Chemoenzymatic Synthesis of *cis*-4-Hydroxy-D-proline

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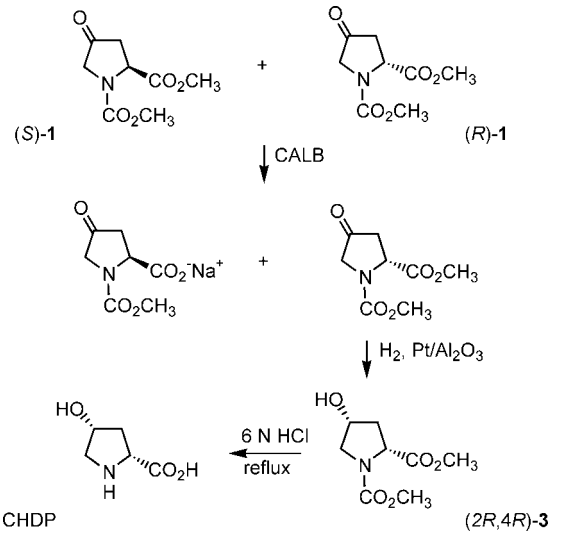
Abstract: *Candida antarctica* lipase fraction B (CALB) catalyzed the hydrolysis of the (*S*)-enantiomer of racemic 4-oxo-1,2-pyrrolidinedicarboxylic acid dimethyl ester with high enantioselectivity (> 99.5% ee at 51% conversion). Regioselective hydrogenation of the isolated (*R*)-4-oxo-1,2-pyrrolidinedicarboxylic acid dimethyl ester produced (2*R*,4*R*)-4-hydroxy-1,2-pyrrolidinedicarboxylic acid dimethyl ester in 98% yield, and subsequent hydrolysis of the ester and *N*-(alkoxycarbonyl) groups produced *cis*-4-hydroxy-D-proline in 98% yield and 96% de. Diastereomeric mixtures of 4-hydroxy-1,2-pyrrolidinedicarboxylic acid dimethyl esters were also resolved using CALB to produce *cis*-4-hydroxy-D-proline or *trans*-4-hydroxy-L-proline in 93 to > 99.5% diastereomeric excess.

Keywords: asymmetric synthesis; *Candida antarctica* lipase B; enzyme catalysis; *cis*-4-hydroxy-D-proline; *trans*-4-hydroxy-L-proline

cis-4-Hydroxy-D-proline (CHDP) is one of four 4-hydroxyproline diastereomers useful for the preparation of pharmaceuticals and agrochemicals,[1] and has been most conveniently prepared by the epimerization of *trans*-4-hydroxy-L-proline (THLP).[2] THLP is routinely isolated from hydrolyzed gelatin (12 – 13 wt % THLP) using a chromatographic separation which generates an excessive amount of waste relative to the amount of THLP produced.[2] More recently, THLP has been prepared by the fermentative oxidation of L-proline,[3] but this method uses a relatively-expensive starting material and produces only 4.1 wt % THLP in the fermentation broth.[3a] A chemoenzymatic synthesis of CHDP has now been developed which employs inexpensive starting materials, and which utilizes an enantiomeric resolution and a regioselective hydrogenation to produce CHDP in high yield and diastereomeric excess (Scheme 1).

Racemic 4-oxo-1,2-pyrrolidinedicarboxylic acid dimethyl ester (*R/S*)-**1** was prepared by reaction of *N*-(methoxycarbonyl)glycine methyl ester with fumaric acid dimethyl ester, followed by decarboxylation of the reaction product and subsequent re-esterification;[4] distillation and recrystallization from acetone gave ca. 99% pure (*R/S*)-**1**. The individual enantiomers (*R*)-**1** and (*S*)-**1** were prepared from CHDP or THLP, respectively, in three steps by esterification with methanol/thionyl chloride,[5] reaction of the resulting methyl ester with methyl chloroformate,[5] and subsequent oxidation of the 4-hydroxy group with sodium periodate/ruthenium trichloride.[6] Racemic 4-oxo-1,2-pyrrolidinedicarboxylic acid diethyl ester (*R/S*)-**2** was prepared by reacting *N*-(ethoxycarbonyl)glycine ethyl ester with fumaric acid diethyl ester.[4]



Scheme 1.

The use of enzymatic resolution to prepare enantiomerically pure α-amino acids has been previously reported.[7] Prior to screening enzymes for enantioselective hydrolysis of (*R/S*)-**1**, the substrate stability from pH 2 to 7 was determined. No change in concentration of 100 mM (*R/S*)-**1** was observed after 48 h in aqueous buffers at 25 °C and at a pH from 3 to 5 (acetate buffer), while a significant decrease in concentration occurred over this time at pH 6 (acetate buffer) or pH 7 (phosphate buffer). The rate of disappearance of (*R/S*)-**1** at > pH 6 increased with increasing (*R/S*)-**1** concentration, and no hydrolysis

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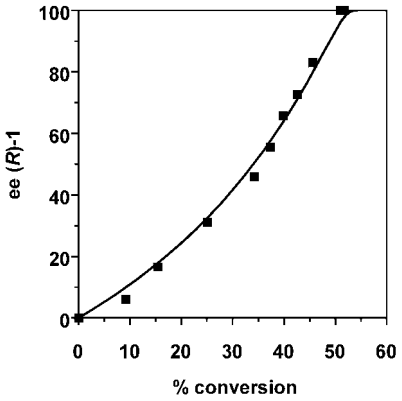


Figure 1. Enantiomeric excess of (*R*)-**1** versus conversion of (*R/S*)-**1** using *Candida antarctica* lipase B (■), and calculated E = 100 (line). Reaction conditions: 0.100 M (*R/S*)-**1** and 10 mg CALB/mL in 0.20 M sodium acetate (pH 5.0) at 25 °C.

products were observed, indicating possible base-catalyzed aldol condensation of substrate. Reactions were therefore run at pH 5 in acetate buffer to maximize substrate stability, enzyme activity, and reaction rate. Of the forty-three different commercial lipases, esterases, and proteases examined, only *Candida antarctica* lipase B[8] (CALB, Roche Molecular Biochemicals) was found to hydrolyze (*R/S*)-**1** with high enantioselectivity.[9] Mixing (*R/S*)-**1** (0.10 M in 0.20 M acetate buffer, pH 5.0) at 25 °C for 24 h with 10 mg/mL of CALB produced an $ee_{(substrate)}$ for *R*-**1** of > 99.5% at 51% conversion (Figure 1; E[10] = 100). The rate of enzymatic hydrolysis of 50 mM (*S*)-**1** alone was ca. 125% of the rate with 50 mM (*R*)-**1** also present, indicating the possible competitive inhibition of (*S*)-**1** hydrolysis by (*R*)-**1**.

The stability of 50 mM (*S*)-**1** after 6 h in 0.20 M acetate at pH 5.0 and at 15 °C to 45 °C was checked, and a 100% recovery was obtained at temperatures up to 35 °C, with 97% recovery at 45 °C. The recovered activity of CALB after 97 h at 35 °C and 45 °C was 95% and 71%, respectively. The effect of increasing (*R/S*)-**1** concentration and/or temperature on reaction rate and $ee_{(substrate)}$ was then determined (Table 1); increasing the reaction temperature resulted in high $ee_{(substrate)}$ over a range of (*R/S*)-1 concentrations. (*R*)-**1** was recovered from reaction mixtures in 99% isolated yield by extraction into dichloromethane; chemical or enzymatic racemization of hydrolysis product (*S*)-4-oxo-1,2-pyrrolidinedicarboxylic acid 1-methyl ester, followed by esterification, would allow recycling of the hydrolysis product back into the resolution step. The CALB-catalyzed hydrolysis of (*R/S*)-**2** (pH 5.0, 0.20 M acetate buffer) also resulted in an $ee_{(substrate)}$ for (*R*)-**2** of > 99.5% at 51% conversion, but the rate of hydrolysis of the (*S*)-enantiomer of (*R/S*)-**2** was ca. 25% of that of (*R/S*)-**1**, and a higher concentration of enzyme or lower concentration of substrate was required to achieve 50% conversion in 24 h.

Several additional 4-oxoproline derivatives were prepared and screened as substrates for enzyme-catalyzed resolution. Hydrolysis of racemic 1-benzoyl-4-oxoproline methyl ester[11] (10 mg/mL) by penicillin G amidase in aqueous suspension was examined at 25 °C and at pH 5.0 (0.2 M acetate), 6.0 (0.2 M succinate), and 7.0 (phosphate); no reaction was observed at pH 5.0, and at either pH 6.0 or 7.0 there was conversion of both (*R*)- and (*S*)-enantiomers with extremely low enantioselectivity for the (*S*)-enantiomer (E = 2). CALB hydrolyzed the (*S*)-enantiomer of this same racemate with only moderate enantioselectivity (55% ee at 38% conversion; E = 28). Racemic 1-acetyl-4-oxoproline methyl ester[12] and 4,4-dimethoxy-1,2-pyrrolidinedicarboxylic acid dimethyl ester [prepared by refluxing (*R/S*)-**1** in methanol] could not be resolved with high enantioselectivity using CALB, or any of the other commercially available lipases, esterases, or proteases examined.

Hydrogenation of (*R*)-**1** over platinum oxide did not produce (2*R*,4*R*)-4-hydroxy-1,2-pyrrolidinedicarboxylic acid dimethyl ester (2*R*,4*R*)-**3** in high yield, as was previously reported,[4] but instead gave (*R*)-1,2-pyrrolidinedicarboxylic acid dimethyl ester as the major product [from hydrogenolysis of the 4-hydroxy group of (2*R*,4*R*)-**3**]. Initial screening of hydrogenation catalysts and reaction conditions at 27 °C and 70 psig hydrogen indicated that 5% Pt/alumina (5 mg/mL) produced (2*R*,4*R*)-**3** with ca. 98.7% regioselectivity to the *cis*-isomer at 100% conversion of (*R*)-**1** (25 mg/mL) in ethyl acetate in 3 h; ca. 1% of the *trans*-isomer (2*R*,4*S*)-**3** was also produced. Ethyl acetate was preferred as solvent when compared to THF or methanol, resulting in fewer reaction by-products. 5% Pt/carbon produced significantly more

Table 1. Enantiomeric excess (ee) of (*R*)-**1** and conversion for the enzymatic resolution of (*R/S*)-**1** by CALB.[a]

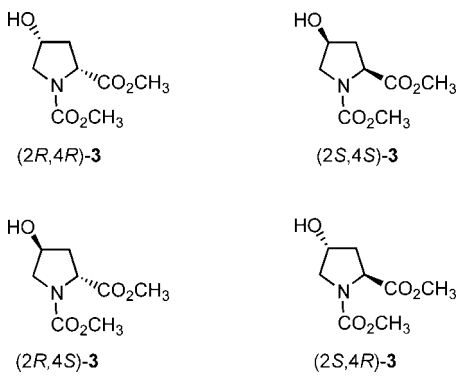
(*R/S*)-**1** (mM)	CALB (mg/mL)	Temperature (°C)	Time (h)	(*R/S*)-**1** conversion (%)	ee (*R*)-**1** (%)
100	20	15	24	51	>99.5
100	10	25	24	51	>99.5
200	6.7	35	29	51	97
100	20	45	6	50	>99.5
300	31	45	23	48	99

[a] Reactions performed in 0.20 M aqueous sodium acetate buffer (pH 5.0).

hydrogenolysis and more *trans*-isomer product than 5% Pt/alumina, while Raney nickel and 5% Ru/carbon had no activity at these low temperatures and pressures, and 5% Pd/carbon produced only 2% conversion in 3 h.

Additional screening of hydrogenation catalysts was also performed at higher temperatures (80 – 100 °C) and pressures (500 – 1000 psig hydrogen), where Raney nickel, supported Ni, and Ru/alumina have better activity. No significant hydrogenation of (*R*)-**1** (2.5 – 5 wt % in ethyl acetate) was achieved with any of the Raney Ni or supported Ni or Ru catalysts at higher pressures (500 psig) and/or temperatures (100 °C). With 5% Pt/alumina, the selectivity to (2*R*,4*R*)-**3** at 100% conversion of (*R*)-**1** decreased from 98.7% at 25 °C and 70 psig H_2 to 97.3% at 25 °C and 500 psig, and to 97.9% at 100 °C and 70 psig H_2. After isolation of (2*R*,4*R*)-**3** from a hydrogenation run for 18 h at 25 °C and 70 psig H_2, conversion to CHDP was simply performed by refluxing a 0.125 M solution of (2*R*,4*R*)-**3** in 6 N HCl for 16 h; the yields of CHDP and THLP were 98.0% and 2.0%, respectively, at 100% conversion.

The chemical synthesis of diastereomeric mixtures of 4-hydroxyproline derivatives from inexpensive achiral or chiral starting materials has also been reported.[13] Diastereomeric mixtures of the 4-hydroxy-1,2-pyrrolidinedicarboxylic acid dimethyl esters (2*R*,4*R*)-**3**, (2*R*,4*S*)-**3**, (2*S*,4*R*)-**3**, and (2*S*,4*S*)-**3** (Scheme 2) were therefore also screened against the same collection of commercial enzymes, and again only CALB was found to hydrolyze the methyl ester of one diastereomer in several pairs of diastereomers with > 95% diastereomeric excess [$de_{(substrate)}$]; these substrates were all stable at pH 7.0. Of six possible pairs of diastereomers of **3**, three could be resolved with >99.5% de at 50–52% conversion, and an additional two pairs could be resolved with a de of >99.5% or 93% at 57% conversion (Table 2). The ability to resolve several combinations of diastereomers with CALB was attributed to the large difference in the relative rates of methyl ester hydrolysis of each of the four diastereomers, according to the following order: (2*S*,4*R*)-**3** > (2*S*,4*S*)-**3** >> (2*R*,4*S*)-**3** >>> (2*R*,4*R*)-**3**, where no hydrolysis of (2*R*,4*R*)-**3** by CALB was observed. Because of the relatively slow rate of hydrolysis of (2*R*,4*S*)-**3** by CALB, no attempt was made to resolve diastereomeric mixtures of (2*R*,4*R*)-**3** and (2*R*,4*S*)-**3**, although such a resolution with high diastereomeric excess for (2*R*,4*S*)-**3** is theoretically possible if the concentration of CALB employed was significantly increased.



Scheme 2.

Experimental Section

Materials and Methods

CHDP, THLP, and 4-hydroxy- and 4-oxoproline derivatives were prepared according to literature procedures, or were purchased from Sigma or BACHEM. Absolute configurations for all substrates and products were determined by chiral gas chromatographic analysis using authentic materials of known configuration. All other chemicals, enzymes, and catalysts were purchased from commercial suppliers. A Chiraldex GTA GC column (Advanced Separation Technologies, 50 m × 0.32 mm ID) was used to determine ee of mixtures of (*R*)-**1** and (*S*)-**1**, and (*R*)-**2** and (*S*)-**2**, and de of mixtures of (*R*,*R*)-**3** and (*S*,*S*)-**3**, and (*S*,*R*)-**3** and (*R*,*S*)-**3**. Analyses of mixtures of *cis*- and *trans*-diastereomers of **3** and their hydrolysis products were performed by HPLC using a Bio-Rad HPX-87H column (30 cm × 7.8 mm diameter) and 0.005 N sulfuric acid as solvent, with RI detection or UV detection at 210 nm. Analysis of CHDP and THLP was performed using a Beckman Model 6300 amino acid analyzer with post-column ninhydrin derivatization.

Table 2. Diastereomeric excess (de) of remaining substrate and conversion for the enzymatic resolution of diastereomeric mixtures of **3** by CALB.[a]

1:1 mixture of diastereomers	CALB (mg/mL)	time (h)	conversion (%)	% de, substrate
(2*R*,4*R*)-**3**:(2*S*,4*R*)-**3**	2.1	24	50	>99.5%, (2*R*,4*R*)-**3**
(2*R*,4*R*)-**3**:(2*S*,4*S*)-**3**	50	47	52	>99.5%, (2*R*,4*R*)-**3**
(2*R*,4*S*)-**3**:(2*S*,4*R*)-**3**	4.0	24	52	>99.5%, (2*R*,4*S*)-**3**
(2*S*,4*S*)-**3**:(2*S*,4*R*)-**3**	3.0	24	57	>99.5%, (2*S*,4*S*)-**3**
(2*S*,4*S*)-**3**:(2*R*,4*S*)-**3**	50	24	57	93%, (2*R*,4*S*)-**3**

[a] Reactions performed at 25 °C in 0.10 M aqueous sodium/potassium phosphate (pH 7.0) containing 0.10 M of the diastereomeric mixture of **3**.

Enzymatic Resolution of (*R*/*S*)-1

An aqueous solution (5.0 mL) of 0.100 M (*R*/*S*)-**1** and 10 mg CALB/mL in 0.20 M sodium acetate (pH 5.0) was mixed at 25 °C. Samples (0.20 mL) were mixed with an equivalent volume of 20 mM hexadecane (GC internal standard) in dichloromethane for 3 minutes, which completely extracted unreacted (*R*/*S*)-**1**, and the extract was analyzed for conversion and $ee_{(substrate)}$ by chiral gas chromatography. After 24 h, the conversion of (*R*/*S*)-**1** was 51%, and $ee_{((R)\text{-}1)}$ was >99.5%. Repeating the reaction at a 200-mL scale using 6.6 mg CALB/mL, the conversion of (*R*/*S*)-**1** was 50% after 24 h. The reaction mixture was extracted with dichloromethane, and the product recovered by rotary evaporation of the extract to produce (*R*)-**1** in 99% isolated yield (99% purity, 97% ee).

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