Supporting Information for

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

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Contents

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.[a]

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
(1*S*,2*S*)-(−)-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
allylbenzene	98	300-57-2
1,2-dihydronaphthalene	98	447-53-0
2-tetralone	98	530-93-8
indene	≥99	95-13-6
2-indanone	98	615-13-4
2-vinylpyridine	97	100-69-6

[a] 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	ABS64560	*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_{254} aluminum sheets (EMD Millipore, Merck, Darmstadt, Germany). Organic solutions were concentrated under reduced pressure with a rotary evaporator (*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 $CDCl_3$ or D_2O. Chemical shifts (δ 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 *Pseudomonas* sp. VLB120,[1] and StyA1 from *Rhodococcus opacus*,[2] were produced and purified as previously described.[3] The *styA* gene (accession number: AJA07151; coding for *Sf*StyA) originating from *Sphingopyxis fribergensis* Kp5.2 was obtained and transformed into *E. coli* BL21(DE3)pLysS cells.

2.1.1. Sequence information and accession numbers

StyA from *Pseudomonas* sp. VLB120
accession number: O50214

```
>sp|O50214|STYA_PSESP Styrene monooxygenase StyA OS=Pseudomonas sp. OX=306
GN=styA PE=1 SV=1
MKKRIGIVGAGTAGLHLGLFLRQHDVDVTVYTDRKPDEYSGLRLLNTVAHNAVTVQREVA
LDVNEWPSEEFGYFGHYYYVGGPQPMRFYGDLKAPSRAVDYRLYQPMLMRALEARGGKFC
YDAVSAEDLEGLSEQYDLLVVCTGKYALGKVFEKQSENSPFEKPQRALCVGLFKGIKEAP
IRAVTMSFSPGHGELIEIPTLSFNGMSTALVLENHIGSDLEVLAHTKYDDDPRAFLDLML
EKLGKHHPSVAERIDPAEFDLANSSLDILQGGVVPAFRDGHATLNNGKTIIGLGDIQATV
DPVLGQGANMASYAAWILGEEILAHSVYDLRFSEHLERRRQDRVLCATRWTNFTLSALSA
LPPEFLAFLQILSQSREMADEFTDNFNYPERQWDRFSSPERIGQWCSQFAPTIAA
```

StyA1 from *Sphingopyxis fribergensis* Kp5.2
accession number: A0A0A7PAQ5

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>tr|A0A0A7PAQ5|A0A0A7PAQ5_9SPHN Styrene monooxygenase StyA OS=Sphingopyxis
fribergensis OX=1515612 GN=styA PE=4 SV=1
MSKKIGIIGAGTAGLKLGLHLLKNGVEVKLFTDRRPEEYAGMRLLNTVAHHHVTVEREDK
LGVNHWPDVGYKGHYYYIGTPEPLQFYGDLVAPSRAVDYRIYQPQLMQDFIDRGGDIEYG
QIAHEDLDAIADEFDLLVVCTGKGPFGQMFTHEPAYSPFDRPQRALCVGLFKGIREPETR
ALTMYFSPGHGEMIEIPTLSFNGMVNALVIENHIGGDLEILAKTKYDDDPKAFIALLLEK
LQKHYPTCYERIDLEEFDLANGPLDILQGGVTPTVRNSYAKLPNGKIAVALGDVQAVVDP
VLGQGANMASYAAIILGEEIVANDVLDERFMEKVDARRRDRVLSATRWTNYMLSSLATLD
PNLLQFIGAVSQNPKLADEFTENFNFPEKQWDCFSSPERVQAWIQARLGTPANDAEELVA
AE
```

***Sf*StyA 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 CTG CAT CTG TTA AAG AAC GGC GTA GAG GTG AAA CTG TTT ACT GAT CGT CGT
CCC GAA GAA TAT GCC GGA ATG CGC CTG CTG AAT ACC GTT GCT CAT CAC CAT GTC
ACG GTT GAA CGG GAG GAT AAA CTC GGT GTG AAT 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 GTG GCT CCG TCA CGT GCT GTC GAT TAC CGC ATT TAC CAG CCT CAA CTG ATG
CAG GAC TTT ATC GAT CGC GGA GGT GAC ATC GAA TAT GGC CAA ATT GCG CAT GAA
GAT CTG GAT GCG ATT GCT GAC GAA TTC GAC CTC CTG GTG GTG TGT ACC GGC AAA
GGT CCG TTT GGC CAG ATG TTT ACC CAT GAA CCA GCC TAT TCG CCG TTT GAT CGC
CCA CAA CGC GCA CTG TGC GTG GGT CTG TTC AAA GGC ATT CGT GAA CCG GAA ACG
CGC GCA TTG ACT ATG TAC TTT AGC CCA GGT CAT GGG GAG ATG ATT GAG ATT CCG
ACG TTA AGC TTC AAT GGC ATG GTG AAT GCT CTC GTC ATT GAA AAC CAC ATT GGC
GGT GAT TTG GAA ATC CTG GCC AAA ACC AAA TAT GAC GAT GAT CCG AAG GCG TTC
ATT GCC TTG CTG TTG GAG AAA CTG CAG AAA CAC TAC CCC ACC TGT TAC GAA CGC
ATC GAT CTC GAA GAA TTC GAC TTG GCA AAC GGG CCG CTT GAT ATC CTT CAG GGT
GGC GTA ACC CCG ACA GTT CGG AAT TCT TAT GCC AAG CTG CCA AAC GGC AAA ATT
GCG GTA GCG TTA GGC GAT GTT CAA GCG GTC GTT GAT CCC GTT CTG GGT CAA GGC
GCA AAC ATG GCC AGC TAT GCG GCT ATC ATC CTC GGG GAG GAA ATC GTG GCC AAT
GAT GTG CTG GAT GAA CGC TTT ATG GAG AAA GTT GAC GCA CGC CGT CGC GAT CGC
GTG CTG TCT GCG ACG CGT TGG ACC AAC TAC ATG CTG AGT AGC CTT GCC ACA TTA
GAT CCG AAC TTA TTA CAG TTC ATT GGT GCA GTC TCG CAG AAT CCG AAA CTG GCG
GAC GAA TTT ACC GAA AAC TTC AAC TTT CCG GAG AAA CAG TGG GAC TGC TTT TCC
AGT CCT GAA CGT GTG CAG GCA TGG ATT CAA GCG CGT TTG GGT ACG CCG GCA AAT
GAT GCC GAA GAG CTT GTA GCC GCG GAA TAA GCG GCC GC

accession number: AJA07151

>AJA07151.1 Styrene monooxygenase StyA [Sphingopyxis fribergensis]
MSKKIGIIGAGTAGLKLGLHLLKNGVEVKLFTDRRPEEYAGMRLLNTVAHHHVTVEREDKLGVNHWPDVG
YKGHYYYIGTPEPLQFYGDLVAPSRAVDYRIYQPQLMQDFIDRGGDIEYGQIAHEDLDAIADEFDLLVVC
TGKGPFGQMFTHEPAYSPFDRPQRALCVGLFKGIREPETRALTMYFSPGHGEMIEIPTLSFNGMVNALVI
ENHIGGDLEILAKTKYDDDPKAFIALLLEKLQKHYPTCYERIDLEEFDLANGPLDILQGGVTPTVRNSYA
KLPNGKIAVALGDVQAVVDPVLGQGANMASYAAIILGEEIVANDVLDERFMEKVDARRRDRVLSATRWTN
YMLSSLATLDPNLLQFIGAVSQNPKLADEFTENFNFPEKQWDCFSSPERVQAWIQARLGTPANDAEELVA
AE

2.1.2. Protein production and purification

A preculture of *E. coli* BL21(DE)pLysS transformed with p*Sf*StyA 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 $MgCl_2$, one tablet of cOmplete™ EDTA-free protease inhibitor cocktail, and DNaseI, 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 *Sf*StyA 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 N_2 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 *Sf*StyA.

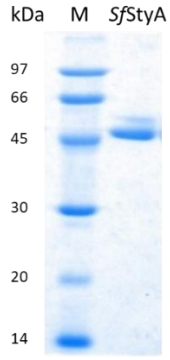
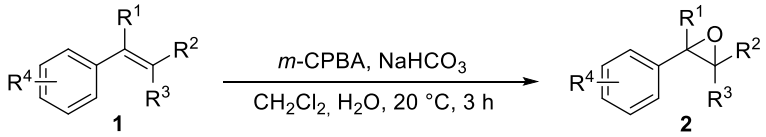


Figure S1. SDS-PAGE of a protein marker M (left column) and purified *Sf*StyA (right column). The gel was stained with Coomassie Brilliant Blue.

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-dihydronaphthalene, 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**).

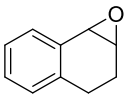


Scheme S1. Chemical aromatic alkene epoxidation.

The non-commercially available racemic epoxides were available in our group, synthesized as previously described.[3] 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

by TLC. 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 **2** were technically pure by ^{1}H NMR or further purified by column chromatography. NMR and GC-MS analyses were consistent with literature.[3] The 1,2-dihydronaphthalene oxide was newly synthesized in this study.

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



1,2-Dihydronaphthalene (0.52 g, 4 mmol) afforded 1,2-dihydronaphthalene oxide as a colorless oil (0.49 g, 84%).[4] **^{1}H NMR** (400 MHz, $CDCl_3$) 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); **^{13}C NMR** (100 MHz, $CDCl_3$) 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-SO_4 pH 7.0), BNAH (15 mM), catalase (650 U), FAD (50 µM), *Sf*StyA (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		
*Sf*SMO + BNAH	1300	this study
StyA1 + BNAH	433	[3]
StyA + $[Cp^*Rh(bpy)(H_2O)]^{2+}$	662[a]	[5]
enzymatic[b]		
StyA1 + StyB	175	[3]
Two-component StyA/StyB	5820	[6]
Natural fused StyA2B	78	[7]
Fus-SMO	5700	[8]

[a] Extrapolated from a 15 min reaction due to the mutual inactivation of the StyA and Rh complex.
[b] 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**).

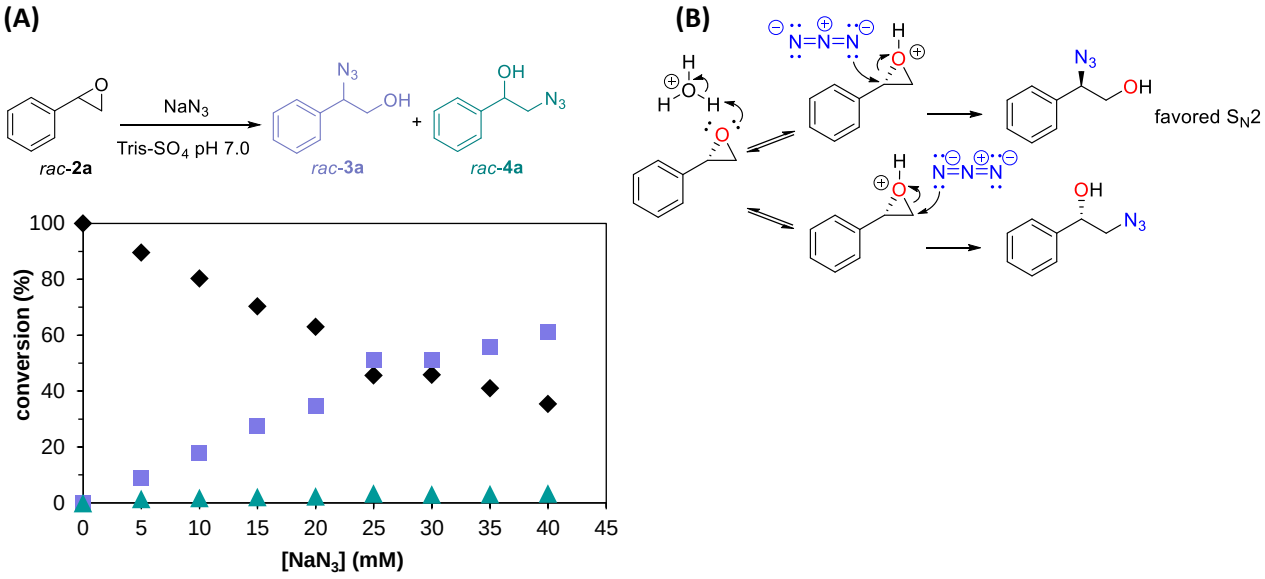


Figure S2. A) Formation of 2-azido-2-phenylethan-1-ol, starting with 5 mM racemic styrene oxide with increasing sodium azide concentration in 50 mM Tris-SO_4 buffer pH 7.0 at 30 °C and 900 rpm for 1 h. Products analyzed by GC. Styrene oxide [♦], 2-azido-2-phenylethan-1-ol [■], 2-azido-1-phenylethan-1-ol [▲]. **B**) Schematic representation of the simplified S_N2 mechanism of aromatic epoxide azidolysis.

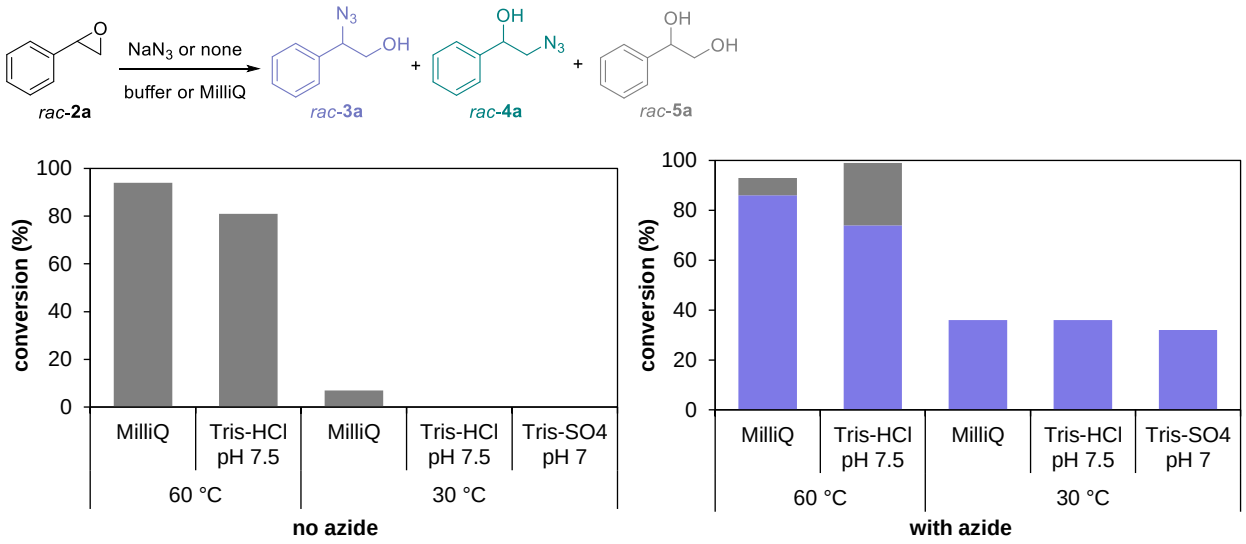


Figure S3. Influence of temperature and pH on chemical epoxide ring opening. Diol product *rac*-**5a** observed in grey. Azido alcohol product in blue. Conversion (%) = 100 - remaining substrate (%).

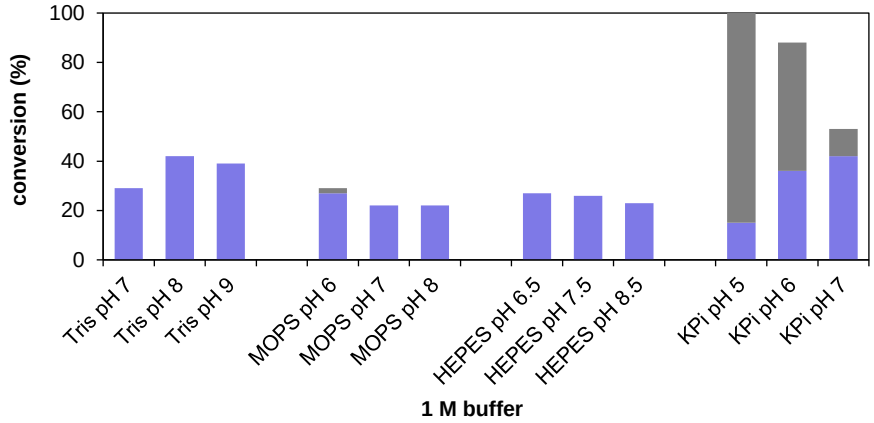


Figure S4. Effect of high buffer concentration and pH on enzyme-catalyzed epoxide formation and ring opening to diol. Azido alcohol product in blue. Diol *rac*-**5a** observed in grey.

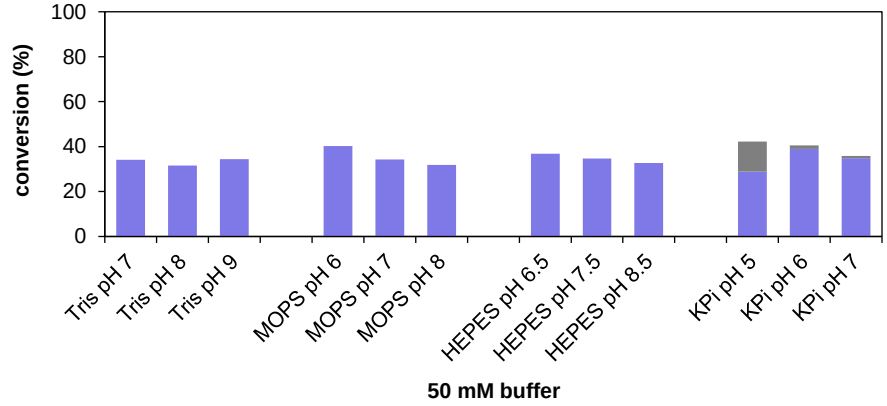


Figure S5. Effect of buffer and pH on enzyme-catalyzed epoxide formation and ring opening to diol. Azido alcohol product in blue. Diol *rac*-**5a** observed in grey.

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), *Sf*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.

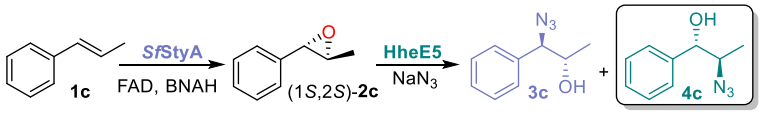
Semi-preparative scale

In a 500 mL glass Erlenmeyer with 200 mL Tris-SO_4 pH 8.0 buffer was added *trans*-β-methylstyrene (260 µL, 10 mM), FAD (8.2 mg, 50 µM), *Sf*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.Bienzymatic cascade

Lyophilized HHDH (10 mg) was rehydrated in buffer for 30 min before use. Bienzymatic 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), *Sf*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 bienzymatic cascade from *trans*-β-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*-β-methylstyrene] = 5 mM, [*Sf*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.

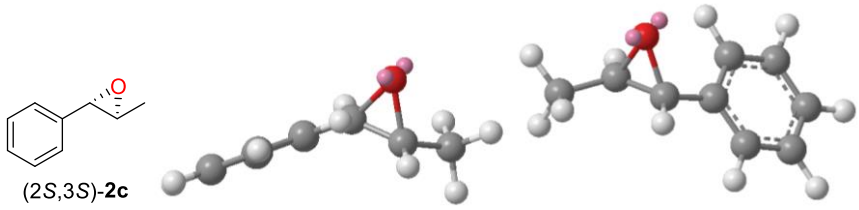


Figure S6. (2*S*,3*S*)-2-methyl-3-phenyloxirane [(2*S*,3*S*)-**2c**].

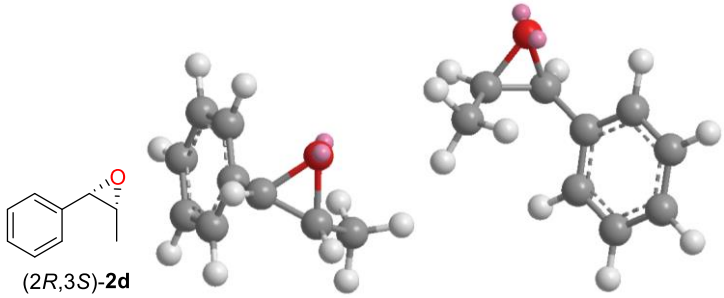


Figure S7. (2*R*,3*S*)-2-methyl-3-phenyloxirane [(2*R*,3*S*)-**2d**].

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.

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*). *Sf*StyA 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).

Table S5. GC methods and compound retention times for epoxide 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 2.16 mL/min	- 15.00	110.0 170.0	15.00 2.00	styrene **1a** dodecane (*S*)-styrene oxide **2a** (*R*)-styrene oxide **2a**	3.9 7.7 9.5 11.0
B	split 50 linear velocity 36.8 cm/s column flow 2.05 mL/min	- 20.00	100.0 220.0	15.00 1.00	styrene **1a** dodecane (*S*)-styrene oxide **2a** (*R*)-styrene oxide **2a**	4.3 8.3 12.0 13.1

A	split 100 linear velocity 40.1 cm/s column flow 2.44 mL/min	- 5.00 5.00 10.0	80.0 90.0 100.0 170.0	5.00 5.00 15.00 1.00	α-methylstyrene **1b** dodecane (*R*)-α-methylstyrene oxide **2b** (*S*)-α-methylstyrene oxide **2b**	9.2 15.5 20.3 20.9
B	split 100 linear velocity 36.8 cm/s column flow 2.04 mL/min	- 20.00	100.0 220.0	15.00 1.00	*trans*-methylstyrene **1c** dodecane (2*S*,3*S*)-2-methyl-3-phenyloxirane **2c** (2*R*,3*R*)-2-methyl-3-phenyloxirane **2c**	6.5 8.2 11.3 12.5
B	split 100 linear velocity 36.8 cm/s column flow 2.04 mL/min	- 20.00	100.0 220.0	15.00 1.00	*cis*-methylstyrene **1d** dodecane benzaldehyde phenylacetone (2*R*,3*S*)-2-methyl-3-phenyloxirane **2d** (2*S*,3*S*)-2-methyl-3-phenyloxirane **2c** (2*R*,3*R*)-2-methyl-3-phenyloxirane **2c** (2*S*,3*R*)-2-methyl-3-phenyloxirane **2d**	5.5 8.2 8.9 16.6 10.7 11.3 12.5 13.5
D	split 100 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 15.00 10.00 25.0	100.0 175.0 205.0 250.0	4.00 2.20 2.00 2.00	dodecane 4-bromostyrene **1e** (*R*)-4-bromostyrene oxide **2e** (*S*)-4-bromostyrene oxide **2e**	9.8 11.0 14.8 14.9
C	split 20 linear velocity 25.0 cm/s column flow 1.19 mL/min	- 10.00 10.00 25.0	100.0 120.0 130.0 225.0	2.00 15.20 2.00 1.00	dodecane 3-bromostyrene **1f** (*R*)-3-bromostyrene oxide **2f** (*S*)-3-bromostyrene oxide **2f**	7.4 7.6 19.1 19.4
C	split 20 linear velocity 25.9 cm/s column flow 1.30 mL/min	- 5.00 5.00 5.00 25.00	80.0 100.0 120.0 160.0 225.0	2.00 8.00 5.00 1.00 1.00	2-bromostyrene **1g** dodecane (*R*)-2-bromostyrene oxide **2g** (*S*)-2-bromostyrene oxide **2g**	13.5 14.2 24.0 24.3
B	split 100 linear velocity 37.4 cm/s column flow 2.14 mL/min	- 5.00 5.00 5.00 20.00	90.0 100.0 110.0 120.0 220.0	5.00 5.00 5.00 5.00 1.00	dodecane 4-chlorostyrene **1h** (*S*)-4-chlorostyrene oxide **2h** (*R*)-4-chlorostyrene oxide **2h**	9.8 10.9 25.5 26.0
B	split 75 linear velocity 35.5 cm/s column flow 1.87 mL/min	- 20.00	120.0 220.0	23.00 1.00	dodecane 3-chlorostyrene **1i** (*S*)-3-chlorostyrene oxide **2i** (*R*)-3-chlorostyrene oxide **2i**	5.3 5.8 15.8 20.5
B	split 75 linear velocity 35.5 cm/s column flow 1.87 mL/min	- 5.00 20.00	120.0 150.0 220.0	5.00 5.00 1.00	dodecane 2-chlorostyrene **1j** (*R*)-2-chlorostyrene oxide **2j** (*S*)-2-chlorostyrene oxide **2j**	5.3 5.8 9.4 10.1
B	split 75 linear velocity 36.8 cm/s column flow 2.04 mL/min	- 20.00	100.0 220.0	15.00 1.00	4-fluorostyrene **1k** dodecane (*S*)-4-fluorostyrene oxide **2k** (*R*)-4-fluorostyrene oxide **2k**	4.4 8.2 11.2 12.8
C	split 50 linear velocity 25.9 cm/s column flow 1.30 mL/min	- 5.00 25.00	80.0 100.0 225.0	10.00 10.00 1.00	3-fluorostyrene **1l** (*R*)-3-fluorostyrene oxide **2l** (*S*)-3-fluorostyrene oxide **2l** dodecane	6.5 17.2 17.4 20.5
C	split 50 linear velocity 26.4 cm/s column flow 1.37 mL/min	- 5.00 5.00	70.0 80.0 90.0	5.00 5.00 5.00	2-fluorostyrene **1m** (*R*)-2-fluorostyrene oxide **2m** (*S*)-2-fluorostyrene oxide **2m**	7.5 18.2 18.8

		5.00 25.00	100.0 225.0	5.00 1.00	dodecane	24.6
B	split 100 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 20.00	70.0 75.0 80.0 85.0 90.0 100.0 220.0	5.00 5.00 5.00 5.00 5.00 10.00 1.00	4-methylstyrene **1n** dodecane (*S*)-4-methylstyrene oxide **2n** (*R*)-4-methylstyrene oxide **2n**	12.1 19.2 34.1 35.5
A	split 100 linear velocity 40.1 cm/s column flow 2.44 mL/min	- 5.00 5.00 10.0	80.0 90.0 100.0 170.0	5.00 5.00 15.00 1.00	4-methylstyrene **1n** dodecane (*R*)-4-methylstyrene oxide **2n** (*S*)-4-methylstyrene oxide **2n**	9.7 15.4 26.6 27.1
B	split 75 linear velocity 36.8 cm/s column flow 2.04 mL/min	- 20.00	100.0 220.0	24.00 1.00	3-methylstyrene **1o** dodecane 3-methylphenylacetaldehyde (*S*)-3-methylstyrene oxide **2o** (*R*)-3-methylstyrene oxide **2o**	5.9 8.4 15.3 19.5 21.7
D	split 100 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 25.0	100.0 120.0 130.0 140.0 250.0	2.00 2.00 5.00 8.00 1.00	allylbenzene **1p** dodecane (*R*)-allylbenzene oxide **2p** (*S*)-allylbenzene oxide **2p**	7.3 13.1 17.8 17.9
C	split 100 linear velocity 29.8 cm/s column flow 1.41 mL/min	- 10.00 20.00	120.0 140.0 225.0	8.00 5.00 1.00	dodecane 1,2-dihydronaphthalene **1q** (*S*)-1,2-dihydronaphthalene oxide **2q** (*R*)-1,2-dihydronaphthalene oxide **2q** 2-tetralone	5.1 6.0 12.3 12.9 15.4
D	split 50 linear velocity 36.6 cm/s column flow 2.00 mL/min	- 5.00 5.00 25.0	110.0 135.0 140.0 250.0	4.00 2.00 8.00 1.00	indene **1r** dodecane (*S*)-indene oxide **2r** (*R*)-indene oxide **2r** 2-indanone	9.4 11.8 18.6 19.1 22.1
A	split 100 linear velocity 38.0 cm/s column flow 2.13 mL/min	- 15.00	110.0 170.0	15.00 2.00	2-vinylpyridine **1s** dodecane (*R*)-2-vinylpyridine oxide **2s** (*S*)-2-vinylpyridine oxide **2s**	5.2 7.8 15.9 16.3

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	- 5.00 5.00 5.00 10.00	110.0 130.0 150.0 160.0 170.0	15.00 5.00 5.00 5.00 3.00	styrene **1a** dodecane (*S*)-styrene oxide **2a** (*R*)-styrene oxide **2a** α-azido alcohols: (*S*)-2-azido-2-phenylethan-1-ol **3a** (*R*)-2-azido-2-phenylethan-1-ol **3a** β-azido alcohols: (*R*)-2-azido-1-phenylethan-1-ol **4a** (*S*)-2-azido-1-phenylethan-1-ol **4a**	3.9 7.8 9.6 11.0 36.0 36.5 38.0 38.4

D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	α-methylstyrene **3b** dodecane α-azido alcohol (*R*)-**3b** diol diol β-azido alcohol (*S*)-**3b** β-azido alcohol (*R*)-**3b**	7.8 17.0 42.6 43.3 43.7 44.6 44.9
A	split 100 linear velocity 38.0 cm/s column flow 2.13 mL/min	- 5.00 5.00 5.00 10.00	110.0 130.0 150.0 160.0 170.0	15.00 5.00 5.00 5.00 3.00	*trans*-methylstyrene **1c** (1*S*,2*S*)-1-azido-1-phenylpropan-2-ol **3c** (1*R*,2*S*)-1-azido-1-phenylpropan-2-ol **3c** (1*S*,2*R*)-2-azido-1-phenylpropan-2-ol **3c**	5.9 34.1 35.2 38.4
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	dodecane (1*S*,2*S*)-1-azido-1-phenylpropan-2-ol **3d** (1*R*,2*S*)-1-azido-1-phenylpropan-2-ol **3d** (1*S*,2*S*)-1-azido-1-phenylpropan-2-ol **3c** (1*R*,2*S*)-1-azido-1-phenylpropan-2-ol **3c** (*trans*-methylstyrene is present in small amounts in the *cis*-methylstyrene starting material)	17.0 39.5 40.2 34.1 35.2
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	dodecane 4-bromostyrene **1e** 4-bromostyrene oxide **2e** (*R*)-2-azido-2-(4-bromophenyl)ethan-1-ol **3e**	17.0 20.5 34.3 59.2
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	dodecane 3-bromostyrene **1f** 3-bromostyrene oxide **2f** (*R*)-2-azido-2-(3-bromophenyl)ethan-1-ol **3f** β-azido alcohol (*S*)-2-azido-1-(3-bromophenyl)ethan-1-ol **3f**	17.0 18.6 34.3 57.7 60.9
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	dodecane 2-bromostyrene **1g** 2-bromostyrene oxide **2g** (*R*)-2-azido-2-(2-bromophenyl)ethan-1-ol **3g** (*S*)-2-azido-2-(2-bromophenyl)ethan-1-ol **3g** β-azido alcohol (*S*)-2-azido-1-(2-bromophenyl)ethan-1-ol **3g**	17.0 17.5 31.0 53.8 54.4 58.7
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	4-chlorostyrene **1h** dodecane (*R*)-2-azido-2-(4-chlorophenyl)ethan-1-ol **3h** (*S*)-2-azido-2-(4-chlorophenyl)ethan-1-ol **3h** β-azido alcohol (*S*)-2-azido-1-(4-chlorophenyl)ethan-1-ol **3h**	13.8 17.0 55.36 55.4 58.4
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	3-chlorostyrene **1i** dodecane 3-chlorostyrene oxide **2i** (*R*)-2-azido-2-(3-chlorophenyl)ethan-1-ol **3i** β-azido alcohol (*S*)-2-azido-1-(3-chlorophenyl)ethan-1-ol **3i**	12.0 17.0 28.0 53.6 58.3

D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	2-chlorostyrene **1j** dodecane 2-chlorostyrene oxide **2j** (*R*)-2-azido-2-(2-chlorophenyl)ethan-1-ol **3j** (*S*)-2-azido-2-(2-chlorophenyl)ethan-1-ol **3j** β-azido alcohol (*S*)-2-azido-1-(2-chlorophenyl)ethan-1-ol **3j**	11.0 17.0 25.0 49.2 50.0 55.1
A	split 100 linear velocity 38.0 cm/s column flow 2.13 mL/min	- 5.00 5.00 5.00 10.00	110.0 130.0 150.0 160.0 170.0	15.00 5.00 5.00 5.00 3.00	dodecane (*R*)-2-azido-2-(4-fluorophenyl)ethan-1-ol **3k** (*S*)-2-azido-1-(4-fluorophenyl)ethan-1-ol **3k**	7.8 44.5 48.0
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	4-fluorostyrene **1k** dodecane β-azido alcohol (*S*)-2-azido-1-(4-fluorophenyl)ethan-1-ol **3k** α-azido alcohol (*R*)-2-azido-2-(4-fluorophenyl)ethan-1-ol **3k**	5.8 17.0 45.3 49.6
A	split 100 linear velocity 38.0 cm/s column flow 2.13 mL/min	- 5.00 5.00 5.00 10.00	110.0 130.0 150.0 160.0 170.0	15.00 5.00 5.00 5.00 3.00	dodecane (*R*)-2-azido-2-(3-fluorophenyl)ethan-1-ol **3l** (*S*)-2-azido-1-(3-fluorophenyl)ethan-1-ol **3l**	7.8 36.1 39.0
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	3-fluorostyrene **1l** 3-fluorostyrene oxide **2l** dodecane (*R*)-2-azido-2-(3-fluorophenyl)ethan-1-ol **3l** (*S*)-2-azido-1-(3-fluorophenyl)ethan-1-ol **3l**	5.6 15.8 17.0 44.5 50.1
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	2-fluorostyrene **1m** dodecane (*R*)-2-azido-2-(2-fluorophenyl)ethan-1-ol **3m** (*S*)-2-azido-1-(2-fluorophenyl)ethan-1-ol **3m** β-azido alcohol **3m**	5.1 17.0 41.3 41.9 46.8
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	dodecane (*S*)-4-methylstyrene oxide β-azido alcohol **3n** (*R*)-2-azido-2-(*p*-tolyl)ethan-1-ol **3n** α-azido alcohol (*S*)-2-azido-2-(*p*-tolyl)ethan-1-ol **3n**	17.0 21.9 45.6 46.6 46.8
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	3-methylstyrene **1o** dodecane α-azido alcohol (*R*)-2-azido-2-(*m*-tolyl)ethan-1-ol **3o**	7.9 17.0 45.6
D	split 50 linear velocity 38.0 cm/s	- 5.00	110.0 130.0	15.00 5.00	dodecane	17.0

	column flow 2.16 mL/min	5.00 5.00 5.00 5.00 25.00	150.0 170.0 190.0 210.0 250.0	5.00 5.00 5.00 2.00 1.00	α-azido alcohol (1*R*,2*R*)-1-azido-1,2,3,4-tetrahydronaphthalen-2-ol **3q** β-azido alcohol **3q**	56.2 57.6
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	dodecane (1*S*,2*R*)-indene oxide **2r** α-azido alcohol (1*R*,2*R*)-1-azido-2,3-dihydro-1H-inden-2-ol **3r** β-azido alcohol **3r**	17.0 31.8 51.7 53.6
D	split 50 linear velocity 38.0 cm/s column flow 2.16 mL/min	- 5.00 5.00 5.00 5.00 5.00 25.00	110.0 130.0 150.0 170.0 190.0 210.0 250.0	15.00 5.00 5.00 5.00 5.00 2.00 1.00	2-vinylpyridine **1s** dodecane 2-vinylpyridine oxide **2s** α-azido alcohol (*R*)-2-azido-2-(pyridin-2-yl)ethan-1-ol **3s** β-azido alcohol (*S*)-2-azido-1-(pyridin-2-yl)ethan-1-ol **3s**	6.9 17.0 19.0 44.2 44.4
A	split 100 linear velocity 38.0 cm/s column flow 2.13 mL/min	- 5.00 5.00 5.00 10.00	110.0 130.0 150.0 160.0 170.0	15.00 5.00 5.00 5.00 3.00	dodecane α-azido alcohol (*R*)-2-azido-2-(pyridine-2-yl)ethan-1-ol **3s** β-azido alcohol (*R*)-2-azido-2-(pyridine-2-yl)ethan-1-ol **3s**	7.8 37.5 41.5

4.2. GC chromatograms of epoxide products

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

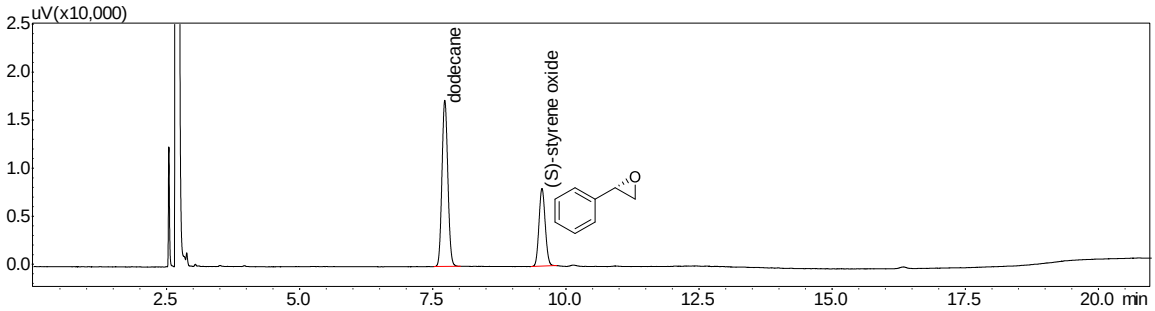


Figure S8. GC chromatogram of full reaction product (*S*)-styrene oxide **2a** on column **A**.

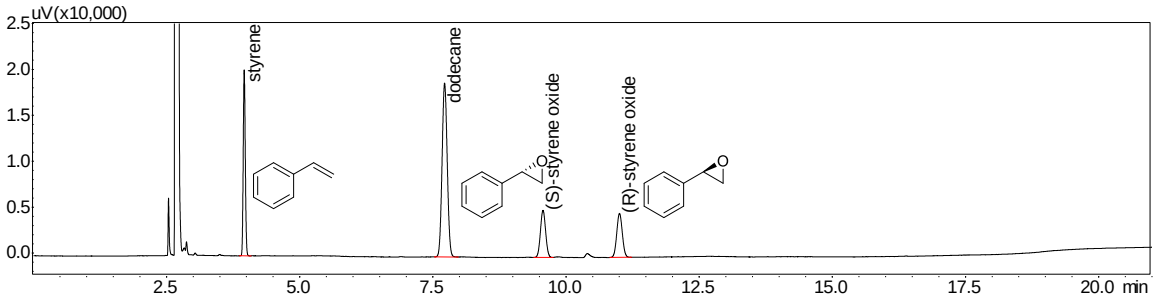


Figure S9. GC chromatogram of commercial racemic styrene oxide **2a** on column **A**.

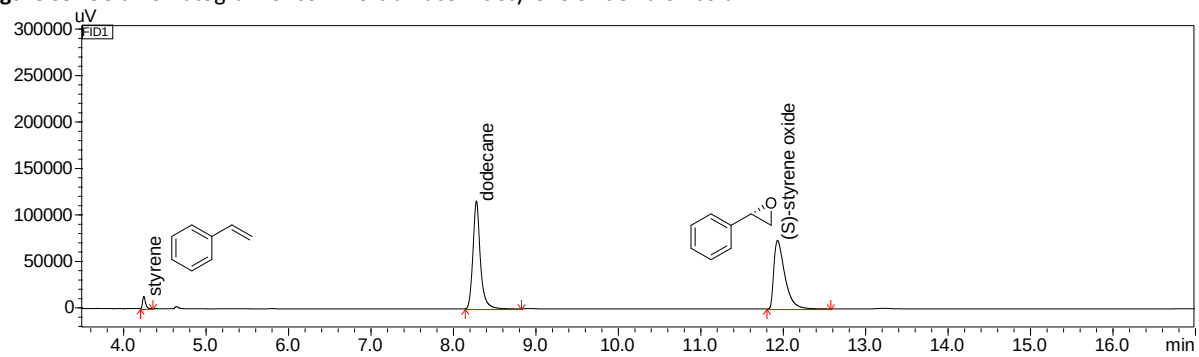


Figure S10. GC chromatogram of reaction product (*S*)-styrene oxide **2a** on column **B**.

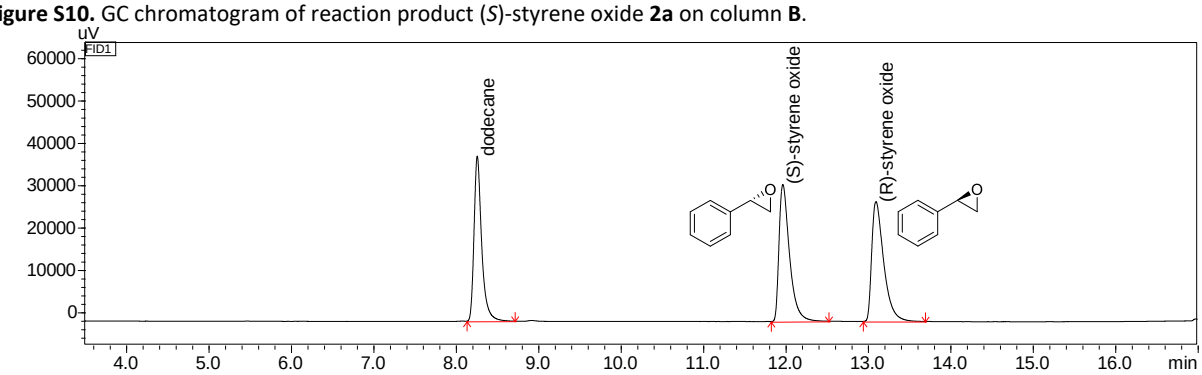


Figure S11. GC chromatogram of commercial racemic styrene oxide **2a** on column **B**.

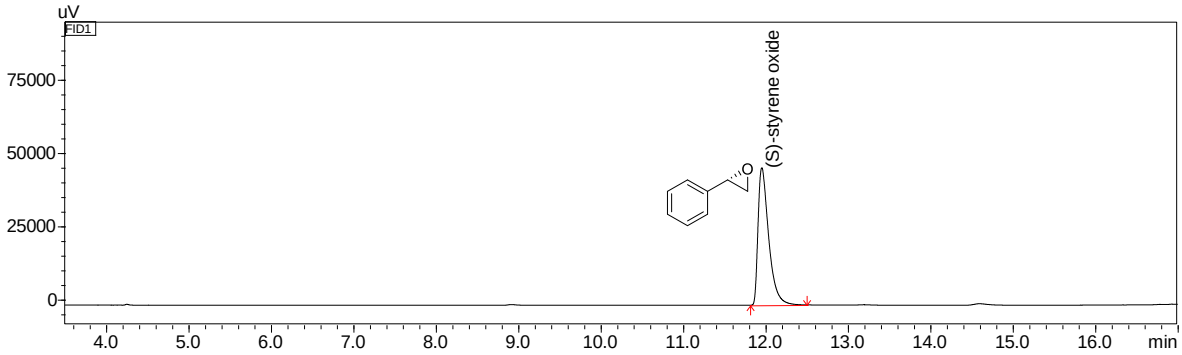


Figure S12. GC chromatogram of commercial (*S*)-styrene oxide **2a** on column **B**.

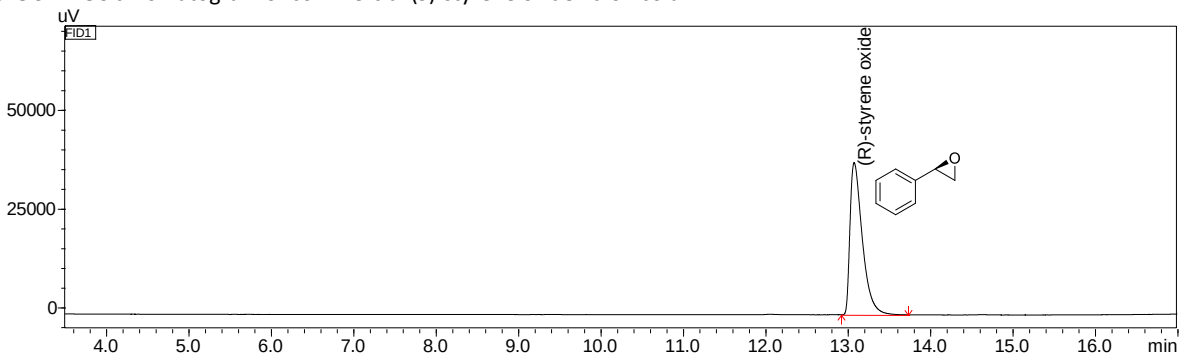


Figure S13. GC chromatogram of commercial (*R*)-styrene oxide **2a** on column **B**.

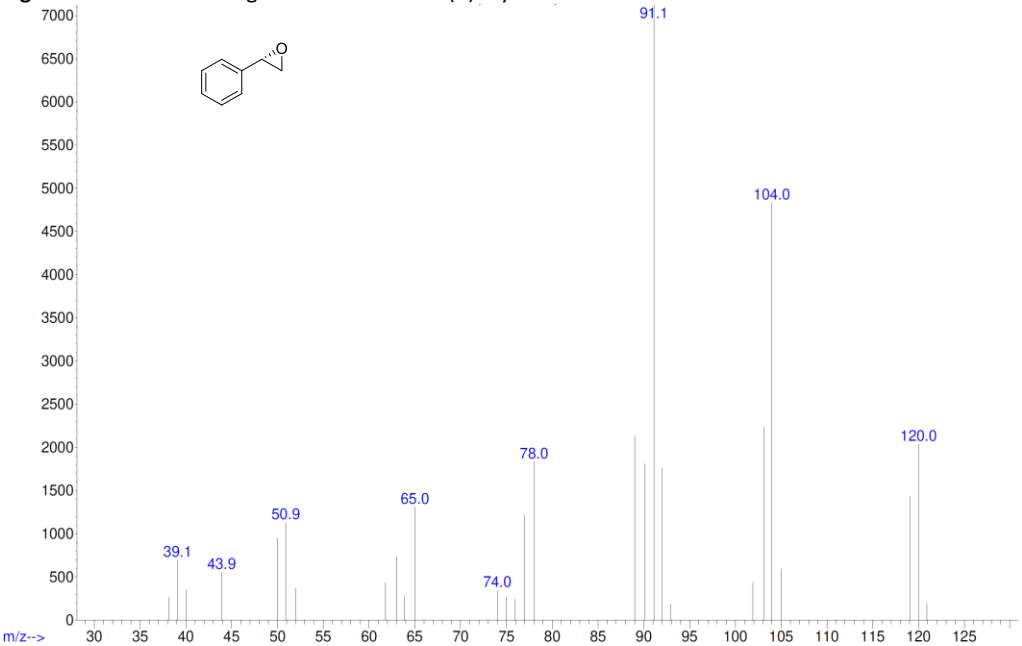


Figure S14. GC-MS mass spectrum of reaction product (*S*)-styrene oxide **2a**.

(*S*)-α-methylstyrene oxide (2-methyl-2-phenyloxirane) **2b**

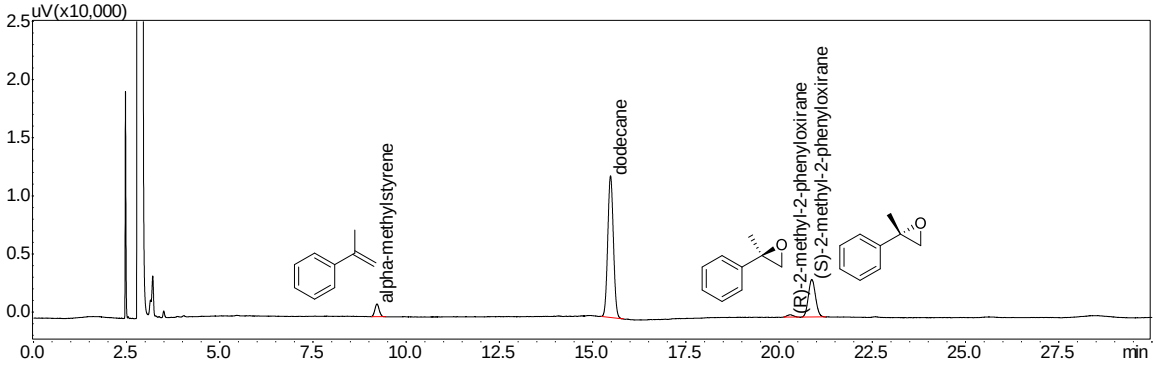


Figure S15. GC chromatogram of reaction products **2b** on column **A**.

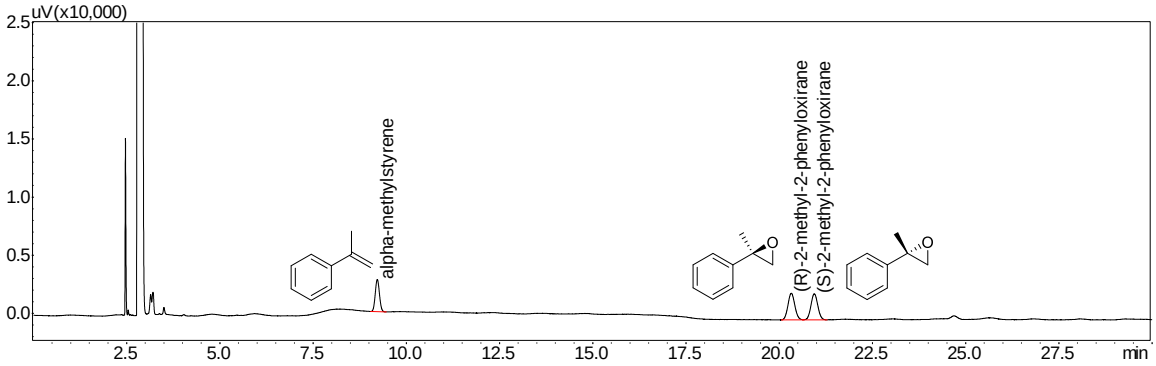


Figure S16. GC chromatogram of synthesized racemic α-methylstyrene oxide **2b** on column **A**.

(1*S*,2*S*)-1-phenylpropylene oxide ((2*S*,3*S*)-2-methyl-3-phenyloxirane; *trans*-β-methylstyrene oxide) **2c**

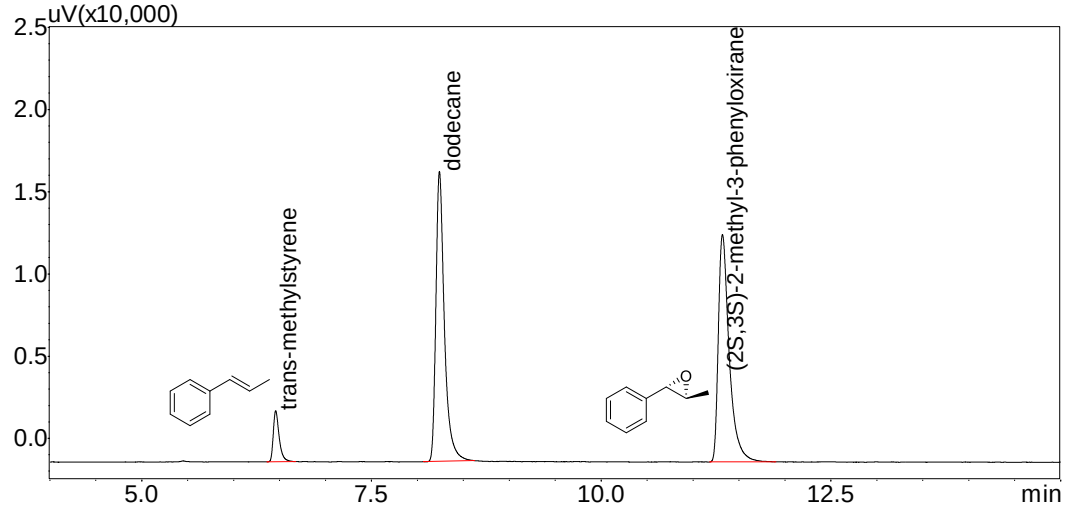


Figure S17. GC chromatogram of reaction product (1*S*,2*S*)-1-phenylpropylene oxide **2c** on column **B**.

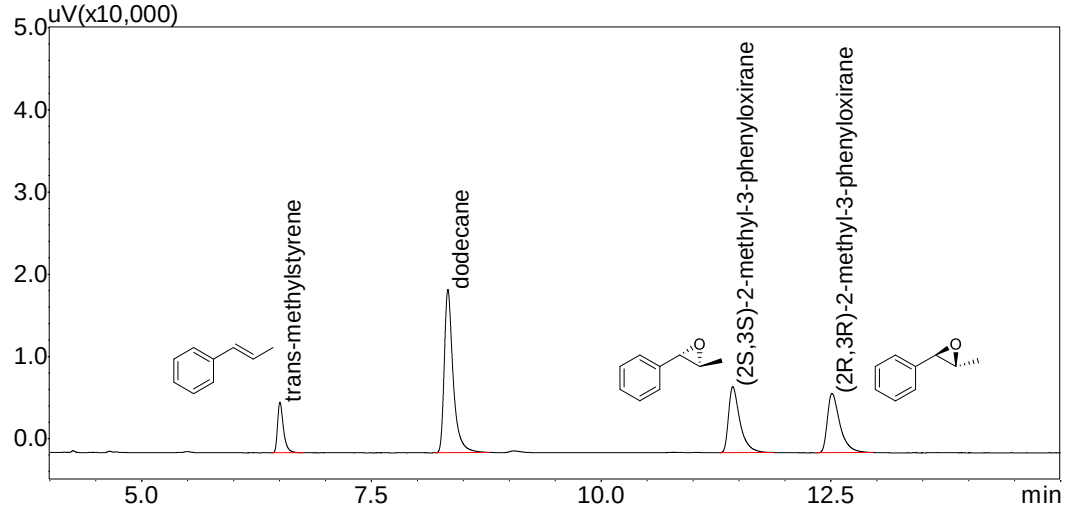


Figure S18. GC chromatogram of synthesized racemic *trans*-β-methylstyrene oxide **2c** on column **B**.

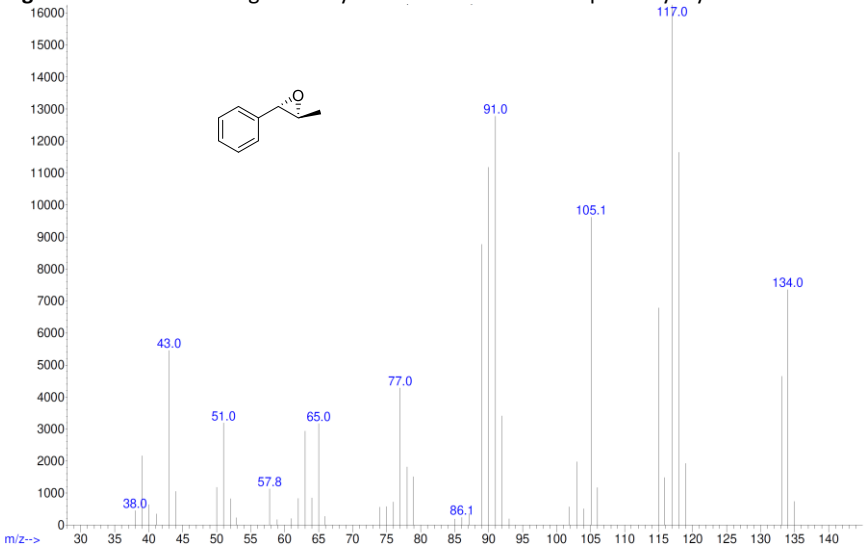


Figure S19. GC-MS spectrum of reaction product (1*S*,2*S*)-1-phenylpropylene oxide **2c**.

(1*S*,2*R*)-1-phenylpropylene oxide ((2*R*,3*S*)-2-methyl-3-phenyloxirane; *cis*-β-methylstyrene oxide) **2d**

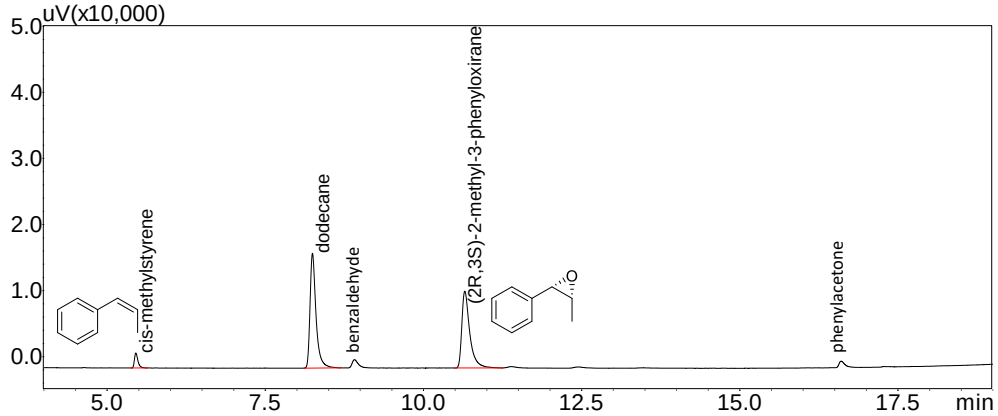


Figure S20. GC chromatogram of reaction products **2d** on column **B**: small peaks are compounds (benzaldehyde, phenylacetone, *trans*-β-methylstyrene oxides) present in the starting material.

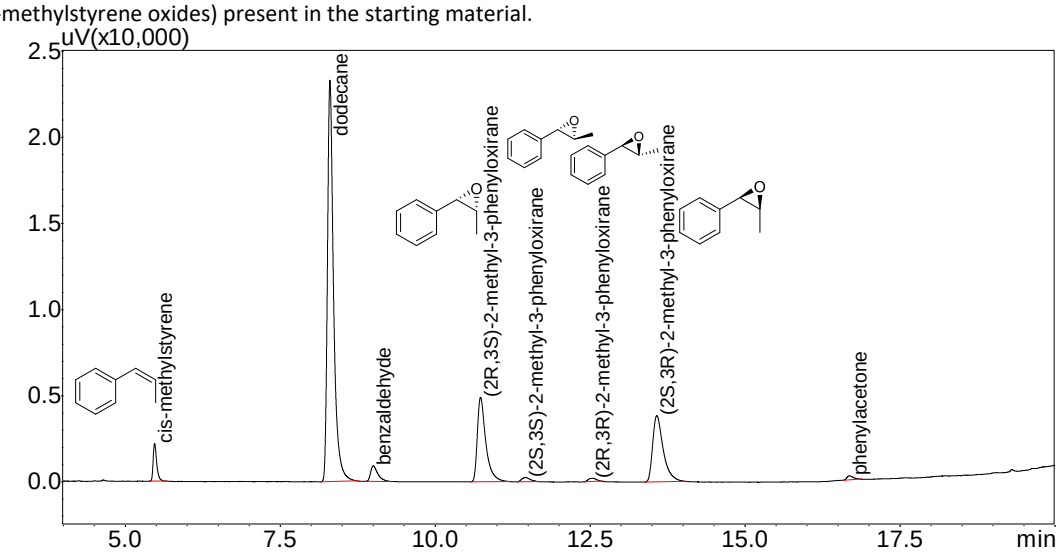


Figure S21. GC chromatogram of synthesized racemic *cis*-β-methylstyrene oxide **2d**, with impurities (benzaldehyde, *trans*-β-methylstyrene oxide, phenylacetone) from the starting material, on column **B**.

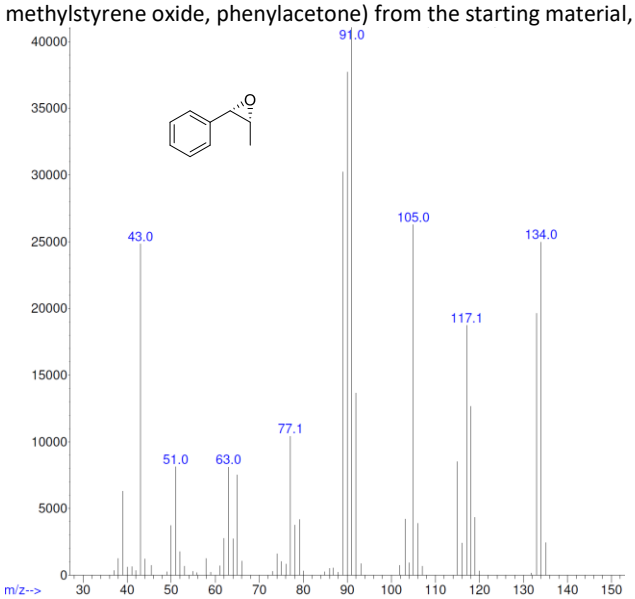


Figure S22. GC-MS mass spectrum of reaction product (1*S*,2*R*)-1-phenylpropylene oxide **2d**.

(*S*)-4-bromostyrene oxide (2-(4-bromophenyl)oxirane) **2e**

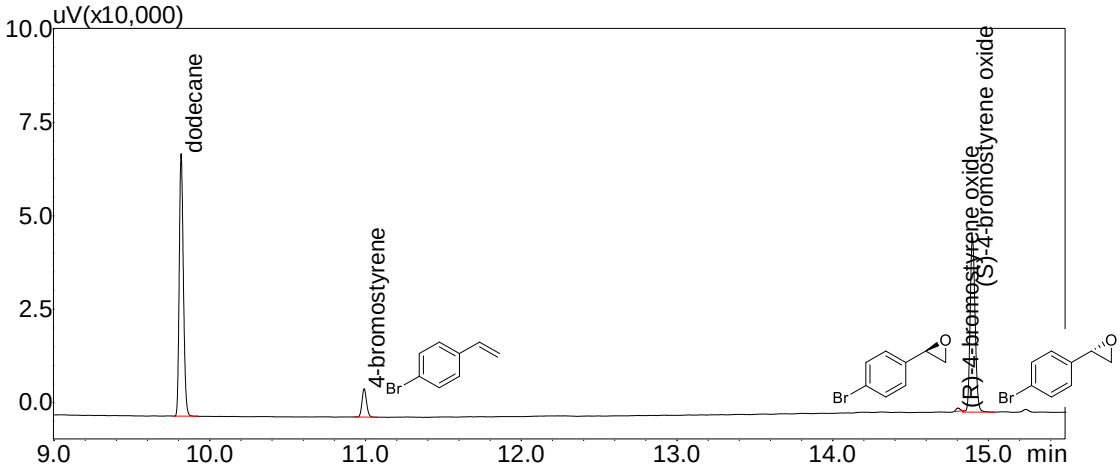


Figure S23. GC chromatogram of reaction products **2e** on column **D**.

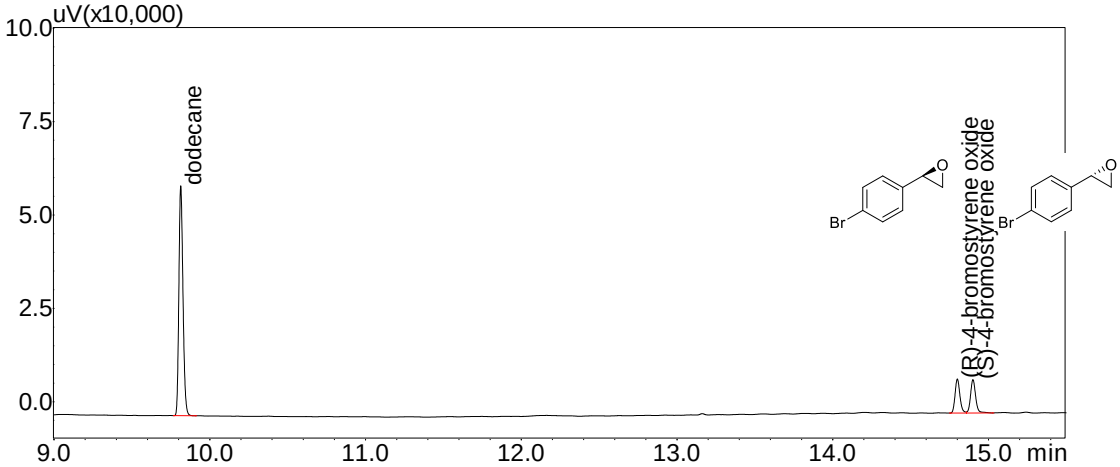


Figure S24. GC chromatogram of commercial racemic 4-bromostyrene oxide **2e** on column **D**.

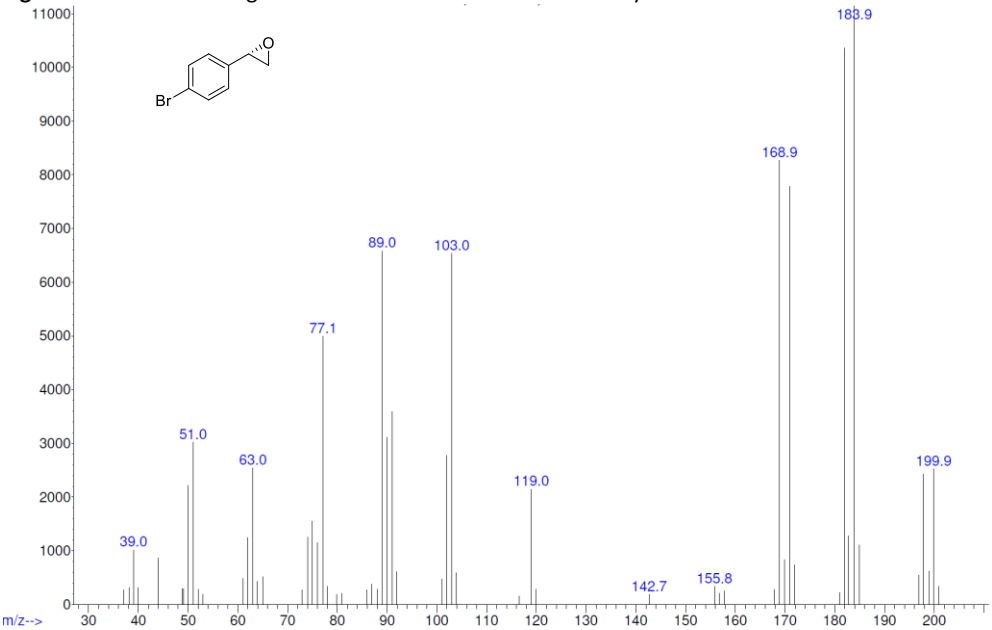


Figure S25. GC-MS mass spectrum of reaction product (*S*)-4-bromostyrene oxide **2e**.

(*S*)-3-bromostyrene oxide (2-(3-bromophenyl)oxirane) **2f**

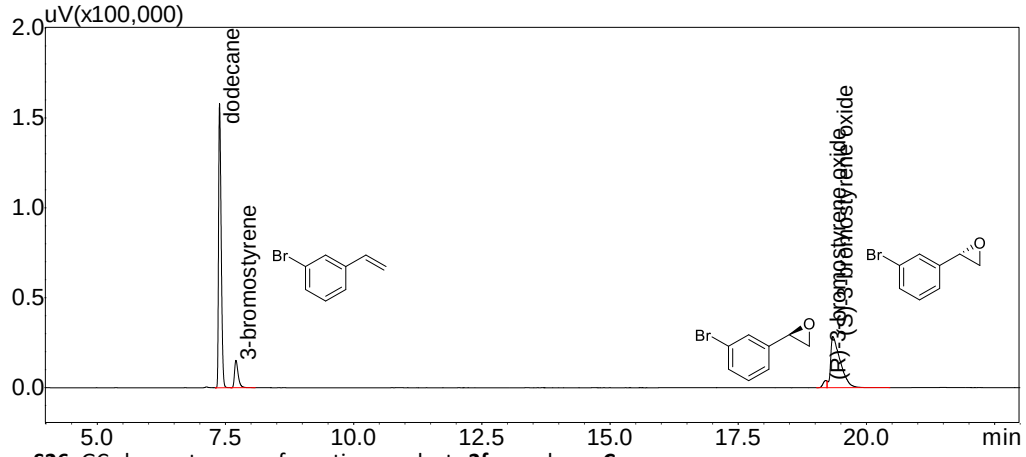


Figure S26. GC chromatogram of reaction products **2f** on column **C**.

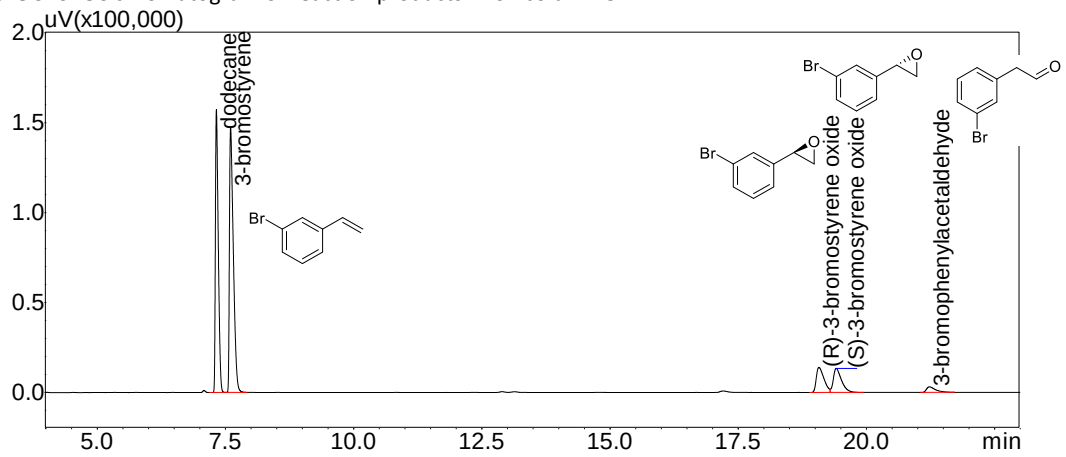


Figure S27. GC chromatogram of synthesized racemic 3-bromostyrene oxide **2f** and side product 3-bromophenylacetaldehyde on column **C**.

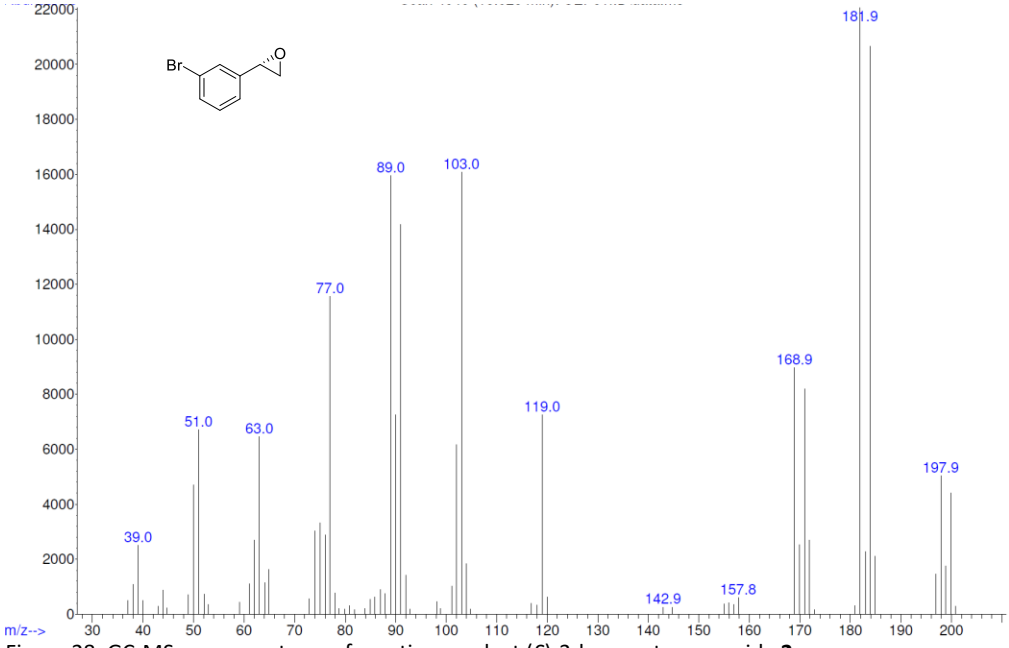


Figure 28. GC-MS mass spectrum of reaction product (*S*)-3-bromostyrene oxide **2e**.

(*S*)-2-bromostyrene oxide (2-(2-bromophenyl)oxirane) **2g**

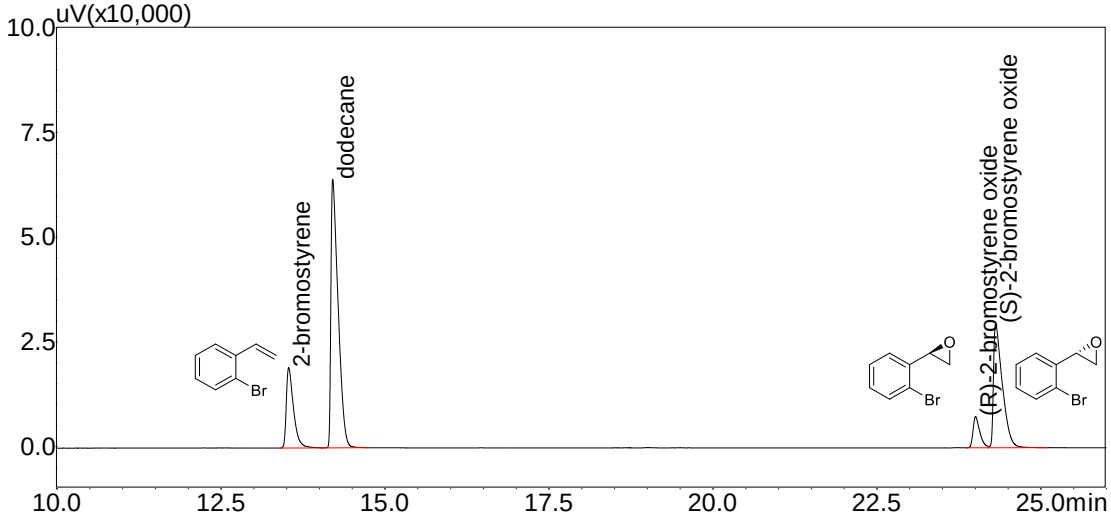


Figure S29. GC chromatogram of reaction products **2g** on column **C**.

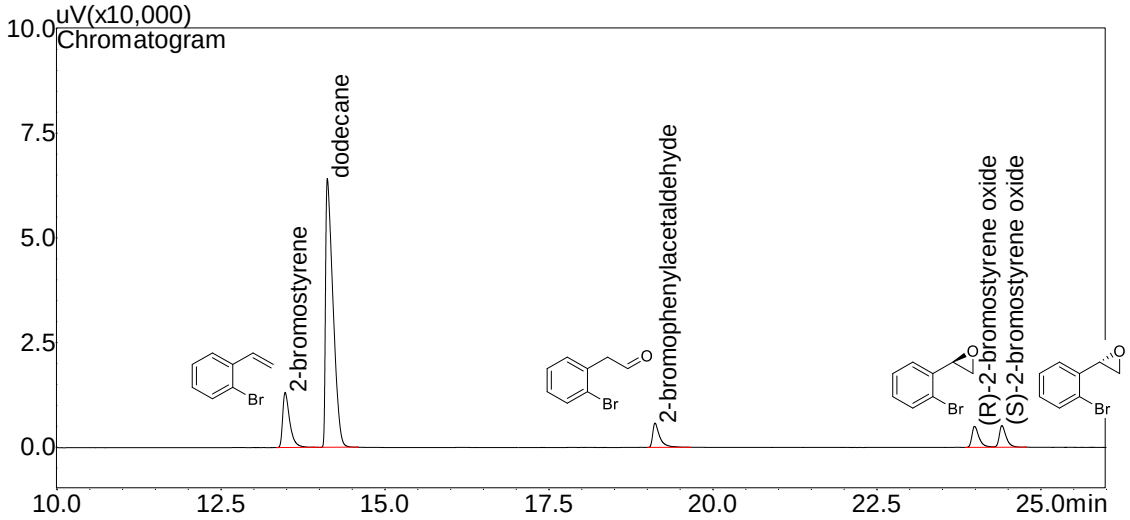


Figure S30. GC chromatogram of synthesized racemic 2-bromostyrene oxide **2g** with 2-bromophenylacetaldehyde side product on column **C**.

(*S*)-4-chlorostyrene oxide (2-(4-chlorophenyl)oxirane) **2h**

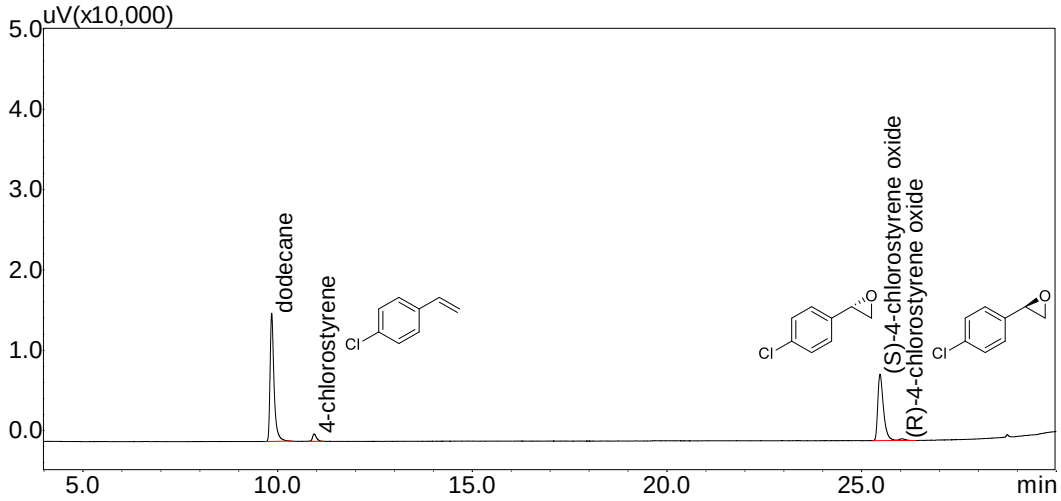


Figure S31. GC chromatogram of reaction products on column **B**.

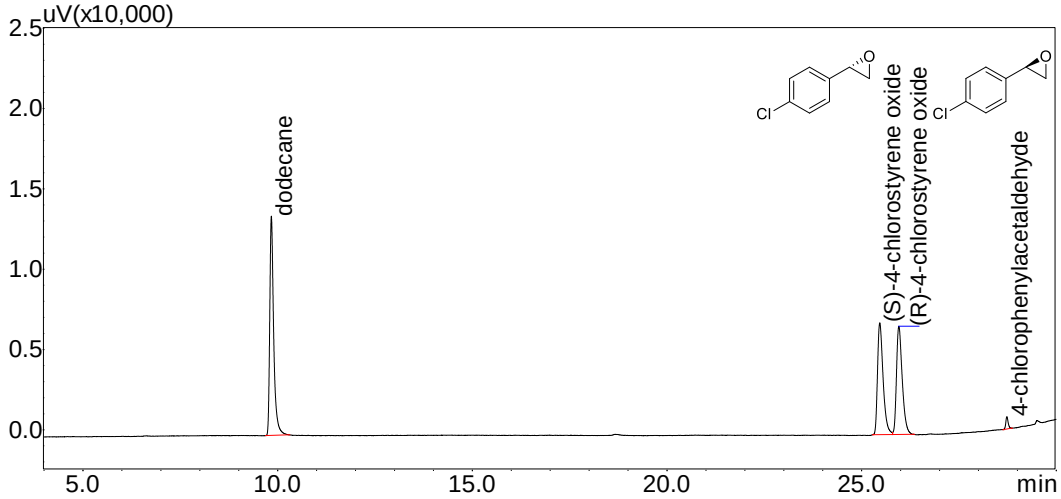


Figure S32. GC chromatogram of synthesized racemic 4-chlorostyrene oxide with 4-chlorophenylacetaldehyde side product on column **B**.

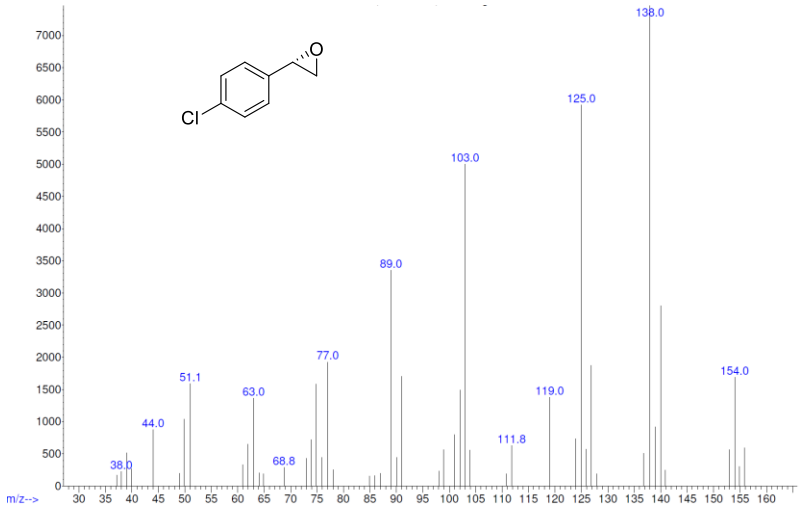


Figure S33. GC-MS mass spectrum of reaction product (*S*)-4-chlorostyrene oxide **2h.**

(*S*)-3-chlorostyrene oxide (2-(3-chlorophenyl)oxirane) **2i**

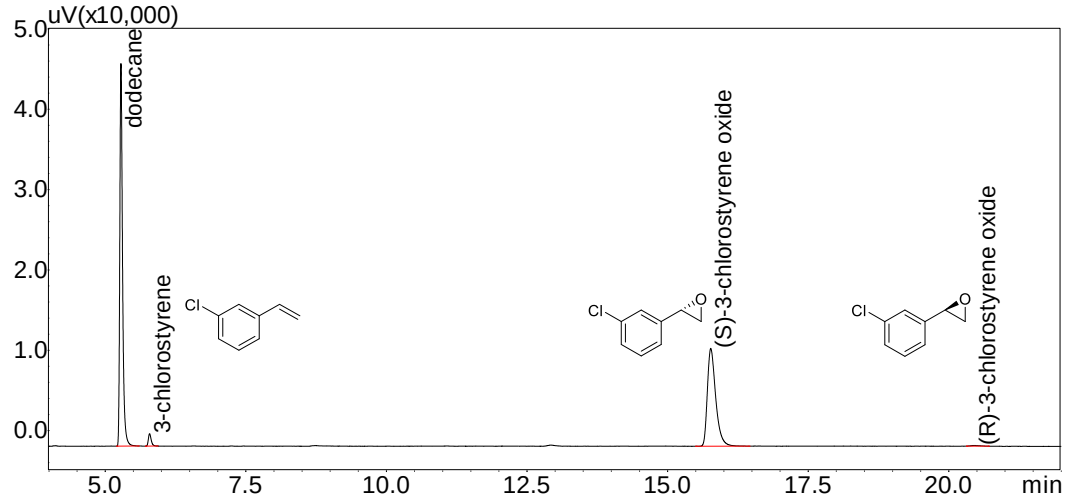


Figure S34. GC chromatogram of reaction products **2i** on column **B**.

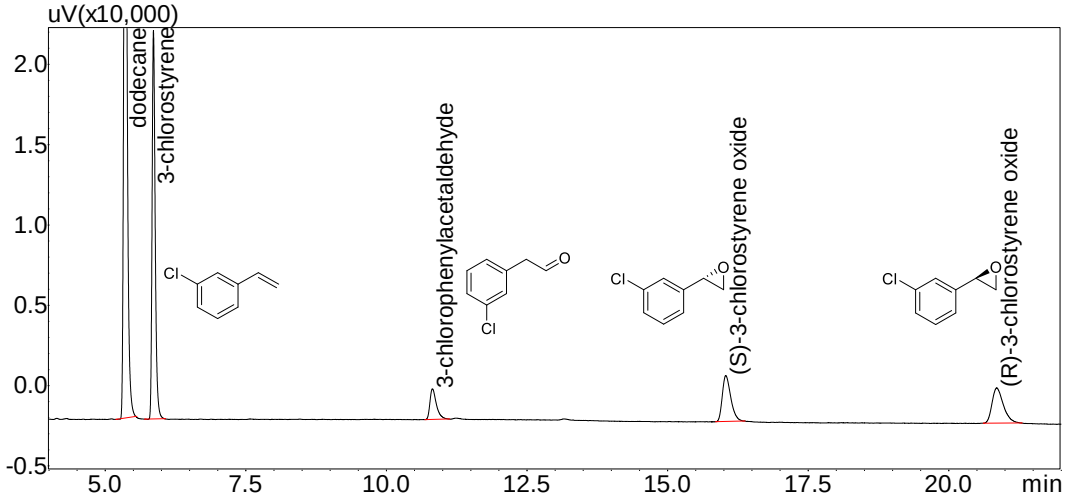


Figure S35. GC chromatogram of synthesized racemic 3-chlorostyrene oxide **2i** with 3-chlorophenylacetaldehyde side product on column **B**.

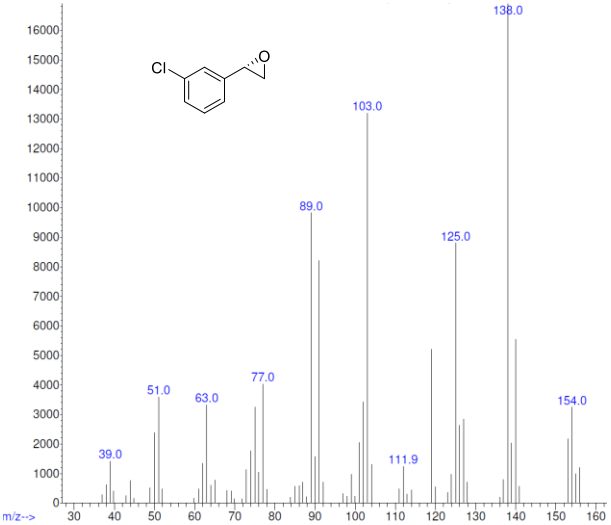


Figure S36. GC-MS mass spectrum of reaction product (*S*)-3-chlorostyrene oxide **2i**.

(*S*)-2-chlorostyrene oxide (2-(2-chlorophenyl)oxirane) **2j**

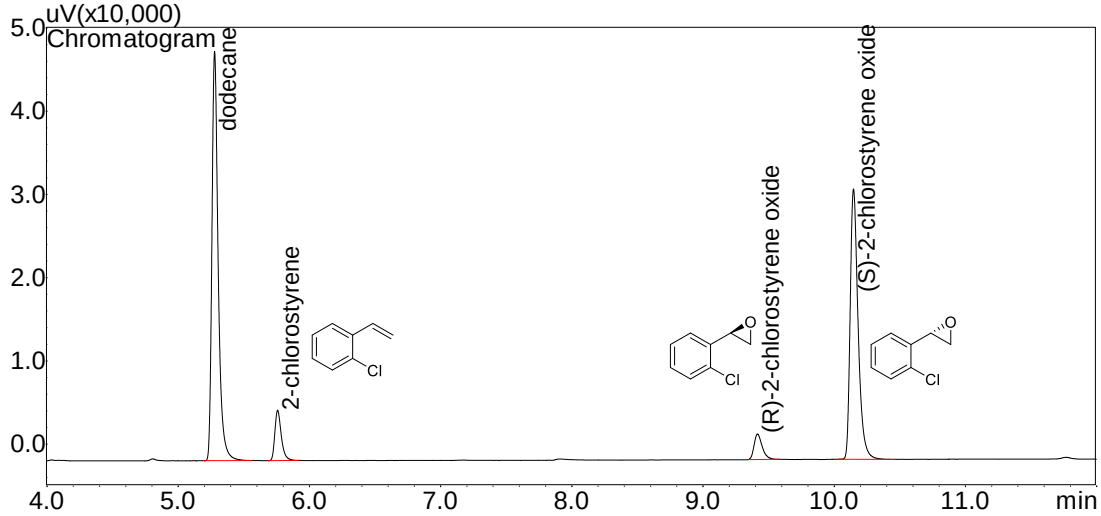


Figure S37. GC chromatogram of reaction products **2j** on column **B**.

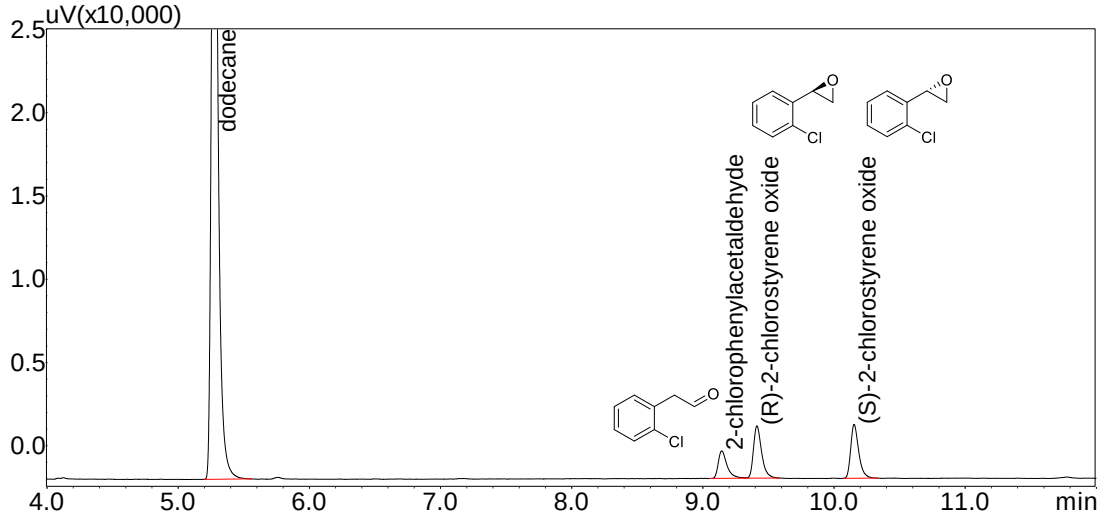


Figure S38. GC chromatogram of synthesized racemic 2-chlorostyrene oxide **2j** with 2-chlorophenylacetaldehyde side product on column **B**.

(*S*)-4-fluorostyrene oxide (2-(4-fluorophenyl)oxirane) **2k**

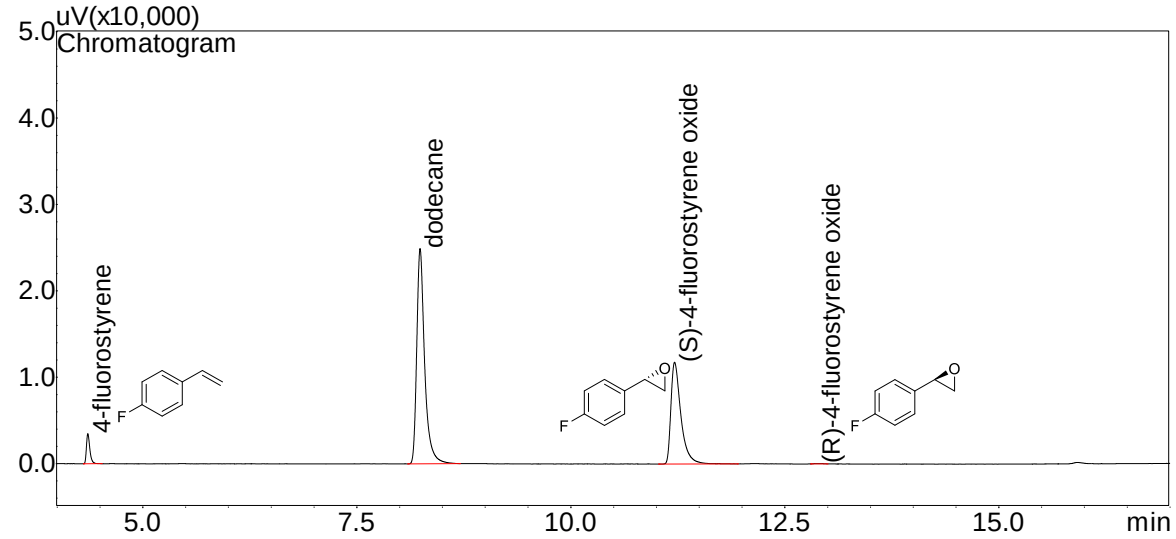


Figure S39. GC chromatogram of reaction products **2k** on column **B**.

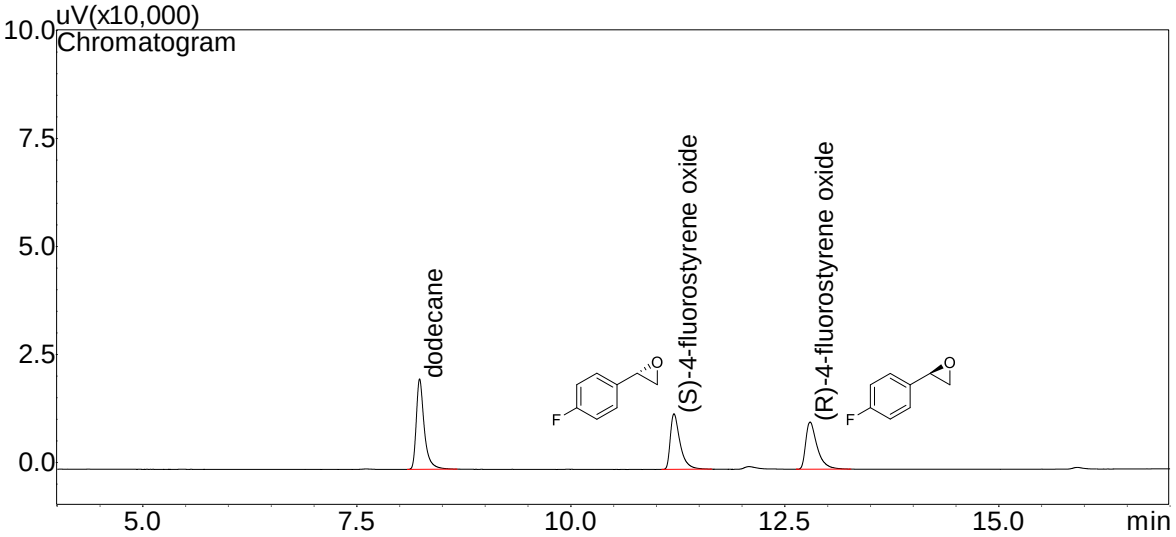


Figure S40. GC chromatogram of commercial racemic product **2k** on column **B**.

(*S*)-3-fluorostyrene oxide (2-(3-fluorophenyl)oxirane) **2l**

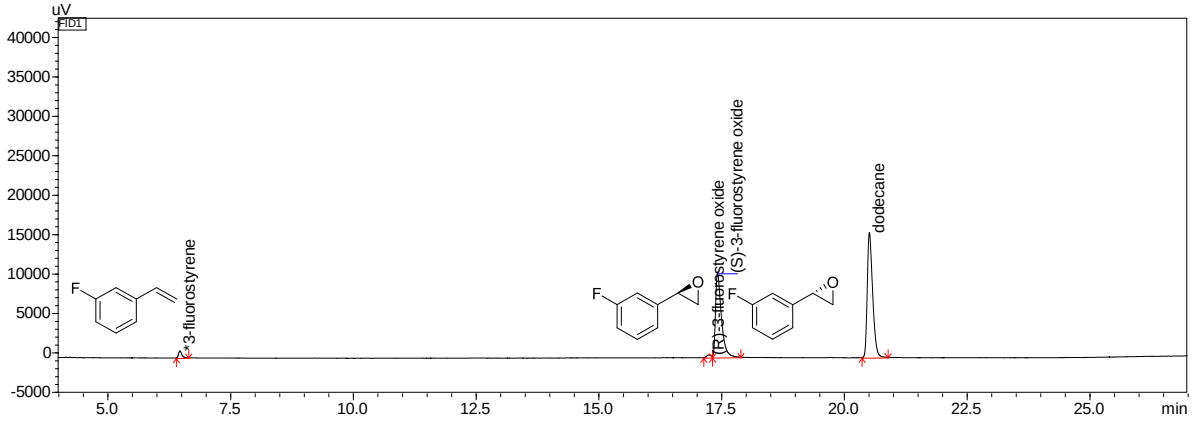


Figure S41. GC chromatogram of reaction products **2l** on column **C**.

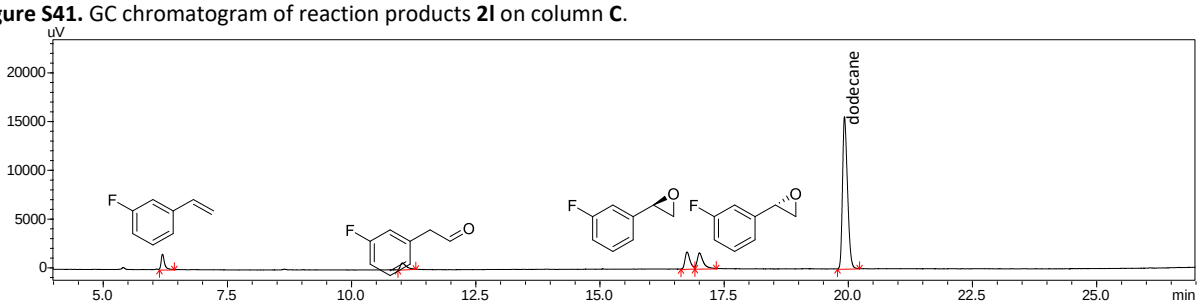


Figure S42. GC chromatogram of synthesized products **2l** with 3-fluorophenylacetaldehyde side produce on column **C** (retention times slightly shifted to the left).

(*S*)-2-fluorostyrene oxide (2-(2-fluorophenyl)oxirane) **2m**

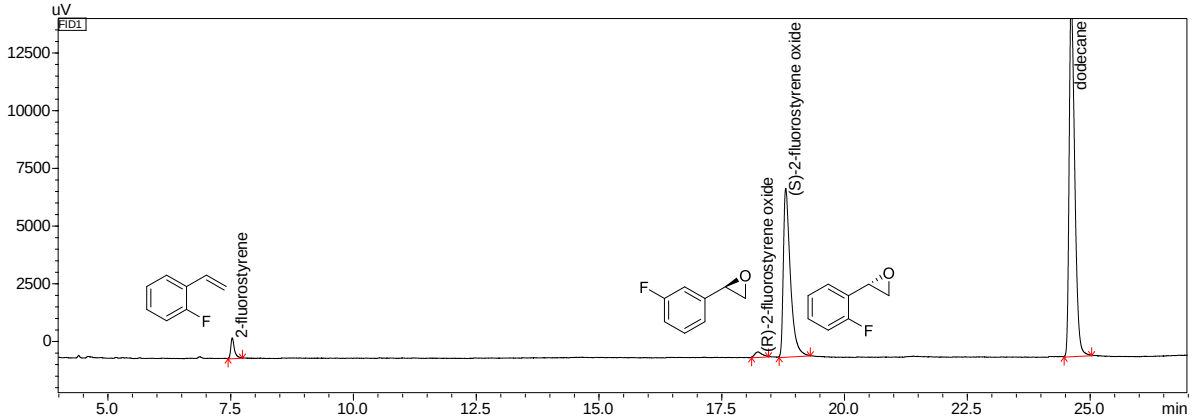


Figure S43. GC chromatogram of reaction products **2m** on column **C**.

(*S*)-4-methylstyrene oxide (2-(*p*-tolyl)oxirane) (*S*)-**2n**

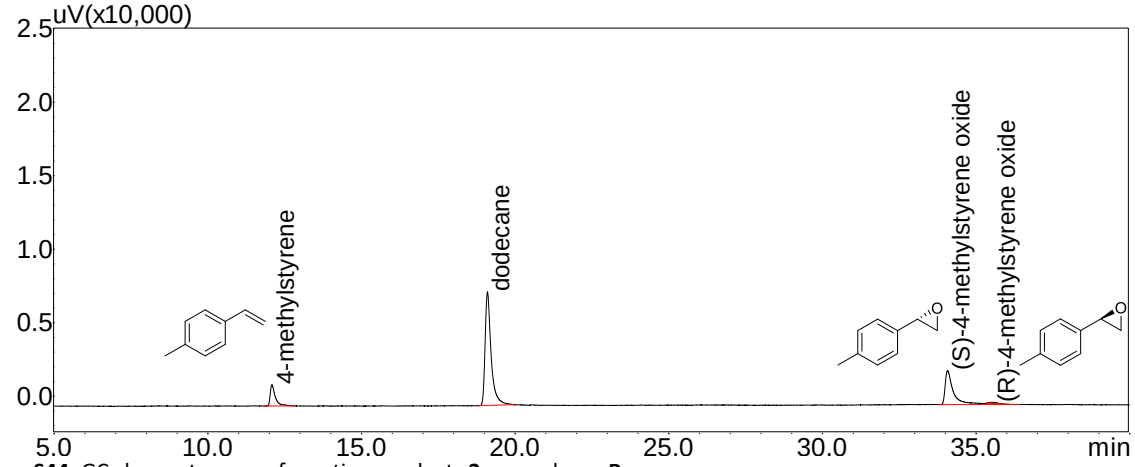


Figure S44. GC chromatogram of reaction products **2n** on column **B**.

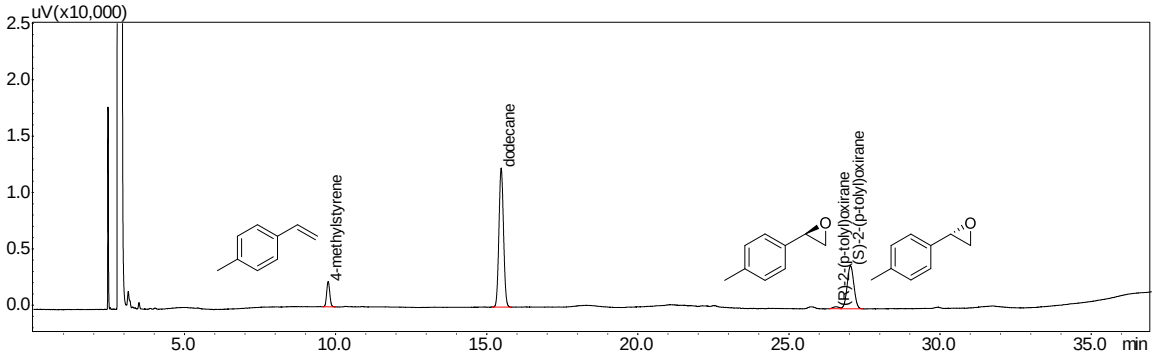


Figure S45. GC chromatogram of reaction products **2n** on column **A**.

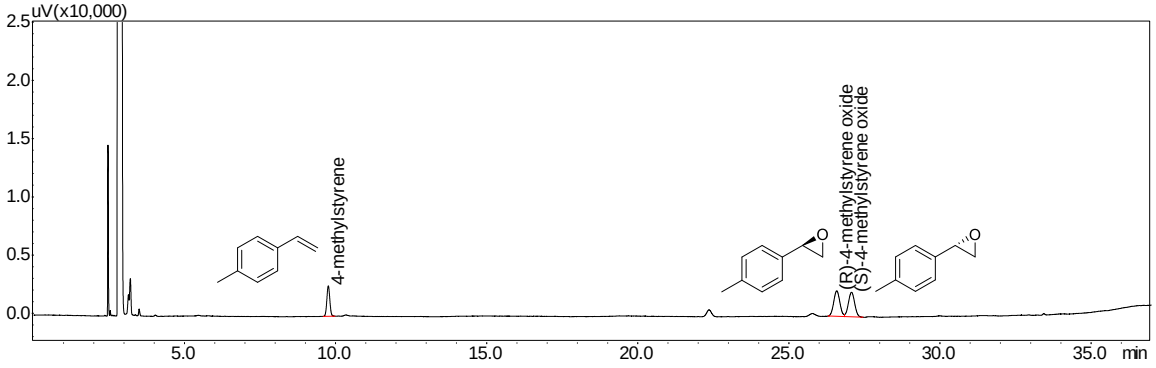


Figure S46. GC chromatogram of synthesized racemic product on column **A**.

(*S*)-3-methylstyrene oxide (2-(*m*-tolyl)oxirane) (*S*)-**2o**

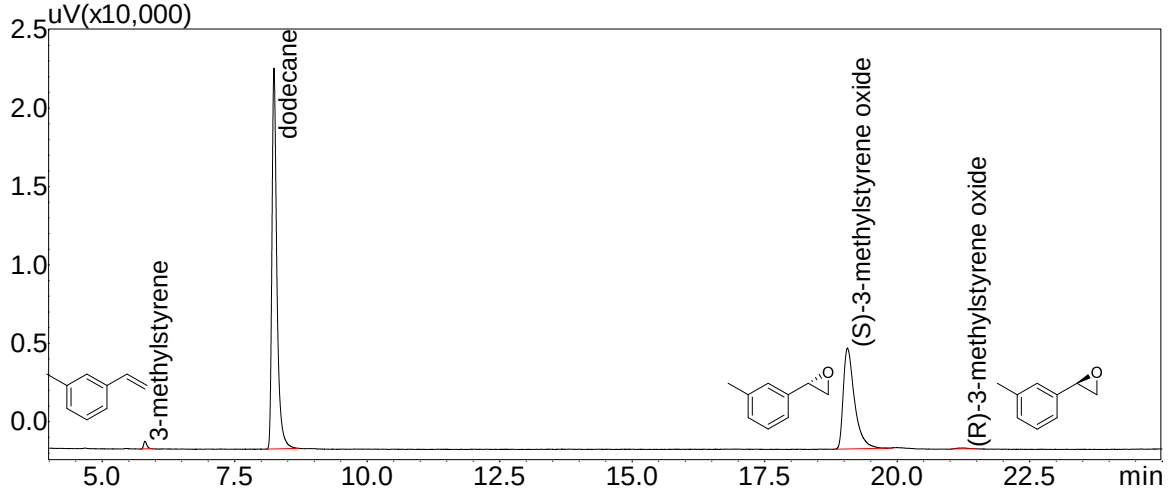


Figure S47. GC chromatogram of reaction products **2o** on column **B**.

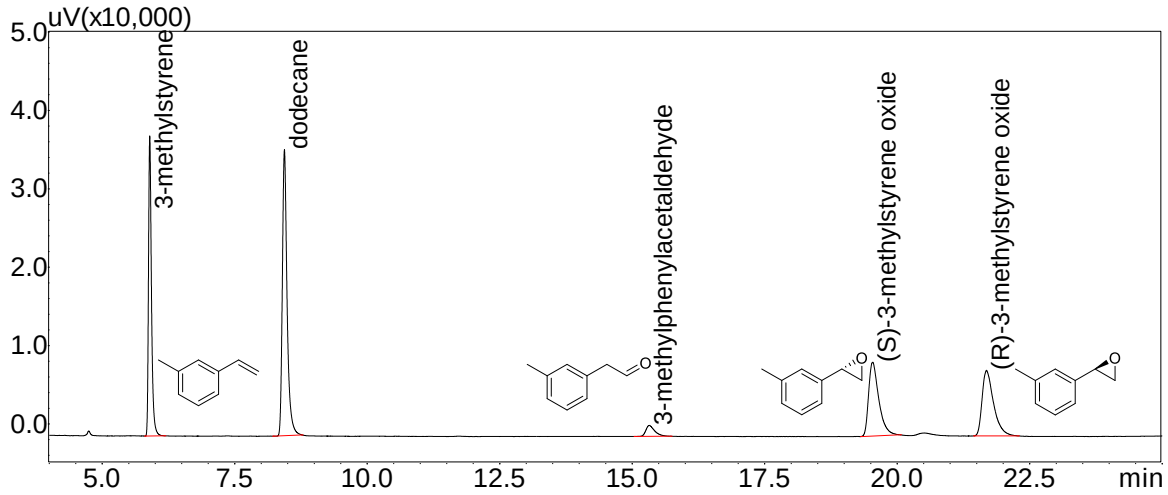


Figure S48. GC chromatogram of synthesized racemic products **2o** and side product 3-methylphenylacetaldehyde on column **B**.

(*S*)-2-benzyloxirane (allylbenzene oxide) (*S*)-**2p**

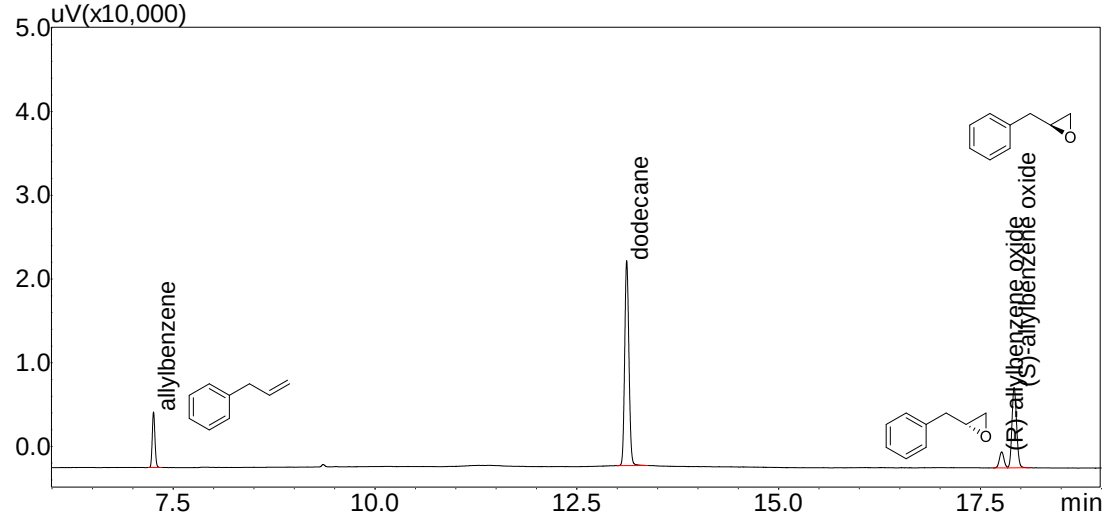


Figure S49. GC chromatogram of reaction products **2p** on column **D**.

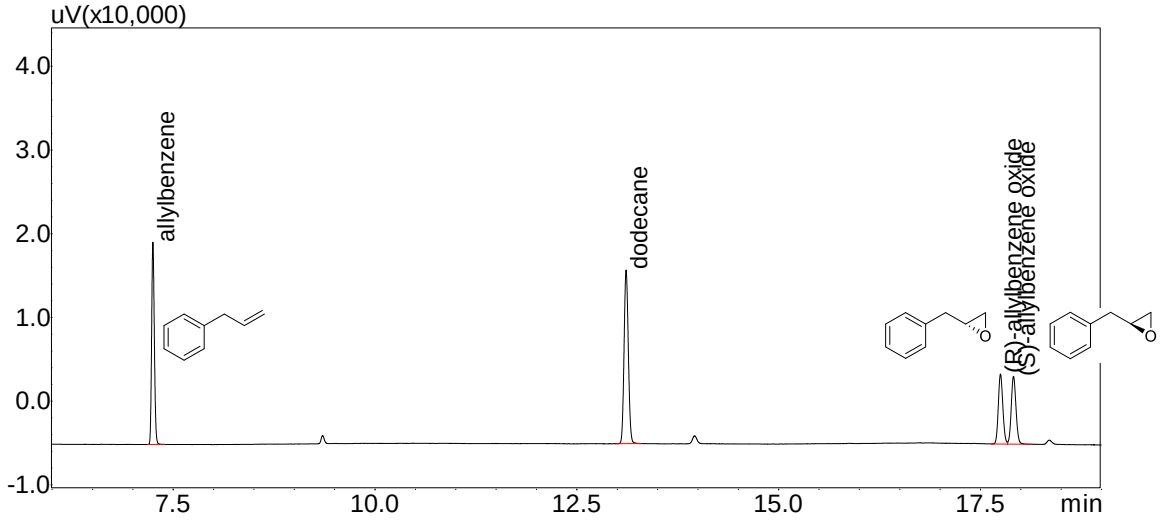


Figure S50. GC chromatogram of synthesized racemic product **2p** on column **D**.

(1*S*,2*R*)-1,2-dihydronaphthalene oxide (1*S*,2*R*)-**2q**

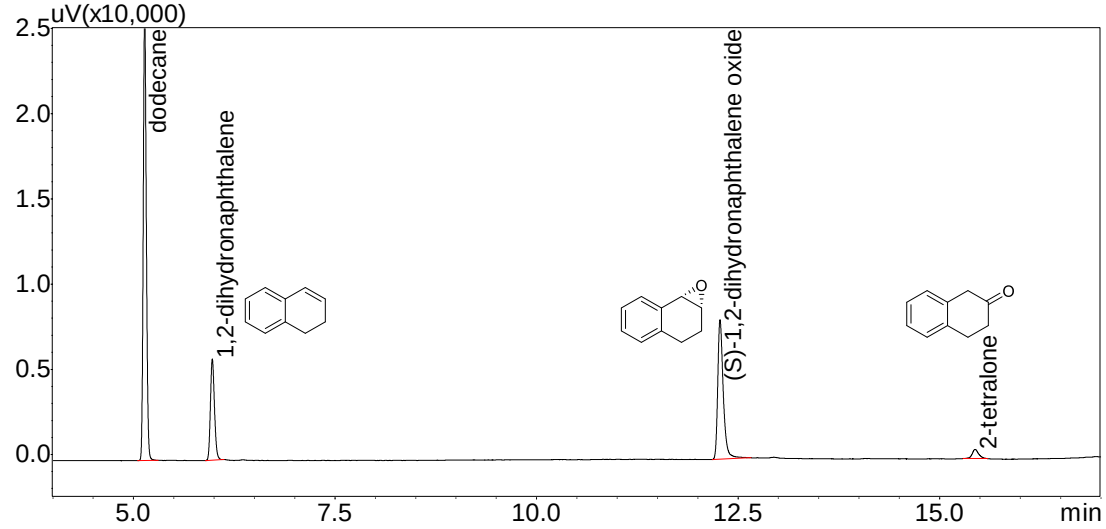


Figure S51. GC chromatogram of reaction products **2q** on column **C**.

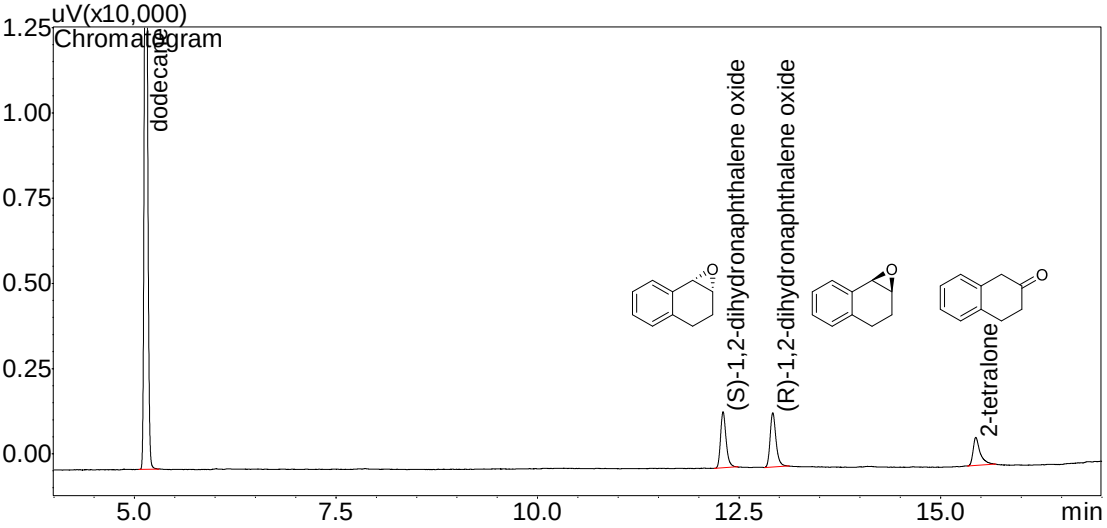


Figure S52. GC chromatogram of synthesized racemic product **2q** and side-product 2-tetralone on column **C**.

(1*S*,2*R*)-1,2-indene oxide **2r**

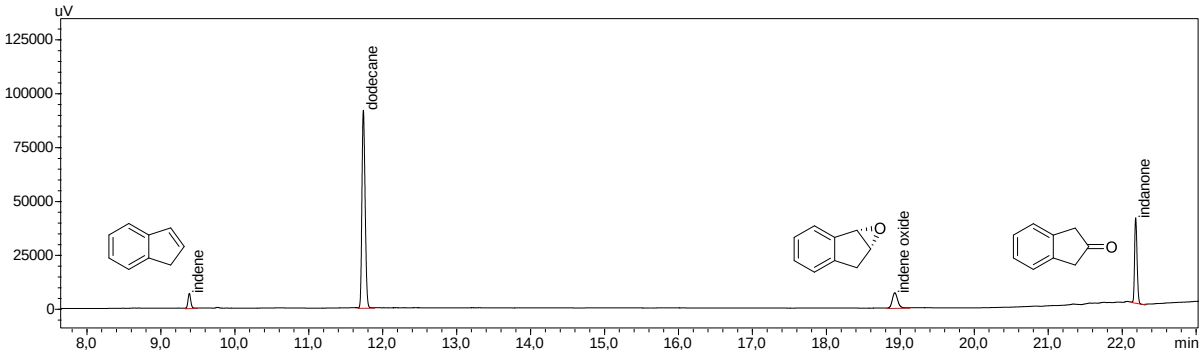


Figure S53. GC chromatogram of reaction products **2r** on column **D**.

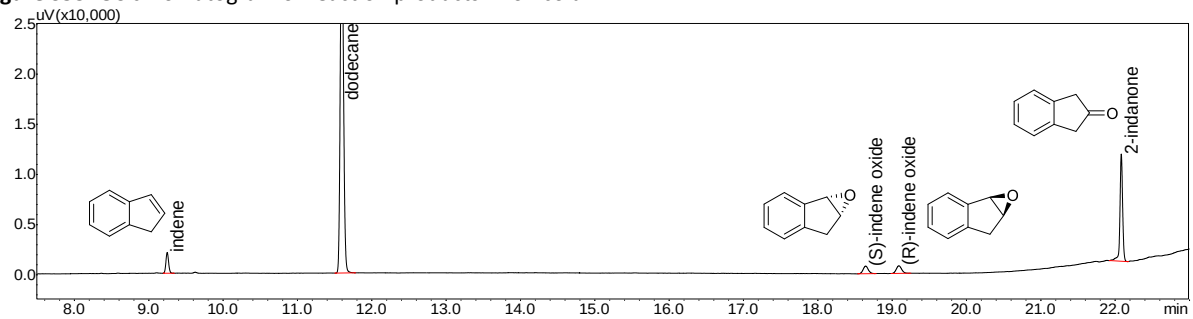


Figure S54. GC chromatogram of synthesized racemic product **2r** and side-product 2-indanone on column **D**.

(*S*)-2-vinylpyridine oxide **2s**

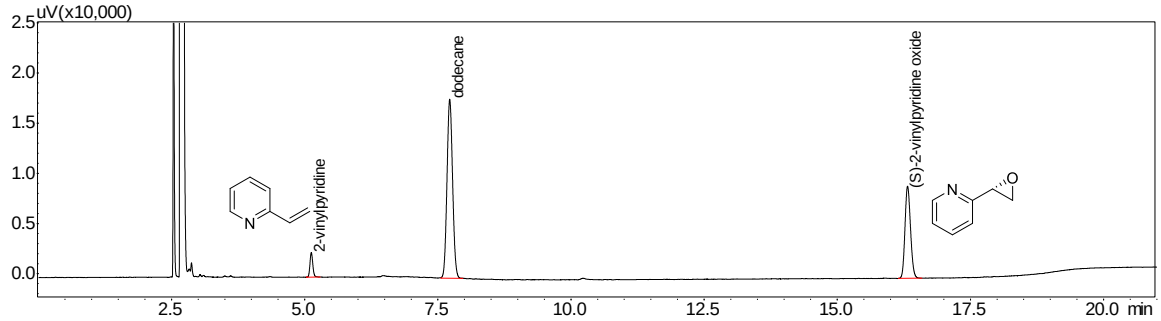


Figure S55. GC chromatogram of reaction products **2s** on column **A**.

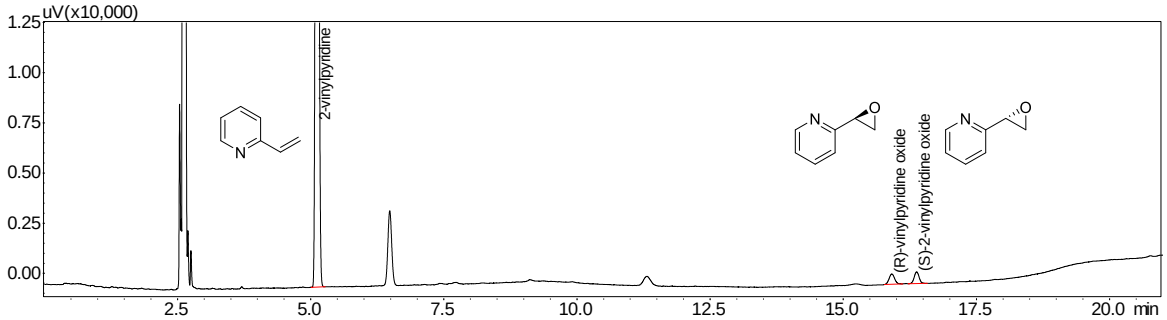


Figure S56. GC chromatogram of synthesized racemic product **2s** on column **A** (peaks at 6.5 and 11.3 min are impurities from the chemical synthesis).

4.3. GC chromatograms of azido alcohol products

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

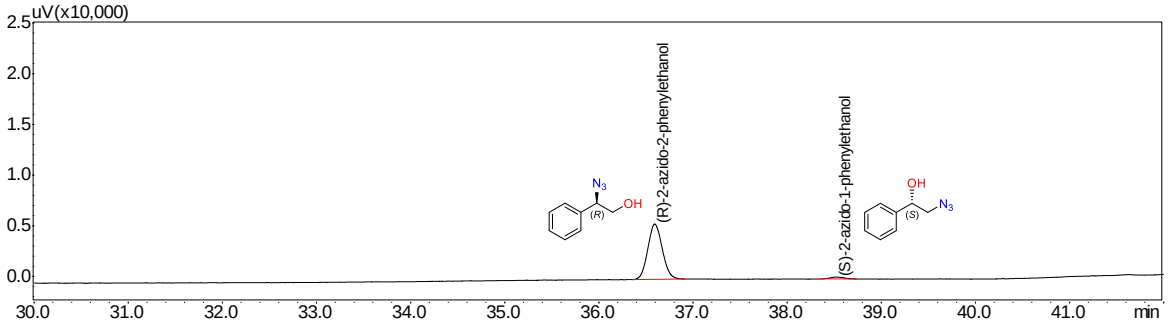


Figure S57 . GC chromatogram of reaction products **3a** on column **A**.

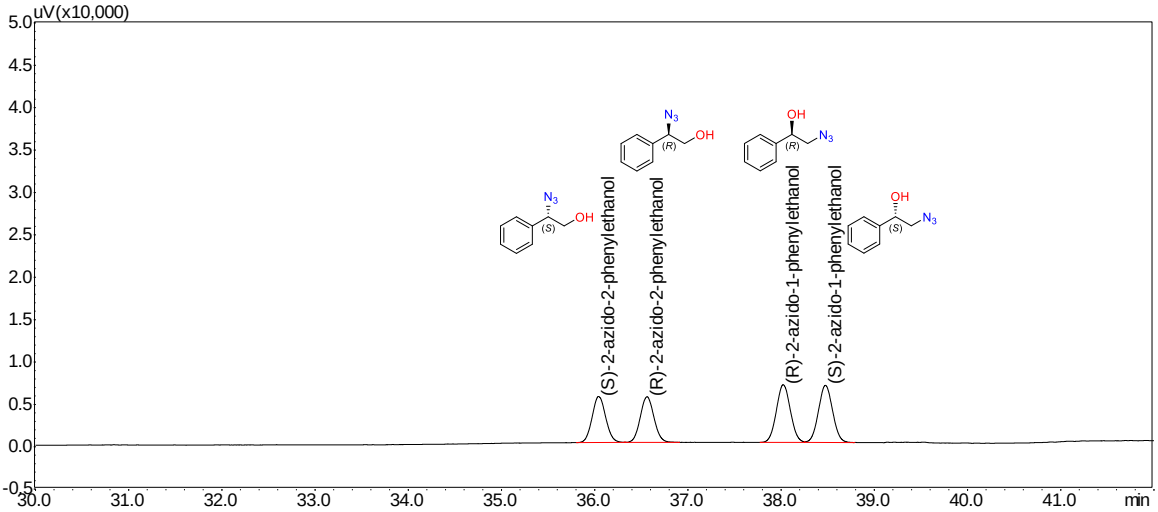


Figure S58. GC chromatogram of synthesized racemic products **3a** and **4a** on column **A**.

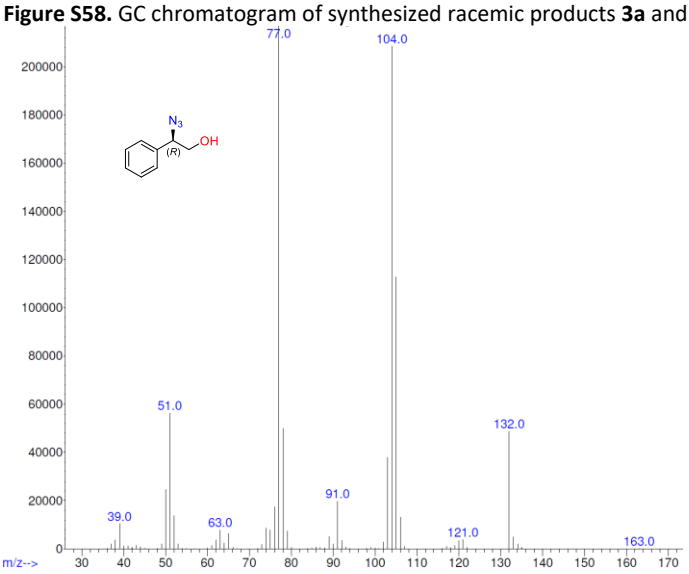


Figure S59. GC-MS mass spectrum of (*R*)-2-azido-2-phenylethan-1-ol **3a**.

Chemoenzymatic cascade to 1-azido-2-phenylpropan-2-ol **3b**

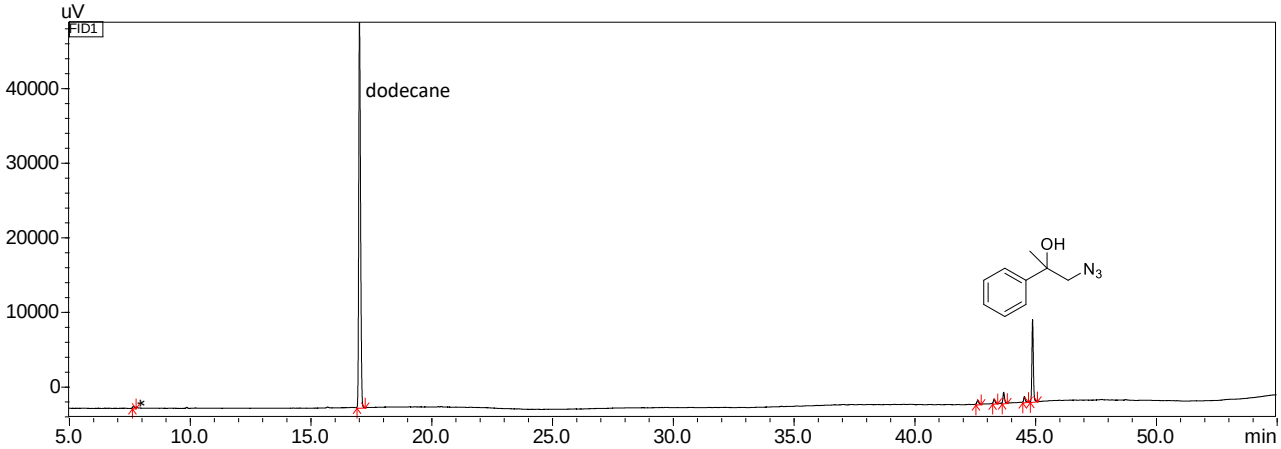


Figure S60. GC chromatogram of reaction products **3b** on column **D**.

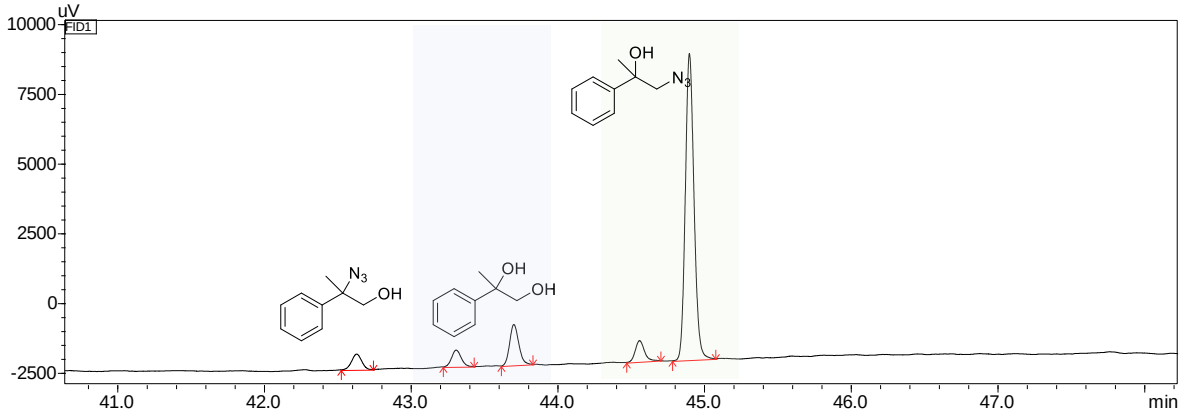


Figure S61. Zoom in of GC chromatogram of reaction products **3b** on column **D**.

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

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

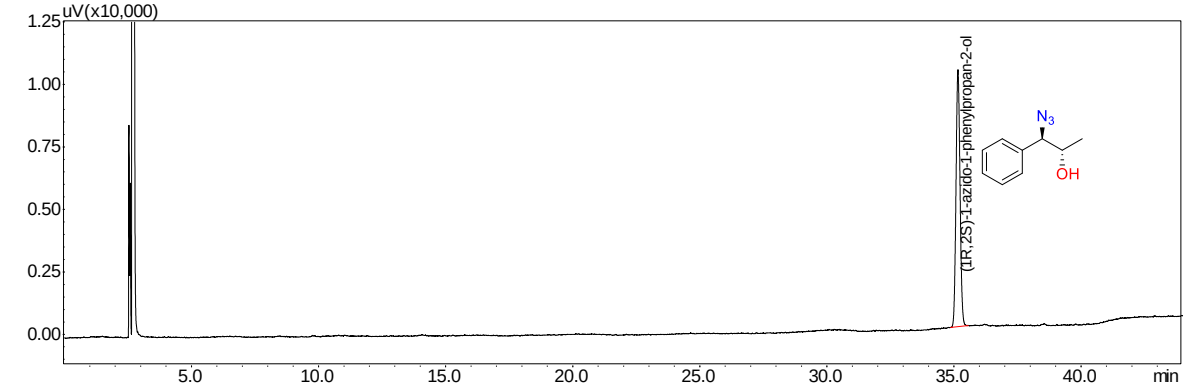


Figure S62. GC chromatogram of reaction products **3c** on column **A**.

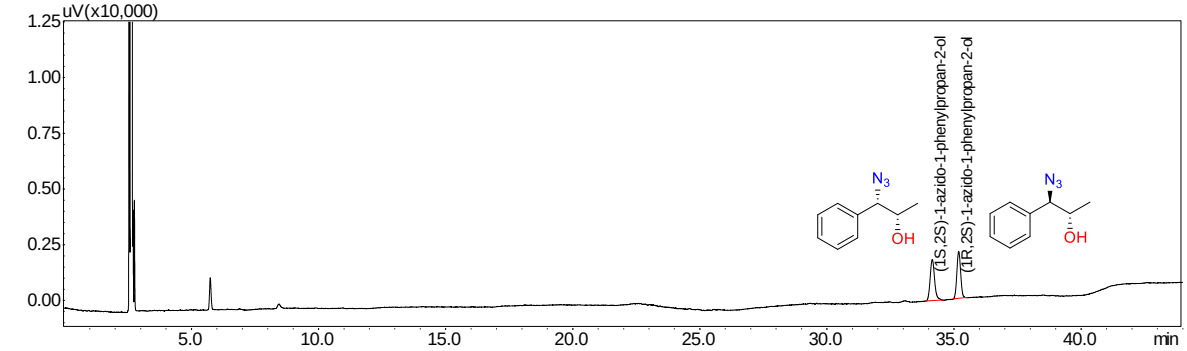


Figure S63. GC chromatogram of synthesized racemic product **3c** on column **A**.

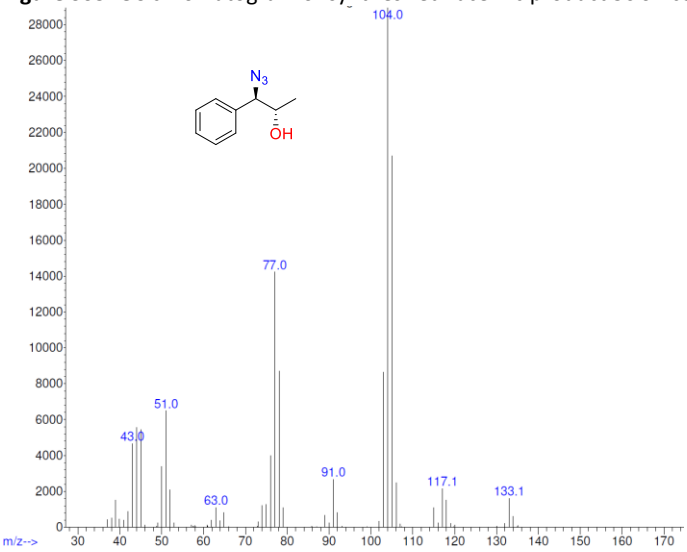


Figure S64. GC-MS mass spectrum of (1*R*,2*S*)-1-azido-1-phenylpropan-2-ol **3c**.

Chemoenzymatic cascade to (1*R*,2*R*)-1-azido-1-phenylpropan-2-ol **3d**

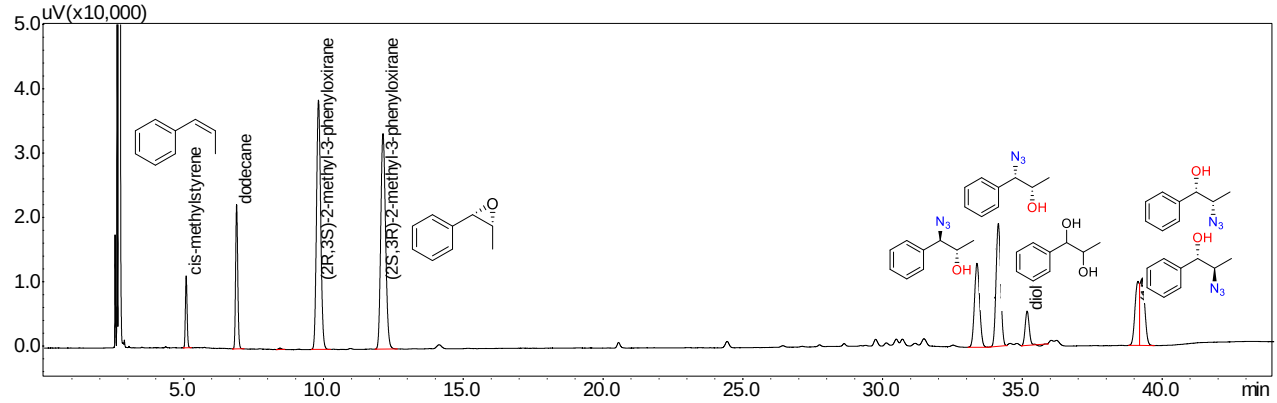


Figure S65. GC chromatogram of reaction products **3d** on column **A**, with products of *trans*-β-methylstyrene due to the impure *cis*-β-methylstyrene starting material.

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

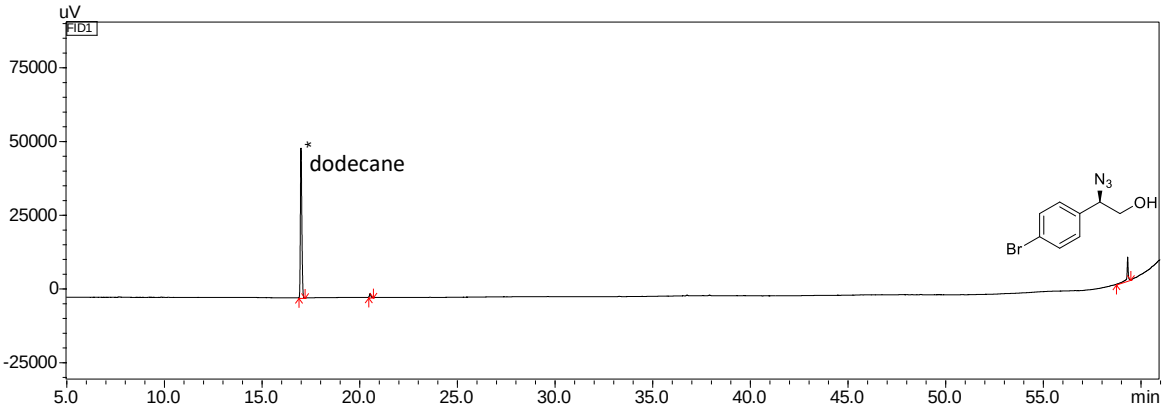


Figure S66. GC chromatogram of reaction products **3e** on column **D**.

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

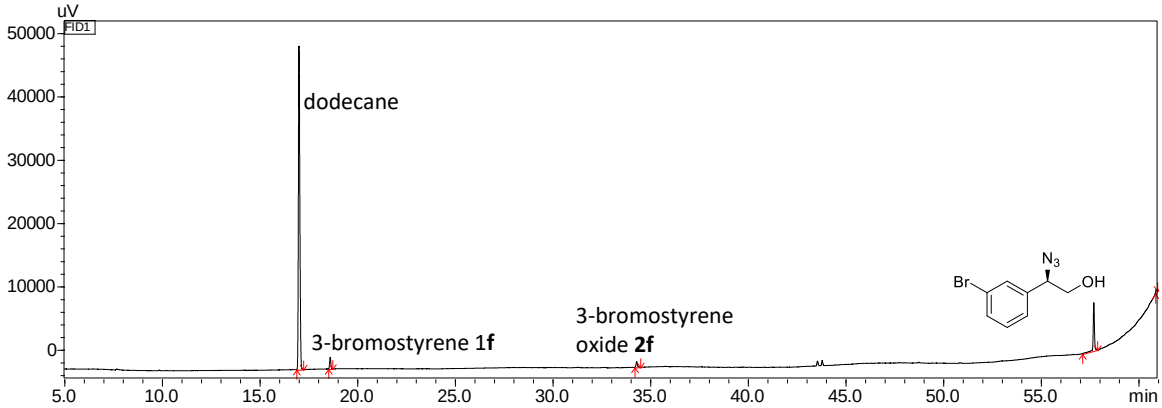


Figure S67. GC chromatogram of reaction products **3f** on column **D**.

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

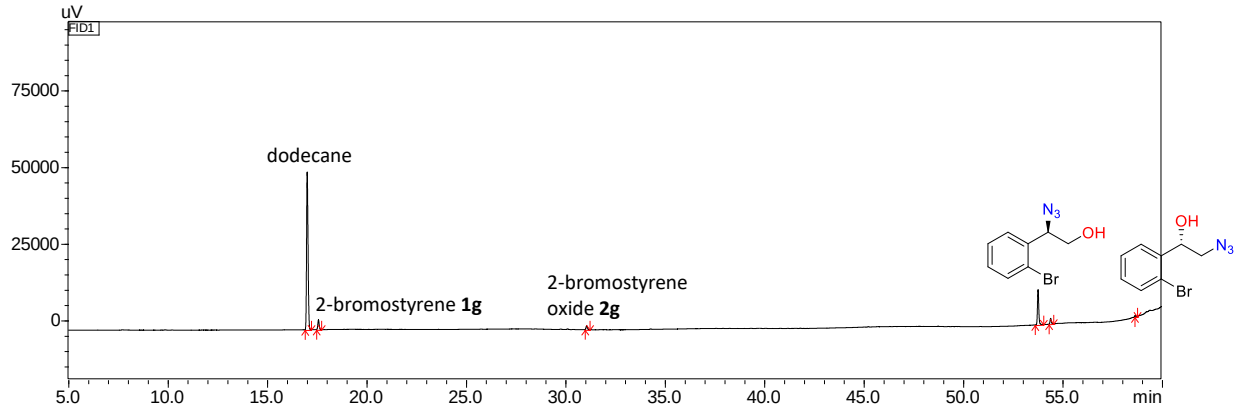


Figure S68. GC chromatogram of reaction products **3g** on column **D**.

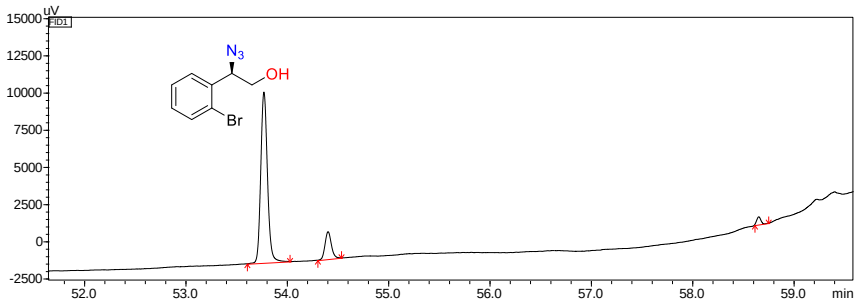


Figure S69. Zoom in of GC chromatogram of reaction products **3g** on column **D**.

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

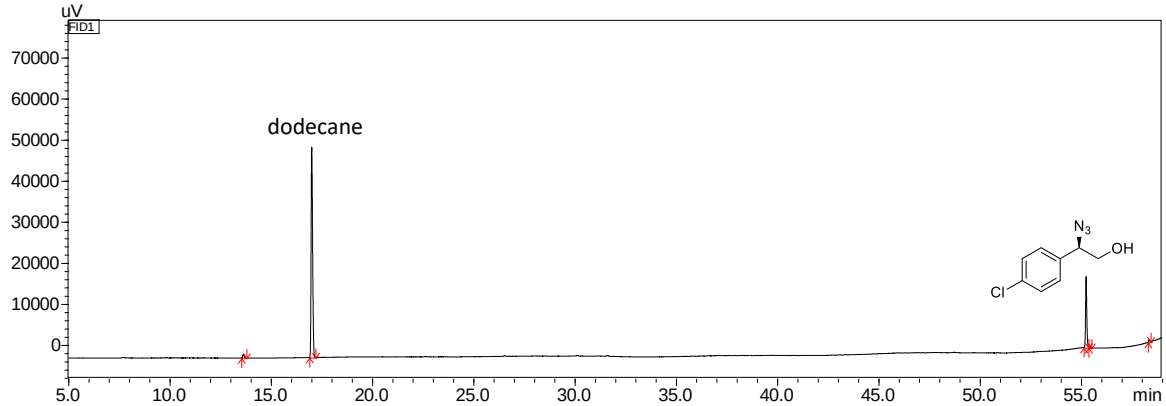


Figure S70. GC chromatogram of reaction products **3h** on column **D**.

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

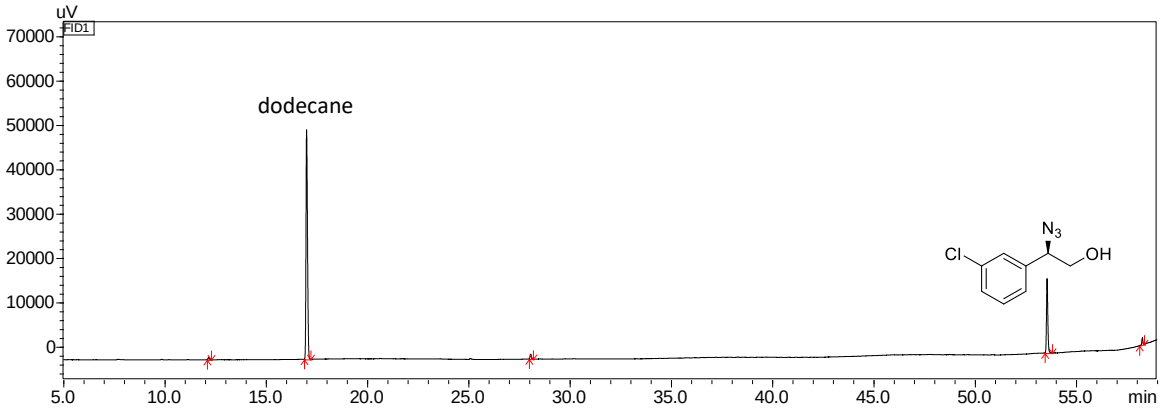


Figure S71. GC chromatogram of reaction products **3i** on column **D**.

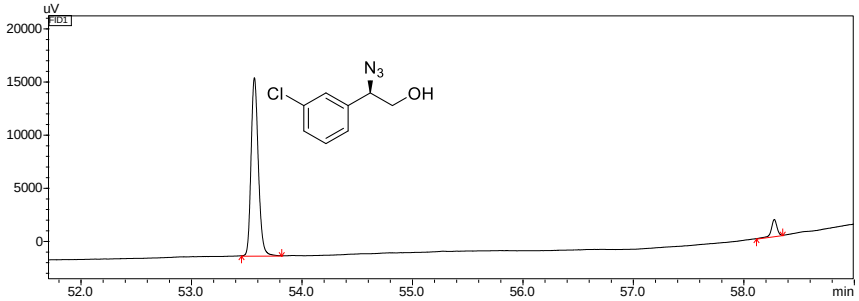


Figure S72. Zoom in of GC chromatogram of reaction products **3i** on column **D**.

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

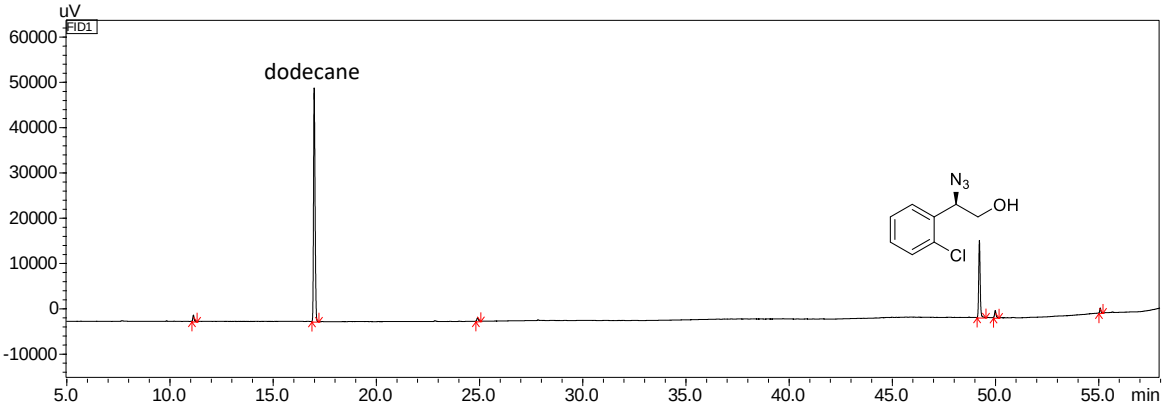


Figure S73. GC chromatogram of reaction products **3j** on column **D**.

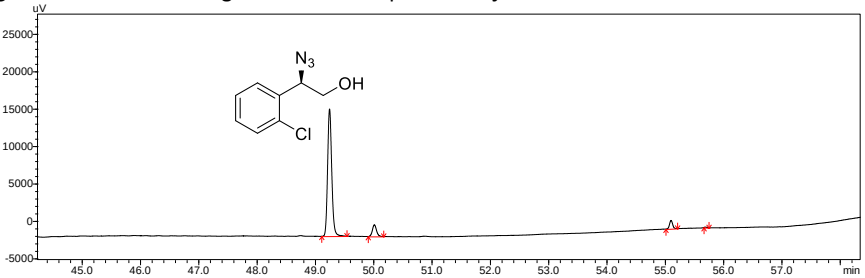


Figure S74. Zoom in of GC chromatogram of reaction products **3j** on column **D**.

Chemoenzymatic cascade to (*R*)-2-azido-2-(4-fluorophenyl)ethan-1-ol **3k**

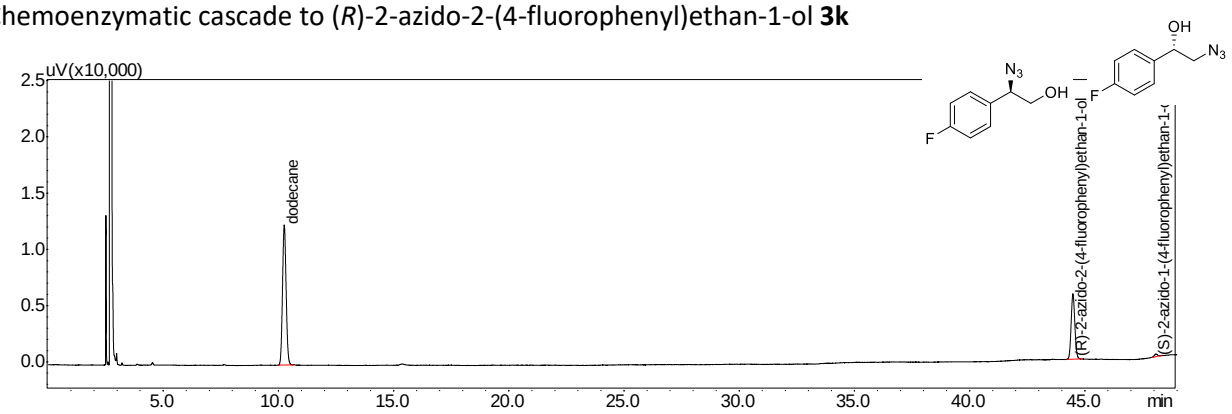


Figure S75. GC chromatogram of reaction products **3k** on column **A**.

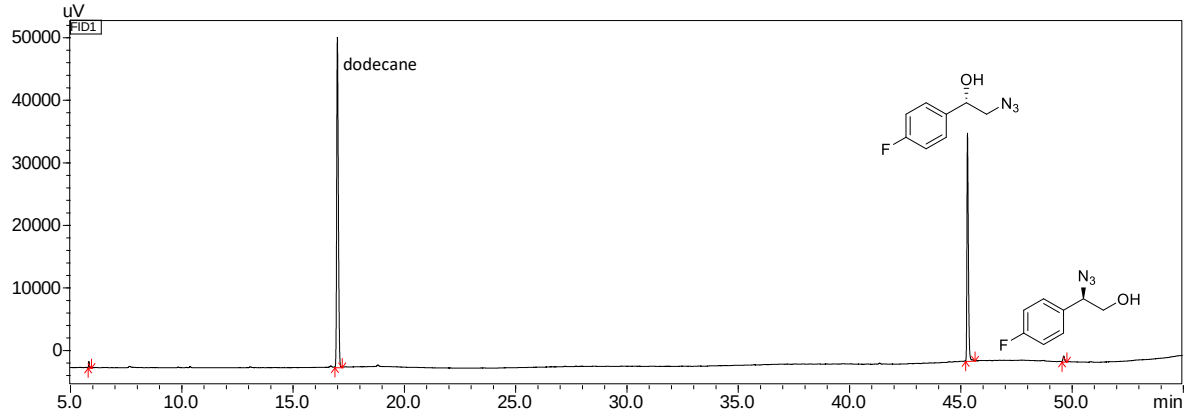


Figure S76. GC chromatogram of reaction products **3k** on column **D**.

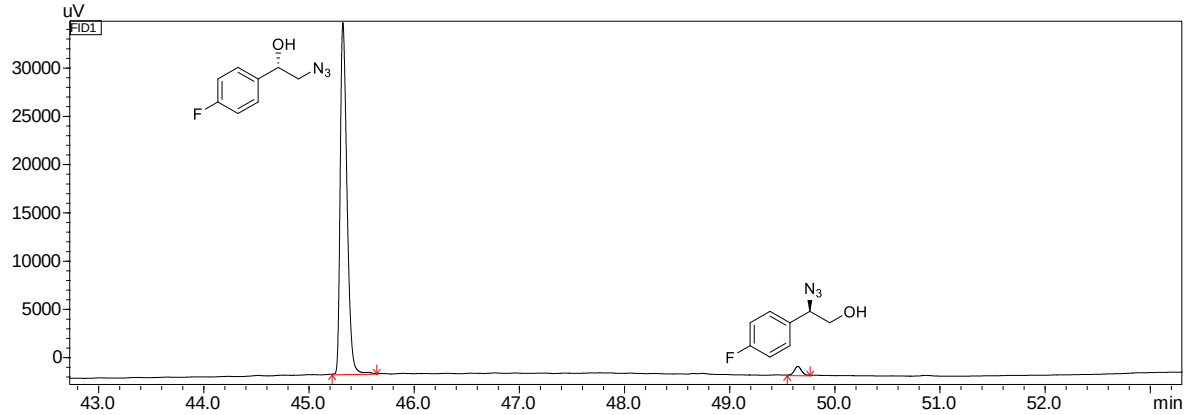


Figure S77. Zoom in of GC chromatogram of reaction products **3k** on column **D**.

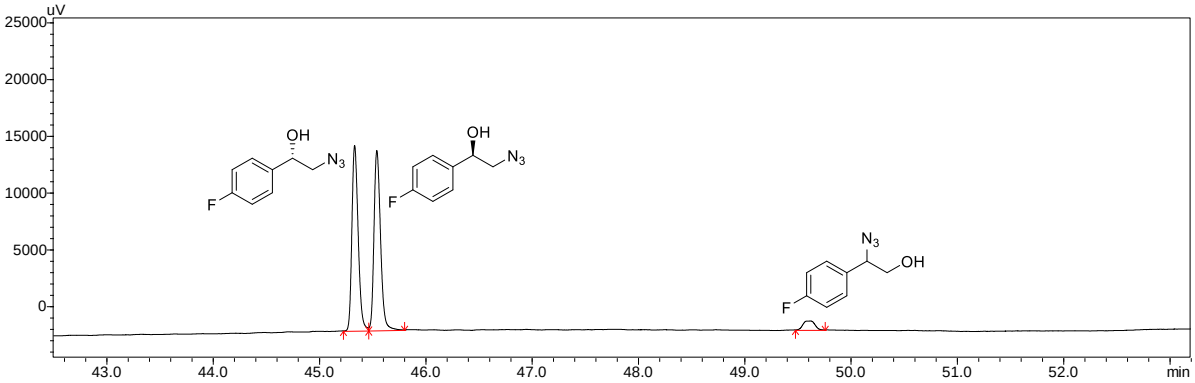


Figure S78. GC chromatogram of synthesized racemic products **3k** on column **D**.

Chemoenzymatic cascade to (*R*)-2-azido-2-(3-fluorophenyl)ethan-1-ol **3l**

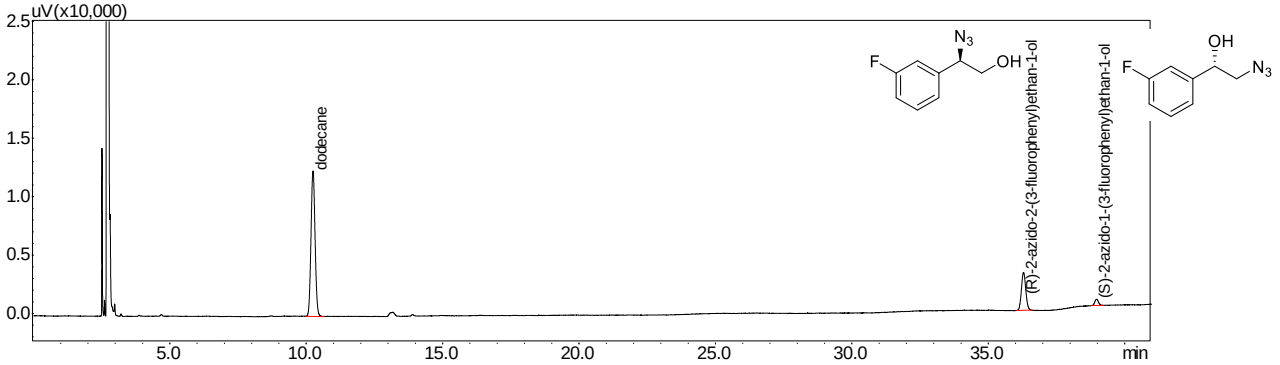


Figure S79. GC chromatogram of reaction products **3l** on column **A**.

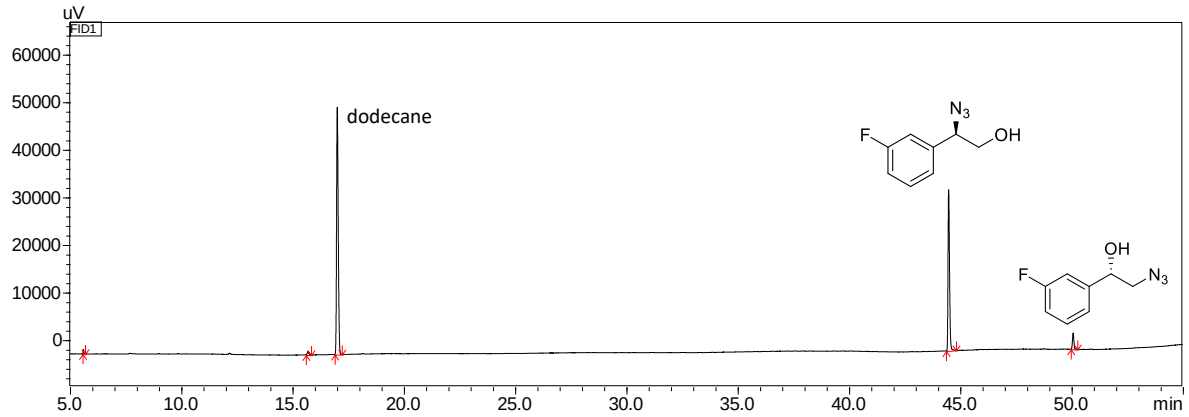


Figure S80. GC chromatogram of reaction products **3l** on column **D**.

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

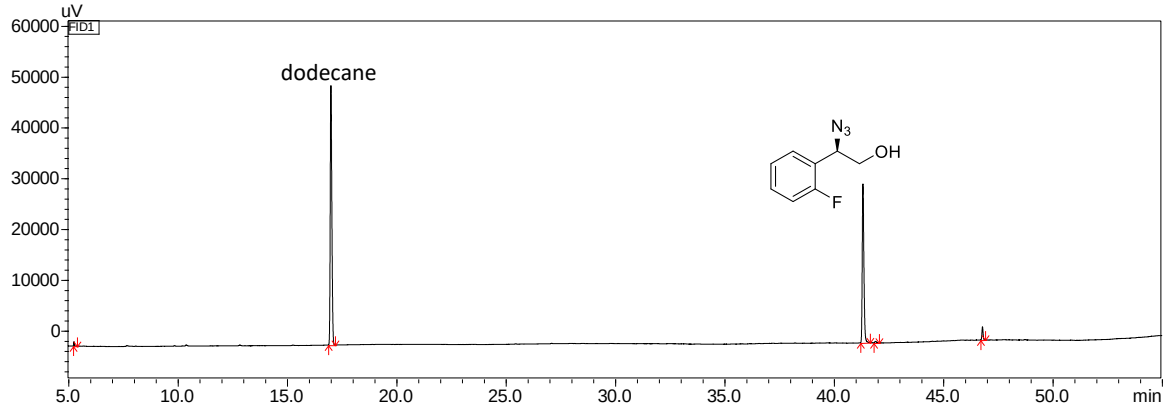


Figure S81. GC chromatogram of reaction products **3m** on column **D**.

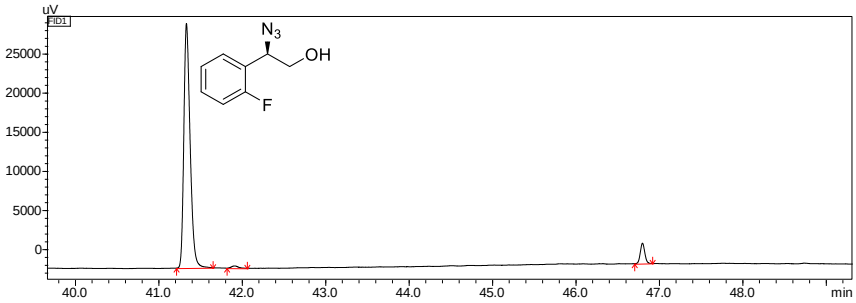


Figure S82. Zoom in of GC chromatogram of reaction products **3m** on column **D**.

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

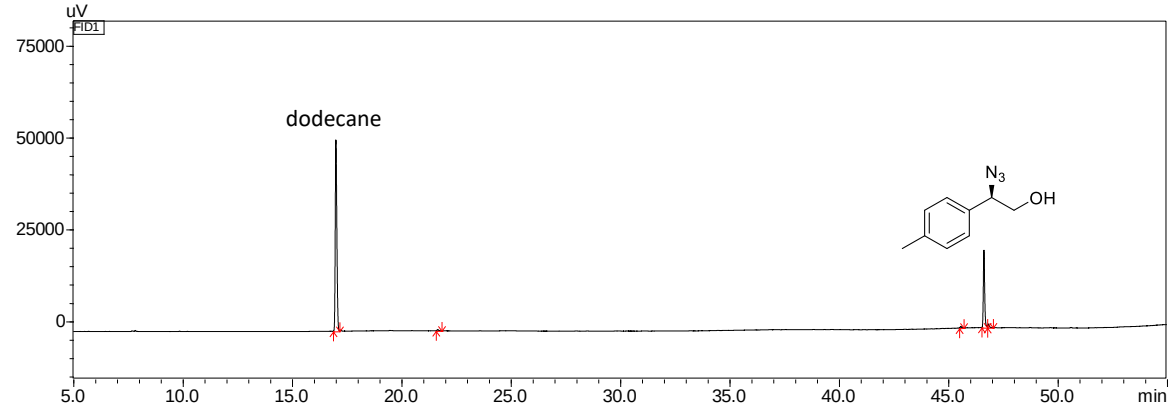


Figure S83. GC chromatogram of reaction products **3n** on column **D**.

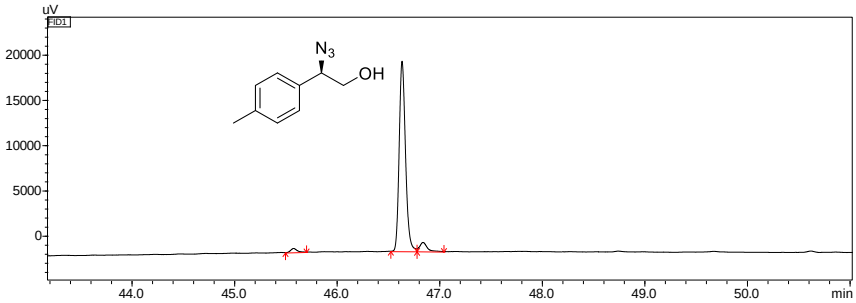


Figure S84. Zoom in of GC chromatogram of reaction products **3n** on column **D**.

Chemoenzymatic cascade to (*R*)-2-azido-2-(*m*-tolyl)ethan-1-ol **3o**

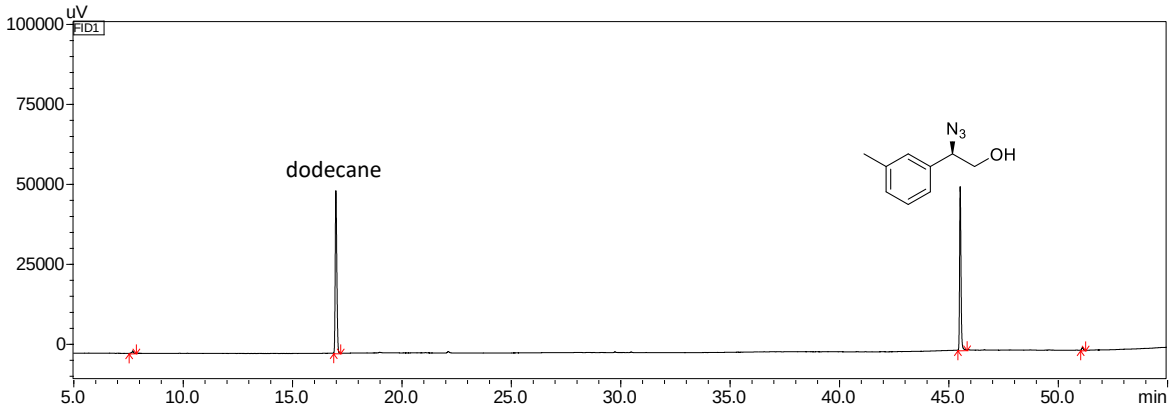


Figure S85. GC chromatogram of reaction products **3o** on column **D**.

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

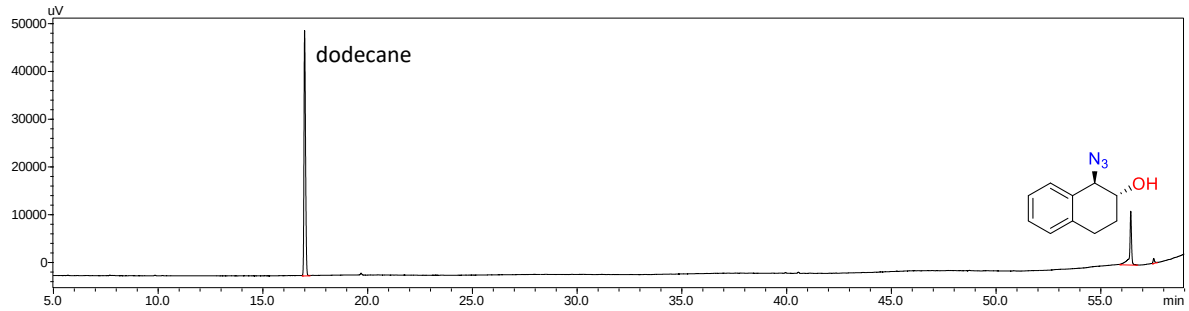


Figure S86. GC chromatogram of reaction products **3q** on column **D**.

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

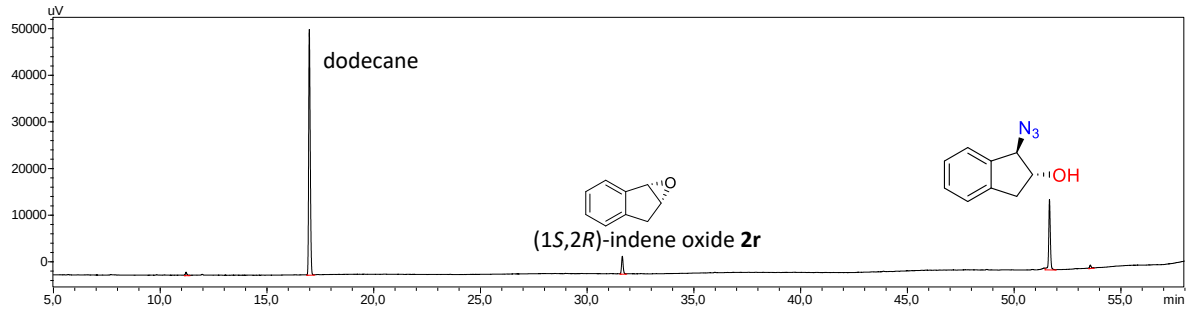


Figure S87. GC chromatogram of reaction products **3r** on column **D**.

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

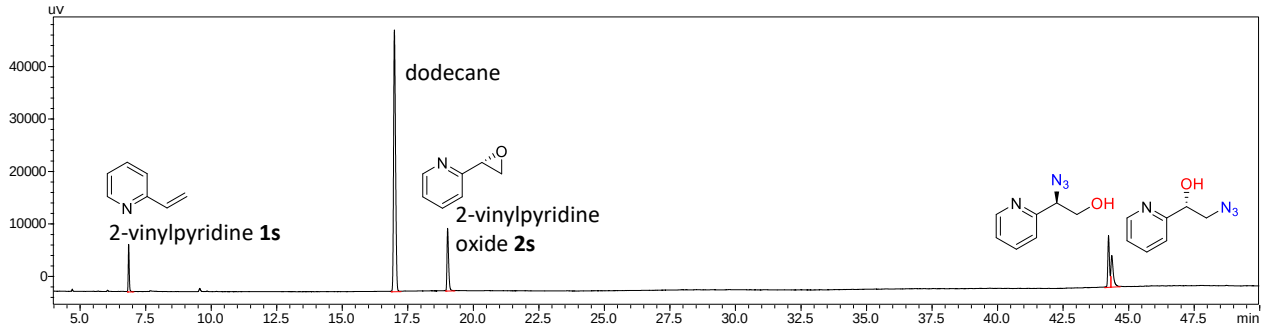


Figure S88. GC chromatogram of reaction products **3s** on column **D**.

Bienzymatic cascade to (*S*)-2-azido-1-phenylethan-1-ol **3a**

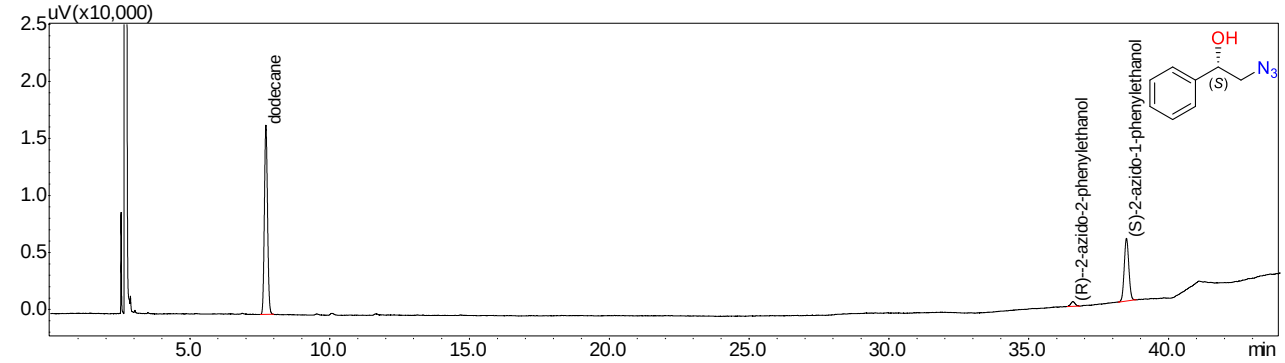


Figure S89. GC chromatogram of reaction products **3a** on column **A**.

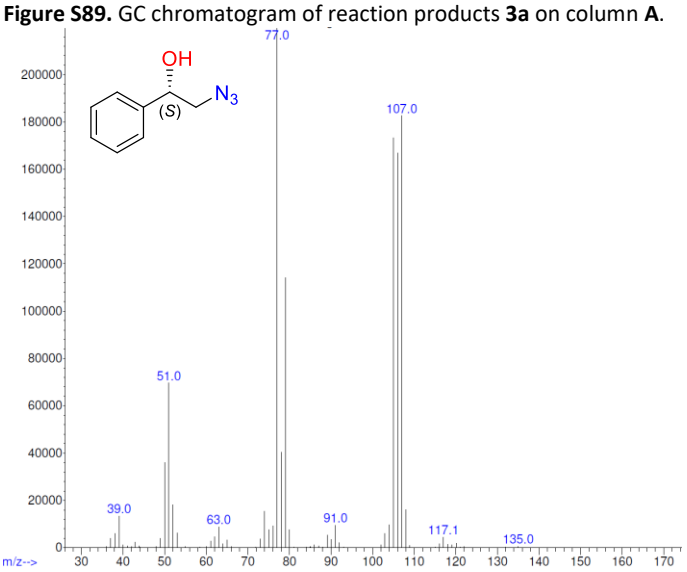


Figure S90. GC-MS mass spectrum of (*S*)-2-azido-2-phenylethan-1-ol **3a**.

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

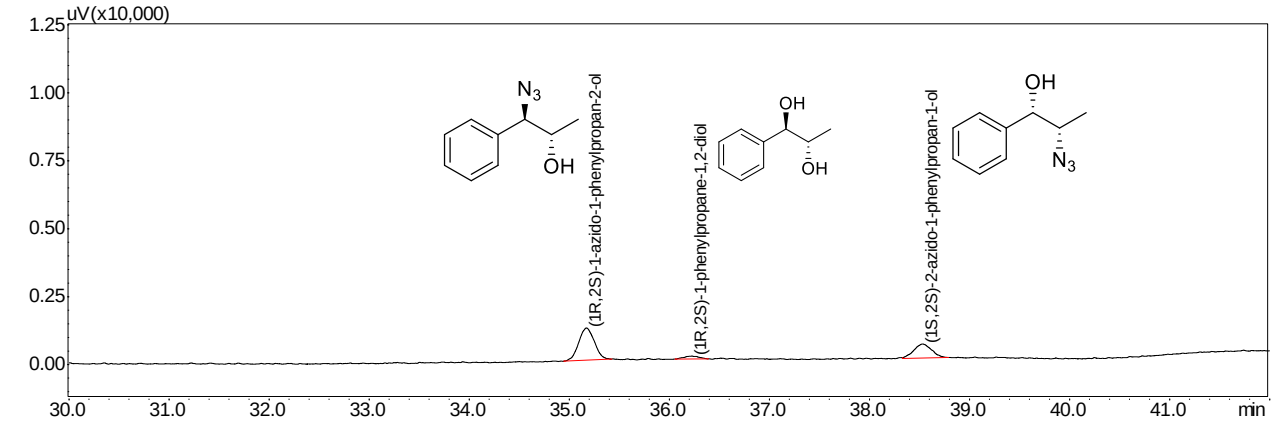


Figure S91. GC chromatogram of reaction products **3c** on column **A**. Trace amounts <1% of diol observed.

Bienzymatic cascade to (*S*)-2-azido-1-(4-fluorophenyl)ethan-1-ol **3k**

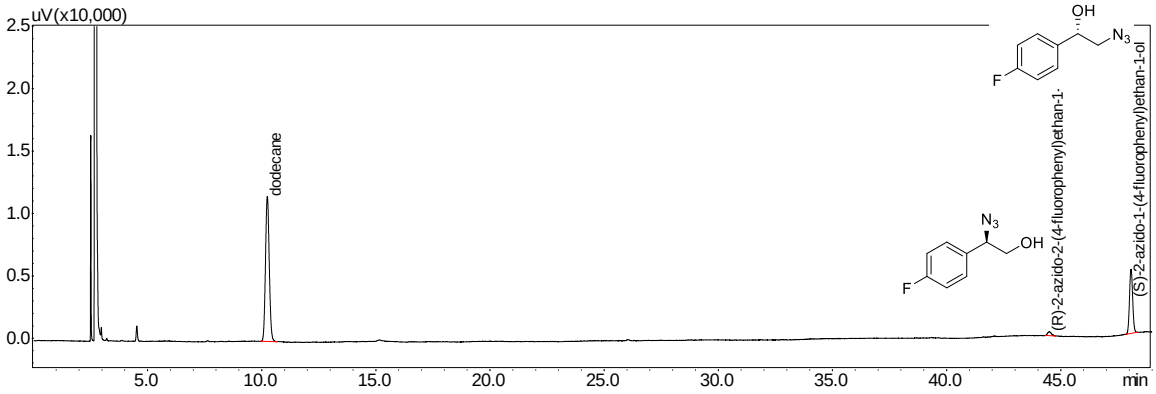


Figure S92. GC chromatogram of reaction products **3k** on column **A**.

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

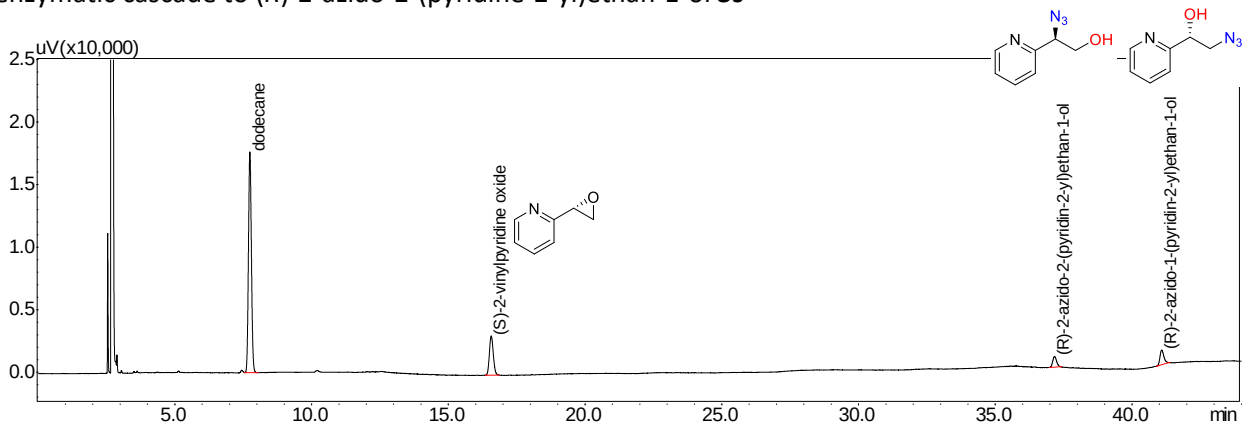


Figure S93. GC chromatogram of reaction products **3s** on column **A**.

5. References

1. S. Panke, M. Held, M. G. Wubbolts, B. Witholt and A. Schmid, *Biotechnol. Bioeng.*, 2002, **80**, 33-41.
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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.
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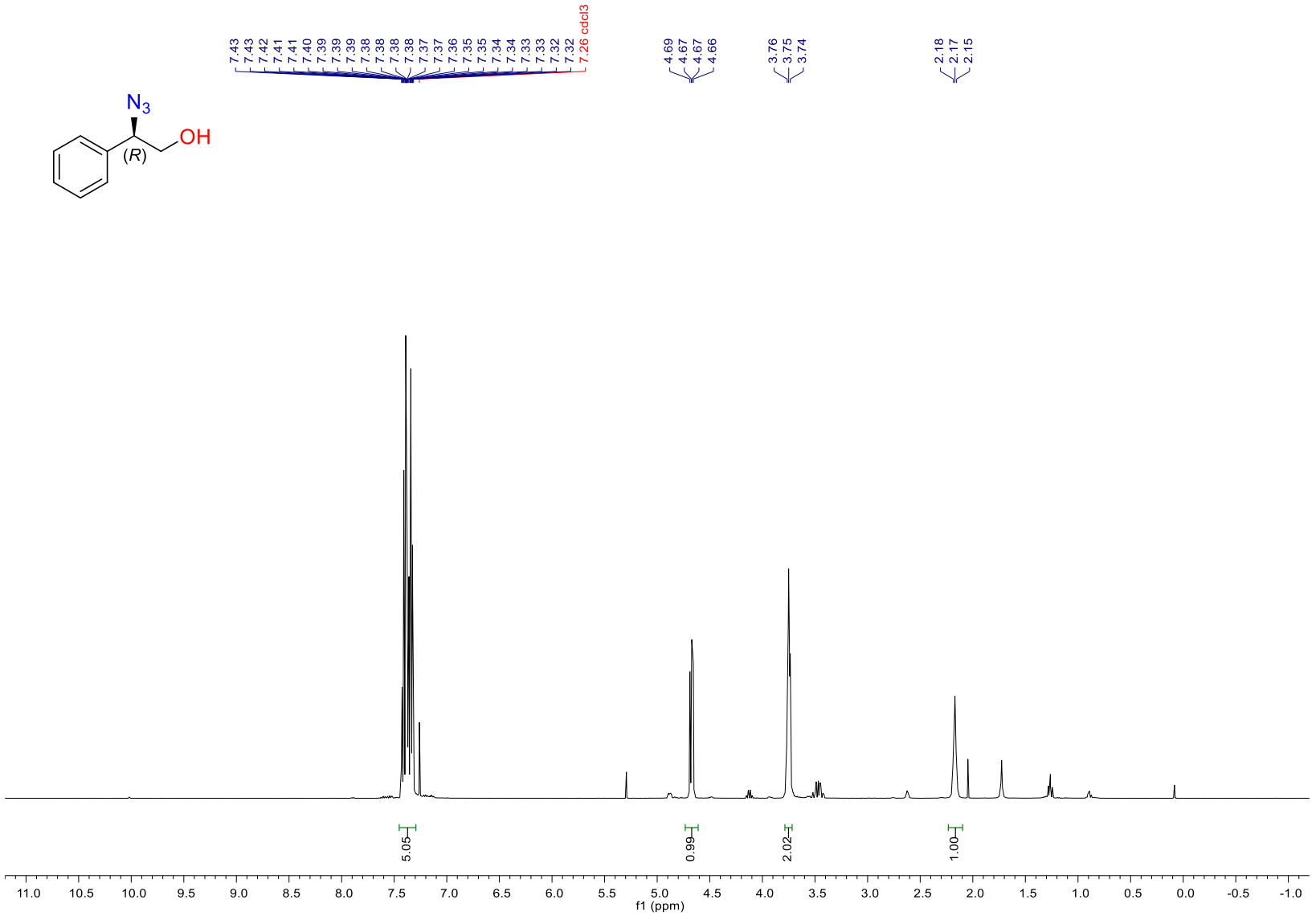
6. K. Otto, K. Hofstetter, M. Rothlisberger, B. Witholt and A. Schmid, *J. Bacteriol.*, 2004, **186**, 5292-5302.
7. D. Tischler, D. Eulberg, S. Lakner, S. R. Kaschabek, W. J. H. van Berkel and M. Schlomann, *J. Bacteriol.*, 2009, **191**, 4996-5009.
8. M. L. Corrado, T. Knaus and F. G. Mutti, *ChemBioChem*, 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.

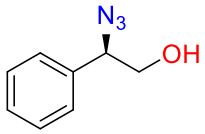
6. NMR spectra

NMR spectra are displayed on the next pages for the upscaled reactions of the chemoenzymatic cascade, crude product extracted without further purification.

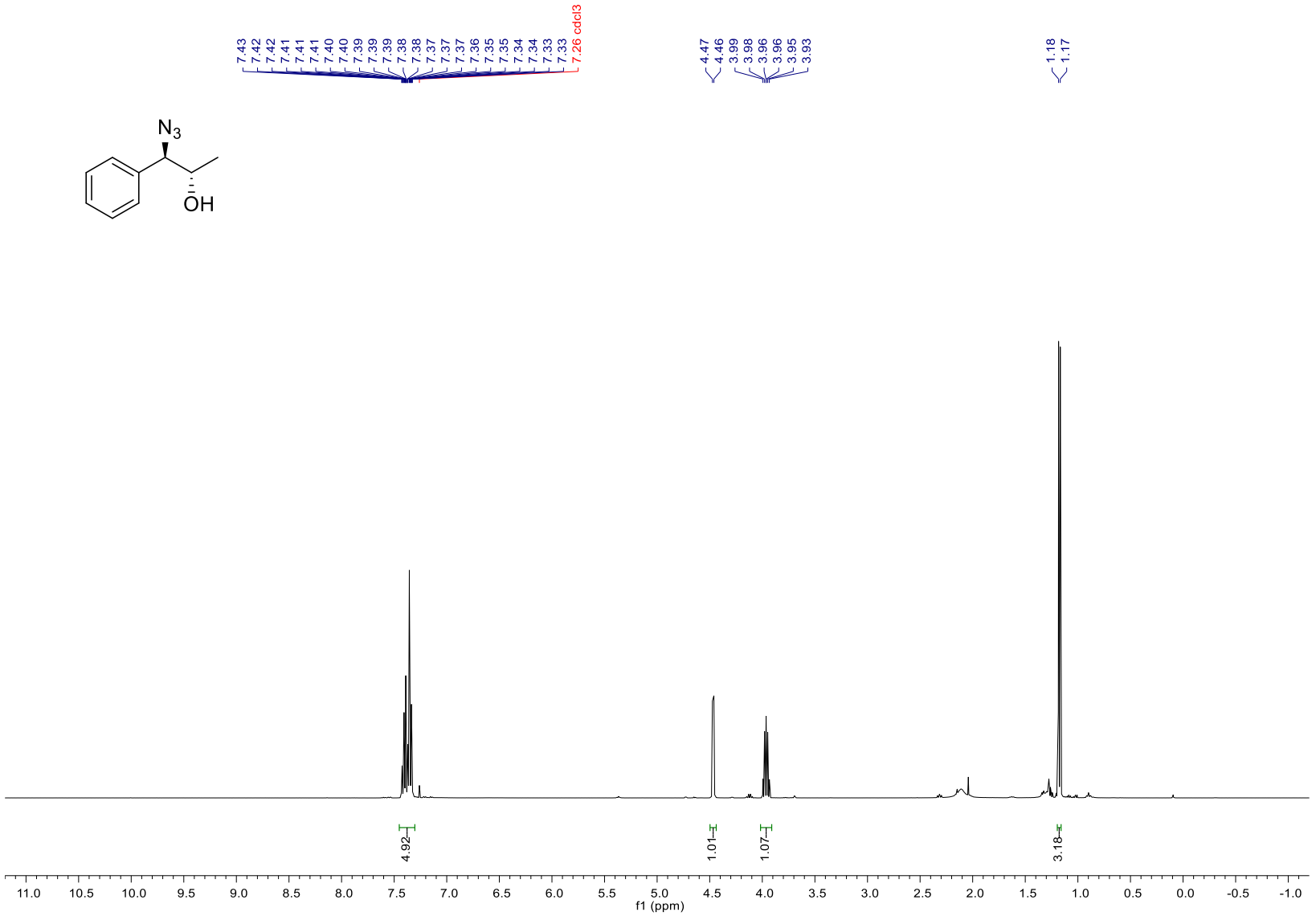
^{1}H NMR (400 MHz, $CDCl_3$) Sty-cascade 15 mg scale

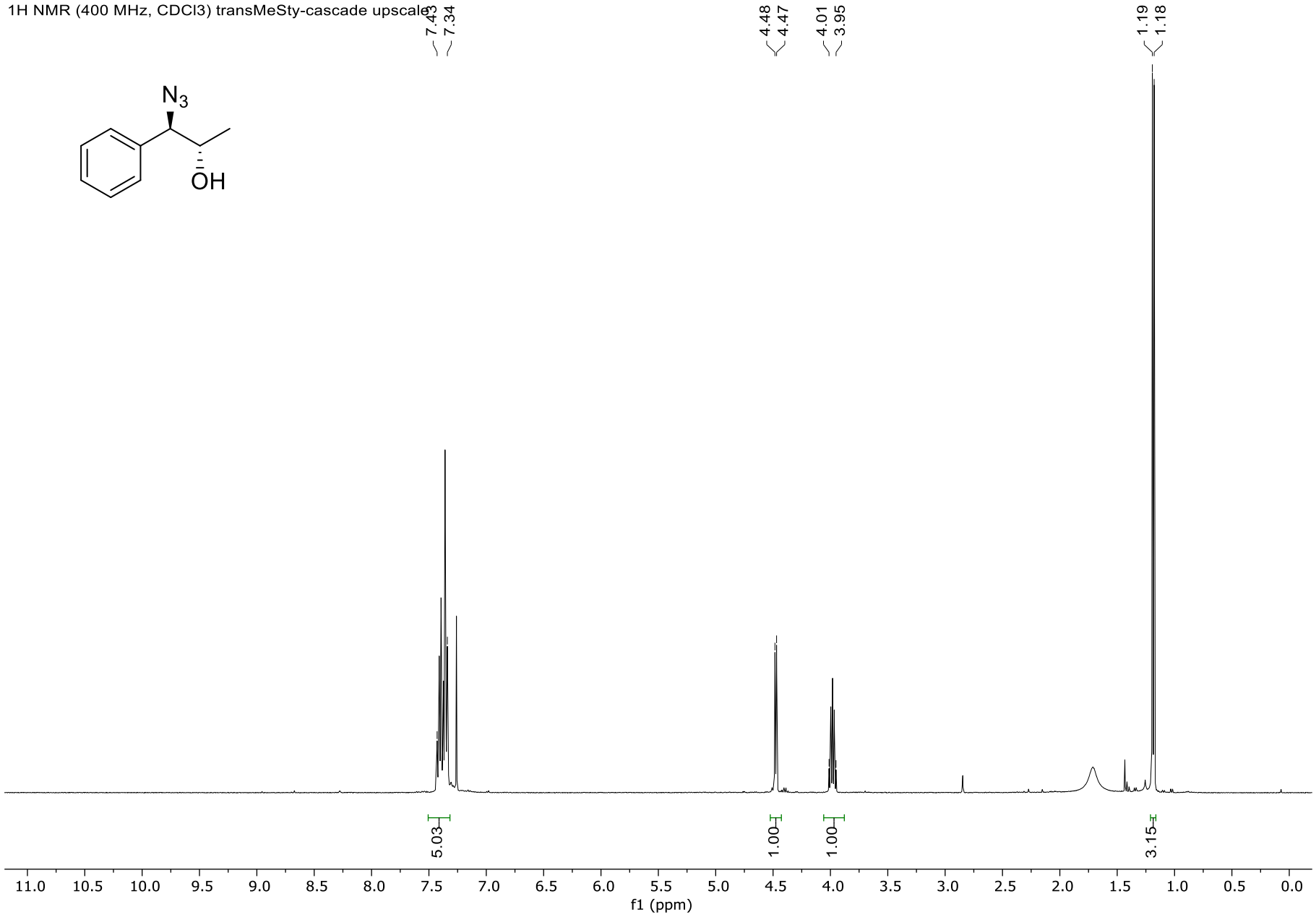


1H NMR (400 MHz, CDCl3) Sty-cascade upscale

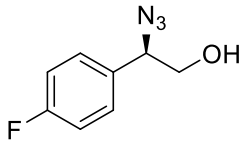


^{1}H NMR (400 MHz, $CDCl_3$) *trans*-methylstyrene-chemo-enzymatic cascade, 15 mg scale

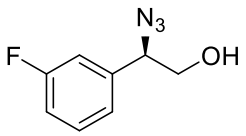



1H NMR (400 MHz, CDCl3) transMeSty-cascade upscale3
7.43
7.34
4.48
4.47
4.01
3.95
1.19
1.18
N3
OH
5.03
1.00
1.00
3.15
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

1H NMR (400 MHz, CDCl3) 4-FSty-cascade



1H NMR (400 MHz, CDCl3) 3FSty-cascade



1H NMR (400 MHz, CDCl3) 4ClSty-cascade