

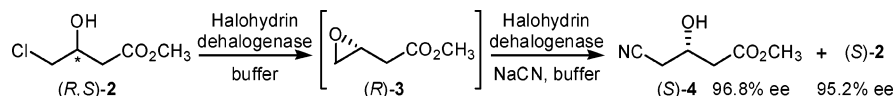
Sequential Kinetic Resolution Catalyzed
by Halohydrin DehalogenaseMaja Majerić Elenkov,[†] Lixia Tang,[†] Bernhard Hauer,[‡] and Dick B. Janssen^{*,†}

Biochemical Laboratory, Groningen Biomolecular Sciences and Biotechnology
Institute, University of Groningen, Nijenborgh 4, 9747 AG, Groningen,
The Netherlands, and BASF AG, Fine Chemicals & Biocatalysis Research,
Ludwigshafen, Germany

d.b.janssen@rug.nl

Received June 5, 2006

ABSTRACT



A sequential kinetic resolution catalyzed by halohydrin dehalogenase was employed for the synthesis of two valuable enantiopure building blocks. Resolution of methyl 4-chloro-3-hydroxybutanoate methyl ester ((R,S)-2) with use of a Trp249Phe mutant of halohydrin dehalogenase yielded methyl 4-cyano-3-hydroxybutanoate methyl ester ((S)-4) with 96.8% ee (40% yield) and (S)-2 with 95.2% ee (41% yield). This reaction is carried out in aqueous solution under mild conditions and provides access to a useful statin side-chain building block.

Halohydrin dehalogenases are bacterial enzymes that catalyze the conversion of vicinal halohydrins into their corresponding epoxides. Alternatively, they can catalyze the kinetic resolution of racemic epoxides by enantioselective ring opening, which provides a new biocatalytic strategy for the preparation of optically active compounds. In this nonnatural reaction, various nucleophiles are accepted, such as cyanide,^{1,2} azide,³ and nitrite,⁴ making it possible to obtain different enantiopure β -substituted alcohols. The enzyme from *Agrobacterium radiobacter* AD1 (HheC) is the best studied halohydrin dehalogenase. Its crystal structure and catalytic mechanism have been determined. Earlier, we showed that HheC is an efficient biocatalyst for the enantioselective and regioselective formation of β -azido alcohols³ and β -hydroxynitriles.² In this paper we explore the potential of halohydrin dehalogenase to catalyze the synthesis of optically pure methyl 4-cyano-3-hydroxybutanoate (4). This is a versatile building block containing three functional groups. The *R*-enantiomer

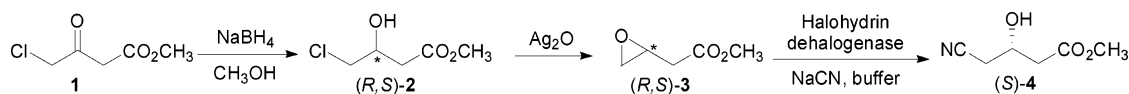
can be applied as an intermediate in the production of cholesterol-lowering drugs of the statin type. Various chemoenzymatic strategies have been developed for the synthesis of statin side-chain building blocks.⁵ Biocatalysts that are used include alcohol dehydrogenase,⁶ α -chymotrypsin,⁷ nitrilase,⁸ and aldolase.⁹

The route to cyanohydrin 4 that we report here is based on a sequential kinetic resolution of racemic 4-chloro-3-hydroxybutyric acid methyl ester ((R,S)-2).

Initially, the racemic epoxide ((R,S)-3) was tested as a substrate for a reaction with cyanide in the presence of wild-type halohydrin dehalogenase. The starting compound ((R,S)-3) was prepared from commercially available methyl 4-chloroacetoacetate (1). Reduction of 1 to ((R,S)-2) was followed by ring closure to the epoxide (Scheme 1). When ((R,S)-3) was converted by cyanide-mediated ring opening catalyzed by wild-type HheC, a moderate *E* value of 15 was found and the enantiopurity of the product was low (Table 1).

[†] University of Groningen.[‡] BASF AG, Fine Chemicals & Biocatalysis Research.(1) Nakamura, T.; Nagasawa, T.; Yu, F.; Watanabe, I.; Yamada, H. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 124–130.(2) Majerić Elenkov, M.; Hauer, B.; Janssen, D. B. *Adv. Synth. Catal.* **2006**, *348*, 579–585.(3) Lutje Spelberg, J. H.; van Hylckama Vlieg, J. E. T.; Tang, L.; Janssen, D. B.; Kellogg, R. M. *Org. Lett.* **2001**, *3*, 41–43.(4) Hasnaoui, G.; Lutje Spelberg, J. H.; de Vries, E.; Tang, L.; Hauer, B.; Janssen, D. B. *Tetrahedron: Asymmetry* **2005**, *16*, 1685–1692.(5) Müller, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 362–365.(6) Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Tetrahedron: Asymmetry* **2001**, *12*, 1713–1718.(7) Ohrlein, R.; Baisch, G. *Adv. Synth. Catal.* **2003**, *345*, 713–715.(8) DeSantis, G.; Zhu, Z.; Greenberg, W. A.; Wong, K.; Chaplin, J.; Hanson, S. R.; Farwell, B.; Nicholson, L. W.; Rand, C. L.; Weiner, D. P.; Robertson, D. E.; Burk, M. J. *J. Am. Chem. Soc.* **2002**, *124*, 9024–9025.(9) Gijzen, H. J. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 8422–8423.

Scheme 1. Preparation and Biocatalytic Conversion of 3,4-Epoxybutanoic Acid Methyl ester



The Trp249Phe mutant of HheC (HheC-W249F), obtained by site-directed mutagenesis,¹⁰ has improved properties such as a higher catalytic rate in halohydrin dehalogenation and enhanced enantioselectivity in nitrite-mediated epoxide ring opening.⁴ Residue Trp249 is positioned in the loop that forms the halide-binding site. The loop is stabilized by hydrogen bonds which connect the side chains of Asn176 and Tyr187 and of Tyr187 with Trp249. Trp249 is donated by the opposite subunit in the tetrameric protein and its replacement destabilizes the halide-binding site, leading to faster halide release and a higher rate. The Trp249Phe mutant showed significantly higher enantioselectivity and activity in the kinetic resolution of (*R,S*)-3 with cyanide than the wild-type enzyme (Table 1). The *E*-value of 60 that was found with the mutant is sufficiently high to be practically useful.

Table 1. Cyanide-Mediated Kinetic Resolution of (*R,S*)-3 Catalyzed by Halohydrin Dehalogenase^a

HheC	<i>t</i> (h)	conv (%)	ee 3 (%)	ee 4 (%)	<i>E</i>
WT	4	54	85 (<i>S</i>)	71 (<i>S</i>)	15
Trp249Phe	1.5	49	88 (<i>S</i>)	90 (<i>S</i>)	60

^a Reactions were carried out at room temperature in 10 mL of Tris-SO₄ buffer (0.2 M, pH 7.5) containing (*R,S*)-3 (5 mM), NaCN (15 mM), and 1 mg of purified enzyme.

Since the natural role of halohydrin dehalogenases is the dehalogenation of vicinal halohydrins with formation of epoxides, the possibility to use HheC for the ring closure of (*R,S*)-2 to prepare (*R,S*)-3 was subsequently considered (Scheme 2). When HheC-W249F was tested for conversion of (*R,S*)-2 in the absence of cyanide (Scheme 2, step a), conversion of (*R*)-2 to the epoxide appeared to be much faster than conversion of (*S*)-2, allowing a kinetic resolution (Figure 1). However, since this enzymatic reaction is reversible, the liberated Cl[−] opens the epoxide and as a consequence equilibrium was reached within 1 h, with formation of

racemic products (Figure 1). The reversibility of the HheC-catalyzed ring closure in general is a disadvantage for kinetic

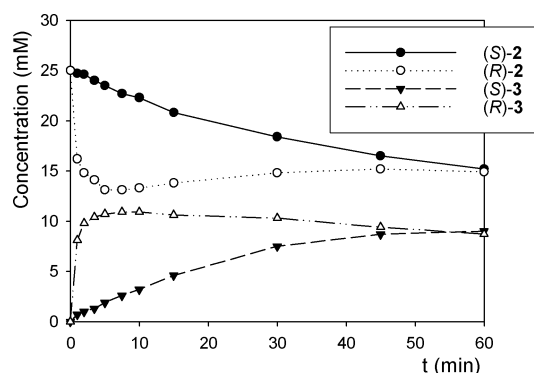
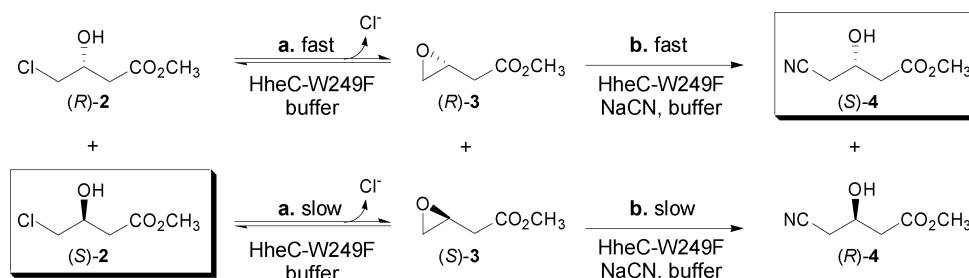


Figure 1. Progress curves for the ring closure of (*R,S*)-2 with the formation of (*R,S*)-3. The reaction was carried out under the following conditions: (*R,S*)-2 (0.11 mmol, 50 mM) in 2.2 mL of buffer (0.5 M Tris-SO₄, pH 7.5), 1 mg of purified enzyme (HheC-W249F).

resolutions because if reactions do not run to completion the enantiopurity of the remaining substrate will be low. This problem can be overcome by removing the produced chloride or epoxide. Trapping away the epoxide was tested earlier by using an epoxide hydrolase that converts the product to a diol, and this resulted in a high optical purity of the remaining halohydrin (>99% ee).¹¹ Here, we tested the ring-closure reaction of HheC in the presence of NaCN, allowing the HheC mutant itself to trap away the epoxide by catalyzing a successive step. This type of sequential resolution has the advantage that the enantioselectivity of the second step is amplified by the first.¹² The synthesis of epoxide (*R,S*)-3 can be avoided, and (*S*)-4 is prepared in the presence of cyanide in one pot starting from racemic (*R,S*)-2 (Scheme 2, steps a and b).

Scheme 2. Sequential Kinetic Resolution



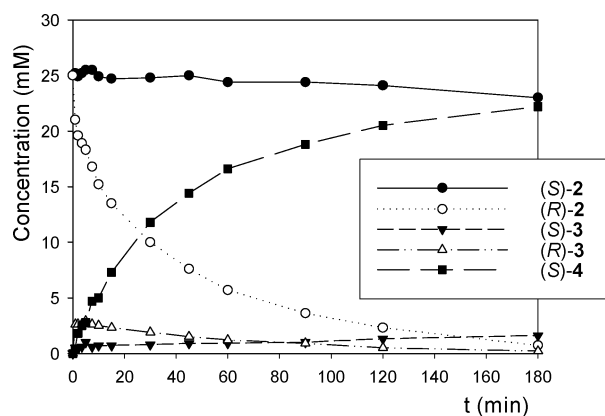


Figure 2. Progress curves for ring closure of (*R,S*)-**2** in the presence of NaCN with formation of (*S*)-**4**. The reaction was carried out under the following conditions: (*R,S*)-**2** (0.11 mmol, 50 mM), 2 equiv of NaCN in 2.2 mL of buffer (0.5 M Tris- SO_4 , pH 7.5), 1 mg of purified enzyme (HheC-W249F).

When this reaction was performed, conversion of (*R,S*)-**2** was very fast, with rapid formation of (*S*)-**4** (Figure 2). Only a low amount of (*R,S*)-**3** transiently accumulated. Since the intermediate epoxide (*R*)-**3** was efficiently and irreversibly converted by the enzyme to (*S*)-**4**, the conversion of (*R*)-**2** was drawn to completion. The other substrate enantiomer, (*S*)-**2**, remained unconverted and from the reaction mixture both highly enantiomerically enriched products (*S*)-**2** and (*S*)-**4** could easily be isolated and separated by column chromatography.

(10) Tang, L.; Torres Pazmiño, D. E.; Fraaije, M. W.; de Jong, R. M.; Dijkstra, B. W.; Janssen, D. B. *Biochemistry* **2005**, *44*, 6609–6618.

(11) Lutje Spelberg, J. H.; van Hylckama Vlieg, J. E. T.; Bosma, T.; Kellogg, R. M.; Janssen, D. B. *Tetrahedron: Asymmetry* **1999**, *10*, 2863–2870.

(12) Caron, G.; Kazlauskas, R. J. *J. Org. Chem.* **1991**, *56*, 7251–7256.

(13) Davis, S. C.; Grate, J. H.; Gray, D. R.; Gruber, J. M.; Huisman, G. W.; Ma, S. K.; Newman, L. M.; Sheldon, R.; Wang, L. A. PCT WO 2004/015132 A2.

To further test the applicability of this reaction, it was performed on a 0.5 g scale (50 mM (*R,S*)-**2**, 2 equiv of NaCN, 15 mg of HheC-W249F, 22 °C). After 5 h, (*S*)-**4** was isolated in 40% yield (190 mg), 96.8% ee, and (*S*)-**2** was obtained in 41% yield (208 mg), 95.2% ee. The enzyme tolerates substrate concentrations exceeding 50 mM, and conversion of 200 mM (*R,S*)-**2** with the HheC mutant yielded (*S*)-**4** of 93% ee at the same extent of conversion.

In conclusion, we have shown that halohydrin dehalogenase catalyses a sequential kinetic resolution that can be applied to the synthesis of useful chiral building blocks. This procedure provides an attractive method for generating two desired compounds (*S*)-**2** and (*S*)-**4** in good yield (81% total yield from the kinetic resolution) and highly enantioenriched form (>95% ee). Recently, a similar conversion route toward **4** was described by Davis et al.¹³ wherein halohydrin dehalogenase activity was used for transformation of optically pure ethyl (*R*)-4-chloro-3-hydroxybutanoate ester to ethyl (*S*)-4-cyano-3-hydroxybutanoate ester via an epoxide intermediate. Here, we describe an alternative for this conversion employing a mutant with improved catalytic properties and enantioselectivity, using a racemic substrate as starting compound. By sequential kinetic resolutions the product ee was improved (ee from ca. 90% to ca. 97%) at complete conversion. The results also emphasize the versatility of this relatively unexplored biocatalyst¹⁴ in the kinetic resolution of *vic*-halohydrins, enantioselective epoxide ring opening, dynamic kinetic resolution, and, as described here, tandem ring closure and ring opening reactions with different nucleophiles.

Supporting Information Available: Biocatalytic procedures, analytical methods, and chemical synthesis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL061369H

(14) Janssen, D. B.; Majeric Elenkov, M.; Hasnaoui, G.; Hauer, B.; Lutje Spelberg, J. H. *Biochem. Soc. Trans.* **2006**, *34*, 291–295.