Electronic Supporting Information

for

Simultaneous Cyclic Deracemisation and Stereoinversion of Alcohols Using Orthogonal Biocatalytic Oxidation and Reduction Reactions

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Experimental Section

General. Sodium borohydride, $NADP^+$, NAD^+, sodium formate, formate dehydrogenase (FDH), (*rac*)-**1b**, (*rac*)-**1d**, (*R*)-**1b**, (*S*)-**1b**, 4-(4′-hydroxyphenyl)-2-butanone (**2a**), 4-(4′-methoxyphenyl)-2-butanone (**2c**) and acetophenone (**2d**) were purchased from commercial sources and used without further treatment; whereas (*rac*)-**1a** and (*rac*)-**1c** were prepared from their corresponding ketones **2a** and **2c**, respectively, using sodium borohydride.[1] (*R*)-**1a** was prepared by *Ca*LB-catalyzed KR of (*rac*)-**1a**.[2] All gas chromatography (GC) analyses were conducted using a capillary GC equipped with an HP chiral-20B column (30 m, 0.32 mm [i.d.], 0.25 μm film thickness) using helium as the carrier gas with a flame ionization detector. GC–MS analysis was performed using an instrument combined with an Agilent 5975C inert mass-selective detector with a triple- Agilent GC 7890A axis detector. NMR spectra were recorded on an FT-NMR at 500 MHz (^{1}H) and at 125 MHz (^{13}C) at room temperature, using deuterated chloroform ($CDCl_3$) peak as an internal standard.

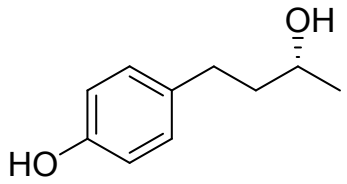
Determination of enantiomeric excess. The produced alcohols were converted to their corresponding acetate esters by treatment with two drops of acetic anhydride and three drops of pyridine prior to their analysis by the chiral GC. The following method was used in the GC analysis: Initial oven temperature was 100 °C for 10 min to 180 °C for 20 min at 5 °C/min; injector 220 °C, detector 230 °C; and the helium at 15 mL/min. The volume injected was 1.0 μL with split ratio of 10:1.

Determination of absolute configuration of alcohols. The absolute configurations of the produced alcohols were elucidated by comparing the chiral GC retention time of their acetate derivatives with either their commercially available (*S*)- or (*R*)-acetate enantiomer, or with the acetate derivatives of alcohols prepared by W110A *Te*SADH-

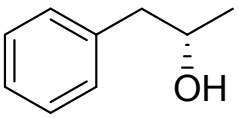
catalysed asymmetric reduction of their ketones, which are reported to produce (*S*)-alcohols.[3] (*R*)-Configured alcohols that are not commercially available were produced by *Candida antarctica* lipase B (*Ca*LB)-catalysed KR of racemic alcohols.[2]

GC–MS analysis. The following temperature program was used for the GC oven: 60 °C (initial, hold time 10 min) to 240 °C (final, hold time 20 min) at a rate of 5 °C/min. The injector temperature was 300 °C and the detector temperature was 310 °C. The gas flow rates for air, H_2, and He were 300, 30, and 15 mL/min, respectively. The split mode with a split ratio of 10:1 was used. Because of the absence of by-products (confirmed by NMR), the relative areas of ketones as well as (*S*)- and (*R*)-alcohols were used to determine percent ketone and ee.

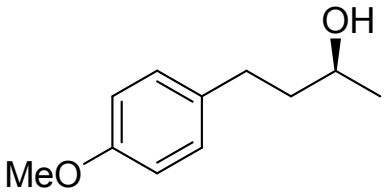
Characterization of Products



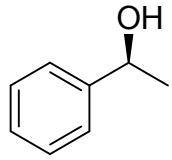
(*R*)-4-(4'-Hydroxyphenyl)-2-butanol [(*R*)-1a]: Both the ^1H and ^{13}C NMR data were consistent with previously reported spectroscopic data.[4]



(*S*)-1-Phenyl-2-propanol [(*S*)-1b]: Both the ^1H and ^{13}C NMR data were consistent with previously reported spectroscopic data.[5]



(*S*)-4-(4′-Methoxyphenyl)-2-butanol [(*S*)-1c]: Both the ^{1}H and ^{13}C NMR data were consistent with the previously reported spectroscopic data.[6]



(*S*)-1-Phenyl-2-propanol [(*S*)-1d]: Both the ^{1}H and ^{13}C NMR data were consistent with the previously reported spectroscopic data.[4]

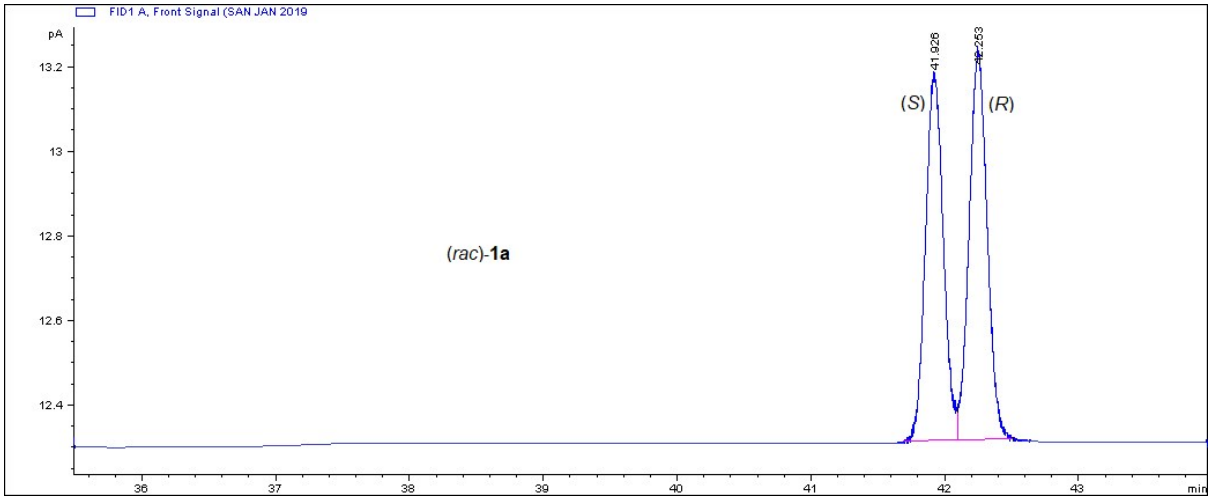


Figure S1. Gas chromatogram of the acetate derivative of (*rac*)-**1a**

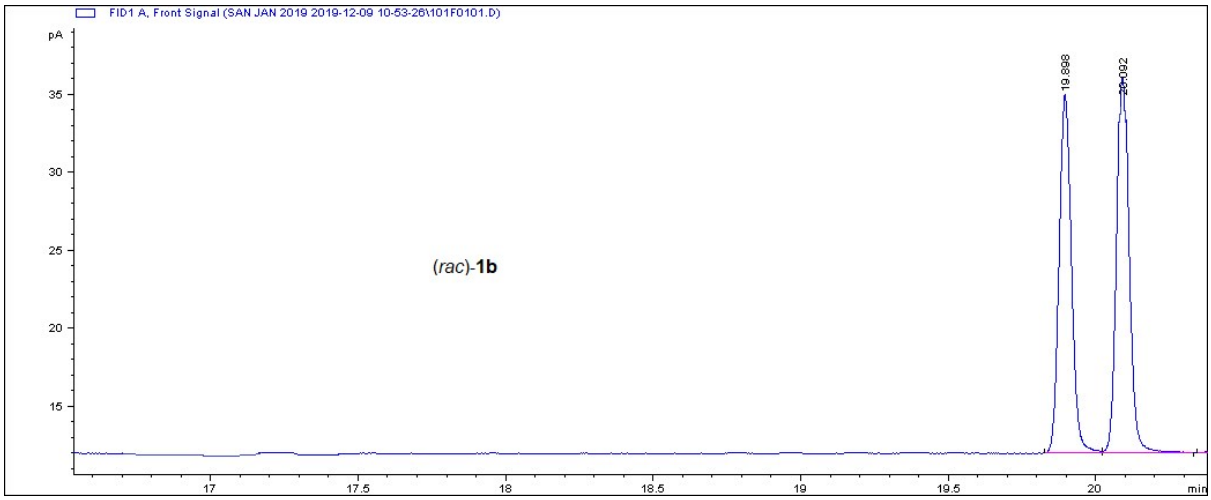


Figure S2. Gas chromatogram of the acetate derivative of (*rac*)-**1b**

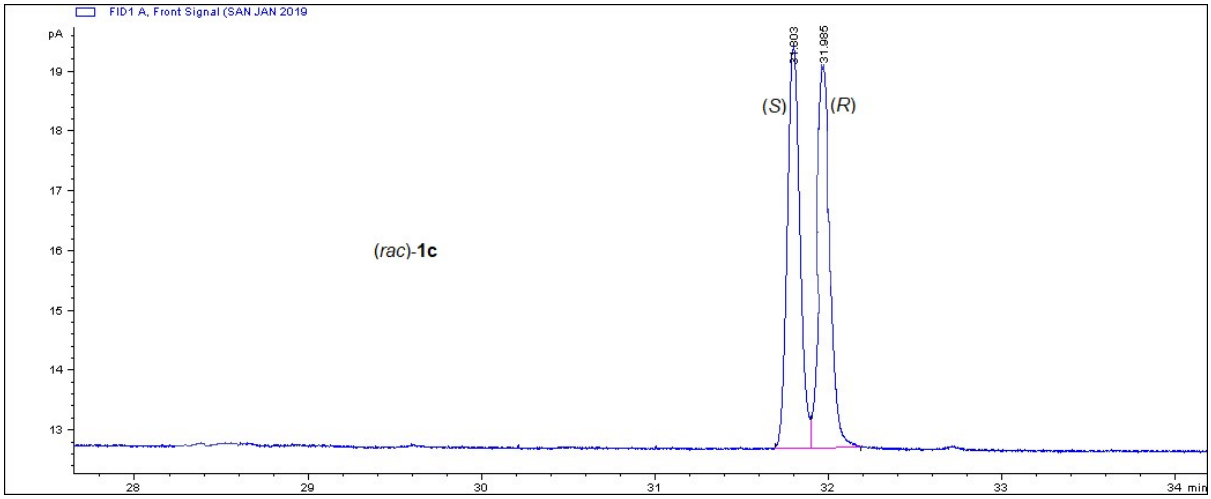


Figure S3. Gas chromatogram of the acetate derivative of (*rac*)-**1c**

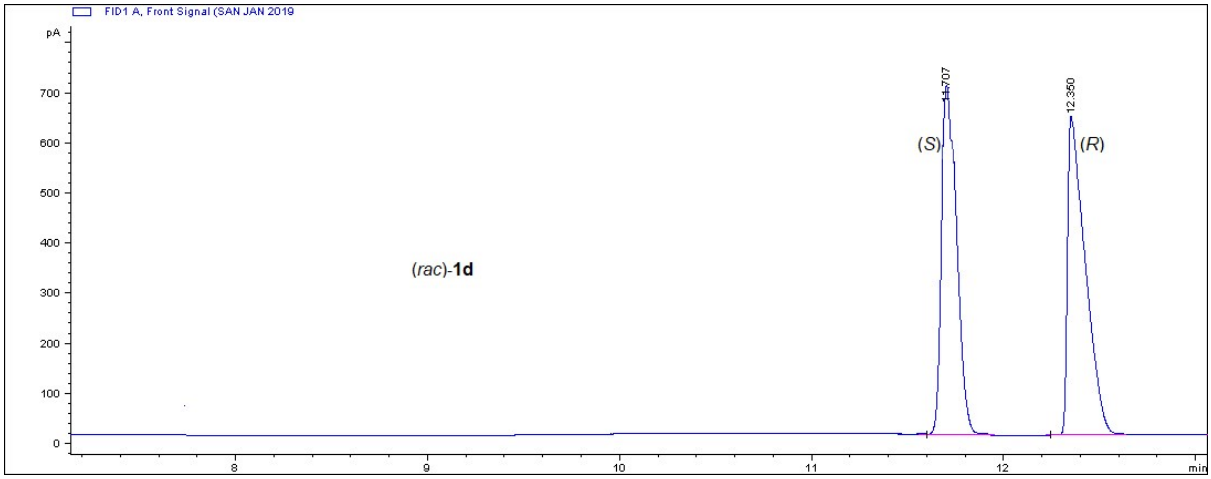


Figure S4. Gas chromatogram of the acetate derivative of (*rac*)-**1d**.

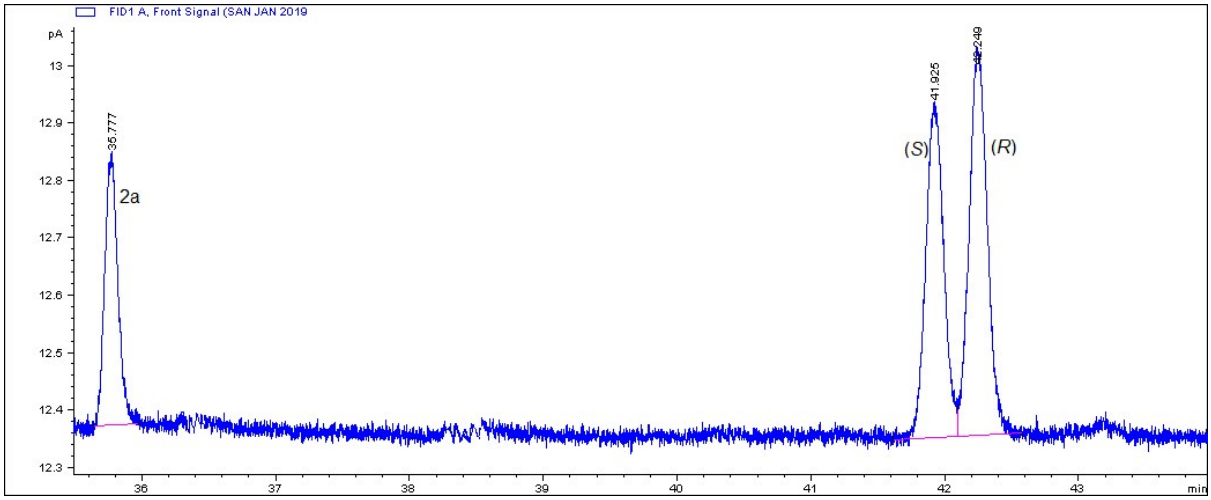


Figure S5. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1a** (see Table 1 in the main text, entry 1).

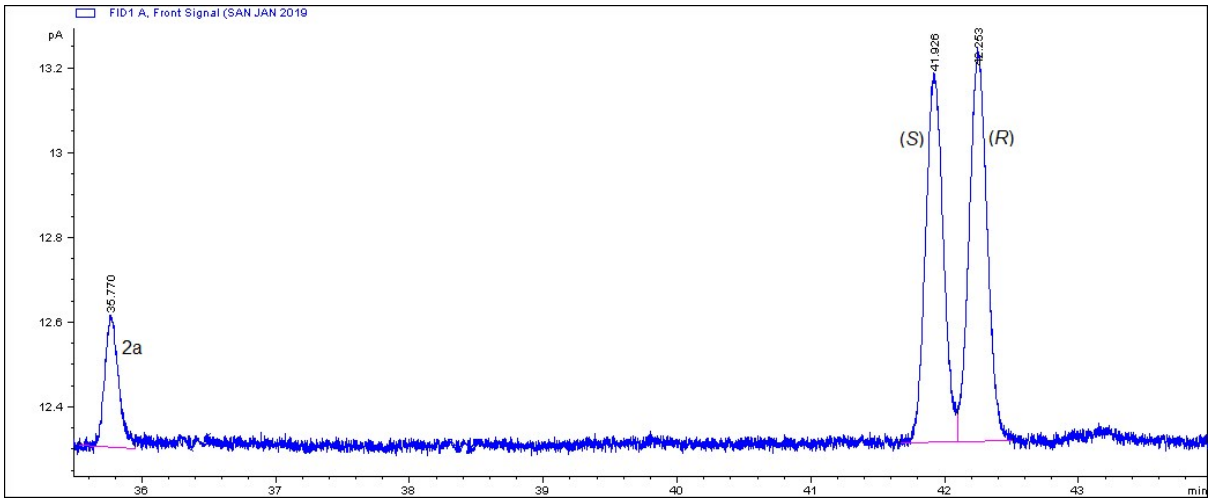


Figure S6. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1a** (see Table 1 in the main text, entry 2).

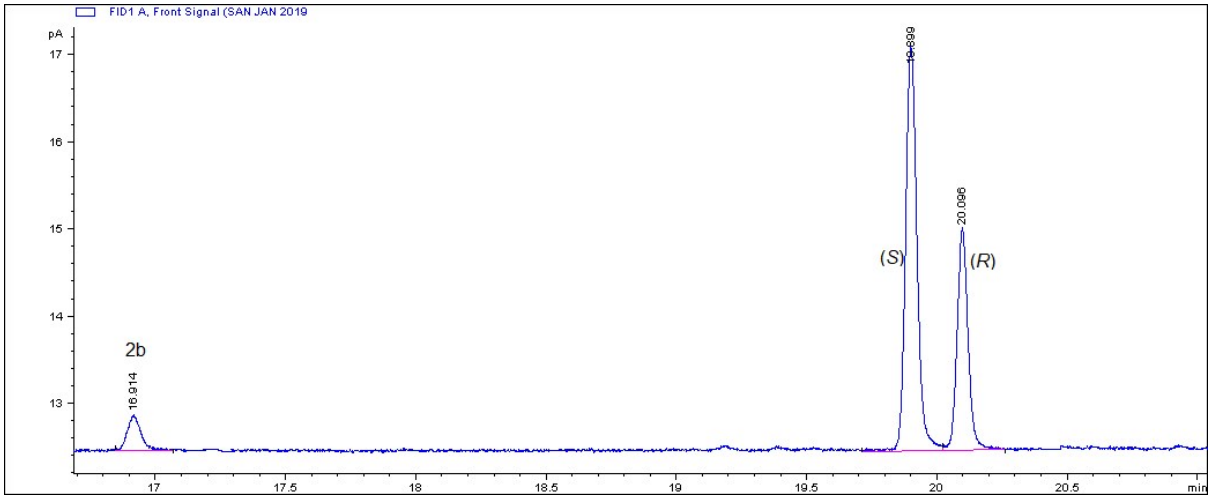


Figure S7. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1b** (see Table 1 in the main text, entry 3).

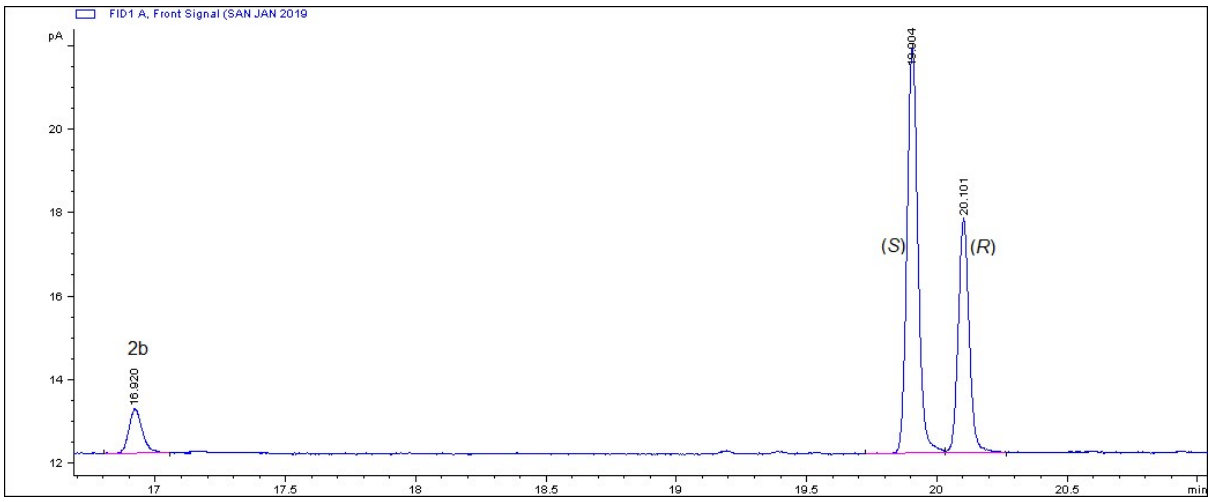


Figure S8. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1b** (see Table 1 in the main text, entry 4).

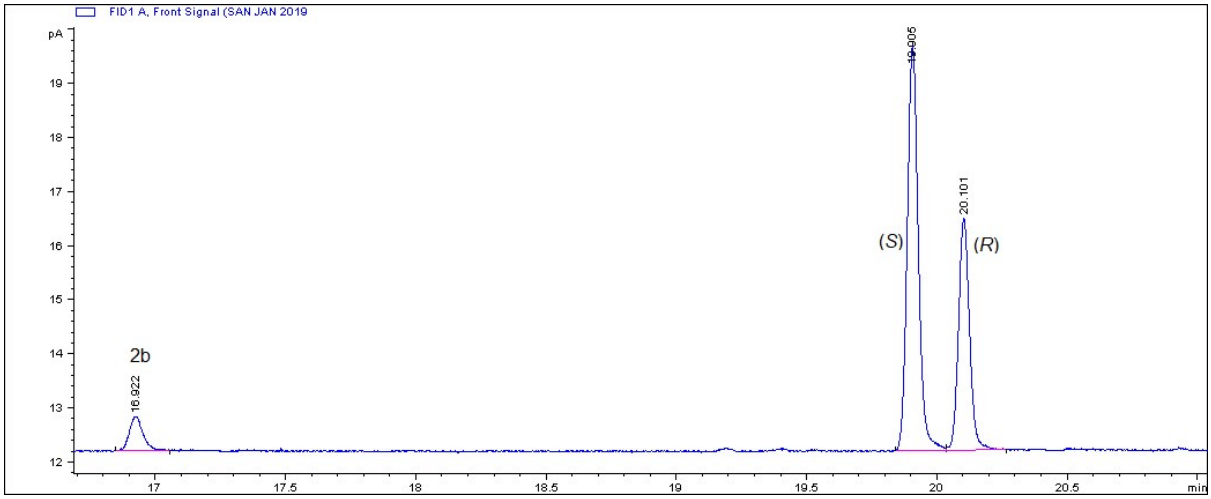


Figure S9. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1b** (see Table 1 in the main text, entry 5).

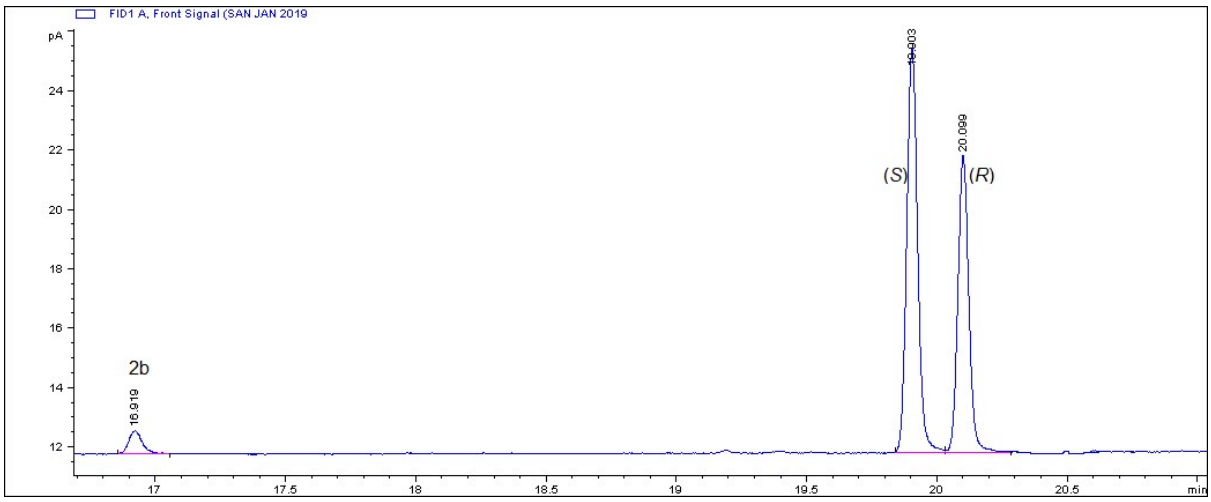


Figure S10. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1b** (see Table 1 in the main text, entry 6).

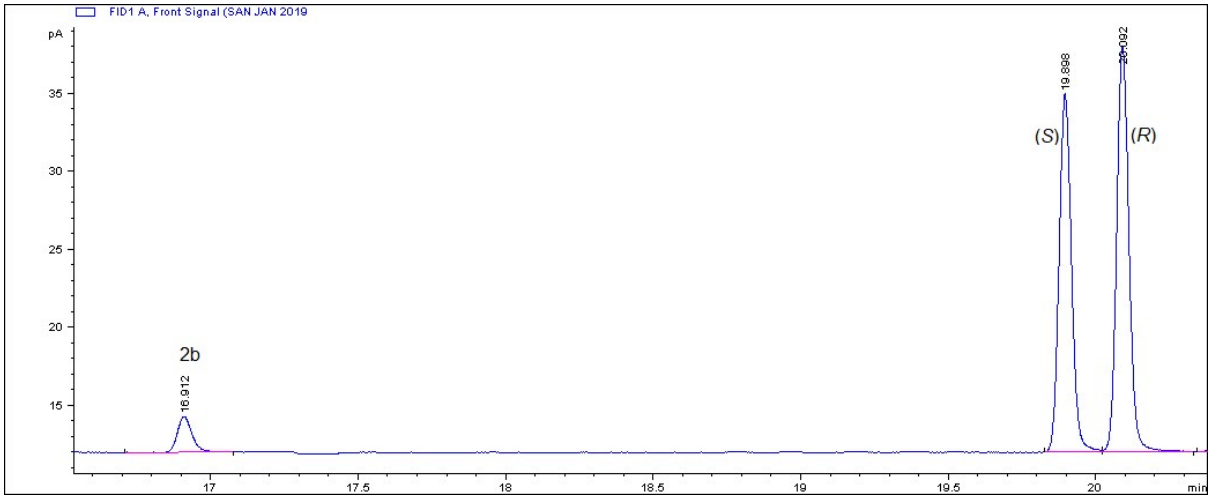


Figure S11. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1b** (see Table 1 in the main text, entry 7).

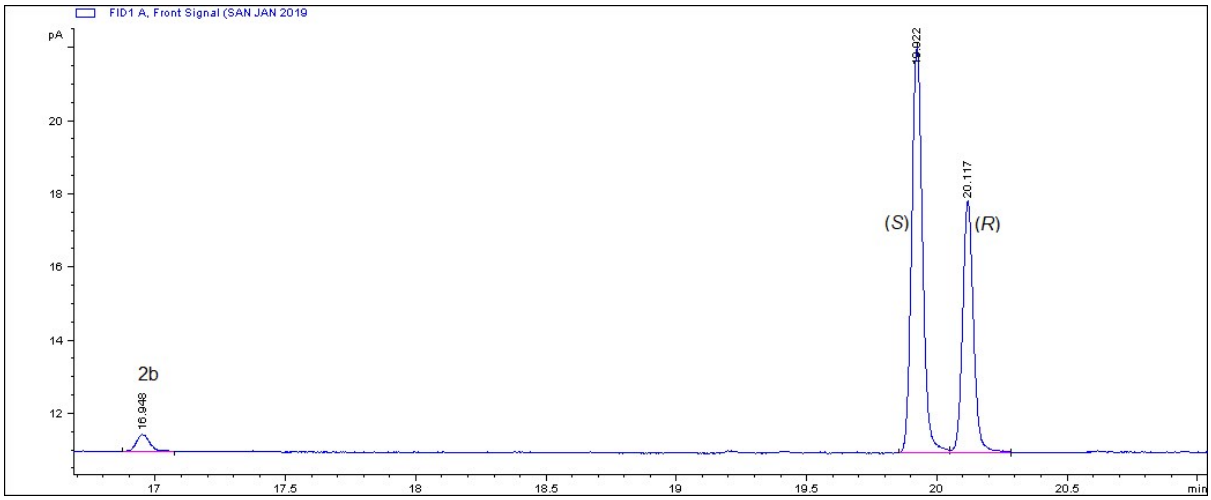


Figure S12. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1b** (see Table 1 in the main text, entry 8).

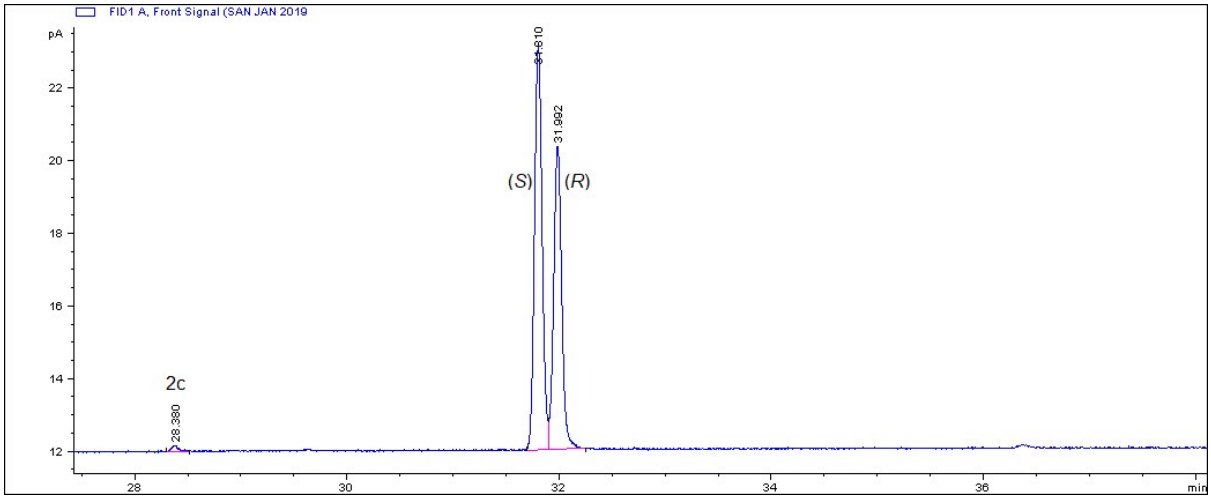


Figure S13. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1c** (see Table 1 in the main text, entry 9).

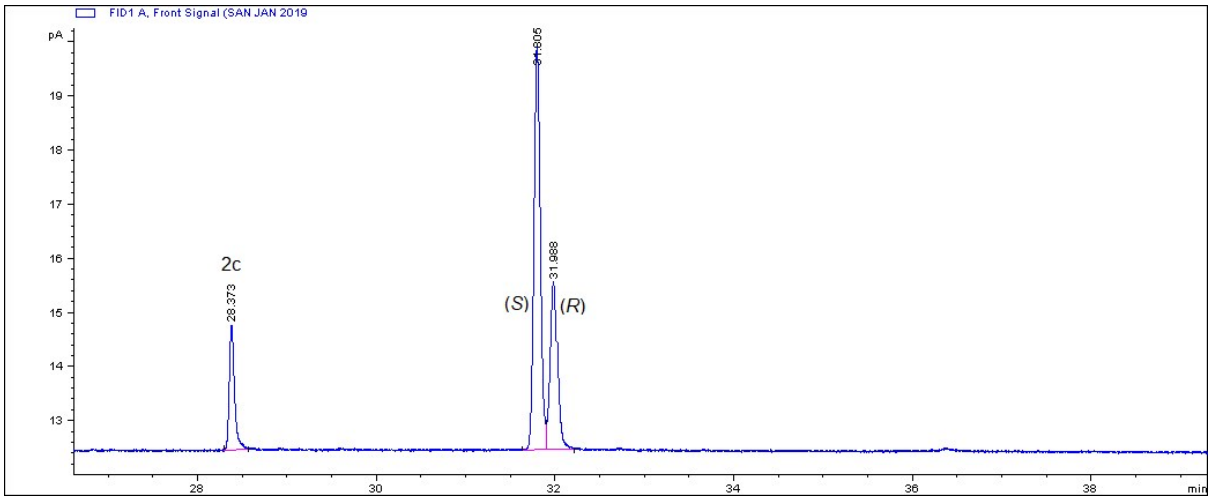


Figure S14. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1c** (see Table 1 in the main text, entry 10).

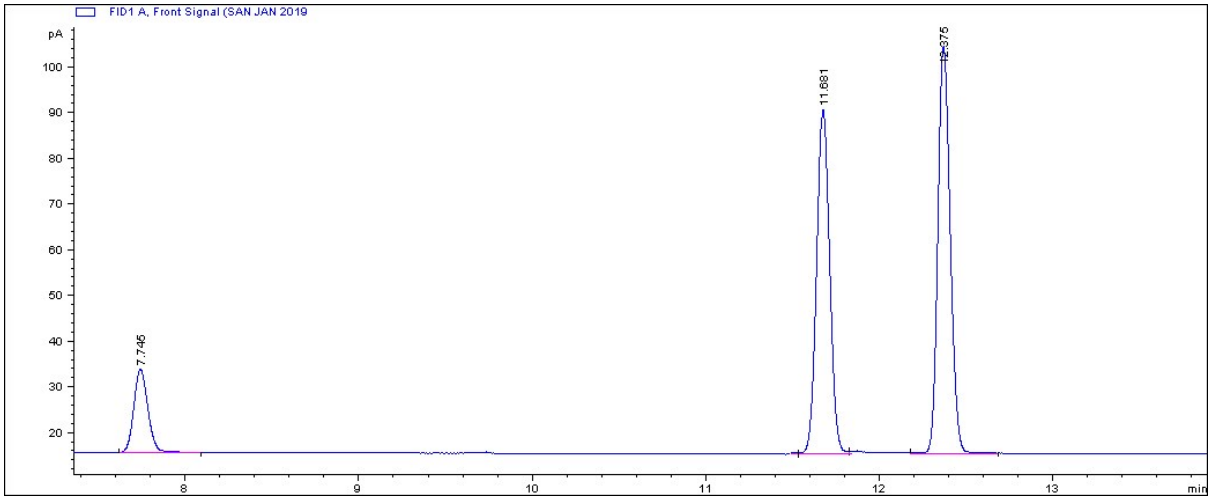


Figure S15. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1d** (see Table 1 in the main text, entry 11).

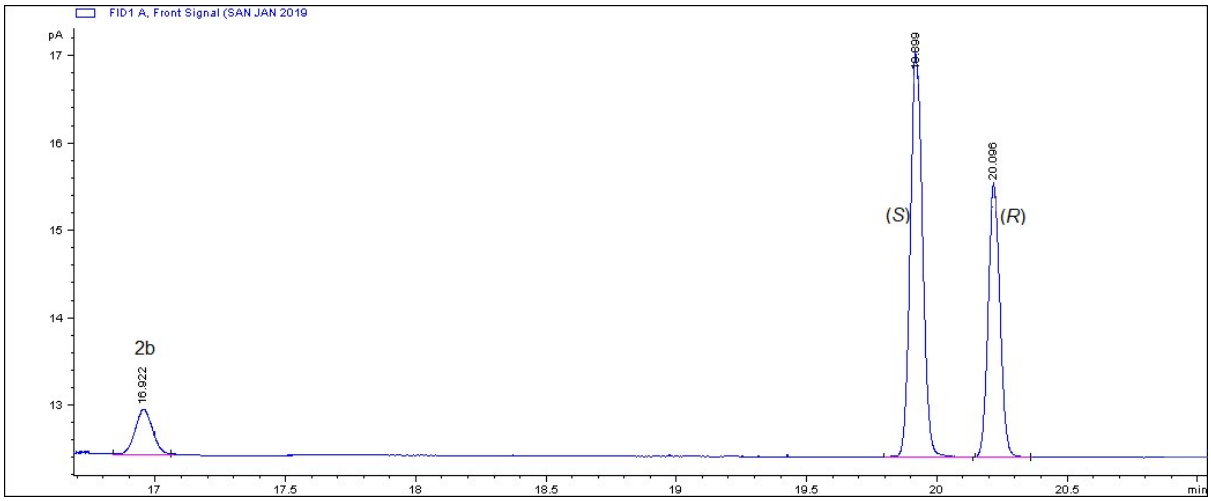


Figure S16. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1b** (see Table 2 in the main text, entry 1).

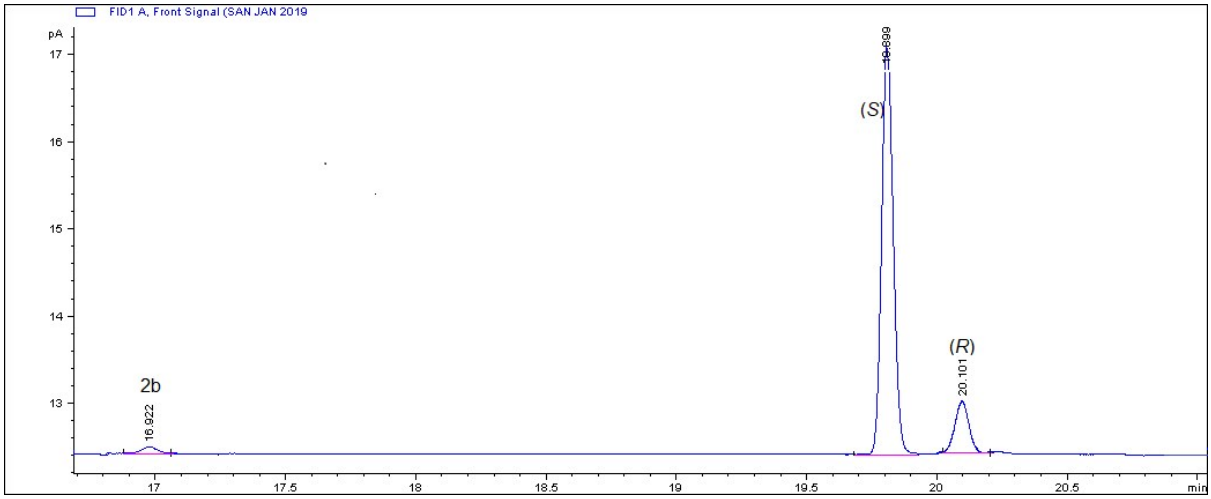


Figure S17. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1b** (see Table 2 in the main text, entry 2).

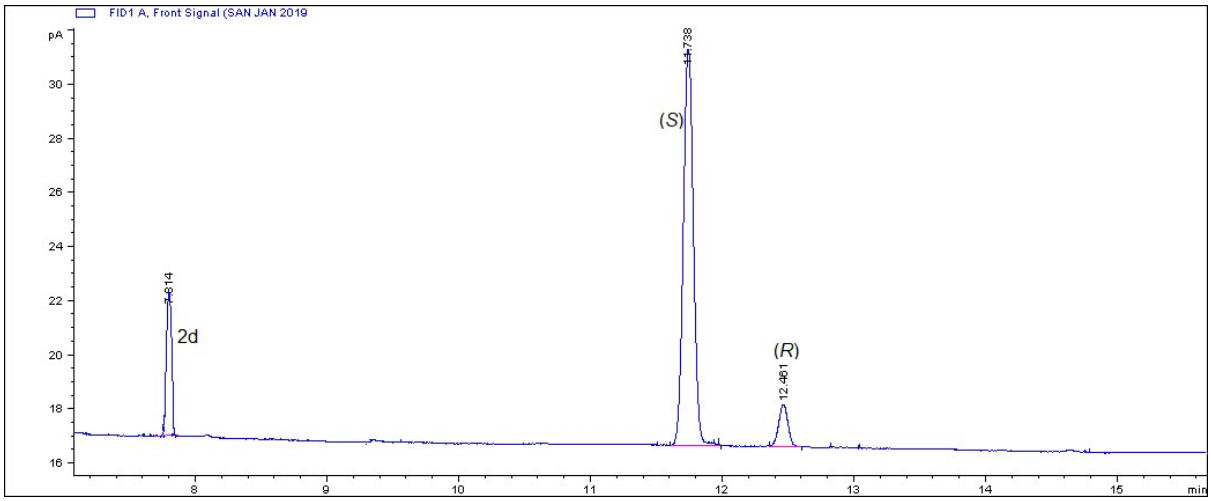


Figure S18. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1d** (see Table 2 in the main text, entry 3).

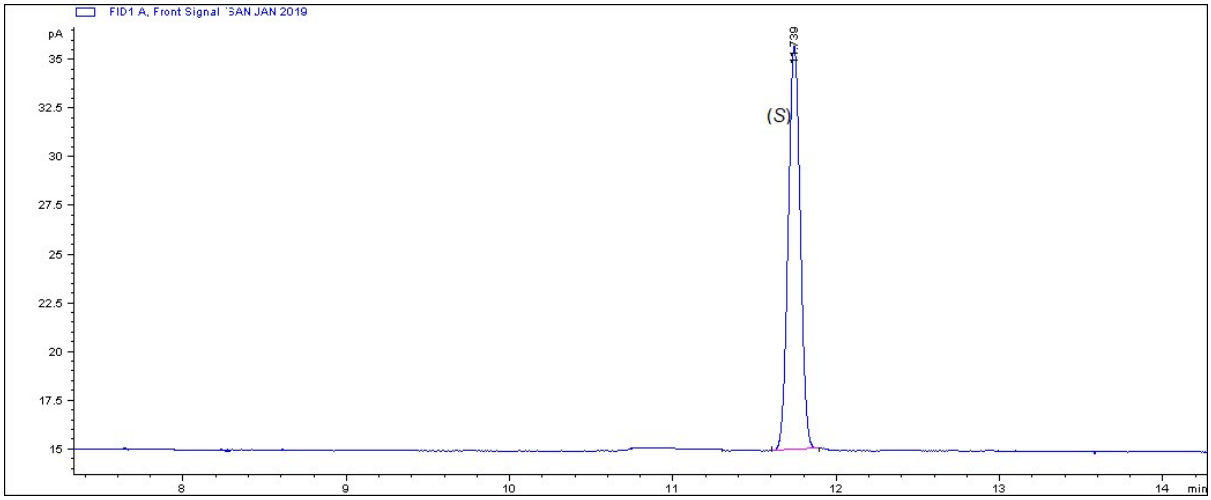


Figure S19. Gas chromatogram of the acetate derivative of the product of the deracemization of (*rac*)-**1d** (see Table 2 in the main text, entry 4).

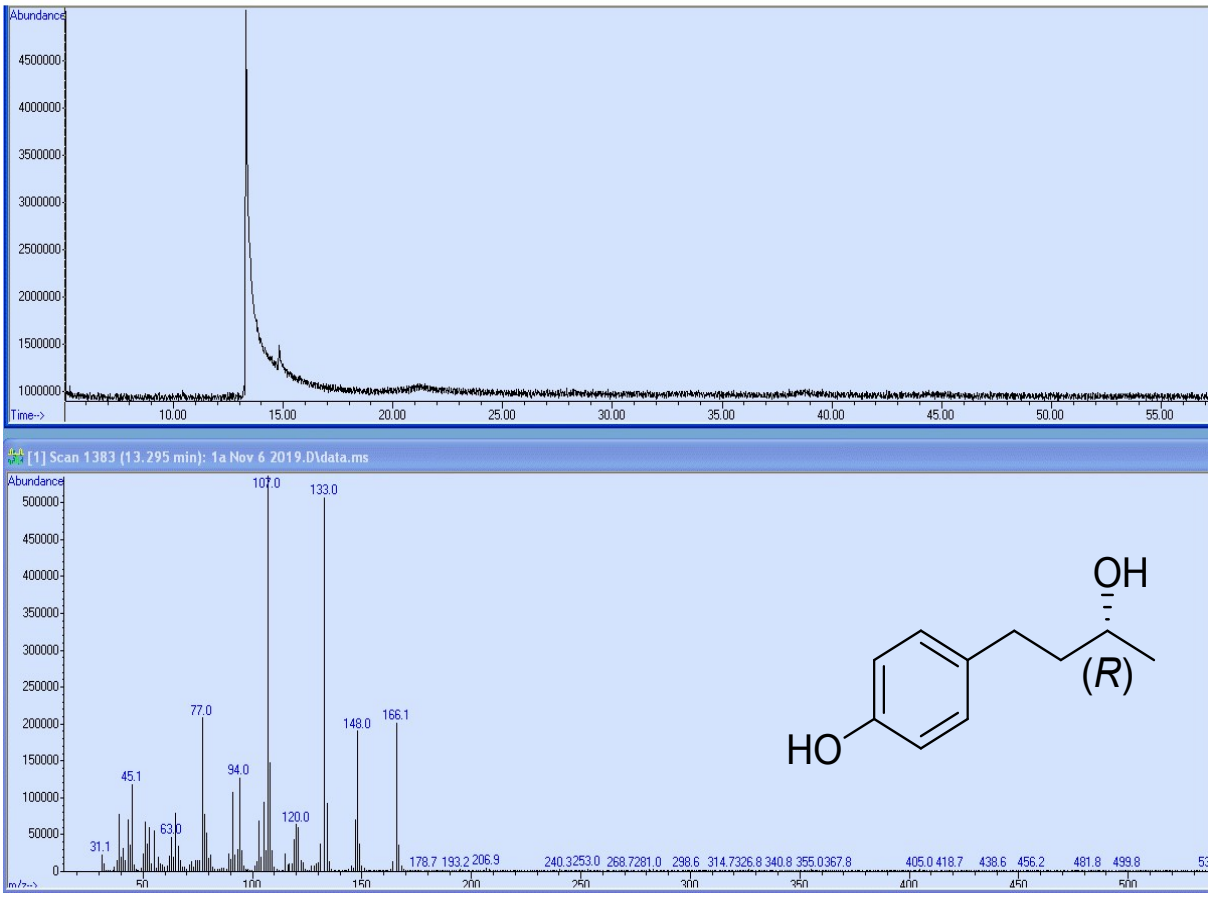


Figure S20. Gas chromatogram (top) of the product of concurrent cyclic deracemization of (*rac*)-**1a** using both W110G and W110V/G198D *Te*SADH and mass spectrum (bottom) of the deracemization product.

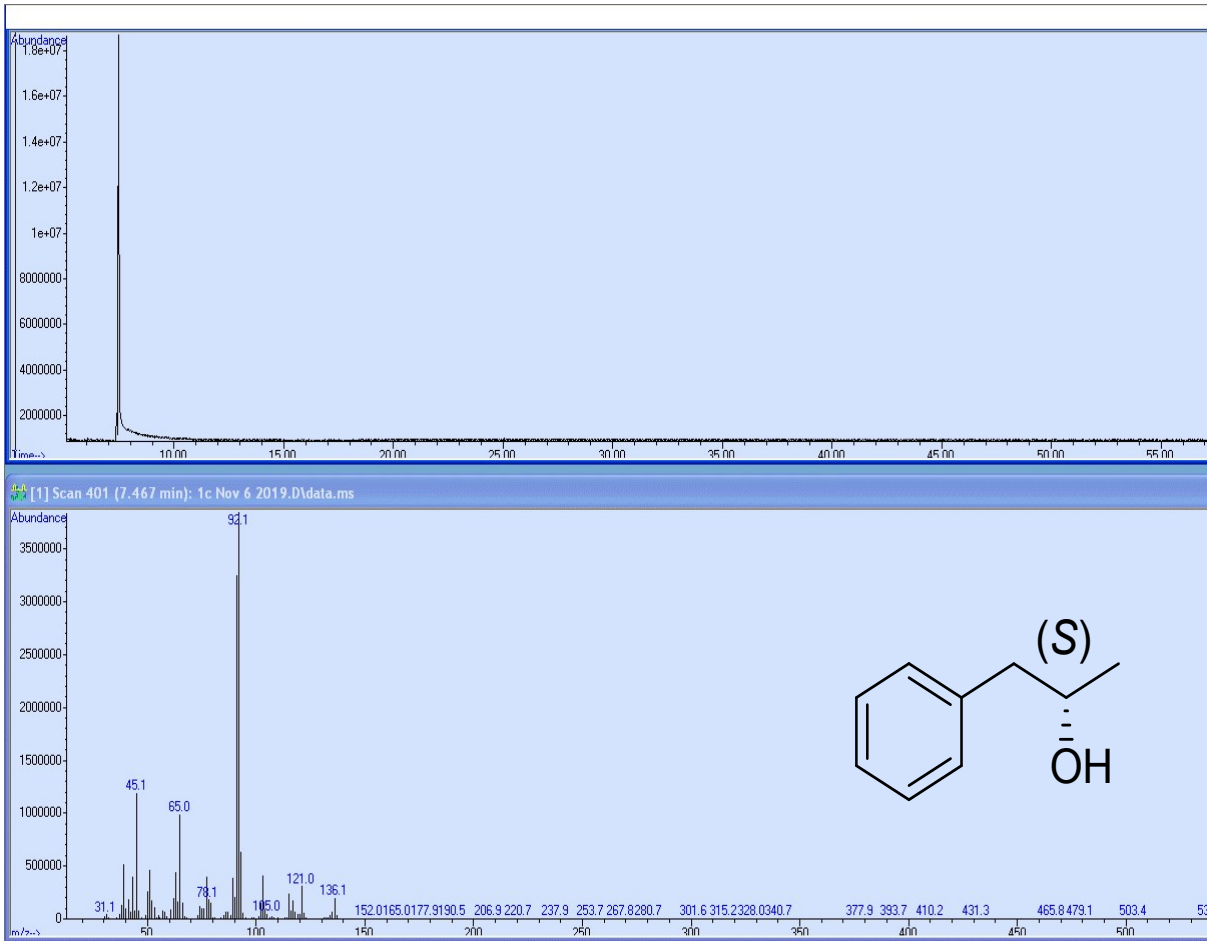


Figure S21. Gas chromatogram (top) of the product of concurrent cyclic deracemization of (*rac*)-**1b** using both W110G and W110V/G198D *Te*SADH and mass spectrum (bottom) of the deracemization product.

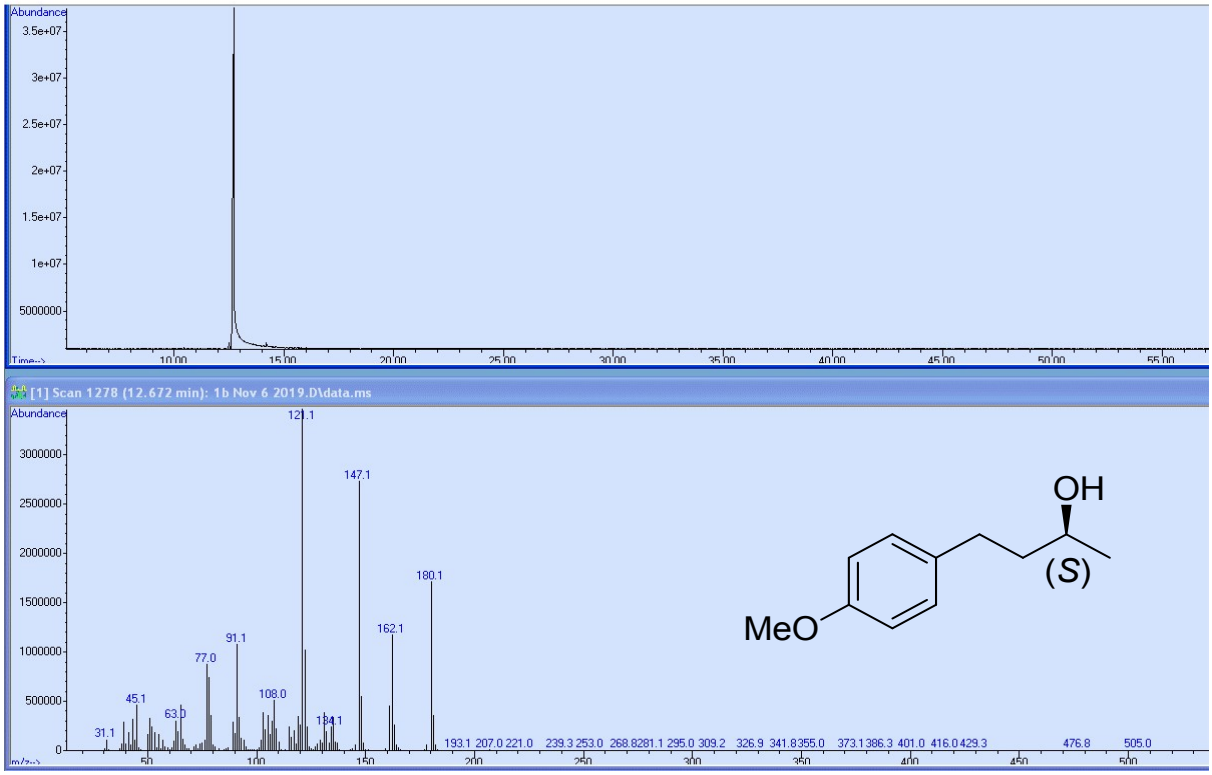


Figure S22. Gas chromatogram (top) of the product of concurrent cyclic deracemization of (*rac*)-**1c** using both W110G and W110V/G198D *Te*SADH and mass spectrum (bottom) of the deracemization product.

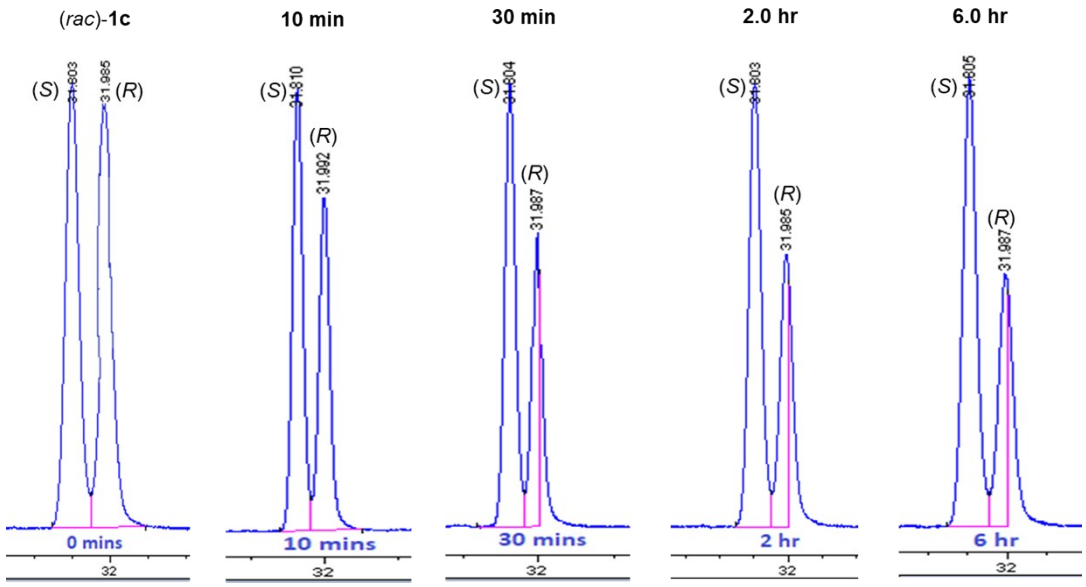


Figure S23. Reaction progress for concurrent CD-RS of (*rac*)-4-(4′-methoxyphenyl)-2-butanol [(*rac*)-**1c**] using W110G and W110V/G198D *Te*SADH mutants. GC chromatograms are for the acetate derivatives of the alcohol.

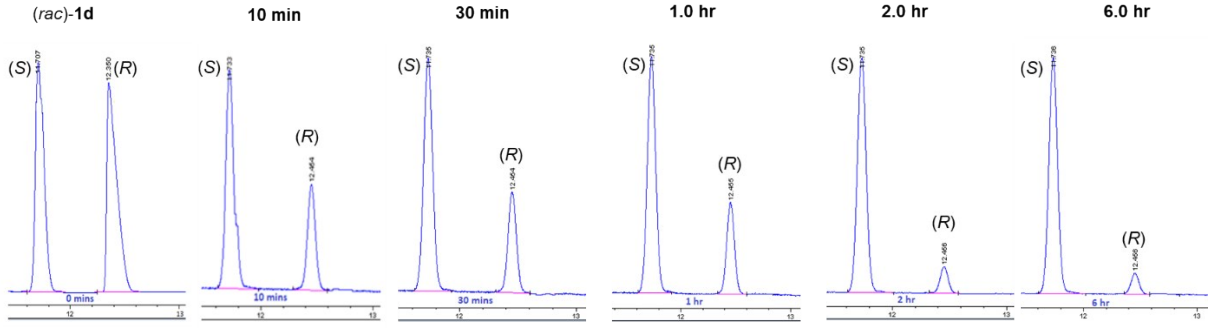


Figure S24. Reaction progress for concurrent deracemization of (*rac*)-1-phenylethanol [(*rac*)-**1d**] via stereoinversion using I86A and W110V/G198D *Te*SADH mutants. GC chromatograms are for the acetate derivatives of the alcohol.

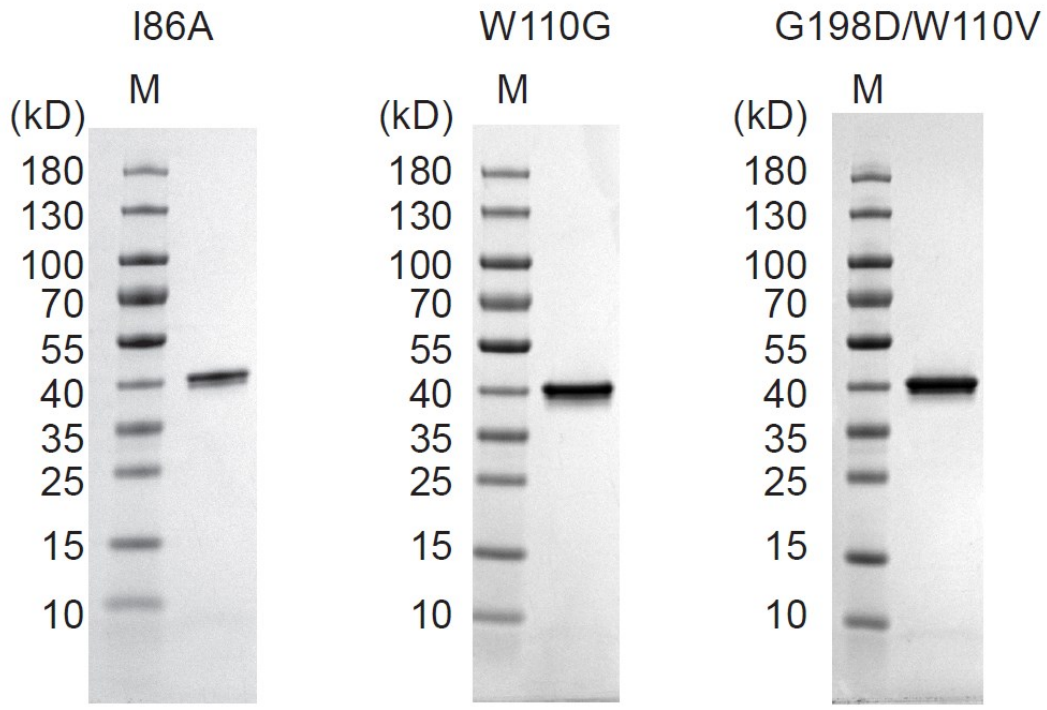


Figure S25. SDS-PAGE analysis of *Te*SADH mutant proteins. Purified I86A, W110G, and W110V/G198D proteins were analyzed on the 4-20 % gradient polyacrylamide gel (Bio-rad). M: protein marker.

REFERENCES

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(3) M. M. Musa, K. I. Ziegelmann-Fjeld, C. V. Vieille, J. G. Zeikus, R. S. Phillips, *J. Org. Chem.* 2007, **72**, 30–34.

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