Enantiocomplementary Decarboxylative Hydroxylation
Combining Photocatalysis and Whole-Cell Biocatalysis
in One-pot Cascade Process

Jian Xu, Mamatjan Arkin, Yongzhen Peng, Weihua Xu, Huilei Yu†, Xianfu Lin and Qi Wu*

*Department of Chemistry, Zhejiang University, Hangzhou 310027, People’s Republic of China.

*corresponding author: wuqi1000@163.com, llc123@zju.edu.cn
Experimental procedures

General information

All reagents were obtained commercially unless otherwise noted. The \(^1\)H and \(^{13}\)C NMR spectra were recorded with a Bruker AMX400 MHz spectrometer using TMS as an internal standard. Chiral GC was performed with Agilent CP-chirasil-Dex CB using dodecane or pentadecane as internal standard. Chiral HPLC was performed with a Chiralpak OB-H, OJ-H column (250 mm\(\times\)4.6 mm, n-hexane/2-propanol as the mobile phase) and a UV detector (220 or 254 nm). All known products were characterized by comparison of \(^1\)H and \(^{13}\)C NMR data with those reported in the literature. Absolute configuration confirmed by comparison with literature values. The power of the LEDs is 8W.

Preparation of ketoreductases \(^1\) (KtCR and YueD)

100 \(\mu\)L Stored bacteria was first incubated in 5 mL LB media with Kanamycin (50 \(\mu\)g/mL) and then shaked at 37 °C overnight. A fresh 200 mL of LB medium with 50 \(\mu\)g/mL Kanamycin was inoculated from 5 mL preculture. The cultures were allowed to grow at 37 °C until OD\(_{600}\) at 0.6. After cooling at 4 °C for 30 min, isopropyl \(\beta\)-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM to induce ketoreductases expression at 25 °C (OD\(_{600}\) at 6, when using for whole-cell reaction system). Cells were harvested by centrifugation, and resuspended in buffer (50 mM sodium phosphate buffer, pH 6.5 for KtCR and pH 7.4 for YueD), then repeated freezing and thawing for 3 times, and released the target proteins by sonication. The cell lysate was removed by centrifugation. The supernatant was concentrated by freeze drying and stored in -20 °C for next reaction.

Preparation of racemic alcohols

The given ketone (2 mmol) was added into a stirred solution of NaBH\(_4\) (5 mmol) in methanol (10 mL) at room temperature (20 °C). The clear solution was stirred until the complete disappearance of ketone substrate indicated by TLC. The crude product was evaporated in vacuo and diluted in dichloromethane (20 mL), and then washed with water (10 mL). The organic phase was separated and dried over anhydrous magnesium sulfate, and then evaporated in vacuo.

General procedure for decarboxylative hydroxylation

SAS (3 mM, 10% mol) was dissolved in 2 ml water containing 1a (30 mM). After irradiation with Blue LEDs for 18 hours (the reaction time could be extended to ensure no residual alcohols), 10 mL whole-cell culture medium of ketoreductase (OD\(_{600}\)=6) with glucose (0.3 mmol) was added into the photocatalytic mixture and shaken at 30 °C over night. The solution was extracted with equal volume of ethyl acetate for three times, and the stereoselectivity was then determined by chiral GC or HPLC.

Scaling-up one-pot decarboxylative hydroxylation catalyzed by photocatalyst and ketoreductase.

The scale-up reaction was performed as follows: A solution of SAS (0.3 mmol, 20 mol%) in 50 mL water with 1a (1.5 mmol, dissolved in 500 \(\mu\)L acetonitrile). The reaction mixture was irradiated with Blue LEDs for 18 hours (the reaction time could be extended to ensure no residual alcohols). 150 mL whole-cell culture medium of ketoreductase (OD\(_{600}\)=6) with glucose (8 mmol) was added into the photocatalytic mixture and shaken at 30 °C over night. The reaction solution was extracted with ethyl acetate for three times, then the organic phase was dried over anhydrous sodium sulfate and concentrated in vacuum. The obtained crude product was further separated and purified by using flash column chromatography.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Substrate</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Expense ($/mg)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Catalyst: 10%SAS, solvent: H₂O, hv</td>
<td>1α</td>
<td>2α</td>
<td>98</td>
<td>0.025</td>
<td>This work</td>
</tr>
<tr>
<td>2</td>
<td>Catalyst: 10%SAS, solvent: H₂O, hv</td>
<td>1j</td>
<td>2j</td>
<td>80</td>
<td>0.013</td>
<td>This work</td>
</tr>
<tr>
<td>3</td>
<td>Catalyst: 2eq K₂S₂O₈, solvent: H₂O, 90 °C</td>
<td>1j</td>
<td>2j</td>
<td>89</td>
<td>0.08</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Solvent: CH₃CN, hv</td>
<td>1α</td>
<td>2α</td>
<td>27</td>
<td>0.91</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Catalyst: FSM-16, solvent: Hexane, hv</td>
<td>1α</td>
<td>2α</td>
<td>47</td>
<td>0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Catalyst: HgO, solvent: CH₃CN/CH₃OH, hv</td>
<td>1α</td>
<td>2α</td>
<td>77</td>
<td>0.09</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Catalyst: HgF₂, solvent: CH₃CN, hv</td>
<td>1α</td>
<td>2α</td>
<td>94</td>
<td>0.25</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>Catalyst: 4eq K₅Co⁵W₁₂O₄₀, solvent: CH₃CN/H₂O, hv</td>
<td>1α</td>
<td>2α</td>
<td>95</td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7</td>
</tr>
<tr>
<td>9</td>
<td>Catalyst: 10% Cu(OAC)₂, solvent: DMSO, 120 °C</td>
<td>1j</td>
<td>2j</td>
<td>31</td>
<td>0.11</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>Catalyst: NaIO₄/Mn(salophen), solvent: CH₃CN/H₂O</td>
<td>1α</td>
<td>2α</td>
<td>93</td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9</td>
</tr>
</tbody>
</table>

<sup>a</sup> The rough estimation of reaction expenses is based on the price of commercial reagents in Sigma-Aldrich, the consumption of electricity was considered. <sup>b</sup> The expense calculated in entries 5, 8, 10 did not include FSM-16, K₅Co⁵W₁₂O₄₀ and Mn(salophen), respectively.
Figure S1. Ultraviolet-visible spectrum of SAS

Figure S2. Stern–Volmer plot for the decarboxylation reaction
Characterization data

1-phenylethanol (3a)
\[
\text{H NMR} (500 \text{ MHz, CDCl}_3): \delta 7.39–7.32 (m, 4H), 7.30–7.24 (m, 1H), 4.88 (q, J = 6.3 Hz, 1H), 1.48 (d, J = 6.5 Hz, 3H). \text{^{13}C NMR} (126 \text{ MHz, CDCl}_3): \delta 145.83, 128.51, 127.48, 125.41, 70.42, 25.17.
\]

1-phenylpropan-1-ol (3b)
\[
\text{H NMR} (400 \text{ MHz, CDCl}_3): \delta 7.37–7.25 (m, 5H), 4.58 (t, 1H), 1.92–1.65 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H). \text{^{13}C NMR} (101 \text{ MHz, CDCl}_3): \delta 144.59, 128.42, 127.52, 126.00, 76.04, 31.89, 10.18.
\]

1-(p-tolyl)ethanol (3c)
\[
\text{H NMR} (400 \text{ MHz, CDCl}_3): \delta 7.19 (d, J = 7.8 Hz, 2H), 7.09 (d, J = 7.9 Hz, 2H), 4.79 (q, J = 6.3 Hz, 1H), 2.27 (s, 3H), 1.41 (d, J = 6.5 Hz, 3H). \text{^{13}C NMR} (101 \text{ MHz, CDCl}_3): \delta 141.82, 136.15, 128.14, 124.33, 69.24, 24.04, 20.07.
\]

1-(4-fluorophenyl)ethanol (3d)
\[
\text{H NMR} (400 \text{ MHz, CDCl}_3): \delta 7.37–7.30 (m, 2H), 7.06–6.99 (m, 2H), 4.88 (q, J = 6.3 Hz, 1H), 1.47 (d, J = 6.4 Hz, 3H). \text{^{13}C NMR} (101 \text{ MHz, CDCl}_3): \delta 163.33, 160.90, 141.52, 141.49, 127.09, 127.01, 115.37, 115.16, 69.79, 25.29.
\]

1-(4-chlorophenyl)ethanol (3e)
\[
\text{H NMR} (400 \text{ MHz, CDCl}_3): \delta 7.43–7.16 (m, 4H), 4.86 (qd, J = 6.4, 3.1 Hz, 1H), 1.46 (d, J = 6.5 Hz, 3H). \text{^{13}C NMR} (101 \text{ MHz, CDCl}_3): \delta 144.25, 133.06, 128.61, 126.81, 69.74, 25.27.
\]

1-(2-chlorophenyl)ethanol (3f)
\[
\text{H NMR} (400 \text{ MHz, CDCl}_3): \delta 7.60 (dd, J = 7.7, 1.6 Hz, 1H), 7.31 (ddd, J = 14.5, 7.8, 1.1 Hz, 2H), 7.20 (td, J = 7.6, 1.7 Hz, 1H), 5.30 (qd, J = 6.4, 3.6 Hz, 1H), 1.50 (d, J = 6.4 Hz, 3H). \text{^{13}C NMR} (101 \text{ MHz, CDCl}_3): \delta 143.04, 131.66, 129.42, 128.43, 127.23, 126.41, 67.00, 23.52.
\]
1-(3-chlorophenyl)ethanol (3g)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.37 (s, 1H), 7.30 – 7.21 (m, 3H), 4.87 (q, J = 6.0 Hz, 1H), 1.48 (d, J = 6.5 Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 147.86, 134.37, 129.81, 127.54, 125.64, 123.55, 69.81, 25.25.

1-phenylethane-1,2-diol (3h)

$^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.39–7.28 (m, 5H), 4.82 (dd, J = 8.2, 3.4 Hz, 1H), 3.76 (dd, J = 11.3, 3.3 Hz, 1H), 3.66 (dd, J = 11.3, 8.3 Hz, 1H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 140.51, 128.57, 128.04, 126.08, 74.70, 68.10.

1-(4-methoxyphenyl)ethanol (3i)

$^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.29 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 4.84 (q, J = 6.4 Hz, 1H), 3.80 (s, 3H), 1.47 (d, J = 6.5 Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$): $\delta$ 158.98, 138.03, 126.68, 113.86, 69.98, 55.31, 25.02.

1-(4-isobutylphenyl)ethanol (3j)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.28 (d, J = 8.0 Hz, 2H), 7.13 (d, J = 8.1 Hz, 2H), 4.87 (q, J = 6.4 Hz, 1H), 2.46 (d, J = 7.2 Hz, 2H), 1.88–1.84 (m, 1H), 1.49 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 6.6 Hz, 6H). $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 143.05, 141.03, 125.21, 70.33, 45.09, 30.25, 25.02, 22.38.

1-(6-methoxynaphthalen-2-yl)ethanol (3k)

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.77–7.69 (m, 3H), 7.51–7.41 (m, 1H), 7.19–7.07 (m, 2H), 5.28–4.86 (m, 1H), 3.92 (s, 3H), 1.57 (d, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 129.41, 127.19, 124.37, 123.79, 118.98, 105.69, 70.55, 55.32, 25.08.
NMR spectra

1-phenylethanol (3a)
1-phenylpropan-1-ol (3b)
1-(p-tolyl)ethanol (3c)
1-(4-fluorophenyl)ethanol (3d)
1-(4-chlorophenyl)ethanol (3e)
1-(2-chlorophenyl)ethanol (3f)
1-(3-chlorophenyl)ethanol (3g)
1-phenylethane-1,2-diol (3h)
1-(4-methoxyphenyl)ethanol (3i)
1-(4-isobutylphenyl)ethanol (3j)
1-(6-methoxynaphthalen-2-yl)ethanol (3k)
GC or HPLC data

3a, chiral GC (Agilent CP-chirasil-Dex CB, T\text{ Internal Standard} = 6.7 \text{ min}, T_R = 10.3 \text{ min}, T_S = 11.0 \text{ min}, Temperature conditions: initial temperature 100 ^\circ \text{C}, 2 \degree \text{C/min to 126 }^\circ \text{C, then 40 }^\circ \text{C/min to 200 }^\circ \text{C, holding 1min).}
3b, chiral GC (Agilent CP-chirasil-Dex CB, T<sub>Internal Standard</sub> = 18.0 min, T<sub>R</sub> = 13.9 min, T<sub>S</sub> = 14.4 min, Temperature conditions: initial temperature 100 °C, 2 °C/min to 132 °C, then 10 °C/min to 200 °C, holding 1 min).
3c, chiral GC (Agilent CP-chirasil-Dex CB, \( T_{\text{Internal Standard}} = 13.5 \text{ min}, T_R = 9.3 \text{ min}, T_S = 10.0 \text{ min}, \) Temperature conditions: initial temperature 110 \(^\circ\)C, 2 \(^\circ\)C/min to 140 \(^\circ\)C, then 40 \(^\circ\)C/min to 200 \(^\circ\)C, holding 1 min).
3d, chiral GC (Agilent CP-chirasil-Dex CB, T<sub>Internal Standard</sub> = 6.9 min, T<sub>R</sub> = 11.8 min, T<sub>S</sub> = 12.8 min, Temperature conditions: initial temperature 100 °C, 2 °C/min to 130 °C, then 40 °C/min to 200 °C, holding 1min).
3e, chiral GC (Agilent CP-chirasil-Dex CB, $T_{\text{Internal Standard}}$ = 11.3 min, $T_R = 12.4$ min, $T_S = 13.2$ min, Temperature conditions: initial temperature 120 °C, 2 °C/min to 150 °C, then 40 °C/min to 200 °C, holding 1min).
3f, chiral GC (Agilent CP-chirasil-Dex CB, T<sub>internal standard</sub> = 11.2 min, T<sub>S</sub> = 11.4 min, T<sub>R</sub> = 13.0 min, Temperature conditions: initial temperature 120 °C, 2 °C/min to 150 °C, then 40 °C/min to 200 °C, holding 1 min).
3g, chiral HPLC (OJ-H, hexane: iopropanol=95: 5, λ=220 nm, 1 ml/min, $T_S = 9.5$ min, $T_R = 10.9$ min).
3h, chiral HPLC (OB-H, hexane: iopropanol=95: 5, λ=220 nm, 1 ml/min, T_<sub>S</sub> = 13.7 min, T_<sub>R</sub> = 17.6 min).

Table 3, (S)-3h
3i, chiral HPLC (OJ-H, hexane: iopropanol=90: 10, λ=220 nm, 1 ml/min, $T_s = 15.0$ min, $T_R = 15.7$ min).

Table 3, (R)-3i

<table>
<thead>
<tr>
<th>Peak #</th>
<th>ID#</th>
<th>Name</th>
<th>Ret. Time</th>
<th>Conc.</th>
<th>Area</th>
<th>Height</th>
<th>Mark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>14.902</td>
<td>0.0000</td>
<td>18005220</td>
<td>721547</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>15.732</td>
<td>0.0000</td>
<td>18035034</td>
<td>652279</td>
<td></td>
</tr>
</tbody>
</table>

Table 4, (S)-3i

<table>
<thead>
<tr>
<th>Peak #</th>
<th>ID#</th>
<th>Name</th>
<th>Ret. Time</th>
<th>Conc.</th>
<th>Area</th>
<th>Height</th>
<th>Mark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>14.902</td>
<td>0.0000</td>
<td>18704209</td>
<td>61255</td>
<td></td>
</tr>
</tbody>
</table>
3j. chiral GC (Agilent CP-chiral-Dex CB, *T*<sub>internal std</sub> = 12.0 min, *T*<sub>R</sub> = 14.4 min, *T*<sub>S</sub> = 14.9 min. Temperature conditions: initial temperature: 120 °C, 2 °C/min to 160 °C, then 40 °C/min to 200 °C, holding 1 min).
3k, chiral HPLC (OJ-H, hexane: isopropanol=50:50, λ=220 nm, 0.7 ml/min, T<sub>S</sub> = 15.5 min, T<sub>R</sub> = 19.5 min).
References


