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Recent Advances in the Chemoenzymatic Synthesis of Bioactive Natural Products

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Abstract

The field of organic chemistry has recently witnessed a rapid rise in the use of chemoenzymatic strategies for the synthesis of complex molecules. Under this paradigm, biocatalytic methods and contemporary synthetic methods are used synergistically in a multi-step approach towards a target molecule. In light of the unparalleled regio- and stereoselectivity of enzymatic transformations and the reaction diversity of contemporary organic chemistry, chemoenzymatic strategies hold enormous potential for streamlining access to important bioactive molecules. This review covers recent demonstrations of chemoenzymatic approaches in chemical synthesis, with special emphasis on the preparation of medicinally relevant natural products.

Introduction

Biocatalysis is increasingly becoming an indispensable tool in chemical synthesis. Due to their unique selectivity profiles, enzymes are especially useful for the preparation of novel building blocks or late-stage modification of complex molecules. However, enzymes only catalyze a small subset of organic transformations and designing novel non-natural transformations using biological systems remains non-trivial [1]. Furthermore, producing synthetic small molecules using purely biological means is still highly challenging as biological systems are highly complex and their refactoring often results in a dramatic decrease in product titer or complete abolition of product formation [2].

In recent years, organic chemists have begun exploring the combination of biocatalytic and synthetic organic methods to provide efficient access to important bioactive small molecules [3,4,5]. Such a chemoenzymatic approach allows practitioners of the field to harness the unparalleled site- and stereoselectivity of enzymatic transformations, while also taking advantage of various organic transformations to forge chemical bonds that are otherwise unattainable using purely enzymatic means. Herein, we highlight recent developments in the

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chemoenzymatic synthesis of bioactive small molecules, paying special attention to cases in natural product synthesis, whereby the use of this hybrid approach has provided significant improvement in synthesis economy. The select case studies are organized according to types of chemical bonds (C–C or C–X) that are formed enzymatically. For each case study, we also provide a brief discussion on the main advantages that the chemoenzymatic approach has to offer over contemporary chemical approaches.

Chemoenzymatic Synthesis Featuring Enzymatic C–C Bond Formation

Jorunnamycin A and saframycin A

Tetrahydroisoquinoline (THIQ) alkaloids, represented by the saframycins, jorunnamycins and ecteinascidins, are a family of tyrosine-derived molecules with potent antitumor activity [6]. Recently, the Oikawa and Oguri groups collaborated to accomplish a concise chemoenzymatic synthesis of these alkaloids [7]. The authors utilized a phosphopantetheinylated SfmC, a dual Pictet-Spengler enzyme discovered by the Oikawa group [8], to construct the common pentacyclic core of these molecules from a tyrosine analogue (**1**) and corresponding aldehydes **2** and **3** (Figure 1A) . Strikingly, two C–C bonds, three C–N bonds, and reduction of the thioester were accomplished in one enzymatic step. The secondary amine was methylated by treatment of the crude mixture with excess formaldehyde and 2-picoline borane (**4**) to give 18% and 13% yield of **5** and **6** from tyrosine. Further hydrolysis to remove the fatty acid and oxidation state adjustment gave jorunnamycin A (**7**) and saframycin A (**8**), respectively. Through a combination of chemical and enzymatic steps, this work achieved the shortest syntheses of jorunnamycin A and saframycin A to date.

Dihydroartemisinic acid

Artemisinin is a sesquiterpenoid endoperoxide widely used to treat malaria [9]. Recently, Allemann and co-workers reported a chemoenzymatic route to dihydroartemisinic acid (**9**) [10], a precursor for the production of artemisinin [11]. The synthesis commenced with a Riley oxidation of commercially available *trans,trans*-farnesyl chloride (**10**) to provide **11** in 62% yield (Figure 1B) . Diphosphorylation of chloride **11** was accomplished in 51% yield by S_N2 substitution with $(Bu_4N)_3HP_2O_7$. In the biosynthesis of artemisinin, the sesquiterpene cyclase amorphadiene synthase (ADS) acts upon (*E,E*)-farnesyl diphosphate to regio- and stereoselectively form two 6-membered rings, four stereocenters and two double bonds. Oxidation of the C11 methyl group to the corresponding acid occurs later in the biosynthesis. Allemann and co-workers believed a more concise synthesis of dihydroartemisinic acid (**9**) could be achieved by reversing the reaction order. To this end, ADS was optimized for the cyclization of non-native substrate **12** which yielded **13** in 78% yield as a 3:2 mixture of C11 epimers. Pinnick oxidation and methylation gave dihydroartemisinic acid and dihydroartemisinic acid methyl ester (**14**) in high yields. This work is complementary to a previous synthetic biology approach [12] and offers an alternative “reversed” chemoenzymatic approach for the production of artemisinin.

Kainic acid

Isolated from the Japanese marine alga *Digenea simplex* in 1953 [13], kainic acid (**15**) is an important neuropharmacological agent due to its agonism of kainate receptors. Although kainic acid is a monocyclic compound, it poses a significant synthetic challenge due to the presence of three contiguous stereocenters [14]. Moore and co-workers recently reported a concise chemoenzymatic synthesis of kainic acid [15] through an α-ketoglutarate(αKG)-dependent dioxygenase-induced oxidative cyclization. In previous studies [16], the authors had identified an αKG-dependent dioxygenase DabC that catalyzes the conversion of N-geranyl-L-glutamic acid to domoic acid. Here, they identified a DabC homolog, DsKabC, that catalyzes similar conversion of "prekainic acid" (**16**) to kainic acid (**15**). To rapidly access **16**, a simple solution to the synthesis of prekainic acid was developed (Figure 1C), featuring a reductive amination between L-glutamic acid (**17**) and 3-methylcrotonaldehyde (**18**). Purified DsKabC could convert **16** to kainic acid in 46% yield on 10 mg scale. To improve the scalability of this key reaction, the authors used *E. coli* expressing DsKabC to directly convert the crude "prekainic acid" to kainic acid in 57% yield on gram-scale. This two-step sequence represents a significant improvement over previous synthetic approaches, which required at least six steps to complete.

Podophyllotoxin

Podophyllotoxin (**19**) is an aryltetralin lignan with potent tubulin depolymerizing activity [17]. This activity has inspired the development of semi-synthetic derivatives etoposide and teniposide as immunotherapy agents. Earlier this year, Chien and co-workers confirmed the role of an αKG-dependent dioxygenase 2-ODD-PH in the biosynthesis of deoxypodophyllotoxin (**20**) from yatein (**21**) [18]. Shortly after, two different groups reported two independent strategies to synthesize podophyllotoxin [19,20]. Fuchs' strategy relied on a biocatalytic kinetic resolution of racemic hydroxyyatein with 2-ODD (Figure 2A). Reductive allylation of aldehyde **22** with bromide **23** afforded homoallylic alcohol **24**, which was converted to *rac*-hydroxyyatein (*rac*-**25**) via Rh-catalyzed 1,4-additon. The oxidative kinetic resolution of *rac*-**25** with 2-ODD-PH gave 39% yield of **26** in 95% ee on gram-scale and 45% recovery of the unreactive enantiomer in 66% ee. An oxidation/reduction sequence was used to invert the stereocenter of the C7 alcohol to give podophyllotoxin. Contemporaneously, our laboratory employed Baran's oxidative enolate coupling [21] to synthesize enantiopure yatein (**20**, Figure 2B). Using lysate of *E. coli* expressing 2-ODD-PH, the biotransformation of enantiopure yatein provided **21** in 95% yield on gram-scale. Benzylic oxidation with CrO_3 and subsequent reduction completed the synthesis in 58% yield. Both groups tested the substrate scope of 2-ODD-PH and synthesized analogs and related natural products. Both chemoenzymatic approaches provide improved overall yields and superior stereocontrol relative to previously developed synthetic strategies to the aryltetralin lignans.

Chemoenzymatic Synthesis Featuring Enzymatic C–X Bond Formation

Sorbicillinoids

The sorbicillinoids are a family of fungal natural products with promising antiviral activities [22]. They also possess intriguing molecular architectures that arise from an asymmetric oxidative dearomatization of highly substituted phenols and subsequent coupling reactions. Significant advances have been made in achieving the former transformation chemically, but they require stoichiometric chiral hypervalent iodine reagents or chiral Cu^{I} salts [23,24]. Independent reports from the Gulder and Narayan groups [25,26] showed that an FAD-dependent monooxygenase from the sorbicillinol biosynthesis can catalyze a highly regioselective oxidative dearomatization of a suite of highly-substituted phenols (*e.g.*, **29** to **30**) with exquisite enantioselectivity (Figure 3A). Both groups further showed that the resulting products constitute useful intermediates in the chemoenzymatic synthesis of various sorbicillinoids, including rezishanone C (**31**), sorbicatechol A (**32**), epoxysorbicillinol (**33**) and urea sorbicillinoid (**34**). For example, the quinone moiety of **30** readily underwent a Diels-Alder cycloaddition to give **31**, **32** or **34**, and the tertiary alcohol of **30** could direct a Weitz-Scheffer epoxidation to give rise to **33**. Overall, the use of this biocatalytic oxidation strategy results in a dramatic improvement in synthesis economy over previous approaches to the sorbicillinoids, both in terms of step counts and reaction yields. Furthermore, the biocatalytic dearomatization step obviates the need for stoichiometric chiral reagents in the synthesis of key intermediate **30**.

Aspergillomarasmine A and Related Aminocarboxylic Acids

The fungal natural product aspergillomarasmine A (AMA, **35**) was identified to potently and selectively inhibit New Delhi metallo-β-lactamase-1 via zinc chelation. AMA and related compounds are therefore promising candidates for the recovery or potentiation of β-lactam antibiotic activity [27]. Fu *et al.* recently developed the first chemoenzymatic route to a suite of these challenging aminocarboxylic acids using a promiscuous ethylenediamine-N,N’-disuccinic acid (EDDS) lyase [28].

EDDS lyase catalyzes the stepwise, stereoselective C–N bond formation between ethylenediamine and two molecules of fumaric acid (**36**). Toxin A (**37**), an intermediate in the biosynthesis of AMA, bears close resemblance to the native intermediate of EDDS lyase, prompting the evaluation of this enzyme’s substrate scope. Indeed, 0.05 mol % of EDDS lyase catalyzed the regio- and stereoselective amination of fumaric acid by **38** to provide toxin A in 52% yield and >98% de (Figure 2B). Compound **38** was then elaborated to aspergillomarasmine B (AMB, **39**) by regioselective N-alkylation in 84% yield. Finally, AMA could also be directly prepared via amination of fumaric acid with dipeptide **40** (Figure 3B). Thus, the exceptional promiscuity of EDDS lyase allows for the expedient asymmetric synthesis of diverse aminocarboxylic acids for further biological studies.

Tetrahydroquinoline Alkaloids

The tetrahydroquinoline (THQ) motif is present in many biologically active natural products and pharmaceuticals, often with a defined stereoconfiguration at the 2 position [29]. Cosgrove *et al.* directly synthesized a variety of 2-substituted dihydroquinolines (DHQs) by

a rhodium-catalyzed conjugate addition/condensation of ortho-amino phenylboronic acids with enone acceptors [30]. The intermediate DHQs were reduced in one pot with $NaBH(OAc)_3$ to provide the racemic natural product precursors (*rac*-**41**–**44**) for biocatalytic kinetic resolution (Figure 4A).

In recent years, flavin-dependent amine oxidases have been used for the deracemization of acyclic and cyclic amines [31,32]. Namely, a cyclohexylamine oxidase (CHAO) was engineered by Lau *et al.* to provide an array of 2-substituted THQs via *in situ* ammonia-borane reduction to accumulate the desired amine enantiomer [33]. Here, whole-cell *E. coli* expressing CHAO was used to perform the deracemization of **41**–**44** in 64–84% yield and up to >99% ee. *Rac*-cuspareine and *rac*-galipeine (**42** and **43**) stalled at ~50% e.e. while **44** gave full deracemization, suggesting that the 3-methoxy group is a limiting factor. The merging of chemocatalytic C–C bond formation and biocatalytic deracemization provides rapid access to these high-value alkaloids at preparative scale.

C19-Hydroxylated Steroids

Direct C19 functionalization of steroids is an unsolved challenge in the chemical literature that has traditionally required multiple steps to realize [34]. Recently, Wang *et al.* reported the multi-gram scale C19 hydroxylation of cortexolone (**45**, Figure 4B) [35]. Biotransformation of **45** to 19-OH-**45** (**46**) using *Thanatephorus cucumeris* NBRC 6298 has been reported [36,37], but the conversion rate was low (20%), prompting cloning of the responsible P450 genes. While providing improved yield of the C19-hydroxylated product (48%), biotransformation of **45** with heterologously expressed TcP450-1 also resulted in the formation of multiple byproducts. As a workaround, the authors optimized the biotransformation conditions with *T. cucumeris* to increase conversion to 45%. Subsequent screening identified 17-acetyl-cortexolone (**47**) as an optimal substrate, providing significantly higher conversion (80%) in the biotransformation and a drastically reduced amount of side products. Furthermore, the product underwent *in situ* hydrolysis to the desired product **46**. Thus, over 5 g of **46** could be prepared and its elaboration to six C19-hydroxylated pregnanes (e.g., **48**–**51**) could be achieved in 4–9 steps. This work set the stage for rapid access to numerous C19-functionalized steroids and future engineering studies to further expand the substrate scope of the enzymatic reaction.

Conclusions

The case studies outlined above demonstrate the transformative power of chemoenzymatic approaches in simplifying synthetic access to bioactive small molecules. Recent explosion in genomic data [38] and advances in bioinformatics [39], analytical techniques [40] and enzyme engineering [41] should provide even more opportunities to develop novel enzymatic transformations and integrate them strategically in multi-step syntheses. Many times, however, practitioners of organic chemistry are not fully aware of the types of enzymatic transformations that are at their disposal. Further formulation of key guidelines for applying biocatalytic retrosynthetic analysis [42,43] in synthesis planning and general education in biocatalysis will prove invaluable in addressing this gap. These efforts will

foster the next generation of chemists who are well-versed in both chemical and biological synthesis and ultimately allow us to realize the full potential of chemoenzymatic techniques.

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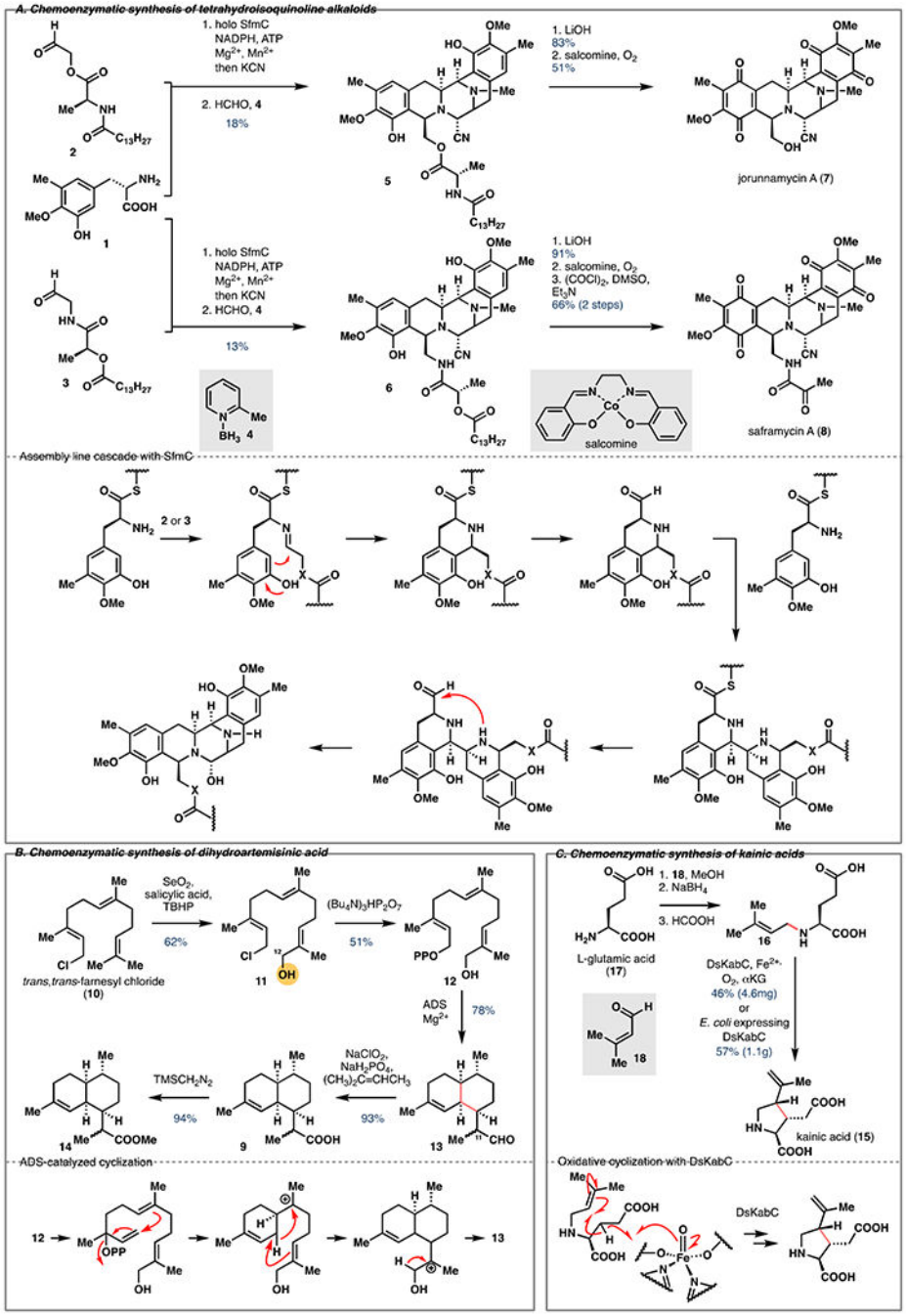

Figure 1.

A. Chemoenzymatic synthesis of jorunnamycin A (**7**) and saframycin A (**8**) featuring construction of their pentacyclic core with SfmC. **B**. Chemoenzymatic synthesis of dihydroartemisinic acid (**9**) via enzymatic cyclization of **12** with amorphadiene synthase (ADS). **C.** Chemoenzymatic synthesis of kainic acid (**15**) via oxidative cyclization of prekainic acid (**16**) with DsKabC.

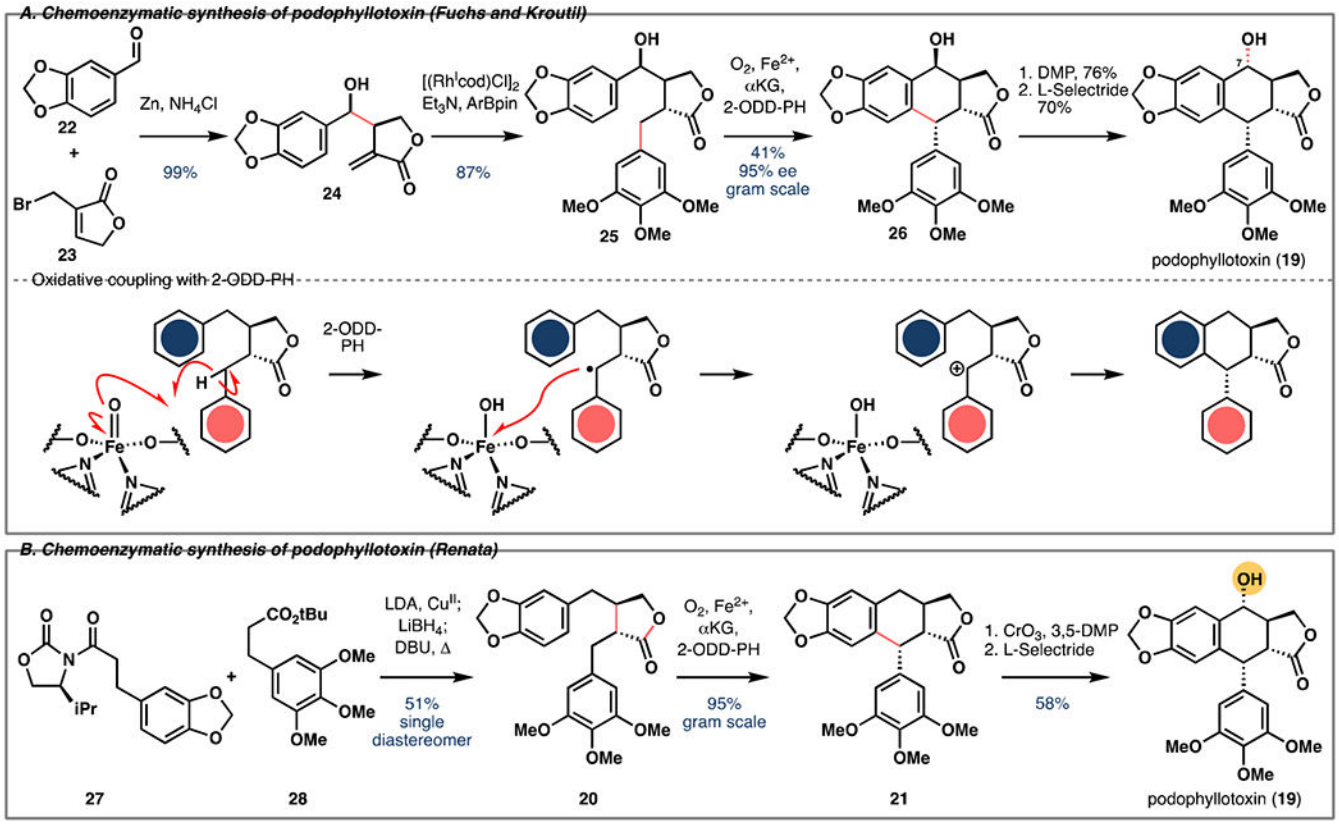

Figure 2.

A. Chemoenzymatic synthesis of podophyllotoxin (**19**) via oxidative kinetic resolution of **26** with 2-ODD-PH. **B.** Chemoenzymatic synthesis of podophyllotoxin (**19**) via oxidative cyclization of enantiopure yatein (**20**) with 2-ODD-PH and benzylic oxidation.

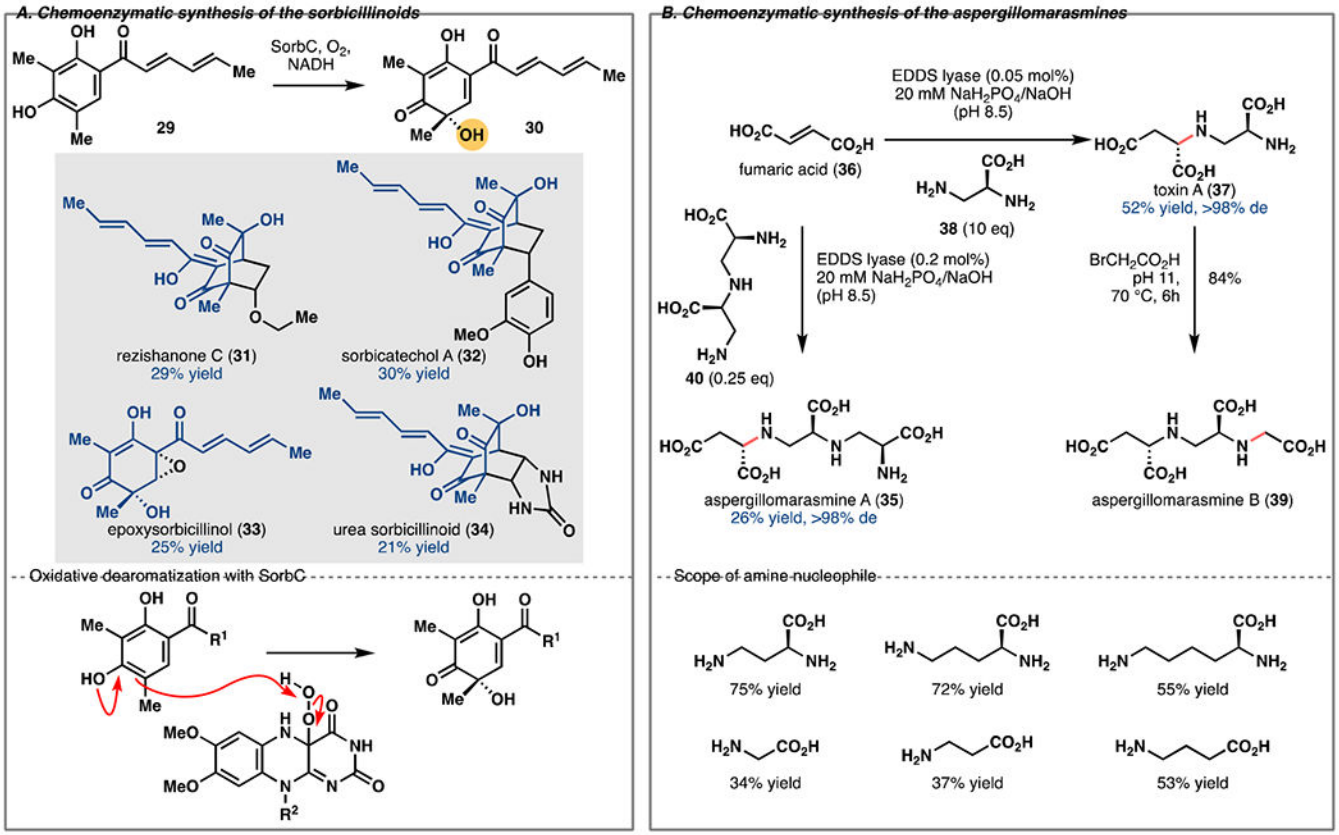

Figure 3.

A. Chemoenzymatic synthesis of the sorbicillinoids via enantioselective oxidative dearomatization of **29** with SorbC. **B.** Chemoenzymatic synthesis of the aspergillomarasmines featuring biocatalytic amination of fumaric acid (**36**) using EDDS lyase.

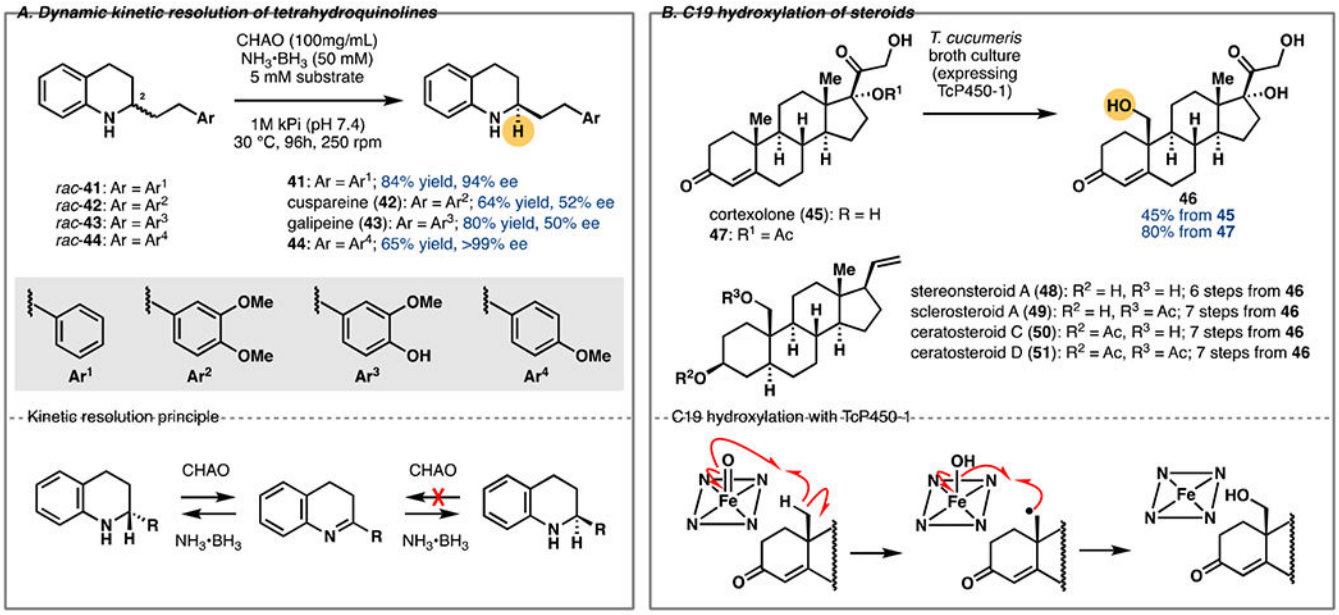

Figure 4.

A. Asymmetric synthesis of tetrahydroquinoline alkaloids via dynamic kinetic resolution with CHAO. **B.** Chemoenzymatic synthesis of 19-hydroxy steroids (e.g., **48**–**51**) via enzymatic hydroxylation with Tc450-1.