

Application of ‘smart’ amine donors for rapid screening and scale-up of transaminase-mediated biotransformations

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THE 'SMART' AMINE DONORS *O*-XYLYLENEDIAMINE AND CADAVERINE WERE EMPLOYED FOR THE RAPID SCREENING OF A LARGE KETONE LIBRARY AND SUBSEQUENT PREPARATIVE-SCALE SYNTHESIS OF SELECTED COMPOUNDS USING THE COMMERCIALLY AVAILABLE AMINE TRANSAMINASE, ATA256. THE METHODOLOGY ENABLES BOTH SCREENING AND PREPARATIVE-SCALE BIOTRANSFORMATIONS TO BE PERFORMED WITH A SINGLE ENZYME AND SIMPLIFIES THE GENERATION OF SP³-RICH SMALL MOLECULE LIBRARIES.

Chiral amines are ubiquitous in pharmaceutical drugs, bioactive natural products and privileged scaffolds.^[1-2] Asymmetric introduction of amines into small molecule libraries represents an attractive way of increasing their complexity.^[3-5] It has been demonstrated that 'sp³-rich' compounds possessing one or more stereocentres have favourable properties for drug discovery processes.^[6-7] In order to produce diverse libraries of optically pure chiral amines, robust strategies for their preparation must be developed.

Current methods for asymmetric amine synthesis typically rely on chiral auxiliaries or precious metal catalysts and often require harsh reaction conditions (high temperatures, pressures etc).^[8] The application of enzymes for their synthesis represents a sustainable alternative, as biotransformations are normally carried out under mild conditions and often display superior regio- and stereoselectivity to the traditional chemical approaches.

Amine transaminase (ATA) enzymes have emerged as powerful catalysts for the production of chiral amines, with many impressive examples of their application present in the scientific literature.^[9-12] ATAs are pyridoxal-5-phosphate-dependent (PLP-dependent) and can catalyse the reversible formation of chiral amines from pro-chiral ketones using a suitable sacrificial amine donor. The reversibility of this transformation means that efficient methods to displace the often challenging reaction equilibrium are necessary to achieve high conversion of substrate to product.

Commonly employed strategies include the use of a large excess of amine donor coupled with *in situ* co-product removal or enzymatic degradation of the reaction by-product.^[9,10, 13-16] We previously demonstrated that *ortho*-xylylenediamine can be used to effectively displace the reaction equilibrium of some of the most challenging substrates, using near stoichiometric equivalents of the 'smart' diamine donor.^[17] Additionally, the co-products formed upon transamination with this donor are highly coloured, suggesting that it could be employed for high-throughput screening of both large enzyme and ketone libraries. We have also extended this methodology to other low-cost diamine donors, including cadaverine.^[18] The efficient conversion achieved using these diamines can be attributed to the spontaneous cyclisation and subsequent oligimerization/polymerization of the co-products, which effectively displaces the reaction equilibrium. This methodology has since been shown to be effective with other

ATAs and importantly, with other low-cost, bulk diamine donors.^[19] The development of biocatalytic approaches for the synthesis of chiral amines that exploit 'smart' low-cost diamine donors, in place of IPA coupled with complex co-product removal, is of great interest.

We have now demonstrated that *o*-xylylenediamine can be employed for the rapid screening of a large library of commercially available ketones and aldehydes, enabling expedient identification of transaminase substrates. Based on this high-throughput screening data, a selection of ketones were converted to the corresponding chiral primary amines on a preparative-scale, using cadaverine. The approach demonstrates that these two smart donors can be used in combination for efficient screening and subsequent scale-up, using lower cost diamine donors.

A library containing 400 ketones/aldehydes was supplied by our industrial partner, Key Organics. Compounds within this library were structurally diverse and many of the corresponding amines were of commercial interest. Screening was conducted in 96-well plates and results analysed after 1 and 24 hours (figure 1). The formation of a dark precipitate in the wells enabled qualitative identification of enzyme substrates.

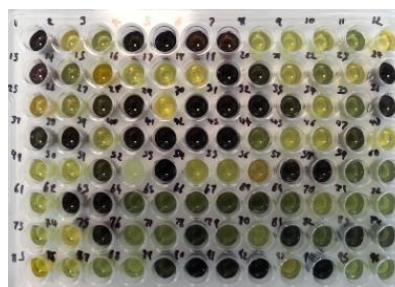


Figure 1. Representative assay plate showing the screening of a ketone library using *o*-xylylenediamine. Wells containing dark precipitate indicate ATA activity.

Analysis of the assay data (figure. 2) revealed that smaller, low molecular weight compounds (<225 Da) are readily accepted by commercially available ATA 256; an enzyme developed by Codexis.^[20] This data demonstrates that in a library of diverse compounds, larger molecules are less likely to be accepted. However, molecular weight and size are not the only important factors and electronic effects, as well as the nature of the substituents on the ketone are also extremely relevant. As expected, the data showed that alpha aryl-substituted methyl ketones were less amenable to transamination compared to those with an alkyl substituent (figure. 3). Cyclic ketones were well tolerated whereas α,β unsaturated ketones were poorer substrates.^[21]

Several ketones were then selected for quantitative analysis (Figure 3) based on our interest in producing the corresponding chiral amines on a preparative scale. The amines selected were either not commercially available, were extremely expensive or were difficult to produce using traditional chemical approaches. Analytical scale reactions

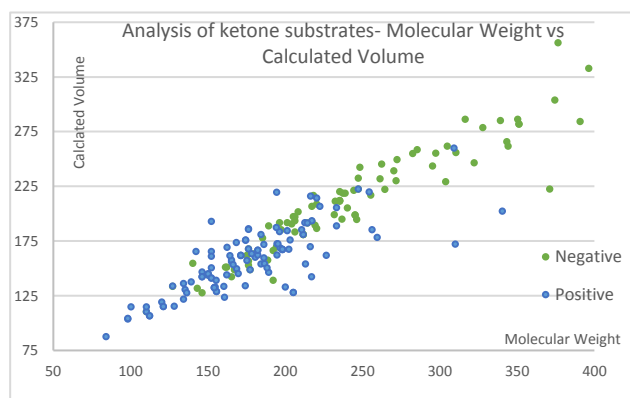


Figure 2. Characterisation of ketones used in the library screening and a plot of their molecular weight vs volume.^[22, 22]

were performed using our recently reported diamine donor cadaverine^[17] and compared to transformations using isopropylamine (the current industry standard amine donor). As expected, reactions employing cadaverine outperformed isopropylamine in almost all cases (figure 3). Using 3 equivalents of cadaverine was sufficient to achieve a 98% conversion of challenging ketone **1** to the corresponding amine. A conversion of only 70% was achieved using IPA at the same concentration. The diamine donor also significantly outperformed IPA in the biotransformations involving the low molecular weight ketones, **5-8**. These ketones (**5-8**) have particularly low boiling points and therefore using high concentrations of IPA in combination with *in situ* acetone co-product removal is likely to be challenging, as the volatile ketone starting material would also be evaporated under these conditions. For this reason, we choose to demonstrate the utility of the 'smart' amine donor, cadaverine, and by extension similar low-cost diamine donors, by performing preparative-scale biotransformations with ketones **1** and **12** and volatile ketones **6-8**.

Initial studies showed that using DMSO as a co-solvent was likely to complicate chromatographic purification when reactions were performed on a preparative scale. A variety of other co-solvents were screened and biotransformations containing methanol and ethanol reached similar conversions to those with DMSO, indicating that these were good alternative solvents. Acetone was the only co-solvent that gave poor conversions, with some of the biotransformations being completely inhibited (see *supplementary data*).

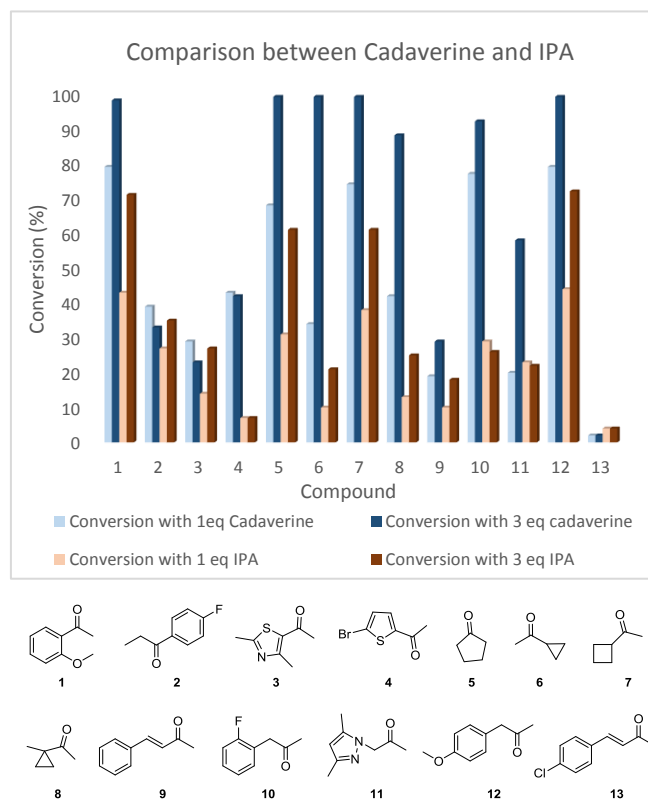


Figure 3: Analytical scale biotransformations with a selection of ketone substrates **1-13**, utilising either 1 or 3 equivalents of the amine donors, cadaverine or IPA. Conversions of compounds **1-13** (20 mM) to the corresponding amine were carried out using ATA256 (2.5 mg/ml), PLP (1 mM) and cadaverine or IPA (20mM). Conversions are an average of three replicates.

Preparative biotransformations were carried out with ketones **6-8** in HEPES buffer at pH 10.9, using DMSO as a co-solvent and were purified by distillation (see ESI for further details). All reactions proceeded with excellent ee (>99%), which was established by the synthesis and NMR analysis of the corresponding Mosher's acid chlorides (see supporting information for details).^[24] Compounds **1** and **12** were extracted and purified via column chromatography in 70% and 39% yield respectively. In all cases, the compounds were of sufficient purity for commercialisation.

The synthesis and application of 'sp³-rich' fragments remains highly important in modern drug-discovery processes and it is expected that this methodology can provide synthetic and medicinal chemists with a facile route for the synthesis of chiral amine fragments. There is increasing interest from industry in the development of sustainable, bio-derived chiral molecules and the use of commercially available enzymes and mild reaction conditions make this approach attractive for accessing these synthetically challenging compounds. Work is on-going to directly evolve new enzymes that possess a broader

substrate scope and that can utilise ‘smart’ amine donors. This will expand the use of the biocatalytic toolbox in modern synthesis.

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