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Stereoconvergent Reduction of Activated Alkenes by a Nicotinamide Free Synergistic Photobiocatalytic System

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ABSTRACT: There is a growing interest in developing cooperative chemoenzymatic reactions to harness the reactivity of chemical catalysts and the selectivity of enzymes for the synthesis of non-racemic chiral compounds. However, existing chemoenzymatic systems with more than one chemical reaction and one enzymatic reaction working cooperatively are rare. Moreover, the application of oxidoreductases in cooperative chemoenzymatic reactions is limited by the necessity of using expensive and unstable redox equivalents such as nicotinamide cofactors. Here we report a light-driven cooperative chemoenzymatic system comprised of a photoinduced electron transfer reaction (PET) and a photosensitized energy transfer reaction (PEnT) with enzymatic reduction in one-pot to synthesize chiral building blocks of bioactive compounds. As a proof of concept, ene-reductase was directly regenerated by PET in the absence of external cofactors. Meanwhile, enzymatic reduction worked cooperatively with photocatalyst-catalyzed energy transfer that continuously replenished the reactive isomer from the less reactive one. The whole system stereoconvergently reduced *E/Z* mixtures of alkenes to the enantiopure products. Additionally, enantioselective enzymatic reduction worked competitively with photocatalyst-catalyzed racemic background reaction and side reactions to channel the overall electron flow to the single enantiopure product. Such a light-driven cooperative chemoenzymatic system holds great potential for asymmetric synthesis using inexpensive petroleum or biomass derived alkenes.

Enzymes have been increasingly used as catalysts for chemical synthesis in both academia and industry owing to their high selectivity and mild reaction conditions.[1-4] Notably, there is a growing interest in developing cooperative chemoenzymatic processes that combine the high selectivity of enzymes with the diverse reactivity of chemical catalysts.[5-10] Unlike building artificial multistep enzymatic reactions, performing cooperative chemoenzymatic synthesis is challenging because chemical catalysis and biocatalysis are generally conducted under very different conditions. Up to now, cooperative chemoenzymatic reactions normally consist of a reversible reaction catalyzed by a chemical catalyst in combination with an irreversible enzymatic reaction (Figure 1a), such as dynamic kinetic resolution of amines and alcohols[5] or chemical catalysts used to regenerate the nicotinamide cofactors NAD(P)H of enzymes (Figure 1b). The transformation with multiple cooperative chemoenzymatic reactions is rare (Figure 1c),[8] but attractive since it largely mimics the simultaneous reactions catalyzed by compatible and selective enzymes to synthesize complex metabolites in vivo in a highly efficient manner[11] and has great potential to precisely control the selective synthesis of abiotic chemicals in a clean and cost-effective way.[12] However, compared with the single cooperative chemoenzymatic reaction, it is more challenging to deal with compatability issues arising from multilple chemical catalysts and enzymes in one-pot and optimize the system by increasing the selectivity of the desired products and reducing the formation of side products.

Recently, we developed a cooperative photoenzymatic system by coupling alkene isomerization catalyzed by a

photocatalyst with enzymatic reduction catalyzed by an oxidoreductase called ene-reductase (ER) to synthesize enantiopure products.[13] Although oxidoreductases are versatile catalysts for asymmetric synthesis due to their intrinsic enantioselectivity and specificity,[14] their application in cooperative chemoenzymatic transformation is limited by using expensive and unstable nicotinamide cofactors such as reduced nicotinamide adenine dinucleotide (phosphate), NAD(P)H.[15] Various methods such as enzymatic, chemical, electrochemical, and photochemical approaches have been developed to regenerate the reduced nicotinamide cofactors.[16-17] Among them, the enzymatic method is still the only strategy industrially employed due to its high turnover frequency and is the most common methods used in chemoenzymatic transformations to regenerate the oxidoreductases.[7, 13] Inspired by recent works using photochemical strategies to directly activate oxidoreductase without involving NAD(P)H,[18-25] we aimed to develop a chemoenzymatic system consisting of multiple reactions working synergistically to stereoconvergently reduce a mixture of alkenes to a single enantiopure product without using nicotinamide cofactors (Figure 1c & d).

In this study, we describe a photobiocatalytic system that consists of multiple reactions working in a synergistic manner: photoinduced electron transfer (PET) for the regeneration of the ligand of ER, flavin mononucleotide ($FMNH^-$), photosensitized energy transfer (PEnT) for alkene isomerization from the less reactive one (typically *Z*-alkenes) to reactive isomer (typically *E*-alkenes), and ER-catalysed stereoconvergent reduction of C=C bond. Meanwhile, the enzymatic reduction works competitively with the photocatalyst-catalysed racemic background reduction and other side reactions to ensure high yields and high enantiomeric excess of the reduced products. This represents a novel cooperative chemoenzymatic system in which more than one chemical reaction works cooperatively with enzymatic reaction, and chemical catalysis and enzymatic catalysis benefit from each other simultaneously.

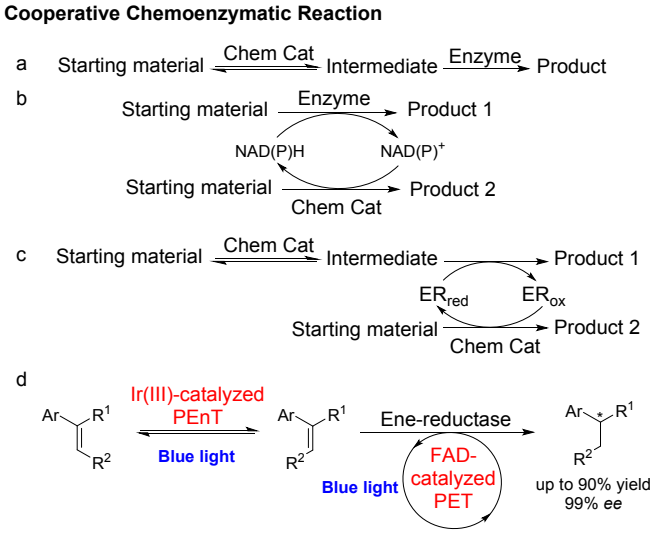


Figure 1. Cooperative chemoenzymatic reactions. a-c, Types of cooperative chemoenzymatic reactions. ER is the abbreviation of ene-reductase. "red" and "ox" indicate the reduction and oxidation state of enzyme. **d**, Combination of PET, PEnT, and enzymatic reduction of alkenes without using NAD(P)H. The asterisk indicates the chiral center; Ar, aryl; Chem Cat, chemical catalyst; PEnT, photosensitized energy transfer reaction; PET, photoinduced electron transfer reaction

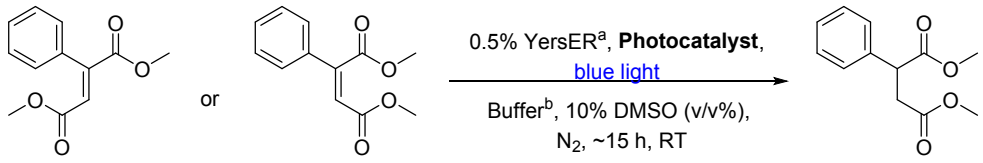
As a proof of concept, we sought to build a light-driven stereoconvergent reduction of an isomeric mixture of acceptor-substituted alkenes. Model substrate 2-phenylbut-2-enedioic acid dimethyl ester **1** was used to set up reactions to investigate suitable photocatalysts and electron donors for the direct regeneration of FMN by PET without compromising ERs' selectivity (Table S1). YersER was used since it exclusively reduced (*E*)-**1** to dimethyl 2-phenylsuccinate (**1p**) in high yield with excellent enantiomeric excess (*ee*) in the presence of a glucose dehydrogenase (GDH), $NADP^+$, and glucose for FMN regeneration.[26] Notably, although Eosin Y[27] was used to activate ER for asymmetric reduction of 2-methylcyclohexone under an aerobic condition[19], it cannot efficiently regenerate YersER for the more challenging substrate (*E*)-**1**(Table 1, entry 1).[28] Additionally, the excited state of Eosin Y is a strong reductant and can directly reduce activated C=C bond to the racemic products,[29] which interfered with the enantioselective enzymatic reduction step (entries 2-4 & 11). Unlike Eosin Y, flavins, especially flavin adenine dinucleotide (FAD) could reactivate YersER in the presence of electron donor ethylenediaminetetraacetic acid (EDTA) to reduce (*E*)-**1** to **1p** in high yield with excellent *ee* (entry 5) comparable to the enzymatic reduction with well-developed GDH catalyzed NADPH regeneration system (Table S1).[13] However, the enzymatic system with FAD alone was not able to reduce (*Z*)-**1** to **1p** in high yield (entry 12) because very few (Z)-**1** was isomerized to (*E*)-**1** in the presence of the electron donor EDTA (entries 13-14). This might be because in the presence of EDTA, the photoexcited FAD preferred to catalyze the photoredox reaction rather than the energy transfer reaction.[30-32] Differently, 1% cationic iridium complex $[Ir(dmppy)_2(dtbbpy)]PF_6$ (**Ir-16**) could isomerize (*Z*)-**1** to (*E*)-**1** efficiently in the presence of EDTA through PEnT mechanism (Figure S4, S5 & S14), but it could not regenerate $FMNH^-$ of YersER under blue light illumination (entries 7-8 & 15-16). The enzymatic system with 9.4% FAD and 1% (**Ir-16**) could efficiently reduce (*Z*)-**1** to **1p** with 99% *ee* within 12 hours (entry 17, Figure S9), where FAD executed electron transfer to regenerate $FMNH^-$ and **Ir-16** isomerized (*Z*)-**1** to (*E*)-**1** in the presence of blue light. Little or no products were produced when the reactions were performed without YersER but under blue light (entry 18) or in the dark condition (entry 19). We further tested the stability of photocatalysts by increasing the concentration of (*Z*)-**1** up to 20 mM while keeping the total amount of photocatalysts (0.47 mM FAD and 0.05 mM **Ir-16**) and enzyme (0.025 mM) unchanged. The yield of **1p** dropped to 26% and all unreduced **1** was *E* isomers (Table S4). Adding more enzymes or FAD in the system after 24 hours did not improve the yield further, which indicated the degradation and damage of YersER and FAD, respectively. However, the low yield was mainly due to the low turnover

rate of YersER. Further increasing the YersER to 0.1 mM improved the yield to 68% (Table S4). The more detailed discussion can be found in SI.

Next, we evaluated the performance of the light-driven enzymatic reduction system with 12 additional 2-aryl substituted alkenes and their corresponding ERs[13] that preferentially reduce *E* isomers of **2** to **12** and *Z* isomers of **13** to the enantioenriched reduced product. A successful light-driven enzymatic reduction system should consist of a photocatalyst and an electron donor that can: 1) quickly regenerate the enzymatic cofactor FMN without interfering with enzymatic activity and selectivity; 2) isomerize the less reactive isomer to the more reactive isomer; and 3) ensure appropriate reaction kinetics where the cooperative enzymatic reduction step must be much faster than the background reactions including FAD catalyzed alkene reduction. Therefore, we first tested the light-driven enzymatic reduction on additional reactive isomers (Table S2) and the isomerization of less reactive isomers in the presence of FAD, **Ir-16**, and EDTA or TEOA under the blue light, respectively (Table S3). For all tested ERs, FAD could efficiently catalyze PET to regenerate ER-FMN_{red} for enantioselective reduction of reactive alkene isomers at high yields and *ee*'s. For all tested substrates, less reactive isomers could be isomerized to active isomers under the blue light and in the presence of FAD. However, additional **Ir-16** was still preferred to achieve faster isomerization and obtain more active isomers at the photostationary phase.

Although it is not straightforward to measure the kinetics of every chemical reaction occurring in the system, enzymatic reduction of less reactive isomers, either *Z* or *E*, to enantiopure products with high yields and *ee*'s could be a strong proof of the above-mentioned criteria 3 for a successful light-driven enzymatic reduction system. As shown in Figure 2, our system could convert unreactive *Z* isomers of aryl-diesters with either electron withdrawing groups (*Z*)-**2** and (*Z*)-**3** or electron donating group (*Z*)-**4** to reduced products **2p, 3p** and **4p** with high yields and at good to excellent *ee*'s with FAD and Ir-16 as photocatalysts, EDTA as electron donors and different ERs. The light-driven enzymatic system would also give high yields and enantioselectivities with alkenes containing diverse combinations of functional groups, including β-cyano-α,β unsaturated ester (*Z*)-**6**, α-cyano-α,β unsaturated ester (*Z*)-**7** and (*E/Z*)-**8**, cyanoketone (*Z*)-**9**, β-ketone-α,β unsaturated ester (*Z*)-**10**, α-ketone-α,β unsaturated ester (*Z*)-**11**, amidoacrylate (*Z*)-**12** and trifluoromethylcyanate (*E*)-**13**. Except for (*Z*)-**6**, for which $CaCl_2$ in TEOA solution was used as electron donor, all other reactions were performed in 100 mM sodium phosphate buffer at pH 7.4, with EDTA as the electron donor. For (*Z*)-**10**, $CaCl_2$ in TEOA solution can result in the reduced product at relatively lower yield 65% but excellent *ee* (>99%). (*E/Z*)-**5p** can undergo enzymatic reduction with FAD as the single photocatalyst by using EDTA as the electron donor and under blue light irradiation. Without ene-reductases, racemates and side products were generated for most of the substrates. The enantioenriched products that were obtained from the light-driven enzymatic reduction system have the potential to be transformed into a variety of biologically active molecules and valuable synthetic intermediates as illustrated in our previous study.[13]

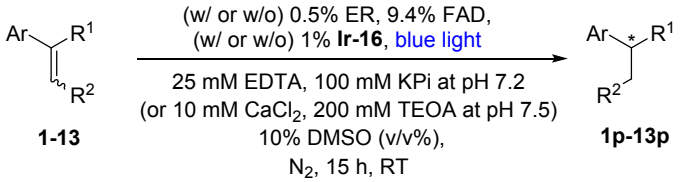
Table 1. Reaction development and condition optimization

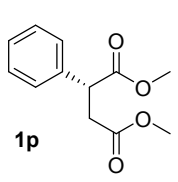


(*E*)-**1** (*Z*)-**1** (*R*)-**1p**

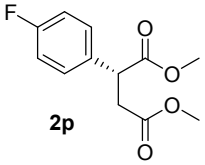
Entry	Substrate	Enzyme	Photocatalyst	e⁻ donor	Yield of **1p**	*ee* of **1p**	*E/Z*[c]
1[d]	(*E*)-**1**	YersER	1% Eosin Y	TEOA	34%	-	-
2	(*E*)-**1**	YersER	1% Eosin Y	TEOA	75%	63%	-
3	(*E*)-**1**	YersER	9.4% Eosin Y	TEOA	60%	47%	
4	(*E*)-**1**	-	1% Eosin Y	TEOA	78%	0%	-
5	(*E*)-**1**	YersER	9.4% FAD	EDTA	86%	99%	-
6	(*E*)-**1**	-	9.4% FAD	EDTA	0%	-	-
7	(*E*)-**1**	YersER	1% **Ir-16**	EDTA	0%	-	83:17
8	(*E*)-**1**	-	1% **Ir-16**	EDTA	0%	-	82:18
9	(*E*)-**1**	YersER	9.4% FAD & 1% **Ir-16**	EDTA	74%	99%	-
10	(*E*)-**1**	-	9.4% FAD & 1% **Ir-16**	EDTA	3%	-	88:12
11	(*Z*)-**1**	YersER	9.4% Eosin Y	TEOA	50%	4%	-
12	(*Z*)-**1**	YersER	9.4% FAD	EDTA	40%	43%	0
13	(*Z*)-**1**	-	9.4% FAD	EDTA	0%	-	7:93
14	(*Z*)-**1**	-	9.4% FAD	-	0%	-	83:17
15	(*Z*)-**1**	YersER	1% **Ir-16**	EDTA	0%	-	82:18
16	(*Z*)-**1**	-	1% **Ir-16**	EDTA	0%	-	85:15
17	(*Z*)-**1**	YersER	9.4% FAD & 1% **Ir-16**	EDTA	88%	99%	-
18	(*Z*)-**1**	-	9.4% FAD & 1% **Ir-16**	EDTA	2%	-	85:15
19[e]	(*Z*)-**1**	-	various	EDTA	0%	-	0

[a]Without specifications, all % means molar percentage, [b]Solution with 200 mM triethanolamine (TEOA), 10 mM $CaCl_2$ at pH 7.5 or solution with 25 mM EDTA, 100 mM sodium phosphate buffer (KPi), at pH 7.2. [c]The *E/Z* ratio of the remaining substrates after reaction. [d]Aerobic reaction. [e]Control reactions were performed under dark with FAD, **Ir-16** or FAD & **Ir-16**, respectively.

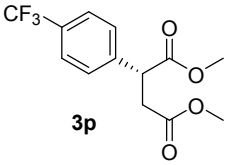




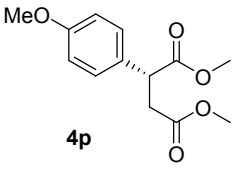
FAD & **Ir-16**, EDTA
YersER: 82% yield, 99% *ee*
w/o: 3% yield, 64% conv.



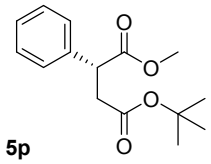
FAD & **Ir-16**, EDTA
XenB: 85% yield, 99% *ee*
w/o: 11% yield, 35% conv.



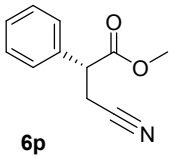
FAD & **Ir-16,** EDTA
YersER: 83% yield, 95% *ee*
w/o: 2% yield, 10% conv.



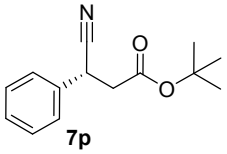
FAD & **Ir-16**, EDTA
YersER: 80% yield, 98% ee
w/o: 0% yield, 0% conv.



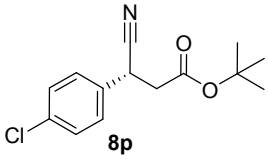
FAD, EDTA
OPR1: 77% yield, >99% *ee*
w/o: 0% yield, 67% conv.



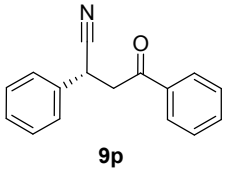
FAD & **Ir-16**, TEOA
TOYE: 64% yield, 93% *ee*
w/o: 14% yield, 74% conv.



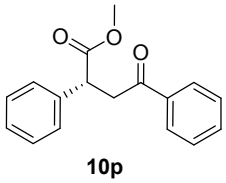
FAD & **Ir-16**, EDTA
OYE2: 94% yield, 95% *ee*
w/o: 9% yield, 96% conv.



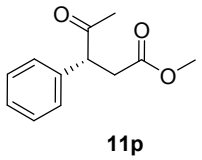
FAD & **Ir-16**, EDTA
OYE2: 82% yield, 90% *ee*
w/o: 6% yield, 53% conv.



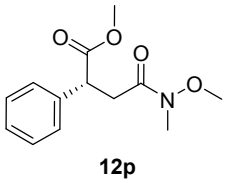
FAD & **Ir-16**, EDTA
OYE2: 78% yield, 92% *ee*
w/o: 40% yield, 60% conv.



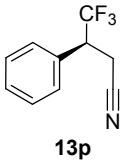
FAD & **Ir-16**, EDTA
OPR1: 92% yield, 93% *ee*
w/o: 79% yield, 95% conv.



FAD & **Ir-16**, EDTA
YersER: 74% yield, 91% *ee*
w/o: 3% yield, 24% conv.



FAD & **Ir-16**, EDTA
OPR1: 60% yield, >99% *ee*
w/o: 7% yield, 100% conv.



FAD & **Ir-16**, EDTA
OYE2: 78% yield, 89% *ee*
w/o: 1% yield,76% conv.

Figure 2. Substrate scope of light-driven, photoenzymatic reduction of less or non-reactive alkenes of isomers to enantioenriched products. Substrate **5** is a mixture of isomers with 62:38 *E/Z* ratio and **8** is a mixture of isomers with 23:77 *E/Z* ratio where *Z* isomers cannot be reduced by ERs for both cases. The starting material of **13** is the *E* isomer. The starting materials of other substrates are *Z* isomers. The yields of **7p**, **8p**, and **13p** were determined by NMR, while the yields of others were determined by GC-MS. Abbreviations: w/o: without adding enzymes; conv.: conversion.

Our light-driven cooperative enzymatic system is able to fully convert non-reactive or less reactive alkene isomers that are usually the major products from organic synthesis to enantiopure products without using external cofactors. It is also able to stereoconvergently reduce mixtures of *E/Z* alkenes in the tested substrates, such as a 62:38 *E/Z* mixture of **5** and 23:77 *E/Z* mixture of **8**, to the reduced products with high yields and *ee*'s. In the presence of an electron donor, more than half of the substrates only have 60% or less reactive isomers generated at the photostationary phase. The cooperative enzymatic reductions of those substrates such as (*Z*)-**7** and (*E*)-**13** shown in Figure 3 highlighted the advantage of a cooperative system over two sequential reactions. The photoisomerization of non-reactive isomer (*Z*)-**7** or (*E*)-**13** with **Ir-16** resulted in *E/Z* mixtures in which active isomers were less than 10%. As a result, low yields were observed if isomerization and reduction were performed in a sequential manner (Figure 3). In this study, ERs preferentially reduce *E* isomers rather than *Z* isomers to the enantioenriched products with the R configuration for most cases, but OYE2 selectively reduces (*Z*)-**13** to (*S*)-**13p** with 89% *ee.* So, another advantage of the

cooperative system is that the dynamic equilibrium enables the isomerization of either *E* or *Z* isomer with simultaneous enzymatic reduction of the *Z* or *E* respectively to the enantioenriched product in high yields.

Furthermore, in our light-driven cooperative enzymatic system, the enzymatic reduction works not only cooperatively with the photocatalyst-catalyzed isomerization reaction (Figure 4, route 2), but also competitively with photoinduced racemic reduction (route 1) and other background reactions (Supporting Information GC-MS traces). Under illumination, FAD is excited to high-redox-potential FAD* that is capable of oxidizing EDTA to form semi-quinones, FADH·. Synproportionation of two FADH· generates fully reduced flavin $FADH_2$ that could proportionate with FMN further in ene-reductase to regenerate ER-FMNH⁻ (route 2, Figures S13).[33-34] $FADH_2$ is a moderate reductant ($E_{1/2}$ = ~ -0.6 V versus saturated calomel electrode (SCE))[35-36] and thermodynamically unable to transfer electron(s) to the substrate ($E_{1/2}$ = -1.055 V and -1.315 versus SCE for (*Z*)-**9** and (*Z*)-**10**, respectively). However, under blue light, FAD mediated photoreduction of activated alkenes may occur with electron donor and result in the reduced racemic products[37] (Figure 2, **9p** and **10p**). Additionally, without adding ERs, the substrate is relatively unstable in the presence of the photocatalysts and blue light irradiation. Most of the substrates were consumed with less than 10% racemic target products generated (Figure 2, **1p**, **5-8p** & **10p**). Based on the time course study of light-driven cooperative enzymatic reduction of (*Z*)-**1** versus reactions without YersER (Figure S9 & S10), It showed that the rate of route 2 was much faster than that of route 1 and other background reactions, which ensure the high yield and *ee* of the reduced product. Generally speaking, with ERs, all other substrates should be also preferentially reduced by the regenerated ER-$FMNH_2$ into enantioenriched products through hydride transfer mechanism (route 2 & Figure 2).[38]

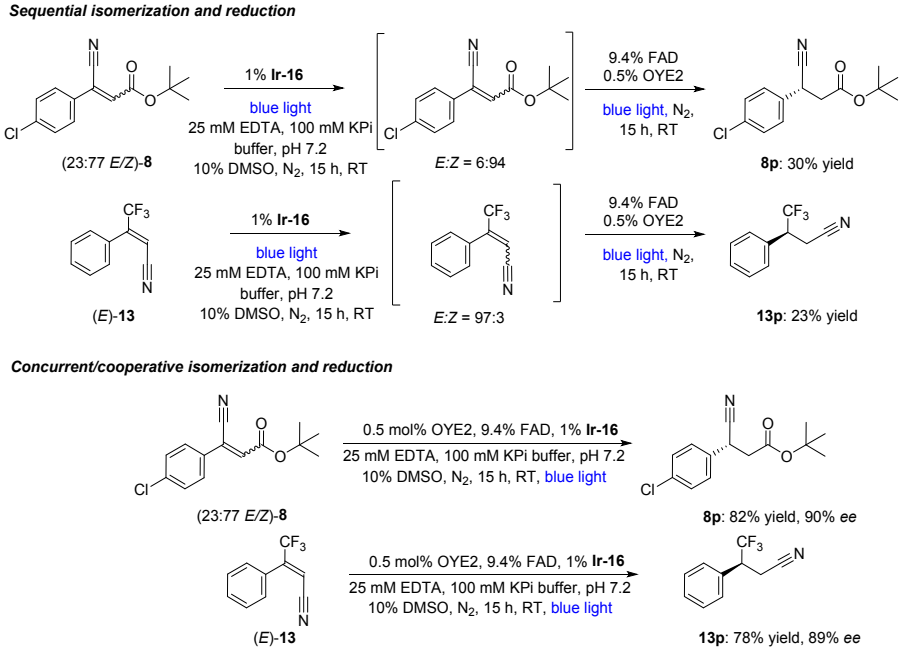


Figure 3. Comparison of sequential isomerization and enzymatic reduction versus cooperative isomerization and reduction.

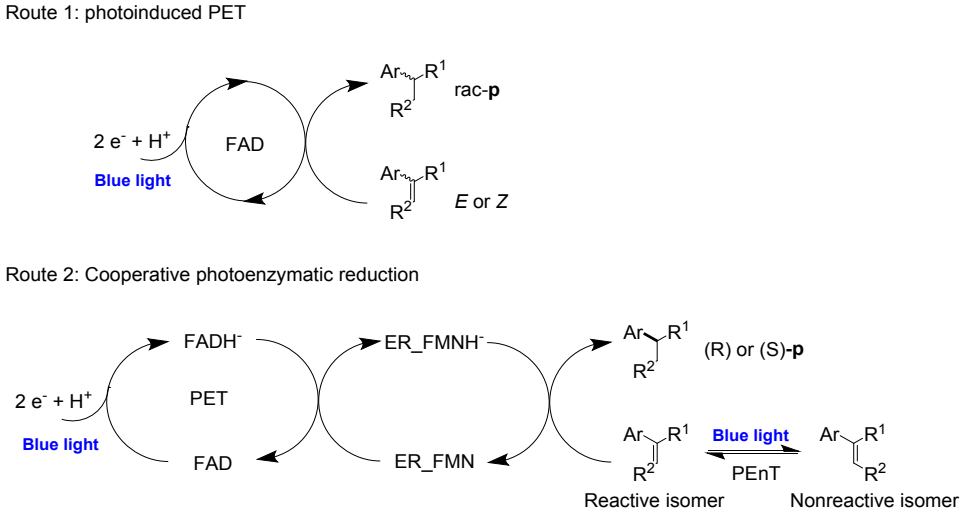


Figure 4. Proposed pathways: FAD-catalyzed directed electron transfer illustrated in route 1 versus cooperative photoenzymatic reduction illustrated in route 2.

In conclusion, we developed a light-driven cooperative chemoenzymatic system to synthesize value-added chiral molecules from a mixture of alkenes without the use of expensive external nicotinamide cofactors. Under blue light irradiation, FAD regenerates $FMNH^-$ ERs by directly transferring electrons from cheap electron donors to the FMN, and **Ir-16** isomerizes the unreactive isomer to the reactive isomer for enzymatic reduction through the energy transfer machinery. Meanwhile, cooperative enzymatic reduction competes with photocatalyst-catalyzed direct background racemic reduction and other side reactions to ensure the high yields and *ee*'s of the products. More generally, this study demonstrates the feasibility of building cooperative chemoenzymatic reactions consisting of more than one chemical reaction and one enzymatic reaction, as well as the potential of combining photocatalysts with oxidoreductases to develop a wide range of new cooperative chemoenzymatic transformations without adding extra expensive nicotinamide cofactors.

ASSOCIATED CONTENT

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Author Contributions

YW and HZ conceived the work, designed and conducted experiments, interpreted the data and wrote the manuscript. HS, XH, and LV performed partial of the experiments. All authors reviewed and approved the manuscript.

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Figures S1-S14 and Tables S1-S4 , materials and methods, detailed optimization studies, experimental procedures, mechanistic rationales, GC-MS spectra, HPLC spectra and NMR spectra.

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