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One-pot chemoenzymatic access to a cefuroxime precursor *via* C1 extension of furfural†

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In this work, one-pot three-step synthesis of (*Z*)-2-methoxyimino-2-(furan-2-yl)acetic acid (SMIA), a key precursor for cefuroxime, was reported from biomass-derived furfural in 81% yield *via* sequential whole-cell catalytic hydroxymethylation by *Escherichia coli* harboring pyruvate decarboxylase, chemoenzymatic oxidation by the laccase-TEMPO system, and spontaneous oximation. In gram-scale production, SMIA was obtained in 63% isolated yield.

Cefuroxime, one of the second-generation semi-synthetic cephalosporins, is of high importance in clinical prevention and treatment of bacterial infection, because of its high antimicrobial activity against a wide spectrum of Gram-positive and Gram-negative microorganisms and good stability against β-lactamases.[1,2] The antibiotic is clinically used in the forms of the sodium salt and the ester prodrug cefuroxime axetil (Scheme 1). (*Z*)-2-Methoxyimino-2-(furan-2-yl)acetic acid (SMIA) is a key intermediate for the manufacture of cefuroxime.[3] Currently, SMIA is commercially produced *via* a sequence of Friedel–Crafts acylation, oxidation (comprising oximation, Beckmann rearrangement and amide hydrolysis), and oximation, starting from furan that may be derived from non-renewable fossil resources or renewable furfural (Scheme 1A); the overall yield of the target product approaches 34%.[4,5] Also, the laboratory-scale synthesis of 2-(furan-2-yl)-2-oxoacetic acid (FOAc), the key intermediate for producing SMIA, was described from renewable 2-furoic acid *via* a series of chemical reactions in a total yield of approximately 77%, but highly toxic NaCN was involved (Scheme 1B).[4] Recently, Chen *et al.* presented an attractive route toward FOAc *via* the dehydration of 2-keto-L-gulonic acid that may be obtained by D-glucose fermentation (Scheme 1C).[6] Notably, the reaction under the optimized conditions (at 130 °C in methanol for 0.5 h) provided access to the methyl ester of FOAc, with 79% yield. Herein, we present an alternative chemoenzymatic route toward FOAc under mild conditions (Scheme 1D), where furfural is converted into 1-(furan-2-yl)-2-hydroxyethan-1-one (FHEO) *via* whole-cell catalytic one-carbon (C1) extension with formaldehyde, followed by aerobic oxidation by the laccase-TEMPO (2,2,6,6-tetramethylpiperidine-*N*-oxyl) system (LTS).

Recently, the production of renewable chemicals from biomass and C1 feedstocks (*e.g.*, CO_2 and its derivatives) has attracted great attention,[7–9] because of growing concerns over non-renewable fossil carbon resources risk and environmental issues such as global warming. Furfural obtained *via* xylose dehydration has been recognized as one of the top value-added biobased platform chemicals,[10] which can be converted into a variety of commercially interesting chemicals.[11] The large-scale production of furfural has been running for approximately a century from agroindustrial waste, like corncob and oat hull.[12–14] Its global market was estimated to reach approximately USD 523 million in 2021, with the volume of more than 300 kilotons, and might grow at a compound annual growth rate of 6.5% from 2022 to 2030.[15] Formaldehyde is an important C1 chemical, which may be obtained *via* CO_2 reduction. From a sustainability viewpoint, the C1 extension strategy is highly attractive for the synthesis of SMIA, because both feedstocks are renewable, along with operating under mild conditions and use of environmentally friendly catalysts.

Given its powerful C–C ligation ability,[16–18] thiamine diphosphate (ThDP)-dependent benzaldehyde lyase from *Pseudomonas fluorescens* (*Pf*BAL) was initially used for the hydroxymethylation of furfural with formaldehyde (Fig. S1, ESI†). Indeed, FHEO was furnished with high yields (>97%) at 10 mM furfural loading. In addition, 3 equiv. formaldehyde seemed to be optimal for FHEO synthesis (Fig. S2, ESI†). When the substrate loading was increased to 30 mM, 81% furfural conversion was observed within 4 h in the presence of 1 g L^{-1} purified *Pf*BAL; nonetheless, the FHEO yield was approximately 59% (data not shown), because the formation of the by-product furoin resulted from enzymatic self-ligation of furfural.[18,19] Considering the limited

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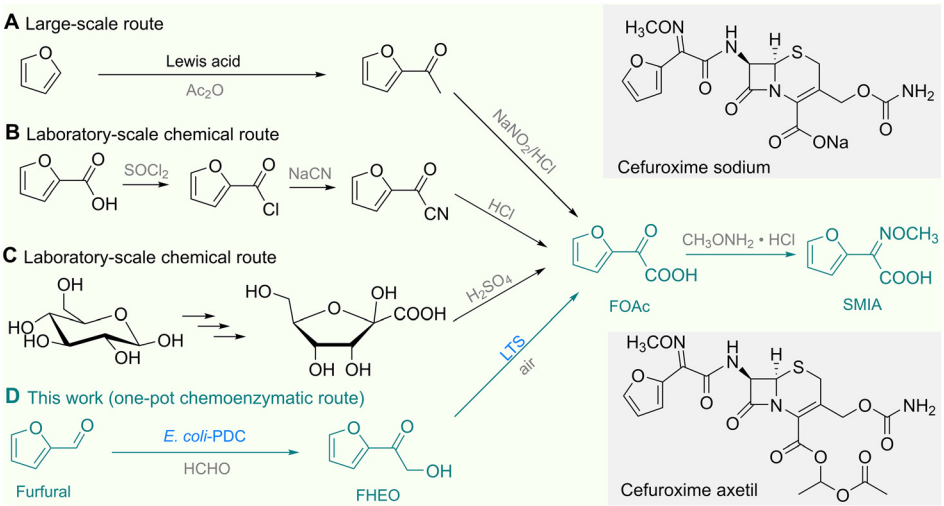

Scheme 1 Synthetic routes toward SMIA and structures of cefuroxime sodium and cefuroxime axetil.

potential of *Pf*BAL in the hydroxymethylation of furfural, we switched our attention to pyruvate decarboxylase from *Sulfobacillus* sp. hq2 (*Sul*PDC), because the enzyme was recently reported for efficient hydroxymethylation of aldehydes.[20–22] The gene of the enzyme linked with maltose binding protein (to enhance the soluble expression of the target protein in *Escherichia coli*) was synthesized, and its heterologous expression was performed in *E. coli*. Based on the SDS-PAGE analysis (Fig. S3, ESI†), good soluble expression of *Sul*PDC was realized in *E. coli*. Then, the resting cells of *E. coli-Sul*PDC were exploited for the hydroxymethylation of furfural (Fig. 1). Strikingly, FHEO was produced within 10 h in approximately 89% yield at 75 mM substrate loading in the presence of 1.5 equiv. formaldehyde. And even at 100 mM substrate loading, 85% yield was achieved, in spite of requiring a longer reaction period. The results suggest that *Sul*PDC is a powerful enzyme for C1 extension of furfural, which is in good agreement with a recent report.[20]

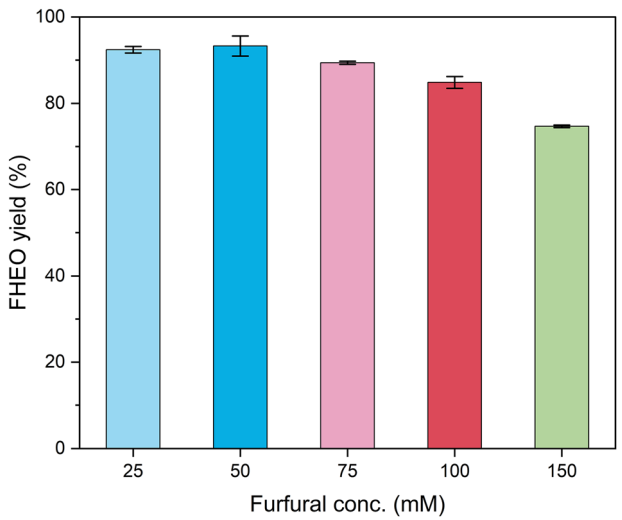

Fig. 1 Biocatalytic FHEO synthesis at different substrate loadings. Reaction conditions: 25–150 mM furfural, 1.5 equiv. formaldehyde, 25 g L^{-1} *E. coli-Sul*PDC cells (wet weight), 0.15 mM ThDP, 2.5 mM Mg^{2+}, 1 mL phosphate buffer (50 mM, pH 6), 35 °C, 150 rpm, 10 h. Notably, the reaction time is 24 h at 100–150 mM substrate loadings.

Then, identification of suitable catalysts was performed for the oxidation of FHEO into FOAc. The oxidation of alcohol into carboxylic acid comprises two consecutive oxidation reactions, namely the oxidation of alcohol into aldehyde and the oxidation of the resulting aldehyde into carboxylic acid. Generally, two catalysts such as alcohol dehydrogenase/oxidase and aldehyde dehydrogenase/oxidase are required for implementing such transformations.[23] Interestingly, single-catalysts such as horse liver alcohol dehydrogenase (HLADH),[24] and LTS[25] were reported to be capable of directly oxidizing alcohols into the corresponding carboxylic acids. Considering the sluggish kinetics of HLADH-catalyzed oxidations, LTS was examined for HEFO oxidation (Fig. 2). In fact, LTS is a well-known catalytic system for aerobic oxidation of alcohols, and usually cannot accept aldehydes as substrates. However, some aldehydes, like 2-(furan-2-yl)-2-oxoacetaldehyde (FOAa) here, may be hydrated under specific reaction conditions (*e.g.* pH 6), resulting in the formation of *gem*-diols that act as the actual substrates for further enzymatic oxidation (Fig. 2A).[25] As shown in Fig. 2B, the two-step oxidation of FHEO proceeded smoothly in the presence of LTS. After 2 h, FOAc was obtained in a quantitative yield.

Considering the equal solvents and pH between the two individual reactions, a concurrent chemobiocatalytic cascade toward FOAc was performed by the combination of *E. coli-Sul*PDC and LTS, starting from furfural. The consumption of furfural and formation of FHEO were observed within 2 h, but furfural instead of FHEO and the target product FOAc was detected after the reaction of 6 h (data not shown). To unveil the underlying reasons for this, the following experiments were designed and performed. Like pyruvate, FOAc is an α-keto acid; thus, it is assumed that the chemical may be a substrate of *Sul*PDC, and the enzyme may promote the decarboxylation of the chemical. Accordingly, the incubation of FOAc was conducted in the presence of *E. coli-Sul*PDC. Indeed, furfural was

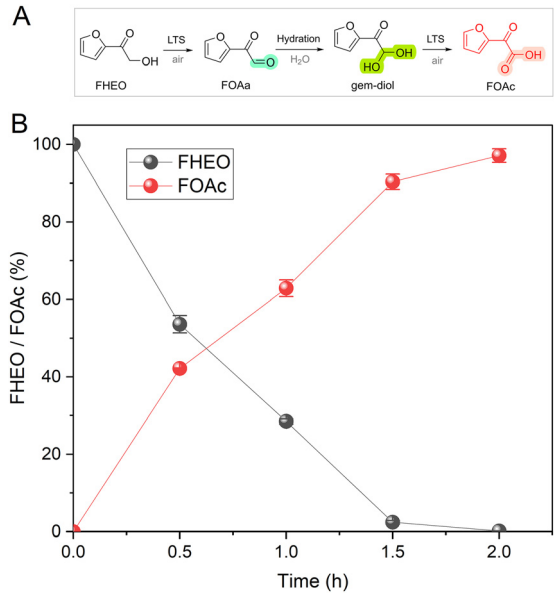

Fig. 2 Oxidation of FHEO into FOAc by LTS: (A) the reaction scheme; (B) the reaction curve. Reaction conditions: 20 mM FHEO, 3 g L^{-1} crude laccase, 50 mol% TEMPO, 35 °C, 150 rpm, 1 mL phosphate buffer (50 mM, pH 6).

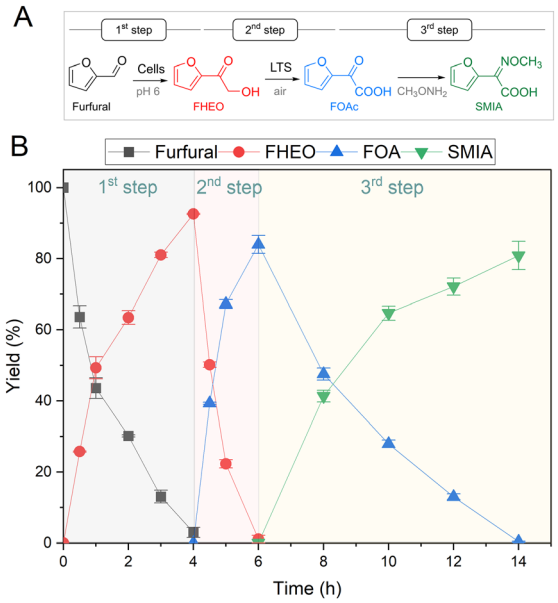

Fig. 3 One-pot three-step synthesis of SMIA from furfural: (A) the reaction scheme; (B) the reaction curve. Reaction conditions: 20 mM furfural, 1.5 equiv. formaldehyde, 25 g L^{-1} *E. coli-Sul*PDC cells (wet weight), 0.15 mM ThDP, 2.5 mM Mg^{2+}, 1 mL phosphate buffer (50 mM, pH 6), 35 °C, 150 rpm, 4 h; removing cells, adding 3 g L^{-1} crude laccase, 50 mol% TEMPO, 35 °C, 150 rpm, 2 h; adding 1 mL CH_3OH, 1.2 equiv. CH_3ONH_2·HCl, 4 °C, no stirring, 8 h.

produced in 83% yield after 2 h. Therefore, FOAc would be rapidly degraded into furfural by *Sul*PDC in the concurrent mode once it is formed, which may be a rational explanation for the above results. On the other hand, the effect of TEMPO on whole-cell catalytic hydroxymethylation of furfural was studied (Fig. S4, ESI†), because we previously found that TEMPO exerted negative or positive effects on whole-cell catalytic transformations.[26,27] It was found that the FHEO yield in the presence of 4 mM TEMPO was approximately half of that of the control without TEMPO. Besides, decreased yields of FHEO were observed with the increment of the TEMPO concentrations. This indicates that TEMPO has a greatly detrimental effect on the *Sul*PDC-based whole-cell catalysis.

Therefore, one-pot sequential synthesis of SMIA was performed *via* hydroxymethylation by *E. coli-Sul*PDC cells, oxidation by LMS and spontaneous oximation (Fig. 3). As shown in Fig. 3B, furfural was completely consumed within 4 h, affording FHEO in 93% yield. Then, the cells were removed by centrifugation, followed by the addition of laccase and TEMPO to initiate the aerobic oxidation of FHEO. After 2 h, FOAc was obtained with 84% yield. Finally, methoxyamine hydrochloride was supplemented, followed by reaction at 4 °C. The target product SMIA was produced in 81% total yield.

To showcase the practicability of the process, scale-up synthesis of FOAc was performed on a 20-mL scale, starting from 20 mM furfural. It was found that the target product FOAc was produced in approximately 92% yield after 8 h. Upon extraction with ethyl acetate, the product was obtained in 62% isolated yield. The structure of the product isolated was verified by ^1H and ^{13}C NMR analysis (Fig. S5 and S6, ESI†), and its purity is good. Encouraged by the above results, gram-scale synthesis of SMIA was conducted at 0.1 M substrate loading on a 50 mL-scale. Likewise, furfural was completely transformed within 4 h. However, the second step (namely FHEO oxidation) became much slower at the high substrate loading (100 mM) than at the low substrate loading (20 mM). Consequently, the reaction period of 20 h was required for the complete oxidation of the intermediate. It is well known that hydrophobic O_2 is scarcely dissolved in aqueous solutions (approximately 0.25 mM from air at 25 °C).[28] The sluggish oxidation in preparative-scale synthesis might be attributed to the great mass transfer limitation of O_2. It is envisioned that the oxidation process may be significantly intensified by enhancing O_2 supply, for example, using continuous flow reactors.[28–31] Pleasingly, the spontaneous oximation proceeded rapidly, and FOAc was completely converted within 6 h. Upon solvent exaction, the target product SMIA (around 0.53 g) was isolated with 63% yield. Its structure was confirmed by ^1H (Fig. S7, ESI†) and ^{13}C NMR spectra (Fig. S8, ESI†), and high-resolution mass spectrometry (Fig. S9, ESI†), along with good purity. Also, the gram-scale synthesis of (*E*)-2-(methoxyimino)-2-phenylacetic acid (MIPA), an analog of SMIA, was implemented *via* the one-pot three-step protocol, starting from benzaldehyde. After 46 h, 0.61 g of the solid was obtained with approximately 69% isolated yield *via* solvent extraction. Based on NMR analysis (Fig. S15 and S16, ESI†), it was assigned as MIPA. However, minor impurities are present in the isolated product according to HPLC (Fig. S14, ESI†) and NMR. Anyway, this indicates the universality of the protocol, and it may be applied to produce a variety of SMIA analogs.

In conclusion, a sustainable route toward SMIA, a key precursor for cefuroxime, was developed in this work, where biobased furfural was subjected to whole-cell catalytic hydroxymethylation with

formaldehyde by *E. coli-Sul*PDC cells, chemoenzymatic oxidation by LTS, and spontaneous oximation. Compared to *Pf*BAL, *Sul*PDC was a better catalyst for the hydroxymethylation of furfural, but the presence of the latter could result in the degradation of the key intermediate FOAc. Therefore, removal of the *E. coli-Sul*PDC cells is necessary prior to the FHEO oxidation by LTS. As a result, one-pot synthesis of SMIA was implemented in a stepwise manner, in 81% yield. More importantly, the practicability of the process was demonstrated by successful gram-scale production of SMIA. The discovery and engineering of high-activity BALs may allow for simultaneous synthesis of FOAc, since this type of enzyme cannot degrade the chemical. Overall, the findings of the work may pave the way for sustainable manufacture of cefuroxime.

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Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

References

1 C. H. O'Callaghan, R. B. Sykes, A. Griffiths and J. E. Thornton, *Antimicrob. Agents Chemother.*, 1976, **9**, 511–519.
2 C. M. Perry and R. N. Brogden, *Drugs*, 1996, **52**, 125–158.
3 S. Kumar Singh, R. P. Tiwari and P. Jain, *Synth. Commun.*, 2003, **33**, 2475–2482.
4 L. Hu, B. Hu, X. Yang and W.-B. Jia, *Shandong Chem. Ind.*, 2010, **39**, 22–24.
5 J.-R. Jiang, X.-F. Liu and J.-J. Chen, *Chin. J. Antibiot.*, 2005, **30**, 304.
6 J. Chen, Z. Zhu, J. Cai, M. Cao, F. Sha, S. Tao, H. Yang, H. Gan, H. Wu, F. Cao, L. Zhao and P. Ouyang, *Green Chem.*, 2022, **24**, 2000–2009.
7 R. A. Sheldon, *Curr. Opin. Green Sustainable Chem.*, 2018, **14**, 89–95.
8 W. Jiang, D. Hernández Villamor, H. Peng, J. Chen, L. Liu, V. Haritos and R. Ledesma-Amaro, *Nat. Chem. Biol.*, 2021, **17**, 845–855.
9 Y. Li, X. Cui, K. Dong, K. Junge and M. Beller, *ACS Catal.*, 2017, **7**, 1077–1086.
10 J. J. Bozell and G. R. Petersen, *Green Chem.*, 2010, **12**, 539–554.
11 N. Li and M.-H. Zong, *ACS Catal.*, 2022, **12**, 10080–10114.
12 D. T. Win, *Assumpt. Univ. J. Technol.*, 2005, **8**, 185–190.
13 A. Jaswal, P. P. Singh and T. Mondal, *Green Chem.*, 2022, **24**, 510–551.
14 R. Mariscal, P. Maireles-Torres, M. Ojeda, I. Sadaba and M. Lopez Granados, *Energy Environ. Sci.*, 2016, **9**, 1144–1189.
15 S.-M. Zou, J.-P. Wang, M.-H. Zong, Z.-L. Wang, Z.-J. Zheng and N. Li, *Green Chem.*, 2023, **25**, 6892–6900.
16 P. Domínguez de María, T. Stillger, M. Pohl, M. Kiesel, A. Liese, H. Gröger and H. Trauthwein, *Adv. Synth. Catal.*, 2008, **350**, 165–173.
17 A. S. Demir, P. Ayhan, A. C. Igdir and A. N. Duygu, *Tetrahedron*, 2004, **60**, 6509–6512.
18 S. Shanmuganathan, D. Natalia, L. Greiner and P. Dominguez de Maria, *Green Chem.*, 2012, **14**, 94–97.
19 P. Dominguez de Maria, T. Stillger, M. Pohl, S. Wallert, K. Drauz, H. Groger, H. Trauthwein and A. Liese, *J. Mol. Catal. B: Enzym.*, 2006, **38**, 43–47.
20 X. Zhang, H. Wei, X. Wei, T. Qi, X. Zong, Z. Liu, J. Qin, X. Gao, G. Zheng and Q. Ma, *Green Chem.*, 2023, **25**, 4713–4722.
21 Y. Liu, Z. Fu, H. Dong, J. Zhang, Y. Mao, M. Zheng and C. Liao, *Angew. Chem., Int. Ed.*, 2023, **62**, e202300906.
22 Q. Li, Y.-C. He and Y. Deng, *Ind. Crops Prod.*, 2024, **222**, 119892.
23 S. Wu, Y. Zhou, T. Wang, H.-P. Too, D. I. C. Wang and Z. Li, *Nat. Commun.*, 2016, **7**, 11917.
24 H.-Y. Jia, M.-H. Zong, H.-L. Yu and N. Li, *ChemSusChem*, 2017, **10**, 3524–3528.
25 A.-D. Cheng, M.-H. Zong, G.-H. Lu and N. Li, *Adv. Sustainable Syst.*, 2021, **5**, 2000297.
26 Z.-C. Wu, W.-W. Li, M.-H. Zong and N. Li, *Chem. – Eur. J.*, 2024, **30**, e202400269.
27 Z.-Y. Yang, M. Wen, M.-H. Zong and N. Li, *Catal. Commun.*, 2020, **139**, 105979.
28 G.-H. Lu, J. Yu and N. Li, *Chin. J. Catal.*, 2024, **67**, 102–111.
29 M. R. Chapman, S. C. Cosgrove, N. J. Turner, N. Kapur and A. J. Blacker, *Angew. Chem., Int. Ed.*, 2018, **57**, 10535–10539.
30 J. M. Bolivar, A. Mannsberger, M. S. Thomsen, G. Tekautz and B. Nidetzky, *Biotechnol. Bioeng.*, 2019, **116**, 503–514.
31 S. C. Cosgrove and A. P. Mattey, *Chem. – Eur. J.*, 2022, **28**, e202103607.

