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Asymmetric Biocatalytic Amination of Ketones at the Expense of NH_3 and Molecular Hydrogen

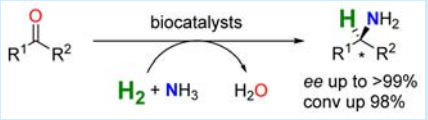
Anja K. Holzer,[†,§] Katharina Hiebler,[†,§] Francesco G. Mutti,[†,⊥] Robert C. Simon,[†] Lars Lauterbach,[‡] Oliver Lenz,[‡] and Wolfgang Kroutil*[,†]

[†]Department of Chemistry, University of Graz, NAWI Graz, Heinrichstraße 28, 8010 Graz, Austria

[‡]Department of Chemistry, Technische Universität Berlin, Straße des 17. Juni 135, 10623 Berlin, Germany

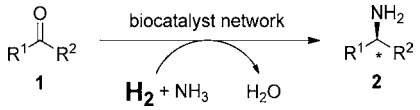
Supporting Information

ABSTRACT: A biocatalytic system is presented for the stereoselective amination of ketones at the expense of NH_3 and molecular hydrogen. By using a NAD^+-reducing hydrogenase, an alanine dehydrogenase, and a suitable ω-transaminase, the *R*- as well as the *S*-enantiomer of various amines could be prepared with up to >99% ee and 98% conversion.



The spectrum of catalytic methods to prepare optically pure α-chiral primary amines encompasses organo-,[1] metal-organo-,[2] and biocatalysts.[3,4] Various biocatalytic methods using ω-transaminases[5,6] have been developed requiring different amine sources[7] such as 2-propylamine, amino acids (e.g., alanine), or some special donors.[6a,e] A biocatalytic system just using ammonia as the amine source required the use of reducing agents such as formate or glucose.[8] Here an alternative option is shown using molecular hydrogen as reducing agent (Scheme 1), thereby improving the atom economy of the asymmetric reductive amination in comparison to the NH_3/glucose or formate system.

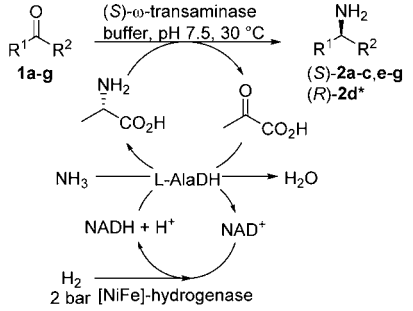
Scheme 1. Asymmetric Bioamination of Ketones at the Expense of NH_3 and Molecular Hydrogen

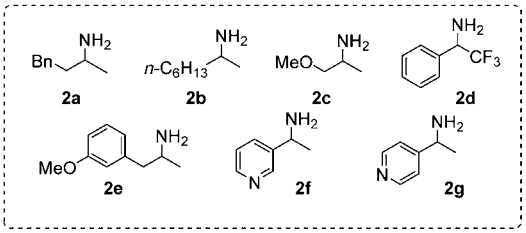


To design a biocatalytic system for the asymmetric amination of ketones at the expense of ammonia and hydrogen, alanine was used as a direct amine donor for the ω-transaminase, leading to the formation of pyruvate as the side product (Scheme 2). To remove pyruvate from the equilibrium, it was recycled back to alanine; this step is catalyzed by an alanine dehydrogenase that consumes ammonia and reduced nicotinamide adenine dinucleotide (NADH). The NADH is finally recycled by the hydrogenase at the expense of molecular hydrogen. The O_2-tolerant NAD^+-reducing hydrogenase from *Ralstonia eutropha* H16 was employed as an efficient catalyst for the H_2-driven reduction of NAD^+ to NADH.[9]

In contrast to commonly used cofactor regeneration systems, H_2-based NADH recycling by hydrogenase is 100% atom-efficient and relies on a cheap, carbon-free reducing agent.[9b] In contrast to most other hydrogenases, the *R. eutropha* enzyme is O_2-tolerant; that is, it sustains full catalytic activity even in the presence 20% O_2,[9c] which makes its utilization in cofactor regeneration particularly convenient.

Scheme 2. Biocatalytic System for the Asymmetric *S*-Amination of Ketones Consuming NH_3/H_2 (*Switch in Cahn-Ingold-Prelog Priority)





The L-alanine dehydrogenase (L-AlaDH) from *Bacillus subtilis*[10] was used for the recycling of L-alanine from pyruvate. When various ω-transaminases in the system were tested with the hydrogenase and the L-AlaDH for the amination of various ketones **1a**–**g**, the following *S*-selective transaminases turned out to be the most suitable: For instance, the ω-transaminase from *Pseudomonas fluorescens* (PF-ω-TA)[10b,11] transformed ketone **1a** to the corresponding (*S*)-amine **2a** with 86% conversion at 10 mM substrate concentration within 8 h (Table 1). The ω-

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Table 1. Asymmetric *S*-Amination of Ketones 1a–g at the Expense of NH_3 and H_2[a]

substrate	ω-TA[b]	conversion[c] (%)	ee[d] (%)
1a	Pf	86	92 (*S*)
1b	Bf	96	93 (*S*)
1c	Cv	90	>99 (*S*)
1d	Bm	92	>99 (*R*)[e]
1e	Cv	98	92 (*S*)
1f	Cv	76	>99 (*S*)
1g	Vf	77	>99 (*S*)

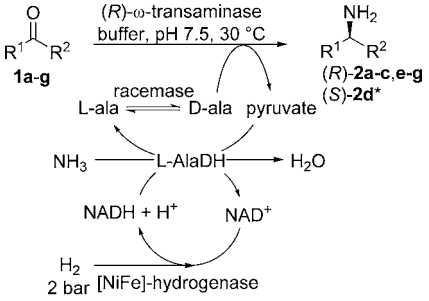
[a]Reaction conditions: substrate (10 mM), lyophilized *Escherichia coli* cells containing the overexpressed ω-TA (20 mg), K-phosphate buffer (100 mM, pH 7.5), PLP (1 mM), NAD^+ (1 mM), FMN (0.5 mM), L-alanine (250 mM), AlaDH (12 U), NH_4Cl (150 mM), hydrogenase (1.81 U), 8 h, 30 °C, 120 rpm, 2 bar $p(H_2)$. [b]ω-TA from Pf, *Pseudomonas fluorescens*; Bm, *Bacillus megaterium*; Cv, *Chromobacterium violaceum*; Vf, *Vibrio fluvialis*. [c]Conversions were measured by GC on an achiral phase. [d]Enantiomeric excess was determined by GC on a chiral phase after derivatization to the corresponding acetamide. [e]Switch in Cahn-Ingold-Prelog priority.

transaminase from *Chromobacterium violaceum* (CV-ω-TA)[12] transformed **1c** and **1e** with over 90% conversion and reached an ee of >99% for **1c**. Also, the transaminase from *Bacillus megaterium*[13] successfully converted **1d** to the optically pure chiral amine **2d** with 92% conversion. Amine (*S*)-**2g** was obtained with >99% ee by employing the ω-transaminase from *Vibrio fluvialis*.[14]

The experiments were performed at 2 bar $p(H_2)$ as the optimum because higher (3 bar H_2) as well as lower pressure (1 bar H_2) led to decreased conversion within the reaction time.

To apply the NH_3/H_2 system for the preparation of (*R*)-amines, *R*-selective transaminases were investigated (Scheme 3).

Scheme 3. Biocatalytic System for the *R*-Amination of Ketones at the Expense of NH_3/H_2 (*Switch in Cahn-Ingold-Prelog Priority)



Since these enzymes accept D-alanine as a direct amine donor, ideally, a D-alanine dehydrogenase would be required. Since no suitable enzyme was at hand, the formed pyruvate was instead removed to L-alanine by L-AlaDH. Alanine racemase(s) present in the *E. coli*[15] preparation of the ω-TAs allows the racemization of alanine to a certain extent.[16]

Using the NH_3/H_2 biocatalytic system for the amination of ketones **1a–g**, all amines **2a–g** were obtained in optically pure form (>99% ee) using the *R*-selective ω-transaminase from either *Arthrobacter* sp.[17] or *Aspergillus terreus*[18] (Table 2). Conversion up to 94% was reached in the best case (entry 5).

A preparative transformation of ketone **1a** (50 mg) with the ω-TA from *P. fluorescens* reached 97% conversion within 8 h under

Table 2. Biocatalytic *R*-Amination at the Expense of NH_3 and H_2[a]

substrate	ω-TA[b]	conversion[c] (%)	ee[d] (%)
1a	ArR	70	>99 (*R*)
1b	At	69	>99 (*R*)
1c	ArR	87	>99 (*R*)
1d	ArR	51	>99 (*S*)[e]
1e	ArR	94	>99 (*R*)
1f	ArR	32	>99 (*R*)
1g	ArR	49	>99 (*R*)

[a]Reaction conditions: Same as described in Table 1, but using D-alanine instead of L-alanine. [b]ω-TA from ArR, (*R*)-transaminase from *Arthrobacter* sp.; At, *Aspergillus terreus*. [c]Conversions were measured by GC on an achiral phase. [d]Enantiomeric excess was determined by GC on a chiral phase after derivatization to the corresponding acetamide. [e]Switch in Cahn-Ingold-Prelog priority.

hydrogen (2 bar). The corresponding amine was isolated with 62% yield.

In conclusion, it was successfully shown that the asymmetric amination of ketones can be performed with NH_3 as the nitrogen source and H_2 as the reducing agent by employing a biocatalytic system comprising a hydrogenase, an alanine dehydrogenase, and a ω-transaminase. Conversion up to 98% was reached, giving the corresponding amines with up to >99% ee.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and characterization data. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01154.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: wolfgang.kroutil@uni-graz.at.

Present Address

⊥F.G.M.: Manchester Institute of Biotechnology, Faculty of Life Sciences, University of Manchester, Manchester M1 7DN, UK.

Author Contributions

§A.K.H. and K.H. contributed equally.

Notes

The authors declare no competing financial interest.

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