Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting Information

Sequential chemo-biocatalytic synthesis of aroma compounds

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List of abbreviations

*Bd*ADH2	Alcohol dehydrogenase from *Bradyrhizobium diazoefficiens* USDA 110
GC	Gas chromatography
IS	Internal standard
*Lb*BVMO	Baeyer-Villiger monooxygenase from *Leptospira biflexa*

Supporting figures

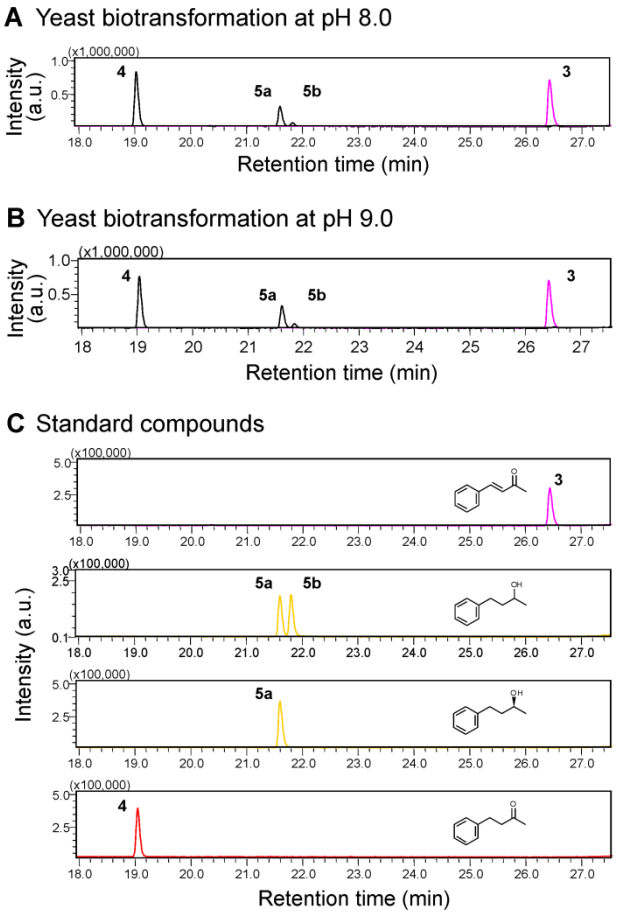

Fig. S1 GC chromatograms of Baker´s yeast biotransformation of benzalacetone. Baker´s yeast-catalysed biotransformation of benzalacetone (**3**) at 0 h (pink) and after 16 h (black) at pH 8.0 (A) and pH 9.0 (B). (C) GC chromatograms of standard compounds are shown: **4**, benzylacetone; **5a**, S-(+)-4-phenyl-2-butanol; **5b**, R-(-)-4-phenyl-2-butanol. The region of the chromatograms between 18.0–27.4 min is shown. Retention time of the internal standard: 4.72 min.

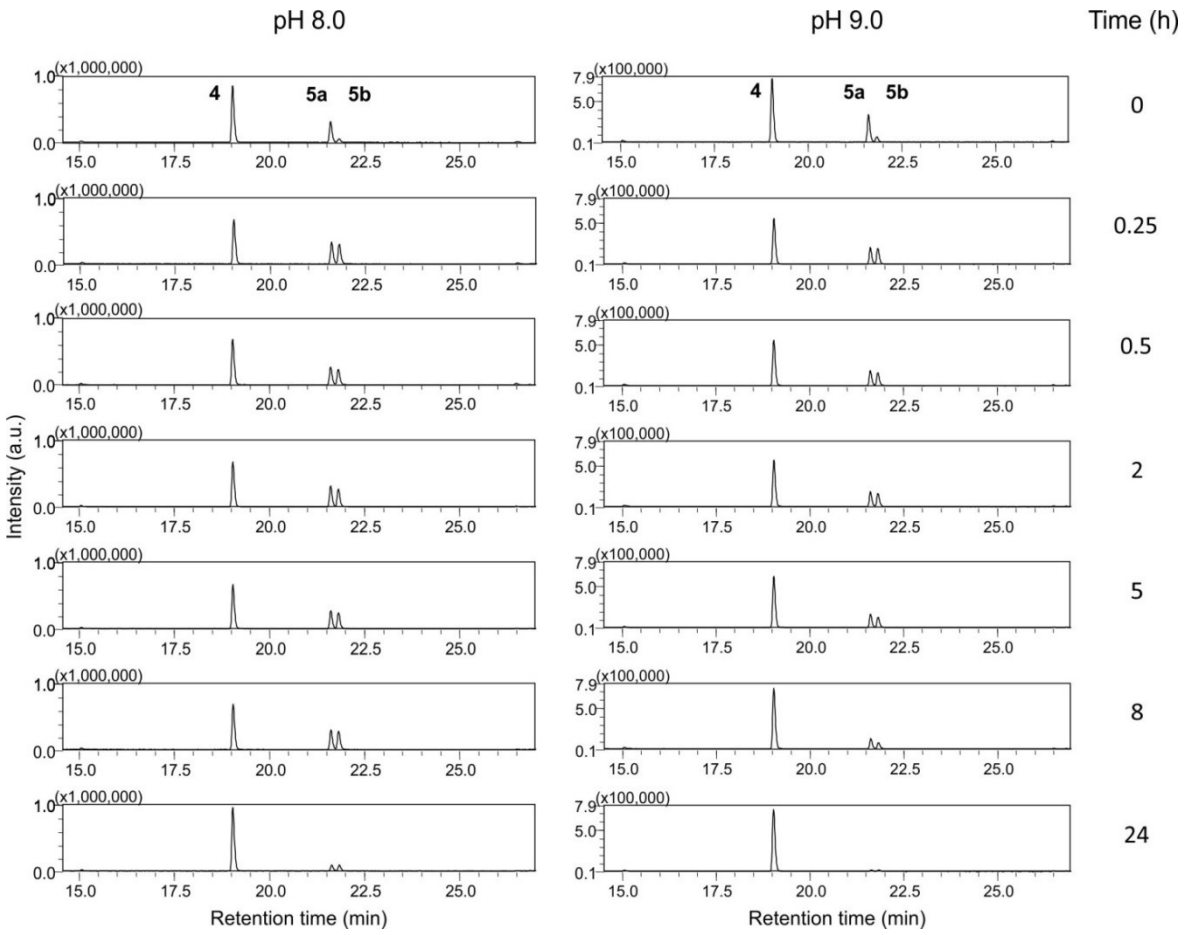

Fig. S2 GC chromatograms of biotransformations of a mixture of benzylacetone and 4-phenyl-2-butanol catalysed by *Escherichia coli* cells expressing *Bd*ADH2. Reactions were stopped after different periods of incubation (0 h, 0.25 h, 0.5 h, 2 h, 5 h, 8 h and 24 h) at either pH 8.0 or 9.0. The starting material was obtained after 16 h of Baker´s yeast biotransformation of benzalacetone at the corresponding pH condition. The region of the chromatograms between 14.5–27.0 min is shown. Retention time of the internal standard: 4.72 min. **4**, benzylacetone; **5a**, *S*-(+)-4-phenyl-2-butanol; **5b**, *R*-(-)-4-phenyl-2-butanol.

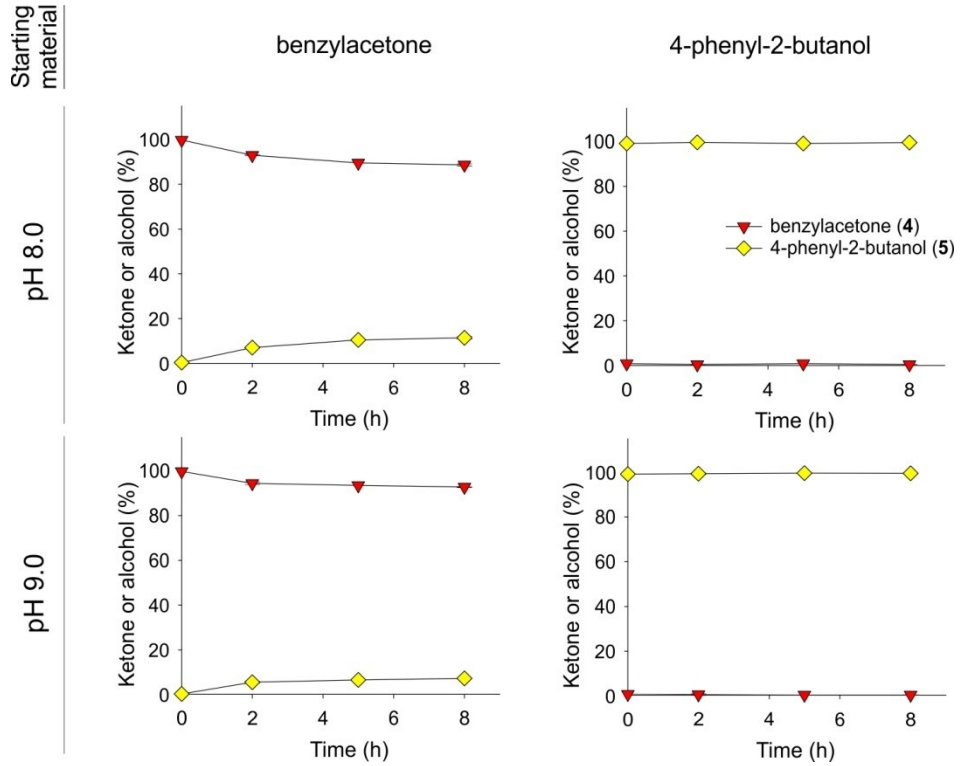

Fig. S3 Biotransformation of benzylacetone or 4-phenyl-2-butanol by non-recombinant *E. coli* cells. The starting material was benzylacetone (**4**) or 4-phenyl-2-butanol (**5**). The effect of two different pH conditions (8.0 and 9.0) was evaluated.

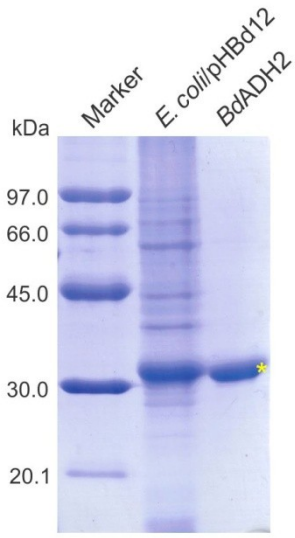

Fig. S4 Purification of recombinant *Bd*ADH2. The soluble fraction corresponding to the protein extract of *E. coli* BL21(DE3) transformed with pHBd12 and expressing *Bd*ADH2, and the purified *Bd*ADH2 (yellow asterisk) were subjected to 12% SDS-PAGE followed by Coomassie Brilliant Blue staining. Marker, molecular mass marker.

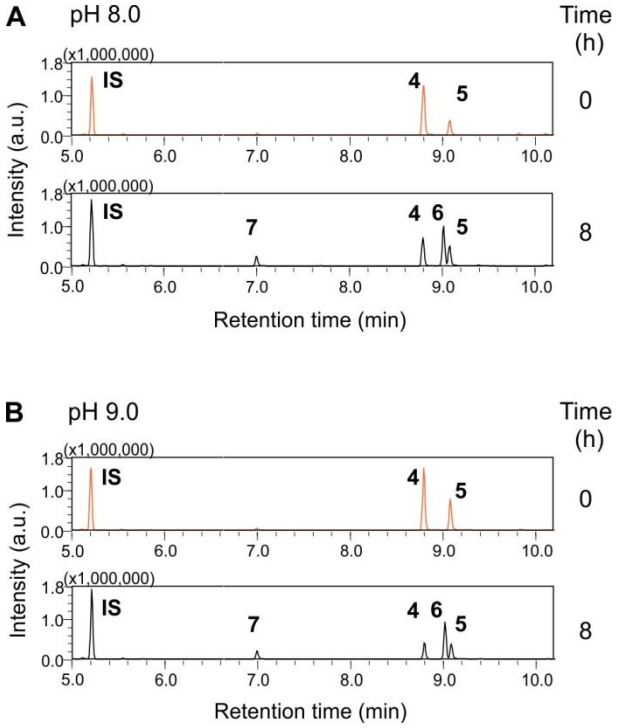

Fig. S5 Representative GC chromatograms of biotransformations of a mixture of benzylacetone and 4-phenyl-2-butanol catalysed by *E. coli* cells expressing individually *Lb*BVMO and *Bd*ADH2. Chromatograms correspond to 0 h (orange) and 8 h (black) of reaction at either pH 8.0 (A) or pH 9.0 (B). The starting material was obtained after 16 h of Baker´s yeast biotransformation of benzalacetone at the corresponding pH condition. The region of the chromatograms between 5.0–10.2 min is shown. **4**, benzylacetone; **5**, 4-phenyl-2-butanol; **6**, 2-phenylethyl acetate; **7**, 2-phenylethanol; **IS**, internal standard.

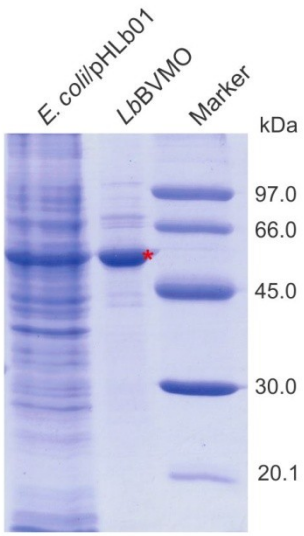

Fig. S6 Purification of recombinant *Lb*BVMO. The soluble fraction of protein extracts of *E. coli* BL21(DE3) transformed with pHLb01 and expressing *Lb*BVMO, and the purified *Lb*BVMO (red asterisk) were subjected to 12% SDS-PAGE followed by Coomassie Brilliant Blue staining. Marker, molecular mass marker.

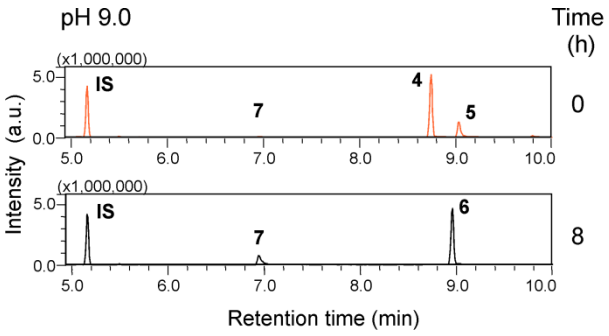

Fig. S7 Representative GC chromatograms of biotransformations of a mixture of benzylacetone and 4-phenyl-2-butanol catalysed by *E. coli* cells coexpressing *Lb*BVMO and *Bd*ADH2. Chromatograms correspond to 0 h (orange) and 8 h (black) of reaction at pH 9.0. The starting material was obtained after 16 h of Baker´s yeast biotransformation of benzalacetone. The region of the chromatograms between 5.0–10.2 min is shown. **4**, benzylacetone; **5**, 4-phenyl-2-butanol; **6**, 2-phenylethyl acetate; **7**, 2-phenylethanol; **IS**, internal standard.

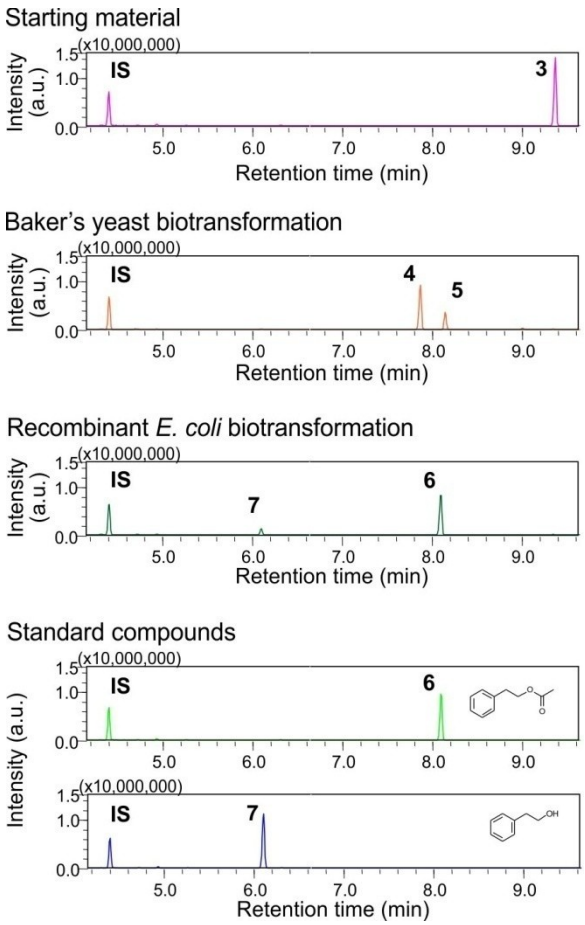

Fig. S8 Representative GC chromatograms of the biotransformations involved in the proposed route under preparative conditions. Baker´s yeast biotransformation of benzalacetone at pH 9.0, at 0 h (pink) and 16 h (orange) of reaction. Biotransformation catalysed by *E. coli* cells coexpressing *Lb*BVMO and *Bd*ADH2 at pH 9.0 after 8 h of reaction (dark green). GC chromatograms of standards of products are shown. The region of the chromatograms between 4.2–9.6 min is shown. **3**, benzalacetone; **4**, benzylacetone; **5**, 4-phenyl-2-butanol; **6**, 2-phenylethyl acetate; **7**, 2-phenylethanol; **IS**, internal standard.

Supporting tables

Table S1 Environmental impact assessment of the aldol condensation between benzaldehyde and acetone using NaOH as catalyst

	E-factor					Yield (%)
	reaction	reaction solvent	work-up	purification	total	
Kad *et al.*, 1999	10.4	77.0	593.3	14.0	694.8	85
Tatsuzaki *et al.*, 2006	15.1	19.9	247.5	30.1	312.6	34
Xiao *et al.*, 2016	14.0	0	63.4	941.8	1019.1	53
Nam *et al.*, 2020	3.6	2.2	88.8	811.3	905.8	66
our procedure A	7.8	0	0.5	1174.9	1183.2	51
our procedure B	12.2	0	9.6	531.1	552.8	51
our procedure C	4.1	0	6.1	212.1	222.3	56

If no information on the amount of auxiliary materials employed was available, we made the following assumptions: 50 mL g^{-1} of crude product of solvents for extraction; 20% of extract volume of aqueous solutions for washing; 10% of extract volume of brine; 0.02 g mL^{-1} of extract volume of anhydrous salts for drying extracts; 10 mL g^{-1} of crude product of solvents for recrystallisation; the amount of catalyst, as well as the amounts of solvents and silica used for flash chromatography were comparable to our procedure A. The *E*-factor values calculated with estimated quantities are indicated in blue.

Table S2 Bioproduction of 2-phenylethyl acetate and 2-phenylethanol in microbial systems

Biocatalyst	**Substrate**	**Products**		**Reference**
Submerged fermentation		**2-PEA** (mgL^{-1})	**2-PE** (mgL^{-1})	
Hanseniaspora guilliermondii	2-PE	164	n.a.	Rojas *et al.*, 2001
Kluyveromyces marxianus CBS 600				
i) batch at 35 °C	*L*-phe (molasses	i) 1270	i) 2200	Etschmann *et al.*, 2005
ii) in situ product removal at 45°C	medium)	ii) 4000	ii) 1600	Etschmann *et al.*, 2005
iii) fed-batch + polypropylene glycol 1200		iii) 6100	iii) 26500	Etschmann *et al.*, 2006
Kluyveromyces marxianus KY3				
i) aerobic conditions	Raw milk	i) 1	i) 469	Chang *et al.*, 2014
ii) anaerobic conditions		ii) 435	ii) 965	Chang *et al.*, 2014
Kluyveromyces marxianus				
i) ITD0211 strain	*L*-phe	i) 203	i) 630	Adame-Soto *et al.*, 2019
ii) ITD0090 strain		ii) 177	ii) 1024	Adame-Soto *et al.*, 2019
Candida glabrata				
i) DNP medium	i) *L*-phe	i) 35	n.d.	Rodríguez-Romero *et al.*, 2020
ii) ECP medium	ii) *L*-phe	ii) 665		Rodríguez-Romero *et al.*, 2020
iii) supplemented tequila vinasse	iii) tequila vinasse	iii) 60		Rodríguez-Romero *et al.*, 2020
Solid-state fermentation		(mg g^{-1} TS)	(mg g^{-1} TS)	
Kluyveromyces marxianus				
i) batch	Sugarcane bagasse	i) 3.9[a]; 7.0[b]	i) 12.1[a]; 10.0[b]	Martínez *et al.*, 2018;
	+*L*-phe			Martínez-Ávila *et al.*, 2019
ii) fed-batch		ii) 8.2[a]; 5.7[b]	ii) 10.2[a]; 13.5[b]	Martínez *et al.*, 2018;
				Martínez-Ávila *et al.*, 2019
iii) sequential-batch		iii) 8.6	iii) 11.5	Martínez-Ávila *et al.*, 2019

L-phe, *L*-phenylalanine; n.a., not applicable; n.d., not determined; TS, initial total solid content of substrate. [a]Ref. Martínez *et al.*, 2018; [b]Ref. Martínez-Ávila *et al.*, 2019.