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Biphasic Reaction System Allows for Conversion of Hydrophobic Substrates by Amine Dehydrogenases

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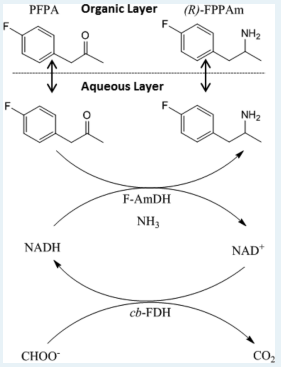
Supporting Information

ABSTRACT: A novel amine dehydrogenase, "F-AmDH", catalyzes the reversible reduction of prochiral ketones to chiral amines. However, many targeted hydrophobic substrates of F-AmDH show little to no solubility in an aqueous medium. The introduction of water-miscible organic solvents was unsuccessful because of AmDH deactivation. In a biphasic aqueous−organic system, F-AmDH, coupled with formate dehydrogenase (FDH), and hydrophilic co-factors are envisioned to remain in the aqueous phase while the hydrophobic substrate partitions between the phases. The advantages include a larger amount of total substrate present in the system, straightforward product removal, and reduced or negligible substrate and product inhibition. We succeeded in generating chiral amines from hydrophobic substrates that were previously unattainable because of the low solubility of the ketone substrate in aqueous medium. The partition coefficient played an important role in establishing optimal reaction conditions. Specific activity was found to be comparable between aqueous and biphasic reaction systems for substrates that showed some solubility. Thus, biphasic reaction conditions widen the range of substrates for the production of chiral amines using amine dehydrogenases.



KEYWORDS: *chiral amines, biocatalysis, organic solvents, biphasic system, amine dehydrogenases*

I. INTRODUCTION

Enantiomerically pure amines are commonly used as precursors for active pharmaceutical ingredients (APIs). Examples include sitagliptin (Januvia and Janumed) and rasagiline (Azilect). In 2010, 80% of the 200 most prescribed brand name drugs in the United States contain nitrogen, with a chiral amine present in 40% of these compounds.[1] Heterogeneous catalysts developed to create chiral amine compounds can be difficult to synthesize, and they often feature low selectivity, less environmentally friendly solvents, and difficult amine product separation.[2] One current enzymatic route used in industrial processes involves ω-transaminase, which requires a sacrificial amine donor and often suffers equilibrium limitations.[3] More recently, amine dehydrogenases (AmDHs) have been developed to catalyze the reductive amination of prochiral ketones into chiral amines with the addition of the co-factor NADH and ammonia, the latter being incorporated into the reaction buffer. Previously developed AmDHs in our laboratory include leucine amine dehydrogenase (L-AmDH),[4] phenylalanine amine dehydrogenase (F-AmDH),[5] and a chimeric amine dehydrogenase (cFL1-AmDH).[6]

The main challenge of the newly developed amine dehydrogenases is their limited substrate acceptance. It is our goal to expand the range of substrates for AmDH toward intermediates important for pharmaceuticals or crop protection. The limited substrate acceptance is largely due to the hydrophobicity and, thus, low solubility of potential substrates in aqueous medium. Reactions in organic solvents have many advantages beyond increased solubility of substrates, including suppression of hydrolysis side reactions, easy product removal, and overcoming unfavorable equilibria.[7] However, reactions involving organic solvents often accelerate deactivation of enzymes.[8] Despite this notion, many reactions have been successfully achieved, including amination of prochiral ketones by transaminases performed in water/DMSO.[9] Dehydrogenases, however, have been scarcely employed in these water-miscible systems, because of their limited stability in organic solvents, and low solubility and stability of the co-factor has prevented successful applications.[10] To incorporate biocatalysts into organic solvents, work can be either performed on the biocatalyst to improve its stability or on the medium to optimize reaction conditions.[11] Optimization of reaction conditions in the presence of organic solvents is the focus of the current work.

In biphasic aqueous−organic solvent systems, enzymes and hydrophilic co-factors, such as NAD(P)H, are envisioned to remain in the aqueous phase while the hydrophobic substrate preferentially partitions into the organic phase (Scheme 1). Enzyme interaction with the organic solvent should be minimal.[12] Alcohol dehydrogenase has been used successfully

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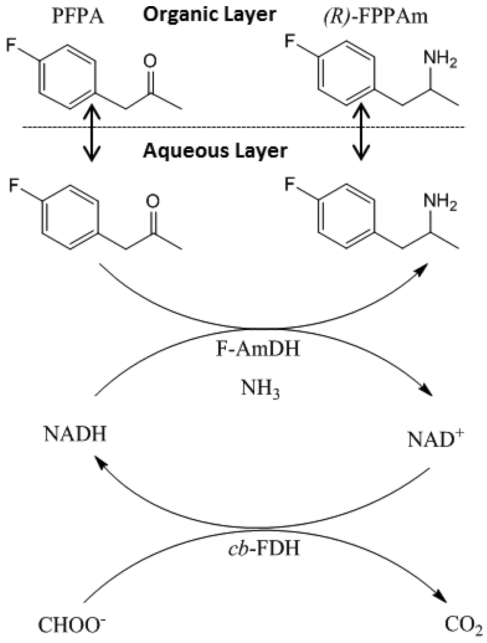
Scheme 1. Biphasic System Reaction Involving F-AmDH and *p*FPA



in a biphasic reaction system coupled with co-factor regeneration using *Candida boidinii* FDH (*Cb*-FDH).[13] Previous work specifically involving phenylalanine dehydrogenases in organic solvents has included immobilization of the enzyme[14] or additional mutations[15] to improve stability, none of which will be included in this work.

This current work focuses on the incorporation of water-immiscible organic solvents to establish an efficient biocatalytic route using AmDH to produce chiral amines, often scaffolds for active pharmaceutical intermediates (APIs), from hydrophobic ketones. The substrate range for AmDH is expanded because the biphasic reaction system allows for conversion of desired substrates that previously could not be converted to chiral amines as a result of their insolubility (Figure 1, substrates **3** and **4**). The amine products of *p*FPA (**1a**) and 1-adamantyl methyl ketone (**3a**), *p*-fluorophenylisopropylamine (**1b**), and 1-adamantyl-ethyl-1-amine (**3b**), respectively, mimic APIs of sitagliptin (Januvia)[16] and saxagliptin (Onglyza),[17] respectively, two anti-Type II diabetes drugs.

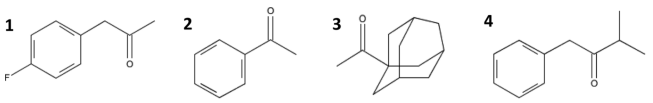


Figure 1. Hydrophobic substrates of the amine dehydrogenases: **1**, *para*-fluoro phenyl acetone (*p*FPA); **2**, acetophenone; **3**, 1-adamantyl methyl ketone; and **4**, 3-methyl-1-phenyl-2-butanone.

II. RESULTS AND DISCUSSION

The model substrate of F-AmDH is *para*-fluoro phenyl acetone (*p*FPA). *p*FPA has limited solubility (10 mM at pH 9.6 and 25 °C) in aqueous ammonium formate buffer, compared to phenylalanine, because of the replacement of the carboxyl group with a methyl group (solubility decreases over 10-fold). The necessity to increase solubility of hydrophobic substrates initially led us first to monophasic aqueous−organic solvent reaction systems. In the presence of organic co-solvents that previously achieved success, including acetone, methanol, dimethyl sulfoxide (DMSO), and ethylene glycol (10%−30%, v/v),[18] F-AmDH quickly lost most of its catalytic activity toward *p*FPA (see the Supporting Information). This led to a transition into biphasic aqueous−organic solvent reaction systems.

The first step toward developing a biphasic organic solvent reaction system is to identify potential solvents that are immiscible with water, achieved previous success,[10b,13] and exhibit minimal environmental impact to maintain benefits of biocatalysts, according to the ACS GCI PR solvent selection guide.[19] Heptane was chosen as a representative example of aliphatic hydrocarbons, toluene for aromatic compounds, methyl *tert*-butyl ether (MTBE) for ethers and butyl acetate for esters.[20] Chloroform was also additionally chosen, because of its ease of extraction of the amine product, especially involving analytical-scale work. Ketones were excluded because of probable cross-reactivity as a substrate.

According to the solvents' log *P* values (Table 1), heptane is the least-polar solvent (highest organic-aqueous partition coefficient).[21] Hydrophilic solvents are known to remove water molecules from the surface of the enzyme essential for catalysis and, subsequently, often lead to enzyme deactivation.[7a] Therefore, heptane did not deactivate the enzymes compared to other solvents, likely because less of it enters the aqueous phase.[22] In addition, we measured the log *P* value of *p*FPA in all four organic solvents between the aqueous reaction buffer and organic solvent (see Table 1 and the Supporting Information). The partition coefficient of *p*FPA in heptane is relatively small, compared to other solvents, so that a good portion of the substrate distributes into the aqueous phase. In addition, the cofactor regeneration enzyme *Cb*-FDH is known to have low stability in the presence of common water-immiscible organic solvents, such as toluene and MTBE, but was previously found to show stability around aliphatic hydrocarbons such as hexane and heptane.[13] Since the enzyme may come into contact with the interface between the organic and aqueous phases during the reaction, it is beneficial for the enzymes to have high stability in the presence of organic solvents. Heptane was ultimately chosen as the organic solvent for the biphasic reaction system, based on these partition coefficient results.

Next, the partition coefficients are experimentally determined for desired hydrophobic substrates and their corresponding amines in heptane (Table 2). Larger partition coefficients indicate that less of the substrate moves into the aqueous phase and conversion becomes harder to achieve. The partition of

Table 1. Partition Coefficient of *p*FPA in Various Organic Solvents

	heptane	MTBE	butyl acetate	toluene	chloroform
*p*FPA	1.11	2.12	2.25	2.27	2.56
log *P* values of solvents (reported in literature)[a]	4.27	1.29	1.80	2.72	1.94

[a]Estimated with Advanced Chemistry Development (ACD/Labs) Software V11.02.[21]

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amines is important to determine the ease of product extraction, and the transition of amine into the organic layer can help avoid product inhibition. While the partition coefficient is the most common criterion to predict enzyme success in organic solvents,[23] prediction of enzyme behavior within a biphasic system likely cannot be captured by just a single variable.[20] Enzymes can easily deactivate or perform poorly in various nonoptimal reaction conditions (such as temperature, pH, enzyme concentrations, substrate concentrations).

Partition coefficients are reported here for varying phase ratios for the case of stirred solution (see Table 2). Previous work in biphasic media had found the optimal phase ratio to be 1:4 for heptane to water.[10b,13] The substrate concentration decreases with a smaller ratio of organic to aqueous phase, because more of the substrate is partitioned into the aqueous phase (see Table 2 and Figure 2). A phase ratio of 1:4 is employed for the remaining biphasic reactions. 1-Adamantyl methyl ketone (**2a**) is the exception because the log *P* values are extremely skewed, as very little of the adamantyl substrate partitioned into the aqueous phase. Therefore, a more concentrated organic phase is observed. This result explains also the issue of low conversion of the 1-adamantyl methyl ketone to 1-(1-adamantyl) ethylamine, since very little of the substrate diffuses into the aqueous phase, regardless of the solvent.

Table 2. Partition Coefficient of Substrates in Heptane[a]

phase ratio	**1a**	**1b**	**2a**	**2b**	**3a**	**3b**[b]	**4a**
1:1	1.11	−0.02	1.35	−0.14	3.28	2.65	2.21
1:2	1.12	−0.03	1.37	−0.15	3.72	2.56	2.1
1:4	1.22	−0.1	1.35	−0.17	4.95	2.28	1.75
1:4, stirring	1.21	−0.04	1.39	−0.18	4.94	2.36	1.79

[a]Reaction conditions (1 mL): concentration, 25 mM, buffer: NH_4HCO_2/OH (5 M, pH 9.6); T = 30 °C. Partitioning was allowed to equilibrate for 5 h. Suffix "a" denotes ketone, whereas suffix "b" denotes amine. [b]Average of two log *P* values: racemic 1-(1-adamantyl ethylamine) is split into two peaks with two retention times on the chiral GC column. For 3-methyl-1-phenyl-2-butanone (**4a**), the respective amine (*α*-(1-methylethyl)-benzethanamine)), compound **4b**, is not available for partition coefficient data.

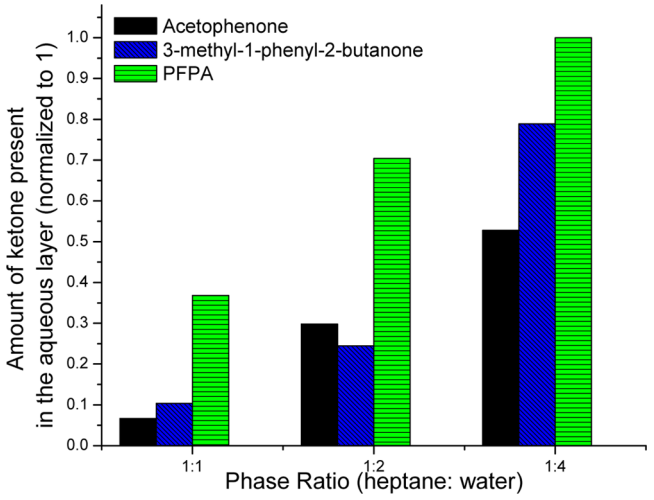

Figure 2. Amount of hydrophobic substrates in the aqueous layer as a function of phase ratio (normalized to **1a**, the amount of *p*FPA in the aqueous phase).

The effect of stirring on the system was also investigated. Previous biphasic reaction systems were gently stirred to improve distribution of the hydrophobic substrate at the interface.[24] However, stirring did not show a significant difference in specific activity and is not critical to the overall reaction because mass transfer, as expected, is fast, compared to kinetics. The rate-limiting step of mass transfer is the movement of the substrate across the interface. This interfacial mass transfer occurs fast enough to ensure the reaction is always saturated with the adequate amount of substrate to proceed in the absence of mass-transfer limitations (see the Supporting Information). Stirring can also lead to enzyme precipitation caused by shear stress. Therefore, stirring is not incorporated into future reactions.

Conversion of all substrates to their corresponding amines is observed via high-performance liquid chromatography (HPLC), except 1-adamantyl methyl ketone, where conversion is measured on the gas chromatography (GC) system, because the ketone and amine both lack UV absorbance. F-AmDH exhibits activity toward *p*FPA (**1a**) and 3-methyl-1-phenyl-2-butanone (**4a**) because it requires a methyl group between the phenyl ring and the ketone.[5] cFL1-AmDH exhibits activity toward acetophenone (**2a**) and 1-adamantyl methyl ketone (**3a**), because it can accept benzylic carbonyls.[6] Co-factor regeneration enzymes employed in the biphasic reaction system are the *Candida boidinii* formate dehydrogenase (*Cb*-FDH) and a thermostable variant of the *Bacillus subtilis* glucose dehydrogenase (*Bs*-GDH).[25]

Enzymatic reactions of the substrates *p*FPA and acetophenone, which still show some solubility, were first determined in purely aqueous reaction. Substrate conversion and thus specific activity are measured via HPLC. Substrate specificity is comparable to activity observed via UV spectrometry (Table 3). The experiment was conducted to show how solubility was limiting the overall volume productivity and the influence of the biphasic system and organic solvents on specific activity (see Figure 3 and the Supporting Information). A reaction system that does not include organic solvents cannot be used for substrates **3** and **4** because of little to no solubility in aqueous medium. This again emphasizes the importance and necessity of a biphasic organic solvent system for these substrates.

Table 3. Specific Activity, Calculated over One Hour

	Specific Activity (U mg^{-1})			
	1	**2**	**3**	**4**
biphasic (HPLC)	3.7	0.45	0.035	0.17
monophasic (HPLC)	4.3	0.55	ND[a]	ND[a]
monophasic (UV-spec)[b]	3 to 7	0.3	ND[a]	ND[a]

[a]ND = not detected (because of solubility issues). [b]Specific activity obtained from the literature and assay are described in the Supporting Information.[5,6] For 3-methyl-1-phenyl-2-butanone, the respective amine (*α*-(1-methylethyl)-benzethanamine)) is not available and specific activity was determined by the amount of ketone that was reacted.

The next step was to implement a biphasic organic solvent system involving heptane (based in Scheme 1). The amount of amine present in the system is shown to increase over time, and successful conversion to amine is achieved for all hydrophobic substrates (see Figure 4 and the Supporting Information).

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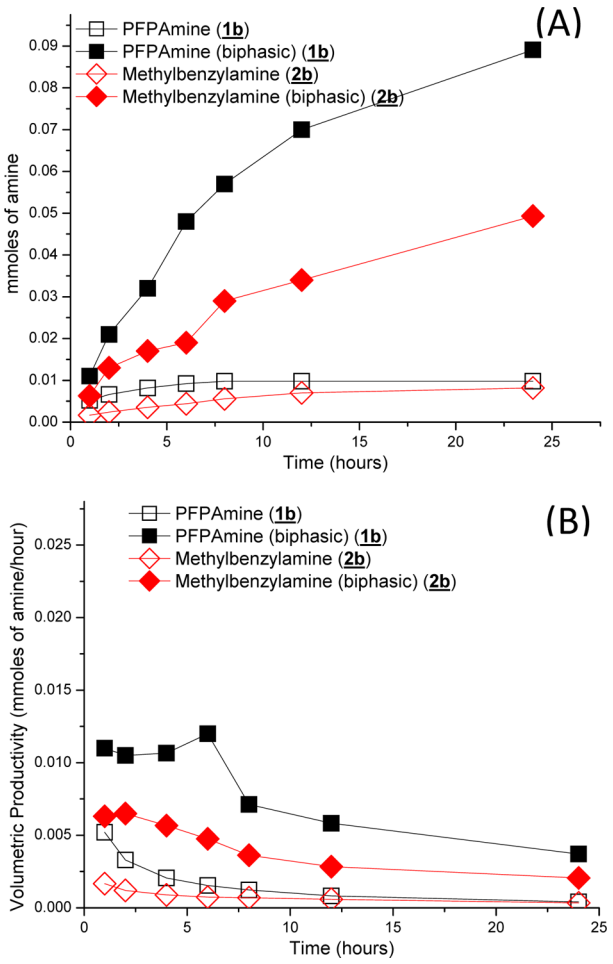

Figure 3. Amount of amine product over time for *p*FPA and acetophenone in a biphasic organic solvent reaction system. (A) Increased volumetric productivity over time for *p*FPA and acetophenone in a biphasic organic solvent reaction system. (B) Reaction conditions for *p*FPA without organic solvents (5 mL): c(*p*FPA) = 10 mM, c(NADH/NAD$^+$) = 1 mM, 0.1 mg F-AmDH, buffer: NH_4HCO_2/OH (5 M, pH 9.6), T = 35 °C. Reaction conditions for *p*FPA in biphasic (1 mL): c(*p*FPA) = 150 mM, c(NADH/NAD$^+$) = 1 mM, 0.1 mg F-AmDH, $V_{organic}/V_{aqueous}$ = 0.25, buffer: NH_4HCO_2/OH (5 M, pH 9.6), T = 35 °C. Reaction conditions for acetophenone without organic solvents (5 mL): c(acetophenone) = 30 mM, c(NADH/NAD$^+$) = 1 mM, 0.25 mg cFL1-AmDH, buffer: NH_4HCl/OH (5 M, pH 9.6), T = 50 °C. Reaction conditions for acetophenone in biphasic (1 mL): c(acetophenone) = 150 mM, c(NADH/NAD$^+$) = 1 mM, 0.25 mg cFL1-AmDH, $V_{organic}/V_{aqueous}$ = 0.25, buffer: NH_4HCl/OH (5 M, pH 9.6), T = 50 °C. [Note: The monophasic reaction system was performed in a 5-mL glass vial. However, the concentration of amine is assumed to be consistent throughout the given volume and the number of millimoles reported in panel (A) shows the theoretical number of millimoles in a 1-mL reaction volume (we divided the number of millimoles observed in the 5-mL reaction by 5).]

Organic solvents are necessary to obtain conversion. An increased volumetric productivity is observed for *p*FPA and acetophenone in a biphasic reaction system, compared to a reaction without organic solvents (Figure 3). *p*FPA exhibits limited solubility (up to 10 mM) without the presence of organic solvent; consequently, the limit on the amine that can be produced similarly is ∼10 mM in purely aqueous systems. However, this problem is resolved through the incorporation of

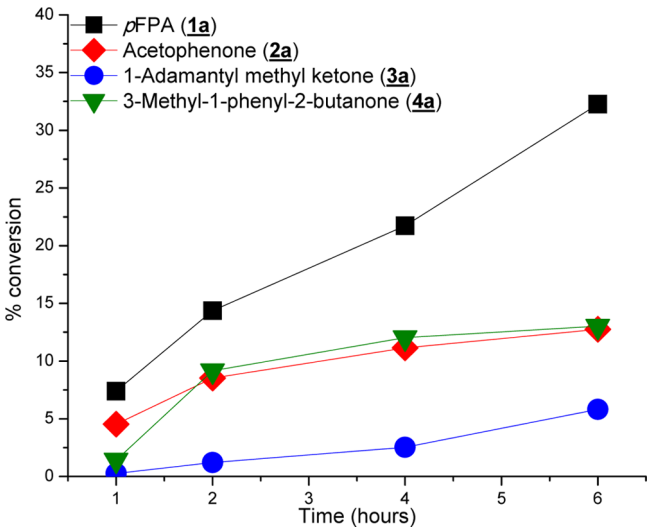

Figure 4. Percent conversion over time in a biphasic organic solvent reaction system. Reaction conditions for *p*FPA (1 mL): c(*p*FPA) = 150 mM, c(NADH/NAD$^+$) = 1 mM, 0.1 mg F-AmDH, $V_{organic}/V_{aqueous}$ = 0.25, buffer: NH_4HCO_2/OH (5 M, pH 9.6), T = 35 °C. Reaction conditions for 3-methyl-1-phenyl-2-butanone are the same as *p*FPA. Reaction conditions for acetophenone (1 mL): c(acetophenone) = 150 mM, c(NADH/NAD$^+$) = 1 mM, 0.25 mg cFL1-AmDH, $V_{organic}/V_{aqueous}$ = 0.25, buffer: NH_4HCl/OH (5 M, pH 9.6), T = 50 °C. Reaction conditions for 1-adamantyl methyl ketone are the same as acetophenone. All reactions performed with heptane as the organic solvent.

organic solvents and the volumetric productivity doubles for *p*FPA and is very apparent after 6 h.

Specific activity of the amine dehydrogenase toward its respective substrates was measured over 1 h. Comparable specific activity involving the model substrates is achieved between reaction systems with and without the presence of organic solvents (Table 3, biphasic versus monophasic). In addition, similar specific activity is observed between time point measurements by the HPLC system and the UV spectrometer. For highly hydrophobic substrates (substrates **3** and **4**), specific activity can only be measured in a biphasic reaction system. The specific activity of cFL1-AmDH with respect to 1-adamantyl methyl ketone is lower because very little of the substrate diffuses into the aqueous phase, because of its high hydrophobicity. In addition, biphasic systems had no negative impact on enantioselectivity, compared to a monophasic system (see the Supporting Information).

III. CONCLUSION

The present work demonstrates the development of a biphasic aqueous−organic solvent reaction system for ketone reduction to enantiomerically pure amines catalyzed by novel amine dehydrogenases and FDH/GDH for regeneration. Because of deactivation of most dehydrogenases by organic solvents and low solubility of the nicotinamide cofactor, dehydrogenases rarely have been incorporated into water-miscible organic systems. By developing biphasic aqueous/water-immiscible organic solvents reaction media for AmDH/(FDH/GDH), we achieved two goals: (i) for moderately water-soluble ketones substrates, we increase overall solubility and volumetric productivity, as enzymatic activity is comparable between aqueous and biphasic media, and (ii) for highly hydrophobic ketones with very low water solubility, several of them reagents

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Table 4. HPLC Method and Retention Times for Ketones and Their Respective Amines

	para-fluoro phenyl acetone (*p*FPA)	acetophenone	3-methyl-1-phenyl-2-butanone
method	methanol−water−(0.5 M sodium acetate−glacial acetic acid buffer, pH 3.40) (60:35:5) with 0.1% TFA[27]	methanol−water−(0.5 M sodium acetate−glacial acetic acid buffer, pH 3.40) (40:55:5) with 0.1% TFA[27]	acetonitrile (0.1% TFA)−water (30:70)
retention times	ketones: 4.3 min amines: 3 min	ketones: 10.2 min amines: 3.8 min	ketones: 9.1 min amines: 3.9 min

for APIs, we are able to demonstrate that they are substrates of AmDH. The results of this work can be applied toward further development of a biphasic organic solvent system for additional substrates of amine dehydrogenases.

IV. EXPERIMENTAL SECTION

General. Substrates were obtained from suppliers and used without further purification. NADH was obtained from Amresco (Solon, OH). *p*FPA, methylbenzylamine, and butyl acetate were obtained from Acros Organic (Morris Plains, NJ). (*R*/*S*)-*p*FPAm and 3-methyl-1-phenyl-2-butanone were obtained from Alfa Aesar (West Hill, MA). Acetophenone, 1-adamantyl methyl ketone, (*R*/*S*)-1-(1-adamantyl) ethylamine hydrochloride, 4-(dimethylamino)pyridine, NAD^+, chloroform, and trifluoroacetic acid anhydride were obtained from Sigma−Aldrich (St. Louis, MO). Heptane and methyl-*tert*-butyl ether (MTBE) was obtained from J.T. Baker (Phillipsburg, NJ). HPLC grade methanol, toluene and acetonitrile were obtained from Fisher Scientific (Fairlawn, NJ).

Protein Expression and Purification. The same protocol as previously described was used to express and purify the amine dehydrogenases.[5] Both amine dehydrogenases were expressed in a pET 28a, BL21 (DE3) (Invitrogen) system at 18 °C in Luria−Bertani medium (United States Biological). Expression was induced with 0.5 mM IPTG at an OD_{600} value of 0.5 and continued for 12 h. Overexpression was determined by observing a thick band at 44 kDa on an SDS-PAGE gel. His-tagged proteins were purified by employing immobilized metal affinity chromatography (IMAC) through a Ni-NTA resin (Thermo Scientific).

Spectrophotometric and Activity Analysis. Specific activity is determined using an NADH-dependent (340 nm, λ_{340} = 6220 M^{-1} cm^{-1}) spectrophotometric assay.[26] Activity is calculated from the stoichiometric oxidation of NADH to NAD^+ as measured by the change in absorbance over time. One unit of activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of amine in 1 min.

High-Performance Liquid Chromatography (HPLC). Liquid chromatography was performed on a Shimadzu UFLC-2010 with a Beckman Coulter Ultrasphere column (4.6 mm × 25 cm). The samples are run at 1 mL min^{-1} with a column temperature at 40 °C. Detection occurs at 255 nm. Methods for detecting conversion to amine are summarized in Table 4.

Gas Chromatography (GC). Gas chromatography was performed on a Shimadzu GC-2010 GC apparatus with a Restek Rt-BDEXcst column (length, 30 m; inner diameter, 0.32 mm; film thickness, 0.25 μm). 1-Adamantyl methyl ketone does not absorb in the ultraviolet (UV) range, so detection of amine was determined by gas chromatography (GC) with flame ionization detection (FID). The reaction mixture is derivatized by trifluoroacetic acid anhydride (TFAA).[28] To derivatize the compounds, 150 mM (approximately one crystal) of 4-(dimethylamino) pyridine, which acts as a catalyst, and 2 μL of TFAA were added to 200 μL of the sample. The derivatized sample was allowed to react for 10 min before GC analysis. The linear velocity is 60 cm/s and the carrier gas was helium. When the aqueous layer was analyzed, chloroform is added to extract any leftover ketone and amine into the organic layer.

Partition Coefficient. The ratio of the amount of the compound present in the organic phase compared to the aqueous phase was calculated to determine the partition coefficient. The aqueous medium is ammonium formate (5 M NH_4HCO_2/OH, pH 9.6). Twenty-five millimolar (25 mM) of the hydrophobic substrate was added to the biphasic mixture. The partitioning was conducted in a glass vial (diameter, 0.5 cm; height, 4 cm) and allowed to equilibrate at 30 °C for 5 h. Different phase ratios were observed in a total reaction volume of 1 mL. When stirring was required, the sample was stirred at 150 rpm. Both the organic and aqueous layers were analyzed via HPLC. As for the 1-adamantyl methyl ketone, both layers were analyzed via GC.

Conversion in Purely Aqueous Systems (Without the Presence of Organic Solvents). To determine conversion to the amine, reactions were performed in the presence of the respective amine dehydrogenase and the regeneration enzyme in a 5-mL glass vial. Time points were taken up to 24 h. Only 10 mM of *p*FPA was added due to solubility issues while 30 mM of acetophenone was added into the reaction. In the case of *p*FPA, 0.1 mg of F-AmDH and 0.25 mg of *Cb*-FDH were added to the aqueous reaction medium of 5 M NH_4HCO_2/OH, pH 9.6. The reaction was performed at 35 °C. In the case of acetophenone, 0.25 mg of cFL1-AmDH and 0.5 mg of the thermostable *Bs*-GDH was added to the reaction medium of 5 M NH_4Cl/OH, pH 9.6. The reaction was conducted at 50 °C and 100 mM of glucose was added to the reaction. One millimolar (1 mM) NADH/NAD^+ was added to all reactions. All reactions were stopped by diluting with methanol and analyzed using HPLC for amine peaks. After 24 h, the product amine was extracted with MTBE and analyzed using ^1H NMR (20 Hz) in $CDCl_3$.

Biphasic Conversion. To determine conversion to the amine, reactions were performed in the presence of the respective amine dehydrogenase and the regeneration enzyme in a 1-mL volume and time points were taken. One hundred fifty millimolar (150 mM) of the substrate was added to the organic solvent. Then, 0.1 mg and 0.25 mg of F-AmDH was added for the *p*FPA and 3-methyl-1-phenyl-2-butanone reactions, respectively. The aqueous reaction medium was 5 M NH_4HCO_2/OH, pH 9.6, and the reaction was performed at 35 °C. 0.25 mg of cFL1-AmDH was added to the reactions involving acetophenone and 1-adamantyl methyl ketone. The aqueous reaction medium was 5 M NH_4Cl/OH, pH 9.6, and the reaction was performed at 50 °C. Twice as much regeneration enzyme activity (*Cb*-FDH, *Bs*-GDH) was added to ensure that (i) the reversible reaction would not occur and (ii) a sufficient amount of co-substrate NADH was always present during the reaction. One millimolar (1 mM) NADH/NAD^+ was added to the reactions. All reactions were stopped by adding methanol and analyzed by HPLC to detect of amine

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peaks. After 24 h, the products *p*FPAm and methylbenzylamine were extracted with MTBE and analyzed via ^{1}H NMR in $CDCl_3$. Mass spectrometry was used to determine product amine with reactions involving 1-adamantyl methyl ketone and 3-methyl-1-phenyl-2-butanone.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information contains HPLC, NMR, and mass spectrometry data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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