chromatography of **8a,b** (0.085 g)[14] through a grade III alumina column with toluene eluant. The front of the red brown band typically contains >98% **8a** (ca. 0.005 g), and further crops can be obtained by recycling material. The separation of **8a** and **8b** suggests that related diastereomers may also be separable in this system and that detailed stereochemical studies will be feasible for many of the important reactions known to occur in tungstenocene complexes.[4]

Diastereomers and enantiomers of pseudotetrahedral bent metallocene complexes have been prepared previously by the Dijon group[15] and have been used to probe the mechanisms of insertion of SO_2 into Ti–C bonds[16] and of σ-ligand exchange[17] in titanocene complexes. Their approach, however (sequential addition of unsubstituted and substituted cyclopentadienides to transition-metal halides), is limited in terms of the accessible functionalities and the halides which are suitable substrates. We have not, for example, been able to prepare chiral tungstenocene derivatives in this way.[18] Nucleophilic addition to **1** represents a fundamentally new approach to the preparation of complexes with functionalized cyclopentadienyl ligands, and similar methods may be applicable in other systems.

Acknowledgment. We thank the National Science Foundation for financial support.

Supplementary Material Available: Spectroscopic and analytical data for compounds **2**, **3**, **4**, **5**, **6**, **7**, **8a**, and **8b** (all new complexes gave satisfactory analyses) (2 pages). Ordering information is given on any current masthead page.

(14) Chromatography was considerably simplified if the mixture was first enriched to ca. 2:1 **8a**:**8b** by selective crystallization of **8b** enriched solids from toluene at low temperature to leave an **8a** enriched supernatant.

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Macrolide Biosynthesis. 5. Intact Incorporation of a Chain-Elongation Intermediate into Nargenicin†

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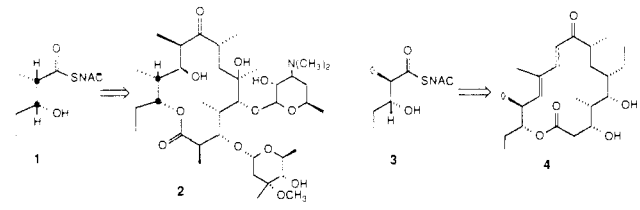
It is generally accepted that the formation of the polyketide carbon skeleton of macrolide antibiotics takes place by a mechanism analogous to the well-studied chain-elongation steps of fatty acid biosynthesis. Substantial indirect evidence, based on the origin of the carbon skeleton, oxygen atoms, and hydrogen atoms of macrolide and polyether antibiotics,[1] has led to the reasonable conclusion that the oxidation level and stereochemistry of the growing reduced polyketide chain are adjusted subsequent to each condensation step involving successive units of malonyl-, methylmalonyl-, and ethylmalonyl-CoA, as required. The absence of any detectable intermediates of the chain-elongation process even in mutants blocked in the biosynthesis of the parent polyketide chains[2] has been a significant impediment to further progress as has the lack of any viable cell-free preparations mediating the formation of any macrolide or polyether.

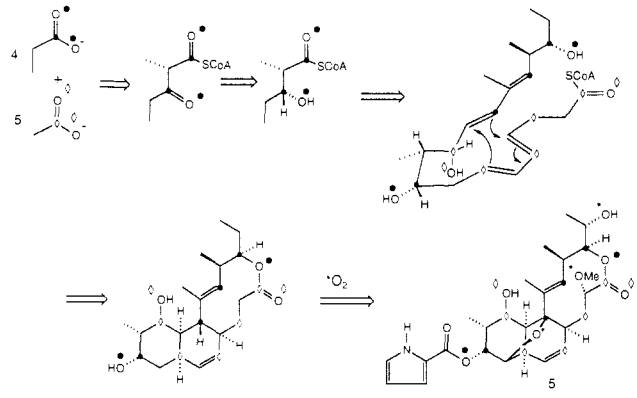
Recently, we reported the intact incorporation of an intermediate of polyketide chain elongation into the macrolide erythromycin.[3] Thus feeding of the *N*-acetylcysteamine (NAC) thioester of (2*S*,3*R*)-[2,3-$^{13}C_2$]-2-methyl-3-hydroxypentanoic acid (**1**) to cultures of *Saccharopolyspora erythraea* (formerly *Streptomyces erythreus*) gave erythromycin B (**2**) labeled at the expected positions C-12 and C-13, as determined by ^{13}C NMR analysis. Simultaneously, Hutchinson and his co-workers reported the analogous incorporation of the NAC thioester of (2*R*,3*R*)-2-methyl-3-hydroxypentanoate (**3**) into tylactone (**4**), the parent aglycone of the 16-membered ring macrolide tylosin.[4] Together, the results from the two laboratories have opened up the possibility of systematic analysis of macrolide and polyether chain-elongation by stepwise incorporation of successive polyketide intermediates.

In earlier work, we have established the origin of the carbon skeleton and oxygen atoms of nargenicin (**5**), a metabolite of *Nocardia argentinensis*,[5] by incorporation of a variety of [^{13}C]- and [$^{13}C^{18}O$]-labeled acetates and propionates as well as $^{18}O_2$ gas. These results, summarized in Scheme II, ruled out plausible epoxy-olefin cyclization schemes and suggested that the characteristic octalin ring system of nargenicin might be generated by an intramolecular Diels–Alder reaction of a reduced polyketide

Scheme I



Scheme II



† This paper is dedicated to Professor E. J. Corey on the occasion of his 60th birthday.

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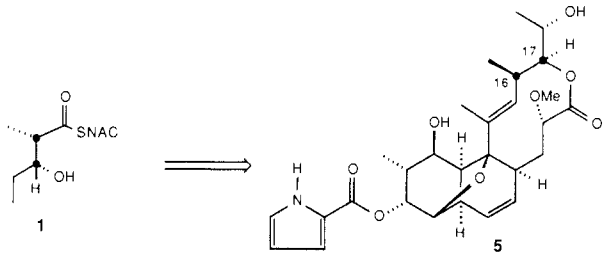
Scheme III



intermediate. Although nargenicin bears little structural resemblance to erythromycin, a consideration of the biosynthetic origins of each of these metabolites would indicate several close parallels in the elaboration of the parent polyketide chain. In order to probe these apparent biosynthetic similarities, we have investigated the incorporation of (2*S*,3*R*)-[2,3-$^{13}C_2$]-2-methyl-3-hydroxypentanoyl NAC-thioester (**1**)[6] into nargenicin.

Ten 70-mL fermentation cultures of *Nocardia agentinesis* Huang ATCC 31306 were incubated in 500-mL flasks at 30 °C and 250 rpm[5,8] for 24 h before administration of a total of 40 mg of [2,3-$^{13}C_2$]-**1** dissolved in 5 mL of 20% ethanol. Additional quantities of precursor were added after 48 h (20 mg) and 72 h (40 mg). After 96 h, the resulting crude nargenicin was extracted with ethyl acetate and purified by a combination of flash column chromatography and preparative TLC on silica gel. The 100.6 MHz ^{13}C NMR spectrum of the labeled nargenicin A_1 (7.1 mg) displayed the predicted set of enhanced and coupled doublets (J_{CC} = 36.2 Hz, 0.2 atom% enrichment), centered at 32.76 and 78.79 ppm, corresponding to C-16 and C-17, respectively.[5]

The observation of coupled ^{13}C NMR signals establishes the intact incorporation of the labeled thioester **1** into nargenicin, indicating that the polyketide synthetase of *N. argentinesis* can utilize a partially elaborated intermediate of the chain elongation process. These results are consistent with a chain elongation scheme involving adjustment of functionality and stereochemistry of the growing polyketide chain prior to each condensation reaction. The observed incorporation of the (2*S*,3*R*)-enantiomer of **1** is expected based on the previously determined absolute configuration of nargenicin.[5] Incorporation experiments involving more advanced intermediates of the chain-elongation process are in progress.

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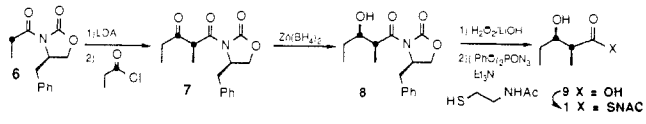
(6) The preparation of 1 was carried out by using the methods of Evans[7] for the erythroselective construction of aldol intermediates. Thus [2′-^{13}C]-(4*R*)-*N*-propionyl-4-benzyloxazolidinone (**6**), prepared as described[7] from (4*R*)-4-benzyloxazolidinone and [2-^{13}C]propionyl chloride,[3] was treated with lithium diisopropylamide and the resulting *Z* enolate was acylated with 0.9 equiv of [1-^{13}C]propionyl chloride. Reduction of the β-ketoimide **7** with 1.1 equiv of $Zn(BH_4)_2$ (Et_2O, 20 °C, 15 min) gave (2′*S*,3′*R*)-[2′,3′-$^{13}C_2$]-*N*-(2′-methyl-3′-hydroxypentanoyl)oxazolidinone (**8**) in 67% yield. Hydrolytic removal of the chiral auxilliary (3 equiv of LiOH, 12 equiv of 30% H_2O_2, THF, 20 °C, 15 min) gave the enantiomerically pure acid **9** (64% yield) which was converted to **1** as previously described.[3]



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An Antibody-Catalyzed Claisen Rearrangement

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Considerable effort is being focused on the design of highly selective catalysts for use in chemistry and biology. Two well-established approaches have involved the introduction of catalytic groups into cavity-containing hosts, such as crown ethers and cyclodextrins, and the modification of enzyme specificity by site-directed mutagenesis. More recently, it was demonstrated that the binding affinity and specificity of antibodies[3-7] could be exploited in the generation of selective catalysts for acyl transfer and carbon–carbon bond-cleaving reactions.[4-7] Because antibodies can be generated to virtually any molecule of interest,[8] this approach may not only lead to the development of tailor-made catalysts but also provide an opportunity to test general notions of enzymatic catalysis. We report here the generation and characterization of a monoclonal antibody which catalyzes the Claisen rearrangement of chorismic acid to prephenic acid (Scheme I).

The thermal 3,3-sigmatropic rearrangement of chorismate to prephenate has been demonstrated to occur through an asymmetric chairlike transition in which the carbon–oxygen bond is substantially broken, while carbon–carbon bond formation has not occurred to any appreciable extent.[9-12] The entropy and enthalpy for the uncatalyzed reaction are 20.71 kcal/mol and −12.85 eu, respectively.[11] The unimolecular rearrangement is also catalyzed approximately 10^6-fold by the enzyme chorismate mutase at the branch point in the biosynthesis of aromatic amino acids in bacteria and plants.[11,13-15] Although the enzymatic reaction has also been demonstrated to proceed through a chairlike transition state,[16] the mechanism by which chorismate mutase accelerates the rearrangement is not fully understood. It has been argued that the rate of the enzyme-catalyzed rearrangement can be attributed to immobilization of the substrate in a conformation appropriate for rearrangement.[13] However, recent mechanistic studies of the enzyme-catalyzed reaction have been interpreted in favor of an intermediate which is covalently linked to the enzyme.[17] An

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