ORGANIC LETTERS

2001
Vol. 3, No. 1
41−43

Highly Enantioselective and Regioselective Biocatalytic Azidolysis of Aromatic Epoxides

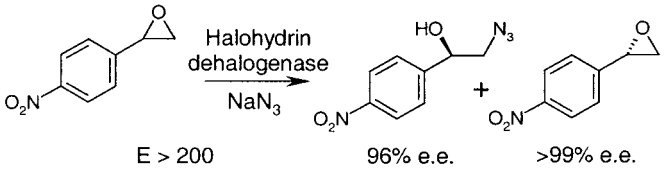
Jeffrey H. Lutje Spelberg,[†,‡] Johan E. T. van Hylckama Vlieg,[†] Lixia Tang,[†] Dick B. Janssen,*[,†] and Richard M. Kellogg[‡,§]

Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, and Department of Organic and Molecular Inorganic Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

d.b.janssen@chem.rug.nl

Received October 19, 2000

ABSTRACT



The halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 catalyzed the highly enantioselective and *β*-regioselective azidolysis of (substituted) styrene oxides. By means of kinetic resolutions the remaining epoxide and the formed azido alcohol could be obtained in very high ee. In a large scale conversion, the decrease in yield and selectivity due to the uncatalyzed chemical side reaction could be overcome by slow addition of azide.

Chiral nonracemic 1,2-azido alcohols are precursors for a wide variety of 1,2-amino alcohols as well as useful intermediates in carbohydrate chemistry.[1] A common method for the synthesis of an 1,2-azido alcohol is ring opening of the corresponding epoxide whereby, if the epoxide is unsymmetrical, azidolysis occurs via a bimolecular reaction at the least substituted carbon atom.[2] Aryl epoxides such as styrene oxide form an exception, because the phenyl group stabilizes the formation of a positive charge at the benzylic carbon atom (C_α) in the transition state, favoring bimolecular attack at this position.[3] Variation of the metal counterion of azide or addition of a catalytic amount of an organometallic reagent increases the rate of azidolysis, but noteworthy change of regioselectivity toward the less substituted *β*-carbon atom is not observed.[2,4] A substantial increase in the *β*-selectivity of the azidolysis of styrene oxide can be obtained with LiN_3/HMPA (94% *β*-attack)[5] and with LiN_3 in the presence of *β*-cyclodextrin.[6] In the latter case a partial kinetic resolution leads to 1-phenyl-2-azido-ethanol, enantiomerically enriched to a low extent. Enantioselective azidolysis of *meso*-epoxides and various terminal epoxides using chiral salen complexes has been described by the group of Jacobsen, but the regioselectivity of ring opening of styrene oxide is obscured by decomposition of the products.[7,8]

Recently we described the use of the halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 to obtain optically active epoxides and halohydrins such as (*S*)-2,3-

† Groningen Biomolecular Sciences & Biotechnology Institute.
‡ Department of Organic and Molecular Inorganic Chemistry.
§ Syncom BV, Kadijk 3, 9747 AT Groningen, The Netherlands.

(1) *The Chemistry of the Azido Group*; Patai, S., Ed.; Wiley: New York, 1971.
(2) Chini, M.; Crotti, P.; Macchia F. *Tetrahedron Lett.* **1990**, *31*, 5641.
(3) Blumenstein, J. J.; Ukachukwu, V. C.; Mohan, R. S.; Whalen, D. L. *J. Org. Chem.* **1993**, *58*, 924.
(4) (a) Saito, S.; Yamashita, S.; Nishikawa, T.; Yokoyama, Y.; Inaba, M.; Moriwake, T. *Tetrahedron Lett.* **1989**, *30*, 4153 (b) Leung, W.; Chow, E. K. F.; Wu, M.; Kum, P. W. Y.; Yeung, L. *Tetrahedron Lett.* **1995**, *36*, 107.
(5) Guy, A.; Dubuffet, T.; Doussot, J.; Godefroy-Falguieres, A. *Synlett* **1991**, 403.
(6) Guy, A.; Doussot, J.; Garreau, R.; Godefroy-Falguieres, A. *Tetrahedron: Asymmetry* **1992**, *3*, 247.
(7) Martinez, L. E.; Leighton, J. L.; Carsten, D. H.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1995**, *117*, 5897.
(8) Farrow, J. F.; Schaus, S. E.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1996**, *118*, 7420.

10.1021/ol0067540 CCC: $20.00
Published on Web 12/13/2000

dichloro-1-propanol ($E > 100$) and (*S*)-2-chloro-1-phenylethanol ($E = 73$) by means of kinetic resolution of halohydrins.[9] Here we show that azide can be used as a nucleophile in the reverse reaction. Furthermore, this enzyme-catalyzed azidolysis is both highly enantioselective and β-regioselective toward (substituted) styrene oxides.

Using the chromogenic substrate *p*-nitrostyrene oxide (*R*)-**1a**, we determined the equilibrium constant (K_{eq}) of the reversible reaction catalyzed by the halohydrin dehalogenase with a variety of halides and nucleophiles. The nucleophilic ring opening of *p*-nitrostyrene oxide leads to a decrease in the extinction coefficient at 310 nm, which made it possible to monitor the enzymatic ring opening online by the decrease of absorbance. Using chiral HPLC, it was established that the enzymatic ring opening is highly regioselective toward the β-carbon atom. With chloride and bromide the equilibrium is predominantly on the side of the epoxide (Table 1).

Table 1. Equilibrium Constants of the Reaction of Various Sodium Salts with (*R*)-**1a** by the Halohydrin Dehalogenase from *Agrobacterium radiobacter* AD1

sodium salt	K_{eq} (mM)[a]
NaBr	480
NaCl	40
NaF	no reaction
NaN_3	<0.03[b]

[a] The equilibrium constant is defined as:

$$K_{eq} = \frac{[\text{NaX}] \cdot [(R)\text{-}\mathbf{1a}]}{[(R)\text{-alcohol}]}$$

[b] The exact equilibrium constant was too low to be determined using the chromogenic substrate.

This limits a practical application of this reaction since a large excess of halide would be necessary to favor the formation of the halohydrin over the epoxide. With azide as nucleophile, the equilibrium lies on the side of the product of ring opening, although the exact equilibrium constant was too small to be determined accurately using the chromogenic substrate. This implies that a total enzymatic conversion of the epoxide to the azido alcohol is possible with only a small excess of sodium azide.

The recombinant halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 catalyzed the highly enantioselective and regioselective azidolysis of substituted styrene oxides (Table 2). The enzyme converted *p*-nitrostyrene oxide **1a** to the corresponding azido alcohol **1b** with high regioselectivity and enantioselectivity. The remaining (*S*)-**1a** was obtained with an ee > 99% and the azido alcohol (*R*)-**1b** was formed with an ee of 96%. The corresponding *E*-value was calculated to be higher than 200 either from the ee's of the epoxide and the azido alcohol or by using conversion and ee of the epoxide.[10] The initial activity with (*R*)-**1a** at a substrate concentration of 1 mM was 180 mU mg^{-1}. Even at initial substrate concentrations higher than 1 mM the curve showed first order kinetics, indicating a considerably higher K_m value of the substrate. The initial activity of (*S*)-**1a** was too low to be measured (<1 mU mg^{-1}). The epoxides **2a** and **3a** were also converted with high enantioselectivity leading to optically pure epoxides (ee > 99%) and the almost optically (98% ee) pure azido alcohols **2b** and **3b**. A complication is chemical azidolysis leading mainly to the azido alcohols **2c** and **3c**. The intrinsic *E*-value (Table 2, ee_s, ee_p) of the enzymatic resolutions based on the ee's of **a** and **b** was higher than 200, but the apparent *E*-value (conv, ee_s), which takes the unwanted chemical conversion into account, was considerably lower.

The high β-regioselectivity of the enzyme-catalyzed reaction is striking and is, as mentioned, opposite to the observed selectivity in the non-catalyzed azide ion ring opening. For the kinetic resolution of **2a**, β-attack took place to the extent of 89% at 55% conversion compared to chemical azidolysis, which involves only 3% reaction at the β-position. During the enzymatic reaction the *apparent* selectivity percentage is continuously lowered as a result of the chemical side reaction. When the reaction is performed with an excess of enzyme, the initial β-regioselectivity for all substrates was higher than 98%, indicating an almost absolute opposite regioselectivity compared to that of the chemical reaction.

Would the chemical azidolysis leading to product **c** derail a practical application of this halohydrin dehalogenase as a biocatalyst for large scale conversions? To determine the optimal conditions for a large scale synthesis, the influence of increasing azide concentrations on the conversion of **1a** to **1b** was investigated by monitoring the initial activities (concentration **1a**, 250 μM) on-line at 310 nm. The apparent K_m value for azide was determined to be 0.2 mM, from which we may expect that above a concentration of approximately 0.5 mM the enzymatic activity will become independent of the azide concentration. If in a kinetic resolution, azide is present in a higher concentration, only an increase in the disadvantageous chemical azidolysis will be observed. To circumvent the problem of excessive volumes owing to the low solubility (3 mM) of **1a** we added the substrate (0.47 g) as a second solid phase to 60 mL of buffer (MOPS, pH = 7.0) containing 29 mg of purified enzyme. The amount of substrate was 7.8 g/L, which is 17-fold the solubility. Sodium azide (0.6 molar equiv) was slowly added over 24 h keeping the azide concentration around 0.5−1 mM.

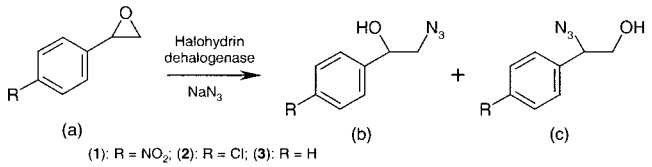
After isolation, the yields and ee's of the compounds present in the mixture were determined using chiral HPLC. The isolated product consisted of (*S*)-**1a** in 46% yield (98% ee) and **(***R***)-1b** in 47% yield (97% ee). Compound **1c**, the result of the nonenzymatic reaction, formed a total of 4% of the reaction mixture, and the product of chemical hydrolysis of the epoxide, *p*-nitrophenylethanediol was present in 3% yield.

In conclusion, we describe here for the first time a highly enantioselective ($E > 200$) and β-regioselective azidolysis of (substituted) styrene oxides using the halohydrin de-

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

(10) Straathof, A. J. J.; Jongejan, J. A. *Enzyme Microb. Technol.* **1997**, *21*, 559.

Table 2. Kinetic Resolution of Epoxides **1a**, **2a**, and **3a** by the Recombinant Halohydrin Dehalogenase from *Agrobacterium radiobacter* AD1



epoxide	conv **a**[a] (%)	ee **a** (%)	ee **b** (%)	*E*-value: ee_s, ee_p	*E*-value: conv, ee_s	β-attack[b] (%)	init. activity[c] (mU mg^{-1})	k_{az}[d] (M^{-1} s^{-1})
1a	51	>99 (*S*)	96 (*R*)	>200	>200	98 (37)	180	4.3×10^{-4}
2a	55	>99 (*S*)	98 (*R*)	>200	51	89 (3)	640	2.2×10^{-3}
3a	64	>99 (*S*)	98 (*R*)	>200	15	79 (2)	190	2.9×10^{-3}

[a] Substrate conc. 2 mM in Tris-SO_4 (50 mM, pH = 7.3, 3 mM mercaptoethanol, 22 °C); NaN_3 concn 1.3 mM. [b] Value between parentheses is the % β-attack of the chemical conversion. [c] Initial activity of (*R*)-enantiomer in kinetic resolution; 1 mU equals 1 nmol min^{-1}. [d] Bimolecular reaction constant of chemical azidolysis of the epoxide.

halogenase from *Agrobacterium radiobacter* AD1. This selectivity appears to be limited to the enzyme from the above-mentioned organism. We have also examined two distinct halohydrin dehalogenases from other organisms in this investigation.[11] The recombinant halohydrin dehalogenases from *Mycobacterium* sp. GP1 and *Arthrobacter* sp. AD2 also catalyzed the azidolysis of **1a**, but the *E*-value with both enzymes was lower than 5.

This method provides at low azide concentration a suitable synthesis of an optically pure aromatic β-azido alcohol under the very mild conditions of room temperature, aqueous medium, and neutral pH. Although **a** and **b** could be obtained in a very high ee, an intrinsic limitation of a kinetic resolution is the maximum yield of 50% for both remaining substrate and formed product. It gives direct access to optically active 2-amino-1-arylethanols, which are building blocks for a wide range of pharmaceutical products, via the inexpensive and easily accessible racemic epoxides. Recently, the gene encoding the halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 has been cloned and the enzyme has been brought to overexpression.[11] This makes the enzyme available in multigram quantities, and thus an application on an industrially interesting scale may become feasible.

Acknowledgment. This research was financially supported by the Innovation Oriented Research Program (IOP) on Catalysis (no. 94007a) of the Dutch Ministry of Economic Affairs.

Supporting Information Available: Experimental procedures and characterizations of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0067540

(11) van Hylckama Vlieg, J. E. T.; Tang, L.; Lutje Spelberg, J. H.; Smilda, T.; Poelarends, G. J.; Bosma, T.; van Merode, A. E. J.; Fraaije, M. W.; Janssen, D. B. Submitted for publication.