Asymmetric *C*-Alkylation of Nitroalkanes via Enzymatic Photoredox Catalysis

Haigen Fu,[‡] Tianzhang Qiao,[‡] Jose M. Carceller, Samantha N. MacMillan, Todd K. Hyster[*]

Supporting Information Placeholder

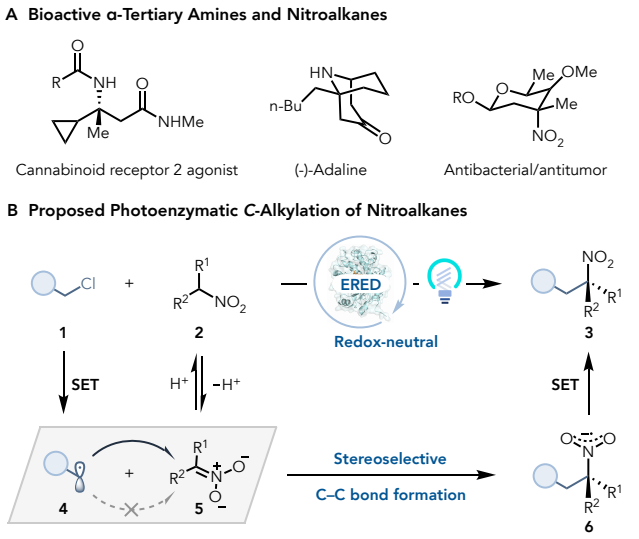
ABSTRACT: Tertiary nitroalkanes and the corresponding α-tertiary amines represent important motifs in bioactive molecules and natural products. The *C*-alkylation of secondary nitroalkanes with electrophiles is a straightforward strategy for constructing tertiary nitroalkanes, however, controlling the stereoselectivity of this type of reaction remains challenging. Here we report a highly chemo- and stereoselective *C*-alkylation of nitroalkanes with alkyl halides catalyzed by an engineered flavin-dependent 'ene'-reductase (ERED). Directed evolution of the old yellow enzyme from *Geobacillus kaustophilus* provided a triple mutant, GkOYE-G7, capable of synthesizing tertiary nitroalkanes with high yield and enantioselectivity. Mechanistic studies indicate that the excitation of an enzyme-templated charge-transfer complex formed between the substrates and cofactor is responsible for radical initiation. Moreover, a single-enzyme two-mechanism cascade reaction was developed to prepare tertiary nitroalkanes from simple nitroalkenes, highlighting the potential to use one enzyme for two mechanistically distinct reactions.

α-Tertiary amines are an important motif in pharmaceuticals and natural products (Figure 1A).[1] While there are countless methods for preparing this motif as a racemate, there are relatively few catalytic asymmetric strategies.[2] Tertiary nitroalkanes can be easily converted to the corresponding amines through nitro group reduction. Consequently, methods for preparing tertiary nitroalkanes stereoselectively are highly attractive, but underdeveloped, typically involving transition metal-catalyzed allylic alkylation or organocatalytic Michael additions and Aza-Henry reactions.[3]

One potential strategy to construct fully substituted nitroalkanes is the *C*-alkylation of secondary nitronates with alkyl halides, however, these reactions are complicated by competing *O*-alkylation to form carbonyl side products.[4] Single-electron transfer (SET) mechanism with specific electron-deficient electrophiles has been explored to favor *C*-alkylation.[5] Watson and co-workers demonstrated a series of general and convenient transition metal-catalyzed (Cu or Ni) *C*-alkylation of nitronates with simple alkyl halide electrophiles via a radical mechanism.[6] Later, the Nickel-catalyzed *C*-alkylation of primary nitronates was rendered asymmetric by the same group, providing enantioenriched secondary nitroalkanes.[7] However, the catalytic asymmetric construction of tertiary nitroalkanes via *C*-alkylation of nitronates with alkyl halides has not yet been reported.

We question whether an enzyme could catalyze asymmetric *C*-alkylation of nitronates to form tertiary nitroalkanes. The high selectivity and evolvability associated with enzyme catalysis make them attractive platforms for this challenge.[8] While various biocatalytic methods use nitronates as nucleophiles in conjugate addition or Henry reaction, none can build chiral tertiary nitroalkanes.[9] Notably, natural enzymes do not catalyze the *C*-alkylation of nitroalkanes with alkyl halides, we envisioned a non-natural catalytic mechanism is required to address this challenge. Recently, we reported that flavin-dependent 'ene'-reductases (EREDs) can catalyze asymmetric cross-electrophile coupling (XEC) between alkyl halides and nitroalkanes.[10] This reaction involved the formation of an alkyl radical reacting with the nitronate to form a nitro radical anion. Enzyme-mediated mesolytic cleavage forms a tertiary radical that could be stereoselectively quenched via hydrogen atom transfer (HAT).[10] We envisioned the identification and evolution of an enzyme that could favor oxidation of the nitro radical anion to synthesize tertiary nitroalkanes (Figure 1B). Photoinduced reduction of the alkyl halide **1** gives an alkyl radical **4** that can add to an *in situ*-generated nitronate **5** to forge a new C–C bond and a nitro radical anion **6**, which can be terminated through single-electron oxidation to afford the *C*-alkylated nitroalkane **3** (Figure 1B). The stereodetermining step of this reaction would be C–C bond formation. In our previous studies exploring the coupling of alkenes with alkyl halides, stereocenters formed during C–C bond formation were challenging to control, possibly due to the need for precise substrate orientation prior to radical formation.[11] We hypothesized that the nitronate could hydrogen bond to the protein scaffold to help favor one binding orientation (Figure 1B).

We initiated our investigation by exploring the coupling of 2-nitropropylbenzene **2a** with α-chloroamide **1a** catalyzed by a series of EREDs under cyan LEDs irradiation (λ_{max} = 497 nm) (Figure 1C and SI Table 1). To our delight, many EREDs can catalyze the designed reaction and provide the desired tertiary nitroalkane product **3a**, with no denitration product observed (SI Table 1). While most of the tested EREDs afforded racemic product, the thermostable old yellow enzyme from *Geobacillus kaustophilus* (GkOYE) afforded product as a 78:22 enantiomeric ratio (er) favoring the (*R*)-enantiomer in 56% yield under the optimized reaction conditions (Figure 1C and SI Table 2).[12] Control experiments confirmed that ERED and cyan light are crucial for the desired reactivity (Figure 1C).



entry	variation	yield[b]	er[c]
1	none[a]	56%	78:22
2	0.5 mol% GkOYE	33%	78:22
3	FMN instead of GkOYE	0%	n.d.[d]
4	no enzyme	0%	n.d.
5	no light	0%	n.d.

Figure. 1. Photoenzymatic asymmetric *C*-alkylation of nitroalkanes. [a] Standard condition: **1a** (10 μmol, 1 equiv), **2a** (20 μmol, 2 equiv), GkOYE (1 mol%) in Tricine buffer (100 mM, pH 9.0), 10% DMSO, cyan LED, rt, 24 h. [b] Yield determined via LCMS relative to an internal standard 1,3,5-tribromobenzene. [c] er (*R*:*S*) determined by HPLC on a chiral stationary phase. [d] not determined.

Next, wild-type GkOYE was subjected to iterative saturation mutagenesis (ISM), targeting residues that line the active site of GkOYE (SI Figure 1).[12,13] For each round of ISM, enzyme libraries were expressed using *Escherichia coli* cells and screened in 96-well plates in the form of cell-free extracts (SI Figure 2-4). After three rounds of protein engineering, a triple mutant (D73C/A104H/Y264W, namely GkOYE-G7) was found that delivered product with excellent yield and enantioselectivity (96% yield, 96:4 er) with 0.5 mol% of biocatalyst loading (Figure 2). Notably, this reaction can be run on a preparative 1.0 mmol scale and provide **3a** in 78% isolated yield (196 mg) with no decrease in stereoselectivity.

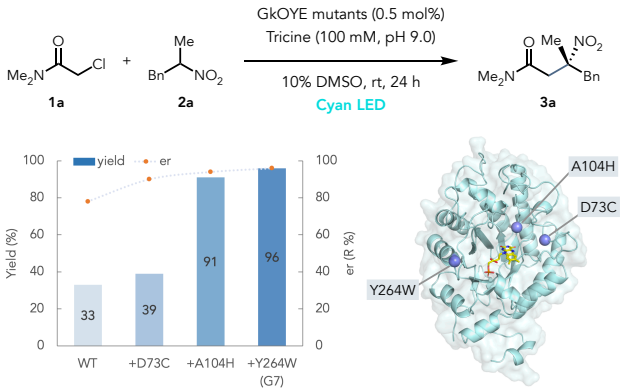
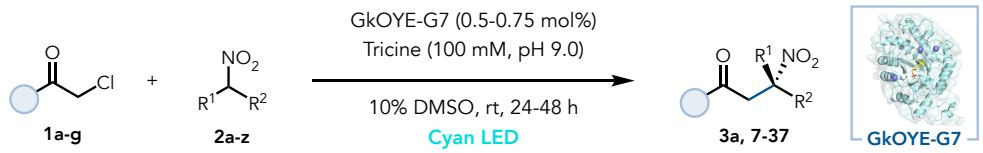


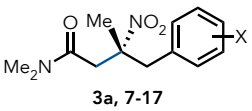
Figure 2. Protein engineering. The crystal structure of wild-type GkOYE (PDB: 3GR8) with three beneficial mutations is shown.

With the engineered GkOYE-G7 in hand, we explored the scope and limitations of the reaction. A variety of α-benzyl nitroalkanes are well accepted as *C*-alkylation acceptors with α-chloroamide **1a**. α-Benzyl nitroalkanes possessing electron-donating or electron-withdrawing substituents at the *ortho*, *meta*, and *para* positions were efficiently converted to the desired enantioenriched β-stereogenic tetrasubstituted nitroamides (**7**–**16**) in yields of 56–98% with high levels of enantioselectivity (>93:7 er, Figure 3). Furthermore, GkOYE-G7 also accommodates the larger α-naphthalenylmethyl substituted nitroalkane, providing the corresponding product **17** (60% yield, 93:7 er,). However, this engineered enzyme was limited to small alkyl substituents at the α-position, with larger ethyl and propyl groups affording product affording product in low yield (**19**, 19% yield, 92:8 er) (Figure 3 and SI Figure 7). Importantly, GkOYE-G7 could accept a variety of heterocycles, including the electron-deficient pyridine, pyrazine, and electron-rich thiophene, affording the respective heterocycle-substituted nitroamides (**20**–**26**) in high yields and enantioselectivities (91–98% yield, up to 95:5 er). Moreover, both linear and cyclic aliphatic nitroalkanes can be tolerated by the engineered enzyme, giving the fully substituted nitroamide products (**27**–**31**) in yields of 22–96% with modest enantioselectivities (up to 88:12 er). Note that most of these enzymatic reactions can be run at 0.1 mmol preparative scale, highlighting the synthetic utility of this method.

Tertiary amides, including Weinreb amide, α,α-difluoroamide, and pyrrolidine amide are accepted by GkOYE-G7 in the reaction, providing the corresponding products (**32**–**34**) in moderate yields and enantioselectivities (33–54% yields, up to 91:9 er). However, secondary amides or larger tertiary amides afforded products with lower yield and enantioselectivity (SI Figure 7). Beyond amide substrates, α-halo ketones can also be accepted to form a racemic β-nitroketone product **35** (70% yield), but α-halo esters or sulfones were poorly reactive with GkOYE-G7 (Figure 3 and SI Figure 7). Pleasingly, we found an alternative ERED from *Caulobacter segnis* (CsER) which accommodates an α-bromoester and α-bromosulfone as alkylating agents, giving β-nitroester **36** and β-nitro sulfone **37**, respectively (Figure 3 and SI Figure 7). Although wild-type CsER cannot control the enantioselectivity of this reaction, protein engineering could be applied to improve the stereoselectivity.

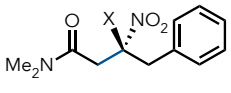


Nitroalkane scope

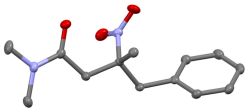


3a, 7-17

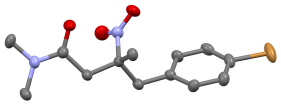
3a	X = H	96%[a] (84%)[b] yield, 96:4 er	**12**	X = 4-Me	98% (80%) yield, 94:6 er
7	X = 2-Me	56% (53%) yield, 93:7 er	**13**	X = 4-OMe	98% (85%) yield, 95:5 er
8	X = 3-Me	84% (80%) yield, 94:6 er	**14**	X = 4-Cl	94% (78%) yield, 95:5 er
9	X = 3-OMe	97% (86%) yield, 94:6 er	**15**	X = 4-Br	83% (73%) yield, 95:5 er
10	X = 3-Cl	89% (81%) yield, 94:6 er	**16**	X = 4-CF_3	61% (50%) yield, 95:5 er
11	X = 4-F	89% (83%) yield, 94:6 er	**17**	X = 2-naphthyl	60% (53%) yield, 93:7 er



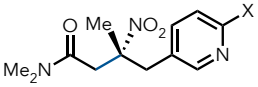
18	X = H	76% (72%) yield
19	X = Et	19% yield, 92:8 er



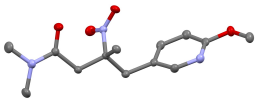
3a (x-ray)



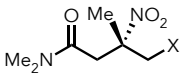
15 (x-ray)



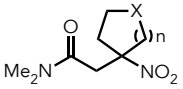
20	X = H	97% (92%) yield, 92:8 er
21	X = Me	92% (90%) yield, 92:8 er
22	X = OMe	91% (86%) yield, 94:6 er
23	X = Br	98% (91%) yield, 95:5 er



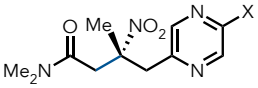
22 (x-ray)



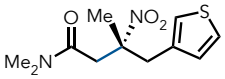
27	X = H	92% (86%) yield
28	X = cyclohexyl	22% yield, 88:12 er



29	n = 1, X = CH_2	96% (84%) yield
30	n = 2, X = CH_2	80% (75%) yield
31	n = 1, X = O	96% (78%) yield, 58:42 er

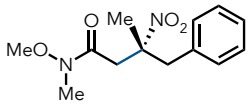


24	X = H	93% (84%) yield, 80:20 er
25	X = Me	94% (83%) yield, 70:30 er

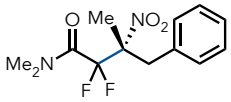


26 98% (90%) yield, 95:5 er

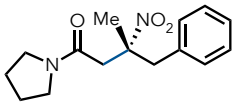
Carbonyl scope



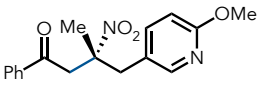
32 45% (41%) yield, 89:11 er



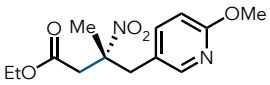
33 54% (50%) yield, 91:9 er



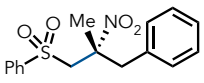
34 33% yield, 80:20 er



35 78% (70%) yield, 50:50 er



36[c] 72% (64%) yield, 50:50 er



37[c] 97% (60%) yield, 50:50 er

Figure. 3. Substrate scope. [a] Analytical yields of 10 µmol-scale reaction. [b] Isolated yields of 0.10 mmol-scale reaction. [c] CsER (0.75 mol%) and α-bromo ester or sulfone were used.

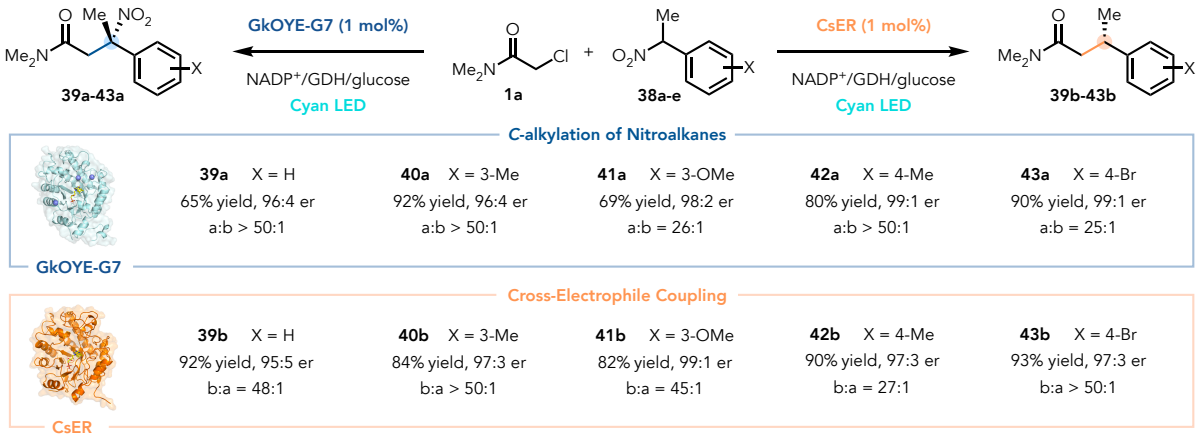


Figure 4. Enzyme-controlled reactivity. Reaction conditions: nitroalkane (10 µmol, 1 equiv), **1a** (20 µmol, 2 equiv), GDH-105 (0.6 mg), $NADP^+$ (0.1 µmol, 1 mol%), glucose (50 µmol) and 'ene'-reductases (0.1 µmol, 1 mol%) in Tricine buffer (100 mM, pH 9.0), 10% DMSO, cyan LED, rt, 24 h.

One of the most appealing features of biocatalysts is their excellent specificity, especially for those highly evolved biocatalysts.[14] We recently demonstrated that CsER can efficiently catalyze the reductive XEC between **1a** and α-aryl nitroalkanes **38** to provide β-stereogenic amides **39b-43b** as the major products in high yields and enantioselectivities (82–93% yield, up to 99:1 er), only a minimal amount of *C*-alkylated products **39a-43a** were observed (**b**/**a** > 27:1, Figure 4).[10] Remarkably, when the engineered GkOYE-G7 was used under identical reaction conditions, the product selectivity is reversed, forming the *C*-alkylated nitroalkanes **39a-43a** as the major products (65–92%, up to 99:1 er) and negligible formation of the XEC products **39b-43b** (**a**/**b** > 25:1, Figure 4). Note that the NADPH turnover system ($NADP^+$/GDH/glucose) is not required for the GkOYE-G7-catalyzed redox-neutral *C*-alkylation of nitroalkanes (SI page 49–53), nevertheless, it was supplied to enable direct comparison. As such, two highly specific EREDs with controllable reactivities were developed. While CsER is efficient for catalyzing XEC, GkOYE-G7 is superior for catalyzing *C*-alkylation of nitroalkanes, demonstrating the unparallel chemoselectivity of biocatalysts that could be difficult to achieve using small molecule catalysts.

EREDs are known to be capable of reducing nitroalkenes to nitroalkanes.[15] We envisioned a single engineered ERED with natural catalytic activity via hydride-transfer mechanism and non-natural catalytic activity via photoredox radical mechanism that could streamline the biocatalytic synthesis of tertiary nitroalkanes (Figure 5A). When nitroalkenes **44**, **1a**, and an NADPH turnover system were subjected to the reaction using GkOYE-G7 as the biocatalyst, we observed full conversion of nitroalkenes **44** and the desired tertiary nitroalkane products with high levels of yield and stereoselectivity (86–96% yield, up to 96:4 er, Figure 5A). Notably, broad substrate scope was observed for this single-enzyme two-mechanism cascade reaction, demonstrating the catalytic promiscuity and synthetic capability of EREDs. Finally, the enantioenriched tertiary nitroalkane **3a** can be readily reduced to the corresponding α-tertiary amine **45** in 65% yield without any erosion of stereoselectivity (96:4 er, Figure 5B).

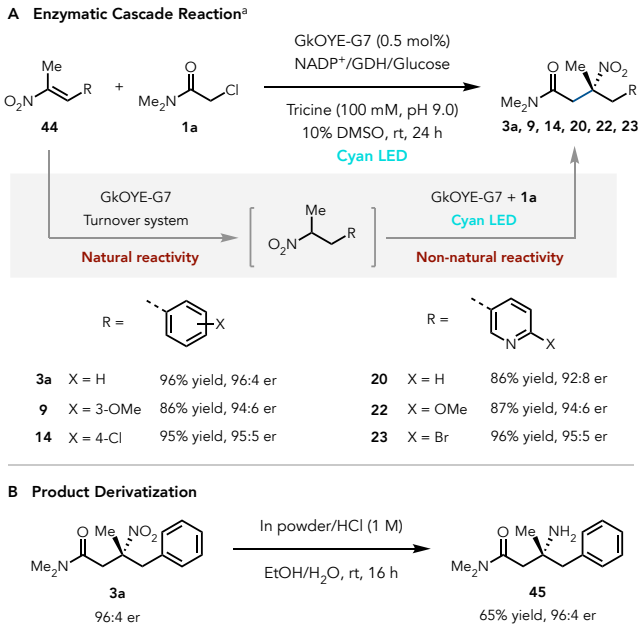


Figure 5. Enzymatic cascade reaction and product derivatization. [a] Same condition as in Figure 4 with 0.5 mol% of GkOYE-G7.

Mechanistic experiments were conducted to elucidate the nuances of this reaction. Previous studies have demonstrated that flavin hydroquinone (FMN_{hq}) and flavin semiquinone (FMN_{sq}) are formed within the active sites of EREDs upon visible light irradiation in the presence of electron donors.[16] Indeed, we observed a rapid photoreduction of GkOYE-G7 in tricine buffer with cyan light irradiation, forming a mixture of FMN_{hq} and FMN_{sq} monitored by ultraviolet-visible (UV-Vis) spectroscopy (Figure 6A). When the photoreduced GkOYE-G7 protein was subjected to the model substrates **1a** and **2a** in the absence of light, no product **3a** was observed, indicating neither the ground-state FMN_{sq} nor ground-state FMN_{hq} was responsible for radical initiation (Figure 6A and SI Figure 8-9). Additionally, reactions with 200-fold excess of reductant (NADPH or sodium dithionite with respect to enzyme) under dark conditions were performed, and neither reaction provided any product **3a**, confirming ground-state FMN_{hq} is not responsible for

reduction (SI Table 4).[17] Moreover, when a 455 nm longpass filter (blocking light with wavelengths less than 455 nm) was added to the model reaction excited by cyan light, comparable yield and enantioselectivity were observed to those of the standard conditions, suggesting that direct excitation of the cofactor FMN_{hq} is not responsible for reduction (SI Table 4).[18]

Having ruled out ground-state FMN_{sq}, ground-state, or excited-state FMN_{hq} as a reductant, we hypothesized that an enzyme-templated charge-transfer (CT) complex was responsible for reducing α-chloroamide **1a** over the thermodynamically favored nitroalkane **2a**.[10,11,19] To probe this possibility, when flavin mononucleotide (FMN) in GkOYE-G7 was fully reduced to FMN_{hq} with sodium dithionite, a diagnostic FMN_{hq} spectrum with minimal absorption longer than 460 nm was observed (Figure 6B). Upon the addition of chloroamide **1a**, a new broad absorption band (450–470 nm) was observed, suggesting the formation of a CT complex between the FMN_{hq} and **1a** (Figure 6B). Interestingly, when nitrone **46**, a mimic of nitronate **5**, was added to the mixture, a further enhanced broadband was observed, indicating a possible quaternary CT complex (Figure 6B). We suggest that the formation of a high-order CT complex in the active site not only facilitates the photoinduced initial electron transfer from FMN_{hq} to **1a**, but also the following addition of the resulting radical **4** to nitronate **5** to give the nitro radical anion **6**, which FMN_{sq} can oxidize to provide the desired tertiary nitroalkane product **3a** and regenerating FMN_{hq} (Figure 1B).

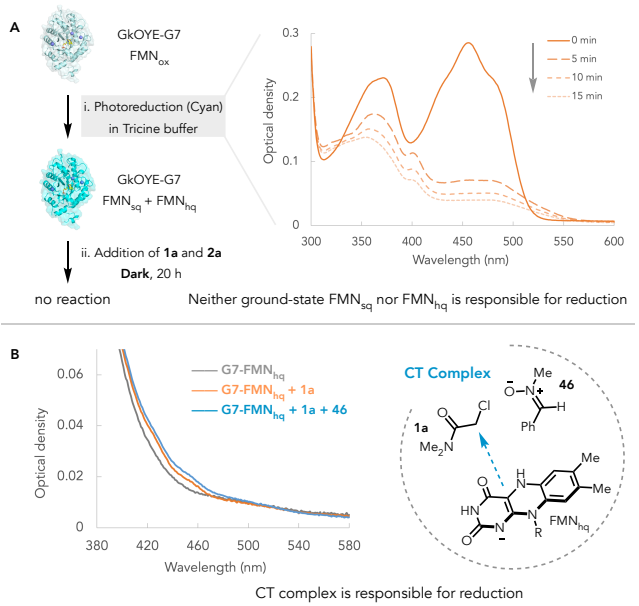


Figure 6. Mechanistic experiments.

In summary, we have established an unprecedented photoenzymatic asymmetric *C*-alkylation of nitroalkanes to access difficult tertiary nitroalkanes. This reaction is enabled by an engineered ERED (GkOYE-G7), featuring an enantioconvergent Csp^3–Csp^3 bond-forming step to construct tetrasubstituted stereogenic centers. While the evolved GkOYE-G7 showed high specification for catalyzing *C*-alkylation of nitroalkanes rather than XEC, it retains the natural reductive reactivity, enabling a unique one-enzyme two-mechanism cascade to synthesize chiral tertiary nitroalkanes from readily available nitroalkenes. By harnessing the power of directed evolution to optimize the catalytic promiscuity of EREDs, our work addresses long-standing challenges in transition metal-catalyzed asymmetric *C*-alkylation of nitroalkanes, thus expanding the boundary of biocatalysis.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization data, NMR spectra, HPLC traces, and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

CCDC 2218379–2218382 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

AUTHOR INFORMATION

Corresponding Author

Todd K. Hyster – *Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14850, United States; orcid.org/0000-0003-3560-355X; Email: thyster@cornell.edu*

Authors

Haigen Fu – *Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14850, United States*

Tianzhang Qiao –*Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14850, United States*

Jose M. Carceller –*Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14850, United States; Institute of Chemical Technology (ITQ) - Valencia, Universitat Politècnica de València, Spain, Camino de Vera s/n*

Samantha N. MacMillan –*Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14850, United States*

Author Contributions

‡These authors contributed equally. The manuscript was written through the contributions of all authors.

Notes

The authors declare no competing financial interest.

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TOC Graphic


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Photoenzymatic
C-Alkylation of Nitroalkanes
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