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Dynamic Kinetic Resolution Process Employing Haloalkane Dehalogenase

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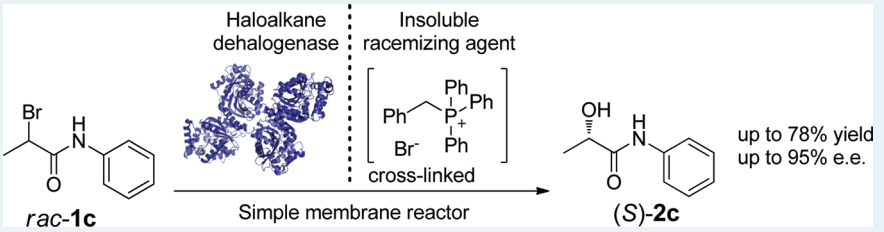
ABSTRACT:



The first dynamic kinetic resolution process with a haloalkane dehalogenase is described, allowing the efficient preparation of enantiopure α-hydroxyamides from racemic α-bromoamides. A simple membrane reactor is used to separate the enzyme from the nonsoluble, polymer-based, and metal-free racemizing agent. A model substrate, *N*-phenyl-2-bromopropionamide, was converted to (*S*)-*N*-phenyl-2-hydroxypropionamide either with 63% yield and 95% e.e or with 78% yield and 88% e.e.

KEYWORDS: dynamic kinetic resolution, enantioselective biocatalysis, haloalkane dehalogenases, α-hydroxyamides, racemization methods

INTRODUCTION

The use of green, chemoenzymatic processes for the preparation of valuable, enantiopure building blocks has been gathering increasing attention in recent years because of the high activity and selectivity offered by enzymes, in combination with the simple way of catalyst production and the use of environmentally benign process conditions.[1−3] The majority of the enzyme-catalyzed conversions that are used for the preparation of enantiopure α-substituted esters and amides employ hydrolytic enzymes in an enantioselective kinetic resolution. In these processes one enantiomer of the substrate is hydrolyzed by an amidase, esterase, or lipase, while the other enantiomer remains unconverted by the enzyme.

The intrinsic limitation of kinetic resolution processes stems from the fact that in a perfectly enantioselective reaction the enantiopure product still can be obtained with a yield of only 50%. One of the most widely applied methods to overcome this limitation is the use of dynamic kinetic resolution (DKR) processes, in which the enantioselective kinetic resolution is combined with a fast in situ racemization of the substrate (Scheme 1).[4,5] In an optimal situation a yield of 100% of optically pure product can be obtained in a DKR, as the nonpreferred enantiomer of the substrate is constantly converted to the preferred one. The in situ racemization of the substrate is performed either in a chemical way[6] or by using an additional enzyme (racemase).[7] A chemoenzymatic DKR can be achieved with a base-catalyzed or transition metal-catalyzed racemization, or through racemization involving a nucleophilic displacement.[8]

The α-hydroxyamide scaffold is present in a variety of compounds with confirmed biological activity, such as pantothenic acid (vitamin B5, **A**, Figure 1),[9] the cholesterol-lowering drug bestatin (**B**, Figure 1),[10] and the antibiotics amikacin (**C**, Figure 1)[11] and cefamandole (**D**, Figure 1).[12] Furthermore, chiral α-hydroxyamides are precursors of α-hydroxy-lactams,[13] α-amino acids,[14] and small, non-natural peptides.[15] Chemical routes for the preparation of enantiopure hydroxyamides are mainly based upon asymmetric hydrogenation with rhodium[16] or ruthenium[17] complexes and enantioselective ene-reactions.[18] The main biocatalytic routes to enantiopure α-hydroxyamides rely on the kinetic resolution of racemic α-acyloxamides[19] and α-bromoamides.[20] Chemoenzymatic DKR processes aiming at chiral alcohols rely on (i) enzymatic enantioselective alcohol acylation combined with in situ substrate racemization by ruthenium complexes[21−23] or (ii) enzymatic enantioselective hydrolysis of the allylic alcohol esters combined with in situ racemization of substrate via π-allyl−palladium complexes.[24] In these processes, transition metal complexes are used, which limits their potential application because of environmental and economic reasons.

Recently, it was described that haloalkane dehalogenases can perform kinetic resolution of α-haloesters[25] and α-haloamides,[20] with E-values of up to >200. In preparative conversions, yields of up to 50% and e.e. values of up to 99% in the conversions

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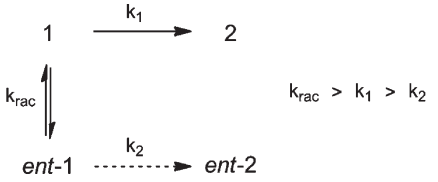
Scheme 1. Schematic Overview of a DKR



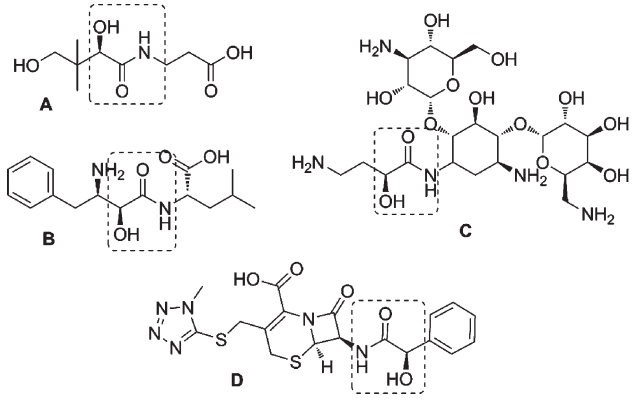


Figure 1. Bioactive compounds bearing an α-hydroxyamide scaffold.

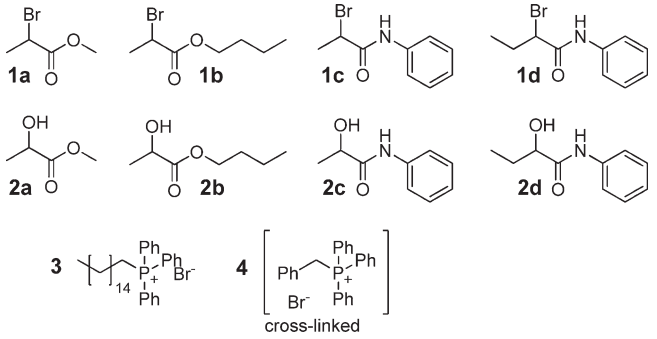


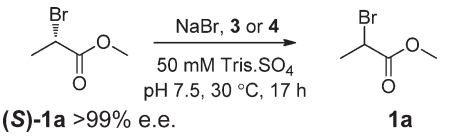
Figure 2. Compounds used in this research: substrates for the enzyme-catalyzed reactions **1**, products of these reactions **2**, and racemization agents **3** and **4**.

catalyzed by haloalkane dehalogenases DbjA and LinB were reported. Here we describe our efforts toward increasing the efficiency of the haloalkane dehalogenase-catalyzed process by designing a reaction setup for the DKR, using racemic α-bromoesters and α-bromoamides as substrates. To obtain a suitable system, key parameters were optimized, including the enzyme type, the racemization agent, and reaction conditions.

RESULTS AND DISCUSSION

Haloalkane Dehalogenase-Catalyzed Kinetic Resolution of Model Compounds. For the current study, we used the α-haloesters (**1a** and **1b**), and α-haloamide **1c** and **1d** shown in Figure 2. This choice was inspired by the high enantioselectivity observed in haloalkane dehalogenase-catalyzed conversions of compounds **1a**−**1c**. A high enantioselectivity (E > 200) was reported previously by Prokop et al.[25] for the conversion of **1a** catalyzed by the bacterial haloalkane dehalogenases DbjA and DhaA.

Table 1. Influence of the Concentration of Racemizing Agents on the Decrease of the Enantiomeric Excess of an Initially Enantiopure Sample of Compound 1a



entry	racemization agent	[Br] (mM)	Br^- equivalents per mol of (*S*)-**1a**	e.e. of **1a** (%)
1	none			>99%
2	Na Br	200	20	85
3	Na Br	400	40	73
4	Na Br	600	60	64
5	Na Br	800	80	59
6	**3**	0.15	0.05	98
7	**3**	0.45	0.15	92
8	**3**	1.5	0.5	59
9	**3**	3	1	19
10	**4**	2.6	0.65	28
11	**4**	10	2.5	0
12	**4**	53	13	0

We observed that the same level of selectivity is obtained in DbjA-catalyzed conversions of compounds **1b**, **1c**,[20] and **1d**.

Selection of the Racemizing Agent. Compound (*S*)-**1a**, obtained by kinetic resolution of *rac*-**1a**, was used as a model substrate in studies aimed at optimizing the racemization process. Inspired by a literature report[26] on the racemization of α-haloesters in lipase-catalyzed DKR, we envisioned the use of bromide ions as racemizing agents, expecting the S_N2 reaction-based shuffling of the bromide group. In our initial attempt, sodium bromide was used as the most simple source of bromide ions (Table 1, entries 2−5).

Under the tested reaction conditions bromoester (*S*)-**1a** does not racemize spontaneously, but racemization occurred when NaBr was added (Table 1, entry 1). The degree of racemization depended on the concentration of sodium bromide, which supports the assumption that bromide salts can be used for racemization of compound **1a** and act through nucleophilic displacement (Table 1, entries 2−5). However, we did not observe full racemization, even when 800 mM salt was used (Table 1, entry 5).

Subsequently, the activity of DbjA was tested in the presence of 400 mM NaBr, but no conversion of substrate was observed. Since a bromide ion is a product of the enzymatic reaction, this lack of activity could be caused by product inhibition. To test this, the binding affinity for bromide ions to DbjA was determined by fluorescence quenching experiments. Such measurements, based on the fluorescent tryptophan(s) in the active site that bind(s) the halide during the dehalogenase reaction, have been used before to assay the binding of the halide for other haloalkane dehalogenases.[27] DbjA has one tryptophan residue at position 104,[28] which flanks the active site and is involved in bromide binding during catalysis,[25] and accordingly the protein was susceptible to fluorescence quenching, in agreement with the product inhibition. From the titration curve, a dissociation constant (K_D) of (1.9 ± 0.2) × 10^2 mM was found. Since bromide ions at high concentrations apparently block the activity

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of the enzyme, NaBr cannot be used as an effective racemization agent in a dynamic resolution. Therefore, an additional activation of substrate toward nucleophilic attack of Br^- is needed to enable the lowering of salt concentration.

The report from Jones and Williams[26] suggests the use of two systems for the efficient racemization of the substrate: a lipophilic phosphonium bromide (compound **3** in Figure 2) and a polymer-based phosphonium bromide (compound **4** in Figure 2). We therefore tested the racemization of (*S*)-**1a** with compounds **3** and **4** as racemization agents. The reaction with 1 equiv of **3** yielded (*S*)-**1a** with an enantiomeric excess that has dropped from 99% to 19% (Table 1, entry 9). When the amount of racemizing agent is lowered (Table 1, entries 6−8) the racemization proceeds slower, and at 0.05 equiv (Table 1, entry 6), the effect is negligible. However, when we tested the activity of the enzyme in the presence of 1 equiv of compound **3**, no conversion of substrate **1a** with DbjA or DhaA was detected. In conclusion, compound **3** cannot be used as a racemization agent in a DKR process with the studied dehalogenases.

Therefore, we continued to test the racemization efficiency of polymer **4** as a Br^- source, in the form of a finely powdered, water-insoluble solid, which forms a suspension upon agitation in buffer. We expected that because of its insolubility it might have a smaller negative effect on enzymatic activity as compared to compound **3**.

Efficient racemization of (*S*)-**1a** was achieved already at low amounts of **4**. When 0.65 equiv of bromide ions (per mol of **1a**) were added in the form of salt **4**, a drop in e.e. from >99 to 28% was observed (Table 1, entry 10). The use of 2.5 equiv of **4** (Table 1, entry 11) results in complete racemization of **1a**. Upon comparison to the data collected in Table 1, entries 2−5, we conclude that Br anions alone cannot cause the racemization, as much higher concentrations of NaBr are needed to obtain results comparable to the ones using compound **4**. No explanation is provided in the literature for the fact that compound **4** dramatically increases the racemization rate as compared to free bromide.[26] We suggest that the polymer acts as a Lewis acid that polarizes the carbon-bromide bond in the substrate, resulting in higher rate of an S_N2-type attack of the free bromide ions. Alternatively, the interaction of the polymer with the substrate can be based around the carbonyl group of the ester moiety, which would also result in the substrate activation toward an S_N2 process.

Subsequently, the activity of the enzyme in the presence of compound **4** was tested in a DKR process (Figure 3). In this process the product was formed with high e.e. (99% during the whole course of the experiment), showing that the enzyme was still active in the presence of polymer **4** and that its enantioselectivity was not affected. In the beginning of the process the enzymatic conversion of (*R*)-**1a** was faster than the racemization, resulting in high enantiomeric excess of the remaining (*S*)-**1a** at the first measured time (Figure 3). In later stages the e.e. of (*S*)-**1a** dropped because of its racemization, which was accompanied by further conversion of the substrate. However, we suspected that the conversion of the substrate might also be caused by spontaneous hydrolysis of the ester bond in the substrate. To test this, the kinetic resolution of **1a** with DbjA was followed in a time course experiment. The concentration of the nonpreferred substrate decreased, while the e.e. of product **2a** stayed >99%, which supported the assumption that **1a** was susceptible to nonenzyme catalyzed ester hydrolysis (Figure 4).

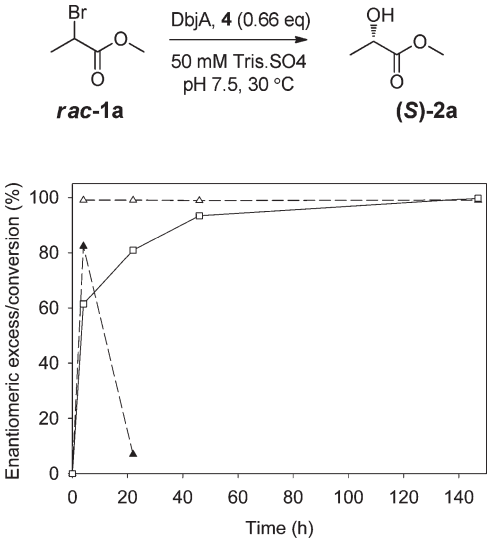

Figure 3. DKR with **1a**. Symbols: (▲) enantiomeric excess of **1a**; (Δ) enantiomeric excess of **2a**; (□) conversion. Concentrations and e.e. values were determined using chiral GC. During later stages of the reaction (>30 h) the e.e. of remaining **2a** could not be reliably determined because of its low concentration.

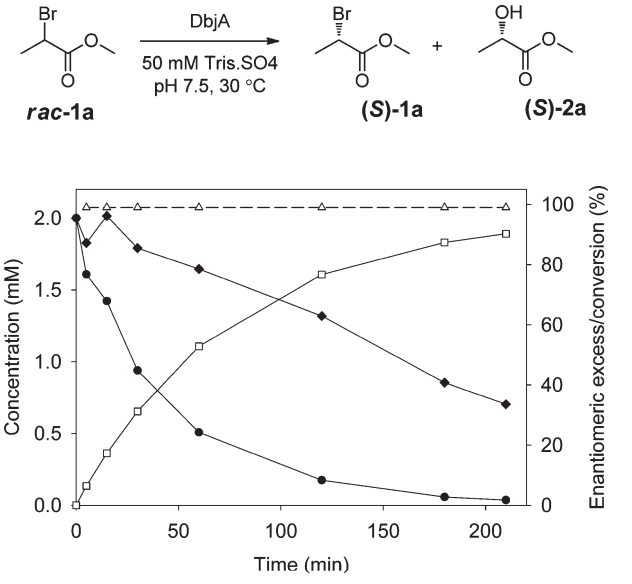

Figure 4. Kinetic resolution of **1a**. Symbols: (●) concentration of (*R*)-**1a**; (♦) concentration of (*S*)-**1a**; (Δ) enantiomeric excess of **2a**; (□) conversion. Concentrations and e.e. values were determined using chiral GC.

On the basis of these results, we chose compound **4** as an efficient racemizing agent that did not abolish the enzymatic activity and did not affect the enantioselectivity of the biotransformation. We also selected the stable substrates butyl 2-bromopropionate (**1b**) and *N*-phenyl-2-bromopropionamide (**1c**) for further optimization experiments.

Optimization of the Reaction Conditions. Subsequently, the ability to achieve DKR using substrate **1b** and racemizing agent **4** was tested (Figure 5). Conversion of **1b** in the presence of **4** was observed and enantiopure **2b** was formed. Furthermore,

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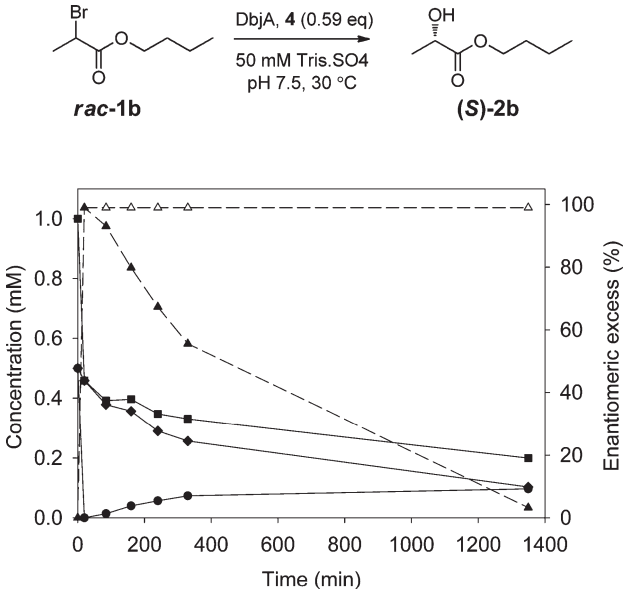

Figure 5. DKR with **1b**. Symbols: (●) concentration of (*R*)-**1b**; (♦) concentration of (*S*)-**1b**; (■) total concentration of **1b**; (▲) enantiomeric excess of **1b**; (Δ) enantiomeric excess of **2b**. Concentrations and e.e. values were determined using chiral GC.

racemization by **4** occurred, as the e.e. of **1b** decreased over time. We concluded that a DKR was achieved, albeit with low efficiency. The rate of nonenzyme catalyzed hydrolysis was found to be much smaller than in the case of compound **1a**, as the slow drop in the concentration of (*S*)-**1b** (Figure 5) was accompanied by an increase of (*R*)-**1b** (Figure 5).

We suspected that either a too low concentration of compound **4** or inactivation of the enzyme could result in the observed slow reaction. Increasing the concentration of **4** led to faster racemization, but the activity of the enzyme was reduced. Addition of an extra amount of the enzyme still failed to bring the reaction to full conversion. These results suggested that compound **4** might inactivate the enzyme during the course of the reaction. To test whether this was a reversible phenomenon, we conducted an experiment in which compound **4** was removed from the reaction mixture by centrifugation. No further conversion of (*R*)-**1a** was observed. However, the addition of a new amount of enzyme to this reaction mixture resulted in full conversion of (*R*)-**1a**, showing that the polymer inactivates the enzyme in a nonreversible manner.

As the enzymatic activity was reduced by the presence of compound **4**, we decided to further optimize the reaction setup by taking advantage of the insolubility of polymer **4** and separating the enzyme and **4** using a membrane reaction system. Two variants of such processes can be performed in which either the polymer, which has the particle size of 37−74 μm (reaction setup A), or the enzyme (reaction setup B) are enclosed in a membrane container of which the pore size excludes the possibility of protein or polymer leakage, but allows for the substrate/product transport across the membrane.

In reaction setup A, the membrane bag was filled with the suspension of racemization polymer **4** and was placed into a solution containing the substrate and the enzyme. In reaction setup B, the membrane compartment was filled with enzyme, placed in a solution containing substrate and compound **4**, and attached to the cap of the reaction container. Both reaction setups

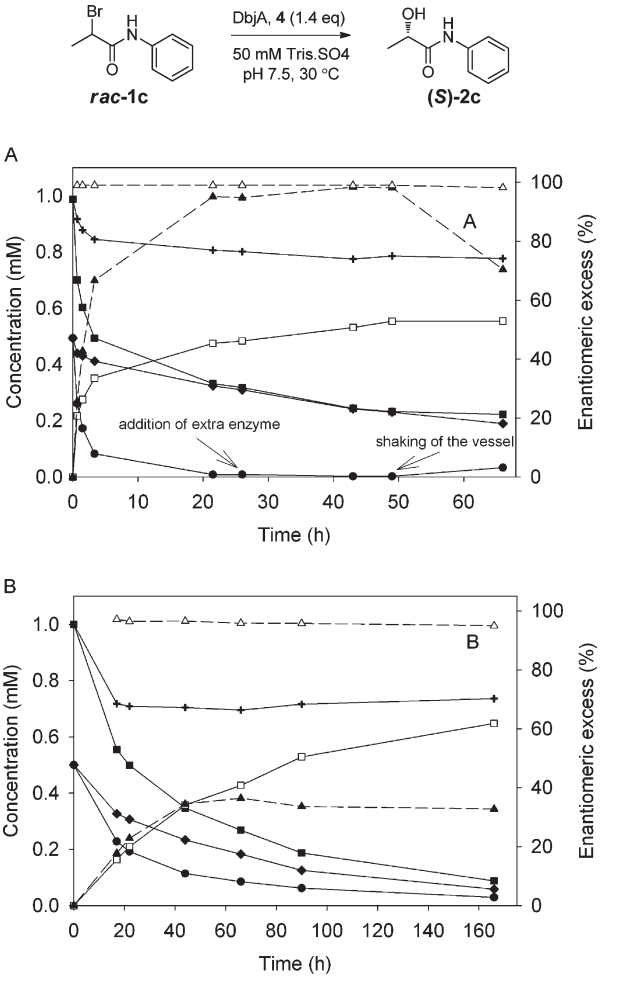

Figure 6. DKR with **1c** using different reaction setups. Symbols: (●) concentration of (*R*)-**1c**; (♦) concentration of (*S*)-**1c**; (■) total concentration of **1c**; (▲) enantiomeric excess of **1c**; (□) concentration of **2c**; (Δ) enantiomeric excess of **2c**; (+) total concentration of **1c** and **2c**. Concentrations were determined by reverse-phase HPLC and e.e. values were determined using chiral HPLC.

were tested in the DKR of compound **1c** (Figure 6A and 6B). The agitation of the heterogeneous reaction system, which was found to be required for the efficient mass transfer to and from the nonsoluble racemization agent, was achieved either with "head over tail" rotation (reaction setup A) or stirring with a magnetic stirrer (reaction setup B).

The reaction in setup A (Figure 6A) was fast in the beginning. However, after 20 h, it seems that the racemization is the limiting factor, as the concentration of the preferred substrate is very low and the conversion is slow (Figure 6A). To confirm this, extra enzyme was added after 27 h, which did not significantly increase the reaction rate. To test whether a different mode of agitation could result in a more efficient racemization process, the reaction mixture was shaken after 49 h. Indeed, a drop in ee of the substrate was observed, which confirmed that the agitation of **4** plays a crucial role in the DKR process. On the other hand, when the system was shaken, no more product was formed between 49 and 66 h, which observation hints at the possibility of enzyme inactivation during the shaking process. In conclusion, the rate of mass transfer to catalytic racemization site is too low if the racemization catalyst is in the membrane compartment.

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Since the ratio of racemization should be higher than the resolution, mass transfer limitation should be prevented. This was efficiently achieved in the alternative experimental setup B, in which compound **4** is stirred with the substrate without affecting the enzyme separated by the membrane (Figure 6B). The use of this setup indeed improves the rate of racemization, as the enantiomeric excess of **1c** is kept below 40% during the whole reaction time. In this preparative scale experiment the product was isolated after 7 days with a yield of 61% and an e.e. value of 95%.

We observed that the total concentration of substrate and product (Figure 6B) decreases in the first 17 h, limiting the yield of the reaction. Incubating the substrate or product with either compound **4** or the membrane revealed that this drop in concentration is caused by the binding of **1a** by the racemization catalyst **4**. Inspired by these results we reduced the amount of **4** four times to increase the yield of a preparative scale reaction. To avoid the further loss of product, which might happen during the extraction of the reaction mixture with an organic solvent, the whole reaction mixture was lyophilized prior to flash chromatography purification. The isolated yield indeed increased to 78%, but the e.e. of (*S*)-**2c** dropped to 88%, which is most likely caused by a limiting racemization rate. Unconverted substrate **1c** was also recovered (22%) with an e.e. of 55%.

A DKR was also observed with compound **1d**. After 7 days of incubation with 25 wt %, a conversion of 33% was observed and the e.e. of (*S*)-**2d** was 91% while the e.e. of the remaining **1d** was 14%. In conclusion, the polymer was active in the racemization of compound **1d**, and the enzymatic reaction was proceeding. However, the activity of the biocatalyst was not sufficient to obtain a complete conversion. The results suggest that expanding the substrate range of haloalkane dehalogenease may contribute to broaden the scope of this DKR.

CONCLUSIONS

In this paper we present the first successful DKR process employing haloalkane dehalogenases, aimed at the preparation of enantiopure α-hydroxyamides, important precursors in medicinal chemistry. We have optimized the system to produce alcohols from bromoalkanes using a haloalkane dehalogenase (DbjA) and racemizing agents. Because the enzyme is susceptible to interfacial inactivation, we used a semipermeable membrane for the physical separation of the enzyme and the suspended racemizing agent. In this final setup bromoamide (*S*)-**2c** could be produced with 63% isolated yield and 95% e.e. or with 78% yield and a lower e.e. of 88%. Together with the DKR of haloalcohols described by Haak et al,[29] this report lays the foundation for the use of a new chemoenzymatic DKR processes aimed at chiral alcohols which does not employ expensive and environmentally hazardous heavy metal complexes.

EXPERIMENTAL SECTION

General Procedures. Production and purification of the haloalkane dehalogenases was performed as described before.[20]

Methyl *rac*-2-bromopropionate, methyl *rac*-lactate, methyl (*S*)-lactate, butyl *rac*-lactate, butyl (*S*)-lactate, *N*-phenyl-2-bromopropionamide and triphenylphosphine, polymer-bound, 200−400 mesh (2% cross-linked phosphine polymer) were purchased from Sigma-Aldrich. (1-Hexadecyl)triphenylphosphonium bromide was purchased from Alfa Aesar. Butyl *rac*-2-bromopropionate was purchased from TCI. *N*-phenyl-2-hydroxypropionamide was synthesized as described before.[20] The membrane bag used was purchased from Spectrumlabs and had a cut off of 3.5 kD (vol/length: 1.1 mL/cm).

Preparation of *N*-phenyl-2-bromobutanamide (1d). 2-Bromobutyric acid bromide (2.0 mmol) and aniline (2.0 mmol) were stirred in DCM (8 mL) at 5 °C. Triethylamine (2.2 mmol) in DCM (2 mL) was added dropwise, and the cooling was removed. After 2−4 h the volatiles were evaporated. Solvents were evaporated, and the product was purified by precipitation from AcOEt/pentane. Yield 91%. White powder. R_f = 0.82 (pentane/AcOEt. 1:1, v/v). mp. 99−100 °C (lit.[30] 98−99). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.11 (t, ^3J = 7.6 Hz, 3H, C**H**$_3$), 2.11−2.30 (m, 2H, CH$_3$C**H**$_2$), 4.27 (dd, ^3J = 7.6 Hz, ^3J = 5.2 Hz, 1H, C**H**Br), 7.15 (t, ^3J = 7.6 Hz, 1H, Ar**H** *para*), 7.35 (app t, ^3J = 7.6 Hz, 2H, Ar**H** *meta*), 7.35 (d, ^3J = 7.6 Hz, 2H, Ar**H** *ortho*), 8.09 (br, s, 1H, N**H**). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 11.8, 29.4, 54.1, 120.0, 125.0, 129.1, 137.1, 166.5; HRMS (ESI+) calc. for $C_{10}H_{13}BrNO$: 242.0175, found: 242.0166. Chiral HPLC analysis (Chiralcel AD, heptane/*i*-PrOH, 95/5, v/v), retention times (min): 9.9 (*R*) and 11.3 (*S*).

Preparation of *N*-phenyl-2-hydroxybutanamide (2d), Using a Modified Literature[31] Procedure. A solution of *N*-phenyl-2-bromobutanamide (1.00 mmol, 242 mg), formic acid (4.00 mmol, 151 μL), NaOH (4.00 mmol, 160 mg), and tetrabutylammonium bromide (31 mg) in 4 mL of a 1:1 (v/v) mixture of toluene and water was stirred and refluxed for 1 h. The reaction mixture was cooled, diluted with AcOEt (30 mL), and washed with water (2 × 20 mL). The organic phase was dried ($MgSO_4$), and the solvent was evaporated. The residue was dissolved in methanol (10 mL), and NaOH (100 mg) was added in one portion. After 10 min of stirring, the volatiles were evaporated, and the residue was dissolved in AcOEt (30 mL) and washed with 1N aq. HCl (20 mL). The organic phase was dried, and the product was purified by flash chromatography (Silicagel 40−63 μm, pentane/AcOEt) and subsequent precipitation from Et_2O/pentane. Yield: 22%. White powder. R_f = 0.66 (pentane/AcOEt. 1:1, v/v). mp. 89−90 °C (lit.[32] 88−89). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.02 (t, ^3J = 7.6 Hz, 3H, C**H**$_3$), 1.74−1.99 (m, 2H, CH$_3$C**H**$_2$), 3.04 (d, ^2J = 4.8 Hz, 1H, O**H**), 4.17−4.21 (m, 1H, C**H**OH), 7.12 (t, ^3J = 7.6 Hz, 1H, Ar**H** *para*), 7.32 (app t, ^3J = 7.6 Hz, 2H, Ar**H** *meta*), 7.55 (d, ^3J = 7.6 Hz, 2H, Ar**H** *ortho*), 8.46 (br, s, 1H, N**H**). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 9.16, 27.8, 73.5, 119.8, 124.6, 129.0, 137.2, 171.9; HRMS (ESI+) calc. for $C_{10}H_{14}NO_2$: 180.1019, found: 180.1010. Chiral HPLC analysis (Chiralcel AD, heptane/*i*-PrOH, 95/5, v/v), retention times (min): 18.3 (*R*) and 22.3 (*S*). The absolute configuration was determined by comparison with the retention time of pure (*S*)-enantiomer, obtained by reacting (*S*)-2-hydroxybutyric acid with *N*-sulfinylaniline, according to a published procedure.[32]

Determination of Concentration and Enantiomeric Excess of Compound 1a−c and 2a−c. 1a/2a: Chiral GC, Chiraldex B-PM (0.9 mL/min, 5 °C/min from 50 to 100 °C, 10 °C/min from 100 to 150 °C, 10 °C/min from 150 to 50 °C), retention times (min): 12.0 (*R*) and 12.7 (*S*) (**2a**); 13.2 (*S*) and 14.5 (*R*) (**1a**).

1b/2b: Chiral GC, CP chiralsil (0.7 mL/min, 50 °C (40 min), 3 °C/min from 50 to 150 °C, 10 °C/min from 150 to 50 °C), retention times (min): 59.3 (*R*) and 59.5(*S*) (**2b**); 61.1 (*S*) and 61.5 (*R*) (**1b**).

1c/2c: Chiral HPLC, Chiralpak ASH, heptane/*i*-PrOH, 97/3, v/v. Compound **1c**: retention times (min): 22.1 (*S*) and 26.7 (*R*). Compound **2c**: retention times (min): 37.2 (*R*) and 43.9

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(*S*). Quantification of **1c**/**2c** was performed by reverse-phase HPLC, using a calibration curve (Nucleosil 100 (C18) column, 50% acetonitrile and 50% 50 mM potassium phosphate buffer, pH 2.0, flow 1 mL/min (retention times (min): 8.5 (**2c**); 29.5 (**1c**)).

1d/**2d:** Chiral HPLC, Chiralpak ADH, heptane/*i*-PrOH, 95/5, v/v. Compound **1d**: retention times (min): 17.8 (*R*) and 20.8 (*S*). Compound **2d**: retention times (min): 28.8 (*R*) and 48.5 (*S*).

Preparation of Enantiopure (*S*)-1a for the Racemization Studies. Enantiopure (*S*)-**1a** was prepared via kinetic resolution by haloalkane dehalogenase DbjA. DbjA (400 μg) was added to a solution of **1a** (1.20 mmol, 200 mg) in 120 mL of 50 mM Tris·SO_4 (pH 8.2) and incubated at 30 °C. After 17 h, the reaction mixture was extracted with diethyl ether (3 × 75 mL), the organic phases were dried with $MgSO_4$ and evaporated. The enantiomeric excess was determined by Chiral GC, as described before, and was >99% for (*S*)-**1a**.

Racemization of (*S*)-1a by Sodium Bromide. To enantiopure (*S*)-**1a** (~10 mM) in 7.5 mL of 50 mM Tris·SO_4 pH 7.5, different amounts of NaBr (200−800 mM) were added. After 17 h at 30 °C, the reaction mixture was extracted with 5 mL of diethyl ether, the organic phases were dried, evaporated, and enantiomeric excess was determined by chiral GC as described before.

Fluorescence Quenching Experiment for K_D Determination. Tryptophan fluorescence was measured using a Spex Fluorlog 322 fluorescence spectrophotometer (Jobin Yvon) in a stirred quartz cuvette, at 25 °C. The excitation wavelength used was 280 nm, and the emission wavelength was 350 nm. To 800 μL of 0.7 μM DbjA in 50 mM Tris·SO_4, pH 8.2, NaBr solution (6 M in 50 mM Tris·SO_4), pH 8.2) was titrated in steps of 10 μL to a final concentration of 2 M. Data for fluorescence quenching were corrected for the dilution, and curves were fitted using Sigmaplot.

Racemization of (*S*)-1a by Racemizing Agent 3. To a 10 mL samples of 3.0 mM solution of (*S*)-**1a** in 50 mM Tris·SO_4 (pH 7.5), different amounts of **3** were added and the mixtures were incubated at 30 °C. After 17 h, the reaction mixtures were extracted with 10 mL diethyl ether, dried, evaporated, and enantiomeric excess was determined by chiral GC as described before.

Synthesis of Polymer-Based Phosphonium Bromide (Compound 4)[33]. Benzyl bromide (700 μL, 6.0 mmol) was added to 1.0 g of 2% cross-linked phosphine polymer in 10 mL of dimethylformamide (DMF), and the mixture was stirred for 48 h at 70 °C. The mixture was cooled, the solid was filtered, washed with toluene (2 × 10 mL), dichloromethane (2 × 10 mL), and diethylether (2 × 10 mL), and subsequently dried under vacuum (at 60 °C), yielding 1.4 g of phosphonium salt. The bromide content was determined by elemental analysis as 17% Br, corresponding to a bromide loading of 0.0021 mol per gram of the prepared polymer.

Racemization of (*S*)-1a by Racemizing Agent 4. To 4.0 mL of 4 mM solution of (*S*)-**1a** in 50 mM Tris·SO_4 (pH 7.5), 0.65, 2.5, or 13 equiv of **4** were added and the suspensions incubated at 30 °C. After 17 h, the reaction mixtures were extracted with 10 mL of diethyl ether, dried, and the enantiomeric excess was determined by chiral GC as described before.

Enzymatic Reactions. *Test Experiment for DKR with **1a** and Compound **3**.* To 30 mL of a 0.3 or 3 mM solution of **1a** in 50 mM Tris·SO_4, pH 7.5, 1 equiv of **3** and 400 μg of DbjA/320 μg DhaA were added and incubated at 30 °C. After 16 h, the reaction mixtures were extracted with 10 mL of diethyl ether, the organic phases were dried, and the enantiomeric excess was determined by chiral GC.

*DKR with **1a** and Compound **4**.* To 25 mL of a 3 mM solution of **1a** in 50 mM Tris·SO_4, pH 7.5, compound **4** (0.66 equiv) and 1.4 mg of DbjA were added, and the bottle was agitated at 30 °C. At various times, samples of 5 mL were taken and extracted with 5 mL of diethyl ether (with 3 mM dodecane as an internal standard), the organic phases were dried, and the enantiomeric excess was determined by chiral GC.

*Kinetic Resolution of **1a**.* To 45 mL of a 4 mM solution of **1a** in 50 mM Tris·SO_4, pH 7.5, 100 μg of DbjA was added and incubated at 30 °C. At various time points, samples of 5 mL were taken and extracted with 5 mL of diethyl ether (with 1 mM dodecane as internal standard), the organic phases were dried, and the enantiomeric excess was determined by chiral GC.

*DKR with **1b** and Compound **4**.* To 45 mL of a 1 mM solution of **1b** in 50 mM Tris·SO_4, pH 7.5, 0.59 equiv of **4** and 100 μg of DbjA were added and agitated at 30 °C. At various time points, samples of 5 mL were taken and extracted with 5 mL of diethyl ether (with 1 mM dodecane as internal standard), the organic phases were dried, and the enantiomeric excess was determined by chiral GC.

*DKR with **1c** and Compound **4** in a Membrane Bag.* To 100 mL of a 1 mM solution of **1c** in 50 mM Tris·SO_4, pH 7.5, a membrane bag filled with 67 mg of compound **4** in 5 mL of 50 mM Tris·SO_4, pH 7.5 (1.4 equiv) and 500 μg of DbjA was added, and the bottle was rotated head over tail at 30 °C. An extra portion of 500 μg DbjA was added after 27 h, and after 49 h the system was shaken at 140 rpm. At various time points, samples of 2 mL were taken and extracted with 2 mL of ethyl acetate, the organic phases were dried, evaporated, and enantiomeric excess and the concentration were determined by chiral HPLC.

*DKR with **1c** and Compound **4**, with DbjA in a Membrane Bag.* To 100 mL of a 1 mM solution of **1c** in 50 mM Tris·SO_4, pH 7.5, with 67 mg (1.4 equiv) of compound **4**, a membrane bag filled with 500 μg of DbjA in 4 mL of 50 mM Tris·SO_4, pH 7.5 was added. The membrane was attached to the cap of the bottle; the racemizing agent **4** was added to the solution, which was stirred and incubated at 30 °C. Samples were analyzed by chiral HPLC and reverse-phase HPLC, as described before. After 7 d the reaction mixture was filtered over a Schott funnel, and the residue was washed with ethyl acetate. Subsequently the reaction mixture was extracted with 5 × 50 mL of ethyl acetate. The collected organic phases were dried, and the volatiles were evaporated. Product **2c** was purified by flash chromatography (Silicagel, 40−63 μm, eluents: **1c:** pentane/diethyl ether (6:4); **2c**: pentane/ethyl acetate (3:7). The yield of (*S*)-**2c** was 61% with an enantiomeric excess of 95%.

The same conversion was done with 17 mg (0.35 equiv) of compound **4** and 1.2 mg of DbjA. After 14 d the reaction mixture was lyophilized. Products **1c** and **2c** were purified by flash chromatography. The yield of (*S*)-**2c** was 78% with an enantiomeric excess of 88%, 22% of **1c** was recovered (e.e. 55%).

*DKR with **1d** and Compound **4**, with DbjA in a Membrane Bag.* To 100 mL of a 0.75 mM solution of **1c** in 50 mM Tris·SO_4, pH 7.5, with 50 mg (1.4 equiv) of compound **4**, a membrane bag filled with 4.5 mg of DbjA in 4 mL of 50 mM Tris·SO_4, pH 7.5, was added. The membrane was attached to the cap of the bottle; the racemizing agent **4** was added to the solution, which was stirred and incubated at 30 °C. After 7 d the reaction mixture was filtered over a Schott funnel and the residue was washed with ethyl acetate. Subsequently the reaction mixture was extracted with 4 × 50 mL of ethyl acetate and 2 × 50 mL of chloroform. The collected organic phases were dried, and the volatiles were evaporated. The conversion was 33%, e.e of (*S*)-**2d** was 91%, and the e.e. of recovered **2c** was 14%.

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