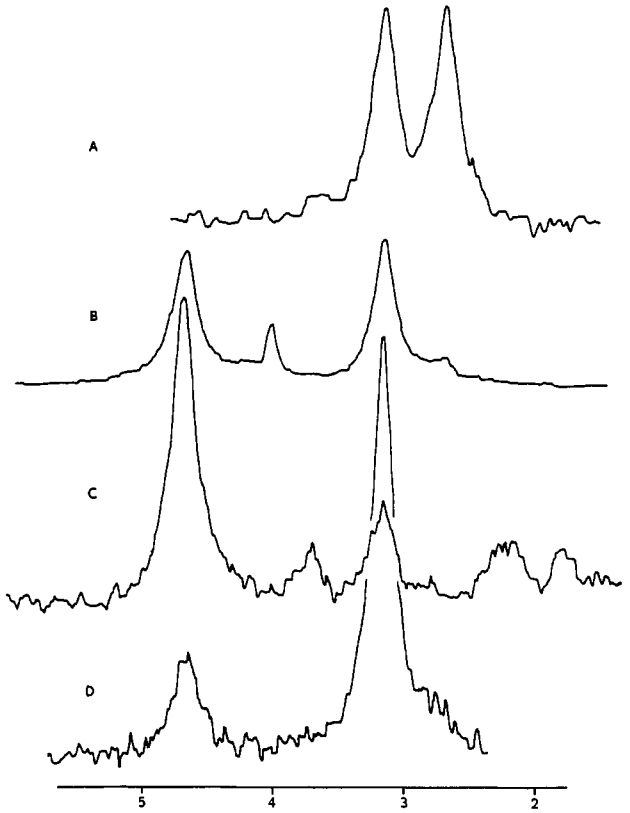
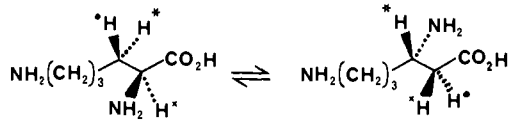


Figure 2. 41.44-MHz ^2H NMR spectra (Bruker HX270), 4000 data points, 90° pulse, line broadening 0.5 Hz; samples in 1.5 mL of $CHCl_3$ with internal $CDCl_3$ reference, δ 7.27: (A) synthetic (2*RS*)-**3c**-d_1 + **3d**-d_1, 394 transients; (B) biosynthetic product **3b**, from **1b**, 1675 transients; (C) biosynthetic product **3f**, from *1d*, 1250 transients; (D) biosynthetic product **3c**, from **1c**, 4682 transients.

From this signal assignment, it therefore follows that the analogous β-lysine derivative produced biosynthetically from **1b** is labeled as in **3b**, since the C-2 *deuterium* resonance appeared at δ 3.18. Thus, transfer of deuterium from C-3 of α-lysine to C-2 of β-lysine proceeds with *inversion* of configuration at C-2.

We then turned our attention to the identification of the hydrogen (deuterium) atom transferred from C-3 to C-2. For this purpose (2*RS*, 3*R*)-lysine-*3*-d_1 (**1c**) and (2*RS*, 3*S*)-lysine-*3*-d_1 (**1d**) were synthesized (Scheme II). Ethyl 4-chlorobutyrate (**9a**) was reduced ($LiAlD_4$, ether, −10 °C) to 4-chloro-1-butanol-*1,1*-d_2 (**9b**) which was then oxidized (pyridinium chlorochromate[27]) to 4-chlorobutyraldehyde-*1*-d_1 (**9c**). This was reduced with either (+)- or (−)-pinanyl-9-BBN[28,29] to yield (1*S*)-4-chloro-1-butanol-*1*-d_1 (**10a**) or (*1R*)-4-chloro-1-butanol-*1*-d_1 (**10b**), respectively. The absolute configurations of **10a** and **10b** were assigned by NMR analysis of the corresponding (−)-camphanate esters, which also showed that their configurational purities were ca. 90%.[30] The alcohols were then converted to the corresponding mesylates, **10c** or **10d**, which, in turn, were treated with the sodium salt of ethyl acetamidocyanoacetate to give in moderate yield the condensation products **11a** or **11b**, mp 92–94 °C, respectively.[31] These were converted with NaI/acetone into the 6-iodo analogues, **11c** or **11d**, mp 94–95 °C, and thence with potassium phthalimide into the 6-phthalimido derivatives[32] **11e** or **11f**, respectively. Finally, acidic hydrolysis gave the required lysines **1c** and **1d**. Incubation of these as before with a cell-free extract from *Clostridium SB4* yielded, after workup, samples of di-*N*-phthaloyl-β-lysine ethyl ester whose deuterium NMR spectra (Figure 2C,D) establish that they are labeled primarily as shown in **3c** (from (3*R*)-lysine-d_1) and **3f** [from (3*S*)-lysine-d_1 (**1d**)]. The fact that both products show some deuterium at both C-2 (pro-*S*) and C-3 is probably a result of incomplete stereospecific labeling in the precursors. In any case it is clear that for the great majority of the product formed, the 3 pro-*R* hydrogen of α-lysine is transferred to C-2, and the 3-pro-*S* hydrogen retained at C-3. Thus, replacement of the transferred hydrogen by the amino group occurs with inversion of configuration at C-3, **12** ⇄ **13**. The stereochemical course of the lysine



12 **13**

2,3-aminomutase reaction thus parallels the cryptic stereochemistry elucidated for the coenzyme-B_{12}-dependent β-lysine mutase reaction in which the 6-amino group of L-β-lysine replaces the C-5 pro-*S* hydrogen to form (3*S*,5*S*)-3,5-diaminohexanoic acid with inversion of configuration at C-5.[33,34]

Acknowledgment. We are especially indebted to Dr. Stuart Shapiro for instructing us in the methods for handling anaerobic bacteria. We also thank Dr. S. S. Welankiwar for some preliminary experiments, Cliff McDonald for some of the ^1H NMR spectra, and 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. We also thank Dr. Steven J. Gould, University of Connecticut, for stimulating discussions and for agreeing to simultaneous publication of our results. This work was supported by Grant GM 25919 from the National Institute of General Medical Sciences.

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(31) Both **11a** and **11b** showed in the ^2H NMR spectra two partially resolved, broad singlets, δ_1 2.11, δ_2 2.26. The spectrum of **11b** showed the presence of minor impurities at δ 3.53 and δ 4.33 (total ca. 0.1 ^2H).
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Studies of Nitrogen Metabolism Using ^{13}C NMR Spectroscopy. 3. Synthesis of DL-[3-^{13}C,2-^{15}N]Lysine and Its Incorporation into Streptothricin F[1]

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One of the major questions we have addressed in our study of streptothricin F (**1**) biosynthesis[3,4] concerns the mechanism of β-lysine (**2**) formation. Evidence has been reported for incorporation of α-lysine (**3**) into the β-lysine portion of streptothricin,[3,5] viomycin,[6] and the polymycins.[7] In the last case, much of the

(1) This is part 3 in the series "Biosynthesis of Streptothricin F".
(2) Career Development Awardee of the National Cancer Institute (CA00627), 1979–1984.
(3) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *J. Am. Chem. Soc.* **1981**, *103*, 2871–2872.
(4) Gould, S. J.; Martinkus, K. J.; Tann, C.-H. *J. Am. Chem. Soc.* **1981**, *103*, 4639–4640.
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