Enantioselective Biocatalytic Reduction of Non-protected Hydroxyacetophenones

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Dedicated to Professor Rolf W. Saalfrank on the occasion of his 70th birthday

Direct enantioselective reduction of 2′-, 3′- and 4′-hydroxyacetophenone without protection of the hydroxy moiety was carried out in the presence of (*R*)- and (*S*)-alcohol dehydrogenases as biocatalysts. Whereas reduction of 2′-hydroxyacetophenone gave only low to medium conversions, reduction of 3′- and 4′-hydroxyacetophenone proceeded efficiently leading to the resulting 1-(3-hydroxyphenyl)ethan-1-ol and 1-(4-hydroxyphenyl)ethan-1-ol with high conversion (up to $> 95\,\%$) and excellent enantioselectivity (up to $> 99\,\%$ *ee*).

Key words: Alcohols, Asymmetric Synthesis, Biocatalysis, Enzymes, Reduction

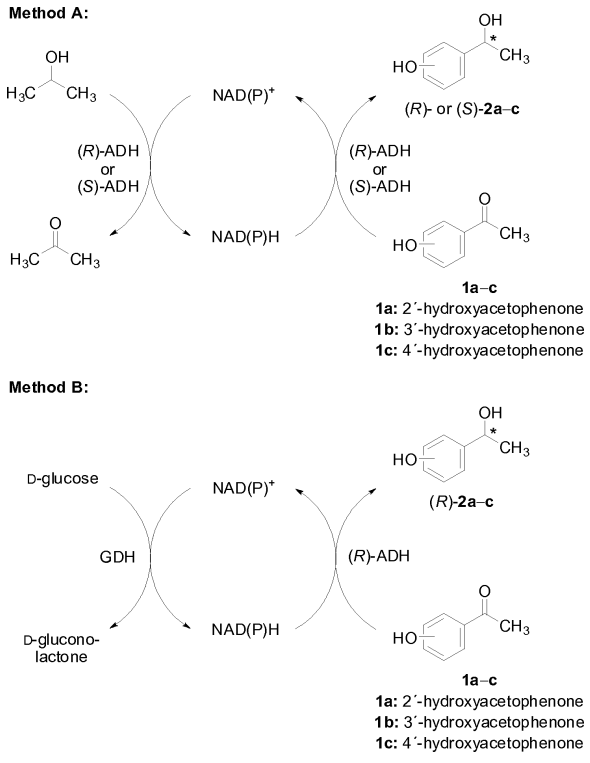
Introduction

In multi-step synthesis of complex molecules such as, *e. g.*, natural products and drugs, the use of protecting groups often is a prerequisite to achieve highly efficient transformations in the presence of functional group(s) which are not involved in the desired reaction [1]. Such a protection of functional groups contributes to a decrease of side-reactions of substrate and product, but also helps to maintain the performance of the catalyst when the catalyst would react with a specific functional group. At the same time, however, the need for protecting groups requires additional reagents and causes additional reaction steps, thus decreasing atom efficiency and economic attractiveness of the overall process [2]. Accordingly, efficient synthetic transformations in the presence of non-protected sensitive functional groups such as, *e. g.*, hydroxy and amino groups, which avoid the requirement for protecting groups are desirable, and their development represents an important (and still challenging) task in organic process chemistry.

In continuation of our studies on enzymatic redox processes [3, 4] herein we report the enantioselective reduction of prochiral acetophenones bearing a non-protected hydroxy group at the aromatic ring as a substituent (**1a** – **c**) by means of an alcohol dehydrogenase (ADH) as (bio-)catalyst. Such a biocatalytic one-step concept appears to be superior over a typical "classic chemical" synthetic strategy comprising the three steps of (i) protection of the hydroxy group, followed by (ii) asymmetric reduction of the ketone and (iii) subsequent removal of the protecting group [5]. Although biocatalytic reduction is recognized as a highly efficient approach towards enantiomerically pure alcohols [6], studies on enzymatic reduction of non-protected hydroxyacetophenones **1** under enantioselective formation of hydroxy-substituted 1-phenylethan-1-ols [(*R*)- or (*S*)-**2**], which represent substructures in a range of drugs [7], are so far surprisingly rare [8].

Results and Discussion

For our studies, 2′-, 3′-, and 4′-hydroxyacetophenone (**1a** – **c**) served as substrates. As biocatalysts we used (*R*)-enantioselective alcohol dehydrogenases from *Lactobacillus brevis* [9] and *Lactobacillus kefir* [10] as well as an (*S*)-enantioselective ADH from *Rhodococcus* sp. [11], which are prepared in the advantageous recombinant form. The enzymatic reductions of ketones of type **1** have been carried out by apply-



Scheme 1. Concepts for enzymatic reduction of hydroxyacetophenones **1a – c**.

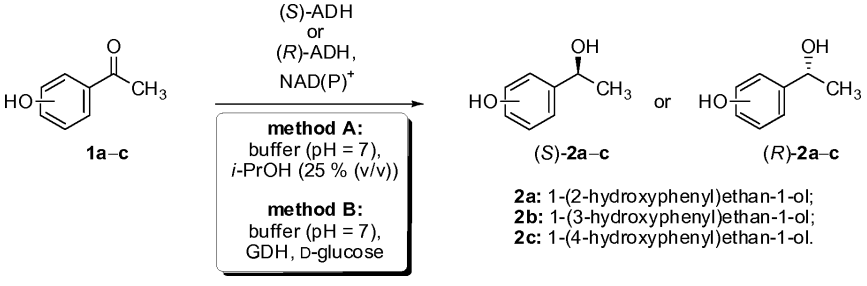
ing the concept of substrate-coupled cofactor regeneration or alternatively enzyme-coupled cofactor regeneration (Scheme 1) [6]. In the substrate-coupled cofactor regeneration process (Scheme 1, method A), the cofactor NAD(P)H is regenerated *in situ* by means of an oxidation of isopropanol (*i*-PrOH) to acetone. This enables the use of the expensive cofactor in catalytic amount only, whereas the economically attractive isopropanol represents the reducing agent used in stoichiometric amount. The enzyme-coupled cofactor regeneration (Scheme 1, method B) is based on the use of a glucose dehydrogenase for *in situ*-regeneration of the cofactor NAD(P)H *via* consumption of a stoichiometric amount of D-glucose under formation of D-gluconolactone.

When using 2′-hydroxyacetophenone (**1a**; *ortho*-hydroxyacetophenone) as a substrate, reduction of **1a** in the presence of an ADH from *L. brevis* (LB-ADH) gave the resulting (*R*)-alcohol (*R*)-**2a** with excellent enantioselectivity of > 98 % *ee* but low conversion of 5 % after a reaction time of 24 h (Table 1, entry 1). The difficulty in reducing 2′-hydroxyacetophenone has also been encountered when using the (*S*)-ADH from *Rhodococcus* sp. (Rsp.-ADH) as a biocatalyst, leading to a somewhat increased but still low conversion of 22 % (entry 2). At a prolonged reaction time of 72 h and higher biocatalyst loading conversion increased to 49 %, and enantioselectivity was excellent with > 99 % *ee* (entry 3). When starting from 3′-hydroxyacetophenone (**1b**; *meta*-hydroxyacetophenone) the enantioselective reduction proceeded successfully in the presence of both (*R*)- and (*S*)-enantioselective enzymes (entries 4 – 6). The use of the (*R*)-enantioselective ADH from *L. brevis* as a biocatalyst resulted in the formation of (*R*)-1-(3-hydroxy)phenylethan-1-ol ((*R*)-**2b**) with > 95 % conversion and > 99 % *ee* after a reaction time of 24 h (entry 4). The reduction of **1b** by means of an (*R*)-ADH from *L. kefir* (LK-ADH) as a biocatalyst also gave (*R*)-**2b** with excellent enantioselectivity of > 99 % *ee*, but with a decreased conversion of 76 % in spite of a prolonged reaction time of 120 h and high amount of enzyme (entry 5). In the presence of the (*S*)-ADH from *Rhodococcus* sp. a high conversion of 95 % was achieved accompanied with an excellent enantioselectivity of > 99 % *ee* after 24 h reaction time (entry 6). In addition, we were pleased to find that 4′-hydroxyacetophenone (**1c**; *para*-hydroxyacetophenone) can also be transformed into the corresponding alcohol (**2c**) with both high conversion and enantioselectivity. The enantioselective reduction of 4′-hydroxyacetophenone (**1c**) catalyzed by the (*R*)-ADH from *L. brevis* led to the formation of the desired (*R*)-alcohol (*R*)-**2c** with > 95 % conversion and > 95 % *ee* after 24 h reaction time (entry 7). The opposite (*S*)-enantiomer (*S*)-**2c** has been formed with 58 % conversion and an enantioselectivity of > 99 % *ee* after a prolonged reaction time of 72 h when using the (*S*)-ADH from *Rhodococcus* sp. as a biocatalyst (entry 8).

Conclusion

In conclusion, direct reduction of 2′-, 3′- and 4′-hydroxyacetophenone (**1a – c**) without protection of the hydroxy moiety was carried out by means of (*R*)-enantioselective ADHs from *L. kefir* and *L. brevis* and an (*S*)-enantioselective ADH from *Rhodococcus* sp. as biocatalysts. Whereas reduction of 2′-hydroxyacetophenone (**1a**) only gave low to medium conversions, a direct reduction of 3′- and 4′-hydroxyacetophenone (**1b**, **c**) proceeded efficiently without the need to protect the hydroxy moiety. The resulting products 1-(3-hydroxyphenyl)ethan-1-ol (**2b**) and 1-(4-hydroxyphenyl)ethan-1-ol (**2c**) were formed with high conver-

Table 1. Enzymatic reduction of **1a – c**.



Entry[a]	Substrate	Enzyme[e]	Method	Product	Conversion (%)	*ee* (%)
1	**1a**	LB-ADH	B	(*R*)-**2a**	5	> 98
2	**1a**	Rsp.-ADH	A	(*S*)-**2a**	22	> 99
3[b]	**1a**	Rsp.-ADH	A	(*S*)-**2a**	49	> 99
4	**1b**	LB-ADH	B	(*R*)-**2b**	> 95	> 99
5[c]	**1b**	LK-ADH	A	(*R*)-**2b**	76	> 99
6	**1b**	Rsp.-ADH	A	(*S*)-**2b**	95	> 99
7	**1c**	LB-ADH	B	(*R*)-**2c**	> 95	> 95
8[d]	**1c**	Rsp.-ADH	A	(*S*)-**2c**	58	> 99

[a] For experimental protocols, see Experimental Section; the reactions were carried out at room temperature, and unless otherwise stated the reaction time was 24 h; [b] prolonged reaction time of 72 h; [c] prolonged reaction time of 120 h; [d] prolonged reaction time of 72 h; [e] LB-ADH: (*R*)-ADH from *L. brevis*; Rsp.-ADH: (*S*)-ADH from *Rhodococcus sp.*; LK-ADH: (*R*)-ADH from *L. kefir*.

sion (up to > 95 %) and excellent enantioselectivity (up to > 99 % *ee*). Based on these encouraging preliminary results for the reactions with 2′-, 3′- and 4′-hydroxyacetophenone current work is in progress to apply this enantioselective enzymatic reduction method for the direct, protection group-free synthesis of related pharmaceutically active compounds bearing a hydroxy-substituted 1-phenylethan-1-ol scaffold.

Experimental Section

*General procedure 1: Reduction of **1a–c** via substrate-coupled cofactor recycling (method A)*

In a 25 mL flask the ketone of type **1** (entries 2, 3, 6, 8: 0.5 mmol, entry 5: 0.25 mmol) and NAD(P)$^+$ (0.02 mmol; NADP$^+$ when using LK-ADH and NAD$^+$ when using Rsp.-ADH) are dissolved in a mixture of phosphate buffer (7.5 mL, pH = 7, 0.05 M) and isopropanol (2.5 mL), followed by addition of the alcohol dehydrogenase (entries 2, 3, 6, 8: 72 U, entry 5: 155 U; U refers to acetophenone as standard substrate for LB-ADH and LK-ADH, and 4-chloroacetophenone as standard substrate for Rsp.-ADH) under stirring. After stirring the resulting reaction mixture for 24 h (entries 2, 6), 72 h (entries 3, 8) or 120 h (entry 5) at r. t., the aqueous phase was extracted three times with methylene chloride (80 mL). The combined organic phases were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The conversion of this reaction was determined from the resulting crude product by means of ^{1}H-NMR spectroscopy. The enantiomeric excess of the product **2** was determined by means of chiral HPLC (**2a**: Chiralcel® column OJ-H, hexane : isopropanol 90 : 10, flow 0.8 mL min^{-1}, 230 nm; retention times t_R = 14.0 min, 16.7 min, or Chiralpak® column IB, hexane : isopropanol 95 : 5, flow 1.0 mL min^{-1}, 230 nm; retention times t_R = 11.6 min, 12.3 min; **2b**: Chiralcel® column OJ-H, hexane : isopropanol 90 : 10, flow 0.8 mL min^{-1}, 230 nm; retention times t_R = 20.8 min, 23.5 min, or Chiralpak® column IB, hexane : isopropanol 95 : 5, flow 0.8 mL min^{-1}, 230 nm; retention times t_R = 30.3 min, 34.6 min; **2c**: Chiralcel® column OJ-H, hexane : isopropanol 90 : 10, flow 0.8 mL min^{-1}, 230 nm; retention times t_R = 44.7 min, 49.7 min, or Chiralpak® column IB, hexane : isopropanol 95 : 5, flow 1.0 mL min^{-1}, 230 nm; retention times t_R = 41.3 min, 42.3 min).

*General procedure 2: Reduction of **1a–c** via enzyme-coupled cofactor recycling (method B)*

In a 100 mL Methrom Titrino reaction apparatus the ketone of type **1** (entries 1, 4, 7: 0.25 mmol) and NADP$^+$ (0.02 mmol) are dissolved in a mixture of phosphate buffer (5 mL, pH = 7, 0.05 M) and distilled water (95 mL), followed by adjustment of the pH at pH = 7 using a NaOH solution (0.2 M) and addition of the alcohol dehydrogenase LB-ADH (16 U; U refers to acetophenone as standard substrate) and glucose dehydrogenase from *Bacillus* sp. (from Amano Enzyme Inc., 8 U). After stirring the resulting reaction mixture for 24 h at room temperature at constant pH (pH = 7, adjusted by automatical dosage of a NaOH solution (0.2 M)), the aqueous phase was extracted three times with methylene chloride (100 mL). The combined organic phases were dried

over magnesium sulfate, filtered and concentrated *in vacuo*. The conversion of this reaction was determined from the resulting crude product by means of ^{1}H-NMR spectroscopy. The enantiomeric excess of the product **2** was determined by means of chiral HPLC (for chiral HPLC methods in detail, see general procedure 1).

Acknowledgement

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[1] Overview: P. J. Kocienski, *Protecting Groups*, Thieme, Stuttgart, **2000**.

[2] a) P. T. Anastas, J. C. Warner, *Green Chemistry – Theory and Practice*, Oxford University Press, Oxford, **2000**; b) R. A. Sheldon, I. Arends, U. Hanefeld, *Green Chemistry and Catalysis*, Wiley-VCH, Weinheim, **2007**.

[3] a) W. Hummel, K. Abokitse, K. Drauz, C. Rollmann, H. Gröger, *Adv. Synth. Catal.* **2003**, *345*, 153 – 159; b) H. Gröger, W. Hummel, S. Buchholz, K. Drauz, T. V. Nyugen, C. Rollmann, H. Hüsken, K. Abokitse, *Org. Lett.* **2003**, *5*, 173 – 176; c) H. Gröger, W. Hummel, C. Rollmann, F. Chamouleau, H. Hüsken, H. Werner, C. Wunderlich, K. Abokitse, K. Drauz, S. Buchholz, *Tetrahedron* **2004**, *60*, 633 – 640; d) H. Gröger, F. Chamouleau, N. Orologas, C. Rollmann, K. Drauz, W. Hummel, A. Weckbecker, O. May, *Angew. Chem.* **2006**, *118*, 5806 – 5809; *Angew. Chem. Int. Ed.* **2006**, *45*, 5677 – 5681; e) H. Gröger, C. Rollmann, F. Chamouleau, I. Sebastien, O. May, W. Wienand, K. Drauz, *Adv. Synth. Catal.* **2007**, *349*, 709 – 712; f) A. Berkessel, C. Rollmann, F. Chamouleau, S. Labs, O. May, H. Gröger, *Adv. Synth. Catal.* **2007**, *349*, 2697 – 2704.

[4] a) M. Kraußer, W. Hummel, H. Gröger, *Eur. J. Org. Chem.* **2007**, 5175 – 5179; b) E. Burda, W. Hummel, H. Gröger, *Angew. Chem.* **2008**, *120*, 9693 – 9696; *Angew. Chem. Int. Ed.* **2008**, *47*, 9551 – 9554; c) K. Baer, M. Kraußer, E. Burda, W. Hummel, A. Berkessel, H. Gröger, *Angew. Chem.* **2009**, *121*, 9519 – 9522; *Angew. Chem. Int. Ed.* **2009**, *48*, 9355 – 9358.

[5] Such a strategy based on protection of the hydroxy group in 4′-hydroxyacetophenone, asymmetric reduction with a chiral chlorodialkylborane and *in situ*-deprotection has been applied for the synthesis of (*S*)-1-(4-hydroxyphenyl)ethan-1-ol: E. T. Everhart, J. C. Craig, *J. Chem. Soc., Perkin Trans.* **1991**, *1*, 1701 – 1707.

[6] Overview: S. Buchholz, H. Gröger in: *Biocatalysis in the Pharmaceutical and Biotechnology Industries* (Ed.: R. N. Patel), CRC Press, New York, **2006**, chapter 32, pp. 757 – 790.

[7] Overview: A. Kleemann, J. Engel, B. Kutscher, D. Reichert, *Pharmaceutical Substances*, 5. ed., Thieme, Stuttgart, **2009**.

[8] a) W. Stampfer, B. Kosjek, K. Faber, W. Kroutil, *J. Org. Chem*. **2003**, *68*, 402 – 406; b) G. Kumaraswamy, S. Ramesh, *Green Chem.* **2003**, *5*, 306 – 308; c) J. S. Yadav, B. V. S. Reddy, C. Sreelakshmi, A. B. Rao, *Synthesis* **2009**, 1881 – 1885.

[9] a) M. Müller, M. Wolberg, T. Schubert, W. Hummel, *Adv. Biochem. Eng. / Biotechnol*. **2005**, *92*, 261 – 287; b) K. Niefind, B. Riebel, J. Müller, W. Hummel, D. Schomburg, *Acta Crystallogr.* **2000**, *D56*, 1696 – 1698; c) N. H. Schlieben, K. Niefind, J. Müller, B. Riebel, W. Hummel, D. Schomburg, *J. Mol. Biol.* **2005**, *349*, 801 – 813.

[10] A. Weckbecker, W. Hummel, *Biocat. Biotransf.* **2006**, *24*, 380 – 389.

[11] Available as commercial product (product number 1.1.030) from evocatal GmbH, Merowinger Platz 1a, 40225 Düsseldorf, Germany. See also: http://www.evocatal.com.