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Imine Reductase-Catalyzed Intermolecular Reductive Amination of Aldehydes and Ketones

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Imine reductases (IREDs) have emerged as promising biocatalysts for the synthesis of chiral amines. In this study, the asymmetric imine reductase-catalyzed intermolecular reductive amination with NADPH as the hydrogen source was investigated. A highly chemo- and stereoselective imine reductase was applied for the reductive amination by using a panel of carbonyls with different amine nucleophiles. Primary and secondary amine products were generated in moderate to high yields with high enantiomeric excess values. The formation of the imine intermediate was studied between carbonyl substrates and methylamine in aqueous solution in the pH range of 4.0 to 9.0 by ^{1}H NMR spectroscopy. We further measured the kinetics of the reductive amination of benzaldehyde with methylamine. This imine reductase-catalyzed approach constitutes a powerful and direct method for the synthesis of valuable amines under mild reaction conditions.

Chiral amines are an important class of organic compounds that serve as key intermediates in the synthesis of a variety of biologically active molecules.[1] The most straightforward strategy for the generation of chiral amines comprises the formation of C=N bonds by the condensation of carbonyls and amines, followed by reduction of the in situ formed imine. The chemoselectivity of the reducing agent, however, is crucial, as it should efficiently reduce the C=N bond while leaving the potentially reducible carbonyl compound unaffected. Tremendous efforts have been made in the areas of organometallic catalysis, organocatalysis, and biocatalysis to develop efficient chemo-, regio-, and enantioselective strategies for this process.[2,3] In the last several decades, most work has focused on the transition-metal-catalyzed asymmetric hydrogenation of imines with particular attention paid to the reductive amination of carbonyl compounds with primary amines. Several reagents and combinations have been reported through the years with modified borohydride reagents playing a predominant role.[4] Inspired by biological systems in nature that use NAD(P)H, the Hantzsch ester was employed as a hydrogen donor in combination with chiral phosphoric acids for the enantioselective reductive amination of aliphatic ketones to furnish amines with excellent enantioselectivities.[5–7] In most cases, these reactions were performed in different organic solvents that favored reversible imine formation by shifting the equilibrium towards the condensation product.

The application of biocatalysis is an important complement to chemical catalysis for chiral amine synthesis in water. To date, two main types of enzymes have been investigated for the transformation of carbonyl groups into amines: Amino acid dehydrogenases and ω-transaminases. Amino acid dehydrogenases catalyze the amination of carbonyl compounds, usually α-keto acids and α-keto esters with NADH as the hydrogen source.[8] Using existing amino acid dehydrogenase scaffolds, the group of Bommarius successfully altered the substrate specificity through several rounds of protein engineering to create amine dehydrogenases.[9,10] However, the application of amine dehydrogenases still suffers from a poor substrate scope. In contrast, ω-transaminases catalyze the transfer of amino groups from co-substrates to carbonyl compounds to form new chiral amines. These enzymes have proven to be powerful catalysts and are frequently used in the synthesis of a broad range of chiral amines.[11–13] Challenges associated with ω-transaminases are the disfavored reaction equilibrium for reductive aminations of ketones and inhibition by the formed byproduct. Both ω-transaminases and amine dehydrogenases are inherently restricted to the transfer of ammonia as amine substrate[14] and thus, do not offer access to pharmaceutically important chiral secondary and tertiary amine building blocks. In this light, imine reductases represent a promising alternative and extension to reported biocatalysts.

Previously, imine reductases were investigated for the asymmetric reduction of C=N bonds with NADPH as the hydride donor.[15–24] To date, both (*R*)- and (*S*)-selective reductases have been screened for the transformation of five-, six-, and seven-membered cyclic imines, dihydroisoquinolines, β-carbolines, and iminium ions. They were shown to possess high catalytic activities and stereoselectivities. This makes them promising biocatalysts for chiral amine synthesis. However, whereas intramolecular reductions with the use of imine reductases have been reported, only a few intermolecular ones have been developed. Evidently, intermolecular processes would represent an important advancement in this methodology. Recent work showed that the (*S*)-selective imine reductase from *Streptomyces* sp. GF3546 catalyzed the conversion of 4-phenyl-2-butanone with methylamine into the corresponding product with 8.8% conversion and 76% *ee*.[24] Furthermore, Codexis/Merck have developed and patented an engineered opine dehydrogenase from *Arthrobacter* sp. possessing imine reductase activity for the conversion of various ketone and amine substrates into secondary and tertiary amines under industrially applicable conditions.[25] Despite these preliminary works, biocatalytic reductive aminations to afford chiral amines in high yields with

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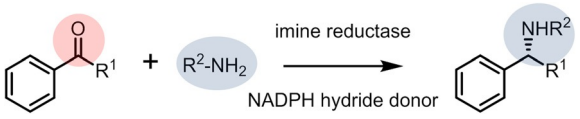


Scheme 1. Imine reductase-catalyzed asymmetric reductive amination.

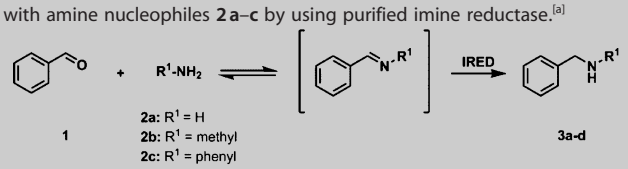
high enantiomeric excesses in one step are scarcely reported. Recently, we described that the (*R*)-selective imine reductase from *Streptosporangium roseum* (*R*)-IRED-*Sr* is a promising novel biocatalyst for the asymmetric transfer hydrogenation of cyclic imines to produce amines with excellent enantioselectivities.[15] As a result of continuing efforts towards the development of selective imine reductase-catalyzed reactions, we now report the reductive amination of a set of carbonyl substrates with various amines by using this enzyme (Scheme 1).

To approach the challenge of the chemo- and enantioselective formation of new carbon–nitrogen bonds, a substrate panel comprising aromatic and cyclic carbonyls and aliphatic and aromatic amines was chosen. As carbonyl compounds, benzaldehyde (**1**), acetophenone (**4**), and cyclohexyl methyl ketone (**6**) were selected. The set of amine nucleophiles consisted of ammonia (**2a**), methylamine (**2b**), and aniline (**2c**).

Although general features of imine-forming reactions are well understood, the available data from the scientific literature on imine formation in water is rather limited.[26] The position of the equilibrium for imines formed from carbonyl compounds and amines by elimination of water is expected to be highly disfavored in aqueous buffer systems.[27] Hence, we first studied the reduction of the commercially available imine *N*-benzylidenemethylamine (corresponding imine species of **3b** shown in Table 1). In the initial screening, enzyme activity was detected, but the imine also rapidly decomposed at pH values below 8. To ensure activity of the biocatalyst at elevated pH levels, the pH profile of the enzyme was recorded by using the cyclic imine substrate 2-methylpyrroline. The activity of the IRED was maximum at pH 7.0 (Figure S7, Supporting Information); however, at pH 8.0 the conversion of *N*-benzylidenemethylamine to the amine was approximately 75% (Table S4). The hydrolytic product benzaldehyde was also reduced to the corresponding benzyl alcohol byproduct (<6%) in these biotransformations.

All IREDs reported so far demonstrated high chemoselectivities towards C=N bonds. Attempts to detect activities for aldehydes, ketones, and keto acids failed.[18,19,21] To ensure that alcohol formation was not catalyzed by the IRED, biotransformations with *N*-benzylidenemethylamine were performed with a previously described IRED mutant. In the variant (*R*)-IRED-*Sr* D191A, the proposed catalytically important amino acid is replaced by alanine. In comparison to the wildtype enzyme, the mutant displays strongly decreased reduction rates in whole-cell biotransformations.[15] In purified form, it showed activity for the reduction of cyclic 2-methylpyrroline that was approximately 100-fold lower than that of the wildtype (Figure S6 and Table S3). Both wildtype and variant possess the same level of purity of approximately 90%, as judged by size-exclusion chromatography (SEC) analysis on HPLC (Figures S1–S5). Moreover,

Table 1. Conversion of benzaldehyde (**1**) into amination products **3a–d** with amine nucleophiles **2a–c** by using purified imine reductase.[a]



2a: R^1 = H
2b: R^1 = methyl
2c: R^1 = phenyl

1 **3a-d**

Carbonyl acceptor	Amine nucleophile	Nucleophile [equiv.]	Formed product [%] 1 h	8 h	24 h
1	**2a**	1[b]	<1[b]	1/<1[b]	7/2[b]
1	**2a**	10[b]	<1[b]	21/<1[b]	51/3[b]
1	**2a**	50[b]	<1[b]	48/<1[b]	61/1[b]
1	**2b**	1	6	35	51
1	**2b**	10	33	72	66
1	**2b**	50	58	73	69
1	**2c**	1	4	13	25

[a] Reaction conditions: carbonyl substrate **1** (10 mM), purified IRED (2.5 mg mL^{-1}) and cofactor regeneration system in Tris HCl buffer (50 mM, pH 8.0). To compare conversion rates and to evaluate formation of byproducts, all reactions were performed with the same batch of purified biocatalyst. Reduction of **1** to benzyl alcohol depended on the nature and the excess amount of the amine nucleophile. Further details are given in the Supporting Information. [b] Equivalents of nucleophile **2a** used in the reaction setup with product **3a** itself acting as an additional nucleophile. Higher levels of product **3a** were obtained; however, **3a** reacted further with benzaldehyde (**1**) to give the dialkylation product dibenzylamine (**3d**).

in the reduction of *N*-benzylidenemethylamine no amine product was observed with the mutant; however <3% of benzyl alcohol was detected. Although we cannot exclude the possibility that IREDs possess weak promiscuous activities towards carbonyls, it seems very likely that byproduct formation results from contaminations of co-purified ketoreductases in the enzyme preparations.

To prove the applicability of the (*R*)-selective IRED also for intermolecular reductive aminations, carbonyl **1** was treated with the three different amine nucleophiles. In control reactions with the inactive enzyme as well as bovine serum albumin (BSA) by using the nicotinamide cofactor regeneration system, no amine products were detected.

Benzaldehyde (**1**) was readily converted by the purified imine reductase into the corresponding amination products with all three amine nucleophiles. Depending on the nature of the amine nucleophile, moderate to good conversions were obtained. In these biotransformations, a definite trend was suggested by the increasing nucleophilicity of the amines leading to higher formations of the amination products (Table 1).[28] Further, the direct comparison of transformations by using nucleophiles **2b** and **2c** also indicates the substrate specificity of the applied IRED. The preference of (*R*)-IRED-*Sr* towards smaller amines and imines might be responsible for the fact that the formation of product **3b** was twofold higher than that of **3c** after 24 h under identical conditions.

By increasing the amine equivalents from an equimolar ratio to 10-fold and 50-fold excess amounts, reaction rates strongly increased, and overall, much higher conversion rates were ob-

tained. It is assumed that the excess amount of the amine boosts the equilibrium of the reaction intermediate towards imine formation, which thus leads to higher saturation of the biocatalyst with its substrate.

During the reductive amination of benzaldehyde (**1**) with **2a**, minor reductions to the alcohol byproduct and a small amount of dialkylation product dibenzylamine (**3d**) were observed.

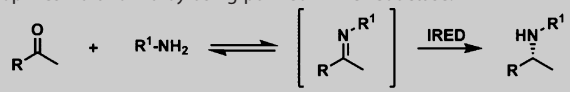
Next, the generality of the developed reductive amination protocol was explored for reactions with aromatic and cyclic ketones. In contrast to aldehydes, the direct reductive amination of ketones provides access to chiral amines in a single step. However, they are far less reactive with aromatic unsaturated ketones being described to be challenging substrates.[29] To explore the transformation of such compounds, ketones **4** and **6** differing in their reactivity were selected.

First screening results with nucleophiles **2a** and **2b** and the reaction conditions previously employed for benzaldehyde indicated only low product formation (<2%) with acetophenone (**4**). Owing to the preference for smaller nucleophiles in the reductive aminations with benzaldehyde (**1**), aniline **2c** was not employed in this set of reactions. The best conversion rates were obtained with a 50-fold excess amount of amine donors **2a** and **2b**, which resulted in 5% of amination product **5a** and 9% of **5b**, respectively. To further increase product formations, conditions were again adjusted to raise the concentration of the imine intermediate at its equilibrium. This was done by slightly increasing the pH value, which thereby enhanced the concentration of the reactive amine species.

The stepwise increase in the pH of the reaction medium resulted in enhanced transformations of acetophenone (**4**) into amination products **5a** and **5b** with approximately 50% more amination product formed. Remarkably, the imine reductase-catalyzed formation of the new C–N bond proceeded for products **5a** and **5b** with very high to excellent enantioselectivities (up to 98% *ee*) under all conditions (Table 2). In control reactions with the inactivated enzyme or BSA, no amine products were detected. Furthermore, alcohol formation was observed only in trace amounts (<1%) with ketone substrates. In contrast, the amination of nonaromatic ketone **6** to give cyclohexylethylamine (**7a**) did not require extensive optimization, as at the starting conditions considerable amounts of the amination product were already formed with good selectivity. Reductive amination of ketones **4** and **6** was then also explored by employing slightly higher catalyst loadings (increase from 0.78 to 3.1 mol%). This ultimately increased the conversion rates by approximately twofold to 16, 39, and 53% for products **5a**, **5b**, and **7a**, respectively, while maintaining the high selectivity (Table 2).

As demonstrated by the optimization of our amination setup, the formation of imines represents a potential bottleneck for enzyme-catalyzed intermolecular reductive aminations in an aqueous environment. First, the amount of nucleophile in the mixture was varied and then the pH was also raised. We reasoned that increasing the nucleophile concentration and pH should result in higher amounts of the condensation products.

Table 2. Conversion of acetophenone (**4**) and cyclohexyl methyl ketone (**6**) into their different amination products **5a–b** and **7a** with amine nucleophiles **2a** and **2b** by using purified imine reductase.[a]



4: R = phenyl
6: R = cyclohexyl

2a: R^1 = H
2b: R^1 = methyl

5a-b
7a

Carbonyl acceptor	Amine nucleophile	pH	Formed product [%][b]	*ee* [%][c]
4	**2a**	8	5	97 (*R*)
4	**2a**	8.5	7	98 (*R*)
4	**2a**	9	10	98 (*R*)
4	**2a**	9	16[d]	97 (*R*)
4	**2b**	8	9	87 (*R*)
4	**2b**	8.5	15	86 (*R*)
4	**2b**	9	19	86 (*R*)
4	**2b**	9	39[d]	87 (*R*)
6	**2a**	8	16	74 (*R*)
6	**2a**	8.5	18	78 (*R*)
6	**2a**	9	19	77 (*R*)
6	**2a**	9	53[d]	78 (*R*)

[a] Reaction conditions: carbonyl substrate **4** or **6** (10 mM), purified IRED (2.5 mg mL^{-1}), amine nucleophiles **2a** and **2b** (500 mM) and cofactor regeneration system in Tris HCl buffer (50 mM, pH 8.0 to 9.0). To compare conversion rates and to evaluate the formation of byproducts, all reactions were performed with the same batch of purified biocatalyst. Trace amounts of the alcohol reduction products of carbonyls **4** and **6** were detected (<1% for all reactions). [b] Conversions were determined after 24 h by GC analysis. [c] Enantiomeric excess was determined after derivatization with acetic anhydride by GC on a chiral stationary phase as detailed in the Supporting Information. [d] Reactions were performed with a fourfold increased enzyme loading (0.78 mol% catalyst to 3.1 mol%).

To obtain further insight into this process, the condensations of benzaldehyde (**1**) as well as acetophenone (**4**) with methylamine (**2b**) were monitored by ^{1}H NMR spectroscopy.[30] For benzaldehyde (**1**) at pH ≥ 8.2 (corresponds to a pD ≥ 8.6), substantial imine formation was observed (Figure 1, see also Figures S19–S24). The course of the condensation reactions was followed by the appearance of the CH signal of the imine bond in the ^{1}H NMR spectrum, which is shifted downfield with respect to the signal of the parent aldehyde. The molar ratio of imine was determined by integration of the resonances of the aldehyde and imine moieties. However, imine formation for acetophenone (**4**) was not detectable (Figure S25). Considering that the reductive amination of **4** resulted in good conversions, at least some levels of the imine intermediate are expected to be formed. Estimating the detection limit of the ^{1}H NMR spectrometer to be <500 μM, it becomes clear that (*R*)-IRED-*Sr* is a very effective catalyst in withdrawing already low levels of imine intermediates from the equilibrium while leaving the potential reducible carbonyl compound untouched.

In addition, we measured the kinetics for the reductive amination reaction of benzaldehyde (**1**) with a 50-fold excess amount of **2a** at pH 8 and at pH 9. The limited imine availability that was also assigned by ^{1}H NMR spectroscopy is reflected by an apparent affinity in the low millimolar range (Table S11). As shown by the Michaelis–Menten plots (Figures S9 and S11),

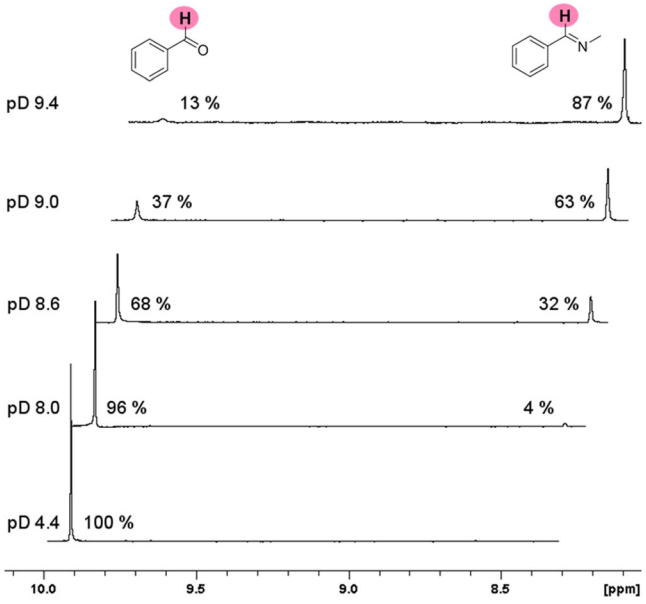


Figure 1. ^{1}H NMR spectra for the condensation of benzaldehyde (**1**) with methylamine (**2b**) to form the corresponding imine with increasing pH conditions (pH 4.0–9.0). D_2O was used as the solvent alternative to H_2O. The pD was calculated by employing pD = pH + 0.4.[31] At pD 8.6 and above, substantial amounts of the condensation product were detectable.

an increase in the concentration of the substrates leads to higher reaction rates, which illustrates the higher conversion rates observed in our biotransformations.

In summary, we showed that the (*R*)-selective imine reductase from *Streptosporangium roseum* displays broad applicability as a biocatalyst for chemo- and stereoselective intermolecular reductive aminations. This approach represents a simple and complementary strategy for the generation of novel carbon–nitrogen bonds in aqueous reaction media. These reductive amination reactions proceeded efficiently for a variety of carbonyl compounds and amine nucleophiles. Low amounts of the imine intermediate are sufficient for the formation of valuable amines in good yields with high selectivities. We are currently optimizing this methodology and exploring its application in organic solvents, as imine reductases represent a unique class of biocatalysts for the synthesis of chiral amines.

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Keywords: asymmetric synthesis · biocatalysis · chiral amines · imine reductase · reductive amination

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