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Cooperative Tandem Catalysis by an Organometallic Complex and a Metalloenzyme**

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Abstract: *Although chemical and enzymatic catalysts have been combined, reactions in which an organometallic catalyst and a metalloenzyme work cooperatively to create products, which cannot be generated with either catalyst alone or in comparable yields by sequential reactions of the two catalysts, have not been reported. Such reactions are challenging to achieve, in part because the milieu in which these catalysts operate are typically different. Herein, two classes of catalysts are demonstrated to react cooperatively in the same system. Combination of a metathesis catalyst and a P450 enzyme lead to a dynamic equilibration of alkenes and a selective epoxidation of the cross-metathesis products. These results show the potential of combining the two classes of catalysts for synthetic transformations.*

Organometallic chemistry and bioinorganic chemistry are two prominent scientific disciplines with each area providing highly efficient, yet contrasting, examples of catalytic transformations. While transition-metal complexes lie at the core of both disciplines, synthetic chemistry that exploits the complementary reactivity of the two systems in a cooperative fashion has rarely been developed. These two classes of catalysts often facilitate completely different types of reactions and are conducted in different vessels, if used in the same synthetic sequence. However, the union of these two types of catalysis has the potential to produce highly efficient and selective reactions which were not previously possible. Herein, we report a cooperative tandem catalytic system, whereby the combination of an olefin metathesis catalyst and cytochrome P450 enzyme converts a mixture of alkenes into a single epoxide. The metathesis catalyst leads to an equilibrating mixture of alkenes in the organic phase, and the P450 enzyme converts one member of the mixture of alkenes into the corresponding epoxide in the aqueous phase. The high yields from this cooperative reaction indicate that the two previously distinct classes of catalyst can be used simultaneously to form products which would not be formed by either catalyst alone or in comparable yields by conducting the two reactions sequentially.

Recently, synthetic catalysts and enzymes have been combined in new ways. In some cases, artificial enzymes have been generated by incorporating an organometallic site into a protein[1] or using a supramolecular approach.[2] In other cases, one-pot reactions containing synthetic catalysts and enzymes have been conducted to avoid isolation of a synthetic intermediate.[3] However, organometallic catalysts have rarely been combined with metalloenzymes because most metalloenzymes, which are involved in the bond construction steps of catalytic reactions, utilize cofactors or reagents which are incompatible with the organometallic system. When they have been combined, it is rare that the organometallic complex and the enzyme react cooperatively.[2,4] We define cooperative reactivity in this context as a process in which two species catalyze a reaction that would not occur with either catalyst alone and would occur in lower yield if the process were conducted as two sequential reactions. The most prominent example of the cooperative reactivity of an organometallic catalyst and an enzyme is the dynamic kinetic resolution of secondary alcohols and amines.[5,6] A system in which an organometallic system and a metalloenzyme react cooperatively has not been reported.

We sought to investigate whether metalloenzymes and organometallic complexes could catalyze a multistep process in a cooperative fashion. In recent years, a number of air- and water-stable organometallic catalysts have been developed.[7] In addition, many enzymes are stable toward or engineered to operate in organic solvents.[8] However, attempts to conduct chemoenzymatic one-pot reactions have often led to catalyst inactivation when the two catalysts are in the same medium.[9] Many organometallic complexes catalyze addition, isomerization, and metathesis reactions of alkenes,[10] whereas many enzymes catalyze the oxidation of alkenes to form epoxides or allylic alcohols.[11] Thus, one could envision a range of reactions catalyzed by organometallic complexes, leading to an equilibrating mixture of alkene isomers or to the production of a mixture of alkenes having different chain lengths, in

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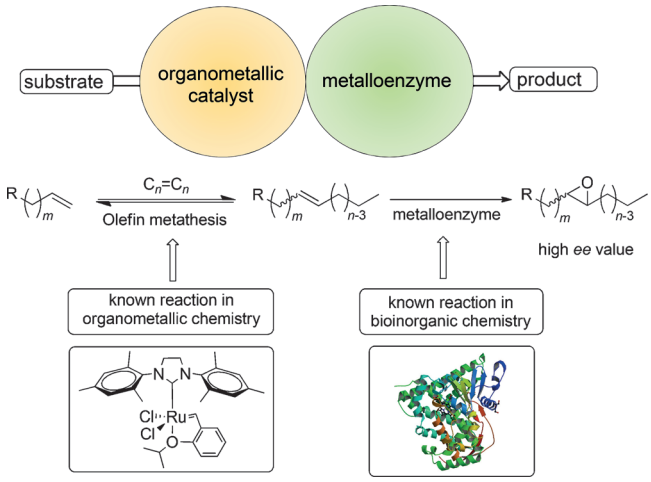


Figure 1. General scheme for tandem reaction.

tandem with the selective reaction of one member of the alkene mixture catalyzed by a metalloenzyme (Figure 1).

To begin our studies, we assessed reaction conditions that could be amenable to the combination of an olefin metathesis catalyst and a P450 enzyme. Our target cooperative reaction required that the metathesis catalyst be stable to the buffer components, NADPH regeneration system, oxygen, and the P450 enzyme. Similarly, the P450 enzyme must be stable to the ruthenium catalyst. However, if the reaction were to be run in a biphasic system, then the catalysts only need to be stable to the conditions under which their partner reacts.[5f,12] We considered that the known selectivity of certain P450 enzymes for the oxidation of fatty acids, alkenes, and alkanes, possessing narrow ranges of chain lengths, could be exploited for the selective epoxidation of one member of a mixture of alkenes, as shown in Figure 1. One example is the regioselective oxidation of C_{12}–C_{20} fatty acids by the wild-type P450 enzyme from *Bacillus megaterium* (P450 BM3).[13] A cross-metathesis reaction between an unsaturated fatty acid, which is too short for epoxidation by the enzyme, and a symmetrical alkene could create an unsaturated fatty acid having an appropriate chain length for epoxidation by P450 BM3. We found that P450 BM3 reacts with more than a fivefold preference for oxidation of the C_{13} chain over the C_{11} chain and with 82 % selectivity for hydroxylation of the ω-2 and ω-3 carbon atoms of tridecanoic acid (see Table S1 in the Supporting Information). On the basis of this result, we hypothesized that a C_{13} fatty acid containing an alkene at the ω-2 position would undergo selective epoxidation when present in an equilibrium mixture of 10-undecenoic acid and a nonreactive symmetrical alkene such as *trans*-3-hexene (see Figure S1).

To find a metathesis catalyst suitable for the cooperative catalytic process, we evaluated five variants of the Hoveyda–Grubbs second generation complexes (Figure 2), which have been shown to be air stable and to operate in protic media.[14] The activity of these catalysts was tested toward cross-metathesis in a biphasic medium containing the P450 in buffered water as the aqueous phase. Isooctane was used as the organic phase because it has been shown to be both

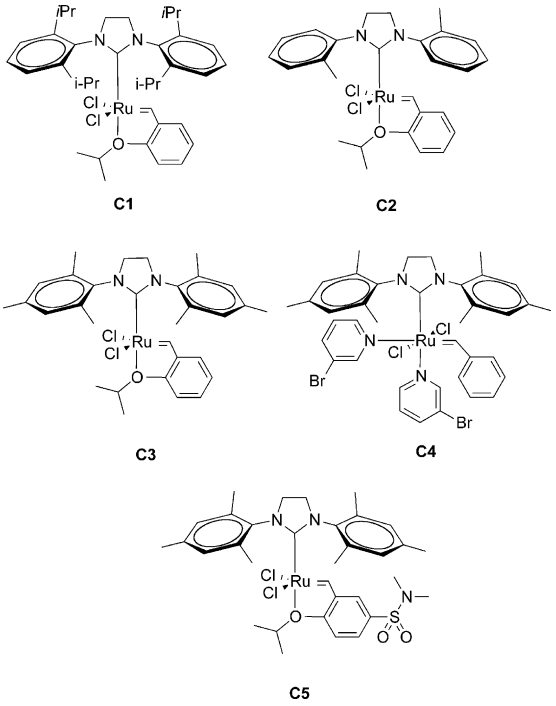


Figure 2. Catalysts tested in this study.

a suitable solvent for olefin metathesis,[15] and a biocompatible solvent in biphasic reactions involving P450 enzymes.[16] The reactivity and relative stability of the five ruthenium carbene catalysts for the cross-metathesis of 10-undecenoic acid and *trans*-3-hexene in this biphasic medium are shown in Figure 3.

These reactions produced an equilibrating mixture of cross-metathesis products, *cis* and *trans*-10-tridecenoic acids, as well as the product of self-metathesis, 10-undecenoic acid. We quantified the relative stability of each catalyst by measuring the conversion of 10-undecenoic acid after its addition in several batches. The catalysts **C4** and **C5** did not induce the metathesis reaction under these conditions, but the

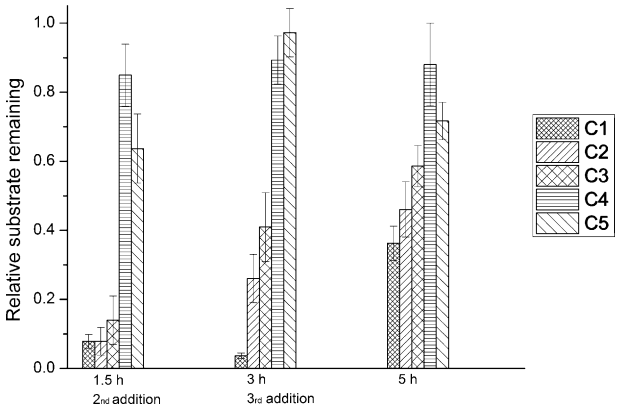


Figure 3. Evaluation of catalyst stability in 1:4 v/v isooctane: buffer (buffer contained 5 μM P450 BM3 lysate, 0.2 mM $NADP^+$). Cross-metathesis reactions were started with 25 mM 10-undecenoic acid and 5 equiv of *trans*-3-hexene. At 1.5 h and 3 h, an additional batch of 10-undecenoic acid (25 mM final concentration relative to the isooctane phase) was added. The reaction was monitored by GC.

catalyst **C3** led to 50% conversion of the unsaturated acid added in two batches. The catalysts **C1** and **C2** lead to conversion of the unsaturated fatty acid into the metathesis products, even after addition of a third batch of alkene. Because both **C1** and **C2** performed almost equally, subsequent experiments were conducted with the more soluble catalyst **C1**.

To confirm that the P450 enzyme retains catalytic activity in the presence of the ruthenium metathesis catalyst, we conducted the hydroxylation of tridecanoic acid using P450 BM3 and a previously engineered phosphite dehydrogenase (PTDH) regeneration system[17] in a biphasic medium in the presence of 5 mol% of **C1**. Additionally, the oxidation of tridecanoic acid was performed in pure buffer in the presence of **C1**. In this case, **C1** was made soluble in the buffer by adding 2.5 wt% of the amphiphile PEG-600/α-Tocopherol-based diester of sebacic acid (PTS).[18] Under both reaction conditions, the activities of the P450 enzyme and phosphite dehydrogenase regeneration system seemed unaffected by the presence of the 5 mol% of **C1**.[19]

Having found two catalysts that act on their respective substrates in the presence of the other catalyst and its medium, we assessed whether the two systems could work cooperatively. The yields of sequential metathesis and epoxidation reactions are constrained because the concentration of the C_{13} alkene is limited by the equilibrium ratio of the different alkenes. However, in a cooperative, dynamic reaction, the C_{13} alkene would be regenerated to allow additional epoxidation by the P450 enzyme. Thus, the yield of a cooperative process can be higher than the yield of these two sequential reactions.

Reactions containing **C1**, 10-undecenoic acid, and *trans*-3-hexene were monitored over time (Figure 4A). At a low ratio of hexene to acid substrate **1** (1.5:1), the product from self-metathesis of **1** was formed preferentially.[20] In the absence of P450 BM3, 18% yield of the cross-metathesis product **2** and 77% of the self-metathesis product **3** were obtained. In the presence of the two starting alkenes and **C1**, the P450 BM3 enzyme reacted with the cross-metathesis product **2** to form the epoxide **4** with 100% selectivity over 1,2-epoxyundecanoic acid, whereas the C_{13} alkene was continuously regenerated by the cross-metathesis process (Figure 4B).[21] Both the *cis*- and *trans*-10-tridecenoic acids were converted into the corresponding epoxides with greater than 95% selectivity over formation of the products from hydroxylation at the ω-1 or ω-4 carbon atoms (see Figures S2–S6). At the end of the reaction, the concentration of the product from the self-metathesis of **1** (compound **3**) was about 40% lower in the dual catalyst system than it was in the metathesis reaction without the P450 enzyme (Table 1, entries 1a and 1b, and Figure S7A). Moreover, the concentration of the fatty acid **1** at 15 hours in the dual catalyst system was 20% lower than it was after the metathesis reaction alone (Figure S7B). These data provide evidence that the cross-metathesis product was siphoned from the metathesis equilibrium to form the epoxide by reaction of the C_{13} alkene with the P450 BM3 enzyme. Under these initial conditions, the final yield of 10,11-epoxytridecanoic acid was 27%. This yield is 1.5 times higher than the hypothetical yield of 18% from a stepwise process that would result from the isolation of alkenes from the metathesis reaction and 100% yield of epoxide from the enzymatic epoxidation of the cross-metathesis product (Table 1, entries 1a and 1b).

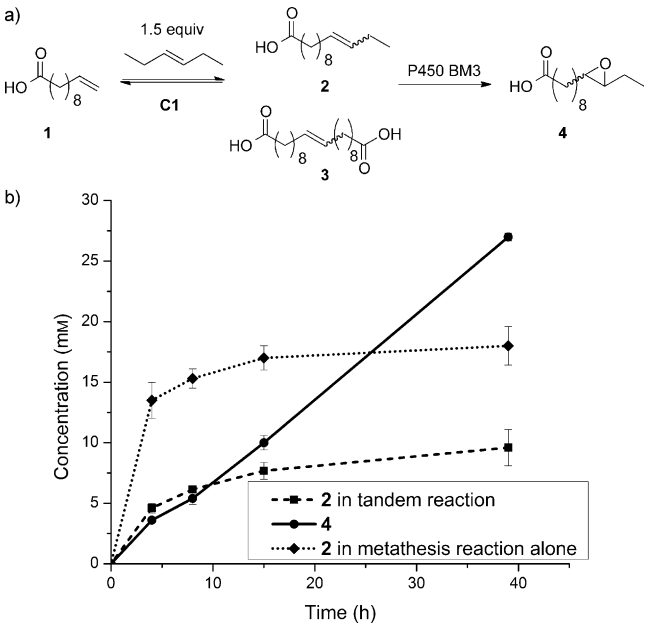


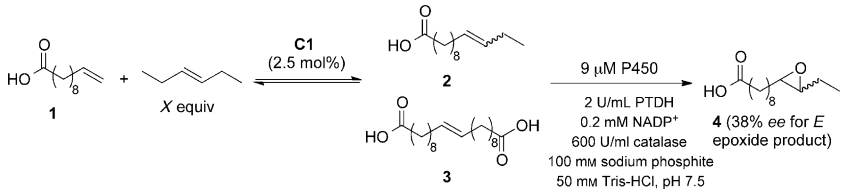
Figure 4. Tandem cross-metathesis-epoxidation monitored over time. A) Reaction scheme. B) Olefin metathesis-epoxidation reaction monitored over time. This reaction corresponds to the time course for entries 1a and 1b in Table 1.

Table 1 also shows the yields of **4** from a series of reactions conducted with the two catalysts together and varied ratios of *trans*-3-hexene and 10-undecenoic acid. These data allow comparison of the yields of **4** from the tandem process to the highest possible yields which could be obtained if the two reactions were run sequentially. Decreasing the concentration of **1** resulted in an increase in the yield of **4** from 35% (50 mM; entry 2a) to 70% (12.5 mM; entry 4a). As shown in entry **4b**, only a 26% theoretical yield of **4** could be obtained if the two reactions were run sequentially.

The concentration of the cross-metathesis product remaining in the reactions detailed in entries 1a and 2a of Table 1 was significantly lower than that of diacid **3**. The compound **3** progressively precipitated as the reaction proceeded, thus becoming less available to regenerate the cross-metathesis product. Only 10–15% of **3** was dissolved in the two layers after 39 hours, as determined by centrifugation of aliquots of the two layers, extraction with ethyl acetate, and GC analysis. As a result, further addition of **C1** did not significantly increase the yield of **4** relative to those shown in entries 1a and 2a.

By increasing the ratio of *trans*-3-hexene to **1** from 3:1 to 10:1, the accumulation of the self-metathesis product was suppressed. Under these reaction conditions, yields of up to 90% of **4** were obtained within 12 hours (Table 1, entries 5a, 6a, and 7a, and see Figure S8). An assessment of the stability of the P450 enzyme under the tandem reaction conditions suggested that it is inactive by the end of this particular reaction.

Table 1: Tandem olefin cross-metathesis/epoxidation of 10-undecenoic acid with *trans*-3-hexene.



Entry	Reaction	Loading **1** [mM]	X Equiv.	**2**[b,c] [mM]	**3**[b] [mM]	**4**[b,c] [mM]	**4** Yield [%]	Yield increase[d]	TTN[e]
1a[f,g]	tandem	100	1.5	9.5	50.1[h]	27.3	27.3	1.5	3000
1b	metathesis[i]	100	1.5	18.0	77.0	–	–	–	–
2a	tandem	50	1.5	6.0	25.4	17.4	35.0	1.4	1930
2b	metathesis	50	1.5	12.7	35.3	–	–	–	–
3a	tandem	25	1.5	3.3	8.6	12.0	48.0	1.6	1328
3b	metathesis	25	1.5	7.4	16.7	–	–	–	–
4a	tandem	12.5	1.5	1.5	2.3	8.7	–	2.6	970
4b	metathesis	12.5	1.5	3.3	9.2	–	–	–	–
5a	tandem	25	3	1.1	1.8	22.1	–	2.0	2460
5b	metathesis	25	3	11.3	12.9	–	–	–	–
6a	tandem	25	5	1.3	1.5	20.6	–	1.2	2288
6b	metathesis	25	5	17.4	6.9	–	–	–	–
7a	tandem	25	10	0.43	2.0	22.6	–	1.4	2510
7b	metathesis	25	10	16.2	6.6	–	–	–	–

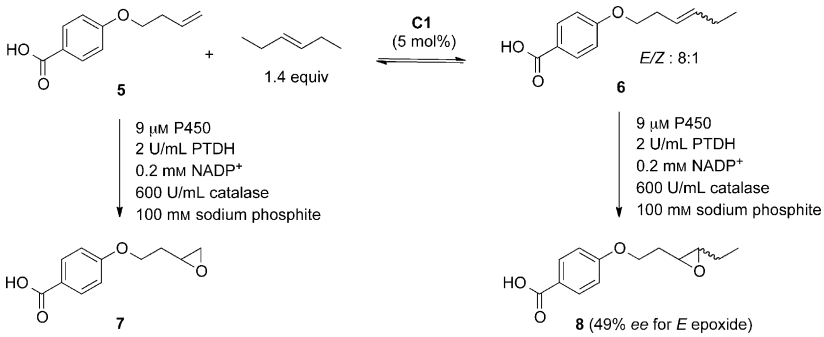
[a] 1:4 v/v isooctane: buffer, 20 mM eicosane as internal standard, RT, 180 rpm, 12 h. [b] Determined by GC-MS using synthesized authentic standards. [c] *E*/*Z* = 6:1. [d] [**4**/**2** in metathesis reaction alone]. [e] [mmol **4**/mmol P450]. [f] 8 mM of **1** unreacted. [g] P450 BM3 and PTDH were added in equal batches at 0 h, 8 h, and 15 h to a final concentration of 9 mM and 2 U mL^{-1}, respectively. **1** was added in two batches (0 h and 15 h), reaction time was 39 h. [h] 10–20% of **3** was found to be dissolved in the isooctane phase prior to extraction. [i] Metathesis reaction without P450.

This cooperative catalysis extends beyond the two alkenes shown in Figure 3 A. Studies on the reactions of unsaturated alkenyloxybenzoic acid derivatives showed that the P450 BM3 enzyme epoxidized (*E*)-4-(hex-3-en-1- yloxy)benzoic acid (**6**), an unnatural substrate, with a rate (judged by the consumption of NADPH) of 151 nmol NADPH·nmol P450^{-1} min^{-1}. Oxidation of an equimolar concentration of 4-(but-3-en-1-yloxy)benzoic acid (**5**) and **6** in the presence of P450 BM3 predominantly formed the epoxide **8** from alkene **6** with only traces of the epoxide **7** from oxidation of **5** (see Figures S9, S12, and S13).[22] Moreover, the cross-metathesis of **5** with 1.4 equivalents of *trans*-3-hexene in a biphasic system consisting of dioctyl phthalate and buffer, formed the 3-alkene **6** in 58% yield with little formation of the product from self-metathesis of **5** (Table 2, entry 2, and see Figures S10–12).

On the basis of these background studies, we conducted the tandem metathesis/epoxidation reaction with **5** and *trans*-3-hexene in the presence of **C1** and P450 BM3. The yields of the epoxides **7** and **8** from the oxidations of **5** and the cross-metathesis product **6** in this tandem system were 18% and 75%, respectively.[20] This yield of **8** is higher than the theoretical 58% yield when the two reactions are performed sequentially (Table 2, and Figure S16). This result shows that the process can be conducted with alkenes which are unnatural substrates for epoxidation by P450 BM3.

The strategy revealed in this work should be applicable to a number of chemical transformations. For example, a transition-metal complex that catalyzes the *cis–trans* isomerization of alkenes, followed by selective epoxidation of one alkene isomer could lead to a single epoxide isomer if an enzyme can be developed that stereospecifically epoxidizes internal alkenes. Likewise, equilibration of internal and

Table 2: Tandem olefin cross-metathesis/epoxidation of 4-butenyloxybenzoic acid with *trans*-3-hexene.



Entry[a]	Reaction	Loading **5** [mM]	**6**[b,c] [mM]	**7**[b] [mM]	**8**[b,c] [mM]	Yield **8** [%]	Yield increase[d]
1	tandem	15	0.7	3.1	11.3	74	1.3
2	metathesis	15	8.7[e]	–			

[a] 1:4 v/v dioctylphthalate: buffer, RT, 180 rpm, 24 h. [b] Determined by HPLC at $\lambda = 254$ nm using synthesized authentic standards. [c] *E*/*Z* = 8:1, determined by GC. [d] [**8**/**6** in metathesis]. [e] The yield for the self-metathesis of **5** was less than 5%.

terminal alkenes could lead to a single terminal oxidation product if a P450 enzyme can be developed to be selective for reaction of a terminal alkene over the internal alkene. Furthermore, catalysts for alkane metathesis could be combined with a P450 enzyme which is selective for the terminal hydroxylation of alkanes having certain chain lengths to form detergent-length alcohols from a mixture of alkanes of varying chain length. By marrying different classes of catalysts containing transition-metal centers, we envision the potential to develop catalytic systems containing both small and macromolecular catalysts which begin to resemble the assemblies of enzymes in biosynthetic systems.

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[21] The compound **4** was produced in 38% *ee* for the *E*-configured epoxide. The *ee* value of **4** obtained in the absence of the metathesis catalyst was a similar, but slightly higher 57%. The origin of this small difference in enantioselectivity is not clear, given that **8** gave essentially the same *ee* value in the presence or the absence of the metathesis catalyst.

[22] Compound **8** was produced in 49% *ee* for the *E* epoxide. The *ee* of **8** obtained in the absence of the metathesis catalyst was 51%.

