REVIEW

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Recent developments in the enzymatic modifications of steroid scaffolds

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Steroids are an important family of bioactive compounds. Steroid drugs are renowned for their multifaceted pharmacological activities and are the second-largest category in the global pharmaceutical market. Recent developments in biocatalysis and biosynthesis have led to the increased use of enzymes to enhance the selectivity, efficiency, and sustainability for diverse modifications of steroids. This review discusses the advancements achieved over the past five years in the enzymatic modifications of steroid scaffolds, focusing on enzymatic hydroxylation, reduction, dehydrogenation, cascade reactions, and other modifications for future research on the synthesis of novel steroid compounds and related drugs, and new therapeutic possibilities.

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1. Introduction

Thousands of steroids have been discovered in microorganisms, plants, and animals, and these compounds display a variety of pharmacological activities, including anti-inflamma-
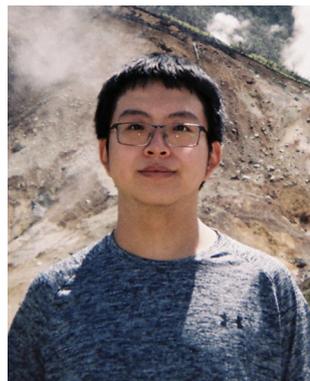
Huibin Wang received his B.S. degree from Shenyang Pharmaceutical University in 2021 and M.S. degree from the University of Tokyo in 2023. He is currently a Ph.D. candidate in the University of Tokyo under the supervision of Prof. Ikuro Abe. His research interest focuses on understanding and reprogramming nature's biosynthetic machinery based on structural enzymology.

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Ikuro Abe received his B.S. (1984) and Ph.D. (1989) from The University of Tokyo, where he studied chemistry and biochemistry of natural products biosynthesis. He did his postdoctoral research with Professors Guy Ourisson and Michel Rohmer mostly at the Ecole Nationale Supérieure de Chimie de Mulhouse (1989–1991), and with Professor Glenn D. Prestwich at the State University of New York at Stony Brook (1991–1996) and

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then at the University of Utah (1996–1998). In 1998, he moved back to Japan to join the faculty at University of Shizuoka (1998–2009), and then was appointed as Professor at the Graduate School of Pharmaceutical Sciences, The University of Tokyo (2009–). His research interests mostly focus on exploring and engineering the natural products biosynthesis. He received the Japanese Society of Pharmacognosy Award (2017), Sumiki Umezawa Memorial Award (2017), The Pharmaceutical Society of Japan Award (2019), Prizes for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology, Japan (2019), and The Society for Actinomycetes Japan, Omura Award (2023). He is a Fellow of The Royal Society of Chemistry (FRSC) and a former President of The Japanese Society of Pharmacognosy.

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tory, antitumor, antimicrobial, antiandrogenic, and immuno-suppressive effects.[1–3] The diverse biological activities attributed to the steroid nucleus stem from its array of functionalities, through the presence of hydroxyl, carbonyl, halide, and glycosyl groups at various positions.[4,5]

Classical steroids are characterized by the 6/6/6/5- tetracyclic ring system, with methyl groups at C-10 and C-13 and a substituent group at C-17. Many bioinspired total syntheses of biologically active steroids have been reported.[6,7] Meanwhile, the use of enzymes in biocatalytic processes offers high selectivity, efficiency, and sustainability.[8–11] Enzymatic modifications of steroids are being pursued, and efforts toward the identification of novel enzymes and protein engineering are crucial for enhancing enzyme selectivity and activity.[12,13] Besides, the chemoenzymatic method presents an effective and concise approach for the synthesis of steroidal products and has been recently reviewed by Qu and coworkers.[14]

In this review, we highlight the developments during the past five years in the enzymatic modifications of steroid scaffolds. We provide a comprehensive overview of advancements in enzymatic hydroxylation, epoxidation, ketoreduction, dehydrogenation, halogenation, glycosylation, side-chain cleavage, methylation and demethylation, ring system reconstruction, acylation, amination, amidation, isomerization, sulfonation, novel biosynthetic pathways, and artificial multi-enzyme cascades and biotransformations.
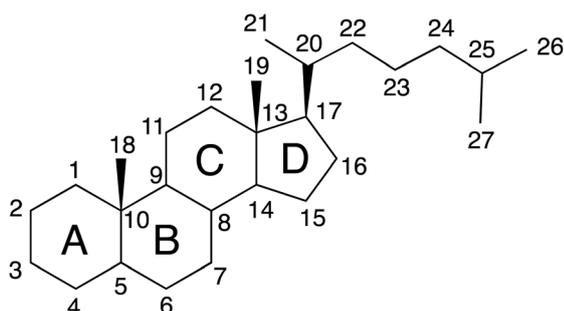
2. Hydroxylation

Hydroxylation of steroids stands as one of the most pivotal reactions in modifications of the steroid scaffold.[15–17] The addition of hydroxy groups not only influences physiological functions, polarity, solubility, and toxicity but also serves as a synthetic starting point for the preparation of valuable steroid drugs.[18,19] In this section, we discuss recent advances in the discovery and engineering of cytochrome P450 oxygenases (P450s), non-heme iron- and α-ketoglutarate-dependent oxygenases (αKG OXs), Rieske oxygenases, and other enzymes for site-specific hydroxylations. For a comparative summary elucidating the preference sites for enzymatic hydroxylation of steroids, readers are directed to the 2020 review by Zhang *et al.*, the 2022 review by Zhu *et al.* and the 2023 review by Abas *et al.*[15–17]

2.1 P450s

C-14 functionalized steroids, encompassing 14α-OH and 14β-OH substituted steroids, are widely distributed in nature and exhibit significant biological activities, as evidenced by drugs such as the cardiac glycosides digoxin (**1**), digitoxigenin (**2**), and bufotalin (**3**), and the veterinary drug proligestone (**4**) (Fig. 1A).[20]

In 2019, Zhang and coworkers utilized the fungal P-450$_{lun}$ from *Cochliobolus lunatus* for C-14α hydroxylation.[21] P-450$_{lun}$ catalyzed the C-11β and C-14α hydroxylation of 11-deoxycortisol (INN, **5**), producing hydrocortisone (**6**) and 14α-OH-INN (**7**), respectively (the final **6**/**7** product ratio is about 3 : 2), while it mainly hydroxylated androstenedione (ASD, **8**) to yield 14α-OH-ASD (**9**) (regiospecificity over 99%) (Fig. 1B). Additionally, in 2019, Ichinose and coworkers characterized CYP5312A4 for the C-14α hydroxylation of testosterone (TES, **10**), to generate 14α-OH-TES (**11**) (95% substrate conversion) (Fig. 1C).[22]

In 2022, Ge and coworkers employed metabolomic and transcriptomic analyses to identify the C-14α hydroxylase CYP11411 from the plant *Calotropis gigantea* and CYP44476 from the toad *Bufo gargarizans*.[23] They conducted a C-14α hydroxylation reaction towards ASD (**8**) and obtained over 5 g of 14α-OH-ASD (**9**) through culture optimization of *C. lunata* CGMCC 3.9012 (70% isolation rate of **8**) (Fig. 1D). Recently, Qu and coworkers identified the highly efficient and promiscuous 14α hydroxylase CYP14A from *Cochliobolus lunatus*.[24] Through semi-rational engineering, the enzyme variants I111L/M115K and I111L/V124W displayed improved C-14 specificity and activity towards various steroids (**12**–**17**) (90–95% site selectivity, 72–78% conversion) (Fig. 1E).

The hydroxylation of C-19 in the steroid nucleus is crucial for the preparation of bioactive C-19 hydroxyl steroids, such as ouabagenin (**18**) and 19-nor steroidal drugs (Fig. 2A).[25–27] However, this process presents a significant challenge due to the steric hindrance from the proximity of the C-19 methyl group between the A and B rings of steroids.[26] In 2019, Zhu and coworkers identified the P450 enzyme STH10 from *Thanatephorus cucumeris* NBRC 6298, which catalyzed 19- and 11β-hydroxylations of INN (**5**) (Fig. 2B).[28] Subsequently, Zhou and coworkers improved the C-19 hydroxylation activity of *T. cucumeris* towards **5** and 17-acetyl-INN (**17**) by optimizing the fermentation conditions (80% conversion at the multi-gram scale) (Fig. 2C).[29]

The valuable bile acid, ursodeoxycholic acid (UDCA, **22**), is utilized for the treatment of primary biliary cholangitis.[30] It features a unique C-7β hydroxy group. The only difference between UDCA and chenodeoxycholic acid (CDCA) is the configuration of the C-7 hydroxy group, which imparts hydrophilicity to UDCA.[31] Direct hydroxylation at C-7 through chemical synthesis is challenging, due to the spatial distance of the C-7 position from any functional group facilitating C–H activation.[32]

The current synthetic approach for UDCA (**22**) involves a chemo-enzymatic pathway employing bile acids as initial
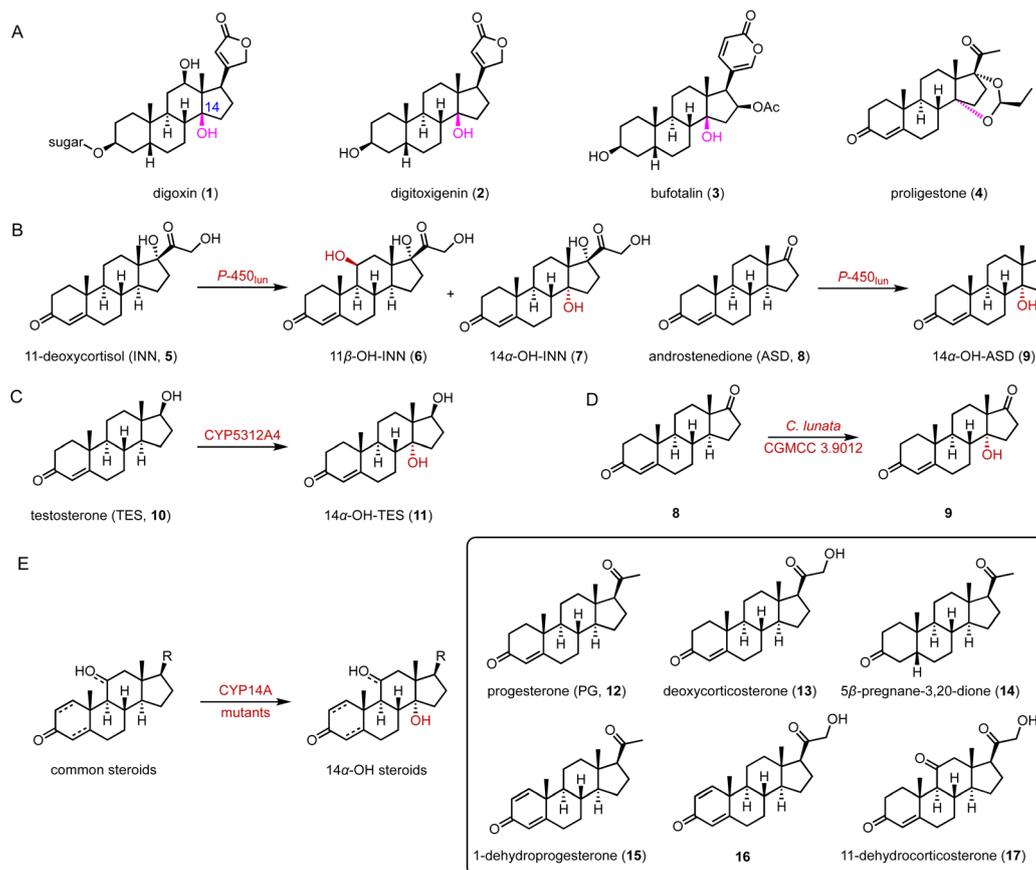
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Fig. 1 C-14 hydroxylation mediated by P450s. (A) Structures of C-14 functionalized steroid drugs. Hydroxylation reactions catalyzed by (B) P-450lun, (C) CYP5312A4, (D) *C. lunata*, and (E) CYP14A enzyme variants.
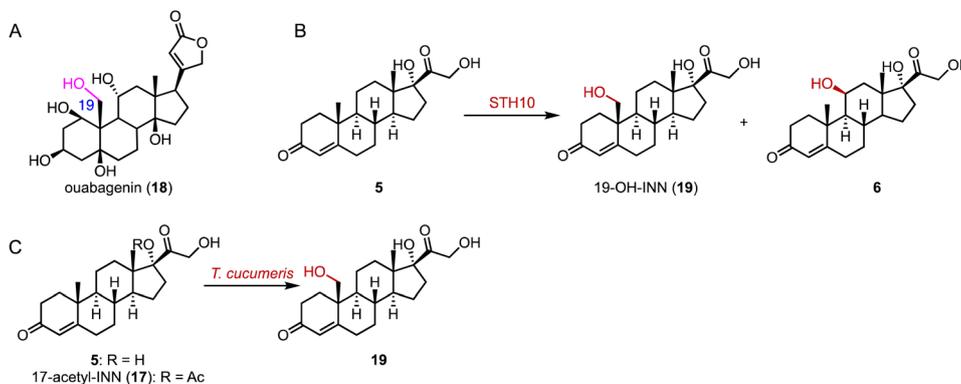

Fig. 2 C-19 hydroxylation mediated by P450s. (A) The structure of ouabagenin (**18**). Hydroxylation reactions catalyzed by (B) STH10 and (C) *T. cucumeris*.

materials.[33] In contrast, lithocholic acid (LCA, **20**), a cost-effective byproduct, could be directly converted to **22**. Bornscheuer and coworkers engineered CYP107D1 (OleP) to catalyze the C-7β hydroxylation of **20** to produce **22**, and the triple enzyme variant (F84Q/S240A/V291G) showed nearly perfect regio- and stereo-selectivity (over 95% selectivity), while the wild-type OleP catalyzed C-6β hydroxylation towards **20** to generate murideoxycholic acid (MDCA, **21**) (0.04 mmol L^{-1} h^{-1} space time yield) (Fig. 3A).[34,35]

Reetz and coworkers performed the directed evolution of P450 BM3 and identified the enzyme variant LG-23, containing 14 mutations compared with the wild-type enzyme.[36] LG-23 efficiently catalyzed the C-7β hydroxylation of different steroid substrates, including TES (**10**), nandrolone (**23**), ASD (**8**), adre-

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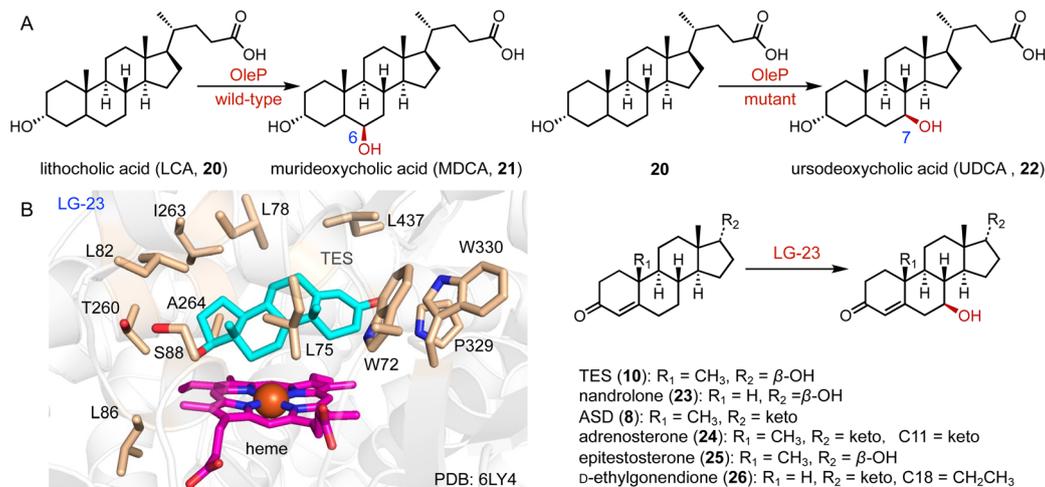*Org. Biomol. Chem.*, 2024, **22**, 3559–3583 | **3561**

Fig. 3 C-7 hydroxylation mediated by P450s. Hydroxylation reactions catalyzed by (A) OleP and (B) LG-23 and the binding mode of TES in the active site of LG-23. The TES, heme, and residues in the TES-binding site are colored cyan, magenta, and wheat, respectively.

nosterone (**24**), epitestosterone (**25**), and D-ethylgonendione (**26**) (75–95% selectivity, 55–99% conversion, 32–82% yield). The co-crystal structure of LG-23 and **10** showed that TES binds to various residues in the active site *via* van der Waals and hydrophobic interactions, revealing the binding mode for selective C-7β hydroxylation (Fig. 3B).

Protein engineering of P450s aims to improve the activity, thermostability, and/or alter the selectivity to conduct new-to-nature reactions.[37–42] Schallmey and coworkers engineered the C-16α hydroxylase CYP154C5 based on its crystal structure, and found that the CYP154C5 F92A variant catalyzed the hydroxylation of progesterone (PG, **12**), generating the

21-hydroxylated product 11-deoxycorticosterone (**28**) in addition to 16α-OH PG (**27**) (Fig. 4A).[43] Bernhardt and coworkers conducted the semi-rational protein engineering of the C-1α hydroxylase CYP260A1.[44] The S276N variant catalyzed the 1α-hydroxylation of PG (**12**), while the S276I variant led to the C-17α hydroxylation of PG (**12**) due to substrate orientation (Fig. 4B).

Li and coworkers engineered the C-16β steroid-hydroxylase CYP109B4 based on its crystal structure, and identified three crucial residues (V84, V292, and S387) involved in regio-selectivity control.[45] The B4-M7 variant (L240V/S387F/V84L/V292S/I291T/M290F/F294I) was characterized for its ability to
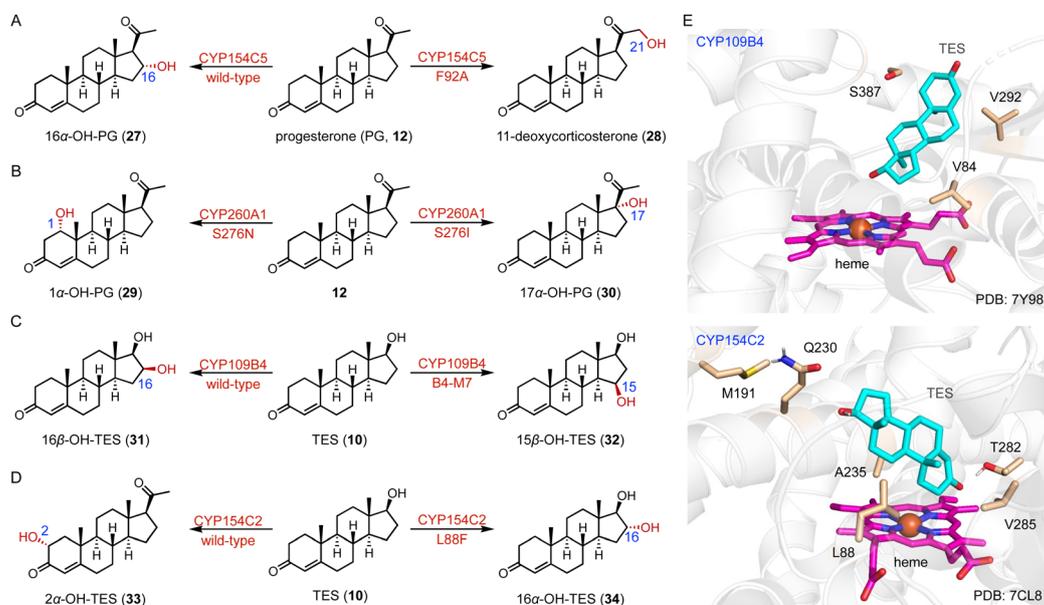

Fig. 4 Protein engineering of P450s for steroid synthesis. The protein engineering of (A) CYP154C5, (B) CYP260A1, (C) CYP109B4, and (D) CYP154C2. (E) The binding mode of TES in the active sites of CYP109B4 and CYP154C2. The TES, heme, and hot-spot residues are colored cyan, magenta, and wheat, respectively.

switch the regioselectivity to C-15β hydroxylation (Fig. 4C and E). Li and coworkers mutated the arginine residues around the substrate entrance and active site of the C-16β hydroxylase CYP105D7, and the conversion rate of the CYP105D7 R70A/R190A variant increased almost 9-fold towards TES (**10**) compared with the wild-type.[46] Recently, they utilized a structure-guided rational design strategy to improve the C-2α hydroxylation activity of CYP154C2.[46] The double variants L88F/M191F and M191F/V285L improved the activities towards TES (**10**) and ASD (**8**), respectively, while all enzyme variants containing L88F generated the 16α-hydroxylation product (Fig. 4D and E).

The dependence of the P450 catalytic function on the cofactor NAD(P)H and redox partner proteins limits its practical applications.[47] One useful strategy is to engineer P450 monooxygenases into the peroxizyme forms, peroxygenase and peroxidase.[48] Using this approach, Bell and coworkers altered the C-16α hydroxylase CYP154C8 to a peroxygenase (over 100-fold peroxygenase activity) by the T258E variant.[49]

Fungal organisms harbor numerous P450 enzymes capable of the highly selective production of valuable hydroxysteroids.[50–53] For example, CYP5150AP3 and CYP5150AN1 from *Thanatephorus cucumeris* NBRC 6298 catalyzed the C-7β hydroxylation of INN (**5**) and TES (**10**), and the C-2β hydroxylation of 11-deoxycortisol (**5**), respectively.[54] P450$_{cur}$ from *Curvularia* sp. VKM F-3040 possessed C-7β hydroxylase activity toward ASD (**8**), dehydroepiandrosterone (DHEA), and androstenediol, and C-7α hydroxylase activity toward 1-dehydrotestosterone.[55] CYP68J5_*Fusarium graminearum* (CYP68J5_fg) showed C-12β hydroxylation (31% yield), 15α-hydroxylation (48% yield) and C-12β and C-15α di-hydroxylation (21% yield) activities towards PG (**12**).[56] CYP68J5 from *Aspergillus ochraceus* TCCC41060 catalyzed the C-11α hydroxylation of both 16,17α-epoxyprogesterone and D-ethylgonendione.[57] The promiscuous CYP68BE1 from *Beauveria bassiana* accomplished mono-hydroxylation on C-11α, C-1α, and C-6β and di-hydroxylation on C-1β, C-11α and C-6β,11α of six steroids, including PG (**12**), TES (**10**), ASD (**8**), estrone, estra-4,9-diene-3,17-dione and 1,4-androstadiene-3,17-dione with more than 70% conversion rate.[58] CYP68JX from *Colletotrichum lini* ST-1 catalyzed the hydroxylation of DHEA to generate 3β,7α,15α-trihydroxy-5-androstene-17-one, a key intermediate for the synthesis of drospirenone, a progestin and antiandrogen medication.[59]

Novel steroid hydroxylases were also characterized from bacteria, animals, and other kingdoms.[60] For example, CYP154C2 from *Streptomyces avermitilis* MA4680 catalyzed the C-2α hydroxylation of TES (**10**).[61] The novel CYP17A2 from *Mastacembelus armatus* (MA_CYP17A2), identified by genome mining, efficiently performed the C-17α hydroxylation of PG (**12**) (120.9 ± 4.2 mg L^{-1} in 7 days).[62] CYP109A2 from *Bacillus megaterium* DSM319 catalyzed the 16β-hydroxylation of TES (**10**),[63] while the human P450 11B2 catalyzed the C-11β hydroxylation of 11-deoxycorticosterone (**28**) and PG (**12**).[64]

2.2 αKG OXs

α-Ketoglutarate-dependent oxygenases (αKG OXs) are metalloenzymes with a non-heme Fe^{2+} ion in the active center, and are widely distributed in both primary and secondary metabolic pathways.[65,66] They perform various reactions, including hydroxylation, epoxidation, desaturation, epimerization, halogenation, cyclization, demethylation, and aziridination.[67,68] Industrially, αKG OXs are used for the regioselective hydroxylation of amino acids, such as proline derivatives, and for the biosynthesis of antibiotics.[69,70]

Mizutani and coworkers reported that 22,26-oxycholesterol 16α-hydroxylase (16DOX) stereoselectively catalyzed the C-16α hydroxylation of (22S)-22,26-dihydroxy-cholesterol (**35**) (Fig. 5A).[71] Moreover, our group discovered the multifunctional αKG OX SptF, involved in the fungal meroterpenoid emervari-
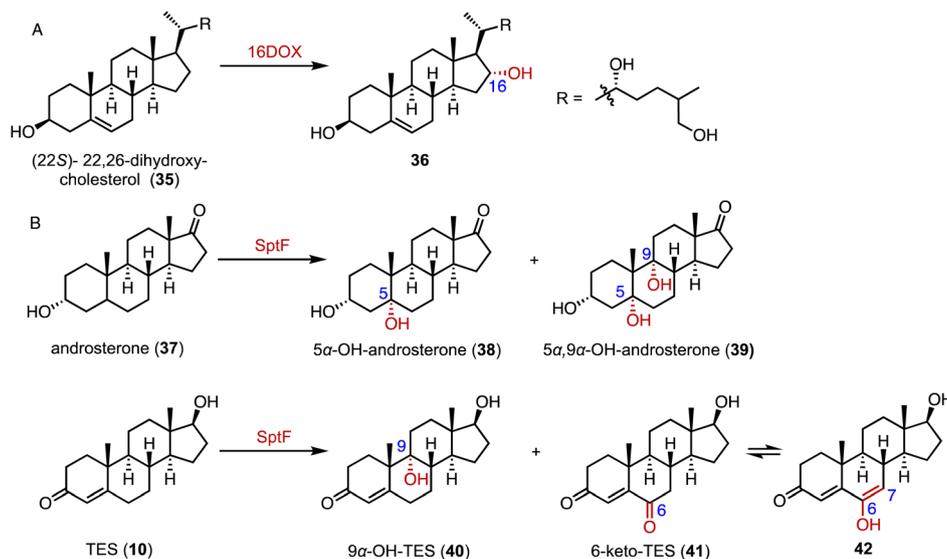

Fig. 5 Hydroxylation reactions mediated by αKG OXs. Hydroxylation reactions catalyzed by (A) 16DOX and (B) SptF.

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done biosynthesis pathway.[72–74] It promiscuously catalyzed α-hydroxylation at the C-5 and C-9 positions with conversion rates of 34%–53% over 24 h, demonstrating the biocatalytic potential of αKG OXs for modifications of the steroid nucleus (Fig. 5B).

2.3 Rieske oxygenases

3-Ketosteroid 9α-hydroxylase (KSH), an essential enzyme in bacterial steroid degradation, consists of two components: a Rieske oxygenase (KshA) and a ferredoxin reductase (KshB).[75–78] It catalyzes the 9α-hydroxylation of ASD (**8**) to produce 9α-OH-ASD (**43**). Qin and coworkers reconstituted the NADH regeneration system, utilizing the fusion expression of toluene 2,3-oxidoreductase TDO-R from *Pseudomonas putida* F17 instead of KshB, along with a Rieske [2Fe–2S] iron-oxidore-ductant protein, TDO-F.[79] The reconstitution significantly enhanced the electron transfer efficiency by 176.3%. Optimization led to the final production of 5.24 g L^{-1} of 9α-OH-ASD (**35**) from ASD (**8**), with a yield of 99.3% (Fig. 6).

Recently, Liu and coworkers employed KSH as an efficient biocatalyst to cleave the C9–C10 bond and aromatize the A-ring of steroids, by C-9α hydroxylation and a fragmentation cascade.[80] Investigation of the substrate scope revealed the acceptance of a broad range of dienone steroids with variable C-17 side chains to generate the corresponding 9,10-secoster-oid products (**44** to **49**) (87–97% yield). In addition, KSH mainly converted the non-dienone substrates that lack the C1–C2 double bond to the corresponding C-9α hydroxylated products (**50** to **52**) (64–94% yield) (Fig. 7).

2.4 Other oxygenases

Fungal unspecific peroxygenases (UPOs) offer significant advantages in the stereo- and regio-selective oxidation of ali-phatic C–H bonds, such as hydroxylation, epoxidation, and sulfoxidation.[81–83] Kiebist and coworkers characterized
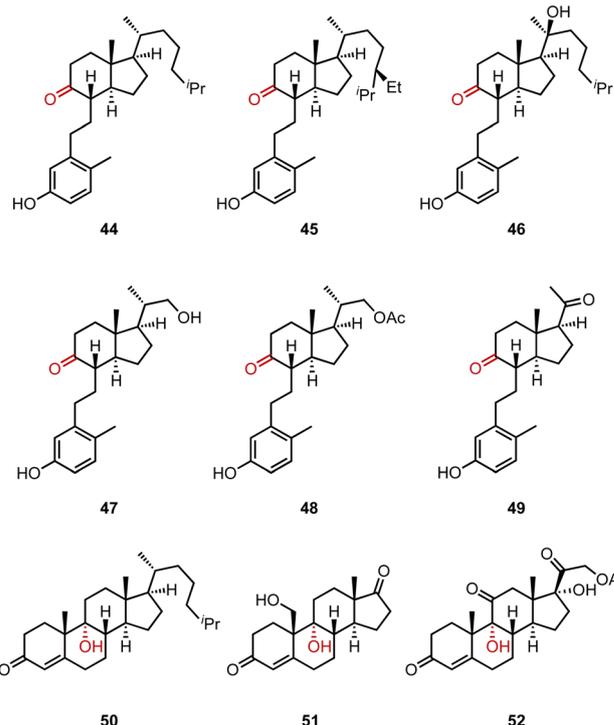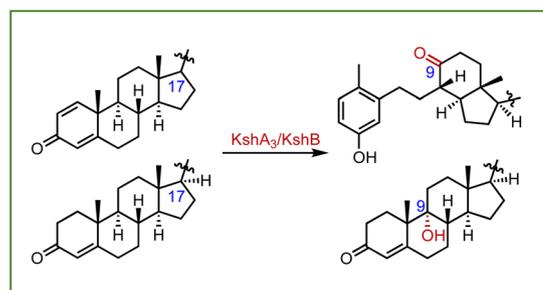

Fig. 7 The substrate scope of C-9α hydroxylation and the fragmenta-tion cascade catalyzed by KshA₃/KshB.
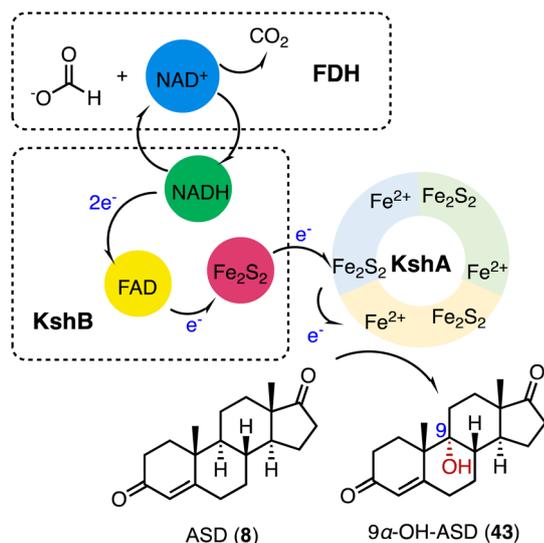
*Cgl*UPO, from the ascomycetous fungus *Chaetomium globosum*, which converted TES (**10**) to 4,5 epoxy-TES (**53**) and 16α-OH-TES (**34**) with a total turnover number (TTN) of up to 7000 (Fig. 8).[84]

3. Epoxidation

Epoxides are valuable organic intermediates with a reactive oxirane group capable of nucleophilic opening, elimination, reduction, or rearrangement.[85,86] In terms of steroid epoxi-dation, Auchus and coworkers reported that CYP17A1 cata-lyzed the novel C-16α,17 epoxidation of 16,17-dehydroproges-terone (**54**) in addition to the minor C-21 hydroxylation to generate **55** and **56**, respectively (Fig. 9A).[87] Besides, CYP17A1 A105L variant gave a 1 : 5 ratio of **55** : **56**. Wang and coworkers performed the directed evolution of P450 BM3 and identified the variant P450 BM-3 5-B10 (A221V/H940Q based on the P450 BM3 enzyme variant 139-3), which catalyzed the 16α,17-epoxi-
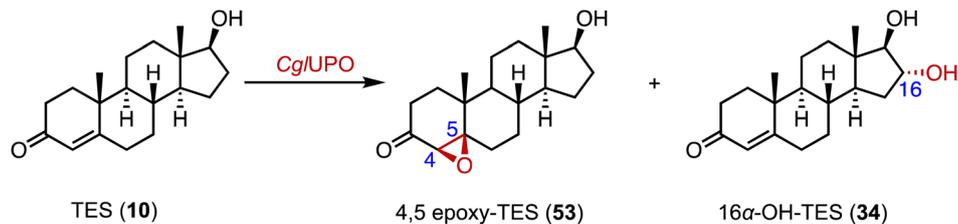

Fig. 6 C-9 hydroxylation mediated by KSH.

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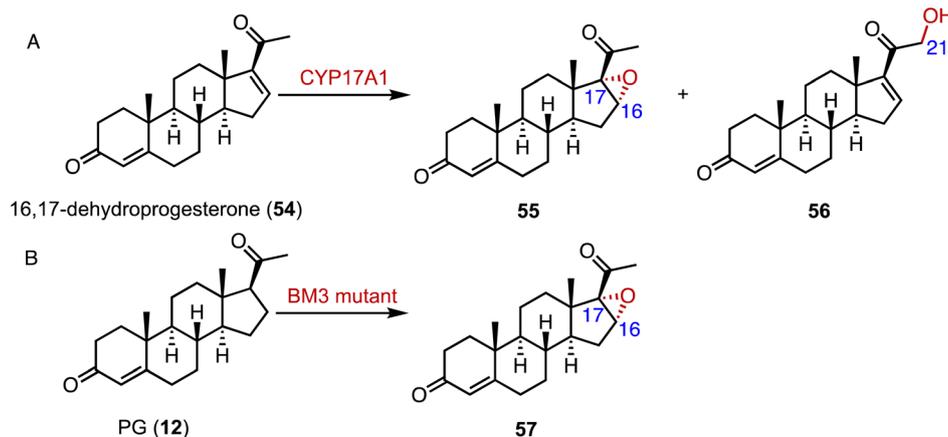**Fig. 8** Hydroxylation and epoxidation mediated by *Cgl*UPO.

Fig. 9 Epoxidation mediated by P450s. Epoxidation reactions catalyzed by (A) CYP17A1 and (B) the P450 BM3 enzyme variant.

dation of PG (**12**) (Fig. 9B).[88] As mentioned previously, *Cgl*UPO also catalyzed the 4,5-epoxidation of TES (**10**) (Fig. 8).[84]

4. Ketoreduction

The biocatalytic reduction of prochiral ketones into the corresponding chiral alcohols represents a valuable strategy in asymmetric synthesis.[89–91] Hydroxysteroid dehydrogenase (HSDH) plays a pivotal role in the metabolic processes and synthetic pathways for the ketoreductions of steroid hormones.[90–93] Typical HSDHs are C-7α hydroxysteroid dehydrogenase (7α-HSDH) and C-7β hydroxysteroid dehydrogenase (7β-HSDH), used for the synthesis of UDCA (**22**). 7α-HSDH catalyzes the C-7 oxidation of chenodeoxycholic acid (CDCA, **58**) to 7-oxo LCA (**59**), and then 7β-HSDH converts **59** to UDCA (**22**) (Fig. 10).[94–96] Considering the atom economy of the HSDH

cofactor system, Xu and coworkers switched the cofactor preference of 7β-HSDH from NADPH to NADH for cost-effective biotechnical applications.[97] In addition, these researchers recently characterized a novel NADH-dependent *Rs*7β-HSDH from *Roseococcus* sp. that showed high specific activity towards **59** (63.3 U per mg_{protein}).[98]

The C-3 hydroxyl substituent is an important moiety in various steroid drugs, such as allopregnanolone (brand name: Zulresso, treatment of postpartum depression) (**60**), dehydroepiandrosterone (brand name: Intrarosa, treatment of moderate to severe dyspareunia) (**61**), and alfaxalone (brand name: Alfaxan, a neurosteroid anesthetic) (**62**) (Fig. 11A).[99] Recently, Xu and coworkers developed an efficient platform for the stereo-complementary synthesis of 3-hydroxy-5-hydrogen steroids.[100] They utilized *Ct*3α-HSDH and *Ss*3β-HSDH, which demonstrated exclusive chemo-, regio-, and enantio-selectivities for the reduction of 3-ketones across 25 examples (up to
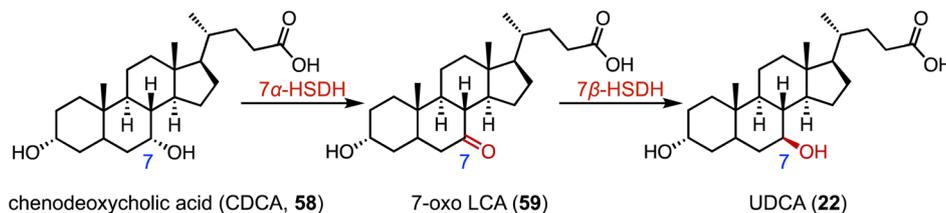

Fig. 10 The synthesis of UDCA (**22**) through C-7 hydroxylation mediated by 7α-HSDH and 7β-HSDH.

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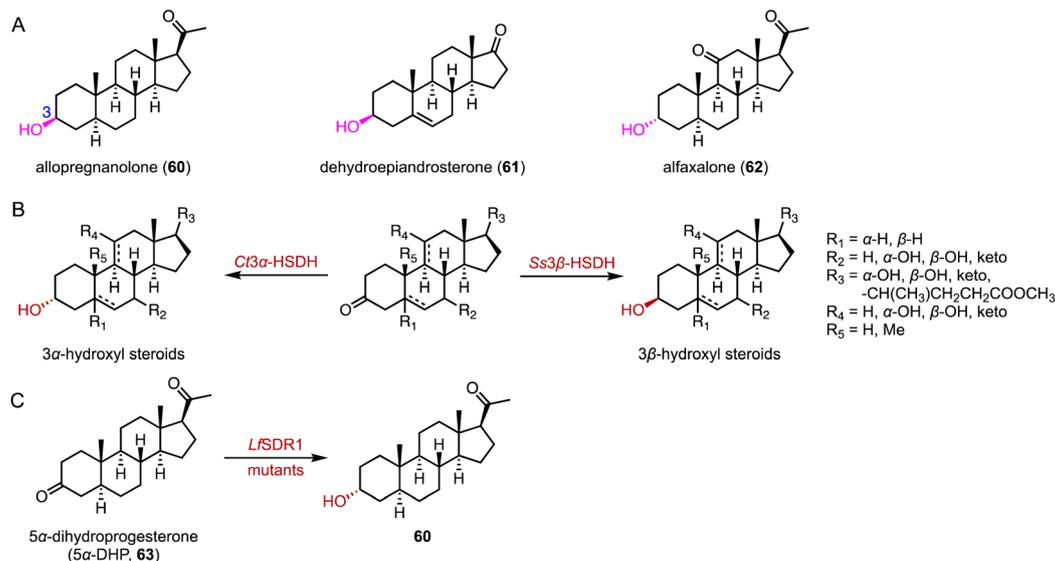*Org. Biomol. Chem.*, 2024, **22**, 3559–3583 | **3565**

Fig. 11 C-3 reduction mediated by reductases. (A) The structure of C-3 hydroxy steroid drugs. (B) The stereo-complementary synthesis of C-3 hydroxy steroids by *Ct*3α-HSDH and *Ss*3β-HSDH. (C) Reduction reaction catalyzed by *Lf*SDR1.

99% conversion and >99% ee) (Fig. 11B). In a separate effort, Qin and coworkers identified *Lf*SDR1 E141L/I192V variant by screening and engineering ketoreductases for the synthesis of allopregnanolone (**60**) with >97% conversion and a 90% isolated yield (Fig. 11C).[101]

An unusual dual-role reductase, mnOpccR, involved in phytosterol catabolism, was discovered by Qu and coworkers in 2020.[102] It reduced 3-oxo-4-pregnene-20-carboxyl-CoA (3-OPC-CoA, **64**) in either a four-electron manner to form 20-hydroxymethyl pregn-4-ene-3-one (4-HBC, **65**) or a two-electron manner to 3-oxo-4-pregnene-20-carbaldehyde (3-OPA, **66**) to form **65**. The strain mJTU8 (ΔkstD-Δhsd4A and pG13-mnOpccR) harboring mnOpccR from *Mycobacterium neoaurum* resulted in the exclusive generation of **65** from phytosterols (Fig. 12).

5. Dehydrogenation

The flavin adenine dinucleotide (FAD)-dependent 3-ketosteroid Δ^1-dehydrogenase (KstD) introduces a double bond between the C-1 and C-2 atoms in the A ring of the C-3 ketosteroids, a key feature in several glucocorticoids such as dexamethasone (**67**), betamethasone dipropionate (**68**), clobetasol propionate (**69**), and beclomethasone dipropionate (**70**) (Fig. 13A).[103] In recent years, various KstDs with high activities have been discovered and applied.[104–112] For instance, Guo and coworkers used *Nocardioides* for the $\Delta^{1,2}$-dehydrogenation of **71** to produce **72**, which is an important intermediate for the synthesis of fluocinolone acetonide (Fig. 13B).[113] In 2022, Chen and coworkers adopted a Focused Site-directed Iterative Saturation Mutagenesis (FSISM) strategy for the engineering of
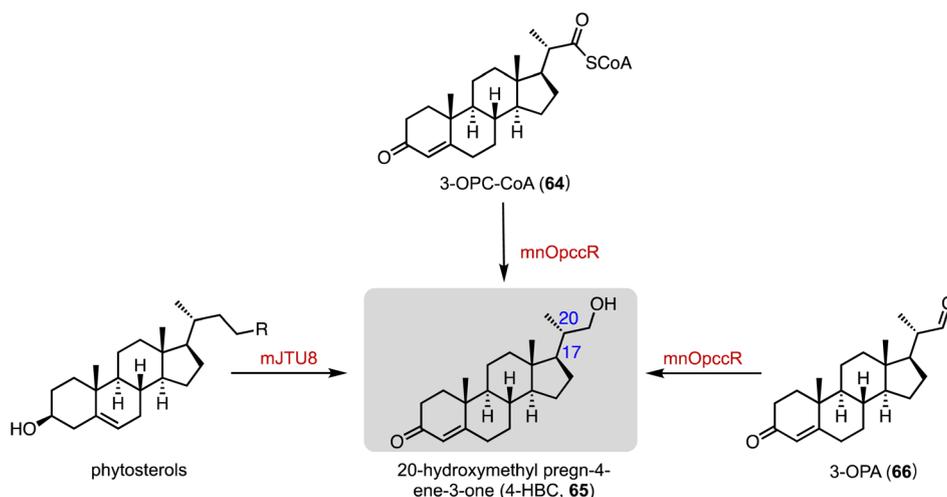

Fig. 12 Reduction mediated by mnOpccR.

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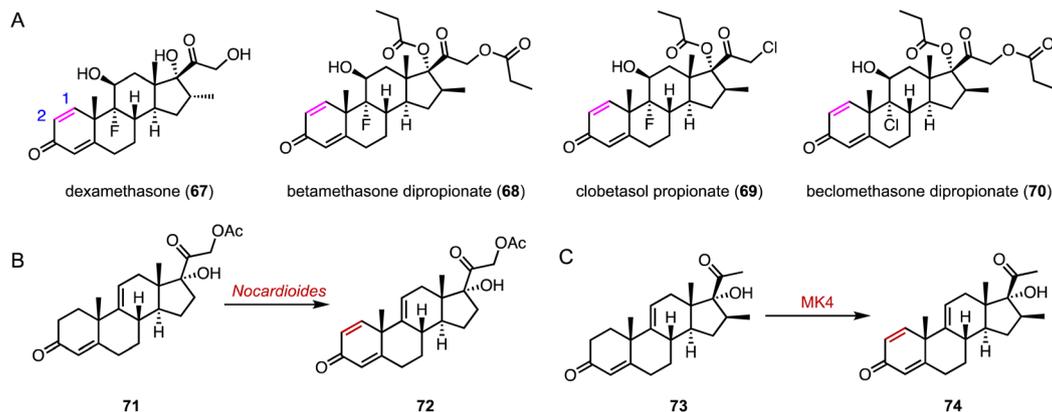This journal is © The Royal Society of Chemistry 2024

Fig. 13 Dehydrogenation mediated by KstDs. (A) Structures of glucocorticoids. Dehydrogenation reactions catalyzed by (B) *Nocardioides* and (C) MK4.

MsKstD1, and the quadruple enzyme variant MK4 (H132M/L113F/V419W/M51L) exhibited 10-fold higher activity towards hydrocortisone than wild-type MsKstD1.[114] Furthermore, MK4 catalyzed the desaturation of **73** to generate **74**, a key intermediate in the synthesis of 16β-methylcorticoids (Fig. 13C).[115]

6. Halogenation

The incorporation of halogens significantly alters the physico-chemical properties of compounds and may affect their biological activities, metabolic pathways, and pharmacokinetic profiles.[116] In addition, carbon–halogen bonds are a prominent class of synthetic building blocks in organic synthesis, which can be used for subsequent metal-catalyzed cross-coupling reactions and nucleophilic substitution reactions.[117,118] In terms of the enzymatic halogenation of steroids, Lewis and coworkers performed family-wide activity profiling of flavin-

dependent halogenases (FDHs) and identified 1-F11, which catalyzed the bromination of β-estradiol 17-(β-D-glucuronide) (**75**) at the C-4 position in 57% yield (Fig. 14A).[119] In addition, Ayala used a fungal chloroperoxidase for the di-halogenation of estradiol (**76**) at the C-2 and C-4 positions to generate **78** (Fig. 14B).[120]

7. Glycosylation

Steroid glycosylation plays a multifaceted role in biology and medicine, influencing the solubility, bioactivity, and therapeutic potential of steroid drugs.[121–123] A notable example is cardiotonic steroids, which feature glycosylation at the C-3 position, leading to lower cardiotoxicity.[124] Chemical glycosylation of steroids faces limitations due to the protection and deprotection processes, poor regio- and stereo-selectivities, and low yields.[125,126] An alternative solution is enzymatic glycosylation, by enzymes such as *Pp*UGT6, UGT80A40, UGT80A41,
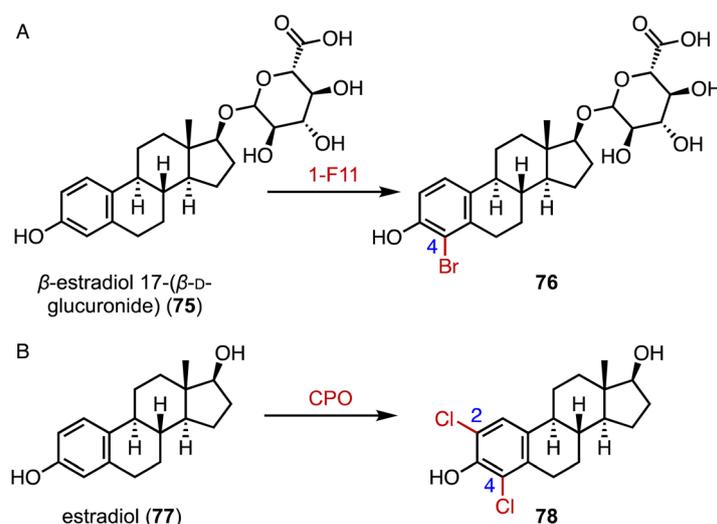

Fig. 14 Halogenation mediated by halogenases. Halogenations catalyzed by (A) 1-F11 and (B) CPO.

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UGT73CR1, UGT80A33, and UGT80A34 from the medicinal plant *Paris polyphylla*, and TfS3GT2 from the pharmacologically important herb fenugreek, which are involved in the biosynthesis of steroidal saponins.[121,127–130]

Feng and coworkers reported the structures of the first sterol glycosyltransferase, UGT51 from *Saccharomyces cerevisiae* and its complex with uridine diphosphate glucose (UDPG, **79**).[131] The UDPG binding pocket features hydrogen and nonpolar bonds (Fig. 15A). UGT51 catalyzed the glycosylation of ergosterol to generate ergosteryl-glucoside.[132] Guided by semi-rational protein engineering, the enzyme variant M7_1 (S801A/L802A/V804A/K812A/E816K/S849A/N892D) was identified and converted protopanaxadiol to the ginsenoside Rh2.[133]

Long and coworkers conducted a structure–function analysis of the plant steroid 3-*O*-glycosyltransferase UGT74AN3, from the medicinal plant *Calotropis gigantea*.[134–136] It mainly catalyzes the C-3 glycosylation of steroids. The overall structure displays a classic GT-B fold structure, characterized by two Rossmann-like β/α/β domains connected by a hinge linker. The N-terminal domain (NTD) and the C-terminal domain (CTD) play primary roles in recognizing and binding the sugar acceptor and donor, respectively. UGT74AN3 showed broad substrate promiscuity towards 78 acceptors and 6 sugar donors (**80–85**).[135,136] UGT74AN3 also exhibited *N*-/*S*-glycosylation activities towards aromatic compounds, expanding the biocatalytic toolbox for producing valuable glycosides (Fig. 15B).

8. Side-chain cleavage

The side-chain cleavage products, androstenedione (ASD, **8**) and androst-1,4-diene-3,17-dione (ADD), derived from cholesterol (**86**) or phytosterols through biological fermentation, serve as key intermediates in the industrial production of steroid drugs.[137–139] In humans, cholesterol (**86**) undergoes a series of three hydroxylation steps catalyzed by CYP11A1, leading to the cleavage of the C-20, 22 bond and the formation of pregnenolone (**87**) (Fig. 16A).[140,141]

Recently, Wang and coworkers reported that CYP87A4 is involved in sterol side-chain cleavage in digoxin (**1**) biosynthesis.[142] Sonawane and coworkers also found that CYP87A exhibited side-chain cleavage activity on both cholesterol (**86**) and phytosterols (campesterol **88** and β-sitosterol **89**), resulting in the formation of pregnenolone (**87**).[143] This marks the initial committed step in cardenolide biosynthesis (Fig. 16B).

In the biosynthetic pathway of the furanosteroid demethoxy-viridin, Gao and coworkers identified a novel pregnane side-chain cleavage pathway mediated by a Baeyer–Villiger mono-
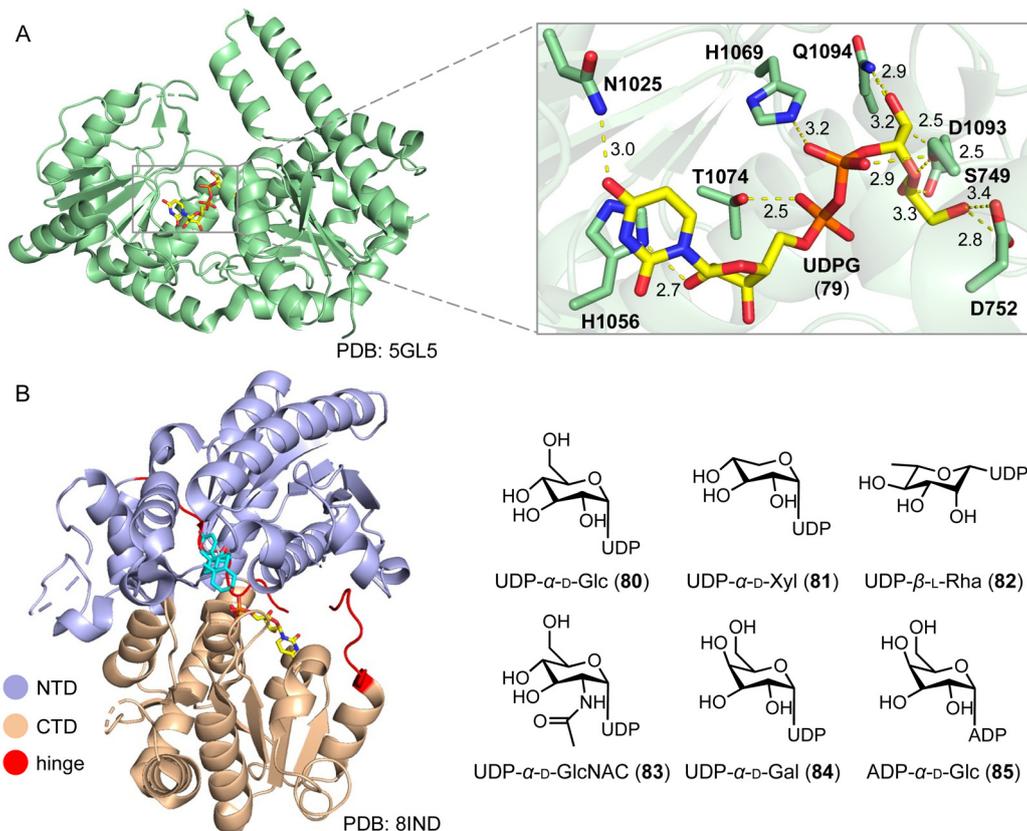
Fig. 15 Glycosylation mediated by glycosyltransferases. (A) Overall structure and active site of UGT51. The protein and the ligand UDPG are shown in pale green and yellow, respectively. (B) Overall structure of UGT74AN3 and sugar donor promiscuity of UGT74AN3. The NTD domain, CTD domain, and hinge motif are colored light blue, wheat, and red, respectively.

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Fig. 16 Side-chain cleavage mediated by P450s in humans (A) and in plants (B).

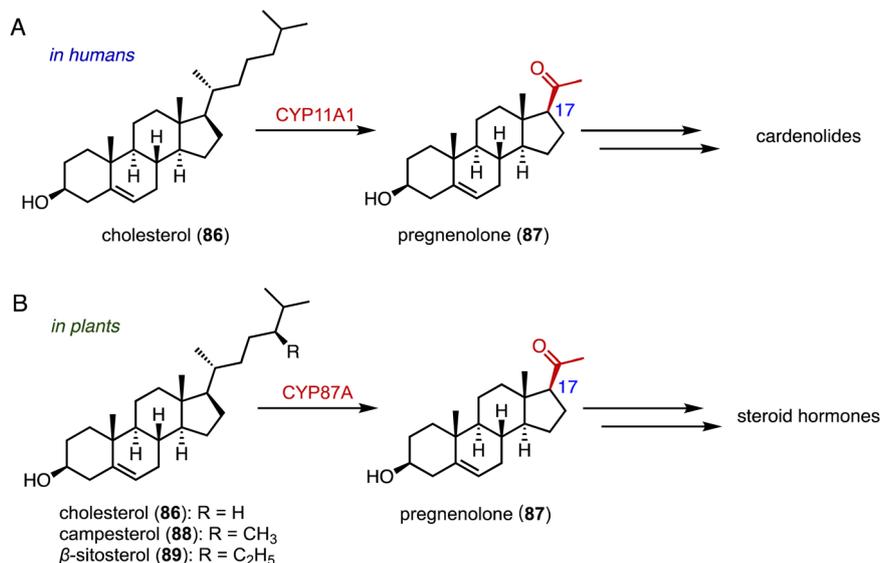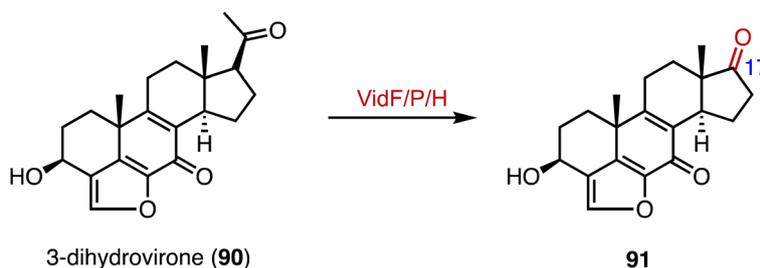**Fig. 17** Side-chain cleavage mediated by VidF/P/H.

oxygenase (VidF), an esterase (VidP), and a dehydrogenase (VidH).[144] These three enzymes work together to cleave the C-17 and C-20 side chain of 3-dihydrovirone (**90**) to generate **91** (Fig. 17).

9. Methylation and demethylation

Methyltransferases (MTs) are ubiquitous in nature and serve in a wide range of vital life processes, including signal transduction, transcriptional regulation, and natural product biosynthesis.[145] Recently, Qu and coworkers identified the novel carboxyl methyltransferase (CbMT) OPCMT, from *Mycobacteria*.[146] It exhibited broad substrate specificity (**92** to **104**), converting carboxylic acids to the corresponding carboxylic acid methyl esters (Fig. 18A). Haslinger and coworkers characterized StrAOMT, an *O*-methyltransferase from *Streptomyces avermitilis*, which catalyzed the methylation of 2-hydroxyestradiol (**105**) to generate 2-methoxyestradiol (**106**) and **107** (Fig. 18B).[147]

Welander and coworkers reported sterol C-24 methyltransferases (SMTs) from both sponges and yet-uncultured bacteria that catalyzed the methylation of desmosterol (**108**) to generate C-24 methyl sterols, including 24-methyl-enecholesterol and (*epi*)codisterol (**109** to **111**) (Fig. 18C).[148,149] Furthermore, Liebeke and coworkers found that gutless marine annelids predominantly synthesized sitosterol, a plant sterol, by a noncanonical SMT, indicating the broader occurrence of plant-like sterol biosynthesis in animals.[150,151]

Chiang and coworkers reported a B_{12}-dependent methyltransferase system, EmtAB, from an anaerobic β-proteobacterium, *Denitratisoma* sp. strain DHT3, which converted estradiol (**77**) to 1-dehydrotestosterone (**112**) (Fig. 18D).[152,153] These results suggested a novel metabolic connection between cobalamin and steroid metabolism. The ratio of 4α/4β-methyl sterane is used as an important indicator of sediment maturity in crude oil and natural gas exploration.[154] The 4β-methyl steranes have long been considered to be formed from 4α-methyl steroids during diagenesis.[155] However, Lu and coworkers discovered that BmSTRM, a sterol A-ring methylase (STRM) from *B. minutum*, catalyzed the biosynthesis of both C-4α and C-4β methyl sterols from cholestanone (**113**) (Fig. 18E).

The C-14α demethylation catalyzed by P450 51 is a crucial step in eukaryotic sterol biosynthesis, such as lanosterol conversion in mammals.[156,157] Guengerich and coworkers solved the crystal structure of the human P450 51A1, and the application of

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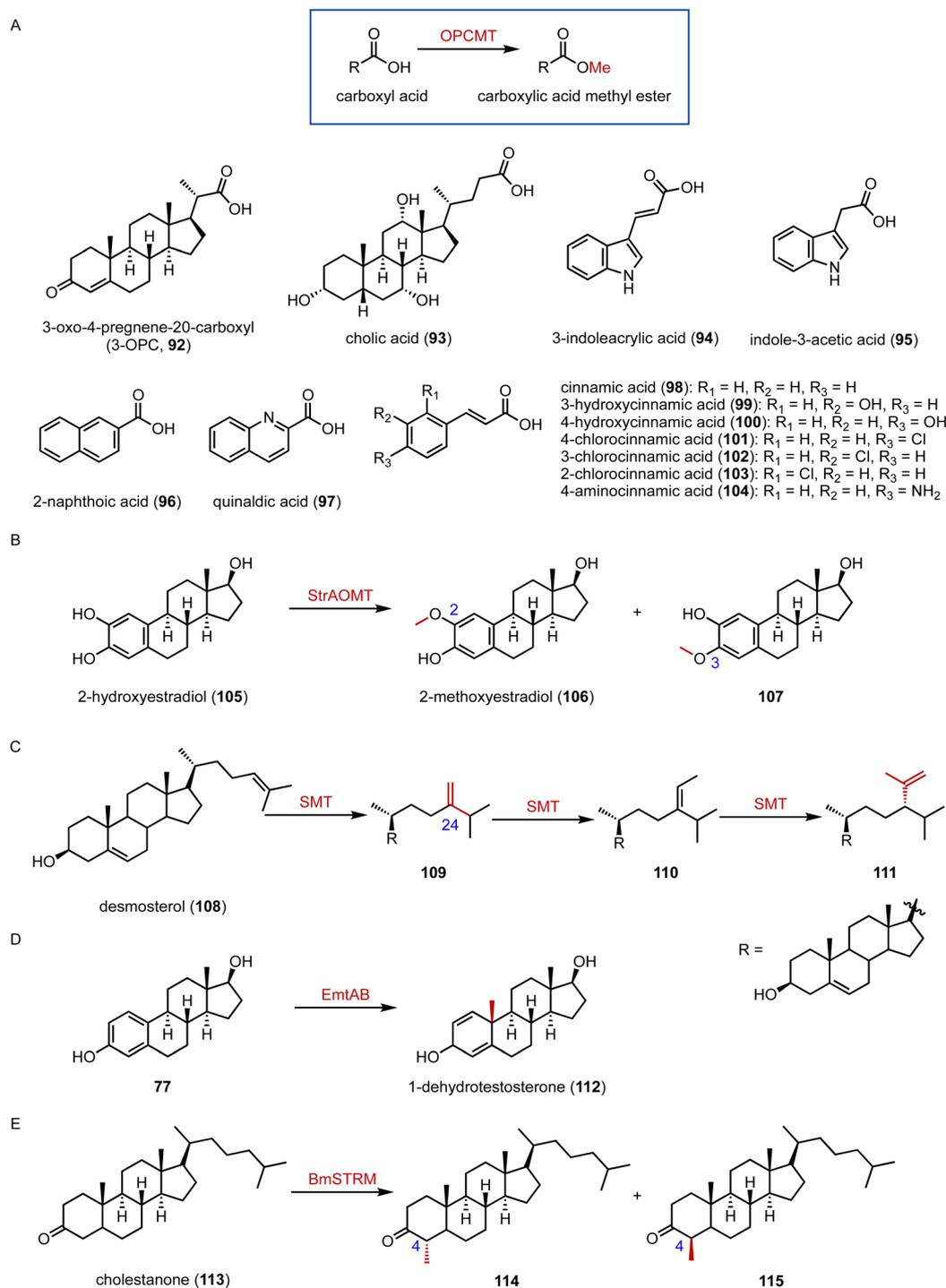*Org. Biomol. Chem.*, 2024, **22**, 3559–3583 | **3569**

Fig. 18 Methylation reactions. (A) The substrate specificity of OPCMT. Methylations catalyzed by (B) StrAOMT, (C) SMT, (D) the EmtAB system, and (E) BmSTRM.

those data in isotopic experiments supported the major contribution of Compound 0 (ferric peroxide anion, FeO_2^-) in 24,25-dihydrolanosterol (**116**) C–C cleavage (Fig. 19A).[158] In addition, a cytochrome P450 aromatase (CYP19A1) is responsible for the biosynthesis of estrogens from androgens, by C-10 demethylation and A-ring aromatization.[159–161] Similarly, in the biosynthetic

pathway of demethoxyviridin, the cytochrome P450 monooxygenase VidA catalyzes the C-13 demethylation and C-ring aromatization processes.[144]

Welander and coworkers highlighted bacterial sterol biosynthesis as distinct from eukaryotes, and identified the C-4 demethylation reaction in *Methylococcus capsulatus*.[162] In detail,

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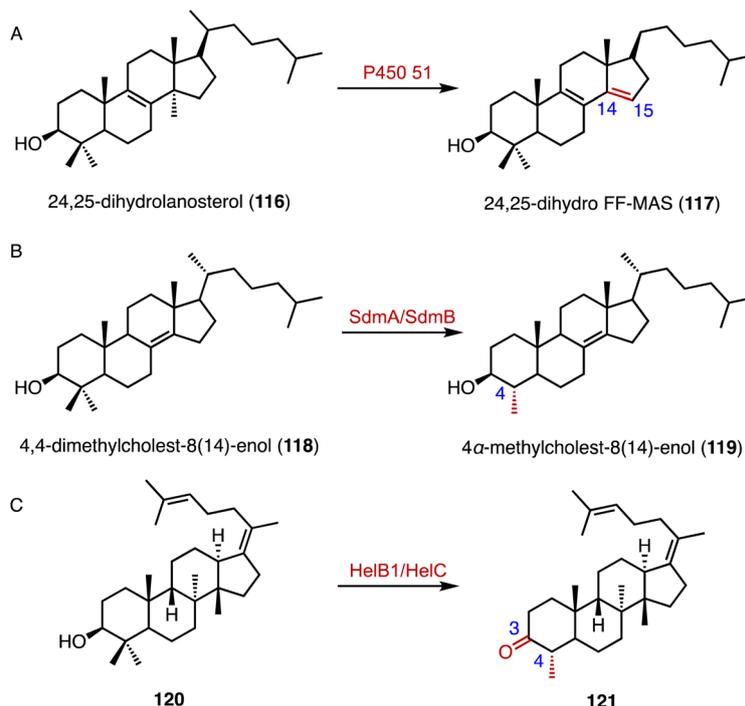This journal is © The Royal Society of Chemistry 2024

Fig. 19 Demethylation reactions catalyzed by (A) P450 51, (B) SdmA/SdmB, and (C) HelB1/HelC.

the Rieske-type oxygenase SdmA catalyzed the oxidation of the C-4β methyl group of 4,4-dimethylcholest-8(14)-enol (**118**), and the NAD(P)H-dependent enzyme SdmB catalyzed the dehydrogenation to generate 4α-methylcholest-8(14)-enol (**119**) (Fig. 19B). Yao and coworkers identified an unusual C-4 demethylation process in the biosynthesis of helvolic acid.[163] The cytochrome P450 enzyme HelB1 oxidized the C-4β methyl group of **120**, and the multifunctional SDR HelC removed the methyl group *via* a β-keto carboxylic acid intermediate to produce **121** (Fig. 19C).

10. Ring system reconstruction

Reconstruction of ring systems plays vital roles in natural product biosynthesis, particularly in the production of complex molecules like steroids.[164] These processes enable the generation of structural diversity, which is crucial for the adaptation and survival of organisms in their environments. For example, in the biosynthesis of demethoxyviridin, three P450 enzymes (VidE, VidG, VidR), a short chain oxidoreductase (VidO), a hypothetical protein (VidN), and the glyoxalase VidQ are responsible for the installation of the typical furan ring (Fig. 20A).[144] In diosgenin biosynthesis, CYP90 catalyzes the dihydroxylation of cholesterol (**86**), allowing the further oxidative cyclization to generate the spiroketal moiety (Fig. 20B).[165] In addition, the flavin-containing Baeyer–Villiger monooxygenase (BVMO) PockeMO, from *T. thermophila*, catalyzes the insertion of a single oxygen atom into the D ring system of ASD (**8**) to generate **125** (Fig. 20C).[166]

Chiang and coworkers identified the *aed* (actinobacterial oestrogen degradation) gene cluster, encoding estrogen-degrading genes, in the actinobacterium *Rhodococcus* sp. Strain B50.[167] In the degradation pathway, the cytochrome P450 monooxygenase AedA is responsible for estrone (**126**) C-4 hydroxylation, and the 4-hydroxyestrone 4,5-dioxygenase AedB subsequently catalyzed the meta-cleavage of the estrogen A ring to generate pyridinestrone acid (PEA, **129**) (Fig. 21).

11. Acylation

Acylation serves as a crucial strategy for enhancing the biological activities of steroid drugs, and stands as a significant avenue for drug modification.[168] Achieving site-specific acylation through chemical methods remains challenging, due to the similar chemical environments of the hydroxyl groups in steroids.[169,170] In contrast, enzymatic acylation provides a sustainable and alternative approach. For instance, Qiao and coworkers discovered a highly regio- and stereo-selective acetyltransferase, *Am*AT19, from *Astragalus membranaceus*.[171] It catalyzed the 6α-OH acetylation of various steroids, producing the hyodeoxycholic acid 6-*O*-acetates **130**, **131**, and **132** (Fig. 22). Zhou and coworkers reported the structure of a human diacylglycerol *O*-acyltransferase 1 (DGAT1), which facilitates the conversion of cholesterol to cholesteryl ester and is crucial for cholesterol storage and transport.[172] The cryogenic electron microscopy (cryo-EM) structure of human ACAT1 revealed a dimeric structure with nine transmembrane segments,

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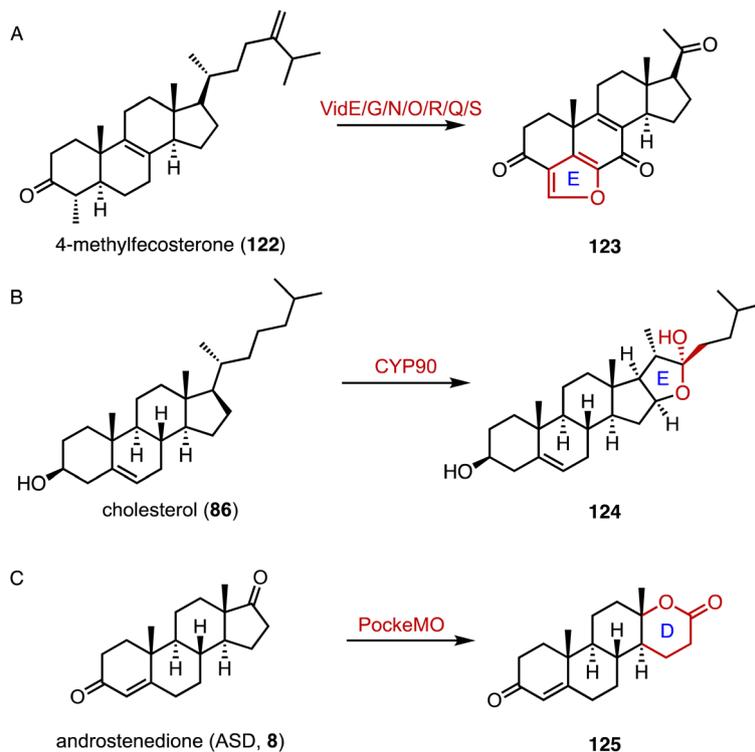*Org. Biomol. Chem.*, 2024, **22**, 3559–3583 | **3571**

Fig. 20 Ring system reconstruction. Ring contractions catalyzed by (A) VidE/G/N/O/R/Q/S and (B) CYP90. (C) Ring expansion catalyzed by PockeMO.
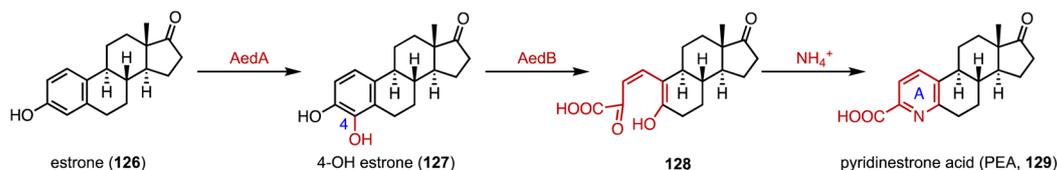

Fig. 21 Ring reconstruction mediated by AedA/B.
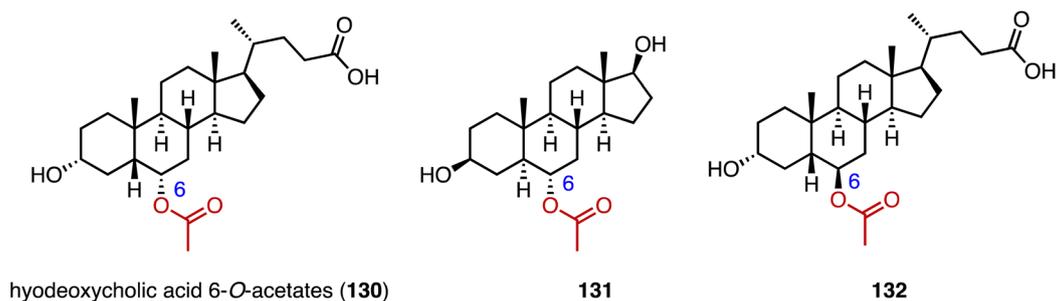
Fig. 22 Structures of acylated steroids mediated by AmAT19.

forming a cytosolic and transmembrane tunnel for acyl-coenzyme A and cholesterol entry, respectively.[173]

Bacteria utilize aminoacyl-transfer RNAs (tRNAs) to aminoacylate membrane lipids, thus enhancing antimicrobial resistance and virulence, in reactions that have not yet been observed in eukaryotes.[174–176] Becker and coworkers discovered

that most fungi, including pathogens like *Aspergillus fumigatus*, utilize tRNA-dependent mechanisms to attach aspartate to ergosterol (**133**), forming ergosteryl-3β-*O*-L-aspartate (Erg-Asp, **134**), a major sterol in fungal membranes.[177] Aspartylation is catalyzed by ergosteryl-3β-*O*-L-aspartate synthase (ErdS), which transfers aspartate from Asp-tRNA^{Asp} onto **133**, and the

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Fig. 23 Aspartylation mediated by ErdS/ErdH.
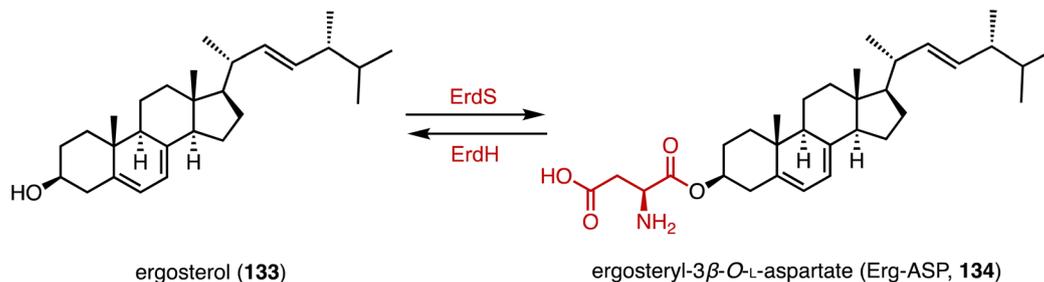
removal of the Asp modifier from **134** is catalyzed by a dedicated hydrolase, ErdH (Fig. 23). The discovery of tRNA-dependent sterol aspartylation highlights a previously unrecognized aspect of fungal biology and offers potential targets for combating fungal infections.

12. Amination and amidation

Chiral amines are important building blocks in many pharmaceuticals and fine chemicals.[178] Aminotransferases utilize the pyridoxal-5'-phosphate (PLP) cofactor to transfer an amino group from the amino donor to the targeted amino acceptor in a stereoselective fashion.[179–181] For the efficient synthesis of 17α-amino steroids, Hailes and coworkers employed the ω-transaminase ArRMut1 for the transamination of **135** to **137** (88–90% yield, 83–89 isolated yield) (Fig. 24A).[182] Prodanovic and coworkers also used this ω-transaminase for the conversion of dihydrotestosterone (**138**) to generate **139** (50% yield) (Fig. 24B).[183] In the biosynthetic pathway of steroidal glycoalk-

aloids (SGAs), Mizutani and coworkers characterized PGA4, a C-26 aminotransferase, which catalyzes the transamination of **140** to produce **141** by introducing the nitrogen into the cholesterol backbone (Fig. 24C).[184]

Bacteria in the gut produce amino acid bile acid amidates that are important in host-mediated metabolic processes.[185] Recently, Patterson's and Quinn's groups reported that bile salt hydrolase (BSH) not only plays a role in bile acid metabolism but also acts as an amine N-acyltransferase, forming bacterial bile acid amidates (BBAAs).[186,187] BSH belongs to the N-terminal nucleophile superfamily of enzymes and catalyzes the hydrolysis of glycocholic acid (**142**) and taurocholic acid (**143**) to generate BBAAs (Fig. 25).

13. Isomerization

Ketosteroid isomerase (KSI) catalyzes the rearrangement of the double bond at C-5 and C-6 in ketosteroids through an enolate intermediate, and a typical KSI reaction is shown in Fig. 26.[188–190] Understanding how enzymes adapt to temperature is crucial for grasping molecular evolution and enzyme function. Herschlag and coworkers found that the temperature adaptation of KSI primarily stems from one residue change, indicating widespread parallel adaptation across organisms.[191,192] Boxer and colleagues reported that an electric field in the active site of KSI aligns its substrate in the ground state, minimizing structural changes during activation to the transition state. These results support the proposal that the active site electric field in KSI is preorganized to enhance catalysis, offering a model for measuring electrostatic preorganization in enzymatic systems.[193] Alexandrova and coworkers analyzed the electrostatic preorganization of KSI, and found that topologically similar local electric fields had similar reaction barriers.[194]

14. Sulfonation

The human gut flora performs a variety of chemical modifications on endogenous and exogenous substances entering the gut, including hydrolysis, chemical bond breaking, and reductive reactions.[195–197] The host-derived enzymes typically modify molecules through binding and oxidation reactions. An
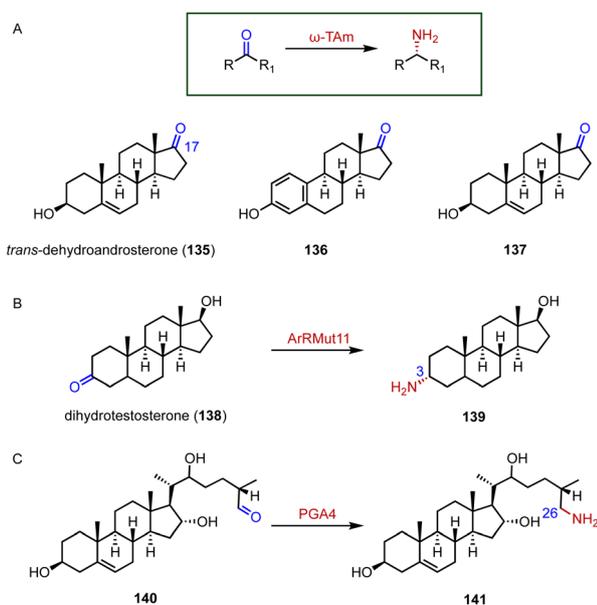

Fig. 24 Aminations mediated by aminotransferases. (A) The substrate specificity of ω-TAm. Amination reactions catalyzed by (B) ArRMut1 and (C) PGA4.
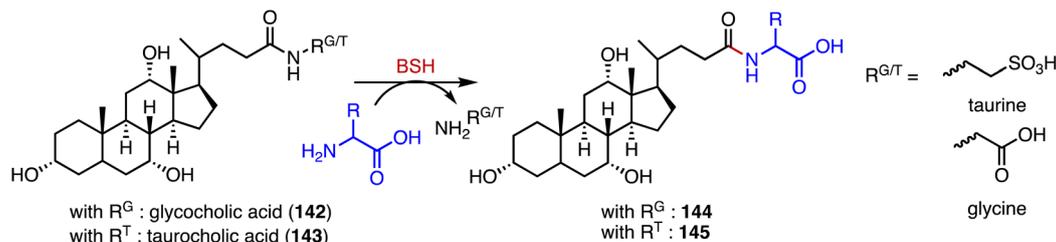
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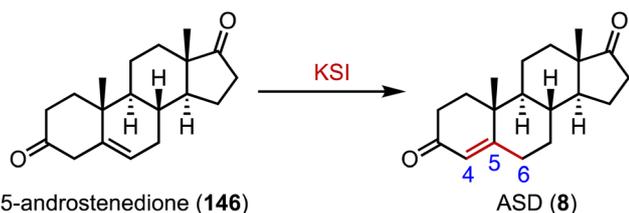**Fig. 25** Amidation mediated by bile salt hydrolase (BSH).

Fig. 26 Isomerization mediated by ketosteroid isomerase (KSI).

important example is sulfonation, the conversion of alcohols to sulfates *via* sulfotransferases (SULTs), which help organisms remove toxic substances and produce signaling molecules.[198,199] Recently, Devlin's and Johnson's groups reported cholesterol (**86**) sulfonation by gut bacteria (Fig. 27).[200,201] Johnson's group determined that cholesterol sulfate entered the circulation and assisted in regulating cholesterol levels,[201] while Devlin's group found an immunoregulatory function of bacterially-produced cholesterol sulfate and the SULT-dependent inhibition of T cell migration.[200]

15. Novel biosynthetic pathways

Natural products refer to the molecules created by microorganisms, plants, and animals through secondary metabolism. Compared with primary metabolites, they possess structural complexity and diversity, as well as attractive biological activities. Recently, novel biosynthetic pathways of steroid-related natural products, including furanosteroids, steroidal glycoalkaloids (SGAs), and diosgenin, have been clarified.
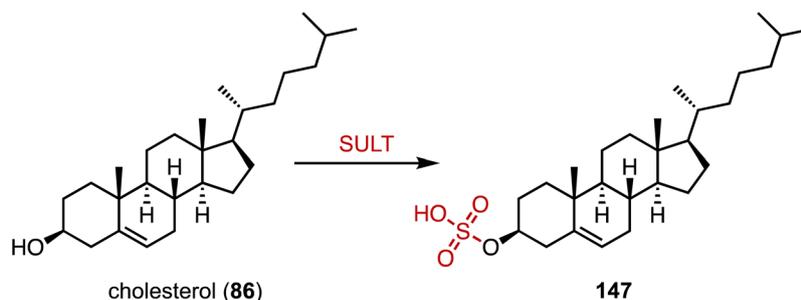
15.1 Furanosteroids

Furanosteroids are a class of fungal secondary metabolites represented by demethoxyviridin (**148**), demethoxyviridiol (**149**), viridin, and wortmannin,[202,203] which are nanomolar-potency inhibitors of phosphatidylinositol 3-kinase (PI3K).[204] Gao and coworkers characterized the biosynthetic pathway of **148** and **149**.[144] It shares a similar early step in the biosynthetic pathway to ergosterol (**133**). VidE, VidG, VidR, VidO, and VidN are responsible for converting **122** or **153** to **154** or **159**. Subsequently, VidQ oxidizes the C-7 position to produce **154** or **158**. VidS mediates the 1,4-dehydration reaction to form the typical furan ring. Afterwards, a Baeyer–Villiger monooxygenase (VidF), an esterase (VidP) and a dehydrogenase (VidH) cleave the C-17, 20 side chain to produce the intermediate **91** or **164** with a carbonyl group at C-17. Two P450 oxidases (VidD and VidA) catalyze the aromatization of the C-ring, and finally a P450 oxidase (VidK) catalyzes the 1β-hydroxylation of **166** or **162** to generate demethoxyviridin (**148**) or demethoxyviridiol (**149**), respectively. The oxidase (VidJ) and the dehydrogenase (VidM) are responsible for the mutual conversion between the 3-OH and 3-keto groups in the pathway (Fig. 28). The biosynthetic pathway of **148** and **149** is widespread and conserved in fungi.

15.2 Steroidal glycoalkaloids (SGAs)

SGAs are toxic specialized metabolites found in members of the Solanaceae, such as tomato, potato, and eggplant.[205–209] They are classified into solanidane and spirosolane, based on their skeleton derived from the cholesterol side chain.[210,211] Recently, Mizutani's, Aharoni's, and other research groups identified the biosynthetic pathway of SGAs.[184,212–218] As shown in Fig. 29, two P450 enzymes (PGA2, PGA1) and the

Fig. 27 Sulfonation mediated by sulfotransferase (SULT).

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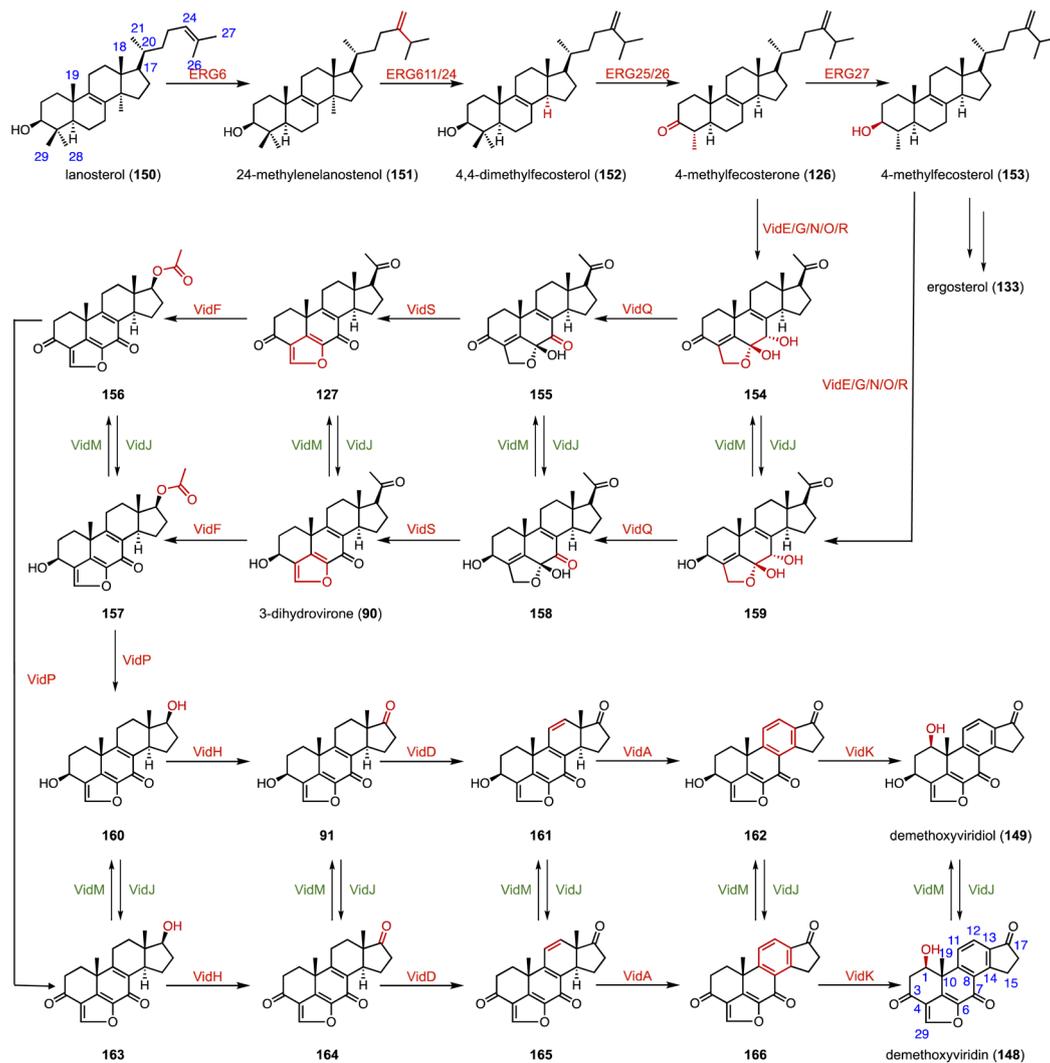This journal is © The Royal Society of Chemistry 2024

Fig. 28 Biosynthetic pathway of demethoxyviridin and demethoxyviridiol.

αKG OX 16DOX catalyze the hydroxylation of cholesterol (**86**) to generate **167**, which is then converted to **168** by a series of enzymes, including PGA3 and PGA4. In addition, diverse uridine diphosphate-dependent glycosyltransferases (UGTs) are responsible for the glycosylation of SGAs to produce α-tomatine (**170**), dehydrotomatine (**171**), α-solamarine (**172**), and β-solamarine (**173**). Notably, the spirosolane moiety is tailored by diverse enzymes for branching the biosynthetic pathways of SGAs. DPS (dioxygenase for potato solanidane synthesis) catalyzes the C-16 hydroxylation of spirosolane-type SGAs, resulting in desaturation *via* E/F ring arrangement to generate the solanidane scaffold. GAME34 (glycoalkaloid metabolism34), an αKG OX, catalyzes the conversion of α-tomatine (**170**) to habrochaitoside (**178**). GAME31 catalyzes the C-23 hydroxylation of α-tomatine (**170**), and the product spontaneously isomerized to **179**. The intermediate **162** was then converted to **180** by a putative acetyltransferase. GAME40 functions as a C-27 hydroxylase for the efficient detoxification of α-tomatine (**170**) during tomato fruit ripening.

15.3 Diosgenin

Diosgenin (**185**), a vital precursor in the steroid hormone industry, is derived from plants.[219] Weng and coworkers clarified the biosynthetic pathway of **185**.[165] Pairs of P450 enzymes, such as CYP90, CYP94, CYP72, and CYP82, are recruited for the formation of the characteristic 5,6-spiroketal moiety. In brassinosteroid biosynthesis, CYP90 mainly catalyzes the C-22β hydroxylation of **86** to generate **183** (Fig. 30).

16. Artificial multi-enzyme cascades and biotransformations

Drawing inspiration from nature, multi-enzymatic cascades provide a cost-effective alternative to single-step reactions.[220,221] These cascades integrate multiple catalytic steps within a single reaction vessel, streamlining the production of a diverse array of chemicals.[222] The numbers of

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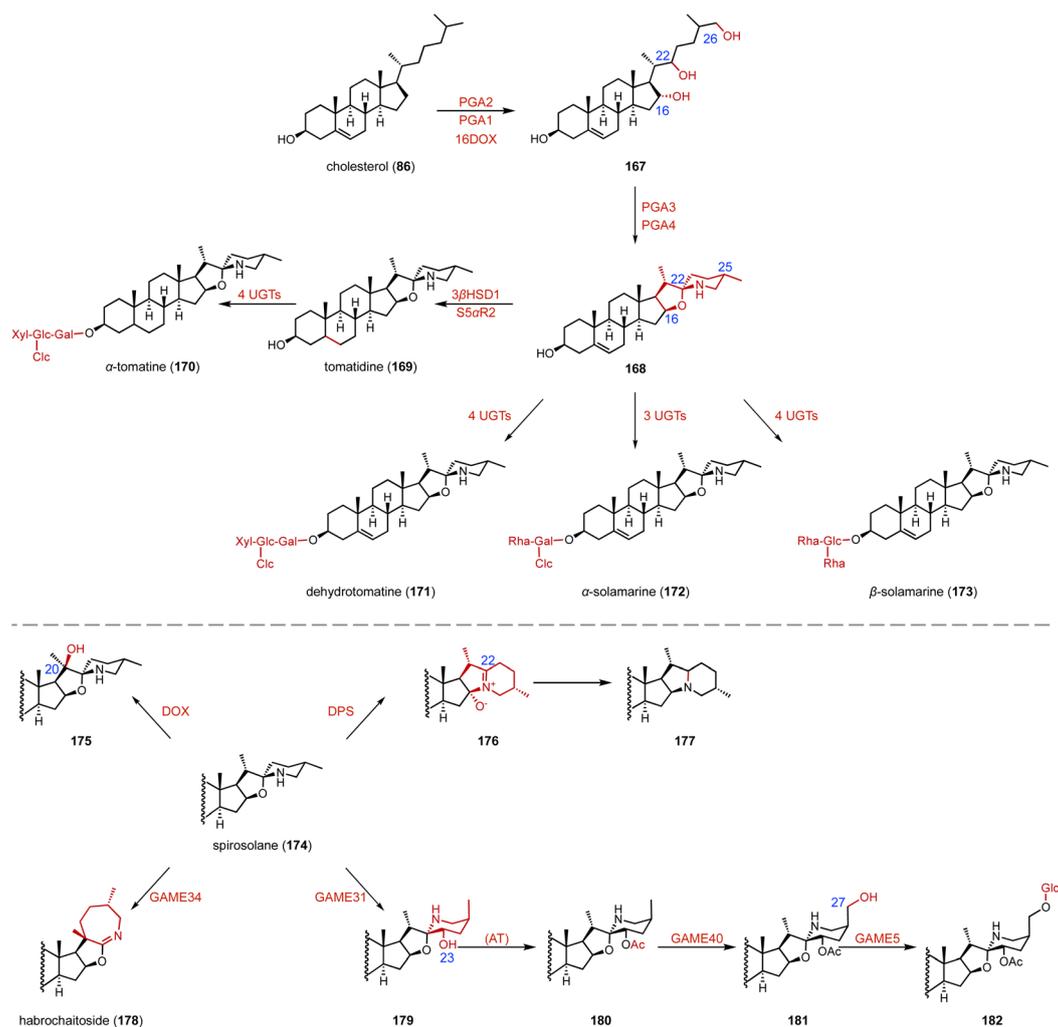
Fig. 29 Biosynthetic pathway of steroidal glycoalkaloids (SGAs).
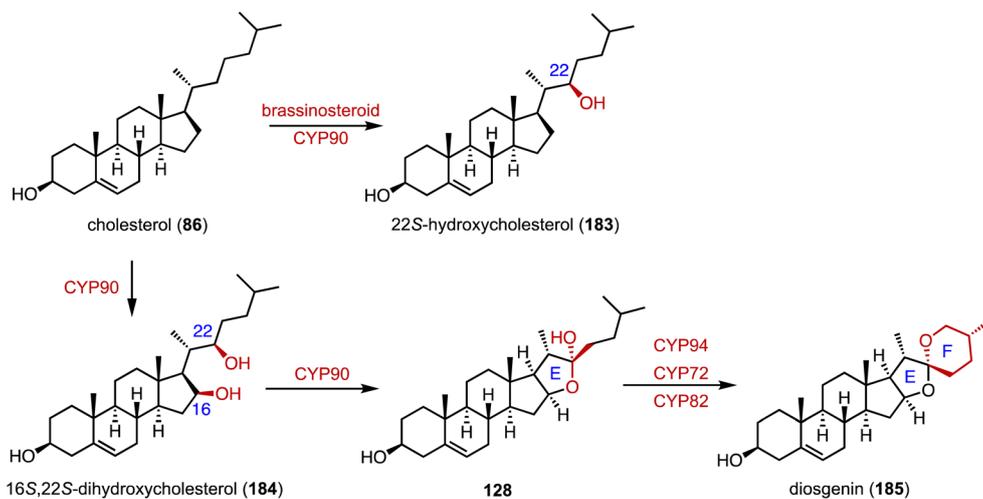
Fig. 30 Biosynthetic pathway of diosgenin.

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reports on enzymatic cascade reactions in the synthesis of steroid drugs continue to grow.[223]

Kong and coworkers discovered and functionally characterized a steroidal glycosyltransferase (SGT) from *Ornithogalum saundersiae* (OsSGT1) and a steroidal glycoside acyltransferase (SGA) from *Escherichia coli* (EcSGA1) for the biosynthesis of acylated steroidal glycosides, and demonstrated their improved cytotoxic activities against human tumor cell lines (Fig. 31A).[224] Chang and coworkers developed a one-pot artificial cascade reaction involving three different P450 modules (17AM, 21M, and 11BM) for the biosynthesis of hydrocortisone (**6**).[225] The optimized three-P450-module co-expression system, named hyg4, achieved the direct production of hydrocortisone (**6**) from PG (**12**) with a total yield of 16.82 μmol L^{-1} within 24 hours (Fig. 31B). Recently, Zhou and coworkers performed the protein engineering of P450 BM3 and a series of P450 BM3 enzyme variants were identified for C-16β, C-17α, C-21, and C-17α/21 hydroxylation of PG (**12**) and C-11α oxidation of **5**.[226]

The developed P450 variants were utilized in the one pot reaction with 1 g L^{-1} **12** as the starting material, resulting in C-11α/β-hydrocortisone production at a molar conversion rate of 81 and 84%, respectively (Fig. 31C).

Chen and coworkers utilized the engineered KstD ReM2 (I51L/I350T) and the newly discovered carbonyl reductase 17β-CR to synthesize (+)-boldenone (BD, **190**) from ASD (**8**), which is a key intermediate for the synthesis of (+)-boldenone undecylenate (**191**) (Fig. 31D).[227]

Li and coworkers performed the biocatalytic one-pot C-11α hydroxylation (LG-23/T438S) and 17β-ketoreduction (17β-HSDcl) of estra-4,9-diene-3,17-dione (**192**).[228] The product **193** is a key intermediate for the synthesis of the veterinary medicine trenbolone acetate (**194**) (Fig. 31E). Meanwhile, Li and coworkers established a biocatalytic cascade reaction including the C-7β hydroxylation (LG-23) and C-17β ketoreduction (17β-HSDcl/V161G) of 19-norandrostenedione (**195**) to synthesize 7β-hydroxynandrolone (**196**), which is an important

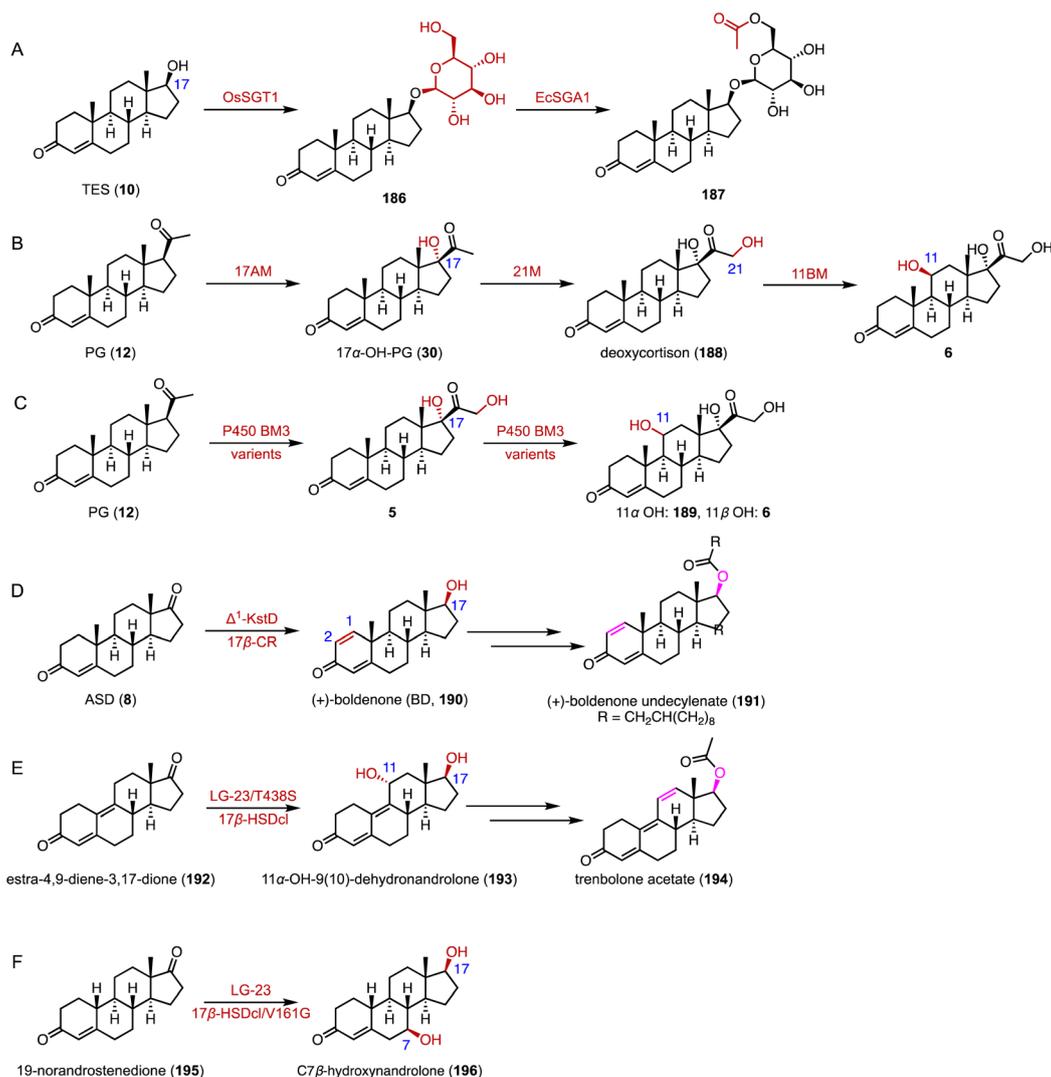**Fig. 31** Enzymatic cascade reactions for the synthesis of steroid drugs. Enzymatic cascades for the synthesis of (A) the acylated steroidal glycoside **187**, (B) hydrocortisone (**6**), (C) **6** and **189**, (D) (+)-boldenone (**190**), (E) 11α-OH-9(10)-dehydronandrolone (**193**), and (F) 7β-hydroxynandrolone (**196**).

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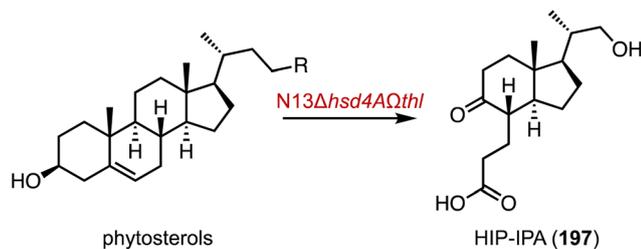*Org. Biomol. Chem.*, 2024, **22**, 3559–3583 | **3577**

Fig. 32 Biotransformation of phytosterols into **197**.

precursor for the synthesis of C-7 functionalized steroid drugs (Fig. 31F).[229]

Finally, microbiological transformation is a powerful and green tool in the manufacturing of steroids.[230] Recently, Zhu and coworkers knocked out of a β-hydroxyacyl-CoA dehydrogenase gene and introduced a sterol aldolase gene in the genetically modified strains of *Mycobacterium fortuitum* N13, resulting in the strain N13Δ*hsd4A*Ω*thl*.[231] It transformed phytosterols into 3-((1*R*,3a*S*,4*S*,7a*R*)-1-((*S*)-1-hydroxypropan-2-yl)-7a-methyl-5-oxooctahydro-1*H*-inden-4-yl) propanoic acid (HIP-IPA, **197**) in 62% isolated yield, which is a key intermediate for the synthesis of dydrogesterone (Fig. 32).

17. Conclusions

Over the past five years, diverse enzymatic modifications of steroids have attracted keen interest, significantly advancing our understanding of biocatalytic steroid drug synthesis. These enzymatic transformations offer precise control of stereochemistry and regioselectivity, particularly the enzymatic hydroxylation, ketoreduction, glycosylation and acylation of steroids. For example, the recently discovered C-14α hydroxylase could be utilized for the preparation of the important intermediates for the synthesis of cardiac glycosides. Meanwhile, the substrate promiscuity of hydroxylases, such as the αKG OX and UPO, provides an alternative strategy for the site-specific hydroxylation of steroids.

Synthetic biology is revolutionizing the industrial production of steroid drugs through concerted efforts in metabolic engineering, enzyme discovery, and enzyme engineering that are new to both biology and chemistry. The enzymatic synthesis of steroids has not only broadened the substrate scope but also opened avenues for the generation of novel derivatives with improved therapeutic properties. Future research on steroid biosynthesis will characterize the intriguing biochemical reactions, the cryptic enzymatic mechanisms, and the biological significance of diverse steroid modifications, such as the sterol aspartylation in fungi and sulfonation in gut bacteria.

In summary, harnessing the power of enzymes is paving the way toward streamlining and enhancing the synthetic routes of steroids, ultimately accelerating the discovery and development of unique steroid drugs.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

References

1 N. Sultana, *Steroids*, 2018, **136**, 76–92.
2 W. Ericson-Neilsen and A. D. Kaye, *Ochsner J.*, 2014, **14**, 203–207.
3 F. Mao, W. Ni, X. Xu, H. Wang, J. Wang, M. Ji and J. Li, *Molecules*, 2016, **21**, 75.
4 D. E. Yerien, S. Bonesi and A. Postigo, *Org. Biomol. Chem.*, 2016, **14**, 8398–8427.
5 S. Gorog, *Anal. Sci.*, 2004, **20**, 767–782.
6 Z. Zhang, X. Qian, Y. Gu and J. Gui, *Nat. Prod. Rep.*, 2024, **41**, 251–272.
7 Y. Wang and J. Gui, *Acc. Chem. Res.*, 2024, **57**, 568–579.
8 J. B. Pyser, S. Chakrabarty, E. O. Romero and A. R. H. Narayan, *ACS Cent. Sci.*, 2021, **7**, 1105–1116.
9 W. Y. Tong and X. Dong, *Recent Pat. Biotechnol.*, 2009, **3**, 141–153.
10 S. K. Wu, R. Snajdrova, J. C. Moore, K. Baldenius and U. T. Bornscheuer, *Angew. Chem., Int. Ed.*, 2021, **60**, 88–119.
11 E. Romero, B. S. Jones, B. N. Hogg, A. Rue Casamajo, M. A. Hayes, S. L. Flitsch, N. J. Turner and C. Schnepel, *Angew. Chem., Int. Ed.*, 2021, **60**, 16824–16855.
12 J. Chen, F. Fan, G. Qu, J. Tang, Y. Xi, C. Bi, Z. Sun and X. Zhang, *Metab. Eng.*, 2020, **57**, 31–42.
13 W. J. Sun, L. Wang, H. H. Liu, Y. J. Liu, Y. H. Ren, F. Q. Wang and D. Z. Wei, *Metab. Eng.*, 2019, **56**, 97–110.
14 M. M. Zheng, Z. Lin, S. J. Lin and X. D. Qu, *Eur. J. Org. Chem.*, 2024, **27**, e202301066.
15 R. Zhu, Y. Liu, Y. Y. Yang, Q. Min, H. Li and L. X. Chen, *Adv. Synth. Catal.*, 2022, **364**, 2701–2719.
16 H. Abas, P. Blencowe, J. L. Brookfield and L. A. Harwood, *Chem. – Eur. J.*, 2023, **29**, e202301066.
17 X. Zhang, Y. Peng, J. Zhao, Q. Li, X. Yu, C. G. Acevedo-Rocha and A. Li, *Bioresour. Bioprocess.*, 2020, **7**, 2.
18 J. Cramer, C. P. Sager and B. Ernst, *J. Med. Chem.*, 2019, **62**, 8915–8930.
19 J. Münch, P. Püllmann, W. Y. Zhang and M. J. Weissenborn, *ACS Catal.*, 2021, **11**, 9168–9203.

3578 | *Org. Biomol. Chem.*, 2024, **22**, 3559–3583

This journal is © The Royal Society of Chemistry 2024

20 L. P. Zhao, Z. Y. Bo and Y. Yang, *Nat. Synth.*, 2023, **2**, 699–700.

21 J. Chen, J. Tang, Y. Xi, Z. Dai, C. Bi, X. Chen, F. Fan and X. Zhang, *Appl. Microbiol. Biotechnol.*, 2019, **103**, 8363–8374.

22 D. Permana, K. Niesel, M. J. Ford and H. Ichinose, *ACS Omega*, 2022, **7**, 13932–13941.

23 Y. Zhao, B. Zhang, Z. Q. Sun, H. Zhang, W. Wang, Z. R. Wang, Z. K. Guo, S. Y. Yu, R. X. Tan and H. M. Ge, *ACS Catal.*, 2022, **12**, 9839–9845.

24 F. Z. Song, M. M. Zheng, J. L. Wang, H. H. Liu, Z. Lin, B. B. Liu, Z. X. Deng, H. J. Cong, Q. H. Zhou and X. D. Qu, *Nat. Synth.*, 2023, **2**, 729–739.

25 T. Terasawa and T. Okada, *Tetrahedron*, 1986, **42**, 537–545.

26 H. Renata, Q. H. Zhou, G. Dünstl, J. Felding, R. R. Merchant, C. H. Yeh and P. S. Baran, *J. Am. Chem. Soc.*, 2015, **137**, 1330–1340.

27 J. Lia, A. Amatuni and H. Renata, *Curr. Opin. Chem. Biol.*, 2020, **55**, 111–118.

28 W. Lu, X. Chen, J. Feng, Y. J. Bao, Y. Wang, Q. Wu and D. Zhu, *Appl. Environ. Microbiol.*, 2018, **84**, e00503-18.

29 J. L. Wang, Y. N. Zhang, H. H. Liu, Y. Shang, L. J. Zhou, P. L. Wei, W. B. Yin, Z. X. Deng, X. D. Qu and Q. H. Zhou, *Nat. Commun.*, 2019, **10**, 3378.

30 D. Suraweera, H. Rahal, M. Jimenez, M. Viramontes, G. Choi and S. Saab, *Liver Int.*, 2017, **37**, 1877–1886.

31 S. Fiorucci and E. Distrutti, *Handb. Exp. Pharmacol.*, 2019, **256**, 265–282.

32 Z. Wang and C. Hui, *Org. Biomol. Chem.*, 2021, **19**, 3791–3812.

33 F. Tonin and I. W. C. E. Arends, *Beilstein J. Org. Chem.*, 2018, **14**, 470–483.

34 S. Grobe, C. P. S. Badenhorst, T. Bayer, E. Hamnevik, S. Wu, C. W. Grathwol, A. Link, S. Koban, H. Brundiek, B. Grossjohann and U. T. Bornscheuer, *Angew. Chem., Int. Ed.*, 2021, **60**, 753–757.

35 S. Grobe, A. Wszolek, H. Brundiek, M. Fekete and U. T. Bornscheuer, *Biotechnol. Lett.*, 2020, **42**, 819–824.

36 A. Li, C. G. Acevedo-Rocha, L. D'Amore, J. Chen, Y. Peng, M. Garcia-Borras, C. Gao, J. Zhu, H. Rickerby, S. Osuna, J. Zhou and M. T. Reetz, *Angew. Chem., Int. Ed.*, 2020, **59**, 12499–12505.

37 B. Ma, Q. Wang, H. Ikeda, C. Zhang and L. H. Xu, *Appl. Environ. Microbiol.*, 2019, **85**, e01530-19.

38 C. G. Acevedo-Rocha, C. G. Gamble, R. Lonsdale, A. T. Li, N. Nett, S. Hoebenreich, J. B. Lingnau, C. Wirtz, C. Fares, H. Hinrichs, A. Deege, A. J. Mulholland, Y. Nov, D. Leys, K. J. McLean, A. W. Munro and M. T. Reetz, *ACS Catal.*, 2018, **8**, 3395–3410.

39 X. D. Zhang, Y. Hu, W. Peng, C. H. Gao, Q. Xing, B. J. Wang and A. T. Li, *Front. Chem.*, 2021, **9**, 649000.

40 W. Y. Chen, M. J. Fisher, A. Leung, Y. Cao and L. L. Wong, *ACS Catal.*, 2020, **10**, 8334–8343.

41 J. Yang, Y. Liu, D. Zhong, L. Xu, H. Gao, J. D. Keasling, X. Luo and H. H. Chou, *Metab. Eng.*, 2023, **80**, 119–129.

42 Y. Yang and F. H. Arnold, *Acc. Chem. Res.*, 2021, **54**, 1209–1225.

43 P. Bracco, H. J. Wijma, B. Nicolai, J. A. R. Buitrago, T. Klünemann, A. Vila, P. Schrepfer, W. Blankenfeldt, D. B. Janssen and A. Schallmey, *ChemBioChem*, 2021, **22**, 1099–1110.

44 Y. Khatri, I. K. Jozwik, M. Ringle, I. A. Ionescu, M. Litzenburger, M. C. Hutter, A. M. W. H. Thunnissen and R. Bernhardt, *ACS Chem. Biol.*, 2018, **13**, 1021–1028.

45 X. D. Zhang, P. P. Shen, J. Zhao, Y. Y. Chen, X. Li, J. W. Huang, L. L. Zhang, Q. Li, C. H. Gao, Q. Xing, C. C. Chen, R. T. Guo and A. T. Li, *ACS Catal.*, 2023, **13**, 1280–1289.

46 Q. Gao, B. Ma, Q. Wang, H. Zhang, S. Fushinobu, J. Yang, S. Lin, K. Sun, B. N. Han and L. H. Xu, *Appl. Environ. Microbiol.*, 2023, **89**, e0218622.

47 N. Kumar, J. He and J. F. Rusling, *Chem. Soc. Rev.*, 2023, **52**, 5135–5171.

48 S. Fan and Z. Cong, *Acc. Chem. Res.*, 2024, **57**, 613–624.

49 J. Akter, E. F. Hayball and S. G. Bell, *Catal. Sci. Technol.*, 2023, **13**, 6355–6359.

50 X. W. Zhang, J. W. Guo, F. Y. Cheng and S. Y. Li, *Nat. Prod. Rep.*, 2021, **38**, 1072–1099.

51 S. L. Li, Y. W. Chang, Y. N. Liu, W. Tian and Z. X. Chang, *J. Steroid Biochem. Mol. Biol.*, 2023, **227**, 106236.

52 G. Yi, H. Zou, T. Long, T. Osire, L. Wang, X. Wei, M. Long, Z. Rao and G. Liao, *Bioresour. Technol.*, 2023, **394**, 130244.

53 S. Mao, X. Wang, Z. Zhang, S. Wang, K. Li, F. Lu and H. Qin, *Biochem. Eng. J.*, 2020, **164**, 107781.

54 W. Lu, J. H. Feng, X. Chen, Y. J. Bao, Y. Wang, Q. Q. Wu, Y. H. Ma and D. M. Zhu, *Appl. Environ. Microbiol.*, 2019, **85**, e01182-19.

55 V. Kollerov, S. Tarlachkov, A. Shutov, A. Kazantsev and M. Donova, *Int. J. Mol. Sci.*, 2023, **24**, 17256.

56 L. Wang, X. Wu, C. Gao, L. Wei, Q. Li and A. Li, *Appl. Environ. Microbiol.*, 2023, **89**, e0196322.

57 X. Wang, X. Yang, X. Jia, P. Jin, Z. Wang, F. Lu and X. Liu, *Ann. Microbiol.*, 2020, **70**, 45.

58 Y. Peng, Y. Wang, T.-J. Chen, J.-J. Chen, J.-L. Yang, T. Gong and P. Zhu, *Chin. Chem. Lett.*, 2024, **35**, 108818.

59 P. He, H. Li, J. Sun, X. M. Zhang, J. S. Gong, J. S. Shi and Z. H. Xu, *J. Steroid Biochem. Mol. Biol.*, 2022, **220**, 106096.

60 P. Subedi, K. H. Kim, Y. S. Hong, J. H. Lee and T. J. Oh, *J. Microbiol. Biotechnol.*, 2021, **31**, 464–474.

61 Q. Wang, B. Ma, S. Fushinobu, C. Zhang and L. H. Xu, *Biochem. Biophys. Res. Commun.*, 2020, **522**, 355–361.

62 C. Liu, K. Chen, Y. Wang, M. Shao, Z. Xu and Z. Rao, *Biochem. Eng. J.*, 2022, **177**, 108264.

63 I. K. Jozwik, E. Bombino, A. Abdulmughni, P. Hartz, H. J. Rozeboom, H. J. Wijma, R. Kappl, D. B. Janssen, R. Bernhardt and A. W. H. Thunnissen, *FEBS J.*, 2023, **290**, 5016–5035.

64 S. M. Glass, M. J. Reddish, S. A. Child, C. J. Wilkey, D. F. Stec and F. P. Guengerich, *J. Steroid Biochem. Mol. Biol.*, 2021, **208**, 105787.

65 S. S. Gao, N. Naowarojna, R. Cheng, X. Liu and P. Liu, *Nat. Prod. Rep.*, 2018, **35**, 792–837.

This journal is © The Royal Society of Chemistry 2024

Org. Biomol. Chem., 2024, **22**, 3559–3583 | **3579**

66 C. Q. Herr and R. P. Hausinger, *Trends Biochem. Sci.*, 2018, **43**, 517–532.

67 R. Ushimaru and I. Abe, *ACS Catal.*, 2022, 1045–1076, DOI: **10.1021/acscatal.2c05247**.

68 C. R. Zwick and H. Renata, *ACS Catal.*, 2023, **13**, 4853–4865.

69 A. Amatuni and H. Renata, *Org. Biomol. Chem.*, 2019, **17**, 1736–1739.

70 W. L. Cheung-Lee, J. N. Kolev, J. A. McIntosh, A. A. Gil, W. Pan, L. Xiao, J. E. Velasquez, R. Gangam, M. S. Winston, S. Li, K. Abe, E. Alwedi, Z. E. X. Dance, H. Fan, K. Hiraga, J. Kim, B. Kosjek, D. N. Le, N. Salehi Marzijarani, K. Mattern, J. P. McMullen, K. Narsimhan, A. Vikram, W. Wang, J. X. Yan, R. S. Yang, V. Zhang, W. Zhong, D. A. DiRocco, W. J. Morris, G. S. Murphy and K. M. Maloney, *Angew. Chem., Int. Ed.*, 2024, e202316133, DOI: **10.1002/anie.202316133**.

71 M. Nakayasu, N. Umemoto, K. Ohyama, Y. Fujimoto, H. J. Lee, B. Watanabe, T. Muranaka, K. Saito, Y. Sugimoto and M. Mizutani, *Plant Physiol.*, 2017, **175**, 120–133.

72 H. Tao, T. Mori, H. Chen, S. Lyu, A. Nonoyama, S. Lee and I. Abe, *Nat. Commun.*, 2022, **13**, 95.

73 T. Bai, Y. Matsuda, H. Tao, T. Mori, Y. Zhang and I. Abe, *Org. Lett.*, 2020, **22**, 4311–4315.

74 T. Mori, Z. Yu, H. Tao and I. Abe, *Org. Lett.*, 2022, **24**, 1737–1741.

75 M. Petrusma, R. van der Geize and L. Dijkhuizen, *Antonie Van Leeuwenhoek*, 2014, **106**, 157–172.

76 H. H. Liu, L. Q. Xu, K. Yao, L. B. Xiong, X. Y. Tao, F. Q. Wang and D. Z. Wei, *Appl. Environ. Microbiol.*, 2018, **84**, e02777-17.

77 H. Sun, J. Yang, K. He, Y.-P. Wang and H. Song, *Chem. Eng. Sci.*, 2021, **230**, 116195.

78 S. Baldanta, J. M. Navarro Llorens and G. Guevara, *Microorganisms*, 2021, **9**, 1171.

79 Z. L. Zhu, X. Gao, Z. Song, C. Li, F. P. Lu, M. Tanokura and H. M. Qin, *ACS Sustainable Chem. Eng.*, 2020, **8**, 16720–16730.

80 H. Song, Z. Zhang, C. Cao, Z. Tang, J. Gui and W. Liu, *Angew. Chem., Int. Ed.*, 2024, e202319624, DOI: **10.1002/anie.202319624**.

81 C. Aranda, J. Carro, A. Gonzalez-Benjumea, E. D. Babot, A. Olmedo, D. Linde, A. T. Martinez and A. Gutierrez, *Biotechnol. Adv.*, 2021, **51**, 107703.

82 A. Kinner, K. Rosenthal and S. Lutz, *Front. Bioeng. Biotechnol.*, 2021, **9**, 705630.

83 A. Beltran-Nogal, I. Sanchez-Moreno, D. Mendez-Sanchez, P. Gomez de Santos, F. Hollmann and M. Alcalde, *Curr. Opin. Struct. Biol.*, 2022, **73**, 102342.

84 J. Kiebist, K. U. Schmidtke, J. Zimmermann, H. Kellner, N. Jehmlich, R. Ullrich, D. Zander, M. Hofrichter and K. Scheibner, *ChemBioChem*, 2017, **18**, 563–569.

85 E. J. de Vries and D. B. Janssen, *Curr. Opin. Biotechnol*, 2003, **14**, 414–420.

86 S. Meninno and A. Lattanzi, *ACS Org. Inorg. Au*, 2022, **2**, 289–305.

87 F. K. Yoshimoto, H. M. Peng, H. Zhang, S. M. Anderson and R. J. Auchus, *Biochemistry*, 2014, **53**, 7531–7540.

88 D. Jiang, R. Tu, P. Bai and Q. Wang, *Biotechnol. Lett.*, 2013, **35**, 1663–1668.

89 G. W. Huisman, J. Liang and A. Krebber, *Curr. Opin. Chem. Biol.*, 2010, **14**, 122–129.

90 E. E. Ferrandi, S. Bertuletti, D. Monti and S. Riva, *Eur. J. Org. Chem.*, 2020, **2020**, 4463–4473.

91 M. J. Badran, N. Bertoletti, A. Keils, A. Heine, G. Klebe and S. Marchais-Oberwinkler, *J. Steroid Biochem. Mol. Biol.*, 2019, **189**, 135–144.

92 Z. Y. Liu, R. Z. Zhang, W. C. Zhang and Y. Xu, *Crit. Rev. Biotechnol.*, 2023, **43**, 770–786.

93 S. C. Shi, Z. N. You, K. Zhou, Q. Chen, J. Pan, X. L. Qian, J. H. Xu and C. X. Li, *Adv. Synth. Catal.*, 2019, **361**, 4661–4668.

94 Y. Q. Zhao, Y. J. Liu, W. T. Ji, K. Liu, B. Gao, X. Y. Tao, M. Zhao, F. Q. Wang and D. Z. Wei, *Microb. Cell Fact.*, 2022, **21**, 59.

95 W. L. Liu, W. Shen, Y. Y. Xia, X. Z. Chen and H. Q. Yang, *ACS Sustainable Chem. Eng.*, 2024, **12**, 1333–1342.

96 F. Tonin, L. G. Otten and I. Arends, *ChemSusChem*, 2019, **12**, 3192–3203.

97 Z. N. You, Q. Chen, S. C. Shi, M. M. Zheng, J. Pan, X. L. Qian, C. X. Li and J. H. Xu, *ACS Catal.*, 2019, **9**, 466–473.

98 B.-Y. Yang, Z.-N. You, J.-T. Xue, J. Pan, C.-X. Li and J.-H. Xu, *Mol. Catal.*, 2023, **537**, 112946.

99 K. A. Scott, M. H. Qureshi, P. B. Cox, C. M. Marshall, B. C. Bellaire, M. Wilcox, B. A. R. Stuart and J. T. Njardarson, *J. Med. Chem.*, 2020, **63**, 15449–15482.

100 C. Zeng, S. Xu, J. Shen, S. Zhao, X. Xu and L. Peng, *Org. Lett.*, 2024, **26**, 127–131.

101 Y. Hou, J. Lv, Y. Guo, J. Fang, C. Huang, W. Zhang, X. Jia, S. You and B. Qin, *Mol. Catal.*, 2023, **548**, 113433.

102 H. Peng, Y. Wang, K. Jiang, X. Chen, W. Zhang, Y. Zhang, Z. Deng and X. Qu, *Angew. Chem., Int. Ed.*, 2021, **60**, 5414–5420.

103 B. Zhang, D.-F. Zhou, M.-J. Li, J.-H. Lan, H. Li, M.-L. Shao, Z.-Q. Liu and Y.-G. Zheng, *Syst. Microbiol. Biomanuf.*, 2024, **74**, 631–660.

104 S. Mao, J. Sun, L. Wang, X. Gao, X. Liu, F. Lu and H.-M. Qin, *Biochem. Eng. J.*, 2022, **181**, 108383.

105 Y. Wang, R. Zhang, J. Feng, Q. Wu, D. Zhu and Y. Ma, *Microorganisms*, 2022, **10**, 508.

106 R. Zhang, X. Xu, H. Cao, C. Yuan, Y. Yuminaga, S. Zhao, J. Shi and B. Zhang, *Appl. Microbiol. Biotechnol.*, 2019, **103**, 6605–6616.

107 J. M. Luo, H. L. Cui, H. C. Jia, F. Li, H. J. Cheng, Y. B. Shen and M. Wang, *J. Agric. Food Chem.*, 2020, **68**, 9496–9512.

108 S. Mao, Y. Chen, J. Sun, C. Wei, Z. Song, F. Lu and H. M. Qin, *Enzyme Microb. Technol.*, 2021, **146**, 109777.

109 X. C. Liu, R. J. Zhang, Z. W. Bao, C. Y. Yuan, H. J. Cao, J. P. Shi, J. S. Sun and B. G. Zhang, *Microb. Cell Fact.*, 2023, **22**, 53.

3580 | *Org. Biomol. Chem.*, 2024, **22**, 3559–3583

This journal is © The Royal Society of Chemistry 2024

110 M. Tataruch, P. Wójcik, A. M. Wojtkiewicz, K. Zaczyk, K. Szymanska and M. Szaleniec, *Catalysts*, 2020, **10**, 1460.

111 B. M. D'Arcy, M. R. Swingle, L. Schambeau, L. Pannell, A. Prakash and R. E. Honkanen, *Sci. Rep.*, 2019, **9**, 5969.

112 X. Li, T. Chen, F. Peng, S. Song, J. Yu, D. N. Sidoine, X. Cheng, Y. Huang, Y. He and Z. Su, *Microb. Cell Fact.*, 2021, **20**, 158.

113 J. Tang, C. L. Zeng, L. Y. Xie, J. H. Wang, M. Tian and C. C. Guo, *Chem. Lett.*, 2018, **47**, 110–112.

114 Y. Zhang, M. Liu, H. Wang, J. Lin and F. Chen, *Mol. Catal.*, 2022, **531**, 112661.

115 J. H. Wei, Y. J. Zhang, M. J. Liu, Y. T. Ning, Y. R. Cao and F. E. Chen, *Angew. Chem., Int. Ed.*, 2024, **63**, e2023139.

116 R. Wilcken, M. O. Zimmermann, A. Lange, A. C. Joerger and F. M. Boeckler, *J. Med. Chem.*, 2013, **56**, 1363–1388.

117 W. J. Chung and C. D. Vanderwal, *Angew. Chem., Int. Ed.*, 2016, **55**, 4396–4434.

118 D. C. Blakemore, L. Castro, I. Churcher, D. C. Rees, A. W. Thomas, D. M. Wilson and A. Wood, *Nat. Chem.*, 2018, **10**, 383–394.

119 B. F. Fisher, H. M. Snodgrass, K. A. Jones, M. C. Andorfer and J. C. Lewis, *ACS Cent. Sci.*, 2019, **5**, 1844–1856.

120 E. Undiano, R. Roman, A. Miranda-Molina and M. Ayala, *Nat. Prod. Res.*, 2022, **36**, 5353–5357.

121 C. Wen, W. Huang, M. M. He, W. L. Deng and H. H. Yu, *Biotechnol. Lett.*, 2020, **42**, 135–142.

122 C. Wen, W. Huang, X. L. Zhu, F. Zhang and R. W. Jiang, *Org. Lett.*, 2018, **20**, 534–537.

123 P. Chaturvedi, P. Misra and R. Tuli, *Appl. Biochem. Biotechnol.*, 2011, **165**, 47–68.

124 P. Mladenka, L. Applova, J. Patocka, V. M. Costa, F. Remiao, J. Pourova, A. Mladenka, J. Karlickova, L. Jahodar, M. Voprsalova, K. J. Varner, M. Sterba, O. E. R. Tox, C. H. K. Researchers and Collaborators, *Med. Res. Rev.*, 2018, **38**, 1332–1403.

125 G. Bati, J. X. He, K. B. Pal and X. W. Liu, *Chem. Soc. Rev.*, 2019, **48**, 4006–4018.

126 B. He, X. Bai, Y. Tan, W. Xie, Y. Feng and G. Y. Yang, *Synth. Syst. Biotechnol.*, 2022, **7**, 602–620.

127 W. Song, C. C. Zhang, J. L. Wu, J. Z. Qi, X. Hua, L. P. Kang, Q. Yuan, J. F. Yuan and Z. Y. Xue, *ACS Synth. Biol.*, 2022, **11**, 1669–1680.

128 J. Gao, Y. Xu, C. Hua, C. Li and Y. Zhang, *Front. Plant Sci.*, 2021, **12**, 809579.

129 M. He, S. Guo, Y. Yin, C. Zhang and X. Zhang, *Mol. Biol. Rep.*, 2023, **50**, 2137–2146.

130 S. Upadhyay, G. S. Jeena, Shikha and R. K. Shukla, *Planta*, 2018, **248**, 519–544.

131 L. Chen, Y. Zhang and Y. Feng, *J. Struct. Biol.*, 2018, **204**, 371–379.

132 D. Warnecke, R. Erdmann, A. Fahl, B. Hube, F. Muller, T. Zank, U. Zahringer and E. Heinz, *J. Biol. Chem.*, 1999, **274**, 13048–13059.

133 Y. Zhuang, G. Y. Yang, X. Chen, Q. Liu, X. Zhang, Z. Deng and Y. Feng, *Metab. Eng.*, 2017, **42**, 25–32.

134 W. Huang, Y. He, R. W. Jiang, Z. X. Deng and F. Long, *ACS Catal.*, 2022, **12**, 2927–2937.

135 W. Huang, X. L. Zhang, J. H. Li, J. X. Lv, Y. H. Wang, Y. He, J. Song, H. Ågren, R. W. Jiang, Z. X. Deng and F. Long, *ACS Catal.*, 2023, **14**, 475–488.

136 W. Huang, C. Wen, Z. R. Zhou, Z. H. Fu, A. Katz, A. Plotnikov, S. J. D. Karlish and R. W. Jiang, *Adv. Synth. Catal.*, 2019, **361**, 3114–3119.

137 A. Zhao, X. Zhang, Y. Li, Z. Wang, Y. Lv, J. Liu, M. A. Alam, W. Xiong and J. Xu, *Biotechnol. Adv.*, 2021, **53**, 107860.

138 R. Ullrich, M. Poraj-Kobielska, S. Scholze, C. Halbout, M. Sandvoss, M. J. Pecyna, K. Scheibner and M. Hofrichter, *J. Inorg. Biochem.*, 2018, **183**, 84–93.

139 T. N. Yuan, M. Yang, K. Gehring and N. S. Sampson, *Biochemistry*, 2019, **58**, 4224–4235.

140 E. R. Simpson and G. S. Boyd, *Eur. J. Biochem.*, 1967, **2**, 275–285.

141 C. Groschel, S. Tennakoon and E. Kallay, *Adv. Pharmacol.*, 2015, **74**, 413–458.

142 E. Carroll, B. Ravi Gopal, I. Raghavan, M. Mukherjee and Z. Q. Wang, *Nat. Commun.*, 2023, **14**, 4042.

143 M. Kunert, C. Langley, R. Lucier, K. Ploss, C. E. Rodriguez Lopez, D. A. Serna Guerrero, E. Rothe, S. E. O'Connor and P. D. Sonawane, *Nat. Plants*, 2023, **9**, 1607–1617.

144 G. Q. Wang, G. D. Chen, S. Y. Qin, D. Hu, T. Awakawa, S. Y. Li, J. M. Lv, C. X. Wang, X. S. Yao, I. Abe and H. Gao, *Nat. Commun.*, 2018, **9**, 1838.

145 E. Abdelraheem, B. Thair, R. F. Varela, E. Jockmann, D. Popadic, H. C. Hailes, J. M. Ward, A. M. Iribarren, E. S. Lewkowicz, J. N. Andexer, P. L. Hagedoorn and U. Hanefeld, *ChemBioChem*, 2022, **23**, e202200212.

146 Z. Lin, Z. Hu, L. Zhou, B. Liu, X. Huang, Z. Deng and X. Qu, *Proc. Natl. Acad. Sci. U. S. A.*, 2023, **120**, e2301389120.

147 N. Sokolova, L. Zhang, S. Deravi, R. Oerlemans, M. R. Groves and K. Haslinger, *ChemBioChem*, 2023, **24**, e202300076.

148 M. O. Brown, B. O. Olagunju, J. L. Giner and P. V. Welander, *Nat. Commun.*, 2023, **14**, 1859.

149 A. K. Lee, J. H. Wei and P. V. Welander, *Nat. Commun.*, 2023, **14**, 2904.

150 D. Michellod, T. Bien, D. Birgel, M. Violette, M. Kleiner, S. Fearn, C. Zeidler, H. R. Gruber-Vodicka, N. Dubilier and M. Liebeke, *Science*, 2023, **380**, 520–526.

151 J. Brocks and I. Bobrovskiy, *Science*, 2023, **380**, 455–456.

152 P. H. Wang, Y. L. Chen, S. T. Wei, K. Wu, T. H. Lee, T. Y. Wu and Y. R. Chiang, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, **117**, 1395–1403.

153 M. Elias-Arnanz, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, **117**, 1833–1835.

154 G. A. Wolff, N. A. Lamb and J. R. Maxwell, *Org. Geochem.*, 1986, **10**, 965–974.

155 N. Robinson, G. Eglinton, S. C. Brassell and P. A. Cranwell, *Nature*, 1984, **308**, 439–442.

156 K. D. McCarty, M. E. Sullivan, Y. Tateishi, T. Y. Hargrove, G. I. Lepesheva and F. P. Guengerich, *J. Biol. Chem.*, 2023, **299**, 104841.

This journal is © The Royal Society of Chemistry 2024

Org. Biomol. Chem., 2024, **22**, 3559–3583 | **3581**

157 S. Kalita, S. Shaik and K. D. Dubey, *ACS Catal.*, 2022, **12**, 5673–5683.

158 K. D. McCarty, Y. Tateishi, T. Y. Hargrove, G. I. Lepesheva and F. P. Guengerich, *Angew. Chem., Int. Ed.*, 2024, e202317711, DOI: 10.1002/anie.202317711.

159 D. Hu, Y. H. Gao, X. S. Yao and H. Gao, *Curr. Opin. Chem. Biol.*, 2020, **59**, 47–53.

160 G. Di Nardo, C. Zhang, A. G. Marcelli and G. Gilardi, *Int. J. Mol. Sci.*, 2021, **22**, 631.

161 F. K. Yoshimoto and F. P. Guengerich, *J. Am. Chem. Soc.*, 2014, **136**, 15016–15025.

162 A. K. Lee, A. B. Banta, J. H. Wei, D. J. Kiemle, J. Feng, J. L. Giner and P. V. Welander, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, 5884–5889.

163 J. M. Lv, D. Hu, H. Gao, T. Kushiro, T. Awakawa, G. D. Chen, C. X. Wang, I. Abe and X. S. Yao, *Nat. Commun.*, 2017, **8**, 1644.

164 M. C. Tang, Y. Zou, K. Watanabe, C. T. Walsh and Y. Tang, *Chem. Rev.*, 2017, **117**, 5226–5333.

165 B. Christ, C. Xu, M. Xu, F. S. Li, N. Wada, A. J. Mitchell, X. L. Han, M. L. Wen, M. Fujita and J. K. Weng, *Nat. Commun.*, 2019, **10**, 3206.

166 M. J. Furst, S. Savino, H. M. Dudek, J. R. Gomez Castellanos, C. Gutierrez de Souza, S. Rovida, M. W. Fraaije and A. Mattevi, *J. Am. Chem. Soc.*, 2017, **139**, 627–630.

167 T. H. Hsiao, Y. L. Chen, M. Meng, M. R. Chuang, M. Horinouchi, T. Hayashi, P. H. Wang and Y. R. Chiang, *Microb. Biotechnol.*, 2021, **14**, 1212–1227.

168 L. L. Wang, Z. H. Jiang, J. H. Zhang, K. Chen, M. Zhang, Z. L. Wang, B. J. Wang, M. Ye and X. Qiao, *Nat. Commun.*, 2023, **14**, 5969.

169 Z. Chang, Y. Li, Y. H. Lu and H. Xiao, *Enzyme Microb. Technol.*, 2023, **162**, 110148.

170 J. A. Lara, A. Burciaga-Monge, A. Chávez, M. Revés, R. Lavilla, M. Arró, A. Boronat, T. Altabella and A. Ferrer, *Front. Plant Sci.*, 2018, **9**, 588.

171 L. L. Wang, K. Chen, Z. L. Wang, Y. Yi, M. Zhang, A. Hasan, Y. Kuang, S. Shaker, R. Yu, H. T. Wang, H. Y. Liu, M. Ye and X. Qiao, *Org. Biomol. Chem.*, 2021, **19**, 7186–7189.

172 L. Wang, H. Qian, Y. Nian, Y. Han, Z. Ren, H. Zhang, L. Hu, B. V. V. Prasad, A. Laganowsky, N. Yan and M. Zhou, *Nature*, 2020, **581**, 329–332.

173 T. Long, E. W. Debler and X. Li, *Curr. Opin. Struct. Biol.*, 2022, **74**, 102369.

174 E. M. Fozo and E. A. Rucks, *Adv. Microb. Physiol.*, 2016, **69**, 51–155.

175 R. N. Fields and H. Roy, *RNA Biol.*, 2018, **15**, 480–491.

176 A. M. Smith, J. S. Harrison, C. D. Grube, A. E. Sheppe, N. Sahara, R. Ishii, O. Nureki and H. Roy, *Mol. Microbiol.*, 2015, **98**, 681–693.

177 N. Yakobov, F. Fischer, N. Mahmoudi, Y. Saga, C. D. Grube, H. Roy, B. Senger, G. Grob, S. Tatematsu, D. Yokokawa, I. Mouyna, J. P. Latge, H. Nakajima, T. Kushiro and H. D. Becker, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, **117**, 14948–14957.

178 L. Banoth, N. S. Thakur, J. Bhaumik and U. C. Banerjee, *Chirality*, 2015, **27**, 382–391.

179 S. A. Kelly, S. Pohle, S. Wharry, S. Mix, C. C. R. Allen, T. S. Moody and B. F. Gilmore, *Chem. Rev.*, 2018, **118**, 349–367.

180 M. Fuchs, J. E. Farnberger and W. Kroutil, *Eur. J. Org. Chem.*, 2015, 6965–6982.

181 K. Koper, S. W. Han, D. C. Pastor, Y. Yoshikuni and H. A. Maeda, *J. Biol. Chem.*, 2022, **298**, 102122.

182 N. Richter, R. C. Simon, W. Kroutil, J. M. Ward and H. C. Hailes, *Chem. Commun.*, 2014, **50**, 6098–6100.

183 N. Kaličanin, G. Kovačević, M. Spasojević, O. Prodanović, S. Jovanović-Šanta, D. Škorić, D. Opsenica and R. Prodanović, *Process Biochem.*, 2022, **121**, 674–680.

184 M. Nakayasu, N. Umemoto, R. Akiyama, K. Ohyama, H. J. Lee, H. Miyachi, B. Watanabe, T. Muranaka, K. Saito, Y. Sugimoto and M. Mizutani, *Plant J.*, 2021, **108**, 81–92.

185 R. A. Quinn, A. V. Melnik, A. Vrbanac, T. Fu, K. A. Patras, M. P. Christy, Z. Bodai, P. Belda-Ferre, A. Tripathi, L. K. Chung, M. Downes, R. D. Welch, M. Quinn, G. Humphrey, M. Panitchpakdi, K. C. Weldon, A. Aksenov, R. da Silva, J. Avila-Pacheco, C. Clish, S. Bae, H. Mallick, E. A. Franzosa, J. Lloyd-Price, R. Bussell, T. Thron, A. T. Nelson, M. Wang, E. Leszczynski, F. Vargas, J. M. Gauglitz, M. J. Meehan, E. Gentry, T. D. Arthur, A. C. Komor, O. Poulsen, B. S. Boland, J. T. Chang, W. J. Sandborn, M. Lim, N. Garg, J. C. Lumeng, R. J. Xavier, B. I. Kazmierczak, R. Jain, M. Egan, K. E. Rhee, D. Ferguson, M. Raffatellu, H. Vlamakis, G. G. Haddad, D. Siegel, C. Huttenhower, S. K. Mazmanian, R. M. Evans, V. Nizet, R. Knight and P. C. Dorrestein, *Nature*, 2020, **579**, 123–129.

186 B. Rimal, S. L. Collins, C. E. Tanes, E. R. Rocha, M. A. Granda, S. Solanki, N. J. Hoque, E. C. Gentry, I. Koo, E. R. Reilly, F. Hao, D. Paudel, V. Singh, T. Yan, M. S. Kim, K. Bittinger, J. P. Zackular, K. W. Krausz, D. Desai, S. Amin, J. P. Coleman, Y. M. Shah, J. E. Bisanz, F. J. Gonzalez, J. P. Vanden Heuvel, G. D. Wu, B. S. Zemel, P. C. Dorrestein, E. E. Weinert and A. D. Patterson, *Nature*, 2024, **626**, 859–863.

187 D. V. Guzior, M. Okros, M. Shivel, B. Armwald, C. Bridges, Y. Fu, C. Martin, A. L. Schilmiller, W. M. Miller, K. M. Ziegler, M. D. Sims, M. E. Maddens, S. F. Graham, R. P. Hausinger and R. A. Quinn, *Nature*, 2024, **626**, 852–858.

188 J. Skerlova, H. Lindstrom, E. Gonis, B. Sjodin, F. Neiers, P. Stenmark and B. Mannervik, *FEBS Lett.*, 2020, **594**, 1187–1195.

189 B. Mannervik, A. Ismail, H. Lindstrom, B. Sjodin and N. H. Ing, *Front. Mol. Biosci.*, 2021, **8**, 765970.

190 Y. Musdal, A. Ismail, B. Sjodin and B. Mannervik, *Biomolecules*, 2023, **13**, 976.

191 M. M. Pinney, D. A. Mokhtari, E. Akiva, F. Yabukarski, D. M. Sanchez, R. Liang, T. Doukov, T. J. Martinez, P. C. Babbitt and D. Herschlag, *Science*, 2021, **371**, 1010.

192 F. Yabukarski, T. Doukov, M. M. Pinney, J. T. Biel, J. S. Fraser and D. Herschlag, *Sci. Adv.*, 2022, **8**, eabn7738.

3582 | *Org. Biomol. Chem.*, 2024, **22**, 3559–3583

This journal is © The Royal Society of Chemistry 2024

193 Y. Wu, S. D. Fried and S. G. Boxer, *J. Am. Chem. Soc.*, 2020, **142**, 9993–9998.

194 M. R. Hennefarth and A. N. Alexandrova, *ACS Catal.*, 2020, **10**, 9915–9924.

195 A. Pant, T. K. Maiti, D. Mahajan and B. Das, *Microb. Ecol.*, 2023, **86**, 97–111.

196 A. Y. M. Woo, M. A. Aguilar Ramos, R. Narayan, K. C. Richards-Corke, M. L. Wang, W. J. Sandoval-Espinola and E. P. Balskus, *Nat. Rev. Chem.*, 2023, **7**, 319–339.

197 M. Funabashi, T. L. Grove, M. Wang, Y. Varma, M. E. McFadden, L. C. Brown, C. Guo, S. Higginbottom, S. C. Almo and M. A. Fischbach, *Nature*, 2020, **582**, 566–570.

198 W. J. Massey and J. M. Brown, *Nat. Microbiol.*, 2022, **7**, 1327–1328.

199 G. D. D'Agostino, S. N. Chaudhari and A. S. Devlin, *Nat. Chem. Biol.*, 2024, **20**, 410–421.

200 L. Yao, G. D. D'Agostino, J. Park, S. Hang, A. A. Adhikari, Y. Zhang, W. Li, J. Avila-Pacheco, S. Bae, C. B. Clish, E. A. Franzosa, C. Huttenhower, J. R. Huh and A. S. Devlin, *Nat. Microbiol.*, 2022, **7**, 1404–1418.

201 H. H. Le, M. T. Lee, K. R. Besler, J. M. C. Comrie and E. L. Johnson, *Nat. Microbiol.*, 2022, **7**, 1390–1403.

202 B. K. Senapati, *Org. Chem. Front.*, 2021, **8**, 2608–2642.

203 P. Luo, J. H. Huang, J. M. Lv, G. Q. Wang, D. Hu and H. Gao, *Nat. Prod. Rep.*, 2024, DOI: 10.1039/d3np00052d.

204 G. Powis, R. Bonjouklian, M. M. Berggren, A. Gallegos, R. Abraham, C. Ashendel, L. Zalkow, W. F. Matter, J. Dodge, G. Grindey, *et al.*, *Cancer Res.*, 1994, **54**, 2419–2423.

205 G. Bianco, P. Schmitt-Kopplin, A. Crescenzi, S. Comes, A. Kettrup and T. R. Cataldi, *Anal. Bioanal. Chem.*, 2003, **375**, 799–804.

206 C. Bailly, *Steroids*, 2021, **176**, 108933.

207 A. A. Egorova, N. A. Chalaya, I. N. Fomin, A. I. Barchuk and S. V. Gerasimova, *Agronomy*, 2022, **12**, 462.

208 P. D. Sonawane, A. Jozwiak, S. Panda and A. Aharoni, *Curr. Opin. Plant Biol.*, 2020, **55**, 118–128.

209 D. K. Zhao, Y. Zhao, S. Y. Chen and E. J. Kennelly, *Nat. Prod. Rep.*, 2021, **38**, 1423–1444.

210 S. E. Milner, N. P. Brunton, P. W. Jones, N. M. O'Brien, S. G. Collins and A. R. Maguire, *J. Agric. Food Chem.*, 2011, **59**, 3454–3484.

211 J. A. Delbrouck, M. Desgagne, C. Comeau, K. Bouarab, F. Malouin and P. L. Boudreault, *Molecules*, 2023, **28**, 4957.

212 P. D. Sonawane, U. Heinig, S. Panda, N. S. Gilboa, M. Yona, S. P. Kumar, N. Alkan, T. Unger, S. Bocobza, M. Pliner, S. Malitsky, M. Tkachev, S. Meir, I. Rogachev and A. Aharoni, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E5419–E5428.

213 P. D. Cardenas, P. D. Sonawane, U. Heinig, A. Jozwiak, S. Panda, B. Abebie, Y. Kazachkova, M. Pliner, T. Unger, D. Wolf, I. Ofner, E. Vilaprinyo, S. Meir, O. Davydov, A. Gal-On, S. Burdman, A. Giri, D. Zamir, T. Scherf, J. Szymanski, I. Rogachev and A. Aharoni, *Nat. Commun.*, 2019, **10**, 5169.

214 R. Akiyama, M. Nakayasu, N. Umemoto, J. Kato, M. Kobayashi, H. J. Lee, Y. Sugimoto, Y. Iijima, K. Saito, T. Muranaka and M. Mizutani, *Plant Cell Physiol.*, 2021, **62**, 775–783.

215 R. Akiyama, B. Watanabe, M. Nakayasu, H. J. Lee, J. Kato, N. Umemoto, T. Muranaka, K. Saito, Y. Sugimoto and M. Mizutani, *Nat. Commun.*, 2021, **12**, 1300.

216 R. Akiyama, B. Watanabe, J. Kato, M. Nakayasu, H. J. Lee, N. Umemoto, T. Muranaka, K. Saito, Y. Sugimoto and M. Mizutani, *Plant Cell Physiol.*, 2022, **63**, 981–990.

217 P. D. Sonawane, A. Jozwiak, R. Barbole, S. Panda, B. Abebie, Y. Kazachkova, S. A. Gharat, O. Ramot, T. Unger, G. Wizler, S. Meir, I. Rogachev, A. Doron-Faigenboim, M. Petreikov, A. Schaffer, A. P. Giri, T. Scherf and A. Aharoni, *New Phytol.*, 2022, **234**, 1394–1410.

218 R. Akiyama, N. Umemoto and M. Mizutani, *Plant Biotechnol.*, 2023, **40**, 185–191.

219 M. Jesus, A. P. Martins, E. Gallardo and S. Silvestre, *J. Anal. Methods Chem.*, 2016, **2016**, 4156293.

220 D. Yi, T. Bayer, C. P. S. Badenhorst, S. Wu, M. Doerr, M. Hohne and U. T. Bornscheuer, *Chem. Soc. Rev.*, 2021, **50**, 8003–8049.

221 E. Ricca, B. Brucher and J. H. Schrittwieser, *Adv. Synth. Catal.*, 2011, **353**, 2239–2262.

222 Y. Zhou, S. K. Wu and U. T. Bornscheuer, *Chem. Commun.*, 2021, **57**, 10661–10674.

223 X. Yin, J. Liu, C. X. Kou, J. J. Lu, H. Zhang, W. Song, Y. H. Li, Z. Y. Xue and X. Hua, *Metab. Eng.*, 2023, **76**, 232–246.

224 M. Liu and J. Q. Kong, *Acta Pharm. Sin. B*, 2018, **8**, 981–994.

225 H. Pan, S. Chang, Y. Qu, M. Liu, W. Tian and Z. Chang, *Biochem. Eng. J.*, 2023, **198**, 109023.

226 Q. Chen, Z. Chao, K. Wang, X. Wang, H. Meng, X. Liu, X. Shan and J. Zhou, *ACS Catal.*, 2024, **14**, 4117–4129.

227 Y. J. Zhang, M. J. Liu, Z. X. Yang, J. Lin, Z. D. Huang and F. E. Chen, *Green Chem.*, 2023, **25**, 3223–3235.

228 Y. Q. Peng, C. H. Gao, Z. L. Zhang, S. J. Wu, J. Zhao and A. T. Li, *ACS Catal.*, 2022, **12**, 2907–2914.

229 Z. L. Zhang, C. H. Gao, J. Zhao, X. G. Peng, Q. Li and A. T. Li, *ACS Catal.*, 2023, **13**, 13111–13116.

230 J. Feng, Q. Wu, D. Zhu and Y. Ma, *ChemSusChem*, 2022, **15**, e202102399.

231 X. Li, R. Zhang, J. Li, N. Liu, X. Chen, Y. Liu, G. Zhao, K. Ding, P. Yao, J. Feng, Q. Wu, D. Zhu and Y. Ma, *JACS Au*, 2024, DOI: 10.1021/jacsau.3c00688.

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Org. Biomol. Chem., 2024, **22**, 3559–3583 | **3583**