Supporting information

High Efficient and Large-scalable Glucoamylase-catalyzed Henry Reactions

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1. Materials and analytical methods

Amyloglucosidase from *Aspergillus niger* [glucoamylase, 1,4-α-D-glucan glucohydrolase, EC 3.2.1.3, ~70 U/mg (One unit corresponds to the amount of enzyme which liberates 1 μmole of glucose per minute at pH 4.8 and 60 °C)] was purchased from Sigma Aldrich. Glucose (HK) Assay Kit (Product Code GAHK 20) was used as received from Sigma-Aldrich. All reagents, 100-kDa cut off centrifugal ultrafilter (Millipore), and PageRuler Prestained Protein Ladder (Thermo) were obtained from different commercial suppliers and were used without further purification. All reactions were monitored by thin-layer chromatography with Haiyang GF254 silica gel plates. Flash column chromatography was performed using 100–200 mesh silica gel at increased pressure. Enzymatic assay of AnGA was completed with the method of Spectrophotometric Stop rate Determination.

2. Procedures of AnGA purification

A centrifugal ultrafiltration strategy was used for the separation and desalting of protein. Briefly, enzyme protein was resolved in ddH2O and passed through 100-kDa cut off centrifugal ultrafilters. The retentates were washed three times with ddH2O and then dissolved in 200 μL of ddH2O. Separated protein was analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) on a 10% gradient gel stained with Coomassie brilliant blue (Fig. 1s). When 1mg of purified AnGA was used to catalyze model Henry reaction, 52% of Henry product was obtained. And its natural activity was 27 U at pH 4.5 and 60 °C.
3. Enzymatic assay of AnGA

Unit definition (U/mg): One unit corresponds to the amount of enzyme which liberates 1 μmole of glucose per minute at pH 4.5 and 60 °C.

The procedures of enzymatic assay were based on literature \(^1\) from Sigma-Aldrich.

4. Characterization of Henry products

1-(2-Methoxyphenyl)-2-nitroethanol (3a) \(^2,3\)

![Chemical structure of 1-(2-Methoxyphenyl)-2-nitroethanol (3a)](image)

The title compound 3a was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 6/1) to give a light yellow oil (86% yield).

\(^1\)\text{HNMR (CDCl}_3, 400 MHz): δ 3.41 (d, \(J = 8\) Hz, 1H), 3.85 (s, 3H), 4.50-4.63 (m, 2H), 5.59 (br s, 1H), 6.89 (d, \(J = 8\) Hz, 1H), 6.98 (t, \(J = 8\) Hz, 1H), 7.31 (t, \(J = 8\) Hz, 1H), 7.40 (d, \(J = 8\) Hz, 1H).

\(^1\)\text{HNMR (CDCl}_3, 100 MHz): δ 55.43, 67.76, 79.91, 110.59, 121.12, 126.12, 127.17, 129.79, 156.04.

Fig. 1s SDS-PAGE analysis of AnGA
1-(3-Methoxyphenyl)-2-nitroethanol (3b)\textsuperscript{4, 5}

\[
\begin{array}{c}
\text{OH} \\
\text{OMe} \\
\text{NO}_2
\end{array}
\]

The title compound 3b was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 6/1) to give a yellow oil (77\% yield).

\textsuperscript{1}HNMR (CDCl\textsubscript{3}, 400 MHz): \(\delta\) 3.23 (br s, 1H), 3.80 (s, 3H), 4.45-4.59 (m, 2H), 5.39 (d, \(J = 8\) Hz, 1H), 6.86-6.94 (m, 3H), 7.29 (t, \(J = 8\) Hz, 1H).

\textsuperscript{13}CNMR (CDCl\textsubscript{3}, 100 MHz): \(\delta\) 55.35, 70.90, 81.25, 111.53, 114.37, 118.12, 130.11, 139.90, 160.02.

2-Nitro-1-(2-nitrophenyl)ethanol (3c)\textsuperscript{5, 7}

\[
\begin{array}{c}
\text{OH} \\
\text{NO}_2
\end{array}
\]

The title compound 3c was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 4/1) to give a brown oil (95\% yield).

\textsuperscript{1}HNMR (CDCl\textsubscript{3}, 400 MHz): \(\delta\) 3.50 (br s, 1H), 4.53-4.59 (m, 1H), 4.84-4.88 (m, 1H), 6.02-6.05 (m, 1H), 7.54-7.57 (m, 1H), 7.75 (t, \(J = 8\) Hz, 1H), 7.95 (d, \(J = 8\) Hz, 1H), 8.06 (d, \(J = 8\) Hz, 1H).

\textsuperscript{13}CNMR (CDCl\textsubscript{3}, 100 MHz): \(\delta\) 66.81, 80.20, 124.93, 128.71, 129.65, 134.36, 134.49, 147.02.

2-Nitro-1-(3-nitrophenyl)ethanol (3d)\textsuperscript{6, 7}

\[
\begin{array}{c}
\text{OH} \\
\text{NO}_2
\end{array}
\]

The title compound 3d was prepared according to the general procedure and purified by column
chromatography (petroleum ether/ethyl acetate = 4/1) to give a white solid (96% yield).

$^1$HNMR (CDCl$_3$, 400 MHz): $\delta$ 3.63 (br s, 1H), 4.62-4.64 (m, 2H), 5.61-5.62 (m, 1H), 7.61 (t, $J = 8$ Hz, 1H), 7.79 (d, $J = 8$ Hz, 1H), 8.19 (d, $J = 8$ Hz, 1H), 8.30 (s, 1H).

$^{13}$CNMR (CDCl$_3$, 100 MHz): $\delta$ 69.86, 80.72, 121.13, 123.69, 130.12, 132.31, 140.52, 148.34.

2-Nitro-1-(4-nitrophenyl)ethanol (3e)$^{6,7}$

The title compound 3e was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 4/1) to give a yellow solid (98% yield).

$^1$HNMR (CDCl$_3$, 400 MHz): $\delta$ 3.13 (br s, 1H), 4.58-4.61 (m, 2H), 5.60-5.62 (m, 1H), 7.63 (d, $J = 8$ Hz, 2H), 8.27 (dd, $J = 8$ Hz, 4 Hz, 2H).

$^{13}$CNMR (CDCl$_3$, 100 MHz): $\delta$ 69.97, 80.58, 124.21, 126.95, 144.90, 148.19.

1-(3-Chlorophenyl)-2-nitroethanol (3f)$^{4,5}$

The title compound 3f was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 8/1) to give a yellow oil (52% yield).

$^1$HNMR (CDCl$_3$, 400 MHz): $\delta$ 3.25 (br s, 1H), 4.48-4.52 (m, 1H), 4.54-4.60 (m, 1H), 5.43 (d, $J = 8$ Hz, 1H), 7.26-7.28 (m, 1H), 7.32-7.34 (m, 2H), 7.41 (s, 1H).

$^{13}$CNMR (CDCl$_3$, 100 MHz): $\delta$ 70.29, 80.97, 124.09, 126.22, 129.08, 130.33, 134.97, 140.17.

1-(4-Chlorophenyl)-2-nitroethanol (3g)$^{4,8}$
The title compound 3g was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 8/1) to give a yellow oil (82% yield).

\(^1\)HNMR (CDCl\(_3\), 400 MHz): \(\delta 3.51 \text{ (br s, 1H)}, 4.44-4.48 \text{ (m, 1H)}, 4.51-4.57 \text{ (m, 1H)}, 5.39 \text{ (d, } J = 8 \text{ Hz, 1H)}, 7.29-7.35 \text{ (m, 4H)}.

\(^1\)CNMR (CDCl\(_3\), 100 MHz): \(\delta 70.30, 81.04, 127.41, 129.17, 134.71, 136.72.

3-(1-Hydroxy-2-nitroethyl)benzonitrile (3h)

The title compound 3h was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 4/1) to give a white solid (97% yield).

\(^1\)HNMR (CDCl\(_3\), 400 MHz): \(\delta 3.39 \text{ (br s, 1H)}, 4.56-4.63 \text{ (m, 2H)}, 5.51-5.55 \text{ (m, 1H)}, 7.52-7.76 \text{ (m, 4H)}.

\(^1\)CNMR (CDCl\(_3\), 100 MHz): \(\delta 69.84, 80.80, 113.10, 118.24, 129.71, 129.86, 130.41, 132.47, 139.84.

4-(1-Hydroxy-2-nitroethyl)benzonitrile (3i)

The title compound 3i was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 4/1) to give a white solid (99% yield).

\(^1\)HNMR (CDCl\(_3\), 400 MHz): \(\delta 3.31 \text{ (d, } J = 4 \text{ Hz, 1H)}, 4.55-4.60 \text{ (m, 2H)}, 5.53-5.57 \text{ (m, 1H)}, 7.56
(d, J = 8 Hz, 2H), 7.70-7.72 (m, 2H).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 70.13, 80.74, 118.28, 126.78, 132.79, 143.41.

1-(2,6-Dichlorophenyl)-2-nitroethanol (3j)$^9$

The title compound 3j was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 12/1) to give a light yellow oil (93% yield).

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 3.64 (d, $J$ = 8 Hz, 1H), 4.53-4.57 (m, 1H), 5.11-5.17 (m, 1H), 6.20-6.25 (m, 1H), 7.22-7.26 (m, 1H), 7.33-7.35 (m, 2H).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 68.51, 77.63, 129.71, 130.64, 132.34, 134.77.

1-(2,4-Dichlorophenyl)-2-nitroethanol (3k)$^{3,6,11}$

The title compound 3k was prepared according to the general procedure and purified by column chromatography petroleum ether/ethyl acetate = 12/1) to give a light yellow oil (98% yield).

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 3.38 (d, $J$ = 4 Hz, 1H), 4.39-4.45 (m, 1H), 4.62-4.66 (m, 1H), 5.78 (d, $J$ = 8 Hz, 1H), 7.33 (d, $J$ = 8 Hz, 1H), 7.39 (s, 1H), 7.60 (d, $J$ = 8 Hz, 1H).

$^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 67.48, 79.20, 127.94, 128.58, 129.41, 132.07, 134.36, 135.08.

1-(4-Bromophenyl)-2-nitroethanol (3l)$^{5,11}$

The title compound 3l was prepared according to the general procedure and purified by column...
chromatography (petroleum ether/ethyl acetate = 8/1) to give a yellow oil (89% yield).

$^1$HNMR (CDCl$_3$, 400 MHz): $\delta$ 3.36 (br s, 1H), 4.46 (dd, $J$ = 12 Hz, 4 Hz, 1H), 4.52-4.58 (m, 1H), 5.40 (d, $J$ = 8 Hz, 1H), 7.26 (d, $J$ = 8 Hz, 2H), 7.50-7.53 (m, 2H).

$^{13}$CNMR (CDCl$_3$, 100 MHz): $\delta$ 70.35, 80.98, 122.88, 127.70, 132.14, 137.23.

1-(2-Furanyl)-2-nitroethanol (3m)$^{2,6}$

![Furan](image)

The title compound 3m was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 7/1) to give a yellow oil (6% yield).

$^1$HNMR (CDCl$_3$, 400 MHz): $\delta$ 2.89 (br s, 1H), 4.66-4.70 (m, 1H), 4.76-4.82 (m, 1H), 5.48 (dd, $J$ = 8 Hz, 4 Hz, 1H), 6.38-6.41 (m, 2H), 7.42-7.43 (m, 1H).

$^{13}$CNMR (CDCl$_3$, 100 MHz): $\delta$ 64.88, 78.40, 108.19, 110.67, 143.19, 150.72.

1-(2-thiophenyl)-2-nitroethanol (3n)$^6$

![Thiophene](image)

The title compound 3n was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 8/1) to give a yellow oil (8% yield).

$^1$HNMR (CDCl$_3$, 400 MHz): $\delta$ 3.07 (br s, 1H), 4.60-4.64 (m, 1H), 4.70-4.75 (m, 1H), 5.73 (d, $J$ = 8 Hz, 1H), 7.01-7.04 (m, 1H), 7.07-7.08 (m, 1H), 7.34-7.35 (m, 1H).

$^{13}$CNMR (CDCl$_3$, 100 MHz): $\delta$ 67.12, 80.79, 125.07, 126.19, 127.24, 141.23.

1-(2-Methoxyphenyl)-2-nitroethanol (3o)$^{12,13}$

![Methoxyphenyl](image)
The title compound 3o was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 15/1) to give a colourless oil (syn 41% yield) and a white solid (anti 31% yield).

$^1$H NMR (CDCl$_3$, 400 MHz): δ _syn_ isomer: 1.34 (d, $J = 8$ Hz, 3H), 3.30 (d, $J = 12$ Hz, 1H), 3.90 (s, 3H), 4.97-5.04 (m, 1H), 5.14 (t, $J = 8$ Hz, 1H), 6.92-7.02 (m, 2H), 7.26-7.36 (m, 2H). _anti_ isomer: 1.49 (d, $J = 4$ Hz, 3H), 3.04 (d, $J = 4$ Hz, 1H), 3.88 (s, 3H), 4.87-4.93 (m, 1H), 5.53-5.55 (m, 1H), 6.90 (d, $J = 8$ Hz, 1 Hz, 1H); 7.00 (t, $J = 8$ Hz, 1H), 7.29-7.33 (m, 1H), 7.41-7.44 (m, 1H).

$^{13}$C NMR (CDCl$_3$, 100 MHz): δ _syn_ isomer: 16.60, 55.45, 74.19, 87.65, 110.99, 121.24, 125.90, 129.02, 130.10, 156.76. _anti_ isomer: 12.59, 55.40, 70.77, 85.07, 110.39, 120.97, 126.25, 127.66, 129.47, 155.79.

1-(4-nitrophenyl)-2-nitropropan-1-ol (3p) $^{12,14}$

The compound 3p was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 8/1) to give a white solid (87% yield).

$^1$H NMR (CDCl$_3$, 400 MHz): δ 1.38 (d, $J = 4$ Hz, 2H), 1.49 (d, $J = 8$ Hz, 1H), 3.29 (br s, 1H ), 4.72-4.80 (m, 1H), 5.18-5.21 (m, 0.58H), 5.57 (s, 0.42H), 7.58-7.60 (m, 2H), 8.23-8.26 (m, 2H).

$^{13}$C NMR (CDCl$_3$, 400 MHz): δ 11.83, 16.20, 72.93, 75.05, 86.83, 87.83, 123.93, 124.09, 127.05, 127.96, 145.37, 145.67, 147.86, 148.24.

4-(1-hydroxy-2-nitropropyl)benzonitrile (3q) $^{12,15}$
The compound 3q was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 6/1) to give colourless oil (89% yield).

$^1$H NMR (CDCl$_3$, 400 MHz): δ 1.34-1.48 (m, 3H), 3.19 (br s, 1H), 4.68-4.75 (m, 1H), 5.12 (d, $J = 8$ Hz, 0.50H), 5.49 (d, $J = 4$ Hz, 0.50H), 7.52-7.55 (m, 2H), 7.67-7.71 (m, 2H).

$^{13}$C NMR (CDCl$_3$, 400 MHz): δ 11.86, 16.18, 73.08, 75.24, 86.90, 87.86, 112.05, 112.67, 118.26, 118.42, 126.89, 127.79, 132.54, 132.71, 143.72.

1-(4-bromophenyl)-2-nitropropan-1-ol (3r)

The compound 3r was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 15/1) to give a white solid (65% yield).

$^1$H NMR (CDCl$_3$, 400 MHz): δ 1.32, (d, $J = 8$ Hz, 2H), 1.48 (d, $J = 8$ Hz, 1H), 2.80-2.88 (m, 1H), 4.62-4.75 (m, 1H), 5.00 (d, $J = 8$ Hz, 0.64H), 5.36 (s, 0.36H), 7.24-7.27 (m, 2H), 7.50-7.55 (m, 2H).

$^{13}$C NMR (CDCl$_3$, 100 MHz): δ 11.99, 16.33, 73.32, 75.55, 87.17, 88.17, 122.47, 123.17, 127.45, 128.63, 131.87, 132.14, 137.32, 137.55.

1-(2-Methoxyphenyl)-2-nitrobutan-1-ol (3s)

The compound 3s was prepared according to the general procedure and purified by column
chromatography (petroleum ether/ethyl acetate = 15/1) to give a yellow oil (anti 31% yield, syn 30% yield).

$^1$HNMR (CDCl$_3$, 400 MHz): $\delta$ syn isomer: 0.87 (t, $J = 6$ Hz, 3H), 1.37-1.47 (m, 1H), 1.85-1.97 (m, 1H), 3.33 (d, $J = 8$ Hz, 1H), 3.89 (s, 3H), 4.81-4.87 (m, 1H), 5.13 (t, $J = 10$ Hz, 1H), 6.92-7.02 (m, 2H), 7.25-7.36 (m, 2H). anti isomer: 0.92 (t, $J = 8$ Hz, 3H), 1.86-1.96 (m, 1H), 2.08-2.20 (m, 1H), 3.37 (d, $J = 8$ Hz, 1H), 3.88 (s, 3H), 4.75-4.80 (m, 1H), 5.24 (t, $J = 6$ Hz, 1H), 6.89-7.02 (m, 2H), 7.26-7.34 (m, 2H).

$^{13}$CNMR (CDCl$_3$, 100 MHz): $\delta$ syn isomer: 10.22, 24.11, 55.47, 73.42, 94.47, 111.00, 121.23, 126.16, 128.93, 130.07, 156.74. anti isomer: 10.48, 21.70, 55.44, 72.10, 92.58, 110.64, 121.00, 126.02, 128.30, 129.67, 156.18.

1-(4-nitrophenyl)-2-nitrobutan-1-ol (3t)$^{13,16}$

The compound 3t was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 8/1) to give a white solid (87% yield).

$^1$HNMR (CDCl$_3$, 400 MHz): $\delta$ 0.89-0.96 (m, 3H), 1.80-2.24 (m, 2H), 3.25-3.29 (m, 1H), 4.57-4.65 (m, 1H), 5.17-5.20 (m, 0.61H), 5.33-5.34 (m, 0.39H), 7.58-7.60 (m, 2H), 8.21-8.26 (m, 2H).

$^{13}$CNMR (CDCl$_3$, 100 MHz): $\delta$ 10.05, 10.33, 21.26, 23.82, 73.27, 74.32, 94.16, 94.63, 123.89, 124.11, 127.30, 127.88, 145.73, 147.94, 148.22.

4-(1-Hydroxy-2-nitrobutyl)benzonitrile (3u)$^{12,15}$
The compound 3u was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 6/1) to give a white solid (87% yield).

$^1$HNMR (CDCl$_3$, 400 MHz): $\delta$ 0.90-0.96 (m, 3H), 1.46-1.50 (m, 1H), 1.81-1.95 (m, 1H), 2.97 (d, $J = 4$ Hz, 1H), 4.53-4.62 (m, 1H), 5.10-5.13 (m, 0.70H), 5.26-5.28 (m, 0.30H), 7.51-7.53 (m, 2H), 7.67-7.72 (m, 2H).

$^{13}$CNMR (CDCl$_3$, 100 MHz): $\delta$ 10.07, 10.34, 21.33, 23.79, 73.42, 74.54, 94.23, 94.68, 112.31, 112.76, 118.24, 118.38, 127.12, 127.71, 132.51, 132.73, 144.00.

1-(4-bromophenyl)-2-nitrobutan-1-ol (3v)$^{12,15}$

The compound 3v was prepared according to the general procedure and purified by column chromatography (petroleum ether/ethyl acetate = 15/1) to give a colorless oil (74% yield).

$^1$HNMR (CDCl$_3$, 400 MHz): $\delta$ 0.86-0.95 (m, 3H), 1.39-1.46 (m, 1H), 1.79-1.89 (m, 1H), 2.69 (s, 1H), 4.50-4.59 (m, 1H), 5.01 (d, $J = 8$ Hz, 0.77H), 5.14 (d, $J = 4$ Hz, 0.23H), 7.24-7.26 (m, 2H), 7.50-7.55 (m, 2H).

$^{13}$CNMR (CDCl$_3$, 100 MHz): $\delta$ 10.06, 10.36, 21.33, 23.88, 73.62, 74.82, 94.44, 94.96, 122.71, 123.18, 127.95, 128.55, 131.90, 132.18, 137.57, 137.66.

5. List of the obvious difference between syn- and anti- isomers on $^1$HNMR

<table>
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<th>Entry</th>
<th>Product</th>
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12
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</table>

6. $^1$HNMR and $^{13}$CNMR spectra for Henry products
3a

3b
7. References


