81.38 and 77.48 (alkyne carbons), 69.03 ($CH_2O$), 31.21, 28.80, 28.40, 28.21, 22.41, 21.44, 18.48, 14.86, and 13.91 (alkane). Anal. Calcd for $C_{18}H_{26}O_3S$: C, 67.08; H, 8.07; S, 9.94. Found: C, 66.96; H, 8.07; S, 10.01.

**4-*tert*-Butylcyclohex-1-yl *p*-toluenesulfonate**: yield 93%; obtained as semisolid[8] from mixture of isomers of 4-*tert*-butylcyclohexan-1-ol; $^{13}C$ NMR ($CDCl_3$) $\delta$ 144.06, 144.0, 134.58, 134.47, 129.49, 127.39 (Ar carbons), 82.11 and 78.86 (CHO), 46.79 and 46.25 (*CH*-*t*-Bu), 32.59, 32.16, 31.87, 30.97, 27.23, 27.12, 25.23, 22.36, 21.25, and 20.84 (alkane).

**4-Hexyn-2-yl *p*-toluenesulfonate**: yield 97%; thick oil; $^1H$ NMR ($CDCl_3$) $\delta$ 1.34 (d, 3 H, $CH_3CH$), 1.69 (d, $J$ = 2.5 Hz, 3 H, $CH_3C\equiv$), 2.44 (s, 3 H, Ar $CH_3$), 4.60 (sextuplet, 1 H, CH), 7.56 (AA'BB', 4 H, Ar H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 144.49, 133.93, 129.62, 127.54 (Ar carbons), 78.40 and 77.40 (alkyne carbons), 77.67 (CH), 26.53 ($CH_2$), 21.35, 19.84, and 3.16 (alkane). Anal. Calcd for $C_{13}H_{16}O_3S$: C, 61.90; H, 6.35; S, 12.70. Found: C, 61.58; H, 6.18; S, 12.72.

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## Biphasic One-Pot Synthesis of Two Useful and Separable Compounds Using Cofactor-Requiring Enzymatic Reactions: Glutamate Dehydrogenase Catalyzed Synthesis of L-α-Aminoadipate Coupled with Alcohol Dehydrogenase Catalyzed Synthesis of a Chiral Lactone

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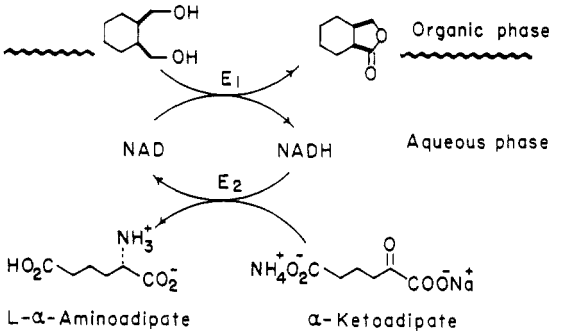
The most generally useful system available for in situ regeneration of NAD(P) from NAD(P)H for use in practical scale enzyme-catalyzed asymmetric oxidation is considered to be the system using 2-oxoglutarate/glutamate dehydrogenase (GluDH).[3,4] Several 70–100-mmol scale syntheses in aqueous solution or in a mixture of water and hexane have been demonstrated.[3,4] The regeneration system, however, requires a second enzyme, (GluDH), and 2-oxoglutarate, the glutamate product of which is not very useful, being currently produced in large quantities by fermentation, and also complicates workup.

We describe here an improved practical procedure which should reduce the cost substantially. This procedure involves an NAD-requiring asymmetric oxidation of a *meso*-diol catalyzed by horse liver alcohol dehydrogenase (HLADH) coupled with an NAD(P)H-requiring asymmetric reductive amination of 2-ketoadipate catalyzed by GluDH (Scheme I). Each of the two enzymatic reactions is *synthetically useful* and generates the proper form of the cofactor for the other. The reactions are carried out

**Scheme I. Coupling of Two Nicotinamide Cofactor-Requiring Enzymatic Syntheses in a Biphasic System. E1, Horse Liver Alcohol Dehydrogenase; E2, Glutamate Dehydrogenase**




in a water–organic solvent biphasic system where the chiral lactone produced is removed from the aqueous phase to separate from the other water soluble product (L-α-aminoadipate) and to minimize product inhibition.[5] The unnatural substrates used in the reactions are either readily available or easily prepared. The preparation of 2-ketoadipate is straightforward and the starting materials used for the preparation are relatively inexpensive. 2-Ketoadipate is a good substrate for GluDH[6] and the product L-α-aminoadipate produced is a component of a linear tripeptide used in the enzymatic synthesis of isopenicillin N and analogues, a precursor of penicillin antibiotics.[7] Although several methods have been described for the preparation of L-α-aminoadipate ($60/g from Sigma), each of them requires several steps and the procedures are quite tedious.[8]

The *meso*-diol used in this representative synthesis is just intended to illustrate the strategy. As shown in Jones work, a number of *meso*-diols have been enantioselectively oxidized to chiral lactones catalyzed by HLADH.[9] They should be good candidates for this system.

Although product inhibition is a severe problem in large-scale HLADH-catalyzed oxidation of alcohols, it can be lessened using *meso*-diols as substrates. The favorable kinetic parameters ($K_m/K_i < 1$) for the diols together with the use of a biphasic system to minimize product inhibition and to accomplish good separation and high turnover numbers for the cofactor and the enzymes allow preparation of chiral lactones on large scales.[3] Further, the NAD regeneration system produces a useful and separable product concurrently and thus reduces the operation cost significantly. The cofactor used in the process is no longer an expensive component.

The synthetic methodology illustrated here should be compatible with that based on non-cofactor-requiring lipase-catalyzed hydrolysis of *meso*-diol diesters which produces products with lower optical purity (67–87%) and requires another chemical step to prepare the lactone.[10]

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## Experimental Section

The materials required for this work are obtained from the respective companies as follows: diethyl oxalate and diethyl glutarate, Aldrich; glutamate dehydrogenase and other biochemicals, Sigma. Instruments required are the Beckman DU-6 UV–vis spectrophotometer for enzyme assays where NADH consumption is monitored at 340 nm. An EM-390 (90 MHz) instrument was used for $^1$H NMR analysis.

**Enzyme Assays.** The method used herein is a modification of the procedures described previously.[3] Determination of the Michaelis–Menten constants is done as follows. Starting with stock solutions of 10 mM NADH, 100 mM 2-ketoadipate, and triethanol amine buffer (pH 7.8, 50 mM) containing ADP (1 mM), ammonium acetate (125 mM), and GluDH (type III dissolved in water, 400 U/mL) cuvette solutions with final volume of 1 mL are made. Thus, to a 1-mL buffer solution was added 25 $\mu$L of NADH, 10 $\mu$L of enzyme, and various amount of substrate (10 to 50 $\mu$L). The change of absorbance at 340 nm ($\epsilon$ at 340 nm for NADH = 6.22 $mM^{-1}$ $cm^{-1}$) was then recorded and used for calculation of velocities. Double reciprocal plots of velocity vs. substrate concentrations gave $K_m$(2-ketoadipate) = 5.3 mM and $V_{max}$ = 3 U/mg, where 1 U is defined as 1 $\mu$mol of product formed per min.

**Preparation of 2-Ketoadipate.** The method used here is a modified procedure of that used in the preparation of 2-oxoglutarate.[11] To 32 mL of diethyl glutarate in 400 mL of anhydrous ether and 15 mL of ethanol is added 12.25 g of sodium ethoxide. The mixture was refluxed for 60 min to allow for dissolution of sodium ethoxide followed by rapid addition of diethyl oxalate (23.6 mL) while vigorously stirring the solution. The reaction is allowed to proceed for 3.5 h and then the mixture concentrated to an oil. The oil is taken up in 275 mL of 3.5 N HCl and the solution quickly extracted with ether until the aqueous layer is almost colorless (4 × 25 mL). The ether is dried over anhydrous $Na_2SO_4$, then concentrated under vacuum. The resulting oil is taken up in 135 mL of concentrated HCl and the mixture kept at room temperature for 18 h. The solution is evaporated to dryness. The solid is dissolved in acetone and decolorized with activated carbon. The acetone is evaporated and the solid dissolved in water and adjusted to pH 4 with NaOH solution. Precipitation is brought about by addition of 2-propanol until precipitation is no longer observed. The mixture is then stored for 1 h at 0–4 °C, the precipitate is filtered off, and further precipitation brought about with more 2-propanol. This step is repeated until precipitate is no longer observed (usually 4–5 volumes of 2-proponal are required). The brown product can be recrystallized by repeating the above procedure. The 2-ketoadipate monosodium salt obtained after recrystallization is slightly tan and has the same $^1$H NMR shifts as a standard sample from Sigma. Yield, 85–90%; mp 175–180 °C dec; $^1$H NMR ($D_2O$) $\delta$ 2.48 (t, 2 H, $C_3$-H), 2.45 (t, 2 H, $C_5$-H), 1.87 (m, 2 H, $C_4$H).

**Preparation of L-$\alpha$-Aminoadipate and (+)-(1*R*,6*S*)-*cis*-8-Oxabicyclononan-7-one.** The procedure is similar to that described previously.[3] To a 900-mL solution containing 2-ketoadipate monosodium salt (50 mmol) and *cis*-1,2-bis(hydroxymethyl)cyclohexane (50 mmol) is added concentrated $NH_4OH$ until pH 8.1 is reached. The enzymes GluDH (400 U) and HLADH (70 U) and the cofactor NAD (1 mmol) are added. Hexane (1 L) is carefully added to the solution without disturbing the water layer. Two days later 40 U GluDH, 100 U of HLADH, and 25 mmol of 2-ketoadipate monosodium salt (in 100 mL of water with pH adjusted to 8.1 with $NH_4OH$) are added. Two days later, the reactions are complete according enzymatic assays. The residual activities of HLADH and GluDH are about 47% and 60%, respectively, of their original activities. The two layers are separated and the aqueous layer is acidified to pH 4 and stored in refrigerator overnight to give the precipitated L-$\alpha$-aminoadipic acid in 60–70% yield. The aqueous solution recovered after filtration is then concentrated to 500 mL and stored at 0 °C for 5 h allowing for further crystallization, and approximately 15% more of theoretical yield is obtained (overall yield = 84%): mp 205–206 °C (same as lit.[8,12] mp 205–210 °C); $^1$H NMR ($D_2O$) $\delta$ 3.8 (t, 1 H, H-2), 2.48 (t, 2 H, H-5), 1.83 (m, 4 H, H-3,4), ($D_2O$, NaOD) $\delta$ 3.2 (t, 1 H, H-2), 2.19 (t, 2 H, H-3), 1.55 (m, 4 H, H-3,4); $[\alpha]^{23}_D$ +24° (*c* 5, 5 N HCl), $[\alpha]^{23}_D$ +12° (*c* 5, 0.5 N NaOH) [lit.[8] +24 (*c* 5, 5 N HCl)]. After filtration of the $\alpha$-aminoadipic acid, the solution is extracted with ether (4 × 55 mL). The ether solution is dried over $MgSO_4$ and added to the reaction hexane layer which has been previously dried. The collected organic layers are concentrated in vacuo. The oil is further purified by distillation, bp 80 °C (0.9 mmHg); $[\alpha]^{23}_D$ +48.8 (*c* 0.5, $CHCl_3$) (100% ee) [lit.[13] $[\alpha]^{25}_D$ +48.8 (*c* 0.5, $CHCl_3$)]; $^1$H NMR ($CDCl_3$) $\delta$ 0.8–2.8 (m, 10 H), 3.8–4.4 (m, 2 H), same as literature values.[13] Yield, 79%.

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# *Communications*

### A Novel Synthesis of $\beta$-Trichlorostannyl Ketones from Siloxycyclopropanes and Their Facile Dehydrostannation Affording 2-Methylene Ketones

*Summary:* Site-selective ring cleavage of siloxycyclopropanes **2** with stannic chloride ($SnCl_4$) leads to good yields of $\beta$-trichlorostannyl ketones **3**. Subsequent treatment of **3** with dimethyl sulfoxide ($Me_2SO$) in chloroform at 60 °C results in the facile dehydrostannation to give good yields of 2-methylene ketones **4**.

*Sir:* Although $\alpha$-metallo ketones or metal enolates have found widespread use in organic synthesis, the use of $\beta$-metallo ketones **1** in synthesis is only just being realized.[1] One of the main reasons for this lies in their limited accessibility, and, in this context, we have been interested in the electrophilic ring opening of siloxycyclopropanes **2** with metal ions as a route to **1**.[2-4] We report here a synthesis and a highly efficient dehydrostannation reaction

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