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Amine–boranes: effective reducing agents for the deracemisation of DL-amino acids using L-amino acid oxidase from *Proteus myxofaciens*

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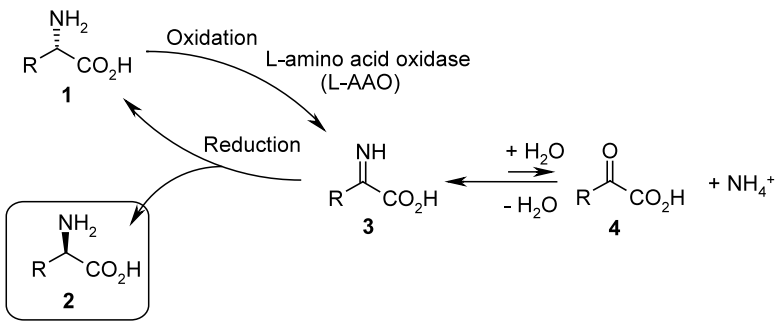
Abstract—The deracemisation of DL-α-amino acids using L-amino acid oxidase from *Proteus myxofaciens* and amine-boranes as chemical reducing agents has been investigated. Amine-boranes were found to be of particular interest in terms of reactivity and chemoselectivity compared to sodium borohydride and cyanoborohydride. Starting from the racemate, a range of D-amino acids were obtained in yields of up to 90% and e.e. >99%. © 2002 Elsevier Science Ltd. All rights reserved.

The kinetic resolution of racemates is a highly successful strategy for the synthesis of enantiomerically pure chiral compounds and has found widespread usage in industry.[1] However, the theoretical yield of each enantiomer can never exceed 50% and thus to avoid the wastage of the unwanted enantiomer, reactions that give >50% yield have received increasing interest.[2] Such reactions can be divided into three types,[2] namely desymmetrisation of *meso* or prochiral compounds,[3] dynamic kinetic resolutions,[4] and less exploited, deracemisation[5] of an enantiomeric mixture. An example of the latter process is the cyclic sequence involving an enantioselective oxidation (e.g. enzymatic) leading to an achiral intermediate, followed by a nonselective reduction (Scheme 1). The repetition of this sequence rapidly leads to a single enantiomer in a theoretical 100% yield and 100% e.e.

Based on the original reports by Hafner et al.[6a] and Soda et al.,[6b,c] we have recently investigated[7] the deracemisation of acyclic and alicyclic α-amino acids using a combination of porcine kidney D-amino acid oxidase



Scheme 1. Deracemisation of DL-amino acids using L-amino acid oxidase (L-AAO).

Keywords: amine–borane; D-amino acid; L-amino acid oxidase; deracemisation.

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and sodium borohydride or sodium cyanoborohydride. For acyclic substrates we have shown that the intermediate imino acid **3** is sufficiently stable to be reduced by sodium borohydride although some hydrolysis to the keto acid **4** is observed. However, both of these hydride reducing agents have certain limitations in terms of practical application. Sodium borohydride is sensitive to water, is easily decomposed at low and neutral pH requiring large excesses to be added which often leads to rapid deactivation of oxidase enzymes.[8] Sodium cyanoborohydride is more stable at low to neutral pH,[9] however its industrial application is limited because of its toxicity and its reduced reactivity compared with sodium borohydride. For these reasons we decided to investigate alternative reducing agents as well as using a new L-amino acid oxidase from *Proteus myxofaciens*,[10] to develop an efficient method for the synthesis of D-amino acids.

The only alternative reducing system that has been reported for deracemisation reactions[11] is Catalytic Transfer Hydrogenation (CTH).[12] The intermediate imino acid **3** is reduced by hydrogen which is released from a metal or metal complex and a hydrogen source as the reaction proceeds. We now report utilisation of amine–boranes as reducing agents in the deracemisation system. These compounds have received relatively little attention compared to their chemical cousins borane–THF, borane–Me_2S, borohydride and cyanoborohydride.[13] Nevertheless, amine–boranes possess interesting properties that could overcome the disadvantages of using $NaBH_4$ and $NaBH_3CN$. Amine–boranes are stable in water[13] (except arylamine–boranes) at neutral or basic pH but they are also soluble and unreactive toward a wide range of protic and aprotic solvents. Their reducing abilities are greatly dependent on the complexing amine, thus a range of reduction capabilities are obtainable providing high versatility and selectivity. Furthermore, most aliphatic amine–boranes are white crystalline solids and stable at room temperature.[13]

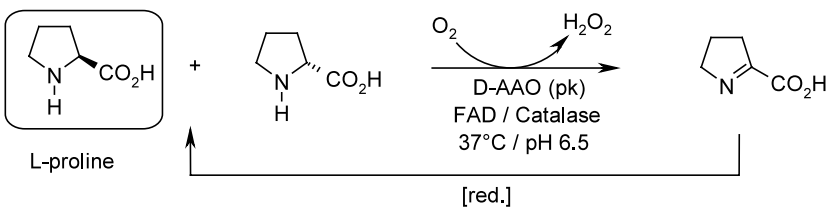
Table 1.

Entry	Reducing agent	Yield (%)[a]	e.e. (%)[a]
a	NH_3–BH_3 (9 equiv.)	97	>99
b	1 M $HCOONH_4$/Pd/C (10%)	82	>99
c	BH_3–Pyridine (6 equiv.)	99	>99
d	*t*-$BuNH_2$–BH_3 (10 equiv.)	92	>99

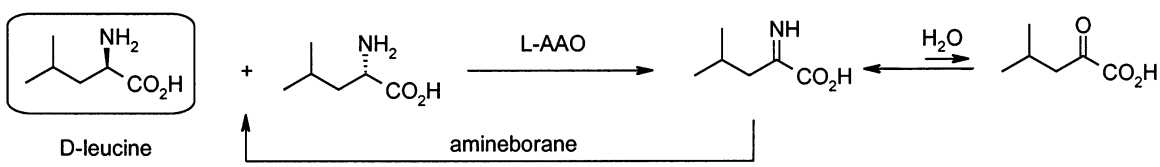
[a] Yield and e.e. based on chiral HPLC analysis.[15]

The deracemisation of DL-proline with D-amino acid oxidase from porcine kidney was initially investigated in order to compare the different reducing systems (Scheme 2). The reactions were performed according to the previously described procedure.[7,14a] HPLC monitoring[15] showed that the reduction step is slower with CTH ($HCOONH_4$/Pd/C) than with the amine–boranes. Ammonia–borane and pyridine–borane gave the best results (see Table 1). Only 6–10 equiv. of amine–boranes, compared to the 500 necessary equivalents for $NaBH_4$,[6] were required to achieve complete deracemisation. Because of the associated toxicity of pyridine–borane, ammonia–borane and *tert*-butylamine–borane were chosen for further studies.

We next examined the ability of the L-amino acid oxidase (L-AAO) from *Proteus myxofaciens*[10] to catalyse the deracemisation of DL-mixtures to produce D-amino acids. The *lad* gene encoding L-amino acid oxidase has been cloned and overexpressed in *Escherichia coli* K12. The enzyme has a broad substrate specificity catalysing the oxidation of both natural and unnatural L-amino acids. The reaction has an absolute requirement for O_2, releases NH_3 but does not produce detectable levels of H_2O_2 and hence the enzyme has also been termed a deaminase. The optimum pH is 7.5.[10] We first studied the deracemisation of DL-leucine using disrupted cells[10,14] of L-AAO. The reactions were performed[14b] at pH 6.7 in 50 mM ammonium formate solution. The variation of the quantity of reducing agent revealed that 40 equiv. of ammonia–borane were needed to afford a 98% yield of optically pure D-leucine (Scheme 3). This result suggests that the concentration of reducing agent must be high enough to ensure immediate reduction of the intermediate α-imino acid



Scheme 2. Deracemisation of DL-proline using D-amino acid oxidase and reducing agents.



Scheme 3. Deracemisation of DL-leucine with L-amino acid oxidase and amine–borane.

and avoid hydrolysis to the keto acid. In order to increase the concentration of the α-imino acid versus keto acid, ammonium formate was added to the reaction (Scheme 1). It appears that the concentration of the α-imino acid must be reasonably high, since an attempt to start from 4-methyl-2-oxo-pentanoic acid to produce D-leucine under the same conditions was unsuccessful. Yields obtained with *tert*-butylamine-borane are also lower than with ammonia–borane as shown in compared reaction profiles (Fig. 1) presumably as a result of the lower reactivity and solubility of *tert*-butylamine-borane in water. Furthermore, ammonia–borane releases ammonia as the reaction proceeds.[16] Experiments using whole cells[14c] of L-amino acid oxidase were also performed and deracemisation of DL-leucine provided D-leucine in 90% yield and e.e. >99% (Table 2).

Several racemic mixtures of natural and unnatural amino acids were then subjected to the deracemisation reaction using ammonia–borane and L-amino acid oxidase.[14] The α-functionality was varied to include

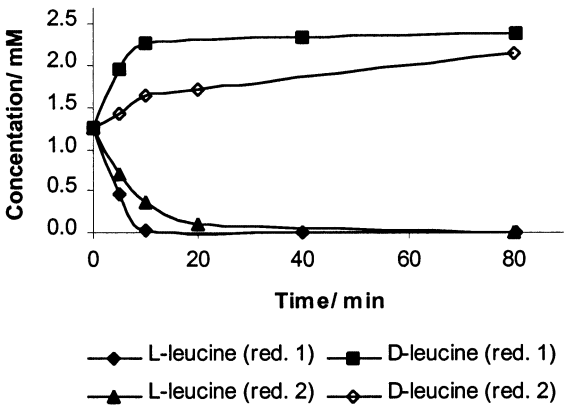


Figure 1. Deracemisation of DL-leucine with L-AAO and 20 equiv. of NH_3–BH_3 (red. 1) or *t*-$BuNH_2$–BH_3 (red. 2).

Table 2.

Entry	Reducing agent	Yield (%)[a]	e.e. (%)[a]
a	NH_3–BH_3 (40 equiv.)	98	>99
b	*t*-$BuNH_2$–BH_3 (20 equiv.)	90	>99
c	1 M $HCOONH_4$/Pd/C (10%)	65	>99

[a] Yield and e.e. based on chiral HPLC analysis.[15]

aromatic, ethylenic, alkyl and thiol containing groups (Table 3). It should be noted that the double bond of allylglycine (entry e) remained unaffected in the deracemisation reaction, showing that ammonia–borane is a poor hydroborating system under these conditions.[13]

In conclusion, we have shown that amine–boranes are efficient reducing agents for deracemisation reactions. Further studies will be focused on their chemoselective reductive abilities in choosing the appropriate amine substituent according to the reductive functionalities in the substrate.

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11. Presented in part at *Chiral USA* organised by Scientific Update, Marriott Longwharf, Boston, USA; May 3–4, **1999**.

Table 3.

Entry	Substrate	Yield (%)[a]	e.e. (%)[a]	Entry	Substrate	Yield (%)[a]	e.e. (%)[a]
a	Norvaline	81	>99	g	*O*-Benzyl serine	87	93
b	Norleucine	86	>99	h	α-Aminobutyric acid	64	96
c	Tryptophan	82	>99	i	Valine	64	28[b]
d	Phenylalanine	82	>99	j	Histidine	67	65[b]
e	Allylglycine	79	>99	k	Cyclopentylglycine	87	>99
f	Methionine	90	>99	l	Tyrosine	79	>99

[a] Yield and e.e. based on chiral HPLC analysis,[15] reaction time 5 h.
[b] Lower e.e. values are due to incomplete deracemisation.

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14. Reaction procedures: (a) using D–AAO of porcine kidney (pk): 100 μL, 25 mM DL–amino acid in $HCOONH_4$ [50 mM or 1 M (CTH)], 100 μL, 0.8 mM FAD solution in $HCOONH_4$, 800 μL $HCOONH_4$, then 1 mg catalase, 2 mg D-AAO (pk) and reducing agent. (b) using disrupted cells of L-AAO: 1.5 mL of 2.5 mM DL-amino acids in $HCOONH_4$ 50 mM, 20 μL L-AAO and reducing agent. (c) using whole cells of L-AAO: 1.5 mL of 2.5 mM DL-leucine in $HCOONH_4$ 50 mM, 20 μL of a whole cells solution (10 mg in 100 μL buffer) and ammonia–borane (40 eq.).
15. Chiral HPLC analyses were performed with Chirex 3126 column (supplied by Phenomenex), using $CuSO_4$ 1.5 mM solution/MeOH as the mobile phase. Yields were determined by integration of the peaks against internal standards.
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