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Stereoinversion of β- and γ-substituted α-amino acids using a chemo-enzymatic oxidation–reduction procedure

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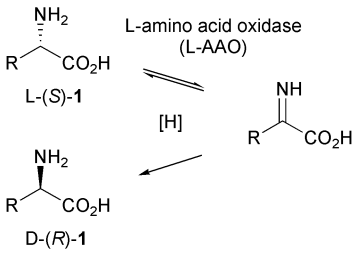
Both D- and L-β- and γ-substituted α-amino acids can be interconverted to their respective L- and D- diastereoisomers by treatment with an enantioselective amino acid oxidase and a chemical reducing agent.

Enantiomerically pure β-substituted α-amino acids are valuable building blocks for the synthesis of peptidomimetics and enzyme inhibitors.[1] The incorporation of a β-substituent into an α-amino acid (*e.g.* β-methylphenylalanine) generally leads to restricted conformational flexibility in peptides offering the prospect of developing high affinity ligands for receptors. Methods for the preparation of β-substituted α-amino acids include resolution,[2] asymmetric catalysis,[3] and also asymmetric synthesis employing chiral auxiliaries.[4] A particularly versatile approach involves the catalytic asymmetric hydrogenation of β,β-disubstituted dehydroamino acids using chiral phosphine ligands.[5] Although such substrates tend to undergo hydrogenation slowly, Burk *et al.* have developed appropriate ligands (*e.g.* Me-DuPHOS) that result in high enantioselectivities.[6] However even these ligands tend to be more selective and reactive towards (*Z*)-configured β-substituted enamides compared to the (*E*)-isomers resulting in preferential access to only two of the four possible diastereoisomers. In addition, a number of natural β-substituted α-amino acids are more readily available in one diastereoisomeric form than the other (*cf.* L-isoleucine *vs.* D-*allo*-isoleucine). With this in mind we sought to develop methodology for interconverting diastereoisomers of amino acids bearing two stereogenic centres.

Recently we have developed a general and practical method for the stereoinversion of α-amino acids[7,8] and amines.[9,10] The overall process relies upon the combined action of an enantioselective oxidase and a non-selective chemical reducing agent (*e.g.* Pd/C–HCO_2NH_4; amine : borane; sodium borohydride/cyanoborohydride). For example, the use of an L-amino acid oxidase (L-AAO) enables conversion of a racemic mixture of α-amino acids to the optically pure D-enantiomer (Scheme 1).[8] Provided that the amino acid oxidase is highly enantioselective then only 7 oxidation/reduction cycles are required to achieve an e.e. of >99% with yields ranging between 80–99%.[11,12]

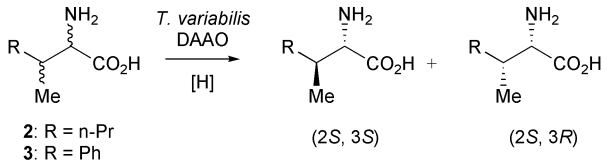
Extension of this approach to α-amino acids containing one or more additional stereogenic centres would provide a method for interconverting diastereoisomers rather than enantiomers. Herein we demonstrate the success of this approach using a range of different amino acid oxidases and chemical reducing agents.



Scheme 1 Deracemisation of an α-amino acid D/L-**1** using a combination of an L-amino acid oxidase (L-AAO) and reducing agent.

Initially it was important to establish that β-substituted α-amino acids were indeed substrates for the various L- and D-amino acid oxidases. Thus (2*RS*,3*RS*)-2-amino-3-methylhexanoic acid **2** and (2*RS*,3*RS*)-β-methyl-phenylalanine **3** were prepared in racemic form, and as a mixture of diastereomers (*ca.* 40% d.e.), from the corresponding racemic aldehydes *via* the Strecker reaction. Each substrate was subjected to stereoinversion using D-amino acid oxidase from *Trigonopsis variabilis* and either $NaBH_4$ or $NaCNBH_3$ as the reducing agent (Scheme 2).[13] The progress of the reactions was monitored by chiral HPLC allowing the concentration of all four stereoisomers to be assessed during the reaction. For both substrates, rapid consumption of the pair of (2*R*)-diastereoisomers was observed, albeit at slightly different rates, with concomitant production of the (2*S*)-isomers (Table 1). For substrate **2**, the reaction was found to be complete (>99% e.e. for each diastereomer) after 18 h using $NaCNBH_3$ and only 3.5 h with the more reactive $NaBH_4$. At the end of the reaction a 1 : 1 mixture of the (2*S*,3*S*)- and (2*S*,3*R*)-isomers was observed (*NB* the final ratio of diastereoisomers is independent of the initial diastereomeric composition generated *via* the Strecker reaction and always approaches 1 : 1). In contrast to **2**, (2*RS*,3*RS*)-β-methylphenylalanine **3** was found to react more slowly although again the use of $NaBH_4$ enabled complete conversion after 6 h. The reduced rate with this substrate may be a consequence of partial inhibition of the reaction by some 3-methylphenylpyruvic acid produced *via* hydrolysis of the intermediate imine.[14]

Having established the basic reaction conditions we next tested the generality of the stereoinversion procedure by examining two different amino acid oxidases and an alternative



Scheme 2 Stereoinversion of β-substituted α-amino acids.

Table 1 Stereoinversion of **2** and **3** using *T. variabilis* D-AAO and $NaBH_4$/$NaCNBH_3$

Substrate	D-AAO (U)	[H] (equiv.)	Time/h	Yield (%)	e.e. (%)
2	0.68	$NaCNBH_3$ (25)	18	63	>99
2	0.68	$NaBH_4$ (40)	3	94	>99
2	0.68	$NaBH_4$ (40)	3.5	99	>99
3	0.68	$NaCNBH_3$ (25)	2	69	33[a]
3	1.70	$NaCNBH_3$ (40)	22	68	48 [a]
3	3.40	$NaCNBH_3$ (60)	22	86	50 [a]
3	0.68	$NaBH_4$ (40)	6	71	>99

[a] denotes e.e. of one of the diastereomers indicating incomplete reaction.

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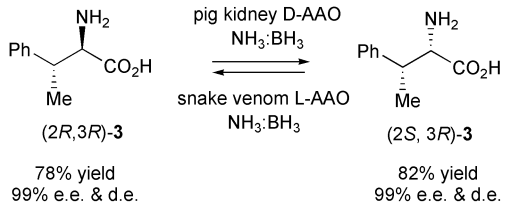
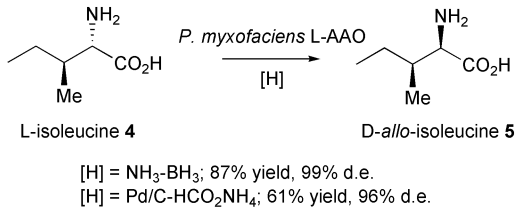
reducing agent (NH_3 : BH_3) which has previously been shown to be effective.[8] In this case we chose to use enantiomerically pure (2*R*,3*R*)-**3** and (2*S*,3*R*)-**3** as substrates and successfully interconverted the pair of diastereoisomers using D-AAO from porcine kidney and L-AAO from snake venom (Scheme 3).

In addition to NH_3 : BH_3, catalytic transfer hydrogenation (Pd/C–HCO_2NH_4) is also compatible with the oxidase reactions.[8] Both reagents were examined for their ability to convert L-isoleucine **4** to D-*allo*-isoleucine **5** in the presence of L-AAO from *Proteus myxofaciens* (Scheme 4). This enzyme has been over-expressed in *Escherichia coli*[15] and can be used either as a whole cell biocatalyst or partially disrupted cells. The use of NH_3 : BH_3 (40 equiv.) with disrupted cells yielded D-*allo*-isoleucine **5** in 87% yield and 99% d.e. whereas by comparison Pd/C–HCO_2NH_4 with intact cells gave **5** in 61% yield and 96% d.e. The use of snake venom L-AAO with NH_3 : BH_3 resulted in a modest improvement (89% yield; > 99% d.e.).

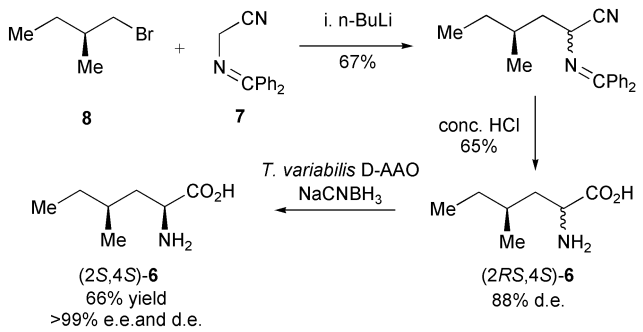
Finally to further explore the range of substrates amenable to stereoinversion, we examined γ-substituted α-amino acids. (2*RS*,4*S*)-2-Amino-4-methylhexanoic acid **6** was prepared by alkylation of imine **7**[16] using (*S*)-(+)-1-bromo-2-methylbutane **8** (e.e. > 98%) followed by hydrolysis (conc. HCl) to give (2*RS*,4*S*)-**6** in 65% yield and 88% d.e (Scheme 5). Treatment of **6** with *T. variabilis* D-AAO and $NaCNBH_3$ resulted in rapid



Scheme 3 Interconversion of (2*R*,3*R*)- and (2*S*,3*R*)-β-methylphenylalanine **3**.



Scheme 4 Conversion of L-isoleucine **4** to D-*allo*-isoleucine **5**.



Scheme 5 Synthesis of (2*S*,4*S*)-4-methyl-2-aminohexanoic acid **6**.

conversion to (2*RS*,4*S*)-**6** (66% yield; > 99% e.e. and d.e.). The D-AAO catalysed oxidation of (2*R*,4*S*)-**6** proceeded rapidly suggesting that the presence of a γ-methyl group has relatively little effect on the reaction rate compared to simple α-amino acids.

In conclusion we have developed a novel procedure for interconverting β- and γ-substituted α-amino acid diastereoisomers using amino acid oxidases in combination with a range of chemical reducing agents. The procedure appears highly versatile in that so far we have examined four different AAOs and four different reducing agents and in all cases the reactions proceeded in good to high yield and excellent stereoselectivities.

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