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Electronic Supporting Information

Enzyme-catalysed Enantioselective Oxidation of Alcohols by Air Exploiting Fast Electrochemical Nicotinamide Cycling in Electrode Nanopores

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Content

Chemicals

(rac)-4-phenyl-2-butanol (Alfa Aesar, 98%), *(R)*-4-phenyl-2-butanol (ChemCruz, 98%, ee $\geq$ 98%), *(S)*-4-phenyl-2-butanol (Alfa Aesar, 98%, ee ≥ 97%), 4-phenyl-2-butanone (Sigma-Aldrich, 98%), $NADP^+$ (monosodium salt, Sigma-Aldrich, 98%), NADPH (tetrasodium salt, Sigma-Aldrich, 93%), Indium Tin oxide nanopowder (ITO, Sigma-Aldrich), [tris(hydroxymethyl)methylamino]propanesulfonic acid (TAPS, Melford, 99%), acetone (ACS Reagent), 2-(*N*-morpholino)ethanesulfonic acid (MES, Melford, 99%), N-Cyclohexyl-2-aminoethanesulfonic acid (CHES, Melford, 99%), 3-(N-morpholino)propanesulfonic acid (MOPS, Melford, 99%), 3-(Trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (TMSP, Alfa Aesar, 98%) were used without further purification.

Experimental Methods

Purification of Ferredoxin $NADP^+$-Reductase (FNR)

Purification of the His-tagged FNR used in this paper was carried out as described previously.[1]

Purification of the secondary alcohol dehydrogenase TeSADH W110A

The vector pET28 containing the gene for the alcohol dehydrogenase from Thermoanerobacter ethanolicus with mutation W110A (TeSADH W110A) was transformed into *E. coli* BL21 (DE3) (New England Biolabs) following the manufacturer's protocol. Colonies were cultivated on LB agar containing kanamycin at 50 μg mL^{-1}. A single colony was used to innoculate 5 mL LB media containing kanamycin at 50 μg mL^{-1} and grown overnight at 37 °C, 250 rpm. This was used to innoculate 600 mL TB autoinduction media (Formedium) containing kanamycin at 50 μg mL^{-1} in a 2 L flask and left to grow for 16 h at 37 °C, 250 rpm. Cells were harvested by centrifugation (4000 rpm, 20 mins) and resuspended in ~ 20 mL buffer A (buffer A = 100 mM potassium phosphate, pH 7.7 + 300 mM NaCl + 20 mM imidazole). 0.1 mg mL^{-1} Lysozyme from chicken egg white (Sigma-Aldrich) was added and cells left to lyse at 30 °C, 250 rpm for 30 mins. Cells were further lysed by sonication (20 s pulse on, 20 s pulse off x 20 cycles). The suspension was

centrifuged to remove insoluble components (18000 rpm, 20 mins) and the supernatant was passed through a 0.45 µm filter. The supernatant was loaded onto a 1 mL HisTrap FF column (GE Healthcare) previously equilibrated with 10 cv buffer A. The column was then washed with 10 cv buffer A and the enzyme eluted with a gradient 0-100% buffer B (buffer B = 100 mM potassium phosphate, pH 7.7 + 300 mM NaCl + 1 M imidazole) over 10 cv. Fractions containing TeSADH were collected and dialysed overnight at 4 °C into 50 mM Tris buffer, pH 9 followed by concentration using a 10 kDa MWCO spin column (GE Healthcare) to >10 mg mL^{-1} and stored at −80 °C until use.

Electrochemical measurements

All electrochemical measurements were made using a Multi-Channel Palmsens potentiostat. For cyclic voltammetry (CV), the reference and counter electrodes were a calibrated saturated calomel electrode (SCE) and Pt wire respectively. Potentials were adjusted to the standard hydrogen electrode (SHE) scale using $E_{SHE} = E_{SCE} + 0.241$ V at 25 °C. For reversible hydrogen electrode (RHE), the relationship between E_{RHE} and E_{SCE} is $E_{RHE} = E_{SCE} + 0.241\ V + 0.059 \times pH$. No iR correction was used in all experimental measurements. Experiments under hydrodynamic control were carried out with a rotating disc pyrolytic graphite 'edge' (PGE) electrode of area 0.08 cm^2. For the air-driven enzyme-catalyzed electrosynthesis experiments, the FNR@ITO/Ti anode was connected to the Pt/carbon cathode *via* the potentiostat and the current was monitored at 0 additional applied voltage. The total charge passed for the electrosynthesis was determined by integration using the 0 value as the baseline. The compressed air flow rate was 2.6 scc min^{-1}, controlled by a mass flow controller (MFC, Sierra Instruments). For the H_2-driven electrosynthesis experiments, the experimental conditions are available in Wan *et al*.[2]

Fabrication of a platinized carbon paper (PCP) cathode

See Wan *et al*. for detailed electrodeposition process.[2]

Fabrication of the reactor of an air-driven electrochemical cell

The reactor consists of two components which are thereafter referred to as the main compartment and the PCP-containing compartment. The main compartment was a 6.5 cm long, 1.7 cm diameter glass vial into which the FNR@ITO/Ti anode was located and reactions other than O_2 reduction take place. The PCP-containing compartment was a 0.7 cm diameter glass tube within which the PCP was housed for the O_2 reduction reaction. On the bottom of this tube a piece of Nafion N115 proton exchange membrane (PEM, Ion Power) was fixed tightly using epoxy (Loctite EA9466). Figure S1 shows the completed reactor.

UV-VIS spectrophotometric analysis

These experiments were carried out using a Perkin Elmer UV/VIS/NIR Spectrometer (Lambda 19) to determine the characteristic adsorption of NADPH at 340 nm. The concentration of NADPH was obtained using Beer-Lambert Law. The mode of the spectrometer was set at 'Timedrive' as a curve corresponding to the NADPH signal was continuously monitored. To obtain the catalytic rate of the reaction '4-Phenyl-2-butanol + $NADP^+$ → 4-Phenyl-2-butanone + NADPH + H^+' (in which the alcohol can either be S or R enantiomer), the slope of the curve corresponding to such a value was calculated through curve fitting.

^{1}H NMR analysis

General procedure

Each NMR sample consisted of cell solution and D_2O in a 90:10 ratio. The ^{1}H spectra with water suppression (in unlocked mode) were recorded using a Bruker AVIIIHD 400 instrument. The concentrations determined from ^{1}H NMR were calculated by comparing the ratio between the characteristic peak area of the chemical of interest and that from a standard solution of known concentration. In our case, the standard solution for calibration and quantification was 3-(Trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (TMSP) whose chemical shift (δ) in the ^{1}H spectra is around 0 ppm, with 9 H. TMSP was sealed inside a fully watertight caterpillar that was placed on the bottom of the NMR tube when measuring. The effective concentration of TMSP is 1.24 mM. The formula for calculating the product is therefore: A/numbers of H corresponding to

the peak)/(B/9/1.24) (A = peak area of a characteristic peak of the solution in question, B = peak area of TMSP at δ 0.0). The unit of concentration for this calculation is mM. It should be noted that for ^{1}H NMR using an internal standard under unlocked mode, the chemical shift for TMSP sometimes will shift by around ±0.1. This shift will apply to all peaks in those spectra.

^{1}H NMR analysis for 4-phenyl-2-butanol

The peak corresponding to 4-phenyl-2-butanol and not convoluted with TAPS buffer, TMSP, and 4-phenyl-2-butanone is located at δ 1.18 (m, 3H). The formula for quantifying 4-phenyl-2-butanol (4P2B) is thus A/3/(B/9/1.24) (A = peak area of 4P2B at δ 1.18 (m, 3H), B = peak area of TMSP around δ 0.0 (m, 9H)). A ^{1}H NMR spectrum of pure 4-phenyl-2-butanol in TAPS buffer (pH =9.0, 0.2 M) using TMSP as internal standard is shown in Figure S3.

^{1}H NMR analysis for 4-phenyl-2-butanone

The peak corresponding to 4-phenyl-2-butanone and not convoluted with TAPS buffer, TMSP, and 4-phenyl-2-butanol is located at δ 2.18 (m, 3H). The formula for quantifying 4-phenyl-2-butanone is thus A/3/(B/9/1.24) (A = peak area of 4-phenyl-2-butanone at δ 2.18 (m, 3H), B = peak area of TMSP around δ 0.0 (m, 9H)). A ^{1}H NMR spectrum of pure 4-phenyl-2-butanone in TAPS buffer (pH =9.0, 0.2 M) using TMSP as internal standard is shown in Figure S4.

GC-FID analysis

Conversions and enatiomeric excesses (ee) were analyzed by GC-FID using a GC 6850 instrument fitted with an autosampler (Agilent) and a chiral BetaDex™ 325 column (30 m x 0.25 mm x 0.25 µm) (Supelco). GC program: inlet temperature 200 °C, detector temperature 250 °C, oven 110 °C/hold 20 mins; 10 °C/min to 200 °C. R_t (4-phenyl-2-butanone) = 20.8 minutes, R_t (*(R)*-4-phenyl-2-butanol) = 23.6 minutes, R_t (*(S)*-4-phenyl-2-butanol) = 23.4 minutes.

Figures S1–S3 (Excluding those for ^{1}NMR and GC-FID analyses)

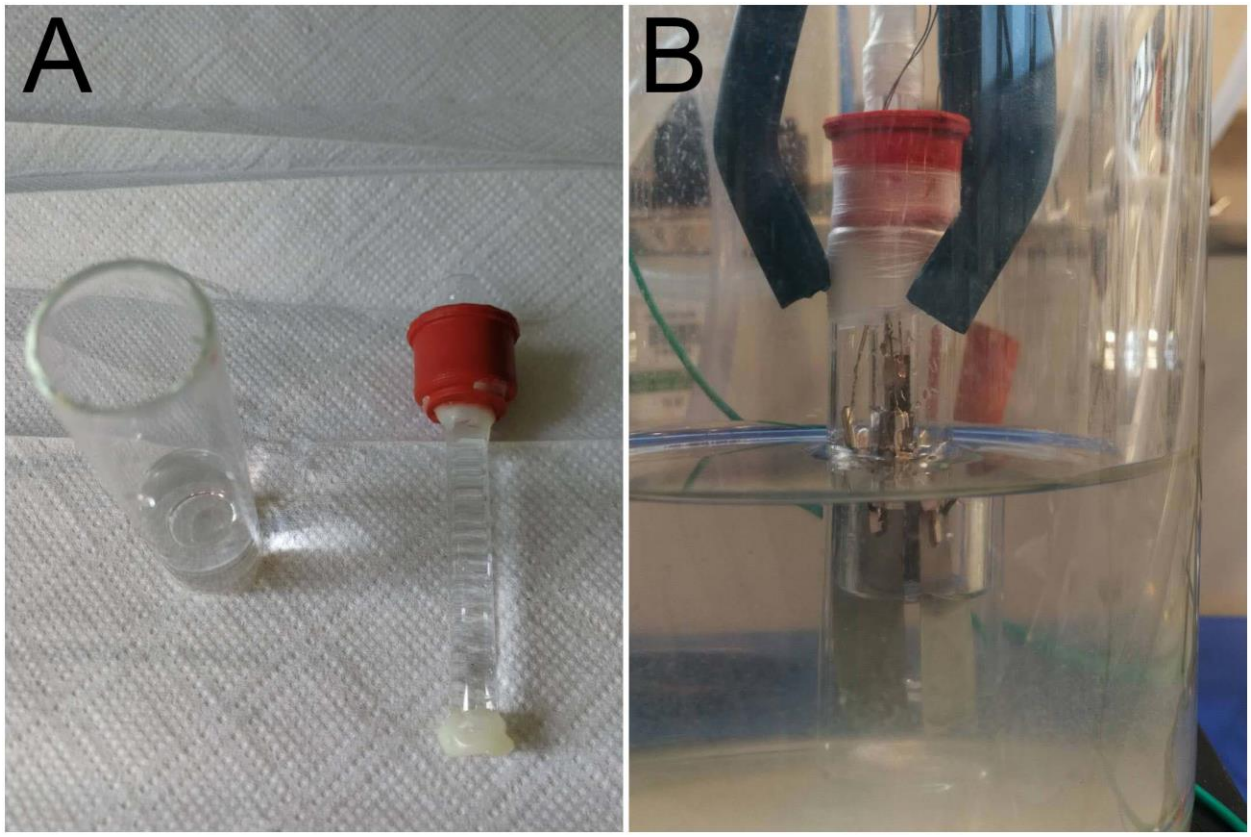


Figure S1. (A) The glass vial shown left is the main compartment and the smaller one shown right is the PCP-containing compartment; **(B)** Assembly of the air-driven bioorganic synthesis fuel cell. Inside the main compartment, two pieces of FNR@ITO/Ti electrodes (7.2 cm^2) flank the PCP-containing compartment and perform NADPH oxidation, while the PCP electrode in the Pt compartment catalyzes O_2 reduction. The cell solution in the main compartment is stirred using a magnetic stirrer.

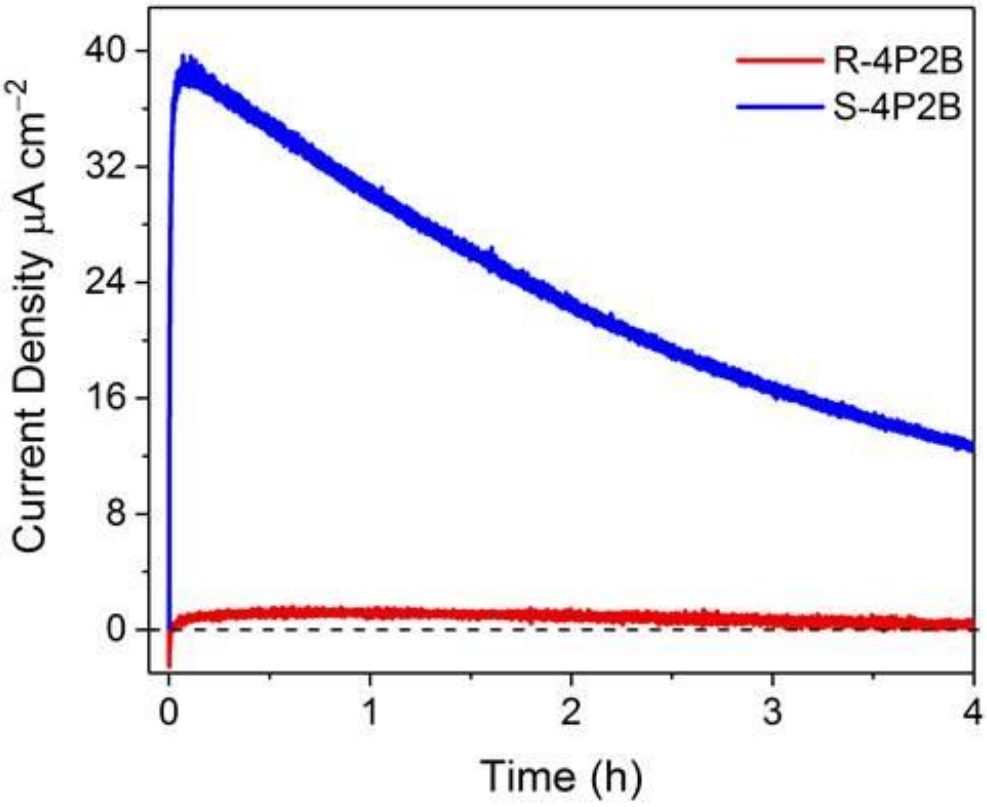


Figure S2. Chronoamperograms showing oxidations of (*S*)- and (*R*)-4-phenyl-2-butanol (4P2B). A 3 μL mixture of FNR and ADH of ratio 22:1 (858 μM and 38.2 μM) was dropcast for 3 min. on the electrode whch was then rinsed with MQ water. Experimental conditions: 20 °C, [$NADP^+$] = 5 μM, [(*S*)-4P2B] = 10 mM, [(*R*)-4P2B] = 10 mM, 1000 rpm, pH = 9.0, buffer = 50 mM MOPS + 50 mM TAPS + 50 mM CHES, applied potential = −0.175 V vs SCE.

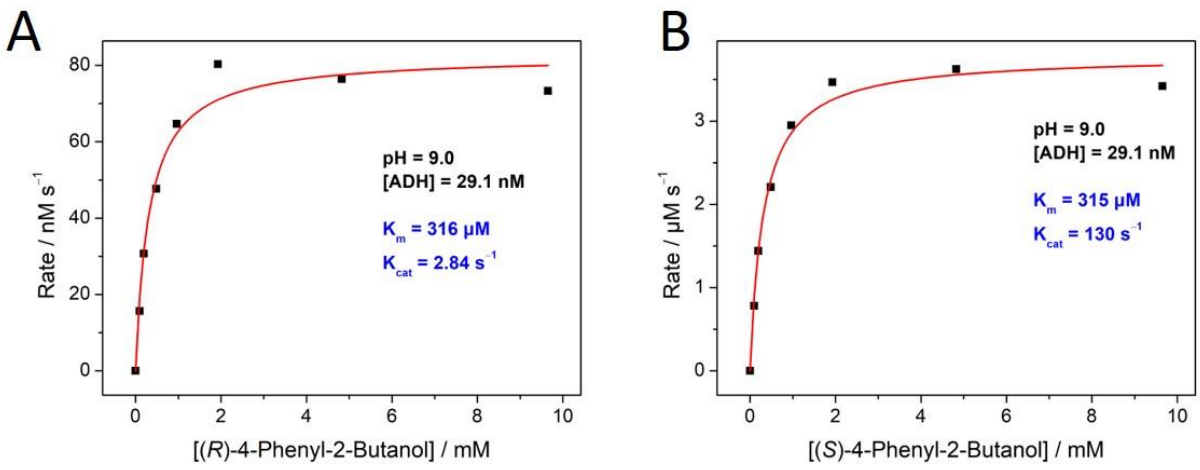


Figure S3. Michaelis-Menten plots for the reactions (A) (*R*)-4-Phenyl-2-butanol + $NADP^+$ → 4-Phenyl-2-butanone + NADPH + H^+ and (B) (*S*)-4-Phenyl-2-butanol + $NADP^+$ → 4-Phenyl-2-butanone + NADPH + H^+. The results were obtained by steady-state solution kinetics using UV-VIS spectrophotometry at 340 nm. Experimental conditions: pH = 9.0, [ADH] = 29.1 nM, temperature = 25 ± 2 °C, $[NADP^+]_{init}$ = 300 µM. The kinetic parameters to calculate enantiomeric ratio E = $(k^S_{\mathrm{cat}}/K^S_{\mathrm{m}})/(k^R_{\mathrm{cat}}/K^R_{\mathrm{m}})$ were obtained using curve fitting. The resultant parameters are k^R_{cat} = 2.84 s^{-1}, k^S_{cat} = 130s^{-1}, K^R_m = 316 µM, K^S_m = 315 µM, respectively.

Figures S4–S8 showing ^{1}H NMR spectra

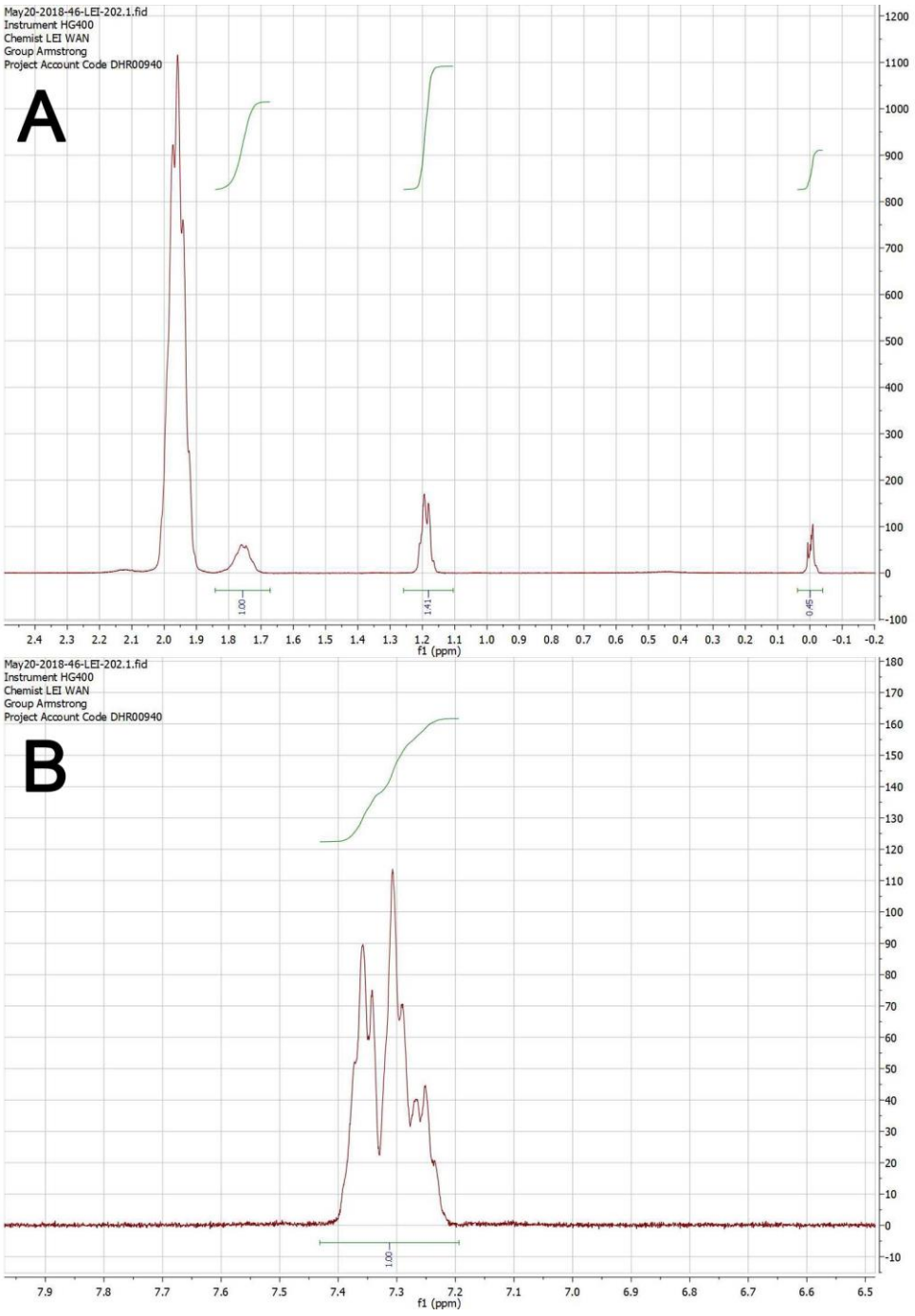


Figure S4. ^{1}H NMR spectrum of 4-phenyl-2-butanol (11.6 mM) in TAPS (pH 9.0, 0.2 M) buffer using TMSP (1.24 mM) as internal standard. The peaks listed as follows are those not convoluted with peaks for TAPS buffer. **A)** δ 1.18 (m, 3H), 1.75 (m, 2H). **B)** δ 7.3 (m, 5H).

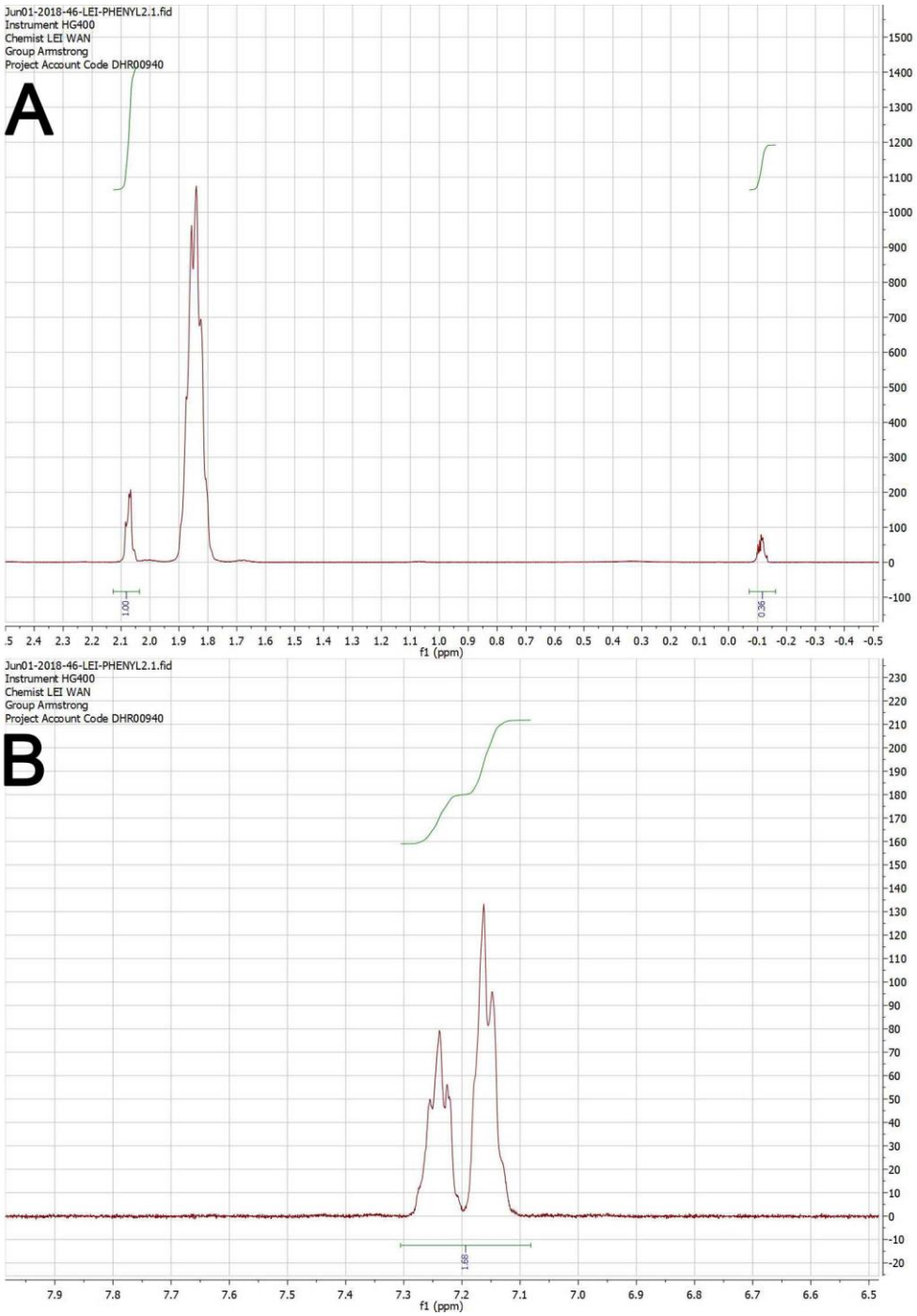


Figure S5 ^{1}H NMR spectrum of 4-phenyl-2-butanone (10.2 mM) in TAPS (pH 9.0, 0.2 M) buffer using TMSP (1.24 mM) as internal standard. The peaks listed as follows are those not convoluted with peaks for TAPS buffer. **A)** δ 2.08 (m, 3H). **B)** δ 7.2 (m, 5H). (Because δ shifts somewhat for TMSP in this spectrum, all peaks involved shift accordingly by approximately –0.1.)

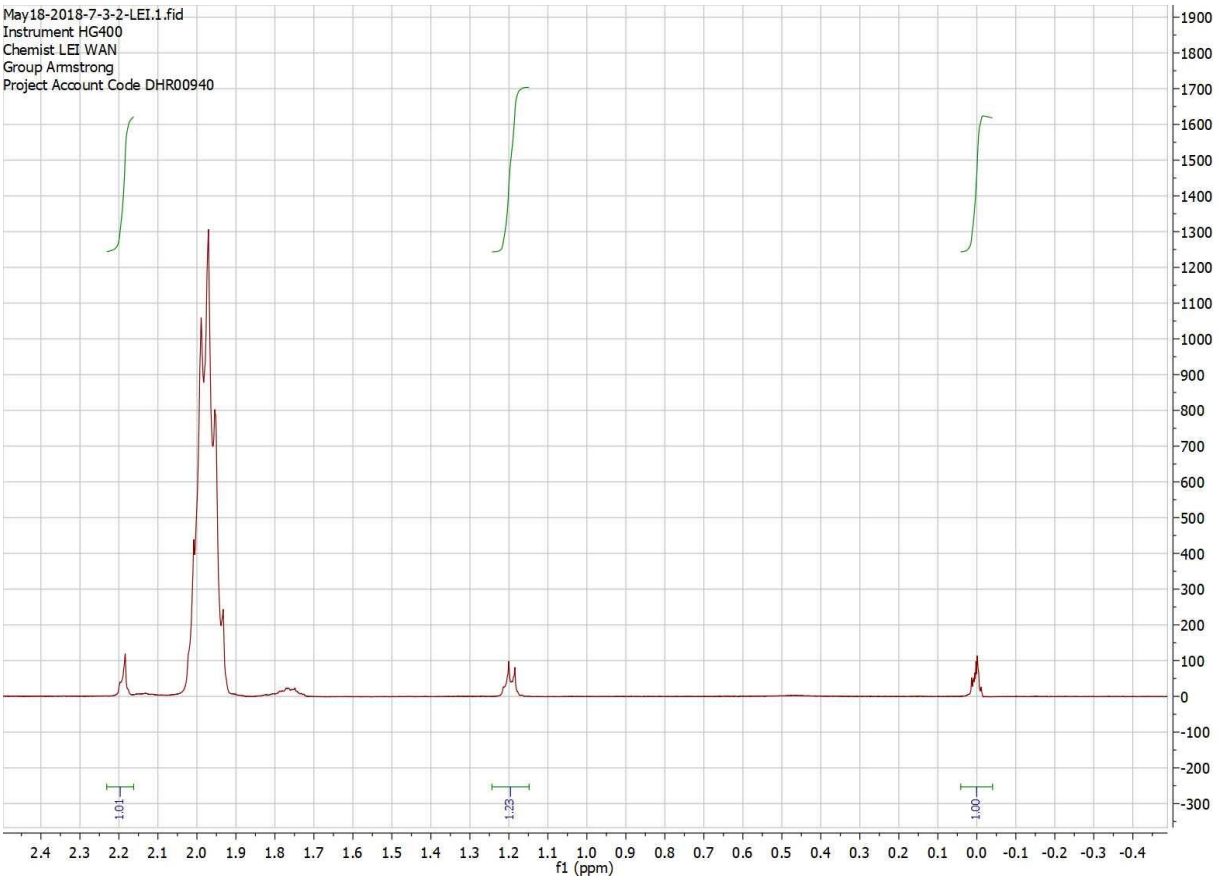


Figure S6 ^{1}H NMR spectrum of the cell solution for entry A in Tables 1 and S1. The peak at δ 1.18 (m, 3H) corresponds to 4-phenyl-2-butanol and the peak at δ 2.18 (m, 3H) corresponds to 4-phenyl-2-butanone.

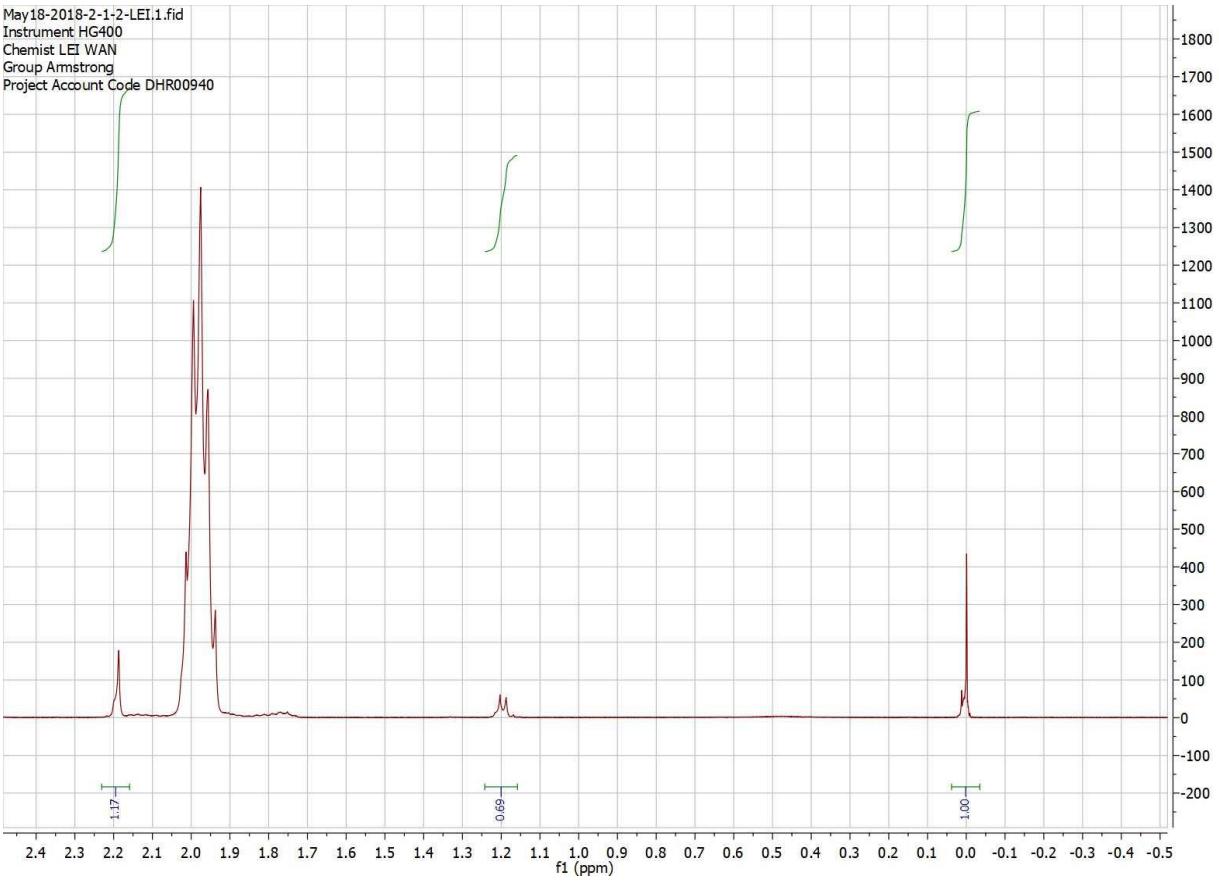


Figure S7. ^{1}H NMR spectrum of the cell solution for entry B in Tables 1 and S1. The peak at δ 1.18 (m, 3H) corresponds to 4-phenyl-2-butanol and the peak at δ 2.18 (m, 3H) corresponds to 4-phenyl-2-butanone.

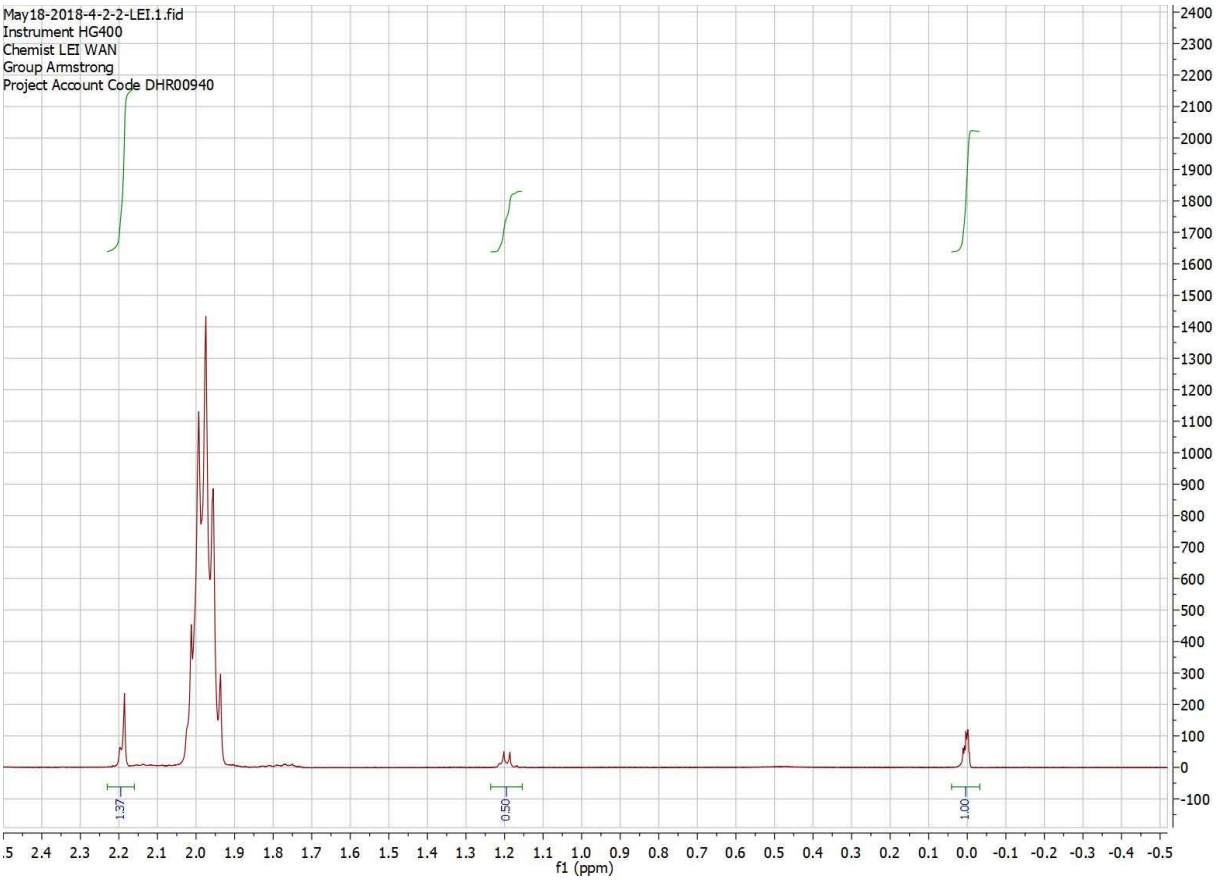


Figure S8. ^{1}H NMR spectrum of the cell solution for entry C in Tables 1 and S1. The peak at δ 1.18 (m, 3H) corresponds to 4-phenyl-2-butanol and the peak at δ 2.18 (m, 3H) corresponds to 4-phenyl-2-butanone.

Figures S9–S12 showing GC-FID results

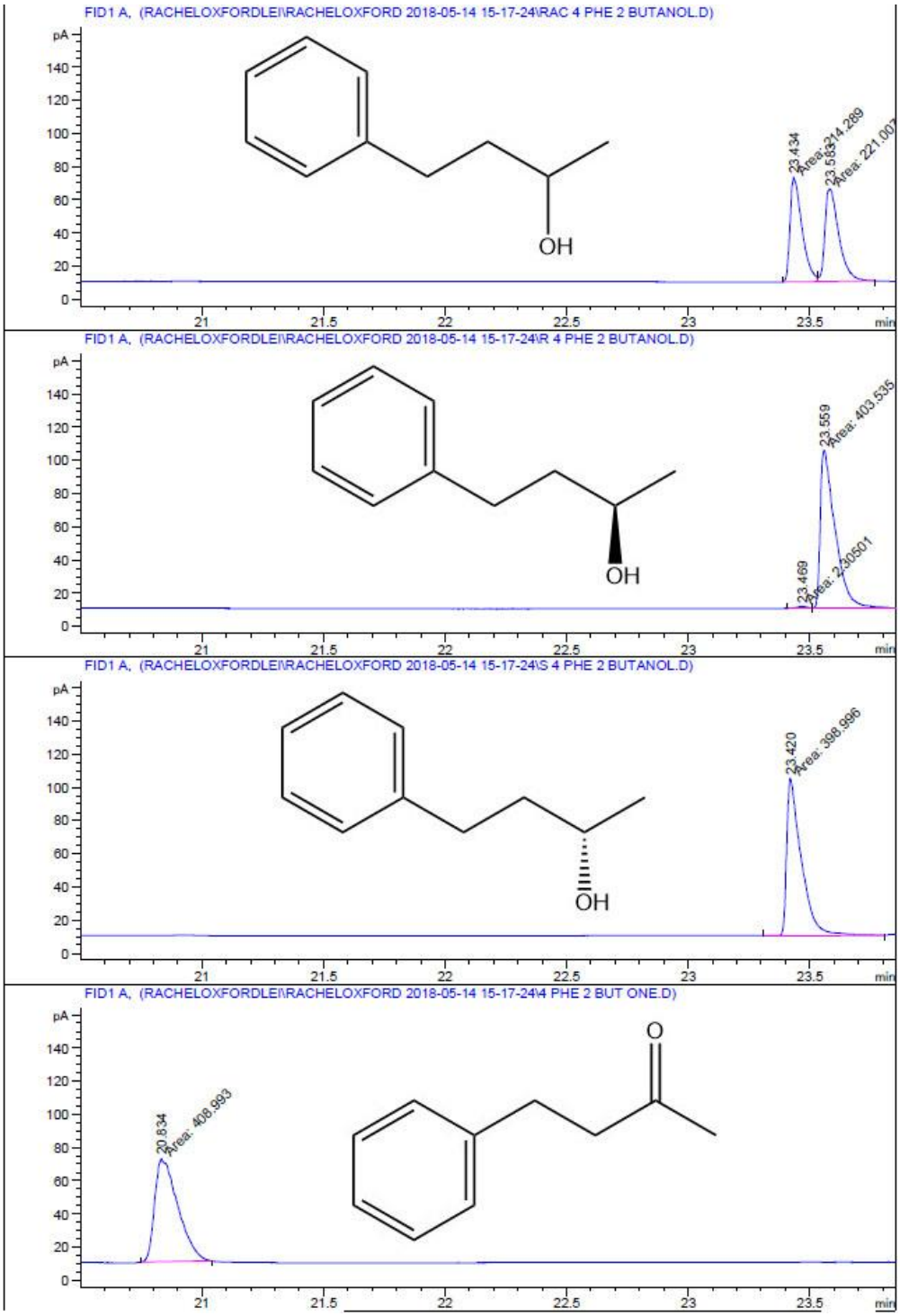


Figure S9. GC-FID spectra (top to bottom) for *(rac)*-4-phenyl-2-butanol (11.6 mM), *(R)*- 4-phenyl-2-butanol (10.5 mM), *(S)*- 4-phenyl-2-butanol (9.9 mM) and 4-phenyl-2-butanone (10.2 mM).

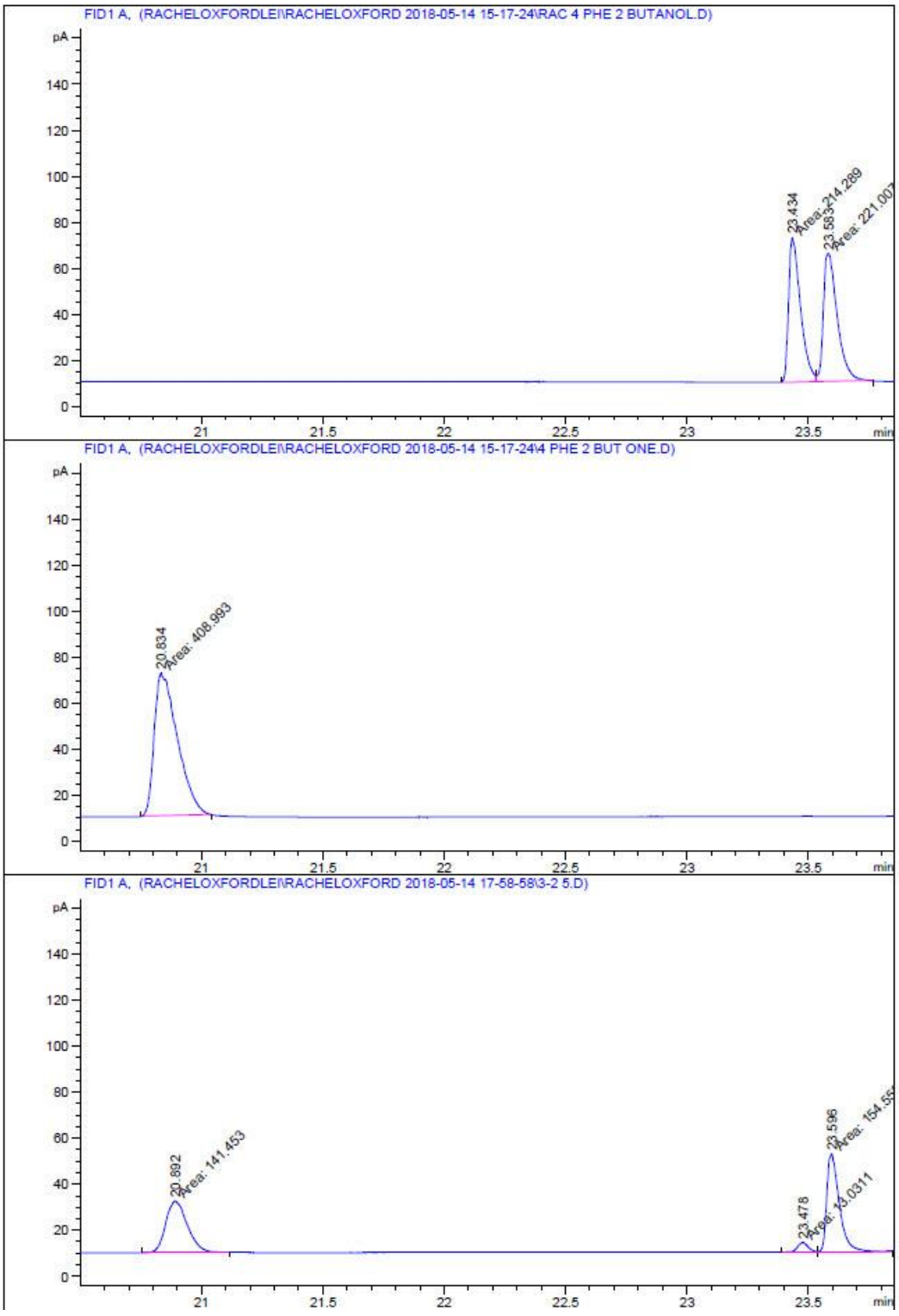


Figure S10. GC-FID spectra (top to bottom) for *(rac)*-4-phenyl-2-butanol, 4-phenyl-2-butanone and cell solution for entry A in Tables 1 and S1.

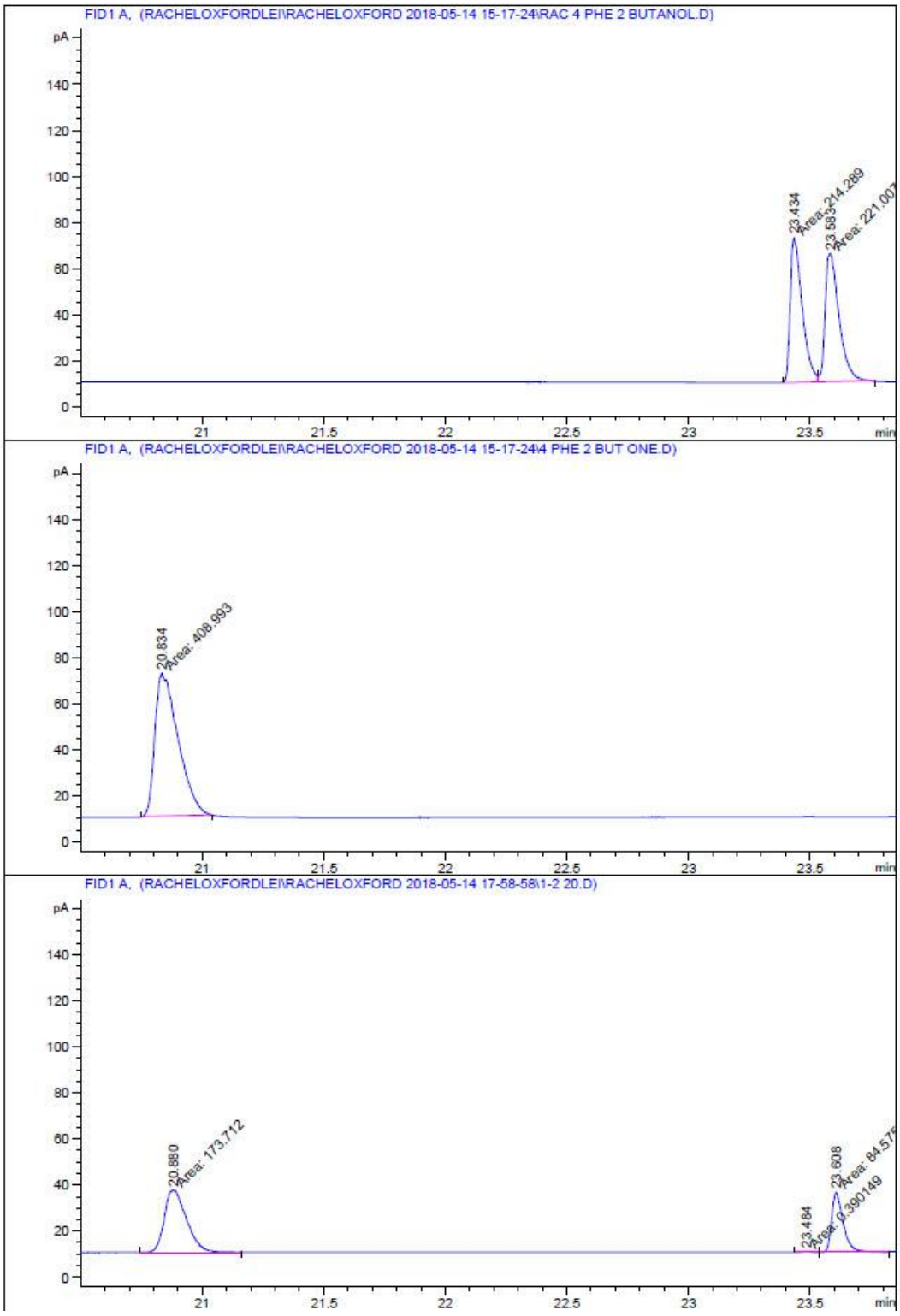


Figure S11. GC-FID spectra (top to bottom) for *(rac)*-4-phenyl-2-butanol, 4-phenyl-2-butanone and cell solution for entry B in Tables 1 and S1.

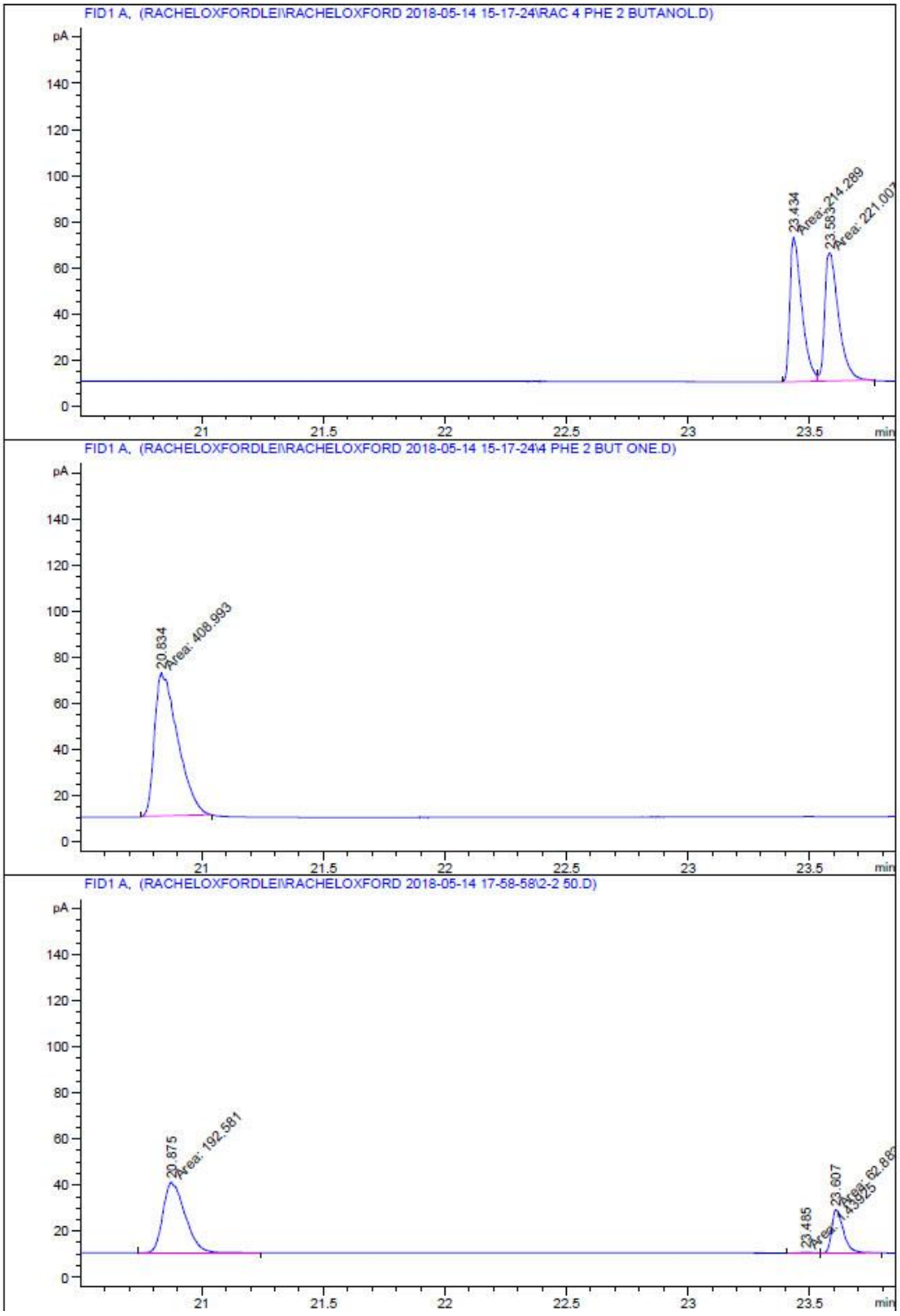
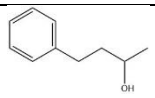
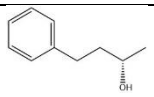
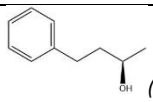


Figure S12. GC-FID spectra (top to bottom) for *(rac)*-4-phenyl-2-butanol, 4-phenyl-2-butanone and cell solution for entry C in Tables 1 and S1.

Table S1. Additional supplementary data corresponding to Table 1.

	Reactant	Final cell volume[a] / mL	[Ketone] / mM		Normalised[b] [Ketone] / mM		[Alcohol] / mM		Normalised[b] [Alcohol] / mM		Loss[c] / %	
			NMR	GC	NMR	GC	NMR	GC	NMR	GC	NMR	GC
A	 *(rac)*	6.3 ± 0.1	3.66	3.53	4.61	4.44	4.57	4.47	5.75	5.63	10.69	13.19
B		6.5 ± 0.1	4.34	4.33	5.63	5.63	2.55	2.27	3.32	2.95	22.84	26.03
C		6.4 ± 0.1	5.03	4.80	6.44	6.15	1.88	1.72	2.41	2.23	23.71	27.76
D	 *(S)*	6.45 ± 0.15	5.57	4.94	7.19	6.38	0.56	0.45	0.73	0.59	24.57	33.61
E	 *(R)*	6.2 ± 0.1	2.94	2.68	3.65	3.32	3.47	3.32	4.31	4.11	19.60	24.95

Entries in this table correspond to those in Table 1. [a] The final cell volume consists of contributions from the PCP-containing compartment and main compartment after chronoamperometry. The total volume is subject to some volume change because the air bubbled into the cell is humidified with pure water. [b] The term 'normalized' means that [ketone] or [alcohol] are the measured concentrations calculated from either GC-FID or ^{1}H NMR spectra multiplied by a volume factor 'final cell volume/5 mL. Initially, all reactants were injected into the main compartment which has a 5 ml cell volume. When ^{1}H NMR and GC-FID were carried out, the solution in the main compartment was mixed with that in the PCP-containing compartment. [c] Defined by (1 – ([Normalized ketone] + [Normalized alcohol])/[initial reactant]] × 100%.

Notes and References

1 B. Siritanaratkul, C. F. Megarity, T. G. Roberts, T. O. Samuels, M. Winkler, J. H. Warner, T. Happe and F. A. Armstrong, *Chem. Sci.*, 2017, **8**, 4579-4586.

2 L. Wan, C. F. Megarity, B. Siritanaratkul and F. A. Armstrong, *Chem. Commun.*, 2018, **54**, 972-975.