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An Asymmetric sp^3–sp^3 Cross-Electrophile Coupling Using 'Ene'-Reductases

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Summary paragraph:

The catalytic asymmetric construction of Csp^3–Csp^3 bonds remains one of the foremost challenges in organic synthesis.[1] Metal-catalyzed cross-electrophile couplings (XEC) have emerged as a powerful tool for C–C bond formation.[2–5] However, coupling two distinct Csp^3-electrophiles with high cross- and stereoselectivity continues as an unmet challenge. Here, we report a highly chemo- and enantioselective Csp^3–Csp^3 XEC between alkyl halides and nitroalkanes catalyzed by flavin-dependent 'ene'-reductases. Photoexcitation of the enzyme-templated charge-transfer complex between an alkyl halide and flavin cofactor enables the chemoselective reduction of alkyl halide over the thermodynamically favored nitroalkane partner. The key C–C bond-forming step occurs via the reaction of an alkyl radical with an *in situ* generated nitronate to form a nitro radical anion that collapses to form nitrite and an alkyl radical. An enzyme-controlled hydrogen atom transfer affords high levels of enantioselectivity. This reactivity is unknown in small molecule catalysis and highlights the potential for enzymes to use new mechanisms to address long-standing synthetic challenges.

Catalytic cross-couplings to forge Csp^2–Csp^2 bonds have revolutionized organic synthesis, enabling the rapid construction of molecules for the pharmaceutical and agrochemical industries.[6,7] As target compounds become more complex — correlating with a higher percentage of sp^3-hybridized atoms and stereocenters — there is a need for technologies to forge Csp^3–Csp^3 bonds stereoselectively.[8,9] Cross-electrophile couplings (XEC) involving two distinct Csp^3-electrophiles are an attractive alternative to the traditional cross-couplings because they have broad functional group tolerance and avoid the need for sensitive organometallic reagents.[10–12] However, these reactions often form homo-coupled products because the metal catalysts struggle to distinguish between the two Csp^3-electrophiles.

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Author contributions: H.F. and J.C. performed and analyzed the experiments. T.Q. performed the DFT calculations. Y.Q and S.J.C performed metagenomic mining and prepared the CsER enzyme. S.G. collected the crystallographic data of CsER. H.F. and T.K.H. designed the experiments. T.K.H. directed the project. The manuscript was prepared with feedback from all the authors.

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This issue can be diminished using alkyl halides that react at different rates with the metal catalyst.[13,14] Moreover, while there has been significant progress toward catalytic asymmetric Csp^2–Csp^3 XEC,[4,5] stereoselective Csp^3–Csp^3 XEC is underdeveloped (Fig. 1a).[15,16] To overcome these limitations, previously unappreciated mechanistic steps and catalytic strategies need to be explored.[17–19]

We questioned whether an enzyme could catalyze an asymmetric Csp^3–Csp^3 XEC. The high level of selectivity associated with biocatalytic reactions makes them attractive scaffolds for this challenge.[20,21] However, as natural enzymes do not catalyze cross-coupling reactions, we needed to develop a novel XEC mechanism that is compatible with existing enzymatic machinery.[22,23] Nitroalkanes are unique and ubiquitous reagents in organic synthesis but are not used as electrophiles for cross-couplings.[24] We recognized that the reactivity of nitronates could be used for a biocatalytic XEC. Nitronates react with open-shell electrophiles to forge a C–C bond and a nitro radical anion.[25,26] If this intermediate were to cleave mesolytically, the resulting radical could be quenched via hydrogen atom transfer (HAT) to afford the cross-coupled product.[27] The key to achieving this previously unknown reaction is identifying an enzyme to facilitate C–C bond formation, C–N bond mesolytic cleavage, and HAT (Fig. 1b). We envision a mechanism where reduction of the alkyl halide **1** forms an alkyl radical **4** that can react with an *in situ* generated nitronate **5** to forge a new C–C bond and a nitro radical anion **6**. Enzyme-mediated homolytic cleavage of the C–N bond generates nitrite and an alkyl radical **7** that can be terminated via HAT to afford the cross-coupled product **3** (Fig. 1b). The proposed mechanism is attractive because the orthogonal reactivity of nitroalkanes and alkyl halides avoids undesired dimerization products.

Precise control over the chemoselectivity of the electron transfer events is required for the proposed reaction. Reduction of the nitroalkanes is thermodynamically favored by comparison to all but the most electronically activated alkyl halides (nitroalkanes $E_{p/2}$ = ~ −0.9 V vs. saturated calomel electrode, SCE;[28] alkyl halides $E_{p/2}$=−1.1 V to −2.5 V vs. SCE[29]).[30] To achieve the desired reaction, we require a catalyst that will preferentially reduce alkyl halides instead of the nitroalkanes. We and others recently demonstrated that flavin-dependent ‘ene’-reductases (EREDs) can reduce alkyl halides using protein templated charge-transfer (CT) complexes.[31–33] Protein templated complexes provide the opportunity for substrate binding to override the inherent thermodynamic preference in electron transfer events. If the protein only forms the CT complex with the alkyl halide, it would be selectively reduced over the nitroalkane. Finally, EREDs can precisely control the radical terminating HAT step, enabling the formation of products with high levels of enantioselectivity.[34,35]

We initiated our studies by exploring the photoenzymatic coupling of α-chloroamide **1a** ($E_{p/2}$ = −1.65 V vs. SCE)[31] with 1-nitroethylbenzene **2a** ($E_{p/2}$ = −0.89 V vs. SCE)[28] catalyzed by a panel of EREDs under cyan light irradiation (λ_{max} = 497 nm) (Supplementary Table 1). To our delight, many of the enzymes provided the desired cross-coupled product **3a**, with none providing the nitrated product (Supplementary Table 1). The most promising catalyst was the ‘ene’-reductase from *Caulobacter segnis* (CsER), providing product **3a** with moderate yield (28%) but excellent enantioselectivity, with

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95:5 enantiomeric ratio (er) of (*S*)- to (*R*)-enantiomer of the product. We hypothesized that the modest yield is likely due to inadequate concentration of the nitronate at pH 8.0 (nitroalkane/nitronate = 200:1, pKa of **2a** is 10.3).[36] Indeed, in moving to the more basic reaction conditions at pH 9.0 (nitroalkane/nitronate = 20:1), the desired product is formed in high yield and excellent enantioselectivity (92% yield, 95:5 er) for the (*S*)-enantiomer, outperforming other tested EREDs (Fig. 1c and Supplementary Table 1). The ERED variant from *Gluconobacter oxydans* (GluER-T36A) favors the formation of the (*R*)-enantiomer of the product (51% yield, 10:90 er), providing a complimentary catalyst for accessing both enantiomers of the product (Fig. 1c). Control experiments confirmed that ERED, cyan light, and NADPH regeneration system (GDH/$NADP^+$/glucose) are crucial for the desired reactivity (Supplementary Table 2). Notably, this reaction can be run on a preparative scale and afford product **3a** in 72% isolated yield from a 0.1 mmol-scale reaction with no changes in enantioselectivity. We solved the crystal structure of wild-type CsER, and the docking model of **3a** with CsER suggests the (*S*)-preference of CsER in this reaction (Supplementary Fig. 4). Notably, the coupled product is not formed when the same reaction is attempted using photoredox catalysts.[37] Instead, we observed nitroalkane reduction to the oxime using $Ir(ppy)_3$ as a photoredox catalyst, highlighting that this reactivity is unique to biocatalysis.

With the optimized reaction parameters in hand, we sought to explore the scope and limitations of this photoenzymatic transformation (Fig. 2). A variety of α-aryl nitroalkanes are well accepted as XEC partners with α-chloroamide **1a**. α-Aryl nitroethanes possessing electron-donating or electron-withdrawing substituents at the *meta*- and *para*-position were efficiently converted to the desired enantioenriched β-stereogenic amide products (**9-16**) in yields of 80–98% with excellent enantioselectivity (>95:5 er). *Ortho*-substituted α-aryl nitroalkanes were less tolerated in the reaction, only the *ortho*-fluoro substituted nitroalkane was accepted to provide product **8** in 28% yield and 94:6 er (Fig. 2, and Supplementary Fig. 2). In addition, the larger substrate α-naphthalenyl nitroethane was well tolerated in this reaction, providing the corresponding product **17** with 58% yield and excellent enantioselectivity (99:1 er). This enzyme, however, was limited to relatively small alkyl substituents at the α-position. While the ethyl group was well accepted (**19**, 64% yield and 98:2 er), larger groups, such as *n*-propyl, were poorly reactive. Pleasingly, CsER could also accommodate heterocycles, including the electron-rich α-thiophene and the electron-deficient α-pyridine nitroethanes, providing the respective β-heterocycle substituted chiral amide products (**22-24**) in 40–95% yield and high enantioselectivity (up to 99:1 er). The compatibility of this photoenzyme was further highlighted by the acceptance of α-nitroester as a coupling partner, giving β-stereogenic 1,4-dicarbonyl product **25** in 96% yield and 82:18 er (Fig. 2).

As for the alkyl halide scope, secondary amides with methyl or benzyl are well accepted when coupled with α-(*p*-CF_3)phenyl nitroethane (**2j**) respectively, affording the products (**26-27**) in 53-75% yields and good enantioselectivities (up to 95:5 er) (Fig. 2). Tertiary amides are also well tolerated by the reaction, with cyclic pyrrolidine, piperidine, morpholine, and linear Weinreb amides providing the corresponding products (**28-33**) in moderate to good yields and enantioselectivities (42%-95% yields, up to 99:1 er). Pleasingly, different classes of carbonyls as coupling electrophiles are also feasible, as

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exemplified by the coupling of α-halo ester and α-halo ketones with α-aryl nitroalkanes, giving the respective enantioenriched β-chiral substituted esters and ketones (**34**-**37**, Fig. 2). Notably, the β-chiral amide **3a** can be reduced to the corresponding γ-chiral amine **41** with good yield and no erosion of stereoselectivity (70% yield, 95:5 er, Fig. 3). Additionally, the enzymatic product **16** can be hydrolyzed to give β-chiral acid **42** and further reduced to γ-chiral alcohol **43** in good yield and excellent stereoretention (99:1 er, Fig. 3).

Mechanistic studies were conducted to determine the mode of radical initiation. Specifically, we were interested in understanding why the less oxidizing α-chloroamide (**1a**, $E_{p/2}$ = −1.65 V vs. SCE) is reduced preferentially to the nitroalkane (**2a**, $E_{p/2}$ = −0.89 V vs. SCE). We hypothesized that an enzyme templated charge-transfer (CT) complex controlled the electron transfer events.[26,27] To probe this possibility, the cofactor FMN in CsER was entirely reduced to FMN_{hq} with sodium dithionite, which revealed negligible absorption around 500 nm (Fig. 4a). Upon the addition of chloroamide **1a**, a new broad absorption band (λ_{max} = 480 nm) was observed, suggesting the formation of a CT complex between the FMN_{hq} and **1a**. Interestingly, we found nitroalkane **2a** can oxidize the ground state FMN_{hq} to generate a flavin feature with an absorption band around 450 – 500 nm. We attribute this feature to a mixture of flavin quinone and flavin semiquinone (Supplementary Fig. 10). Interestingly, when nitroalkane **2a** is mixed with CsER and cofactor turnover mix under visible light irradiation, we do not observe reduction to the oxime, hydroxylamine, or hydrodenitrated products. This suggests that initial reduction of the nitroalkane can occur, in contrast to examples with photoredox catalysts,[31] but subsequent electron transfers to form oximes and hydroxylamines do not transpire, making this single electron reduction event reversible under the reaction conditions. As the first irreversible step is the reduction of the alkyl halide, reversible nitroalkane reduction does not have a detrimental effect on the reaction.

Another exciting feature of these reactions is the minimal formation of the enzyme-dependent hydrodehalogenated product (Supplementary Table 2), suggesting that alkyl halide reduction occurs when the nitronate is present in the enzyme active site. However, oxidation of FMN_{hq} by the nitroalkane obscures the observation of a higher-order CT complex (Supplementary Fig. 10). To avoid this issue, nitrone **38**, a close analog of nitronate **5**, was used in the UV-Vis spectra experiments because **38** can also readily react with **1a** (Fig. 4a), mimicking the radical initiation step of the model XEC reaction. Intriguingly, a further enhancement of the CT complex spectra was observed when nitrone **38** was added to a sample containing **1a** and the reduced CsER, indicating a quaternary CT complex was formed to enable efficient radical formation and prolongation. No CT complex was observed using free FMN_{hq} with **1a** and **38**, suggesting the CT complex is formed within the enzyme active site (Supplementary Fig. 11).

Next, we were interested in understanding the denitration step of the reaction. While we propose a mechanism where the alkyl radical reacts with the nitronate to form the unstable radical anion, which rapidly undergoes mesolytic cleavage, we recognized the possibility of a two-step mechanism where the coupled nitroalkane is included in a redox-neutral process. The coupled nitroalkane intermediate is a substrate for reductive denitration in this scenario. To probe this possibility, we considered the reaction using nitromethylbenzene (**2l**), which

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under standard photoenzymatic conditions forms a 1:1 mixture of cross-coupling product **18** (28% yield) and compound **40** retaining a NO_2 group (29% yield) (Fig. 4b). When the nitroalkane product **40** is resubjected to the reaction conditions, no cross-coupling product **18** was observed, indicating that the nitroalkane is not an intermediate in the denitrative coupling reaction. As nitro radical anions are proposed intermediates in non-enzymatic radical reactions but are not reported to undergo mesolytic cleavage,[21] we postulate the protein facilitates the mesolytic cleavage event.

The reaction with nitromethylbenzene **2l** indicates a competition between denitration and electron transfer to FMN_{sq} under the reaction conditions. To better understand the distinctive feature of this reaction, we conducted density functional theory (DFT) calculations on the model reaction (Fig. 4c) and the one involving nitromethylbenzene **2l** (Supplementary Fig. 13). We found the initial addition step of α-amidyl radical **Int-1** to nitronate **5** is occurring rapidly with a free energy barrier of only 9.9 kcal mol^{-1} for the model reaction. The resulting radical anion **Int-2** readily undergoes irreversible denitration (a free energy barrier of 9.6 kcal mol^{-1}) to give the radical **Int-3**, which is terminated by HAT from FMN_{sq}, as supported by deuterium labeling experiments (Supplementary Fig. 8), to provide the final product **3a** (Fig. 4c). Next, the same DFT calculations were conducted with nitromethylbenzene **2l** (Supplementary Fig. 13). While a similar free energy barrier (10.0 kcal mol^{-1}) was observed for the initial addition step, we found a higher energy barrier (13.4 kcal mol^{-1}) for the denitration step, indicating a slower denitration step when compared to the model reaction with **2a**. Thus, as a competitive pathway to the desired cross-coupling, the radical anion **Int-2'** can be terminated by oxidation by FMN_{sq} to provide product **40** (Supplementary Fig. 13).

In summary, we have established an unprecedented photoenzymatic enantioselective sp^3–sp^3 cross-electrophile coupling between the readily available alkyl halides and nitroalkanes. This new enantioconvergent Csp^3–Csp^3 bond formation reaction is powered by EREDs, highlighting the unparallel capability of biocatalysts in differentiating Csp^3 electrophile substrates and controlling stereoselectivity. By using non-traditional coupling partners and mechanisms, our work addresses the long-standing selectivity challenge in transitional-metal catalyzed cross-electrophile couplings by exploiting the promiscuous unnatural reactivity of EREDs, thus expanding the biocatalyst toolbox for asymmetric C–C bond formations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data and materials availability:

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 7TNB for CsER.

References

1. Choi J & Fu GC Transition metal-catalyzed alkyl-alkyl bond formation: another dimension in cross-coupling chemistry. Science 356, eaaf7230 (2017). [PubMed: 28408546]
2. Everson DA & Weix DJ Cross-electrophile coupling: principles of reactivity and selectivity. J. Org. Chem 79, 4793–4798 (2014). [PubMed: 24820397]
3. Gu J, Wang X, Xue W & Gong H Nickel-catalyzed reductive coupling of alkyl halides with other electrophiles: concept and mechanistic considerations. Org. Chem. Front 2, 1411–1421 (2015).
4. Lucas EL & Jarvo ER Stereospecific and stereoconvergent cross-couplings between alkyl electrophiles. Nat. Rev. Chem 1, 0065 (2017).
5. Poremba KE, Dibrell SE & Reisman SE Nickel-catalyzed enantioselective reductive cross-coupling reactions. ACS Catal. 10, 8237–8246 (2020). [PubMed: 32905517]
6. Biffis A, Centomo P, Del Zotto A & Zecca M Pd metal catalysts for cross-couplings and related reactions in the 21st century: a critical review. Chem. Rev 118, 2249–2295 (2018). [PubMed: 29460627]
7. Magano J & Dunetz JR Large-scale applications of transition metal-catalyzed couplings for the synthesis of pharmaceuticals. Chem. Rev 111, 2177–2250 (2011). [PubMed: 21391570]
8. Lovering F, Bikker J & Humblet C Escape from flatland: increasing saturation as an approach to improving clinical success. J. Med. Chem 52, 6752–6756 (2009). [PubMed: 19827778]
9. Lovering F Escape from flatland 2: complexity and promiscuity. Medchemcomm 4, 515–519 (2013).
10. Qian X, Auffrant A, Felouat A & Gosmini C Cobalt-catalyzed reductive allylation of alkyl halides with allylic acetates or carbonates. Angew. Chem. Int. Ed 50, 10402–10405 (2011).
11. Liu JH et al. Copper-catalyzed reductive cross-coupling of nonactivated alkyl tosylates and mesylates with alkyl and aryl bromides. Chem. – A Eur. J 20, 15334–15338 (2014).
12. Sanford AB et al. Nickel-catalyzed alkyl-alkyl cross-electrophile coupling reaction of 1,3-dimesylates for the synthesis of alkylcyclopropanes. J. Am. Chem. Soc 142, 5017–5023 (2020). [PubMed: 32129601]
13. Yu X, Yang T, Wang S, Xu H & Gong H Nickel-catalyzed reductive cross-coupling of unactivated alkyl halides. Org. Lett 13, 2138–2141 (2011). [PubMed: 21434609]
14. Xu H, Zhao C, Qian Q, Deng W & Gong H Nickel-catalyzed cross-coupling of unactivated alkyl halides using bis(pinacolato)diboron as reductant. Chem. Sci 4, 4022–4029 (2013).
15. Erickson LW, Lucas EL, Tollefson EJ & Jarvo ER Nickel-catalyzed cross-electrophile coupling of alkyl fluorides: stereospecific synthesis of vinylcyclopropanes. J. Am. Chem. Soc 138, 14006–14011 (2016). [PubMed: 27706939]
16. Tollefson EJ, Erickson LW & Jarvo ER Stereospecific intramolecular reductive cross-electrophile coupling reactions for cyclopropane synthesis. J. Am. Chem. Soc 137, 9760–9763 (2015). [PubMed: 26230365]
17. Smith RT et al. Metallaphotoredox-catalyzed cross-electrophile Csp^3-Csp^3 coupling of aliphatic bromides. J. Am. Chem. Soc 140, 17433–17438 (2018). [PubMed: 30516995]
18. Zhang W et al. Electrochemically driven cross-electrophile coupling of alkyl halides. Nature 604, 292–297 (2022). [PubMed: 35189623]
19. Jana SK, Maiti M, Dey P & Maji B Photoredox/nickel dual catalysis enables the synthesis of alkyl cyclopropanes via $C(sp^3)$-$C(sp^3)$ cross electrophile coupling of unactivated alkyl electrophiles. Org. Lett 24, 1298–1302 (2022). [PubMed: 35133153]
20. Bell EL et al. Biocatalysis. Nat. Rev. Methods Primers 1, 46 (2021).

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21. Zhou Q, Chin M, Fu Y, Liu P & Yang Y Stereodivergent atom-transfer radical cyclization by engineered cytochromes. Science 374, 1612–1616 (2021). [PubMed: 34941416]
22. Chatterjee A et al. An enantioselective artificial Suzukiase based on the biotin–streptavidin technology. Chem. Sci 7, 673–677 (2015). [PubMed: 29896353]
23. Pierron J et al. Artificial metalloenzymes for asymmetric allylic alkylation on the basis of the biotin–avidin technology. Angew. Chem. Int. Ed 47, 701–705 (2008).
24. Ballini R, Bosica G, Fiorini D, Palmieri A & Petrini M Conjugate additions of nitroalkanes to electron-poor alkenes: recent results. Chem. Rev 105, 933–972 (2005). [PubMed: 15755081]
25. Gildner PG, Gietter AAS, Cui D & Watson DA Benzylation of nitroalkanes using copper-catalyzed thermal redox catalysis: toward the facile C-alkylation of nitroalkanes. J. Am. Chem. Soc 134, 9942–9945 (2012). [PubMed: 22691127]
26. Gietter AAS, Gildner PG, Cinderella AP & Watson DA General route for preparing β-nitrocarbonyl compounds using copper thermal redox catalysis. Org. Lett 16, 3166–3169 (2014). [PubMed: 24870052]
27. Kornblum N, Carlson SC & Smith RG Replacement of the nitro group by hydrogen. J. Am. Chem. Soc 100, 289–290 (1978).
28. Tanner DD et al. On the mechanism of the radical chain transformation of nitroalkanes to alkanes using triaryl- or trialkyltin hydrides. J. Org. Chem 55, 3321–3325 (1990).
29. Roth HG, Roth HG, Romero NA & Nicewicz DA Experimental and calculated electrochemical potentials of common organic molecules for applications to single-electron redox chemistry. Synlett. 27, 714–723 (2016).
30. Durchschein K et al. Reductive biotransformation of nitroalkenes via nitroso-intermediates to oxazetes catalyzed by xenobiotic reductase A (XenA). Org. Biomol. Chem 9, 3364–3369 (2011). [PubMed: 21409264]
31. Biegasiewicz KF et al. Photoexcitation of flavoenzymes enables a stereoselective radical cyclization. Science 364, 1166–1169 (2019). [PubMed: 31221855]
32. Page CG et al. Quaternary charge-transfer complex enables photoenzymatic intermolecular hydroalkylation of olefins. J. Am. Chem. Soc 143, 97–102 (2020). [PubMed: 33369395]
33. Huang X et al. Photoenzymatic enantioselective intermolecular radical hydroalkylation. Nature 584, 69–74 (2020). [PubMed: 32512577]
34. Fu H et al. Ground-state electron transfer as an initiation mechanism for biocatalytic C-C bond forming reactions. J. Am. Chem. Soc 143, 9622–9629 (2021). [PubMed: 34114803]
35. Sandoval BA, Meichan AJ & Hyster TK Enantioselective hydrogen atom transfer: discovery of catalytic promiscuity in flavin-dependent ‘ene’-reductases. J. Am. Chem. Soc 139, 11313–11316 (2017). [PubMed: 28780870]
36. Fukuyama M et al. The thermodynamic and kinetic acidity properties of nitroalkanes. correlation of the effects of structure on the ionization constants and the rate constants of neutralization of substituted 1-phenyl-1-nitroethanes. J. Am. Chem. Soc 92, 4689–4699 (1970).
37. Cai S, Zhang S, Zhao Y & Wang DZ New approach to oximes through reduction of nitro compounds enabled by visible light photoredox catalysis. Org. Lett 15, 2660–2663

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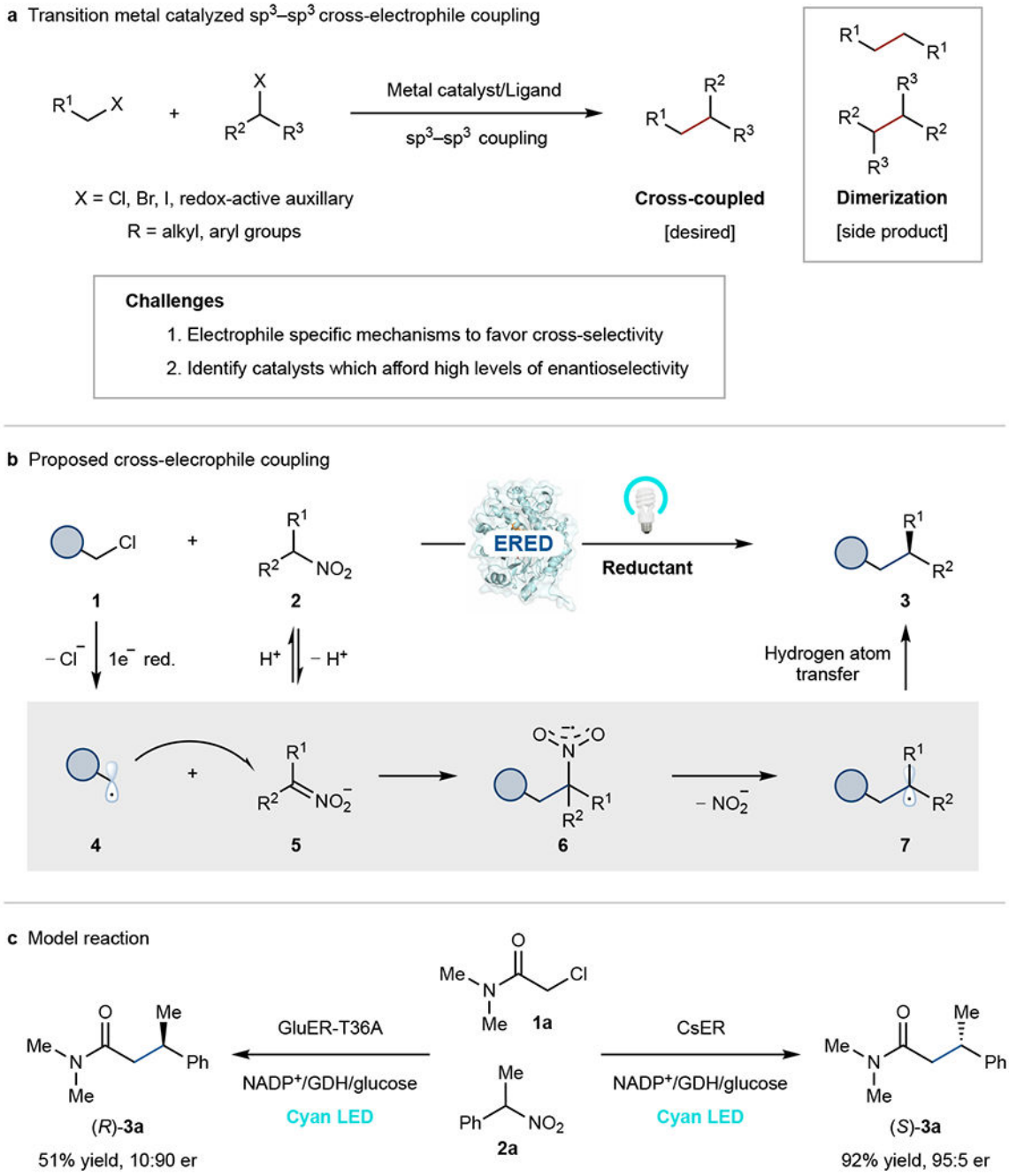

Fig. 1. Photoenzymatic asymmetric cross-electrophile coupling reactions.

a, Challenges associated with sp^3–sp^3 XEC. **b**, Proposed photoenzymatic asymmetric XEC. **c**, Two stereocomplimentary 'ene'-reductases catalyze the model reaction at pH 9.0. Enantiomeric ratio (er) refers to as the ratio of (*S*)- to (*R*)-enantiomer. ERED, 'ene'-reductase; GDH, glucose dehydrogenase; $NADP^+$, nicotinamide adenine dinucleotide phosphate.

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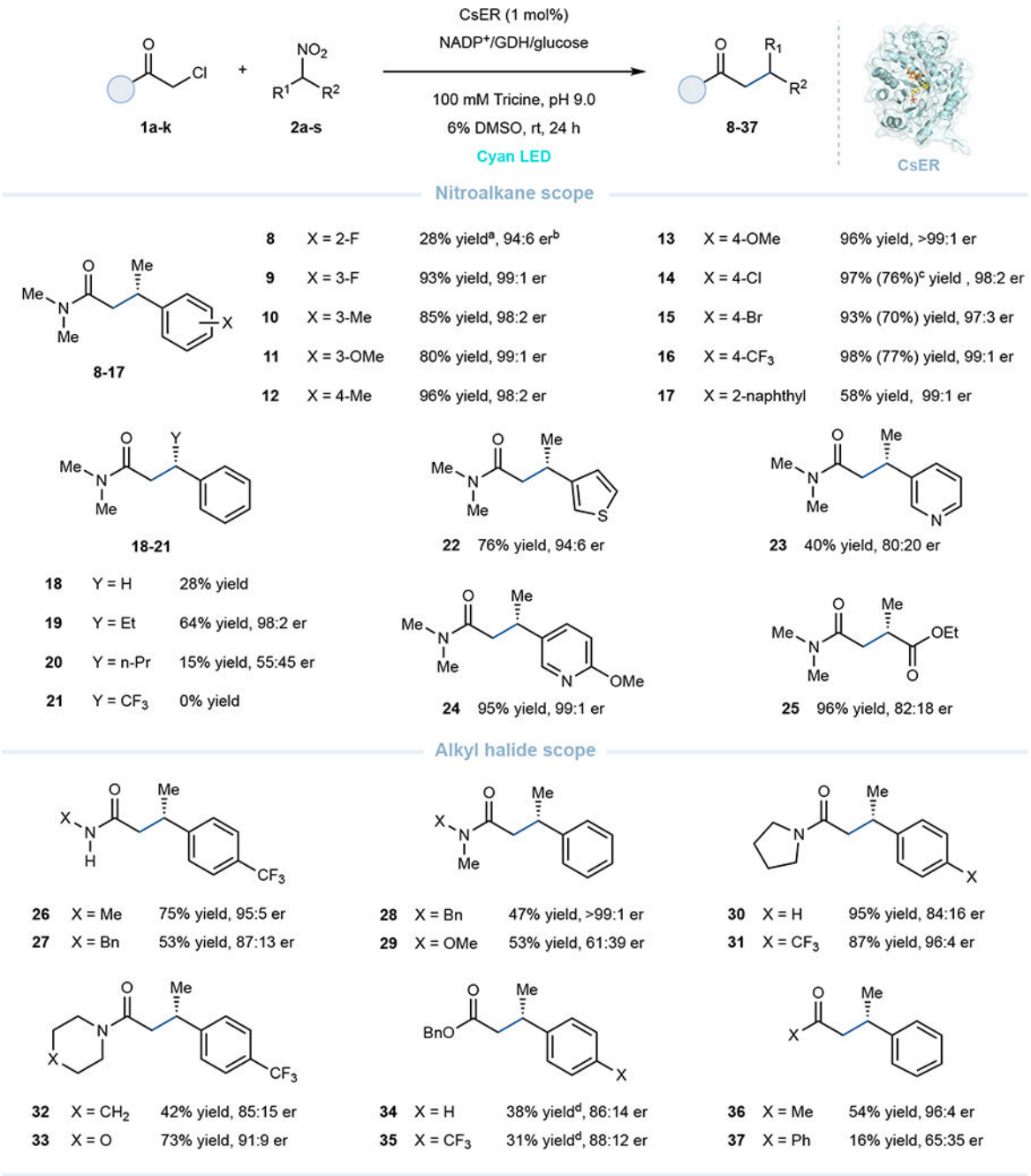

Fig. 2. Scope of the photoenzymatic cross-electrophile couplings.

Reaction conditions: α-chloro carbonyl substrate (10 μmol, 2 equiv), nitroalkane (5 μmol, 1 equiv), GDH-105 (0.3 mg), $NADP^+$ (0.05 μmol, 1 mol%), glucose (25 μmol) and purified 'ene'-reductases (0.05 μmol, 1 mol% based on nitroalkane) in tricine buffer (100 mM, pH 9.0), with 6% DMSO as cosolvent, final total volume is 800 μL. Reaction mixtures were irradiated with cyan LEDs under anaerobic conditions at room temperature for 24 h. [a] Yields determined *via* LCMS relative to an internal standard (TBB). [b] Enantiomeric ratio (er) refers to as the ratio of (*S*)- to (*R*)-enantiomer, er determined by HPLC on a chiral stationary

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phase. [c] Isolated yields were based on 0.10 mmol-scale reaction. [d] 3 equiv of α-bromo ester (15 μmol) were used.

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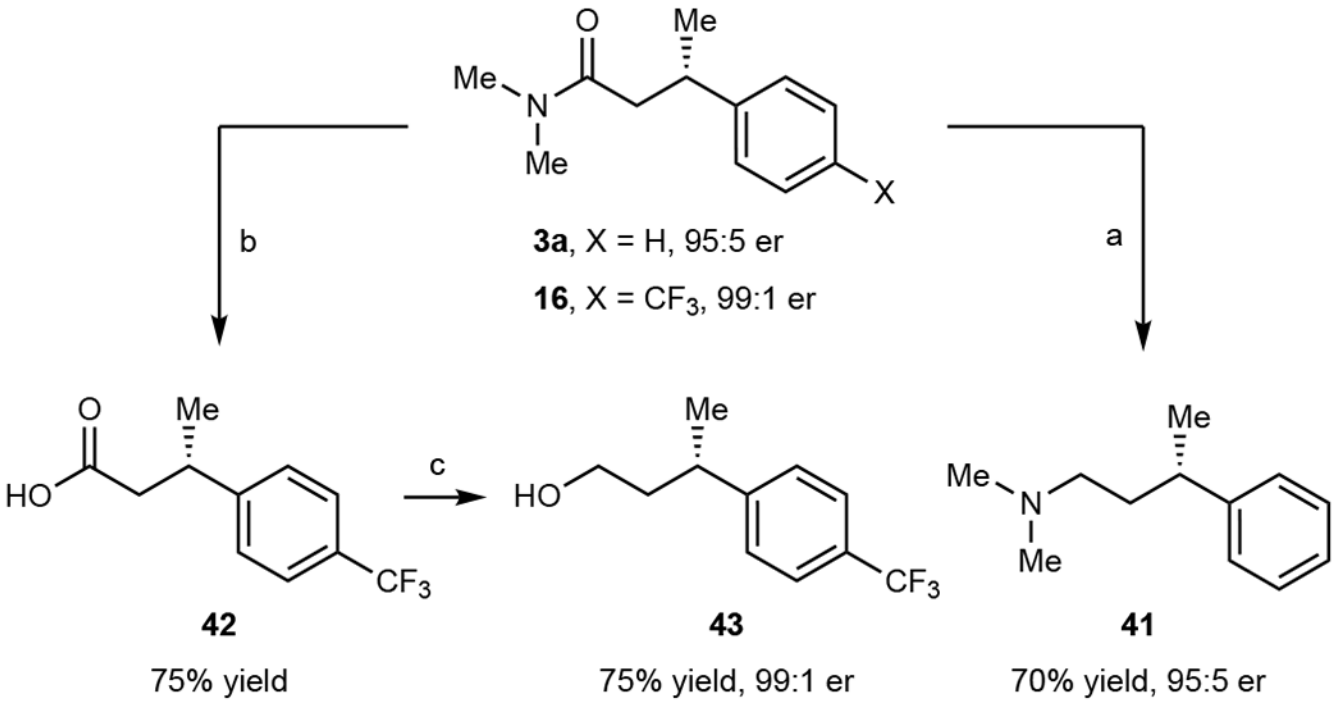

Fig. 3. Derivatization of the enzymatic products.

Reaction conditions: [a] $BH_3 \cdot Me_2S$ (3.0 equiv), THF, 0 to 65 °C, 5 h. [b] H_2SO_4 (4 M)/HOAc, 150 °C, 16 h. [c] $BH_3 \cdot Me_2S$ (3.0 equiv), THF, 0 to 45 °C, 5 h.

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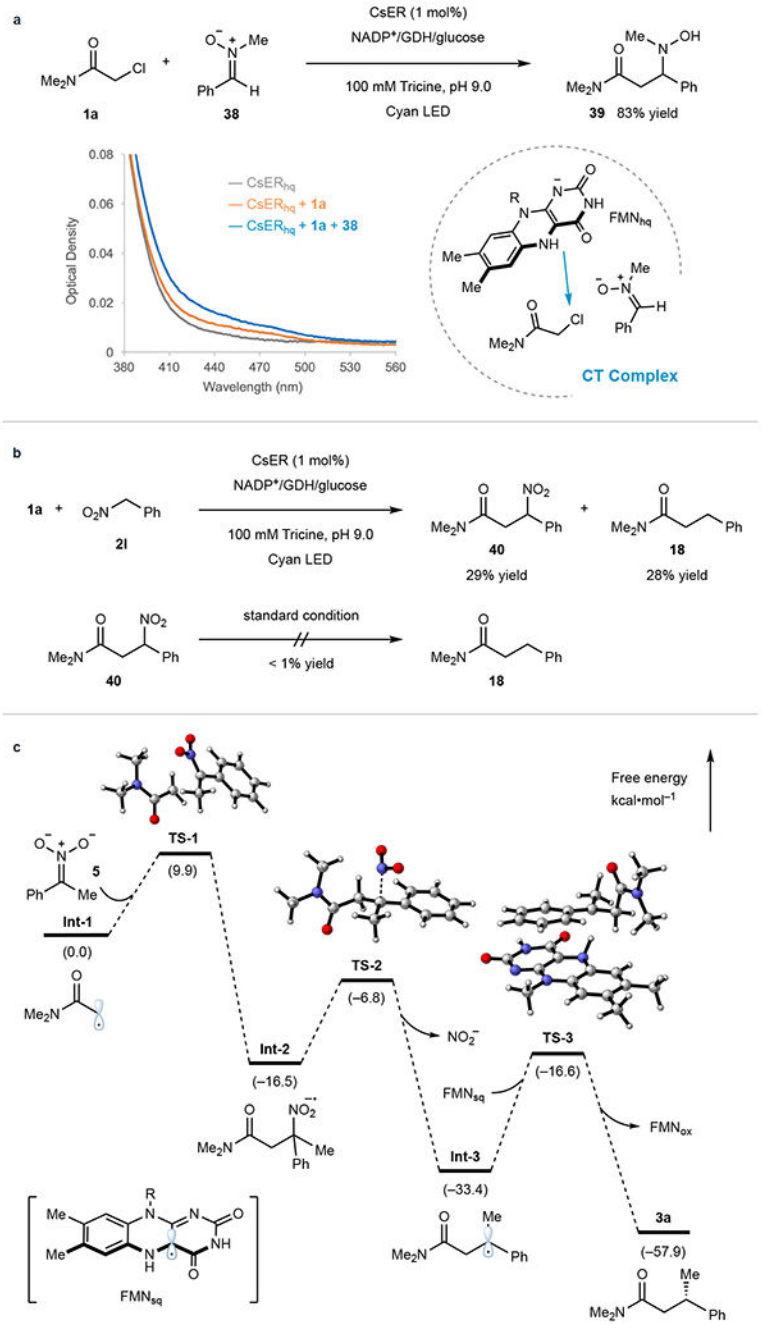

Fig. 4. Mechanistic experiments.

a, UV-Vis spectra of reduced CsER (FMN*hq*) in the presence of substrates. **b**, Feed experiment. **c**, Density functional theory (DFT) calculations of the model reaction.