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Deracemisation and stereoinversion of α-amino acids using D-amino acid oxidase and hydride reducing agents

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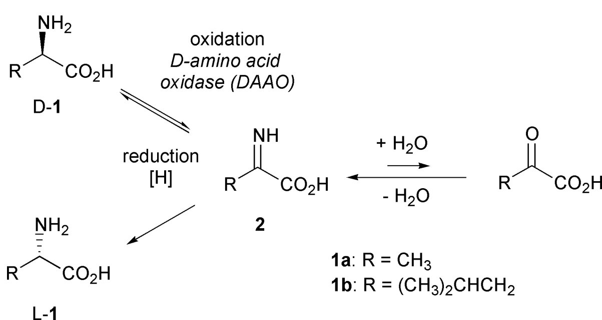
The deracemisation and stereoinversion of both cyclic and acyclic DL-α-amino acids, using porcine kidney D-amino acid oxidase (DAAO) and a hydride reducing agent ($NaCNBH_3$–$NaBH_4$), has been investigated.

Enzyme-catalysed processes have proven to be extremely useful for the synthesis of enantiopure amino-acids[1] and indeed a number of commercially important amino acids are now manufactured using enzymatic methods.[2] The most efficient reactions are those that result in > 50% yield and in general this outcome can be achieved by either the asymmetric transformation of a prochiral substrate[3] or by a dynamic kinetic resolution.[4] A third approach involves the *deracemisation* of a mixture of enantiomers in which a racemic mixture is converted to the single enantiomer form.[5] Deracemisation has been achieved in two ways, either (i) by using two or more enzymes (*e.g*. dehydrogenases) with complementary enantioselectivity[6] or (ii) by combining an enantioselective enzyme-catalysed oxidation reaction with a non-selective, non-enzymatic reduction reaction. The latter approach is the subject of this communication.

In 1971, Hafner and Wellner reported the generation of L-alanine **1a** and L-leucine **1b** from the corresponding D-enantiomers by the use of porcine kidney D-amino acid oxidase (DAAO) (EC 1.4.3.3.) and $NaBH_4$ (Scheme 1). Although the yield was low the principle of stereoinversion was established by enzyme catalysed oxidation of the D-enantiomer to the corresponding achiral imino acid **2** followed by reduction *in situ* by the borohydride generating a mixture of D- and L-amino acids.[7]

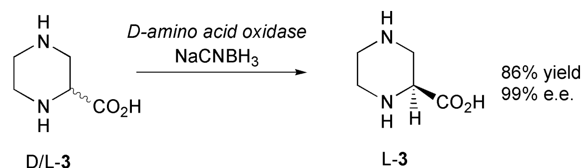
Subsequently Soda *et al*. extended this method to the deracemisation of DL-proline[8a] and DL-pipecolic acid,[8b] again using a combination of D-amino acid oxidase and $NaBH_4$. In both cases the yields and optical purities were high (> 98%). The high yields obtained with cyclic substrates are a consequence of the increased stability of the respective imino acid intermediates allowing for a sufficient number of oxidation–reduction cycles to occur leading to complete deracemisation.[9]

Herein we now report some significant developments of this deracemisation process, in particular that the reaction (i) can be made more efficient for cyclic substrates by the use of $NaCNBH_3$ rather than $NaBH_4$, (ii) can be applied to the stereoinversion of cyclic substrates and also used to prepare α-^{2}H-L-amino acids and (iii) can be used to deracemise a wide range of *acyclic* amino acids in high yield and optical purity provided that $NaBH_4$ is used.



Scheme 1 Stereoinversion of D- to L-amino acids.

We selected proline as the substrate for initial optimisation experiments. One problem associated with the original procedure[7,8] was the requirement for large excesses of $NaBH_4$ (up to 500 equiv.) owing to its high reactivity with water. In addition, the use of $NaBH_4$ results in an operating pH of ~ 10 which is close to the pH at which the enzyme begins to undergo irreversible denaturation.[10] $NaCNBH_3$ is a milder, water stable, hydride reducing agent[11] and was able to deracemise proline (> 99% yield; > 99% ee) with as little as 3 molar equivalents. We were able to apply this improved methodology[12] to the deracemisation of DL-piperazine-2-carboxylic acid **3**, a component of the HIV-protease inhibitor Crixivan,[13] in 86% yield and 99% ee (Scheme 2).



Scheme 2 Deracemisation of DL-piperazine-2-carboxylic acid **3**.

Interestingly, replacement of $NaBH_4$ by $NaCNBH_3$ caused a change in the rate limiting step of the reaction. With $NaBH_4$ as reducing agent, the total concentration of D and L-proline remained almost constant throughout the reaction indicating that the imino acid intermediate did not accumulate to any significant degree. However, with $NaCNBH_3$, the increase of the L enantiomer did not follow the decrease in D enantiomer suggesting that the reduction of the imine was slower than with $NaBH_4$ (Fig. 1), although by the end of the reaction the yield of the L-isomer was > 98%. The $NaCNBH_3$ concentration was varied (3–100 equivalents) but did not significantly affect the overall rate of reduction.

$NaCNBH_3$ was found to be equally effective for the stereoinversion of D- to L-α-amino acids. Thus in the presence of DAAO and $NaCNBH_3$, D-proline was converted to L-proline in > 99% yield and > 99% ee. This transformation could be particularly useful in cases where the D-isomer is more readily available than the racemate as a starting material.

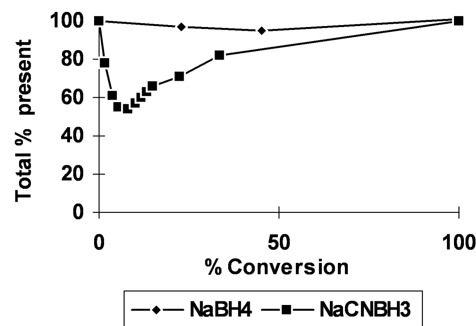


Fig. 1 Comparison of $NaCNBH_3$ with $NaBH_4$.

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The stereoinversion of D- to L-proline suggested that it should be possible to prepare L-^{2}H-proline by replacing $NaCNBH_3$ with $NaCNB^2H_3$ although we recognised that the production of D-^{2}H-proline early on in the reaction might slow down the oxidation reaction. However no significant decrease in the rate of consumption of the D-enantiomer was observed suggesting that DAAO only exhibits a small primary kinetic isotope effect with proline as the substrate. The use of $NaCNB^2H_3$ did cause a slight decrease in the rate of formation of the L-enantiomer due to slower reduction of the imine intermediate. This observation is consistent with the fact that reduction of the imino acid is the rate limiting step and suggests that cleavage of the B–^{2}H bond is slower than cleavage of the B–H bond. The L-^{2}H-proline derived from this experiment was found to have a 96% incorporation of ^{2}H (82% yield, >99% ee); ESI-MS: *m/z* (%) 117 (100) [M^+ + 1].

To date there have been no reports of the deracemisation of *acyclic* amino acids using DAAO. We have found that acyclic substrates give good to excellent yields (75–90%) of high ee (>98%) products indicating that the corresponding acyclic imino acids **2** are sufficiently stable to undergo reduction rather than hydrolysis to the α-keto acid. However it was necessary to use $NaBH_4$ in these reactions rather than $NaCNBH_3$ because the lower reactivity of the latter led to decreased yields of the L-amino acid. The α-functionality was varied (Table 1) to include side-chains containing aromatic **1c**–**1e**, thiol **1f**, alkyl (straight chained **1g** and branched **1b**) and cyclic **1h** groups. *tert*-Leucine **1i** failed to act as a substrate, presumably due to the bulky nature of the *tert*-butyl group. The reaction profile for the deracemisation of DL-leucine **1b** (90% yield; >99% ee) is given in Fig. 2.

The use of $NaBH_4$ was found to result in partial inactivation of the DAAO presumably due to reduction of the Schiff's base formed between some adventitious α-keto acid and an important amino group on the enzyme. This problem could be reduced by addition of bovine serum albumin (BSA) to the reaction which sacrificially reacted with the α-keto acid thereby removing it from solution.

Table 1 Deracemisation of $RCH(NH_2)CO_2H$

R		Yield (%)	Ee (%)
Ph	**1c**	75	99
$PhCH_2$	**1d**	82	99
Indolyl-CH_2	**1e**	76	99
$HSCH_2$	**1f**	77	99
CH_3CH_2	**1g**	87	99
$(CH_3)_2CHCH_2$	**1b**	90	99
Cyclopentyl	**1h**	79	99
tBu	**1i**	Not a substrate	—

For conditions see reference 12.

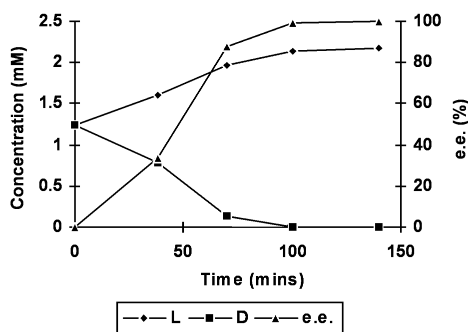

Fig. 2 Profile for deracemisation of DL-leucine **1b**.

In conclusion we have developed a highly efficient chemoenzymatic method for the deracemisation and stereoinversion of cyclic and acyclic α-amino acids. The reaction is attractive in that only a single enzyme is required together with the readily available enzyme DAAO and $NaCNBH_3$ or $NaBH_4$ We are currently examining additional facets of this reaction namely the identification of a wider range of D- and L-amino acid oxidases from microbial sources and exploring the use of alternative chemical reducing agents.

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12 *General procedure*: deracemisation of cyclic amino acids was carried out in phosphate buffer (pH 8.0, 40 mM) containing amino acid (2.5 mM), FAD (80 μM), porcine kidney DAAO (5U, Sigma), catalase (2000U) and $NaCNBH_3$ (3 equivalents) and the solution incubated at 37 °C. Acyclic amino acids were deracemised using the same methodology except that BSA (1 mg/1 ml) was added and the $NaCNBH_3$ was replaced by $NaBH_4$. Samples were removed, filtered and analysed by chiral HPLC (Chirex 3126 supplied by Phenomenex, using $CuSO_4$–MeOH as the mobile phase). For the deuteration experiments the $NaCNBH_3$ was replaced by $NaCNBD_3$ and the DAAO was removed through a 10000 molecular weight cut off prior to analysis by ES-MS.

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