Unified Strategy to Prostaglandins:

Chemoenzymatic Total Synthesis of Cloprostenol, Bimatoprost, PGF$_{2\alpha}$, Fluprostenol, and Travoprost Guided by Biocatalytic Retrosynthesis

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ABSTRACT: Development of efficient and stereoselective synthesis of prostaglandins (PGs) is of utmost importance, owing to their valuable medicinal applications and unique chemical structures. We report here a unified synthesis of PGs cloprostenol, bimatoprost, PGF$_{2\alpha}$, fluprostenol, and travoprost from the readily available dichloro-containing bicyclic ketone 6a guided by biocatalytic retrosynthesis, in 11-12 steps with 2.9-6.5% overall yields. A Baeyer-Villiger monooxygenase (BVMO)-catalyzed stereoselective oxidation of 6a (99% ee), and a ketoreductase (KRED)-catalyzed diastereoselective reduction of enones 12 (87 : 13 to 99 : 1 dr) were utilized in combination for the first time to set the critical stereochemical configurations under mild conditions. Another key transformation was the copper (II)-catalyzed regioselective $p$-phenylbenzoylation of the secondary alcohol of diol 10 (3.8 : 1 rr). This study not only provides an alternative route to the highly stereoselective synthesis of PGs, but also showcases the usefulness and great potential of biocatalysis in construction of complex molecules.
Prostaglandins (PGs) are hormone-like lipid compounds often found in animals and human-beings\textsuperscript{1}, and they are shown to display a multitude of biological functions. To date, more than 20 PG analogs have been developed as the marketed medicines, such as the veterinary drugs cloprostenol (1) and fluprostenol (4), antiglaucoma drugs bimatoprost (2) and travoprost (5) (Fig. 1)\textsuperscript{1}. In particular, bimatoprost has recently become a block-buster drug. Because of their valuable medicinal applications and unique chemical structures, tremendous efforts have been devoted to the efficient synthesis of PGs\textsuperscript{1-3}. In fact, since Corey’s landmark synthesis of prostaglandin F\textsubscript{2α} (PGF\textsubscript{2α}, 3) in the late 1960s\textsuperscript{4}, PGs have become one touchstone of state-of-the-art synthetic methodologies\textsuperscript{5-23}, as exemplified in the elegant synthesis recently reported by the groups of Aggarwal\textsuperscript{3,5-7}, Hayashi\textsuperscript{8-12}, Stoltz\textsuperscript{13}, Fletcher\textsuperscript{14}, and Baran\textsuperscript{23}. Very recently, our group has developed an efficient and modular synthesis of PGs (Scheme S1)\textsuperscript{24}. Crucial to our success was the stereocontrolled synthesis of the key lactone intermediate 7a via a chiral spiro-phosphoric acid-catalyzed Baeyer-Villiger (B-V) oxidation of bicyclic ketone 6a (Scheme S1).

![Fig. 1 Structure of select prostaglandins: cloprostenol (1), bimatoprost (2), PGF\textsubscript{2α} (3), fluprostenol (4), and travoprost (5).](image_url)
The past two decades have witnessed the rapid development of biocatalysis into a sophisticated technology, mainly thanks to the ever-increasing bioinformatics and protein engineering tools. Compared to chemocatalysis, biocatalysis usually offers unparalleled chemo-, regio-, and stereoselectivity pivotal for efficient synthesis of complex molecules like pharmaceuticals and bioactive natural products. To harness the synthetic potential of these extraordinary selectivities and other advantageous properties inherent to biocatalysis (e.g. mild reaction conditions) more productively, Turner introduced the concept of biocatalytic retrosynthesis to the scientific community about seven years ago, which has since been widely and increasingly utilized when planning synthetic routes to target molecules. From retrosynthetic viewpoint, a biocatalytic reaction can be adopted in two distinct ways: either being used as an alternative approach to replace an equivalent chemical step, or being employed to fulfill a disconnection which is impossible to realize by traditional chemical means. As an example in the former scenario, ketoreductase (KRED)-catalyzed stereoselective reduction was demonstrated to be a milder and more sustainable process compared to (-)-DIP-Cl-mediated reduction, in the synthesis of a key chiral alcohol intermediate to the active pharmaceutical ingredient (API) montelukast. So far, the latter scenario has been less exploited. Recently, a regio- and stereoselective transformation from prochiral ketoenones to 2,6-disubstituted piperidines, a formidable task for classical chemical methods, was accomplished via an ω-transaminase triggered intramolecular aza-Michael reaction and subsequent epimerization. Continuing our interests in the development of efficient, stereoselective, and green synthesis of PGs, as well as application of biocatalysis to organic synthesis, herein we report a tractable, chemoenzymatic total synthesis of prostaglandins guided by biocatalytic retrosynthesis.
Results

**Biocatalytic retrosynthesis.** In our biocatalytic retrosynthesis (Fig. 2), cloprostenol (1), bimatoprost (2), PGF\(_2\alpha\) (3), and fluprostenol (4), prostaglandins containing an allylic alcohol moiety on the \(\omega\)-side-chain, could be synthesized from lactones 13a-13d through a three-step sequence of \(p\)-phenylbenzoyl (PPB) ester hydrolysis, DIBAL-H reduction, and Wittig olefination. We envisioned that the crucial stereogenic center of the \(\omega\)-side-chain of 13a-13d could be installed via a KRED-catalyzed diastereoselective reduction of enones 12a-12d, which were prepared from \(C_{11}\)-OH-PPB-protected Corey alcohol 11 (prostaglandin numbering) through primary alcohol oxidation, followed by Horner-Wadsworth-Emmons (HWE) olefination. It was hoped that 11 might be furnished via a catalyst-controlled regioselective \(p\)-phenylbenzoylation of the \(C_{11}\)-OH of the known diol 10\(^{46-49}\), which was synthesized from lactone 7a via a three-step sequence of dechlorination, Prins reaction, and deformylation. At this stage, the key lactone 7a was envisioned to be accessible through a Baeyer-Villiger monooxygenase (BVMO)-catalyzed stereoselective oxidation of bicyclic ketone 6a. Notably, our previous study on chiral spiro-phosphoric acid-catalyzed B-V oxidation suggested it was beneficial to introduce the dichloro functionality into the cyclobutanone ring of 6a. Firstly, formation of normal lactone (NL) 7a becomes more favored because of the electron-withdrawing effect of the dichloro group (Scheme S1). Secondly, the formed abnormal lactone (AL) 8 could be readily converted to the easily removable cyclopentene dicarboxylic acid 9 during the reaction workup, thus facilitating the isolation of the desired 7a (Scheme S1). Finally, travoprost (5) can be synthesized from fluprostenol (4) via a late-stage \(i\)-propyl ester formation reaction\(^6\).
**Fig. 2 Biocatalytic retrosynthesis of prostaglandins 1-4.**

**CHMO\textsubscript{Rhodol1}-catalyzed oxidation of bicyclic ketones 6.** Our study commenced with the development of a feasible BVMO-catalyzed stereoselective oxidation of 6a, which has not yet been reported, to the best of our knowledge. In contrast, bicyclo[3.2.0]hept-2-en-6-one (14), a bicyclic ketone lacking the dichloro functionality in comparison to 6a, was routinely employed as the model substrate in BVMO-catalyzed oxidation reactions (Scheme S2)\textsuperscript{50-52}. A regiodivergent conversion of 14 has been observed for most BVMOs, resulting in the formation of the enantioenriched NL 15 and AL 16 in similar amounts (Scheme S2). Under such circumstance, the separation of 15 and 16 is troublesome. Occasionally, BVMO with regio- plus enantioselectivity towards 14 has been disclosed. For instance, the Grogan group has reported that BVMO-MO14, an enzyme originating from *Rhodococcus jostii*, was able to catalyze the resolution of 14 to furnish NL 15 and the residual enantiomer of 14 both with excellent enantiomeric purity (Scheme S2)\textsuperscript{53}. In the present study, three “regiodivergent” BVMOs, namely CHMO\textsubscript{Rhodol1}\textsuperscript{54}, CHMO\textsubscript{Arthro}\textsuperscript{54}, and
CHMOBrev1, as well as the “regioselective” enzyme BVMO-MO14, are examined in the oxidation of 6a. On one hand, moderate to high conversion of 6a and formation of trace-amounts of the desired 7a (≤ 5%) were observed in the CHMOArthro-, CHMOBrevil-, and BVMO-MO14-catalyzed reactions (Table S2). On the other hand, 6a was completely consumed in the CHMORhodol-catalyzed reaction, but the yield of 7a was only 25% (Table 1, entry 1, also see Table S2), much lower than 50% maximum theoretical yield for a resolution event. To account for this mass imbalance, we investigated the stability of 6a and racemic-7a. In the absence of BVMO, it was found that 6a and racemic-7a decomposed readily, with only 58% and 66% remaining, respectively, after incubation for 90 minutes (Figure S5). Presumably, the electron-withdrawing dichloro group makes the carbonyl more electron-deficient, hence rendering 6a and 7a more susceptible to hydrolysis. To improve the yield of 7a, CHMORhodol-catalyzed oxidation reactions were carried out under different pHs (Table 1, entry 2 and 3) or at different temperatures (Table 1, entry 4 and 5). No significant improvement was achieved. Next, five organic solvents were screened in an effort to mitigate decomposition and increase product yield (Table 1, entry 6-10). To our delight, the use of methyl tert-butyl ether (MTBE) and 2-methoxyethanol (MME) significantly increased the yield of 7a to 38% and 36%, respectively. Not only the yield, but the enantiomeric purity of 7a were revealed to be dependent on the co-solvents employed. While the use of MTBE resulted in an inferior enantiomeric purity (73% ee), no adverse effect was observed when using MME. Hence, the latter solvent was chosen for the remaining study. Gratifyingly, a preparative-scale oxidation of 6a under the above optimized condition furnished the desired 7a in 38% isolated yield with 99% ee (Table 1, Entry 11). Notably, thus generated 7a possessed a superior enantiomeric purity to that prepared using our chiral spiro-phosphoric acid catalysis method (99% ee versus 95% ee), highlighting the stereoselectivity advantage of using enzyme.
catalysis. The chemical structure and the stereochemical assignment of 7a were unambiguously established by X-ray crystallography. Cyclopentene dicarboxylic acid 9, resulting from the hydrolysis of abnormal lactone 8, was isolated in 35% yield.

Table 1. CHMO_{Rhodo1}-catalyzed oxidation of 6a.\textsuperscript{a}

| Entry | Co-solvent | pH  | 6a remained (%)\textsuperscript{b} | yield of 7a (%)\textsuperscript{b} | Ee of 7a (%)\textsuperscript{c} |
|-------|------------|-----|-----------------------------------|-----------------------------------|---------------------------------|
| 1     | N.A.\textsuperscript{d} | 7.5 | 0                                 | 25                                | 99                              |
| 2     | N.A.       | 7.0 | 0                                 | 26                                | N.D.\textsuperscript{e}        |
| 3     | N.A.       | 6.5 | 6                                 | 26                                | N.D.                            |
| 4\textsuperscript{f} | N.A.   | 7.5 | 0                                 | 22                                | N.D.                            |
| 5\textsuperscript{g} | N.A.   | 7.5 | 0                                 | 22                                | N.D.                            |
| 6     | Cyclohexane| 7.5 | 23                                | 23                                | N.D.                            |
| 7     | DMSO       | 7.5 | 0                                 | 28                                | N.D.                            |
| 8     | MTBE       | 7.5 | 0                                 | 38                                | 73                              |
| 9     | Dioxane    | 7.5 | 0                                 | 32                                | 82                              |
| 10    | MME        | 7.5 | 0                                 | 36                                | 99                              |
| 11\textsuperscript{h} | MME   | 7.5 | 0                                 | 40 (38\textsuperscript{i})       | 99                              |

\textsuperscript{a}Reaction conditions (1 mL): 6a (10 mM), glucose (60 mM), NADP\textsuperscript{+} (0.2 mM), FAD (0.05 mM), 0.79 mL of 20\% (w/v) cell-free extract (CFE) of CHMO\textsubscript{Rhodo1} in NaPi buffer (50 mM), 0.016 mL of 15\% (w/v) CFE of glucose dehydrogenase (GDH) in NaPi buffer (50 mM, pH 7.0), and 0.1 mL co-solvent (if applicable) in NaPi buffer (50 mM). Reaction mixtures were incubated at 25 °C with 200 rpm shaking for 90 min. \textsuperscript{b}Determined by GC analysis using undecane as the internal standard. \textsuperscript{c}Determined by SFC analysis. \textsuperscript{d}Not Applicable (N.A.). \textsuperscript{e}Not Determined (N.D.). \textsuperscript{f}The reaction temperature was 30 °C. \textsuperscript{g}The reaction temperature was 35 °C. \textsuperscript{h}The reaction volume was 50 mL. \textsuperscript{i}Isolated yield.
Other bicyclic cyclobutanones were employed as substrates to evaluate the feasibility of CHMO_Rhodo1-catalyzed oxidation reaction (Fig. 3). Firstly, ketone 6b without an olefin moiety was well recognized by CHMO_Rhodo1, furnishing the desired normal lactone 7b in 30% isolated yield with 99% ee. Secondly, the size of the fused ring seemed to play an important role in this enzyme-catalyzed B-V oxidation process. For instance, the oxidation of substrates containing a 5-membered or 6-membered fused ring (6b, 6c, 6d, 6e) occurred smoothly, affording the corresponding lactones 7b, 7c, 7d, and 7e in 22-34% isolated yield with 91-99% ee. However, ketone 6f with an 8-membered fused ring could not be converted by CHMO_Rhodo1 and no desired lactone 7f was detected. It is possible that the overlarge size of this fused ring prevents a proper binding of 6f to CHMO_Rhodo1.

**Fig. 3** Substrate scope of CHMO_Rhodo1-catalyzed B-V oxidation of bicyclic cyclobutanones.

**Synthesis of diol 10 in continuous flow.** The transformation of 7a into the known diol 10 was realized via a three-step sequence in flow chemistry, which was significantly more time-
economical compared to batch reactions (Fig. 4). Firstly, a continuous flow dechlorination of 7a was accomplished in a packed bed reactor (Shenzhen E-zheng tech Co., Ltd) filled with zinc power at 70 °C under 7 bar back-pressure with 10 min residence time, giving NL 15 in 90% yield. Then NL 15 was dissolved in a 10 : 1 HCOOH/H2SO4 solution containing pre-dissolved paraformaldehyde, and the mixture was pumped into a 0.5 mL PTFE reactor coil (i.d. = 0.8 mm) at 70 °C under 17 bar back-pressure with 15 min residence time to afford crude 17 as the major product with full conversion by Prins reaction. After neutralization and removing the inorganic salt, the crude 17 in MeOH combined with NaOMe was pumped into a 0.5 mL PTFE reactor coil via a T-junction to complete deformylation followed by quenching with AcOH to give diol 10 smoothly in 81% yield over two steps (Fig. 4).

**Fig. 4 Conversion of lactone 7a into diol 10 in continuous flow.**
Regioselective \( p \)-phenylbenzoylation of diol 10. With access to diol 10, the site-selective protection of C\(_{11}\)-OH could now commence. In the traditional total synthesis of PGs, the three-step choreography of protecting group manipulations of diol 10 would establish a C\(_{11}\)-OH-protected Corey alcohol motif present within PGs, aiding purification and imparting chemoselectivity in subsequent transformations\(^{56-58}\). However, this strategy is not step-economic for PG synthesis. To circumvent this limitation, we recently developed a directly regioselective oxidation of the primary alcohol of 10 by using a modified TEMPO/PhI(OAc)\(_2\) protocol into C\(_{11}\)-OH non-masked Corey aldehyde for joining the \( \omega \)-side-chain via Horner-Wadsworth-Emmons (HWE) olefination\(^{24,59}\). While seemingly straightforward, successful implementation of our strategy would not be attractive and practical in the large-scale synthesis of prostaglandins, because the most of oil prostaglandin intermediates obtained in this protecting-group-free chemistry are unstable. Therefore, designing a one-step method for catalyst-controlled site-selective \( p \)-phenylbenzoylation of C\(_{11}\)-OH in 10 would represent a valuable goal that promises to the streamlined access to the stable and nicely crystalline C\(_{11}\)-OH-PPB-protected Corey alcohol 11 (Fig. 2)\(^{60,61}\).

With this assumption in mind, the \( p \)-phenylbenzoylation of diol 10 was first conducted under a typical acylation condition as reported by the Dong group\(^{62}\). As summarized in Table 2, monoesters 11 and 18 were both formed in 8% and 16% yields, respectively (entry 1). When the reaction temperature was lowered to -20 °C, only trace amount of 18 (<5%) was obtained (Table 2, entry 2). The use of copper salt and appropriate ligands/additives were previously demonstrated to promote regioselective acylation of substrates containing multiple hydroxyl groups\(^{46-49}\). In the present study, we found CuCl\(_2\) alone could accelerate the reaction, and importantly, the desired 11 was afforded in 48% yield as the major product (Table 2, entry 3). To further improve the
regioselectivity, several additives were tested. While the use of additives Ad1 and Ad2 shifted the selectivity to favor the formation of 18 (Table 2, entry 4 and 5), to our delight, the ratio of 11 to 18 was improved to 3.8 : 1 when the N-methylated diphenylprolinol silyl ether Ad3 was attempted (Table 2, entry 6, also see Figure S6 and S7). The desired monoester 11 was isolated in 57% yield. Intriguingly, the application of additives Ad4 and Ad5, the enantiomer and the desilylated analog of Ad3, respectively, also gave comparable results (Table 2, entry 7 and 8). The detailed mechanism of this copper (II)-catalyzed regioselective acylation reaction warrants investigation in the future.

**Table 2. Regioselective p-phenylbenzoylation of diol 10.**

| Entry | Metal  | Additive | Yield of 11 (%) | Yield of 18 (%) | Yield of 19 (%) | Regioisomeric ratio (rr), 11 : 18 |
|-------|--------|----------|-----------------|----------------|----------------|----------------------------------|
| 1 c   | N.A.   | N.A.     | 8               | 16             | 1              | 1 : 2                            |
| 2     | N.A.   | N.A.     | 0               | <5             | 0              | N.A.                             |
| 3     | CuCl₂  | N.A.     | 48              | 18             | 4              | 2.7 : 1                          |
| 4     | CuCl₂  | Ad1      | 13              | 63             | 0              | 1 : 4.8                          |
| 5     | CuCl₂  | Ad2      | 32              | 45             | 3              | 1 : 1.4                          |

a. Conditions: Metal, additive, PPBCl, DIPEA, MeCN, DCM.

b. Yields are isolated.

c. N.A.: Not available.

1: Ph: Phenyl.
6  CuCl₂  Ad3  58 (57°)  15  3  3.8 : 1
7  CuCl₂  Ad4  57  16  4  3.6 : 1
8  CuCl₂  Ad5  48  14  6  3.4 : 1

 Reaction conditions (1.5 mL): 10 (0.1 mmol), PPBCl (0.1 mmol), DIPEA (0.1 mmol), CuCl₂ (0.1 equiv., if applicable), additive (0.1 equiv., if applicable) in MeCN (1 mL) and DCM (0.5 mL). Reaction mixtures were stirred at -20 ℃ for 14 h. 

 Reaction run at room temperature. 

 Chemoenzymatic total synthesis of cloprostenol (1), bimatoprost (2), PGF₂α (3), fluprostenol (4), and travoprost (5). With the key C₁₁-OH-PPB-protected Corey alcohol 11 in hand, we then turned our attentions to efficiently install the ω-side-chain of PGs (Fig. 5). Oxidation of 11 with TEMPO and trichloroisocyanuric acid (TCCA) in ethyl acetate and dimethyl carbonate at 0 ℃ provided the desired crude C₁₁-OH-PPB-protected Corey aldehyde 20, and set the stage for the C₁₃-C₁₄ HWE olefination. Addition of the crude aldehyde 20 to the solutions of phosphonates 21a-21d in dichloromethane in the presence of 30% aq. NaOH at 0 ℃ afforded the corresponding enones 12a-12d in good isolated yields (71-75% over two steps) as a single geometric isomer with an (E)-configuration at the newly formed C₁₃-C₁₄ double bond judged by the ¹H NMR spectra analysis.

 Stereoselective reduction of the keto functionality of prostaglandin’s ω-side-chain is dominated by chemical approaches, with biocatalytic reduction being rarely exploited. Recently, the Romano group reported an efficient and highly diastereoselective synthesis of a key allylic alcohol-containing intermediate to bimatoprost via the yeast Pichia anomala-mediated reduction. However, careful optimization of the biotransformation conditions was necessary in order to suppress the competing reduction of the carbon-carbon double bond by the enoate reductase.
present in the same yeast. To alleviate this inconvenience, use of KREDs recombinantly over-expressed in *E. coli* should be a viable option. Due to their excellent stereoselectivity, broad substrate spectrum, good stability, and high volumetric productivity, such over-expressed KREDs either in isolated form (purified enzyme or crude-cell lysate) or in whole-cell form, have been widely adopted in the synthesis of pharmaceuticals and bioactive molecules\textsuperscript{43,45,70-72}. To the best of our knowledge, recombinantly over-expressed KRED-catalyzed stereoselective reduction has not yet been reported for PG synthesis.

**Table 3. ChKRED20-catalyzed reduction of 12a.**

| Entry | DMSO (v/v, %) | 12a (mM) | Conv. (%)\textsuperscript{b} | dr (C-15, α : β)\textsuperscript{b} |
|-------|--------------|---------|-----------------|-----------------|
| 1     | 3            | 10      | 43              | 97.8 : 2.2      |
| 2     | 5            | 10      | 58              | 97.4 : 2.6      |
| 3     | 10           | 10      | 71              | 96.7 : 3.3      |
| 4     | 20           | 10      | 83              | 93.9 : 6.1      |
| 5     | 10           | 5       | 90              | 97.9 : 2.1      |
| 6\textsuperscript{c} | 10           | 5       | 96 (91\textsuperscript{d}) | 97.9 : 2.1 |

\textsuperscript{a}Reaction conditions (0.5 mL): 12a (5 mM or 10 mM), glucose (2 equiv. relative to 12a), NADP\textsuperscript{+} (0.2 mM), ChKRED20 (1 mg/mL), 0.1 mL of 15% (w/v) cell-free extract (CFE) of GDH, and DMSO (v/v, 3-20%) in KP\textsubscript{i} buffer (100 mM, pH 7.0). Reaction mixtures were incubated at 30 °C with 200 rpm shaking for 17 h. \textsuperscript{b}Determined by SFC analysis. \textsuperscript{c}The reaction volume was 40 mL and the reaction time was 33 h. \textsuperscript{d}Isolated yield.

To this end, we examined the bioreduction of 12a using a small library of in-house preserved KREDs (Table S3). Out of 15 enzymes tested, ten could not transform 12a at all. The conversion of the rest five enzymatic reactions was in the range of 4-43%, with ChKRED20 giving the best
performance (Table 3, entry 1, also see Table S3)\textsuperscript{73,74}. It was noticed that the low conversion of 12a was to some extent due to its apparently poor solubility under current reaction conditions, which probably arises from its hydrophobic structure. To help solubilize 12a and improve the conversion, reactions with larger amount of the co-solvent DMSO were attempted. Indeed, the conversions of 12a were increased to 58% and 71% (Table 3, entry 2 and 3), respectively, when 5% and 10% of DMSO were used. Although using 20% of DMSO further improved the conversion to 83%, an inferior diastereoselectivity (93.9 : 6.1 dr) was obtained (Table 3, entry 4). On the other hand, decrease of the substrate concentration from 10 mM to 5 mM boosted the reaction conversion to 90%, and importantly the excellent diastereoselectivity (97.9 : 2.1 dr) was retained (Table 3, entry 5). We believe efficient reduction of higher concentration of 12a would become possible by optimization of the reaction and protein engineering in the future\textsuperscript{28,75}. Pleasingly, ChKRED20-catalyzed reduction of 12a under the optimized condition at a preparative-scale occurred smoothly, delivering the desired allylic alcohol 13a in 91% isolated yield with 97.9 : 2.1 dr (Table 3, entry 6). The configuration of the newly generated stereogenic center (C-15, prostaglandin numbering) was assigned to $\alpha$ by comparing to that of 13a prepared using (-)-DIP-Cl-mediated reduction\textsuperscript{76}. From 13a, hydrolysis of the PPB ester to alcohol, followed by the DIBAL-H mediated reduction of the lactone to the hemiacetal, and a final Wittig olefination furnished cloprostenol (1) in 44% over three steps (Fig. 5). The applicability of our developed route was further demonstrated by the synthesis of another four PGs: bimatoprost, PGF$_{2\alpha}$, fluprostenol, and travoprost (Fig. 5). When enones 12b, 12c, and 12d with different $\omega$-side-chains were subjected to ChKRED20-catalyzed reduction, the desired allylic alcohols 13b, 13c, and 13d were isolated in 80-90% yields with 87 : 13 to 99 : 1 dr. Analogous to the above synthesis of cloprostenol (1), bimatoprost (2), PGF$_{2\alpha}$ (3), and fluprostenol (4) were prepared from 13b, 13c, and 13d in a three-step sequence with 31%,
63%, and 51% yields, respectively. Finally, the transformation from fluprostenol to travoprost was accomplished in 68% yield by using 2-iodopropane and Cs$_2$CO$_3$ in the mixed solvent of DCM and DMF$^6$. Our chemoenzymatic synthesis of cloprostenol, bimatoprost, PGF$_{2\alpha}$, fluprostenol, and travoprost were completed in 11-12 steps from bicyclic ketone 6a with 2.9-6.5% overall yields.

**Fig. 5** Completion of the chemoenzymatic total synthesis of cloprostenol (1), bimatoprost (2), PGF$_{2\alpha}$ (3), fluprostenol (4), and travoprost (5).

**Discussion**

In summary, a unified, biocatalytic retrosynthesis-guided route has been developed for the synthesis of cloprostenol (1), bimatoprost (2), PGF$_{2\alpha}$ (3), fluprostenol (4), and travoprost (5) from
the readily available dichloro-containing bicyclic ketone 6a in 11-12 steps with 2.9-6.5% overall yields, featuring a BVMO-catalyzed stereoselective oxidation of 6a (99% ee), a copper (II)-catalyzed regioselective p-phenylbenzoylation of the secondary alcohol of diol 10 (3.8 : 1 rr), and a KRED-catalyzed diastereoselective reduction of enones 12 (87 : 13 to 99 : 1 dr). Compared to chemocatalytic reactions, these two key enzymatic transformations were performed under milder conditions and meanwhile exhibited superior stereoselectivity. Our study not only provides an alternative route to the highly stereoselective synthesis of prostaglandins, but also showcases the usefulness and great potential of biocatalysis in construction of complex molecules.

**Methods**

**General.** MeCN was freshly distilled from CaH₂ under N₂. THF was freshly distilled from Na under N₂ using benzophenone as the indicator. Unless otherwise specified, all reagents and solvents were purchased from commercial sources and used as received. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 spectrometer in CDCl₃ using tetramethyl silane (TMS) as internal standards. Coupling constant (J) values are given in Hz. Multiplicities are designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, multiplet. Melting points were measured on MP450 full-automatic melting-point apparatus. Products were purified by flash column chromatography on silica gel purchased from Qingdao Haiyang Chemical Co., Ltd. Optical rotations were measured by a Rudolph AUTOPOL I Automatic Polarimeter. EI-MS were recorded on an Agilent 6890N/5975 spectrometer and ESI-MS were recorded on a Waters Micromass Quattro Micro spectrometer. HRMS (ESI) were recorded on a Bruker micrOTOF spectrometer. HPLC analysis were performed with SFC (Agilent 1260 Infinity II) using Daicel Chiralpak IA column (25 cm × 4.6 mm × 5 µm),
Chiralpak IF-3 column (25 cm × 4.6 mm × 5 µm), Chiralpak AD-H column (25 cm × 4.6 mm × 5 µm) and Chiralpak OD-H column (25 cm × 4.6 mm × 5 µm).

Data availability

Experimental procedures and characterization data of new compounds are available in Supplementary Information. The X-ray crystallographic data for compound 7a reported in this study has been deposited at the Cambridge Crystallographic Data Centre (CCDC) under deposition numbers CCDC 1975866. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Any further relevant data are available from the authors upon reasonable request.

References

1. Peng, H. & Chen, F. Recent advances in asymmetric total synthesis of prostaglandins. *Org. Biomol. Chem.* **15**, 6281-6301 (2017).

2. Das, S., Chandrasekhar, S., Yadav, J. S. & Grée, R. Recent developments in the synthesis of prostaglandins and analogues. *Chem. Rev.* **107**, 3286-3337 (2007).

3. Bennett, S. H., Coulthard, G. & Aggarwal, V. K. Prostaglandin total synthesis enabled by the organocatalytic dimerization of succinaldehyde. *Chem. Rec.* **20**, 936-947 (2020).

4. Corey, E. J., Weinshenker, N. M., Schaaf, T. K. & Huber, W. Stereo-controlled synthesis of dl-prostaglandins F2α and E2. *J. Am. Chem. Soc.* **91**, 5675-5677 (1969).
5. Pelss, A. et al. Reoptimization of the organocatalyzed double aldol domino process to a key enal intermediate and its application to the total synthesis of Δ^{12}-prostaglandin J₃. *Chem. Eur. J.* **24**, 9542-9545 (2018).

6. Prévost, S. et al. Synthesis of prostaglandin analogues, latanoprost and bimatoprost, using organocatalysis via a key bicyclic enal intermediate. *Org. Lett.* **17**, 504-507 (2015).

7. Coulthard, G., Erb, W. & Aggarwal, V. K. Stereocontrolled organocatalytic synthesis of prostaglandin PGF₂α in seven steps. *Nature* **489**, 278-281 (2012).

8. Umekubo, N. & Hayashi, Y. Pot-economical total synthesis of clinprost. *Org. Lett.* **22**, 9365-9370 (2020).

9. Umekubo, N., Suga, Y. & Hayashi, Y. Pot and time economies in the total synthesis of corey lactone. *Chem. Sci.* **11**, 1205-1209 (2020).

10. Umekubo, N. & Hayashi, Y. Asymmetric synthesis of corey lactone and latanoprost. *Eur. J. Org. Chem.* 6221-6227 (2020).

11. Kawauchi, G., Umemiya, S., Taniguchi, T., Monde, K. & Hayashi, Y. Enantio- and diastereoselective synthesis of latanoprostone using an organocatalyst. *Chem. Eur. J.* **24**, 8409-8414 (2018).

12. Hayashi, Y. & Umemiya, S. Pot economy in the synthesis of prostaglandin A₁ and E₁ methyl esters. *Angew. Chem. Int. Ed.* **52**, 3450-3452 (2013).
13. Li, J., Ahmed, T. S., Xu, C., Stoltz, B. M. & Grubbs, R. H. Concise syntheses of Δ^{12}-prostaglandin J natural products via stereoretentive metathesis. *J. Am. Chem. Soc.* **141**, 154-158 (2019).

14. Kučera, R., Goetzke, F. W. & Fletcher, S. P. An asymmetric Suzuki–Miyaura approach to prostaglandins: synthesis of tafluprost. *Org. Lett.* **22**, 2991-2994 (2020).

15. Nicolaou, K. C. et al. Synthesis and biological investigation of Δ^{12}-prostaglandin J₃ (Δ^{12}-PGJ₃) analogues and related compounds. *J. Am. Chem. Soc.* **138**, 6550-6560 (2016).

16. Nicolaou, K. C. et al. Total Synthesis of Δ^{12}-Prostaglandin J₃, a Highly Potent and Selective Antileukemic Agent. *Angew. Chem. Int. Ed.* **53**, 10443-10447 (2014).

17. Egger, J. et al. Total synthesis of prostaglandin 15d-PGJ₂ and investigation of its effect on the secretion of IL-6 and IL-12. *Org. Lett.* **17**, 4340-4343 (2015).

18. Egger, J., Bretscher, P., Freigang, Kopf, S. & Carreira, M. E. M. Discovery of a highly potent anti-inflammatory epoxyisoprostane-derived lactone. *J. Am. Chem. Soc.* **136**, 17382-17385 (2014).

19. Danishefsky, S. J., Cabal, M. P. & Chow, K. Novel stereospecific silyl group transfer reactions: practical routes to the prostaglandins. *J. Am. Chem. Soc.* **111**, 3456-3457 (1989).

20. Stork, G., Sher, P. M. & Chen, H. L. Radical cyclization-trapping in the synthesis of natural products. a simple, stereocontrolled route to prostaglandin F₂α. *J. Am. Chem. Soc.* **108**, 6384-6385 (1986).
21. Achmatowicz, B., Baranowska, E., Daniewski, A. R., Pankowski, J. & Wicha, J. BF₃-mediated reaction of a sulphone with aldehydes: a method for sterospecific construction of prostaglandin ω-chain. *Tetrahedron* **44**, 4989-4998 (1988).

22. Corey, E. J., Arnold, Z. & Hutton, J. Total synthesis of prostaglandins E₂ and F₂α (dl) via a tricarbocyclic intermediate. *Tetrahedron Lett.*, **11**, 307-310 (1970).

23. Edwards, J. T. et al. Decarboxylative alkenylation. *Nature* **545**, 213-218 (2017).

24. Zhu, K., Hu, S., Liu, M., Peng, H. & Chen, F. Access to a key building block for the prostaglandin family via stereocontrolled organocatalytic Baeyer–Villiger oxidation. *Angew. Chem. Int. Ed.* **58**, 9923-9927 (2019).

25. Turner, N. J. & Reilly, E. O. Biocatalytic retrosynthesis. *Nat. Chem. Biol.* **9**, 285-288 (2013).

26. Höning, M., Sondermann, P., Turner, N. J. & Carreira, E. M. Enantioselective chemo- and biocatalysis: partners in retrosynthesis. *Angew. Chem. Int. Ed.* **56**, 8942-8973 (2017).

27. De Souza, R. O. M. A., Miranda, L. S. M. & Bornscheuer, U. T. A retrosynthesis approach for biocatalysis in organic synthesis. *Chem. Eur. J.* **23**, 12040-12063 (2017).

28. Arnold, F. H. Directed evolution: bringing new chemistry to life. *Angew. Chem. Int. Ed.* **57**, 4143-4148 (2018).

29. Sheldon, R. A., Brady, D. & Bode, M. L. The Hitchhiker's guide to biocatalysis: recent advances in the use of enzymes in organic synthesis. *Chem. Sci.* **11**, 2587-2605 (2020).

30. Wu, S., Snajdrova, R., Moore, J. C., Baldenius, K. & Bornscheuer, U. T. Biocatalysis: enzymatic synthesis for industrial applications. *Angew. Chem. Int. Ed.* **60**, 88-119 (2021).
31. Bornscheuer, U. T. et al. Engineering the third wave of biocatalysis. *Nature* **485**, 185-194 (2012).

32. McKinnie, S. M. K. et al. Total enzyme syntheses of napyradiomycins A₁ and B₁. *J. Am. Chem. Soc.* **140**, 17840-17845 (2018).

33. Ryan, J. et al. Transaminase triggered aza-Michael approach for the enantioselective synthesis of piperidine scaffolds. *J. Am. Chem. Soc.* **138**, 15798-15800 (2016).

34. Fu, H. et al. Chemoenzymatic asymmetric synthesis of the metallo-β-lactamase inhibitor aspergillomarasmine A and related aminocarboxylic acids. *Nature Catal.* **1**, 186-191 (2018).

35. Zhang, X., King-Smith, E. & Renata, H. Total synthesis of tambromycin by combining chemocatalytic and biocatalytic C-H functionalization. *Angew. Chem. Int. Ed.* **57**, 5037-5041 (2018).

36. Xu, J., Green, A. P. & Turner, N. J. Chemo-enzymatic synthesis of pyrazines and pyrroles. *Angew. Chem. Int. Ed.* **57**, 16760-16763 (2018).

37. Lazzarotto, M. et al. Chemoenzymatic total synthesis of deoxy-, epi-, and podophyllotoxin and a biocatalytic kinetic resolution of dibenzylbutyrolactones. *Angew. Chem. Int. Ed.* **58**, 8226-8230 (2019).

38. H. Eastman, J. Ryan, B. Maciá, V. Caprio, E. O’Reilly, Alcohol dehydrogenase-triggered oxa-Michael reaction for the asymmetric synthesis of disubstituted tetrahydropyran and tetrahydrofurans. *ChemCatChem* **11**, 3760-3762 (2019).
39. Parmeggiani, F. et al. Biocatalytic retrosynthesis approaches to d-(2,4,5-trifluorophenyl)alanine, key precursor of the antidiabetic sitagliptin. *Green Chem.* **21**, 4368-4379 (2019).

40. Li, J., Li, F., King-Smith, E. & Renata, H. Merging chemoenzymatic and radical-based retrosynthetic logic for rapid and modular synthesis of oxidized meroterpenoids. *Nature Chem.* **12**, 173-179 (2020).

41. Taday, F. et al. Asymmetric construction of alkaloids by employing a key ω-transaminase cascade. *Chem. Eur. J.* **26**, 3729-3732 (2020).

42. Xu, Z. et al. Biocatalytic access to 1,4-diazepanes via imine reductase-catalyzed intramolecular asymmetric reductive amination. *ACS Catal.* **10**, 8780-8787 (2020).

43. Liang, J. et al. Development of a biocatalytic process as an alternative to the (-)-DIP-Cl-mediated asymmetric reduction of a key intermediate of montelukast. *Org. Process Res. Dev.* **14**, 193-198 (2010).

44. Wu, X. et al. Asymmetric synthesis of a key dextromethorphan intermediate and its analogues enabled by a new cyclohexylamine oxidase: enzyme discovery, reaction development, and mechanistic insight. *J. Org. Chem.* **85**, 5598-5614 (2020).

45. Hu, C., Liu, M., Yue, X., Huang, Z. & Chen, F. Development of a practical, biocatalytic synthesis of tert-butyl (R)-3-hydroxyl-5-hexenoate: a key intermediate to the statin side chain. *Org. Process Res. Dev.* **24**, 1700-1706 (2020).
46. Chen, I.-H., Kou, K. G. M., Le, D. N., Rathbun, C. M. & Dong, V. M. Recognition and site-selective transformation of monosaccharides by using copper(II) catalysis. *Chem. Eur. J.* **20**, 5013-5018 (2014).

47. Matsumura, Y., Maki, T., Murakami, S. & Onomura, O. Copper ion-induced activation and asymmetric benzylation of 1,2-diols: kinetic chiral molecular recognition. *J. Am. Chem. Soc.* **125**, 2052-2053 (2003).

48. Mazet, C., Roseblade, S., Köhler, V. & Pfaltz, A. Kinetic resolution of diols and pyridyl alcohols by Cu(II)(borabox)-catalyzed acylation. *Org. Lett.* **8**, 1879-1882 (2006).

49. Allen, C. L. & Miller, S. J. Chiral copper(II) complex-catalyzed reactions of partially protected carbohydrates. *Org. Lett.* **15**, 6178-6181 (2013).

50. Mihovilovic, M. D. Enzyme mediated Baeyer-Villiger oxidations. *Curr. Org. Chem.* **10**, 1265-1287 (2006).

51. Bucko, M. et al. Baeyer-Villiger oxidations: biotechnological approach. *Appl. Microbiol. Biotechnol.* **100**, 6585-6599 (2016).

52. Fürst, M. J. L. J., Gran-Scheuch, A., Aalbers, F. S. & Fraaije, M. W. Baeyer–Villiger monooxygenases: tunable oxidative biocatalysts. *ACS Catal.* **9**, 11207-11241 (2019).

53. Summers, B. D. et al. *E. coli* cells expressing the Baeyer–Villiger monooxygenase ‘MO14’ (ro03437) from Rhodococcus jostii RHA1 catalyse the gram-scale resolution of a bicyclic ketone in a fermentor. *Org. Biomol. Chem.* **13**, 1897-1903 (2015).
54. Brzostowicz, P. C., Walters, D. M., Thomas, S. M., Nagarajan, V. & Rouvière, P. E. mRNA differential display in a microbial enrichment culture: simultaneous identification of three cyclohexanone monooxygenases from three species. *Appl. Environ. Microbiol.* **69**, 334-342 (2003).

55. Brzostowicz, P. C., Gibson, K. L., Thomas, S. M., Blasko, M. S. & Rouvière, P. E. Simultaneous identification of two cyclohexanone oxidation genes from an environmental brevibacterium isolate using mRNA differential display. *J. Bacteriol.* **182**, 4241-4248 (2000).

56. González-González, C. A. et al. Corey lactone as key precursor for a facile synthesis of novel 1,2,3-triazole carbocyclic nucleosides via click chemistry. *Tetrahedron Lett.* **54**, 2726-2728 (2013).

57. González-Calderón, D. et al. Antifungal activity of 1’-homo-N-1,2,3-triazol-bicyclic carbonucleosides: a novel type of compound afforded by azide-enolate (3+2) cycloaddition. *Bioorganic Chem.* **69**, 1-6 (2016).

58. Jackson, M. et al. Preparation of lubiprostone. U.S. Patent 2013/0184476 A1 (2013).

59. De Mico, A., Margarita, R., Parlanti, L., Vescovi, A. & Piancatelli, G. A versatile and highly selective hypervalent iodine (III)/2,2,6,6-tetramethyl-1-piperidinyloxyl-mediated oxidation of alcohols to carbonyl compounds. *J. Org. Chem.* **62**, 6974-6977 (1997).

60. Corey, E. J., Albonico, S. M., Koelliker, U., Schaaf, T. K. & Varma, R. K. New reagents for stereoselective carbonyl reduction. improved synthetic route to the primary prostaglandins. *J. Am. Chem. Soc.* **93**, 1491-1493 (1971).
61. Corey, E. J., Varma, R. K. & Becker, K. B. Efficient generation of the 15$S$ configuration in prostaglandin synthesis. attractive interactions in stereochemical control of carbonyl reduction. *J. Am. Chem. Soc.* **94**, 8616-8618 (1972).

62. Lv, J., Ge, J., Luo, T. & Dong, H. An inexpensive catalyst, Fe(acac)$_3$, for regio/site-selective acylation of diols and carbohydrates containing a 1,2-cis-diol. *Green Chem.* **20**, 1987-1991 (2018).

63. Ohta, C. et al. An improved synthesis of the selective EP4 receptor agonist ONO-4819. *J. Org. Chem.* **74**, 8298-8308 (2009).

64. De Luca, L., Giacomelli, G. & Porcheddu, A. A very mild and chemoselective oxidation of alcohols to carbonyl compounds. *Org. Lett.* **3**, 3041-3043 (2001).

65. Chambournier, G., Kornilov, A., Mahmoud, H. M., Vesely, I. & Barrett, S. D. Process for the preparation of F-series prostaglandin. U.S. Patent 2012/0283451 A1 (2012).

66. Tolstikov, G. A. et al. Direct oxidation of alkyl trimethyl- and triethylsilyl ethers to carbonyl compounds. Application to synthesis of prostanoids. *Synthesis*, 940-942 (1989).

67. Gutman, A. et al. Process for the preparation of prostaglandin derivatives. U.S. Patent 2005/0209337 A1 (2005).

68. Gutman, A. et al. Bimatoprost Crystalline Form I. U.S. Patent 2009/0163596 A1 (2009).

69. Contentea, M. L. et al. A new chemoenzymatic approach to the synthesis of latanoprost and bimatoprost. *J. Mol. Catal. B-Enzym.* **114**, 7-12 (2015).

70. Ma, S. K. et al. A green-by-design biocatalytic process for atorvastatin intermediate. *Green Chem.* **12**, 81-86 (2010).
71. Gong, X. et al. Development of an engineered ketoreductase with simultaneously improved thermostability and activity for making a bulky atorvastatin precursor. *ACS Catal.* **9**, 147-153 (2019).

72. Su, B. et al. Redesign of a short-chain dehydrogenase/reductase for asymmetric synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutanoate based on per-residue free energy decomposition and sequence conservatism analysis. *Green Synth. Catal.* **1**, 150-159 (2020).

73. Liu, Y., Tang, T., Pei, X., Zhang, C. & Wu, Z. Identification of ketone reductase ChKRED20 from the genome of chryseobacterium sp. CA49 for highly efficient anti-prelog reduction of 3,5-bis(trifluoromethyl)acetophenone. *J. Mol. Catal. B-Enzym.* **102**, 1-8 (2014).

74. Zhao, F. et al. Crystal structure and iterative saturation mutagenesis of ChKRED20 for expanded catalytic scope. *Appl. Microbiol. Biotechnol.* **101**, 8395-8404 (2017).

75. Qu, G., Li, A., Acevedo-Rocha, C. G., Sun, Z. & Reetz, M. T. The crucial role of methodology development in directed evolution of selective enzymes. *Angew. Chem. Int. Ed.* **59**, 13204-13231 (2020).

76. Chen, Y., Yan, H., Chen, H., Weng, J. & Lu, G. An improved and efficient process for the preparation of (+)-cloprostenol. *Chirality* **27**, 392-396 (2015).

**Acknowledgements**

Financial supports from the National Natural Science Foundation of China (no. 22071033 and 21801047) and Shanghai Sailing Program (18YF1402100) are greatly appreciated.

**Author contributions**
Z.H. and F.C. conceived and directed the project and wrote the paper with assistance from K.Z. K.Z., M.J., B.Y., G.Z., W.L., P.T. performed the experiments and analyzed the data. All authors discussed the results and commented on the paper.

**Additional information**

Supplementary information, enzyme information, and chemical compound information are available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to Z.H or to F.C.

**Competing interests**

The authors declare no competing interests.
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