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Majeric Elenkov, Maja; Tang, Lixia; Hauer, B.; Janssen, Dick

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Sequential Kinetic Resolution Catalysed by Halohydrin Dehalogenase - Supporting Information

Maja Majerić Elenkov,[a] Lixia Tang,[a] Bernhard Hauer[b] and Dick B. Janssen[a]*

[a] Biochemical Laboratory, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands,

[b] BASF AG, Fine Chemicals & Biocatalysis Research, Ludwigshafen, Germany

*e-mail: d.b.janssen@rug.nl

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General methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 300 (^{1}H 300 MHz and ^{13}C 75.5 MHz) spectrometer in $CDCl_3$. 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 ^{o}C) and a split injector (set at 225 ^{o}C), using N_2 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 $NaBH_4$ 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 ºC in 10 mL volumes containing 0.2 M Tris-SO_4 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 Na_2SO_4 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-SO_4 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 Na_2SO_4, 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-SO_4 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 ºC 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 Na_2SO_4 and removed by rotary evaporation. Flash chromatography of the residue using hexane/ethylacetate (7:3) as eluent yielded (*S*)-**2** and (*S*)-**4**.

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Methyl (*S*)-4-cyano-3-hydroxybutanoate ((*S*)-**4**) was obtained in 40% yield (190 mg), 96.8% ee, $[\alpha]^{24}_{D}$ +26.3º (c 1.1 $CHCl_3$). HRMS calcd. for $C_6H_9NO_3$: 143.05824. Found: 143.05933. ^{1}H NMR ($CDCl_3$) δ 2.61-2.64 (4H, m), 3.72 (3H, s), 4.30-4.39 (1H, m). ^{13}C NMR ($CDCl_3$) 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, $[\alpha]^{24}_{D}$ –18.9º (c 1.27 $CHCl_3$). HRMS calcd. for $C_5H_9ClO_3$: 152.02402. Found: 152.02505. ^{1}H NMR ($CDCl_3$) 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). ^{13}C NMR ($CDCl_3$) 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 $TMSN_3$ 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]

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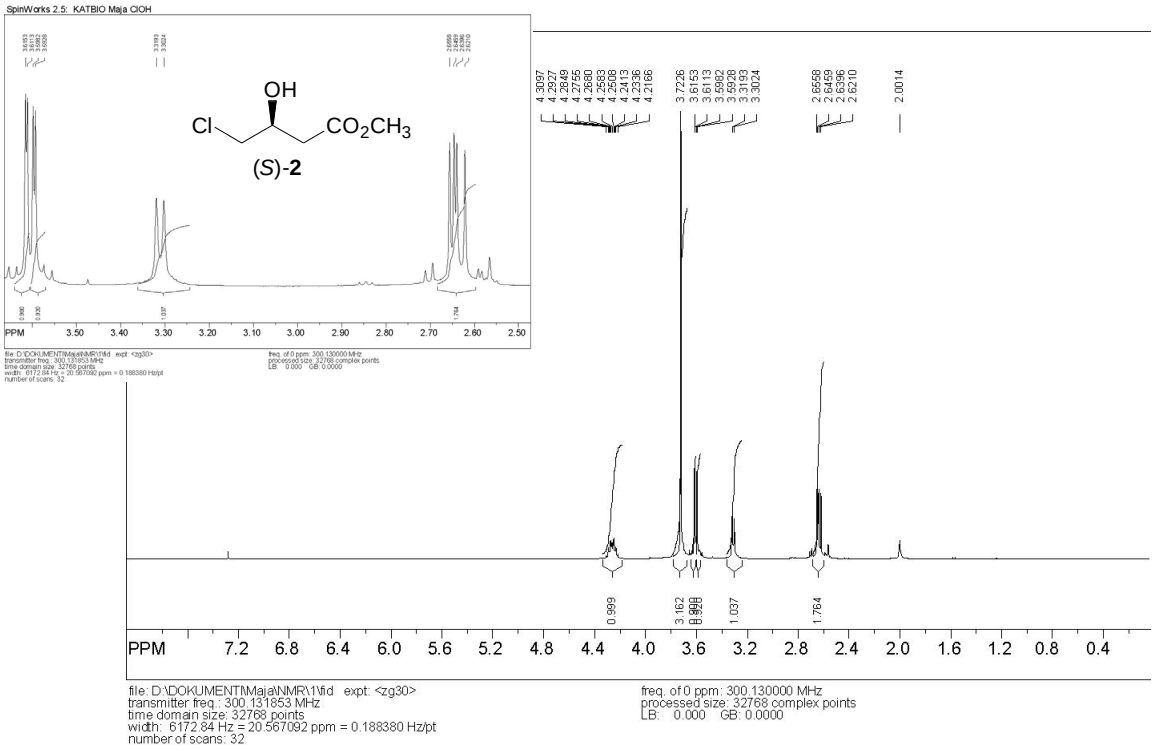
^{1}H NMR ($CDCl_3$)



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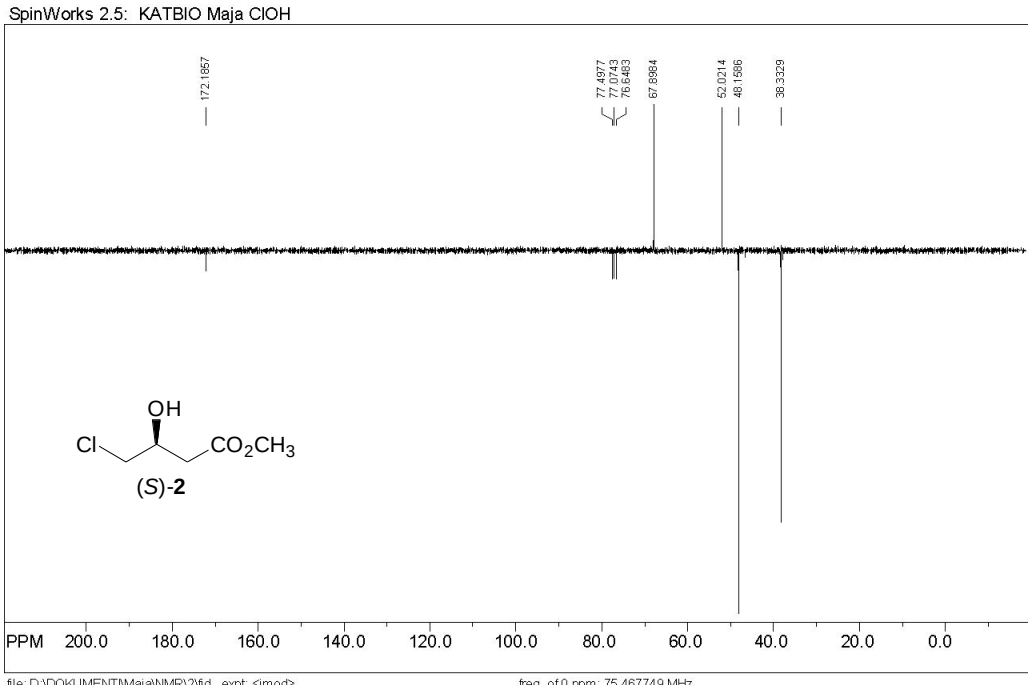

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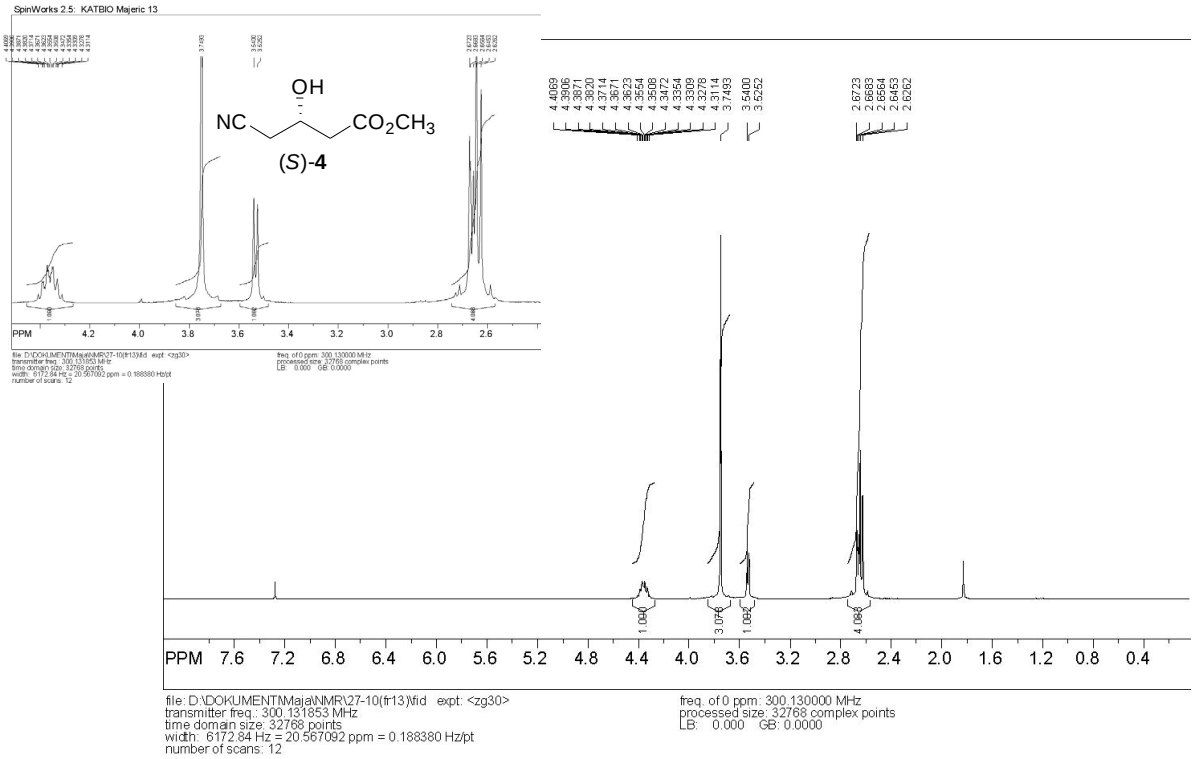

^{13}C NMR ($CDCl_3$)