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Resolution of fused bicyclic ketones by a recombinant biocatalyst expressing the Baeyer–Villiger monooxygenase gene Rv3049c from *Mycobacterium tuberculosis* H37Rv

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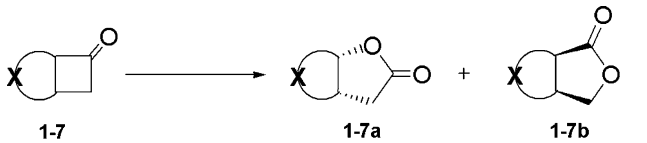
Abstract—Recombinant *Escherichia coli* B834 (DE3) pDB5 expressing the Rv3049c gene encoding a Baeyer–Villiger monooxygenase from *Mycobacterium tuberculosis* H37Rv was used for regioselective oxidations of fused bicyclic ketones. This whole-cell system represents the first recombinant Baeyer–Villiger oxidation biocatalyst that effectively resolves the racemic starting materials in this series. Within biotransformations using this organism one substrate enantiomer remains in high optical purity, while the second enantiomer is oxidized to one type of regioisomeric lactone preferably.

Biocatalysis offers efficient and sustainable access to chiral building blocks on bulk, fine, and specialty chemical scale. It is a highly interdisciplinary and tremendously active field at the cross roads of organic synthesis, fermentation technology, microbiology, and molecular biology. Due to the exponentially growing number of potential biocatalysts identified by genome mining, biocatalysis is offering an increasing number of enzyme platforms with novel reactivities and complementary properties.[1]

In particular, biological oxygenation reactions offer attractive options to synthetic chemists, as the corresponding enzymes utilize molecular oxygen as oxidant.[2] Recombinant overexpression systems represent easy-to-use catalytic entities, which can be applied by synthetic chemists without complicated cofactor recycling techniques and elaborate enzyme purification. In addition, biocatalysts originating from pathogenic organisms can be studied in a benign host system for their potential in asymmetric synthesis. Among this enzyme group, Baeyer–Villiger monooxygenases (BVMOs) have received increasing interest in recent years due to the availability of several new enzymes.[3–7]

The regiodivergent biooxidation of fused bicyclic ketones bearing a cyclobutanone structural motif by BVMOs has been identified as one of the more remarkable transformations by the enzyme class. Racemic starting material is oxidized to two regioisomers with either the more substituted (and, hence, more nucleophilic) or the less substituted carbon center undergoing migration, consequently leading to the 'normal' or the 'abnormal' lactone (Scheme 1).[8–11] A mechanistic rationale has been proposed for this behavior based on stereoelectronic effects.[12]

Consistent with our recently discovered clustering of BVMOs into two distinct groups,[13] we observed different behavior by cyclohexanone (CHMO)- and cyclopentanone (CPMO)-type monooxygenases, with the prior



Scheme 1. Regioselective Baeyer–Villiger oxidations of fused bicyclic ketones.

Keywords: Baeyer–Villiger oxidation; Biocatalysis; Biotransformation; Lactone; Monooxygenase.
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yielding equal amounts of both oxidation products in usually good optical purity and the latter only providing essentially racemic normal lactones.[14]

Very recently, a ligation independent cloning (LIC) strategy was reported to give rapid access to a family of recombinant biocatalysts by exploiting the genome of a sequenced species.[15] In this study, the methodology was applied to express BVMOs originating from *Mycobacterium tuberculosis* H37Rv—a class 3 pathogen—in benign and easily cultured *Escherichia coli*. One of the recombinant *E. coli* B834 (DE3) strains, expressing gene Rv3049c from *M. tuberculosis* ($BVMO_{Mtb5}$), was found to perform a preparative kinetic resolution of racemic bicyclo[3.2.0]hept-2-en-6-one **1**.

Whilst the selective formation of 'abnormal' lactone **1b** from ketone **1** was reported for a wild-type strain of *Cunninghamella*,[16] a recent re-investigation of this biotransformation indicated that it was not, in our hands, a useful kinetic resolution process, as no enantiomerically enriched substrate could be recovered.[17]

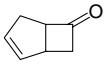
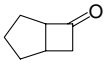
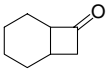
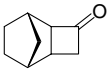
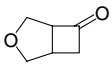
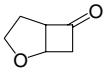
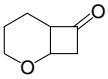
In order to explore this interesting behavior in detail, we focused on biotransformations by *E. coli* expressing Rv3049c using structurally similar ketones as substrates. Successful resolution of such racemic ketones would enable access to valuable optically pure precursors for further synthetic sequences to bioactive compounds.[18]

Results obtained from microbial Baeyer–Villiger oxidations of racemic ketones **1**–**7** after 48 h of biotransformation time using recombinant whole-cells expressing $BVMO_{Mtb5}$ are compiled in Table 1. Experiments were performed in parallel format using multi-well plastic dishes.[19,20] Enantioselectivity values (*E*; determined by computer fitting)[21] and regioisomeric excess re[22] were calculated to evaluate the efficiency of kinetic resolution and the regioselectivity of the oxygen insertion process.

Ketones **1**–**3** were almost fully converted to the product lactones within 48 h. In all cases, a single substrate enantiomer ('better-fitting' enantiomer within the active site of enzyme) was oxidized at slightly higher velocity than the antipodal ('less fitting') enantiomer. The rather low *E* values indicate limited quality of the kinetic resolution, although reasonable yields of enantiopure starting material had been recovered when the oxidation of **1** was stopped at lower conversions.[15] In the new examples, high enantiomeric excess values of ketones (ee_S) were observed only at a high degree of conversion. Bio-oxidations led to the formation of 'abnormal' lactone, preferably, with a regioisomeric excess of around 70%.

The structurally more demanding ketone **4** was converted with significantly higher *E* value, hence providing an

Table 1. Microbial Baeyer–Villiger oxidations of fused bicyclic ketones **1**–**7** using recombinant whole-cells of *Escherichia coli* expressing monooxygenase from *Mycobacterium tuberculosis*

Substrate	ee_S (%) of substrate	*E*[a]	Conversion (%)[b]	re (%)[c]	ee_N (%) of normal lactone[d,e]	ee_A (%) of abnormal lactone[d,e]
 **1**	>99	2	95	72	56 (1*S*,5*R*)	86 (1*R*,5*S*)
 **2**	98	2	91	68	95 (1*S*,5*S*)	76 (1*R*,5*S*)
 **3**	92	3	93	76	87 (1*S*,6*S*)	79 (1*S*,6*R*)
 **4**	>99	12	68	96	23 (n.d.)	54 (n.d.)
 **5**	>99	7	78	38	93 (1*R*,5*R*)	86 (1*S*,5*R*)
 **6**	82	14	54	29	19 (1*S*,5*S*)	95 (1*R*,5*S*)
 **7**	>99	>200	50	100	n.a.	>99 (1*S*,6*R*)

n.d., not determined; n.a., not applicable.

[a] Enantioselectivity values (*E*) were determined by computer fitting of GC data to an equation derived from the theoretical expression relating the enantiomeric excess of residual substrates (ee_S) with the fractional conversion.

[b] Conversion after 48 h of biotransformation determined by chiral phase chromatography.

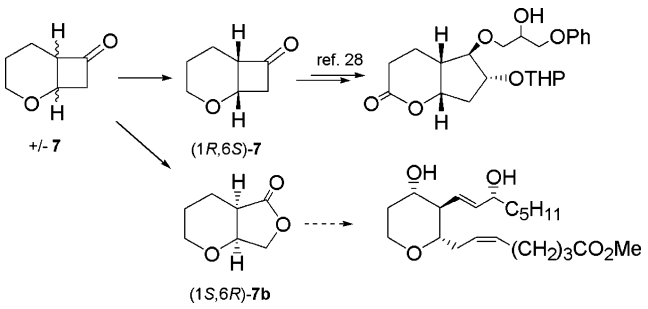
[c] Percent regioisomeric excess in favor of 'abnormal' lactone determined by chiral phase GC.

[d] ee_N and ee_A determined by chiral phase GC; racemic reference was prepared by mCPBA oxidation of ketones **2**–**7**.

[e] GC data were compared to data of CHMO mediated biotransformation and assigned according to the literature.[10,11]

improved kinetic resolution. Excellent ee_S was obtained already after 68% conversion.

Hetero-bicyclic fused ketones **5**–**7** containing oxygen were even better precursors for the kinetic resolution. Such substrates did not reach complete conversion within the standard biotransformation times of the screening protocol and only marginal reaction was observed during the second 24 h. While tetrahydrofuran based systems **5** and **6** gave comparable results to carbobicyclic substrate **4**, tetrahydropyran ketone **7** was only converted to the corresponding 'abnormal' lactone; biotransformation stopped at 50% and both product and remaining substrate were isolated in excellent optical purity. Such a transformation is very interesting from the point of view of possible synthetic applications, as enantioselectivity values above 200 ensure rapid and effective processes.[23]



Scheme 2. Ketone (+)-**7** and lactone (−)-**7b** as key precursors for transformations toward natural products.

In order to underscore the uniqueness of $BVMO_{Mtb5}$, we compared the progress of biotransformation of ketone **7** with recombinant *E. coli* strains expressing $CHMO_{Acineto}$ from *Acinetobacter*[24] and $CPMO_{Coma}$ from *Comamonas*.[25] Those strains can be regarded as 'benchmark' catalysts reflecting the key features of the two family clusters among BVMOs.

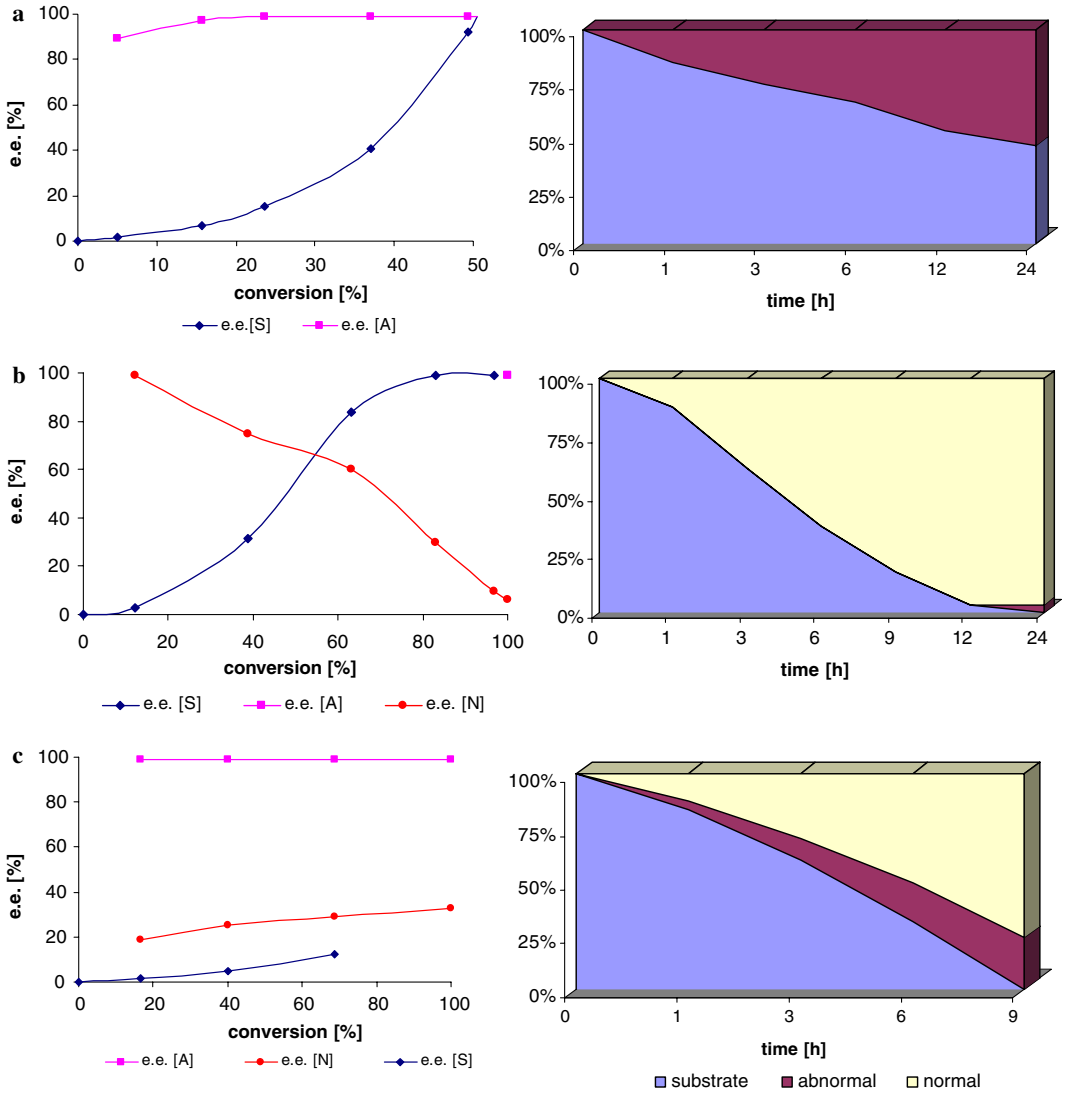


Figure 1. Regioselective Baeyer–Villiger oxidations of **7** using engineered *Escherichia coli* expressing: (a) BVMO from *Mycobacterium tuberculosis* ($BVMO_{Mtb5}$); (b) $CPMO_{Coma}$ from *Comamonas*; (**c**) $CHMO_{Acineto}$ from *Acinetobacter*. S, substrate; N, normal lactone; A, abnormal lactone.

Scheme 2 outlines the different behavior of these three whole-cell biocatalysts for the biooxidation of ketone **7** over the timeframe until the end of conversion. Engineered cells expressing $CHMO_{Acineto}$ did not resolve starting material sufficiently; highest value of ee_S was approx. 10% at 70% conversion. Both normal and abnormal lactone were formed from the beginning of the biooxidation. The prior lactone was generated predominantly, however, in low optical purity, while abnormal lactone was obtained in excellent enantiomeric excess (Fig. 1c).

$CPMO_{Coma}$ converted (+)-**7** to one enantiomer of normal lactone, preferably. The second enantiomer (−)-**7** was oxidized to the antipodal normal lactone also, however with lower velocity. Consequently, biooxidation with this strain allows isolation of (−)-substrate in good ee at a conversion above approx. 70% (Fig. 1b). Formation of abnormal lactone was detected only in minor amounts (approx. 6% of total lactone quantity) in the last stage of transformation, but with excellent ee (ee_A = >99%).

$BVMO_{Mtb5}$ displays excellent kinetic resolution with formation of abnormal lactone in high optical purity from the very beginning of the biotransformation. The reaction stops at 50% conversion, and at this stage ee_S of remaining (+)-**7** is also >99% (Fig. 1a). It is noteworthy that this whole-cell system provides access to the antipodal ketone compared to $CPMO_{Coma}$ mediated transformations.

A preparative scale biotransformation using **7** as starting material and $BVMO_{Mtb5}$ as biocatalyst was performed to confirm assignment of absolute stereochemistry from GC based screening experiments.[26] Data for optical rotation were compared to the literature values.[11] After purification of products, we obtained (1*R*,6*S*)-(+)-2-oxabicyclo[4.2.0]octan-7-one (+)-**7** and (1*S*,6*R*)-(−)-3-oxabicyclo[3.3.0]octan-2-one (−)-**7b** in >99% ee and in good yields.[27]

Based on our observations, biooxidations using $BVMO_{Mtb5}$ display general, novel, and very valuable properties for the kinetic resolution of racemic ketone substrates for the first time in enzyme mediated Baeyer–Villiger oxidations. We consider this new whole-cell system as superior biocatalyst compared to previous catalytic entities for such transformations based on the simplicity to grow and handle recombinant *E. coli* together with the high control of biocatalyst production by simultaneously minimizing possible side reactions in living cells. The above results open up new possible pathways toward synthesis of natural products. Ketone (+)-**7** represents a key intermediate for preparation of potentially important prostanoid synthons,[28] while lactone (−)-**7b** is envisioned to become an interesting precursor for natural products bearing a tetrahydropyran structural core as the central feature (Scheme 2).[29]

$BVMO_{Mtb5}$ displays a very different biocatalytic behavior to previously reported Baeyer–Villiger monooxygenases. We are presently trying to extend our family clustering hypothesis to this enzyme based on protein sequence information and active site models. Future synthetic applications of the recombinant expression system are currently addressed in our laboratory.

Acknowledgment

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20. *Typical procedure for bacterial cultivation.* Fresh LB_{amp} ($CHMO_{Acineto}$, $CPMO_{Coma}$) or LB_{kan} ($BVMO_{Mtb5}$) was inoculated with 1% of an overnight preculture of the appropriate recombinant *E. coli* strain in a baffled Erlenmayer flask. The culture was incubated at 120 rpm at 37 °C on an orbital shaker until the culture reached the OD_{600} of 0.5.
Typical procedure for screening experiments in multi-well dishes. Each well was charged with $LB_{amp/kan}$ grown bacterial culture (2 mL); IPTG was added (final concentration of 0.025 mM) together with substrate (1 mg) and

β-cyclodextrin (1 equiv). The plate was shaken at rt and after certain time periods (1, 3, 6, 9, 12, 24, and 48 h) samples were analyzed by chiral phase GC measurement (after extraction with EtOAc supplemented with internal standard).

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22. Percent regioisomeric excess (% re) is defined as the percentage of the major regioisomer (both enantiomers) minus the percentage of the minor regioisomer (both enantiomers).
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26. *Typical procedure for biotransformation on preparative scale.* Baffled Erlenmayer flask was charged with LB_{kan} grown bacterial culture (250 mL). IPTG was added (final concentration of 0.025 mM) together with substrate (100 mg) and β-cyclodextrin (1 equiv). The flask was shaken at rt until the conversion was complete (24 h). The biomass was removed by centrifugation; the aqueous phase was repeatedly extracted with dichloromethane. The combined organic layers were dried over sodium sulfate and concentrated. Ketone **7** and lactone **7b** were purified by flash column chromatography.
27. *Physical and spectral data of (+)-***7**. Yellow oil; yield 64% (32 mg); δ_H (200 MHz, $CDCl_3$) 1.46–1.64 (m, 3H), 2.04–2.12 (m, 1H), 2.74 (d, $J = 17$ Hz, 1H), 3.17–3.38 (m, 3H), 3.81–3.87 (m, 1H), 4.45 (t, $J = 6$ Hz, 1H). δ_C (50 MHz, $CDCl_3$) 18.7 (t), 22.1 (t), 53.4 (t), 57.1 (d), 64.0 (t), 65.1 (t), 207.5 (s). $[\alpha]_D^{20}$ +15.27 (*c* 0.81, $CHCl_3$). *Physical and spectral data of* (−)-**7b**. Colorless oil; yield 71% (40 mg); δ_H (200 MHz, $CDCl_3$) 1.48–1.62 (m, 2H), 1.74–1.93 (m, 1H), 2.26–2.32 (m, 1H), 2.55–2.60 (m, 1H), 3.30–3.43 (m, 1H), 3.87–3.93 (m, 1H), 4.24–4.31 (m, 3H). δ_C (50 MHz, $CDCl_3$) 20.0 (t), 22.0 (t), 39.9 (d), 66.0 (t), 72.4 (t), 73.5 (d), 176.8 (s). $[\alpha]_D^{20}$ −24.50 (*c* 0.82, $CHCl_3$).
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