ACS Catalysis

Article

Development of a one-pot tandem reaction combining ruthenium-catalyzed alkene metathesis and enantioselective enzymatic oxidation to produce aryl epoxides

Carl A. Denard, Mark J. Bartlett, Yajie Wang, Lu Lu, John F. Hartwig, and Huimin Zhao

ACS Catal., **Just Accepted Manuscript** • DOI: 10.1021/acscatal.5b00533 • Publication Date (Web): 12 May 2015

ACS Catalysis is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Development of a One-Pot Tandem Reaction Combining Ruthenium-Catalyzed Alkene Metathesis and Enantioselective Enzymatic Oxidation to Produce Aryl Epoxides

Carl A. Denard,[a]‡ Mark J. Bartlett,[b]‡ Yajie Wang,[a] Lu Lu,[c] John F. Hartwig,[b] and Huimin Zhao*,[a,d]*

[a]Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801

[b]Department of Chemistry, University of California-Berkeley, Berkeley CA 94720

[c]Department of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

[d]Departments of Chemistry, Biochemistry, and Bioengineering, Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

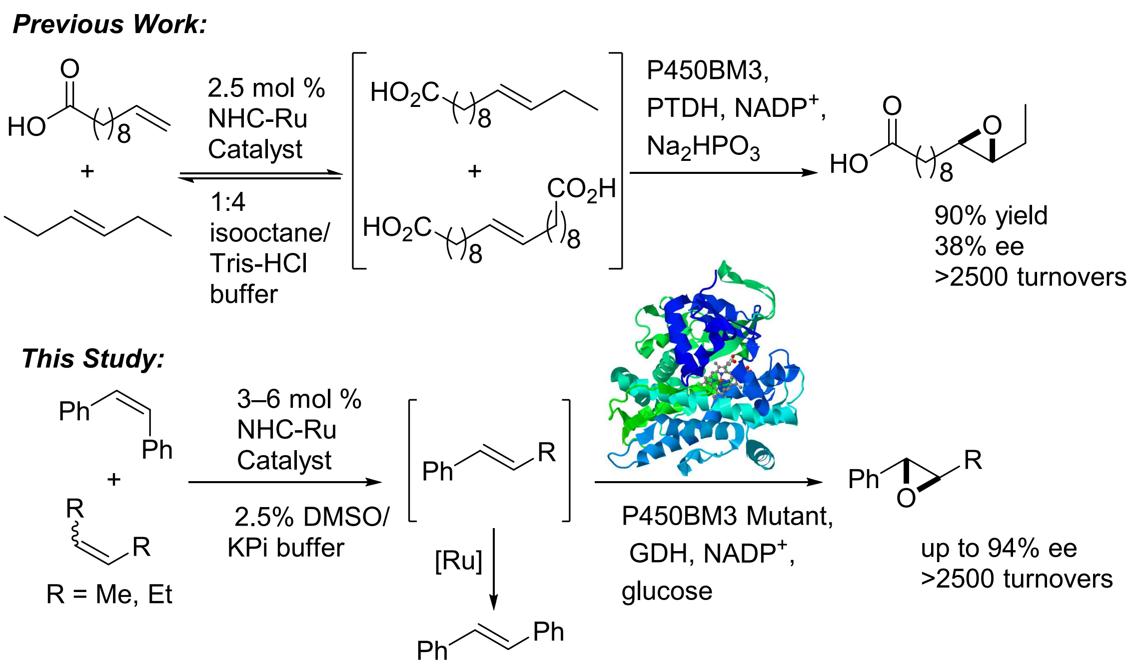
We report the development of a tandem chemoenzymatic transformation that combines alkene metathesis with enzymatic epoxidation to provide aryl epoxides. The development of this One-pot reaction required substantial protein and reaction engineering to improve both selectivity and catalytic activity. Ultimately, this reaction converts a mixture of alkenes into a single epoxide product in high enantioselectivity and moderate yields and illustrates both the challenges and benefits of tandem catalysis combining organometallic and enzymatic systems.

KEYWORDS: Chemo-enzymatic catalysis, cytochrome P450, organometallic catalysis, biocatalysis, tandem catalysis, olefin metathesis, biocatalysis.

Introduction

The utility of biocatalysis in the synthesis of fine chemicals and medicinal compounds has grown significantly in recent times.[1] Furthermore, the development of one-pot multistep reactions containing both transition-metal catalysts and enzymes has proven appealing in terms of both selectivity and synthetic efficiency.[2] These tandem reactions exploit the broad reactivity of Transition-metal catalysts and the high selectivity of enzymes simultaneously. Work in this area has resulted in several well-established dynamic kinetic resolutions involving metal catalysts and enzymes[3], along with a number of sequential and One-pot cascade reactions.[2a, 4] Nonetheless, combining Small-molecule organometallic catalysts and biocatalysts remains challenging, in part because the milieu in which these catalysts operate are typically different.[5]

Besides dynamic kinetic resolutions, there have been few reports in which an Enzyme-catalyzed transformation occurs with one substrate of a dynamic equilibrium or a transient product which is consumed in a subsequent side reaction. Yet, these scenarios could lead to cooperative catalytic reactions in which one catalytic transformation aids the efficiency of the other and provides a higher yield of the final product than would be obtained by two sequential reactions. For such processes, cytochromes P450 are particularly appealing, due to their ability to catalyze the oxidization of C–H and C=C bonds in substrates of a specific size and shape.[6, 7] These transformations utilize molecular oxygen and NADPH to form a reactive Iron-oxo intermediate and often provide alcohols or epoxides with high regio- and stereoselectivity.[8] In previous work, we developed a cooperative tandem reaction in which P450-BM3 and an alkene metathesis catalyst work together to convert an equilibrium mixture of alkenes into a single epoxide on the basis of chain length (Scheme 1).[9] The yields of this One-pot reaction were higher than those obtained when the reactions were run sequentially. In the present study, we sought to evolve this strategy to convert stilbene selectively to aryl epoxides. We show that alkene metathesis and enzymatic epoxidation can be used in a tandem reaction to selectively provide aryl epoxides in high enantioselectivity.

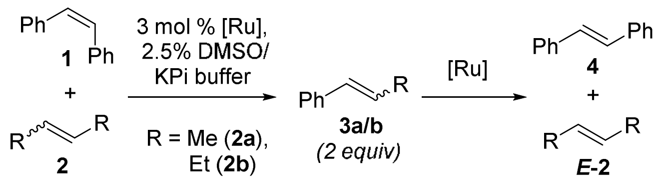


Scheme 1. Alkene Metathesis and Oxidation in Cooperative Tandem Catalysis. The $P450_{BM3}$ image is based on the crystal structure reported previously (P450 KT2 crystal structure PDB ID: 3PSX).[7]

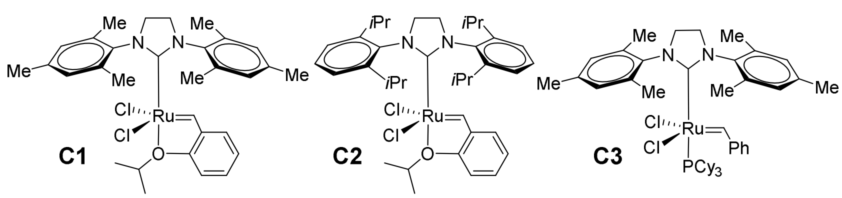
Results and Discussion

We investigated the metathesis reaction of *Z*-stilbene and a symmetrical alkene as shown in Scheme 1. NHC-based ruthenium complexes are known to catalyze alkene metathesis in the aerobic aqueous conditions often required for enzymatic transformations.[10] Three Ru-catalysts (Entries 3–5) were tested for the Cross-metathesis of *Z*-stilbene **1** and *Z*-2-butene (*Z*-**2a**) (Table 1). In all three cases, only moderate yields (32–35%) of *E*-β-methylstyrene (*E*-**3a**) were obtained, but with very high *E*/*Z* selectivity (>30:1). The same reaction catalyzed by **C2** provided a low yield of *E*-stilbene **4** (Entries 4 and 8). The lower reactivity of **C2**, compared with that of **C1** and **C3**, is likely due to the greater steric encumbrance from the isopropyl groups in this catalyst.[11] Control experiments revealed that *E*-stilbene (**4**) does not undergo cross metathesis with *E*-3-hexene (*E*-**2b**) in the presence of either Ru-catalyst **C1** or **C2** (Entries 1 and 2).[12] Furthermore, the Self-metathesis of *Z*-stilbene (**1**) to form *E*-stilbene **4** is slow. In contrast to our previous work,[8] these results suggest that an irreversible pathway leads to the conversion of *Z*-stilbene (**1**) to β-alkylstyrene (**3**), and the conversion of **3** to *E*-stilbene (**4**). The reaction of **1** with the liquid alkene *E*-**2b**, as opposed to the gaseous *Z*-2-butene, provided higher yield of β-alkylstyrene (Entries 3 and 6). Reaction with **1** equiv. of *E*-**2b** instead of 3 equiv. resulted in lower yield of β-ethylstyrene (*E*-**3b**) while the amount of *E*-stilbene side product was relatively unchanged (Entry **7**). Similar results were observed when reactions between **1** and *E*-**2b** were run in an isooctane: buffer biphasic, where in the metathesis reaction occurred in the organic phase (Table S2). The formation of *E*-stilbene, presumably, is slowed by competing unproductive metathesis between **3** and **2**. Thus increasing the equivalents of **2b** provides a higher ratio of alkylstryene **3b** to stilbene **4** after 16 hours. Overall, these results suggest that in order to epoxidize **3** efficiently in a tandem system, the self-metathesis of *Z*-stilbene **3** to produce *E*-stilbene **4** must be mitigated. This reduction of the formation of **4** could be achieved by using the selective, but less reactive, Ru-catalyst **C2** or by maintaining a low concentration of alkylstyrene by the simultaneous use of an epoxidation catalyst that causes this reaction to be faster than the metathesis reaction.

Table 1. Cross-Metathesis of Stilbene on Water.



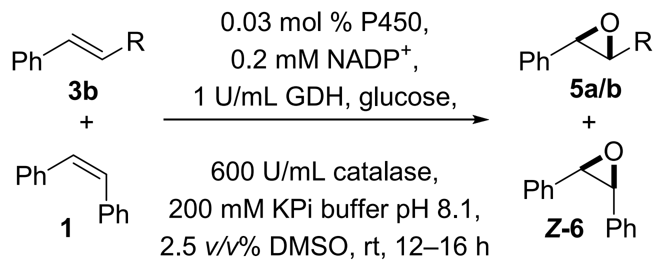
Entry	[Ru] Catalyst	Alkenes (equiv)	Yield[a] 3a/b[b]	4
1[c]	**C1**	**4 + *E*-2b** (3)	0%	-
2	**C1**	**1** (1)	-	8%
3[d]	**C1**	**1 + *Z*-2a** (10, 8 psig)	33%	40%
4[d]	**C2**	**1 + *Z*-2a** (10, 8 psig)	35%[d]	8%
5[d]	**C3**	**1 + *Z*-2a** (10, 8 psig)	32%	20%
6[c]	**C1**	**1 + *E*-2b** (3)	50%	47%
7[c]	**C1**	**1 + *E*-2b** (1)	33%	43%
8[c]	**C2**	**1 + *E*-2b** (3)	47%(32%)	4%



[a] Determined by GC analysis. Isolated yield shown in parentheses. Remaining mass balance consists of unreacted **1**. [b] *E/Z*>30:1. [c] Reactions run for 16 h. [c] Reactions run in triplicate, average yields are reported. [d] 23% yield after 4 h.

To establish these relative rates, we investigated the activity and selectivity of three P450-BM3 variants: RLYF,[13] KT2,[14] and RH47[15] (Table S1), previously shown to catalyze the oxidation of arenes.[16] Each P450 variant was evaluated using a competition experiment between *Z*-stilbene (**1**) and alkylstyrene **3a** or **3b**, while determining the *ee* of the resulting epoxides **5a**/**b** (Table 2). In addition, a glucose dehydrogenase (GDH) system was used to regenerate NADPH by cloning and expressing the *gdhIV* gene from *Bacillus megaterium*.[17] This system is more productive than the previously used phosphite dehydrogenase (PTDH) system.[18] A reaction conducted with 3 nmol of a P450 KT2 lysate and a glucose-driven GDH-$NADP^+$ system led to the selective epoxidation of *E*-**3a** to form *trans*-**5a** in 41% yield with more than 4000 turnovers (Entry 3). Less than 3% yield of *Z*-stilbene oxide (*Z*-**6**) was observed. The aqueous solubility of *Z*-stilbene is higher than that of *E*-β-methylstyrene; therefore, the observed selectivity does not result from differences in aqueous solubility. Lower yields were typically obtained from reactions conducted with purified P450, presumably due to the presence of stabilizing agents in the lysate.[19] Interestingly, performing the bioepoxidation of *E*-**3b** in a biphasic isooctane: buffer system proved to be mass-transfer limited and produced *trans*-**5b** in <2% yield, even in emulsions created by sodium docusate salt[20] or methyl-β-cyclodextrin.[21] All three P450 variants formed epoxide (*R*,*R*)-*trans*-**5a** with >80% *ee*.[11b] Furthermore, initial rates revealed that the reactions of **3a** catalyzed by all three metalloenzymes occurred with a high degree of coupling between NADPH consumption and rate of product formation. The epoxidation of *E*-β-ethylstyrene catalyzed by RLYF, KT2 and RH47 occurred in significantly lower yields and NADPH coupling (Entries 7–10) than epoxidation of β-methylstyrene. However, epoxide **5b** was formed in >90% *ee* with all three variants. In contrast to epoxidation of *Z*- and *E*-β-methylstyrene, the epoxidation of *E*-β-ethylstyrene has proven challenging for a number of asymmetric epoxidation catalysts.[22] Furthermore, P450 metalloenzymes typically display only moderate *ee* in the epoxidation of aryl alkenes.[23]

Table 2. Enzymatic Epoxidation of β-Alkyl Styrenes.



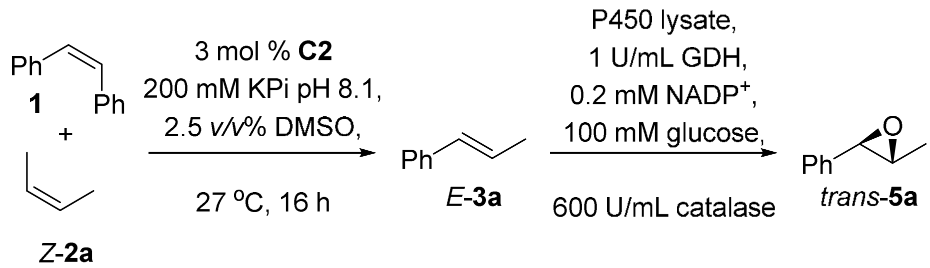
Entry	P450	R	equiv **1**	Yield[a] **5**	Initial Rate (Coupling)[b]	*ee*[c]	Yield[a]**6**
1	RLYF	Me	1	19%	-	-	5%
2			0	18%[d] (15%)[e]	630 (98%)	83% (*R*,*R*)	-
3[f]	KT2	Me	1	41%	-	-	3%
4			0	34%[d] (38%)[e]	815 (95%)	88% (*R*,*R*)	-
5	RH47	Me	1	29%	-	-	3%
6			0	26%[d]	960 (80%)	86% (*R*,*R*)	-
7	RLYF	Et	1	14%	311 (75%)	94%	7%
8	KT2	Et	1	8%	-	91%	3%
9	RH47	Et	0	11%[d]	-	93%	-

[a] Yield determined by GC. Reactions performed on a 0.027 mmol scale. [b] Initial rate in nmol•min^{-1}•nmol P450^{-1}, see SI for details. [c] Enantiomeric excess determined by chiral SFC analysis. [d] NMR yield. [e] Isolated yield, average yield of four 0.08 mmol reactions. [f] Reaction performed with 0.01 mol % P450.

Having developed a single set of reaction conditions that accommodate both catalytic reactions, we investigated the two reactions in One-pot. These tandem reactions required careful layering of *Z*-stilbene and the Ru-catalyst on top of the aqueous layer to ensure adequate contact between the Ru-catalyst and gaseous *Z*-2-butene (Figure S3). In addition, the aqueous solubility of *E*-β-methylstyrene and *Z*-stilbene were determined to be 0.9 and 5.2 mM, respectively.[24] This low aqueous solubility highlights the need for a highly active epoxidation enzyme. To maximize reaction yields and maintain reproducibility, several reaction parameters were investigated, including substrate loading, organic cosolvent, loading of the regeneration enzyme, reaction volume and buffer concentration (Table S4). To assess the possibility of mutual catalyst inactivation, several control reactions were performed. These experiments showed that the alkene metathesis was unaffected by the volume of the aqueous phase, the nature of the cosolvent, or the presence of enzymatic components (Tables S6–S8). Furthermore, enzymatic oxidation was not inhibited by the presence of 3 mol % of the Ru-complex **C2** (Figure S2) or 8 psig of *Z*-**2a**.

Epoxide *trans*-**5a** was initially obtained in 13% yield in a tandem reaction with 0.06 mol % (15 nmol) P450 RLYF (Table 3, Entry **2**). However, decreasing the volume of the reaction from 5 mL to 3 mL improved the yield of *trans*-**5a** to 22%, with only 0.03 mol % (9 nmol) P450 (Entries **4** and **5**). When KT2 was used as the epoxidation catalyst, the yield of *trans*-**5a** increased to 40%, and over 2400 turnovers were achieved (Entry **6**). Reactions conducted with twice the loading of P450/GDH did not result in significantly higher yields of *trans*-**5a** (Figure S4), however, the addition of a second batch of enzyme after the initial 16 h of reaction gave a slight increase in the conversion of *E*-**3a**, providing *trans*-**5a** in 50% yield (Entry **7**). Similar results have been observed when increasing the loading of P450 to improve the yield of styrene epoxidation.[25]

Table 3. Tandem Metathesis–Oxidation with *Z*-2-Butene.



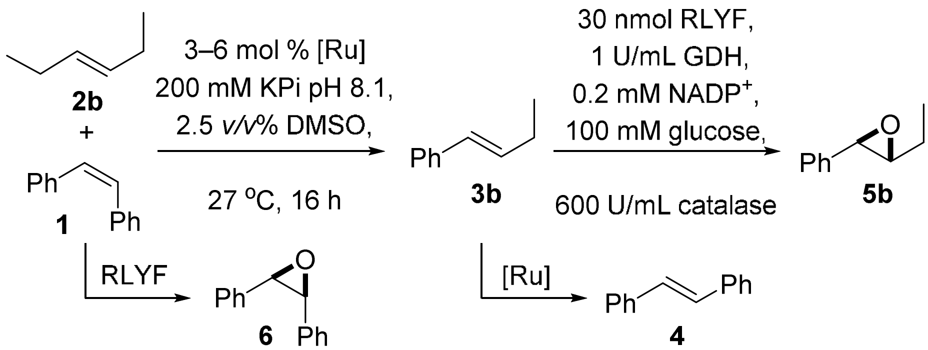
Entry	P450 (nmol)	psig *Z*-**2a**	Reaction Volume	*E*-**3a**	Yield[a] *trans*-**5a**	**4**	P450 TTN
1	-	6	3 mL	37%	-		-
2	RLYF (15)	6	5 mL	28%	13%	14%	936
3	RLYF (9)	6	3 mL	24%	22%	25%	2640
4[b]	RLYF (9)	6	4 mL	43%	19% (23%)[c]	26%	2280
5	RLYF (9)	8	5 mL	33%	20%	15%	2400
6[d]	KT2 (9)	6	6 mL	21%	41%[e]	10%	2460
7[d]	KT2 (27)[f]	6	3 mL	13%	50%	9%	2000
8[g]	KT2 (9)	6	3 mL	15%	38%	10%	4560

[a] Yield determined by GC and is an average of multiple experiments, see Table S10 for details. Isolated yield in parentheses. Reactions performed on a 0.054 mmol scale. [b] Reaction performed with 5 mol % C2. [c] 83% *ee*. [d] 0.027 mmol **1** was used. [e] 87% *ee*. [f] After 16 h, 19 nmol P450 and 0.5 U/mL GDH were added (24 h reaction). [g] One-pot sequential reaction (0.027 mmol). 10 h alkene metathesis, 16 h epoxidation.

Performing the two transformations as a sequential One-pot reaction afforded *trans*-**5a** in 38% yield (Entry 8), which is approximately the same yield as both the tandem reaction (Entry 6) and the isolated epoxidation of *E*-**3a** with KT2. These results indicate a lack of cooperativity between the two catalytic cycles.[26]

Several additional observations are noteworthy. First, there was increased consumption of *Z*-stilbene (**1**) in all of the tandem reactions compared to the isolated metathesis reaction (Entry 1). These results suggest that the selective removal of **3a** from the system by epoxidation is compensated by further production of **3a**. Although higher yields of *E*-stilbene (**4**) were observed in tandem reactions, compared to isolated metathesis reactions, this is attributed to longer reaction times and the viscous P450 lysate. The viscous P450 lysate is believed to partially inhibit contact between the Ru-catalyst and gaseous *Z*-2-butene, which in turn favors the self-metathesis of *E*-**3a** to form *E*-stilbene (Figure S3). In general, tandem reactions with higher yields of *trans*-**5a** also produced less *E*-stilbene (*i.e.* Entries **4** and **6**). There was little or no formation of products from epoxidation of *Z*-β-methylstyrene, *Z*-stilbene or *E*-stilbene (**1** and **4**, respectively) in these tandem reactions. Yet, separate oxidations of *Z*-β-methylstyrene showed that this alkene undergoes epoxidation in >65% yield with RLYF (Table S9). Thus, the excellent *E*-selectivity of the metathesis catalyst leads to the absence *cis*-**5a**. Overall, the selectivity of both the ruthenium catalyst and P450 variant combine to make this tandem system highly selective for the epoxidation of *E*-β-methylstyrene.

Tandem reactions involving **1** and *E*-3-hexene (*E*-**2b)** were also conducted (Scheme 2). These reactions were conducted with RLYF and the desired epoxide *trans*-**5b** was formed in low yield, with the majority of the mass balance consisting of *E*-**3b** and *E*-stilbene (**4)**. These results highlight the lower activity of the P450 variants towards *E*-β-ethylstyrene. Furthermore, *cis*-stilbene oxide (**6**) was obtained in 7% yield (Entry **1**), indicating only moderate selectivity for *E*-β-ethylstyrene over *Z*-stilbene in the alkene epoxidation. Nonetheless, the desired epoxidation occurred with more than 300 turnovers, providing *trans*-**5b** in up to 22% yield (Entry **1**). Similar yield was obtained in a sequential reaction (Entry **2**) and also an isolated epoxidation (Entry **3**), once again suggesting that the enzymatic reaction is not adversely affected by the metathesis reaction.



Entry	[Ru] Catalyst	Yield[a] 5b	3b	4	6	P450 TTN
1	**C2**	22%	26%	21%	7%	198
2[b]	**C2**	17%	30%	10%	-[c]	153
3[d]	-	25%	-	-	-	225
4[e]	**C3**	10%	43%	40%	-[c]	270

[a] Yield determined by GC. Reactions performed on a 0.027 mmol scale. [b] Reaction was run sequentially in one-pot. 10 h alkene metathesis, 16 h enzymatic epoxidation. [c] Yield not determined. [d] Epoxidation only. [e] 0.054 mmol reaction with 20 nmol P450 in 2 mL reaction volume.

Scheme 2. Tandem Metathesis/Oxidation with *E*-3-Hexene.

Conclusion

In summary, we have developed a One-pot alkene Metathesis–enzymatic epoxidation reaction to convert a mixture of stilbene-derived alkenes selectively into a single epoxide. Simultaneous alkene metathesis and epoxidation has the potential to disfavor the irreversible formation of *E*-stilbene and provide improved conversion of *Z*-stilbene. Ultimately, moderate yield and excellent enantioselectivity were obtained in the formation of a number of aryl epoxides. Current efforts are directed towards developing P450 BM3 variants with improved catalytic activity and the use of emulsions to increase the enzymatic reaction rates.

Experimental Details

Expression and Purification of P450 Variants

The cytochrome P450 BM3 variants were expressed as follows. Overnight cultures of DH5α-pCWori+-BM3 variant were inoculated in 500 mL TB medium supplemented with 100 µg/mL ampicillin. After 12 h of growth at 30 ºC and 250 rpm, protein expression was induced with 0.5 mM δ-aminolevulinic acid and 1 mM IPTG and allowed to grow for a further 24 h at 30 ºC and 180 rpm, after which the cells were harvested by centrifugation (6000 rpm, 4 ºC, 10 min). The cell pellets were resuspended in 27–30 mL of 0.1 M phosphate buffer (pH 8.1) and 1 mg/mL of lysozyme was added. After a freeze-thaw cycle at -80 ºC, the cells were disrupted by sonication (5s on, 5 off, 40% amplitude) for 5 minutes, and the lysate was clarified multiple times by centrifugation (20,000 rpm, 4 ºC, 15 min). The clarified lysate was filtered through a 0.22 µM Amicon filter. The P450 concentration was measured by the carbon monoxide binding assay.[27] Typical concentrations of 40 µM P450 were readily obtained. This lysate was used as is in tandem reactions.

For purification of the P450 BM3 variants, the cell lysate was purified as follows. The lysate was loaded onto a column packed with DEAE 650-M resin (Toyopearl, Los Angeles, CA) coupled to a fast-performance liquid chromatography. A wash step with 15% NaCl in 25 mM phosphate buffer, pH 8.1 was applied. The protein eluted at 25% NaCl in the phosphate buffer. Purity of the protein was estimated to around 70% using SDS-PAGE. At this purity, the protein was judged to be pure enough for biocatalysis.

General Procedure for Enzymatic Epoxidation

To a solution of D-glucose (300 μL of 1M stock in 200 mM KPi), $NADP^+$ (30 μL of a 20 mM stock in 100 mM KPi), GDH (20 uL, 1 U/mL), and catalase (30 μL, 600 U/mL) in 200 mM phosphate buffer pH 8.1 (to 3 mL) was added *E*-β-methylstyrene (3.51 μL, 3.19 mg, 27 μmol) in 75 μL DMSO. Freshly prepared P450 lysate (9 nmol, 0.033 mol %) was added and the reaction was incubated at 25–27 ºC, 100 rpm, overnight (12–16 h). Reactions were performed in 27 mL crimp cap vials to avoid the loss of volatile compounds. Final concentrations are as follows; 3.3 μM P450, 100 mM glucose, 0.2 mM $NADP^+$, 1 U/mL GDH, 600 U/mL catalase, 9 mM *E*-β-methylstyrene. 3 mL of EtOAc (or Et_2O) and 200 μL of a dodecane stock (20 μL/mL in EtOAc) were added to the reaction and thoroughly mixed. An aliquot was removed for GC analysis. Isolated yields were obtained by extraction with Et_2O (x2), the combined organic layers dried over $MgSO_4$, filtered and concentrated *in vacuo* (>400 mbar, room temperature). The crude product was purified as described below.

General Protocol for Tandem Metathesis–Epoxidation with *Z*-Stilbene (1) and *Z*-2-Butene (*Z*-2a)

All reactions were set up open to air in 27-mL headspace crimp cap vials (Sigma-Aldrich). In general, it was found that the minimum substrate loading required to obtain reliable and reproducible results was 0.054 mmol. To a solution of D-glucose (300 μL of 1M stock in 200 mM KPi), $NADP^+$ (30 μL of a 20 mM stock in 100 mM KPi), GDH (20 uL, 1 U/mL), catalase (30 μL, 600 U/mL) and 75 μL DMSO (2.5 *v/v*%) in 200 mM phosphate buffer pH 8.1 (to 3 mL) was added freshly prepared P450 RLYF lysate (9 nmol, 0.017 mol %). *Z*-Stilbene (1, 9.6 μL, 9.7

mg, 0.054 mmol, 1 equiv.) was added to the top of the aqueous reaction mixture (avoid vial walls). The ruthenium catalyst C2 (2 mg, 0.0033 mmol, 6 mol %) was carefully added to the small organic layer, avoiding agitation that might cause the catalyst solution to sink (see Figure S3 in SI) or stick to the walls. The vial was quickly sealed with a crimp cap and pressurized with *Z*-2-butene gas, *Z*-2a (6 psig, 7.4 equiv.). The reaction was run in a shaking incubator at 100 rpm, 27 °C for 16 h. It is important to note that, in order to keep enough oxygen in the system for the epoxidation reaction, the vial was not evacuated prior to addition of *Z*-2-butene. In addition, using *E*-2-butene or a mixture of *E*- and *Z*-2-butene affords low yields of *E*-3a, presumably because these two gases contain traces of 1,3-butadiene, a compound that is poisonous to alkylidene-based metathesis catalysts.[28] Final concentrations are as follows: 3.3 μM P450, 100 mM glucose, 0.2 mM $NADP^+$, 1 U/mL GDH, 600 U/mL catalase, 18 mM *Z*-stilbene, 6 psig *Z*-2-butene. Reactions were extracted with 9 mL of ethyl acetate containing 1 mM eicosane, dried over Na_2SO_4, filtered and analyzed by GC. The crude products were purified as described below.

General Protocol for Tandem Reaction Using *Z*-Stilbene (1) and *E*-3-Hexene (*E*-2b)

All reactions were set up open to air in either 10-mL or 27-mL headspace crimp cap vials. To a solution of D-glucose (300 μL of 1M stock in 200 mM KPi), $NADP^+$ (30 μL of a 20 mM stock in 100 mM KPi), GDH (20 uL, 1 U/mL), catalase (30 μL, 600 U/mL), and DMSO (75 μL, 2.5 *v/v*%) in 200 mM phosphate buffer pH 8.1 (to 3 mL) was added freshly prepared P450 RLYF lysate (30 nmol, 0.1 mol %). *E*-3-Hexene (20 μL, 13.6 mg, 0.16 mmol, 3 equiv) and *Z*-Stilbene (**1**, 9.6 μL, 9.7 mg, 0.054 mmol, 1 equiv.) were carefully added to the top of the aqueous reaction mixture (avoiding vial walls). The ruthenium catalyst **C2** (1 mg, 0.0033 mmol, 3 mol %) was carefully added to the small organic layer, avoiding agitation that might cause the catalyst solution to sink (see picture in SI) or stick to the walls. The vial was sealed with a crimp cap and the reaction was run in a shaking incubator at 100 rpm, 27°C for 16 h. Reactions were extracted with 9 mL of ethyl acetate containing 1 mM eicosane, dried over Na_2SO_4, filtered and analyzed by GC.

ASSOCIATED CONTENT

Supporting Information.

This material is available free of charge via the Internet at http://pubs.acs.org.

Supplemental tables and figures, and additional information of experimental procedures and methods, characterization data, and NMR spectra of organic products.

AUTHOR INFORMATION

Corresponding Author

Huimin Zhao: zhao5@illinois.edu

John F. Hartwig: jhartwig@berkeley.edu

Present Addresses

Carl Denard: University of Texas-Austin, Chemistry Department, 2500 Speedway, Austin Texas, 78712

Mark J. Bartlett: Gilead Sciences, Inc., 333 Lakeside Dr., Foster City, CA 94404

Lu Lu: Civil and Environmental Engineering, 4146 Newmark, 205 N. Mathews Ave. Urbana, IL 61801

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

ACKNOWLEDGMENT

This work was supported by NSF under the CCI Center for Enabling New Technologies through Catalysis (CENTC) Phase II Renewal, CHE-1205189. Electronic Supplementary Information (ESI) available: supplementary tables and figures, experimental procedures and product characterization data. See DOI: 10.1039/c000000x/

References

1 Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K. *Nature*, **2012**, *485*, 185-194.

2 (a) Denard, C. A.; Hartwig, J. F.; Zhao, H. *ACS Catal*, **2013**, *3*, 2856-2864; (b) Wallace, S.; Balskus, E. P. *Curr Opin Biotechnol*, **2014**, *30*, 1-8.

3 (a) Pamies, O.; Bäckvall, J. E. *Chem Rev*, **2003**, *103*, 3247-3262; (b) Turner, N. J. *Curr Opin Chem Biol*, **2004**, *8*, 114-119; (c) Engström, K.; Johnston, E. V.; Verho, O.; Gustafson, K. P. J.; Shakeri, M.; Tai, C.-W.; Bäckvall, J.-E. *Angew Chem Int Ed*, **2013**, *52*, 14006-14010.

4 Tenbrink, K.; Seßler, M.; Schatz, J.; Gröger, H. *Adv Synth Catal*, **2011**, *353*, 2363-2367.

5 For representative examples, see: (a) Köhler V.; Wilson, Y. M.; Dürrenberger M.; Ghislieri D.; Churakova E.; Quinto T.; Knörr L.; Häussinger D.; Hollmann F.; Turner, N. J.; Ward, T. R. *Nat Chem*, **2013**, *5*, 93-99; (b) Wang, Z. J.; Clary, K. N.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. *Nat Chem*, **2013**, *5*, 100-103; (c) Lee, Y.; Umeano, A.; Balskus, E. P. *Angew Chem Int Ed*, **2013**, *52*, 11800-11803.

6 Whitehouse, C. J.; Bell, S. G.; Wong, L. L. *Chem Soc Rev*, **2012**, *41*, 1218-1260.

7 Whitehouse, C. J. C.; Yang, W.; Yorke, J. A.; Tufton, H. G.; Ogilvie, L. C. I.; Bell, S. G.; Zhou, W.; Bartlam, M.; Rao, Z.; Wong, L.-L. *Dalton Trans*, **2011**, *40*, 10383-10396.

8 For representative examples, see: (a) Kille, S.; Zilly, F. E.; Acevedo, J. P.; Reetz, M. T. *Nat Chem*, **2011**, *3*, 738-743; (b) Lewis, J. C.; Bastian, S.; Bennett, C. S.; Fu, Y.; Mitsuda, Y.; Chen, M. M.; Greenberg, W. A.; Wong, C. H.; Arnold, F. H. *Proc Natl Acad Sci U S A*, **2009**, *106*, 16550-16555.

9 Denard, C. A.; Huang, H.; Bartlett, M. J.; Lu, L.; Tan, Y.; Zhao, H.; Hartwig, J. F. *Angew Chem Int Ed Engl*, **2014**, *53*, 465-469.

10 (a) Connon, S. J.; Blechert, S. *Angew Chem Int Ed Engl*, **2003**, *42*, 1900-1923; (b) Binder, J. B.; Raines, R. T. *Curr Opin Chem Biol*, **2008**, *12*, 767-773.

11 (a) Stewart, I. C.; Douglas, C. J.; Grubbs, R. H. *Org Lett*, **2008**, *10*, 441-444; (b) Sanford, M. S.; Love, J. A.; Grubbs, R. H. *J Am Chem Soc*, **2001**, *123*, 6543-6554.

12 Chatterjee, A. K.; Choi, T. L.; Sanders, D. P.; Grubbs, R. H. *J Am Chem Soc*, **2003**, *125*, 11360-11370.

13 Carmichael, A. B.; Wong, L. L. *Eur J Biochem*, **2001**, *268*, 3117-3125.

14 Li, Q. S.; Ogawa, J.; Schmid, R. D.; Shimizu, S. *Appl Environ Microbiol*, **2001**, *67*, 5735-5739.

15 Kubo, T.; Peters, M. W.; Meinhold, P.; Arnold, F. H. *Chemistry*, **2006**, *12*, 1216-1220.

16 See the Supplementary Information for details of the point mutations that comprise these P450-BM3 variants.

17 Nagao, T.; Mitamura, T.; Wang, X. H.; Negoro, S.; Yomo, T.; Urabe, I.; Okada, H. *J Bacteriol*, **1992**, *174*, 5013-5020.

18 (a) Johannes, T. W.; Woodyer, R. D.; Zhao, H. *Appl Environ Microbiol*, **2005**, *71*, 5728-5734; (b) Johannes, T. W.; Woodyer, R. D.; Zhao, H. *Biotechnol Bioeng*, **2007**, *96*, 18-26; (c) Liu, D. F.; Ding, H. T.; Du, Y. Q.; Zhao, Y. H.; Jia, X. M. *Appl Biochem Biotechnol*, **2012**, *166*, 1301-1313; (d) McLachlan, M. J.; Johannes, T. W.; Zhao, H. *Biotechnol Bioeng*, **2008**, *99*, 268-274; (e) Woodyer, R.; van der Donk, W. A.; Zhao, H. *Comb Chem High Throughput Screen*, **2006**, *9*, 237-245.

19 Kühnel, K.; Maurer, S. C.; Galeyeva, Y.; Frey, W.; Laschat, S.; Urlacher, V. B. *Adv Synth Catal*, **2007**, *349*, 1451-1461.

20 Ryan, J. D.; Clark, D. S. *Biotechnol Bioeng*, **2008**, *99*, 1311-1319.

21 Tee, K. L.; Dmytrenko, O.; Otto, K.; Schmid, A.; Schwaneberg, U. *J Mol Catal B: Enz*, **2008**, *50*, 121-127.

22 The absolute configuration of epoxide 5a was determined by chiral SFC co-injection with authentic (*S,S*)-5a.

23 Koya, S.; Nishioka, Y.; Mizoguchi, H.; Uchida, T.; Katsuki, T. *Angew Chem Int Ed Engl*, **2012**, *51*, 8243-8246.

24 (a) Huang, W. C.; Cullis, P. M.; Raven, E. L.; Roberts, G. C. *Metallomics*, **2011**, *3*, 410-416; (b) Tee, K. L.; Schwaneberg, U. *Angew Chem Int Ed Engl*, **2006**, *45*, 5380-5383; (c) Fruetel, J. A.; Mackman, R. L.; Peterson, J. A.; Ortiz de Montellano, P. R. *J Biol Chem*, **1994**, *269*, 28815-28821.

25 Lin, M.; Tesconi, M.; Tischler, M. *Int J Pharm*, **2009**, *369*, 47-52.

26 Alcalde, M.; Farinas, E. T.; Arnold, F. H. *J Biomol Screen*, **2004**, *9*, 141-146.

27 The yield obtained in the tandem reactions is significantly higher than the theoretical yield from separate metathesis (37%) and epoxidation (34%) reactions. We hypothesize that this is a result of the low aqueous solubility of E-β-methylstyrene (ca. 0.11 mg/mL), which means that the epoxidation of 27 μmol (3.19 mg E-3a) and 10 μmol (37% of 27 μmol) of E-3a have the same amount of substrate available for epoxidation at a given time. This is also reflected in the higher yield obtained with KT32 on a smaller scale (Table 3, Entry 6). However, in general it was found that the minimum substrate loading required to obtain reliable and reproducible metathesis reactions was 0.027–0.054 mmol.

28 Omura, T.; Sato, R. *J Biol Chem*, **1964**, *239,* 2370-2378.

29 Patel, J.; Elaridi, J.; Jackson, W. R.; Robinson, A. J.; Serelis, A. K.; Such, C. *Chem Commun (Camb)*, **2005**, *44*, 5546-5547.

Insert Table of Contents Graphic and Synopsis Here

An organometallic catalyst and a metalloenzyme working together in one-pot