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Asymmetric Reduction and Oxidation of Aromatic Ketones and Alcohols Using W110A Secondary Alcohol Dehydrogenase from *Thermoanaerobacter ethanolicus*

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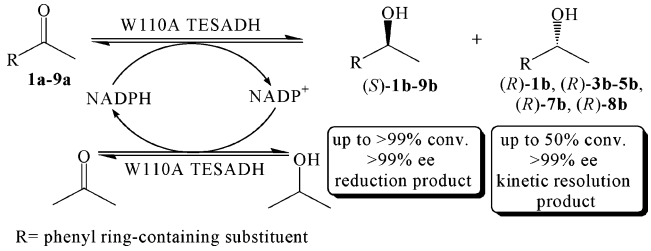

R= phenyl ring-containing substituent

An enantioselective asymmetric reduction of phenyl ring-containing prochiral ketones to yield the corresponding optically active secondary alcohols was achieved with W110A secondary alcohol dehydrogenase from *Thermoanaerobacter ethanolicus* (W110A TESADH) in Tris buffer using 2-propanol (30%, v/v) as cosolvent and cosubstrate. This concentration of 2-propanol was crucial not only to enhance the solubility of hydrophobic phenyl ring-containing substrates in the aqueous reaction medium, but also to shift the equilibrium in the reduction direction. The resulting alcohols have *S*-configuration, in agreement with Prelog's rule, in which the nicotinamide-adenine dinucleotide phosphate (NADPH) cofactor transfers its *pro*-R hydride to the *re* face of the ketone. A series of phenyl ring-containing ketones, such as 4-phenyl-2-butanone (**1a**) and 1-phenyl-1,3-butadione (**2a**), were reduced with good to excellent yields and high enantioselectivities. On the other hand, 1-phenyl-2-propanone (**7a**) was reduced with lower ee than 2-butanone derivatives. (*R*)-Alcohols, the *anti*-Prelog products, were obtained by enantiospecific oxidation of (*S*)-alcohols through oxidative kinetic resolution of the *rac*-alcohols using W110A TESADH in Tris buffer/acetone (90:10, v/v).

Introduction

Tremendous efforts have been made in recent years to establish enantioselective routes to enantiomerically pure compounds, due to their importance in pharmaceutical, agricultural, and food industries.[1] Recent developments in medicine have shown that a single enantiomer is biologically active in most chiral drugs.[2] Optically active alcohols are one of the most important synthons. They can be produced from their corresponding prochiral ketones via asymmetric reduction, or from their racemic alcohols via enantiospecific kinetic resolution (KR).[3,4] Chiral metal complexes have been used as catalysts for these purposes;[5] however, these methods produce toxic residual metals that create environmental problems. Enzymes are recognized to be among the most effective catalysts for producing optically active alcohols. Among their advantages are their chemo-, regio-, and stereoselectivities due to the strict recognition of a particular substrate by a given enzyme.

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Biocatalytic processes also are less hazardous and energy consuming than conventional chemistry methodologies. They are normally carried out under mild conditions, which minimize problems of product isomerization, racemization, or epimerization. Biocatalysts are easily produced at low cost and with minimum waste, and they can be decomposed in the environment after use. Unfortunately, they do have some disadvantages. For example, many enzymes are thermally unstable. Another disadvantage is the limited solubility of most organic substrates in water; this leads to larger reaction volumes, a need for cosolvents, and complicated product recovery.[6]

Alcohol dehydrogenases (ADHs, EC 1.1.1.*X*, $X = 1$ or 2) are enzymes that catalyze the reversible reduction of ketones and aldehydes to the corresponding alcohols. The asymmetric reduction of ketones using the commercially available yeast ADH and horse liver ADH is limited not only due to their temperature sensitivity, but also due to their sensitivity toward organic solvents and their loss of activity upon immobilization. An additional disadvantage of horse liver ADH is its low affinity for acyclic ketones.[1b,7] Secondary ADH from *Thermoanaerobacter ethanolicus* (TESADH, EC 1.1.1.2), a highly thermostable enzyme, has been isolated and characterized.[8] NADPH is required by this enzyme, from which the hydride is transferred to the carbonyl carbon. Because NADPH is a costly cofactor, alcohols like 2-propanol or ketones like acetone are used as hydrogen source or hydrogen sink to regenerate the cofactor and therefore make both processes catalytic. This enzyme is stable at temperatures up to 80 °C, and it exhibits high activity in the asymmetric reduction of ketones.[9] Because of its thermostability, resistance to organic solvents, and reactivity for a wide variety of substrates, it is a useful biocatalyst for synthetic applications.[10]

A series of ethynyl ketones and ethynylketoesters were reduced enantioselectively to the corresponding nonracemic propargyl alcohols using wild-type TESADH.[10a] The behavior of TESADH has been shown to be similar to results obtained from reductions with a very highly homologous (99% identity),[8b] NADPH-dependent, *Thermoanaerobium brockii* ADH (TBADH).[11] For TBADH, Keinan et al. suggested that the two alkyl groups of substrates occupy two hydrophobic sites, which differ from one another in volume and also in their affinities toward the alkyl groups (Figure 1).[11] It was also shown that the small site, which has higher affinity toward the alkyl groups of the ketone, can accommodate up to three carbon substituents, like the isopropyl group.[10a,b,11]

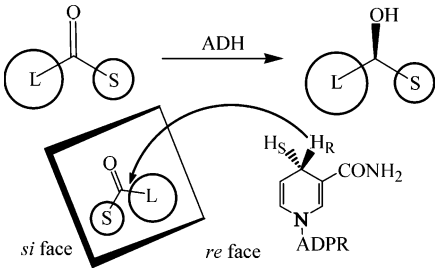

FIGURE 1. Prelog's rule for predicting the stereochemistry of alcohols formed from their corresponding ketones by asymmetric reduction with ADHs.

We have recently reported a new mutant of TESADH, where tryptophan-110 was substituted by alanine (W110A TESADH).[12] This replacement makes the large pocket able to accommodate phenyl ring-containing substrates that are not substrates for wild-type TESADH.[10b] Its modified substrate range makes this mutant enzyme useful for the enantioselective reduction of phenyl ring-containing ketones such as 4-phenyl-2-butanone (**1a**) and, in the reverse direction, for the enantioselective oxidation via KR of racemic phenyl ring-containing secondary alcohols.

Results and Discussion

A series of phenyl ring-containing ketones, which could not be reduced by wild-type TESADH, were reduced by W110A TESADH to produce the corresponding nonracemic alcohols with good yields and high optical purities (Table 1). The reductions were carried out in Tris buffer containing 30% (v/v) 2-propanol, which serves as both cosolvent and hydride source to reduce the oxidized coenzyme. The use of such a high percentage of 2-propanol was crucial not only to enhance the solubility of the hydrophobic phenyl ring-containing ketone substrates in aqueous media, but also to shift the equilibrium into the reduction direction. The produced alcohols had *S* configuration, in agreement with Prelog's rule, in which the NADPH cofactor transfers its *pro*-R hydride to the *re* face of the ketone (Figure 1).[1b,c,13]

Phenyl ring-containing 2-butanone derivatives were reduced to the corresponding (*S*)-alcohols with excellent stereoselec-

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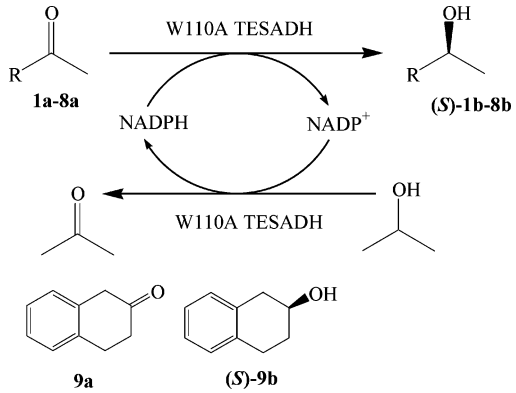
TABLE 1. Asymmetric Reduction of Phenyl Ring-Containing Ketones Using W110A TESADH



substrate	R	product[a]	conv. (%)[b]	ee (%)[d]
1a	$PhCH_2CH_2$	(*S*)-**1b**	99	>99
2a	$Ph(C{=}O)CH_2$	(*S*)-**2b**	98	>99
3a	(*E*)-Ph−HC═CH	(*S*)-**3b**	64	>99
4a	*p*-$MeOC_6H_4(CH_2)_2$	(*S*)-**4b**	87	91
5a	$PhOCH_2$	(*S*)-**5b**	>99	>99[e]
6a	*p*-$ClC_6H_4CH_2CHCl$	(2*S*,3*R*)-**6b**	83[c]	>99
7a	$PhCH_2$	(*S*)-**7b**	95	37[e]
8a	*p*-$MeOC_6H_4CH_2$	(*S*)-**8b**	97	>99[e]
9a		(*S*)-**9b**	>99	71[e]

[a] The absolute configurations of the products were determined by comparison of the signs of the optical rotation with those reported previously. [b] % conversion was determined by GC. [c] Isolated yield. [d] Unless otherwise mentioned, ee was determined by chiral stationary phase GC for the produced alcohol. [e] The ee was determined for the corresponding acetate derivative (see the Supporting Information).

tivities and moderate to excellent yields (Table 1). 4-Phenyl-2-butanone (**1a**) was reduced stereoselectively to produce (*S*)-4-phenyl-2-butanol ((*S*)-**1b**) with excellent chemical and optical yields. The *β*-diketone 1-phenyl-1,3-butadione (**2a**) was reduced regio- and stereoselectively to furnish the monohydroxy ketone (*S*)-3-hydroxy-1-phenyl-1-butanone ((*S*)-**2b**) with excellent yield and ee, leaving the other keto group at C-1 intact. (*E*)-4-Phenyl-3-butene-2-one (**3a**) was reduced with moderate yield and excellent optical purity to produce the allylic alcohol (*S*)-4-phenyl-3-butene-2-ol ((*S*)-**3b**). The presence of the methoxy group at the para position of the phenyl ring in 4-(4-methoxyphenyl)-2-butanone (**4a**) affected the ee of the produced (*S*)-4-(4-methoxyphenyl)-2-butanol ((*S*)-**4b**) (91% ee), which is lower than for (*S*)-**1b**. Phenoxy-2-propanone (**5a**) was reduced with very high yield and optical purity to produce the corresponding (*S*)-phenoxy-2-propanol ((*S*)-**5b**). When the α-chloroketone, 3-chloro-4-(4-chlorophenyl)-2-butanone (**6a**), was reduced with W110A TESADH, (+)-(2*S*,3*R*)-3-chloro-4-(4-chlorophenyl)-2-butanol ((+)-(2*S*,3*R*)-**6b**) was produced with high enantioselectivity (>99% ee) and diastereoselectivity (92:8 mixture of *anti*- and *syn*-α-chlorohydrins). The absolute configuration of (+)-(2*S*,3*R*)-**6b** was confirmed by comparing the sign of the optical rotation with that reported previously for the very similar compound, (+)-(2*S*,3*R*)-4-phenyl-3-bromo-2-butanol) ($[\alpha]^{20}_D$ +29.2, *c* 2.08, $CHCl_3$; lit.[14] $[\alpha]^{25}_J$ +37, *c* 0.06, $CHCl_3$, 95% ee). In a separate experiment, reduction of **6a** with $NaBH_4$, which is expected to give mainly the syn product,[15] afforded a mixture of four diastereomers ((±)-**6b**) (88:12

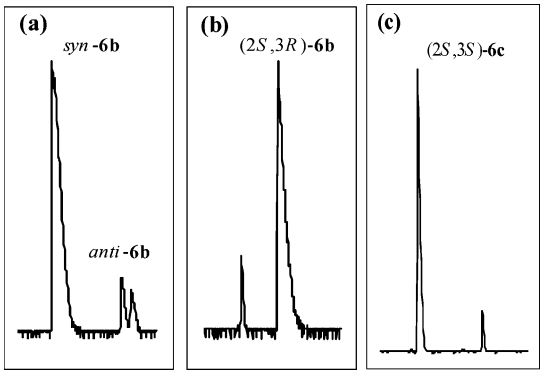
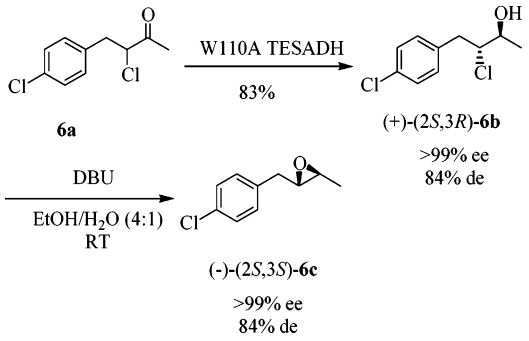


FIGURE 2. GC chromatograms illustrating (a) the products of $NaBH_4$ reduction of **6a**, (b) the products of W110A TESADH reduction of **6a**, and (c) (−)-(2*S*,3*S*)-**6c** produced from (+)-(2*S*,3*R*)-**6b**.

SCHEME 1. Conversion of (+)-(2*S*,3*R*)-6b into (−)-(2*S*,3*S*)-6c



mixture of *syn*- and *anti*-α-chlorohydrins), in which the *syn*-**6b** had a different retention time than (+)-(2*S*,3*R*)-**6b** by injection in a chiral column GC (Figure 2a,b). Reduction of **6a** to almost a single stereoisomer, (+)-(2*S*,3*R*)-**6b**, using W110A TESADH indicated that the process involves a KR, and this should be combined with isolation of (*S*)-**6a** as unreacted enantiomer and a maximum yield of 50% of the produced α-chlorohydrin. We have noticed that the yield is higher than 50%, and the isolated unreacted **6a** is a racemic mixture. This indicates that the reduction of **6a** with W110A TESADH proceedes by dynamic kinetic resolution via a facile buffer-catalyzed enolization, which enables the unreacted enantiomer (*S*)-**6a** to racemize after the depletion of (*R*)-**6a** starts.[16] The α-chlorohydrin (+)-(2*S*,3*R*)-**6b** was then converted quantitatively to the corresponding epoxide, (−)-(2*S*,3*S*)-4-(4-chlorophenyl)-2,3-epoxybutane ((−)-(2*S*,3*S*)-**6c**), without racemization using 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (Scheme 1, Figure 2c).[3d] The absolute configuration of (−)-(2*S*,3*S*)-**6c** was confirmed by the comparison of the sign of optical rotation with that reported for the

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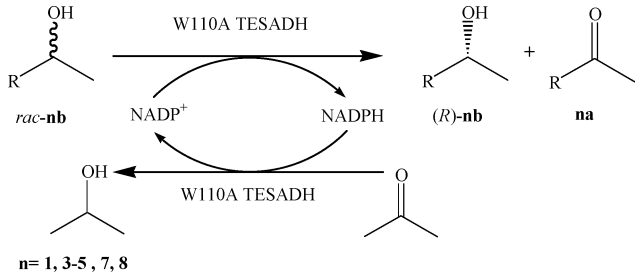
TABLE 2. Enantiospecific Kinetic Resolution of Phenyl Ring-Containing *rac*-Alcohols Using W110A SADH



substrate	R	product[a]	conv. (%)[b]	ee (%)[c]
rac-**1b**	$PhCH_2CH_2$	(*R*)-**1b**	50	>99
rac-**3b**	(*E*)-Ph−HC═CH	(*R*)-**3b**	50	>99
rac-**4b**	*p*-$MeOC_6H_4(CH_2)_2$	(*R*)-**4b**	75	77[d]
rac-**5b**	$PhOCH_2$	(*R*)-**5b**	19	25
rac-**7b**	$PhCH_2$	(*R*)-**7b**	49	39[d]
rac-**8b**	*p*-$MeOC_6H_4CH_2$	(*R*)-**8b**	48	92[d]

[a] The absolute configurations of the unreacted alcohols were confirmed by co-injection in a chiral column GC with their *S* enantiomers prepared by the asymmetric reduction of the corresponding ketones employing W110A TESADH (Table 1). [b] % conversion was determined by GC. [c] Unless otherwise mentioned, ee was determined by a chiral stationary phase GC for the alcohols. [d] The ee was determined for the corresponding acetate derivative (see the Supporting Information).

very similar compound (−)-(2*S*,3*S*)-4-phenyl-2,3-epoxybutane ($[\alpha]^{20}_D$ −26.2, *c* 2.32, $CHCl_3$; lit.[14] $[\alpha]^{25}_J$ −27, *c* 0.04, $CHCl_3$, >98% ee).

Unexpectedly, 1-phenyl-2-propanone (**7a**) was reduced to produce (*S*)-1-phenyl-2-propanol ((*S*)-**7b**) with poor enantioselectivity, indicating that **7a** can fit in alternative modes in the active site within the large pocket, allowing the NADPH cofactor to deliver its *pro*-R hydride from either *re* or *si* faces. 1-(4-Methoxyphenyl)-2-propanone (**8a**) was reduced to produce (*S*)-1-(4-methoxyphenyl)-2-propanol ((*S*)-**8b**) with excellent chemical yield and ee, which means that the sterically bulky para methoxy substituent in **8a** restricts the substrate to only a single binding mode within the active site. The cyclic ketone 2-tetralone (**9a**) was reduced with high yield and moderate stereopreference to produce (*S*)-2-tetralol ((*S*)-**9b**). Enzymatic asymmetric reduction of substrates with sterically hindered groups on both sides of the carbonyl, like **9a**, is of great interest because these substrates are typically either poor or non-substrates for ADHs; therefore, very few ADHs are able to achieve such asymmetric reductions.[1b,3c]

Oxidation via KR of phenyl ring-containing *rac*-alcohols was used to produce their (*R*)-alcohols, the *anti*-Prelog configurated alcohols, as unreacted enantiomers with moderate to high enantiomeric ratios using W110A TESADH. The reactions were carried out in Tris buffer containing 10% (v/v) acetone. The amount of acetone needed was less than the amount of 2-propanol used in the reduction pathway simply because alcohols are more soluble than their corresponding hydrophobic ketones in aqueous media. As with all KRs, these reactions suffer from the limitation that the maximum theoretical yield with high enantiomeric ratio of a single enantiomer, (*R*) in this case, is 50% (Table 2). As expected, the substrates reduced with high ee showed high stereospecificities in the oxidation pathway and vice versa.

The enantiospecific oxidation via KR using W110A TESADH exclusively oxidized the *S* enantiomers of *rac*-**1b** and *rac*-**3b** to the corresponding ketones **1a** and **3a**, respectively, leaving their (*R*)-alcohols as unreacted enantiomers with excellent enantiomeric ratios (Table 2). The production of optically active **1b** is important as it is a precursor for antihypertensive agents, such as bufeniode and labetalol.[3b,17] For *rac*-**4b**, it was resolved by oxidative KR to furnish (*R*)-**4b** with moderate stereopreference (77% ee at 75% conversion). Under the same conditions, KR of *rac*-**5b** furnished (*R*)-**5b** with 25% ee at only 19% conversion, indicating that the KR of this alcohol takes place with high enantiomeric discrimination. Even with addition of more enzyme and acetone, we were not able to push the reaction to higher yield. The racemic 1-phenyl-2-propanol (*rac*-**7a**) was resolved, as expected, with low enantiospecificity because it was reduced with low ee. (*S*)-1-(4-Methoxyphenyl)-2-propanol ((*S*)-**8b**) was oxidized with excellent enantioselectivity to its corresponding ketone **8a**, leaving (*R*)-**8b** as enantiomerically pure unreacted enantiomer. Although **9a** was reduced with high yield and moderate ee, *rac*-**9b** was not oxidized by W110A TESADH. The same results for *rac*-**9b** were obtained by Stampfer et al. using *Rhodococcus ruber* DSM 44541.[3b]

Resistance of TESADH to organic cosolvents allowed the redox reactions in both directions to be carried out at relatively high substrate concentration (35 mM in the reduction pathway and 70 mM in the oxidation pathway). The design of new TESADH mutants such as W110A TESADH in addition to TESADH's resistance to organic solvents and high concentrations of substrates make this enzyme useful for synthetic applications.

Conclusion

We have been able to produce both enantiomers of a series of phenyl ring-containing secondary alcohols by asymmetric reduction and enantioselective oxidation via KR using W110A TESADH. (*S*)-Alcohols were produced via asymmetric reduction with high chemical and moderate to high optical yields using 2-propanol as a cosubstrate for coenzyme regeneration and as a cosolvent. A number of racemic phenyl ring-containing alcohols were resolved with W110A TESADH using acetone as a hydrogen acceptor and a cosolvent. These reactions produced a mixture of (*R*)-alcohols as unreacted enantiomer with good enantiomeric ratios and the corresponding ketones, which could be recycled. The use of 2-propanol (30%, v/v) and acetone (10% v/v) in high concentration in the reduction and oxidation pathways was crucial not only to enhance the solubility of hydrophobic phenyl ring-containing substrates, but also to shift the equilibrium to the desired direction. It is of great interest to produce optically active alcohols of both enantiomers using the same enzyme because the two enantiomers are often of equal importance and only a few *anti*-Prelog enzymes are available. W110A TESADH will be of great interest to organic chemists for the preparation of optically active phenyl ring-containing alcohols because of its thermal stability and high tolerance to organic cosolvents.

Experimental Section

General Procedures. Capillary gas chromatographic measurements were performed on a GC equipped with a flame ionization detector and a Supelco β-Dex 120 chiral column (30 m, 0.25 mm [i.d.], 0.25 μm film thickness) using helium as the carrier gas. ^{1}H

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NMR and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer at room temperature in $CDCl_3$ using either solvent peak or tetramethylsilane as internal standard. Column chromatographies were carried out on standard grade silica gel (60 Å, 32−63 μm) with ethyl acetate in hexane as eluent.

Materials. Commercial grade solvents were used without further purification. $NADP^+$, Novozyme 435, and $NaBH_4$ were used as purchased from commercial sources. Substrates **1a**−**6a**, **9a**, *rac*-**1b**, *rac*-**7b**, (*R*)-**7b**, and (*S*)-**7b** were used as purchased from commercial suppliers. **7a** and **8a** were prepared as described previously.[18] *rac*-**3b**, *rac*-**4b**, *rac*-**5b**, *rac*-**8b**, and *rac*-**9b** were prepared by reducing the corresponding ketones with $NaBH_4$.[19]

Gene Expression and Purification of W110A TESADH. W110A TESADH was expressed in recombinant *E. coli* HB101-(DE3) cells and purified as described.[12]

General Procedure for Asymmetric Reduction of Phenyl Ring-Containing Ketones with W110A TESADH. Reactions were conducted with 0.34 mmol of substrate, 2 mg of $NADP^+$, and 0.75 mg of W110A TESADH in 10.0 mL of 50 mM Tris-buffer (pH 8.0)/2-propanol (70:30, v/v). The reaction mixture was stirred at 50 °C for 10 h, and then it was extracted with 3 × 5 mL of CH_2Cl_2. The combined organic layers were dried with Na_2SO_4 and concentrated under vacuum. The remaining residue was analyzed by chiral column GC to determine the percent conversion and ee of the produced alcohols and then purified with silica gel using hexane/ethyl acetate (85/15) (95/5 for **6b**).

(+)-(2*S*,3*R*)-3-Chloro-4-(4-chlorophenyl)-2-butanol ((+)-(2*S*,3*R*)-6b). $[\alpha]^{20}_D$ +29.2 (*c* 2.08, $CHCl_3$) >99% ee, 84% de. ^1H NMR, δ: 1.33 (d, 3H, J = 6.4 Hz), 1.91 (brs, 1H), 2.91 (dd, 1H, J = 14.6 Hz, J = 9.8 Hz), 3.10 (dd, 1H, J =14.6 Hz, J = 4.2 Hz), 3.96 (qd, 1H, J = 6.4, J = 4.0), 4.14 (td, 1H, J = 9.6, J = 4.0), 7.17 (d, 2H, J = 8.0), 7.29 (d, 2H, J = 8.0). ^{13}C NMR, δ: 18.8, 39.2, 69.4, 70.3, 128.9, 130.8, 132.9, 136.3. HRMS calcd for $C_{10}H_{12}OCl_2$ [M + H]$^+$, 219.0343; found, 219.0347.

General Procedure for Kinetic Resolution of Phenyl Ring-Containing Racemic Alcohols with W110A TESADH. Reactions were conducted with 0.34 mmol of substrate, 1 mg of $NADP^+$, and 0.38 mg of W110A TESADH in 5.0 mL of 50 mM Tris-buffer/acetone (90:10) (v/v). The reaction mixture was stirred at 50 °C for 12 h, and then it was extracted with 3 × 5 mL of CH_2Cl_2. The combined organic layers were dried with Na_2SO_4 and concentrated under vacuum. The remaining residue was analyzed by chiral stationary phase GC to determine the percent conversion to ketone and ee of the unreacted (*R*)-alcohol.

Synthesis of (−)-(2*S*,3*S*)-4-(4-Chlorophenyl)-2,3-epoxybutane ((−)-(2*S*,3*S*)-6c). It was prepared from (2*S*,3*R*)-**6b** using a previously reported procedure for epoxidation.[3d] $[\alpha]^{20}_D$ −26.2 (*c* 2.32, $CHCl_3$) >99% ee, 84% de. ^1H NMR, δ: 1.23 (d, 3H, J = 5.2 Hz), 2.71−2.80 (m, 4H), 7.10 (d, 2H, J = 8.4), 7.20 (d, 2H, J = 8.8). ^{13}C NMR, δ: 17.1, 37.9, 54.6, 59.6, 128.8, 130.5, 132.6, 136.1. HRMS calcd for $C_{10}H_{11}OCl$ [M + H]$^+$, 183.0576; found, 183.0571.

Determination of Absolute Configuration. The absolute configurations of the following compounds were determined by comparing of the sign of the optical rotation with that reported in the literature: (*S*)-**1b**,[20] (*S*)-**2b**,[21] (*S*)-**3b**,[22] (*S*)-**4b**,[23] (*S*)-**5b**,[24] (*S*)-**7b**,[25] (*S*)-**8b**,[26] and (*S*)-**9b**.[3b] The absolute configuration of (*S*)-**7b** was also demonstrated by co-injection on a chiral column GC with commercially available (*R*)-**7b** and (*S*)-**7b**. The absolute configuration of (*S*)-**1b** was confirmed by co-injection on a chiral column GC with (*R*)-**1b**, which was prepared by KR of *rac*-**1b** using Novozyme 435.[27] The absolute configurations of (*R*)-**1b**, (*R*)-**3b**, (*R*)-**4b**, (*R*)-**5b**, (*R*)-**7b**, and (*R*)-**8b** were elucidated by co-injection on GC using a chiral stationary phase with their *S* enantiomers prepared from asymmetric reduction of the corresponding ketones using W110A TESADH.

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Supporting Information Available: Chiral GC chromatogram for both enantiomers of the acetate derivatives of alcohols **1b**−**5b** and **7b**−**9b**. Optical rotation values for (*S*)-**1b**-**5b** and (*S*)-**7b**-**9b**. ^1H NMR and ^{13}C NMR spectra for compounds (*S*)-**1b**-**5b**, (*S*)-**7b**-**9b**, (+)-(2*S*,3*R*)-**6b**, and (−)-(2*S*,3*S*)-**6c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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