Enzymatic Synthesis

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Exploiting the Regioselectivity of Baeyer–Villiger Monooxygenases for the Formation of β-Amino Acids and β-Amino Alcohols**

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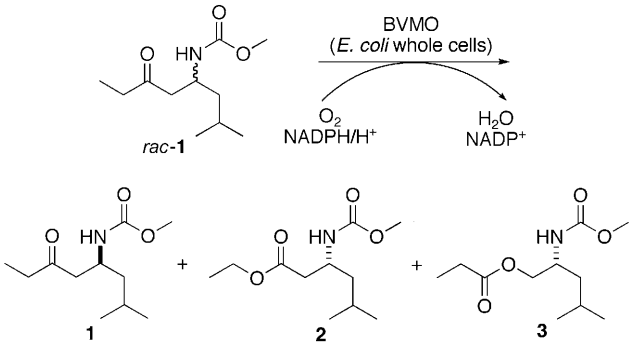
β-Amino acids are of growing interest because of their pharmaceutical importance as building blocks of β-peptides, alkaloids, terpenoids, and β-lactam antibiotics.[1] Biologically active β-peptides in particular have great potential because of their enhanced stability towards proteolytic enzymes; this offers new possibilities in the creation of drugs that are not rejected or degraded by the human body. Therefore, a plethora of chemical methods for the preparation of β-amino acids have been developed, with a focus on asymmetric synthesis.[2] However, chemical syntheses are often not as selective as enzymatic syntheses. So far, the main enzymatic routes for obtaining highly enantiopure β-amino acids have been based on kinetic resolution exploiting enzymes acting on either C–N bonds (e.g. acylases,[3] amidases,[4] and aminopeptidases[5]) or C–O bonds (e.g. hydrolytic enzymes such as lipases[6] and esterases[7]). Optically pure β-amino acids can also be obtained from α-amino acids by using aminomutases[8] and by reductive amination of ketones with β-aminotransferases.[9] We herein report a new enzymatic route to enantiopure β-amino acids under mild conditions using Baeyer–Villiger monooxygenases. For this, N-protected β-amino ketones serve as racemic substrates in a kinetic resolution, leading to an enrichment of N-protected β-amino esters, which are hydrolyzed in a second step to furnish optically pure N-protected β-amino acids. As a further product, N-protected β-amino alcohols can be formed.

Baeyer–Villiger monooxygenases (BVMOs, 1.14.13.x) are flavoenzymes and belong to the class of oxidoreductases. They convert cyclic ketones to lactones and aromatic, linear or aryl alkyl ketones into esters using molecular oxygen instead of the peracids typically utilized in the chemical route.[10] In addition to the flavin cofactor they require reduction equivalents in the form of NAD(P)H. The high regio-, chemo-, and enantioselectivity of enzyme-mediated Baeyer–Villiger reactions represents a valuable advantage over (metal)-based catalysts[11] used in chemical oxidations. Until recently, mainly cyclic and bicyclic ketones have been subjected to approaches based on desymmetrization and kinetic resolution using BVMOs as biocatalysts.[12] In previous work we demonstrated that linear aliphatic ketones such as 4-hydroxy-2-ones and 5-hydroxy-3-ones can also serve as substrates for BVMOs in kinetic resolutions.[13] Only a few results have been published on the use of aryl ketone converting enzymes for the conversion of aryl alkyl compounds in which the carbonyl group is in the side chain.[14]

In the present work we investigated whether linear aliphatic ketones bearing an amino substituent in the β position are also accepted as substrates by BVMOs. Interestingly, we found that certain BVMOs incorporate the oxygen on the less sterically hindered carbon atom to furnish the regioisomeric ester **2**, which after hydrolysis then yields a β-amino acid (Scheme 1). Oxygen insertion at the higher substituted carbon center leads to the "normal" ester **3**, which can be hydrolyzed to furnish a β-amino alcohol. Usually the regiochemistry of oxygen insertion can be predicted by assuming that the carbon atom best able to support a positive charge will migrate preferentially.[15] Changes in the reaction conditions with respect to peracid used, temperature, substrate concentration, and solvent can influence the regioselectivity of a chemical Baeyer–Villiger oxidation. In addition to electronic effects, steric effects arising from the substrate have also been identified as affecting energy differences in the transition state.[16] Synthesizing the "abnormal" ester by chemical methods remains challenging, and thus the imple-



Scheme 1. BVMO-catalyzed kinetic resolution of the aliphatic N-protected β-amino ketone **1** using whole cells of *E. coli*. For details regarding the BVMOs used, see the text and the Supporting Information.

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mentation of a BVMO-mediated ketone oxidation offers a distinct advantage.

From a collection of 16 BVMOs of various bacterial origins, four enzymes (cyclododecanone monooxygenase from *Rhodococcus ruber* SC1 (CDMO), cyclohexanone monooxygenases from *Arthrobacter* sp. ($CHMO_{Arthro}$), from *Brachymonas* sp. ($CHMO_{Brachy}$), and from *Xanthobacter* sp. ZL5 ($CHMO_{Xantho}$)) were active against 5-amino-3-one **1** and showed high enantioselectivities (Table 1). In prescreening

Table 1: Results of kinetic resolutions of **1** using various BVMOs.[a]

Product	BVMO	Conv. [%][b]	ee_P [%][c]	E[d]	RP [%][e]
2	CDMO	12	>99 (+)[f]	>200	42
	$CHMO_{Arthro}$	24	>99 (−)[f]	>200	37
	$CHMO_{Brachy}$	7	>99 (−)[f]	>200	66
	$CHMO_{Xantho}$	13	>99 (−)[f]	>200	42
3	CDMO	12	81	10	58
	$CHMO_{Arthro}$	24	>99	>200	63
	$CHMO_{Brachy}$	7	>99	>200	34
	$CHMO_{Xantho}$	13	>99	>200	58

[a] Biotransformations were carried out at 24 °C using whole cells of *E. coli* expressing the desired BVMO. Screenings were performed in 24-well microplates; reactions were stopped after 24 hours and analyzed by GC on a chiral stationary phase. [b] Total conversion of substrate calculated from ee_S (enantiomeric excess of substrate) and ee_P (enantiomeric excess of product). [c] ee_P values were determined by GC on a chiral stationary phase and calculated according to Chen et al.[17] [d] Enantioselectivity values were determined by computer fitting of GC data[18] from ee_S and ee_P values. [e] Percentage of the regioisomer **2** or **3** in total product mixture. [f] Sign of optical rotation is given in parenthesis. Further details are given in the Supporting Information.

experiments performed in 24-well microplates all four enzymes proved capable to insert oxygen on either side of the ketone functionality, allowing access to both Baeyer–Villiger esters, which is less likely in chemical oxidations using peracids or hydrogen peroxide. Whereas $CHMO_{Arthro}$, $CHMO_{Brachy}$, and $CHMO_{Xantho}$ generated both regioisomers **2** and **3** with high enantiomeric excess (>99% *ee*), CDMO showed rather low enantioselectivity for **3** ($E = 10$), although selectivity for **2** was very high ($E > 200$). Still, it is important that, in contrast to the other three enzymes, CDMO oxidized the (+)-enantiomer of **1** generating the (+)-enantiomer of the "abnormal" **2**. Thus, biotransformations with *rac*-**1** using $CHMO_{Arthro}$ and CDMO yield (−)-**2** and (+)-**2**, respectively, both with high optical purity. Interestingly, the nonprotected β-amino ketones were not converted by any of the BVMOs tested (data not shown).

The biotransformation of *rac*-**1** into (+)-**2** using CDMO as the biocatalyst in a baffled shake flask (0.35 mmol substrate) progressed steadily over 96 hours (Figure 1), whereas the reaction in the 24-well microplates did not exceed 12% conversion (data not shown). This was explained by the increased oxygen supply in the shake flask as a result of the greater surface area and better mixing conditions, and better substrate distribution. One advantage for the synthesis of the β-amino acids was noted: the ratio between "normal" and "abnormal" esters changed from 1:1 in the 24-well microplates to 1:4 in the baffled shake flask. A similar observation

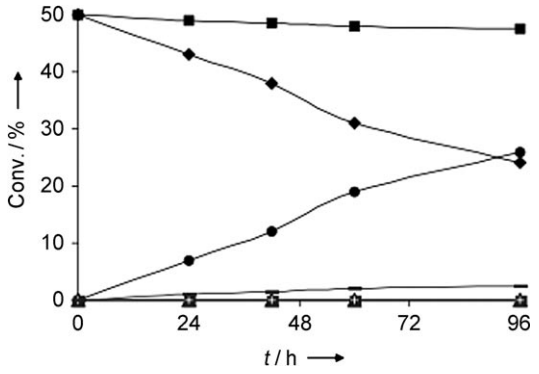
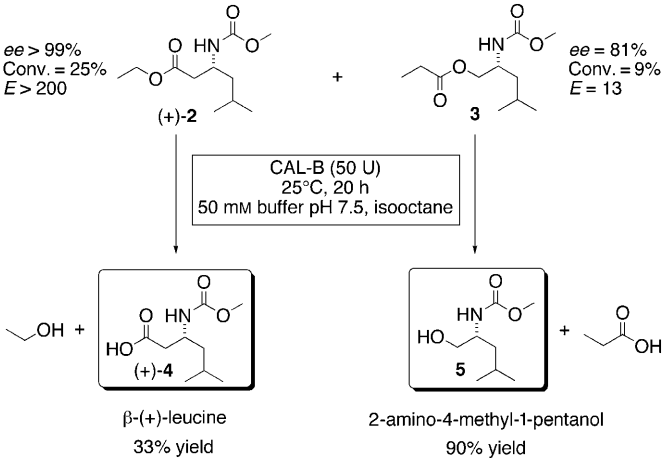


Figure 1. Time-dependent conversion of *rac*-**1** into **2** and **3** at 24 °C using whole cells of *E. coli* expressing CDMO from *Rhodococcus ruber* SC1. ■: (−)-**1**, ◆: (+)-**1**, ●: (+)-**2**, ▲: (−)-**2**, +: (+)-**3**, –: (−)-**3**.

was made for the CHMO-catalyzed oxidation of racemic bicyclo[3.2.0]hept-2-en-6-one. In this case higher substrate concentration led to an increase in the amount of the "abnormal" ester. It was postulated that this resulted from the allosteric binding of the substrate molecule and resulting steric hindrance.[19] This allosteric effect was recently used in a directed-evolution approach of a phenylacetone monooxygenase to generate mutants able to convert different 2- and 4-substituted cyclohexanone derivatives.[20]

After isolation and purification of both Baeyer–Villiger products from the shake flask experiment, ester hydrolysis was performed using *Candida antarctica* lipase B (CAL-B) yielding N-protected β-leucine and N-protected β-amino-4-methyl-1-pentanol (Scheme 2), which in principle can be further oxidized to α-leucine. Since ester hydrolysis proceeds without any conformational changes, both products possess the same configuration as their corresponding esters. Interestingly, we also observed the spontaneous autohydrolysis of **3** and concurrent formation of **5** starting 30 hours after substrate addition. Since *E. coli* does not overexpress endoge-



Scheme 2. Lipase-catalyzed hydrolysis of Baeyer–Villiger esters leading to the formation of the N-protected β-amino acid **4** and the N-protected β-amino alcohol **5**. (Conv.: conversion; yield refers to methyl ester after derivatization of the β-amino acid with methanol and TMS/diazomethane).

nous hydrolytic enzymes like esterases or lipases, we assume that the decrease in pH resulting from metabolism might lead to ester bond cleavage. However, this autohydrolysis was not detected for the "abnormal" ester.

These studies demonstrate the broad applicability of Baeyer–Villiger monooxygenases in synthetic chemistry through the formation of highly valuable building blocks previously not associated with Baeyer–Villiger oxidations. Indeed, kinetic resolution of β-amino ketones revealed that oxygen insertion into a carbon–carbon bond is an efficient method to generate five chemically different compounds, which also differ in their configuration. Owing to the regioselectivity of Baeyer–Villiger monooxygenases, valuable optically active synthons can be synthesized, all of high importance for the pharmaceutical industry. Furthermore, generating an "abnormal" Baeyer–Villiger ester and therefore allowing access to β-amino acids is particularly interesting since common chemical strategies still lack this possibility. In this particular example four different enzymes generate the regioisomeric Baeyer–Villiger esters in enantiocomplementary form and therefore allowed access to both β-amino acid enantiomers. Thus, the enzymatic Baeyer–Villiger reaction opens up new synthetic routes for the formation of β-amino acids, and this offers a useful alternative to already described enzymatic processes.[3–8]

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