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Enzymatic dynamic kinetic resolution of epihalohydrins

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Abstract—The haloalcohol dehalogenase from *Agrobacterium radiobacter* AD1 catalyses the reversible ring closure of vicinal haloalcohols to produce epoxides and halides. In the ring opening of epoxides, nonhalide nucleophiles such as N_3^- are accepted. The enantioselective irreversible ring opening of an epihalohydrin by N_3^-, combined with racemisation caused by a reversible ring opening by a halide, resulted in an enzymatic dynamic kinetic resolution yielding optically active (*S*)-1-azido-3-halo-2-propanol. With epichlorohydrin as a substrate, the rate of ring opening by N_3^- was higher than the rate of racemisation, resulting in a mixed kinetic resolution and dynamic kinetic resolution. With epibromohydrin as the substrate, the racemisation rate was higher than the rate of ring opening, resulting in an efficient dynamic kinetic resolution. By optimising the pH of the medium and the concentrations of N_3^- and Br^-, the product (*S*)-1-azido-3-bromo-2-propanol could be obtained in 84% yield and 94% ee. An (*R*)-enantiomer selective ring closure of this bromoalcohol, catalysed by the same enzyme, caused a simultaneously occurring kinetic resolution, yielding when the conversion progressed, an increase in enantiopurity of (*S*)-1-azido-3-bromo-2-propanol to >99% ee with a yield of 77%. This compound and the ring-closed product glycidyl azide can be used as chiral synthetic building blocks.

1. Introduction

Kinetic resolution of racemic mixtures of chiral compounds is a useful method for obtaining enantiomerically pure compounds, but suffers from the drawbacks that the maximum yield is only 50% of the starting material and that labourious separation of an optically active substrate and product is required. This limitation can be overcome via a dynamic kinetic resolution process, in which the slower reacting enantiomer racemises during the conversion.[1] This process can theoretically result in a single product enantiomer with 100% yield. Several processes have been described in which the kinetic resolution and/or in situ racemisation is catalysed by an enzyme.[2]

Recently, the biocatalytic potential of the epoxide hydrolase and haloalcohol dehalogenase produced by the microorganism *Agrobacterium radiobacter* AD1 has been investigated. The epoxide hydrolase (EchA) was used to obtain a variety of enantiomerically pure epoxides by kinetic resolution.[3] The haloalcohol dehalogenase (HheC) catalyses the reversible enantioselective ring closure of aliphatic and aromatic vicinal haloalcohols, yielding optically active haloalcohols and epoxides.[4] Haloalcohols are direct precursors of epoxides since ring closure of a haloalcohol yields an epoxide with retention of configuration. Recently it has been shown that HheC catalyses the highly enantioselective ring opening of *para*-nitrostyrene oxide by N_3^-, CN^- and NO_2^-.[5]

Epihalohydrins are widely used as intermediates in the preparation of various products such as resins, polymers, adhesives and pharmaceuticals.[6] The carbon atom bearing the halide and especially the oxirane carbon atoms are highly reactive. Generally, the nucleophilic ring opening of an epihalohydrin is directed towards the terminal position of the epoxide, yielding the corresponding 3-substituted 1-halo-2-propanol. Ring closure of this haloalcohol yields hydrogen halide and an epoxide that can undergo another nucleophilic attack and thus serve as a useful synthon. Enantiomerically pure epichlorohydrin was used as a building block for biologically active compounds such as (*S*)-atenolol and (*R*)-carnitine.[6] In the preparation of these compounds, the epoxide ring of enantiopure epichlorohydrin is opened by a nucleophile. An attractive nucleophile for epoxide ring opening is an azide, which in turn affords

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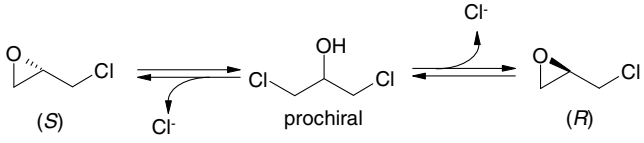
an azidoalcohol. Optically active azidoalcohols have been prepared using various biocatalytic methods such as lipase-catalysed esterification[7,8] and microbial reduction of azidoketones.[9] The combination of a lipase-catalysed enzymatic resolution of azidoalcohols with ruthenium-catalysed racemisation resulted in a dynamic kinetic resolution process in which the corresponding azide substituted acetate esters were obtained in high yields and high enantiomeric excesses.[10] Enantiomerically pure azidoalcohols can be used as precursors for aminoalcohols and aziridines. Several chiral aminoalcohols such as 2-amino-1-phenylethanols are biologically active and have been used as building blocks for pharmaceutical products or product candidates.

A requirement for a dynamic kinetic resolution is rapid in situ racemisation of the substrate. Previous work described the enzymatic racemisation of epichlorohydrin, which was produced during the enantioselective conversion of 2,3-dichloro-1-propanol.[4] The β-regioselective cleavage of epichlorohydrin by a chloride, yielded 1,3-dichloro-2-propanol. Ring closure of this prochiral compound produced racemic epichlorohydrin, causing racemisation to occur (Scheme 1).



Scheme 1. Racemisation of epichlorohydrin.

An obvious requirement for a dynamic kinetic resolution process is an irreversible enantioselective ring opening of the epoxide. Previous research has shown that the HheC-catalysed azidolysis of substituted styrene oxides is highly enantioselective and β-regioselective, yielding the corresponding (*R*)-azidoalcohols.[11] The combination of the racemisation of epihalohydrin and the enantioselective irreversible ring opening by N_3^-, yielding one enantiomer of the 1-azido-3-halo-2-propanol, is the concept of the dynamic kinetic resolution that is reported herein. This 1-azido-3-halo-2-propanol and glycidyl azide, the ring-closed product thereof, have been used as synthetic building blocks.[12,13]

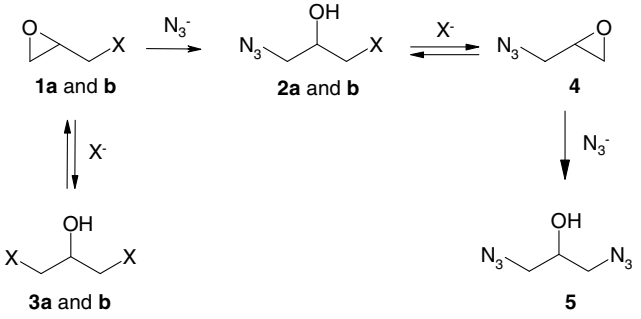
Four epoxides can be suitable substrates for the above proposed principle: epifluorohydrin, epichlorohydrin, epibromohydrin and epiiodohydrin. Alkyl iodides have a high chemical reactivity, making the occurrence of various unwanted side-reactions likely. HheC shows no activity towards vicinal fluoroalcohols. As a consequence, the epoxides epifluorohydrin and epiiodohydrin were considered to be unfavourable substrates for investigating the possibility of a dynamic kinetic resolution. HheC catalyses the conversion of a wide variety of chloroalcohols and bromoalcohols. These haloalcohols are generally reasonably stable in a neutral aqueous environment. Epichlorohydrin and epibromohydrin are the most suitable substrates for investigating the above-proposed dynamic kinetic resolution. The goal was to achieve complete conversion of an epihalohydrin in order to obtain the corresponding 1-azido-3-halo-2-propanol in high enantiomeric purity and yield.

2. Results and discussion

2.1. Dynamic kinetic resolution with epichlorohydrin and N_3^-

Epichlorohydrin was chosen as the substrate to investigate the dynamic kinetic resolution process. The separate enantiomers of this epoxide are commercially available, making it feasible to determine several aspects of the reaction, such as the rate of racemisation and ring opening. The reactions that can occur starting from an epihalohydrin and N_3^- are depicted in Scheme 2. In theory, all these reactions can be catalysed by a haloalcohol dehalogenase. The ring opening of epihalohydrin **1** by N_3^- yields 1-azido-3-halo-2-propanol **2**, which can again be used as a substrate for the enzyme since a vicinal haloalcohol moiety is present. Ring closure of **2** results in the formation of glycidyl azide **4** and a halide (X^-). Ring opening of epoxide **4** by N_3^- results in the formation of the diazido product **5**.

The reaction steps shown in Scheme 2 can be influenced by several factors such as temperature, pH, product inhibition and the concentrations of organic substrates and nucleophiles. The pH of the reaction medium influences the rate of enzyme-catalysed ring-closure and ring-opening reaction and the position of the equilibrium between these reactions. The rate of chemical ring opening of epoxides by a nucleophilic compound is also pH-dependent.[14,15] The chemical hydrolysis of epichlorohydrin **1a** to yield 3-chloro-1,2-propanediol could trigger the unwanted enzyme-catalysed formation of glycidol and, in the presence of N_3^-, 3-azido-1,2-propanediol. To determine suitable reaction conditions, the influence of the pH on all aspects of the dynamic kinetic resolution process was investigated.



Scheme 2. Summary of possible reactions catalysed by a haloalcohol dehalogenase starting from epihalohydrin and N_3^- (a) X = Cl, (b) X = Br.

No noteworthy chemical hydrolysis of epichlorohydrin **1a** was observed between pH 4.5 and 7.5 (<3% h^{-1}). At pH 8.5 (6% h^{-1}) and especially pH 9.5 (45% h^{-1}),

chemical hydrolysis was significant. The rate of HheC-catalysed ring opening of epichlorohydrin (*S*)-**1a** by N_3^- displayed an optimum lower than pH 6. The high rate of the ring-opening reaction is remarkable. The maximum activity (470 μmol min^{-1} mg^{-1}) at pH 5 was more than 20-fold higher than the observed rate of ring closure of 1,3-dichloro-2-propanol (at pH 7.5). The rate of HheC-mediated ring closure of 1,3-dichloro-2-propanol **3a** was highest around pH 8 (Fig. 1). The latter compound was considered to be an optimal substrate for the HheC.[16] The ring opening of epichlorohydrin was more than a factor of 2000 higher than the earlier described ring opening of styrene oxide.[5]

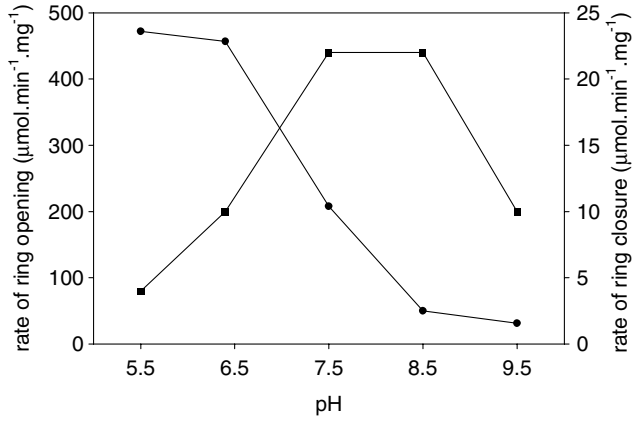


Figure 1. Effect of pH on the initial rate of ring opening by N_3^- (-●-) and ring closure (-■-) reactions catalysed by HheC. Incubations contained: ring opening, 20 mM (*S*)-epichlorohydrin **1a** and 30 mM NaN_3; ring closure, 5 mM 1,3-dichloro-2-propanol **3a**.

The rate of racemisation was determined both by the conversion of epihalohydrin **1** to 1,3-dihalo-2-propanol **3** and the reverse reaction. For an efficient dynamic kinetic resolution to take place, racemisation of the substrate **1** must occur faster than the ring opening by N_3^-. If this is the case, the enantiomeric purity of remaining epihalohydrin will remain low and that of the formed 1-azido-3-halo-2-propanol **2** (ee_p) will be constant throughout the course of the reaction. In such an ideal dynamic kinetic resolution, the enantiomeric purity of the desired product is determined by the *E*-value of the kinetic resolution (Eq. 1).

$$ee_p = \frac{(E-1)}{(E+1)} * 100\% \qquad (1)$$

The enzyme-catalysed racemisation of 10 mM (*R*)-**1a** was measured in the presence of a catalytic amount (0.1 M equiv) of Cl^- (Fig. 2). At pH 4.5, no racemisation occurred, since the rate of ring closure at this pH was very low. Between pH 6.5 and 8.5 fast racemisation of (*R*)-**1a** occurred, resulting in almost racemic epichlorohydrin (ee <10%) within 30 min.

The HheC-catalysed dynamic kinetic resolution, based on the combination of the above-described ring opening and the racemisation of **1a**, was investigated at various pH-values. The progress of the reaction between racemic epichlorohydrin **1a** and N_3^- was monitored by periodically taking samples from the reaction mixture and determining the enantiomeric excess of substrate **1a** and product **2a** using chiral gas chromatography.

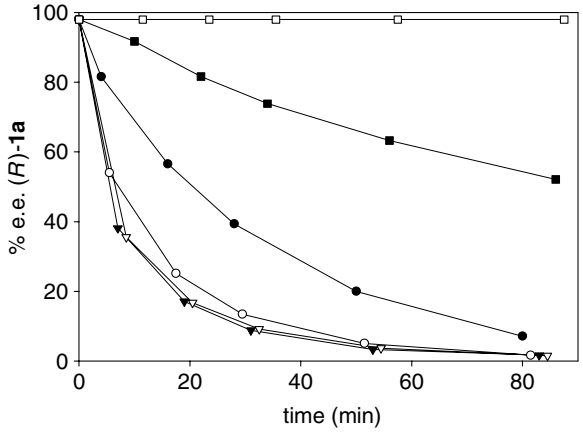


Figure 2. Effect of pH on the chloride-mediated racemisation of (*R*)-epichlorohydrin **1a** catalysed by HheC. Incubations contained 10 mM (*R*)-epichlorohydrin **1a**, 1 mM NaCl and 0.7 μM enzyme. Symbols: (-□-), pH 4.5; (-■-), pH 5.5; (-▽-), pH 6.5; (-▼-), pH 7.5; (-○-), pH 8.5; (-●-), pH 9.5.

The ring opening of epichlorohydrin by N_3^-, catalysed by HheC, was selective towards (*S*)-epichlorohydrin, yielding (*S*)-1-azido-3-chloro-2-propanol as the main product. At pH 4.5 a (nondynamic) kinetic resolution occurred, resulting in an increase in the enantiomeric purity of the remaining (*R*)-**1a** to higher than 99% ee (Fig. 3). The enantiomeric purity of product **2a** gradually decreased during the conversion. An E-value of 21 was calculated using conversion and enantiomeric excess of the remaining substrate **1a**.[17] At pH 5.5 and higher, the enantiomeric excess of (*R*)-**1a** initially, temporarily increased to a maximum at around 50% substrate conversion (Fig. 3A). The maximum transient enantiomeric purity of (*R*)-**1a** decreased from around 90% ee at pH 5.5 to less than 20% ee at pH 8.5 indicating that the kinetic resolution became more dynamic at higher pH-values. After the initial increase, the enantiomeric excess of (*R*)-**1a** decreased again, indicating that the relative rate of racemisation increased.

The above results indicate that at a pH lower than 8.5 the rate of epoxide racemisation was lower than the rate of ring opening, resulting in a mixed kinetic and dynamic kinetic resolution. At pH 8.5, the enantiomeric purity of the epoxide **1a** remained below 20%, indicating predominantly a dynamic kinetic resolution. However, at this pH the yield of the desired product (*S*)-**2a** was less than 20% at complete conversion of epichlorohydrin **1a**. This was caused by the ring closure of 1-azido-3-chloro-2-propanol **2a** to glycidyl azide **4**, which proceeded faster at increasing pH values.

2.2. Dynamic kinetic resolution with epibromohydrin and N_3^-

The dynamic kinetic resolution with epibromohydrin **1b** was investigated between pH 4.5 and 8.5. Identical to the

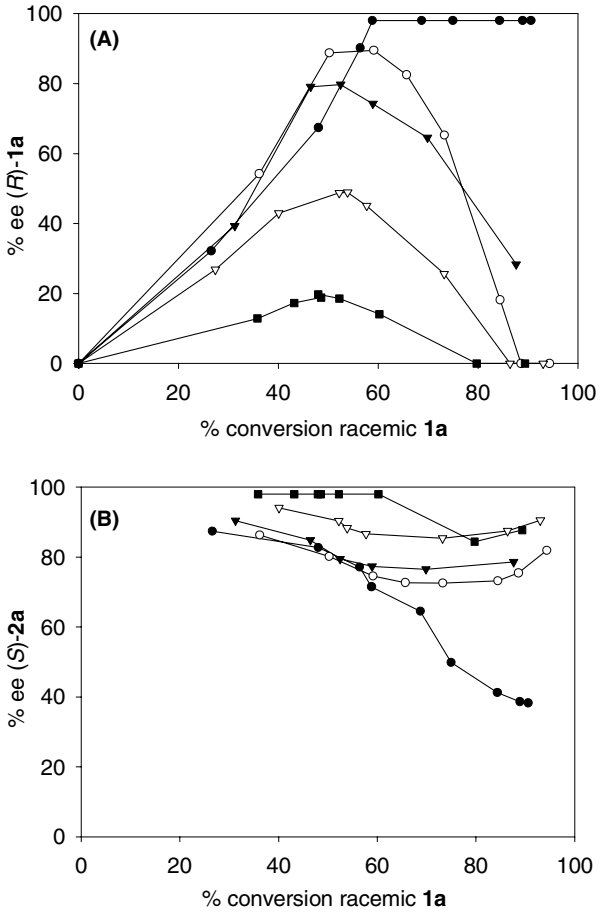


Figure 3. Effect of pH on enantiomeric purity of substrate (**A**) and product (**B**) during the HheC catalysed ring opening of epichlorohydrin **1a** by N_3^-. Incubations contained: 20 mM racemic epichlorohydrin **1a** and 30 mM NaN_3. Symbols: (-●-), pH 4.5; (-○-), pH 5.5; (-▼-) pH 6.5; (-▽-), pH 7.5; (-■-), pH 8.5.

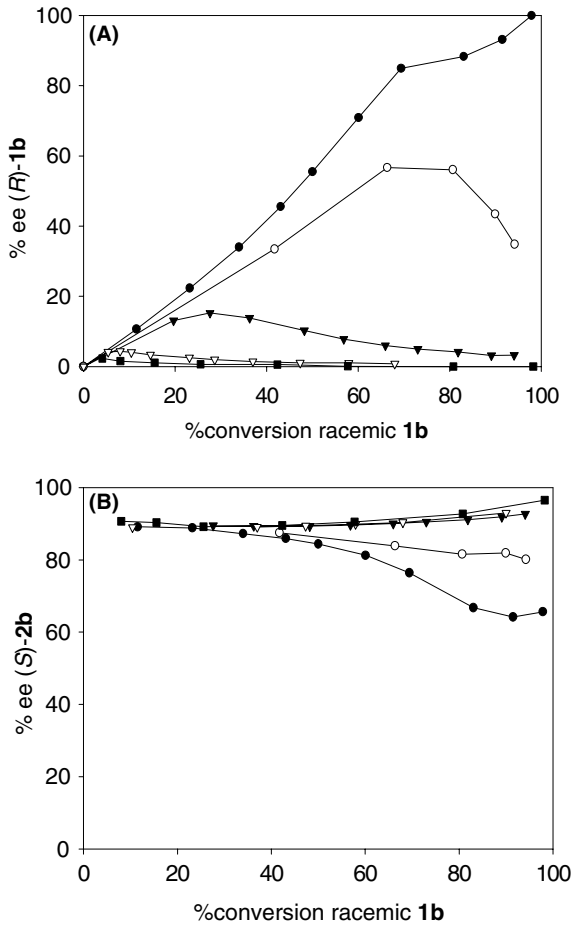


Figure 4. Effect of pH on enantiomeric purity of substrate (**A**) and product (**B**) during the HheC catalysed ring opening of epibromohydrin **1b** by N_3^-. Incubations contained: 20 mM racemic epibromohydrin **1b** and 30 mM NaN_3. Symbols: (-●-), pH 4.5; (-○-), pH 5.5; (-▼-), pH 6.5; (-▽-), pH 7.5; (-■-), pH 8.5.

process described above for epichlorohydrin, the dynamic character of the resolution improved with increasing pH value (Fig. 4). Below pH 6.5 the reaction showed predominantly the character of a kinetic resolution resulting in an increase of the enantiomeric purity of the remaining substrate (*R*)-**1b** and a decrease in the enantiomeric purity of product (*S*)-**2b** during the course of the reaction.

At pH 6.5 and higher, the enantiomeric excess of (*R*)-**1b** remained below 15%, showing that an almost optimal dynamic kinetic resolution was obtained. The initial enantiomeric excess of (*S*)-**2b** was around 90% at all pH-values, indicating that the kinetic resolution occurred with an E-value of approximately 20.

At pH 6.5 and higher, the enantiomeric purity of the product **2b** remained constant but increased slightly after 50% substrate conversion. These results indicate that epibromohydrin **1b** is a better substrate than epichlorohydrin **1a** for the dynamic kinetic resolution since at pH 6.5 or higher, the rate of racemisation was higher than the rate of ring opening. The reaction with epibromohydrin can be carried out at lower pH-values, which also has the added advantage of a lower rate of chemical conversion.

According to Scheme 1, the broad substrate range of the enzyme makes the formation of various side products possible. During the resolution of epibromohydrin **1b** at pH 6.5, 1-azido-3-bromo-2-propanol **2b**, glycidyl azide **4** and 1,3-dibromo-2-propanol **3b** were initially formed at an almost equal rate (Fig. 5). The concentration of the intermediate product **3b**, causing racemisation of epibromohydrin, decreased as the reaction proceeded with only a trace being left at total substrate conversion. The rate of formation of **5**, which is the product of the reaction of N_3^- with glycidyl azide **4**, accelerated towards the end of the conversion of the substrate. This indicated that HheC also catalyses this ring-opening reaction.

In Table 1, the yields of the various products at several pH-values are shown at the first data point at which the conversion of **1b** was higher than 98%. Between pH 4.5 and 7.5, the azidoalcohol **2b** was formed as the major product. Generally, with increasing pH, the yield of epoxide **4** increased while that of haloalcohols **2b** and **3b** decreased. This can be explained by the high pH opti-

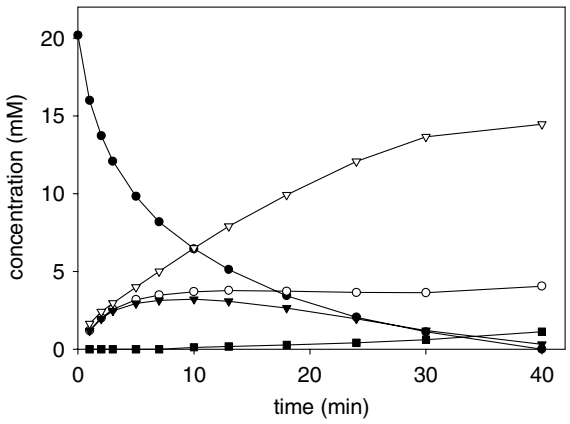


Figure 5. Progress curves of the dynamic kinetic resolution of epibromohydrin **1b** and the formation of products catalysed by HheC. The incubation contained 20 mM epibromohydrin **1b**, 30 mM NaN_3 and 1.8 μM enzyme at pH 6.5. Symbols: (-●-), epibromohydrin **1b**; (-▽-), 1-azido-3-bromo-2-propanol **2b**; (-▼-), 1,3-dibromo-2-propanol **3b**; (-○-), glycidyl azide **4**; (-■-), 1,3-diazido-2-propanol **5**.

Table 1. Effect of pH on the formation of the products during the dynamic kinetic resolution of epibromohydrin **1b**[a]

pH	% Yield **2b**	% Ee (*S*)-**2b**	% Yield **4**	% Yield **3b**	% Yield **5**
4.5	67	65	8	18	7
5.5	81	87	6	8	6
6.5	72	94	20	2	6
7.5	65	95	19	0	16
8.5	14	97	72	0	12

[a] Incubations contained 20 mM epibromohydrin **1b** and 30 mM NaN_3. Reaction mixtures were analysed at the first data point at which >98% conversion of epibromohydrin **1b** was observed.

mum of the ring-closure reaction (around pH 8.0). At pH 8.5 the formation of **2b** was slower than the sequential ring closure to glycidyl azide **4** resulting in the formation of the latter as the major product.

2.3. Optimisation of reactant concentrations

The results described above showed that the dynamic kinetic resolution of epibromohydrin with N_3^- as a nucleophile is feasible. At pH 6.5 the azidoalcohol (*S*)-**2b** was obtained in 72% yield at a moderate enantiomeric excess of 94%. The experiments were performed with 20 mM epibromohydrin **1b** and 30 mM N_3^-. No Br^- was added since the catalytic amount of Br^-, which was formed by the conversion of **2b** to **4,** was sufficient enough to cause racemisation. To test if variation of the initial concentrations of Br^- and N_3^- would have any effect on enantiomeric excess and yield of the desired product (*S*)-**2b**, the concentrations of these anions were varied. These experiments were performed at pH 6.5 since a pH lower than 6.5 resulted in a less dynamic character of the kinetic resolution and a pH higher than 6.5 resulted in an increase in the amount of side products **4** and **5**. Increasing the concentration of Br^- had no influence on the initial rate of product **2b** formation, but resulted in an increase of product yields (Fig. 6A and Table 2, exp. no 1, 5, 6 and 7). These increases in product yields were accompanied by a decrease in the formation of the side product **4**. Apparently, the higher yield of **2b** at increasing Br^- concentrations was caused by the inhibition of the conversion of **2b** to **4**.

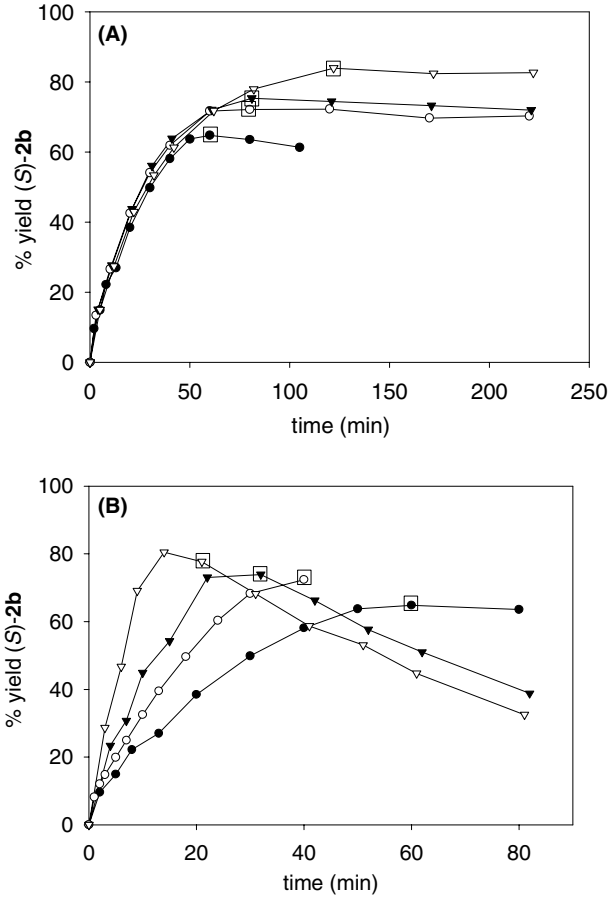


Figure 6. Effect of NaBr (A) and NaN_3 (B) concentration on the formation of product (*S*)-**2b** at pH 6.5. The incubations contained: (A) 20 mM epibromohydrin **1b**, 22.5 mM NaN_3 and various concentrations NaBr; (B) 20 mM epibromohydrin **1b** and various concentrations NaN_3. Symbols: (A) (-●-), 0 mM NaBr; (-○-), 10 mM NaBr; (-▼-), 20 mM NaBr; (-▽-), 50 mM NaBr and (B) (-●-), 22.5 mM NaN_3; (-○-), 30 mM NaN_3; (-▼-), 45 mM NaN_3; (-▽-), 90 mM NaN_3. Rectangles around data points correspond to entries in Table 2.

The rate of substrate conversion increased with a higher concentration of N_3^- resulting in an increased transient yield of (*S*)-**2b** (Fig. 6B). Complete conversion of **1b** (20 mL, 20 mM) was achieved in 60 min with 22.5 mM N_3^- and in less than 20 min with 90 mM N_3^-, using 1.8 μM enzyme. However, after the initial rapid accumulation of **2b**, its concentration slowly decreased. The combination of both a higher N_3^- and higher Br^- concentration did not yield an increase in yield of **2b** (Table 2, exp. no 8 and 9). Varying the Br^- or N_3^- concentrations did not have an effect on the enantioselectivity of the ring opening since all conversions yielded (*S*)-**2b** in 94% to 95% ee at total conversion of the substrate.

Table 2. Effect of NaBr and NaN_3 concentration on product formation during the dynamic kinetic resolution of epibromohydrin **1b**[a]

Exp. no	Concd NaBr (mM)	Concd NaN_3 (mM)	% Yield **2b**	% Ee (*S*)-**2b**	% Yield **4**	% Yield **3b**	% Yield **5**
1	0	22.5	65	94	26	3	4
2	0	30	72	94	20	2	6
3	0	45	74	95	17	1	8
4	0	90	78	94	12	1	9
5	10	22.5	72	95	19	2	5
6	20	22.5	75	95	17	3	5
7	50	22.5	84	95	10	3	5
8	50	30	84	94	9	3	4
9	50	45	84	94	8	3	5

[a] Incubations contained 20 mM epibromohydrin **1b** and 1.8 μM enzyme. Reaction mixtures were analysed at the first datapoint at which >98% conversion of epibromohydrin **1b** was observed.

2.4. Increased enantiomeric excess by a sequential kinetic resolution

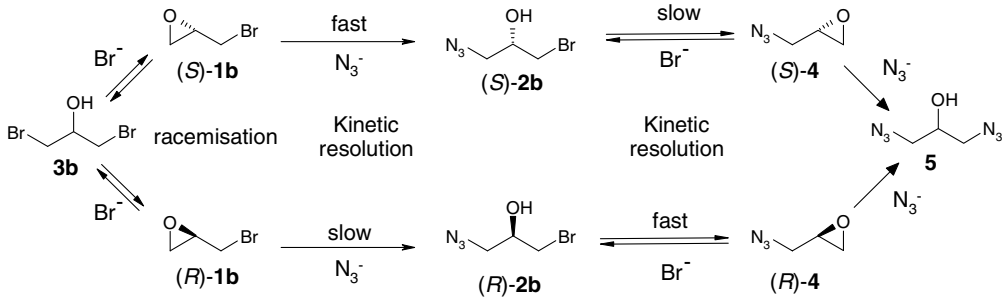
During the conversion of epibromohydrin **1b**, the enantiomeric purity of (*S*)-**2b** remained almost constant during the first 50% of the conversion and steadily increased during the second 50%. In a normal dynamic kinetic resolution process, the enantiomeric purity of the product remained constant throughout the course of the reaction. The increase in enantiomeric purity of (*S*)-**2b** can be explained by the occurrence of a sequential kinetic resolution, the (*R*)-selective ring closure of 1-azido-3-bromo-2-propanol **2b** yielding glycidyl azide **4**. This reaction caused a decrease in the yield of **2b**, but an increase in enantiomeric excess. However, when racemic **2b** was subjected to HheC in a separate experiment, only 20% conversion occurred. This can be explained by the position of the equilibrium between **2b** and **4**, which is apparently positioned towards **2b**. During a dynamic kinetic resolution, HheC also catalyses the ring opening of glycidyl azide **4** by N_3^- and thereby draws the conversion of (*R*)-**2b** to completion. When the enzymatic conversions of **2a** or **2b** were performed in the presence of an equimolar amount of N_3^- the (*R*)-enantiomers were converted preferentially resulting in kinetic resolutions with E-values of 15 and 8, respectively. Due to this sequential kinetic resolution, (*S*)-1-azido-3-bromo-2-propanol **2b** can be obtained enantiomerically pure if the reaction is allowed to proceed after the dynamic kinetic resolution of epibromohydrin is completed (Table 3). With the addition of an excess of N_3^- and no Br^- (exp. no 3 and 4), a prolonged incubation resulted in a disadvantageous decrease in the yield of (*S*)-1-azido-3-bromo-2-propanol **2b**. This was due to the HheC-catalysed ring opening of glycidyl azide **4** by N_3^- yielding 1,3-diazido-2-propanol **5**. This shifted the equilibrium between **2b** and **4** in the direction of **4**, and increased the degree of conversion of **2b**.

The optimal reaction conditions (pH 6.5, 50 mM Br^- and 30 mM N_3^-), combined with a prolonged incubation after complete conversion of substrate **1b**, resulted in (*S*)-1-azido-3-bromo-2-propanol **2b** in higher than 99% ee with a yield of 77%. The overall reaction scheme of

Table 3. Increase of enantiomeric purity of (*S*)-**2b** due to the sequential kinetic resolution[a]

Exp. no	Concd NaBr (mM)	Concd NaN_3(mM)	% Yield of **2b** at 94.5% (±0.5%) ee of (*S*)-**2b**	% Yield of **2b** at 97% (±0.5%) ee of (*S*)-**2b**	% Yield of **2b** at 99% ee of (*S*)-**2b**
3	0	45	74	66	58
4	0	90	78	68	51
8	50	30	84	80	77
9	50	45	84	77	71

[a] Incubations contained 20 mM epibromohydrin **1b** and 1.8 μM enzyme.



Scheme 3. Overall reaction scheme of the combined dynamic kinetic resolution process of epibromohydrin **1b** and subsequent kinetic resolution of the formed product 1-azido-3-bromo-2-propanol. All 12 reaction steps are catalysed by the haloalcohol dehalogenase HheC.

the dynamic kinetic resolution with the sequential kinetic resolution is shown in Scheme 3.

3. Discussion and conclusion

An efficient dynamic kinetic resolution was developed for the conversion of racemic epibromohydrin to enantiomerically pure (*S*)-1-azido-3-bromo-2-propanol **2b**. The reactions depicted in Scheme 3 occurred simultaneously and were all catalysed by the haloalcohol dehalogenase HheC. With epibromohydrin **1b** at pH 6.5 only a slight transient enantiomeric excess of the remaining substrate was observed during the reaction, indicating that the rate of racemisation was higher than the rate of ring opening. Optimisation of the reaction conditions resulted in 1-azido-3-bromo-2-propanol **2b** in 84% yield and 94% ee. Due to an in situ sequential kinetic resolution by ring closure of **2b** catalysed by the same enzyme, the product can be obtained enantiomerically pure (ee >99%).

This sequential kinetic resolution did not take place if the product, glycidyl azide **4** was not converted to the diazido product **5**. The HheC catalysed conversion of **4** to **5** removes the product and draws the equilibrium between **2b** and **4** towards the latter. This explains that the optimal concentration of N_3^- is between 1.2- and 1.5-fold higher than the concentration of epibromohydrin. If an equimolar amount of azide were to be used, no N_3^- would be left to react with glycidyl azide **4**. However if a too large excess of N_3^- was used (Table 3, exp. no 3 and 4) the rate of conversion of **2b** became very fast, resulting in a lower yield of **2b** and the formation of **5** as the major side product. A considerable loss in yield of **2b** was observed since the E-value of this second kinetic resolution is only 8.

Jacobsen and Schaus have reported a similar dynamic kinetic resolution process. In this reaction, the enantioselective ring opening (k_{rel} >100) of epichlorohydrin by $TMSN_3$ was catalyzed by a (Salen)Cr(III)N_3 complex resulting in almost enantiomerically pure 3-azido-1-chloro-2-trimethylsiloxypropane (ee >97%) in a 76% yield.[13] A sequential kinetic resolution was not described. Similar to the results described above for epichlorohydrin, the limiting factor in this process was the slow racemisation of epoxide (*R*)-**1a**. This problem could be circumvented by a controlled addition of azide, thereby increasing the relative rate of racemisation.

This is the first description of a dynamic kinetic resolution process using a haloalcohol dehalogenase. Although the overall reaction scheme was somewhat complicated, since various reactions occurred simultaneously (Scheme 3), choosing the optimal conditions allowed an efficient conversion of racemic epibromohydrin to enantiomerically pure (*S*)-1-azido-3-bromo-2-propanol **2b**. In this research, the substrate was limited to one type of epoxide and one nucleophile (N_3^-). The substrate range of this reaction is not limited to epihalohydrins and N_3^-. A recent evaluation of nucleophiles showed that the enzyme accepts as well as Cl^-, Br^- and N_3^- but also CN^- and NO_2^-.[5] Any compound that contains an epihalohydrin as a substructure, forms a prochiral intermediate after ring opening by a halide and can be accepted by the enzyme, is expected to be a substrate for the described dynamic kinetic resolution process.

4. Experimental

4.1. General

The enantiomeric excesses (% ee's) and the yields of all the aliphatic compounds were determined with a Hewlett–Packard 5890 gas chromatograph equipped with a FID-detector, using a Chiraldex G-TA capillary column (col I, 50 m, Astec) or a Chiraldex A-TA capillary column (col II, 25 m, Astec) all of 0.25 mm inside diameter. NMR-spectra were recorded in $CDCl_3$ or DMSO-d_6. Epichlorohydrin **1a**, (*R*)-**1a**, (*S*)-**1a** and epibromohydrin **1b** were purchased from Aldrich. The haloalcohol dehalogenase from *A. radiobacter* AD1 (HheC) was overexpressed and purified as described before.[3,4]

4.2. General procedure for biocatalytic conversions

Stock solutions of NaCl, NaBr and NaN_3 were freshly prepared. The substrate was dissolved in 20 mL buffer and incubated at 22 °C. The proper volumes of stock solutions were added to the substrate solution. The reaction was started by the addition of the enzyme. The reaction was monitored by periodically taking 0.5 mL samples from the closed reaction vessel. The samples were extracted with 2 mL diethyl ether containing 1-chlorohexane as an internal standard. Prior to analysis by chiral GC, the samples were dried by passing them through a small column containing $MgSO_4$. The following initial substrate concentrations were used: epoxides **1a**, (*S*)-**1a** and **1b**, 20 mM; (*R*)-**1a** and **2a**, 10 mM; 1,3-dichloro-2-propanol **3a**, 5 mM. The effect of pH on the conversions was determined using the following buffers: pH 4.5, 100 mM sodium acetate; pH 5.5, 100 mM sodium citrate; pH 6.5, 100 mM sodium phosphate; pH 7.5 and pH 8.5, 100 mM Tris-sulfate; pH 9.5, 100 mM glycine–NaOH.

4.3. Synthesis of racemic reference compounds

Compounds **2a** and **2b** were prepared from the corresponding epoxides, **1a** and **1b**.[15] The epoxide (2.5 mmol) was dissolved in an aqueous solution of sodium azide (13.0 mmol in 4.0 mL), 2.3 mL acetic acid then added and the solution stirred for 5 h at 30 °C. The solution was extracted with diethyl ether (3×4 mL). The combined organic phase was washed five times with 5 mL portions of sodium phosphate buffer (50 mM, pH 6.5). The organic phase was dried and the diethyl ether removed on a rotary evaporator. NMR data: Compound **2a** (DMSO-d_6): ^{1}H NMR δ 3.32 (m, 2H), 3.60 (m, 2H), 3.85 (m, 1H), 5.7 (br s, 1H); ^{13}C NMR δ 43.9 (C-1), 50.6 (C-3), 67.1 (C-2). Compound **2b**

(DMSO-d_6): ^{1}H NMR δ 3.34 (m, 2H), 3.47 (m, 2H) 3.86 (m, 1H), 5.8 (d, 1H); ^{13}C NMR δ 33.5 (C-1), 51.3 (C-3), 66.8 (C-2). Compound **4** was prepared from racemic **2b**. The haloalcohol was dissolved in a diluted aqueous NaOH solution and stirred for 0.2 h at 22 °C. The solution was extracted with diethyl ether. The organic phase was dried and the diethyl ether removed on a rotary evaporator yielding compound **4** as an oil. NMR data: Compound **4** ($CDCl_3$): ^{1}H NMR δ 2.68 (dd, 1H), 2.81 (dd, 2H), 3.16 (m, 1H), 3.26 (dd, 1H), 3.53 (dd, 1H); ^{13}C NMR δ 42.2, 47.7, 49.7. Compound **5** was prepared by the ring opening of **4** with an azide, according to the procedure described above for **2a** and **2b**. Since the diazido compound **5** is an unstable and explosive substance, it was not isolated in a pure form, but a solution containing **5** was analysed using gas chromatography.

4.4. Absolute configuration and chiral analysis

The absolute configurations of 1-azido-3-chloro-2-propanol **2a** and glycidyl azide **4** were determined by co-injection with the enantiomerically pure compounds. Enantiomerically pure (*S*)-**2a** was prepared from (*S*)-**1a** as described above. Ring closure of (*S*)-**2a** under basic conditions yielded (*S*)-**4**. During the dynamic kinetic resolution of epibromohydrin **1a** and epibromohydrin **2a**, (*S*)-**4** was formed as major product. From this result we concluded that the enantiopreference towards epibromohydrin must be identical to that of epichlorohydrin. The retention times of the analysed compounds are as follows: Col I: temperature program: 5 min at 80 °C, 10 °C/min to 170 °C, 1 min at 170 °C. (*S*)-**1a**, 3.1 min; (*R*)-**1a**, 3.3 min; (*S*)-**1b**, 4.4 min; (*R*)-**1b**, 4.9 min; (*R*)-**2a**, 10.8 min; (*S*)-**2a**, 11.0 min. Col I: temperature program: 100 °C; (*R*)-**2b**, 20.0 min; (*S*)-**2b**, 20.9 min. Col II: temperature program: 65 °C; (*S*)-**4**, 22.3 min; (*R*)-**4**, 22.6 min.

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