Supporting Information for

Asymmetric azidohydroxylation of styrene derivatives mediated by a biomimetic styrene monooxygenase enzymatic cascade

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## 1. General information

All chemicals were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany), TCI Chemicals Europe (Tokyo Chemical Industry, Tokyo, Japan), abcr GmbH (Karlsruhe, Germany) or Alfa Aesar (Thermo Fisher Scientific, Ward Hill, MA, USA) and were used without further purification (Table S1). Catalase (2100 U/mg) from bovine liver (EC 1.11.1.6) was purchased from Sigma-Aldrich.

### Table S1. Purity of commercially available chemicals bought from chemical vendors.

| Chemical                          | Purity (%, ee (%), by GC) | CAS     |
|----------------------------------|---------------------------|---------|
| styrene                          | ≥99                       | 100-42-5|
| (±)-styrene oxide                | 98                        | 96-09-3 |
| (R)-(+)-styrene oxide            | 97, 97                    | 20780-53-4|
| (S)-(−)-styrene oxide            | 98, 98                    | 20780-54-5|
| α-methylstyrene                  | 99                        | 98-83-9 |
| trans-β-methylstyrene            | 99                        | 873-66-5|
| (1S,2S)-(−)-1-phenylpropylene oxide | 98                    | 4518-66-5|
| cis-β-methylstyrene              | 98                        | 766-90-5|
| 4-bromostyrene                   | 97                        | 2039-82-9|
| (±)-4-bromostyrene oxide         | 96                        | 32017-76-8|
| 3-bromostyrene                   | 97                        | 2039-86-3|
| 2-bromostyrene                   | 97                        | 2039-88-5|
| 4-chlorostyrene                  | 97                        | 1073-67-2|
| (±)-4-chlorostyrene oxide        | 96                        | 2788-86-5|
| 3-chlorostyrene                  | 98                        | 2039-85-2|
| 2-chlorostyrene                  | 96                        | 2039-87-4|
| 4-fluorostyrene                  | 99                        | 405-99-2 |
| (±)-4-fluorostyrene oxide        | 97                        | 18511-62-1|
| (R)-4-fluorostyrene oxide        | ≥98, 98                   | 134356-73-3|
| 3-fluorostyrene                  | 97                        | 350-51-6 |
| 2-fluorostyrene                  | 98                        | 394-46-7 |
| 4-methylstyrene                  | 96                        | 622-97-9 |
| 3-methylstyrene                  | 98                        | 100-80-1 |
| allylbenezene                    | 98                        | 300-57-2 |
| 1,2-dihydronaphthalene           | 98                        | 447-53-0 |
| 2-tetralone                      | 98                        | 530-93-8 |
| indene                           | ≥99                       | 95-13-6 |
| 2-phenanalone                    | 98                        | 615-13-4 |
| 2-vinylpyridine                  | 97                        | 100-69-6 |

*Sigma-Aldrich in white, TCI Europe in yellow, Alfa Aesar in blue, abcr GmbH in green.

The HHDH enzyme screening kits were kindly provided by Enzymicals AG (Greifswald, Germany, Table S2).

### Table S2. Enzyme abbreviation, accession number and sources of HHDH enzymes.

| Enzyme abbrev. | Protein accession number | Origin (strain)                   |
|----------------|--------------------------|-----------------------------------|
| HheA3          | AB564560                 | *Parvibaculum lavamentivorans* DS-1|
| HheA5          | AFK51877                 | *Tistrella mobilis* KA081020-065  |
| HheB5          | ECR06649                 | marine metagenome (Burkholderia)  |
| HheD3          | ABM93639                 | *Methylibium petroleiphilum* PM1  |
| HheD4          | ECY18578                 | marine metagenome (Haliangium)    |
| HheD5          | YP_002355872             | *Thauera* sp. MZ1T                |
| HheD6          | ENO15189                 | *Marinobacter nanhaiticus* D15-8W |
| HheE4          | EDH34310                 | marine metagenome (Catenulispora) |
| HheE5          | EGG28524                 | gamma proteobacterium strain IMCC3088|
| HheF           | BAH89601                 | uncultured bacterium              |
HPLC-grade ethyl acetate (EtOAc) was used for extractions. Thin-layer chromatography (TLC) was performed on silica gel 60 F\textsubscript{254} aluminum sheets (EMD Millipore, Merck, Darmstadt, Germany). Organic solutions were concentrated under reduced pressure with a rotary evaporator (\textit{in vacuo}). Flash column chromatography was carried out with Silicycle SiliaFlash P60 silica gel (40-63 μm, 230-400 mesh) with mixtures of distilled petroleum ether (boiling range 40-60 °C) and EtOAc as eluent.

\(^1\)H and \(^{13}\)C nuclear magnetic resonance (NMR) spectra were recorded on a Varian 400 or a Bruker Avance III 400 NMR spectrometer, internally referenced to residual proton signals in CDCl\textsubscript{3} or D\textsubscript{2}O. Chemical shifts (\(\delta\) in ppm) are reported with multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (J values in Hz), integration and assignment.

Gas chromatography (GC) measurements were carried out on a Shimadzu GC-2010 gas chromatograph (Shimadzu corporation, Kyoto, Japan) equipped with a flame ionization detector (FID). GC mass spectrometry (MS) measurements were obtained on a Hewlett Packard HP6890 and 5973 MSD.

2. Experimental procedures

2.1. Molecular genetics, protein production and purification

Styrene monooxygenases StyA from \textit{Pseudomonas} sp. VLB120,\(^1\) and StyA1 from \textit{Rhodococcus opacus},\(^2\) were produced and purified as previously described.\(^3\) The \textit{styA} gene (accession number: AJA07151; coding for \textit{SfStyA}) originating from \textit{Sphingopyxis fribergensis} Kp5.2 was obtained and transformed into \textit{E. coli} BL21(DE3)pLysS cells.

2.1.1. Sequence information and accession numbers

\textbf{StyA from \textit{Pseudomonas} sp. VLB120}

accession number: O50214

>sp|O50214|STYA_PSESP Styrene monooxygenase StyA OS=Pseudomonas sp. OX=306
GN=styA PE=1 SV=1
MKKRIGIVGATLHLGLFLRQHDVTVYTDORPDYESGLRULLNTVHAHNAVTQREVA
LDVNEWSEPSEGFGHYYYYGQPIMRFGYDLKAPSRAVYLYQPMRLEARGGKFC
YAVASDLEGELSEQDVECTKVALKYVKVEKQSENPEFEKPRALCVGLFKGHEAP
IRAVTMEFGHGLIEIPTGMSALFVLENHIAGDLEVAHLYDAPARRFPLDLML
EKLGKHPSVAERIDPAEFDLNASLDDILQGQGVPAPRDGHDATLNNNGTILGIDIATV
DPVLQGQANMASYAAWILGEELAHSVYLRFSELERRQDRVLCATRWTLNSALSA
LPFEPLAFQILQSREMADEFTDNNFYPERQWDRFSSPERIGQWSQFAPTTAA

\textbf{StyA1 from \textit{Sphingopyxis fribergensis} Kp5.2}

accession number: A0A07PAQ5

>tr|A0A07PAQ5|A0A07PAQ5_9SPHN Styrene monooxygenase StyA OS=Sphingopyxis fribergensis OX=1515126 GN=styA PE=4 SV=1
MSKKIGIIGATGLKLHLLNKQVEKLFDTDRPQEEYAGMRLLLNTVAHHVHVTREDK
LGTVNEPSEGEMNYYQIPPEFLQYPHDVLVAPSRAVDYRYQPMQDFIDRGGIEYG
QIACEDLDAIEFDPDLVCTKGFPQFQMPTHEPAYSFDFPQRALCVGLFKGIREPETR
ALTMYFSGHGEMIEIPTLSFSMNYALVENIHIGDLEIARKYDDDPKAFIALLLEK
LQKHYPTCYERIDLEFDPDNLGQILQGVTPTVRNSYAKLPGNKIAVALGVDQAVVDP
VLPQGQANMASYAAWILGEELAHSVYLRFSELERRQDRVLCATRWTLNSALSA
PQNLQIGAAMQPKLATESTENFNPFEKQWDCSSPERVQAWIQARLGTANDAEELVA
AE
**SfStyA from *Sphingopyxis fribergensis* Kp5.2**

>styA (codon optimized gene with adjacent sites of restriction enzymes NdeI and NotI which are underlined; length: 1280 bp)

```
CAT ATG TCC AAG AAA ATT GGT ATC ATT GGA GCG GGG ACT GCG GGT CTG AAA CTG
GGA CTT GAA CGG CAT GGG TAT GGT ATT CAC TGG CCT GAC GTC GGC TAT
AAA GGG CAC TAC TAT TAT ATC GGC ACA CCG GAG CCT CTG CAG TTC TAT GGC GAC
CTG GAT TGG TCT CCA TGG GCT ATC ATC GGC ATG GAC GGG ATC GAA TAT GGC ACT GAA
CAG TAT GCC GAT ATT GAC GAA ATT CGG GAC GGG GTC GAC GGG CCG GCG GAC
GCT GAT GAT ACC TGG GCT GAT TGT ATC AAC ATT GGC GAA GTT GAG AGT GGA
GCT GAT GAT GCG ATT GCT GAC GAA TTC GAC TCT GCA GAC GGG GAG GAA ATC GTG
GTC AAT GCT CTC GAT GAA AAG CAA GAC CTA GTG GCT GAT GCT TAT TGT TAT TG
GAG AGT TAA
```

accesion number: AJA07151

>AJA07151.1 Styrene monooxygenase StyA [*Sphingopyxis fribergensis*]

MSKKIGIIGAGTAGLKLGLHLLKNGVEVKLFTDRRPEEYAGMRLLNTVAHHHVTEVDKLVNHWFDPVG
YKGHYIYGTPEPLQYFLGLVLSPASDVRDIYIQQPMQDFIDRDGDIYGGIAHEDLDAIDEFDLLVVC
TGKGPFGQAMFTPEAYSPFPDFPQARLCVGLFVGIREPETRALMYFSCHGEMIEIPTLSFGMVNAVLY
ENHIGGDILEILAKTYDDPPKAFLALALLLEKLKHYPTCYERIDEEFLANGLDLQGVTPTVRNSYAC
KLPHGKIAVALGVDVAVDPVQLQGAMASYAAILGEEIVANDVIDERFMEKVDARRRDRVLSTSATRTNW
YMLSSLATLDPNLQLQIGAVSQNPKLADEFTENFNFPEKQWDCFSPERVQAVIQARLGPANDAEELVA
AE

### 2.1.2. Protein production and purification

A preculture of *E. coli* BL21(DE)pLysS transformed with pSfStyA was grown overnight at 37 °C in LB (lysogeny broth) medium with 100 µg/mL ampicillin and 34 µg/mL chloramphenicol. Four 2-L shake flasks containing 500 mL Overnight Express™ Instant TB (Terrific broth) Medium (Novagen, Merck, Darmstadt, Germany), with the appropriate antibiotics as above, were each inoculated with 5 mL of preculture and shaken at 30 °C and 180 rpm for 24 hours.

Cells were harvested by centrifugation (10,000 rpm, 20 min) re-suspended with 10 mM Tris-HCl pH 7.5 buffer, centrifuged (10,000 rpm, 20 min) and stored at -80 °C. The cell pellet was then thawed, re-suspended with buffer, supplemented with MgCl₂, one tablet of cOmplete™ EDTA-free protease inhibitor cocktail, and DNase, to be lysed with a cooled Multi Shot Cell Disruption System (Constant Systems Ltd, Daventry, UK) and centrifuged for 30 min at 4 °C and 10,000 rpm.
The supernatant containing the enzyme SfStyA was passed through a nickel column 5 mL HisTrap HP (GE Healthcare, Chicago, IL, USA) on a Pharmacia Biotech Äkta system (GE Healthcare, Chicago, IL, USA). The collected fractions containing protein were dialyzed (molecular cut-off 12 kDa) in 10 mM Tris-HCl pH 7.0 buffer, concentrated using an Amicon® Ultra-15 Centrifugal Filter Device (cut-off 30 kDa) and flash-frozen in liquid N₂ to be stored at -80 °C.

Pure enzyme concentration was determined to be 239 µM (11.75 mg/mL) using the bicinchoninic acid (BCA) protein assay with bovine serum albumin (BSA) for calibration.

The purified enzyme was analyzed by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, Figure S1 right lane), confirming a molecular weight of 47 kDa for SfStyA.

![Figure S1. SDS-PAGE of a protein marker M (left column) and purified SfStyA (right column). The gel was stained with Coomassie Brilliant Blue.](image)

### 2.2. Asymmetric epoxidation

#### 2.2.1. Preparation of racemic epoxides

The following reagents were bought from the chemical suppliers mentioned in the general information: styrene, α-methylstyrene, cis-β-methylstyrene, trans-β-methylstyrene, 4-bromostyrene, 3-bromostyrene, 2-bromostyrene, 4-chlorostyrene, 3-chlorostyrene, 2-chlorostyrene, 4-fluorostyrene, 3-fluorostyrene, 2-fluorostyrene, 4-methylstyrene, 3-methylstyrene, 1,2-dihydropyran, allylbenzene, 2-phenyloxirane (styrene oxide), 2-methyl-3-phenyloxirane (trans-methylstyrene oxide), 2-(4-bromophenyl)oxirane (4-bromostyrene oxide), 2-(4-chlorophenyl)oxirane (4-chlorostyrene oxide), 2-(4-fluorophenyl)oxirane (4-fluorostyrene oxide), (R)-(4-fluorophenyl)oxirane ((R)-fluorostyrene oxide), 2-tetralone, 2-indanone. The corresponding racemic oxiranes were either commercially available or chemically prepared using 3-chloroperbenzoic acid described below (Scheme S1).

![Scheme S1. Chemical aromatic alkene epoxidation.](image)

The non-commercially available racemic epoxides were available in our group, synthesized as previously described. In a 25 mL round-bottom flask, the alkene (1, 2 mmol) was dissolved in dichloromethane (10 mL), sodium bicarbonate (1 g) in deionized water (10 mL) was added, 3-chloroperbenzoic acid (2.2 mmol) was carefully added and the reaction mixture was stirred at room temperature (20 °C) for 3 h or longer, monitored...
The reaction was quenched through the addition of aqueous sodium sulfite (1.3 g in 10 mL) and stirred for an additional 20 min. The mixture was extracted with dichloromethane (2 × 10 mL), the organic phase washed with saturated sodium bicarbonate (2 × 25 mL) and distilled water (25 mL), dried over anhydrous magnesium sulfate and the solvent evaporated in vacuo. In most cases the racemic epoxides were technically pure by ^1H NMR or further purified by column chromatography. NMR and GC-MS analyses were consistent with literature. The 1,2-dihydronaphthalene oxide was newly synthesized in this study.

**rac-1,2-Dihydronaphthalene oxide rac-2r**

1,2-Dihydronaphthalene oxide (0.52 g, 4 mmol) afforded 1,2-dihydronaphthalene oxide as a colorless oil (0.49 g, 84%). ^1H NMR (400 MHz, CDCl₃) δ 7.40 (dd, J = 7.2, 1.6 Hz, 1H), 7.28–7.17 (m, 2H), 7.09 (d, J = 7.3 Hz, 1H), 3.85 (d, J = 4.2 Hz, 1H), 3.78–3.70 (m, 1H), 2.86–2.72 (m, 1H), 2.55 (ddt, J = 15.8, 6.0, 1.4 Hz, 1H), 2.42 (dddd, J = 14.5, 6.6, 2.9, 1.7 Hz, 1H), 1.81–1.73 (m, 1H); ^13C NMR (100 MHz, CDCl₃) δ 136.8, 132.7, 129.7, 128.6, 128.5, 126.2, 55.2, 52.9, 24.5, 21.9.

**2.2.2. Enzyme-catalyzed epoxidations**

Stock solutions were made fresh in buffer: catalase from bovine liver (65,000 U/mL), FAD (5 mM), alkene (2.5 M in DMSO). Reaction conditions: in a 2 mL microcentrifuge plastic tube, buffer (50 mM Tris-SO₄ pH 7.0), BNAH (15 mM), catalase (650 U), FAD (50 µM), SfStyA (3 µM), alkene (5 mM, final 2% v/v DMSO), final volume 1 mL, shaken at 900 rpm and 30 °C for 1 h. Product concentrations and ee were determined with a calibration curve and standards by gas chromatography (GC) with a chiral column (see section 3).

**2.2.3. Comparison of styrene monooxygenases turnover frequency**

The turnover frequency for the epoxidase component (StyA) of different SMOs, two component systems (StyA + StyB) and fused SMO (StyAB) were obtained or calculated from literature values as a comparison to our study (Table S3).

**Table S3. Comparison of styrene monooxygenase-catalyzed epoxidation of styrene depending on its FAD reduction system.**

| SMO system                  | TOF (h⁻¹) | Reference |
|-----------------------------|-----------|-----------|
| non-enzymatic               |           |           |
| SfSMO + BNAH                | 1300      | this study |
| StyA1 + BNAH                | 433       | ³          |
| StyA + [Cp*Rh(bpy)(H₂O)]²⁺ | 662²      | ⁵          |
| enzymatic b                 |           |           |
| StyA1 + StyB                | 175       | ³          |
| Two-component StyA/StyB     | 5820      | ⁶          |
| Natural fused StyA2B        | 78        | ⁷          |
| Fus-SMO                     | 5700      | ⁸          |

² Extrapolated from a 15 min reaction due to the mutual inactivation of the StyA and Rh complex.

⁶ Calculated from the specific epoxidation activity (min⁻¹).

**2.3. Epoxide ring opening**

**2.3.1. Chemical epoxide ring opening**

Standards 2-azido-2-phenylethan-1-ol (rac-3c) and 2-azido-1-phenylethan-1-ol (rac-4c) were synthesized as previously reported,⁹ and used as standards for GC analyses (section 3). The chemical epoxide ring opening of styrene oxide was investigated with different equivalents of sodium azide (Figure S2) and with water at different temperatures in various buffered conditions (Figure S3, Figure S4, Figure S5).
Reaction conditions: in a 2 mL microcentrifuge plastic tube, buffer or MilliQ was added as stated, with styrene oxide (5 mM, final 2% v/v DMSO), sodium azide (5 mM or otherwise stated), final volume 1 mL, shaken at 900 rpm and 30 °C for 1 h (or otherwise stated). Product concentrations were determined by gas chromatography (GC) with a chiral column (see section 3).

Upon mixing styrene oxide and azide, formation of the rac-3c azido alcohol was predominantly observed (Figure S2). When leaving styrene oxide in MilliQ water or Tris buffer without azide at 30 °C no diol was observed, at 60 °C 81% diol was observed with buffer at pH 7.5, and 94% diol in MilliQ, which is slightly acidic (Figure S3). In the presence of azide at 30 °C no diol was observed, but at 60 °C 25% of diol was observed in Tris-HCl buffer at pH 7.5, and 7% in MilliQ. The type of buffer, concentration of buffer, and pH had an effect on diol formation (Figure S4 and Figure S5).

Figure S2. A) Formation of 2-azido-2-phenylethan-1-ol, starting with 5 mM racemic styrene oxide with increasing sodium azide concentration in 50 mM Tris-SO₄ buffer pH 7.0 at 30 °C and 900 rpm for 1 h. Products analyzed by GC. Styrene oxide [●], 2-azido-2-phenylethan-1-ol [▲], 2-azido-1-phenylethan-1-ol [▲]. B) Schematic representation of the simplified Sₙ₂ mechanism of aromatic epoxide azidolysis.
Figure S3. Influence of temperature and pH on chemical epoxide ring opening. Diol product rac-5a observed in grey. Azido alcohol product in blue. Conversion (%) = 100 - remaining substrate (%).

Figure S4. Effect of high buffer concentration and pH on enzyme-catalyzed epoxide formation and ring opening to diol. Azido alcohol product in blue. Diol rac-5a observed in grey.

Figure S5. Effect of buffer and pH on enzyme-catalyzed epoxide formation and ring opening to diol. Azido alcohol product in blue. Diol rac-5a observed in grey.
2.3.2. Chemoenzymatic cascade

Chemoenzymatic cascade reactions (1 mL in volume unless otherwise noted) were performed in buffer (50 mM Tris-SO\(_4\) pH 7.0) containing the styrene derivative (5 mM), \(S_f\)StyA (3 µM), FAD (50 µM), BNAH (15 mM), NaN\(_3\) (35 mM). The reaction mixtures were agitated in a Thermomixer (Eppendorf) at 30 °C and 900 rpm. Product identification was performed by both comparing retention times with authentic standards and GC-MS (see section 3, analytical methods). Conversion and enantiomeric excess were determined by GC analysis.

Semi-preparative scale

In a 500 mL glass Erlenmeyer with 200 mL Tris-SO\(_4\) pH 8.0 buffer was added trans-\(\beta\)-methylstyrene (260 µL, 10 mM), FAD (8.2 mg, 50 µM), \(S_f\)StyA (2.4 mL, 3 µM), BNAH (640 mg, 30 mM), NaN\(_3\) (900 mg, 70 mM). The reaction mixture was shaken on a platform incubator at 30 °C and 180 rpm for 24 h.

2.3.3. Bi enzymatic cascade

Lyophilized HHDH (10 mg) was rehydrated in buffer for 30 min before use. Bi enzymatic cascade reactions (1 mL in volume unless otherwise noted) were performed in buffer (50 mM Tris-SO\(_4\) pH 7.0) containing the styrene derivative (5 mM), \(S_f\)StyA (3 µM), FAD (50 µM), BNAH (15 mM), NaN\(_3\) (5 mM) and HHDH (10 mg/mL). The reaction mixtures were agitated in a Thermomixer (Eppendorf) at 30 °C and 900 rpm. Conversion and enantiomeric excess were determined by GC analysis.

Table S4. Enzymicals HHDH screening kit for the bi enzymatic cascade from trans-\(\beta\)-methylstyrene 1c

| entry | HHDH  | ratio 3:4 |
|-------|-------|----------|
| 1     | HheA3 | 17:83    |
| 2     | HheA5 | 16:84    |
| 3     | HheD3 | 14:86    |
| 4     | HheD5 | 14:86    |
| 5     | HheD6 | 15:85    |
| 6     | HheE5 | 13:87    |
| 7     | HheB5 | 15:85    |

Reaction conditions: Tris-SO\(_4\) buffer (150 mM, pH 7.5), [trans-\(\beta\)-methylstyrene] = 5 mM, [\(S_f\)StyA] = 3 µM, [FAD] = 50 µM, [BNAH] = 10 mM, [NaN\(_3\)] = 15 mM, HHDH (10 mg), mixed at 200 rpm and 30 °C for 16 h 30 min.
3. MM2 energy minimization with Chem3D

With the epoxide product originating from trans-methylstyrene, in the most stable conformation, the π-bonds of the benzene ring align with the epoxide C-O bond (Figure S6). As in an $S_N2$ reaction the nucleophile aligns with the leaving group, it will in this case also align with the π bonds of the ring, enabling electrostatic interaction between them, favoring the nucleophilic attack.

![Figure S6. (2S,3S)-2-methyl-3-phenyloxirane [(2S,3S)-2c].](image)

With the epoxide formed from cis-methylstyrene, in the most stable conformation the phenyl ring is rotated to avoid steric hindrance (Figure S7). The π-bonds are now perpendicular to the epoxide C-O bond and thus to the incoming azide, hampering electrostatic interaction and thereby leading to lower reaction rate and decreased regioselectivity.

![Figure S7. (2R,3S)-2-methyl-3-phenyloxirane [(2R,3S)-2d].](image)
4. Analytical methods

4.1. GC analyses

All biocatalytic reactions were followed by GC. Analyses were carried out on a Shimadzu GC-2010 gas chromatograph (Shimadzu corporation, Kyoto, Japan) equipped with a flame ionization detector (FID). Products were confirmed by reference standards and GC-MS. Product concentrations were obtained with calibration curve equations using 5 mM dodecane as an internal standard in the EtOAc used to extract all compounds.

The FID response factor was assumed to be the same for regioisomers of the styrene derivatives. Authentic samples were used to determine the absolute configuration of the product enantiomers of styrene oxide, trans-β-methyl styrene oxide, and 4-fluorostyrene oxide, determining the major enantiomer product of the enzymatic reaction to be (S). SfStyA was assumed to be (S)-selective on other regioisomers of the styrene substrates.

The following chiral columns were used to determine enantiomeric excess of chiral products. Details on the injection temperature, linear velocity, column flow, oven temperature program and retention times can be found for each compound in section 3.2.

A: Chiraldex G-TA (Astec), injection at 250 °C
   50 × 0.25 mm × 0.12 µm
   2,6-di-O-pentyl-3-trifluoroacetyl-γ-cyclodextrin;
B: Lipodex E (Macherey-Nagel), injection at 200 °C
   50 m × 0.25 mm × 0.25 µm
   octakis-(2,6-di-O-pentyl-3-O-butyryl)-γ-cyclodextrin;
C: CP-Chirasil-Dex CB (Agilent J&W), injection at 250 °C
   25 m × 0.32 mm × 0.25 µm
   heptakis (2,3,6-tri-O-methyl)-β-cyclodextrin;
D: Hydrodex β-6TBDM (Macherey-Nagel), injection at 250 °C
   50 m × 0.25 mm × 0.15 µm
   heptakis-(2,3-di-O-methyl-6-O-t-butyldimethyl-silyl)-β-cyclodextrin.

GC-MS measurements were obtained on a Hewlett Packard HP6890 and 5973 MSD, with a HP-5MS column (Agilent J & W, 30 m × 0.25 mm × 0.25 µm).

| Column | Method and oven temperature program: | Ramp (°C/min) | Temp. (°C) | Hold time (min) | Compound | Ret. time (min) |
|--------|-----------------------------------|--------------|------------|----------------|----------|----------------|
| A      | split 100                         | -            | 110.0      | 15.00          | styrene 1a | 3.9            |
|        | linear velocity 38.0 cm/s         | 15.00        | 170.0      | 2.00           | dodecane  | 7.7            |
|        | column flow 2.16 mL/min           |              |            |                | (S)-styrene oxide 2a | 9.5 |
|        |                                   |              |            |                | (R)-styrene oxide 2a | 11.0 |
| B      | split 50                          | -            | 100.0      | 15.00          | styrene 1a | 4.3            |
|        | linear velocity 36.8 cm/s         | 20.00        | 220.0      | 1.00           | dodecane  | 8.3            |
|        | column flow 2.05 mL/min           |              |            |                | (S)-styrene oxide 2a | 12.0 |
|        |                                   |              |            |                | (R)-styrene oxide 2a | 13.1 |
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| Column Flow | Linear Velocity | Time | Retention Time | Compound | Column
|-------------|-----------------|------|----------------|----------|-------|
| A           | 40.1 cm/s       | 5.00 | 80.0, 5.00     | α-methylstyrene 1b | Dodecane 9.2 |
|             |                 |      | 90.0, 5.00     | (R)-α-methylstyrene oxide 2b | 15.5 |
|             |                 | 5.00 | 100.0, 15.00   | (S)-α-methylstyrene oxide 2b | 20.3 |
|             |                 | 10.0 | 170.0, 1.00    | trans-methylstyrene 1c | Dodecane 8.2 |
|             | 2.04 mL/min     |      | 100.0, 15.00   | cis-methylstyrene 1d | Dodecane 5.5 |
|             |                 | 20.0 | 220.0, 1.00    | benzaldehyde | 8.9 |
|             |                 |      | 100.0, 15.00   | (2S,3S)-2-methyl-3-phenyloxirane 2c | 11.3 |
|             |                 |      | 200.0, 2.00    | (2S,3S)-2-methyl-3-phenyloxirane 2c | 12.5 |
| B           | 36.8 cm/s       | 5.00 | 100.0, 4.00    | 4-bromostyrene 1e | Dodecane 9.8 |
|             | 2.04 mL/min     |      | 15.00, 175.0, 2.20 | (R)-4-bromostyrene oxide 2e | 11.0 |
|             |                 | 10.0 | 205.0, 2.00    | (S)-4-bromostyrene oxide 2e | 14.8 |
|             |                 | 25.0 | 250.0, 2.00    | 3-bromostyrene 1f | Dodecane 7.4 |
|             |                 |      | 100.0, 4.00    | 2-bromostyrene 1g | Dodecane 14.2 |
|             |                 |      | 120.0, 15.20   | (R)-2-bromostyrene oxide 2g | 19.1 |
|             |                 |      | 130.0, 2.00    | (S)-3-bromostyrene oxide 2g | 19.4 |
| B           | 37.4 cm/s       | 5.00 | 90.0, 5.00     | 4-chlorostyrene 1h | Dodecane 9.8 |
|             | 2.14 mL/min     |      | 5.00, 100.0, 8.00 | (S)-4-chlorostyrene oxide 2h | 10.9 |
|             |                 | 5.00 | 120.0, 5.00    | (R)-3-chlorostyrene oxide 2h | 25.5 |
|             |                 | 5.00 | 160.0, 1.00    | (R)-2-chlorostyrene oxide 2j | 26.0 |
| B           | 35.5 cm/s       | 5.00 | 120.0, 5.00    | 3-chlorostyrene 1i | Dodecane 5.3 |
|             | 1.87 mL/min     |      | 5.00, 100.0, 5.00 | (S)-3-chlorostyrene oxide 2i | 5.8 |
|             |                 | 5.00 | 110.0, 5.00    | (R)-3-chlorostyrene oxide 2i | 15.8 |
|             |                 | 20.0 | 220.0, 1.00    | 2-chlorostyrene 1j | Dodecane 5.3 |
|             |                 |      | 120.0, 23.00   | (R)-2-chlorostyrene oxide 2j | 5.8 |
|             |                 |      | 150.0, 5.00    | (S)-2-chlorostyrene oxide 2j | 9.4 |
|             |                 |      | 200.0, 1.00    | 4-fluorostyrene 1k | Dodecane 4.4 |
|             |                 |      | 100.0, 15.00   | (S)-4-fluorostyrene oxide 2k | 8.2 |
|             |                 |      | 220.0, 1.00    | (R)-4-fluorostyrene oxide 2k | 11.2 |
| C           | 26.4 cm/s       | 5.00 | 80.0, 10.00    | 3-fluorostyrene 1l | Dodecane 6.5 |
|             | 1.37 mL/min     |      | 5.00, 100.0, 10.00 | (R)-3-fluorostyrene oxide 2l | 17.2 |
|             |                 | 5.00 | 100.0, 10.00   | (S)-3-fluorostyrene oxide 2l | 17.4 |
|             |                 | 25.0 | 225.0, 1.00    | 2-fluorostyrene 1m | Dodecane 20.5 |
| C           | 25.9 cm/s       | 5.00 | 70.0, 5.00     | (R)-2-fluorostyrene oxide 2m | 7.5 |
|             | 1.30 mL/min     |      | 5.00, 80.0, 5.00 | (S)-2-fluorostyrene oxide 2m | 18.2 |

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Table S6. GC methods and compound retention times for azido alcohol products.

| Column | Method and oven temperature program: | Ramp (°C/min) | Temp. (°C) | Hold time (min) | Compound                                  | Ret. time (min) |
|--------|-------------------------------------|---------------|------------|----------------|-------------------------------------------|-----------------|
| A      | split 100 linear velocity 38.0 cm/s column flow 2.13 mL/min | -             | 110.0      | 15.00          | styrene 1a                               | 3.9             |
|        |                                     | 5.00          | 130.0      | 5.00           | dodecane                                 | 7.8             |
|        |                                     | 5.00          | 150.0      | 5.00           | (S)-styrene oxide 2a                     | 9.6             |
|        |                                     | 5.00          | 160.0      | 5.00           | (R)-styrene oxide 2a                     | 11.0            |
|        |                                     | 10.00         | 170.0      | 3.00           | α-azido alcohols:                         |                 |
|        |                                     |               |            |                | (S)-2-azido-2-phenylethanol-1-ol 3a       | 36.0            |
|        |                                     |               |            |                | (R)-2-azido-2-phenylethanol-1-ol 3a       | 36.5            |
|        |                                     |               |            |                | β-azido alcohols:                         |                 |
|        |                                     |               |            |                | (R)-2-azido-1-phenylethanol-1-ol 4a       | 38.0            |
|        |                                     |               |            |                | (S)-2-azido-1-phenylethanol-1-ol 4a       | 38.4            |
| Column | Linear Velocity | Flow Rate | Analyte Description | Value |
|--------|----------------|-----------|---------------------|-------|
| D      | 38.0 cm/s      | 2.1 mL/min| α-methylstyrene 3b  | 7.8   |
|        |                |           | dodecane            | 17.0  |
| D      | 38.0 cm/s      | 2.1 mL/min| α-azido alcohol (R)-3b | 42.6 |
|        |                |           | diol                | 43.6  |
| D      | 38.0 cm/s      | 2.1 mL/min| α-azido alcohol (S)-3b | 44.6 |
|        |                |           | β-azido alcohol (R)-3b | 44.9 |
| A      | 38.0 cm/s      | 2.13 mL/min| trans-methylstyrene 1c | 5.9  |
|        |                |           | (1S,2S)-1-azido-1-phenylpropan-2-ol 3c | 35.2 |
|        |                |           | (1R,2S)-1-azido-1-phenylpropan-2-ol 3c | 35.2 |
|        |                |           | (1S,2R)-1-azido-1-phenylpropan-2-ol 3c | 35.2 |
| A      | 38.0 cm/s      | 2.1 mL/min| dodecane            | 17.0  |
|        |                |           | 4-bromostyrene 1e   | 20.5  |
|        |                |           | 4-bromostyrene oxide 2e | 34.3 |
|        |                |           | (R)-2-azido-2-(4-bromophenyl)ethan-1-ol 3e | 59.2 |
| A      | 38.0 cm/s      | 2.1 mL/min| dodecane            | 17.0  |
|        |                |           | 3-bromostyrene 1f   | 18.6  |
|        |                |           | 3-bromostyrene oxide 2f | 34.3 |
|        |                |           | (R)-2-azido-2-(3-bromophenyl)ethan-1-ol 3f | 57.7 |
|        |                |           | β-azido alcohol (S)-2-azido-1-(3-bromophenyl)ethan-1-ol 3f | 60.9 |
| A      | 38.0 cm/s      | 2.1 mL/min| dodecane            | 17.0  |
|        |                |           | 2-bromostyrene 1g   | 17.5  |
|        |                |           | 2-bromostyrene oxide 2g | 31.0 |
|        |                |           | (R)-2-azido-2-(2-bromophenyl)ethan-1-ol 3g | 53.8 |
|        |                |           | (S)-2-azido-2-(2-bromophenyl)ethan-1-ol 3g | 54.4 |
|        |                |           | β-azido alcohol (S)-2-azido-1-(2-bromophenyl)ethan-1-ol 3g | 58.7 |
| A      | 38.0 cm/s      | 2.1 mL/min| dodecane            | 17.0  |
|        |                |           | 4-chlorostyrene 1h   | 13.8  |
|        |                |           | dodecane            | 17.0  |
|        |                |           | (R)-2-azido-2-(4-chlorophenyl)ethan-1-ol 3h | 55.36 |
|        |                |           | (S)-2-azido-2-(4-chlorophenyl)ethan-1-ol 3h | 55.4 |
|        |                |           | β-azido alcohol (S)-2-azido-1-(4-chlorophenyl)ethan-1-ol 3h | 58.4 |
| A      | 38.0 cm/s      | 2.1 mL/min| dodecane            | 17.0  |
|        |                |           | 3-chlorostyrene 1i   | 12.0  |
|        |                |           | dodecane            | 17.0  |
|        |                |           | 3-chlorostyrene oxide 2i | 28.0 |
|        |                |           | (R)-2-azido-2-(3-chlorophenyl)ethan-1-ol 3i | 53.6 |
|        |                |           | β-azido alcohol (S)-2-azido-1-(3-chlorophenyl)ethan-1-ol 3i | 58.3 |
| Column | Split | Linear Velocity | Column Flow | Description |
|--------|-------|-----------------|-------------|-------------|
| D      | 50    | 38.0 cm/s       | 2.1 mL/min  | 2-chlorostyrene 1j 11.0 |
|        |       |                 |             | dodecane 17.0 |
| A      | 50    | 38.0 cm/s       | 2.1 mL/min  | 2-chlorostyrene oxide 2j 25.0 |
|        |       |                 |             | (R)-2-azido-2-(2-chlorophenyl)ethan-1-ol 3j 49.2 |
|        |       |                 |             | (S)-2-azido-2-(2-chlorophenyl)ethan-1-ol 3j 50.0 |
|        |       |                 |             | β-azido alcohol (S)-2-azido-1-(2-chlorophenyl)ethan-1-ol 3j 55.1 |
| D      | 100   | 38.0 cm/s       | 2.13 mL/min | dodecane 7.8 |
|        |       |                 |             | (R)-2-azido-2-(4-fluorophenyl)ethan-1-ol 3k 44.5 |
|        |       |                 |             | (S)-2-azido-1-(4-fluorophenyl)ethan-1-ol 3k 48.0 |
| D      | 50    | 38.0 cm/s       | 2.16 mL/min | 4-fluorostyrene 1k 5.8 |
|        |       |                 |             | dodecane 17.0 |
|        |       |                 |             | β-azido alcohol (S)-2-azido-1-(4-fluorophenyl)ethan-1-ol 3k 45.3 |
|        |       |                 |             | α-azido alcohol (R)-2-azido-2-(4-fluorophenyl)ethan-1-ol 3k 49.6 |
| D      | 100   | 38.0 cm/s       | 2.13 mL/min | dodecane 7.8 |
|        |       |                 |             | (R)-2-azido-2-(3-fluorophenyl)ethan-1-ol 3l 36.1 |
|        |       |                 |             | (S)-2-azido-1-(3-fluorophenyl)ethan-1-ol 3l 39.0 |
| D      | 50    | 38.0 cm/s       | 2.16 mL/min | 3-fluorostyrene 1l 5.6 |
|        |       |                 |             | 3-fluorostyrene oxide 2l 15.8 |
|        |       |                 |             | dodecane 17.0 |
|        |       |                 |             | (R)-2-azido-2-(3-fluorophenyl)ethan-1-ol 3l 44.5 |
|        |       |                 |             | (S)-2-azido-1-(3-fluorophenyl)ethan-1-ol 3l 50.1 |
| D      | 50    | 38.0 cm/s       | 2.16 mL/min | 2-fluorostyrene 1m 5.1 |
|        |       |                 |             | dodecane 17.0 |
|        |       |                 |             | (R)-2-azido-2-(2-fluorophenyl)ethan-1-ol 3m 41.3 |
|        |       |                 |             | (S)-2-azido-1-(2-fluorophenyl)ethan-1-ol 3m 41.9 |
|        |       |                 |             | β-azido alcohol 3m 46.8 |
| D      | 50    | 38.0 cm/s       | 2.16 mL/min | 2-fluorostyrene 1m 5.1 |
|        |       |                 |             | dodecane 17.0 |
|        |       |                 |             | (R)-2-azido-2-(2-fluorophenyl)ethan-1-ol 3m 41.3 |
|        |       |                 |             | (S)-2-azido-1-(2-fluorophenyl)ethan-1-ol 3m 41.9 |
|        |       |                 |             | β-azido alcohol 3m 46.8 |
| D      | 50    | 38.0 cm/s       | 2.16 mL/min | 2-fluorostyrene 1m 5.1 |
|        |       |                 |             | dodecane 17.0 |
|        |       |                 |             | (R)-2-azido-2-(2-fluorophenyl)ethan-1-ol 3m 41.3 |
|        |       |                 |             | (S)-2-azido-1-(2-fluorophenyl)ethan-1-ol 3m 41.9 |
|        |       |                 |             | β-azido alcohol 3m 46.8 |
| D      | 50    | 38.0 cm/s       | 2.16 mL/min | dodecane 17.0 |
|        |       |                 |             | (S)-4-methylstyreren oxide 22.1 |
|        |       |                 |             | β-azido alcohol 3n 45.6 |
|        |       |                 |             | (R)-2-azido-2-(p-tolyl)ethan-1-ol 3n 46.6 |
|        |       |                 |             | α-azido alcohol (S)-2-azido-2-(p-tolyl)ethan-1-ol 3n 46.8 |
| D      | 50    | 38.0 cm/s       | 2.16 mL/min | dodecane 17.0 |
|        |       |                 |             | (S)-4-methylstyreren oxide 22.1 |
|        |       |                 |             | β-azido alcohol 3n 45.6 |
|        |       |                 |             | (R)-2-azido-2-(p-tolyl)ethan-1-ol 3n 46.6 |
|        |       |                 |             | α-azido alcohol (S)-2-azido-2-(p-tolyl)ethan-1-ol 3n 46.8 |
| D      | 50    | 38.0 cm/s       | 2.16 mL/min | dodecane 17.0 |
|        |       |                 |             | (S)-4-methylstyreren oxide 22.1 |
|        |       |                 |             | β-azido alcohol 3n 45.6 |
|        |       |                 |             | (R)-2-azido-2-(p-tolyl)ethan-1-ol 3n 46.6 |
|        |       |                 |             | α-azido alcohol (S)-2-azido-2-(p-tolyl)ethan-1-ol 3n 46.8 |
|                  | column flow 2.16 mL/min | 5.00 | 150.0 | 5.00 | α-azido alcohol (1R,2R)-1-azido-1,2,3,4-tetrahydrodronaphthalene-2-ol 3q | 56.2 |
|------------------|-------------------------|------|-------|------|--------------------------------------------------------------------------------|------|
|                  |                         | 5.00 | 170.0 | 5.00 | β-azido alcohol 3q                                                                | 57.6 |
|                  |                         | 5.00 | 190.0 | 5.00 |                                                                                   |      |
|                  |                         | 5.00 | 210.0 | 2.00 |                                                                                   |      |
|                  |                         | 25.00| 250.0 | 1.00 |                                                                                   |      |
|                  | split 50                | -    | 110.0 | 15.00| dodecane                                                                          |      |
|                  | linear velocity 38.0 cm/s|      |       |      | (15,2R)-indene oxide 2r                                                            | 17.0 |
|                  | column flow 2.16 mL/min | 5.00 |     130.0 | 5.00 | α-azido alcohol (1R,2R)-1-azido-2,3-dihydro-1H-inden-2-ol 3r                      | 51.7 |
|                  |                         | 5.00 |     150.0 | 5.00 | β-azido alcohol 3r                                                                 | 53.6 |
|                  |                         | 5.00 |     170.0 | 5.00 |                                                                                   |      |
|                  |                         | 5.00 |     190.0 | 5.00 |                                                                                   |      |
|                  |                         | 5.00 |     210.0 | 2.00 |                                                                                   |      |
|                  |                         | 25.00|     250.0 | 1.00 |                                                                                   |      |
|                  | split 50                | -    | 110.0 | 15.00| 2-vinylpyridine 1s                                                                 | 6.9  |
|                  | linear velocity 38.0 cm/s|      |       |      | dodecane                                                                          | 17.0 |
|                  | column flow 2.16 mL/min | 5.00 |     130.0 | 5.00 | 2-vinylpyridine oxide 2s                                                            | 19.0 |
|                  |                         | 5.00 |     150.0 | 5.00 | α-azido alcohol (R)-2-azido-2-(pyridin-2-yl)ethan-1-ol 3s                          | 44.2 |
|                  |                         | 5.00 |     170.0 | 5.00 | β-azido alcohol (S)-2-azido-1-(pyridin-2-yl)ethan-1-ol 3s                          | 44.4 |
|                  |                         | 5.00 |     190.0 | 5.00 |                                                                                   |      |
|                  |                         | 5.00 |     210.0 | 2.00 |                                                                                   |      |
|                  |                         | 25.00|     250.0 | 1.00 |                                                                                   |      |
|                  | split 100               | -    | 110.0 | 15.00| dodecane                                                                          | 7.8  |
|                  | linear velocity 38.0 cm/s|      |       |      | α-azido alcohol (R)-2-azido-2-(pyridine-2-yl)ethan-1-ol 3s                         | 37.5 |
|                  | column flow 2.13 mL/min | 5.00 |     130.0 | 5.00 | β-azido alcohol (R)-2-azido-2-(pyridine-2-yl)ethan-1-ol 3s                         | 41.5 |
|                  |                         | 5.00 |     150.0 | 5.00 |                                                                                   |      |
|                  |                         | 5.00 |     160.0 | 5.00 |                                                                                   |      |
|                  |                         | 10.00|     170.0 | 3.00 |                                                                                   |      |
4.2. GC chromatograms of epoxide products

(S)-styrene oxide (2-phenyloxirane) 2a

Figure S8. GC chromatogram of full reaction product (S)-styrene oxide 2a on column A.

Figure S9. GC chromatogram of commercial racemic styrene oxide 2a on column A.

Figure S10. GC chromatogram of reaction product (S)-styrene oxide 2a on column B.

Figure S11. GC chromatogram of commercial racemic styrene oxide 2a on column B.
Figure S12. GC chromatogram of commercial (S)-styrene oxide 2a on column B.

Figure S13. GC chromatogram of commercial (R)-styrene oxide 2a on column B.

Figure S14. GC-MS mass spectrum of reaction product (S)-styrene oxide 2a.
(S)-α-methylstyrene oxide (2-methyl-2-phenyloxirane) 2b

Figure S15. GC chromatogram of reaction products 2b on column A.

Figure S16. GC chromatogram of synthesized racemic α-methylstyrene oxide 2b on column A.
(1S,2S)-1-phenylpropylene oxide ((2S,3S)-2-methyl-3-phenyloxirane; trans-β-methylstyrene oxide) 2c

Figure S17. GC chromatogram of reaction product (1S,2S)-1-phenylpropylene oxide 2c on column B.

Figure S18. GC chromatogram of synthesized racemic trans-β-methylstyrene oxide 2c on column B.

Figure S19. GC-MS spectrum of reaction product (1S,2S)-1-phenylpropylene oxide 2c.
\((1S,2R)-1\text{-phenylpropylene oxide} \ ((2R,3S)-2\text{-methyl-3-phenyloxirane}; \text{cis-} \beta\text{-methylstyrene oxide}) \ 2d\)

**Figure S20.** GC chromatogram of reaction products \(2d\) on column B: small peaks are compounds (benzaldehyde, phenylacetone, \textit{trans-}\(\beta\)-methylstyrene oxides) present in the starting material.

**Figure S21.** GC chromatogram of synthesized racemic \textit{cis-} \(\beta\)-methylstyrene oxide \(2d\), with impurities (benzaldehyde, \textit{trans-}\(\beta\)-methylstyrene oxide, phenylacetone) from the starting material, on column B.

**Figure S22.** GC-MS mass spectrum of reaction product \((1S,2R)-1\text{-phenylpropylene oxide} \ 2d\).
(S)-4-bromostyrene oxide (2-(4-bromophenyl)oxirane) 2e

Figure S23. GC chromatogram of reaction products 2e on column D.

Figure S24. GC chromatogram of commercial racemic 4-bromostyrene oxide 2e on column D.

Figure S25. GC-MS mass spectrum of reaction product (S)-4-bromostyrene oxide 2e.
(S)-3-bromostyrene oxide (2-(3-bromophenyl)oxirane) 2f

Figure S26. GC chromatogram of reaction products 2f on column C.

Figure S27. GC chromatogram of synthesized racemic 3-bromostyrene oxide 2f and side product 3-bromophenylacetaldehyde on column C.

Figure 28. GC-MS mass spectrum of reaction product (S)-3-bromostyrene oxide 2e.
(S)-2-bromostyrene oxide (2-(2-bromophenyl)oxirane) 2g

Figure S29. GC chromatogram of reaction products 2g on column C.

Figure S30. GC chromatogram of synthesized racemic 2-bromostyrene oxide 2g with 2-bromophenylacetaldehyde side product on column C.
(S)-4-chlorostyrene oxide (2-(4-chlorophenyl)oxirane) 2h

Figure S31. GC chromatogram of reaction products on column B.

Figure S32. GC chromatogram of synthesized racemic 4-chlorostyrene oxide with 4-chlorophenylacetaldehyde side product on column B.

Figure S33. GC-MS mass spectrum of reaction product (S)-4-chlorostyrene oxide 2h.
(S)-3-chlorostyrene oxide (2-(3-chlorophenyl)oxirane) 2i

Figure S34. GC chromatogram of reaction products 2i on column B.

Figure S35. GC chromatogram of synthesized racemic 3-chlorostyrene oxide 2i with 3-chlorophenylacetaldehyde side product on column B.

Figure S36. GC-MS mass spectrum of reaction product (S)-3-chlorostyrene oxide 2i.
(S)-2-chlorostyrene oxide (2-(2-chlorophenyl)oxirane) 2j

Figure S37. GC chromatogram of reaction products 2j on column B.

Figure S38. GC chromatogram of synthesized racemic 2-chlorostyrene oxide 2j with 2-chlorophenylacetaldehyde side product on column B.
(S)-4-fluorostyrene oxide (2-(4-fluorophenyl)oxirane) 2k

Figure S39. GC chromatogram of reaction products 2k on column B.

Figure S40. GC chromatogram of commercial racemic product 2k on column B.
**Figure S41.** GC chromatogram of reaction products 2l on column C.

**Figure S42.** GC chromatogram of synthesized products 2l with 3-fluorophenylacetaldehyde side produce on column C (retention times slightly shifted to the left).

**Figure S43.** GC chromatogram of reaction products 2m on column C.
(S)-4-methylstyrene oxide (2-(p-tolyl)oxirane) (S)-2n

Figure S44. GC chromatogram of reaction products 2n on column B.

Figure S45. GC chromatogram of reaction products 2n on column A.

Figure S46. GC chromatogram of synthesized racemic product on column A.
(S)-3-methylstyrene oxide (2-(m-tolyl)oxirane) (S)-2o

Figure S47. GC chromatogram of reaction products 2o on column B.

Figure S48. GC chromatogram of synthesized racemic products 2o and side product 3-methylphenylacetaldehyde on column B.
(S)-2-benzylxirane (allylbenzene oxide) (S)-2p

Figure S49. GC chromatogram of reaction products 2p on column D.

Figure S50. GC chromatogram of synthesized racemic product 2p on column D.
(1S,2R)-1,2-dihydronaphthalene oxide (1S,2R)-2q

Figure S51. GC chromatogram of reaction products 2q on column C.

Figure S52. GC chromatogram of synthesized racemic product 2q and side-product 2-tetralone on column C.
(1S,2R)-1,2-indene oxide 2r

Figure S53. GC chromatogram of reaction products 2r on column D.

(S)-2-vinylpyridine oxide 2s

Figure S55. GC chromatogram of reaction products 2s on column A.

Figure S56. GC chromatogram of synthesized racemic product 2s on column A (peaks at 6.5 and 11.3 min are impurities from the chemical synthesis).
4.3. GC chromatograms of azido alcohol products

Chemoenzymatic cascade to (R)-2-azido-2-phenylethan-1-ol 3a

Figure S57. GC chromatogram of reaction products 3a on column A.

Figure S58. GC chromatogram of synthesized racemic products 3a and 4a on column A.

Figure S59. GC-MS mass spectrum of (R)-2-azido-2-phenylethan-1-ol 3a.
Chemoenzymatic cascade to 1-azido-2-phenylpropan-2-ol 3b

Figure S60. GC chromatogram of reaction products 3b on column D.

Figure S61. Zoom in of GC chromatogram of reaction products 3b on column D.
Chemoenzymatic cascade to (1R,2S)-1-azido-1-phenylpropan-2-ol 3c

Chemoenzymatic cascade from trans-methylstyrene to (1R,2S)-1-azido-1-phenylpropan-2-ol [(1R,2S)-3c] 50 mg reaction scale:

Figure S62. GC chromatogram of reaction products 3c on column A.

Figure S63. GC chromatogram of synthesized racemic product 3c on column A.

Figure S64. GC-MS mass spectrum of (1R,2S)-1-azido-1-phenylpropan-2-ol 3c.
Chemoenzymatic cascade to $(1R,2R)$-1-azido-1-phenylpropan-2-ol 3d

![GC chromatogram of reaction products 3d on column A, with products of trans-β-methylstyrene due to the impure cis-β-methylstyrene starting material.](image)

Chemoenzymatic cascade to (R)-2-azido-2-(4-bromophenyl)ethan-1-ol 3e

![GC chromatogram of reaction products 3e on column D.](image)

Chemoenzymatic cascade to (R)-2-azido-2-(3-bromophenyl)ethan-1-ol 3f

![GC chromatogram of reaction products 3f on column D.](image)
Chemoenzymatic cascade to \((R)\)-2-azido-2-(2-bromophenyl)ethan-1-ol \(3g\)

![Figure S68. GC chromatogram of reaction products \(3g\) on column D.](image)

Chemoenzymatic cascade to \((R)\)-2-azido-2-(4-chlorophenyl)ethan-1-ol \(3h\)

![Figure S70. GC chromatogram of reaction products \(3h\) on column D.](image)
Chemoenzymatic cascade to (R)-2-azido-2-(3-chlorophenyl)ethan-1-ol 3i

Figure S71. GC chromatogram of reaction products 3i on column D.

Figure S72. Zoom in of GC chromatogram of reaction products 3i on column D.

Chemoenzymatic cascade to (R)-2-azido-2-(2-chlorophenyl)ethan-1-ol 3j

Figure S73. GC chromatogram of reaction products 3j on column D.

Figure S74. Zoom in of GC chromatogram of reaction products 3j on column D.
Chemoenzymatic cascade to (R)-2-azido-2-(4-fluorophenyl)ethan-1-ol 3k

Figure S75. GC chromatogram of reaction products 3k on column A.

Figure S76. GC chromatogram of reaction products 3k on column D.

Figure S77. Zoom in of GC chromatogram of reaction products 3k on column D.

Figure S78. GC chromatogram of synthesized racemic products 3k on column D.
Chemoenzymatic cascade to $(R)$-2-azido-2-(3-fluorophenyl)ethan-1-ol $3l$

**Figure S79.** GC chromatogram of reaction products $3l$ on column A.

- **Figure S80.** GC chromatogram of reaction products $3l$ on column D.

Chemoenzymatic cascade to $(R)$-2-azido-2-(2-fluorophenyl)ethan-1-ol $3m$

**Figure S81.** GC chromatogram of reaction products $3m$ on column D.
Figure S82. Zoom in of GC chromatogram of reaction products 3m on column D.

Chemoenzymatic cascade to (R)-2-azido-2-(p-tolyl)ethan-1-ol 3n

Figure S83. GC chromatogram of reaction products 3n on column D.

Figure S84. Zoom in of GC chromatogram of reaction products 3n on column D.

Chemoenzymatic cascade to (R)-2-azido-2-(m-tolyl)ethan-1-ol 3o
Figure S85. GC chromatogram of reaction products 3o on column D.

Chemoenzymatic cascade to $(1R,2R)$-1-azido-1,2,3,4-tetrahydronaphthalen-2-ol 3q

Figure S86. GC chromatogram of reaction products 3q on column D.

Chemoenzymatic cascade to $(1R,2R)$-1-azido-2,3-dihydro-1H-inden-2-ol 3r

Figure S87. GC chromatogram of reaction products 3r on column D.

Chemoenzymatic cascade to (R)-2-azido-2-(pyridin-2-yl)ethan-1-ol 3s

Figure S88. GC chromatogram of reaction products 3s on column D.
Bienzymatic cascade to (S)-2-azido-1-phenylethan-1-ol 3a

Figure S89. GC chromatogram of reaction products 3a on column A.

Figure S90. GC-MS mass spectrum of (S)-2-azido-2-phenylethan-1-ol 3a.

Bienzymatic cascade to (1S,2R)-2-azido-1-phenylpropan-2-ol 3c

Figure S91. GC chromatogram of reaction products 3c on column A. Trace amounts <1% of diol observed.
Bienzymatic cascade to (S)-2-azido-1-(4-fluorophenyl)ethan-1-ol 3k

![Figure S92. GC chromatogram of reaction products 3k on column A.](image1)

Bienzymatic cascade to (R)-2-azido-2-(pyridine-2-yl)ethan-1-ol 3s

![Figure S93. GC chromatogram of reaction products 3s on column A.](image2)

5. References

1. S. Panke, M. Held, M. G. Wubbolts, B. Witholt and A. Schmid, *Biotechnol. Bioeng.*, 2002, **80**, 33-41.
2. D. Tischler, R. Kermer, J. A. D. Groning, S. R. Kaschabek, W. J. H. van Berkel and M. Schloemann, *J. Bacteriol.*, 2010, **192**, 5220-5227.
3. C. E. Paul, D. Tischler, A. Riedel, T. Heine, N. Itoh and F. Hollmann, *ACS Catal.*, 2015, **5**, 2961-2965.
4. M. M. C. H. van Schie, C. E. Paul, I. W. C. E. Arends and F. Hollmann, *Chem. Commun.*, 2019, **55**, 1790-1792.
5. F. Hollmann, P. C. Lin, B. Witholt and A. Schmid, *J. Am. Chem. Soc.*, 2003, **125**, 8209-8217.
6. K. Otto, K. Hofstetter, M. Rothlisberger, B. Witholt and A. Schmid, *J. Bacteriol.*, 2004, **186**, 5292-5302.
7. D. Tischler, D. Eulberg, S. Lakner, S. R. Kaschabek, W. J. H. van Berkel and M. Schloemann, *J. Bacteriol.*, 2009, **191**, 4996-5009.
8. M. L. Corrado, T. Knaus and F. G. Mutti, *ChemBioChem*, 2018, **19**, 679-686.
9. J. H. Schrittwieser, F. Coccia, S. Kara, B. Grischek, W. Kroutil, N. d’Alessandro and F. Hollmann, *Green Chem.*, 2013, **15**, 3318-3331.
6. NMR spectra

NMR spectra are displayed on the next pages for the upscaled reactions of the chemoenzymatic cascade, crude product extracted without further purification.
$^1$H NMR (400 MHz, CDCl$_3$) Sty-cascade 15 mg scale
1H NMR (400 MHz, CDCl3) Sty-cascade upscale

![Chemical structure with N3 and OH groups](image)

Integrations and Chemical Shifts:
- 7.42 ppm (7H, H-8 and H-9)
- 7.33 ppm (H-4)
- 4.88 ppm (H-2 and H-3)
- 3.74 ppm (H-1)

f1 (ppm): 11.0 to 0.0
$^1$H NMR (400 MHz, CDCl$_3$) trans-methylstyrene-chemo-enzymatic cascade, 15 mg scale
1H NMR (400 MHz, CDCl3) transMeSty-cascade upscald
$^1$H NMR (400 MHz, CDCl$_3$) 4-FSty-cascade
1H NMR (400 MHz, CDCl3) 3FSty-cascade

F
\[ \text{N}_3 \]
\[ \text{OH} \]

\[ \begin{array}{c}
7.49 \\
7.36 \\
7.13 \\
7.03
\end{array} \]
\[ \begin{array}{c}
4.90 \\
4.87 \\
4.66 \\
3.79 \\
3.70 \\
3.47 \\
3.46
\end{array} \]
