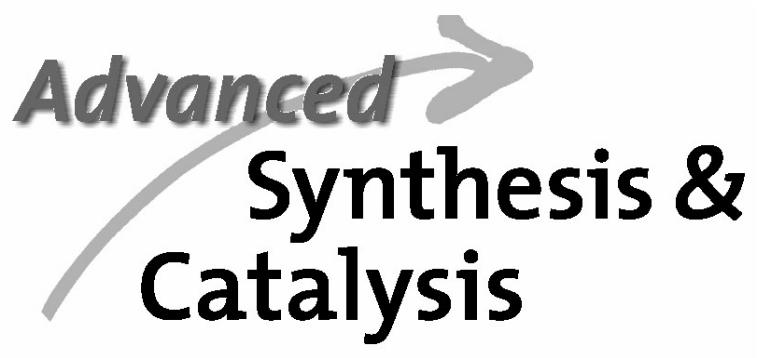


Supporting Information

Comparing the Stereoselective Biooxidation of Cyclobutanones by Recombinant Strains Expressing Bacterial Baeyer-Villiger Monooxygenases

Florian Rudroff, Joanna Rydz, Freek H. Ogink, Michael Fink, Marko D. Mihovilovic*

Supporting Material – Experimental Details

1 General

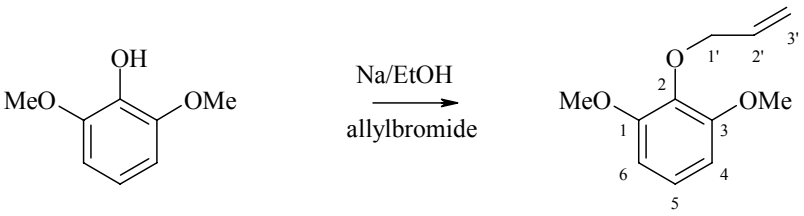
Unless otherwise noted, chemicals and microbial growth media were purchased from commercial suppliers and used without further purification. All solvents were distilled prior to use. Flash column chromatography was performed on silica gel 60 from Merck (40-63µm). Melting points were determined using a Kofler-type Leica Galen III micro hot stage microscope and are uncorrected.

NMR-spectra were recorded from $CDCl_3$ or DMSO-d_6 solutions on a Bruker AC 200 (200 MHz) or Bruker Advance UltraShield 400 (400 MHz) spectrometer and chemical shifts are reported in ppm using TMS as internal standard. Peak assignment is based on correlation experiments. Ambiguous assignment is marked with an asterix.
Combustion analysis was carried out in the Microanalytic Laboratory, University of Vienna.
General conversion control and examination of purified products were performed with GC Top 8000 / MS Voyager (quadrupol, EI+) using a standard capillary column BGB5 (30mx0.32mm ID). Enantiomeric excess was determined *via* GC using a BGB 175 column (30mx0.25mm ID, 0.25µm film) and a BGB 173 column (30mx0.25mm ID, 0.25µm film) on a ThermoQuest Trace GC 2000 and a Thermo Focus GC.

Specific rotation $[\alpha]_D^{20}$ was determined using a Perkin Elmer Polarimeter 241 by the following equation: $[\alpha]_D^{20} = 100*\alpha / [c]*l$; c[g/100mL], l[dm]

2. Synthesis of 3-(3,4,5-Trimethoxy-benzyl)-cyclobutanone

2.1 Preparation of 2-(Allyloxy)-1,3-dimethoxybenzene (4)



Procedure: Allyl bromide was distilled prior to use.

Small pieces of sodium (1.79g, 78.0mmol, 1.2equiv.) were suspended in dry EtOH (150mL) and stirred under argon atmosphere until completely dissolved. Then a solution of 2,6-dimethoxyphenole (10.0g, 65mmol) in dry EtOH (120mL) was added dropwise *via* a syringe and the mixture was stirred for one hour. Pure allyl bromide (9.44g, 78.0mmol, 1.2equiv.) was added dropwise and the reaction mixture was stirred over night at rt.

The reaction mixture had turned into a grey slurry. More allyl bromide (1.00g, 8.3mmol) was added and the reaction was stirred until GC/MS showed full conversion.

The reaction mixture was concentrated at reduced pressure and the remaining suspension was then diluted with water (400mL). After extraction at pH 12 with Et_2O (approx. 800mL), the combined organic layers were dried over Na_2SO_4. Evaporation of the solvent provided the allyl ether as golden yellow oil in very good purity (>99% according to GC/MS) with good yield (10.5g, 54.1mmol, 83%).

Yield: 10.5g (83%)

MW: 194.2; $C_{11}H_{13}O_3$

golden yellow oil (Lit.[1])

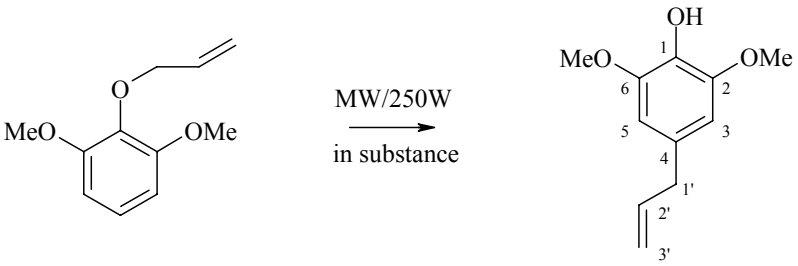
^{1}H NMR ($CDCl_3$): δ 3.84 (s, 6H, 2xO-CH_3), 4.52 (d, J=6Hz, 2H, H-1'), 5.14-5.36 (m, 2H, H-3'), 6.02-6.22 (m, 1H, H-2'), 6.57 (d, J=8Hz, 2H, H-4/H-6), 6.98 (t, J=8Hz, 1H, H-5);

^{13}C NMR ($CDCl_3$): δ 55.9 (q, 2xO-CH_3), 74.0 (t, C-1'), 105.1 (2xd,C-4/C-6), 117.5 (t, C-3'), 123.5 (d, C-5), 134.5 (d, C-2'), 136.6 (s, C-2), 153.6 (2xs, C-1/C-3)

m/z: 194 (61, M^+), 153 (100), 125 (48), 110 (56), 95 (38), 93 (40).

[1] Jing, X.; Gu, W.; Bie, P.; Ren, X.; Pan, X. *Synthetic Comm.* **2001**, 31, 861-867.

2.2 Preparation of 4-Allyl-2,6-dimethoxyphenole (5)



Procedure: Pure 2-allyloxy-1,3-dimethoxybenzene (2.0g, 10.3mmol) was sealed in a microwave tube and irradiated for 75 min (250 W, 180°C). Reaction control *via* GC/MS indicated complete conversion.

Yield: 2.00g (100%)

MW: 194.2; $C_{11}H_{13}O_3$

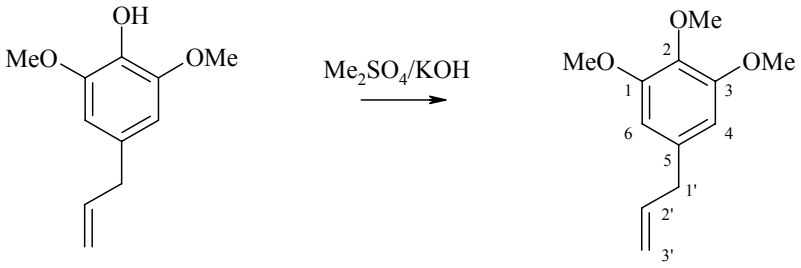
brown oil, (Lit.[1])

^{1}H NMR ($CDCl_3$): δ 3.23 (d, J=6Hz, 2H, H-1'), 3.78 (s, 6H, 2xO-CH_3), 4.96-5.05 (m, 2H, H-3'), 5.35 (s, 1H, aryl-OH), 5.77-5.97 (m, 1H, H-2'), 6.33 (s, 2H, H-3/H-5);

^{13}C NMR ($CDCl_3$): δ 40.1 (t, C-1'), 56.0 (2xq, 2xO-CH_3), 104.9 (2xd, C-3, C-5), 115.5 (t, C-3'), 130.8 (s), 132.8 (s), 137.4 (d, C-2'), 147.8 (2xs, C-2, C-6);

m/z: 194 (100, M^+), 179 (11), 147 (10), 131 (14), 119 (20), 91 (25), 77 (13).

2.3 Preparation of 5-Allyl-1,2,3-trimethoxybenzene (6)



Procedure: Dimethyl sulfate was distilled prior to use (73.5 °C/18mbar).

4-Allyl-2,6-dimethoxyphenole (8.23g, 42.4mmol) was solved in 10% aqueous potassium hydroxide solution (2.97g KOH, 53.0mmol, 1.25equiv.) under vigorous magnetic stirring. The reaction mixture turned dark blue and later to a greenish yellow and became a slurry. An ice cooling bath was installed and dimethyl sulafte (5.88g, 46.7mmol, 1.1equiv.) was added slowly; no temperature change was observed. The cooling bath was removed and the mixture was stirred for 4 hours. For completion of the reaction more dimethyl sulfate was added (0.54 g, 0.1equiv.) and the reaction was continued at reflux temperature. After 30 min reaction control *via* TLC and GC-MS showed complete conversion.

The reaction solution was extracted with Et_2O (5 x 60mL), the combined organic layers were dried over anhydrous sodium sulfate and concentrated *in vacuo.*

Kugelrohr distillation of the crude product yielded the desired compound as colorless oil in good yield (8.20g, 39.4mmol, 93%).

Yield: 8.20g (93%, Lit.[2] 47%)

MW: 208.3; $C_{12}H_{16}O_3$

colorless oil, b.p.: 106-108°C/1mbar, (Lit.[3] 106-107°C/10mbar)

^{1}H NMR ($CDCl_3$): δ 3.26 (d, J=7Hz, 2H, H-1'), 3.78 (s, 9H, 3xO-CH_3), 4.99-5.09 (m, 2H, H-3'), 5.79-5.99 (m, 1H, H-2'), 6.34 (s, 2H, H-4/H-6);

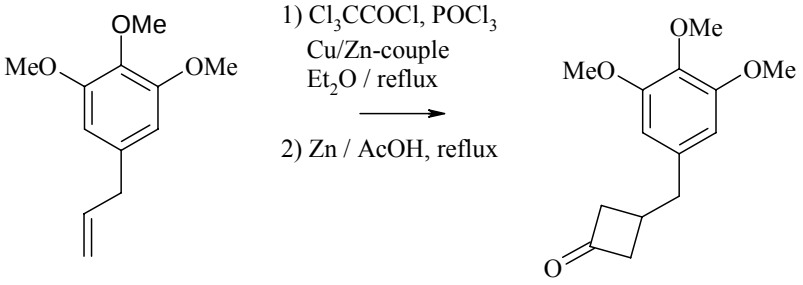
^{13}C NMR ($CDCl_3$): δ 39.4 (t, C-1'), 54.8 (2xq, C-1, C-3-OMe), 59.6 (q, C-2-OMe), 104.1 (2xd, C-4, C-6), 114.8 (t, C-3'), 134.6 (s), 135.0 (s), 151.9 (2xs, C-1, C-3);

m/z: 208 (100, M^+), 193 (62), 177 (13), 133 (17), 118 (13), 91 (15), 77 (18).

[2] Medina, A.L.; Lucero, M.E.; Holguin, F.O.; Estell, R.E.; Posakony, J.J.; Simon, J.; O'Connell, M.A. *J. Agric. Food Chem*. **2005**, *53*, 8694-8698.

[3] Gunasekaran, A.; Balasubramanian, K: Indian *J. Chem. Sect. B* **1988**, *27*; 308-310.

2.4 Preparation of 3-(3,4,5-Trimethoxybenzyl)-cyclobutanone (1i)



2.4.1 Synthesis of 2,2-Dichloro-3-(3,4,5-trimethoxybenzyl)-cyclobutanone

Procedure: A stirred suspension of zinc dust (10g, 0.15mol) in water (40mL) was degassed by passing through N_2 for 15 minutes. Subsequently, $CuSO_4$ (750mg, 4.7mmol) was added at once and the black suspension was stirred while N_2 was passed through for an additional 45 minutes. The Cu/Zn-couple was collected on a sintered glass funnel and was washed successively with 100mL degassed water and acetone. The Cu/Zn-couple was dried in vacuum and stored (maximum 2 days) under N_2.

Freshly prepared Cu/Zn-couple (0.56g, 8.64mmol, 1.2equiv.) was suspended in dry diethyl ether (20mL). 5-Allyl-3,4,5-trimethoxybenzene (1.50g, 7.20mmol) was added and the reaction mixture was set under nitrogen atmosphere. A mixture of both freshly distilled Cl_3CCOCl (1.57g, 8.64mmol, 1.2equiv.) and $POCl_3$ (1.32g, 8.64mmol, 1.2equiv.) dissolved in dry diethyl ether (10mL) was added subsequently over a period of one hour. Then, the reaction mixture was refluxed for six hours, was cooled to rt, and a mixture of Cl_3CCOCl, $POCl_3$ (1.2eq) and Cu/Zn-couple (1.2equiv.) was added again.

After 12 hours complete conversion was observed via TLC (GC/MS reaction control did not give satisfactory results; detection of the chloro compound was not efficient enough). The crude reaction mixture was diluted with CH_2Cl_2 and extracted with satd. bicarbonate solution. The aqueous phase was extracted three times with ethyl acetate. The combined organic layers were dried over sodium sulfate. After evaporation of the solvent the desired compound was isolated in quantitative yield (2.25g, 7.20mmol, quant.). The crude product (>95% according to GC/MS) was used without further purification in the next step.

2.4.2 Synthesis of 3-(3,4,5-Trimethoxybenzyl)-cyclobutanone

Procedure: Crude 2,2-dichloro-3-(3,4,5-trimethoxyphenyl)methylcyclobutane (2.25g, 7.20mmol) was dissolved in glacial acetic acid (20mL). Then zinc dust (1.4g, 21.6mmol, 3equiv.) was added. The reaction mixture was refluxed o/n. After TLC control showed complete conversion the reaction mixture was cooled to rt and diluted with ethyl acetate.
After extraction with saturated aqueous sodium bicarbonate solution the organic layer was dried and the solvent was evaporated. The crude compound was purified *via* flash column chromatography (LP/EtOAc=4/1-2/1).

Yield: 864mg (48% over 2 steps)
MW: 250.3; $C_{14}H_{18}O_4$

colorless oil

^{1}H NMR ($CDCl_3$): δ 2.67–2.86 (m, 5H), 3.08–3.24 (m, 2H), 3.83 (s, 3H p-OCH_3), 3.86 (s, 6H, 2 x m-OCH_3), 6.41 (s, 2H, H-2`/H-6`);

^{13}C NMR ($CDCl_3$): δ 25.1 (d, C-3), 42.2 (t, $\underline{C}H_2$-Ph), 52.3 (2xt, C-2/C-4), 56.1 (2xq, m-OCH_3), 60.8 (q, p-OCH_3), 105.4 (2xs, C-2`/C-6`), 135.7 (2xs, C-3`/C-5`), 136.4 (s, C-4`), 150.9 (s, C-1`), 207.7 (C=O);

Calc.: C 67.18%, H 7.25%
Found.: C 67.05%, H 7.25%

m/z: 250 (60, M^+), 208 (22), 193 (41), 181 (100), 91 (19), 77 (25).

3 Baeyer-Villiger Oxidation

3.1 Chemical Baeyer-Villiger Oxidation (Method A)

Procedure: *m*-Chloroperoxybenzoic acid (1.5 equiv., 70% purity) was added to a solution of the corresponding ketone (1 equiv.) in dry dichloromethan (10% solution) and the mixture was stirred overnight at rt. Complete conversion was determined by TLC. When the reaction was complete triethylamine (excess) was added and the mixture was stirred for 15 minutes. Subsequently, water was added and the organic layer was separated. The aqueous layer was extracted two times with dichloromethane. The combined organic phases were washed with satd. sodium bicarbonate solution, dried over sodium sulfate, filtered and evaporated.

GC-analysis: Chemical oxidation of ketones was mandatory for the preparation of racemic reference material. Due to calculation and description of enantioselectivity of the obtained lactone, baseline separation of the racemic reference material performed by chiral GC analysis was necessary.

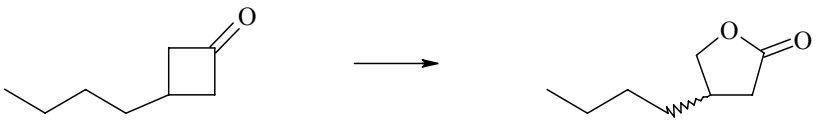
Method A	80°C-2min→80-160°C, 5°C/min, 160°C-1min→160-220°C, 10°C/min, 220°C-8min
Method B	80°C-2min→80-220°C, 2°C/min, 220°C-8min
Method C	80°C-2min→80-220°C, 1°C/min, 220°C-8min
Method D	160°C-2min→160-230°C, 2°C/min, 230°C-15min

3.2 Microbial Baeyer-Villiger Oxidation (Method B)

Procedure: Fresh LB_{amp} medium (250mL) was inoculated with 1% (2.5mL) of an overnight preculture of recombinant *E. coli* strains: $CHMO_{Acineto}$, $CPMO_{Pseudo}$, $CHMO_{Brevi\ I}$, $CHMO_{Brevi\ II}$, $CHMO_{Rhodo\ I}$, $CHMO_{Rhodo\ II}$, $CHMO_{\textit{Brachy}}$, $CHMO_{Arthro}$, in a baffled Erlenmeyer flask. The culture was incubated at 120 rpm at 37°C on an orbital shaker for 2 hours, and then 50µl IPTG was added to a final concentration of 0.004 wt/v. The substrate (100mL) was added neat along with β-cyclodextrin (1 equiv.). The culture was incubated o/n at 120 rpm at rt until GC (sample of 700µl) showed complete conversion of the ketone (24 hours). After complete conversion the biomass was separated by centrifugation (15 min., 4000 rpm). The supernatant was filtered through a bed of Celite, which was subsequently washed with the extraction solvent.

The aqueous layer was extracted with the same solvent (2x200mL). The combined organic layers were dried over sodium sulfate, filtered, and the solvent was removed in *vacuo*. The crude lactones were purified by flash column chromatography.

3.3 4-Butyl-dihydro-furan-2(3H)-one (2a)



Methode A: 3-Butylcyclobutanone (299mg, 2.37mmol) was oxidized to 4-butyl-dihydro-furan-2-one according to the general procedure. Purification of the product by column chromatography on silica gel (LP/EtOAc = 7/1) yielded in 55% of the desired lactone.[4] (GC: BGB 175, method B)

Yield: 186mg (55%)

MW: 142.2, $C_8H_{14}O_2$

Method B: 3-Butylcyclobutanone was oxidized with recombinant *E. coli* cells according to general procedure. The crude product was purified *via* column chromatography (silica gel, LP/EtOAc=7/1) and was obtained as yellow oil.

Enzyme	1a	2a	*ee*	abs. config.[5]	$[\alpha]^D_{20}$
$CHMO_{Acineto}$	150mg	105mg (62%)	17%	(S)	-2.05 (*c* 2.10, $CHCl_3$)
$CHMO_{Arthro}$	150mg	87mg (51%)	32%	(S)	-1.84 (*c* 1.74, $CHCl_3$)
$CHMO_{Brachy}$	150mg	93mg (55%)	14%	(S)	-1.20 (*c* 1.86, $CHCl_3$)
$CHMO_{Brevi1}$	150mg	110mg (65%)	>99%	(S)	-5.95 (*c* 2.20, $CHCl_3$)
$CHMO_{Brevi2}$	150mg	125mg (74%)	69%	(S)	-3.68 (*c* 2.50, $CHCl_3$)
$CPMO_{Coma}$	150mg	121mg (72%)	76%	(S)	-4.46 (*c* 2.42, $CHCl_3$)
$CHMO_{Rhodo1}$	150mg	91mg (54%)	rac.	-	n.d.
$CHMO_{Rhodo2}$	150mg	98mg (58%)	rac.	-	n.d.

yellow oil

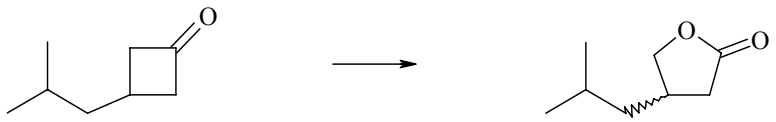
^{1}H-NMR ($CDCl_3$): δ 0.91 (t, J=5Hz, 3H, CH_3), 1.21-1.56 (m, 6H, $(CH_2)_3$), 2.11-2.28 (m, 1H, H-3), 2.46-2.68 (m, 2H, H-3), 3.92 (dd, J_1=7Hz, J_2=9Hz, 1H, H-5,), 4.40 (dd, J_1=7Hz, J_2=9Hz, 1H, H-5);

^{13}C-NMR ($CDCl_3$): δ 13.8 (q, CH_3), 22.4 (t, CH_2), 29.4 (t), 32.7 (t), 34.4 (t, C-3), 35.6 (d, CH), 73.3 (t, C-5), 177.4 (s, C=O);

m/z: 142 (1, M^+), 114 (8), 111 (8), 84 (12), 70 (15), 69 (29), 56 (100), 55 (46).

[4] Röder, E.; Krauß, H. *Liebigs Ann. Chem.,* **1992**, 177-181.
[5] Assignment based on: Gagnon, R.; Grogan, G.; Goussain, E.; Pedragosa-Moreau, S.; Richardson, P.F.; Roberts, S.M.; Willets, A.J.; Lebreton, R.; Furstoss, R. *J.Chem. Soc. Perkin Trans 1* **1995**, 2527-2528.

3.4 *i*-Butyl-dihydro-furan-2(3H)-one (2b)



Method A: 3-*i*-Butylmethylcyclobutanone (305mg, 2.46mmol) was oxidized to 4-benzyloxy methyl-dihydro-furan-2-one, according to the general procedure. After purification *via* flash column chromatography (LP/EtOAc = 14/1) the desired lactone was isolated in 29% yield.[6] (GC: BGB 175, method B)

Yield: 103mg (29%)

MW: 142.2, $C_8H_{14}O_2$

Method B: 3-(2-Methylpropyl)cyclobutanone was oxidized with *E. coli* expressing cells according to general procedure. The crude product was purified *via* flash column chromatography (LP/EtOAc = 35/1) and the brown odorant oil was isolated in the yields and properties specified below.

Enzyme	1b	2b	*ee*	abs. config.[5]	$[\alpha]^D_{20}$
$CHMO_{Acineto}$	172mg	103mg (53%)	rac	n.d.	n.a.
$CHMO_{Arthro}$	93mg	43mg (41%)	74%	(S)	-0.44 (*c* 0.67, $CHCl_3$)
$CHMO_{Brachy}$	93mg	25mg (24%)	77%	(S)	-1.46 (*c* 0.41, $CHCl_3$)
$CHMO_{Brevi1}$	172mg	58mg (30%)	>99%	(S)	-1.47 (*c* 1.16, $CHCl_3$)
$CHMO_{Brevi2}$	93mg	45mg (43%)	22%	(S)	-0.44 (*c* 0.67, $CHCl_3$)
$CPMO_{Coma}$	172mg	123mg (63%)	76%	(S)	-1.02 (*c* 2.46, $CHCl_3$)
$CHMO_{Rhodo1}$	93mg	38mg (35%)	49%	(S)	-2.16(*c* 0.28, $CHCl_3$)
$CHMO_{Rhodo2I}$	93mg	42mg (40%)	45%	(S)	-0.72 (*c* 0.84, $CHCl_3$)

brown odorant oil

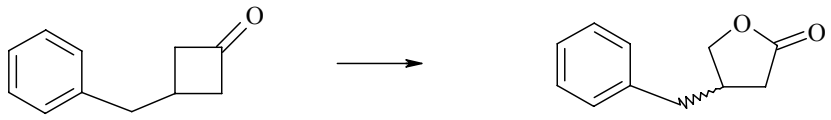
^{1}H NMR ($CDCl_3$): δ 0.92 (d, J=6Hz, 6H, 2x CH_3), 1.32-1.50 (m, 2H, CHC$\underline{H_2}$CH), 1.50-1.57 (m, 1H, C$\underline{H}(CH_3)_2$), 2.05-2.26 (m, 1H, H-3), 2.51-2.72 (m, 2H, H-3, H-4), 3.83-3.94 (m, 1H, H-5), 4.35-4.48 (m, 1H, H-5);

^{13}C NMR ($CDCl_3$): δ 22.4, 22.6 (q, 2x CH_3), 26.3 (d, $\underline{C}H(CH_3)_2$), 33.8 (d, $\underline{C}H(CH_2)_3$, 34.8 (t, CH$\underline{C}H_2$CH), 42.2 (t, C-3), 73.5 (t, C-5), 177.2 (s, C=O);

m/z: 142 (3, M^+), 112 (11), 100 (12), 84 (16), 69 (40), 56 (100), 55 (34).

[6] Bode, J.W.; Doyle, M.P; Protopopova, M.N.; Zhou, Q.-L. *J. Org. Chem.* **1996**, *61*, 9146-9155.

3.5 Dihydro-4-(phenylmethyl)-furan-2(3H)-one (2c)



Method A: Cyclobutanone (137mg, 0.87mmol) was oxidized according to the general procedure. The product did not require additional purification. (GC: BGB 175, method A)

Yield: 133 mg (87%)

MW: 176.2, $C_{11}H_{12}O_2$

Method B: 3-(Phenylmethyl)cyclobutanone was oxidized with all nine *E. coli* expressing cells according to general procedure. The crude product was purified *via* column chromatography (LP/EtOAc = 10/1) and the pink odorant oil was isolated in the yields and properties specified below.

Enzyme	1c	2c	*ee*	abs. config.[5,7]	$[\alpha]^{D}_{20}$
$CHMO_{Acineto}$	106mg	37mg (32%)	88%	(S)	-7.77 (c 0.75, $CHCl_3$)
$CHMO_{Arthro}$	106mg	65mg (56%)	93%	(S)	-5.35 (c 1.31, $CHCl_3$)
$CHMO_{Brachy}$	106mg	45mg (38%)	84%	(S)	-6.40 (c 0.89, $CHCl_3$)
$CHMO_{Brevi1}$	106mg	35mg (30%)	93%	(S)	-12.8 (c 0.70, $CHCl_3$)
$CHMO_{Brevi2}$	106mg	32mg (27%)	59%	(S)	-8.66 (c 0.36, $CHCl_3$)
$CPMO_{Coma}$	106mg	43mg (37%)	31%	(S)	-2.20 (c 0.86, $CHCl_3$)
$CHMO_{Rhodo1}$	106mg	38mg (33%)	87%	(S)	-5.60 (c 0.54, $CHCl_3$)
$CHMO_{Rhodo2}$	106mg	36mg (31%)	87%	(S)	-15.1 (c 0.48, $CHCl_3$)

pink odorant oil

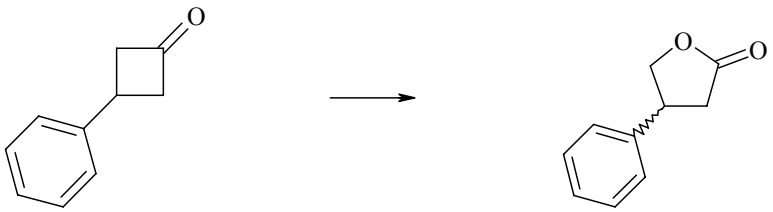
^{1}H-NMR ($CDCl_3$): δ 2.43 (dd, J_1=9Hz, J_2=17Hz, 1H, H-3), 2.72-2.93 (m, 3H, C<u>H</u>C<u>H</u>$_2$), 4.19 (dd, J_1=5Hz, J_2=9Hz, 1H, H-5), 4.32 (dd, J_1=5Hz, J_2=9Hz, 1H, H-5), 7.11-7.36 (m, 5H, Ph);

^{13}C-NMR ($CDCl_3$): δ 34.2 (t, C-3), 37.1 (d), 38.9 (t, <u>C</u>H$_2$Ph), 72.6 (t, C-5), 126.8 (d, C-4`), 128.6 (d, C-3`), 127.7 (d, C-2`), 138.3 (s, C-1`), 176.8 (s, C=O);

m/z: 176 (80, M^+), 117 (47), 115 (29), 92 (82), 91 (100), 65 (42).

[7] Assignment based on: Alphand, V.; Mazzini, C.; Lebreton, J.; Furstoss, R. *J. Mol. Catal. B: Enzym.* **1998**, *5*, 219-221.

3.6 Dihydro-4-phenyl-furan-2(3H)-one (2d)



Method A: Cyclobutanone (183mg, 1.25mmol) was oxidized according to the general procedure. The product did not require additional purification. (GC: BGB 173, method A)

Yield: 243 mg (99%)

MW: 162.19, $C_{10}H_{10}O_2$

Method B: 3-Phenylcyclobutanone was oxidized with recombinant *E. coli* cells according to general procedure. After purification *via* column chromatography (silica gel, LP/EtOAc=15/1) lactone (2d) was obtained as colorless crystals.

Enzyme	1d	2d	*ee*	abs. config.[7]	$[\alpha]^{D}_{20}$
$CHMO_{Acineto}$	110mg	65mg (53%)	62%	(R)	-30.0 (*c* 1.20, MeOH)
$CHMO_{Arthro}$	110mg	66mg (54%)	87%	(R)	-43.2 (*c* 1.20, MeOH)
$CHMO_{Brachy}$	110mg	55mg (45%)	93%	(R)	-45.0 (*c* 1.00, MeOH)
$CHMO_{Brevi1}$	110mg	89mg (73%)	98%	(R)	-47.3 (*c* 1.80, MeOH)
$CHMO_{Brevi2}$	110mg	61mg (50%)	39%	(S)	+18.0 (*c* 1.10, MeOH)
$CPMO_{Coma}$	110mg	81mg (66%)	37%	(S)	+16.9 (*c* 1.50, MeOH)
$CHMO_{Rhodo1}$	110mg	71mg (58%)	52%	(R)	-24.0 (*c* 1.30, MeOH)
$CHMO_{Rhodo2}$	110mg	77mg (63%)	50%	(R)	-21.2 (*c* 1.50, MeOH)

colorless crystals, m.p.=50-55°C, (Lit.[8]: 47-48.5°C)

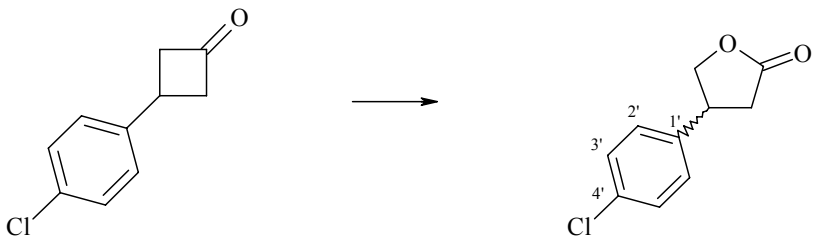
^{1}H-NMR ($CDCl_3$): δ 2.59-2.72 (dd, J=18Hz, 1H, H-3), 2.85-2.98 (dd, J=18Hz, 1H, H-3), 3.70-3.86 (pent, J=17Hz, 1H, H-4), 4.21-4.30 (dd, J=9Hz, 1H, H-5), 4.61-4.70 (dd, J=9Hz, 1H, H-5), 7.19-7.41 (m, 5H,-Ph);

^{13}C-NMR ($CDCl_3$): δ 35.6 (t, C-3), 41.0 (d, C-4), 73.9 (t, C-5), 126.6 (2xd, C-Ph), 127.6 (d, C-Ph), 129.0 (2xd, C-Ph), 139.4 (s, Ph), 176.3 (s, C=O);

m/z: 162 (29, M^+), 105 (9), 104 (100), 78 (11), 77 (11), 51 (11).

[8] Sato, M. *Chem. Pharma. Bull.* **1981**, *29*, 2885-2892.

3.7 4-(4-Chlorophenyl)-dihydrofuran-2(3H)-one (2e)



Method A: According to the general procedure 3-(4-chlorophenyl)-cyclobutanone (100mg, 0.55mmol) converted to the desired racemic lactone. After flash chromatography (15g SiO_2, LP/EtOAc=3/1) the desired product was isolated in 93% yield. (GC: BGB 173, method B)

Yield: 100mg (93%)

MW: 196.6, $C_{10}H_9ClO_2$

Method B: 3-(4-chlorophenyl)-cyclobutanone was oxidized with recombinant *E. coli* cells according to general procedure. The crude product was purified *via* column chromatography (silica gel, LP/EtOAc=3/1) and was obtained as yellow oil.[9]

Enzyme	1e	2e	*ee*	abs. config.[9]	$[\alpha]^D_{20}$
$CHMO_{Acineto}$	100mg	73mg (67%)	81%	(S)	+31.1 (*c 0.94*, $CHCl_3$)
$CHMO_{Arthro}$	100mg	55mg (50%)	87%	(S)	+20.2 (*c* 1.04, $CHCl_3$)
$CHMO_{Brachy}$	100mg	67mg (57%)	68%	(S)	+18.5 (*c* 1.14, $CHCl_3$)
$CHMO_{Brevi1}$	100mg	51mg (47%)	87%	(R)	-37.6 (*c* 0.54, $CHCl_3$)
$CHMO_{Brevi2}$	100mg	55mg (50%)	42%	(S)	+16.8 (*c* 1.00, $CHCl_3$)
$CPMO_{Coma}$	100mg	85mg (78%)	44%	(S)	+20.8 (*c* 1.56, $CHCl_3$)
$CHMO_{Rhodo1}$	100mg	56mg (51%)	95%	(S)	+42.1 (*c* 1.26, $CHCl_3$)
$CHMO_{Rhodo2}$	100mg	69mg (63%)	95%	(S)	+44.2 (*c* 1.02, $CHCl_3$)

yellow oil

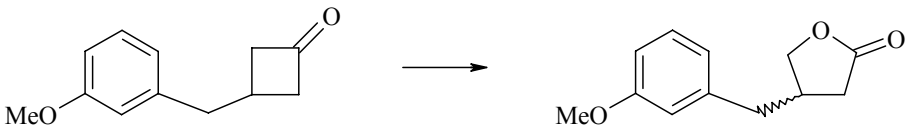
^{1}H-NMR ($CDCl_3$): δ 2.63 (dd, J_1=9Hz, J_2=17Hz, 1H, H-3/H-5), 2.94 (dd, J_1=9Hz, J_2=17Hz, 1H, H-3/H-5), 3.77 (quin, J=8Hz, 1H, H-4,), 4.24 (dd, J_1=8Hz, J_2=9Hz, 1H, H-3/H-5), 4.67 (dd, J_1=8Hz, J_2=9Hz, 1H, H-3/H-5), 7.18 (d, J=8Hz, 2H, Ph), 7.35 (d, J=8Hz, 2H, Ph);

[9] Assignment based on: Mazzini, C.; Lebreton, J.; Alphand, V.; Furstoss, R. *Tetrahedron Lett.* **1997**, 7, 1195-1196.

^{13}C-NMR ($CDCl_3$): δ 35.6 (t, C-3), 40.5 (d, C-4), 73.8 (t, C-5), 128.0, 129.3 (2xd C-2', C-3'), 133.6, 137.9 (2xs, C-1', C-4'), 176.1 (s, C=O);

m/z: 196 (25, M^+), 140 (27), 138 (100), 103 (18).

3.8 Dihydro-5-(3-methoxyphenyl)-furan-2(3H)-one (2f)



Method A: Cyclobutanone (131mg, 0.69mmol) was oxidized according to the general procedure. The crude product was purified *via* flash column chromatography (LP/EtOAc = 6/1). (GC: BGB 173, method B)

Yield: 32.0 mg (23%)

MW: 206.24, $C_{12}H_{14}O_3$

Mehod B: 3-[(3-Methoxyphenyl)methyl]cyclobutanone was biooxidized according to general procedure. The crude product was purified *via* column chromatography (LP/EtOAc = 8/1) and the yellow odorant oil was isolated.

Enzyme	1f	2f	*ee*	abs. config.[5]	$[\alpha]^D_{20}$
$CHMO_{Acineto}$	108mg	60mg (50%)	>99%	(S)	-3.80 (c 1.20, $CHCl_3$)
$CHMO_{Arthro}$	108mg	51mg (43%)	93%	(S)	-5.85 (c 0.91, $CHCl_3$)
$CHMO_{Brachy}$	108mg	54mg (45%)	93%	(S)	-5.45 (c 0.95, $CHCl_3$)
$CHMO_{Brevi1}$	108mg	80mg (74%)	35%	(S)	-2.10 (c 1.60, $CHCl_3$)
$CHMO_{Brevi2}$	108mg	59mg (50%)	45%	(S)	-1.85 (c 0.27, $CHCl_3$)
$CPMO_{Coma}$	108mg	83mg (70%)	45%	(S)	-3.70 (c 0.98, $CHCl_3$)
$CHMO_{Rhodo1}$	108mg	72mg (60%)	98%	(S)	-6.50 (c 1.42, $CHCl_3$)
$CHMO_{Rhodo2}$	108mg	87mg (72%)	98%	(S)	-6.32 (c 1.71, $CHCl_3$)

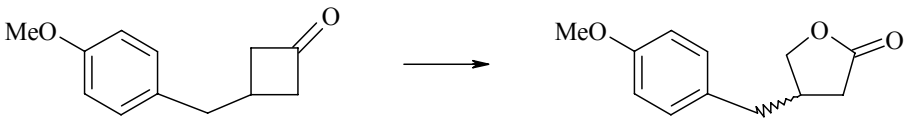
yellow odorant oil

^{1}H-NMR ($CDCl_3$): δ 2.15-2.27 (dd, J=17Hz, 1H, H-3), 2.47-2.60 (dd, J=17Hz, 1H, H-3), 2.62-2.86 (m, 3H, C<u>H</u>C<u>H$_2$</u>), 3.72 (s, 3H, OCH_3), 3.87-3.99 (dd, J=9Hz, 1H, H-5), 4.22-4.30 (dd, J=9Hz, 1H, H-5), 6.62-6.74 (m, 3H, H-2`, H-4`, H-6`, Ph), 7.12-7.20 (t, J=8Hz, 1H, H-5`, Ph);

^{13}C-NMR ($CDCl_3$): δ 34.2 (t, C-3), 37.0 (d), 38.9 (t, CH_2Ph), 55.2 (q, OCH_3) 72.6 (t, C-5), 111.8 (d, C-6`), 114.6 (d, C-2`), 120.9 (d, C-4`), 129.8 (d, C-5`), 139.8 (s, C-3`), 159.9 (s, C-1`), 176.8 (s, C=O);

m/z: 206 (31, M^+), 122 (100), 121 (33), 107 (9), 91 (22), 77 (12).

3.9 Dihydro-4-[(4-methoxyphenyl)methyl]-2(3H)-furanone (2g)



Method A: 3-(4-Methoxybenzyl)-cyclobutanone (100mg, 0.53mmol) was converted according to the general procedure and gave the desired lactone as a yellow oil after 19 hours of reaction time and purification *via* column chromatography (LP/EtOAc=7/1, 15g, SiO_2). (GC: BGB 173, method B)

Yield: 50.0mg (46%)

MW: 206.2; $C_{12}H_{14}O_3$

Method B: 3-[(4-Methoxyphenyl)methyl]cyclobutanone was oxidized according to general method B. The crude product was purified *via* column chromatography (LP/EtOAc = 10/1) and the yellow odorant oil was isolated in the yields and properties specified below.

Enzyme	1g	2g	*ee*	abs. config.[10]	$[\alpha]^D_{20}$
$CHMO_{Acineto}$	100mg	60mg (55%)	97%	(S)	-5.45 (c 0.95, $CHCl_3$)
$CHMO_{Arthro}$	69mg	69mg (89%)	97%	(S)	-6.11 (c 0.95, $CHCl_3$)
$CHMO_{Brachy}$	69mg	48mg (64%)	90%	(S)	-5.27 (c 0.74, $CHCl_3$)
$CHMO_{Brevi1}$	100mg	80mg (73%)	26%	(S)	-2.10 (c 1.60, $CHCl_3$)
$CHMO_{Brevi2}$	69mg	48mg (64%)	24%	(R)	+1.08 (c 0.74, $CHCl_3$)
$CPMO_{Coma}$	69mg	42mg (56%)	24%	(R)	+1.29 (c 0.62, $CHCl_3$)
$CHMO_{Rhodo1}$	69mg	40mg (53%)	80%	(S)	-3.97 (c 0.68, $CHCl_3$)
$CHMO_{Rhodo2}$	69mg	42mg (56%)	95%	(S)	-8.83 (c 0.60, $CHCl_3$)

yellow odorant oil

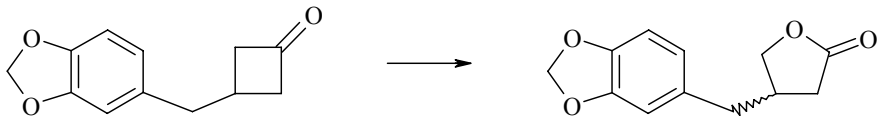
^1H ($CDCl_3$): δ 2.27 (dd, J_1=17Hz, J_2=7Hz, 1H, H-3), 2.59 (d, J_1=8Hz, 1H, H-3,), 2.67-2.86 (m, 4H), 3.79 (s, 3H, OCH_3), 4.02 (dd, J_1=9Hz, J_2=6Hz, 1H, H-5), 4.32 (dd, J_1=9Hz, J_2=7Hz, 1H, H-5), 6.85 (d, J=9Hz, 2H, H-3', H-5'), 7.07 (d, J=9Hz, H-2', H-6');

^{13}C ($CDCl_3$): δ 34.7 (t, C-3), 37.9 (d, C-4), 38.6 (t), 55.8 (q, OCH_3), 114.7 (d, C-3'), 130.1 (d, C-2'), 130.7 (s, C-1'), 159.0 (s, C-4'), 177.4 (s, C=O);

m/z: 206 (15, M^+), 122 (9), 121 (100), 77 (8).

[10] Shiotani, S.; Okada, H.; Yamamoto, T.; Nakamata, K.; Adachi, J.; Nakamoto, H. *Heterocycles* **1996**, *43*, 113-126.

3.9 Dihydro-4-(1,3-benzodioxol-5-ylmethyl)-furan-2(3H)-one (2h)



Method A: 3-(1,3-Benzodioxol-5-ylmethyl)-cyclobutanone (165mg, 0.81mmol) was oxidized according to the general procedure. The crude product was purified *via* column chromatography (LP/EtOAc=7/1) to give a brown odorant oil. (GC: BGB 173, method B)

Yield: 104 mg (59%)

MW: 220.2, $C_{12}H_{12}O_4$

Method B: 3-(1,3-Benzodioxol-5-ylmethyl)-cyclobutanone was oxidized with *E.coli* expressing cells according to general procedure. The crude product was purified *via* column chromatography (LP/EtOAc = 6/1) and the brown odorant oil was isolated in the yields and properties specified below.

Enzyme	1h	2h	*ee*	abs. config.[5,7]	$[\alpha]^D_{20}$
$CHMO_{Acineto}$	94mg	35mg (35%)	97%	(S)	-6.16 (*c* 0.70, $CHCl_3$)
$CHMO_{Arthro}$	94mg	35mg (35%)	98%	(S)	-3.71 (*c* 0.70, $CHCl_3$)
$CHMO_{Brachy}$	94mg	61mg (60%)	98%	(S)	-4.95 (*c* 1.21, $CHCl_3$)
$CHMO_{Brevi1}$	94mg	62mg (61%)	75%	(R)	+2.38 (*c* 1.51, $CHCl_3$)
$CHMO_{Brevi2}$	94mg	53mg (53%)	37%	(S)	-2.15 (*c* 1.30, $CHCl_3$)
$CPMO_{Coma}$	94mg	57mg (56%)	40%	(S)	-2.64 (*c* 1.14, $CHCl_3$)
$CHMO_{Rhodo1}$	94mg	45mg (45%)	98%	(S)	-3.74 (*c* 0.91, $CHCl_3$)
$CHMO_{Rhodo2}$	94mg	53mg (52%)	98%	(S)	-4.24 (*c* 0.75, $CHCl_3$)

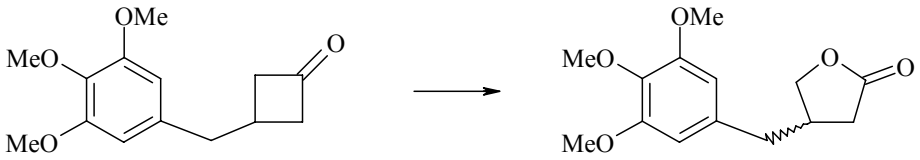
brown odorant oil

^{1}H-NMR ($CDCl_3$): δ 2.13-2.25 (dd, J=17Hz, 1H, H-3), 2.46-2.80 (m, 4H, H-3, H-4, $CHC\underline{H}_2$), 3.90-3.98 (dd, J=9Hz, 1H, H-5), 4.21-4.29 (dd, J=9Hz, 1H, H-5), 5.87 (s, 2H, OCH_2O), 6.50-6.56 (m, 2H, H-6`, H-2`), 6.66-6.70 (d, J=8Hz, 1H, H-5`);

^{13}C-NMR ($CDCl_3$): δ 31.7 (t, C-3), 34.9 (d), 36.2 (t, CH_2Ph), 70.1 (t, C-5), 98.6 (OCH_2O), 106.0 (d, C-2`, Ph), 106.5 (d, C-5`, Ph), 119.2 (d, C-6`, Ph), 129.5 (d, C-1`, Ph), 144.0 (s, C-4`, Ph), 145.6 (s, C-3`, Ph), 174.4 (s, C=O);

m/z: 220 (44, M^+), 136 (20), 135 (100), 105 (7), 77 (29), 51 (15).

3.10 Dihydro-4-(3,4,5-Trimethoxy-benzyl)-furan-2(3H)-one (2i)



Method B: 3-(3,4,5-trimethoxy-benzyl)-cyclobutanone was oxidized with *E.coli* expressing cells according to general procedure. The crude product was purified *via* column chromatography (LP/EtOAc = 2/1, 5g SiO_2) and the colorless crystals were isolated in the yields and properties specified below. [11] (GC: BGB 173, method D)

Enzyme	1i	2i	*ee*	abs. config.[11]	$[\alpha]^D_{20}$
$CHMO_{Acineto}$	50mg	48mg (90%	90%	(S)	-5.60 (c 0,96, $CHCl_3$)
$CHMO_{Arthro}$	65mg	50mg (72%)	94%	(S)	-6.10 (*c* 1.00, $CHCl_3$)
$CHMO_{Brachy}$	65mg	40mg (58%)	94%	(S)	-8.88 (*c* 0.80, $CHCl_3$)
$CHMO_{Brevi1}$	65mg	50mg (72%)	79%	(R)	+4.38 (*c* 1.00, $CHCl_3$)
$CHMO_{Brevi2}$	65mg	n.c.	n.a.	-	n.a.
$CPMO_{Coma}$	65mg	n.c.	n.a.	-	n.a.
$CHMO_{Rhodo1}$	65mg	46mg (67%)	95%	(S)	-4.75 (*c* 0.80, $CHCl_3$)
$CHMO_{Rhodo2}$	65mg	38mg (55%)	92%	(S)	-6.58 (*c* 0.76, $CHCl_3$)

MW: 266.3, $C_{14}H_{18}O_5$

colorless crystals, m.p.: 93-96°C (Lit.[11] 98-99°C)

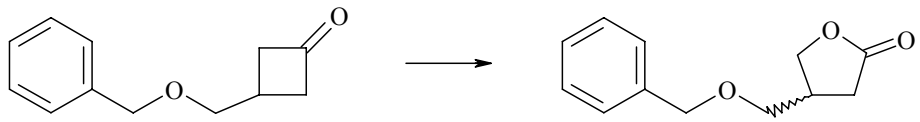
^{1}H-NMR ($CDCl_3$): δ 2.30 (dd, J_1=7Hz, J_2 = 17Hz, 1H, H-3), 2.55-2.95 (m, 4H), 3.83 (s, 3H, OCH_3), 3.85 (s, 6H, 2xOMe), 4.05 (dd, J_1=6Hz, J_2=9Hz, 1H, H-5), 4.36 (dd, J_1=7Hz, J_2=9Hz, 1H, H-5);

^{13}C-NMR ($CDCl_3$): δ 34.3 (t), 34.9 (d), 37.2 (d, C-4), 56.1 (2xq, OMe), 60.8 (q, OMe), 72.6 (t, C-5), 105.2 (2xd, Ph), 134.0 (s, Ph), 136.8 (s, Ph), 153.4 (s, Ph), 176.8 (s, C=O);

m/z: 266 (39, M^+), 182 (41), 181 (100), 167 (14), 151 (19).

[11] Tanaka, M.; Mitsuhashi, H.; Maruno, M.; Wakamatsu, T. *J. Org. Chem.* **1995**, 4339-4352.

3.11 4-Benzyloxymethyl-dihydro-furan-2(3H)-one (2j)



Method A: 3-Benzyloxymethylcyclobutanone (312mg, 1.64mmol) was oxidized to the desired lactone according to the general procedure. After purification *via* flash column chromatography (LP/EtOAc = 2/1) the desired lactone was obtained as a yellow oil.[12] (GC: BGB 175, method B)

Yield: 178mg (53%)

MW: 206.2, $C_{12}H_{14}O_3$

Method B: 3-Benzyloxymethylcyclobutanone was oxidized with recombinant *E. coli* cells according to general procedure. The crude product was purified *via* column chromatography (silica gel, LP/EtOAc=2/1) and **2a** was obtained as yellow oil.

Enzyme	1j	2j	*ee*	abs. config.[5,7]	$[\alpha]^{D}_{20}$
$CHMO_{Acineto}$	116mg	52mg (41%)	53%	(S)	+16.8 (*c* 1.04, $CHCl_3$)
$CHMO_{Arthro}$	116mg	23mg (18%)	58%	(R)	-19.1 (*c* 0.40, $CHCl_3$)
$CHMO_{Brachy}$	116mg	28mg (22%)	rac.	-	n.a.
$CHMO_{Brevi1}$	116mg	33mg (26%)	55%	(S)	+17.1 (*c* 0.66, $CHCl_3$)
$CHMO_{Brevi2}$	116mg	65mg (52%)	62%	(R)	-20.7 (*c* 1.30, $CHCl_3$)
$CPMO_{Coma}$	116mg	67mg (53%)	63%	(R)	-18.9 (*c* 1.34, $CHCl_3$)
$CHMO_{Rhodo1}$	116mg	25mg (45%)	6.0%	(S)	+1.80 (*c* 0.50, $CHCl_3$)
$CHMO_{Rhodo2}$	116mg	29mg (23%)	9.2%	(S)	+2.07 (*c* 0.58, $CHCl_3$)

yellow oil

^{1}H-NMR (CDCl3): δ 2.36 (dd, J_1=6Hz, J_2=18Hz, 1H, H-3), 2.61 (dd, J_1=9Hz, J_2=18Hz, 1H, H-3), 2.84 (sept, J=8Hz, 1H, H-4), 3.47 (m, 2H, CH_2O), 4.17 (dd, J_1=6Hz, J_2=9Hz, 1H, H-5), 4.41 (dd, J_1=7Hz, J_2=9Hz, 1H, H-5), 4.52 (s, 2H, Ph$C\underline{H}_2$O), 7.25-7.36 (m, 5H, Ph);

^{13}C-NMR ($CDCl_3$): δ 31.0 (t, C-3), 35.2 (d, C-4), 70.2 (t, CH_2O), 70.6 (t, CH_2O), 73.2 (t, CH_2O), 127.5 (2xd, C-Ph), 127.7 (d, C-Ph), 128.3 (2xd, C-Ph), 137.5 (s, C-Ph), 176.8 (s, C=O);

m/z: 206 (5, M^+), 205 (10), 177 (9). 120 (46), 105 (15), 92 (16), 91 (100), 65 (17).

[12] Hon, Y.-S.; Lee, C.-F. *Tetrahedron,* **2001**, *57*, 6181-6188.