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A new chemoenzymatic approach to the synthesis of Latanoprost and Bimatoprost

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ABSTRACT

Bimatoprost (**1**) and Latanoprost (**2**) are prostaglandin analogues widely used for glaucoma treatment. We have developed a new chemoenzymatic synthesis for **1** and **2**, which utilizes a highly stereoselective sequence of biotransformations catalyzed by enzymes belonging to a single microorganism (the yeast *Pichia anomala*). The original synthesis, starting from (−)-Corey lactone benzoate (3a*R*,4*R*,5*R*,6a*S*)-**3**, was modified by replacing three synthetic steps (C=C reduction, stereoselective C=O reduction and hydrolysis/deprotection of the benzoate ester) with a one-pot, three-enzymes reaction. The overall biotransformation gave good yields and it was highly stereoselective; noteworthy, by engineering the reaction medium, C=C reduction could be modulated so that unsaturated (3a*R*,4*R*,5*R*,6a*S*,3′*S*)-**6** or saturated intermediate (3a*R*,4*R*,5*R*,6a*S*,3′*R*)-**7** could be preferentially obtained.

1. Introduction

Bimatoprost (**1**) and Latanoprost (**2**) (Scheme 1) are prostaglandin analogues used for controlling the progression of glaucoma by reducing intraocular pressure and have become billion-dollar drugs [1,2]. The industrial manufacture of Bimatoprost and Latanoprost is mostly based on variants of the original strategy developed by Corey [3–9], although different synthetic strategies have been proposed, including a shorter stereocontrolled organocatalytic synthetic procedure recently reported [10]. In the conventional route, the key ketoprostaglandin intermediate (3a*R*,4*R*,5*R*,6a*S*)-**4** is obtained by Horner-Wadsworth–Emmons (HWE) condensation of (−)-Corey lactone benzoate (3a*R*,4*R*,5*R*,6a*S*)-**3** with the suited ketophosphonate. Key intermediate (3a*R*,4*R*,5*R*,6a*S*)-**4** is then reduced by chemoselective hydrogenation (i.e. lithium selectride at low temperature) to give the unsaturated secondary alcohol (3a*R*,4*R*,5*R*,6a*S*,3′*S*)-**5** that can be used for the synthesis of Bimatoprost after hydrolysis to (3a*R*,4*R*,5*R*,6a*S*,3′*S*)-**6** (also known as **Lactondiol B**); alternatively, (3a*R*,4*R*,5*R*,6a*S*,3′*S*)-**5** can be reduced at the double bond with Pd/C catalytic hydrogenation and hydrolyzed at the ester moiety to furnish (3a*R*,4*R*,5*R*,6a*S*,3′*R*)-**7** (also known as **Lactondiol L**), the actual intermediate for Latanoprost synthesis (Scheme 1).

The transformations occurring in the overall conversion of (3a*R*,4*R*,5*R*,6a*S*)-**4** into (3a*R*,4*R*,5*R*,6a*S*,3′*S*)-**6** (C=O reduction and ester hydrolysis) or into (3a*R*,4*R*,5*R*,6a*S*,3′*R*)-**7** (C=O and C=C reduction, ester hydrolysis) can be also enzymatically catalyzed (Scheme 1, in blue colour); more specifically, enoate reductases catalyze the reduction of C=C conjugated to an electron withdrawing group (such as C=O groups) [11], dehydrogenases (carbonyl reductases) catalyze the reduction of C=O groups [12] and esterases catalyze the hydrolysis of esters [13]. Microbial reduction of (3a*R*,4*R*,5*R*,6a*S*)-**4** into the corresponding alcohol (3a*R*,4*R*,5*R*,6a*S*,3′*S*)-**5** has been already reported using different yeasts, with *Kloeckera jensenii* ATCC 20110 giving the best yields [14].

In this work, we have studied the possibility to set up a one-pot biocatalytic method for the stereocontrolled transformation of (3a*R*,4*R*,5*R*,6a*S*)-**4** directly into (3a*R*,4*R*,5*R*,6a*S*,3′*S*)-**6** (Lactondiol B) or (3a*R*,4*R*,5*R*,6a*S*,3′*R*)-**7** (Lactondiol L) by using different yeasts.

2. Experimental

2.1. General experimental methods

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Merck Silica Gel 60 F_{254} plates were used for analytical TLC; ^{1}H and ^{13}C

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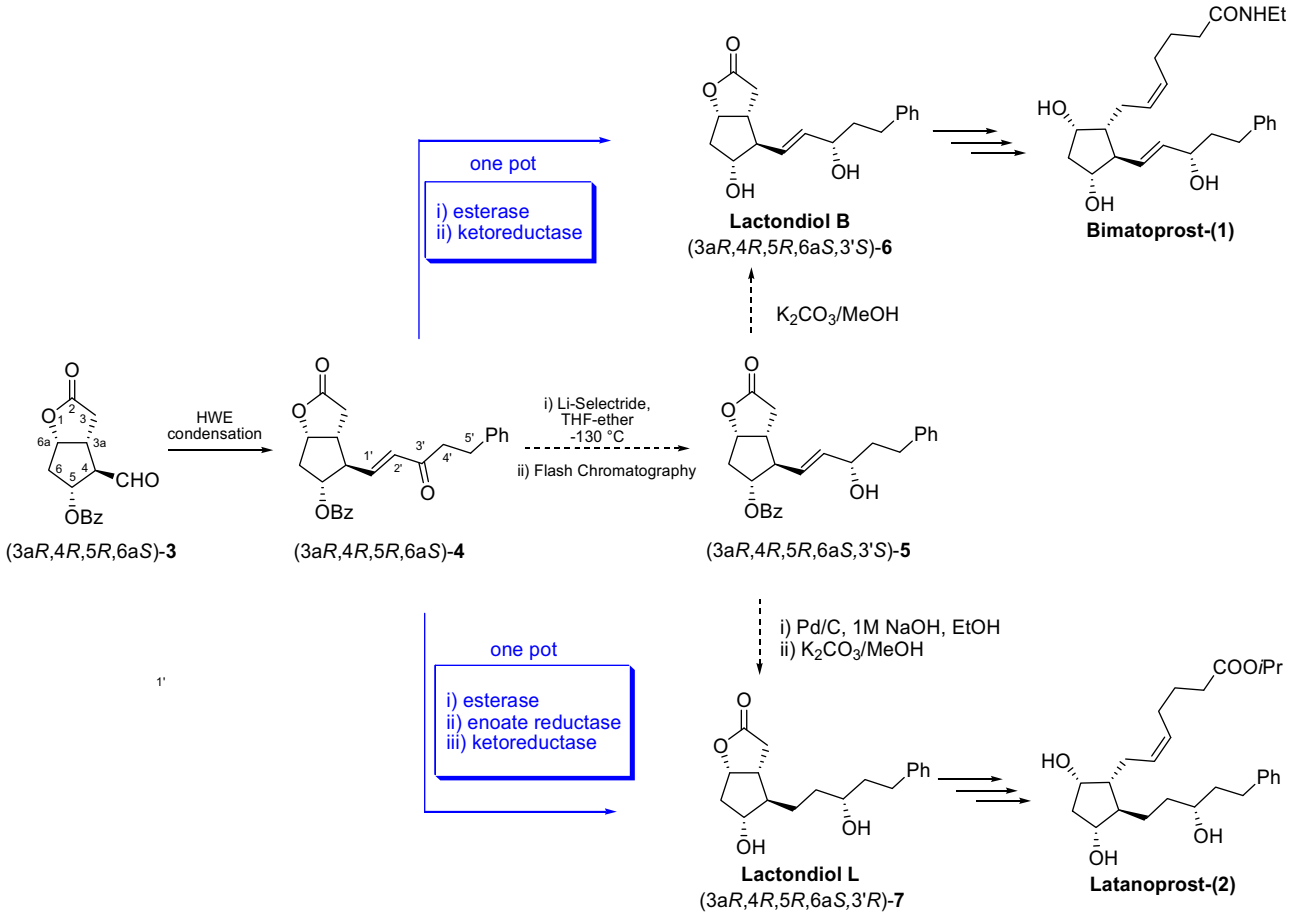

Scheme 1. Synthetic routes to Bimatoprost and Latanoprost.

NMR spectra were recorded on a Varian-Gemini 200 spectrometer. Flash column chromatography was performed on Merck Silica Gel (200–400 mesh). ^{1}H and ^{13}C chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hertz (Hz). MS analyses were performed on a Thermo-Finnigan LCQ ADVANTAGE mass spectrometer equipped with an electron spray ionization (ESI) source. Microanalyses (C, H, N) of new compounds were within 0.4% of theoretical values.

2.2. General procedures for microbial screening of transformation of **4**

Eighteen yeasts were used in the primary screening for LB enone **4** modification; the strains were chosen among 12 yeasts known for esterase and/or ketoreductase activity (*Candida boidini* CBS6056, *Kluyveromyces lactis* CBS2359, *Kluyveromyces marxianus* CBS1553, *K. marxianus* var. *lactis* CL69 *Pichia anomala* CBS110, *Pichia etchellsii* MIM, *Pichia glucozyma* CBS 5766, *S. cerevisiae* CBS1782, *S. cerevisiae* CBS3093, *S. cerevisiae* CBS3081, *S. cerevisiae* NCYC 73, *S. cerevisiae* Zeus)[11] and 6 strains with ketoreductase and/or enoate reductases activity (*S. cerevisiae* BY4741, *S. cerevisiae* BY4741ΔOye1, *S. cerevisiae* BY4741ΔOye2, *S. cerevisiae* BY4741ΔOye2Cc, *S. cerevisiae* BY4741ΔOye2Ks, *S. cerevisiae* L12) [16,17,21].

Strains from official collections or from our collection (Microbiologia Industriale Milano) were routinely maintained on M5YE slants (Barley malt flour 100 g/L (Diagermal), 5 g/L yeast extract (Difco), agar 15 g/L, pH 5.6). To obtain cells for biotransformations, the microorganisms were cultured in 2 L Erlenmeyer flasks containing 300 mL of M5YE liquid medium (Barley malt flour 100 g/L (Diagermal), 5 g/L yeast extract (Difco), distilled water pH 5.6), incubated for 48 h at 28 °C on a reciprocal shaker (150 spm). Fresh cells from submerged cultures were centrifuged (5000 rpm, 20 min) and washed with 0.1 M phosphate buffer, pH 7.0, prior to use.

Biotransformations were carried out in 10 mL screw-capped test tubes with a reaction volume of 3 mL with cells (20 g/L, dry weight) suspended in 0.1 M phosphate buffer, pH 7, containing 5% of glucose and 1 g/L of **4**, at 28 °C under magnetic stirring (500 rpm).

2.3. Analyticals

Biotransformations were monitored by HPLC. Samples (0.5 mL) were taken at regular intervals, centrifuged and the aqueous phase was extracted with an equal volume of ethyl acetate; substrate and product concentrations were determined by HPLC using a Purospher Star RP18e250*4.6 mm (5 μm) (Merck, Darmstadt, Germany), UV detection at 220 nm with a Merck-Hitachi 655-22 detector and CH_3CN/H_2O/MeOH (18/72/10) as eluent with a flow rate of 0.8 mL/min.

The retention time of substrates and products was: **4** = 29.0 min; **5** = 37.8 min; **6** = 58.2 min; **7/11** = 61.6 min; **8** = 22.7 min; **9** = 69.8 min; **10** = 76.7 min.

The stereochemical composition of **6** was determined by HPLC using a Pinnacle II silica 250*4.6 mm (4 μm, Restek, Bellefonte, PA, US), UV detection at 220 nm with a Merck-Hitachi 655-22 detector and *n*-heptane/iPrOH (90/10) as eluent with a flow rate of 1.5 mL/min.

Separation of isomers **7** and **11** was performed by HPLC using a Phenomenex Lux Cellulose-1 column 250*4.6 mm (5 μm) (Phenomenex, Torrance, CA, US), UV detection at 220 nm with a Merck-Hitachi 655-22 detector and cyclohexane/*i*PrOH (85/15) as