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Monooxygenase-catalyzed Baeyer–Villiger oxidations: CHMO versus CPMO

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Abstract

Cyclopentanone monooxygenase (CPMO) from *Comamonas* sp. NCIMB 9872 expressed in *E. coli* was evaluated as a potential new bioreagent for Baeyer–Villiger oxidations of 4-alkoxy- and halo-substituted cyclohexanones (10 examples). The results were compared with those obtained in oxidations catalyzed by an engineered *E. coli* strain expressing cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp. CPMO was found to have modest to good stereoselectivity and broader substrate acceptability than CHMO. The stereoselectivities of the two enzymes were generally opposite. It appears, therefore, that the two engineered strains can be useful and complementary reagents for enantioselective Baeyer–Villiger oxidations of certain prochiral ketones.

Keywords: Baeyer–Villiger oxidations; Cyclopentanone monooxygenase; Cyclohexanone monooxygenase

1. Introduction

Biological Baeyer–Villiger oxidations of cyclic and bicyclic ketones provide simple routes to homochiral lactones [1–3]. These compounds are of great interest as important synthetic intermediates for the construction of natural products, pharmaceuticals and agrochemicals [4]. Although many bacterial species carry monooxygenases capable of performing Baeyer–Villiger oxidations, cyclohexanone monooxygenase (E.C. 1.14.13.22; CHMO) from *Acinetobacter* NCIMB 9871 has been, by far, the most extensively investigated [5,6]. The early transformations performed with a parent organism or isolated enzyme demonstrated the broad substrate acceptability and high enantioselectivity of CHMO, but the problems associated with cell growth (*Acinetobacter* sp. is class II pathogen) on the one hand, and enzyme isolation and recycling of the co-factor on the other hand, were the major obstacles to adopting it as a general "reagent" for organic synthesis. The construction of baker's yeast (*S. cerevisiae*) and *E. coli* strains overexpressing CHMO [7–9] made it simpler and more attractive for application in organic synthesis, and several reports focused on developing these recombinant organisms for use as bioreagents have now been published [8–11].

In the last 3 years, several other oxygenases have been cloned in *E. coli* [12–15]; these should eventually enrich the repertoire of biological Baeyer–Villiger oxidizing "reagents" accessible to organic chemists. Recently, we have reported the cloning and preliminary

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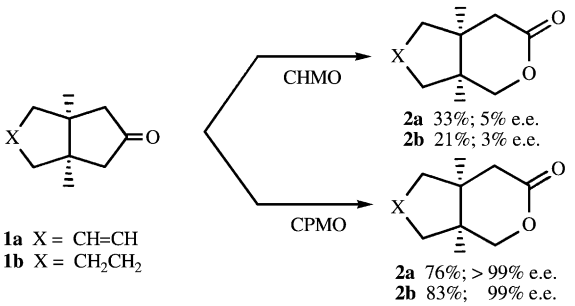
profiling of cyclopentanone monooxygenase (CPMO) (E.C. 1.14.13.) from *Comamonas* (previously *Pseudomonas*) sp. NCIMB 9872 [16]. The recombinant *E. coli* (CPMO) engineered as described [16] is a companion strain to the versatile *E. coli* (CHMO) discussed earlier.

In the past, CPMO was relatively little used in enzymatic Baeyer–Villiger oxidations, probably because the early results indicated that it might be less enantioselective than CHMO [3,17–19]. The newly engineered *E. coli* strain for CPMO allowed us to conduct more easily a comparative study of substrate–enantioselectivity profiles for the two enzymes. Although the first experiments showed CPMO to possess broad substrate acceptability, they also confirmed the suspicion that in the case of many substrates common to both CHMO and CPMO enantioselectivity of CPMO was low, frequently significantly lower than that of CHMO [16,20]. Fortunately, the discovery that the CPMO-catalyzed biooxidations of bicyclo[4.3.0]ketones **1a** and **1b** not only produced the enantiomers of the opposite absolute configuration than those generated by CHMO, but also with dramatically better yields and higher optical purity (Scheme 1), saved this enzyme from instant obscurity [21].

The results from the oxidation of prochiral ketones **1a** and **1b** encouraged further comparison of the two enzymes vis à vis prochiral ketones since these compounds appeared to be the most likely substrates,



1a X = CH=CH
1b X = CH_2CH_2

2a 33%; 5% e.e.
2b 21%; 3% e.e.

2a 76%; > 99% e.e.
2b 83%; 99% e.e.

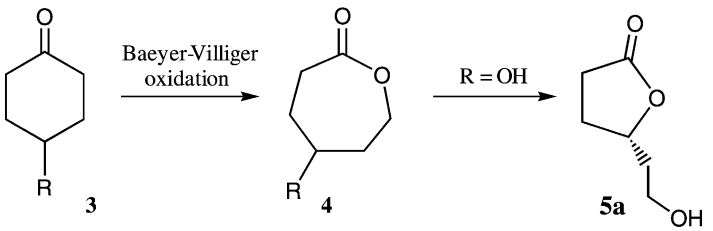
Scheme 1.

where the two enzymes might complement each other [21]. In this paper, we report the results from comparative use of the two monooxygenases in oxidation of 10 mesomeric 4-substituted cyclohexanones (Scheme 2 and Table 1).

2. Results

2.1. Bioreagents

The construction of the *E. coli* strain overexpressing CHMO and CPMO was described in detail elsewhere [16,22]. The *Acinetobacter sp.* NCIMB 9871 CHMO gene was cloned downstream of the strong T7 promoter in the plasmid PMM4, and this plasmid was used to transform *E. coli* strain BL21(DE3) to create



3 **4** **5a**

3a R = OH
3b R = OMe
3c R = OEt
3d R = OAllyl
3e R = OBn
3f R = OAc
3g R = COOEt
3h R = I
3i R = Br
3j R = Cl

Scheme 2. Baeyer–Villiger oxidation of 4-substituted cyclohexanones.

Table 1
Baeyer–Villiger oxidation of 4-substituted cyclohexanones catalyzed by CHMO and CPMO

Substrate R	CHMO (*E. coli*)			CPMO (*E. coli*)			Ref.
	Yield (%)[a]	ee (%)[b]	$[\alpha]_D$[c]	Yield (%)[a]	ee (%)[b]	$[\alpha]_D$[c]	
OH (a)	61	9 (*R*)	−6.9 c 2.62	73	85 (*S*)	+64.0 c 1.4	[10][d]
OMe (b)	84	78 (*S*)	+10.0 c 10.8	71	28 (*S*)	+3.7 c 0.5	[10][d]
OEt (c)	NR	–		90	37[e]	−7.1 c 1.4	
Oallyl (d)	NR	–	–	80	45 (*S*)[e]	+0.8 c 1.2	
OBn (e)	NR	–	–	95	75 (*S*)[e]	−2.9 c 1.3	[28]
OAc (f)	NR	–	–	81	5	ND	
COOEt (g)	15[f]	93[g]	ND	83	64[g]	+37.5 c 2.0	
I (h)	61	97 (*S*)	−26.0 c 1.05	65	82 (*R*)	+25.4 c 3.1	[10][d]
Br (i)	63	97 (*S*)	−10.0 c 6.43	70	64 (*R*)	+6.9 c 2.6	[10][d]
Cl (j)	56	95 (*S*)	−4.7 c 2.1	64	34 (*R*)	+1.7 c 1.1	

[a] Isolated yield of chromatographically purified product.
[b] Values for enantiomeric excess from chiral-phase GC analyses.
[c] Rotation measured in CH_2Cl_2 at room temperature.
[d] Published data for CHMO-catalyzed oxidation; NR: no reaction; ND: not determined.
[e] Signal not resolved on chiral GC.
[f] Conversion from GC analysis.
[g] Opposite enantiomers obtained for the two biotransformations.

the engineered strain [BL21(DE3)(pMM4)] that was used in biotransformation [10]. New strains for CPMO expression in *E. coli* BL21(DE3) and DH5α were constructed and the two were found to be comparable in terms of their performance in Baeyer–Villiger oxidations [16]. Since the latter construct, however, appeared to be slightly more robust, it was selected as a principal reagent for this study. All reactions reported here were carried out with BL21(DE3)(pMM4) for the expression of CHMO and DH5α(pCMP201) for the expression of CPMO.

In both strains, the production of the enzyme (CHMO or CPMO) is induced by the addition of isopropyl-thio-β-D-galactoside (IPTG) to the growth medium; the substrates are then added during the maximum enzyme-producing phase. Since the recombinant strains were constructed to produce a large proportion of the desired proteins, approximately 25–30% total cytosolic protein content, biotransformations were faster and more efficient than with the wild-type parent organisms (*Acinetobacter* and *Comamonas*). Furthermore, over metabolism common in the reactions catalyzed by the wild-type strains, was minimized in the transformations with engineered whole cells. The reactions were shaken at 30 °C per 200 rpm and the conversion of 80–100%, was achieved in 18–24 h for most ketones. To overcome substrate hydrophobicity (and in some cases, toxicity) stoichiometric amounts of β-cyclodextrin (relative to the ketone) were included in most biotransformation mixtures [23]. A control experiment in which DH5α or BL21(DE3) carrier strains were substituted for the engineered strains showed no lactone formation, confirming that CPMO or CHMO are the enzymes responsible for the observed Baeyer–Villiger oxidations. Unless otherwise specified, all yields reported in Table 1 refer to isolated and chromatographically purified products.

2.2. Substrate ketones and product lactones

We have reported previously Baeyer–Villiger oxidations of ketones **3a**, **3b**, **3h**, and **3i** catalyzed by the engineered *E. coli* (CHMO) cells [10]. For comparison with the CPMO oxidations these results are included in Table 1. The remaining ketones listed in Table 1 were prepared as described in Section 4.

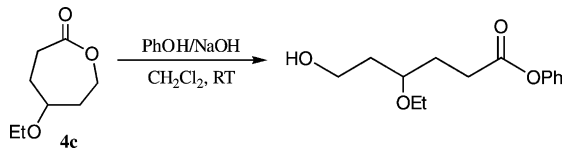
2.3. Absolute configurations

The chemical and optical purity of the lactone products were determined by chiral GC analyses, and were compared with racemic lactones from chemical oxidation. Absolute configurations for all lactones, except

4c and **4g**, were assigned by comparison with the previously reported optical rotation values for actual compounds or for their derivatives as described later.

The absolute configuration of **4b** was determined by reducing the lactone with lithium aluminum hydride and comparing the specific rotation value of the diol ($[\alpha]_D = +15^\circ$, $c = 0.51$, acetone) to that reported by Ackermann et al. [24], who prepared the same diol from (−)(*S*)-malic acid. Lactones **4c**, **4d**, **4e**, and **4f**, not accepted as substrates for CHMO, were readily oxidized by CPMO and were isolated in good yields (Table 1). Since **4c**, **4d**, and **4e** could not be resolved by chiral-phase GC but did have optical rotations we converted them to the known hydroxy lactone **4a**. To remove the allyl group, lactone **4d** was treated with SeO_2, AcOH/dioxane or Pd/C and TsOH/MeOH. The resulting sample **4a** was found to have rotation opposite to that obtained from the CHMO oxidation. This and matching of the traces from chiral GC, allowed us to establish the enantiomeric excess as 45% and the absolute configuration as **4d** (*S*). Similarly, hydrogenation of **4e** (Pd/C, H_2/EtOH) to **4a** provided the enantiomeric excess value and confirmed the absolute configuration of the major enantiomer as being (*S*) (Table 1).

We were unable to convert cleanly compounds **4c** to **4a**, and the absolute configuration of this lactone could not be determined unambiguously, although by analogy with the related compounds discussed earlier one may tentatively assign (*S*) configuration to the major enantiomer. To complicate matters, **4c** was not resolved on chiral GC, and neither was the diol from chemical reduction of **4c**. In the end, the enantiomeric excess was determined when lactone **4c** and a chemically prepared racemate were transformed into chiral GC-resolvable phenol esters (Scheme 3). Ketone **3g** was oxidized by CHMO with high enatioselectivity (93% ee), but even after prolonged fermentation the conversion was only 15%. On the other hand, in the CPMO-catalyzed reaction, **3g** was consumed completely within 24 h yielding **4g** lactonic product of the opposite absolute configuration but, unfortunately, lower optical purity (64% ee).



Scheme 3.

4-Halo substituted cyclohexanones are good substrates for both enzymes, and the products are antipodes. Thus, in the case of 4-iodocyclohexanone CHMO-catalyzed oxidation produced **4h** (*S*) while CPMO oxidation gave **4h** (*R*). Here, both enantiomers were obtained with high ee values (Table 1). For bromo- and chloro-substituted substrates the same trend was maintained, but CPMO oxidations yielded lactones of lower optical purity. The absolute configuration of (−)-5-iodooxepan-2-one **4h** was determined by X-ray crystallographic analysis [10]. The configurations of **4i** and **4j** were assigned by analogy with **4h**, rotation signs and peak matching of chiral GC traces.

3. Discussion

Although CPMO has been recognized for three decades as an enzyme capable of performing Baeyer–Villiger oxidations of unnatural substrates, its scope remained unexplored, most likely because a few results available at the time suggested that it might be similar, but less enantioselective, than the extensively investigated CHMO [3]. The recent availability of engineered *E. coli* strains overexpressing CPMO, combined with an indication that CPMO may occasionally have selectivity opposite to that of CHMO [18] and may catalyze oxidation of conjugated ketones (not accepted by CHMO) [16,19], reopened interest in this enzyme.

Our earlier experiments designed to compare the substrate base and enantioselectivity of CPMO with that of CHMO showed that CPMO-catalyzed reactions are usually fast and efficient, and that the substrate acceptability might be broader than that of CHMO. At the same time, CPMO proved to be essentially non-discriminating in the oxidation of several racemic 2-substituted cyclopentanones [20] and less selective in transformation of 4-alkyl substituted cyclohexanones [16]. But, while *E. coli* (CHMO)-catalyzed oxidations of several 4-alkyl substituted cyclohexanones were shown to be highly enantioselective, only substrates with relatively small substituents were accepted; also, oxidation of 4-hydroxycyclohexanone occurred readily enough but stereoselectivity was very

low (9% ee) [10]. Considering these limitations of CHMO, we had a second look at the CPMO-catalyzed oxidations of 4-substituted cyclohexanones.

The results listed in Table 1 show that CPMO readily accepts cyclohexanones with larger substituents in 4-position, and the trend suggests that selectivity might improve with the increasing size of a group (the series 4-OMe, 4-OEt, 4-Oallyl, 4-Obenzyl). Another useful discovery is that 4-halocyclohexanones **3h**, **3i**, and **3j** are converted by the two enzymes to the lactones with opposite absolute configurations. Thus, the transformations of **3h** by the two enzymes gave products **4h** (*S*) and **4h** (*R*) with adequately high enantiomeric excess values to be synthetically useful. 4-Hydroxycyclohexanone **3a** was oxidized by CPMO to lactone **4a** (*S*) with 85% optical purity; in this case, however, highly optically pure antipode is not available since CHMO is not selective (9% ee). It should be noted that **4a** lactones undergo spontaneous rearrangement to the corresponding butyrolactones, as shown in Scheme 2.

In conclusion, a number of substrates suitable for CPMO-catalyzed Baeyer–Villiger oxidation has been identified. Several of the biotransformations are enantioselective and, in some cases, provide antipodes of the lactones produced by CHMO. Our study shows that the two enzymes can be considered as complementary and that both engineered systems can and should become useful reagents for organic synthesis.

4. Experimental

4.1. General

Optical rotations were measured on a Perkin-Elmer 241 polarimeter operating at room temperature. IR spectra were recorded on a Mattson Satellite FT–IR spectrometer. ^{1}H and ^{13}C NMRs were recorded in $CDCl_3$ solution at room temperature, unless otherwise stated, on Varian XL-200 or Bruker AMX-400 FT–NMR spectrometers. Capillary gas chromatography separations utilized a 0.32 mm × 30 m × 0.25 μm column. Chiral GC was performed on a Shimadzu GC-9A gas chromatography employing a β-Dex 225 column (Supelco Inc.). The injector and detector temperatures were maintained at 225 and 300 °C, respectively. Thin-layer chromatography was performed on pre-coated silica gel 60 plates. Reaction products were purified by flash chromatography using 200–425 mesh silica gel. Tetrahydrofuran was distilled from sodium metal in the presence of benzophenone. Acetone was dried over $CaSO_4$ and distilled from $KMnO_4$. Methylene chloride was dried over anhydrous potassium carbonate, distilled and stored over 3 Å molecular sieves. All solvents were purified by fractional distillation. Other reagents were obtained from commercial suppliers and used as received.

4.2. Preparations of substrates

4-Ethoxycyclohexanone **3c** [25]: 4-Hydroxycyclohexanone ethylene ketal (0.8 g, 5 mmol), NaH (2 g), and ethyl bromide (10 ml added in four portions) reacted according to the literature procedure [26] to give 4-ethoxycyclohexanone ethylene ketal, 0.9 g. IR ν_{max} (neat): 2954 (m), 2881 (m), 1381 (m), 1117 (s), 1045 (m), 920 (m) cm^{-1}; ^{1}H NMR δ: 3.74–3.68 (4H, m), 3.45 (2H, q, $J = 7.0$), 3.39–3.32 (1H, m), 1.85–1.74 (2H, m), 1.72–1.62 (2H, m), 1.55–1.46 (2H, m), 1.32–1.22 (2H, m), 1.16 (3H, t, $J = 7.0$) ppm; ^{13}C NMR δ: 108.4, 74.5, 67.9, 64.2, 31.5, 28.7, 15.6 ppm. Subsequent deketalization [27] followed by flash chromatography using petroleum ether:ethyl acetate (20:1) as eluent gave **3c** as a colorless oil, 0.42 g (58%). IR ν_{max} (neat): 2967 (s), 2940 (s), 2868 (s), 1723 (vs), 1341 (w), 1111 (s), 1085 (m), 966 (w) cm^{-1}; ^{1}H NMR δ: 3.66(1H, heptet, $J = 2.9$ Hz), 3.51 (2H, q, $J = 7.0$ Hz), 2.57–2.49 (2H, m), 2.25–2.17 (2H, m), 2.06–1.97 (2H, m), 1.94–1.85 (2H, m), 1.19 (3H, t, $J = 7.0$ Hz) ppm; ^{13}C NMR δ: 211.3, 72.5, 63.6, 37.2, 30.6, 15.6 ppm.

4-Allyloxycyclohexanone **3d**: 4-Hydroxycyclohexanone ethylene ketal (1.0 g, 6.3 mmol), NaH (1 g), and allyl bromide (1 ml, 12 mmol) reacted according to the literature procedure [26] to give 4-allyloxycyclohexanone ethylene ketal as pale yellow oil, 1.12 g (89%). ^{1}H NMR δ: 5.94–5.83 (1H, m), 5.24 (1H, dq, $J_1 = 17.3$, $J_2 = 1.7$), 5.12 (1H, m), 3.95 (2H, dt, $J_1 = 5.47$, $J_2 = 1.7$), 3.90 (4H, dd, $J_1 = 3.9$, $J_2 = 2.9$), 3.42 (1H, heptet, $J = 3.2$), 1.84–1.75 (4H, m), 1.70(2H, q, $J = 7.7$), 1.55–1.48 (2H, m) ppm; ^{13}C NMR δ: 135.4, 116.3, 108.5, 74.1, 68.9, 64.3, 31.4, 28.5 ppm. Subsequent deketalization [27] followed by flash chromatography using petroleum ether:ethyl acetate (20:1) as eluent gave **3d** as a colorless oil, 0.87 g

(>99%). IR ν_{max} (neat): 3090 (w), 3010 (w), 2950 (s), 2870 (s), 1720 (vs), 1650 (w), 1420 (m), 1340 (m), 1100 (s), 1060 (s), 930 (m) cm^{-1}; ^1H NMR δ: 5.92–5.81 (1H, m), 5.23 (1H, dq, $J_1 = 17.3$ Hz, $J_2 = 1.7$ Hz), 5.10 (1H, dq, $J_1 = 10.4$ Hz, $J_2 = 1.4$ Hz), 3.96 (2H, dt, $J_1 = 5.5$ Hz, $J_2 = 1.7$ Hz), 3.68 (1H, heptet, $J = 2.9$ Hz), 2.55–2.46 (2H, m), 2.21–2.14 (2H, m), 2.05–1.96 (2H, m), 1.91–1.82 (2H, m) ppm; ^{13}C NMR δ: 210.9, 134.9, 116.4, 72.0, 69.1, 37.0, 30.4 ppm.

4-Benzyloxycyclohexanone **3e** [28]: Ketone **3a** (0.8 g, 70 mmol) reacted with benzyl bromide (2 ml, excess) in Ag_2O/THF. Flash chromatography of the crude product using petroleum ether:acetone (4:1) as eluent gave **3e** as colorless oil 0.35 g (45%) as well as 0.4 g of recovered starting material.

4-Acetoxycyclohexanone **3f**: A mixture of 4-hydroxycyclohexanone (**3a**, 0.5 g), acetic anhydride (3 ml), and pyridine (0.5 ml) was stirred at room temperature for 12 h. The solution, concentrated under vacuum to a half volume, showed 95% GC conversion. The concentrated solution was added to ethyl acetate and acidified to pH ~5 with conc. HCl. The layers were separated and the aqueous layer was extracted twice with ethyl acetate (50 ml). Combined organic layers were dried over anhydrous sodium sulfate; the solvent was removed and the product was purified by flash chromatography on silica gel using hexane:ethyl acetate (2:1) as eluent to give **3f** as colorless oil, 0.49 g (71% yield); IR ν_{max} (KBr): 2959.4 (s), 2873.0 (m), 1731.4 (vs), 1730.0 (vs), 1437.9 (m), 1367.7 (s), 1241.0 (vs), 1040.2 (m), 956.1 (m) cm^{-1}; ^1H NMR δ: 5.13(1H, m), 2.51(2H, m), 2.34 (2H, m), 2.05 (4H, m), 1.35 (3H, s) ppm; ^{13}C NMR δ: 210.1, 170.6, 68.9, 37.4 (2C), 30.6 (2C), 21.4 ppm.

4-Chlorocyclohexanone **3j** [25]: 4-Chlorocyclohexanol [29] was prepared from 7-oxabicyclo-[2, 2, 1]heptane by ring opening reaction with HCl catalyzed by $ZnCl_2$ in CH_2Cl_2, yield 81%; ^1H NMR δ: 3.94 (1H, m), 3.72 (1H, m), 2.16 (2H, m), 1.98 (2H, m), 1.65 (2H, m), 1.38 (2H, m) ppm; ^{13}C NMR δ: 68.2, 58.5, 33.3, 32.9 ppm. 4-Chlorocyclohexanol was oxidized with Jones reagent in acetone. The product was purified by flash chromatography on silica gel, using petroleum ether:acetone (10:1) as eluent to give **3j** as colorless oil, 68% yield. IR ν_{max} (KBr): 2957 (s), 2871 (m), 1714 (s), 1437 (m), 1324 (m), 1255 (m), 1129 (m), 775 (m) cm^{-1}; ^1H NMR δ: 4.43 (1H, m), 2.66 (2H, m), 2.29 (2H, m), 2.20 (4H, m) ppm; ^{13}C NMR δ: 208.8, 56.3, 37.3 (2C), 34.7 (2C) ppm.

4.3. Protocols for E. coli-mediated reactions

Propagation of E. coli strains: *E. coli* strain BL21(DE3)(pMM04) (or DH5α[pCMP201]) was streaked from a frozen stock on LB-Ampicillin plates and incubated at 37 °C until colonies were 1–2 mm in size, usually overnight. One colony was used to inoculate 10 ml of LB-Ampicillin medium in a 50 ml Erlenmeyer flask and shaken at 30 °C, 250 rpm overnight. Sterile glycerol was added (15% v/v) and the mixture was divided into 0.5 ml aliquots and stored in a −80 °C freezer. The frozen stock should be kept below −40 °C at all times, vials that had been thawed were discarded.

Protocol for E. coli-mediated reactions: The *E. coli* strain BL21(DE3)(pMM04) (or DH5α[pCMP201]) was streaked from a frozen stock on LB-Ampicillin plates and incubated at 37 °C until colonies were 1–2 mm in size. One colony was used to inoculate 10 ml of LB-Ampicillin medium in a 50 ml Erlenmeyer flask and shaken at 30 °C, 250 rpm overnight. This culture was used at a 1:100 (v/v) ratio to inoculate an LB-Ampicillin medium supplemented with 2% glucose in a baffled Erlenmeyer flask. The culture was incubated at 30 °C, 250 rpm until OD_{600} was approximately 0.3–0.4. IPTG stock solution (200 mg/ml in water) was added (0.1 μl/ml of medium) and the flask was shaken for another 30 min. The substrate was then added; 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 brine and dried with anhydrous Na_2SO_4 or $MgSO_4$. The solvent was removed on a rotary evaporator and the residue was purified by flash chromatography on silica gel.

5(2-Hydroxyethyl)oxolane-2-one **4a**: *E. coli* DH5α[pCMP201]-mediated oxidation of **3a** (100 mg, 0.88 mol) was performed according to general procedure. Chromatography on silica gel using petroleum ether:acetone (2:1) as eluent gave **4a** as a colorless oil, 84 mg (73%); chiral-GC showed 85% ee (*S*), $[\alpha]_D = +64°$, c 2.8 CH_2Cl_2.

5-Methoxyoxepan-2-one **4b**: *E. coli* DH5α[pCMP-201]-mediated oxidation of **3b** (100 mg, 0.78 mol) was performed according to general procedure. Chromatography on silica gel using petroleum ether:acetone (5:1) as eluent gave **4b** as a colorless oil, 81 mg (71%), $[\alpha]_D = +3.7$, c 0.5 CH_2Cl_2.

5-Ethoxyoxepan-2-one **4c**: *E. coli* DH5α[pCMP-201]-mediated oxidation of **3c** (97 mg, 0.68 mol) was performed according to general procedure. Chromatography on silica gel using hexane:ethyl acetate (5:1) as eluent gave lactone **4c** (91 mg, 84% yield); IR (neat) ν_{max}: 2947 (s), 1743 (s), 1446 (m), 1301 (m), 1097(s) cm^{-1}; ^1H NMR δ: 4.47 (1H, ddd, $J_1 = 13$ Hz, $J_2 = 9.5$ Hz, $J_3 = 1.2$ Hz), 4.03 (1H, ddd, $J_1 = 13$ Hz, $J_2 = 6$ Hz, $J_3 = 1.8$ Hz), 3.63 (1H, heptet $J = 2.8$ Hz), 3.46 (2H, dq, $J_1 = 7.0$ Hz, $J_2 = 2.0$ Hz), 2.94 (1H, ddd, $J_1 = 14$ Hz, $J_2 = 12$ Hz, $J_3 = 1.5$ Hz), 2.39 (1H, ddd, $J_1 = 14$ Hz, $J_2 = 8.5$ Hz, $J_3 = 1.3$ Hz), 2.00–1.76 (4H, m), 1.17 (3H, t, $J = 7$ Hz) ppm; ^{13}C NMR δ: 176.4, 73.9, 63.8, 63.7, 34.3, 28.1, 27.7, 15.7 ppm; MS: *m/e* 159 ($M + 1$), 158 (M), 130 ($M - 48$), 85 (75%), 57 (100%).

5-Allyloxyoxepan-2-one **4d**: *E. coli* DH5α[pCMP-201]-mediated oxidation of **3d** (105 mg, 0.68 mol) was performed according to general procedure. Chromatography on silica gel using hexane:ethyl acetate (5:1) as eluent gave lactone **4d** as a colorless oil (93 mg, 80% yield); IR (neat) ν_{max}: 2932(m), 2868 (m), 1732 (s), 1255 (m), 1171 (m), 1078 (m) cm^{-1}; ^1H NMR δ: 5.91 (1H, m), 5.24 (2H, dd, $J_1 = 17$ Hz, $J_2 = 10$ Hz), 4.52 (1H, dd, $J_1 = 13$ Hz, $J_2 = 10$ Hz), 4.08 (1H, dd, $J_1 = 6.5$ Hz, $J_2 = 1.8$ Hz), 4.00 (2H, m), 3.73 (1H, m), 3.00 (1H, t, $J = 13$ Hz), 2.43 (1H, m), 2.09–1.81 (4H, m) ppm; ^{13}C NMR δ: 176.1, 135.0, 117.0, 73.6, 69.4, 63.6, 34.3, 28.1, 27.7 ppm; MS *m/e*: 113 ($M - 57$, 11%), 85 (16%), 55(100%).

5-Benzyloxyoxepan-2-one **4e** [27]: *E. coli* DH5α-[pCMP201]-mediated oxidation of **3e** (50 mg, 0.25 mmol) was performed according to general procedure. Chromatography on silica gel using hexane:ethyl acetate (4:1) as eluent gave lactone **4e** as a colorless oil (51 mg, 95% yield), $[\alpha]_D = -2.9$, c 1.3, CH_2Cl_2.

5-Acetoxyoxepan-2-one **4f**: *E. coli* DH5α[pCMP-201]-mediated oxidation of **3f** (100 mg, 0.64 mmol) was performed according to general procedure. Chromatography on silica gel using hexane:ethyl acetate (9:1) as eluent gave lactone **4f** (89 mg, 81% yield); IR (neat) ν_{max}: 2954 (m), 1736 (s), 1739 (s), 1442 (m), 1373 (m), 1250 (s), 1062 cm^{-1}; ^1H NMR δ: 5.09 (1H, m), 4.40 (1H, ddd, $J_1 = 13$ Hz, $J_2 = 8.5$ Hz, $J_3 = 2$), 4.13 (1H, ddd, $J_1 = 13$ Hz, $J_2 = 6.3$ Hz, $J_3 = 2$ Hz), 2.88 (1H, m), 2.52 (1H, m), 2.07 (3H, s), 2.15–1.90 (4H, m) ppm; ^{13}C NMR δ: 175.2, 170.1, 70.3, 63.7, 34.2, 28.7, 27.8, 21.3 ppm.

Ethyl ester 5-oxepan-2-one carboxylic **4g**: *E. coli* DH5α[pCMP201]-mediated oxidation of **3g** (100 mg, 0.59 mmol) was performed according to general procedure to afford lactone **4g** (93 mg, 83% yield, 64% ee); IR (neat) ν_{max}: 2980 (m), 2935 (m), 1729 (s), 1255 (m), 1189 (m), 1071(m) cm^{-1}; ^1H NMR δ: 4.34 (1H, dd, $J_1 = 17$ Hz, $J_2 = 7$ Hz), 4.18 (1H, dd, $J_1 = 19$ Hz, $J_2 = 9$ Hz), 4.15 (2H, q, $J = 7.3$ Hz), 2.80–2.57 (3H, m), 2.20–1.90 (4H, m), 1.25 (3H, t, $J = 7.3$ Hz) ppm. ^{13}C NMR δ: 175.2, 173.8, 66.9, 61.1, 44.3, 32.3, 31.6, 25.0, 14.3 ppm.

5-Iodooxepan-2-one **4h**: *E. coli* DH5α[pCMP201]-mediated oxidation of **3h** (100 mg, 0.45 mmol), was performed in the presence of 0.5 g β-cyclodextrin, according to general procedure. Chromatography on silica gel using petroleum ether:ethyl acetate (4:1) as eluent gave **4h** (*R*) as white crystals 66 mg (65% yield and 82% ee), $[\alpha]_D = +25.4^\circ$, c 3.13, CH_2Cl_2.

5-Bromooxepan-2-one **4i**: *E. coli* DH5α[pCMP-201]-mediated oxidation of **3i** (100 mg, 0.56 mmol), was performed in the presence of 0.5 g β-cyclodextrin, according to general procedure. Chromatography on silica gel using petroleum ether:ethyl acetate (4:1) as eluent gave **4i** (*R*) as a colorless oil, 76 mg (70% yield and 64% ee), $[\alpha]_D = +6.9^\circ$, c 2.6, CH_2Cl_2.

5-Chlorooxepan-2-one **4j**: *E. coli* DH5α[pCMP-201]-mediated oxidation of **4j** (100 mg, 0.74 mmol), was performed in the presence of 0.5 g β-cyclodextrin, according to general procedure. Chromatography on silica gel using petroleum ether:ethyl acetate (4:1) as eluent gave **4j**(*R*) as a colorless oil, 72 mg (64% yield and 34% ee) $[\alpha]_D = +1.7^\circ$, c 1.1, CH_2Cl_2; IR ν_{max} (neat): 2954 (w), 1736 (vs), 1200 (m), 1065 (m) cm^{-1}. ^1H NMR δ: 4.55 (2H, m), 4.16 (1H, ddd, $J_1 = 13.8$ Hz, $J_2 = 5.0$ Hz, $J_3 = 2.5$ Hz), 3.12 (1H, m), 2.55 (1H, m), 2.28 (4H, m) ppm; ^{13}C NMR δ: 164.0, 63.5, 58.5, 37.9, 31.5, 29.1 ppm. *E. coli* BL21(DE3)[pMM04]-mediated oxidation of **3j** (100 mg) performed as above gave product **4j** (*S*) 63 mg (56% yield and 95% ee), $[\alpha]_D = -4.7$, c 2.1, CH_2Cl_2.

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