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Baker's yeast mediated enantiospecific synthesis of *anti*-(2*R*,3*R*)-*p*-chloro-3-hydroxytyrosine: an α-amino-β-hydroxy acid of Vancomycin †

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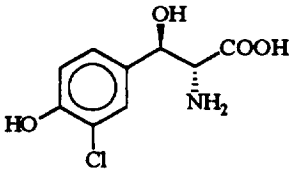
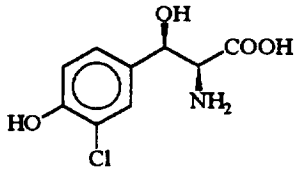
Abstract: Baker's yeast mediated reduction of α-azido-β-keto ester lead to reduction of the carbonyl group with high enantiospecificity and diastereoselectivity at low pH (4.0, e.e. >99%, d.e. 79%). At pH ~7, although the enantioselectivity is maintained, the diastereoselectivity is lost. © 1997 Published by Elsevier Science Ltd. All rights reserved.

α-Amino-β-hydroxy acids are important constituents of various biologically active cyclic peptides possessing a wide range of biological activity such as antibiotic and immunosuppressive properties; and also serve as precursors of β-lactam antibiotics.[1] Recently, we have reported the reduction of α-hydroxy-β-ketoesters by immobilised baker's yeast to obtain the corresponding diol esters with high enantio- and diastereoselectivity.[2] Here we present the application of this methodology in the asymmetric synthesis of *anti*-(2*R*,3*R*)-**1** and *syn*-(2*S*,3*R*)-*p*-chloro-3-hydroxytyrosine **2**. These two diastereomeric β-hydroxy amino acids are directly linked with *p*-hydroxy-α-phenyl glycine in the glycopeptide antibiotic Vancomycin.[3]



1



2

Asymmetric syntheses of **1** and **2** have been reported earlier with several approaches such as nucleophilic opening of epoxides,[4] electrophilic amination,[5] and reduction of N-protected-α-amino-β-ketoesters with chiral catalysts.[6] In the simple chemoenzymatic synthesis of **1** presented here, the α-azido-β-keto ester **6** is reduced in the key step to the corresponding azido hydroxy ester by immobilised baker's yeast with high enantioselectivity (e.e. >99%) and also high diastereoselectivity (d.e. 79%).

p-Hydroxybenzaldehyde **3** was chlorinated with N-chlorosuccinimide (85%) and the phenol was protected with benzyl bromide (95%). The resulting aldehyde **4** underwent Reformatsky reaction smoothly to give β-hydroxy ester (81%) which was further oxidised with PDC to β-ketoester **5** (90%). Treatment of **5** with [hydroxy(tosyloxy)iodoso]benzene and trimethylsilylazide[7] gave the required α-azido-β-keto ester **6** (67%). Reduction of **6** with baker's yeast immobilised in calcium alginate at pH 4 gave the corresponding *anti*-(2*R*,3*R*)-2-azido-3-(4-benzyloxy-3-chlorophenyl)-3-hydroxypropanoate **7a** with high diastereomeric purity[8] (d.e. 79%) and high enantioselectivity (e.e. >99%). Reduction of the azide group by $SnCl_2$ and protection of the amino group with ditertiarybutyl dicarbonate gave the protected amino acid **8** (79%) (Scheme 1).

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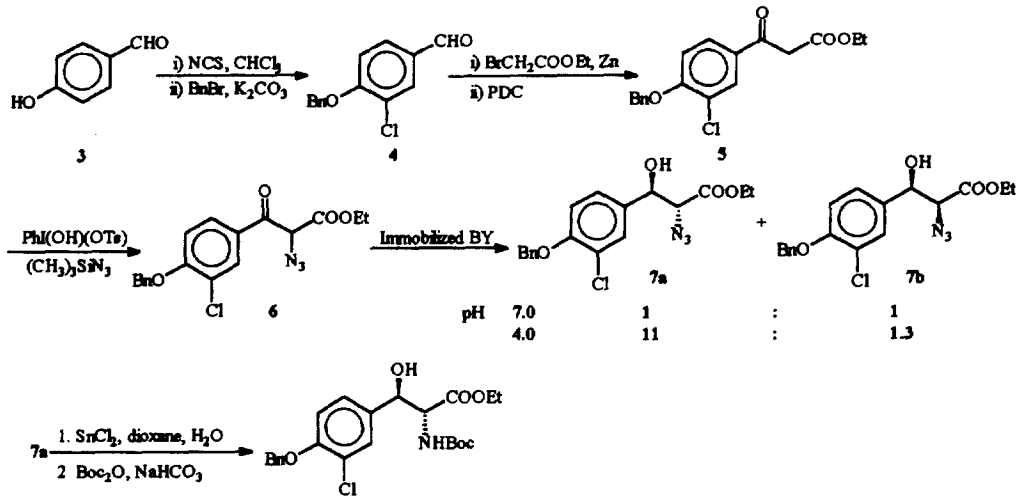
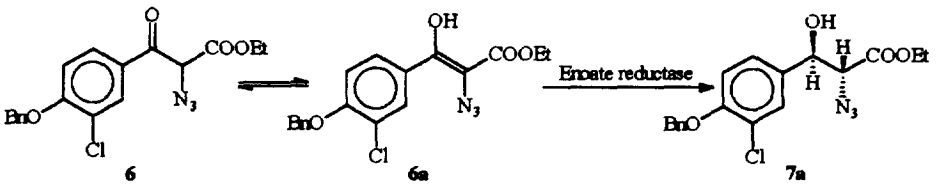


Scheme 1.

As observed in the case of 3-azido-2-octanone,[9] the reduction of the carbonyl group is highly enantioselective (e.e. >99%). In accordance with our earlier results on β-ketoester reductions with baker's yeast[2] we find a strong dependence of the diastereoselectivity on the pH of the medium in the present case. Although the enantioselectivity remains high, the d.e. changes from 79% at pH 4 to 0% at pH 7–8. Similarly at pH 4, addition of a divalent metal salt such as $MgCl_2$ (10 mM) caused loss of diastereoselectivity. The α-azido-β-ketoester **4** is very much prone to enolization in aqueous solutions and in principle, the diastereoselectivity in the reaction could be observed due to an enantiotopic face differentiation in the hydrogenation of the carbon–carbon double bond of the enolic intermediate by enzyme enoate reductase of baker's yeast[10] (which reduces a carbon–carbon double bond in a *trans* manner). In this case, in analogy to the reduction of an activated carbon–carbon double bond, the *anti* product should be formed (Scheme 2).



Scheme 2.

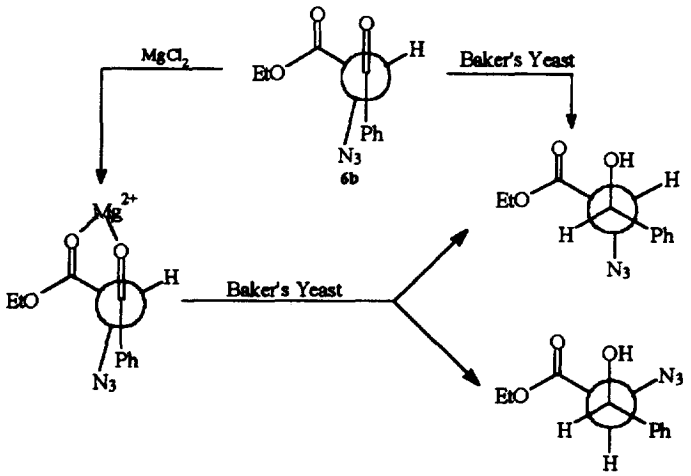
To check this possibility, **6** was reduced with isolated alcohol dehydrogenase from baker's yeast (which is free from enoate reductase). Here again, the product **7a** (e.e. >99%) with a diastereomeric excess of 84.6% was obtained at pH 4 while at pH 7 a 1:1 diastereomeric mixture of *anti*-(2*R*,3*R*) and *syn*-(2*S*,3*R*) was obtained. Since alcohol dehydrogenase does not reduce C=C, the possibility of formation of **7a** via reduction of the carbon–carbon double bond of the enolic intermediate **6a** can be ruled out. Interestingly, when **6** was reduced with either the whole cells or the isolated enzyme at pH 4 in the presence of 10 mM $MgCl_2$, a 1:1 diastereomeric mixture was obtained (Table 1) although the enantiomeric purity of the azido alcohol did not change.

Our results in Table 1 can be rationalised as follows: the α-azido-β-ketoester prefers a configuration in which the azido group is away from the carbonyl. Since the azido group is electron withdrawing, epimerization occurs at C_2 via the enol form **6a**, and the *anti* product **7a** with high diastereoselectivity is obtained at low pH through a dynamic kinetic resolution in which the ketoester with *R* configuration

Table 1.

Reaction Conditions	Anti-(2R, 3R)/ syn-(2S, 3R)
Baker's Yeast pH 7.0	1 :1
Baker's Yeast pH 4.0	11:1.3
Alcohol Dehydrogenase pH 4.0	12:1
Baker's Yeast / Alcohol Dehydrogenase pH 4.0 + 10 mM $MgCl_2$	1:1

at C_2 (**6b**, Scheme 3) is reduced faster than its antipode. At low pH (pH 4) the rate of enolization is fast and the anti-alcohol formed from the more rapidly reduced enantiomer is the predominant product. At neutral pH, when enolization becomes slow, enantioselectivity prevails but optically pure diastereomers are formed in a 1:1 ratio. Competition between the rate of epimerization at the C_2-position and the rate of enzymatic hydrogen transfer at C_3 (both pH dependent) determine the diastereomeric ratio of the product.



Scheme 3.

Divalent metal ions such as Mg^{2+} can form a six-membered chelate ring and reduce the rate of epimerization resulting in loss of diastereoselectivity (Scheme 3).

Acknowledgements

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8. The diastereomeric excess of the crude product was determined from the NMR (carbinol proton at δ 4.85 ppm for *anti* and 5.05 ppm for *syn*). The enantiomeric excess of the product was determined by chiral HPLC after conversion to corresponding phenylacetyl derivative by treatment of the crude with phenylacetyl chloride (Chiralcel O.J. column, 25×0.46 cm, Daicel, Japan; solvent system hexane:isopropanol=80:20; flow rate 0.6 ml/min; detection 254 nm; retention times: 2*R*,3*R*, 8.1 min.; 2*S*,3*S*, 10.4 min.). **7a:** $[\alpha]_D^{25}$ −1.8 (c 1.0, $CHCl_3$). The *syn* and *anti* diastereomers can be separated by column chromatography as TBDMS derivatives.[3a] **8**: $[\alpha]_D^{25}$ −51.8 (c 1.0, $CHCl_3$). The absolute configuration was determined by comparing the optical rotation with that of an authentic sample prepared as per the reported procedure.[3a]
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