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COMMUNICATION

Oxidation-hydroxymethylation-reduction: a one-pot three-step biocatalytic synthesis of optically active α-aryl vicinal diols

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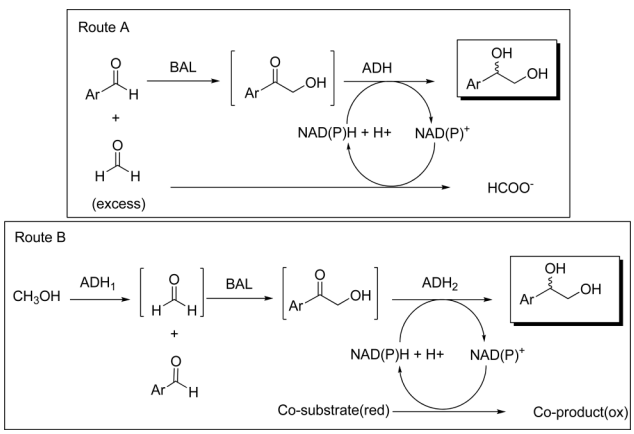
A one-pot multi-step approach comprising enzymatic oxidation-hydroxymethylation-reduction enables the synthesis of optically active α-aryl vicinal diols with high yields and enantioselectivities. Formaldehyde required for the hydroxymethylation step is enzymatically produced *in situ* using less hazardous methanol as substrate.

The production of enantiopure building blocks for manufacturing pharmaceuticals or biologically-active compounds usually involves elaborate multi-step synthetic protocols. Typically, isolation of intermediates and tedious protection-deprotection steps are needed, often leading to low yields and high E-factors. One powerful approach towards sustainable organic synthesis lies in the development of one-pot multi-step syntheses, *e.g.* enzyme cascades inspired by the *in vivo* biosynthesis. For the successful implementation of one-pot catalytic multi-step reaction systems, stringent conditions need to be orchestrated for optimal results. Reaction rates need balancing; compatibility of catalysts has to be achieved; and productivity has to be optimized.[1–3] A few multi-step catalytic processes have been reported, comprising excellent examples of enzymatic and metal-enzyme combinations.[4–10] Furthermore, recent elegant approaches combine organo- and biocatalysis as well.[11–12]

In this communication, we disclose a one-pot multi-enzymatic concept utilizing oxidoreductases and benzaldehyde lyase (BAL), a thiamine-diphosphate-dependent (ThDP) lyase, for the production of optically active α-aryl vicinal diols, which are valuable building blocks for fine chemicals and pharmaceuticals.[13] The use of multi-step biocatalytic reactions for the production of these important building blocks has been testimonial, with only few examples involving lyases and oxidoreductases (in two-pot reactions),[14] or chemo-catalysis.[15,16] Herein, the core step – a C–C bond formation – is enabled by the capability of BAL to catalyze the hydroxymethylation of aromatic aldehydes with formaldehyde to afford α-aryl-2-oxo-1-hydroxy ketones.[17–20] Alternatively, this step could also be conducted *via* carbene-based organocatalysis,[21–23] mimicking the function of ThDP-lyases.[24–26] Yet, in itself, the efficient organocatalytic approach requires either stoichiometric amounts of bases or elevated temperatures, thus combining this with enzymatic steps in one-pot could prove difficult.

In modern sustainable synthesis the required efficient and selective protocols need to be aligned with minimal waste production. Hazardous chemicals must be avoided or at least efficiently converted to safer chemicals. To combine these requirements with the supply of formaldehyde for the BAL-catalyzed hydroxymethylation[17,18] two strategies were devised: A) Co-utilization of formaldehyde for cofactor regeneration in the subsequent alcohol dehydrogenase (ADH) catalyzed enantioselective ketone reduction (Scheme 1, route A); or B) Enzymatic *in situ* formation of formaldehyde through oxidation of the less hazardous methanol (Scheme 1, route B).

As first step, the BAL-catalyzed carboligation of benzaldehyde and formaldehyde was carefully assessed to minimize the excess of formaldehyde needed to allow quantitative conversion in **1**, as the competitive formation of benzoin potentially reduces



Scheme 1 Conceptual outline for the multi-step biocatalytic reactions.

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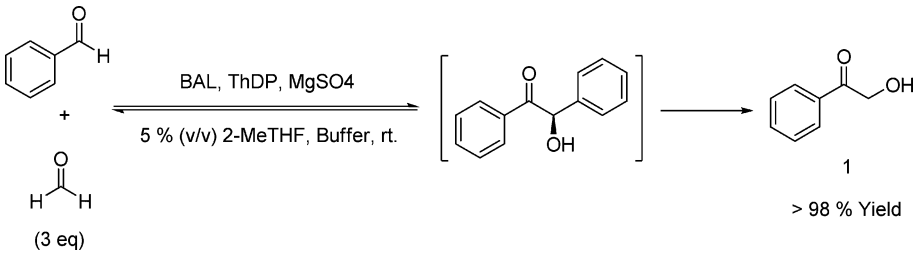
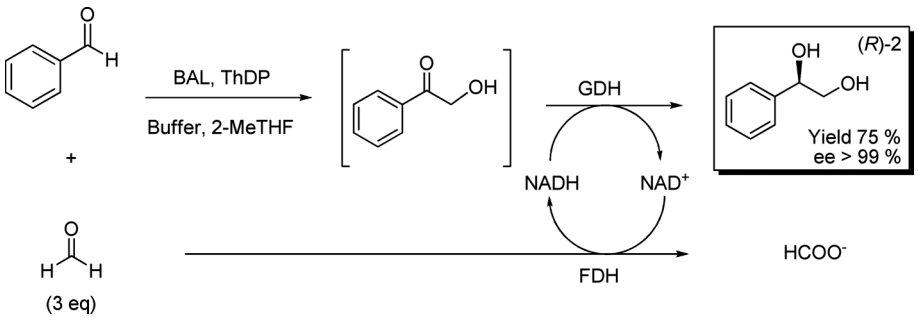


Scheme 2 BAL-catalyzed hydroxymethylation. 20 mL phosphate buffer 50 mmol L^{-1}, pH 8.0, 5% v/v 2-MeTHF, 0.25 mmol benzaldehyde, 0.75 mmol formaldehyde, 2.5 mmol L^{-1} $MgSO_4$, 0.15 mmol L^{-1} ThDP, 10 U BAL.



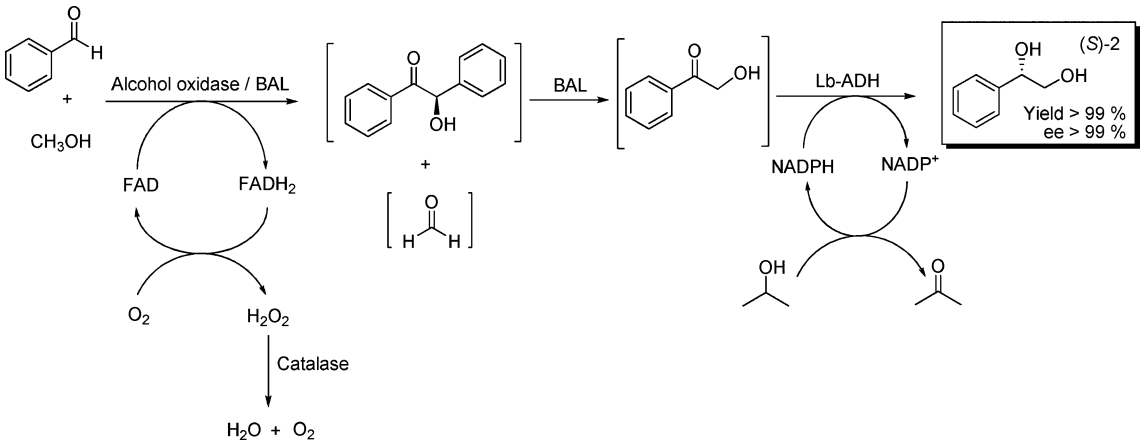
Scheme 3 Enzymatic multi-step process for the enantioselective synthesis of (*R*)-**2**. 10 mL phosphate buffer 50 mmol L^{-1}, pH 8.0, 5% v/v 2-MeTHF, 0.13 mmol benzaldehyde, 0.4 mmol formaldehyde, 2.5 mmol L^{-1} $MgSO_4$, 0.15 mmol L^{-1} ThDP, 10 U BAL, 354 U GDH, 21 U FDH, NADH/NAD^+ 0.5 mmol L^{-1} each.

overall selectivity. 2-Methyltetrahydrofuran (2-MeTHF) was employed as (bio-based) co-solvent, since it provides an excellent operational window for BAL, while avoiding significant formation of wastewater and further environmental issues associated with other co-solvents (*e.g.* DMSO, MTBE), leading to a diminished E factor in the process.[27] Thus, a 3-fold excess of formaldehyde assured quantitative conversion in α-aryl-2-oxo-1-hydroxy ketone **1** (Scheme 2).

Once the reaction conditions of the enzymatic hydroxymethylation were optimized, the next step was to *capitalize* the excess of formaldehyde (Scheme 2) as ancillary substrate (cofactor regeneration) for the enantioselective ketone reduction. To this end, glycerol dehydrogenase from *Cellulomonas* sp. (GDH) was selected for the enantioselective reduction of **1**.[28] For cofactor regeneration (NADH), formaldehyde dehydrogenase from *Pseudomonas putida* was chosen.[29] After careful optimization of absolute and relative enzyme and cofactor loadings with reaction times, high conversion and excellent enantioselectivity for the desired product diol (*R*)-**2** were achieved (isolated yield 75%, ee > 99%) (Scheme 3).

Alternatively, to avoid issues related to toxicity inherent to formaldehyde (still one equivalent present after hydroxymethylation and oxidation steps), the enzymatic *in situ* formaldehyde formation and consumption was assessed, thus providing the basis for a "formaldehyde-free" hydroxymethylation. To this end, the FAD-dependent alcohol oxidase from *Hansenula* sp. proved to be an efficient catalyst for the oxidation of methanol with oxygen.[30,31] The coupled product H_2O_2 was converted by catalase, regenerating oxygen in solution and avoiding detrimental effects to the enzymes. Firstly reactions were separated in time, that is, enzymatic methanol oxidation being conducted for 8 h, followed by BAL and benzaldehyde addition for additional 16 h. **1** was isolated in ~93% yield along with traces of benzoin. Presumably, enzymatic oxidation steps are slower compared to the C–C ligation reaction. By systematically varying enzyme loadings, yield of **1** was quantitative, and remarkably colored impurities were diminished, compared to route A (with addition of excess of formaldehyde). Finally, since no deactivation of BAL in the presence of methanol or alcohol oxidase was observed, oxidation and hydroxymethylation were combined in one step. Gratifyingly, **1** was again formed in quantitative conversion. Consequently, all three reactions were conducted in a one-pot sequential array: first the *in situ* oxidation of methanol to formaldehyde, followed by concomitant BAL-catalyzed cross condensation with benzaldehyde, and followed by *Lb*-ADH addition to afford (*S*)-**2** quantitatively (isolated yield) with *ee* > 99% (Scheme 4). It is important to mention that in this case NADPH-dependent alcohol dehydrogenase from *Lactobacillus brevis* (*Lb*ADH) was used,[32] affording the (*S*)-enantiomer. Thus, by smartly using different enzymes of a toolbox, access to both enantiomers might be achieved.

Finally, the methodology was extended to more challenging aromatic aldehydes like furfural. Furan ethane diols can be used as building blocks for the preparation of levoglucosenone, a valuable synthon for biologically-sound intermediates.[33–35] Traditional synthetic approaches involve Lewis acid-catalyzed glucal rearrangement, typically with poor enantioselectivity.[33–35] Since furfural-based diols are unstable, they were chemically dibenzoylated *in situ*. The first approach following route A



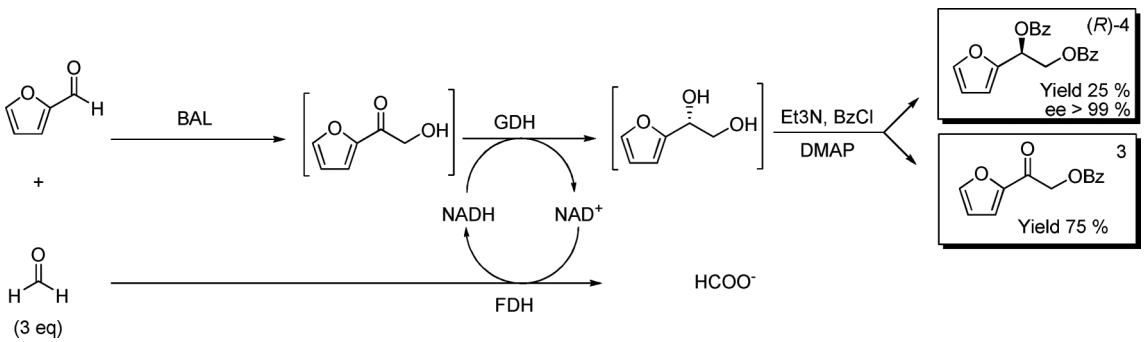
Scheme 4 Four enzymes in one-pot reaction: *In situ* oxidation-hydroxymethylation-reduction. 20 mL, phosphate buffer 50 mmol L^{-1}, pH 8.0, 5% v/v 2-MeTHF, 0.12 mmol benzaldehyde, 0.90 mmol methanol, 33 U alcohol oxidase, 14 U catalase, 2.5 mmol L^{-1} $MgSO_4$, 0.15 mmol L^{-1} ThDP, 40 U BAL, 10 U *Lb*ADH. 0.5 mmol L^{-1} NADPH, 0.1 mmol L^{-1} FAD.

(Scheme 1) provided a successful proof-of-principle, with low isolated yields (25%) albeit with excellent (*R*)-enantioselectivity (Scheme 5).

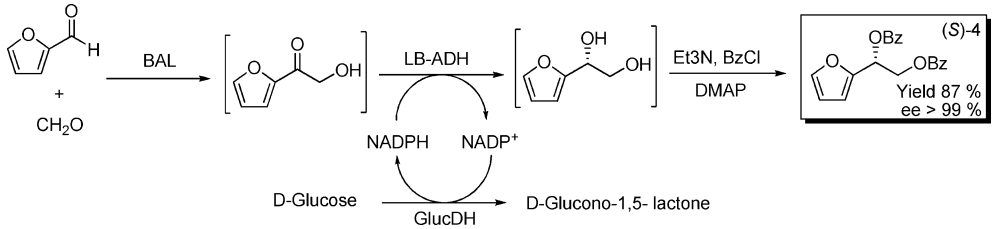
With the considerable amount of **3** as intermediate despite the higher amount of GDH applied, the bottleneck was supposed to be at low activity of GDH towards furan compounds. In fact, when *Lb*ADH was applied, with glucose dehydrogenase (GlucDH) for cofactor regeneration, this resulted in excellent isolated yields (87%) and enantioselectivity (>99% ee, (*S*)) (Scheme 6). Therefore, the herein reported conceptual approach may accept other enzymes with different enantioselectivity or different bias towards organic molecules. Due to the ample portfolio of oxidoreductases already available,[31,36] further successful combinations may be expected.

By means of these strategies a significant reduction of the E factor can be achieved, since the amount of organic solvent (for product extraction), as well as water for the enzymatic process can be reduced to one third, due to the setup of just one final downstream processing. Importantly, these inherent environmental advantages of the herein reported concept still need to be complemented with optimized reaction times, substrate loadings and less enzyme concentrations, eventual biocatalyst recyclability, *etc.* In this respect, a promising approach for future research may be the incorporation of whole-cells with overexpressed enzymes, as it has been successfully reported for lyases or oxidoreductases in processes with enhanced enzyme stability and higher substrate loadings.[37,38] With such implementation at hand, the smart combination of enzymatic cascade processes together with high productivities and efficiencies may be reached.

In summary one-pot multi-step biocatalytic concepts have been reported, comprising oxidation, hydroxymethylation, and a further reduction to afford chiral α-aryl vicinal diols. By *in situ* production or its subsequent utilization as reducing agent, the use of hazardous formaldehyde is addressed. Another advantage is that the concept allows the use of different oxidoreductases, providing access to both enantiomers ("on-demand") and furan derivatives. Future sustainable (industrial) processes will adopt more and more such highly integrated multi-catalytic steps with diminished waste formation, together with optimized substrate concentration and enzyme loadings.



Scheme 5 Biocatalytic formation of chiral furane-diols *via* route A. 40 mL phosphate buffer 50 mmol L^{-1}, pH 8.0, 5% v/v 2-MeTHF, 1.6 mmol furaldehyde, 4.8 mmol formaldehyde, 2.5 mmol L^{-1} $MgSO_4$, 0.15 mmol L^{-1} ThDP, 168 U BAL, 1500 U GDH, 95 U FDH, NADH/NAD^+ 1 mmol L^{-1} each.



Scheme 6 Biocatalytic formation of chiral furan-diols. 40 mL phosphate buffer 50 mM, pH 8.0, 5% v/v 2-MeTHF, 1.6 mmol furaldehyde, 3.6 mmol glucose, 4.4 mmol $CaCO_3$, 4.8 mmol formaldehyde, 2.5 mmol L^{-1} $MgSO_4$, 0.15 mmol L^{-1} ThDP, 168 U BAL, 50 U *Lb*-ADH, 2800 U GlucDH, 0.5 mmol L^{-1} NADPH.

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