

## Supplementary Materials:

Continuous-flow biocatalytic process for the synthesis of the best stereoisomers of the commercial fragrances Leather Cyclohexanol (4-(isopropyl)cyclohexanol) and Woody Acetate (4-(*tert*-butyl)cyclohexyl acetate)

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### Overexpression of GDH in *E. coli* BL21(DE3).

Glucose dehydrogenase (GDH from *Bacillus megaterium*) was overexpressed in *E. coli* BL21(DE3) strains harboring the plasmid pKTS-GDH [1]. LB medium (5 mL) containing the appropriate antibiotic (100 µg mL<sup>-1</sup> ampicillin) was inoculated with a single colony from a fresh plate and grown for 8 h at 37°C and 220 rpm. This starter culture was used to inoculate 200 mL medium, which was incubated for 8 h at the same conditions and used to inoculate 1.5 L medium. The latter culture was shaken at 37 °C and 220 rpm until OD<sub>600</sub> reached 0.4-0.5, then enzyme expression was induced by the addition of 0.1 mM IPTG and 50 ng mL<sup>-1</sup> anhydrotetracycline. After 5-6 h the cells were harvested by centrifugation (5000 g, 20 min, 4°C), resuspended in 50 mL of lysis buffer (20 mM potassium phosphate buffer pH 7.0, 300 mM NaCl, 10 mM imidazole) and disrupted by sonication (Omni Ruptor 250 ultrasonic homogenizer, five sonication cycles, 15 s each, 50% duty). The cell-free extract, after centrifugation (20000 g, 20 min, 4°C), was chromatographed on IMAC stationary phase (Ni-Sepharose Fast Flow, GE Healthcare) with a mobile phase composed of 20 mM potassium phosphate buffer pH 7.0, 300 mM NaCl and a 10-300 mM imidazole gradient. Protein elution was monitored at 280 nm, the fractions were collected according to the chromatogram and dialysed twice against 1.0 L of 50 mM potassium phosphate buffer pH 7.0 (12 h each, 4°C) to remove imidazole and salts. Purified protein aliquots were stored frozen at –80°C.

### References:

1. Bechtold, M.; Brenna, E.; Femmer, C.; Gatti, F.G.; Panke, S.; Parmeggiani, F.; Sacchetti, A. *Org. Process Res. Dev.* **2012**, *16*, 269–276.

**Table S1.** ADH-mediated reduction of 4-alkylcyclohexanones **4** and **5** to 4-alkylcyclohexanols **1** and **3** (preliminary screening)<sup>1</sup>

ADH	4-isopropylcyclohexan-1-one		4-( <i>tert</i> -butyl)cyclohexan-1-one	
	c (%)	<i>de</i> (%)	c (%)	<i>de</i> (%)
10	60	77 ( <i>trans</i> )	7	71 ( <i>trans</i> )
20	45	91 ( <i>trans</i> )	4	>99 ( <i>trans</i> )
30	>99	89 ( <i>cis</i> )	>99	>99 ( <i>cis</i> )
40	20	90 ( <i>trans</i> )	5	>99 ( <i>trans</i> )
130	16	80 ( <i>trans</i> )	5	>99 ( <i>trans</i> )
140	32	50 ( <i>trans</i> )	<1	-
190	16	75 ( <i>trans</i> )	<1	-
200	>99	87 ( <i>cis</i> )	>99	>99 ( <i>cis</i> )
210	27	48 ( <i>trans</i> )	8	50 ( <i>trans</i> )
250	31	23 ( <i>trans</i> )	34	94 ( <i>cis</i> )
260	<1	-	<1	-
270	>99	73 ( <i>cis</i> )	>99	67 ( <i>cis</i> )
380	63	17 ( <i>trans</i> )	42	>99 ( <i>trans</i> )
420	>99	81 ( <i>cis</i> )	>99	97 ( <i>cis</i> )
430	5	60 ( <i>trans</i> )	16	>99 ( <i>trans</i> )
440	>99	87 ( <i>trans</i> )	>99	>99 ( <i>trans</i> )
441	>99	87 ( <i>cis</i> )	>99	>99 ( <i>cis</i> )
442	31	61 ( <i>trans</i> )	65	78 ( <i>trans</i> )

<sup>1</sup> 5 mM substrate, 20 mM glucose, ADH (200  $\mu\text{g mL}^{-1}$ ), GDH (5 U  $\text{mL}^{-1}$ ), NADH and NADP<sup>+</sup> (0.1 mmol each), 1% DMSO, phosphate buffer pH 7.0, 30°C, 18 h; conversion (c %) and diastereoisomeric excess (*de* %) calculated by GC/MS.

**Table S2.** ADH-mediated reduction of 4-alkylcyclohexanones **6** and **7** to 4-alkylcyclohexanols **8** and **9** (preliminary screening)<sup>1</sup>

ADH	4-methylcyclohexan-1-one		4-phenylcyclohexan-1-one	
	c (%)	de (%)	c (%)	de (%)
10	11	64 ( <i>trans</i> )	32	>99 ( <i>trans</i> )
20	12	>99 ( <i>trans</i> )	53	>99 ( <i>trans</i> )
30	47	96 ( <i>cis</i> )	>99	>99 ( <i>cis</i> )
40	<1	-	7	>99 ( <i>trans</i> )
130	<1	-	6	>99 ( <i>trans</i> )
140	<1	-	<1	-
190	<1	-	9	33 ( <i>cis</i> )
200	46	91 ( <i>cis</i> )	>99	>99 ( <i>cis</i> )
210	23	39 ( <i>trans</i> )	<1	-
250	18	78 ( <i>cis</i> )	48	92 ( <i>cis</i> )
260	<1	-	<1	-
270	78	92 ( <i>cis</i> )	95	83 ( <i>cis</i> )
380	86	7 ( <i>trans</i> )	76	61 ( <i>trans</i> )
420	85	90 ( <i>cis</i> )	57	79 ( <i>cis</i> )
430	11	45 ( <i>cis</i> )	17	88 ( <i>trans</i> )
440	90	98 ( <i>trans</i> )	95	>99 ( <i>trans</i> )
441	77	95 ( <i>cis</i> )	77	64 ( <i>cis</i> )
442	<1	-	97	94 ( <i>trans</i> )

<sup>1</sup> 5 mM substrate, 20 mM glucose, ADH (200  $\mu\text{g mL}^{-1}$ ), GDH (5 U  $\text{mL}^{-1}$ ), NADH and NADP<sup>+</sup> (0.1 mmol each), 1% DMSO, phosphate buffer pH 7.0, 30°C, 18 h; conversion (c %) and diastereoisomeric excess (*de* %) calculated by GC/MS.