Baeyer–Villiger Oxidation of Bridged *endo*-Tricyclic Ketones with Engineered *Escherichia coli* Expressing Monooxygenases of Bacterial Origin

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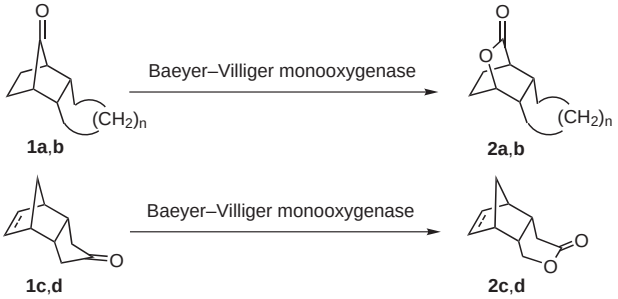
Abstract: Whole-cell biotransformations using engineered strains of *Escherichia coli* expressing cyclopentanone (CPMO) and cyclohexanone monooxygenases (CHMO) of various bacterial origins have been tested for substrate acceptance on tricyclic ketones. Based on the stereopreference of the biocatalytic Baeyer–Villiger oxidation, our recent clustering of this library of enzymes into two distinct groups based on protein sequence was confirmed. Together with short and facile reaction sequences for the production of the substrate ketones, microbial biooxidation enables access to antipodal product lactones as versatile building blocks in natural product and bioactive compound synthesis.

Key words: biocatalysis, biooxidation, recombinant whole-cell biotransformation, Baeyer–Villiger oxidation, monooxygenase, stereoselectivity

The stereoselective Baeyer–Villiger (BV) oxidation to chiral lactone intermediates has received considerable attention in recent years.[1] Both organometal complexes[2] and enzymes (BV monooxygenases – BVMOs)[3] are available as enantioselective catalysts fulfilling the requirements of sustainable and green chemistry strategies.

Very recently, we introduced a platform of recombinant strains of *Escherichia coli* overexpressing BVMOs of various microbial origin.[4,5] This small library of enzymes possesses overlapping substrate specificity for the biooxidation of various ketones and displays enantiocomplementary[6] and regio-divergent biotransformations.[7] The utilization of recombinant whole-cells minimizes potential enzymatic side reactions[8] and simultaneously allows for a facile application of cofactor dependent biocatalysts.[9] Our current research program aims at providing an easy-to-use biocatalytic toolbox of renewable catalytic entities for stereoselective BV oxidations[10] to organic chemists and to demonstrate its potential in natural product synthesis.

Enzyme-mediated BV biooxidation of tricyclic bridged ketones of type **1** as prochiral precursors potentially generates four new chiral centers (**2**) in a single desymmetrization step (Scheme 1).[11]



Scheme 1 Whole-cell biotransformation of tricyclic ketones (n = 1, 2).

Tricyclic bridged ketones **1a**,**b** were prepared according to the literature[12] with minor modifications in a short and efficient sequence: initial Diels–Alder cyclization of the appropriate alkenes with activated diene **3** to **5a**,**b** was followed by reductive removal of the chlorines (**6a**,**b**), acid-catalyzed hydrolysis of ketal (**7a**,**b**), and subsequent hydrogenation using Pd/C (Scheme 2).

The synthetic route to compounds of type **1c**,**d** utilized carbic anhydride **8** to generate diol **9**.[13] Subsequent transformations involved activation of the hydroxyls (**10**) and Kolbe nitrile formation (**11**).[14] Cyclization to ketone **1c** was performed via diacid **12**, following a route recently developed by our group for the preparation of fused bicycloketones (Scheme 3).[6c] Alkene **1c** was easily converted to compound **1d** by atmospheric pressure hydrogenation.

Only related bicyclo-substrates of type **1a**,**b** were previously oxidized by a cyclohexanone monooxygenase (CHMO) using isolated enzyme.[15] The stereopreference of monooxygenases originating from *Acinetobacter* ($CHMO_{Acineto}$),[16] *Arthrobacter* ($CHMO_{Arthro}$),[17] *Brachymonas* ($CHMO_{Brachy}$),[18] *Brevibacterium* ($CHMO_{Brevi1}$, $CHMO_{Brevi2}$),[19] *Comamonas* ($CPMO_{Coma}$),[20] and *Rhodococcus* ($CHMO_{Rhodo1}$, $CHMO_{Rhodo2}$)[17] species in whole-cell mediated BV oxidations utilizing *E. coli* as host organism was investigated in this study for the biooxidation of *endo*-tricyclic ketones **1a**–**d**.[21]

Protein expression of BVMOs at high level was triggered by addition of isopropyl-β-D-galactopyranoside to the growing culture of recombinant *E. coli* strains. Fermentations with substrates **1a**,**b** were carried out according to our previously reported procedure on 100 mg scale in Erlenmeyer flasks.[4,22] Biooxidation of ketones **1c**,**d** was carried out in parallel format using 12- and 24-well plastic dishes.[23] A crucial aspect of such a mini-scale screen for

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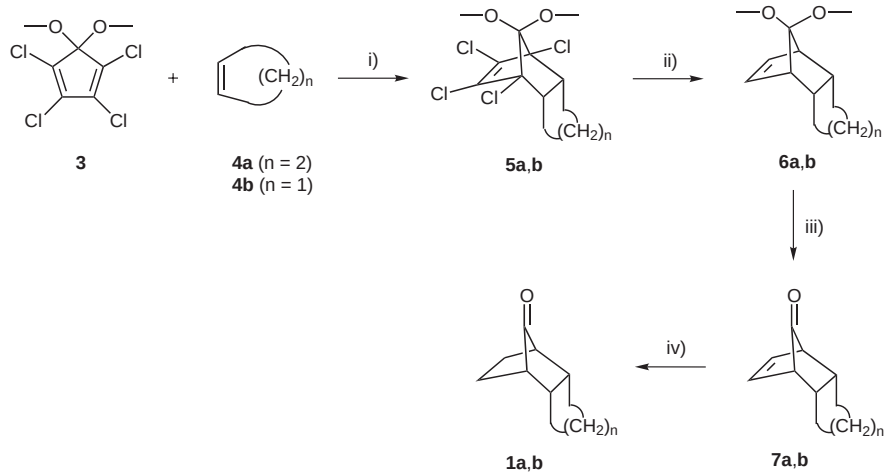
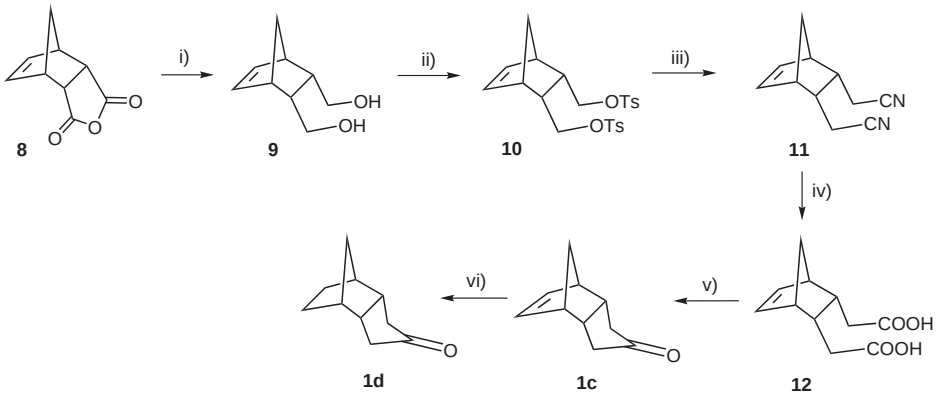


Scheme 2 (i) Δ, 82–85%; (ii) Na, *t*-BuOH, THF, reflux, 67–88%; (iii) *p*-TSA, dry acetone, r.t., 73–82%; (iv) H_2 (5 bar), Pd/C, EtOAc, r.t., 85–87%.



Scheme 3 (i) LAH, THF, reflux, 75–95%; (ii) TsCl, pyridine, –35 °C, 75%; (iii) NaCN, DMSO, 150 °C, 56%; (iv) KOH, H_2O, EtOH, reflux, 46%; (v) pyridine, Ac_2O, 1 N HCl, reflux, 71%; (vi) H_2 (1 bar), Pd/C, r.t., 84%.

this study was the comparability of the multi-well experiment with fermentation results in shake-flasks. Our optimization efforts took advantage of previous studies for the use of microscale processing technologies in kinetic studies and bioprocess design.[24]

The parallel screening format was designed to provide an easy-to-use methodology for the assessment of recombinant whole-cell biocatalysts, implementing reproducible and optimized conditions for cell growth, expression of recombinant biocatalyst, and the biotransformation itself. Standardized experiments were carried out with 0.5 mg of substrate per mL of broth in 12- or 24-well dishes in the presence of 1 equivalent of β-cyclodextrin to facilitate biooxidation of slowly converted substrates. Transformations were analyzed after 24 hours of fermentation time at 24 °C by extraction of the sample with EtOAc supplemented by an internal standard. This fermentation temperature was determined as optimum value to achieve good protein expression and to minimize loss of biocatalyst due to limited enzyme stability.

These screening conditions provided conversion results that were comparable with shake-flask experiments. Single preparative biotransformations were carried out to determine physical and spectral data together with specific optical rotation of isolated lactones **2c**,**d**. Racemic reference material of lactones **2a**, **2b**, and **2d** was prepared by chemical oxidation of the corresponding ketones with MCPBA.

Results of the biotransformations of substrates **1a**–**d** to lactones **2a**–**d** with all eight overexpression systems of *E. coli* are summarized in Table 1.[25]

Substrates **1a** and **1b** were oxidized by all BVMOs and gave the respective lactones **2a**,**b** in excellent stereoselectivity. Our recently discovered trend for enantiodivergent biotransformations was also observed for this compound type, with $CPMO_{Coma}$ and $CHMO_{Brevi2}$ ('CPMO group') providing antipodal products to the 'CHMO group'.[4]

In the case of tricyclo-ketones **1c** and **1d** the BVMOs displayed a selective acceptance of biooxidation precursors. Compound **1c** was essentially no substrate for CHMO-type enzymes, with conversion rates below 10%. In contrast, CPMO-type proteins readily gave the expected lactone **2c**. With respect to stereopreference, $CPMO_{Coma}$ and $CHMO_{Brevi2}$ produced (–)-**2c** in moderate to poor stereoselectivity, while trace amounts of lactones obtained from

Table 1 Biotransformations of *endo*-Tricyclic Ketones with Whole-Cells of Recombinant *E. coli* Expressing Bacterial BVMOs

	2a	**2b**	**2c**	**2d**
Strain	Yield (%),[a] ee[c] (+/–, %)	Yield (%),[a] ee[c] (+/–, %)	Conv. (%),[b] ee[c] (+/–, %)	Conv. (%),[b] ee[c] (+/–, %)
$CHMO_{Acineto}$	63 97 (–)	47 97 (–)	n.c.[d] n.a.	3 92 (+)
$CHMO_{Arthro}$	46 99 (–)	40 99 (–)	6 92 (+)	49 93 (+)
$CHMO_{Brachy}$	46 99 (–)	42 99 (–)	n.c. n.a.	12 94 (+)
$CHMO_{Brevi1}$	56 93 (–)	49 94 (–)	n.c. n.a.	n.c. n.a.
$CHMO_{Brevi2}$	78 94 (+)	67 92 (+)	68[a] 74 (–)	3 74 (–)
$CPMO_{Coma}$	57 83 (+)	45 91 (+)	73[a] 36 (–)	62[a] 23 (–)
$CHMO_{Rhodo1}$	58 99 (–)	50 99 (–)	2 91 (+)	63[a] 95 (+)
$CHMO_{Rhodo2}$	54 99 (–)	63 99 (–)	4 90 (+)	26 95 (+)

[a] Isolated yield after chromatographic purification.
[b] Conversion of substrate in parallel screening according to GC.
[c] The ee values were determined by chiral phase GC; the sign of specific rotation is given; racemic reference material prepared by MCPBA oxidation of ketones **1a**,**b**,**d**.
[d] Abbreviations: n.c. = no conversion; n.a. = not applicable.

biooxidations with CHMO-type enzymes showed good selectivity for the generation of (+)-lactone **2c**. The high chemoselectivity of the enzymatic BV oxidation should be noted, as no indication of epoxidation at the C=C double bond was observed in the biotransformations.

Substrate **1d** was more readily accepted by CHMO-type BVMOs and $CHMO_{Rhodo1}$ gave good isolated yields of (+)-**2d** in excellent optical purity. Again, CPMO-type biocatalysts produced the antipodal lactone, however, either with poor conversion ($CHMO_{Brevi2}$) or in low stereoselectivity ($CPMO_{Coma}$).

In summary, the present study demonstrates the potential of a library of recombinant whole-cell expression systems for bacterial BVMOs to produce antipodal lactones with a tricyclic structural core. Both the stereopreference of the enzymatic oxidation together with the substrate acceptance is in good agreement with our previous hypothesis of two BVMO groups, which is based on protein sequence and biocatalyst performance.[4] Furthermore, we have optimized our previous efforts in characterizing novel BVMOs as versatile biocatalysts by using a parallel screening system for the rapid determination of substrate profiles and stereopreference.

The obtained compounds represent versatile building blocks for natural and bioactive compound synthesis. In particular, lactone **2c** bearing an additional alkene functionality seems suitable as precursor for the total synthesis of various prostaglandines.[26] Currently, the further elaboration of the chiral products obtained is addressed in our laboratories together with the determination of their absolute configuration.

We consider our approach to utilize the natural diversity of BVMOs in enantiodivergent transformations as highly complementary to recent advances in the random[27] and knowledge-based[28] modification of these enzymes based on the previous publication of the first structure of this protein family.[29] Taken together, these strategies are aiming at the design of a toolbox for BV biooxidations, which should ultimately become a standard technique in day-to-day synthetic chemistry.

Acknowledgment

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(21) ***endo*-Tricyclo[6.2.1.0^{2,7}]undecan-11-one (1a)**: yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.81–1.89 (m, 14 H), 2.06–2.14 (m, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 17.1 (t), 18.1 (t), 19.5 (t), 32.9 (d), 43.4 (d), 216.7 (s).
***endo*-Tricyclo[5.2.1.0^{2,6}]decan-10-one (1b)**: yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.63–1.75 (m, 12 H), 2.46 (m, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 27.7 (t), 29.2 (t), 29.9 (t), 38.2 (d), 43.6 (d), 214.9 (s).
***endo*-Tricyclo[5.2.1.0^{2,6}]dec-8-en-4-one (1c)**: beige amorphous solid; mp = 97–99 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.30–1.55 (m, 2 H), 1.70–1.90 (m, 2 H), 2.05–2.35 (m, 2 H), 2.70–2.95 (m, 4 H), 6.00–6.15 (m, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 39.7 (d), 41.0 (t), 47.0 (d), 49.7 (t), 136.1 (d), 219.9 (s).
***endo*-Tricyclo[5.2.1.0^{2,6}]decan-4-one (1d)**: colorless amorphous solid; mp = 97–100 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.16–1.59 (m, 6 H), 2.03–2.40 (m, 6 H), 2.51–2.74 (m, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.1 (t), 38.6 (d), 39.3 (t), 40.6 (t), 41.3 (d), 221.0 (s).

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(23) **Typical Procedure for Screening Experiment in Multi-Well Dishes (12 or 24 Wells).**
Each well was charged with LB-amp medium (2 mL/12-well format or 1 mL/24-well format) and inoculated with 1% of an overnight preculture of recombinant *E. coli* strains. A plate was incubated at 120 rpm at 37 °C on an orbital shaker for 2 h. IPTG was added (final concentration of 0.025 mM) together with substrate (1 mg or 0.5 mg) and β-cyclodextrin (1 equiv). The plate was shaken at r.t. for 24 h and then analyzed by chiral phase GC after extraction of the sample with EtOAc.

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(25) **Physical and Spectroscopic Data of Lactones 2a–d.**
***endo*-9-Oxatricyclo[6.2.2.0^{2,7}]dodecan-10-one (2a)**: beige crystals, mp 76–78 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.09–2.06 (m, 14 H), 2.39–2.41 (q, *J* = 2.7 Hz, 1 H), 4.36–4.41 (q, *J* = 3.3 Hz, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 16.9 (t), 19.3 (t), 19.5 (t), 20.0 (t), 20.5 (t), 20.8 (t), 32.3 (d), 36.2 (d), 39.8 (d), 78.9 (d), 177.3 (s). Specific optical rotation ($CHMO_{Brachy}$): $[\alpha]_D^{20}$ –31.8 (*c* 0.95, $CHCl_3$); 99% ee.
***endo*-8-Oxatricyclo[5.2.2.0^{2,6}]undecan-9-one (2b)**: colorless crystals, mp 78–80 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.66–1.90 (m, 12 H), 2.55 (q, *J* = 3.1 Hz, 1 H), 4.55 (q, *J* = 3.7 Hz, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 16.7 (t), 20.6 (t), 27.7 (t), 27.9 (t), 28.1 (t), 37.7 (d), 39.2 (d), 41.2 (d), 78.9 (d), 177.3 (s). Specific optical rotation ($CHMO_{Rhodo2}$): $[\alpha]_D^{20}$ –17.3 (*c* 1.70, $CHCl_3$); 99% ee.
***endo*-5-Oxatricyclo[6.2.1.0^{2,7}]undec-9-en-4-one (2c)**: colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.45 (d, *J* = 8.0 Hz, 1 H), 1.64 (d, *J* = 8.0 Hz, 1 H), 1.93–2.98 (m, 6 H), 3.70 (m, 1 H), 4.30 (m, 1 H), 6.05–6.28 (m, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 33.4 (t), 35.9 (d), 38.6 (d), 44.2 (d), 46.0 (d), 50.6 (t), 69.9 (t), 134.9 (d), 136.3 (d), 173.7 (s). Specific optical rotation ($CHMO_{Brevi2}$): $[\alpha]_D^{20}$ –12.7 (*c* 1.72, $CHCl_3$); 74% ee.
***endo*-5-Oxatricyclo[6.2.1.0^{2,7}]undecan-4-one (2d)**: colorless solid, mp 78–80 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.30–1.59 (m, 6 H), 2.12–2.55 (m, 6 H), 4.02–4.32 (m, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 21.9 (t), 23.3 (t), 30.4 (t), 35.9 (d), 37.0 (d), 39.0 (d), 40.7 (d), 41.2 (t), 68.2 (t), 174.3 (s). Specific optical rotation ($CHMO_{Rhodo1}$): $[\alpha]_D^{20}$ +32.2 (*c* 3.53, $CHCl_3$); 95% ee.

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