Supporting Information for

Enzymatic reduction of acetophenone derivatives with a benzil reductase from *Pichia glucozyma* (KRED1-Pglu): electronic and steric effects on activity and enantioselectivity

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Materials
All reagents and solvents were obtained from Sigma–Aldrich–Fluka and used without further purification or drying. TLC was performed with Merck silica gel 60 F\(_{254}\) pre-coated plates. Silica gel column chromatography was carried out on silica gel 60 M (40–63 mm). Recombinant KRED1-Pglu was produced in *Escherichia coli* and purified to apparent homogeneity as described earlier [19]. Recombinant glucose dehydrogenase (GDH) was a kind gift from Dr. Daniela Monti. For each compound, the alcohol racemic mixture was obtained by NaBH\(_4\) reduction (0.25 mMol of substrate).
Analyticals
$^1$H-NMR and $^{13}$C-NMR spectra were recorded with a Varian Mercury 300 (300 MHz) spectrometer. Chemical shifts ($\delta$) are expressed in ppm, and coupling constants ($J$) are expressed in Hz. GC analyses were performed with a Dani 6500 gas chromatograph: gas carrier H$_2$ (0.6 bar, T=100°C), detector FID (Flame Ionization Detector), $T_{\text{max}} = 300$°C. Chiral capillary GC columns used: DMePeBeta-CDX-PS086 (diameter 0.25 mm, length 25 m, thickness 0.25 $\mu$m, MEGA) and Mega-Dex DET beta (diameter 0.25 mm, length 25 m, thickness 0.25 $\mu$m, MEGA). HPLC analyses were performed with a Jasco Pu-980 equipped with a UV-vis detector Jasco UV-975. Chiral HPLC columns used: Chiralcel OD-H (250x4mm, Daicel), Chiralcel OD (250x4mm, Daicel), Lux cellulose-2 column (4.6 x150 mm, Phenomenex) and Lux cellulose-3 column (4.6 x150 mm, Phenomenex). Rotary power determinations were carried out using a Jasco P-1010 spectropolarimeter, coupled with a Haake N3-B thermostat.

Enzymatic activity
Enzymatic activity was measured spectrophotometrically at 340 nm by determining the consumption of NADPH during the reduction of acetophenones at 22°C in a half-micro cuvette (total volume 1 mL) for 5 min. The cuvette contained the ketone (0.47 mM) dissolved in DMSO (final concentration 0.1%) and NADPH (0.25 mM) in Tris-HCl 50 mM pH 8.0 buffer in a 1 mL volume. The reaction was initiated by the addition of KRED1-Pglu (0.07 mg). The reaction rates were calculated from measurements determined in triplicate. One unit (U) of activity is defined as the amount of enzyme which catalyses the consumption of 1 µmol of NADPH per minute. Relative rates were calculated taking into account the maximal activity registered towards 4'-nitroacetophenone.

Preparative reduction of acetophenones
General procedure for the ketone reduction using a NADPH recycle system was as follows: reductions were carried out in 10 mL screw-capped test tubes with a reaction volume of 5 mL with KRED1-Pglu (20 mU/mL), GDH (1 U/mL), NADP$^+$ (0.1 mM), substrate (1 g/L), glucose (4 x mMol of substrate) suspended in 50 mM Tris/HCl buffer pH 8.0. The samples were extracted with ethyl acetate (3x5 mL). The organic extracts were dried over anhydrous sodium sulfate and subjected to chiral GC or HPLC analysis.

Characterization of the products
(S)-2b (1-(4’-methoxyphenyl)ethanol): $[\alpha]_D^{25} = -25.0^\circ$ (c 0.2 chloroform) lit.[23] $[\alpha]_D^{25} = -51.9^\circ$ (c 0.718 chloroform). $^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 7.25-7.30 (m, 2H), 6.83-6.87 (m, 2H), 4.82 (q, $J = 6.5$ Hz, 1H), 3.78 (s, 3H), 1.44 (d, $J = 6.5$ Hz, 3H) ppm. $^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta$ 158.5, 138.0, 126.4, 114.0, 69.7, 55.1, 25.0 ppm. The enantiomeric excess (% ee) was determined by HPLC using Chiralcel OD-H column (n-hexanes/i-PrOH 99:1, 1 mL/min, 210 nm): $t_r$ (R)-1-(4’-methoxyphenyl)ethanol min, $t_r$ (S)-1-(4’-methoxyphenyl)ethanol 22.6 min.

(S)-2c (1-(4’-tolyl)ethanol): $[\alpha]_D^{20} = -51.4.0^\circ$ (c 0.2 chloroform) lit.[24] $[\alpha]_D^{20} = -53.4^\circ$ (c 0.85 chloroform). $^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 7.20-7.24 (m, 2H), 7.10-7.15 (m, 2H), 4.8 (q, $J = 6.5$ Hz, 1H), 2.32 (s, 3H), 1.45 (d, $J = 6.5$ Hz, 3H) ppm. $^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta$ 143.0, 136.5, 129.1, 128.3, 124.7, 68.6, 25.1, 21.0 ppm. The enantiomeric excess (% ee) was determined by GC using Mega-dex DET Beta column, gas carrier: H$_2$ (0.6 bar, T = 100°C), detector FID (flame ionization detector), temperature: 70-170°C 4°/min: $t_r$ (S)-1-(4-tolyl)ethanol 14.1 min, $t_r$ (R)-1-(4-tolyl)ethanol 14.6 min.

(R)-2d (1-phenylethanol): $[\alpha]_D^{25} = +40.0^\circ$ (c 0.5 chloroform) lit.[25] $[\alpha]_D^{25} = +36.0^\circ$ (c 1.0 chloroform). $^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 7.20–7.35 (m, 5H), 4.85 (q, $J = 6.4$ Hz, 1H), 1.46 (d, $J = 6.5$ Hz, 3H) ppm. $^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta$ 145.9, 128.5, 127.5, 125.4, 70.4, 25.2 ppm. The enantiomeric excess (% ee) was determined by HPLC using Chiralcel OD column (n-hexanes/i-PrOH 95:5, 0.7 mL/min, 254 nm): $t_r$ (R)-1-phenylethanol 14.2 min, $t_r$ (S)-1-phenylethanol 18.0 min.

(S)-2e (1-(4’-fluorophenyl)ethanol): $[\alpha]_D^{20} = -78.6.0^\circ$ (c 0.2 chloroform) lit.[24] $[\alpha]_D^{20} = -81.5^\circ$ (c 1.58 chloroform). $^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 7.30-7.35 (m, 2H), 7.00-7.05 (m, 2H), 4.85 (q, $J = 6.5$ Hz, 1H), 1.45 (d, $J = 6.5$ Hz, 3H) ppm. $^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta$ 162.0 (d, $J = 242.5$ Hz), 148.9, 129.5 (q, $J = 32.0$ Hz), 124.3, 125.4 (q, $J = 3.9$ Hz), 123.1, 70.0 (CH), 25.2 ppm. The enantiomeric excess (% ee) was determined by GC using Mega-dex DET Beta column, gas carrier: H$_2$ (0.6 bar, T = 100°C), detector FID (flame ionization detector), temperature: 70-170°C 4°/min: $t_r$ (S)-1-(4-Fluorophenyl)ethanol 12.9 min, $t_r$ (R)-1-(4-Fluorophenyl)ethanol 13.5 min.

(S)-2f (1-(4’-trifluoromethylphenyl)ethanol): $[\alpha]_D^{20} = -40.50^\circ$ (c 0.2 chloroform) lit.[24] $[\alpha]_D^{20} = -32.0^\circ$ (c 0.86 chloroform). $^1$H-NMR (300 MHz, CDCl$_3$): $\delta$ 7.58 (d, $J = 8.0$ Hz, 2H), 7.45 (d, $J = 8.0$ Hz, 2H), 4.95 (q, $J = 6.5$ Hz, 1H), 1.46 (d, $J = 6.5$ Hz, 3H) ppm. $^{13}$C-NMR (75 MHz, CDCl$_3$): $\delta$ 148.9, 129.5 (q, $J = 32.0$ Hz), 124.3, 125.4 (q, $J = 3.9$ Hz), 123.1, 70.0 (CH), 25.2 ppm. The
enantiomeric excess (% ee) was determined by HPLC using Chiralcel OD-H column (n-hexane/EtOH 98:2, 0.8 mL/min, 220 nm): t_r (S)-1-(4-trifluoromethylphenyl)ethanol 16.5 min, t_r (R)-1-(4-trifluoromethylphenyl)ethanol 17.25 min.

(S)-2g (1-(4'-cyanophenyl)ethanol): [α]_D^20 = - 45.2° (c 0.2 chloroform ) lit.[24] [α]_D^20 = - 46.4° (c 2.40 chloroform). ¹H-NMR (300 MHz, CDCl₃): δ 7.61 (d, J = 8.5 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 4.98 (q, J = 6.5 Hz, 1H), 1.48 (d, J = 6.5 Hz, 3H) ppm. ¹³C-NMR (75 MHz, CDCl₃): δ 151.5, 133.4, 126.1, 119.0, 111.0, 69.5, 25.2 ppm. The enantiomeric excess (% ee) was determined by GC using Mega-dex DET Beta column, gas carrier: H₂ (0.6 bar, T = 100°C), detector FID (flame ionization detector), temperature: 70-170°C 4°/min: t_r (S)-1-(4-cyanophenyl)ethanol 23.7 min, t_r (R)-1-(4-cyanophenyl)ethanol 24.0 min.

(S)-2h (1-(4'-nitrophenyl)ethanol): [α]_D^25 = - 25.0° (c 0.2 chloroform ) lit.[26] [α]_D^20 = - 22.6° (c 1.0 chloroform). ¹H-NMR (300 MHz, CDCl₃): δ 8.18 (d, J = 8.5 Hz, 2H), 7.51 (d, J = 8.0 Hz, 2H), 4.95 (q, J = 6.5 Hz, 1H), 1.50 (d, J = 6.5 Hz, 3H) ppm. ¹³C-NMR (75 MHz, CDCl₃): δ 153.1, 147.1, 126.1, 123.7, 69.4, 25.4 ppm. The enantiomeric excess (% ee) was determined by HPLC using Phenomenex LUX Cellulose-3 column (n-hexane/i-PrOH 90:10, 0.5 mL/min, 254 nm): t_r (S)-1-(4-nitrophenyl) ethanol 23.4 min, t_r (R)-1-(4-nitrophenyl) ethanol 24.9 min.

(S)-2j (1-(3'-methoxyphenyl)ethanol): [α]_D^20 = - 40.2° (c 0.2 chloroform ) lit.[24] [α]_D^20 = - 38.9° (c 1.27 chloroform). ¹H-NMR (300 MHz, CDCl₃): δ 7.20-7.25 (m, 1H), 6.90-6.95 (m, 2H), 6.80 (ddd, J = 8.5 Hz, 1.5 Hz and 1.0 Hz, 1H), 4.84 (q, J = 6.5 Hz, 1H), 3.80 (s, 3H), 1.46 (d, J = 6.5 Hz, 3H) ppm. ¹³C-NMR (75 MHz, CDCl₃): δ 159.8, 147.6, 130.0, 116.5, 113.0, 111.0 , 70.3, 56.2, 25.1 ppm. The enantiomeric excess (% ee) was determined by HPLC using Chiralcel OD column (n-hexane/i-PrOH 95:5, 0.5 mL/min, 216 nm): t_r (R)-1-(3-methoxyphenyl)ethanol 23.1 min, t_r (S)-1-(3-methoxyphenyl)ethanol 26.4 min.

(S)-2k (1-(3'-tolyl)ethanol): [α]_D^20 = - 45.4.0° (c 0.2 chloroform ) lit.[24] [α]_D^20 = - 47.2° (c 1.09 chloroform). ¹H-NMR (300 MHz, CDCl₃): δ 7.00-7.25 (m, 4), 4.84 (dq, J = 6.5 Hz and 3.5 Hz, 1H), 2.35 (s, 3H), 1.47 (d, J = 6.5 Hz, 3H) ppm. ¹³C-NMR (75 MHz, CDCl₃): δ 144.0, 137.1, 128.4, 128.2, 126.1, 122.4, 68.4, 25.1, 21.4 ppm. The enantiomeric excess (% ee) was determined by GC using Mega-dex DET Beta column, gas carrier: H₂ (0.6 bar, T = 100°C), detector FID (flame ionization detector), temperature: 70-170°C 4°/min: t_r (S)-1-(3-tolyl)ethanol 23.1 min, t_r (R)-1-(3-tolyl)ethanol 26.4 min.
ionization detector), temperature: 70-170°C 4°/min: t<sub>r</sub> (S)-1-(3’-tolyl)ethanol 14.8 min, t<sub>r</sub> (R)-1-(3’-tolyl)ethanol 15.1 min.

(S)-2l (1-(3’-fluorophenyl)ethanol): [α]₀<sup>20</sup> = - 25.60° (c 0.2 chloroform) lit.[27] [α]₀<sup>20</sup> = - 24.4° (c 1.24 chloroform). <sup>1</sup>H-NMR (300 MHz, CDCl₃): δ 7.25-7.32 (m, 1H), 7.10-7.15 (m, 1H), 6.88-6.98 (m, 2H), 4.85 (q, J = 6.5 Hz, 1H ), 1.45 (d, J = 6.5 Hz, 3H ) ppm. <sup>13</sup>C-NMR (75 MHz, CDCl₃): δ 163.0 (d, J = 246.1 Hz), 148.5 (d, J = 6.4 Hz), 129.9 (d, J = 7.7 Hz), 120.9 (d, J = 2.6 Hz), 114.2 (d, J = 21.2 Hz), 112.3 (d, J = 21.2 Hz), 69.7 (d, J = 2.1 Hz), 25.2 ppm. The enantiomeric excess (% ee) was determined by GC using Mega-dex DET Beta column, gas carrier: H₂ (0.6 bar, T = 100°C), detector FID (flame ionization detector), temperature: 70-170°C 4°/min: t<sub>r</sub> (S)-1-(3’-fluorophenyl)ethanol 17.0 min, t<sub>r</sub> (R)-1-(3’-fluorophenyl)ethanol 17.5 min.

(S)-2m (1-(3’-trifluoromethylphenyl)ethanol): [α]₀<sup>20</sup> = - 30.50° (c 0.2 chloroform) lit.[24] [α]₀<sup>20</sup> = - 27.6° (c 1.05 chloroform). <sup>1</sup>H-NMR (300 MHz, CDCl₃): δ 7.63-7.25 (m, 4H), 4.91 (q, J = 6.5 Hz, 1H), 1.48 (d, J = 6.5 Hz, 3H) ppm. <sup>13</sup>C-NMR (75 MHz, CDCl₃): δ 146.7, 130.8 (q, J = 32.5 Hz), 128.9, 128.8, 124.2 (q, J = 4.6 Hz), 124.1 (q, J = 270.8 Hz), 122.2 (q, J = 4.4 Hz), 69.8, 25.2 ppm. The enantiomeric excess (% ee) was determined by HPLC using Chiralcel OD-H column (n-hexane/EtOH 98:2, 0.8 mL/min, 220 nm): t<sub>r</sub> (S)-1-(3’-trifluoromethylphenyl)ethanol 18.5 min, t<sub>r</sub> (R)-1-(3’-trifluoromethylphenyl)ethanol 19.3 min.

(S)-2n (-1-(3’-cyanophenyl)ethanol): [α]₀<sup>20</sup> = - 25.2° (c 0.2 chloroform) lit.[28] [α]₀<sup>20</sup> = - 26.6° (c 0.3 chloroform). <sup>1</sup>H-NMR (300 MHz, CDCl₃): δ 7.66 (s, 1H,), 7.60 (d, J = 7.3 Hz, 1H,), 7.52-7.55 (m, 1H,), 7.40-7.45 (m, 1H), 4.90 (q, J = 6.1 Hz, 1H,), 1.48 (d, J = 6.1 Hz, 3H,) ppm. <sup>13</sup>C-NMR (75 MHz, CDCl₃): δ 146.5, 130.5, 129.9, 129.0, 128.5, 119.0, 111.0, 69.5, 25.2 ppm. The enantiomeric excess (% ee) was determined by GC using Mega-dex DET Beta column, gas carrier: H₂ (0.6 bar, T = 100°C), detector FID (flame ionization detector), temperature: 70-170°C 4°/min: t<sub>r</sub> (S)-1-(3’-cyanophenyl)ethanol 23.7 min, t<sub>r</sub> (R)-1-(3’-cyanophenyl)ethanol 24.0 min.

(S)-2o 1-(3’-nitrophenyl)ethanol: [α]₀<sup>25</sup> = - 22.0° (c 0.2 chloroform) lit.[29] [α]₀<sup>25</sup> = - 14.5° (c 1.0 chloroform). <sup>1</sup>H-NMR (300 MHz, CDCl₃): δ 8.18 (t, J = 1.8. Hz, 1H,), 8.05 (ddd, J = 8.3, 2.1 and 0.9 Hz, 1H,), 7.65 (d, J = 7.8, 1H,), 7.50 (t, J = 7.8. Hz, 1H,), 4.95 (q, J = 6.5 Hz, 1H,), 1.50 (d, J = 6.5 Hz, 3H,) ppm. <sup>13</sup>C-NMR (75 MHz, CDCl₃): δ 149.1, 147.1, 131.5, 129.1, 123.7, 120.2, 69.4, 25.4 ppm. The enantiomeric excess (% ee) was determined by HPLC using Phenomenex LUX Cellulose-2 column (n-hexane/i-PrOH 98:2, 0.5 mL/min, 254 nm): t<sub>r</sub> (R)-1-(3’-nitrophenyl)ethanol 60.0 min, t<sub>r</sub> (S)-1-(3’-nitrophenyl)ethanol 67.5 min.
(S)-2s (1-(2′-fluorophenyl)ethanol): \([\alpha]_D^{20} = -43.1^\circ\) (c 0.2 chloroform) lit.[30] \([\alpha]_D^{25} = -47.8^\circ\) (c 1.46 chloroform). \(^1\)H-NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.45-7.50 (m, 1H), 7.27-7.20 (m, 1H), 7.15–7.05 (m, 1H), 7.04-6.98 (m, 1H), 4.85 (q, \(J = 6.5\) Hz, 1H ), 1.43 (d, \(J = 6.5\) Hz, 3H ) ppm. \(^1^3\)C-NMR (75 MHz, CDCl\(_3\)): \(\delta\) 159.9 (d, \(J = 246.1\) Hz), 132.5 (d, \(J = 13.4\) Hz), 128.9 (d, \(J = 8.2\) Hz), 126.9 (d, \(J = 4.6\) Hz), 124.2 (d, \(J = 3.4\) Hz), 115.3 (d, \(J = 21.2\) Hz), 65.7, 24.2 ppm. The enantiomeric excess (\% ee) was determined by GC using Mega-dex DET Beta column, gas carrier: H\(_2\) (0.6 bar, T = 100°C), detector FID (flame ionization detector), temperature: 70-170°C 4°/min: \(t_r\) (S)-1-(2′-fluorophenyl)ethanol 12.09 min, \(t_r\) (R)-1-(2′-fluorophenyl)ethanol 13.9 min.

(S)-2u (1-(2′-cyanophenyl)ethanol): \([\alpha]_D^{20} = -45.6^\circ\) (c 0.2 dichloromethane) lit.[31] \([\alpha]_D^{20} = -47.6^\circ\) (c 1.0 dichloromethane). \(^1\)H-NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.60-7.70 (m, 1H,), 7.55 (d, \(J = 7.3\) 2H,), 7.20-7.36 (m, 1H,), 5.20 (q, \(J = 6.4\) Hz, 1H,), 1.48 (d, \(J = 6.4\) Hz, 3H,) ppm. \(^1^3\)C-NMR (75 MHz, CDCl\(_3\)): \(\delta\) 148.9, 133.1, 132.5, 127.8, 125.4, 116.5, 109.0, 66.7, 24.2 ppm. The enantiomeric excess (\% ee) was determined by GC using Mega-dex DET Beta column, gas carrier: H\(_2\) (0.6 bar, T = 100°C), detector FID (flame ionization detector), temperature: 70-170°C 4°/min: \(t_r\) (S)-1-(2′-cyanophenyl)ethanol 26.4 min, \(t_r\) (R)-1-(2′-cyanophenyl)ethanol 27.2 min.

Figure S1. LUMO frontier molecular orbitals for all the investigated substrates.