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Supplementary Information

Improving the Toolbox of Bioreductions by the Use of Continuous Flow Systems

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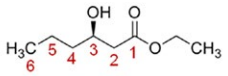
Characterisation NMR: ethyl 3-hydroxyhexanoate



^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ (ppm): 0.92 (t, 3H, H6); 1.26 (t, 3H, $COOCH_2\underline{CH_3}$); 1.43 (m, 4H, H4 and H5); 2.44 (m, 2H, H2); 3.15 (s, 1H, O<u>H</u>); 3.99 (m, 1H, H3); 4.16 (m, 2H, $COO\underline{CH_2}CH_3$); .

^{13}C NMR (50 MHz, $CDCl_3$, TMS) δ (ppm): 14.1 (C6); 14.3 ($COOCH_2\underline{C}H_3$); 18.8 (C5); 38.8 C4); 41.5 (C2); 60.8 ($COO\underline{C}H_2CH_3$); 68.0 (C3); 173.3 ($\underline{C}OOCH_2CH_3$).

DEPT 135 (50 MHz, $CDCl_3$, TMS) δ (ppm): 14.1 (C6); 14.3 ($COOCH_2\underline{C}H_3$); 18.8 (C5); 38.8 C4); 41.5 (C2); 60.8 ($COO\underline{C}H_2CH_3$); 68.0 (C3).

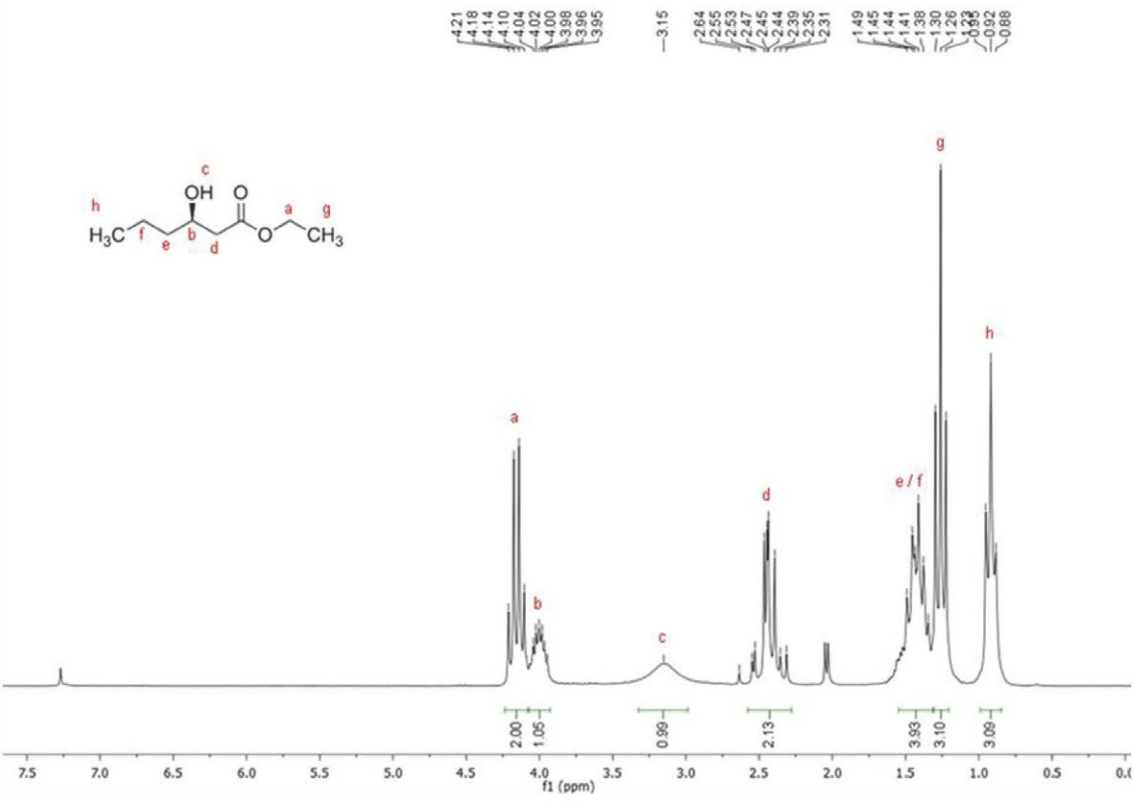


Figure S1. ^{1}H NMR (200 MHz, $CDCl_3$): ethyl 3-hydroxyhenoate (racemate obtained via $NaBH_4$ reduction).

*e-mail: souzarod21@gmail.com

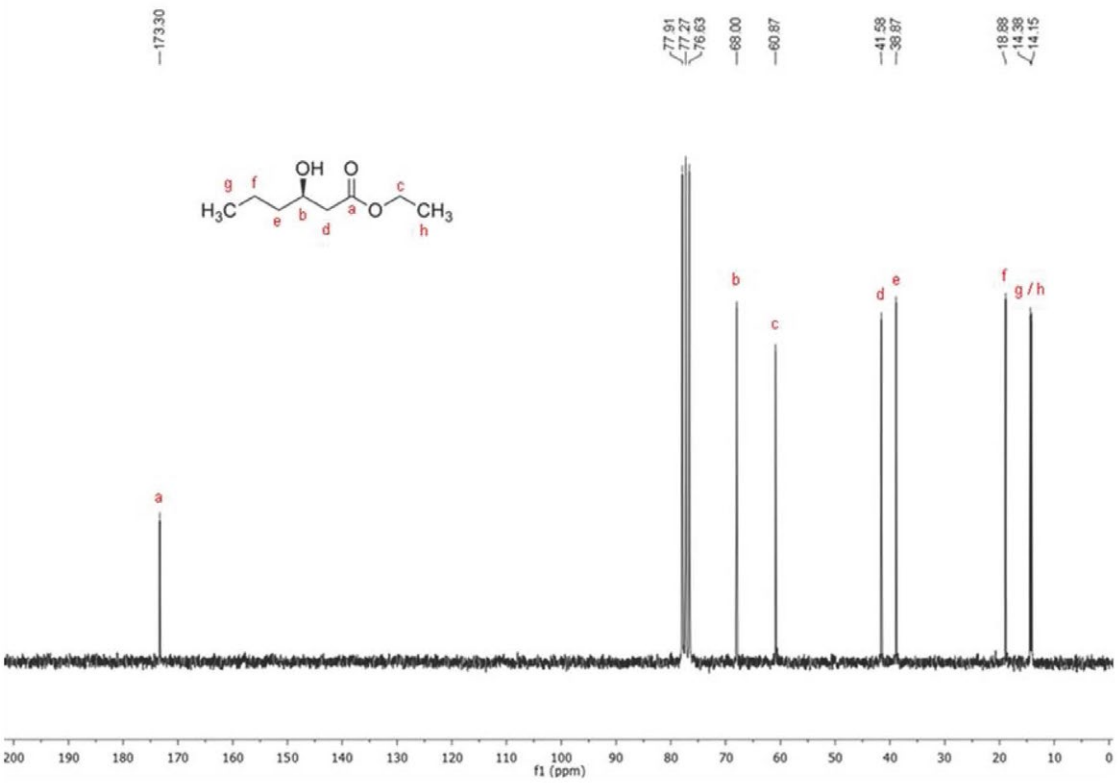


Figure S2. ^{13}C NMR (50 MHz, $CDCl_3$): ethyl 3-hydroxyhexanoate (racemate obtained via $NaBH_4$ reduction).

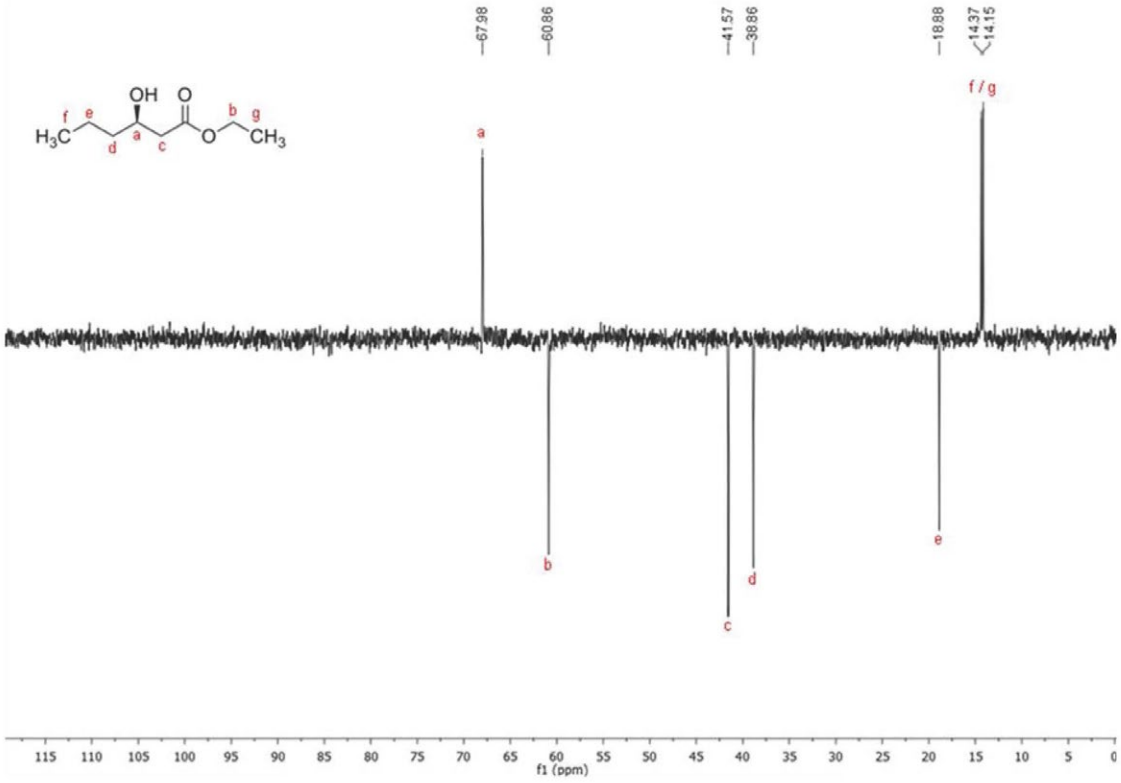


Figure S3. DEPT 135 (50MHz, $CDCl_3$): ethyl 3-hydroxyhexanoate (racemate obtained via $NaBH_4$ reduction).

CG-FID – Ethyl 3-oxohexanoate

Column:
Beta DEX325
Length: 30.0 m
Film thickness: 0.25 µm
Inner diameter: 0.25 mm ID

Method:
SPL1
Temp: 230.0 °C
Injection mode: split
Flow control mode: pressure
Pressure: 142.0 Kpa
Total flow: 68.5 mL/min
Column flow: 3.12 mL/min
Linear velocity: 80 cm/sec
Purge flow: 3.0 mL/min
Split ratio: 20.0
FID 1
Temp: 220.0 °C
Sampling rate: 40 msec
Make up gas: He
H_2 flow: 40 mL/min
Air flow: 400.0 mL/min
COLUMN:
Temp: 90.0 °C
Equilibration time: 1 min

	Rate	Final temperature (°C)	Hold time (min)
0		90.0	23.00
1	0.00	0.0	0.00
2	0.00	0.0	0.00
3	0.00	0.0	0.00

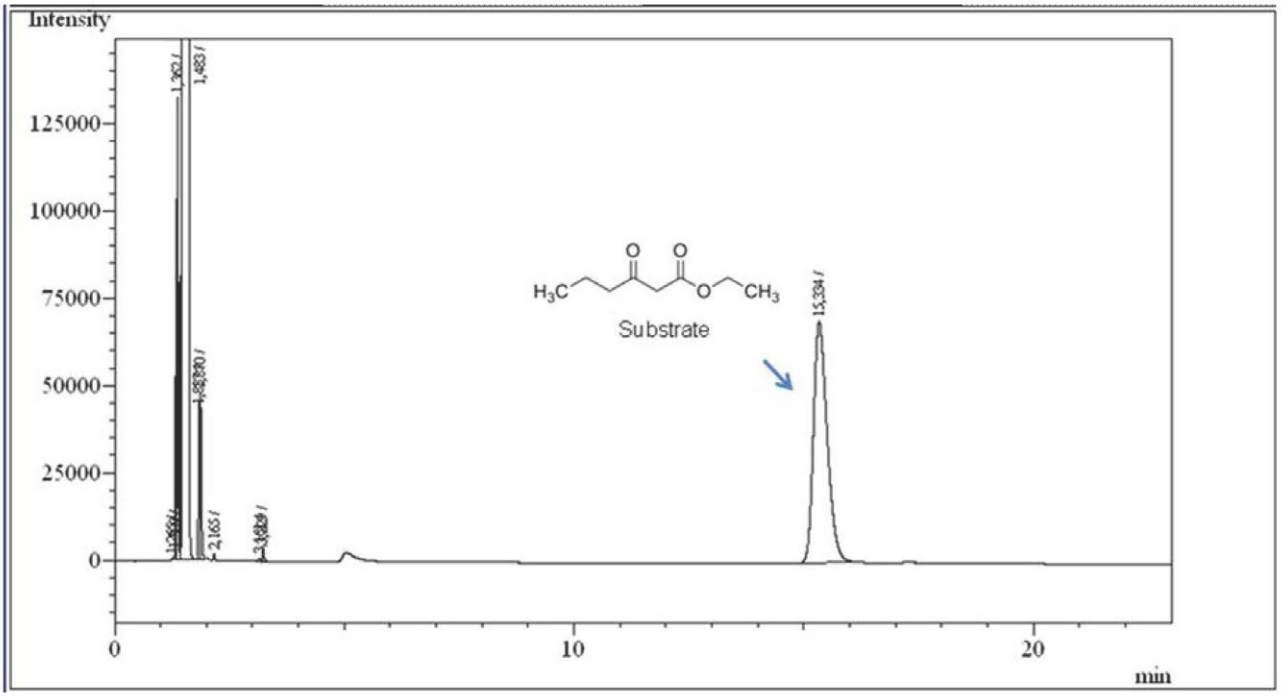


Figure S4. Chromatogram: ethyl 3-oxohexanoate.

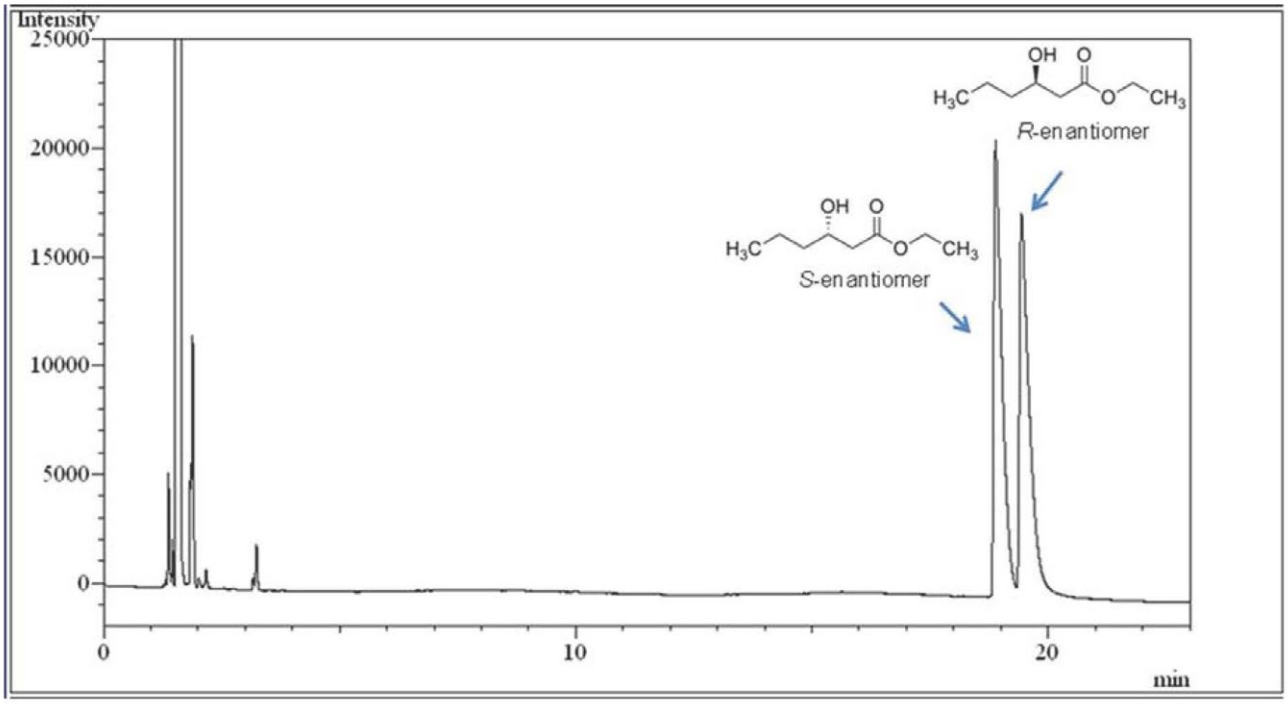


Figure S5. Chromatogram: ethyl 3-hydroxyhexanoate (racemate obtained via $NaBH_4$ reduction).

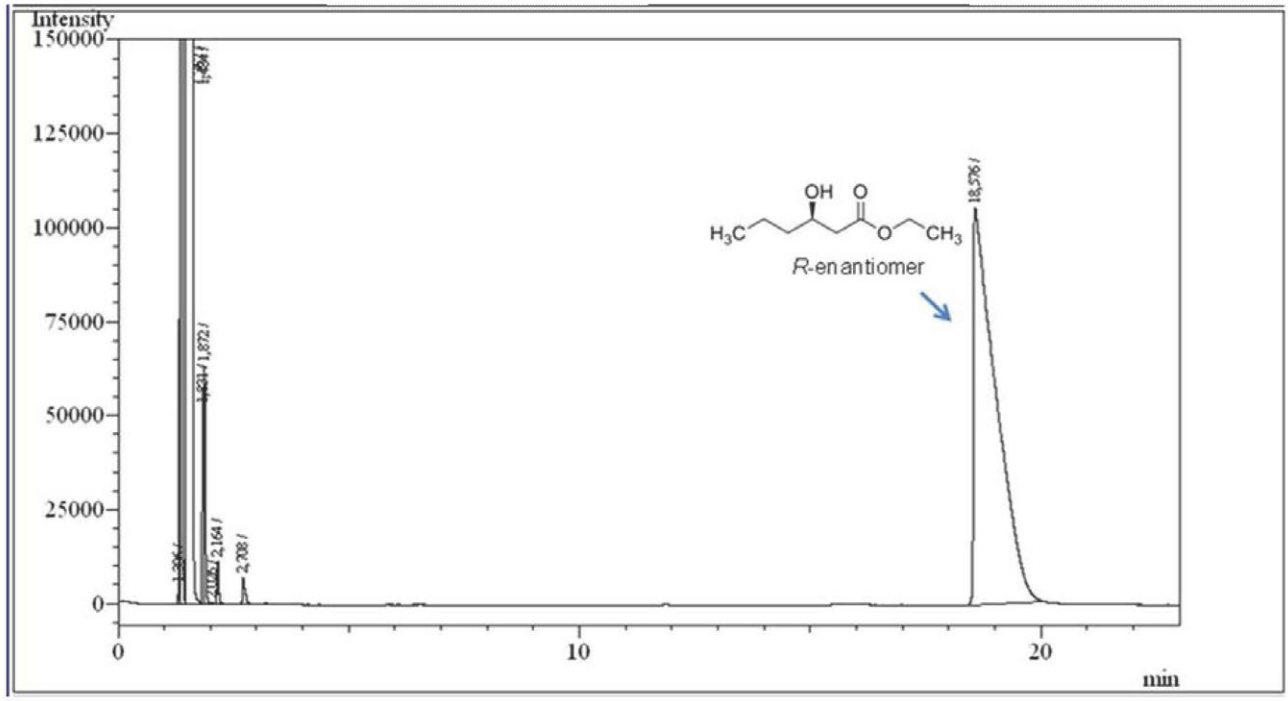


Figure S6. Chromatogram: ethyl 3 R-hydroxyhexanoate. Optical rotations were measured from $CHCl_3$ solutions using a JASCO DIP-370 polarimeter at the sodium D line (589 nm) operating at 25 °C.

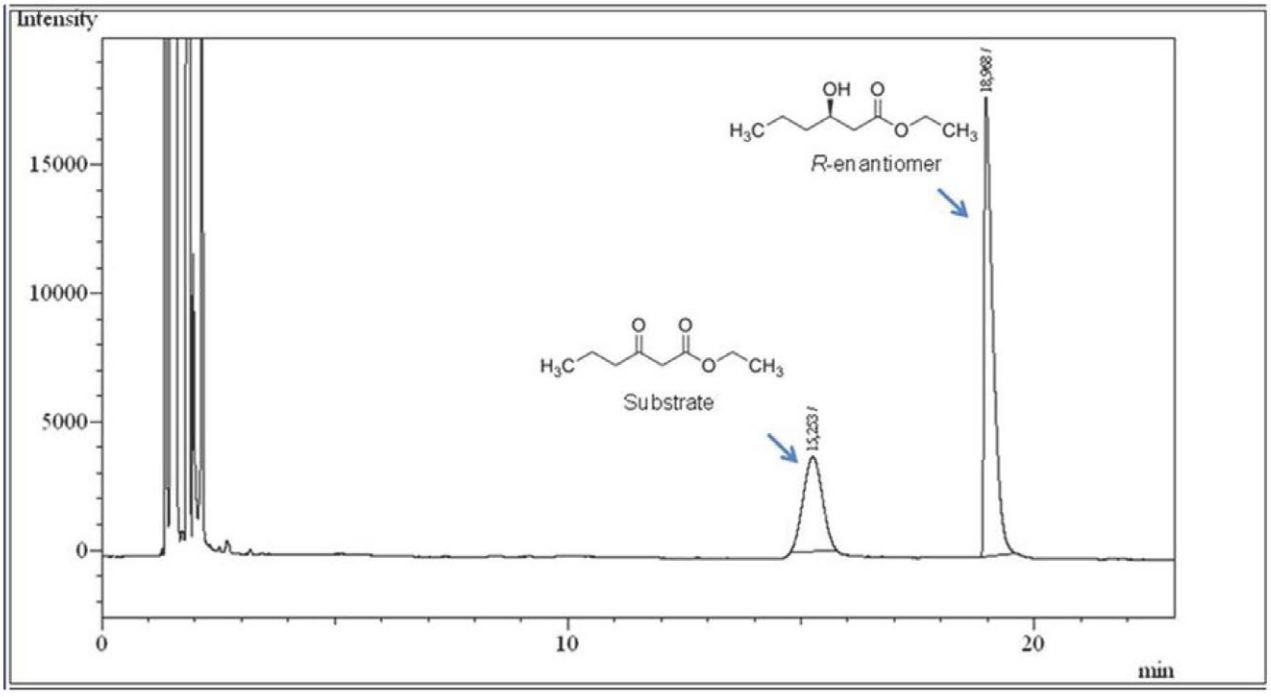


Figure S7. Bioreduction of ethyl 3-oxohexanoate (19 mM) to ethyl 3-hydroxyhexanoate by *Kluyveromyces marxianus* immobilized in calcium alginate spheres in continuous flow (0.2 mL min^{-1}).

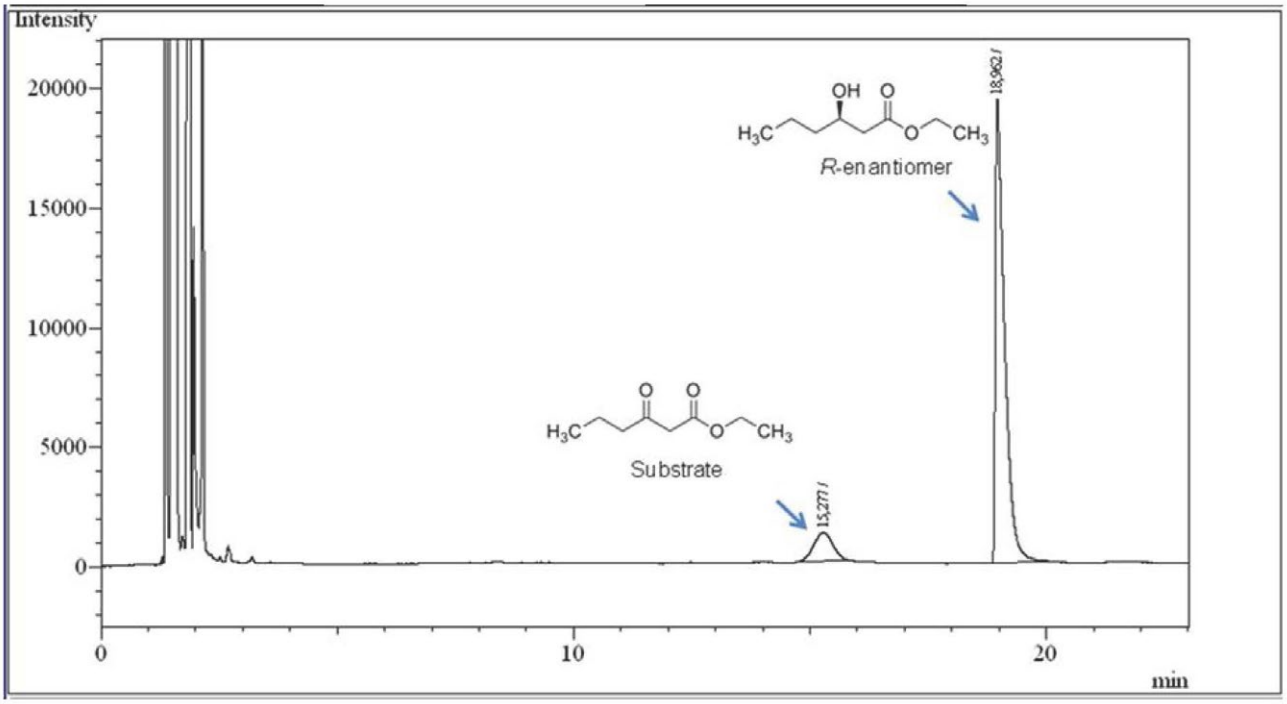


Figure S8. Bioreduction of ethyl 3-oxohexanoate (19 mM) to ethyl 3-hydroxyhexanoate by *Kluyveromyces marxianus* immobilized in calcium alginate spheres in continuous flow (0.1 mL min^{-1}).

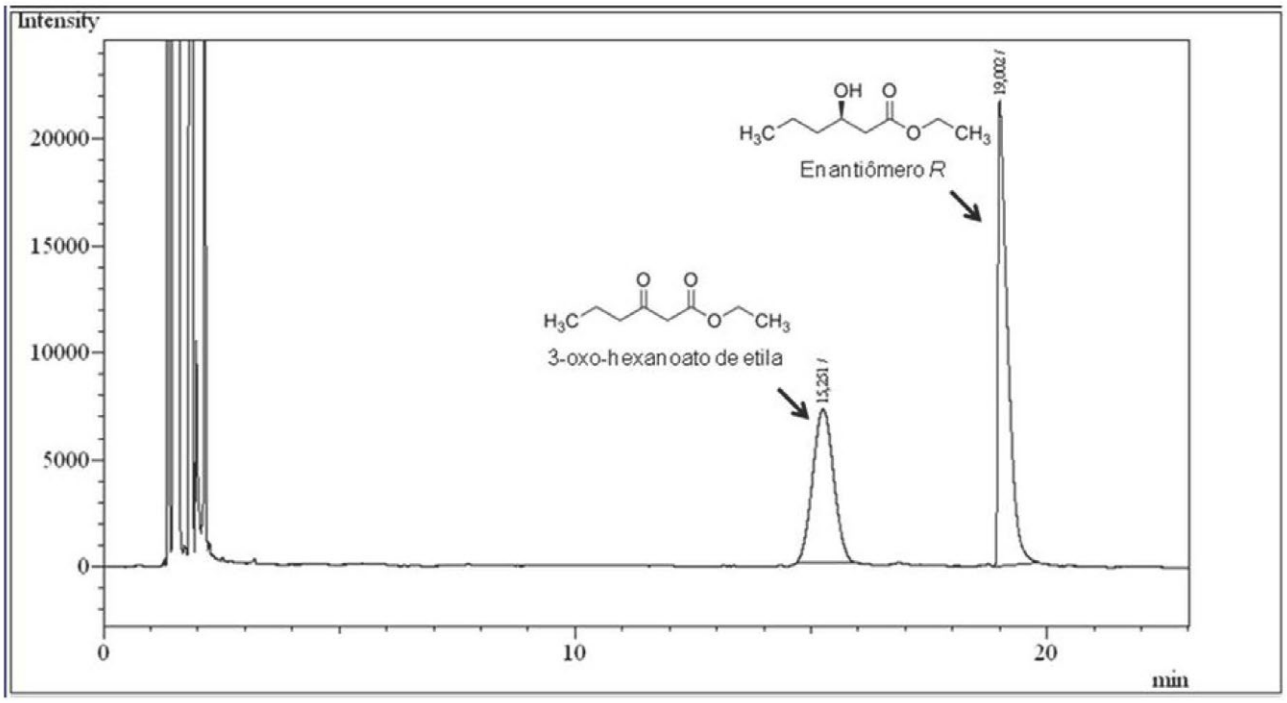


Figure S9. Bioreduction of ethyl 3-oxohexanoate (25 mM) to ethyl 3-hydroxyhexanoate by *Kluyveromyces marxianus* immobilized in calcium alginate spheres in continuous flow (0.2 mL min^{-1}).

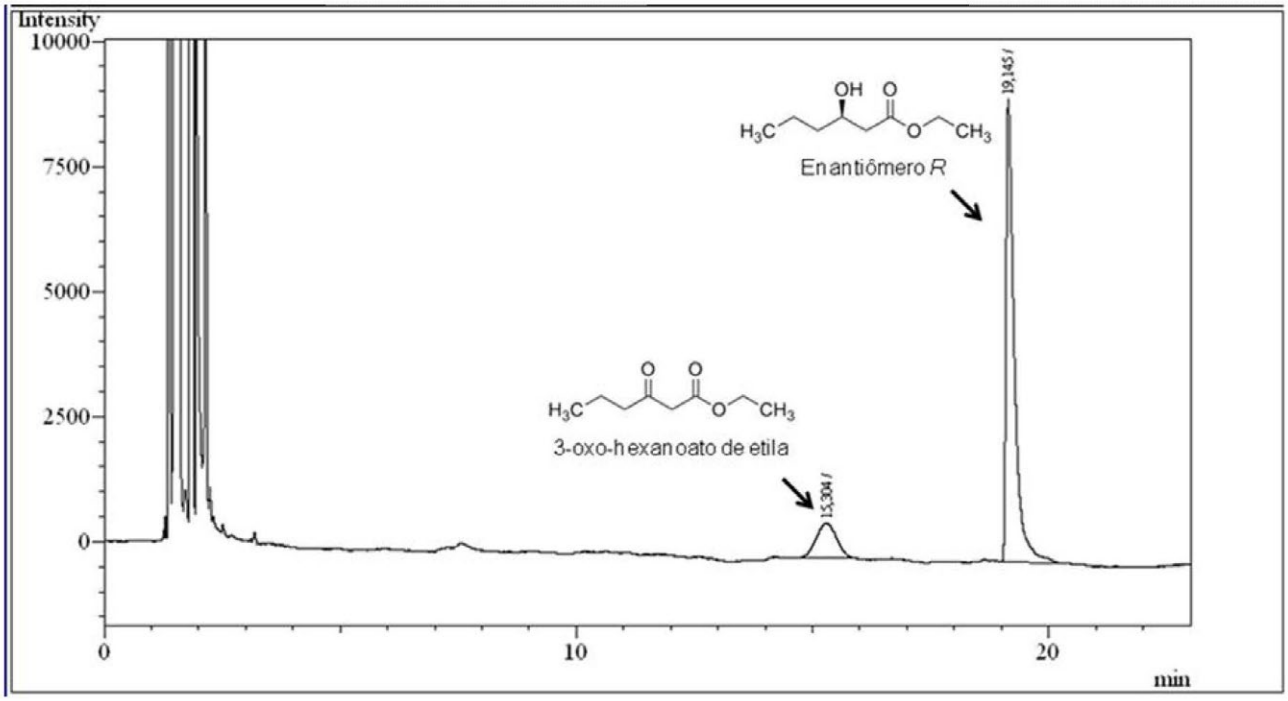


Figure S10. Bioreduction of ethyl 3-oxohexanoate (25 mM) to ethyl 3-hydroxyhexanoate by *Kluyveromyces marxianus* immobilized in calcium alginate spheres in continuous flow (0.1 mL min^{-1}).

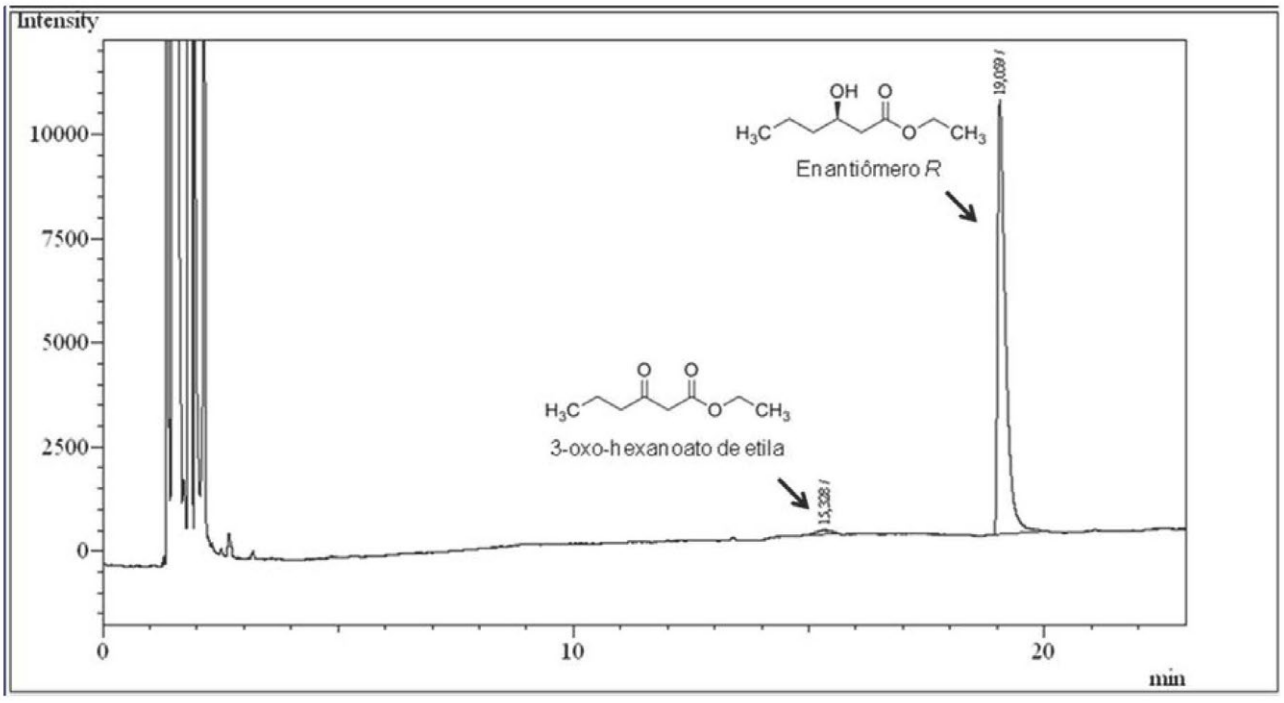
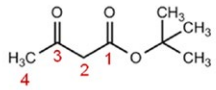


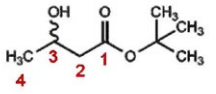
Figure S11. Bioreduction of ethyl 3-oxohexanoate (25 mM) to ethyl 3-hydroxyhexanoate by *Kluyveromyces marxianus* immobilized in calcium alginate spheres in continuous flow (0.075 mL min^{-1}).

Characterisation NMR: *tert*-butyl 3-oxobutanoate



^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ (ppm): 1.47 [s, 9H, $COOC(C\underline{H}_3)_3$]; 2.25 (s, 3H, H4); 3.35 (s, 2H, H2).

Characterisation NMR: *tert*-butyl 3-hydroxybutanoate



^{1}H NMR (200 MHz, $CDCl_3$, TMS) δ (ppm): 1.20 (d, 3H, *J* = 6 Hz, H4); 1.74 [s, 9H, $COOC(C\underline{H}_3)_3$]; 2.39 (d, 2H, *J* = 4 Hz, H2); 3.17 (s, 1H, O<u>H</u>); 4.15 (m, 1H, H3).

^{13}C NMR (50 MHz, $CDCl_3$, TMS) δ (ppm): 22.5 (C4); 28.3 [$COOC(\underline{C}H_3)_3$]; 44.0 (C2); 64.5 (C3); 81.4 [$COO\underline{C}(CH_3)_3$]; 172.6 [$\underline{C}OOC(CH_3)_3$].

DEPT 135 (50 MHz, $CDCl_3$, TMS) δ (ppm): 22.5 (C4); 28.3 [$COOC(\underline{C}H_3)_3$]; 44.0 (C2); 64.5 (C3).

GC-FID – *tert*-butyl 3-hydroxybutanoate

Column:
Beta DEX325
Length: 30.0 m
Film thickness: 0.25 µm
Inner diameter: 0.25 mm ID

Method:
SPL1
Temp: 230.0 °C
Injection mode: split
Flow control mode: pressure
Pressure: 142.0 Kpa
Total flow: 68.5 mL/min
Column flow: 3.12 mL/min
Linear velocity: 80 cm/sec
Purge flow: 3.0 mL/min
Split ratio: 20.0
FID 1
Temp: 220.0 °C
Sampling rate: 40 msec
Make up gas: He
H_2 flow: 40 mL/min
Air flow: 400.0 mL/min
COLUMN:
Temp: 90.0 °C
Equilibration time: 1 min

	Rate	Final temperature (°C)	Hold time (min)
0		90.0	11.00
1	0.00	0.0	0.00
2	0.00	0.0	0.00
3	0.00	0.0	0.00

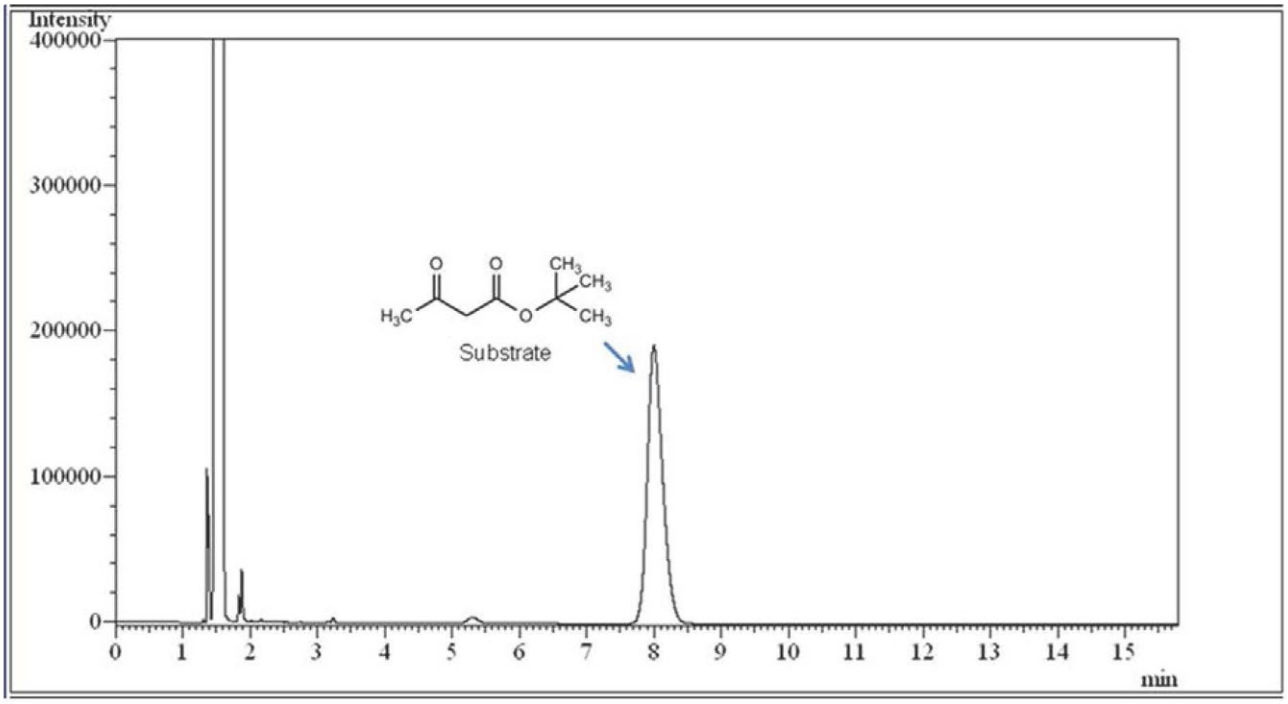


Figure S12. Chromatogram : tert-butyl 3-oxobutanoate.

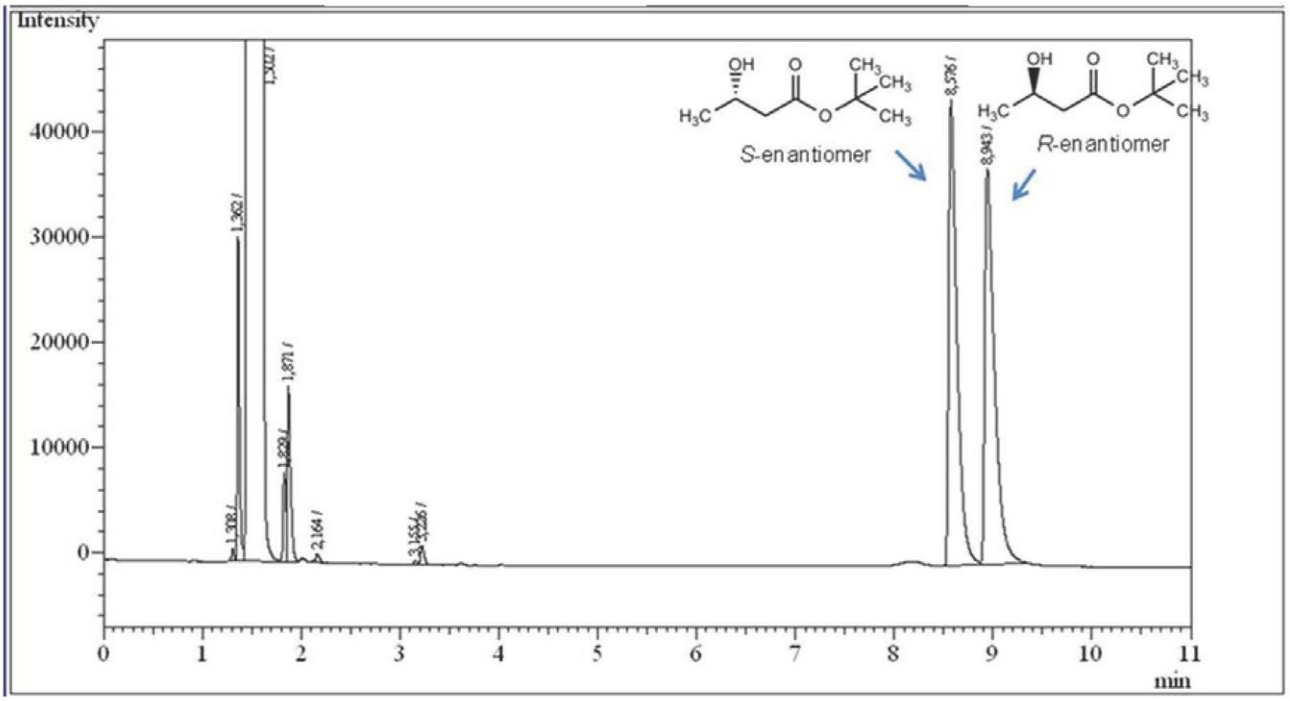


Figure S13. Chromatogram: tert-butyl 3-hydroxybutanoate (racemate obtained via $NaBH_4$ reduction).

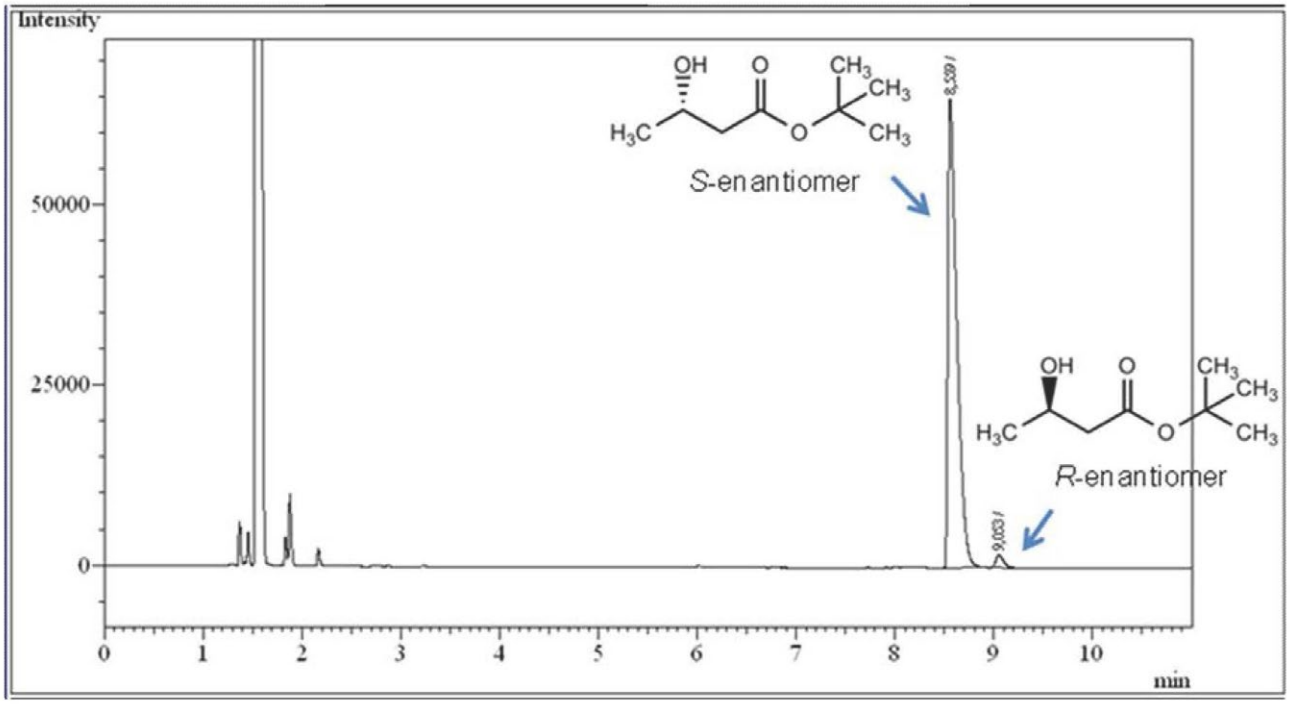


Figure S14. Optical rotations were measured from $CHCl_3$ solutions using a JASCO DIP-370 polarimeter at the sodium D line (589 nm) operating at 23 °C.

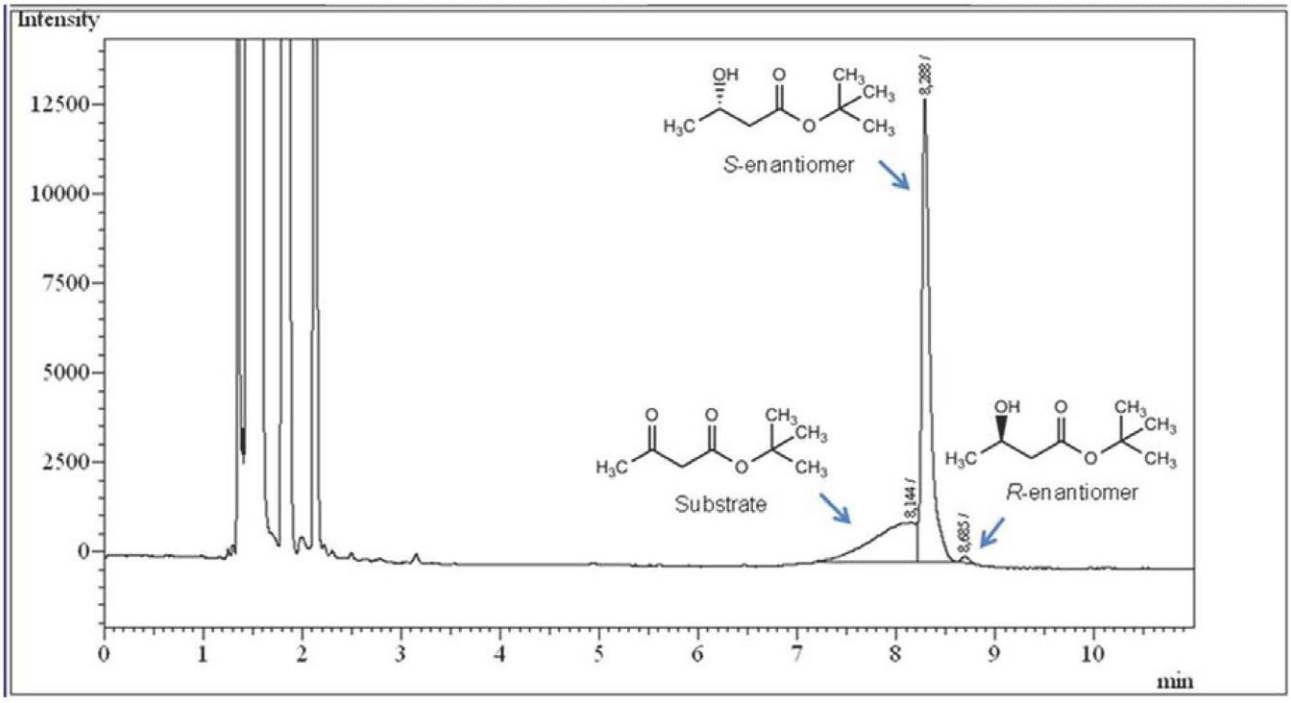


Figure S15. Bioreduction of tert-butyl 3-oxobutanoate (19 mM) to tert-butyl 3-hydroxybutanoate by *Rhodotorula rubra* immobilized in calcium alginate spheres in continuous flow (0.2 mL min^{-1}).

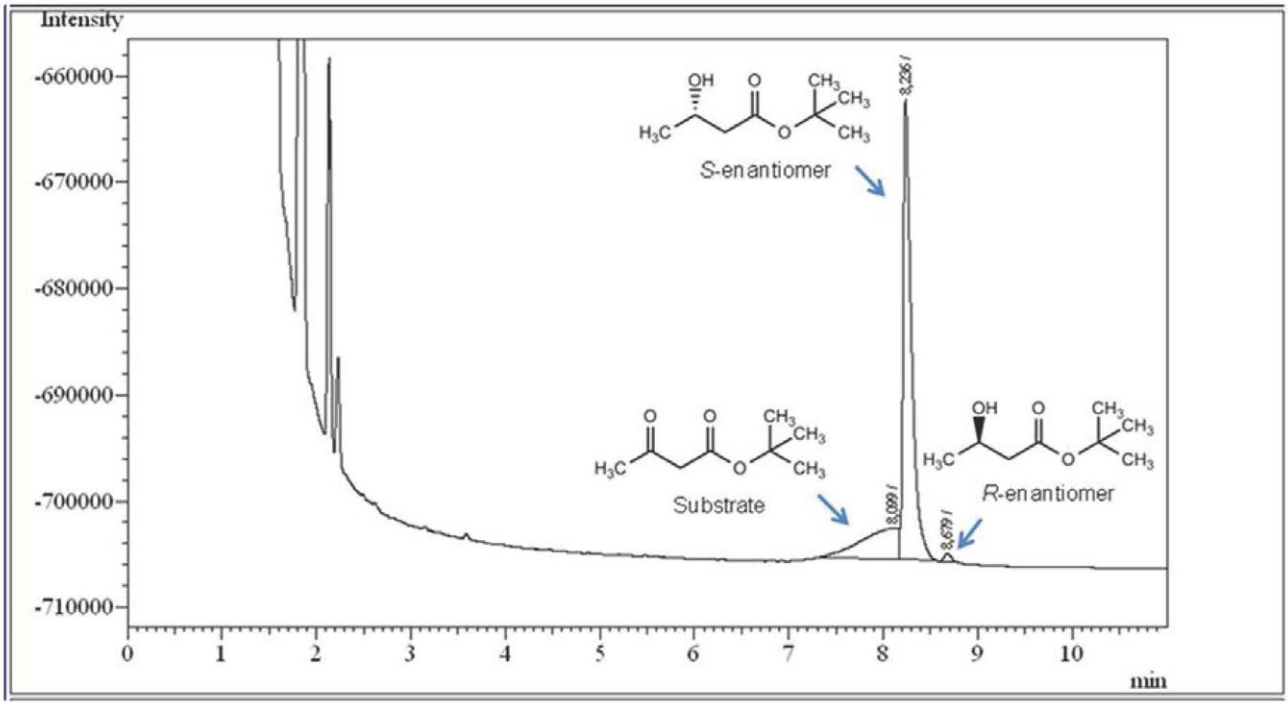


Figure S16. Bioreduction of tert-butyl 3-oxobutanoate (19 mM) to tert-butyl 3-hydroxybutanoate by *Rhodotorula rubra* immobilized in calcium alginate spheres in continuous flow (0.1 mL min^{-1}).

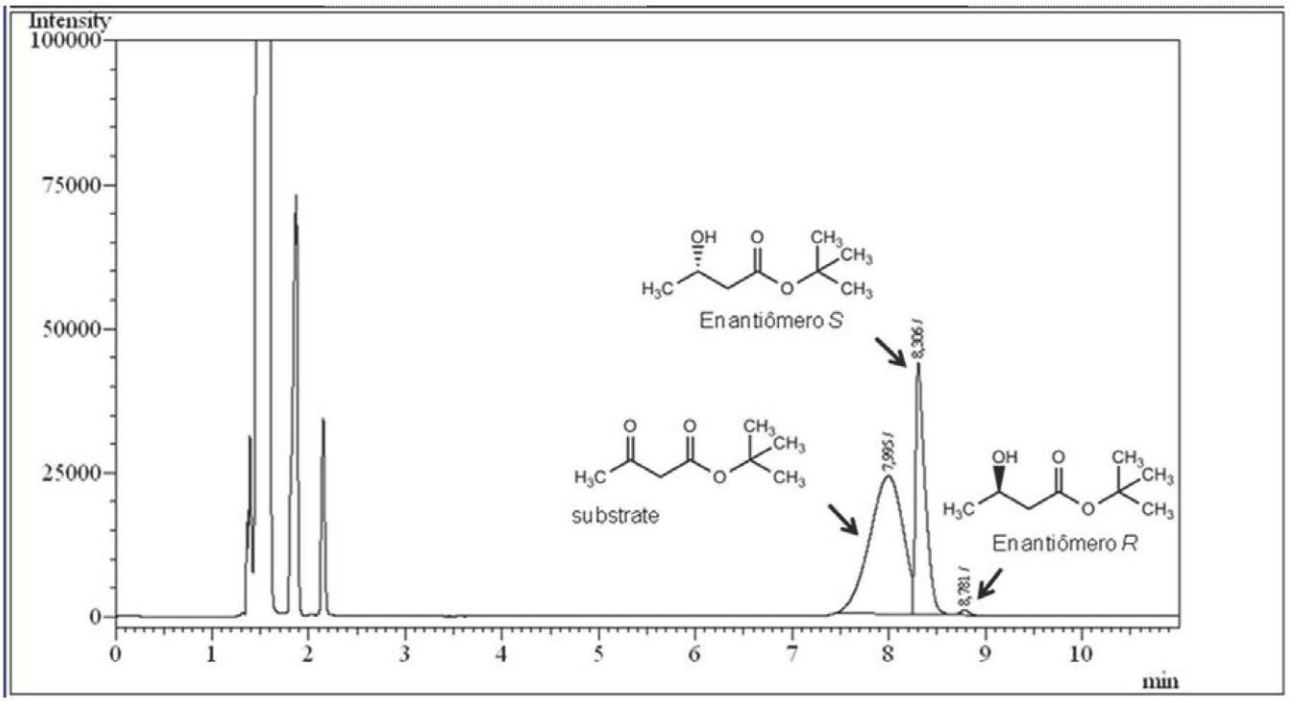


Figure S17. Bioreduction of tert-butyl 3-oxobutanoate (25 mM) to tert-butyl 3-hydroxybutanoate by *Rhodotorula rubra* immobilized in calcium alginate spheres in continuous flow (0.2 mL min^{-1}).

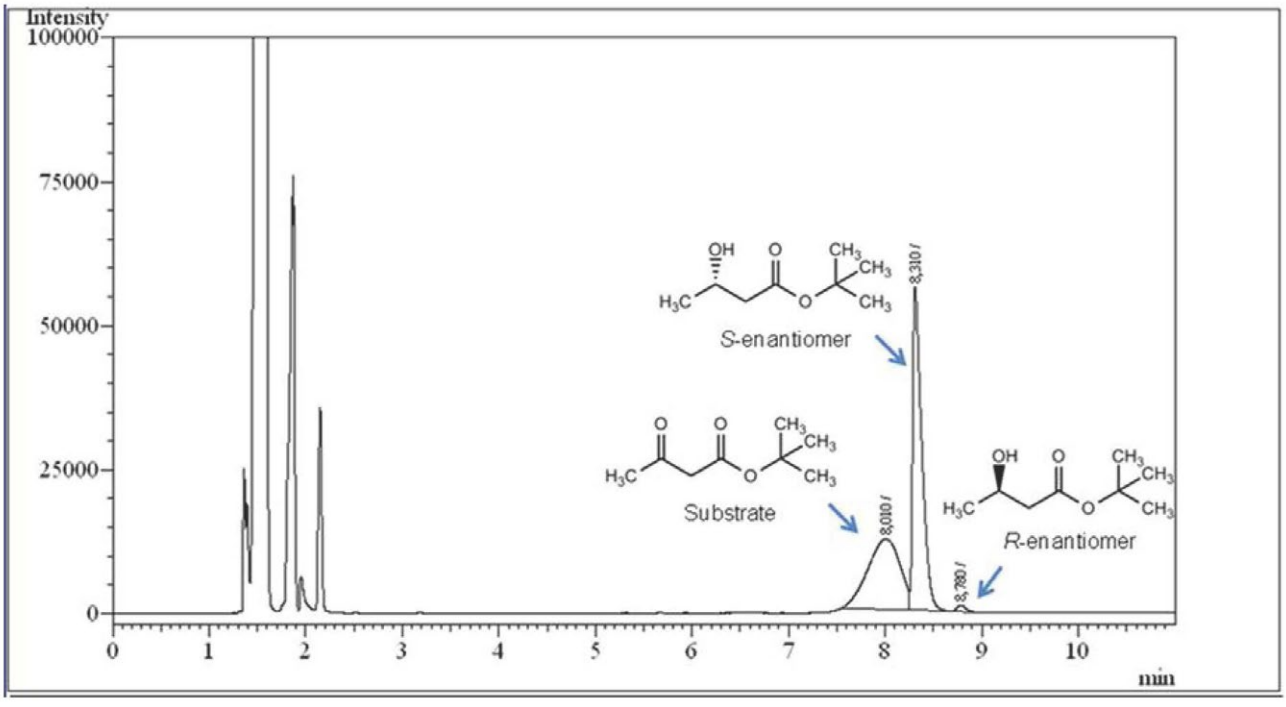


Figure S18. Bioreduction of tert-butyl 3-oxobutanoate (25 mM) to tert-butyl 3-hydroxybutanoate by *Rhodotorula rubra* immobilized in calcium alginate spheres in continuous flow (0.1 mL min^{-1}).

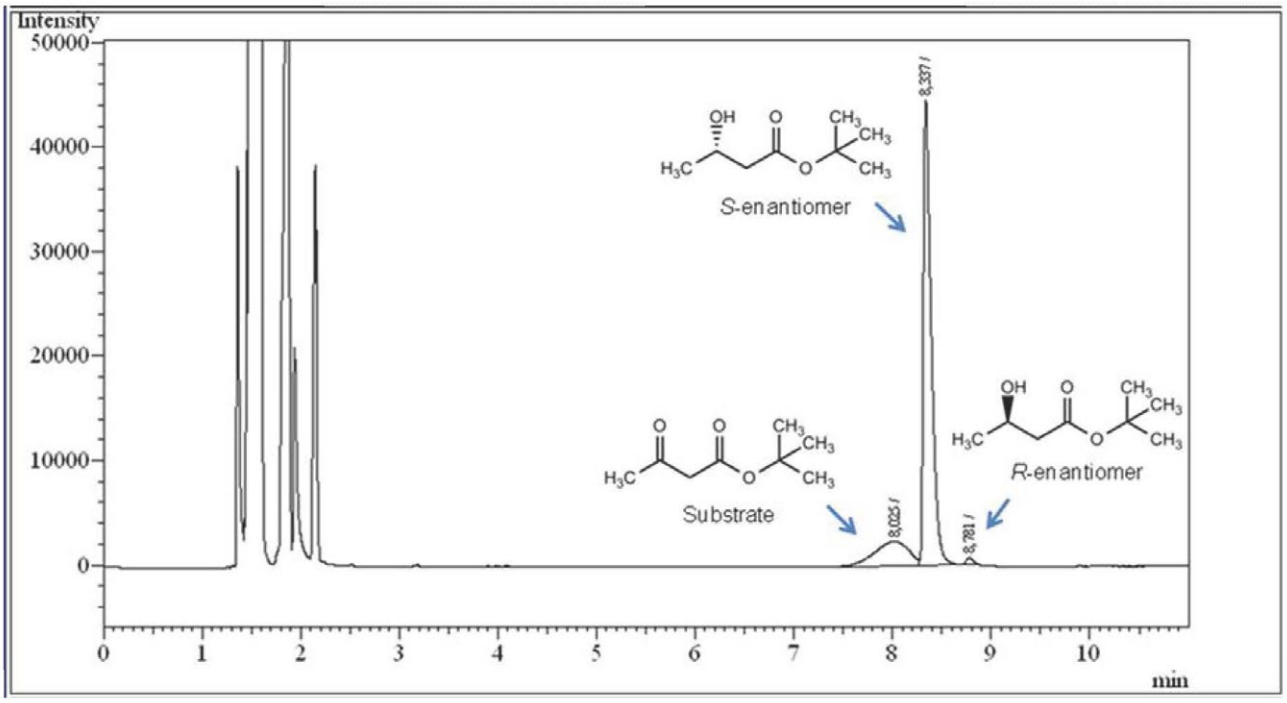


Figure S19. Bioreduction of tert-butyl 3-oxobutanoate (25 mM) to tert-butyl 3-hydroxybutanoate by *Rhodotorula rubra* immobilized in calcium alginate spheres in continuous flow (0.075 mL min^{-1}).

Reference

1. Ramos, A. S.; Ribeiro, J. B.; Lopes, R. O.; Souza, R. O. M. A.; *Synth. Commun.* **2013**, *43*, 1611.