Supplementary Information

Dual Biocatalytic One-Pot Deracemization of Aryl-Ring-Containing Secondary Alcohols via Sequential Oxidation-Reduction

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1. Experimental Procedures

1.1. General information

Sodium borohydride, NADP$^+$, (*rac*)-**1a**, (*rac*)-**4a**, and (*rac*)-**8a** were obtained from commercial sources and used without further purification, while (*rac*)-**2a**, (*rac*)-**3a**, (*rac*)-**5a**, (*rac*)-**6a**, (*rac*)-**7a,** (*rac*)-**9a,** and (*rac*)-**10a** were synthesized from their corresponding ketones using sodium borohydride.[1] H_2O_2 was obtained as a 30% (w/w) solution in water. Gas chromatography (GC) analyses were performed on a capillary GC equipped with an HP Chiral-20 B column (30 m, 0.32 mm i.d., 0.25 μm film thickness) using helium as the carrier gas and a flame ionization detector (FID). GC-FID analysis for all substrates was conducted using a chiral column with the following conditions: initial oven temperature of 70 °C, ramped at 10 °C/min to 120 °C with a 10-minute hold, further ramped at 10 °C/min to 180 °C with another 10-minute hold, with a total run time of 80 minutes and a split ratio of 10:1. For substrates (*rac*)-**2a**, (*rac*)-**7a**, and (*rac*)-**10a**, initial oven temperature of 70 °C, ramped at 10 °C/min to 100 °C with a 15-minute hold, further ramped at 10 °C/min to 180 °C with another 15-minute hold, with a total run time of 150 minutes and a split ratio of 10:1. Full oxidation was confirmed by GC-MS using a split ratio of 100:1, with the following conditions: an initial oven temperature of 70 °C held for 10 minutes, followed by a temperature ramp of 5 °C/min to 120 °C over 20 minutes, then a final increase to 220 °C, where the temperature was held for 20 minutes. All nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ using 400 MHz (^{1}H NMR) and 101 MHz (^{13}C NMR) or 600 MHz (^{1}H NMR) and 151 MHz (^{13}C NMR) instruments. Chemical shifts (ppm) were recorded as the signal frequency relative to the solvent peak. Multiplicities are given as: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), dt (doublet of triplets), qt (quintet of triplets), or m (multiplet).

1.2. Quantification of pure enantiomer of alcohols produced by bi-enzymatic deracemization.

For alcohol quantification, samples were periodically collected and analyzed by GC-FID using a standard calibration curve. The calibration curve was prepared under conditions similar to the reaction medium by varying substrate concentration (4 mM to 12 mM) in 1.5 mL Eppendorf tubes, each containing 1.0 mL of an aqueous solution containing 20% (v/v) acetonitrile and 5% isopropanol. From each standard solution, 50 μL was transferred to another 1.5 mL Eppendorf tube, and 500 µL of chloroform was added. The mixture was thoroughly mixed, and a drying agent was added to absorb residual water, followed by centrifugation at 5000 rpm for 2 minutes. The chloroform solution was then transferred to a GC vial for analysis.

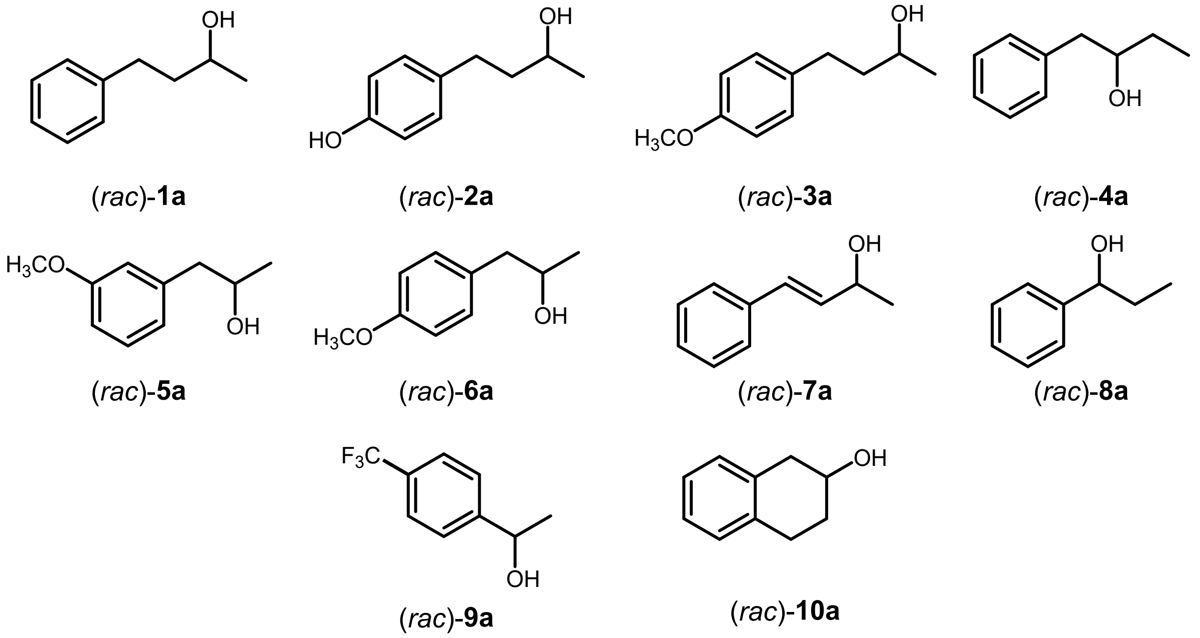
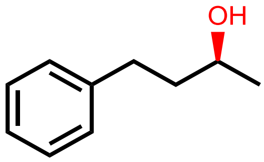

(*rac*)-**1a** (*rac*)-**2a** (*rac*)-**3a** (*rac*)-**4a**
(*rac*)-**5a** (*rac*)-**6a** (*rac*)-**7a** (*rac*)-**8a**
(*rac*)-**9a** (*rac*)-**10a**

Figure S1. Substrate scope.

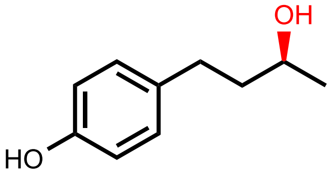
2. Characterization of products

(*S*)-4-Phenylbutan-2-ol [(*S*)-(1a)]



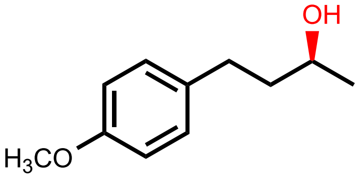
^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.31 – 7.27 (m, 2H), 7.20 (dd, *J* = 14.0, 7.1 Hz, 3H), 3.82 (dq, *J* = 12.4, 6.2 Hz, 1H), 2.78 – 2.63 (m, 2H), 1.82 – 1.70 (m, 2H), 1.24 (d, *J* = 6.2 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 142.2, 128.6, 128.5, 126.0, 67.7, 41.0, 32.3, 23.8. The data are consistent with those reported in previous studies.[1]

(*S*)-4-(3-Hydroxybutyl)-phenol [(*S*)-(2a)]



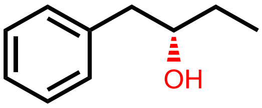
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.07 (d, *J* = 8.6 Hz, 2H), 6.75 (d, *J* = 8.6 Hz, 2H), 3.82 (dq, *J* = 13.1, 6.2 Hz, 1H), 2.72 – 2.57 (m, 2H), 1.77 – 1.70 (m, 2H), 1.22 (d, *J* = 6.2 Hz, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 153.8, 134.3, 129.6, 115.4, 67.7, 41.2, 31.4, 23.8. The data are consistent with those reported in previous studies.[2]

(*S*)-4-(4´-Methoxyphenyl)-butan-2-ol [(*S*)-(3a)]



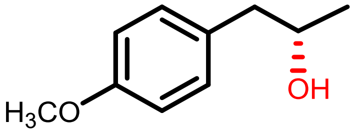
^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.12 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 3.82 (dt, *J* = 12.4, 6.1 Hz, 1H), 3.79 (s, 3H), 2.74 – 2.56 (m, 2H), 1.81 – 1.68 (m, 2H), 1.22 (d, *J* = 6.2 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 157.9, 134.2, 129.5, 113.9, 67.6, 55.4, 41.2, 31.3, 23.8.

(*S*)-1-Phenylbutan-2-ol [(*S*)-(4a)]



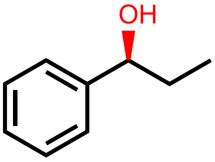
^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.34 – 7.30 (m, 2H), 7.25 – 7.21 (m, 3H), 3.81 – 3.72 (m, 1H), 2.84 (dd, *J* = 13.6, 4.3 Hz, 1H), 2.65 (dd, *J* = 13.6, 8.4 Hz, 1H), 1.59 – 1.49 (m, 2H), 1.00 (t, *J* = 7.5 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 138.8, 129.6, 128.7, 126.6, 74.2, 43.7, 29.7, 10.2.

(*S*)-1-(4-Methoxyphenyl)-propan-2-ol [(*S*)-(6a)]



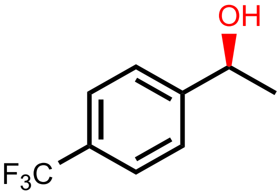
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.13 (d, *J* = 8.5 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 4.03 – 3.93 (m, 1H), 3.79 (s, 3H), 2.80 – 2.54 (m, 2H), 1.23 (d, *J* = 6.2 Hz, 3H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 158.4, 130.6, 130.5, 114.1, 69.1, 55.4, 44.9, 22.8.

(*S*)-1-Phenylpropan-1-ol [(*S*)-(8a)]



^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.38 – 7.22 (m, 5H), 4.59 – 4.53 (m, 1H), 1.85 – 1.65 (m, 2H), 0.89 (t, *J* = 6.0 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 144.5, 128.4, 127.4, 125.9, 75.9, 31.8, 10.1. The data are consistent with those reported in previous studies.[3]

(*S*)-1-(4′-trifluoromethylphenyl)-ethanol [(*S*)-(9a)]



^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.61 (d, *J* = 8.1 Hz, 2H), 7.49 (d, *J* = 8.1 Hz, 2H), 4.97 (q, *J* = 6.5 Hz, 1H), 1.51 (d, *J* = 6.5 Hz, 3H). **^{13}C NMR** (151 MHz, Chloroform-*d*) δ 149.8 (d, *J* = 1.5 Hz), 129.8 (d, *J* = 32.4 Hz), 125.8, 125.6 (q, *J* = 3.8 Hz), 124.3 (d, *J* = 271.8 Hz), 70.0, 25.5. The data are consistent with those reported in previous studies.[3]

4. Calibration curves of substrates

4-Phenyl-2-butanol (1a)

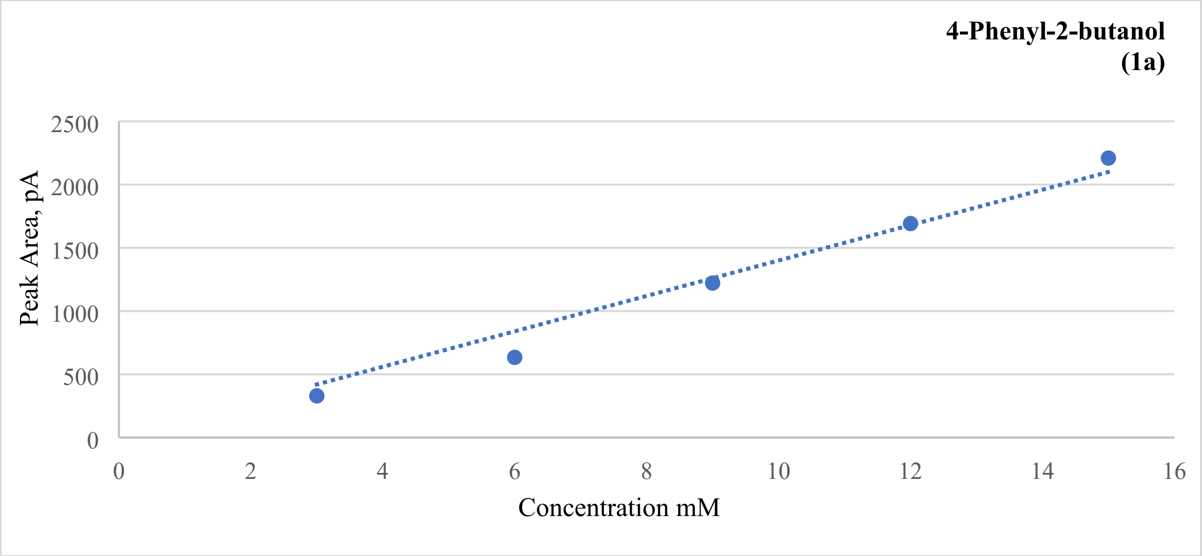


Figure S2. Calibration curve for 4-phenyl-2-butanol (**1a**) obtained by GC-FID.

4-(3-Hydroxybutyl)-phenol (2a)

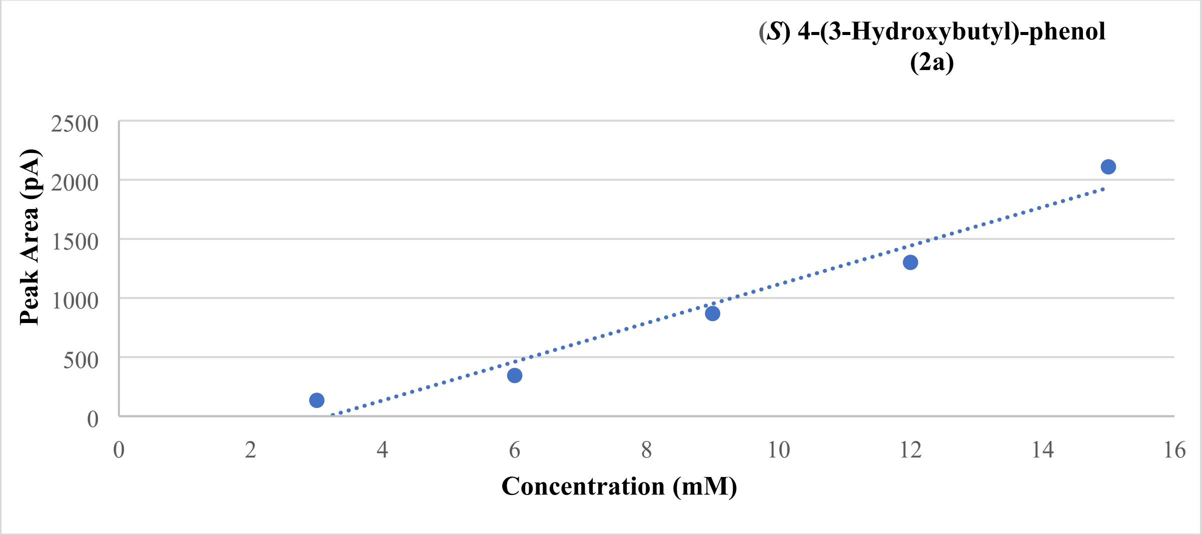


Figure S3. Calibration curve for (*S*) 4-(3-hydroxybutyl)-phenol (**2a**) obtained by GC-FID.

4-(4´-Methoxyphenyl)-butan-2-ol (3a)

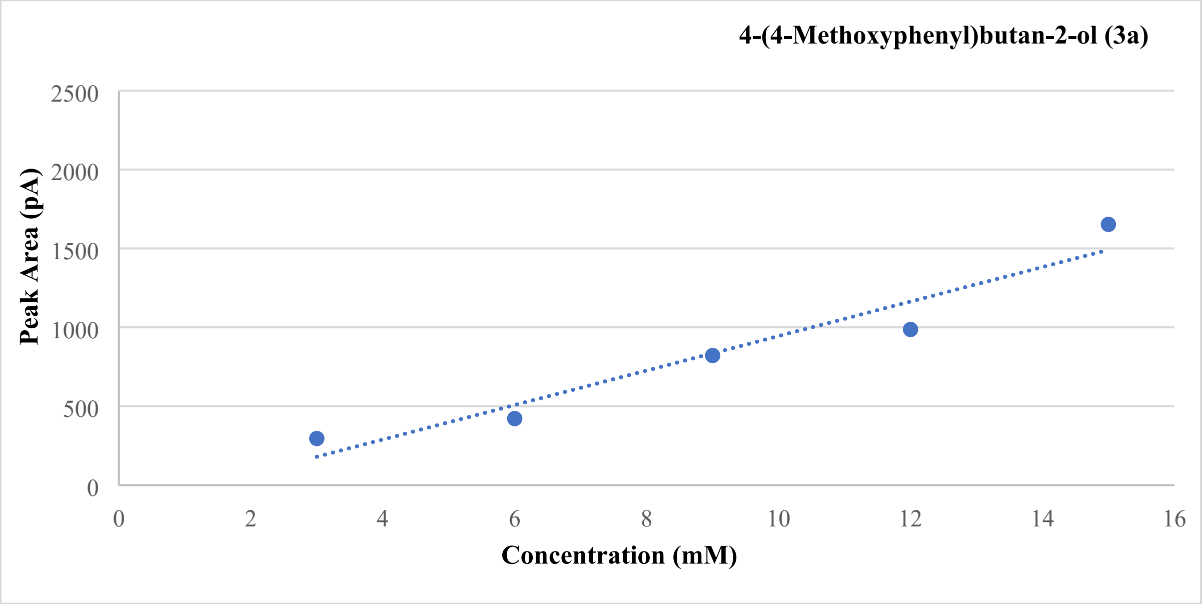


Figure S4. Calibration curve for 4-(4'-methoxyphenyl)-2-butanol (**3a**) obtained by GC-FID.

1-Phenylbutan-2-ol (4a)

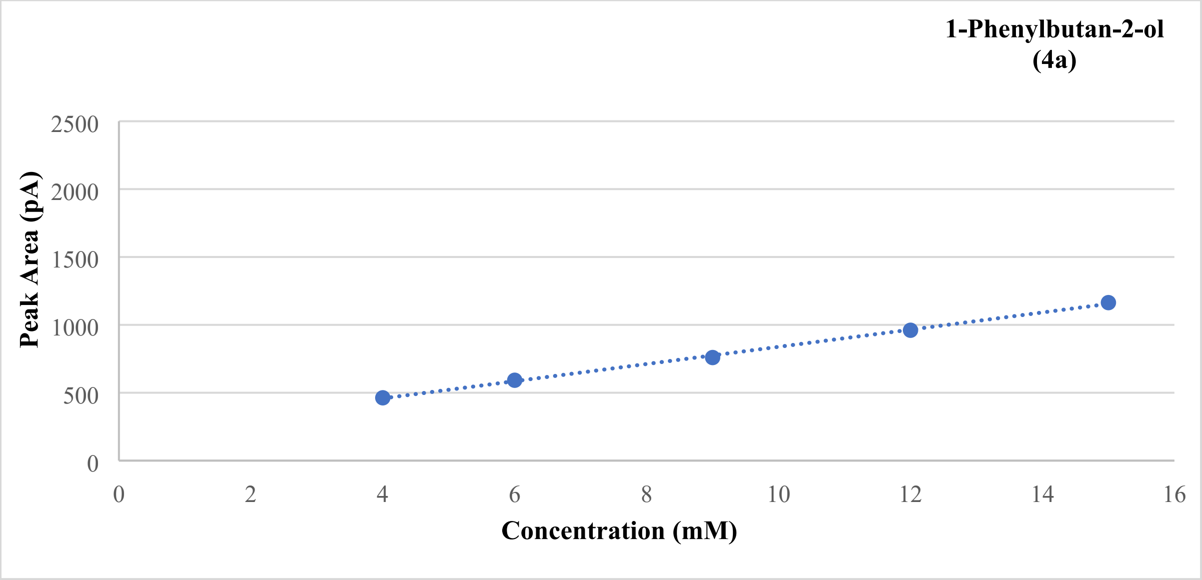


Figure S5. Calibration curve for 1-phenylbutan-2-ol (**4a**) obtained by GC-FID.

1-(3-Methoxyphenyl)-propan-2-ol (5a)

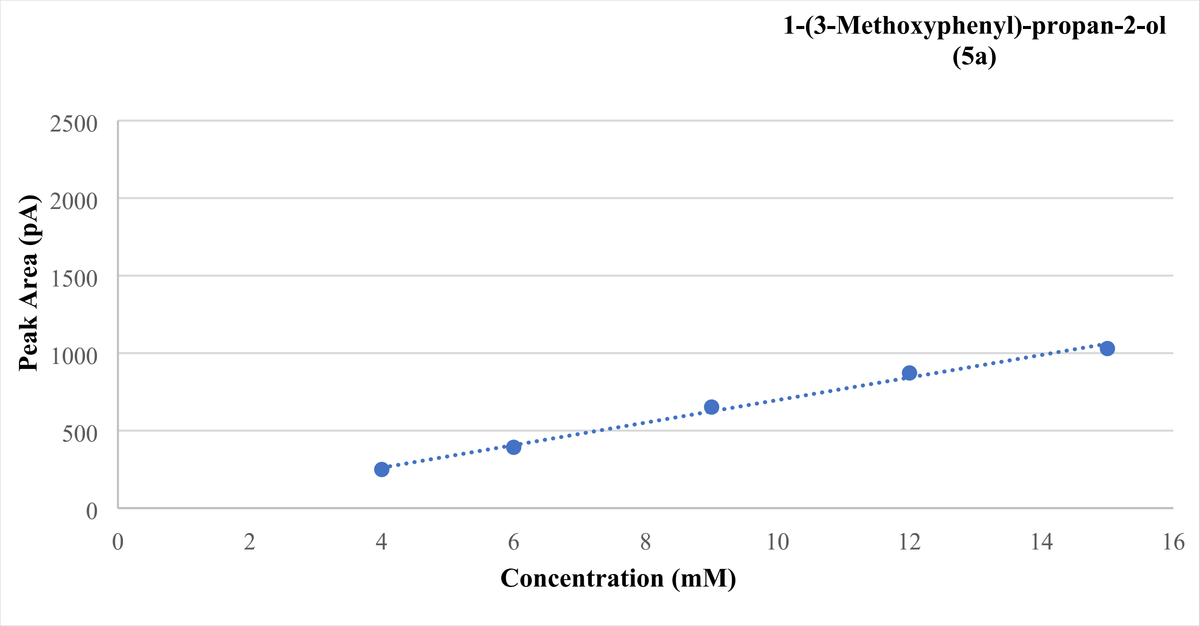


Figure S6. Calibration curve for 1-(3-methoxyphenyl)-propan-2-ol (**5a**) obtained by GC-FID.

1-(4-Methoxyphenyl)-propan-2-ol (6a)

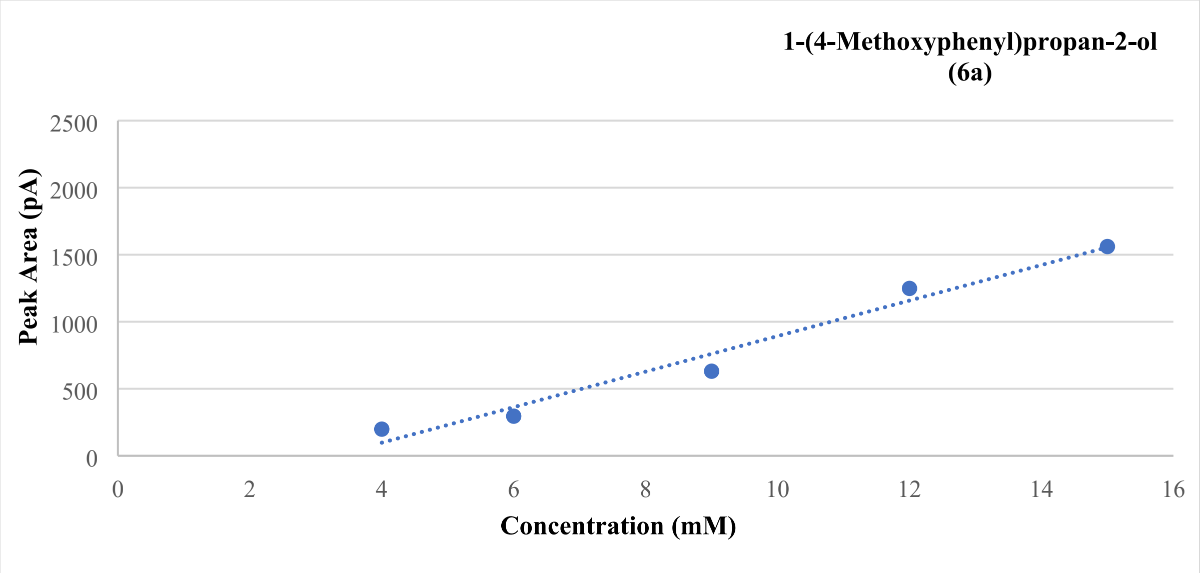


Figure S7. Calibration curve for 1-(4-methoxyphenyl)-propan-2-ol (**6a**) obtained by GC-FID.

(*E*)-4-Phenylbut-3-en-2-ol (7a)

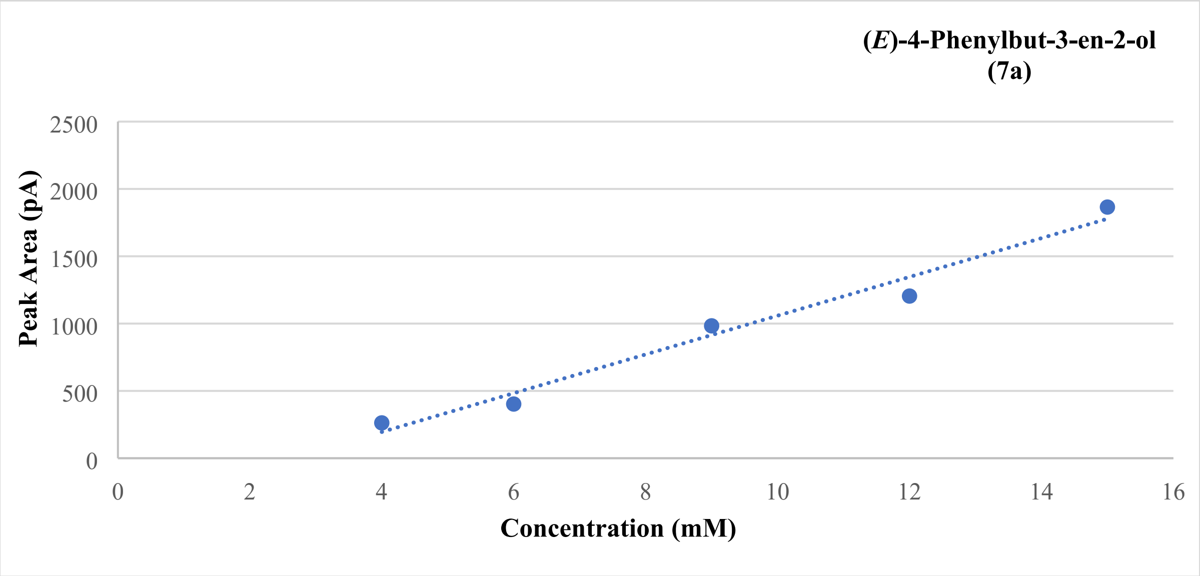


Figure S8. Calibration curve for (*E*)-4-phenylbut-3-en-2-ol **(7a)** obtained by GC-FID.

1-Phenylpropan-2-ol (8a)

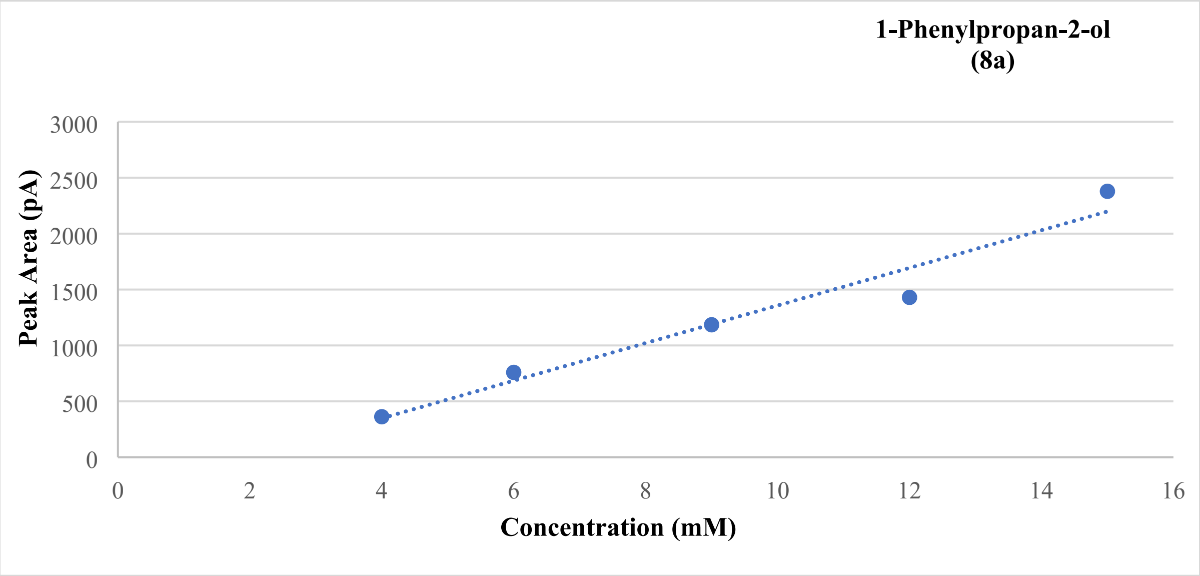
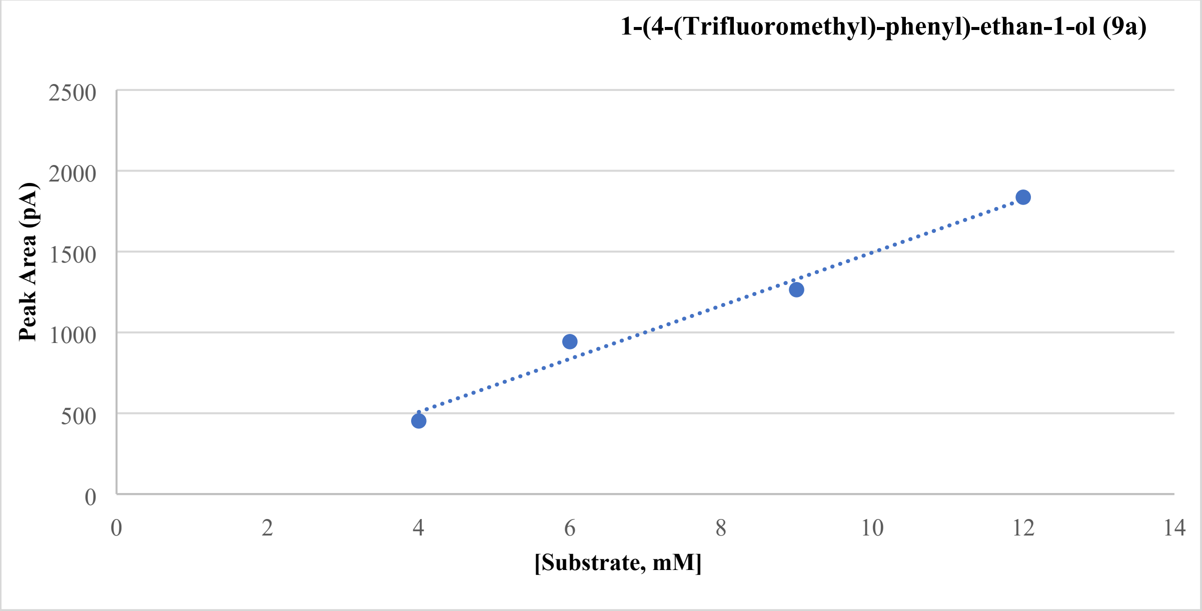


Figure S9. Calibration curve for 1-phenylpropan-2-ol **(8a)** obtained by GC-FID.

1-(4′-trifluoromethylphenyl)-ethanol (9a)



2-Tetralone (10a)

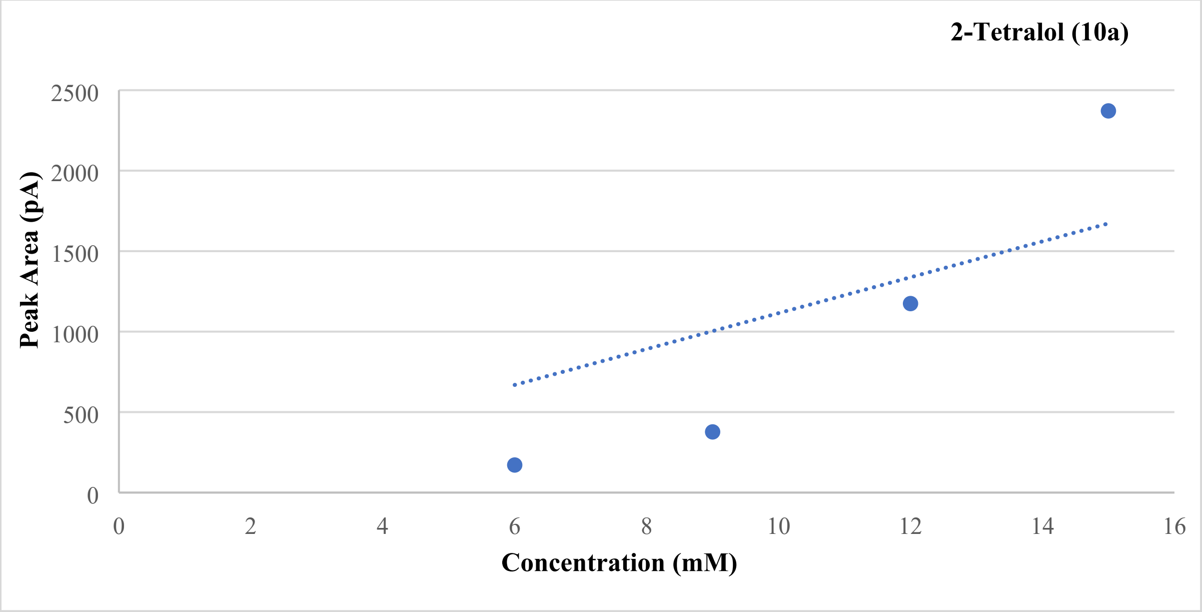
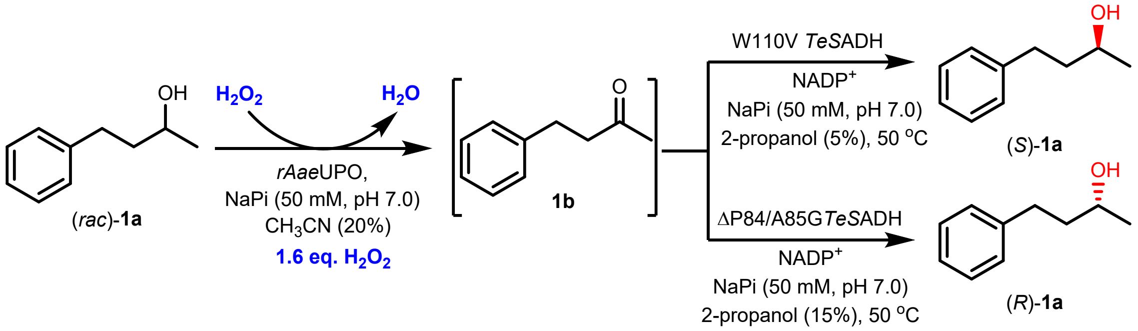


Figure S10. Calibration curve for 2-Tetralone obtained by GC-FID.

5. Characterization data copies of GC-MS, ^{1}H, and ^{13}C NMR spectra of products:

(*rac*)-4-Phenyl-2-butanol [(*rac*)-1a]



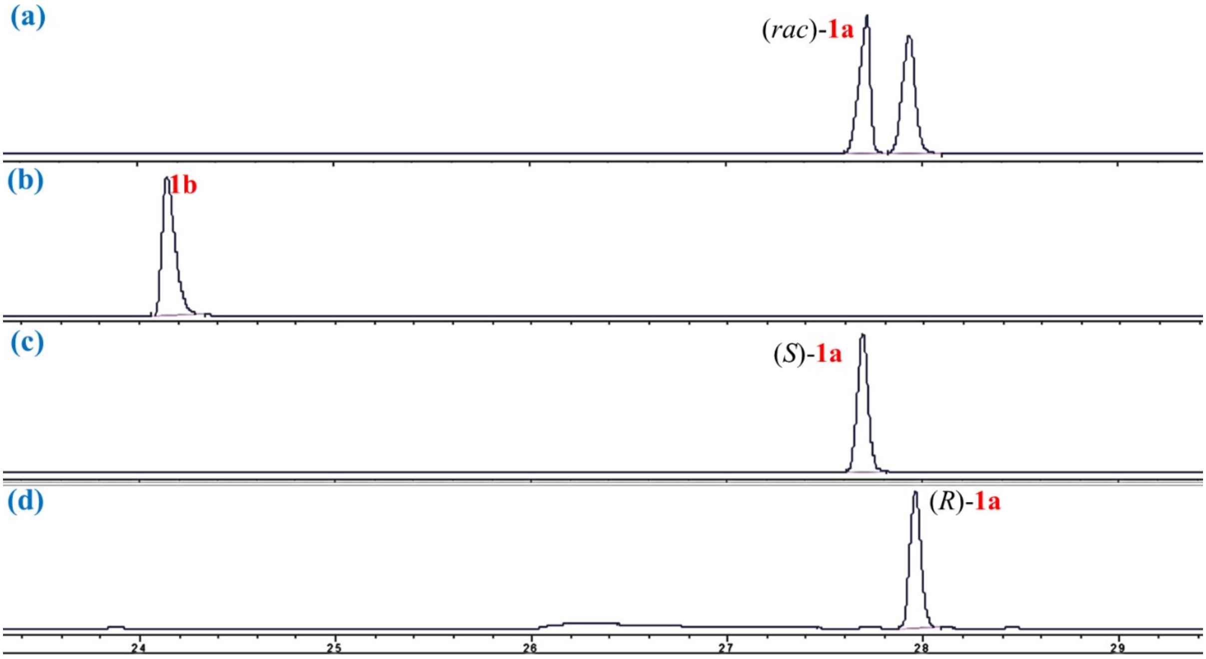
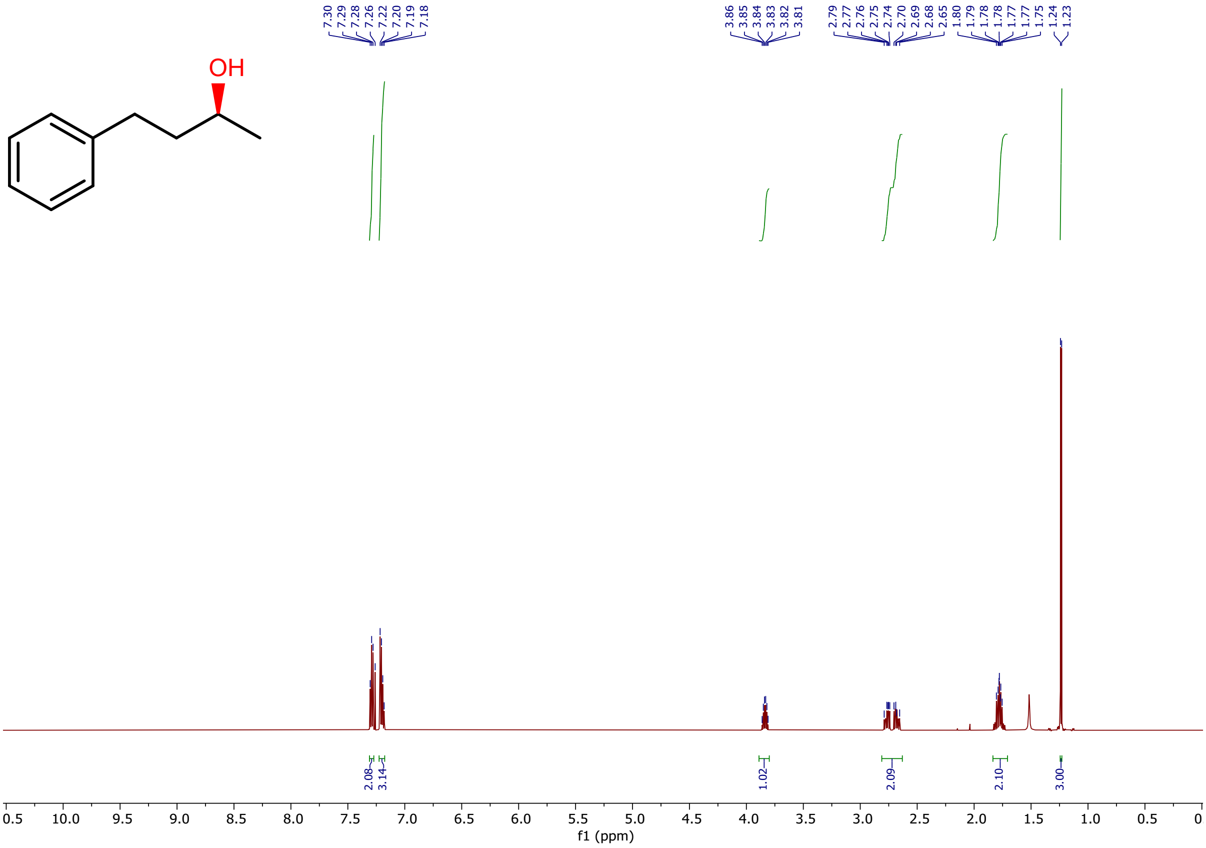
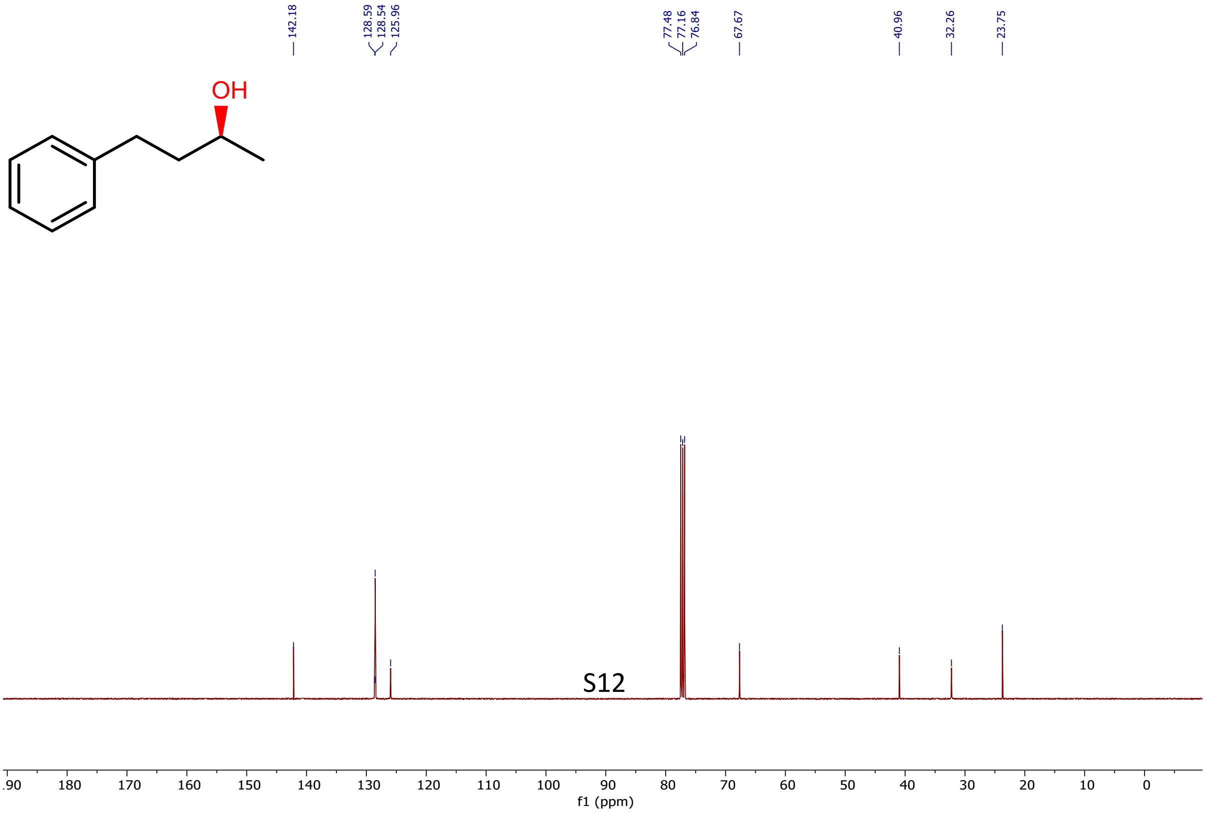


Figure S11. Gas chromatograms showing (a) the acetate derivative of (*rac*)-**1a**; (b) compound **1b** produced by *Aae*UPO-catalyzed oxidation of (*rac*)-**1a**, and the acetate derivatives obtained from the two-step one-pot deracemization process: (c) The oxidation by *Aae*UPO, followed by a reduction using W110V *TeS*ADH produced (*S*)-**1a**. (d) while the variant ΔP84/A85G *TeS*ADH yielded (*R*)-**1a**.

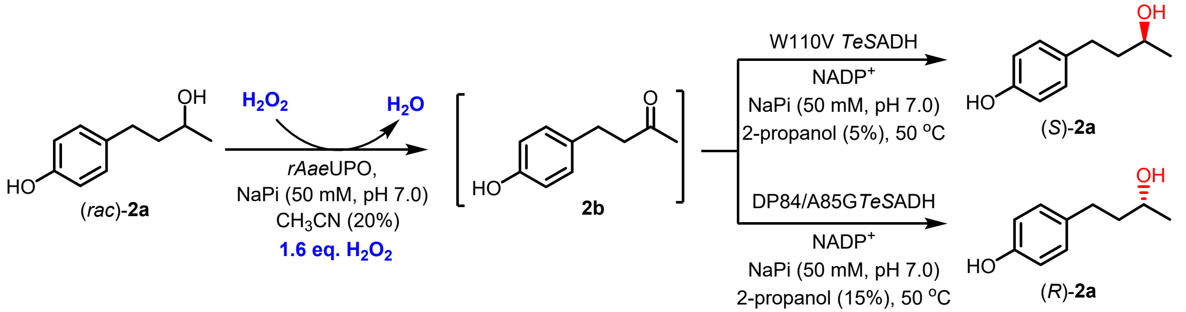
¹H NMR (600 MHz, Chloroform-*d*): spectrum of (*S*)-**1a** produced by a two-step one-pot deracemization process of (*rac*)-**1a**.



¹³C { ¹H } NMR (151 MHz, Chloroform-*d*): spectrum of (*S*)-**1a** produced by a two-step one-pot deracemization process of (*rac*)-**1a**.



(*rac*)- 4-(3-hydroxybutyl)-phenol [(*rac*)-2a]



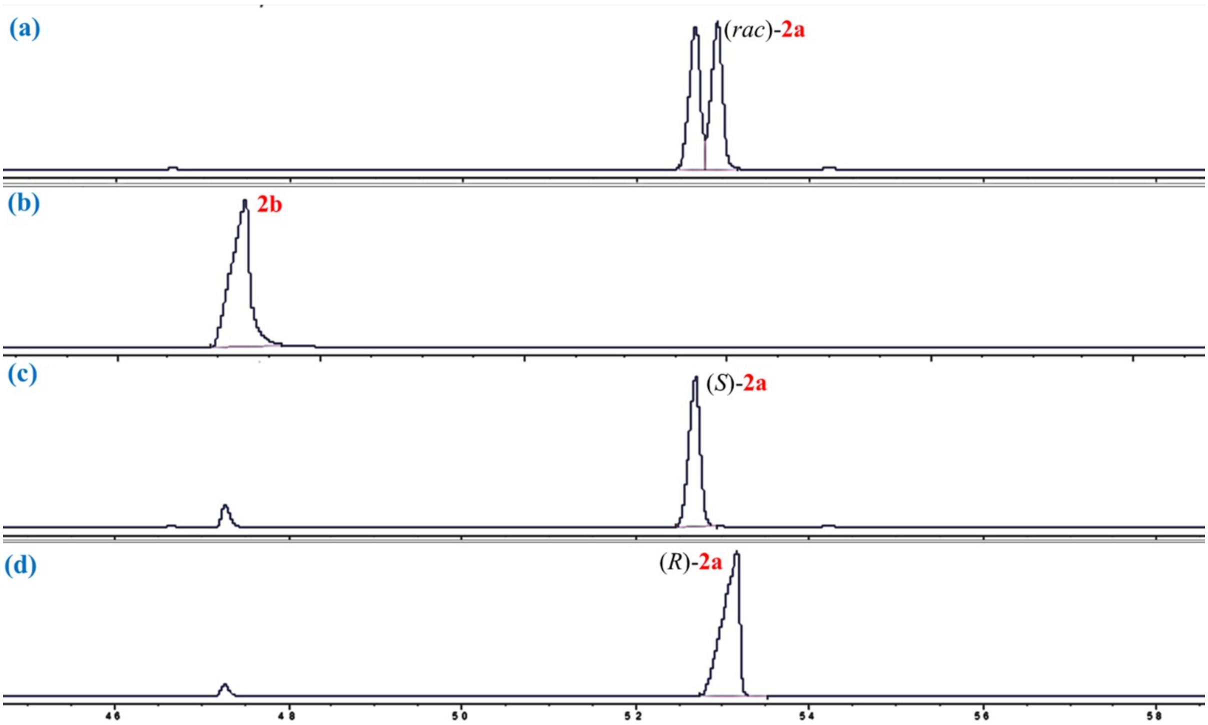
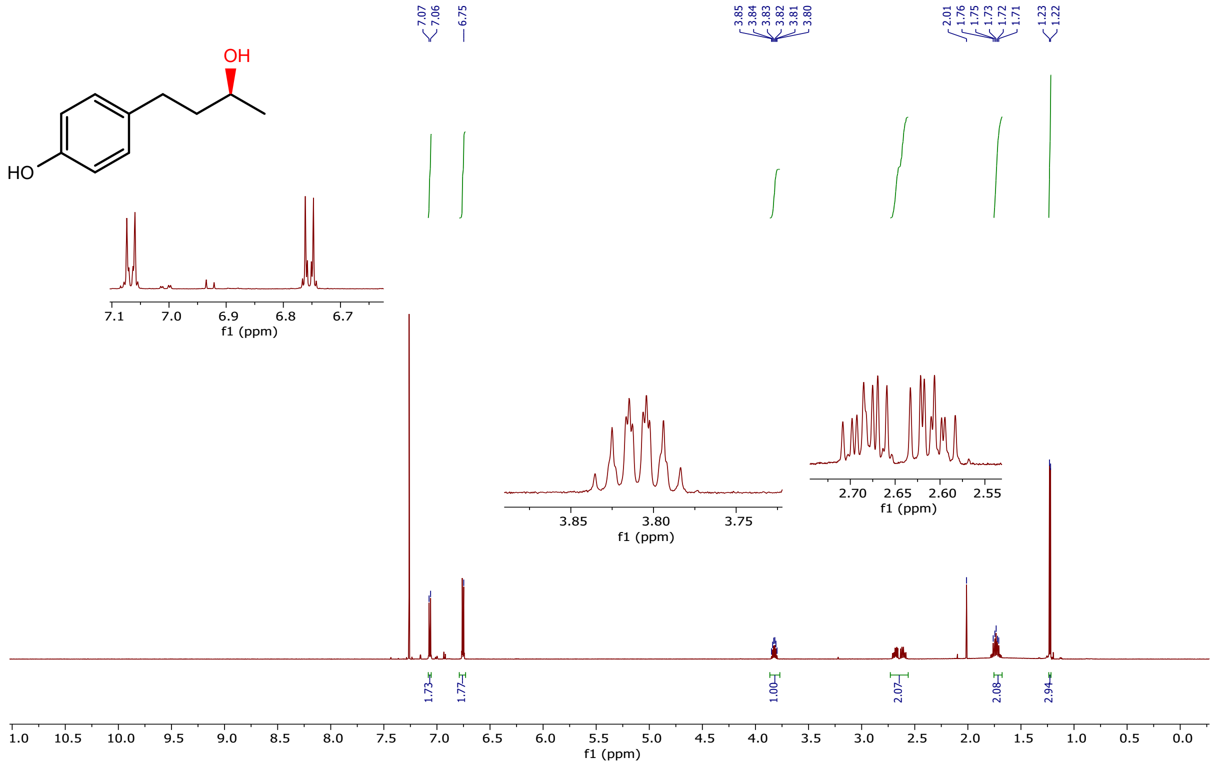
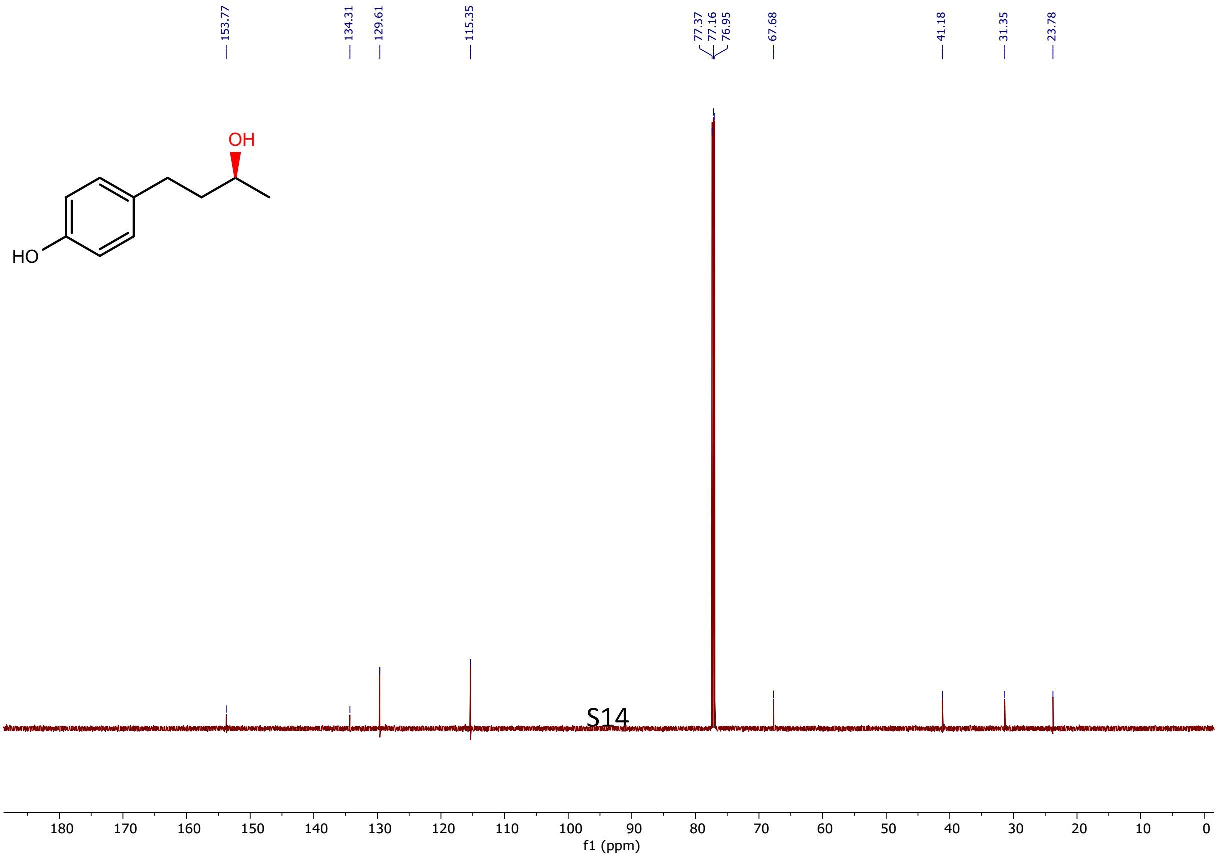


Figure S12. Gas chromatograms showing (a) the acetate derivative of (*rac*)-**2a**, (b) compound **2b** produced by *Aae*UPO-catalyzed oxidation of (*rac*)-**2a**, and the acetate derivatives obtained from the two-step one-pot deracemization process: (c) The oxidation by *Aae*UPO followed by a reduction using W110V *TeS*ADH produced (*S*)-**2a**. (d) while the variant ΔP84/A85G yielded (*R*)-**2a.**

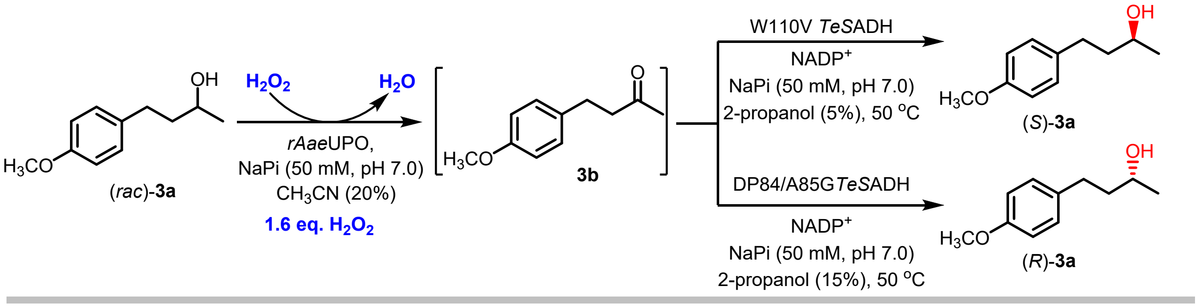
¹H NMR (600 MHz, Chloroform-*d*): spectrum of (*S*)-**2a** produced by a two-step one-pot deracemization process of (*rac*)-**2a**.



¹³C{ ¹H } NMR (151 MHz, Chloroform-*d*): spectrum of (*S*)-**2a** produced by a two-step one-pot deracemization process of (*rac*)-**2a**.



(*rac*)-4-(4´-Methoxyphenyl)-butan-2-ol [(*rac*)-3a]



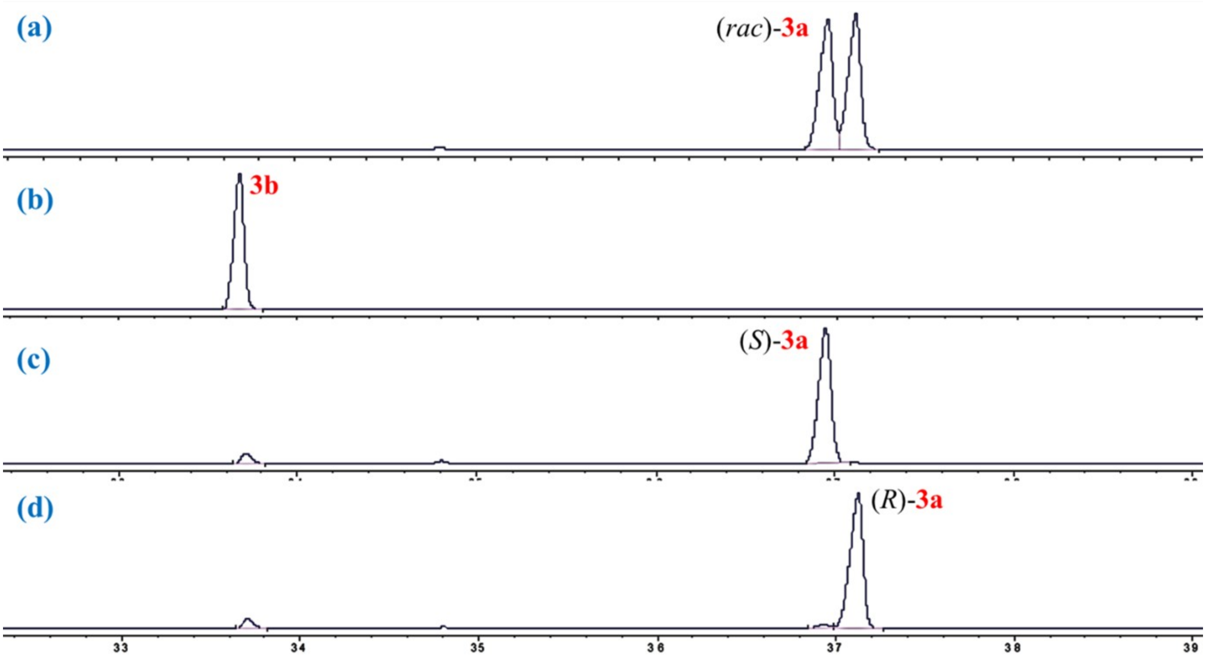
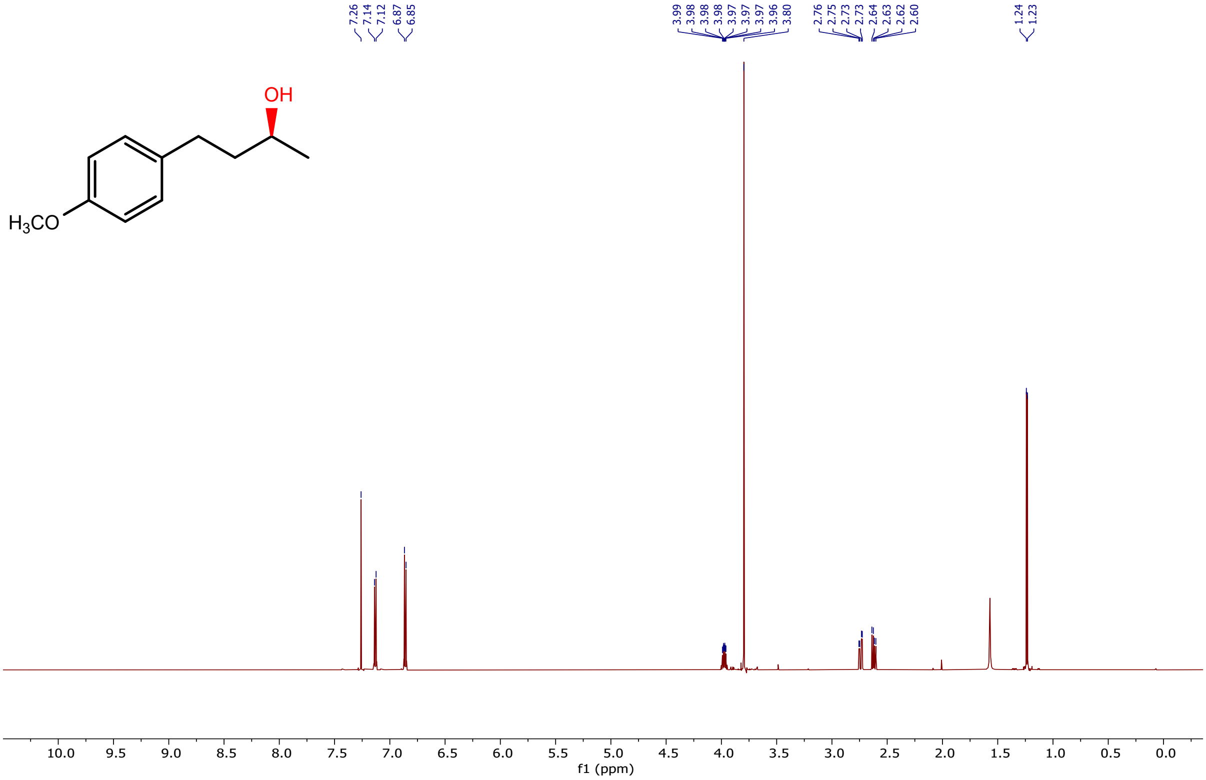
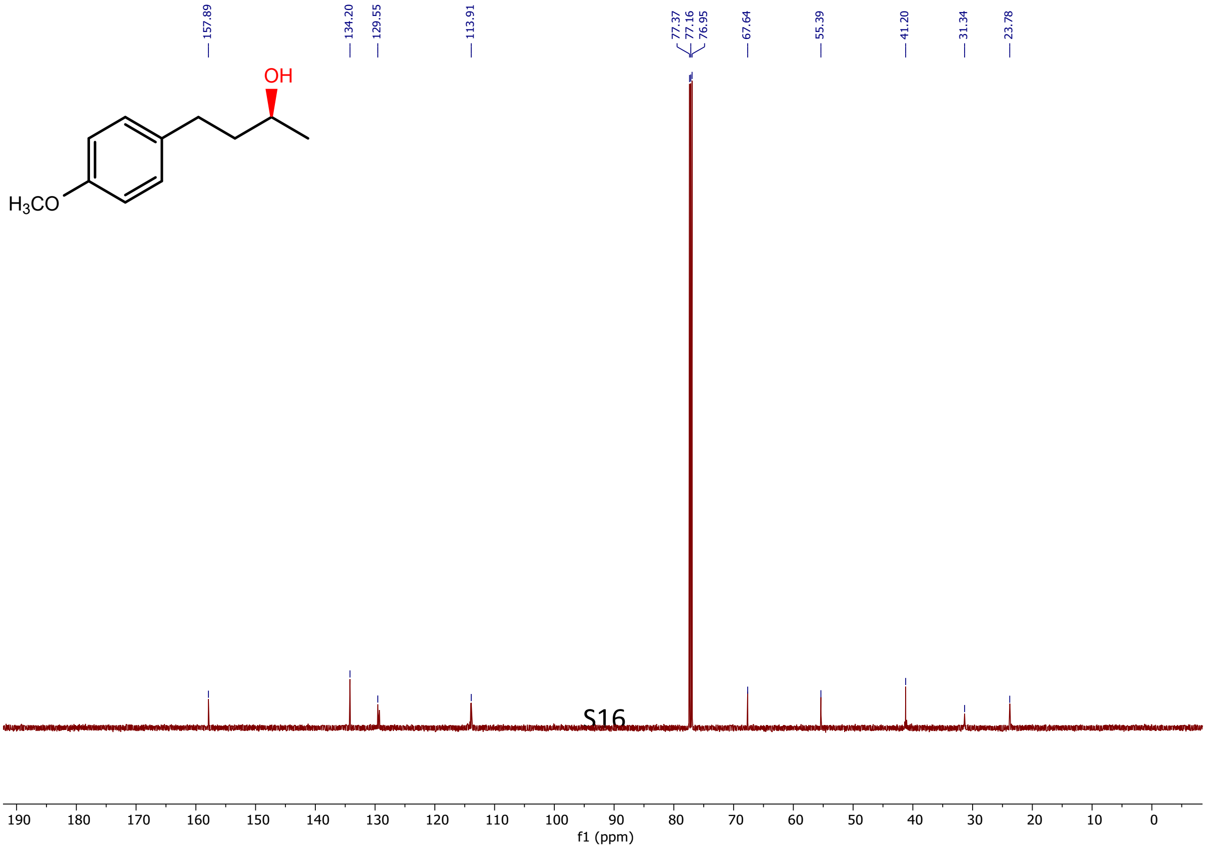


Figure S13. Gas chromatograms showing (a) the acetate derivative of (*rac*)-**3a**, (b) compound **3b** produced by *Aae*UPO-catalyzed oxidation of (*rac*)-**3a**, and the acetate derivatives obtained from the two-step one-pot deracemization process: (c) The oxidation by *Aae*UPO followed by a reduction using W110V *TeS*ADH produced (*S*)-**3a**. (d) while the variant ΔP84/A85G yielded (*R*)-**3a**.

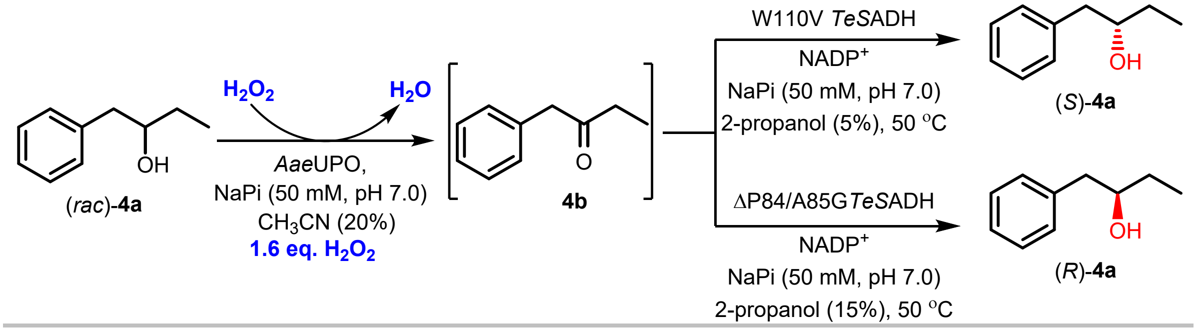
^{1}H NMR (600 MHz, Chloroform-*d*): spectrum of (*S*)-**3a** produced by a two-step one-pot deracemization process of (*rac*)-**3a**.



^{13}C{ ^{1}H } NMR (151 MHz, Chloroform-*d*): spectrum of (*S*)-**3a** produced by a two-step one-pot deracemization process of (*rac*)-**3a**.



(*rac*)-1-Phenylbutan-2-ol [(*rac*)-4a]



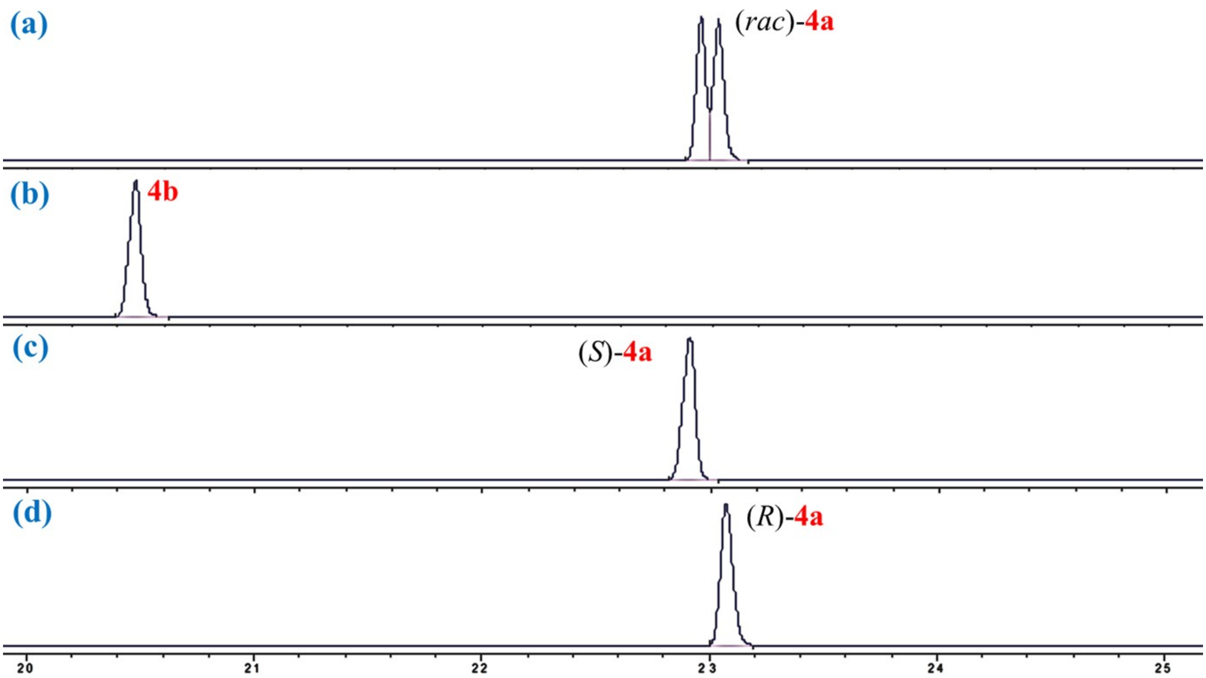
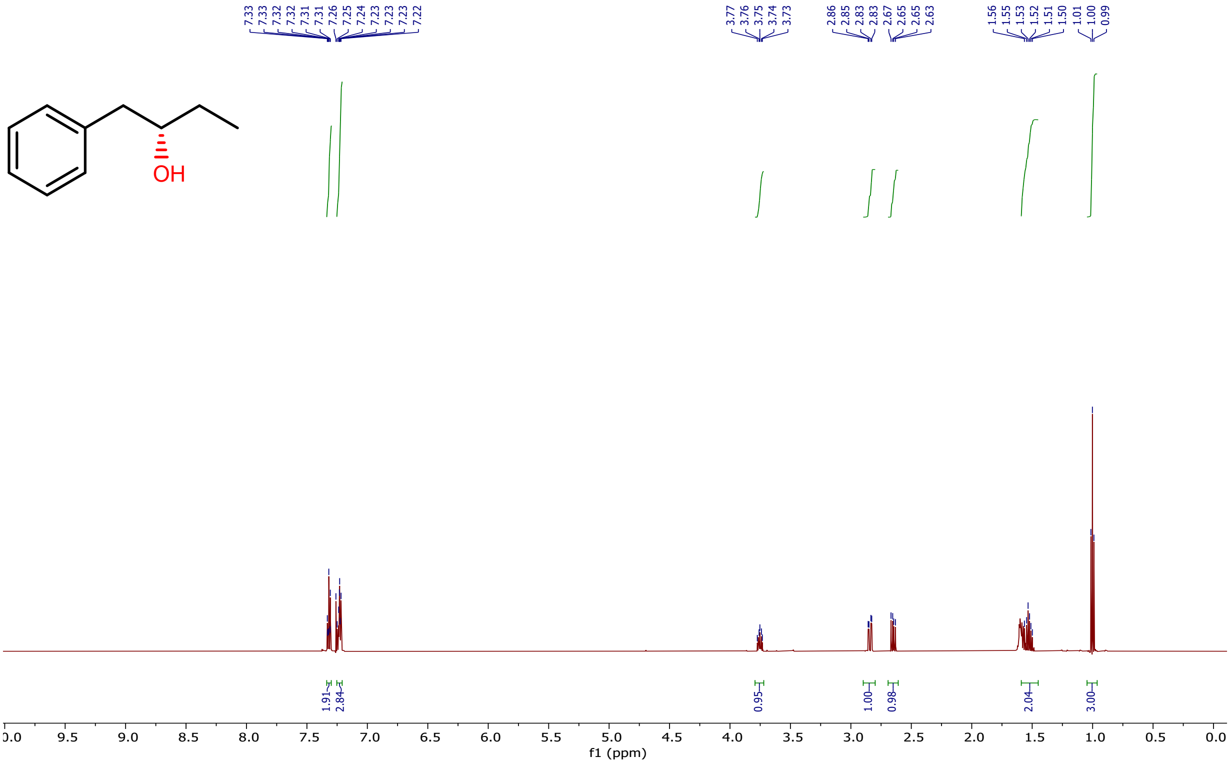
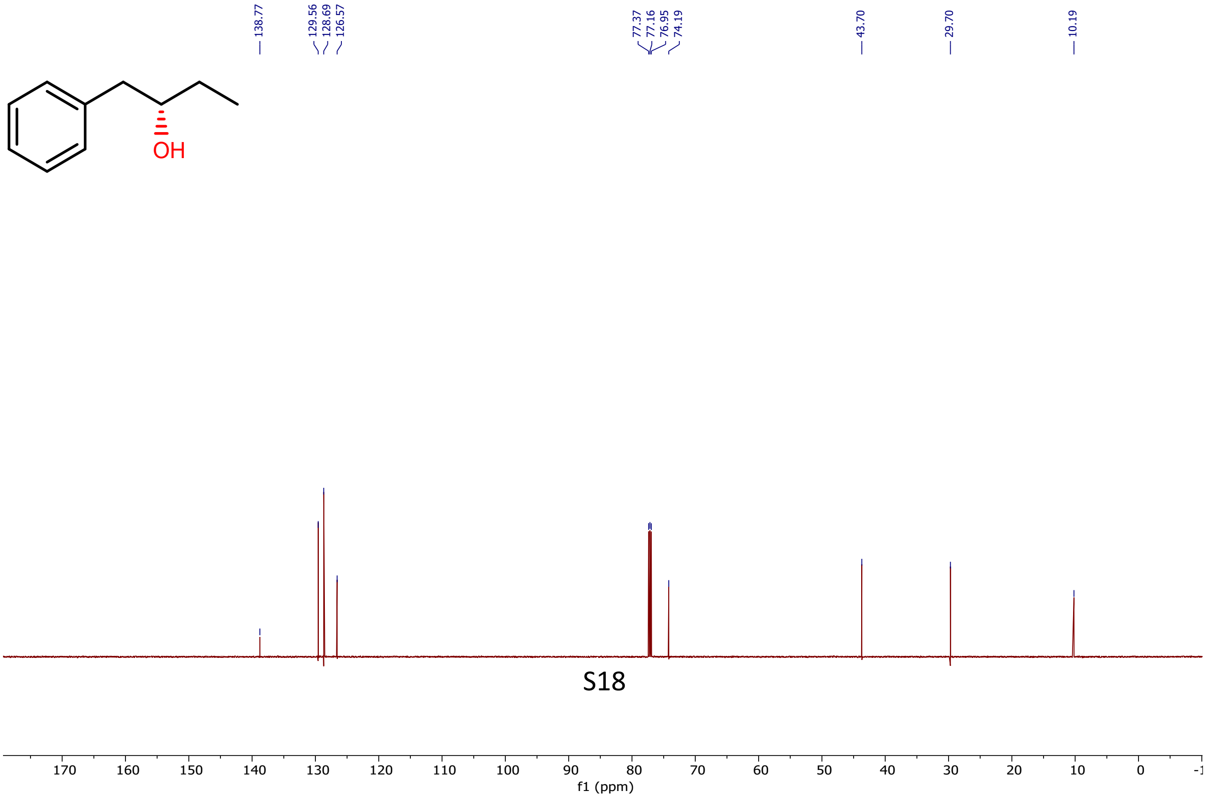


Figure S14. Gas chromatograms showing (a) the acetate derivative of (*rac*)-**4a**, (b) compound **4b** produced by *Aae*UPO-catalyzed oxidation of (*rac*)-**4a**, and the acetate derivatives obtained from the two-step one-pot deracemization process: (c) The oxidation by *Aae*UPO followed by a reduction using W110V *TeS*ADH produced (*S*)-**4a**. (d) while the variant ΔP84/A85G yielded (*R*)-**4a**.

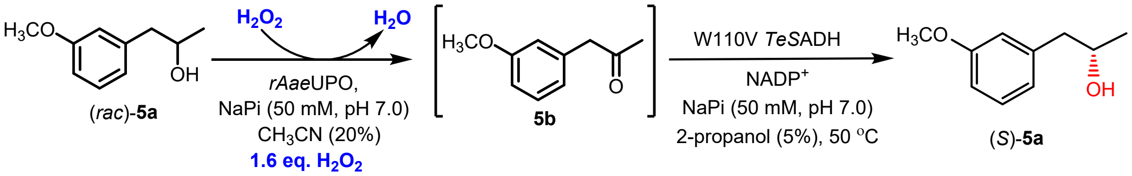
¹H NMR (600 MHz, Chloroform-*d*): spectrum of (*S*)-**4a** produced by a two-step one-pot deracemization process of (*rac*)-**4a**.



¹³C{¹H} NMR (151 MHz, Chloroform-*d*): spectrum of (*S*)-**4a** produced by a two-step one-pot deracemization process of (*rac*)-**4a.**



(*rac*)-1-(3'-Methoxyphenyl)-propan-2-ol [(*rac*)-5a]



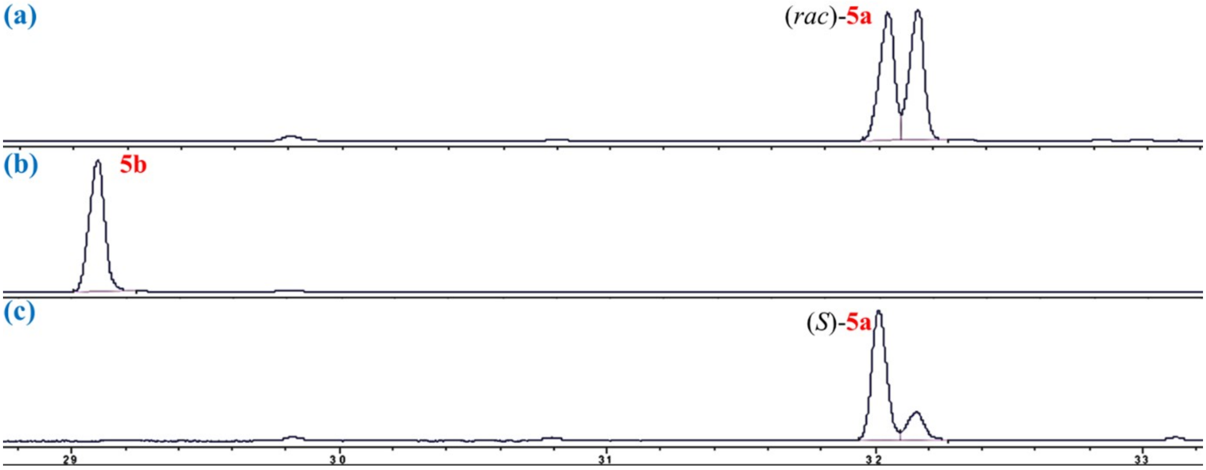
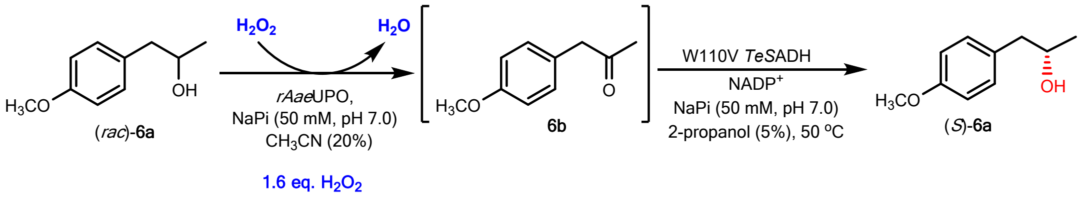


Figure S15. Gas chromatograms showing (a) the acetate derivative of (*rac*)-**5a**, (b) compound **5b** produced by *Aae*UPO-catalyzed oxidation of (*rac*)-**5a**, and the acetate derivatives obtained from the two-step one-pot deracemization process: (c) The oxidation by *Aae*UPO, followed by a reduction using W110V *TeS*ADH produced (*S*)-**5a**.

(*rac*)-1-(4'-Methoxyphenyl)-propan-2-ol [(*rac*)-6a]



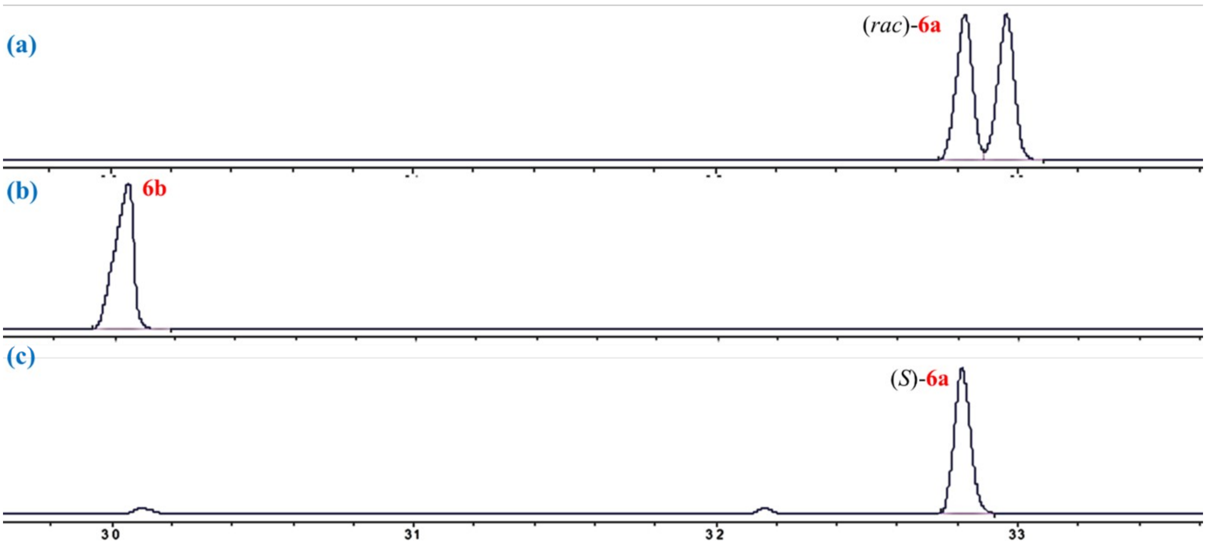
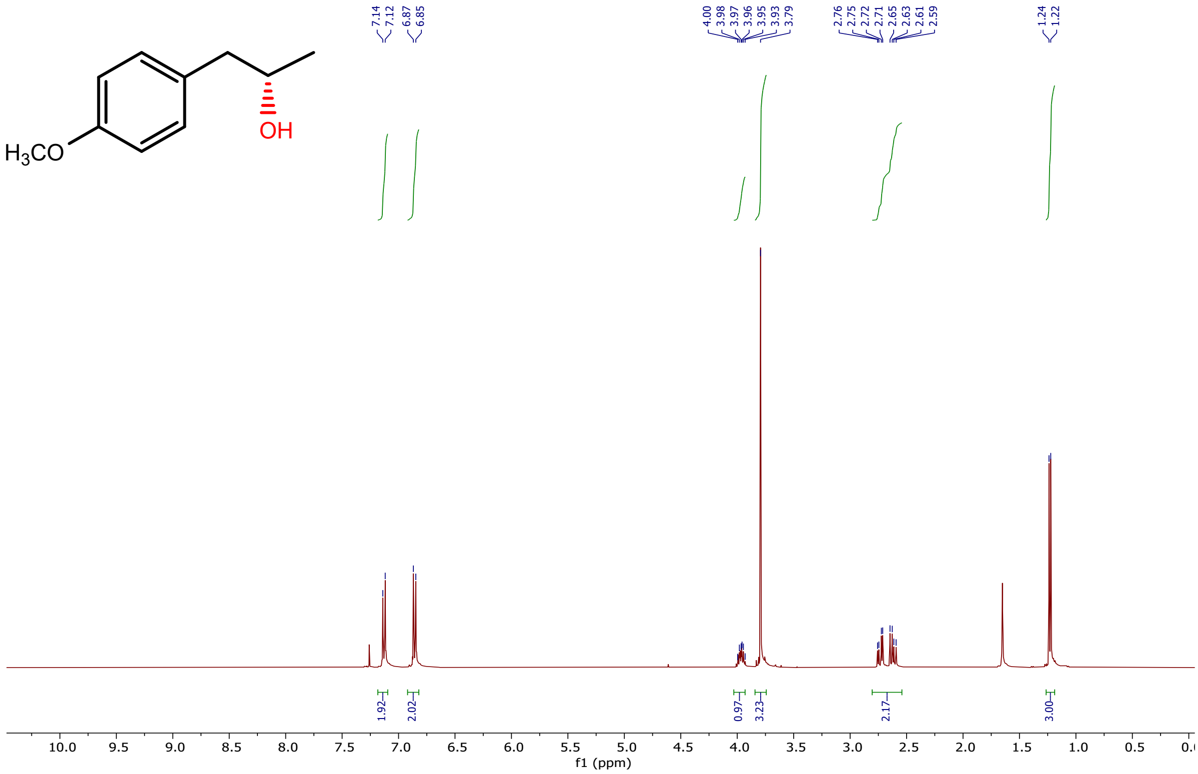
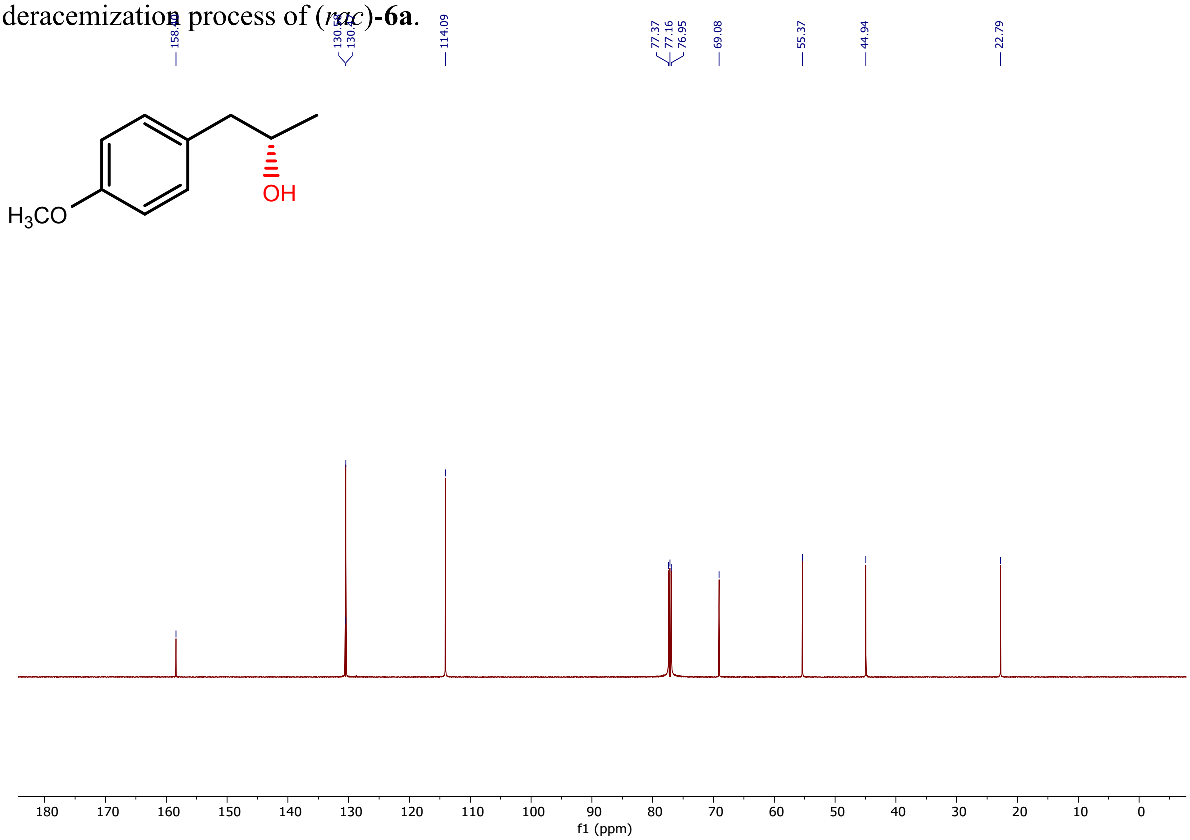


Figure S16. Gas chromatograms showing (a) the acetate derivative of (*rac*)-**6a**, (b) compound **6b** produced by *Aae*UPO-catalyzed oxidation of (*rac*)-**6a**, and the acetate derivatives obtained from the two-step one-pot deracemization process: (c) The oxidation by *Aae*UPO, followed by a reduction using W110V *TeS*ADH produced (*S*)-**6a**.

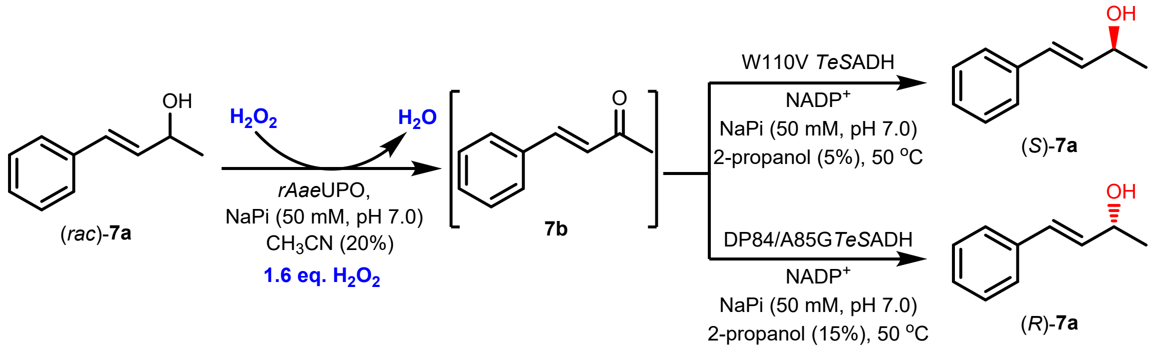
^1H NMR (600 MHz, Chloroform-*d*): spectrum of (*S*)-**6a**. Produced via a two-step, one-pot deracemization of (*rac*)-**6a**.



$^{13}C\{ ^1H \}$ NMR (151 MHz, Chloroform-*d*): spectrum of (*S*)-**6a** produced by a two-step one-pot deracemization process of (*rac*)-**6a**.



(*rac*)-(*E*)-4-Phenylbut-3-en-2-ol [(*rac*)-7a]



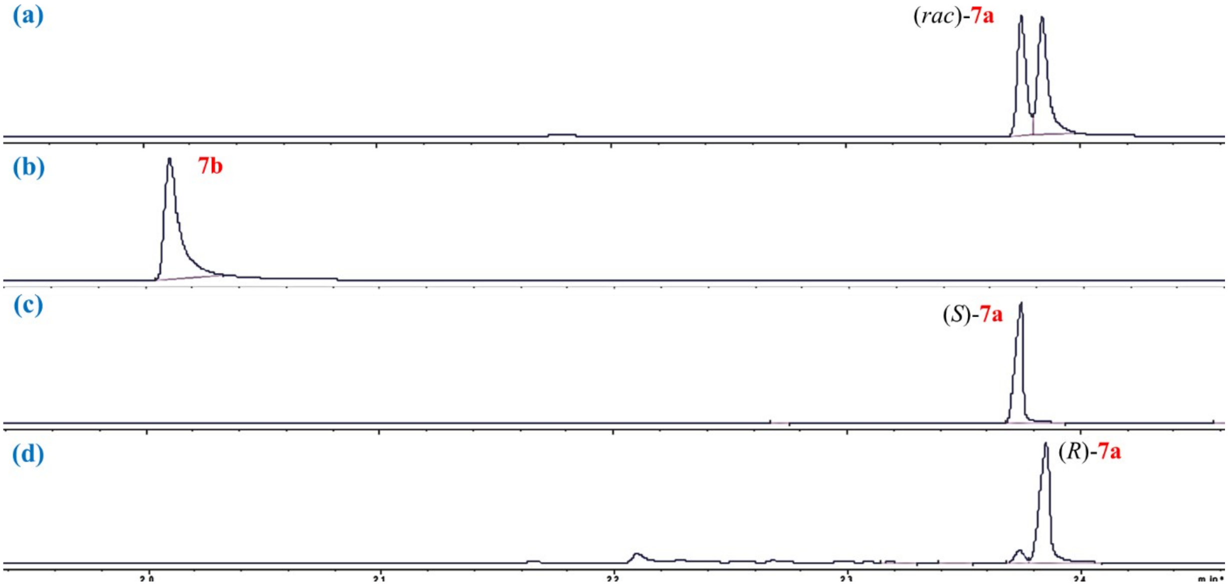
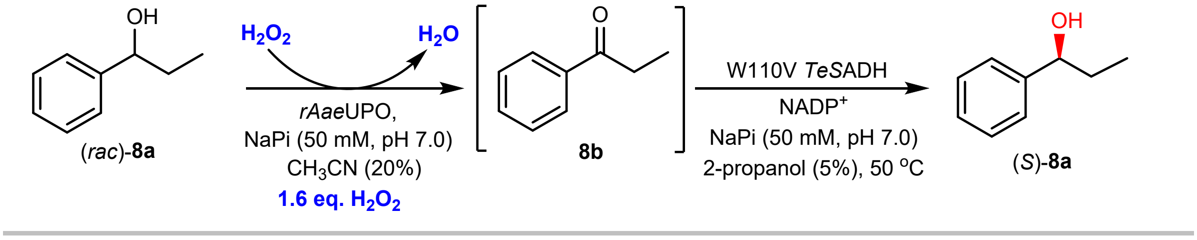


Figure S17. Gas chromatograms showing (a) the acetate derivative of (*rac*)-**7a**, (b) compound **7b** produced by *Aae*UPO-catalyzed oxidation of (*rac*)-**7a**, and the acetate derivatives obtained from the two-step one-pot deracemization process: (c) The oxidation by *Aae*UPO, followed by a reduction using W110V *TeS*ADH, produced (*S*)-**7a**.(d) while the variant ΔP84/A85G yielded (*R*)-**7a**.

(*rac*)-1-Phenylpropan-1-ol [(*rac*)-8a]



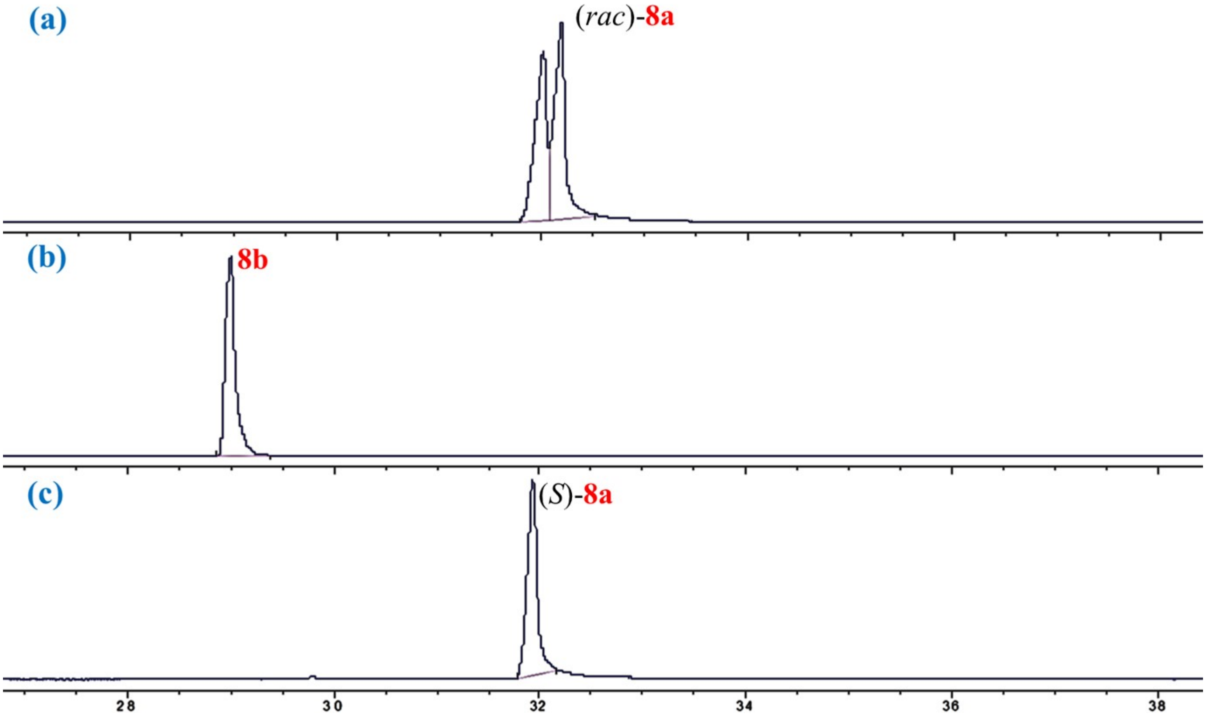
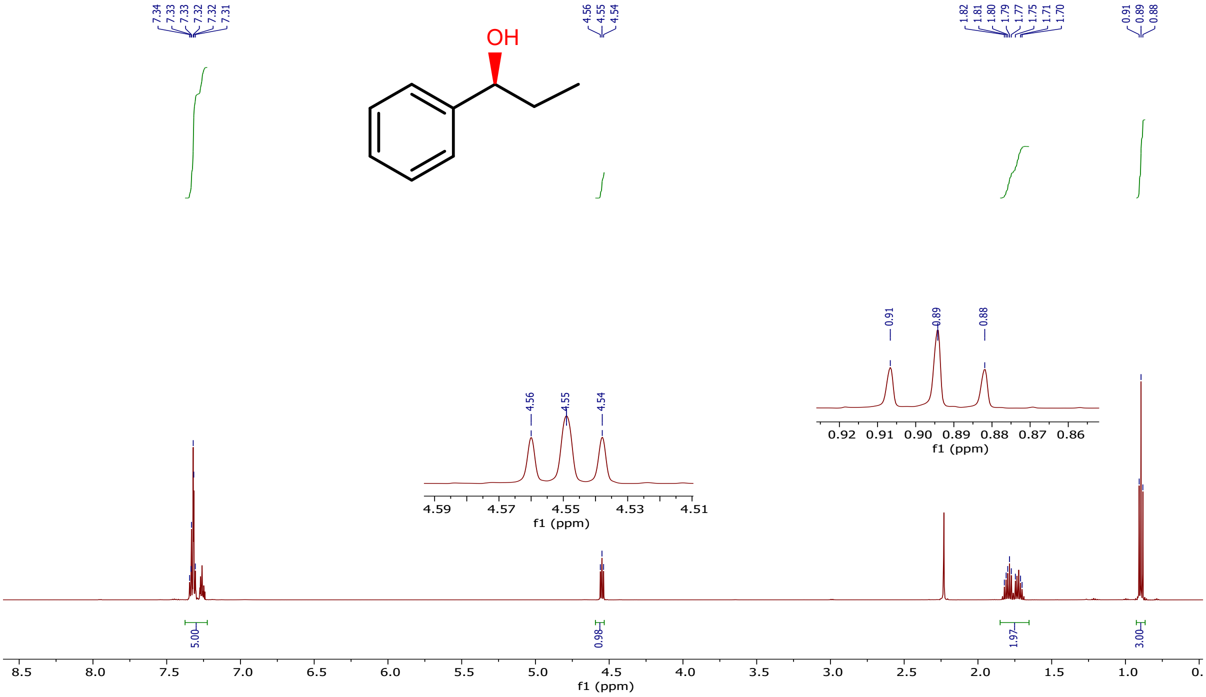
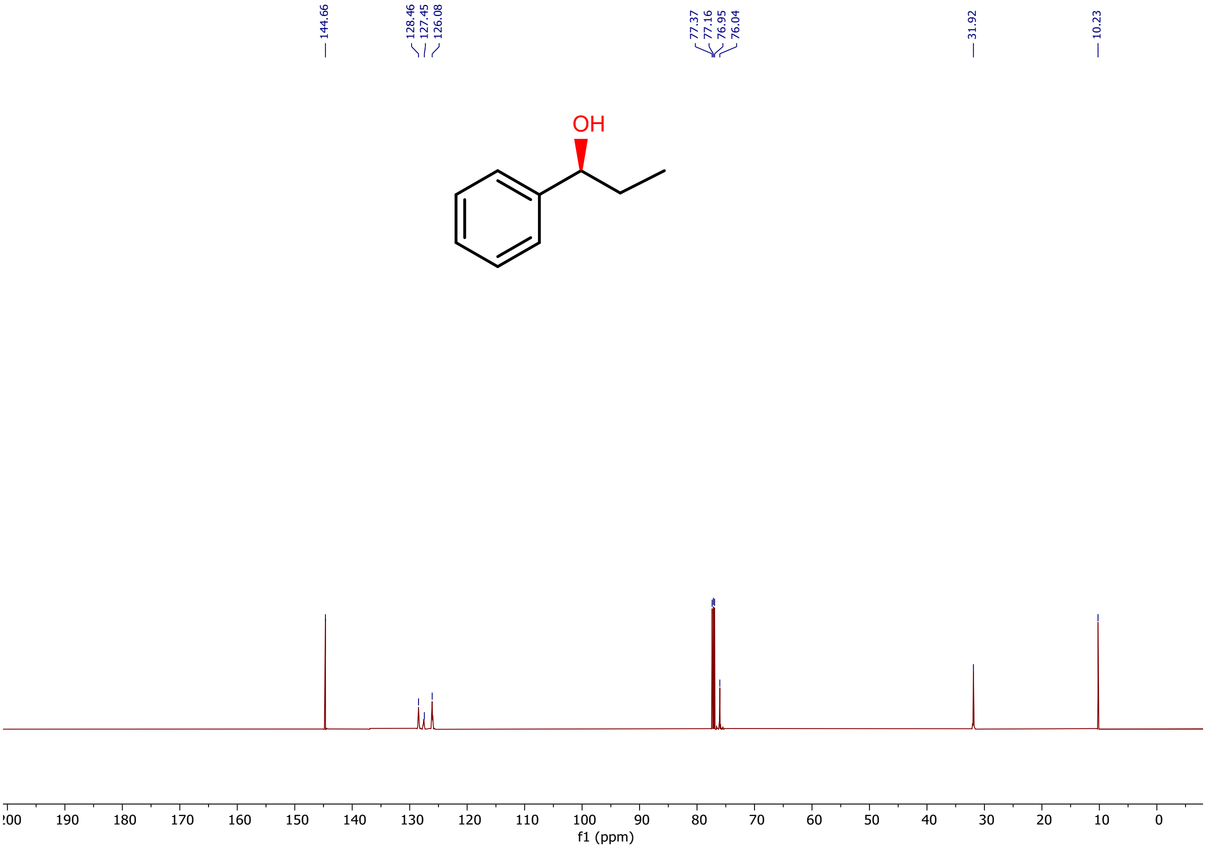


Figure S18. Gas chromatograms showing (a) the acetate derivative of (*rac*)-**8a**, (b) compound **8b** produced by *Aae*UPO-catalyzed oxidation of (*rac*)-**8a**, and the acetate derivatives obtained from the two-step one-pot deracemization process: The oxidation by *Aae*UPO, followed by a reduction using W110V *TeS*ADH, produced (*S*)-**8a** (c).

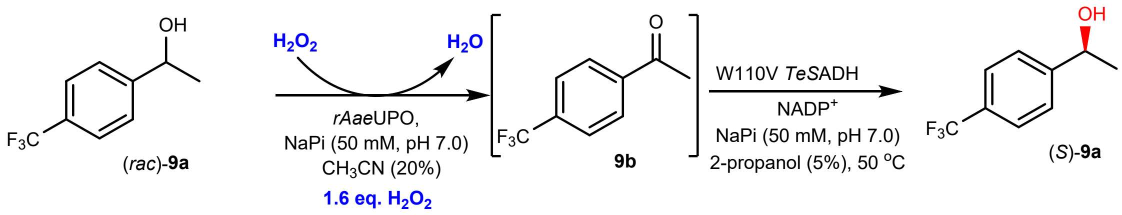
^{1}H NMR (600 MHz, Chloroform-*d*): spectrum of (*S*)-**8a** produced by a two-step one-pot deracemization process of (*rac*)-**8a**.



^{13}C {^{1}H} NMR (151 MHz, Chloroform-*d*) spectrum of (*S*)-**8a** obtained via a two-step, one-pot deracemization of (*rac*)-**8a**.



(*rac*)-1-(4′-trifluoromethylphenyl)-ethanol [(*rac*)-9a]



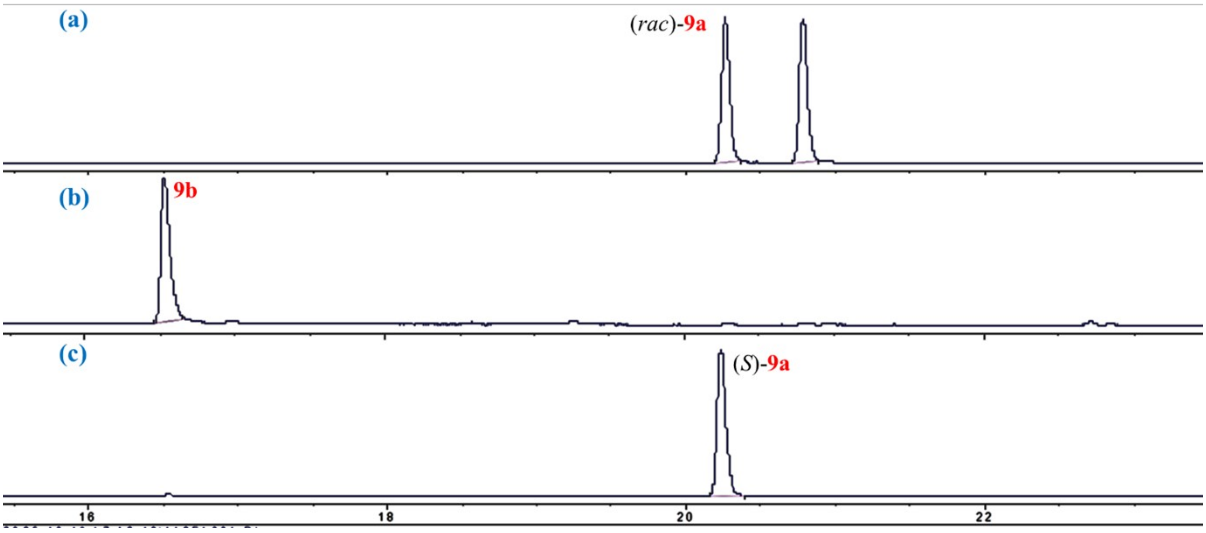
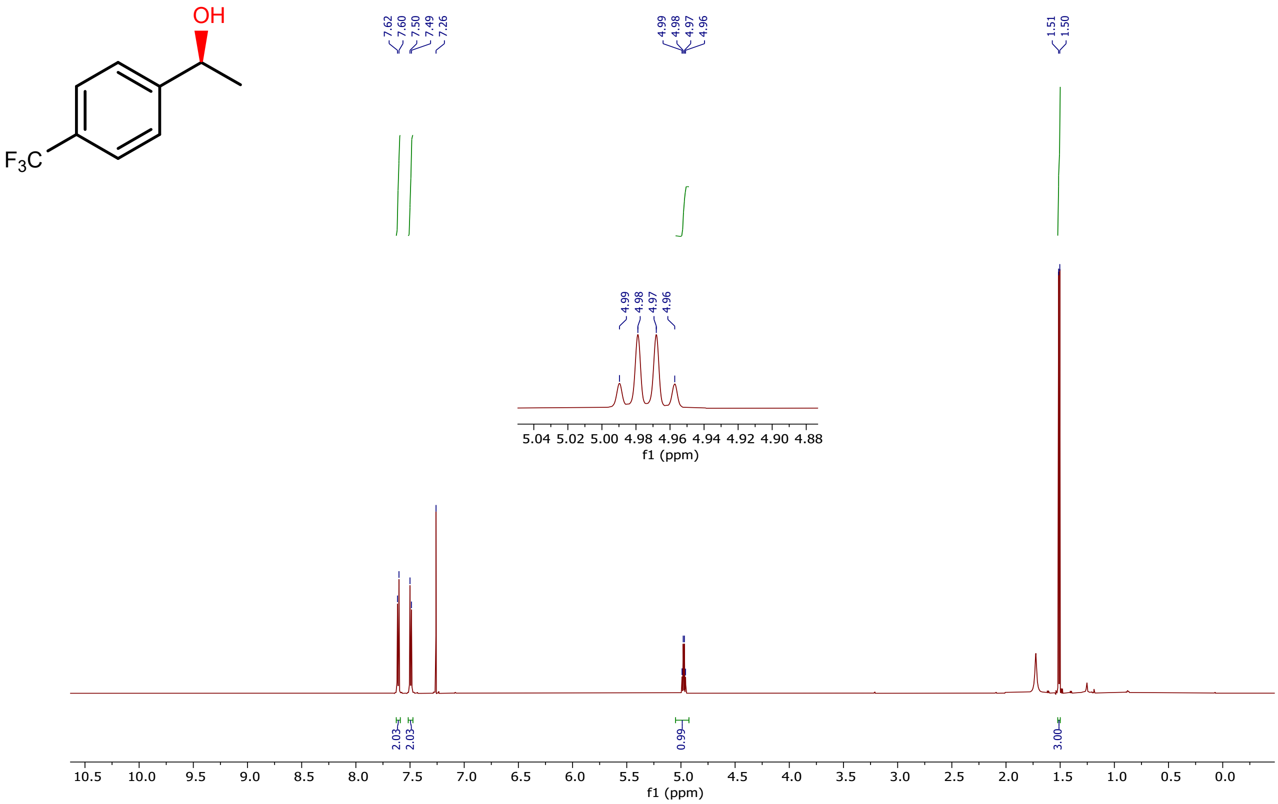
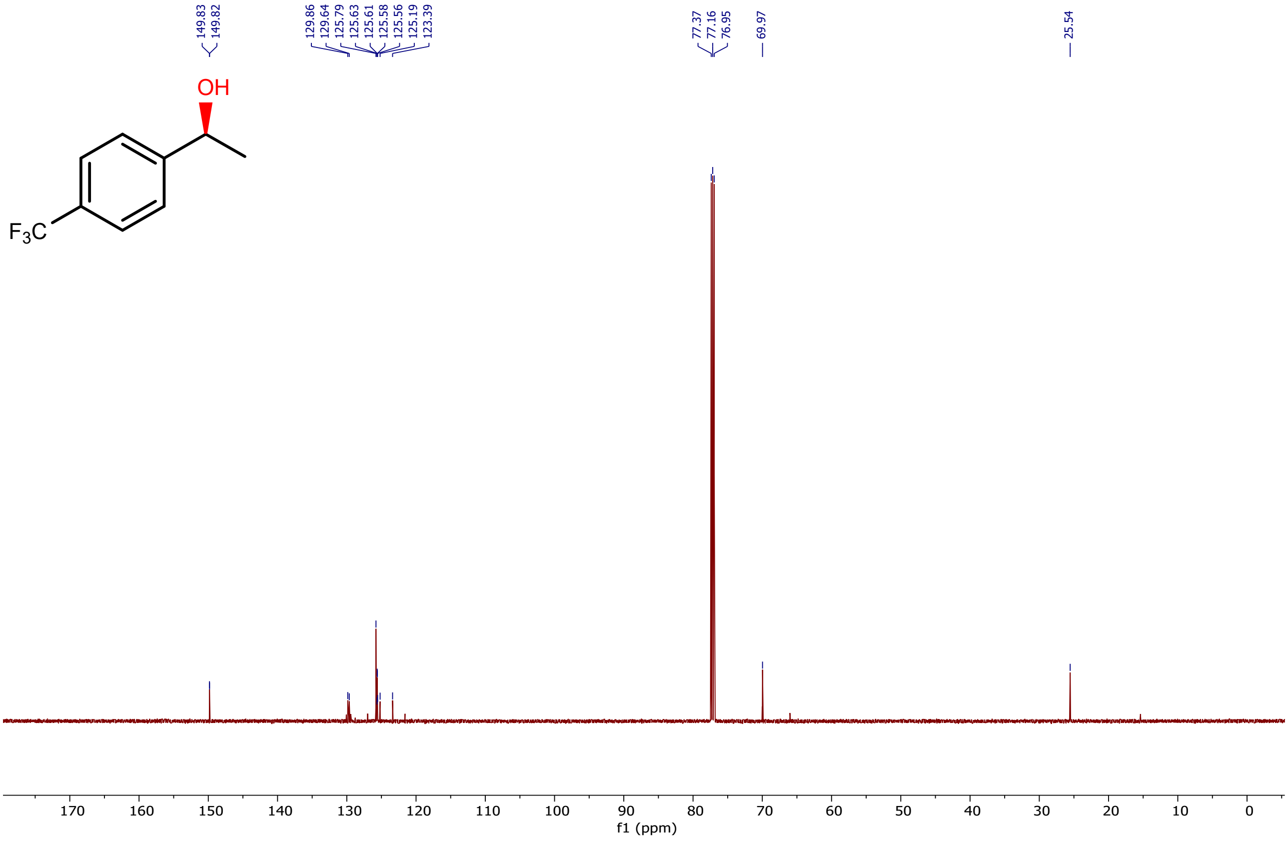


Figure S19. Gas chromatograms showing (a) the acetate derivative of (*rac*)-**9a**, (b) compound **9b** produced by *Aae*UPO-catalyzed oxidation of (*rac*)-**9a**, and the acetate derivatives obtained from the two-step one-pot deracemization process**:** The oxidation by *Aae*UPO, followed by a reduction using W110V *TeS*ADH produced (***S***)-**9a** (c).

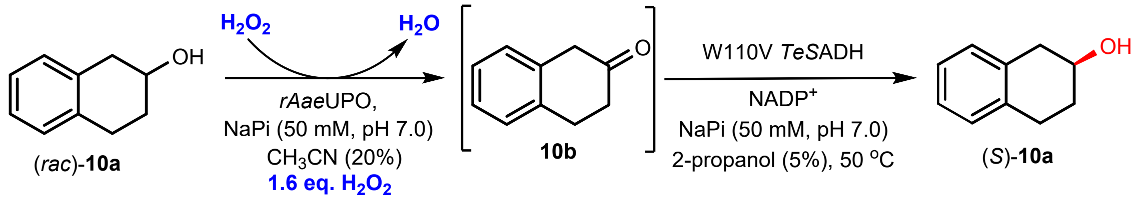
^{1}H NMR (600 MHz, Chloroform-*d*): spectrum of (*S*)-**9a**. Produced via a two-step, one-pot deracemization of (*rac*)-**9a**.



^{13}C {^{1}H} NMR (151 MHz, Chloroform-*d*): spectrum of (*S*)-**9a**. Produced via a two-step, one-pot deracemization of (*rac*)-**9a**.



(*rac*)-2-Tetralol [(*rac*)-10a]



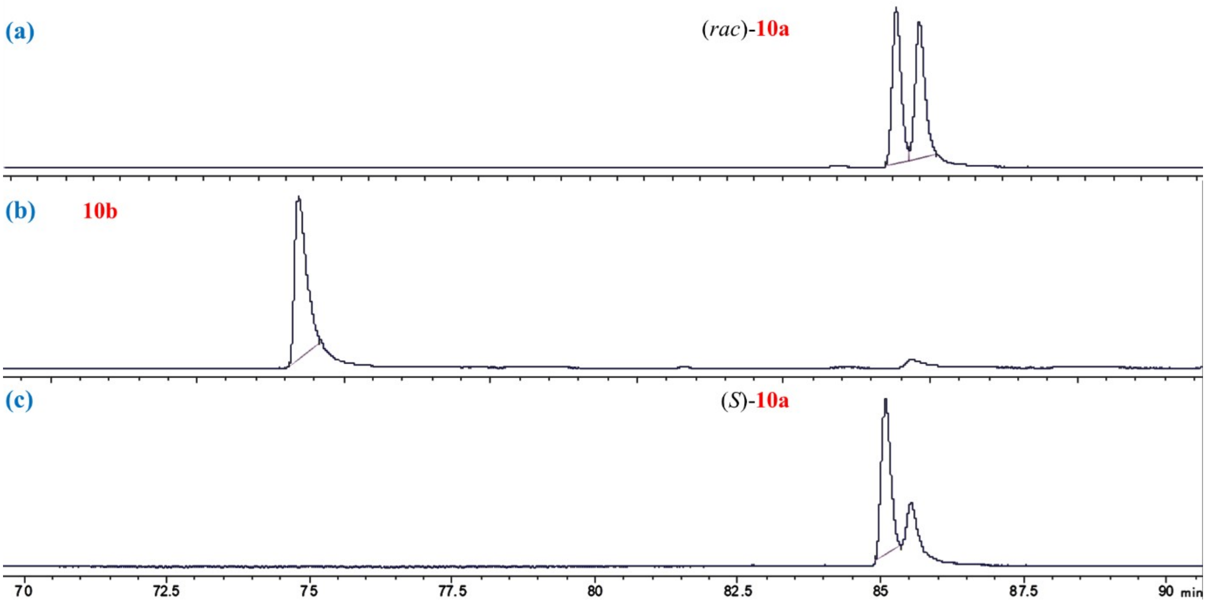


Figure S20. Gas chromatograms showing (a) the acetate derivative of (*rac*)-**10a**, (b) compound **10b** produced by *Aae*UPO-catalyzed oxidation of (*rac*)-**10a**, and the acetate derivatives obtained from the two-step one-pot deracemization process**:** The oxidation by *Aae*UPO, followed by a reduction using W110V *TeS*ADH produced (***S***)-**10a** (c).

5. References

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(3.) M. Li, B. Li, H.-F. Xia, D. Ye, J. Wu and Y. Shi, *Green Chem.*, 2014, **16**, 2680–2688.