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Enantioselective Enzymatic Reduction of Acrylic Acids

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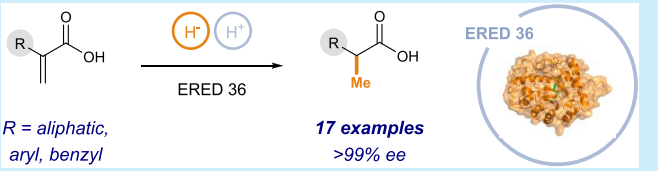
ABSTRACT: An ene-reductase (ERED 36) with broad substrate specificity was identified, and optimization studies led to the development of an enzymatic protocol for the reduction of α,β-unsaturated acids under mild, aqueous conditions. The substrate scope includes aromatic- and aliphatic-substituted acrylic acids, as well as cyclic α,β-substituted acrylic acids, yielding chiral α-substituted acids with exquisite levels of enantioselectivity (>99% ee).



Chiral α-substituted carboxylic acids are found in a wide array of biologically active molecules, including non-steroidal anti-inflammatory compounds such as ibuprofen, naproxen, and indan-1-carboxylic acids (Figure 1).[1−4] Recent

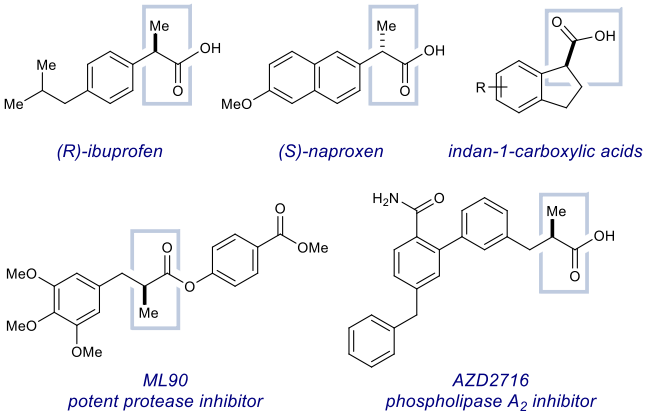


Figure 1. Chiral substituted carboxylic acids and derivatives.

reports outlining the discovery of 2-benzylpropanoic acid derivatives with important biological properties highlight the importance of developing enantioselective methods that provide synthetic chemists with simple and efficient approaches for accessing this structural motif.[5,6]

Despite the importance of these scaffolds, many current methods fail to uphold green chemistry principles: low step count, sustainable and environmentally benign conditions, and minimal associated safety risks.[7] Large-scale syntheses often rely on chiral auxiliaries[8] or kinetic resolution,[9,10] approaches that require additional steps or limit the theoretical yield to 50%. Asymmetric hydrogenation protocols, with finely tuned catalyst systems, can be employed to provide chiral acids in excellent yields and enantioselectivities from comparably simple unsaturated substrates.[11−14] However, the use of expensive transition metals, safety hazards associated with the use of hydrogen gas and chemoselectivity challenges, render this approach nonideal. Over the past decade, biocatalysis has come to the forefront of synthetic chemistry, enabling mild, green, and highly enantio- and chemoselective syntheses of diverse compounds. Herein, we outline the development of a green synthetic approach to deliver enantioenriched carboxylic acids via enzymatic reduction of readily accessible acrylic acid derivatives.

Ene-reductases (EREDs) are a class of enzymes that catalyze the reduction of activated alkenes such as α,β-unsaturated aldehydes, ketones, esters, and nitroalkenes, often with exquisite stereoselectivity (Scheme 1).[15−17] Although the synthetic applicability of nicotinamide-dependent flavoproteins from the "Old Yellow Enzyme" (OYE) family is well-established, the use of these enzymes for reduction of less activated C=C bonds (e.g., α,β-unsaturated carboxylic acids) remains underexplored. Mechanistically, it is understood that the activated alkene binds in the active site of the enzyme and undergoes a stereoselective *anti*-reduction via hydride conjugate addition from a flavin mononucleotide ($FMNH_2$) cofactor, followed by protonation from a tyrosine residue.[18,19] Given that carboxylic acids are poor Michael acceptors, it is perhaps unsurprising that EREDs possess limited activity on unactivated acids. Indeed, previous literature reports are restricted to enzymatic reduction of activated bisacids,[20−22] employ EREDs that require anaerobic conditions,[23−26] or display narrow scope and low activity (typically on 2-

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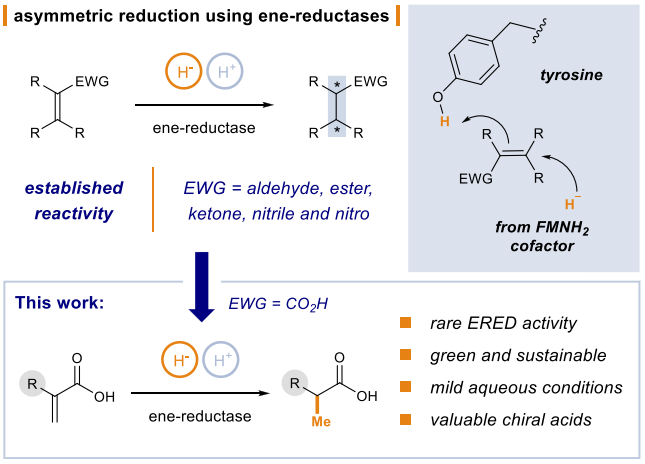
Scheme 1. Ene-Reductase-Mediated Synthesis of Chiral Acids

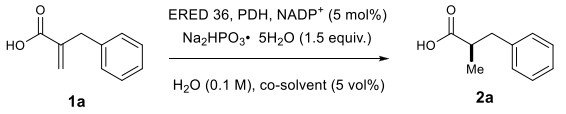


arylpropanoic acids).[27,28] Scrutton and co-workers circumvented the poor reactivity of ene-acids by subjecting more reactive enals to a biocatalytic reduction/oxidation cascade sequence.[29] Considering the scarcity of the literature on the enzymatic reduction of unsaturated acids and the value this synthetic strategy could offer industrial chemists, we were motivated to investigate this transformation.

We selected 2-benzylacrylic acid **1a** as a model substrate for our investigations. To the best of our knowledge, enzymatic reduction of 2-benzylacrylic acid derivatives has not been reported, likely due to insufficient activation of the alkene toward hydride addition. To this end, we evaluated two panels of commercially available EREDs (Codexis and Prozomix panels, see Supporting Information Table S1) for the enantioselective reduction of acid **1a**. From over 130 ene-reductases screened, only three enzymes displayed appreciable activity (>2% conversion). Catalysis by Prozomix ERED 36 delivered chiral acid (*R*)-**2a** with the highest level of conversion (52%) and >99% ee, with the minor (*S*)-enantiomer undetectable by chiral GC.

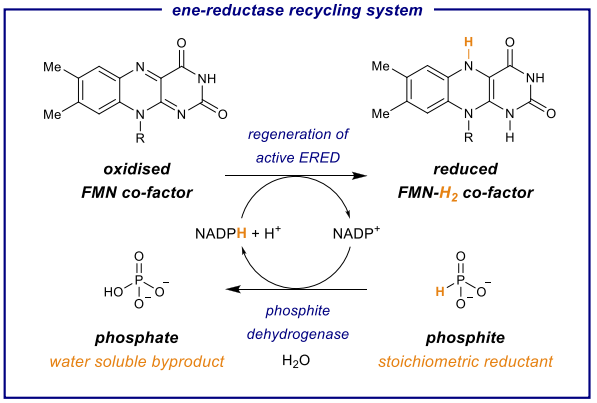
With this result in hand, we conducted further optimization of the reaction conditions using ERED 36 (Table 1). Regeneration of the reduced flavin cofactor bound within the ERED active site requires the addition of a second reducing cofactor nicotinamide adenine dinucleotide phosphate (NADPH). However, nicotinamide cofactors are extremely costly, and using these reagents on scale in stoichiometric quantities is not feasible. As such, we introduced a second enzyme, phosphite dehydrogenase (PDH), to regenerate NADPH.[30] This recycling system uses inexpensive sodium phosphite as the stoichiometric reductant, generates innocuous inorganic phosphate as the byproduct, and has no requirement for active pH control (unlike glucose dehydrogenase recycling systems).[31] Using the outlined enzyme system, we established that temperature and pH are critical parameters for achieving high activity (entries 1–6). ERED 36 was most active at slightly acidic to neutral conditions (pH 6.5–7), and outside this range a dramatic reduction in rate was observed. Likewise, while excellent yields could be achieved by performing the reaction between 25 and 30 °C, the limited thermostability of ERED 36 led to a considerably decreased reaction rate at 35 °C. The low solubility of lipophilic organic molecules in water is a key challenge for the application of enzymatic transformations in industrial chemical synthesis; therefore, we investigated organic cosolvent tolerance as a key reaction parameter (entries 7–11). Pleasingly, ERED 36 remains highly active in the presence of 5 vol % of DMSO or MeOH. The use of other cosolvents, including DMF, IPA, and MeCN, resulted in a significant reduction or complete loss of catalytic activity. Lastly, to ensure synthetic utility, we demonstrated gram-scale synthesis and isolation of acid **2a** in 97% yield and >99% ee.

Table 1. pH, Temperature, and Cosolvent Screening[a]



entry	pH[b]	temperature	cosolvent	yield[c]
1	6.0	25 °C	none	30%
2	6.5	25 °C	none	97%
3	7.0	25 °C	none	96%
4	7.5	25 °C	none	66%
5	6.5	30 °C	none	98%
6	6.5	35 °C	none	43%
7	7.0	30 °C	DMSO	94%
8	7.0	30 °C	DMF	6%
9	7.0	30 °C	IPA	48%
10	7.0	30 °C	MeOH	88%
11	7.0	30 °C	MeCN	3%

[a]Reaction conditions: 2-benzylacrylic acid **1a** (0.1 M), sodium phosphite dibasic pentahydrate (1.5 equiv), ERED 36 (50 mg/mmol of **1a**), $NADP^+$ (5 mol %), PDH (8 mg/mmol **1a**), H_2O (0.1 M), cosolvent (5 vol %), 24 h. [b]pH is adjusted with 1 N NaOH. [c]Yield determined by HPLC.



With optimized conditions in hand, we next investigated the substrate scope of ERED 36 by exploring differentially substituted 2-benzylacrylic acids **1b–1h** (Table 2). *Ortho*- and *para*-substituted benzyl acrylic acids **1b–g** were well tolerated (42–88% yield of **2b–g**, >99% ee), although higher enzyme loading was required (vs unsubstituted 2-benzylacrylic acid, **1a**) with more sterically encumbered substrates. The exquisite functional group tolerance offered by enzymatic reduction protocols was demonstrated through formation of product **2g** containing a ketone substituent (**2g**, 42% yield, >99% ee), functionality that would likely suffer from competitive reduction when subjected to transition metal-catalyzed hydrogenation. Under standard conditions, no reactivity was observed for substrate **1h** containing an unprotected indole. We noted that acid **1h** was poorly soluble in the aqueous reaction medium and postulated that increased

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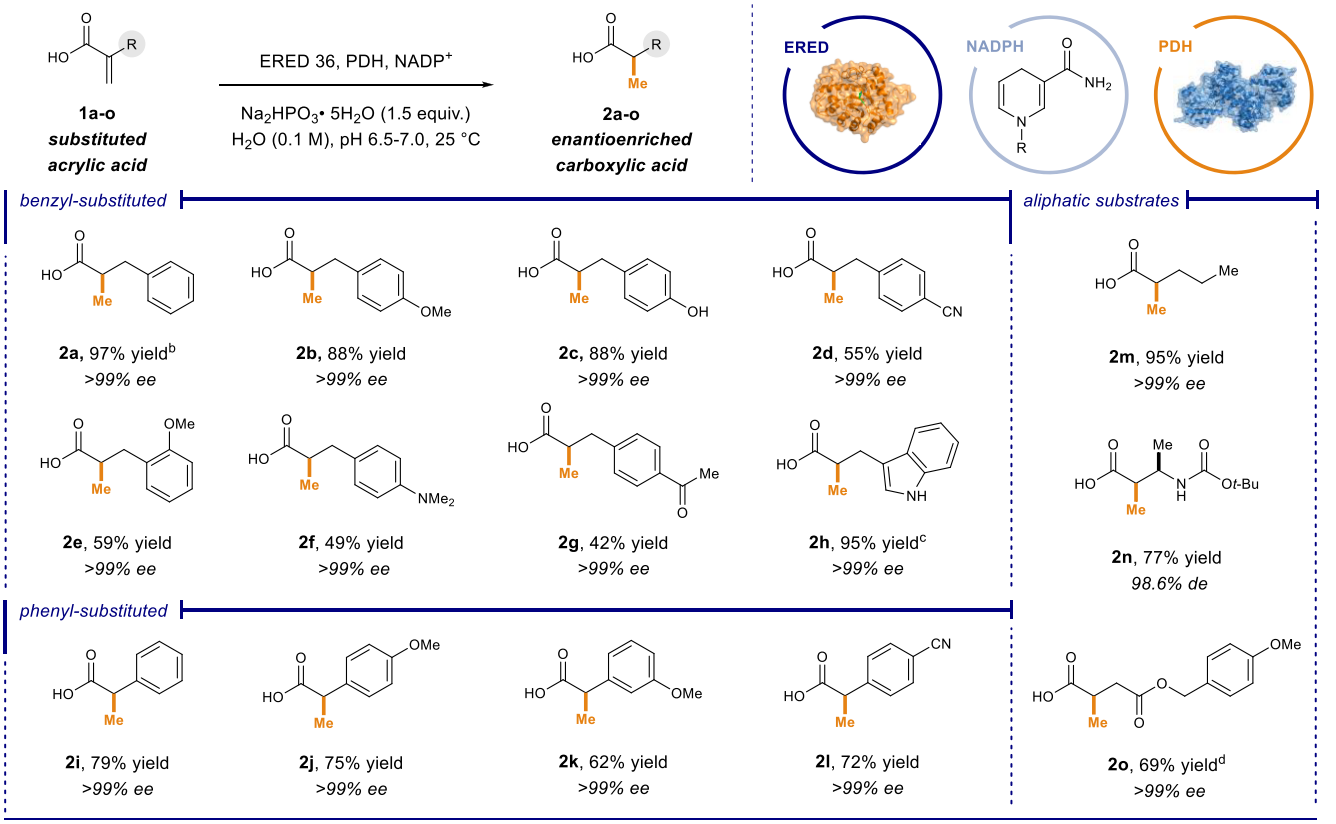
Table 2. Substrate Scope of Enzymatic Reduction with ERED 36[a]



[a]Reaction conditions unless otherwise stated: acid **1** (1.5 mmol), ERED 36 (50−300 mg/mmol of **1**), $NADP^+$ (5 mol %), PDH (8 mg/mmol of **1**), sodium phosphite dibasic pentahydrate (1.5 equiv), H_2O (0.1 M), 18−24 h. Isolated yields reported. ee values and absolute configuration for **2a** and **2i** were determined by chiral SFC or GC analysis. Unless otherwise stated, the absolute stereochemistry of other products was assigned by analogy. [b]Reaction performed on 1.6 g (10 mmol) scale. [c]10 vol % of DMSO. [d]ee value and absolute stereochemistry were determined by conversion to 2-methylsuccinic acid and subsequent analysis by chiral SFC.

pH and/or inclusion of an organic cosolvent could aid dissolution and recover reactivity. Indeed, when increasing to pH 7 and including 10 vol % of DMSO as a cosolvent, full conversion to the desired saturated carboxylic acid was observed (**2h**, 95% yield, >99% ee).

Ene-reductase 36 catalyzes efficient and highly enantioselective reduction of activated 2-phenylacrylic acids (**2i**−**2l**, 62−79% yield). Here, we evaluated the influence of alkene electronics on reactivity; substrates containing electron-withdrawing and electron-donating substituents on the aryl ring were both converted to optically pure acids in excellent yields. Next, we explored the reactivity of aliphatic acrylic acids and found that ERED 36 accepts substrates of varying steric bulk with the reduction proceeding in a highly stereoselective manner (**2m**, **2n**, and **2o**, 95%, 77%, and 69% yield, respectively). Formation of chiral acid **2n** highlights the applicability of this method to the diastereoselective synthesis of unnatural β-amino acids. Finally, for substrate **1o**, chemoselective alkene reduction in preference to 4-methoxybenzyl ester deprotection demonstrates a clear advantage over hydrogenation protocols.

Exploration of the substrate scope highlighted two key features of ERED 36: (i) rare activity toward unactivated α,β-unsaturated acids and (ii) broad substrate specificity, with ERED 36 accepting substrates with electronically and sterically diverse substituents. The discovery of this reactivity motivated us to design further experiments probing the scope and mechanism of enzymatic reduction. While there have been reports of ene-reductases with limited activity on 2-aryl-substituted acids,[27,28] we were particularly surprised to observe activity on less activated aliphatic and benzylic acrylic acids (**1a**−**1h** and **1m**−**1o**). We considered a mechanistic scenario in which isomerization to the α,β-substituted ene-acid occurs prior to reduction (Scheme 2A).[32] Cinnamic acids can be reduced by oxygen-sensitive ene-reductases,[25,26] though comparable activity with ene-reductases applicable to industrial processes (e.g., oxygen-tolerant EREDs from the OYE family) has not been demonstrated.[28,29] To probe this hypothesis, we tested ERED 36 on (*E*)-α-methylcinnamic acid and both geometric isomers of cinnamic acid (Scheme 2A). Regardless of the alkene geometry, we failed to observe reduction products **2a** and **2p**, invalidating the intermediacy of these conjugated acids along the reaction pathway.

The outlined studies, in accordance with prior reports,[28] highlight the challenge of identifying ene-reductases from the OYE family capable of reducing β-substituted ene-acids and prompted us to explore other substrates in this class (Scheme 2B). Initially, we probed the impact of alkene geometry on enzymatic activity by evaluating the reduction of (*E*)- and (*Z*)-2-methyl-2-butenoic acid. Interestingly, while ERED 36 exhibited minimal activity on the *cis*-alkene isomer (*Z*-**1q**), *trans*-isomer *E*-**1q** undergoes reduction to deliver saturated acid **2q** with high enantioselectivity (98.4% ee) albeit in low yield (Table S5). Having discovered that unhindered β-

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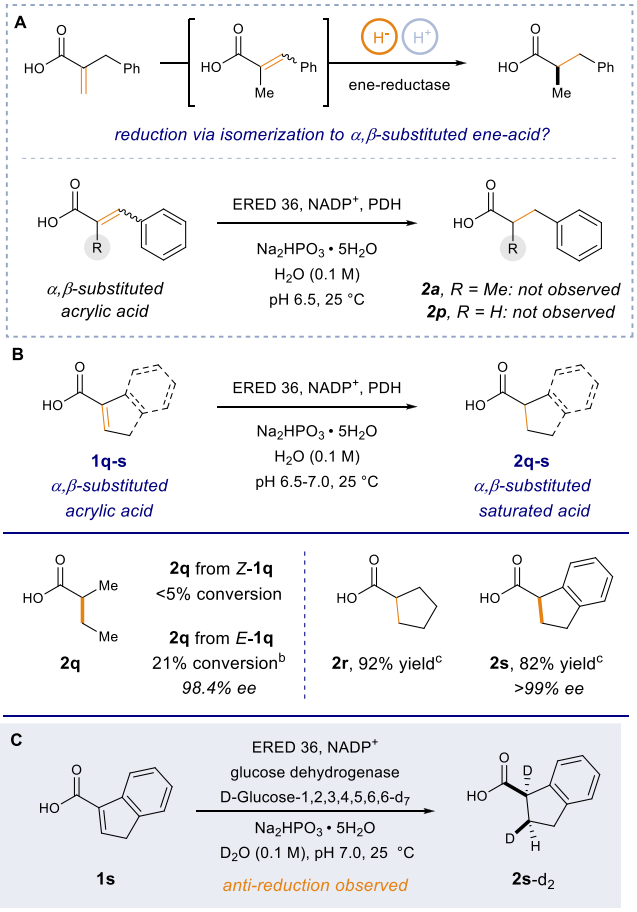
Scheme 2. Reactivity of β-Substituted Unsaturated Acids: (A) Experiments Probing the Viability of an Isomerization−Reduction Mechanistic Pathway; (B) Reduction of β-Substituted Unsaturated Acid Substrates; and (C) Deuterium Labeling Experiment Probing the Reduction Mechanism[a]



[a]Reaction conditions: acid **1** (1.5 mmol), ERED 36 (50−300 mg/mmol **1**), $NADP^+$ (5 mol %), PDH (8 mg/mmol **1**), sodium phosphite dibasic pentahydrate (1.5 equiv), water (0.1 M). ee values and absolute stereochemistry were determined by chiral SFC or GC analysis. [b]Conversion determined by NMR. [c]Isolated yields reported.

substituents are tolerated (*E*-**1q**, Me group), we anticipated that cyclic α,β-substituted ene-acids might display enhanced reactivity due to the limited steric bulk of the constrained β-substituent and relief of ring strain upon reduction. Indeed, exposure of 1-cyclopentene carboxylic acid **1r** and 1*H*-indene-3-carboxylic acid **1s** to ERED 36 resulted in formation of the corresponding saturated acids in excellent yields (**2r** and **2s**, 92% and 82% yield, respectively).

Since ERED 36 exhibits remarkable activity on unactivated carboxylic acids generating saturated products with excellent enantioselectivity, we utilized cyclic substrate **1s** to probe whether the reduction mechanism is consistent with the canonical *anti*-reduction pathway of the OYE family. We performed a deuterium labeling experiment with 1*H*-indene-3-carboxylic acid **1s** in the presence of D_2O and *in situ* generated NADPD (from $NADP^+$, D-glucose-d_7, and glucose dehydrogenase) to produce bisdeuterated **2s**-d_2 (Scheme 2C).[33] NMR analysis (see Supporting Information Figures S2 and S3) revealed that the relative stereochemistry of the deuterium atoms was *trans*, indicating that the reactivity conferred by ERED 36 follows the typical *anti*-reduction mechanism. Overall, the studies described above probing the reactivity of ERED 36 toward β-substituted ene-acids (i) indicate that products **2a−o** (Table 2) are likely generated via direct *anti*-reduction of the 1,1-disubstituted ene acids and (ii) further highlight the reactivity of ERED 36 with respect to the reduction of challenging ene-acid substrates.

In summary, enzymatic reduction of a wide range of unsaturated acids was demonstrated with exceptional levels of enantioselectivity. We identified a commercially available ERED (Prozomix ERED 36) that reduces unactivated ene-acids such as benzyl- and aliphatic-substituted acrylic acids. Although α,β-substituted ene-acids are typically challenging substrates for EREDs, we successfully demonstrated reduction of cyclic α,β-substituted ene-acids. The utilization of an environmentally benign catalyst under mild aqueous conditions with unrivaled chemoselectivity illustrates clear advantages of enzymatic reduction over asymmetric hydrogenation. Finally, mechanistic studies indicated that ERED 36 reduces ene-acids via the canonical *anti*-reduction pathway. Future studies will focus on understanding the remarkable reactivity of ERED 36 toward ene-acids with the goal of further expanding the scope of enzymatic reduction.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c02959.

Experimental procedures, compound characterization, NMR spectra, and chiral assays (PDF)

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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