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Asymmetric Bioreductions of β-Nitro Acrylates as a Route to Chiral β²-Amino Acids

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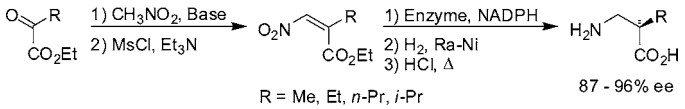
ABSTRACT



Reductions of β-nitroacrylates by *Saccharomyces carlsbergensis* old yellow enzyme is the key step in a concise route to optically active β²-amino acids. The enzymatic reductions occur with 87–96% ee, with larger substrates providing greater stereoselectivities. This work extends enantioselective enzymatic alkene reductions to include acyclic systems with weakly coordinating substituents.

2-Substituted derivatives of 3-aminopropionic acid (β^2-amino acids) have become important synthetic targets since peptides containing these residues can assume different structures than those of α-amino acids and their peptide bonds resist protease degradation.[1] In contrast to α-amino acids, for which a variety of reliable, high-yielding methods have been devised, β^2-amino acids remain challenging synthetic targets.[2] Recent attention has focused on catalytic strategies involving conjugate additions to amino-[3] and nitroacrylates.[4] Asymmetric hydrogenation of aminoacrylates has also been reported,[5,6] and this can provide very high enantioselectivities in favorable cases.[7] We[8] and others[9−11] have investigated the potential of enzyme-mediated alkene reductions to supply chiral intermediates. Here, we describe how the best-studied enzyme in this class—*Saccharomyces carlsbergensis* old yellow enzyme—can be used in stereoselective β^2-amino acid synthesis.

The required β-nitroacrylates **3a**−**g** were prepared by a two-step sequence (Scheme 1). Henry reactions were carried out using a solid-phase base along with the appropriate α-keto ester (either commercially available or prepared by the method of Macritchie et al.[12]) and nitroalkane.[13a] Spectral data for nitroaldol adducts **2a**−**e**,**g** matched those reported previously.[13,14] These were converted to the corresponding

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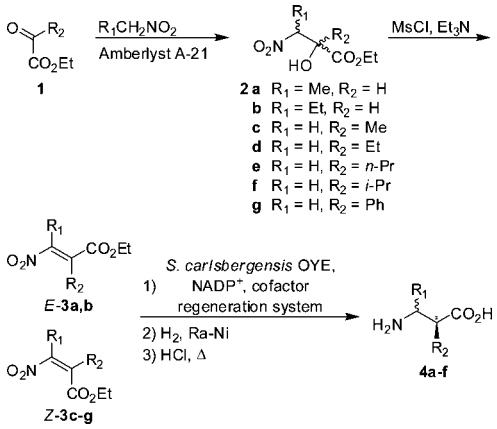
Scheme 1



nitroacrylates via mesylate derivatives.[15] Both 2-alkyl-substituted nitroacrylates **3a** and **b** were obtained predominantly in the (*E*)-form, whereas the (*Z*)-isomers predominated for 3-substituted alkenes **3c**−**g**.[16] Because olefin geometry directly impacted the stereoselectivity of enzymatic reductions, the major alkene isomers were chromatographically enriched (>95% geometric purity).

Biocatalytic reductions utilized *S. carlsbergensis* old yellow enzyme that had been purified by affinity chromatography[17] to avoid possible complications from *Escherichia coli* alkene reductases. NADPH was supplied by a cofactor regeneration system (glucose-6-phosphate/baker's yeast glucose-6-phosphate dehydrogenase).

Preliminary studies had revealed that olefin isomerization was more rapid under alkaline conditions, and pH 6.93 was selected to minimize this side-reaction while maintaining acceptable enzyme efficiency. A 2-fold molar excess of β-cyclodextrin (relative to the nitroacrylate) was also included to enhance substrate solubility under aqueous conditions. Unfortunately, the solubility of the β-cyclodextrin/**3g** complex was still too low for efficient reduction, and no reaction was observed in this case. Both the substrates (**3a**−**f** and glucose-6-phosphate) and the two enzymes were added portionwise to enhance the longevity of the processes, which were carried out at room temperature.[18] Reactions were monitored by GC/MS, and complete substrate consumption was observed after *ca*. 8 h in all cases except for (*E*)-**3b**.

NMR and GC analysis of the crude reaction products verified that only the olefins had been reduced and the nitro groups remained intact. No significant levels of side products were observed, and yields ranged from 74 to 98%. Because it was not possible to determine the optical purities of the reduction products by chiral-phase chromatography, the crude materials were hydrogenated in the presence of Raney-Ni to the corresponding amines (75−85% yield).[19] Enantiomer separations were then possible by chiral-phase GC following derivatization with trifluoroacetic anhydride. Good optical purities were obtained from 2-alkyl-substituted nitroalkenes **3c**−**f**; by contrast, 3-alkyl-substituted products were obtained in essentially racemic form (Table 1). The absolute configurations were assigned by the direction of optical rotations of the free β^2-amino acids as their hydrochloride salts (obtained by acid hydrolysis in 88−95% yields). Overall yields of β^2-amino acids from β-nitroalkenes ranged from 57 to 73%.

Table 1. β-Nitroacrylate Reductions by *S. carlbergensis* Old Yellow Enzyme

starting compd	conversion (%) after 8 h	% ee (absolute config)[a]	$[\alpha]_D$[b]
(*E*)-**3a**	>98	8 (*R*)	
(*E*)-**3b**	50		
(*Z*)-**3c**	>98	8 (*R*)	−13.0 (*c* 0.94)
(*Z*)-**3d**	>98	91 (*R*)	−2.7 (*c* 1.0)
(*Z*)-**3e**	>98	94 (*R*)	+1.2 (*c* 1.0)
(*Z*)-**3f**	>98	96 (*R*)	−1.6 (*c* 1.0)

[a] Determined by chiral-phase GC following nitro group reduction and derivatization with trifluoroacetic anhydride. [b] Measured in aqueous solution at the indicated concentrations from hydrochloride salts at room temperature.enzyme[8] under the same conditions after 48 h.[20a]

The results of our previous studies of alkyl-substituted 2-cyclohexenone reductions by old yellow enzyme[8] were consistent with the net *trans*-addition of H_2 mechanism elucidated by Massey and Karplus (Scheme 2).[20] Hydride β-addition (from reduced FMN) occurs from the bottom face

(14) Spectral data for **2f**: ^{1}H NMR ($CDCl_3$) δ 4.85 (d, J = 13.5 Hz, 1H), 4.70 (d, J = 13.3 Hz, 1H), 4.39 (m, 2H), 3.61 (s, 1H), 2.0 (m, 1H), 1.36 (t, J = 7.1 Hz, 3H), 1.0 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.1, 80.3, 77.7, 63.1, 34.3, 17.0, 16.4, 14.2.

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(16) Olefin configurations for **3c**−**g** were assigned based on the chemical shift values of the allylic protons, e.g., 2.11 ppm for (*Z*)-**3c** versus 2.60 ppm for the methyl ester of (*E*)-**3c** (ref 15a), as described by Denmark and Marcin (*J. Org. Chem.* **1993**, *58*, 3850−3856.).

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(18) Reaction mixtures contained final concentrations of $NADP^+$ (20 μmoles, 15 mg), glucose-6-phosphate (1.27 mmoles, 429 mg), glucose-6-phosphate dehydrogenase (500 μg), nitroacrylate (25mM), and purified OYE (20−40 μg) in 100 mM KP_i, pH 6.95 in total volumes of 50 mL. Conversions were carried out at room temperature. Reaction components (except for KP_i buffer) were added in 10 equal portions every 45 minutes and the mixtures were sampled for GC analysis periodically. After nearly all of the substrate had been consumed, the reaction mixture was extracted with Et_2O (3 × (5 × reaction volume)). The combined organic extracts were washed with brine (1 volume) and water (1 volume), dried with $MgSO_4$, and concentrated in vacuo.

(19) Crude reduction products (ca. 1.5 mmol) were hydrogenated at 500 psi in the presence of Raney nickel (200 mg) in EtOH (50 mL) at room temperature. After 16 h, the resulting solution was filtered through Celite and the solvent was evaporated. A portion of the residue (50 mg) was dissolved in 6 M HCl and the solution was held at reflux overnight. The solution was concentrated under reduced pressure to afford a yellow oil, which was washed with EtOAc to remove any nonpolar impurities. Water was removed by rotary evaporator to yield the β^2-amino acids as hydrochloride salts. Spectral data matched those reported previously: Nejman, M.; Sliwinska, A.; Zwierzak, A. *Tetrahedron* **2005**, *61*, 8536−8541. Gangadhar, N.; Huber, V. J.; Lum, C.; Goodman, B. A. *Org. Lett.* **2000**, *2*, 3527−3529. Sammis, G. M.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2003**, *125*, 4442−4443. Lee, H. S.; Park, J. D.; Kim, D. H. *Bull. Korean Chem. Soc.* **2003**, *24*, 467−472.

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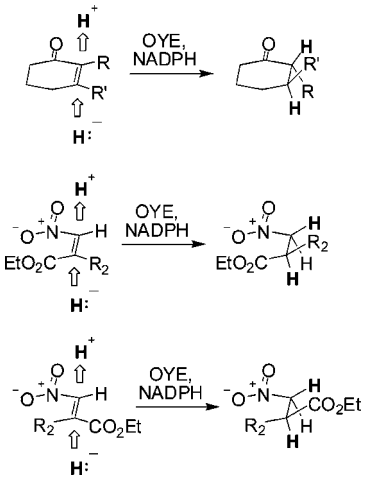
Scheme 2



while α-protonation likely involves the phenol side-chain of Tyr 196. Carbonyl activation is achieved via hydrogen bonding by the side chains of His 191 and Asn 194 (not shown). For the acyclic *β*-nitroacrylates investigated here, analogous binding could occur in which one nitro oxygen occupies the same location as the carbonyl oxygen and the alkene is positioned similarly (Scheme 2). This substrate binding orientation was verified by carrying out the enzymatic reductions of (*E*)-**3b** and (*E*)-**3c** in D_2O. All MS and NMR data were consistent with deuterium incorporation only on the nitro-bearing carbon in both cases. Isotope wash-in following reduction was ruled out by observing that the alkene reduction product of (*E*)-**3a** failed to incorporate detectable levels of deuterium under the same conditions after 48 h.

Net *trans*-addition of H_2 to **3c**−**f** would lead to the observed (*R*)-products, and this model provides a useful method for predicting the outcomes of additional nitroalkene reductions by old yellow enzyme.[21] High stereoselectivity requires alkene configurational stability and this could be the main reason that *β*-nitroacrylates with smaller alkyl substituents afforded slightly lower enantiopurities. Within 7h, initially pure (*Z*)-**3d** incubated in buffer alone afforded three byproducts in an aggregate yield of ca. 50%.[22] These observations underscore the need to reduce *β*-nitroacrylates rapidly and minimize their exposure to the aqueous reaction conditions. Fortunately, the reduction products are stable to the reaction milieu. Conversions were not optimized during these preliminary investigations, and it is likely that adjusting the catalyst level and/or reaction pH might further improve the biotransformations.

Old yellow enzyme-mediated reductions of 3-alkyl-substituted *β*-nitroacrylates **3a**,**b** yielded essentially racemic products. Given the highly stereoselective α-protonation observed in 2-cyclohexenone reductions, this result was surprising. Post-reduction racemization is unlikely since Kawai has shown that similar α-alkyl-substituted nitro compounds are configurationally stable at neutral pH[23] and our own studies in D_2O buffer further support this notion. Instead, we suspect that α-protonation likely occurs *after* product release from the active site.[24] The pK_a values of the nitronate intermediates and the phenol of Tyr 196 make proton transfer thermodynamically unfavorable. Indeed, Massey has shown that mutation of Tyr 196 to Phe rendered old yellow enzyme incapable of enone reductions while having minimal impact on nitroalkene reductions.[20a] Stereoselective protonation of the nitronate intermediates will require an active site general acid with a better-matched pK_a value near 7.0.

In conclusion, our results have uncovered a new application for *S. carlsbergensis* old yellow enzyme in synthesizing optically active *β*[2]-amino acids. The synthetic route is concise and utilizes inexpensive starting materials. The major difficulties lie in suppressing alkene isomerization prior to reduction and ensuring active site protonation of the nitronate intermediate. Efforts to accomplish both of these goals are underway.

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Supporting Information Available: Experimental procedures, spectral and chromatographic data used to determine chemical and optical purities of the *β*[2]-amino acid final products, and spectral data for new compounds **3c**−**g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) In a preliminary experiment, *E*-**3d** was reduced by old yellow enzyme with largely (*S*)-stereoselectivity, as would be expected from the model in Scheme 2. This suggests that it may be possible to prepare the enantiomeric series of *β*-amino acids by using the alternate olefin isomers.

(22) The three byproducts were identified by GC/MS as (*E*)-**3d**, the alternate acrylate alkene regioisomer of **3d**, and the water addition product from **3d** (in racemic form).

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