

# Efficient kinetic resolution of racemic amines using a transaminase in combination with an amino acid oxidase†

Matthew D. Truppo,\*<sup>a</sup> Nicholas J. Turner\*<sup>a</sup> and J. David Rozzell<sup>b</sup>

Received (in Cambridge, UK) 12th February 2009, Accepted 5th March 2009

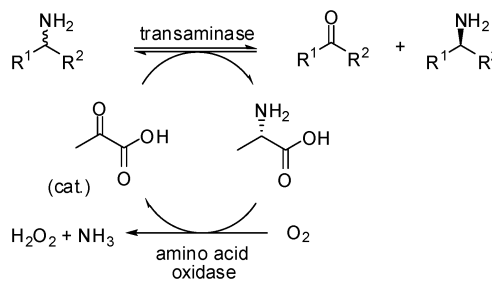
First published as an Advance Article on the web 16th March 2009

DOI: 10.1039/b902995h

A range of enantiomerically pure (*R*)- and (*S*)-configured chiral amines has been prepared in excellent e.e. (99%) by combining a transaminase enzyme with an amino acid oxidase and catalytic quantities of pyruvate.

Enantiomerically pure chiral amines are key intermediates in a number of pharmaceutical compounds that possess a wide range of biological activities.<sup>1</sup> Biocatalytic approaches to these building blocks have traditionally relied upon kinetic resolution of racemic substrates using hydrolytic enzymes<sup>2</sup> such as lipases, acylases and proteases and indeed some of these processes have been operated successfully at large scale.<sup>3</sup> In certain cases it is possible to racemise the unreactive enantiomer by addition of a racemisation catalyst leading to a dynamic kinetic resolution (DKR) process.<sup>4</sup> Alternative approaches based upon deracemisation have also been developed in which enantioselective amine oxidases are combined with non-selective reducing agents resulting in yields of >50% and high enantioselectivities.<sup>5</sup> Recently transaminases have emerged as viable biocatalysts for chiral amine production.<sup>6</sup> Transaminases can be used in two complementary ways, either (i) in the asymmetric synthesis<sup>7</sup> of chiral amines from ketones using a suitable amine donor (e.g. L-alanine) or (ii) in the kinetic resolution<sup>8</sup> of racemic amines using a suitable amine acceptor (e.g. pyruvate). Although the former process is attractive in terms of the potential for high yields of product, it often suffers from poor conversion due to the equilibrium favouring the ketone and also product inhibition of the transaminase by the amine product.<sup>9,10</sup> Thus the kinetic resolution of amines using transaminases remains an option in many instances and has been applied in some cases on a large scale.<sup>11</sup> However, it is necessary to add stoichiometric quantities of pyruvate which in addition to the cost can also result in inhibition of the reaction. In this communication, we report a new approach in which catalytic quantities of pyruvate are used in combination with an amino acid oxidase.

Scheme 1 outlines the process in which a catalytic amount of amine acceptor (pyruvate) is added to the reaction together with an amino acid oxidase (AAO). Transaminase catalysed kinetic resolution of the amine leads to conversion of pyruvate



**Scheme 1** Resolution of a racemic amine using a transaminase with catalytic quantities of pyruvate and an amino acid oxidase.

to alanine which undergoes *in situ* oxidation back to pyruvate catalysed by AAO in the presence of molecular oxygen. In addition to significantly reducing the quantities of pyruvate required, we reasoned that this system should also overcome the unfavourable equilibrium, since one of the components (pyruvate) is being removed. Reduction of pyruvate concentration should in addition lead to reduced inhibition of the transaminase enzyme.

To establish the basis for this approach, the kinetic resolution of ( $\pm$ )- $\alpha$ -methylbenzylamine (MBA **1**) at 25 mM concentration was carried out using the (*R*)-selective transaminase ATA-117 together with D-amino acid oxidase (D-AAO) and a catalytic amount of pyruvate (2 mM; 8 mol%).<sup>12</sup> The reaction proceeded to 50% conversion after 1 h yielding (*S*)-**1** (99% e.e.) (Fig. 1). A control reaction was also run in which the D-AAO was omitted. The reaction proceeded to only 7.5% conversion after 1 h affording (*S*)-**1** in 8% e.e. This result corresponds well to a maximum theoretical conversion of 8% based on a 25 mM substrate charge and a 2 mM amine acceptor concentration.

The resolution of ( $\pm$ )-**1** was then attempted at concentrations up to 100 mM using ATA-117, D-amino acid oxidase, and a catalytic amount of pyruvate (8 mol%). All reactions proceeded to 50% conversion and yielded (*S*)-**1** in 99% e.e. (Fig. 2).

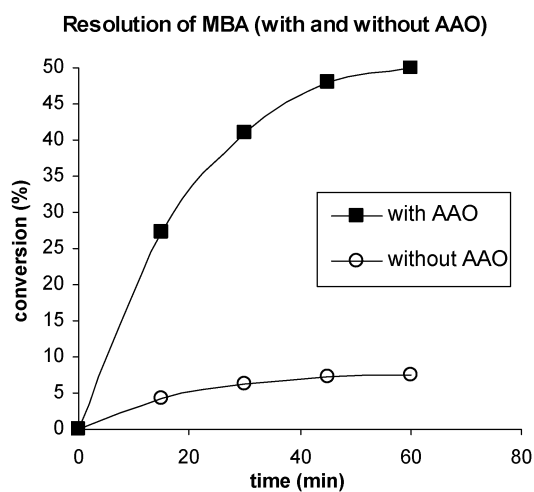
To improve the efficiency of the reaction we decided to explore the effect of reducing the concentration of pyruvate. Kinetic resolutions were run with ( $\pm$ )-**1** (100 mM) and pyruvate loadings from 2 mM (2 mol%) to 0.05 mM (0.05 mol%). As expected the reaction rate decreased with decreasing concentrations of pyruvate, although even with 0.1 mM pyruvate (0.1 mol%) the reaction proceeded to completion in 12 h (Fig. 3).

To examine the generality of the process, two transaminases ATA-113 [(*S*)-selective] and ATA-117 [(*R*)-selective] were applied to the resolution of a variety of racemic amine substrates **1–6**. Reactions were run with amine substrate (25 mM)

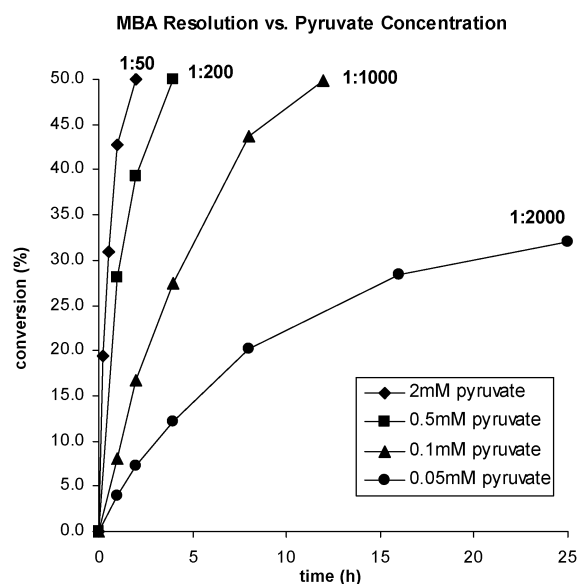
<sup>a</sup> School of Chemistry, University of Manchester, Manchester Interdisciplinary Biocentre, 131 Princess Street, Manchester, UK M1 7DN. E-mail: Nicholas.turner@manchester.ac.uk; Fax: (+44)161 306 5173

<sup>b</sup> Codexis Inc., 129 N. Hill Avenue, Pasadena, CA 91106, USA

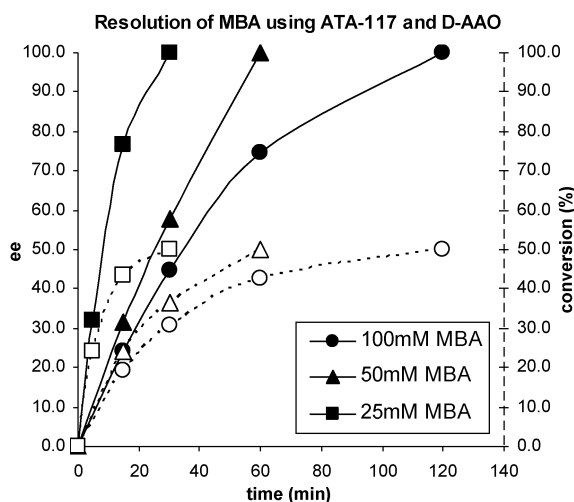
† Electronic supplementary information (ESI) available: HPLC assay conditions; resolution conditions; HPLC chromatograms. See DOI: 10.1039/b902995h



**Fig. 1** Transaminase (ATA-117) catalyzed resolution of  $\alpha$ -methylbenzylamine **1** (25 mM) using a catalytic amount (2 mM) of amine acceptor (pyruvate), with and without D-amino acid oxidase (D-AAO).



**Fig. 3** Resolution of  $\alpha$ -methylbenzylamine **1** (100 mM) using a catalytic amount of amine acceptor (pyruvate). The molar ratio of amine acceptor to substrate is shown in bold.



**Fig. 2** Resolution of various concentrations of  $\alpha$ -methylbenzylamine **1** using a catalytic amount (8 mol%) of amine acceptor (pyruvate). Closed lines and symbols show enantiomeric excess (e.e.). Open lines and symbols show conversion.

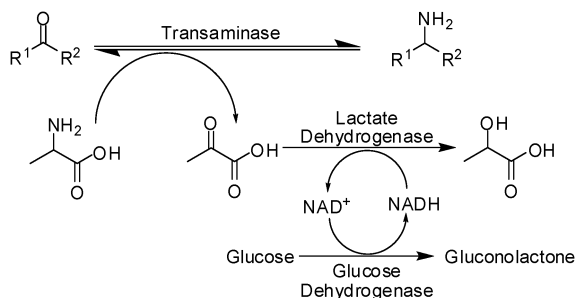
and a catalytic amount of pyruvate (2 mM; 8 mol%). L-Amino acid oxidase and D-amino acid oxidase were used in the ATA-113 and ATA-117 catalysed reactions in view of their known specificities for L- and D-alanine respectively. Reactions were run for 3 h and monitored by HPLC to determine % conversion and e.e. (Table 1). All six amines were successfully resolved by transaminase ATA-113, giving the corresponding (*R*)-amine products with 99% e.e. at 50% conversion. Transaminase ATA-117 successfully resolved amines **1–4**, giving the (*S*)-amine product with >99% e.e. at 50% conversion. However, ATA-117 showed no activity towards the more sterically demanding amines **5** and **6** and hence we sought to prepare the (*S*)-enantiomers of these two amines from the corresponding ketones according to the procedure that we have previously reported.<sup>13</sup>

Amines (*S*)-**5** and (*S*)-**6** were prepared in 99% e.e. from the corresponding ketones using ATA-113 and L-alanine as the

**Table 1** Synthesis of (*R*)- and (*S*)-amines **1–6** using transaminases ATA-113 and ATA-117

#	Compound Structure	Product ( <i>R</i> )-amine	Product ( <i>S</i> )-amine
<b>1</b>		>99% ee (ATA113)	>99% ee (ATA117)
<b>2</b>		>99% ee (ATA113)	>99% ee (ATA117)
<b>3</b>		>99% ee (ATA113)	>99% ee (ATA117)
<b>4</b>		>99% ee (ATA113)	>99% ee (ATA117)
<b>5</b>		>99% ee (ATA113)	>99% ee (ATA113) synthesis direction
<b>6</b>		>99% ee (ATA113)	>99% ee (ATA113) synthesis direction

amine donor (Scheme 2). Lactate dehydrogenase (LDH) was added to drive the reaction to completion by reduction of the pyruvate formed to lactate and the recycling of NADH was achieved by using glucose dehydrogenase (GDH). It is interesting to note that a single broad specificity transaminase ATA-113 can be used to prepare both (*R*)-(kinetic resolution) and (*S*)-amines (asymmetric synthesis) in high enantiomeric excess illustrating the benefit of the two complementary approaches.



**Scheme 2** Asymmetric synthesis of amines from ketones using transaminase and lactate dehydrogenase to drive the reaction to completion.

In summary, we have developed a novel system for the resolution of racemic amines using a transaminase (ATA) coupled with an amino acid oxidase (AAO). In contrast to previously reported approaches that use a stoichiometric amount of amine acceptor, the system described here employs a catalytic amount of amine acceptor (pyruvate) that is continuously recycled *in situ* by an amino acid oxidase and molecular oxygen. The effective replacement of a molar quantity of amine acceptor with oxygen will result in significant cost savings when this process is applied on a large scale. Additionally, the AAO system drives the reaction equilibrium and significantly reduces transaminase inhibition by the co-substrate pyruvate. Finally, we have demonstrated two complementary systems for the synthesis of chiral amines. Given a single transaminase with broad substrate specificity and high enantioselectivity, both the (*R*)- or (*S*)-amine product can be obtained *via* either resolution of racemic amine substrates or asymmetric synthesis of the chiral amine through transamination of the corresponding prochiral ketone substrate.

## Notes and references

1 N. J. Turner and R. Carr, in *Biocatalysis in the Pharmaceutical and Biotechnology Industries*, ed. R. N. Patel, CRC Press, Boca Raton,

- Fl., 2007, pp. 743–755; S. Bräse, T. Baumann, S. Dahmen and H. Vogt, *Chem. Commun.*, 2007, 1881.
- 2 H. Ismail, R. M. Lau, F. van Rantwijk and R. A. Sheldon, *Adv. Synth. Catal.*, 2008, **350**, 1511.
- 3 M. Breuer, K. Ditrich, T. Habicher, B. Hauer, M. Keßeler, R. Stürmer and T. Zelinski, *Angew. Chem., Int. Ed.*, 2004, **43**, 788.
- 4 C. Roengpithya, D. A. Patterson, A. G. Livingston, P. C. Taylor, J. L. Irwin and M. R. Parrett, *Chem. Commun.*, 2007, 3462; A. N. Parvulescu, P. A. Jacobs and D. E. De Vos, *Adv. Synth. Catal.*, 2008, **350**, 113; C. E. Hoben, L. Kanupp and J.-E. Bäckvall, *Tetrahedron Lett.*, 2008, **49**, 977; M.-J. Kim, W.-H. Kim, K. Han, Y. K. Choi and J. Park, *Org. Lett.*, 2007, **9**, 1157.
- 5 C. J. Dunsmore, R. Carr, T. Fleming and N. J. Turner, *J. Am. Chem. Soc.*, 2006, **128**, 2224.
- 6 K. Faber and W. Kroutil, *Curr. Opin. Chem. Biol.*, 2005, **9**, 181; J. D. Stewart, *Curr. Opin. Chem. Biol.*, 2001, **5**, 120; D. Koszelewski, I. Lavandera, D. Clay, G. M. Guebitz, D. Rozzell and W. Kroutil, *Angew. Chem., Int. Ed.*, 2008, **47**, 9337; U. Kaulmann, K. Smithies, M. E. B. Smith, H. C. Hailes and J. M. Ward, *Enzyme Microb. Technol.*, 2007, **41**, 628.
- 7 D. Koszelewski, I. Lavandera, D. Clay, D. Rozzell and W. Kroutil, *Adv. Synth. Catal.*, 2008, **350**, 2761; H. Yun and B.-G. Kim, *Biosci., Biotechnol., Biochem.*, 2008, **72**, 3030; A. Iwasaki, Y. Yamada, N. Kizaki, Y. Ikenaka and J. Hasegawa, *Appl. Microbiol. Biotechnol.*, 2006, **69**, 499; J.-S. Shin and B.-G. Kim, *Biotechnol. Bioeng.*, 1999, **65**, 206.
- 8 M. Höhne, K. Robins and U. T. Bornscheuer, *Adv. Synth. Catal.*, 2008, **350**, 807; J.-S. Shin and B.-G. Kim, *Biotechnol. Bioeng.*, 1997, **55**, 348; J.-S. Shin, B.-G. Kim and D.-H. Shin, *Enzyme Microb. Technol.*, 2001, **29**, 232; J.-S. Shin and B.-G. Kim, *Biotechnol. Bioeng.*, 1998, **60**, 534; J.-S. Shin, B.-G. Kim, A. Liese and C. Wandrey, *Biotechnol. Bioeng.*, 2001, **73**, 179.
- 9 Y. B. Tawari, R. N. Goldberg and J. D. Rozzell, *J. Chem. Thermodyn.*, 2000, **32**, 1381.
- 10 For approaches to overcome the problems associated with equilibrium see: M. Höhne, S. Kühn, K. Robins and U. T. Bornscheuer, *ChemBioChem*, 2008, **9**, 363.
- 11 R. L. Hanson, B. L. Davis, Y. Chen, S. L. Goldberg, W. L. Parker, T. P. Tully, M. A. Montana and R. N. Patel, *Adv. Synth. Catal.*, 2008, **350**, 1367.
- 12 All enzymes, including transaminases (ATA-113 and ATA-117), L-amino acid oxidase (L-AAO) and D-amino acid oxidase (D-AAO), glucose dehydrogenase (GDH CDX-901) and lactate dehydrogenase (LDH-102), were generously supplied by Codexis (Redwood City, CA).
- 13 M. D. Truppo, D. J. Rozzell, J. C. Moore and N. J. Turner, *Org. Biomol. Chem.*, 2009, **7**, 395.