Baeyer-Villiger oxidations catalyzed by engineered microorganisms: Enantioselective synthesis of δ-valerolactones with functionalized chains

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Abstract: Cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp NCIMB 9871 expressed in baker's yeast and in *E. coli* and cyclopentanone monooxygenase (CPMO) from *Comamonas* (previously *Pseudomonas*) sp. NCIMB 9872 expressed in *E. coli* are new bioreagents for Baeyer-Villiger oxidations. These engineered microorganisms, requiring neither biochemical expertise nor equipment beyond that found in chemical laboratories, were evaluated as reagents for Baeyer-Villiger oxidations of cyclopentanones substituted at the 2-position with polar and nonpolar chains suitable for further modifications. Two such functionalized substrates that can be transformed into highly enantiopure lactones were identified. The performance and the potential of these bioreagents are discussed.

Key words: enantioselective Baeyer-Villiger oxidations, biotransformations, cyclohexanone monooxygenase, cyclopentanone monooxygenase, engineered baker's yeast, recombinant *E. coli*, optically pure 2-substituted cyclopentanones, optically pure lactones.

Résumé : La monooxygénase de la cyclohexanone (« CHMO ») obtenue à partir d'*Acinetobacter* sp NCIMB 9871 extraite de la levure pâtissière et d'*E. coli* et la monooxygénase de la cyclopentanone (« CPMO ») de *Comamonas* (antérieurement *Pseudomonas*) sp. NCIMB 9872 extraite d'*E. coli* sont les nouveaux bioréactifs pour les oxydations de Baeyer-Villiger. Ces microorganismes, qui ne nécessitent ni expertise biochimique ni équipement supplémentaire par rapport à ce que l'on retrouve dans les laboratoires chimiques, ont été évalués comme réactifs pour des oxydations de Baeyer-Villiger de cyclopentanones substituées en position 2 par des chaînes polaires ainsi que non polaires susceptibles d'être modifiées ultérieurement. On a identifié deux de ces substrats fonctionnalisés qui peuvent être éventuellement transformés en lactones de grande pureté énantiomérique. On discute de la performance et du potentiel de ces bioréactifs.

Mots clés : oxydations de Baeyer-Villiger, biotransformations, monooxygénase de la cyclohexanone, monooxygénase de la cyclopentanone, levure pâtissière, *E. coli* recombinant, cyclopentanone substituée en position 2 optiquement pure, lactones optiquement pures.

[Traduit par la Rédaction]

Introduction

The transformation of cyclic ketones to lactones is most frequently performed using peroxyacids. The lactones are important intermediates in organic synthesis, and hence the reaction has been studied and extensively employed for over a hundred years since its discovery in 1899 by Baeyer and Villiger (1). Many developments and changes to the reaction occurred over the years (2), and the accepted two-step mechanism is familiar to chemists (2, 3). In general, this is a successful reaction and problems only arise when scaling-up is required because peracid oxidants are toxic and intrinsically unstable. For this reason, new oxidizing agents continue to be investigated and several have been introduced over the years (4). The growing interest in the preparation of optically pure compounds has stimulated interest in creating enantioselective Baeyer-Villiger oxidations, and a number of transition-metal-based oxidants have been described (5). The yields and enantiomeric excesses (ee) of the lactone products varied, but were typically in the order of 30–70% ee. "Biological" Baeyer-Villiger oxidants, recognized for their use for several decades, perform these oxidations on a broad variety of compounds, frequently with good yields and with superior enantioselectivity (6). Although many microorganisms produce enzymes that perform Baeyer-Villiger reactions, two bacterial species *Acinetobacter* sp. and *Pseudomona*, have received the most attention in recent years. These

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We would like to dedicate this paper to J. Bryan Jones whose vision inspired us all.

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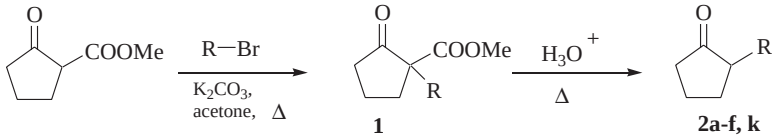
Scheme 1.



R : a = allyl
b = n-butyl
c = cyclopentyl
d = isobutyl
e = benzyl
f = 3-bromopropyl
g = hydroxymethyl
h = methoxymethyl
i = allyloxymethyl
j = acetoxymethyl
k = ethoxycarbonylmethyl

organisms and (or) the Baeyer-Villiger enzymes (flavin monooxygenases) that they produce are biological reagents of real value in organic synthesis.

Pseudomonas sp. NCIMB 10007, which carries genes inducible by growth on (+)- or (–)-camphor to produce the camphore flavin monooxygenase enzymes, has been investigated in numerous reactions. The crude-enzyme extracts from this organism were separated on the basis of the co-factor requirement to give two fractions: NADH-dependent MO1 and NADPH-dependent MO2. These fractions were used as selective catalysts in reactions with various unnatural substrates (6*b*, *c*, 7, 8). Elective culture grown on cyclohexanol allowed Donoghue and Trudgill (9) to identify *Acinetobacter* sp. NCIMB 9871. Since then, CHMO has been the subject of several mechanistic and biochemical studies (10–12). Extraction of cyclohexanone monooxygenase from this strain (CHMO EC 1.14.13.22) was optimized by Trudgill (13), and both the microorganism and enzyme continued to be extensively investigated (see reviews listed in ref. 6). The high enantioselectivity and broad spectrum of substrate acceptability made the enzyme and the organism the most widely used bioreagents for Baeyer-Villiger oxidations. Since, however, *Acinetobacter* sp. NCIMB 9871 is a class 2 pathogen and the isolated CHMO enzyme is relatively unstable and requires the NADPH co-factor, these biotransformations were not embraced enthusiastically by nonspecialists. The recently constructed recombinant strains of baker's yeast [15C(pKR001)] and *E. coli* [BL21(DE3)(pMM4)] overexpressing CHMO, which can be used to perform enantioselective Baeyer-Villiger oxidations in ordinary shake flasks in any organic laboratory (14, 15), should place these versatile reagents at the disposal of all organic chemists.

Elective culture grown on cyclopentanol yielded *Pseudomonas* sp. NCIMB 9872, now identified as *Comamonas* (16). This organism was able to oxidize cyclopentanol, via cyclopentanone and δ-valerolactone, to glutarate and, presumably, to acetate by an inducible set of enzymes (17). Purified cyclopentanone monooxygenase (CPMO; EC 1.14.13.16) is NADPH-specific (17*b*, *c*). Compared with NCIMB 9871, it has been studied relatively little in Baeyer-Villiger oxidations of unnatural substrates, probably because it was assumed to have stereoselectivity similar to that of cyclohexanone monooxygenase from *Acinetobacter* (6*b*, *c*, 7, 18, 19). We have now cloned and overexpressed the gene for cyclopentanone monooxygenase in the laboratory *E. coli* strain DH5α, [*E. coli* (CPMO)] making it as accessible as *E. coli* (CHMO) for development as another reagent for biological Baeyer-Villiger oxidations (16).

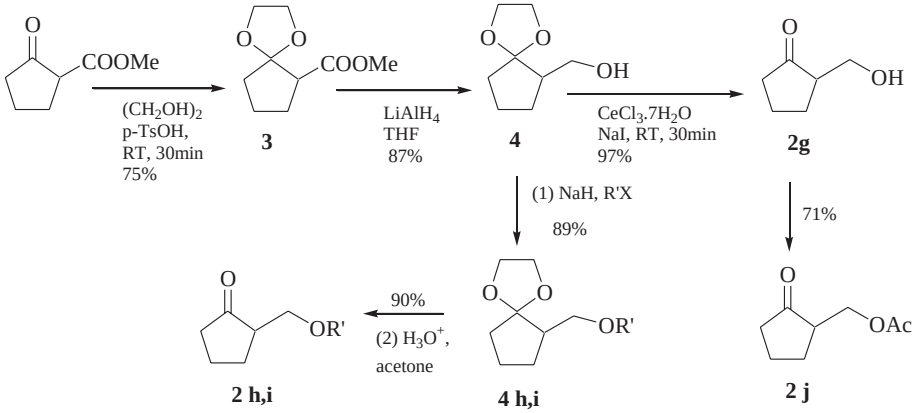
In an earlier paper, we reported several successful Baeyer-Villiger oxidations of 2-alkyl cyclopentanones mediated by the recombinant yeast (CHMO) (20). Our objective in the current project was to evaluate the recombinant CHMO- and CPMO-expressing strains as reagents for Baeyer-Villiger oxidations of 2-substituted cyclopentanones with functional groups on the side chain. Several such substrates were prepared and subjected to biotransformations. The stereochemical results of this work are discussed in this paper.

Results

2-Cyclopentylcyclopentanone (**2c**) was purchased from Aldrich, and compounds **2d**–**f** were prepared in good yields by alkylation and decarboxylation of methyl cyclopentanone-2-carboxylate as shown in Scheme 1. 2-Allylcyclopentanone (**2a**) was prepared by direct alkylation of cyclopentanone. 2-Oxocyclopentyl-1-methanol (**2g**) and 2-alkoxymethylcyclopentanones **2h** and **2i** were synthesized in 3 and 4 steps, respectively, from methyl cyclopentanone-2-carboxylate. In these reactions, the ketone was protected as an ethylene ketal, and the ester group was reduced by $LiAlH_4$ to give hydroxyketal **4**. Subsequent hydrolysis of the ketal with a mild Lewis acid catalyst preserved the hydroxyl group and yielded hydroxyketone **2g**. Alkylation of **4** followed by hydrolysis with a dilute aqueous acid gave alkoxyketones **2h** and **2i** (Scheme 2). The synthesis of ester **2k** paralleled that for simple alkyl side chains. Coupling of ethyl cyclopentanone-2-carboxylate with bromoacetate, followed by acidic hydrolysis and decarboxylation, afforded the desired product **2k** (Scheme 1).

Whole-cell mediated oxidations were carried out in shake flasks as previously described (15*a*, 21). In most cases, one equivalent of β-cyclodextrin (relative to the ketone) was included in the reaction medium to diminish any of the toxic effects of the substrates. The reactions were monitored by chiral GC, and the concentrations of the ketone and the lactone were measured against an internal standard (methyl benzoate). Only the results that were consistent in terms of conversion, enantioselectivity, time, and isolated yields over the course of several fermentations are listed in Tables 1*a* and 1*b*. Controlled chemical-oxidation experiments were performed for all substrates, and it was possible to achieve baseline resolution for the majority of ketones and (or) lactones. The absolute configuration of ketones **2a** and **2b** were deduced by comparison with literature values as described in our earlier paper (20). The absolute configurations for **2c**–**f** were tentatively assigned by analogy with prior results (22)

Scheme 2.



and with the demonstrated selectivity of the CHMO for (*S*)-cyclohexanones and cyclopentanones substituted with *n*-alkyl chains (6, 23). It should be noted that because of changes in numbering priority the *R*/*S* nomenclature is reversed for compounds **2a** and **2c**–**k**.

A majority of the reactions reported in Tables 1*a* and 1*b* were performed with the recombinant *E. coli* (CHMO); however, the same reactions that were re-investigated with the yeast (CHMO) gave virtually identical results, apart from small variations in the isolated yields. Compound **2d** appeared to be toxic to *E. coli* even in the presence of cyclodextrin and the successful transformation could only be achieved with the yeast (CHMO) strain. As can be seen in Table 1*a*, branching and increased bulk of the 2-substituents (**2c**–**e**) resulted in diminished selectivity of the CHMO oxidations. Parallel *E. coli* (CPMO) catalyzed reactions were generally faster and the lactones were isolated in excellent yields; however, the enantioselectivity of the transformation was consistently lower than that with CHMO. Extending the three-carbon chain by a bromine atom in compound **2f** restored CHMO's selectivity and the racemic ketone was kinetically resolved to give the lactone **5f** ($[\alpha]_D^{25}$ –63 (*c* 0.4, EtOAc), 95% ee) and the unreacted ketone **2f** ($[\alpha]_D^{25}$ –144 (*c* 1.65, CH_2Cl_2), 92% ee) in excellent isolated yields. The (*S*)-**2f** ketone was subsequently chemically oxidized to the corresponding lactone without loss of optical purity. Thus, readily prepared racemic 2-(3′-bromopropyl)-cyclopentanone (**2f**) introduces the possibility of further modification of the highly enantiopure δ-valerolactones.

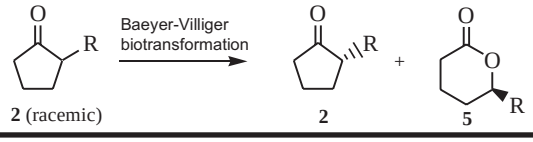
The *E. coli* (CPMO) catalyzed oxidations, shown in Table 1*a*, were intentionally interrupted at low conversion levels to estimate the enantioselectivity values (*E*) (24), which turned out to be low, but consistently showed a slight preference for the *same* ketone enantiomer as the CHMO-catalyzed transformations. Uninterrupted *E. coli* (CPMO) mediated reactions rapidly produced racemic lactones in excellent yields. All starting materials were generally consumed, and neither by-products nor metabolites were evident in the GC traces of the crude-reaction mixtures. Only in the case of ketone **2f** did the CPMO-mediated transformation show significant enantioselectivity.

The 2-cyclopentanones substituted with polar oxygen-containing groups on the side chain were acceptable substrates for both CHMO and CPMO (Table 1*b*). Ketone **2g**, with an unprotected hydroxy group on the side chain, reacted very slowly with yeast (CHMO) to give (*R*)-**5g** (34% ee, $[\alpha]_D^{25}$ –11 (*c* 4.5, EtOAc)). The absolute configuration for this compound was confirmed by comparison with the previously reported rotation (25). The difficulty in transporting the more polar substrates across cell membranes could be partially responsible for the sluggishness of this transformation. When the hydroxy group in **2g** was masked in the form of its ether derivatives **2h** and **2i,** the oxidations were faster, which supports the transport-through-membranes hypothesis. Furthermore, the enantioselectivity of the latter transformations was improved, particularly in the case of ketone **2i** bearing a long allyloxy chain. In this case, the lactone product (*R*)-**5i** was obtained in 93% ee. When the allyl group in lactone **5i** was removed (Pd/C in *p*-toluenesulfonic acid–H_2O), the product **5g** was shown to have the same configuration (*R*) as the major lactone enantiomer obtained from the oxidation of ketone **2g** by yeast (CHMO) or *E. coli* (CPMO). Again, the two enzymes showed a preference for the *same* enantiomer, and again, the CHMO enzyme proved to be the more selective of the two. Ketone **2j** was transformed by *E. coli* (CPMO) to the known lactone (*R*)-**5j** (26). At 41% conversion and 53% ee the reaction was stopped, the lactone product was isolated, and the optical rotation was determined ($[\alpha]_D^{25}$ –15 (*c* 2.5, EtOAc)). In a parallel reaction catalyzed by *E. coli* (CHMO), the **2j** substrate was also consumed and at 49% conversion an unidentified optically active product was formed in 74% ee. Lactone **5j** from the CPMO oxidation was subsequently chemically hydrolyzed ($NaHCO_3$, MeOH, pH 11) to (*R*)-**5g** without a change in optical purity. Ketone **2k** was rapidly oxidized by *E. coli* (CPMO), but the *E*-value was only 8. At 52% conversion, the product mixture consisted of lactone **5k** (61% ee, $[\alpha]_D^{25}$ –10 (*c* 1.2, EtOAc)) and residual ketone **2k** (66% ee, $[\alpha]_D^{25}$ –12.5 (*c* 0.8, EtOAc)). The transformation with *E. coli* (CHMO) was very slow and apparently nonselective and, since large quantities of by-products and metabolites were formed in the early stages of the reaction, we did not pursue this transformation any further.

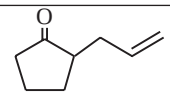
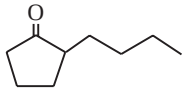
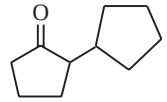
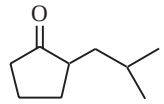
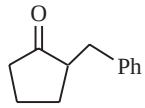
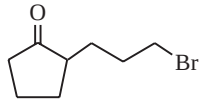
Discussion

Cyclopentanones substituted at the 2-position with alkyl chains of five carbons or longer are substrates to several Baeyer-Villiger monooxygenases. *Acinetobacter* species NCIB 9871 and TD 63 (23), *Pseudomonas* sp. NCIMB

Table 1a. Biological Baeyer-Villiger oxidations of 2-substituted cyclopentanones.



2 (racemic) **2** **5**

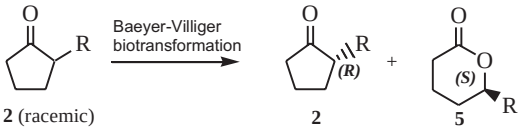
	Organism	Time (hours)	Conv. %[a]	Ketone **2** yield %[b] (ee %)[c]	Lactone **5** yield %[b] (ee %)[c]	E[d]
2a 	*E. coli* / **CHMO**	8	27	10 (51 *S*)	54 (34 *R*)	2.3
	E. coli / **CPMO**	3	95	5[a] (- -)	95[a] (- -)	0
2b 	*E. coli* / **CHMO**	12	50	32 (98 *R*)	18 (95 *S*)	200
	E. coli / **CPMO**	2.5	11	89[a] (2 *R*)	11[a] (19 *S*)	1.5
2c 	*E. coli* / **CHMO**	33	37	28 (--)[e]	20 (9)	1.3
	E. coli / **CPMO**	24	46	54[a] (--)[e]	46[a] (16)	1.6
2d 	*Yeast* / **CHMO**	19	35	18 (--)[e]	23 (83)	16
	E. coli / **CPMO**	--	ND			
2e 	*E. coli* / **CHMO**	16	34	56 (58)	28 (--)[e]	7.3
	E. coli / **CPMO**	16	85	15[a] (26)	85[a] (--)[e]	1.3
2f 	*Yeast* / **CHMO**	24	49	47 (92)	47 (95)	128
	E. coli / **CPMO**	36	36	64[a] (34)	36[a] (61)	5.7

[a] Conversion from G.C.; [b] Isolated yield of chromatographically purified product; [c] Determined from chiral G.C.(Supelco β-Dex 225 column); [d] Calculated according to Ref.(24); [e] Not resolved on chiral GC; **Note** that because of change in numbering priority the R/S nomenclature for ketones and and lactones **a**, c-f is reversed *vis a vis* that of **2b** and **5b**; also absolute configurations indicated are only tentatively assigned for compounds c-f (see text).

10007, and enzyme fractions MO1 and MO2 from that organism were used in oxidations of these compounds. The highest enantioselectivities were reported in reactions catalyzed by the MO2 fraction (7). *Acinetobacter*-catalyzed reactions were found to be highly enantioselective in several of the compounds studied, but the yields were low because the lactonic products were further metabolized by a hydrolase present in the organism. The problem was minimized by adding tetraethyl pyrophosphate to the reaction medium to inhibit the hydrolytic enzyme, and the reactions were re-investigated using *Acinetobacter* TD 63, a related species, which does not produce lactone hydrolase (23). Using our nonpathogenic yeast (CHMO) strain, we were able to achieve excellent kinetic resolution of the 2-alkylcyclopentanones, provided that the chains were at least four-carbon atoms long (20). It was previously observed that cyclohexanone monooxygenase exhibits virtually complete enantioselectivity for the (*S*)-enantiomer of 2-alkyl cyclohexanones, with the exception of 2-methyl cyclohexanone (10, 23, 27). With cyclopentanones, however, the chains must be longer to obtain comparable enantioselectivities. Cyclopentanones and cyclohexanones with functionalized chains have not been evaluated as substrates for either *Acinetobacter* sp. or the isolated cyclohexanone monooxygenase enzyme, but they were used as substrates in oxidations with the MO2 fraction from the species NCIMB 10007 (7, 19) and NCIMB 9872 (7, 18, 19). These compounds were of particular interest to us, since the oxidations with our easy-to-use bioreagents could provide convenient routes to optically pure lactones, ready for further modification through classical chemical reactions.

Our earlier experiments (20) with the yeast (CHMO) have shown that, although 2-*n*-butylcyclopentanone was fully kinetically resolved over the course of the Baeyer-Villiger oxidation, the 2-allylcyclopentanone was not, giving at best the (*R*)-lactone with 51% ee. To investigate the effect of branching and of polar substituents on the selectivity of CHMO and CPMO, we evaluated several potential substrates

Table 1b. Biological Baeyer-Villiger oxidations of 2-substituted cyclopentanones.



2 (racemic) **2** **5**

	Organism	Time (hours)	Conv. %[a]	Ketone **2** yield %[b] (ee %)[c]	Lactone **5** yield %[b] (ee %)[c]	E[d]
2g	*Yeast* / **CHMO**	96	24	20 (11 *S*)	10 (34 *R*)	2.3
	E. coli / **CPMO**	4	5	95[a] (12 *S*)	5[a] (43 *R*)	2.8
2h	*E. coli* / **CHMO**	36	14	66 (--)[e]	14 (44)	2.8
	E. coli / **CPMO**	6	78	22[a] (--)[e]	78[a] (56)	3.7
2i	*E. coli* / **CHMO**	36	45	19 (-- *S*)[e]	33 (93 *R*)	69
	E. coli / **CPMO**	19	30	70[a] (-- *S*)[e]	30[a] (81 *R*)	13
2j	*E. coli* / **CHMO**	--	--	--	--	--
	E. coli / **CPMO**	5	41	59[a] (-- *S*)[e]	41[a] (53 *R*)	4.6
2k	*E. coli* / **CHMO**	--	--	--	--	--
	E. coli / **CPMO**	10	52	48[a] (66)	52[a] (61)	8
				Ketone **6a,b** yield %[b] (ee %)	Lactone **7a,b** yield %[b] (ee %)	
6	*purified* **MO2**[f]	3.5	--	13 (75 *S*)	34 (83 *R*)	17
	purified **CPMO**[g]	0.5	61	37 (68 *R*)	59 (42 *S*)	5

[a] Conversion from G.C.; [b] Isolated yield of chromatographically purified product; [c] Determined from chiral G.C.(Supelco β-Dex 225 column); [d] Calculated according to Ref.(24); [e] Not resolved on GC; [f] Ref. 23; [g] Ref. 7, 19. Note that because of change in numbering priority the R/S nomenclature for ketones and lactones **g-k** is reversed *vis a vis* that of compounds **2b** and **5b.**

listed in Table 1. The ability of CHMO to accommodate polar chains was intimated by the fact that both isolated enzyme (28) and our recombinant organisms successfully oxidized cyclohexanones with polar chains in the 3- and 4-positions (29). To the best of our knowledge, however, these compounds have not been tested as substrates for CPMO.

The point that needs to be stressed involves generalizations applied to the enantioselectivity trends of various oxygenases. Although these can be useful when one chooses an enzyme or an organism for a specific transformation, wide speculation, resulting in nonconfirmed assignments of absolute configuration, are unwarranted. We experienced this when comparing oxidations by CHMO and CPMO with those performed by MO2. In an earlier study, Adger et al. (18) showed that monooxygenase MO2 from camphor-grown NCIMB 10007 species converted racemic 2-(2′-acetoxyethyl)cyclohexanone (**6**) to the corresponding *R*-(–)-lactone **7a** (40% conversion, 83% ee), while purified CPMO from cyclopentanol-grown NCIMB 9872 transformed the same substrate to its *S*-(+) antipode **7b** (59% conversion, 42% ee), as shown in Table 1*b*. The rotations were reported and the absolute configurations were unambiguously confirmed after the two lactones were converted to the (*R*)- and (*S*)-lipoic acids. MO2 was also used to oxidize cyclopentanones **2j** and **2k** to lactones **5j** and **5k**, respectively, in better than 98% ee (7). The two lactones were reported as having the *R* configuration. Unfortunately, neither rotations nor references were specified. We were able to unambiguously identify lactone **5j** from the CPMO oxidation as the (*R*) enantiomer (vide supra), but the absolute configuration

of lactone **5k** has not yet been confirmed. Although it was shown (19) that enantioselectivity of CPMO (NCIMB 9872) can be opposite to that of MO2 (Table 1*b*, example 6), it appears that, at least for cyclopentanone **2j**, the enantioselectivities are the same and that both enzymes preferentially oxidize the (*R*) ketone.

In summary, the goals of this project were to determine (*i*) if cyclopentanones with more polar functionalized substituents in the 2-position are substrates for CHMO and CPMO; (*ii*) if highly optically enriched lactone (or lactones) suitable for further chain modifications could be identified; and (*iii*) if the performance of CPMO was adequately different from the CHMO to attempt its development as an alternative bioreagent for biological Baeyer-Villiger reactions. From the results reported here it is clear that while Baeyer-Villiger oxidations of 2-substituted cyclopentanones by CHMO- and CPMO-expressing strains are frequently nonselective, some compounds are oxidized with a very high enantiselectivity; in the present survey three of the candidates investigated (**2b**, **2f**, and **2i**) were found to meet the selectivity criteria. In general, of the two enzymes studied, CHMO was shown to be the more selective. On the other hand, oxidations with CPMO are appealing for another reason: these transformations are fast, clean, and there are no by-products or metabolites. Where enantioselectivity is not a primary concern, the *E. coli* (CPMO) strain (16) can serve as an excellent biological equivalent of organic peracids.

In conclusion, the results presented here indicate that "designer microbes" expressing various Baeyer-Villiger monooxygenases can contribute significantly to "developing synthetic methodology and "green" processes that meet criteria of a sustainable, environmentally conscious development (30)."

Experimental

Optical rotations were measured on a PerkinElmer 241 polarimeter operating at room temperature. IR spectra were recorded from thin films on a Mattson Satellite FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian XL-200 or Bruker AMX-400 FT-NMR spectrometers. All spectra were recorded in $CDCl_3$ solutions unless otherwise specified. Chemical shifts are reported in ppm with TMS as internal standard. EI-MS spectra and HRMS were obtained on Kratos MS 50 mass spectrometer. Packed-column gas chromatography was performed on a Shimadzu GC-9A gas chromatograph employing a customer-packed column (1/8" × 1 m, 5% OV-101 on 100/120 Supelcoport, Supelco Inc.). Capillary gas chromatography was performed on a Hewlett Packard 5890 instrument employing a 0.54 μm × 1.00 mm × 15 m DB-1301 column (J&W) or Shimadzu GC-9A using a 0.32 μm × 0.25 mm × 30 m β-Dex 225 column (Supelco). All the GC instruments used flame-ionization detectors and helium as the carrier gas. The injector and detector temperatures were maintained at 225°C and 300°C, respectively. Thin-layer chromatography was performed on precoated silica gel 60 plates (Aldrich). Flash chromatography was performed on silica gel (200–425 mesh, Fisher Scientific or SiliCycle). Tetrahydrofuran was distilled from Na 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.

Protocols for biotransformations

Preparation of yeast cells (pKR001) and the general procedure for yeast-catalyzed Baeyer-Villiger oxidations were previously described (14, 21, 22). Maintenance and biotranformation protocols for the reactions with *E. coli* [BL21(DE3)(pMM4)] are outlined in refs. 15 and 29.

Protocol for *E. coli*-(CPMO) mediated oxidations

The *E. coli* strain 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 was shaken at 30°C and 250 rpm overnight. This culture was used at a 1:100 (v/v) ratio to inoculate an LB-ampicillin medium supplemented with 10% glucose in a baffled-Erlenmeyer flask. The culture was incubated at 30°C and 250 rpm until OD_{600} was approximately 1. IPTG (isopropyl thio-β-D-galactoside) stock solution (200 mg per mL in water) was added (0.1 μL per mL of medium) and the flask was shaken for another 30 min. The substrate was then added, and, if solubility was a problem, cyclodextrin was introduced at this stage. The culture was agitated at 30°C at 250 rpm and monitored by GC or TLC. 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.

Compounds 2d and 2e

Compounds **2d** and **2e** were prepared according to modified literature procedure (31). Anhydrous K_2CO_3 (5.5 g, 40 mmol) and acetone (20 mL) were placed in a three-neck round-bottom flask equipped with reflux condenser. After flushing the system with nitrogen, methyl cyclopentanone-2-carboxylate (20 mmol) and alkyl halide (20 mmol) were added, and the stirred reaction mixture, maintained under nitrogen, was gently heated at reflux for two days until all starting material was consumed or a significant amount of by-product was formed as shown by GC. The mixture was cooled to room temperature and poured into ice water. The aqueous phase was extracted with ethyl acetate (3 × 200 mL) and the combined extracts were washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent on a rotary evaporator the crude product **1d** (or **1e**) was purified by flash chromatography on silica gel using a mixture of hexane and ethyl acetate as eluent. The resulting 2-alkylketoester (**1d** or **1e**, 10 mmol) was dissolved in a mixture of glacial acetic acid (20 mL) and HCl (6 M, 10 mL) and gently heated at reflux under nitrogen until all starting material was consumed as shown by GC (usually 4–6 h). The reaction mixture was cooled to room temperature, poured into ice water, and extracted with ethyl acetate or petroleum ether (3 or 4 × 150 mL). The combined extracts were washed with water and then with saturated $NaHCO_3$. After drying over anhydrous Na_2SO_4, the solvent was

removed and the crude product **2** was purified by vacuum distillation.

2*-iso-*Butyl cyclopentanone (2d)

bp 120–140°C (18–25 Torr). IR ν_{max} (neat) (cm^{-1}): 2957.5 (s), 2869.1 (m), 1739.4 (s), 1467.1 (m), 1154.0 (m). ^{1}H NMR δ: 2.20 (m, 2H), 2.10 (m, 1H), 2.09–1.90 (m, 3H), 1.72 (m, 1H), 1.58 (m, 1H) 1.42 (m, 1H), 1.08, (m, 1H), 0.86 (d, *J* = 3.2 Hz, 3H), 0.82 (d, *J* = 3.0 Hz, 3H). ^{13}C NMR δ: 221.8, 47.4, 38.9, 37.9, 30.0, 26.1, 23.3, 21.4, 20.7.

2-Benzylcyclopentanone (2e)

Obtained after chromatography (hexane–ethyl acetate 10:1) in 85% yield as pale yellow oil: IR υ_{max} (neat) (cm^{-1}): 3000 (m), 2960 (s), 2870 (m), 1730 (vs), 1600 (w), 1490 (m), 1450 (s), 1400 (m), 1150 (s), 690 (s). ^{1}H NMR δ: 7.27 (m, 2H), 7.17 (m, 3H), 3.15 (dd, *J* = 7 and 3 Hz, 1H), 2.54 (dd, *J* = 7 and 6 Hz, 1H), 2.37 (m, 2H), 2.12 (m, 2H), 1.97 (m, 1H), 1.71 (m, 1H), 1.60 (m, 1H). ^{13}C NMR δ: 220.1, 140.0, 128.8, 128.4, 126.1, 51.0, 38.2, 35.6, 29.1, 20.5.

2-(3′-Bromopropyl)cyclopentanone (2f)

The racemic ketone (32) was purified by flash chromatography on silica gel using 10:1 petroleum ether–acetone as the eluant to give **2f** as colourless oil (2.6 g, 41% yield after two steps). IR ν_{max} (neat) (cm^{-1}): 2960 (m), 2875 (m), 1743 (s), 1374 (w), 1242 (m), 1157 (w). ^{1}H NMR δ: 4.00 (t, *J* = 3 Hz, 2H), 2.23 (m, 2H), 2.03 (m, 1H), 1.80–1.60 (m, 4H), 1.46 (m, 2H), 1.27 (m, 2H). ^{13}C NMR δ: 220.7, 64.1.3, 48.6, 38.0, 29.5, 26.6, 26.0, 20.6. MS *m*/*z* (%): 204/206 (1), 125 (100), 107 (38), 83 (26), 67 (37), 55 (85).

6-Cyclopentyltetrahydropyran-2-one (5c)

Ketone **2c** (100 mg, 0.66 mmol) was oxidized using *E. coli* (CHMO) and was purified by flash chromatography on silica gel (petroleum ether–ethyl acetate, 5:1) to afford **5e** (22 mg, 20%, 9% ee). IR ν_{max} (neat) (cm^{-1}): 2952 (s), 2869 (m), 1731 (s), 1243 (m), 1176 (m). ^{1}H NMR δ: 4.10, (m, 1H), 2.57 (m, 1Ha), 2.45 (m, 1Hb); 2.07 (m, 2H), 1.90 (m, 3H), 1.75–1.50 (m, 4H), 1.42 (m, 2H), 1.28 (m, 2H). ^{13}C NMR δ: 172.3, 84.5, 45.2, 29.7, 28.9, 28.8, 27.1, 25.6, 25.5, 18.7.

6-*iso*-Butyltetrahydropyran-2-one (5d)

Biotransformation of **2d** using *E. coli* (CHMO) followed by flash chromatography (petroleum ether–acetone, 7:1 then 5:1) gave **5d** (23%, 83% ee). IR ν_{max} (neat) (cm^{-1}): 2928 (s), 2855 (m), 1741 (s), 1463 (w), 1246 (m), 1047(m). ^{1}H NMR δ: 4.31 (m, 1H), 2.51 (m, 1H), 2.43 (m, 1H), 1.87 (m, 4H), 1.63 (m, 1H), 1.47 (m, 1H), 1.28 (m, 1H), 0.90 (d, *J* = 1 Hz, 3H), 0.88 (d, *J* = 1 Hz, 3H). ^{13}C NMR δ: 172.0, 78.7, 44.9, 29.4, 28.3, 23.9, 23.0, 22.0, 18.4.

6-Benzyltetrahydropyran-2-one (5e)

Biotransformation of **2e** (100 mg, 0.57 mmol) using *E. coli* (CHMO) followed by flash chromatography (petroleum ether–ethyl acetate, 4:1) gave **5e** pale yellow oil (28%, 61% ee). IR ν_{max} (neat) (cm^{-1}): 3060 (w), 3027 (w), 2951 (m), 2874 (m), 1733 (vs), 1496 (m), 1455 (m), 1238 (s), 1178 (m), 1040 (m), 749 (w), 700 (m). ^{1}H NMR δ: 7.33–7.14 (m, 5H), 4.51 (m, 1H), 3.09–2.88 (d ABquartet, *J* = 27 and 11 Hz, 2H), 2.57 (m, 1H), 2.44 (m, 1H), 1.89 (m, 2H), 1.52 (m, 2H). ^{13}C NMR δ: 171.6, 136.4, 129.5, 128.5, 126.8, 81.0, 42.1, 29.4, 27.0, 18.4.

(*R*)-6-(3′-Bromopropyl)tetrahydropyran-2-one (5f) (33)

Ketone **2f** (100 mg, 0.45 mmol) and γ-cyclodextrin (0.5 g) were added to YP-Gal (100 mL) in a 250-mL baffled-conical flask. The mixture was then shaken at 30°C at 250 rpm for 5–10 min to obtain a uniform dispersion. A 1-mL portion of yeast cells was added to the reaction flask and the culture was shaken at 30°C (250 rpm). The reaction monitored by GC and chiral-GC, and was stopped at 50% conversion. After saturating the mixture with NaCl, the organic layer was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated by rotary evaporation. Separation of chiral lactone and ketone by flash chromatography (hexane–acetone, 3:1) gave lactone **5f** as colorless oil (51 mg, 47% yield, 94% ee) and ketone (*S*)-**2f** also as colorless oil (53 mg, 47% yield, 92% ee). (*R*)-**5f**: $[\alpha]_D^{25}$ –63 (*c* 0.4, EtOAc, 95% ee (GC)). IR ν_{max} (neat): 2956, 1734 (s), 1242, 1944. ^{1}H NMR δ: 4.29 (m, 1H), 4.08 (m, 2H), 2.57 (m, 1H) 2.42 (m, 1H), 1.88–1.71 (dm, 6H), 1.51 (m, 2H). ^{13}C NMR δ: 171.3, 80.0, 64.1, 32.5, 28.0, 25.1, 24.5, 18.7. MS *m*/*z*: 223 ($[M + 1]^+$), 141, 112, 99, 55. HRMS calcd. for $C_8H_{13}BrO_2$: 220.0099 / 222.0078; found: 220.0099 / 222.0079. Unreacted ketone (42 mg, 42%) (*S*)-**2f**: $[\alpha]_D^{25}$ –144 (*c* 1.65, CH_2C1_2, 92% ee (GC)). An analytical sample of (*S*)-**2d** was oxidized with *m*-chloproperbenzoic acid (TFA, CH_2Cl_2) to give (*S*)-**5f** 92% ee (GC).

1,4-Dioxa-spiro[4.4]nonane-6-carboxylic acid methyl ester (3)

Methyl cyclopentanone-2-carboxylate (5.0 mL, 40 mmol) was added to a solution of *p*-toluene sulfonic acid (5.6 g, 40 mmol) in ethylene glycol (20 mL) and stirred at room temperature for 30 min. The mixture was poured into 50 mL 1 M KOH solution saturated with NaCl and extracted with ether (4 × 50 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed on a rotary evaporator to give **3** as a colourless oil, 4.73 g (75%). IR ν_{max} (neat) (cm^{-1}): 2950 (s), 2890 (m), 1700 (vs), 1480 (m), 1350 (m), 1210 (s), 1040 (m). ^{1}H NMR δ: 4.05–4.00 (m, 1H), 3.97–3.88 (m, 3H), 3.70 (s, 3H), 2.92 (t, *J* = 7.7 Hz, 1H), 2.12 (m, 1H), 1.98–1.79 (m, 4H), 1.67 (m, 1H). ^{13}C NMR δ: 172.8, 118.3, 65.1, 64.5, 52.2, 51.7, 51.6, 36.7, 26.9, 22.0.

1,4-Dioxa-spiro[4.4]non-6-yl-methanol (4)

1,4-Dioxa-spiro[4.4]nonane-6-carboxylic acid methyl ester (**3**, 3.8 g in 10 mL THF, 20 mmol) was added to a vigorously stirred suspension of $LiAlH_4$ (0.8 g, 21 mmol) in THF (60 mL) at 0°C. The mixture was stirred for 1.5 h and allowed to warm up to room temperature. The reaction was quenched by pouring it into an ice cold saturated tartaric acid solution and was then extracted with ethyl acetate (5 × 40 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvent was removed on a rotary evaporator to give **4** as a colourless oil, 2.81 g (87%). IR ν_{max} (neat) (cm^{-1}): 3500 (m, br), 2950 (s), 2890 (s), 2390 (s), 2280 (m), 1460 (m), 1390 (m), 1330 (m), 1160 (s), 1100 (m), 1020 (s). ^{1}H NMR δ: 3.96–3.84 (m, 4H), 3.66–3.54 (m,

2H), 2.64 (br s, 1H), 2.34–2.05 (m, 1H), 1.84–1.76 (m, 1H), 1.74–1.46 (m, 5H). ^{13}C NMR δ: 119.0, 64.5, 64.0, 62.5, 47.0, 35.6, 25.6, 21.3.

2-Hydroxymethylcyclopentanone (2g) (34)

$CeCl_3 \cdot 7H_2O$ (1.86 g, 5 mmol) and KI (0.3 g, 1.8 mmol) were mixed with 18 g silica gel (200–425 mesh) in acetone (30 mL) and ground in a mortar. Compound **4** (0.96 g, 6 mmol) was added and the mixture was stirred at room temperature for 30 min. The silica was filtered off and the solid was washed with acetone. The solvent was removed on a rotary evaporator and the residue was purified by flash chromatography (hexane–ethyl acetate, 3:1) to give **2g** as a colourless oil, 0.67 g (97%). IR ν_{max} (neat) (cm^{-1}): 3427 (s, br), 2966 (s), 2877 (s), 1734 (vs), 1404 (m), 1154 (m), 1054 (m). ^{1}H NMR (acetone-d_6) δ: 3.73–3.64 (m, 3H, including an overlapping OH peak), 2.26–2.12 (m, 3H), 2.10–1.95 (m, 2H), 1.96–1.75 (m, 2H). ^{13}C NMR (acetone-d_6) δ: 219.5, 61.6, 51.7, 39.0, 27.1, 21.4.

6-Hydroxymethyltetrahydropyran-2-one ((*R*)-5g)

Ketone **2g** oxidized using yeast (CHMO) was purified by flash chromatography (petroleum ether–acetone, 2:1) to give colorless oil (16 mg, 14%, 34% ee). $[\alpha]_D^{25}$ –11 (*c* 4.5, EtOAc. Lit. (25) (*R*)-enantiomer: $[\alpha]_D^{25}$ –32 (*c* 1.3, $CHC1_3$)). IR ν_{max} (neat) (cm^{-1}): 3394 (m, br), 2947 (m), 2881 (w), 1723 (vs), 1249 (s), 1058 (s). ^{1}H NMR δ: 4.42–4.35 (m, 1H), 3.76 (dd, *J* = 12.1 and 3.2 Hz, 1H), 3.66 (dd, *J* = 12.3 and 5.5 Hz, 1H), 2.64–2.54 (m, 1H), 2.48–2.39 (m, 1H), 2.00–1.90 (m, 1H), 1.90–1.81 (m, 2H), 1.81–1.64 (m, 2H). ^{13}C NMR δ: 171.4, 81.0, 64.9, 29.6, 23.6, 18.3.

6-Methoxymethyl-1,4-dioxa-spiro[4.4]nonane (4h)

NaH (2 g, 50% dispersion in mineral oil, excess) was added to a 100-mL two-neck round-bottom flask and the system was flushed with nitrogen. NaH was washed 3 times with THF, and then was finally suspended in THF (10 mL). MeI (2 mL, excess) was added, followed by 1,4-dioxa-spiro[4.4]non-6-yl-methanol **4** (1.58 g in 40 mL THF, 10 mmol) with vigorous stirring. After reacting overnight, the mixture was poured onto ice cubes and extracted with ethyl acetate (3 × 35 mL). The combined extracts were washed once with 0.2 M KOH, once briefly with 0.5 M HCl, and once with brine. After drying over anhydrous K_2CO_3, the solvent was removed on a rotary evaporator to give the title product as a colourless oil, 1.11 g (89%). IR ν_{max} (film) (cm^{-1}): 2950 (vs), 2870 (vs), 1450 (s), 1380 (s), 1320 (s), 1200 (s), 1100 (vs), 750 (s). The crude product was used in the next step without further characterizations.

2-Methoxymethylcyclopentanone (2h)

6-Methoxymethyl-1,4-dioxa-spiro[4.4]nonane (**4h**, 0.8 g, 4.7 mmol) was dissolved in acetone (10 mL). In a separate flask $PdCl_2$ (30 mg) was dissolved in acetone (1 mL) and acetonitrile (0.5 mL). The two solutions were combined and TFA (1 mL) was added. The mixture was stirred at room temperature for 3 h, poured into brine, and extracted with ethyl acetate (3 × 35 mL). The combined extracts were dried over anhydrous Na_2SO_4 and the solvent was removed on a rotary evaporator. The residue was purified by flash chromatography (hexane–ethyl acetate, 10:1) to give **2h** as a colourless oil, 0.56 g (94%). IR ν_{max} (neat) (cm^{-1}): 2970 (s), 2920 (s), 2870 (s), 1730 (s), 1150 (m), 1120 (m). ^{1}H NMR δ: 3.53 (m, 2H), 3.30 (s, 3H), 2.34–1.96 (m, 5H), 1.80 (m, 2H). ^{13}C NMR δ: 219.5, 71.5, 59.0, 49.4, 38.6, 27.1, 20.8.

6-Methoxymethyltetrahydropyran-2-one (5h)

Ketone **2h** was oxidized using *E. coli* (CHMO) to give **5h**, which was purified by flash chromatography (petroleum ether–acetone, 5:1, then petroleum ether–acetone, 3:1) to give a colorless oil (16 mg, 14%, 44% ee). IR ν_{max} (neat) (cm^{-1}): 2940 (m), 2881 (m), 2815 (w), 1736 (vs), 1249 (s), 1071 (s). ^{1}H NMR δ: 4.46–3.90 (m, 1H), 3.55–3.47 (m, 1H), 3.36 (s, 3H), 3.28–3.18 (m, 1H), 2.62–2.53 (m, 1H), 2.43–2.34 (m, 1H), 1.98–1.87 (m, 2H), 1.74–1.62 (m, 2H). ^{13}C NMR δ: 172.6, 79.0, 74.4, 59.4, 29.6, 24.5, 18.3. The unreacted **2h** was also isolated: 7% ee; $[\alpha]_D^{25}$ –17 (*c* 1.3, CH_2Cl_2).

6-Allyloxymethyl-1,4-dioxa-spiro[4.4]nonane (4i)

Compound **4i** was synthesized according to the same protocol as **4h**, except allyl bromide was used instead of methyl iodide. The crude product was used in the following step without purification.

2-Allyloxymethylcyclopentanone (2i)

Crude **4i** dissolved in a mixture of acetone (20 mL) and HCl (5 mL, 2 M) was stirred at room temperature for 2 h. Acetone was removed by rotary evaporation and the residue was extracted with CH_2Cl_2. Combined extracts were dried over anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by flash chromatography (hexane–ethyl acetate, 6:1) to give **2j** as a colorless oil, 0.35 g (90% from **4i**). IR ν_{max} (neat): 3081 (w), 2967 (s), 2877 (s), 1737 (vs), 1647 (w), 1152 (s), 1088 (s), 926 (s). ^{1}H NMR δ: 5.84–5.73 (m, 1H), 5.20–5.04 (m, 2H), 3.87 (dt, *J* = 5.5 and 1.4 Hz, 2H), 3.57–3.48 (m, 2H), 2.31–2.10 (m, 3H), 2.12–2.00 (m, 1H), 2.00–1.91 (m, 1H), 1.88–1.76 (m, 1H), 1.76–1.65 (m, 1H). ^{13}C NMR δ: 219.3, 134.5, 116.6, 71.9, 68.9, 49.2, 38.5, 27.0, 20.7.

6-Allyloxymethyltetrahydropyran-2-one ((*R*)-5i)

Ketone **2i** oxidized using *E. coli* (CHMO) was purified by flash chromatography (petroleum ether–acetone 5:1 followed by petroleum ether–acetone, 3:1). $[\alpha]_D^{25}$ = –14.1 (*c* 2.2, $CDCl_3$). IR ν_{max} (neat) (cm^{-1}): 3078 (w), 2947 (m), 2876 (m), 1726 (vs), 1453 (m), 1347 (m), 1240 (s), 1062 (s), 932 (m). ^{1}H NMR δ: 5.91–5.80 (m, 1H), 5.25 (dq, *J* = 17.3 and 1.5 Hz, 1H), 5.16 (qd, *J* = 10.4 and 1.2 Hz, 1H), 4.46–4.39 (m, 1H), 4.01 (dd, *J* = 5.5 and 1.5 Hz, 2H), 3.56 (m, 2H), 2.60–2.52 (m, 1H), 2.48–2.39 (m, 1H), 1.98–1.88 (m, 2H), 1.87–1.76 (m, 1H), 1.75–1.63 (m, 1H). ^{13}C NMR δ: 171.2, 134.2, 117.3, 79.1, 72.5, 71.8, 29.6, 24.6, 18.2. MS *m*/*z* (%): 171 ($[M + 1]^+$, 0.2), 114 (48), 99 (58), 71 (100), 55 (28). The isolated chiral ketone **2i**, 19% yield, 81% ee: $[\alpha]_D^{25}$ – 56 (*c* 1.9, CH_2Cl_2). Lactone (*R*)-**5i** was hydrogenated (5% Pd/C in methanol–water (2:1) containing 1% *p*-toluene sulfonic acid, 5 h) to give (*R*)-**5g** (60%, 93% ee) (25).

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