

Asymmetric Synthesis of α -Chloroamides via Photoenzymatic Hydroalkylation of OlefinsYi Liu,¹ Sophie G. Bender,¹ Damien Sorigue, Daniel J. Diaz, Andrew D. Ellington, Greg Mann, Simon Allmendinger, and Todd K. Hyster*Cite This: *J. Am. Chem. Soc.* 2024, 146, 7191–7197

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ABSTRACT: Photoenzymatic intermolecular hydroalkylations of olefins are highly enantioselective for chiral centers formed during radical termination but poorly selective for centers set in the C–C bond-forming event. Here, we report the evolution of a flavin-dependent “ene”-reductase to catalyze the coupling of α,α -dichloroamides with alkenes to afford α -chloroamides in good yield with excellent chemo- and stereoselectivity. These products can serve as linchpins in the synthesis of pharmaceutically valuable motifs. Mechanistic studies indicate that radical formation occurs by exciting a charge-transfer complex templated by the protein. Precise control over the orientation of molecules within the charge-transfer complex potentially accounts for the observed stereoselectivity. The work expands the types of motifs that can be prepared using photoenzymatic catalysis.

Enantioenriched α -haloamides are attractive linchpins for preparing many chiral motifs found in molecules used by the agrochemical and pharmaceutical industries (Figure 1).^{1–6}

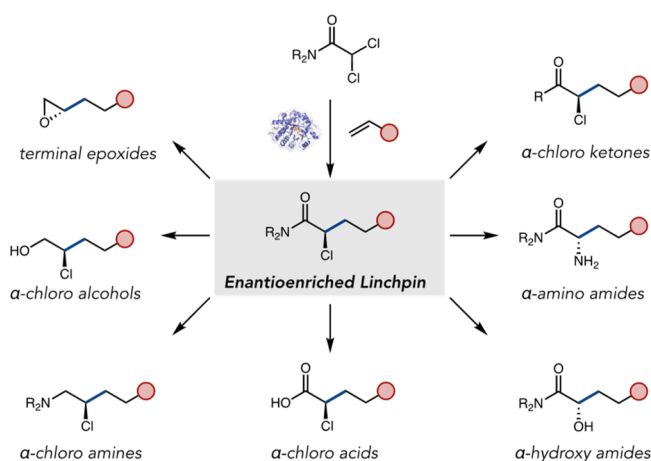


Figure 1. α -Chloroamides as a linchpin for synthesizing various important motifs.

For instance, the chloride functionality can readily undergo S_N2 reactions to afford α -amino and α -oxyamides with the inversion of stereochemistry. Alternatively, the amide motif can be diversified to yield an array of diverse alkyl chlorides. Asymmetric synthesis of α -halocarbonyl compounds is most commonly achieved through selective redox reactions such as asymmetric halogenation of enolizable substrates or ketenes^{7–24} or hydrodehalogenation of dihalocarbonyl compounds.^{25,26} In the realm of molecular complexity building reactions, asymmetric alkylations of enolizable α -halocarbonyls can be achieved using chiral auxiliaries^{27–30} or small-molecule catalysts.^{31,32} We envisioned the reductive coupling of α,α -

dihaloamides with alkenes using radical intermediates as an attractive alternative to these methods, as it would enable the preparation of α -haloamides on less activated substrates. However, small molecule catalysts struggle to achieve high levels of enantioselectivity for this type of reaction and are plagued by hydrodehalogenation of the α -haloamide product.^{32–38} We recognized that an enzyme would be ideally suited to overcome these selectivity challenges because their active sites can be tailored using protein engineering.^{39–41}

Our group and others previously demonstrated that flavin-dependent “ene”-reductases (EREDs) could catalyze the intermolecular hydroalkylations of olefins using α -halocarbonyl compounds as radical precursors.^{42–45} Building upon these foundational methods has enabled a variety of stereoselective reactions involving the formation of C–C,^{46–50} C–N,^{51,52} C–O,⁵³ and C–S bonds.⁵⁴ These reactions proceed via the single electron reduction of the alkyl halide by flavin hydroquinone (FMN_{Hq}) to afford a radical intermediate that reacts with the alkene to afford an alkyl radical. This prochiral radical is reductively terminated via hydrogen atom transfer (HAT) from the flavin semiquinone (FMN_{sq}) to afford the product. In these reports, radical termination occurs with high enantioselectivity.⁴¹ Control over stereocenters generated in the C–C bond-forming step has proved to be elusive, with radical additions into nitronates being the only known example.^{55,56} To achieve high selectivity, the alkyl halide and alkene need to be preorganized in the protein active site prior to radical formation. Under this hypothesis, engineering the active site to

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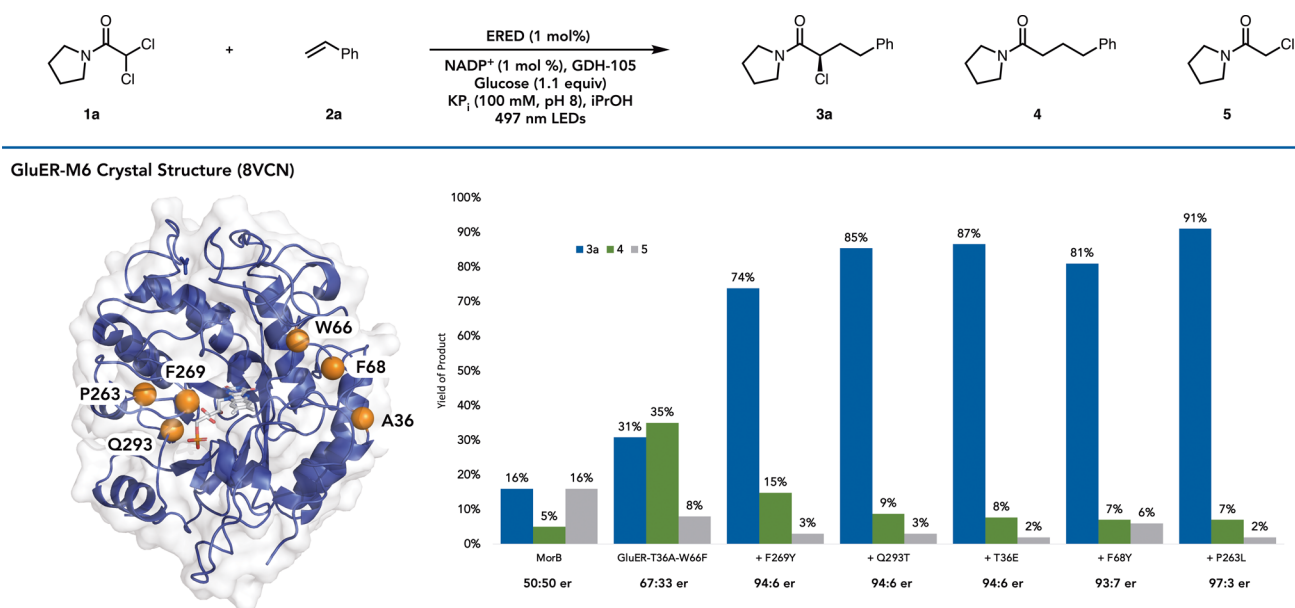


Figure 2. Protein engineering of GluER-T36A-W66F for synthesizing α -chiral chloroamides.

favor a particular binding orientation should enable C–C bond formation with high enantioselectivity. Beyond controlling the stereoselectivity, this method requires a protein that exhibits a preference for hydroalkylation over the competing hydrodehalogenation of the starting material or the product.

We began by exploring the coupling of α,α -dichloropyrrolidine amide **1a** with styrene **2a** to prepare the corresponding α -chloroamide **3a**. After evaluating a collection of EREDs under cyan LED irradiation ($\lambda_{\text{max}} = 497 \text{ nm}$), we identified two catalysts that exhibited promising performance. The ERED from *Gluconobacter oxydans* with previously identified mutations (GluER-T36A-W66F) furnished the product with 31% yield and moderate enantioselectivity (65:35 er), albeit with 26% and 8% of the hydrodehalogenated byproducts **4** and **5**, respectively. This catalyst was selected from a library of diverse GluER-T36A active site mutants for its improved activity for α -substituted amide substrates (Figure S1).⁵⁷ The morphinone reductase from *Pseudomonas putida* (MorB) furnished a superior ratio of the desired product to the undesired coupled hydrodehalogenated product (3.2:1), however with the product formed as a racemate (Figure 2). Control experiments revealed that the ERED, the cofactor turnover system (GDH-105, NADP⁺, and glucose), and light are essential for the reaction (Table S1).

Given the orthogonal strengths and weaknesses of the two most promising catalysts, we initiated protein engineering campaigns on both enzymes, aimed at enhancing yield, enantioselectivity, and chemoselectivity (Figure 2). In each protein, we targeted 10–15 residues lining the protein active site for iterative site-saturation mutagenesis (ISM). For MorB, the first round of evolution led to the identification of two mutants, MorB-N189H and MorB-Y72S, with improved ability of suppressing both dehalogenated byproducts. However, this enhancement was accompanied by a reduction in yield and no improvement in enantioselectivity (Table S2). In contrast, mutations to GluER-T36A-W66F had a more significant effect on the reaction outcome. Introduction of a tyrosine residue at position 269 (F269Y) significantly boosted the yield of the desired product **3a** to 74%, diminishing the formation of the dehalogenated side product **4** to 15% and **5** to 3%, while

enhancing the enantioselectivity to 94:6 er. A second round of ISM identified a Q293T mutation, which further improved the yield to 85%, decreased the yield of **4** to 9%, and maintained the low yield of **5** (3%).

With the goal of identifying mutations distal to the protein active site, we used MutComputeX, a 3D self-supervised machine learning (ML) framework trained to identify residues where the wild-type amino acid is chemically incongruent with its local environment, for ML-guided rational design of surface mutations based on the GluER-T36A crystal structure.^{58–62} We anticipated that these mutations could enhance the stability or catalytic performance of the protein. Among the residues predicted, we found that mutating alanine (originally threonine) at position 36 to aspartic acid (T36E) and phenylalanine at position 68 to tyrosine (F68Y) led to variants that provided product in comparable yields and enantioselectivities but with decreased formation of the hydrodehalogenated product **4**. Contrary to our initial hypothesis for these ML mutations, they did not thermally stabilize the protein, as has been previously observed (Figure S13), but rather served to decrease formation of the hydrodehalogenated product.^{59–62} This observation is aligned with the learning objective of self-supervised protein ML frameworks: they are trained to predict the extant wild-type amino acids of natural proteins, which have been evolutionarily selected to optimize overall fitness rather than a particular phenotype. Thus, residues where the wild-type amino acid is poorly predicted are primed for identifying gain-of-function mutations, and it is up to the experimentalist to screen these ML predictions and select for the phenotype to optimize, such as decreased shunt production formation. Finally, we subjected the new gene to a final round of ISM, leading to the identification of P263L. This variant increased the yield of **3a** to 91% and er to 97:3, with a mere 7% yield of **4** and 2% of **5**. While these results were achieved using purified enzyme, reactions can be run using lyophilized enzyme lysate as the catalyst, giving a 60% yield and excellent enantioselectivity (99:1 er) (Figure S2). The enhanced enantioselectivity could be attributed to the more stabilized protein in lysate form than in its purified form.

Finally, the reaction can be run on a 1.0 mmol scale in 65% yield with 97:3 er (Figure S2).

To investigate the structural effects of introduced mutations, we grew crystals of GluER-M6 and obtained a high-resolution structure (PDB: 8VCN). The overall structure of GluER-M6 is similar to that of GluER-T36A (PDB: 6MYW) with an RMSD of 0.198 Å. Notable in this structure is the introduction of a weak hydrogen-bonding contact between F269Y and Y343 (3.6 Å) at the solvent-exposed face of the active site.

With the evolved catalyst in hand, we investigated the generality of this method. Substituted pyrrolidinyl amides, including 3-hydroxyl and 2-bromo pyrrolidinyl amides, emerged as outstanding substrates for this transformation, affording α -stereogenic chloro products with remarkable yields and enantioselectivity (Figure 3, 3b,c). Moreover, 2,5-dihydropyrrole (3d) and azetidiny (3e) substrates proved to be highly amenable to the process. When a chloro-fluoro substrate was employed, the reaction delivered a product (3f) featuring an α -fluoro chiral center. Although the yield was moderate (44%), the enantioselectivity remained high (94:6 er), showcasing the potential of this method to access the α -fluoro chiral center. Of note, there was no hydrodefluorinated product observed in the reaction. Although substrates featuring larger cyclic amides exhibited reactivity, the yields were slightly diminished, and enantioselectivities reduced (3g). Notably, acyclic amides, such as Weinreb amides and *N*-methyl benzyl amides, were found to be active substrates within this catalytic system (3h and 3i). These findings offer valuable starting points for future protein engineering endeavors aimed at enhancing the performance of selected target substrates.

We then explored the alkene substrates. Styrenes bearing *para*-methyl, -methoxy, -fluoro, and -chloro groups exhibited moderate to good reactivity, yielding products with exceptional enantioselectivities (Figure 3, 3j–m). Beyond substituted styrenes, the system is also compatible with heterocyclic vinyl substrates. Vinylpyridines, featuring diverse substituents, delivered products with moderate yields and moderate to good enantioselectivities (3n–p). Notably, when α -methylstyrene was subjected to the reaction, a product featuring both α - and γ -stereocenters was obtained with impressive enantioselectivity and diastereoselectivity (3q). Finally, employing 2-phenyl allylic alcohol as a coupling partner led to the corresponding 1,3-disubstituted tetrahydrofuran product (3r) in 42% yield with 89:11 dr and 98:2 er. For both substrates, the α -stereocenter formed in the C–C bond-forming event is set with high enantioselectivity, while the γ -stereocenter set in the HAT event is less precisely controlled.

To unravel the intricacies of this catalytic system, we conducted a series of mechanistic experiments. Notably, a small degree of catalytic activity was observed with dichloroamide substrate 1a under ambient light conditions (Figure S3). This observation was associated with the generation of flavin quinone (FMN_{ox}), which absorbs light in a spectral region akin to the anticipated charge-transfer complex we were investigating.⁶³ Consequently, we employed the corresponding difluoro amide 1j for UV–vis spectroscopic studies, as this substrate does not display any reactivity under ambient conditions but is reactive under the reaction conditions (Figure S3). Initially, we generated FMN_{hq} through titration with sodium dithionite (Figure 4a, green trace). Upon the addition of either styrene 2a or amide 1j, no significant changes in absorbance were observed in the visible light region (Figure 4a, purple and orange traces, respectively). However,

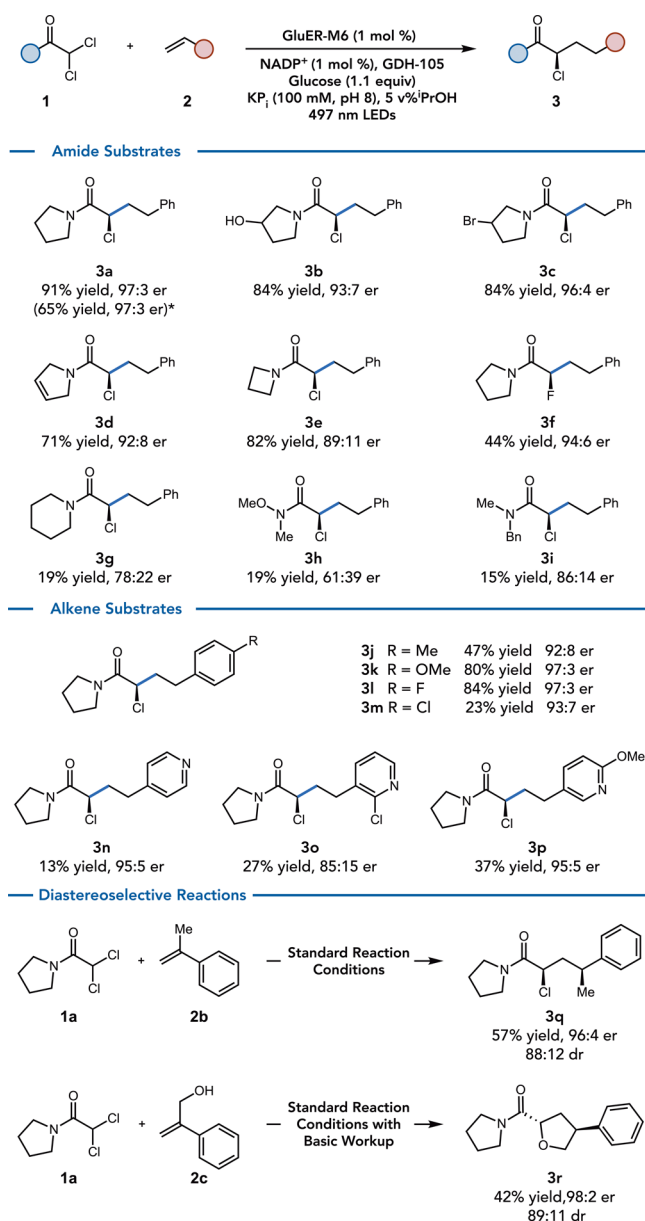
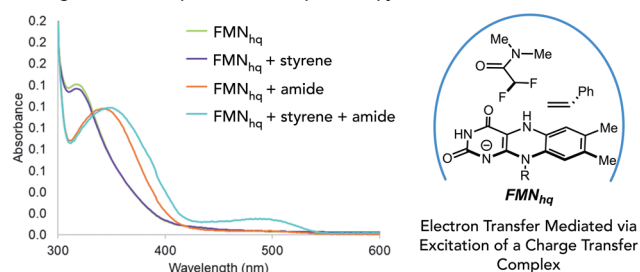


Figure 3. Substrate scope. Analytic reactions were performed on a 20 μ mol scale, and yields were determined by NMR analysis using benzyl benzoate as the internal standard. The enantioselectivities were determined by isolating the products via preparative TLC or reverse phase preparative HPLC from a 0.2 mmol scale enzymatic reaction. *1 mmol reaction scale.

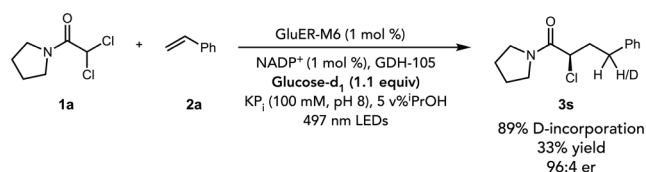
when both the amide and styrene substrates were concurrently present, a highly absorbing species emerged, predominantly in the cyan light region (Figure 4a, cyan trace). These findings support the hypothesis that the ERED templates a ternary charge-transfer complex responsible for light absorption and subsequent radical initiation. As both the amide and alkene are necessary for complex formation, undesired hydrodehalogenation of the starting material is avoided.^{43,52,64} Of note, we conducted similar UV–vis spectroscopic studies on the starting variant, GluER-T36A-W66F, and no obvious charge-transfer complex was observed (Figure S14).

To investigate the radical termination step, we conducted deuterium incorporation experiments. Deuterated FMN_{hq} was generated in situ by using D-glucose-1-*d*₁. A high level of

A. Charge Transfer Complex via UV/Vis Spectroscopy



B. Isotope Incorporation Experiments



C. Product Reactivity

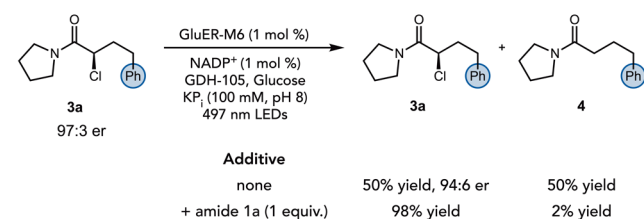


Figure 4. Mechanistic studies. (a) UV-vis studies; (b) deuterium incorporation studies; (c) competition experiments with the product.

deuterium incorporation (89%) was observed at the benzylic position, consistent with HAT from the FMN_{sq} to the radical intermediate (Figure 4b).

Next, we were interested in determining how hydrodehalogenation product 4 is formed (Figure 4c). We considered two hypotheses, the first is that the dichloroamide 1a is dehalogenated to form a monochloroamide, which engages in the intermolecular coupling to generate product 4. Alternatively, the chloroamide product 3a could be hydrodehalogenated under the reaction conditions. When chloroamide 3a is subjected to the standard reaction conditions without additional substrates, 50% is hydrodehalogenated, indicating that the enzyme can react with the product (Figure 4c). However, addition of dichloroamide 1a decreased the consumption of chloroamide 3a to only 2%, indicating that the enzyme preferentially reacts with the more electronically activated dichloroamide (Figure S4). Consequently, we hypothesize that coupling of styrene to the monochloroamide is the primary mechanism by which the hydrodehalogenated product is formed.

Finally, we sought to demonstrate that these α -chloroamides can serve as linchpins for the synthesis of a diverse range of valuable motifs through a selection of representative transformations (Figure 5). Treatment of the α -chloroamides with sulfuric acid cleanly afforded the corresponding α -chlorocarboxylic acid with no erosion in enantioselectivity. Alternatively, employing NaN_3 followed by PPh_3 enabled synthesis of the α -aminoamide. Utilizing LiAlH_4 under refluxing conditions on the α -chloroamide led to the preparation of the chiral α -methyl amine with minimal loss in enantioselectivity.⁶⁵ Finally, reduction of the amide with DIBAL-H affords the corresponding α -chloroaldehyde, which can be further reduced to the

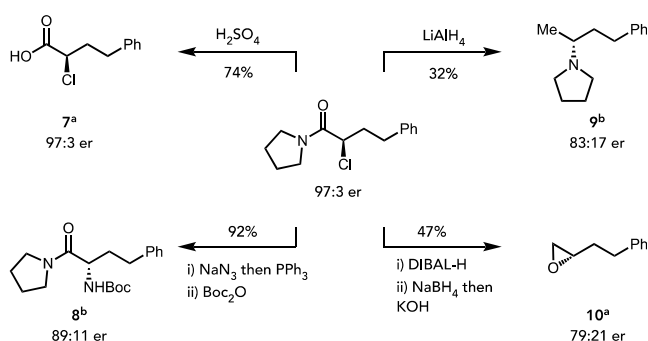


Figure 5. Product derivatization. Enantiomeric ratios were determined by HPLC (major:minor). ^aReactions performed with a starting material er of 97:3; ^breactions performed with a starting material er of 88:12.

halohydrin and upon cyclization affords the chiral terminal epoxide.

In summary, an engineered photoenzyme has enabled the efficient synthesis of linchpin α -chiral amides from readily available dichloro amides and alkenes. The high enantioselectivity achieved stems from the enzyme-controlled radical addition to alkenes, complementing the previously reported enantioselective HAT mechanism. Leveraging protein engineering strategies and machine learning, we successfully enhanced various facets of the reaction including yield, enantioselectivity, and chemoselectivity. Overall, this method demonstrates that intermolecular C–C bond formation can be rendered asymmetric within photoenzymatic catalysis, expanding the synthetic utility of the field.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.4c00927>.

Raw MutComputeX predictions file (XLSX)

Experimental procedures and characterization data, including Supplemental Figures S1–S21 and Supplemental Tables S1–S5 (PDF)

Accession Codes

The data that support the findings in this study are available from the corresponding author upon reasonable request. Crystallographic models and structure factors have been deposited in the Protein Data Bank with accession number 8VCN for GluER-M6.

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Notes

The authors declare no competing financial interest.

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