Microbial Baeyer–Villiger oxidation of terpenones by recombinant whole-cell biocatalysts—formation of enantiocomplementary regioisomeric lactones†

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Recombinant whole-cell expression systems for Baeyer–Villiger monooxygenases of various bacterial origin were utilized in the regiodivergent biooxidation of cyclic terpenones enabling access to enantio- and regioisomeric lactones on preparative scale.

Introduction

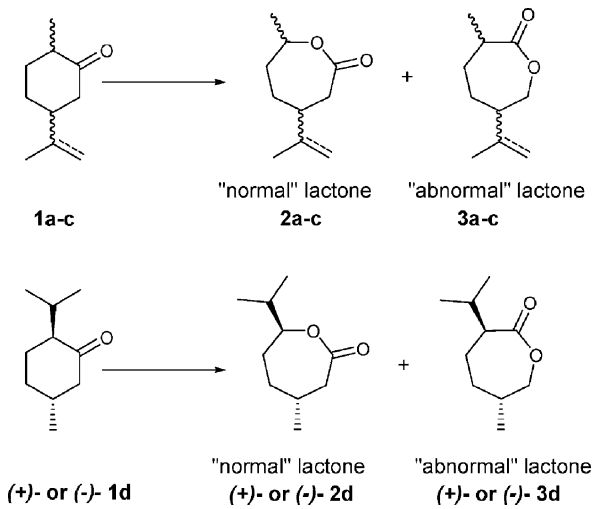
Baeyer–Villiger oxidation of cyclic ketones to chiral lactones allows access to highly interesting and versatile intermediates in the synthesis of bioactive or natural products.[1–4] The biocatalytic approach to realize this transformation with high regio- and enantioselectivity takes advantage of the high promiscuity of flavin dependent monooxygenases to accept a large diversity of non-natural substrates. Moreover, this "green chemistry" strategy utilizes molecular oxygen for the oxidation process as most sustainable oxidant. An increasing number of such Baeyer–Villiger monooxygenases (BVMOs) has become available in recent years. Due to the application of recombinant overexpression systems for the production of biocatalyst as well as the direct implementation of such systems in whole-cell mediated biotransformations, the microbial Baeyer–Villiger biooxidation has become a highly attractive methodology to access optically pure lactones in both kinetic resolution and desymmetrization processes.[4]

While the regioselectivity of the Baeyer–Villiger oxidation is governed predominantly by electronic effects leading to preferred migration of the more nucleophilic and higher substituted carbon center, stereoelectronic effects can override this rule-of-thumb in reactions catalyzed by salen-type complexes with Zr as the central atom[5] or, more frequently, in enzymatic transformations.[6] In particular, in the series of fused bicycloketones incorporating a cyclobutanone structural motif, regiodivergent biooxidation of the antipodal substrate ketones to enantiomerically pure "normal" and "abnormal" lactone regioisomers was observed as one of the remarkable behaviors of BVMOs.[7]

However, such a behavior was found only in a few cases of the cyclohexanone series. Alphand and Furstoss reported that wild-type cells of *Acinetobacter* NCIMB 9871 or the mutant strain *Acinetobacter* TD63 convert (−)-*trans*-dihydrocarvone to the expected normal lactone, while (+)-*trans*-dihydrocarvone afforded abnormal lactone.[8] In contrast, metabolism of carveol and dihydrocarveol in *Rhodococcus erythropolis* DCL14 displayed transformation of (+)-*trans* and (−)-*cis*-dihydrocarvone to normal lactones while opposite enantiomers led to the formation of the abnormal lactones.[9]

Normal lactones of α-substituted cyclohexanones can be also synthesized by chemical Baeyer–Villiger oxidation using *m*-chloroperoxybenzoic acid, trifluoroperoxyacetic acid, peroxyacetic acid and hydrogen peroxide as oxidants. However, starting from unsaturated ketones such as dihydrocarvone, competitive oxidation of the carbon–carbon double bond can take place. Within non-chiral reactions, utilization of Sn-beta zeolites and platinum complexes in H_2O_2 or PSA (monopersuccinic acid) in water enabled complete control of the parallel oxidation processes, ultimately giving selective Baeyer–Villiger oxidation upon reaction of bifunctional substrates.[10]

Herein, we are reporting a preliminary study on regiodivergent and enantiocomplementary biooxidations in the cyclohexanone series. Utilizing a collection of BVMO producing recombinant *E. coli* strains we were able to provide access to all regio- and enantiomeric lactones for the first time starting from terpenone precursors. In particular we have studied the biooxidation of pure enantiomers of *trans*- and *cis*-dihydrocarvone **1a/b**, carvomenthone **1c** and menthone **1d** (Scheme 1). We compared substrate acceptance profiles and stereopreferences of cyclohexanone (CH) and cyclopentanone (CP) monooxygenases originating from *Acinetobacter* ($CHMO_{Acineto}$),[11] *Arthrobacter* ($CHMO_{Arthro}$),[12] *Brachymonas* ($CHMO_{Brachy}$),[13] *Brevibacterium* ($CHMO_{Brevi1}$, $CHMO_{Brevi2}$),[14] *Rhodococcus* ($CHMO_{Rhodo1}$, $CHMO_{Rhodo2}$),[12] and *Comamonas*



Scheme 1 Baeyer–Villiger oxidations of ketones **1a–d** to "normal" lactones **2a–d** and "abnormal" lactones **3a–d**.

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(CPMO$_{Coma}$)[15] species in whole-cell mediated Baeyer–Villiger oxidations with recombinant *E. coli* as the host organism.[16]

Results and discussion

Enantiomerically pure biooxidation substrates were either purchased (menthone **1d**) or prepared according to literature protocols: reduction of (−)- and (+)-carvone was performed with Zn dust to give *cis*- and *trans*-isomers of enantiomerically pure dihydrocarvone in a ratio of 15 : 85, which were separated by flash column chromatography; reduction with a mixture of zinc dust and nickel chloride hexahydrate led to enantiomerically pure carvomenthone.[17]

The regiopreference of Baeyer–Villiger biooxidation was first investigated for (−)- and (+)-*trans*-dihydrocarvone with all eight overexpression systems of *E. coli*. Initial mini-scale screening experiments using 24-well plastic dishes previously standardized in our laboratory[18] gave results with only very low reproducibility because of the high volatility of substrates, hence making baffled flask biotransformations mandatory. The optimized experiments were carried out with 10 mg of substrate in 25 cm^3 of medium. Biotransformations were stopped after 24 hours of fermentation time at 24 °C and analyzed by chiral phase GC after extraction of the sample with EtOAc supplemented by an internal standard. Results of this screening program are summarized in Table 1.

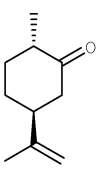
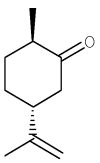
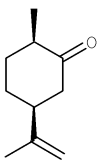
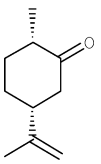
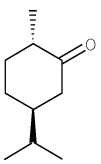
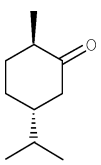
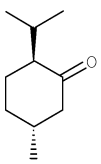
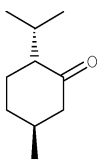
Recently, we have proposed the existence of two distinct sub-clusters of cycloketone oxidizing BVMOs based on protein sequence alignment, substrate acceptance, and stereopreference.[19] Baeyer–Villiger oxidations of (−)-*trans*-dihydrocarvone (−)-**1a** and (−)-carvomenthone (−)-**1c** catalyzed with "CHMO"-type enzymes (CHMO$_{Acineto}$, CHMO$_{Arthro}$, CHMO$_{Brachy}$, CHMO$_{Rhodo1}$, CHMO$_{Rhodo2}$) led to the formation of "normal" lactones **2a**,**c** with good to excellent conversions. In contrast, the opposite enantiomers (+)-**1a** and (+)-**1c** were transformed exclusively into "abnormal" lactones **3a**,**c** with "CHMO"-type proteins. Biooxidation with "CPMO"-type enzymes (CHMO$_{Brevi2}$, CPMO$_{Coma}$) showed in both cases preferred formation of "abnormal" lactones **3a**,**c**, however, with low conversions. A somewhat different outcome was observed for the biotransformation of (+)-**1a** and (+)-**1c** with CHMO$_{Brevi1}$, where both "normal" and "abnormal" lactones were obtained in ratios of 55 : 45 and 41 : 59.

Biooxidation of *cis*-dihydrocarvone **1b** was also investigated: "CHMO"-type enzymes converted (−)-**1b** to "normal" lactone (−)-**2b** with excellent conversions, while "abnormal" lactone (−)-**3b** was generated with "CPMO"-type enzymes, however in low conversions. While these findings are in line with biooxidations of (−)-**1a**,**c**, the opposite enantiomer (+)-**1b** was only poorly to moderately converted by all studied strains displaying decreased regioselectivities.

Host strain mediated reduction to the corresponding alcohol (10–30%) was observed whenever BVMO mediated biooxidation was sluggish; this side reaction became even more prominent when replacing LB media with TB media. Another possible side reaction was observed for the *cis*-substrates: these compounds can undergo chemical isomerization to *trans*-products and the presence of cells seems to further promote this effect (below 20% of side product).

It is important to point out that both lactones ("normal" and "abnormal") can be prepared selectively by our BVMO collection from each enantiomer of *trans*- and *cis*-dihydrocarvone as well as carvomenthone.

Table 1 Screening for Baeyer–Villiger oxidations of ketones **1a–d** by recombinant *E. coli* cells producing BVMOs of bacterial origin

	trans-Dihydrocarvone		*cis*-Dihydrocarvone		Carvomenthone		Menthone	
								
	(−)-**1a**	(+)-**1a**	(−)-**1b**	(+)-**1b**	(−)-**1c**	(+)-**1c**	(−)-**1d**	(+)-**1d**
	Conversion[a]							
Strain	"normal" : "abnormal" lactone ratio							
CHMO$_{Acineto}$	+++ 100 : 0	+++ 0 : 100	+++ 100 : 0	+ 64 : 36	++ 100 : 0	++ 0 : 100	n.c. —	+++ 100 : 0
CHMO$_{Arthro}$	++ 100 : 0	+++ 0 : 100	+++ 100 : 0	+(traces) 0 : 100	+ 100 : 0	+ 0 : 100	n.c. —	+++ 100 : 0
CHMO$_{Brachy}$	+++ 100 : 0	++ 0 : 100	+++ 100 : 0	+ 57 : 43	+++ 100 : 0	++ 0 : 100	n.c. —	+++ 100 : 0
CHMO$_{Brevi1}$	+++ 100 : 0	++ 55 : 45	+++ 100 : 0	+ 100 : 0	++ 100 : 0	++ 41 : 59	n.c. —	+++ 100 : 0
CHMO$_{Brevi2}$	+ 0 : 100	+(traces) 0 : 100	+ 0 : 100	n.c. —	+(traces) 0 : 100	n.c. —	n.c. —	n.c. —
CPMO$_{Coma}$	+ 0 : 100	+ 0 : 100	+ 0 : 100	+ 37 : 63	+ 0 : 100	++ 0 : 100	n.c. —	n.c. —
CHMO$_{Rhodo1}$	+++ 100 : 0	++ 0 : 100	+++ 100 : 0	+ 27 : 73	++ 100 : 0	++ 0 : 100	n.c. —	+++ 100 : 0
CHMO$_{Rhodo2}$	+++ 100 : 0	+++ 0 : 100	++ 100 : 0	+ 28 : 72	+++ 100 : 0	+++ 0 : 100	n.c. —	+++ 100 : 0

[a] Conversion according to GC: +++: >90%, ++: 50–90%, +: <50%.

Table 2 Baeyer–Villiger oxidations of ketones **1a–d** on preparative scale

Substrate	Strain	Time/h	Yield (%)[a]		$[\alpha]_D^{20}$ ($CHCl_3$)	
			2 (normal)	**3** (abnorm.)	**2** (normal)	**3** (abnorm.)
(−)-**1a**	$CHMO_{Acineto}$	19	77 (80)[8]	—	+45.5 (*c* 1.48)	—
	$CPMO_{Coma}$	72	—	18	—	+34.1 (*c* 0.46)
(+)-**1a**	$CHMO_{Acineto}$	19	—	70 (73)[8]	—	−34.6 (*c* 0.90)
	$CHMO_{Brevil}$	24	34	28	−42.1 (*c* 1.12)	−36.2 (*c* 0.73)
(−)-**1b**	$CHMO_{Acineto}$	22	77	—	−3.7 (*c* 3.78)	—
	$CPMO_{Coma}$	53	—	28	—	−45.8 (*c* 0.62)
(+)-**1b**	$CHMO_{Brevil}$	72	60	—	+3.3 (*c* 0.98)	—
	$CHMO_{Brachy}$	48	—	7	—	—
(−)-**1c**	$CHMO_{Brevil}$	24	71	—	+16.9 (*c* 1.15)	—
	$CPMO_{Coma}$	46	—	60	—	+34.3 (*c* 2.35)
(+)-**1c**	$CHMO_{Brevil}$	44	34	44	−17.2 (*c* 1.04)	−35.1 (*c* 1.36)
	$CHMO_{Brachy}$	24	—	76	—	−33.9 (*c* 1.55)
(+)-**1d**	$CHMO_{Acineto}$	24	82 (90)[8]	—	+19.9 (*c* 1.58)	—

[a] Isolated yield after FCC.

In the menthone series (**1d**) the findings by the group of Furstoss[8] for $CHMO_{Acineto}$ could be generalized for the (+)-substrate: "CHMO"-type enzymes give "normal" lactone **2d** with excellent conversions while "CPMO"-type enzymes did not accept this precursor. None of the enzymes expressed within this strain collection accepted (−)-**1d**.

To confirm the results of the screening, some representative biotransformations were also performed on preparative scale in order to isolate and characterize the biooxidation products; results are summarized in Table 2.

This set of experiments confirms the principal access to both regioisomers of the biooxidation process, in enantiocomplementary form, of the *trans*-dihydrocarvone and carvomenthone series for the first time. In the *cis*-dihydrocarvone series three out of the possible four isomers are accessible on preparative scale. In these cases the biooxidation represents the only means to access the "abnormal" products *via* a Baeyer–Villiger process. Moreover, the enzymes are highly chemoselective and tolerate the presence of olefinic substituents, which would undergo epoxidation upon chemical oxidation.

While the obtained yields (18–82%) are not optimized, advanced fermentation techniques have recently been applied in similar cases to improve the overall performance of the whole-cell mediated biooxidation reaction.[20] Adaptations of these techniques to counter the high volatility of these precursors as the major challenge of this substrate class are currently underway in our laboratories.

Conclusions

The observed substrate acceptance pattern and stereopreference of the BVMOs investigated within this study is in good agreement with our recently published hypothesis of two groups of cycloketone oxidizing BVMOs.[19] Within this class of substrates "CHMO"-type enzymes displayed very good substrate acceptance, while "CPMO"-type BVMOs usually gave poorer conversions. "CHMO"-Type enzymes showed regiodivergent oxidations to both "normal" and "abnormal" lactones depending on the absolute configuration of the ketone precursor; "CPMO"-type BVMOs usually yielded "abnormal" lactone products. A similar influence of the absolute configuration of enantiopure substrate ketones was recently reported for the biooxidation of β-substituted cyclopentanones and -hexanones determining migratory preference to yield proximal or distal lactone products.[21] The CHMO originating from *Brevibacterium* ($CHMO_{Brevil}$) displayed a distinctly different biooxidation profile, which underscores its "borderline" position in the phylogenetic relationship relative to "CHMO"-type and "CPMO"-type enzymes of this collection.

Considering the obtained results, we can conclude that both "normal" and "abnormal" terpene derived lactones can be prepared by enzyme mediated Baeyer–Villiger oxidation. Currently, additional studies to determine the scope and limitation of this behavior by BVMOs as well as the influence of various functional substituents are being carried out by our group.

Experimental

Unless otherwise noted, chemicals and microbial growth media were purchased from commercial suppliers and used without further purification. All solvents were distilled prior to use. Shake flask fermentations were performed in a Gerhard THO5 orbital thermoshaker. Flash column chromatography was performed on silica gel 60 from Merck (40–63 μm). NMR spectra were recorded from $CDCl_3$ solutions on a Bruker AC 200 (200 MHz) spectrometer and chemical shifts are reported in ppm using TMS as internal standard. Enantiomeric purity was determined by chiral phase GC using a BGB 175 column (30 m × 0.25 mm ID, 0.25 μm film) on a ThermoFinnigan Trace or Focus chromatograph and compared to reference material obtained by chemical *m*-chloroperoxybenzoic acid oxidation where applicable. GC-MS analyses were carried out on a GC-MS Voyager 8000Top with standard capillary column DB5 (30 m × 0.32 mm ID, 1.0 μm film). Specific rotation $[\alpha]_D^{20}$ was determined using a Perkin Elmer Polarimeter 241.

Since no comprehensive characterization of the "normal" and "abnormal" lactones of *cis*- and *trans*-dihydrocarvone, carvomenthone and menthone has been published until now, we provide experimental data of all synthesised lactones.

Typical procedure for screening experiment

Precultures were inoculated with a single colony from a plate and incubated at 37 °C on an orbital shaker at 120 rpm overnight.

LB_{amp} (25 cm^3: 1% peptone, 0.5% yeast extract, 1% NaCl in dion. water, supplemented by 200 μg mL^{-1} of ampicillin) was inoculated with the overnight preculture (250 μL) and incubated for 2–3 hours at 37 °C. After reaching an optical density $OD_{590} = 0.6$, 4 μL of IPTG stock solution was added (final concentration of 0.134 mM) to start the expression of the corresponding monooxygenase, followed by addition of 10 mg of substrate. Transformations were analyzed after 24 hours of fermentation time at 24 °C by extraction of the sample with EtOAc supplemented by an internal standard.

Typical procedure for biotransformation on preparative scale

Fresh LB_{amp} medium (1% peptone, 0.5% yeast extract, 1% NaCl in dion. water, supplemented by 200 μg mL^{-1} of ampicillin) was inoculated with 1% of an overnight preculture of the corresponding recombinant *E. coli* strain in a baffled Erlenmeyer flask. The culture was incubated at 120 rpm at 37 °C on an orbital shaker for 2–3 hours. After reaching an optical density $OD_{590} = 0.6$, IPTG stock solution was added to a final concentration of 0.134 mM. The substrate (3–6 mM) was added neat along with β-cyclodextrin (1 equiv.). The culture was incubated at rt for 19–72 hours. The biomass was removed by centrifugation, saturated with sodium chloride, and repeatedly extracted with the corresponding solvent (EtOAc, diethylether or dichloromethane). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed *in vacuo*. The crude material was purified by standard flash column chromatography.

7-Methyl-4-isopropenyl-2-oxo-oxepanone 2a.

(4S,7S)-(+). (−)-*trans*-Dihydrocarvone **1a** (60 mg in 125 cm^3 of LB_{amp}) was oxidized with $CHMO_{Acineto}$ according to the general procedure. The crude product was purified by column chromatography (silica gel, petroleum ether (PE) : Et_2O = 6 : 1) and (+)-**2a**[8] was obtained as a colorless oil (51 mg, 77%). $[\alpha]_D^{20}$ +45.5 (*c* 1.48, $CHCl_3$) (lit.,[8] +46.2, *c* 1.1, $CHCl_3$).

(4R,7R)-(−). (+)-*trans*-Dihydrocarvone **1a** (92 mg in 250 cm^3 of LB_{amp}) was oxidized with $CHMO_{Brevi1}$ according to the general procedure. The crude product, a mixture of normal and abnormal lactones, was separated by column chromatography (silica gel, PE : Et_2O = 6 : 0.5; 6 : 1) and (−)-**2a**[9] was obtained as a colorless oil (35 mg, 34%). $[\alpha]_D^{20}$ −42.1 (*c* 1.12, $CHCl_3$)

δ_H(200 MHz; $CDCl_3$; Me_4Si) 1.37 (3 H, d, *J* 6.4 Hz), 1.58–1.97 (4 H, m), 1.73 (3 H, s), 2.24–2.35 (1 H, m), 2.58–2.80 (2 H, m), 4.41–4.54 (1 H, m) and 4.74–4.76 (2 H, m); δ_C(50 MHz; $CDCl_3$; Me_4Si) 20.1 (q), 22.6 (q), 34.3 (t), 35.8 (t), 40.2 (t), 41.8 (d), 76.2 (d), 110.1 (t), 148.4 (s) and 174.6 (s); *m/z* 168 (M^+, 6%), 125 (33), 108 (43), 81 (34), 67 (100).

3-Methyl-6-isopropenyl-2-oxo-oxepanone 3a.

(3S,6R)-(+). (−)-*trans*-Dihydrocarvone **1a** (95 mg in 250 cm^3 of LB_{amp}) was oxidized with $CPMO_{Coma}$ according to the general procedure. The crude product was purified by column chromatography (silica gel, PE : Et_2O = 6 : 1) and (+)-**3a**[9] was obtained as a colorless oil (19 mg, 18%). $[\alpha]_D^{20}$ +34.1 (*c* 0.46, $CHCl_3$).

(3R,6S)-(−). (+)-*trans*-Dihydrocarvone **1a** (40 mg in 125 cm^3 of LB_{amp}) was oxidized with $CHMO_{Acineto}$ according to the general procedure. The crude product was purified by column chromatography (silica gel, PE : Et_2O = 6 : 1) and (−)-**3a**[8] was obtained as a colorless oil (31 mg, 70%). $[\alpha]_D^{20}$ −34.6 (*c* 0.9, $CHCl_3$) (lit.[8] −35.8, *c* 1.6, $CHCl_3$).

δ_H(200 MHz; $CDCl_3$; Me_4Si) 1.22 (3 H, d, *J* 6.7 Hz), 1.50–2.07 (4 H, m), 1.82 (3 H, s), 2.22–2.34 (1 H, m), 2.68–2.85 (1 H, m), 4.15–4.18 (2 H, m) and 4.72–4.83 (2 H, m); δ_C(50 MHz; $CDCl_3$; Me_4Si) 18.4 (q), 21.8 (q), 31.8 (t), 34.1 (t), 37.2 (d), 46.4 (d), 71.5 (t), 111.1 (t), 145.7 (s) and 177.7 (s); *m/z* 168 (M^+, 0.4%), 138 (23), 110 (100), 95 (46), 68 (89).

7-Methyl-4-isopropenyl-2-oxo-oxepanone 2b.

(4S,7R)-(−). (−)-*cis*-Dihydrocarvone **1b** (100 mg in 250 cm^3 of LB_{amp}) was oxidized with $CHMO_{Acineto}$ according to the general procedure. The crude product was purified by column chromatography (silica gel, PE : Et_2O = 3 : 1) and (−)-**2b**[9] was obtained as a colorless oil (85 mg, 77%). $[\alpha]_D^{20}$ −3.7 (*c* 3.78, $CHCl_3$).

(4R,7S)-(+). (+)-*cis*-Dihydrocarvone **1b** (50 mg in 125 cm^3 of LB_{amp}) was oxidized with $CHMO_{Brevi1}$ according to the general procedure. The crude product was purified by column chromatography (silica gel, PE : Et_2O = 3 : 1) and (+)-**2b** was obtained as a colorless oil (33 mg, 60%). $[\alpha]_D^{20}$ +3.3 (*c* 0.98, $CHCl_3$).

δ_H(200 MHz; $CDCl_3$; Me_4Si) 1.37 (3 H, d, *J* 6.4 Hz), 1.72–2.05 (4 H, m), 1.79 (3 H, s), 2.52–2.59 (1 H, m), 2.75–3.04 (2 H, m), 4.44–4.55 (1 H, m) and 4.84–4.86 (2 H, m); δ_C(50 MHz; $CDCl_3$; Me_4Si) 21.3 (q), 21.8 (q), 29.7 (t), 33.3 (t), 38.4 (t), 38.6 (d), 75.5 (d), 111.4 (t), 146.3 (s) and 173.8 (s); *m/z* 168 (M^+, 6%), 139 (13), 108 (98), 81 (39), 67 (100).

3-Methyl-6-isopropenyl-2-oxo-oxepanone 3b[9].

(3R,6R)-(−). (−)-*cis*-Dihydrocarvone **1b** (95 mg in 250 cm^3 of LB_{amp}) was oxidized with $CPMO_{Coma}$ according to the general procedure. The crude product was purified by column chromatography (silica gel, PE : Et_2O = 3 : 1) and (−)-**3b** was obtained as a colorless oil (30 mg, 28%). $[\alpha]_D^{20}$ −45.8 (*c* 0.62, $CHCl_3$).

δ_H(200 MHz; $CDCl_3$; Me_4Si) 1.20 (3 H, d, *J* 6.7 Hz), 1.64–2.14 (4 H, m), 1.80 (3 H, s), 2.40–2.50 (1 H, m), 2.76–2.93 (m, 1H), 4.32–4.49 (2 H, m) and 4.88–4.90 (2 H, m); δ_C(50 MHz; $CDCl_3$; Me_4Si) 18.0 (q), 21.6 (q), 28.6 (t), 29.5 (t), 36.7 (d), 43.2 (d), 69.0 (t), 112.3 (t), 144.1 (s) and 177.2 (s); *m/z* 168 (M^+, 3%), 153 (9), 138 (12), 110 (98), 95 (51), 68 (100).

7-Methyl-4-isopropyl-2-oxo-oxepanone 2c[22].

(4R,7S)-(+). (−)-Carvomenthone **1c** (50 mg in 125 cm^3 of LB_{amp}) was oxidized with $CHMO_{Brevi1}$ according to the general procedure. The crude product was purified by column chromatography (silica gel, PE : EtOAc = 9 : 1) and (+)-**2c** was obtained as a colorless oil (39 mg, 71%). $[\alpha]_D^{20}$ +16.9 (*c* 1.15, $CHCl_3$).

(4S,7R)-(−). (+)-Carvomenthone **1c** (93 mg in 250 cm^3 of LB_{amp}) was oxidized with $CHMO_{Brevi1}$ according to the general procedure. The crude product, a mixture of normal and abnormal lactone, was separated by column chromatography (silica gel, PE : EtOAc = 9.5 : 0.5) and (−)-**2c** was obtained as a colorless oil (36 mg, 34%). $[\alpha]_D^{20}$ −17.2 (*c* 1.04, $CHCl_3$).

δ_H(200 MHz; $CDCl_3$; Me_4Si) 0.87 (3 H, d, *J* 6.7 Hz), 0.90 (3 H, d, *J* 6.7 Hz), 1.35 (3 H, d, *J* 6.4 Hz), 1.41–1.96 (6 H, m), 2.46–2.49 (1 H, m) and 4.36–4.50 (1 H, m); δ_C(50 MHz; $CDCl_3$; Me_4Si) 18.5 (q), 18.8 (q), 22.6 (q), 31.3 (t), 33.5 (d), 35.8 (t), 38.1 (t), 40.3 (d), 76.6 (d) and 175.7 (s); *m/z* 171 (M^+, 0.4%), 155 (1), 126 (58), 83 (100), 69 (29), 55 (52).

3-Methyl-6-isopropyl-2-oxo-oxepanone 3c.

(3S,6R)-(+). (−)-Carvomenthone **1c** (90 mg in 250 cm^3 of LB_{amp}) was oxidized with $CPMO_{Coma}$ according to the general

procedure. The crude product was purified by column chromatography (silica gel, PE : EtOAc = 9 : 1) and (+)-**3c** was obtained as a colorless oil (60 mg, 60%). $[\alpha]_D^{20}$ +34.3 (*c* 2.35, $CHCl_3$).

(3R,6S)-(−). (−)-Carvomenthone **1c** (50 mg in 125 cm^3 of LB_{amp}) was oxidized with $CHMO_{Brachy}$ according to the general procedure. The crude product was purified by column chromatography (silica gel, PE : EtOAc = 9 : 1) and (−)-**3c** was obtained as a colorless oil (42 mg, 76%). $[\alpha]_D^{20}$ −33.9 (*c* 1.55, $CHCl_3$).

δ_H(200 MHz; $CDCl_3$; Me_4Si) 0.90 (3 H, d, *J* 6.8 Hz), 0.91 (3 H, d, *J* 6.8 Hz), 1.19 (3 H, d, *J* 6.8 Hz), 1.50–1.89 (6 H, m), 2.70–2.78 (1 H, m) and 4.03–4.20 (2 H, m); δ_C(50 MHz; $CDCl_3$; Me_4Si) 18.4 (q), 19.1 (q), 19.4 (q), 31.0 (d), 31.1 (t), 31.8 (t), 37.0 (d), 44.6 (d), 71.6 (t) and 178.1 (s); *m/z* 171 (M^+, 0.4%), 140 (3), 125 (5), 98 (100), 82 (14), 69 (30), 55 (32).

(4*S*,7*R*)-(+)-4-Methyl-7-isopropyl-2-oxo-oxepanone 2d. (+)-Menthone **1d** (50 mg in 125 cm^3 of LB_{amp}) was oxidized with $CHMO_{Acineto}$ according to the general procedure. The crude product was purified by column chromatography (silica gel, PE : EtOAc = 9 : 0.5) and (+)-**2d**[8] was obtained as a colorless oil (45 mg, 82%). $[\alpha]_D^{20}$ +19.9 (*c* 1.58, $CHCl_3$) (lit.,[8] +20.5, *c* 1.4, $CHCl_3$).

δ_H(200 MHz; $CDCl_3$; Me_4Si) 0.97 (3 H, d, *J* 6.8 Hz), 0.98 (3 H, d, *J* 6.8 Hz), 1.04 (3 H, d, *J* 6.6 Hz), 1.20–1.99 (6 H, m), 2.42–2.62 (2 H, m), 4.04 (1 H, dd, *J* 9.0 and 4.4 Hz); δ_C(50 MHz; $CDCl_3$; Me_4Si) 17.0 (q), 18.3 (q), 23.9 (q), 30.3 (d), 30.9 (t), 33.2 (d), 37.3 (t), 42.4 (t), 84.6 (d) and 174.9 (s); *m/z* 171 (M^+, 2%), 127 (93), 99 (85), 81 (100), 69 (96), 55 (86).

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