A new chemoenzymatic approach to the synthesis of Latanoprost and Bimatoprost

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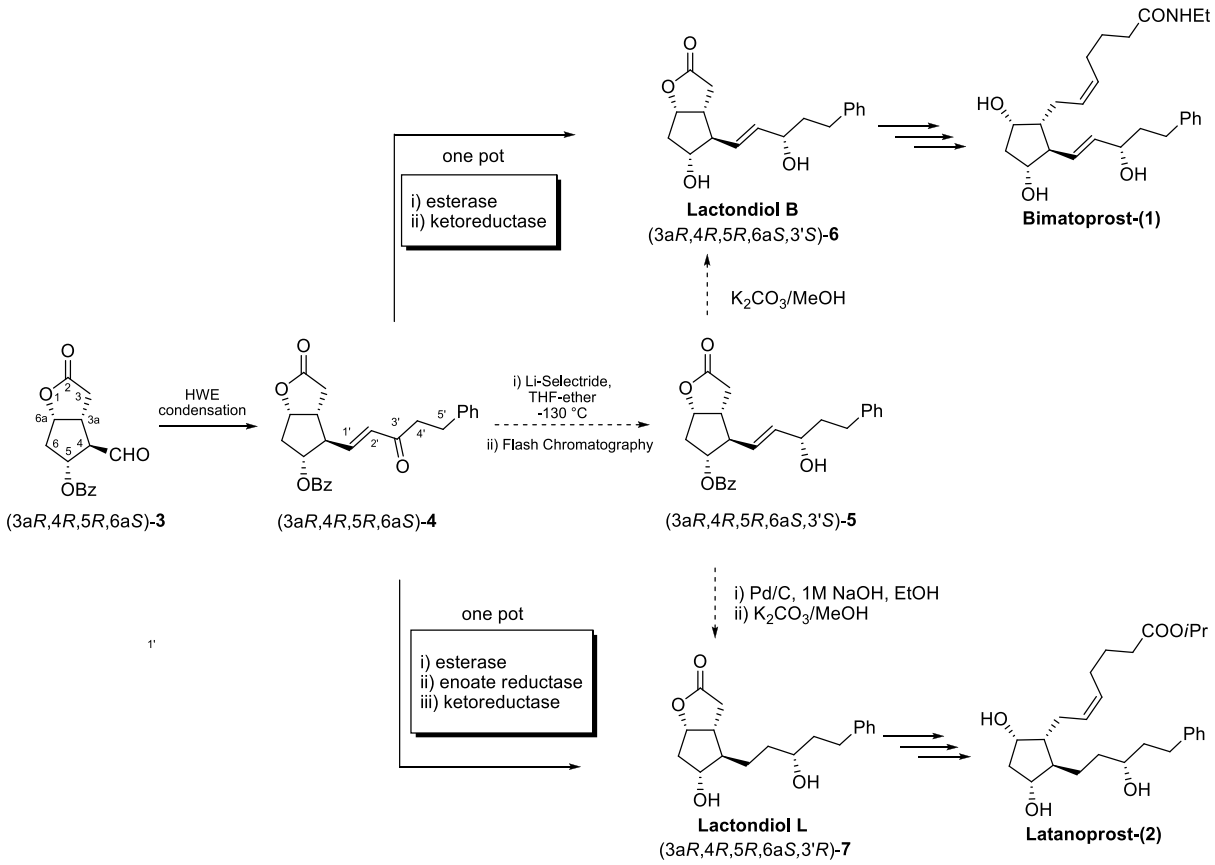
ABSTRACT

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.

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 give (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7** (also known as **Lactondiol L**), the actual intermediate for Latanoprost synthesis (Scheme 1).



Scheme 1. Synthetic routes to Bimatoprost and Latanoprost.

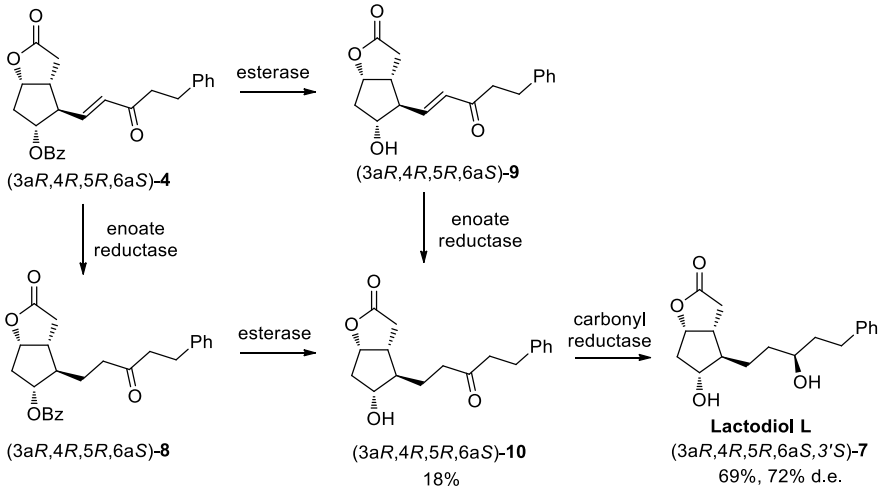
The transformations occurring in the overall conversion of (3a*R*,4*R*,5*R*,6a*S*)-**4** into (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** (C=O reduction and ester hydrolysis) or into (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7** (C=O and C=C reduction, ester hydrolysis) can be also enzymatically catalyzed (Scheme 1); 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.

RESULTS AND DISCUSSIONS

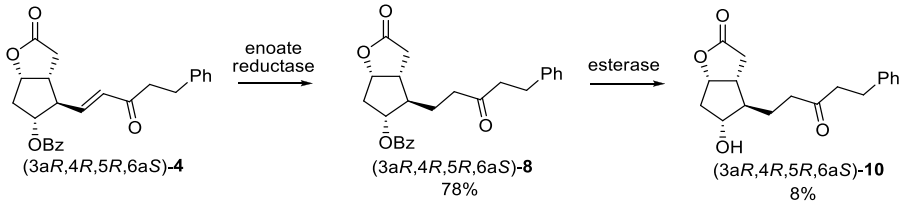
A screening for the identification of yeasts (see materials and methods for the complete list) able to transform (3a*R*,4*R*,5*R*,6a*S*)-**4** into (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** and/or (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7** was carried out using resting cells suspended in phosphate buffer in the presence of 5% glucose and neat (3a*R*,4*R*,5*R*,6a*S*)-**4** (1g/L). Two wild-type strains (*Pichia anomala* CBS110[15,16] and *Pichia glucozyma* CBS 5766[17-20]) and the recombinant strain *Saccharomyces cerevisiae* BY4741ΔOye2Ks (which has the original OYE2 deleted and bears the highly expressed enoatereductase gene from *Kazachstania spencerorum*)[21] gave molar conversion of (3a*R*,4*R*,5*R*,6a*S*)-**4** above 30%.

Pichia glucozyma CBS 5766 gave ketone (3a*R*,4*R*,5*R*,6a*S*)-**10** (18% yield) and Lactondiol L (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**7** (69% yield, 72% d.e.) as final products. During the reaction, traces of the transient intermediates (3a*R*,4*R*,5*R*,6a*S*)-**8** and (3a*R*,4*R*,5*R*,6a*S*)-**9** were observed, indicating that the overall transformation likely occurred as reported in Scheme 2. The reduction of the carbonyl was partially enantioselective, furnishing (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**7** with 72% d.e.



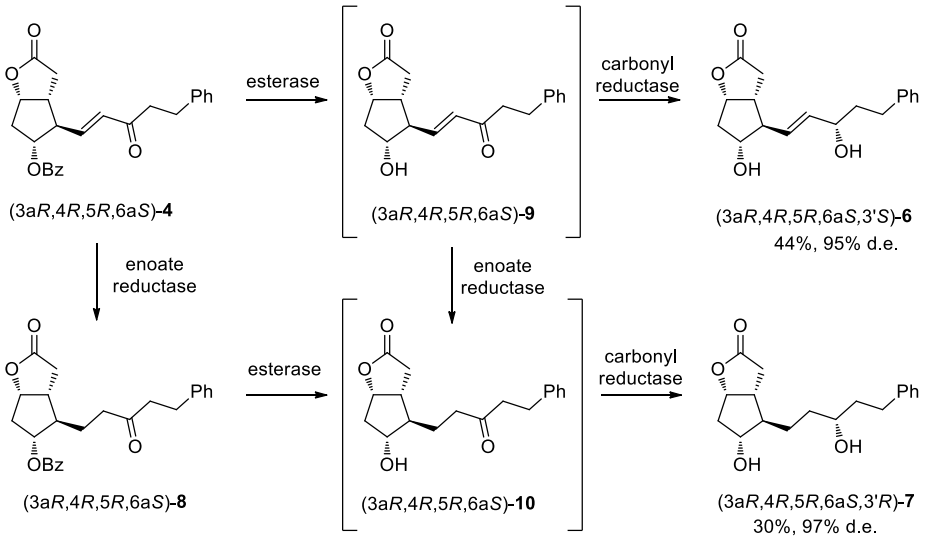
Scheme 2. Biotransformation of **4** with *Pichia glucozyma*.

Saccharomyces cerevisiae BY4741ΔOye2Ks showed, as expected, high enoate reductase activity giving the saturated ketone (3a*R*,4*R*,5*R*,6a*S*)-**8** as the main product (78%) and minor amounts of **10**, deriving from subsequent ester hydrolysis (Scheme 3).



Scheme 3. Biotransformation of **4** with *Saccharomyces cerevisiae* BY4741ΔOye2Ks.

Pichia anomala CBS 110 gave a mixture of Lactondiol B (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** (44% yield, 95% d.e.) and Lactondiol L (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7** (30% yield, 97% d.e.), the useful epimers for Bimatoprost and Latanoprost synthesis, respectively. Scheme 4 shows the biotransformations involved in the conversion of (3a*R*,4*R*,5*R*,6a*S*)-**4** with *P. anomala*, based on the different intermediates observed during the overall transformation.



Scheme 4. Biotransformation of **4** with *Pichia anomala*

It is known that the use of whole cells of yeasts generally gives a mixture of saturated and unsaturated alcohols in the reduction of α,β-unsaturated carbonyls, depending on the strain and conditions employed;[22] this is due to the relative rates of enoatereductase and carbonyl reductase activities, which determine the final ratio of the products. A crucial role in the activity of the different reductases can be played by the type of co-substrates added for favouring the regeneration of the co-factors involved in the reductions. Thus, the biotransformation with *P. anomala* was carried out using various conventional co-substrates (glucose, ethanol, glycerol, xylose) showing the predominant formation of Lactondiol B (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** with d.e. in the range of 95-97%; the use of glucose or glycerol allowed for the highest conversion (44-62%) and diastereoselectivity (d.e. 95-97%). The use of fumaric acid gave higher amounts of Lactondiol L (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7**; fumaric acid is an unconventional co-substrate for cofactor regeneration and it is assumed that the yeast like *Pichia anomala* may oxidize fumaric acid in Krebs cycle, therefore allowing for regeneration of NAD(P)H. The amount of fumaric acid was varied to check its influence on the overall conversion of (3a*R*,4*R*,5*R*,6a*S*)-**4** by whole cells of *Pichia anomala* and it was observed that,

when a 5/1 molar ratio of fumaric acid/substrate was employed, (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7** was produced in high yields (82%) with only traces of (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** in the final reaction mixture after 7 days. Table 1 summarizes the results obtained using different co-substrates.

			6		**7**	
entry	**co-substrate**	**concentration of co-substrate g/L**	**yield (%)**	**d.e. (%) ***	**yield (%)**	**d.e. (%)***
1	Ethanol	5	5	n.d.	12	92
2	Ethanol	50	10	97	20	92
3	Fumaric acid	5	8	n.d.	75	97
4	Fumaric acid	50	13	97	70	97
5	Glucose	5	38	95	25	97
6	Glucose	50	44	95	30	97
7	Glycerol	5	57	97	29	97
8	Glycerol	50	62	97	30	97
9	Xylose	5	-	n.d.	21	96
10	Xylose	50	-	n.d.	33	95

Table 1

*determined by HPLC analysis

The biotransformation of (3a*R*,4*R*,5*R*,6a*S*)-**4** catalyzed by *Pichia anomala* was optimized using the Multimplex® experimental design, which simultaneously evaluates different parameters of the biotransformation and had previously shown to be suited for the fast optimization of different biotransformations.[23,24] The response parameters were product yields and d.e., while the control variables were temperature, pH, concentration of cells, concentration of DMSO and co-substrate. The best performance for the preparation of (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7** (82% yield, 97% d.e., 7 days) was obtained using 0.1 mM phosphate buffer at pH 7.0, 28 °C, 1 g/L substrate concentration in the presence of 1.5 g/L fumaric acid, while the best conditions for the preparation of (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** (62% yield, 97% d.e., 7 days) were found using 0.1 mM phosphate buffer at pH 7.2, 28 °C, 1 g/L substrate concentration in the presence of 50 g/L glycerol. When the

biotransformation carried out in the presence of glycerol was stopped after 3 days, a significant amount (34%) of the intermediate (3a*R*,4*R*,5*R*,6a*S*)-**9** could be recovered.

CONCLUSIONS

In conclusion, here we propose an alternative chemoenzymatic approach for the synthesis of Bimatoprost and Latanoprost, based on the biotransformation of 15-ketoprostaglandin (3a*R*,4*R*,5*R*,6a*S*)-**4** with whole cells of non-conventional yeast *Pichia anomala*. This appears like a useful method for preparing optically pure Lactondiol B (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** (62%) and Lactondiol L (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7**. The use of *Pichia anomala* allows to obtain Lactondiol B (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** or Lactondiol L (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7**, depending on the co-substrate employed: Lactondiol B (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** was obtained with 44-62% yield using glucose or glycerol, whereas the use of fumaric acid gave 82% yield of Lactondiol L (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**8** in a simple one-pot, three-step modification of substrate (3a*R*,4*R*,5*R*,6a*S*)-**4**. The stereoselectivity of the carbonyl reduction depends on the yeast employed, as shown in the case of *Pichia glucozyma*, which furnishes Lactondiol L (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7** as the main epimer.

Experimental Section

General experimental information

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 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 Varian 320-MS triple quadrupole mass spectrometer equipped with an electron spray ionization (ESI) source.

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, *Kluyveromyces marxianus* var. *lactis* CL69 *Pichia anomala* CBS110, *Pichia etchellsii* MIM, *Pichia glucozyma* CBS 5766, *Saccharomyces cerevisiae* CBS1782, *Saccharomyces cerevisiae* CBS3093, *Saccharomyces cerevisiae* CBS3081, *Saccharomyces cerevisiae* NCYC 73, *Saccharomyces*

cerevisiae Zeus)[11] and 6 strains with ketoreductase and/or enoate reductases activity (*Saccharomyces cerevisiae* BY4741, *Saccharomyces cerevisiae* BY4741ΔOye1, *Saccharomyces cerevisiae* BY4741ΔOye2, *Saccharomyces cerevisiae* BY4741ΔOye2Cc, *Saccharomyces cerevisiae* BY4741ΔOye2Ks, *Saccharomyces 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 (150spm). 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 (3a*R*,4*R*,5*R*,6a*S*)-**4**, at 28 °C under magnetic stirring (500 rpm).

Analyticals

The 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 times of substrates and products were: (3a*R*,4*R*,5*R*,6a*S*)-**4** = 29.0 min; (3a*R*,4*R*,5*R*,6a*S*)-**5** = 37.8 min; (3a*R*,4*R*,5*R*,6a*S*)-**6** = 58.2 min; (3a*R*,4*R*,5*R*,6a*S*)-**7** = 61.6 min; (3a*R*,4*R*,5*R*,6a*S*)-**8** = 22.7 min; (3a*R*,4*R*,5*R*,6a*S*)-**9** = 69.8 min; (3a*R*,4*R*,5*R*,6a*S*)-**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, Pennsylvania, U.S.), 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. The retention times of the two epimers were: (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** = 21.7 min; (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**6** = 25.9 min.

The stereochemical composition of **7** was determined by HPLC using a Phenomenex Lux Cellulose-1 column 250*4.6 mm (5 μm) (Phenomenex, Torrance, California, U.S), UV detection at 220 nm with a Merck-Hitachi 655-22 detector and cyclohexane/*i*PrOH (85/15) as eluent with a flow

rate of 1.0 mL/min. The retention times of the two epimers were: (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7** = 16.4 min; (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**7** = 26.6 min.

Diastereoisomers were identified by comparison with authentic reference materials chemically synthesized.[6]

Optimization studies

Optimization studies were carried out with *Pichia anomala* CBS 110. The desired amount of cells was suspended in different 0.1 M phosphate buffers containing co-substrates and neat substrate was added to reach the desired concentration; the suspensions obtained were shaken (reciprocal shaker, 150spm) at different temperatures. Samples of 100 μL were withdrawn from the reaction mixture, centrifuged, extracted with EtOAc (100 μL) and analysed by HPLC.

[(3a*R*,4*R*,5*R*,6a*S*)-5-hydroxy-4-((*S*,*E*)-3-hydroxy-5-phenylpent-1-en-1-yl)hexahydro-2*H*-cyclopenta[*b*]furan-2-one] (Lactondiol B, (3*S*)-6)

Pichia anomala CBS 110 was grown in a 3.0 L fermenter with 1.5 L of M5YE liquid medium for 48 h, 28 °C and agitation speed 150 rpm. Fresh cells from submerged cultures were centrifuged and washed with 0.1 M phosphate buffer, pH 7.0, resuspended in 0.5 L of phosphate buffer to reach 25 $g_{dry\ cells}$/L concentration of cells and used for biotransformations. Reactions were carried out in 2 L Erlenmeyer flask by adding glycerol (25 g) and (3a*R*,4*R*,5*R*,6a*S*)-**4** (500 mg) dissolved in 1% of DMSO and keeping the reaction mixture under reciprocal shaking (150 spm) at 28 °C. After 7 days, the biotransformation mixture was centrifuged, and the aqueous supernatant was extracted twice with EtOAc (350 mL); the organic extract was dried over Na_2SO_4 and the solvent was evaporated to give a crude mixture and the single products were purified by flash chromatography (gradient 7:3; 5:5; 2:8 *n*-hexane/EtOAc), furnishing (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**6** (232 mg, 62%).

Colourless oil. R_f= 0.085 (*n*-hexane/EtOAc 4:6); $[\alpha]^{24}_D$= –5.2 (c = 1.00 CH_3CN);

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.78-1.98 (m, 2H), 2.23-2.36 (m, 1H), 2.44-2.78 (m, 7H), 3.91 (q, *J* = 7.70, 1H, $CH_2CHOHCH_2$), 4.07 (q, *J* = 6.97, 1H, C*H*OH), 4.83-4.92 (m, 1 H, C*H*OC=O), 5.44 (dd, J^1= 8.06, J^2 = 15.40, 1H, C_{sp2}-H), 5.62 (dd, J^1= 6.60, J^2= 15.40, 1H, C_{sp2}-H), 7.16-7.29 (m, 5H, C_{sp2}-H).

^{13}C-NMR ($CDCl_3$, 50.4 MHz): δ 177.06 (C=O), 141.79 (C_{sp2}quat.), 136.65, 130.51 (C_{sp2}-H), 128.68, 128.63, 126.20 (C_{sp2}-H), 82.69 (O*C*HCH_2), 76.74 (*C*HOH), 72.17 (CH_2*C*HOHCH_2), 56.39 (CH_2CH*C*HCHOH), 42.69 (CH_2*C*HCHCHOH), 40.05, 38.89, 34.38, 31.95 (CH_2).

MS (ESI + m/z %): 325.3 [M^++Na^+] (100).

[(3a*R*,4*R*,5*R*,6a*S*)-5-hydroxy-4-((*R*)-3-hydroxy-5-phenylpentyl)hexahydro-2*H*-cyclopenta[*b*]furan-2-one)] (Lactondiol L, (3*R*)-7)

Pichia anomala CBS 110 was grown in a 3.0 L fermenter with 1.5 L of M5YE liquid medium for 48 h, 28 °C and agitation speed 150 rpm. Fresh cells from submerged cultures were centrifuged and washed with 0.1 M phosphate buffer, pH 7.0, resuspended in 0.5 L of phosphate buffer to reach 25 $g_{dry\ cells}$/L of cells and used for biotransformations. Reactions were carried out in 2 L Erlenmeyer flask by adding fumaric acid (719 mg) and (3a*R*,4*R*,5*R*,6a*S*)-**4** (500 mg) dissolved in 1% of DMSO and keeping the reaction mixture under reciprocal shaking (150 spm) at 28 °C. After 7 days, the biotransformation mixture was centrifuged, and the aqueous supernatant was extracted twice with EtOAc (350 mL); the organic extract was dried over Na_2SO_4 and the solvent evaporated to give a crude mixture and the products were purified by flash chromatography (gradient 7:3; 5:5; 2:8 *n*-hexane/EtOAc), furnishing (3a*R*,4*R*,5*R*,6a*S*,3'*R*)-**7** (308 mg, 82%).

Colourless oil. R_f= 0.087 (*n*-hexane/EtOAc 4:6); $[\alpha]^{24}_D$= –25.0 (c = 2.00 CH_3CN);

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.22-1.28 (m, 1H), 1.46-1.59 (m, 3H), 1.73-1.81 (m, 3H), 2.21-2.28 (m, 1H), 2.43-2.50 (m, 2H), 2.64-2.80 (m, 2H), 3.60-3.66 (m, 1H, $CH_2CHOHCH_2$), 3.97 (q, *J* = 5.1 1H, C*H*OH), 4.88-4.96 (m, 1 H, C*H*OC=O), 7.16-7.27 (m, 5H, C_{sp2}-H).

^{13}C-NMR($CDCl_3$, 50.4 MHz): δ 177.95 (C=O), 142.13 (C_{sp2}quat.), 128.68, 128.62, 126.15 (CH_{sp2}), 84.20 (O*C*HCH_2), 77.65 (*C*HOH), 71.52 (CH_2*C*HOHCH_2), 54.16 (CH_2CH*C*HCHOH), 43.43 (CH_2*C*HCHCHOH), 40.69, 39.31, 36.22, 35.43, 32.26, 29.18 (CH_2).

MS (ESI + m/z %): 327.3 [M^++Na^+] (100).

[(3a*R*,4*R*,5*R*,6a*S*)-5-hydroxy-4-((*S*)-3-hydroxy-5-phenylpentyl)hexahydro-2*H*-cyclopenta[*b*]furan-2-one] (Lactondiol L, (3*S*)-7)

Pichia glucozyma CBS 5766 was grown in a 3.0 L fermenter with 1.5 L of M5YE liquid medium for 48 h, 28°C and agitation speed 150 rpm. Fresh cells from submerged cultures were centrifuged and washed with 0.1 M phosphate buffer, pH 7.0, resuspended in 0.5 L of phosphate buffer to reach 25 $g_{dry\ cells}$/L concentration of cells and used for biotransformations. Reactions were carried out in 2 L Erlenmeyer flask by adding glycerol (25 g) and (3a*R*,4*R*,5*R*,6a*S*)-**4** (500 mg) dissolved in 1% of DMSO and keeping the reaction mixture under reciprocal shaking (150 spm) at 28°C. After 7 days, the biotransformation mixture was centrifuged, and the aqueous supernatant was extracted twice with EtOAc (350 mL); the organic extract was dried over Na_2SO_4 and the solvent was evaporated to give a crude mixture and the single products were purified by flash chromatography (gradient 7:3; 1:1; 2:8 *n*-hexane/EtOAc), furnishing (3a*R*,4*R*,5*R*,6a*S*,3'*S*)-**7** (260 mg, 69%).

Colourless oil. R_f= 0.087 (*n*-hexane/EtOAc 4:6); $[\alpha]^{24}_D$= + 18.9 (c = 2.00 CH_3CN).

[(3a*R*,4*R*,5R,6a*S*)-5-hydroxy-4-((*E*)-3-oxo-5-phenylpent-1-en-1-yl)hexahydro-2*H*-cyclopenta[b]furan-2-one] (9).

Compound (3a*R*,4*R*,5*R*,6a*S*)-**9** was obtained starting from intermediate (3a*R*,4*R*,5*R*,6a*S*)-**4,** following the same procedure described for the synthesis of compound (3a*R*,4*R*,5*R*,6a*S*3'*R*)-**7** and stopping the reaction after 3 days. The reaction mixture was purified by flash chromatography (gradient 7:3; 1:1; 2:8 *n*-hexane/EtOAc), furnishing (3a*R*,4*R*,5*R*,6a*S*)-**9** (126 mg, 34%).

Brown oil. R_f= 0.28 (*n*-hexane/EtOAc 4:6);

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.50-1.85 (m, 2H), 2.20-2.30 (m, 1H), 2.30-2.50 (m, 3H), 2.58-2.72 (m, 2H), 2.90-3.0 (m, 2H), 4.10 (q, *J* = 6.90 1H, C*H*OH), 4.83-4.92 (m, 1 H, C*H*OC=O), 6,20 (d, *J* = 15.30, 1H,C_{sp2}-H), 6.60 (dd, J^1 = 6.23, J^2 = 15.33, 1H, C_{sp2}-H), 7.15-7.30 (m, 5H, C_{sp2}-H).

[(3a*R*,4*R*,5*R*,6a*S*)-2-oxo-4-(3-oxo-5-phenylpentyl)hexahydro-2*H*-cyclopenta[*b*]furan-5-yl benzoate] (8) and [(3a*R*,4*R*,5*R*,6a*S*)-5-hydroxy-4-(3-oxo-5-phenylpentyl)hexahydro-2*H*-cyclopenta[*b*]furan-2-one] (10).

Saccharomyces cerevisiae BY4741ΔOye2Ks was grown in a 3.0 L fermenter with 1.5 L of M5YE liquid medium for 48 h, 28°C and agitation speed 150 rpm. Fresh cells from submerged cultures were centrifuged and washed with 0.1 M phosphate buffer, pH 7.0, resuspended in 0.5 L of phosphate buffer to reach 20 $g_{dry\ cells}$/L concentration of cells and used for biotransformations. Reactions were carried out in 2 L Erlenmeyer flask by adding glucose (50 g) and (3a*R*,4*R*,5*R*,6a*S*)-**4** (500 mg) dissolved in 1% of DMSO and keeping the reaction mixture under reciprocal shaking (150 spm) at 28°C. After 7 days, the biotransformation mixture was centrifuged, and the aqueous supernatant was extracted twice with EtOAc (350 mL); the organic extract was dried over Na_2SO_4 and the solvent was evaporated to give a crude mixture and the single products were purified by flash chromatography (gradient 7:3; 1:1; 2:8 *n*-hexane/EtOAc), furnishing 392 mg of (3a*R*,4*R*,5*R*,6a*S*)-**8** (392 mg, 78%) and (3a*R*,4*R*,5*R*,6a*S*)-**10** (30 mg, 8%).

(3a*R*,4*R*,5*R*,6a*S*)-**8**: White solid. R_f= 0.55 (*n*-hexane/EtOAc 4:6);

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.58-1.79 (m, 2H), 2.00-2.20 (m, 1H), 2.40-2.60 (m, 3H), 2.62-2.80 (m, 3H), 2.82-2.98 (m, 5H), 5.05-5.12 (m, 1H, C*H*OC=O), 5.14-5.20 (m, 1H, PhOCOC*H*), 7.10-7.32 (m, 5H, C_{sp2}-H), 7.40-7.50 (m, 2H, C_{sp2}-H), 7.52-7.60 (m, 1H, C_{sp2}-H), 8.10 (d, *J* = 6.98, 2H, C_{sp2}-H).

(3a*R*,4*R*,5*R*,6a*S*)-**10**: Colourless oil. R_f= 0.25 (*n*-hexane/EtOAc 4:6);

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 1.40-1.50 (m, 1H), 1.55-1.72 (m, 3H), 1.95-2.10 (m, 2H), 2.30-2.55 (m, 4H), 2.70-2.82 (m, 2H), 2.87-2.98 (m, 2H), 3.82 (q, *J*= 5.6, 1H, C*H*OH), 4.79-4.86 (m, 1H, C*H*OC=O), 7.16-7.30 (m, 5H, C_{sp2}-H).

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