J|A|C|S
ARTICLES
Published on Web 03/01/2002

Nonactin Biosynthesis: The Initial Committed Step Is the Condensation of Acetate (Malonate) and Succinate

Michael E. Nelson and Nigel D. Priestley*

Contribution from the Department of Chemistry, The University of Montana, Missoula, Montana 59812-1656

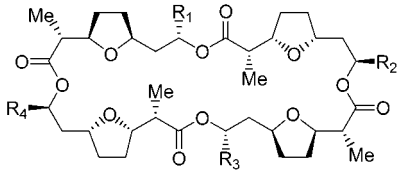
Received August 29, 2001

Abstract: Nonactin is a macrotetrolide antibiotic produced by *Streptomyces griseus* subsp. *griseus* ETH A7796 that has shown activity against the P170-glycoprotein efflux pump associated with multiple drug resistant cancer cells. Nonactin is a polyketide, albeit a highly atypical one. The structure is composed of two units of each of the enantiomers of nonactic acid, arranged in a macrocycle, so that the molecule has S4 symmetry and is achiral. The monomer units, (+)- and (−)-nonactic acid, are derived from acetate, succinate, and propionate, although the exact details of the assembly process are quite unclear. We have used feeding experiments with a series of multiple stable isotope labeled precursors to elucidate the details of the first committed step of nonactic acid biosynthesis. We have found that the ^{13}C label from 3-ketoadipate is incorporated specifically into both nonactic acid and its homologue, homononactic acid. The data conclusively show that the first committed step of nonactin biosynthesis is the coupling of a succinate derivative with either acetate or malonate. The differentiation into either nonactate or homononactate occurs after the initial condensation; the homologues are not derived from use of a different "starter unit" by the nonactate polyketide synthase. The first step of nonactin biosynthesis involves achiral intermediates; differentiation between the known enantiocomplementary biosynthesis pathways to form each enantiomer of the precursor monomer units likely occurs after the initial condensation reaction.

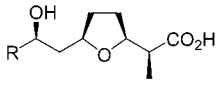
Introduction

Streptomyces griseus subsp. *griseus* ETH A7796 (DSM40695) produces a group of macrocyclic, ionophore antibiotics known as the macrotetrolides.[1−8] The parent compound, nonactin (Figure 1, **1**), has been shown to possess antitumor activity both against mammalian cell lines in vitro and against Crocker Sarcoma 180 in studies in mice.[1] Nonactin has also been shown to be an effective inhibitor of the 170-kDa-P-glycoprotein-mediated efflux of 4-*O*′-tetrahydropyranyldoxorubicin in multidrug resistant erythroleukemia K562 cells at subtoxic concentrations.[9] The macrotetrolide antibiotics also act as ionophores being effective against Gram positive bacteria, mycobacteria, and fungi.

The macrotetrolide homologues (Figure 1) are derived from the sequential substitution of ethyl groups for methyl groups on the macrocyclic backbone. The naturally occurring homologues exhibit a wide range of potency.[1,2] The minimum inhibitory concentration of nonactin against *Staphylococcus aureus* and *Mycobacterium bovis* is over an order of magnitude greater than that of dinactin; the differences in activity follow the differences in ion-binding affinity. The association constant for the K^+ complex of dinactin is 7-fold greater than that of nonactin.[10]



	R_1	R_2	R_3	R_4
Nonactin (**1**)	Me	Me	Me	Me
Monactin (**2**)	Me	Me	Et	Me
Dinactin (**3**)	Et	Me	Et	Me
Trinactin (**4**)	Et	Et	Et	Me
Tetranactin (**5**)	Et	Et	Et	Et



R = Me **6** (+)-nonactate

R = Et **7** (+)-homononactate

R = Pr **8** (+)-bishomononactate

Figure 1. Structures of the naturally occurring macrotetrolides and the monomer precursor acids.

(1) Bennett, R. E.; Brindle, S. A.; Giuffre, N. A.; Jackson, P. W.; Kowald, J.; Pansy, F. E.; Perlman, D.; Trejo, W. H. *Antimicrob. Agents Chemother.* **1961**, 169−172.
(2) Corbaz, R.; Ettinger, L.; Gaumann, E.; Keller-Schlierlein, W.; Kradolfer, F.; Kyburz, E.; Neipp, L.; Prelog, V.; Zahner, H. *Helv. Chim. Acta* **1955**, *38*, 1445.
(3) Dobler, M. *Helv. Chim. Acta* **1972**, *55*, 1371−1384.
(4) Dutcher, J. D. *Antimicrob. Agents Chemother.* **1961**, *173*−*177*.
(5) Gerlach, H.; Hutter, R.; Keller-Schlierlein, W.; Seibl, J.; Zahner, H. *Helv. Chim. Acta* **1967**, *50*, 1782−1793.
(6) Keller-Schierlein, W.; Gerlach, H. *Fortschr. Chem. Org. Naturst.* **1968**, *26*, 161−189.
(7) Menshikov, G. P.; Rubinsthein, M. M. *J. Gen. Chem. USSR* **1956**, *26*, 2267.
(8) Meyers, E.; Pansy, F. E.; Perlman, D.; Smith, D. A.; Weisenborn, F. L. *J. Antibiot.* **1965**, *18*, 128.
(9) Borrel, M. N.; Pereira, E.; Fiallo, M.; Garnier-Suillerot, A. *Eur. J. Biochem.* **1994**, *223*, 125−133.

10.1021/ja016965f CCC: $22.00

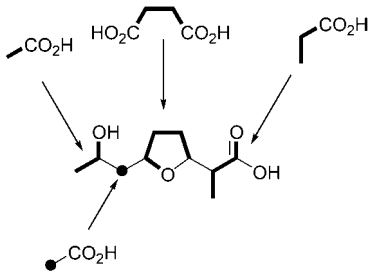


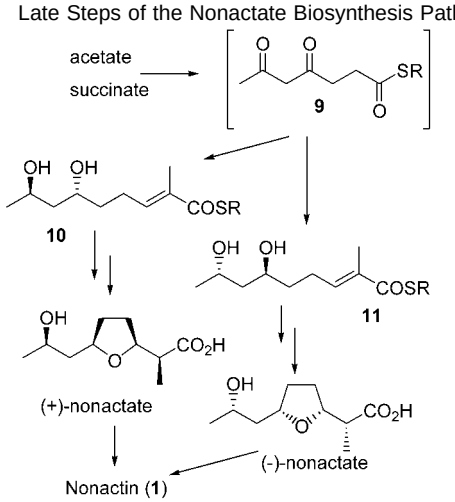
Figure 2. Biosynthesis origin of the atoms in the nonactate monomer.

The generic macrotetrolide structure is a cyclic tetraester composed of monomers of either nonactate **6**, homononactate **7**, or bishomononactate **8** (Figure 1). For example, the 32-membered macrocyclic ring of nonactin is composed of two units of (+)-nonactate and two units of (−)-nonactate, joined alternately (+)−(−)−(+)−(−) around the macrocycle so that the whole structure has S4 symmetry and is, therefore, achiral.

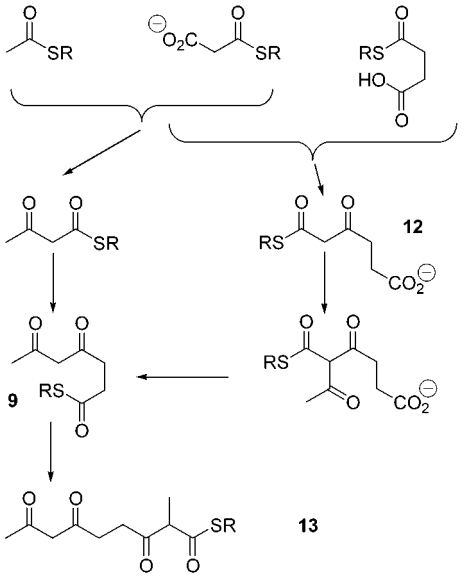
Resolving the details of macrotetrolide biosynthesis is a significant task as any credible hypothesis has to account for the production of both enantiomers of the precursor monomer units (**6**−**8**). Further, the incorporation of a succinate into the precursor backbone is an unusual twist on conventional polyketide biosynthesis.

Initial biosynthesis studies utilized ^{14}C-labeled compounds and established that acetate, propionate, and succinate were the primary metabolic precursors of the macrotetrolides.[11−13] The early work was confirmed and greatly extended by Robinson and co-workers who used extensive feeding studies with both stable and radiolabeled compounds (Figure 2).[14−19] Robinson postulated a pathway for nonactate biosynthesis that was based upon polyketide biosynthesis and demonstrated the highly unusual nature of the pathway. The late steps of nonactate biosynthesis were confirmed by Robinson and Spavold who synthesized the acyclic, late intermediates **10** and **11** (Scheme 1). The compounds were enantioselectively incorporated into nonactin; **10** was converted into (+)-nonactate, and **11** was converted into (−)-nonactate. The data showed that the late steps of nonactate biosynthesis occur via a pair of enantiocomplementary pathways.[19] To this date, however, the details of the conversion of the primary metabolites acetate, propionate, and succinate into **10** and **11** remain unconfirmed. Robinson and co-workers had proposed a pathway in which the coupling of an acetate (or malonate) with an acetate to generate acetoacetate was the first committed step (Scheme 2). The acetoacetate was then coupled to a succinate whereupon a rather unusual rearrangement reaction involving a decarboxylation step affords the diketoheptanoate **9**. It was originally thought that differentiation into the pair of enantiocomplementary pathways would occur quite late in the overall pathway; extension with methylmalonate, following typical polyketide chemistry, would generate the achiral intermediate **13** (Scheme 2). The enantiocomplementary pathways were believed to branch at this point, dependent upon the specificity of ketoreductase enzymes. The reductive steps, together with an elimination of water, would generate the confirmed intermediates **10** and **11**. The pathway was consistent with all the data derived from direct incorporation of labeled acetate, propionate, and succinate. One difficulty, noted by Robinson, was in the interpretation of data obtained in a feeding study with ethyl [1,2,3,4-^{13}C$_4$]acetoacetate.[16,18] While observing no direct, intact incorporation of label into the expected positions, labeling of atoms derived from propionate and methylmalonate was observed. Robinson postulated that the acetoacetate was first reduced to butyrate. The action of a coenzyme B_{12}-dependent mutase would convert the butyrate into isobutyrate; subsequent oxidation would generate methylmalonate, thereby explaining the observed incorporation of label into positions of nonactate formally derived from propionate and methylmalonate. The indirect result, based upon a lack of

Scheme 1. Late Steps of the Nonactate Biosynthesis Pathway

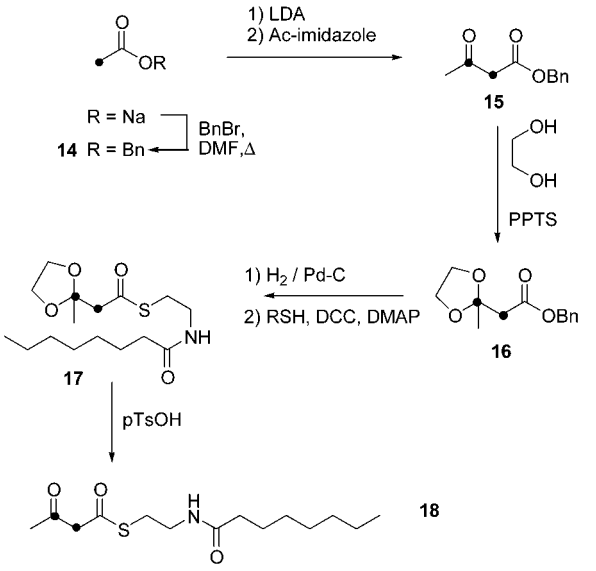


Scheme 2. Early Steps of the Nonactate Biosynthesis Pathway Showing the Two Alternate Hypotheses of Robinson; Initial Coupling of a C2 Unit (Acetate or Malonate) with Either Acetate or Succinate

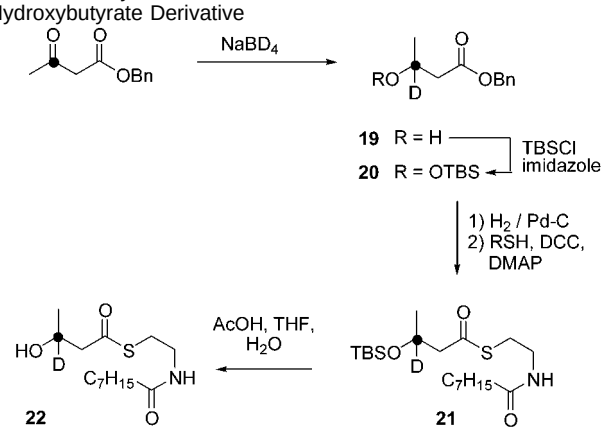


(10) Izatt, R. M.; Bradshaw, J. S.; Nielsen, S. A.; Lamb, J. D.; Christensen, J. J.; Sen, D. *Chem. Rev.* **1985**, *85*, 271−339.
(11) Pape, H. *Arch. Microbiol.* **1972**, *82*, 254−264.
(12) Stahl, P.; Pape, H. *Arch. Microbiol.* **1972**, *85*, 239−248.
(13) Stahl, P. O. *Untersuchungen zur Biosynthese der Macrotetrolide bei Streptomyces griseus*; Eberhard-Karls-Universitat: Tubingen, 1975.
(14) Ashworth, D. M.; Robinson, J. A.; Turner, D. L. *J. Chem. Soc., Chem. Commun.* **1982**, 491−493.
(15) Ashworth, D. M.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1983**, 1327−1329.
(16) Ashworth, D. M.; Robinson, J. A.; Turner, D. L. *J. Chem. Soc., Perkin Trans. 1* **1988**, 1719−1727.
(17) Ashworth, D. M.; Clark, C. A.; Robinson, J. A. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1461−1467.
(18) Clark, C. A.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1985**, 1568−1569.
(19) Spavold, Z. M.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 4−6.

Scheme 3. Synthesis of a ^{13}C-Labeled, Thioester-Activated Acetoacetate Derivative



Scheme 4. Synthesis of a $^{13}C/^{2}H$-Labeled, Thioester-Activated Hydroxybutyrate Derivative



direct incorporation, argued against Robinson's first biosynthesis hypothesis. Robinson suggested that if the lack of direct incorporation of acetoacetate were not due to the specificity of a nonactate polyketide synthase then an alternative pathway for the initial stages of nonactate biosynthesis might be operative. The latter pathway, as proposed by Robinson, was based upon the coupling of an acetate (or malonate) with succinate as the first committed step (Scheme 2). Intermediate **12**, formed by such a condensation, in one of a number of possible pathways, would then be acylated with either acetate (to eventually form nonactate, **6**) or propionate (to eventually form homononactate, **7**). After acylation, transposition of the thioester would allow for spontaneous decarboxylation generating **9**, a central intermediate in both of Robinson's hypotheses. The evidence for the latter pathway, however, was based upon indirect evidence − the lack of direct incorporation of label from ethyl acetoacetate. In this current report, we describe the synthesis and use of dual isotope-labeled, thioester-activated analogues of acetoacetate, hydroxybutyrate, and 3-ketoadipate (**12**). The incorporation of label, or lack thereof, from the compounds into nonactate **6** and homononactate **7** conclusively shows that Robinson's second hypothesis is operative in *S. griseus*. The coupling of acetate (or malonate) with succinate is the first committed step of nonactate biosynthesis.

Results and Discussion

Synthesis of a [2,3-$^{13}C_2$]Acetoacetate Thioester. We sought to synthesize a dual ^{13}C-labeled acetoacetate thioester to confirm Robinson's results from the ethyl acetoacetate feeding experiment. The thioester-activated target was chosen as it has been observed that incorporation into a polyketide of a putative, thioester-activated intermediate can be achieved even when the corresponding ester or acid failed to show incorporation.

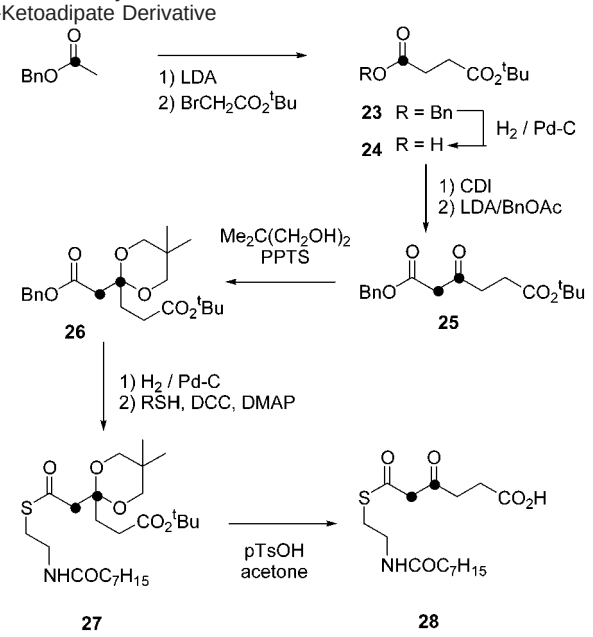
The synthesis of the labeled acetoacetate was based heavily upon chemistry we had previously used for the synthesis of [5,6-$^{13}C_2$]-4,6-diketoheptanoate.[20] Benzyl [2-$^{13}C_1$]acetate was prepared from commercially available sodium [2-$^{13}C_1$]acetate by reaction with benzyl bromide in DMF (Scheme 3). The corresponding enolate was generated at low temperatures and quenched with an imidazolide preformed from [1-$^{13}C_1$]acetic acid and 1,1-carbonyldiimidazole. The conditions used represent an optimized method in our hands; many variants of the coupling were evaluated subject to the constraints on reagent stoichiometry imposed by using two ^{13}C-labeled fragments. Currently, we obtain consistent yields between 50 and 75%. The ketone **15** was protected using standard conditions as a dioxolane derivative **16**, whereupon the free acid was unmasked by hydrogenolysis. Standard coupling to *N*-caprylcysteamine gave **17**. Deprotection of the ketone gave the target **18** compound in 30% overall yield.

Synthesis of a [3-$^{2}H_1$,3-$^{13}C_1$]Hydroxybutyrate Thioester. It was conceivable that nonactate biosynthesis could be initiated with a thioester-activated hydroxybutyrate. We sought to synthesize [3-$^{13}C_1$,3-$^{2}H_1$]hydroxybutyrate activated as its *N*-caprylcysteamine thioester **22** to investigate the possibility (Scheme 4). The dual label pattern was chosen to determine if any observed ^{13}C-label incorporation was due to oxidation of **22** to acetoacetate. Such an oxidation would separate the ^{13}C and ^{2}H labels. Generation of the appropriately labeled benzyl acetoacetate was accomplished using lithium enolate chemistry. Reduction of benzyl [3-$^{13}C_1$]acetoacetate with $NaBD_4$ readily generated the racemic hydroxybutyrate, **19**. Protection of the alcohol as the *tert*-butyldimethylsilyl ether (**20**), replacement of the benzyl ester with the thioester (**21**), and liberation of the target compound (**22**) proceeded relatively uneventfully to give the labeled target in approximately 37% overall yield.

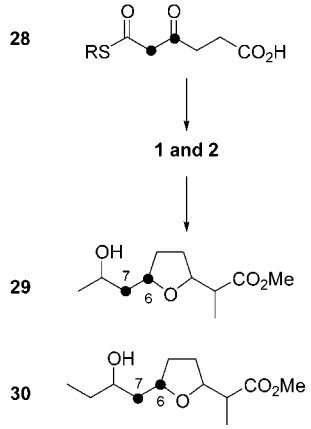
Synthesis of a [2,3-$^{13}C_2$]-3-Ketoadipate Thioester. The 3-ketoadipate derivative **28** (Scheme 5) was chosen as a target as the compound is the product of a first biosynthesis step coupling of acetate (or malonate) with succinate, that is, the first intermediate on the alternative pathway proposed by Robinson. The route to **28** required an unsymmetrically ^{13}C-labeled succinate derivative. Labeled succinate derivatives are available but usually have a symmetrical disposition of the ^{13}C-labels. The labeling in the succinate in our work would remain unscrambled by judicious choice of protecting groups for the carboxyl groups. The enolate derived from benzyl [1-$^{13}C_1$]-acetate was quenched with unlabeled *tert*-butyl bromoacetate to afford the succinate diester **23**. The benzyl group was removed by hydrogenolysis, and the free carboxyl group (**24**) was activated as an imidazolide. The electrophile so generated was used to quench an enolate generated from benzyl [2-$^{13}C_1$]-acetate. In this manner, the contiguous, dual ^{13}C labels were

(20) Nelson, M. E.; Derrer, A.; Priestley, N. D. 1999, unpublished work.

Scheme 5. Synthesis of a ^{13}C-Labeled, Thioester-Activated 3-Ketoadipate Derivative



Scheme 6. Interpretation of Observed ^{13}C-Label Incorporation[a]



[a] The dual label is incorporated intact into nonactin and monactin and is observed, after degradation and derivatization, in the methyl ester derivatives of nonactate **29** and homononactate **30**.

introduced into **25**. Protection of the ketone **25** as a dioxane proved surprisingly problematic. Reaction rates were quite slow with a number of 1,3- and 1,2-diols; attempts to force the reaction caused extensive decomposition presumably through loss of the *tert*-butyl ester under the acidic conditions. Using bis-silyl ether diol derivatives with TMSOTf as catalyst also proved ineffective. Eventually, reaction at reflux for 20 h with neopentyl glycol using PPTS as catalyst proved to be the most efficient procedure. Replacement of the benzyl ester with the thioester was accomplished as described for our other syntheses (**20** to **21**). Removal of the dioxane and *tert*-butyl ester protecting groups was accomplished by reflux with pTsOH in acetone to afford the target molecule in 4% overall yield.

General Feeding Study Protocol and Analysis Procedures. The labeled compounds were administered in ethanol solution to fermentative cultures of *S. griseus* at the onset of macrotetrolide production. Care was taken to ensure that the sample of *S. griseus* used was capable of the synthesis of macrotetrolides. Any strain that produced less than 250 mg L^{-1} of macrotetrolides in control culture was considered to be compromised, and a fresh set of spore suspensions was prepared from our original standard culture. In this manner, yields of macrotetrolides of 1000−3000 mg L^{-1} could be consistently achieved. At 48 h following administration of the labeled compound, the macrotetrolides were recovered by acetone extraction of the culture cell paste.[16,17] A preliminary fractionation of the extract to obtain a macrotetrolide sample was achieved by chromatography on silica gel. Screening of the preliminary mixture for ^{13}C-label incorporation was done by ^{13}C NMR. The macrotetrolide fraction, being mainly a mixture of nonactin and monactin, was subjected to methanolysis to afford a mixture of methyl nonactate **29** and methyl homononactate **30** (Scheme 6); the esters were readily separable by chromatography on silica gel.[16,17] Analysis by ^{13}C NMR was carried out on the cleaner, less complicated, methyl ester samples. The procedures, of course, generate mixtures of the enantiomers of **29** and **30**. Further analysis of the incorporation of label into the individual enantiomers was not carried out as the compounds fed were either achiral (**18** and **28**) or racemic (**22**), and we logically assume that no discrimination between enantiocomplementary biosynthesis pathways occurs.

Feeding Results. In the case of the thioesters of acetoacetate **18** and hydroxybutyrate **22**, no dual ^{13}C-^{13}C label incorporation, in the former case, or ^{2}H-^{13}C dual label incorporation, in the latter case, was observed in either nonactate or homononactate. No significant single ^{13}C enrichments were observed in either case. The amount of compound fed was lower than that used by Robinson. The indirect incorporation of label from ethyl [1,2,3,4-$^{13}C_4$]acetoacetate observed by Robinson was obtained in part because of the relatively large sample fed and the presumed slow, continuous release of acetoacetate from the ethyl ester by nonspecific hydrolysis.

In the case of the thioester of [2,3-$^{13}C_2$]-3-ketoadipate **28**, however, the results were strikingly different. In the ^{13}C NMR spectra of *both* methyl nonactate and methyl homononactate clear doublets arising from ^{13}C-^{13}C coupling were observed at positions assigned to C6 and C7 (Figure 3). The ^{13}C-^{13}C dual label of the precursor is incorporated intact. The position of label incorporation is entirely consistent with Robinson's alternate biosynthesis hypothesis, but unlike the previous results of the indirect ^{13}C incorporation from acetoacetate, the current data support *direct* incorporation of the label. The data support the notion that the first committed step of nonactate biosynthesis is the coupling of an acetate (or malonate) unit with succinate to generate 3-ketoadipate (**12**; Scheme 2). The anomalous incorporation derived from ethyl acetoacetate observed by Robinson, and the lack of incorporation from the acetoacetate and hydroxybutyrate thioesters, while being negative evidence, also support the latter pathway. Because label from 3-ketoadipate is found in both nonactate and homononactate, it is evident that the differentiation of the pathways to form the homologues occurs after the first committed step. Naturally, acylation of the 3-ketoadipate with acetyl-CoA would lead to nonactate; acylation with propionyl-CoA would lead to homononactate. The synthesis of nonactate and homononactate is not controlled, therefore, by a choice of either acetate or propionate as a "starter unit" by the nonactate polyketide synthase. The divergence of the biosynthesis pathways to form both enantiomers of nonactate most likely occurs after the first committed step as the intermediate **12** is achiral. The result, therefore, has great

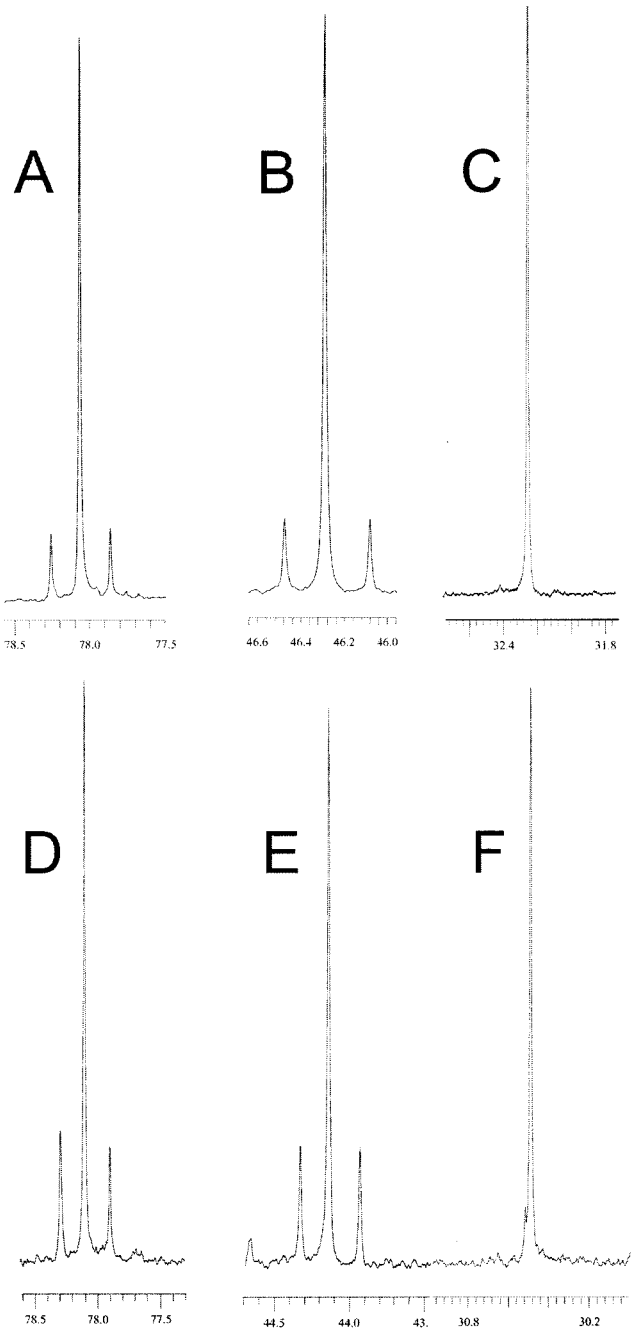


Figure 3. Expansions of the ^{13}C NMR spectra of methyl nonactate **29** and methyl homononactate **30** derived from macrotetrolides isolated from a culture to which **28** had been added. A: C-6 of **29** (0.26 ± 0.02% specific incorporation; 1.4 ± 0.1% absolute incorporation). B: C-7 of **29** (0.30 ± 0.02% specific; 1.6 ± 0.1% absolute incorporation). C: C-5 of **29** (peak with no enrichment shown for comparison). D: C-6 of **30** (0.51 ± 0.04% specific; 1.4 ± 0.1% absolute incorporation). E: C-7 of **30** (0.43 ± 0.04% specific; 1.2 ± 0.1% absolute incorporation). F: C-5 of **30** (peak with no enrichment shown for comparison).

implication for hypotheses which relate the deduced functions of gene products of the nonactin biosynthesis gene cluster to chemical conversions occurring in the biosynthesis pathway.[21−24]

In conclusion, feeding experiments with three ^{13}C-labeled, thioester-activated potential precursors have been used to demonstrate that the first committed step of macrotetrolide biosynthesis is the coupling of a succinate unit with an acetate (or malonate). Differentiation to form the homologues of nonactate occurs after the initial step potentially by acylation of the β-ketothioester product. Partition between the enantiocomplementary pathways most likely occurs after the initial committed step.

Experimental Section

General. Chemical Synthesis. Solvents were obtained from Fisher Scientific. All other chemicals, unless noted otherwise, were obtained from the Aldrich Chemical Co. (Milwaukee, WI). The solvents CH_2Cl_2 (over CaH_2), diethyl ether (over Na/K-benzophenone), and THF (over Na/K-benzophenone) were dried and distilled prior to use. $MgSO_4$ refers exclusively to anhydrous $MgSO_4$. Solutions were concentrated by evaporation in vacuo. All synthesis procedures, unless noted otherwise, were carried out under a slight positive pressure of dry argon gas. Column chromatography was performed using Merck Silica Gel 60. NMR spectra were acquired at 400 MHz (^1H) and 100 MHz (^{13}C), were referenced to the residual solvent, and are reported as chemical shift (δ/ppm), splitting pattern, coupling constant (J/Hz), and intensity.

General. Strains. *Streptomyces griseus* subsp. *griseus* ETH A7796 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM 40695). *S. griseus* was grown in 2 × TSB liquid medium[25] or on R2YE solid medium.[25] For macrotetrolide production, *S. griseus* was propagated by previously reported methods.[26] Macrotetrolides were isolated as described previously.[26] In each of the feeding experiments, 100 mg of a 1:1 mixture of labeled compound and its unlabeled counterpart were added to a 250 mL shake flask fermentative culture of *S. griseus.*

Benzyl Acetate (14). Benzyl bromide (5.9 mL, 50 mmol) was added via syringe to a suspension of sodium acetate (2.0 g, 24 mmol) in DMF (50 mL). The stirred mixture was heated at 96 °C for 3 days. Water (200 mL) and Et_2O (300 mL) were added to the cooled mixture, mixed, and the organic layer was recovered. The water fraction was further extracted with Et_2O (2 × 300 mL). The pooled Et_2O fractions were dried and concentrated, and the product was purified by chromatography on silica gel, eluting with EtOAc−hexanes (9:1), to give **14** (3.5 g, 94.8%), which was shown to be identical to authentic material.

Benzyl [2-$^{13}C_1$]Acetate. This was prepared according to the method for the unlabeled material from benzyl bromide and sodium [2-$^{13}C_1$]-acetate (4.86 g, 89.1%). ^1H NMR (400 MHz, $CDCl_3$): δ 2.08 (3H, d, J_{1H-13C} = 134.5 Hz), 5.10 (2H, s), 7.30 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.1, 66.5, 128.5, 128.8, 136.3, 171.0 (d, $J_{13C-13C}$ = 59.4 Hz).

Benzyl 3-Oxobutanoate (15). Acetic acid (0.95 mL, 16.4 mmol) was added to a stirred suspension of CDI (2.7 g, 16.6 mmol) in dry THF (16 mL). Gas was evolved, and the CDI was dissolved over 10 min at room temperature to generate a solution of the imidazolide. *n*-BuLi (35.3 mmol) in hexanes (25 mL) was added dropwise to a solution of diisopropylamine (5.4 mL, 38.4 mmol) in dry THF (30 mL) and stirred in a dry ice−acetone bath until the temperature reached −60 °C. Benzyl acetate (4.8 mL, 33.2 mmol) was added via syringe, maintaining the temperature below −20 °C, and then stirred until the reaction temperature fell to −60 °C. The acetylimidazole solution was transferred directly into the reaction vessel, again maintaining the temperature below −20 °C. The reaction was stirred for 1 min,

(21) Smith, W. C.; Xiang, L.; Shen, B. *Antimicrob. Agents Chemother.* **2000**, *44*, 1809−1817.
(22) Walczak, R. J.; Woo, A. J.; Strohl, W. R.; Priestley, N. D. *FEMS Lett.* **2000**, *183*, 171−175.
(23) Woo, A. J.; Strohl, W. R.; Priestley, N. D. *Antimicrob. Agents Chemother.* **1999**, *43*, 1662−1668.
(24) Kwon, H.-J.; Smith, W. C.; Xiang, L.; Shen, B. *J. Am. Chem. Soc.* **2001**, *123*, 3385−3386.
(25) Kieser, T.; Bibb, M. J.; Buttner, M. J.; Chater, K. F.; Hopwood, D. A. *Practical Streptomyces Genetics*; The John Innes Foundation: Norwich, 2000.
(26) Priestley, N. D. *Studies on Enzymes Involved in the Primary and Secondary Metabolism of Antibiotic Producing Streptomyces*; Southampton University: Southampton, 1991.

quenched by addition of water (100 mL), acidified to pH 4 with concentrated HCl, and then partitioned between water and $CHCl_3$ (200 mL). The aqueous portion was washed with $CHCl_3$ (2 × 200 mL), and the pooled organic portions were dried with Na_2SO_4, filtered, and concentrated. The product was recovered by chromatography on silica gel, eluting with EtOAc−hexanes (15:85), as an oil (2.1 g, 66.4%), which was shown to be identical to authentic material.

Benzyl [3-$^{13}C_1$]-3-Oxobutanoate. This was prepared as was the unlabeled material from [1-$^{13}C_1$]acetic acid (2.6 g, 82.1%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.24 (3H, d, J_{1H-13C} = 6.4 Hz), 3.49 (2H, d, J_{1H-13C} = 6.4 Hz), 5.1 (2H, s), 7.35 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.15 (d, $J_{13C-13C}$ = 42.7 Hz), 50.0 (d, $J_{13C-13C}$ = 36.7 Hz), 67.1, 128.35, 128.5, 128.6, 135.2, 166.9 (enol, enriched), 175.9, 200.4 (keto, enriched).

Benzyl (2-Methyl-1,3-dioxolan-2-yl)acetate (16). Ketoester **15** (0.94 g, 4.9 mmol), ethylene glycol (0.86 mL, 15.4 mmol), and PPTS (0.32 g, 1.3 mmol) were dissolved in benzene (50 mL), and the mixture was heated at reflux for 11 h with azeotropic removal of water by means of a Dean−Stark trap. The mixture was concentrated and suspended in Et_2O (100 mL) and washed first with aqueous saturated $NaHCO_3$ solution (100 mL) and then brine (100 mL). The organic phase was dried, filtered, and concentrated; the dioxolane product was then recovered by chromatography on silica gel, eluting with EtOAc−hexanes (1:4), as an oil (1.02 g, 88.2%). IR (liquid film): 1736 cm^{-1}. UV (acetonitrile): 257 (ϵ = 190 dm^3 mol^{-1} cm^{-1}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (3H, s), 2.71 (2H, s), 3.92 (4H, m), 5.13 (2H, s), 7.35 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.5, 44.1, 64,7, 66.3, 107.5, 128.10, 128.2, 128.4, 135.4, 169.2. ESI−HRMS calcd for $C_{13}H_{16}O_4$Na $[M + Na]^+$, 259.0946; found, 259.0957. Anal. Calcd for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83. Found: C, 65.86; H, 6.87.

Benzyl [2-$^{13}C_1$]-([2-$^{13}C_1$]-2-Methyl-1,3-dioxolan-2-yl)acetate. This was prepared as described for the unlabeled material (0.86 g, 81.1%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.49 (3H, dd, J_{1H-13C} = 4.5, 2.6 Hz), 2.72 (3H, dd, J_{1H-13C} = 130.0, 5.8 Hz), 3.94 (4H, m), 5.14 (2H, s), 7.36 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.5 (dt, $J_{13C-13C}$ = 24.4, 6.1 Hz), 44.1 (enriched, d, $J_{13C-13C}$ = 44.3 Hz), 64.7, 66.3, 107.5 (enriched, d, $J_{13C-13C}$ = 42.7 Hz), 128.1, 128.2, 128.4, 135.4, 169.3 (t, $J_{13C-13C}$ = 27.5 Hz).

***S*-[2-(Octanoylamino)ethyl] (2-Methyl-1,3-dioxolan-2-yl)ethanethioate (17).** Pd on carbon catalyst (10%, 0.10 g) was added to a stirred solution of **16** (1.02 g, 4.3 mmol) in THF (22 mL) under an atmosphere of Ar. The Ar was replaced by H_2 (balloon), and the suspension was stirred overnight at room temperature. Celite was added, and the suspension was filtered. The Celite was washed with $CHCl_3$ (100 mL). The combined organic fractions were concentrated and then dissolved in dry CH_2Cl_2 (34 mL). *N*-Caprylcysteamine (1.89 g, 9.3 mmol), DCC (2.27 g, 11.0 mmol), and DMAP (0.13 g, 1.1 mmol) were added, and the suspension was stirred at room temperature for 8 h. Oxalic acid (0.5 g, 5.6 mmol) was added, and the mixture was concentrated, resuspended in Et_2O, and the solid matter was removed by filtration. The target thioester was recovered by chromatography on silica gel, eluting with EtOAc−hexanes (3:2), as an oil (1.02 g, 71.5%). IR (liquid film): 3293, 1691, 1646, 1546 cm^{-1}. UV (acetonitrile): 235 nm (ϵ = 2383 dm^3 mol^{-1} cm^{-1}). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, t, J_{1H-1H} = 6.4 Hz), 1.25 (8H, m), 1.44 (3H, s), 1.57 (2H, m), 2.11 (2H, t, J_{1H-1H} = 8.0 Hz), 2.88 (2H, s), 3.01 (2H, t, J_{1H-1H} = 6.4 Hz), 3.41 (2H, dt, J_{1H-1H} = 6.0, 6.4 Hz), 3.95 (4H, s), 5.84 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.5, 24.6, 25.6, 28.9, 28.9, 29.2, 31.6, 36.6, 39.3, 52.5, 64.7, 107.4, 173.3, 195.5. ESI−HRMS calcd for $C_{16}H_{29}NO_4SNa$ $[M + Na]^+$, 354.1715; found, 354.1716. Anal. Calcd for $C_{16}H_{29}NO_4SNa$: C, 58.8; H, 7.9; N, 4.2; S, 9.7. Found: C, 58.08; H, 8.90; N, 4.44; S, 9.44.

***S*-[2-(Octanoylamino)ethyl] [2-$^{13}C_1$]-([2-$^{13}C_1$]-2-Methyl-1,3-dioxolan-2-yl)ethanethioate.** This was prepared as described for the unlabeled material from benzyl [2-$^{13}C_1$]-([2-$^{13}C_1$]-2-methyl-1,3-dioxolan-2-yl)acetate (0.8 g, 69.3%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (3H, t, J_{1H-1H} = 7.1 Hz), 1.26 (8H, m), 1.46 (3H, dd, J_{1H-13C} = 4.5, 2.6 Hz), 1.59 (2H, m), 2.14 (2H, t, J_{1H-1H} = 7.8 Hz), 2.88 (2H, dd, J_{1H-13C} = 115.8, 5.2 Hz), 3.05 (2H, t, J_{1H-1H} = 7.1 Hz), 3.45 (2H, dt, J_{1H-1H} = 6.5, 5.8 Hz), 3.98 (4H, d, J_{1H-13C} = 3.2 Hz), 5.84 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.6, 24.6 (dt, $J_{13C-13C}$ = 22.9, 6.1 Hz), 25.6, 28.9, 29.0, 29.2, 31.7, 36.7, 39.4, 52.6, 64.8, 107.5 (enriched, d, $J_{13C-13C}$ = 42.7 Hz), 174.4, 195.7 (d, $J_{13C-13C}$ = 45.8 Hz).

***S*-[2-(Octanoylamino)ethyl] 3-Oxobutanethioate (18).** The ketal **17** (1.02 g, 3.1 mmol) and pTsOH (0.20 g, 1.1 mmol) were dissolved in acetone (30 mL), and the stirred mixture was heated at reflux for 18 h. The mixture was concentrated, resuspended in Et_2O−$CHCl_3$ (1:1, 200 mL), and washed with 5% (w/v) aqueous $NaHCO_3$ solution and then brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. The target compound was obtained by chromatography on silica gel, eluting with EtOAc−hexanes (3:2), as a white solid (0.68 g, 76.2%). IR (powder-ATR): 3283, 1714, 1684, 1627, 1561 cm^{-1}. UV (acetonitrile): 236 (ϵ = 3800 dm^3 mol^{-1} cm^{-1}), 281 nm (2300). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (3H, t, J_{1H-1H} = 6.8 Hz), 1.25 (8H, m), 1.58 (2H, m), 1.92 (1H, enol, s), 2.12 (0.67H, enol, t, J_{1H-1H} = 7.8 Hz), 2.13 (1.33H, keto, t, J_{1H-1H} = 7.9 Hz), 2.24 (2H, keto, s), 3.06 (0.67, enol, t, J_{1H-1H} = 6.0 Hz), 3.07 (1.33H, keto, t, J_{1H-1H} = 6.2 Hz), 3.45 (1.33H, keto, q, J_{1H-1H} = 6 Hz), 3.45 (0.67H, enol, q, J_{1H-1H} = 5.8 Hz), 3.68 (1.5H, keto, s), 5.44 (0.3H, enol, s), 5.94 (0.66H, keto, br s), 5.99 (0.33H, enol, br s), 12.57 (0.5H, enol-H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 21.0, 22.6, 25.6, 25.6, 27.7, 28.95, 29.2, 29.25, 30.4, 31.6, 36.6, 36.7, 38.9, 39.7, 58.0, 99.85, 173.4, 173.5, 173.95, 192.2, 194.3, 199.7. ESI−HRMS calcd for $C_{14}H_{26}NO_3S$ $[M + H]^+$, 288.1633; found, 288.1617. Anal. Calcd for $C_{14}H_{26}NO_3S$: C, 58.5; H, 8.77; N, 4.87; S, 11.16. Found: C, 58.40; H, 8.74; N, 4.82; S, 11.16.

***S*-[2-(Octanoylamino)ethyl] [2,3-$^{13}C_2$]-3-Oxobutanethioate.** This was prepared as described for the unlabeled material (0.65 g, 94.6%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (3H, t, J_{1H-1H} = 6.8 Hz), 1.25 (8H, m), 1.58 (2H, m), 1.92 (0.4H, enol, dd, J_{1H-13C} = 6.2, 3.8 Hz), 2.14 (2H, t, J_{1H-1H} = 7.3 Hz), 2.25 (2.6H, keto, dd, J_{1H-13C} = 6.0, 1.5 Hz), 3.07 (2H, t, J_{1H-1H} = 6.6 Hz), 3.44 (2H, q, J_{1H-1H} = 6.4 Hz), 3.67 (1.9H, keto, dd, J_{1H-13C} = 131.1, 6.2 Hz), 5.44 (0.1H, enol, ddd, J_{1H-13C} = 167.5, 4.4 Hz, J_{1H-1H} = 0.7 Hz), 5.91 (1H, b), 12.57 (0.1H, enol-H, t, J_{1H-13C} = 4.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.03, 21.02 (enol, dt, $J_{13C-13C}$ = 23.3, 6.9 Hz), 22.6, 25.6, 25.65, 27.7, 29.0, 29.2, 29.3, 30.4 (keto, dt, $J_{13C-13C}$ = 19.8, 14.12 Hz), 31.6, 36.6, 36.7, 38.9, 39.7, 58.0 (keto, enriched, d, $J_{13C-13C}$ = 36.6 Hz), 99.8 (enol, enriched, d, $J_{13C-13C}$ = 69.8 Hz), 173.4, 173.5, 173.9 (enol, enriched, d, $J_{13C-13C}$ = 69.4 Hz), 192.2 (enol, dt, $J_{13C-13C}$ = 22.9, 2.3 Hz), 194.3 (keto, t, $J_{13C-13C}$ = 30.9 Hz), 199.7 (keto, enriched, d, $J_{13C-13C}$ = 36.6 Hz).

Benzyl 3-Hydroxybutyrate (19). Acetoacetate **15** (2.1 g, 11.0 mmol) was dissolved in THF (110 mL) and water (22 mL) and placed in an ice bath. $NaBH_4$ (0.13 g, 3.3 mmol) was added and stirred for 30 min at 0 °C. More $NaBH_4$ (0.04 g, 1.1 mmol) was added every 20 min until no starting material was present (40 min total). The reaction was diluted with water (100 mL) and partitioned between water and Et_2O (3 × 200 mL). The pooled Et_2O portions were dried with $MgSO_4$, filtered, and concentrated. The product was recovered by chromatography on silica gel, eluting in EtOAc−hexanes (1:4), as an oil (1.63 g, 76.1%), which was shown to be identical to authentic material.

Benzyl [3-$^{13}C_1$,3-$^2H_1$]-3-Hydroxybutanoate. This was prepared as described for the unlabeled material from benzyl [3-$^{13}C_1$]-3-oxobutanoate (2.26 g, 85.7%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21 (3H, d, J_{1H-13C} = 4.4 Hz), 2.5 (2H, m), 2.82 (1H, s), 5.14 (2H, s), 7.35 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.3 (d, $J_{13C-13C}$ = 39.7 Hz), 42.7 (d, $J_{13C-13C}$ = 36.6 Hz), 63.9 (enriched, t, $J_{13C-13C}$ = 22.8 Hz), 67.5, 128.25, 128.4, 128.6, 135.55, 172.7.

Benzyl 3-{[*tert*-Butyl(dimethyl)silyl]oxy}butanoate (20). Alcohol **19** (1.63 g, 8.4 mmol), *tert*-butyldimethylsilyl chloride (2.37 g, 15.7 mmol), and imidazole (1.89 g, 27.7 mmol) were dissolved in dry DMF

(4 mL) and stirred for 18 h at room temperature. The mixture was partitioned between water (100 mL) and $CHCl_3$ (3 × 100 mL). The pooled $CHCl_3$ portions were dried with $MgSO_4$, filtered, and concentrated. The product was recovered by chromatography on silica gel, eluting with EtOAc−hexanes (5:95), as an oil (1.91 g, 73.8%). IR (liquid film): 1738 cm^{-1}. UV (acetonitrile): 258 nm (ϵ = 180 dm^3 mol^{-1} cm^{-1}). ^1H NMR (400 MHz, $CDCl_3$): δ 0.02 (3H, s), 0.05 (3H, s), 0.85 (9H, s), 1.19 (3H, d, J_{1H-1H} = 6.2 Hz), 2.42 (1H, dd, J_{1H-1H} = 9.38, 5.18 Hz), 2.53 (1H, dd, J_{1H-1H} = 6.8, 7.8 Hz), 4.3 (1H, m), 5.10 (2H, d, J_{1H-1H} = 5.5 Hz), 7.35 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.0, −4.5, 17.9, 23.9, 25.7, 44.85, 65.8, 66.15, 128.2, 128.2, 128.5, 135.9, 171.45. ESI−HRMS: calcd for $C_{17}H_{28}O_3NaSi$ $[M + Na]^+$, 331.1705; found, 331.1710. Anal. Calcd for $C_{17}H_{28}O_3Si$: C, 66.19; H, 9.15. Found: C, 66.29; H, 9.27.

Benzyl [3-$^{13}C_1$,3-$^2H_1$]-3-*tert*-Butyldimethylsilyloxybutanoate. This was prepared as described for unlabeled material (3.59 g, 100.0%). ^1H NMR (400 MHz, $CDCl_3$): δ 0.02 (3H, s), 0.05 (3H, s), 0.85 (9H, s), 1.18 (3H, d, J_{13C-1H} = 4.3 Hz), 2.41 (1H, dd, J_{13C-1H} = 3.8, J_{1H-1H} = 14.5 Hz), 2.52 (1H, dd, J_{13C-1H} = 6.2, J_{1H-1H} = 14.7 Hz), 5.10 (2H, d, J_{1H-1H} = 5.4 Hz), 7.34 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.0, −4.5, 17.9, 23.7 (d, $J_{13C-13C}$ = 38.9 Hz), 25.7, 44.7 (d, $J_{13C-13C}$ = 38.9 Hz), 65.4 (enriched, t, $J_{13C-13C}$ = 22.1 Hz), 66.1, 128.1, 128.2, 128.5, 135.9, 171.4.

***S*-[2-(Octanoylamino)ethyl] 3-{[*tert*-Butyl(dimethyl)silyl]oxy}butanoate (21).** Butanoate **20** (1.91 g, 6.2 mmol) was dissolved in dry THF (25 mL). Pd−C catalyst (10%, 0.19 g) was added and stirred under a H_2 atmosphere (balloon) for 2 h. The reaction was filtered through Celite, and the Celite was washed with $CHCl_3$ (150 mL). The mixture was concentrated and dissolved in dry CH_2Cl_2 (40 mL) together with *N*-caprylcysteamine (2.54 g, 12.5 mmol), DCC (2.47 g, 12 mmol), and DMAP (0.28 g, 2.3 mmol). The mixture was stirred at room temperature for 18 h. The suspension that formed was filtered, and the solid was washed with CH_2Cl_2 (100 mL). The filtrate was concentrated, resuspended in CH_2Cl_2 (20 mL), and filtered. The product was recovered by chromatography on silica gel, eluting with EtOAc−hexanes (35:65), as an oil (1.63 g, 62.6%). IR (neat): 3281, 1692, 1651 cm^{-1}. UV (acetonitrile): 233 nm (ϵ = 1000 dm^3 mol^{-1} cm^{-1}). ^1H NMR (400 MHz, $CDCl_3$): δ 0.01 (3H, s), 0.04 (3H, s), 0.84 (9H, s), 0.86 (3H, t, J_{1H-1H} = 6.6 Hz), 1.17 (3H, d, J_{1H-1H} = 6.1 Hz), 1.26 (8H, m), 1.59 (2H, m), 2.13 (2H, t, J_{1H-1H} = 7.4 Hz), 2.57 (1H, dd, J_{1H-1H} = 14.2, 4.9 Hz), 2.74 (1H, dd, J_{1H-1H} = 14.2, 10.6 Hz), 3.0 (2H, m), 3.42 (2H, m), 4.27 (1H, m), 5.80 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.1, −4.5, 14.0, 17.95, 22.6, 23.8, 25.6, 25.7, 28.7, 29.0, 29.2, 31.6, 36.7, 39.4, 53.8, 65.9, 173.25, 198.1. ESI−HRMS calcd for $C_{20}H_{42}NO_3SSi$ $[M + H]^+$, 404.2655; found, 404.2671. Anal. Calcd for $C_{20}H_{41}NO_3SSi$: C, 59.5; H, 10.24; N, 3.47; S, 7.94. Found: C, 59.38; H, 10.36; N, 3.49; S, 7.88.

***S*-[2-(Octanoylamino)ethyl] [3-$^{13}C_1$,3-$^2H_1$]-3-{*tert*-Butyldimethylsilyloxy}butanoate.** This was prepared as described for unlabeled material from benzyl [3-$^{13}C_1$,3-$^2H_1$]-3-*tert*-butyldimethylsilyloxybutanoate (2.58 g, 53.1%). ^1H NMR (400 MHz, $CDCl_3$): δ 0.02 (3H, s), 0.04 (3H, s), 0.85 (9H, s), 0.86 (3H, t, J_{1H-1H} = 6.8 Hz), 1.17 (3H, d, J_{1H-13C} = 4.2 Hz), 1.26 (8H, m), 1.59 (2H, m), 1.61 (1H, s), 2.13 (2H, t, J_{1H-1H} = 7.2 Hz), 2.57 (1H, dd, J_{13C-1H} = 3.7, J_{1H-1H} = 14.1 Hz), 2.74 (1H, dd, J_{13C-1H} = 6.0, J_{1H-1H} = 14.1 Hz), 3.0 (2H, m), 3.42 (2H, m), 5.77 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ −5.05, −4.5, 14.1, 18.0, 22.6, 23.7 (d, $J_{13C-13C}$ = 39.7 Hz), 25.7, 25.7, 31.7, 36.75, 39.4, 53.7 (d, $J_{13C-13C}$ = 38.2 Hz), 65.6 (enriched, t, $J_{13C-13C}$ = 21.3 Hz), 173.3, 198.1.

***S*-[2-(Octanoylamino)ethyl] 3-Hydroxybutanethioate (22).** Butanoate **21** (1.63 g, 3.9 mmol) was dissolved in THF (13 mL), water (13 mL), and AcOH (42 mL), and stirred at room temperature for 48 h. The mixture was partitioned between water (100 mL) and $CHCl_3$ (200 mL). The organic phase was recovered, dried with $MgSO_4$, filtered, and concentrated. The product was recovered by chromatography, eluting with EtOAc−hexanes (6:4), as a white solid (0.93 g, 82.8%). IR (powder-ATR): 3298, 1684, 1638 cm^{-1}. UV (acetonitrile): 233 nm (ϵ = 4700 dm^3 mol^{-1} cm^{-1}). ^1H NMR (400 MHz, $CDCl_3$): δ 0.84 (3H, t, J_{1H-1H} = 6.8 Hz), 1.2 (3H, t, J_{1H-1H} = 6.4 Hz), 1.24 (8H, m), 1.57 (2H, m), 2.12 (2H, t, J_{1H-1H} = 7.5 Hz), 2.69 (2H, m), 3.01 (2H, dt, J_{1H-1H} = 5.9, 1.5 Hz), 3.02 (1H, br s), 3.42 (2H, dq, J_{1H-1H} = 6.2, 1.8 Hz), 4.22 (1H, m), 6.01 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.5, 22.65, 25.6, 28.8, 28.9, 29.2, 31.3, 36.6, 38.95, 52.45, 64.9, 173.6, 199.3. ESI−HRMS calcd for $C_{14}H_{27}NO_3SNa$ $[M + Na]^+$, 312.1609; found, 312.1590. Anal. Calcd for $C_{14}H_{27}NO_3S$: C, 58.1; H, 9.4; N, 4.84; S, 11.08. Found: C, 58.18; H, 9.42; N, 4.73; S, 11.02.

***S*-[2-(Octanoylamino)ethyl] [3-$^{13}C_1$,3-$^2H_1$]-3-Hydroxybutanethioate.** This was prepared as described for unlabeled material from *S*-[2-(octanoylamino)ethyl] [3-$^{13}C_1$,3-$^2H_1$]-3-{*tert*-butyldimethylsilyloxy}-butanoate (1.45 g, 81.6%). ^1H NMR (400 MHz, $CDCl_3$): δ 0.85 (3H, t, J_{1H-1H} = 6.8 Hz), 1.2 (3H, d, J_{1H-13C} = 4.5 Hz), 1.25 (8H, m), 1.57 (2H, m), 2.13 (2H, t, J_{1H-1H} = 7.5 Hz), 2.69 (2H, m), 2.92 (1H, s), 3.01 (2H, dt, J_{1H-1H} = 6.0, 1.6 Hz), 3.42 (2H, dq, J_{1H-1H} = 6.2, 2.1 Hz), 5.94 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.5 (d, $J_{13C-13C}$ = 38.9 Hz), 22.6, 25.6, 28.8, 28.95, 29.2, 31.7, 36.65, 39.0, 52.3 (d, $J_{13C-13C}$ = 35.9 Hz), 64.6 (enriched, t, $J_{13C-13C}$ = 22.1 Hz), 173.6, 199.3.

1-Benzyl 4-*tert*-Butyl Succinate (23). A clean, dry three-neck flask equipped with an addition funnel was charged with dry THF (60 mL) and diisopropylamine (4.8 mL, 29.2 mmol) and cooled in a dry ice−acetone bath. *n*-Butyllithium (29.2 mmol) in hexanes (11.4 mL) was added via syringe and stirred until the temperature dropped below −70 °C. Benzyl acetate (4.2 mL, 29.1 mmol) in dry THF (30 mL) was added dropwise to the reaction flask, maintaining the temperature below −70 °C. When complete, the flask was transferred to a liquid N_2−EtOH slush bath and stirred until the temperature dropped below −100 °C, while the addition funnel was charged with *tert*-butyl 2-bromoacetate (7 mL, 43.4 mmol) in THF (30 mL). The bromide solution was added dropwise to the reaction, maintaining the temperature below −100 °C, then stirred for 30 min at −100 °C. The reaction was quenched by addition of water (100 mL) and allowed to warm to room temperature. The mixture was partitioned between water (200 mL) and Et_2O (200 mL). The organic portion was dried with $MgSO_4$, filtered, and concentrated. The product was recovered by chromatography on silica gel, eluting with EtOAc−hexanes (5:95), as an oil (5.2 g, 67.6%). IR (liquid film): 1727 cm^{-1}. UV (acetonitrile): 258 nm (ϵ = 180 dm^3 mol^{-1} cm^{-1}). ^1H NMR (400 MHz, $CDCl_3$): δ 1.42 (9H, s), 2.59 (4H, m), 5.12 (2H, s), 7.34 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0, 29.4, 30.3, 66.4, 80.7, 128.2, 128.5, 135.8, 171.4, 172.25. ESI−HRMS calcd for $C_{15}H_{20}O_4Na$ $[M + Na]^+$, 287.1259; found, 287.1260. Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 67.80; H, 7.65.

1-Benzyl 4-*tert*-Butyl [1-$^{13}C_1$]Succinate. This was prepared as described for the unlabeled material from benzyl [1-$^{13}C_1$]acetate (4.74 g, 58.6%). ^1H NMR (400 MHz, $CDCl_3$): δ 1.41 (9H, s), 2.58 (4H, m), 5.12 (2H, d, J_{1H-13C} = 3.1 Hz), 7.34 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0, 29.3 (d, $J_{13C-13C}$ = 58.4 Hz), 30.3 (d, $J_{13C-13C}$ = 1.5 Hz), 66.4 (d, $J_{13C-13C}$ = 2.5 Hz), 80.7, 128.2, 128.5, 138.8 (d, $J_{13C-13C}$ = 2.3 Hz), 171.4, 172.3 (enriched).

***tert*-Butyl Succinate (24).** Succinate **23** (5.2 g, 19.7 mmol) was dissolved in dry THF (40 mL). Pd−C catalyst (10%, 0.52 g) was added and stirred under a H_2 atmosphere (balloon) for 3 h. The reaction mixture was filtered through Celite, and the Celite was washed with $CHCl_3$ (200 mL). The filtrate was concentrated to give the product as an oil which crystallized upon refrigeration (2.87 g, 84.5%). The product was identical to authentic material.

***tert*-Butyl [1-$^{13}C_1$]Succinate.** This was prepared as described for the unlabeled material (2.51 g, 81.1%). ^1H NMR (400 MHz, $CDCl_3$): δ 1.42 (9H, s), 2.52 (2H, m), 2.61 (2H, m), 10.35 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0, 29.1 (d, $J_{13C-13C}$ = 56.5 Hz), 30.05 (d, $J_{13C-13C}$ = 2.3 Hz), 81.0, 171.4, 178.6 (enriched).

1-Benzyl 6-*tert*-Butyl 3-Oxohexanedioate (25). *tert*-Butyl succinate (3.45 g, 20.1 mmol) was dissolved in dry THF (20 mL). Recrystallized

CDI (3.25 g, 20.1 mmol) was added, and the reaction was stirred at room temperature, forming a solution of the imidazolide in 10 min. A clean, dry, three-neck flask equipped with a thermometer and addition funnel was charged with dry THF (70 mL) and diisopropylamine (9.4 mL, 57.2 mmol) and was placed in a dry ice−acetone bath to cool. *n*-Butyllithium (56.2 mmol) in hexanes (21.6 mL) was added slowly and then stirred until the temperature dropped to below −70 °C. Benzyl acetate (5.2 mL, 36.0 mmol) in THF (36 mL) was added dropwise from the addition funnel, maintaining the temperature below −70 °C. The reaction was stirred for 5 min while the imidazolide solution was transferred into the addition funnel via cannula. The imidazolide solution was added dropwise, maintaining the temperature below −70 °C, and then stirred for 5 min before the reaction was quenched by addition of water (100 mL), and allowed to warm to room temperature. The mixture was partitioned between water (200 mL) and Et_2O (200 mL). The Et_2O portion was collected, dried with Na_2SO_4, filtered, and concentrated. The product was recovered by chromatography, eluting with EtOAc−hexanes (1:9), as an oil (3.83 g, 62.3%). IR (liquid film): 1720 cm^{-1}. UV (acetonitrile): 251 nm (ϵ = 410 dm^3 mol^{-1} cm^{-1}). ^1H NMR (400 MHz, $CDCl_3$): δ 1.41 (9H, s), 2.50 (2H, t, J_{1H-1H} = 6.6 Hz), 2.77 (2H, t, J_{1H-1H} = 6.2 Hz), 3.53 (2H, s), 5.16 (2H, s), 7.34 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0, 29.1, 49.2, 37.55, 67.1, 80.75, 128.3, 128.4, 128.55, 135.2, 166.8, 171.5, 200.9. ESI−HRMS calcd for $C_{17}H_{22}O_5Na$ $[M + Na]^+$, 329.1365; found, 329.1352. Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.48; H, 7.28.

1-Benzyl 6-*tert*-Butyl [2,3-$^{13}C_2$]-3-Oxohexanedioate. This was prepared as described for the unlabeled material from *tert*-butyl [1-$^{13}C_1$]-succinate and benzyl [2-$^{13}C_1$]acetate (1.32 g, 63.0%). ^1H NMR (400 MHz, $CDCl_3$): δ 1.41 (9H, s), 2.50 (2H, m), 2.77 (2H, m), 3.53 (2H, dd, J_{1H-13C} = 130.5, 6.3 Hz), 5.16 (2H, s), 7.34 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.0, 29.1 (dd, $J_{13C-13C}$ = 2.1, 0.96 Hz), 37.55 (dd, $J_{13C-13C}$ = 41.2, 14.11 Hz), 49.2 (keto, enriched, d, $J_{13C-13C}$ = 38.5 Hz), 67.1 (d, $J_{13C-13C}$ = 0.95 Hz), 80.8, 89.2 (enol, enriched, d, $J_{13C-13C}$ = 72.4 Hz), 128.3, 128.4, 128.6, 135.2, 166.9 (d, $J_{13C-13C}$ = 2.3 Hz), 171.5 (d, $J_{13C-13C}$ = 2.5 Hz), 177.2 (enol, enriched, d, $J_{13C-13C}$ = 73.1 Hz), 201.0 (keto, enriched, d, $J_{13C-13C}$ = 38.5 Hz).

***tert*-Butyl 3-{2-[2-(Benzyloxy)-2-oxoethyl]-5,5-dimethyl-1,3-dioxan-2-yl}propanoate (26).** Hexanedioate **25** (1.11 g, 3.6 mmol), neopentyl glycol (4.49 g, 43.1 mmol), and PPTS (1.45 g, 5.8 mmol) were dissolved in benzene (30 mL), and the mixture was heated at reflux for 20 h with azeotropic removal of water by means of a Dean−Stark trap. The mixture was concentrated and resuspended in Et_2O (100 mL) and washed first with aqueous saturated $NaHCO_3$ solution (100 mL) and then brine (100 mL). The organic phase was dried, filtered, and concentrated; the dioxane product was recovered by chromatography on silica gel, eluting with EtOAc−hexanes (1:9), as an oil (0.71 g, 50.4%). IR (liquid film): 1723 cm^{-1}. UV (acetonitrile): 257 nm (ϵ = 190 dm^3 mol^{-1} cm^{-1}). ^1H NMR (400 MHz, $CDCl_3$): δ 0.81 (3H, s), 1.05 (3H, s), 1.41 (9H, s), 2.17 (2H, t, J_{1H-1H} = 8.1 Hz), 2.41 (2H, t, J_{1H-1H} = 8.1 Hz), 2.85 (2H, s), 3.41 (2H, d, J_{1H-1H} = 12.0 Hz), 3.60 (2H, d, J_{1H-1H} = 11.7 Hz), 5.12 (2H, s), 7.34 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.3, 22.9, 28.0, 29.4, 29.5, 32.2, 38.0, 66.4, 70.4, 79.8, 97.9, 128.1, 128.4, 135.7, 169.05, 172.7. ESI−HRMS calcd for $C_{22}H_{32}O_6Na$ $[M + Na]^+$, 415.2097; found, 415.2098. Anal. Calcd for $C_{22}H_{32}O_6$: C, 67.32; H, 8.22. Found: C, 67.53; H, 8.33.

***tert*-Butyl [2,3-$^{13}C_2$]-3-{2-[2-(Benzyloxy)-2-oxoethyl]-5,5-dimethyl-1,3-dioxan-2-yl}propanoate.** This was prepared as described for the unlabeled material from 1-benzyl 6-*tert*-butyl [2,3-$^{13}C_2$]-3-oxohexanedioate (0.69 g, 40.8%; 53.3% based on recovered starting material). ^1H NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, s), 1.05 (3H, s), 1.41 (9H, s), 2.18 (2H, m), 2.41 (2H, m), 2.86 (2H, dd, J_{1H-13C} = 130.1, 5.7 Hz), 3.42 (2H, dd, J_{1H-13C} = 5.7 Hz, J_{1H-1H} = 11.8), 3.61 (2H, dd, J_{1H-13C} = 2 Hz, J_{1H-1H} = 11.8), 5.12 (2H, s), 7.34 (5H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.4, 22.9, 28.0, 29.4, 29.6 (d, $J_{13C-13C}$ = 2.5 Hz), 32.2 (dd, $J_{13C-13C}$ = 47.7, 3.8 Hz), 38.05 (enriched, d, $J_{13C-13C}$ = 43.2 Hz), 66.5 (d, $J_{13C-13C}$ = 1.0 Hz), 70.4, 79.9, 97.9 (enriched, d, $J_{13C-13C}$ = 43.2 Hz), 128.1, 128.1, 128.5, 135.8, 169.1 (d, $J_{13C-13C}$ = 1.0 Hz), 172.8 (d, $J_{13C-13C}$ = 4.0 Hz).

***tert*-Butyl 3-[5,5-Dimethyl-2-(2-{[2-(octanoylamino)ethyl]sulfanyl}-2-oxoethyl)-1,3-dioxan-2-yl]propanoate (27).** Compound **26** (2.26 g, 5.77 mmol) was dissolved in dry THF (10 mL). Pd−C catalyst (10%, 0.23 g) was added and stirred at room temperature under a H_2 atmosphere (balloon) for 3 h. The suspension was filtered through Celite, the Celite was washed with $CHCl_3$ (100 mL), and the filtrate was concentrated. The residue was dissolved in dry CH_2Cl_2 (20 mL). *N*-Caprylcysteamine (1.78 g, 8.8 mmol), DCC (1.80 g, 8.8 mmol), and DMAP (0.21 g, 1.7 mmol) were added, and the mixture was stirred at room temperature for 18 h. The suspension was diluted with $CHCl_3$ (50 mL), water (30 mL), and aqueous saturated $NaHCO_3$ (30 mL) and stirred. Iodine, to remove the excess thiol, was added in small portions to the stirred mixture until a red-yellow color persisted; aqueous saturated $Na_2S_2O_3$ solution was then added until the color just disappeared. The layers were separated, and the organic portions were dried with $MgSO_4$, filtered, and concentrated. The product was recovered by chromatography on silica gel, eluting with EtOAc−hexanes (2:3), as an oil (1.4 g, 51.6%). IR (liquid film): 3298, 1727, 1682, 1650 cm^{-1}. UV (acetonitrile): 236 nm (ϵ = 4000 dm^3 mol^{-1} cm^{-1}). ^1H NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, s), 0.84 (3H, t, J_{1H-1H} = 6.8 Hz), 1.05 (3H, s), 1.25 (8H, m), 1.40 (9H, s), 1.57 (2H, m), 2.12 (4H, m), 2.40 (2H, t, J_{1H-1H} = 7.4 Hz), 3.01 (2H, t, J_{1H-1H} = 7.4 Hz), 3.06 (2H, s), 3.40 (2H, q, J_{1H-1H} = 5.8 Hz), 3.42 (2H, d, J_{1H-1H} = 10.7 Hz, 2H), 3.60 (d, J_{1H-1H} = 12.0 Hz), 6.15 (1H, t, J_{14N-1H} = 5.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.4, 22.55, 22.9, 25.6, 28.0, 29.0, 29.2, 29.6, 31.65, 32.4, 36.5, 39.1, 46.2, 70.5, 80.1, 97.8, 172.95, 173.5, 192.2. ESI−HRMS calcd for $C_{25}H_{45}NO_6SNa$ $[M + Na]^+$, 510.2865; found, 510.2881. Anal. Calcd for $C_{25}H_{45}NO_6S$: C, 61.57; H, 9.3; N, 2.87; S, 6.58. Found: C, 61.75; H, 9.33; N, 2.99; S, 6.65.

***S*-[2-(Octanoylamino)ethyl] [2,3-$^{13}C_2$]-3-[2-(2-*tert*-Butyloxy-2-oxoethyl)-5,5-dimethyl-1,3-dioxan-2-yl]propanethioate.** This was prepared as described for the unlabeled material (0.66 g, 78.6%). ^1H NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, s), 0.84 (3H, t, J_{1H-1H} = 6.8 Hz), 1.05 (3H, s), 1.25 (8H, m), 1.40 (9H, s), 1.57 (2H, m), 2.12 (4H, m), 2.39 (2H, dt, J_{1H-1H} = 7.3 Hz, J_{1H-13C} = 3.4 Hz), 3.02 (2H, t, J_{1H-1H} = 5.8 Hz), 3.02 (2H, dd, J_{1H-13C} = 130.0, 5.5 Hz), 3.40 (2H, q, J_{1H-1H} = 6.3 Hz), 3.41 (2H, dd, J_{1H-1H} = 12.0, J_{1H-13C} =5.8 Hz), 3.60 (2H, dd, J_{1H-13C} = 1.6 Hz, J_{1H-1H} = 11.8), 6.16 (1H, t, J_{14N-1H} = 5.5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.4, 22.55, 22.9, 25.6, 28.0, 29.0, 29.2 (d, $J_{13C-13C}$ = 2.7 Hz), 29.55 (d, $J_{13C-13C}$ = 2.3 Hz), 31.6, 32.3 (dd, $J_{13C-13C}$ = 47.7, 3.4 Hz), 36.5, 39.05, 47.3 (enriched, d, $J_{13C-13C}$ = 42.4 Hz), 70.51, 80.1, 98.9 (enriched, d, $J_{13C-13C}$ = 42.3 Hz), 174.1 (d, $J_{13C-13C}$ = 3.8 Hz), 174.6, 196.3.

6-{[2-(Octanoylamino)ethyl]sulfanyl}-4,6-dioxohexanoic acid (28). Thioester **27** (1.42 g, 2.98 mmol) was dissolved in acetone (30 mL). pTsOH (0.19 g, 1.0 mmol) was added, and the reaction was stirred at reflux for 24 h. The cooled mixture was partitioned between water (50 mL) and EtOAc (3 × 100 mL). The pooled EtOAc fractions were dried over $MgSO_4$, filtered, and concentrated. The product was recovered by chromatography on silica gel, eluting with EtOAc−hexanes−AcOH (40:58:2). The column eluate was concentrated down to a minimal volume, mostly comprised of AcOH, and the product was then precipitated out by addition of water (50 mL). The suspension was refrigerated, filtered, washed with 0.1 M HCl, and then dried under vacuum to give a white solid (0.28 g, 26.7%). IR (powder-ATR): 3283, 1719, 1689, 1632, 1556 cm^{-1}. UV (acetonitrile): 236 (ϵ = 3200 dm^3 mol^{-1} cm^{-1}), 281 nm (79). ^1H NMR (400 MHz, CD_3OD): δ 0.89 (3H, t, J_{1H-1H} = 6.8 Hz), 1.30 (8H, m), 1.58 (2H, m), 2.15 (2H, t, J_{1H-1H} = 7.4 Hz), 2.53 (2H, t, J_{1H-1H} = 6.5 Hz), 2.83 (2H, t, J_{1H-1H} = 6.5 Hz), 3.05 (2H, t, J_{1H-1H} = 6.5 Hz), 3.34 (2H, q, J_{1H-1H} = 6.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 23.3, 26.6, 28.2, 29.5, 29.8, 29.9, 32.6, 36.7, 38.2, 39.4, 175.7, 176.2, 193.0, 202.4. ESI−HRMS calcd for $C_{16}H_{28}NO_5S$ $[M + H]^+$, 346.1688; found, 346.1698. Anal. Calcd for

$C_{16}H_{27}NO_5S$: C, 55.63; H, 7.88; N, 4.05; S, 9.28. Found: C, 55.58; H, 7.88; N, 3.96; S, 9.13.

6-{[2-(Octanoylamino)ethyl]sulfanyl}-[4,5-$^{13}C_2$]-4,6-dioxohexanoic acid. This was prepared as described for the unlabeled material (0.14 g, 30.3%). ^{1}H NMR (400 MHz, CD_3OD): δ 0.89 (3H, t, J_{1H-1H} = 6.8 Hz), 1.30 (8H, m), 1.57 (2H, m), 2.15 (2H, t, J_{1H-1H} = 7.4 Hz), 2.53 (2H, m), 2.83 (2H, m), 3.04 (2H, t, J_{1H-1H} = 6.5 Hz), 3.34 (2H, t, J_{1H-1H} = 6.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 23.35, 26.6, 28.2, 29.5, 29.8, 29.9, 32.6, 36.7, 38.2 (dd, $J_{13C-13C}$ = 41.2, 13.7 Hz), 39.4, 57.1 (keto, enriched, sept, $J_{13C-13C}$ = 20.0 Hz), 99.4 (enol, enriched, dt, $J_{13C-13C}$ = 70.2, 25.2 Hz, J_{13C-2H} = 20.0 Hz), 175.7 (enol, enriched, d, $J_{13C-13C}$ = 70.2 Hz), 175.7, 176.2, 193.0 (d, $J_{13C-13C}$ = 2.3 Hz), 202.4 (keto, enriched, d, $J_{13C-13C}$ = 37.4 Hz).

Acknowledgment. We acknowledge the financial support of the NIH (CA77347) and NSF (MCB-0111054). M.E.N. thanks the AFPE for a graduate fellowship. We also thank one of the referees for insightful and helpful comments.

JA016965F