Nargenicin Biosynthesis. Incorporation of Polyketide Chain Elongation Intermediates and Support for a Proposed Intramolecular Diels-Alder Cyclization

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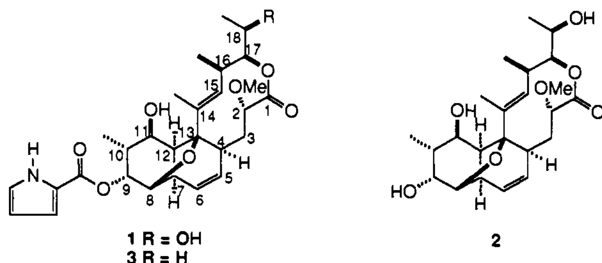
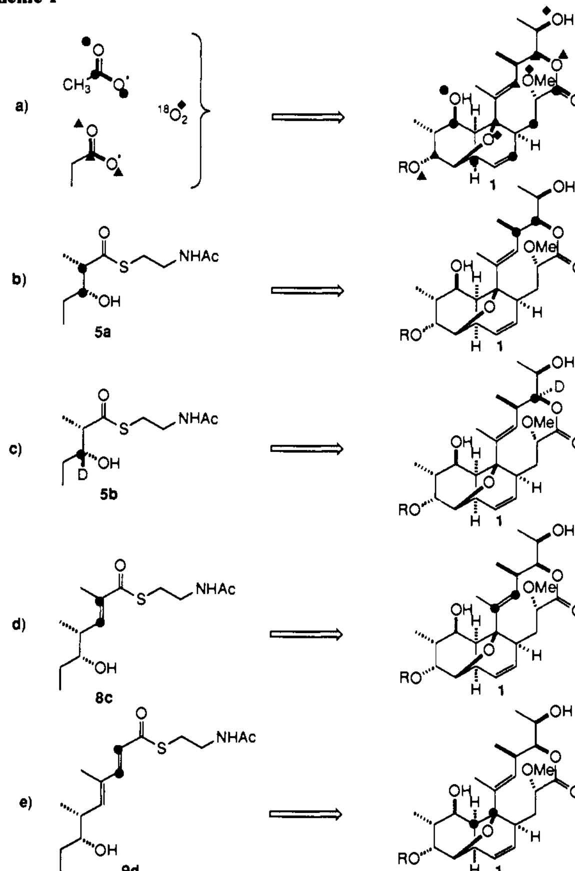
Abstract: A series of proposed polyketide chain elongation intermediates has been synthesized as the *N*-acetylcysteamine (NAC) thioesters and incorporated into nargenicin (**1**) in ^{13}C-labeled form by administration to both actively fermenting and resting cultures of *Nocardia argentinensis*. Thus, feeding of (2*S*,3*R*)-[2,3-$^{13}C_2$]-2-methyl-3-hydroxypentanoyl NAC thioester **5a** gave nargenicin bearing contiguous labels at C-16 and C-17, as established by ^{13}C NMR. In a complementary experiment, (2*S*,3*R*)-[3-^{2}H,3-^{13}C]-2-methyl-3-hydroxypentanoyl NAC thioester **5b** was incorporated into nargenicin without loss of deuterium. Incorporation of the triketide substrate (2*E*,4*R*,5*R*)-[2,3-$^{13}C_2$]-2,4-dimethyl-5-hydroxy-2-heptenoyl NAC thioester **8c** gave nargenicin labeled at C-14 and C-15. Finally, feeding of the tetraketide dienoic ester, (2*E*,4*E*,6*R*,7*R*)-[2,3-$^{13}C_2$]-4,6-dimethyl-7-hydroxy-2,4-nonadienoic acid NAC thioester **9d**, gave nargenicin labeled, as expected, at C-12 and C-13. These results provide further confirmation for a processive model of polyketide chain elongation in which the stereochemistry and oxidation level are adjusted prior to each successive round of condensation-chain extension. The incorporation of the tetraketide precursor also lends support to a proposed intramolecular Diels-Alder mechanism for formation of the characteristic octalin ring system of **1**.

Nargenicin (**1**) is a member of a group of naturally occurring reduced alicyclic polyketides containing a characteristic cis-fused octalin ring system. The antibiotic, which is active against *Staphylococcus aureus*, was first isolated from cultures of *Nocardia argentinensis* Huang ATCC 31306 by researchers at Pfizer[1] and proved to be the pyrrolecarboxylate ester of the closely related metabolite nodusmicin (**2**), isolated by investigators at Upjohn from *Saccharopolyspora hirsuta*.[2] The structures and relative configurations of **1** and **2** were established by a combination of chemical, spectroscopic, and X-ray crystallographic methods. The absolute configuration of **1** was subsequently determined by CD exciton chirality analysis of the derived 11-(*p*-nitrobenzoyl)-18-acetyl ester.[3] Cultures of *S. hirsuta* have also been shown to produce the deoxygenated derivative 18-deoxynargenicin (**3**).[4]



The polyketide origin of nargenicin was originally confirmed by biosynthetic studies which established that nargenicin is derived from five acetate and four propionate building blocks.[5] Analogous results were obtained concurrently by Rinehart for the biosynthesis of nodusmicin.[6] Further incorporation experiments with [1-$^{18}O_2$,1-^{13}C]acetate established that the oxygen atoms at C-1 and C-11 of **1** originated from the carboxylate of the acetate precursor[5] (Scheme Ia). Similarly, incorporation of [1-$^{18}O_2$,1-^{13}C]propionate revealed that the C-9 and C-17 oxygen atoms of nargenicin were derived from propionate.[5] Finally, complementary experiments with $^{18}O_2$ established that the oxygen atoms at C-2 and C-18, as well as the C-8,13 bridging ether atom, were all derived from molecular oxygen.[3]

Scheme I



The formation of nargenicin can be envisaged as taking place in three conceptually and mechanistically distinct phases: (1) the elaboration of a branched-chain, partially saturated polyketide fatty acid which undergoes (2) cyclization with generation of the macrolide and ring systems followed by (3) late-stage oxidations, methylation, and esterification with pyrrolecarboxylic acid. The biosynthetic results obtained to date are fully consistent with the currently favored model of polyketide chain elongation in which

(1) Celmer, W. D.; Chmurny, G. N.; Moppett, C. E.; Ware, R. S.; Watts, P. C.; Whipple, E. B. *J. Am. Chem. Soc.* **1980**, *102*, 4203. Celmer, W. D.; Cullen, W. P.; Moppett, C. E.; Jefferson, M. T.; Huang, L. H.; Shibakawa, R.; Tone, J. U.S. Patent 4,148,883, 1979.
(2) Whaley, H. A.; Chidester, C. G.; Mizsak, S. A.; Wnuk, R. J. *Tetrahedron Lett.* **1980**, *21*, 3659.
(3) Cane, D. E.; Yang, C. *J. Antibiot.* **1985**, *38*, 423.
(4) Whaley, H. A.; Coats, J. H. *Abstr. Intersci. Conf. Antimicrob. Agents Chemother.* **1981**, Abstract No. 187. Magerlein, B. J.; Reid, R. J. *J. Antibiot.* **1982**, *35*, 254.
(5) Cane, D. E.; Yang, C. *J. Am. Chem. Soc.* **1984**, *106*, 784.
(6) Snyder, W. C.; Rinehart, K. L. *J. Am. Chem. Soc.* **1984**, *106*, 787.

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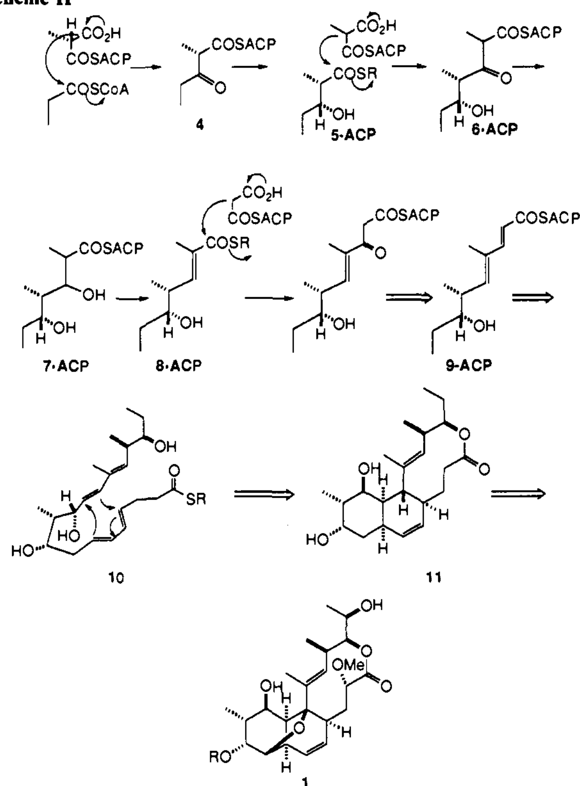
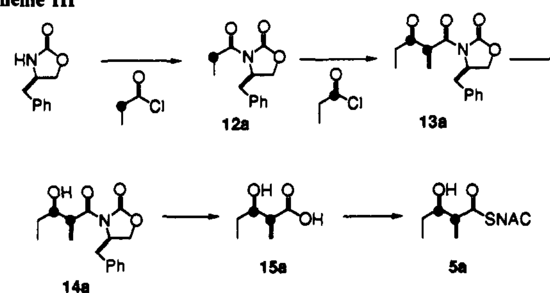
Scheme II



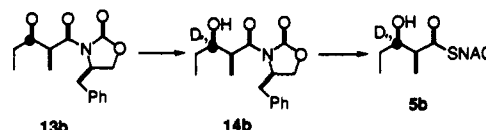
the oxidation level and stereochemistry of the growing reduced polyketide chain are adjusted subsequent to each condensation step and prior to the addition of the next malonyl or methylmalonyl CoA building block, as appropriate.[7-9] Furthermore, the demonstrated origins of the various oxygen atoms have ruled out plausible epoxy-olefin cyclization mechanisms and suggested that the characteristic octalin ring system is generated by an intramolecular Diels-Alder reaction.[3,5] The key steps in this mechanism are illustrated in Scheme II.

The finding that diketide and triketide chain elongation intermediates, administered as the corresponding N-acetylcysteamine (NAC) thioesters, could be incorporated intact into the macrolides erythromycin[7b] and tylactone[8] has provided a direct and powerful means of probing the detailed sequence of intermediates in the elaboration of complex polyketide natural products. These techniques have subsequently been used by our own and other groups to examine the biosynthesis of a variety of polyketides, including methymycin,[10] nonactin,[11] dehydrocurvularin,[12] and aspyrone.[13] We now report experiments based on the intact incorporation of presumptive intermediates of polyketide chain elongation, which provide further support for the proposed

Scheme III



Scheme IV



mechanisms of both polyketide chain assembly and cyclization in nargenicin biosynthesis.[14]

Results

According to the mechanism shown in Scheme II, the biosynthesis of the parent polyketide chain of nargenicin should be initiated by condensation of a propionyl thioester starter unit with an equivalent of (2*R*)-methylmalonyl-ACP thioester. Reduction of the resultant β-keto ester **4** will give (2*S*,3*R*)-2-methyl-3-hydroxypentanoate as an enzyme-bound thioester (**5**-ACP). Interestingly, the same intermediate has also been implicated in the formation of both the 12- and 14-membered macrolides methymycin[10] and erythromycin.[7b] The requisite NAC thioester substrate **5a** was readily prepared by the method of Evans for erythroselective aldol synthesis[15] (Scheme III). Acylation of (4*R*)-4-benzyloxazolidinone with [2-^{13}C]propionyl chloride gave (4*R*)-*N*-[2′-^{13}C]propionyl-4-benzyloxazolidinone (**12a**), which was metalated with lithium diisopropylamide. The resulting (*Z*)-enolate was acylated with [1-^{13}C]propionyl chloride with formation of the configurationally stable β-keto imide **13a**, which underwent stereospecific reduction with $Zn(BH_4)_2$ to give (4*R*,2′*S*,3′*R*)-[2′,3′-$^{13}C_2$]-*N*-(2′-methyl-3′-hydroxypentanoyl)-4-benzyloxazolidinone (**14a**) in 67% yield. Hydrolysis with LiOH/30% H_2O_2 gave the enantiomerically pure acid **15a**, which was converted to the NAC thioester **5a** by successive treatment with diphenylphosphoryl azide and *N*-acetylcysteamine.

The labeled diketide precursor **5a** was administered in 20% ethanol in portions of 40, 20, and 40 mg at 24, 48, and 72 h, respectively, to 10 70-mL fermentation cultures of *N. argentinensis*. After a total of 96 h, the cultures were harvested, and the resulting labeled nargenicin (7.1 mg) was isolated and then analyzed by 100.6-MHz ^{13}C NMR. The ^{13}C NMR spectrum of **5a** showed a set of enhanced and coupled satellite doublets (J_{CC} = 36.2 Hz, 0.2 atom % enrichment) for the signals arising from C-16 (32.76 ppm) and C-17 (78.79 ppm), as predicted for the intact incorporation of the doubly-^{13}C-labeled precursor and consistent with the portion that the β-hydroxy thioester is an intermediate in the elaboration of the polyketide chain (Scheme Ib). On the other hand, these results alone did not rule out the alternative possibility that incorporation of **5a** involves initial reoxidation of the exogenously administered precursor to the corresponding 2-methyl-3-oxopentanoyl thioester **4**, which would serve as the true substrate for loading onto the polyketide synthase.

In order to rule out the possibility of interfering oxidation of **5a**, we prepared the corresponding deuterated substrate, (2*S*,3*R*)-[3-^{2}H,3-^{13}C]-2-methyl-3-hydroxypentanoyl NAC thioester, **5b**, by using $Zn(BD_4)_2$ in the reduction of the β-keto imide

(7) (a) Cane, D. E.; Hasler, H.; Liang, T. *J. Am. Chem. Soc.* **1981**, *103*, 5960. Cane, D. E.; Hasler, H.; Taylor, P. B.; Liang, T. *Tetrahedron* **1983**, *39*, 3449. Cane, D. E.; Liang, T.; Taylor, P. B.; Chang, C.; Yang, C. *J. Am. Chem. Soc.* **1986**, *108*, 4957. (b) Cane, D. E.; Yang, C. *J. Am. Chem. Soc.* **1987**, *109*, 1255. (c) Cane, D. E.; Prabhakaran, P. C.; Tan, W.; Ott, W. R. *Tetrahedron Lett.* **1991**, *32*, 5457.

(8) Yue, S.; Duncan, J. S.; Yamamoto, Y.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1987**, *109*, 1253.

(9) (a) Cane, D. E.; Yang, C.; Ott, W. R. *Proceedings of the Seventh International Symposium on Biology of Actinomycetes*; Japan Antibiotics Research Assocation: Tokyo, 1988; p 395. (b) Staunton, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1302.

(10) Cane, D. E.; Lambalot, R. H.; Prabhakaran, P. C.; Ott, W. R. *J. Am. Chem. Soc.*, preceding article in this issue.

(11) Spavold, Z. M.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 4.

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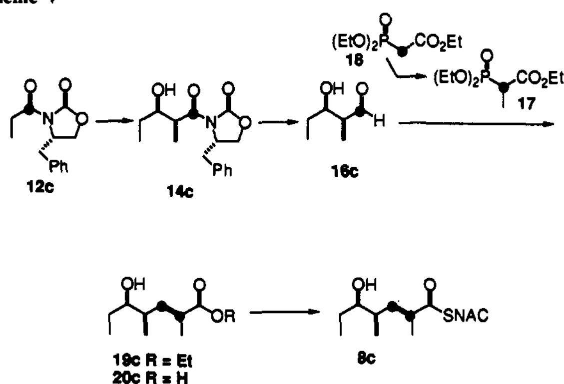
(13) Staunton, J.; Sutkowski, A. C. *J. Chem. Soc., Chem. Commun.* **1991**, 1108. Staunton, J.; Sutkowski, A. C. *J. Chem. Soc., Chem. Commun.* **1991**, 1110. Jacobs, A.; Staunton, J.; Sutkowski, A. C. *J. Chem. Soc., Chem. Commun.* **1991**, 1113.

(14) Portions of this work have been reported in preliminary form: Cane, D. E.; Ott, W. R. *J. Am. Chem. Soc.* **1988**, *110*, 4840. Reference 7c.

(15) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127. Evans, D. A.; Ennis, M. D.; Le, T.; Mandel, N.; Mandel, G. *J. Am. Chem. Soc.* **1984**, *106*, 1154. Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141.

Scheme V



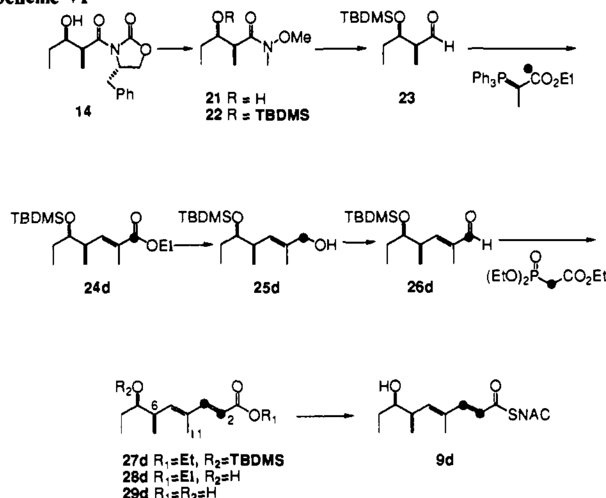
13b. Intact incorporation of **5b** was achieved by feeding 80 mg to a 200-mL replacement culture of *N. argentinensis*, prepared by resuspension of cells from 200 mL of a 72-h production culture in fresh production medium and incubation for an additional 96 h in the presence of 15 mg of 4-pentynoic acid (Scheme Ic). The latter compound, an inhibitor of fatty acid β-oxidation, was originally shown by Vederas to be critical for the successful incorporation of advanced precursors into dehydrocurvularin[12] and has subsequently been used by us in achieving the successful incorporation of intact precursors into both methylmycin[10] and erythromycin.[7c] The ^{13}C NMR spectrum of labeled nargenicin (3.5 mg) obtained from incorporation of deuterated **5b** displayed the expected 1:1:1 ^{13}C–^{2}H triplet (J_{CD} = 22.4 Hz) located 0.41 ppm upfield of the signal for C-17 at 79.1 ppm. Upon ^{2}H decoupling, the triplet collapsed to a singlet, while in the difference ^{13}C{^{1}H,^{2}H} spectrum the only signals apparent were the peak centered at 78.7 ppm and the expected inverted signals at ±22.4 ppm. Similar incorporation results were also obtained by direct feeding of **5b** to actively fermenting cultures of *N. argentinensis*.

The successful incorporation of ^{13}C–^{13}C- and ^{13}C–^{2}H-labeled dipropionate substrates into nargenicin is consistent with the notion that the parent polyketide chain is built up by adjustment of the oxidation level and stereochemistry of each intermediate prior to the next cycle of chain elongation. According to this model, the next step in the chain elongation process is proposed to be the condensation of **5**-ACP with one or the other enantiomer of methylmalonate so as to generate the tripropionate derivative **6**-ACP. Reduction of the latter compound will give one or the other diastereomer of the corresponding β-hydroxy thioester **7**-ACP, which in turn will undergo dehydration to the α,β-unsaturated ester **8**-ACP. In principle, any of the tripropionate intermediates could be tested as the corresponding NAC thioesters as potential precursors of nargenicin. In practice, however, the β-keto ester **6** suffers from its inherent configurational instability at C-2, while uncertainties about the stereochemistry of the actual β-hydroxy ester intermediate, as well as the expected propensity of **7** to lactonize, make these compounds less attractive as substrates for intact cell feeding experiments. We therefore turned to the preparation of **8c** for testing as a presumptive triketide intermediate in the formation of nargenicin.

Treatment of [1′-^{13}C]propionyl imide **12c** with dibutylboron triflate in the presence of Hunig's base, followed by addition of propionaldehyde, gave (4*S*,2′*S*,3′*R*)-[1′-^{13}C]-3-(2′-methyl-3′-hydroxypentanoyl)-4-benzyl-2-oxazolidinone (**14c**) in 91% yield after oxidative workup (Scheme V). After recrystallization, the imide **14c** was treated with sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al),[16] and the resulting crude aldehyde **16c** was treated immediately with triethyl [2-^{13}C]phosphonopropionate **17** at −78 °C, itself prepared by treatment of triethyl [2-^{13}C]-phosphonoacetate **18** with potassium *tert*-butoxide followed by addition of methyl iodide at 0 °C. The unsaturated (*E*)-[2,3-$^{13}C_2$] ester **19c**, which was readily separated from the isomeric (*Z*) olefin, was obtained in 30% overall yield from **14c**.[17,18] Hydrolysis of

(16) Meyers, A. I.; Spohn, R. F.; Linderman, R. J. *J. Org. Chem.* **1985**, *50*, 3633.

Scheme VI



the conjugated ester **19c** was performed under mild basic conditions (K_2CO_3, 3:1 $MeOH/H_2O$, reflux, 3 h) to avoid unwanted epimerization. Acidification to pH 2.2 followed by ether extraction afforded the unsaturated acid **20c** as a single diastereomer in 92% yield. Treatment of **20c** with diethyl phosphorochloridate in the presence of triethylamine followed by addition of thallous *N*-acetylcysteamine[19] afforded the desired NAC thioester **8c** in 79% yield.

A mixture of 30 mg of (2*E*,4*R*,5*R*)-[2,3-$^{13}C_2$]-2,4-dimethyl-5-hydroxy-2-heptanoyl NAC thioester **8c** and 15 mg of 4-pentynoic acid in 0.5 mL of ethanol was administered in one portion to a 24-h production culture of *N. argentinensis*, corresponding to the previously established maximum in the rate of nargenicin production. After fermentation for an additional 4 days, the culture broth was harvested to give, after extraction and purification, 4 mg of nargenicin. Examination of the 100.6-MHz ^{13}C NMR spectrum of the labeled nargenicin revealed the presence of the expected two sets of enhanced and coupled doublets (J_{CC} = 73.0 Hz, 0.2 atom % enrichment) centered at 134.8 and 131.8 ppm, corresponding to C-14 and C-15, respectively (Scheme Id).

Having incorporated an intact triketide substrate, we next turned our attention to the tetraketide intermediates. The structures of the putative diketide and triketide chain elongation intermediates could be more or less directly inferred from the structure of the nargenicin ultimately formed, after taking into account late-stage oxidation at C-18. The precise structure of a presumptive tetraketide intermediate, however, was not as readily apparent from the structure of the octalin ring system and was further obscured by the presence of the ether oxygen atom at C-13. Nonetheless, the results of our earlier investigations utilizing [^{13}C]- and [^{13}C,^{18}O]-labeled acetates and propionates did suggest a possible dienoic ester structure for the key tetraketide intermediate, consistent with the previously proposed intramolecular Diels–Alder cyclization (Scheme Ia). We therefore undertook the preparation of [2,3-$^{13}C_2$]-**9d**.

Reaction of unlabeled **14** with *N,O*-dimethylhydroxylamine and trimethylaluminum[20] gave the corresponding *N*-methyl-*N*-methoxy amide **21**, which was protected as the *tert*-butyldimethylsilyl ether **22** by treatment with TBDMSOTf and triethylamine in CH_2Cl_2 (Scheme VI). Reduction of **22** with DIBAL[20] in THF at −50 °C gave the corresponding aldehyde **23**. Although **23** could be purified by SiO_2 chromatography and fully characterized, it was normally used directly without further purification. Reaction of **23** with [[1-^{13}C]carbethoxyethylidene]triphenylphosphorane in

(17) The synthesis of the enantiomeric (2*E*,4*S*,5*S*)-2,4-dimethyl-5-hydroxy-2-heptenoate has been reported: Shimizu, I.; Hayashi, K.; Ide, N.; Oshima, M. *Tetrahedron* **1991**, *47*, 2991.

(18) β-Olefinic protons are deshielded by 0.5–0.9 ppm by a *cis*-alkoxycarbonyl group. Cf. Jackman, L. M.; Wiley, R. H. *J. Chem. Soc.* **1960**, 2881. Jackman, L. M.; Wiley, R. H. *J. Chem. Soc.* **1960**, 2886.

(19) Schwab, J. M.; Klassen, J. B. *J. Am. Chem. Soc.* **1984**, *106*, 7217.

(20) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.

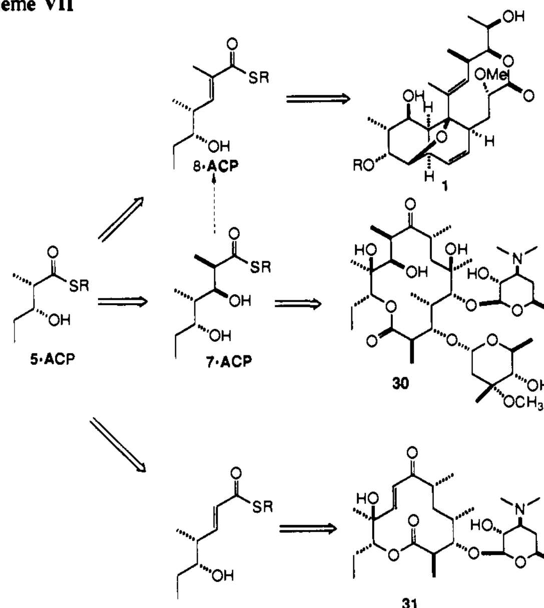
refluxing THF gave a 55% yield of the α,β-unsaturated ester **24d**, which was shown to be exclusively trans by ^{1}H NMR analysis.[18,21] Reduction with LAH in ether gave the desired allylic alcohol **25d** (90% yield), which was oxidized with pyridinium chlorochromate (PCC) on neutral activated aluminum oxide at room temperature. After passage through a short column of Florisil, the conjugated aldehyde **26d** was obtained in 92% yield without detectable epimerization of the chiral centers, as evidenced by ^{1}H NMR spectroscopy. The aldehyde **26d** was reacted at −78 °C in THF with the Emmons reagent generated by treatment of triethyl [2-^{13}C]phosphonoacetate with potassium *tert*-butoxide at room temperature. The reaction mixture was allowed to warm to room temperature overnight to afford the desired [2,3-$^{13}C_2$]-dienoic ester **27d** in 95% yield. The trans,trans geometry of **27d** was evident from the characteristic 15.5 Hz coupling constant between H-2 and H-3 and was further confirmed by a difference ^{1}H NOE experiment in which irradiation of the H-11 methyl protons gave rise to enhancements of the resonances corresponding to H-2 and H-6 with no effect on the signals for H-3 and H-5. Desilylation with tetrabutylammonium fluoride (TBAF) at 25 °C in THF for 8 h gave the hydroxy ester **28d**, which was hydrolyzed under mild basic conditions (K_2CO_3, $MeOH/H_2O$, reflux, 3 h) to give the dienoic acid **29d** in 92% overall yield. Finally, treatment of **29d** with diethyl phosphorochloridate in the presence of triethylamine in THF at 0 °C for 3 h, followed by addition of TlSNAC gave the desired (2*E*,4*E*,6*R*,7*R*)-[2,3-$^{13}C_2$]-4,6-dimethyl-7-hydroxy-2,4-nonadienoic acid NAC thioester **9d** in 55% yield.

For the incorporation experiment, a mixture of 50 mg of **9d** and 14.6 mg of 4-pentynoic acid in 0.75 mL of ethanol was fed in one portion to a 24-h production culture of *N. argentinensis*. After an additional incubation of 4 days, the resulting nargenicin was isolated and analyzed by ^{13}C NMR. Intact incorporation of the precursor was confirmed by the observation of the expected pair of enhanced and coupled doublets (J_{CC} = 35.4 Hz, 0.25 atom % enrichment) for C-12 and C-13 centered at 49.0 and 89.0 ppm, respectively (Scheme Ie).

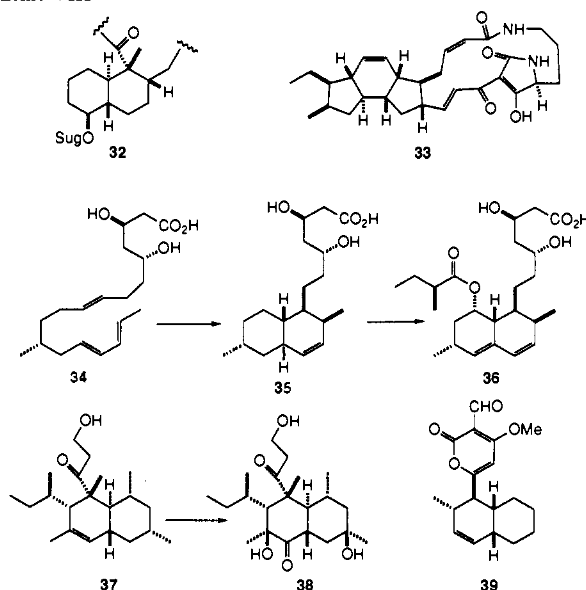
Discussion

The intact incorporation of diketide, triketide, and tetraketide intermediates into nargenicin is fully consistent with the processive model of polyketide chain elongation in which the oxidation level and stereochemistry of each intermediate are adjusted prior to each new ketosynthase-catalyzed condensation. This model has recently received solid support at the molecular genetic level by the demonstration by two independent laboratories that the structural genes for 6-deoxyerythronolide B synthase, which mediates the assembly of one propionate and six methylmalonates into the parent erythromycin aglycone, consist of a series of domains with strong homologies to analogous genes of fatty acid biosynthesis, organized in a manner apparently collinear with the presumed steps of polyketide chain assembly. Interestingly, although the gross structures of erythromycin A and nargenicin are very different, there is a striking similarity in the first several steps of polyketide chain assembly.[22] Thus the NAC thioester of the dipropionate substrate **5** has been shown to act as an intact precursor of both nargenicin (**1**) and erythromycin A (**30**)[7b,c] (as well as the closely related 12-membered-ring macrolide methymycin (**31**)[10] (Scheme VII). Moreover, both **6**-ACP and **7**-ACP are likely tripropionate intermediates in the formation of erythromycin. The two pathways would appear to branch at this stage, however, with erythromycin formation involving direct condensation of **7** with an enzyme-bound methylmalonyl-ACP, while nargenicin biosynthesis requires dehydration to the unsaturated ester **8**-ACP prior to condensation with a malonyl thioester. It will be of great interest to compare the detailed organization of the first two polyketide biosynthetic modules of the nargenicin pathway with that already established for erythromycin and that under investigation for methymycin.

Scheme VII



Scheme VIII



Although genetic evidence has suggested that the macrolide polyketide synthases are organized in a manner that corresponds to the sequence of individual synthetic transformations of reduced fatty acid chain assembly, the successful incorporation of NAC thioesters of various intermediates of polyketide chain elongation into nargenicin, as well as erythromycin, methymycin, and other complex polyketides, establishes that there is an important element of molecular recognition in the action of each biosynthetic module which allows each presumptive intermediate to be correctly bound and processed by the appropriate ketosynthase. Precisely which structural features of the substrate are critical to this molecular recognition and whether this discrimination resides in the acyltransferase, ketosynthase, or ACP components of each chain elongation module or some combination of all three remain to be established.

The incorporation of the dienoyl thioester **9d** into nargenicin lends support to the postulated intramolecular Diels–Alder cyclization mechanism involving reaction of an (*E*,*E*)-diene with an (*E*) dienophile, which has been proposed to account for the

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formation of the characteristic cis-fused octalin ring system (Scheme II). According to this picture, the proton at C-13 of the originally formed ring system would be replaced by oxygen with net retention of configuration. Although there are no confirmed examples of bona fide enzyme-catalyzed Diels–Alder reactions in natural products biosynthesis, this mechanism has several attractive mechanistic and stereochemical features. We have previously pointed out that closely related mechanisms can account for the formation of the octalin ring systems of chlorothricin (**32**), kijanimicin, and tetrocarcin, all of which exhibit a common cis relationship between the allylic protons of the product octalin ring system.[23] A similar intramolecular cyclization may well be involved in the biosynthesis of the tricyclic perhydrophenanthrene core of ikarugamycin (**33**).[24] These ideas can be extended to the formation of monacolin K (mevinolin) (**36**), which is derived from 4a,5-dihydromonacolin L (**35**).[25] The latter metabolite would appear to be formed by intramolecular Diels–Alder condensation of the hypothetical (*E,E,E*)-triene acid **34**. Further support for the operation of biological Diels–Alder cyclizations of polyketide chains has come from the work of Oikawa and Sakamura, who have implicated the deoxygenated intermediate probetaenone I (**37**) in the biosynthesis of the fungal metabolite betaenone B (**38**)[26] and have established the origins of the carbon skeleton and oxygen atoms of the presumptive intramolecular Diels–Alder product solanapyrone A (**39**).[27] Work is currently in progress to test further many of these ideas by the incorporation of more advanced intermediates of polyketide chain elongation into nargenicin as well as the screening of extracts of *N. argentinensis* for abortive chain-elongation products which may accumulate in the mycelium or fermentation broth.

Experimental Section

Materials and Methods. Nuclear magnetic resonance (NMR) spectra were recorded as solutions in the indicated solvents on Bruker WM 250 or AM 400 FT NMR spectrometers. The $^{13}C\{^1H,^2H\}$ decoupling experiments were performed with a B-SV3 BX broad-band amplifier at 62.85 MHz, using a fixed-frequency decoupler and normal spectral acquisition parameters. Chemical shifts are reported in parts per million relative to tetramethylsilane, using $CHCl_3$ or CH_2Cl_2 as internal standards. Coupling constants are reported in hertz. Spectral splitting patterns are designated as follows: s, singlet; t, triplet; q, quartet, br, broad; and m, multiplet. Infrared spectra were recorded on a Perkin-Elmer Model 1600 FT-IR spectrophotometer. Mass spectra were obtained by chemical ionization with NH_4^+ or electron impact using a Kratos MS80RFA instrument and are reported as m/z. Radioactivity measurements utilized a Beckman LS-5801 liquid scintillation counter and were carried out in 10-mL Opti-Fluor solutions. Optical rotations were measured on a Perkin-Elmer 241 polarimeter using the Na D line at 25 °C. Optical rotations are reported as follows: $[\alpha]^D$ (concentration (c (g/100 mL)), solvent). Fermentations were carried out in a New Brunswick G-25 gyrotory shaker. Doubly deionized (nanopure) water obtained from a Barnstead Nanopure II water purification system was used to prepare all culture media. Flash chromatography was performed with EM reagent silica gel 60 (230–240 mesh) and the indicated solvent systems. Data are reported as follows: column size, solvent system. Tetrahydrofuran (THF) and diethyl ether (ether) were distilled from sodium metal/benzophenone. Benzene was distilled from sodium metal. Dichloromethane, diisopropylethylamine, triethylamine, diisopropylamine, and acetonitrile were distilled from calcium hydride. Dimethylformamide (DMF) was distilled under reduced pressure from calcium hydride and stored over activated 5-Å molecular sieves under an argon atmosphere. Chloroform was passed through a column of activity 1 basic alumina immediately before use as a solvent for optical rotation measurements. Commercial *N,O*-dimethylhydroxylamine (Aldrich) was recrystallized from 2-propanol. Air- and/or moisture-sensitive reactions were carried out under an atmosphere of argon with the use of flame-dried glassware. All other reagents and solvents were obtained from Aldrich Chemical Co., Cambridge Isotope Laboratories, or the Los Alamos National Laboratories.

Cultivation of *Nocardia argentinensis*. *Nocardia argentinensis* (ATCC 31306) was purchased from ATCC as a lyophilized pellet. Half of the pellet was dissolved in 1 mL of sterile water. This solution was shaken well and transferred to a sterile YME solution of the following composition: yeast extract (Difco), 200 mg; malt extract (Difco), 500 mg; dextrose, 200 mg; water, 50 mL; pH 7.0. The inoculated solution was incubated with shaking at 250 rpm at 26 °C for 3 days. Half of the resulting cell suspension was stored in 20 1.5-mL plastic vials under liquid nitrogen. The remaining half was used to inoculate YME agar slants, prepared from 200 mg of yeast extract, 500 mg of malt extract, 200 mg of dextrose, and 1 g of agar in 50 mL of water (3 mL of agar solution per slant). To these slants (predried for 6 days at 28 °C prior to use) was added 3 drops of *N. argentinensis* YME inoculum. The agar slants were then incubated at 26 °C for 6 days, before storage at 4 °C with the cover tightly closed. These preparations remained viable for at least a year. Fresh slants could also be generated from the frozen cell suspension. Thawed inoculum was transferred into autoclaved YME broth at 26 °C, and the resulting suspension was shaken for 96 h at 26 °C before being used as the slant inoculum. After 10 days, the resulting slants sporulated and could be stored at 4 °C.

A 0.5 × 0.5 cm agar plug from a sporulated slant culture was used to inoculate sterile medium of the following composition: glucose, 1 g; starch (soluble), 2 g; yeast extract, 500 mg; enzymatic digest of casein, 500 mg; K_2HPO_4, 50 mg; $CoCl_2$, 0.2 mg; water, 100 mL; pH adjusted to 7.05 by addition of 1 N NaOH; calcium carbonate, 400 mg. This vegetative inoculum was incubated at 28 °C at 250 rpm for 72 h, after which time 6 mL was transferred to a 1-L Delong flask containing 200 mL of sterilized medium of the same composition. The production culture was incubated with shaking at 250 rpm and 28 °C for 5 days before harvest. The production culture gradually thickened over the first 3 days and maintained a relatively constant cell density during the final 2 days of fermentation. The pH of the culture changed from 7.1 to 8.7 during the 5-day period.

For isolation of nargenicin, the mycelia and fermentation broth from the production medium were centrifuged at 8000 rpm for 20 min in a Sorvall GS3 rotor (1816 g). The supernatant was decanted, the pH was determined, and the solution was saturated with NaCl and extracted with ethyl acetate. The mycelia were also rinsed with 200 mL of water and recentrifuged, and the resulting supernatant was saturated with NaCl and extracted with ethyl acetate. The combined ethyl acetate extracts were concentrated on a rotary evaporator and dried for 15 min in vacuo. The orange-yellow residue (30–50 mg) contained nargenicin, as indicated by TLC analysis (R_f 0.25, cyclohexane/ethyl acetate, 1:1, UV detection). The nargenicin was purified by silica gel column (11 cm × 11 mm diameter) chromatography. The column was preequilibrated with 30% EtOAc/70% hexanes and then overlaid with 20 mL of hexane. The sample was dissolved in 3 mL of ethyl acetate and applied to the column. A mixture of 50 mL of 3:7 ethyl acetate/hexane was used to elute the yellowish impurities. Finally, a 1:1 EtOAc/hexane mixture (150 mL) was used as eluent under nitrogen to fractionate the remaining compounds in the column. Nargenicin was monitored by TLC (UV detection), and the fractions that contained the pure band of nargenicin were collected to afford a colorless solution. Concentration under reduced vacuum yielded **1** as a white solid (5 mg), which was characterized by ^1H and ^{13}C NMR spectroscopy, IR, mass spectrometry, and melting point analysis. All analytical data were in complete agreement with those from an authentic sample and previously reported.[1,3]

For the preparation of replacement cultures, the mycelium from 200 mL of a 72-h production culture of *N. argentinensis* was collected by centrifugation under sterile conditions. The cells were resuspended in 200 mL of broth of the same composition as the normal fermentation medium. After an additional 3 days, the replacement culture was harvested and the nargenicin purified in the usual manner.

[1-^{13}C]Propionyl Chloride. A flame-dried 50-mL 1-neck round-bottom flask charged with 1.03 g (10.4 mmol) of sodium [1-^{13}C]propionate (99 atom % ^{13}C) and 2.10 mL (14.5 mmol) of phthaloyl dichloride was connected through a bent glass tube to a 2-neck receiving flask in a dry ice/acetone bath. The reaction mixture was maintained at 150 °C with magnetic stirring for 1.5 h. [1-^{13}C]Propionyl chloride was allowed to

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distill into the receiving flask as it was formed (87%). The product was used immediately in the next step.

(4*R*,2′*S*)-[3′-^{13}C]-3-(2′-Methyl-3′-oxopentanoyl)-4-benzyl-2-oxazolidinone (13b). To a stirred solution of 6.19 g (26.5 mmol) of (4*R*)-3-propionyl-4-benzyl-2-oxazolidinone (**12**) in 100 mL of THF was added 13.25 mL (26.5 mmol) of a freshly-prepared LDA solution at −78 °C under nitrogen atmosphere. The mixture was stirred at −78 °C for 1.5 h and treated with the freshly-prepared [1-^{13}C]propionyl chloride. After 30 min at −78 °C, the reaction was quenched with 25 mL of saturated NH_4Cl solution and washed with H_2O to remove solids. The aqueous layer was extracted with 4 × 100 mL of CH_2Cl_2. The combined organic extracts were washed with 2 × 50 mL of H_2O and 2 × 20 mL of 1 N NaOH, dried over Na_2SO_4, and concentrated under vacuum. Flash chromatography gave white crystalline needles of **13b** (86%): R_f 0.24 (40% EtOAc/hexane); mp 96–97 °C; IR (neat) ν 2983, 1778, 1703, 1635, 1454, 1392, 1361, 1215, 1126, 1080, 752 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.25 (m, 5 H, aromatic), 4.68 (m, 1 H, C_4-H), 4.54 (m, 1 H, $C_{2'}$-H), 4.18 (m, 2 H, C_5-H), 3.47 (dd, J = 3.34, 13.57 Hz, 1 H, C_{6a}-H), 2.8–2.6 (m, 3 H, C_{6b}-H + $C_{4'}$-H), 1.42 (dd, J = 4.44, 7.27 Hz, 3 H, $C_{2'}$-CH_3), 1.10 (dt, J = 5.02, 7.23 Hz, 3 H, $C_{5'}$-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.1 (enriched), 170, 154, 135.5, 129.4, 128.9, 127.2, 66.4, 55.4, 52.3 (d, J_{CC} = 36.9 Hz), 37.4, 33.9 (d, J_{CC} = 41.2 Hz), 12.7, 7.5; $[\alpha]_D$ +36° (*c* 1.3, $CHCl_3$); CIMS (NH_4^+) *m/z* (M + H)$^+$ 291.

Zinc Borodeuteride. Zinc chloride was fused in a crucible over a flame and poured into a tared oven-dried 100-mL round-bottom flask to yield 4.15 g (29.6 mmol) of dry solid, after cooling to room temperature. The solid was dissolved in 100 mL of ether at reflux for 3 h. The suspension was allowed to stand at room temperature until the homogeneous solution was separated from the residue. The solution was cannulated slowly into a stirred suspension of 5.5 g (131.1 mmol, 98 atom %, Aldrich) of sodium borodeuteride in 100 mL of ether. The resulting mixture was allowed to stir for 24 h at room temperature. The residue was separated, and the homogeneous solution was cannulated into a sealed flask and stored at 4 °C.

(4*R*,2′*S*,3′*R*)-[3′-^{2}H,3′-^{13}C]-3-(2′-Methyl-3′-hydroxypentanoyl)-4-benzyl-2-oxazolidinone (14b). In a 250-mL round-bottom, 1-neck flask, 700 mg (2.41 mmol) of (4*R*,2′*S*)-[3′-^{13}C]-3-(2′-methyl-3′-oxopentanoyl)-4-benzyl-2-oxazolidinone (**13b**) was dissolved in 150 mL of Et_2O with magnetic stirring under N_2 at room temperature. After cooling to −20 °C with dry ice/CCl_4, 25 mL, of the $ZnBD_4$ solution (3.62 mmol) was added and the resulting mixture was stirred at −20 °C for 45 min. The reaction mixture was poured into 200 mL of ice water in a 1-L flask and allowed to stand for 1 h. The organic layer was separated. The aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with 50 mL of 2 N HCl and 50 mL of brine. The combined organic extracts were dried over $MgSO_4$. Recrystallization from Et_2O yielded white crystalline needles of **14b** (89%): mp 95–96 °C; IR ($CHCl_3$) ν 3535, 3019, 2970, 1779, 1686, 1384 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.21 (m, 5 H, aromatic), 4.70 (m, 1 H, C_4-H), 4.2 (m, 2 H, C_5-H), 3.86 (dq, J = 2.88, 7.01 Hz, 1 H, $C_{2'}$-H), 3.30 (dd, J = 3.4, 13.33 Hz, 1 H, C_{6a}-H), 2.77 (dd, 1 H, J = 9.66, 13.38 Hz, C_{6b}-H), 2.69 (b, 1 H, OH), 1.68–1.51 (m, 2 H, $C_{4'}$-H), 1.19 (dd, J = 4.1, 7.02 Hz, 3 H, $C_{2'}$-CH_3), 1.01 (dt, J = 5.33, 7.42 Hz, 3 H, $C_{5'}$-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 171.9, 153, 135.1, 129.3, 128.9, 127.3, 73.2 (natural abundance), 72.7 (^{13}C–^{2}H enriched, t, J_{CD} = 22.0 Hz), 66.1, 55.3, 41.6 (d, J_{CC} = 35.5 Hz), 38.0, 26.7 (d, J_{CC} = 39.1 Hz), 10.4, 10.0; $[\alpha]_D$ −44.5° (*c* 0.89, $CHCl_3$); CIMS (NH_4^+) *m/z* (M + H)$^+$ − H_2O 276.

(2*S*,3*R*)-[3′-^{2}H,3′-^{13}C]-2-Methyl-3-hydroxypentanoic Acid (15b). To a stirred solution of 1.60 g (5.51 mmol) of **14b** in 150 mL of THF at 0 °C was added 690 mg (16.5 mmol) of LiOH·H_2O. After the mixture was stirred for 15 min, 10 mL (82.5 mmol) of 30% H_2O_2 was added dropwise. The reaction was stirred for 3 h at 0 °C. The excess H_2O_2 was quenched by adding 50 mL of 10% Na_2SO_3. After removal of THF by rotary evaporation, the basic aqueous phase was extracted with 5 × 50 mL of benzene. The aqueous phase was acidified to pH 2.5 with 5% HCl and saturated with NaCl. This solution was continuously extracted with Et_2O at room temperature for 24 h to yield 700 mg (96%) of the acid **15b** as an oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.62 (dq, J = 3.49, 7.2 Hz, 1 H, C_2-H), 1.52 (m, 2 H, C_4-H), 1.17 (ddd, J = 1.13, 4.26, 7.15 Hz, 3 H, C_2-CH_3), 0.97 (dt, J = 5.47, 7.34 Hz, 3 H, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.4 (d, J = 55.8 Hz), 73.5 (natural abundance), 73.0 (^{13}C–^{2}H enriched, t, J_{CD} = 22.0 Hz), 43.5 (d, J_{CC} = 36.5 Hz), 26.4 (d, J_{CC} = 38.7 Hz), 10.3, 10.0.

Unlabeled 15. (2*S*,3*R*)-2-Methyl-3-hydroxypentanoic acid (**15**) was prepared in the same manner as described for labeled **15b**. **15**: IR (neat) ν 3313, 2973, 1710, 1461, 1209, 972 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.91–3.87 (m, 1 H, C_3-H), 2.64–2.58 (dq, J = 3.6, 7.2 Hz, 1 H, C_2-H), 1.57–1.47 (m, 2 H, C_4-H), 1.20 (d, J = 7.21 Hz, 3 H, C_2-CH_3), 0.948 (t, J = 7.42 Hz, 3 H, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.0, 73.4, 43.7, 26.6, 10.3, 10.2; $[\alpha]_D$ +2.52° (*c* 1.8, $CHCl_3$); CIMS (NH_4^+) *m/z* (M + H)$^+$ 133.

***N*-Acetylcysteamine Thioester of (2*S*,3*R*)-[3′-^{2}H,3′-^{13}C]-2-Methyl-3-hydroxypentanoic Acid (5b).** Under an argon atmosphere, 425 mg (3.17 mmol) of **15b** was dissolved in 10 mL of DMF in a 25-mL round-bottom flask. After cooling to 0 °C, the solution was treated with diphenylphosphoryl azide (1.37 mL, 6.34 mmol) and 1.77 mL (12.7 mmol) of Et_3N. This mixture was stirred at 0 °C for 2 h, and the freshly-prepared *N*-acetylcysteamine (3.77 g, 31.7 mmol) was added. The reaction was allowed to stir at room temperature for 24 h. Addition of 25 mL of H_2O followed by extraction with 4 × 50 mL of EtOAc gave a combined organic extract which was dried over Na_2SO_4. Concentration followed by flash chromatography (silica, 50 g, 6% MeOH/94% $CHCl_3$) yielded 325 mg (52%) of the product **5b** as a colorless oil: IR ν 3302, 3088, 2973, 2878, 1660, 1552, 1453, 1372, 1291, 947, 757 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.4 (broad, 1 H, NH), 3.39 (m, 2 H, NCH_2), 3.00 (m, 2 H, SCH_2), 2.72 (dq, J = 3.4, 7.0 Hz, 1 H, $C_{2'}$-H), 1.9 (s, 3 H, C(O)CH_3), 1.45 (m, 2 H, $C_{4'}$-H), 1.17 (dd, J = 4.13, 7.04 Hz, 3 H, $C_{2'}$-CH_3), 1.26 (dt, J = 5.3, 7.41 Hz, 3 H, $C_{5'}$-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.8, 170.6, 73.6 (natural abundance), 73.1 (enriched, t, J_{CD} = 21.9 Hz), 52.94 (d, J_{CC} = 35.5 Hz), 39.2, 28.4, 27.0 (d, J_{CC} = 38.8 Hz), 23.0, 11.1, 10.3; $[\alpha]_D$ +7.90° (*c* 7.3, $CHCl_3$); CIMS (NH_4^+) *m/z* (M + H)$^+$, 236.

Unlabeled 5. The *N*-acetylcysteamine thioester of (2*S*,3*R*)-2-methyl-3-hydroxypentanoic acid (**5**) was prepared following the same procedure described for the labeled **5b**: oil; R_f 0.35 (10% MeOH/0.1% NH_4OH/$CHCl_3$); IR (neat) ν 3310, 3087, 2972, 2934, 2879, 1662, 1549, 1454, 1372, 1291, 1220, 963, 757 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.8 (b, 1 H, NH), 3.85 (m, 1 H, $C_{3'}$-H), 3.49–3.43 (m, 2 H, CH_2N), 3.06–3.02 (m, 2 H, CH_2S), 2.74 (dq, J = 3.66, 7.06 Hz, 1 H, $C_{2'}$-H), 1.97 (s, 3 H, CH_3CO), 1.6–1.4 (m, 2 H, $C_{4'}$-H), 1.22 (d, J = 7.07 Hz, 3 H, $C_{2'}$-CH_3), 0.98 (t, J = 7.42 Hz, $C_{5'}$-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.1, 170.4, 73.6, 53.0, 39.4, 28.6, 27.1, 23.2, 11.1, 10.4; $[\alpha]_D$ +11.31° (*c* 10, $CHCl_3$); CIMS (NH_4^+) *m/z* (M + H)$^+$ 234.

Incorporation of 5b. (1) Administration of 5b to Replacement Cultures of *N. argentinensis*. A mixture of 80 mg of the *N*-acetylcysteamine thioester of (2*S*,3*R*)-[3′-^{2}H,3′-^{13}C]-2-methyl-3-hydroxypentanoic acid (**5b**) and 15 mg of 4-pentynoic acid was dissolved in 1 mL of 100% ethanol and fed in one portion to a 24-h replacement culture of *N. argentinensis*. The fermentation was continued for 3 additional days before harvesting. The final pH of the replacement medium was 9.6. The final pH of a control fermentation conducted without addition of precursors was 9.8. Extraction of the broth gave 28 mg of a crude yellow solid. This was subjected to chromatography, which afforded 3.5 mg of pure nargenicin. The purified labeled nargenicin was analyzed by 100.61-MHz ^{13}C NMR.

(2) Administration of 5b to Actively Fermenting Cultures of *N. argentinensis*. A mixture of 80 mg of **5b** and 15 mg of 4-pentynoic acid was dissolved in 1 mL of 100% ethanol and fed in one portion to a 24-h production culture of *N. argentinensis*. The fermentation was continued for 4 additional days before harvesting. The final pH of the fermentation medium was 8.3. The final pH of a control fermentation conducted without addition of precursors was 9.7. Extraction of the broth gave 17 mg of a crude yellow solid. This was subjected to chromatography, which afforded 4.5 mg of pure nargenicin. The purified labeled nargenicin was analyzed by 100.61-MHz ^{13}C NMR.

(4*S*)-3-[1′-^{13}C]Propionyl-4-benzyl-2-oxazolidinone (12c). To a flame-dried 250-mL 3-necked round-bottom flask under nitrogen were added *S*-(−)-4-benzyl-2-oxazolidinone (3.05 g, 17.2 mmol) and 100 mL of THF. With magnetic stirring, the mixture was placed in a dry ice/acetone bath for 10 min. *n*-Butyllithium (10.8 mL, 17.2 mmol, 1.6 M in hexane) was added via syringe. This mixture was stirred for 10 min and [1-^{13}C]propionyl chloride (1.61 g, 17.0 mmol) was added. After 10 min the cooling bath was removed and the reaction was allowed to warm to 0 °C over 30 min. The reaction was quenched with 20 mL of saturated NH_4Cl solution. The THF was removed by rotary evaporation and the mixture was extracted with 3 × 50 mL of CH_2Cl_2. The combined organic extract was washed with 20 mL of 1 N NaOH and subsequently with 20 mL of brine. The organic phase was dried with Na_2SO_4 and concentrated to yield a yellow oil. Chromatography of this oil (5 × 20 cm, silica, 40% EtOAc/60% hexanes) afforded 3.8 g (95%) of the desired product **12c** as white crystals: mp 43.5–44.5 °C; IR ($CHCl_3$) ν 3027, 1778, 1684, 1384, 1090, 1013 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.18 (m, 5 H, aromatic), 4.65 (m, 1 H, C_4-H), 4.17 (m, 2 H, C_5-H), 3.27 (dd, J = 3.2, 13.4 Hz, 1 H, C_{6a}-H), 3.01–2.86 (m, 2 H, $C_{2'}$-H), 2.77 (dd, J = 3.2, 13.4 Hz, 1 H, C_{6b}-H), 1.18 (dt, J = 5.56, 7.32 Hz, 3 H, $C_{3'}$-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8 (enriched), 153.4, 135.3, 129.3, 128.8, 127.2, 66.0, 55.0, 37.3, 29.0 (d, J = 50 Hz), 8.1; R_f 0.69 (35% EtOAc/hexane); $[\alpha]_D$ +59.5° (*c* 2, $CHCl_3$); EIMS *m/z* M$^+$ 234.

(4*S*,2′*S*,3′*R*)-[1′-^{13}C]-3-(2′-Methyl-3′-hydroxypentanoyl)-4-benzyl-2-oxazolidinone (14c). To 125 mL of dry CH_2Cl_2 at 0 °C were added 938

mg (4 mmol) of benzyloxazolidinone **12c**, 4.4 mL (4.4 mmol, 1 M in CH_2Cl_2) of Bu_2BOTf, and 0.8 mL (4.6 mmol) of diisopropylethylamine. The mixture was stirred for 30 min at 0 °C followed by stirring at −78 °C for 30 min, and then 3.6 mL (4.8 mmol) of distilled propionaldehyde was added in one portion. The reaction mixture was stirred at −78 °C for 30 min and then allowed to warm to room temperature over 2 h. After recooling to 0 °C, the reaction was quenched by the addition of 4.6 mL of phosphate buffer (pH 7.4), poured into a 250-mL flask containing 20 mL of MeOH, cooled to 0 °C, and treated with a solution of 25 mL of precooled 30% aqueous H_2O_2 for 1 h. The organic solvents were removed in vacuo, aqueous $NaHCO_3$ (50 mL) was added, and the resultant solution was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated to a colorless oil. Flash chromatography (50 g of silica, 10% EtOAc/$CHCl_3$) afforded 1.06 g (91%) of the desired product **14c** as a white solid, which was recrystallized from ether: mp 94–95 °C; IR ($CHCl_3$) ν 3481, 2970, 2878, 1779, 1649, 1455, 1386, 1213, 1112, 969, 757 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35–7.15 (m, 5 H, aromatic), 4.71 (m, 1 H, C_4-H), 4.20 (m, 2 H, C_5-H), 3.76 (m, 1 H, $C_{3'}$-H), 3.69 (m, 1 H, $C_{2'}$-H), 3.25 (dd, 1 H, J = 13.4, 3.31 Hz, C_{6a}-H), 2.86 (br, 1 H, $C_{3'}$-OH), 2.79 (dd, 1 H, J = 13.4, 9.4 Hz, C_{6b}-H), 1.62–1.41 (m, 2 H, $C_{4'}$-H), 1.25 (dd, 3 H, J = 6.98, 1.75 Hz, $C_{2'}$-CH_3), 0.98 (t, 3 H, J = 7.43 Hz, $C_{5'}$-H); ^{13}C NMR (100 MHz, $CDCl_3$) 177.0 (enriched), 152.9 (d, J_{CC} = 4.5 Hz), 134.9, 129.2, 128.7, 127.2, 70.3 (d, J_{CC} = 2.2 Hz), 54.9, 41.7 (d, J_{CC} = 49.3 Hz), 37.5, 26.8 (d, J_{CC} = 3.6 Hz), 10.2; $[\alpha]_D$ +50.7° (c 1.1, $CHCl_3$); CIMS (NH_4^+) m/z (M + H)$^+$ 293.

Unlabeled 14. Unlabeled (4*S*,2′*S*,3′*R*)-3-(2′-methyl-3′-hydroxypentanoyl)-4-benzyl-2-oxazolidinone (**14**) was prepared according to the same procedure described for the labeled **14c**: R_f 0.35 (10% EtOAc in $CHCl_3$); IR ν 3486, 3013, 2990, 1778, 1702, 1384, 1243, 1226, 744 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.19 (m, 5 H, aromatic) 4.73–4.69 (m, 1 H, C_4-H), 4.25–4.17 (m, 2 H, C_5-H), 3.87 (m, 1 H, $C_{3'}$-H), 3.79 (dq, J = 2.8, 7.0 Hz, 1 H, $C_{2'}$-H), 3.26 (dd, J = 3.35, 13.4 Hz, 1 H, C_{6a}-H), 2.87 (b, 1 H, $C_{3'}$-OH), 2.79 (dd, J = 9.43, 13.4 Hz, 1 H, C_{6b}-H), 1.58–1.47 (m, 2 H, $C_{4'}$-H), 1.25 (d, J = 7.03 Hz, 3 H, $C_{2'}$-CH_3), 0.98 (t, J = 7.40 Hz, 3 H, $C_{5'}$-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 152.9, 134.9, 129.4, 129.0, 127.4, 73.3, 66.2, 55.3, 41.8, 38.0, 26.9, 10.4, 10.1; $[\alpha]_D$ +53.8° (c 2.1, $CHCl_3$); CIMS (NH_4^+) m/z (M + H)$^+$ 292.

Ethyl (2*E*,4*R*,5*R*)-[2,3-$^{13}C_2$]-2,4-Dimethyl-5-hydroxy-2-heptenoate (19c). To a 100-mL flame-dried flask under nitrogen charged with 25 mL of dry THF was added 0.79 mL (2.7 mmol, 1.1 equiv, 3.4 M in toluene) of sodium bis(2-methoxyethoxy)aluminum hydride. Following cooling to −78 °C, 720 mg (2.47 mmol, 1.0 equiv) of the β-hydroxy imide **14c** in 10 mL of THF was added slowly. The evolution of gas could be seen as the solution was stirred for 15 min at −78 °C and then warmed to −55 °C (dry ice with $CHCl_3$) and stirred between −50 and 60 °C for 1 h. The reaction was quenched at −50 °C with 4 mL of EtOAc and 1 mL of MeOH and then poured into a mixture of 14 mL of 5% HCl and 30 mL of Et_2O at −20 °C and stirred for 15 min. The frozen aqueous layer separated as a gel. The organic layer was decanted, and the gel was quickly rinsed twice (40 mL, Et_2O). The combined organic extracts were dried (K_2CO_3) and concentrated in vacuo to an oil to give 870 mg of the crude aldehyde (**16c**). This aldehyde was carried on immediately to the olefination step. **16c**: ^1H NMR (250 MHz, $CDCl_3$) δ 9.75 (d, J_{CH} = 175 Hz, 1 H CHO), 7.30 (m, aromatic), 4.5 (m, C_4-H, oxazolidinone), 4.1 (m, C_5-H, oxazolidinone), 3.50 (m, 1 H, C_3-H), 3.35 (s, methoxyethanol), 2.85 (m, C_6-H, oxazolidinone), 1.5–1.7 (m, 2 H, C_4-H), 1.15 (dd, 3 H, C_2-CH_3), 0.9 (m, C_5-H).

To 25 mL of THF in a 100-mL flask under nitrogen was added 500 mg (2.17 mmol, 1.15 equiv) of triethyl [2-^{13}C]-2-phosphonopropionate (**17**) (99 atom % ^{13}C), followed by 231 mg (2.06 mmol, 1.1 equiv) of potassium *tert*-butoxide.[16] This mixture was stirred at room temperature for 5 min and then cooled to −78 °C. The crude hydroxy aldehyde (**16c**) (870 mg, 1.0 equiv) in 25 mL of THF was then slowly added and the mixture was stirred overnight while warming to room temperature. The mixture was poured into brine, extracted with Et_2O (5 × 50 mL), dried (K_2CO_3), and concentrated in vacuo. Flash chromatography (20 g silica, 60% Et_2O/hexane) gave the ester **19c** (151 mg, 30.2% yield based on **14c**): R_f = 0.49 (60% Et_2O/hexane); $[\alpha]_D$ +22.9° (c 1.9, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.62 (dd, J = 10.1, 156 Hz, 1 H, $^{13}C_3$-H), 4.19 (q, 2 H, J = 7.1 Hz, OCH_2CH_3), 3.41 (m, 1 H, C_5-H), 2.58 (m, 1 H, C_4-H), 1.87 (m, 3 H, C_2-CH_3), 1.55 (m, 2 H, C_6-H), 1.30 (t, 3 H, J = 7.1 Hz, OCH_2CH_3), 1.06 (dd, 3 H, J = 4.9, 4.9 Hz, C_4-CH_3), 0.97 (t, 3 H, J = 7.4 Hz, C_7-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 143.9 (enriched, d, J_{CC} = 71.8 Hz), 127.7 (enriched, d, J_{CC} = 71.8 Hz), 76.7, 60.5, 39.3, 27.8, 15.3, 14.3, 12.7, 10.2.

Unlabeled 19. The unlabeled ethyl (2*E*,4*R*,5*R*)-2,4-dimethyl-5-hydroxy-2-heptenoate (**19**) was prepared according to the same procedure described for **19c**: R_f 0.49 (60% Et_2O/hexane); $[\alpha]_D$ +22.9° (c 1.9, $CHCl_3$) (lit.[17] $[\alpha]_D$ −25.0° (c, 1.6, $CHCl_3$) for (2*E*,4*R*,5*R*)-**19**); IR (neat) ν 3458, 2964, 2876, 2360, 1711, 1650, 1462, 1368, 1271, 1143, 1098, 975, 751; ^1H NMR (400 MHz, $CDCl_3$) δ 6.62 (dd, 1 H, J = 1.43, 10.3 Hz, C_3-H), 4.19 (q, 2 H, J = 7.1 Hz, OCH_2CH_3), 3.41 (m, 1 H, C_5-H), 2.58 (m, 1 H, C_4-H), 1.87 (d, 3, H, J = 1.46 Hz, C_2-CH_3), 1.55 (m, 2 H, C_6-H), 1.30 (t, 3 H, J = 7.1 Hz, OCH_2CH_3), 1.06 (d, 3 H, J = 6.74 Hz, C_4-CH_3), 0.97 (t, 3 H, J = 7.4 Hz, C_7-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 143.9, 127.7, 76.7, 60.5, 39.3, 27.8, 15.3, 14.3, 12.7, 10.2; CIMS (NH_4^+) m/z (M + H)$^+$ 201; exact mass calcd for $C_{11}H_{21}O_3$ 201.1412, found 201.1490.

(2*E*,4*R*,5*R*)-[2,3-$^{13}C_2$]-2,4-Dimethyl-5-hydroxy-2-heptenoic Acid (20c). To a 100-mL flask under nitrogen charged with 30 mL of MeOH and 10 mL of distilled H_2O were added 200 mg (0.99 mmol) of the ester **19c** and 420 mg (3.04 mmol, 3 equiv) of K_2CO_3. The solution was refluxed for 3 h. Methanol was removed by rotary evaporation and the residue was extracted with 60% ether in hexane. The aqueous layer was separated and acidified to pH 2.2. Extraction with ether (4 × 50 mL) followed by concentration afforded 159 mg of the acid **20c** (92%) as a colorless oil: ^1H NMR (250 MHz, $CDCl_3$) δ 6.74 (dd, 1 H, J = 11, 155 Hz, $^{13}C_3$-H), 3.48 (m, 1 H, C_5-H), 2.59 (m, 1 H, C_4-H), 1.86 (dd, 3 H, J = 5.5, 5.5 Hz, C_2-CH_3), 1.6–1.3 (m, 2 H, C_6-H), 1.07 (dd, 3 H, J = 5.7, 5.7 Hz, C_4-CH_3), 0.95 (t, 3 H, J = 7.3 Hz, C_7-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 146.7 (enriched, d, J_{CC} = 71.3 Hz), 126.8 (enriched, d, J_{CC} = 71.3 Hz), 76.6, 39.5, 27.8, 15.2, 12.3, 10.2.

Unlabeled 20. The unlabeled (2*E*,4*R*,5*R*)-2,4-dimethyl-5-hydroxy-2-heptenoic acid (**20**) was prepared following the same procedure described for labeled **20c**: IR ($CHCl_3$) ν 3501, 3033, 2965, 2933, 2874, 2589, 1723, 1655, 1386, 1276, 1123, 987 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 6.78 (dd, 1 H, J = 1.34, 10.41 Hz, C_3-H), 3.48 (m, 1 H, C_5-H), 2.59 (m, 1 H, C_4-H), 1.86 (d, 3 H, J = 1.36 Hz, C_2-CH_3), 1.6–1.3 (m, 2 H, C_6-H), 1.07 (d, 3 H, J = 6.76 Hz, C_4-CH_3), 0.95 (t, 3 H, J = 7.3 Hz, C_7-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 146.7, 126.8, 76.6, 39.5, 27.8, 15.2, 12.3, 10.2; $[\alpha]_D$ +34.5° (c 1.3, $CHCl_3$); CIMS (NH_4^+) m/z (M + H)$^+$ 173; exact mass calcd for $C_9H_{17}O_3$ 173.1178, found 173.1165.

(2*E*,4*R*,5*R*)-[2,3-$^{13}C_2$]-2,4-Dimethyl-5-hydroxy-2-heptenoic Acid *N*-Acetylcysteamine Thioester (8c).[19,28] To a solution of 159 mg (0.914 mmol) of the acid **20c** and 0.14 mL (1.01 mmol, 1.1 equiv) of triethylamine in 30 mL of THF was added a solution of 0.14 mL (0.96 mmol, 1.05 equiv) of diethyl phosphorochloridate in 2 mL of THF dropwise at room temperature under an argon atmosphere. The mixture was stirred at room temperature for 3 h, and the precipitated triethylamine hydrochloride was removed by filtration and washed with 2 × 10 mL of THF. To the combined filtrates and washings was added 9.6 mL (0.96 mmol, 1.05 equiv, 0.1 M in THF) of Tl (I) *N*-acyl-2-aminoethanethiolate (NAC thiolate), and the mixture was stirred at room temperature for 5 h. During this period the bright yellow color was discharged gradually and finally turned milky white. The mixture was filtered, THF was evaporated under reduced pressure, and ethyl acetate was added to the residue. The organic phase was rinsed with brine, dried with Na_2SO_4, and then concentrated in vacuo. Flash chromatography afforded 218 mg (79%) of the triketide thioester **8c** as a colorless oil: ^1H NMR (250 MHz, $CDCl_3$) δ 6.64 (dd, 1 H, J = 10.1, 150.6 Hz, $^{13}C_3$-H), 6.04 (br, 1 H, $NHCOCH_3$), 3.45 (m, 3 H, C_5-H and $CH_2NHCOCH_3$), 3.07 (t, 2 H, J = 6.3 Hz, SCH_2), 2.60 (m, 1 H, C_4-H), 1.98 (s, 3 H, $NCOCH_3$), 1.88 (dd, 3 H, J = 6.14, 6.14 Hz, C_2-CH_3), 1.6–1.3 (m, 2 H, C_6-H), 1.10 (dd, 3 H, J = 5.8, 5.8 Hz, C_4-CH_3), 0.98 (t, 3 H, J = 7.4 Hz, C_7-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.1, 170.3, 143.5 (enriched, d, J = 72.1 Hz), 135.5 (enriched, d, J = 72.1 Hz), 76.5, 39.8, 39.4, 28.6, 27.8, 23.2, 15.1, 12.8, 10.2.

Unlabeled 8. Unlabeled (2*E*,4*R*,5*R*)-2,4-dimethyl-5-hydroxy-2-heptenoic acid *N*-acetylcysteamine thioester (**8**) was prepared according to the same procedure described for **8c**: $[\alpha]_D$ +18.8° (c 2, $CHCl_3$); IR (neat) ν 3295, 3088, 2965, 2933, 2875, 1711, 1659, 1553, 1434, 1374, 1291, 1241, 1202, 1111, 1023, 976, 917, 874, 756, 705, 666 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.64 (d, 1 H, J = 10.1 Hz, C_3-H), 6.04 (br, 1 H, $NHCOCH_3$), 3.45 (m, 3 H, C_5-H and $CH_2NHCOCH_3$), 3.07 (t, 2 H, J = 6.3 Hz, SCH_2), 2.60 (m, 1 H, C_4-H), 1.98 (s, 3 H, $NCOCH_3$), 1.88 (dd, 3 H, J = 1.40, 6.14 Hz, C_2-CH_3), 1.6–1.3 (m, 2 H, C_6-H), 1.10 (d, 3 H, J = 6.75 Hz, C_4-CH_3), 0.98 (t, 3 H, J = 7.4 Hz, C_7-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.1, 170.3, 143.7, 135.4, 76.5, 39.8, 39.4, 28.6, 27.8, 23.2, 15.1, 12.8, 10.2; CIMS (NH_4^+) m/z (M + H)$^+$ 274; exact mass calcd for $C_{13}H_{24}NO_3S$ 274.1477, found 274.1490.

Incorporation of 8c. A mixture of **8c** (30 mg, 0.11 mmol) and 15 mg of 4-pentynoic acid was dissolved in 0.5 mL of 100% ethanol and fed in one portion to a 24-h production culture of *N. argentinensis*. This fermentation was continued for an additional 4 days before the culture was harvested. The final pH of the production medium was 8.2. Extraction of the broth gave 17 mg of crude yellow solid, from which 4 mg of pure

(28) Masamune, S.; Kamata, S.; Diakur, J.; Sugihara, Y.; Bates, G. S. *Can. J. Chem.* **1975**, *53*, 3693.

nargenicin could be obtained after chromatography. A control fermentation to which no precursor had been added yielded 4.5 mg of nargenicin. Labeled precursor **8c** (2 mg) could also be recovered from the fermentation broth. Examination of the 100.6-MHz ^{13}C NMR spectrum of labeled nargenicin revealed two sets of enhanced and coupled doublets (J = 73.0 Hz, 0.2 atom % enrichment) centered at δ 134.8 and 131.8 ppm, corresponding to C-14 and C-15, respectively.

(2*S*,3*R*)-3-Hydroxy-2-methyl-*N*-methoxy-*N*-methylpentanamide (21). To a suspension of 1.68 g (17.2 mmol) of *N*,*O*-dimethylhydroxylamine hydrochloride in 60 mL of CH_2Cl_2 at 0 °C was added 8.6 mL (17.2 mmol) of 2.0 M $AlMe_3$ in hexane over a 5-min period (**caution**: vigorous gas evolution).[20] After the addition was completed, the cooling bath was removed, and the clear solution was stirred for 30 min at room temperature. The solution was recooled to −15 °C, and a solution of 2.51 g (8.6 mmol) of the imide **14** in 15 mL of CH_2Cl_2 was added via cannula followed by an additional 10-mL rinse. The cloudy reaction mixture was stirred at 0–10 °C, at which temperature gas evolved steadily and the mixture slowly cleared. After 30 min, the clear solution was cooled to −20 °C and allowed to warm slowly overnight (16 h). The solution was then cannulated into 50 mL of 1 M aqueous tartaric acid, and the mixture was stirred vigorously for 1 h. The layers were separated, and the aqueous layer was extracted with 3 × 50 mL of CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. A majority (1.34 g, 88% recovery) of the recovered (4*S*)-4-benzyl-2-oxazolidinone could be recycled by crystallization from ether. Concentration of the supernatant gave the crude amide, which was purified by flash chromatography (silica, 50 g, 5% ethyl acetate in hexane) to afford **21** as a colorless oil (1.38 g, 92%): $[\alpha]_D$ +21° (c 2.2, $CHCl_3$); IR (neat) ν 3436, 1966, 1637, 1461, 1420, 1384, 1237, 1173, 991, 756 cm^{-1}; NMR (400 MHz, $CDCl_3$) δ 3.58 (s, 3 H, OCH_3), 3.07 (s, 3 H, NCH_3), 2.79 (m, 1 H, C_2-H), 1.42–1.38 (m, 2 H, C_4-H), 1.03 (d, J = 7.0 Hz, 3 H, C_2-CH_3), 0.84 (t, J = 7.4 Hz, 3 H, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 73.8, 61.2, 38.3, 32.6, 10.2, 10.1; R_f 0.23 (50% ethyl acetate in hexane); CIMS (NH_4^+) m/z $(M + H)^+$ 176; exact mass calcd for $C_8H_{18}O_3N$ 176.1286, found 176.1298.

(2*S*,3*R*)-2-Methyl-3-(*tert*-butyldimethylsiloxy)-*N*-methoxy-*N*-methylpentanamide (22). To a stirred solution of 870 mg (4.97 mmol) of the amide **21** in 20 mL of CH_2Cl_2 was added 1.71 mL (7.5 mmol) of TBDMSOTf at −15 °C. After 10 min, 1.74 mL (12.5 mmol) of triethylamine was added. The reaction was warmed slowly to room temperature over 1.5 h. After addition of 20 mL of brine, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with saturated $NaHCO_3$ solution, dried with Na_2SO_4, and concentrated in vacuo. Flash chromatography (50 g of silica, 5% EtOAc/hexane) afforded 1.41 g (98%) of the desired product **22**: R_f 0.78 (35% EtOAc/hexane); IR (neat) ν 3460, 2943, 2860, 1649, 1384, 1249, 1114, 1049, 997, 867, 832, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 3.90 (m, 1 H, C_3-H), 3.70 (s, 3 H, OCH_3), 3.17 (s, 3 H, NCH_3), 2.98 (m, 1 H, C_2-H), 1.50 (m, 2 H, C_4-H), 1.15 (d, J = 7 Hz, C_2-CH_3), 0.90 (m, 12 H, C_5-H + $SiC(CH_3)_3$), 0.07 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) 170.8, 74.0, 61.1, 60.3, 40.0, 28.3, 26.8, 18.1, 14.9, 8.6; $[\alpha]_D$ +3.46° (c 1.3, $CHCl_3$); CIMS (NH_4^+) m/z $(M + H)^+$ 290; calcd for $C_{14}H_{32}O_3NSi$: 290.2151, found: 290.2167.

(2*S*,3*R*)-2-Methyl-3-(*tert*-butyldimethylsiloxy)pentanal (23). A solution of 744 mg (2.57 mmol) of **22** in 20 mL of THF was stirred and cooled at −78 °C as 5.2 mL (5.2 mmol) of 1.0 M DIBAL in hexane was added slowly. The solution was stirred for an additional 30 min at −78 °C and allowed to warm to −50 °C over 30 min. The excess hydride was consumed by addition of 0.2 mL of acetone, and after 5 min the solution was poured into a vigorously stirred mixture of 50 mL of 0.5 M tartaric acid and 50 mL of hexane at 0 °C. After 30 min, the layers were separated, and the organic layer was washed with 20 mL each of water and brine. The aqueous layers were extracted with 2 × 40 mL of CH_2Cl_2, and the combined organic layers were dried (Na_2SO_4) and concentrated. Chromatography (50 g of silica gel, 10% Et_2O in hexane, high flow rate) yielded 573 mg (97%) of the aldehyde **23** as a colorless oil: R_f 0.81 (60% ether in hexane); IR (neat) ν 2957, 2858, 1711, 1471, 1386, 1255, 1102, 1045, 836, 755 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 9.75 (s, 1 H, CHO), 4.03 (m, 1 H, C_3-H), 2.48 (m, 1 H, C_2-H), 1.52 (m, 2 H, C_4-H), 1.04 (d, 3 H, J = 7.2 Hz, C_2-CH_3), 0.85 (m, overlapping, 3 H (C_5-H) and 9 H ($C(CH_3)_3$)); ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.5, 73.4, 50.8, 27.4, 25.7, 18.03, 10.1, 7.56, −4.24, −4.72; $[\alpha]_D$ +22.7° (c 2.4, $CHCl_3$); CIMS (NH_4^+) m/z $(M + H)^+$ 231; exact mass calcd for $C_{12}H_{27}O_2Si$ 231.1702, found 231.1713.

Ethyl (2*E*,4*R*,5*R*)-[1-^{13}C]-5-(*tert*-Butyldimethylsiloxy)-2,4-dimethyl-2-heptenoate (24d). The aldehyde **23** (540 mg, 2.34 mmol) was dissolved in 30 mL of THF and treated with 850 mg (2.34 mmol) of [[1-^{13}C]carbethoxyethylidene]triphenylphosphorane (99 atom % ^{13}C). The mixture was stirred at reflux for 24 h. After cooling to room temperature, the THF was removed by rotary evaporation and the residue was subjected to silica filtration with Et_2O to remove triphenylphosphine oxide. The filtrates were washed with 1 mL of $NaHSO_3$/ethanol solution (10 g of $NaHSO_3$ mixed with 3.8 mL of MeOH and 15 mL of H_2O) to remove the unreacted aldehyde. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to afford a colorless oil (402 mg, 54.3%): R_f 0.78 (60% Et_2O in hexane); ^1H NMR (400 MHz, $CDCl_3$) δ 6.69 (dd, 1 H, J = 1.4, 10.3 Hz, C_3-H), 4.20 (m, 2 H, $COOCH_2CH_3$), 3.51 (m, 1 H, C_5-H), 2.60 (m, 1 H, C_4-H), 1.85 (dd, 3 H, J = 1.44, 4.21 Hz, C_2-CH_3), 1.6–1.3 (m, 2 H, C_6-H), 1.29 (t, 3 H, J = 7.13 Hz, $COOCH_2CH_3$), 0.98 (d, 3 H, J = 6.76 Hz, C_4-CH_3), 0.88–0.80 (s and t, overlapping, J = 7.41 Hz, 12 H, $C(CH_3)_3$ and C_7-H), 0.05 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4 (enriched), 145.4, 126.5, 76.1, 60.4, 37.4, 27.7, 25.9, 18.2, 14.7, 14.3, 12.5, 9.1, −4.20, −4.64; $[\alpha]_D$ +13° (c 1.1, $CHCl_3$).

Unlabeled 24. The aldehyde **23** (1.5 g, 6.5 mmol) was dissolved in 100 mL of THF and treated with 7.25 g (20 mmol, Aldrich) of (carbethoxyethylidene)triphenylphosphorane. The mixture was stirred at reflux for 24 h. After the mixture was cooled to room temperature, the THF was removed by rotary evaporation and the residue was subjected to silica filtration with Et_2O to remove triphenylphosphine oxide. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to afford ethyl (2*E*,4*R*,5*R*)-5-(*tert*-butyldimethylsiloxy)-2,4-dimethyl-2-heptenoate (**24**) as a colorless oil (1.74 g, 85%): R_f 0.78 (60% Et_2O in hexane); IR (neat) ν 2931, 2858, 1713, 1651, 1463, 1367, 1255, 1085, 1018, 836, 775, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.69 (dd, 1 H, J = 1.38, 10.21 Hz, C_3-H), 4.20 (q, 2 H, J = 7.21 Hz, $O_2CH_2CH_3$), 3.51 (m, 1 H, C_5-H), 2.60 (m, 1 H, C_4-H), 1.85 (d, 3 H, J = 1.36 Hz, C_2-CH_3), 1.33–1.61 (m, 2 H, C_6-H), 1.29 (t, 3 H, J = 7.1 Hz, OCH_2CH_3), 0.98 (d, 3 H, J = 6.76 Hz, C_4-CH_3), 0.88–0.80 (s and t, overlapping, 12 H, J = 7.4 Hz, $C(CH_3)_3$ and C_7-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 145.4, 126.5, 76.1, 60.4, 37.4, 27.7, 25.9, 18.2, 14.7, 14.3, 12.5, 9.1, −4.20, −4.64; $[\alpha]_D$ +13° (c 1.2, $CHCl_3$); CIMS (NH_4^+) m/z $(M + H)^+$ 315; exact mass calcd for $C_{17}H_{35}O_3Si$ 315.2355, found 315.2343.

(2*E*,4*R*,5*R*)-[1-^{13}C]-5-(*tert*-Butyldimethylsiloxy)-2,4-dimethyl-2-heptenol (25d). To a cooled (−78 °C), stirred solution of the ester **24d** (300 mg, 0.95 mmol) in 20 mL of ether was added $LiAlH_4$ (3 mL, 1 M in THF) in one portion. The reaction was held at −78 °C for 30 min and at −20 °C for 30 min. After recooling to −78 °C, the reaction was quenched with $Na_2SO_4 \cdot H_2O$ (3 g). After 10 min the reaction mixture was filtered through a silica layer with Et_2O. The filtrate was concentrated. Flash chromatography (40 g of silica, 30% Et_2O in hexane) afforded **25d** as a colorless oil (234 mg, 90.2%): R_f 0.41 (60% Et_2O in hexane); ^1H NMR (250 MHz, $CDCl_3$) δ 5.28 (dd, 1 H, J = 8.29, 8.29 Hz, C_3-H), 4.00 (d, 2 H, J = 95 Hz, $^{13}C_1$-H), 3.44 (m, 1 H, C_5-H), 2.50 (m, 1 H, C_4-H), 1.68 (dd, 3 H, J = 1.16, 4.13 Hz, C_2-CH_3), 1.50 (m, 2 H, C_6-H), 0.94 (d, 3 H, J = 6.88 Hz, C_4-CH_3), 0.90 (s, 9 H, $SiC(CH_3)_3$), 0.85 (t, 3 H, J = 7.44 Hz, C_7-H), 0.05 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.5, 130.1, 76.7, 69.1 (enriched), 36.4, 27.3, 25.9, 25.7, 18.2, 16.1, 13.8, 9.05, −4.21, −4.46.

Unlabeled 25. Unlabeled (2*E*,4*R*,5*R*)-5-(*tert*-butyldimethylsiloxy)-2,4-dimethyl-2-heptenol (**25**) was prepared according to the same procedure described for **25d**: IR (neat) ν 3332, 2958, 2857, 1463, 1253, 1016, 939, 834, 773, 667 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.28 (dd, 1 H, J = 1.31, 10.13 Hz, C_3-H), 4.00 (s, 2 H, C_1-H), 3.44 (m, 1 H, C_5-H), 2.50 (m, 1 H, C_4-H), 1.68 (d, 3 H, J = 1.36 Hz, C_2-CH_3), 1.50 (m, 2 H, C_6-H), 0.94 (d, 3 H, J = 6.75 Hz, C_4-CH_3), 0.90 (s, 9 H, $SiC(CH_3)_3$), 0.85 (t, 3 H, J = 7.46 Hz, C_7-H), 0.05 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.5, 130.1, 76.7, 69.1, 36.4, 27.3, 25.9, 25.7, 18.2, 16.1, 13.8, 9.05, −4.21, −4.46; $[\alpha]_D$ +6.58° (c 1.22, $CHCl_3$); CIMS (NH_4^+) m/z $(M + H)^+$ 273, exact mass calcd for $C_{15}H_{33}O_2Si$ 273.2250, found 273.2238.

(2*E*,4*R*,5*R*)-[1-^{13}C]-5-(*tert*-Butyldimethylsiloxy)-2,4-dimethyl-2-heptenal (26d). A suspension of pyridinium chlorochromate (278 mg, 1.29 mmol) and activated neutral alumina (Aldrich, 1.36 g, 13.3 mmol) in CH_2Cl_2 (20 mL) was mixed for 15 min at room temperature. To the vigorously stirred dispersion was added dropwise a solution of **25d** (234 mg, 0.86 mmol) in CH_2Cl_2 (5 mL). After 4 h, the reaction mixture was passed through a short column of Florisil (30 mesh, elution with ether), and the filtrate was concentrated in vacuo to afford the aldehyde **26d** (220 mg, 94%): ^1H NMR (250 MHz, $CDCl_3$) δ 9.40 (d, 1 H, J = 175 Hz, CHO), 6.42 (dd, 1 H, J = 10.2, 10.1 Hz, C_3-H), 3.56 (m, 1 H, C_5-H), 2.82 (m, 1 H, C_4-H), 1.76 (dd, 3 H, J = 1.1, 3.60 Hz, C_2-CH_3), 1.51 (m, 2 H, C_6-H), 1.03 (d, 3 H, J = 6.82 Hz, C_4-CH_3), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.87 (t, 3 H, J = 7.44 Hz, C_7-H), 0.05 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.6 (enriched), 157.9, 138.1, 76.0, 37.8, 27.4, 25.9, 18.1, 14.6, 9.35, 9.19, −4.21, −4.52.

Unlabeled 26. Unlabeled (2*E*,4*R*,5*R*)-5-(*tert*-butyldimethylsiloxy)-2,4-dimethyl-2-heptenal (**26**) was prepared according to the procedure described for **26d**: IR (neat) ν 2931, 2360, 1711, 1644, 1451, 1377, 1120,

1029, 891 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.40 (s, 1 H, CHO), 6.42 (dd, 1 H, J = 1.34, 10.0 Hz, C_3-H), 3.56 (m, 1 H, C_5-H), 2.82 (m, 1 H, C_4-H), 1.76 (dd, 3 H, J = 1.1, 3.6 Hz, C_2-CH_3), 1.62–1.40 (m, 2 H, C_6-H), 1.03 (d, 3 H, J = 6.78 Hz, C_4-CH_3), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.87 (t, 3 H, J = 7.45 Hz, C_7-H), 0.05 (s, 6 H, $Si(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.6, 157.9, 138.1, 76.0, 37.8, 27.4, 25.9, 18.1, 14.6, 9.35, 9.19, –4.21, –4.52; $[\alpha]_D$ +4.36° (c 1.1, $CHCl_3$); CIMS (NH_4^+) m/z $(M + H)^+$ 271; exact mass calcd for $C_{15}H_{31}O_2Si$ 271.2093, found 271.2079.

Ethyl (2*E*,4*E*,6*R*,7*R*)-[2,3-$^{13}C_2$]-7-(*tert*-Butyldimethylsiloxy)-4,6-dimethyl-2,4-nonadienoate (27d). To a stirred, cooled (–78 °C) solution of 183 mg (0.81 mmol) of triethyl [2-^{13}C]phosphonoacetate (99 atom % ^{13}C) was added 0.77 mL (Aldrich, 1.0 M in THF, 0.77 mmol, 1.1 equiv) of potassium *tert*-butoxide at room temperature. After 10 min, the mixture was cooled to –78 °C and reacted with 200 mg (0.74 mmol) of the aldehyde **26d** in 5 mL of THF, followed by an additional 5-mL rinse. The reaction was allowed to warm to room temperature overnight. The mixture was poured into brine and extracted with 60% Et_2O/hexane (3 × 30 mL). The organic layer was concentrated and passed through a short layer of silica with Et_2O elution. The filtrate was concentrated in vacuo to afford **27d** as a colorless oil (231 mg, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (ddd, 1 H, J = 2.27, 15.7, 160 Hz, $^{13}C_3$-H), 5.80 (dd, 1 H, J = 15.7, 160 Hz, $^{13}C_2$-H), 5.75 (dd, 1 H, J = 9.48, 9.54 Hz, C_5-H), 4.21 (q, 2 H, J = 7.11 Hz, $COOCH_2CH_3$), 3.48 (m, 1 H, C_7-H), 2.65 (m, 1 H, C_6-H), 1.78 (dd, 3 H, J = 1.18, 3.82 Hz, C_4-CH_3), 1.48 (m, 2 H, C_8-H), 1.30 (t, 3 H, J = 7.14 Hz, $COOCH_2CH_3$), 0.98 (d, 3 H, J = 6.74 Hz, C_6-CH_3), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.85 (t, 3 H, J = 7.44 Hz, C_9-H), 0.05 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 150.0 (enriched, d, J = 70.4 Hz), 145.4, 131.6, 115.8 (enriched, d, J = 70.4 Hz), 76.6, 60.1, 37.7, 27.5, 25.9, 18.1, 15.7, 14.3, 12.4, 8.96, –4.24, –4.50.

Unlabeled 27. Unlabeled ethyl (2*E*,4*E*,6*R*,7*R*)-7-(*tert*-butyldimethylsiloxy)-4,6-dimethyl-2,4-nonadienoate (**27**) was prepared according to the same procedure described for **27d**: R_f 0.83 (60% Et_2O/hexane); IR (neat) ν 2958, 2930, 2857, 1719, 1626, 1463, 1368, 1307, 1256, 1173, 1028, 836, 774 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, 1 H, J = 15.7 Hz, C_3-H), 5.80 (d, 1 H, J = 15.7 Hz, C_2-H), 5.75 (d, 1 H, J = 9.42 Hz, C_5-H), 4.21 (q, 2 H, J = 7.11 Hz, $COOCH_2CH_3$), 3.48 (m, 1 H, C_7-H), 2.65 (m, 1 H, C_6-H), 1.78 (d, 3 H, J = 1.15 Hz, C_4-CH_3), 1.48 (m, 2 H, C_8-H), 1.30 (t, 3 H, J = 7.07 Hz, $COOCH_2CH_3$), 0.98 (d, 3 H, J = 6.74 Hz, C_6-CH_3), 0.89 (s, 9 H, $SiC(CH_3)_3$), 0.85 (t, 3 H, J = 7.43 Hz, C_9-H), 0.05 (s, 6 H, $Si(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167, 150.0, 145.4, 131.6, 115.8, 76.6, 60.1, 37.7, 27.5, 25.9, 18.1, 15.7, 14.3, 12.4, 8.96, –4.24, –4.50; $[\alpha]_D$ 6.79° (c 1.4, $CHCl_3$); CIMS (NH_4^+) m/z $(M + H)^+$ 341; exact mass calcd for $C_{19}H_{37}O_3Si$ 341.2512, found 341.2501.

Ethyl (2*E*,4*E*,6*R*,7*R*)-[2,3-$^{13}C_2$]-7-Hydroxy-4,6-dimethyl-2,4-nonadienoate (28d). A solution of 230 mg (0.67 mmol) of **27d** in THF was treated with 0.77 mL (Aldrich, 1 M in THF, containing 5% H_2O) of n-$Bu_4N^+F^-$ at room temperature. The mixture was stirred for 16 h. After they were washed with brine and extracted with EtOAc (3 × 50 mL), the organic layers were dried with Na_2SO_4 and concentrated in vacuo. Flash chromatography (20 g of silica, 60% ether in hexane) afforded 131 mg of the ester **28d** (97%): R_f = 0.44 (60% Et_2O/hexane); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.30 (ddd, 1 H, J = 2.26, 15.6, 150 Hz, C_3-H), 5.81 (dd, 1 H, J = 15.6, 167.5 Hz, C_2-H), 5.75 (dd, 1 H, J = 9.40, 9.44 Hz, C_5-H), 4.20 (q, 2 H, J = 7.11 Hz, $COOCH_2CH_3$), 3.38 (m, 1 H, C_7-H), 2.61 (m, 1 H, C_6-H), 1.81 (dd, 3 H, J = 1.22, 3.78 Hz, C_4-CH_3), 1.50 (m, 2 H, C_8-H), 1.30 (t, 3 H, J = 7.14 Hz, $COOCH_2CH_3$), 1.05 (d, 3 H, J = 6.74 Hz, C_6-CH_3), 0.95 (t, 3 H, J = 7.40 Hz, C_9-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 150.0 (enriched, J_{CC} = 70.3 Hz), 145.3, 131.4, 115.8 (enriched, J_{CC} = 70.3 Hz), 76.7, 60.1, 38.0, 27.6, 15.7, 14.4, 12.4, 8.97.

Unlabeled 28. Unlabeled ethyl (2*E*,4*E*,6*R*,7*R*)-7-hydroxy-4,6-dimethyl-2,4-nonadienoate (**28**) was prepared according to the procedure described for **28d**: $[\alpha]_D$ +6.79° (c 1.4, $CHCl_3$); IR (neat) ν 3427, 2961, 2875, 2360, 2095, 2076, 1714, 1622, 1435, 1372, 1284, 1171, 1116, 1024, 978, 847, 730; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (dd, 1 H, J = 0.70, 14.88 Hz, C_3-H), 5.81 (dd, 1 H, J = 0.29, 15.7 Hz, C_2-H), 5.75 (d, 1 H, J = 10.1 Hz, C_5-H), 4.20 (q, 2 H, J = 7.13 Hz, $COOCH_2CH_3$), 3.38 (m, 1 H, C_7-H), 2.61 (m, 1 H, C_6-H), 1.81 (d, 3 H, J = 1.21 Hz, C_4-CH_3), 1.60 (m, 2 H, C_8-H), 1.30 (t, 3 H, J = 7.16 Hz, $COOCH_2CH_3$), 1.05 (d, 3 H, J = 6.74 Hz, C_6-CH_3), 0.95 (t, 3 H, J = 7.39 Hz, C_9-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 150.0, 145.3, 131.4, 115.8, 76.7, 60.1, 38.0, 27.6, 15.7, 14.4, 12.4, 8.97; CIMS (NH_4^+) m/z $(M + H)^+$ 227; exact mass calcd for $C_{13}H_{23}O_3$ 227.1647, found 227.1633.

(2*E*,4*E*,6*R*,7*R*)-[2,3-$^{13}C_2$]-7-Hydroxy-4,6-dimethyl-2,4-nonadienoic Acid (29d). To a 100-mL flask under nitrogen charged with 18 mL of MeOH and 6 mL of distilled H_2O was added 349 mg (2.53 mmol) of potassium carbonate and 116 mg (0.51 mmol) of ester **28d**. The solution was heated under reflux for 3 h. Methanol was removed by rotary evaporation. The remaining aqueous layer was acidified to pH 2 with 2.5 N HCl, followed by saturation with NaCl and extraction with Et_2O (4 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated by rotary evaporation. The desired product **29d** (94 mg, 92%) was obtained as a colorless oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.40 (ddd, 1 H, J = 2.37, 15.8, 150 Hz C_3-H), 5.82 (dd, 1 H, J = 9.25, 9.47 Hz, C_5-H), 5.81 (dd, 1 H, J = 15.8, 155 Hz, C_2-H), 3.40 (m, 1 H, C_7-H), 2.61 (m, 1 H, C_6-H), 1.83 (dd, J = 1.07, 13.2 Hz, C_4-CH_3), 1.65–1.35 (m, 2 H, C_8-H), 1.06 (d, 3 H, J = 6.74 Hz, C_6-CH_3), 0.95 (t, 3 H, J = 7.39 Hz, C_9-H).

Unlabeled 29. Unlabeled (2*E*,4*E*,6*R*,7*R*)-7-hydroxy-4,6-dimethyl-2,4-nonadienoic acid (**29**) was prepared according to the procedure described for **29d**: IR ($CHCl_3$) ν 3490, 3045, 2978, 2931, 2602, 1678, 1625, 1455, 1267, 1238, 1110, 967, 755; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.40 (d, 1 H, J = 15.7 Hz, C_3-H), 5.82 (d, 1 H, J = 10.9 Hz, C_5-H), 5.80 (d, 1 H, J = 15.7 Hz, C_2-H), 3.40 (m, 1 H, C_7-H), 2.61 (m, 1 H, C_6-H), 1.83 (dd, J = 1.07, 13.2 Hz, C_4-CH_3), 1.65–1.35 (m, 2 H, C_8-H), 1.06 (d, 3 H, J = 6.72 Hz, C_6-CH_3), 0.96 (t, 3 H, J = 7.38 Hz, C_9-H); ^{13}C NMR (100 MHz) δ 172.4, 151.7, 145.3, 132.6, 115.4, 77.0, 39.2, 27.7, 15.6, 12.5, 10.2; $[\alpha]_D$ +12.3° (c 2.2, $CHCl_3$); CIMS (NH_4^+) m/z $(M + H)^+$ 199; exact mass calcd for $C_{11}H_{19}O_3$ 199.1329, found 199.1313.

(2*E*,4*E*,6*R*,7*R*)-[2,3-$^{13}C_2$]-7-Hydroxy-4,6-dimethyl-2,4-nonadienoic Acid *N*-Acetylcysteamine Thioester (9d). To a solution of 94 mg (0.47 mmol) of **29d** and 0.072 mL (0.52 mmol, 1.1 equiv) of triethylamine in 30 mL of THF was added a solution of 0.071 mL (0.49 mmol, 1.05 equiv) of diethyl phosphorochloridate in 1 mL of THF dropwise at room temperature under argon atmosphere. The mixture was stirred at room temperature for 3 h, and the precipitated triethylamine hydrochloride was removed by filtration and washed with 2 × 5 mL of THF. To the combined filtrates and washings was added 6 mL (0.6 mmol, 1.05 equiv, 0.1 M in THF) of Tl(I) *N*-acyl-2-aminoethanethiolate (NAC thiolate), and the mixture was stirred at room temperature for 6 h. During this period the bright yellow color was discharged gradually. The milky white mixture was filtered, the THF was evaporated under reduced pressure, and EtOAc was added to the residue. The organic phase was rinsed with brine, dried over Na_2SO_4, and concentrated in vacuo. Flash chromatography afforded 78 mg (55%) of the tetraketide thioester **9d** as a colorless oil: R_f 0.20 (80% EtOAc/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (ddd, 1 H, J = 1.51, 15.6, 148 Hz, C_3-H), 6.13 (dd, 1 H, J = 15.7, 172 Hz, C_2-H), 5.95 (br, 1 H, $NHCOCH_3$), 5.86 (d, 1 H, J = 9.1 Hz, C_5-H), 3.48 (q, 2 H, J = 6.01 Hz, CH_2NCOCH_3), 3.40 (m, 1 H, C_7-H), 3.12 (t, 2 H, J = 6.57 Hz, SCH_2), 2.65 (m, 1 H, C_6-H), 1.98 (s, 3 H, $NHCOCH_3$), 1.81 (dd, 3 H, J = 1.12, 3.84 Hz, C_4-CH_3), 1.70 (br, 1 H, C_7-OH), 1.6 (m, 1 H, C_{8a}-H), 1.35 (m, 1 H, C_{8b}-H), 1.08 (d, 3 H, J = 6.73 Hz, C_6-H), 0.98 (t, 3 H, J = 7.39 Hz, C_9-H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 190.4, 170.0, 146.7 (d, J = 69.8 Hz), 146.5, 132.4, 123.0 (d, J = 69.9 Hz), 76.7, 40.0, 39.5, 28.3, 27.8, 23.2, 15.6, 12.5, 10.2; CIMS (NH_4^+) m/z $(M + H)^+$ 302; exact mass calcd for $C_{13}{}^{13}C_2H_{26}O_3NS$ 302.1695, found 302.1701.

Unlabeled 9. Unlabeled (2*E*,4*E*,6*R*,7*R*)-7-hydroxy-4,6-dimethyl-2,4-nonadienoic acid *N*-acetylcysteamine thioester (**9**) was prepared according to the procedure described for **9d**: IR (neat) ν 3302, 2968, 2934, 1658, 1549, 1440, 1375, 1288, 1104, 1025, 976 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (dd, 1 H, J = 0.71, 15.5 Hz, C_3-H), 6.12 (dd, 1 H, J = 0.30, 15.5 Hz, C_2-H), 5.95 (br, 1 H, $NHCOCH_3$), 5.87 (d, 1 H, J = 10.11 Hz, C_5-H), 3.48 (q, 2 H, J = 5.93 Hz, CH_2NCOCH_3), 3.40 (m, 1 H, C_7-H), 3.12 (t, 2 H, J = 6.58 Hz, SCH_2), 2.65 (m, 1 H, C_6-H), 1.98 (s, 3 H, $NHCOCH_3$), 1.81 (d, J = 1.20 Hz, C_4-CH_3), 1.70 (br, 1 H, C_7-OH), 1.6 (m, 1 H, C_{8a}-H), 1.35 (m, 1 H, C_{8b}-H), 1.08 (d, 3 H, J = 6.73 Hz, C_6-H), 0.98 (t, 3 H, J = 7.38 Hz, C_9-H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 190.4, 170.0, 146.7, 146.5, 132.4, 123.0, 76.7, 40.0, 39.5, 28.3, 27.8, 23.2, 15.6, 12.5, 10.2; $[\alpha]_D$ +16.9° (c 0.55, $CHCl_3$).

Incorporation of 9d. A mixture of 50 mg (0.17 mmol) of **9d** and 14.6 mg of 4-pentynoic acid was dissolved in 0.75 mL of 100% ethanol and fed in one portion to a 24-h production culture of *N. argentinensis*. The fermentation was continued for 4 more days before harvesting. The final pH of the production medium was 8.1. The final pH of a control fermentation conducted without addition of precursors was 9.7. Extraction of the broth gave 22 mg of a crude yellow solid. This was subjected to chromatography, which afforded 4.9 mg of pure nargenicin. Analysis by ^{13}C NMR (100 MHz, CD_2Cl_2) revealed the presence of two sets of enhanced and coupled doublets (J_{CC} = 35.4 Hz) centered at δ 49.0 and 89.0 ppm, corresponding to C-12 and C-13, respectively.

Acknowledgment. This work was supported by a grant from the National Institutes of Health, GM22172, and by the NIH (RR02231)–USDOE/OHER Stable Isotope Resource program at Los Alamos.