Considerable effort has been spent to develop efficient, catalytic methods for the preparation of enantiomerically pure chiral amines as pharmaceutical building blocks. Biocatalytic approaches\(^1\) have largely relied on kinetic resolution of racemic amines using lipases.\(^2–3\) In some instances, situ racemization of the unreactive enantiomer has permitted dynamic kinetic resolutions to be performed.\(^4–6\) Racemic amines can also be deracemized using an amine oxidase combined with a nonselective reducing agent.\(^7\) The emergence of transaminases, which catalyze the direct amination of prochiral ketones, has provided an attractive alternative option for the synthesis of chiral amines.\(^8–10\) Tufvesson et al. have published an excellent review of the process challenges encountered when using transaminases for the synthesis of chiral amines.\(^11\)

We previously reported on the creation of an active and stable \(R\)-selective amine transaminase for the synthesis of sitagliptin by using a combination of modeling and directed evolution (Scheme 1).\(^12\) A limitation of the protocol in its current form is the restriction to the use of DMSO/water solvent systems, which may not always be ideal. Enzyme immobilization is an approach that has been employed (primarily with hydrolases) to enable the use of a much wider array of solvent choices. Additionally, enzyme immobilization can allow for catalyst reuse, resulting in a lowering of the effective enzyme loading. Herein we report on the successful immobilization of a transaminase on a hydrophobic support. There have been isolated reports on the immobilization of transaminases in the literature, though these have been used exclusively in aqueous reaction systems.\(^13–15\) We report here the first immobilized transaminase that is active and stable in organic solvent.

Several Mitsubishi sourced polymer-based resins (SEPABEADS) were evaluated for the immobilization of the Januvia transaminase. The resins selected included three epoxide functionalized supports for covalent immobilization (EC-EP, EC-HFA/S, and EXE119), and two adsorption supports for immobilization through hydrophobic interaction (EXA252 and EXE120).

The lyophilized transaminase along with its pyridoxal-5’-phosphate cofactor was dissolved in buffer and incubated with each resin for 24 h. The resins were then recovered, rinsed with buffer, and dried under vacuum with a nitrogen sweep. Each resin was then evaluated for activity on the sitagliptin ketoamide substrate in isopropyl acetate (IPAc). The lyophilized non-immobilized transaminase was also tested as a control. Although all of the immobilization supports tested showed activity, SEPABEAD EXE120, a highly hydrophobic octadecyl functionalized polymethacrylate resin, provided the highest specific activity (Figure 1). As much as 45% of the enzyme activity charged to the immobilization process was recovered on the EXE120 resin, resulting in a 4 wt% loading of transaminase on the resin (40 mg transaminase per 1 g solid support). Not surprisingly, the lyophilized non-immobilized enzyme showed no activity and was completely denatured in the organic solvent environment immediately after addition.

Selecting the SEPABEADS EXE120 resin, we optimized the immobilization procedure to provide for maximum specific activity and stability. Increasing the immobilization time from 24 h to 48 h doubled the specific activity of the immobilized prep.

Immobilized transaminase activity and stability in various solvents and at a range of temperatures was then investigated. The enzyme was shown to be active in a variety of solvents (Figure 2). However, IPAc gave the best results, owing to the
favorable solubility of the ketone substrate and the increased stability of the immobilized transaminase compared to the other solvents. A study of the effect of reaction temperature showed that reaction rate increased linearly with increasing temperature, up to 60°C at which point it leveled off (Figure 3).

Next, the stability of the immobilized transaminase was evaluated and found to be remarkably robust in neat solvent. Proceeding in dry iPrAc at 50°C, the enzyme showed a deactivation rate of only 0.5%/h over 6 days. However, when the reaction solvent was switched to water saturated iPrAc, no deactivation was observed over the same time period. We then demonstrated 10 consecutive recycles of the enzyme over a 200 hour time period with no detectable loss of activity (Figure 4). This stability along with the ability to reuse the immobilized enzyme enable a >90% reduction in the amount of transaminase enzyme required (on a unit activity basis) compared to the soluble enzyme process.

Finally, we demonstrated the generality of this immobilized transaminase in the synthesis of several amines starting from their prochiral ketone precursors (Figure 5). The ability to perform transaminations in organic solvent represents a significant advantage compared to soluble enzyme reactions performed in aqueous systems. Aqueous reactions require the use of buffer and necessitate continuous pH control throughout the course of the reaction. At the end of an aqueous/soluble enzyme reaction, organic solvent is added to extract the product. The mixture is then filtered to remove denatured enzyme...
that occurs as a result of the organic solvent added. The phases are split; and the organic phase containing the desired product is recovered and dried down. The aqueous phase must then be disposed of as solvent contaminated waste. The ability to perform the reaction in an organic solvent with an immobilized transaminase eliminates the need for buffer, continuous pH control, and difficult filtration of a denatured enzyme. This greatly simplifies the workup, reducing the processing time needed and the waste generated. Critically, in contrast to an aqueous/soluble enzyme reaction for which the deactivated enzyme is discarded, the immobilized enzyme can be reused over and over again.

In summary, we have demonstrated for the first time the successful immobilization of a transaminase that is capable of operating in organic solvent. The transaminase, immobilized on SEPABEADS EXE120, exhibited excellent activity and stability under the optimized process conditions and allowed for multiple rounds of enzyme reuse with no detectable loss of activity. Finally, the immobilized transaminase has been shown to be a generally useful tool for the facile synthesis of amines from ketones.

**Experimental Section**

Commercial grade reagents and solvents were purchased from Sigma–Aldrich and used without further purification. The transaminase (CDX-017 from Codexis) was made in 100 mM potassium phosphate buffer (pH 7.0) containing 0.5 % H3PO4) mobile phase at 1 mL min⁻¹ and 25 °C. UV absorbance was monitored at 210 nm. The 50 μL reaction samples were taken and diluted in 950 μL of mobile phase. Both ketone substrates and amine products were readily visible with this method (amine product standards were purchased from Sigma–Aldrich). The racemic amine product standards were used to develop analytical methods for the determination of enantiomeric excess (ee).

**Immobilized transaminase reaction protocol**

30 g L⁻¹ ketone substrate and 80 mL⁻¹ isopropylamine were added to neat isopropyl acetate (iAPc). 200 g L⁻¹ of immobilized transaminase was then added to the substrate solution. The reaction was heated to 60 °C and mixed with an orbital shaker for 24 h. Optimized reaction conditions developed for the synthesis of sitagliptin used 200 g L⁻¹ ketone substrate, 300 g L⁻¹ immobilized transaminase (equivalent to 6 wt % transaminase to substrate loading), and water saturated iAPc, which resulted in excellent enzyme stability. For immobilized transaminase reuse testing, the resin was filtered from the reaction solution and rinsed with 3 x volumes of water saturated iAPc to ensure removal of starting material and product. It was then used directly in the subsequent reaction with no further manipulation. Most reactions were performed on a 1 mL scale. The optimized sitagliptin reaction was scaled to 100 mL.

Analysis of the extent of conversion was performed by isotopic reverse phase Agilent (Palo Alto, CA) HPLC using a Zorbax SB-C18 (75 mm × 4.6 mm) column and a 70 % acetonitrile/30 % water (containing 0.5 % H3PO4) mobile phase at 1 mL min⁻¹ and 25 °C. UV absorbance was monitored at 210 nm. The 50 μL reaction samples were taken and diluted in 950 μL of mobile phase. Both ketone substrates and amine products were readily visible with this method (amine product standards were purchased from Sigma–Aldrich). The racemic amine product standards were used to develop analytical methods for the determination of enantiomeric excess (ee).

ChiralPak IC 150 × 3 mm, 5 micron, 25 °C, 1 mL min⁻¹, 210 nm; mobile phase: 15 % 25 mM IBA in MeOH in 3 mL min⁻¹; ¹H NMR (400 MHz, CDCl₃): δ = 1.39 (3H, d), 4.12 (1H, q), 7.22 (1H, m), 7.34 (3H, m), 7.45 ppm (1H, m); ¹³C NMR (500 MHz, CDCl₃): δ = 133.20, 128.65, 127.14, 126.14, 51.38, 25.15 ppm.[16]

AD-RH 150 × 4.6 mm, 5 micron, 25 °C, 1.5 mL min⁻¹, 210 nm; mobile phase: A: pH 9 Borate Buffer (5 mM), B: MCN, 10 % B to 30 % B in 10 min, ramp to 80 % in 15 min and hold 2 min; ¹H NMR (400 MHz, CDC3): δ = 0.84 (3H, t), 1.70 (2H, m), 2.81 (2H, bs), 3.85 (1H, t), 7.25–7.48 ppm (5H, m); ¹³C NMR (500 MHz, CDCl₃): δ = 137.04, 128.33, 127.38, 126.42, 61.68, 31.90, 10.63 ppm.[17]

AD-RH 150 × 4.6 mm, 5 micron, 25 °C, 1.5 mL min⁻¹, 210 nm; mobile phase: A: pH 9 Borate Buffer (5 mM), B: MCN, 10 % B to 30 % B in 10 min, ramp to 80 % in 15 min and hold 2 min; ¹H NMR (400 MHz, CDC3): δ = 1.38 (3H, d), 2.85 (2H, bs), 3.87 (3H, s), 4.12 (1H, q), 6.98 (2H, d), 7.28 ppm (2H, d); ¹³C NMR (500 MHz, CDCl₃): δ = 159.18, 128.36, 127.70, 113.79, 55.56, 50.86, 25.27 ppm.[18]

OD-H 150 × 4.6 mm, 5 micron, 25 °C, 1.0 mL min⁻¹, 210 nm; mobile phase: A: isopropanol, B: hexanes (with 1 % diethylamine); 5 % A and 95 % B Run time: 30 min; ¹H NMR (400 MHz, CDC3): δ = 4.98 (1H, q), 7.54 (3H, m), 7.62 ppm (2H, m); ¹³C NMR (500 MHz, CDC3): δ = 135.64, 129.00, 128.65, 128.14, 126.19, 61.66 ppm.[19]

ChiralPak AD-H 150 × 3 mm, 5 micron, 25 °C, 1 mL min⁻¹, 210 nm; mobile Phase: 6 % 25 mM IBA in MeOH 4 mL min⁻¹; ¹H NMR (400 MHz, CDCl₃): δ = 1.11 (3H, d), 1.64 (2H, q), 2.40 (2H, bs), 2.64 (2H, t), 2.95 (1H, m), 7.13 (3H, m), 7.29 ppm (2H, m); ¹³C NMR (400 MHz, CDCl₃): δ = 142.03, 128.40, 128.35, 125.71, 46.65, 40.85, 32.68, 22.99 ppm.[16]

ChiralPak IC 150 × 3 mm, 5 micron, 25 °C, 1 mL min⁻¹, 210 nm; mobile phase: 15 % 25 mM IBA in MeOH in 3 mL min⁻¹; ¹H NMR (400 MHz, CDCl₃): δ = 1.6–2.1 (4H, m), 2.61–2.70 (2H, m), 4.01 (1H, t), 7.14–7.32 (3H, m), 7.44–7.49 ppm (1H, m); ¹³C NMR (400 MHz, CDCl₃): δ = 186.18, 28.2, 30.9, 49.2, 126.1, 126.4, 127.2, 128.5, 128.9, 132.6 ppm.[16]

OD-H 150 × 4.6 mm, 5 micron, 25 °C, 1.0 mL min⁻¹, 210 nm; mobile phase: A: isopropanol, B: hexanes (with 1 %
diethylamine); 5% A and 95% B; 1H NMR (400 MHz, CDCl₃): $\delta = 1.12 (3 \text{ H}, \text{ d}), 4.51 (1 \text{ H}, \text{ q}), 7.30 (1 \text{ H}, \text{ m}), 7.49 (1 \text{ H}, \text{ m}), 7.62 \text{ ppm} (2 \text{ H}, \text{ m}); 13C NMR (400 MHz, CDCl₃): $\delta = 138.3, 133.2, 131.7, 128.4, 126.5, 123.4, 48.7, 25.4 \text{ ppm}$. [20]

OD-RH 150 × 4.6 mm, 5 micron, 25 °C, 1 mL min⁻¹, 210 nm; mobile phase: A: 0.02% HClO₄ 150 mM NaClO₄ (aq), B: MeCN, 20% B to 60% B in 30 min; 1H NMR (CDCl₃, 400 MHz): $\delta = 1.36 (3 \text{ H}, \text{ d}), 4.08 (1 \text{ H}, \text{ q}), 7.16 (1 \text{ H}, \text{ m}), 7.24 (1 \text{ H}, \text{ m}), 7.38 (1 \text{ H}, \text{ m}), 7.49 \text{ ppm} (1 \text{ H}, \text{ m}); 13C NMR (400 MHz, CDCl₃): $\delta = 148.58, 132.59, 130.27, 129.30, 124.87, 122.64, 50.96, 24.73 \text{ ppm}$. [21]

Key words: chiral amine · enzyme · immobilized · transaminase