Microbial Baeyer–Villiger Oxidation of Prochiral Polysubstituted Cyclohexanones by Recombinant Whole-Cells Expressing Two Bacterial Monooxygenases

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The microbial Baeyer–Villiger oxidation of prochiral 3,5-dimethylcyclohexanones bearing various functionalities with recombinant *E. coli* cells overexpressing cyclohexanone monooxygenase from *Acinetobacter* sp. NCIMB 9871 and cyclopentanone monooxygenase from *Comamonas* sp. NCIMB 9872 has been investigated. A distinct difference in substrate specificity and stereoselectivity of the two enzymes was observed, and enantiocomplementary products were obtained in some cases. The biocatalytic systems enabled access to chiral lactones as valuable intermediates for the total synthesis of various natural compounds. Substituents with varying lipophilicity and hybridization have been prepared by a diastereoselective synthetic route and subsequently biotransformed for the investigation of conformational and electronic effects on the biooxidation,.

Introduction

Baeyer–Villiger oxidation of cyclic ketones offers a facile entry to chiral lactones, which are interesting intermediates in enantioselective chemistry and are frequently encountered as precursors in natural compound synthesis.[1] Over recent years, asymmetric, metal-catalyzed Baeyer–Villiger oxidations have been continuously improved and represent a promising strategy for future process developments.[2] A complementary methodology is offered by biocatalysis. This "green chemistry" alternative usually gives products with optical purities that are still unattainable by artificial catalytic entities.

Flavin-dependent Baeyer–Villiger monooxygenase (BVMO)-catalyzed biotransformations play a key role in degradative pathways of microorganisms.[3–6] The action of such enzymes in cell metabolism is restricted to a single compound, but a remarkable acceptance for nonnatural substrates has been observed. Together with high enantioselectivity, this protein family represents a powerful catalytic system for chiral synthesis.

Cyclohexanone monooxygenase (CHMO) is a bacterial flavoprotein, which was found in *Acinetobacter* sp. NCIMB 9871 by Donoghue and Trudgill in 1975.[7] By applying selective growth conditions in cyclohexanol, a degradative pathway was triggered involving the Baeyer–Villiger oxidation of cyclohexanone to ε-caprolactone by CHMO. The oxygen-transfer process requires NADPH and FAD as cofactors.

CHMO is currently the best studied Baeyer–Villigerase, and its remarkably broad substrate specificity has been established in several studies.[4,5,8] One of the most important advantages of the enzyme is its ability to perform this transformation in an enantioselective way with nonnatural substrates. In addition, the enzyme is capable of performing stereoselective oxidation of heteroatoms, with the conversion of thioethers to optically pure sulfoxides being an especially valuable biotransformation.[9]

In order to provide a simple and convenient catalytic system for synthetic chemists, we designed an *E. coli*-based overexpression system for CHMO[10,11] as a second-generation recombinant version of the previously reported "designer yeasts".[12–14] By performing whole-cell fermentations, all required cofactors, especially NADPH, are recycled by the organism and it is not necessary to use artificial regeneration systems.[15]In addition the laborious process of enzyme isolation, which is complicated by protein stability, especially in the case of CHMO, can be avoided by whole-cell fermentations. It is the aim of this approach to take away the nimbus of using "exotic reagents" and to make recombinant cells an ordinary tool in synthetic chemistry.

Whole-cell fermentation protocols have previously been optimized for CHMO, with promising results reported with nongrowing recombinant cells.[16,17] The first dynamic kinetic resolution of a racemic precursor was reported using a high-pH-tolerant recombinant strain.[18] Very recently, significant progress has been made to scale-up monooxygenase-mediated biooxidations to large-scale synthetic applications.[19,20]

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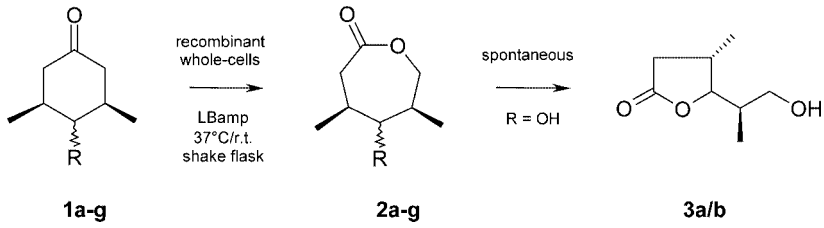
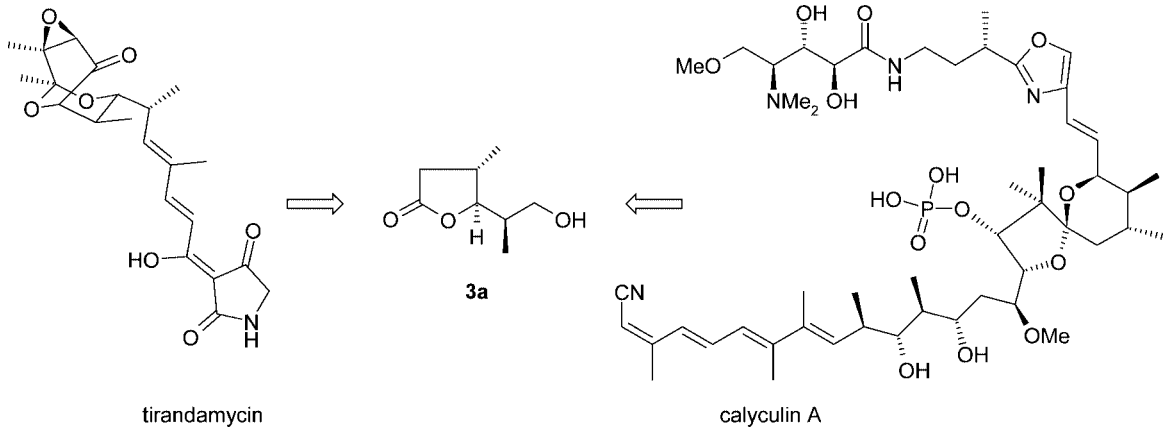


Scheme 1. Biotransformation of functionalized cyclohexanone systems with CHMO-expressing recombinant cells



Scheme 2. Lactone **3a** as a key intermediate for the synthesis of tirandamycin and calyculin A

With the recent construction of a whole-cell overexpression system for cyclopentanone monooxygenase (CPMO) [21] from *Comamonassp.* NCIMB 9872 (EC 1.14.13.16),[22] we started a comparative substrate-profiling program. CPMO has received considerably less attention in biocatalytic Baeyer–Villiger oxidations, as a biocatalytic behavior similar to CHMO was initially assumed.[23,24] However, recent studies have revealed some differences in substrate acceptance as well as stereospecificity of the two enzymes, with the enantiodivergent biooxidation of some ketones being the most prominent example.[25–27]

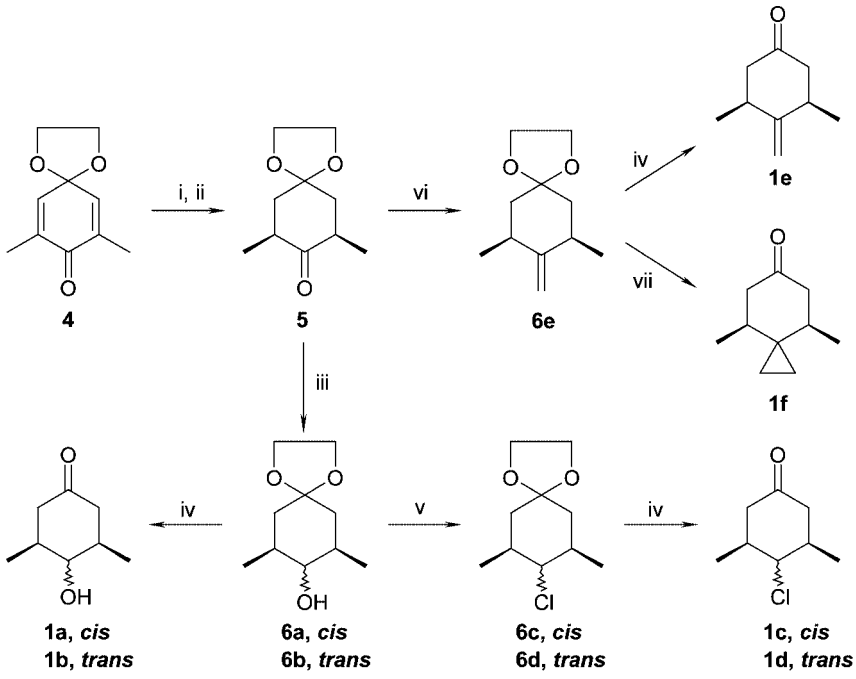
In our previous studies with recombinant whole cells expressing CHMO we observed a significant dependence of conversion and enantioselectivity on conformational aspects of the substrate.[26,28,29] These findings prompted us to reinvestigate such effects on polysubstituted cyclohexanones. Ketones **1a**–**g** (Scheme 1) are highly interesting model compounds for such a study, since the corresponding lactones **2/3** are versatile building blocks for subsequent natural compound synthesis: Taschner and coworkers have established **3a**, which is derived from the intermediate lactone **2a** by a rearrangement process after a biotransformation with isolated CHMO,[30] as a key intermediate for the synthesis of calyculin (Scheme 2).[31] This compound has also been utilized in approaches towards tirandamycin A.[32–34]

Results and Discussion

This study focuses on the 3,5-dimethyl ketones **1**, bearing various substituents in position 4, as probes to study the effect of polarity and steric strain on the substrate specificity of CHMO and CPMO. Although a crystallographic structure determination has been reported very recently for a rather distantly related BVMO from a *Thermobifida* sp. with a very different substrate-acceptance pattern,[35] so far, active-site models for enzymes that convert cycloketones are only available based on substrate profiling.[36–40] The quality of predictions derived from such "super-substrate models" depends heavily on the size and diversity of the data set they are based on.

The functionalities contained in substrate ketones **1a**–**g** involve both hydrophilic (R = OH) and lipophilic groups (R = Cl) in *cis* (**1a/c**) and *trans* substitution patterns (**1b/d**). Since we have observed a significant effect of hybridization on the enantioselectivity of CHMO when transforming prochiral bicyclo[3.3.0]ketones,[26,29] both sp^3 (**1a**–**d**) and sp^2 centers (**1e**) at position 4 were studied. We also became interested in a 4,4-disubstituted substrate (**1f**), superimposing the structural features of *cis*- and *trans*-ketones **1a**–**e**. In order to compare the effect of substitution at position 4 with the unsubstituted case, we included ketone **1g** (R = H) in the study as a reference point.[41]

Access to the model substrates proceeded in a straightforward manner. The mono-protected quinone **4** is a readily available precursor for the subsequent functionalization.[42] Reduction of the double bonds by catalytic hydrogenation using palladium on charcoal gave a mixture of *cis*- and *trans*-isomers **5** (Scheme 3). This mixture of diastereomers was converted into the thermodynamically favored *cis* conformer, with both methyl groups adopting an equatorial position, by base-promoted epimerization. Taking advantage



Scheme 3. (i) H_2, Pd/C, 5 bar; (ii) Na/MeOH, room temp., 69% (overall); (iii) $NaBH_4$, dry MeOH, 0 °C, 90%; (iv) 1% H_2SO_4/acetone, (1:1), room temp. to 40 °C, 88–92%; (v) PPh_3, NCS, THF, 61–80%; (vi) $MePPh_3^+Br^-$, BuLi, dry THF, 91%; (vii) $ZnEt_2$, CH_2I_2, CF_3COOH, CH_2Cl_2, 0 °C, then 2 N HCl, room temp., 50% (overall)

of the acidity of the α-protons adjacent to the carbonyl group, repeated deprotonation and reprotonation in a protic solvent[43] gave *cis*-**5** in greater than 95% purity as a key intermediate for subsequent functionalization. The hydrogenation and epimerization was performed without purification. The final step gave pure intermediate **5** in an overall yield of 69% after column chromatography.

Reduction of compound **5** with sodium borohydride at 0 °C led to *cis*- and *trans*-alcohols **6a** and **6b** in a ratio of 1:3 as the first model substrates for the substrate profiling. The two diastereomers could be separated by flash column chromatography. Since both compounds were required for the subsequent substrate acceptance studies, we preferred this rapid route to both compounds to a selective reduction to **6a** using L-selectride[44] and subsequent Mitsunobu inversion, a protocol recently utilized by us on similar systems.[26]

The introduction of a chloro substituent as representative nonpolar substrate for the subsequent biotransformations was performed by a nucleophilic substitution of the protected alcohols under Mitsunobu-type conditions in the presence of triphenylphosphane and NCS.[26,45] The transformation gave clean inversion of the stereocenter to products **6c** and **6d** in good to excellent yields.

Introduction of the exocyclic methylene group towards compound **6e** was performed under standard Wittig conditions.[46] We preferred this two-step route to the method reported in the literature,[42] since reproducing the in situ epimerization under the basic conditions of the Wittig reaction was troublesome.

Access to the cyclopropyl spiro system was investigated from compound **6e**. Standard heterogeneous Simmons–Smith reaction conditions using the Cu/Zn couple[28,47] or activated Zn[42] failed to give reproducible yields. Consequently, we changed to $ZnEt_2$[48] to generate the required carbene species, and best results were obtained with CF_3COOH as additive.[49] Due to the high volatility of the ketal intermediate, we preferred to convert precursor **6e** directly into ketone **1f** in a single operation.

In general, deprotection was carried out under transketalization conditions using a mixture of acetone and 1% sulfuric acid at 25–40 °C to give ketones **1a**–**e**.

Expression of CHMO or CPMO in growing cultures of recombinant *E. coli* strain *BL21(DE3)(pMM4)* or *DH5α(pCMO206)* was triggered by addition of isopropyl-β-D-thiogalactopyranoside (IPTG). Fermentations were carried out according to our previously reported procedure on a 250 mL scale in Erlenmeyer flasks;[26] β-cyclodextrin was added to facilitate the biotransformations.[50] Racemic reference samples of lactones were prepared by chemical oxidation with *m*-CPBA where applicable (**2c/d/f/g**, **3a/b**).

The results of the biotransformations of substrates **1a**–**g** with recombinant whole-cells are summarized in Table 1.

All substrates **1a**–**g** were converted into the corresponding lactones (**2c**–**g**, **3a/b**) by CHMO-expressing cells with good to very good yields and excellent enantiomeric excess. The biotransformation of *cis*-ketone **1a** with recombinant whole-cells gave the rearranged lactone (–)-{4*S*-[4α,5β(*S**)]}-**3a** similar to previous reports on biooxidation with the isolated enzyme.[30] The seven-membered-ring intermediate was not observed, and intramolecular attack of the 4-hydroxy group gave the corresponding γ-lactone exclusively.

The isomeric *trans* substrate **1b** showed similar behavior, and the rearranged compound (+)-**3b** was isolated as the

Table 1. Biotransformation of functionalized cyclohexanone substrates with CHMO- and CPMO-expressing recombinant cells

Substrate	R	Product	Enzyme	Yield[a]	*ee*[b]	$[\alpha]_D^{20}$
1a	*cis*-OH	**3a**	CHMO	77%	99%	–24.5 (*c* = 1.71, $CHCl_3$)
			CPMO	n.c.[c]	n.a.[d]	n.a.[d]
1b	*trans*-OH	**3b**	CHMO	80%	96%	+53.7 (*c* = 1.76, $CHCl_3$)
			CPMO	n.c.[c]	n.a.[d]	n.a.[d]
1c	*cis*-Cl	**2c**	CHMO	53%	99%	–67.1 (*c* = 0.76, $CHCl_3$)
			CPMO	n.c.[c]	n.a.[d]	n.a.[d]
1d	*trans*-Cl	**2d**	CHMO	40%	>99%	–81.5 (*c* = 1.30, $CHCl_3$)
			CPMO	n.c.[c]	n.a.[d]	n.a.[d]
1e	$=CH_2$	**2e**	CHMO	54%	92%	+8.4 (*c* = 0.80, $CHCl_3$)
			CPMO	63%	99%	–8.6 (*c* = 0.64, $CHCl_3$)
1f	cyclopropyl	**2f**	CHMO	57%	>99%	+73.0 (*c* = 1.00, $CHCl_3$)
			CPMO	n.c.[c]	n.a.[d]	n.a.[d]
1g	H	**2g**	CHMO	65%	>99%	–12.7 (*c* = 2.0, $CHCl_3$)
			CPMO	58%	91%	+7.7 (*c* = 1.3, $CHCl_3$)

[a] Yield of product isolated after purification by column chromatography. [b] Enantiomeric excess was determined by chiral-phase gas chromatography; racemic reference material was prepared by oxidation of ketones **1a**–**d** and **1f/g** with *m*-CPBA. [c] No conversion. [d] Not applicable.

only product of the biooxidation. Biotransformation of the lipophilic substrates bearing a chloro substituent gave the expected lactones (–)-**2c/d**, also in high optical purity and acceptable yields. Only olefin **1e**, which bears an sp^2 center in position 4, gave lactone (+)-**2e** in slightly decreased optical purity. However, enzymatic oxidation was highly chemoselective for the Baeyer–Villiger process and no undesired attack at the double bond was observed.

Substrate **1f** combines structural features of lipophilic ketones **1c** and **1d** by bearing two substituents in the 4-position. This compound was readily accepted by CHMO and was converted into (+)-**2f** with excellent enantioselectivity.

Biotransformation of **1g** has previously been carried out using isolated CHMO and gave good yields using the whole-cell fermentation protocol. The enantioselectivity was comparable to literature reports and the absolute configuration of (–)-**2** was assigned as (4*S*,6*R*).[30]

Biooxidations with CPMO were only successful in two cases to yield the expected lactones: ketones **1e** and **1g** were readily converted by recombinant CPMO-producing cells. Obviously, in the case of 3,5-dimethyl precursors only limited flexibility of the active site of CPMO seems to exist. Substitution at the axial or equatorial position at carbon 4 of the substrate prevents conversion by the enzyme. This is the case for both lipophilic and hydrophobic groups. Only a methylene substituent seems to be accommodated within the active site simultaneously with the presence of groups in the 3- and 5-positions of the substrate. Consequently, the 4-unsubstituted ketone **1g** is also oxidized by CPMO.

It is remarkable that the biooxidation with CPMO leads to antipodal lactones **2e/g** as obtained in CHMO-mediated transformations. Both microbial reactions proceed in excellent stereoselectivity. This extends the number of substrates where enantiodivergent transformation is observed for this pair of enzymes.

Conclusions

Based on an initial study by Taschner et al. for CHMO,[30] this work represents an extended systematic survey of a group of prochiral 3,4,5-trisubstituted cyclohexanones for the microbial oxidation to the corresponding lactones. A diastereoselective route to substrates with both hydrophilic (**1a/b**) and lipophilic **(1c**–**f)** substituents has been developed.

In the case of CHMO-mediated biooxidations, the different stereochemistry of the substrates does not have a significant effect on the efficiency of the biooxidation or the enantioselectivity of the enzymatic reaction. In the case of trisubstituted cyclohexanone substrates, CHMO seems to be less susceptible to conformational aspects than we had previously found for a series of bicyclo[3.3.0]ketones.[26] Even the presence of a quaternary center with spiro-disubstitution at position 4 does not influence the efficiency or stereoselectivity of the enzymatic oxidation. This might provide valuable insights to further refine existing active-site models. Position 4 is particularly tolerant towards various types of hybridization, as substituents can adopt axial and equatorial positions for sp^3 centers. Occupation of both substitution sites does not hamper transformation by the whole-cell expression system, even in the presence of two additional substituents in the vicinity. The steric flexibility of the active site also allows the presence of an sp^2 methylene group.

While substrate acceptance of CHMO and CPMO was observed to significantly overlap in previous studies using recombinant whole-cells, the latter expression system displays some restrictions for the conversion of polysubstituted cyclohexanones in the present survey. Only sterically less challenging substrates were transformed, with enantiocomplementary selectivity compared to CHMO. This further extends the array of ketones oxidized to enantiodivergent lactones and underscores the high potential of this enzyme pair for future applications.

The complementary biocatalytic performance of CHMO and CPMO with respect to stereoselectivity is similar to a recently discovered pair of BVMOs from *Brevibacterium*, which also display enantiodivergent biotransformation for several structurally diverse substrate ketones.[51] Further

substrate profiling of the recombinant expression systems for these enzymes is currently underway in our laboratories in order to establish a toolbox of BVMO biocatalysts for a broad range of synthetic applications.

Experimental Section

Unless otherwise noted, chemicals and microbial growth media were purchased from commercial suppliers and used without further purification. All solvents were distilled prior to use (LP: light petroleum, EtOAc: ethyl acetate). Flash column chromatography was performed on silica gel 60 from Merck (40–63 μm). Melting points were determined with a Kofler-type Leica Galen III micro hot stage microscope and are uncorrected. NMR spectra were recorded from $CDCl_3$ or [D_6]DMSO solutions on a Bruker AC 200 (200 MHz) or Bruker Avance UltraShield 400 (400 MHz) spectrometer and chemical shifts are reported in ppm relative to TMS as internal standard. Combustion analysis was carried out in the Microanalytical Laboratory of the University of Vienna. Enantiomeric excess was determined by GC using a BGB 175 column (30 m × 0.25 mm ID, 0.25 μm film) on a HP 6890 Series chromatograph. Biotransformation progress and conversion control was performed with a ThermoQuest Trace GC 2000 using a standard capillary column DB5 (30 m × 0.32 mm ID). Specific rotation $[\alpha]_D^{20}$ was determined with a Perkin–Elmer Polarimeter 241.

***cis*-7,9-Dimethyl-1,4-dioxaspiro[4.5]decan-8-one (5):** 7,9-Dimethyl-1,4-dioxaspiro[4.5]deca-6,9-dien-8-one (**4**; 9.11 g, 50.5 mmol) and Pd/C catalyst (1 g, 10%) suspended in EtOAc (200 mL) were placed into a Parr apparatus. Reduction was carried out under a hydrogen pressure of 5 bar at room temp. overnight. Once GC indicated complete conversion, the catalyst was removed by filtration through a bed of Celite® and the solvent was evaporated. A *cis*/*trans* mixture of **5** was obtained as a yellow oil and used without purification in the epimerization step.

The crude *cis,trans*-7,9-dimethyl-1,4-dioxaspiro[4.5]decan-8-one (**5**) from the above reduction (7.16 g, 38.9 mmol) was added to a solution of NaOMe, prepared from sodium (447 mg, 19.5 mmol) by the usual method, in dry MeOH (40 mL) and the reaction mixture was stirred at room temp. for 12 h. The progress of the epimerization was determined by GC. The reaction mixture was poured into water and extracted three times with EtOAc. The combined organic layers were dried with sodium sulfate and the solvent was evaporated. Flash column chromatography (LP/EtOAc, 5:1, NEt_3 impregnated silica gel) gave pure *cis*-**5** as a yellowish liquid (6.42 g, overall yield: 69%). The ^{1}H NMR spectroscopic data were identical to reports in the literature.[44] ^{13}C NMR ($CDCl_3$): δ = 14.1 (q), 41.0 (d), 43.6 (t), 64.6 (t), 107.2 (s), 213.1 (s) ppm. *trans*-**5** (spectroscopic data obtained as a mixture with *cis*-**5**). ^{1}H NMR ($CDCl_3$): δ = 1.01 (d, J = 6.7 Hz, 3 H), 1.14 (d, J = 6.6 Hz, 3 H), 1.61–1.90 (m, 2 H), 2.00–2.17 (m, 2 H), 2.59–2.87 (m, 2 H), 3.95–4.12 (m, 4 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 16.0 (q), 40.0 (d), 41.1 (t), 64.2 (t), 107.7 (s), 215.6 (s) ppm.

***cis*-7,9-Dimethyl-8-methylene-1,4-dioxaspiro[4.5]decane (6e):** Methyltriphenylphosphonium bromide(6.00 g, 16.8 mmol) was washed with dry diethyl ether and dried in vacuo for about 15 min. Freshly washed Wittig salt (5.80 g, 16.3 mmol) was suspended in dry THF (50 mL) under an argon atmosphere and cooled to –5 °C. Then, *n*BuLi (16.8 mL, 2.14 M solution in hexane) was added dropwise at such a rate to keep the reaction temperature between –5 °C and 0 °C. After complete addition of *n*BuLi the temperature was increased to room temp. and the mixture was stirred for 1 h. During this period the reaction mixture became an orange-red clear solution. Subsequently, pure ketone **5** was added slowly with a syringe. After 12 h stirring at room temp., GC indicated complete conversion. The mixture was hydrolyzed with saturated NH_4Cl solution, extracted three times with diethyl ether, and washed with brine. The combined organic layers were dried with sodium sulfate and the solvent was evaporated. The very volatile product **6e** (1.80 g, 91%, colorless volatile liquid) was used directly for the subsequent transformation without further purification. ^{1}H NMR spectroscopic data identical to those described in the literature.[42] ^{13}C NMR ($CDCl_3$): δ = 18.0 (q), 34.7 (d), 44.7 (t), 64.2 (t), 64.4 (t), 102.5 (t), 108.8 (s), 156.0 (s) ppm.

Reduction with $NaBH_4$ in dry MeOH: Compound **5** (500 mg, 2.70 mmol) was dissolved in dry MeOH (20 mL) and cooled to 0 °C. Then, $NaBH_4$ (2 equiv.) was added in portions whilst maintaining the temperature at 0 °C. After complete addition of $NaBH_4$ the reaction went to completion within 1 h. Unreacted $NaBH_4$ was slowly hydrolyzed by addition of 0.1 N NaOH and the aqueous layer was extracted three times with diethyl ether. The combined organic phases were washed with brine, dried over sodium sulfate, and the solvent was evaporated. The two diastereomers formed were separated by column chromatography (LP/EtOAc, 10:1, silica gel).

(7α,8α,9α)-7,9-Dimethyl-1,4-dioxaspiro[4.5]decan-8-ol (6a): Reduction according to the above procedure gave pure alcohol **6a**[44] (113.2 mg, 23%) as colorless crystals after chromatographic separation. M.p.: 68–72 °C.

(7α,8β,9α)-7,9-Dimethyl-1,4-dioxaspiro[4.5]decan-8-ol (6b): Pure alcohol **6b** (339.4 mg, 67%) was isolated as colorless crystals after separation by column chromatography. M.p.: 65–68 °C. ^{1}H NMR ($CDCl_3$): δ = 1.02 (d, J = 6.1 Hz, 6 H), 1.25–1.44 (m, 3 H), 1.52–1.80 (m, 4 H), 2.75 (t, J = 9.3 Hz, 1 H), 4.93 (s, 4 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 18.5 (q), 36.7 (t), 42.0 (d), 64.2 (t), 64.4 (2 t), 80.9 (d), 108.1 (s) ppm.

Chlorination with PPh_3/NCS: Triphenylphosphane (2 equiv.) in dry THF (10% solution) was treated dropwise with *N*-chlorosuccinimide (1 equiv.) in dry THF (5% solution). Alcohol **6a/b** (1 equiv.) in dry THF (10% solution) was added to the resulting suspension. The mixture was stirred at room temp. until the solution became clear (1–3 h). After evaporation of the volatiles the residue was taken up in water and extracted with diethyl ether. The organic layer was washed with brine, dried over anhydrous sodium sulfate and then the solvents were evaporated. The crude product was purified by flash chromatography on silica gel (LP/EtOAc, 10:1).

(7α,8α,9α)-8-Chloro-7,9-dimethyl-1,4-dioxaspiro[4.5]decane (6c): *trans*-**6b** (100 mg, 0.50 mmol) was converted according to the above procedure into **6c**. Colorless liquid (180 mg, 80%). ^{1}H NMR ($CDCl_3$): δ = 1.02 (d, J = 6.1 Hz, 6 H), 1.22–2.28 (m, 6 H), 3.94 (br. s, 4 H), 4.11 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 19.0 (q), 35.4 (d), 36.3 (t), 64.2 (2t), 71.8 (d), 110.6 (s) ppm.

(7α,8β,9α)-8-Chloro-7,9-dimethyl-1,4-dioxaspiro[4.5]decane (6d): *cis*-**6a** (930 mg, 5.00 mmol) was converted following the above procedure to give **6d** as a colorless liquid (625 mg, 61%). ^{1}H NMR ($CDCl_3$): δ = 1.12 (d, J = 6.3 Hz, 6 H), 1.27–1.44 (m, 2 H), 1.75–1.88 (m, 2 H), 1.91–2.10 (m, 3 H), 3.13 (t, J = 10.5 Hz, 1 H), 3.94 (br. s, 4 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 20.3 (q), 38.1 (d), 42.8 (t), 64.3 (t), 64.5 (t), 73.1 (d), 107.4 (s) ppm.

General Procedure for Ketal Deprotection: The corresponding ketal was stirred at the specified temperature in a 1:1 mixture (20% solution) of 1% sulfuric acid and acetone until complete conversion (as determined by GC). The reaction mixture was hydrolyzed with saturated sodium hydrogen carbonate solution and then extracted

three times with diethyl ether. The combined organic layers were washed with brine, dried with sodium sulfate, and the solvent was evaporated. Pure ketones were obtained after purification by column chromatography.

(3α,4α,5α)-4-Hydroxy-3,5-dimethylcyclohexanone (1a): Ketal **6a** (50 mg, 0.27 mmol) gave 35.6 mg (92%) of **1a** as a colorless oil after 24 h at room temp.[30] ^{1}H NMR ($CDCl_3$): δ = 1.09 (d, J = 6.7 Hz, 6 H), 1.87–2.16 (m, 4 H), 2.45 (t, J = 13.7 Hz, 2 H), 2.54 (br. s, 1 H), 3.68 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 18.1 (q), 37.6 (d), 42.9 (t), 72.7 (d), 212.5 (s) ppm.

(3α,4β,5α)-4-Hydroxy-3,5-dimethylcyclohexanone (1b): Ketal **6b** (50 mg, 0.27 mmol) gave 33.6 mg (88%) of **1b** as colorless crystals after 24 h at room temp. M.p.: 90–93 °C. ^{1}H NMR ($CDCl_3$): δ = 1.13 (d, J = 6.5 Hz, 6 H), 1.71–1.94 (m, 3 H), 2.15 (t, J = 13.5 Hz, 2 H), 2.32–2.44 (m, 2 H), 3.18 (t, J = 10.8 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 18.9 (q), 39.2 (d), 47.5 (t), 79.7 (d), 209.1 (s) ppm. $C_8H_{14}O_2$ (142.2): calcd. C 67.57, H 9.92; found C 67.34, H 9.69.

(3α,4α,5α)-4-Chloro-3,5-dimethylcyclohexanone (1c): Ketal **6c** (100 mg, 0.48 mmol) gave 69.4 mg (90%) of **1c** as a colorless oil after stirring the reaction mixture for 48 h at 40 °C. ^{1}H NMR ($CDCl_3$): δ = 1.14 (d, J = 6.2 Hz, 6 H), 2.08–2.56 (m, 6 H), 4.18 (br. s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 19.2 (q), 38.1 (d), 43.1 (t), 71.2 (d), 209.3 (s) ppm, $C_8H_{13}ClO$ (160.7): calcd. C 59.81, H 8.16; found C 60.10, H 8.07.

(3α,4β,5α)-4-Chloro-3,5-dimethylcyclohexanone (1d): Ketal **6d** (100 mg, 0.48 mmol) gave 61.7 mg (80%) of **1d** as a colorless oil after stirring the reaction mixture for 24 h at 40 °C. ^{1}H NMR ($CDCl_3$): δ = 1.23 (d, J = 6.1 Hz, 6 H), 2.00–2.26 (m, 4 H), 2.34–2.51 (m, 2 H), 3.49 (t, J = 9.9 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 20.7 (q), 40.6 (d), 48.2 (t), 70.9 (d), 207.6 (s) ppm. $C_8H_{13}ClO$ (160.7): calcd. C 59.81, H 8.16; found C 60.05, H 8.25.

***cis*-3,5-Dimethyl-4-methylenecyclohexanone (1e):** Crude compound **6e** (996 mg, 5.40 mmol) was stirred at 40 °C overnight to give 760 mg (90%) of **1e** as a colorless oil. ^{1}H NMR ($CDCl_3$): δ = 1.18 (d, J = 6.3 Hz, 6 H), 2.07 (t, 2 H), 2.36–2.68 (m, 4 H), 4.95 (s, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 18.5 (q), 36.8 (d), 49.9 (t), 105.1 (t), 153.4 (s), 209.9 (s) ppm.

***cis*-4,8-Dimethylspiro[2.5]octan-6-one (1f):** Diethylzinc (13.72 mL, 13.72 mmol, 1 M solution in hexane) was added to dry dichloromethane (10 mL). The solution was cooled with an ice-bath and trifluoroacetic acid (1.57 g, 13.72 mmol) in dichloromethane (5 mL) was added dropwise to the solution. The mixture was stirred for 30 min in the ice-bath. Then, methylene iodide (3.67 g, 13.72 mmol) diluted in dichloromethane (5 mL) was added to the mixture. After stirring for 30 min olefin **6e** (1.25 g, 6.86 mmol, in 5 mL of dry dichloromethane) was added to the mixture. Conversion was monitored by GC. Due to the volatility of the intermediate ketal, deprotection was initiated directly after complete transformation. The reaction mixture was quenched with 2 N HCl (25 mL) and stirred overnight until deprotection was complete. The two phases were then separated and the aqueous phase was extracted with dichloromethane. The combined organic phases were washed with satd. $NaHCO_3$ solution, brine, and water. The combined organic phases were dried with sodium sulfate and concentrated. The crude product was purified by column chromatography (LP/EtOAc, 12:1) to give **1f** as a yellow oil (512 mg, 50%).^{1}H NMR identical to data described in the literature.[42] ^{13}C NMR ($CDCl_3$): δ = 1.5 (t), 7.6 (t), 17.3 (q), 26.2 (s), 36.7 (d), 48.8 (t), 211.0 (s) ppm.

Microbial Baeyer–Villiger Oxidation: Fresh LB-amp medium (250 mL) was inoculated with 1% of an overnight preculture of recombinant *E. coli* in a baffled Erlenmeyer flask. The culture was incubated at 120 rpm at 37 °C on an orbital shaker for 2 h, and then IPTG was added to a final concentration of 0.025 mM. The substrate (3–6 mM) was added neat along with β-cyclodextrin (1 equiv.). The culture was incubated at room temp. until GC showed complete conversion of the ketone (24–96 h). The biomass was removed by centrifugation, the fermentation broth was filtered through a bed of Celite®, saturated with sodium chloride, and repeatedly extracted with the corresponding solvent (EtOAc, diethyl ether). The combined organic layers were dried with sodium sulfate, filtered, and the solvent was removed in vacuo.

(4α,5α,6α)-5-Chloro-4,6-dimethyl-2-oxepanone (2c): Microbial oxidation of (3α,4α,5α)-4-chloro-3,5-dimethylcyclohexanone (**1c**; 100 μL, 92.0 mg, 0.56 mmol) according to the general procedure with CHMO-producing cells gave the desired lactone **2c** (53 mg, 53%) as a colorless oil after purification by column chromatography (LP/Et_2O, 5:1). ^{1}H NMR ($CDCl_3$): δ = 1.08 (d, J = 7.0 Hz, 3 H), 1.20 (d, J = 6.4 Hz, 3 H), 2.16–2.43 (m, 3 H), 3.15 (dd, J_1 = 11.3, J_2 = 14.5 Hz, 1 H), 3.70–3.84 (m, 1 H), 4.21 (br. s, 1 H), 4.47 (dd, J_1 = 9.1, J_2 = 13.2 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 7.0 (q), 21.3 (q), 35.2 (t), 35.6 (d), 42.4 (d), 67.1 (t), 72.8 (d), 174.1 (s) ppm, $C_8H_{13}ClO_2$, (176.5): calcd. C 54.40, H 7.42; found C 54.61, H 7.55.

(4α,5β,6α)-5-Chloro-4,6-dimethyl-2-oxepanone (2d): (3α,4β,5α)-4-Chloro-3,5-dimethylcyclohexanone (**1d**; 100 μL, 92.0 mg, 0.56 mmol) was oxidized with CHMO-producing cells according to the general procedure. The crude product was purified by column chromatography (LP/Et_2O, 5:1) to give lactone **2d** as colorless crystals (40 mg, 40%). M.p.: 96–99 °C. ^{1}H NMR ($CDCl_3$): δ = 1.22 (d, J = 7.0 Hz, 3 H), 1.30 (d, J = 6.7 Hz, 3 H), 2.09–2.30 (m, 2 H), 2.52–2.79 (m, 2 H), 3.48 (t, J = 8.8 Hz, 1 H), 4.01 (dd, J_1 = 8.6 Hz, 1 H), 4.25 (dd, J_1 = 2.0, J_2 = 13.4 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 17.4 (q), 22.1 (q), 37.4 (d), 37.7 (t), 42.4 (d), 70.0 (t), 72.1 (d), 173.4 (s) ppm. $C_8H_{13}ClO_2$ (176.5): calcd. C 54.40, H 7.42; found C 54.70, H 7.62.

***cis*-4,6-Dimethyl-5-methylene-2-oxepanone (2e):** *cis*-3,5-Dimethyl-4-methylenecyclohexanone (**1e**; 100 μL, 92 mg, 0.66 mmol) was oxidized according to the general procedure with CHMO-producing cells to give **2e**[51] (55 mg, 54%) as a colorless oil after column chromatography (LP/EtOAc, 10:1). Biooxidation with CPMO yielded 64 mg (63%) of **2e**.

***cis*-4,9-Dimethyl-6-oxaspiro-[2.6]-nonan-7-one (2f):** *cis*-4,8-Dimethylspiro[2.5]octane-6-one (**1f**; 100 μL, 98 mg, 0.644 mmol) was oxidized with CHMO-producing cells according to the general procedure to give **2f** (62 mg, 57%) as colorless crystals after column chromatography (LP/Et_2O, 4:1). M.p.: 126–127 °C. ^{1}H NMR ($CDCl_3$): δ = 0.25–0.43 (m, 2 H), 0.44–0.47 (m, 2 H), 1.12 (d, J = 10 Hz, 8 H), 2.51 (dd, J = 5.9, J = 7.6 Hz, 1 H), 2.97 (dd, J = 2.5, J = 10.8 Hz, 1 H), 4.06 (dd, J = 4.5, J = 8 Hz, 1 H), 4.44 (d, J = 12.5 Hz, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 11.9 (t), 15.3 (q), 17.3 (t) 18.2 (q), 28.4 (s), 37.7 (d), 39.5 (t), 42.4 (d), 72.1 (t), 174.9 (s) ppm.

***cis*-4,6-Dimethyl-2-oxepanone (2g):** *cis*-3,5-Dimethylcyclohexanone (**1g**) (200 μL, 184 mg, 1.38 mmol) was oxidized according to the general procedure with CHMO-producing cells to give **2g**[30] (128 mg, 65%) as a colorless oil after column chromatography (LP/EtOAc, 4:1). Biooxidation with CPMO yielded 114 mg (58%) of product.

(4α,5β)-4,5-Dihydro-5-(2-hydroxy-1-methylethyl)-4-methyl-2(3*H*)-furanone (3a): Ketone **1a** (100 μL, 100 mg, 0.70 mmol) was oxidized according to the general procedure with CHMO-producing cells

to give lactone **3a** (85 mg, 77%) as a colorless oil after column chromatography (LP/EtOAc, 2:1).[30][1] ^{1}H NMR ($CDCl_3$): δ = 0.96 (d, J = 7.0 Hz, 3 H), 1.11 (d, J = 6.7 Hz, 3 H), 1.78–1.94 (m, 1 H), 2.1 (dd, J_1 = 8.8, J_2 = 17.2 Hz, 1 H), 2.26–2.52 (m, 2 H), 2.65 (dd, J_1 = 7.4, J_2 = 17.2 Hz, 1 H), 3.55–3.65 (m, 2 H), 3.94–4.04 (m, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.9 (q), 19.1 (q), 32.8 (d), 36.6 (t), 39.4 (d), 64.2 (t), 88.9 (d), 176.4 (s) ppm.

(4*α*,5α)-4,5-Dihydro-5-(2-hydroxy-1-methylethyl)-4-methyl-2(3*H*)-furanone (3b): Ketone **1b** (100 μL, 100 mg, 0.70 mmol) was oxidized according to the general procedure with CHMO-producing cells to give lactone **3b** (88 mg, 80%) as a pale-yellow oil after column chromatography (LP/EtOAc, 2:1). ^{1}H NMR ($CDCl_3$): δ = 1.01–1.14 (m, 6 H), 1.94–2.31 (m, 3 H), 2.61–2.81 (m, 2 H), 3.51–3.69 (m, 2 H), 4.36–4.45 (m, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 15.5 (q), 14.0 (q), 32.7 (d), 35.6 (t), 38.3 (d), 64.4 (t), 84.8 (d), 177.0 (s) ppm, $C_8H_{14}O_3$ (158.2): calcd. C 60.74, H 8.92; found C 60.83, H 8.89.

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