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Oxazolidinone Synthesis through Halohydrin Dehalogenase-Catalyzed Dynamic Kinetic Resolution

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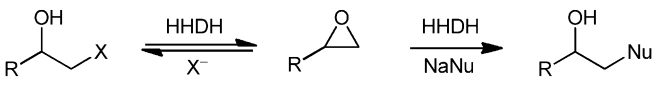
Abstract: An efficient dynamic kinetic resolution protocol using a single enzyme is described. Both the kinetic resolution and substrate racemization are catalyzed by halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 (HheC). The HheC-catalyzed reaction of epibromohydrin and 2-bromomethyl-2-methyloxirane with sodium cyanate afforded 5-substituted 2-oxazolidinones in high yields (97% and 87%) and high optical purity (89% and >99% *ee*) in the presence of catalytic amounts of bromide ion. These compounds are valuable building blocks with diverse synthetic applications.

Keywords: cyanates; dynamic kinetic resolution; enzyme catalysis; epoxides; halohydrin dehalogenase; oxazolidinones

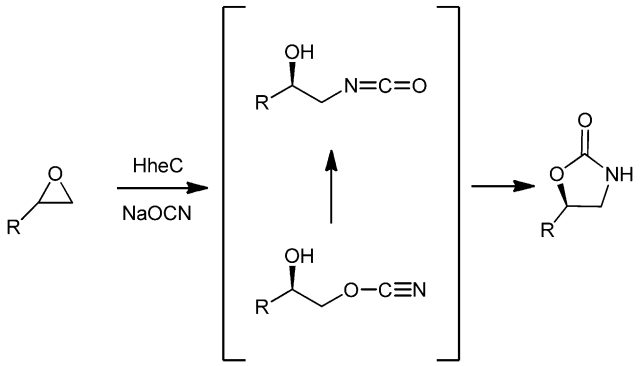
The quantitative transformation of enantiomeric mixtures into enantiopure products is a challenging task in asymmetric synthesis. One method that can be applied for deracemization is dynamic kinetic resolution (DKR).[1] DKR is based on kinetic resolution (KR) combined with *in situ* racemization of the slower reacting substrate enantiomer. In such a process, mild racemization methods are needed, which can be achieved by using an (organo)metallic catalyst or an enzyme.[2] Enzyme-catalyzed racemization is attractive since it takes place at moderate temperature, neutral pH, atmospheric pressure and usually is highly chemoselective.[3] This usually involves racemases, a small group of enzymes capable of racemizing α-hydroxycarbonyl and α-amino acid derivatives.[3] However, there are only a few examples where enzymatic resolution is combined with enzymatic racemization catalyzed by racemases.[4,5]

Halohydrin dehalogenases (HHDH) are enzymes that catalyze the formation and conversion of epoxides (Scheme 1).[6–8] They are unique in the sense that they can simultaneously catalyze KR and racemization of certain substrates. This applies to epihalohydrin derivatives.[9] The ability of HHDH to catalyze both a ring-closure reaction of halohydrins (the natural reaction) as well as a ring-opening reaction of epoxides with anionic nucleophiles makes DKR feasible if an achiral intermediate can be formed.

The concept of DKR catalyzed by HHDH from *Agrobacterium radiobacter* AD1 (HheC) has been reported for the ring-opening of epihalohydrins by azide ion.[9] Epibromohydrin was established as the most suitable substrate to perform DKR, compared to other epihalohydrins. HheC shows no activity towards vicinal fluoro alcohols, while the high chemical reactivity of alkyl iodides makes unwanted side reactions possible. Moreover, the rate of HheC-catalyzed racemization of epibromohydrin is higher than that of epichlorohydrin.[9] A DKR of epibromohydrin performed under optimized reaction conditions on an analytical scale gave (*S*)-1-azido-3-bromo-2-propanol with >99% *ee* and 77% yield.[9] Due to the broad substrate range of HheC, the ring-opening with azide



Scheme 1. Ring-closure and ring-opening reactions catalyzed by halohydrin dehalogenases.



Scheme 2. HheC-catalyzed transformation of epoxides to oxazolidinones.

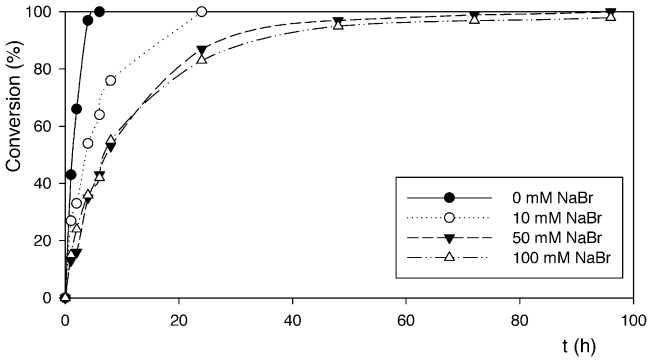


Figure 1. Effect of the NaBr concentration on the conversion of **1a**.

and bromide and ring-closure reactions occurred simultaneously during this process.

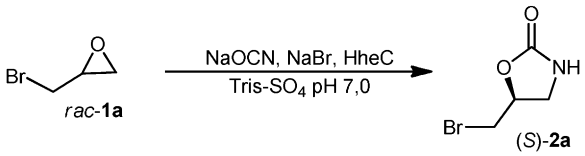
While HheC-catalyzed ring-opening of epoxide with azide, nitrite and cyanide generates β-substituted alcohols,[10] the reaction with cyanate (OCN^-) proceeds *via* an isocyanate–cyanate species that undergoes rapid cyclization, affording a 2-oxazolidinone as the final product (Scheme 2).[11] This reaction is highly regioselective, with no observable by-products and therefore could provide an attractive method for the preparation of chiral oxazolidinones.

DKR and complete conversion of racemic epichlorohydrin to the corresponding oxazolidinone was observed in a reaction catalyzed by HheC.[11] The substrate was completely consumed within 3 h and the product was isolated in 54% yield and 69% *ee*. The modest product yields were assigned partially to hydrolytic instability of epichlorohydrin and partially to formation of polymeric material.

Herein, we report the optimized DKR of racemic epibromohydrins to provide highly enantioenriched oxazolidinones. Based on previous data[9,11] epibromohydrin (**1a**) and 2-bromomethyl-2-methyl-oxirane (**1b**) were chosen as substrates for DKR in the presence of cyanate ion. We initially checked the ability of HheC to convert **1a** into 2-oxazolidinone **2a** and found good activity leading to enantioenriched product. In a typical experiment, reactions were carried out in Tris-SO_4 buffer (2.5 mL) with 0.25 mmol of **1a**, 1.5 mol equivalents of NaOCN and different concentrations of bromide ion. To make the protocol simpler, instead of purified HheC a cell-free extract was used to catalyze the reaction. Progress of the reaction was followed in the absence of added bromide ion and in the presence of 0.1, 0.5 and 1.0 mol equiv. of NaBr (Figure 1, Table 1). The optical purity of the product was determined upon completion (100% conversion).

When the reaction was performed without an extra bromide, complete conversion to enantioenriched **2a** (89% *ee*) was reached over 5 h (Table 1, entry 1). In the presence of increasing concentrations of bromide,

Table 1. HheC-catalyzed conversion of *rac*-**1a** to (*S*)-**2a**.



Entry[a]	NaBr [mM]	Time [h]	Conversion [%][b]	ee_P [%]
1	0	5	100	89
2	10	24	100	89
3	50	96	100	86
4	100	168	100	85

[a] *Conditions: rac*-**1a** (34 mg, 0.25 mmol, 100 mM), NaOCN (0.375 mmol, 150 mM), 200 μL cell-free extract, Tris-SO_4 buffer (2.3 mL, 0.5 M, pH 7.0), different concentrations of NaBr.

[b] Determined by GC.

reactions were slower (Figure 1), but still continued to completion and *ee* values were slightly lower (Table 1, entries 2–4). The complete conversion to **2a** achieved in the absence of added NaBr indicates that traces of Br^-, probably present as the impurity in the starting material, were sufficient to catalyze racemization. Both enantiomers of **1a** were consumed at approximately the same rate pointing to a very fast racemization of the slower reacting substrate enantiomer (*R*)-**1a** (Figure 2).

Accordingly, a particularly simple experimental procedure for the preparation of (*S*)-**2a** could be established by simply mixing epoxide **1a**, NaOCN and enzyme in buffer solution at room temperature. On a 0.5-g scale, (*S*)-**2a** was prepared in high optical purity (89% *ee*) and very high chemical yield (97%) after a short reaction time (5 h).

In previous studies we observed the HheC-catalyzed enantioselective conversion of epoxide **1b** into highly enantioenriched 2-oxazolidinone (*S*)-**2b**.[11] Conversion of **1b** proceeded rapidly to 50%, but after that drastically slowed down. (*S*)-**2b** was isolated in

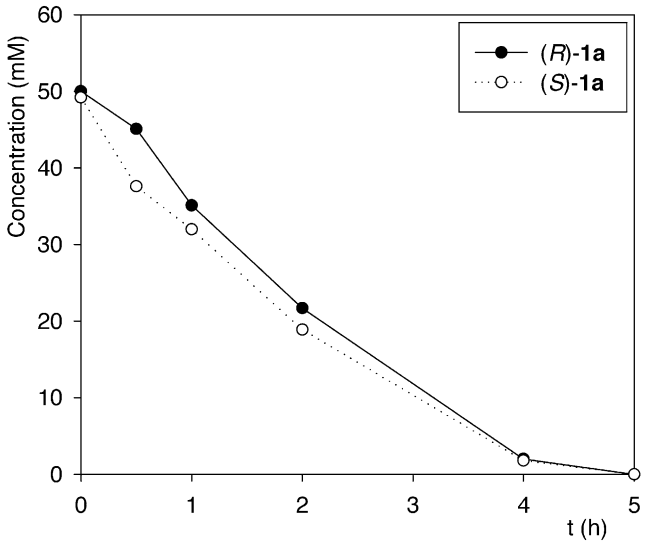
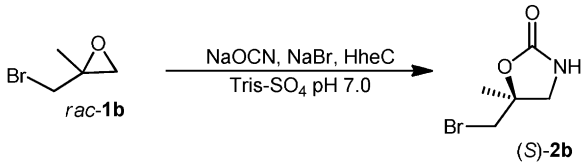


Figure 2. Progress curves of the conversion of both enantiomers of **1a** in the presence of NaOCN and HheC (Table 1, entry 1).

47% yield and 98% *ee* when **1b** (0.5 g, 100 mM) reacted with NaOCN (1 mol equiv.) in the presence of purified HheC for 1 h (Scheme 3, KR).[11] Clearly, under these conditions the reaction had the character of a KR rather than a DKR and addition of bromide ions was necessary to increase the rate of racemization relative to the rate of ring-opening.

To establish the concentration of bromide needed for efficient racemization, DKR experiments were performed at the 0.25-mmol scale in the presence of 1.5 mol equiv. of NaOCN and different concentrations of NaBr (Table 2). All reactions were initiated by adding a cell-free extract containing HheC to Tris-SO_4 buffer (total volume 2.5 mL). As a control, the reaction was performed without NaBr (Table 2, entry 1). The results matched those obtained with purified enzyme.[11]

Table 2. HheC-catalyzed conversion of of *rac*-**1b** to (*S*)-**2b**.



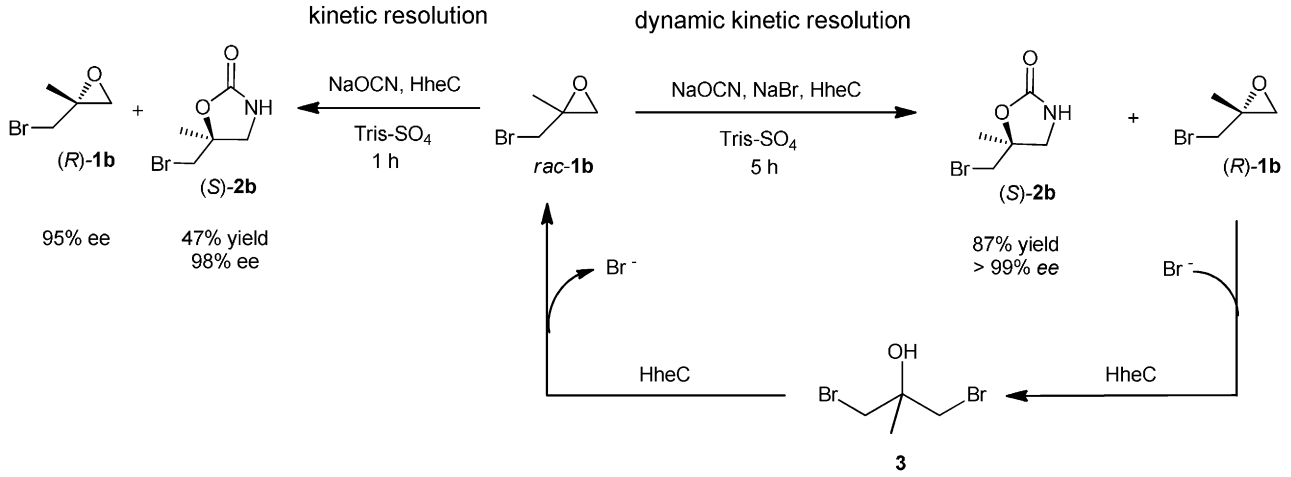
Entry[a]	NaBr [mM]	Time [h]	Conversion [%][b]	ee_P [%]
1	0	0.5	48	98
2	10	4	100	>99
3	50	3	100	>99
4	100	4	100	>99

[a] *Conditions: rac*-**1b** (38 mg, 0.25 mmol, 100 mM), NaOCN (0.375 mmol, 150 mM), 200 µL cell-free extract, Tris-SO_4 buffer (2.3 mL, 0.5 M, pH 7.0), different concentrations of NaBr.
[b] Determined by GC.

As shown in Table 2, the DKR of **1b** resulted in 100% conversion and excellent product *ee*, already in the presence of 10 mM NaBr (Table 2, entry 2). While both enantiomers were completely consumed within 4 h, conversion of the (*S*)-enantiomer of **1b** was significantly faster than conversion of the (*R*)-enantiomer (Figure 3). We attribute this to very high enantioselectivity of HheC toward (*S*)-**1b** resulting in slower racemization of (*R*)-**1b** compared to (*R*)-**1a**.

With higher concentrations of bromide ion, complete conversion of **1b** was achieved within 3 h (50 mM NaBr, entry 3) or 4 h (100 mM NaBr, entry 4), showing an insignificant effect on the reaction rate and overall DKR process. Since 0.1 mol equiv. of NaBr was sufficient to complete substrate racemization, it was considered a suitable concentration to perform a preparative reaction.

The reaction was performed with 0.5 g of *rac*-**1b**, 1.5 mol equiv. of NaOCN, 0.1 mol equiv, of NaBr and



Scheme 3. KR and DKR of **1b**.

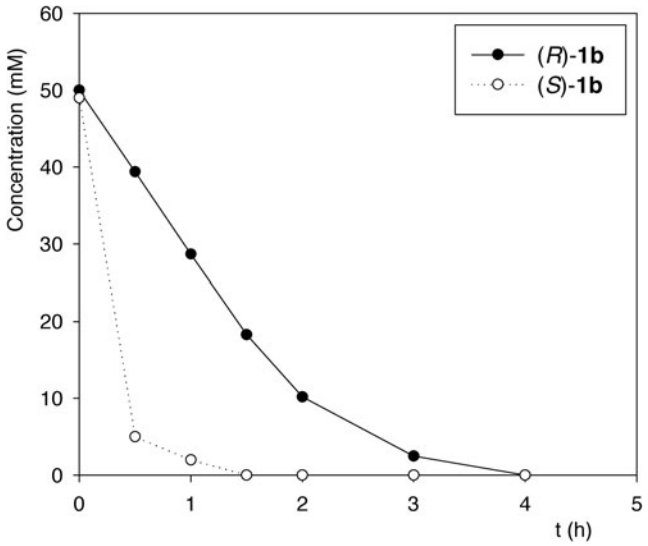


Figure 3. Progress curves of the conversion of both enantiomers of **1b** in the presence of NaOCN and HheC (Table 2, entry 2).

a cell-free extract containing HheC. The substrate was completely consumed over 5 h and highly enantioenriched product (*S*)-**2b** (>99% *ee*) was isolated in 87% yield (Scheme 3, DKR). Besides a higher yield, the product also had a higher *ee* (>99% from DKR *vs.* 98% from KR). The higher optical purity of (*S*)-**2b** (>99% *ee*) compared to (*S*)-**2a** (89% *ee*) can be attributed to the high enantioselectivity of HheC toward 2,2-disubstituted epoxides.[12]

The racemization of the slower reacting epoxide enantiomer in these conversions can be explained by the HheC-catalyzed interconversion of epoxide to achiral halohydrin in the presence of halide ion. Racemization of (*R*)-**1b** thus occurs *via* the prochiral intermediate 1,3-dibromo-2-methyl-2-propanol **3** (Scheme 3). The very fast and enantioselective ring-closure reaction of **3** leads to (*S*)-**1b** which is continuously transformed into (*S*)-**2b**. The slowest among these simultaneous reactions is the ring-opening of (*R*)-**1b** with bromide, which limits the rate of racemization.

In conclusion, a highly efficient and simple DKR protocol using a single enzyme was developed for oxazolidinones (*S*)-**2a** and (*S*)-**2b**. These highly functionalized compounds were prepared in high yields and high optical purity by a simple and fast procedure that took place at room temperature and neutral pH with crude enzyme prepared from recombinant cells. Due to high substrate tolerance and good enzyme stability, reactions are readily performed on a preparative scale.

Although HheC proved to be an advantageous catalyst for the preparation of 2-oxazolidinones, the main limitation of this method is the rather modest range of epoxides that can be converted this way and the fact that only this HHDH catalyzes the reaction that is presented. Extension of this methodology to other wild-type and engineered HHDHs is ongoing and will be reported in due course.

Experimental Section

General Remarks

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 300 (^{1}H 300 MHz and ^{13}C 75 MHz) spectrometer in $CDCl_3$. Chemical shifts (δ) are given in ppm downfield from TMS as the internal standard. Coupling constants are given in Hz. IR spectra were recorded as thin films on KBr on a Bruker ABB Bomen and peaks reported in cm^{-1}. High resolution mass spectrometry (HR-MS) was performed on 4800 Plus MALDI TOF/TOF Analyzer. Melting points were determined on an Electrothermal 9100 apparatus in open capillaries and are not corrected. Optical rotations were measured using an Optical Activity AA-10 automatic polarimeter. Enzymatic reactions were monitored by gas chromatography (GC) using an instrument equipped with an FID detector (set at 300 °C) and a split injector (set at 250 °C) and N_2 as carrier gas. The following columns were used, Alpha DEX 120 (30 m × 0.25 mm × 0.25 μm, Supelco), Beta DEX 225 (30 m × 0.25 mm × 0.25 μm, Supelco) and CP-Chirasil-Dex CB (25 m × 0.25 mm × 0.25 μm, Varian). The enantiomeric excess of the formed products and remaining epoxides was determined by chiral GC analyses under conditions described in Table 3. Absolute configurations were assigned by chiral GC analysis using reference compounds as previously described.[9,11]

Commercial grade reagents and solvents were used without further purification. Epibromohydrin (**1a**), NaBr and NaOCN were supplied by Sigma–Aldrich. 2-Bromomethyl-2-methyloxirane (**1b**) was prepared by *m*-CPBA oxidation of the corresponding alkene.[11] Racemic oxazolidinones **2a** and **2b** were prepared in a three-step procedure starting from racemic epoxides **1a** and **1b** as described before.[11]

Table 3. Chiral GC analyses.

Compound	Column	Conditions	Retention times [min]
1a	Beta DEX 225	100 °C 3 min 15 °C min^{-1} to 200 °C	6.1 (*R*) and 6.3 (*S*)
1b	Alpha DEX 120	70 °C 10 min 15 °C min^{-1} to 200 °C	8.4 (*R*) and 8.6 (*S*)
2a	CP-Chirasil-DEX CB	190 °C isothermal	11.4 (*R*) and 12.0 (*S*)
2b	CP-Chirasil-DEX CB	185 °C isothermal	12.8 (*R*) and 13.2 (*S*)

Enzyme Production

The enzyme was prepared according to a modified literature procedure.[13] A derivative of plasmid pBAD containing the gene *hheC* from *Agrobacterium radiobacter* AD1 was used for expression of the halohydrin dehalogenase. This construct was transformed into *E. coli* strain MC1061, and grown on LB agar plates supplemented with 100 μg mL^{-1} ampicillin, at 37 °C, overnight. Several colonies of transformed cells were inoculated into 20 mL LB medium supplemented with 100 μg mL^{-1} ampicillin and grown overnight at 37 °C with shaking at 250 rpm. The culture was then added to 0.5 L of LB medium supplemented with 100 μg mL^{-1} ampicillin. To induce protein expression, L-arabinose was added to a final concentration of 0.02%, and the culture was incubated for 24 h at 30 °C with shaking at 200 rpm. Cells were harvested by centrifugation at 5,000 × *g* at 4 °C for 15 min, and stored at −20 °C until protein isolation. The pellets was resuspended in TEMG buffer (10 mM Tris-SO_4, 1 mM EDTA, 1 mM β-mercaptoethanol, 10% glycerol, pH 7.5) containing a protease inhibitor (10 mL g^{-1}, Complete Protease Inhibitor Cocktail Tablets, Roche). Following sonication (10 × 10 sec, Labsonic M), the lysate was centrifuged (11,000 × *g*, 40 min, 4 °C) and the resulting supernatant was used as cell-free extract and stored at −20 °C until further use. The protein profile of the obtained extract was analyzed by SDS-PAGE, which indicated that HheC was highly overexpressed to about 70–80% of the total protein in cell-free extract.

General Procedure for Enzymatic Ring-Opening of Epoxides 1a and 1b

To 2.3 mL of Tris-SO_4 buffer (0.5 M, pH 7.0) at room temperature, racemic epoxide was added (0.25 mmol, 100 mM) followed by addition of sodium cyanate (0.375 mmol, 150 mM). In parallel experiments NaBr was added at different concentrations (10, 50 and 100 mM final concentration). Reactions were initiated by addition of 200 μL of cell-free extract in TEMG buffer (*ca.* 1 mg of HheC), final volume 2.5 mL. Conversion was followed by periodically taking samples (100 μL) from the reaction mixture. Samples were extracted with MTBE (1 mL) containing mesitylene as internal standard, dried over anhydrous Na_2SO_4, and analyzed by GC. When the substrate was consumed the reaction mixture was extracted with ethyl acetate, dried over anhydrous Na_2SO_4, and analyzed by chiral GC to determine the optical purity of the products. Non-enzyme-catalyzed ring-opening reactions with cyanate were followed by monitoring epoxide consumption in the absence of enzyme.

DKR on a Preparative Scale

(*S*)-5-Bromomethyloxazolidin-2-one (2a): Racemic substrate **1a** (0.50 g, 3.65 mmol) was dissolved in 30 mL Tris-SO_4 buffer (0.5 M, pH 7.0) followed by addition of NaOCN (4.95 mmol, 150 mM) and 3.1 mL of cell-free extract in TEMG buffer (*ca.* 15 mg of HheC). The mixture was stirred at room temperature and after 5 h, when the substrate was completely consumed, extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and evaporated. Pure (*S*)-**2a** was isolated as a white solid; yield: 640 mg (97%); 89% *ee*; mp 86.8–87.6 °C; $[\alpha]_D^{20}$: +40.5 [*c* 0.37 $(CH_3)_2CO$]. IR (KBr): ν = 3326, 1741, 1419, 1244, 1100 cm^{-1}. HR-MS (MALDI): m/z = 201.9477, calcd. for $[M+Na]^+$: 201.9474; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.47–3.53 (2H, s), 3.57 (1H, dd, J_1 = 10.5 Hz, J_2 = 4.5 Hz), 3.77 (1H, ddd, J_1 = 9.0 Hz, J_2 = 8.5 Hz, J_3 = 0.5 Hz), 4.80–4.89 (1H, m), 6.19 (1H, bs); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 32.3, 44.7, 74.5, 159.1.

(*S*)-5-Bromomethyl-5-methyloxazolidin-2-one (2b): Racemic substrate **1b** (0.50 g, 3.31 mmol) was dissolved in 30 mL Tris-SO_4 buffer (0.5 M, pH 7.0) followed by addition of NaOCN (4.95 mmol, 150 mM), NaBr (0.33 mmol, 10 mM) and 3.1 mL of cell-free extract in TEMG buffer (*ca.* 15 mg of HheC). The mixture was stirred at room temperature and after 5 h, when the substrate was completely consumed, extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and evaporated. Pure (*S*)-**2b** was isolated as a white solid; yield: 560 mg (87%); >99% *ee*; mp 116.5–117.7 °C; $[\alpha]_D^{20}$: +28.5 [*c* 0.38 $(CH_3)_2CO$]. IR (KBr): ν = 3262, 1738, 1705, 1307, 1235, 1094 cm^{-1}; HR-MS (MALDI): m/z = 231.9368, calcd. for $[M+K]^+$: 231.9370; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.63 (3H, s), 3.36 (1H, d, J = 9 Hz), 3.45 (1H, d, J = 10.5 Hz), 3.56 (1H, d, J = 10.5 Hz), 3.68 (1H, d, J = 9 Hz), 6.35 (1H, bs); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.5, 37.7, 49.9, 81.0, 158.8. NMR data are in accordance with literature data.[11]

Acknowledgements

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