C-23 were not enhanced, it appears that **6** was not catabolized to [3-$^{13}$C]propionate by *S. fradiae*.

Although further experiments using the NAC thioesters of D–G must be done to confirm the implication that tylactone formation is a processive biochemical event, we are encouraged by the above results to make this conclusion. We also predict that the biosynthesis of other macrolide lactones, e.g., 6-deoxyerythronolide B, will be proven to occur in an analogous manner.[13] Consequently, the results indicate that the biochemistry of macrolactone formation can be studied systematically with the expectation of learning how the enzymology controls the sequence of precursor assembly and creates the absolute stereochemistry of these structurally complex natural products.

**Acknowledgment.** This research was supported by a grant from Eli Lilly and Co. We thank Professor D. Seebach for a gift of ethyl (−)-3-hydroxypentanoate; Dr. Bruce Adams, Department of Chemistry, University of Wisconsin—Madison, for the 125-MHz $^{13}$C NMR spectra; Dr. Narayan Punekar for feeding **5** and **6**; and Drs. E. T. Seno and R. H. Baltz for helpful discussions, bacterial strains, fermentation media constituents, and a sample of tylactone.

**Supplementary Material Available**: Experimental data for the synthesis of compounds **2–6**, the precursor feedings, Figure 2, and the method of calculating the intact-to-catabolic incorporation ratios for **4** and **5** (10 pages). Ordering information is given on any current masthead page.

(13) See the following paper by Cane and Yang *J. Am. Chem. Soc.*, following paper in this issue.

## Macrolide Biosynthesis. 4. Intact Incorporation of a Chain-Elongation Intermediate into Erythromycin

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Although the general outlines of erythromycin biosynthesis are by now well accepted, the details of the key chain-elongation process by which the macrolide carbon skeleton is assembled are still obscure.[1] Extensive investigations in our own[2] and other laboratories[1] have demonstrated the role of propionate as the basic building block for the macrolide aglycone and established that the oxygen atoms at C-1, C-3, C-5, C-9, C-11, and C-13 are all derived from the propionate precursor. These results have been interpreted in terms of a mechanism analogous to the chain-elongation steps of fatty acid biosynthesis in which the oxidation level and stereochemistry of the growing polyketide chain are adjusted subsequent to each condensation step involving successive units of methylmalonate (Scheme I). Further support for the analogy to fatty acid biosynthesis has come from the finding that incorporation of [2-$^{2}$H$_2$,2-$^{13}$C]propionate gave erythromycin A (**1**) and B (**2**), which retained deuterium at C-2, C-4, and C-10, consistent with the direct utilization of (2*S*)-[2-$^{2}$H,2-$^{13}$C]-methylmalonyl-CoA (**3b**) in those condensation steps leading to the generation of D-methyl groups,[3] as required by a process involving decarboxylative inversion.[4] Unfortunately, all of these experiments, limited to simple three- and four-carbon precursors, have provided only indirect insights into the details of the chain-elongation process itself. Indeed, none of the intermediates between methylmalonate and the parent aglycone, 6-deoxyerythronolide B (**8**), have ever been directly detected, either in antibiotic-producing cultures of *Streptomyces erythreus* or in mutants blocked in the formation of **8**, nor do any of the latter mutants act as secretors in cosynthesis experiments.[5] Furthermore, although the structural genes for erythromycin biosynthesis have recently been cloned,[6] a viable cell-free synthetase mediating formation of the macrolide skeleton has so far eluded numerous attempts at isolation. We now report the first successful incorporation of a partially elaborated polyketide into the intact macrolide ring of erythromycin.

According to the proposed mechanism for erythromycin biosynthesis (Scheme I), chain elongation is initiated by condensation of (2*R*)-methylmalonyl-CoA (**3a**) (or an enzymatically equivalent thioester) with propionyl-CoA to give (2*S*)-2-methyl-3-ketopentanoyl-CoA (**4**). Stereospecific reduction of **4** will then give (2*S*,3*R*)-2-methyl-3-hydroxypentanoyl-CoA (**5**), which serves as the substrate for the next condensation reaction. We sought, therefore, to test this mechanism by attempted incorporation of (2*S*,3*R*)-[2,3-$^{13}$C$_2$]-2-methyl-3-hydroxypentanoic acid (**9**), readily available by the versatile methodology for enantiospecific aldol condensations recently introduced by Evans.[7,8] The use of the double $^{13}$C label not only provides greater sensitivity in the detection of low levels of enrichment, it also allows recognition of incorporation of the intact precursor against the anticipated background due to substrate degradation and random incorporation of derived propionate. Thus [2′-$^{13}$C]-*N*-propionyloxazolidone (**10**), prepared as described from [2-$^{13}$C]propionyl chloride[9] and (4*S*)-4-(2-propyl)oxazolidone,[7] was treated with di-*n*-butylboryl triflate and diisopropylethylamine, and the derived *Z* boron enolate was condensed with anhydrous [1-$^{13}$C]propionaldehyde[10] in $CH_2Cl_2$ followed by oxidative workup (Scheme II). The resulting (2′*S*,3′*R*)-[2′,3′-$^{13}$C$_2$]-*N*-(2-methyl-3-hydroxypentanoyl)oxazolidone (**11**), obtained in 65% yield, appeared as a single diastereomer by $^{1}$H and $^{13}$C NMR analysis, displaying a characteristic $J_{H2,H3}$(erythro) = 2.9 Hz.[7,12,13] Hydrolysis of

(1) (a) Corcoran, J. W. In *Antibiotics IV. Biosynthesis*; Corcoran, J. W., Ed.; Springer-Verlag: New York, 1981; pp 132–174. (b) Omura, S.; Tanaka, Y. In *Macrolide Antibiotics: Chemistry, Biology, and Practice*; Omura, S., Ed.; Academic: New York, 1984; pp 199–299.

(2) (a) Cane, D. E.; Hasler, H.; Liang, T.-C. *J. Am. Chem. Soc.* **1981**, *103*, 5960. (b) Cane, D. E.; Hasler, H.; Taylor, P. B.; Liang, T.-C. *Tetrahedron* **1983**, *39*, 3449.

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(5) Weber, J. M.; Wierman, C. K.; Hutchinson, C. R. *J. Bacteriol.* **1985**, *164*, 425.

(6) Stanzak, R.; Matsushima, P.; Baltz, R. H.; Rao, R. N. *Bio/Technology* **1986**, *4*, 229.

(7) (a) Evans, D. A.; Bartoli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127. (b) Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 3099. (c) Evans, D. A. *Aldrichimica Acta* **1982**, *15*(2), 23.

(8) The preparation of **9** has been reported by Masamune: Masamune, S.; Choy, W.; Kerdesky, F. A. J.; Imperiali, B. *J. Am. Chem. Soc.* **1981**, *103*, 1566.

(9) [2-$^{13}$C]Propionyl chloride was prepared by treatment of sodium [2-$^{13}$C]propionate (99 atom % $^{13}$C, MSD Isotopes) with 1.4 equiv of phthaloyl chloride (150 °C, 1.5 h) followed by heating to 200 °C and collection of the distillate.

(10) Reduction of [1-$^{13}$C]propionic acid (99 atom % $^{13}$C, Cambridge Isotopes) with 1.2 equiv of borane–dimethyl sulfide ($Et_2O$, reflux, 6 h) gave the [1-$^{13}$C]tripropyloxyboroxine[11] trimer which was oxidized with 1.1 equiv of PDC in a minimum volume of $CH_2Cl_2$ (50 °C, 2 h). Distillation of the reaction mixture and passage of the distillate through Florosil gave [1-$^{13}$C]-propionaldehyde (70 mg/mL, 40% yield). Success in the subsequent aldol condensation reaction required that the propionaldehyde solution be no more dilute than 10% as well as free of water and contaminating propanol and propionic acid.

(11) Brown, H. C.; Rao, C. G.; Kulkarni, S. U. *Synthesis* **1979**, 704.

(12) The configurational assignments were based on the known stereochemical course of the boron enolate mediated aldol condensation of **10**, derived from L-valine,[7] as well as the characteristic $^{1}$H and $^{13}$C NMR parameters: ($J$(erythro) = 3–6 Hz, $J$(threo) = 7–9 Hz; δ C-2 methyl (erythro) 9–13, δ C-2 methyl (threo) 12–18). Cf.: Evans, D. A.; Nelson, J. V.; Taber, T. R. In *Topics in Stereochemistry*; Allinger, N. L., Eliel, E. L., Wilen, S. H., Eds.; Wiley: New York, 1982; pp 1–115. Heathcock, C. H. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, 1984; Vol. III, pp 111–212.

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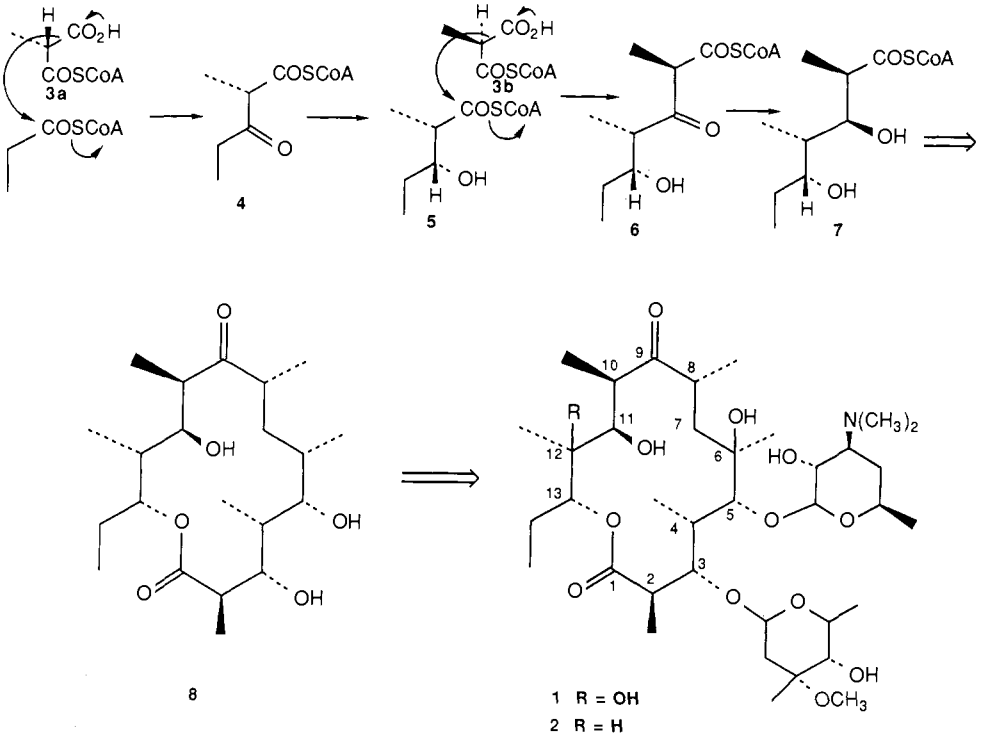
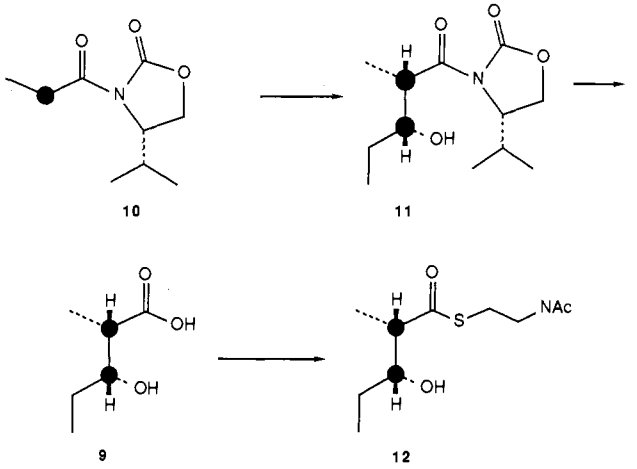
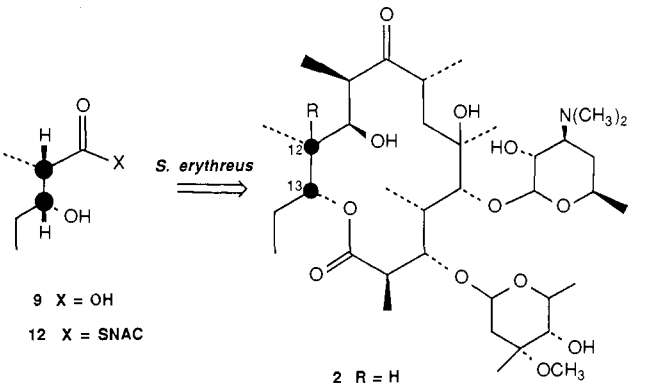
**Scheme I**




**Scheme II**




**Scheme III**




**11** (4 equiv of 2 N KOH in MeOH, 0 °C, 40 min) gave the desired (2*S*,3*R*)-[2,3-$^{13}C_2$]-2-methyl-3-hydroxypentanoic acid (**9**), $J_{H2,H3}$(erythro) = 3.5 Hz, in 73% purified yield.[8,12,13]

When 0.093 g of [2,3-$^{13}C_2$]-**9**, mixed with 0.135 g of unlabeled **9** and containing 2.4 × $10^6$ dpm of [1-$^{14}$C]-**9** as internal standard, was administered in portions to cultures of *S. erythreus* at 48 (20%), 72 (30%), and 96 h (50%) by previously described procedures,[2,3] analysis of the resulting erythromycin A by liquid scintillation indicated an apparent overall specific incorporation of 15%. Disappointingly, however, no significant enrichments or couplings could be detected by $^{13}$C NMR, indicating that the precursor had most likely been degraded to a mixture of [1-$^{13}$C]- and [2-$^{13}$C]propionate followed by general incorporation into the macrolide. Fortunately, the problem could be overcome by converting [2,3-$^{13}C_2$]-**9**, containing [1-$^{14}$C]-**9** as internal standard, to the corresponding *N*-acetylcysteamine (NAC) thioester **12**.[13,14]

Administration of 0.100 g of **12**, dissolved in 0.3 mL of $Me_2SO$, to a 100-mL culture of *S. erythreus* at 48 (40%), 72 (30%), and 96 h (30%) gave labeled erythromycin B (**2**), which was purified by a combination of HPLC and silica gel chromatography.[3] The 100.6-MHz $^{13}$C NMR spectrum of **2** displayed a set of enhanced and coupled doublets ($J_{CC}$ = 38.95 Hz, 2% enrichment), centered at 39.8 and 75.2 ppm, corresponding to C-12 and C-13, respectively[2,17] (Scheme III). The coupling between the two signals was further confirmed by 1D $^{13}$C INADEQUATE analysis of **2**, which showed only the expected two sets of signals.

The above results, which constitute the first intact incorporation of an intermediate of chain elongation into erythromycin, are fully consistent with the postulated intermediacy of (2*S*,3*R*)-2-methyl-3-hydroxypentanoyl-CoA (**5**) or an equivalent thioester derivative. Completely analogous results have been obtained by Hutchinson et al. for the intact incorporation of the NAC thioester of (2*R*,3*R*)-2-methyl-3-hydroxypentanoate into tylactone, the parent aglycone of the 16-membered ring macrolide tylosin.[18] In

(13) $^{13}$C NMR: **11**, C-2′ methyl, 10.67; C-2′, 41.82; C-3′, 72.88 ($J_{CC}$ = 37 Hz). **9**, C-2 methyl, 10.07; C-2, 43.82; C-3, 73.4 ($J_{CC}$ = 36.9 Hz). **12**, C-2 methyl, 11.10; C-2, 53.16; C-3, 73.71 ($J_{CC}$ = 35.5 Hz).

(14) The NAC thioester was conveniently prepared (87% yield) by treatment of **9** with diphenylphosphoryl azide[15] (2 equiv) and *N*-acetylcysteamine[16] (6 equiv) in the presence of $Et_3N$ (4 equiv, room temperature, 24 h).

(15) Yamada, S.; Yokoyama, Y.; Shioiri, T. *J. Org. Chem.* **1974**, *39*, 3302.

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

(17) (a) Nourse, J. G.; Roberts, J. D. *J. Am. Chem. Soc.* **1975**, *97*, 4584. (b) Terui, Y.; Tori, K.; Nagashima, K.; Tsuji, N. *Tetrahedron Lett.* **1975**, 2583. (c) Omura, S.; Neszelyi, A.; Sangare, M.; Lukacs, G. *Tetrahedron Lett.* **1975**, 2939.

(18) Yue, S.; Duncan, J. S.; Yamamoto, Y.; Hutchinson, C. R. *J. Am. Chem. Soc.*, preceding paper in this issue.

the latter case also, attempted incorporation of the corresponding free acid, as well as the derived ethyl ester, was unsuccessful. The success of the *N*-acetylcysteamine derivative is presumably related to the ability of the thioester substrate to undergo facile exchange with a thiol residue at the active site of the macrolide synthetase.[19] Taken together, the results from the two laboratories have provided the first direct glimpse of the individual steps leading to generation of the macrolide carbon skeleton and open up the possibility of systematic analysis of the chain-elongation process by stepwise incorporation of successive polyketide intermediates. Further investigations along these lines are in progress.

**Acknowledgment.** We thank Professor C. Richard Hutchinson for informing us of his results, cited in ref 18, and generously offering to delay publication of his own manuscript so as to allow simultaneous publication of the work from our two laboratories. We also thank Professor David A. Evans for useful discussions. This work was supported by the National Institutes of Health, Grant GM 22172. The Bruker AM-400 used in this work was purchased with funds provided by the NIH Division of Research Resources, RR 02458.

(19) NAC thioesters are frequently used as substitutes for the corresponding physiologically relevant CoA and ACP esters for in vitro studies of fatty acid biochemistry.

## A Remarkable Steric Effect in Palladium-Catalyzed Grignard Coupling: Regio- and Stereoselective Monoalkylation and -arylation of 1,1-Dichloro-1-alkenes[1]

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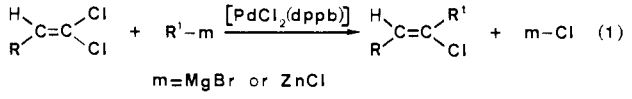
Functionalized carbon chain elongation has been a central concern in transition-metal complex catalyzed carbon–carbon bond-forming reactions.[2] As part of our continued studies on the palladium–phosphine complex catalyzed selective monoalkylation of organic polyhalides,[3] we report here the first success in the regio- and stereoselective monoalkylation and -arylation of 1,1-dichloro-1-alkenes by Grignard or organozinc reagents in the presence of $[PdCl_2(dppb)]$, dppb = $Ph_2P(CH_2)_4PPh_2$, as a catalyst to produce 1-substituted (*Z*)-1-chloro-1-alkenes as shown in eq 1.




(1)

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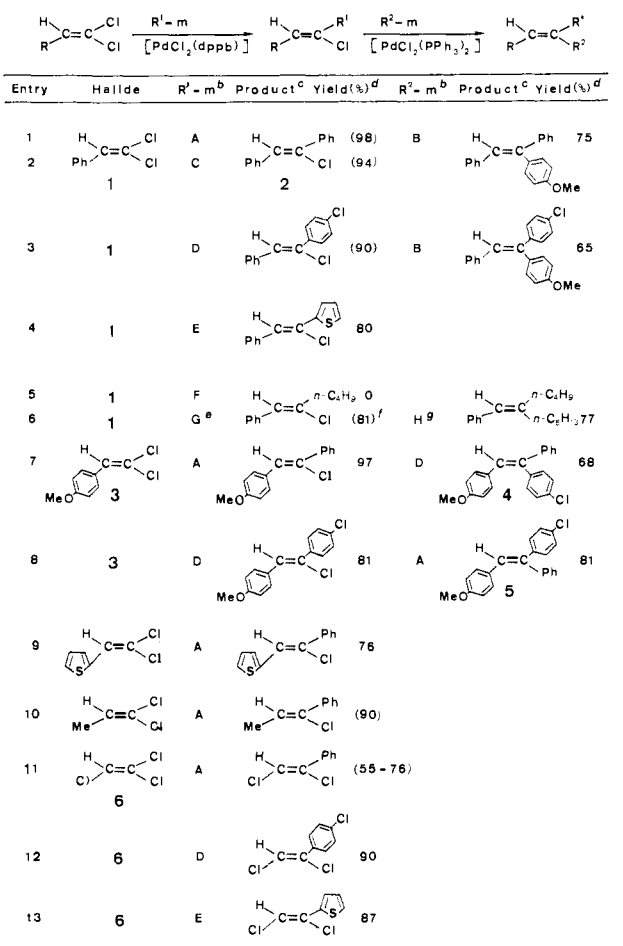
(3) (a) Minato, A.; Tamao, K.; Hayashi, T.; Suzuki, K.; Kumada, M. *Tetrahedron Lett.* **1980**, *21*, 845. (b) Minato, A.; Suzuki, K.; Tamao, K.; Kumada, M. *J. Chem. Soc., Chem. Commun.* **1984**, 511.

**Table I.** Stepwise Coupling of 1,1-Dichloro-1-alkenes with Grignard and Organozinc Reagents Catalyzed by Palladium–Phosphine Complexes[a]




[a] Unless otherwise stated, the reactions were carried out under the following conditions. First step: dihalide/$R^1$-m/$[PdCl_2(dppb)]$ = 1/1/0.01, ether reflux, 2 h. Second step: halide/$R^2$-MgBr/$[PdCl_2(PPh_3)_2]$ = 1/1.2/0.01, ether reflux, 3 h. [b] A = PhMgBr; B = 4-$MeOC_6H_4MgBr$; C = PhZnCl; D = 4-$ClC_6H_4MgBr$; E = 2-thienylMgBr; F = *n*-$C_4H_9MgBr$; G = *n*-$C_4H_9ZnCl$; H = *n*-$C_6H_{13}MgBr$. [c] All new compounds gave satisfactory spectral (IR, $^1H$ NMR, $^{13}C$ NMR, and mass) data and elemental analyses and/or exact mass molecular weights. [d] Isolated yields; yields in parentheses were determined by GLC. [e] Carried out with 2.5 equiv of G in THF at 40 °C for 4 h. [f] Dibutylation product was formed in 11% yield. [g] Performed in the presence of $[NiCl_2(dppp)]$ (1 mol %) under ether reflux for 6 h.

The representative results are listed in Table I. Typically, when 1,1-dichloro-2-phenylethene (**1**) was allowed to react with 1 equiv of phenylmagnesium bromide in the presence of 1 mol % of $[PdCl_2(dppb)]$ under ether reflux for 2 h, monoarylation product (*Z*)-1-chloro-1,2-diphenylethene (**2**)[4] was obtained exclusively in 98% yield (entry 1): the structure of **2** was unambiguously characterized by converting it into (*E*)-stilbene via $[Pd(PPh_3)_4]$-catalyzed hydrogenolysis.[5] A variety of 1,1-dichloro-1-alkenes coupled with alkyl,[6] aryl, and heteroaryl Grignard and/or

(4) Mp 51–52 °C (EtOH); lit. mp 52–54 °C: Cristol, S. J.; Bly, R. S., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 4027.

(5) Helquist, P. *Tetrahedron Lett.* **1978**, *22*, 1913. We have confirmed that this reduction proceeds with retention of configuration of olefin stereochemistry. For example, authentic (*E*)- and (*Z*)-1-chloro-1,2-diphenylethene were reduced to (*Z*)- and (*E*)-stilbene, respectively. Details will be reported in due course.

(6) Selective monobutylation occurred effectively by using organozinc reagent[7] instead of Grignard reagent (entry 6 compared with entry 5). The structure of the monobutylation product was also determined by reduction[5] and a large NOE between the olefin proton and the allylic methylene protons.

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