

Baeyer–Villiger Monooxygenases (BVMOs) as Biocatalysts

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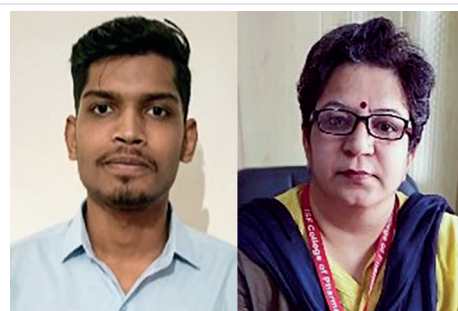
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Natural or artificial enzymes are used in biocatalytic processes to produce high-value fine chemicals, most notably chiral pharmaceutical intermediates. On the other hand, there are few instances of the enzymatic production of bulk compounds.¹ In particular production of polymer precursors such as ϵ -caprolactone, currently obtained from cyclohexanone utilizing peracetic acid; where Baeyer–Villiger monooxygenases (BVMOs) are potential alternative catalysts to carry out the reaction under much milder conditions.² Bulk manufacturing of feedstock chemicals utilizing biocatalysts such as BVMO has yet to be accomplished due to a number of reasons.³ The versatility of BVMOs is highlighted in this Spotlight, along with various instances of how protein engineering has been employed to circumvent some of the disadvantages of BVMO use.

BVMOs are flavin-reliant enzymes that utilize molecular oxygen and NAD(P)H to catalyze a number of oxidation processes, including Baeyer–Villiger oxidations (Table 1).^{4,5} The genes to encode them were discovered at the beginning of this century.⁶ Even though the biochemical reason for the



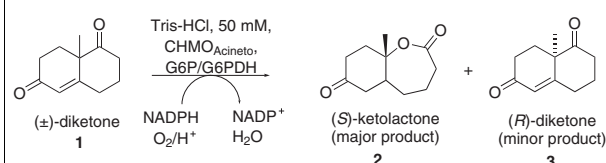
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retention of these residues was unclear until recently, the sequence pattern has shown to be quite useful for mining genomes for new BVMOs.⁷ Although the genomes of higher animals and plants do not include any type I BVMOs, bacteria are rich in BVMOs, with one BVMO per genome on average.⁸ These enzymes are notably common among the *Actinomyces*, making them an intriguing source of new BVMOs.⁹ Fungal genomes are also rather rich in BVMOs but have not yet been fully investigated.¹⁰ The crucial functions that BVMOs play in microbial metabolic pathways have recently been confirmed by investigations on the biotransformation of natural compounds.^{11–13}

Table 1 Baeyer–Villiger Monooxygenases (BVMOs) as Biocatalysts

(A) Despite the fact that it is been demonstrated that CHMO_{Acineto} could be utilized to carry out Baeyer–Villiger oxidation of a range of ketones, little attention has been put into exploring potential chemoselectivity. This is because the focus has mostly been on establishing regio- and/or enantioselectivity.¹⁴ It has been shown recently that oxygenation happens preferentially at the carbonyl group in the presence of various oxidizable functional groups such as alkenes and thioethers.¹⁵ A crude form of CHMO_{Acineto} produced in *E. coli* has recently been used to catalyze the chemoenzymatic Baeyer–Villiger oxidation of bicyclic diketones.¹⁶ For example, Wieland–Miescher ketone **1** was highly selectively oxidized by CHMO to produce the corresponding enantiopure (S)-ketolactone, **2** from the saturated carbonyl group, while the unreacted (R)-diketone **3** was retrieved in 43% yield with an enantiomeric excess of 80% (Scheme 1).



Scheme 1 Oxidation of Wieland–Miescher ketone by CHMO

<p>(B) In the Baeyer–Villiger oxidation, the more highly substituted or highly nucleophilic carbon center migrates preferentially and the majority of Baeyer–Villiger oxidations catalyzed by enzymes likewise exhibit this migratory propensity. However, there are certain instances when using BVMOs has resulted in the creation of ‘unexpected’ lactones. It is hypothesized that the chiral surroundings of BVMOs place constraints on which moiety will migrate. Thus, by employing BVMOs as catalysts, various unexpected regioselective Baeyer–Villiger oxidations have resulted.¹⁷ Recently, Zhang <i>et al.</i> engineered a novel BVMO (GsBVMO) with high expected regioselectivity and demonstrated that long-chain aliphatic keto acids 4 transformed into medium-chain ω-hydroxy fatty acids 5 with good regioselectivity and catalytic efficiency (Scheme 2).¹⁸</p>	<p>Scheme 2 Conversion of 10-ketostearic acid (4) into 9-(nonanoyloxy)-nonanoic acid (5) using the variant GsBVMO_{C308L}</p>
<p>(C) Use of the β-amino acids to create β-lactam antibiotics, alkaloids, terpenoids, and β-peptides has generated significant industrial interest. Various bacterial BVMOs have been used for oxidation of linear aliphatic ketones with a β-amino substituent.¹⁹ All of the BVMOs tested showed no reaction when the amino group was unprotected, but four of the enzymes – cyclodecanone monooxygenase (CDMO) from <i>R. ruber</i> SC1, CHMO_{Xantho}, CHMO_{Brachy}, and CHMO_{Arthro} – were active on (\pm)-methyl(2-methyl-6-oxooctan-4-yl) carbamate (6) and produced both regioisomeric lactones with excellent enantioselectivities. After isolation and purification of both protected β-amino esters 7 and 8 and hydrolysis by <i>Candida antarctica</i> lipase B, <i>N</i>-protected-β-leucine 9 and <i>N</i>-protected β-amino-4-methyl-1-pentanol 10 (α-leucine precursor) were obtained (Scheme 3).²⁰</p>	<p>Scheme 3 Synthesis of <i>N</i>-protected-β-leucine 9 and <i>N</i>-protected β-amino-4-methyl-1-pentanol 10 via biotransformation of (\pm)-methyl(2-methyl-6-oxooctan-4-yl) carbamate (6)</p>
<p>(D) Although all of the BVMOs that have been used so far only catalyze Baeyer–Villiger reactions in nature, their capacity to catalyze sulfoxidations has been demonstrated,²¹ giving access to chiral sulfoxides.²² The Walsh group initially used CHMO_{Acineto} to study the oxidation of organic sulfides.²³ Various chiral aromatic sulfoxides have recently been synthesized using purified PAMO. This enzyme is capable of catalyzing sulfoxidation of alkyl phenyl sulfides. Chen <i>et al.</i> used an <i>E. coli</i> strain for oxidation of dithianes 11 into (<i>R</i>)-sulfoxides 12 with improved yields (Scheme 4). The high optical purities attained from oxidizing sulfide substrates are likely the result of the asymmetric oxidation of the initial sulfide in conjunction with the oxidative kinetic resolution of the formed sulfoxide to the sulfone.²⁴ For instance, Liu <i>et al.</i> realized the asymmetric synthesis of (<i>R</i>)-lansoprazole and other pyrazole-derived sulfoxides 14 using CbBVMO from <i>Cupriavidus basilensis</i> (Scheme 5).²⁵</p>	<p>Scheme 4 Oxidation of dithianes using an <i>E. coli</i> strain</p> <p>Scheme 5 Asymmetric synthesis of pyrazole-derived sulfoxides 14 using CbBVMO</p>
<p>(E) The biotransformation of selenides into selenoxides, boronic acids into phenols, tertiary amines into <i>N</i>-oxides, and even the epoxidation of double bonds to the corresponding epoxides have been demonstrated to be among alternative oxidation processes that BVMOs are capable of carrying out.²⁶ CHMO_{Acineto} has been used to oxidize secondary amines 15 under mild conditions, leading to the corresponding nitrones 16, 17, and 18 via the corresponding intermediate hydroxylamines (Scheme 6).²⁷ With increasing knowledge of the structure of the implicated biocatalysts and their dynamic behavior during biotransformation, it is becoming feasible to anticipate and change the catalytic activity of BVMOs.^{11a,28} Nevertheless, in terms of making this enzyme class even more enticing for potential industrial use in the future, important issues of enzyme stability and enhanced efficiency must yet be satisfactorily addressed and resolved.²⁹</p>	<p>Scheme 6 Conversion of <i>N</i>-methylbenzylamine into hydroxylamine and regioisomeric nitrones</p>

Abbreviations

BVMOs Bayer–Viliger monooxygenases
 CbBVMO BVMO from *Cupriavidus basilensis*
 CDMO cyclododecanone monooxygenase
 CHMO cyclohexanone monooxygenase
 CHMO_{Acineto} CHMO from *Acinetobacter* sp.
 CHMO_{Arthro} CHMO from *Arthrobacter* sp.

CHMO_{Brachy} CHMO from *Brachymonas* sp.
 CHMO_{Xantho} CHMO from *Xanthobacter* sp.
 CPMO cyclopentanone monooxygenase
 FAD flavin adenine dinucleotide
 GsBVMO BVMO from *Gordonia sihwensis*
 NADPH nicotinamide adenine dinucleotide phosphate hydrogen
R. ruber SC1 *Rhodococcus ruber* SC1

Conflict of Interest

The authors declare no conflict of interest.

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