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Enantiopure (*S*)-4-Phenyl-3-butyn-2-ol and (*S*)-1-Phenyl-2-butanol Through an Enzymatic Reduction

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This paper describes an enzymatic approach to producing (*S*)-4-phenyl-3-butyn-2-ol [(*S*)-**3**] and (*S*)-1-phenyl-2-butanol [(*S*)-**4**] from their corresponding ketones. W110A secondary alcohol dehydrogenase from *Thermoanaerobacter ethanolicus* (W110A *Te*SADH), a Prelog mutant of *Te*SADH, is used as a biocatalyst in this approach. The enantioselective reduction of α,β-alkynyl ketone 4-phenyl-3-butyn-2-one produced (*S*)-**3** in an excellent yield and high levels of both chemo- and enantio-selectivities keeping the alkynyl moiety intact. The reduction of 1-phenyl-2-butanone (**2**) produced (*S*)-**4** of higher enantioselectivity than that observed previously in the W110A *Te*SADH-catalyzed reduction of phenylacetone, a ketone with one methylene unit less than compound **2**.

Keywords: Alcohol dehydrogenase, Asymmetric reduction, Biotransformations, (*S*)-1-Phenyl-2-butanol, (*S*)-4-Phenyl-3-butyn-2-ol.

INTRODUCTION

Extensive efforts have been dedicated to establishing new methods for generating optically active compounds, including alcohols. Optically active alcohols are crucial synthons that are used in agricultural, food and pharmaceutical industries. One of the simplest and most efficient methods for producing non-racemic alcohols involves asymmetric reduction of their corresponding prochiral ketones. An attractive reduction method involves using biocatalysis[1]. Enzymes are known for their high chemo-, regio- and stereoselectivities. They also have potential for use as green catalysts because they are natural catalysts and their reactions are generally conducted in aqueous media. One shortcoming of using enzymes in organic synthesis is their limited substrate specificity. However, recent advancements in biotechnology have made biocatalysis a practical choice for organic chemists[2].

Alcohol dehydrogenases (ADHs, EC 1.1.1.1 and 1.1.1.2) catalyze the reversible reduction of aldehydes and ketones to their corresponding alcohols[3-6]. Alcohol dehydrogenases have been used successfully to produce optically active alcohols. Secondary ADH from *Thermoanaerobacter ethanolicus* (*Te*SADH, EC 1.1.1.2), a nicotinamide-adenine dinucleotide phosphate (NADPH)-dependent ADH, is a thermally stable enzyme that has been used to synthesize optically active alcohols. In addition to its high thermal stability, this enzyme tolerates high concentrations of organic solvents, making it an ideal choice in organic synthesis. A noteworthy mutant of *Te*SADH is W110A *Te*SADH, which can accommodate phenyl-ring-containing ketones and their corresponding alcohols, which are not substrates for wild-type *Te*SADH[7]. This mutant obeys Prelog's rule[8], according to which the pro-*R* hydride is delivered from the *Re* face of prochiral ketone substrates, thus producing *S*-configured alcohols (Fig. 1).

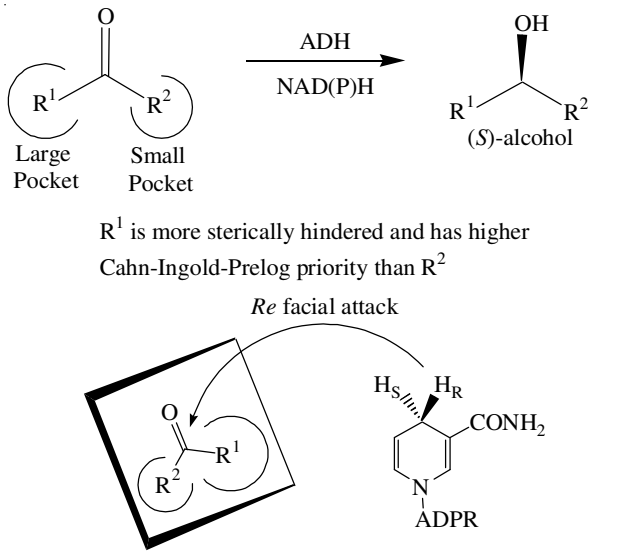


Fig. 1. Prelog's rule for predicting the stereochemical outcome for ADH-catalyzed asymmetric reduction of prochiral ketones. ADPR: adenosine diphosphoribose

In this study, W110A *Te*SADH was used to produce (*S*)-4-phenyl-3-butyn-2-ol [(*S*)-**3**], an important building block

for Fenleuton which is a 5-lipoxygenase inhibitor[9] and (*S*)-1-phenyl-2-butanol [(*S*)-**4**] through enantioselective reduction of their corresponding ketones.

EXPERIMENTAL

Commercial grade solvents were used without further purification. 4-Phenyl-3-butyn-2-one (**1**), 1-phenyl-2-butanone (**2**), $NaBH_4$ and $NADP^+$ were used as purchased from commercial suppliers. (*rac*)-1-Phenyl-2-butanol was produced[10] by reducing compound **1** with $NaBH_4$. All buffer solutions were adjusted to pH 8 at room temperature. Capillary GC measurements were performed on a GC equipped with a flame ionization detector and an HP chiral-20B column (30 m, 0.32 mm [i.d.], 0.25 μm film thickness) using helium as the carrier gas. Nuclear magnetic resonance spectra were recorded in $CDCl_3$ on a 500 MHz spectrometer at 500 MHz for ^1H and at 125 MHz for ^{13}C at room temperature using either the solvent peak or tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are reported in ppm. Optical rotation values were measured at 24 °C with a cell of 1 dm path length. The concentration *c* is reported in g/100 mL.

Gene expression and purification of W110A *Te*SADH: Plasmid of W110A *Te*SADH was obtained from Prof. Claire Vieille, from the Department of Microbiology and Molecular Genetics at Michigan State University. W110A *Te*SADH was expressed in recombinant *Escherichia coli* BL21(DE3) cells and purified as reported[11].

W110A *Te*SADH-catalyzed reduction of compounds 1 and 2: Reactions were conducted by mixing compounds **1** or **2** (0.365 mmol), Tris-HCl buffer solution (7 mL, 50 mM, pH 8), 2-propanol (3 mL), $NADP^+$ (1 mg) and W110A *Te*SADH (0.7 mg in 100 μL of 50 mM Tris-HCl buffer solution) in a round-bottomed flask equipped with a magnetic stirrer and a condenser. The reaction mixture was stirred for 24 h at 50 °C. It was then extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with brine solution (5 mL), dried with Na_2SO_4 and then concentrated under vacuum. About 5 μL of the residue was treated with pyridine and acetic anhydride, as described below, to convert the produced alcohol to the corresponding acetate. For compound **2**, the alcohol obtained from W110A *Te*SADH-catalyzed reduction was purified by silica gel using hexane/ethyl acetate.

(*S*)-4-Phenyl-3-butyn-2-ol [(*S*)-3]: 99.7 % conversion, > 99.9 % ee; $[\alpha]_D^{24}$ -35.3 (*c* 0.58, $CHCl_3$), lit.[12] $[\alpha]_D^{25}$-46.8 (*c* 1, Et_2O) > 99 % ee; ^1H NMR ($CDCl_3$), δ: 1.55 (d, *J* = 6.7 Hz, 3H), 2.15 (bs, 1H), 4.76 (q, *J* = 6.7 Hz, 1H), 7.29-7.43 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 24.4, 58.8, 84, 90.9, 122.6, 128.2, 128.3, 131.6.

(*S*)-1-Phenyl-2-butanol [(*S*)-4]: 49.7 % conversion, 78.6 % ee; $[\alpha]_D^{24}$+7.4 (*c* 0.61, $CHCl_3$), lit.[13] $[\alpha]_D^{25}$+14.7 (*c* 2.2, Et_2O) 94 % ee; ^1H NMR ($CDCl_3$) δ: 1.01 (t, *J* = 7.3 Hz, 3 H), 1.50-1.59 (m, 3H), 2.66 (dd, *J* = 13.5, 8.4 Hz, 1H), 2.85 (dd, *J* = 13.5, 4.3 Hz, 1H), 3.76 (p, *J* = 3.9 Hz, 1H), 7.19-7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ: 10, 29.6, 43.6, 74.0, 126.4, 128.5, 129.4, 138.6.

Acetylation of (*S*)-3 and (*S*)-4: A mixture of (*S*)-**3** or (*S*)-**4** (0.033 mmol, about 5 μL), acetic anhydride (one drop) and pyridine (two drops) was placed in a 1.5 mL plastic tube. The tube was capped and left at room temperature for 2 h. The mixture was then diluted with $CHCl_3$ and then analyzed by a GC equipped with a chiral column to calculate % conversion of compounds **1** and **2** to (*S*)-**3** and (*S*)-**4**, respectively and % ee of (*S*)-**3** and (*S*)-**4**.

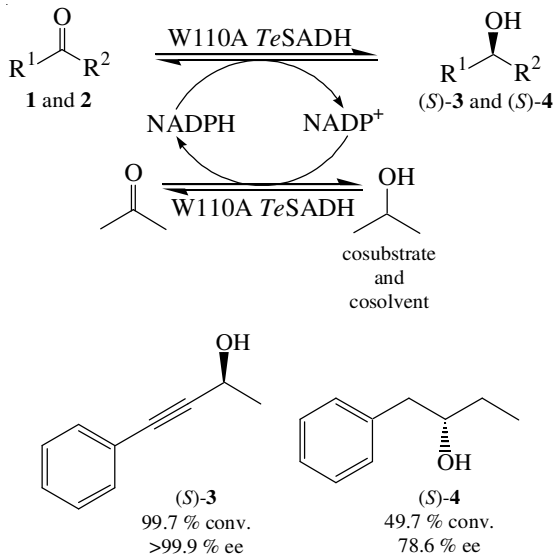
Determination of absolute configuration and optical purity for (*S*)-3 and (*S*)-4: The absolute configurations of (*S*)-**3** and (*S*)-**4** were determined by comparing their signs of optical rotation with reported values. Their % ee were calculated from GC chromatograms obtained for their corresponding acetates. (*rac*)-**3** and (*rac*)-**4**, prepared by $NaBH_4$ reduction of their corresponding ketones, were also acetylated and analyzed by CG equipped with a chiral column for comparison.

Production of (*rac*)-3 through $NaBH_4$ reduction of compound 1: 4-Phenyl-3-butyn-2-one (0.2 g, 1.39 mmol) was dissolved in MeOH (5 mL). The mixture was cooled to 0 °C. $NaBH_4$ (53 mg, 1.39 mmol) was added and the mixture was stirred at room temperature for 0.5 h. Volatile components were removed under vacuum. The remaining residue was dissolved in ethyl acetate (10 mL) and washed with water (5 mL) then brine solution (5 mL). The organic layer was concentrated under vacuum and the remaining residue was filtered through a very short silica gel column (Pasteur pipette) to produce a colorless liquid, 91 % yield.

RESULTS AND DISCUSSION

The enantioselective reduction of 4-phenyl-3-butyn-2-one (**1**), an α,β-alkynyl ketone, using W110A *Te*SADH, produced the corresponding propargylic alcohol (*S*)-**3** at 99.7 % conversion and > 99.9 % ee (**Scheme-I**). The reaction was conducted in a Tris-HCl buffer solution (50 mM, pH 8) containing 2-propanol (70:30, v/v), which is used as a cosubstrate to regenerate NADPH, thus rendering the process catalytic. A high percentage of 2-propanol is crucial for enhancing the solubility of the substrate ketone and for directing the equilibrium toward the reduction pathway. The resultant alcohol has an *S* configuration, in agreement with Prelog's rule. This enzymatic asymmetric reduction is not only highly stereoselective but also highly chemoselective, because the carbonyl of the α,β-alkynyl ketone **1** is reduced, leaving the triple bond intact (*i.e.*, no 1,4-addition is observed). This high chemoselectivity can be explained according to the ideal fit of the prochiral ketone **1** at the active site of W110A *Te*SADH, which allows NADPH to deliver its hydride exclusively to the carbonyl carbon. This represents an advantage of using biocatalysts over other methods that might not exhibit such high chemoselectivity. It is worth mentioning that ketone **1**, known as a difficult substrate[14], is tough to be reduced with high enantioselectivity using non-enzymatic methods[15-17].

Under the same conditions described for ketone **1**, the asymmetric reduction of 1-phenyl-2-butanone (**2**) resulted in approximately 50 % conversion to (S)-**4** at 78.6 % ee (**Scheme-I**). The stereochemical outcome of this reaction is also consistent with Prelog's rule. In a previous study[13], (*S*)-1-phenyl-2-propanol was produced at 37 % ee from phenylacetone by using W110A *Te*SADH under the same conditions used in the current study for the reduction of compound **2**. Such poor enantioselectivity was explained by the ability of phenylacetone



Scheme-I: Asymmetric production of (S)-**3** and (S)-**2** using W110A TeSADH-catalyzed reduction. Conditions: reactions were performed at 50 °C using compounds **1** or **2** (0.365 mmol), $NADP^+$ (1 mg), Tris-HCl buffer solution (7 mL, 50 mM, pH 8), 2-propanol and W110A TeSADH (0.7 mg)

to adapt in alternative modes within the large pocket of W110A *Te*SADH, which allows NADPH to deliver the hydride from either the *Re* face or *Si* face of phenylacetone, thus lowering the enantioselectivity of the reaction. The higher enantioselectivity in the reduction of compound **2** indicates that a small change in the substrate (a methylene moiety in this case) induces the substrate to fit in the expected mode (*i.e.*, in Prelog's fashion), thus significantly tuning the stereoselectivity of the reduction of compound **2** in comparison with phenylacetone. This observation is consistent with results obtained by Keinan *et al*. and others regarding the asymmetric reduction of aliphatic ketones by using *Thermoanaerobium brockii* ADH (TbADH)[18,19]. In that study, the authors proposed two hydrophobic pockets which are different in volume within the active site of *Tb*ADH, with the small pocket having higher affinity toward alkyl groups; *i.e.*, the small pocket has higher affinity toward ethyl in comparison with methyl. This finding facilitates understanding the mode of substrate-fitting at the active site and therefore assists in designing new reactions catalyzed by *Te*SADH. It also helps to expand substrate specificity for *Te*SADH by designing new mutants.

The chemical identities of (*S*)-**3** and (*S*)-**4** were confirmed using both ^{1}H NMR and ^{13}C NMR. Their *S* configuration was confirmed by comparing their signs of optical rotation with reported values. Furthermore, W110A *Te*SADH is a Prelog ADH and was used to produce *S*-configured phenyl-ring-containing alcohols exhibiting similar structures to those of (*S*)-**3** and (*S*)-**4**. The resultant alcohols (*S*)-**3** and (*S*)-**4** were converted to the corresponding acetate esters prior to analyzing them by using GC equipped with a chiral column to determine their optical purities as explained in the experimental section.

Expanding substrate specificity for *Te*SADH by continually searching for new substrates and by designing new mutants is crucial for the success of this enzyme as a biocatalyst. This fact, accompanied by the unique characteristics of *Te*SADH, makes this enzyme a useful choice in organic synthesis.

Conclusion

An asymmetric reduction catalyzed by W110A *Te*SADH was performed to generate (*S*)-4-phenyl-3-butyn-2-ol in excellent yield demonstrating high chemo- and enantioselectivity. Under the same reaction conditions, (*S*)-1-phenyl-2-butanol was produced from its corresponding ketone in a medium yield and favorable enantioselectivity. We showed that an extra methylene moiety in a prochiral ketone substrate can tune the reaction selectivity, as indicated by the enhanced stereoselectivity of the W110A *Te*SADH-catalyzed reduction of 1-phenyl-2-butanone, in comparison with phenylacetone. Expanding substrate specificity for *Te*SADH is of considerable relevance, making this enzyme a useful biocatalyst.

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