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Recent Advances in Photoenzymatic Catalysis

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Abstract

Photoenzymatic catalysis has become an emerging field in organic synthetic chemistry that provides eco-friendly alternatives to traditional methods. This comprehensive review examines the developing field of photoenzymatic catalysis, categorized by reaction types and focusing on its application in organic synthesis. This article highlights recent advances in the use of photoenzymatic reactions in carbon-carbon cross-coupling, ketone and alkene reduction, hydroamination, and hydrosulfonylation, mostly by flavin-dependent "ene"-reductases and nitroreductases. In each case, we exemplified the substrate scope that produces products with high yield and enantioselectivity. Additionally, the emerging trends in developing new enzymatic variants and novel reaction pathways that broaden the scope and enhance yield of these reactions were discussed.

Keywords

Photoenzymatic, Biocatalysis, Biocatalysts, ERED, "Ene"-Reductases, Nitroreductases, Flavin

1. Introduction

In recent years, application of photo-induced enzymatic reactions for organic synthesis has emerged as a vibrant field of research, not only for clean and abundant energy source of light with the principles of green chemistry [1] but also for its remarkable selectivity and specificity [2] [3], enabling the targeted transformation of substrates into desired products with minimal by-products. Biocatalysis, the use of enzymes to catalyze the chemical reaction under mild conditions (aqueous solvent system, pH, temperature, and pressure), is more environmentally benign than the extreme conditions required in the traditional chemical reactions performed in organic solvents, which in most cases would be the use of metal catalysts. [4] [5] Additionally, enantiomerically pure compounds can be obtained with the unparalleled chemo-, regio-, and stereoselectiv-

ity of enzymes. [6] [7]

Driven in part by the characteristic advantages of photoenzymatic reactions described above, this surge of interest has led to the development of an increasing number of photoenzymatic methodologies. In this review, we covered the latest (mostly within the past five years) innovations of reaction development-carbon-carbon cross coupling formation with intermolecular reaction and intramolecular reactions, reduction reaction mainly with ketone reduction and alkene reduction, hydroamination reaction, and hydrosulfonylation reaction, and pioneering developments at the principle and methodology aspects. We aim to showcase the versatility of the photoenzymatic reaction with different types of biocatalysis by exploring the substrate scope with moderate to high yields and high levels of enantioselectivity.

2. Carbon-Carbon Cross Coupling

By promoting the formation of asymmetric radical intermediates with the photoexcitation of flavin-dependent "ene"-reductases (EREDs), a single electron transfer occurs through an electron donor-acceptor complex, which forms between the substrate and the reduced flavin cofactor at the enzyme active site, followed by hydrogen atom transfer (HAT). This naturally present photo-induced enzymatic ligation of carbon-carbon bonds has now been applied to synthetic organic chemistry for a wide range of substrates.

2.1. Intermolecular Reaction

2.1.1. Alkene Functionalization

In 2023, Li group demonstrated remote stereocontrol with azaarenes **1-1** via a flavin mononucleotide (FMN)-dependent ERED system through HAT under the reduction of the glucose dehydrogenase (GDH)/nicotinamide adenine dinucleotide phosphate ($NADP^+$)/glucose (Glu) system, yielding γ-stereocenter **1-3**. [8] In the Glu/GDH/$NADP^+$ system, Glu functions as a substrate for GDH, facilitating the conversion of $NADP^+$ into its reduced form, NADPH, thus reducing FMN into FMN hydroquinone (FMNhq) conversely within the OYE1 cofactor recycling framework. The reaction utilized *in situ* generated deuterated FMND, derived from D-Glu, achieving products with high yields and high level of enantioselectivity. Examining the scope, reaction tolerates α-methyl styrene with different substituents, different substituted 2-bromomethyl pyridines, and 4-bromomethyl pyridine with different substituents and the groups (-Me and -Cl) at the 2-position (**Figure 1**).

Another study reported an enantioselective photoinduced radical hydroalkylation of alkenes catalyzed by EREDs with Glu/GDH/$NADP^+$ framework, achieving synthesis of γ-chiral carbonyl compounds **2-3** [9]. For substrate scope, the enzyme is tolerant to a variety of α-bromo and α-chloro aryl ketones **2-4**, amides, esters, and different classes of alkenes (α-methyl aryl substituted ethylenes); however, an aliphatic alkene and an internal alkene furnished with poor enantioselectivity (**Figure 2**).

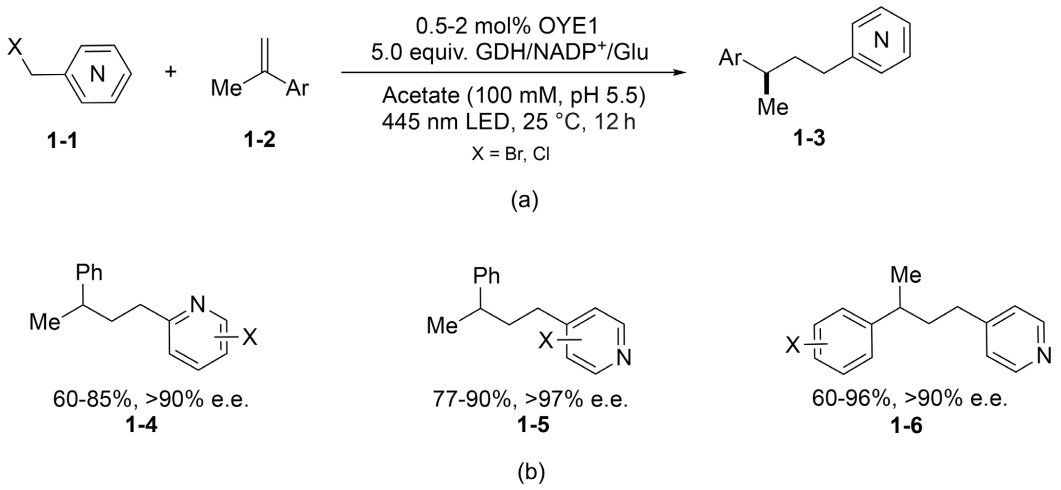

Figure 1. Enantioselective radical hydroalkylation of azaarenes. (a) Reaction conditions and (b) Representative reaction scope.

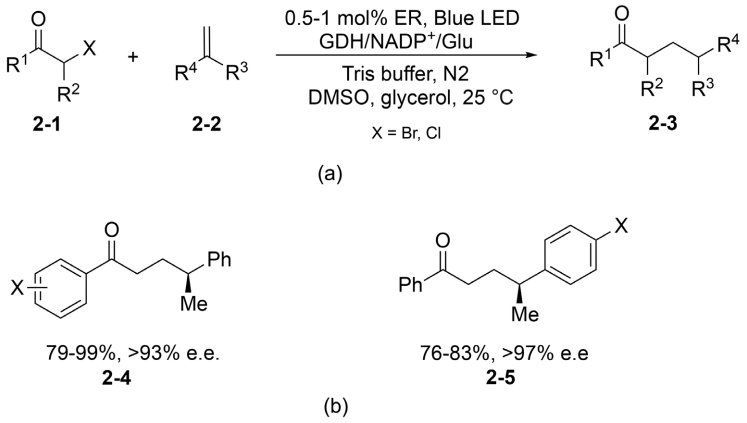

Figure 2. Enantioselective intermolecular radical hydroalkylation of *α*-halo carbonyls. (a) Reaction conditions and (b) Representative reaction scope.

Another enantioselective intermolecular radical conjugate addition under synergistics interaction of light excitation and nicotinamide-dependent ketoreductases (KREDs) is reported by Zhao's group. [10] The reaction starts the formation of an EDA complex between KRED-bound NADPH and the electron-deficient radical precursor under visible light, generating the radical within the KRED active site, thus conjugating addition to terminal alkene, followed by HAT to yield the chiral stereocentres **3-3**. The substrates bearing different radicals from primary to ternary achieve moderate yields in high levels of enantioselectivity (**Figure 3**).

In 2024, Fu and co-workers reported a decarboxylative alkylation with an exogenous $Ru(bpy)_3^{2+}$ cofactor and flavin-dependent EREDs (old yellow enzyme 3 (OYE3) for the formation of *S*-enantiomer **4-3** and GluER-T36A for the formation of *R*-enantiomer **4-4**). [11] Investigating the scope, Fu team explores substrates bearing different substituents at different positions on pyridine ring, and pyrazines, which afford *S*-enantiomers (**Figure 4**).

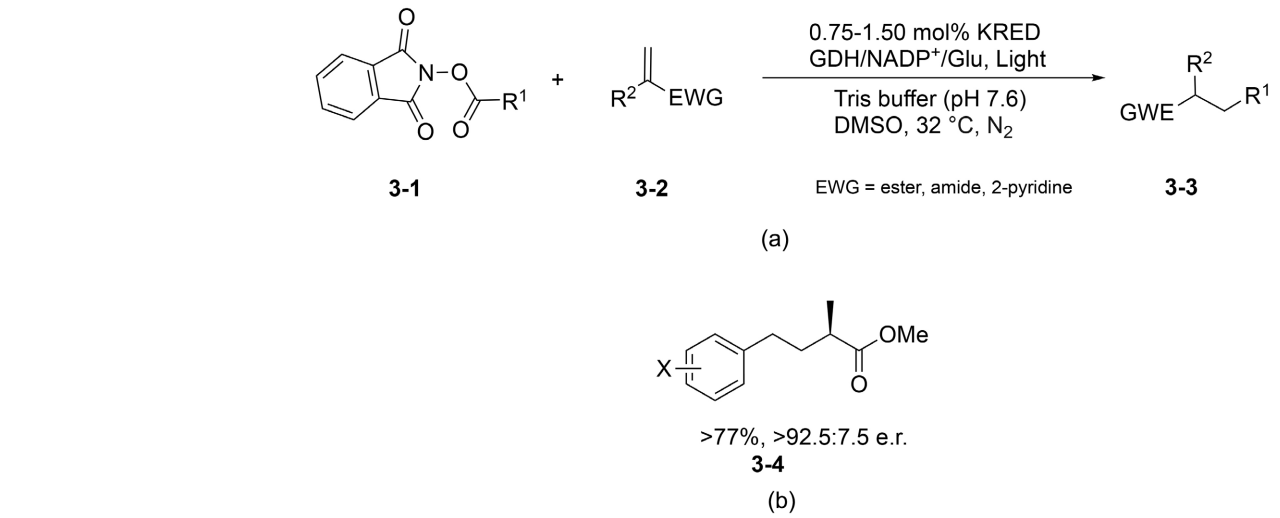

Figure 3. Enantioselective intermolecular radical conjugate addition of *N*-(Acyloxy)phthalimides. X for H, MeO, etc. (a) Reaction conditions and (b) Representative reaction scope.

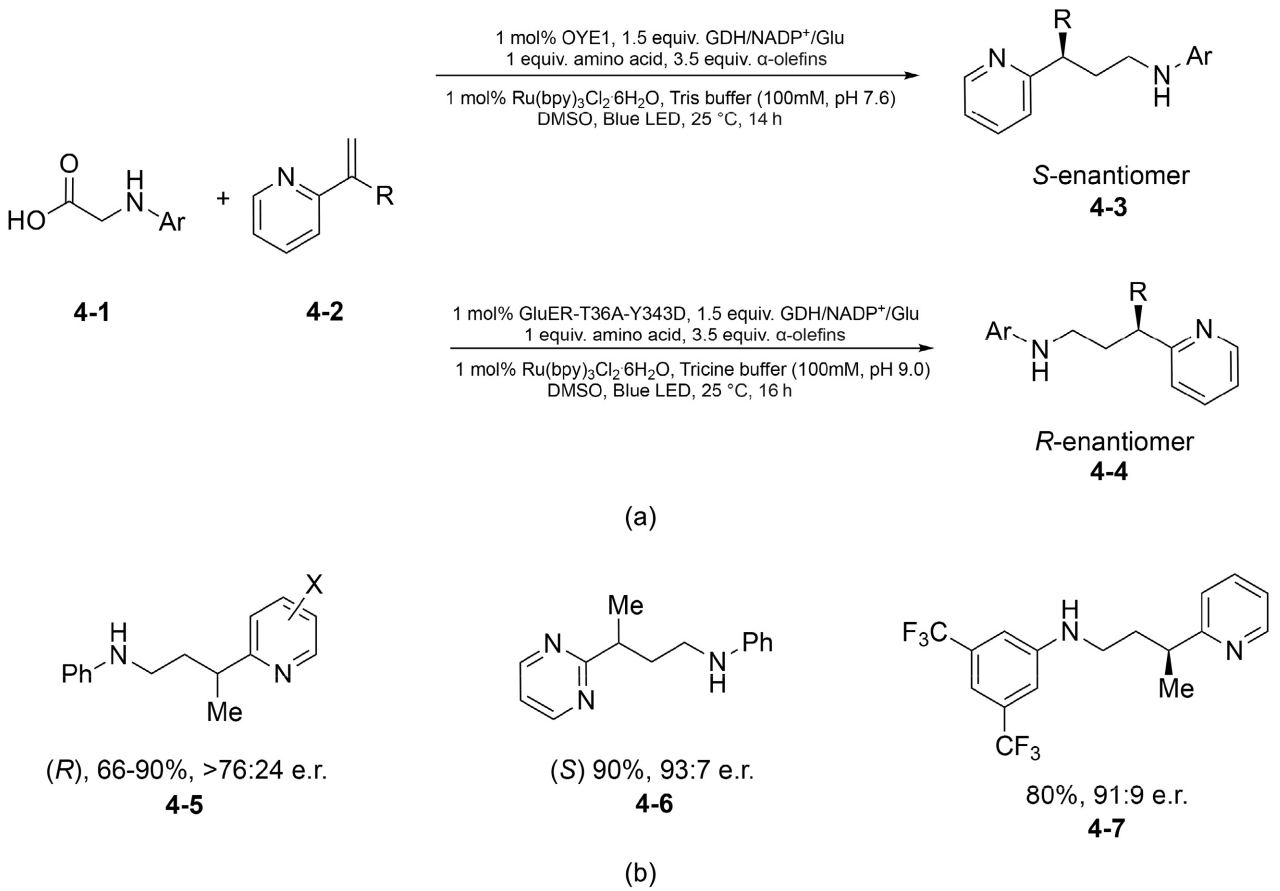

Figure 4. Enantioselective decarboxylative alkylation. (a) Reaction conditions and (b) Representative reaction scope.

In 2023, Bender and Hyster presented the enantioselective alkene hydroalkylation with electron-deficient heterocycles **5-1** by using pyridylmethyl radicals and EREDs under visible light, yielding *γ*-stereocenter **5-3**. [12] The enzyme is

limited to work on the substrates bearing a methyl substituent on the pyridyl ring and the 2,6-dimethyl substituent **5-4** (Figure 5).

The Ouyang group reported an asymmetric carbohydroxylation of alkenes for the production of tertiary alcohols, catalyzed by flavin-dependent EREDs and light irritation. [13] The methodology uses a 5-*endo*-trig cyclization mechanism to terminate radicals and form the tertiary alcohols, differing from HAT. The substrates encompass olefins with EDGS at the *para* positions **6-4**, and alkenes with various electronic substituents **6-5**, provide products in modest to good yields with high levels of enantioselectivity; however, bulky aromatic groups (naphthyl groups and benzofurans) led to lower yields (Figure 6).

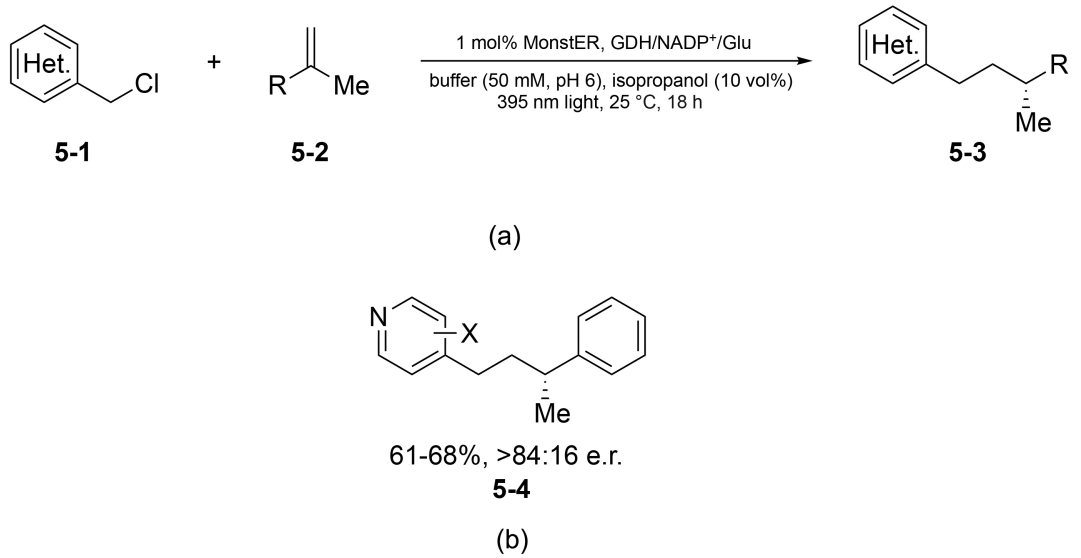

Figure 5. Alkene hydroalkylation. X for H and 2,6-Me. (a) Reaction conditions and (b) Representative reaction scope.

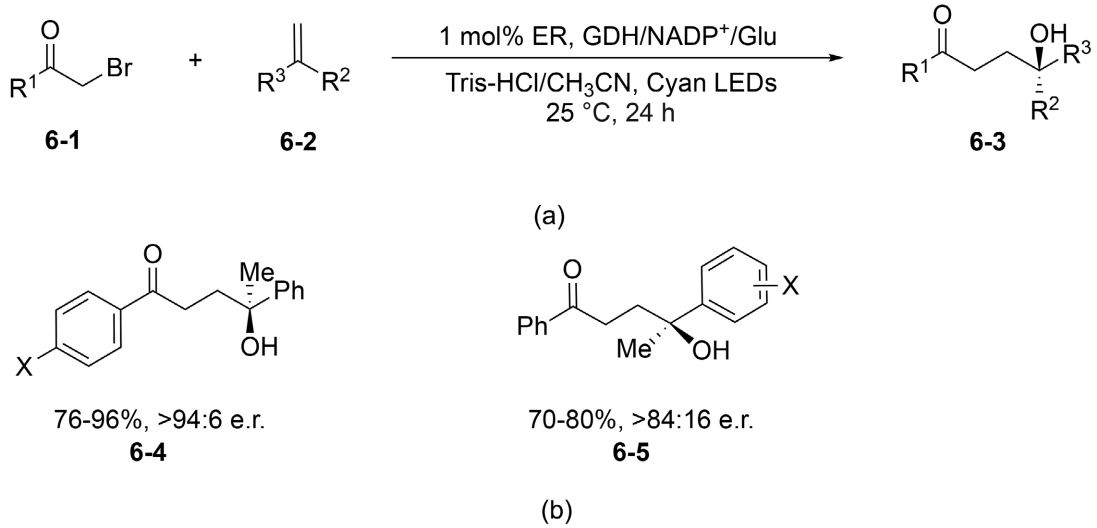

Figure 6. Asymmetric Carbohydroxylation of Alkenes. (a) Reaction conditions and (b) Representative reaction scope.

In 2023, Huang group reported stereocontrolled intermolecular radical hydroarylation of alkenes with electron-rich arenes under direct visible-light-excitied flavoproteins (**Figure 7**). [14] The reaction proceeds through a single electron oxidation of arenes catalyzed by flavin-dependent EREDs under visible-light activation **7-4**, generating aryl radical cations, followed by C(sp^2)–C(sp^3) bond-forming **7-7**, thus yielding products **7-8** in a redox-neutral. The substrate scope accommodates a variety of methyl styrenes with different substituents (functional groups at the para/meta/ortho position of phenyl group **7-9**, thienyl, and benzofuranyl) and electron-rich arenes, using different ERED variants for both enantiomers (GluER, GluER_T36A-Y177F, OYE1_F296A, and OYE1_F296G).

2.1.2. Acylation

Acylation is the addition of an acyl group to a structure. In 2024, Huang's group reported an unnatural enantioselective radical acyl transfer with synergistic combination of thiamine diphosphate (ThDP)-dependent enzymes and an organophotoredox catalyst (**Figure 8**). [15] The reaction proceeds through single electron oxidation to form enzyme-bound ThDP-derived ketyl radicals, cross-coupling with prochiral alkyl radicals, thus forming chiral ketones **8-3** with high enantioselectivity. Moreover, Huang's group extends the scope of reaction under the substrates bearing aromatic aldehydes with different substituents (both electron-donating (EDGs) and electron-withdrawing groups (EWGs) at *para* position **8-4**, and halogens).

2.1.3. Alkylation

The general alkylation is a chemical process that substitutes halogen atom to form bond between two reactant molecules. In photoenzymatic reactions, it will form radicals for transferring alkyl groups after losing halogen atoms. By photoexcitation of the enzyme-templated charge-transfer (CT) complex between an alkyl halide, conjugation partner, and flavin cofactors, the reaction proceeds through reduction of alkyl halide to generate corresponding radicals, yielding the cross-coupled product. In 2023, Page group reported a biocatalyst-controlled method for the regioselective radical alkylation of both electron-rich and electron-deficient heteroarenes **9-2** upon the evolved ERED variants. [16] The reaction alkylates the C4 position of indole with radical formation to obtain the desired products. The substrate scope explored a variety of α-chloroamides, electron-rich, and electron-deficient arenes, alkylating at elusive positions of indole and quinoline (**Figure 9**).

Another parallel study, conducted by Fu and co-workers, reported a highly chemo- and stereoselective C-alkylation for tertiary nitroalkanes **10-2** and alkyl halides **10-1** with EREDs [17] Investigating scope, an array of α-benzyl nitroalkanes possessing different substituents and larger ethyl groups, heterocycles (pyridine, pyrazine, and thiophene), and linear and cyclic aliphatic nitroalkanes, were obtained in high levels of enantioselectivity. Moreover, substrates possessing different EDGs or EWGs at the *para*/*meta*/*ortho* position **10-4** and a variety of heterocycles **10-5** are investigated for expanding the scope (**Figure 10**).

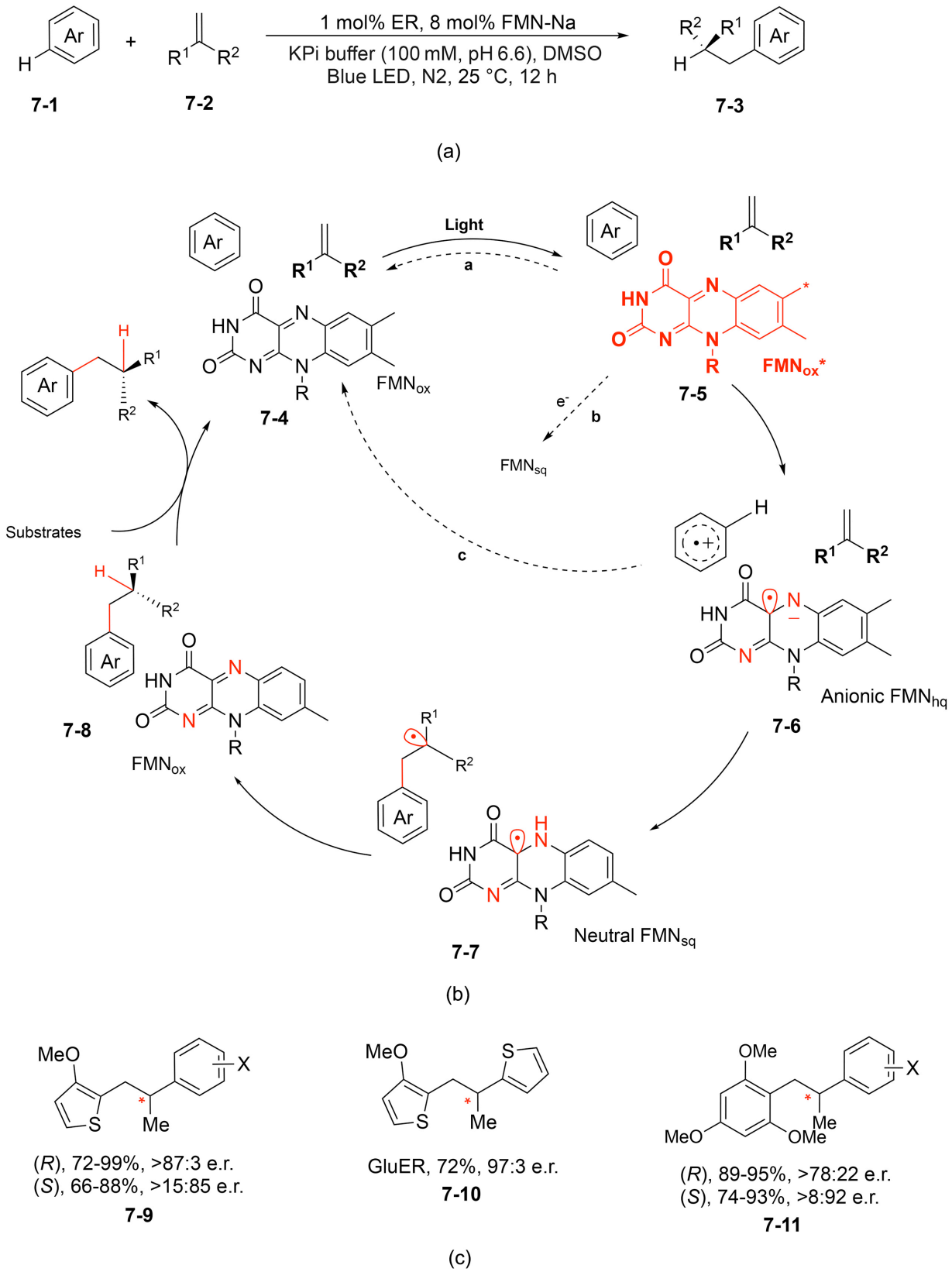

Figure 7. Redox-neutral asymmetric radical hydroarylation. (a) Reaction conditions; (b) Proposed Mechanism; (c) Representative reaction scope.

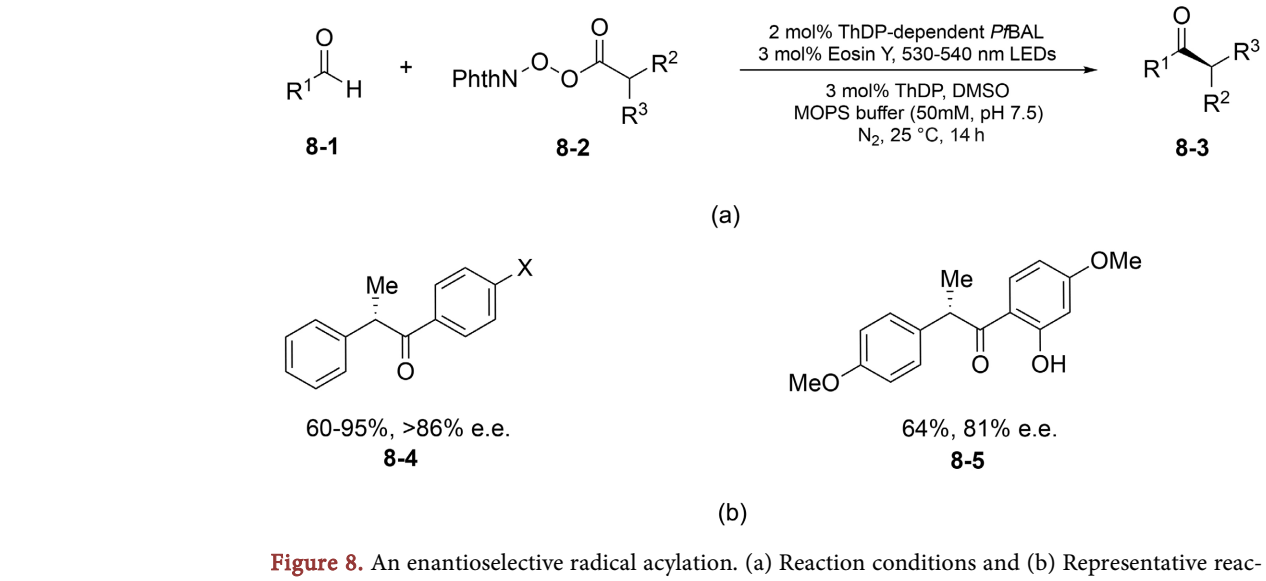

Figure 8. An enantioselective radical acylation. (a) Reaction conditions and (b) Representative reaction scope.

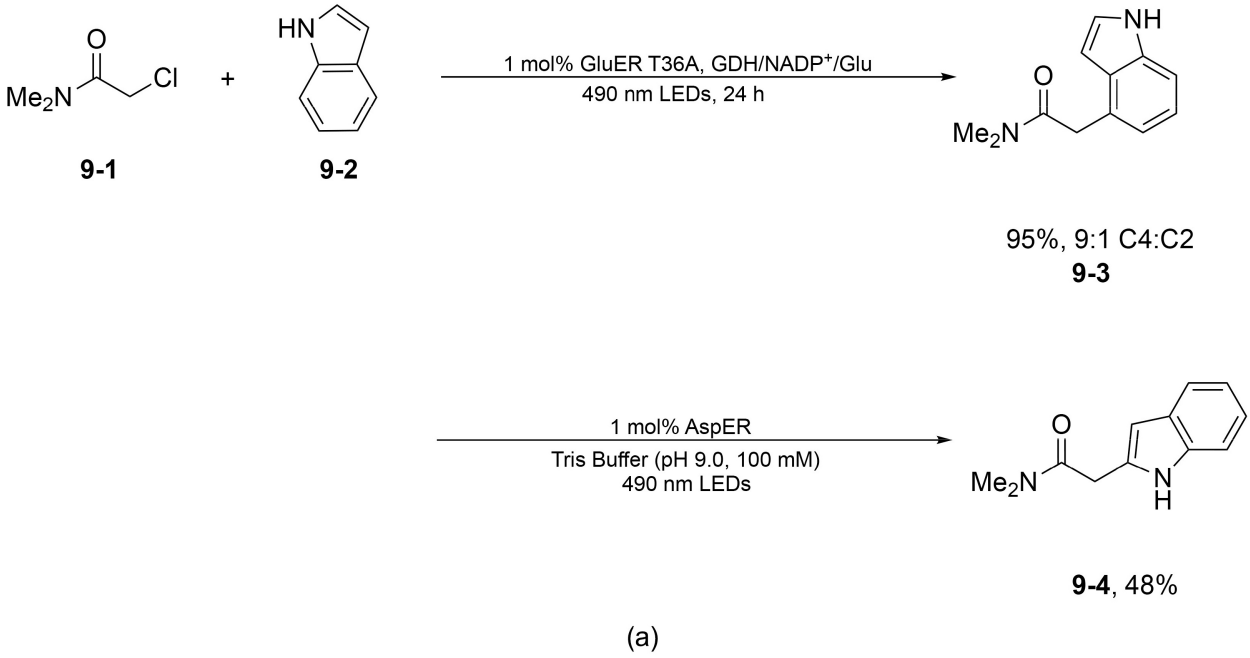

Figure 9. alkylation of heteroarenes. (a) Reaction conditions.

2.1.4. Cross-Electrophile Couplings (XECs)

A novel photoenzymatic asymmetric C(sp^3)–C(sp^3) XEC using flavin-dependent EREDs, facilitating chemoselective and enantioselective coupling between alkyl halides **11-1** and nitroalkanes **11-2**, is presented by Fu *et al.* [18] The reaction proceeds through alkyl radical reacts with *in situ*-generated nitronate to form a nitro radical anion, thus yielding the cross-coupled product **11-3**. The substrate scope encompasses various *α*-aryl nitroethanes possessing different substituents at *meta* or *para* positions **11-4**, extending to various heterocycles **11-5**, and ternary amides **11-6** (Figure 11).

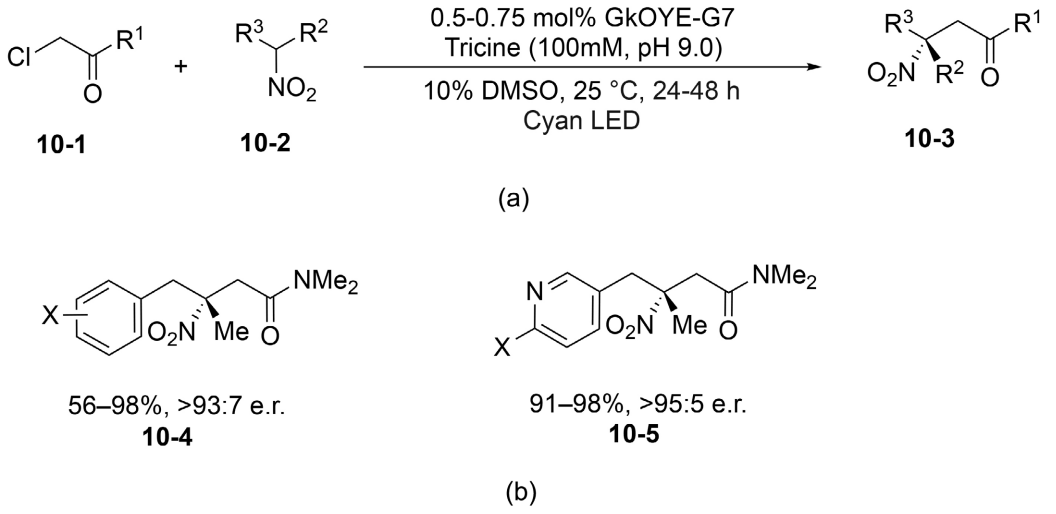

Figure 10. C-alkylation of nitroalkanes. (a) Reaction conditions and (b) Representative reaction scope.

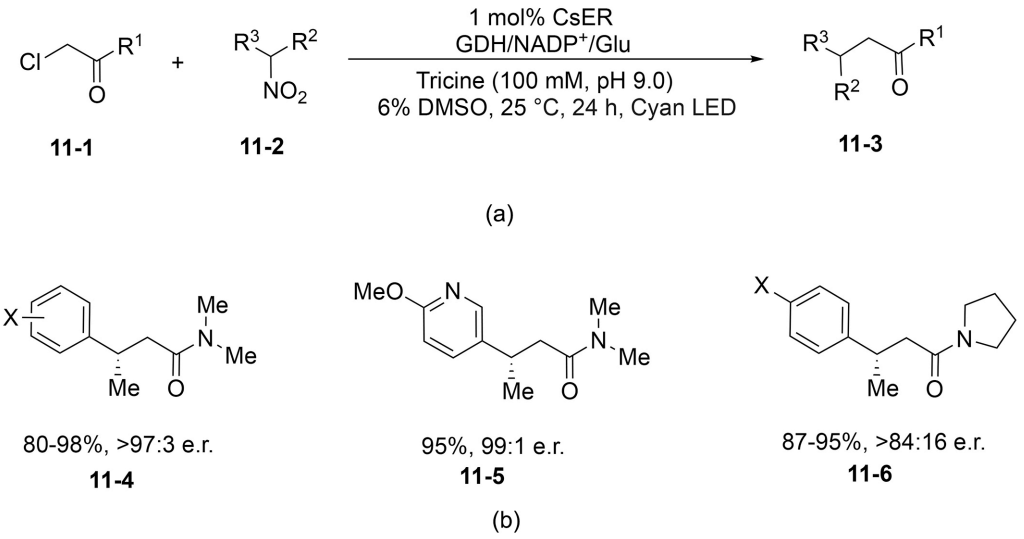

Figure 11. C(sp^3)–C(sp^3) XEC. (a) Reaction conditions and (b) Representative reaction scope.

2.2. Intramolecular Cyclization

Intramolecular cyclization in this context involves the reaction between an alkene moiety and a halogen-attached carbon within the same molecule to form a cyclic structure. In 2020, Hyster, Black, and co-workers found a redox-neutral radical cyclization of *α*-haloamides **12-1**. [19] This reaction **12-3** occurs via electron transfer from flavin hydroquinone (FMNhq) to the substrate, reducing *α*-halo-*β*-amidoesters, forming an *α*-acyl radical, upon oxidation of the resulting vinylogous *α*-amido radical, followed by hydrogen atom transfer from the flavin semiquinone (FMNsq), affording the product, 3,3-disubstituted oxindoles. The FMNsq needs to serve as oxidant for desired transformation. Furthermore, Hyster's team focuses on the scope of this cyclization for substrates bearing longer linear groups **12-4**, a variety of easter substituents, and different electron-donating substituents on *para*-substituted aromatic rings **12-5** (**Figure 12**).

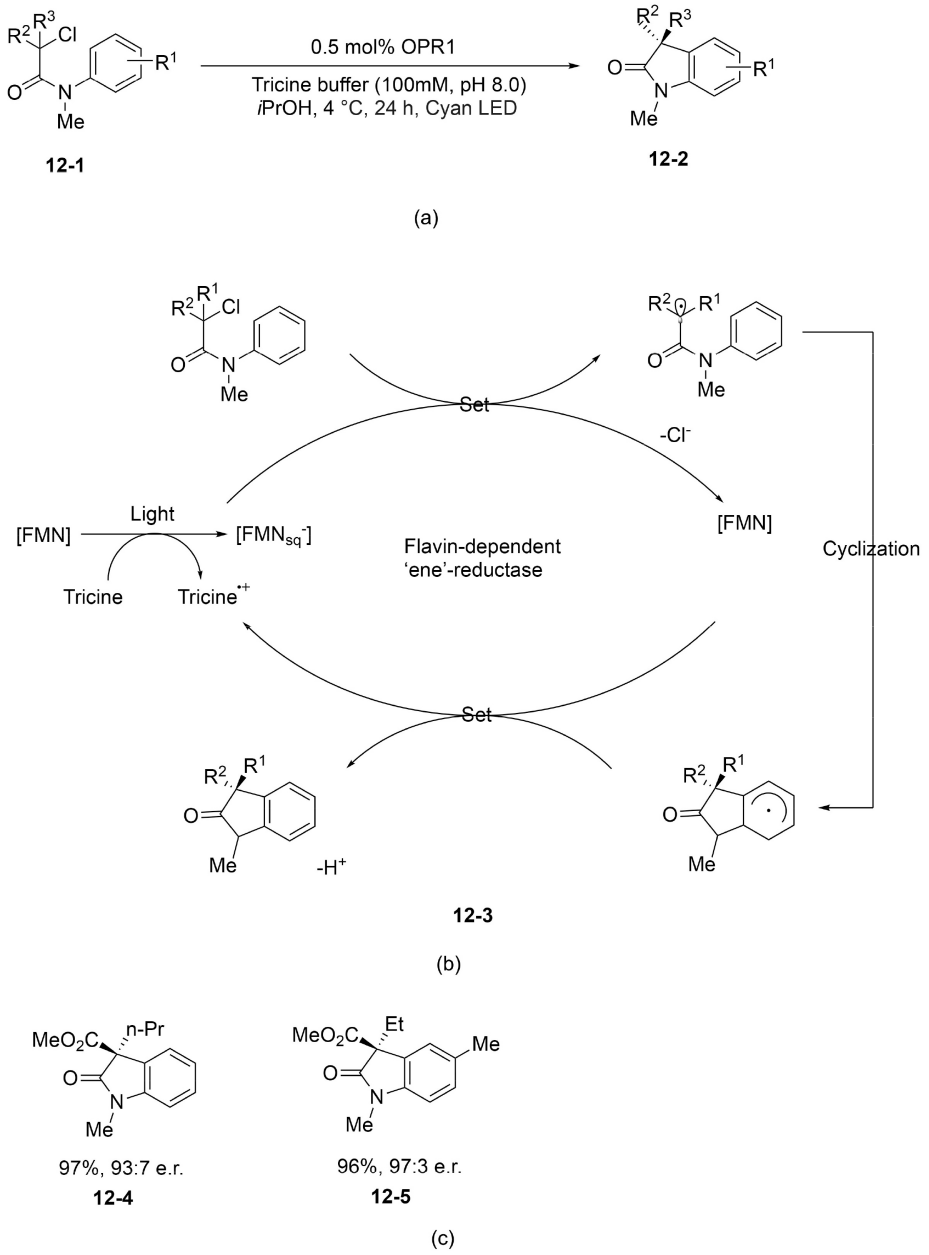

Figure 12. Asymmetric redox-neutral radical cyclization. SET, single electron transfer. (a) Reaction conditions; (b) Proposed Mechanism; (c) Representative reaction scope.

In 2019, Hyster's team demonstrated a novel approach where photoexcitation of flavin-dependent EREDs enables asymmetric radical cyclization (**Figure 13**). [20] This methodology enables the precise construction of lactams with various ring sizes with stereochemical preferences dictated by the active site of the enzyme. Hyster's team extended scope to access several different cyclization modes that encompass a range of aromatic and alkyl-substituted alkenes, effectively producing five- **13-3** to eight-membered lactams and spanning from *exo*- to *endo*-trig. Various catalysts (GluER-T36A and LacER (*Lactobacillus casei*)) were used for desired transformation (**Figure 13**).

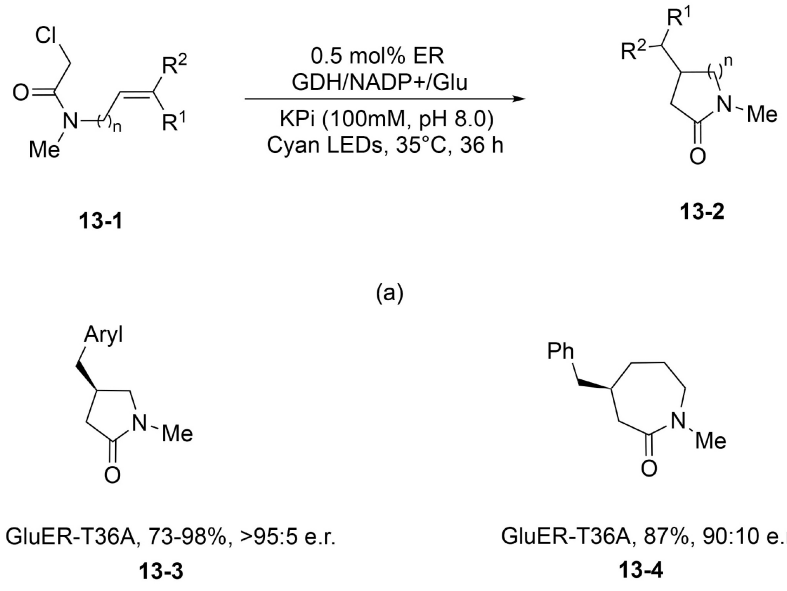

Figure 13. A stereoselective radical cyclization. (a) Reaction conditions and (b) Representative reaction scope.

A study of the photoenzymatic generation of unstabilized alkyl radicals for asymmetric reductive cyclization, using alkyl iodides **14-1** as precursors, is presented (Clayman & Hyster, 2020) (**Figure 14**). [21] The reaction utilizes flavin-dependent EREDs as catalysts, irradiated with light, to facilitate electron transfer within a CT complex formed between flavin and substrate. Optimizing the scope of reactions, the reaction is explored within GluER-Y177F from five- to six-membered substrates, possessing the different substituents, functional groups (an alkene and alkyl bromide) at the *α*-position, and heteroatoms provide 5-*exo*-trig substrates **14-3** and 6-*exo-trig* substrates **14-4** (**Figure 14**).

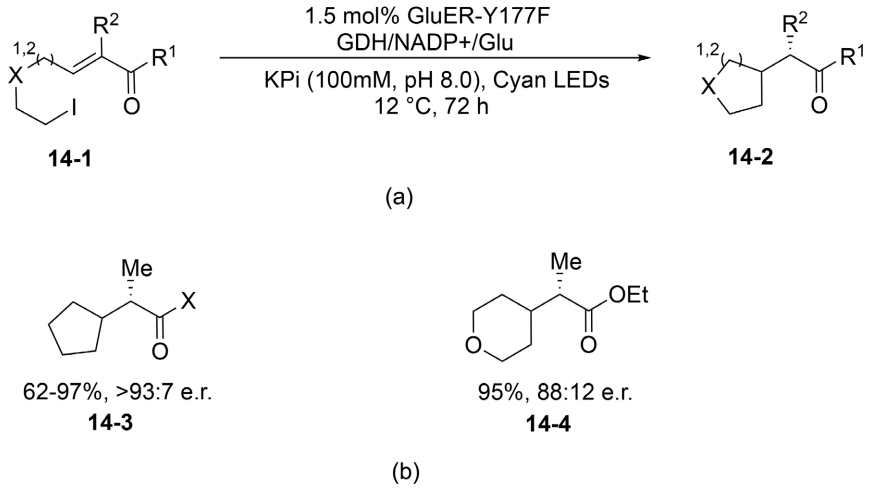

Figure 14. An asymmetric reductive cyclization. (a) Reaction conditions and (b) Representative reaction scope.

In 2023, Rao and co-workers reported a novel radical-mediated photoenzymatic synthesis of oxygen-containing benzo-fused heterocycles with flavin-dependent ERED, GluER (Figure 15). [22] Leveraging structure-guided engineering of GluER, it effectively synthesizes various benzoxepinones, chromanone, and indanone. Investigating the substrate scope, Rao's team further studies the substrates bearing different methyl group substituents at the *para* **15-3** or *meta* **15-4** position and the acetophenone moiety **15-5** at different positions (Figure 15).

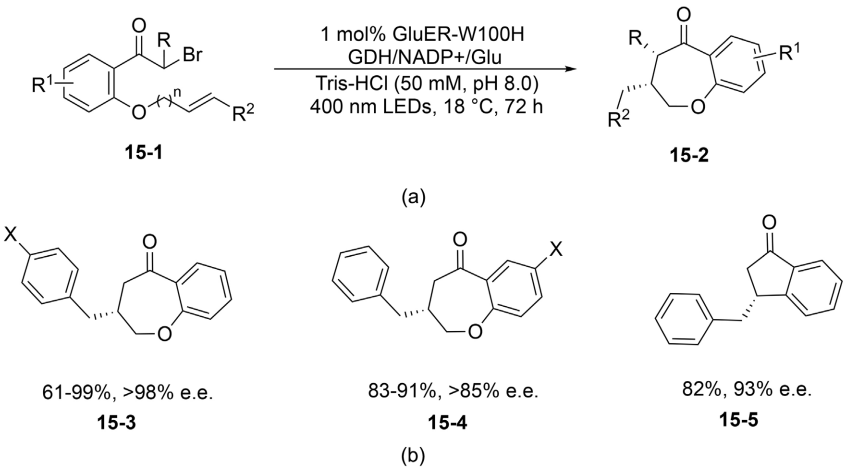

Figure 15. Enantioselective synthesis of oxygen-containing benzo-fused heterocycles. (a) Reaction conditions and (b) Representative reaction scope.

3. Reduction Reaction

3.1. Ketone Reduction

Ketone reduction is a chemical process of converting ketones into alcohols. The mechanism of currently developed reactions capitalizes on the synergistic interplay between photocatalysis and biocatalysis, where a photocatalyst, upon excitation by blue light, donates an electron to the bound ketone, generating a ketyl radical that is subsequently quenched by a hydrogen atom from the reduced flavin, thus forming the alcohol product. In 2022, Poelarends *et al.* showcases the chemo- and enantioselective photoenzymatic ketone reductions facilitated by a promiscuous flavin-dependent nitroreductase, BaNTR1. [23] The methodology provides products in high level of enantiopurity without affecting the C=C or C≡C bonds. Expanding the scope, the enzyme is tolerant with substrates encompassing various substituted acetophenones **16-3** and *α*,*β*-unsaturated ketone **16-4** (Figure 16).

In a parallel vein of research, Sandoval *et al.* demonstrated the reduction of aromatic ketones **17-1** into alcohol **17-2** in presence of photoredox catalysts with flavin-dependent EREDs, allowing native and non-natural mechanisms to occur simultaneously without modification of enzymes. [24] For the substrate scope, substrates are examined with MorB and $Ru(bpy)_3Cl_2$, bearing different EWGs **17-3** at *para* and *meta* positions and naphthyl ketone **17-4** (Figure 17).

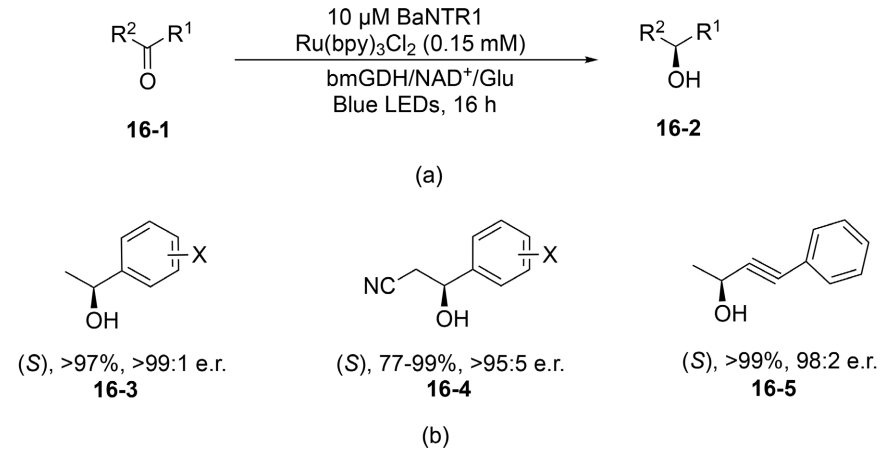

Figure 16. Ketone reductions catalyzed by a promiscuous flavin-dependent nitroreductase. (a) Reaction conditions and (b) Representative reaction scope.

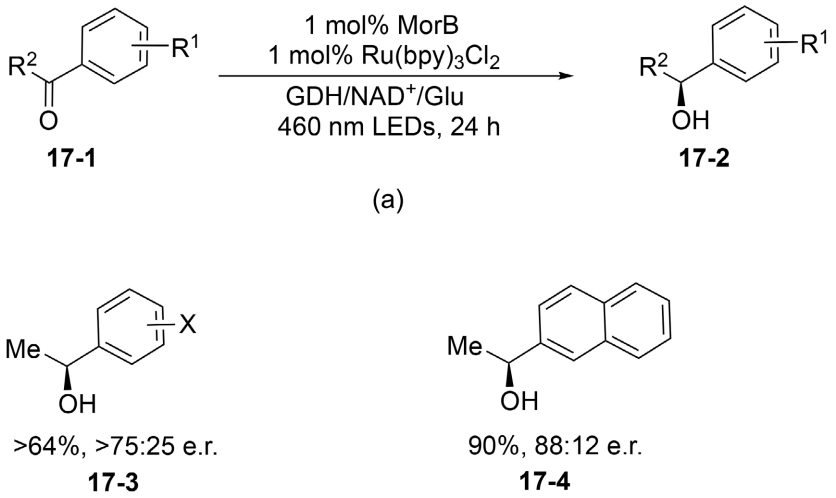

Figure 17. Radical-mediated ketone reduction. (a) Reaction conditions and (b) Representative reaction scope.

3.2. Alkene Reduction

Alkene reduction is a chemical process of reducing alkene into alkane. In 2020, Nakano *et al.* showcases a novel approach to the photoenzymatic reduction of vinyl pyridines **18-1** through a radical mechanism without depending on native hydride transfer (Figure 18). [25] The reaction is achieved through a synergistic combination of flavin-dependent EREDs and photoredox catalysis under visible light, proceeding via a radical mechanism initiated by single electron reduction to the form a radical, followed by HAT, resulting in the reduced product **18-2**. The reaction is further explored the substrates, bearing a variety of vinyl pyridines with EDGs or EWGs at *para* or *meta* positions on the aromatic ring **18-3** and different groups (3-thiophenyl groups **18-4**), and heteroaromatic substituents (vinyl and thiazoles groups **18-5**).

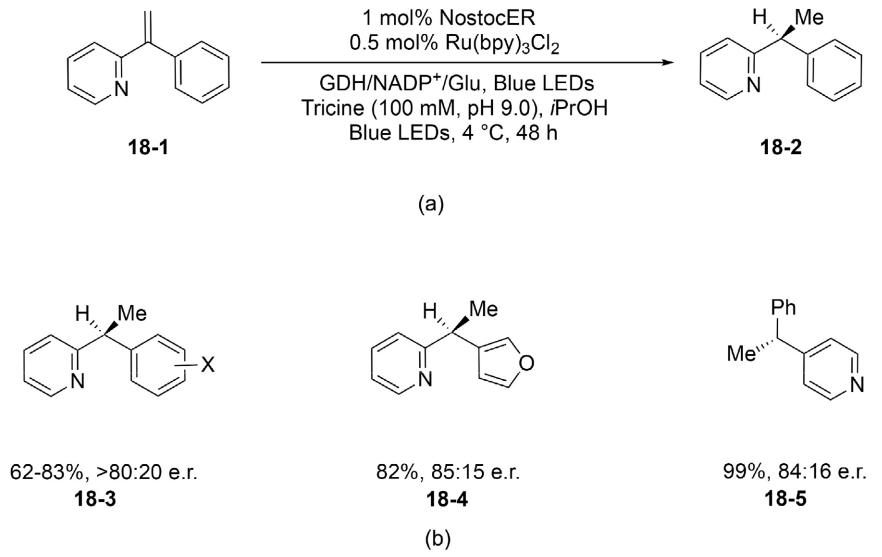

Figure 18. Hydrogenation of heteroaromatic olefins. (a) Reaction conditions and (b) Representative reaction scope.

Another study, conducted by Sandoval *et al.*, introduces a method for alkene reduction via direct excitation of flavin hydroquinone within the active sites of EREDs. [26] The key innovation here is leveraging photoinduced electron transfer as a means to generate radical intermediates from alkenes, which are then reduced in an enzymatically controlled environment. The enzyme is tolerant to a variety of cyclic **19-3** and acyclic amides **19-4**, and aliphatic substituents **19-5** at *para* or *meta* position (**Figure 19**).

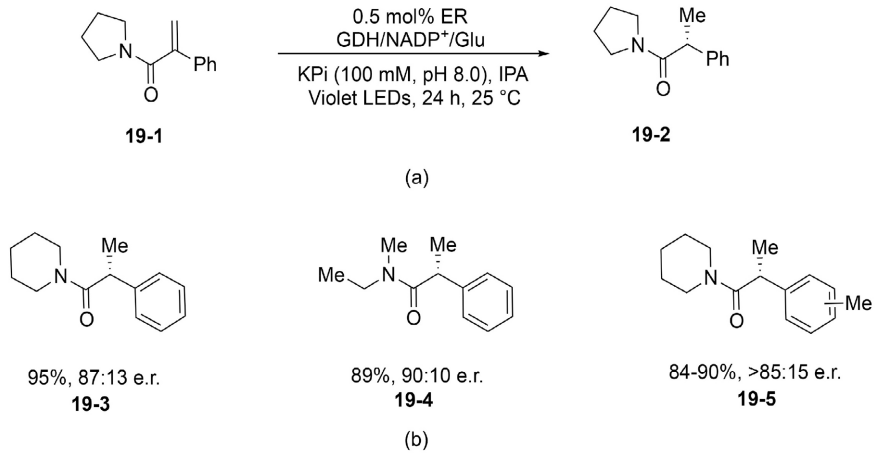

Figure 19. Alkene reduction. (a) Reaction conditions and (b) Representative reaction scope.

3.3. Reduction of Nitro Compounds

The study by Alejandro Prats Luján *et al.* presents a light-powered photoenzymatic synthesis of both aliphatic and aromatic nitro products. [27] The reaction proceeds through reduction of nitro compounds catalyzed by light irritation and

nitroreductase from BaNTR1 and Enterobacter cloacae (EcNR) with a photocatalytic system based on chlorophyll, thus yielding the desired series of products (Figure 20).

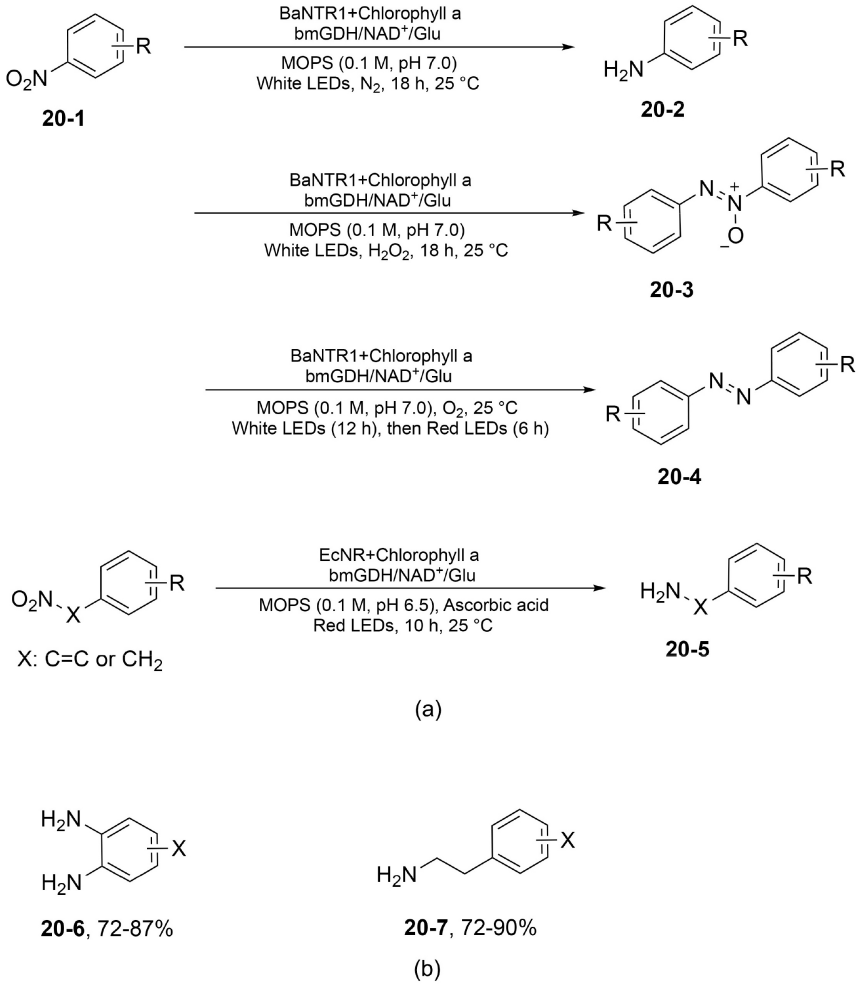

Figure 20. Selective reduction of aliphatic and aromatic nitro compounds. (a) Reaction conditions and (b) Representative reaction scope.

3.3.1. Synthesis of Aromatic Amines

The reaction reduces various nitroarenes **20-1** into aromatic amines **20-2**, employing BaNTR1 with chlorophyll under white light. This process is tolerant to electronically diverse nitroarenes bearing EDGs or EWGs, dinitrobenzenes **20-6**, and heteroarenes, with conversion up to 99%.

3.3.2. Synthesis of Aromatic Azoxy and Azo Compounds

The reduction proceeds through the formation of azoxy intermediates **20-3** from nitroarenes, which can be further selectively reduced to azo compounds **20-4** with chlorophyll and BaNTR1 under red light, employing an oxidant (O2 or H_2O_2). For desired aromatic azoxy and azo products, the reaction achieved moderate to high yields and promising levels of conversions (>99%) with substrates contain EDGs or EWGs.

3.3.3. Synthesis of Aliphatic Amines

The reduction of nitroaliphatic into aliphatic amines **20-5** employs EcNR with chlorophyll and addition of ascorbic acid under red light. Selecting a variety of nitroalkenes **20-7** and nitroalkanes for scope, substrates possessing EDGs and EWGs at para or meta positions give production with conversion up to 97% and moderate to high yields (>72%).

3.4. Reductive Dehalogenation

Reductive dehalogenation is a reduction process that eliminates halogen atoms. In 2016, Emmanuel and co-workers demonstrated enzyme promiscuity through photoexcitation of KREDs. [28] The reaction proceeds through photoexcitation of KREDs with racemic lactone **21-1**, transforming into initiators of radical species through enantioselective radical dehalogenation of lactones with cofactor regeneration from $NADP^+$ into NADPH, yielding chiral lactone **21-3**. Exploring the scope, the six-membered lactones with different substituents (fluoro- or chloro-substituted and methyl at ortho and para position) **21-4** and five-membered lactones **21-5** are investigated with the presence of KRED-3 (**Figure 21**).

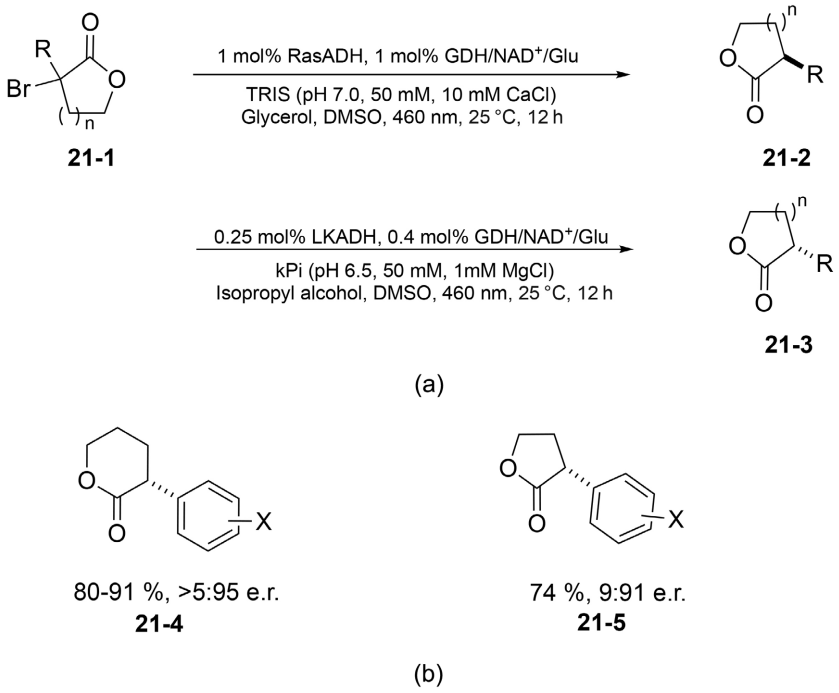

Figure 21. Enantioselective radical dehalogenation of lactones. (a) Reaction conditions and (b) Representative reaction scope.

A novel approach, conducted by Peng *et al.* in 2022, discovered a photoinduced synthesis of *α*-fluoroketones **22-2** using cyclohexanone monooxygenase (CHMO) through reductive dehalogenation via electron transfer/proton transfer mechanism under mild conditions. [29] For the exploration of scope, the substrates handle different substituents (EDGs or EWGs on the phenyl ring), and five-membered ring (**Figure 22**).

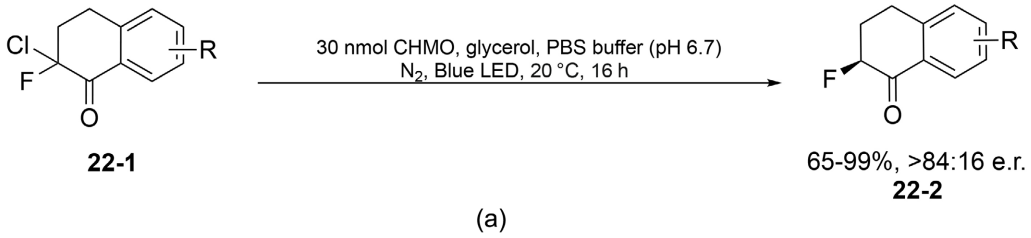

Figure 22. Enantioselective reductive dehalogenation of *α*, *α*-halofluoroketones. (a) Reaction conditions.

4. Carbon-Heteroatom Bond Formation

4.1. Hydroamination Reaction

Hydroamination reaction is a chemical process that adds an amine across an unsaturated bond to form a new C–N bond. In 2023, an innovative approach for hydroamination was developed by Ye *et al*., focusing on taming nitrogen-centred radicals (NCRs) with flavin-dependent EREDs. [30] The reaction starts with the selective generation of amidyl radicals within the active site of flavin-dependent EREDs, facilitated by an exogenous photoredox catalyst under visible light, followed by HAT to form the desired C-N bond. The enzymes, YqjM and GluER, can catalyze both intramolecular and intermolecular hydroamination reactions with high enantioselectivity. The enzyme is tolerant to a variety of cyclizations from six- to seven-membered *endo-trig* lactams with halogens, EDGs, and EWGs **23-7** (**Figure 23**).

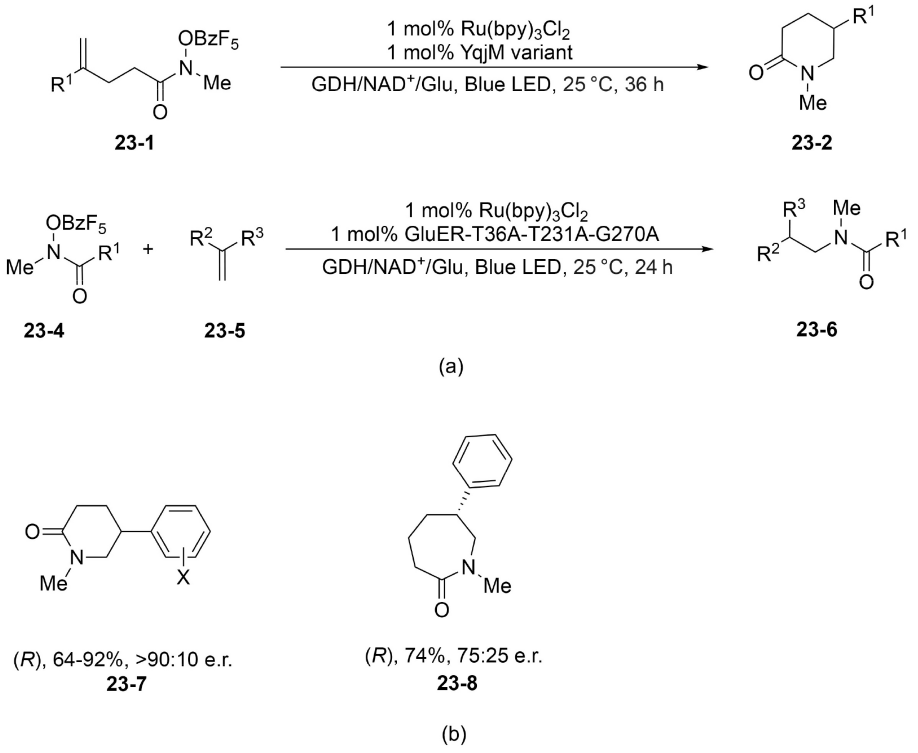

Figure 23. Enantioselective hydroamination. (a) Reaction conditions and (b) Representative reaction scope.

4.2. Hydrosulfonylation Reaction

Hydrosulfonylation reaction is a chemical process that involves the addition of sulfonyl groups to alkenes or alkynes, synthesizing chiral sulfones. In 2023, Xu's team introduced a photoenzymatic approach for radical-mediated stereoselective hydrosulfonylation, employing engineered variants of EREDs as biocatalysts under visible light irradiation. [31] The reaction generates sulfonyl radicals from sulfonyl chloride substrates, then captured by alkenes positioned within the enzyme's active site, adding to the alkenes to form *β*-chiral sulfonyl compounds **24-3**, expanding scope of styrenes with different EDGs and EWGs at meta and para positions **24-4**, heteroaromatic substructure **24-5** (Figure 24).

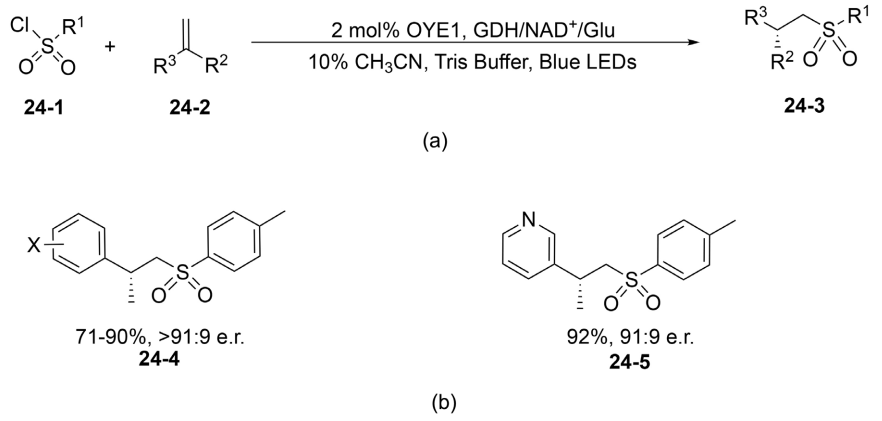

Figure 24. Radical-mediated stereoselective hydrosulfonylation. (a) Reaction conditions and (b) Representative reaction scope.

Another innovative oxidation-initiated photoenzymatic enantioselective hydrosulfonylation of olefins was developed by Ye and co-workers, using a novel mutant of GluER-W100F-W342F without formation of electron donor-acceptor complex and addition of cofactor regeneration mixture. [32] The reaction proceeds through single-electron oxidation to generate sulfonyl radicals, then adding to styrene to form prochiral radicals, followed by HAT to form chiral sulfones **25-3**. The scope accommodates diverse olefins and sulfinates, with olefins bearing different substituents (EDGs and EWGs) across different positions on the aryl ring **25-4** and bulkier groups (naphthyl and benzothienyl). Both aryl sulfinates bearing EDGs (Me, Et, NHAc, and OMe) or EWGs (F, OCF3, and Cl) at the *para* or *meta* position of arenes **25-5** and alkyl sulfinates (Figure 25).

5. Technical Advances

Due to the building interest in the combination of organic reactions and photoenzymatic reactions, efforts have been made to investigate novel approaches to improve the utilization of photoenzymatic reaction under different conditions by testing catalysis, EREDs, etc. Discovering regeneration of active reduced form of cofactor, an example of photoenzymatic selective epoxidation, hydroxylation or halogenation reactions, using two-component-diffusible-flavomonooxygenases,

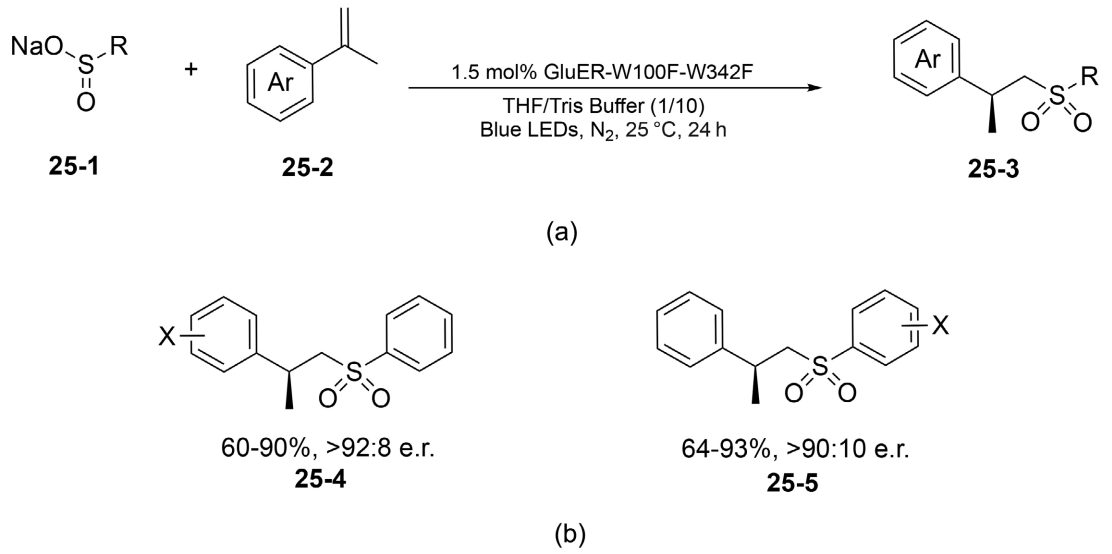

Figure 25. Oxidation-initiated photoenzymatic enantioselective hydrosulfonylation. (a) Reaction conditions and (b) Representative reaction scope.

achieved by direct photochemical regeneration of the reduced form of flavin adenine dinucleotide [33]. Another innovation for regenerating the reduced flavin directly through ketone reduction enables enzymes to catalyze reduction of conjugated C=C double bonds continuously without the use of nicotinamide cofactors [34].

Testing new creations of ERED for the future design, gluconobacter ERED conjugates, covalently linking a light antenna, facilitate efficient energy transfer from the light-harvesting components to the photoenzyme, resulting in a substantial reduction of required light intensity and high yields for both intermolecular and intramolecular reaction [35].

Exploring new use of enzymes, researchers propose the potential development of photoenzymatic reactions, including new reaction pathways and new reactions. For new reaction pathways to offer alternatives, one is the synthesis of hydrocarbon from carboxylic acid with photodecarboxylase from *Chlorella variabilis* NC64A by employing the decoy molecule approach beyond its natural preference for long-chain fatty acid [36] Another new reaction pathway for light-induced electron transfer from histidine to flavin behind the photodecarboxylation catalyzed by lactate monooxygenase [37]. It claimed that other flavoproteins could facilitate light-driven oxidative decarboxylation chemistry if the relationship between substrate, bearing carboxyl groups, and cofactor satisfies certain spatial criteria. In 2019, Yang group demonstrated a novel reaction pathway of generating homolytic S–CH_3 bond cleavage of S-adenosyl-L-methionine (SAM) under low-temperature photoinduced electron transfer with a Radical SAM enzyme could generate a methyl radical directly from SAM. [38] For new reactions, an environmental friendly Hunsdiecker-Borodin-type halodecarboxylation of producing vinyl halides from α,β-unsaturated carboxylic acids with vanadate-dependent chloroperoxidase, serving as biocatalyst, blue-LED, oxygen

and organic solvent in a biphasic system presented the possibility of photoenzymatic generation of electrophilic halide species *in situ* [39].

One innovation for enhancing the conversion of efficient photoenzymatic catalysis is the use of covalent organic frameworks (COFs), achieving a dramatic improvement of NADH regeneration [40]. It transforms two imine-linked COFs into ultrastable and π-conjugated fused-aromatic thieno [3,2-c]pyridine-linked COFs through a postoxidative cyclization process.

6. Conclusion and Outlook

The recent research showcases a significant promotion and attention to the applications of convergence of enzymatic catalysis and photochemistry in photoenzymatic reactions. Such advancements highlighted in this review investigate the catalytic activities of photoenzymes, particularly in the realm of EREDs and photodecarboxylase, expand the scope of reactivities–such as Carbon-Carbon and C-N/S bond formation, alkene functionalization, and C-H activation–and improve efficiency with moderate to high yields and high enantiomeric ratios. The Glu/GDH/$NADP^+$ framework has proven to be a viable pathway for regenerating the reduced cofactor to supply efficient photoenzymatic catalysis, which rarely works in the intramolecular reactions.

Albeit with the developments, we expect the broader use of photoenzymatic reactions in new chemical spaces to furnish alternatives to pressing challenges in sustainable synthesis. For example, the challenging C-C formation involving long aliphatic chain substrates for study of fundamental biological processes relies on metal catalysis to date as used by Zhang *et al.*, photoenzymatic catalysis could be the beneficial and sustainable alternatives for the further investigation. [41] From the perspective of this review, we see several emerging research directions to advance, creating new ERED variants from existing enzymes, exploring more cross-coupling reactions with generating radicals or activating inert chemical bond, investigating the effects of synergistic combination of photoenzymes and organocatalysis, and maximizing light penetration.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Candish, L., Collins, K.D., Cook, G.C., Douglas, J.J., Gómez-Suárez, A., Jolit, A., *et al.* (2021) Photocatalysis in the Life Science Industry. *Chemical Reviews*, **122**, 2907-2980. https://doi.org/10.1021/acs.chemrev.1c00416

[2] Xu, J., Hu, Y., Fan, J., Arkin, M., Li, D., Peng, Y., *et al.* (2019) Light-Driven Kinetic Resolution of α-Functionalized Carboxylic Acids Enabled by an Engineered Fatty Acid Photodecarboxylase. *Angewandte Chemie International Edition*, **58**, 8474-8478. https://doi.org/10.1002/anie.201903165

[3] Santner, P., Szabó, L.K., Chanquia, S.N., Merrild, A.H., Hollmann, F., Kara, S., *et al.*

(2021) Optimization and Engineering of Fatty Acid Photodecarboxylase for Substrate Specificity. *ChemCatChem*, **13**, 4038-4046. https://doi.org/10.1002/cctc.202100840

[4] Dhakshinamoorthy, A., Asiri, A.M. and Garcia, H. (2020) Catalysis in Confined Spaces of Metal Organic Frameworks. *ChemCatChem*, **12**, 4732-4753. https://doi.org/10.1002/cctc.202001188

[5] Ji, P., Feng, X., Oliveres, P., Li, Z., Murakami, A., Wang, C., *et al.* (2019) Strongly Lewis Acidic Metal-Organic Frameworks for Continuous Flow Catalysis. *Journal of the American Chemical Society*, **141**, 14878-14888. https://doi.org/10.1021/jacs.9b07891

[6] Shultz, C.S. and Krska, S.W. (2007) Unlocking the Potential of Asymmetric Hydrogenation at Merck. *Accounts of Chemical Research*, **40**, 1320-326. https://doi.org/10.1021/ar700141v

[7] Lucas, E.L. and Jarvo, E.R. (2017) Stereospecific and Stereoconvergent Cross-Couplings between Alkyl Electrophiles. *Nature Reviews Chemistry*, **1**, Article No. 0065. https://doi.org/10.1038/s41570-017-0065

[8] Li, M., Harrison, W., Zhang, Z., Yuan, Y. and Zhao, H. (2023) Remote Stereocontrol with Azaarenes via Enzymatic Hydrogen Atom Transfer. *Nature Chemistry*, **16**, 277-284. https://doi.org/10.1038/s41557-023-01368-x

[9] Huang, X., Wang, B., Wang, Y., Jiang, G., Feng, J. and Zhao, H. (2020) Photoenzymatic Enantioselective Intermolecular Radical Hydroalkylation. *Nature*, **584**, 69-74. https://doi.org/10.1038/s41586-020-2406-6

[10] Huang, X., Feng, J., Cui, J., Jiang, G., Harrison, W., Zang, X., *et al.* (2022) Photoinduced Chemomimetic Biocatalysis for Enantioselective Intermolecular Radical Conjugate Addition. *Nature Catalysis*, **5**, 586-593. https://doi.org/10.1038/s41929-022-00777-4

[11] Sun, S., Nicholls, B.T., Bain, D., Qiao, T., Page, C.G., Musser, A.J., *et al.* (2023) Enantioselective Decarboxylative Alkylation Using Synergistic Photoenzymatic Catalysis. *Nature Catalysis*, **7**, 35-42. https://doi.org/10.1038/s41929-023-01065-5

[12] Bender, S.G. and Hyster, T.K. (2023) Pyridylmethyl Radicals for Enantioselective Alkene Hydroalkylation Using "Ene"-Reductases. *ACS Catalysis*, **13**, 14680-14684. https://doi.org/10.1021/acscatal.3c03771

[13] Ouyang, Y., Turek-Herman, J., Qiao, T. and Hyster, T.K. (2023) Asymmetric Carbohydroxylation of Alkenes Using Photoenzymatic Catalysis. *Journal of the American Chemical Society*, **145**, 17018-17022. https://doi.org/10.1021/jacs.3c06618

[14] Zhao, B., Feng, J., Yu, L., Xing, Z., Chen, B., Liu, A., *et al.* (2023) Direct Visible-Light-Excited Flavoproteins for Redox-Neutral Asymmetric Radical Hydroarylation. *Nature Catalysis*, **6**, 996-1004. https://doi.org/10.1038/s41929-023-01024-0

[15] Xu, Y., Chen, H., Yu, L., Peng, X., Zhang, J., Xing, Z., *et al.* (2023) A Light-Driven Enzymatic Enantioselective Radical Acylation. *Nature*, **625**, 74-78. https://doi.org/10.1038/s41586-023-06822-x

[16] Page, C.G., Cao, J., Oblinsky, D.G., MacMillan, S.N., Dahagam, S., Lloyd, R.M., *et al.* (2023) Regioselective Radical Alkylation of Arenes Using Evolved Photoenzymes. *Journal of the American Chemical Society*, **145**, 11866-11874. https://doi.org/10.1021/jacs.3c03607

[17] Fu, H., Qiao, T., Carceller, J.M., MacMillan, S.N. and Hyster, T.K. (2023) Asymmetric *C*-Alkylation of Nitroalkanes *via* Enzymatic Photoredox Catalysis. *Journal of the American Chemical Society*, **145**, 787-793. https://doi.org/10.1021/jacs.2c12197

[18] Fu, H., Cao, J., Qiao, T., Qi, Y., Charnock, S.J., Garfinkle, S., *et al.* (2022) An Asymmetric sp^3-sp^3 Cross-Electrophile Coupling Using 'Ene'-Reductases. *Nature*, **610**, 302-307. https://doi.org/10.1038/s41586-022-05167-1

[19] Black, M.J., Biegasiewicz, K.F., Meichan, A.J., Oblinsky, D.G., Kudisch, B., Scholes, G.D., *et al.* (2019) Asymmetric Redox-Neutral Radical Cyclization Catalysed by Flavin-Dependent 'Ene'-Reductases. *Nature Chemistry*, **12**, 71-75. https://doi.org/10.1038/s41557-019-0370-2

[20] Biegasiewicz, K.F., Cooper, S.J., Gao, X., Oblinsky, D.G., Kim, J.H., Garfinkle, S.E., *et al.* (2019) Photoexcitation of Flavoenzymes Enables a Stereoselective Radical Cyclization. *Science*, **364**, 1166-1169. https://doi.org/10.1126/science.aaw1143

[21] Clayman, P.D. and Hyster, T.K. (2020) Photoenzymatic Generation of Unstabilized Alkyl Radicals: An Asymmetric Reductive Cyclization. *Journal of the American Chemical Society*, **142**, 15673-15677. https://doi.org/10.1021/jacs.0c07918

[22] Zhu, C., Yuan, Z., Deng, Z., Yin, D., Zhang, Y., Zhou, J., *et al.* (2023) Photoenzymatic Enantioselective Synthesis of Oxygen-Containing Benzo-Fused Heterocycles. *Angewandte Chemie International Edition*, **62**, e202311762. https://doi.org/10.1002/anie.202311762

[23] Prats Luján, A., Bhat, M.F., Saravanan, T. and Poelarends, G.J. (2022) Chemo- and Enantioselective Photoenzymatic Ketone Reductions Using a Promiscuous Flavin-Dependent Nitroreductase. *ChemCatChem*, **14**, e202200043. https://doi.org/10.1002/cctc.202200043

[24] Sandoval, B.A., Kurtoic, S.I., Chung, M.M., Biegasiewicz, K.F. and Hyster, T.K. (2019) Photoenzymatic Catalysis Enables Radical-Mediated Ketone Reduction in Ene-Reductases. *Angewandte Chemie International Edition*, **58**, 8714-8718. https://doi.org/10.1002/anie.201902005

[25] Nakano, Y., Black, M.J., Meichan, A.J., Sandoval, B.A., Chung, M.M., Biegasiewicz, K.F., *et al.* (2020) Photoenzymatic Hydrogenation of Heteroaromatic Olefins Using 'Ene'-Reductases with Photoredox Catalysts. *Angewandte Chemie International Edition*, **59**, 10484-10488. https://doi.org/10.1002/anie.202003125

[26] Sandoval, B.A., Clayman, P.D., Oblinsky, D.G., Oh, S., Nakano, Y., Bird, M., *et al.* (2020) Photoenzymatic Reductions Enabled by Direct Excitation of Flavin-Dependent "Ene"-Reductases. *Journal of the American Chemical Society*, **143**, 1735-1739. https://doi.org/10.1021/jacs.0c11494

[27] Luján, A.P., Bhat, M.F., Tsaturyan, S., van Merkerk, R., Fu, H. and Poelarends, G.J. (2023) Tailored Photoenzymatic Systems for Selective Reduction of Aliphatic and Aromatic Nitro Compounds Fueled by Light. *Nature Communications*, **14**, Article No. 5442. https://doi.org/10.1038/s41467-023-41194-w

[28] Emmanuel, M.A., Greenberg, N.R., Oblinsky, D.G. and Hyster, T.K. (2016) Accessing Non-Natural Reactivity by Irradiating Nicotinamide-Dependent Enzymes with Light. *Nature*, **540**, 414-417. https://doi.org/10.1038/nature20569

[29] Peng, Y., Wang, Z., Chen, Y., Xu, W., Hu, Y., Chen, Z., *et al.* (2022) Photoinduced Promiscuity of Cyclohexanone Monooxygenase for the Enantioselective Synthesis of α-Fluoroketones. *Angewandte Chemie International Edition*, **61**, e202211199. https://doi.org/10.1002/anie.202211199

[30] Ye, Y., Cao, J., Oblinsky, D.G., Verma, D., Prier, C.K., Scholes, G.D., *et al.* (2022) Using Enzymes to Tame Nitrogen-Centred Radicals for Enantioselective Hydroamination. *Nature Chemistry*, **15**, 206-212. https://doi.org/10.1038/s41557-022-01083-z

[31] Chen, X., Zheng, D., Jiang, L., Wang, Z., Duan, X., Cui, D., *et al.* (2023) Photoen-

zymatic Hydrosulfonylation for the Stereoselective Synthesis of Chiral Sulfones. *Angewandte Chemie International Edition*, **62**, e202218140. https://doi.org/10.1002/anie.202218140

[32] Shi, Q., Kang, X., Liu, Z., Sakthivel, P., Aman, H., Chang, R., *et al.* (2024) Single-Electron Oxidation-Initiated Enantioselective Hydrosulfonylation of Olefins Enabled by Photoenzymatic Catalysis. *Journal of the American Chemical Society*, **146**, 2748-2756. https://doi.org/10.1021/jacs.3c12513

[33] van Schie, M.M.C.H., Paul, C.E., Arends, I.W.C.E. and Hollmann, F. (2019) Photoenzymatic Epoxidation of Styrenes. *Chemical Communications*, **55**, 1790-1792. https://doi.org/10.1039/c8cc08149b

[34] Höfler, G.T., Fernández-Fueyo, E., Pesic, M., Younes, S.H., Choi, E., Kim, Y.H., *et al.* (2018) A Photoenzymatic NADH Regeneration System. *ChemBioChem*, **19**, 2344-2347. https://doi.org/10.1002/cbic.201800530

[35] Cesana, P.T., Page, C.G., Harris, D., Emmanuel, M.A., Hyster, T.K. and Schlau-Cohen, G.S. (2022) Photoenzymatic Catalysis in a New Light: *Gluconobacter* "Ene"-Reductase Conjugates Possessing High-Energy Reactivity with Tunable Low-Energy Excitation. *Journal of the American Chemical Society*, **144**, 17516-17521. https://doi.org/10.1021/jacs.2c06344

[36] Zhang, W., Ma, M., Huijbers, M.M.E., Filonenko, G.A., Pidko, E.A., van Schie, M., *et al.* (2019) Hydrocarbon Synthesis via Photoenzymatic Decarboxylation of Carboxylic Acids. *Journal of the American Chemical Society*, **141**, 3116-3120. https://doi.org/10.1021/jacs.8b12282

[37] Li, X., Page, C.G., Zanetti-Polzi, L., Kalra, A.P., Oblinsky, D.G., Daidone, I., *et al.* (2023) Mechanism and Dynamics of Photodecarboxylation Catalyzed by Lactate Monooxygenase. *Journal of the American Chemical Society*, **145**, 13232-13240. https://doi.org/10.1021/jacs.3c02446

[38] Yang, H., Impano, S., Shepard, E.M., James, C.D., Broderick, W.E., Broderick, J.B., *et al.* (2019) Photoinduced Electron Transfer in a Radical SAM Enzyme Generates an *S*-Adenosylmethionine Derived Methyl Radical. *Journal of the American Chemical Society*, **141**, 16117-16124. https://doi.org/10.1021/jacs.9b08541

[39] Zippilli, C., Bartolome, M.J., Hilberath, T., Botta, L., Hollmann, F. and Saladino, R. (2022) A Photochemoenzymatic Hunsdiecker-Borodin-Type Halodecarboxylation of Ferulic Acid. *ChemBioChem*, **23**, e202200367. https://doi.org/10.1002/cbic.202200367

[40] Wang, Y., Liu, H., Pan, Q., Wu, C., Hao, W., Xu, J., *et al.* (2020) Construction of Fully Conjugated Covalent Organic Frameworks via Facile Linkage Conversion for Efficient Photoenzymatic Catalysis. *Journal of the American Chemical Society*, **142**, 5958-5963. https://doi.org/10.1021/jacs.0c00923

[41] Zhang, B., Yu, Y., Fox, B.W., Liu, Y., Thirumalaikumar, V.P., Skirycz, A., *et al.* (2024) Amino Acid and Protein Specificity of Protein Fatty Acylation in *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the United States of America*, **121**, e2307515121. https://doi.org/10.1073/pnas.2307515121