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# Chemoenzymatic C,C-Bond Forming Cascades by Cryptic Vanadium Haloperoxidase Catalyzed Bromination

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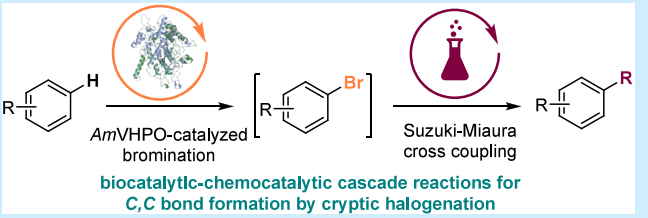
**ABSTRACT:** Inspired by natural cryptic halogenation in *C,C*-bond formation, this study developed a synthetic approach combining biocatalytic bromination with transition-metal-catalyzed cross-coupling. Using the cyanobacterial *Am*VHPO, a robust and sustainable bromination-arylation cascade was created. Genetic modifications allowed enzyme immobilization, enhancing the compatibility between biocatalysis and chemocatalysis. This mild, efficient method for synthesizing biaryl compounds provides a foundation for future biochemo cascade reactions harnessing halogenation as a traceless directing tool.






Halogen-containing compounds are well-known for their vast applications in pharmaceutical and agricultural industries.[1] In organic synthesis, installing a halogen atom can open a plethora of further transformations, particularly carbon−carbon (*C,C*)coupling reactions. Nature also takes advantage of the beneficial properties of *C,X*-bonds and uses them as a strategic tool for assembling structurally complex frameworks.[2] Biochemical reactions triggered by cryptic halogenations have recently been observed in the biosynthesis of cyclindrophanes **1** (Friedel−Crafts alkylation),[3] marinopyrrole A (**2**, *C,N*-biaryl coupling),[4] and merochlorine D (**3**, *α*-hydroxy-ketone rearrangement).[5] By harnessing in situ generated *C,X*-bonds for *C,C*-bond formations, highly efficient multistep sequences are performed in a straightforward way.

Inspired by nature's synthetic strategy, our group aims to emulate such processes.[6] In this article, we establish halogenation-triggered cascade reactions as a tool in organic synthesis by integrating *AmVHPO*-catalyzed bromination in a one-pot reaction with a Pd-catalyzed cross-coupling reaction. Such pathways are highly rewarding,[7] as they offer improved step economics[8] while reducing resources compared to conventional organic synthesis. Integrating biocatalytic processes into chemosynthetic regimes offers multiple advantages.[9] However, successfully integrating enzymes with chemocatalysts is still challenging because of their inherently different operating conditions, often requiring compartmentalization of at least one of the catalysts.[10] In chemical synthesis, halogenations of $sp^2$-carbons proceed mostly via electrophilic halogenations using stoichiometric amounts of either toxic, often difficult-to-handle, corrosive dihalogens or organic electrophilic reagents that produce large quantities of organic waste. Biocatalytic halogenations serve as an exciting alternative.[11] Haloperoxidases constitute here a promising enzyme class because of their ability to convert a broader substrate range and their cost- and atom-economic cofactors vanadate and $H_2O_2$ (Figure 1b).[12] These characteristics inherently position VHPOs as versatile choices for catalytic applications in biotechnology.

Vanadium-dependent haloperoxidases (VHPO) have lately gained recognition for their applications in organic synthesis,[13] even catalyzing reactions beyond their natural scope. The catalytic oxidative bromination requires cheap halide salts, sodium vanadate as a prosthetic group, and $H_2O_2$ as an

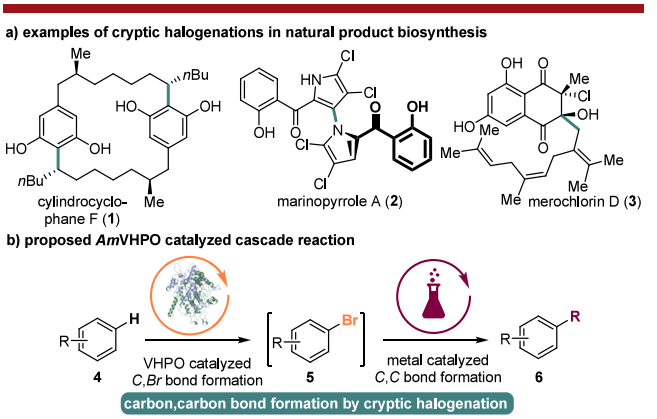



**Figure 1.** Cryptic halogenations (a) in nature during the synthesis of cyclindrophanes, marinopyrroles, and merochlorines and (b) their application in the arylation and alkenylation of (hetero)aromatic compounds.

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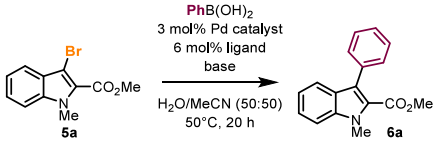
oxidant, providing a cost-effective and atom-economic system. Our group has previously investigated the haloperoxidase *Am*VHPO from the cyanobacterium *Acaryochloris marina*.[13i,14] *Am*VHPO exhibits exceptional robustness toward organic solvents, temperature, and $H_2O_2$, making it ideally suited for its application in organic transformations.[13h,i] We established a protocol for *Am*VHPO-catalyzed brominations of diverse aromatic compounds, achieving good to excellent yields and selectivities. Combining *Am*VHPO with photochemically generated $H_2O_2$ gave access to even milder reaction conditions and further showed the potential of this biocatalyst.[13h,13,15]

Generally, fundamental differences must be overcome between enzymatic transformations and transition-metal catalysis to effectively combine a Pd-catalyzed cross-coupling reaction with an enzymatic transformation. The aqueous environment and high dilution needed for enzymatic reactions are vastly untypical for most chemical conversions. For the enzymatic bromination with *Am*VHPO, the highest conversion was achieved at 7.5 mM,[13h,i] a substrate concentration substantially lower than the concentration used in most organic reactions. In our study, we mainly focused on converting (hetero)aromatic compounds since these are prevalent structural motifs found ubiquitously in bioactive drugs and pharmaceuticals.[16] Therefore, we started our investigations by selecting indole **5a** as our model substrate. As an optimized procedure for *Am*VHPO-triggered aromatic bromination has already been established before,[13h,i] we first focused on adjusting the reaction conditions of the Suzuki–Miyaura cross-coupling to those needed for the enzymatic bromination. Initially, screenings were performed in a highly diluted (7.5 mM), degassed aqueous environment, employing different Pd catalysts, ligands, and bases (Table 1 and Supporting Information (SI)). Product **6a** was afforded in quantitative yields using the Pd(II) catalysts $PdCl_2(PPh_3)_2$ and $Pd(OAc)_2$ in combination with the water-soluble trisodium 3,3′,3″-phosphanetriyltri(benzene-1-sulfonate) (TPPTS) ligand and $K_2CO_3$ as a base at 50 °C (Table 1, entries 3 and 4). Because of the superior solubility in the water/acetonitrile (50:50) mixture, further investigations were conducted with $Pd(OAc)_2$.

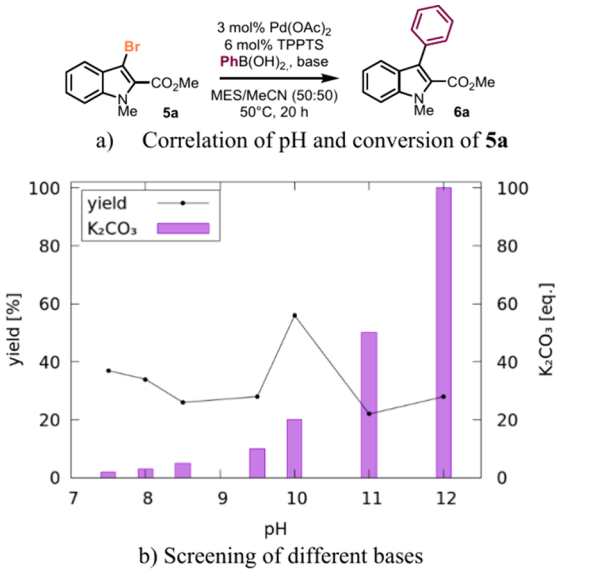
**Table 1. Optimization of the Suzuki–Miyaura Cross-Coupling Reaction in Water/Acetonitrile**[a]




| entry | Pd catalyst | base | ligand[d] | yield[b] [%] |
|---|---|---|---|---|
| 1 | $Na_2PdCl_4$ | $K_2CO_3$ | – | 58 |
| 2 | $Pd(PPh_3)_4$ | $K_2CO_3$ | – | 68 |
| 3 | $PdCl_2(PPh_3)_2$[c] | $K_2CO_3$ | TPPTS | quant. |
| 4 | $Pd(OAc)_2$ | $K_2CO_3$ | TPPTS | quant. |
| 5 | $Pd(OAc)_2$ | $K_3PO_4$ | TPPTS | 59 |
| 6 | $Pd(OAc)_2$ | $K_2CO_3$ | TPPTS | 85 |

[a]The reactions were carried out using 3-bromoindole **5a** (12.0 μmol, 1.0 equiv), $PhB(OH)_2$ (14.4 μmol, 1.2 equiv), ligand (0.72 μmol, 0.06 equiv), base (0.24 mmol, 2.0 equiv), and Pd catalyst (0.36 μmol, 0.03 equiv) in 1.6 mL degassed solvent mixture (50:50, 7.5 mM) at 50 °C for 20 h. [b]The yield was determined by GC MS from the crude mixture using dodecane as internal standard. [c]Suspension in $H_2O$ and MeCN. [d]Solvent mixture not degassed. TPPTS = trisodium 3,3′,3″-phosphanetriyltri(benzene-1-sulfonate).

The optimized aqueous conditions needed further adjustments when reacting in buffered solutions. An increase in pH was needed when turning from biocatalytic bromination to Pd-catalyzed *C,C*-coupling. While the *Am*VHPO requires pH 6.0 to achieve optimal halogenation activity, basic conditions are warranted for the Suzuki–Miyaura reaction. Therefore, the optimum pH for the second step was evaluated by varying the amount of $K_2CO_3$ (Figure 2a). The best yields were obtained using 20 equiv of $K_2CO_3$ at pH 10 (56%). Subsequent screening of bases showed that with an excess of $NEt_3$, the buffer capacity was likewise overcome (pH 10), and **6a** was isolated in 90% yield (Figure 2b, cf. SI chapter S4).




| entry | base | yield[b] [%] |
|---|---|---|
| 1 | $K_2CO_3$ | 56 |
| 2 | $K_3PO_4$ | 41 |
| 3 | $Cs_2CO_3$ | 17 |
| 4 | CsF | 34 |
| 5 | KF | 21 |
| 6 | $NEt_3$ | quant. (90)[b] |
| 7 | DIPEA | 26 |
| 8 | $NaHCO_3$ | 53 |
| 9 | $KHCO_3$ | 46 |

**Figure 2.** Influence of the base on the cross-coupling reaction.[a] [a]The reactions were carried out using 3-bromoindole **5a** (12.0 μmol, 1.0 equiv), $PhB(OH)_2$ (1.2 equiv), TPPTS (0.06 equiv), and $Pd(OAc)_2$ (0.03 equiv) in degassed MES buffer (pH 6.0, 50 mM) and MeCN (50:50, v/v, 7.5 mM) at 50 °C for 20 h. (a) Influence of pH on the conversion of the aqueous Pd-catalyzed cross-coupling reaction in MES buffer with 2–100 equiv $K_2CO_3$ as base. (b) Screening of different bases using 20 eq base. [a]GC yield was determined from the crude using dodecane as the internal standard. [b]Isolated yield. MES = 2-(*N*-morpholino)ethanesulfonic acid, DIPEA = diisopropylamine, TPPTS = trisodium 3,3′,3″-phosphanetriyltri(benzene-1-sulfonate).

Next, we tested the compatibility of the *C,C*-bond forming reaction with enzymatic bromination (see Table 2). In our initial attempt using soluble *Am*VHPO,[13h,i] we observed no conversion to the desired cross-coupling product **6a** (Table 2, entry 1). This lack of conversion may be attributed to the


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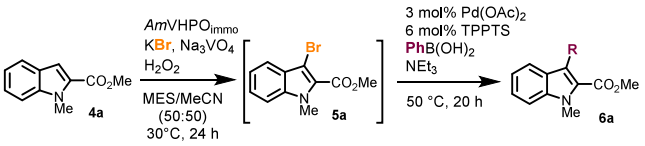
**Table 2. Optimization of the Cascade Reaction Combining Enzymatic Bromination and a Pd-Catalyzed Cross-Coupling Reaction**[a]



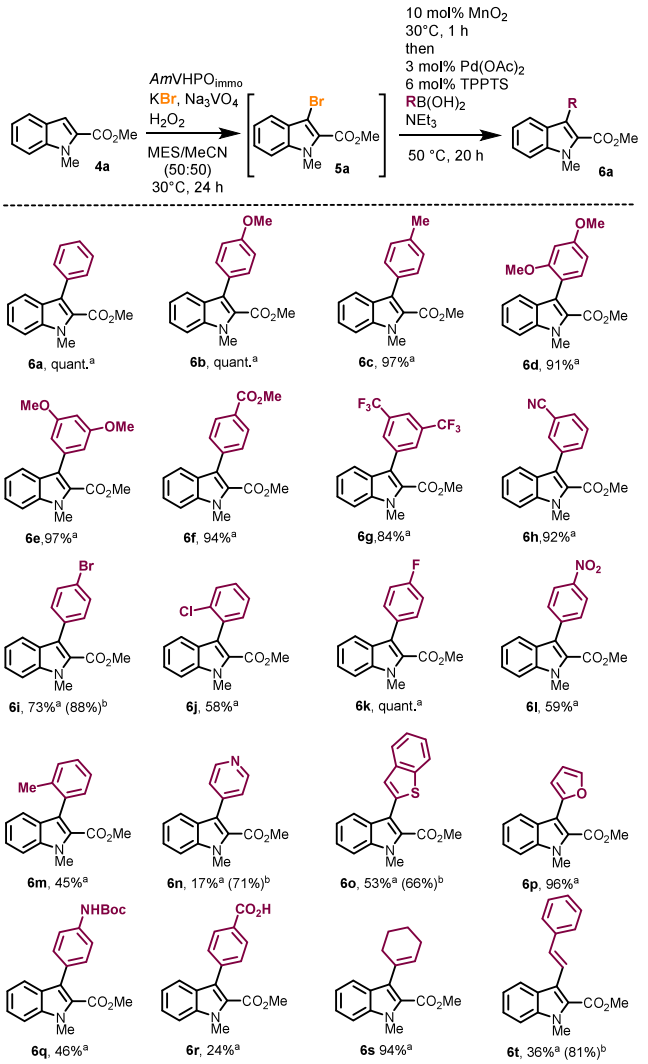

| entry | enzyme | $H_2O_2$ deactivation | yield[b] [%] |
|---|---|---|---|
| 1 | *Am*VHPO | – | – |
| 2 | *Am*VHPO$_{immo}$[c] | – | 41 |
| 3[d] | *Am*VHPO$_{immo}$[c] | – | 52 |
| 4[e] | *Am*VHPO$_{immo}$[c] | – | 69 |
| 5 | *Am*VHPO$_{immo}$[c] | 10 mol % $MnO_2$, 30 °C, 1 h | quant. |
| 6 | *Am*VHPO$_{immo}$[c] | 1.1 equiv $FeCl_3$, rt, 1 h | 94 |

[a]The reactions were carried out using indole **4a** (7.50 mM; 12.0 μmol), KBr (1.1 equiv), $H_2O_2$ (1.1 equiv), $Na_3VO_4$ (190 μM), *Am*VHPO (50 μL; 3 mg mL$^{-1}$; 6 U; preincubated with 300 μL of 30 mM $K_3VO_4$) in MES buffer (pH 6.0, 50 mM) and MeCN (1:1, 7.5 mM, total volume = 1.6 mL) were shaken at 30 °C and 1200 rpm for 24 h. After degassing the reaction mixture, boronic acid (1.2 equiv), TPPTS (0.06 equiv), $NEt_3$ (20 equiv), and $Pd(OAc)_2$ (0.03 equiv) were added, and the reaction mixture was stirred at 50 °C for 20 h. . [b]GC yield determined from the crude reaction mixture using dodecane as an internal standard. [c]HaloTag-HaloLink *Am*VHPO complex immobilized on HaloLink resin. [d]Additional 3 mol % $Pd(OAc)_2$ and 6 mol % TPPTS were added after 1 h. [e]Additional 3 mol % $Pd(OAc)_2$, 6 mol % TPPTS, and 1.2 equiv of $PhB(OH)_2$ were added after 1 h and 2 h. MES = 2-(*N*-morpholino)ethanesulfonic acid; TPPTS = trisodium 3,3′,3″-phosphanetriyltri(benzene-1-sulfonate).

inhibitory effect of Lewis basic amino acid side chains within the protein such as those in cysteines and histidines. Such moieties are known to coordinate the metal and thus hinder its catalytic ability.[17] To address this problem, we covalently attached the biocatalyst to solid support.[18] Therefore, we genetically fused a HaloTag construct into *Am*VHPO[19] and immobilized the tagged enzyme with the commercially available HaloLink resin.[19c] For details on cloning, protein production, purification, immobilization protocol, and enzyme loading, refer to SI 3.1 and 3.3 and 3.4, respectively. Despite *Am*VHPO's huge size due to its dodecameric structure, *Am*VHPO loading could be increased to 0.51 ± 0.03 mg mg$^{-1}$. The *Am*VHPO$_{HaloTag}$ complex exhibited comparable bromination activity in the standard monochlorodimedon assay to the soluble HisTagged enzyme (see SI chapter S3.3). After immobilizing *Am*VHPO$_{HaloTag}$ onto HaloLink resin (*Am*VHPO$_{immo}$), the enzyme did not interfere with the transition metal catalyst after simply centrifuging the reaction mixture after the enzymatic bromination step. This procedure increased the overall yield to 41% (Table 2, entry 2). Another increase in yield (52% and 69% from **4a**, respectively) was observed by adding additional portions of palladium, ligand, and boronic acid after each hour (Table 2, entries 3 and 4). Such observations hint at a decomposition of these reactants over time under the applied conditions. This behavior could be caused by residual $H_2O_2$ from the bromination step that interferes with the Pd species through oxidation. As lowering the $H_2O_2$ concentration diminished the yield of brominated indole **5a**, we set out to destroy the residual oxidant from the reaction mixture before adding the Pd species. The addition of reductants, such as $MnO_2$ (10 mol %) or $FeCl_3$ (1.1 equiv), and incubating the reaction mixture for another hour afforded the final product **6a** in quantitative and 94% overall yield, respectively (Table 2, entries 5 and 6). As $MnO_2$ needed to be employed only in catalytic amounts, it was used for all further transformations.

With a working one-pot method on hand, the scope of the reaction was explored. Regarding boronic acids, various substrates were compatible with our transformation (Scheme 1). Both electron-rich and electron-poor aryl boronic acids

**Scheme 1. Substrate Scope: Variation of the Boronic Acid Moiety**




[a]Isolated yield. [b]Yield based on recovered bromoindole **5a**. MES = 2-(*N*-morpholino)ethane sulfonic acid; TPPTS = trisodium 3,3′,3″-phosphanetriyltri(benzene-1-sulfonate).

could be employed, giving the corresponding products **6a**–**6t** in up to quantitative yields. Only the *p*-nitro-benzene **6l** showed a decreased yield of 59% due to solubility issues of the boronic acid. Furthermore, the sterically hindered *ortho*-chlorinated and *ortho*-methylated boronic acid resulted in a 58% and 45% yield of indoles **6j** and **6m**, respectively. Heterocyclic boronic acids were also successfully converted. For instance, furanyl boronic acid yielded indole **6p** with an impressive 96% yield, whereas boronic acids with 4-pyridyl and 2-benzothiophene functionalities showed binding to the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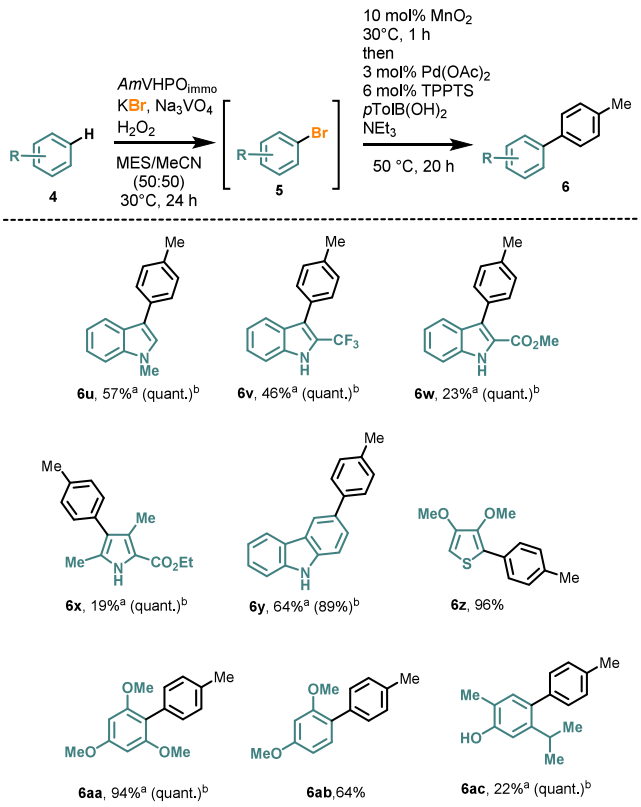
protein, thus hampering the conversion of intermediate **5a**. Nevertheless, the biaryl products **6n** and **6o** were obtained in 71% and 66% yield based on the recovered intermediate (bri) **6a**. Simple cyclohexenyl boronic acids afforded product **6s** in 94% isolated yield, and even the styrenyl derivative produced the desired product **6t** in 36% (81% bri) despite the styrenyl reactant's tendency to undergo polymerization.

The versatility of the cascade reaction prompted us to expand our exploration to include starting materials beyond the 2-carboxylic methyl ester indole **4a** (Scheme 2).[13h,i] For

**Scheme 2. Substrate Scope: Variation of the Aryl Moiety**




[a]Isolated yield. [b]Yield based on recovered starting material **4**. MES = 2-(*N*-morpholino)ethane sulfonic acid; TPPTS = trisodium 3,3′,3″-phosphanetriyltri(benzene-1-sulfonate).

instance, *N*-methylated indole **4u** was seamlessly converted to the desired monoarylated product **6u** in 57% yield. Despite the inherent instability of substrate **4u** and its brominated derivative **5u**, we did not observe any dibromination or oxidative decomposition of the indole moiety, underscoring the mildness of the reaction conditions. Indoles lacking substituents at the nitrogen atom were transformed into the corresponding products **6v** and **6w** only when electron-withdrawing substituents were positioned at C2. The biocatalytic cascade reaction was applicable to other heterocyclic substrates such as pyrroles (→**6x**), carbazoles (→**6y**), and thiophenes (→**6z**). The latter stood out as the electron-rich bis methoxylated thiophene **4z** forms a delicate, acid- and heat-sensitive brominated intermediate **5z** that is tough to handle in stepwise transformations. Notably, electron-rich benzene derivatives served as substrates, giving the biaryl products **6aa** and **6ab** in 94% and 64% yield, respectively. Also, monoterpene carvacrol (**4ac**) was successfully converted to corresponding biphenyl **6ac**.

Nature provides numerous examples of cryptic halogenations in complex natural product synthesis as an efficient way to forge *C,C* bonds. Inspired by this natural strategy, we elaborated a synthetic method combining biocatalytic bromination with transition-metal catalyzed cross-coupling reactions. In our setup, the cyanobacterial haloperoxidase *Am*VHPO was employed to activate the C−H bond through cryptic halogenation assisting *C,C* bond formation in a bromination-arylation cascade. The employed biocatalytic system is robust, simple, and easy to handle, thus making it a cost-effective, atom-economical, and sustainable alternative for halogenations. Simple genetic modification of the *Am*VHPO was applied to enable enzyme immobilization on resin to overcome incompatibility issues between the biocatalytic and chemocatalytic steps. The two-step-one-pot procedure presented here is proof of principle for the versatility of vanadium-dependent haloperoxidases in chemoenzymatic transformations and the enzymes' compatibility with organic synthetic setups. Overall, the mild, generally applicable, and efficient method for converting various electron-rich (hetero)aromatic scaffolds into biaryl compounds laid the foundation for future biocascade reactions, ranging from other transition-metal catalyzed transformations to photocatalytic and electrochemical reactions.

## ■ ASSOCIATED CONTENT

### Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.4c04108.

Experimental procedures, characterization of all compounds, and further information on the substrate scope are presented in the Supporting Information. (PDF)

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## Notes

The authors declare no competing financial interest.

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