Comparison of Fatty Acid and Polyketide Biosynthesis: Stereochemistry of Cladosporin and Oleic Acid Formation in *Cladosporium cladosporioides*

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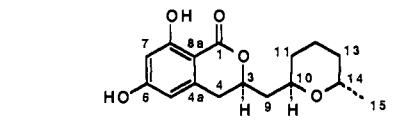
Abstract: Stereochemical aspects of the biosynthesis of the polyketide cladosporin (**1**) and of oleic acid (**2**) by *Cladosporium cladosporioides* NRRL 5507 were compared by use of stable isotope labeling and 2D NMR spectrometry. The absolute stereochemistry of **1** was confirmed by degradation to (2*R*,6*S*)-(−)-(6-methyltetrahydropyran-2-yl)acetic acid (**12**). Incorporations of sodium [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, [1-^{13}C,$^{18}O_2$]-, [1-^{13}C,$^{2}H_3$]-, and [2-^{13}C,$^{2}H_2$]acetates into **1** followed by ^{13}C NMR analysis of its diacetate **13** provided the biosynthetic pattern of all bonds derived intact from acetate. Deuterium-decoupled ^{1}H,^{13}C shift correlation NMR spectra of **13** derived from [2-^{13}C,$^{2}H_3$]acetate showed that deuterium occupies the *pro-R* position at C-9 and the *pro-S* position at C-11. Oleic acid (**2**) obtained from the same incorporation experiment was degraded and derivatized with methyl (*S*)-(+)-mandelate to afford esters **20** and **21a**. Stereospecifically deuterated standard samples **21b** and **21c** were synthesized. Deuterium-decoupled ^{1}H,^{13}C shift correlation NMR spectra of these compounds demonstrated that during fatty acid biosynthesis in *C. cladosporioides* the intact carbon–hydrogen bond of acetate is in the *pro-R* position, the opposite of that at C-11 for the polyketide **1**. The possible mechanism of tetrahydropyran ring formation during biosynthesis of cladosporin (**1**) is discussed.

Biosynthesis of polyketide secondary metabolites involves assembly of the basic carbon skeleton from acetate, propionate, and/or butyrate by a route that closely resembles fatty acid formation.[1] A basic difference between the two processes is that with polyketide synthase enzymes addition of the next building block may occur before reduction of the preceding unit is complete (Figure 1). This leads to incorporation of double bonds, hydroxyls, or keto functionalities into the growing chain; these may in turn initiate further transformations (e.g., cyclization) or eventually provide sites for attack by other (often oxidative) enzymes. Labeling experiments with stable isotopes[1b,2-4] and incorporations of advanced (e.g., di- and triketide) precursors[5] have supported this hypothesis. However, examination of the absolute configurations of polyketide carbon–oxygen bonds derived intact from acetate or propionate[2] shows that reduction of a carbonyl to an alcohol can often occur with stereochemistry opposite (*S*) to that (*R*) thought to be general[6] for fatty acid synthase enzymes. Further investigation of the stereochemistry of polyketide and fatty acid biosyntheses involves comparison of the configurations of acetate-derived hydrogens in both types of metabolites in a single organism.[4,7] This appears particularly useful because the stereochemistry of the last reductive enzyme of fatty acid biosynthesis (enoyl thiol ester reductase) varies with its source.[6,7a] The present report describes stable isotope labeling and NMR experiments to examine stereochemical details of formation of the polyketide cladosporin (**1**) and of oleic acid (**2**) by *Cladosporium cladosporioides* NRRL 5507.[8] The accompanying paper utilizes similar methods to compare production of two other reduced polyketides and fatty acids in different fungi.[9]



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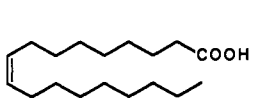

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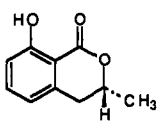

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Cladosporin (**1**) (also known as asperentin) is an antifungal antibiotic and plant growth inhibitor produced by various fungal sources.[10,11] Researchers at Merck assigned the absolute stere-

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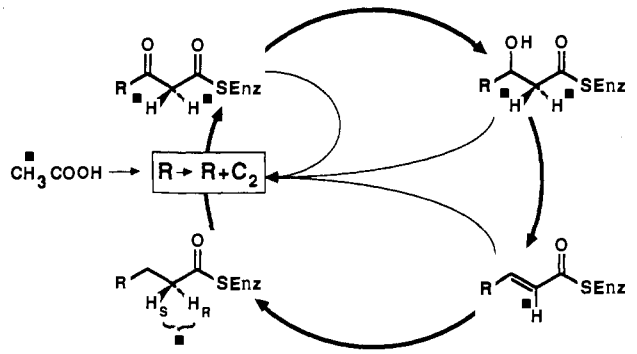


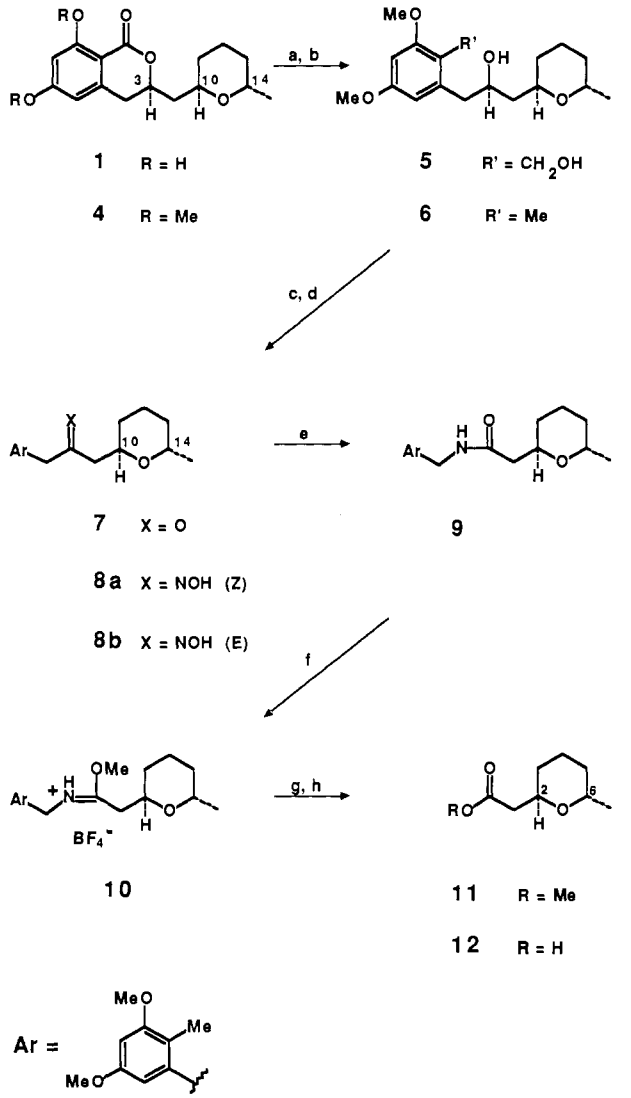
Figure 1. Sequence of oxidation states in each cycle of two-carbon addition by fatty acid synthase (dark circle only) and by polyketide synthase enzymes.

ochemistry of **1** isolated from *Aspergillus repens* on the basis of X-ray analysis for *relative* configurations and comparison of its circular dichroism spectrum with that of (*R*)-(−)-mellein (**3**).[11e] Grove and co-workers have previously studied the general features of its biosynthesis in *Aspergillus flavus* by incorporation of [1-^{13}C]acetate and [2-^{14}C]malonate.[12] However, in order to identify all bonds derived intact from acetate and to define the stereochemical outcome of the assembly of cladosporin (**1**) in *C. cladosporioides*, it was essential to confirm its absolute configuration and to fully assign ^{1}H and ^{13}C NMR spectra. It also appeared useful to develop a rapid method for analysis of deuterium stereochemistry both in **1** and in oleic acid (**2**). Application of ^{2}H-decoupled ^{1}H,^{13}C shift correlation NMR spectroscopy[4,13] after incorporation of a ^{13}C,^{2}H doubly labeled precursor seemed ideal for this purpose.

Results

Determination of the Absolute Stereochemistry of 1. Samples of cladosporin (**1**) obtained from cultures of *C. cladosporioides* NRRL 5507 gave circular dichroism spectra which matched those reported[10b] for **1** produced by *A. repens*. To rigorously confirm the absolute stereochemistry, cladosporin (**1**) was degraded to (2*R*,6*S*)-(−)-(*trans*-6-methyltetrahydropyran-2-yl)acetic acid (**12**), a known compound,[14] as shown in Scheme I. The dimethyl ether **4** was reduced to the diol **5** which was hydrogenolyzed to **6**. Swern oxidation[15] gave the ketone **7** (52% overall yield from **4**). Compounds of this type with a carbonyl at C-3 are somewhat sensitive and tend to epimerize at C-10 to the more stable diequatorial cis-substituted tetrahydropyranyl system via an α,β-elimination/Michael addition process.[14,16] Baeyer–Villiger oxidation of these systems is known to be difficult, and such reactions with **7** produced complex mixtures even with the recommended oxidant, trifluoroperacetic acid.[14] Hence, the ketone **7** was transformed to the separable (*Z*)- and (*E*)-oximes **8a** and **8b**, respectively. Beckmann rearrangement of the (*Z*)-oxime **8a** afforded amide

Scheme I. Degradation of Cladosporin (**1**)[a]



[a] (a) $LiBH_4$; (b) H_2, Pd/C; (c) DMSO, $(COCl)_2$, Et_3N; (d) H_2NOH; (e) TsCl, pyridine; (f) Me_3OBF_4; (g) HCOOH, H_2O; (h) NaOH, HCl.

9 in 64% yield. To avoid epimerization at C-10, the amide was cleaved by conversion to the imidate salt **10** followed by acidic hydrolysis to the ester **11** (56% yield). Basic hydrolysis[17] of **11** produced the known acid **12**[14] in optically pure form. Since the relative stereochemistry of **1** from *A. repens* was known from X-ray analysis,[11e] the absolute stereochemistry of cladosporin (**1**) from both organisms was confirmed to be 3*R*,10*R*,14*S*.

Assignment of ^{1}H and ^{13}C NMR Spectra. Since cladosporin (**1**) is relatively insoluble, all NMR experiments were performed on the corresponding diacetate **13**.[10b] The aromatic hydrogen resonances are easily distinguished by an NOE between H-5 and the benzylic protons at C-4. Standard ^{1}H-decoupling, COSY, and ^{1}H,^{13}C NMR shift correlation experiments[18] identified most of the proton and carbon signals except for the C-11/C-13 and C-10/C-14 groups. Facile assignment of these carbon resonances was achieved by a homonuclear ^{13}C COSY experiment on a sample of **13** derived from incorporation (see below) of sodium [1,2-$^{13}C_2$]acetate.[19] Strong correlation signals for carbons com-

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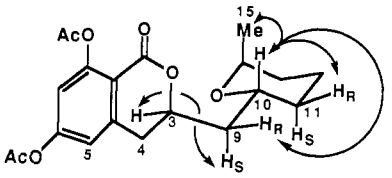
(19) For a review see ref 2a. For a discussion of related 2D INADEQUATE NMR experiments in biosynthetically enriched samples see ref 3i.

Table I. ^{13}C and ^{1}H Chemical Shifts for Cladosporin Diacetate (**13**) and Isotope Shifts in ^{13}C NMR Spectra[a]

carbon	^{13}C δ		^{1}H δ		^{2}H isotope shift, ppb (type)[b]	^{18}O isotope shift, ppb[c]
	$CDCl_3$	CD_3CN	$CDCl_3$	CD_3CN		
1	161.80	162.13				33
6	155.06	155.76			46 (β)	21
8	153.57	153.84			15 (β)	18
4a	143.20	144.40			76 (β)	
5	118.62	119.57	7.00	7.02	274 (α)	
7	116.84	117.42	6.88	6.85	244 (α)	
8a	116.00	116.70				
3	76.04	76.73	4.73	4.65	*d*	32
14	67.86	67.70	3.97	3.87	46 (β), 92 (2β), 137 (3β)	26
10	67.00	67.56	4.10	4.06	*d*	none
9	36.69	39.07	2.01[e]	2.11[e]	366 (α)[g]	
			1.83[f]	1.70[f]		
4	34.89	34.80	3.02	3.08	336 (α), 702 (2α)	
			3.00	3.02	366 (α)	
13	31.55	32.39	1.73	1.68	428 (α), 840 (2α)	
			1.36	1.28	412 (α)	
11	31.35	31.24	1.73[f]	1.70[f]	427 (α)[g]	
			1.36[e]	1.36[e]		
15	19.38	20.05	1.20	1.17	280 (α), 564 (2α), 840 (3α)	
12	18.74	19.12	1.74	1.72	106 (β), 213 (2β)	
			1.62	1.60	106 (β)	

[a] Obtained at 100.6 MHz for ^{13}C and 400 MHz for ^{1}H on Bruker WH400 spectrometers. [b] Samples of **13** derived from sodium [1-^{13}C,$^{2}H_3$]acetate (β shifts) and [2-^{13}C,$^{2}H_3$]acetate (α shifts). All isotope shifts ±10 ppb. [c] Sample of **13** derived from sodium [1-^{13}C,$^{18}O_2$]acetate. [d] No β-isotope shift observed; see ref 2a. [e] *pro-S* hydrogen. [f] *pro-R* hydrogen. [g] See Figure 4.



13

$J_{3\text{-}9R}$ = 8.7 $J_{3\text{-}9S}$ = 4.1

$J_{10\text{-}9R}$ = 2.7 $J_{10\text{-}9S}$ = 10.4

$J_{10\text{-}11R}$ = 2.6 $J_{10\text{-}11S}$ = 7.7

$J_{9R\text{-}9S}$ = 14.5

Figure 2. Approximate solution conformation of cladosporin diacetate (**13**). Arrows indicate important NOE enhancements (see Supplementary Material). Key ^{1}H NMR coupling constants are given ($^{1}J_{H\text{-}H}$).

prising a single biosynthetic acetate unit showed that C-15 (already assigned) coupled to C-14 and that C-10 coupled to C-11. Because of the increased level of carbon-13, weak signals for *interunit* couplings (e.g., C-4 to C-3 and C-9 to C-10) could also be detected.[19] The remaining proton resonances were then assigned from the ^{1}H,^{13}C shift correlation spectra.

Stereochemical assignment of the signals for hydrogens at C-9 and C-11 was essential for the present study. Although the ^{13}C NMR signals for C-11 and C-13 overlap in most solvents except CD_3CN, the ^{1}H NMR spectra are best dispersed in $CDCl_3$. Hence, NMR measurements were also done in systematically varied mixtures of CD_3CN and $CDCl_3$ to ensure unambiguous assignment of all hydrogen and carbon peaks in each pure solvent. Extensive ^{1}H-decoupling, difference NOE, and 2D-ROESY[20,21] experiments in $CDCl_3$ provided the approximate major solution conformation (Figure 2) as well as the required coupling constants and chemical shifts (Table I). The coupling constants for the H-3, H-9, H-10, and H-11 system of cladosporin diacetate (**13**) were refined by input of estimated values into the Bruker PANIC program and iteration to match experimental spectra. The assignment of the axial (*pro-S*) and equatorial (*pro-R*) hydrogens at C-11 is based on their respective ~7.7-Hz and ~2.6-Hz coupling to the C-10 axial hydrogen as well as on chemical shifts. These values are well precedented for oxygen-containing rings.[22] Identification of the 9-*pro-R* hydrogen follows from its couplings of 2.7 Hz to H-10 and 8.7 Hz to H-3 as well as corresponding NOE enhancements at those sites. The 9-*pro-S* hydrogen displays couplings of 10.4 Hz to H-10 and 4.1 Hz to H-3 and shows no significant NOE from H-10 but strongly enhances H-3. The magnitudes of the coupling constants are in excellent agreement with values obtained by Kishi and co-workers for a series of closely related C-glycosides.[23]

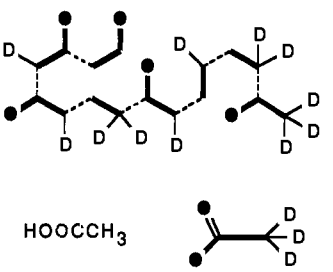


Figure 3. Bonds in cladosporin (**1**) derived intact from acetate.

Biosynthetic Origin of Atoms in Cladosporin (1). Sodium [1-^{13}C]-, [2-^{13}C]-, [1,2-$^{13}C_2$]-, [1-^{13}C,$^{2}H_3$]-, and [1-^{13}C,$^{18}O_2$]-acetates were separately administered to growing cultures of *C. cladosporioides*, and the resulting cladosporins were isolated and converted to the corresponding diacetates for NMR analysis by well-established procedures.[2] Incorporation rates for the labeled acetates are sometimes very high and can range up to 15% per enriched site. Two-dimensional ^{13}C COSY is especially effective for detecting coupled carbons comprising an intact acetate unit in the sample derived from [1,2-$^{13}C_2$]acetate.[2a,24] As mentioned above, this method also aids spectral assignment through observation of both the intense intraunit and weak interunit couplings

(20) We are grateful to Professor George Kotovych and Dr. Albin Otter for a preprint of their work[21c] and assistance in obtaining 2D-ROESY spectra.

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(24) For some recent examples see: (a) Ubukata, M.; Uzawa, J.; Isono, K. *J. Am. Chem. Soc.* **1984**, *106*, 2213–2214. (b) Parry, R. J.; Mafoti, R. *J. Am. Chem. Soc.* **1986**, *108*, 4681–4682. (c) Stoessl, A.; Stothers, J. B.; Zimmer, R. C. *J. Chem. Soc., Chem. Commun.* **1987**, 508–509.

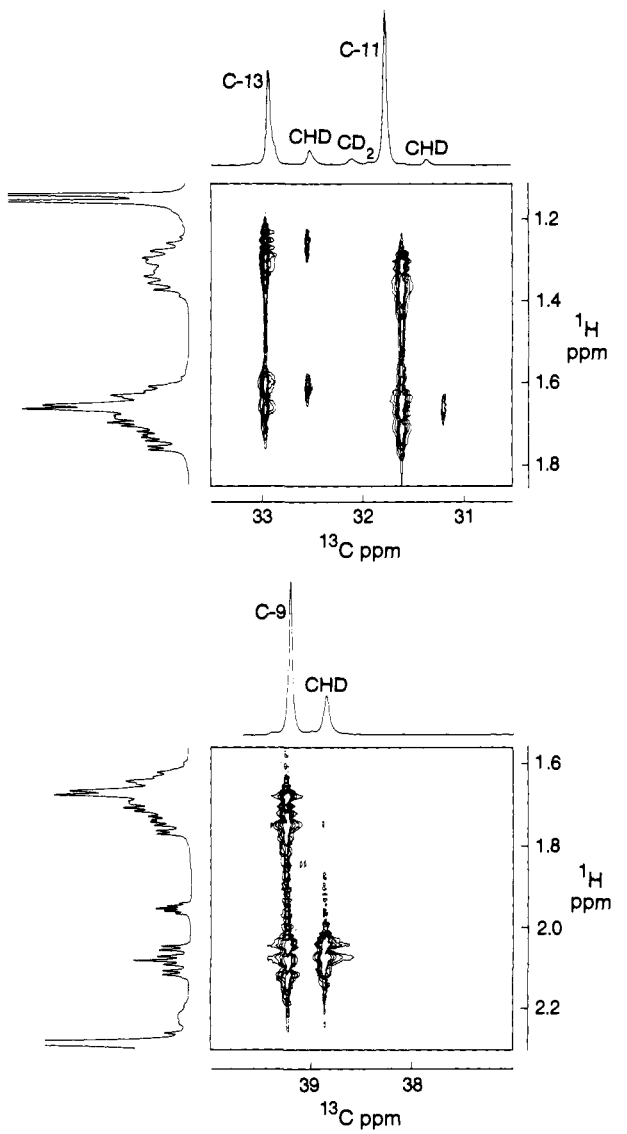
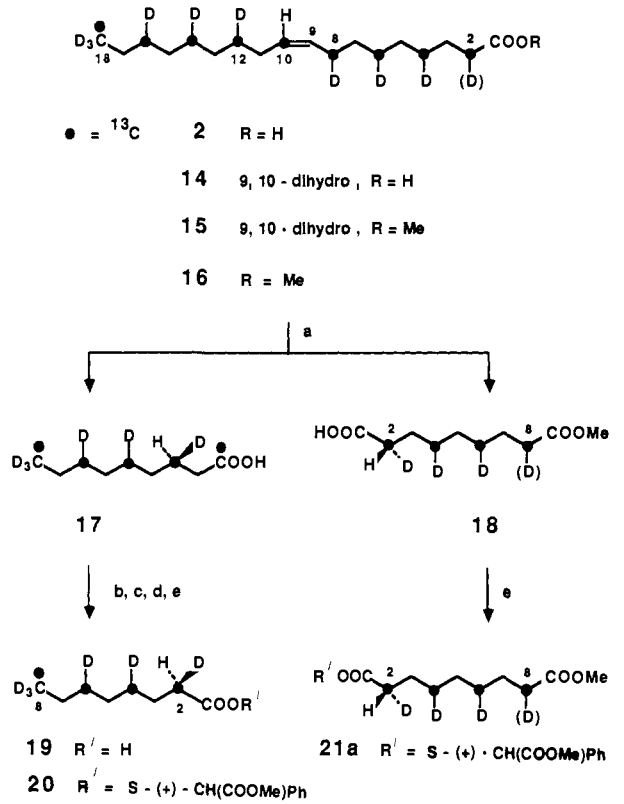


Figure 4. ^{2}H-Decoupled ^{1}H,^{13}C chemical shift correlation plots[13] of cladosporin diacetate (**13**) derived from sodium [2-^{13}C,^{2}H$_3$]acetate. Upfield CHD correlations show C-9 and C-11 are stereospecifically labeled but C-13 is not. Spectra were obtained on a Bruker WH400 on ca. 0.2 M solutions of **13** in CD_3CN.

of adjacent carbon-13 nuclei. Because of the high incorporations and consequent long-range ^{13}C couplings, ^{18}O-induced isotope shifts (Table I) in samples derived from [1-^{13}C,$^{18}O_2$]acetate were best determined by the SEFT technique.[2a,25] However, carbon coupling was not problematic in detection of intact carbon–deuterium bonds by the β-isotope shift approach,[2,26] possibly because of lower incorporation of [1-^{13}C,^{2}H$_3$]acetate. Our experiments confirmed the results obtained by Grove and co-workers with *A. flavus*[12] and identified all bonds derived intact from acetate in cladosporin (**1**) as shown in Figure 3.

The configuration of the single carbon–hydrogen bond derived intact from acetate at C-11 of **1** provides key information about the stereochemistry of enzymatic reduction during polyketide assembly. Similarly, the stereochemistry of acetate hydrogen at C-9 affords insight into the mechanism of tetrahydropyran ring formation. To determine these configurations, sodium [2-^{13}C,^{2}H$_3$]acetate was incorporated (ca. 2%) into cladosporin (**1**). Deuterium-decoupled ^{1}H,^{13}C NMR shift correlation[13] of the derived acetate **13** (Figure 4) shows that the hydrogen of the CHD

Scheme II[a]



[a] (a) $KMnO_4$, $NaIO_4$; (b) CH_2N_2, PhMgCl; (c) TsOH; (d) $NaIO_4$, $RuCl_3$; (e) DCC, DMAP, methyl *S*-(+)-mandelate.

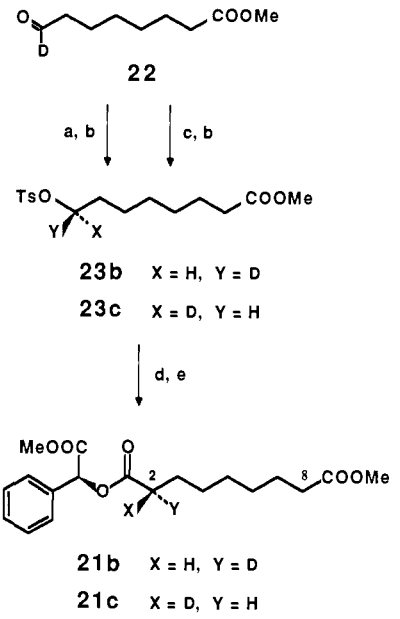
groups at C-11 occupies the downfield *pro-R* position (equatorial). Hence, the acetate deuterium is axial at C-11 and the *methylene configuration is S on the polyketide chain during assembly.* The one-dimensional ^{2}H,^{1}H-decoupled ^{13}C NMR spectrum of **13** (top of Figure 4) shows that, in contrast to C-11, C-13 bears two deuteriums in some molecules but has one in others. The presence of two CHD signals for C-13 in the two-dimensional correlation spectrum clearly demonstrates that deuterium labeling is nonstereospecific at the site and results from partial exchange of acetate deuterium with the medium (possibly via malonate). At C-9 the single deuterium occupies exclusively the *pro-R* position as shown by the correlation of the downfield *pro-S* hydrogen with the isotopically shifted CHD carbon.

Stereochemistry of Acetate-Derived Deuteriums in Oleic Acid (2). Since the stereochemistry of enoyl thiol ether reductase varies in different organisms,[6,7a] the single acetate-derived hydrogen at alternate methylene groups in fatty acids from *C. cladosporioides* could in principle possess either *R* or *S* configuration. Hence, the fats obtained from the experiment with sodium [2-^{13}C,^{2}H$_3$]acetate described above were hydrolyzed, methylated, and separated to afford fractions containing the methyl ester **15** of stearic acid (**14**) and pure methyl oleate (**16**). Oleic acid (**2**) is known to be formed by enzymatic dehydrogenation of **14** which, in the cases examined, involves removal of the 9*R* and 10*R* hydrogens.[6c,h,7a,27] The absence of a deuterium isotope-shifted peak for C-10 in the ^{2}H,^{1}H-decoupled ^{13}C NMR spectrum of **16** suggested that acetate-derived deuterium occupies the *pro-R* positions at even-numbered carbons in stearic acid (**14**).

To confirm this, the methyl oleate (**16**) was oxidatively degraded to octanoic acid (**19**) [via nonanoic acid (**17**)] and monomethyl 1,9-nonanedioate (**18**) (Scheme II). These were converted to their corresponding (*S*)-(+)-(methoxycarbonyl)benzyl esters **20** and **21a** for NMR analysis because the 2-*pro-R* hydrogens are known

(25) Vederas, J. C. *Can. J. Chem.* **1982**, *60*, 1637–1642 and references cited therein.
(26) Abell, C.; Staunton, J. *J. Chem. Soc., Chem. Commun.* **1981**, 856–858.
(27) (a) Schroepfer, G. J., Jr.; Bloch, K. *J. Biol. Chem.* **1965**, *240*, 54–63. (b) Morris, L. J.; Harris, R. V.; Kelly, W.; James, A. T. *Biochem. Biophys. Res. Commun.* **1967**, *28*, 904–908. (c) Morris, L. J. *Biochem. J.* **1970**, *118*, 681–693. (d) White, R. H. *Biochemistry* **1980**, *19*, 9–15.

Scheme III[a]



[a] (a) (−)-Pinene, 9-BBN; (b) TsCl, pyridine; (c) (+)-pinene, 9-BBN; (d) $Na_2Fe(CO)_4$; (e) I_2, methyl (*S*)-(+)-mandelate.

to generally appear downfield of the 2-*pro-S* hydrogens in such esters.[28] Direct esterification of oleic acid (**2**) with methyl (*S*)-(+)-mandelate for analysis of its C-2 position did not seem feasible because NMR examination of **15** and **16** showed major loss of deuterium at that site, possibly due to exchange during basic hydrolysis of the crude fats.

To verify that the 2-*pro-R* hydrogen of the functionalized ester **21a** would indeed resonate downfield as expected,[28] the deuterated diastereomers **21b** and **21c** were independently synthesized as shown in Scheme III. A mixture (~2:1) of deuterated and unlabeled aldehydes **22** was subjected to Midland reduction[29] using (−)-pinene to produce a stereospecifically labeled alcohol which was isolated as its tosylate **23b**. Reaction of this with disodium tetracarbonylferrate[30] followed by iodine and methyl (*S*)-(+)-mandelate gave the 2*S* ester **21b**. The substitution reaction is known to proceed with inversion of configuration.[30c] Use of (+)-pinene for the Midland reduction[29] in the same reaction sequence afforded the 2*R* diastereomer **21c**.

The deuterium-decoupled $^1H,^{13}C$ shift correlation spectra of **21a**, **21b**, and **21c** (Figure 5) demonstrate that the 2-*pro-R* hydrogen does indeed appear downfield. More importantly, these spectra show that the deuterium configuration at C-2 of the biosynthetically labeled **21a** [corresponding to C-8 of the oleic acid (**2**)] is *S*. Because of a priority change, this corresponds to *R on the growing chain* of the saturated fatty acid **14**. Examination of analogous 2D NMR spectra of the octanoate ester **20** confirms that, as expected,[6a,f,h] its C-2 carbon (corresponding to C-12 of oleate **2**) has *R* configuration. Since the stereochemistry of acetate-derived hydrogens in fatty acids from a single organism is the same at all sites,[6] these results support the proposal that dehydrogenation of stearic acid (**14**) to oleic acid (**2**) in *C. cladosporioides* involves loss of the 10-*pro-R* hydrogen.

Discussion

Once the absolute stereochemistry of cladosporin (**1**) was verified by degradation to optically pure **12**, the combination of ^1H-decoupling experiments and NOE studies provides the necessary stereochemical assignment of proton NMR resonances. The results also indicate that the major solution conformation of diacetate **13** (Figure 2) closely resembles the crystal structure of cladosporin (**1**).[11e] This fits well with extensive studies on orientation of C-glycosides;[23] for example, the C-10 to C-11 bond is roughly antiperiplanar to the C-3 to C-9 bond. The proposed conformation about the latter bond derives support from the strong NOE between the hydrogen at C-3 and that at the 9-*pro-S* position, but not with the one at the 9-*pro-R* site.

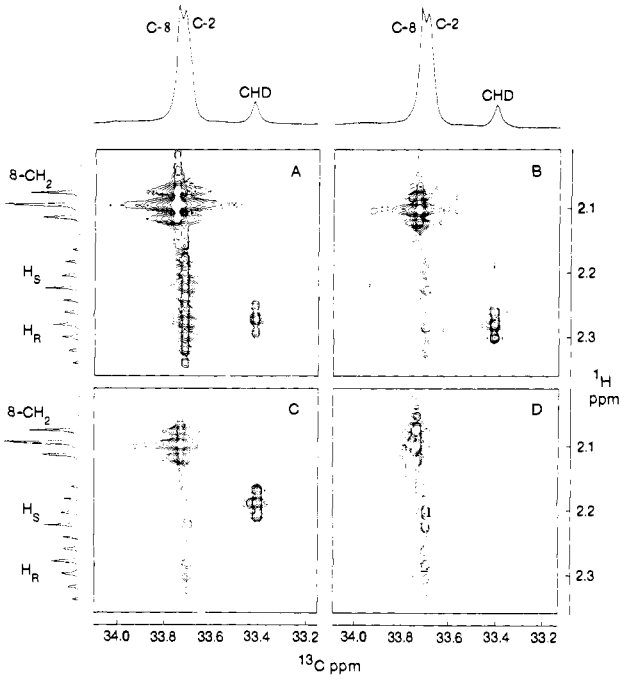


Figure 5. ^2H-Decoupled $^1H,^{13}C$ chemical shift correlation plots[13] of the C-8 and C-2 regions of **21a** (A), **21b** (B), **21c** (C), and unlabeled material (D). Spectra were measured on a Bruker WH400 on ca. 0.3 M solutions in C_6D_6.

The eight acetate units in cladosporin (**1**) have the expected head to tail connection typical of polyketide biogenesis.[2,3] An important feature apparent from the pattern of intact carbon–oxygen and carbon–hydrogen bonds is that the number of acetate-derived hydrogens at any nonaromatic site corresponds to that predicted from the oxidation state achieved by the *previous* acetate unit (i.e., one closer to the starter unit) during an assembly process (Figure 1) akin to fatty acid biogenesis. For example, two acetate hydrogens are present at C-13 because C-14 is reduced only to the alcohol stage, whereas only one is found at C-11 which follows the completely reduced methylene at C-12. Thus, the location of the acetate hydrogen at C-11 would be determined by enzymatic reduction of an intermediate six carbon conjugated enoyl thiol ester to the corresponding saturated derivative (5-hydroxyhexanoyl thiol ester, Figure 6). This is in analogy to the construction of stearic acid (**14**) and its dehydrogenation product, oleic acid (**2**).[6,27]

However, despite the similarities in the labeling patterns, there are important differences in the actual stereochemistry. The carbon–oxygen bonds derived from acetate at C-14 and C-3 of **1** possess configurations which would be *S on the growing chain*.[31] This is opposite to the *R* stereochemistry believed to be universal for hydroxy intermediates in fatty acid biosynthesis.[6] Furthermore, the present work demonstrates that enoyl thiol ester reduction (Figure 1) in *C. cladosporioides* leaves acetate-derived hydrogen in the *R* configuration on growing fatty acids but in the *S* position at C-11 on the growing polyketide chain enroute to **1**. Understanding the significance and generality of this phenomenon requires additional stereochemical comparisons of fatty acid and polyketide biosynthesis.[4,7,9] Incorporation of $^{13}C,^2H$ doubly labeled precursors follwed by stereochemical analysis of the products with

(28) (a) Parker, D. *J. Chem. Soc., Perkin Trans. 2* **1983**, 83–88. (b) Schwab, J. M.; Klassen, J. B. *J. Am. Chem. Soc.* **1984**, *106*, 7217–7227.
(29) Midland, M. M.; Greer, S.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1979**, *101*, 2352–2355.
(30) (a) Collman, J. P.; Winter, S. R.; Komoto, R. G. *J. Am. Chem. Soc.* **1973**, *95*, 249–250. (b) Collman, J. P. *Acc. Chem. Res.* **1975**, *8*, 342–347. (c) Collman, J. P.; Winter, S. R.; Clark, D. R. *J. Am. Chem. Soc.* **1972**, *94*, 1788–1789. (d) Cooke, M. P. *J. Am. Chem. Soc.* **1970**, *92*, 6080–6082.

(31) The configuration at C-3 of **1** becomes *R* upon formation of the aromatic ring because of a priority change.

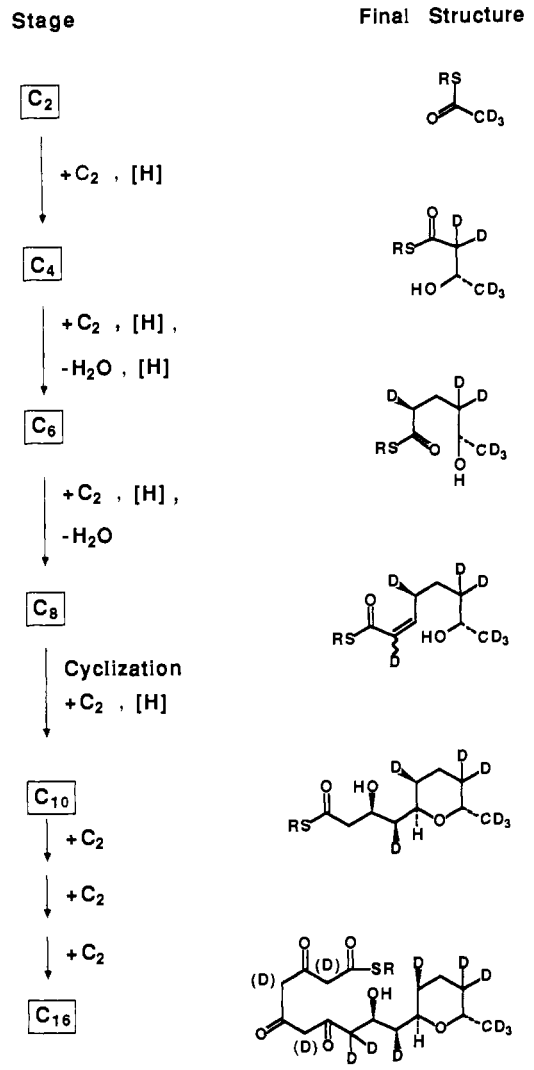


Figure 6. Proposed structures of enzyme-bound intermediates at the completion of each cycle of two-carbon addition (cf. Figure 1). Tetrahydropyran cyclization may also occur immediately after addition of the C_2 unit to the C_8 stage.

^{2}H-decoupled ^{1}H,^{13}C shift correlation NMR spectroscopy[13] is a powerful technique for this purpose. This is especially evident in the spectra of **21** (Figure 5), wherein the chemical shift separation of the H_R and H_S protons at C-2 is less than 0.1 ppm and the C-8 and C-2 carbon resonances overlap extensively. Since incorporation of acetate in this case is only about 2% per site and some deuterium loss occurs through biological exchange with the aqueous media, double labeling with ^{13}C and ^{2}H is essential for intact bond detection.[2a]

The deuterium configuration at C-9 cladosporin (**1**) derived from [2-^{13}C,^{2}H$_3$]acetate almost certainly reflects the mechanism of tetrahydropyran ring formation. The most likely process is Michael addition of a hydroxyl group at C-14 on a $\Delta^{9,10}$-conjugated intermediate (Figure 7). Such ring closures on α,β-unsaturated ketones or esters are well precedented to occur spontaneously, although without enzyme the major product is usually the more stable diequatorial cis-substituted tetrahydropyran system.[14,16] This type of unsaturated compound would be the expected enzyme-bound intermediate at the C_8 stage of cladosporin biosynthesis (Figure 6). Attachment of another two carbons by the polyketide synthase would initially produce a C_{10} β-keto γ,δ-unsaturated thiol ester which would be even more prone to intramolecular Michael cyclization.[32] Regardless of whether tetrahydropyran formation occurs at the C_8 or C_{10} stage, the stereochemistry of acetate deuterium at C-9 (*R*) and the *R* configuration at C-10 requires either syn addition on a trans double bond or anti addition on a cis one (Figure 7). Experiments to test the intermediacy of such compounds are in progress.

(32) Failure of ϵ-hydroxy α,β-unsaturated *thiol esters* to spontaneously cyclize to tetrahydrofuran systems[5c] suggests that uncatalyzed ring closure of a C_8 *thiol ester* to the tetrahydropyran may be slow.

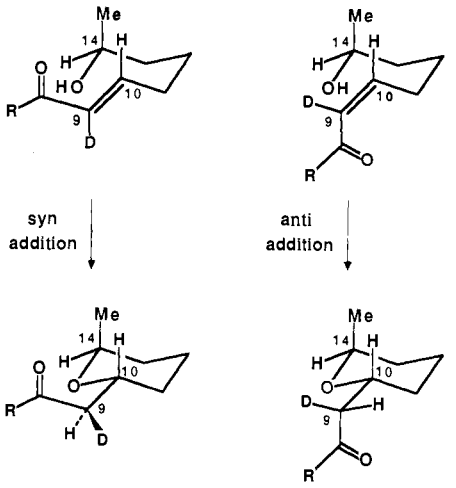


Figure 7. Possible modes of tetrahydropyran ring formation consistent with observed stereochemistry at C-9 and C-10.

In summary, this work has confirmed the absolute stereochemistry of cladosporin (**1**), determined the approximate major conformation of its diacetate **13** in solution, and identified all bonds remaining intact from acetate during biosynthesis (Figure 3). The combination of ^{13}C,^{2}H double labeling and analysis by ^{2}H-decoupled ^{1}H,^{13}C shift correlation NMR spectroscopy provides a rapid approach for elucidation of the stereochemistry of acetate-derived hydrogens in fatty acids and polyketides; it should also be applicable to other classes of natural products (e.g., terpenoids). The results show that although the pattern of intact acetate bonds in the polyketide cladosporin (**1**) supports its formation by a process very closely related to fatty acid biosynthesis, the stereochemistry at several sites (C-3, C-14, C-11) is opposite. Additional examples of this phenomenon are discussed in the accompanying paper.[9]

Experimental Section

General Methods. Most general procedures, instrumentation, and NMR methods have been previously described.[3i,13b] Labeled precursors (>98% isotopic purity) were purchased from Cambridge Isotope Laboratories (Woburn, MA) unless otherwise indicated. Flash chromatography employed the method of Still on Merck silica gel (230–420 mesh).[33] Two-dimensional ^{2}H-decoupled ^{1}H,^{13}C shift correlation NMR spectra[13b] (nonselective) of chiral esters **20** and **21a–c** in C_6D_6 (C_6F_6 lock, 5%) used the Bruker XHCORR·AU program on a WH400 instrument with a relaxation delay (D_1) of 2.0 s, sweep widths of 1136 Hz in the F_2 (^{13}C) and 600 Hz in the F_1 (^{1}H) dimensions, and 256 experiments of size 1K. The spectra were processed as power spectra (with zero filling once in the F_1 dimension) with Gaussian windows. NOE experiments on cladosporin diacetate (**13**) were done in degassed $CDCl_3$ with the Bruker NOEMULT program on an AM300 instrument with irradiation of tetramethylsilane as standard and subtraction of fid's to obtain difference spectra. ROESY spectra were obtained by literature procedures.[20,21c]

Fermentation of *C. cladosporioides* To Produce Cladosporin (1) and Incorporation Experiments. A procedure similar to that used by Scott and Van Walbeek[10a] was employed. Potato dextrose slants (240 mg of Difco potato dextrose broth and 200 mg of Difco Bacto-agar in 10 mL of deionized water per slant) were inoculated with *C. cladosporioides* NRRL 5507. The slants were grown at 25 °C for 7 days and then stored at 4 °C until required. Spore suspensions from these slants were used to inoculate media containing yeast extract (20 g/L) and sucrose (150 g/L of deionized water). In a typical labeled acetate incorporation experiment, eight Erlenmeyer flasks (500 mL) containing the yeast extract–sucrose broth (125 mL) were inoculated with a spore suspension in water and then shaken (160 rpm) at 25 °C. An aqueous solution of labeled sodium acetate (total 1.0 g in ca. 10 mL of water) was administered in equal aliquots after 12, 24, 36, and 48 h. After 132 h, the

(33) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

mycelium was filtered, air-dried, and homogenized with chloroform (500 mL) in a Waring blender. This homogenate was stirred overnight at 25 °C and then filtered. Drying and solvent removal in vacuo gave the crude extract (1.2 g). Purification by flash chromatography (50% EtOAc/hexane) gave a triglyceride fraction (928 mg) with subsequent elution of cladosporin (**1**) (89 mg, 0.30 mmol): mp 187.5–188.5 °C (lit.[10a] mp 188.5–189 °C); $[\alpha]_D$ = −18.1° (*c* 1.2, 98% ethanol) [lit.[10a] $[\alpha]$ −24.8° (*c* 0.9, ethanol)]; IR ($CHCl_3$ cast) 3230 (br), 2937 (m), 1620 (vs), 1592 (s), 1161 (m), 1117 (m), 1035 (w) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 11.17 (br s, 1 H, O*H*), 6.29 (d, 1 H, 2 Hz, Ar*H*), 6.18 (d, 1 H, 2 Hz, Ar*H*), 5.65 (br s, 1 H, O*H*), 4.75–4.58 (m, 1 H), 4.12–4.06 (m, 1 H), 4.01–3.94 (m, 1 H), 2.93–2.81 (ABX, 2 H, C*HH*Ar), 1.97 (ddd, 1 H, 15, 10, and 4 Hz, H-9), 1.84 (ddd, 1 H, 16, 9, and 3 Hz, H-9), 1.75–1.59 (m, 4 H), 1.39–1.31 (m, 2 H), 1.22 (d, 3 H, 7 Hz, CH_3); ^{13}C NMR (100 MHz; $CDCl_3$) δ 169.7, 164.5, 162.5, 141.9, 128.4, 106.6, 102.0, 76.4, 67.9, 66.5, 39.5, 33.7, 31.00, 30.97, 19.0, 18.3; exact mass 292.1312 (292.1311 calcd for $C_{16}H_{20}O_5$).

Cladosporin Dimethyl Ether (4). An ether solution of diazomethane was added to a solution of cladosporin (**1**) (87.4 mg, 0.299 mmol) in methanol (50 mL) until a yellow color persisted. The mixture was stirred for 24 h with further additions of diazomethane until thin-layer chromatography (TLC) (50% EtOAc/hexane) showed that the rapidly formed monomethyl ether (R_f 0.7) was converted to the dimethyl ether (R_f 0.2). Removal of solvent in vacuo and chromatography on silica gel (50% EtOAc/hexane) gave **4** (75.1 mg, 78%): mp 115–116 °C (lit.[10b] mp 118 °C); $[\alpha]^{25}_D$ +48.1° (*c* 1.3, MeOH) [lit.[10b] $[\alpha]_D$ +48.0° (*c* 0.05, MeOH)]; IR ($CHCl_3$ cast) 1718 (s), 1602 (s), 1580 (m), 1341 (m), 1238 (m), 1220 (m), 1160 (m), 1078 (m), 1040 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.40 (d, 1 H, 2 Hz, Ar*H*), 6.29 (d, 1 H, 2 Hz, Ar*H*), 4.60–4.53 (m, 1 H), 4.12–4.04 (m, 1 H), 3.97–3.90 (m, 1 H), 3.92 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 2.94–2.75 (m, 2 H), 1.93–1.77 (m, 2 H), 1.72–1.56 (m, 4 H), 1.36–1.25 (m, 2 H), 1.19 (d, 3 H, 7 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 163.1, 162.7, 144.1, 107.2, 104.1, 97.8, 74.5, 67.4, 66.3, 56.1, 55.6, 39.5, 35.5, 31.0 (2 C), 19.0, 18.3; exact mass 320.1625 (320.1624 calcd for $C_{18}H_{24}O_5$).

Reduction of 4 to Diol 5. Lithium borohydride (1.00 g, 45.9 mmol) was added slowly to a solution of cladosporin dimethyl ether (**4**) (1.02 g, 3.19 mmol) in THF (30 mL). The mixture was warmed to 60 °C for 3.5 h, cooled, and added to water (100 mL). THF was removed in vacuo, and the resulting mixture was extracted with dichloromethane (3 × 75 mL). The extracts were dried (Na_2SO_4), concentrated in vacuo, and purified by flash chromatography (66% EtOAc/hexane) to afford diol **5** as an oil (919 mg, 89%): $[\alpha]^{25}_D$ −27.0° (*c* 1.2, $CHCl_3$); IR ($CHCl_3$ cast) 3350 (br), 2954 (s), 1606 (s), 1592 (s), 1463 (m), 1455 (m), 1424 (m), 1314 (m), 1203 (s), 1148 (s), 1097 (m), 1059 (m), 1005 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.39 (d, 1 H, 2 Hz, Ar*H*), 6.36 (d, 1 H, 2 Hz, Ar*H*), 4.81 (d, 1 H, 12 Hz, C*H*HOH), 4.57 (d, 1 H, 12 Hz, CH*H*OH), 4.14–4.00 (m, 2 H, 2 × C*H*–O), 3.86 (s, 3 H, OCH_3), 3.83 (s, 3 H, OCH_3), 3.75–3.60 (m, 1 H, C*H*–O), 2.93 (dd, 1 H, 12 and 9 Hz, ArC*H*H), 2.77 (dd, 1 H, 12 and 3 Hz, ArCH*H*), 1.97–1.86 (m, 1 H, CHC*H*HCH–O), 1.80–1.60 (m, 4 H), 1.50–1.30 (m, 3 H), 1.15 (d, 3 H, 7 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.9, 159.1, 140.4, 121.4, 106.3, 96.6, 69.8, 67.9, 67.6, 55.2, 55.1 (2 C), 40.7, 40.6, 30.5, 30.3, 18.3, 18.1; exact mass 324.1931 (324.1937 calcd for $C_{18}H_{28}O_5$).

Hydrogenolysis of 5 to Alcohol 6. A suspension of palladium on carbon (5%, 250 mg) in a solution of diol **5** (909 mg, 2.8 mmol), methanol (4 mL), and glacial acetic acid (2.0 mL) was stirred under hydrogen overnight. The mixture was filtered through Celite, solvent was removed in vacuo, and the residue was purified by flash chromatography (25% EtOAc/hexane) to give alcohol **6** (628 mg, 72%): $[\alpha]_D$ −17.4° (*c* 4.5, $CHCl_3$); IR ($CHCl_3$ cast) 3430 (s), 2933 (s), 1606 (s), 1592 (s), 1490 (m), 1484 (s), 1203 (s), 1149 (s), 1109 (m), 1060 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.35 (br s, 2 H, Ar*H*), 4.15–4.01 (m, 2 H, 2 × C*H*–O), 4.00–3.92 (m, 1 H, C*H*–O), 3.79 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 2.84–2.72 (m, 2 H, ArC*HH*), 2.63 (br d, 1 H, O*H*), 2.11 (s, 3 H, ArCH_3), 1.95–1.84 (m, 1 H, CH*H*CHOH), 1.72–1.58 (m, 4 H), 1.57–1.46 (m, 1 H, C*H*HCHOH), 1.42–1.26 (m, 2 H), 1.19 (d, 3 H, 6 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.2, 158.5, 139.1, 117.7, 106.7, 96.7, 69.0, 67.8, 67.5, 55.4, 55.2, 41.6, 39.5, 30.7, 30.3, 18.6, 18.1, 10.8; exact mass 308.1981 (308.1987 calcd for $C_{18}H_{28}O_4$).

Oxidation of 6 to Ketone 7. Dimethyl sulfoxide (1.7 mL, 24 mmol) was added to a cooled (−60 °C) solution of oxalyl chloride (1.0 mL, 11 mmol) in dichloromethane (25 mL). After 2 min, a solution of alcohol **6** (620 mg, 2.01 mmol) in dichloromethane (5.0 mL) was added. The mixture was stirred 20 min at −50 °C, triethylamine (7.0 mL, 50 mmol) was added, and the temperature was raised to 25 °C over 1 h. The mixture was added to water (200 mL) and extracted with dichloromethane (3 × 75 mL). The combined organic extracts were washed with hydrochloric acid (1%, 200 mL) followed by sodium carbonate solution (5%, 200 mL), dried ($MgSO_4$), and concentrated in vacuo. Purification by flash chromatography (15% EtOAc/hexane) gave ketone **7** (498 mg, 81%): $[\alpha]^{25}_D$ −38.3° (*c* 3.1, $CHCl_3$); IR ($CHCl_3$ cast) 2934 (s), 2867 (m), 2840 (m), 1716 (s), 1607 (s), 1593 (s), 1492 (m), 1464 (s), 1381 (m), 1317 (m), 1309 (m), 1203 (s), 1149 (s), 1110 (m), 1056 (s), 1010 (m), 831 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.37 (d, 1 H, 2 Hz, Ar*H*), 6.28 (d, 1 H, 2 Hz, Ar*H*), 4.30–4.22 (m, 1 H, C*H*–O), 3.92–3.84 (m, 1 H, C*H*–O), 3.77 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 3.71 (s, 2 H, ArCH_2), 2.78 (dd, 1 H, 15 and 8 Hz, C*H*HCO), 2.42 (dd, 1 H, 15 and 5 Hz, CH*H*CO), 2.00 (s, 3 H, ArCH_3), 1.70–1.50 (m, 4 H), 1.30–1.22 (m, 2 H), 1.15 (d, 3 H, 6 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.0, 159.1, 158.7, 134.7, 118.2, 106.8, 97.3, 67.4 (2 C), 55.3, 55.1, 49.1, 45.9, 30.8, 29.8, 18.9, 17.8, 10.8; exact mass 306.1827 (306.1831 calcd for $C_{18}H_{26}O_4$). Anal. Calcd for $C_{18}H_{26}O_4$: C, 70.56; H, 8.55. Found: C, 70.16; H, 8.51.

Conversion of 7 to (*Z*)-Oxime 8a and (*E*)-Oxime 8b. Hydroxylamine hydrochloride (750 mg, 10.8 mmol) was added to a solution of ketone **7** (332 mg, 1.08 mmol) in ethanol (20 mL) and water (20 mL). The pH was maintained at 3.5 for 2 h by addition of dilute sodium hydroxide solution as necessary. The mixture was extracted with dichloromethane (3 × 70 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography (20% EtOAc/hexane) gave the (*Z*)-oxime **8a** as a solid (189 mg, 54%; R_f 0.12 in 20% EtOAc/hexane) and the (*E*)-oxime **8b** as an oil (117 mg, 33%; R_f 0.09).

For (*Z*)-oxime **8a**: mp 92–93 °C; $[\alpha]^{25}_D$ −48.2° (*c* 2.9, $CHCl_3$); IR ($CHCl_3$ cast) 3300 (br), 2935 (m), 1606 (m), 1593 (m), 1462 (m), 1454 (m), 1204 (s), 1147 (s), 1056 (m), 1045 (m) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.36 (d, 1 H, 2.4 Hz, Ar*H*), 6.32 (d, 1 H, 2.4 Hz, Ar*H*), 4.20–4.10 (m, 1 H, C*H*–O), 4.02–3.95 (m, 1 H, C*H*–O), 3.79 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 3.55 (m, 2 H, ArC*HH*), 2.69 (dd, 1 H, 13.3 and 8.8 Hz, C*H*HC=N), 2.46 (dd, 1 H, 13.3 and 5.1 Hz, CH*H*C=N), 2.09 (s, 3 H, ArCH_3), 1.70–1.50 (m, 4 H), 1.40–1.20 (m, 2 H), 1.16 (d, 3 H, 6.5 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.6, 158.1, 157.8, 136.7, 117.9, 106.2, 96.9, 68.7, 67.0, 55.4, 55.2, 39.0, 31.6, 30.8, 29.9, 19.8, 18.3, 10.9; exact mass 321.1935 (321.1940 calcd for $C_{18}H_{27}NO_4$).

For (*E*)-oxime **8b**: $[\alpha]^{25}_D$ +3.5° (*c* 2.2, $CHCl_3$); IR ($CHCl_3$ cast) 3300 (br), 2934 (s), 1607 (s), 1593 (s), 1491 (m), 1464 (s), 1204 (s), 1148 (s), 1090 (m), 1056 (m), 1047 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.40 (m, 2 H, Ar*H*), 4.05–3.91 (m, 1 H, C*H*–O), 3.93–3.83 (m, 1 H, C*H*–O), 3.85 (d, 1 H, 15 Hz, ArC*HH*), 3.77 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 3.63 (d, 1 H, 15 Hz, ArCH*H*), 2.48 (dd, 1 H, 14 and 8 Hz, CHC*H*HC=N), 2.12 (dd, 1 H, 14 and 6 Hz, CHCH*H*C=N), 2.08 (s, 3 H, ArCH_3), 1.68–1.46 (m, 4 H), 1.30–1.18 (m, 2 H), 1.11 (d, 3 H, 6 Hz, $CHCH_3$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 158.6, 158.3, 157.6, 136.5, 117.7, 106.1, 96.8, 68.3, 67.0, 55.6, 55.3, 36.7, 31.5 (2 C), 29.5, 19.7, 18.2, 10.8; exact mass 321.1943 (321.1940 calcd for $C_{18}H_{27}NO_4$).

Rearrangement of Oxime 8a to Amide 9. *p*-Toluenesulfonyl chloride (315 mg, 1.65 mmol) and dry pyridine (0.222 mL, 2.76 mmol) were added to a solution of oxime **8a** (177 mg, 0.550 mmol) in chloroform (10 mL). The reaction mixture was stirred at 40 °C for 11 h. Concentration in vacuo and purification of the residue by flash chromatography (50% EtOAc/hexane) gave solid amide **9** (113 mg, 64%): mp 103–104 °C; $[\alpha]^{25}_D$ −24.1° (*c* 2.5, $CHCl_3$); IR ($CHCl_3$ cast) 3298 (br), 2934 s), 1644 (s), 1610 (m), 1592 (m), 1544 (m), 1465 (m), 1314 (m), 1201 (s), 1150 (s), 1058 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.91 (br, 1 H, N*H*), 6.43 (d, 1 H, 2 Hz, Ar*H*), 6.37 (d, 1 H, 2 Hz, Ar*H*), 4.42–4.32 (m, 2 H, C*HH*NH), 4.15–4.06 (m, 1 H, C*H*–O), 3.98–3.90 (m, 1 H, C*H*–O), 3.77 (s, 3 H, OCH_3), 3.76 (s, 3 H, OCH_3), 2.75 (s, 3 H, OCH_3), 2.59 (dd, 1 H, 15 and 9 Hz, C*H*HC=O), 2.24 (dd, 1 H, 15 and 3 Hz, CH*H*C=O), 2.09 (s, 3 H, ArCH_3), 1.70–1.54 (m, 4 H), 1.34–1.23 (m, 2 H), 1.10 (d, 3 H, 7 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 158.6, 158.3, 137.7, 116.9, 104.9, 97.3, 67.6, 67.5, 55.4, 55.1, 41.7, 40.6, 30.6, 30.2, 18.6, 17.9, 10.3; exact mass 321.1937 (321.1940 calcd for $C_{18}H_{27}NO_4$). Anal. Calcd for $C_{18}H_{27}NO_4$: C, 67.26; H, 8.47; N, 4.36. Found: C, 66.98; H, 8.42; N, 4.26.

Conversion of 9 via Imidate 10 to Methyl (2*R*,6*S*)-(6-Methyltetrahydropyran-2-yl)acetate (11). Trimethyloxonium tetrafluoroborate[34] (62.0 mg, 0.419 mmol) was added to a solution of amide **9** (62.3 mg, 0.194 mmol) in $CDCl_3$ (10 mL). The suspension was heated to reflux for 2.5 h, and the progress of the reaction was followed by ^1H NMR; three more portions of oxonium salt (21 mg, 0.14 mmol each) were added at 30-min intervals before all amide **9** was consumed. The NMR spectra indicated the product was about 80% unstable imidate salt **10**: ^1H NMR (400 MHz, $CDCl_3$) δ 10.06 (br, 1 H, N*H*), 6.44 (d, 1 H, 2 Hz, Ar*H*), 6.39 (d, 1 H, 2 Hz, Ar*H*), 4.65–4.47 (m, 2 H, C*HH*N), 4.31 (s, 3 H,

(34) Meerwein, H. In *Organic Syntheses*; Baumgarten, H. E., Ed.; Wiley: New York, 1973; Collect. Vol. V., pp 1096–1098.

$COCH_3$), 4.30–4.24 (m, 1 H, C*H*–O), 3.92–3.84 (m, 1 H, C*H*–O), 3.74 (s, 6 H, 2 × OCH_3), 3.18 (dd, 1 H, 18 and 10 Hz, MeOCCH*H*), 2.89 (dd, 1 H, 3 and 18 Hz, MeOCCH*H*), 2.06 (s, 3 H, $ArCH_3$), 1.80–1.70 (m, 1 H), 1.67–1.55 (m, 3 H), 1.45–1.35 (m, 1 H), 1.30–1.19 (m, 1 H), 0.90 (d, 3 H, 7 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.3, 159.3, 132.8, 117.5, 105.3, 99.0, 68.1, 68.0, 60.5, 55.4, 55.3, 44.7, 31.4, 30.3, 29.2, 18.6, 17.1 (2 C), 10.1.

A solution of water (1 mL) acidified to pH 4 with formic acid and acetone (1 mL) was added to the solution of imidate salt **10**. The mixture was stirred 16 h, acetone was removed in vacuo, and the residue was diluted with water (10 mL) and extracted with dichloromethane (3 × 10 mL). The extract was dried (Na_2SO_4), carefully concentrated in vacuo, and purified by column chromatography on silica gel (230–420 mesh, 5 mm × 25 mm) with ethyl acetate/hexane (20%, 5 mL) to give the crude ester **11** and with pure ethyl acetate (5 mL) to afford recovered amide **9** (11.8 mg, 19%). The ester fraction was carefully concentrated in vacuo and distilled in a kugelrohr apparatus (7 mmHg) to give ester **11** (18.6 mg, 56%): $[\alpha]^{25}_D$ −40.9° (*c* 1.2, $CHCl_3$); IR ($CHCl_3$ cast) 2935 (s), 1739 (s), 1288 (m), 1212 (m), 1168 (m), 1048 (m), 756 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.29–4.22 (m, 1 H, C*H*–O), 3.98–3.90 (m, 1 H, C*H*–O), 3.68 (s, 3 H, OCH_3), 2.68 (dd, 1 H, 15 and 8 Hz, C*H*HC=O), 2.45 (dd, 1 H, 15 and 6 Hz, CH*H*C=O), 1.76–1.59 (m, 4 H), 1.41–1.24 (m, 2 H), 1.18 (d, 3 H, 6 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 67.9, 67.4, 51.4, 38.7, 30.9, 29.4, 19.2, 17.9.

(2*R*,6*S*)-(6-Methyltetrahydropyran-2-yl)acetic Acid (12). The procedure of Ley et al. was adapted.[17] The ester **11** (13.4 mg, 77.9 μmol) was dissolved in methanol (1 mL), treated with aqueous sodium hydroxide (4 mL, 10% w/v), and heated to reflux for 2 h. The cooled solution was diluted with water (40 mL), acidified to pH 3 with HCl, and extracted with ether (40 mL). The dried (Na_2SO_4) extracts were concentrated in vacuo to give the acid **12** (7.8 mg, 64%): $[\alpha]^{25}_D$ −42.6° (*c* 0.8, $CHCl_3$) [lit.[14] $[\alpha]_D$ −39.5° (*c* 1.45, $CHCl_3$)]; IR ($CHCl_3$ cast) 3050 (br), 2970 (m), 2935 (s), 1735 (s), 1708 (s), 1296 (m), 1212 (m), 1176 (m), 1136 (m), 1049 (m), 1011 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 4.31–4.16 (m, 1 H, C*H*–O), 4.15–3.99 (m, 1 H, C*H*–O), 2.71 (dd, 1 H, 15 and 9 Hz, C*H*HC=O), 2.46 (dd, 1 H, 15 and 5 Hz, CH*H*C=O), 1.83–1.56 (m, 4 H), 1.50–1.30 (m, 2 H), 1.24 (d, 3 H, 7 Hz, $CHCH_3$); ^{13}C NMR (100 MHz, $CHCl_3$) δ 175.6, 68.1, 67.4, 38.7, 30.5, 29.5, 18.7, 17.6; exact mass 158.0943 (158.0943 calcd for $C_8H_{14}O_3$).

Diacetyl Cladosporin (13).[10b] Acetic anhydride (1.0 mL, 10.6 mmol) was added to a solution of cladosporin (**1**) (100 mg, 0.34 mmol) in dry pyridine (2 mL). The reaction mixture was stirred at 25 °C for 24 h and was then poured into ice–water. This mixture was extracted with ethyl acetate, the combined organic layers were dried ($MgSO_4$), and the solvent was removed in vacuo to give a gum (135 mg). Purification by preparative thin-layer chromatography (toluene/EtOAc/formic acid, 6:3:1) gave the diacetate (**13**) (103 mg, 80%): $[\alpha]^{25}_D$ 39.8° (*c* 1.6, $CHCl_3$); IR ($CHCl_3$ cast) 2930 (m), 1777 (s), 1723 (s), 1614 (m), 1369 (m), 1251 (m), 1189 (s), 1133 (s), 1088 (m), 1052 (s) cm^{-1}; ^1H and ^{13}C NMR data given in Table I; exact mass 376.1526 (376.1522 calcd for $C_{20}H_{24}O_7$).

Isolation and Purification of Methyl Oleate (16). Water (0.162 mL, 9.0 mmol) was added to a solution of potassium *tert*-butoxide (1.0 g, 8.9 mmol) in dry THF (50 mL). The solution was stirred at 0 °C for 5 min, a solution of the fatty acid ester fraction (928 mg) (isolated from the fermentation described above) in THF (10 mL) was added, and the resulting mixture was stirred at room temperature for 5 h. The solvent was removed in vacuo, hexane (50 mL) was added, and the solvent was removed in vacuo to give a residue which was dissolved in water (100 mL). Acidification to pH 3 with concentrated hydrochloric acid and extraction with ether (3 × 75 mL) gave a semisolid (874 mg). Purification by flash chromatography with 7% EtOAc/hexane gave a neutral fraction and with 20% EtOAc/hexane gave an acidic fraction (665 mg). A solution of the acidic fraction (665 mg) in dichloromethane (10 mL) at 0 °C was treated with excess diazomethane in ether. Solvent removal in vacuo gave a mixture of fatty acid methyl esters (721 mg). Purification[7a] by preparative TLC (20% $AgNO_3$; 1% methanol/chloroform) gave methyl linoleate (19 mg), methyl palmitate contaminated with methyl stearate (**15**) (209 mg), and methyl oleate (**16**) (271 mg). ^1H NMR, ^{13}C NMR, and gas chromatography–mass spectral comparisons with authentic materials confirmed the structural assignments.

Degradation of 16 to Nonanoic Acid (17) and Monomethyl 1,9-Nonanedioate (18). To a solution of methyl oleate (**16**) (230 mg, 0.776 mmol) in *tert*-butyl alcohol (90 mL) was added a solution of potassium carbonate (100 mg) in water (190 mL). The oxidizing reagent was prepared by the addition of sodium periodate (2.086 g, 9.75 mmol) and potassium permanganate (0.039 g, 0.25 mmol) to water (100 mL). This solution was added over 5 min to the reaction mixture to give a purple solution which became fainter as stirring was continued for 66 h. Excess sodium bisulfite (2.85 g, 20.6 mmol) was added, and the solution was neutralized with saturated aqueous sodium bicarbonate. The volume of the reaction mixture was reduced in vacuo to ca. 250 mL, acidified to pH 2 with concentrated hydrochloric acid, and extracted with chloroform (3 × 100 mL). The extracts were dried (Na_2SO_4) and concentrated in vacuo. The resulting oil (260 mg) was purified by preparative thin-layer chromatography (hexane/ether/methanol/acetic acid, 90:10:5:5, eluted twice) to give recovered methyl oleate (**16**) (4 mg; upper band), monomethyl 1,9-nonanedioate (**18**) (62.0 mg, 40%; lower band), and nonanoic acid (**17**) (75.2 mg, 62%; middle band). The chromatographic and spectral properties of **17** and **18** were consistent with those of the commercially available unlabeled compounds (Aldrich). For labeled nonanoic acid (**17**) derived from sodium [2-^{13}C,$^2H_3$]acetate: ^{13}C NMR (100 MHz, $CDCl_3$; ^2H decoupled) δ 180.05 (C-1), 33.93 (C-2), 31.58 (C-7), 31.16 (C-7, CHD), 28.95 (C-4), 28.85 (C-5, C-6), 28.54 (C-5, CHD), 24.42 (C-3), 24.06 (C-3, CHD), 22.35 (C-8), 13.70 (C-9), 13.40 (C-9, CH_2D), 13.10 (C-9, CHD_2), 12.82 (C-9, CD_3).

Degradation of Nonanoic Acid (17) to Octanoic Acid (19). Excess ethereal diazomethane was added to a solution of nonanoic acid (1.45 g, 9.18 mmol) in chloroform (10 mL). Removal of the solvent in vacuo gave the methyl ester (1.53 g, 97%). For unlabeled material: IR ($CHCl_3$ cast) 2956 (m), 2925 (s), 2858 (s), 1743 (s), 1460 (m), 1436 (m), 1251 (m), 1196 (m), 1167 (7); ^1H NMR (400 MHz, $CDCl_3$) δ 3.66 (s, 3 H, OCH_3), 2.32 (t, 2 H, 8 Hz, CH_2C=O), 1.67–1.60 (m, 2 H, CH_2CH_2CO), 1.34–1.24 (m, 10 H), 0.89 (t, 3 H, 7 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 51.0, 33.9, 31.7, 29.06, 29.04, 28.96, 24.8, 22.5, 13.8; exact mass 172.1463 (172.1463 calcd for $C_{10}H_{20}O_2$).

To a solution of phenylmagnesium chloride (2.0 M in THF; 7.5 mL, 15 mmol) at 0 °C was added a solution of the methyl nonanoate (519 mg, 3.02 mmol) in THF (6 mL). The reaction mixture was warmed to 25 °C, stirred for 4 h, and then poured into ice-cold ammonium chloride solution (5% w/v; 200 mL). Extraction with chloroform and purification by flash chromatography (7% EtOAc/hexane) gave 1,1-diphenylnonan-1-ol as a clear liquid (735 mg, 82%). For unlabeled material: IR ($CHCl_3$ cast) 3560 (br), 3470 (br), 2922 (s), 2852 (m), 1448 (m), 700 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.45–7.37 (d, 4 H, 7 Hz, *o*-Ar*H*), 7.32–7.26 (t, 4 H, 7 Hz, *m*-Ar*H*), 7.24–7.18 (t, 2 H, 7 Hz, *p*-Ar*H*), 2.28–2.22 (m, 2 H, CH_2C–OH), 2.11 (s, 1 H, O*H*), 1.34–1.18 (m, 12 H), 0.84 (t, 3 H, 7 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.6 (2 C), 128.3 (4 C), 126.9 (2 C), 126.3 (4 C), 78.3, 41.9, 31.6, 29.8, 29.2, 29.0, 23.5, 22.4, 13.7; exact mass 296.2132 (296.2140 calcd for $C_{21}H_{28}O$). Anal. Calcd for $C_{21}H_{28}O$: C, 85.08; H, 9.52. Found: C, 85.09; H, 9.52.

To a solution of 1,1-diphenylnonan-1-ol (457 mg, 1.55 mmol) in dichloromethane (25 mL) was added *p*-toluenesulfonic acid monohydrate (110 mg, 0.579 mmol). The progress of the reaction was followed by TLC (15% EtOAc/hexane), and after 2 h the reaction mixture was added to saturated sodium carbonate solution (50 mL) and extracted with chloroform (3 × 30 mL). Purification by flash chromatography (hexane) gave 1,1-diphenyl-1-nonene (351 mg, 81%). For unlabeled material: IR ($CHCl_3$ cast) 2955 (m), 2922 (s), 2853 (s), 1494 (m), 1442 (m), 759 (m), 700 (s) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.15 (m, 10 H, Ar*H*), 6.09 (t, 1 H, 8 Hz, C*H*), 2.13 (dt, 2 H, 8 and 8 Hz, allylic CH_2), 1.46–1.38 (m, 2 H, homoallylic CH_2), 1.30–1.20 (m, 8 H), 0.86 (t, 3 H, 7 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.0, 141.5, 140.4, 130.3, 129.9 (2 C), 128.1 (2 C), 128.0 (2 C), 127.2 (2 C), 126.8, 126.7, 31.8, 29.9, 29.7, 29.2, 29.1, 22.6, 14.0; exact mass 278.2033 (278.2034 calcd for $C_{21}H_{26}$). Anal. Calcd for $C_{21}H_{26}$: C, 90.59; H, 9.41. Found: C, 90.38; H, 9.36.

To the 1,1-diphenyl-1-nonene (234 mg, 0.842 mmol) was added carbon tetrachloride (2 mL), acetonitrile (2 mL), water (3 mL), sodium periodate (885 mg, 4.14 mmol), and ruthenium(III) chloride trihydrate (7.2 mg, 0.028 mmol). The reaction mixture was stirred vigorously for 2 h and then added to water (10 mL; pH 2). Extraction with dichloromethane (3 × 10 mL) and solvent removal in vacuo gave a black residue, which was added to ether (10 mL). Filtration through Celite and purification by flash chromatography (15% EtOAc/hexane) gave octanoic acid (**19**) (65.0 mg, 54%). For unlabeled material: IR ($CHCl_3$ cast) 3040 (br), 2928 (s), 2878 (m), 1711 (s), 1411 (m), 1285 (m), 1275 (m), 935 (m) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.36 (t, 2 H, 6 Hz, CH_2CO), 1.68–1.60 (m, 2 H), 1.38–1.24 (m, 8 H), 0.89 (t, 3 H, 7 Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.9, 34.1, 31.6, 29.0, 28.9, 24.7, 22.6, 13.9; exact mass 144.1150 (144.1150 calcd for $C_8H_{16}O_2$).

(*S*)-(Methoxycarbonyl)benzyl Octanoate (20). To a solution of octanoic acid (**19**) (973 mg, 6.76 mmol) in dry dichloromethane (10 mL) at 0 °C was added 4-(dimethylamino)pyridine (5 mg, 0.04 mmol), dicyclohexylcarbodiimide (1.54 g, 7.50 mmol), and methyl *S*-(+)-mandelate (1.22 g, 7.38 mmol). The reaction mixture was warmed to room temperature and stirred for 24 h. It was then filtered and concentrated in vacuo. Purification by flash chromatography (2% EtOAc/hexane) gave **20** (1.72 g, 87%) as a clear liquid. For unlabeled

material: $[\alpha]^{25}_D$ +97.0° (*c* 4.4, $CHCl_3$); IR ($CHCl_3$ cast) 2958 (m), 2930 (m), 2858 (m), 1760 (s), 1748 (s), 1458 (m), 1438 (m), 1218 (s), 1157 (s), 698 (m) cm^{-1}; ^1H NMR (400 MHz, C_6D_6, C_6F_6 lock) δ 7.45–7.40 (m, 2 H, Ar*H*), 7.11–7.02 (m, 3 H, Ar*H*), 6.07 (s, 1 H, C*H*Ph), 3.21 (s, 3 H, OCH_3), 2.34–2.15 (m, 2 H, $CHHCO_2$), 1.63–1.52 (m, 2 H, $CH_2CH_2CO_2$), 1.23–1.09 (m, 8 H), 0.83 (t, 3 H, 8 Hz, CH_3); ^{13}C NMR (100 MHz, C_6D_6, C_6F_6 lock) δ 173.0, 169.6, 134.9, 129.2, 128.9 (2 C), 128.1 (2 C), 74.6, 51.5, 33.63, 31.55, 28.79 (2 C), 24.66, 22.44, 13.63; exact mass 292.1675 (292.1675 calcd for $C_{17}H_{24}O_4$). Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.84; H, 8.27. Found: C, 69.93; H, 8.16. Compound **20** prepared from nonanoic acid (**17**) (75 mg, 0.47 mmol) derived from sodium [2-^{13}C,$^2H_3$]acetate was obtained in 30% yield (40 mg, 0.14 mmol) and showed identical chromatographic properties. The spectral properties were similar to those of unlabeled material: partial ^{13}C NMR (100 MHz, C_6D_6, ^2H decoupled) δ 33.64, 33.34 (CH–D), 31.52, 31.09 (CH–D), 28.78, 28.75, 28.37 (CH–D), 24.65, 22.41, 13.62, 13.32 (CH_2–D), 13.02 (CH–D_2).

(*S*)-(Methoxycarbonyl)benzyl Methyl 1,9-Nonanedioate (21a) from Monomethyl 1,9-Nonanedioate (18). To a solution of **18** (61 mg, 0.30 mmol) in dichloromethane (2.5 mL) at −10 °C was added dicyclohexylcarbodiimide (64 mg, 0.31 mmol), 4-(dimethylamino)pyridine (2 mg), and methyl (*S*)-(+)-mandelate (51 mg, 0.31 mmol). The reaction mixture was stirred and allowed to warm to room temperature overnight. Filtration and removal of the solvent in vacuo gave an oil. Purification by preparative thin-layer chromatography ($CHCl_3$, eluted twice) gave the mandelate ester **21a** (95 mg, 88%), whose chromatographic properties were identical with those of **21b** and **21c**. It displayed the expected spectral properties which were very similar to those of **21b** described below except for the following: partial ^{13}C NMR (100 MHz, C_6D_6, ^2H decoupled) δ 33.3 (C-8, CH_2), 33.69 (C-2, CHH), 33.40 (C-2, CHD), 28.83 (C-6, CHH), 28.75, 28.70 (C-4, CHH), 28.42 (C-6, CHD), 28.28 (C-4, CHD).

Methyl [8-$^2H_1$]-8-Oxooctanoate (22). A ca. 2:1 mixture of the labeled and unlabeled aldehydes was prepared from cyclooctanone by a standard sequence of Baeyer–Villiger oxidation,[35] methanolysis to the known methyl 8-hydroxyoctanoate,[36] Swern oxidation[15] to known unlabeled methyl 8-oxooctanoate,[37] reduction with sodium boro[$^2H_4$]hydride, and reoxidation.[15] The material was distilled (84–87 °C; 0.4 mmHg). For unlabeled material: IR ($CHCl_3$ cast) 2938 (m), 1737 (vs), 1199 (m), 1173 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 9.74 (t, 1 H, 2 Hz, C*H*O), 3.68 (s, 3 H, OCH_3), 2.44 (dt, 2 H, 2 and 7 Hz, CH_2CHO), 2.32 (t, 2 H, 7 Hz, CH_2CO_2), 1.72–1.55 (m, 4 H), 1.40–1.30 (m, 4 H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 202.3, 173.9, 51.3, 43.6, 33.8, 28.7 (2 C), 24.6, 21.8; CI (NH_3) MS 191 (11.5), 190 (100, MNH_4^+), 173 (4, MH^+). Anal. Calcd for $C_9H_{16}O_3$: C, 62.77; H, 9.36. Found: C, 62.85; H, 9.36. The 2:1 mixture of the labeled and unlabeled aldehydes **22** showed identical chromatographic properties and similar spectral data except for CI (NH_3) MS: 192 (11), 191 (100), 190 (63), 173 (14).

Methyl (8*R*)-[8-$^2H_1$]-8-Hydroxyoctanoate *p*-Toluenesulfonate Ester (23b). (−)-Pinene (1.33 g, 9.78 mmol) was added to a solution of 9-borabicyclo[3.3.1]nonane dimer (1.09 g, 4.47 mmol) in THF (16 mL), and the reaction mixture was gently refluxed for 2 h. The mixture was cooled to room temperature, and a solution of the above mixture of deuterated and undeuterated aldehydes **22** (1.25 g, 7.23 mmol) in THF (3 mL) was added. The reaction mixture was refluxed for 1 h and cooled to room temperature. Acetaldehyde (0.20 mL, 3.58 mmol) was added, and after 5 min the volatiles were removed in vacuo (40 °C, 0.05 mmHg, 2 h). The residue was cooled to 0 °C; ether (6 mL) was added, followed by 2-aminoethanol (0.575 mL, 9.50 mmol). The resulting white suspension was stirred for 5 min and filtered. The filtrate was dried ($MgSO_4$), and the solvent was removed in vacuo to give a yellow oil (1.73 g). Kugelrohr distillation (0.04 mmHg; oven temperature 88–90 °C) gave the crude *R* alcohol as a colorless liquid (816 mg), estimated to be ca. 85% pure by ^1H NMR comparison with unlabeled material. *p*-Toluenesulfonyl chloride (1.52 g, 7.97 mmol) was added to a solution of the crude alcohol (706 mg) in dry pyridine (8 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and then kept at 4 °C overnight. The mixture was diluted with water (100 mL) and extracted with ether (3 × 65 mL). The combined ethereal extracts were washed with saturated aqueous copper sulfate (4 × 40 mL), water (2 × 50 mL), and sodium carbonate solution (5%; 2 × 50 mL). The extracts were dried ($MgSO_4$), and the solvent was removed in vacuo. Purification by flash chromatography ($CHCl_3$) yielded the (8*R*)-tosylate **23b** (861 mg, 42%) as an oil. For unlabeled material: IR (thin film) 2935 (s), 2859 (m), 1738 (s), 1359 (s), 1180 (s), 1177 (s), 1098 (m), 951 (m), 930 (m), 819 (m), 662 (m), 555 (m) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.80 (d, 2 H, 7 Hz, Ar*H*), 7.36 (d, 2 H, 7 Hz, Ar*H*), 4.01 (t, 2 H, 6 Hz, CH_2O), 3.67 (s, 3 H, OCH_3), 2.45 (s, 3 H, $ArCH_3$), 2.28 (t, 2 H, 7 Hz, CH_2CO), 1.67–1.53 (m, 4 H), 1.35–1.20 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 144.5, 132.8, 129.7 (2 C), 127.8 (2 C), 70.4, 51.2, 33.9, 28.8 (2 C), 28.5, 25.1, 24.7, 21.4; CI (NH_3) MS 348 (11), 347 (22), 346 (100, MNH_4^+). Anal. Calcd for $C_{16}H_{24}O_5S$: C, 58.51; H, 7.37. Found: C, 58.68; H, 7.33. The (8*R*)-tosylate **23b** showed identical chromatographic properties and similar spectral characteristics except for the following: partial ^1H NMR (400 MHz; $CDCl_3$) δ 4.06–3.96 (m, 1.3 H, C*H*D and CH_2CO); partial ^{13}C NMR (50 MHz; $CDCl_3$) δ 70.41 (CH_2O), 70.09 (CHDO, t, 23 Hz); CI (NH_3) MS 348 (20), 347 (100), 346 (56).

Methyl (8*S*)-[8-$^2H_1$]-8-Hydroxyoctanoate *p*-Toluenesulfonate Ester (23c). Substitution of (+)-pinene for the (−)-pinene in the procedure used above to prepare the enantiomeric tosylate **23b** gave the desired compound **23c**. Chromatographic properties were identical, and spectral data were very similar.

(*S*)-(+)-(Methoxycarbonyl)benzyl Methyl (2*S*)-[2-$^2H_1$]-1,9-Nonanedioate (21b). A solution of disodium tetracarbonylferrate (compound with 1,4-dioxane; 700 mg, 2.02 mmol), triphenylphosphine (310 mg, 1.18 mmol), and the (8*R*)-tosylate **23b** (220 mg, 0.671 mmol) in THF (25 mL) and hexamethylphosphoric triamide (HMPA; 5 mL) was stirred at room temperature under argon for 12 h. The reaction mixture was then cooled to 0 °C, and methyl (*S*)-(+)-mandelate (1.00 g, 6.02 mmol) was added. After 1 min, iodine (2.5 g, 9.85 mmol) was cautiously added, causing the reaction mixture to foam. After being stirred for 30 min with warming to room temperature, the reaction mixture was added to water (200 mL) and extracted with ether (3 × 100 mL). The combined ether fractions were washed with water (100 mL), washed repeatedly with saturated sodium bisulfite until no more iodine was present in the ether layer, then washed with water (100 mL), and dried ($MgSO_4$). The residue was purified by flash chromatography (10% EtOAc/hexane) to give the (2*S*)-mandelate ester **21b** (56.3 mg, 24%). For unlabeled material: $[\alpha]_D$ +78.4° (*c* 4.8, $CHCl_3$); IR ($CHCl_3$ cast) 2934 (m), 1755 (s), 1741 (s), 1216 (m), 1171 (m) cm^{-1}; ^1H NMR (400 MHz, C_6D_6) δ 7.50–7.46 (m, 2 H, Ar*H*), 7.20–7.08 (m, 3 H, Ar*H*), 6.07 (s, 1 H, PhC*H*), 3.41 (s, 3 H, OCH_3), 3.28 (s, 3 H, OCH_3), 2.36–2.12 (m, 2 H, $CHHCO_2CHPh$), 2.10 (t, 2 H, 7 Hz, CH_2CO_2Me), 1.59–1.43 (m, 4 H), 1.07–1.01 (m, 6 H); ^{13}C NMR (100 MHz, C_6D_6) δ 173.3, 172.7, 169.4, 134.8, 129.2, 128.9 (2 C), 128.1 (2 C), 74.7, 51.9, 50.9, 33.96 (C-8, CH_2CO_2Me), 33.93 (C-2, CH_2CO_2CHPh), 29.06 (C-6), 28.98 (C-5), 28.92 (C-4), 25.04 (C-7), 24.93 (C-3); EI MS 318 (8), 186 (11), 185 (100), 149 (15), 125 (25), 121 (13), 105 (10), 97 (16), 83 (11), 79 (9). Anal. Calcd for $C_{19}H_{26}O_6$: C, 65.13; H, 7.48. Found: C, 65.05; H, 7.37. The 2*S*-2-$^2H_1$ compound **21b** showed identical chromatographic properties and displayed similar spectral properties except for the following: ^{13}C NMR (100 MHz, C_6D_6, ^2H decoupled) δ 33.56 (2 C, C-2, C-8, 2 × CH_2), 33.24 (C-2, CHD), 28.64, 28.56, 28.50 (C-4, CHH), 28.48 (C-4, CH_2CH_2CHD), 24.61, 24.50 (C-3, CHH), 24.43 (C-3, CH_2CHD); ^2H NMR (61.4 MHz, C_6H_6) δ 2.12.

(*S*)-(+)-(Methoxycarbonyl)benzyl Methyl (2*R*)-[2-$^2H_1$]-1,9-Nonanedioate (21c). A procedure similar to that used to prepare **21b** was employed except that **23c** was used as the starting material. Spectral data were similar to those of **21b** except for ^2H NMR (61.4 MHz, C_6H_6): δ 2.20.

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Supplementary Material Available: ^{13}C COSY spectra of **13** derived from sodium [1,2-$^{13}C_2$]acetate, deuterium-decoupled ^1H,^{13}C shift correlation spectrum of **20**, and table of NOE enhancements (3 pages). Ordering information is given on any current masthead page.

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