J. prakt. Chem. 339 (1997) 754–757

Journal für praktische Chemie
Chemiker-Zeitung

Das Reagenz · The Reagent

Horse Liver Alcohol Dehydrogenase (HLADH); Biocatalytic Redox-Transformations in Organic Synthesis

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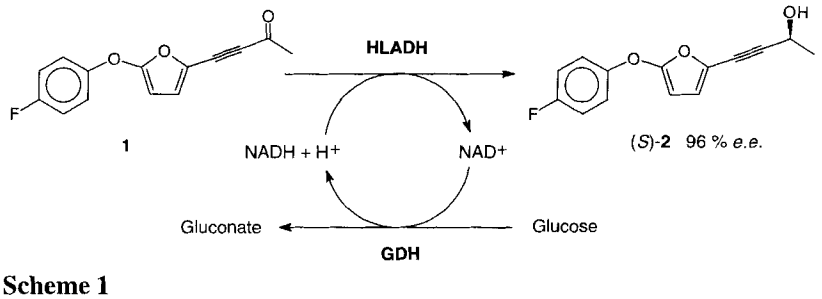
Eingegangen am 9. September 1997

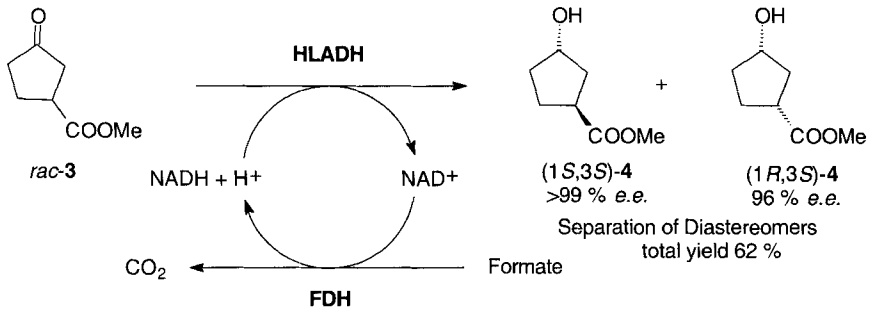
Biocatalysis has become a standard tool for the synthesis of enantiomerically pure compounds or for the economic preparation of large amounts of chiral building blocks [1, 2]. Of particular value are hydrolytic enzymes like esterases and lipases [3], since these enzymes do not require cofactors. Next to the hydrolases, the oxido-reductases cover the second largest area of synthetic operations towards chiral compounds [1]. One of the most prominent enzymes out of this class is the horse liver alcohol dehydrogenase, HLADH (E.C. 1.1.1.1.), a commercially available, NAD(H)-dependent biocatalyst [1, 2]. The enzyme catalyses the transfer of hydrogen atoms from one substrate to another by using the cofactor as a molecular shuttle for the hydrogen atoms. HLADH exhibits a unique combination of a very broad substrate tolerance with an almost invariable and predictable stereochemical course [4] allowing the discrimination of enantiotopic faces of prochiral compounds. In the reductive mode, the enzyme can be used for the kinetically controlled resolutions of racemic ketones and the production of chiral alcohols from prostereogenic ketones and isotopically labeled alcohols. Following Prelog's rule, the hydride is exclusively transferred from NADH or NAD^{2}H to the *re*-face of the carbonyl group [5] yielding homochiral (*S*)-alcohols. In the oxidative mode, the enzyme has found broadest application for the regio- and enantioselective oxidation of primary 1,4-diols to chiral γ-lactones (*vide infra*). Other functional groups like alkynes, alkenes, (thio)ethers, nitriles and esters are tolerated, and even the bioconversion of organosilicon [6] and sensitive organometallic [7] compounds has been described. Only amines and chelating agents may provide difficulties by complexing the essential Zn^{2+} metal at the active sites of the dimeric enzyme.

While the enormous potential of HLADH-mediated oxido-reductions was discovered early, the major disadvantage which hampered a general use in preparative organic synthesis is the need for a coupled, continuous cofactor recycling. In the simplest case this is achieved by addition of a co-substrate to the system which delivers or consumes the hydride produced or needed by the enzymatic transformation (coupled-substrate-recycling). However, the efficiency of the overall process is seriously limited by the equilibrium constants of the coupled reactions. More effective is the coupled-system-recycling which combines two different oxido-reductases, *e.g.* HLADH and GDH (glucose dehydrogenase), a substrate and a co-substrate (*e.g.* glucose) for integrated cofactor recycling. In Scheme 1 the irreversible oxidation of glucose into gluconate shifts the equilibrium between NAD^+ and NADH towards the reduced cofactor and, hence, facilitates the enantioselective reduction of the ketone **1** to (*S*)-**2**. The alcohol (*S*)-**2** served as a key-intermediate for the synthesis of one enantiomer of a lipoxygenase inhibitor [8].

A regeneration system using HLADH together with formate dehydrogenase (FDH), *e.g.* from *Candida boidinii*, that catalyses the irreversible oxidation of formate to CO_2 (Scheme 2) is even more advantageous [9]. The system is economic for large scale reductions and can be used for the preparation of NADH and NAD^{2}H as well (from $^2HCOONH_4$) [10].The



Scheme 1

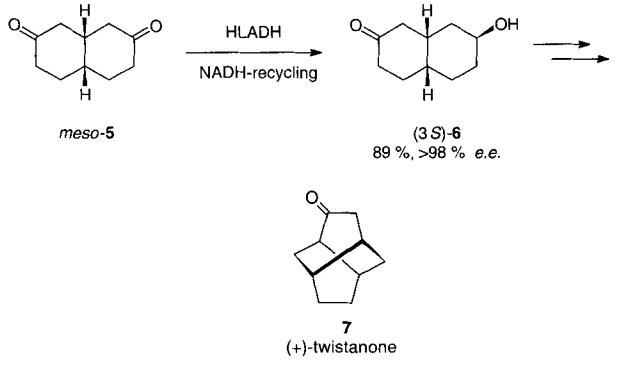


Scheme 2

reduction of pyruvate to lactate by LDH (lactate dehydrogenase) can be employed for the generation of NAD^+ [11].

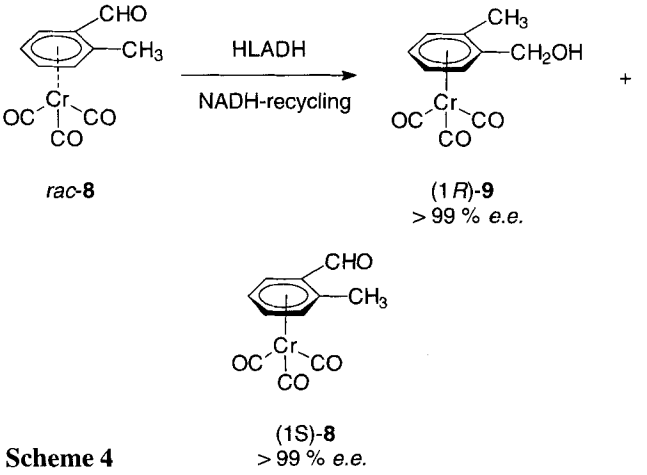
Owing to the well defined and predictable orientation of the substrate at the active center of the enzyme [4], the keto-ester *rac*-**3** is transformed into a pair of diastereomers, (1*S*, 3*S*)-**4** and (1*R*,3*S*)-**4**, that is readily separated (Scheme 2) [12]. Frequently, only one enantiomer is reduced, yielding optically active alcohol and ketones.

Besides simple cyclic and polycyclic ketones [13], also a great number of *meso*-diketones has been reduced to the corresponding mono-alcohols in good yield and high *e.e.* In these examples the regio- and enantioselectivity, as well as the enantioface differentiation are controlled concurrently, and isomerically pure starting materials for the synthesis of terpenoids or cage-shaped molecules become available. Thus, a convenient route to (+)-twistanone **7** has been realised *via* dissymmetrisation of the bicyclic *meso*-decalindione **5** by reduction with HLADH to the key intermediate (3*S*)-**6** in high chemical and optical yield [14]. Unsaturated decalins of type **5** give lower yields (ca. 20%) than the saturated substrates (Scheme 3).



Scheme 3

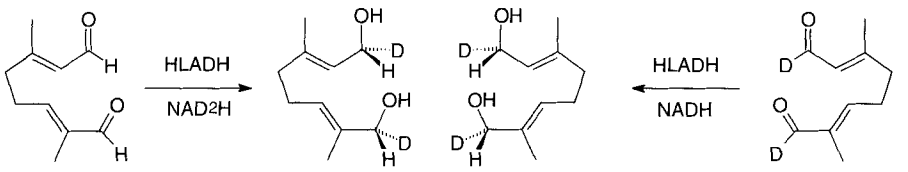
An impressive example for the capacity and the broad substrate tolerance of HLADH is provided by the enantioselective reduction of organometallic compounds like *rac*-**8** (Scheme 4). The planar chiral tricarbonyl chromium complexes (1*R*)-**9** and (1*S*)-**8** are obtained with exceedingly high enantiomeric excess (>99% *e.e.*) [7].



Scheme 4

HLADH mediated reductions provide a convenient access to deuterium or tritium labeled alcohols of high enantiomeric excess (>98% *e.e.*), that are required for the evaluation of the stereochemical course of unknown redox-transformations in metabolism. As a consequence of the strict *re*-facial transfer of the hydride to the carbonyl group, (1*S*)-[1-$^2H_1$]alcohols are obtained from HLADH promoted reduction of 1-^2H labeled aldehydes (R-C^{2}HO). (1*R*)-[1-$^2H_1$]alcohols result from a transfer of a deuteride (*via* NAD^2H, *vide supra*) to the unlabeled aldehyde (R–CHO). Along this permutation concept the homochiral hydroxygeraniols (1*R*,8*R*)-[1,8-$^2H_2$]-**11** and (1*S*,8*S*)-[1,8-$^2H_2$]-**11** were recently synthesised from the dialdehydes **10** and [1,8-$^2H_2$]-**10**, respectively (Scheme 5). The compounds served as chiral probes in iridoid biosynthesis of leaf beetle larvae [15].

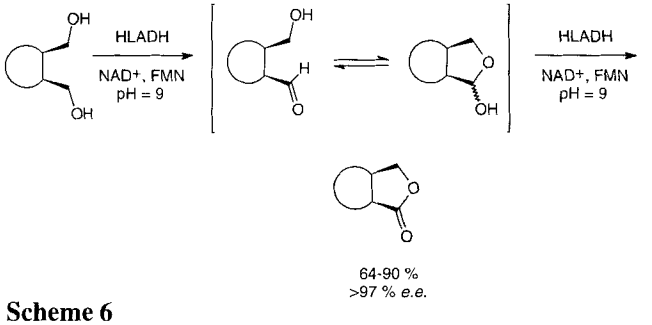
In the oxidative mode, HLADH has been most frequently used for the enantioselective oxidation of *meso-diols* to homochiral lactols and lactones, which is not trivial to perform with classical synthetic methods. In general, HLADH accepts primary and secondary racemic alcohols as well as prostereogenic mono- and dihydroxy compounds. For small scale transformations technical grade flavine mononucleotide (FMN) can be used for coupled-substrate-recycling. A coupled-system-regeneration, based on GluDH (glutamate dehydrogenase) which reduces 2-oxoadipic acid to L-α-amino adipic acid with consumption of hydride equivalents is the method of choice for large scale oxidations [16]. The ideal substrates for HLADH-catalysed oxidations are the *cis meso*-



10 (1*R*,8*R*)-[1,8-$^2H_2$]-**11** ≥ 95 % *e.e.* (1*S*,8*S*)-[1,8-$^2H_2$]-**11** ≥ 95 % *e.e.* [1,8-$^2H_2$]-**10**

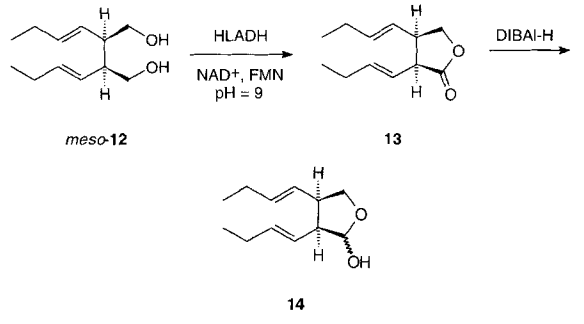
Scheme 5

1,4- and *meso*-1,5-diols depicted in Scheme 6. Without exception, these substrates are attacked at the pro-*S* hydroxymethyl group providing hydroxy-aldehydes which immediately cyclise to hemiacetals followed by further oxidation to the stable (1*S*)-γ- and (1*S*)-δ-lactones. This convenient and stereochemi-cally predictable one-step synthesis of chiral γ- and δ-lactones has been successfully carried out with a wide structural range of acyclic and cyclic *meso*-diols, including heterocyclic and bridged [17] substrates. The chemical (64–90%) and optical yields (>97%) are generally high and independent of the ring size or the substitution pattern [18].



Scheme 6

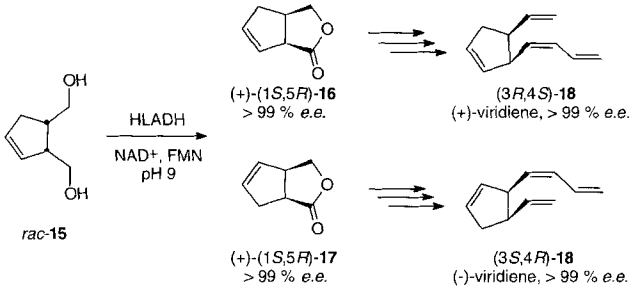
Following this methodology, the absolute configuration of **14**, the sexual pheromone of the spined citrus bug (*Biprorulus bibax*) was firmly established. As the key reaction, the diol **12** was oxidised by HLADH to the lactone **13**. Subsequent reduction provided the enantiomerically enriched hemiacetal **14** [19] (Scheme 7).



Scheme 7

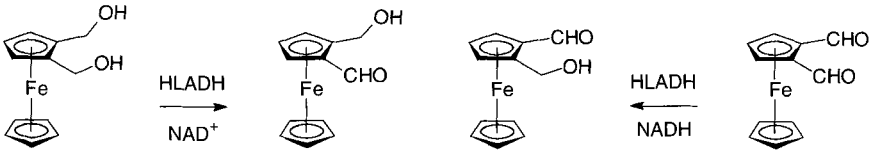
In an enantiodivergent fashion, immobilised HLADH [20] was used to oxidise both enantiomers of the non-*meso* diol *rac*-**15** into a mixture of two diastereoisomeric chiral lactones, (+)-(1*S*,2*R*)-**16** and (+)-(1*S*,2*R*)-**17**, respectively. Chromatographic separation provided the two isomeric lactones in good yield and >99% *e.e.* and individual transformations furnished the enantiopure (+)-viridiene, (+)-**18**, a sexual pheromone of several marine brown algae and its antipode, (–)-**18** [21] (Scheme 8).

The HLADH promoted oxidation of the ferrocene diol *meso*-**19** and the reduction of the corresponding dialdehyde **20** provided the first examples of planar chiral organometallic compounds obtained with a biocatalyst [22, 7] (Scheme 9). Again, the invariant stereochemical course of the HLADH-catalysed transformations is responsible for the same enantiotopos differentiation of the *meso*-compounds in both directions, *i.e.* oxidation and reduction. [22].



Scheme 8

In summary, the HLADH-catalysed transformations provide an experimentally simple and rapid access to chiral precursors with predictable absolute stereochemistry. The reactions proceed very cleanly, and often the products can be isolated by simple extraction into organic solvents. The problem of cofactor regeneration is principally solved, and a number of approved chemical [6, 23], electrochemical [24–27] and enzymatic methods are available today. Artificial cofactors, that mimic the adenine moiety of NAD and allow the monitoring of the reaction [28], or poly(ethylene glycol)-bound NAD (PEG-NAD$^+$) [29] for the use in continuous flow reactors have been successfully developed. Moreover, the application of biphasic systems, immobilized or noncovalent entrapped enzymes [30, 31] in anhydrous organic solvents represent other strategies by which high chemical yields of thermodynamically unfavored products may be achieved. Finally, it should be noted that besides HLADH, many other alcohol dehydro-



meso-**19** (1*R*)-**21** 81 %, 86 % *e.e.* (1*S*)-**21** 80 %, 94 % *e.e.* *meso*-**20**

Scheme 9

genases are commercially available [1]. Owing to their individual substrate tolerance and sometimes complementary stereoselectivity, biocatalytic oxido-reductions provide a generally applicable method for the (large scale) synthesis of optically active starting materials.

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