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Sequential Kinetic Resolution Catalysed by Halohydrin Dehalogenase - Supporting Information

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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. Mass spectra (HRMS) were recorded on an AEI MS-902. 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 Hewlett-Packard 6890 series gas chromatograph equipped with FID detector (set at 225 °C) and a split injector (set at 225 °C), using N₂ as the carrier gas and an autosampler. 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)sulphate (7.5 g): sulphuric acid (25 mL): water (495 mL) and subsequent heating.

Materials

Wild-type halohydrin dehalogenase (HheC) and Trp249Phe mutant HheC were produced and purified as described before.¹ Commercial grade reagents and solvents were used without further purification. Epoxide **3** was prepared by reduction of methyl 4-chloroacetoacetate (**1**) (purchased from Acros Organics) with NaBH₄ to racemic alcohol **2**, followed by ring closure to methyl 3,4-epoxybutyrate according to a literature procedure.² Racemic alcohol **4** was prepared by ring opening reactions of epoxide according to a standard procedure.³

Kinetic resolution of (*R,S*)-**3**

Reactions were carried out at 22 °C in 10 mL volumes containing 0.2 M Tris-SO₄ buffer, pH 7.5, 5 mM epoxide, 15 mM NaCN (added from a 200 mM stock solution in water), and 0.5% DMSO. Reactions were initiated by addition of purified enzyme (1.0 mg). The progress of the reaction was followed by periodically taking samples (1 mL) from the reaction mixture. Samples were extracted with diethyl ether (1 mL) containing an internal standard, dried over Na₂SO₄ and analysed by GC.

Kinetic resolution of (*R,S*)-**2**

Substrate (*R,S*)-**2** (0.11 mmol, 50 mM final concentration) was dissolved in Tris-SO₄ buffer (2.2 mL, 0.5 M, pH 7.5). Reactions were performed at ambient temperature (22 °C) and initiated by addition of purified enzyme (1.0 mg). For the ring closure experiment with the formation of (*S*)-**4** (Fig. 2 in the manuscript) NaCN (0.22 mmol, 100 mM final concentration) was used. The progress of the reactions was followed by periodically taking samples (100 µL) from the reaction mixture. Samples were extracted with diethyl ether (1 mL) containing an internal standard, dried over Na₂SO₄, and analysed by GC.

Preparation of methyl (*S*)-4-chloro-3-hydroxybutanoate ((*S*)-**2**) and methyl (*S*)-4-cyano-3-hydroxybutanoate ((*S*)-**4**)

To a solution of methyl 4-chloro-3-hydroxybutanoate (0.50 g, 3.3 mmol) in 62 mL Tris-SO₄ buffer (0.5 M, pH 7.5) NaCN was added (322 mg, 6.6 mmol) followed by addition of 15 mg purified enzyme (Trp249Phe HheC in 3 mL buffer). The reaction was carried out at 22 °C with stirring. After 5 h of incubation the reaction mixture was saturated with NaCl and extracted with ethylacetate (4 x 70 mL). The combined organic extracts were dried with Na₂SO₄ and removed by rotary evaporation. Flash chromatography of the residue using hexane/ethylacetate (7:3) as eluent yielded (*S*)-**2** and (*S*)-**4**.

¹ Tang, L.; van Merode, A. E.; Lutje Spelberg, J. H.; Fraaije, M. W.; Janssen, D. B. *Biochemistry* **2003**, *42*, 14057-14065.

² McClure, J. D. *J. Org. Chem.* **1967**, *32*, 3888-3894.

³ Dexter, A. F.; Lakner, F. J.; Campbell, R.A.; Hager, L. P. *J. Am. Chem. Soc.* **1995**, *117*, 6412-6413.

Methyl (*S*)-4-cyano-3-hydroxybutanoate ((*S*)-**4**) was obtained in 40% yield (190 mg), 96.8% ee, $[\alpha]_D^{24} +26.3^\circ$ (c 1.1 CHCl₃). HRMS calcd. for C₆H₉NO₃: 143.05824. Found: 143.05933. ¹H NMR (CDCl₃) δ 2.61-2.64 (4H, m), 3.72 (3H, s), 4.30-4.39 (1H, m). ¹³C NMR (CDCl₃) δ 25.1, 39.9, 52.1, 64.0, 117.1, 171.8.

The remaining methyl (*S*)-4-chloro-3-hydroxybutanoate ((*S*)-**2**) was obtained in 41% yield (208 mg), 95.2% ee, $[\alpha]_D^{24} -18.9^\circ$ (c 1.27 CHCl₃). HRMS calcd. for C₅H₉ClO₃: 152.02402. Found: 152.02505. ¹H NMR (CDCl₃) δ 2.60 (1H, d, *J*= 7.5 Hz), 2.62 (1H, d, *J*=4.5 Hz), 3.56 (1H, d, *J*= 1Hz), 3.58 (1H, d, *J*= 1Hz), 3.69 (3H, s), 4.19-4.27 (1H, m). ¹³C NMR (CDCl₃) δ 38.3, 48.1, 51.9, 67.8, 172.1.

Determination of enantiomeric purity

Enantiomeric excess (ee) was determined by chiral GC analysis on a Chiraldex G-TA column. The temperature program was isothermal at 100°C for 6 min, increase at 10°C/min to 170°C, final 3 min at 170°C. Retention times were: *R*_t = 6.7 min for (*R*)-**3**, *R*_t = 7.5 min for (*S*)-**3**, *R*_t = 10.5 min for (*R*)-**2**, *R*_t = 10.6 min for (*S*)-**2**, *R*_t = 14.7 min for (*S*)-**4** and *R*_t = 15.2 min for (*R*)-**4**.

Determination of absolute configuration

Absolute configurations were assigned by chiral GC analysis using reference compounds. The enantiomerically enriched epoxide (*R*)-**3** was prepared by (*R,R*)-(salen)CrCl catalysed ring opening with TMSN₃ according to Label and Jacobsen.⁴

Calculation of enantioselectivity

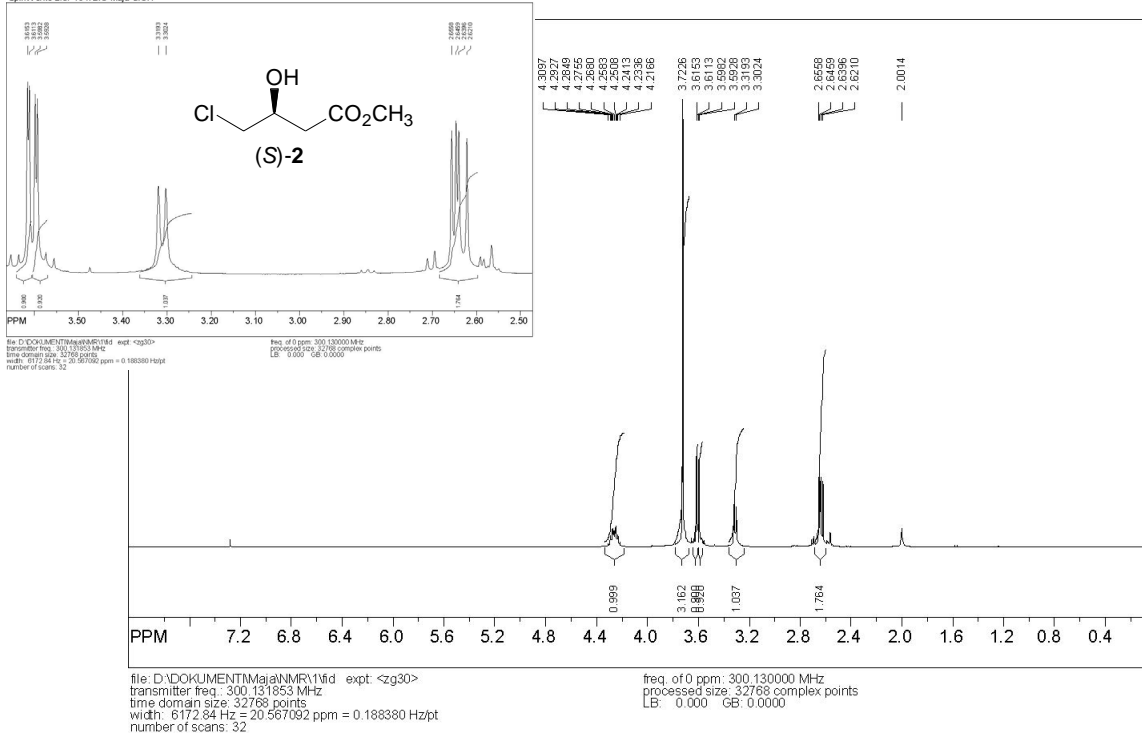
E-values were calculated from ee_p and ee_s according to formula
 $E = \ln[(1-ee_s)/(1+ee_s/ee_p)]/\ln[(1+ee_s)/(1+ee_s/ee_p)]$.⁵

⁴ Label, H.; Jacobsen, E. N. *Tetrahedron Lett.* **1999**, 40, 7303-7306.

⁵ Chen, C.-S.; Fujimoto, Y.; Girdauskas, G.; and Sih, C.J. *J. Am. Chem. Soc.* **1982**, 102, 7294-7298.

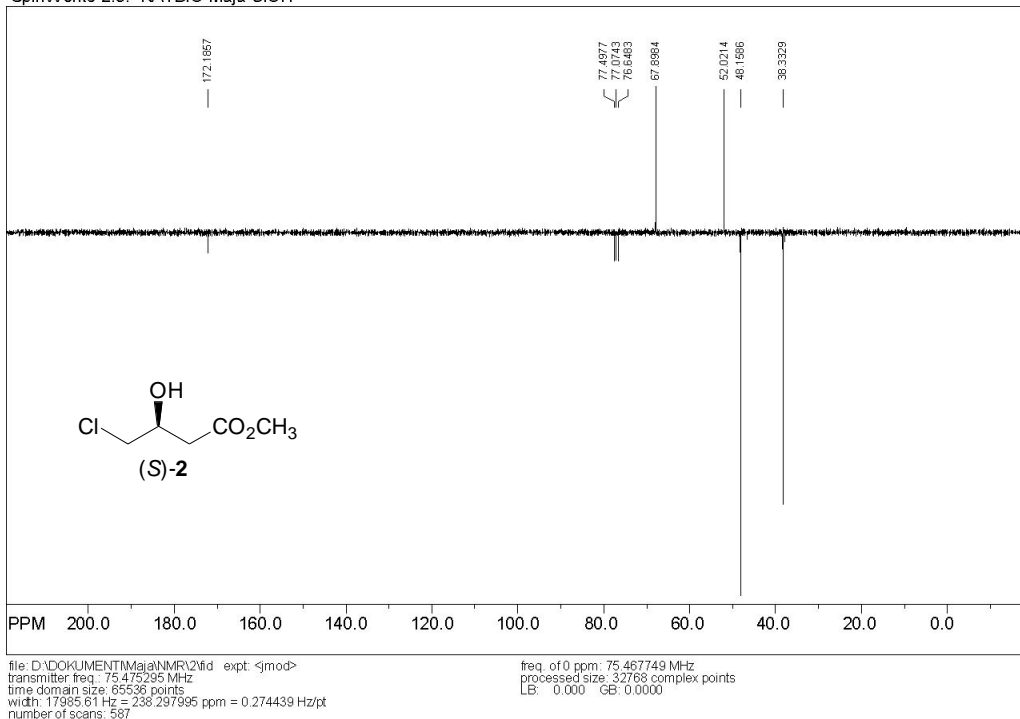
¹H NMR (CDCl₃)

SpinWorks 2.5: KATBIO Maja ClOH



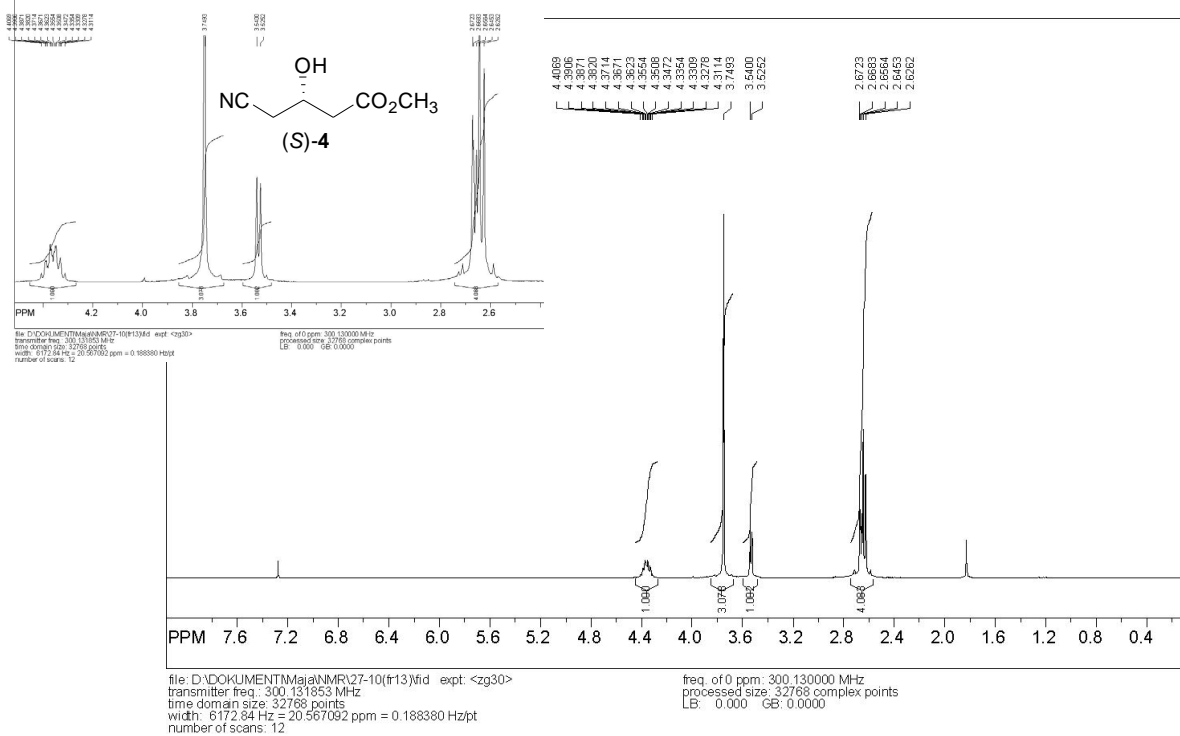
¹³C NMR (CDCl₃)

SpinWorks 2.5: KATBIO Maja ClOH



¹H NMR (CDCl₃)

SpinWorks 2.5: KATBIO Majoric 13



¹³C NMR (CDCl₃)

SpinWorks 2.5: KATBIO Maja CNOH

