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Catalytic Promiscuity of Halohydrin Dehalogenase and its Application in Enantioselective Epoxide Ring Opening

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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 (*d*) are given in ppm downfield from TMS as the internal standard. Mass spectra (HRMS) were recorded on an AEI MS-902 instrument. Gas chromatography (GC) was performed on Hewlett-Packard 6890 series gas chromatographs equipped with FID detectors (*T* = 200°C) by using He as the carrier gas and autosampler injection (*T* = 200°C). Chiral analyses were done using a Chiraldex G-TA column (30 m x 0.25 mm x 0.25 µm, Astec) and Chiraldex A-TA column (30 m x 0.25 mm x 0.25 µm, Astec).

Materials: Racemic 1,2-epoxybutane (99% purity) and enantiomerically pure (*R*) and (*S*)-1,2-epoxybutane were purchased from Aldrich Chemie. Racemic 1,2-epoxybutane was distilled under vacuum prior to utilization in enzymatic reactions. Racemic butane-1,2-diol, sodium salts of azide, cyanide, nitrite, chloride, bromide, iodide, cyanate, thiocyanate and formate were obtained from commercial sources and used without further purification.

Synthesis of standard compounds: Racemic a-substituted alcohols **1**[1], **4-6**[2], **8**[3] and **9**[4] (see manuscript, Figure 1) were prepared from 1,2-epoxybutane according to liter-

ature procedures. In the case of **4**, **5**, **6** and **9**, a mixture of the two regioisomers was obtained, while **1** and **8** were obtained as a single regioisomer.

1-Nitrobutan-2-ol (**2**) was obtained by a typical Henry condensation[5] of nitromethane and propionaldehyde in the presence of *t*BuOK. The regioisomer 2-nitrobutan-1-ol (**2a**) was obtained by condensation of nitroethane and acetaldehyde.

1-Cyanobutan-2-ol (**3**) and 2-cyanobutanol (**3a**) were obtained separately by condensation of propionaldehyde on acetonitrile[6] and by alkylation of ethyl cyanoacetate[7] followed by a reduction with $NaBH_4$[8], respectively.

Oxazolidinones **7** and **7a**[9] as well as oxazolidine-2-thiones **8b** and its regioisomer 4-ethyloxazolidine-2-thione (**8c**)[10] were prepared from the corresponding aminoalcohols according to literature procedures. Compound **8a** was obtained by spontaneous rearrangement of **8**.

(*S*)-butane-1,2-diol was obtained by base catalyzed hydrolyzis of enantiopure (*S*)-epoxybutane. Prior to analysis, the diol was derivatized to the acetonide upon reaction with dimethoxypropane promoted with proton-activated Amberlite resin.

Preparation of (*R*)-5-ethyl-5-methyloxazolidin-2-one was done as follows. Racemic 1,2-epoxy-3-methylbutane (0.50 g, 5.8 mmol, 250 mM) was dissolved in Tris-SO_4 buffer (23 mL, 0.5 M, pH 7.5) followed by addition of NaOCN (185 mg, 2.84 mmol) and purified halohydrin dehalogenase (5 mg of HheC). The mixture was stirred at room temperature for 7 h, then saturated with NaCl and extracted with ethylacetate. The organic phase was dried over Na_2SO_4 and solvent evaporated together with unreacted epoxide. Without further purification (*R*)-5-ethyl-5-methyloxazolidin-2-one was obtained in 40 % yield and 97 % *ee* (chemically pure according to GC and NMR). $[a]_D^{20}$ +10.9° (c 1.33 $CHCl_3$). ^{1}H NMR ($CDCl_3$): d = 0.86 (3H, t, J= 7.5 Hz), 1.31 (3H, s), 1.61 (2H, q, J= 7.5 Hz), 3.16 (1H, d, J= 8.5 Hz) 3.28 (1H, d, J= 8.5 Hz). ^{13}C NMR ($CDCl_3$): d = 7.1, 24.5, 32.4, 50.2, 82.9, 159.5. NMR data of synthesized compounds were identical with spectra published in the literature.

Enzyme preparation: Halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 was expressed in *E. coli* and isolated as described earlier[11].

Screening for nucleophile acceptance: Screening for nucleophile acceptance by HheC was done by adding purified enzyme (HheC, final concentration of 10 µM) to a mixture of 25 mM of racemic 1,2-epoxybutane and 25 mM of the nucleophile to be

tested in 2 mL of Tris-SO_4 buffer (100 mM, pH 7.5) and DMSO (0.5 % *v/v*) to facilitate solubilisation of the epoxide. After 2 h the reaction mixture was extracted with diethyl ether containing 1-chlorohexane as the internal standard. Samples were dried over an $MgSO_4$ column.and analyzed by GC. Conditions and retention times are given in Table S1.

Table S1. Chiral GC analysis.

Compound	Column	Conditions[a]	t_R [min][b]
1-azidobutan-2-ol (**1**)	A-TA	I	12.8 (*S*)/13.0 (*R*)
1-nitrobutan-2-ol (**2**)	G-TA	II	13.8 (*S*)/13.9 (*R*)
1-cyanobutan-2-ol (**3**)	G-TA	II	13.9 (*R*)/14.1 (*S*)
1-chlorobutan-2-ol (**4**)	G-TA	II	9.9 (*R*)/10.0 (*S*)
2-chlorobutan-1-ol (**4a**)	G-TA	II	10.6/10.7
1-bromobutan-2-ol (**5**)	G-TA	II	11.0 (*R*)/11.1 (*S*)
2-bromobutan-1-ol (**5a**)	G-TA	II	11.6/11.7
1-iodobutan-2-ol (**6**)	G-TA	II	12.1 (*R*)/12.2 (*S*)
2-iodobutan-1-ol (**6a**)	G-TA	II	12.7
5-ethyloxazolidin-2-one (**7**)	G-TA	III	20.6 (*R*)/23.1(*S*)
4-ethyloxazolidin-2-one (**7a**)	G-TA	III	20.3/20.6
1-isocyanatobutan-2-ol (**8**)	G-TA	II	15.2
2-ethylthiirane (**8a**)	G-TA	II	8.4
5-ethyloxazolidine-2-thione (**8b**)	G-TA	II	27.4/30.6
1-formyloxybutan-2-ol (**9**)	G-TA	II	11.8 (*R*)/11.9 (*S*)
2-formyloxybutan-1-ol (**9a**)	G-TA	II	12.4
1,2-epoxybutane	G-TA	IV	3.9 (*R*)/4.2 (*S*)
butane-1,2-diol[c]	G-TA	I	4.9 (*S*)/5.0 (*R*)
5-ethyl-5-methyloxazolidin-2-one	G-TA	III	20.7 (*R*)/ 21.5(*S*)

[a] GC conditions were: (I) 50ºC, 15ºC/min to 150ºC; (II) 40ºC for 5 min, 15ºC/min to 120ºC for 5 min; (III) 40ºC for 5 min, 15ºC/min, 180ºC for 10 min; (IV) 40ºC for 5 min. [b] Absolute configurations were derived from the optical configuration of the remaining epoxide and the known retention of configuration at the secondary carbon atom during HheC-catalyzed ring opening of an epoxide. [c] Measured as dimethoxy derivative.

Biocatalytic conversions with HheC: To determine initial rates, enantioselectivities, and the nature of the formed products with the accepted nucleophiles, kinetic resolutions of 5 mM epoxybutane were done in 20 mL of a buffered solution (Tris.SO_4, 100 mM, pH 7.5) containing appropriate concentrations of the sodium salt of an anionic nucleophile (10-100 mM). After addition of enzyme, samples were collected at regular times, extracted with diethyl ether, dried with $MgSO_4$, and analyzed by chiral GC under the conditions described in Table **1**. Initial rates were calculated from the initial slope of the epoxide depletion curve. Enantioselectivies were calculated from the *ee* and the conversion of the epoxide according to the formula:

$$E = [\ln[(1-c)(1-ee_S)]/[(1-c)(1+ee_S)]$$

Product identification and quantification was done by co-injection of samples with standard compounds. Ratios between regioisomers of products were determined by GC analysis of concentrated samples from kinetic resolution experiments. The regioselectivity is expressed as the percentage of the major regioisomer over the total amount of product.

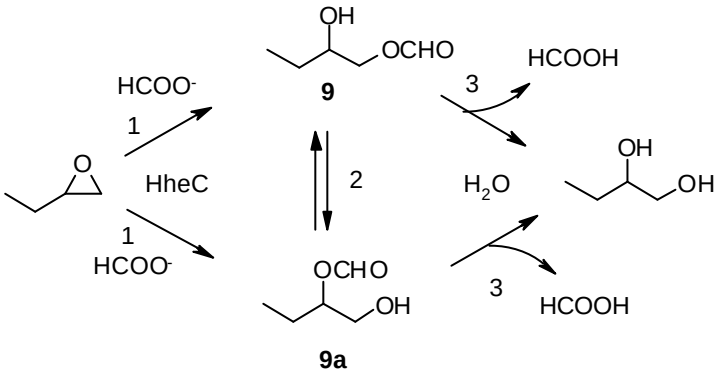

Figure S1: Ring opening of epoxybutane with HheC and formate. A possible route to the formation of both formate regioisomers and butanediol consists of 1) epoxide attack with low regioselectivity and/or 2) spontaneous transesterification, and 3) hydrolysis of formate ester. See text for details.

The ring opening of epoxybutane by HheC in presence of formate resulted in the formation of both regiosomers **9** and **9a** (Figure S1). These products were further hydrolyzed to butane-1,2-diol. To determine if the formation of both regioisomer was due to attack by formate on both carbon atoms of the epoxide (Figure S1a) or by spontaneous transesterification of the a-ring opened product (Figure S1b), enantiopure (*R*)- and (*S*)-epoxybutane (2.5 mM) were ring opened by HheC (1 mg / 5 mL) in the pre-

sence of sodium formate (100 mM) in a buffered solution (5 mL, 100 mM Tris-SO_4, pH 7.5). After complete depletion of the epoxide, and spontaneous hydrolysis of the obtained esters to the corresponding butane-1,2-diol, a sample of 1 mL of the reaction medium was extracted with 2 mL dimethoxypropane. The dimethoxypropane solution was subsequently treated with proton-activated Amberlite resin to promote derivatisation to the acetal of butane-1,2-diol, which was then analyzed by chiral GC. The absolute configuration of the formed diol was determined by co-injection of the derivatized samples with standard acetal of (S)-butane-1,2-diol. The analysis showed that the ring opening of both enantiopure (*R*) and (*S*)-epoxybutane occurred with retention of configuration, indicating that formate attacked the epoxide on the terminal carbon.

Enzymatic ring opening of styrene oxide with azide to produce (*R*)-1-azido-2-hydroxy-styrene was done as described previously for aromatic azidoalcohols[12]. Epoxide ring opening with cyanide and product isolation were done as described by Majeric-Elenkov et al.[13] Details on the production of nitroalcohols with halohydrin dehalogenase will be published elsewhere.

References

[1] F. Fringuelli, O. Piermatti, F. Pizzo, L. Vaccaro. *J. Org. Chem*. **1999**, *64*, 6094-6096.

[2] B.-I. Halperin, H.B. Danahoe, J. Kleinberg, C.A. Vanderwerf. *J. Org. Chem.* **1952**, *17*, 623-629.

[3] N. Iranpoor, G.A. Kohmareh. *Phosphorus, Sulfur and Silicon* **1999**, *152*, 135-139.

[4] H. Hagiwara, K. Morohashi, H. Sokai, H, T. Suzuki, M. Ando. *Tetrahedron* **1998**, *54*, 5845-5852.

[5] C.R. Henry *Acad. Sci. Paris* **1985**, *120*, 1265.

[6] T. Itoh, K. Mitsukura, W. Kanphai, Y. Takagi, H. Kihara, H. Tsukube. *J. Org. Chem.* **1997**, *62*, 9165-9172.

[7] A. Ghaib, S. Ménager, P. Vérité, O. Lafont. *Il Farmaco* **2002**, *57*, 109-116.

[8] J.A. Marshall, R.D. Carroll. *J. Org. Chem*. **1965**, *30*, 2748-2754.

[9] J. R. Gage, D.A. Evans. *Organic Syntheses, Coll.* **1993**, *8*, 528-530.

[10] Y. Wu, Y. Yang, Q. Hu. *J. Org. Chem*. **2004**, *69*, 3990-3992.

[11] L. Tang, J.E.T. van Hylckama Vlieg, M.W. Fraaije, D.B. Janssen. *Enz. Microb. Technol.* **2002**, *30*, 251-258.

[12] J.H. Lutje Spelberg, J.E.T. Van Hylckama Vlieg, L. Tang. D.B. Janssen, R.M. Kellogg, R.M. *Org. Lett.* **2001**, 3, 41.

[13] a) M. Majeric Elenkov, B. Hauer, D.B. Janssen. *Adv. Synth. Catal*. **2006**, *348*, 579. b) M. Majeric Elenkov, W. Hoeffken, L. Tang, B. Hauer, D.B. Janssen. *Adv. Synth. Catal.* **2007** 349, 2279-2285.