General methods.

Recombinant DNA procedures were carried out essentially as described by Sambrook et al.[1] Restriction endonucleases were purchased from New England Biolabs or Promega. T4 DNA ligase was obtained from New England Biolabs. Standard media and techniques for routine growth and maintenance of *E. coli* and *S. cerevisiae* strains were used. YPD contained 1% Bacto-Yeast Extract and 2% Bacto-Peptone. Yeast expression vector pYES2 was purchased from Invitrogen. Oligonucleotides were obtained from Integrated DNA Technologies or Gemini Biotechnology. The knockout was constructed according to the micro-homology PCR method[2] and confirmed by PCR and Southern blot hybridization. *S. cerevisiae* were transformed by the lithium acetate method.[3] Substrate **1a** was obtained from Aldrich and ketones **1b** and **1c** were synthesized as reported by Dauben et al,[4] with slight modifications.

General procedure for biotransformations.

The different strains of *S. cerevisiae* were maintained on SD plates with the appropriate nutrients added (*e.g.*, ATCC 26403 requires fatty acid supplementation). Fresh plates were streaked from a frozen stock and a single colony was used to inoculate 50 mL of YPD in a sterile 250 mL Erlenmeyer flask. The culture was incubated at 30 °C in a rotary shaker at 200 rpm until the OD_{600} value was between 4 and 6. Cells were then harvested by centrifuging at 3000 × *g* for 10 min. The cell pellet was resuspended in 20 mL of 10 mM Tris-Cl, 1 mM EDTA (pH 7.5) by vortexing. This washing procedure was repeated an additional two times. The final cell pellet was resuspended in 10 mM Tris-Cl, 1 mM EDTA (pH 7.5), 15% glycerol at a concentration of 0.1 g/mL (wet weight). At this stage,

cells were either used directly for a reaction or frozen in aliquots at –80 °C for later use. Standard reaction mixtures for preparative biotransformations contained 90 mL YEP, 10 mL 20% galactose and 10 mM substrate. Freshly prepared or frozen cells were added to a final concentration of 2 mg/mL. Reaction flasks were shaken at 200 rpm and 30 °C, and the conversion was monitored by GC. Analytical samples were prepared by mixing 200 μL of the reaction mixture with 600 μL of ethyl acetate. After vortexing for 10 sec, the mixture was centrifuged in a microcentrifuge for 2 min, the organic layer was removed and 1 μL was used for GC analysis. After the reaction was complete, the reaction mixture was centrifuged at 3000 × *g* for 10 min at 4 °C to remove yeast cells. The supernatant was extracted with ethyl acetate (3 × 50 mL). The cell pellet was extracted with 20 mL of ethyl acetate and the organic extracts were combined, washed with brine and dried with Na_2SO_4. After concentrating the sample by rotary evaporation, the β-hydroxy esters were purified by flash chromatography on a 1 × 15 cm silica column using 1:1 ether:hexanes as mobile phase. Enantiomeric and diasteromeric excess values were determined by chiral GC (Chrompack 0.25 mm × 25 m CP chirasil-Dex CB). Chiral separations used the following conditions: 70 °C (2 min) to 130 °C (5 min) at 1.0 °C/min, followed by a 10 °C/min to 180 °C (10 min). The injector and detector temperatures were maintained at 250 °C and 220 °C, respectively.

References:

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