DOI: 10.1002/cctc.200((will be filled in by the editorial staff))

Enantioselective preparation of δ-valerolactones using horse liver alcohol dehydrogenase

Alba Díaz-Rodríguez,[a] Javier Iglesias-Fernández,[b] Carme Rovira[b,c] and Vicente Gotor-Fernández*[a]

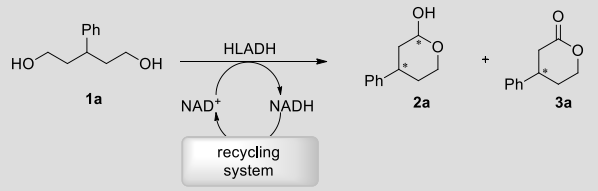
Alcohol dehydrogenases (ADHs) are widely used to catalyze the reduction and oxidation of carbonyl groups and alcohols, respectively. Recently, these oxidorreductases have found important applications in both academia and industry for the production of pharmaceuticals, fragrances and high added value derivatives.[1] Although the stereoselective bioreduction of prochiral ketones is a very attractive transformation for organic chemists, the oxidation of alcohols is less appealing because in most of the cases the chirality is lost. Thus, there are a limited number of biocatalytic examples reporting on the oxidation of diols to the corresponding lactones.[2] Remarkably, 1,4-butanediol has been recently used as an irreversible cosubstrate for redox biocatalysis leading to γ-butyrolactone as a thermodynamically stable and kinetically inert coproduct.[3] In this context, the biocatalytic enantioselective oxidation of diols,[4] and, in particular *meso* and prochiral diols, has been scarcely investigated. Conceptually, this approach is a powerful strategy for the preparation of optically active compounds since the substrate loses one or more elements of symmetry.[5] To the best of our knowledge, only a few examples in the literature apart from early examples by Jones and co-workers deal with the enzymatic desymmetrization of prochiral 1,5-pentanediols through oxidative processes.[4a,c,e]

Lactones are important compounds because of their structural implications as basic chemicals but also they are relevant building blocks found in natural products.[6] With the growing demand for enantiomerically pure synthons, the biocatalyzed preparation of chiral lactones remains a challenging goal and different (chemo)enzymatic routes have been successfully applied making use of lipase-mediated hydrolysis of carboxylic acid derivatives,[7] Baeyer-Villiger biooxidations of the corresponding cyclic ketones,[8] or ADH-catalyzed bioreduction of ketones.[9]

Herein, we report an asymmetric approach for the synthesis of enantioenriched lactones based on the desymmetrization of the corresponding 3-arylpentane-1,5-diols. Enantioselective oxidation of prochiral diols for the preparation of these relevent compounds using ADHs could represent an interesting alternative to the commonly employed protocols.

In a first set of experiments, we screened a panel of ADHs in the one-pot oxidation of 3-phenylpentane-1,5-diol as model substrate (**1a**, Table 1) to prepare the corresponding enantioenriched δ-valerolactone **3a** (Table 1). Among them, HLADH showed the highest activity (see SI for details) and therefore it was selected for further optimization of the biocatalytic process.

Table 1. Optimization of the enzymatic reaction of **1a**



Entry[a]	pH	cosolvent	c (%)[b]	(*S*)-**3a** *ee*(%)[c]
1[d]	9	-	52 (1:1.8)	48
2	7.5	-	100 (1:16)	49
3	6.5	-	80 (1:4)	50
4	9	-	66 (1: 5.2)	38
5[e]	7.5	-	70 (0:1)	44
6[f]	7.5	-	46 (1:3)	50
7[g]	7.5	-	34 (1:1)	45
8	7.5	2% THF	84 (1:5.4)	54
9	7.5	5% THF	72 (1:3)	64
10	7.5	7% THF	61 (1:2)	74
11	7.5	2% CH_3CN	73 (1:3.2)	50
12	7.5	7% CH_3CN	62 (1:2.6)	60

[a] Reactions were carried out using 25 mM concentration of **1a,** 0.84 mM of NAD^+, 2 U of HLADH and the recycling system in Tris buffer for 72 h at 30 ºC unless noted. [b] Conversion was determined by GC considering the formation of hemiacetal **2a** and lactone **3a**, which ratio appears in parentheses. [c] Enantiomeric excesses of **3a** were determined by HPLC. [d] FMN was used as recycing system. Reaction performed at [e] 37 ºC, [f] 20 ºC and [g] 4 ºC.

[a] *A. Díaz-Rodríguez, V. Gotor-Fernández*
Department of Organic and Inorganic Chemistry
University of Oviedo
C/Julián Clavería 33006 Oviedo (Spain)
Fax: (+) 34 985103448
E-mail: vicgotfer@uniovi.es
[b] *J.Iglesias-Fernández, C. Rovira*
Department of Organic Chemistry.
Institut de Quimica Computational (IQTACUB)
Martí i Franquès 1. 08028 Barcelona (Spain)
[c] *C. Rovira*
Institució Catalana de Recerca i Estudis Avancats (ICREA)
Passeig Lluis Companys 23, 08020 Barcelona (Spain)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cctc.200xxxxxx

Formation of the valerolactone occurs by desymmetrization of **1a**, which is oxidized generating an unstable hydroxy aldehyde that cyclised affording the corresponding hemiacetal (**2a**). The subsequent reoxidation of the latter gave access to the stable lactone **3a**.[10] Based on these initial results and in order to use catalytic amounts of NAD^+ cofactor, we next attempted the enantioselective oxidation of diol **1a** by incubation with HLADH using flavin mononucleotide (FMN) as recycling system.[4a] Reaction was monitored by GC observing a slow oxidation of diol **1a**. Under the above mentioned conditions, a mixture of hemiacetal **2a** and lactone **3a** was observed in a ratio 1:1.8, yielding the lactone **3a** with a modest 48% *ee* after 72 h (Table 1, entry 1). It is worth mentioning that previously reported enantiomeric excess for the desymmetrization of **1a** was 21% *ee*.[4a] The biotransformation proceeded with stereoselectivity towards the pro-*S* hydroxy group, and preferentially, after reoxidation to the (*S*)-lactone.

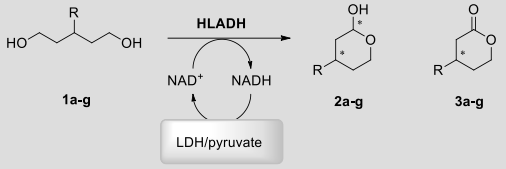
In order to improve the conversion values towards the lactone, we explored other recycling systems and reaction conditions. Firstly, the addition of acetaldehyde to the reaction mixture was attempted. Unfortunately, no reaction was observed probably due to inhibition issues. Based on this unsuccessful result with the coupled substrate approach, L-lactate dehydrogenase (LDH) was used. This enzyme catalyzes the reduction of pyruvate to lactate with the concomitant oxidation of NADH to regenerate NAD^+.[11] Remarkably, this regeneration system led to total conversion of the process, yielding the (*S*)-lactone in moderate enantiomeric excess after 72 h (Table 1, entry 2). The experimental results clearly indicate that the enantioselection in the **1a**→**2a** step is the most important contribution. The enantiomeric excess of final lactone **3a** remains mainly unaltered when the conversion values are between 50% and 100% (see SI for detailed time course study).

At this point, different parameters were investigated in order to enhance the *ee* of this biotransformation. As expected, a change in the pH of the reaction drastically affected the yield of the process (Table 1, entries 3 and 4) without improvement of the *ee* and in detriment of the **2a**:**3a** ratio. Next, the effect of the temperature was studied, and although an increase of the temperature favoured the formation of the termodynamically stable lactone **3a** (note that hemiacetal **2a** was not detected at 37 ºC, entry 5), the conversion of the process drastically decreased. Yet, when the reaction was carried out at lower temperatures (20 ºC and 4 ºC, entries 6 and 7), only lower conversions were observed indicating that these parameters are not tuneable to achieve better *ee* values.

Motivated by the fact that oxidoreductases can work in organic solvents with interesting changes in their properties,[12] a percentage of cosolvent with respect to the buffer solution was added to the reaction mixture (Table 1, entries 8-12). Gratifyingly, a significant improvement in the enantioselectivity was observed. The best results in terms of conversion and enantiomeric excess were obtained when THF was used (Table 1, entries 8, 9 and 10) observing a 74% *ee* when 7% of THF was present. However, we should mention here that the yield of **3a** dropped considerably in the lattest case. Percentages of cosolvent higher than 15% inhibited the oxidation reaction (data not shown). Solvent engineering studies demonstrated that when acetonitrile was used as the cosolvent, conversions were slighly lower than with THF (Table 1, entries 11 and 12). Moreover, the addition of a non-miscible cosolvent such as hexane allowed the addition of higher percentages of cosolvent, finding similar conversions and *ee* values than in aqueous medium (data not shown).

We also extended this methodology to other 3-arylpentane-1,5-diols **1b**-**g**[13] (Table 2) in order to study the effect of the pattern substitution in the aromatic ring on the lactonization reaction. From the results in Table 2, it becomes clear that the introduction of a group in the aromatic ring at the *para*- position (**1b** and **1c**) led to a slight improvement of the *ee* (Table 2, entries 3 and 5). This could be explained by additional interactions in the active site of the HLADH. Nevertheless, the conversions for the oxidation of these substrates dropped.

Table 2. Enantioselective preparation of δ-valerolactones **3a–g**: effect of the substituent position and cosolvent



Entry[a]	R	THF(%)	c (%)[b]	(*S*)-**3a**–**g** *ee*(%)[c]
1	C_6H_5 (**a**)	----	100 (1:9)	52
2	C_6H_5 (**a**)	7	61 (1:2.5)	74
3	4-MeO-C_6H_4 (**b**)	----	92 (1:17)	60
4	4-MeO-C_6H_4 (**b**)	7	55 (1:3)	68
5	4-F-C_6H_4 (**c**)	----	72 (1:2.6)	65
6	4-F-C_6H_4 (**c**)	7	39 (2:1)	76
7	3-MeO-C_6H_4 (**d**)	----	82 (1:9)	40
8	3-MeO-C_6H_4 (**d**)	7	43 (1:1.8)	53
9	3-F-C_6H_4 (**e**)	----	71 (1:3.5)	52
10	3-F-C_6H_4 (**e**)	7	31 (1:0.8)	75
11	2-MeO-C_6H_4 (**f**)	----	62 (1:15)	92
12	2-MeO-C_6H_4 (**f**)	7	21 (1:3)	97
13	2-F-C_6H_4 (**g**)	----	76 (1:12)	50
14	2-F-C_6H_4 (**g**)	7	32 (1:2)	55

[a] Reactions were carried out using 25 mM concentration of substrates **1a**-**g**, 0.84 mM of NAD^+, 68 mM of pyruvate, LDH and 2 U of HLADH in Tris buffer pH= 7.5 at 30 ºC during 72 h. [b] Conversion values were determined by GC considering the formation of the corresponding hemiacetal and lactone, which ratio appears in parentheses. [c] Enantiomeric excesses of lactones were determined by HPLC.

We next turned our attention to diols **1d-g** possesing substituents closer to the prochiral center (*meta*- and *ortho*-positions). We can observe that 3-(*m*-methoxyphenyl)pentanediol (**1d**) reacts slower in comparison to the corresponding *para*-substituted diol (**1b**), the *ee* being also lower (Table 2, entry 7). This outcome may suggest that the substrate binds less efficiently in the active site. The same tendency was observed with **1e** (Table 2, entry 9). Moreover, when the methoxy group was placed at the *ortho*- position (**1f**) it had a remarkable effect on the enantioselectivity observing a 92% *ee* (Table 2, entry 11). In light of these results, we can hypothesize that the enantioselectivity of the process is not simply influenced by the presence of a substituent but also by its position that may interact with residues in the HLADH active site. However, for the substrate **1g**

containing a fluorine atom in *ortho*-position, the *ee* dropped again probably due to the small size of the substituent (Table 2, entry 13).

In order to further rationalize the relation between the diol structure and the enantioselectivity, docking studies were performed on substrates **1a, 1g** and **1f**.[14] For the sake of simplicity, we only focused on the desymmetrization process. It was found that whereas compound **1a** and **1g** form a E-S complex where the pro-*R* and pro-*S* conformations resulted in the same structure, compound **1f** shows important differences in both conformers. In particular an intramolecular hydrogen bond interaction is observed for the pro-*S* conformation (see Figure 1 and SI for more details), supporting the experimental observations. Furthermore, we would like to highlight that as a general trend although conversion values decreased considerably when adding THF, the optical purities for the final valerolactones improved in all cases (Table 2, entries 2, 4, 6, 8, 10, 12 and 14).

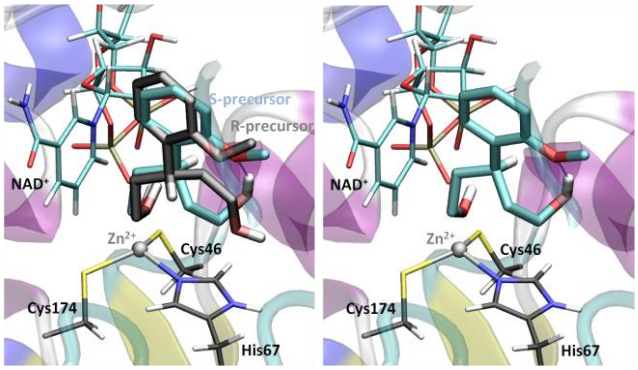


Fig 1. Substrate **1f** in complex with HLADH. Black and cyan ligands are precursors of the *R* and *S* enantiomers, respectively (left) and intramolecular hydrogen bond interaction for the pro-*S* enantiomer (right).

Overall, the versatility of desymmetrization processes have been demonstrated over a series of 3-aryl-pentane-1,5-diols through their selective oxidation using HLADH, leading to interesting lactones in good to excellent optical purities. This method expands the enzyme toolbox, as hydrolases were previously employed for the highly stereoselective desymmetrizarion of similar diols by means of acylation reactions,[13] but also providing straightforward access to related lactones via hydrolytic processes of diesters, followed by chemical intramolecular cyclization.[7] Our study shows that HLADH can be also applied for desymmetrization oxidation reactions of bulky diols to prepare enantioenriched lactones expanding the substrate scope hitherto reported.[4a] Optical purities can be tuned by adding organic cosolvents, where THF has shown a beneficial effect in terms of enantiodiscrimination while aqueous systems lead to the isolation of the lactones in higher yields. The synthesis of substituted diols has allowed the evaluation of a certain structure-activity relationship in the active site of HLADH. We are currently investigating further opportunities in this area, considering new target substrates and focusing on the improvement of the conversion values.

Experimental Section

General procedure for the enantioselective preparation of valerolactones 3a-g. In a typical experiment, a diol **1a-g** (25 mM) was suspended in Tris buffer (50 mM, pH 7.5, 0.5 mL) and treated with NAD^+ (50 μL, 10 mM stock, 0.84 mM), sodium pyruvate (40 μL, 1 M stock, 68 mM) and LDH (2 U). This mixture was shaken for 5 min and, finally, HLADH was added (2 U). The mixture was shaken at 250 rpm in a rotatory shaker at 30 ºC, extracted with EtOAc (2 x 0.5 mL), dried over Na_2SO_4 and analyzed by GC in order to determine the conversion value. HPLC analyses were carried out to determine the enantiomeric excesses. Control experiments in the absence of enzyme were performed for all substrates, not observing oxidation reaction after long periods of time.

Acknowledgements

This research is part of BIONEXGEN project (grant agreement 266025) sponsored by the European Union inside the 7th Framework Programme (FP7 2007-2013).

Keywords: asymmetric synthesis • biocatalysis • enantiopure lactones • molecular modelling • recycling system

[1] a) T. Matsuda, R. Yamanaka, K. Nakamura, *Tetrahedron: Asymmetry* **2009**, *20*, 513–517; b) G. W. Huisman, J. Liang, A. Krebber, *Curr. Opin. Chem. Biol*. **2010**, *14*, 122–129; c) F. Hollmann, I. W. C. E. Arends, D. Holtmann, *Green Chem*. **2011**, *13*, 2285–2313; d) M. M. Musa, R. S. Phillips, *Catal. Sci. Technol*. **2011**, *1*, 1311–1323; e) M. Hall, A. S. Bommarius, *Chem. Rev*. **2011**, *111*, 4088–4110; f) Y. Ni, J.-H. Xu, *Biotechnol. Adv*. **2012**, *30*, 1279–1288.

[2] For recent articles of non steroselective synthesis see: a) S. Gargiulo, I. W. C. E. Arends, F. Hollmann, *ChemCatChem* **2011**, *3*, 338–342; b) A. Díaz-Rodríguez, I. Lavandera, S. Kanbak-Aksu, R. A. Sheldon, V. Gotor, V. Gotor-Fernández, *Adv. Synth. Catal*. **2012**, *354*, 3405–3408.

[3] S. Kara, D. Spickermann, J. H. Schrittwieser, C. Leggewie, W. J. H. van Berkel, I. W. C. E. Arends, F. Hollmann, *Green Chem.* **2013**, *15*, 330–335.

[4] a) A. J. Irwin, J. B. Jones, *J. Am. Chem. Soc.* **1977**, *99*, 556–561; b) J. B. Jones, K. P. Lok, *Can. J. Chem.* **1979**, *57*, 1025–1032; c) I. J. Jakovac, G. Ng, K. P. Lok, J. B. Jones, *J. Chem. Soc., Chem. Commun.* **1980**, 515-516; d) I. J. Jakovac, H. B. Goodbrand, K. P. Lok, J. B. Jones, *J. Am. Chem. Soc.* **1982**, *104*, 4659–4665; e) H. Ohta, H. Tetsukawa, N. Noto, *J. Org. Chem*. **1982**, *47*, 2400–2404; f) J. B. Jones, I. J. Jakovac, *Can. J. Chem.* **1982**, *60*, 19–28; g) G. S. Y. Ng, L.-C. Yuan, I. J. Jakovac, J. B. Jones, *Tetrahedron* **1984**, *40*, 1235–1243; h) A. J. Bridges, P. S. Raman, G. S. Y. Ng, J. B. Jones, *J. Am. Chem. Soc.* **1984**, *106*, 1461-1467; i) J. B. Jones, I. J. Jakovac, *Org. Synth.* **1985**, *63*, 10-17; j) K. P. Lok, I. J. Jakovac, J. B. Jones, *J. Am. Chem. Soc.* **1985**, *107*, 2521–2526; k) M.-E. Gourdel-Martin, C. Comoy, F. Huet, *Tetrahedron: Asymmetry* **1999**, *10*, 403–404; l) F. Boratyński, G. Kiełbowicz, C. Wawrzeńczyk, *J. Mol. Catal. B: Enzym.* **2010**, 65, 30–36; m) D. Romano, M. Contente, T. Granato, W. Remelli, P. Zambelli, F. Molinari, *Monatsh. Chem.* **2013**, *144*, 735–737.

[5] For revisions about enzymatic desymmetrization of diols see a) J. B. Jones, *Tetrahedron* **1986**, *42*, 3351–3403; b) E. Schoffers, A. Golebiowski, C. R. Johnson, *Tetrahedron* **1996**, *52*, 3769–3826; c) E. García-Urdiales, I. Alfonso, V. Gotor, *Chem. Rev.* **2011**, *111*, PR110–PR180.

[6] See for instance a) Y. Wang, R. L. Tennyson, D. Romo, *Heterocycles* **2004**, *64*, 605–658; b) F. Faye, I. Linossier, V. Langlois, E. Renard, K. Vallee-Rehel, *Biomacromolecules* **2006**, *7*, 851–857; c) E. Brenna, C. Fuganti, F. G. Gatti, S. Serra, *Chem. Rev*. **2011**, *111*, 4036–4072; d) P. Azadi, R. Carrasquillo-Flores, Y. J. Pagán-Torres, E. I. Gürbüz, R. Farnood, J. A. Dumesic, *Green Chem*. **2012**, *14*, 1573–1576.

[7] For the preparation of δ-valerolactones by desymmetrization processes, see for instance: a) L. K. P. Lam, R. A. H. F. Hui, J. B. Jones, *J. Org. Chem*. **1986**, *51*, 2047–2050; b) A. Fryszkowska, M. Komar, D. Koszelewski, R. Ostaszewski, *Tetrahedron: Asymmetry* **2005**, *16*, 2475–2485.

[8] a) G. de Gonzalo, M. D. Mihovilovic, M. W. Fraaije, *ChemBioChem* **2010**, *11*, 2208–2231; b) G. de Gonzalo, M. W. Fraaije, *ChemCatChem* **2013**, *5*, 403–415.

[9] a) T. Fischer, J. Pietruszka, *Adv. Synth. Catal.* **2012**, *354*, 2521–2530; b) J. Mangas-Sánchez, E. Busto, V. Gotor-Fernández, V. Gotor, *Catal. Sci. Technol.* **2012**, *2*, 1590–1595; c) S. E. Franz, R. R. Watkins, L. A. Wright, B. A. Weaver, R. C. Hartage, I. Ghiviriga, G. Gumina, B. D. Feske, *Synthesis* **2013**, *45*, 2171–2178.

[10] Overoxidation of **1a** or hydrolysis of **3a** were not detected after acidic work-up of the aqueous phase.

[11] H. K. Chenault, G. M. Whitesides, *Bioorg. Chem*. **1989**, *17*, 400–409.

[12] a) A. M. Klibanov, *Nature* **2001**, *409*, 241–249; b) A. M. Klibanov, *Curr. Opin. Biotechnol.* **2003**, *14*, 427–431; for HLADH example see c) D.

Giacomini, P. Galletti, A. Quintavalla, G. Gucciardo, F. Paradisi, *Chem. Commun.* **2007**, 4038–4040.
[13] N. Ríos-Lombardía, V. Gotor-Fernández, V. Gotor, *J. Org. Chem.* **2011**, *76*, 811–819 (see supporting information for the description of their chemical synthesis).
[14] Docking calculations were performed with the Autodock 4.1.5.1 program, on the monomeric form of the enzyme (PDB code 4DWV). See for more details the SI.

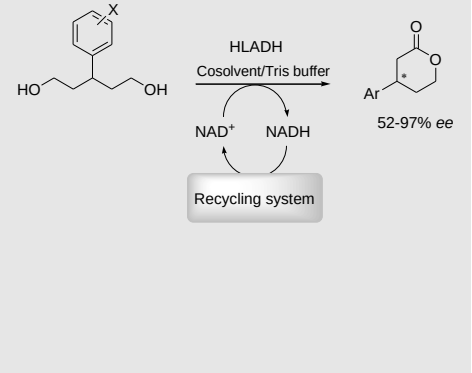
Received: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

Entry for the Table of Contents (Please choose one layout only)

Layout 1:

COMMUNICATION

Horse liver alcohol dehydrogenase has been found to be a versatile biocatalyst for the desymmetrization of 3-arylpentane-1,5-diols based on a two-step one-pot oxidation. The catalytic performance of HLADH has been studied using several cofactor regeneration systems and cosolvents. Docking studies has revealed the pattern substitution importance in the selectivity and activity of this biotransformation.



*A. Díaz-Rodríguez, J. Iglesias-Fernández, C. Rovira, V. Gotor-Fernández**

Page No. – Page No.

Enantioselective preparation of δ-valerolactones using horse liver alcohol dehydrogenase