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Current Opinion in Chemical Engineering

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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Current Opinion in Chemical Engineering 2015, **10**:35–41

This review comes from a themed issue on **Biotechnology and bioprocess engineering**

Edited by **Eleftherios Terry Papoutsakis** and **Nigel J Titchener-Hooker**

For a complete overview see the Issue and the Editorial

Available online 27th August 2015

http://dx.doi.org/10.1016/j.coche.2015.07.007

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

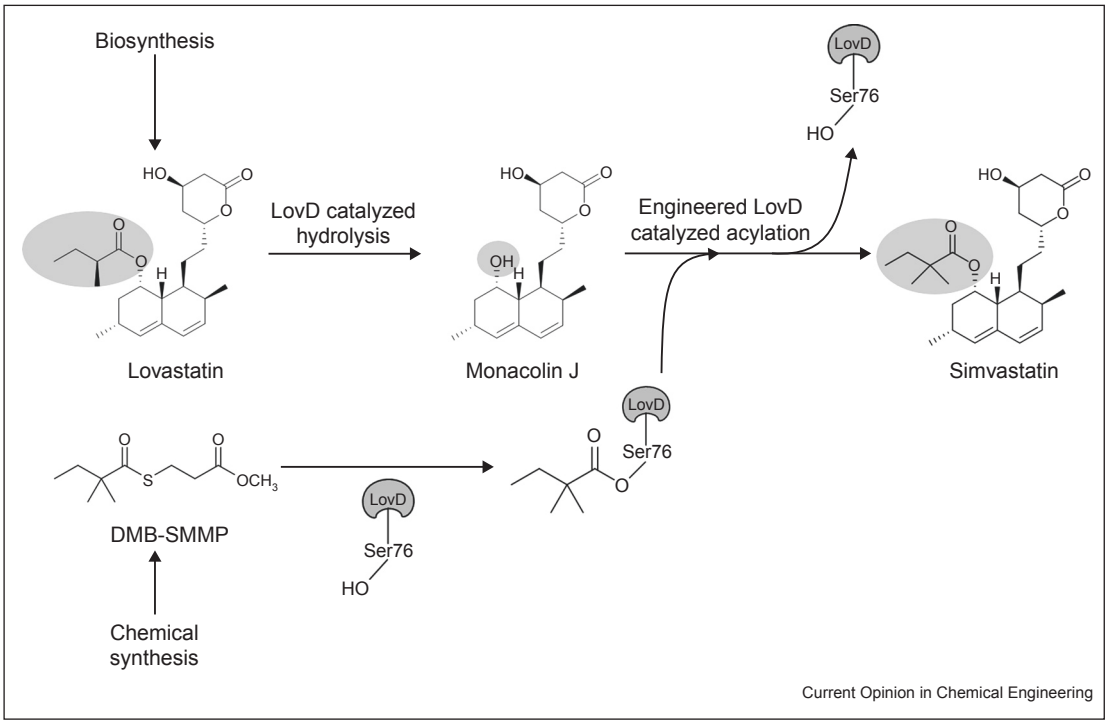
esterification of C8 alcohol with dimethylbutyryl chloride [14–16]. In an effort to improve the overall efficiency of the process, Xie *et al.* demonstrated the ability to use the acyl transferase homolog, LovD, which catalyzes the last step of lovastatin biosynthesis, to selectively acylate monacolin 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••].

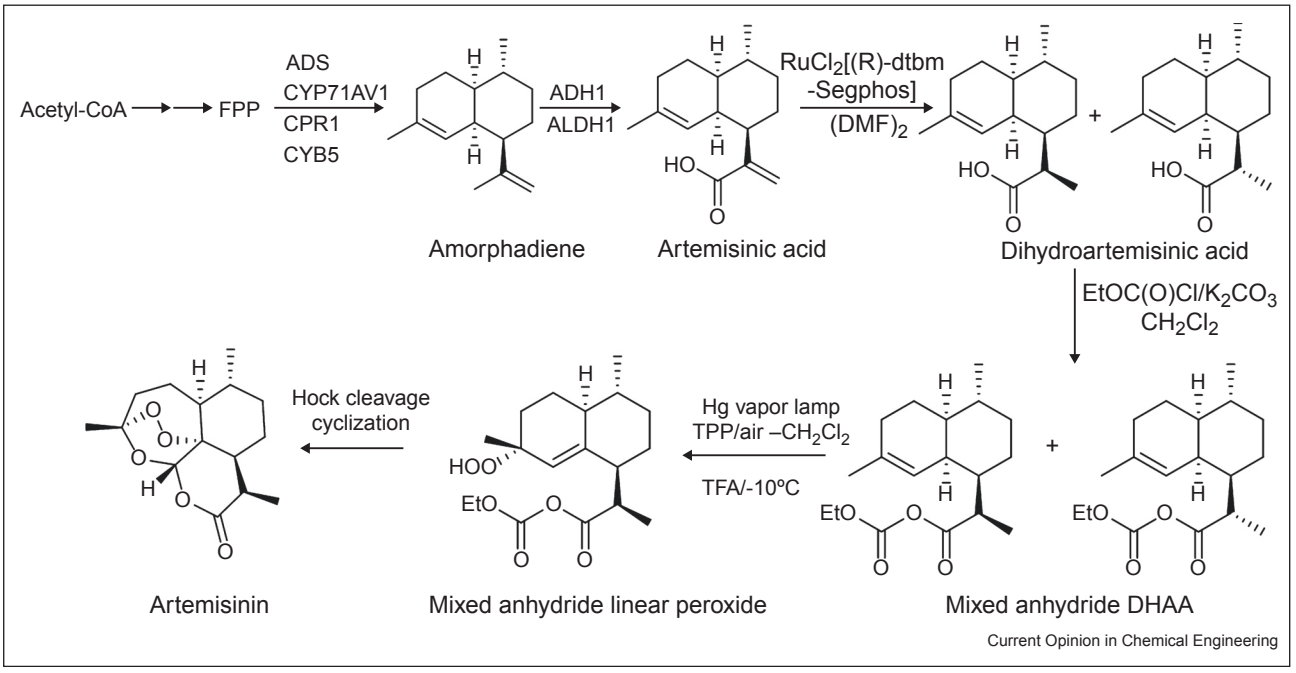
The chemical process for converting artemisinic acid to artemisinin involves the following steps: first, hydrogenation of artemisinic acid (AA) to dihydroartemisinic acid (DHAA); second, esterification of DHAA to avoid formation

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.

Figure 2



Semi-synthetic pathway for production of artemisinin. Biological route in *S. cerevisiae* for synthesis of the precursor, artemisinic acid using the mevalonate pathway. Chemical conversion of artemisinic acid to artemisinin developed by Sanofi which includes diastereoselective hydrogenation of artemisinic acid to dihydroartemisinic acid, followed by its esterification to mixed anhydrides and finally a Schenck ene reaction and Hock cleavage cyclization to produce artemisinin.

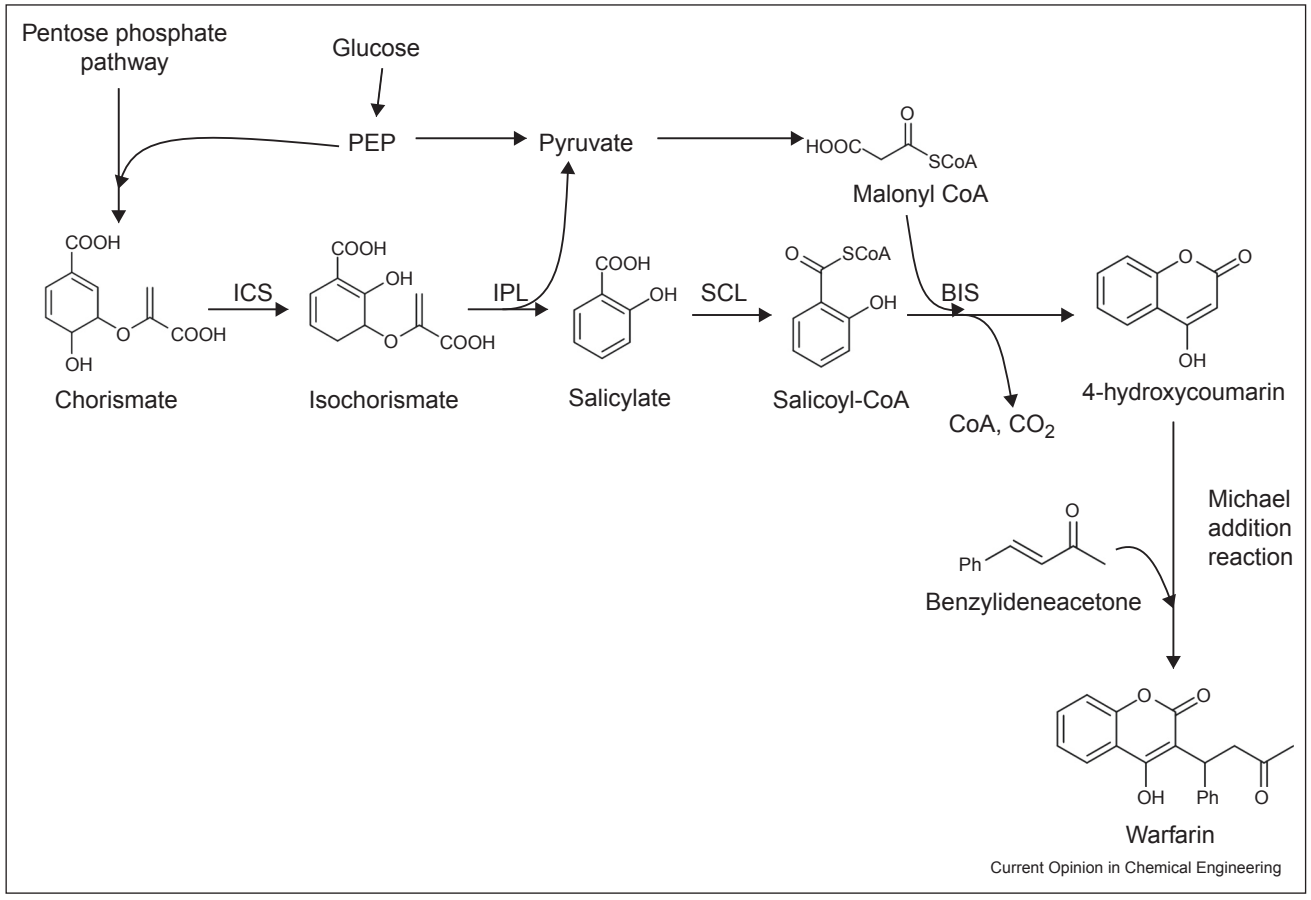
of by-products; and third, generation of a singlet oxygen by chemical or photochemical means to convert DHAA methyl ester to artemisinin [29••]. For stereoselective conversion of AA to DHAA, several catalysts have been screened [29••,30] to achieve high diastereoselectivities, and recently, work performed by researchers at Sanofi provided $RuCl_2$ [(R)-DTBM-Segphos] $(DMF)_n$ catalyst which yielded 95:5 selectivity [31,32]. The conversion of DHAA ester to artemisinin involves regioselective Schenck ene reaction between a singlet oxygen (either derived by chemical reaction or photochemically) and the double bond of DHAA, followed by a Hock cleavage catalyzed by a strong Lewis acid and a subsequent addition of triplet oxygen and cyclization [33••]. Sanofi designed a one-pot synthesis route to convert a DHAA derivative (mixed anhydride) to artemisinin and they obtained an overall yield of 55% of artemisinin starting with artemisinic acid. This semi-synthetic route has capacity to produce 60 tons of artemisinin annually, which corresponds to a third of the global annual need for the drug [33••].

Warfarin

Warfarin is one of the most commonly prescribed 4-hydroxycoumarin (4HC) type anticoagulant used in the prevention of thrombosis or thromboembolism, which is one of the leading causes of morbidity and mortality worldwide. Recently, a *de novo* biosynthetic pathway was designed in *E. coli* for the production of 4HC [34••] as shown in Figure 3 by employing a biphenyl synthases (BIS) to catalyze the decarboxylative condensation of salicoyl-CoA with malonyl-CoA to form a diketide intermediate which undergoes intracellular cyclization and enolization to form 4HC [35]. The pathway used EntC from *E. coli* and PfPchB from *Pseudomonas fluorescens* as isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL) respectively, to convert chorismate to salicylate and a salicylate: CoA ligase (SCL) SdgA from *Streptomyces* sp. to convert salicylate to salicoyl-CoA, the substrate for BIS enzyme [34••]. After identifying BIS catalyzed step as the bottleneck of the pathway, they identified 4-hydroxy-2 (1H)-quinolone synthase (PqsD) by function-based bioprospecting and this enzyme allowed >99% yield for 4HC synthesis and with further engineering they achieved ~483 mg/L 4HC, a ~11-fold increase compared to their parent strain [34••].

The synthesis of warfarin proceeds via Michael addition reaction of 4HC and benzylideneacetone [36] and while most of the studies involve the use of harmful organic solvents, Rogozińska *et al.* carried out the reaction on water using commercially available amines ((*S*,*S*)-diphenylethylenediamine) and achieved 70% *ee* with reasonable yield

Figure 3



Chemoenzymatic pathway for warfarin production. 4-Hydroxycoumarin (4HC) is first biologically produced in *E. coli* from chorismate, derived via the shikimic acid pathway. 4HC is then converted to warfarin by a Michael addition reaction with benzylideneacetone.

(~30%) [37]. When ultrasound bath was applied to the reaction, yields were significantly improved and this method was later used by Lin *et al.* to demonstrate *in situ* semi-synthesis of warfarin using biologically derived 4HC [34••]. In more recent reports, a novel chiral porous metal organic framework (MOF) [38] and a novel polystyrene bound 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) [39], was used for Michael addition of 4HC to α,β-unsaturated ketones to synthesize (*S*)-warfarin and its analogs.

Commodity polymers

Apart from pharmaceuticals, semi-synthesis has also been used successfully for the industrial production of high-volume commodity chemicals such as polyethylene, acrylic acid, and butanediene, by adopting a biological route for synthesis of their precursors–ethanol [4], 3-hydroxypropionic acid [40], and 1,4-butanediol [41,42] respectively from biomass-derived sugars. This approach addresses the growing concern associated with use of fossil-based feedstocks and helps to establish a sustainable and more environment-friendly route for the production of these compounds. In this section we will discuss the recent work done in establishing a semi-synthetic pathway for two monomers — β-methyl-δ-valerolactone (βMδVL) and butadiene — both of which can be polymerized to produce bio-based high-performance polymers.

β-Methyl-δ-valerolactone (βMδVL)

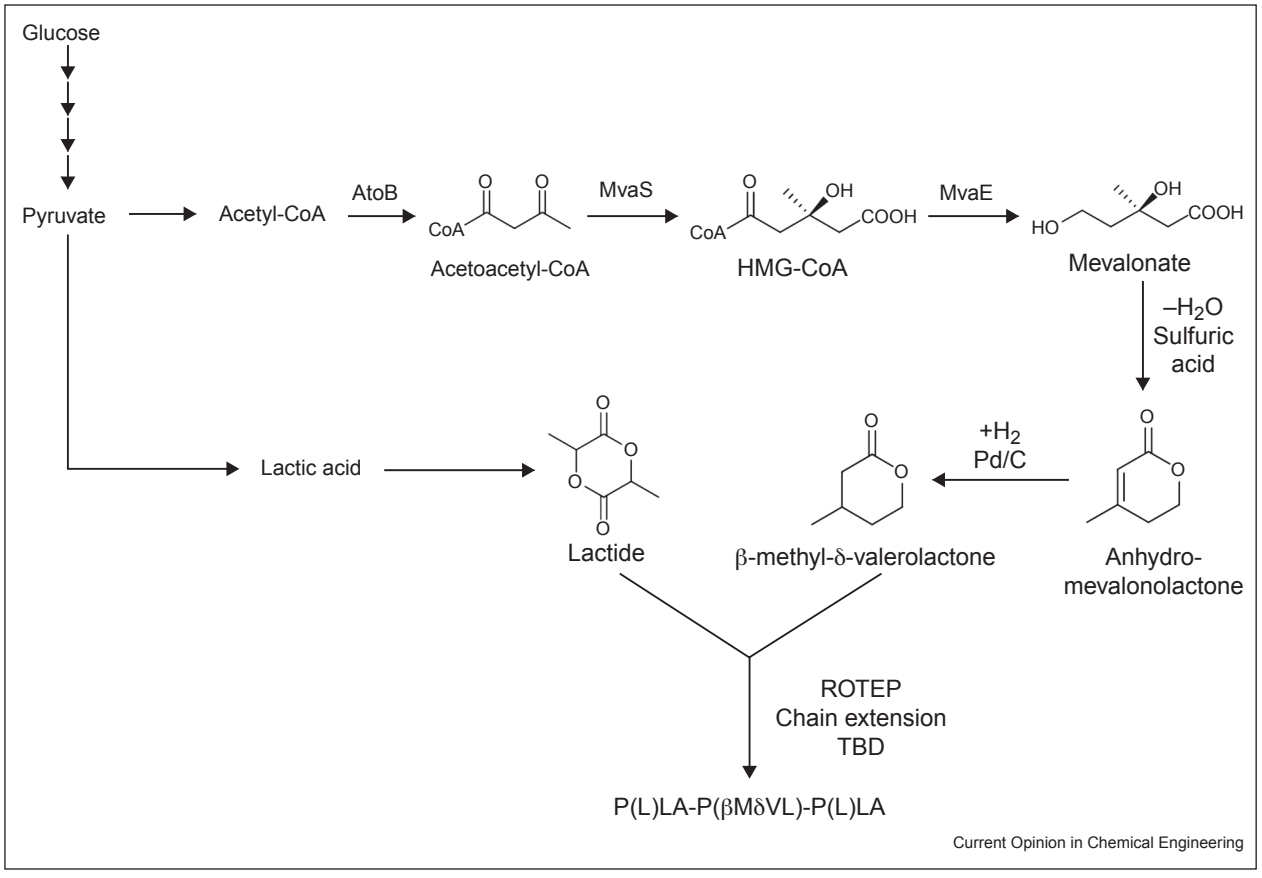
Polymer industry is the third largest manufacturing industry in US with an annual market of nearly $400 billion. The stiff nature of current biodegradable polyesters such as polylactic acid (PLA) and polyhydroxybutyrate (PHB) has limited their applications in the polymer industry. It is envisioned that this challenge can be addressed by developing ABA type triblock polymers with a rigid, glassy end block A (such as PLA) and a soft, rubbery block B in center with low glass transition temperature. However, there is no biobased soft block available, and to this end, Xiong *et al.* recently developed a biosynthetic route to produce β-methyl-δ-valerolactone followed by block copolymerization of βMδVL with lactide (LA) to yield

P(L)LA-PβMδVL-P(L)LA with mechanically tunable properties [7••] (Figure 4). They designed a semi-synthetic approach to βMδVL, which included a biological pathway to synthesize the key intermediate, mevalonate, followed by its chemical conversion to βMδVL. The authors employed the endogenous enzyme AtoB of *E. coli* to produce acetoacetyl-CoA and the HMG-CoA synthase (MvaS) and HMG-CoA reductase (MvaE) from *Lactobacillus casei* to produce 88 g/L mevalonate from acetyl-CoA with a productivity of 2 g/L/h in a 1.3 L fermentor [7••]. Mevalonate was dehydrated to anhydromevalonolactone using sulfuric acid and the unsaturated lactone was hydrogenated to βMδVL using Pd/C as catalyst. The polymerization of βMδVL was carried out in bulk monomer at room temperature using triazabicyclodecene (TBD) as organocatalyst and subsequently chain extension with lactide yielded triblock P(L)LA-P(βMδVL)-P(L)LA whose mechanical and thermal properties could be tuned by controlling molar mass, architecture and end block tacticity [7••].

Butadiene

1,3-Butadiene is used as feedstock for synthetic rubbers and for Nylon production, making it one of the most important conjugated dienes in the petrochemical industry [5•]. Because of the recent shale gas revolution, there has been lightening of the feedstock [43] and this has resulted in an increased interest in exploring catalytic conversion of bio-derived ethanol and C4 alcohols and diols to butadiene. Recently there have been several studies investigating the use of different metal catalysts to carry out the conversion of ethanol to BD [44,45] and in 2013, Axens, IFPEN and Michelin launched a joint research program to develop an economically competitive process for bio-synthetic rubber from bioethanol [5•]. Apart from ethanol, bio-derived C4 alcohols could also be used for sustainable BD production. The biological production of *n*-butanol is via ABE fermentation (acetone–butanol–ethanol) of biomass-derived sugars in *Clostridia* species [46,47], and subsequent dehydration using acid-catalyzed gas phase reaction produces 1-butene

Figure 4



Semi-synthetic pathway for production of branched lactone, β-methyl-δ-valerolactone (βMδVL), and its copolymerization with lactide. Mevalonate was first produced in *E. coli*, followed by sulfuric acid catalyzed dehydration and hydrogenation to βMδVL. βMδVL and lactide were then copolymerized using ring-opening transesterification polymerization (ROTEP) to produce a triblock polymer P(L)LA-P(βMδVL)-P(L)LA with mechanically tunable properties.

which can further be dehydrogenated to yield BD [48,49]. Because of prior commercialization of ABE fermentation [46] and dehydrogenation of butenes [50], this route shows great potential for production of bio-based BD. Butanediols (1,4-BDO, 2,3-BDO, and 1,3-BDO) could also be used as substrate to produce BD through double dehydration reactions. Recently, Genomatica developed a bio-based route for the synthesis of 1,4-BDO from biomass-derived sugars [41] and this process has been tested to produce five million pounds of BDO in 2012. Additionally, 2,3-BDO production has also been reported in *Clostridia* sp. from CO-containing industrial waste gas or syngas via Wood–Ljungdahl pathway [51,52] and this has been commercialized by LanzaTech and INVISTA. The final BDO isomer, 1,3-BDO is an intermediate of the old BD synthesis pathway based on acetaldehyde. Although there are several groups focusing on fermentative production of this diol from biomass sugars, none of the pathways have been commercialized or licensed for industrial production. All three BDOs undergo double dehydration reactions to produce BD, but they have different by-products owing to different dehydration mechanisms [5•].

Conclusion

Merging chemical and biological methods have improved overall efficiencies and allowed production of much higher yields of certain compounds by reducing the total number of steps involved in synthesis [2••]. In some cases, it has also made some processes more environment friendly by reducing waste and use of hazardous chemicals [34••]. Furthermore, when biosynthesis is used for the production of a chiral precursor from biomass-derived sugar, it allows high enantioselectivities and regioselectivities, important in case of drugs, and also eliminates dependence on fossil-based feedstocks. In this review, we have covered the recent developments in the semi-synthesis of three widely prescribed pharmaceutical drugs — simvastatin, artemisinin, and warfarin — and two commodity chemicals — βMδVL, which shows great potential in production of high-performance ABA-type bio-based polyesters and butadiene with wide applications in synthetic rubber industry.

Acknowledgement

This work was supported by the National Science Foundation (NSF) under the Center for Sustainable Polymers CHE-1413862.

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