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Regiodivergent Baeyer–Villiger oxidation of fused ketone substrates by recombinant whole-cells expressing two monooxygenases from *Brevibacterium*

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Abstract—Microbial Baeyer–Villiger oxidations of fused bicyclic ketones with a cyclobutanone structural motif were investigated using recombinant *Escherichia coli* cells expressing two monooxygenases from *Brevibacterium*. In a kinetic resolution process fused ketones were transformed to regioisomeric lactones: 'normal' lactones were generated by migration of the more substituted carbon atom and 'abnormal' lactones resulted from migration of the less substituted carbon atom. The two Baeyer–Villigerases demonstrated a significantly different stereoselectivity for the regiodivergent biotransformation.
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Baeyer–Villiger-type oxidation reactions using biocatalysts represent a powerful methodology for the one-step asymmetric synthesis of chiral lactones.[1–3] Flavin dependent monooxygenases have been established as powerful chiral catalysts for this biooxidation and these enzymes are able to transform a large spectrum of nonnatural substrates in high enantioselectivity. The utilization of recombinant living cells circumvents the obstacle to recycle noncovalently bound cofactors such as NAD(P)H and provides easy to handle catalytic entities.[4–6] In addition, the required proteins are produced in high concentration by strong promoters and become the dominant fraction in the cell's proteome. As a consequence, such high performance overexpression systems minimize the chance of unwanted side reactions caused by competing enzymes.[7,8]

Previous substrate profiling studies have focused on cyclohexanone monooxygenase ($CHMO_{Acineto}$)[9] from *Acinetobacter* NCIMB 9871.[10] However, with a number of additional recombinant whole-cell expression systems becoming available, further Baeyer–Villiger monooxygenases (BVMOs) have received considerable attention, but hardly any information on their potential as biocatalysts has been published, so far.[11] Recently, an enantiodivergent trend in stereoselectivity for $CHMO_{Acineto}$ and cyclopentanone monooxygenase (CPMO) from *Comamonas*[12] has been observed.[13–15] Very recently, we found enantiodivergent biooxidation[16] for two novel BVMOs from a single *Brevibacterium*[17] species. With this contribution we continue our substrate profiling studies of the latter two enzymes.

Bicyclic ketones **1** with a cyclobutanone structural motif represent convenient substrates for native organisms producing BVMOs. Some biotransformations of various fused ketones using native *Acinetobacter* NCIMB 9871 and mutant *Acinetobacter* TD 63 have been reported previously.[18,19] In addition to bacterial organisms some fungi also produce BVMOs that accept such substrates.[20,21] Racemic compounds **1** are transformed in a resolution process into two types of regioisomeric lactones: the expected 'normal' lactone **2** is generated by migration of the more substituted carbon atom; 'abnormal' lactone **3** is formed by migration of the less substituted carbon atom (Scheme 1). Each product is derived from one enantiomeric substrate and a mechanistic concept for this resolution process was outlined, previously.[22]

Intrigued by the substantially different behavior of the two *Brevibacterium* BVMOs $CHMO_{Brevi1}$ and $CHMO_{Brevi2}$ in the biocatalytic asymmetrization of prochiral substrates,[16] we became interested in this

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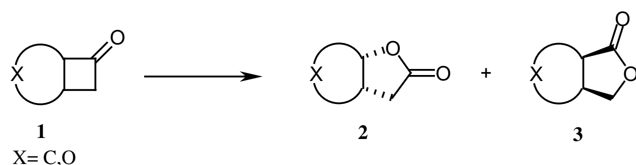


Scheme 1. Regiodivergent biooxidation of racemic bicycloketones **1**.

'benchmark' reaction for chiral Baeyer–Villiger oxidations (Scheme 1). An array of carbo- and heterocylic substrates **1a**–**g**[19,23,24] was investigated in order to study the effect of ring size, hybridization, and polarity on the biooxidation.

Microbial Baeyer–Villiger oxidation with recombinant *Escherichia coli* expressing $CHMO_{Brevi1}$ and $CHMO_{Brevi2}$ gave complete conversion of substrates to lactone products in all cases. Biotransformation results are summarized in Table 1.

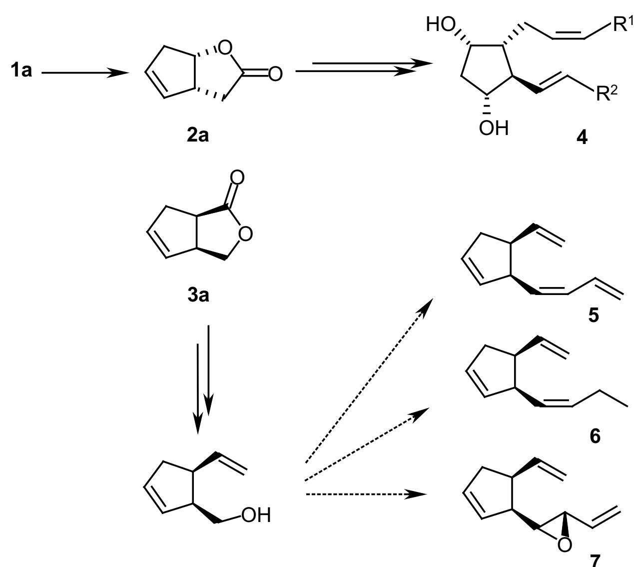
Five-membered ring ketones **1a**,**b**,**e**,**f** were converted by $CHMO_{Brevi1}$ to the corresponding regioisomeric lactones **2** and **3** in a ratio of approximately 1:1 with excellent enantioselectivity. These results are comparable with previous $CHMO_{Acineto}$ biooxidations.[18,19] However, six-membered ring ketones **1c**,**g** were generally converted at superior stereoselectivity by $CHMO_{Brevi1}$. Especially optical purity of 'normal' lactones **2** was significantly higher in all cases as compared to $CHMO_{Acineto}$. Assignment of absolute configuration of products **2** and **3** is based on comparison with biotransformations by $CHMO_{Acineto}$. We also investigated the biooxidation of bridged substrate **1d** as an interesting scaffold for the first time and observed a comparable regiodivergent reaction. Based on the stereochemistry of the previous biotransformation products, we tentatively assign the same general sense of chirality for the two asymmetric centers at the site of anellation (1*S*,2*R*,5*S*,6*R*-**2d**; 1*S*,2*R*,5*S*,6*R*-**3d**).

Biotransformations using recombinant microorganisms producing $CHMO_{Brevi2}$ showed a very different picture: To our surprise, most bicyclic ketones were transformed to 'normal' lactones **2** with very low enantioselectivity. 'Abnormal' lactones **3** were formed in only minor amounts, but high enantioselectivity. Hence, in the majority of cases investigated so far, $CHMO_{Brevi2}$ displayed only poor resolution capability, hence, yielding the thermodynamically favored 'normal' lactone **2** in racemic form.

Table 1. Microbial Baeyer–Villiger oxidations to lactones **2** and **3** using recombinant whole-cells expressing monooxygenases from *Brevibacterium*

Ketone	Strain	Total yield (%)[a]	'Normal' lactones **2a**–**g**		Ratio	'Abnormal' lactones **3a**–**g**	
			% Ee[b]	Absolute configuration		% Ee[b]	Absolute configuration
1a	$CHMO_{Brevi1}$	85	96	1*S*,5*R*	51:49	>99	1*R*,5*S*
	$CHMO_{Brevi2}$	61	0		98:2	>99	
1b	$CHMO_{Brevi1}$	78	>99	1*S*,5*S*	50:50	>99	1*R*,5*S*
	$CHMO_{Brevi2}$	78	76		55:45	91	
1c	$CHMO_{Brevi1}$	83	78	1*S*,6*S*	60:40	>99	1*S*,6*R*
	$CHMO_{Brevi2}$	79	2		96:4	>99	
1d	$CHMO_{Brevi1}$	77	89	n.d.	52:48	>99	n.d.
	$CHMO_{Brevi2}$	86	44		69:31	>99	
1e	$CHMO_{Brevi1}$	74	99	1*R*,5*R*	65:35	>99	1*S*,5*R*
	$CHMO_{Brevi2}$	79	6		98:2	84	
1f	$CHMO_{Brevi1}$	72	97	1*S*,5*S*	46:54	>99	1*R*,5*S*
	$CHMO_{Brevi2}$	83	57		62:38	97	
1g	$CHMO_{Brevi1}$	64	97	1*S*,6*S*	75:25	>99	1*S*,6*R*
	$CHMO_{Brevi2}$	71	0		96:4	>99	

[a] Combined isolated yield of **2** and **3** after purification by flash column chromatography.
[b] Ee determined by chiral phase gas chromatography.



Scheme 2. Application of biooxidation products **2a** and **3a** in natural product total synthesis.

It is interesting to note, that CHMO$_{Brevi2}$ seems to be very susceptible to moderate structural and electronic modifications in the substrate: While ketone **1a** gives almost exclusively racemic **2a**, the fully saturated analog **1b** again leads to a regiodivergent biotransformation in reasonable stereoselectivity. This is also the case for the more hydrophilic furan derivative **1f**. These results might give valuable hints for an active site model for CHMO$_{Brevi2}$, which has yet to be developed.

The synthetic value of such a regiodivergent biooxidation has been demonstrated, previously. Lactones **2a** and **3a** represent key intermediates for the synthesis of various natural compounds, such as prostaglandins **4** or algae pheromones viridiene **5**, multifidene **6**, and caudoxirene **7** (Scheme 2).[25–28]

Biotransformations were carried out according to our typical fermentation protocols for recombinant whole-cell biotransformations published previously.[13] Products were isolated by flash column chromatography to give mixtures of **2** and **3**, which were analyzed by chiral phase GC. Separation of regioisomers was accomplished by a second flash column chromatography.

All lactones were identified by comparison of their ^{1}H and ^{13}C NMR spectra. All enantio- and regioisomers were assignment by independent NMR and GC analysis of biooxidations using recombinant CHMO$_{Acineto}$ producing *E. coli*[4] and comparing the data with published results.[18,19] Physical data and specific rotation of lactones **2** and **3** were in agreement with published data.[18,19,29]

In summary, CHMO$_{Brevi1}$ and CHMO$_{Brevi2}$ display a very different stereoselectivity in the biooxidation of racemic bicycloketones **1**. We observed significant similarity in the regiodivergent behavior of CHMO$_{Brevi1}$ compared to CHMO$_{Acineto}$, with the *Brevibacterium* BVMO giving superior enantioselectivity in several cases. CHMO$_{Brevi2}$ demonstrated a substantially different selectivity and the majority of substrates gave 'normal' lactone **2** as major product in essentially racemic form.

Previously we reported enantiodivergent Baeyer–Villiger oxidations by these two BVMOs on several prochiral substrates. Taken together with these novel results for a resolution process of racemic precursors, this underscores the fundamental difference of the two *Brevibacterium* enzymes with respect to biocatalytic performance. Additional experiments are currently underway in our laboratory to further evaluate the potential of whole-cells expressing these two BVMOs as enantioselective catalysts.

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