Green Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Green Chem.*, 2021, **23**, 838

Rapid biosynthesis of phenolic glycosides and their derivatives from biomass-derived hydroxycinnamates†

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Received 23rd October 2020,
Accepted 10th December 2020

DOI: 10.1039/d0gc03595e

rsc.li/greenchem

Biomass-derived hydroxycinnamates (mainly including *p*-coumaric acid and ferulic acid), which are natural sources of aromatic compounds, are highly underutilized resources. There is a need to upgrade them to make them economically feasible. Value-added phenolic glycosides and their derivatives, both belonging to a class of plant aromatic natural products, are widely used in the nutraceutical, pharmaceutical, and cosmetic industries. However, their complex aromatic structures make their efficient biosynthesis a challenging process. To overcome this issue, we created three novel synthetic cascades for the biosynthesis of phenolic glycosides (gastrodin, arbutin, and salidroside) and their derivatives (hydroquinone, tyrosol, hydroxytyrosol, and homovanillyl alcohol) from *p*-coumaric acid and ferulic acid. Moreover, because the biomass-derived hydroxycinnamates directly provided aromatic units, the cascades enabled efficient biosynthesis. We achieved substantially high production rates (up to or above 100-fold enhancement) relative to the glucose-based biosynthesis. Given the ubiquity of the aromatic structure in natural products, the use of biomass-derived aromatics should facilitate the rapid biosynthesis of numerous aromatic natural products.

Introduction

Lignocellulosic biomass-derived aromatic monomers, which are natural sources of aromatic compounds, are highly underutilized resources.[1–3] Hydroxycinnamates (including *p*-coumaric acid and ferulic acid) are phenolic compounds and occur widely in grasses, such as wheat, rice, sugarcane, and maize.[4–8] Grasses are readily available and cheap resources and can serve as prominent lignocellulosic feedstock.[9–13] In the cell wall of grasses, ferulic acid is predominantly esterified to polysaccharides and can also be covalently linked to lignin by ether bonds, while *p*-coumaric acid is primarily ester-linked to lignin.[6,14,15] *p*-Coumaric acid and ferulic acid are commercially obtained by the saponification of their esters from grassy biomass.[14,16–18] For the valorization of hydroxycinnamates to make them economically feasible, they need to be upgraded for the synthesis of value-added bioproducts,[2,3] especially complex natural products which are broadly used in the nutraceutical, pharmaceutical, and cosmetic industries.[19,20] Recently, *p*-coumaric acid and ferulic acid have been exploited to synthesize a value-added commodity product called muconic acid[21,22] and natural products (such as vanillin[23] and flavonoids[24,25]). However, the biosynthesis starting from biomass-derived hydroxycinnamates is limited to a few bioproducts. Thus, there is a demand for alternatives for the bioconversion of biomass-derived hydroxycinnamates to valuable products.

Phenolic glycosides, which contain a sugar unit bound to a phenol aglycone, belong to a class of aromatic plant natural products (PNPs). Some of them such as gastrodin, arbutin, and salidroside (studied here) are used as food additives, nutraceuticals, and therapeutic drugs (Table S1†). Gastrodin (4-hydroxymethylphenyl β-D-glucopyranoside) has been reported to exhibit several biological effects (such as anti-inflammatory and anti-oxidative activities), and is widely used in Asian countries for treating various diseases (such as vertigo, dizziness, convulsion, and headache).[26–28] Arbutin (4-hydroxyphenyl β-D-glucopyranoside) is known to be a skin-lightening agent with anti-oxidative properties.[29,30] Salidroside (8-O-β-D-glucoside of tyrosol), which is extracted from a Chinese herb, is a potential medicine and nutraceutical supplement used for anoxia resistance, anti-aging, brain cell protection and so on.[31–33] Phenolic glycoside derivatives hydroquinone, tyrosol, hydroxytyrosol, and homovanillyl alcohol are also PNPs and known for their antioxidant activities with manifold biological effects (Table S1†).[34–37] The production of

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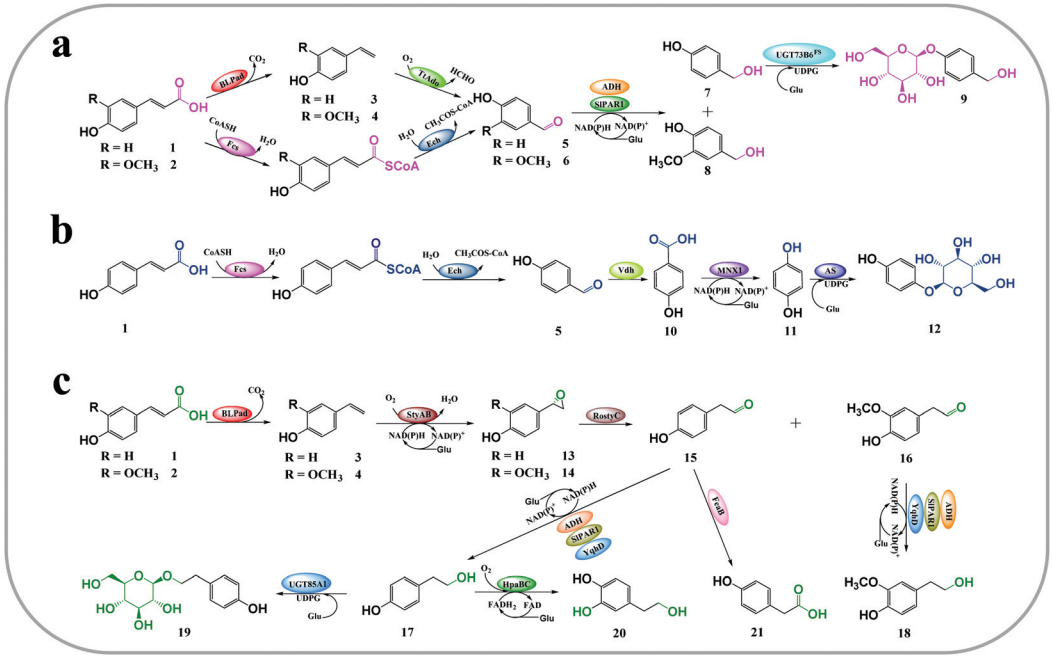
†Electronic supplementary information (ESI) available. See DOI: 10.1039/d0gc03595e



Scheme 1 Synthetic cascades for the biosynthesis of phenolic glycosides **9** (a), **12** (b), and **19** (c). Fcs: feruloyl-CoA synthetase; Ech: enoyl-CoA hydratase/aldolase; BLPad: phenolic acid decarboxylase; Ado: aromatic dioxygenase; ADH: alcohol dehydrogenase; SlPAR1: phenylacetaldehyde reductase; UGT73B6^{FS}: a mutant of *Rhodiola*-derived glycosyltransferase; Vdh: vanillin dehydrogenase; MNX1: 4-hydroxybenzoate 1-hydroxylase; AS: arbutin synthase; StyAB: styrene monooxygenase; RostyC: styrene oxide isomerase; FeaB: phenylacetaldehyde dehydrogenase; HpaBC: 4-hydroxyphenylacetate 3-hydroxylase; UGT85A1: glycosyltransferase.

these promising PNPs is critical for their applications. Traditionally, they are extracted from plants, which is time-consuming, labor-intensive, and vulnerable to environmental conditions. Recently, the synthesis of some phenolic glycosides from glucose *via* biocatalysts has been reported. The biocatalysts use glycolysis and shikimate pathways to transform glucose into aromatic compounds and further convert them into phenolic glycosides by glycosylation.[38–40] However, the multi-step biosynthesis involves plenty of enzymes, often resulting in low efficiency and slow production rates.[41,42]

Biomass-derived *p*-coumaric acid and ferulic acid are cheap renewable aromatics. They are expected to be directly used as aromatic units for the biosynthesis of high-value aromatic natural products. In this study, we designed and constructed three novel synthetic cascades to use *p*-coumaric acid and ferulic acid for the biosynthesis of phenolic glycosides (gastrodin, arbutin, and salidroside) and their derivatives (hydroquinone, tyrosol, hydroxytyrosol, and homovanillyl alcohol) (Scheme 1). Using hydroxycinnamates enabled efficient biosynthesis, especially presenting substantially high productivities ranging from 1.5 to over 100-fold increase compared to the glucose-based biosynthesis.

Results and discussion

Synthetic enzyme cascade for gastrodin (9) biosynthesis

To synthesize **9**, we devised a synthetic enzyme cascade for one-pot synthesis from *p*-coumaric acid (**1**). Given that *p*-hydroxybenzylalcohol (**7**) can be converted into **9** by one-step glycosylation *via* glycosyltransferases (Scheme 1a), we first selected a well-studied glycosyltransferase UGT73B6^{FS} obtained from the *Rhodiola* plant for this glycosylation.[38] This glycosyltransferase was then validated for the synthesis of **9** from **7** under our conditions (Fig. S4†).

Two cascades (coenzyme-dependent and coenzyme-free) were reported for the conversion of **1** into **5**.[43] The coenzyme-free cascade includes a phenolic acid decarboxylase (Pad, catalyzing the decarboxylation of **1** to 4-vinylphenol (**3**) or ferulic acid (**2**) to 4-vinylguaiacol (**4**)) and a rate-limiting aromatic dioxygenase (Ado, catalyzing the conversion of **3** into **5** or **4** into vanillin (**6**)).[43] To test the Ado enzymes catalyzing the oxidation of **3** to **5**, four Ados from various microorganisms were compared, namely, a reported Ado from *Thermothelomyces thermophila*[43] and three unidentified Ados obtained from a BLAST search with high similarity (70–85%) to the Ado (TtAdo from *Thielavia terrestris* NRRL 8126 (XP_003653923), CgAdo from *Chaetomium globosum* CBS 148.51 (XP_001219451), and PaAdo from *Podospora anserina* S mat+ (XP_001905181)). The results showed that only Ado and TtAdo catalyzed the oxidation under the current conditions (pH 7.0 and 37 °C) with conversion yields of 16% and 32%, respectively (Fig. S1†). Therefore, we used TtAdo along with a well-known Pad (called BLPad) from *Bacillus licheniformis* CGMCC7172[44] to prepare a coenzyme-free biocatalyst, *E. coli* (pET28a-TtAdo-BLPad) (Fig. S2 and Table S3†). On the other hand, for the well-studied coenzyme-dependent cascade,[43] a feruloyl-CoA synthe-

tase (Fcs) and an enoyl-CoA hydratase/aldolase (Ech) both from *Pseudomonas putida* KT2440 were used to construct a biocatalyst *E. coli* (pET28a-Fcs-Ech) (Fig. S2 and Tables S2, S3†). Meanwhile, we discovered that **5** and **6** were reduced by endogenous dehydrogenases (ADHs) in *E. coli* into **7** and vanillyl alcohol (**8**), respectively.[23] This means that **7** and **8** can be detected during the biosynthesis of **5** and **6** in *E. coli*.

Then, we evaluated two *E. coli* biocatalysts using **1** and **2** (1000 mg L^{-1}) as substrates (Scheme 1a). Within 6 h, the coenzyme-free biocatalyst accumulated large amounts of styrenes **3** (675 mg L^{-1}) and **4** (707 mg L^{-1}), and trace amounts of aromatic aldehydes (**5** and **6**) and alcohols (**7** and **8**) (all less than 40 mg L^{-1}) (Fig. 1a). This suggested that the biocatalyst could not completely convert the substrates into aldehydes. Conversely, the coenzyme-dependent biocatalyst achieved high titers of aromatic aldehydes and alcohols (Fig. 1b), converting **1** into 307 mg L^{-1} **5** and 446 mg L^{-1} **7**, and transforming **2** into 11 mg L^{-1} **6** and 793 mg L^{-1} **8**. These results demonstrated that the coenzyme-dependent cascade was better than the coenzyme-free cascade under the current conditions. Thus, we selected the coenzyme-dependent cascade for the following experiments.

Notably, compared to the high production of **8**, the production of **7** was low and a large amount of **5** remained, implying the need to overexpress ADHs for the reduction of **5** to **7**. Five ADHs from different sources were selected, namely, ADHP from *E. coli* MG1655,[45] YahK and YqhD from *E. coli* BL21 (DE3),[45,46] ScADH6 from yeast *Saccharomyces cerevisiae*,[47] and SlPAR1 from tomato *Solanum lycopersicum*[48] (Fig. 1c). The overexpressions of YahK, YqhD, ScADH6, and SlPAR1 greatly increased the reduction (yield of all >90%), especially for SlPAR1, which performed the best (98% yield). Therefore, SlPAR1 was selected along with Fcs and Ech to construct the Fcs-Ech-SlPAR1 cascade for the transformation of **1** into **7** (Fig. S3†). Next, UGT73B6^{FS} was combined with the cascade to form a Fcs-Ech-SlPAR1-UGT73B6^{FS} cascade. To verify the feasibility of the cascade for the conversion of **1** into **9**, we carried out time course experiments using the corresponding *E. coli* (Fcs-Ech-SlPAR1-UGT73B6^{FS}) with 2000 mg L^{-1} **1** (Fig. 1d). Within the first 4 h, **1** was rapidly consumed and 1451 mg L^{-1} **7** accumulated. To enhance the glycosylation, extra 10 g L^{-1} glucose was provided after 8 h, resulting in considerable enhancement in the biosynthesis of **9**. After 12 h, the production of **9** reached 1461 mg L^{-1} in titer and 122 mg L^{-1} h^{-1} in productivity, which is significantly superior to those for the reported glucose-based biosynthesis of **9** (545 mg L^{-1} in 48 h)[38] (Table S4†).

Synthetic enzyme cascade for arbutin (12) biosynthesis

To synthesize **12** from **1**, we first developed a synthetic enzyme cascade for the one-pot conversion of **1** into hydroquinone (**11**), which can later be converted into **12** by one-step glycosylation.[39] As shown in Scheme 1b, **1** can be converted into **5** by

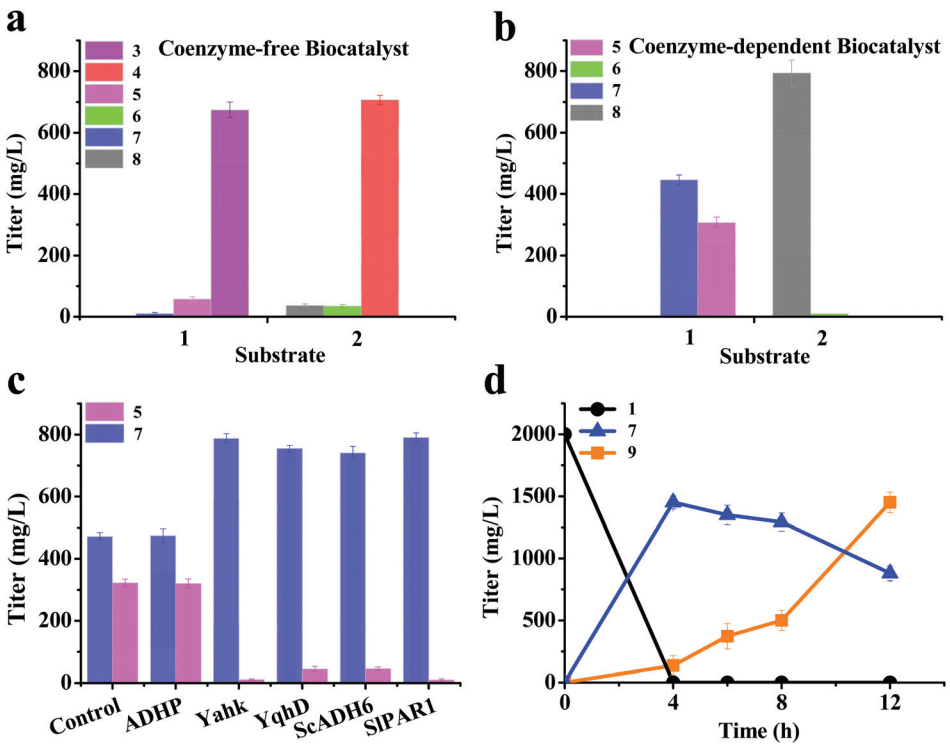


Fig. 1 Biosynthesis of **9**. (a) Coenzyme-free biocatalyst for the synthesis of aromatic aldehydes with *E. coli* (pET28a-TtAdo-BLPad) from 1000 mg L^{-1} **1** or **2** in a KPi buffer for 6 h. (b) Coenzyme-dependent biocatalyst for the synthesis of aromatic aldehydes with *E. coli* (pET28a-Fcs-Ech) from 1000 mg L^{-1} **1** or **2**. (c) ADHs improve the conversion of **5** to **7**. Five ADHs from different sources were selected to test the synthesis of **7** from 800 mg L^{-1} **5**. Control is a wild-type strain without ADH expression. (d) Time course of the biosynthesis of **9** from 2000 mg L^{-1} **1** by the Fcs-Ech-SlPAR1-UGT73B6^{FS} cascade in 12 h.

the abovementioned Fcs-Ech cascade, and then **5** is converted into *p*-hydroxybenzoic acid (**10**) with a known vanillin dehydrogenase (Vdh) from *P. putida*.[23] The next step of converting **10** into **11** was catalyzed by MNX1 (a 4-hydroxybenzoate 1-hydroxylase) from yeast *Candida parapsilosis* CDC317[49] or vibMO1 (a monooxygenase) from basidiomycete fungus *Boreostereum vibrans*.[50] The results showed that MNX1 catalyzed the conversion with almost 100% yield, whereas vibMO1 resulted in less than 5% conversion (Fig. S5†). Thus, MNX1 was used to construct an Fcs-Ech-Vdh-MNX1 cascade to synthesize **11** from **1**. We tested the cascade using 3000 mg L^{-1} **1** (Fig. 2a). Within 6 h, **1** was quickly converted into **11** (1902 mg L^{-1}), and a little amounts of **10** (50 mg L^{-1}) and **7** (55 mg L^{-1}) remained. After another 2 h, **10** was further converted into 1951 mg L^{-1} **11** (97% yield and 244 mg L^{-1} h^{-1} productivity). These results suggested a high efficiency of the cascade for the biosynthesis of **11**. As an important industrial intermediate (Table S1†), **11** is often synthesized from fossil-fuel-based benzene using heavy metal catalysts.[37,51] We herein demonstrated an environmentally friendly route for the synthesis of **11** from renewable resources.

Next, we used a well-studied glucosyltransferase (arbutin synthase, AS) from *Rauvolfia serpentina*[52] for the conversion of **11** into **12** (Fig. S6†). To construct an enzyme cascade for the synthesis of **12** from **1**, enzymes Fcs, Ech, Vdh, MNX1 and AS (Scheme 1b) were co-expressed in five *E. coli* biocatalysts (SArbutin 1–5) in which the corresponding genes were cloned into different plasmids in various organizations (Fig. S7†). Starting from 2000 mg L^{-1} **1**, the titers of **12** using SArbutin 1–5 were 911, 1430, 513, 1762, and 2462 mg L^{-1}, respectively (Fig. 2b). Among these, SArbutin 5, which presented the highest production titer, was selected for the time course experiment using 2000 mg L^{-1} **1** (Fig. 2c). Within 6 h, **1** was quickly consumed, and the production titers of **10**, **11**, and **12** were 250, 900, and 1450 mg L^{-1}, respectively. After 12 h, the intermediates **10** and **11** were reduced to less than 250 mg L^{-1}, while **12** reached 2500 mg L^{-1}. By the end of 24 h, 3051 mg L^{-1} **12** was synthesized in 92% yield and 127 mg L^{-1} h^{-1} productivity. This is faster than the previously reported biosynthesis of **12** from glucose with productivities of 87 mg L^{-1} h^{-1} and 59.7 mg L^{-1} h^{-1} (Table S4†).[39,53]

Synthetic enzyme cascade for tyrosol (17), homovanillyl alcohol (18) and salidroside (19) biosynthesis

Similarly, to synthesize **19**, we first focused on the biosynthesis of precursor **17**, which can later be converted into phenolic glycoside **19** by one-step glycosylation.[40] Inspired by the biocatalytic formal anti-Markovnikov hydration of aryl alkenes,[54] we designed a decarboxylation–epoxidation–isomerization–reduction cascade to convert **1** into **17** (Scheme 1c). As mentioned above, **3** was easily synthesized from **1** by one-step decarboxylation *via* BLPad (Scheme 1c). **3** was supposed to be epoxidized into **13** by styrene monooxygenase, followed by isomerization to 4-hydroxyphenylacetaldehyde (**15**) by styrene oxide isomerase, and further reduced to **17** by endogenous or heterologous dehydrogenases (Scheme 1c and Fig. S8†). To explore this concept, we selected a styrene monooxygenase (StyAB) from *Pseudomonas* sp. strain VLB120[55] and a styrene oxide isomerase (RostyC) from *Rhodococcus opacus* 1CP,[56] resulting in biocatalyst *E. coli* (StyAB-RostyC) with endogenous dehydrogenases. The feasibility of the biocatalyst was verified by converting 1000 mg L^{-1} **3** into 1145 mg L^{-1} **17** (Fig. S8†), demonstrating that the cascade of anti-Markovnikov alkene hydration worked efficiently using **3** as the substrate under our design.

Next, we developed a cascade for the conversion of **1** into **17** using BLPad, StyAB, and RostyC, along with an alcohol dehydrogenase (YahK, YqhD or SlPAR1) which was expected to further help the reduction of **15** to **17** (Fig. 3a). To obtain an efficient biocatalyst, the related genes were cloned into different plasmids in various organizations, resulting in eight *E. coli* biocatalysts (Styrosol 1–8) (Fig. 3b and Fig. S9†). To evaluate the biocatalysts, 2000 mg L^{-1} **1** was used for the conversion, and Styrosol 1–8 produced **17** at 1101, 1250, 1639, 1383, 1422 1484, 1640, and 1431 mg L^{-1}, respectively. The biocatalysts (Styrosol 3–8) with the overexpression of dehydrogenases resulted in higher production than those (Styrosol 1 and 2) without the overexpression, demonstrating that overexpressing the dehydrogenase can further improve the production of **17**. Among the eight biocatalysts, Styrosol 3 and 7 showed the highest catalytic production and were, thus, selected for the following experiments. The time courses of the conversion of **1**

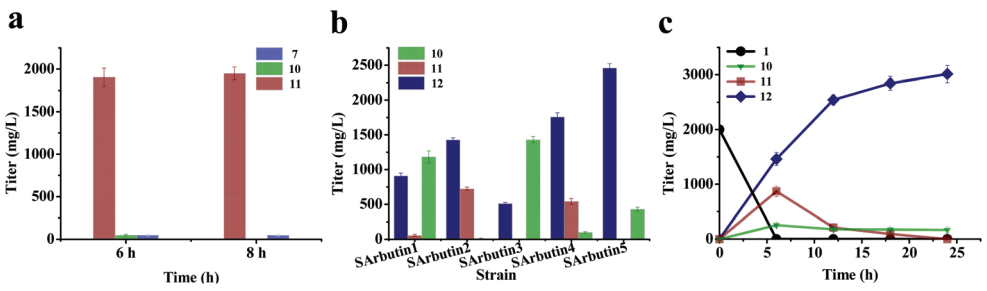


Fig. 2 Biosynthesis of **11** and **12**. (a) Biosynthesis of **11** from 3000 mg L^{-1} **1** with *E. coli* (Fcs-Ech-Vdh-MNX1) for 8 h. (b) Different biocatalysts SArbutin 1–5 were constructed for the biosynthesis of **12** from 2000 mg L^{-1} **1** for 12 h. (c) Time course of converting 2000 mg L^{-1} **1** into **12** using SArbutin 5 for 24 h.

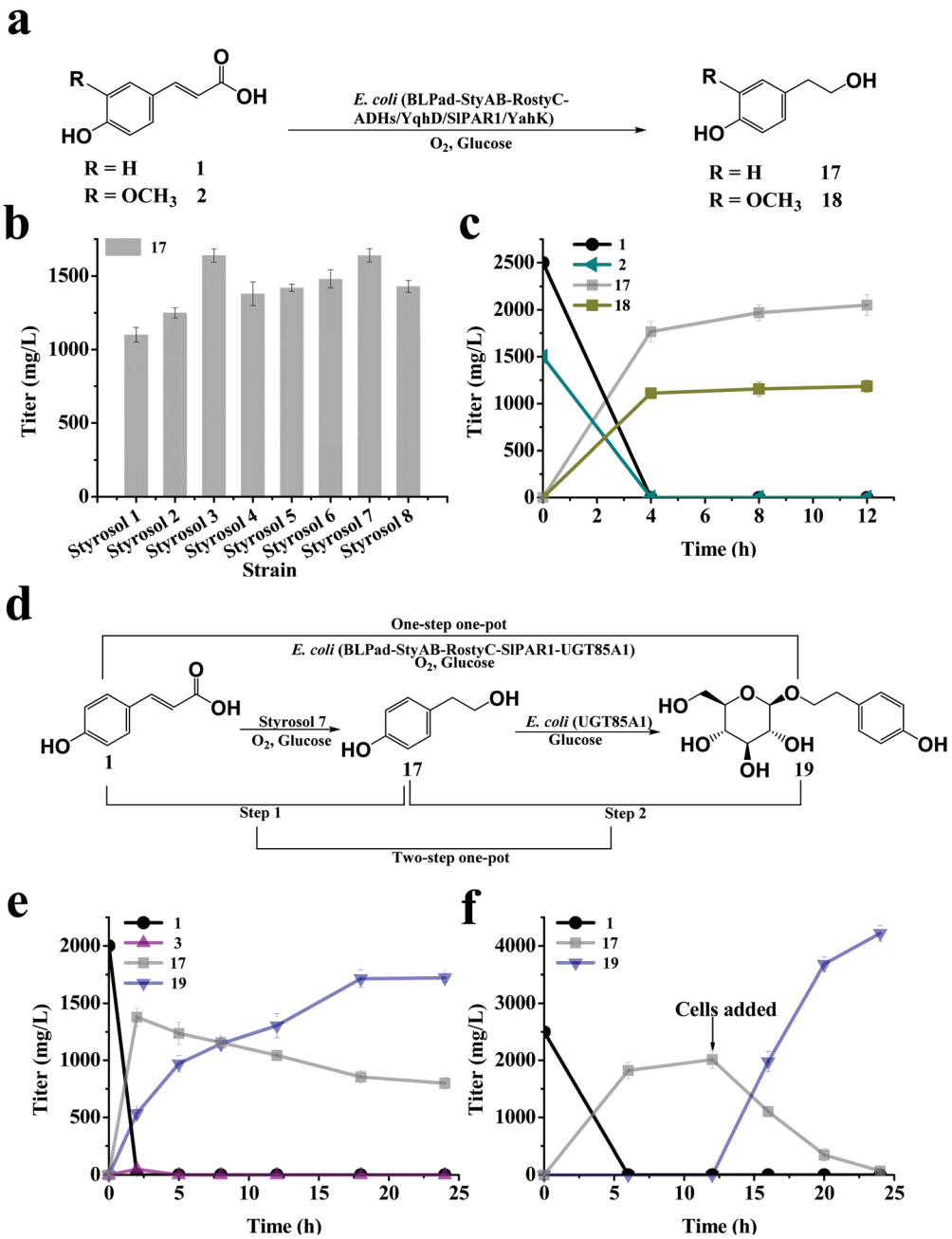


Fig. 3 Biosynthesis of **17**, **18** and **19**. (a) *E. coli* strains expressing *blpad*, *styAB*, *RostyC*, *yqhD*, *SlPAR1* and *yahK* for the biosynthesis of **17** and **18** from **1** and **2**, respectively. (b) Conversion of 2000 mg L^{-1} **1** into **17** with different biocatalysts Styrosol 1–8 for 12 h. (c) Time course of converting 2500 mg L^{-1} **1** into **17** or 1500 mg L^{-1} **2** into **18** using Styrosol 7 in 12 h. (d) *E. coli* strains expressing *blpad*, *styAB*, *RostyC*, *yqhD*, *SlPAR1* and *ugt85a1* for the biosynthesis of **19** from **1** by a one-step or two-step method. (e) Time course of converting 2000 mg L^{-1} **1** into **19** by a one-step one-pot method using *E. coli* (BLPad-StyAB-RostyC-SlPAR1-UGT85A1) for 24 h. (f) Time course of converting 2500 mg L^{-1} **1** into **19** by a two-step one-pot method. Styrosol 7 was first used to convert **1** into **17** for 12 h, and then *E. coli* (UGT85A1) was added for the conversion of **17** to **19** for another 12 h.

and **2** into **17** and **18** by Styrosol 7 were investigated (Fig. 3c): 2500 mg L^{-1} **1** and 1500 mg L^{-1} **2** were quickly converted into **17** (1750 mg L^{-1}) and **18** (1050 mg L^{-1}) within the first 4 h. By the end of 12 h, the conversion reached 2046 mg L^{-1} for **17** (97.4% yield and 171 mg L^{-1} h^{-1} productivity) and 1189 mg L^{-1} for **18** (91.9% yield and 99.1 mg L^{-1} h^{-1} productivity). During the conversion, a byproduct 4-hydroxyphenylacetic acid (**21**) was detected in a small amount (35 mg L^{-1}), which might be catalyzed by endogenous phenylacetaldehyde dehydrogenase (FeaB)[57] from **15** (Scheme 1c).

To synthesize **19** from **17**, we tested three glycosyltransferase (UGT73C5 and UGT85A1 from *Arabidopsis thaliana*;[58,59] UGT73B6 from *Rhodiola sachalinensis*[60]) (Fig. S10†). The best-performing UGT85A1 was selected and combined into the

cascade catalyzing **1** to **17**, to construct a new cascade BLPad-StyAB-RostyC-SlPAR1-UGT85A1 in a single *E. coli* biocatalyst (Fig. 3d and Fig. S11†). We first investigated the one-step one-pot synthesis of **19** from **1** by this biocatalyst. As shown in Fig. 3e, within the first 2 h, 2000 mg L^{-1} **1** was quickly converted into **17** (1380 mg L^{-1}), and the concentration of **19** reached 536 mg L^{-1}, and a small amount of **3** (50 m L^{-1}) was detected. By the end of 24 h, 1724 mg L^{-1} **19** was obtained in 46.7% conversion, but 804 mg L^{-1} **17** still remained, implying the low efficiency of glycosylation in one-step conversion. To improve the conversion efficiency, we next tested a two-step one-pot conversion,[61] in which two biocatalysts Styrosol 7 (*E. coli* (BLPad-StyAB-RostyC-YqhD)) and *E. coli* (UGT85A1) (Fig. S11†) were used for the sequential conversions of **1** to **17** and **17** to **19**. The conversion started with 2500 mg L^{-1} **1** using resting cells of Styrosol 7. Within 12 h, **1** was totally consumed and converted into 2046 mg L^{-1} **17** (Fig. 3f). At that point of time, the other *E. coli* (UGT85A1) was added into the culture to initiate the conversion of **17** into **19**. After another 12 h, **17** was almost completely converted from **1** into **19** (4221 mg L^{-1}) with 176 mg L^{-1} h^{-1} productivity and 94.9% yield. In comparison to the productivities of **17** and **19** generated from glucose, we obtained the highest productivities of **17** and **19** so far (Table S4†).[40,59,62–64]

In addition, we tested the reuse performances of the biocatalysts for the biosynthesis of **9**, **12**, and **19**. Compared to their initial bioconversions, the reuse performance showed 6 to 100-fold decrease (Fig. S14†), indicating that the reuses of the biocatalysts were not ideal under the current conditions.

Synthetic enzyme cascades for hydroxytyrosol (20) and β-glucoside of *p*-coumaric acid (22) biosynthesis

On the basis of the developed cascade for the biosynthesis of **17** from **1**, we designed a synthetic enzyme cascade for the biosynthesis of **20** from **1** (Fig. 4a). The cascade included BLPad, StyAB, RostyC, SlPAR1, and a reported 4-hydroxyphenylacetate 3-hydroxylase HpaBC from *E. coli* BL21(DE3)[65] (Fig. S12†). To construct a cascade BLPad-StyAB-RostyC-SlPAR1-HpaBC, we combined HpaBC into Styrosol 3 (Fig. S13†). To evaluate the cascade for the synthesis of **20** from **1**, the conversion was investigated (Fig. 4a). Using this cascade, **1** (2000 mg L^{-1}) was completely consumed and converted into **20** (1599 mg L^{-1}) with the accumulation of a little amount of **17** (251 mg L^{-1}) within the first 4 h. By the end of 8 h, 1832 mg L^{-1} **20** was obtained in 97.5% conversion and 229 mg L^{-1} h^{-1} productivity. The productivity achieved using this cascade was considerably superior to the productivity obtained from the production of **20** using glucose as the starting material (Table S4†).[66,67] Additionally, compared to a recently developed tyrosine-based biosynthesis of **20**,[65] biomass-derived aromatics provide a low-cost substrate for this biosynthesis.

Additionally, glycosyltransferase $UGT_{BL}1$ from *Bacillus licheniformis* strain ZSP1 was reported to catalyze the glycosylation of **2** to **23**.[68] Out of curiosity, we tested whether $UGT_{BL}1$ could convert **1** into its glucoside **22**. As expected (Fig. 4b), within 12 h, 1000 mg L^{-1} **1** and 1500 mg L^{-1} **2** were converted into

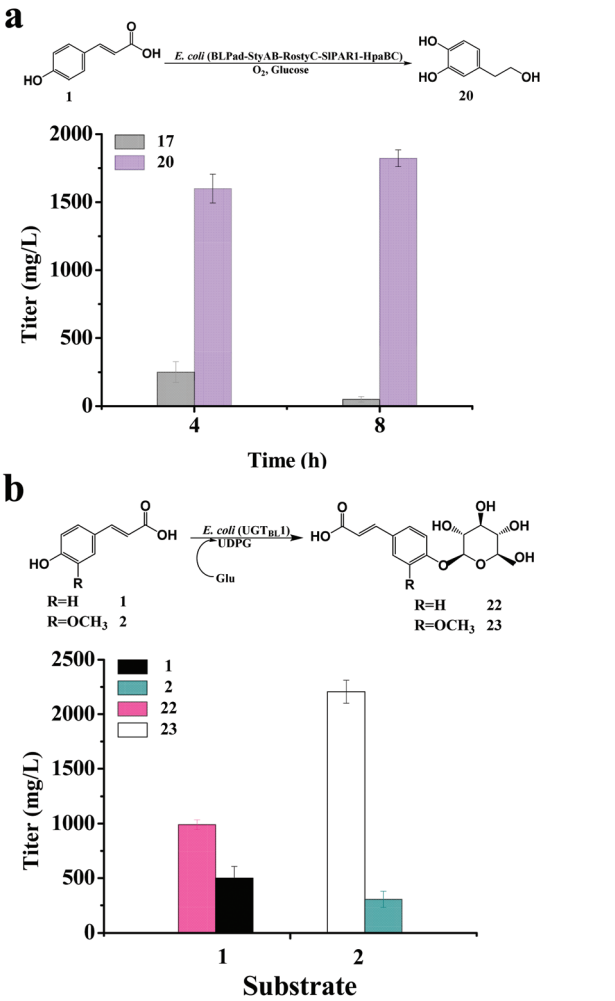


Fig. 4 Biosynthesis of **20** and **22**. (a) Conversion of 2000 mg L^{-1} **1** into **20** with *E. coli* (BLPad-StyAB-RostyC-SlPAR1-HpaBC) within 4 and 8 h. (b) Conversion of 1000 mg L^{-1} **1** and 1500 mg L^{-1} **2** into **22** and **23**, respectively.

991 mg L^{-1} **22** in 50.2% conversion and 2207 mg L^{-1} **23** in 84.8% conversion, respectively. This implied that there should be more alternative cascades for the conversion of biomass-derived aromatic monomers into manifold phenolic glycosides.

Conclusion

Herein, biomass-derived **1** and **2** were exploited to efficiently produce value-added phenolic glycosides (gastrodin, arbutin, and salidroside) and their derivatives (hydroquinone, tyrosol, hydroxytyrosol, and homovanillyl alcohol). We constructed three synthetic cascades using **1** and **2** as starting materials, resulting in the efficient biosynthesis of gastrodin (1461 mg L^{-1}, 42.5% yield, 122 mg L^{-1} h^{-1} productivity), arbutin (3051 mg L^{-1}, 92% yield, 127 mg L^{-1} h^{-1} productivity), salidroside (4221 mg L^{-1}, 94.9% yield, 176 mg L^{-1} h^{-1} productivity), hydroquinone (1951 mg L^{-1}, 97% yield, 244 mg L^{-1} h^{-1} pro-

ductivity), tyrosol (2046 mg L^{-1}, 97.3% yield, 171 mg L^{-1} h^{-1} productivity), hydroxytyrosol (1832 mg L^{-1}, 97.5% yield, 229 mg L^{-1} h^{-1} productivity) and homovanillyl alcohol (1189 mg L^{-1}, 91.9% yield, 99.1 mg L^{-1} h^{-1} productivity). To the best of our knowledge, these synthetic cascades are the first ones to be reported. We used *p*-coumaric acid and ferulic acid as substrates to biosynthesize phenolic glycosides and their derivatives. Notably, the cascades achieved the biosynthesis of homovanillyl alcohol, which has never been reported to be synthesized by bioconversion previously. Moreover, compared to the glucose-based biosynthesis of related phenolic glycosides, our cascades contained fewer steps to give higher production rates (ranging from 1.5 to over 100-fold increase).

This study also provided a new strategy for the utilization of lignocellulosic biomass. **1** and **2** are renewable aromatic resources and can be easily obtained from the saponification of their esters in grass biomass. Given the ubiquity of the aromatic structure in natural products, we believe biomass-derived aromatics can be broadly used for the rapid biosynthesis of high-value aromatic natural products.

Experimental section

Chemicals and reagents

All the chemicals were purchased from commercial companies as follows. Gastrodin (**9**), arbutin (**12**), salidroside (**19**), and ferulic acid (**2**) were purchased from Macklin Biochemical Co., Ltd; *p*-coumaric acid (**1**), *p*-hydroxybenzyldehyde (**5**), *p*-hydroxybenzylalcohol (**7**), methanol, and acetonitrile used for HPLC from Adamas Reagent Co., Ltd; 4-vinylphenol (**3**) and 4-vinylguaiacol (**4**) from Ark Pharm; hydroquinone (**11**) and hydroxytyrosol (**20**) from Aladdin; tyrosol (**17**) from Bidepharm; and homovanillyl alcohol (**18**) from ChemFaces. A DNA gel extraction kit, plasmid purification kit, genome DNA extraction kit, Phusion® High-Fidelity DNA Polymerase, and dNTP mix (10 mM each) were obtained from Thermo Fisher Scientific. Restriction endonucleases and T4 DNA ligase were from New England Biolabs.

Genes, plasmids and strains

Genes were either amplified from the DNA genomes of various microorganisms or synthesized with DNA codon-optimization by GENEWIZ, Inc.

Genes *fcs* (encoding feruloyl-CoA synthetase), *ech* (encoding enoyl-CoA hydratase/aldolase) and *vdh* encoding (vanillin dehydrogenase) were amplified from the genome of *Pseudomonas putida* KT2440 (NC_002947.4).[69] *yahK* (encoding aldehyde reductase),[45] *yqhD* (encoding aldehyde reductase)[46] and *hpaBC* (encoding 4-hydroxyphenylacetate 3-hydroxylase)[70] were amplified from the genome of BL21 (DE3) (NC_012971.2).

The other genes were synthesized with codon optimization by GENEWIZ (Suzhou, China) as follows: *blpad* (KM267085.1) from *Bacillus licheniformis* strain CGMCC 7172 encoding phenolic acid decarboxylase,[44] *ado* (XM_003665537.1) from *Thermothelomyces thermophila* encoding aromatic dioxygenase,[43] *Ttado* (NC_016459.1) from *Thielavia terrestris* NRRL 8126, *Cgado* (NT_165976.1) from *Chaetomium globosum* CBS 148.51, *Paado* (NW_001914846.1) from *Podospora anserina* S mat+, *slpar1* (EF613490.1) from *Solanum lycopersicum* encoding phenylacetaldehyde reductase,[48] *Scadh6* (NC_001145.3) encoding alcohol dehydrogenase from *Saccharomyces cerevisiae* S288C,[47] *ugt73b6* (AY547304.1) encoding glycosyltransferase from *Rhodiola sachalinensis*,[60] *ugt73c5* (Q9ZQ94.1) encoding glycosyltransferase[58] and *ugt85a1* (NC_003070.9) encoding glycosyltransferase from *Arabidopsis thaliana*,[59] *MNX1* (HE605203.1) encoding 4-hydroxybenzoate 1-hydroxylase from *Candida parapsilosis* strain CDC317,[49] *vibMO1* (KU668560) encoding monooxygenase from *Boreostereum vibrans*,[50] *AS* (Q9AR73.1) encoding arbutin synthase from *Rauvolfia serpentina*,[52] *styAB* (AF031161.1) encoding styrene monooxygenase from *Pseudomonas* sp. VLB120,[55] *RostyC* (KF540254.1) from *Rhodococcus opacus* 1CP encoding styrene oxide isomerase,[56] and $ugt_{BL}1$ (KP123426.1) encoding glycosyltransferase from *Bacillus licheniformis* strain ZSP1.[68]

All the plasmids were constructed using Golden Gate assembly[71] methods and are presented in Table S2.† Plasmids pET28a (+) (Novagen), pACYCDuet-1 (Novagen) and pA7a-RFP (a BglBrick plasmid) were used as vectors for gene expression. The constructed plasmids were confirmed by sequencing. *E. coli* BL21 (DE3) was used as the host cell for bioconversion, and DH5α was used for cloning. Related plasmids were introduced into strain BL21 (DE3) to give various biocatalysts. All the biocatalysts constructed are listed in Table S3.†

E. coli strain cultivation

E. coli strains were cultivated in LB medium with appropriate antibiotics (50 μg mL^{-1} kanamycin, 100 μg mL^{-1} ampicillin and/or 25 μg mL^{-1} chloramphenicol). The strains were first inoculated into 2 mL of LB, and cultivated for 12 h (37 °C and 250 rpm). Then, they were transferred to 100 mL of LB medium in a 500 mL shaking flask. When the cell cultures reached an OD_{600} of 0.8, they were cooled down to 22 °C, and 0.5 mM IPTG was added for protein expression. After 12 h of induction at 22 °C, the cells were harvested by centrifugation at 4 °C and 3800*g* for 10 min. Next, bioconversions were performed in a K_2HPO_4–KH_2HPO_4 (KPi) buffer (100 mM, pH 7.0, 1% glucose) or conversion medium (pH 7.0, M9Y medium supplemented with 1% glucose). M9Y medium: 3 g L^{-1} KH_2PO_4, 6.78 g L^{-1} Na_2HPO_4, 0.5 g L^{-1} NaCl, 1.0 g L^{-1} NH_4Cl, 0.493 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.011 g L^{-1} $CaCl_2$ and 1.5 g L^{-1} yeast extract.

Constructing *E. coli* for bioconversion

E. coli (TtAdo-BLPad) with pET28a-TtAdo-BLPad and *E. coli* (Fcs-Ech) with pET28a-Fcs-Ech were investigated for the synthesis of **5**. *E. coli* (Fcs-Ech-SlPAR1) with pET28a-Fcs-Ech-SlPAR1 was used for the synthesis of **7**. *E. coli* (Fcs-Ech-SlPAR1-$UGT73B6^{FS}$) with pET28a-Fcs-Ech-SlPAR1 and pA7a-$UGT73B6^{FS}$ was used for the synthesis of **9**.

E. coli (Fcs-Ech-Vdh-MNX1) with pET28a-Fcs-Ech-Vdh and pA7a-MNX1 was used for the synthesis of **11**. Strains SArbutin

1–5 were constructed for the synthesis of **12**, and the details are shown in Table S3 and Fig. S7.†

E. coli (StyAB-RostyC) with pET28a-StyAB-RostyC was used to investigate the anti-Markovnikov hydration of aryl alkenes. Strains Styrosol 1–8 were constructed for the synthesis of **17** or **18** (Table S3 and Fig. S9†). *E. coli* (BLPad-StyAB-RostyC-SlPAR1-UGT85A1) with pET28a-StyAB-RostyC-SlPAR1 and pA7a-BLPad-7-UGT85A1 was engineered for the synthesis of **19**.

E. coli (StyAB-RostyC-SlPAR1-BLPad-HpaBC) with pET28a-StyAB-RostyC-SlPAR1 and pA7a-BLPad-7-HpaBC was engineered for the synthesis of **20**. *E. coli* ($UGT_{BL}1$) with pET28a-$UGT_{BL}1$ was used for the synthesis of β-glucosides from **2** and **1**. The constructed *E. coli* strains were used for bioconversion at 37 °C and 250 rpm unless otherwise noted. The biosynthesized compounds were determined by HPLC and LC-MS or LC-MS/MS.

Bioconversion for gastrodin 9 synthesis

The harvested cells of *E. coli* (TtAdo-BLPad) and *E. coli* (Fcs-Ech) were resuspended in 10 mL of KPi buffer with an OD_{600} of 10. Then, 1000 mg L^{-1} **3** or **4** was used for the synthesis of **5** or vanillin **6**, respectively. Similarly, *E. coli* (Fcs-Ech-SlPAR1) was used to investigate the synthesis of **7** using 2000 mg L^{-1} **1**. The conversion reaction was performed for 6 h.

For the synthesis of **9**, cell pellets of *E. coli* (Fcs-Ech-SlPAR1-$UGT73B6^{FS}$) were resuspended in 10 mL of a conversion medium to reach an OD_{600} of 20 by adding 2000 mg L^{-1} **1**. Then the reaction was performed for 12 h, and extra 1% glucose was added after 8 h.

Bioconversion for the synthesis of hydroquinone 11 and arbutin 12

The harvested cells of *E. coli* (Fcs-Ech-Vdh-MNX1) were resuspended in 10 mL of KPi buffer to reach an OD_{600} of 20 by adding 3000 mg L^{-1} **1** for the synthesis of **11**, and the reaction was performed for 8 h. SArbutin 1–5 cells were resuspended in 10 mL of a conversion medium at an OD_{600} of 20 by adding 2000 mg L^{-1} **1**, and the reaction was performed for 24 h by adding extra 10 g L^{-1} glucose after 12 h.

Bioconversion for the synthesis of tyrosol 17, homovanillyl alcohol 18, hydroxytyrosol 20 and salidroside 19

To investigate the anti-Markovnikov hydration of aryl alkenes, the harvested cells of *E. coli* (StyAB-RostyC) were resuspended in 10 mL of a conversion medium (OD_{600} of 10) with 1000 mg L^{-1} **3**, and then used for bioconversion for 6 h. For the synthesis of **17** or **18**, cell pellets of strains Styrosol 1–8 were resuspended in 10 mL of a conversion medium to reach an OD_{600} of 20 upon adding 2500 or 1500 mg L^{-1} **1** or **2**, respectively. For the synthesis of **20**, harvested cells of *E. coli* (StyAB-RostyC-SlPAR1-BLPad-HpaBC) were used for converting 2000 mg L^{-1} **1** for 12 h.

For the one-pot one-step synthesis of **19**, harvested cells of *E. coli* (BLPad-StyAB-RostyC-SlPAR1-UGT85A1) were resuspended in 10 mL of a conversion medium to reach an OD_{600} of 20 upon adding 2000 mg L^{-1} **1**. The reaction was performed for 24 h by adding extra 10 g L^{-1} glucose after 12 h. To further improve the synthesis of **19**, a two-step, one-pot method was used with strain Styrosol 7 and *E. coli* (UGT85A1). The harvested cells of Styrosol 7 were first resuspended in 10 mL of the conversion medium to reach an OD_{600} of 20 by adding 2500 mg L^{-1} **1** to start the synthesis of **17** for 12 h, and then the cell pellets of *E. coli* (UGT85A1) along with extra 1% glucose were added into the conversion system for another 12 h to synthesize **19**.

For the synthesis of their corresponding β-glucosides, harvested cells of strain *E. coli* ($UGT_{BL}1$) were resuspended in 10 mL of a conversion medium to reach an OD_{600} of 20 by adding 1000 mg L^{-1} **1** or 1500 mg L^{-1} **2**. The reaction was performed for 12 h.

To test the reuse performances of the biocatalysts, *E. coli* (Fcs-Ech-SlPAR1-$UGT73B6^{FS}$), SArbutin5, and *E. coli* (BLPad-StyAB-RostyC-SlPAR1-UGT85A1) were selected to synthesize **9**, **12** and **19**, respectively. The bioconversions were performed in 10 mL of a conversion medium with 1000 mg L^{-1} **1** for 12 h using each biocatalyst with an OD_{600} of 10. After 12 h, the biocatalysts were collected and washed with a KPi buffer and then resuspended to test the reuse experiments under the same conditions. The substrates and products were measured using the abovementioned method.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was sponsored by the National Key R&D Program of China (2019YFA0904800), the National Natural Science Foundation of China (31870071), and the Science and Technology Commission of Shanghai Municipality (18JC1413600). We thank the Instrumental Analysis Center, Shanghai Jiao Tong University, for help with LC-MS/MS analyses.

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