Asymmetric Baeyer−Villiger Oxidations of 4-Mono- and 4,4-Disubstituted Cyclohexanones by Whole Cells of Engineered *Escherichia coli*

Marko D. Mihovilovic,[†,‡] Gang Chen,[‡,§] Shaozhao Wang,[‡,§] Brian Kyte,[||] Fernande Rochon,[⊥] Margaret M. Kayser,*[,§] and Jon D. Stewart*[,||,#]

Departments of Chemistry, University of New Brunswick, P.O. Box 5050, Saint John, NB E2L 4L5, Canada, University of Florida, P.O. Box 117200, Gainesville, Florida 32611, and Université du Québec à Montréal, Montréal, Québec H3C 3P8, Canada

jds2@chem.ufl.edu

Received August 28, 2000

Whole cells of an *Escherichia coli* strain that overexpresses *Acinetobacter* sp. NCIB 9871 cyclohexanone monooxygenase have been used for the Baeyer−Villiger oxidations of a variety of 4-mono- and 4,4-disubstituted cyclohexanones. In cases where comparisons were possible, this new biocatalytic reagent provided lactones with chemical yields and optical purities that were comparable to those obtained from the purified enzyme or a strain of bakers' yeast that expresses the same enzyme. The efficient production of cyclohexanone monooxygenase in the *E. coli* expression system (ca. 30% of total soluble protein) allowed these oxidations to reach completion in approximately half the time required for the engineered bakers' yeast strain. Surprisingly, 4,4-disubstituted cyclohexanones were also accepted by the enzyme, and the enantioselectivities of these oxidations could be rationalized by considering the conformational energies of bound substrates along with the enzyme's intrinsic enantioselectivity. The enzyme expressed in *E. coli* cells also oxidized several 4-substituted cyclohexanones bearing polar substituents, often with high enantioselectivities. In the case of 4-iodocyclohexanone, the lactone was obtained in >98% ee and its absolute configuration was assigned by X-ray crystallography. The crystal belongs to the monoclinic $P2_1$ space group with $a = 5.7400(10)$, $b = 6.1650(10)$, $c = 11.377(2)$ Å, $b = 99.98(2)°$, and $Z = 2$. Taken together, these results demonstrate the utility of an engineered bacterial strain in delivering useful chiral building blocks in an experimentally simple manner.

Introduction

Asymmetric Baeyer−Villiger oxidations of substituted cyclohexanones provide a simple and attractive route to chiral ϵ-caprolactone building blocks that have found wide use in organic synthesis. Several reagents based on metal complexes have been described, and these provide lactones in high chemical and optical purities, although the range of substrates is somewhat limited.[1−5] Enzymes catalyzing asymmetric Baeyer−Villiger oxidations have also been described (for a recent review, see 6). These enzymes are usually part of degradative pathways that allow microorganisms to utilize non-carbohydrate compounds as sources of carbon and energy.[7,8] Interestingly, while their physiological role is associated with degradation of specific compounds, such enzymes often display remarkably broad substrate acceptance and high enantioselectivities. This combination of properties makes these Baeyer−Villiger enzymes ideal for applications to asymmetric organic synthesis. Cyclohexanone monooxygenase from *Acinetobacter* sp. NCIB 9871[9] is the best-known Baeyer−Villiger enzyme, and it has been shown to oxidize more than 80 different ketones, often with very high enantioselectivities.[6] The major difficulty lies in devising simple methods that allow these enzymes to be used by nonspecialists. For example, the use of purified cyclohexanone monooxygenase is complicated by the relatively low protein yield from pathogenic *Acinetobacter* cells and the need to supply reduced nicotinamide adenine dinucleotide phosphate (NADPH), whose high cost and low stability discourages large-scale reactions.[10]

To overcome these obstacles and make cyclohexanone monooxygenase accessible to nonspecialists, we have created a strain of bakers' yeast (*Saccharomyces cerevisiae*) that expresses this enzyme.[11] Whole cells of this

[†] Present address: Vienna University of Technology, Institute for Organic Chemistry, Getreidemarkt 9/A-1060 Vienna, Austria.
[‡] These authors contributed equally to this work.
[§] University of New Brunswick. Phone (506) 648-5576. Fax (506) 648-5650. E-mail for M.M.K.: kayser@unbsj.ca.
[||] University of Florida. Phone (352) 846-0743. Fax (352) 846-2095.
[⊥] Université du Québec à Montréal.
[#] Camille and Henry Dreyfus Foundation New Faculty Awardee, 1994−1999.

(1) Bolm, C.; Schlingloff, G.; Weickhardt, K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1848−1849.
(2) Gusso, A.; Baccin, C.; Pinna, F.; Strukul, G. *Organometallics* **1994**, *13*, 3442−3451.
(3) Bolm, C.; Schlingloff, G.; Bienewald, F. *J. Mol. Catal. A: Chem.* **1997**, *117*, 347−350.
(4) Bolm, C.; Luong, T. K. K.; Schlingloff, G. *Synlett* **1997**, 1151.
(5) Strukul, G.; Varagnolo, A.; Pinna, F. *J. Mol. Catal. A: Chem.* **1997**, *117*, 413−423.
(6) Stewart, J. D. *Curr. Org. Chem.* **1998**, *2*, 211−232.
(7) Trudgill, P. W. In *Microbial Degradation of Organic Compounds*; Gibson, D. T., Ed.; Marcel Dekker: New York, 1984; Vol. 13, pp 131−180.
(8) Grogan, G.; Roberts, S.; Wan, P.; Willetts, A. *Biotechnol. Lett.* **1993**, *15*, 913−918.
(9) Donoghue, N. A.; Norris, D. B.; Trudgill, P. W. *Eur. J. Biochem.* **1976**, *63*, 175−192.
(10) Abril, O.; Ryerson, C. C.; Walsh, C. T.; Whitesides, G. M. *Bioorg. Chem.* **1989**, *17*, 41−52.

10.1021/jo001292p CCC: $20.00
Published on Web 01/18/2001

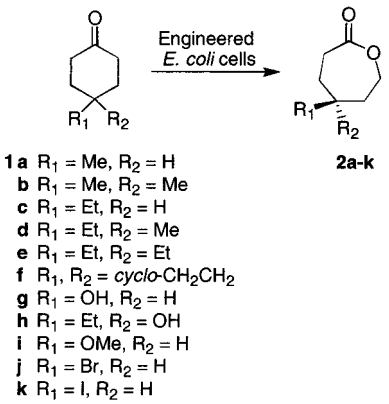
recombinant yeast can be used directly to carry out asymmetric Baeyer−Villiger oxidations on synthetically useful scales, and we have shown that a variety of chiral lactone building blocks can be prepared in high chemical and optical yields by this method.[12−14] More recently, we have expressed *Acinetobacter* sp. cyclohexanone monooxygenase in *Escherichia coli* and shown that whole cells of these engineered bacteria carry out asymmetric oxidations of thioethers to provide chiral sulfoxides.[15] Preliminary studies have also shown that the same strain catalyzes Baeyer−Villiger oxidations of heterocyclic ketones.[16] The present studies had three major goals. First, we hoped to show that engineered *E. coli* cells would be a simple and useful reagent for carrying out asymmetric Baeyer−Villiger oxidations, similar to the engineered yeast strain described previously. Protein expression is generally higher in *E. coli* than bakers' yeast, which should facilitate the reactions by reducing the time required. In addition, we hoped to provide a straightforward route to useful chiral building blocks by oxidations of mesomeric 4-mono- and 4,4-disubstituted cyclohexanones, most of which had not been investigated previously as substrates for the enzyme. Finally, we hoped that the outcomes of these oxidations might better illustrate the origin of the very high enantioselectivity displayed by cyclohexanone monooxygenase, an enzyme whose physiological role is to convert cyclohexanone into ϵ-caprolactone, an achiral reaction.

Results and Discussion

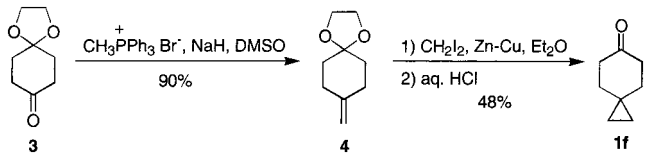
The construction of the *E. coli* strain overexpressing cyclohexanone monooxygenase has been described in detail elsewhere.[15] The monooxygenase gene was cloned downstream from the strong T7 promoter in plasmid pMM4, and this plasmid was used to transform *E. coli* strain BL21(DE3) to create the strain used for biotransformations [BL21(DE3)(pMM4)]. Production of cyclohexanone monooxygenase was induced by adding isopropyl-thio-β-D-galactoside (IPTG) to the growth medium, which allowed the protein to accumulate to ca. 30% of total protein after a few hours.[17] These levels are significantly higher than those observed by Walsh and co-workers,[18] primarily because of the development of more efficient bacterial promoters in recent years.[19] Virtually all of the cyclohexanone monooxygenase produced by BL21(DE3)-(pMM4) was soluble and catalytically active.[17]

The ability of whole cells of the *E. coli* strain to carry out the Baeyer−Villiger oxidation of cyclohexanone was tested by adding the ketone at a concentration of 10 mM to a growing culture of *E. coli* BL21(DE3)(pMM4) to which IPTG had been introduced 30 min prior to addition

Scheme 1



Scheme 2



of the substrate. GC analysis indicated that all of the ketone was consumed within 7 h and ϵ-caprolactone was the sole extractable product present in the reaction mixture. A control experiment in which BL21(DE3) cells were substituted for the engineered strain showed no lactone formation, demonstrating that cyclohexanone monooxygenase was responsible for the observed Baeyer−Villiger oxidation. No carbonyl reduction was observed with either the engineered or control *E. coli* strains.

The ketones oxidized by the engineered *E. coli* strain are summarized in Scheme 1. All of these ketones were mesomeric, which avoided the loss of material inherent in kinetic resolutions. Moreover, the oxidations of ketones **1a**, **1c**, **1g**, and **1i** by one or more forms of cyclohexanone monooxygenase (isolated enzyme, whole *Acinetobacter* cells or our engineered bakers' yeast strain) had been reported previously, making these substrates particularly useful in comparing different experimental methods with the engineered *E. coli* strain. Ketones such as **1b**, **1d**, and **1e** were designed to test the possibility that the enzyme might accept cyclohexanones with two alkyl substituents at the 4-position while the others were designed to provide useful chiral intermediates for synthesis. While most of the ketones were available commercially, cyclohexanones **1b**, **1d**, and **1e** were synthesized by a two-step Robinson annelation[20]/reduction sequence. Ketone **1f** was synthesized in two steps from commercially available ketal **3** (Scheme 2), which is a simpler route than that reported previously.[21] Cyclohexanone **1h** was prepared by Grignard addition to **3** followed by acidic hydrolysis, and ketones **1j** and **1k** were obtained by reacting 4-tosyloxycyclohexanone with bromide and iodide, respectively.

Oxidations of ketones **1a**−**k** were performed by adding these ketones at concentrations ranging between 6.3 and 10 mM to growing cultures of recombinant *E. coli* (Table 1). In some cases, cyclodextrins were added along with the substrate to improve its solubility.[22] After all of the

(11) Stewart, J. D.; Reed, K. W.; Kayser, M. M. *J. Chem. Soc., Perkin Trans. 1* **1996**, 755−757.
(12) Stewart, J. D.; Reed, K. W.; Zhu, J.; Chen, G.; Kayser, M. M. *J. Org. Chem.* **1996**, *61*, 7652−7653.
(13) Stewart, J. D.; Reed, K. W.; Martinez, C. A.; Zhu, J.; Chen, G.; Kayser, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 3541−3548.
(14) Kayser, M. M.; Chen, G.; Stewart, J. D. *Synlett* **1999**, 153−158.
(15) Chen, G.; Kayser, M. M.; Mihovilovic, M. D.; Mrstik, M. E.; Martinez, C. A.; Stewart, J. D. *New J. Chem.* **1999**, 827−832.
(16) Mihovilovic, M. D.; Müller, B.; Kayser, M. M.; Stewart, J. D.; Fröhlich, J.; Stanetty, P.; Spreitzer, H. *J. Mol. Catal. B.* In press.
(17) Martinez, C. A. Ph.D. Dissertation, University of Florida, 2000.
(18) Chen, Y.-C. J.; Peoples, O. P.; Walsh, C. T. *J. Bacteriol.* **1988**, *170*, 781−789.
(19) Studier, F. W.; Moffatt, B. A. *J. Mol. Biol.* **1986**, *189*, 113−130.
(20) Flaugh, M. E.; Crowell, T. A.; Farlow, D. S. *J. Org. Chem.* **1980**, *45*, 5399−5400.
(21) Wuesthoff, M. T.; Rickborn, B. *J. Org. Chem.* **1968**, *33*, 1311−1312.

Table 1. Baeyer−Villiger Oxidations of 4-Substituted Cyclohexanones by Engineered *E. coli* Cells

substrate	R_1	R_2	yield,[a] %	ee,[b] %	$[\alpha]_D$[c]
1a	Me	H	61	≥98	−44.0°, *c* 8.16
1b	Me	Me	61	*d*	
1c	Et	H	91	97	−38.9°, *c* 11.7
1d	Et	Me	91	75	−9.61°, *c* 14.2
1e	Et	Et	60		
1f	*cyclo*-CH_2CH_2		74		
1g	OH	H	61[e]	9.1	−6.9°, *c* 2.64
1h	Et	OH	54	94	−3.8°, *c* 1.0
1i	OMe	H	84	78	+10.0°, *c* 1.08
1j	Br	H	63	97	−10.1°, *c* 6.43
1k	I	H	60	97	−26.0°, *c* 1.05

[a] Isolated yield of purified products. [b] Values for enantiomeric excess were determined by chiral-phase GC analysis. [c] Optical rotations were determined from $CHCl_3$ or $CDCl_3$ solutions at the indicated concentrations (expressed in g/100 mL) at room temperature. [d] Not applicable. [e] Isolated as the rearranged γ-lactone.

substrate had been consumed, the cells were removed by centrifugation and the lactone was isolated by extraction from the culture medium and then purified by flash chromatography. Small quantities of indole were observed after extended reaction times;[23] no other contaminants were found in the organic extracts. The yields and optical purities of lactones known to be substrates for cyclohexanone monooxygenase matched previously reported values, demonstrating that whole cells of engineered *E. coli* produce a catalyst with properties indistinguishable from those of the purified enzyme or the engineered yeast strain.

The chemical and optical purities of the lactone products were determined by chiral-phase GC analysis. Absolute configurations were assigned to lactones **2a**, **2c**, and **2g** by comparison to previously reported optical rotation values. The absolute configuration of **2i** was assigned as (*S*) by reducing the lactone with lithium aluminum hydride and comparing the specific rotation value of the diol ($[\alpha]_D = +15°$, $c = 0.51$, acetone) to that reported by Ackermann et al., who prepared the same diol from (−)-(*S*)-malic acid.[24,25] The absolute configuration of **2k** was determined by X-ray crystallography, and that of **2j** was assigned by analogy to **2k**. The absolute configurations of **2d** and **2h** are unknown, and we have assigned their structures tentatively by analogy to other substrates.

All ketones except **1d** and **1g** and **1i** gave the corresponding lactones in very high enantiomeric purities. This was precedented for lactone **2g**, which was obtained in low optical purity and as the spontaneously rearranged γ-lactone, consistent with the results reported by Taschner[26] for this oxidation using purified cyclohexanone monooxygenase. The unusual results observed for

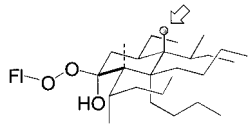


Figure 1. Diamond lattice representation of the allowed positions for alkyl substituents in cyclohexanone monooxygenase-mediated oxidations of 2-, 3-, and 4-substituted cyclohexanones. The arrow denotes the location of axial substituents derived from 4-substituted cyclohexanones. Note that the precise conformations of the extended alkyl chains are not known. Reprinted with permission from Stewart, J. D.; Reed, K. W.; Martinez, C. A.; Zhu, J.; Chen, G.; Kayser, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 3541−3548. Copyright 1998 American Chemical Society.

this ketone may signal unique interactions with the enzyme active site made possible by the hydroxyl substituent. On the other hand, the high stereoselectivity for 4-methoxy-substituted cyclohexanone **1i** was consistent with expectations based on analogous 4-alkyl-substituted cyclohexanones. This was also the case for halogen-substituted substrates **1j** and **1k**. Taken together, these results demonstrate that the engineered *E. coli* strain provides a simple method for obtaining these valuable chiral lactone building blocks.

The natural function for cyclohexanone monooxygenase is to provide ε-caprolactone for subsequent degradation to acetate and succinate.[7] This role does not demand chiral discrimination, yet as is clear from the results above and those obtained in other studies,[6] the enzyme is often highly enantioselective for a diverse range of substrates. Our previous studies have suggested that the enantioselectivity of cyclohexanone monooxygenase arises from a combination of modest intrinsic chiral discrimination coupled with the conformational preferences of bound substrates.[13] The mesomeric substrates studied in this paper provided an excellent means to explore this hypothesis further.

No lactone products derived from a Criegee-like intermediate possessing an axial substituent at the 4-position had been reported prior to our studies (Figure 1).[13] Cyclohexanone **1b** was designed specifically to determine whether cyclohexanone monooxygenase could accommodate an alkyl substituent at this location, and our results clearly indicated that **1b** is a suitable substrate for the enzyme. Moreover, the successful oxidation of **1e** demonstrates that an axial ethyl group can be accommodated as well. On the other hand, attempted enzymatic oxidation of spiro[5.5]undecan-3-one gave no lactone product and only starting material was observed, even after extended reaction times. Taken together, these observations argued that the enzyme should also have been able to accommodate axial methyl or ethyl groups during the oxidations of monosubstituted ketones such as **1a** and **1c**, respectively; however, we were unable to detect the enantiomeric lactones that would have resulted from these alternate Criegee-like intermediates.

By analogy with substituted cyclohexanes, the alternate Criegee-like intermediates derived from **1a** and **1c** are separated by approximately 1.7 kcal/mol (Scheme 3).[27] According to the diamond-lattice model, these conformational energies would augment the enzyme's stereochemical preference to favor the observed lactone enantiomers. We therefore designed ketone **1d**, which

(22) Bar, R. *Trends Biotechnol.* **1989**, *7*, 2−4.

(23) *E. coli* tryptophanase produces indole and serine by tryptophan degradation, and this activity is well-known for *E. coli* grown in a medium containing excess tryptophan such as LB (for example, see: Zylstra, G. J.; Gibson, D. T. *Genet. Eng.* **1991**, *13*, 183−203).

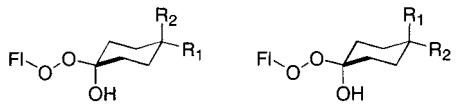
(24) Ackermann, P.; Tobler, H.; Ganter, C. *Helv. Chim. Acta* **1972**, *55*, 2731−2743.

(25) Oxidation of **1i** by cyclohexanone monooxygenase has been reported to afford a lactone with a negative specific rotation value (ref 39); by contrast, we reproducibly obtained a positive value for this compound. While the source of this discrepancy is not clear, our correlation with the stereochemically unambiguous diol prepared by Ackermann argues strongly that the configuration of **2i** has been assigned correctly.

(26) Taschner, M. J.; Black, D. J.; Chen, Q.-Z. *Tetrahedron: Asymmetry* **1993**, *4*, 1387−1390.

(27) Hirsch, J. A. *Top. Stereochem.* **1967**, *1*, 199−222.

Scheme 3



5 a R_1 = Me, R_2 = H
b R_1 = Me, R_2 = Me
c R_1 = Et, R_2 = H
d R_1 = Et, R_2 = Me
e R_1 = Et, R_2 = Et

6 a-e

would yield two virtually isoenergetic Criegee-like intermediates. The absence of conformational bias between the two Criegee-like intermediates was predicted to decrease the enantioselectivity of the enzymatic Baeyer−Villiger oxidation, and indeed, lactone **2d** was isolated in 75% ee.[28] On the basis of our previously published analysis,[13] the observed optical purity corresponds to a difference of 1.2 kcal/mol between the free energies of the two transition states for the rearrangements of the alternate Criegee-like intermediates derived from **1d**. It is tempting to speculate that a similar intrinsic energetic preference by the enzyme also applies to the oxidations of other 4-substituted cyclohexanones. This rather modest level of chiral discrimination might be expected for an enzyme that has evolved to catalyze the oxidation of an achiral substrate. However, when this intrinsic preference is reinforced by the energetic differences that separate the alternative conformations of the Criegee-like intermediates, synthetically useful levels of enantiomeric discrimination can result. On the other hand, when such substrate conformational biases are absent, for example, in alkyl-substituted cyclopentanones, much lower levels of enantiomeric purity would be expected, and this has been observed experimentally.[29]

It is more difficult to apply this analysis quantitatively to the oxidations of ketones containing polar substituents such as **1g**−**k** since their conformational behavior is complex and influenced by the presence of the carbonyl group dipole.[30−32] Moreover, specific interactions between the substituent and the protein (i.e., hydrogen bonds) may introduce additional complications. Nontheless, the high or relatively high enantioselectivities observed for the oxidations of ketones **1i**, **1j**, and **1k** makes this methodology useful for preparative purposes.

Conclusions

Whole cells of *E. coli* overexpressing *Acinetobacter* sp. cyclohexanone monooxygenase catalyze enantioselective Baeyer−Villiger oxidations. Where comparisons are possible, the enzyme expressed in *E. coli* demonstrates essentially identical enantioselectivities as the purified monooxygenase or the engineered yeast strain. The *E. coli* strain is particularly well-suited for large-scale fermentations, and its availability expands the possibilities for applying cyclohexanone monooxygenase to organic synthesis. Compared with our earlier engineered bakers' yeast strain, the *E. coli* cells grow approximately twice as quickly, they express a higher level of cyclohexanone monooxygenase, and the reaction times are corresponding shorter. The cost of the inducer (IPTG) is the major drawback to using the engineered *E. coli* strain on large scales; however, other, more economical expression systems are also available.

Our results also demonstrate that the enzyme can accommodate 4,4-dialkyl-substituted cyclohexanones, and the enantioselectivities displayed by these reactions underscore the importance of substrate conformational behavior in augmenting the modest intrinsic enantioselectivity of cyclohexanone monooxygenase. This feature can be used to design appropriate substrates for the enzyme, and the engineered whole cells provide a simple means for its use by nonspecialists.

Experimental Section

Reagents and bacterial growth media were purchased from commercial suppliers and used as received. Synthetic reactions were carried out in oven-dried glassware under argon or nitrogen atmospheres unless otherwise indicated. Biotransformations were monitored by GC using a DB-17 column (0.25 mm × 25 m, 0.25 μm film thickness) with flame ionization or MS detection. Chiral-phase GC analyses were performed on a Chirasil-Dex column (0.25 mm × 25 m, 0.25 μm film thickness) using helium as the carrier gas and flame ionization detection. Standards of racemic lactones of all lactones except **2g** and **2k** were prepared by oxidation of ketones with *m*-CPBA,[33] and conditions that allowed base-line resolution of the two enantiomers were achieved for all chiral lactones. It was not possible to isolate lactones **2g** or **2k** using this procedure, although the enzymatic Baeyer−Villiger oxidations proceeded smoothly.

The engineered *E. coli* strain [BL21(DE3)(pMM4)] was cultured routinely in liquid LB medium (1% Bacto-Tryptone, 0.5% Bacto-Yeast Extract, 1% NaCl) containing 200 μg/mL of ampicillin at 37 °C with shaking at 150−200 rpm. Frozen stocks were prepared by adding sterile glycerol (final concentration 15%) to a culture that had grown overnight; then 1 mL portions were stored at −80 °C. Fresh plates were streaked weekly from frozen stocks and grown overnight at 37 °C on LB plates that also contained 1.5% Bacto-Agar and 200 μg/mL ampicillin.

General Procedure for Preparing 4,4-Dialkyl-Substituted Ketones 1b, 1d, and 1e. A 100 mL round-bottom flask was charged with the appropriate aldehyde (69.3 mmol), methyl vinyl ketone (69.3 mmol, 5.77 mL), concentrated sulfuric acid (3.5 mmol, 0.18 mL) and benzene (50 mL). A Dean−Stark trap and reflux condenser were added, and the mixture was held at reflux until the theoretical amount of water (1.24 mL) had been collected. After cooling, the reaction mixture was washed with saturated $NaHCO_3$ (3 × 40 mL), dried with $MgSO_4$, and concentrated by rotary evaporator. The enone was purified by distillation under reduced pressure. The saturated ketone was prepared by catalytic hydrogenation of the enone (2 g) in EtOAc (100 mL) using 5% Pd/C (165 mg) and a hydrogen-filled balloon or Parr shaker apparatus. After GC/MS analysis indicated that all the starting material had been consumed, the catalyst was removed by filtration through Celite and the solvent was removed by rotary evaporator to afford analytically pure ketone that was used without further purification.

Spiro[2.5]octan-6-one 1f. A 250 mL round-bottom flask equipped with a reflux condenser was charged with alkene **4**[34]

(28) A control experiment indicated that the optical purity of the lactone was unchanged over the course of the reaction, which indicated that the observed 75% ee was due solely to the Baeyer−Villiger oxidation and that secondary processes such as enantioselective lactone hydrolysis did not interfere.

(29) Kayser, M. M.; Chen, G.; Stewart, J. D. *J. Org. Chem.* **1998**, *63*, 7103−7106.

(30) Stolow, R. D.; Groom, T. *Tetrahedron Lett.* **1968**, 5781−5784.

(31) Stolow, R. D.; Giants, T. W. *J. Chem. Soc. Chem. Commun.* **1971**, 528−529.

(32) Dosen-Micovic, L.; Jeremic, D.; Allinger, N. L. *J. Am. Chem. Soc.* **1983**, *105*, 1723−1733.

(33) Meinwald, J.; Tufariello, J. J.; Hurst, J. J. *J. Org. Chem.* **1964**, *29*, 2914−2919.

(34) Nicolaou, K. C.; Magolda, R. L.; Claremon, D. A. *J. Am. Chem. Soc.* **1980**, *102*, 1404−1409.

(4.8 mmol, 0.74 g), CH_2I_2 (24.0 mmol, 6.43 g, 1.93 mL), and Et_2O (45 mL). A zinc−copper couple prepared according to Shank and Schechter[35] (30 mmol, 1.96 g) was added, and the mixture was held at reflux for 12 h. After this time, additional portions of CH_2I_2 (24.0 mmol, 6.43 g, 1.93 mL) and zinc−copper couple (30 mmol, 1.96 g) were added and the reaction was continued at reflux for an additional 17 h. After this time, the reaction mixture was cooled to room temperature, solids were removed by filtration, and the filtrate was washed with 60 mL of Et_2O. The combined organics were placed in a 500 mL round-bottom flask along with 100 mL of 1 M HCl. The mixture was stirred vigorously at room temperature for 1 h; then the layers were separated and the aqueous fraction was extracted with Et_2O (4 × 100 mL). The combined organics were dried with $MgSO_4$ and concentrated by rotary evaporator; then the crude product was purified by silica gel chromatography to afford 0.29 g (48% yield) of the title compound as a light yellow oil whose spectral data were identical to those reported previously by Rickborn.[21]

4-Ethyl-4-hydroxycyclohexanone 1h. Magnesium metal (55 mmol, 1.3 g) and 25 mL of anhydrous Et_2O were placed in a 150 mL flask equipped with a dropping funnel and reflux condenser. Ethyl bromide (45 mmol, 4.9 g, 3.8 mL) dissolved in 25 mL of anhydrous Et_2O was added at a rate sufficient to maintain a gentle reflux; this was continued for an additional hour at room temperature. Ketal **3** (7.5 mmol, 1.2 g) dissolved in 20 mL of anhydrous Et_2O was added dropwise; then stirring was continued for an additional 1.5 h at room temperature. The reaction was quenched by adding 50 mL of water. The organic layer was separated, and the aqueous phase was extracted with Et_2O (3 × 150 mL). The combined organics were dried with $MgSO_4$, and the solvent was removed by rotary evaporator. The crude product was dissolved in 100 mL of aqueous HCl (pH ∼ 3) and stirred at room temperature for 4 h. After being extracted with EtOAc (3 × 180 mL), the combined organics were dried with $MgSO_4$ and the solvent was removed by rotary evaporation. The crude product was purified by chromatography on silica gel using 2:3 EtOAc:petroleum ether to afford the title compound as colorless crystals (0.76 g, 70% yield): mp 48−49 °C. IR (neat) ν 3429, 1710 cm^{-1}. ^1H NMR δ 2.80 (2H, ddd, J = 13.9, 13.7, 6.4 Hz), 2.25 (2H, dm, J = 14.5 Hz), 1.98 (2H, dm, J = 13.0 Hz), 1.79 (2H, ddd, J = 13.4, 13.3, 4.9 Hz), 1.66 (2H, q, J = 9.5 Hz), 0.98 (3H, t, J = 9.5 Hz) ppm. ^{13}C NMR δ 212.5, 70.3, 37.0, 36.3, 35.0, 7.7 ppm.

4-Bromocyclohexanone 1j. Lithium bromide (112 mmol, 10 g) was dissolved in 50 mL of acetone in a flask equipped with a reflux condenser; then 4-tosyloxycyclohexanone (15 mmol, 4.0 g) was added and the mixture held at reflux until TLC showed complete consumption of the starting material. The solvent was removed by rotary evaporator, and the residue was dissolved in a minimum amount of water and extracted with EtOAc (3 × 50 mL). The combined organics were washed with 0.1 M KOH and brine, dried with $MgSO_4$, and concentrated by rotary evaporation. Silica gel chromatography using 1:3 Et_2O:petroleum ether followed by CH_2Cl_2 afforded the title compound as a colorless oil (1.58 g, 59% yield) whose spectral data agreed with literature values.[36]

4-Iodocyclohexanone 1k. The general procedure for preparation of **1j** was followed. Lithium iodide (50 mmol, 7.0 g) was reacted with 4-tosyloxycyclohexanone (10 mmol, 2.7 g) to afford the title compound as white crystals (1.1 g, 47% yield): mp 43−43.5 °C, lit. mp 62 °C.[37] Spectral data agreed with literature values.[37]

General Procedure for Baeyer−Villiger Oxidations by Whole Cells of *E. coli* BL21(DE3)(pMM4). A 1 mL aliquot from an overnight culture of BL21(DE3)(pMM4) (optical density at 600 nm [$O.D._{600}$] of 4 to 5) was added to one or two 100 mL portion of LB medium supplemented with 200 μg/mL of ampicillin in a 500 mL Erlenmeyer flask. The cultures were shaken at 150−200 rpm at 37 °C until they reached an $O.D._{600}$ between 0.3 and 0.4; then IPTG was added to a final concentration of 0.10 mM. If cyclodextrins were required, they were added at this time. The cultures were shaken at 150 rpm at room temperature for 30 min; then the ketone was added neat and the cultures were shaken at room temperature at 150 rpm. Samples for GC analysis were prepared by mixing 100 μL of the reaction mixture with 100 μL of EtOAc and then vortexing vigorously for ca. 30 s. A 1 μL portion of the organic extract was analyzed by GC. When >95% of the starting ketone had been consumed, the reaction mixture was centrifuged at 5000 × g for 10 min at 4 °C. The pellet was extracted with 20 mL of EtOAc. The supernatant was saturated with NaCl and extracted with EtOAc (4 × 60 mL); then the combined organic extracts were dried with $MgSO_4$ and concentrated by rotary evaporator. The lactone was purified by flash chromatography on silica gel.

(*S*)-5-Methyl-2-oxepanone 2a. Using the general procedure outlined above, 1.0 mmol (113 mg) of ketone **1a** was oxidized in a 100 mL culture over 28.5 h to afford lactone **2a** (78 mg, 61% yield). Spectral data agreed with those reported previously.[13]

5,5-Dimethyl-2-oxepanone 2b. Using the general procedure outlined above, 1.0 mmol (126 mg) of ketone **1b** was oxidized in a 100 mL culture over 28 h to lactone **2b** (86 mg, 61% yield). Spectral data agreed with those reported previously.[38]

(*S*)-5-Ethyl-2-oxepanone 2c. Using the general procedure outlined above, 1.0 mmol (126 mg) of ketone **1c** was divided into equal portions and oxidized in two 100 mL cultures over 6 h in the presence of 1.0 equiv (1.14 g) of β-cyclodextrin to afford lactone **2c** (129 mg, 91% yield). Spectral data agreed with those reported previously.[13]

5-Ethyl-5-methyl-2-oxepanone 2d. Using the general procedure outlined above, 1.0 mmol (140 mg) of ketone **1d** was divided into equal portions and oxidized in two 100 mL cultures over 27 h in the presence of 1.0 equiv (1.14 g) of β-cyclodextrin to afford lactone **2d** (142 mg, 91% yield). IR (neat) ν 1737 cm^{-1}. ^1H NMR δ 4.15−4.28 (2H, m), 2.53−2.69 (2H, m), 1.48−1.74 (4H, m), 1.30−1.43 (2H, m), 0.96 (3H, s), 0.85 (3H, t, J = 7.6 Hz) ppm. ^{13}C NMR δ 176.3, 103.3, 64.5, 39.8, 34.2, 33.4, 29.6, 24.1, 7.7 ppm. MS: m/e 156 (M^+, 1.5%), 111, (75%), 97 (76%), 69 (100%), 55 (89%).

5,5-Diethyl-2-oxepanone 2e. Using the general procedure outlined above, 1.0 mmol (154 mg) of ketone **1e** was divided into equal portions and oxidized in two 100 mL cultures over 28.5 h in the presence of 1.0 equiv (1.14 g) of β-cyclodextrin to afford lactone **2e** (101 mg, 60% yield). IR (neat) ν 1735 cm^{-1}. ^1H NMR δ 4.19−4.23 (2H, m), 2.57−2.61 (2H, m), 1.64−1.67 (2H, m), 1.54−1.58 (2H, m), 1.30−1.43 (4H, m), 0.80 (6H, t, J = 7.6 Hz) ppm. ^{13}C NMR δ 176.4, 64.2, 37.7, 36.2, 31.1, 29.2, 28.3, 7.3 ppm. MS: m/e 170 (M^+, 0.7%), 141 (50%), 69 (41%), 55 (100%).

6-Oxaspiro[2.6]nonan-7-one 2f. Using the general procedure outlined above, 1.0 mmol of ketone **1e** was oxidized in a 100 mL culture to lactone **2f** (103 mg, 74% yield). IR (neat) ν 1728 cm^{-1}. ^1H NMR δ 4.30 (2H, m), 2.73 (2H, m), 1.66 (2H, m), 1.56 (2H, m), 0.43 (4H, br d, J = 1.4 Hz) ppm. ^{13}C NMR δ 176.0, 68.4, 38.7, 33.7, 32.5, 21.5, 12.8 ppm. MS: m/e 140 (M^+, 1.4%), 83 (51%), 67 (100%), 54 (42%).

4,6-Dihydroxyhexanoic acid, γ-Lactone 2g. Using the general procedure outlined above, 0.88 mmol (100 mg) of ketone **1g** was oxidized in a 100 mL culture to afford lactone **2g** (73 mg, 64% yield). Spectral data agreed with those reported previously.[39]

5-Ethyl-5-hydroxy-2-oxepanone 2h. Using the general procedure outlined above, 0.35 mmol (50 mg) of ketone **1h** was oxidized in the presence of 0.44 mmol (0.5 g) of β-cyclodextrin in a 100 mL culture to afford lactone **2h** (30 mg, 54% yield). IR (neat) ν 3435, 1764 cm^{-1}. ^1H NMR δ 3.80 (2H, m), 2.61 (2H,

(35) Shank, R. S.; Schechter, H. *J. Org. Chem.* **1959**, *24*, 1825−1826.
(36) Engel, P. S.; Kitamura, A.; Keys, D. E. *J. Org. Chem.* **1987**, *52*, 5015−5021.
(37) Curran, D. P.; Chang, C.-T. *J. Org. Chem.* **1989**, *54*, 3140−3157.
(38) Heslinga, L.; van der Linde, R.; Pabon, H. J. J.; van Dorp, D. A. *Recl. Trav. Chim. Pays-Bas* **1975**, *94*, 262−273.
(39) Taschner, M. J.; Black, D. J. *J. Am. Chem. Soc.* **1988**, *110*, 6892−6893.

m), 2.15 (2H, m), 1.97 (2H, m), 1.74 (2H, m), 0.96 (3H, t, J = 8.9 Hz) ppm. ^{13}C NMR δ 177.0, 88.6, 58.3, 40.2, 31.6, 30.7, 28.9, 7.9 ppm. MS: *m/e* 129 ($M^+ - 29$, 33%), 113 (71%), 101 (100%), 57 (72%), 55 (60%).

(*S*)-5-Methoxy-2-oxepanone 2i. Using the general procedure outlined above, 0.78 mmol (100 mg) of ketone **1i** was oxidized in a 100 mL culture in the presence of 0.40 equiv (0.40 g) of γ-cyclodextrin to afford lactone **2i** (95 mg, 85% yield). Spectral data agreed with those reported previously.[39]

(*S*)-5-Bromo-2-oxepanone 2j. Using the general procedure outlined above, 0.80 mmol (140 mg) of ketone **1j** was oxidized in a 100 mL culture over 7 h in the presence of 0.38 equiv (0.40 g) of γ-cyclodextrin to afford lactone **2j** (121 mg, 79% yield). IR (neat) ν 1736 cm^{-1}. ^{1}H NMR δ 4.57 (1H, m), 4.49 (1H, ddd, J = 13.3, 8.0, 2.1 Hz), 4.13 (1H, dddd, J = 13.2, 5.8, 2.7, 0.5 Hz), 3.02 (1H, m), 2.54 (1H, m), 2.31 (2H, m), 2.20 (2H, m) ppm. ^{13}C NMR δ 174.6, 64.6, 51.5, 38.6, 32.1, 30.5 ppm. MS: *m/e* 194, 192 (M^+, 0.8%, 0.9%), 113 (4.0%), 109 (3.5%), 107 (3.8%), 85 (100%).

(*S*)-5-Iodo-2-oxepanone 2k. Using the general procedure outlined above, 0.63 mmol (140 mg) of ketone **1k** was oxidized in a 100 mL culture over 11 h in the presence of 0.60 equiv (0.40 g) of γ-cyclodextrin to afford lactone **2k** (91 mg, 60% yield). IR (neat) ν 1736 cm^{-1}. ^{1}H NMR δ 4.67 (1H, s, br), 4.35 (1H, dd, J = 13.3, 8.7 Hz), 4.16 (1H, ddd, J = 13.5, 7.2, 1.4 Hz), 2.90 (1H, t, br, J = 13.7 Hz), 2.58 (1H, ddd, J = 14.5, 8.9, 1.5 Hz), 2.34−2.10 (4H, m) ppm. ^{13}C NMR δ 174.6, 66.9, 40.5, 34.1 (br), 33.4 ppm. MS: *m/e* 240 (M^+, 15%), 127 (36%), 113 (52%), 83 (52%), 69 (74%), 55 (100%).

Acknowledgment. Support by the Natural Sciences and Engineering Research Council and the University of New Brunswick (M.M.K.), the National Science Foundation (J.D.S.; CHE-9513349, CHE-9816318), and the FWF for a Schödinger Fellowship (M.D.M.; J1471-CHE) is gratefully acknowledged. The authors also thank Cerestar, Inc. for supplying the cyclodextrins used in this study.

Supporting Information Available: Details of the crystallographic studies of **2k** and table of bond lengths and angles. This material is available free of charge via the Internet at http://pubs.acs.org. The CIF table of the crystal has been deposited in the Cambridge Date File (CCDC 148195).

JO001292P