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Biooxidation of Bridged Cycloketones Using Baeyer−Villiger Monooxygenases of Various Bacterial Origin

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Bridged cycloketones were synthesized and utilized as substrates to study biooxidations mediated by Baeyer−Villiger monooxygenases (BVMO) of various bacterial origin. The required enzymes were heterologously produced by recombinant overexpression systems based on *Escherichia coli* to enable facile recycling of the required nicotinamide cofactors during the whole-cell biotransformations. Ketone precursors of various structural demands were chosen to evaluate steric limitations and flexibility of the active site of BVMOs. By desymmetrization of the prochiral substrates, four to six stereogenic centers were generated within a single biooxidation step. The enzyme library investigated in this study allowed access to antipodal lactone products with excellent enantioselectivity in several cases. Together with a distinct substrate acceptance profile, the recently proposed classification into two groups of cycloketone converting BVMOs was supported by the biotransformation results obtained within this study.

Introduction

Biocatalysis has become a frequently applied strategy in synthetic routes to complex molecules of industrial interest due to the usually high regio-, chemo-, and enantioselectivity of enzyme mediated conversions combined with a production method compatible with the concepts of green and sustainable chemistry. The enzymes used for this purpose may be contained within an intact microbial cell, as well as used as crude or purified protein. A highly facile and simple manner for laboratory-scale applications in particular is to perform whole-cell biotransformations. This methodology offers multiple advantages: the growing organism represents a renewable source for the required catalytic entity; the biocatalyst remains in its natural environment, hence, optimum operational efficiency and stability may be expected; tedious protein purification of non-commercial enzymes is circumvented, and selective biotransformations can be realized by using genetically modified strains; and within living cells, all required cofactors are supplied, consequently avoiding the setup of artificial recycling systems.

Stereoselective biooxygenations represent a highly active field in biocatalysis within recent years.[1] In particular, enzyme mediated Baeyer−Villiger oxidations have experienced a remarkable renaissance since the turn of the century with genome sequencing projects providing access to a large diversity of novel Baeyer−Villiger monooxygenases (BVMOs).[2,3] The recent identification of BVMO groups enabling access to enantio- and regiocomplementary lactone products further emphasized the versatility of the presently available biocatalyst platform.[4] In addition to the exploitation of the natural diversity of this enzyme family, evolutionary[5] and knowledge based strategies[6]

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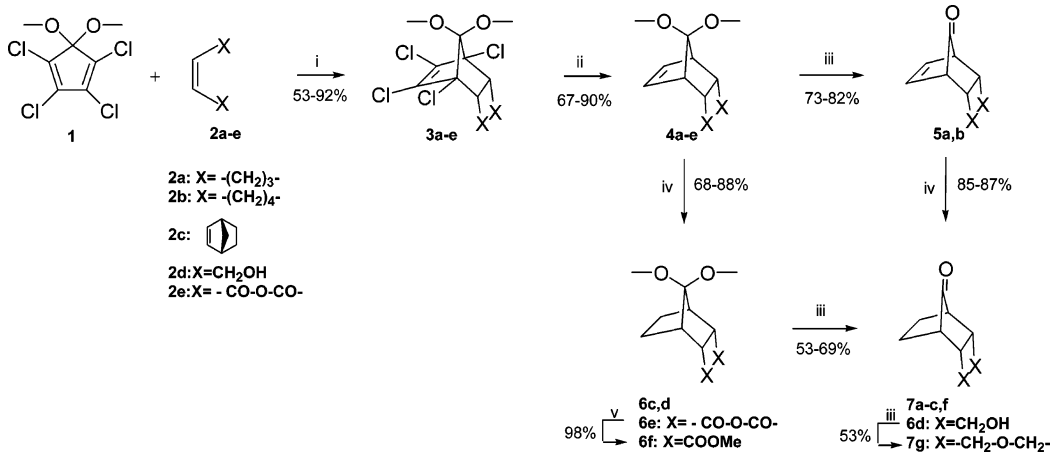
SCHEME 1. Synthesis of Functionalized Bridged Ketones 7a−d,f,g[a]



[a] (i) [4 + 2] cyclization under thermal or microwave conditions. (ii) Dechlorination using Birch conditions or Na/*t*-BuOH. (iii) Ketal deprotection under various conditions (see Experimental Section). (iv) Catalytic hydrogenation: Pd(C)/H_2, EtOAc. (v) Esterification using sulfuric acid in methanol.

were utilized, in connection with the first structure determination of a thermostable representative of this enzyme group,[7] to modify stereoselectivity and catalyst performance, further extending the catalytic repertoire of BVMOs. These developments resulted in an increasing number of applications of BVMOs in the synthesis of bioactive compounds and natural products within this decade.[8]

Herein, we report on BVMO mediated oxidation of polycyclic ketones bearing a norbornanone structural motif. Starting from prochiral substrates, the generation of four to six chiral centers has been achieved within a single desymmetrization step.[9] In addition, the lactone group can easily be further transformed into other functionalities (e.g., by hydrolysis to give access to appropriate δ-hydroxycarboxylic acid, which can be further utilized in the synthesis of enantiomerically pure bicyclo[4.2.0]-octanon-2-ol and bicyclo[4.2.0]octan-2-one, as proposed previously).[4b]

BVMO mediated oxidations were initially accomplished in screening scale using a 12- and 24-well plastic dishes parallel format.[10] On the basis of screening results, single preparative biotransformations were conducted subsequently with selected biocatalysts. Biooxidations were routinely carried out using *Escherichia coli* recombinant overexpression systems for BVMOs as previously described.[11,12] In addition to the previous outlined benefits of whole-cell based biotransformations, the application of heterologous recombinant producers moreover offers the advantage to study biocatalysts originating from hazardous organisms in a benign and easy to cultivate host.

Results and Discussion

Synthesis of Ketones. Cyclic ketones were prepared in short and efficient sequences (Scheme 1) based in part on literature protocols, which were modified and optimized in particular cases. The initial chlorinated adducts **3a**−**e** were synthesized by Diels−Alder cyclization of the appropriate cyclic olefin **2a**−**e** and highly reactive 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene **1**. Appropriate starting materials were heated either without any solvent (**2a**−**c**) or in the presence of toluene (**2d**,**e**). All products were isolated in very good yields (**3b**: 82%; **3c**: 92%; **3d**: 53%; and **3e**: 87%). Proof of the stereochemistry of **3c** was based on comparison with a structurally similar and

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stereochemically well-defined tetracyclic compound, readily available through an inverse electron demand Diels−Alder reaction between **1** and bicyclo[2.2.1]hepta-2,5-diene.[13]

Reaction with cyclopentene **2a** required additional optimization of reaction conditions. In this case, it was necessary to carry out the reaction at a lower temperature (100 °C), otherwise the thermodynamically favored but unwanted exo product was formed in significant amounts (140 °C; *exo*-**3a**/*endo*-**3a** = 5:1). To achieve sufficient conversion at lower temperatures, a prolonged reaction time was essential (88% conversion after 65 h). Cyclization was also performed in a microwave reactor. A conversion of 80% was obtained after 2.5 h irradiation at 100 °C. If the temperature was increased to 120 °C, the formation of *exo*-**3a** (*exo*-**3a**/*endo*-**3a** = 3:1) was observed. Best results were obtained when 20 equiv of **2a** was added and the reaction temperature was maintained at 100 °C under microwave irradiation. In this case, full conversion was reached within 16 h, and only *endo*-**3a** was formed. For small-scale preparations, the microwave protocol was most efficient; however, due to volume limitations (7 mL), larger scale experiments were conducted using classical heating for prolonged reaction times (100 °C, 20 equiv of **2a**, 85% yield)

Subsequently, **4a**−**c** were prepared by dechlorination analogous to Dauben and Kellogg[14] using sodium and *t*-butanol in good yields (**4a**: 67%; **4b**: 88%; and **4c**: 90%). However, these conditions failed for substrates **3d**,**e**, and a previously reported protocol using tri-*n*-butyltinhydride (Bu_3SnH)[15] led to only partial dehalogenation.[16] Better results were achieved by utilizing Birch conditions using sodium metal dissolved in liquid ammonia.[17,18] The reaction gave satisfactory yields (**4d**: 71% and **4e**: 78%), and **4d**,**e** were obtained in good purity (>95% according to NMR) without the need for further purification.

Compounds **4a**,**b** were submitted to acidic hydrolysis of the ketal functionality to prepare unsaturated ketone substrates, which could be used for subsequent BVMO mediated oxidation. Ketones **7c**,**f**,**g** were prepared by applying the hydrogenation step prior to ketal hydrolysis because of the higher yield obtained by this order of reaction steps (>50%). When hydrolysis was performed prior to hydrogenation, ketones were isolated in yields of only approximately 20%. Catalytic hydrogenation gave smooth conversion and excellent yields in all cases (**6c**: 68%; **6d**: 85%; **6e**: 88%; **7a**: 85%; and **7b**: 87%).

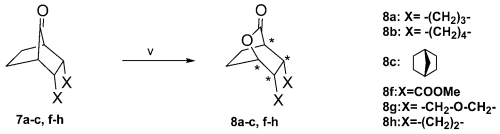
To implement additional functionality in biotransformation substrates stable at aqueous fermentation conditions, anhydride **6e** was esterified under acidic conditions in methanol. The reaction led to the formation of **6f** bearing two methyl ester groups in quantitative yield within 48 h.

For the hydrolysis of the ketal protecting group, acidic conditions were used applying different reagents optimized for each compound. Reaction times reported in the literature for the deprotection of **4a**,**b** with an aqueous 10% sulfuric acid solution[14] (7 days) could be drastically shortened (12 h) when employing *p*-toluenesulfonic acid (pTSA) dissolved in dry acetone.[19] Final purification of **5a**,**b** by column chromatography required using triethylamine pretreated silica gel; otherwise, yields decreased dramatically. Ketal **6c** was hydrolyzed to **7c** using the same procedure. All compounds were prepared in good yields (**5a**: 82%; **5b**: 73%; and **7c**: 63%).

Literature protocols for the deprotection of ketals **6d**,**f**[20,21] failed in our hands, and full conversion to ketone **7f** was only achieved within reasonable reaction times and in acceptable yields (67%) by applying substantially harsher conditions (boiling dioxane, 3 N HCl).[22] Ketal **6d** was ultimately deprotected using Amberlyst-15 in dry acetone,[23] and complete conversion was reached after stirring the reaction mixture at room temperature overnight (53% yield). However, using this strongly acidic resin led to cyclization of the dihydroxyl groups to form a tetrahydrofuran ring. The cyclization was finally confirmed after BVMO mediated oxidation to the corresponding lactone, which was obtained as a crystalline product and was subjected to X-ray structure analysis (see Supporting Information).

The synthesis of ketone **7h** was performed starting from 1,3-divinyl-2-cyclopentanol by a Cu catalyzed [2 + 2] photocycloaddition reaction. While both the unprotected and the *t*-butyldimethylsilyl protected substrate underwent photocycloaddition, the reaction proceeded more cleanly and with an improved diastereoselectivity in the latter case (72% chemical yield). Cleavage of the protecting group with tetrabutylammoniumfluoride, followed by oxidation with 2-iodoxybenzoic acid, afforded ketone **7h** in 89% yield.[4b]

Biooxidation of Cycloketones. Enzyme mediated Baeyer−Villiger oxidations of tricyclic ketones as prochiral precursors potentially generated four to six new chiral centers in a single desymmetrization step (eq 1).



Equation 1: BVMO-mediated oxidation of functionalized bridged ketones **7a-c,f-h**.

On the basis of experience from our lab, only certain BVMOs are able to oxidize ketones with ring systems larger than cyclohexanone or cyclopentanone. For biotransformations of tricyclic and tetracyclic cycloketones, it was necessary to select a specific type of BVMO that could realize oxidations of such sterically demanding substrates. Successful biooxidations of some structurally similar cycloketones were already reported using isolated cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* 9871 ($CHMO_{Acineto}$).[24] On the basis of these

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data, an engineered strain of *E. coli* expressing $CHMO_{Acineto}$ was selected as a promising candidate for the oxidation of polycyclic ketones.

Recently, clustering of BVMOs into two groups was proposed based on protein sequence and enantiopreference.[4g] The first group contains CHMO-type enzymes: cyclohexanone monooxygenases from *A. calcoaceticus* 9871 ($CHMO_{Acineto}$),[25] from *Brevibacterium* DPR#14 ($CHMO_{Brevi1}$),[26] from *Rhodococcus* DPR#455 ($CHMO_{Rhodo1}$) and *Rhodococcus* DPR#460 ($CHMO_{Rhodo2}$),[27] from *Arthrobacter* DPR#453 ($CHMO_{Arthro}$),[27] and from *Brachymonas* DPR#192 ($CHMO_{Brachy}$).[28] The second group consists of CPMO-type enzymes and contains cyclopentanone monooxygenase (CPMO) from *Comamonas* NCIMB 9872 ($CPMO_{Coma}$)[29] and a second CHMO from *Brevibacterium* DPR#399 ($CHMO_{Brevi2}$).[26] For oxidation studies of bicyclic, tricyclic, and tetracyclic ketones, all members from both enzyme families were compiled into a collection of polycycloketone oxidizing BVMOs.

Screening experiments were realized in multi-well plastic dishes prior to biotransformations on a preparative scale. The parallel screening format was designed to provide an easy-to-use methodology for the assessment of recombinant whole-cell catalysts, implementing reproducible and optimized conditions for cell growth, expression of recombinant biocatalysts, and the biotransformation itself.[10] These screening conditions provided rapid information about substrate profile and stereopreference.

Single preparative biotransformations were carried out to determine physical and spectral data together with specific optical rotation of isolated lactones. Racemic reference material of lactones was prepared by chemical oxidation of the corresponding ketones with mCPBA.

Cycloketones were oxidized with all eight overexpression systems. It was found that only saturated ketones **7a**−**c**,**f**,**g** were converted to the corresponding lactones. Biotransformation of unsaturated analogues **5a**,**b** did not lead to lactone products after prolonged incubation time. Only the starting material was detected by GC in the fermentation broth. It was confirmed after comparison to a GC standard that no biooxidation occurred. These results were unexpected since it was already published by Taschner and Peddada that purified CHMO from *Acinetobacter* NCIMB 9871 showed good conversion with excellent enantioselectivity.[24]

A similar behavior was also observed in experiments when structurally analogous ketones were utilized. On the other hand, saturated analogues were accepted with all expression systems.[10] To test whether poor cell membrane permeability was the reason for unreactivity of the unsaturated substrates, a crude cell extract of *E. coli* overexpressing a modified $CHMO_{Acineto}$ biocatalyst was used. Under these conditions, it was confirmed by GC/MS analysis that unsaturated ketone **5a** was converted to the corresponding lactone.[30] On the basis of this observation, only saturated ketones were further used for recombinant whole-cell mediated biooxidation reactions.

Conversion of ketone **7a** was complete after a standard biooxidation time (24 h) with all used recombinant strains overexpressing cycloketone oxidizing BVMOs within screening experiments. The biotransformation of ketone **7a** on a preparative scale with recombinant *E. coli* strains gave lactone **8a** in good yield in 24 h apart from $CPMO_{Coma}$, $CHMO_{Brevi2}$, and $CHMO_{Brachy}$, which required a prolonged incubation time of ca. 72 h to reach full conversion. Additionally, $CPMO_{Coma}$ and $CHMO_{Brevi2}$ displayed opposite enantiopreference (according to chiral phase GC and specific rotation) as compared to the other expression systems (Tables 1 and 2).

As in the previous case, ketone **7b** was fully converted within 24 h with all used recombinant strains overexpressing cycloketone oxidizing BVMOs in the screening experiment. On the basis of those results, oxidation of *endo*-tricyclo[5.2.1.$0^{2,6}$]decan-11-one **7b** was carried out with all eight strains on a preparative scale. Full conversion was achieved within 24 h with all strains apart from *E. coli* producing $CPMO_{Coma}$, $CHMO_{Brevi2}$, and $CHMO_{Brachy}$. Again, members of the CPMO-type family converted the substrate with opposite enantiopreference (Tables 1 and 2). These results nicely correspond to the previously reported hypothesis of two clusters of cycloketone converting BVMOs as proposed by our group.[4g]

Full conversion of tetracyclic ketone **7c** was achieved only with $CHMO_{Acineto}$, $CHMO_{Rhodo1}$, $CHMO_{Rhodo2}$, $CHMO_{Brachy}$, and $CHMO_{Arthro}$. Members of the CPMO family did not convert the substrate at all. Fermentation with $CHMO_{Brevi1}$ reached 83% conversion within the standard experiment time (24 h). With this compound, substrate acceptance differs between enzyme families, which again outlines the intrinsic difference in catalyst behavior between the two protein groups. One family fully converts the substrate in an enantioselective manner, while representatives of the CPMO-type family do not accept this ketone at all. $CHMO_{Brevi1}$ converts the substrate with the same enantioselectivity as the other CHMO-type enzymes, however, with a lower efficiency than the remaining recombinant systems. Biooxidation was realized with $CHMO_{Acineto}$ also on a preparative scale according to the general procedure, and lactone **(−)-8c** was isolated in 51% yield.

The bifunctional precursor 3-oxo-2-oxa-bicyclo[2.2.2]octane-5,6-dicarboxylic acid dimethyl ester **7f** was not a suitable substrate for any recombinant expression system of this BVMO collection (no conversion was observed according to GC). Racemic lactone **8f** was obtained via chemical Baeyer−Villiger oxidation using mCPBA as the oxidizing agent in a reference experiment. Probably, the bulkiness of the carboxylate groups eventually in combination with increased polarity prevented the compound from properly fitting into the active site of BVMOs, and therefore, steric hindrance did not permit the required oxidation. However, it has been demonstrated on structurally simpler substrates that BVMOs can also tolerate more polar substituents.

Biotransformation with ketone **7g** displayed full conversion with representatives of the CHMO-type family, while CPMO-type biocatalysts gave only incomplete conversion (Table 1) within the standard biotransformation time. Both clusters differ

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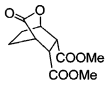
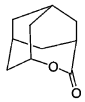
TABLE 1. Screening-Scale Biotransformations of Ketones 7a−c,f−i

	 **8a**	 **8b**	 **8c**	 **8f**	 **8g**	 **8h**	 **8i**
	Conversion[a] *e.e.*[b]	Conversion[a] *e.e.*[b]	Conversion[a] *e.e.*[b]	Conversion[a] *e.e.*[b]	Conversion[a] *e.e.*[b]	Conversion[a] *e.e.*[b]	Conversion[a] *e.e.*[b]
$CHMO_{Acineto}$	100% *97 %* (-)	100% *97 %* (-)	100% *98 %* (-)	n.c.	100% *92 %* (-)	100% *84%* (-)	100% n.a.
$CPMO_{Coma}$	100% *91%* (+)	100% *83%* (+)	n.c.	n.c.	84% *71 %* (+)	56% *86%* (+)	n.c.
$CHMO_{Brevi1}$	100% *94%* (-)	100% *93%* (-)	83% *78%* (-)	n.c.	100% *93%* (-)	100% *96%* (-)	83% n.a.
$CHMO_{Brevi2}$	100% *92%* (+)	100% *94%* (+)	n.c.	n.c.	28% *35 %* (+)	30% *75%* (+)	n.c.
$CHMO_{Rhodo1}$	100% *99%* (-)	100% *99%* (-)	100% *98%* (-)	n.c.	100% *92%* (-)	100% *60%* (-)	100% n.a.
$CHMO_{Rhodo2}$	100% *99%* (-)	100% *99%* (-)	100% *98%* (-)	n.c.	100% *92%* (-)	100% *60%* (-)	100% n.a.
$CHMO_{Arthro}$	100% *99%* (-)	100% *99%* (-)	100% *98%* (-)	n.c.	100% *93%* (-)	100% *80%* (-)	100% n.a.
$CHMO_{Brachy}$	100% *99%* (-)	100% *99%* (-)	100% *98%* (-)	n.c.	100% *93%* (-)	100% *92%* (-)	100% n.a.

[a] Conversion determined by GC (internal standard). [b] Enantiomeric excess determined by chiral phase GC; value of optical rotation given in parentheses. n.c.: no conversion and n.a.: not applicable.

TABLE 2. Yields and Optical Rotation Values of Selected Preparative-Scale Biotransformations to Lactones 8

	strain	yield (%)	e.e. (%)	$[\alpha]_{20}^{D}$ ($CHCl_3$)
8a	$CHMO_{Rhodo2}$	63	99	−17.30 (c = 1.70)
	$CHMO_{Brevi2}$	67	92	+13.04 (c = 1.43)
8b	$CHMO_{Rhodo1}$	58	99	−30.42 (c = 1.06)
	$CHMO_{Brevi2}$	78	94	+28.74 (c = 1.44)
8c	$CHMO_{Acineto}$	51	98	−39.63 (c = 0.97)
8g	$CHMO_{Acineto}$	53	92	−17.68 (c = 0.57)
	$CPMO_{Coma}$	49	71	+15.66 (c = 0.49)
8h	$CHMO_{Brevi1}$	72	96	−41.5 (c = 1.00)
8i	$CHMO_{Rhodo2}$	60	n.a.	n.a.

also in enantiopreference: CHMO-type enzymes convert ketone **7g** to lactone **8g** in high enantioselectivity, while biooxidation with $CPMO_{Coma}$ and $CHMO_{Brevi2}$ provides access to optical antipodes, however, with only moderate to low enantiomeric excess. Again, the results are in agreement with the proposed theory of two family clusters. Biooxidations of **7g** with $CHMO_{Acineto}$ and $CPMO_{Coma}$ were performed also on a preparative scale to establish a specific rotation value of both antipodal product lactones (Table 2).

Enantiodivergent behavior of both enzyme families was also observed with ketone **7h**. $CPMO_{Coma}$ and $CHMO_{Brevi2}$ gave access to the antipodal lactone as compared to the CHMO-type family (Table 1) with high optical purity for each lactone stereoisomer.[4b] Preparative biotransformation was carried out with $CHMO_{Brevi1}$ as the biocatalyst, and lactone **8h** was isolated in a yield of 72% (Table 2).

Determination of absolute configuration for lactones obtained by recombinant strain biooxygenation was exemplified for **8h** using the Mosher ester method, as outlined in a previous publication.[4b] Since all ketones **7a**−**c**,**g** were converted by CHMO-type biocatalysts to lactones possessing the same sign of optical rotation as lactone **8h** (1*R*,2*R*,5*S*,6*S*) obtained with $CHMO_{Brevi1}$, we propose the same absolute configuration for lactones **7a**−**c**,**g** (as displayed in eq 1 and Table 2). It is worth mentioning that a similar set of substrates differing only by the presence of a C═C double bond in the bridged cyclic system

was reported to give opposite enantioselectivity when using isolated $CHMO_{Acineto}$.[24]

It has already been published that intact cells of (±)-camphor-grown *Pseudomonas putida* ATCC 17453(CAM) oxidize adamanthanone to the expected lactone. However, mono- and dihydroxylated products were formed as well.[31] Since it was shown previously that polycycloketone oxidizing BVMOs are able to oxidize bi-, tri-, and tetracyclic ketones, sterically demanding adamantanone **7i** was included in this study as a potential substrate for all eight recombinant strains of *E. coli*. Results of the screening experiment are summarized in Table 1.

CHMO family BVMOs converted adamantanone **7i** completely within 24 h. The only exception was $CHMO_{Brevi1}$ achieving only 83% conversion under standard screening conditions. Representatives of the CPMO-type family did not accept this substrate at all; only the starting material remained unchanged in the fermentation broth after standard biotransformation times. Preparative biotransformation was realized with $CHMO_{Rhodo2}$, and spectral data of the isolated product were found to be identical to the chemical reference and literature data. No hydroxylated products were obtained; ketone **7i** was converted to the desired lactone **8i** exclusively. The presented results are again in agreement with the proposed theory of the two BVMO clusters.

Conclusion

Microbial Baeyer−Villiger oxidations of polycyclic substrates with the ketone functionality located at a bridge of the bicyclic compounds was successfully realized with a library of recombinant whole-cells expressing BVMOs of bacterial origin. Biotransformations were conducted in screening scale to identify suitable candidates for subsequent preparative experiments, and isolated yields of chiral lactones ranged between 50 and 78%. In the majority of the examples, both enantiomers of the product lactones could be obtained in moderate to excellent stereoselectivities. The classification of this group of BVMOs into two clusters could be confirmed both with respect to stereospecificity as well as with respect to substrate acceptance.

All preparative-scale experiments were carried out in shake flasks to demonstrate the principal applicability of the methodology for accessing chiral lactone products. While such experiments may be limited in fermentation volume to a low 100 mg scale, up-scaling of microbial Baeyer−Villiger oxidations was reported in recent contributions utilizing advanced fermentation techniques to demonstrate the potential of this technology for the production of multigram quantities of optically pure lactones for subsequent applications in bioactive compound and natural product synthesis.[32]

Experimental Section

General Procedures for Thermal Cyclization. Compound **1** and the corresponding olefin **2a** (20 equiv) or **2b**−**e** (5 equiv) were heated neat (**3a**−**c**)[13,33] or in solution with toluene (**3d**,**e**).[21,34] After complete conversion and eventual removal of solvent, products were optionally purified by Kugelrohr distillation (**3a**,**b**,**d**).

General Procedures for Microwave-Assisted Cyclization. Compound **1** and cyclopentene **2a** (20 equiv) were dissolved in dry THF (10% solution), and the reaction mixture was irradiated at 100 °C in a CEM Explorer PLS microwave synthesizer for 2.5 h (noninvasive IR sensor). Product **3a** was purified as described previously.

General Procedure for Dechlorination. Procedure A. The procedure of Gassman and Marshall for the preparation of 7,7-dimethoxynorbonene was followed in the case of **3a**−**c**.[33] A solution containing the corresponding tetrachloro compound in THF was added dropwise to a vigorously stirred and gently refluxing mixture of THF, sodium spheres (15.5 equiv), and *t*-butanol (5.7 equiv) over a period of 3 h under nitrogen. The mixture was heated at reflux for an additional 12 h. Then, the solution was filtered, water was added, and the mixture was extracted with Et_2O. The combined ethereal extracts were washed twice with saturated NaCl and once with water, dried over Na_2SO_4, and concentrated.

Procedure B. Dechlorination of **3d**,**e** was carried out under Birch conditions according to literature procedures.[17,18] In a 1 L four-necked round-bottomed flask fitted with a condenser for gaseous ammonia, deep-temperature thermometer, a flask for adding solid compounds, and an outlet for gases, liquid ammonia (200−300 mL) was collected while cooling with liquid nitrogen to −80 °C. Small pieces of freshly cut sodium were added to the reaction mixture until a permanent blue color appeared. A solution of tetrachloro compound **3d**,**e** (1.00 g) dissolved in THF (3 mL) and EtOH (1 mL) was added to the reaction mixture dropwise at −80 °C. The reaction mixture was stirred at this temperature for 1 h. Then, the reaction was quenched with NH_4Cl (0.20 g). Ammonia was allowed to evaporate, and crushed ice was added to the residue, which was extracted several times with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to obtain **4d**,**e**, which was used in the next step without further purification.

General Procedure for Catalytic Hydrogenation. Compounds **4c**−**e** and **5a**,**b** were dissolved in EtOAc, and Pd/C (10% Pd on C; 10% w/w) was added. Hydrogenation was carried out in a Parr apparatus at approximately 60−75 psi overnight at room temperature. The suspension was filtered through a pad of Celite and concentrated to give crude product. If necessary, purification was carried out by flash column chromatography.

General Procedures for Ketal Hydrolysis. Procedure A. Hydrolysis of ketals **4a**,**b** and **6c** was carried in the presence of catalytic amount of pTSA and dry acetone according to the procedure of Yadav and Sasmal.[19] pTSA (0.1 equiv) was added to a solution of the corresponding ketal in dry acetone, and the mixture was stirred at room temperature for 6 h. Acetone was removed on a rotary evaporator, and the residue was diluted with Et_2O. The ethereal layer was washed with aq $NaHCO_3$ solution, water, and brine and dried over Na_2SO_4. Concentration and chromatographic purification furnished products **5a**,**b** and **7c.**

Procedure B. Ketone **7f** was prepared in analogy to a literature protocol used for the hydrolysis of a related ketal utilizing 3 N HCl with dioxane.[22] A solution of **6f** (0.48 g, 1.76 mmol) dissolved in dioxane (60 mL) and 3 N HCl (15 mL) was heated at reflux overnight. The mixture was cooled to room temperature, diluted with brine, and extracted 3 times with EtOAc. The organic layers were washed with saturated bicarbonate and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2 = 1:50, LP/EtOAc = 3:1) to give **7f**[20] as a yellow oil (0.25g; 67% yield).

Procedure C. Ketal **6d** was deprotected using Amberlyst-15 in dry acetone.[23] Compound **6d** (0.30 g, 1.38 mmol) was dissolved in dry acetone, and Amberlyst-15 was added. The mixture was stirred

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overnight at room temperature. The ion-exchange resin was separated by filtration over a bed of Celite, and the solvent was evaporated. Crude product was purified by column chromatography (SiO_2 = 1:50, LP/EtOAc = 6:1), which gave pure **7g**[21] as a colorless solid in 53% yield.

***endo*-Tricyclo[5.2.1.0^{2,6}]decan-10-one (7a).** Ketone **7a** was prepared according to the general procedure for catalytic hydrogenation using *endo*-tricyclo[5.2.1.0^{2,6}]dec-8-en-10-one **5a** (1.00 g, 6.70 mmol) as the starting material. Crude product was purified by column chromatography (SiO_2, LP/EtOAc = 100:1) to give **7a**[35] as a yellow oil (0.86 g) in 85% yield. ^{1}H NMR ($CDCl_3$): δ 1.63−1.75 (m, 12H), 2.45−2.51 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 27.7 (t), 29.2 (t), 29.9 (t), 38.2 (d), 43.6 (d), 214.9 (s).

***endo*-Tricyclo[6.2.1.0^{2,7}]-undecan-11-one (7b).** Ketone **7b** was prepared according to the general procedure for catalytic hydrogenation using *endo*-tricyclo[6.2.1.0^{2,7}]undec-9-en-11-one (**5b**) (1.00 g, 6.2 mmol) as the starting material. The crude product was purified by column chromatography (SiO_2, LP/EtOAc = 100:1) to give **7b** as a yellow oil (0.89 g) in 87% yield. ^{1}H NMR ($CDCl_3$): δ 0.81−1.89 (m, 14H), 2.06−2.14 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 17.2 (t), 18.1 (t), 19.5 (t), 32.9 (d), 43.4 (d), 216.7 (s). Calcd C 80.44%, H 9.82%, found C 80.49%, H 9.43%.

(1α,4α,5β,8β)-1,2,3,4,4a,5,6,7,8,8a-Decahydro-dimethanonaphthalen-10-one (7c). Compound **6c** (1.71 g, 7.71 mmol) was converted according to the general procedure for ketal hydrolysis using pTSA. Chromatographic purification of crude product (SiO_2, LP/EtOAc = 100:1) provided **7c** as a colorless oil (0.85 g) in 63% yield. ^{1}H NMR ($CDCl_3$): δ 1.11−1.20 (m, 2H), 1.54−1.72 (m, 4H), 1.79−2.06 (m, 8H), 2.29−2.36 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 18.3 (t), 31.3 (t), 36.4 (t), 37.2 (d), 43.0 (d), 46.9 (d), 215.3 (s). Calcd C 81.77%, H 9.15%; found C 81.52%, H 9.12%.

General Procedures for Screening Experiments in Plates. Plates with either 12 or 24 wells were utilized. Each well (2 or 1 mL, respectively) was charged with LB_{amp} medium (5.0 g of peptone, 2.5 g of yeast extract, 5.0 g of NaCl, 500 mL of deionized water, 2 mL of ampicilline stock solution −(50 mg/mL)) and inoculated with 1% of an overnight preculture of recombinant *E. coli* overexpressing BVMOs from different bacterial origin. A plate was incubated at 120 rpm at 37 °C on an orbital shaker for 2 h. Isopropyl-β-D-galactopyranoside (IPTG) was added (final concentration of 0.025 mM) together with substrate (final concentration of 3−6 mM). The plate was shaken at room temperature. After 24 h, samples for GC measurement were taken (700 μL of culture was centrifuged, and the supernatant was extracted with 700 μL of EtOAc supplemented with a defined concentration of methyl benzoate as the internal standard).

General Procedures for Biotransformations on Preparative Scale. Fresh LB_{amp} medium (250 mL) was inoculated with 1% of an overnight preculture of recombinant *E. coli* strains overexpressing the appropriate BVMO 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 (100 mg) was added neat along with β-cyclodextrin (1 equiv). The culture was incubated at room temperature until GC showed complete conversion of the ketone (24−72 h).

General Procedure for Chemical Baeyer−Villiger Oxidation. mCPBA (1.5 equiv, 70% purity) was added to a solution of the corresponding ketone in dry dichloromethane (10% solution), and the mixture was stirred overnight. Complete conversion was determined by TLC and gas chromatography. Excess triethylamine was added, and the mixture was stirred for 15 min. Afterward, water was added, and the organic layer was separated. The aqueous layer was extracted 2 times with dichloromethane. The combined organic phases were washed with saturated bicarbonate and brine, dried over sodium sulfate, and evaporated

Oxa-tricyclo[5.2.2.0^{2,6}]undecan-9-one (8a). *endo*-Tricyclo[5.2.1.0^{2,6}]decan-11-one **7a** (100 mg, 0.67 mmol) was oxidized with *E. coli* overexpressing $CHMO_{Rhodo2}$ and $CHMO_{Brevi2}$ according to the general procedure. The crude product was purified via column chromatography (SiO_2, LP/EtOAc = 24:1), and **8a** was isolated as colorless crystals in the following yields: $CHMO_{Rhodo2}$ in 63% yield (70 mg) and $CHMO_{Brevi2}$ in 67% yield (74 mg). $[\alpha]_{20}^{D}$ ($CHCl_3$) ($CHMO_{Rhodo2}$): −17.30, c = 1.70 g/100 mL; $[\alpha]_{20}^{D}$ ($CHCl_3$) ($CHMO_{Brevi2}$): +13.04, c = 1.43 g/100 mL. mp: 78−80 °C. ^{1}H NMR ($CDCl_3$): δ 1.66−1.90 (m, 12H), 2.49−2.55 (m, 1H), 4.54−4.59 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 16.7 (t), 20.6 (t), 27.8 (t), 27.9 (t), 28.1 (t), 37.7 (d), 39.2 (d), 41.2 (d), 78.9 (d), 177.3 (s). Calcd C 72.26%, H 8.49%, found C 72.48%, H 8.78%.

(1α,4α,5α,8α)-1,4-Ethano-5,8-methano-1,4,4a,5,6,7,8,8a-octahydro-3H-2-benzopyran-3-one (8c). Tetracyclic ketone **7c** (100 mg, 0.57 mmol) was oxidized with *E. coli* overexpressing $CHMO_{Acineto}$ according to the general procedure. The crude product was purified by column chromatography (SiO_2, LP/EtOAc = 20:1), and **8c** (56 mg, 51% yield) was isolated as colorless crystals. mp: 52−54 °C. $[\alpha]_{20}^{D}$ ($CHCl_3$) ($CHMO_{Acineto}$): −39.63; c = 0.965 g/100 mL. ^{1}H NMR ($CDCl_3$): δ 1.10−1.22 (m, 3H), 1.58−2.25 (m, 11H), 2.63−2.68 (m, 1H), 4.61−4.66 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 18.4 (t), 22.7 (t), 30.3 (t), 30.6 (t), 34.7 (t), 36.2 (d), 37.9 (d), 38.9 (d), 42.1 (d), 46.3 (d), 78.6 (d), 176.8 (s). Calcd C 74.97%, H 8.39%; found C 74.88%, H 8.34%.

4,8-Dioxa-tricyclo[5.2.2.0^{2,6}]undecan-9-one (8g). 4-Oxatricyclo[5.2.1.0^{2,6}]decan-10-one **7g** (100 mg, 0.57 mmol) was oxidized with *E. coli* overexpressing $CHMO_{Acineto}$ and $CPMO_{Coma}$ according to the general procedure. The crude product was purified by column chromatography (SiO_2, LP/EtOAc = 3:1), and the colorless crystals were isolated in the following yields: $CHMO_{Acineto}$ in 53% yield (58 mg) and $CPMO_{Coma}$ in 49% yield (54 mg). $[\alpha]_{20}^{D}$ ($CHCl_3$) ($CHMO_{Acineto}$): −17.68, c = 0.57 g/100 mL; $[\alpha]_{20}^{D}$ ($CHCl_3$) ($CPMO_{Coma}$): +15.66, c = 0.49 g/100 mL. mp: 116−118 °C. ^{1}H NMR ($CDCl_3$): δ 1.55−2.13 (m, 4H), 2.54−2.58 (m, 1H), 2.64−2.80 (m, 2H), 3.49−3.65 (m, 2H), 3.78 (d, J = 10.2 Hz, 1H), 3.96 (d, J = 9.8 Hz, 1H), 4.56−4.60 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 17.3 (t), 21.1 (t), 38.1 (d), 38.6 (d), 41.3 (d), 69.2 (t), 70.5 (t), 77.9 (d), 175.9 (s). Calcd C 74.97%, H 8.39%; found C 74.88%, H 8.34%.

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Note Added after ASAP Publication. At the top of page 4, $CHMO_{Acineto}$ was missing from the sentence in the version published ASAP on November 15, 2007; the corrected version was published November 16, 2007.

Supporting Information Available: ^{1}H, ^{13}C, and DEPT NMR spectra of ketones **7a**−**c**,**h** and lactones **8a**−**c**,**f**−**h** and ^{1}H NMR spectra of previously reported compounds; CIF file for X-ray structure determination of **(−)-8g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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