Supplementary Information

Chemoenzymatic access to enantiopure N-containing furfuryl alcohol from chitin-derived N-acetyl-D-glucosamine

Cheng Hao, a Min-Hua Zong, a Zhi-Lin Wang, b* Ning Li a*

a School of Food Science and Engineering, South China University of Technology, 381 Wushan Road, Guangzhou 510640, China
b Agro-biological Gene Research Center, Guangdong Academy of Agricultural Sciences, 20 Jinying Road, Guangzhou, 510640, China
* Corresponding authors.
Z.L. Wang, Email: wangzhilin@gdaas.cn
Dr. N. Li, Email: lining@scut.edu.cn

**Fig. S1.** SDS-PAGE analysis of purified ScCR and YueD. Lane M: protein marker

| Sample       | Specific rotation | Used dehydrogenase |
|--------------|-------------------|--------------------|
| (R)-3A5HEF   | 6.7               | ScCR               |
| (S)-3A5HEF   | -6.8              | YueD               |

Conditions: 179 mg of the corresponding sample was added to 10 mL ethanol and measured at 589 nm and 25 °C in 1 dc polarimeter tube.
**Fig. S2.** Effect of 2-propanol concentrations on asymmetric reduction of 3A5AF catalyzed by *E. coli* ScCR cells. Reaction conditions: 10 mM 3A5AF, 20 mg/mL cells, 50-1000 mM 2-propanol, 1 mL sodium phosphate buffer (0.1 M, pH 7.0) containing 5% DMSO (v/v), 30 °C, 150 r/min.

**Scheme S1.** Construction of recombinant pET28a-GDH-ScCR plasmid.
**Fig. S3.** SDS-PAGE analysis of protein present in the supernatant and precipitant
Lane M: protein marker; Lane 1: supernatant of *E. coli*/pET28a; Lane 2: precipitant of *E. coli*/pET28a; Lane 3: supernatant of *E. coli* GDH ScCR; Lane 4: precipitant of *E. coli* GDH ScCR; Lane 5: supernatant of *E. coli* ScCR; Lane 6: precipitant of *E. coli* ScCR; Lane 7: supernatant of *E. coli* GDH; Lane 8: precipitant of *E. coli* GDH

**Fig. S4.** Effect of substrate concentrations (A) and product concentrations (B) on ScCR-catalyzed synthesis of 3A5HEF. Conditions for Figure S4A: 1-10 mM 3A5AF, 1 mg/mL ScCR, 0.05 mg/mL GDH cell-free extract, 0.1 mM NADH, Molglucose : Mol3A5AF= 2:1, 1 mL sodium phosphate buffer (0.1 M, pH 7.0) containing 10% DMSO (v/v), 35 °C, 150 r/min, 0.5 h; conditions for Figure S4B: 10 mM 3A5AF, 1 mg/mL ScCR, 0.05 mg/mL GDH, 0.1 mM NADH, 20 mM glucose, 1 mL sodium phosphate buffer (0.1 M, pH 7.0) containing 10% DMSO (v/v), 35 °C, 150 r/min, 12-120 mM 3A5HEF. The relative activities were based on the changes in the substrate concentrations after the reaction of 0.5 h.
Fig. S5. HPLC analysis of (R)-3A5HEF obtained on a preparative-scale experiment. General conditions: the mobile phase: a mixture of acetonitrile and 0.4% (NH₄)₂SO₄ aqueous solution (pH 3.5, 5/95, v/v); flow rate: 0.6 mL/min.

1.1.1 Expression and purification of RalADH

The recombinant plasmid pET22b-RalADH was synthesized by Nanjing GenScript Biotechnology Co., Ltd. with codon optimization for E. coli. The enzyme expression was performed according to the method described previously (Kulig, et al., 2012, Lavandera, et al., 2008). Similarly, the enzyme purification steps were the same as those for purifying ScCR, with the exception of the elution buffer (300 mM imidazole, 500 mM NaCl, pH 7.0, 100 mM sodium phosphate buffer). The purified enzyme was subjected to SDS-PAGE analysis (Fig. S6).

Fig. S6. SDS-PAGE analysis of purified RalADH. Lane M: protein marker

1.1.2 Expression and purification of AceCR

The expression of AceCR was performed according to the method previously described (Wei, et al., 2017). The enzyme purification steps were the same as the previous report (Wei, et al., 2017), with the exception of using pH 7.0 sodium phosphate buffer (instead of pH 6.5) in binding, elution and desalting buffers. The purified enzyme was subjected to SDS-PAGE analysis (Fig. S7).
1.1.3 Expression and purification of SynADH

The expression of SynADH was performed as described by Jia et al (Jia, et al., 2019) with slight modifications in the induction conditions (0.5 mM IPTG at 20 °C and 160 r/min). Besides, the purification steps of SynADH were the same as those for purifying ScCR. The purified enzyme was subjected to SDS-PAGE analysis (Fig. S8).

1.1.4 Expression and purification of HLADH

The expression of HLADH was performed as described by Jia et al (Jia, et al., 2019). Similarly, the enzyme purification steps were the same as those for purifying ScCR. The purified enzyme was subjected to SDS-PAGE analysis (Fig. S9).
1.1.5 Expression and purification of ADH434 and AAD1669

*Pichia pastoris* X-33/pPICZα A-ADH434 and *P. pastoris* X-33/pPICZα A-
AAD1669 (from *Meyerozyma guilliermondii* SC1103) were previously constructed by
our laboratory (Xia, et al., 2020). Prior to use, cells were inoculated on YPD plates and
cultivated at 30 °C for 3 d. A single colony was picked out and pre-cultivated in 30 mL
BMGY medium at 30 °C and 230 r/min until the OD_{600} of the culture reached 2.0 ~ 6.0
(approximately 24-36 h). Upon centrifugation, the yeast cells were resuspended with
100 mL of BMMY medium, so that the OD_{600} of the culture was 1. The culture was
cultivated at 30 °C and 230 r/min to induce the enzymes expression for approximately
3 d. Methanol was added every 24 h until its final concentration reached 1% (v/v).

Upon centrifugation (15285 × g, 20 min) at 4 °C, the supernatant was collected.
At °C, ammonium sulfate powder was slowly added to 80% saturation, continue stirring
for another 2 hours. After centrifugation for 15 min (15285 × g, 4 °C), the protein
precipitates were collected. The precipitates were resuspended with pH 7.0 sodium
phosphate buffer (100 mM). The crude enzyme solutions were obtained after dialysis
and concentration. Fig. S10 shows SDS-PAGE analysis of the crude enzyme.

![Fig. S9. SDS-PAGE analysis of purified HLADH. Lane M: protein marker](image)

![Fig. S10. SDS-PAGE analysis of crude ADH434 and AAD1669. Lane M: protein marker](image)
1.2 Enzyme assay

Carbonyl reductase/alcohol dehydrogenase activities were determined by monitoring the changes in the absorbances at 340 nm within 3 min using a Shimadzu UV2550 spectrophotometer (Japan). The enzyme-catalyzed reduction of benzaldehyde/COBE was conducted with NAD(P)H at 30 °C in 0.4 mL sodium phosphate buffer (0.1 M, pH 7.0). One U corresponds to the amount of enzyme which oxidizes 1 μmol NAD(P)H per minute at 30 °C and pH 7.0. The specific activities of ScCR, YueD, RalADH, AceCR, SynADH and HLADH were presented in Table S2.

Table S2. Specific activities of various CRs/ADHs.

| Entry | Enzyme | Substrate | Cofactor       | Specific activity (U/mg) |
|-------|--------|-----------|----------------|-------------------------|
| 1     | ScCR   | COBE      | 0.1 mM NADH    | 23.4                    |
| 2     | YueD   | COBE      | 0.1 mM NADPH   | 1.1                     |
| 3     | RalADH | benzaldehyde | 0.5 mM NADPH | 0.2                     |
| 4     | AceCR  | COBE      | 0.5 mM NADH    | 17.5                    |
| 5     | SynADH | benzaldehyde | 0.5 mM NADPH | 2.4                     |
| 6     | HLADH  | benzaldehyde | 0.5 mM NADH | 6.6                     |

Reaction conditions: 5 mM substrate, 0.1/0.5 mM NAD(P)H, 0.4 mL sodium phosphate buffer (0.1 M, pH 7.0), 30 °C

Table S3. Kinetic parameters of two enzymes using 3A5AF as a substrate.

| Enzyme | $k_{\text{cat}}$ (s$^{-1}$) | $K_m$ (mM) | $K_{\text{cat}}/K_m$ (M$^{-1}$ s$^{-1}$) |
|--------|-----------------------------|-------------|--------------------------------------|
| ScCR$^a$ | 0.1                          | 6.3         | 15.9                                 |
| YueD$^b$ | $0.7 \times 10^{-3}$       | 0.5         | 1.4                                  |

$^a$Reactions were performed in sodium phosphate buffer (0.1 M, pH 7.0) at 25 °C; the native molecular mass of ScCR: 326 kDa. $^b$Reactions were performed in sodium phosphate buffer (0.1 M, pH 8.0) at 25 °C; the native molecular mass of YueD: 59.3 kDa.

Fig. S11. $^1$H NMR of 3A5AF (DMSO-$d_6$, 600 MHz)
**Fig. S12.** $^{13}$C NMR of 3A5AF (DMSO-$d_6$, 125 MHz)

**Fig. S13.** $^1$H NMR of 3A5HEF (DMSO-$d_6$, 600 MHz)
Fig. S14. $^{13}$C NMR of 3A5HEF (DMSO-$d_6$, 125 MHz)

Fig. S15. The chiral HPLC spectrum of 3A5HEF prepared by chemical method. The retention time of the products as follows: $t \ [(R)-3A5HEF] = 19.7 \text{ min}$, $t \ [(S)-3A5HEF] = 17.2 \text{ min}$.
Fig. S16. The chiral HPLC spectrum of \((R)-3\text{A5HEF}\) obtained on a preparative-scale experiment.

Fig. S17. The chiral HPLC spectrum of \((S)-3\text{A5HEF}\)

Fig. S18. HPLC analysis of the reaction mixture in enzymatic reduction of \(3\text{A5AF}\)
General conditions: the mobile phase: a mixture of acetonitrile and 0.4% (NH₄)₂SO₄ aqueous solution (pH 3.5, 2/8, v/v); flow rate: 0.6 mL/min.

Fig. S19. The chiral HPLC spectrum of the reaction mixture in enzymatic reduction of 3A5AF to (R)-3A5HEF

Fig. S20. The chiral HPLC spectrum of the reaction mixture in enzymatic reduction of 3A5AF to (S)-3A5HEF

RalADH gene sequence (Accession number: EU485985)

atgtatcacttattttatatatgacctgctataacccggttggtgtggttgaagttccggagagcgctggcgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtttgtgtggagacacctagttacgaggtt
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