Supporting information

General

The enantiomeric excesses (e.e.) and/or the yields of **1a**, **1b** and **1c** and *para*-nitrophenylethanediol were determined by HPLC using a Chiralpak AS column from Daicel. The enantiomeric excesses (e.e.) and/or yields of **2a**, **2b**, **2c**, **3a**, **3b**, and **3c** were determined by chiral GC using a Chiralsil Dex CB column from Chrompack.
Substrate depletion curves of epoxide **1a** were recorded on a Perkin Elmer Lambda BIO 40 spectrophotometer provided with a temperature-controlled cell holder. NMR-spectra were recorded in $CDCl_3$.

Production of halohydrin dehalogenase

A gene library of *A. radiobacter* AD1 was constructed in the cosmid vector pLAFR3. After in vitro packaging, the library was transduced to *E. coli* HB101. Transconjugants were screened for dehalogenase activity with 1,3-dichloro-2-propanol. The halohydrin dehalogenase gene, designated hheC, was sequenced and subsequently amplified by PCR and cloned behind the T7 promotor of the expression vector $pGEF^+$,[1] yielding pGEFhheC. The halohydrin dehalogenase gene was overexpressed up to 30% of soluble protein by introduction of pGEFhheC in *E. coli* BL21(DE3).
For the described kinetic resolutions purified enzyme was used. Plasmid DNA was transformed by electroporation to competent *E. coli* BL21 (DE3) cells, which were then plated out on LB medium containing tetracycline and incubated overnight at 30 °C. A preculture was started by inoculating 100 ml of LB medium containing tetracycline with the transformants from a plate to an initial OD_{600} of 0.1. The culture was incubated at 30 °C until an OD_{600} of 1-2 was reached, diluted in 1 l of LB medium containing tetracycline and incubated overnight at 20 °C. The cells were subsequently centrifuged, washed and resuspended. A crude extract was prepared by ultrasonic disruption and centrifugation of the cells. This was followed by a purification step with a Resource Q column. The overexpression and production of the halohydrin dehalogenases from *Mycobacterium* sp. GP1 and *Arthrobacter* sp. AD2 will be published in a separate paper.[2]

Synthesis of substrates and reference compounds

Racemic *para*-nitrostyrene oxide 1a

To a cooled solution of ω-bromo-*para*-nitro-acetophenone (5.0 g, 20 mmol) in MeOH (50 ml), sodium borohydride was added (1.0 g, 26 mmol) and stirred for 3 h. Water (50 ml) was added and the mixture was extracted with diethyl ether. After separating, the organic phase was washed with brine, dried with $MgSO_4$ and the solvent wasremoved by a rotary evaporator yielding 4.1 gram of a orange solid. To 1.0 gram of the solid dissolved in diethyl ether 15 ml of an aqueous solution (1 M) of KOH was added. The mixture was refluxed for 15 min, cooled, diluted with sulfuric acid (20 ml, 1 M) and

extracted with diethyl ether. After separation, the organic layer was dried with $MgSO_4$, and removed by a rotary evaporator. Recrystallisation in ethanol yielded 0.76 gram of **1a**. ^{1}H NMR, δ: 2.72 (dd, 1H, *J* = 2.6 Hz and 5.5 Hz); 3.17 (dd, 1H, *J* = 4.0 Hz and 5.5 Hz); 3.91 (dd, 1H, *J* = 2.6 Hz and 4.0 Hz); 7.40 (d, 2H_{ar}, *J* = 8.8 Hz); 8.16 (d, 2H_{ar}, *J* = 8.8 Hz) ^{13}C NMR δ: 48.9 (C-1); 49.2 (C-2); 121.3; 123.7; 142.7; 145.3 (C_{ar}).

Racemic *para*-chlorostyrene oxide 2a

To a solution of *para*-chlorobenzaldehyde (2.8 gram, 19.9 mmol) in CH_2Cl_2 (20 ml) trimethylsulfonium methylsulphate was added (4.4 gram, 23.3 mmol). Aqueous NaOH (50 %, 10 ml) was added and the reaction mixture was stirred overnight. Water was added and the organic phase was separated. The water phase was extracted twice with CH_2Cl_2. The combined organic phase was washed twice with 20 ml portions of water, subsequently shaken for 20 minutes with a saturated solution of sodium metabisulphite and finally washed twice with 20 ml portions of water. The organic phase was dried and the CH_2Cl_2 was removed on a rotary evaporator. The *para*-chlorostyrene oxide was obtained by flash chromatography on silica 60 H.
^{1}H NMR, δ: 2.88 (dd, 1H), 3.27 (dd, 1H), 3.96 (dd, 1H), 7.32-7.46 (m, 4H), ^{13}C-NMR δ: 51.0 (C-2), 51.5 (C-1), 126.7, 128.6, 133.8, 136.1 (C_{ar}).

Racemic azido alcohols

To a 400 mM solution of sodium azide in water (300 ml), 200 mg of racemic **1a** was added and the mixture was stirred for 15 h at room temperature. The water phase was extracted four times with diethyl ether. After separating, the organic phase was dried with $MgSO_4$ and removed by a rotary evaporator yielding an orange oil that consisted of **1b** and **1c** in a 37:63 ratio. Flash chromatography on silica 60 H using heptane/ethyl acetate (ratio 7:3) yielded pure **1b** and **1c**.
2-azido-1-(*para*-nitro-phenyl)-ethanol **1b**: ^{1}H NMR, δ: 2.49 (d, 1H, OH, *J* = 3.7 Hz); 3.45 (m, 2H); 4.95 (m, 1H); 7.51 (d, 2H_{ar}, *J* = 8.8 Hz); 8.17 (d, 2H_{ar}, *J* = 8.8 Hz). ^{13}C NMR δ: 55.3 (C-1); 70.0 (C-2); 121.3; 124.3; 145.1; 145.3 (C_{ar}).
2-azido-2-(*para*-nitro-phenyl)-ethanol **1c**: ^{1}H NMR, δ: 1.99 (t, 1H, OH, *J* = 5.5 Hz); 3.75 (m, 2H); 4.74 (dd, 1H, *J* = 4.4 Hz and 7.3 Hz); 7.49 (d, 2H_{ar}, *J* = 8.8 Hz); 8.21 (d, 2H_{ar}, *J* = 8.8 Hz).^{13}C NMR δ: 63.9 (C-1); 64.2 (C-2); 121.5; 125.6; 141.2; 145.3(C_{ar}).
The same procedure was used for the azidolysis of epoxides **2** and **3**. The product mixture was identified by NMR and GC. The product ratio of the two regio-isomers **b** and **c** were close to identical to previous reports.[3]

Optically pure (R) and (S) *para*-nitrostyrene oxide 1a

Optically pure (**R**)-**1a** and (**S**)-**1a** were obtained from racemic **1a** by preparative HPLC using an analytical Chiralpak AS column with hexane/isopropanol (95:5) as eluent (1 ml/min). Yields and retention times of the individual enantiomers: (**R**)-**1a**, yield 39.2 mg, retention time 17.2 min, e.e.>99%; (**S**)-**1a**, yield 37.0 mg, retention time 25.3 min.

Influence of pH and sodium concentration on initial activity

A small volume of freshly prepared stock solution of (**R**)-**1a** in DMSO (< 0.5% v/v) was injected in a cuvet containing 1.00 ml of the appropriate buffer at 30°C to a concentration of 250 μM. The reaction was started by adding the purified enzyme and 50 μl of a stock solution of sodium azide to a final concentration of 0.1 mM to 20 mM. The enzymatic azidolysis rate was monitored by the decrease in absorbance at 310 nm. The following buffers were used: pH 6.1 and pH 6.8, 50 mM potassium phosphate; pH 7.0, 50 mM MOPS-NaOH; pH 7.4 and pH 8.5, 50 mM Tris-SO_4; pH 9.0 and pH 10.0, 50 mM glycine-NaOH.

Enzymatic conversions

Typical kinetic resolution experiment: to 20 ml of Tris-SO_4 buffer (50 mM, pH = 7.3, 30 °C) containing 2 mM of the epoxide and 1.3 mM of NaN_3, purified enzyme was added to a concentration of 8 μM. The reaction was monitored by periodically taking 1 ml samples and extracting them with 1.5 ml of diethyl ether containing an internal standard. The organic phase was analysed by chiral HPLC and GC. The bimolecular reaction constants (K_{az}) of chemical azidolysis of the epoxides were determined from the slope of a $k_{observed}$ vs [NaN_3] plot.

Large scale conversion: To 60 ml of MOPS buffer (50mM, pH = 7.0), 0.47 gram (3.2 mmol) of racemic **1a** was added and the suspension was stirred for 60 min. After addition of 29 mg of the enzyme, a prepared stock solution of 0.6 molar equivalents sodium azide in 5 ml MOPS buffer was slowly added over a period of 24 hours. The reaction was stopped and the suspension was extracted three times with diethyl ether. After separation, the organic phase was dried with $MgSO_4$, and removed by a rotary evaporator yielding an orange oil. This mixture was redissolved in diethyl ether and the composition and e.e. of the products were determined by chiral HPLC. The yields given in the text are calculated yields. Flash chromatography on silica 60 H using heptane/ethylacetate (ratio 7:3) yielded pure epoxide and azido alcohols. The NMR data were identical with the synthesized racemic compounds.

Absolute configurations and enantioselectivity

The absolute configurations of the azido alcohols were established by chemical azidolysis of the optically pure epoxide to the corresponding azido alcohols. The products and side product were analysed by chiral HPLC. Retention times eluent hexane/isopropanol (92:8): (**R**)-**1a**, 10.8 min; (**S**)-**1a**, 15.9 min; (**R**)-**1b**, 41.1 min; (**S**)-**1b**, 26.2 min; (**R**)-**1c**, 18.7 min; (**S**)-**1c**, 22.6 min; *para*-nitrophenylethanediol 30.9 and 35.6 min. The enantiomeric excess (e.e.) and yields of **2a**, **2b**, **3a**, and **3b** were determined by chiral GC using a Chiralsil Dex CB column from Chrompack.
The regioselectivity of formation of **1b** versus **1c** was calculated using Formula 3.

$$\beta - \text{regioselectivity} = \frac{\beta_{attack}}{\beta_{attack} + \alpha_{attack}} \qquad (3)$$

The enantioselectivity of the kinetic resolutions was calculated using Formula (1) (ee_s is the e.e. of the substrate **a** and ee_p that of azido alcohol **b** and Formula (2) (c equals the conversion of substrate **a**. [4,5]

$$E = \frac{\ln[(1-ee_S)/(1+ee_S/ee_P)]}{\ln[(1+ee_S)/(1+ee_S/ee_P)]} \qquad (1)$$

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} \qquad (2)$$

The outcome of the above formulas will be the same if no non-enzymatic side reaction occurs. In the case of the kinetic resolutions described in this article, a chemical side reaction to product **c** occurs. This will result in an overestimated enzymatic conversion and thus an erroneous E-value(apparent E-value) when formula (2) is used. The use of formula (1) is insensitive to the chemical conversion and results in the intrinsic E-value of the enzyme catalysed kinetic resolution.

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(1) Schanstra, J. P.; Rink, R.; Pries, F.; Janssen, D. B. *Protein Exp. Purif.* **1993**, 4, 479

(2) van Hylckama Vlieg, J. E. T.; Tang, L.; Lutje Spelberg, J. H.; Smilda, T.; Poelarends, G. J.; Bosma, T.; van Merode, A. E. J.; Janssen, D. B. *manuscript in preparation*

(3) a) Guy, A.; Doussot, J.; Dallamaggiore, A.; Garreau, R. *Can. J. Chem.* **1995**, 73, 599 b) Fringuelli, F.; Piermatti, O.; Pizzo, F.; Vaccaro, L. *J. Org. Chem.* 1999, 64, 6094

(4) Straathof, A. J. J.; Jongejan, J. A. *Enzyme Microb. Technol.* **1997**, *21*, 559

(5)C. S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294