groups are protonated and not capable of coordinating to the $Ca^{2+}$ ion. In the crystals, the charge of the $Ca^{2+}$ ion is balanced by that of the halide ions. The $Ca(Glu)_2 \cdot 4H_2O$ crystals were also obtained by recrystallization of commercially available material from water solution,[10] and again the pH must have been relatively low. On the other hand, crystals of the four remaining complexes were obtained from solutions of pH 10 or higher, usually because free $Ca(OH)_2$ was involved in the preparation. At such high pH values, the $NH_2$ is not protonated and thus available for coordination to the calcium ion. It is these four complexes that indeed exhibit nitrogen coordination.

**Registry No.** $Ca(C_5H_8NO_3)_2 \cdot 5H_2O$, 97860-73-6.

**Supplementary Material Available:** Tables of parameters of the hydrogen atoms, thermal parameters of non-hydrogen atoms, least-squares planes, and observed and calculated structure factors (13 pages). Ordering information is given on any current masthead page.

# Stereochemical Course of an Enzyme-Catalyzed Allene–Acetylene Isomerization

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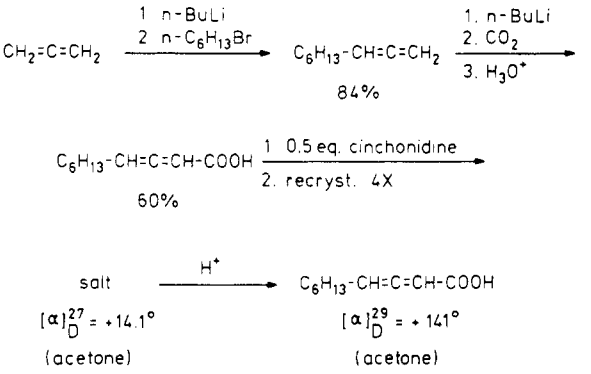
**Abstract:** An allene–acetylene isomerase (AAI) isolated from hog liver interconverts the *N*-acetylcysteamine thio ester of 3-decynoic acid (3-decynoyl-NAC) and (+)-2,3-decadienoyl-NAC. It is thus far the only enzyme that has been isolated that produces (or utilizes) an allenic compound and that is not inactivated by that same allene. Although physical and kinetic characteristics of the enzyme have previously been obtained, details of its mechanism of action remain unknown. The stereochemical course of the AAI-catalyzed propargylic rearrangement has now been determined, by synthesis of 2,3-[2-$^2$H]- and 2,3-[4-$^2$H]decadienoyl-NAC and enzymatic conversion of these substrates to 3-[2-$^2$H$_1$]decynoyl-NAC. Derivatization of the chirally labeled acetylenes, followed by $^2$H NMR analysis, has shown that protonation occurs on the *si* face at C-2 of the allene. X-ray crystallographic analysis of a derivative has revealed that (+)-2,3-decadienoic acid possesses the *S* configuration. The enzyme-mediated propargylic rearrangement is therefore a suprafacial process, apparently involving a single active-site base.

Although nature elaborates a substantial variety of allenic natural products,[2] the enzymology of allene biosynthesis is virtually unexplored. To date, knowledge of biological allene formation is confined exclusively to those allenes that are formed transiently from acetylenic "suicide" substrates.[3]

In 1975 Miesowicz and Bloch reported[4] the isolation from hog liver of an enzyme capable of converting the (2-mercaptoethyl)amine (*N*-acetylcysteamine; NAC) thio ester of 3-decynoic acid (3-decynoyl-NAC) into the corresponding allenic thio ester, 2,3-decadienoyl-NAC. This is precisely the process that transpires in the course of the mechanism-based ("suicide") inactivation of β-hydroxydecanoylthioester dehydrase.[5-9] Dehydrase is inactivated almost instantaneously by 2,3-decadienoyl-NAC;[6] remarkably, the allene–acetylene isomerase (AAI) is unaffected by this allenic thio ester. Moreover, the allene is the predominant species at equilibrium. AAI is thus far the only enzyme that has been isolated that produces (or utilizes) an allenic compound and that is not inactivated by that same allene.

AAI's physical properties and substrate specificity have been rigorously characterized.[10] However, understanding of its mechanism of action has been hampered owing to the enzyme's failure to react in a specific manner with any of a number of reagents.[11]

Scheme I. Synthesis and Resolution of 2,3-Decadienoic Acid




We now report the results of experiments demonstrating that the AAI-catalyzed propargylic rearrangement is a suprafacial process, thus implicating a single active-site base.[12,13] In the course of these studies the absolute configurations of the allenic inactivators of β-hydroxydecanoylthioester dehydrase have also been determined.[7]

## Results

Elucidation of the overall steric course of the AAI-catalyzed propargylic rearrangement required determination of (a) the

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(2) Robinson, C. H.; Covey, D. F. In "The Chemistry of Ketenes, Allenes, and Related Compounds"; Patai, S., Ed.; Wiley: Chichester, England, 1980; Part 1, pp 451-485.
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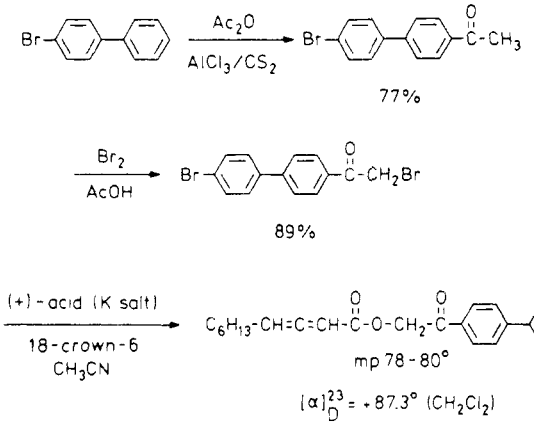
Scheme II. Derivatization of (+)-2,3-Decadienoic Acid




absolute configuration of the allene enantiomer that is turned over by the enzyme and (b) which enantiotopic hydrogen is removed from C-2 of 3-decynoyl-NAC (or, conversely, which hydrogen at C-2 of 3-decynoyl-NAC was originally at C-2 of 2,3-decadienoyl-NAC and which has come either from C-4 of the allene or from the medium).

**Synthesis and Resolution of 2,3-Decadienoic Acid.** The desired enzymatically active allene enantiomer had previously[10,11] been obtained from AAI-catalyzed equilibration of 3-decynoyl-NAC and 2,3-decadienoyl-NAC, though complete separation of these isomeric thio esters from one another was achieved only with difficulty.[11] More importantly, determination of the configuration of purified 2,3-decadienoyl-NAC itself was not expected to be straightforward. On the other hand, Morisaki had previously[14] resolved 2,3-decadienoic acid by fractional crystallization of its amphetamine salt. It therefore seemed most expedient to determine the configuration of the allene by X-ray crystallographic analysis of a salt made from a chiral amine and a single enantiomer of the acid.

Racemic 2,3-decadienoic acid was synthesized from 1,2-propadiene by the route portrayed in Scheme I.[15-19] After purification by low-temperature recrystallization, the acid was added to one-half molar equivalent of (−)-cinchonidine, and the resulting salt was crystallized 4 times from acetone. At this point the rotation of the salt was constant and maximal, and acidification afforded (+)-2,3-decadienoic acid with a rotation essentially identical with that which had been reported by Morisaki and Bloch.[14]

The cinchonidine salt, a powder, could not be made to crystallize in a form suitable for X-ray crystallography. Accordingly, a heavy-atom derivative was sought, facilitating determination of the absolute configuration by the anomalous scattering technique. Unfortunately, while the *p*-bromophenacyl ester of (±)-2,3-decadienoic acid melted at 43 °C, the corresponding derivative of the dextrorotatory acid melted at only 34 °C. Neither ester afforded single crystals of appropriate dimensions.

A suitable solution (Scheme II) was provided through the use of a modified derivatizing reagent, 4-(4′-bromophenyl)phenacyl bromide (2-bromo-1-(4′-bromo-1,1′-biphenyl-4-yl)ethanone). This substance was readily obtained from biphenyl by Friedel–Crafts acetylation, followed by bromination. Reaction of (+)-2,3-decadienoic acid with 4-(4′-bromophenyl)phenacyl bromide under phase-transfer conditions[20] provided the requisite (dextrorotatory) ester, with a melting point of 78–80 °C. Moreover, the ester readily produced large single crystals.

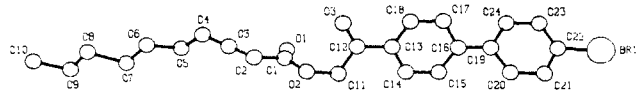


**Figure 1.** Computer-generated perspective drawing of the final X-ray model of 1-oxo-1-(4′-bromo-1,1′-biphenyl-4-yl)eth-2-yl (+)-2,3-decadienoate (4-(4′-bromophenyl)phenacyl (+)-2,3-decadienoate). Hydrogens are omitted for clarity. The absolute configuration was determined using anomalous dispersion effects of the bromine atom.

X-ray crystallographic analysis of 4-(4′-bromophenyl)phenacyl (+)-2,3-decadienoate afforded the structure portrayed in Figure 1. (A detailed description of the structure determination has already been published,[7] and the data have been deposited with the Cambridge Crystallographic Data Centre.) Clearly, the allene bears the *S* configuration.[46,47]

Although only the configuration of the acid, and not that of the enzymatically active thio ester, has been determined, the latter almost certainly has the *S* configuration as well (vide infra). Both the dextrorotatory acid and thio ester inactivate β-hydroxydecanoylthioester dehydrase far more effectively than do the corresponding racemates.[10,14] Furthermore, the empirical rules formulated by Lowe[21] and Brewster[22] predict the *S* configuration for each of these dextrorotatory allenes.

**Synthesis of Labeled 2,3-Decadienoyl-NAC.** Samples of 2,3-[2-$^2$H]- and 2,3-[4-$^2$H]decadienoyl-NAC were synthesized as shown in Scheme III. Reaction[23] of heptanoyl chloride with bis(trimethylsilyl)acetylene in the presence of aluminum chloride, followed by desilylation,[24] gave 1-nonyn-3-one. The ketone was reduced[25] to the propargylic alcohol, which was reacted with triphenoxyphosphonium methiodide,[26] affording 1-iodo-1,2-nonadiene. Sequential treatment of the latter with *n*-butyllithium and carbon dioxide[15,27] gave 2,3-decadienoic acid (along with a substantial quantity of 2-ethynyloctanoic acid, formed by rearrangement of the allenic carbanion). At this point, an improved thioesterification procedure was devised, based on Rosenquist and Chapman's method for preparation of diazomethyl allenyl ketones.[28] Thus, the allenic acid was hydrobrominated, using hydrogen bromide in ether, giving 3-bromo-3-decenoic acid. The latter was treated first with oxalyl chloride, and then with the thallium(I) derivative of *N*-acetylcysteamine, affording the protected thio ester. Finally, dehydrobromination provided 2,3-decadienoyl-NAC. The desired deuterium substitution was effected by exchange of the acetylenic proton of 1-nonyn-3-ol (leading to 2,3-[2-$^2$H]decadienoyl-NAC) and by reduction of 1-nonyn-3-one with lithium borodeuteride (giving 2,3-[4-$^2$H]decadienoyl-NAC).

**Preparation of AAI and Incubation of Labeled Substrates.** AAI was prepared from fresh hog liver, by the procedure of Miesowicz.[10,11] Enzyme purified through the first DEAE cellulose chromatography step converted 5.6 μmol of 3-decynoyl-NAC into 2,3-decadienoyl-NAC per minute per milligram of protein. This degree of purity was judged sufficient for our purposes.

As a control, a spectrophotometric assay was carried out with 2,3-decadienoyl-NAC as substrate, and under the conditions used, equilibrium was achieved in 1–2 min. In preparative-scale incubations, the proportion of enzyme to allenic substrate was twice that which had been used in the spectrophotometric assay. These reactions were allowed to proceed for 2–4 min, affording a maximal yield of the rearranged acetylenic product. Short reaction times were crucial to the success of these experiments, as Miesowicz had observed[10,11] that the C-2 protons of 3-decynoyl-NAC are completely exchanged after 30 min in $^2H_2O$. (There was,

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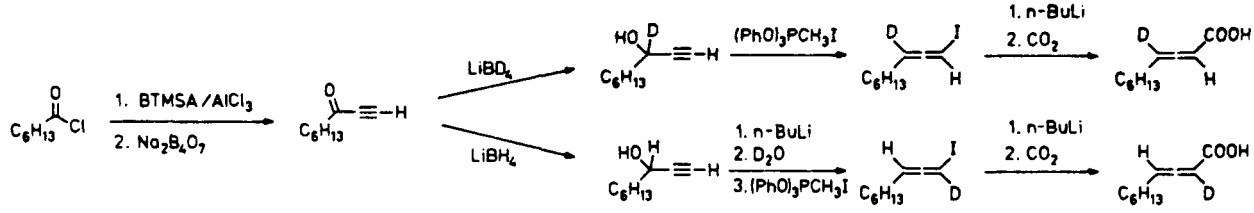
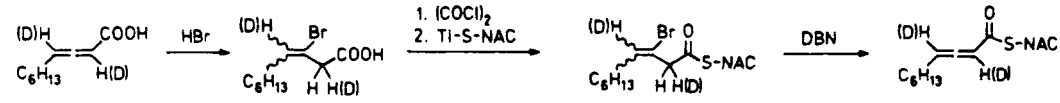
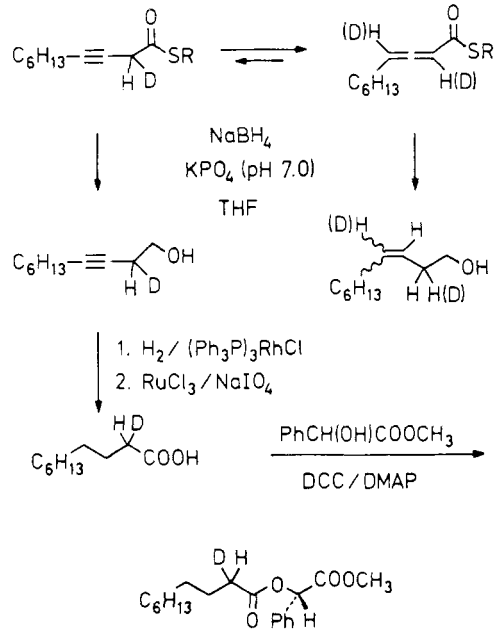
Scheme III. Synthesis of Specifically Labeled 2,3-Decadienoyl-NAC




Scheme IV. Workup of AAI Incubations and Derivatization of Labeled Products




however, less than 5% exchange after 1 min.)

**Derivatization and Analysis of Labeled Acetylenic Thio Esters.** Incubation workups, derivatization of products, and analysis of the chirality of labeling closely paralleled the procedures that were employed for the determination of the steric course at carbon 2 in the allylic rearrangement catalyzed by β-hydroxydecanoyl-thioester dehydrase[8,29] (see Scheme IV). Thus, incubations of labeled allenic thio esters with AAI were terminated reductively, by addition of potassium phosphate buffer (pH 7.0), tetrahydrofuran, and then a large excess of sodium borohydride. Under these conditions, 3-decynoyl-NAC and 2,3-decadienoyl-NAC were reduced quickly to 3-decyn-1-ol and 3-decen-1-ol, respectively. (The latter compound arises through initial 1,4-attack of hydride on the allenic thio ester.) As a control, a mixture of 3-decynoyl-NAC and 2,3-decadienoyl-NAC was dissolved in THF/$^2H_2O$/$KPO_4$ (pH 7.0), and excess $NaBH_4$ was added. (The pH was maintained at 7.0 by addition of 2 N HCl.) Following purification, the resulting alcohols were examined by proton and deuterium NMR spectroscopy and were found not to have suffered any detectable incorporation of deuterium.

Following chromatographic purification, each sample of 3-[2-$^2H_1$]decyn-1-ol was subjected to homogeneous hydrogenation,[30]

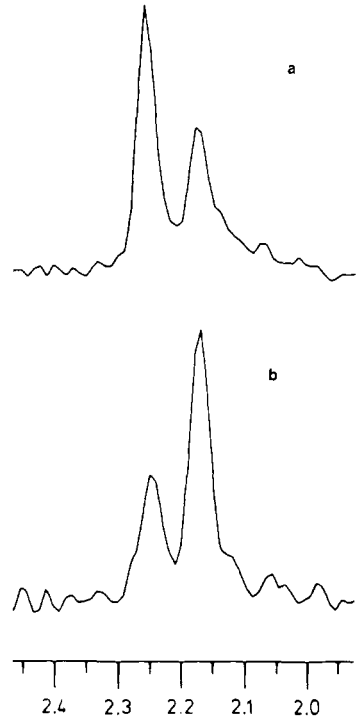



**Figure 2.** Deuterium NMR spectra (at 76.77 MHz) of mandelic acid diesters derived from incubation of (a) 2,3-[2-$^2$H]decadienoyl-NAC and (b) 2,3-[4-$^2$H]decadienoyl-NAC with AAI. The spectra are resolution enhanced, with exponential and Gaussian multiplication factors of −2.000 and 0.200 Hz, respectively. Chemical shifts are referenced to deuteriobenzene (7.15 ppm) at natural abundance in the solvent ($C_6H_6$).

and the resulting 1-[2-$^2H_1$]decanol was oxidized[31] to labeled decanoic acid. Previous studies[32] had shown that the diastereotopic C-2 protons of carboxylic acids (including decanoic acid[7,29]) can be differentiated by NMR spectroscopy performed on the corresponding methyl (*S*)-mandelate diester derivatives. In every diester of this type thus examined, the *pro*-2*R* proton has been found to resonate at lower field (by ca. 0.1 ppm) than does the *pro*-2*S* proton. Accordingly, each sample of labeled decanoic acid was esterified[32] to (*S*)-α-hydroxybenzeneacetic acid (methyl mandelate), and the diesters were examined by deuterium NMR spectroscopy, at 76.77 MHz. The upper and lower spectra in Figure 2 are of the decanoyl C-2 region of diester derived from incubation of 2,3-[2-$^2$H]- and 2,3-[4-$^2$H]decadienoyl-NAC, respectively, with AAI. Clearly, the predominant site of deuterium substitution is the *pro*-2*R* position in the upper spectrum and the *pro*-2*S* position in the lower spectrum. It is therefore clear that

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(30) Osborn, J. A.; Jardine, F. H.; Young, J. F.; Wilkinson, G. *J. Chem. Soc. A* **1966**, 1711–1732.
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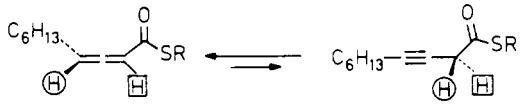
proton or deuteron transfer in the course of the AAI-catalyzed propargylic rearrangement has been to the *si* face at C-2. It is also clear that some epimerization of label has taken place. This was not unexpected, owing to the extraordinary acidity of the C-2 protons of 3-decynoyl-NAC.[11]

## Discussion

Since the absolute configuration of the dextrorotatory enantiomer of 2,3-decadienoyl-NAC (i.e., the enantiomer acted upon by AAI) had already been shown to be *S*, the fact that this allene is protonated on the *si* face at C-2 leads to the conclusion that *the AAI-catalyzed propargylic rearrangement is a suprafacial process, apparently mediated by a single active-site base.*[12,13]



For several reasons the stereochemical outcome is not unexpected. First, it is consistent with the stereochemical courses of all enzyme-catalyzed allylic (and aza-allylic) rearrangements that ostensibly involve carbanion intermediates.[8] Second, Miesowicz and Bloch previously demonstrated that the AAI-catalyzed proton transfer is largely intramolecular,[10,11] a fact that we have exploited in our experimental design. This observation in itself is suggestive of a single-base mechanism. If there were two active-site bases, there would have to be transfer of the substrate proton from one base to the other. This scenario is possible, albeit mechanistically cumbersome.

The fact that in short-term incubations there is exchange of only $^1/_3$–$^1/_2$ of a proton[11] indicates that the active-site base must be monoprotic (i.e., it cannot be lysine or arginine). The most likely candidates are aspartate, glutamate, and histidine, although cysteine, and tyrosine, with their higher p$K_a$ values, merit consideration.

The specific reason that 2,3-decadienoyl-NAC fails to inactivate AAI remains unknown. There is copious precedent in the literature[2,3] for the inactivation of a wide range of enzymes by allenic substrate analogues and reaction intermediates. In many cases, the enzyme inactivation is believed to be caused by the attack of an active-site nucleophile on a particularly electrophilic allene. Conversely, it has been suggested[2,3] that some flavoproteins may suffer inactivation via attack of an enzyme-generated allenic anion on the oxidized cofactor. In any case, the number of enzymes that will function successfully with an allenic substrate is miniscule compared to those that are inactivated by allenic substrate analogues.

In addition to AAI, other allene-resistant enzymes include bovine crotonase[33] and (to an extent) $\Delta^5$-3-ketosteroid isomerase, from *Pseudomonas testosteroni*.[34,35] Crotonase does not lose crotonyl-CoA hydratase activity following preincubation with a large excess of 2,3-decadienoyl-NAC. In fact, the enzyme apparently hydrates the allenic thio ester to 3-oxodecanoyl-NAC.

The steroid isomerase, on the other hand, suffers complete loss of activity upon incubation with certain acetylenic secosteroids. The mode of inactivation involves propargylic rearrangement of a $\beta,\gamma$-acetylenic ketone function to the corresponding conjugated allene. The allene undergoes attack at the sp-hybridized carbon atom by an enzyme nucleophile, an asparagine residue.[36,37] Interestingly, the fit between the enzyme and the secosteroid is apparently rather loose. Thus, the allene is more often than not released into solution, and, remarkably, the allenic product is a mixture of isomers that differ in the configuration of the allenic linkage.

Although both AAI and the steroid isomerase equilibrate acetylenes with the isomeric allenes, AAI is unaffected by its product, whereas the steroid isomerase ultimately is completely and irreversibly inactivated.

Unfortunately, it is not possible to comment on the enantiomeric purity of 2,3-decadienoyl-NAC produced by AAI. While Miesowcz and Bloch reported[10] a specific rotation of +52° for the AAI product, the purity of this material evidently was not determined. Both Morisaki and Bloch[14] and we[7] have obtained enantiomerically pure (+)-2,3-decadienoic acid. The specific rotation of the chemically derived NAC thio ester, however, is only +5.88°. This low value is apparently attributable to epimerization during the thioesterification process. Neverthless, (a) the synthetic allenic thio ester's positive (clearly nonzero) rotation, (b) the fact that it was derived from an allenic acid with the *S* configuration, and (c) the fact that the Miesowicz thio ester is dextrorotatory strongly suggest that the enzymatically active thio ester also has the *S* configuration.

The detailed mechanism of action of AAI as well as the reason that the enzyme fails to react with 2,3-decadienoyl-NAC remain obscure. As noted above, despite numerous attempts, Miesowicz failed to observe specificity in reactions between AAI and active-site-directed reagents. Apparently the active-site Lewis base that facilitates the propargylic rearrangement is not a particularly potent nucleophile or it is sterically indisposed toward attacking the allene. It is worth noting in this connection that in model studies we have observed adduct formation between an allenic thioester and *N*-acetyltyrosine methyl ester *only* after prior treatment of the tyrosine derivative with sodium hydride.[38]

As mentioned previously, the biosynthesis of naturally occurring allenes is virtually unexplored. Interestingly, in most allenic natural products, the allene linkage is either completely isolated or is conjugated only to a carbon–carbon double bond, and not a carbonyl group. Nevertheless, the existence and function of AAI suggest that allenes may arise via isomerization of homopropargylic carbonyl precursors, with subsequent additional elaboration of the carbon skeleton and functionality.

## Experimental Section

**General.** NMR spectra were obtained on a JEOL FX-90Q(II) ($^1$H,$^2$H), a Varian EM-360A ($^1$H), and a Bruker WM-500 ($^2$H). Unless otherwise stated, 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). Perkin-Elmer Model 257 and 298 spectrophotometers were 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 on Chromosorb W (AW-DMCS). Optical rotations were measured with a Rudolph Autopol II digital polarimeter. Analytical silica gel TLC plates and silica gel for flash chromatography[39] 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 were performed on a component system which included an Altex 110A pump, a Rheodyne 7125 injector, and an ISCO 1840 absorbance monitor. A 10-$\mu$m 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-$\mu$m silica gel by Alltech and by Altex.

All chemicals used were of reagent grade. Methylene chloride was distilled from $P_2O_5$ and stored over 3A molecular sieves. Tetrahydrofuran was routinely distilled from $LiAlH_4$ immediately before use. Pyridine and triethylamine were stirred over KOH overnight, distilled from BaO, and stored over 3A molecular sieves. HMPT was stirred over $CaH_2$ overnight and then distilled at reduced pressure immediately before use. Isotopically labeled compounds were purchased from Aldrich or from Alfa. Standardization of *n*-BuLi was by the method of Winkle et al.[40]

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Unless otherwise noted, anhydrous $MgSO_4$ was used for drying organic extracts, and, following filtration, samples were concentrated via rotary evaporation (using a water aspirator), with gentle warming.

**1,2-Nonadiene.** The title compound was prepared from propadiene (5.0 g; 125 mmol) by the method of Linstrumelle,[16] as described by Brandsma.[19] The product, a pale yellow oil (10.33 g; 84%), was used without further purification (GC analysis showed it to be 95% pure): $^1$H NMR $\delta$ 0.9 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.5 (m, 8 H, $CH_2$), 1.6–2.2 (m, 2 H, $CH_2CH$=), 4.5–5.3 (m, 3 H, CH=C=$CH_2$); IR (neat film) 2940, 2850, 1950, 1450, 1250, 710, 620 cm$^{-1}$.

**2,3-Decadienoic Acid.** 1,2-Nonadiene (3.0 g; 24.2 mmol) was converted to the title compound by the procedure of Clinet and Linstrumelle,[15] as modified by Brandsma.[19] The crude yellow oil was recrystallized from petroleum ether at −80 °C, leading to the isolation of 3.14 g (77%) of pure acid: $^1$H NMR $\delta$ 0.9 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.7 (m, 8 H, $CH_2$), 1.9–2.3 (m, 2 H, $CH_2CH$=), 5.5–5.9 (m, 2 H, CH=C=CH), 10.4 (br s, 1 H, COOH); IR (neat film) 3200, 2960, 2850, 2630, 1950, 1700, 1440, 1280, 1180, 920, 830, 700 cm$^{-1}$.

**Resolution of 2,3-Decadienoic Acid.** A hot ethanolic solution (20 mL) of (−)-cinchonidine (3.4 g; 11.55 mmol) was added to a solution of 2,3-decadienoic acid (3.88 g; 23.1 mmol) in 5 mL of EtOH, and the mixture was allowed to stand in the dark for 30 min. After this time the solvent was removed in vacuo, and the resulting light brown solid was fractionally crystallized from acetone 4 times, until the optical rotation of the salt had become constant ($[\alpha]^{27}_D$ +14.1°, acetone). The diastereomerically pure salt was decomposed by partitioning between 4% aqueous HCl and ether. The ether-soluble fraction was washed first with 4% HCl and then with saturated aqueous NaCl. Drying over $MgSO_4$ and evaporation of solvent afforded (+)-2,3-decadienoic acid (142 mg) ($[\alpha]^{29}_D$ +141°, acetone; the published[14] rotation, measured under unspecified conditions, was +145°).

**1-Oxo-1-(4′-bromophenyl)eth-2-yl 2,3-Decadienoate (4′-Bromophenacyl 2,3-Decadienoate).** 2,3-Decadienoic acid was reacted with 4-bromophenacyl bromide under phase-transfer conditions.[20] The melting points of the esters derived from racemic and dextrorotatory acid were 43 and 34 °C, respectively. $^1$H NMR $\delta$ 0.85 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.5 (m, 8 H, $CH_2$), 1.9–2.3 (m, 2 H, $CH_2CH$=), 5.3 (s, 2 H, $OCCH_2CO$), 5.6–5.8 (m, 2 H, CH=C=CH), 7.5–7.9 (m, 4 H, Ar H).

**1-(4′-Bromo-1,1′-biphenyl-4-yl)ethanone.**[41-43] A solution of 5 g (21.5 mmol) of 4′-bromo-1,1′-biphenyl and 6.43 g (48.3 mmol) of $AlCl_3$ in 20 mL of $CS_2$ (freshly distilled from $P_2O_5$) was heated to a gentle reflex, and acetic anhydride (21.5 mmol) was added to this solution over a period of 30 min. After an additional 1 h at reflux, the reaction mixture was cooled to room temperature and then poured into ice-cold dilute HCl. The resulting tan precipitate (mp 110–112 °C; 4.57 g; 77%) was collected by filtration and dried in vacuo over $P_2O_5$. A portion of this material was recrystallized from absolute EtOH, affording flakelike tan crystals, mp 131–133 °C (lit. 131,[41] 129–130 °C[44]): $^1$H NMR $\delta$ 2.63 (s, 3 H, $CH_3$), 7.4–7.7 (m, 6 H, Ar H), 8.03 (d, $J$ = 9 Hz, 2 H, C-3′,5′ H); IR (KBr pellet) 1680, 1602, 1480, 1430, 1375, 1360, 1265, 1070, 1000, 958, 810, 768, 598 cm$^{-1}$.

**2-Bromo-1-(4′-bromo-1,1′-biphenyl-4-yl)ethanone.** The substituted acetophenone (mp 110–112 °C) was brominated by standard procedures,[43] using bromine in glacial acetic acid. From 4.5 g (16.3 mmol) of starting material was obtained 5.03 g (14.2 mmol; 87%) of brominated ketone, as a pale yellow powder (mp 135–138 °C). Recrystallization of a portion of the crude product from absolute EtOH gave a white powder, mp 138–140 °C. $^1$H NMR $\delta$ 4.47 (s, 2 H, $CH_2Br$), 7.4–7.75 (m, 6 H, Ar H), 8.07 (d, $J$ = 8.5 Hz, 2 H, C-3′,5′ H); IR (KBr pellet) 2950, 1690, 1601, 1550, 1480, 1450, 1430, 1375, 1270, 1190, 1070, 1000, 806, 680, 625 cm$^{-1}$. Anal. Calcd for $C_{14}H_{10}Br_2O$: C, 47.5; H, 2.85; Br, 45.14. Found: C, 47.29; H, 2.85; Br, 44.87.

**1-Oxo-1-(4′-bromo-1,1′-biphenyl-4-yl)ethan-2-yl (+)-2,3-Decadienoate (4-(4′-Bromophenyl)phenacyl (+)-2,3-Decadienoate).**[20] To a solution of 142 mg (0.84 mmol) of (+)-2,3-decadienoic acid in 5 mL of THF was added 85 mg (0.84 mmol) of $KHCO_3$, dissolved in a small amount of water. The potassium salt separated immediately, as a white powder. After removal of the volatile components at reduced pressure, the salt, dissolved in 35 mL of $CH_3CN$, was combined with 330 mg (0.93 mmol) of 2-bromo-1-(4′-bromo-1,1′-biphenyl-4-yl)ethanone and 90 mg (0.34 mmol) of 18-crown-6. The resulting solution was stirred overnight. After workup and chromatographic purification, 283 mg (76%) of the ester was obtained, as small white crystals. Recrystallization from ether afforded colorless prisms, mp 78–80 °C. $^1$H NMR $\delta$ 0.88 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.6 (m, 8 H, $CH_2$), 2.0–2.3 (m, 2 H, $CH_2C$=), 5.39 (s, 2 H, $OCH_2CO$), 5.6–5.8 (m, 2 H, CH=C=CH), 7.4–7.8 (m, 6 H, Ar H), 7.99 (d, $J$ = 8 Hz, C-3′,5′ H); IR (KBr pellet) 2910, 2850, 1950, 1690, 1604, 1475, 1450, 1360, 1245, 1178, 1078, 1008, 978, 800, 708, 560, 525 cm$^{-1}$.

**(+)-2,3-Decadienethioic Acid (*S*)-2-(Acetylamino)ethyl Ester ((+)-2,3-Decadienoyl-NAC).** (+)-2,3-Decadienoic acid (147 mg; 0.875 mmol) was dissolved in 6 mL of dry $CH_2Cl_2$ and cooled to −20 °C. $Et_3N$ (88.5 mg; 0.875 mmol) and 186 mg (0.875 mmol) of $PhOPOCl_2$ were then added via syringe, with stirring. After 15 min, the cooling bath was removed, and the resulting mixture was stirred at room temperature for another 50 min. At this time 10 mL of dry $Et_2O$ was added via syringe, causing the immediate formation of a white precipitate. The mixture was filtered through a small pad of Celite into an oven-dried 100-mL round-bottomed flask, and the solvent was removed at reduced pressure. The resulting mixed anhydride, under a blanket of nitrogen, was dissolved in 16 mL of dry, degassed THF and cooled in an ice bath. A suspension of the thallium(I) derivative of *N*-acetylcysteamine[8] (9.0 mL of a 0.097 M solution in THF; 0.875 mmol) was added via syringe, and the resulting suspension was stirred at ice bath temperature for 2 h. During this 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 gave a pale yellow oil (68 mg) which was purified first by flash chromatography (6 in. × 30 mm; 6:1, $CH_2Cl_2$/acetone) and then by HPLC (7:1, $CH_2Cl_2/CH_3CN$; 5 mL/min; 10 × 250 mm silica gel column; effluent monitored at 263 nm). The product was a colorless oil (8.2 mg; 3.5%), $[\alpha]^{23}_D$ +5.88° ($CH_2Cl_2$; lit.[10] $[\alpha]^{25}_D$ +52° ($CH_2Cl_2$)): $^1$H NMR $\delta$ 0.85 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.6 (m, 8 H, $CH_2$), 1.95 (s, 3 H, $CH_3CO$), 2.0–2.4 (m, 2 H, $CH_2CH$=), 3.0 (t, $J$ = 6 Hz, 2 H, $CH_2S$), 3.4 (d of t, $J$ = 6, 6 Hz, 2 H, $CH_2NH$), 5.6–5.9 (m,3 H, CH=C=CH and $CH_2NH$); IR ($CHCl_3$) 3300, 3000, 2920, 2850, 1930, 1650, 1500, 1180, 750, 660 cm$^{-1}$.

**1-(Trimethylsilyl)nonyn-3-one.** To a flame-dried 250-mL round-bottomed flask was added a solution of heptanoyl chloride (9.21 g, 62 mmol) and bis(trimethylsilyl)acetylene (11.48 g, 67 mmol, Aldrich) in 80 mL of $CH_2Cl_2$. The mixture was stirred and cooled to 0 °C, and then 8.5 g (63.5 mmol) of anhydrous $AlCl_3$ was added to the mixture in one portion. The solution immediately turned from colorless to brown. After 3 min the ice bath was removed, and the reaction mixture was stirred for 2 h at room temperature. The dark brown solution was then poured into 250 mL of ice water, and the resulting mixture was extracted several times with $Et_2O$. After drying of the extracts and removal of solvent, 13.0 g (100%) of a light brown oil was obtained. GC analysis showed the product to be 99.6% pure: $^1$H NMR $\delta$ 0.2 (s, 9 H, $SiMe_3$), 0.9 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.4 (m, 6 H, $CH_2$), 1.5–1.8 (m, 2 H, $CH_2CH_2CO$), 2.55 (t, $J$ = 6 Hz, 2 H, $CH_2CH_2CO$); IR (neat film) 3340, 2930, 2850, 2160, 1680, 1470, 1460, 1410, 1250, 1225, 1130, 1090, 860, 770 cm$^{-1}$.

**1-Nonyn-3-one.** 1-(Trimethylsilyl)nonyn-3-one (13 g, 61.9 mmol) was desilylated by the procedure of Walton and Waugh.[24] The crude product was purified by bulb to bulb distillation under vacuum, providing 7.76 g (90.5%) of a clear colorless oil: $^1$H NMR $\delta$ 0.9 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.4 (m, 6 H, $CH_2$), 1.5–1.9 (m, 2 H, $CH_2CH_2CO$), 2.6 (t, $J$ = 6.5 Hz, 2 H, $CH_2CO$) 3.2 (s, 1 H, C≡CH); IR (neat film) 3260, 2920, 2860, 2100, 1680, 1470, 1460, 1140, 1090, 850 cm$^{-1}$.

**1-Nonyn-3-ol.** 1-Nonyn-3-one (3.58 g; 25.9 mmol) was reduced by the method of Cornforth,[25] using $LiBH_4$ (665 mg; 29.2 mmol). The product, 3.10 g (85.6%) of a pale yellow liquid, was shown by GC analysis to be mostly (95%) the desired alcohol. This material was used without further purification. $^1$H NMR $\delta$ 0.9 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.4 (m, 8 H, $CH_2$), 1.5–1.9 (m, 2 H, $CH_2CHOH$), 2.45 (d, $J$ = 2 Hz, 1 H, C≡CH), 2.55 (br s, 1 H, OH), 4.4 (br t, $J$ = 6 Hz, 1 H, $CH_2CHOH$); IR (neat film) 3400, 3300, 2930, 2860, 1730, 1470, 1130 cm$^{-1}$.

**1-[1-$^2$H]Nonyn-3-ol.** *n*-BuLi (17.3 mL of a 1.6 M solution in hexane; 27.7 mmol) was added dropwise, from a syringe, to a stirred, ice-cold solution of 3.10 g (22.2 mmol) of 1-nonyn-3-ol in dry THF (90 mL). The reaction was conducted under an atmosphere of dry $N_2$. When the addition was complete, the ice bath was removed, and the mixture was stirred for 1 h at room temperature. $^2H_2O$ (20 mL) was then added dropwise, from a syringe, to the reaction mixture. The color of the solution changed to milky white and then back to light yellow. After 30 min, $NH_4Cl$ was added, to the point of saturation, and the mixture was extracted with $Et_2O$ several times. Workup gave 3.12 g of a light yellow liquid (ca. 100%): $^1$H NMR 0.9 (t, $J$ = 5.5 Hz, 3 H, $CH_3$), 1.1–1.4 (m,

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8 H, $CH_2$), 1.6–1.9 (m, 2 H, $CH_2CHOH$), 2.2 (br s, 1 H, OH), 4.35 (t, $J$ = 6.2 Hz, 1 H, $CHOH$); $^2H$ NMR $\delta$ ($CCl_4$, 50 °C) 2.3 (C≡CD); IR (neat film) 3400, 2920, 2860, 2600, 2000, 1640, 1470, 1460, 1040 $cm^{-1}$.

**1-[3-$^2$H]Nonyn-3-ol.** The title compound was made in the same way as was the unlabeled alcohol, except that $LiB^2H_4$ was added as the reducing reagent. From 5.0 g (36.2 mmol) of propargylic ketone was obtained 5.0 g (98%) of the labeled alcohol: $^1H$ NMR $\delta$ 0.9 (t, $J$ = 5.5 Hz, 3 H, $CH_3$), 1.1–1.4 (m, 8 H, $CH_2$), 2.4 (s, 1 H, C≡CH), 2.70 (br s, 1 H, OH); IR (neat film) 3400, 2920, 2860, 2610, 1640, 1475, 1460, 1030 $cm^{-1}$.

**1-Iodo-1,2-[1-$^2$H]nonadiene.** 1-[1-$^2$H]Nonyn-3-ol (2.06 g; 14.6 mmol) was converted to the iodoallene by the method of Landor,[26] as described by Brandsma.[19] The resulting yellow oil was purified by flash chromatography (6 in. × 50 mm; petroleum ether), providing 2.70 g (74%) of the desired product as a pale yellow liquid: $^1H$ NMR ($CCl_4$) $\delta$ 0.9 (t, $J$ = 5.8 Hz, 3 H, $CH_3$), 1.1–1.6 (m, 8 H, $CH_2$), 2.1 (q, $J$ = 6.5 Hz, 2 H, $CH_2CH$=), 5.0 (t, $J$ = 6.8 Hz, 1 H, CH=C=CDI); $^2H$ NMR ($CCl_4$) $\delta$ 5.7 (s, CH=C=CDI); IR (neat film) 2920, 2860, 2300, 1930, 1470, 1460, 1440, 1380, 1150, 1110, 840 $cm^{-1}$.

**1-Iodo-1,2-[3-$^2$H]nonadiene.** 1-[3-$^2$H]Nonyn-3-ol (3.74 g; 26.5 mmol) was converted to the title compound by the same method as described above. Following purification by flash chromatography, 4.0 g (60%) of product was obtained: $^1H$ NMR ($CCl_4$) $\delta$ 0.9 (t, $J$ = 5.5 Hz, 3 H, $CH_3$), 1.1–1.4 (m, 8 H, $CH_2$), 2.13 (br t, $J$ = 6 Hz, 2 H, $CH_2CD$=), 5.6 (br t, $J$ = 2 Hz, 1 H, CD=C=CHI); $^2H$ NMR ($CCl_4$) 5.1 (s, CD=C=CHI); IR (neat film) 3300, 2920, 2860, 1950, 1700, 1470, 1450, 1440, 1380, 1130, 750 $cm^{-1}$.

**2,3-[2-$^2$H]Decadienoic Acid.** 1-Iodo-1,2-[1-$^2$H]nonadiene (3.68 g; 14.7 mmol) was dissolved in 30 mL of dry hexane in a flame-dried 100-mL round-bottomed flask equipped with a magnetic stir bar, capped with a rubber septum, and flushed with $N_2$. The solution was cooled to −75 °C, and 9.2 mL (1.47 mmol) of a 1.6 M solution of *n*-BuLi (in hexane) was added dropwise from a syringe, over a period of 3 min. The resulting milky mixture was stirred for 1 h at −75 °C. Concurrently, 110 mL of dry hexane in a 250-mL round-bottomed flask cooled to −75 °C was saturated with dry $CO_2$ (introduced from a cylinder). The solution containing the lithiated allene was then poured into the vigorously stirred $CO_2$ solution, and the resulting mixture was stirred at −75 °C for another 5 min. After this time, the cooling bath was removed, and the stirring was continued for an additional 20 min. The resulting white suspension was poured into 250 mL of water, and the flask was rinsed with water. After shaking, the organic layer was separated and washed with water. The combined aqueous layers were washed once with pentane and acidified (to pH 1) with 3 N HCl. Extraction with $Et_2O$ and the usual workup provided a crude product, which was recrystallized from petroleum ether/$Et_2O$ (1:1) at −80 °C, leading to the isolation of 950 mg (38.5%) of pure labeled acid: $^1H$ NMR ($CCl_4$) $\delta$ 0.9 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.6 (m, 8 H, $CH_2$), 2.1 (q, $J$ = 6.5 Hz, 2 H, $CH_2CH$=), 5.6 (t, $J$ = 7 Hz, 1 H, $CH$=C=CDCOOH), 11.7 (s, 1 H, COOH); $^2H$ NMR ($CCl_4$) $\delta$ 5.56 (br s, =CDCOOH); IR (neat film) 3100, 2920, 2850, 2640, 2520, 1950, 1690, 1470, 1430, 1280, 1160, 925 $cm^{-1}$. A byproduct, 2-(1-[2-$^2$H]ethynyl)octanoic acid, was also isolated, in 42% yield: $^1H$ NMR $\delta$ 0.9 (t, $J$ = 6, 3 H, $CH_3$), 1.1–1.5 (m, 8 H, $CH_2$), 1.6–1.9 (m, 2 H, $CH_2CH$(COOH)), 3.3 (t, $J$ = 7 Hz, 2 H, $CH_2CH$-(COOH)), 12.0 (br s, 1 H, COOH); $^2H$ NMR $\delta$ ($CCl_4$) 2.15 (br s, C≡CD).

**2,3-[4-$^2$H]Decadienoic Acid.** By use of the above procedure, 1-iodo-1,2-[3-$^2$H]nonadiene (4.72 g; 18.8 mmol) was converted into 1.22 g (38%) of pure labeled acid: $^1H$ NMR ($CCl_4$) $\delta$ 0.9 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.6 (m, 8 H, $CH_2$), 2.1 (br s, 2 H, $CH_2CD$=), 5.5 (t, $J$ = 2.6 Hz, 1 H, =$CHCOOH$), 11.7 (br s, 1 H, COOH); $^2H$ NMR ($CCl_4$) $\delta$ 5.6 (br s, CD=C=CHCOOH); IR (neat film) 3100, 2920, 2860, 2660, 2550, 1955, 1680, 1430, 1290, 1250, 1200, 920, 840 $cm^{-1}$. A byproduct, 2-[2-$^2$H]ethynyloctanoic acid, was also isolated, in 35% yield: $^1H$ NMR ($CCl_4$) $\delta$ 0.9 (t, $J$ = 6, 3 H, $CH_3$), 1.1–1.5 (m, 8 H, $CH_2$), 1.6–1.9 (m, 2 H, $CH_2CD$(COOH)), 2.1 (s, 1 H, C≡C—H), 10.8 (br s, 1 H, COOH); $^2H$ NMR ($CCl_4$) $\delta$ 3.28 (br s, $CH_2CD$(COOH)).

**3-Bromo-3-[2-$^2$H$_1$]decenoic Acid.** 2,3-[2-$^2$H]Decadienoic acid (1.03 g; 60.9 mmol) was hydrobrominated using the procedure described by Rosenquist and Chapman.[28] The resulting light brown oil, 1.42 g (93.8%), a mixture of stereoisomers, was used without further purification: $^1H$ NMR ($CCl_4$) $\delta$ 0.90 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.4 (m, 8 H, $CH_2$), 1.8–2.3 (m, 2 H, =$CHCH_2CH_2$), 3.49 (br s, 1 H, =$CBrCH$DCOOH), 5.8 (t, $J$ = 6.7 Hz, 0.4 H, CH=CBr), 6.0 (t, $J$ = 7.6 Hz, 0.6 H, CH=CBr), 12.0 (s, 1 H, COOH); $^2H$ NMR ($CCl_4$) $\delta$ 3.48 (br s, =CBrCHDCOOH); IR (neat film) 3100, 2920, 2870, 2690, 2600, 1725, 1660, 1470, 1420, 1300, 1220, 950 $cm^{-1}$.

**3-Bromo-3-[4-$^2$H]decenoic Acid.** 2,3-[4-$^2$H]Decadienoic acid (1.17 g; 6.92 mmol) was treated in the same manner as described above, providing 1.72 g (99%) of a mixture of diastereomeric bromo acids: $^1H$ NMR ($CCl_4$) $\delta$ 0.9 (t, $J$ = 6.5 Hz, 3 H, $CH_3$), 1.1–1.6 (m, 8 H, $CH_2$), 2.1 (br t, $J$ = 7 Hz, 2 H, $CH_2CH_2CD$=), 3.40 (s, 0.7 H, =$CBrCH_2COOH$), 3.50 (s, 1.3 H, =$CBrCH_2COOH$), 12.2 (s, 1 H, COOH); $^2H$ NMR ($CCl_4$) $\delta$ 6.0 (br s, CD=CBr).

**3-Bromo-3-[2-$^2$H$_1$]decenethioic Acid (*S*)-2-(Acetylamino)ethyl Ester.** 3-Bromo-3-[2-$^2$H$_1$]decenoic acid (615 mg; 2.47 mmol) was dissolved in 12 mL of dry benzene, in a 100-mL flame-dried round-bottomed flask outfitted with a reflux condenser and a magnetic stir bar. To this solution was added an excess of oxalyl chloride (4 mL). The resulting mixture was then heated at 60 °C under $N_2$ overnight. After removing the solvent and excess oxalyl chloride under vacuum, the resulting light yellow oil was blanketed with $N_2$ and dissolved in 30 mL of dry, degassed THF. To this solution, cooled in an ice bath, was added 25.1 mL (2.47 mmol) of a suspension of thallium–NAC in THF[8] (via syringe), and the resulting mixture was stirred at ice bath temperature for 4–5 h. During this time the color changed from bright yellow to white. After the reaction mixture had been concentrated (in vacuo), the residue was taken up in $Et_2O$ and filtered through Celite. The filtrate was washed with saturated aqueous NaCl, dried with $MgSO_4$, filtered, and concentrated in the usual manner, giving a pale yellow oil. After purification by flash chromatography (6 in. × 30 mm; 6:1, $CH_2Cl_2$/acetone), 740 mg (85%) of product was recovered, as a colorless oil: $^1H$ NMR ($CCl_4$) $\delta$ 0.8 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.4 (m, 8 H, $CH_2$), 1.80 (s, 3 H, $CH_3CO$), 2.05 (br t, $J$ = 6 Hz, 2 H, $CH_2CH_2CH$=), 2.90 (t, $J$ = 6 Hz, 2 H, $CH_2CH_2S$), 3.20 (d of t, $J$ = 6, 6 Hz, 2 H, $CH_2CH_2NH$), 3.55 (br d, $J$ = 3 Hz, 1 H, =CBrCHDCO), 5.7 (t, $J$ = 6.7 Hz, 0.4 H, CH=CBr), 5.9 (t, $J$ = 7.5 Hz, 0.6 H, CH=CBr), 7.4 (br t, $J$ = 5 Hz, 1 H, $CH_2NH$); $^2H$ NMR ($CCl_4$) $\delta$ 3.54 (br s, =CBrCHDCO).

**3-Bromo-3-[4-$^2$H]decenethioic Acid (*S*)-2-(Acetylamino)ethyl Ester.** 3-Bromo-3-[4-$^2$H]decenoic acid (1.19 g; 4.78 mmol) was converted to the thio ester (1.44 g; 86%) by the procedure described above: $^1H$ NMR ($CCl_4$) $\delta$ 0.8 (t, $J$ = 6 Hz, 3 H, $CH_3$), 0.9–1.4 (m, 8 H, $CH_2$), 1.8 (s, 3 H, $CH_3CO$), 2.05 (t, $J$ = 6 Hz, 2 H, $CH_2CH_2CD$=), 2.85 (t, $J$ = 6 Hz, 2 H, $CH_2CH_2S$), 3.0–3.3 (m, 2 H, $CH_2CH_2NH$), 3.5 (d, $J$ = 3 Hz, 2 H, =$CBrCH_2CO$), 7.45 (br t, $J$ = 5 Hz, 1 H, $CH_2NH$); $^2H$ NMR ($CCl_4$) $\delta$ 5.81 (br s, CD=CBr); IR (neat film) 3300, 3080, 2920, 2850, 1940, 1680, 1550, 1430, 1370, 1300, 1200, 1050 $cm^{-1}$.

**2,3-[2-$^2$H]Decadienethioic Acid (*S*)-2-(Acetylamino)ethyl Ester (2,3-[2-$^2$H]Decadienoyl-NAC).**[28] 3-Bromo-3-[2-$^2$H$_1$]decenethioic acid (*S*)-2-(acetylamino)ethyl ester (770 mg; 2.21 mmol) was dissolved in 100 mL of anhydrous $Et_2O$, and the solution was cooled to −60 °C under $N_2$. With vigorous stirring, 270 mg (2.17 mmol) of DBN was added dropwise from a syringe over a period of 15 min. A white precipitate formed immediately. The resulting suspension was stirred at −60 °C for another 20 min and filtered through Celite into a flask, which contained 150 mL of $H_2O$. The organic phase was separated and washed twice with water, and then once with saturated aqueous NaCl. After drying of the extracts and removal of solvent under vacuum, 590 mg of the thio ester (99%) was obtained: $^1H$ NMR ($CCl_4$) $\delta$ 0.8 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.0–1.4 (m, 8 H, $CH_2$), 1.79 (s, 3 H, $CH_3CO$), 2.05 (q, $J$ = 7 Hz, 2 H, $CH_2CH_2CH$=), 2.85 (t, $J$ = 6 Hz, 2 H, $CH_2CH_2S$), 3.20 (d of d, $J$ = 6, 6 Hz, $CH_2CH_2NH$), 5.60 (t, $J$ = 7 Hz, 1 H, $CH_2CH$=), 7.10 (br t, $J$ = 5 Hz, 1 H, $CH_2NH$); $^2H$ NMR ($CCl_4$) $\delta$ 5.78 (br s, =CD); IR (neat film) 3300, 3080, 2920, 2860, 1950, 1680, 1550, 1470, 1440, 1380, 1300, 1200, 1090, 1050, 870, 720 $cm^{-1}$.

**2,3-[4-$^2$H]Decadienethioic Acid (*S*)-2-(Acetylamino)ethyl Ester.** 3-Bromo-3-[4-$^2$H]decenethioic acid (*S*)-2-(acetylamino)ethyl ester (926 mg; 2.65 mmol) was converted to the title compound (708 mg; 99%) by the method described above: $^1H$ NMR ($CCl_4$) $\delta$ 0.85 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.1–1.4 (m, 8 H, $CH_2$), 1.80 (s, 3 H, $CH_3CO$), 1.9–2.1 (m, 2 H, $CH_2CH_2CD$=), 2.9 (t, $J$ = 6 Hz, 2 H, $CH_2CH_2S$), 3.2 (q, $J$ = 6 Hz, 2 H, $CH_2CH_2NH$), 5.70 (t, $J$ = 2.9 Hz, 1 H, $CH_2CD$=C=$CH$), 7.3 (br t, $J$ = 5 Hz, 1 H, $CH_2NH$); $^2H$ NMR ($CCl_4$) $\delta$ 5.61 (br s, CD=); IR (neat film) 3300, 3070, 2920, 2850, 1930, 1650, 1520, 1410, 1350, 1270, 1180, 1020, 890, 720 $cm^{-1}$.

**Incubation of Labeled Thio Ester Substrates with AAI.** To 200 mL of 0.05 M $KPO_4$ (pH 7.0, 20 °C) was added 11 mL of AAI solution (purified through the first DEAE-cellulose chromatography step[10,11] (270 mg of protein, via Bio-Rad assay[45])). After 3 min of gentle stirring, 200 mg (0.74 mmol) of labeled 2,3-decadienoyl-NAC in 5 mL of dioxane was added. The cloudy mixture was stirred at 20 °C for 2 min (2,3-[4-$^2$H]decadienoyl-NAC was stirred for 4–5 min, owing to the isotope effect). At this point, 200 mL of 1.0 M $KPO_4$ (pH 7.0, 20 °C) and 200 mL of THF were added, the solution was cooled quickly to 4 °C, and 7.4 g of $NaBH_4$ was added over a period of 15 min. During this time, the pH of the solution was kept at 6.8–7.0 by addition of 2 N HCl. Following the addition of $NaBH_4$, the reaction mixture was stirred at 4 °C

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

for another 20 min, after which time the excess borohydride was destroyed by the addition of 2 N HCl until the pH had reached 2.0. $(NH_4)_2SO_4$ was added to the saturation point, and the solution was extracted with three 300-mL aliquots of $Et_2O$. The combined organics were dried, filtered, and concentrated (in vacuo), affording a yellow oil. This procedure was repeated 7-8 times with each of the labeled substrates. The combined crude product mixtures were partially purified by flash chromatography (6 in. × 20 mm; 5:1, petroleum ether/EtOAc). Incubations of substrates labeled at C-2 and at C-4 afforded 12.5 and 7.0 mg, respectively, of impure 3-[2-$^2H_1$]decyn-1-ol. These alcohol samples were contaminated with ca. 25-30% (by GC) of 3-decen-1-ol (from reduction of 2,3-decadienoyl-NAC). Even after further purification of a sample by HPLC (19:1, hexane/EtOAc; 1.0 mL/min; 4.6 × 250 mm silica gel column; effluent monitored using a Knauer differential refractometer), GC analysis of the product from the incubation of substrate labeled at C-2 showed the presence of both 3-decen-1-ol (15%) and 3-decyn-1-ol (81%). Accordingly, HPLC purification was not ordinarily employed, and the olefinic impurities were carried through the remainder of the derivatization process.

In a prior control experiment, a mixture of 3-decynoyl-NAC (13 mg) and 2,3-decadienoyl-NAC (34 mg) was dissolved in 20 mL of THF, and this solution was further diluted with 20 mL of $^2H_2O$ and 20 mL of 1.0 M $KPO_4$ (pH 7.0, in 99.8% $^2H_2O$). This solution was cooled and treated with $NaBH_4$ as described above. Extraction of the reaction mixture was followed by purification by flash chromatography, affording 2.5 mg of a mixture of alcohols, identified by GC analysis as 3-decyn-1-ol, (*E*)-3-decen-1-ol, and (*Z*)-3-decen-1-ol. Owing to difficulty in separating these alcohols from one another, a $^2H$ NMR spectrum was run on the mixture. No incorporation of deuterium from the medium was observed.

**Reduction of Chirally Labeled 3-[2-$^2H_1$]Decyn-1-ol.** 3-[2-$^2H_1$]Decyn-1-ol (12.5 mg, from incubation of substrate labeled at C-2) was reduced to 1-[2-$^2H_1$]decanol by homogeneous hydrogenation in benzene solution, in the presence of $(Ph_3P)_3RhCl$.[30] The course of the reaction was followed 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 13.2 mg of labeled 1-decanol, as a light yellow oil. This material was used without further purification.

**Oxidation of Chirally Labeled 1-[2-$^2H_1$]Decanol.** Labeled 1-[2-$^2H_1$]-decanol (13 mg; 0.083 mmol) was oxidized to 1-[2-$^2H_1$]decanoic acid by Sharpless's procedure.[31] After 2 h, the color of the reaction mixture had changed from black to light green. An aliquot was removed for GC analysis, which showed complete conversion of the alcohol to the corresponding acid. The reaction mixture was then extracted 3 times with $CH_2Cl_2$. The combined organics were carefully concentrated, and the black residue was taken up in $Et_2O$ and filtered through a plug of silica gel. Concentration of the filtrate in vacuo gave 7.0 mg of labeled acid, as a white solid. This was esterified without further purification.

**Esterification of Chirally Labeled [2-$^2H_1$]Decanoic Acid (from Incubation of C-2 Labeled Substrate) to Methyl (*S*)-α-Hydroxybenzeneacetate (Methyl Mandelate).** The methyl mandelate ester of chirally labeled 1-[2-$^2H_1$]decanoic acid (7.0 mg) was made by the method described by Parker.[32] Following flash chromatographic purification, 6.5 mg of labeled ester was obtained, as a clear colorless oil: $^1H$ NMR ($CCl_4$) δ 0.9 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.05-1.9 (m, 14 H, $CH_2$), 2.4 (t, $J$ = 8 Hz, 1 H, $CH_2CHDCOO$), 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; see Figure 2a) δ 2.27 (s, ca. 0.64 D, *pro-R* CHDCOO), 2.16 (s, ca. 0.36 D, *pro-S* CHDCOO).

**Conversion of Chirally Labeled 3-[2-$^2H_1$]Decyn-1-ol (from Incubation of C-4 Labeled Substrate) to the Decanoic Acid Ester of Methyl Mandelate.** Labeled 3-[2-$^2H_1$]decyn-1-ol (6 mg), derived from incubation of 2,3-[4-$^2H$]decadienoyl-NAC with AAI, was converted to the corresponding methyl mandelate ester by the procedure described above, except that unlabeled 3-decyn-1-ol (6 mg) was added as carrier. The yield of labeled ester was 8 mg. $^1H$ NMR ($CCl_4$) δ 0.9 (t, $J$ = 6 Hz, 3 H, $CH_3$), 1.05-1.9 (m, 14 Hz, $CH_2$), 2.4 (t, $J$ = 8 Hz, 1 H, $CH_2CHDCOO$), 3.7 (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 2b) δ 2.16 (s, ca. 0.67 D, *pro-S* CHDCOO), 2.25 (s, ca. 0.33 D, *pro-R* CHDCOO).

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# Calixarenes. 13. The Conformational Properties of Calix[4]arenes, Calix[6]arenes, Calix[8]arenes, and Oxacalixarenes

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**Abstract:** The [1$_n$]metacyclophanes known as calixarenes, obtainable in ring sizes containing 4-8 aryl moieties in the macrocyclic array, are conformationally flexible in varying degrees. We have studied the conformational characteristics of these molecules by means of temperature-dependent $^1H$ NMR measurements. On the basis of the data that have been obtained, preferred conformations are suggested, and the energies of activation for conformational inversion are assigned. It is found that the $\Delta G^{\ddagger}$ values in nonpolar solvents such as chloroform and benzene are somewhat higher than those in semipolar solvents such as acetone and acetonitrile and considerably higher than those in pyridine. The $^1H$ NMR data are commensurate with a "cone" conformation for the cyclic tetramers and cyclic pentamers, a "winged" or "hinged" conformation for the cyclic hexamers, and a "pleated-loop" conformation for the cyclic octamers. The conformational implications of the temperature-dependent $^1H$ NMR behavior of a cyclic pentamer, a cyclic heptamer, a dihomooxacalix[4]arene, a tetrahomodioxacalix[4]arene, and a hexahomotrioxacalix[3]arene are also discussed.

Calixarenes, which are [1$_n$]metacyclophanes, can be obtained in ring sizes ranging from 4 to 8 aromatic residues[1] by base-induced condensation of certain para-substituted phenols and formaldehyde. The smallest members of the series, the calix[4]arenes, can exist in several conformations, as first adumbrated by Megson[2] and Ott and Zinke[3] and subsequently made explicit by Cornforth.[4] Space-filling (Corey-Pauling-Koltun) molecular

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