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Efficient Production of Enantiomerically Pure Chiral Amines at Concentrations of 50 g/L Using Transaminases

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Abstract:

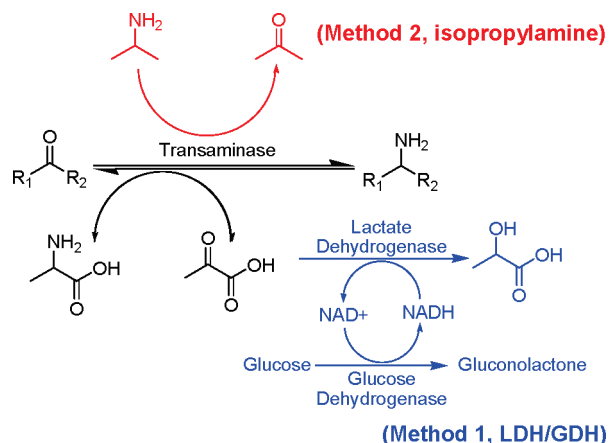
Two methods for the efficient (50 g/L) production of optically pure amines from their corresponding ketones using transaminases have been developed. The first method utilizes an ion-exchange resin for in situ product removal allowing the reaction to be carried out at a substrate concentration of 50 g/L. The second approach relies upon conversion of the initially formed amine, via spontaneous cyclisation, to a noninhibitory product. Both methods have been demonstrated at 50 mL scale. (*R*)- and (*S*)-methylbenzylamine, and (*R*)- and (*S*)-6-methyl-2-piperidone have been produced in >90% isolated yield and >99% ee.

Introduction

In view of the importance of enantiomerically pure chiral amines as building blocks for pharmaceuticals, there is currently considerable effort underway to develop efficient catalytic methods for their preparation. Approaches based on biocatalysis¹ have largely relied upon kinetic resolution of racemic amides or amines using lipases, and indeed such methods have been successfully commercialized at scale.² Some groups have recently addressed the issue of in situ racemization of the unreactive enantiomer by developing catalysts for amine racemization permitting dynamic kinetic resolutions to be carried out.^{3–5} Alternatively, racemic amines can be deracemized in high yield and ee by the combined use of an enantioselective amine oxidase and a nonselective reducing agent.⁶ However, the emergence of transaminases, which catalyse the direct amination of ketones to chiral amines using ammonia, has provided an alternative and highly attractive additional option.⁷

Transaminases typically possess high turnover rate, stability, and a tightly bound pyridoxal cofactor. However, two factors have limited the application of transaminases for chiral amine

Scheme 1. Overview of transaminase-catalysed reaction methods



production on scale.^{8,9} First, the equilibrium constant for the reaction often favors the ketone starting material requiring the development of methods to drive the reaction to completion. Second, transaminases typically suffer significant product inhibition by both the desired amine product and the keto acid byproduct. Although methods have been reported which address the issue of product inhibition,¹⁰ no general procedures have been reported for carrying out productive, scaleable reactions. Generally, for a biocatalyst to be considered for an industrial application, the process must tolerate substrate concentrations of at least 50 g/L.¹¹ Herein, we report a simple procedure for conducting transaminase-catalysed reactions at substrate concentrations of 50 g/L.

Results

Scheme 1 shows two alternative approaches for carrying out transaminase catalysed reactions.¹² In Method 1, alanine is employed as the amine donor, and the pyruvate generated is reduced by lactate dehydrogenase (LDH) to L-lactate.¹³ Removing the pyruvate serves the dual purpose of driving the reaction

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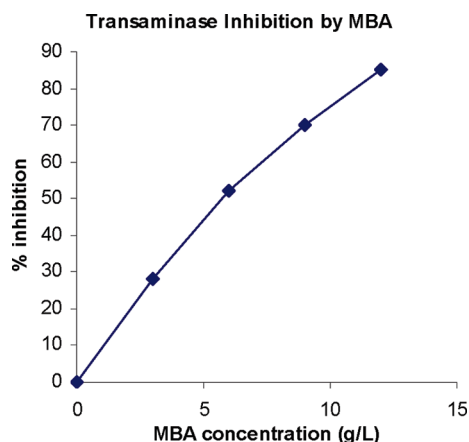


Figure 1. Inhibition of the transaminase enzyme by the product methylbenzylamine (MBA).

equilibrium and also eliminating pyruvate inhibition of the transaminase. Glucose dehydrogenase (GDH) is used to recycle the NADH cofactor. In Method 2, a large excess of isopropylamine (1 M) is employed as the amine donor, instead of alanine.¹⁴

Using these approaches we recently reported the conversion of acetophenone to (*R*)- or (*S*)- α -methylbenzylamine (MBA) (99% ee) at substrate concentrations of up to 6 g/L (Method 1) and 2.5 g/L (Method 2). Significant inhibition of the transaminase by the amine product prevented running the reactions at higher substrate loading (Figure 1). For example, 50% inhibition of the transaminase was observed at an MBA concentration of 6 g/L, with >80% inhibition at a concentration of 12 g/L.

To alleviate product inhibition, in situ product removal using an ion-exchange resin (Amberlite XAD 1180) was examined. To a solution of MBA (50 g/L) in 100 mM potassium phosphate buffer (pH 7.5) were added various concentrations of ion-exchange resin from 0 to 400 g/L. Each solution was stirred for 5 min at 30 °C, after which the ion-exchange resin was removed by filtration and the reaction buffer then sampled to determine the aqueous MBA concentration by HPLC analysis. Figure 2 shows that increasing the resin loading leads to a decrease in the aqueous MBA concentration as expected. A resin loading of 200 g/L resulted in an MBA concentration of 4 g/L, below the 50% transaminase inhibition threshold, and hence this loading was selected for further experiments.

Fifty-milliliter scale reactions were run with automated pH control and 50 g/L of acetophenone. Control of pH is necessary because of acid production from recycling of the NADH cofactor. Three separate reactions were run to demonstrate the effectiveness of the system: (i) no lactate dehydrogenase (LDH) to consume the pyruvate ketoacid byproduct and drive the reaction equilibrium, (ii) with LDH but no ion-exchange resin to alleviate transaminase inhibition, (iii) with both LDH and 200 g/L ion-exchange resin (Figure 3).

The reaction without LDH proceeded to <1% conversion as a result of the equilibrium favoring ketone over amine. The reaction containing LDH but no ion-exchange resin proceeded

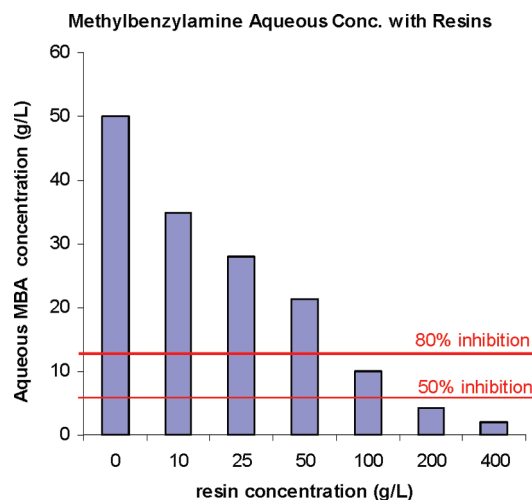


Figure 2. Effect of ion-exchange resin on aqueous concentration of methylbenzylamine (MBA). MBA concentrations corresponding to 80% and 50% transaminase inhibition levels are shown with red lines.

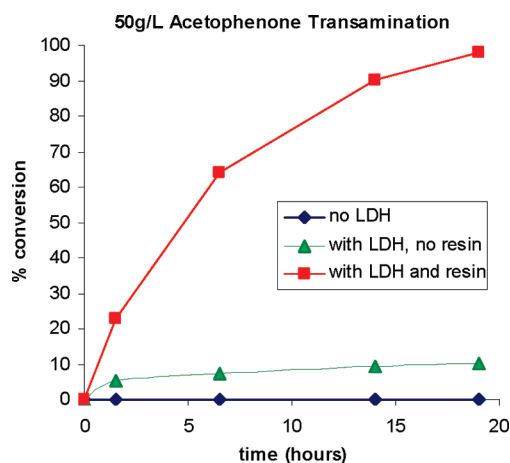


Figure 3. Transamination of acetophenone with and without ion-exchange resin and lactate dehydrogenase (LDH).

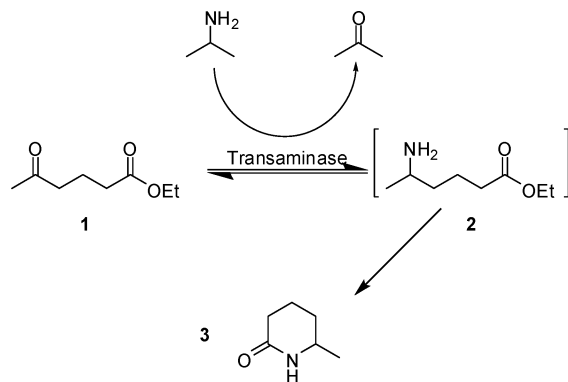
to ~10% conversion highlighting inhibition of the transaminase by the MBA product. Pleasingly, the reaction containing both LDH and the ion-exchange resin proceeded to >99% conversion. Both (*S*)- and (*R*)-MBA were produced using the ion-exchange resin process in >99% ee using transaminases ATA-113 and ATA-117, respectively. The desired MBA product was easily recovered in >90% yield by washing the resin with buffer (pH 11).

As expected, the ion-exchange resin was not effective in selectively binding the product MBA in reactions containing a high concentration (1 M) of isopropylamine as the amine donor (Figure 1, Method 2). The isopropylamine competed with the MBA for binding to the resin, rendering the in situ product removal ineffective. An alternative solution was envisaged in which the product of the transamination reaction underwent spontaneous and irreversible conversion to a secondary product, thereby simultaneously driving the reaction to completion and also eliminating product inhibition. Ethyl 4-acetylbutyrate **1** was chosen as a model substrate. Upon transamination of the ketone, the amine **2** spontaneously cyclizes to the 6-methyl-2-piperidone

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Scheme 2. Transamination of ethyl 4-acetylbutyrate followed by spontaneous cyclisation to 6-methyl-2-piperidone using isopropylamine as the amine donor



3 product (Scheme 2). Optically pure intermediates of this type are found in a variety of natural products.¹⁵

Fifty-milliliter scale reactions were run with 50 g/L ethyl 4-acetylbutyrate and 1 M isopropylamine as the amine donor. The reactions proceeded to >99% conversion in <15 h (Figure 4). Both (*S*)- and (*R*)-6-methyl-2-piperidone were produced with >99% ee using transaminases ATA-113 and ATA-117, respectively. The desired product was isolated in >90% yield via extraction into dichloromethane.

In summary, we have developed transamination procedures that can be run at industrially acceptable substrate concentrations of 50 g/L. The approach is based on the elimination of product inhibition that commonly plagues transaminase reactions by the use of an ion-exchange resin to selectively remove the desired product amine in situ. The method has been successfully applied to the transamination of acetophenone yielding (*S*)- or (*R*)-methylbenzylamine in >90% isolated yield and >99% ee.

A second approach, which is substrate dependent, relies upon spontaneous cyclisation of the initially formed amine to a secondary product. This method has been successfully applied to the transamination of ethyl 4-acetylbutyrate at 50 g/L yielding (*S*)- or (*R*)-6-methyl-2-piperidone in >90% isolated yield and >99% ee.

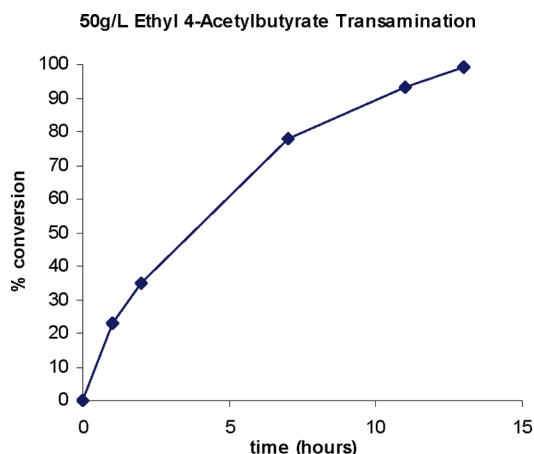


Figure 4. Transamination of ethyl 4-acetylbutyrate using isopropylamine transamination system.

Experimental Section

Conversion Analysis. Reaction conversions were monitored using reverse phase high performance liquid chromatography (HPLC) at 220 nm using an Agilent 1100 series HPLC and a Zorbax Eclipse XDB-C18 (50 mm × 4.6 mm) column with a flow rate of 1 mL/min (60% acetonitrile/40% water) for 3 min.

Enantiomeric Excess Analysis. Enantiomeric excess of methylbenzylamine was determined by normal phase high performance liquid chromatography (HPLC) at 220 nm using an Agilent 1100 series HPLC and a Chiralpak OD-H (250 mm × 4.6 mm) column with a flow rate of 1 mL/min (90% hexanes/10% 2-propanol) for 12 min. The retention times of the (*S*)- and (*R*)-enantiomers were 8.7 and 7.2 min, respectively. Specific rotation of the methylbenzylamine product was established by comparison to known standards that were purchased from Sigma.

The enantiomeric excess of 6-methyl-2-piperidone was determined by normal phase high performance liquid chromatography (HPLC) at 220 nm using an Agilent 1100 series HPLC and a Chiralpak AD-H (250 × 4.6 mm) column with a flow rate of 0.5 mL/min (80% hexanes/20% ethanol) for 20 min. The retention times of the (*S*)- and (*R*)-enantiomer were 17 and 15 min, respectively. The absolute configuration of the 6-methyl-2-piperidone products was established by comparison to that of authentic standards using chiral HPLC.¹⁶

Reagents and Enzymes. Commercial-grade reagents and solvents were purchased from Sigma-Aldrich and used without further purification. All enzymes, including transaminases (ATA-113 and ATA-117), glucose dehydrogenase (GDH CDX-901), and lactate dehydrogenase (LDH-102) are catalogue enzymes of Codexis and were generously supplied by Codexis (Redwood City, CA).

Transamination of Acetophenone. Transaminations of acetophenone were conducted at 50-mL scale using the lactate dehydrogenase/glucose dehydrogenase system under the following conditions: 100 mM potassium phosphate buffer, 1 g/L NAD⁺, 0.5 g/L pyridoxal-5-phosphate, 90 g/L glucose, 90 g/L alanine, 50 g/L acetophenone, 1 g/L glucose dehydrogenase (GDH), 1 g/L lactate dehydrogenase (LDH), 5 g/L transaminase (ATA), and 200 g/L ion-exchange resin (Amberlite XAD 1180). Reactions were run at 30 °C and pH 7.5 in a Multimax reactor system with overhead mechanical stirring at 400 rpm. Reaction pH was controlled through the automated addition of 2 M NaOH.

Upon complete conversion, the ion-exchange resin was recovered from the reaction mixture by filtration and washed with pH 11 buffer to recover the methylbenzylamine product.

Transamination of Ethyl 4-Acetylbutyrate. Transaminations of ethyl 4-acetylbutyrate were conducted at 50 mL scale using the isopropylamine amino donor system under the following conditions: 100 mM potassium phosphate buffer, 0.5 g/L pyridoxal-5-phosphate, 1 M isopropylamine, 50 g/L ethyl 4-acetylbutyrate, and 5 g/L transaminase (ATA). Reactions were run at 20 °C and pH 9.5 in a Multimax reactor system with overhead mechanical stirring at 400 rpm.

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Upon complete conversion, the reaction was extracted with 2 volumes of dichloromethane followed by removal of the solvent by via vacuum distillation.

Acknowledgment

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Supporting Information Available

HPLC chromatograms showing enantiomeric excess analysis of methylbenzylamine products are available. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

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