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Biocatalytic *N*-Alkylation of Amines Using Either Primary Alcohols or Carboxylic Acids via Reductive Aminase Cascades

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Supporting Information

ABSTRACT: The alkylation of amines with either alcohols or carboxylic acids represents a mild and safe alternative to the use of genotoxic alkyl halides and sulfonate esters. Here we report two complementary one-pot systems in which the reductive aminase (RedAm) from *Aspergillus oryzae* is combined with either (i) a 1° alcohol/alcohol oxidase (AO) or (ii) carboxylic acid/carboxylic acid reductase (CAR) to affect *N*-alkylation reactions. The application of both approaches has been exemplified with respect to substrate scope and also preparative scale synthesis. These new biocatalytic methods address issues facing alternative traditional synthetic protocols such as harsh conditions, over-alkylation and complicated workup procedures.

Primary and secondary amines constitute important classes of compound for the chemical industry, with applications including surface coatings, resins, polymers, dyestuffs, resins as well as building blocks for the synthesis of pharmaceuticals and agrochemicals. The two most widely employed methods for their preparation involve either *N*-alkylation or reductive amination; however, these methods are far from ideal and have problems associated with their use.[1−4] Alkylating agents (e.g., alkyl halides, sulfonate esters) are highly genotoxic, and their application is further limited by poor chemoselectivity due to the formation of byproducts resulting from overalkylation. Reductive amination of carbonyl compounds with primary amines requires an excess of the amine reagent in order to prevent overalkylation which lowers the efficiency of the process and complicates workup procedures. Both methods also utilize highly reactive and often nonrenewable feedstocks.

Aldehydes usually need to be prepared *in situ* and are unstable to aerobic oxidation and other side reactions including condensation and aldol type processes. These limitations have prompted the recent development of synthetic methodologies that employ alcohols and carboxylic acids as surrogate reagents for amine alkylation. The use of alcohols requires homogeneous transition-metal catalysis, in an overall redox neutral transformation, where water is the sole byproduct. This approach, termed "chemocatalytic hydrogen borrowing", involves the *in situ* activation of an alcohol to its corresponding carbonyl compound prior to imine formation and subsequent reduction to yield the amine (Figure 1a).[5−9] The "hydrogen borrowing" principle has been exploited using both homogeneous and heterogeneous[10] catalysis and has recently been applied on kilogram scale by Pfizer in the synthesis of an API for the treatment of schizophrenia.[11]

Despite the favorable atom economy of these reactions, their green credentials are somewhat limited by requirements for high temperatures, long reaction times and precious metal catalysts, which may be poisoned by either the substrate or product amine (or both). Carboxylic acids represent attractive alternatives as reagents for alkylation reactions in view of their availability from renewable feedstocks. However, implicit in their use is the selective reduction to aldehydes for application in the alkylation of amines. Two complementary methods utilizing ruthenium catalysis have been reported by Beller et al.[12−14] and a metal free method using a boron catalyst was reported by Fu et al.[15] These approaches elegantly demonstrate the concept of alkylation of amines with carboxylic acids although their suitability for large scale application may be limited by the requirement for hydrogen gas or silanes as a hydride source.

In recent years, the principles of green chemistry have been increasingly applied to chemical synthesis and process design,[16] leading to the emergence of enzymes as alternative catalysts due to their ability to perform a wide range of transformations with outstanding selectivity under benign conditions. Biocatalysis also represents a key enabling technology in the pursuit of more sustainable synthesis, with the inherent ability of enzymes to better utilize natural feedstocks providing more opportunities for renewable synthesis as manufacturing seeks to transition from nonrenewable fossil reserves to a biobased economy.[17]

Several different classes of enzyme have demonstrated potential as biocatalytic tools for amine synthesis including transaminases (TAs),[18] amine dehydrogenases (AmDHs),[16] Pictet-Spenglerases,[17] cytochrome P411s,[18] phenylalanine ammonia lyases,[19] amine oxidases,[20−22] and imine reductases (IREDs).[22,23] We have previously reported the characterization of a reductive aminase from *Aspergillus oryzae* (*Asp*RedAm).[24] *Asp*RedAm was shown to be capable of not only catalyzing imine reduction but also the more challenging step of imine formation through condensation of amine and carbonyl substrates in aqueous media,[25] highlighting the potential of this biocatalyst

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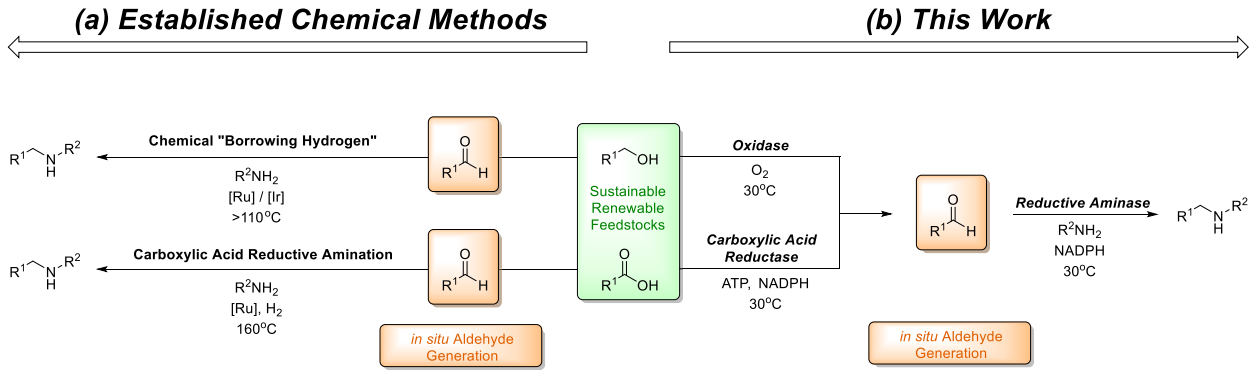

Figure 1. Comparison of (a) previously reported chemocatalytic methods of amine alkylation using sustainable feedstocks via *in situ* aldehyde generation with (b) the one-pot biocatalytic cascades demonstrated in this work.

and other related members of the subclass to be developed further for synthetic application.

We, and others, have also previously described the biocatalytic amination of alcohols through one pot enzymatic systems that mimic chemocatalytic hydrogen borrowing, in which two complementary oxidoreductases perform hydrogen autotransfer through a nicotinamide cofactor. In these studies the conversion of alcohols to primary amines was achieved by the coupling of alcohol dehydrogenases (ADH) with AmDHs[26,27] and a redox self-sufficient combination of an ADH, TA and alanine dehydrogenase (AlaDH).[28] Recently, we reported a system in which *Asp*RedAm was coupled with a panel of nonenantioselective ADHs to demonstrate the first example of primary amine alkylation directly from alcohols using a biocatalytic hydrogen borrowing system.[29] However, while these systems allow for redox neutral amine synthesis, the reversibility of both the ADH and *Asp*RedAm enzyme catalyzed reactions leads to conversions that ultimately are limited by reaction thermodynamics. This leads to a requirement for a large excess of amine substrate and is more pronounced in the synthesis of secondary amines, where the equilibrium is less favorable.[30]

Herein we present two new complementary methods for the direct biocatalytic *N*-alkylation of amines through the simple one-pot combination of *Asp*RedAm with either an engineered choline oxidase variant ($AcCO_6$)[27] or the WT carboxylic acid reductase from *Segniliparus rugosus* (CAR*sr*) (Figure 1b).[28] The ability of these enzymes to generate aldehydes irreversibly allows for *N*-alkylation to be achieved using either alcohol or carboxylic acid substrates in high conversion and isolated yield.

In order to establish the basis for a combined oxidase/RedAm coupling of amines and alcohols, we initially examined the *Asp*RedAm mediated reductive amination of several aldehyde substrates which can be generated from the corresponding primary alcohol using the recently reported engineered choline oxidase variant $AcCO_6$.[31,32] The results of these biotransformations (see Supporting Information) demonstrated a clear overlap between the product scope of the oxidase and the substrate scope of the reductive aminase. Although all aldehydes tested proved to be substrates for *Asp*RedAm, a side reaction was observed in which some of the aldehyde was oxidized to the corresponding carboxylic acid in up to 10% conversion, likely as a result of stirring under an O_2 atmosphere.

Next we examined the direct coupling of amines **1a**−**8a** and alcohols **i**−**v** using a combination of $AcCO_6$ and *Asp*RedAm together with NADPH (cat.) and glucose/GDH for *in situ* cofactor recycling (Table 1). Purified $AcCO_6$ and *Asp*RedAm were applied at 1 mg mL^{-1} with the alcohol concentration kept constant (10 mM) while amine loading was varied (20−100 mM). In general, high conversions (>99%) were obtained using only 2 equiv of amine. Where quantitative conversion was achieved at lower amine loadings, higher concentrations were not examined. Previously we have observed that for *Asp*RedAm catalyzed reductive amination reactions, an increase in amine concentration generally leads to higher conversion to product.[24] However, this phenomenon was not generally observed in this cascade, possibly due to inhibition of the oxidase $AcCO_6$ at higher amine loadings. In fact, the conversions obtained were typically higher than those achieved from direct *Asp*RedAm mediated reductive amination with the equivalent aldehyde, presumably as a result of elimination of the aerobic oxidation side reaction by *in situ* formation of the aldehyde. Benzyl alcohol **7a** gave lower conversions with formation of the conjugated imine often observed.

We next examined a complementary cascade using carboxylic acids as substrates instead of alcohols (Table 2). CAR*sr* was selected, due to its wide substrate scope, and combined with *Asp*RedAm together with glucose/GDH and ATP. As in previous work involving CAR mediated cascades,[33−36] the enzyme was initially as applied as a lyophilized whole cell biocatalyst. However, a significant amount of alcohol byproduct was observed from over-reduction of the carboxylic acid, likely due to endogenous reductase enzymes in *Escherichia coli* intercepting the aldehyde intermediate. Therefore, CAR*sr* was instead applied as a purified enzyme alongside a previously described ATP recycling system (AdK, PAP, polyphosphate).[37] Three carboxylic acid substrates **1b**, **6b**, **8b** were tested with three amine donors **i**, **ii** and **v**. *Asp*RedAm was again applied at 1 mg mL^{-1} and the other enzymes at a concentration of 0.25 mg mL^{-1}. For this cascade, both acid and amine loading were kept constant at 10 mM and 20 mM respectively, while glucose concentration was increased to 100 mM to reflect the requirement for two glucose dehydrogenase turnovers for each alkylation reaction.

The use of purified CAR*sr* significantly reduced the problem of over reduction of the intermediate aldehyde to the corresponding alcohol and resulted in the overall alkylation of primary amines using carboxylic acids as substrates with generally high conversions (>95%). In most cases, crude reaction mixtures were sufficiently pure to enable further use of amine products without purification if desired (see Supporting Information).

Prior to carrying out preparative scale reactions, both cascades were subsequently optimized with respect to substrate (i.e., alcohol, carboxylic acid, amine concentration), biocatalyst

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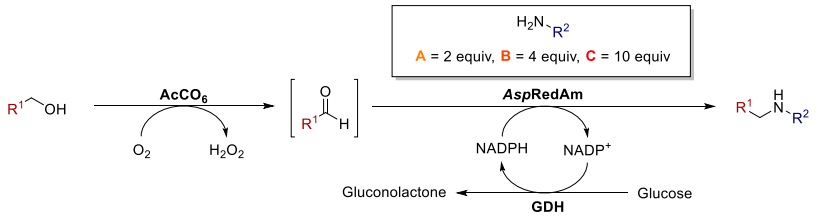
Table 1. Biocatalytic *N*-Alkylation of Amines Using Primary Alcohols[a]



	i	ii	iii	iv	v
1a	1i A: >99% conv.	1ii A: >99% conv.	1iii A: >99% conv.	1iv A: >99% conv.	1v A: >99% conv.
2a	2i A: >99% conv.	2ii A: >99% conv.	2iii A: >99% conv.	2iv A: >99% conv.	2v A: >99% conv.
3a	3i A: >99% conv.	3ii A: 89% conv. B: 78% conv. C: 70% conv.	3iii A: >99% conv.	3iv A: >99% conv.	3v A: >99% conv.
4a	4i A: >99% conv.	4ii A: 52% conv. B: 49% conv. C: 23% conv	4iii A: 69% conv. B: 96% conv. C: 98% conv.	4iv A: 88% conv. B: 93% conv. C: >99% conv.	4v A: >99% conv.
5a	5i A: 89% conv. B: >99% conv.	5ii A: 30% conv. B: 53% conv. C: 21% conv.	5iii A: 46% conv. B: 60% conv. C: >99% conv.	5iv A: 66% conv. B: >99% conv.	5v A: 96% conv. B: 93% conv. C: 79% conv.
6a	6i A: >99% conv	6ii A: >99% conv.	6iii A: 0% conv. B: 0% conv. C: 0% conv.	6iv A: 40% conv. B: 67% conv. C: 76% conv.	6v A: >99% conv
7a	7i A: 25% conv. B: 29% conv. C: 55% conv.	7ii A: 38% conv. B: 74% conv. C: >99% conv.	7iii A: 19% conv. B: 67% conv. C: 60% conv.	7iv A: 38% conv. B: 74% conv. C: >99% conv.	7v A: 67% conv. B: 21% conv. C: 73% conv.
8a	8i A: >99% conv.	8ii A: >99% conv.	8iii A: >99% conv.	8iv A: >99% conv.	8v A: >99% conv.

[a]Reaction conditions: 10 mM alcohol, 20 mM/40 mM/100 mM amine, 0.1 mM $NADP^+$, 50 mM glucose, 1 mg mL^{-1} *Asp*RedAm, 1 mg mL^{-1} $AcCO_6$, 0.3 mg mL^{-1} CDX-901 GDH, 2% (v/v) DMSO, 100 mM pH 7.0 KPi buffer, 500 μL reaction volume, 30 °C, 250 rpm, 24 h.

loading and form (i.e., crude lysate versus purified enzyme), addition of cosolvent (DMSO), buffer concentration, cofactor recycling enzymes (GDH, PAP, AdK), agitation speed and reaction time. Reactions were performed on a 1 mmol scale with

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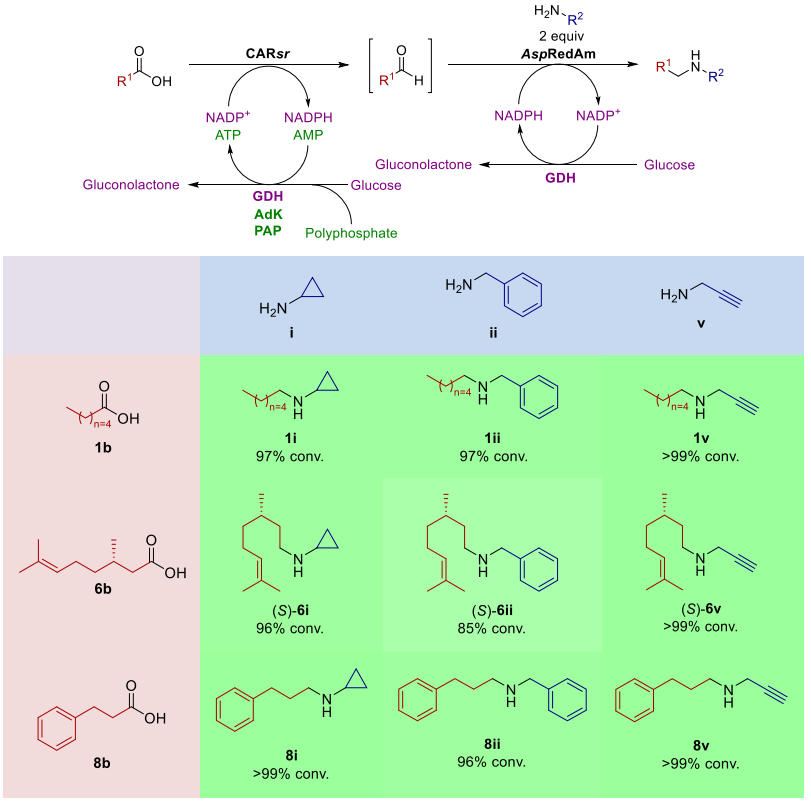
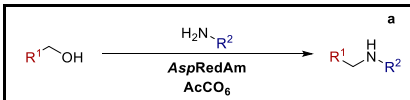
Table 2. Biocatalytic *N*-Alkylation of Amines Using Carboxylic Acids[a]

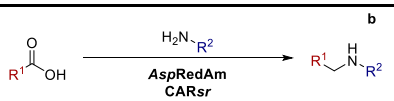


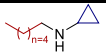
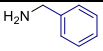
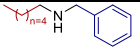
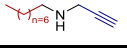
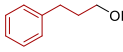
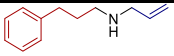
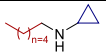
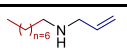
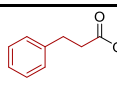
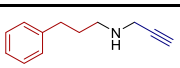
[a]Reaction conditions: 0.1 mM ATP, 10 mM carboxylic acid, 20 mM amine, 0.1 mM $NADP^+$, 100 mM glucose, 100 mM $MgCl_2$, 4 mg mL^{-1} polyphosphate, 1 mg mL^{-1} *Asp*RedAm, 0.25 mg mL^{-1} CAR*sr*, 0.25 mg mL^{-1} Adk, 0.25 mg mL^{-1} PAP, 0.3 mg mL^{-1} CDX-901 GDH, 2% (v/v) DMSO, 100 mM pH 7.5 Tris buffer, 500 μL reaction volume, 30 °C, 250 rpm, 24 h.

Table 3. Preparative Scale Reactions



or



Entry	Alkylating Agent	Amine	Amine Equivalents	Product	Conversion (%)	Yield (%)
1			2		>99	67
2			0.8		>99	72
3			4		>99	62
4			4		>99	70
5			2		>99	49
6			4		84	64
7			4		93	74

[a]Reaction conditions: 25 mM alcohol, 0.2 mM $NADP^+$, 50 mM glucose, 1 mL DMSO, 0.2 mg mL^{-1} *Asp*RedAm, Lysate formed from 3 g *E. coli* expressing $AcCO_6$, 0.3 mg mL^{-1} CDX-901 GDH, 100 mM pH 7.0 KPi buffer, 40 mL reaction volume, 30 °C, 250 rpm, 24 h. [b]Reaction conditions: 0.1 mM ATP, 10 mM acid, 0.08 mM $NADP^+$, 100 mM glucose, 100 mM $MgCl_2$, 4 mg mL^{-1} polyphosphate, 2 mL DMSO, 0.2 mg mL^{-1} *Asp*RedAm, 0.25 mg mL^{-1} CAR*sr*, 0.25 mg mL^{-1} Adk, 0.25 mg mL^{-1} PAP, 0.3 mg mL^{-1} CDX-901 GDH, 100 mM pH 7.5 Tris buffer, 100 mL reaction volume, 30 °C, 250 rpm, 24 h.

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a reaction volume of either 40 mL (alcohol to amine) or 100 mL (carboxylic acid to amine) (Table 3). For all of these preparative reactions, high conversions were observed (84−99%) and product of high purity (>95%) was isolated in good yield (49−74%) through straightforward workup procedures. In examples where full conversion (>99%) to product was observed by GC-FID, the reaction media was simply basified (pH = 12) before extraction into ethyl acetate to yield the product. In cases where reagents remained present, prior acidification and extraction was used to remove impurities. While this workup procedure is suitable for the alkylation of small low molecular weight amines, which when used in excess may be removed under reduced pressure, it cannot provide pure product when less volatile amines such as benzylamine **ii** are used in excess. Therefore, in the biocatalytic alkylation of benzylamine **ii**, the alkylating agent hexanol **1a** was used in excess (1.25 equiv), allowing pure product to again be obtained through acid−base extraction. Although with many chemical methods, an excess of alkylating agent risks byproduct formation through overalkylation, the intrinsic selectivity of these enzyme mediated cascades avoids this issue thereby offering a distinct advantage in terms of selectivity and mildness of reaction conditions.

In summary, we have demonstrated how the recently reported reductive aminase from *Aspergillus oryzae* (*Asp*RedAm) can be combined with either an engineered primary alcohol oxidase ($AcCO_6$) or a carboxylic acid reductase (CAR*sr*) for the development of two simple one-pot biocatalytic systems in which *N*-alkylation of amines may be achieved using either alcohol or carboxylic acid substrates as alkylating agents, respectively. Both of these novel cascades yield product in high conversion and allow for isolation of pure product through simple workup procedures. The reductive aminase displays remarkable complementarity with both of these enzymes with *in situ* aldehyde generation by both cascades leading to higher product conversion than direct biocatalytic reductive amination of aldehyde substrates. The irreversibility of aldehyde formation also offers a marked advantage over previous biocatalytic systems reported for *N*-alkylation that utilize similar feedstocks.[26] The limitations of traditional chemical approaches such as the requirement for harsh conditions and overalkylation are avoided by these systems, and with the recent discovery of new members of reductive aminase enzyme family[38] we envisage further applications of the methodology reported herein for amine synthesis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b11561.

Experimental procedures including characterization of compounds, GC-FID traces of analytical biotransformations, optimization data, and control experiments (PDF)

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Notes

The authors declare no competing financial interest.

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