2-(triphenylmethyl)tetrahydrofuran (TTF) was recrystallized from methanol to give colorless prisms; mp 102 °C.

The spectral data for TTF were as follows: ^{1}H NMR ($CDCl_3$) δ 0.2-2.2 (4 H, m), 3.60 (2 H, d of d, J = 7.8, 5.4 Hz), 5.50 (1 H, d of d, J = 6.0, 7.8 Hz), 7.36 (5 H, m); MS m/e 243, 59. Anal. Calcd for $C_{23}H_{22}O$: C, 87.85; H, 7.06. Found: C, 87.68; H, 7.06.

The remaining residue 0.477 g, was allowed to dissolve in ether methanol, and O_2 was bubbled through the solution for ca. 2 h to oxidize residual triarylmethanes. After solvent removal, the residue was triturated with ether. The undissolved material was identified as tetraphenylmethane by mass spectral analysis: m/e 320, 243, 166, 89.

B. In Liquid Ammonia. Solutions of triphenylmethyllithium in liquid ammonia were prepared by condensing 30 mL of ammonia into a cylindrical irradiation vessel containing 0.390 g (1.60 mmol) of triphenylmethane that had been degassed and filled with argon. A 1.0-mL solution of 1.6 M n-butyllithium was introduced and the resulting red solution allowed to equilibrate for 1 h. Bromobenzene (0.168 g, 1.60 mmol) was added, the solution irradiated or allowed to stand, and the resulting blue solution quenched by addition of solid ammonium chloride. The evaporated residue was taken up in ether and washed with water. Triphenylethylene and (4-biphenylyl)diphenylmethanol were added as internal standards, and the product mixture was quantitated by gas chromatography. The yields of products were as follows.

Run 1 (30-min irradiation): triphenylmethane, 250.2 mg; TPM, 11.3 mg (6.2%); BDM, 11.6 mg (6.4%).

Run 2 (15-min irradiation): triphenylmethane, 269 mg; TPM, 7.54 mg (4.8%); BDM, 9.85 mg (6.3%).

Run 3 (7-min irradiation): triphenylmethane, 320 mg; TPM, 4.9 mg (5.3%); BDM, 9.15 mg (10.0%).

Run 4 (no irradiation): triphenylmethane, 305 mg; TPM, 4.66 mg (4.4%); BDM, 2.52 mg (2.3%).

Run 5 (no irradiation, 2.0 mL (3.2 mmol) of n-butyllithium): triphenylmethane, 140 mg; TPM, 53.1 mg (16.6%); BDM, 19.0 mg (6.0%).

Preparative Thermolysis of (Phenylazo)triphenylmethane (PAT) in Tetrahydrofuran. A 173-mg (0.500 mmol) portion of PAT was dissolved in 50 mL of THF and the mixture refluxed for 2 h. Gas chromatographic analysis indicated the presence of triphenylmethane (5%), TTF (32%), TPM (8%), and BDM (7%) as well as various unidentified less volatile products. The residue was chromatographed on a 20 × 20 × 0.2 cm silica gel plate developed twice with 5:95 ether-hexane. The isolated yields were as follows: triphenylmethane, 12 mg; TTF, 64 mg; TPM, 23 mg; BDM, 19 mg.

2-Chlorotetrahydrofuran. This was prepared by the method of Kratochivil and Hort.[7] Dried THF (82 mL, 1.0 mol) was placed in a low-temperature irradiation vessel with evacuation jacket mounted in a Dewar containing dry ice and saturated $CaCl_2$ both maintained at −48 °C. Chlorine gas was introduced, and the mixture was irradiated for 50 min. The mixture was distilled under reduced pressure; a fraction boiling at 25–37 °C (21 mmHg) contained 2-chlorotetrahydrofuran along with other chlorinated THF products. This fraction was redistilled on a Vigreux column, and two fractions (40–55 °C, 55–80 °C at 47 mmHg) were collected in a chilled receiving flask. Both fractions containing ca. 80% 2-chlorotetrahydrofuran along with dichlorinated impurities were used without further purification.

2-(Triphenylmethyl)-2,3,4,5-tetrahydrofuran (TTF). Trityl anion was generated with 2.44 g (10 mmol) of triphenylmethane and 6.5 mL (10 mmol) of n-butyllithium in 50 mL of THF. A 1.5-mL portion of 2-chlorotetrahydrofuran (fraction 2) was added, causing loss of color. After ether extraction, the oil was dissolved in hexane and chromatographed on a column of silica gel with 5:95 ethyl acetate-hexane as eluent. The yield of 2-(triphenylmethyl)tetrahydrofuran was 0.144 g (5.0%). This material had spectral properties (NMR, IR, MS) and melting point identical with those of the photochemical material.

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Registry No. 1, 40006-86-8; (triphenylmethyl)lithium, 733-90-4; bromobenzene, 108-86-1; iodobenzene, 591-50-4; diphenyl sulfoxide, 945-51-7; 2-chlorotetrahydrofuran, 13369-70-5; triphenylmethyl radical, 2216-49-1; TPM, 630-76-2; BDM, 745-36-8; TTF, 85004-92-8; PAT, 981-18-0; THF, 109-99-9.

Combining Enzymatic and Chemical Steps in the Synthesis of Biochemically Valuable Compounds: Isotopically Chiral Methyl Acetate

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An inexpensive, practical route for preparing isotopically chiral methyl acetic acid by using a combination of chemical and enzymatic steps is described. Chirality is introduced by the stereoselective exchange of the *pro-R* α-protons of [2-^{3}H]cyclohexanone catalyzed by the enzyme acetoacetate decarboxylase (AAD), while chemical steps allow the subsequent preparation of chiral acetate in 70% overall yield. Malates prepared from (*R*)-acetates retained 66% of their tritium label when incubated with fumarase, while those from (*S*)-acetates retained 35%.

Enzymes are becoming increasingly popular as synthetic tools, especially when they are used to synthesize chiral molecules.[1] However, the remarkable stereospecificity of enzymes is often not sufficient to overcome two serious limitations to their use as organic catalysts: the small quantity of material that can normally be prepared enzymatically and the narrow range of substrates accepted by most enzymes.

Recently, we needed to prepare large quantities of chiral methyl groups for enzymatic studies. Of the methods that were available in the literature, none of the purely chemical or purely enzymatic routes seemed well adapted to this goal. The purely chemical routes rely on chemical resolutions to obtain optical purity,[2-4] although in one case a

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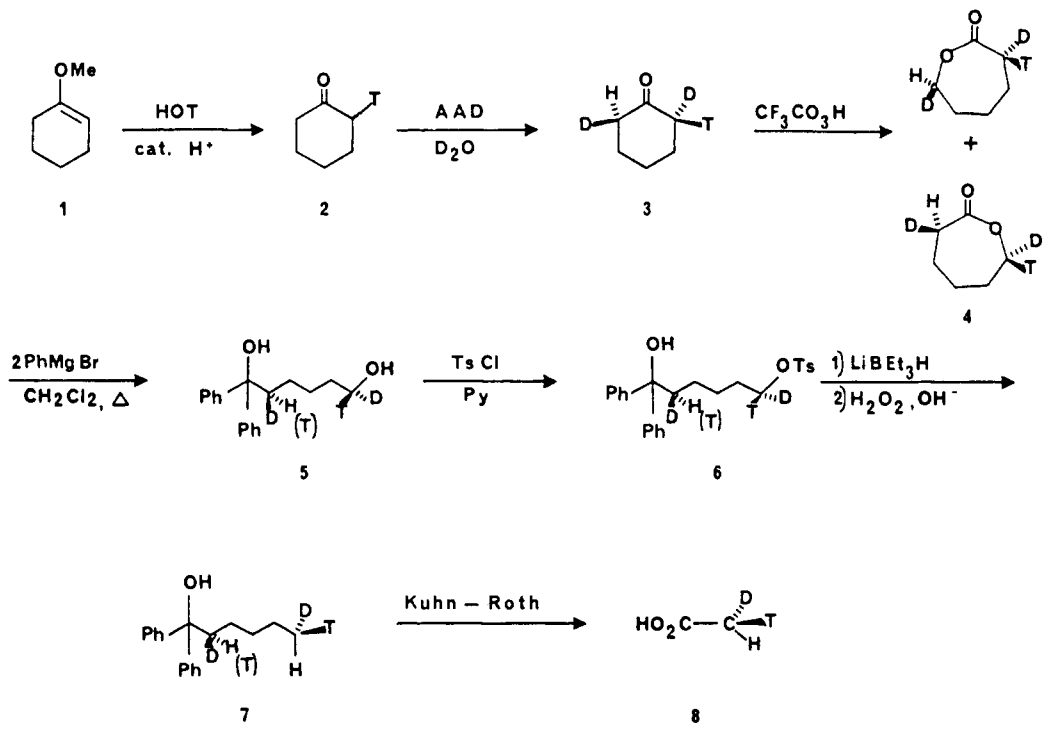
Scheme I



chemical sequence involving an asymmetric hydrogenation has been reported to be useful for the synthesis of chiral lactic acid.[5] The purely enzymatic routes typically yield only small amounts of material, often require dilution with carrier to facilitate handling, and do not offer the convenience of introducing the labels as water.[6]

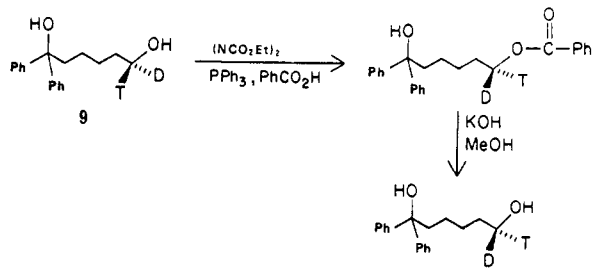
In view of the well-known ability of enzymes to distinguish between enantiotopic groups, it seemed rational to design a synthesis of chiral methyl groups where chirality was introduced by an enzyme-catalyzed exchange with water that was rapid enough to produce inexpensively gram quantities of chirally labeled material. Then, it seemed most advantageous to use chemical steps to convert the isotopically chiral intermediate into the chiral methyl group, choosing those manipulations that facilitated the handling of material, without concern for whether subsequent intermediates were enzymic substrates.

We report here a synthetic scheme based on precisely these rationales which permits the synthesis of large quantities of chiral acetate. Chirality is introduced by the enantioselective exchange of the α-protons of cyclohexanone catalyzed by the enzyme acetoacetate decarboxylase (AAD). The exchanged product is then converted to a stable, crystalline, chirally labeled intermediate, 1,1-diphenyl-1,6-hexanediol, which is in turn converted via a tosylate to chiral methyl 1,1-diphenylhexan-1-ol. Two of the hydrogens are introduced as H^+, while one is introduced as a hydride. Although the chiral methyl groups produced have less than optimal chirality, the facility and low cost of this approach make it likely to be of use in the future for the synthesis of chiral methyl groups.

Results

The synthetic route for the preparation of chiral methyl groups is outlined in Scheme I. 1-Methoxycyclohexene, prepared by a modification of a literature procedure,[7] was converted to randomly labeled 2-tritiocyclohexanone by hydrolysis in tritiated water with a catalytic amount of H_2SO_4. Stereoselective exchange of the *pro-R* α-protons in D_2O with AAD[8] gave a chiral, doubly-labeled cyclohexanone, which was converted to labeled caprolactone by a Baeyer–Villiger oxidation.[9] The Baeyer–Villiger oxidation has been previously shown to occur with retention of configuration at the migrating center.[10-12] Grignard reactions using an excess of freshly prepared PhMgBr in CH_2Cl_2 gave 1,1-diphenyl-1,6-hexanediol[13] as a stable, crystalline solid, which could be isolated in 85% yield from 1-methoxycyclohexene. Selective tosylation of the primary alcohol followed by S_N2 displacement of the tosylate with $LiBEt_3H$[14] gave 1,1-diphenylhexanol with a chiral methyl group now in place at C-6. Kuhn–Roth oxidation degraded 7 to chiral acetic acid,[15] which was isolated as its sodium

Scheme II



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Scheme III

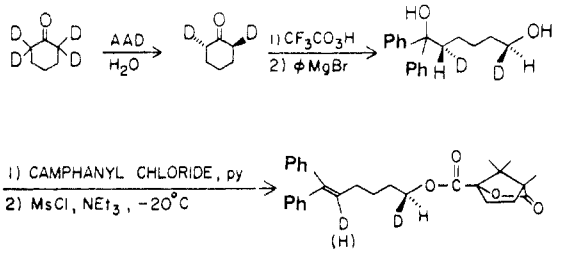

Table I. Analysis of Chirality of Acetates

absolute config	*F* value
R	66
S	35

salt by bulb to bulb distillation at low temperature and pressure, titration with a standardized solution of NaOH, and lyophilization. The overall yield was 70% from the starting enol ether. The enantiomer of **5** could be prepared either by reversing the order in which any two labels were introduced or, more easily, in 90% yield by carrying out a two-step inversion sequence involving formation of the inverted benzoate ester by using triphenylphosphine, diethyl azodicarboxylate, and benzoic acid followed by hydrolysis in methanolic KOH, as shown in Scheme II.[16]

We demonstrated the chirality of the diol **9** by the method of Gerlach as recently applied by Schwab.[17,18] The (−)-camphanyl ester of **9** was prepared, dehydrated with $MsCl/NEt_3$ in CH_2Cl_2 (Scheme III), and examined by 270-MHz NMR in the presence of $Eu(dpm)_3$ in $CDCl_3$. The diasterotopic protons in an unlabeled sample were clearly distinguishable; in a sample derived from **9** the downfield signal was absent, and the assignment of the *pro-R* and *pro-S* protons agrees with the general rule given by Gerlach that the *pro-R* proton corresponds to the downfield NMR signal.

Examination of chiral acetates by the method introduced by Cornforth,[3] Arigoni,[4] and co-workers produced the results shown in Table I. Specimens of (*S*)-malate derived from (*R*)-acetate retained 66% of their tritium label when equilibrated with the enzyme fumarase, while those derived from (*S*)-acetate retained 35%. The percentage of tritium retention in the fumarase incubation is referred to as the *F* value.

Discussion

In the analysis of chiral methyl groups, theoretical values for the fraction of tritium retained in the incubation of malates with fumarase have been cited by Floss as 79% and 21% for the (*R*)- and (*S*)-acetates, respectively,[19] although values commonly reported are around 70% and 30%, and useful values can be as low as 60% for the (*R*)-acetate and as high as 40% for the (*S*)-acetate. The less than optimal chirality observed here most likely reflects the fact that AAD is not completely stereospecific in its exchange of the α-protons of cyclohexanone[8] and the fact that during the exchange step the label accumulates in the solvent. The second of these problems is obviously easily alleviated by using large volumes of water in the exchange step. Nonetheless, this problem is inherent in any exchange process, and we have found that the improved ease of introducing the label from water far outweighs the slightly lower optical activities of the acetates produced.

Of the methods currently available for preparing chiral methyl groups, only two others combine enzymatic and chemical steps. However, once the enzyme is in hand, the method presented here is the cheapest and most practical for preparing significant quantities of chiral methyl groups. While other methods use enzymatic steps to introduce optical activity in the isotopic labeling, the method presented here is the only one to do so by an enzymatic-exchange reaction, which is kinetically faster and more adaptable to scale-up. The alternate methods both introduce the label from hydride donors or from NADH.[20] The principle deficiency of this procedure arises from the fact that AAD is not commercially available.

Of the methods for preparing chiral methyl groups which rely exclusively on enzymatic steps, the method of Rose[6,21] involving all the enzymes in the glycolytic pathway has been useful in the hands of Floss and co-workers.[19] However, none of these methods introduce the label as H^+, and the overall yields of chiral methyl groups are small. Nonetheless, the method developed by Rose, starting with tritiated glucose, which is available with high specific activities, can be used to prepare small quantities of chiral acetate with good enantiomeric purity.

Purely chemical methods for the preparation of chiral methyl groups involve chemical resolutions. In addition to the elegant procedure, based on an ene reaction,[22] developed by Arigoni and co-workers, chemical syntheses include some of the oldest and newest routes to chiral methyl groups.[3-5,23] In each case, our method compares favorably both in terms of yield and overall effort with the best of the purely chemical methods, even after considering the need to prepare AAD.

Experimental Section

General Procedures. All chemicals were reagent grade unless noted. Tritiated water was purchased from New England Nuclear, and radioactivity measurements were performed on a Packard 3320 scintillation counter. Silica gel thin-layer chromatography plates were obtained from EM Reagents or Analtech, DE-52 ion-exchange resins from Whatman, Sephadex G-25 from Pharmacia, and analytical grade cation- and anion-exchange resins form Bio-Rad. Spectrophotometric determinations were performed by using Cary 14, Zeiss, or Gilford 240 spectrophotometers. Infrared spectra were obtained on a Perkin-Elmer 137, proton NMR spectra on a Varian CFT-20 or JEOL 270-MHz NMR spectrometer, and mass specral analyses on an AEI MS9. All compounds listed gave satisfactory NMR and mass spectral data.

Enzymic Work. Acetoacetate decarboxylase (AAD) was prepared from *Clostridium acetobutylicum* by the method of Westheimer.[24] All exchange reactions were run in 50 mM potassium phosphate buffer at pH 6.0 and 25 °C. The analysis of the chirality of acetates was carried out as previously described.[3,4]

[2-^{3}H]Cyclohexanone (2). To 1-methoxycyclohexene (680 mg, 6.0 mmol) in a 5-mL flask capped with a serum cap were added tritiated water (New England Nculear, 200 μL, 5 Ci/g) and a catalytic amount of H_2SO_4. The solution was allowed to stand

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at room temperature for 15 min. In model studies with D_2O, the reaction was found to form deuterated cyclohexanone quantitatively. In tritiated runs, the specific activity of the cyclohexanone was found to be ca. 40 mCi/mmol. Normally, this material was not isolated but was immediately carried through.

(2*S*)-[2-^{3}H,^{2}H; 6-^{2}H]Cyclohexanone (3). The tritiated cyclohexanone prepared as above was added to a solution of AAD in 50 mM potassium phosphate buffer in D_2O (50 mL, 1000 international units). The enzyme is known to catalyze the exchange of the 2-*pro-R* hydrogens of cyclohexanone. The reaction was followed by removing aliquots (10 μL) from the reaction mixture at time intervals and injecting them into a mixture of xylene and water. By comparison of the amount of radioactivity in the water layer to that extracted into the xylene layer, the exchange of the radiolabel from cyclohexanone into water could be determined. When 60% of the label had been exchanged, the reaction mixture was extracted with methylene chloride and dried over sodium sulfate. Although this material was not routinely isolated, the specific activity at this stage was typically 15 mCi/mmol. The dried methylene chloride solution of the chiral, labeled cyclohexanone was used directly in the next step.

(2*S*)-[2-^{3}H]Cyclohexanone. This material was prepared as above, but with AAD in H_2O/potassium phosphate buffer.

Isotopically Chiral Caprolactone (4). To the methylene chloride solution from the preceeding step were added 4.0 g of disodium hydrogen phosphate and CF_3CO_3H (prepared by adding 0.250 mL of 90% H_2O_2 to 1.5 mL of trifluoroacetic anhydride in 5 mL of CH_2Cl_2 at 0 °C) with stirring on ice. The reaction mixture was allowed to warm to room temperature over 1.5 h, was quenched with solid K_2CO_3, and was filtered. The caprolactone was immediately carried through the Grignard reaction to avoid decomposition.

Isotopically Chiral 1,1-Diphenyl-1,6-Hexanediol (5). To the CH_2Cl_2 solution of the caprolactone prepared as described above was added an excess of phenylmagnesium bromide, freshly prepared in a small volume of anhydrous ether. The mixture was refluxed for 4 h and quenched with aqueous ammonium chloride, and the product was purified by chromatography on silica gel with an ether/methylene chloride (30:70) elution (R_f 0.4). The overall yield from 1-methoxycyclohexene was 75–80%. The material could be stored indefinitely at −10 °C and carried through to chiral acetate as needed: mp 64.5–65.0 °C; NMR δ 7.30 (m, 10 H), 3.50 (t, 2 H), 2.5 (s, 2 H), 2.25 (m, 2 H), 1.37 (br s, 6 H).

Chiral Methyl 1,1-Diphenylhexanol (7). To a solution of 1,1-diphenyl-1,6-hexanediol (35 mg, 1.6 mmol) in pyridine (3 mL) was added tosyl chloride (75 mg, 2.5 mmol). After 12 h the mixture was worked up by adding ice–water, allowing the mixture to stand for 15 min, extracting twice with ether, washing the ether extracts extensively with aqueous $CuSO_4$ to remove pyridine, and drying over magnesium sulfate/potassium carbonate. After evaporation of the ether, the tosylate was carried through the hydride reduction immediately to avoid decomposition. To a solution of the tosylate in 4 mL of anhydrous THF was added a 3-fold excess of $LiBEt_3H$ in THF (1.0 M solution from Aldrich). The reaction mixture was stirred at room temperature for 20–30 min and quenched with 10% NaOH, and the BEt_3 was oxidatively decomposed with excess 30% H_2O_2. After the residual H_2O_2 was destroyed with sodium bisulfite, the product was isolated by ether extraction, and the solvent was evaporated to yield 33 mg (1.6 mmol) of a colorless oil: NMR δ 7.25 (m, 10 H), 2.20 (m, 2 H), 2.0 (s, 1 H), 1.25 (br s, 6 H), 0.85 (broadened t, 3 H). In particular, the ethanol produced in the oxidative degradation of the BEt_3 must be completely removed; otherwise, it would be converted to acetate during the subsequent exidation, causing a dilution of the specific activity of the chiral acetates.

Chiral Acetic Acid (8). Kuhn–Roth oxidation of the 1,1-diphenylhexanol (33 mg, 1.6 mmol) was carried out by stirring with CrO_3 (1.0 g) in 2 mL of water at room temperature for 12 h. At the end of this time, a few drops of 85% H_3PO_4 were added, and the acetic acid was isolated by bulb to bulb distillation at low temperature and pressure. The resulting acetic acid solution was titrated with standardized NaOH and lyophilized to leave the chiral acetate as its sodium salt. This could be stored indefinitely at 0 °C.

Inversion at C-6 of the 1,1-Diphenyl-1,6-hexanediol. While the opposite enantiomer of chiral acetate could be obtained by reversing the order in which any two isotopes of hydrogen are introduced (e.g., using H_2O and $LiBEt_3D$ instead of D_2O and $LiBEt_3H$), it proved more convenient to invert the configuration of the 6-position of diol **5**. Reaction of **5** (20 mg, 0.079 mmol) with PPh_3 (74 mg, 0.28 mmol), diethyl azodicarboxylate (48 mg, 0.24 mmol), and benzoic acid (37 mg, 0.25 mmol) produced the inverted benzoate ester in 90% yield after purification by preparative thin-layer chromatography (silica gel plates, CH_2Cl_2 eluent, R_f 0.59). Hydrolysis in methanolic KOH gave the diol inverted in the C-6 position in quantitative yield.

Demonstration of Chirality of Labeled 1,1-Diphenyl-1,6-hexanediol. The diol (330 mg, 0.84 mmol) was reacted with 1.5 equiv of camphanyl chloride in pyridine to produce the camphanic ester of the primary alcohol. The crude product was dehydrated by treatment with CH_3SO_2Cl in CH_2Cl_2 in the presence of excess NEt_3 at −20 °C for 15 min, and the camphanate ester of the 6,6-diphenyl-5-hexen-1-ol (247 mg, 0.57 mmol) was isolated by preparative thin-layer chromatography on silica gel: R_f 0.56 (benzene/Et_2O, 93:7); NMR δ 7.28 (m, 10 H), 6.07 (t, 1 H), 4.20 (t, 2 H), 2.1–1.2 (multiplets, 10 H), 1.15 (s, 3 H), 1.09 (s, 3 H) 1.00 (s, 3 H). By use of the procedure developed by Gerlach and recently applied by Schwab,[17,18] the 270-MHz NMR spectrum of this ester in the presence of $Eu(dpm)_3$ in $CDCl_3$ at 303 K clearly resolved the two diastereotopic protons of the C-1 methylene group. However, in our hands it was necessary to add greater than 1 equiv of shift reagent to clearly resolve the two signals. When the camphanate ester was prepared from 2,2,6,6-tetradeuteriocyclohexanone, as shown in Scheme III, the downfield signal from the diastereotopic pair was absent when examined by 270-MHz NMR, indicating that the isotopically labeled center maintained its chirality at the diol stage.

Acknowledgment. We are grateful to Jerome V. Connors for expert technical assistance in the preparation of acetoacetate decarboxylase. In addition, we are indebted to Professor F. H. Westheimer, in whose laboratories this work was done, for financial support and scientific guidance. Support for this work was also furnished by a National Science Foundation predoctoral fellowship (to J. D.R.), a National Science Foundation grant (PCM-8111659) to S.A.B., and a National Institutes of Health grant (SROI-GM-04712) to F. H. Westheimer.

Registry No. **2**, 28093-51-8; **3**, 84849-27-4; **4**, 84849-28-5; **5**, 84849-29-6; **7**, 84849-30-9; **8**, 23198-06-3; AAD, 9025-03-0; (2*S*)-[2-^{3}H]cyclohexanone, 78680-01-0.