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Direct alkylation of amines with primary and secondary alcohols *via* biocatalytic hydrogen borrowing

Sarah L. Montgomery, [a] Juan Mangas-Sanchez, [a] Matthew P. Thompson, [a] Godwin A. Aleku,[a] Beatriz Dominguez[b] and Nicholas J. Turner[a]*

Abstract: The reductive aminase from *Aspergillus oryzae* (*Asp*RedAm) has been combined with a single alcohol dehydrogenase (either metagenomic ADH-150, an ADH from *Sphingobium yanoikuyae* (SyADH), or a variant of the ADH from *Thermoanaerobacter ethanolicus* (*Te*SADH W110A)) in a redox-neutral cascade for the biocatalytic alkylation of amines using primary and secondary alcohols. Aliphatic and aromatic secondary amines have been obtained in up to 99% conversion, as well as chiral amines directly from the racemic alcohol precursors in up to >97% ee, releasing water as the only by-product.

Amines are important targets for synthesis and are present in a myriad of organic compounds ranging from adhesives and resins to agrochemicals and pharmaceuticals.[1,2] Consequently the development of new methodologies that enable access to this class of compound is a major research goal in synthetic organic chemistry. In particular, the activation of alcohols under mild conditions for nucleophilic substitution has been identified as a key area for method development.[3] Hydrogen borrowing or autotransfer is an elegant, redox-neutral approach for the formation of C-C, C-O and C-N bonds. This strategy has high atom economy as both oxidation and reduction steps are combined in a single pot, removing the need for sacrificial reagents that produce significant waste.[4–9] While a number of chemical methods exist for mediating hydrogen borrowing,[10,11] a frequent limitation is a lack of stereocontrol during the reductive amination step.[12] Only a handful of examples have been reported involving enantioselective transformations with modest enantiomeric excess.[13–15]

Biocatalysis offers a variety of methods for the asymmetric synthesis of amines including transaminases (TAs), monoamine oxidases (MAO-N), phenylalanine ammonia lyases (PALs), amine dehydrogenases (AmDHs) and imine reductases (IREDs).[16–24] These enzymes have also been combined in cascades to carry out multiple transformations in one pot, often with oxidation and reduction steps taking place concurrently.[25–27]. Recently we reported the discovery of a new enzyme, a reductive aminase from *Aspergillus oryzae* (*Asp*RedAm),[28] which catalyses the reductive amination of a variety of carbonyl compounds at low amine loadings with good to excellent stereoselectivity. This discovery prompted us to consider the application of *Asp*RedAm in hydrogen borrowing amination of alcohols.

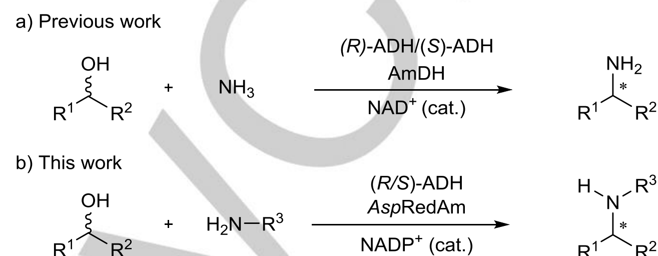

Figure 1. Methods for the asymmetric conversion of alcohols to amines via biocatalytic hydrogen borrowing.

We and subsequently others have reported a method for the conversion of alcohols to primary amines *via* biocatalytic hydrogen-borrowing.[29–31] An alcohol dehydrogenase (ADH) was combined with an amine dehydrogenase (AmDH) and catalytic NAD^+, establishing proof-of-concept but also highlighting important challenges for further development. Firstly, the oxidation of racemic chiral secondary alcohols required the use of two enantiocomplementary alcohol dehydrogenases (ADHs) to convert racemic secondary alcohols (Figure 1). Secondly, the initial system employed an NADH-dependent amine dehydrogenase (AmDH) for the amination step and hence is limited to the formation of primary amines. Herein we describe the combination of single non-selective, NADPH-dependent ADHs with *Asp*RedAm to generate redox-neutral cycles for the conversion of alcohols into primary and secondary amines (Figure 2) with water as the sole by-product.

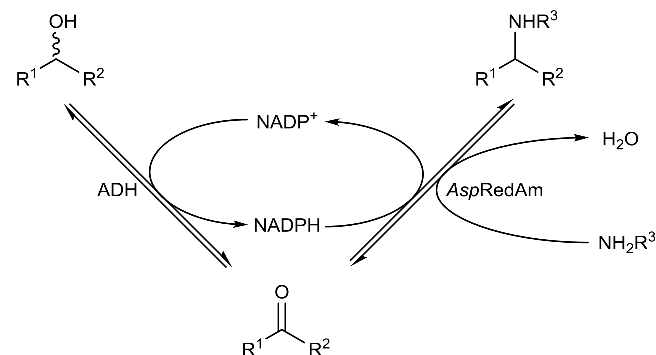

Figure 2. ADH/RedAm catalytic cycle for the biocatalytic alkylation of primary amines with primary and secondary alcohols.

In order to establish the feasibility of the H-borrowing cascade, we selected a variant of the ADH from *Thermoanaerobacter ethanolicus* (*Te*SADH W110A)[32] with known activity for the oxidation of cyclohexanol **1**, and examined its amination using benzylamine **5** in the presence of *Asp*RedAm.[28] A 17% conversion to *N*-benzylcyclohexylamine **12** was observed, which

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was significantly lower than for the corresponding conversion for reductive amination of cyclohexanone with *Asp*RedAm (82%). To optimise the conversion, the *Asp*RedAm loading (1 – 2 mg mL^{-1}), amine concentration (20 – 250 mM) and $NADP^+$ concentration (0.5 – 3.0 mM) were varied in biotransformations also containing 5 mM **1** and 1 mg mL^{-1} of a different ADH, ADH-150, with monitoring of the conversion to both cyclohexanone and the amine product **12**. The amine concentration showed the largest effect on conversion to **12**, with increases in conversion of up to 71% with 100 mM of **5** followed by a decline, reaching negligible conversion at 250 mM amine (Supplementary Information Figure S1).

Whilst higher *Asp*RedAm concentrations improved conversions, enzyme loadings above 2 mg mL^{-1} gave diminishing improvements in conversion to product, perhaps due to decreased enzyme stability at higher concentration. The optimal $NADP^+$ concentration was found to be 1 mM, with higher concentrations resulting in more ketone but no additional amine product in the reaction mixture. The optimised reaction conditions resulted in an improved conversion to amine **12** of 71%.

Next, a time point study was conducted to investigate the relative rates of the oxidation and reductive amination steps (Supplementary Information Figure S2). The concentration of cylohexanone reached a steady state within one hour, thereafter being slowly consumed in the reductive amination step. A further study of different temperatures (20°C and 30°C) and pHs (7.0 and 9.0) indicated that the transformation occured more slowly at lower temperature and neutral pH but with a less noticeable decline in rate (Figure 3). The final conversions after 3 days were remarkably similar, indicating that the optimal conditions required a compromise between enzyme stability and rate of reductive amination.

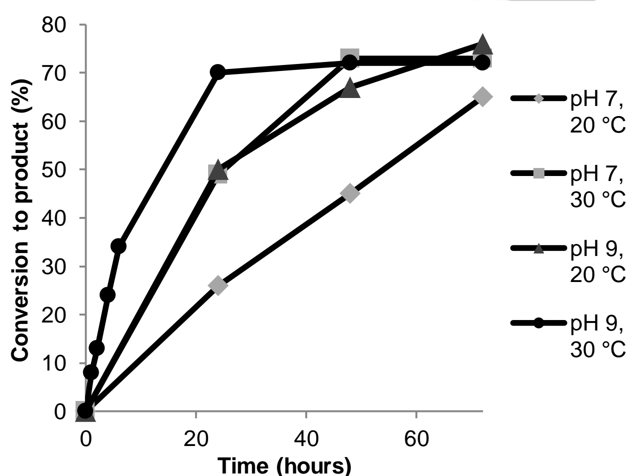
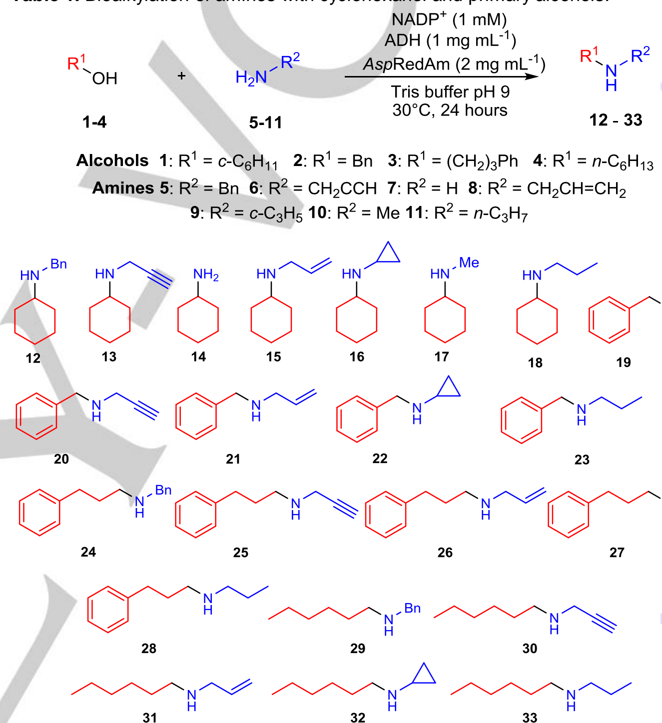


Figure 3. Comparison of time points for the hydrogen borrowing bioalkylation of **A** with **1a** catalysed by ADH-150 and *Asp*RedAm under different conditions.

The optimised conditions were then applied to a different amine, propargylamine **6**, resulting in 88% conversion to *N*-propargylcyclohexylamine **13** with 6% cyclohexanone remaining in the reaction mixture after 24 hours. Increasing the amine loading to 250 mM resulted in a slightly improved conversion (91%).. Several other amines were then studied with cyclohexanol **1**, benzyl alcohol **2**, 3-phenylpropan-1-ol **3** and 1-hexanol **4**. These transformations proceeded in moderate to high conversion (Table 1), except in the case of **2** where substrate inhibition of the ADH lowered the conversion. This problem was alleviated to some degree by running the biotransformations as biphasic systems with 50% v/v cyclohexane, which is well tolerated by *Asp*RedAm. However, *Te*SADH W110A still showed significantly lower conversions than ADH-150 and the ADH from *Sphingobium yanoikuyae* (SyADH).[33,34]

Table 1. Bioalkylation of amines with cyclohexanol and primary alcohols.[a]



Entry	Product	Conversion (%)[b] (ADH-150)	Conversion (%)[b] (SyADH)	Conversion (%)[b] (*Te*SAD W110A)
1	**12**	71	54	58
2	**13**	88	92	90
3	**14**	79	79	44
4	**15**	91	95	89
5	**16**	95	99	95
6	**17**	79	88	86
7	**18**	75	90	85
8	19	63	58	16
9	**20**	21	32	0
10	**21**	79	56	12
11	**22**	60	69	6
12	**23**	0	0	0
13	**24**	95	99	82
14	**25**	99	83	99
15	**26**	99	94	96
16	**27**	99	99	86
17	**28**	99	96	79

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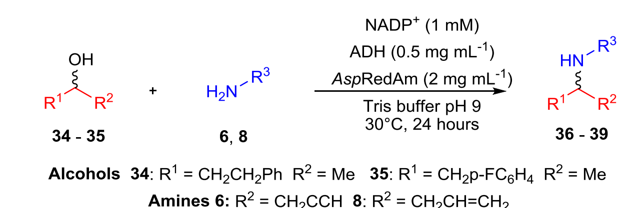
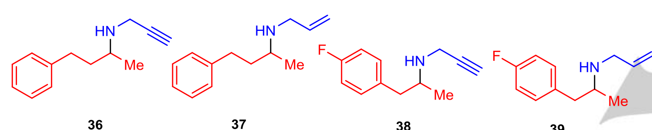
18	**29**	96	99	99
19	**30**	99	95	93
20	**31**	99	99	99
21	**32**	92	99	99
22	**33**	57	74	99

[a] Reaction conditions: 5 mM alcohol, 100 mM amine. [b] Conversion to product determined by GC-FID.

Mixtures of products were observed in *Asp*RedAm-catalysed reductive aminations of **2 – 4** with ammonia **7** and methylamine **10**, so these amines were excluded from the cascade screen (Table 1). The highest conversions (99%) were observed with **3** and **4** using short-chain, linear amines. ADH-150 appears to have the broadest substrate tolerance overall, although SyADH and *Te*SADH W110A gave better conversions in some cases.

Table 2. Bioalkylation of amines with racemic secondary alcohols.[a]





Entry	Product	ADH	Conversion (%)[b]	ee (%)
1	**36**	ADH-150	8	n.d.
2	**36**	SyADH	84	n.d.
3	**36**	*Te*SADH	67	n.d.
4	**37**	ADH-150	9	44 (*R*)[c]
5	**37**	SyADH	70	38 (*R*)[c]
6	**37**	*Te*SADH	52	42 (*R*)[c]
7	**38**	ADH-150	25	96 (*R*)
8	**38**	SyADH	31	96 (*R*)
9	**38**	*Te*SADH	41	95 (*R*)
10	**39**	ADH-150	13	>97
11	**39**	SyADH	27	>97
12	**39**	*Te*SADH	44	>97

[a] Reaction conditions: 5 mM alcohol, 250 mM amine. [b] Conversions and enantiomeric excesses determined by GC-FID on a chiral stationary phase. [c] Enantiomeric excesses determined by HPLC on a chiral stationary phase.

To examine the potential of this new process for the conversion of racemic secondary alcohols into enantiomerically enriched amines, 4-phenyl-2-butanol **34** and 1-(4-fluorophenyl)propan-2-ol **35** were selected as substrates. Under the same conditions used for the *N*-alkylation of amines with **1 – 4**, initial yields in the alkylation of allylamine **7** with **35** were relatively low. We speculate that this could be due to the lower activity of the ADHs with bulky alcohols. A lower ADH:*Asp*RedAm ratio (1:4) afforded higher conversions (Table 2). For **34**, higher yields were observed using SyADH (entries 2 and 5), while for **35**, the best results were obtained when *Te*SADH W110A was used (entries 9 and 12). In the reaction of alcohol **34** with amine **8**, after 24 hours we observed 92% *ee* in the starting material when *Te*SADH was used and 42% *ee* when using SyADH. This indicates that SyADH is less enantioselective, although both ADHs will oxidise both enantiomers of the alcohol.

Prior to studying the scalability of the system, further analytical scale biotransformations were performed in order to intensify the process. For the conversion of **1** to *N*-methylcyclohexylamine **17** (Table S5), it was observed that concentrations of **1** up to 50 mM were tolerated, but at 100 mM **1** precipitation of one or both enzymes occurred. Up to 250 mM of **10** could be added with no reduction in conversion. Lower concentrations of $NADP^+$ were also well tolerated (95% conversion with 5 mol%), as were decreased enzyme loadings (89% conversion with 0.5 mg mL^{-1} ADH-150 and *Asp*RedAm).

The preparative conversion of **1** to *N*-allylcyclohexylamine **15** was then investigated (Supplementary Information Scheme S1, Table S6). Increasing the amine loading to 0.5 M did not further improve conversion, thus the conditions from entry 1 were deemed most promising for scale up, and were replicated on 100 mg scale, resulting in 98% conversion to amine **15** by GC-FID analysis and an isolated yield of 61%.

In conclusion, the ADH/*Asp*RedAm-catalysed alkylation of amines with alcohols has enabled the preparation of a wide range of secondary amines via biocatalytic hydrogen borrowing. This redox-neutral cascade generates water as the sole by-product and to our knowledge constitutes the first example of biocatalytic hydrogen autotransfer for the direct preparation of secondary amines. The use of chiral secondary alcohols has permitted access to optically active amines in up to 84% conversion with ee's up to >97%. The results reported here further demonstrate the potential of reductive aminases (RedAms) as powerful new biocatalysts for the preparation of enantiopure amines under environmentally benign conditions.

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Keywords: biocatalysis • asymmetric amination • enzyme cascades • reductive aminase • synthetic methods.

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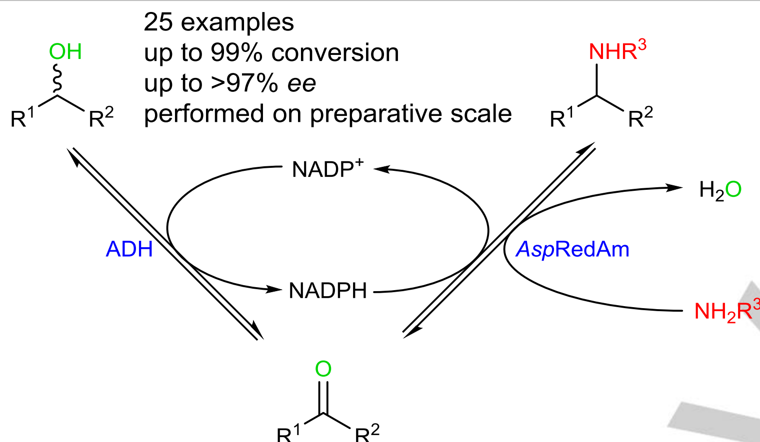

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