An Enantiodivergent Trend in Microbial Baeyer−Villiger Oxidations of Functionalized Pentalenones by Recombinant Whole Cells Expressing Monooxygenases from *Acinetobacter* and *Pseudomonas*

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We have investigated the microbial Baeyer–Villiger oxidations of pentalenones by recombinant whole cells expressing cyclohexanone monooxygenase from *Acinetobacter* and cyclopentanone monooxygenase from *Pseudomonas*. The two enzymes display a distinguishable enantiodivergent trend in the biotransformation of some prochiral substrates. A significant influence of the conformation of the fused-ring system and the polarity of the substituents on the conversion and enantioselectivity of the microbial oxidations was observed and is discussed.

Introduction

Since the discovery of an oxidative ring expansion from ketones to lactones by Adolf Baeyer and Victor Villiger in 1899,[1] many aspects of the reaction, which later was named after these pioneers in chemistry, have received considerable attention. Substantial progress has been made in understanding the mechanism, predicting the migratory preference, and applying this conversion to preparative chemistry.[2,3] The Baeyer−Villiger oxidation is of considerable value to synthetic chemists because it is a powerful method for breaking carbon−carbon bonds with concomitant oxygen atom insertion.

Chiral Baeyer−Villiger oxidation of cyclic ketones allows rapid access to asymmetric lactones, which are valuable intermediates in organic chemistry and frequently encountered precursors in enantioselective synthesis. In recent years, the use of organometallic catalysts has allowed continuous improvements in this reaction and represents a promising approach for its future process development.[4] Complementing this strategy, biocatalysis offers a "green chemistry" alternative for this transformation. Optical purities obtained by microbial Baeyer−Villiger oxidations have yet to be attained by artificial catalytic entities.

Nature has developed a variety of flavin-dependent Baeyer−Villiger monooxygenases (BVMOs) that catalyze this biotransformation, which plays a key role in degradative pathways of microorganisms.[5−10] While the metabolic action of such enzymes in the cell is restricted to a single compound, these biocatalytic entities often display a remarkably broad acceptance for nonnatural substrates. This feature, together with high enantioselectivity, are prerequisites for a powerful catalytic system for chiral synthesis.

Artificial organometallic catalysts can allow access, in a straight forward manner, to both enantiomeric products through chiral catalysis merely by inverting the chirality of the inducing ligands. In contrast, this aspect represents one of the key challenges for biotransformations. Enzymes consist of hundreds of L-amino acids that are responsible for strong chiral induction. With the present state-of-the-art, inverting the enantioselectivity of an enzyme, with a well-defined substrate profile, is not feasible by the act of changing the stereochemistry of its building blocks. Currently, the only options to access the enantiomeric product in biotransformations are directed evolution and screening for an alternative enzyme. As a consequence, such an alternative enzyme does not necessarily possess matching substrate acceptance profiles. Nevertheless, the identification and subsequent characterization of enantiodivergent enzymes with largely overlapping substrate profiles is a crucial aspect for the successful application of biocatalysts in synthetic chemistry.

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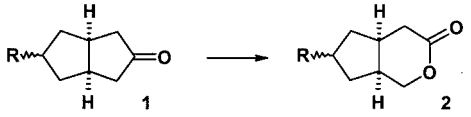
Recently, we started to investigate the oxidation of functionalized pentalenones using a recombinant overexpression system for cyclohexanone monooxygenase (CHMO)[11] from *Acinetobacter sp.* NCIMB 9871 (E.C. 1.14.13.22).[12] This enzyme represents the best studied Baeyer−Villigerase to date and transforms more than 100 nonnatural substrates by both kinetic resolution and desymmetrization with good enantioselectivity.[13] With the recent construction of a whole-cell overexpression system for cyclopentanone monooxygenase (CPMO)[14] from *Pseudomonas sp.* NCIMB 9872 (EC 1.14.13.16),[15] we became interested in expanding this profiling program. CPMO had received considerably less attention in biocatalytic Baeyer−Villiger oxidations, probably because its biocatalytic behavior was assumed to be similar to CHMO.[16,17] In a previous comparative study with cells expressing CHMO and CPMO by one of us (MMK), a matching trend in enantioselectivity was found for the resolution of 2-substituted cyclopentanones with similar optical purity.[18] To our surprise, recently we observed an enantiodivergent trend for CHMO and CPMO in the biotransformation of some mesomeric perhydroindenones, key intermediates for the synthesis of some indole alkaloids.[19]

These findings prompted us to investigate this substrate class further and to study the effects of conformational and electronic aspects on fused-ring systems. Our approach, designed to provide simple catalytic entities for enantioselective Baeyer−Villiger oxidations, utilizes recombinant whole cells that were engineered to produce the required enzyme in substantial quantities. Several such systems for CHMO based on *Saccharomyces cerevisiae*[20−22] and *Escherichia coli*[23−28] host organisms have been developed within the past few years and are, often in contrast to the native organism, safe and simple to cultivate. By performing whole-cell biotransformations the laborious process of enzyme isolation, which is often complicated by limited protein stability, can be avoided. In addition, all cofactors required by the biocatalyst (NADPH in the case of the above BVMOs) are recycled by the living cells. Hence, the construction of artificial regeneration systems[29−31] is not required and an easy-to-use methodology can be offered to preparative chemists who lack special expertise in microbiology.

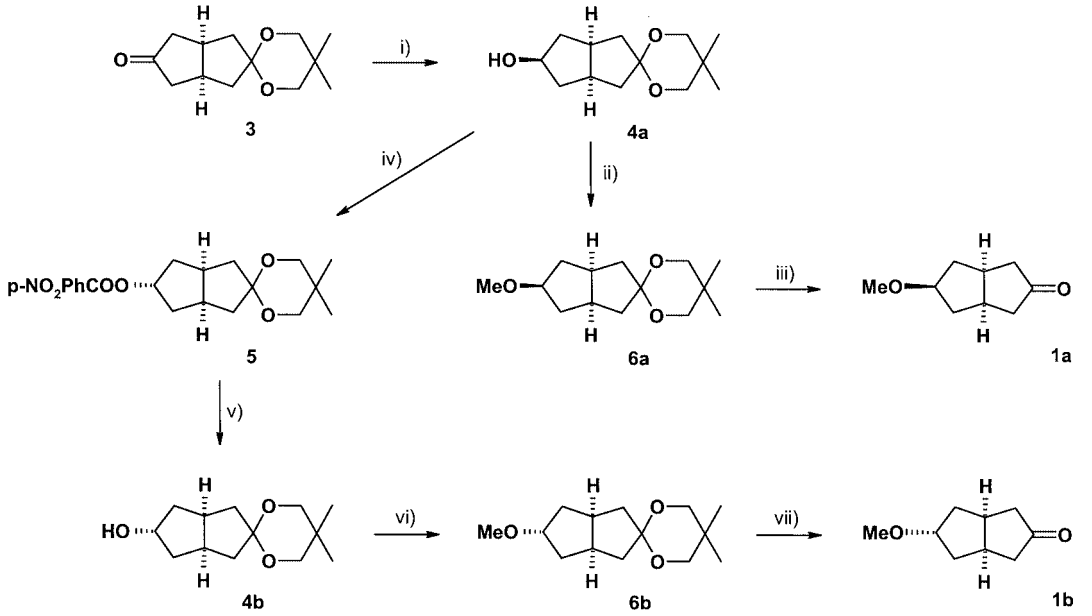
Results and Discussion

We considered ketones **1** bearing various substituents in the 5-position as interesting probes to investigate the effects of conformation and polarity of functional groups on the microbial Baeyer−Villiger oxidation (Scheme 1). Moreover, enzymatic asymmetrization of the prochiral substrates generates optically active products with a theoretical yield of 100%. In contrast, the classical kinetic resolution of racemic samples leads only to a maximum 50% yield of enantiopure product.[32] Lactones of type **2** have been used previously in various natural product syntheses and represent interesting chiral intermediates.[33−36]



Scheme 1. Biotransformation of functionalized fused systems with CHMO- and CPMO-expressing recombinant cells

For the investigation of conformational effects, we were interested in both *endo* (**1a/c**) and *exo* (**1b/d**) substitution



(i) $NaBH_4$, MeOH, 94%; (ii) NaH, DMS, THF, 90%; (iii) 0.1M H_2SO_4, H_2O, acetone, 66%; (iv) PPh_3, DEAD, *p*-nitrobenzoic acid, Et_2O, 77%; (v) KOH, quant.; (vi) NaH, DMS, THF, 98%; (vii) 0.1M H_2SO_4, H_2O, acetone, 80%.

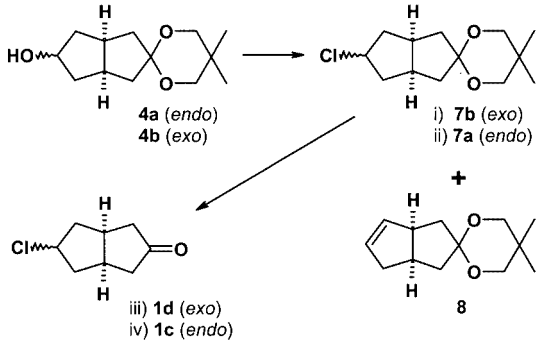
Scheme 2. Synthetic route to methoxy-substituted ketones **1a/b**

patterns at the sp^3-hybridized C-5 carbon atom, together with an sp^2-hybridized C-5 center (**1e**). Methoxy and chloro substituents in the 5-position served as representative functional groups to investigate the aspect of polarity on the biotransformation.

The synthetic strategy towards these substrates followed a straightforward route: Compound **3**, which is readily available by a Weiss-condensation[37] and subsequent selective ketalization,[38] was reduced diastereoselectively to *endo*-alcohol **4a** (Scheme 2). We obtained superior selectivity by using sodium borohydride[39,40] instead of LAH.[41] Methylation yielded ketal **6a** and subsequent deprotection gave *endo*-methoxy substrate **1a** in good yield as a representative polar substrate.

Alcohol **4a** also served as a key intermediate to access the *exo* series: Inversion of the hydroxy group was performed by a Mitsunobu[42] protocol with nitrobenzoic acid as nucleophile, via the corresponding *exo*-ester **5**. Subsequent basic hydrolysis of the crude intermediate afforded *exo*-alcohol **4b**. Methylation and deprotection was performed analogously to the procedure in the *endo* series to give *exo*-methoxy substrate **1b** in excellent overall yield. This sequence is superior to previous inversion strategies that proceeded through tosylation to access compound **4b**.[41]

Synthesis of the chloro-substituted pentalenones as less-polar substrates utilized the protected alcohols **4a/b** (Scheme 3). Halogenation through a Mitsunobu protocol using $ZnCl_2$ as the halogen donor[43] gave a clean inversion of the desired stereocenter to yield the desired products **7a/b**. This reaction was accompanied, however, by formation of substantial amounts of elimination product **8**. This undesired side reaction was reduced to a yield of approx. 10% of **8** by changing the halogenation protocol to the use of triphenylphosphine and NCS.[44] By this method, *endo*-**7a** and *exo*-**7b** ketals were obtained in 52 and 50% yields, respectively. Subsequent acidic deprotection conditions afforded substrates **1c** and **1d**.



(i) PPh_3, NCS, THF, 52%; (ii) PPh_3, NCS, THF, 50%; (iii) 0.1 M H_2SO_4, H_2O, acetone, 68%; (vi) 0.1 M H_2SO_4, H_2O, acetone, 58%.

Scheme 3. Synthesis of chloro ketones **1c/d**

Synthesis of the exocyclic olefin **1e** utilized precursor **3** and proceeded through a sequence of a Wittig reaction followed by deprotection according to the literature.[38,45]

Racemic reference samples of compounds **2a−d** were prepared by abiotic Baeyer−Villiger oxidation with *m*CPBA in dry methylene chloride. Microbial transformation was performed in shake-flask cultures in rich media. Protein production in the two expression systems was under the control of a T7 promoter[46] and was triggered by addition of isopropyl β-D-thiogalactopyranoside (IPTG). Equimolar amounts of β-cyclodextrin were supplemented prior to addition of the substrate to enhance solubility and membrane penetration and decrease its toxicity toward the living cells. Cyclic sugars of this type[47] are known to exert this effect, and we observed improved conversion in most cases.

Table 1 summarizes the results of the biotransformations of substrates **1a−e** with recombinant *E. coli* cells expressing CHMO and CPMO. All substrates were converted into the corresponding lactones with excellent chemoselectivity. Starting from prochiral ketones, two (**2e**) or three (**2a−d**) new chiral centers were introduced in one biocatalytic step. CHMO and CPMO displayed a trend to enantiodivergent conversion for several substrates.

Some general trends for the two expression systems are deduced from the data on biotransformations with the model substrates:

In the case of the CHMO-producing strain, the methoxy-substituted substrates with higher polarity are poorly accepted by CHMO and complete conversions were not observed for substrates **1a** and **1b**. The catalytic activity of recombinant cells usually dropped significantly after reaching the stationary growth phase (24−48 h). This effect was recently described by Stewart and co-workers for the CHMO-producing strain.[48] We attribute the poor substrate acceptance to a feature of the active site of CHMO, since the related *E. coli* host strain producing CPMO gave excellent conversion for these substrates. At the current state of research, we give two possible explanations for this observation: (i) the steric limitations of the active site are reached, and/or (ii) the active site possesses a polar group in the region of the C-5 substituent, imposing a repulsive electrostatic interaction. While the less-polar chloro-substrates **1c** and **1d** were readily accepted by both CHMO and CPMO to give excellent conversions of the corresponding lactones **2c** and **2d**, olefin **1e** also showed only sluggish conversion with CHMO-expressing cells.

The stereochemistry at the 5-position in substrate ketones **1a−e** substantially influenced the selectivity of the CHMO-producing strain. Generally, *endo* compounds were converted into chiral lactones with only low-to-moderate enantiomeric excess. For CHMO, the combination of polar substituent and *endo* substitution pattern is especially unfavorable, leading to almost-racemic product (−)-(*S*)-**2a** (9% *ee*). The assignment of absolute configuration is based on our previous results for perhydroindenones.[19]

Changing to *exo* ketones, however, led to a significant improvement in the optical purity of products. The nature of the functional group in the 5-position of the ketone seems to have only a minor effect on the enantioselectivity of the biotransformation. (*S*)-Methoxy compound **2b** was

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

Substrate	R	Product	Config.	Strain	Yield[a]	*ee*[b]	$[\alpha]_D^{20}$
1a	MeO	**2a**	endo	CHMO	24% (71%)	9%	−3.0 (c = 0.16, EtOAc)
1a	MeO	**2a**	endo	CPMO	81%	34%	+13.2 (c = 0.50, EtOAc)
1b	MeO	**2b**	exo	CHMO	40% (81%)	96%	−36.2 (c = 1.0, $CHCl_3$)
1b	MeO	**2b**	exo	CPMO	75%	11%	−4.3 (c = 1.0, $CHCl_3$)
1c	Cl	**2c**	endo	CHMO	75% (98%)	80%	−23.7 (c = 1.0, $CHCl_3$)
1c	Cl	**2c**	endo	CPMO	79%	60%	−17.2 (c = 1.0, $CHCl_3$)
1d	Cl	**2d**	exo	CHMO	78% (97%)	>99%	−38.9 (c = 0.5, $CHCl_3$)
1d	Cl	**2d**	exo	CPMO	92%	>99%	+33.2 (c = 0.5, $CHCl_3$)
1e	$=CH_2$	**2e**	n.a.	CHMO	43% (88%)	61%	−3.2 (c = 0.3, EtOAc)
1e	$=CH_2$	**2e**	n.a.	CPMO	85%	41%	+2.2 (c = 1.0, $CHCl_3$)

[a] Isolated yield after chromatographic purification; yield in parenthesis is based on consumed starting material. [b] Value of ee determined by chiral-phase gas chromatography; racemic reference material was prepared by oxidation of ketones **1a**−**d** with mCPBA.

obtained in good enantiomeric excess for CHMO-expressing cells. Again, the *endo*-chloro ketone **1c** was converted with moderate stereoselectivity (note, that the chloro substituent requires a change in priority of numbering, which leads to a reversal of the *R*/*S* assignment; in all cases, the sense of chirality for CHMO products was the same) into (*R*)-lactone **2c** by CHMO, while the best results were obtained for *exo* substrate **1d**, where both strains gave excellent chemical yields together with >99% optical purity .

The sp^2-hybridized compound **1e** gives enantioselectivities between those of the *endo* and *exo* substrates. Biooxidations with CHMO-producing cells did not proceed to completion, which indicates a limiting effect imposed by the active site.

Summarizing the results of the CHMO biotransformations, a "stretched" geometry (*exo*) for bicycloketones of type **1** is a prerequisite for high optical purity of product lactones **2**. The enzyme displays a decreased enantioselectivity for the oxidation of substrates adopting a sterically more-demanding "angled" configuration (*endo*). The presence of less-polar functional groups is favorable for both high conversion and stereoselectivity of the biocatalyst. Recently, we have observed a strong influence of conformational aspects on the recognition and induction of chirality by CHMO.[49] The data presented in this study further support this theory.

Biotransformations with CPMO-producing cells do not display such a significant trend that is governed by conformational aspects. In some cases, however, the enzyme produces lactones **2** that are antipodal as compared to those from the CHMO-mediated biotransformations. We have observed this behavior of CPMO recently for the Baeyer−Villiger oxidation of perhydroindenones.[19]

Polar interactions within the active site seem to play a relevant role in the biooxidation of substrates **1**, since both methoxy-substituted ketones **1a** and **1b** gave only poor enantioselectivities for the production of lactones **2**. In the case of the *endo* compound **2a**, we have observed the formation of the enantiomeric lactone (*R*) when compared to CHMO, while *exo*-**1b** gave almost racemic product with a slight preference for the (*S*)-enantiomer. While the less-polar *endo* substrate **1c** gave rise to (−)-lactone **2c** in moderate optical purity and with matching sign of optical rotation as was obtained for the CHMO product, both **1d** and **1e** were converted into antipodal lactones by CPMO and CHMO. The *exo*-chloro compound (+)-**2d** was obtained in excellent optical purity as determined by chiral-phase GC. This substrate (**1d**) is the first example of an efficient enantiodivergent Baeyer−Villiger oxidation by CHMO and CPMO.

When comparing the conversions and yields of CHMO and CPMO biotransformations, it is noteworthy that CPMO seems to be rather tolerant toward steric demands by the substrate.

Enantiodivergent behavior has been observed for two enzymes involved in camphor degradative pathways from *Pseudomonas putida* NCIMB 10007. On some fused substrate compounds, 2-oxo-Δ^3-4,5,5-trimethylcyclopentylacetyl coenzyme A monooxygenase (MO2) and 2,5-diketocamphane monooxygenase (2,5-DKCMO) yielded antipodal enantiomeric products when used in an isolated form.[50] The application of this process in a native whole-cell system was compromised by overlapping substrate acceptance.[51] With the overexpression systems available, we have been able to study microbial biotransformations of enantiocomplementary BVMOs from two different natural sources for the first time.

Conclusions

Expanding previous substrate-profiling studies, we discovered enantiodivergent Baeyer−Villiger oxidation of functionalized pentalenones by CHMO and CPMO in some cases. We identified these substrates as valuable probes to investigate the effects of conformational and polar interactions in determining the yields and optical purities of the product lactones. Our results complement recent findings of an enantiodivergent oxidation by CPMO and CHMO in the 4-substituted cyclohexanone series.[15]

CHMO displayed a significant trend for the enantioselective conversion of ketones of type **1**, governed predominantly by conformational aspects, with beneficial effects exhibited by *exo* geometry and low substrate polarity. Predictions of the optical purities obtained by CPMO-mediated

biooxidation are complicated by obvious additional interactions. Additional substrate profiling is required to draw further conclusions.

Considering the absolute enantiodivergence of CHMO and CPMO for the formation of lactones **2d**, the investigation of the potential of this pair of "antipodal" biocatalysts is an important contribution for the further proliferation of enzyme-mediated Baeyer−Villiger oxidations, which is currently under way in our laboratory.

Experimental Section

General: 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. Flash column chromatography was performed on silica gel 60 from Merck (40−63 μm). Kugelrohr distillation was carried out using a Büchi GKR-51 apparatus. Melting points were determined using a Kofler-type Leica Galen III micro-hot-stage microscope and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, University of Vienna. Chiral-phase GC was performed on a ThermoFinnigan Trace-GC and an HP 6890 Series chromatograph using a BGB 175 cross-linked column (30 m × 0.25 mm ID, 0.25 μm film). Specific rotation was determined with a Perkin−Elmer Polarimeter 241. NMR spectra were recorded from $CDCl_3$ solutions with a Bruker AC 200 (200 MHz) or Bruker Avance UltraShield 400 (400 MHz) spectrometer and chemical shifts are reported in ppm using Me_4Si as internal standard.

(3′aα,5′β,6′aα)-Hexahydro-5,5-dimethylspiro[1,3-dioxane-2,2′(1′*H*)-pentalen]-5′-ol (4a): Ketone **3** (5.19 g, 23.1 mmol) was dissolved in dry methanol (80 mL) and treated portionwise with $NaBH_4$ (1.73 g, 45.8 mmol, 2.0 equiv.) between −2 and 0 °C. The mixture was stirred for 4 h, and then 0.1 N NaOH (100 mL) was added while keeping the temperature at 0−10 °C. The aqueous layer was extracted with diethyl ether and then the combined organic layers were dried over sodium sulfate and concentrated to give **4a**[39,40] as colorless crystals (4.85 g, 93%). M.p. 73−74 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.97 (s, 6 H), 1.45−1.60 (m, 2 H), 1.85−1.98 (m, 2 H), 2.03−2.35 (m, 5 H), 2.45−2.65 (m, 2 H), 3.48 (s, 2 H), 3.52 (s, 2 H), 4.16−4.30 (m, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.5 (q), 30.0 (s), 38.6 (d), 40.8 (t), 42.5 (t), 71.9 (t), 72.2 (t), 74.7 (d), 110.5 (s) ppm.

(3′aα,5′α,6′aα)-Hexahydro-5,5-dimethylspiro[1,3-dioxane-2,2′-(1′*H*)-pentalen]-5′-yl 4-Nitro-benzoate (5): A solution of alcohol **4a** (2.20 g, 9.72 mmol) and triphenylphosphane (2.55 g, 9.72 mmol) in dry diethyl ether (15 mL) was added dropwise at room temp. to a solution of DEAD (1.69 g, 9.72 mmol) and *p*-nitrobenzoic acid (1.63 g, 9.72 mmol). The resulting yellow mixture was stirred at 20 °C for 14 h. After evaporation of the solvent, the residue was subjected to flash chromatography (silica gel; light petroleum/EtOAc, 10:1) to give **5** (13.30 g, 77%) as colorless crystals. M.p. 160−162 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.99 (s, 6 H), 1.61−1.93 (m, 4 H), 2.06−2.32 (m, 4 H), 2.71−2.90 (m, 2 H), 3.50 (s, 4 H), 5.50−5.60 (m, 1 H), 8.17 (d, *J* = 8 Hz, 2 H), 8.29 (d, *J* = 8 Hz, 2 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.5 (q), 30.1 (s), 38.3 (d), 39.3 (t), 39.9 (t), 72.0 (t), 72.1 (t), 80.3 (d), 110.1 (s), 123.4 (d), 130.6 (d), 136.2 (s), 150.4 (d), 164.2 (s) ppm.

(3′aα,5′α,6′aα)-Hexahydro-5,5-dimethylspiro[1,3-dioxane-2,2′-(1′*H*)-pentalen]-5′-ol (4b): The purified benzoate **5** (2.2 g, 6.4 mmol) was dissolved in methanol (30 mL) and treated with a solution of potassium hydroxide (2.0 g) in water (15 mL). The mixture was kept at 40 °C for 15 h, then water was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous potassium carbonate and concentrated. The residue was submitted to column chromatography (silica gel; light petroleum/EtOAc, 10:1) to afford **4b**[39] (1.4 g, quant., 77% overall from **4a**) as colorless crystals. M.p. 78−80 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.97 (s, 6 H), 1.47 (br. s, 1 H), 1.51−1.93 (m, 6 H), 2.15−2.30 (m, 2 H), 2.60−2.80 (m, 2 H), 3.45 (s, 2 H), 3.48 (s, 2 H), 4.36−4.47 (m, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.5 (q), 30.1 (s), 38.0 (d), 40.1 (t), 42.2 (t), 71.8 (t), 72.3 (t), 74.8 (d), 110.0 (s) ppm.

Methylation of Alcohols 4a/b

Oil-free sodium hydride (4 equiv.; washed with light petroleum) was suspended in dry THF under nitrogen and treated with a 5% solution of alcohol **4a/b** (1 equiv.) in dry THF. After heating under reflux for 2 h the mixture was cooled to room temp. and dimethylsulfate (1 equiv.) was added. The mixture was heated under reflux for 48 h and then quenched with satd. NH_4Cl solution. The aqueous layer was extracted with diethyl ether (3 × 50 mL) and the combined organic layers were washed with brine. After drying over sodium sulfate and evaporation, crude **6a/b** was isolated and was used without further purification.

(3′aα,5′β,6′aα)-Hexahydro-5′-methoxy-5,5-dimethylspiro[1,3-dioxane-2,2′(1′*H*)-pentalene] (6a): Alcohol **4a** (0.950 g, 4.46 mmol) was converted according to the above procedure to give **6a** (0.900 g, 90%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.97 (s, 6 H), 1.47−1.80 (m, 4 H), 1.95−2.10 (m, 2 H), 2.22−2.36 (m, 2 H), 2.37−2.53 (m, 2 H), 3.30 (s, 3 H), 3.46, 3.49 (2s, 4 H), 3.68−3.84 (m, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.5 (q), 30.0 (s), 37.8 (d), 38.1 (t), 40.3 (t), 56.7 (q), 71.5 (t), 72.5 (t), 84.3 (d), 110.0 (s) ppm.

(3′aα,5′α,6′aα)-Hexahydro-5′-methoxy-5,5-dimethylspiro[1,3-dioxane-2,2′(1′*H*)-pentalene] (6b): Alcohol **4b** (1.20 g, 5.3 mmol) was converted according to the above procedure to give **6b** (1.26 g, 98%), as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.96 (s, 6 H), 1.50−1.65 (m, 4 H), 1.78−1.95 (m, 2 H), 2.13−2.27 (m, 2 H), 2.50−2.70 (m, 2 H), 3.25 (s, 3 H), 3.43 (s, 2 H), 3.47 (s, 2 H), 3.68−3.95 (m, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.5 (q), 30.0 (s), 37.8 (d), 38.5 (t), 40.2 (t), 56.3 (q), 71.7 (t), 72.3 (t), 83.3 (d), 109.9 (s) ppm.

Chlorination with PPh_3/NCS

Triphenylphosphane (1 equiv.) in dry THF (10% solution) was treated dropwise with NCS (1 equiv.) in dry THF (5% solution). The corresponding alcohol (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 (approx. 1.5 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 submitted to flash chromatography (silica gel; light petroleum/EtOAc, 100:1).

(3′aα,5′β,6′aα)-5′-Chlorohexahydro-5,5-dimethylspiro[1,3-dioxane-2,2′(1′*H*)-pentalene] (7a): *exo*-Alcohol **4b** (0.50 g, 2.2 mmol) was converted according to the above procedure to give *endo*-**7a** (0.25 g, 50%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.97 (s, 6 H), 1.61−1.91 (m, 4 H), 2.12−2.59 (m, 6 H), 3.46 (s, 4 H), 3.98−4.15 (m, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.5 (q), 30.0 (s), 38.6 (d), 39.9 (t), 43.6 (t), 58.8 (d), 71.8 (t), 72.2 (t), 110.1 (s) ppm.

(3′aα,5′α,6′aα)-5′-Chlorohexahydro-5,5-dimethylspiro[1,3-dioxane-2,2′(1′*H*)-pentalene] (7b): *endo*-Alcohol **4a** (0.50 g, 2.2 mmol) was converted according to the above procedure to give *exo*-**7b** (0.28 g, 52%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.90 (s, 6 H), 1.55−1.72 (m, 2 H), 1.73−1.94 (m, 2 H), 1.97−2.20 (m, 4 H), 2.67−2.87 (m, 2 H), 3.38 (s, 2 H), 3.40 (s, 2 H), 4.32−4.48 (m, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.5 (q), 30.0 (s), 38.1 (d), 39.3 (t), 43.7 (t), 62.6 (d), 72.0 (t), 109.8 (s) ppm.

***cis*-(1′,3′a,6′,6′a)-Tetrahydro-5,5-dimethylspiro[1,3-dioxane-2,2′(1′*H*)-pentalene] (8):** Compound **8**[39] was obtained as by-product (10−15%) in the chlorination according to the procedure above. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.97 (s, 6 H), 1.48−1.73 (m, 2 H), 2.11−2.19 (m, 1 H), 2.25−2.45 (m, 2 H), 2.50−2.72 (m, 2 H), 3.10−3.28 (m, 1 H), 3.45 (s, 2 H), 3.52 (s, 2 H), 5.52−5.70 (m, 2 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 22.48 (q), 22.53 (q), 30.1 (s), 36.7 (d), 38.37 (t), 38.45 (t), 40.0 (t), 46.6 (d), 71.8 (t), 72.0 (t), 109.2 (s), 128.5 (d), 133.8 (d) ppm.

Cleavage of the 2,2-Dimethylpropanediol Protecting Group

The pertinent ketal was stirred overnight at room temp. in a 1:1 mixture of 0.1 M H_2SO_4 and acetone. The solution was neutralized with satd. $NaHCO_3$ solution and extracted with diethyl ether. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo.

(3aα,5β,6aα)-Hexahydro-5-methoxy-2(1*H*)-pentalenone (1a): Ketal **6a** (0.880 g, 3.66 mmol) was deprotected according to the above procedure to give **1a** (0.371 g, 66%) as a colorless oil after Kugelrohr distillation. B.p. 160−163 °C/13 mbar. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.58−1.73 (m, 2 H), 2.02−2.20 (m, 4 H), 2.23−2.28 (m, 2 H), 2.43−2.60 (m, 2 H), 3.26 (s, 3 H), 3.83−3.96 (m, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 37.7 (d), 39.1 (t), 45.1 (t), 56.3 (q), 83.5 (d), 220.3 (s) ppm. $C_9H_{14}O_2$ (154.21): calcd. C 70.10, H 9.15; found C 69.86, H 9.31.

(3aα,5α,6aα)-Hexahydro-5-methoxy-2(1*H*)-pentalenone (1b): Compound **6b** (0.750 g, 3.12 mmol) was converted according to the above protocol to yield **1b** (0.383 g, 80%) as a colorless oil after flash column chromatography (silica gel; light petroleum/EtOAc, 10:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.50−1.68 (m, 2 H), 1.95−2.20 (m, 4 H), 2.43−2.65 (m, 2 H), 2.78−3.00 (m, 2 H), 3.30 (s, 3 H), 3.94 (m, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 37.5 (d), 39.1 (t), 44.4 (t), 56.1 (q), 82.9 (d), 220.2 (s) ppm. $C_9H_{14}O_2$ (154.21): calcd. C 70.10, H 9.15; found C 69.96, H 9.23.

(3aα,5β,6aα)-5-Chloro-hexahydro-2(1*H*)-pentalenone (1c): Precursor **7a** (0.27 g, 1.1 mmol) was hydrolyzed by the above procedure to give **1c** (0.10 g, 58%) as a colorless oil after flash column chromatography (silica gel; light petroleum/EtOAc, 5:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.73−1.92 (m, 2 H), 2.20−2.40 (m, 2 H), 2.45−2.70 (m, 4 H), 2.72−2.95 (m, 2 H), 4.18−4.37 (m, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 38.1 (d), 44.2 (t), 44.8 (t), 58.6 (d), 219.3 (s) ppm. $C_8H_{11}ClO$ (158.63): calcd. C 60.57, H 6.99; found C 60.54, H 6.83.

(3aα,5α,6aα)-5-Chloro-hexahydro-2(1*H*)-pentalenone (1d): Ketal **7b** (257 mg, 1.05 mmol) gave ketone **1d** (112 mg, 68%) as a colorless oil following the above procedure after flash column chromatography (silica gel; light petroleum/EtOAc, 10:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.73−2.12 (m, 4 H), 2.30−2.72 (m, 4 H), 3.00−3.25 (m, 2 H), 4.50−4.60 (m, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 37.1 (d), 43.9 (t), 44.5 (t), 61.7 (d), 219.3 (s) ppm. $C_8H_{11}ClO$ (158.63): calcd. C 60.57, H 6.99; found C 60.74, H 6.73.

Biotransformations with Recombinant Cells

Fresh LB-amp medium (250 mL; 1% Bacto-Peptone, 0.5% Bacto-Yeast Extract, 1% NaCl supplemented by 200 ppm ampicillin) was inoculated with a 1:100 aliquot of an overnight preculture of the corresponding construct *BL21(DE3)(pMM4)* or *DH5α(pCMO206)* in a baffled Erlenmeyer flask. The culture was incubated at 37 °C on an orbital shaker at 120 rpm for 2 h, then IPTG was added so as to obtain a final concentration of 0.025 mM. The substrate **1a**−**e** (100 μL) was added neat along with β-cyclodextrin (1 equiv.). The culture was incubated at room temperature for 18−48 h and the conversion was monitored by GC. The biomass was removed by centrifugation (3500 rpm, 10 min), the aqueous layer was passed through a bed of Celite®, and then the product was isolated by repeated extraction with EtOAc. The combined organic layers were dried with sodium sulfate and concentrated. Lactones **2a**−**e** were purified by flash column chromatography.

(4aα,6β,7aα)-Hexahydro-6-methoxycyclopenta[*c*]pyran-3(1*H*)-one (2a): Biotransformation of **1a** (108 mg, 0.7 mmol) with recombinant cells according to the above biotransformation protocol gave lactone **2a** as a colorless oil after chromatographic purification (silica gel; light petroleum/EtOAc, 10:1) in the yield and enantiomeric purity specified in Table 1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.50−1.65 (m, 2 H), 1.82−2.10 (m, 2 H), 2.40−2.65 (m, 4 H), 3.23 (s, 3 H), 3.72−3.83 (m, 1 H), 4.08 (dd, *J* = 14, 7 Hz, 1 H), 4.31 (dd, *J* = 14, 7 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 32.8 (d), 33.8 (t), 34.9 (t), 35.2 (d), 38.0 (t), 56.4 (q), 69.9 (t), 82.3 (d), 173.8 (s) ppm. $C_9H_{14}O_3$ (170.21): calcd. C 63.51, H 8.29; found C 63.67, H 8.04.

(4aα,6α,7aα)-Hexahydro-6-methoxycyclopenta[*c*]pyran-3(1*H*)-one (2b): Microbial oxidation of **1b** (108 mg, 0.7 mmol) yielded lactone **2b** as a colorless oil after chromatographic purification (silica gel; light petroleum/EtOAc, 10:1) in the yield and enantiomeric purity specified in Table 1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.24−1.44 (m, 1 H), 1.52−1.68 (m, 1 H), 2.00−2.90 (m, 6 H), 3.29 (s, 3 H), 3.80−3.92 (m, 1 H), 4.10 (dd, *J* = 14, 7 Hz, 1 H), 4.35 (dd, *J* = 14, 7 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 32.1 (d), 34.1 (t), 34.2 (t), 34.4 (d), 38.4 (t), 55.7 (q), 69.7 (t), 81.4 (d), 173.6 (s) ppm. $C_9H_{14}O_3$ (170.21): calcd. C 63.51, H 8.29; found C 63.17, H 8.16.

(4aα,6β,7aα)-6-Chlorohexahydrocyclopenta[*c*]pyran-3(1*H*)-one (2c): Biooxidation of **1c** (116 mg, 0.73 mmol) yielded lactone **2c** as colorless crystals after chromatographic purification (silica gel; light petroleum/EtOAc, 10:1) in the yield and enantiomeric purity specified in Table 1. M.p. 90−92 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.58−1.68 (m, 1 H), 1.70−1.81 (m, 1 H), 2.30−2.70 (m, 6 H), 4.05−4.15 (m, 1 H), 4.20−4.30 (m, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 32.8 (d), 34.2 (t), 35.1 (d), 38.8 (t), 43.1 (t), 56.6 (t), 69.2 (t), 172.7 (s) ppm. $C_8H_{11}ClO_2$ (174.63): calcd. C 55.02, H 6.35; found C 54.87, H 6.55.

(4aα,6α,7aα)-6-Chlorohexahydrocyclopenta[*c*]pyran-3(1*H*)-one (2d): Biotransformation of **1d** (116 mg, 0.73 mmol) according to the general protocol yielded lactone **2d**[34] as colorless crystals after chromatographic purification (silica gel; light petroleum/EtOAc, 10:1) in the yield and enantiomeric purity specified in Table 1. M.p. 88−90 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.60−1.80 (m, 1 H), 1.92−2.10 (m, 1 H), 2.20−2.88 (m, 6 H), 4.10 (dd, *J* = 14, 7 Hz, 1 H), 4.30 (dd, *J* = 14, 7 Hz, 1 H), 4.45−4.60 (m, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 32.2 (d), 33.7 (t), 34.5 (d), 39.5 (t), 43.5 (t), 61.4 (d), 69.1 (t), 172.8 (s) ppm. $C_8H_{11}ClO_2$ (174.63): calcd. C 55.02, H 6.35; found: C 55.03, H 6.73.

***cis*-Hexahydro-5-methylenecyclopenta[*c*]pyran-3(1*H*)-one (2e):** Biotransformation of **1e** (105 mg, 0.78 mmol) gave lactone **2e** as a colorless oil after chromatographic purification (silica gel; light petroleum/EtOAc, 7:1) in the yield and enantiomeric purity specified in Table 1. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.98−2.70 (m, 8 H), 3.93 (dd, *J* = 14, 7 Hz, 1 H), 4.21 (dd, *J* = 14, 7 Hz, 1 H), 4.80 (br. s, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 33.5 (d), 34.5 (t), 35.2 (t), 35.7 (d), 40.1 (t), 69.9 (t), 107.4 (t), 148.5 (s), 173.8 (s) ppm. $C_9H_{12}O_2$ (152.19): calcd. C 71.03, H 7.95; found C 70.73, H 8.08.

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