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Combining biological and chemical approaches for green synthesis of chemicals

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Recent advances have allowed semi-synthetic production of complex pharmaceutical compounds and commodity chemicals by combining chemical and biological approaches. This approach offers several advantages including synthesis of chirally pure precursors for drugs, design of greener and more sustainable production routes for commodity chemicals by eliminating the use of hazardous chemicals and generation of waste and improving overall process efficiencies by reducing total number of steps involved in synthesis. In this review, we will discuss in detail the synthesis of three pharmaceuticals — simvastatin, artemisinin, and warfarin — and two commodity chemicals — β-methyl-δ-valerolactone and butadiene, all of which have wide applications in the pharmaceutical and polymer industry.

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Introduction

Over the past several years, there have been numerous reports of semi-synthesis of important pharmaceuticals and other commodity chemicals that combines biological and chemical pathways to achieve the final products [1,2••,3,4,5•]. In most of these cases, biological fermentation allows the production of an important precursor/intermediate using renewable biomass-derived sugars, following which the precursor is transformed using single or multi-step chemical reactions to yield the final compound. This hybrid process can convert bio-derived precursors into useful commodity chemicals, thus establishing a more sustainable and greener route for the production of these high-volume compounds. This approach is also useful for the synthesis of chiral drug precursors since biological enzymes offer much better stereoselectivity as compared to chemical catalysts at milder reaction conditions [6].

In this review, we have divided target compounds into two categories: pharmaceuticals and commodity chemicals. In the first section, we discuss the most recent advancements in the semi-synthesis of three widely prescribed drugs — simvastatin, artemisinin, and warfarin. In the second section, we focus on chembiosynthesis of commodity chemicals including monomers for industrially relevant polymers such as β-methyl-δ-valerolactone and butadiene. For few of the compounds discussed in this review, a direct total biosynthetic [7••] or chemical synthetic [8,9] pathway has been established, but the titers obtained are very low for industrial relevance, making semi-synthesis an attractive option at this stage.

Pharmaceuticals

In the past, there have been several successful stories in the pharmaceutical industry where biological route has been used to synthesize an optically pure precursor which is subsequently subjected to chemical reactions to yield the target drug. Examples include the biosynthesis of the taxol precursor, taxadiene [1], and Tamiflu precursor, shikimic acid [10], in engineered *Escherichia coli*. This semi-synthetic approach reduces dependence on isolation of relevant metabolites from natural resources and also significantly improves process economics and sustainability of drug production. In case of drugs such as Lipitor [11] and Sitagliptin [12,13], while pure chemical synthetic routes exist, biosynthesis has been used to replace some of the chemical reactions with the objective of reducing waste and eliminating use of hazardous catalysts. Over the past few years there have been significant advancements in the synthesis of other drugs, some of which are covered in detail in this section. We have reviewed the recent progress made in the field for semi-synthesis of three widely used drugs — first, simvastatin, a cholesterol-lowering drug; second, artemisinin, an antimalarial drug, and third, warfarin, an anticoagulant used for prevention of thrombosis.

Simvastatin

As a derivative of lovastatin, simvastatin has a 2,2-dimethylbutyroloxy side chain at C8 position as against a 2-methylbutyroloxy side chain in its natural counterpart. Traditionally, the semi-synthetic process for producing simvastatin involves isolation of lovastatin from *Aspergillus terreus* fermentation, hydrolysis to yield monacolin J, protection of free alcohol to allow subsequent regioselective

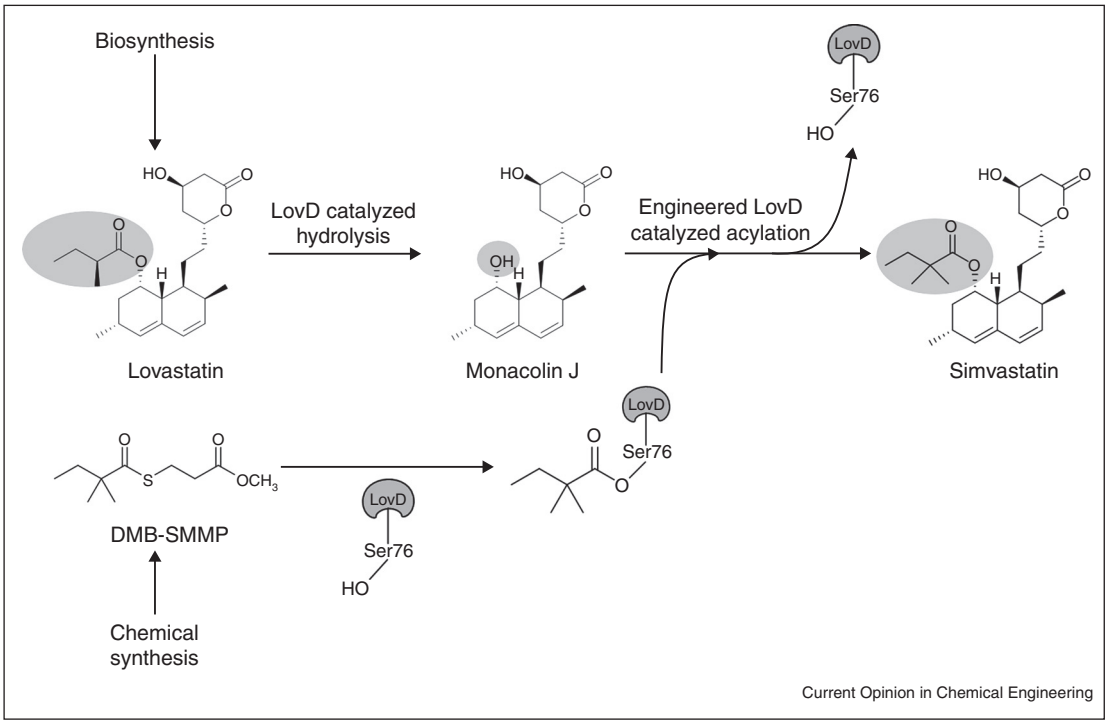
esterification of C8 alcohol with dimethylbutyryl chloride [14–16]. In an effort to improve the overall efficiency of the process, Xie *et al.* demonstrated the ability to use the acyl transferase homolog, LovD, which catalyzes the last step of lovastatin biosynthesis, to selectively acylate monacolin J for the single-step synthesis of simvastatin using chemically synthesized α-dimethylbutyryl-*S*-methylmercaptopropionate (DMB-S-MMP) as the acyl donor [17,18] as shown in Figure 1. This one-step process significantly reduces the number of chemical transformations needed, improves process efficiency and also reduces the cost of manufacturing of simvastatin. In a more recent report, a variant of LovD with 29 mutations was identified by directed evolution, which is 1000-fold more efficient in synthesizing simvastatin than the wild type enzyme. The authors used microsecond molecular dynamics (MD) in solution to explain how distant mutations could improve catalytic efficiency of the active site by lowering the free energy of catalytic conformation of active site [2••].

Artemisinin

Artemisinin is a potent antimalarial drug which is naturally produced by the plant *Artemisia annua* and has a long history of use in Chinese medicine. Because of tremendous fluctuations in the price and supply of this drug as a consequence of inconsistent weather [19,20], the semi-synthetic artemisinin project was started which involved microbial production of artemisinic acid, a chemical precursor of artemisinin, followed by a chemical transformation step to produce artemisinin. After studying the artemisinin pathway in *A. annua* [21], *E. coli* was originally chosen as the chassis organism to produce artemisinic acid [22], but due to the problem of expression of eukaryotic enzymes in *E. coli* [23], the pathway was transferred into a *Saccharomyces cerevisiae* CEN.PK2 strain [24]. Over-expression of mevalonate pathway genes along with expression of amorphadiene synthase (ADS), the P450 enzyme (CYP71AV1) and its cognate reductase (CPR1) allowed the production of 40 g/L of amorphadiene, but artemisinic acid production was still very low [25,26]. Expression of cytochrome *b5* (CYB5) [27] and the aldehyde and alcohol dehydrogenase (ADH1 and ALDH1) [28] from *A. annua* improved P450 activity and increased artemisinic acid titer to 25 g/L as shown in Figure 2, which was the starting goal of the semi-synthetic artemisinin project [29••]. Artemisinic acid was extracted from the fermentation medium with isopropyl myristate (IPM) at high purities and was subsequently used as a substrate for chemical transformation to artemisinin [29••].

The chemical process for converting artemisinic acid to artemisinin involves the following steps: first, hydrogenation of artemisinic acid (AA) to dihydroartemisinic acid (DHAA); second, esterification of DHAA to avoid formation

Figure 1



Conversion of lovastatin to simvastatin using LovD. Biologically produced lovastatin is first hydrolyzed in a reaction catalyzed by LovD, followed by an acylation reaction catalyzed by LovD mutant (LovD9 obtained after nine rounds of evolution). Chemically synthesized α-dimethylbutyryl-*S*-methylmercaptopropionate (DMB-SMMP) acts as an acyl donor for the reaction.