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Enantioselective Synthesis of Isotopically Labeled Homocitric Acid Lactone

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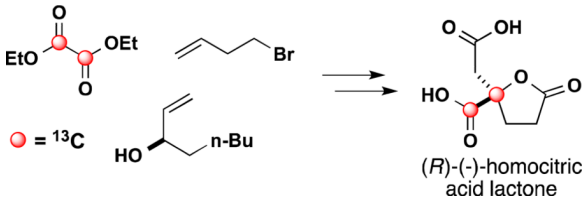
Abstract



A concise synthesis of homocitric acid lactone was developed to accommodate systematic placement of carbon isotopes (specifically ^{13}C) for detailed studies of this co-factor. This new route uses a chiral allylic alcohol, available in multi-gram quantities from enzymatic resolution, as a starting material, which transposes asymmetry through an Ireland-Claisen rearrangement.

Homocitric acid lactone (**1**, Scheme 1) in the hydrolyzed ring-open form is an essential component of nitrogenase, the enzyme responsible for fixation of atmospheric nitrogen by bacteria and archaea. Homocitrate is incorporated into the nitrogenase Fe-Mo cofactor before its introduction into the nifDK protein matrix.[1] Homocitrate coordinates to the Mo site of the Fe-Mo cofactor in a bidentate fashion through alkoxide and carboxylate functionalities (atoms 1 and 6 respectively, Scheme 1), creating a 5-membered ring in one of the most complex metal cofactors found in nature.[2] Replacement of homocitrate by citrate, a deletion of a single methylene group, reduces N_2 reduction activity to only 7% that of wild-type enzyme.[3] Shah and coworkers studied the ability of a wide range of homocitrate analogues to reconstitute nitrogenase activity in mutants lacking homocitrate, and concluded that the minimal requirements were the hydroxyl group, the 1- and 2-carboxyl groups, and the *R* configuration of the stereogenic center.[4] Based on these observations, we devised a synthesis of **1** that incorporated ^{13}C-labels at these positions. Specific labeling of these functionalities would open up a number of NMR, IR, and EPR/ENDOR experiments related to nitrogenase biosynthesis and mechanism.

The difficulty of isolating homocitric acid from natural sources has prompted many synthetic efforts directed toward this compound to support biological studies. Two racemic syntheses have been reported, the latter of which employs a biomimetic approach that efficiently produces (±)-**1** in three steps.[5] Several approaches using chiral pool materials as auxiliaries or for semi-synthesis offer access to (*R*)-**1** and (*S*)-**1** with varying degrees of

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Supporting Information Available Experimental procedures, compound characterization data for all new compounds, and HPLC analysis of **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

efficiency.[6] Finally, two approaches employ asymmetric catalysis for installation of the stereogenic center in **1**.[7] Although these routes are effective for making enantiomerically enriched **1**, they are not easily translated to allow for selective incorporation of carbon isotopes.

We required an efficient, scalable synthesis that would allow for the systematic placement of ^{13}C isotopic labels at positions 5 and 6 on **1**. We initially attempted the route disclosed by Pansare, which assembles (*R*)-**1** from three modular fragments and employs ephedrine as a chiral auxiliary. This route was unsuitable for two reasons. First, the assembly of the morpholinedione[7b] required oxalyl chloride, which worked as described to make the unlabeled intermediate. However, it would be difficult to handle oxalyl chloride as a labeled reagent prepared from oxalic acid, given its air sensitivity. Secondly, and more importantly, ephedrine is a controlled substance that has become difficult to acquire. Our laboratory was unable to identify a vendor that would ship useful quantities to meet our synthetic requirements as a chiral auxiliary. Attempts to circumvent this problem with other amino alcohols (not shown) were not fruitful.

Mindful of the limited commercial availability of isotopically labeled starting materials, a thorough retrosynthetic analysis of **1** (Scheme 1) identified diethyloxalate as the optimal precursor (Scheme 1). This material is readily available with both carbonyl carbons as ^{13}C in high abundance. Moreover, it can be synthesized from per-^{13}C ($_D$)-glucose by an established procedure for large-scale preparations. Use of an ester enolate Claisen rearrangement[8] would install a fragment in the appropriate oxidation state and would also derive asymmetry from a chiral allylic alcohol. In this case, (±)-**4** is commercially available and easily resolved with *C. antarctica* lipase in high yield and with high selectivity.[9] Additionally, alkene placement following this rearrangement is ideal for installing carboxylic acid functionality by oxidative cleavage at a late stage in the synthesis. It is important for this oxidation to occur in the last step because small molecules with multiple carboxylic acid groups can be difficult to purify and isolate. Importantly, if additional labels are required for future studies, they can easily be incorporated into **4** and **6**. For our purposes, carbon atoms 5 and 6 would be labeled by using $(^{13}C)_2$-diethyl oxalate. Herein we present an efficient asymmetric synthesis of (*R*)-**1** that is scalable, suitable for preparing labeled analogs, and derives asymmetry from an enzyme-catalyzed kinetic resolution.

The Claisen rearrangement substrate **3** was assembled in a short sequence (Scheme 2). Commercially available allylic alchol (±)-**4** was resolved to afford (−)-**4** with acrylic bound *C. antarctica* lipase and vinyl propionate in 94% yield (based on a 50% conversion) with enantioselectivity of 99:1 as judged by HPLC analysis of the corresponding 3,5-dinitrobenzoate ester. Diethyl oxalate was treated with freshly-prepared 3-butenylmagnesium bromide to produce **7** in 86% yield,[10] which was then reduced with $NaBH(OAc)_3$ and O-alkylated with PMB-trichloroacetimidate. Saponification and coupling to (−)-**4** yielded **3** in 76% yield as a 50:50 mixture of diastereomers. Similar yields were observed when isotopically labeled diethyl oxalate was used as the starting material (Scheme 2, shown in brackets).

Claisen rearrangement of **4** proceeded in high yield and with a high degree of stereochemical transposition. After initial attempts with various amide bases including LDA and LiHMDS, treatment of **3** with KHMDS followed by silylation with TMSCl and warming to room temperature proceeded with high conversion. This was followed by methylation with CH_3I/K_2CO_3 to produce methyl ester **2** in high yield with no detectable trace of the *Z*-isomer. The enantiomeric ratio was determined to be 95:5 by analysis of a subsequent intermediate (Scheme 4, vide infra), demonstrating only slight loss of enantiomeric purity from (−)-**4**. The sense of stereochemistry of this reaction is explained by

a chair transition state emanating from the *Z*-enolate (Scheme 3). The slight loss of enantiomeric purity can potentially be explained by small quantities of the *E*-enolate of **9**. The high selectivity for formation of the *E*-alkene isomer suggests that transition state **10** is significantly lower in energy than the diastereomeric conformation in which the allylic *n*-butyl group is in an axial position. Although acid **12** could be isolated in high purity by column chromatography, it was taken through to methyl ester **2** in 96% overall yield from **3**.

Conversion of **2** to homocitric acid lactone was straightforward (Scheme 4). Initial attempts at direct oxidative cleavage of **2** followed by removal of the PMB group were low-yielding and required difficult separation of the tri-acid from the aqueous reaction medium. We reasoned that cyclohexene **13** would require fewer oxidizing equivalents and might be cleaved under milder conditions. Diene **2** was smoothly cyclized using Grubbs's second generation catalyst and the PMB group was removed in high yield. At this stage, the enantiomeric purity was measured at 95:5 er (90% ee) by conversion to 3,5-dinitrobenzoate ester **14**, which was analyzed by chiral HPLC. Cyclohexene **14** was oxidized, hydrolyzed and dehydrated to provide **1** in 82% yield. The optical rotation of **1** was −21.4°, which is in agreement with the literature value.

In conclusion, a scalable enantioselective synthesis of homocitric acid lactone, (−)-**1,** in 9 steps including and Ireland-Claisen rearrangement and a ruthenium catalyzed oxidative cleavage is presented. This sequence allows for the incorporation of carbon isotopes at the carbon atoms proximal to molybdenum in the Mo-Fe complex. Isotopically labeled homocitrate analogs made by this route will aid efforts aimed at further elucidating the mechanism and activity of bacterial and archeal nitrogenase.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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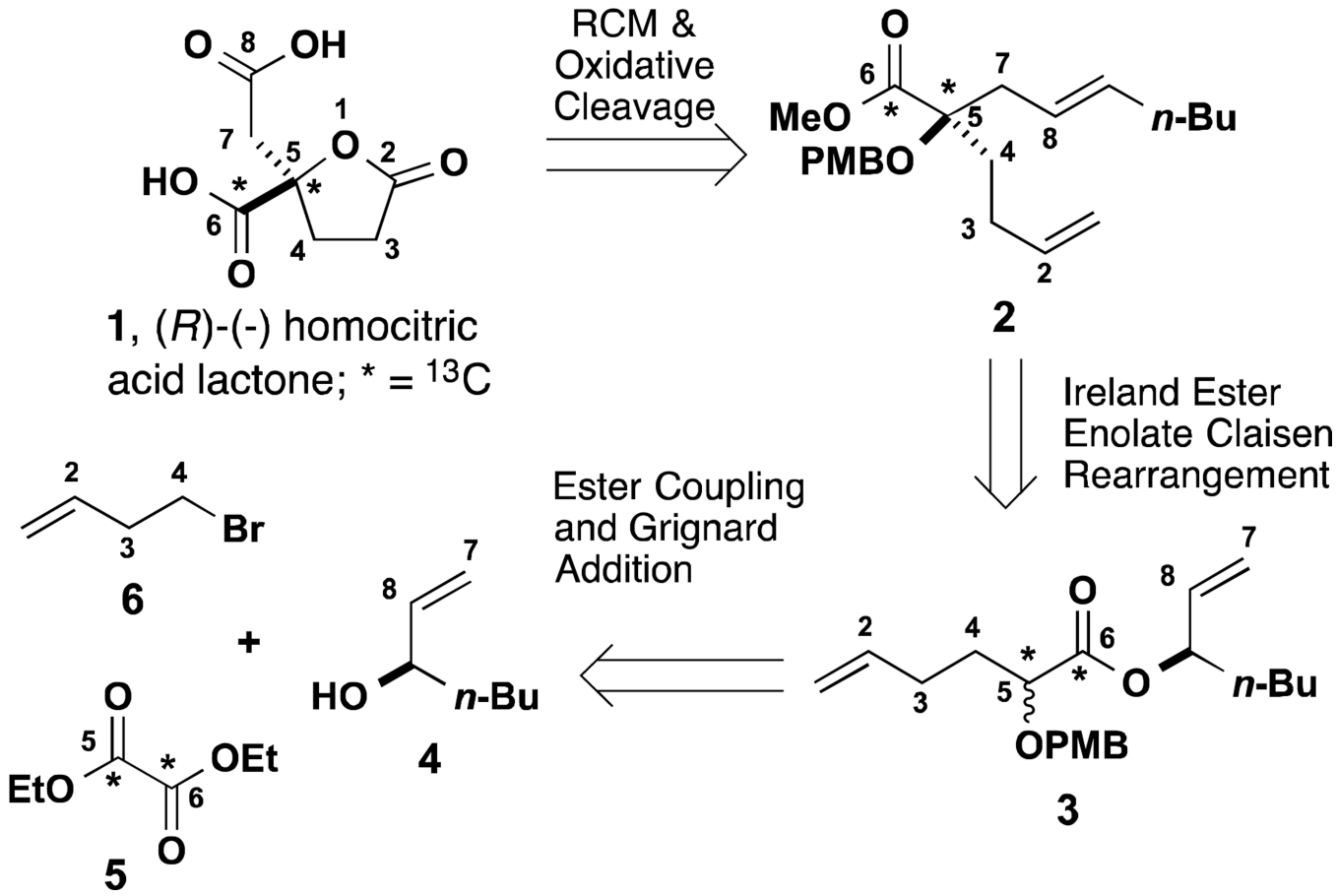
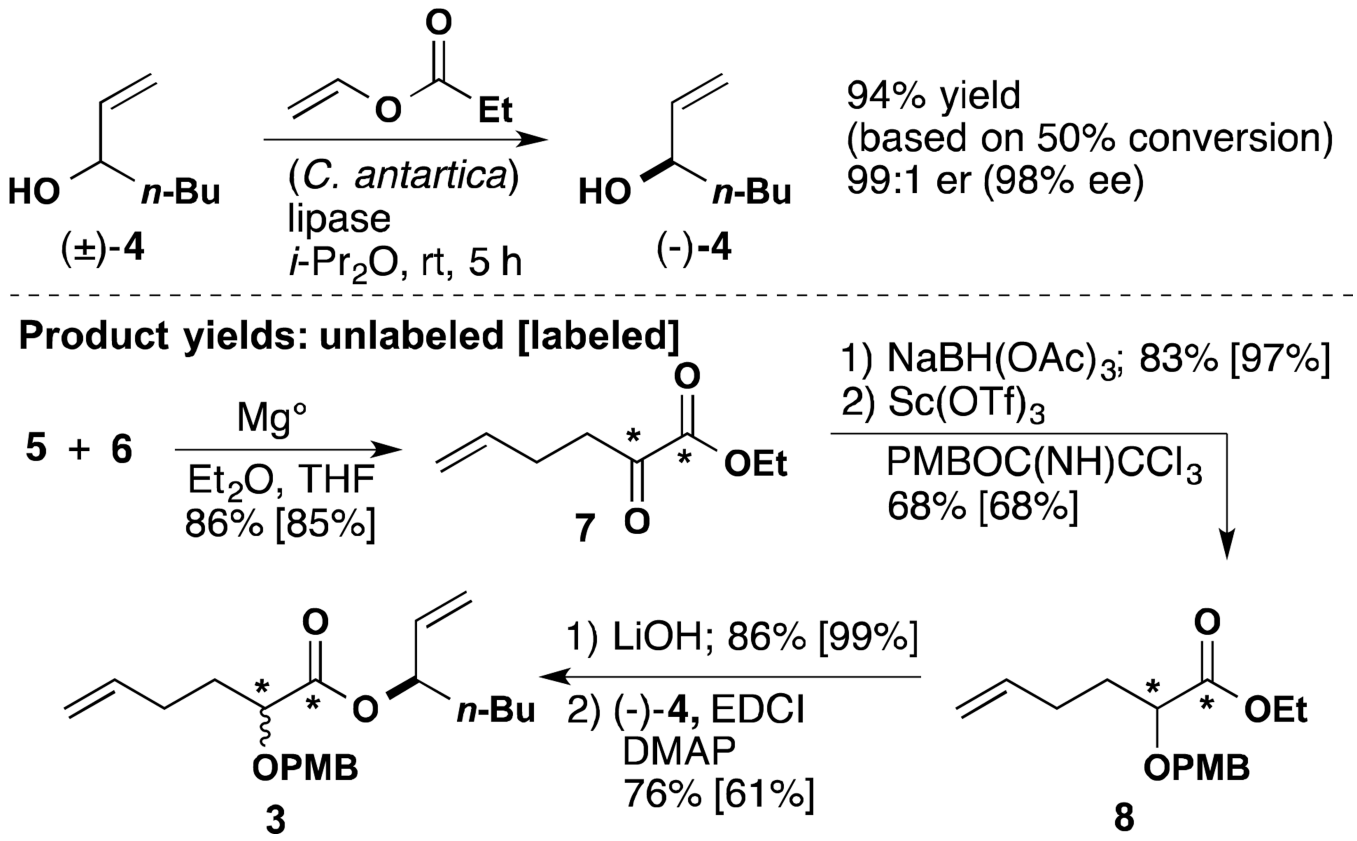
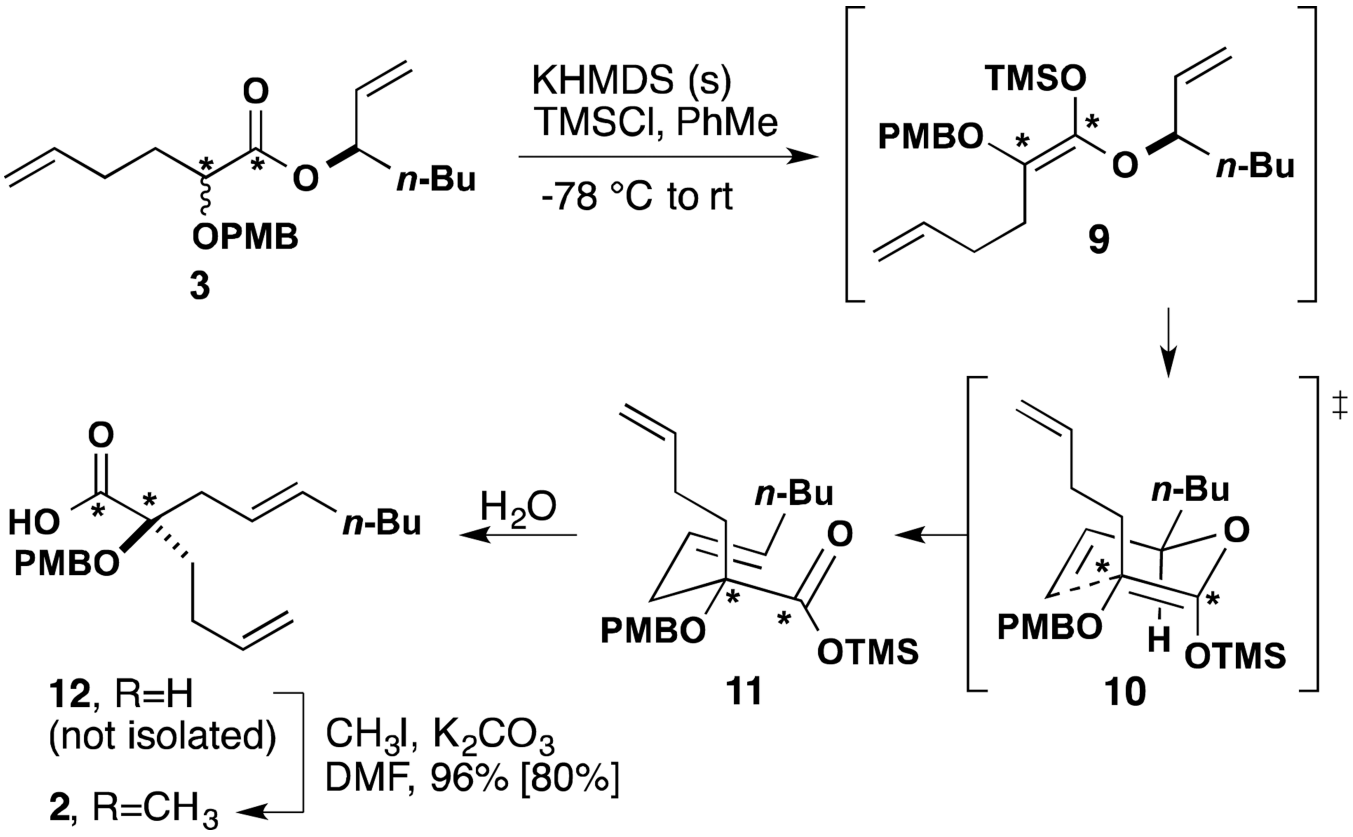


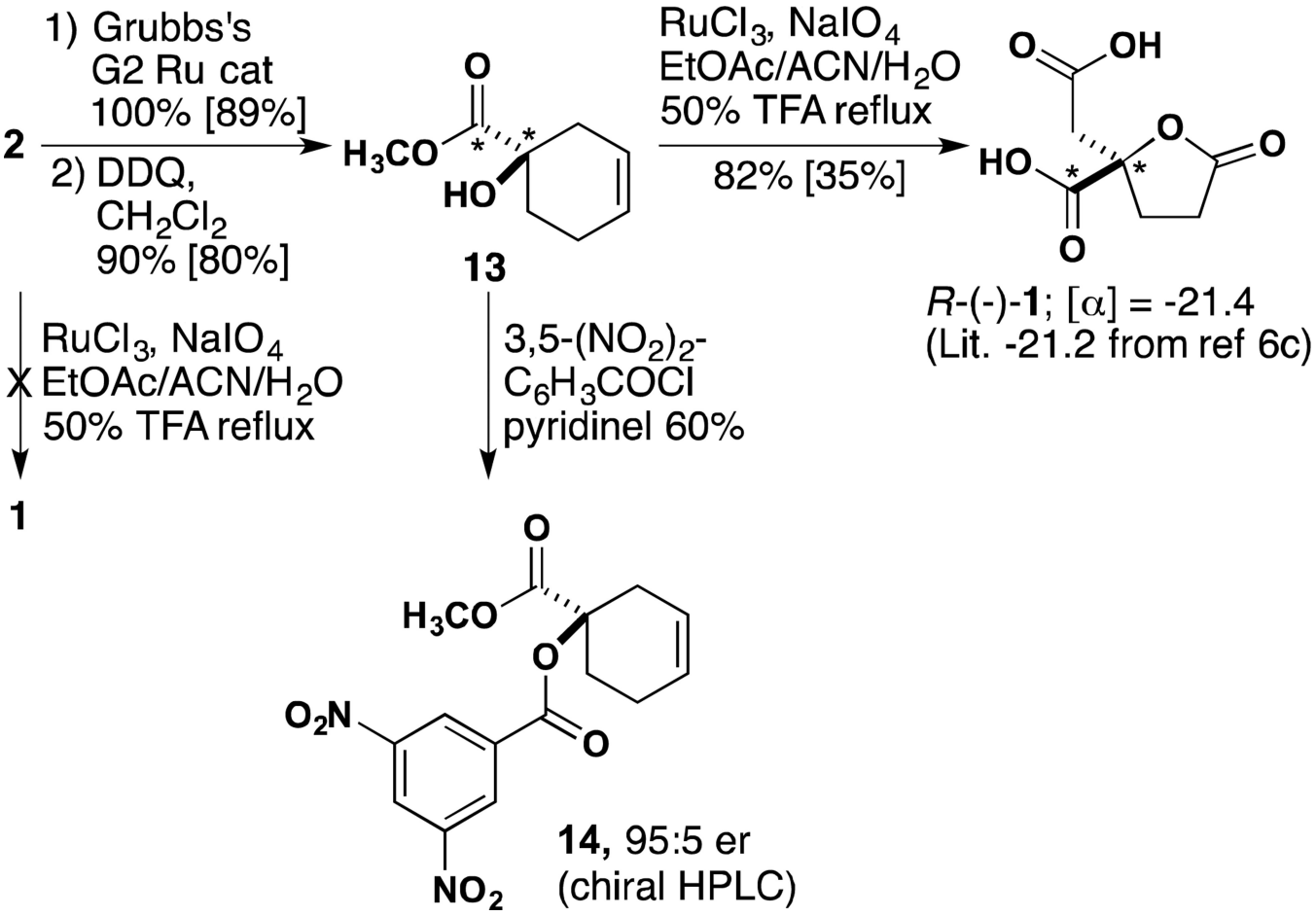
Scheme 1.
Retrosynthetic analysis of (*R*)-1



Scheme 2.
Synthesis of Ireland-Claisen precursor. Yields in brackets refer to ^{13}C-labeled intermediates.



Scheme 3.
Ireland ester enolate Claisen Rearrangment. Yields in brackets refer to ^{13}C-labeled intermediates.



Scheme 4.

RCM, late stage oxidation, and analysis of enantiopurity. Yields in brackets refer to ^{13}C-labeled intermediates and products.