Table VIII. Conformational Constraints from NMR Data for *cyclo*(L-Ala-Gly-L-Pro-D-Phe)$_2$ in Me_2SO

observation (see Table VI)	conformational constraint	ref[a]
$^3J_{HNCH}$	ϕ_{Ala} = −120 ± 15°	12, 13[b]
	ϕ_{Gly} = ca. ±90°	
	ϕ_{Phe} = 120 ± 15°	
$^2J_{HCH}$ (Gly)	ψ_{Gly} near 180°	16
$\Delta\delta_{\beta\gamma}$ (Pro C)	ψ_{Pro} = 165° or −45°	10
NOE Phe H^N – Pro H^α	ψ_{Pro} = 165° rather than −45°	c

[a] References are given to papers describing the relationship used to obtain the conformational constraint from the NMR observation. [b] The J_{HNCH} correlations cited are those we consider to have the best experimental bases for the present purpose. Within the ranges given they give similar dihedral angles, but the true uncertainties are undoubtedly larger. [c] NOE data are not used quantitatively, but to distinguish between pairs of possibilities with significantly different interproton distances.

data do not suggest an obvious mutually consistent interpretation. If a single conformation is important, the NMR constraints on it would be those given in Table VIII. Within those constraints models with the approximate dihedral angles below can be constructed, but they are not suggested with confidence. They differ primarily in the orientation of the Ala–Gly peptide bond.

	Ala		Gly		Pro		D-Phe	
	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
A	−120	0	90	180	−60	165	120	−40
B	−120	−160	−90	180	−60	165	120	−60

Conclusions

Cyclic octapeptides cannot form the complete cyclic β structures that can be approximated by cyclic hexapeptides (two joined β turns) or cyclic decapeptides like gramicidin S (two β turns linked by two extended residues). However, cyclic octapeptides that will adopt C_2 symmetric average conformations with two β turns can apparently be designed. Of the four diastereomers prepared in this work, the two that have the most closely defined conformations in solution are *cyclo*(D-Ala-Gly-L-Pro-D-Phe)$_2$ and *cyclo*(L-Ala-Gly-L-Pro-L-Phe)$_2$. Their conformations are similar: planes formed by the α-carbons of the Ala-Gly-Pro-Phe-Ala sequences, which include Pro-Phe turns, meet along a line joining the Ala α-carbons. The β turns are type I for L-Pro-L-Phe and type II for L-Pro-D-Phe)$_2$, as expected. The fold at the Ala C^α is such as to place the Ala side chains on its convex side, so that very roughly speaking the backbone of the ring containing L-Ala is a reflection of the ring containing D-Ala. To generalize from this result, it may be expected that cyclic octapeptides in which β turns are caused to exist at residues 2,3 and 6,7 will tend to adopt such a dihedral structure folded along the 4–8 line if 4 and 8 are of the same configuration. It would be of interest to test whether stable conformations occur if 4 and 8 are of opposite configurations. Further discussion of this kind of backbone will be offered in a subsequent paper on *cyclo*(D-Ala-Gly-Pro-L-Phe)$_2$.

Acknowledgment. This research was supported by grants from the National Institute of General Medical Sciences, GM 26071 and GM 26071-02S1 (300-MHz NMR instrument). The FAB mass spectra were obtained by Dr. Kenneth B. Tomer of the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, a NSF Regional Instrumentation Facility.

Registry No. *cyclo*(L-Ala-Gly-L-Pro-L-Phe), 91383-23-2; *cyclo*(L-Ala-Gly-L-Pro-D-Phe), 91383-24-3; *cyclo*(D-Ala-Gly-L-Pro-L-Phe), 91383-22-1; *cyclo*(D-Ala-Gly-L-Pro-D-Phe), 91302-75-9; Z-Ala-Gly-Pro-Phe-OMe, 91302-78-2; Z-D-Ala-Gly-Pro-Phe-OMe, 91302-77-1; Z-D-Ala-Gly-Pro-D-Phe-OMe, 91302-76-0; Z-Ala-Gly-Pro-D-Phe-OMe, 91302-79-3; Z-(Ala-Gly-Pro-Phe)$_2$-OMe, 91949-02-9; Z-(D-Ala-Gly-Pro-Phe)$_2$-OMe, 91949-03-0; Z-(D-Ala-Gly-Pro-D-Phe)$_2$-OMe, 91949-04-1.

Steric Course of the Allylic Rearrangement Catalyzed by β-Hydroxydecanoylthioester Dehydrase. Mechanistic Implications

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Abstract: β-Hydroxydecanoylthioester dehydrase, which is the pivotal enzyme in the biosynthesis of unsaturated fatty acids in anaerobic metabolism, catalyzes the equilibration of thio esters of (*R*)-3-hydroxydecanoic acid, (*E*)-2-decenoic acid, and (*Z*)-3-decenoic acid. On the basis of evidence available to date, both two-base and one-base mechanisms of action can be envisioned for dehydrase. In an effort to distinguish between these mechanisms, the stereochemical course of the dehydrase-catalyzed allylic rearrangement has been determined. *N*-Acetylcysteamine (NAC) thio esters of (*R*)- and (*S*)-(*E*)-[4,5,5-$^2H_3$]decanoic acid were synthesized and incubated with dehydrase. The product (*Z*)-3-decenoyl-NAC was derivatized, and ^2H NMR analysis showed that the *pro*-4*R* hydrogen had been removed. (*E*)-2-[2-^2H]Decenoyl-NAC and unlabeled (*E*)-2-decenoyl-NAC were incubated with dehydrase in ^1H_2O and ^2H_2O, respectively. Analysis of a derivative of the resulting labeled (*Z*)-3-decenoyl-NAC showed that protonation had occurred on the *si* face at substrate C-2. The overall steric course of the reaction is therefore suprafacial. The significance of this result is discussed in terms of the mechanisms of the "normal" dehydrase-catalyzed reactions as well as the "suicide" inactivation of the enzyme.

While the overall scheme for biosynthesis of saturated fatty acids[1] is essentially invariant throughout nature, there are fundamental differences in the ways in which unsaturated fatty acids are assembled[2] in aerobic and anaerobic metabolism. In aerobes, a double bond is introduced at an isolated position in a preformed saturated fatty acid (often 16, 18, or 20 carbon atoms in length). The enzyme responsible is a membrane-bound fatty acid desaturase system that requires NADPH and molecular oxygen. Obviously, anaerobes cannot utilize oxygen, and so as an alternative, they synthesize unsaturated fatty acids de novo. The pivotal step in this pathway is catalyzed by β-hydroxydecanoylthioester dehydrase[3] ("dehydrase"), a bifunctional enzyme that mediates

(1) Zubay, G. "Biochemistry"; Addison-Wesley: Reading, MA, 1983; pp 487–495.
(2) Bloch, K. *Acc. Chem. Res.* **1969**, *2*, 193–202.

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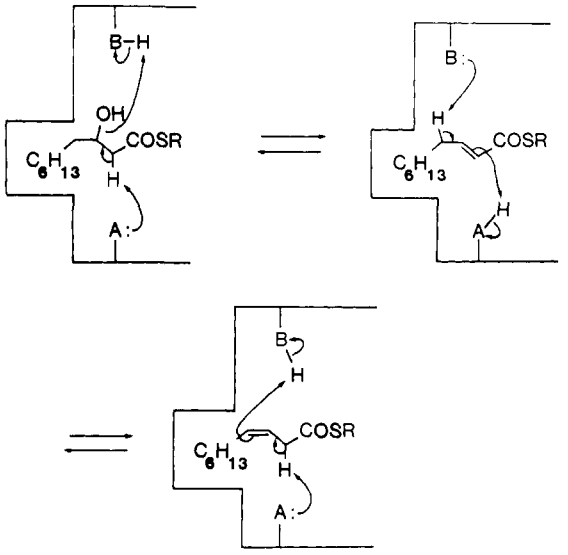
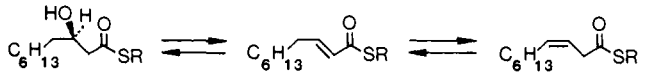
Scheme I. "Two-Base" Mechanism of Action Proposed for Dehydrase[4]



the interconversion of acyl carrier protein (ACP) thio esters of (*R*)-3-hydroxydecanoic acid, (*E*)-2-decenoic acid, and (*Z*)-3-decenoic acid (**1**, **2**, and **3**, respectively). In vivo, **2** functions as



1 **2** **3**

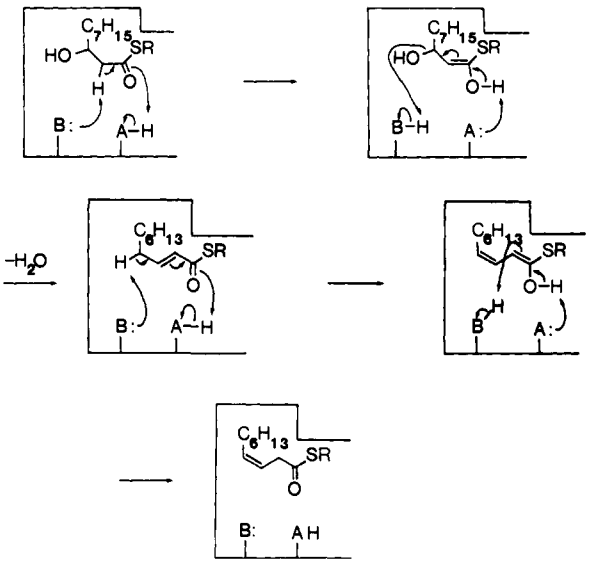
the precursor of saturated fatty acids (via reduction and subsequent homologation), and **3** is chain-extended directly, leading to the common monounsaturated fatty acids.

Dehydrase has been studied quite extensively, primarily in the laboratory of Bloch.[3] Through a particularly important series of experiments[4] (vide infra), Helmkamp and Bloch produced irrefutable evidence for the presence of a histidine residue at the active site of dehydrase. In addition, their experiments suggested that there is an active site tyrosine, although the authors admitted that the evidence was "less than compelling".[4] In light of the foregoing, two general mechanisms of action could be envisioned for dehydrase: a "two-base" mechanism,[4] with tyrosine as well as histidine mediating deprotonation and protonation at unique sites on the substrate (cf. Scheme I), and a "one-base" mechanism, with histidine functioning as the only base and conjugate acid, at substrate C-2, C-3, and C-4 (cf. Scheme II).

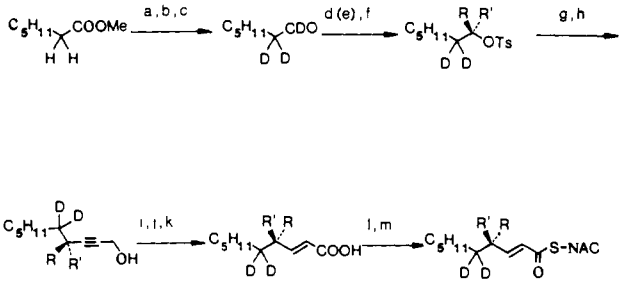
Hanson and Rose have proposed[5] that for enzymes "natural selection for catalytic efficiency has led to (a) the use of the minimal number of acidic and basic catalytic groups, (b) the maximal separation of catalytic groups..., and (c) minimal motion of the substrate". A direct corollary to the foregoing is that overall reaction stereochemistry can be used as a probe of mechanism.[3,4] In particular, for an enzyme-catalyzed allylic rearrangement, such as the one which is catalyzed by dehydrase, a "one-base" mechanism should be accompanied by a suprafacial steric course, while an antarafacial steric course implicates a "two-base" mechanism.

Clearly, important questions remain in regard to the number and the relative orientations of catalytically significant functional groups at dehydrase's active site. In this paper we report the details of experiments[6,7] which have allowed us to define the overall steric course of the dehydrase-catalyzed allylic rearrangement of **2** to **3**. As described below, our findings have implications for the mechanisms of the "normal" reactions catalyzed by dehydrase as well as for the "suicide" inhibition[8] process, involving acetylenic thioester substrates.

Scheme II. "One-Base" Mechanism of Action Proposed for Dehydrase



Scheme III. Synthesis of Substrates Chirally Labeled at C-4[a]



	R	R'
(4R)-[4,5,5-$^2H_3$]**2**	H	D
(4S)-[4,5,5-$^2H_3$]**2**	D	H

[a] (a) NaOMe, EtO^2H; (b) $LiAl^2H_4$, Et_2O; (c) CrO_3, pyridine; (d) 9-BBN, (+)-α-pinene,[9] for R = ^1H, R' = ^2H; (e) 9-BBN, (−)-α-pinene,[9] for R = ^2H, R' = ^1H; (f) *p*-TsCl, pyridine; (g) LiC≡CCH$_2$OTHP, HMPA;[10] (h) *p*-TsOH, MeOH; (i) $LiAlH_4$, NaOMe, THF;[11] (j) MnO_2, pentane;[12] (k) $NaClO_2$, *t*-BuOH;[13] (l) Ph_2POCl, CH_2Cl_2;[14,15] (m) TlSCH$_2$CH$_2$NHAc, THF.[16]

Results

Steric Course at C-4. The majority of previous experiments on dehydrase have utilized *N*-acetyl-2-mercaptoethylamine (*N*-acetylcysteamine, NAC) thio esters of the substrate fatty acids rather than the natural but less easily synthesized ACP derivatives. Our plan was to synthesize NAC thio esters of (*E*)-2-decenoic acid chirally labeled with deuterium at C-4, incubate these samples with dehydrase, isolate (*Z*)-3-decenoyl-NAC, and analyze for deuterium enrichment at C-4 of this material. Deuterium NMR spectroscopy was the method of choice for analysis, and to facilitate quantification of C-4 label, deuterium was incorporated at C-5 as well. As carbon 5 is not involved in the reactions catalyzed by dehydrase, the extent of loss or retention of deuterium at C-4 could be determined by comparison of the C-4/C-5 deuterium *ratio* of the product to that of the corresponding substrate sample.

(1) Synthesis of Labeled Substrates. Syntheses of (*R*)- and (*S*)-(*E*)-2-[4,5,5-$^2H_3$]decenoyl-NAC followed the route shown in Scheme III. A sample of [1,2,2-$^2H_3$]heptanal, synthesized by standard procedures, was divided into two portions for reduction

(3) Bloch, K. In "The Enzymes", 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1971; Vol. 5, pp 441–464.
(4) Helmkamp, G. M., Jr.; Bloch, K. *J. Biol. Chem.* **1969**, *244*, 6014–6022.
(5) Hanson, K. R.; Rose, I. A. *Acc. Chem. Res.* **1975**, *8*, 1–10.
(6) Schwab, J. M.; Klassen, J. B. *J. Chem. Soc., Chem. Commun.* **1984**, 296–297.
(7) Schwab, J. M.; Klassen, J. B. *J. Chem. Soc., Chem. Commun.* **1984**, 298–299.
(8) Walsh, C. *Tetrahedron* **1982**, *38*, 871–909.

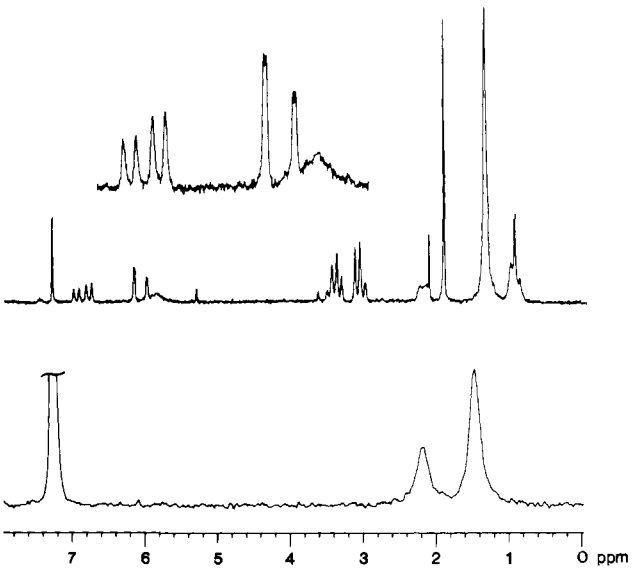


Figure 1. Proton (top) and deuterium (botton) NMR spectra of (*R*)-(*E*)-2-[4,5,5-$^2H_3$]decenoyl-NAC (**2**). The sample was dissolved in CCl_4 and spectra were run on a JEOL FX-90Q(II) spectrometer, using the external ^{7}Li lock. $CHCl_3$ and $CDCl_3$ were used as internal standards (7.26 ppm). Inset: vinyl region of the proton spectrum.

by Midland's procedure;[9] by employment of the 9-BBN complexes of (+)- and (−)-α-pinene, both (*S*)- and (*R*)-[1,2,2-$^2H_3$]heptanol were obtained. Each of these enantiomerically labeled alcohols, as its *p*-toluenesulfonate, was used for alkylation[10] of the THP derivative of propargyl alcohol (with inversion of configuration at C-1 of the tosylate). Following removal of the protecting group, stereospecific reduction of the triple bond was effected by Corey's method,[11] giving the (*E*)-allylic alcohol, which was oxidized[12,13] to the corresponding carboxylic acid in high overall yield via the aldehyde. The two-step thioesterification sequence, which is a variant on published procedures,[14-16] gave consistently high yields of the desired NAC derivatives.

Deuterium NMR analysis of the chirally labeled thio esters (Figures 1 and 2) showed C-4/C-5 deuterium ratios of 0.48 ± 0.01 and 0.51 ± 0.01, for the *R* and *S* forms, respectively. Furthermore, the proton NMR spectra of the samples exhibited vinyl proton coupling patterns and diminished C-4 proton signals that were entirely consistent with the extents and positions of labeling as shown.

(2) Incubations of Substrates with Dehydrase and Derivatization of Products. While *E. coli* strain B has been the usual source of dehydrase,[17,18] *E. coli* strain DM51 A, a cloned mutant, proved superior in this regard.[19] Using this organism and a simplified purification procedure, it was possible in the course of a single day to obtain enough partially purified dehydrase to carry out several preparative-scale incubations.

Substrate samples chirally labeled with deuterium at C-4 were incubated with the partially purified enzyme preparation long enough to achieve equilibrium between **1**, **2**, and **3**. (Modifications in the buffer composition, pH, temperature, and length of the incubation period failed to produce any increase in the proportion of **3** beyond 1–3% of the total product mixture.[20])

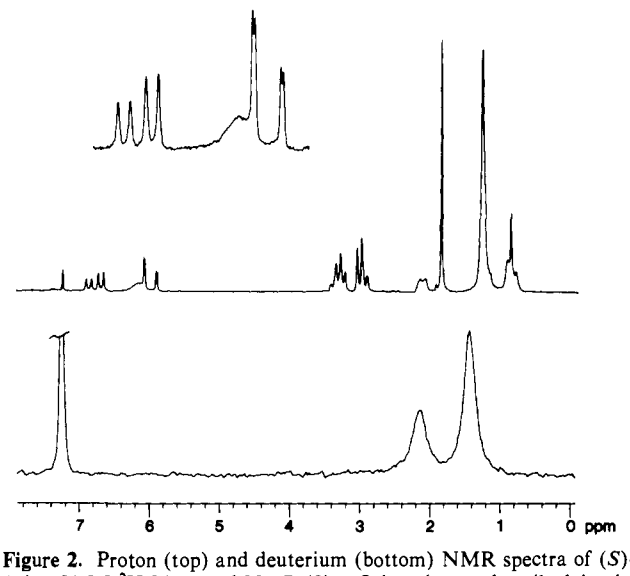


Figure 2. Proton (top) and deuterium (bottom) NMR spectra of (*S*)-(*E*)-2-[4,5,5-$^2H_3$]decenoyl-NAC (**2**). Otherwise as described in the legend to Figure 1.

The incubations of substrates labeled at C-4 were terminated reductively, by sequential addition of Tris buffer (pH 8.5), tetrahydrofuran, and a large excess of sodium borohydride. The organic cosolvent served to enhance the nucleophilicity of the borohydride, such that there was no thio ester remaining after a period of 10 min. This treatment afforded an alcohol mixture that included large amounts of 1,3-decanediol and 1-decanol, accompanied by smaller quantities of (*E*)-2-decen-1-ol and (*Z*)-3-decen-1-ol. The saturated alcohol resulted from initial conjugate attack on **2**, followed by direct reduction of the thio ester moiety. As the unsaturated alcohols were exceedingly difficult to separate from one another, the crude alcohol mixture was treated with a large excess of manganese dioxide, which oxidized[12] the allylic alcohol to the corresponding aldehyde but left the desired homoallylic alcohol unchanged. Finally, the alcohols were aroylated. Since the molecular weight of the *p*-phenylbenzoate (and even the benzoate) is considerably greater than that of the parent alcohol, derivatization increased the mass and facilitated manipulation of the labeled products. In addition, aroylation provided a chromophore, which enabled HPLC monitoring at 230 nm. While it had been hoped that the *p*-phenylbenzoates might prove to be crystalline compounds, all attempts to crystallize (*Z*)-3-decen-1-yl *p*-phenylbenzoate were unsuccessful. Since the *p*-phenylbenzoates of (*Z*)-3-decen-1-ol and 1-decanol proved exceedingly difficult to separate via preparative HPLC, later experiments utilized the more readily separable benzoate esters.

(3) Analysis of Labeled Products. Since the vinyl protons at C-3 and C-4 of (*Z*)-3-decen-1-yl *p*-phenylbenzoate *both* resonate at 5.5 ppm, proton NMR spectroscopy appeared not to be the method of choice for analysis of hydrogen or deuterium loss from C-4 of **2**. Accordingly, *deuterium* NMR spectroscopy was selected as a means of revealing the presence or absence of deuterium in the labeled derivatives of enzymatically produced **3**. In fact, the C-4 and C-5 resonances of (*Z*)-3-decen-1-yl *p*-phenylbenzoate are well separated, the latter appearing at 2.1 ppm. (C-5 is the site of the reference label.)

Figure 3 depicts 13.8-MHz deuterium NMR spectra of the labeled homoallylic esters. The upper spectrum (Figure 3a) is of (*Z*)-3-decen-1-yl *p*-phenylbenzoate obtained from incubation of (*S*)-[4,5,5-$^2H_3$]**2** with dehydrase. Two product resonances are

(9) Midland, M. M.; Greer, S.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1979**, *101*, 2352–2355.
(10) Corey, E. J.; Katzenellenbogen, J. A.; Gilman, N. W.; Roman, S. A.; Erickson, B. W. *J. Am. Chem. Soc.* **1968**, *90*, 5618–5620.
(11) Corey, E. J.; Katzenellenbogen, J. A.; Posner, G. H. *J. Am. Chem. Soc.* **1967**, *89*, 4245–4247.
(12) Corey, E. J.; Gilman, N. W.; Ganem, B. E. *J. Am. Chem. Soc.* **1968**, *90*, 5616–5617.
(13) Balkrishna, S. B.; Childers, W. E., Jr.; Pinnick, H. W. *Tetrahedron* **1981**, *37*, 2091–2096.
(14) Kende, A. S.; Scholz, D.; Schneider, J. *Synth. Commun.* **1978**, *8*, 59–63.
(15) Scholz, D.; Eigner, D. *Monatsh. Chem.* **1979**, *110*, 759–761.
(16) Masamune, S.; Kamata, S.; Diakur, J.; Sugihara, Y.; Bates, G. S. *Can. J. Chem.* **1975**, *53*, 3693–3695.
(17) Reference 3 and references cited therein.
(18) Stein, J. P. Ph.D. Thesis, Harvard University, Cambridge, MA, 1976.
(19) Cronan, J. E., Jr.; Lakshman, M., unpublished results.
(20) Klassen, J. B.; Schwab, J. M., unpublished results. Cf.: Brock, D. J. H.; Kass, L. R.; Bloch, K. *J. Biol. Chem.* **1967**, *242*, 4432–4440.

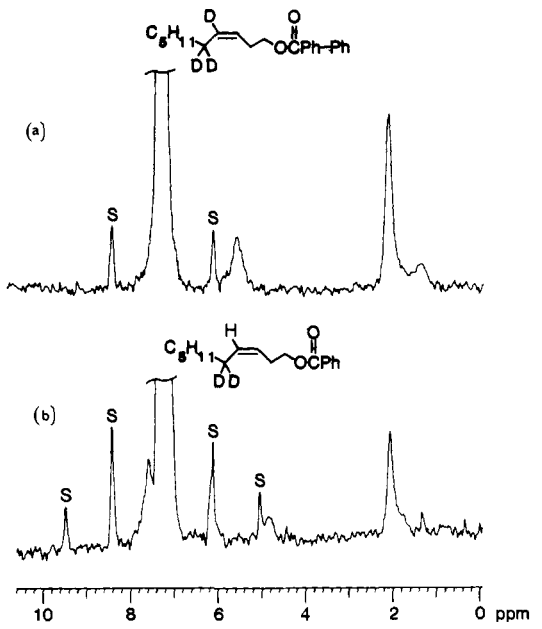


Figure 3. Deuterium NMR spectra, 13.8 MHz, of homoallylic esters derived from incubation of (a) (*S*)- and (b) (*R*)-(*E*)-2-[4,5,5-$^2H_3$]decenoyl-NAC with dehydrase. The upper spectrum was run using external ^{7}Li lock, and the lower one with internal ^{7}Li lock (by means of a capillary tube insert containing saturated LiBr with 10% DMF to promote relaxation). Each ester was dissolved in CCl_4, and $CDCl_3$ was used as the chemical shift internal standard (7.26 ppm). Spinning sidebands are denoted by the symbol "S" In the lower spectrum, extraneous signals are present at 4.8 and 7.6 ppm. These result from the presence of impurities in the (*Z*)-3-decenyl benzoate and not from deuterium substitution in this ester, as shown by the fact that the proton spectrum of unlabeled ester has no resonances at these chemical shifts.

evident at 2.08 and at 5.57 ppm, corresponding to C-5 and C-4 deuterons, respectively. The ratio of integrated signal areas is 0.48 ± 0.01 (C-4/C-5). Taking into account the enantiomeric purity of the labeled substrate (inferred from the optical rotation of the (−)-α-pinene used in the Midland reduction[9]), the C-4/C-5 integral ratios of the *S* substrate and the derived product are within experimental error of each other. By contrast, the spectrum of (*Z*)-3-decen-1-yl benzoate from the incubation involving *R* substrate (Figure 3b) contains only a single peak assignable to this compound, at 2.08 ppm.

It is clear that the hydrogen (or deuterium) atom that is removed from C-4 of **2** en route to **3** is the one that had been in the *pro-R* position.

Steric Course at C-2. Elucidation of the steric course at C-2 during the allylic rearrangement required the incubation of (*E*)-2-[2-^{2}H]decenoyl-NAC with dehydrase in ^1H_2O and of unlabeled (*E*)-2-decenoyl-NAC with dehydrase in ^2H_2O. Each incubation was expected to provide (*Z*)-3-decenoyl-NAC, chirally labeled (but with opposite configurations) at C-2.

(1) Synthesis of Substrates. (*E*)-2-[2-^{2}H]Decenoyl-NAC was made by the same route as had been employed for synthesis of **2** chirally labeled at C-4 (Scheme III). This time, unlabeled propargylic alcohol was reduced with lithium aluminum deuteride in the presence of sodium methoxide,[11] affording (*E*)-2-[2-^{2}H]-decen-1-ol. The allylic alcohol was converted, as previously, to the corresponding NAC thio ester.

(2) Incubations of Substrates with Dehydrase. Incubations of [2-^{2}H]**2** and unlabeled **2** with dehydrase in ^1H_2O and ^2H_2O, respectively, were conducted as described above for incubations involving **2** chirally labeled at C-4, except for the pH of the reaction medium. The incubation of [2-^{2}H]**2** was performed at pH 7.0, and that of unlabeled **2** was carried out at an apparent pH of 6.6.[21] The intent was to minimize the vulnerability to

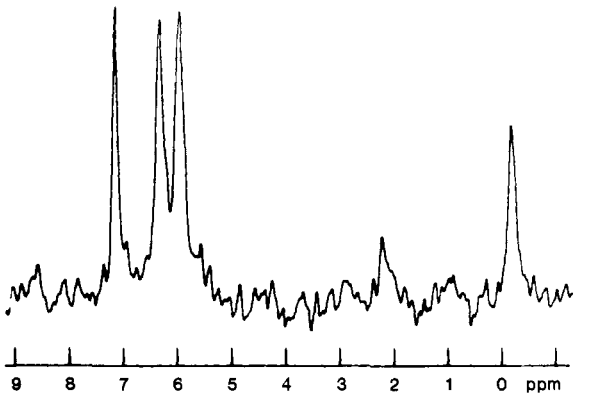
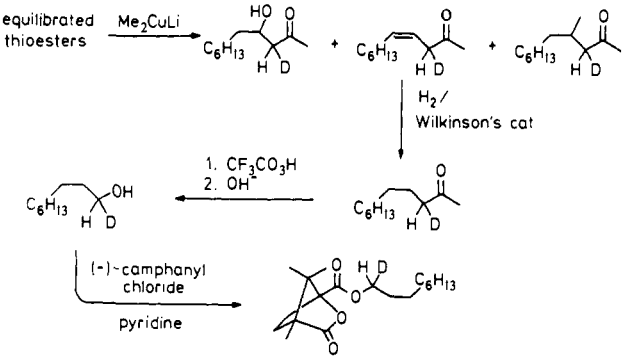


Figure 4. Eu(dpm)$_3$-shifted 46-MHz deuterium NMR spectrum of 1-nonyl camphanate derived from incubation of (*Z*)-2-[2-^{2}H]decenoyl-NAC with dehydrase in ^1H_2O-based buffer. The sample was dissolved in $CHCl_3$, and $CDCl_3$, at natural abundance, was used as the internal standard (7.2 ppm).

Scheme IV. Analysis of Product Chirality at C-2. The Camphanate Ester Approach



epimerization of our [2-$^2H_1$]**3**, as the labeled center would be both allylic and adjacent to a thio ester carbonyl group. Following each enzyme-catalyzed equilibration of **1**, **2**, and **3**, the incubation mixture was saturated with solid ammonium sulfate, and the thio esters were extracted with tetrahydrofuran.

(3) Analysis of Product Chirality at C-2. The Camphanate Ester Approach. An initial attempt to devise an expedient method for determining the configuration of C-2 of **3** is summarized in Scheme IV. The goal was to degrade labeled **3** to 1-nonanol, with analysis by deuterium NMR of the derived camphanate ester, as described by Gerlach.[22]

Thus, treatment of the equilibrated thio esters with lithium dimethylcuprate[23] affords a mixture of methyl ketones. The unsaturated ketone, which is derived from the allylic rearrangement product **3**, is readily separated from the other two by means of flash chromatography.[24] A Baeyer–Villiger[25] sequence then leads to 1-nonanol, which is esterified[22] to (−)-camphanyl chloride.

Accordingly, a mixture of thio esters obtained by incubation of [2-^{2}H]**2** with dehydrase in ^1H_2O was carried through the sequence of reactions as described. A 46-MHz deuterium NMR spectrum (Figure 4) of the resulting [1-^{2}H]nonyl camphanate (to which had been added a portion of Eu(dpm)$_3$), exhibits *two* product signals, at 5.99 and 6.37 ppm. This clearly indicates that there was deuterium in *both* the *pro-R* and the *pro-S* positions at nonyl C-1 of the nonyl camphanate. Since it appeared that the chiral label had been epimerized in the course of the degradation, the following control experiment was performed. A sample

(21) Jencks, W. P., "Catalysis in Chemistry and Enzymology"; McGraw-Hill; New York, 1969; p 276.

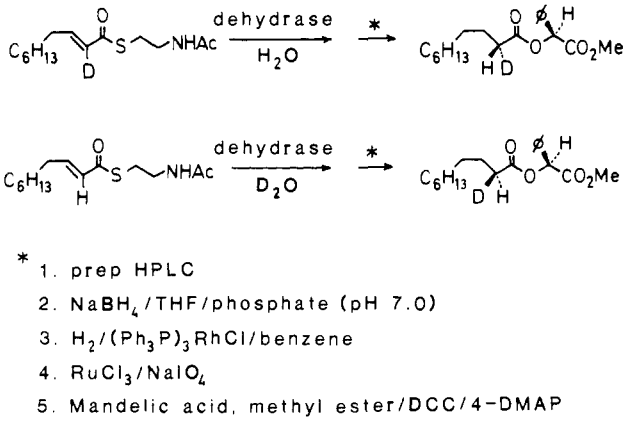
(22) Gerlach, H.; Zagalak, B. *J. Chem. Soc., Chem. Commun.* **1973**, 274–275.

(23) Anderson, R. J.; Henrick, C. A.; Rosenblum, L. D. *J. Am. Chem. Soc.* **1974**, *96*, 3654–3655.

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

(25) (a) Baeyer, A.; Villiger, V. *Ber. Dtsch. Chem. Ges.* **1899**, *32* 3625–3633. (b) Baeyer, A.; Villiger, V. *Ibid.* **1900**, *33*, 858–864.

Scheme V. Analysis of Product Chirality at C-2. The Mandelate Diester Approach



of unlabeled **3** was treated with lithium dimethylcuprate, and the reaction was quenched by the addition of deuterated ammonium chloride. Deuterium and proton NMR spectra clearly showed that the resulting (*Z*)-4-undecen-2-one had undergone substantial exchange at C-3.

(4) Analysis of Product Chirality at C-2. The Mandelate Diester Approach. A second scheme for determining the configuration of [2-$^2H_1$]**3** was based on the method that has recently been described by Parker.[26] Conversion of a carboxylic acid to the corresponding ester of methyl (*S*)-α-hydroxybenzeneacetic acid allows the use of NMR spectroscopy for distinguishing between the *pro-R* and *pro-S* protons (or deuterons) at C-2 of the original acid. Examining the mandelate diester derivatives of five different acids, Parker consistently found that the diastereotopic C-2 proton NMR resonances were separated by approximately 0.1 ppm and that, in each case, the *pro-R* proton appeared at lower field. The suitability of Parker's technique to the present investigation required proof that it would work for decanoic acid. In addition [2-$^2H_1$]**3** would need to be converted to [2-$^2H_1$]decanoic acid, without epimerization of label.

The applicability of the mandelate diester method to distinguishing the α protons of decanoic acid was demonstrated by synthesis and NMR analysis of standard samples. A 76.8-MHz deuterium NMR spectrum of the methyl mandelate ester of [2,2-$^2H_2$]decanoic acid[27] (in benzene solution) displayed two peaks of equal intensity, at 2.17 and 2.26 ppm. A similar spectrum of the ester of (*R*)-[2-$^2H_1$]decanoic acid[28] comprised a single peak, at 2.26 ppm. This result was in perfect agreement with all of Parker's examples.

Further manipulation of the enzymatically equilibrated, labeled thio esters followed the route shown in Scheme V. Following preliminary removal of hydroxy thio ester **1** by flash chromatography, the unsaturated thio esters **2** and **3** were separated from one another by semipreparative HPLC. In this way were obtained 5 mg of [2-$^2H_1$]**3** from the incubation of [2-^2H]**2** (500 mg) with dehydrase in ^1H_2O and 24 mg of [2-$^2H_1$]**3** from incubation of unlabeled **2** (500 mg) with enzyme in ^2H_2O. The unusually high proportion of **3** that was produced in the second incubation can probably be attributed to a solvent deuterium isotope effect, which should suppress the hydration of **2**. In order to facilitate manipulation of intermediates in the analysis, 10 mg of unlabeled **3** was added to the smaller sample of [2-$^2H_1$]**3**.

Chirally labeled **3** was reduced as before, using sodium borohydride in a mixed aqueous/organic medium, buffered at pH 7.0. This pH value was chosen so as to minimize the risk of epimerization of chirally labeled **3**. The double bond of the resulting homoallylic alcohol was reduced by homogeneous hydrogenation,[29]

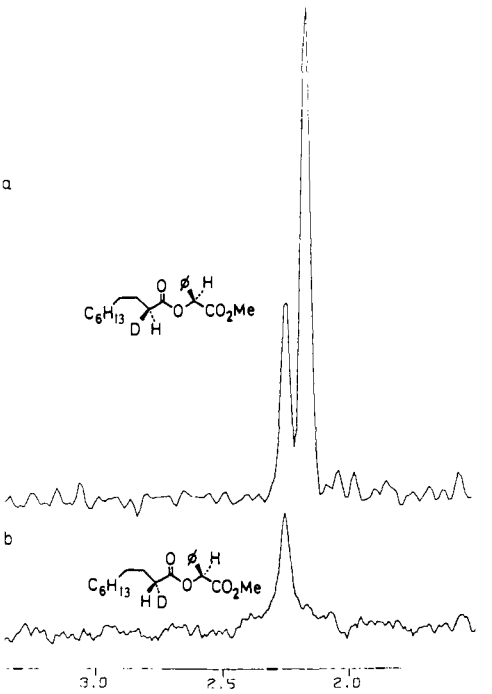


Figure 5. Deuterium NMR spectra, 76.88 MHz, of mandelate esters derived from incubation of (a) unlabeled (*E*)-2-decenoyl-NAC and (b) (*E*)-2-[2-^{2}H]decenoyl-NAC with dehydrase in ^2H_2O and ^1H_2O, respectively. The upper spectrum is resolution-enhanced, leading to some distortion in the relative peak areas. A similar spectrum processed with 1.0 Hz of line broadening suggested that the true ratio of peak areas was ca. 1:2. Chemical shifts are referenced to deuteriobenzene (7.15 ppm) at natural abundance in the solvent.

giving decanol, which was oxidized to the acid, by using ruthenium(VI).[30] Finally, each labeled decanoic acid sample was esterified, as had been described by Parker.

Deuterium NMR spectra of the two methyl mandelate esters are shown in Figure 5. The lower spectrum is of ester derived from the incubation of [2-^{2}H]**2** with dehydrase in ^1H_2O-based buffer. A single product resonance is observed, at 2.27 ppm, corresponding to deuterium in the *pro-R* position at C-2 of the 10-carbon acid. There is no more than a hint of a deuterium in the higher field (*pro-S*) position. The upper spectrum is of the ester sample that was obtained from the incubation of unlabeled **2** with dehydrase in deuterated medium. This time two peaks are evident: a major peak at 2.17 ppm plus a smaller peak at 2.27 ppm. By far the greater portion of the deuterium was in the *pro-S* position. The label epimerization that is evident here presumably occurred in the reduction of chirally labeled **3** with borohydride, a reaction that generates a locally high concentration of strong base. Control of pH was not easily achieved, despite vigorous stirring of the reaction mixture. As C-2 of **3** is both allylic and adjacent to a thio ester carbonyl group, pH control was crucial in maintaining the integrity of the chiral label. This interpretation is supported by the fact that the lower spectrum shows little or no evidence of imperfect stereoselection on the part of the enzyme.

Discussion

The spectra depicted in Figure 5 give complementary views of events at C-2 in the course of the allylic rearrangement of **2** to **3**. In each case, the new hydrogen (or deuterium) atom (the one provided by the buffer) has been added to the *si* face at C-2. When this is considered along with the previously described findings on the steric course at C-4 (i.e., that the *pro-R* hydrogen atom is removed in conversion of **2** to **3**), it is seen that *the dehydrate-catalyzed allylic rearrangement is a suprafacial pro-*

(26) Parker, D. *J. Chem. Soc., Perkin Trans. 2* **1983**, 83–88.
(27) Atkinson, J. G.; Csakvary, J. J.; Herbert, G. T.; Stuart, R. S. *J. Am. Chem. Soc.* **1968**, *90*, 498–499.
(28) Battersby, A. R.; Buckley, D. G.; Staunton, J.; Williams, P. J. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2550–2558.
(29) Osborn, J. A.; Jardine, F. H.; Young, J. F.; Wilkinson, G. *J. Chem. Soc. A* **1966**, 1711–1732.
(30) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936–3938.

Chart I. Suprafacial Steric Courses

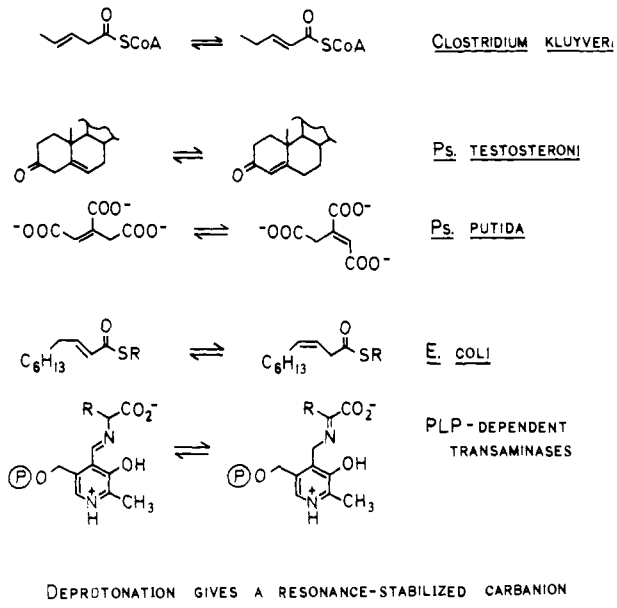
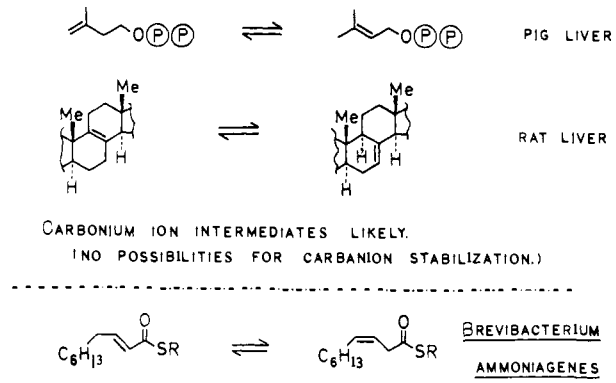
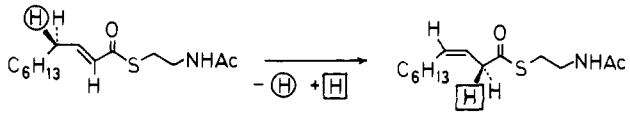


Chart II. Antarafacial Steric Courses



cess. This finding is most consistent[5] with a *single-base* mechanism.



The present result is also consistent with the stereochemical outcomes of the majority of enzyme-catalyzed allylic rearrangements. Chart I depicts a diverse assortment of allylic[31-33] (and aza allylic[34]) rearrangements catalyzed by enzymes from a variety of organisms. All of these reactions proceed suprafacially. Two notable exceptions to the general rule are shown in Chart II. Isopentenyl-pyrophosphate isomerase[35] (a key enzyme in the biosynthesis of terpenes) and Δ^8-Δ^7 steroid isomerase[36,37] (which is involved in the conversion of lanosterol to cholesterol) both mediate antarafacial allylic rearrangements. Upon closer examination of all of these allylic rearrangement substrates, an interesting trend becomes evident. Deprotonation of each of the substrates shown in Chart I will afford a stabilized carbanion intermediate. On the other hand, the substrates of Chart II are not so easily deprotonated; rather, in all likelihood, these antarafacial reactions proceed via carbonium ion intermediates or are concerted. This it is apparent that *by a simple inspection of the gross structural characteristics of an enzyme-catalyzed allylic rearrangement substrate, one can predict the overall reaction stereochemistry as well as the general mechanism.* Furthermore, it is clear that catalytic efficiency (represented by the number of active site acid/base groups) is favored by facile deprotonation of the substrate. In other words, nature has provided two bases (for a "push-pull" effect) *only* when proton removal is particularly difficult.

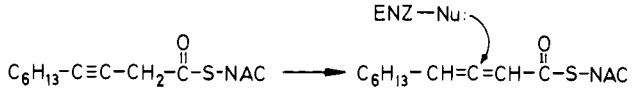
An apparent exception to the foregoing structure–stereochemistry-mechanism rule for allylic rearrangements was described recently[38] by Japanese workers who have been investigating fatty acid metabolism in *Brevibacterium ammoniagenes*. Interestingly, these researchers have isolated a dehydrase which seems functionally identical with that from *E. coli*, but they have concluded that the allylic rearrangement catalyzed by their enzyme proceeds antarafacially. The discrepancy between this finding and ours has led us to scrutinize the methods that they used. Indeed, there are serious shortcomings both in the experimental design and in the interpretability of their data. The entire analysis rests on the use of gas chromatography–mass fragmentography for determining the deuterium content at specific sites in multiply deuterated molecules. Gas chromatographic fractionation of isotopically labeled molecules is well precedented.[39] It is not apparent whether this and other significant, recognized[40] potential sources of error associated with GC–MS were taken into account. Second, in the analysis of the steric course of the rearrangement at C-2 of decenoyl-ACP, the coenzyme A thio ester of the monomethyl oxygen ester of nonanedioic acid was incubated with an acyl-CoA oxidase (i.e., desaturase). Saito and his co-workers *assumed* that desaturation occurred adjacent to the thio ester moiety rather than adjacent to the oxygen ester functionality. There was, however, *no* evidence provided in support of this crucial assumption. Finally, Table I of ref 38 presents two calculated sets of mass spectral ion intensities, each set assuming a different steric outcome at substrate C-2. The experimentally observed ion intensities fall *between* the pairs of calculated values, precluding any meaningful interpretation of the results. For all of the foregoing reasons, it is clear that the *Brevibacterium* enzyme merits reinvestigation.

An additional line of evidence used in support of a "single-base" catalytic mechanism has been the demonstration of intramolecular hydrogen transfer. All but one of the reactions from Chart I is accompanied by at least a small degree of intramolecular hydrogen transfer. The single apparent exception is the *E. coli* dehydrase. Specifically, Rando and Bloch found[41] that in relatively short-term incubations with dehydrase, both hydration of (*Z*)-3-[2(*RS*)-^{3}H,1-^{14}C]decenoyl-NAC and dehydration of 3(*RS*)-[2(*RS*)-^{3}H,1-^{14}C]hydroxydecanoyl-NAC are accompanied by loss of approximately 57% of the tritium label. Indeed, if dehydrase functioned by direct transfer of hydrogen from C-4 of **2** to C-2 of **3** without substantial exchange en route, then we might have expected to observe a signal at 2.5 ppm in the upper spectrum of Figure 3. This is where the C-2 resonances appear in the ester derivatives of **3**. On the other hand, the signal-to-noise ratios of

(31) (a) Malhotra, S. K.; Ringold, H. J. *J. Am. Chem. Soc.* **1965**, *87*, 3228–3236. (b) Viger, A.; Coustal, S.; Marquet, A. *J. Am. Chem. Soc.* **1981**, *103*, 451–458.
(32) Klinman, J. P.; Rose, I. A. *Biochemistry* **1971**, *10*, 2259–2266.
(33) Hashimoto, H.; Günther, H.; Simon, H. *FEBS Lett.* **1973**, *33*, 81–83.
(34) For reviews, see: Floss, H. G.; Vederas, J. C. In "New Comprehensive Biochemistry"; Tamm, C., Ed.; Elsevier: New York, 1982; Vol. 3, pp 161–199. Rêtey, J.; Robinson, J. A. "Stereospecificity in Organic Chemistry and Enzymology"; Verlag Chemie: Weinheim, Germany, 1982; pp 151–184.
(35) Cornforth, J. W.; Clifford, K.; Mallaby, R.; Phillips, G. T. *Proc. R. Soc. London, Ser. B* **1972**, *182*, 277–295.
(36) Lee, W.-H.; Kammereck, R.; Lutsky, B. N.; McCloskey, J. A.; Schroepfer, G. J., Jr. *J. Biol. Chem.* **1969**, *244*, 2033–2040.
(37) Fiecchi, A.; Galli Kienle, M.; Scala, A.; Galli, G.; Grossi Paoletti, E.; Cattanbeni, F.; Paoletti, R. *Proc. R. Soc. London, Ser. B* **1972**, *180*, 147–165.
(38) Saito, K.; Kawaguchi, A.; Seyama, Y.; Yamakawa, T.; Okuda, S. *Tetrahedron Lett.* **1982**, *23*, 1689–1692.
(39) See, for example: (a) McCloskey, J. A.; Lawson, A. M.; Leemans, F. A. J. M. *J. Chem. Soc., Chem. Commun.* **1967**, 285–287 and references therein. (b) Liberti, A. In "Gas Chromatography 1966"; Littlewood, A. B., Ed.; The Institute of Petroleum: London, 1967; pp 95–118.
(40) (a) Eldjarn, L.; Helland, P.; Jellum, E. In "Quantitative Mass Spectrometry in Life Sciences II"; de Leenheer, A. P., Roncucci, R. R., van Peteghem, C., Eds.; Elsevier: Amsterdam, 1978; pp 151–159. (b) Dehennin, L.; Reiffsteck, A.; Scholler, R. In "Stable Isotopes"; Schmidt, H.-L., Förstel, H., Heinzinger, K., Eds.; Elsevier: Amsterdam, 1982; pp 617–622.
(41) Rando, R. R.; Bloch, K. *J. Biol. Chem.* **1968**, *243*, 5627–5634.

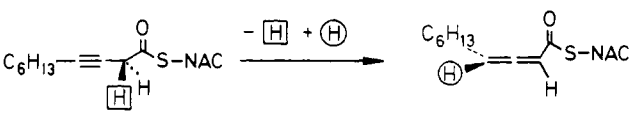
the spectra in Figure 3 are such that we would not be able to detect a relatively small amount (e.g., 10% or less) of intramolecular deuterium transfer. More to the point, however, our reactions were allowed to reach equilibrium, and so each labeled substrate molecule may have undergone many turnovers. Even if a substantial amount of label were transferred intact in *each* turnover, given the conditions of our experiment it is not surprising that we do not observe label at C-2 of the product. On the basis of our stereochemical findings, however, we expect that a single turnover experiment will provide evidence for some intramolecular hydrogen transfer by dehydrase.

One of the most important pieces of evidence in support of histidine's accepted role as a catalytic base at dehydrase's active site came from the incubation of the substrate analogue 3-decynoyl-NAC with the enzyme.[42] Initially, dehydrase treats 3-decynoyl-NAC as a normal substrate; however, the product of the resulting propargylic rearrangement is the potent electrophile 2,3-decadienoyl-NAC. This allenic thio ester alkylates an active



site histidine,[4] thereby killing the enzyme. This, in fact, was the pioneering demonstration of the now-classical technique of "suicide" enzyme inactivation.[8]

Both the acetylenic and allenic thio esters as well as the parent allenic acid will inactivate dehydrase.[43] In fact, Bloch and his associates have shown[44,45] that the inactivation is a highly stereoselective process; the dextrorotatory form of each allene is by far the more potent. Although this stereochemical preference has been recognized for a decade, the absolute configurations of the inhibitory allenes have remained unknown. Knowing the steric course of the "normal" allylic rearrangement, however, we can *predict* the absolute configurations of the inhibitory, dextrorotatory allenes. Thus, if (by analogy) the *pro*-2*S* proton is removed from 3-decynoyl-NAC, and the acetylenic linkage is protonated at C-4 on the same "face", then the allenic thio ester will have the *S* configuration,[46] as shown. In fact, we have recently obtained X-ray crystallographic evidence confirming this prediction.[47]



Experimental Section

NMR spectra were recorded on a JEOL FX-90Q(II) (^{1}H, ^{2}H), a Varian EM-360A (^{1}H), a Bruker WM-300 (^{2}H), a JEOL GX-400 (^{2}H), and a Bruker WM-500 (^{2}H). Generally, proton spectra were run at ambient temperature, with $CDCl_3$ as solvent, and chemical shifts are given in parts per million downfield from tetramethylsilane, added as an internal standard. When CCl_4 was used as solvent, proton and deuteron chemical shifts were referenced to $CHCl_3$ and $CDCl_3$, respectively, each added as an internal standard (7.26 ppm). A Perkin-Elmer Model 257 was employed for measurement of infrared spectra. Melting points are uncorrected and were determined with a Thomas-Hoover apparatus, with samples placed in unsealed capillaries. Analytical gas chromatographic analyses were conducted on Varian 2100 and Packard 428 instruments, using flame ionization detection and equipped with 6 ft × 2 mm (i.d.) columns packed with 7.5% Carbowax 20M AW-DMCS. Optical rotations were measured with a Rudolph Autopol II digital polarimeter. Analytical silica gel TLC plates and silica gel for flash chromatography were purchased from Analtech and Merck, respectively. Visualization of tlc plates was accomplished by UV or by phosphomolybdic acid spray reagent. Analytical and preparative HPLC was performed on a component system which included an Altex 110A pump, a Rheodyne 7125 injector, and an ISCO 1840 absorbance monitor. A 10-μm Lichrosorb SI-60 column (250 × 4.6 mm) packed by Chromanetics was used for analytical separations, while preparative work utilized 250 × 10 mm columns packed with 10-μm silica gel by Alltech and by Altex.

Tetrahydrofuran was routinely distilled from $LiAlH_4$ immediately before use. Pyridine was stirred over KOH overnight, distilled from BaO, and stored over 3A molecular sieves. HMPA was stirred over CaH_2 overnight and then distilled at reduced pressure immediately before use. Standardization of methyllithium was by the method of Winkle et al.[48] All chemicals used were of reagent grade. Isotopically labeled compounds were purchased from Aldrich, Alfa, and Cambridge Isotope Laboratories.

Unless otherwise noted, anhydrous magnesium sulfate was used for drying organic extracts, and following filtration, samples were concentrated via rotary evaporation (using a water aspirator), with gentle warming.

E. coli cells were grown in 2-L flasks using a New Brunswick G-25 incubator–shaker and were broken with a French pressure cell (Aminco). Manipulations of enzyme solutions were performed at 0–4 °C, and deionized water was used throughout.

Ethyl [2,2-$^2H_2$]Heptanoate. Methyl heptanoate (6.8 g, 47 mmol, Aldrich) was added to a solution of 1.4 g (26 mmol) of freshly prepared NaOMe in 100 mL of [*hydroxyl*-$^2H_1$]ethanol. The reaction mixture was heated at reflux, under an atmosphere of nitrogen, for 8 h. Following cooling to room temperature and concentration in vacuo, water was added, and the resulting mixture was extracted several times with ether. The combined ether extracts were washed with brine, dried, filtered, and concentrated to a colorless oil. GC showed one major component, having a retention time identical with that of authentic ethyl heptanoate. ^{1}H NMR showed essentially complete exchange of the C-2 protons. ^{1}H NMR 0.95 (t, J = 6 Hz, 3 H, CH_3); 1.1–1.4 (m, 11 H, heptyl CH_2 and ethyl CH_3), 4.2 (q, J = 6 Hz, 2 H, CH_3CH_2O); IR (neat film) 2950, 2930, 2850, 1705, 1460, 1390, 1160, 1095, 1015 cm^{-1}.

[1,1,2,2-$^2H_4$]Heptanol. Ethyl [2,2-$^2H_2$]heptanoate (5.25 g, 32.8 mmol) in Et_2O was treated with $LiAl^2H_4$ (1.38 g, 32.8 mmol), yielding (after workup[49]) 3.84 g (87%) of labeled heptanol. The GC retention time of this material was identical with that of authentic, unlabeled heptanol, and integration of the trace showed the product to be 94% pure. ^{1}H NMR 0.95 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.4 (m, 8 H, CH_2), 3.5 (br s, 1 H, OH); IR (neat film) 3350, 2925, 2840, 1470, 1425, 1108, 1050, 855, 715 cm^{-1}.

[1,2,2-$^2H_3$]Heptanal. [1,1,2,2-$^2H_4$]Heptanol (3.67 g, 30.6 mmol) was oxidized to the corresponding aldehyde by the procedure of Ratcliffe and Rodehorst.[50] The product was purified by flash chromatography (6 in. × 50 mm); 1:9, Et_2O/pentane), providing 2.27 g of a colorless liquid, shown by GC analysis to comprise mostly the desired aldehyde (87%) plus solvent (8%). This material was used without further purification. ^{1}H NMR 0.95 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.4 (m, 8 H, CH_2); IR (neat film) 2925, 2740, 2695, 1701, 1470, 1440, 1110 cm^{-1}.

(*S*)-[1,2,2-$^2H_3$]Heptanol. [1,2,2-$^2H_3$]Heptanal (1.31 g, 11.2 mmol) was converted to the title alcohol by the method of Midland,[9] using purified[51] (+)-α-pinene ($[\alpha]^{22}_D$ +49.46° (neat), calculated[51] enantiomeric purity = 98.4%). 3.22 g of a yellow oil was recovered, shown by GC to be mostly the desired alcohol. Purification was effected on the derived *p*-toluenesulfonate (vide infra).

(*S*)-[1,2,2-$^2H_3$]Heptyl *p*-Toluenesulfonate. The crude alcohol from the previous reaction was converted to the corresponding *p*-toluenesulfonate in the usual manner.[52] The resulting product was purified by flash chromatography (6 in. × 50 mm; 1:9, EtOAc/hexanes), providing 2.50 g of a clear, colorless oil (82% from the aldehyde). ^{1}H NMR 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.0–1.35 (m, 8 H, CH_2), 2.36 (s, 3 H, Ar CH_3), 4.0 (s, 1 H, CHDO), 7.2–7.8 (AB q, J = 8 Hz, 4 H, Ar H); IR (neat film) 3030, 2930, 1580, 1450, 1425, 1330, 1165, 1088, 810 cm^{-1}.

(*S*)-1-[(2-Tetrahydropyranyl)oxy][4,5,5-$^2H_3$]dec-2-yne.[10,53] Methyllithium (7.2 mL of a 1.4 M solution in Et_2O, 10.1 mmol) was added dropwise, from a syringe, to a stirred, ice-cold solution of 1.6 g (11.1 mmol) of the THP derivative of 2-propyn-1-ol in HMPA (10 mL). The

(42) Kass, L. R.; Bloch, K. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 1168–1173.
(43) Endo, K.; Helmkamp, G. M., Jr.; Bloch, K. *J. Biol. Chem.* **1970**, *245*, 4293–4296.
(44) Morisaki, M.; Bloch, K. *Bioorg. Chem.* **1971**, *1*, 188–193.
(45) Miesowicz, F. M.; Bloch, K. *J. Biol. Chem.* **1979**, *254*, 5868–5877.
(46) (a) Cahn, R. S.; Ingold, C. K.; Prelog, V. *Experientia* **1956**, *12*, 81–94. (b) Cahn, R. S.; Ingold, C.; Prelog, V. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 385–415.
(47) Schwab, J. M.; Lin, D. C. T.; He, C.-h.; Clardy, J. *Tetrahedron Lett.* **1984**, *25*, 4909–4912.
(48) Winkle, M. R.; Lansinger, J. M.; Ronald, R. C. *J. Chem. Soc., Chem. Commun.* **1980**, 87–88.
(49) Fieser, L. F.; Fieser, M. "Reagents for Organic Synthesis"; Wiley: New York, 1967; Vol. 1, p 581.
(50) Ratcliffe, R.; Rodehorst, R. *J. Org. Chem.* **1970**, *35*, 4000–4001.
(51) Brown, H. C.; Yoon, N. M. *Isr. J. Chem.* **1976/1977**, *15*, 12–16.
(52) Jacobs, T. L.; Macomber, R. S. *J. Am. Chem. Soc.* **1969**, *91*, 4824–4837.
(53) Kunitani, M. G., Ph.D. Thesis, Brandeis University, Waltham, MA 1977.

reaction was conducted under an atmosphere of dry nitrogen. After the mixture had been stirred for 0.5 h at 0 °C (during which time the color changed from yellow to deep purple), (*S*)-[1,2,2-$^2H_3$]heptyl *p*-toluenesulfonate (2.50 g, 9.16 mmol) in 5 mL of HMPA was added dropwise over a period of 15 min. The color of the solution changed to green and then to brown. After 2 h at 0 °C, the reaction was terminated by cautious addition of 30 mL of cold water. An additional 100 mL of water was added 15 min later, and the resulting mixture was extracted several times with a 1:3 mixture of Et_2O in hexanes. The organics were washed twice with water and once with brine, then dried, filtered, and concentrated. The resulting brown oil (2.6 g) was purified by flash chromatography (6 in. × 50 mm; 1:40, EtOAc/hexanes; run in two portions). The yield of product (a pale yellow liquid) was 1.55 g (70.5%). ^1H NMR 0.91 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.4 (m, 8 H, CH_2), 1.4–1.8 (m, 6 H, THP C-3–5 H), 2.1–2.25 (m, 1 H, $CD_2CHDC{\equiv}$), 3.37–3.67 (m, 1 H, THP C-6_{ax}), 3.7–4.0 (m, 1 H, THP C-6_{eq}), 4.23, 4.25 (d, J = 1.8 Hz, each 1 H, ≡CCH_2O (diastereotopic)), 4.75–4.87 (m, 1 H, acetal H); IR (neat film) 2910, 2840, 2160, 1460, 1192, 1118, 1110, 892 cm^{-1}.

(*R*)-2-[4,5,5-$^2H_3$]Decyn-1-ol.[54] To a stirred solution of the foregoing THP derivative (1.54 g, 6.40 mmol) in 40 mL of MeOH was added 20 mg of *p*-toluenesulfonic acid. The resulting cloudy reaction mixture became clear after 20 min, and GC analysis at that time showed that the starting material had been consumed. Five milliliters of saturated aqueous $NaHCO_3$ was added, MeOH was removed *in vacuo*, and the remaining aqueous mixture was extracted several times with Et_2O. The combined organics were washed with brine until the washes were neutral to pH paper, then dried, filtered, and concentrated, leaving 994 mg of a clear oil (98%). ^1H NMR (CCl_4) 0.91 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.4 (m, 8 H, CH_2), 2.16 (br s, 1 H, $CD_2CHDC{\equiv}$), 3.30 (s, 1 H, OH), 4.16 (d, J = 6 Hz, 2 H, CH_2OH); ^2H NMR (CCl_4) 1.50 (s, 1.80 D, CH_2CD_2CHD), 2.19 (s, 1.00 D, $CD_2CHDC{\equiv}$); IR (neat film) 3350, 2960, 2930, 2860, 2160, 1350, 1110 cm^{-1}.

(*R*)-(*E*)-2-[4,5,5-$^2H_3$]Decen-1-ol. (*R*)-2-[4,5,5-$^2H_3$]Decyn-1-ol was converted to the corresponding *E* allylic alcohol by the method of Corey,[11] as described by Cane.[55] The resulting yellow oil was purified by flash chromatography (6 in. × 30 mm; 1:4, EtOAc/hexanes), providing 890 mg (89%) of the desired product as a colorless oil. ^1H NMR (CCl_4) 0.95 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.4 (m, 8 H, CH_2) 2.0 (br m, 1 H, $CHDCH{=}$), 2.6 (br s, 1 H, OH), 4.0 (br d, J = 6 Hz, 2 H, $=CHCH_2OH$), 5.55–5.65 (m, 2 H, $CH{=}CH$); ^2H NMR (CCl_4) 1.36 (s, 1.78 D, CD_2CHD), 2.07 (s, 1.00 D, CD_2CHD); IR (neat film) 3350, 2910, 2860, 1440, 1425, 1082, 995, 965 cm^{-1}.

(*R*)-(*E*)-2-[4,5,5-$^2H_3$]Decenoic Acid. (*R*)-(*E*)-2-[4,5,5-$^2H_3$]Decen-1-ol (875 mg, 5.5 mmol) in 150 mL of pentane was oxidized[12] by addition of 7.5 g of activated MnO_2. The suspension was stirred vigorously at room temperature for 1.5 h, after which time GC analysis showed that the reaction had reached completion. The suspension was filtered through Celite, and the filtrate was concentrated by distillation (using a Vigreux column and an oil bath at 55 °C) followed by cooling in an ice bath and application of a water aspirator. GC analysis showed that the product had a retention time identical with that of authentic, unlabeled (*E*)-2-decenal. Oxidation to the corresponding carboxylic acid was by the method of Pinnick,[13] as follows. A portion of the aldehyde (300 mg, 1.85 mmol) was dissolved in (40 mL of *t*-BuOH plus 9 mL of 2-methyl-2-butene. A solution of $NaClO_2$ (1.60 g, 30.7 mmol) and NaH_2PO_4 (1.6 g, 40.8 mmol) in 15 mL of water was added to the stirred aldehyde solution, over a period of 15 min. The pale yellow reaction mixture was stirred vigorously at room temperature for 6 h and was then concentrated at reduced pressure. To the residue was added dilute HCl, and the resulting mixture was extracted several times with hexanes. Workup of the extracts afforded 210 mg of the acid, as a clear, colorless oil, which was purified by flash chromatography (6 in. × 30 mm; 30:70:1, Et_2O/hexanes/HOAc) giving a colorless oil (180 mg, 60%). ^1H NMR 0.91 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.4 (m, 8 H, CH_2), 2.15 (br d, J = 7 Hz, 1 H, $CD_2CHDCH{=}$); 5.8 (d of d, $J_{vicinal}$ = 15 Hz, $J_{allylic}$ = 1 Hz, 1 H, $CHDCH{=}CH$), 7.06 (d of d, J = 7, 15 Hz, 1 H, $CHDCH{=}CH$), 9.5 (br s, 1 H, COOH); IR 2955, 2655, 1690, 945, 925 cm^{-1}.

Ethanethioic Acid (*S*)-[2-(Acetylamino)ethyl] Ester (*N*,*S*-Diacetylcysteamine).[56] To a three-necked 250-mL round-bottomed flask equipped with a pH electrode and two addition funnels was added 5.68 g (50 mmol) of 2-mercaptoethylamine hydrochloride in 40 mL of water. One addition funnel was charged with 14.14 mL (150 mmol) of acetic anhydride and the other with ca. 40 mL of 8 M KOH. After the contents of the flask had been cooled by means of an ice bath and the pH had been adjusted to 8.0 (by addition of a portion of the KOH solution), acetic anhydride was added dropwise along with sufficient KOH solution to maintain the pH at 8.0. After all of the acetic anhydride had been added, the pH of the reaction mixture was brought to 7.0 by addition of 2 N HCl, and the mixture was stirred for 1 h. At this time, NaCl was added (to the point of saturation), and the resulting mixture was extracted with five 50-mL portions of CH_2Cl_2. The combined extracts were dried, filtered, and concentrated, yielding 8.0 g (98%) of a clear, colorless liquid, which GC analysis showed to comprise only one compound. ^1H NMR 2.0 (s, 3 H, CH_3CONH), 2.1 (s, 3 H, CH_3COS), 2.78 (t, J = 6 Hz, 2 H, CH_2S), 3.60 (d of t, J = 6, 6 Hz, 2 H, CH_2NH), 7.35–7.6 (m, 1 H, NH); IR 3280, 3180, 2940, 1655, 1545, 1465, 1390, 1348, 1262, 1225, 1180, 1115 cm^{-1}.

***N*-(2-Mercaptoethyl)acetamide (*N*-Acetylcysteamine).**[57] A 250-mL round-bottomed flask was charged with a solution of 3.06 g (19 mmol) of *N*,*S*-diacetylcysteamine in 100 mL of water. After the solution had been chilled to 10 °C, 3.55 g (63.3 mmol) of solid KOH was added, and the resulting cloudy mixture was stirred at room temperature for 0.5 h, under an atmosphere of nitrogen. During this time the reaction mixture became clear. An aliquot was removed and worked up by adjustment of the pH to 7.0 (2 N HCl), saturation with NaCl, and extraction with Et_2O. GC analysis confirmed that the hydrolysis had reached completion. The remainder of the reaction mixture was worked up in an analogous manner, except that CH_2Cl_2 was used for the extraction. The combined organics were dried, filtered, and concentrated, giving a clear, colorless oil, which was used immediately. ^1H NMR 1.39 (t, J = 8 Hz, 1 H, SH), 2.0 (s, 3 H, CH_3CO), 2.78 (d of t, J = 8, 6 Hz, 2 H, CH_2SH), 3.60 (d of t, J = 6, 6 Hz, 2 H, CH_2NH), 6.45–6.75 (m, 1 H, NH); IR (neat film) 3280, 3080, 2920, 2852, 2540, 1640, 1523, 1455, 1425, 1380, 1275 cm^{-1}.

Thallium(I) Salt of *N*-(2-Mercaptoethyl)acetamide. To a dry, preweighed 250-mL round-bottomed flask equipped with a magnetic stir bar and a rubber septum cap was added 3.72 g (14.9 mmol) of thallium ethoxide. Following the addition to the flask of 145 mL of nitrogen-saturated THF, 1.86 g (15.65 mmol) of freshly prepared *N*-acetylcysteamine was added dropwise, via syringe, over a period of 20 min. The concentration of Tl-NAC in the resulting bright yellow suspension (which was stored at 4 °C) was calculated to be 0.098 M.

(*R*)-(*E*)-2-[4,5,5-$^2H_3$]Decenethioic Acid (*S*)-[2-(Acetylamino)ethyl] Ester ((*E*)-2-Decenoyl-NAC). The mixed diphenylphosphinic carboxylic anhydride of (*R*)-(*E*)-[4,5,5-$^2H_3$]decenoic acid (2.13 mmol) was prepared as described by Kende et al.[14] The oil thus obtained, dissolved in dry, degassed THF (70 mL), was placed under an atmosphere of nitrogen and cooled in an ice bath. Tl-NAC suspension (21.7 mL, 2.13 mmol) was added via syringe, and the resulting suspension was stirred at ice bath temperature for 4 h, during which time the color changed from bright yellow to white. After the reaction mixture had been concentrated (in vacuo), the residue, a thick slurry, was taken up in Et_2O and filtered through Celite. Concentration of the filtrate in vacuo left a pale yellow oil which was purified by flash chromatography (6 in. × 30 mm; 6:1, CH_2Cl_2/acetone). The yield of white crystalline solid (mp 55–55.3 °C) was 525 mg (90%). ^1H NMR (CCl_4, 50 °C; see Figure 1) 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.4 (m, 8 H, CH_2), 1.87 (s, 3 H, CH_3CO), 2.16 (br d, J = 8 Hz, 1 H, $CD_2CHDCH{=}$), 3.02 (t, J = 6 Hz, 2 H, CH_2CH_2S), 3.38 (d of t, J = 6, 6 Hz, 2 H, CH_2CH_2NH), 5.75–5.95 (m, 1 H, NH), 6.05 (d of d, $J_{vicinal}$ = 15, $J_{allylic}$ = 1 Hz, 1 H, $CHDCH{=}C$-*H*CO, 1 H), 6.84 (br d of d, J = 15, 7 Hz, 1 H, $CHDCH{=}CH$); ^2H NMR (CCl_4, 50 °C; see Figure 1) 1.45 (s, 2.00 D, $CD_2CHDCH{=}$), 2.19 (s, 0.96 D, $CD_2CHDCH{=}$); IR (KBr pellet) 3300, 3116, 2940, 2910, 2835, 1618, 1530, 1447, 1374, 1280, 1227, 1182, 1060, 954 cm^{-1}.

(*S*)-(*E*)-2-[4,5,5-$^2H_3$]Decenethioic Acid (*S*)-[2-(Acetylamino)ethyl] Ester. The 4*S* compound was made in the same way as was the 4*R* isomer (vide supra), except that (−)-α-pinene ($[\alpha]^{22}_D$ −40.17° (neat), calculated[51] enantiomeric excess = 78.6%, enantiomeric purity = 89.3%) was substituted for (+)-α-pinene in the Midland reduction of labeled heptanal. ^1H NMR (CCl_4, 50 °C; see Figure 2) 0.89 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.4 (m, 8 H, CH_2), 1.88 (s, 3 H, CH_3CO); 2.15 (br d, J = 8 Hz, 1 H, $CD_2CHDCH{=}$), 3.02 (t, J = 6 Hz, 2 H, CH_2CH_2S), 3.36 (d of t, J = 6, 6 Hz, 2 H, CH_2CH_2NH), 6.04 (d of d, $J_{vicinal}$ = 15, $J_{allylic}$ = 1 Hz, 1 H, $CHDCH{=}CHCO$) 6.05–6.3 (m, 1 H, NH), 6.80 (br d of d, J = 15, 7 Hz, 1 H, $CHDCH{=}CH$); ^2H NMR (CCl_4, 50 °C; see Figure 2) 1.43 (s, 1.96 D, $CD_2CHDCH{=}$), 2.20 (s, 1.0 D, $CD_2CHDCH{=}$); IR (KBr pellet) 3298, 2947, 2903, 2835, 1651, 1617, 1525, 1430, 1062, 954 cm^{-1}.

Growth of *E. coli* and Preparation of β-Hydroxydecanoylthioester Dehydrase. A sample of *E. coli* strain DM51 A and procedures for growth of this strain as well as purification of dehydrase from it were provided by Prof. J. E. Cronan, Jr., and Dr. M. Lakshman (University

(54) Corey, E. J.; Achiwa, K.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1969**, *91*, 4318–4320.
(55) Cane, D. E.; Iyengar, R.; Shiao, M. *J. Am. Chem. Soc.* **1981**, *103*, 914–931.
(56) Gerstein, J.; Jencks, W. P. *J. Am. Chem. Soc.* **1964**, *86*, 4655–4663.
(57) Kass, L. R.; Brock, D. J. H. *Methods Enzymol.* **1964**, *86*, 696–698.

of Illinois). According to these researchers,[19] the dehydrase thus produced is the same as from that which has been isolated from *E. coli* strain B by Bloch and his co-workers.[17]

Incubation of Labeled Thio Ester Substrates with Dehydrase. To 180 mL of 10 mM Tris-HCl (pH 7.0, 37 °C) was added 60 mL of dehydrase solution (1.09 g of protein, via Bio-Rad assay,[58] 90 units of isomerase activity per mg of protein). After 10 min of gentle stirring under a nitrogen atmosphere, 200 mg (0.73 mmol) of labeled (*E*)-2-decenoyl-NAC dissolved in 7.5 mL of dioxane was added dropwise. The cloudy mixture was stirred at 37 °C for 1 h and then poured into an ice-cold mixture of 200 mL of THF and 150 mL of 1M Tris-HCl (pH 8.5). 4.0 g of solid $NaBH_4$ was added in one portion to the solution, which was stirred rapidly. The initially vigorous foaming subsided after 15 min, after which time the reaction mixture was allowed to warm to room temperature. After 30 min, excess borohydride was destroyed by the addition of 2 N HCl until the pH of the reaction mixture had reached 2.0. Solid $(NH_4)_2SO_4$ was added to the saturation point, and the solution was then extracted with five 100-mL aliquots of a 1:3 mixture of Et_2O in hexanes. The combined organics were dried and filtered, and the filtrate was concentrated in vacuo, affording 211 mg of a yellow oil. GC analysis showed the presence of the following: (*E*)-2-decen-1-ol (17% of the total peak area), (*Z*)-3-decen-1-ol (0.8%), and 1-decanol (31%). (1,3-Decanediol was not identified, although it was assumed to be a product of the reductive workup of the incubation.) The crude product mixture was treated with 2.0 g of activated MnO_2 in 20 mL of hexanes. When GC analysis showed oxidation[12] of the allylic alcohol to be complete, the solids were removed by filtration through Celite and washed with CH_2Cl_2. Solvents were removed in vacuo.

Derivatization of Product Alcohols. (1) Benzoate Esters from Incubation of (*R*)-(*E*)-2-[4,5,5-$^2H_3$]Decenoyl-NAC with Dehydrase. The mixture of alcohols (170 mg) from the MnO_2 oxidation was dissolved in 2 mL of dry pyridine, and 250 mg of benzoyl chloride was added via syringe. The reaction mixture was stirred at room temperature under an atmosphere of dry nitrogen for 8 h. After this time the pyridine was removed (oil pump), and the residue was extracted with Et_2O. After drying of the extracts and removal of solvent, the residual yellow solid was triturated with hexanes. Evaporation of the hexanes gave 250 mg of a white solid, which was subjected to preliminary purification by flash chromatography (6 in. × 20 mm; 3:2, hexanes/CH_2Cl_2). The mixture of esters thus obtained (112 mg of a yellow oil) was further fractionated by semipreparative HPLC (7:3, hexanes/CH_2Cl_2; 4 mL/min; 10 × 250 mm silica gel column; effluent monitored at 230 nm). (*Z*)-3-Decen-1-yl benzoate in the ester mixture was identified by comparison of its retention time under the above conditions to that of authentic, unlabeled material.[59] Removal of solvents from the appropriate fractions gave less than a milligram of the desired ester as a viscous, colorless liquid. ^1H NMR (CCl_4) 0.87 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.5 (m, 8 H, CH_2) 2.52 (d of t, J = 6, 6 Hz, 2 H, =CHCH_2CH$_2$O), 4.32 (t, J = 7 Hz, 2 H, CH$_2$CH$_2$OCO), 5.25–5.5 (m, 2 H, CD$_2$CH=CH), 7.2–8.2 (m, 5 H, Ar H); ^2H NMR (CCl_4; see Figure 3) 2.08 (CD_2CH=); IR (neat film, spectrum of unlabeled material) 3055, 2930, 2840, 1704, 1581, 1460, 1432, 1391, 1295, 1252, 1162, 1095, 1015, 695 cm^{-1}.

(2) *p*-Phenylbenzoate Esters from Incubation of (*S*)-(*E*)-2-[4,5,5-$^2H_3$]Decenoyl-NAC with Dehydrase. The mixture of alcohols (130 mg) from the MnO_2 oxidation[12] was dissolved in 3 mL of dry pyridine, 500 mg of *p*-phenylbenzoyl chloride was added, and the resulting solution was stirred under nitrogen for 8 h. Workup, as described above for the benzoate ester, afforded 600 mg of a white solid, which was fractionated by flash chromatography (6 in. × 30 mm; 3:2, hexanes/CH_2Cl_2), followed by HPLC (6:1, hexanes/CH_2Cl_2; 5 mL/min; 10 × 250 mm silica gel column; effluent monitored at 230 nm). From 123 mg of partially purified ester was obtained an estimated 0.5 mg of (*Z*)-3-decen-1-yl *p*-phenylbenzoate, as identified by comparison of its retention time to that of authentic, unlabeled material.[59] ^1H NMR (CCl_4) 0.87 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.4 (m, 8 H, CH_2), 2.52 (d of t, J = 7, 7 Hz, 2 H, =CHCH_2CH$_2$OCO), 4.32 (t, J = 7 Hz, 2 H, CH$_2$CH_2OCO), 5.48 (t, J = 7 Hz, 1 H, CD=CHCH$_2$OCO), 7.3–8.2 (m, 9 H, Ar H); ^2H NMR (CCl_4; see Figure 3) 2.08 (2.00 D, CD_2CD=), 5.57 (0.92 D, CD_2CD=); IR (neat film, spectrum taken on unlabeled material) 2940, 2905, 2830, 1680, 1580, 1460, 1295, 1098, 740, 695 cm^{-1}.

(*E*)-2-[2-^2H]Decenethioic Acid (*S*)-2-(Acetylamino)ethyl Ester ((*E*)-2-[2-^2H]Decenoyl-NAC). 2-Decyn-1-ol (886 mg, 5.75 mmol) dissolved in 140 mL of THF was reduced[11] to (*E*)-2-[2-^2H]decen-1-ol with $LiAlH^2H_4$ (886 mg, 21 mmol) in the presence of freshly prepared NaOMe (2.2 g, 41 mmol) (vide supra for procedural details). Purified allylic alcohol (794 mg, 88%) was obtained. This was converted to the C-2 labeled thio ester by methods described above. ^1H NMR (CCl_4) 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.0–1.65 (m, 10 H, CH_2), 1.87 (s, 3 H, CH_3CO), 2.18 (d of t, J = 7, 6 Hz, 2 H, CH_2CH=), 3.00 (t, J = 6 Hz, 2 H, CH_2S), 3.35 (d of t, J = 6, 6 Hz, 2 H, CH_2NH), 5.8–6.3 (br m, 1 H, NH), 6.81 (t, J = 7 Hz, CH$_2$CH=); ^2H NMR (CCl_4) 6.13 (s, CH=CDCO); IR (KBr pellet) 3305, 3115, 2940, 2910, 2840, 1620, 1530, 1447, 1375, 1280, 1230, 1182, 1060, 954 cm^{-1}.

Incubation of (*E*)-2-[2-^2H]Decenoyl-NAC with Dehydrase in ^1H_2O Buffer. To 450 mL of 10 mM $KHPO_4$ (pH 7.0, 37 °C) was added 60 mL of dehydrase solution (1.15 g of protein via Bio-Rad assay,[58] 90 units of isomerase activity per mg of protein). After 10 min of gentle stirring under a nitrogen atmosphere, 500 mg of (*E*)-2-[2-^2H]decenoyl-NAC dissolved in 15 mL of dioxane was added dropwise. The cloudy mixture was stirred at 37 °C for 45 min and then cooled to 20 °C. Solid $(NH_4)_2SO_4$ was added (to the saturation point), and then the solution was extracted repeatedly with THF. The organics were concentrated in vacuo, and the residue was then partitioned between water and Et_2O. The Et_2O extracts were dried, filtered, and concentrated, providing 535 mg of thio ester mixture as a yellow oil. Removal of 3-hydroxydecanoyl-NAC was effected by flash chromatography (13 in. × 30 mm; 49:1, CH_2Cl_2/CH_3OH), and labeled **3** was further purified from the resulting white solid via HPLC (6:1, CH_2Cl_2/CH_3CN; 5 mL/min; 10 × 250 mm silica gel column; effluent monitored at 230 nm). Labeled (*Z*)-3-decenoyl-NAC (**3**) was identified by comparison of its retention time to that of authentic, unlabeled material. Ultimately, 5 mg of **3** was obtained, as a viscous, colorless oil. ^1H NMR (CCl_4) 0.89 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.6 (m, 8 H, CH_2), 1.86 (s, 3 H, CH_3CO), 1.9–2.2 (m, 2 H, CH_2CH=CHCHD), 2.94 (t, J = 6 Hz, 2 H, CH_2S), 3.1–3.5 (m, 3 H, CH_2NH and CHDCOS), 5.4–5.7 (m, 2 H, CH=CH), 5.7–6.1 (m, 1 H, NH); ^2H NMR (CCl_4) 3.22 (s, CHDCOS).

(*Z*)-4-[3-$^2H_1$]Undecen-2-one. To a flamed 50-mL round-bottomed flask equipped with a rubber septum and a magnetic stir bar were added CuI (1.07 g, 5.62 mmol) and anhydrous Et_2O (10 mL). The stirred suspension was cooled to 0 °C, and then methyllithium (7.95 mL of a 1.4 M ethereal solution, 11.12 mmol) was introduced in a rapid, dropwise manner, via syringe. After it had been stirred for 10 min, the cuprate[61] solution was cooled to −78°C. The enzymatically equilibrated thio esters (from the incubation of [2-^2H]**2** with dehydrase in ^1H_2O) were dissolved in 4 mL of Et_2O, and this solution was added, over a period of a few minutes, to the cuprate reagent. The resulting yellow suspension was stirred at −78 °C for 2 h, after which time 5 mL of saturated aqueous NH_4Cl was added, terminating the reaction. The mixture was allowed to warm to room temperature and was then filtered to remove solids. After the filtrate had been washed with brine, it was dried and concentrated in vacuo at 0 °C. Flash chromatography (two successive columns—13 in. × 30 mm, 12:1, petroleum ether/Et_2O; followed by 13 in. × 10 mm, 19:1, petroleum ether/Et_2O) provided 3 mg of (*Z*)-4-undecen-2-one, identified by comparison of GC retention times between this material and authentic, unlabeled enone. ^1H NMR (CCl_4 solvent, spectrum of unlabeled material) 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.2–1.5 (m, 8 H, CH_2), 1.8–2.1 (m, partially obscured by signal at 2.06, total integral 5 H, CH_2CH_2CH=), 2.06 (s, overlapping multiplet at 1.8–2.1, total integral 5 H, $COCH_3$), 3.0 (br t, J = 5 Hz, 2 H, =CHCH_2CO), 5.2–5.6 (m, 2 H, CH=CH).

2-[3-$^2H_1$]Undecanone. To a dry 10-mL two-necked round-bottomed flask equipped with a magnetic stirbar was added 4 mL of dry CH_2Cl_2 and 20 mg of $(Ph_3P)_3RhCl$.[29] After the flask had been flushed with H_2, (*Z*)-4-[3-$^2H_1$]undecen-2-one (2 mg, 0.012 mmol, in 1 mL of CH_2Cl_2) was added. After 1.5 h, GC analysis showed the reaction to be complete, and the solvent was removed in vacuo (0 °C). An Et_2O solution of the residue was forced through a plug of silica gel, and the filtrate was concentrated to dryness as before. Further purification was not attempted. ^1H NMR (unlabeled material) 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.7 (m, 14 H, CH_2), 2.07 (s, 3 H, $COCH_3$) 2.36 (t, J = 7 Hz, 2 H, CH_2COCH$_3$).

1-[1-$^2H_1$]Nonyl Acetate. Trifluoroperacetic acid was prepared by the dropwise addition of trifluoroacetic anhydride (600 mg, 2.85 mmol) to 90% H_2O_2 (40 mg of H_2O_2, 1.17 mmol) in 0.4 mL of CH_2Cl_2. When vigorous reaction became apparent, the flask was placed in an ice bath, and the remaining anhydride was added. The peracid solution was stirred at 0 °C for 15 min, and then one-third of it was added to a stirred suspension of 2-[3-$^2H_1$]undecanone (ca. 3 mg, 0.018 mmol) and Na_2HPO_4 (200 mg, 1.4 mmol) in 3 mL of CH_2Cl_2. The reaction mixture was heated at reflux for 0.5 h, at which time GC analysis showed that the reaction was complete. Solids were removed by filtration through Celite,

(58) Bulletin 1069, Bio-Rad Laboratories: Richmond, CA, 1979.

(59) 3-Decyn-1-ol (Farchan) was hydrogenated[60] to (*Z*)-3-decen-1-ol by using hydrogen over Lindlar's catalyst in the presence of quinoline, with methanol as solvent. This homoallylic alcohol was converted to the corresponding ester as described in the text.

(60) Cram, D. J.; Allinger, N. L. *J. Am. Chem. Soc.* **1956**, *78*, 2518–2524.

(61) See: Posner, G. H. "An Introduction to Synthesis Using Organocopper Reagents"; Wiley: New York, 1980.

and the filtrate was concentrated in vacuo (0 °C), affording 2 mg of 1-[1-$^2H_1$]nonyl acetate. This ester, which had an identical GC retention time with that of authentic, unlabeled nonyl acetate, was hydrolyzed directly. ^1H NMR (unlabeled material) 0.89 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.7 (m, 14 H, CH_2), 2.03 (s, 3 H, $OCOCH_3$), 4.06 (t, J = 7 Hz, 2 H, CH_2OCOCH_3).

1-[1-$^2H_1$]Nonanol. A solution of 1-[1-$^2H_1$]nonyl acetate (2 mg, 0.011 mmol) in 1 mL of MeOH was stirred with 15 mg (0.38 mmol) of finely divided NaOH. Twelve hours later, the solvent was removed in vacuo, and Et_2O and water were added to the residue. Several extractions with Et_2O were followed by drying, filtration, and concentration of the organic phase. The clear oil that was obtained (1.4 mg) was esterified without further purification. ^1H NMR (unlabeled material) 0.89 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.9 (m, 14 H, CH_2), 1.8–2.1 (br s, 1 H, OH), 3.63 (t, J = 7 Hz, 2 H, CH_2OH).

1-[1-$^2H_1$]Nonyl Camphanate.[22] To a solution of the foregoing labeled alcohol (1.4 mg, 0.01 mmol) in 2 mL of dry pyridine was added 100 mg (0.035 mmol) of (−)-camphanyl chloride. After the reaction mixture had been stirred for 2 h, the pyridine was removed in vacuo, and Et_2O and water were added to the residue. The aqueous phase was extracted with Et_2O, and the organics were washed sequentially with 2 N HCl, 5% aqueous $NaHCO_3$, and brine. The organic solution was dried, filtered, and concentrated, giving a yellow oil, which was subjected to flash chromatography (6 in. × 10 mm; 19:1, petroleum ether/EtOAc), affording a small amount of labeled nonyl camphanate. ^1H NMR (CCl_4 solvent, spectrum of unlabeled material) 0.91 (t, partially obscured by signal at 0.94, J = 6 Hz, nonyl CH_3), 0.94 (s, 3 H, CH_3), 1.03 (s, 3 H, CH_3), 1.08 (s, 3 H, CH_3), 1.2–1.5 (m, 12 H, CH_2), 1.6–1.9 (m, 3 H), 1.9–2.2 (m, 2 H), 2.3–2.5 (m, 1 H), 4.18 (t, J = 6 Hz, 2 H, $COOCH_2$); ^2H NMR ($CHCl_3$) 4.16 (relative to $CDCl_3$, set to 7.2 ppm), shifted to 5.99 and 6.37 ppm in the presence of Eu(dpm)$_3$. The shifted methylene deuteron signals were of equal intensity (see Figure 4).

Cuprate Addition to (*Z*)-3-Decenoyl-NAC. Workup with N^2H_4Cl. Authentic (*Z*)-3-decenoyl-NAC (15 mg, 0.055 mmol) in 0.5 mL of dry Et_2O was added to 0.12 mmol of lithium dimethylcuprate, and the reaction was allowed to proceed as described above. The reaction was terminated by addition of a saturated solution of N^2H_4Cl in ^2H_2O, and workup as before gave 8 mg (80%) of the unsaturated ketone, as a clear oil. ^1H NMR (CCl_4) 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.2–1.5 (m, 8 H, C-7 to C-10 CH_2), 1.8–2.1 (m, partially obscured by signal at 2.06, total integral 5 H, CH_2CH_2CH=), 2.06 (s, overlapping the multiplet at 1.8–2.1, total integral 5 H, $COCH_3$), 3.0 (br t, J = 5 Hz, 0.8 H, =CHCHDCO), 5.2–5.6 (m, 2 H, CH=CH); ^2H NMR (CCl_4) 3.06 (s, =CHCHDCO).

[2,2-$^2H_2$]Decanoic Acid. Decanoic acid, stereorandomly deuterated at C-2, was prepared according to the procedure of Atkinson and coworkers.[27] A solution of 1.0 g of the potassium salt of decanoic acid, dissolved in 30 mL of ^2H_2O, was heated at 160 °C for 30 h in a sealed tube. After workup, ^1H NMR analysis showed that the extent of the exchange was only 20%.

(*S*)-Methyl α-[(1-Oxodecyl-2,2-$^2H_2$)oxy]benzeneacetate (Ester of [2,2-$^2H_2$]Decanoic Acid with the Methyl Ester of (*S*)-Mandelic Acid). The ester was made as described by Parker.[26] ^1H NMR (CCl_4) 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.8 (m, 14 H, CH_2), 2.41 (t, J = 7.5 Hz, 1.6 H, residual CH_2COO), 3.7 (s, 3 H, $COOCH_3$), 5.77 (s, 1 H, Ar CH(OCOR)$COOCH_3$), 7.36 (m, 5 H, Ar H); ^2H NMR (benzene) 2.17, 2.26 (singlets of equal intensity); IR (neat film) 3048, 3010, 2905, 2830, 1720, 1485, 1465, 1414, 1255, 1200, 1150, 1100, 1032, 964, 720, 682 cm^{-1}.

(*R*)-[2-$^2H_1$]Decanoic Acid. The route described by Battersby et al.[28] was used for conversion of 1-[2,2-$^2H_2$]nonanol (made in the same way as was 1-[2,2-$^2H_2$]heptanol, vide supra) into the title compound. The yield of labeled acid, obtained as a white crystalline solid (mp 31–2 °C), was 810 mg (37%, based on starting dideuterated alcohol). ^1H NMR (CCl_4) 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.8 (m, 14 H, CH_2), 2.31 (t, J = 7 Hz, 1 H, CHDCOOH), 11.2–11.6 (br s, 1 H, COOH); ^2H NMR (CCl_4) 2.32 (s, CHDCOOH); IR (KBr pellet) 2952, 2661, 1703, 1481, 1460, 1391, 1369, 1275, 1235, 1201, 1103, 928, 725 cm^{-1}.

[(*S*)-(*R,*S**)]-Methyl α-[(1-Oxodecyl-2-$^2H_1$)oxy]benzeneacetate (Ester of (*R*)-[2-$^2H_1$]Decanoic Acid with the Methyl Ester of (*S*)-Mandelic Acid).** The ester was prepared in the manner described by Parker.[26] Following flash chromatography (6 in. × 10 mm; 19:1, hexanes/EtOAc), 27 mg of product was obtained, as a colorless oil. ^1H NMR (CCl_4) 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.1–1.8 (m, 14 H, CH_2), 2.41 (t, J = 7.5 Hz, 1 H, CHDCOO), 3.7 (s, 3 H, $COOCH_3$), 5.77 (s, 1 H, Ar CH(OCOR)$COOCH_3$), 7.36 (m, 5 H, Ar H). ^2H NMR (benzene) 2.26 (s, *pro-R* CHDCOO), IR (neat film) 3050, 3010, 2910, 1722, 1484, 1257, 1206, 1153, 1104, 1035, 964, 721, 685 cm^{-1}.

Reduction of Chirally Labeled (*Z*)-3-[2-$^2H_1$]Decenoyl-NAC. To a 200-mL beaker equipped with a magnetic stir bar and a pH electrode, charged with 20 mL of 1 M $KHPO_4$ (pH 7.0) and 20 mL of THF and maintained at 10 °C, was added a THF solution of 5 mg of chirally labeled (*Z*)-3-[2-$^2H_1$]decenoyl-NAC (vide supra) and 10 mg of unlabeled (*Z*)-3-decenoyl-NAC. The solution was stirred vigorously, and 1.5 g of $NaBH_4$ was added in 100-mg portions, over a period of 30 min. The pH of the reaction mixture was maintained at 7.0 by periodic addition of 2 N HCl, and the temperature was not allowed to rise above 15 °C. When the addition of $NaBH_4$ was complete, the reaction mixture was warmed to room temperature, and its pH was adjusted to 3.0 by further addition of 2 N HCl. Addition of $(NH_4)_2SO_4$ to the point of saturation caused the mixture to separate into organic and aqueous phases, and the latter was extracted several times with Et_2O. The combined organics were dried, filtered, and concentrated, affording the alcohol as an oil, which was purified by flash chromatography (6 in. × 10 mm; 1:3, EtOAc/petroleum ether). The yield was 6 mg of a clear oil, found by GC analysis to be identical with authentic, unlabeled (*Z*)-3-decen-1-ol.

Reduction of Chirally Labeled (*Z*)-3-[2-$^2H_1$]Decen-1-ol. The title compound was reduced to 1-[2-$^2H_1$]decanol by homogeneous hydrogenation, in the presence of Wilkinson's catalyst.[29] The course of the reaction was followed conveniently by GC. When the reaction was complete, the mixture was filtered through a plug of silica gel, which was rinsed with a 4:1 mixture of petroleum ether/Et_2O. Concentration of the filtrate in vacuo gave an oily yellow residue (6 mg) shown by GC to be 1-decanol. This material was used without further purification.

Oxidation of Chirally Labeled 1-[2-$^2H_1$]Decanol. The labeled alcohol was oxidized to decanoic acid by the procedure of Sharpless.[30] When the color of the reaction mixture had changed from black to light green, an aliquot was removed for GC analysis, which showed complete converion to the acid, with no alcohol remaining. The bulk of the reaction mixture was concentrated, and the residue was taken up in water. Extraction with Et_2O gave a solution which was dried and then filtered through silica gel. Concentration of the filtrate in vacuo gave 7 mg of labeled decanoic acid, as a white solid. This was esterified without further purification.

Esterification of Chirally Labeled [2-$^2H_1$]Decanoic Acid to Methyl (*S*)-α-Hydroxybenzeneacetate (Methyl Mandelate). The methyl mandelate ester of the chirally labeled acid was made in the manner described by Parker.[26] After the reaction mixture had been stirred at −10 °C for 3 h, it was filtered through Celite, and the filtrate was concentrated. Flash chromatography of the residue (6 in. × 10 mm; 19:1, petroleum ether/EtOAc) gave 9 mg of ester, as a clear colorless oil. ^1H NMR (CCl_4) 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.05–1.9 (m, 14 H, CH_2), 2.41 (t, J = 8 Hz, 1 H, CHDCOO), 3.70 (s, 3 H, $COOCH_3$), 5.77 (s, 1 H, Ar CH(OCOR)$COOCH_3$) 7.36 (m, 5 H, Ar H); ^2H NMR (benzene, see Figure 5) 2.27 (s, *pro-R* CHDCOO).

Incubation of Unlabeled (*E*)-2-Decenoyl-NAC with Dehydrase in ^2H_2O Buffer. To 450 mL of ^2H_2O (at 37 °C) was added 871 mg (5 mmol) of K_2HPO_4 and 684 mg of KH_2PO_4. The apparent pH[21] of the solution was adjusted to 6.6 by addition of ca. 20 mg of KH_2PO_4. A 60-mL portion of freshly thawed dehydrase (1.15 g of protein, via Bio-Rad assay,[55] 90 units of isomerase activity per mg of protein) was transferred into the buffer. After the solution had equilibrated for 5 min under a nitrogen atmosphere, 500 mg (1.84 mmol) of (*E*)-2-decenoyl-NAC in 15 mL of dioxane was added dropwise, and the resulting cloudy mixture was stirred at 37 °C for 45 min. At this point, the mixture was cooled to 20 °C, and NaCl was added to the point of saturation. The salt solution was extracted with THF and Et_2O, and the organics were concentrated in vacuo. Water was added to the residue, which was again extracted with Et_2O. The Et_2O extracts were dried and concentrated, affording 540 mg of yellowish crystalline solid, from which chirally labeled [2-$^2H_1$]**3** was isolated and purified as described above (cf. the incubation of [2-$^2H_1$]**2** with dehydrase in ^1H_2O buffer). Ultimately, 24 mg of chirally labeled **3** was obtained, as a viscous, colorless oil.

Conversion of Chirally Labeled [2-$^2H_1$]3 (from the ^2H_2O Incubation) to the Decanoic Acid Ester of Methyl Mandelate. This sequence was performed as described for labeled **3** derived from incubation of [2-^2H]**2** with dehydrase in ^1H_2O, except that addition of unlabeled **3** (to serve as carrier) was unnecessary. The yield of labeled ester was 7 mg. ^1H NMR (CCl_4) 0.90 (t, J = 6 Hz, 3 H, CH_3), 1.05–1.9 (m, 14 H, CH_2), 2.41 (t, J = 8 Hz, 1 H, CHDCOO), 3.70 (s, 3 H, $COOCH_3$), 5.78 (s, 1 H, Ar CH(OCOR)$COOCH_3$), 7.36 (m, 5 H, Ar H); ^2H NMR (benzene, see Figure 5) 2.17 (s, ca. 0.7 D, *pro-S* CHDCOO), 2.27 (s, ca. 0.3 D, *pro-R* CHDCOO).

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Cytidylyl(3′-5′)guanosine Dinucleotides Give Two Platinum Chelates with *cis*-Diamminedichloroplatinum That Are Cytidine Syn-Anti Conformational Isomers

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Abstract: CpG and d(pCpG) react with *cis*-$[PtCl_2(NH_3)_2]$ (*cis*-DDP) or *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ (1 Pt per dinucleotide, 10^{-5}-(5×10^{-4}) M) in water at pH 5.5 to give as single adduct the CN3-GN7 chelate of the *cis*-$Pt(NH_3)_2^{2+}$ moiety. The nature of the ribo- and deoxy-(C-G)·*cis*-Pt chelates is established by atomic absorption spectroscopy, high-pressure gel permeation chromatography, and ^1H NMR. Reaction of CpG with [PtBr(dien)]Br and monitoring of its 10^{-2} M reaction with *cis*-DDP and its diaqua derivative show that the formation of the (CpG)·*cis*-Pt chelate is a two-step process starting with N7-platination of the guanine. The ribo- and deoxy-(G-C)·*cis*-Pt chelates exist as C(anti)-G(anti) and C(syn)-G(anti) isomers (respectively, ca. 20-80% at 20 °C) whose structures are established by ^1H NMR. When separated by HPLC, these two isomers slowly equilibrate at room temperature. The circular dichroism spectra of the two diastereoisomeric C(anti)-G(anti) and C(syn)-G(anti) chelates present a remarkable sign inversion, which can be related to the respective left- and right-handed pseudohelical arrangements of their sugar-phosphate backbones. For CpG the activation parameters of the C(anti)-G(anti) → C(syn)-G(anti) isomerization, determined from the evolution of the CD spectra at different temperatures between -9 and 18.5 °C, are $\Delta H^{\circ\ddagger}$ = 41 (6) kJ mol^{-1} and $\Delta S^{\circ\ddagger}$ = -130 (30) J mol^{-1} K^{-1}. From these values it is concluded that the equilibration process between the C(anti)-G(anti) and C(syn)-G(anti) platinum chelates is actually a conformational isomerization via the rotation of the cytosine about its glycosidic and N3-Pt bonds.

In the cell, DNA is considered as the primary target of the active aquated forms[2] of the antitumor drug *cis*-$[PtCl_2(NH_3)_2]$[3] (*cis*-DDP).[4] Studies with various DNAs have established that intrastrand cross-linking of two adjacent guanines is the major coordination fate of the *cis*-$Pt(NH_3)_2^{2+}$ moiety.[6-11] This is in agreement with model oligonucleotide studies which pointed to the facile GN7-GN7 platinum chelation by the GpG sequence to give only one complex with an anti-anti configuration.[12-16] In the case of DNA, there seems to be conflicting evidence about whether platinum first binds to all guanines with equal probability[17] or by a direct bifunctional attack at preferred sites.[11]

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(2) (a) Lim, M. C.; Martin, R. B. *J. Inorg. Nucl. Chem.* **1976**, *38*, 1911-1914 (b) Martin, R. B. "Platinum, Gold and Other Chemotherapeutic Agents"; Lippard, S. J., Ed.; American Chemical Society: Washington, DC, 1982; ACS Symp. Ser. No. 209, pp 231-244.

(3) (a) Roberts, J. J.; Thomson, A. J. *Prog. Nucleic Acid Res. Mol. Biol.* **1979**, *22*, 71-133. (b) Roberts, J. J.; Pera, M. F., Jr. "Platinum, Gold and Other Metal Chemotherapeutic Agents"; Lippard, S. J., Ed.; American Chemical Society: Washington, DC, 1982; ACS Symp. Ser. No. 209, pp 3-25.

(4) (a) Abbreviations: *cis*-DDP, *cis*-diamminedichloroplatinum(II); dien, diethylenetriamine; en, ethylenediamine; $TSPd_4$, sodium 3-(trimethylsilyl)-1-propionate-2,2,3,3-d_4; HPLC = high-pressure liquid chromatography; NOE = nuclear Overhauser effect; C and G represent cytidine and guanosine; d = deoxy; p to the left of a nucleoside symbol indicates a 5′-phosphate and to the right it indicates a 3′-phosphate; 5′-GMP = guanosine 5′-monophosphate. (b) Nomenclature: The common nomenclature for inorganic compounds is combined with that recommended by the IUPAC-IUB for nucleic acids,[5] i.e., the metal is listed first in formulas, followed by the ligands. The coordinating atom(s) of the ligand, in case of ambiguity, is (are) indicated in italics after the ligand, separated by a hyphen, according to ref 5, e.g., *cis*-$[Pt(NH_3)_2\{d(CpG)$-*N3,N7*$\}]^+$. Bridging ligands are indicated with the prefix μ, e.g., $[cis\text{-}Pt(NH_3)_2]_2\mu\text{-}\{CpG\text{-}N3,N7\}]_2^{2+}$. *cis*-Pt is used as an abbreviation for the *cis*-$Pt(NH_3)_2^{2+}$ moiety. Abbreviations used for the platinum dinucleotide complexes are CpG·*cis*-Pt, d(pCpG)·*cis*-Pt, and more generally (C-G)·*cis*-Pt for both of them.

(5) IUPAC-IUB Joint Commission on Biochemical Nomenclature, Recommendations, 1982; *Eur. J. Biochem.* **1983**, *131*, 9-15.

(6) Kelman, A. D.; Buchbinder, M. *Biochimie* **1978**, *60*, 893-899.

(7) Cohen, G. L.; Ledner, J. A.; Bauer, W. R.; Ushay, H. M.; Caravana, C.; Lippard, S. J. *J. Am. Chem. Soc.* **1980**, *102*, 2487-2488.

(8) Ushay, H. M.; Tullius, T. D.; Lippard, S. J. *Biochemistry* **1981**, *20*, 3744-3748.

(9) Tullius, T. D.; Lippard, S. J. *J. Am. Chem. Soc.* **1981**, *103*, 4620-4622.

(10) Fichtinger-Schepman, A.-M. J.; Lohman, P. H. M.; Reedijk, J. *Nucleic Acids Res.* **1982**, *10*, 5345-5356.

(11) Eastman, A. *Biochemistry* **1983**, *22*, 3927-3933.

(12) (a) Chottard, J.-C.; Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Mansuy, D. *Nouv. J. Chim.* **1978**, *2*, 551-553. (b) Chottard, J.-C.; Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Mansuy, D. *J. Am. Chem. Soc.* **1980**, *102*, 5565-5572.

(13) Marcelis, A. T. M.; Canters, G. W.; Reedijk, J. *Recl. Trav. Chim. Pays-Bas* **1981**, *100*, 391-392.

(14) Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Chottard, J.-C. *Biochemistry* **1982**, *21*, 1352-1356.

(15) Caradonna, J.-P.; Lippard, S. J.; Gait, M. J.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 5793-5795.

(16) (a) Girault, J.-P.; Chottard, J.-C.; Guittet, E. R.; Lallemand, J.-Y.; Huynh-Dinh, T.; Igolen, J. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 1157-1163. (b) Chottard, J.-C.; Girault, J.-P.; Guittet, E.; Lallemand, J.-Y.; Huynh-Dinh, T.; Igolen, J.; Neumann, J.; Tran-Dinh, S. Inorg. *Chim. Acta* **1983**, *79*, 249-250.

0002-7863/84/1506-7227$01.50/0