Asymmetric Catalysis

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Highly Enantioselective Reduction of α-Methylated Nitroalkenes**

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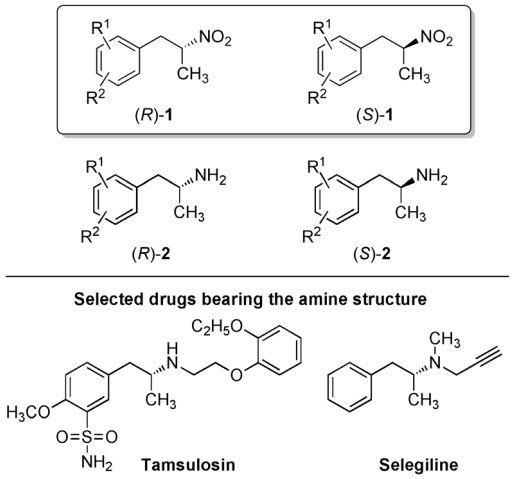
The enantioselective preparation of α-substituted nitroalkanes of type **1** is of high interest due to the use of the corresponding amines **2** in the synthesis of pharmaceuticals.[1] Representative examples for commercial drugs based on such amines are Tamsulosin and Selegiline (Scheme 1). Due to the lack of suitable enantioselective catalytic synthetic methodologies, these drugs are produced in a laborious fashion either by chiral resolution (at the final stage of a suitable amine) or by a reaction relying on a chiral auxiliary (used in stoichiometric amount and not recyclable).[1,2]



Scheme 1. General structure of nitroalkenes of type **1** as precursors for amines of type **2** and selected related drugs.

An attractive alternative synthetic approach would be based on the enantioselective reduction of α-methylated nitroalkenes of type **5** as a key step. The concept of this process as well as its integration in the preparation of amines and related derivatives is shown in Scheme 2. The *trans* substrates **5** are easily accessible starting from economically attractive and readily available industrial chemicals (aldehydes, nitroethane), and methods have been reported for the final transformation of the nitro group into an amino group with retention of the absolute configuration.[3] However, hitherto reported processes for the enantioselective reduction of nitroalkenes **5** in the presence of chemocatalysts as well as enzymes only led to low to moderate and in a few cases good enantioselectivities. For instance, in the presence of metal-containing hydrogenation catalysts maximum enantioselectivities of 58% *ee* are obtained for the nitroalkanes **1**.[4] Alternatively, due to the achievements in the enzymatic reduction of activated C=C bonds of a multitude of substrate classes,[5] the enantioselective reduction of α-methylated nitroalkenes **5** using ene reductases was also studied.[5,6] Despite intensive efforts, however, until recently such enzyme-catalyzed processes for these substrates have given only enantioselectivities of 0–48% *ee* in most cases and 70% *ee* at best.[6a–g] In a current study, the enantioselectivity has been increased up to 84% *ee*.[6h] A range of reasons for this were identified, such as the high CH acidity and the resulting sensitivity of nitroalkanes **1** towards racemization,[7] as well as the general low enantioselectivity of enzymes in the reduction of substrates of type **5**.[6] One difficulty may arise from the fact that the stereogenic center is not formed in the initial addition of the hydride, but in the subsequent protonation of the resulting carbanion;[8] the control of enantioselectivity in such asymmetric protonations is generally considered to be difficult. A further demanding task is avoiding the competing enzymatic Nef reaction,[9] which transforms nitroalkenes **5** into the corresponding ketones. Accordingly (and independent of the type of catalyst), the development of a highly enantioselective C=C reduction of nitroalkenes of type **5** is still regarded as a challenge. Herein we report the first highly enantioselective process for the reduction of α-methylated *trans*-nitroalkenes **5** to provide nitroalkanes **1** with enantiomeric excesses of typically 90–95% *ee*; the catalyst is a particularly suitable enzyme in an efficient enzyme formulation.

For our screening for suitable enzymes for this reduction, we chose a range of enzymes often used in synthesis as well as an ene reductase from *Gluconobacter oxydans*.[10] This enzyme, which was recently identified by Hummel et al., is available in recombinant form but has been rarely used in synthesis so far. Interestingly, in contrast to other utilized ene

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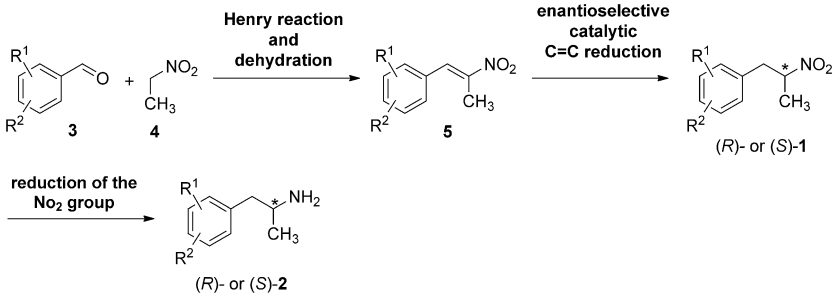
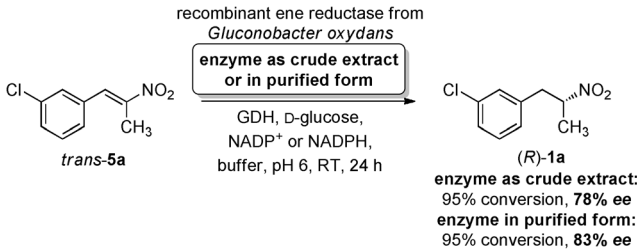


Scheme 2. Synthetic concept for the construction of chiral amines of type (*R*)- or (*S*)-**2** based on an enantioselective catalytic reduction of nitroalkenes **5**.

reductases, this enzyme, which we first used in the form of a crude extract typical for biotransformations, turned out to be very suitable already in the initial experiments for the reduction of *trans*-nitroalkene **5a**. For instance, for the reduction of **5a** the formation of product (*R*)-**1a**[11] with an enantioselectivity of 78% *ee* (which is already high for this type of reaction) was observed (Scheme 3). However, we also found that the enantioselectivity changed with the increasing



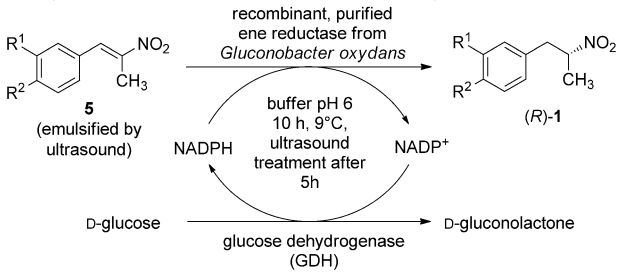
Scheme 3. Dependence of the ene reductase catalyzed reductions on the purity of the biocatalyst. GDH = glucose dehydrogenase.

storage time of the enzyme.[12] We assumed that this surprising result arises from an undesired "background activity" caused by a further, non-enantioselective or minor enantioselective ene reductase.[13] On examination of the known genome of *E. coli* we assigned this background activity to the following enzymes found in *E. coli*: nemA (accession no. NP_416167, "Old Yellow Enzyme" family)[14] and NfsB (YP_002998379, nitroreductase family). This explanation prompted us to study the activity of the crude extract originating from the host organism *E. coli* (without expression of the ene reductase from *G. oxydans*). The use of this crude extract from *E. coli* then led to the expected reduction of **5a** to give **1a** with low enantiomeric excess (< 3% *ee*), which indicates a nearly non-enantioselective reaction course. In order to avoid this undesired background activity of the *E. coli* host organism, we were interested in using the recombinant enzyme component, which is overexpressed in *E. coli*, in purified form. Toward this end, the ene reductase from *G. oxydans* was heterogeneously overexpressed as an N-terminal hexahistidine fusion construct in *E. coli* so that the enzyme could be purified simply by immobilized metal ion affinity chromatography. The use of this purified form of the enzyme resulted in the expected further increase of the enantioselectivity up to 83% *ee* for the formation of product **1a** (Scheme 3).[15]

Surprisingly, during investigation of the synthetic process the adjustment of three more parameters, which are often considered to be less important in asymmetric biocatalysis, led to further remarkable improvements. Thus, the enantioselectivity could be increased by using the purified recombinant ene reductase at decreased reaction temperatures of 5–15°C. Here it should be noted that in contrast to chemocatalytic processes the increase of the enantioselectivity of biotransformations by lowering the reaction temperature is known to a much lesser extent.[16] However, the observed beneficial effect of a decreased reaction temperature of 5–15°C on the *ee* value can also be caused by a decelerated racemization of nitroalkane **1a**. Furthermore, shortening the reaction time also improved the enantioselectivity. Interestingly, we could exclude a racemization of the formed nitroalkane **1a** caused by the reaction medium, the glucose dehydrogenase, and the inactivated ene reductase (for the corresponding experiments, see the Supporting Information). To ensure a high conversion and high robustness of the synthetic process, ultrasound treatment of the mixture of the nitroalkenes in buffer (prior to the addition of enzymes) and further ultrasound treatment of the reaction mixture after a reaction time of 5 h turned out to be advantageous. This could be due to the improved mixing of the heterogeneous reaction mixture containing the nitroalkene **5**, which is hardly soluble in water. Subsequently, these optimized reaction conditions were applied to the reduction of a range of nitroalkenes **5** on an increased 100 mL scale (Table 1). The desired stereoselective enzymatic reductions of the *m*-halogen-substituted nitroalkenes **5a**–**c**, which were used initially as model substrates, proceeded with excellent (overall) conversions of 96 to > 99% and very good enantioselectivities of 93–95% *ee* (Table 1, entries 1–3). The workup used for entries 2 and 3 led to the products (*R*)-**1b**,**c**, in each case in a high yield of 84%. The reaction of the *m*-methoxy-substituted nitroalkene **5d** also proceeded with high (overall) conversion (95%) and a high enantioselectivity of 93% *ee*, which is excellent for this kind of reaction (Table 1, entry 4). This indicates—as desired with respect to a broad application—that the enzymatic process proceeds with high enantioselectivity independent of the type of substituent in *meta* position. The reduction of the *p*-substituted nitroalkenes **5e**,**f**, however, gave decreased enantioselectivities of 81% and 66% *ee*, respectively (Table 1, entries 5 and 6). In turn we were pleased to find a high enantioselectivity of 90% *ee* for the reaction of nitroalkene **5g** bearing a nonsubstituted phenyl group (Table 1, entry 7). Thus, with this practical and easy-to-conduct enzymatic reduction process, α-methyl-substituted nitroalkenes of type **5** can be reduced enzymatically for the first time with enantioselectivities exceeding 90% *ee*.

An additional focus of our work was on the integration of the developed process into an improved total synthesis of nitroalkenes **5** starting from aldehydes as substrates. Here, the

Table 1: Enzymatic reduction of nitroalkenes **5** under optimized conditions.

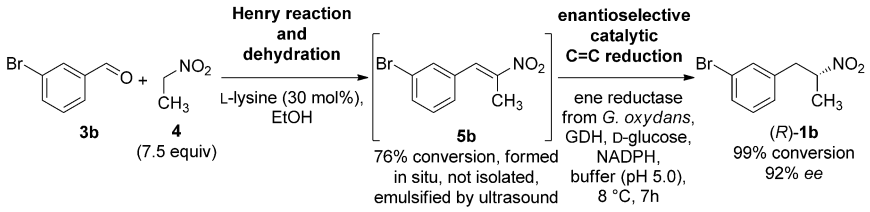


Entry[a]	**1**	R^1	R^2	Overall conv. [%]	Conv. to prod. **1** [%][b,c]	*ee* [%][e]
1	**1a**	Cl	H	96	95	94
2	**1b**	Br	H	>99	>99 (84)[d]	95
3	**1c**	I	H	99	99 (84)[d]	93
4	**1d**	OCH_3	H	95	93	93
5	**1e**	H	Br	31	27	81
6	**1f**	H	OCH_3	53	30	66
7	**1g**	H	H	76	71	90

[a] For the experimental procedure, see the Supporting Information; the corresponding conversions were determined by ^{1}H NMR analysis of the crude product. [b] The conversion to product **1** is defined as the amount of product **1** obtained relative to the amount of substrate consumed (in %). [c] The difference between the overall conversion and the conversion to product **1** corresponds to the amount of the corresponding Nef product formed as a side product in entries 1 (1%), 4 (2%), 5 (4%), 6 (23%), and 7 (5%). [d] The values given in parentheses refer to the yield of the corresponding compound obtained after workup and purification. [e] The enantiomeric excess was determined by HPLC on a chiral stationary phase.

goal was to develop an attractive two-step synthesis of the nitroalkanes (*R*)-**1** through the combination of a simple process for the in situ synthesis of nitroalkenes **5** with a direct subsequent enzymatic enantioselective reduction. This synthetic route, which avoided workup of nitroalkenes **5**, was achieved by the organocatalytic condensation of an aldehyde and nitroethane, followed by the direct use of the resulting reaction mixture in the biocatalytic reduction. A representative example is shown in Scheme 4: Here the condensation of 3-bromobenzaldehyde (**3b**) with nitroethane in the presence of L-lysine as the organocatalyst proceeds with 76% conversion, and the enzyme-catalyzed direct transformation of the resulting nitroalkene **5b** yields the desired product (*R*)-**1b** with 99% conversion and 92% *ee*.

In summary, we have reported the first highly enantioselective process for the reduction of α-methylated *trans*-nitroalkenes **5** to provide desired nitroalkanes of type (*R*)-**1** with enantiomeric excesses of up to 95% *ee*. Furthermore, this synthetically practical process was successfully integrated into a chemoenzymatic total synthesis, which consisted of an organocatalytic conversion of nitroethane and an aldehyde into the nitroalkene **5** and its direct subsequent enzymatic enantioselective reduction. Key aspects of the current research are, besides work on enzyme optimization and process development, the application of this new process for alternative syntheses of pharmaceuticals. In addition, the study of ene reductases, which are known but have not yet displayed high enantioselectivity, in purified form as well as under optimized reaction conditions appears to be worthwile.



Scheme 4. Combination of an in situ synthesis of the nitroalkene **5b** and its enantioselective enzymatic reduction.

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