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Enantiocomplementary Asymmetric Reduction of 2−Haloacetophenones Using *Te*SADH: Synthesis of Enantiopure 2-Halo-1-arylethanols

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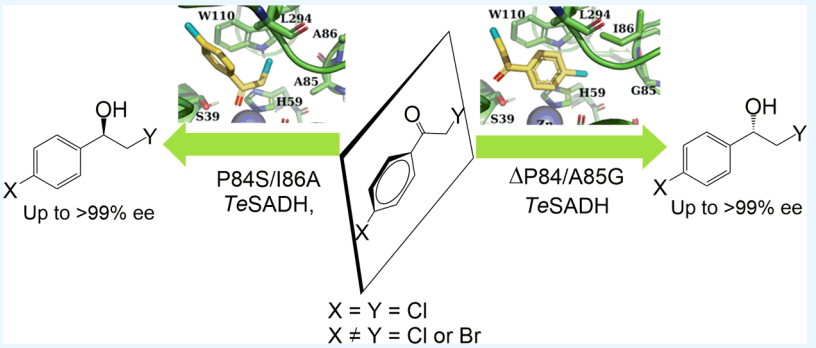

ABSTRACT: Enantiopure 2-halo-1-arylethanols are essential precursors for the synthesis of pharmaceuticals, agrochemicals, and fine chemicals. This study investigates the asymmetric reduction of 2-haloacetophenones and their substituted analogs to obtain their corresponding optically active 2-halo-1-arylethanols using secondary alcohol dehydrogenase from *Thermoanaerobacter pseudethanolicus* (*Te*SADH) mutants. Specifically, the ΔP84/A85G and P84S/A85G *Te*SADH mutants were evaluated for the asymmetric reduction of 2-haloacetophenones, generating their corresponding optically active halohydrins with high enantioselectivities. The asymmetric reduction of 2-haloacetophenones and their substituted analogs using the ΔP84/A85G *Te*SADH mutant yielded their corresponding (*S*)-2-halo-1-arylethanols with high enantiopurity in accordance with the *anti*-Prelog's rule. Conversely, the P84S/A85G *Te*SADH mutant produced (*R*)-alcohols when reducing 2-chloro-4′-chloroacetophenone, 2-chloro-4′-bromoacetophenone, and 2-bromo-4′-chloroacetophenone, while generating the (*S*)-configured halohydrin from 2-chloro-4′-fluoroacetophenone. Asymmetric reduction of the unsubstituted 2-bromoacetophenone, 2-chloroacetophenone, and 2,2,2-trifluoroacetophenone resulted in production of their (*S*)-halohydrins with the tested mutants, which reflects the importance of the nature of the substituent on the substrate's ring in controlling the stereopreference of these *Te*SADH-catalyzed reduction reactions. These findings contribute to the understanding and application of *Te*SADH in synthesizing optically active compounds and aid in the design of further mutants with the desired stereopreference.

INTRODUCTION

The synthesis of optically active 2-halo-1-arylethanols has garnered considerable interest from various research groups.[1−5] These compounds are important building blocks for pharmaceutical drugs. For instance, (*S*)-2-chloro-1-(2′,4′-dichlorophenyl)ethanol is essential in the synthesis of ticonazole, a treatment for vaginal candidiasis and superficial fungal infections of the skin.[6,7] Similarly, (*R*)-2-chloro-1-phenylethanol is used in the synthesis of mirabegron, a *β*-3 adrenergic receptor agonist,[8] and (*S*)-2-chloro-1-(3,4-difluorophenyl)ethanol is used in the synthesis of ticagrelor, a receptor antagonist.[9] Optically active alcohols are typically obtained through asymmetric reduction of prochiral ketones[10,11] or via kinetic resolution (KR) or deracemization of racemates.[12,13] However, KR is limited to 50% yield with high enantiopurity, while deracemization necessitates multiple catalysts with specific stereopreferences operating in the same vessel, hampering the development of new deracemization approaches. Consequently, the asymmetric reduction of prochiral 2-haloacetophenones presents a straightforward option for producing enantiopure 2-halo-1-arylethanols.

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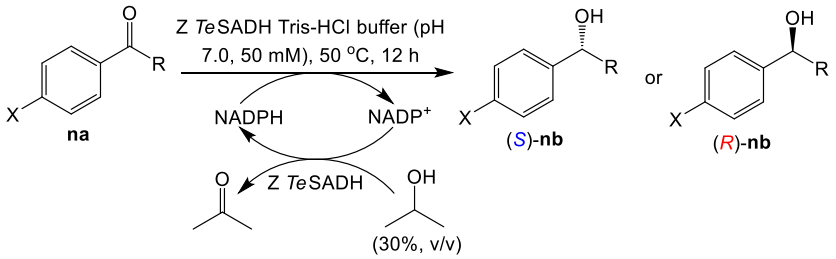
Table 1. Asymmetric Reduction of 2-Haloacetophenone Analogs using *Te*SADH Mutants[a]



X	R	substrate	Z							
			A85G/186A/C295A		P84S/I86A		ΔP84/A85G		I86A	
			conv. (%)[b]	*ee* (%)[c]	conv. (%)[b]	*ee* (%)[c]	conv. (%)[b]	*ee* (%)[c]	conv. (%)[b]	*ee* (%)[c]
H	CH_2Br	**1a**	>99	>99 *S*	>99	>99 *S*	23	>99 *S*	71	>99 *S*
H	CH_2Cl	**2a**	97	>99 *S*	>99	>99 *S*	14	>99 *S*	75	>99 *S*
H	CF_3	**3a**	>99	>99 *S*	43	>99 *S*	low	nd	low	nd
F	CH_2Cl	**4a**	78	>99 *S*	>99	>99 *S*	98	>99 *S*	48	96 *S*
Cl	CH_2Cl	**5a**	10	>99 *R*	66	>99 *R*	37	88 *S*	12	>99 *R*
Br	CH_2Cl	**6a**	16	low	>99	>99 *R*	>99	>99 *S*	10	low
Cl	CH_2Br	**7a**	nr		17	99 *R*	47	82 *S*	nr	
NO_2	CH_2Br	**8a**	nr		46	96 *R*	77	97 *S*	nr	

[a]Unless otherwise stated, reactions were performed in Tris-HCl buffer solution (pH 7.0, 50 mM) containing 2-propanol (30%, v/v) with total reaction volume of 1.0 mL. The following components are expressed as final concentrations in the reaction mixture: ketones substrate (**na**, 10 mM), *Te*SADH mutant (1.6 μM), $NADP^+$ (1.0 mM). The reaction mixture was shaken at 50 °C and 180 rpm for 12 h. [b]Percent conversion was determined by GC. [c]The % *ee* of each of the produced alcohols was determined by GC using a chiral stationary phase. nd: not determined, nr: no reaction detected.

The biocatalytic asymmetric reduction of prochiral ketones is a highly attractive approach for the production of enantiopure secondary alcohols.[14] This is primarily due to the exceptional chemo-, regio-, and stereoselectivity of enzymes, besides being environmentally friendly catalysts, which makes them a sustainable choice.[15−17] Alcohol dehydrogenases (ADHs, EC 1.1.1.X, X = 1 or 2) have been previously employed for the asymmetric reduction of 2-haloacetophenones.[2,4,18−21] An interesting enzyme is secondary ADH from *Thermoanaerobacter pseudethanolicus* (*Te*SADH, EC 1.1.1.2),[22,23] a nicotinamide-adenine dinucleotide phosphate ($NADP^+$)-dependent ADH, which has garnered particular interest because of its thermal stability and tolerance to high concentrations of organic solvents.[24] The latter characteristic makes *Te*SADH suitable for substrate-coupled coenzyme regeneration using 2-propanol. This enzyme is identical to the commercially available secondary ADH from *Thermoanaerobacter brockii* (*Tb*SADH).[25]

The stereopreference of *Te*SADH follows Prelog's rule,[26] in which the NADPH delivers its pro-*R* hydride from the *re* face of prochiral ketones, producing (*S*)-configured alcohols when the large group of the prochiral ketone exhibits a higher Cahn-Ingold-Prelog priority than that of the small group. To expand the substrate scope of *Te*SADH, various mutants of this enzyme have been constructed to accommodate aryl-ring-containing ketones that are not substrates for the wild-type *Te*SADH. Notably, mutations at the W110 site have been shown to enable the accommodation of substrates such as 4-aryl-2-butanones and 1-aryl-2-propanones, resulting in the production of their corresponding (*S*)-configured alcohols (i.e., Prelog mode).[27−29] Furthermore, the I86A *Te*SADH mutant has been reported to accommodate unsubstituted acetophenone, producing the corresponding (*R*)-1-phenylethanol.[30] The construction of mutants such as I86A/C295A, A85G/I86A/C295A, I86A/V115A/C295A, and I86A/T153A/C295A has further expanded the smaller pocket in the active site of *Te*SADH, enabling the reduction of substituted acetophenones to their corresponding (*R*)-alcohols.[31,32] In the current study, we present the asymmetric reduction of 2-haloacetophenone analogs to obtain enantiocomplementary optically active 2-halo-1-arylethanols using various mutants of *Te*SADH.

RESULTS AND DISCUSSION

In this study, we constructed four mutants of *Te*SADH, namely, I86A, A85G/186A/C295A, P84S/186A, and ΔP84/A85G to conduct the asymmetric reduction of 2−haloacetophenone analogs which have never been reported to be reduced using *Te*SADH. The design of these mutants aimed to expand the enzyme's smaller binding pocket[30] and to disrupt the rigidity (imposed by proline-84) of the loop that lines the active site.[33] A85, I86, and C295 line the small pocket of *Te*SADH, and thus mutations at these sites with sterically less demanding amino acids is expected not only to improve the substrate scope of *Te*SADH, but also to switch its stereopreference. I86A and A85G/186A/C295A mutants of *Te*SADH were proven before to reduce acetophenone analogs in *anti*-Prelog mode.[30−32]

To evaluate the performance of these *Te*SADH mutants, we conducted reduction reactions using 2-bromoacetophenone (**1a**) as the substrate. The reactions were carried out in tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer solutions (pH 7.0, 50 mM) containing 2-propanol (30% v/v), which served as both a cosubstrate for NADPH regeneration and a cosolvent to enhance the solubility of aryl-ring-containing hydrophobic substrates, which are sparingly soluble in aqueous media. Reactions under slightly acidic or basic pH conditions resulted in formation of byproducts including the 1-phenyl-1,2-ethanediol and 1-phenylethanol.

All the mutants that were tested successfully reduced **1a**, resulting in production of (*S*)-2-bromo-1-phenylethanol [(*S*)-

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1b] with high conversions and enantioselectivities (>99%), except for ΔP84/A85G, which showed a low conversion, Table 1. Similar results were observed in the asymmetric reduction of 2-chloroacetophenone (**2a**) to (*S*)-2-chloro-1-phenylethanol [(*S*)-**2b**]. The production of (*S*)-**1b** and (*S*)-**2b**, considering the inverted Cahn-Ingold-Prelog priority for all halohydrin substrates reported in the current study, was in line with our expectations based on the design of these *Te*SADH mutants. The production of (*S*)-alcohols using the tested mutants indicates that their ketones **1a** and **2a** fit into the active site of *Te*SADH in a pro-*S* orientation (i.e., *anti*-Prelog mode). This finding is consistent with a previous report of I86A *Te*SADH-catalyzed reduction of acetophenone, which resulted in the production of (*R*)-1-phenylethanol (i.e., *anti*-Prelog mode).[30]

Reduction of 2,2,2-trifluoroacetophenone (**3a**) using A85G/186A/C295A *Te*SADH resulted in quantitative formation of (*S*)-2,2,2-trifluoro-1-phenylethanol [(*S*)-**3b**] with high enantioselectivity. The same enantiomer, yet with a lower yield, was produced when using P84S/I86A *Te*SADH.

Reduction of 2-chloro-4′-fluoroacetophenone (**4a**) using all mutants resulted in formation of (*S*)-2-chloro-1-(4′-fluorophenyl)-1-ethanol [(*S*)-**4b**] with very high enantioselectivity. Notably, P84S/186A and ΔP84/A85G mutants showed improved conversion yields for this substrate. Reduction of 2-chloro-4′-chloroacetophenone (**5a**) using I86A, A85G/186A/C295A and P84S/186A *Te*SADH mutants resulted in production of (*R*)-2-chloro-1-(4′-chlorophenyl)-1-ethanol [(*R*)-**5b**] with high enantioselectivity (>99% ee), but with low to moderate conversions. In contrast, the ΔP84/A85G *Te*SADH produced (*S*)-**5b** with low conversion but high enantioselectivity. Asymmetric reduction of 2-chloro-4′-bromoacetophenone (**6a**) using P84S/I86A and ΔP84/A85G mutants quantitatively yielded (*R*)-2-chloro-1-(4′-bromophenyl)-1-ethanol [(*R*)-**6b**], and (*S*)-**6b**, respectively, with high enantioselectivity (>99% ee). A similar trend was observed in the asymmetric reduction of 2-bromo-4′-chloroacetophenone (**7a**) and 2-bromo-4′-nitrocetophenone (**8a**) using P84S/I86A and ΔP84/A85G mutants, albeit with low to medium conversions.

The results obtained from the asymmetric reduction of **4a**−**8a** underscore the significant impact of the substituent's identity on the phenyl ring in controlling the stereopreference of *Te*SADH-catalyzed asymmetric reductions of substituted 2−haloacetophenones. Furthermore, the performance of P84S/I86A and ΔP84/A85G *Te*SADH mutants surpassed that of A85G/I86A/C295A and I86A *Te*SADH in the asymmetric reduction of the tested para-substituted 2-haloacetophenones.

Remarkably, P84S/I86A *Te*SADH displayed enantiocomplementary stereopreference to ΔP84/A85G *Te*SADH in the asymmetric reduction of **5a**−**8a**, which is aligned with a previous study on asymmetric reduction of bulky−bulky ketones that exhibit aryl-ring-containing groups on both sides of the carbonyl group.[33] Additionally, ΔP84/A85G *Te*SADH consistently exhibited *anti*-Prelog stereopreference, yielding (*S*)-halohydrins in reduction of ketones **1a**-**8a**. Interestingly, despite the para-substituent, substrate **4a** interacted similarly with the tested *Te*SADH mutants as the unsubstituted acetophenones, possibly due to the smaller size of fluorine compared to chlorine and bromine. With substrates **1a**, **2a**, and **4a**, both P84S/I86A and ΔP84/A85G exhibited a similar trend to that observed in I86A and A85G/186A/C295A mutants but with improved conversions and enantioselectivities.

The origin of stereopreference in P84S/I86A and ΔP84/A85G *Te*SADH mutants was investigated by docking substrates **5a** and **2a** into the active sites of these mutants using the AutoDock Vina program.[34] The crystal structure of *Tb*SADH, which is identical to *Te*SADH,[25] complexed with NADP (PDB: 1YKF) was used as the basis for docking analyses.[35] The lowest energy docked conformations are shown in Figure 1. In the P84S/I86A mutant, the aryl ring

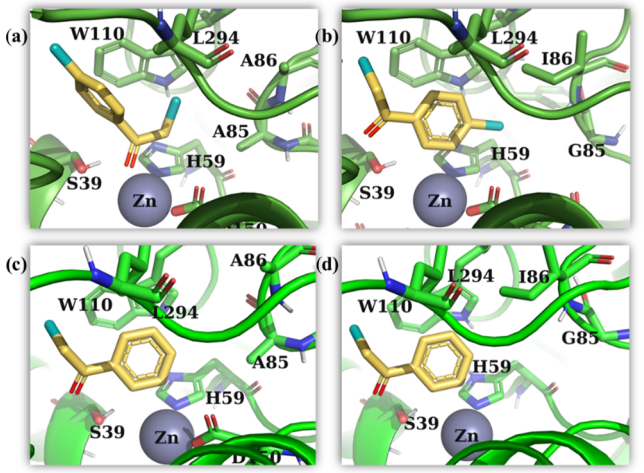

Figure 1. Lowest energy dockings of: (a) **5a** into the binding pocket of P84S/I86A *Tb*SADH, (b) **5a** into the binding pocket of ΔP84/A85G *Te*SADH, (c) **2a** into the binding pocket of P84S/I86A, (d) **2a** into the binding pocket of ΔP84/A85G. The substrate is viewed from the face occupied by NADPH in the *Tb*SADH crystal structure. Enzyme carbon is represented in green, substrate carbon in yellow, nitrogen in blue, oxygen in red, and chlorine in cyan.

of **5a** occupies the space in the large pocket while the halomethylene group is placed in the small pocket (Figure 1a), allowing for a pro-*R* orientation. Placing the aryl ring in the small pocket of P84S/I86A would have resulted in a steric clash with the methyl group of A85 (Figure S1). In contrast, ΔP84/A85G allows the aryl ring to fit in the small pocket and the halomethylene group in the large pocket, enabling a pro-*S* orientation (Figure 1b).

The absence of a substituent on the phenyl ring of **2a** eliminates the possible steric clash with A85's methyl group, resulting in a pro-*S* orientation in both P84S/I86A and ΔP84/A85G (Figure 1c,1d). The docking results indicate that the methyl group of A85 plays critical role in altering the stereopreference of *Te*SADH in asymmetric reduction of para-substituted 2-haloacetophenones. Unsubstituted 2-haloacetophenones fit in the active site of the *Te*SADH mutants tested in this study in an orientation that positions the phenyl ring in the small pocket of the active site, allowing for a pro-*S* orientation (i.e., *anti*-Prelog's mode). For para-substituted 2-haloacetophenones, the aryl ring is ejected from the small pocket due to the clash with A85 in P84S/I86A *Te*SADH, and thus moves to the large pocket allowing for a pro-*R* orientation (i.e., Prelog mode). These results also confirm the higher affinity of the smaller binding pocket of *Te*SADH when compared with that of the larger binding pocket, which is consistent with the original findings for the wild-type *Te*SADH and *Tb*SADH.[36,37]

To further explore the impact of A85G in altering the stereopreference in the asymmetric reduction of para-

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substituted 2-haloacetophenones using ΔP84/A85G *Te*SADH, we created A85G and ΔP84 mutants of *Te*SADH. Subsequently, we carried out reduction reactions of para-substituted 2-chlorocetophenones using A85G and ΔP84 single mutants of *Te*SADH. The results presented in Table 2

Table 2. Asymmetric Reduction of 2-Haloacetophenone Analogs using A85G and ΔP84 Mutants of *Te*SADH[a]

			Z			
			A85G		ΔP84	
X	R	substrate	conv. (%)[b]	*ee* (%)[c]	conv. (%)[b]	*ee* (%)[c]
F	CH_2Cl	**4a**	27	>99 *R*	low	>99 *R*
Cl	CH_2Cl	**5a**	78	>99 *S*	low	>99 *R*
Br	CH_2Cl	**6a**	86	>99 *S*	low	>99 *R*

[a]The same reaction conditions explained as footnote for Table 1 are used here. [b]Percent conversion was determined by GC. [c]The % *ee* of each of the produced alcohols was determined by GC using a chiral stationary phase. nr: no reaction detected.

reveal that when reducing para-substituted 2-chloroacetophenones, **4a**−**6a**, A85G exhibited a stereopreference that is consistent with that of ΔP84/A85G, producing the (*S*)-halohydrins in the reduction of **5a** and **6a**. Interestingly, asymmetric reduction of **4a** using A85G *Te*SADH produced the corresponding (*R*)-halohydrin, unlike all other variants tested. Conversely, ΔP84 exhibited minimal to no activity with these substrates. These results indicate that replacing A85 with the less sterically demanding glycine is crucial in influencing the *Te*SADH's stereopreference in the asymmetric reduction of substituted 2-haloacetophenones and in expanding the binding pocket of *Te*SADH to accommodate these substrates. This observation is consistent with previous findings on asymmetric reduction of substituted acetophenones.[32]

The current study adds to the growing repertoire of available ADHs that exhibit *anti*-Prelog stereopreference in the asymmetric reduction of prochiral ketones. It also provides valuable insights into the factors that influence the stereopreference of *Te*SADH, as demonstrated by the switch in the stereochemical outcome observed when using the P84S/I86A versus ΔP84/A85G variants of *Te*SADH. Expanding the pool of ADHs capable of *anti*-Prelog reduction opens up new opportunities for the selective synthesis of valuable chiral alcohol building blocks.[38]

CONCLUSIONS

In conclusion, this study delved into the asymmetric reduction of 2-haloacetophenone analogs using four mutants of *Te*SADH (I86A, A85G/186A/C295A, P84S/186A, and ΔP84/A85G). Notably, the P84S/I86A and ΔP84/A85G mutants demonstrated improved performance compared to I86A and A85G/186A/C295A mutants, displaying superior conversions and enantioselectivities. Intriguingly, the P84S/I86A and ΔP84/A85G mutants exhibited enantiocomplementary stereopreferences in the reduction of para-substituted 2-haloacetophenones. Modeling studies emphasize the critical role of the interactions between the substituent in the para position of the substituted 2-haloacetopenones and the methyl group of A85 in controlling the stereopreference of P84S/I86A and ΔP84/A85G mutants of *Te*SADH. These findings demonstrate the potential of guided mutagenesis in broadening the substrate scope and stereopreference of *Te*SADH. They also provide valuable insights into how the substituent on the aryl ring of 2-haloacetophenones influences the stereopreference in their *Te*SADH-catalyzed asymmetric reductions. The current study opens up avenues for exploring asymmetric reduction of other substituted 2-haloacetophenone analogs using P84S/I86A and ΔP84/A85G. It should also guide in designing *Te*SADH mutants with large substrate scope and with the desired stereopreference. Overall, this study contributes to the development of more efficient and selective biocatalysts for organic synthesis.

EXPERIMENTAL SECTION

Asymmetric Reduction of Ketones Using *Te*SADH Mutants. The enzymatic reduction reactions of ketones were carried out by using an NADPH recycle system as described previously. Reactions were conducted in 1.5 mL reaction tubes consisting of α-haloacetophenones (1.0 mg), NAD^+ (1.0 mg), Tris-HCl buffer solution (700 μL, pH 8.5 and pH 7.0 for brominated substrates), 2-propanol (300 μL), and 10 μL of ∼160 μM (i.e., 1.6 μM final concentration) enzyme. The mixture was shaken at 180 rpm and 50 °C for 14 h. The reaction progress was monitored using thin layer chromatography. The mixture was then extracted with diethyl ether (500 μL × 2). The organic layer was dried with sodium sulfate, and then concentrated to dryness.

Gene Expression and Purification of *Te*SADH Mutants. The genes encoding *Te*SADH with mutations were synthesized and subcloned into the pET11b vector (GenScript). The resulting expression vectors to express Δ84 *Te*SADH, A85G *Te*SADH, ΔP84/A85G *Te*SADH, A85G/I86A/C295A *Te*SADH, and I86A *Te*SADH are pET11b_*Te*SADH-Δ84, pET11b_*Te*SADH-A85G, pET11b_*Te*SADH-ΔP84 A85G, pET11b_A85G I86A C295A, and pET11b_*Te*SADH-I86A, respectively. Each expression vector was transformed into BL21(DE3) *E. coli* cells. The transformed cells were grown in LB medium at 37 °C until 1.0 of OD_{600} and further incubated for 3 h after adding isopropyl b-d-1-thiogalactophranoside (IPTG, 1 mM). The cells were harvested by centrifugation (5,500 g for 10 min) and resuspended in Lysis buffer (50 mM Tris-HCl pH 8.8, 10 mM BME). The protein purifications were performed as reported previously.[39,40]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c05151.

Experimental procedures for enzymatic reactions and protein expression and purification, representative chiral gas chromatograms, ^1H NMR and ^{13}C NMR spectra (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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