Stereodivergent Preparation of Valuable γ- or δ-Hydroxy Esters and Lactones through One-Pot Cascade or Tandem Chemoenzymatic Protocols

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ABSTRACT. A series of enantiopure hydroxy esters and lactones has been synthesized in a chemodivergent manner *via* alcohol dehydrogenase (ADH) reduction of the corresponding keto esters by means of cascade or tandem protocols. Thus, ADH from *Rhodococcus ruber* (ADH-A) or *Lactobacillus brevis* (LBADH) afforded both antipodes in a very selective way when dealing with small derivatives. With bulkier substrates, ADH from *Ralstonia* sp. (RasADH) was successfully employed to achieve the synthesis of enantioenriched γ- or δ-hydroxy esters. To isolate the corresponding lactones, two different approaches were followed: a cascade reaction by spontaneous cyclization of the hydroxy ester intermediate, or a one-pot two-step tandem protocol. Moreover, a chemoenzymatic route was designed to obtain a chiral brominated lactone

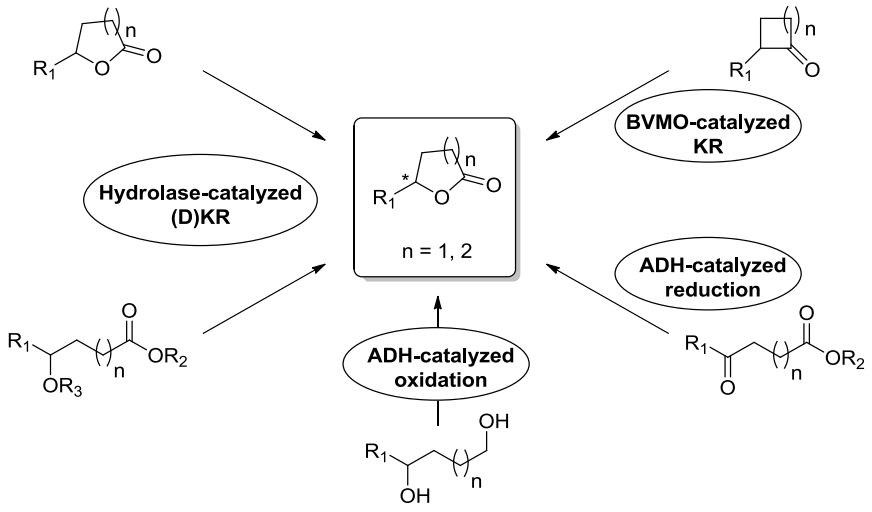
which enabled further modifications in a sequential fashion by Pd-catalyzed reactions affording relevant functionalized lactones.

Introduction

The preparation of γ- and δ-lactones has attracted increasing attention because of their structural implications as basic chemicals but also as valuable building blocks for polymers and natural product synthesis. These compounds display a broad biological profile and are also important flavor and aroma constituents.[1] In particular, γ-valerolactone (GVL) has been identified as a potential intermediate for the production of fuels,[2] while δ-caprolactone is used as monomer to synthesize biodegradable polymers.[3] On the other hand, enantioenriched γ- or δ-hydroxy esters are present in many bioactive molecules such as polyketides, prostaglandins, pheromones and other important compounds.[4]

Owing to their unsurpassed selectivity, biocatalytic approaches (Scheme 1) have rapidly gained ground in the synthesis of these enantioenriched derivatives. Among them, the Baeyer-Villiger monooxygenase (BVMO) oxidation of racemic or prochiral cyclic ketones,[5] the hydrolase-catalyzed kinetic resolutions (KRs) starting from the corresponding racemic lactones[6] or the hydroxy ester precursors,[7] and the enantioselective alcohol dehydrogenase (ADH)-catalyzed oxidation of racemic diols can be mentioned.[8,9] Alternatively, dynamic systems (DKRs) using lipases with a metal-based racemization have also been studied to quantitatively yield the enantiopure products.[10]

Scheme 1. General biocatalytic approaches to achieve γ- or δ-lactones.



Another straightforward route to obtain such enantioenriched derivatives is the direct ADH-catalyzed bioreduction of γ- or δ-keto esters. In this sense, the first protocols were traditionally done employing whole-cell systems,[11] but in general selectivities or substrate concentrations were not satisfactory. Furthermore, in most cases mixtures of the lactone and the corresponding hydroxy ester were detected. Although some of these drawbacks were overcome using isolated enzymes, the hitherto reported methodologies only led to the (*S*)-antipodes in an efficient manner.[12] More recently, Pietruszka and co-workers described the enzymatic synthesis of several γ- or δ-hydroxy esters, and then different lactones were obtained in a multi-step route.[13]

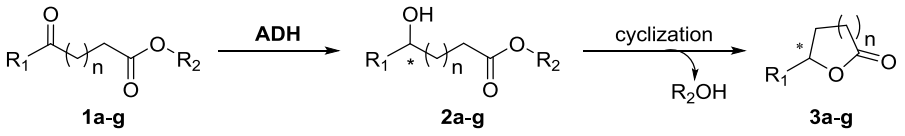
As a part of our ongoing interest in the field of one-pot cascade or sequential transformations,[14] and due to the rise of methodologies that have elegantly combined the use of alcohol dehydrogenases with organo- or metal-catalyst(s),[15] we have considered worthwhile an exploration of the bioreduction of several γ- and δ-keto esters to: (i) find a chemoselective and efficient approach to get access to the lactones or the hydroxy esters; (ii) search for recombinant ADHs able to afford these products in a stereodivergent fashion allowing the synthesis of both antipodes; and (iii) combine a bioreductive cascade with several Pd-based transformations to

expand the scope and potential of these protocols applied to the formation of highly valuable lactones.

Results and Discussion

Thus, we first focused on the bioreduction of several prochiral γ- and δ-keto esters **1a-g** (Scheme 2) studying different biocatalysts and reaction conditions such as pH. In an initial set of experiments, a number of recombinant ADHs were screened for activity towards the bioreduction of methyl 5-oxohexanoate (**1a**, 50 mM) at pH 9.0 and 30ºC. For some of them 2-propanol (2-PrOH) was used as hydrogen donor to recycle the nicotinamide cofactor, while for other glucose and glucose dehydrogenase (GDH) were employed.

Scheme 2. Biosynthesis of enantioenriched γ- and δ-hydroxy esters and lactones using an ADH-based one-pot cascade or tandem strategy.



a, n= 2, R_1= CH_3, R_2= CH_3; **b**, n= 2, R_1= CH_3, R_2= CH_2CH_3
c, n= 1, R_1= CH_3, R_2= CH_3; **d**, n= 1, R_1= CH_3, R_2= CH_2CH_3
e, n= 2, R_1= Ph, R_2= CH_3; **f**, n= 1, R_1= Ph, R_2= CH_3; **g**, n= 1, R_1= *p*-Br-C_6H_4, R_2= CH_3

Although good activities were detected with several alcohol dehydrogenases (see Supporting Information), only ADH from *Rhodococcus ruber* (ADH-A)[16] and ADH from *Lactobacillus brevis* (LBADH)[17] showed excellent stereoselectivities (*ee* >99%). Furthermore, due to the fact that these enzymes display opposite selectivities, the formation of both enantiomers of methyl 5-hydroxyhexanoate (**2a**) and δ-caprolactone (**3a**) was feasible. For these reasons, ADH-A and

LBADH were selected for further investigations. In a subsequent study, the effect of the pH was examined in order to check whether this parameter could shift the equilibrium towards the formation of either of the two possible products. Thus, these bioreductions were performed at pH 5.0, 7.5 and 9.0 (Figure 1). Gratifyingly, neutral pH was found as the best one to form preferentially hydroxy ester **2a** over lactone **3a**, while a higher pH led to noticeable amounts of **3a** with both ADHs (*c* >18%), corresponding to the intramolecular cyclization of the hydroxy ester intermediate in a cascade fashion. A lower pH inhibited the formation of lactone **3a** also at expenses of the enzymatic activity.

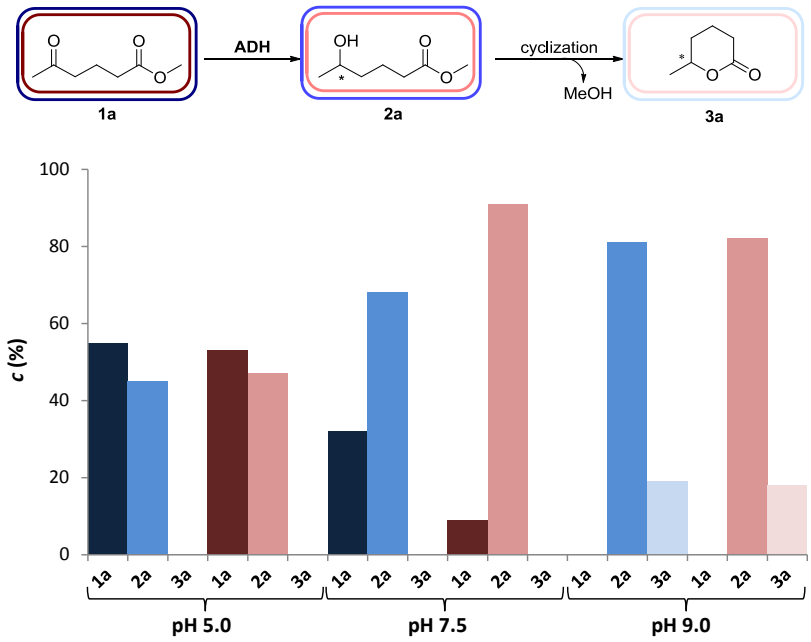


Figure 1. Effect of the pH in the ADH-catalyzed bioreduction of **1a** (50 mM, *t*= 24 h). In blue, results obtained for ADH-A and in red for LBADH. In all cases *ee* of the final products were >99%.

Next, the effect of the chain length (γ- *vs* δ-keto ester) and the ester moiety (Me *vs* Et) were studied with both biocatalysts. Thus, bioreductions were performed over ethyl 5-oxohexanoate

(**1b**), methyl levulinate (**1c**) and ethyl levulinate (**1d**). From these experiments, two trends appeared clear: a) the intramolecular cyclization to afford the desired lactones was highly favored for the 5-membered ring (compare formation of **3a** *vs* **3c** in Figure 2); and b) the cyclization rate was enhanced for methyl ester derivatives (thus producing MeOH), compared to the ethyl esters (where EtOH was formed), since the transformation into lactone **3a** starting from **1a** was faster than from **1b**. The same tendency was observed for γ-valerolactone (**3c**) starting from **1c** or **1d** with both ADHs. Additionally, enantiomeric excess was >99% in all cases, getting access to both antipodes of the corresponding hydroxy ketones **2a** and **2b** or lactones **3a** and **3c**.

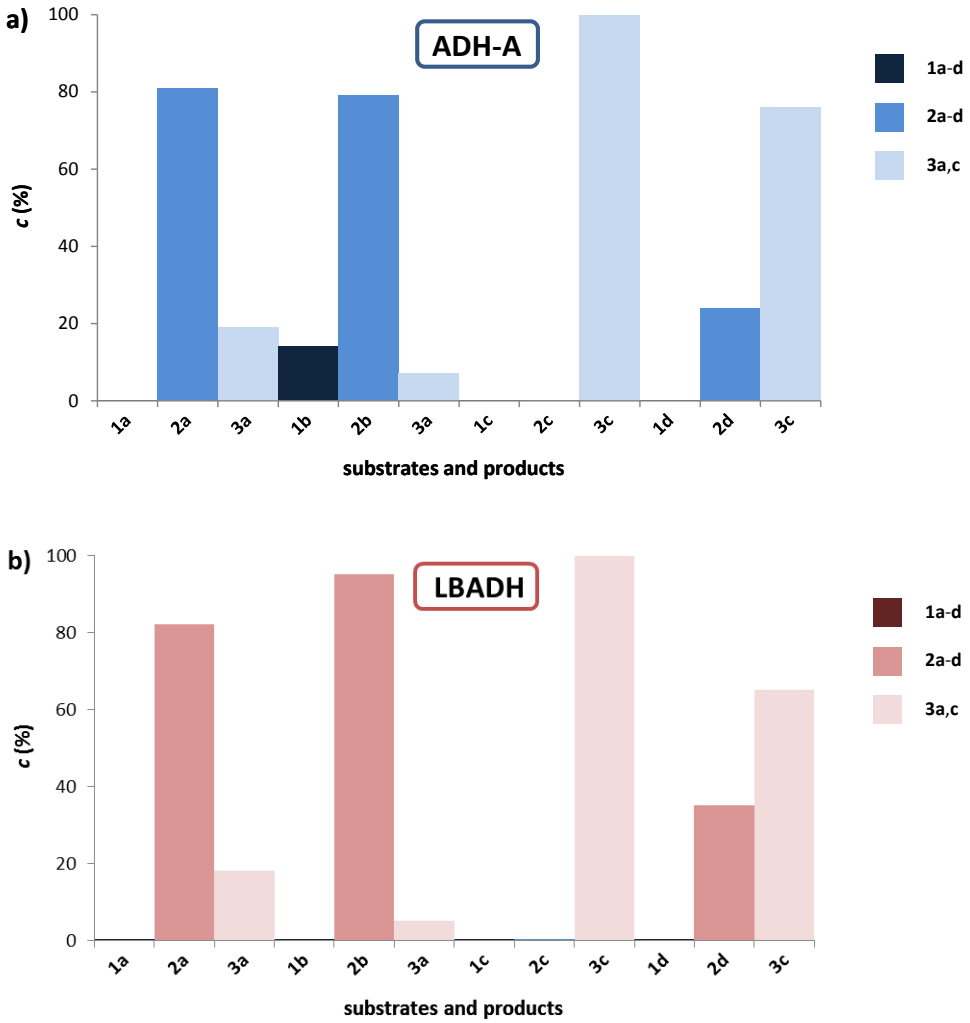


Figure 2. Effect of the alkyl chain length and the ester moiety in the ADH-catalyzed bioreduction of **1a-d** (50 mM) at 30ºC and pH 9.0 for: a) ADH-A; and b) LBADH. In all cases *ee* of the final products were >99%.

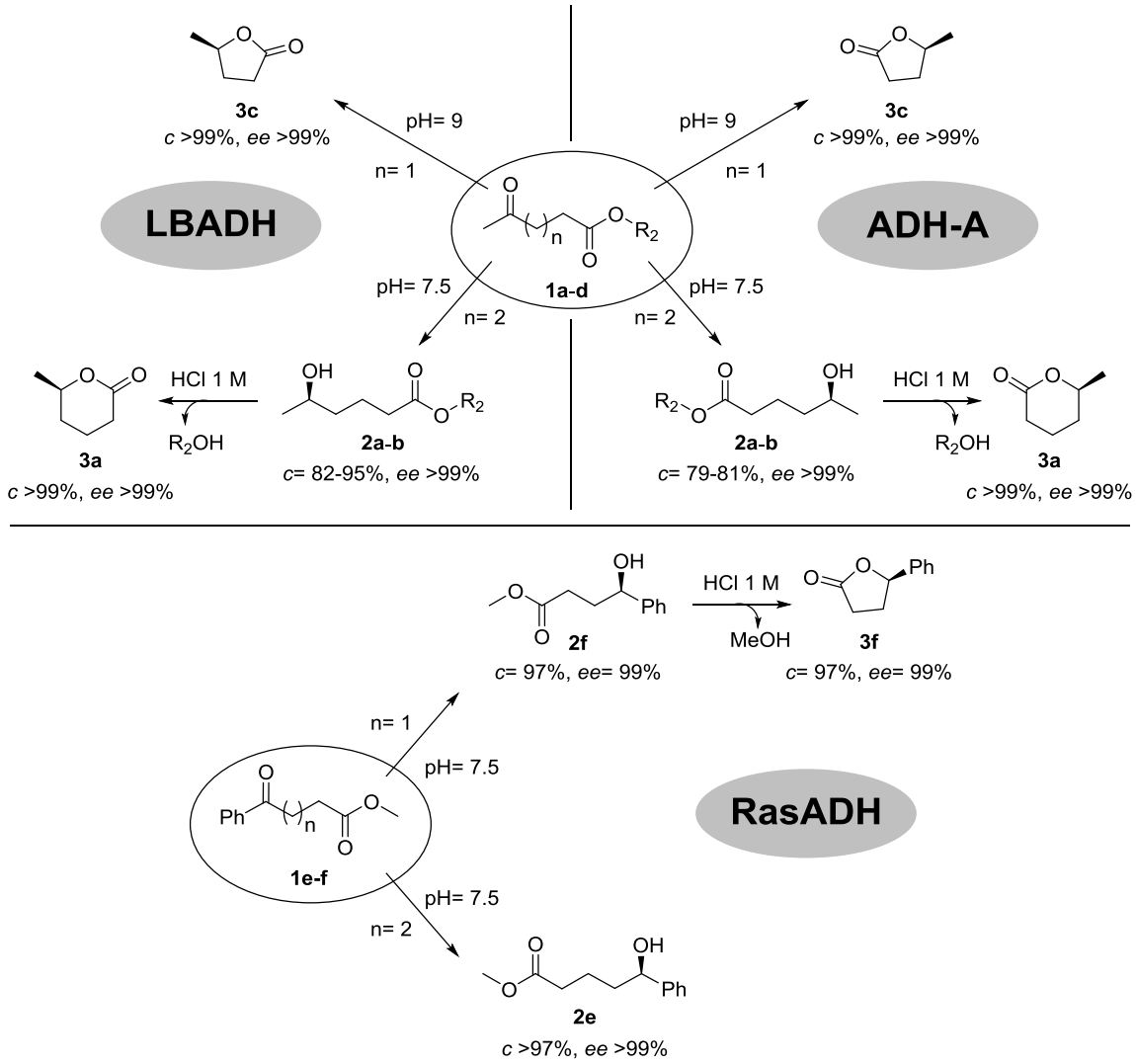
Due to the drastic influence of the pH in the cyclization process, we designed a one-pot two-step tandem protocol to achieve the synthesis of δ-caprolactone **3a**. It was envisaged that acidic treatment of the reaction mixture of **2a** and **3a** after the ADH-catalyzed bioreduction of **1a** could led to **3a** without losing the excellent stereopreference. Thus after enzymatic reduction, a

solution of HCl 1 M was added to the reaction medium, exclusively detecting lactone **3a** after 24 h (*ee* >99%). In the case of γ-keto esters **1c** and **1d**, GVL **3c** was obtained as major product recycling the cofactor by simple addition of 2-PrOH and without the need of adding HCl. It is important to remark that these findings could be reproduced on a 300 mg scale, achieving enantioenriched (*R*)-GVL employing LBADH as biocatalyst in 93% conversion and 97% *ee* (see SI for more details). Using this methodology, both enantiomers of **3c** could be easily obtained in a one-pot cascade fashion performing the bioreduction at 30ºC and pH 9.0. To summarize this part, it is worth mentioning that depending on the reaction conditions we could control the synthesis of the hydroxy esters **2a** or **2b** or the lactone **3a** in a chemo- and stereodivergent manner. In the case of γ-keto esters, the formation of the lactone was highly favored thus synthesizing both enantiomers of GVL **3c** with high efficiency at slightly basic pH. Furthermore, traces coming from the hydrolysis of the staring material could be just detected.[18]

At this point, we decided to test a bulkier substrate such as methyl 5-oxo-5-phenylpentanoate (**1e**, Scheme 3). With this goal in mind, we used ADHs from *Sphingobium yanoikuyae* (SyADH)[19] and *Ralstonia* sp. ADH (RasADH)[20] overexpressed in *E. coli*, since the substrate spectra for these enzymes is known to cover similar 'bulky-bulky' ketones. 2-PrOH was employed as cosubstrate for *E. coli*/SyADH, whereas the glucose/GDH system was chosen in the case of *E. coli*/RasADH. Based on previous results, we performed the reduction of **1e** at pH 7.5 with both enzymes to obtain hydroxy ester **2e** preferentially. Thus, while *E. coli*/RasADH led to the enantiomer (*R*)-**2e**[21] with an excellent conversion (>97%) and *ee* values (>99%), *E. coli*/SyADH led also to (*R*)-**2e** with a moderate conversion of 52% with a high enantiomeric excess (95%). In this case the cyclization process using HCl 1 M did not work properly, attaining less than 25% conversion of lactone **3e**.

When the aromatic γ-keto ester **1f** was used as substrate, in contrast to compounds **1c** and **1d** only hydroxy ester **2f** was detected. Taken together, these results emphasize the relevance of the keto ester structure in order to favor (or not) the lactonization process. In this case, *E. coli*/RasADH was the most effective in terms of conversion and enantioselectivity towards (*R*)-**2f** (*c*= 97%, *ee*= 99%), since SyADH afforded very low conversions (<20%) at pH 7.5. It is noteworthy that **2f** is a valuable precursor for the preparation of γ-peptides[22] and is also present in the structure of cryptophycin,[23] a potent cytotoxic drug. In order to shift the equilibrium towards the formation of lactone **3f**, an acidic treatment with HCl 1 M was required. When these bioreductions were tried at pH 9.0 to favor the cyclization, slightly lower *ee* values (~90%) were attained with both biocatalysts towards hydroxy ester **2f**, due to a racemization issue (*vide infra*), although complete conversion could be achieved with *E. coli*/SyADH in this case. As a matter of fact, depending on the pH or the substrate structure, hydroxy esters **2** or lactones **3** can be preferentially formed in a chemodivergent manner through one-pot cascade or tandem protocols (Scheme 3).

Scheme 3. Chemo- and stereodivergent synthesis of γ- and δ-hydroxy esters and lactones through ADH-catalyzed reactions.



Once found the appropriate conditions to get access to enantioenriched hydroxy esters or lactones, we decided to apply this biocatalytic method to the synthesis of several bioactive derivatives. Therefore, the bioreduction of brominated compound **1g** was studied since lactone **3g** has been used as intermediate for the synthesis of γ-hydroxybutyric acid (GHB) analogues for high-affinity GHB sites and γ-aminobutyric acid (GABA) receptors.[24] Based on our previous

results, *E. coli*/RasADH was selected as the biocatalyst to achieve this transformation with glucose and GDH to recycle the cofactor. In order to obtain the corresponding lactone, the reduction was firstly tried under basic conditions (pH 9.0). Unfortunately, we observed that the corresponding hydroxy ester **2g** and the lactone **3g** were accessed with very low *ee* values. After confirming that other ADHs from the *E. coli* host were not involved in the non-stereoselective bioreduction of this substrate, we decided to study this process in more detail following the reduction within the time (Figure 3). As depicted in Figure 3, *ee* values from both **2g** and **3g** decreased along the reaction. Control experiments indicated that racemization of hydroxy ester **2g** occurred in basic medium while lactone **3g** remained unaltered.

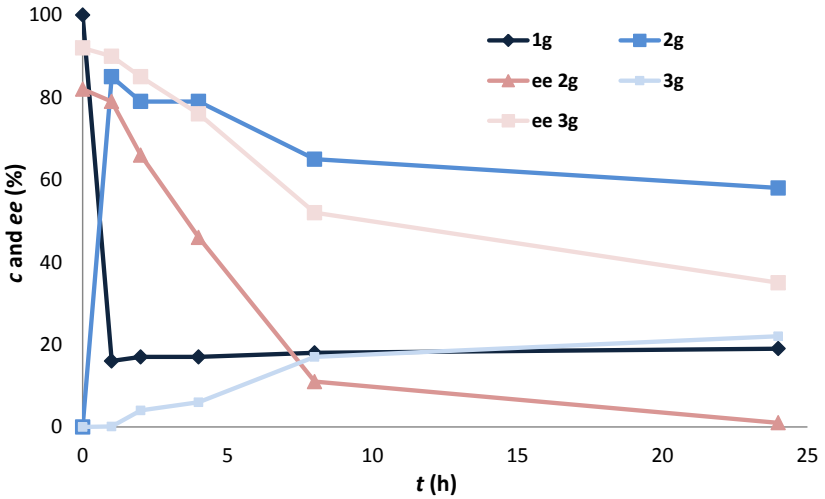
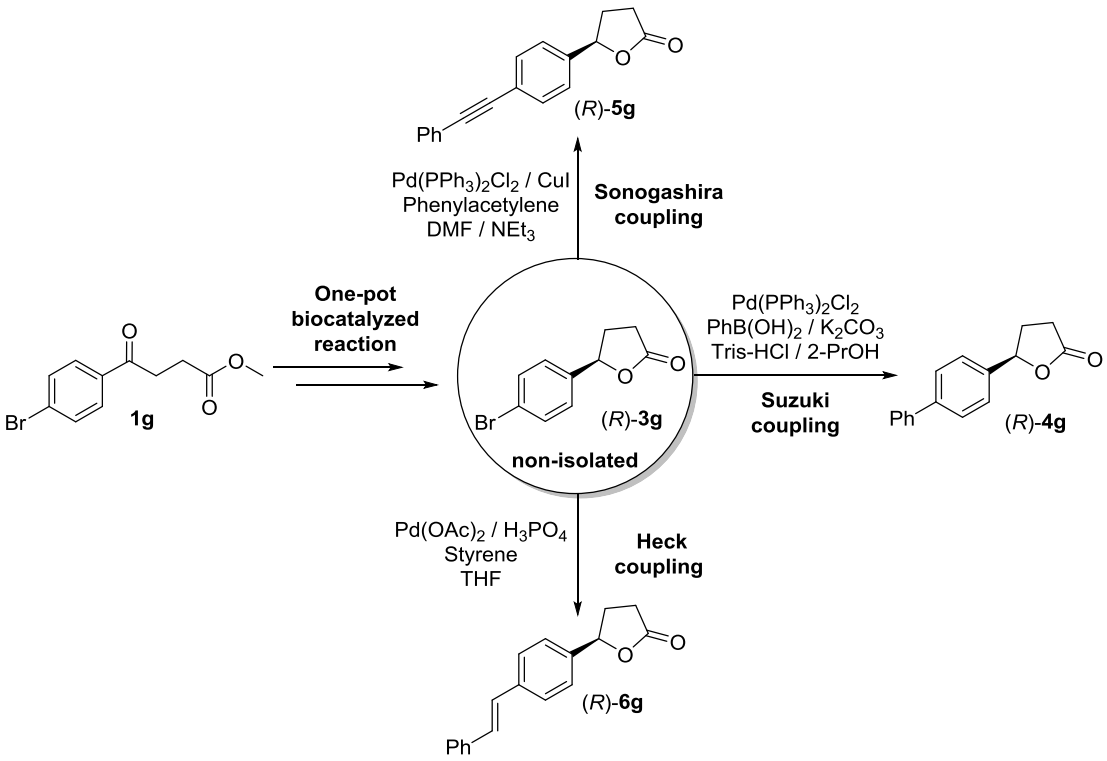


Figure 3. Time study of the *E. coli*/RasADH-catalyzed bioreduction of **1g** at 30ºC and pH 9.0.

With the goal of avoiding this undesired process, the bioreduction was performed at pH 7.5. Under these conditions, RasADH showed high activity (*c* >99%) and (*R*)-**2g** could be obtained with an excellent selectivity (*ee* >97%) after 4 h. Again acidic treatment with HCl 1 M led to the corresponding lactone with identical *ee* value. Due to the easy handling of *E. coli* lyophilized

cells, a preparative preparation of (*R*)-**3g** could be readily achieved in a one-pot two-step tandem protocol on a 200 mg scale (50 mM), thus attaining the desired compound with excellent isolated yield (95%) and *ee* (97%). Notably, this methodology improves the previously described protocols for the preparation of brominated lactone (*R*)-**3g**.[7a,25] Interestingly, SyADH gave access to the opposite enantiomer, (*S*)-**3g**, with complete conversion although with a lower stereoselectivity (*ee*= 60%).

Scheme 4. Chemodiverse preparation of (*R*)-**4g**, (*R*)-**5g** and (*R*)-**6g** combining a one-pot enzymatic protocol with Pd-based catalysis.



After these promising results with RasADH, we focused on the subsequent functionalization of the brominated lactone in order to expand the applicability of this methodology. Based on the

nature of the substrate, we employed Pd-coupling reactions to construct more complex and potentially bioactive lactones (Scheme 4).[24,26] We first attempted the Suzuki coupling starting from racemic **3g** with phenylboronic acid using $Pd(PPh_3)_2Cl_2$ since this catalyst showed good activities in aqueous media.[15b] We were pleased to find that such a reaction was compatible with the stability of the lactone, achieving compound **4g** with an excellent conversion (>98%). However, when both steps were combined in one-pot only traces of the coupled product was detected. This result probably arose from inhibition issues. The reaction was therefore performed in a sequential manner. After bioreduction, the crude was simply extracted and then treated under Suzuki conditions obtaining (*R*)-**4g** in 75% conversion. In a similar manner, Sonogashira and Heck coupling were attempted. In the first case, $Pd(PPh_3)_2Cl_2$ and CuI were used to couple lactone (*R*)-**3g** with phenylacetylene at 100ºC affording derivative (*R*)-**5g** in a 60% isolated yield, while for the Heck transformation, $Pd(OAc)_2$ and styrene were employed at 120ºC to achieve lactone (*R*)-**6g** with a 69% conversion. Remarkably, all three Pd-catalyzed reactions proceeded smoothly getting access to compounds (*R*)-**4g**-**6g** without losing enantioselectivity.

Conclusions

In summary, we have demonstrated that ADHs can be used for the chemo- and stereodivergent synthesis of relevant hydroxy esters and lactones in *ee* >97% through direct bioreduction, or by means of cascade and tandem protocols by simply tuning the reaction conditions. Also, the substrate structure was a key factor in the spontaneous cyclization process. ADH-A or LBADH were used to reduce selectively methyl ketone substrates, whereas levulinate compounds afforded the formation of (*S*)- or (*R*)-GVL, 5-oxohexanoate surrogates preferentially gave access to the corresponding (*S*)- or (*R*)-δ-hydroxy esters. In these latter cases, an acid-catalyzed

cyclization step was necessary in order to isolate the six-membered ring lactones. For bulkier substrates RasADH or SyADH were employed, and after bioreduction the corresponding (*R*)-γ-hydroxy esters were efficiently obtained. After acidic treatment the five-membered ring lactone could be achieved.

These findings have been applied to the preparation of relevant compounds simplifying the previous established methods. Furthermore, the viability of this synthetic route prompted us the one-pot two-step chemoenzymatic preparation of an enantioenriched brominated lactone that allowed the divergent synthesis of several functionalized chiral lactones with potential biological properties by sequential Pd-catalyzed transformations. Our approach is efficient, simple and scalable having great potential as a "greener" protocol.

Experimental Section

General considerations

Glucose dehydrogenase (GDH 106, 54 U mg^{-1}), ADH-A from *Rhodococcus ruber* (20 U mg^{-1}), and LBADH from *Lactobacillus brevis* (80 U mg^{-1}) were obtained from Codexis. ADHs from *Rhodococcus ruber* (ADH-A), *Thermoanaerobacter ethanolicus* (TeSADH), *Sphingobium yanoikuyae* (SyADH) and *Ralstonia* sp. (RasADH) have been obtained from Prof. Wolfgang Kroutil at the University of Graz and have been overexpressed following the methodology previously described.[19,20,27]

NMR spectra were recorded on a 300 MHz, 400 MHz or 600 MHz spectrometers. All chemical shifts (δ) are given in parts per million (ppm) and are referenced to the residual solvent signal as internal standard. The following abbreviation is employed: *m*= multiplet. Gas chromatography (GC) analyses were performed on a standard GC chromatograph equipped with

FID. High performance liquid chromatography (HPLC) analyses were carried out in a standard chromatograph coupled to a UV detector at 210 nm. High resolution mass spectra (HRMS) were obtained using a spectrometer by positive electrospray ionization (ESI^+). IR spectra were recorded as thin films on NaCl plates on a standard FT-IR and are reported in frequency of absorption (cm^{-1}). Thin-layer chromatography (TLC) was conducted with silica gel precoated plates and visualized using a UV lamp and/or potassium permanganate stain. Column chromatography was performed using silica gel (230-400 mesh). Microwave reactions were carried out with a standard microwave apparatus with high stirring. Optical rotations were measured in a polarimeter and values are reported in 10^{-1} deg cm^2 g^{-1} (concentration, solvent).

Protocol using ADH from Lactobacillus brevis *(LBADH)*

In a 10 mL vial, LBADH (7.5 U) was dissolved in Tris-HCl buffer (50 mM, pH 5.0-9.0, 2.28 mL, 1 mM NADPH, 1 mM $MgCl_2$), 2-PrOH (120 μL, 5% v v^{-1}) and the corresponding keto ester (50 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h. The reaction mixture was extracted with Et_2O (3 x 2 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was carefully evaporated and the conversion of the corresponding hydroxy ester or lactone was determined by NMR and the *ee* by GC or HPLC.

Protocol using ADH from Rhodococcus ruber *(ADH-A)*

In a 10 mL vial, ADH-A (3 U) was dissolved in Tris-HCl buffer (50 mM, pH 5.0-9.0, 2.28 mL, 1 mM NADH), 2-PrOH (120 μL, 5% v v^{-1}) and the corresponding keto ester (50 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h. The reaction mixture was extracted with Et_2O (3 x 2 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was carefully evaporated and the conversion of the corresponding hydroxy ester or lactone was determined by NMR and the *ee* by GC or HPLC.

Protocol using ADH from Ralstonia *sp. (*E. coli*/RasADH)*

In a 10 mL vial, *E. coli*/RasADH (50 mg) was resuspended in Tris-HCl buffer 50 mM pH 7.5-9.0 (2.28 mL, 1 mM NADPH), glucose (100 mM), glucose dehydrogenase (GDH, 5 U), and the corresponding keto ester (50 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h and stopped by extraction with Et_2O (3 x 2 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was carefully evaporated and the conversion of the corresponding hydroxy ester or lactone was determined by NMR and the *ee* by GC or HPLC.

*Chemoenzymatic one-pot two-step protocol to obtain enantiopure lactone **3a***

In a 10 mL vial, LBADH (7.5 U) was dissolved in Tris-HCl buffer (50 mM, pH 9.0, 2.28 mL, 1 mM NADPH, 1 mM $MgCl_2$), 2-PrOH (120 μL, 5% v v^{-1}) and the corresponding keto ester **1a-b** (50 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h. Then, a solution of HCl 1 M (2 mL) was added into the reaction mixture and was shaken at 30ºC and 250 rpm for 24 additional hours. The reaction mixture was extracted with Et_2O (3 x 2 mL). The organic layers were combined and dried over Na_2SO_4. The solvent was carefully evaporated and the conversion of the reaction was determined by NMR (>97%) and the *ee* by GC or HPLC (>97%).

*Scale-up of the preparation of (*R*)-GVL using LBADH*

In a 50 mL Erlenmeyer flask, LBADH (140 U) was dissolved in a Tris-HCl buffer solution (50 mM, pH 9, 17 mL, 1 mM NADPH, 1 mM $MgCl_2$) containing 2-PrOH (2.4 mL, 5% v v^{-1}) and methyl levulinate **1a** (295 μL, 100 mM). The reaction was shaken at 30ºC and 250 rpm for 24 h. The reaction mixture was then extracted with Et_2O (3 x 20 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent carefully evaporated. (*R*)-**3a** was obtained in quantitative yield with a purity of 93% (*ee* = 97%).

*Scale-up of the enzymatic reaction to obtain (*R*)-**3g** with* E. coli*/RasADH*

In a 50 mL Erlenmeyer flask, *E. coli*/RasADH cells (250 mg) were resuspended in a Tris-HCl buffer solution (50 mM, pH 7.5, 11.4 mL, 1 mM NADPH) containing glucose (100 mM), GDH (200 U), 2-PrOH (600 μL, 5% v v^{-1}), and **1g** (200 mg, 50 mM). The reaction was shaken at 30ºC and 250 rpm for 24h. After that time, 10 mL of HCl 1M were added and the reaction mixture was shaken at 30ºC and 250 rpm for 24 additional hours. The reaction mixture was then extracted with Et_2O (3 x 10 mL). The organic layers were combined and dried over Na_2SO_4. The crude residue was purified by chromatography column (20% EtOAc/hexane) isolating the corresponding lactone (*R*)-**3g** in 95% yield (*ee* = 97%), $[\alpha]_D^{20}$ = -24.7 (*c* 1.23, $CHCl_3$). Spectral data for **3g** were consistent with those reported in the literature.[24]

*Sequential reaction using the Suzuki coupling for the synthesis of (*R*)-**4g***

In a 50 mL erlenmeyer, *E. coli*/RasADH cells (125 mg) were resuspended in Tris-HCl buffer (50 mM, pH 7.5, 4.5 mL, 1 mM NADPH), containing glucose (400 mM), GDH (20 U), 2-PrOH (300 μL, 5% v v^{-1}), and **1g** (81.3 mg, 0.3 mmol). The reaction was shaken at 250 rpm for 24 h at 30ºC. The resulting mixture was treated with 5 mL of HCl (1 M) and stirred for additional 24 h. After that time, the reaction was centrifuged (5 min, 4000 rpm) and the cell pellet was washed with EtOAc (3 x 20 mL). The organic layers were combined and dried over Na_2SO_4 and the solvent was evaporated. The resulting crude mixture was dissolved in Tris-HCl buffer (16 mL, 50 mM, pH 7.5) and 2-PrOH (1.6 mL, 10% v v^{-1}) and treated with phenylboronic acid (63 mg, 0.50 mmol) and K_2CO_3 (92 mg, 0.66 mmol). Then, $Pd(PPh_3)_2Cl_2$ (11.4 mg, 0.016 mmol) was added and the resulting mixture was stirred for 24 h at 45ºC. The reaction mixture was extracted with EtOAc (3 x 15 mL) and the combined organic phases dried over Na_2SO_4 and concentrated in vacuo. The crude was analyzed by NMR observing the formation of lactone (*R*)-**4g** in a 75%

conversion (*ee* = 97%). Spectral data for **4g** were consistent with those reported in the literature.[28]

*Sequential reaction using the Sonogashira coupling for the synthesis of (*R*)-**5g***

The corresponding brominated lactone *rac*- or (*R*)-**3g** (40 mg, 0.166 mmol), $Pd(PPh_3)_2Cl_2$ (4.7 mg, 0.007 mmol) and CuI (1.9 mg, 0.01 mmol) were added to a sealed tube under a stream of nitrogen and dissolved in a mixture DMF/Et_3N (1 mL, 5:3 v v^{-1}). To the stirred solution phenylacetylene (27 μL, 0.249 mmol) was added. The reaction mixture was heated at 100ºC for 16 h. After that time, the reaction mixture was extracted with EtOAc (3 x 10 mL). The organic layers were combined and dried over Na_2SO_4. The crude residue was purified by column chromatography (20% EtOAc/hexane) isolating the corresponding lactone **5g** in 58-64% isolated yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.21 (*m*, 1H), 2.68 (*m*, 3H), 5.54 (*m*, 1H), 7.36 (*m*, 6H, Ar), 7.55 (*m*, 4H, Ar); ^{13}C NMR (106 MHz, $CDCl_3$): δ 176.6, 139.4, 132.0 (2C), 131.6 (2C), 128.4 (2C), 126.9, 125.2 (2C), 123.5, 123.0, 90.0, 88.7, 80.8, 30.9, 28.9; IR (neat): 3026, 2920, 2845, 1653, 1600, 1494, 1449, 1260, 1068, 1022 cm^{-1}. MS ($APCI^+$, m/z) 263.0 [$(M+H)^+$, 100%].

*Sequential reaction using the Heck coupling for the synthesis of (*R*)-**6g***

The corresponding brominated lactone *rac*- or (*R*)-**3g** (40 mg, 0.166 mmol), $Pd(OAc)_2$ (1.9 mg, 0.008 mmol) and H_3PO_4 (2.4 mmol) were added to a sealed tube under a stream of nitrogen and dissolved in THF (1 mL). To the stirred solution styrene (28 μL, 0.249 mmol) was added. The reaction mixture was heated at 120ºC for 16 h. The reaction mixture was then extracted with EtOAc (3 x 10 mL). The organic layers were combined and dried over Na_2SO_4 and the crude residue was analyzed by NMR observing the formation of lactone (*R*)-**6g** in a 69% conversion (*ee* = 97%). Spectral data for **6g** were consistent with those reported in the literature.[24]

Supporting Information. Experimental procedures, enzymatic protocols at higher substrate concentrations, analytical data, and copies of ^{1}H- and ^{13}C-NMR for **5g** are described. This information is available free of charge via the Internet at http://pubs.acs.org.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡ These authors contributed equally.

Notes

The authors declare no competing financial interest.

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