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

J. David Rozzell, Jr., and Steven A. Benner*

Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

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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 \( \alpha \)-protons of \( [2-\text{H}] \) 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. 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.


Isotopically Chiral Methyl Acetate

Scheme I

Chemical sequence involving an asymmetric hydrogenation has been reported to be useful for the synthesis of chiral lactic acid. 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.

In view of the well-known ability of enzymes to distinguish between enantiomorphic 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 \( \alpha \)-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 \( \mathrm{H}^+ \), 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, was converted to randomly labeled 2-tritiocyclohexanone by hydrolysis in tritiated water with a catalytic amount of \( \mathrm{H}_2\mathrm{SO}_4 \). Stereoselective exchange of the pro-\( R \) \( \alpha \)-protons in \( \mathrm{D}_2\mathrm{O} \) with AAD gave a chiral, doubly-labeled cyclohexanone, which was converted to labeled caprolactone by a Baeyer-Villiger oxidation. The Baeyer-Villiger oxidation has been previously shown to occur with retention of configuration at the migrating center. Grignard reactions using an excess of freshly prepared PhMgBr in \( \mathrm{CH}_2\mathrm{Cl}_2 \) gave 1,1-diphenyl-1,6-hexanediol as a stable, crystalline solid, which could be isolated in 85% yield from 1-methoxycyclohexene. Selective tosylation of the primary alcohol followed by SN2 displacement of the tosylate with LiBE\( \text{t}_2 \) gave 1,1-diphenylhexanol with a chiral methyl group now in place at \( \alpha \). Kuhn–Roth degradation degraded 7 to chiral acetic acid, which was isolated as its sodium salt.

References

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 S 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, di-
ethyl azodicarboxylate, and benzoic acid followed by hy-
drolysis in methanolic KOH, as shown in Scheme II.14

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/NEt3 in CH2Cl2 (Scheme III), and examined by
270-MHz NMR in the presence of Eu(dpm)3 in CDCl3.
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,1 Arigoni,4 and co-workers produced the re-
sults shown in Table I. Specimens of (S)-malate 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 re-
lects the fact that AAD is not completely stereospecific
in its exchange of the α-protons of cyclohexanone8 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 im-
proved ease of introducing the label from water far out-
weighs 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 pres-
ented here is the only one that does so by an isotopic-ex-
change reaction, which is kinetically faster and more adap-
table to scale-up. The alternate methods both in-
roduce 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 Rose21
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. Silicone gel thin-layer chromatography
plates were obtained from EM Reagents or Analtech. DE-52
ion-exchange resins from Whatman, Sephadex G-25 from Phar-
macia, and analytical grade cation- and anion-exchange resins form
Bio-Rad. Spectrophotometric determinations were performed
by using Cary 14, Zeiss, or Gilford 240 spectrophotometers.
Infared spectra were obtained on a Perkin-Elmer 137, proton NMR
spectra on a Varian CPT-20 or JEOL 270-MHz NMR spectrom-
eter, and mass spectral 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 po-
tassium phosphate buffer at pH 6.0 and 25 °C. The analysis of
the chirality of acetates was carried out as previously described.3,4

[2-3H]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 Nuclear, 200 µL, 5 Ci/g) and
a catalytic amount of H2SO4. The solution was allowed to stand

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at room temperature for 15 min. In model studies with D₂O, 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.

(2S)-[2-2H₂, 6-2H]Cyclohexanone (3). The tritiated cyclohexanone prepared as above was added to a solution of AAD in 50 mM potassium phosphate buffer in D₂O (50 mL, 1000 international units). The enzyme is known to catalyze the exchange of the 2-pro-R hydrogen 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 of this material 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.

Chiral Acetic Acid (8). Kuhn–Roth oxidation of the 1,1-diphenylhexanol (33 mg, 1.6 mmol) was carried out by stirring with CrO₃ (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% H₃PO₄ 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 H₂O and LiBEt₃D instead of D₂O and LiBEt₃H), it proved more convenient to invert the configuration of the 6-position of diol 5. Reaction of 5 (20 mg, 0.079 mmol) with PF₃ (74 mg, 0.22 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, CH₂Cl₂ eluent, Rf 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 (350 mg, 0.84 mmol) was reacted with 1.5 equiv of camphorl chloride in pyridine to produce the camphane ester of the primary alcohol. The crude product was dehydrated by treatment with CH₃SO₂Cl in CH₂Cl₂ at 0 °C, and the camphane ester as its sodium salt. This could be stored indefinitely at 0 °C for 15 min, and the camphane ester of the 6,6-diphenyl-5-hexen-1-ol (247 mg, 0.57 mmol) was isolated by preparative thin-layer chromatography (silica gel: Rf 0.56 (benzene/Et₂O, 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, the 270-MHz NMR spectrum of this ester in the presence of Eu(dpm₃) in CDCl₃ 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 camphane ester was prepared from 2,2,6,6-tetra-deutero-cyclohexanone, 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.

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