Formation of enantiopure 5-substituted oxazolidinones through enzyme-catalysed kinetic resolution of epoxides

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General methods

1H and 13C NMR spectra were recorded on a Bruker AV 300 (1H 300 MHz and 13C 75.5 MHz) spectrometer in CDCl3. Chemical shifts (δ) are given in ppm downfield from TMS as the internal standard. Coupling constants are given in Hz. Mass spectra were recorded on Micromass Quattromicro. Capillary voltage 3.5 kV, cone voltage 40eV. Infrared spectra were recorded on a Bruker-Vector 22 spectrometer. Melting points were determined on Electrothermal 9100 apparatus and are uncorrected. Enzymatic reactions were monitored by GC using a chiral column (Chiraldex G-TA, 30 m x 0.25 mm x 0.25 µm, Astec). GC analysis was performed on a Varian 3350 gas chromatograph equipped with FID detector (set at 250 °C) and a split injector (set at 250 °C), using N2 as a carrier gas, set at a column head pressure of 13 psi. HPLC analysis was performed on a Hewlet-Packard instrument Series 1050 with UV detector at 210 nm using a chiral column (Chirallicia PST 1, 250 mm x 4.6 mm x 5 µm, Chirallicia d.o.o.). Optical rotations were measured on an Optical Activity LTD automatic polarimeter AA-10. Column chromatography was done using silica gel (Merck type 9385, 230-400 mesh). TLC was performed on 0.25 mm silica gel 60-F plates (Merck). Spots
were visualised after dipping the TLC plate in a mixture of phosphomolybdic acid (25 g): cerium(II)sulfate (7.5 g): sulfuric acid (25 mL): water (495 mL) and subsequent heating.

Materials
Wild-type halohydrin dehalogenase from Agrobacterium radiobacter (HheC) was produced and purified from recombinant E. coli cells as described before. Commercial grade reagents and solvents were used without further purification. The commercially available substrates 1,2-epoxybutane (1a), epichlorohydrin (1e), glycidol (1g), 2,3-epoxypropyl-benzene (1h) and enantiomerically pure (R)-1a, (R)-1e and (S)-1e, were supplied by Aldrich, as well as 5-chloromethyl-oxazolidin-2-one (2e). 1,2-Epoxy-2-methylbutane (1b) and 2-(chloromethyl)-2-methyloxirane (1c) were purchased from Acros Organics. 3,3-Dimethyl-1,2-epoxybutane (1j) and 1,2-epoxy-3-methylbutane (1k) were purchased from Lancaster, and 2-vinylxylene (1l) from Fluka. 2-Cyclohexyl-oxirane (1i) was prepared from corresponding aldehyde as previously described. Other racemic epoxides (1d and 1f) were prepared by m-CPBA oxidation of the corresponding alkenes. (R)-1d (80% ee) was prepared by HheC catalysed kinetic resolution of rac-1d.

Preparation of racemic oxazolidinones
Racemic 5-ethyl-oxazolidin-2-one (2a) was prepared according to a modified literature procedure. A mixture of 1-amino-2-butanol (1.5 g, 16.8 mmol), anhydrous K₂CO₃ (225 mg, 1.6 mmol) and diethyl carbonate (3 mL) was stirred under reflux. Over a period of 1 h the ethanol was distilled from the reaction mixture. The resulting mixture was cooled to room temperature, diluted with CH₂Cl₂ and washed with water. Purification of the residue by column chromatography (SiO₂, EtOAc: hexane = 2:8) gave product as a white solid (1.1 g, 57% yield).

Racemic 2b, 2c and 2d were prepared in a three-step procedure starting from rac epoxides 1b-1d.

\[
\begin{align*}
1b-d & \quad \text{(a) NaN₃, H₂O pH 4.2, 30 °C, 5 h; (b) PhOCOCl, Pyridine, CH₂Cl₂, rt; (c) H₂, Pd/C, MeOH, rt, 18 h.}
\end{align*}
\]

Representative procedure: To a solution of epoxide 1c (270 mg, 2.5 mmol) in water (4 mL), NaN₃ was added (840 mg, 13.0 mmol) followed by acetic acid (2.3 mL). The reaction mixture was stirred at 30 °C for 5 h. The mixture was extracted with CH₂Cl₂ (2 x 5 mL), saturated with NaCl and extracted again with CH₂Cl₂ (2 x 5 mL). The organic extracts were washed with 10% NaOH and dried over Na₂SO₄. The residue

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was purified by chromatography (SiO₂, EtOAc: hexane =3:7) providing the pure 1-azido-3-chloro-2-methyl-2-propanol as colorless oil (281 mg, 75% yield). ¹H NMR (CDCl₃) δ 1.33 (3H, s), 2.34 (1H, s), 3.40 (1H, d, J= 12.4 Hz), 3.45 (1H, d, J= 12.4 Hz), 3.54 (1H, d, J= 11.2 Hz), 3.58 (1H, d, J= 11.2 Hz). ¹³C NMR (CDCl₃) δ 22.9, 50.6, 57.5, 72.4

The resulting azidoalcohol (150 mg, 1.0 mmol) was dissolved in 8 mL of CH₂Cl₂, followed by addition of pyridine (200 µL, 4.0 mmol) and phenylchloroformate (400 µL, 2.8 mmol). After stirring overnight at room temperature, the reaction mixture was poured on H₂O (30 mL) and extracted with EtOAc (3 x 40 mL). The extracts were washed with H₂O, aqueous bicarbonate solution, dried with Na₂SO₄, and the solvent was evaporated. The crude product was purified by chromatography (SiO₂, CH₂Cl₂: hexane =2:8) providing the pure 2-azido-1-(chloromethyl)-1-methylethyl phenyl carbonate (247 mg, 91% yield). ¹H NMR (CDCl₃) δ 1.68 (3H, s), 3.74 (1H, d, J= 12.9 Hz), 3.81 (1H, d, J= 12.9 Hz), 3.85 (1H, d, J= 11.7 Hz), 4.04 (1H, d, J= 11.7 Hz), 7.18-7.4 (5H, m). ¹³C NMR (CDCl₃) δ 19.8, 45.9, 54.4, 83.6, 120.9, 121.0, 126.2, 126.3, 129.5, 129.6, 150.7.

The obtained carbonate (242 mg, 0.90 mmol) was dissolved in MeOH (2 mL) and 10% Pd/C (50 mg) was added. Hydrogenation was performed in a closed system at room temperature and at atmospheric pressure of H₂, and the mixture was stirred overnight. The crude product was obtained by filtration of the reaction mixture and purified by chromatography (SiO₂, EtOAc: CH₂Cl₂ =1:1). Pure rac-2c was obtained in 78% yield (105 mg). The NMR data were identical with biocatalytically prepared (S)-2c.

Preparation of 3-benzoyl-5-chloromethyl-oxazolidin-2-one (3e)

To a solution of racemic oxazolidinone 2e (135 mg, 1.0 mmol) in dry THF (4 mL), NaH (1.1 mmol) was added under the inert atmosphere, followed by the addition of benzoyl chloride (300 µL, 2.6 mmol) at 0°C. The reaction mixture was stirred at room temperature for 2.5 h, quenched with an aqueous solution of NaHCO₃ and extracted with CH₂Cl₂. The organic phase was dried over Na₂SO₄. The residue was purified by chromatography (SiO₂, EtOAc: hexane =1:9 to EtOAc gradient elution) providing the pure N-benzoyl oxazolidinone (3e) as a white solid (173 mg, 72% yield). ¹H NMR (CDCl₃) δ 3.76 (1H, dd, J₁= 12.0 Hz, J₂= 3.8 Hz), 3.83 (1H, dd, J₁= 12.0 Hz, J₂= 5.0 Hz), 4.08 (1H, dd, J₁= 11.3 Hz, J₂= 5.6 Hz), 4.29 (1H, dd, J₁= 11.3 Hz, J₂= 8.5 Hz), 4.87-4.95 (1H, m), 7.42-7.69 (5H, m). ¹³C NMR (CDCl₃) δ 44.7, 46.3, 71.7, 127.9, 128.5, 129.0, 132.5, 152.2, 169.4.

Following the same procedure N-benzoyl derivative of enantioenriched (S)-2e was prepared. The NMR data were identical to rac-3e.

Enzymatic preparation of chiral 5-substituted oxazolidinones

Racemic substrate (0.50 g, 100-250 mM) was dissolved in Tris-SO₄ buffer (0.5 M, pH 7.5) followed by addition of NaOCN and purified halohydrin dehalogenase (HheC). The mixture was stirred at room temperature and stopped at conversion <50%. The mixture was saturated with NaCl and extracted with ethylacetate. The organic phase was dried over Na₂SO₄.
(R)-5-ethyl-oxazolidin-2-one (2a). The reaction was carried out for 5 h in 27.5 mL buffer, following the general procedure, and using 0.50 g (6.9 mmol, 250 mM) of racemic 1a, 230 mg (3.5 mmol) of NaOCN and 10 mg of HheC. After extraction, the solvent was evaporated together with unreacted epoxide. Without further purification 2a was obtained in 47% yield (374 mg) as a white solid (chemically pure according to NMR); mp 57-58 °C. The product was determined to be in 80% ee by GC analysis. [α]$^2_D$ +24.0 (c 1.04 CHCl₃). MS (ES⁺)[MH]$^+$ m/z 116.0. Anal.: Calcd for C₅H₉NO₂: C, 52.16; H, 7.88; N, 12.17; Found: C, 52.07; H, 7.95; N, 12.14. $^1$H NMR (CDCl₃) δ 1.01 (3H, t, $J = 7.5$ Hz), 1.66-1.87 (2H, m), 3.25 (1H, dd, $J_1 = 8.0$ Hz, $J_2 = 8.0$ Hz), 3.67 (1H, dd, $J_1 = 8.5$ Hz, $J_2 = 8.5$ Hz), 4.54-4.63 (1H, m), 6.06 (1H, bs). $^{13}$C NMR (CDCl₃) 8.3, 27.4, 45.1, 77.7, 159.9. IR (KBr) 3283, 2968, 2921, 1732, 1244, 1085 cm⁻¹.

(S)-5-chloromethyl-5-methyloxazolidin-2-one (2c). The reaction was carried out for 60 min in 23.5 mL buffer, following the general procedure, using 0.50 g (5.8 mmol, 250 mM) of racemic 1c, 305 mg (4.7 mmol) of NaOCN and 10 mg of HheC. After extraction and purification by chromatography (SiO₂, CH₂Cl₂: EtOAc =1:1), pure (S)-2c was obtained in 46% yield (325 mg, 93% ee) as a white solid; mp 124 °C. [α]$^2_D$ = −15.1 (c 0.99 CHCl₃). MS (ES⁺)[MH]$^+$ m/z 150.0, 151.9. Anal.: Calcd for C₅H₈ClNO₂: C, 40.15; H, 5.39; N, 9.36; Found: C, 40.19; H, 5.41; N, 9.33. $^1$H NMR (CDCl₃) δ 1.56 (3H, s), 3.34 (1H, d, $J = 9.0$ Hz), 3.45 (1H, d, $J = 10.5$ Hz), 3.56 (1H, d, $J = 10.5$ Hz), 3.68 (1H, d, $J = 9.0$ Hz), 6.36 (1H, d, $J = 11.5$ Hz). 3.67 (1H, d, $J = 11.5$ Hz). 3.67 (1H, d, $J = 9.0$ Hz), 6.63 (1H, bs). $^{13}$C NMR (CDCl₃) δ 23.9, 49.0, 63.0, 81.4, 159.0. IR (KBr) 3259, 2953, 1738, 1312, 1095, 988, 760 cm⁻¹.

(S)-5-chloromethyl-oxazolidin-2-one (2e). Racemic epichlorohydrin (1e) (250 mg, 2.7 mmol) was dissolved in Tris-SO₄ buffer (13 mL, 0.5 M, pH 7.5) followed by addition of NaOCN (265 mg, 4.0 mmol) and purified HheC (12.4 mg). The mixture was stirred at room temperature for 3 h (stopped when conversion
reached 100%). The mixture was lyophilized and the solid residue was dissolved in ethylacetate. Solids were removed by filtration and the filtrate was purified by chromatography (SiO₂, EtOAc). (S)-2e (199 mg, 54% yield) was isolated as a white solid; [α]²⁵D − 6.7 (c 0.89 CHCl₃) and [α]³⁵D + 12.5 (c 2 CH₂Cl₂). The ee (69%) was determined by HPLC analysis of the corresponding N-benzoyl derivative (S)-3e, prepared as described previously. MS (ES⁺)[MH]⁺ m/z 136.0, 138.0. Anal.: Caled for C₇H₆ClNO₂: C, 35.44; H, 4.46; N, 10.33; Found: C, 35.59; H, 4.44; N, 10.25.

1H NMR (CDCl₃) 3.56 (1H, ddd, J₁ = 9.0 Hz, J₂ = 5.8 Hz, J₃ = 0.9 Hz), 3.70 (1H, dd, J₁ = 11.5 Hz, J₂ = 6.3 Hz), 3.73 (1H, dd, J₁ = 11.5 Hz, J₂ = 6.6 Hz), 3.78 (1H, dd, J₁ = 8.9 Hz, J₂ = 8.8 Hz), 4.82-4.91 (1H, m), 5.75 (1H, bs). 13C NMR (CDCl₃) 43.6, 44.4, 74.7, 159.3. IR (KBr) 3363, 3327, 2965, 2908, 1748, 1240, 1077, 959, 769 cm⁻¹.

(S)-5-chloromethyl-oxazolidin-2-one (2e). (S)-epichlorohydrin ((S)-1e) (100 mg, 1.0 mmol, 97% ee) was dissolved in Tris-SO₄ buffer (10.5 mL, 0.5 M, pH 7.5) followed by addition of NaOCN (249 mg, 3.8 mmol) and purified HheC (3.0 mg). The mixture was stirred at room temperature for 1.5 h (stopped when conversion reached 100 %). The mixture was lyophilized and the solid residue was dissolved in ethylacetate. Solids were removed by filtration and the filtrate was purified by chromatography (SiO₂, EtOAc). (S)-2e (113 mg, 77% yield, 96% ee) was isolated as a white solid, mp 73-74 °C, [α]²⁴D − 9.0 (c 1.06 CHCl₃), and used to prepare a crystal for X-ray analysis.

Racemisation of (R)-1e and (R)-1d.

To 2.5 mL of Tris-SO₄ buffer (0.5 M, pH 7.5) containing optically pure epoxide (100 mM of (R)-1e or 50 mM of (R)-1d) purified HheC was added (0.75 mg). Samples of 100 µL were taken with regular time intervals, extracted with diethyl ether (1 mL), dried over anhydrous Na₂SO₄ and analysed by GC using a chiral column. The racemisation of (R)-1e was repeated in the presence of NaCl (25 mM). The racemisation of (R)-1d was repeated (a) in the presence of NaOCN (150 mM) and (b) in the presence of NaOCN (150 mM) and KBr (100 mM).

Determination of absolute configurations.

Absolute configurations of epoxides were assigned by chiral GC analysis using reference compounds. The enantiomerically enriched epoxides (R)-1b, (S)-1c (77% ee) and (S)-1d (90% ee) were prepared by (R,R)-(salen)CrN₃ catalysed ring opening with TMSN₃ according to Label and Jacobsen.⁶ The measured optical rotation of oxazolidinones 2a and 2e was compared with the literature values ((R)-2a 80% ee, [α]²⁴D + 24.0 (c 1.04 CHCl₃), Lit.⁷ (S)-2a 99.9% ee, [α]³⁵D − 14.7 (c 1.0 CHCl₃); (S)-2e 69% ee, [α]³⁵D + 12.5 (c 2 CH₂Cl₂), Lit.⁸ (S)-2e 100% ee, [α]²⁰D + 18 (c 2 CH₂Cl₂). On the basis of crystal structure determination the absolute configuration of 2e was confirmed. All other absolute configurations were assigned by analogy.

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Crystallographic data

Suitable platelet-shaped crystals were obtained by recrystallisation from dichloromethane/hexane. 

\( \text{C}_7\text{H}_6\text{ClNO}_2 \), \( M_r = 135.55 \), monoclinic, \( P2_1 \), 
\( a = 9.1140(13) \), \( b = 5.6900(8) \), \( c = 10.7825(16) \) Å, \( \beta = 90.5123(18) \)°, 
\( V = 559.14(14) \) Å\(^3\), \( Z = 4 \), \( D_r = 1.610 \text{ gcm}^{-1} \), \( F(000) = 280 \), \( \mu = 5.81 \text{ cm}^{-1} \), \( \lambda(\text{MoK\(\alpha\)}) = 0.71073 \) Å, 
\( T = 100(1) \) K, 5015 reflections measured, \( \text{GooF} = 1.046 \), \( wR(F^2) = 0.1321 \) for 2623 unique reflections and 193 parameters, 1 restraints and \( R(F) = 0.0509 \) for 2535 reflections obeying the \( F_o \geq 4.0 \sigma(F_o) \) criterion of observability. The asymmetric unit consisted of two molecules of the title compound, which were linked by N-H…O hydrogen bonds into an infinite two-dimensional network. The adopted labelling scheme and the molecular geometry of the asymmetric unit are illustrated in the \textit{PLUTO}\(^9\) drawings of Fig. 1. The arrangement of molecules in the unit cell is shown in Fig. 2. Each asymmetric unit contains two formula units (= molecule). The monoclinic unit cell contains four moieties of the title compound. The chiral centers of C12 and C22 both showed the \textit{S}-configuration.\(^{10,11}\)

\[\text{Fig. 1. Perspective } \text{PLUTO}^9 \text{ drawings showing the configuration of molecule 1 and molecule 2 of the asymmetric unit with the adopted labelling scheme.}\]


\(^{11}\) CCDC reference number 680924.
Fig. 2. View of a unit cell with minimal overlap.

Fig. 3. Perspective ORTEP\(^{\theta}\) drawing of the two crystallographic independent molecules 1 and molecule 2 of the title compound with the atom-labelling scheme of the non-hydrogen atoms. All atoms are represented by their displacement ellipsoids drawn at the 50% probability level. The hydrogen atoms are drawn with an arbitrary radius.
Determination of enantiomeric purity

Enantiomeric excesses (ee's) of epoxides and oxazolidinones were determined by chiral GC analysis on a Chiraldex G-TA column (Col I) (30 m x 0.25 mm x 0.25 μm, Astec), or HPLC analysis on a Chirallica PST 1 column (Col II) (250 mm x 4.6 mm x 5 μm, Chirallica d.o.o.) of the corresponding N-benzoyl derivative (3e).

Table 1. Chiral GC and HPLC analysis.

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