# Synthesis of enantiopure chloroalcohols by enzymatic kinetic resolution†

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***Received 26th September 2006, Accepted 2nd November 2006***
***First published as an Advance Article on the web 11th December 2006***
**DOI: 10.1039/b613937j**

3-Alkenyl and heteroaryl chloroalcohols have been obtained in excellent enantiomeric excess (>99%) by enzymatic kinetic resolution using the haloalcohol dehalogenase HheC. Yields were close to the theoretical maximum for all substrates employed. Furthermore, the applicability of this methodology on multigram scale has been established.

## Introduction

Haloalcohol dehalogenases are enzymes that catalyze the interconversion of haloalcohols and epoxides.[1] HheC is a haloalcohol dehalogenase produced by *Agrobacterium radiobacter* AD1, a soil-dwelling bacterium that is able to use halogen-containing organic compounds as its sole carbon source. The enzyme was discovered some years ago.[2] Recently, its structure, kinetics and catalytic mechanism have been elucidated.[3,4] The substrate binds near a catalytic triad (Ser132-Tyr145-Arg149), of which the tyrosine residue activates the hydroxy group of the haloalcohol. Concurrent $S_N2$-type attack on the vicinal carbon atom by the hydroxy group leads to ring closure and expulsion of the halide anion.[3,4]

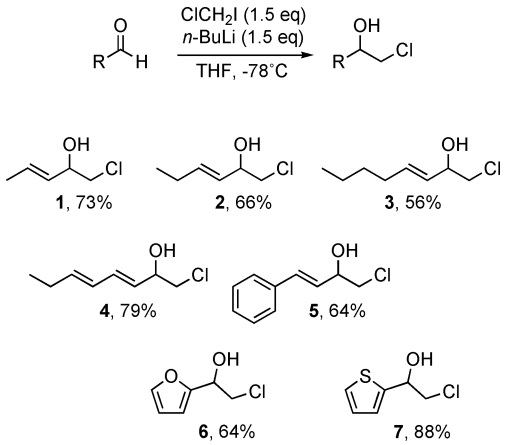
Notably, the biocatalytic potential of HheC has been the subject of investigation[5] and its substrate scope was found to be remarkable.[6] HheC catalyzes the reversible ring-closure of haloalcohols to form epoxides, as well as the irreversible ring-opening of epoxides with a number of non-halide nucleophiles, such as cyanide, nitrite, and azide.

Here, we report the preparation in enantiomerically pure form of functionalized vicinal chloroalcohols. These are highly valuable and frequently used building blocks in synthesis.

A number of strategies have been used to prepare these compounds in enantiomerically pure form. Chloroalcohols **5**–**7** have been prepared by asymmetric transfer hydrogenation of the corresponding chloroketones.[7] Furthermore, enantiomerically pure **6** has been obtained by lipase-catalyzed kinetic resolution of the racemic chloroalcohol,[8] and a route to enantiomerically pure **5** by Red-Al reduction of the enantiomerically pure alkyne is known.[9] Enantiomerically pure **1**–**4** have not been reported before, and a general, convenient, and highly enantioselective method for preparing enantiopure **1**–**7** is lacking.

## Results and discussion

We chose to concentrate on chloroalcohols functionalized with unsaturated and heteraromatic moieties, since these are especially versatile synthetic scaffolds. The compounds prepared[10] and studied are summarized in Scheme 1.




**Scheme 1** Synthesis of functionalized chloroalcohols.[10]

### Kinetic resolution on analytical scale

We studied the synthesized chloroalcohols as substrates for HheC, initially on analytical scale. The results of this screening are summarized in Table 1. Enzymatic activity towards each of the substrates is expressed both as initial enzyme activity ($\mu mol \cdot min^{-1} \cdot mg^{-1}$ of enzyme) and as turnover frequency ($s^{-1}$).[11] In the last column the selectivity factor $E$ is given for each of the substrates.

For the linear substrates, a clear trend in reactivity can be observed: the shorter the chain, the faster the enzymatic conversion. This is expected on the basis of previous observations.[6] We attribute the observed reactivity pattern to increasing difficulty of the substrate to fit in the active site of the enzyme in a reactive conformation as it gets bulkier. Based on such steric arguments, the reactivity pattern of substrates **1**–**5** can be rationalized. Differences in substrate binding, influenced by steric factors, may also partly explain the marked difference in reactivity between the otherwise comparable substrates **6** and **7**.

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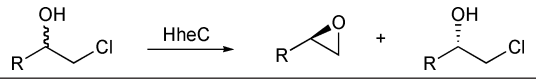
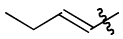
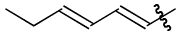
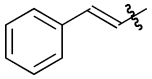
† Electronic supplementary information (ESI) available: Experimental procedures and full spectroscopic data for compounds **1**–**7**. See DOI: 10.1039/b613937j

**Table 1** HheC-catalyzed kinetic resolutions of substrates **1–7** on analytical scale[a]




| Entry | R = | Init. enz. activ.[b] / μmol·min$^{-1}$·mg$^{-1}$ | TOF/s$^{-1}$[c] | $E$[d] |
|---|---|---|---|---|
| 1 |  | 48 | 22.4 | >200 |
| 2 |  | 29 | 13.5 | 177 |
| 3 |  | 8 | 3.7 | >200 |
| 4 |  | 12 | 5.6 | 102 |
| 5 |  | 10 | 4.7 | >200 |
| 6 |  | 47 | 21.9 | >200 |
| 7 |  | 11 | 5.1 | 65 |

[a] General conditions: 0.2 mmol scale, 10 mM in Tris-sulfate pH 8.1. [b] Initial enzyme activity (μmol of product per min per mg of HheC). [c] Per enzyme subunit. [d] Obtained by fitting measured data points (concentration *vs.* time) against the mathematical curves for competitive Michaelis–Menten kinetics using MicroMath® Scientist®.

The enantioselectivity of these transformations is high in all cases, and even the lowest $E$ observed (65, Table 1, entry 7) is excellent for a kinetic resolution.

All of the HheC-catalyzed ring closure reactions described here, show competitive Michaelis–Menten kinetics.[12,13] Initially, epoxide formation of the slower reacting enantiomer is inhibited by the faster reacting enantiomer. When all fast-reacting enantiomer has been consumed, ring closure of the other enantiomer starts to take place, sometimes at an appreciable rate. This behaviour is illustrated in Fig. 1.[14]

In formulae 1a and 1b, depicted in Fig. 1, $R$ and $S$ represent the concentrations of both enantiomers, $V_{max}{}^{R}$, $V_{max}{}^{S}$, $K_m{}^{R}$ and $K_m{}^{S}$ are the relevant Michaelis–Menten parameters, and $k_c$ is the first-order rate constant of chemical hydrolysis. After fitting these equations by numerical integration to the obtained data points,[15] the $E$-value was calculated from formula 2 (Fig. 1).[16]

As is shown in Fig. 1, the reaction rate of the slow-reacting (*S*)-enantiomer can be appreciable. This is the case especially for the linear substrates **1–4**. Naturally, this behavior should be taken into account when these reactions are to be performed on preparative scale (*vide infra*).

### Kinetic resolution on preparative scale

Having established that this enzymatic kinetic resolution is highly efficient, we set out to transform substrates **1** and **3–7** on a preparative scale. In view of the excellent selectivities, our initial aim was to isolate both the enantiomerically pure remaining chloroalcohol and the produced epoxide.

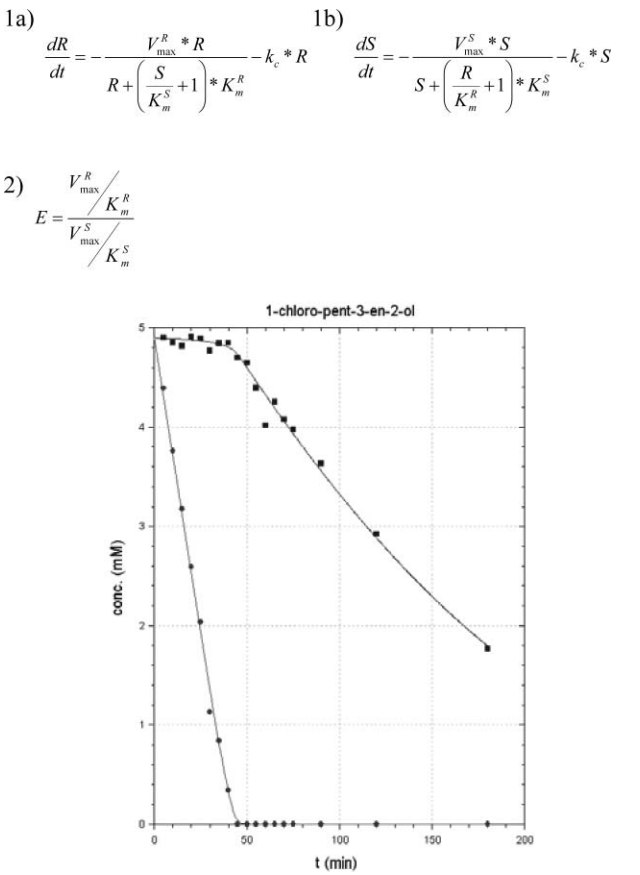



**Fig. 1** Formulae for competitive Michaelis–Menten kinetics displayed by HheC (1a and b), for the enzymatic selectivity $E$ (2), and an example of a typical progress curve for enzymatic conversion of substrate **1**.

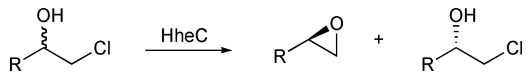
Preparative scale reactions were performed on 2.0 mmol scale at low concentration (10 mM) in Tris-sulfate buffer, *i.e.*, analogous to the analytical reactions. In this manner it proved possible to isolate enantiomerically pure chloroalcohols (ee > 99%) in fair to high yields (Table 2, entries 1–4). Our attempts to isolate also the produced epoxides failed, since it turned out that they rapidly hydrolyze *in situ* to form the corresponding diols.

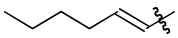
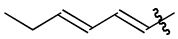
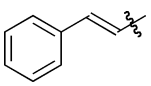
It was attempted to suppress this hydrolysis by performing the reaction in a two-phase system (Table 2, entries 5 and 6), but even in those cases hydrolysis proved inevitable. The role of this spontaneous hydrolysis will be examined in more detail further on.

Accordingly, our efforts focused on obtaining the enantiomerically pure halohydrins, which were isolated in good yields and with excellent enantioselectivities (Table 2).

The absolute configuration of the remaining chloroalcohols was *S* in all cases, in agreement with previous studies which showed that HheC is *R*-selective for most substrates.[5,6] A variety of methods was used to elucidate the absolute configuration of the slow-reacting enantiomer of the chloroalcohols. For (*S*)-(*E*)-1-chloro-4-phenyl-but-3-en-2-ol ((*S*)-**5**) the absolute configuration could be deduced from a crystal structure (CCDC 605888).[17] (*S*)-**6** and (*S*)-**7** could be correlated to known compounds by the sign of their optical rotation.[7,8] Finally, (*S*)-**1**, (*S*)-**3**, and (*S*)-**4** were converted to their saturated analogues, for which specific rotations are known.[18]

**Table 2** Enzymatic kinetic resolutions of unsaturated and heteroaromatic vicinal chloroalcohols on preparative scale




| Entry | Substrate | R = | Chloroalcohol (yield[a], ee, conf.) | Diol (yield[a]) |
|---|---|---|---|---|
| 1 | **1**[b] |  | 40%, >99%, *S* | N.i.[c] |
| 2 | **3**[d] |  | 31%, >99%, *S* | N.i.[c] |
| 3 | **4**[e] |  | 29%, >99%, *S* | 24%[f] |
| 4 | **5**[d] |  | 47%, >99%, *S* | 19%[f] |
| 5 | **6**[g] |  | 42%, 98.5%, *S* | N.i.[c] |
| 6 | **7**[h] |  | 47%[i], >99%, *S* | 49%[i] |

[a] Isolated yield (based on 50% maximum). [b] 1.0 mmol scale, 10 mM in Tris-sulfate buffer pH 8.1. [c] Not isolated. [d] 2.0 mmol scale, 10 mM in Tris-sulfate buffer pH 8.1. [e] 1.5 mmol scale, 10 mM in Tris-sulfate buffer pH 8.1. [f] Mixture of diols. [g] 16 mmol scale, 1 : 1 toluene–Tris-sulfate pH 8.1. [h] 120 mmol scale, 1 : 10 toluene–Tris-sulfate pH 8.1. [i] Crude yield.

We were especially interested in the possibilities of performing this resolution as a preparative procedure. Interestingly, it was possible to perform this kinetic resolution on multigram scale (Table 2, entries 5 and 6). For instance, starting from 20.9 g of racemic 2-chloro-1-thiophen-2-yl-ethanol (**7**), there was obtained 9.8 g (94% of the theoretical yield) of (*S*)-**7** with an excellent ee of >99%. No modifications of the enzyme were needed to achieve these results. To avoid the use of excessive amounts of buffer solution, the latter two reactions were performed in a two-phase system of toluene and Tris-buffer.

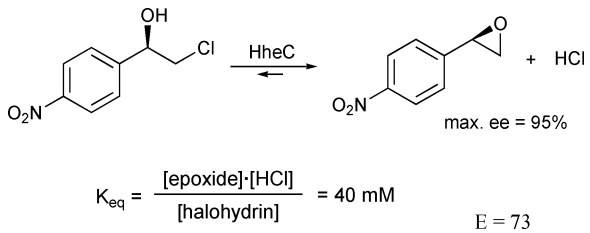
### Role of spontaneous hydrolysis

The spontaneous hydrolysis of the epoxide products mentioned earlier was initially perceived as a drawback of the system. However, comparison of our results with those previously reported for the related compound 2-chloro-1-phenyl-ethanol, made us reconsider.

As illustrated in Scheme 2, when a kinetic resolution is performed on 2-chloro-1-(4-nitrophenyl)-ethanol, the affinity of HheC is larger for the (*R*)- than for the (*S*)-enantiomer. Hence, the (*R*)-enantiomer is converted first. However, the $K_{eq}$ of the equilibrium between (*R*)-2-chloro-1-(4-nitrophenyl)-ethanol and (*R*)-2-(4-nitrophenyl)-oxirane is 40 mM,[6] which indicates that a few percent of the (*R*)-enantiomer will always remain present. Therefore the maximum ee of the remaining chloroalcohol will be about 95%.[5,6]

Presumably, in our system the spontaneous hydrolysis of the formed epoxide pulls the equilibrium to the right side, thus ensuring excellent ee's of the remaining chloroalcohols.

Interestingly, in the case of substrate **7** (Table 2, entry 6) hydrolysis proceeds with almost complete racemization, resulting from equal rates of hydrolysis on the terminal and internal carbon atom of the (enantiomerically pure) epoxide.[19]




**Scheme 2** Incomplete conversion of (*R*)-2-chloro-1-(4-nitrophenyl)-ethanol.

## Conclusions

In conclusion, a highly efficient kinetic resolution protocol was developed for functionalized vicinal chloroalcohols. The majority of these compounds had not been reported before in their enantiomerically pure form. Various unsaturated and heteroaromatic chlorohydrins were resolved in high yields and with excellent enantioselectivities. This resolution was shown to be effective on multigram scale, rendering it highly practical as a preparative method.

## Experimental

### General

Starting materials were purchased from Aldrich or Acros and used as received unless stated otherwise. All solvents were reagent grade and dried and distilled prior to use. Demineralized water was used in the preparation of all aqueous solutions.

Column chromatography was performed on silica gel (Aldrich 60, 230–400 mesh). TLC was performed on silica gel 60/Kieselguhr $F_{254}$ or neutral aluminium oxide 60 $F_{254}$ where indicated.

$^{1}H$ and $^{13}C$ NMR spectra were recorded on a Varian VXR300 (299.97 MHz for $^{1}H$, 75.48 MHz for $^{13}C$) or a Varian AMX400

(399.93 MHz for $^1$H, 100.59 MHz for $^{13}$C) spectrometer in $CDCl_3$ unless stated otherwise. Chemical shifts are reported in $\delta$ values (ppm) relative to the residual solvent peak ($CHCl_3$, $^1$H = 7.24, $^{13}$C = 77.0). Carbon assignments are based on APT $^{13}$C experiments. Splitting patterns are indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad).

Mass spectra (HRMS) were performed on an Jeol JMS-600H. GCMS spectra were recorded on a Hewlett Packard HP6890 equipped with a HP1 column and an HP 5973 Mass Selective Detector.

GC analysis was performed on a Shimadzu GC-17A or a Hewlett Packard HP6890 spectrometer equipped with the columns indicated for each compound separately.

HPLC analysis was performed on a Shimadzu HPLC system equipped with two LC-10AD *vp* solvent delivery systems, a DGU-14A degasser, a SIL-10AD *vp* auto injector, an SPD-M10A *vp* diode array detector, a CTO-10A *vp* column oven, and an SCL-10A *vp* system controller using the columns indicated for each compound separately.

Optical rotations were measured on a Schmidt + Haensch Polartronic MH8 using a 10 cm cell.

### Synthesis of substrates 1–7

Substrates **1–7** were synthesized according to a literature procedure.[10] Spectroscopic data and chromatographic separation conditions will now follow for each of the substrates.

**1-Chloro-pent-3-en-2-ol (1)[20].** Obtained as a colorless oil (2.21 g; 18.3 mmol; 73%) after flash chromatography (pentane–$Et_2O$ 4 : 1); $^1$H NMR ($CDCl_3$) $\delta$ 5.76–5.80 (m, 1H), 5.48 (dd, $J$ = 15.4, 6.6 Hz, 1H), 4.26 (m, 1H), 3.58 ($dd_{ABX}$, $J$ = 11.0, 3.7 Hz, $\Delta\nu_{AB}$ = 45.0 Hz, 1H), 3.47 ($dd_{ABX}$, $J$ = 11.0, 7.3 Hz, $\Delta\nu_{AB}$ = 45.0 Hz, 1H), 2.17 (d, $J$ = 4.0 Hz, 1H), 1.71 (d, $J$ = 6.6 Hz, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 129.7 (d), 129.2 (d), 72.3 (d), 49.6 (t), 17.7 (q); MS (EI+) $m/z$ = 122 ($M^+$), 120 ($M^+$), 107, 105, 71, 53, 41; chiral GC: Chiraldex B-PM, 30 m × 0.25 mm × 0.25 µm, He-flow: 1.1 mL min$^{-1}$, 80 °C isothermic, $T_r$ = 10.2 min (*S*), $T_r$ = 10.9 min (*R*).

**1-Chloro-hex-3-en-2-ol (2).** Obtained as a colorless oil (1.77 g; 13.2 mmol; 66%) after flash chromatography (pentane–$Et_2O$ 6 : 1, gradient to 4 : 1); $^1$H NMR ($CDCl_3$) $\delta$ 5.84 (dtd, $J$ = 15.8, 6.2, 1.1 Hz, 1H), 5.44 (ddt, $J$ = 15.4, 6.6, 1.5 Hz, 1H), 4.28 (br, 1H), 3.59 ($dd_{ABX}$, $J$ = 11.0, 3.7 Hz, $\Delta\nu_{AB}$ = 46.1 Hz, 1H), 3.47 ($dd_{ABX}$, $J$ = 11.0, 7.3 Hz, $\Delta\nu_{AB}$ = 46.1 Hz, 1H), 2.22 (d, $J$ = 3.7 Hz, 1H), 2.06 (qdd, $J$ = 7.3, 6.6, 1.5 Hz, 2H), 0.98 (t, $J$ = 7.3 Hz, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 136.4 (d), 127.0 (d), 72.3 (d), 49.7 (t), 25.2 (t), 13.1 (q); MS (EI+) $m/z$ = 134 ($M^+$), 105, 85, 67, 55; HRMS (EI+) calculated: 134.0498, measured: 134.0491; chiral GC: Chiraldex B-TA, 30 m × 0.25 mm × 0.25 µm, He-flow: 1.0 mL min$^{-1}$, 85 °C isothermic, $T_r$ = 16.7 min (*S*), $T_r$ = 18.0 min (*R*).

**1-Chloro-oct-3-en-2-ol (3)[10,21].** Obtained as a colorless oil (903 mg; 5.55 mmol; 56%) after flash chromatography (pentane–$Et_2O$ 6 : 1, $R_f$ = 0.32); $^1$H NMR ($CDCl_3$) $\delta$ 5.79 (dtd, $J$ = 15.4, 7.0, 1.1 Hz, 1H), 5.44 (ddt, $J$ = 15.4, 6.6, 1.5 Hz, 1H), 4.2–4.35 (m, 1H), 3.58 ($dd_{ABX}$, $J$ = 11.0, 3.7 Hz, $\Delta\nu_{AB}$ = 45.0 Hz, 1H), 3.47 (dd, $J$ = 11.0, 7.7 Hz, $\Delta\nu_{AB}$ = 45.0 Hz, 1H), 2.19 (d, $J$ = 4.2 Hz, 1H), 1.99–2.09 (br, 2H), 1.2–1.4 (br, 4H), 0.87 (t, $J$ = 7.3 Hz, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 135.2 (d), 127.9 (d), 72.4 (d), 49.9 (t), 31.9 (t), 31.0 (t), 22.1 (t), 13.9 (q); MS (EI+) $m/z$ = 162 ($M^+$), 113, 95, 57; chiral GC: CP Chiralsil Dex CB, 25 m × 0.25 mm × 0.25 µm, He-flow: 1.0 mL min$^{-1}$, 120 °C isothermic, $T_r$ = 12.6 min (*S*), $T_r$ = 13.0 min (*R*).

**1-Chloro-octa-3,5-dien-2-ol (4).** Obtained as a colorless oil (1.29 g; 8.0 mmol; 79%) after flash chromatography (pentane–$Et_2O$ 7 : 1, $R_f$ = 0.26); $^1$H NMR ($CDCl_3$) $\delta$ 6.30 (dd, $J$ = 15.0, 10.3 Hz, 1H), 6.02 (dd, $J$ = 15.0, 10.3 Hz, 1H), 5.79 (dt, $J$ = 15.0, 6.6 Hz, 1H), 5.54 (dd, $J$ = 15.4, 5.9 Hz, 1H), 4.35 (br, 1H), 3.60 ($dd_{ABX}$, $J$ = 11.0, 3.7 Hz, $\Delta\nu_{AB}$ = 47.5 Hz, 1H), 3.48 ($dd_{ABX}$, $J$ = 11.0, 7.3 Hz, $\Delta\nu_{AB}$ = 47.5 Hz, 1H), 2.23 (d, $J$ = 3.7 Hz, 1H), 2.09 (dt, $J$ = 13.9, 7.3 Hz, 2H), 0.99 (t, $J$ = 7.3 Hz, 3H); $^{13}$C NMR ($CDCl_3$) $\delta$ 138.4 (d), 133.4 (d), 128.1 (d), 128.0 (d), 72.1 (d), 49.6 (t), 25.6 (t), 13.2 (q); MS (EI+) $m/z$ = 162 ($M^+$), 160 ($M^+$), 111, 93, 55; HRMS (EI+) calculated: 160.06549, measured: 160.06621; chiral GC: CP Chiralsil Dex CB, 25 m × 0.25 mm × 0.25 µm, He-flow: 1.0 mL min$^{-1}$, 125 °C isothermic, $T_r$ = 14.8 min (*S*), $T_r$ = 15.6 min (*R*).

**(*E*)-1-Chloro-4-phenyl-but-3-en-2-ol (5)[7,9,10,21,22].** Obtained after flash chromatography (pentane–$Et_2O$ 5 : 1, $R_f$ = 0.24) as a colorless oil (2.34 g; 12.8 mmol; 64%), which crystallized upon standing; $^1$H NMR ($CDCl_3$) $\delta$ 7.20–7.40 (m, 5H), 6.71 (dd, $J$ = 16.1, 1.1 Hz, 1H), 6.19 (dd, $J$ = 16.1, 6.0 Hz, 1H), 4.52 (br m, 1H), 3.71 ($dd_{ABX}$, $J$ = 11.0, 3.7 Hz, $\Delta\nu_{AB}$ = 48.9 Hz, 1H), 3.58 ($dd_{ABX}$, $J$ = 11.0, 7.3 Hz, $\Delta\nu_{AB}$ = 48.9 Hz, 1H), 2.38 (d, $J$ = 4.4 Hz, 1H); $^{13}$C NMR ($CDCl_3$) $\delta$ 136.0 (s), 132.7 (d), 128.6 (d), 128.1 (d), 127.2 (d), 126.6 (d), 72.3 (d), 49.6 (t); MS (EI+) $m/z$ = 184 ($M^+$), 182 ($M^+$), 133, 115, 105; HRMS (EI+) calculated: 182.0498, measured: 182.0507; chiral HPLC: Chiralcel OD, 40 °C, heptane–IPA 92 : 8, 1.0 mL min$^{-1}$, $T_r$ = 11.5 min (*S*), $T_r$ = 16.0 min (*R*).

**2-Chloro-1-fur-2-yl-ethanol (6)[7,8].** Obtained as a light yellow oil (1.87 g; 12.7 mmol; 64%) after flash chromatography (pentane–$Et_2O$ 4 : 1, gradient to 3 : 1, $R_{f,\,3:1}$ = 0.40); for the resolution on 2.3 g scale, different preparations were combined; $^1$H NMR ($CDCl_3$) $\delta$ 7.39 (s, 1H), 6.36 (s, 2H), 4.93 (m, 1H), 3.83 (m, 2H), 2.53 (d, $J$ = 5.5 Hz, 1H); $^{13}$C NMR ($CDCl_3$) $\delta$ 152.6 (s), 142.6 (d), 110.4 (d), 107.6 (d), 68.0 (d), 47.7 (t); MS (EI+) $m/z$ = 148 ($M^+$), 146 ($M^+$), 97; chiral GC: Chiraldex G-TA, 30 m × 0.25 mm × 0.25 µm, He-flow: 0.5 mL min$^{-1}$, 120 °C isothermic, $T_r$ = 5.1 min (*R*), $T_r$ = 5.4 min (*S*).

**2-Chloro-1-thiophen-2-yl-ethanol (7)[7,23].** Obtained as a colorless oil (2.86 g; 17.6 mmol; 88%) after flash chromatography (pentane–$Et_2O$ 4 : 1, $R_f$ = 0.33); for the 20 g scale resolution, this compound was prepared analogously (64%); $^1$H NMR ($CDCl_3$) $\delta$ 7.29 (dd, $J$ = 5.1, 1.1 Hz, 1H), 7.03 (ddd, $J$ = 3.7, 1.1, 0.7 Hz, 1H), 6.99 (dd, $J$ = 5.1, 3.7 Hz, 1H), 5.15 (ddd, $J$ = 8.1, 4.0, 0.7 Hz, 1H), 3.80 ($dd_{ABX}$, $J$ = 11.4, 4.0 Hz, $\Delta\nu_{AB}$ = 27.4 Hz, 1H), 3.72 ($dd_{ABX}$, $J$ = 11.4, 8.1, $\Delta\nu_{AB}$ = 27.4 Hz, 1H), 2.81 (br, 1H); $^{13}$C NMR ($CDCl_3$) $\delta$ 143.2 (s), 126.9 (d), 125.4 (d), 124.7 (d), 70.2 (d), 50.4 (t); MS (EI+) $m/z$ = 164 ($M^+$), 162 ($M^+$), 113; HRMS (EI+, for $C_6H_7{}^{37}ClOS$) calculated: 163.9877, measured: 163.9881; chiral GC: Chiraldex B-PM, 30 m × 0.25 mm × 0.25 µm, He-flow: 1.1 mL min$^{-1}$, 135 °C isothermic, $T_r$ = 14.2 min (*S*), $T_r$ = 14.8 min (*R*).

### Production and purification of the enzyme

Halohydrin dehalogenase was expressed in *E. coli* MC1061. The *hheC* gene was amplified by PCR from pGEFHheC and cloned

into pBAD/Myc-HisA between *Nco*I and *Pst*I sites. Plasmid DNA was transformed by electroporation to *E. coli* cells, which were then plated out on LB plates containing ampicillin and incubated overnight at 30 °C. A preculture was started by inoculating 100 mL of TB containing 50 μg $mL^{-1}$ ampicillin with the transformants from a plate to a starting OD600 of 0.1. After overnight incubation at 30 °C, the preculture was diluted in 1 L of TB, containing 50 μg $mL^{-1}$ ampicillin, 2.5 mM betaine, 0.5 M sorbitol and 0.02% arabinose, and the culture was incubated for two days at 37 °C. The cells were centrifuged, washed, and resuspended in 50 mL of TEMG buffer (10 mM Tris-$SO_4$, 1 mM EDTA, 1 mM β-mercaptoethanol, and 10% glycerol, pH 7.5) containing a protease inhibitor cocktail (Complete Protease Inhibitor Cocktail Tablets, Roche). Cells were broken by sonication and the extract was centrifuged (50 000 rpm, 45 min, 4 °C). The supernatant was applied on a 50 mL Q-Sepharose anion exchange column and elution was carried out with a gradient of 0 to 0.45 M ammonium sulfate in TEMG. The collected fractions that displayed enzymatic activity were pooled and concentrated. The enzyme was stored at 4 °C or −20 °C.

### General procedure for enzymatic kinetic resolution on analytical scale

To 20 mL of Tris-$SO_4$ buffer (100 mM, pH 8.1) at room temperature, 200 μL of a 1 M stock solution of substrate in DMF were added. Then, 20 μL of a solution of HheC in TEMG[24] were added.[25] Periodically, 1.0 mL aliquots were taken from the reaction mixture, which were extracted with 1.0 mL of toluene containing 5.0 mM of dodecane as an internal standard. The resulting organic solutions were then analyzed by chiral GC.

In the case of substrate **5**, another internal standard was used (cinnamyl alcohol, present in the reaction mixture instead of the extraction solvent) as well as another extraction solvent, heptane. Reactions with this substrate were analyzed by chiral HPLC.

### General procedure for enzymatic kinetic resolution on preparative scale

Typically, reactions were performed analogous to the procedure described for the kinetic resolutions on analytical scale, but on a scale of 1.0–2.0 mmol. This general procedure is for a reaction on 2.0 mmol scale. To 200 mL of Tris-sulfate buffer (100 mM, pH 8.1) at room temperature, 2.0 mL of a 1 M stock solution of substrate in DMF were added. Then, 50 μL of a solution of HheC in TEMG were added.[25] When the reaction had finished, the mixture was extracted with diethyl ether (or ethyl acetate if the aim was to isolate the formed diol as well), the combined organic layers dried on $Na_2SO_4$, filtered, and the solvents evaporated. The crude product(s) obtained were purified by column chromatography, using the conditions described for the racemic substrates.

### Isolated yields and ee's

Specific conditions for the resolutions of substrates **1** and **3–5** are outlined in Table 3. Substrates **6** and **7** were resolved on a larger scale, in a two-phase system consisting of toluene in addition to Tris-sulfate buffer. Although some enzyme deactivation was observed under these conditions, it remained possible to perform these transformations using very low catalyst loadings: $1.5 \cdot 10^{-4}$ mol% and $3.0 \cdot 10^{-4}$ mol% for **6** and **7**, respectively.

**Resolution of substrate 6.** To a mixture of 50 mL Tris-sulfate (2M, pH 8.1) and 50 mL toluene was added a 1 : 1 w/w solution of racemic **6** (2.32 g, 16.1 mmol) in DMF. Then, 225 μg HheC were added. Since after 8 h the conversion turned out to proceed slower than expected, another 255 μg HheC were added, followed by another 176 μg after 32 h (total amount of enzyme: 656 μg). The reaction was stopped after 48 h. Flash chromatography ($SiO_2$, eluent pentane–$Et_2O$ 4 : 1, $R_{f(chloroalc)} = 0.33$) yielded 989 mg (6.75 mmol, 42%) of (*S*)-**5** with an ee of 98.5%.

**Resolution of substrate 7.** A 1 : 1 v/v solution of racemic **7** (20.9 g; 129 mmol) in DMF was added to a mixture of 1 L Tris-sulfate (1M, pH 8.1) and 100 mL toluene. Subsequently, a solution containing 2.68 mg of active HheC was added, followed by another 1.99 mg after 7 h, 0.88 mg after 24.5 h, 1.395 mg after 33.5 h, 1.395 mg after 54 h, 1.53 mg after 76 h, and 0.396 mg after 79.5 h (total amount of enzyme: 10.266 mg). The reaction was worked up after 4 d by extraction with toluene, yielding 9.8 g (60.3 mmol, 47%) of crude (*S*)-**7** with an ee of >99%. To extract the formed diol from the reaction mixture, the residual aqueous layer was evaporated, and the resulting salt slurry extracted with dimethoxypropane and ethyl acetate, yielding 9.1 g (63.1 mmol, 49%) of almost racemic 1-thiophen-2-yl-ethane-1,2-diol.

**1-Thiophen-2-yl-ethane-1,2-diol.**[26] $^1$H NMR ($CDCl_3$) $\delta$ 7.26–7.24 (m, 1H), 7.00–6.96 (m, 2H), 5.03 (dd, $J = 7.3$, 3.7 Hz, 1H), 3.83–3.72 (m, 2H), 3.05 (br, 1H), 2.50 (br, 1H).

### Determination of absolute configuration

The absolute configurations of the remaining enantiomers of the chloroalcohols were determined using several methods.

For (*S*)-(*E*)-1-chloro-4-phenyl-but-3-en-2-ol (**5**), a crystal structure (Cl used as heavy atom) could be obtained[17] (CCDC 605888, CIF available as supplementary information).

Suitable crystals were obtained by slow diffusion of pentane into a concentrated solution of (*S*)-**5** in diethyl ether.

(*S*)-**6** and (*S*)-**7** could be correlated to known compounds by the sign of their optical rotation.[7,8]

**Table 3**

| Substrate | Scale/mmol | Enzyme/μg | Reaction time/h | Isolated yield[a] (mg, mmol, %) |
|---|---|---|---|---|
| (*S*)-**1** | 1.0 | 200 | 5 | 47, 0.40, 40 |
| (*S*)-**3** | 2.0 | 250 | 16 | 100, 0.62, 31 |
| (*S*)-**4** | 1.5 | 300 | 66 | 70, 0.44, 29 |
| (*S*)-**5** | 2.0 | 740[b] | 3 | 173, 0.95, 47 |

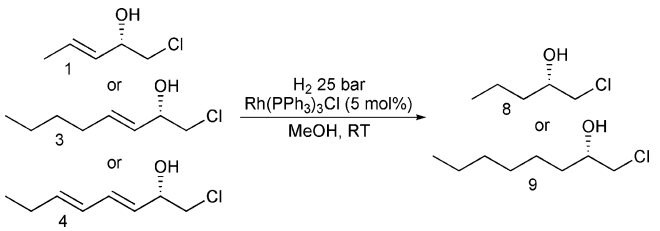
[a] Ee's were >99% in each case. [b] A large amount of enzyme was used to shorten the reaction time.

**6**: $[\alpha]_D^{20} = +29.2$ (*c* 0.452, $CHCl_3$) → *S* (lit. (*S*)-**6** $[\alpha]_D^{20} = +23.0$ (*c* 0.52, $CHCl_3$)).[7]

**7**: Identified as *S*-**7** using the sign of rotation (+). (Lit. (*S*)-**7** $[\alpha]_D^{20} = +28.5$ (*c* 0.53, $CHCl_3$)).[7]

Compounds **1**, **3**, or **4** had not been described before in their enantiomerically pure form. Therefore, they were converted by hydrogenation to their saturated analogues, for which optical rotations are known.[18] Hydrogenation using Pd/C as a catalyst proved unsatisfactory, therefore Wilkinson's catalyst was employed (Reaction 1).




(1)

The unsaturated chloroalcohol (1 mmol) was dissolved in 4 mL methanol together with 5 mol% Wilkinson's catalyst, and this mixture was stirred until the catalyst had dissolved. After various vacuum–$N_2$ cycles, the reaction mixture was put under an atmosphere of $H_2$ (25 bar) and was allowed to react overnight. Then it was filtered over a plug of silica, the solvent evaporated and the residue analyzed. When the product was positively identified as the saturated chloroalcohol, the crude material was purified by flash chromatography, and the optical rotation measured.

**8** (from **1**): $[\alpha]_D^{20} = +1.3$ (*c* 4.6, $CHCl_3$) → *S* (lit. (*S*)-**8** $[\alpha]_D^{20} = +1.1$ (*c* 2.9, $CHCl_3$)).[18]

**9** (from **3**): $[\alpha]_D^{20} = +1.1$ (*c* 6.5, $CHCl_3$) → *S* (lit. (*S*)-**9** $[\alpha]_D^{20} = +1.4$ (*c* 3.1, $CHCl_3$)).[18]

**9** (from **4**): Identified as (*S*)-**9** using the sign of rotation (+).[18]

## Acknowledgements

A. Meetsma is gratefully acknowledged for elucidating the crystal structure of (*S*)-**5**. We thank A. J. A. Gayet, H. J. Heeres, G. N. Kraai, C. Kronenburg, M. Schreuder Goedheijt, and T. Sonke for valuable discussions, and T. D. Tiemersma-Wegman for technical support. Financial support from NWO, the Ministry of Economic Affairs, DSM, and N. V. Organon, administered through the IBOS program, is gratefully acknowledged.

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