Accepted Manuscript

Regio- and stereoselective synthesis of chiral nitrilolactones using Baeyer-Villiger monooxygenases

Michael J. Fink, Radka Snajdrova, Alexander Winninger, Marko D. Mihovilovic

PII: S0040-4020(15)30295-7

DOI: 10.1016/j.tet.2015.12.047

Reference: TET 27373

To appear in: *Tetrahedron*

Received Date: 23 November 2015

Revised Date: 9 December 2015

Accepted Date: 22 December 2015

Please cite this article as: Fink MJ, Snajdrova R, Winninger A, Mihovilovic MD, Regio- and stereoselective synthesis of chiral nitrilolactones using Baeyer-Villiger monooxygenases, *Tetrahedron* (2016), doi: 10.1016/j.tet.2015.12.047.

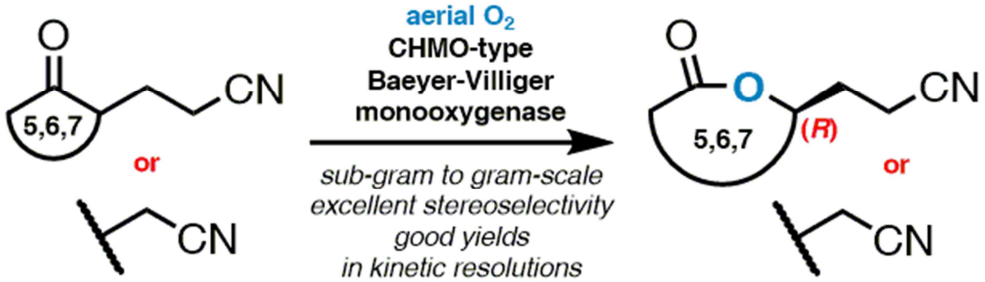
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Regio- and stereoselective synthesis of chiral nitrilolactones using Baeyer-Villiger monooxygenases

Leave this area blank for abstract info.

Michael J. Fink, Radka Snajdrova, Alexander Winninger and Marko D. Mihovilovic
Institute of Applied Synthetic Chemistry, TU Wien, Getreidemarkt 9/163, 1060 Vienna, Austria



Tetrahedron
journal homepage: www.elsevier.com

Regio- and stereoselective synthesis of chiral nitrilolactones using Baeyer-Villiger monooxygenases

Michael J. Fink [a,b] ∗, Radka Snajdrova[a,c], Alexander Winninger[a] and Marko D. Mihovilovic[a]

[a]*Institute of Applied Synthetic Chemistry, TU Wien, Getreidemarkt 9/163, 1060 Vienna, Austria.*
[b]*Present affiliation: Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford St, Cambridge, MA 02138, United States of America.*
[c]*Present affiliation: Medicines Research Centre, GlaxoSmithKline, Gunnel's Wood Road, Stevenage, Herts SG1 2NY, United Kingdom.*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Biocatalysis
BVMO
Redox biotransformation
Chiral building block
Stereoelectronic effects

ABSTRACT

This work describes the regio- and enantioselective synthesis of nitrile-containing chiral lactones from easily accessible ketone precursors using Baeyer-Villiger monooxygenases. These biocatalysts controlled the distribution of regioisomers much more tightly than commonly used stoichiometric reagents, additionally with good to excellent optical purity of products. A surprising case of strong stereoelectronic control was also observed. We tested a library of 14 catalysts using five- to eight-membered cyclic ketones with two different tether lengths to the nitrile group. In all but the largest series we found suitable wild-type enzymes for preparative scale synthesis of the target compounds. The diverse possibilities to further functionalize lactones and nitriles make this method interesting for the generation of chiral building blocks.

∗ Corresponding author. E-mail: michael.j.fink@tuwien.ac.at

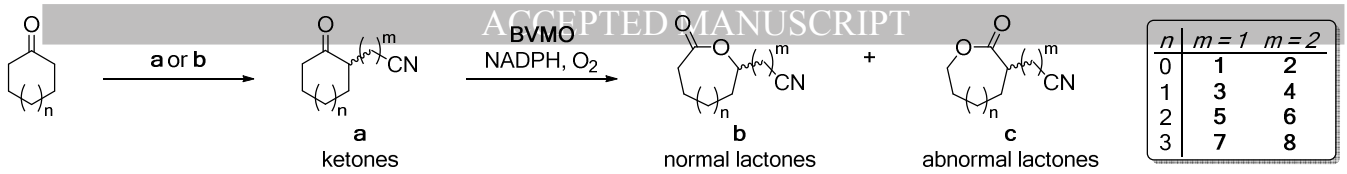
1. Introduction

The widespread use of biocatalysts in organic-synthetic research still much depends on their ease of use, the predictability of substrate acceptance, and to a lesser extent on the availability of stereocomplementary enzymes in asymmetric synthesis. Whereas industrial chemists have long embraced the potential of biocatalysis and have created productive overlaps of biotechnology, biocatalysis and organic synthesis in the production of bulk and fine chemicals, as well as marketed pharmaceuticals[1] — scientists working in exploratory research often shun the option of enzymatic catalysis for its alleged higher initial effort to reach a set goal of selectivity, or scale. Stronger innovation and development is thus needed to bring these powerful methods closer together.[2,3]

We envision two main areas for improvement to facilitate the broader application of enzymes in organic synthesis: catalyst formulation and stability, and development as well as demonstration of reliable use for certain reactions. The first issue is critical to the user, but given the inherent complexity of enzymes the solutions to this problem are usually more elaborate than e.g. simple exclusion of oxygen and moisture for certain metal catalysts. Tremendous effort is invested on this topic.[4-6] The second area addresses the organic chemist's desire to have rules to predict selectivity, or more generally, reaction outcome (cf. Prelog's rule[7] for alcohol dehydrogenases or Kazlauskas's rule[8] for lipases). Alternatively, clear superiority over other catalysts or reagents also promotes usage (e.g. Merck & Co.'s department of research and development has recently restricted their screening of carbonyl reduction reactions to enzymes only).[9] With this work we want to contribute in this way for the class of Baeyer-Villiger monooxygenases (BVMOs).

The first BVMOs were discovered more than 40 years ago; they are so far indisputably the best catalysts for asymmetric Baeyer-Villiger oxidations (BVOx).[10-12] Many enzymes of this class have been characterized in great detail from both biochemical and synthetic perspectives, resulting in knowledge of multiple hundred accepted substrates.[13] Still, most synthetic studies focused on a holistic description of an enzyme's substrate profile using model compounds, or showed the performance of particular BVMOs in the synthesis of certain molecules of interest. This continued attention and the emergence of such publications actually illustrates the lack of a general rule for reactivity and selectivity.

This study aims to establish BVMOs as excellent catalysts for the synthesis of nitrile-containing lactones as chiral building blocks derived from their respective nitriloketones. These starting materials are themselves easily accessible from cheap bulk chemicals. The combination of three functional groups (carboxylate, secondary alcohol and nitrile) with strongly distinct reactivity patterns provides a plethora of possible reactions. Our investigations were based on the discovery of this activity by Alphand and co-workers on two cyclohexanone substrates.[14] We expanded the scope to cyclopentanones, –heptanones and –octanones, identified general trends and independently corroborated the assignment of absolute configuration, all in order to facilitate the planning of synthetic strategies using the products described herein.



Scheme 1. Synthesis of racemic ketones **1a–8a** via Stork enamine synthesis and their biocatalytic Baeyer-Villiger oxidation towards regioisomeric normal lactones **1b-6b** and abnormal lactones **1c-6c**. Reagents and conditions: a) 1. pyrrolidine, benzene or toluene, Dean-Stark trap, 2. chloroacetonitrile, dioxane, then H_2O; b) 1. as in a), 2. acrylonitrile, dioxane, then H_2O. NADPH: nicotinamide adenine dinucleotide, reduced form.

2. Results and Discussion

We synthesized eight racemic ketone substrates **1a–8a** via Stork enamine reactions (**Scheme 1**).[15] First, the respective unsubstituted cycloketones were condensed with pyrrolidine as benzene or toluene solutions in a Dean-Stark trap apparatus. We used the resulting enamines without further purification for alkylation with chloroacetonitrile (for compounds **1a**, **3a**, **5a** and **7a**) or for Michael addition to acrylonitrile (for compounds **2a**, **4a**, **6a** and **8a**). All reactions and subsequent purifications proceeded smoothly; the conjugate additions generally gave higher yields (up to 88% after vacuum distillation, ketone **4a**).

In preparation for the biocatalyst screening reactions, all expected lactone products were synthesized by BVOx using meta-chloroperbenzoic acid (mCPBA) for analytical characterization and GC method development (data not shown). We selected 14 BVMOs for the evaluation: three linear ketone-converting enzymes (phenylacetone monooxygenase, PAMO, from *Thermobifida fusca*, p-hydroxyacetophenone MO, HAPMO, from *Pseudomonas fluorescens*, a steroid MO, STMO, from *Rhodococcus rhodochrous*) and 11 cycloketone-converting enzymes, which are further sub-grouped in seven cyclohexanone MO-type (CHMOs from *Acinetobacter calcoaceticus*, *Arthrobacter* sp. BP2, *Brachymonas petroleovorans*, *Brevibacterium epidermis*, *R. ruber*, *Xanthobacter*sp. ZL5), two cyclopentanone MO-type (CPMO from *Comamonas* sp. NCIMB 9872 and CHMO from *B. epidermis*) and two large ring-converting BVMOs (cyclododecanone MO, CDMO, from *R.* ruber and a *Pseudomonad* cyclopentadecanone MO, CPDMO). The Supplementary Data contains detailed information and references. All enzymes were tested as heterologous whole-cell over-expression systems in *Escherichia coli* BL21(DE3) or TOP10, which harbored standard vectors with a T7 promotor for expression control (except PAMO, HAPMO, STMO and CPDMO: *ara* regulon). The reactions were carried out in multi-well plates with 1 mL initial volume for up to 24 h, starting in the early exponential growth phase of the bacteria. Samples were withdrawn after 4, 8 and 24 h, extracted with EtOAc, and analyzed the extracts on chiral-phase GC-FID. Additional reactions containing acetophenone (PAMO), p-hydroxyacetophenone (HAPMO), 3-benzylcyclobutanone (STMO) and *rac*-bicyclo[3.2.0]hept-2-en-6-one (all other BVMOs) were performed and analyzed as positive controls for enzyme activity (data not shown).

2.1. Results from screening reactions on analytical scale

Already with the first substrate, cyclopentanone **1a**, we found a strongly different trend than expected from the known results[14] with $CHMO_{Acineto}$ and the higher homolog ketone **3a** (1:1 ratio of regioisomers). All active enzymes preferentially catalyzed the formation of the levorotatory normal lactone (–)-**1b** (**Figure 1a**); even at full conversion of the starting material, we only found <20% of abnormal lactone **3c** in any of the reactions. CHMO-type BVMOs were moderately enantioselective, with ee-values ranging from 50–90% (selectivity factor E up to 33). Excellent selectivity was achieved with CPMO-types (up to >99% ee, E>200). CDMO performed sluggishly, and CPDMO gave results similar to the CHMO-group's. In total, we detected both the remaining ketone enantiomer (–)-**1a** and the normal lactone (–)-**1b** with excellent optical purity, but its regioisomer **1c** only with poor optical enrichment.

In strong contrast to the above results, all CHMO-types except $CHMO_{Brevi\ 1}$, plus CDMO and CPDMO converted the enantiomers of cyclohexanone **3a** with almost perfect stereospecificity to the regioisomers **3b** and **3c** (**Figure 1b**), as suggested by the initial finding using $CHMO_{Acineto}$.[14] The CPMO-types had negligible activity, and $CHMO_{Brevi\ 1}$ produced only **3b**. Interestingly, in addition to the high specificity, we observed a pronounced difference in rate constants for the ketone enantiomers. Thus at first one enantiomer was preferentially depleted, before the remaining one was converted via a different transition state mechanism (stereoelectronic control).[16,17] This was a peculiar finding from a kinetic and structural aspect.

Cycloheptanone **5a** was the first substrate in this library to illustrate the known limitations in ring size for substituted cycloketones in BVMO-catalyzed reactions (**Figure 2a**).[18,17,19] Only six enzymes could accommodate this ring in their active site, and in all these biotransformations we only detected the formation of normal lactone **5b**. The large-ring converting BVMOs CDMO[19,20] and CPDMO[17,18,21] were already previously described as not proficient at oxidizing bulky and/or substituted medium to large ring ketones. Neither did CPMO-types measurably oxidize **5a**. Mainly CHMO-type enzymes were active in kinetic resolution of this substrate with moderate selectivity. We measured the best result using $CHMO_{Rhodo\ 2}$ (E = 11 at 39% relative conversion). In general, the reaction rate was also much lower than for the smaller ring sizes we tested before. As we expected after these findings, we could not detect any product formation with both cyclooctanone derivates **7a** and **8a** with any of the 15 tested BVMOs.

All active wild-type BVMOs in our test set converted the three remaining substrates **2a**, **4a** and **6a** to the respective normal lactones, exclusively, in kinetic resolution reactions. It was reported that stoichiometric reagents as mCPBA or trifluoroperacetic acid favored the nucleophilicity-driven rearrangement, but also produced substantial amounts of the abnormal product from **4a** (6–20%).[14] We could corroborate this trend with ketones **2a** and **6a** qualitatively during the synthesis of racemic reference samples (unquantified reactions, data not shown). We further hypothesized that the virtually absolute regioselectivity of BVMOs with these substrates was promoted by the lower inductive effect of the nitrile group on the α-carbonyl carbon atom over three σ-bonds in ketones **2a**, **4a** and **6a** vs. two σ-bonds in **1a**, **3a**. Cycloheptanone **5a** was too poor a substrate to possibly be taken into consideration for this trend.

All cycloketone-converting enzymes accepted cyclopentanone **2a** as a substrate (**Figure 2b**) and produced (*R*)-**2b** exclusively. We measured comparable reaction rates throughout the catalysts (40–50% conversion after 4 h), except for CDMO (only 36% after 24 h). CPMO-types, CPDMO and CDMO could not

discriminate between ketone enantiomers, but most CHMO-types showed excellent enantioselectivity. $CHMO_{Brevi\ 1}$ scored the best and preparatively highly useful result (E>200 at 20%, E = 79 at 38% conversion).

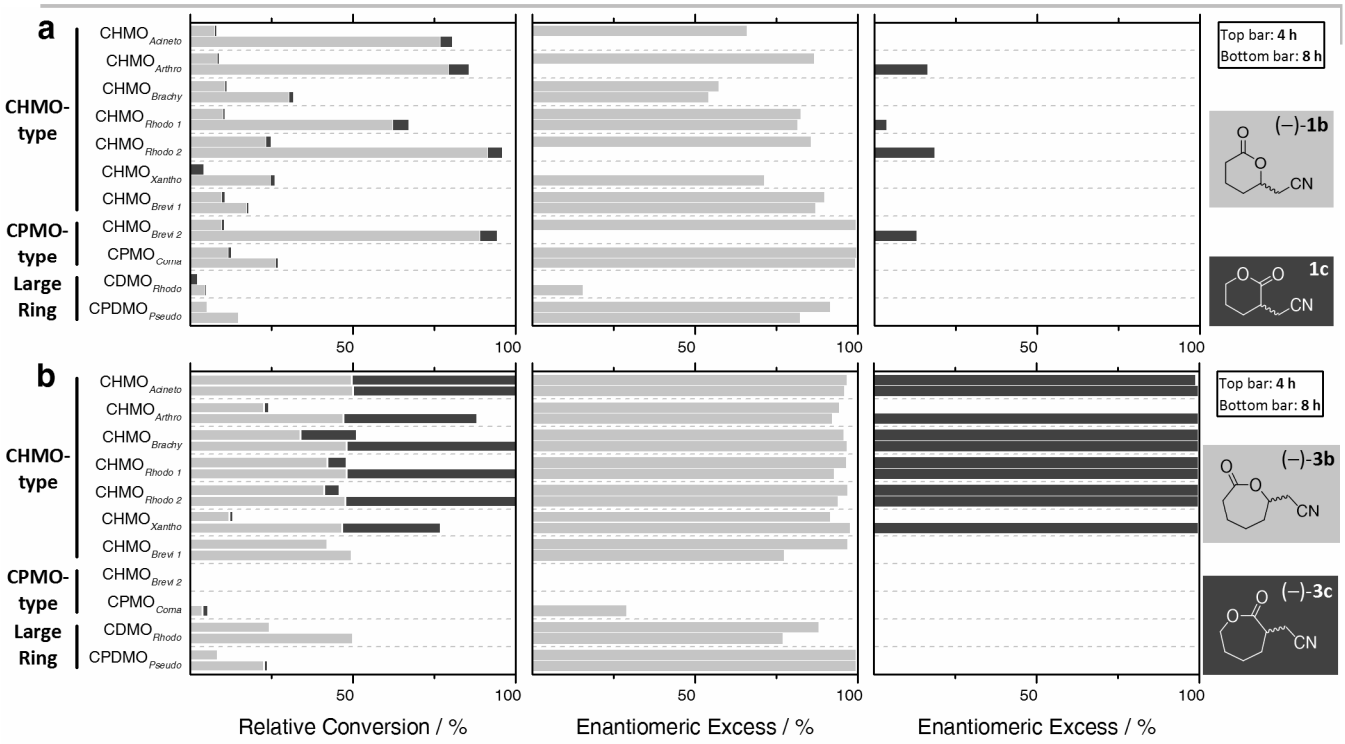


Figure 1. Analytical screening data for BVMO-catalyzed reactions with the partial formation of abnormal lactones **c** (from ketones **1a** and **3b**). The enzymes are grouped by phylogenetic clusters (cf. ref. [22]). For each enzyme, the top bar value was determined after 4 h, the bottom bar value after 8 h. All data is color-coded: light grey for normal lactones, dark grey for abnormal lactones. Left boxes: relative conversion of racemic ketone to regioisomeric products. Center boxes: enantiomeric excess of normal lactone. Right boxes: enantiomeric excess of abnormal lactone.

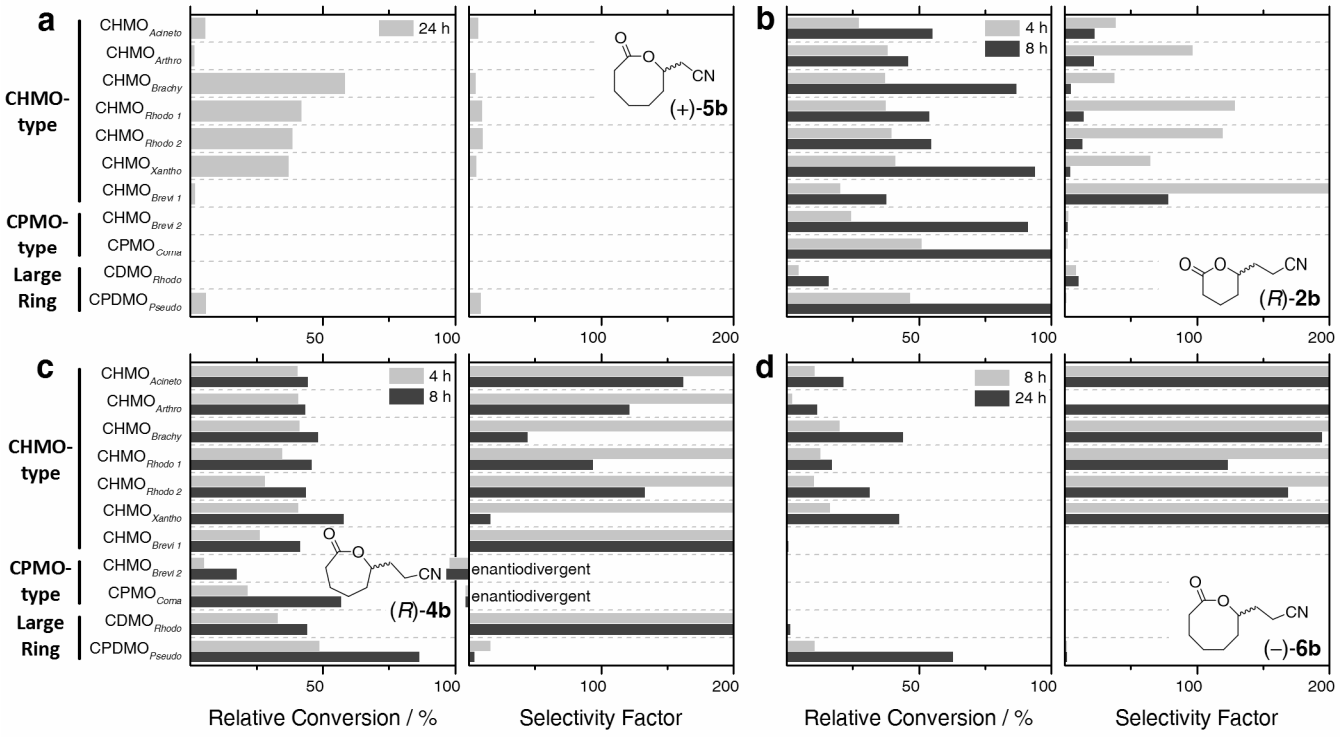
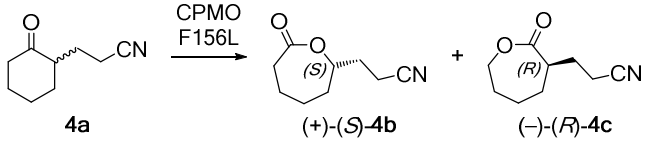


Figure 2. Analytical screening data for BVMO-catalyzed reactions towards normal lactones **b** only (from ketones 5**a**, **2a**, **4a** and **6a**). The enzymes are grouped by phylogenetic clusters (cf. ref. [22]). Left boxes: relative conversion of racemic ketone to normal lactone. Right boxes: selectivity factor for the kinetic resolution, calculated according to Sih's equation $E = [\ \ln(1 - ee_S) - \ln(1 + ee_S/ee_P)\ ] / [\ \ln(1 + ee_S) - \ln(1 + ee_S/ee_P)\ ]$ from ee of substrate (ee_S) and ee of product (ee_P) values. a: values after 24 h only. b, c: values after 4 h and 8 h. d: values after 8h and 24 h.

Again, all cycloketone-converting catalysts efficiently oxidized cyclohexanone **4a** to normal lactone **4b** (**Figure 2c**). CHMO-types and CDMO catalyzed the formation of (*R*)-**4b** with excellent selectivity (E>200), whereas the CPMO-types showed poor, but enantiodivergent selectivity. CPDMO had no apparent preference for the ketone enantiomers over the course of the reaction. We observed a similar reactivity and selectivity pattern for cycloheptanone **6a**. CHMO-types save $CHMO_{Brevi\ 1}$ were active and highly selective for the formation of (–)-**6b**, and CPDMO produced racemic **6b**. In this case, we could not measure any activity with CPMO-types and CDMO.



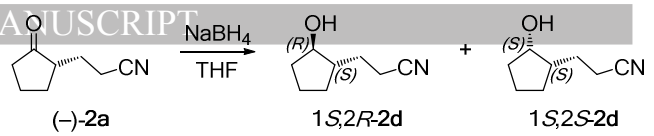
Scheme 2. Formation of abnormal lactone **4c** using variant F156L of $CPMO_{Coma}$.

Inspired by results from a concurrent BVMO project,[23] we found a surprising new activity when screening a library of CPMO variants[24-26] with ketone **4a**. Not only did stereoselectivity improve (although not better than $CHMO_{Brevi\ 2}$), but the F156L variant also preferentially produced the abnormal lactone (–)-**4c** (approx. ratio 3:7 **4b/4c; Scheme 2**), unfortunately with mediocre enantioselectivity (45% ee). A double mutant, F156H-G157L, performed with better selectivity (75% ee, 1:1 **4b/4c**), but at a very low rate (see Supplementary Data). This collection of mutants had already previously been generated with the aim of improving the selectivity and/or of extending the substrate acceptability of wild-type CPMO.[24-26] Four active site positions were varied (F156, G157, G449, F450), to modulate available space and hydrogen bonding properties specifically without randomization, including four double mutants. These results strongly suggest that migratory preference can change drastically with rather small perturbations in catalyst structure; only recently a similar effect was demonstrated on another BVMO in a mutagenesis study addressing regioselectivity.[27]

In summary, we found that CHMO-type BVMOs perform best with nitriloketones. As often, CPMO-types acted as complementary catalysts, either in terms of activity or selectivity. The large ring-converting BVMOs CPDMO and CDMO were not particularly useful in this substrate class. This feature was surprising, given the otherwise wide applicability of CDMO.[19] The linear ketone-converting BVMOs in our test set completely failed to accept any of the substrates with a single exception: HAPMO converted ketone **2a** enantiodivergently to (+)-**2b**, but with poor selectivity (E<10; see Supplementary Data).

2.2. Corroboration of the assignment of absolute configuration

In their initial publication[14] Berezina et al. assigned the absolute configuration of (+)-**2a** by comparison of the sign of optical rotation to a published value. [28] The authors of the cited reference had based their assignment on the reaction mechanism of the alkylation of cyclohexanone (*R*)-prolinol enamine with acrylonitrile. This assignment was unfortunately never cross-validated or confirmed using unambiguous methods, e.g. single-crystal XRD. We opted to obtain a second reference point for the assignment of absolute configuration in this series in order to provide higher fidelity in stereochemical planning. After all, this method should serve as a means to generate chiral building blocks for more elaborate synthetic targets.



Scheme 3. Reduction of the enantiomerically enriched ketone (–)-**2a** to the previously characterized *trans*-configured alcohol 1*S*,2*R*-**2d**.

This goal was achieved by reducing enantioenriched (–)-**2a**, obtained by kinetic resolution using $CHMO_{Brevi\ 1}$, to the corresponding diastereomeric cyclopentanols using sodium borohydride, and subsequent separation of the diastereomers by careful column chromatography. Despite the massive loss in chiral information due to the strongly basic reaction conditions (ee_{2a} = 88%, ee_{2d}<30%), we were able to measure the sign of optical rotation of the *trans*-isomer. By comparison to the published value for this compound[29] we safely assigned the *S*-configuration to (–)-**2a**, as the main topic of the cited study was the assignment of absolute configurations to mono- and polycyclic lactones similar to our compounds. Our independent pathway thus corroborated the previous assignment, and followed the general trend in BVMO-catalyzed oxidations of 2-substituted cycloketones: the preferential oxidation of *R*-ketones.[19]

2.3. Exemplary preparative biotransformations

We performed preparative biotransformations with substrates **1a–6a** to confirm the results we obtained in the screening reactions, and to demonstrate the usefulness of the method in the synthesis of chiral nitrilolactone building blocks (multi-100 mg scale). The reactions were linearly scaled up from the 1 mL scale in standard shake flasks and without pH control. We isolated most products in high to excellent optical purity, and with reasonably good yields for kinetic resolution reactions (**Table 1**).

Table 1. Summary of preparative biotransformation results.

Entry	Ketone	Enzyme	Product 1	Product 2
1	**1a**	$CHMO_{Brevi\ 1}$	(–)-**1a** 46%, 58% ee	(–)-**1b** 41%, 97% ee
2	**2a**	$CHMO_{Brevi\ 1}$ 0.3 g scale	(–)-(*S*)-**2a** 40%, 98% ee	(–)-(*R*)-**2b** 31%, >99% ee
3		$CHMO_{Brevi\ 1}$ 1.2 g scale[a]	(–)-(*S*)-**2a** 47%, 88% ee	(–)-(*R*)-**2b** 33%, 97% ee
4	**3a**	$CHMO_{Brachy}$	(–)-**3b** 19%, >99% ee	(–)-**3c** 34%, >99% ee
5	**4a**	$CHMO_{Arthro}$	(+)-(*S*)-**4a** 38%, 97% ee	(–)-(*R*)-**4b** 39%, 98% ee
6		$CPMO_{F156L}$	(+)-(*S*)-**4b** 18%, 96% ee	(–)-**4c** 47%, 59% ee
7	**5a**	$CHMO_{Rhodo\ 2}$	(–)-**5a** 41%, 68% ee	(+)-**5b** 10%, 66% ee
8	**6a**	$CHMO_{Xantho}$	(–)-**6a** 43%, 99%	(–)-**6b** 42%, 95%

[a] The reaction was performed in a small bench-top bioreactor with pH and aeration control.

We observed substantial racemization of the enantiomerically enriched cyclopentanones and –hexanones upon purification on regular silica, but not of the cycloheptanones (see Experimental Section). We did not further investigate or optimize this issue, but are convinced that other more neutral chromatographic stationary phases would alleviate the problem.

We performed the transformations of ketone **2a** on different scales and reaction vessels (**Table 1**, entries 2 and 3), and found that the controlled environment of a bioreactor improved the results only slightly in yield. In any case, it was convenient and easy to quench the reaction after such time that both starting

material and product could be isolated in highly enantio-enriched form (**Figure 3**).

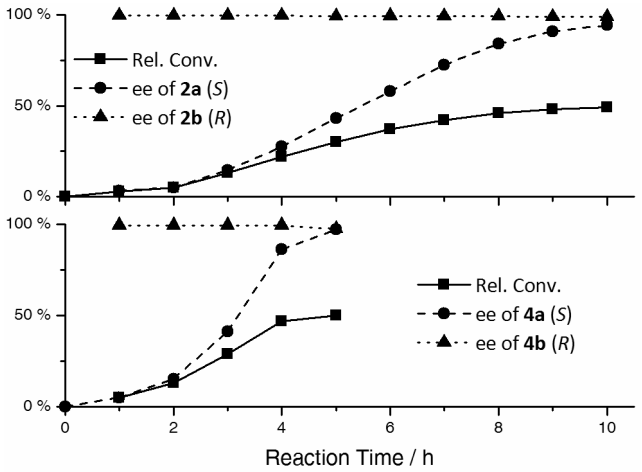


Figure 3. Time course of preparative kinetic resolutions; ee values were deteremined by chiral-phase GC; conversion values were calculated from ee data according to Sih's equations.[30] Top: ketone **2a** using $CHMO_{Brevi\ 1}$. Bottom: ketone **4a** using $CHMO_{Arhtro}$.

3. Conclusion

In this work we demonstrated the potential of BVMOs in the synthesis of chiral nitrilolactones. The respective starting materials were easily accessible from cheap starting materials (cycloketones, pyrrolidine, chloroacetonitrile or acrylonitrile). We identified suitable biocatalysts to generate 6-, 7- and 8-membered lactones with excellent optical purity and good yields on the sub-gram to gram-scale. In total, CHMO-type BVMOs performed best, with CPMO-types serving as a useful extension for different selectivity. Large ring-converting and linear ketone-converting BVMOs were found unsuitable for this substrate class. In most cases, the nucleophilicity-goverened, *normal* lactones were formed with high selectivity. We also observed and interpreted cases which deviated from this trend. We are convinced that the extensive analytical screening reaction data and the exemplary preparative biotransformations we presented are sufficient to make use of this method for the synthesis of such chiral building blocks. Their lactone and nitrile moieties should provide ample opportunity for various synthetic strategies.

4. Experimental

4.1. General — Chemistry

Unless noted otherwise, all reagents were purchased from commercial suppliers and used without further purification. Anhydrous dioxane, THF and toluene was obatained by passing the pre-distilled solvents over Al_2O_3 columns (PURESOLV, Innovative Technology). Chromatography solvents were distilled prior to use. Column chromatography was performed on a Büchi Sepacore Flash System using silica gel from Merck (40-63 µm) using the indicated solvent mixtures. NMR spectra were recorded from $CDCl_3$ solutions on a Bruker AC 200 (200 MHz ^{1}H resonance) spectrometer and chemical shifts are reported in ppm relative to tetramethylsilane. Reaction progress of non-biocatalytic reactions was determined using GC-MS(EI) (Thermo Finnigan Focus GC / DSQ II, capillary column BGB5 (30m x 0.32 mm ID). Enzyme screening data and enantiomeric excess of products was determined via GC-FID using a BGB175 (30 m x 0.25 mm ID, 0.25 µm film) or BGB173 (30 m x 0.25 mm ID, 0.25 µm film) column on a ThermoQuest Trace GC 2000 and a ThermoFocus GC. Specific rotation was measured on an Anton Paar MCP500 polarimeter at the specified conditions. High resolution mass spectrometry was performed on a Thermo Scientific LTQ Orbitrap XL hybrid FT-MS, equipped with a Thermo Fischer Exactive Plus Orbitrap (LC-ESI+) and a Shimadzu IT-TOF Mass Spectrometer.

4.2. General — Cultivation of E. coli

Bacterial cultures were incubated in baffled Erlenmeyer flasks in orbital shakers (InforsHT Multitron 2 Standard) at 200 rpm. All materials and biotransformation media were sterilized by autoclaving at 121 °C for 20 min. Various aqueous stock solutions were sterilized by filtration through 0.20 µm syringe filters. Agar plates were prepared with LB medium (10 g L^{-1} bacto-peptone, 5 g L^{-1} yeast extract, 10 g L^{-1} NaCl) supplemented by 1.5% w/v Agar Agar.

4.3. General procedure for the alkylation of cycloketones via the corresponding pyrrolidine enamines

Following the procedures of Stork,[15] the cycloketone and pyrrolidine (1.1 equiv.) were dissolved in benzene or toluene (300 mL mol^{-1} ketone; toluene for n>1) and refluxed for 5–10 h (cyclopentanone and –hexanone) or 24–48 h (cycloheptanone and –octanone). Excess pyrrolidine and solvent were removed under reduced pressure and the yellow to brown oily residue was directly used for further reactions. For medium-sized rings a small amount of *p*-toluenesulfonic acid was added (n>1).

Next, the enamine (typically 5–20 g) was dissolved in anhydrous dioxane (400 mL mol^{-1}) under an argon atmosphere. Then the electrophile (1.5 equiv.; chloroacetonitrile for compounds **1**, **3**, **5** and **7**, or acrylonitrile for compounds **2**, **4**, **6** and **8**) was added in one shot using a syringe. Occasionally, more electrophile was added in case of stagnant reactions. The mixture was refluxed for 10–48 h until complete conversion of the enamine could be detected by GC-MS. Subsequently, water (200 mL mol^{-1}) was added and the biphasic mixture was heated to reflux for 30–60 min. Dioxane was then removed by rotary evaporation and the residue was partitioned between EtOAc (2 mL $mmol^{-1}$) and 2N HCl (0.5 mL $mmol^{-1}$). The aqueous phase was extracted with EtOAc (4 x same volume as before) and the pooled extracts were dried over sodium sulfate and concentrated under reduced pressure. Purification of the brown crude oils was performed on MPLC or via distillation.

*4.4. 2-(2-Oxocyclopentyl)acetonitrile **1a***

Following the general procedure in section 4.3 with cyclopentanone pyrrolidine enamine (9.0 g, 66 mmol) and chloroacetonitrile and purification via chromatography (LP/EtOAc 4:1) yielded the title ketone as a yellow oil (1.1 g, 14%). The spectral data was in agreement with previous literature.[31] ^{1}H-NMR: δ 1.61-1.89 (m, 2H), 2.00-2.20 (m, 2H), 2.27-2.47 (m, 4H), 2.59-2.72 (m, 1H). ^{13}C-NMR: δ 17.1 (t), 20.1 (t), 28.8 (t), 37.0 (t), 45.3 (d), 118.0 (s), 216.2 (s).

4.5.** 3-(2-Oxocyclopentyl)propanenitrile **2a

Following the general procedure in section 4.3 with cyclopentanone pyrrolidine enamine (7.5 g, 55 mmol) and acrylonitrile and purification via vacuum distillation yielded the title ketone as a pale yellow oil (4.1 g, 54%). b.p.: 123–124 °C at 5 mbar. ^{1}H-NMR: δ 1.37–1.84 (m, 3H), 1.87–2.37 (m, 6H), 2.47 (dt, 2H, J_1 = 3.6 Hz, J_2 = 7.2 Hz). ^{13}C-NMR: δ 15.4 (t), 20.4 (t), 25.5 (t), 29.3 (t), 37.7 (t), 47.5 (d), 119.4 (s), 219.4 (s).

4.6.** 2-(2-Oxocyclohexyl)acetonitrile **3a

Following the general procedure in section 4.3 with cyclohexanone pyrrolidine enamine (15.1 g, 100 mmol) and

chloroacetonitrile and purification via chromatography (LP/EtOAc 4:1) yielded the title ketone as a dark yellow oil (4.7 g, 34%). The spectral data was in agreement with previous literature.[32] ^{1}H-NMR: δ 1.36-2.72 (m, 11H). ^{13}C-NMR: δ 17.9 (t), 24.9 (t), 27.6 (t), 33.4 (t), 41.6 (t), 46.8 (d), 118.6 (s), 208.8 (s).

4.7. *3-(2-Oxocyclohexyl)propanenitrile* ***4a***

Following the general procedure in section 4.3 with cyclohexanone pyrrolidine enamine (454 g, 3 mol) and acrylonitrile and purification via vacuum distillation yielded the title ketone as a colorless liquid (400 g, 88%). b.p.: 144-146 °C at 10 mbar. The spectral data was in agreement with previous literature.[33] ^{1}H-NMR: δ 1.16–2.12 (m, 8H), 2.18–2.49 (m, 5H). ^{13}C-NMR: δ 15.0 (t), 25.0 (t), 25.5 (t), 27.8 (t), 34.1 (t), 42.1 (t), 48.7 (d), 119.7 (s), 211.7 (s).

4.8. *2-(2-Oxocycloheptyl)acetonitrile* ***5a***

Following the general procedure in section 4.3 with cycloheptanone pyrrolidine enamine (4.56 g, 28 mmol) and chloroacetonitrile and purification via chromatography (LP/EtOAc 5:1) yielded the title ketone as a yellow oil (1.28 g, 31%). The spectral data was in agreement with previous literature.[34,35] ^{1}H-NMR: δ 1.23-2.03 (m, 8H), 2.34-2.70 (m, 3H), 2.87-3.00 (m, 1H). ^{13}C-NMR: δ = 19.7 (t), 23.6 (t), 28.9 (t), 29.2 (t), 30.7 (t), 43.1 (t), 48.1 (d), 119.0 (s), 211.6 (s).

4.9. *3-(2-Oxocycloheptyl)propanenitrile* ***6a***

Following the general procedure in section 4.3 with cycloheptanone pyrrolidine enamine (13.6 g, 82 mmol) and acrylonitrile and purification via chromatography (LP/EtOAc 10:1) yielded the title ketone as a pale yellow oil (5.5 g, 40%). The spectral data was in agreement with previous literature.[36] ^{1}H-NMR: δ 1.17–2.12 (m, 10H), 2.21–2.58 (m, 4H), 2.64–2.78 (m, 1H). ^{13}C-NMR: δ 15.2 (t, C-2), 23.7 (t), 27.5 (t), 28.8 (t), 29.0 (t), 31.6 (t), 43.4 (t), 49.9 (d), 119.6 (s), 214.3 (s).

4.10. *2-(2-Oxocyclooctyl)acetonitrile* ***7a***

Following the general procedure in section 4.3 with cyclooctanone pyrrolidine enamine (4.22 g, 24 mmol) and chloroacetonitrile and purification via chromatography (LP/EtOAc 10:1) yielded the title ketone as a pale yellow oil (2.05 g, 53%). The spectral data was in agreement with previous literature.[37] ^{1}H-NMR: δ 2.57-3.10 (m, 2H, CH_2-CN), 1.35-2.5 (m, 13H). ^{13}C-NMR: δ 17.0 (t), 22.6 (t), 22.9 (t), 23.9 (t), 25.7 (t), 29.6 (t), 40.5 (t), 44.7 (d), 117.3 (s), 213.8 (s).

4.11. *3-(2-Oxocyclooctyl)propanenitrile* ***8a***

Following the general procedure in section 4.3 with cyclooctanone pyrrolidine enamine (16.0 g, 89 mmol) and acrylonitrile and purification via chromatography (LP/EtOAc 10:1) yielded the title ketone as a pale yellow oil (4.0 g, 25%). The spectral data was in agreement with previous literature.[36] ^{1}H-NMR: δ 1.00-2.53 (m, 16H), 2.72-2.86 (m, 1H). ^{13}C-NMR: δ 15.4 (t), 24.6 (t), 24.9 (t), 25.0 (t), 27.2 (t), 27.5 (t), 32.9 (t), 42.9 (t), 48.3 (d), 119.4 (s), 218.4 (s).

4.12. General procedure for substrate acceptance and biocatalyst performance screening on analytical scale

A baffled Erlenmeyer flask was charged with LB medium with appropriate antibiotics supplement (10 mL), inoculated with a bacterial single colony from an Agar plate and incubated at 37 °C in an orbital shaker o/n. The biotransformation medium, supplemented with appropriate antibiotics, was then inoculated with 2% v/v of the preculture and incubated for approx. 1–2 h under the same conditions until an optical density of 0.2–0.6 was reached. Inducing agent (see Supplementary Data) and β-cyclodextrin (1 equiv.) were added, the mixture was thoroughly mixed and split in 1.0 mL aliquots into 24-well plates. Substrates were added as 0.8 M solutions in dioxane to a final concentration of 4 mM. The plates were sealed with adhesive film and incubated at the appropriate temperature (see Supplementary Data) in an orbital shaker for up to 24 h. Analytical samples were prepared by extraction of 0.5 mL of biotransformation culture with 1.0 mL EtOAc (supplemented with 1 mM methyl benzoate as internal standard) after centrifugal separation of the cells (approx. 15 kRCF, 1 min, rt).

4.13. General procedure for preparative biotransformations in shake flasks

LB growth medium was inoculated, induced, and charged with additives as described in section 4.12. The substrate was then added directly to the shake flask as approx. 10% (w/v) solution in dioxane. Incubation at the appropriate temperature was carried out until the desired degree of conversion was determined via GC control. The aqueous solution was then centrifuged (17 kRCF, 15 min, 4 °C) and the supernatant was extracted with EtOAc (5x equal volume). The pooled organic layers were washed with brine, dried over sodium sulfate and concentrated. The crude compounds were purified by chromatography on silica using LP/EtOAc mixtures.

*4.14. (–)-2-(2-Oxocyclopentyl)acetonitrile (–)-**1a***

Following the general procedure in section 4.13 with racemic ketone **1a** (100 mg, 812 µmol, 4 mM) and $CHMO_{Brevi\ 1}$, after 10.5 h reaction time and subsequent purification (LP/Et_2O 1:3), the title compound was obtained as a yellow oil (46 mg, 46%). NMR data: see **1a**. ee (GC) = 58%. α_D^{25} = –115.4 (*c* 0.54, $CHCl_3$).

*4.15. (–)-2-(6-oxotetrahydro-2H-pyran-2-yl)acetonitrile (–)-**1b***

Following the general procedure in section 4.13 with racemic ketone **1a** (100 mg, 812 µmol, 4 mM) and $CHMO_{Brevi\ 1}$, after 10.5 h reaction time and subsequent purification (LP/Et_2O 1:3), the title compound was obtained as a yellow oil (46 mg, 41%). ^{1}H-NMR: δ 1.58–2.13 (m, 4H), 2.35–2.79 (m, 4H), 4.46–4.62 (m, 1H). ^{13}C-NMR: δ 18.2 (t), 24.6 (t), 27.0 (t), 29.1 (t), 74.8 (d), 115.9 (s), 169.9 (s). ee (GC) = 97%. α_D^{25} = –4.0 (*c* 0.70, $CHCl_3$). ESI-FT-MS m/z $[M+H]^+$ calc. 140.07061, found 140.07039, Δ 1.54 ppm.

*4.16. (–)-(S)-3-(2-Oxocyclopentyl)propionitrile (–)-(S)-**2a***

Following the general procedure in section 4.13 with racemic ketone **2a** (300 mg, 2.19 mmol, 4 mM) and $CHMO_{Brevi\ 1}$, after 10.5 h reaction time and subsequent purification (LP/EtOAc 2:1), the title compound was obtained as a colorless oil (121 mg, 40%). NMR data: see **2a**. ee (GC) = 98% before purification, 85% after purification. α_D^{25} = –100.8 (*c* 2.42, $CHCl_3$).

*4.17. (–)-(R)-3-(6-Oxotetrahydro-2H-pyran-2-yl)propionitrile (–)-(R)-**2b***

Following the general procedure in section 4.13 with racemic ketone **2a** (300 mg, 2.19 µmol, 4 mM) and $CHMO_{Brevi\ 1}$, after 10.5 h reaction time and subsequent purification (LP/EtOAc 1:1), the title compound was obtained as a yellow oil (104 mg, 31%). ^{1}H-NMR: δ 1.40–1.66 (m, 1H), 1.71–2.01 (m, 5H), 2.32–2.66 (m, 4H), 4.27–4.43 (m, 1H). ^{13}C-NMR: δ 13.2 (t), 18.3 (t), 27.5 (t), 29.2 (t), 31.4 (t), 77.9 (d), 119.0 (s), 171.1 (s). ee (GC) >99% before and after purification. α_D^{25} = -132.9 (*c* 0.38, $CHCl_3$). ESI-

FT-MS: m/z $[M+H]^+$ calc. 154.08626, found 154.08601, Δ 1.59 ppm.

*4.18. 3-(2-Hydroxycyclopropyl)propanenitriles 1R,2S-**2d** and 1S,2S-**2d***

$NaBH_4$ (248 mg, 6.56 mmol, 5 equiv.) was suspended in anhydrous THF (20 mL) under an argon atmosphere. Then a solution of ketone (–)-**2a** in THF (10 mL) was added at 0–5 °C. The mixture was stirred at this temperature for 2 h, but no conversion of starting material was detected. The cooling bath was removed and stirring was continued for 2 d. More $NaBH_4$ was added (5 equiv.) and stirring was continued (24 h). After 3 d total reaction time 50% conversion was reached. Another 10 equiv. $NaBH_4$ and THF (20 mL) were added and stirring was continued at rt. After 4 d the reaction mixture was poured on 2N HCl (150 mL) and extracted with Et_2O (3 x 100 mL). The pooled extracts were dried over sodium sulfate and concentrated under reduced pressure. Careful column chromatography (50 g silica, LP/Et_2O 1:2) separated both diastereomers as colorless oils. The spectral data of the *trans* isomer was in agreement with previous literature.[29] 1*R*,2*S*-**2d** (48 mg, 26%): ^{1}H-NMR: δ 1.07–1.25 (m, 1H), 1.45–2.02 (m, 9H), 2.39–2.47 (m, 2H), 3.80 (q, 1H, *J* = 6.1 Hz). ^{13}C-NMR δ 16.1 (t), 21.5 (t), 29.6 (t, 2C), 34.9 (t), 47.7 (d), 78.9 (d), 120.1 (s). α_D^{20} = –4.0 (*c* 0.96, $CHCl_3$); Lit.: α_D^{25} = –38.9 (*c* 7.5, $CHCl_3$).[29] d.r. (GC) = 2:98 *cis*/*trans*. ee (GC) = 34% ee. 1*S*,2*S*-**2d** (15 mg, 8%): ^{1}H-NMR: δ 1.15–2.04 (m, 10H), 2.31–2.56 (m, 2H), 4.21 (br s, 1H). ^{13}C-NMR: δ 16.3 (t), 21.8 (t), 25.2 (t), 28.6 (t), 35.6 (t), 44.4 (d), 73.9 (d), 120.3 (s). α_D^{20} = +4.9 (*c* 0.1, $CHCl_3$). d.r. (GC) = >99:1 *cis*/*trans*. ee (GC) = approx. 13% (no baseline separation).

*4.19. (–)-2-(7-Oxooxepan-2-yl)acetonitrile (–)-**3b***

Following the general procedure in section 4.13 with racemic ketone **3a** (300 mg, 2.19 mmol, 4 mM) and $CHMO_{Brachy}$, after 4 h reaction time and subsequent purification (LP/EtOAc 1:1), the title compound was obtained as a colorless oil (64 mg, 19%). The spectral data was in agreement with previous literature.[14] ^{1}H-NMR: δ 1.38–1.82 (m, 3H), 1.88–2.13 (m, 3H), 2.50–2.83 (m, 4H), 4.48–4.59 (m, 1H). ^{13}C-NMR: δ 22.5 (t), 25.2 (t), 27.8 (t), 33.9 (t), 34.7 (t), 74.9 (d), 116.4 (s), 173.7 (s). ee (GC) >99%. α_D^{25} = –22.6 (*c* 1.28, $CHCl_3$).

*4.20. (–)-2-(2-oxooxepan-3-yl)acetonitrile (–)-**3c***

Following the general procedure in section 4.13 with racemic ketone **3a** (300 mg, 2.19 mmol, 4 mM) and $CHMO_{Brachy}$, after 4 h reaction time and subsequent purification (LP/EtOAc 1:1), the title compound was obtained as a colorless solid (115 mg, 34%). The spectral data was in agreement with previous literature.[14] ^{1}H-NMR: δ 1.44–1.80 (m, 3H), 1.82–2.11 (m, 3H), 2.49 (dd, 1H, J_1 = 16.8 Hz, J_2 = 7.4 Hz), 2.67 (dd, 1H, J_1 = 16.9 Hz, J_2 = 5.8 Hz), 2.92–3.07 (m, 1H), 4.14–4.37 (m, 2H). ^{13}C-NMR: δ 21.0 (t), 27.7 (t), 28.4 (t), 29.1 (t), 39.9 (d), 68.9 (t), 118.2 (s), 174.6 (s). ee (GC) >99%. α_D^{25} = –27.0 (*c* 2.30, $CHCl_3$).

*4.21. (+)-(S)-3-(2-Oxocyclohexyl)propionitrile (+)-(S)-**4a***

Following the general procedure in section 4.13 with racemic ketone **4a** (300 mg, 1.98 mmol, 4 mM) and $CHMO_{Arthro}$, after 5 h reaction time and subsequent purification (LP/EtOAc 6:1), the title compound was obtained as a pale yellow oil (113 mg, 38%). NMR data: see **4a**. ee (GC) = 97% before purification, 80% after purification. α_D^{25} = +24.6 (*c* 2.26, $CHCl_3$).

*4.22. (–)-(R)-3-(7-Oxooxepan-2-yl)propionitrile (–)-(R)-**4b***

Following the general procedure in section 4.13 with racemic ketone **4a** (300 mg, 1.98 mmol, 4 mM) and $CHMO_{Arthro}$, after 5 h reaction time and subsequent purification (LP/EtOAc 2:1), the title compound was obtained as a yellow oil (131 mg, 39%). The spectral data was in agreement with previous literature.[38] ^{1}H-NMR: δ 1.38–1.68 (m, 3H), 1.74–2.02 (m, 5H), 2.43–2.59 (m, 4H), 4.25–4.35 (m, 1H). ^{13}C-NMR: δ 13.4 (t), 22.6 (t), 27.8 (t), 31.7 (t), 34.2 (t), 34.6 (t), 77.5 (d), 119.0 (s), 174.8 (s). ee (GC) = 98%. α_D^{25} = –100.5 (*c* 2.62, $CHCl_3$).

*4.23. (+)-(S)-3-(7-Oxooxepan-2-yl)propionitrile (+)-(S)-**4b***

Following the general procedure in section 4.13 with racemic ketone **4a** (265 mg, 1.75 mmol, 7 mM) and $CPMO_{Coma\text{-}F156L}$ after 22 h reaction time and subsequent purification (LP/Et_2O 3:1), the title compound was obtained as a colorless solid (52 mg, 18%). m.p.: 48–54 °C. ee (GC) = 96%. α_D^{25} = +56.5 (*c* 1.04, $CHCl_3$).

*4.24. (–)-3-(2-Oxooxepan-3-yl)propanenitrile (–)-**4c***

Following the general procedure in section 4.13 with racemic ketone **4a** (265 mg, 1.75 mmol, 7 mM) and $CPMO_{Coma\text{-}F156L}$ after 22 h reaction time and subsequent purification (LP/Et_2O 3:1), the title compound was obtained as a colorless solid (139 mg, 47%). m.p.: 62–66 °C. ^{1}H-NMR δ 1.46-1.80 (m, 5H), 1.83–2.03 (m, 2H), 2.04–2.22 (m, 1H), 2.48 (dd, 2H, J_1 = 7.6 Hz, J_2 = 6.4 Hz), 2.63-2.85 (m, 1H), 4.17-4.34 (m, 2H). ^{13}C-NMR δ 15.1 (t), 27.9 (t), 28.5 (t), 28.7 (t), 30.1 (t), 41.3 (d), 68.6 (t), 119.4 (s), 176.0 (s). ee (GC) = 59%. α_D^{25} = –9.3 (*c* 1.39, $CHCl_3$). ESI-FT-MS: m/z $[M+H]^+$ calc. 168.10245, found 168.1018, Δ 3.89 ppm.

*4.25. (–)-2-(2-Oxocycloheptyl)acetonitrile (–)-**5a***

Following the general procedure in section 4.13 with racemic ketone **5a** (100 mg, 661 µmol, 4 mM) and $CHMO_{Rhodo\ 2}$, after 24 h reaction time and subsequent purification (LP/EtOAc 3:1), the title compound was obtained as a colorless oil (41 mg, 41%). NMR data: see **5a**. ee (GC) = 68% before purification, 66% after purification. α_D^{25} = –58.8 (*c* 0.82, $CHCl_3$).

*4.26. (+)-2-(8-Oxooxocan-2-yl)acetonitrile (+)-**5b***

Following the general procedure in section 4.13 with racemic ketone **5a** (100 mg, 661 µmol, 4 mM) and $CHMO_{Rhodo\ 2}$, after 24 h reaction time and subsequent purification (LP/EtOAc 3:1), the title compound was obtained as a yellow oil (11 mg, 10%). ^{1}H-NMR: δ 1.47–1.94 (m, 8H), 2.44–2.54 (m, 2H), 2.67 (dd, 1H, J_1 = 16.8 Hz, J_2 = 5.8 Hz), 2.74 (dd, 1H, J_1 = 16.9 Hz, J_2 = 6.3 Hz), 4.87–5.00 (m, 1H). ^{13}C-NMR: δ 24.0 (t), 24.4 (t), 26.6 (t), 29.4 (t), 33.1 (t), 36.9 (t), 73.3 (d), 116.5 (s), 175.8 (s). ee (GC) = 66% before and after purification. α_D^{25} = +31.5 (*c* 0.22, $CHCl_3$). ESI-FT-MS: m/z $[M+H]^+$ calc. 168.10191, found 168.10170, Δ 1.22 ppm.

*4.27. (–)-3-(2-Oxocycloheptyl)propionitrile (–)-**6a***

Following the general procedure in section 4.13 with racemic ketone **6a** (200 mg, 1.21 mol, 4 mM) and $CHMO_{Xantho}$, after 19 h reaction time and subsequent purification (LP/EtOAc 4:1), the title compound was obtained as a colorless oil (85 mg, 43%). NMR data: see **6a**. ee (GC) = 99% after purification. α_D^{25} = -25.9 (*c* 1.70, $CHCl_3$).

*4.28. (–)-3-(8-oxooxocan-2-yl)propionitrile (–)-**6b***

Following the general procedure in section 4.13 with racemic ketone **6a** (200 mg, 1.21 mmol, 4 mM) and $CHMO_{Xantho}$, after 19 h reaction time and subsequent purification (LP/EtOAc 2:1), the title compound was obtained as a yellow oil (91 mg, 42%).

^{1}H-NMR: δ 1.38–2.04 (m, 10H), 2.39–2.63 (m, 4H), 4.60–4.74 (m, 1H). ^{13}C-NMR: δ = 13.8 (t), 23.7 (t), 26.1 (t), 28.8 (t), 31.0 (t), 32.3 (t), 36. 8 (t), 76.2 (d), 119.1 (s), 176.4 (s). ee (GC) = 95% after purification. α_D^{25} = -26.4 (*c* 1.82, $CHCl_3$). ESI-FT-MS: m/z $[M+H]^+$ calc. 182.11756, found 182.11728, Δ 1.51 ppm.

Supplementary Data

A document containing NMR spectra of the lactones isolated from preparative transformations, numerical data from all screening reactions and detailed biocatalyst and *E. coli* strain information can be downloaded from the publisher's website.

Acknowledgements

M.J.F. was funded within the EU-FP7 program Oxygreen (grant # 212281). A.W. received a graduate student fellowship within the DOC-program by the Austrian Academy of Sciences. The project was supported by the Austrian Science Fund (FWF) (project # I19-B10).

5. References

1. Bornscheuer, U. T.; Huisman, G. W.; Kazlauskas, R. J.; Lutz, S.; Moore, J. C.; Robins, K. *Nature* **2012,** *485*, 185.
2. Stewart, J. D.; Goswami, A., Eds. *Organic Synthesis Using Biocatalysis*; Academic Press, 2016.
3. Reetz, M. T. *J. Am. Chem. Soc.* **2013,** *135*, 12480.
4. Bommarius, A. S.; Paye, M. F. *Chem. Soc. Rev.* **2013,** *42*, 6534.
5. Stepankova, V.; Bidmanova, S.; Koudelakova, T.; Prokop, Z.; Chaloupkova, R.; Damborsky, J. *ACS Catal.* **2013,** *3*, 2823.
6. Iyer, P. V.; Ananthanarayan, L. *Process Biochem.* **2008,** *43*, 1019.
7. Prelog, V. *Pure Appl. Chem.* **1964,** *9*, 119.
8. Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991,** *56*, 2656.
9. Truppo, M. (Merck & Co.): *Personal Communication*, Cambridge, MA, **2015**.
10. Leisch, H.; Morley, K.; Lau, P. C. K. *Chem. Rev.* **2011,** *111*, 4165.
11. Balke, K.; Kadow, M.; Mallin, H.; Sass, S.; Bornscheuer, U. T. *Org. Biomol. Chem.* **2012,** *10*, 6249.
12. Rudroff, F.; Fink, M. J.; Mihovilovic, M. D. In *Organic Synthesis Using Biocatalysis;* Stewart, A. G. D. Ed.; Academic Press, 2016; pp. 243-283.
13. de Gonzalo, G.; Mihovilovic, M. D.; Fraaije, M. W. *ChemBioChem* **2010,** *11*, 2208.
14. Berezina, N.; Kozma, E.; Furstoss, R.; Alphand, V. *Adv. Synth. Catal.* **2007,** *349*, 2049.
15. Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrell, R. *J. Am. Chem. Soc.* **1963,** *85*, 207.
16. Noyori, R.; Sato, T.; Kobayashi, H. *Bull. Chem. Soc. Jpn.* **1983,** *56*, 2661.
17. Fink, M. J.; Fischer, T. C.; Rudroff, F.; Dudek, H.; Fraaije, M. W.; Mihovilovic, M. D. *J. Mol. Catal. B: Enzym.* **2011,** *73*, 9.
18. Iwaki, H.; Wang, S.; Grosse, S.; Bergeron, H.; Nagahashi, A.; Lertvorachon, J.; Yang, J.; Konishi, Y.; Hasegawa, Y.; Lau, P. C. K. *Appl. Environ. Microbiol.* **2006,** *72*, 2707.
19. Fink, M. J.; Rial, D. V.; Kapitanova, P.; Lengar, A.; Rehdorf, J.; Cheng, Q.; Rudroff, F.; Mihovilovic, M. D. *Adv. Synth. Catal.* **2012,** *354*, 3491.
20. Kostichka, K.; Thomas, S. M.; Gibson, K. J.; Nagarajan, V.; Cheng, Q. *J. Bacteriol.* **2001,** *183*, 6478.
21. Beneventi, E.; Ottolina, G.; Carrea, G.; Panzeri, W.; Fronza, G.; Lau, P. C. K. *J. Mol. Catal. B: Enzym.* **2009,** *58*, 164.
22. Mihovilovic, M. D.; Rudroff, F.; Grötzl, B.; Kapitan, P.; Snajdrova, R.; Rydz, J.; Mach, R. *Angew. Chem. Int. Ed.* **2005,** *44*, 3609.
23. Fink, M. J.; Mihovilovic, M. D. *Chem. Commun.* **2015,** *51*, 2874.
24. Clouthier, C. M.; Kayser, M. M. *J. Mol. Catal. B: Enzym.* **2007,** *46*, 32.
25. Clouthier, C. M.; Kayser, M. M.; Reetz, M. T. *J. Org. Chem.* **2006,** *71*, 8431.
26. Clouthier, C. M.; Kayser, M. M. *Tetrahedron: Asymmetry* **2006,** *17*, 2649.
27. Balke, K.; Schmidt, S.; Genz, M.; Bornscheuer, U. T. *ACS Chem. Biol.* **2015**.
28. O'Neill, P. M.; Rawe, S. L.; Borstnik, K.; Miller, A.; Ward, S. A.; Bray, P. G.; Davies, J.; Oh, C. H.; Posner, G. H. *ChemBioChem* **2005,** *6*, 2048.
29. Pirkle, W. H.; Adams, P. E. *J. Org. Chem.* **1980,** *45*, 4111.
30. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982,** *104*, 7294.
31. Chen, Y.; Ho, D. M.; Lee, C. *J. Am. Chem. Soc.* **2005,** *127*, 12184.
32. Miura, K.; Fujisawa, N.; Saito, H.; Wang, D.; Hosomi, A. *Org. Lett.* **2001,** *3*, 2591.
33. Cren, S.; Schar, P.; Renaud, P.; Schenk, K. *J. Org. Chem.* **2009,** *74*, 2942
34. Asselin, A. A.; Humber, L. G.; Dobson, T. A., American Home Products Corp., USA, US4057559A, **1977**; CAN 1978:120981.
35. Bailey, D. M.; Bowers, J. E.; Gutsche, C. D. *J. Org. Chem.* **1963,** *28*, 610.
36. Hickmott, P. W.; Jutle, K. K.; Pienar, D. H. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2399.
37. Schlichting, O.; Scheuerer, G., BASF AG, Germany, US2882292, **1959**; CAN 1959:121615.
38. Cotarca, L.; Delogu, P.; Maggioni, P.; Nardelli, A.; Bianchini, R.; Sguassero, S. *Synthesis* **1997**, 328.