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Access to Optically Pure 4- and 5-Substituted Lactones: A Case of Chemical−Biocatalytical Cooperation

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Optically pure or highly enantiomerically enriched 4- and 5-substituted lactones are rather difficult to obtain. Chemical or enzymatic syntheses alone are not particularly successful. A combination of chemical catalysis and biocatalysis, however, provides a convenient route to a variety of these useful chiral compounds. In this paper we describe the synthesis of several optically pure 4- and 5-substituted lactones obtained via whole cell-catalyzed Baeyer−Villiger oxidations of highly enantiomerically enriched 3-alkyl cyclic ketones. Such chiral ketones are readily accessed by recently developed copper-catalyzed asymmetric conjugate reductions of the corresponding enones. A very high proximal regioselectivity and complete chirality transfer was obtained by employing biological Baeyer−Villiger oxidations, using recombinant *E. coli* strains that overexpress cyclopentanone monooxygenase (CPMO). A comparative study showed that CPMO gives superior results to those obtained with cyclohexanone monooxygenase (CHMO) catalyzed oxidations.

Introduction

Chemical Baeyer−Villiger oxidations of racemic 3-substituted cyclic ketones are not particularly useful reactions since they generally lead to the formation of two enantiomeric pairs of regioisomeric lactones;[1,2] the corresponding transformations mediated by cyclohexanone monooxygenase (CHMO) or cyclopentanone monooxygenase (CPMO) are more selective.[3,4] We have observed in the past that the products of CHMO-catalyzed oxidations of 3-substituted cyclohexanones and cyclopentanones depended on the size of the substituents.[5,6] Thus CHMO cleanly converted the antipodes of 3-methyl and 3-ethyl cyclohexanones to divergent regioisomers.[5] Such divergent behavior of enantiomers was noted earlier by Furstoss and co-workers in CHMO-catalyzed oxidation of racemic bicyclo[3.2.0]heptenones and dihydrocarvone.[7] The corresponding oxidations of 3-methyl, ethyl, propyl, and allyl cyclopentanones were nonselective and produced racemic mixtures of both regioisomers.[6]

In the case of 3-cyclohexanones substituted with the propyl or butyl groups, and 3-cyclopentanones with chains longer than four carbon atoms, only the proximal regioisomers were formed, with modest enantioselectivity favoring (*R*) configuration for ϵ-caprolactones,[5] and no significant enantioselectivity for δ-valerolactones.[6] Thus, the access to optically pure 4- and 5-substituted lactones is not easy by either chemical or biological routes.

Since several synthetic methods have been developed to afford optically pure or highly enantioenriched ketones, the combination of chemical synthesis and enzymatic Baeyer−Villiger oxidation allows for regio- and enantioselective synthesis of 4- and 5-substituted lactones. Here we report the preparation of these compounds achieved through the cooperation of chemical and biological catalysis.

Results and Discussion

Chemical Baeyer−Villiger oxidations of 3-substituted cyclic ketones lead to the formation of two enantiomeric

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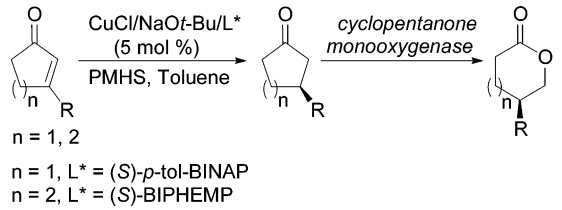
SCHEME 1



pairs of regioisomeric lactones. In contrast to 2-alkyl-substituted ketones, there is no electronic preference for migration of one of the two carbon−carbon bonds. We have reported earlier[5] that oxidations of 3-substitued cyclopentanones and cyclohexanones by CHMO-expressing engineered baker's yeast were more selective and produced one or the other regioisomer depending on the size of a substituent. However, at the outset of this study, the CPMO-catalyzed Baeyer−Villiger oxidations of 3-substituted cyclic ketones[5] were not known.

Our preliminary experiments with CPMO-catalyzed oxidations of several racemic substrates yielded the proximal regioisomer exclusively, regardless of the size of the tether. Unfortunately, no enantio-preference could be detected in any of the CPMO reactions. Although kinetic resolution[8] of 3-alkylcyclohexanones, particularly with longer alkyl chains, can be achieved in the course of the CHMO-catalyzed reactions, the process is tedious and the yields are low.[5,6] Furthermore, no kinetic resolution of 3-alkylcyclopentanones was observed.

Thus, neither of the two enzymes provides a convenient route to optically pure ϵ-caprolactones and δ-valerolactones from racemic 3-substituted cyclic ketones. An obvious way to circumvent this problem was to use optically pure or highly enantioenriched 3-alkyl cyclic ketones. Recently, the latter compounds became more readily available through enantioselective conjugate addition of organometallic reagents[9,10] to α,β-unsaturated cyclic ketones and asymmetric conjugate reduction of cyclic enones.[11] The marriage of chemical and biological catalytic methods provided a convenient route to optically pure 3- and 4-substituted lactones (Scheme 1).

The enantioenriched cyclic ketones[12] used in this study were prepared via asymmetric conjugate reduction of the corresponding enones. This recently developed method employs catalytic amounts of CuCl, NaO*t*-Bu, and a chiral bis-phosphine ligand with polymethylhydrosiloxane (PMHS), as a stoichiometric reductant, to generate a highly enantioselective catalyst for 1,4-reduction.[11]

The Baeyer−Villiger oxidations were performed with two engineered *E. coli* strains, one expressing cyclohexanone monooxygenase (CHMO)[13] and the other expressing cyclopentanone monooxygenase (CPMO).[14] The constructions of the *E. coli* strain expressing CHMO from *Acinetobacter* sp. NCIB 9871 and CPMO from *Comamonas* sp. NCIB 9872 have been described in detail elsewhere.[13,14] Both constructs have a strong promoter and produce an excess of the desired proteins when induced by adding isopropyl thio β-D-galactoside (IPTG) to the growth media. The maintenance of the recombinant organisms and the fermentations are simple to perform. All biotransformations discussed in this paper were carried out in a standard organic laboratory without specialized equipment.

All reactions, monitored by chiral-phase GC, were completed within 18−28 h. In the case of racemic compounds **4c** and **4e**, CHMO-catalyzed biotransformations were allowed to proceed to ca. 50% conversion to assess the degree of kinetic resolution obtainable in isolated products. The parallel reactions mediated by CPMO were allowed to reach 100% conversion since no enantiopreference was observed in the course of these transformations. Control experiments in which the host strains (*E.coli* BL21 and *E.coli* DH5α) were used instead of the plasmid-containing strains were run routinely and showed no lactone formation, confirming that CHMO or CPMO was indeed responsible for the observed Baeyer−Villiger reactions.

In contrast to CHMO-mediated Baeyer−Villiger oxidations of ketones to lactones, small quantities of corresponding hydroxy acids were obtained when CPMO catalyst was used, resulting in lower isolated yields of the desired products.[15] The control experiments with two *E. coli* carrier strains [DH5α] vs [BL21] showed that the *E. coli* [DH5α] contains an enzyme or enzymes that can slowly hydrolyze the proximal valerolactones but not caprolactones. There was no lactone hydrolysis observed with the *E. coli* [BL21] strain.

In addition to compounds listed in Tables 1 and 2, two other optically pure 3-substituted cyclopentanones (*S*)-**4g** and (*S*)-**4h** were tested as possible substrates (Figure 1). In both cases no reaction was observed after 24 h of fermentation with either CHMO or CPMO, and ketones were recovered unchanged.

As in the "designer yeast"- mediated reactions reported earlier,[5] the *E. coli* (CHMO)-catalyzed oxidation of (*R*)-3-methylcyclohexanone **1a** gave exclusively the proximal lactone **2a** while its antipode (*S*)-**1a** was converted to the distal lactone **3a**. Highly enantiomerically enriched (*R*)-**1b** (81% ee) and (*S*)-**1c** (83% ee) yielded (*R*)-**2b** (94% ee) and (*S*)-**2c** (56% ee), respectively. The results obtained

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(10) For recent examples of enantioselective conjugate addition to cyclic enones, see: (a) Arnold, L. A.; Imbos, R.; Mandoli, A.; de Vries, A. H. M.; Naasz, R.; Feringa, B. L. *Tetrahedron* **2000**, *56*, 2865−2878. (b) Escher, I. H.; Pfaltz, A. *Tetrahedron* **2000**, *56*, 2879−2888. (c) Chataigner, I.; Gennari, C.; Ongeri, S.; Piarulli, U.; Ceccarelli, S. *Chem. Eur. J.* **2001**, *7*, 2628−2634. (d) Degrado, S. J.; Mizutani, H.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2001**, *123*, 755−756. (e) Reetz, M. T.; Gosberg, A.; Moulin, D. *Tetrahedron Lett.* **2002**, *43*, 1189−1191.

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(12) Compounds (*R*)-**1b** and (*R*)-**4b** were a gift from Drs. Escher and Pfaltz, who synthesized them via 1,4-additions of organozinc reagents to cyclic enones. See ref 10b.

(13) Mihovilovic, M. D.; Chen, G.; Wang, S.; Kyte, B.; Rochon, F.; Kayser, M. M.; Stewart, J. D. *J. Org. Chem.* **2001**, *66*, 733−738.

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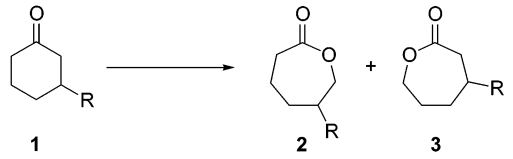
(15) The hydroxy acids were not detectable on GC since at high temperature they undergo condensation to yield the corresponding lactones.

TABLE 1. CHMO- and CPMO-Catalyzed Oxidations of 3-Alkylcyclohexanones

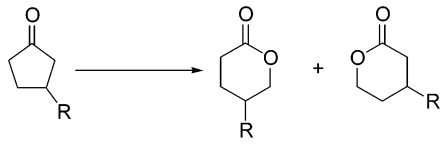


1 **2** **3**

		E. coli CHMO			*E. coli* CPMO		
			2:**3** ratio			**2**:**3** ratio	
R	reactant (abs configur)	conv, % (isolated yield, %)	**2** (% ee)[a]	**3** (% ee)	conv, % (isolated yield, %)	**2** (% ee)	**3** (% ee)
Me	(*R*)-**1a** (100 % ee)	100 (77)	>99 (100 *R*)	–	100 (75)	>99 (100 *R*)	–
Me	(*S*)-**1a** (100 % ee)	100 (60)	–	99 (100 *S*)	ND	ND	
Et	(*R*)-**1b** (81 % ee)	100 (89)	94 (94 *R*)	6 (>99 *S*)	100 (87)	>99 (80 *R*)	–
n-Bu	(*S*)-**1c** (83 % ee)	38 (ND)	>99 (56 *S*)	–	100 (85)	>99 (83 *S*)	–

[a] The ee of starting materials and products were determined by chiral GC.

TABLE 2. CHMO- and CPMO-Catalyzed Oxidations of 3-Alkylcyclopentanones

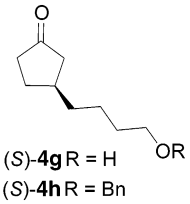


4 **5** **6**

		E. coli CHMO			*E. coli* CPMO		
			5:**6** ratio			**5**:**6** ratio	
R	reactant (abs configur)	conv, % (isolated yield, %)	**5** (% ee)[a]	**6** (% ee)	conv, % (isolated yield, %)	**5** (% ee)	**6** (% ee)
Me	(*R,S*)-**4a** (racemic)	100 (95)	13 (9 *R*)	87 (36 *S*)	100 (68)	>99 (racemic)	–
Me	(*R*)-**4a** (>99% ee)	100 (88)	10 (99 *R*)	90 (99 *R*)	100 (62)	100 (>99 *R*)	–
Et	(*R,S*)-**4b** (racemic)	86 (80)	20 (12 *R*)	80 (32 *S*)	100 (78)	100 (racemic)	–
Et	(*R*)-**4b** (86% ee)	ND	ND		100 (69)	>99 (86 *R*)	–
n-Pr	(*R,S*)-**4c** (racemic)	53 (44)[b]	83 (60 *S*)	17 (33 *R*)	100 (70)	>99 (racemic)	–
i-Pr	(*S*)-**4d** (87% ee)	65 (58)[c]	33 (97 *R*)	67 (93 *S*)	100 (75)	>99 (87 *R*)	–
n-Bu	(*R,S*)-**4e** (racemic)	44 (34)[d]	>99 (38 *S*)	–	100 (76)	>96 (racemic)	–
n-Bu	(*S*)-**4e** (90% ee)	100 (88)	95 (90 *S*)	5	95 (84)	>99 (92 *S*)	–
$(CH_2)_2Ph$	(*S*)-**4f** (88% ee)	81 (80)[e]	>99 (99 *S*)	–	100 (80)	>99 (88 *S*)	–

[a] The ee values of starting materials and products were determined by chiral GC. [b] The remaining ketone was 25% ee (*R*). The absolute configuration in this case was tentatively assigned by comparing the chiral GC traces of the *n*-propyl-substituted ketones and lactones with those with 3-butyl substitution. [c] The remaining ketone was 84% ee. [d] The remaining ketone was 25% ee (R). [e] The remaining ketone was 42% ee.

are consistent with our diamond lattice-active site model for cyclohexanone monooxygenase.[5] This model, illustrated in Figure 2, incorporates effects of the conformational equilibrium of a substrate within the active site on enantioselectivity of CHMO-catalyzed reactions.[5,6] Here, for example, while 3-ethyl substituted (*R*)-**1b** (81% ee) is converted completely and rapidly to a mixture of lactones (*R*)-**2b** and (*S*)-**2b**, both of higher optical purity than the substrate, the 3-*n*-butyl-substituted ketone (*S*)-**1c** (83% ee) reacts slowly (38% conversion after 24 h) to give the proximal lactone (*S*)-**2c** with diminished ee (56% ee). The remaining unreacted ketone (*S*)-**2b** is significantly enantiomerically enriched (99% ee vs 83% ee) in the process.



(*S*)-**4g** R = H
(*S*)-**4h** R = Bn

FIGURE 1. Compounds (*S*)-**4g** and (*S*)-**4h** are not suitable substrates for CHMO and CPMO.

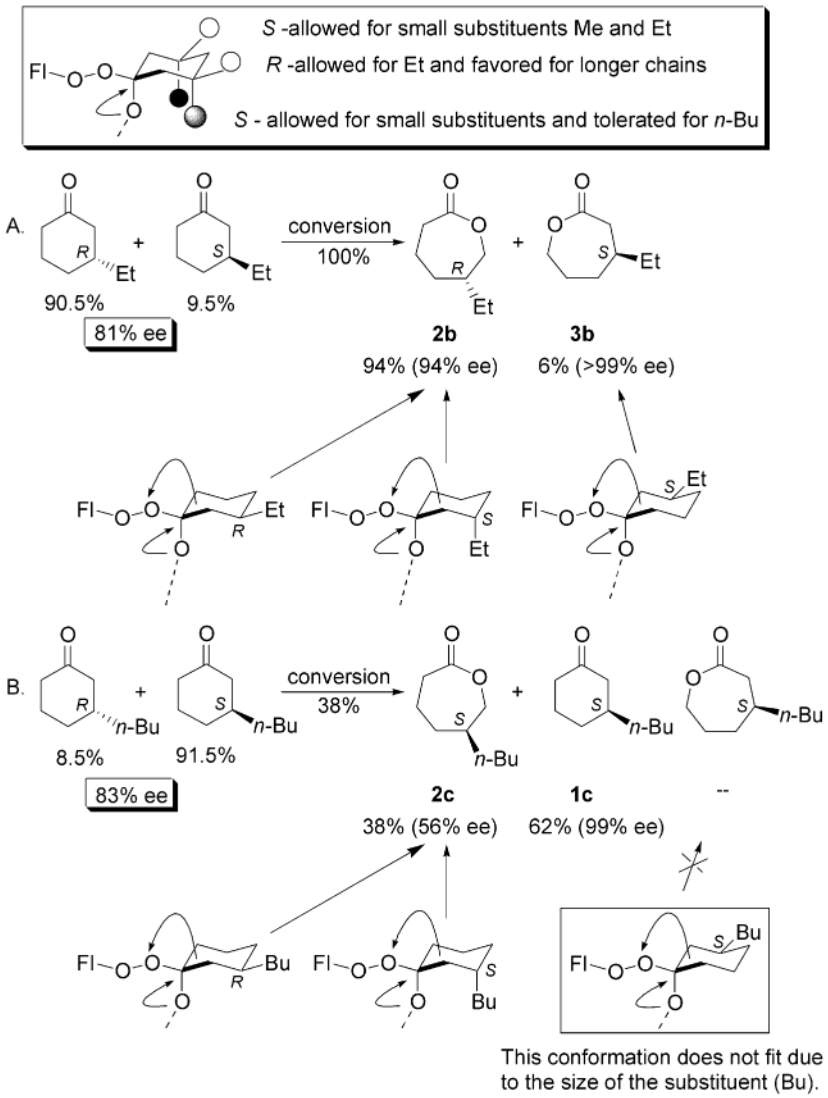


FIGURE 2. Diamond lattice active site model for cyclohexanone monooxygenase (CHMO). (A) According to the model, the major (*R*) enantiomer (90.5%) in equatorial conformation is the preferred (faster reacting) substrate and yields enantioselectively enriched lactone **2b** during fermentation. The antipode (*S*) (9.5%) is preferentially converted to the distal lactone **3b**, which is also enantiomerically enriched in the process. (B) In the case of 3-*n*-butylcyclohexanone, the (*S*)-enantiomer is disfavored. The *n*-butyl chain in the equatorial position (see box) does not fit into the enzyme's active site pocket. Conversely, the *n*-butyl group in the axial position is conformationally disfavored and reacts more slowly than the (*R*)-enantiomer. As a result, only 38% conversion is achieved after 24 h. The minor enantiomer (*R*) reacts faster and is completely converted to the lactone during that time. This process results in enantiomeric enrichment of the remaining (*S*)-ketone; the optical purity of the lactone, however, is diminished.

Compared to 3-alkylcyclohexanones, 3-alkylcyclopentanones show lower selectivity. This behavior has been linked to the near equivalence of pseudoaxial and pseudoequatorial conformations.[6] In the present study, similar effects are evident. The conformational considerations may apply but the effects are weak due to the intrinsic flexibility of five-member rings and the resulting small differences in energy between axial and equatorial substituents. The lack of such preferences is clearly evident in the case of conversions of (*S*)-**4d** to (*R*)-**5d** and (*S*)-**4f** to (*S*)-**5f**, where both lactones have higher optical purity than the original ketones. This may be the result of favorable stabilizing interactions between the substituents and the active site of the enzyme.

In contrast to CHMO, the selectivity of CPMO in the Baeyer−Villiger oxidations of 3-substituted cyclic ketones has not been previously investigated and there is no active site model available at present. The Baeyer−Villiger oxidations with CPMO (whole cells, partly purified enzyme, or an overexpression *E. coli* system) indicate that transformations of 2- and 4-alkyl-substituted cyclic ketones tend to be less enantioselective than the corresponding reactions performed with CHMO.[15,16] The notable exceptions are prochiral bicyclo[4.3.0]ketones which upon fermentation with CHMO produce (+) lactones in excellent enantiomeric excess (>99%).[17] CPMO-mediated oxidations of 3-substituted cyclohexanones (Table 1) and cyclopentanones (Table 2) are usually faster than the corresponding CHMO transformations and reach 100% conversion in less than 28 h.

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(17) Mihovilovic M. D.; Müller, B.; Kayser, M. M.; Stanetty, P. *Synlett* **2002**, 700−703.

All substrates investigated, irrespective of their absolute configurations, were converted to the corresponding proximal lactones. However, the products formed were racemic and no enantiopreference was observed in any of the transformations. The chiral phase GC analyses of samples collected during the reactions showed that both enantiomers were converted at approximately the same rate. This apparent disadvantage of CPMO-catalyzed oxidations is circumvented when optically pure ketones are used and is compensated by a very high and predictable regioselectivity of these reactions.

Conclusion

Although CHMO and CPMO-catalyzed Baeyer−Villiger oxidations of racemic 3-alkyl-substituted cyclohexanones, and particularly 3-alkyl-substituted cyclopentanones alone, do not provide convenient high-yielding routes to optically pure lactones, their high and predictable regioselectities are complementary when optically pure or enantiomerically enriched ketones, prepared via chemical enatioselective catalysis, are available. The combination of two catalytic methodologies, therefore, allows the synthesis of rather difficult to obtain but highly desirable chiral 4- and 5-substituted lactones.

Experimental Section

General Consideration. Toluene, THF, and Et_2O were purchased from J. T. Baker in CYCLE-TAINER solvent delivery kegs, which were vigorously purged with argon for 2 h. Toluene was further purified by passing through two packed columns of neutral alumina and copper(II) oxide under argon pressure. THF and Et_2O were further purified by passing through a column of neutral alumina.[18] For biocatalytical oxidations, all solvents were purified by fractional distillation. For asymmetric conjugate reductions, CuCl (99.995%) was purchased from Strem and NaO*t*-Bu was purchased from Aldrich and stored in a nitrogen-filled drybox. PMHS was purchased from Aldrich, (*S*)-*p*-tol-BINAP was purchased from Strem. All other reagents were available from commercial sources and were used without further purification, unless otherwise noted. All manipulations involving air-sensitive materials were conducted in a Vacuum Atmospheres drybox under an atmosphere of nitrogen. Unless otherwise stated, all reactions were conducted in flasks sealed with a rubber septum under a positive pressure of argon. Analytical thin-layer chromatography was performed using E.M. Reagents 0.25 mm silica gel 60 plates. Flash chromatography was performed on E.M. Science Kieselgel 60 (230−400 mesh). Yields refer to isolated yields of compounds of greater than 95% purity as estimated by capillary GC and ^{1}H NMR. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-200, or a Varian Mercury 300, or a Varian Unity 300, or a Brucker AMX-400FT-NMR, or a Varian Inova 500 spectrometer. Splitting patterns are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; td, triplet of doublets; q, quartet; qd, quartet of doublets; m, multiplet. Infrared (IR) spectra were recorded on an Asi Applied Systems ReactIR 1000 (liquids and solids were measured neat on a DiComp probe) or Mattson Satellite FT-IR spectrometer. Gas chromatography (GC) analyses were performed on a Hewlett-Packard 6890 gas chromatograph with an FID detector, using a 25 m × 0.20 mm or a 30 m × 0.32 mm capillary column with cross-linked methyl siloxane as a stationary phase. All new compounds were characterized by ^{1}H NMR, ^{13}C NMR, and IR spectroscopy, in addition to high-resolution mass spectra (Finnegan MAT System 8200 spectrometer). Chiral GC analyses were performed on a Shimadzu GC-9A gas chromatograph, using a Supelco Inc. FILM β-Dex225 column.

General Procedure for Copper-Catalyzed Asymmetric Conjugate Reductions. A chiral bisphosphine (0.1 mmol) was placed in an oven-dried Schlenk flask and dissolved in toluene (2.0 mL). The Schlenk flask was then moved into a nitrogen-filled drybox. In the drybox, NaO*t*-Bu (10 mg, 0.1 mmol) and CuCl (10 mg, 0.1 mmol) were weighed into a vial. The toluene solution of chiral bisphosphine was added via pipet to the vial to dissolve the solids and the resulting solution was then transferred back into the Schlenk flask. The Schlenk flask was removed from the drybox, the solution was stirred for 10−20 min, and PMHS (0.126 mL, 2.1 mmol) was added to the solution under argon purge and stirred for 10 min at room temperature. The resulting reddish orange solution was then cooled to 0 °C. The solution of cyclic enone (2.0 mmol) in 2.5 mL of toluene was added (on the walls of the Schlenk flask) to the reaction solution. The reaction was stirred for the indicated period of time. Consumption of cyclic enone was monitored by GC. The catalyst-free filtrate was concentrated and analyzed by GC to determine the percent conversion. The Schlenk flask was opened and water (2 mL) was added. The resulting solution was diluted with diethyl ether, washed once with brine, and back extracted with diethyl ether. To the combined organic extracts was added TBAF (2.0 mL, 1.0 M in THF) and the resulting solution was stirred for 1 h. The solution was then washed once with brine and back extracted with diethyl ether and the organic layer was dried over anhydrous $MgSO_4$. The solvent was removed in vacuo and the enantiomerically enriched starting material was purified by silica column chromatography and analyzed by chiral GC to determine the enantiomeric excess.

Precursors to asymmetric conjugate reductions, 3-alkyl cyclic enones, were prepared by literature reported procedures.[19]

3-(*S*)-*n*-Butylcyclohexanone (1c). Following the General Procedure for the asymmetric conjugate reduction with (*S*)-BIPHEMP and 3-butylcyclohexenone (0.304 g, 2.0 mmol) gave, after 2 d at 0 °C and flash chromatography (8:1 hexanes:ethyl acetate), the title compound as a clear oil (0.262 g, 85% yield). Spectroscopic data for the title compound were consistent with the previously reported data for this compound.[20] For ee determination see the procedure for **2c**.

3-(*R*)-Isopropylcyclopentanone (4d). Following the General Procedure for the asymmetric conjugate reduction with (*S*)-*p*-tol-BINAP and 3-isopropylcyclopentenone (0.248 g, 2.0 mmol) gave, after 4 d at 0 °C and flash chromatography (10:1 pentane:diethyl ether), the title compound as a clear oil (0.219 g, 87% yield). Spectroscopic data for the title compound were consistent with the previously reported data for this compound.[21] For ee determination see the procedure for **6d**.

3-(*S*)-*n*-Butylcyclopentanone (4e). Following the General Procedure for the asymmetric conjugate reduction with (*S*)-*p*-tol-BINAP and 3-butylcyclopentenone (0.276 g, 2.0 mmol) gave, after 12 h at 0 °C and flash chromatography (20:1 hexanes:ethyl acetate), the title compound as a clear oil (0.260 g, 93% yield). Spectroscopic data for the title compound were consistent with the previously reported data for this compound.[22] For ee determination see the procedure for **5e**.

3-(*S*)-Phenethylcyclopentanone (4f). Following the General Procedure for the asymmetric conjugate reduction with (*S*)-*p*-tol-BINAP and 3-phenethylcyclopentenone (0.372 g, 2.0

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mmol) gave, after 24 h at 0 °C and flash chromatography (10:1 hexanes:ethyl acetate), the title compound as a clear oil (0.323 g, 86% yield). Spectroscopic data for the title compound were consistent with the previously reported data for this compound.[23] For ee determination see the procedure for **5f**.

Propagation of *E. coli* Strains. The *E. coli* strain BL21(DE3)(pMM04) was streaked from a frozen stock on LB-Ampicillin plates and incubated at 30 °C until colonies were 1−2 mm in size. One colony was used to inoculate 10 mL of an LB-Ampicillin medium in a 50-mL Erlenmeyer flask and incubated at 30 °C, 250 rpm overnight. Sterile glycerol (15% v/v) was added and the mixture was divided into 0.5-mL aliquots and stored in a −80 °C freezer. The propagation of the *E. coli* DH5α(pCMP201) was carried out in the same way. The carrier strains BL21(DE3) and DH5α were propagated by using the above protocol except that no ampicillin was used in the plates and the medium.

Protocol for *E. coli*-Mediated Reactions. A saturated culture of *E. coli* was prepared as above. This culture was used at a 1:100 ratio to inoculate an LB-Ampicillin medium supplemented with 10% glucose in a baffled Erlenmeyer flask. The culture was incubated at 30 °C, 250 rpm until OD_{600} was approximately 0.6. IPTG stock solution (0.84 M/L, 200 mg/mL) was added (0.1 μL per mL of medium) followed by the substrate. If cyclodextrin was necessary to alleviate the solubility problem, it was introduced at this stage. The culture was agitated at 30 °C at 250 rpm and monitored by GC or TLC until the reaction was finished. The culture was saturated with NaCl and extracted with ethyl acetate. Combined extracts were washed once with $NaHCO_3$ (1%) and NaCl brine and dried over anhydrous Na_2SO_4 or $MgSO_4$. The solvent was removed on a rotary evaporator and the residue was purified by flash chromatography.

(*R*)-6-Methyl-2-oxepanone ((*R*)-2a).[5] Biotransformation according to the general protocol of (*R*)-3-methyl cyclopentanone (0.050 g, 0.45 mmol, chiral GC analysis with a Shimadzu GC-9A column indicated >99% ee) mediated by *E. coli* (CPMO) gave, after 20 h and flash chromatography (9:1 hexanes:ethyl acetate), the title compound as colorless oil (0.043 g, 75% yield). Chiral GC analysis with a Shimadzu GC-9A column indicated >99% ee. $[\alpha]_D$ −33 (*c* 1.8, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 (1H, dt, *J* = 12, 1.2 Hz), 4.00 (1H, dd *J* = 12, 8 Hz), 2.65 (1H, t, *J* = 3 Hz), 2.61 (1H, t, *J* = 16 Hz), 1.94 (1H, m), 1.86 (1H, m), 1.67 (1H, m), 1.38 (2H, m), 0.98 (3H, d, *J* = 20 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 73.9, 36.9, 34.4, 33.8, 21.6, 17.7 ppm.

(*R*)-6-Ethyl-2-oxepanone ((*R*)-2b).[5] Biotransformation according to the general protocol of (*R*)-3-ethylcyclohexanone (0.093 g, 0.74 mmol, chiral GC analysis with a Shimadzu GC-9A column indicated 81% ee) mediated by *E. coli* (CPMO) gave, after 24 h and flash chromatography (9:1 hexanes:ethyl acetate), the title compound as a colorless oil (0.085 g, 87% yield). Chiral GC analysis with a Shimadzu GC-9A column indicated 81% ee. $[\alpha]_D$ −26 (*c* 2.1, CH_2Cl_2). IR (neat) ν_{max} 2961, 2876, 1734, 1462, 1169 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.11 (1H, d, *J* = 1.2 Hz), 4.01 (1H, dd, *J* = 12.6, 7.8 Hz), 2.59 (2H, t, *J* = 4.8 Hz), 1.87 (2H, m), 1.63 (2H, m), 1.41 (1H, m), 1.30 (2H, m), 0.92 (3H, t, *J* = 7.4 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 72.7, 40.5, 34.6, 34.5, 24.7, 21.5, 11.6 ppm.

(*S*)-6-*n*-Butyl-2-oxepanone ((*S*)-2c).[5] Biotransformation according to the general protocol of (*S*)-3-*n*-butylcyclohexanone (0.050 g, 0.32 mmol, chiral GC analysis with a Shimadzu GC-9A column indicated 83% ee) mediated by *E. coli* (CPMO) gave, after 28 h and flash chromatography (9:1 hexanes:ethyl acetate), the title compound as a colorless oil (0.047 g, 85% yield). Chiral GC analysis with a Shimadzu GC-9A column indicated 83% ee. $[\alpha]_D$ 2.7 (*c* 1.0, CH_2Cl_2). IR (neat) ν_{max} 2927, 1735, 1458, 1352, 1273, 1165, 1048 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.14 (1H, d, *J* = 8.0 Hz), 4.00 (1H, dd, *J* = 8.5, 5.0 Hz), 2.60 (2H, m), 1.88 (2H, m), 1.75 (1H, m), 1.63 (2H, m), 1.41 (2H, m), 1.26 (4H, m), 0.90 (3H, t, *J* = 3.0 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2, 72.6, 38.6, 34.9, 34.3, 31.3, 29.2, 22.6, 21.3, 14.0 ppm.

(*R*)-5-Methyl Tetrahydropyran-2-one ((*R*)-5a).[24] Biotransformation according to the general protocol of (*R*)-3-methyl cyclopentanone (0.100 g, 1.02 mmol, chiral GC analysis with a Shimadzu GC-9A column indicated >99% ee) using *E. coli* (CPMO) gave, after 28 h and flash chromatography (4:1 hexanes:ethyl acetate), the title compound as a pale yellow oil (0.072 g, 62% yield). Chiral GC analysis with a Shimadzu GC-9A column indicated >99% ee. $[\alpha]_D$ 17.0 (*c* 0.72, CH_2Cl_2). IR (neat) ν_{max} 2962, 2880, 1732, 1462, 1262, 1177, 1113, 994, 777 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.31 (1H,m), 3.91 (1H, t, *J* = 10 Hz), 2.63 (1H, m), 2.51 (1H, m), 2.07 (1H, m), 1.98 (1H, m), 1.55 (1H, m), 1.01 (3H, d, *J* = 6.6 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 74.8, 29.1, 27.9, 27.5, 16.5 ppm.

(*R*)-5-Ethyl Tetrahydropyran-2-one ((*R*)-5b).[25] Biotransformation according to the general protocol of (*R*)-3-ethyltetrahydronpyran-2-one (0.050 g, 0.45 mmol, chiral GC analysis with a Shimadzu GC-9A column indicated 84% ee) mediated by *E. coli* (CPMO) gave, after 18 h and flash chromatography (7:1 hexanes:ethyl acetate), the title compound as a pale yellow oil (0.030 g, 69% yield). Chiral GC analysis with a Shimadzu GC-9A column indicated 84% ee. $[\alpha]_D$ −2.4 (*c* 2.3, CH_2Cl_2). IR (neat) ν_{max} 2967, 2927, 1743, 1262, 1223, 1071 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34 (1H, ddd, *J* = 11, 9, 1 Hz), 4.00 (1H, m), 2.63 (1H, ddd, *J* = 17, 6, 2 Hz), 2.51 (1H, m), 2.02 (1H, m), 1.87−1.60 (2H, m), 1.36 (2H, m), 0.97 (3H, t, *J* = 7.5 Hz) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ 174.0, 73.6, 34.7, 29.9, 25.3, 24.7, 11.5 ppm.

(±)-5-*n*-Propyl Tetrahydropyran-2-one (5c).[6] Biotransformation according to the general protocol of (±)-3-*n*-propylcyclopentanone (0.092 g, 0.73 mmol) mediated by *E. coli* (CPMO) gave, after 24 h and flash chromatography (9:1 hexanes:ethyl acetate), the title compound as a colorless oil (0.079 g, 70% yield). IR (neat) ν_{max} 2960, 2927, 2875, 1743, 1190, 1052 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.31 (1H, ddd, *J* = 15.6, 4.5, 1.9 Hz), 3.91 (1H, t, *J* = 9.7 Hz), 2.56 (1H, m), 2.30 (1H, m), 1.94 (2H, m), 1.51 (1H, m), 1.30 (4H, m), 0.89 (3H, t, *J* = 6.8 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 73.6, 33.6, 32.5, 29.0, 25.4, 19.9, 14.0 ppm.

(*R*)-5-Isopropyl Tetrahydropyran-2-one ((*R*)-5d).[26] Biotransformation according to the general protocol of (*S*)-3-isopropyl cyclopentanone (0.050 g, 0.35 mmol, chiral GC analysis with a Shimadzu GC-9A column indicated 87% ee) mediated by *E. coli* (CPMO) gave, after 20 h and flash chromatography (9:1 hexanes:ethyl acetate), the title compound as a colorless oil (0.038 g, 68% yield). Chiral GC analysis with a Shimadzu GC-9A column indicated 87% ee. $[\alpha]_D$ 37 (*c* 3.8, $CHCl_3$). IR (neat) ν_{max} 2963, 2877, 1739, 1269, 1123, 1048, 772 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.38 (1H, dq, *J* = 18.5, 2.5 Hz), 4.06 (1H, dd, *J* = 12.5, 10 Hz), 2.52 (2H, m), 1.96 (1H, m), 1.80−1.52 (3H, m), 0.96 (6H, d, *J* = 7.5 Hz) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ 172.0, 72.2, 42.6, 39.1, 29.2, 22.7, 20.0, 19.7 ppm.

(*S*)-5-*n*-Butyl Tetrahydropyran-2-one ((*S*)-5e).[6] Biotransformation according to the general protocol of (*S*)-3-*n*-butyl cyclopentanone (0.048 g, 0.34 mmol, chiral GC analysis with a Shimadzu GC-9A column indicated >95% ee) mediated by *E. coli* (CPMO) gave, after 28 h and flash chromatography (7:1 hexanes:ethyl acetate), the title compound as a colorless oil (0.045 g, 84% yield). Chiral GC analysis with a Shimadzu GC-9A column indicated >95% ee. $[\alpha]_D$ −1.6 (*c* 2.3, CH_2Cl_2). IR (neat) ν_{max} 2928, 1734, 1457, 1169 cm^{-1}. ^{1}H NMR (400 MHz,

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$CDCl_3$) δ 4.32 (1H, ddd, J = 11, 5, 2 Hz), 3.93 (1H, t, J = 10 Hz), 2.59 (1H, m), 2.46 (1H, m), 1.98 (1H, m), 1.87 (1H, m), 1.50 (1H, m), 1.28 (6H, m), 0.87 (3H, t, J = 6.0 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 73.8, 32.8, 31.2, 29.0, 28.9, 25.5, 22.7, 13.9 ppm.

(*S*)-5-Phenylethyl Tetrahydropyran-2-one ((*S*)-5f). Biotransformation according to the general protocol of (*S*)-3-phenethyl cyclopentanone (0.030 g, 0.16 mmol, chiral GC analysis with a Shimadzu GC-9A column indicated 92% ee) mediated by *E. coli* (CHMO) gave, after 24 h and flash chromatography (7:1 hexanes:ethyl acetate), the title compound as a colorless oil (0.026 g, 80% yield). Chiral GC analysis with a Shimadzu GC-9A column indicated >95% ee. $[\alpha]_D$ −0.3 (*c* 2.3, CH_2Cl_2). IR (neat) ν_{max} 3026, 2928, 2861, 1732, 1603, 1454, 1244, 1179, 1054, 750, 700 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (2H, dd, J = 15, 7.5 Hz), 7.19 (3H, dd, J = 7.2, 2.0 Hz), 4.35 (1H, m), 3.99 (1H, t, J = 10 Hz), 2.68 (2H, m), 2.51 (1H, m), 2.04 (1H, m), 1.94 (1H, m), 1.66 (4H, m) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 141.2, 128.6, 128.2, 126.2, 73.3, 33.3, 33.0, 32.3, 28.9, 25.4 ppm. HRMS (EI, *m/e*) calcd for $C_{13}H_{16}O_2$: 204.1145 (M^+). Found: 204.1148.

(*S*)-4-Isopropyl Tetrahydropyran-2-one ((*S*)-6d).[27] Biotransformation according to the general protocol of (*R*)-3-isopropyl cyclopentanone (0.050 g, 0.35 mmol, chiral GC analysis with a Shimadzu GC-9A column indicated 87% ee) mediated by *E. coli* (CHMO) gave, after 24 h and flash chromatography (9:1 hexanes:ethyl acetate), the title compound as the major product (2:1, **6d**:**5d** as determined by GC) as a colorless oil (0.033 g, 60% yield). Chiral GC analysis with a Shimadzu GC-9A column indicated 93% ee for **6d** and 97% ee for **5d**. $[\alpha]_D$ −21 (*c* 2.8, $CHCl_3$). IR (neat) ν_{max} 2961, 2876, 1731, 1468, 1403, 1259, 1179, 1083 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.38 (1H, m), 4.23 (1H, ddd, J = 11, 10, 2.5 Hz), 2.62 (1H, m), 2.23 (1H, dd, J = 18, 10 Hz), 1.96 (1H, m), 1.80−1.53 (3H, m), 0.94 (3H, d, J = 1.5), 0.91 (3H, d, J = 1.5 Hz) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ 172.1, 68.7, 37.8, 34.0, 32.3, 26.3, 19.2, 19.1 ppm.

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