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A Biocatalytic Henry Reaction—The Hydroxynitrile Lyase from *Hevea brasiliensis* Also Catalyzes Nitroaldol Reactions**

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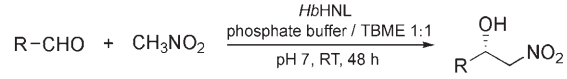
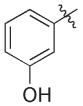
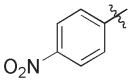
Hydroxynitrile lyases (HNLs) are a family of versatile enzymes that catalyze the reversible cleavage of α-hydroxy nitriles and are utilized for the production of enantiopure cyanohydrins from aldehydes or ketones and HCN.[1] Several HNLs from different sources have been identified, and the HNL-catalyzed synthesis of a large number of cyanohydrins with *R* and *S* configuration with excellent stereoselectivity has been demonstrated.[2] In trying to expand the synthetic applicability of the HNL methodology we considered replacing HCN by other nucleophiles to be added to carbonyl compounds catalyzed by these enzymes. Based on the mechanism of this biotransformation[3] crucial parameters for such alternative reagents would be the molecular size and the pK_a of the CH-acidic portion, which should be similar to that of HCN (p$K_a \approx 9$). One important substance class that meets these criteria are nitroalkanes. Their reaction with carbonyl compounds—known as the nitroaldol or Henry reaction—constitutes a carboligation process of high synthetic value. The Henry reaction furnishes vicinal nitroalcohols, which can easily be transformed to a series of valuable intermediates such as, for example, 1,2-aminoalcohols and α-hydroxycarboxylic acids.[4]

First, we examined the addition of nitromethane to aldehydes in the presence of the hydroxynitrile lyase from *Hevea brasiliensis* (*Hb*HNL). The reaction of benzaldehyde with nitromethane gave 2-nitro-1-phenylethanol in 63% yield with an enantiomeric excess of 92% (Table 1). Besides the expected nitroalcohol product, small amounts (10–15%) of the corresponding elimination product, 1-nitro-2-phenylethene, was detected as the only by-product. By comparing optical rotation data of the product with literature values the absolute configuration of the product was determined to be *S*,[5] which is in agreement with the known stereopreference of *Hb*HNL in cyanohydrin reactions.

Table 1: Stereoselective addition of nitromethane to aldehydes in the presence of *Hb*HNL.[a]



R	Yield [%]	*ee* [%][b]
	63	92
	46	18
	77	28
	57	72
$CH_3(CH_2)_5$	25	89

[a] TBME = *tert*-butyl methyl ether. [b] The absolute configuration of the products was assigned by comparison of the sign of optical rotation with literature data (see the Supporting Information).

Although the nitroaldol reaction has been known for more than a century,[6] stereoselective protocols started to evolve only a few decades ago. In these studies various nonenzymatic catalysts have been utilized.[7] Our results represent the first example of a biocatalytic asymmetric Henry reaction.

The *Hb*HNL-catalyzed addition of nitromethane to benzaldehyde was carried out under standard conditions after adjustment of the aqueous enzyme solution to pH 7.[8] The yields and selectivity are comparable when either *tert*-butyl methyl ether (TBME) or toluene are used as the organic phase. In contrast to *Hb*HNL-catalyzed cyanohydrin reactions, spontaneous unselective product formation does not play a crucial role even at elevated pH values. This can be explained in part by the fact that the partition coefficient of nitromethane in the water/organic phase system is lower than that of HCN, owing to the reduced solubility of nitromethane in the aqueous phase.[9] On the other hand, this also constitutes a limiting factor since the amount of nitro compound available for the enzyme in the aqueous phase cannot be increased arbitrarily.

The productivity of *Hb*HNL in the nitroaldol reaction is much lower than its activity in cyanohydrin reactions. On average several hundred units of enzyme are sufficient to transform carbonyl compounds into the corresponding α-hydroxy nitriles within a few hours, whereas 4000 units are

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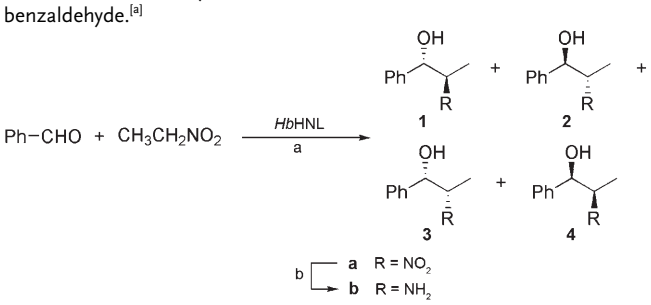
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

required to perform nitroaldol reactions and reach acceptable yields within 48 h. Regarding the scope of the reaction with respect to the carbonyl acceptor, several representative aldehydes were converted into the corresponding nitroalcohols in the presence of *Hb*HNL (Table 1).

The corresponding addition of nitroethane to benzaldehyde introduces two new stereocenters simultaneously (Table 2) and requires good control of the diastereoselectivity, which has been shown to be difficult in Henry reactions.[10] In the *Hb*HNL-catalyzed reaction of nitroethane and benzaldehyde we obtained a diastereomeric mixture of 2-nitro-1-phenylpropanol (**1a**–**4a**) in 67% yield (Table 2).

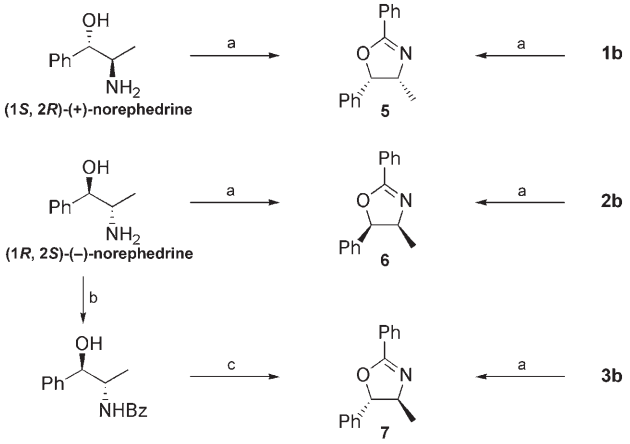
Table 2: *Hb*HNL-catalyzed stereoselective addition of nitroethane to benzaldehyde.[a]



		Config.	Relative yield [%]	*ee* [%]
anti	**1a**	1*S*,2*R*	88	95
	2a	1*R*,2*S*	2	
syn	**3a**	1*S*,2*S*	8	53
	4a	1*R*,2*R*	2	

[a] Reaction conditions: a) *Hb*HNL, phosphate buffer (pH 7)/TBME 1:1, RT, 48 h, 67%; b) H_2, Pd/C, EtOH, RT, 6 h, 90%.

The absolute configuration of the products **1a**–**4a** was determined by reduction to aminoalcohols **1b**–**3b** followed by derivatization to give oxazolines **5**–**7** (Scheme 1); these were compared to reference compounds of known configuration.



Scheme 1. Determination of the absolute configuration of aminoalcohols **1b**, **2b**, **3b**. Conditions: a) triethyl orthobenzoate, trifluoroacetic acid, 1,2-dichloroethane, reflux, 3 h, 74%; b) Et_3N, PhCOCl, CH_2Cl_2, RT, 3 h, 80%; c) diisopropyl azodicarboxylate, PPh_3, THF, RT, 16 h, 63%.

Optically pure reference compounds were prepared starting from commercially available *anti*-norephedrins. To get access to the *syn* series we inverted the configuration at C-1 via the corresponding benzamide to give oxazoline **7** (Scheme 1). Based on this analysis, the main product of the *Hb*HNL-catalyzed addition of nitroethane to benzaldehyde is (1*S*,2*R*)-2-nitro-1-phenylpropanol (**1a**). Nitroalcohols **1a**–**4a** were obtained with an *anti*/*syn* ratio of 9:1 and an enantiomeric excess of the *anti* isomer **1a** of 95%. Thus, the product mixture contains almost 90% of the main product (1*S*,2*R*)-**1a**. Assignment of the absolute configuration of the *anti* isomers using a similar derivatization method was accomplished recently.[11]

Possible binding modes of 2-nitro-1-phenylethanol to the active site of *Hb*HNL were investigated by molecular modeling. *Hb*HNL has been studied extensively with respect to its three-dimensional structure[12] and mechanism of catalysis.[13] A wealth of experimental structural information on substrate complexes of this enzyme is available.[3,13a,14] Complexes of *Hb*HNL with both enantiomers of 2-nitro-1-phenylethanol were modeled by molecular docking simulations.[15] In analogy to studies with cyanohydrins, these calculations predicted the *S* enantiomer to bind more favorably to the enzyme than the corresponding *R* enantiomer. In the complex with the *S* enantiomer, the substrate OH group forms hydrogen bonds to the side chains of Ser80 and Thr11 and the nitro group interacts with Lys236 (Figure 1, yellow structure). Thus, all mechanistically important polar interactions with active-site residues are preserved.[3] The phenyl ring is bound in the same hydrophobic pocket as that observed in the complex with mandelonitrile.[14] The nitro and phenyl groups occupy similar positions in the complex with the *R* enantiomer as in the complex with the *S* enantiomer, but the crucial hydrogen bond with Ser80 is lost (data not shown).

As a result of the equivalent substrate binding modes and the conservation of important polar interactions, the mechanism for the transformation of cyanohydrins by *Hb*HNL very likely applies to the nitroaldol reaction as well. The

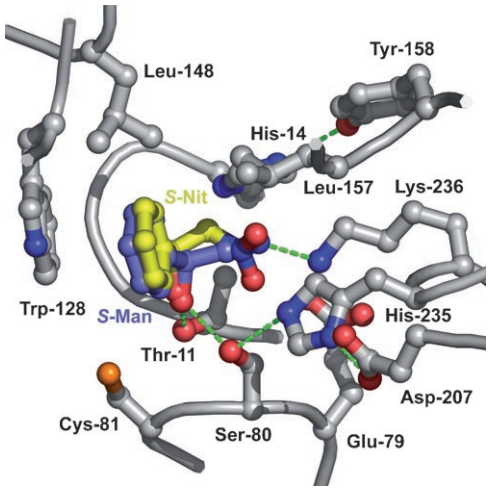


Figure 1. Modeled complex of *Hb*HNL with (*S*)-2-nitro-1-phenylethanol (yellow) in comparison with the binding mode of (*S*)-mandelonitrile (blue) observed experimentally.

nitroalcohol would then be deprotonated at the hydroxy function by the catalytic triad Ser80/His235/Asp207 in the cleavage direction, and the positive charge contributed by Lys236 would facilitate the deprotonation of the nitroalkane in the synthesis direction.[3] Whether enzyme kinetics follow an ordered "uni–bi" mechanism as observed for cyanohydrin substrates[16] is under investigation. The substrate binding site of *Hb*HNL is spacious enough to accept the more voluminous nitro compounds in a similar fashion to the binding of the corresponding cyanohydrins (Figure 1). Steric interactions, however, may still be responsible for the observed reduced catalytic rate.

For more information about the mechanism of the enzymatic Henry reaction, we carried out experiments using deuterated nitroalkanes. Although definitive conclusions will have to await results from proper kinetics experiments, the reduced yield obtained for the addition of [1,1-D_2]nitroethane to benzaldehyde already indicates the existence of a kinetic isotope effect and suggests the deprotonation of the nitroalkane to be the rate-limiting step. In contrast, in the *Hb*HNL-catalyzed cyanohydrin reaction, the C–C bond formation was found to be rate limiting.[17] In enzyme kinetics studies of nitroalkane oxidases, true kinetic isotope effects of 8–9 were measured for the formation of a nitroethane anion.[18] This is in reasonable agreement with our rough estimate for the equivalent effect—on the order of 10—for the *Hb*HNL-catalyzed Henry reaction. These findings are also in line with the well-known nitroalkane anomaly, which describes the fact that the deprotonation rate of nitroalkanes is lower than that expected based on their pKa value.[19] Thus, a combination of steric and electronic effects may explain the reduced rate of the *Hb*HNL-catalyzed Henry reaction compared to the rate of cyanohydrin formation.

In summary, Henry reactions catalyzed by the hydroxynitrile lyase from *Hevea brasiliensis* involving aldehydes and either nitromethane or nitroethane yielded the corresponding nitroalcohols in good yields and reasonable-to-high enantiomeric excess. In the case of the reaction with nitroethane, two stereocenters are generated simultaneously with good diastereo- and enantioselectivity, granting access to substances of the ephedrine family. We are currently testing other carbonyl compounds and nitroalkanes as starting materials for this reaction, and studies to improve the efficiency of the process with respect to both yields and stereoselectivity are underway.

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[8] General procedure: Wt-*Hb*HNL (4000 U mmol^{-1} aldehyde; activity determined for the cleavage of mandelonitrile; the enzyme was kindly provided by DSM) was stirred in phosphate buffer (pH 7, 50 mM) and TBME (1:1) until an emulsion was established. Freshly distilled aldehyde (1–10 mmol) was added to the mixture. The mixture was stirred for 5 min before the nitroalkane (10 mmol mmol^{-1} aldehyde) was added. The reaction mixture was stirred for 48 h at room temperature. After centrifugation and separation of the layers, the aqueous phase was extracted with TBME. The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. The crude products were purified by column chromatography.

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