Photoenzymatically-Induced Asymmetric Hydroarylation of Alkenes with (Hetero)aryl Halides

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Abstract

Herein we describe a set of privileged and stereocomplementary ene-reductase enzymes which, when induced by light and aided by an exogenous photocatalyst, catalyze the coupling of (hetero)aryl halides and alkenes in an asymmetric intermolecular hydroarylation process. Thus, carbon scaffolds containing C(sp^2)-C(sp^3) bonds are synthesized enzymatically from simple precursors in excellent enantiomeric excess. Furthermore, an intramolecular coupling is achieved through tethering of (hetero)aryl halides to their alkene reaction partners and in this manner problematic side reactions are suppressed and yields improved. This work extends the utility of photo-induced biocatalysis through the addition of the novel and pharmaceutically important (hetero)aromatic halide class of radical precursors.

Introduction

Stereodefined C(sp^2)-C(sp^3) bonds are ubiquitous in bioactive pharmaceutical and agrochemical compounds (Figure 1)[1, 2] with numerous methods of synthesis reported.[3,4] Of these approaches, the direct asymmetric coupling of (hetero)aryl halides and alkenes is particularly attractive due to its efficiency and broad starting material availability. Chemically, enantioselective hydro(hetero)arylations of alkenes have been accessed through chiral Brønsted acid[5] and, more commonly, metal catalysis.[6, 7] While these approaches are powerful, they do not offer the unique benefits of biocatalysis,[8, 9] particularly enzyme engineering for improved reaction performance.[10,11] To the best of our knowledge, enzyme-catalyzed examples of this reaction class are limited to the impressive recent work of Zhao *et al.* who employed single-electron oxidation of electron-rich (hetero)arenes by visible-light-excited flavoproteins and subsequent asymmetric radical hydroarylation of alkenes.[12] A limitation of the approach, however, is the inherent substrate-controlled regioselectivity, which may not allow alkylation of different C-H positions on the (hetero)arene. Furthermore, in general, the position of reactivity of aryl radical intermediates generated by single-electron oxidation can be difficult to predict *a priori.*[13] Other examples of molecular scaffold building through enzymatic (sp^2)-(sp^3) carbon-carbon bond formation[14] include the use of engineered tryptophan synthases,[15] phenyl transferases,[16] ene-reductases,[17] CylK alkylating enzymes[18] and methyl transferases.[19] Whilst highly enabling for their respective products, these transformations are generally limited to narrow substrate scopes and do not employ (hetero)aryl halides as coupling partners. The extension of biocatalytic methods to include the utilization of (hetero)aryl halides as coupling partners

has the potential to overcome many of these drawbacks, allowing access to the wide diversity and halide-controlled regioselectivity of this broad set of chemical building blocks.[20]

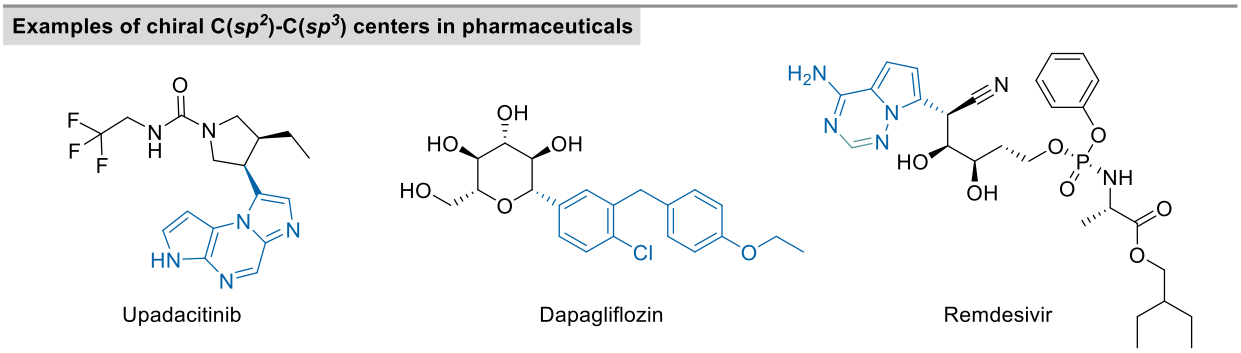


Figure 1: Selected examples of stereo-defined (hetero)aromatic-C(sp^3) structure in drug products.

Groundbreaking work by Hyster and co-workers has shown that flavin-dependent ene-reductases (EREDs) are able to catalyze promiscuous radical reactions in which the flavin hydroquinone co-factor acts as a single electron reductant. This strategy has been applied to the coupling of alkenes with a variety of radical precursors including α-halo esters and amides,[21-24] ketones,[25] alkyl iodides,[26] redox active esters,[26, 27] vinyl pyridines,[28] aza-benzyl halides,[29, 30] hydroxamic esters,[31, 32] and O-benzoyl hydroxylamines[33] (Figure 2A). Whilst (hetero)aryl halides have not yet been described as substrates in enzyme-induced hydroarylation reactions, it is noteworthy that their reduction potentials typically fall within or close to the range of established radical precursors (Figure 2B). Work by Jui and co-workers[34-37] has demonstrated that the desired (hetero)aryl radicals can be generated chemically through a photocatalyst-mediated single-electron reduction of aryl halides. These aryl radicals are versatile synthetic intermediates[38] which engage in radical alkene addition reactions to form new C(sp^2)-C(sp^3) bonds,[34-37] though an enantioselective version of this coupling does not exist to our knowledge. To this end, we envisaged that flavin-dependent ene-reductases may induce activation of (hetero)aryl halides to their corresponding electrophilic radical intermediates with subsequent addition across alkenes (Figure 2C). The activation could potentially be achieved either through irradiation of a charge-transfer complex formed between the reactants and the flavin hydroquinone,[21, 23] direct excitation of the flavin co-factor,[39] or with the aid of an exogeneous photocatalyst.[25, 31] Herein we report the establishment of (hetero)aryl halides as radical precursors for photo-induced biocatalysis, demonstrating their coupling to alkenes in asymmetric intermolecular and intramolecular hydroarylation processes.

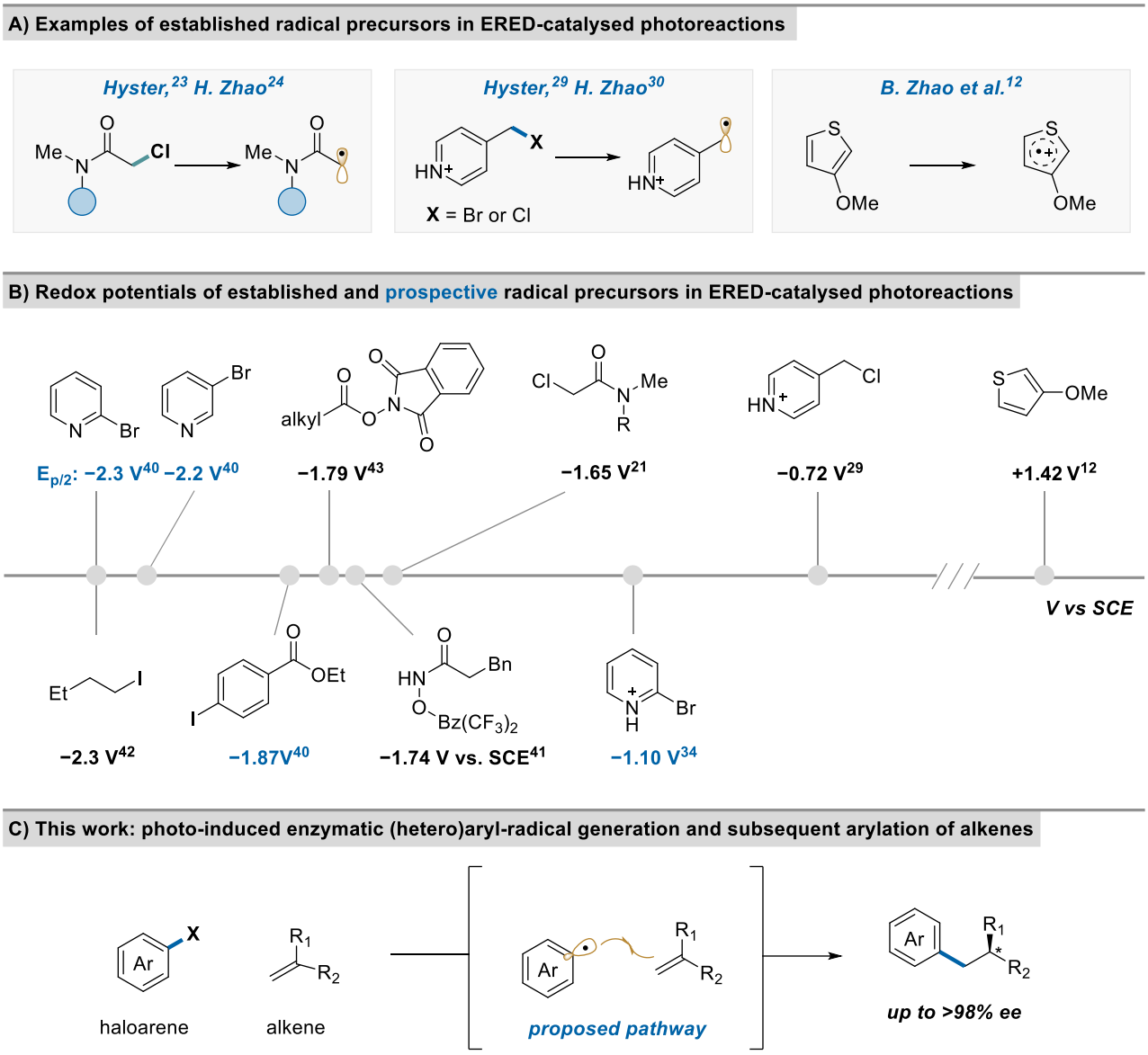
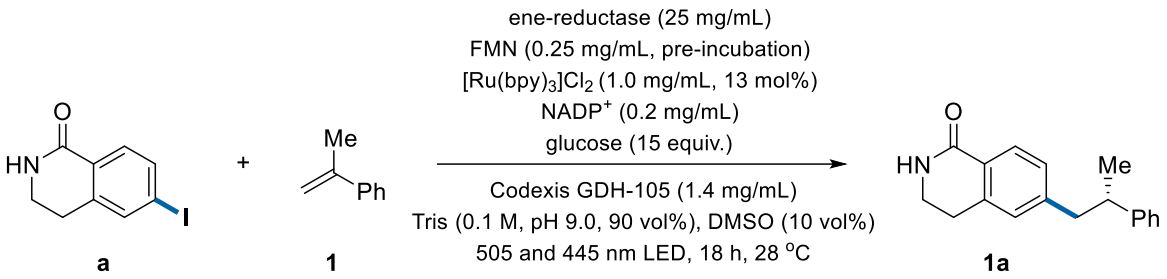


Figure 2: Established and novel photo-induced enzymatic reactions.

Results and discussion

Through initial investigations, we identified iodophenyl lactam **(a)** as a potential radical precursor in the desired intermolecular hydroarylation process utilizing *α*-methylstyrene **1** as a model radical acceptor and an ene-reductase as catalyst (see SI for details). An exogeneous photocatalyst, $[Ru(bpy)_3]Cl_2$, was required to achieve measurable product generation. In order to establish whether the reactivity was indeed enzyme-induced, rather than a non-enzymatic background reaction, we sought to determine the reaction product's enantiomeric excess. Pleasingly, with initial optimization including the choice of ene-reductase, we established that the (*S*)-enantiomer of the desired product could be synthesized in 78% ee using 12-oxo-phytodienoic acid reductase from *Zea mays* (OPR1) and the (*R*)-enantiomer could be generated in 62% ee employing GluER-T36A-W66L (Table 1). With enantioselectivity established, an enzyme-induced pathway was confirmed.



Entry	ene-reductase	%Yield of 1a*	% ee (*R*/*S*)†	LED (nm)
1	OPR1	11%	78% (*S*)	445 (blue)
2	GluER-T36A-W66L	7%	62% (*R*)	505 (cyan)
3	YersER	14%	16% (*S*)	445
4	None	0%	N/A	505/445

*Table 1. Photo-induced enzymatic hydroarylation of α-methylstyrene **1** with aryl iodide **a**. Conditions: aryl halide **a** (10 mM) and alkene **1** (30 mM), ERED (25 mg/mL), NADP+ (0.2 mg/mL), Codexis GDH-105 (1.4 mg/mL), glucose (27 mg/mL, 15 eq.), [Ru(bpy)$_3$]Cl$_2$ (1.0 mg/mL, 13 mol%), DMSO (10 vol%), and buffer (0.1M TRIS pH 9.0, 90 vol% to a total reaction volume of 200 µL). Reaction irradiated for 18 h at an internal reaction temperature of 28°C. ERED incubated in a solution FMN cofactor prior to reaction and made up to a final reaction concentration of 0.25 mg/mL (see SI for details). *†See SI for method(s) of yield and ee determination through calibrated chiral SFC-UV-(MS) and details of reaction equipment set-up. Absolute determination of stereoconfiguration by comparison to independently synthesized enantiopure product (R)-**1a** (see SI for further details). N/A = not applicable. OPR1: 12-oxo-phytodienoic acid reductase from Zea mays; GluER-T36A-W66L: alkene reductase from Gluconobacter oxydans mutant T36A-W66L; YersER: alkene reductase from Yersenia bercovieri.*

Next, seeking to improve enantioselectivity and employ a substrate class of high importance to the synthesis of pharmacologically active compounds,[44] we investigated the performance of pyridyl and related heteroaryl halides. Recently, both Hyster[29] and Zhao[30] reported that benzylic radicals of N-heterocycles could be generated within an ERED active site, postulating that the binding of a basic heterocyclic nitrogen in the ERED active site orientates the reaction intermediates towards highly enantioselective H-atom transfer from the bound flavin cofactor. Hoping to benefit from similar active site binding, we screened a set of ene-reductases from literature, in-house and commercial sources against two pyridyl bromide radical precursors (Figure 3, **b** and **c**). Pleasingly, >98% ee (*S*)-configured product **1c** was generated from 3-bromopyridine **c** and 96% ee (*R*)-configured product **1b** from 2-bromopyridine **b** (Table 3). Additionally, we identified a set of privileged enantiocomplementary enzymes which served as a focused enzyme panel in subsequent studies (see SI for further details). To the best of our knowledge, several of the privileged EREDs have not been reported to participate in photo-induced biocatalysis previously, including alkene reductase from *Klebsiella variicola* At-22 (ER024), alkene reductase from *Citrobacter koseri* (ER025), and alkene reductase from *Acetobacter pomorum* (ER056).

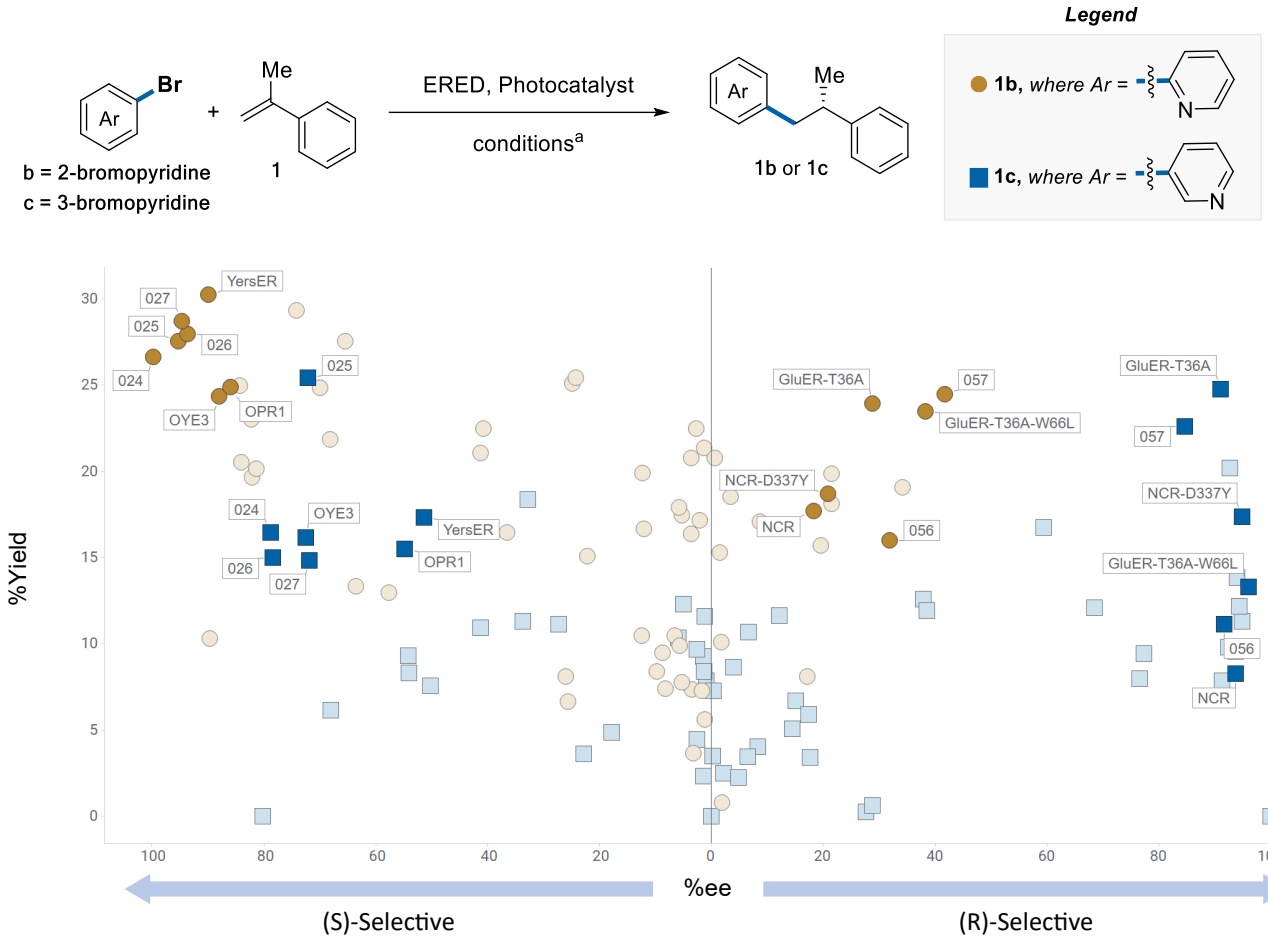
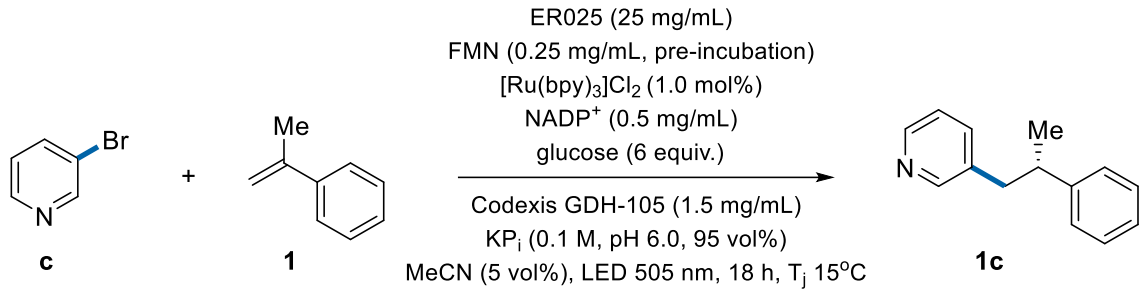


*Figure 3: Screening of 2- and 3-bromopyridine radical precursors against a set of ene-reductases. [a]Conditions: aryl halides **b** or **c** (10 mM) and alkene **1** (30 mM), ERED (25 mg/mL), NADP⁺ (0.5 mg/mL), Codexis GDH-105 (1.5 mg/mL), glucose (11 mg/mL, 6 eq.), $[Ru(bpy)_3]Cl_2$ (1.0 mg/mL, 13 mol%), MeCN (5 vol%), and buffer (0.1M KP_i pH 6.0, 95 vol% to a total volume of 150 µL). Reaction irradiated at 505 nm (cyan) for 18 h at an external chiller temperature of 15°C (internal reaction temperature < 30°C). ERED incubated in a solution of FMN cofactor prior to reaction and made up to a final reaction concentration of 0.25 mg/mL (see SI for details). See SI for method(s) of yield and ee determination through calibrated chiral SFC-UV-(MS) and details of reaction equipment set-up. Absolute determination of stereoconfiguration by comparison to independently synthesized enantiopure products (R)-**1b** and (R)-**1c** (see SI for further details). See SI for further details on the ene-reductases labelled.*

We next performed a series of control reactions with 3-bromopyridine **c** and found that the presence of both an ERED and a photocatalyst, as well as cofactor recycling and the correct choice of wavelength, were crucial to the success of the transformation (Table 2). With this information in hand, we explored the substrate scope of our selected set of EREDs.

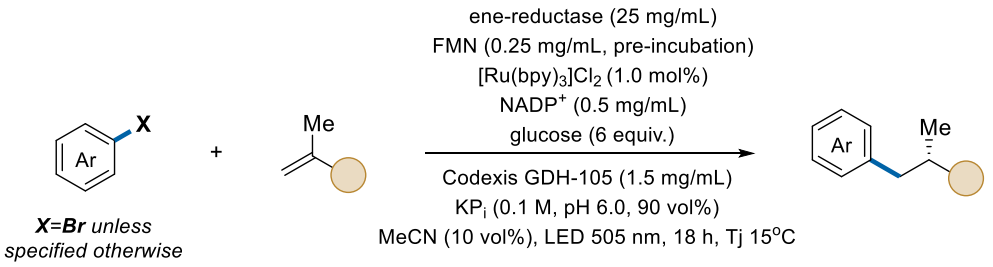


c + **1** → **1c**

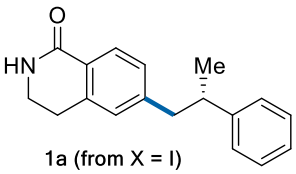
entry	variations from standard conditions	%Yield of 1c*	ee (%)†
1	none	18	97 (*S*)
2	445 nm light	9	98 (*S*)
3	no GDH	0	-
4	no glucose	0	-
5	no $[Ru(bpy)_3]Cl_2$	0	-
6	no GDH, no glucose and no $NADP^+$	0	-
7	no light	0	-
8	FMN + ERED only	0	-
9	FMN only	0	-
10	no ERED	0	-

*Table 2. Control reactions. Reactions performed in triplicate. Conditions: aryl halide **c** (10 mM) and alkene **1** (30 mM), ERED (25 mg/mL), $NADP^+$ (0.5 mg/mL), Codexis GDH-105 (1.5 mg/mL), glucose (11 mg/mL, 6 eq.), $[Ru(bpy)_3]Cl_2$ (1.0 mol%), MeCN (5 vol%), and buffer (0.1M KP_i pH 6.0, 95 vol% to a total volume of 150 µL). Reaction irradiated at 505 nm (cyan) for 18 h at an external chiller temperature of 15°C (internal reaction temperature < 30°C). ERED incubated in a solution of FMN cofactor prior to reaction and made up to a final reaction concentration of 0.25 mg/mL (see SI for details). *†See SI for method(s) of yield and ee determination through calibrated chiral SFC-UV-(MS) and details of reaction equipment set-up. Absolute determination of stereoconfiguration by comparison to independently synthesized enantiopure product (R)-***1c*** (see SI for further details). N/A = not applicable. ER025: alkene reductase from Citrobacter koseri.*

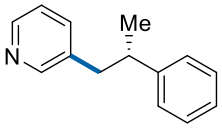
Whilst yields were low, were pleased to observe product formation and high enantioselectivity for many of the desired targets (Table 3, see SI for further details). Good tolerance for halide position was observed with 2-, 3-, and 4-halogenated pyridines all achieving >98% ee for at least one product enantiomer (Table 3: **1b**, **1c**, and **1d** respectively), as well as flexibility in choice of halide. Products **1d** from 4-bromopyridine and **1d** from 4-iodopyridine gave similar product enantioselectivies suggesting a common reaction pathway at the enantiodetermining step. Electronic effects were investigated by positioning electron donating (**1f**) and electron withdrawing (**1g**) groups *ortho* to the reactive halide. In both cases, the substrates were accepted and high enantioselectivity (>98% ee) achieved by at least one enzyme within the panel. Similarly, the scope of the alkene radical acceptor was investigated through incorporation of electron donating and withdrawing phenyl ring substituents *para* to the olefin (**2b**, **3b** respectively). Furthermore, quinoline (**1e**) was also accepted but with slightly lower selectivity (maximum 88% ee (*S*) and 73% ee (*R*)). Inactive (hetero)aryl halides included 5-bromoquinoline, 2-bromo-4-fluorobenzonitrile, 3-bromopyrimidine, bromobenzene as well as pseudohalide phenyltriflate (Table 3).



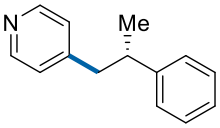
aryl halide scope



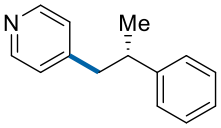
1a (from X = I)
OPR1
2% yield, 80% ee (*S*)[a]
5% yield, 78% ee (*S*)[b]
GluER-T36A-W66L
4% yield, 68% ee (*R*)[a]
7% yield, 62% ee (*R*)[b]



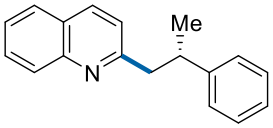
1c
ER025
18% yield, >98% ee (*S*)
GluER-T36A
13% yield, 15% ee (*R*)



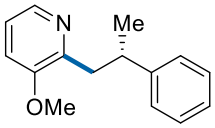
1d (from X = Br)
ER025
21% yield, >98% ee (*S*)[c]
ER057
12% yield, 84% ee (*R*)[c]
NCR
12% yield, 89% ee (*R*)[c]



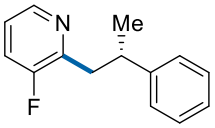
1d (from X = I)
ER025
20% yield, >98% ee (*S*)[d]
ER057
19% yield, 85% ee (*R*)[d]
NCR
15% yield, 82% ee (*R*)[d]



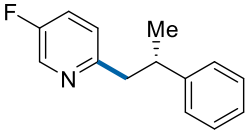
1e
OYE3
17% yield, 88% ee (*S*)
GluER-T36A
13% yield, 77% ee (*R*)



1f
OPR1
5% yield, 40% ee (*S*)[a]
ER056
14% yield, >98% ee (*R*)[a]

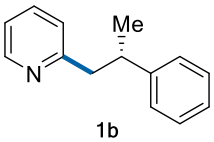


1g
ER025
4% yield, >98% ee (*S*)
GluER-T36A
9% yield, 95% ee (*R*)

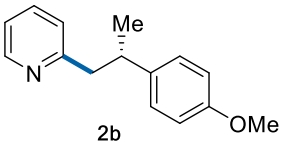


1h
ER026
11% yield, 48% ee (*S*)
GluER-T36A
16% yield, 97% ee (*R*)

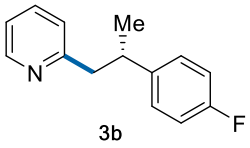
alkene scope



1b
ER026
12% yield, 85% ee (*S*)
GluER-T36A
11% yield, >98% ee (*R*)



2b
ER024
9% yield, 65% ee (*S*)[a]
GluER-T36A-W66L
12% yield, 89% ee (*R*)[a]



3b
OYE3
4% yield, >98% ee (*S*)
NCR-N33Y
4% yield, 77% ee (*R*)

Examples of inactive haloarenes

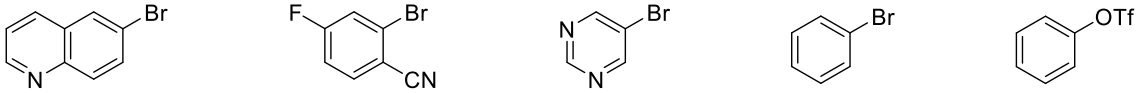
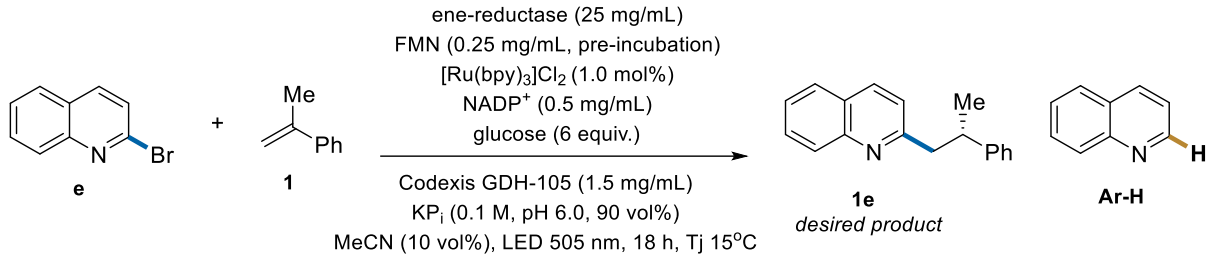


Table 3. Substrate scope of intermolecular reaction with results from the best-performing EREDs shown. Conditions unless otherwise specified: aryl halide (10 mM) and alkene (30 mM), ERED (25 mg/mL), NADP+ (0.5 mg/mL), Codexis GDH-105 (1.5

*mg/mL), glucose (11 mg/mL, 6 eq.), [Ru(bpy)$_3$]Cl$_2$ (1.0 mol%), MeCN (10 vol%), and buffer (0.1M KP$_i$ pH 6.0, 90 vol% to a total volume of 150 µL). Reaction irradiated at 505 nm (cyan) for 18 h at an external chiller temperature of 15°C (internal reaction temperature < 30°C). ERED incubated in a solution of FMN cofactor prior to reaction and made up to a final reaction concentration of 0.25 mg/mL (see SI for details). See SI for method(s) of yield and ee determination through calibrated chiral SFC-UV-(MS) and details of reaction equipment set-up. Absolute stereoconfiguration determined by analogy based upon ERED enantioselectivities observed for (R)-***1a***, *(R)-**1b*** and *(R)-**1c** (independently synthesized authentic samples, see SI for further details). [a] [Ru(bpy)$_3$]Cl$_2$ (13 mol%). [b] See Table 1 for conditions. [c]4-bromopyridine.HCl salt rather than free base, [Ru(bpy)$_3$]Cl$_2$ (13 mol%), DMSO (7 vol%), buffer (0.1M KP$_i$ pH 6.0, 93 vol% to a total volume of 150 µL). [d] DMSO (10 vol%), buffer (0.1M KP$_i$ pH 6.0, 90 vol% to a total volume of 150 µL).*

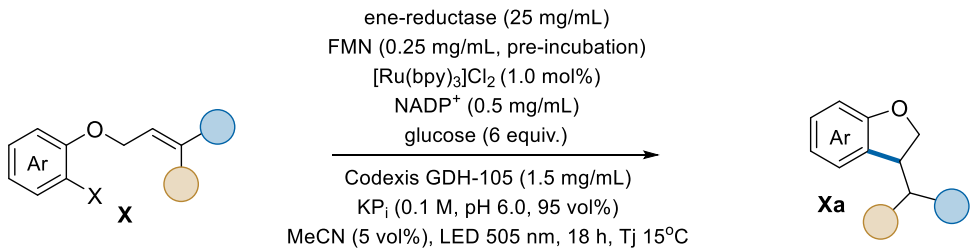
To better understand the reasons for the modest yields observed, we investigated the mass balance of the coupling of 2-bromoquinoline with α-methylstyrene for which the unreacted substrate and potential byproducts could be quantified by SFC-UV-(MS). Representative product distributions are given for the reactions employing NADPH dehydrogenase from *Saccharomyces cerevisiae* (OYE3) and alkene reductase from *Gluconobacter oxydans* mutant T36A (GluER-T36A) (Table 4). Intriguingly, for both systems we observed significant levels of quinoline generation (18% and 14% assay yield, respectively), indicating that protodehalogenation competes with the desired reaction. Such protodehalogenation of (hetero)aryl halides represents another novel photo-induced biocatalytic transformation uncovered in this study, to the best of our knowledge. With the exception of desired product, quinoline and unreacted starting materials, significant levels of other compounds were not observed by chromatographic analysis under either extractive or acetonitrile-quenched work-up. To account for the remaining mass balance loss, we postulate that the activated quinoline-radical intermediate may couple to the enzyme itself or its bound flavin, and thus become unavailable to standard work-up and analytical techniques.



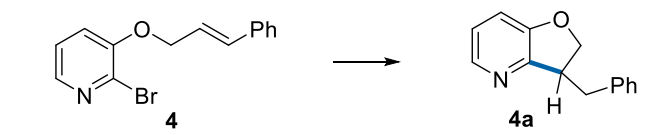
ene-reductase	% Yield (1e)*	% ee (1e)†	% ArBr#	% ArH	% Mass recovery
OYE3	17%	88% (*S*)	30%	18%	65%
GluER-T36A	13%	77% (*R*)	30%	14%	57%

*Table 4. Mass balance determination for the reaction of 2-bromoquinoline and α-methylstyrene. Conditions: aryl halide (10 mM) and alkene (30 mM), ERED (25 mg/mL), NADP$^+$ (0.5 mg/mL), Codexis GDH-105 (1.5 mg/mL), glucose (11 mg/mL, 6 eq.), [Ru(bpy)$_3$]Cl$_2$ (1.0 mol%), MeCN (10 vol%), and buffer (0.1M KP$_i$ pH 6.0, 90 vol% to a total volume of 150 µL). Reaction irradiated at 505 nm (cyan) for 18 h at an external chiller temperature of 15°C (internal reaction temperature < 30°C). ERED incubated in a solution of FMN cofactor prior to reaction and made up to a final reaction concentration of 0.25 mg/mL (see SI for details). *†See SI for method(s) of yield and ee determination through calibrated chiral SFC-UV-(MS) and details of reaction equipment set-up. Absolute stereoconfiguration determined by analogy based upon ERED enantioselectivities observed for (R)-**1a**, (R)-**1b** and (R)-**1c** (independently synthesized authentic samples, see SI for further details). #Unreacted 2-bromoquinoline.*

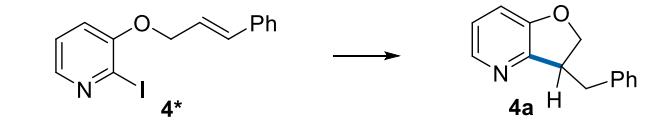
Hoping to improve the propensity of the radical (hetero)arene electrophile to couple with the desired alkene partner, rather than a proton source or other unproductive components of the reaction mixture, we decided to tether the two coupling partners to each other and establish an intramolecular variant of this reaction. Pleasingly, significantly improved yields and mass recoveries were observed with this approach (Table 5), including 80% assay yield achieved through extended reaction times for bromopyridyl-alkene **4** (Table 5, entry *i*). Unfortunately, however, the high levels of enantioselectivity observed under intermolecular coupling did not translate to the examples of intramolecular coupling reactions tested. Control reactions (Table 5, entries *iv, viii, xii, xvi*) indicated that the presence of an ene-reductase was required to achieve higher yields, although some background reaction occurs. Low but measurable enantioselectivity was achieved in many cases, the highest being in the absence of photocatalyst for amide **6** (Table 5, entry *xiv*). Combined, these results indicate that the low enantioselectivity observed is not primarily due to background, non-enzymatic, reaction, but instead due to lower enantio-differentiation within an enzyme-induced process.



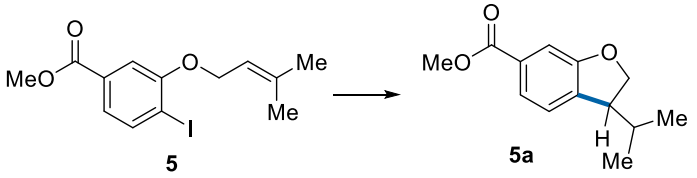
intramolecular cyclization scope



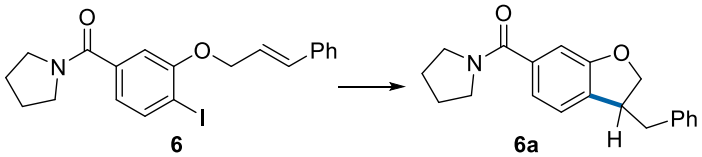
ene-reductase:	4a
i. ER057[a, b]	80% yield, 4% ee
ii. ER024	53% yield, 8% ee
iii. ER056	21% yield, 21% ee
iv. No ERED[a, b]	2% yield, 0% ee



ene-reductase:	4a
v. ER025	50% yield, 2% ee
vi. ER025[b]	47% yield (isolated), 0% ee
vii. ER057	51% yield, 8% ee
viii. No ERED	4% yield, 0% ee



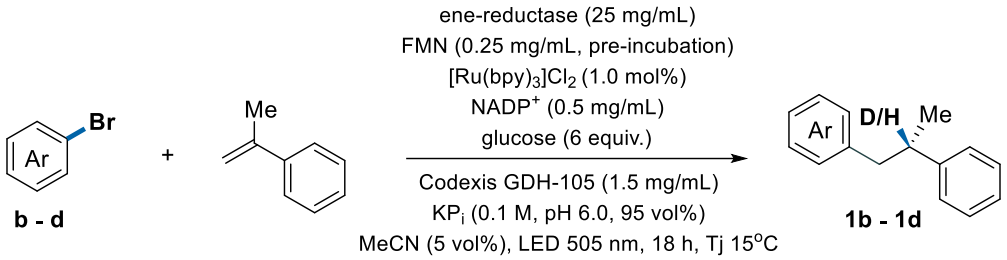
ene-reductase:	5a
ix. OPR1[a, b]	43% yield, 1% ee
x. NCR-N33Y[a, b]	43%yield, 5% ee
xi. ER025	15% yield, 0% ee
xii. No ERED	6% yield. 0% ee



ene-reductase:	6a
xiii. GluER-T36A[c, d]	70% yield, 20% ee
xiv. GluER-T36A, no photocat.[c]	31% yield, 41% ee
xv. ER056[c]	22% yield, 21% ee
xvi. No ERED[c]	0% yield, 0% ee

*Table 5. Investigation of intramolecular cyclization. Conditions unless otherwise specified: aryl halide (10 mM), ERED (25 mg/mL), $NADP^+$ (0.5 mg/mL), Codexis GDH-105 (1.5 mg/mL), glucose (11 mg/mL, 6 eq.), $[Ru(bpy)_3]Cl_2$ (13 mol%), MeCN (5 vol%), and buffer (0.1M KP_i pH 6.0, 95 vol% to a total volume of 150 µL). Reaction irradiated at 505 nm (cyan) for 18 h at an external chiller temperature of 15°C (internal reaction temperature < 30°C). ERED incubated in a solution of FMN cofactor prior to reaction and made up to a final reaction concentration of 0.25 mg/mL (see SI for details). See SI for method(s) of yield and ee determination through calibrated chiral SFC-UV-(MS) and details of reaction equipment set-up. Absolute stereoconfiguration determined by analogy based upon ERED enantioselectivities observed for (R)-***1a***, (R)-***1b*** and (R)-***1c*** (independently synthesized authentic samples, see SI for further details). [a] Reaction time 115 h. [b] Isolated yield, see SI for preparative scale conditions. [c] Codexis GDH-105 (1.4 mg/mL), glucose (27 mg/mL, 15 eq.), $[Ru(bpy)_3]Cl_2$ (13 mol%), DMSO (10 vol%), and buffer (0.1M TRIS HCl pH 9.0, 90 vol% to a total volume of 150 µL). [d] Reaction time 90 h, external chiller temperature 25°C.*

In photo-induced enzymatic alkene addition reactions, literature suggests that the enantiodetermining step is typically a terminal hydrogen atom transfer from the bound flavin cofactor (FMN) to a prochiral radical intermediate.[21] Isotopic labelling experiments were performed in order to determine whether our system also follows this mechanism. A selection of haloarenes (**b**, **c**, and **d**) were treated with (*D*)-glucose-1-d_1 in non-deuterated aqueous buffer, which forms deuterated FMN *in situ* (conditions A). Haloarenes **b**, **c** and **d** were also treated with non-deuterated (*D*)-glucose in a D_2O-based buffer, which would allow for deuteration of exchangeable protons in the active site (conditions B). Should the HAT occur primarily from the bound flavin as described, we would expect to see product deuteration in the reactions employing (*D*)-glucose-1-*d*1 (conditions A) but not in those employing standard glucose in a D_2O-based buffer (conditions B). In both cases, however, moderate deuterium incorporation into the product was observed, ranging from 2 to 25% under conditions A (Table 6, entries *i* - *ix*) and 9 to 41% under conditions B (Table 6, entries *x* - *xviii*). Additionally, the degree of deuterium incorporation was both substrate and enzyme dependent. These results imply a set of competing mechanisms, whereby hydrogen atom transfer occurs from multiple H-atom sources including flavin.



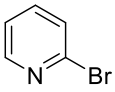
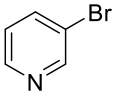
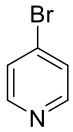
	Substrate	ERED[d]	Conditions A: FMN$_{hq}$ as deuterium source[a] entry	%Yield	%ee	%D-labelled[c]	Conditions B: Buffer as deuterium source[b] entry	%Yield	%ee	%D-labelled[c]
b		OPR1	i.	9%	56%	7%	x.	3%	>98%	33%
		ER025	ii.	6%	>98%	2%	xi.	3%	>98%	25%
		ER056	iii.	6%	>98%	2%	xii.	4%	>98%	9%
c		OPR1	iv.	17%	74%	11%	xiii.	12%	92%	41%
		ER025	v.	12%	93%	4%	xiv.	12%	>98%	28%
		ER056	vi.	<1%	N/A	9%	xv	3%	31%	18%
d		OPR1	vii.	16%	48%	25%	xvi	20%	78%	23%
		ER025	viii.	18%	91%	25%	xvii	20%	>98%	23%
		ER056	ix.	13%	18%	11%	xviii	9%	54%	21%

Table 6: Deuterium incorporation experiments. Results are an average of duplicates. Conditions: aryl halide (10 mM) and prop-1-en-2-ylbenzene (30 mM), ERED (25 mg/mL), NADP$^+$ (0.5 mg/mL), Codexis GDH-105 (1.5 mg/mL), glucose (11 mg/mL, 6 eq.), [Ru(bpy)$_3$]Cl$_2$ (1.0 mol%), MeCN (10 vol%), and buffer (0.1M KP$_i$ pH 6.0, 90 vol% to a total volume of 150 µL). Reaction irradiated at 505 nm (cyan) for 18 h at an external chiller temperature of 15°C (internal reaction temperature < 30°C). ERED incubated in a solution of FMN cofactor prior to reaction and made up to a final reaction concentration of 0.25 mg/mL (see SI for details). See SI for method(s) of yield and ee determination through calibrated chiral SFC-UV-(MS) and details of reaction equipment set-up. [a] Deuterated FMN was generated in situ using (D)-glucose-1-d$_1$ [b] D$_2$O used to prepared deuterated buffer solutions. [c] Deuterium incorporation was determined by analysis with LC-HRMS using DGet! software (see SI for full details). [d] OPR1: 12-oxo-phytodienoic acid reductase from Zea mays, ER025: alkene reductase from Citrobacter koseri, ER056: alkene reductase from Acetobacter pomorum.

Conclusions

In summary, a set of privileged and stereocomplementary ene-reductase enzymes have been identified which, when induced by light and aided by an exogenous photocatalyst, catalyze the reaction between (hetero)aryl halides and alkenes in an asymmetric intermolecular hydroarylation process. In this manner, carbon scaffolds containing C(sp^2)-C(sp^3) bonds are synthesized enzymatically from simple, commercially available, precursors in excellent enantiomeric excess. The reaction was exemplified by fifteen substrate systems including intermolecular and higher yielding but poorly enantioselective intramolecular transformations. Additionally, a number of the privileged ene-reductases have been identified which had not previously been described to perform photo-induced biocatalysis. The work serves as a proof of

concept, establishing the utility of photo-induced biocatalysis through the addition of the novel and pharmaceutically important (hetero)aromatic halide class of radical precursors.

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Enzyme Abbreviation and Species Name

Abbreviation	Enzyme Name
OYE3	NADPH dehydrogenase from *Saccharomyces cerevisiae* (OYE3)
NCR	NAD(P)H-dependent 2-cyclohexen-1-one reductase (NCR) from *Zymomonas mobilis*
OPR1	12-oxo-phytodienoic acid reductase from *Zea mays* (OPR1, maize plant)
ER024	Alkene reductase from *Klebsiella variicola* At-22
ER025	Alkene reductase from *Citrobacter koseri*
ER026	Alkene reductase from *Citrobacter sp.*
ER027	N-ethylmaleimide reductase from *Citrobacter rodentium*
ER056	Alkene reductase from *Acetobacter pomorum*
ER057	Alkene reductase from *Gluconobacter morbifer*
NCR-D337Y[45]	NAD(P)H-dependent 2-cyclohexen-1-one reductase (NCR) from *Zymomonas mobilis* mutant D37Y
GluER-T36A[21]	Alkene reductase from *Gluconobacter oxydans* mutant T36A
YersER	Alkene reductase from *Yersenia bercovieri*
GluER-T36A-W66L[46]	Alkene reductase from *Gluconobacter oxydans* mutant T36A-W66L