Biocatalytic C–H Oxidation Meets Radical Cross-Coupling: Simplifying Complex Piperidine Synthesis

Authors: Jiayan He[1†], Kenta Yokoi[2†], Breanna Wixted[2], Benxiang Zhang[1], Yu Kawamata*[1], Hans Renata*[2], Phil S. Baran*[1]

Affiliations:

[1] Department of Chemistry, Scripps Research, 10550 North Torrey Pines Road, La Jolla, CA, 92037, United States.

[2] Department of Chemistry, BioScience Research Collaborative, Rice University, Houston, TX, 77005, United States.

[†]These authors contributed equally to this work.

[*]Correspondence to: yukawama@scripps.edu, hr28@rice.edu, pbaran@scripps.edu

Abstract:

Medicinal chemists in the modern era are targeting molecules with greater complexity to address increasingly challenging biological targets, a drive to enhance on-target specificity as well as physiochemical properties. As such, structures with greater fraction sp^3 (Fsp^3) character,[1–3] reminiscent to those found in nature,[4] are being synthesized. Many decades of synthetic methodology development have democratized access to flat, high sp^2 (for example biaryl linkages) which has led to the commercialization of innumerable medicines. Those approaches rely heavily on electrophilic aromatic substitution (such as halogenation) followed by Pd-based cross coupling.[5] In contrast, methods and strategies that allow for similarly modular and rapid construction of three-dimensional saturated molecules are less well developed. Here we exemplify a new approach for the rapid, modular, enantioselective construction of piperidine frameworks (the saturated analog of pyridine) that combines robust, tunable, and scalable biocatalytic methods with the logic of radical cross coupling. Thus, a set of reliable enzymatic systems (analogous to site-selective aromatic functionalization) provides scalable access to enantiopure hydroxyacid-containing piperidine derivatives that can be utilized to dramatically simplify routes to medicinally

important molecules and natural products by employing recently developed electrocatalytic couplings[6–10] (analogous to Pd-based cross couplings in aromatic systems). This study points to a different approach to rapidly access complex architectures that may appeal to both medicinal and process chemists alike.

Introduction:

From the standpoint of modern medicine, pyridines (and their benzannulated analogs) are perhaps the most important heteroaromatic unit due to their prevalence in FDA approved drugs.[11] Generally, these flat two-dimensional structures are diversified through sequential functionalization (halogenation, borylation, etc.) followed by transition-metal catalyzed cross-coupling (Figure 1A).[12] Complex piperidines, the fully saturated analog of pyridine, are becoming more popular as drug discovery campaigns gradually move towards more topologically complex, three-dimensional space (increased Fsp^3).[13,14] To be sure, polysubstituted piperidines are a frequently occurring motif in both medicinal leads and natural products such as compounds **1**-**5**, with disubstituted piperidines making up for 61% of current piperidine-containing pharmaceuticals.[15] Unlike their unsaturated pyridine relatives, the modular synthesis of such scaffolds with precise stereochemical control can be a complex puzzle.[16] Notwithstanding stereochemical considerations, the retrosynthesis of such compounds largely relies on polar bond disconnections that are often plagued with functional group (FG), protecting group (PG) and redox manipulations. Disconnections are often non-intuitive and derived on a case-by-case basis, with a heavy reliance on carbonyl and olefin chemistry (Figure 1B). Meanwhile, enzymatic C-H oxidation facilitated by directed evolution techniques is now recognized as one of the most powerful methods to scalably and site-specifically install a functional group onto unactivated Csp^3-H bonds.[17–19] In some sense this is analogous to tried-and-true functionalizations that are routine on pyridines, such as electrophilic aromatic substitution. Radical cross-coupling has also recently emerged as a versatile platform to couple common C-sp^3 bearing functional groups such as acids[20] and alcohols[21] in the same vein that Suzuki and related couplings are employed on pyridines. The potential power of combining these two strategies is vividly illustrated with the aspirational synthesis of disubstituted piperidine **8** as outlined in Figure 1B. Using conventional polar-bond analysis, this structure is accessed through a laborious sequence (8 steps) where the majority of steps do not make strategic C–C bonds.[22,23] In principle, direct access to piperidines such as **8**

might instead be achieved in two simple stages: biocatalytic C–H oxidation of inexpensive **10** followed by two sequential radical cross-couplings of the resulting acid and alcohol functional groups. Herein we present a proof of concept for this generalizable logic with scalable biocatalytic access to numerous hydroxylated piperidines followed by modular radical cross couplings. In this fashion, the synthesis of numerous high-value enantiopure piperidines can be dramatically simplified.

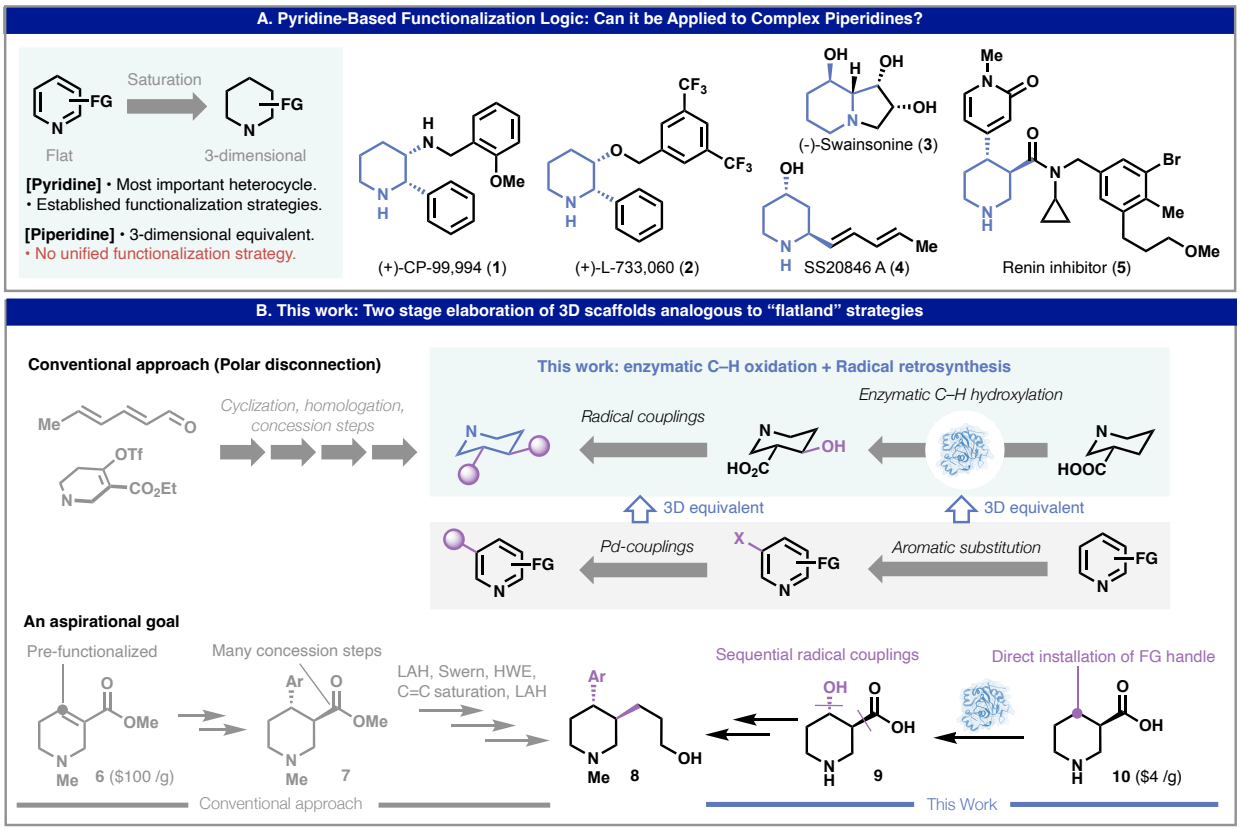

Figure 1. Combination of enzymatic C–H oxidation and radical couplings streamlines access to functionalized saturated heterocycles. This logic is analogous to halogenation/Pd-coupling sequence for flat molecule synthesis.

Scalable, Biocatalytic Synthesis of Hydroxylated Piperidines:

The first stage of this study required scalable enzymatic methods for site-specific C–H hydroxylation of inexpensive 2- and 3-carboxylated piperidines **11** and **10**. We have previously reported the gram scale syntheses of **14** and **17**.[24,25] However, a scalable and inexpensive synthesis of other building blocks with alternative hydroxylation patterns was still needed. *trans*-4-Proline hydroxylase (*trans*-P4H), a 2-ketoglutarate (α-KG) dependent dioxygenase with C4 hydroxylation

activity on L-proline, was previously reported to produce *trans*-5-hydroxy-L-Pip **12** in 68-78% conversion and 61% isolated yield on a preparative scale (35 mg) by using a purified enzyme or a recombinant *E. coli*.[26] Our initial attempt to reproduce this hydroxylation only resulted in 39% conversion, which was ultimately improved to 100% after significant optimization of the reaction parameters (equivalence of α-KG and Fe^{2+} and buffer pH, Fig. 2 and Table S1 in the Supporting Information). The optimized condition was amenable to gram scale synthesis, with higher substrate concentration than previously reported conditions (8 mM vs 1 mM). Reasoning that the stereochemical pattern on the hydroxylated building blocks would have significant influence on the stereochemical outcome of subsequent functionalizations, the synthesis of *cis*-5-hydroxy-L-Pip **13** was sought. Toward this end, an engineered α-KG-dependent L-pipecolic acid hydroxylase from *Xenorhabdus doucetiae* (XdPH YR)[27] and a proline-4-hydroxylase (P4H810) developed by Codexis[28] were examined. While the former negligibly converted L-Pip to the desired product, the latter produced 5-hydroxylated L-Pip **13** in a regio- and stereoselective manner with 80% conversion. Subsequent optimization, notably of the reaction pH, improved the conversion to 100% (Fig. 2 and Table S2 in the Supporting Information).

To date, there are no examples of enzymes that catalyze the hydroxylation of 3-carboxylated piperidine (nipecotic acid). To address this gap, a select pool of enzymes were screened for reaction on nipecotic acid (Fig. 2 and Table S3 in the Supporting Information). *trans*-P4H and P4H810 under the above-described optimized conditions and two $P450_{BM3}$ variants, which were reported to induce hydroxylation of *N*-protected heterocycles,[29] were examined, but exhibited no hydroxylation activities against (*S*)-nipecotic acid (L-Nip), (*R*)-nipecotic acid (D-Nip), and their protected derivatives. In light of the structural resemblance of D-Nip (**10**) to ectoine and their similar charge distributions, ectoine 5-hydroxylase from *Sphingopyxis alaskensis* (*Sa*EctD), an ectoine hydroxylase that has been identified to convert ectoine to 5-hydroxyectoine in its biosynthetic pathway,[30,31] was next tested for the reaction. Initial attempt resulted in the production of *trans*-4-hydroxy-D-Nip with 90% conversion, whose structure was confirmed by COSY NMR analysis and X-ray crystallography of a dinitrobenzamide derivative. (see Supporting Information for detail). Increasing amount of α-KG from 2.5 eq. to 5.0 eq. improved the conversion to 100%, which was also amenable to use at higher substrate concentration (15 mM). The Michaelis-Menten kinetic parameters of EctD toward D-Nip were analyzed, and k_{cat} and K_M were determined to be

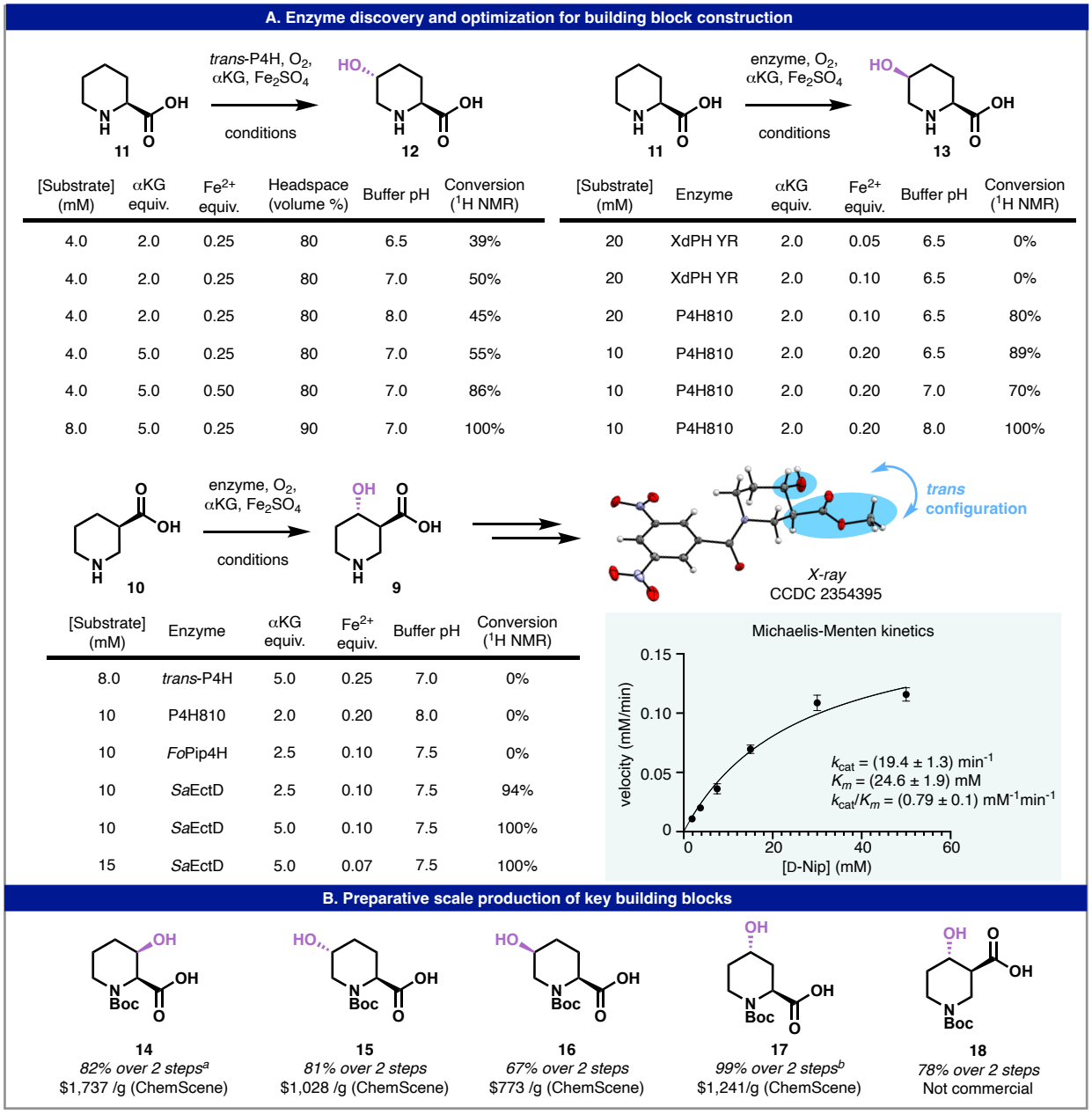

Figure 2. Development of scalable biocatalytic hydroxylation.[a]Ref 24, [b]Ref 25

19.4 ± 1.3 min^{-1} and 24.6 ± 1.9 mM, respectively, and k_{cat}/K_M was calculated to be 0.79 ± 0.1 $mM^{-1}min^{-1}$, which is approximately one order of magnitude higher than the reported value for the native substrate (Table S5). Preliminary docking studies suggest that D-Nip exhibits a different binding mode than ectoine in the active site, specifically by relying on the polar interactions between the carboxylate motif and Gln127, Arg129 and Arg280 (Figure S1). With the aforementioned building blocks at hand, routine Boc protection completed the synthesis of five key piperidines **14-18**, which form the basis of subsequent synthetic explorations. While four out of these five building

blocks are commercially available, their purchase costs are prohibitively expensive ($773–$1737/g).

Modular Radical Cross Coupling to Simplify Complex Piperidine Synthesis:

With robust biocatalytic access to the key hydroxylated piperidines in hand, their downstream modular functionalization was explored using radical cross-coupling methods. Thus, a set of 2-substituted-3-hydroxypiperidine containing molecules were selected (Figure 3A) as this structural class is present in a variety of natural products and bioactive compounds. *N*-Boc-3-hydroxy-2-phenylpiperidine **19** was utilized as a key intermediate in the synthesis of neurokinin NK1 receptor antagonists, such as (+)-L-733,060 and (+)-CP-99,994.[32,33] These lead compounds are known to exhibit a variety of biological activities, including neurogenic inflammation, pain transmission and regulation of the immune response. They have been implicated in a variety of disorders such as migraine, rheumatoid arthritis, and pain. The main synthetic strategy employed involves the use of chiral pool derived intermediates or chiral auxiliaries to achieve diastereoselective synthesis of the 1,2-amino alcohol fragment using polar-bond disconnections, followed by ring-closure.[33–37] Among the >10 routes reported (see Supporting Information for summary), most suffer from a lack of diastereoselectivity, lengthy routes (7-17 steps), cryogenic temperatures, and expensive transition metals. In contrast, hydroxypiperidine **14** can simply engage in chemoselective Ni-electrocatalytic decarboxylative cross coupling with iodobenzene to afford **19** directly (48% isolated yield, single diastereomer). If a medicinal chemist chose to explore the structure-activity relationship (SAR) of the arene moiety this could now be easily accomplished rather than starting from scratch.

The same approach can be employed to access swainsonine (**3**), a naturally occurring indolizidine alkaloid isolated from the fungal plant pathogen *Rhizoctonia leguminicola*.[38] This natural product is a potent inhibitor of α-D-mannosidase and mannosidase II with anticancer and other useful biological activities that are most likely associated with its ability to inhibit the processing of glycoproteins. More than 40 syntheses of swainsonine have been reported to date that range in length from six to >20 steps (average approximately 14 steps).[39] The shortest of these syntheses[39] commences from 5-chloro-pentanal and involves an asymmetric chlorination that requires a low temperature (-35^{o}C) reaction for 19 days to afford 82% ee of aldehyde **23**. Subsequent

alkynyllithium addition and Lindlar reduction affords *Z*-olefin **24**. After removal of the Boc group, strict control of the equivalents and the rate of addition of NaOH to the alkenylchlorohydrin is necessary to avoid the formation of the undesired tetrahydrofuran byproduct; dihydroxylation of **22** delivers the natural product (**3**). The current approach is also six steps but is far simpler to perform as the same hydroxypiperidine **14** used to procure **21** can be divergently processed using electrocatalytic decarboxylative alkenylation with vinyliodide **20** to afford **21** in high dr. Subsequent one-pot Appel reaction and cyclization affords the same intermediate **22** that can be converted to **3** after dihydroxylation thereby avoiding experimentally time-consuming steps, cryogenic temperatures, and Pd-catalysis.

2,4-disubstituted piperidines are important building blocks in synthesis and a core scaffold in many bioactive molecules. Figure 3B illustrates how biocatalytic access to **17** can dramatically reduce the effort needed to obtain such molecules. One such example of this can be seen in a revised approach to the natural product SS20846 A (**4**), isolated from *Steptomyces* sp.[40] S20846 as a proposed intermediate in the biosynthesis of the potent antimicrobial agent streptazolin. Previous syntheses required 6-17 steps, all of which rely on polar-bond disconnections.[41–44] The shortest of these[40] involves asymmetric construction of 1,3-amino alcohol **28** through sequential enolate chemistry and diastereoselective reduction from a Davis sulfinimine derived from sorbaldehyde, followed by intramolecular cyclization to obtain the core scaffold. Reduction of the intermediate β-keto ester exhibits poor diastereoselectivity (ca. 3:1) and LAH is used to exhaustively reduce the lactam to piperidine. A far simpler approach can be realized using radical based Ni-electrocatalytic decarboxylative alkenylation of **17** with vinylbromide **29** to directly access **4** in high dr (7:1, 51% isolated yield) thereby avoiding pyrophoric reagents, chiral auxiliaries, and non-strategic reductions.

LNP023, also known as Iptacopan, is an FDA-approved medicine developed by Novartis for the treatment for paroxysmal nocturnal hemoglobinuria (PNH).[45] It is a first-in-class, highly potent, orally administered targeted factor B inhibitor of the alternative complement pathway that is also in numerous clinical trials for other indications. A 9-step route was used by medicinal chemists to obtain key intermediate **38**, utilizing Comins' pyridinium dearomatization of 4-methoxypyridine

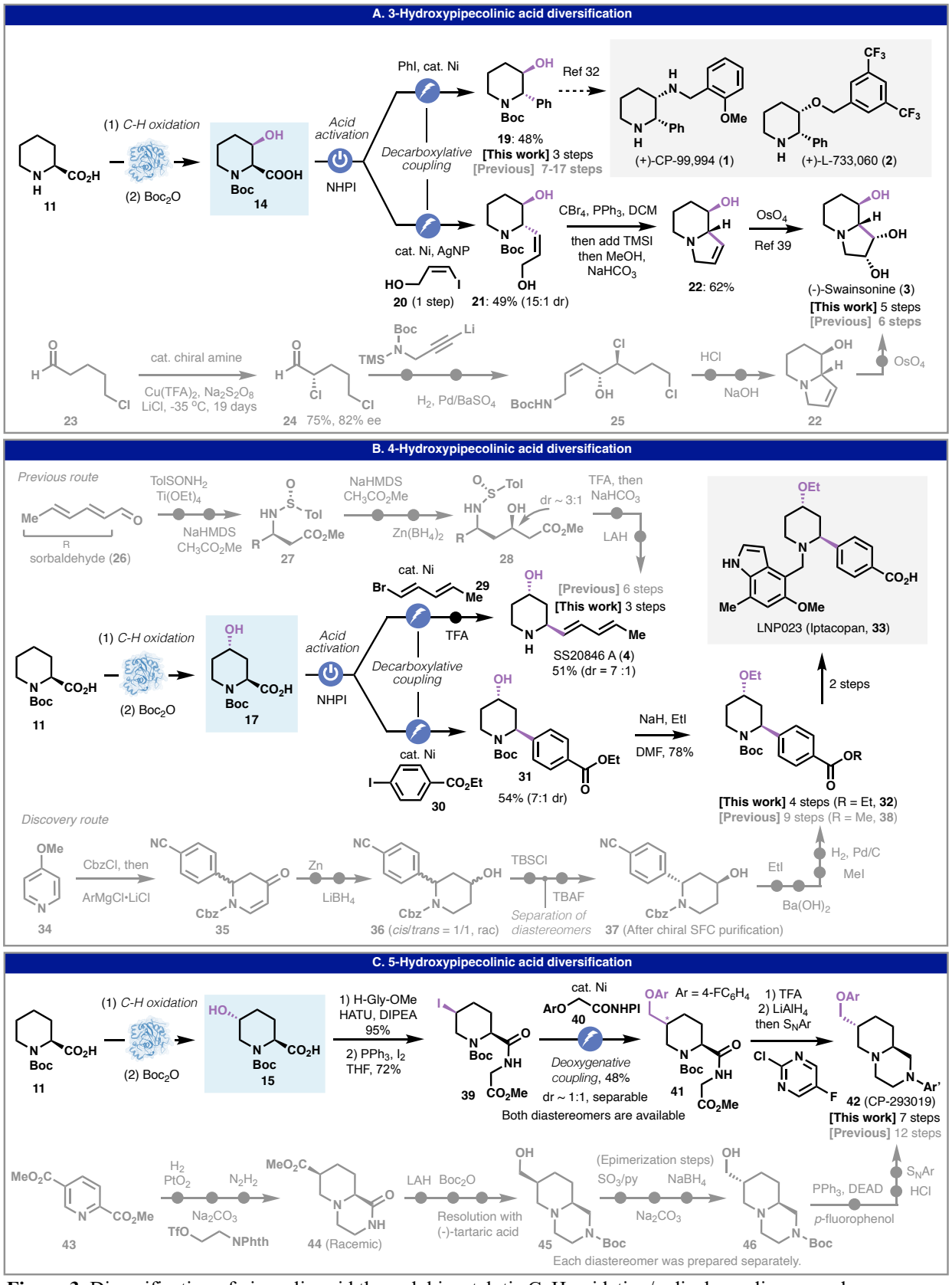

Figure 3. Diversification of pipecolic acid through biocatalytic C–H oxidation/radical coupling cascade.

with an aryl Grignard reagent to forge the 1,4-disubstituted piperidine followed by chiral SFC

separation and multiple FG and redox manipulations.[46] A recently reported improvement to this route relied on a similar logic utilizing a Rh-catalyzed Hayashi-type conjugate addition.[47] In contrast, Ni-electrocatalytic cross-coupling of **17** with iodoarene **30** followed by ethylation arrives at intermediate **32** directly in high dr without protection of the OH group (two known steps are subsequently used to convert **32** to **33**, see Supporting Information). From the standpoint of a medicinal chemistry effort this is attractive as SAR can be interrogated directly at the end of the synthesis through skeletal bond forming reactions (C–O and C–C).

CP-293019 is a potent, selective antagonist of dopamine D4 receptor developed by Pfizer.[48] The previous medicinal chemistry route commenced from 2,5-dipyridylmethyl ester **43** with the diamine intermediate accessed through hydrogenation and cyclization. After reduction, chiral resolution, epimerization, Mitsunobu reaction, and S_NAr, a total of 12 steps were required to obtain the target molecule. Importantly, in the early stages of this program both diastereomers at C-X were targeted and evaluated. In contrast to this route, **15** could be smoothly coupled to glycine methyl ester followed by a non-diastereoselective Ni-electrocatalytic cross coupling of the corresponding alkyl iodide with RAE **40** (48%, 1:1 dr). Whereas the Weix group reported the chemical reductive coupling of alkyl bromides with RAEs,[49] to our knowledge, this is the first example of a coupling between a secondary alkyl iodide and an RAE under electrochemical conditions. Finally, through a one-pot amide reduction and S_NAr reaction, CP-293019 can be obtained in 7 steps. This pathway avoids chiral resolution, epimerization, and the use of expensive metal catalysts to deliver both diastereomeric series for biological evaluation.

3,4-disubstituted piperidines are common fragments in medicinal chemistry, and their syntheses often require laborious multi-step processes. Biocatalytic access to 4-hydroxy-nipecotic acid serves as a gateway to access various 3,4-disubstituted piperidine derivatives easily through consecutive radical cross-coupling reactions as outlined in Figure 4. For example, **8** is a highly active monoamine transporter ligand.[22] In the previous synthesis (Figure 1B), addition of a Grignard reagent to an expensive unsaturated piperidine (**6**), followed by chiral resolution and epimerization delivers piperidine **7**.[22] Subsequent ester reduction, oxidation, Horner-Wadsworth-Emmons (HWE) reaction, and another ester reduction were required, delivering the target molecule in eight steps (of which only two steps were strategic). In contrast, biocatalytically

derived enantiopure **18** can be enlisted in doubly decarboxylative cross coupling (dDCC) with **47** to afford intermediate **48** in high dr (>20:1). A dDCC was applied here rather than Giese-type addition to acrylate[50] to showcase how a medicinal chemist could use such an approach to easily modulate the length of the carbon chain if desired. Subsequently, **48** can undergo in situ bromination using CEBO/TBAB followed by highly diastereoselectivie Ni-electrocatalytic coupling with **49**. This in situ activation/electrocatalytic coupling protocol was modeled after the elegant studies of the Gong group[51] and, in our hands, led to superior conversion relative to the use of chemical reductants (see Supporting Information). Simultaneous Boc and ester reduction yields the target product in only 5 steps from **10**, thereby avoiding the need for chiral resolution, pyrophoric reagents (Grignard, LAH x2) and multiple redox manipulations. From a strategic perspective the versatility of installing the arene near the end of the synthesis rather than at the outset is attractive.

The complex chiral 3,4-disubstituted piperidine **61**, invented by Merck scientists, is an intermediate used to produce a potent and selective inhibitor of renin with a suitable profile for further clinical development.[52,53] Although the process-scale synthesis of **61** allowed for Kg-scale production of this compound, the overall route relies exclusively on polar bond disconnections and precious metal catalysis for both the piperidine subunit and aryl side chain. For the aryl fragment **57**, a four-step reaction process is employed, two of which involve precious metal catalyzed coupling and hydrogenation. In contrast, dibromobenzoic acid **55** can be subjected to amide bond formation with cyclopropyl amine followed by mono-selective Ni/Ag-electrocatalytic decarboxylative arylation[7] to furnish the same arene **57**, which can then be subjected to borane-mediated reduction to afford benzyl amine **58**. The process-scale route to the piperidine subunit starts from commercially available benzyl-protected β-keto ester **62** and traverses a total of 10 reaction steps to obtain the target molecule, only three of which make skeletal bonds (C–C and C–N). These steps include Pd-catalyzed hydrogenation, borylation, and Suzuki coupling, along with a Ru-catalyzed asymmetric hydrogenation. These precious metal catalyzed reactions significantly increase cost and require a high-pressure hydrogenation. In stark contrast, the same product can be obtained through the amide coupling of **18** with benzylic amine **58**, and an Appel reaction to generate the alkyl iodide which subsequently is coupled to iodopyridone **60** under Ag/Ni-electrocatalysis.[7] The chemoselectivity of the pivotal cross-coupling is notable as the aryl bromide

is untouched. In this five-step sequence, four of the steps are skeletal bond forming (C–C, C–O, C–N) and precious metals are completely removed. A conservative estimate of cost savings just based on the raw cost of metals employed (not including ligand cost) is profound and differs by three orders of magnitude (ca. $1 for Ni vs. ca. $1765 for the Pd, Pt, and Ru). Overall, the route outlined above is considerably shorter (5 steps, 8 steps total) than the current process route (10, 14

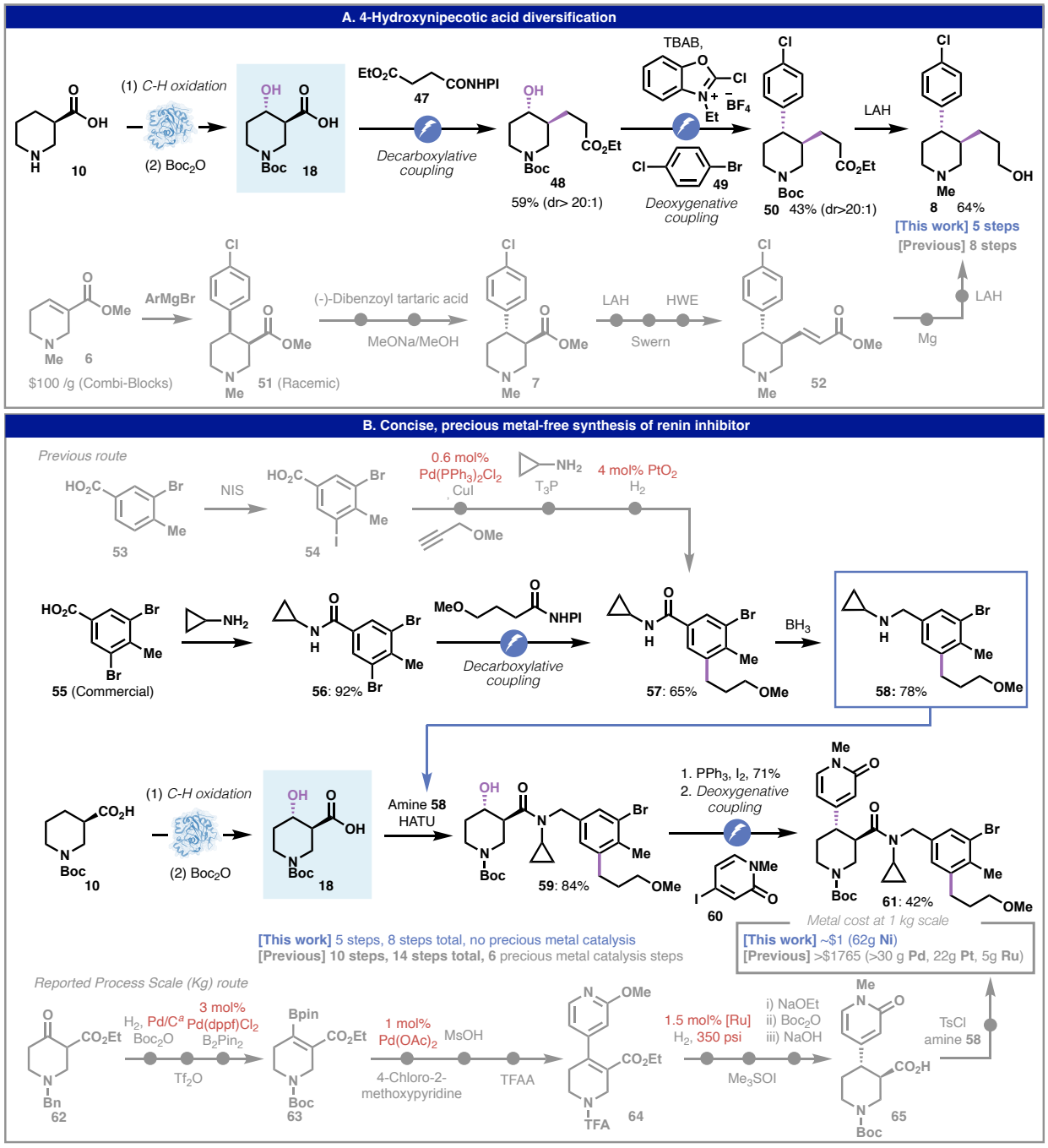

Figure 4. Diversification of nipecotic acid through biocatalytic C–H oxidation/radical coupling sequence. [a]No detail of Pd loading was provided in Ref 52.

steps total), and deletes six steps that employ precious metal catalysis. It is a good example of the power of combining the efficiency of biocatalytic C–H oxidation with simplifying radical based transforms.

Conclusion:

Tunable enzymatic catalysts for C–H oxidation represent perhaps the best and most cost-effective solution to stereo- and regio-controlled preparation of high-value hydroxylated building blocks for organic synthesis. The downstream functionalization of such intermediates has historically relied exclusively on polar bond disconnections thereby diminishing the strategic benefit incurred by their rapid access. By leveraging the power of radical retrosynthesis with biocatalysis incredibly concise and modular pathways to complex three-dimensional architectures are now within reach. The analogy to two-dimensional heteroarene functionalization, the zeitgeist for over fifty years, is apt. In that world, electrophilic aromatic halogenation (EAH) followed by cross-coupling is a tried and true approach that has and continues to be employed on a daily basis. Proposed herein and demonstrated through this proof-of-concept study is a model wherein biocatalytic C–H oxidation and radical based cross couplings on three dimensional systems are akin to EAH and polar cross couplings (mostly Pd-based) in two dimensions. This study points to a uniquely powerful approach to both medicinal and process chemists that maximizes synthetic ideality and rapid SAR exploration and minimizes chiral resolution steps, expensive chiral ligands, and precious metal catalysts.

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Corresponding Author

*yukawama@scripps.edu

* hr28@rice.edu

*pbaran@scripps.edu

Author contributions

Conceptualization: YK, PSB, HR

Experimental investigation: JH, KY, BW, BZ

Data analysis: JH, KY, BW, BZ, YK, PSB, HR

Manuscript writing: JH, KY, BW, BZ, YK, PSB, HR

Funding acquisition: PSB, HR

Project administration: YK, PSB, HR

Competing interest

The authors declare no competing financial interest.

Supporting Information

Experimental graphical procedures, additional experimental data, NMR characterization data and X-ray characterization detail.