1 PERKIN

# Asymmetric reduction of ethynyl ketones and ethynylketoesters by secondary alcohol dehydrogenase from *Thermoanaerobacter ethanolicus* †

**Christian Heiss ‡[a] and Robert S. Phillips *[abc]**

[a] *Department of Chemistry,* [b] *Department of Biochemistry and Molecular Biology, and* [c] *Center for Metalloenzyme Studies, University of Georgia, Athens, Georgia 30602-2556, USA. Phone: (706) 542-1996; Fax: (706) 542-9454; E-mail: rsphillips@sunchem.chem.uga.edu*

***Received (in Cambridge, UK) 17th February, Accepted 23rd May 2000***
***Published on the Web 10th July 2000***

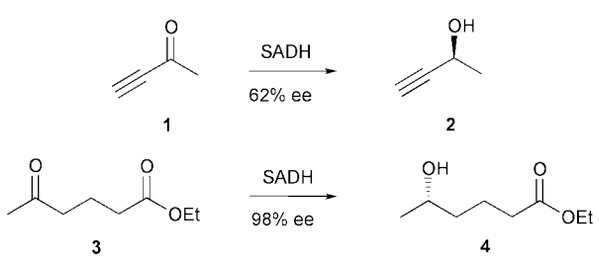


Secondary alcohol dehydrogenase (SADH) from *Thermoanaerobacter ethanolicus*, an NADP-dependent, thermostable oxidoreductase, reduces ethynyl ketones and ethynylketoesters enantioselectively to the corresponding propargyl (propargyl = prop-2-ynyl) alcohols. Ethynyl ketones, in general, are reduced with moderate enantioselectivity (with the exception of 4-methylpent-1-yn-3-one, which gives the (*S*)-alcohol with >98% ee). Although ethynyl ketones bearing a small (up to *n*-propyl) alkyl substituent are reduced to (*S*)-alcohols, larger ethynyl ketones give (*R*)-alcohols. In contrast, ethynylketoesters are converted to (*R*)-ethynylhydroxyesters of excellent optical purity. Unexpectedly, isopropyl ethynylketoesters give higher chemical yields and higher enantioselectivities of ethynylhydroxyesters than methyl or ethyl ethynylketoesters. The optically pure ethynylhydroxyesters may serve as useful chiral building blocks for asymmetric synthesis.

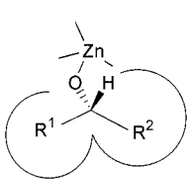
Due to their high chemoselectivity, their environmentally benign character, and their increasing availability as a result of rapid progress in genetic engineering, enzymes play an increasingly important role in organic synthesis. Dehydrogenases from various sources have been widely applied to the asymmetric reduction of ketones to give optically active secondary alcohols. Among these enzymes, horse liver alcohol dehydrogenase (HLADH)[1] and yeast alcohol dehydrogenase (YADH)[2] have been the most extensively studied. Unfortunately, both HLADH and YADH exhibit some disadvantages that limit their routine usage in asymmetric synthesis. HLADH gives good results only with cyclic ketones, and YADH reduces ketones with usually low, unreproducible, and hardly predictable enantioselectivity. Furthermore, both enzymes have low thermal stability and tend to lose their activity in the presence of organic solvents or upon immobilization. In contrast, the NADP-dependent alcohol dehydrogenase from *Thermoanaerobium brockii* (TBADH),[3] a highly thermophilic, ethanologenic bacterium, is stable to these conditions and reduces ketones with excellent enantioselectivity.

We have been studying a very similar NADP-dependent enzyme, secondary alcohol dehydrogenase (SADH) from *Thermoanaerobacter ethanolicus*, which was first described by Bryant *et al.*[4] We found that the enantioselectivity with which it reduces ketones depends upon temperature,[5] the nature of the employed cofactor,[6] and substrate structure.[7] The reduction of small ketones like butan-2-one, but-3-yn-2-one (**1** in Scheme 1), and pentan-2-one affords alcohols of low optical purity whereas larger ketones and ketoesters (*e.g.* **3**) give alcohols with ee's ranging from 92 to 99%.

This behavior of SADH is similar to results obtained from reductions with TBADH. Keinan *et al.*[3] put forth a model of




**Scheme 1**




**Scheme 2**

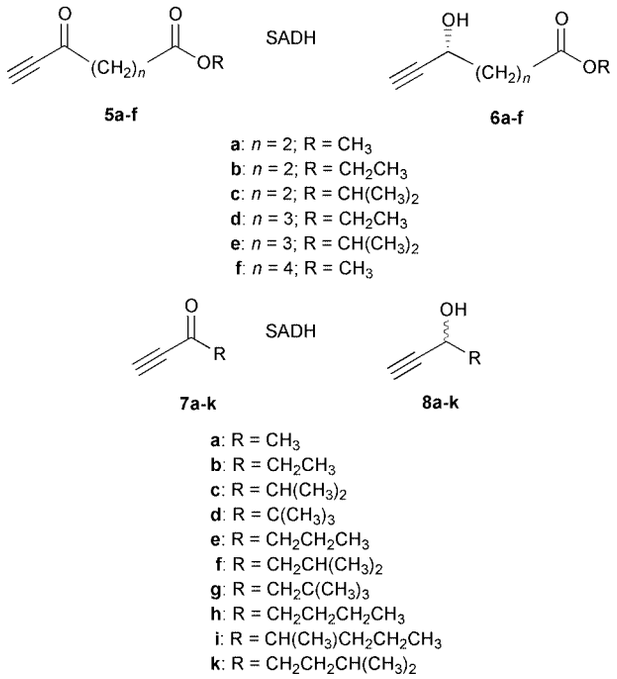
the active site of TBADH that proposed a large and a small alkyl binding pocket (Scheme 2). They suggested that the substrate binds in an orientation determined by the best fit of the two groups attached to the carbonyl into these pockets. The nicotinamide cofactor then transfers its *pro-R* hydride to the *Re* face of the ketone, producing secondary alcohols with (*S*)-configuration (assuming that the larger substituent has higher Cahn–Ingold–Prelog priority), *i.e.*, the enzyme follows Prelog's rule.[8]

Based on the high degree of sequence identity (~99%) of TBADH and SADH,[9] Keinan's active site model can also be applied to SADH. On this basis, the high enantioselectivity with which SADH reduces the various ketoesters together with the low ee in the case of but-3-yn-2-one led us to believe that if the triple bond in the ketone is "counterbalanced" with an ester function on the opposite side of the carbonyl as in **5** (Scheme 3), it could be forced into the small pocket giving rise to (*R*)-hydroxyalkynoates (**6**) in high optical purity. These repre-

† Experimental details for the preparation of compounds **7a**–**k** are available as supplementary data. For direct electronic access see http://www.rsc.org/suppdata/p1/b0/b001329n/

‡ Present address: McNeil Specialty Products Company, Athens, Georgia.

DOI: 10.1039/b001329n




**Scheme 3**

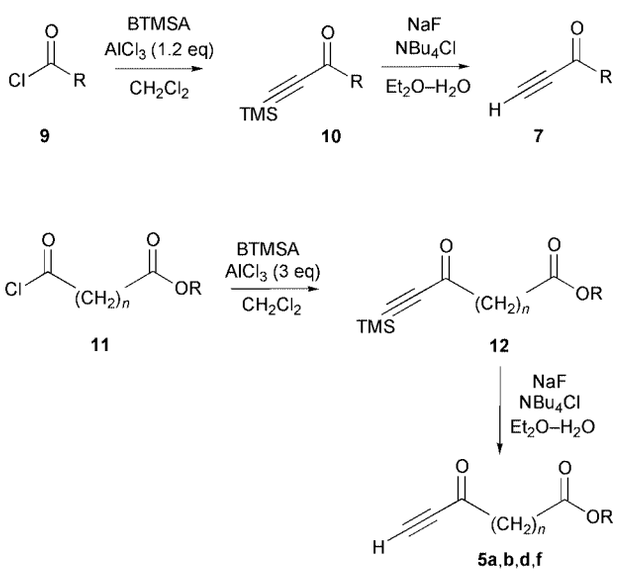
sent potentially useful chiral building blocks for asymmetric syntheses, since they possess three functional groups that can undergo further transformation independently of each other.

In order to obtain a more detailed model and greater predictability of the scope and stereochemistry of SADH-reductions, we also subjected simple alkyl-substituted ethynyl ketones (**7**) to reduction by the enzyme. We were especially interested in the minimum size of the alkyl substituent required to give predominantly (*R*)-alcohols (**8**), as well as how branching of the alkyl group would affect the reaction.

## Results and discussion

### Synthesis of ethynyl ketones and ketoesters

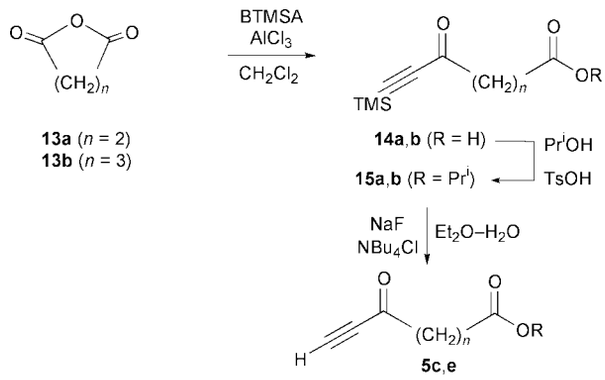
The ethynyl ketones (**7a**–**k**) were prepared by a modification of the method by Walton and Waugh[10] (Scheme 4). Bis(trimethylsilyl)acetylene (BTMSA) was acylated with the appropriate acyl chloride (**9**) in the presence of $AlCl_3$ to give the TMS-protected ethynyl ketones (**10**), followed by phase-transfer catalyzed removal of the TMS group with NaF and $NBu_4Cl$. Essentially




**Scheme 4**

the same procedure[11] was employed in the synthesis of the methyl and ethyl esters, **5a**,**b**,**d**, and **f**.

The isopropyl esters, **5c** and **e**, had to be made in a different manner (Scheme 5) because the acylation was accompanied by formal ester hydrolysis (presumably by an AAL1 type mechanism). The resulting acids, **14a** and **b**, however, could easily be re-esterified with propan-2-ol yielding the TMS-protected ethynylketoesters, **15a** and **b**, and since they are readily prepared from succinic or glutaric anhydride (**13a** and **b**),[12] respectively, this offered a convenient route to the isopropyl ethynylketoesters.




**Scheme 5**

### Enzyme reactions

The enzyme reductions were carried out in aqueous buffer containing 15% propan-2-ol, which serves as both co-solvent and sacrificial substrate for coenzyme regeneration. We found that ethynyl ketones require higher concentrations of SADH for reaction than unconjugated ketones because they decompose slowly ($t_{1/2} \sim 1$ day) under the reaction conditions, and, more significantly, they cause irreversible inactivation of the enzyme. We ran the reduction of isopropyl 4-oxohex-5-ynoate (**5c**) and 4-methylpent-1-yn-3-one (**7c**) with varying amounts of enzyme and found that the minimum concentration of SADH to give reasonable chemical conversions (>90%) is 54 U $cm^{-3}$ for **5c** and less than 10 U $ml^{-1}$ for **7c** (compared to 2 U $cm^{-3}$ for unconjugated ketones[7]). The enzyme inactivation is possibly caused by conjugate addition of a nucleophilic residue in the active site of the enzyme to the triple bond of the substrate, which is in conjugation with the carbonyl. Product inhibition can be excluded as an explanation because the inactivation takes place even if no measurable product is formed.

Fortunately, the need for large amounts of enzyme poses no major problem since Burdette *et al.* recently cloned SADH and prepared a plasmid to overexpress it in *E. coli*.[9] This new source offers major advantages over cells of *T. ethanolicus*: *E. coli* does not require high temperature anaerobic growth conditions and can therefore be grown easily in large quantities; SADH is produced in larger amounts in the recombinant cells than in *T. ethanolicus*; and, due to the thermal stability of SADH, its purification is greatly simplified because the *E. coli* proteins can be selectively precipitated by heat treatment of the cell-free extract, resulting in sufficiently pure enzyme for the reductions.

The reductions of the ethynyl ketones (**7a**–**k**) are summarized in Table 1. Initially, increasing the size of the alkyl substituent from methyl to ethyl to isopropyl (compounds **7a**–**c**) leads to higher amounts of the (*S*)-enantiomer, with the latter being the only observed product when the alkyl group is isopropyl. Similar behavior was observed in TBADH-reductions of dialkyl ketones by Keinan for TBADH,[3] and was explained by assuming that the small pocket has higher affinity for hydrophobic groups. The observations that **7c** is reduced with an ee >98% and that **7i** does not react indicate that the geometry of the

**Table 1** SADH reduction of ethynyl ketones

| Compound | Yield (%) | Abs. conf. | Ee (%) | Reaction time/h |
|---|---|---|---|---|
| **7a**[a] | 30 | *S* | 60 | 0.5 |
| **7b** | 32 | *S* | 80 | 1 |
| **7c** | 50 | *S* | >98 | 2 |
| **7d** | — | *S* | 85[b] | 24 |
| **7e** | 28 | *S* | 51 | 1 |
| **7f** | 20 | *R* | 50 | 1 |
| **7g** | — | *R* | 66[b] | 7 |
| **7h** | 32 | *R* | 42 | 4 |
| **7i** | — | — | — | — |
| **7k** | 55 | *R* | 80 | 5 |

[a] From ref. 7. [b] No product was isolated but the absolute configuration and ee were determined by GC.

**Table 2** SADH reduction of ethynylketoesters

| Compound | Yield (%) | Abs. conf. | Ee (%) | Reaction time/h |
|---|---|---|---|---|
| **5a** | 35 | *R* | 82 | 2.5 |
| **5b** | 51 | *R* | 90 | 1.5 |
| **5c** | 88 | *R* | >98 | 1.0 |
| **5d** | 76 | *R* | 97 | 1.5 |
| **5e** | 76 | *R* | >98 | 1.5 |
| **5f** | 68 | *R* | >98 | 2.5 |

α-carbon partly determines the binding orientation. It seems likely that the α-substitution of the alkyl group leads to unfavorable interaction with a putative steric restriction in the large pocket close to the catalytic site. With its linear geometry, the sp-carbon of the ethynyl group is less likely to be subject to this kind of steric hindrance. Starting with **7e**, substrates substituted with longer alkyl groups give increasingly higher amounts of the (*R*)-enantiomer, as is to be expected from Keinan's model.

Whereas secondary carbons are tolerated well in substituents (compounds **7c**, **f**, and **k**), tertiary carbons in the substrates result in very slow reaction rates and marginal yields (compounds **7d** and **g**), the ketones decomposing before significant conversion has taken place. The moderate yields in the other cases can be ascribed to losses due to evaporation and to some decomposition of the substrates under the reaction conditions.

Table 2 lists the results of the SADH-reduction of the ethynylketoesters (**5a**–**f**). The yields are higher the faster the reactions take place, in accordance with both the slow decomposition of the substrates and the competition of substrate reduction with enzyme inactivation. As expected, the enantioselectivity of the reduction depends on the number of methylenes between the carbonyl and the ester moiety as well as on the size of the latter. Due to the high enzyme concentration employed, the initial rates of reduction were too high to be measured accurately. However, the time required for complete conversion gives some indication of the relative reaction rates. In contrast to earlier results involving the reduction of aliphatic methyl ketones, where each additional methylene decreased the reaction rate by a factor of 3,[7] and also to the observations made with the simple ethynyl ketones (**7a**–**k**), increasing the size of the ester group results in faster reaction rates in the 4-oxohex-5-ynoate series (**5a**–**c**) and has no effect on the rates in the 5-oxohept-6-ynoate series (**5d** and **e**). Although methyl 5-oxohept-6-ynoate was not examined, it would be expected to show similar ee values to the ethyl and isopropyl esters (**5d** and **e**). Elongation of the tether also seems to have no marked effect on the rate. The higher enantioselectivities with which the ethynylketoesters are reduced compared to the simple ethynyl ketones and especially the high enantioselectivity, high yield, and the short reaction time in the case of **5c**, cannot be explained by simply invoking the size of the substituents. Apparently, the ester group plays a significant role in substrate binding to the active site. This assumption is supported by a revised model of the active site of SADH published by Burdette and Zeikus,[13] which is based on evidence for acetyl-coenzyme A being the physiological substrate for SADH. In this model, the large alkyl binding pocket is replaced by an open-ended channel that accommodates part of the pantotheine moiety of acetyl-CoA. This part of acetyl-CoA possesses an amide function in the approximate position of the ester group in methyl 6-oxooct-7-ynoate. The notable influence of the isopropyl ester on the enantioselectivity may be due to favorable hydrophobic interaction with part of the binding channel of the enzyme.

We observed that **7c**, which is reduced to (*S*)-**8c**, inactivates the enzyme much more slowly than **5c**, which yields (*R*)-**6c**. This suggests that the putative nucleophilic residue responsible for the inactivation by ethynyl ketones may be located in the small alkyl binding pocket. However, we have found that mutation of Cys295, a potential nucleophile in the small pocket, to alanine does not prevent inactivation of SADH by ethynyl ketones.[14]

There is at least one hydroxyalkynoate that is obtained in high yield and excellent optical purity for each length of the tether between the carbonyl and ester groups ($n = 2, 3, 4$). Therefore, by choosing the appropriate ester, potentially useful chiral building blocks can be readily prepared with this enzymatic reduction methodology. The nature of the alkyl portion of the ester group should have only minor influence on further transformations. The achiral starting materials are obtained in two (**7a**–**k** and **5a**,**b**,**d**,**f**) or three (**5c**,**e**) steps from commercially available compounds.

## Experimental

### General

UV-Vis spectra and enzyme assays were recorded on a Varian Cary 1E spectrophotometer equipped with a thermoelectric 6 × 6 cell cuvette changer. Capillary GC was performed on a Varian 3300 gas chromatograph with FI detection (Supelco β-Dex 120 chiral column, 30 m × 0.250 mm id, 0.25 μm film thickness) programmed between 40 and 175 °C. $^1$H and $^{13}$C NMR spectra were taken on a Bruker AC-250 or AC-300 spectrometer, respectively, using the residual $CHCl_3$ signal ($\delta$ 7.26 ppm) as internal reference. Optical rotations were measured on a Rudolph Autopol IV polarimeter. IR spectra were obtained with NaCl plates on a Perkin-Elmer Paragon 1000PC FT-IR spectrometer. Pet. ether refers to the fraction boiling in the range 35–60 °C.

### Enzyme assay

SADH was assayed following the increase in absorbance at 340 nm due to the formation of NADPH ($\Delta\varepsilon_{340} = 6.22$ mM$^{-1}$ cm$^{-1}$) at 50 °C. The assay mixture contained 200 mM Pr$^i$OH and 1.25 mM NADP in 50 mM Tris, pH 8.0. One unit (U) is the amount of enzyme required to reduce 1 μmol of NADP per minute.

### Purification of SADH

*E. coli* cells expressing SADH were grown as described.[9] The wet cells (150 g) were suspended in 50 mM tris, pH 8.0, (375 cm$^3$) containing 0.1 mM dithiothreitol, sonicated, and centrifuged (30 min at 10000 × g, all of the following centrifugations were done under these conditions). The supernatant was heated in a capped bottle at 70 °C for 1 h, 100 cm$^3$ buffer was added and the thick suspension again centrifuged. $(NH_4)_2SO_4$ (48.0 g, 20% saturation) was added to the supernatant in portions, and the mixture was stirred for 1 h at room temperature. After removal of the precipitate by centrifugation, an additional 110 g $(NH_4)_2SO_4$ (60% saturation) were added and stirring was

continued for 90 min. The mixture was centrifuged, the pellet was dissolved in Tris (50 $cm^3$), and the resulting solution was dialyzed against 50 mM Tris, pH 8.0, (3 × 4 $dm^3$). The solution was then heated at 70 °C for 1 h, centrifuged, and lyophilized yielding 2.12 g of SADH as a tan powder with the specific activity of 27 U $mg^{-1}$ solid.

### Determination of minimum enzyme concentration required for complete conversion

SADH (2.0, 13.5, 27, 54, 81, 108 U) and NADP (0.1 mg, 0.12 μmol) were dissolved in 50 mM Tris, pH 8.0, (0.85 $cm^3$). After pre-incubating the solution for 10 min at 50 °C, a solution of **5c** (16.8 mg, 0.10 mmol) or **7c** (9.6 mg, 0.10 mmol) in $Pr^iOH$ (0.15 $cm^3$) was added in one portion. The reactions were monitored by extracting an aliquot (40 $mm^3$) with $CH_2Cl_2$ (40 $mm^3$) followed by GC analysis (40–125 °C, 2 °C $min^{-1}$ for **7c**, 40–175 °C, 2 °C $min^{-1}$ for **5c**). The presence or absence of enzyme activity was assessed by incubating an aliquot (0.2 $cm^3$) with pentan-2-one (1.7 mg, 0.02 mmol) for 10 min and checking for the presence of pentan-2-ol by GC.

### Enzymatic reduction of ethynyl ketones and ethynylketoesters with SADH

SADH (1000 U) and NADP (1 mg, 1.2 μmol) were dissolved in 50 mM Tris, pH 8.0, (8.5 $cm^3$). After pre-incubation for 10 min at 50 °C, the appropriate ketone (**5** or **7**) (1.0 mmol) was added in one portion and the mixture was kept at 50 °C. When complete conversion was obtained (GC), the reaction mixture was saturated with NaCl and extracted with $Et_2O$ (3 × 4 $cm^3$). Due to their tendency to form emulsions, the extractions had to be centrifuged (10 min at 4000 rpm) prior to separation. The combined extracts were dried with $Na_2SO_4$, the solvent was removed *in vacuo*, and the residue chromatographed.

### Determination of optical purity of the alcohols

The alcohol (0.02 mmol) was dissolved in $CDCl_3$ (0.3 $cm^3$) in an NMR tube and a 50 mM solution of $Eu(hfc)_3$ in $CDCl_3$ (0.5 $cm^3$) was added in portions of 0.1 $cm^3$. A $^1H$ NMR spectrum was taken after each addition, and the two signals corresponding to the diastereotopic methanol protons were integrated. If only one signal was visible, the enantiomeric excess was assumed to be greater than 98%.

### Spectral data of isolated products

**(*S*)-Pent-1-yn-3-ol (8b).** Yield 30.3 mg (36%), 80% ee, $R_f$ 0.20 (10% ether–pet. ether), $[\alpha]_D^{20}$ −19.2 (*c* = 12, dioxane) (lit.[15] $[\alpha]_D^{25}$ +23.15 (*c* = 2, dioxane), 86% ee for (*R*)-enantiomer), $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 4.32 (dt, *J* = 6.5 Hz, 2.0 Hz, 1H), 2.46 (d, *J* = 1.8 Hz, 1H), 1.74 (m, 3H), 1.02 (t, *J* = 7.4 Hz, 3H).

**(*S*)-4-Methylpent-1-yn-3-ol (8c).** Yield 49.1 mg (50%), >98% ee, $R_f$ 0.22 (10% ether–pet. ether), $[\alpha]_D^{20}$ −15.7 (*c* = 13, dioxane) (lit.[15] $[\alpha]_D^{20}$ +13.8 (*c* = 2, dioxane), 86% ee for (*R*)-enantiomer), $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 4.17 (dd, *J* = 5.7 Hz, 2.0 Hz, 1H), 2.45 (d, *J* = 2.3 Hz, 1H), 1.89 (m, 2H), 1.01 (m, 6H).

**(*S*)-Hex-1-yn-3-ol (8e).** Yield 27.5 mg (28%), 51% ee, $R_f$ 0.22 (10% ether–pet. ether), $[\alpha]_D^{20}$ −4.5 (*c* = 1.5, $CHCl_3$) (lit.[15] $[\alpha]_D^{20}$ +9.0 (*c* = 1.0, $CHCl_3$), 75% ee for (*R*)-enantiomer), $^1H$ NMR (250 MHz, $CDCl_3$) $\delta$ 4.39 (dt, *J* = 6.5 Hz, 2.2 Hz, 1H), 2.47 (d, *J* = 2.1 Hz, 1H), 1.71 (m, 3H), 1.50 (m, 2H), 0.97 (t, *J* = 7.0 Hz, 3H).

**(*R*)-5-Methylhex-1-yn-3-ol (8f).** Yield 22.4 mg (20%), 50% ee, $R_f$ 0.20 (10% ether–pet. ether), $[\alpha]_D^{20}$ +18.3 (*c* = 1.2, dioxane) (lit.[15] $[\alpha]_D^{25}$ +28.8 (*c* = 3, dioxane), 88% ee for (*R*)-enantiomer), $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 4.42 (dt, *J* = 7.4 Hz, 2.3 Hz, 1H), 2.46 (d, *J* = 2.1 Hz, 1H), 1.87 (m, 1H), 1.60 (m, 3H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.93 (d, *J* = 6.8 Hz, 3 H).

**(*R*)-Hept-1-yn-3-ol (8h).** Yield 30.3 mg (27%), 42% ee, $R_f$ 0.19 (10% ether–pet. ether), $[\alpha]_D^{20}$ +3.8 (*c* = 5.8, $CHCl_3$) (lit.[16] $[\alpha]_D^{20}$ −5.5 (*c* = 0.9, $CHCl_3$), 70% ee for (*S*)-enantiomer), $^1H$ NMR (250 MHz, $CDCl_3$) $\delta$ 4.37 (dt, *J* = 7.3 Hz, 2.1 Hz, 1H), 2.47 (d, *J* = 2.1 Hz, 1H), 1.87 (br s, 1H), 1.72 (m, 2H), 1.41 (m, 4H), 0.92 (t, *J* = 7.0 Hz, 3H).

**(*R*)-6-Methylhept-1-yn-3-ol (8k).** Yield 61.8 mg (49%), 80% ee, $R_f$ 0.21 (10% ether–pet. ether), $[\alpha]_D^{20}$ +9.2 (*c* = 1.2, $CHCl_3$), +13.8 (*c* = 2.0, dioxane), $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 4.35 (dt, *J* = 6.6 Hz, 1.9 Hz, 1H), 2.47 (d, *J* = 1.9 Hz, 1H), 2.03 (br s, 1H), 1.71 (m, 2H), 1.57 (m, 1H), 1.33 (m, 2H), 0.89 (d, *J* = 6.5 Hz, 6H).

**(*R*)-Methyl 4-hydroxyhex-5-ynoate (6a).** Yield 49.8 mg (35%), 82% ee, $R_f$ 0.20 (20% ethyl acetate–hexanes), $[\alpha]_D^{20}$ +9.9 (*c* = 1.8, $CHCl_3$), $^1H$ NMR (250 MHz, $CDCl_3$) $\delta$ 4.49 (dt, *J* = 6.0 Hz, 2.0 Hz, 1H), 3.69 (s, 3H), 2.55 (m, 2H), 2.49 (d, *J* = 2.1 Hz, 1H), 2.21–1.98 (m, 3H).

**(*R*)-Ethyl 4-hydroxyhex-5-ynoate (6b).** Yield 79.6 mg (51%), 90% ee, $R_f$ 0.18 (15% ethyl acetate–hexanes), $[\alpha]_D^{20}$ +17.9 (*c* = 2.0, $CHCl_3$), IR (neat) $\nu_{max}$ 3443, 3291, 2112, 1732 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 4.49 (dt, *J* = 6.1 Hz, 2.1 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 2.52 (m, 2H), 2.48 (d, *J* = 1.7 Hz, 1H), 2.21 (br s, 1H), 2.04 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 173.9, 97.6, 84.0, 73.4, 61.3, 60.7, 32.1, 29.8, 14.1.

**(*R*)-Isopropyl 4-hydroxyhex-5-ynoate (6c).** Yield 150 mg (88%), >98% ee, $R_f$ 0.21 (15% ethyl acetate–hexanes), $[\alpha]_D^{20}$ +13.1 (*c* = 2.0, $CHCl_3$), IR (neat) $\nu_{max}$ 3442, 3293, 2114, 1731 $cm^{-1}$; $^1H$ NMR (250 MHz, $CDCl_3$) $\delta$ 5.02 (m, 1H), 4.49 (dt, *J* = 6.1 Hz, 2.0 Hz, 1H), 2.51 (m, 3H), 2.03 (m, 2H), 1.24 (d, *J* = 6.4 Hz, 6H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 173.2, 84.0, 73.3, 68.1, 61.2, 32.2, 30.2, 21.7.

**(*R*)-Ethyl 5-hydroxyhept-6-ynoate (6d).** Yield 129 mg (76%), 97% ee, $R_f$ 0.22 (20% ethyl acetate–hexanes), $[\alpha]_D^{20}$ +16.9 (*c* = 1.66, $CCl_4$), +7.8 (*c* = 3.8, $CHCl_3$) (lit.[17] $[\alpha]_D^{20}$ −16.4 (*c* = 3.30, $CCl_4$), 96% ee for (*S*)-enantiomer); $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 4.38 (dt, *J* = 6.2 Hz, 1.8 Hz, 1H), 4.12 (q, *J* = 7.2 Hz, 2H), 2.46 (d, *J* = 2.2 Hz, 1H), 2.35 (t, *J* = 7.0 Hz, 2H), 2.24 (br s, 1H), 1.78 (m, 4H), 1.24 (t, *J* = 7.1 Hz, 3H).

**(*R*)-Isopropyl 5-hydroxyhept-6-ynoate (6e).** Yield 139 mg (76%), >98% ee, $R_f$ 0.24 (20% ethyl acetate–hexanes), $[\alpha]_D^{20}$ +10.3 (*c* = 2.6, $CHCl_3$), IR (neat) $\nu_{max}$ 3433, 3291, 2112, 1727 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 5.01 (m, 1H), 4.40 (dt, *J* = 6.0 Hz, 2.6 Hz, 1H), 2.47 (d, *J* = 2.6 Hz, 1H), 2.33 (t, *J* = 6.9 Hz, 2H), 2.03 (br s, 1H), 1.78 (m, 4H), 1.23 (d, *J* = 6.2 Hz, 6H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 173.0, 84.5, 73.0, 67.7, 61.7, 36.7, 34.0, 21.8, 20.4.

**(*R*)-Methyl 6-hydroxyoct-7-ynoate (6f).** Yield 115 mg (68%), >98% ee, $R_f$ 0.22 (20% ethyl acetate–hexanes), $[\alpha]_D^{20}$ +8.8 (*c* = 2.2, $CHCl_3$), IR (neat) $\nu_{max}$ 3434, 3289, 2111, 1734 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 4.36 (dt, *J* = 6.5 Hz, 2.1 Hz, 1H), 3.65 (s, 3H), 2.45 (d, *J* = 2.5 Hz, 1H), 2.32 (t, *J* = 7.4 Hz, 2H), 2.22 (br s, 1H), 1.79–1.60 (m, 4H), 1.50 (m, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 174.1, 84.8, 73.0, 62.0, 51.6, 37.1, 33.9, 24.5 (2×).

## Acknowledgements

We thank Professor J. Gregory Zeikus and Dr Douglas S. Burdette for generously providing the microorganisms expressing SADH.

## References


1 C. Wong and G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Tetrahedron Organic Chemistry Series, vol. 12, Elsevier Science, Oxford, 1994.
2 (*a*) S. Servi, *Synthesis*, 1990, 1; (*b*) R. Csuk and G. Brigitte, *Chem. Rev.*, 1991, **91**, 49.
3 E. Keinan, E. K. Hafeli, K. K. Seth and R. Lamed, *J. Am. Chem. Soc.*, 1986, **108**, 162.
4 F. O. Bryant, J. Wiegel and L. G. Ljungdahl, *Appl. Environ. Microbiol.*, 1988, 460.
5 V. T. Pham and R. S. Phillips, *J. Am. Chem. Soc.*, 1990, **112**, 3629.
6 C. Zheng and R. S. Phillips, *J. Chem. Soc.*, *Perkin Trans. 1*, 1992, 1083.
7 (*a*) C. Zheng, V. T. Pham and R. S. Phillips, *Bioorg. Med. Chem. Lett.*, 1992, **2**, 619; (*b*) C. Zheng, V. T. Pham and R. S. Phillips, *Catal. Today*, 1994, **22**, 607.
8 V. Prelog, *Pure Appl. Chem.*, 1964, **9**, 119.
9 D. S. Burdette, C. Vieille and J. G. Zeikus, *Biochem. J.*, 1996, **316**, 115.
10 D. R. M. Walton and F. Waugh, *J. Organomet. Chem.*, 1972, **37**, 45.
11 R. A. Earl and K. P. C. Vollhardt, *J. Org. Chem.*, 1984, **49**, 4786.
12 N. K. Nayyar, D. R. Hutchinson and M. J. Martinelli, *J. Org. Chem.*, 1997, **62**, 982.
13 D. S. Burdette and J. G. Zeikus, *Biochem. J.*, 1994, **302**, 163.
14 C. Heiss, M. Lavenieks, J. G. Zeikus and R. S. Phillips, *Biochemistry*, submitted.
15 J. Vigneron and V. Bloy, *Tetrahedron Lett.*, 1979, 2683.
16 M. D. De Marquez, P. J. Rowland, P. M. Scopes and V. Thaller, *J. Chem. Res.* (*M*), 1986, 1348.
17 B. I. Glänzer, K. Königsberger, B. Berger, K. Faber and H. Griengl, *Chem. Phys. Lipids*, 1990, **54**, 43.