

Electronic Supporting Information (ESI)

A new clade of styrene monooxygenases for (*R*)-selective epoxidation

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General Methods

The substrates **1a-13a** were purchased from Alfa-Aesar (Tianjin, China) or Sigma-Aldrich (St. Louis, MO, USA). Racemic oxides (*rac*)-**2b-13b** were synthesized with >95% purity from the corresponding substrates by *m*-chloroperoxybenzoic acid oxidation.¹ Racemic oxides **1b** was purchased from Sigma-Aldrich. Restriction enzymes, High-Fidelity DNA polymerase and T4 DNA ligase were purchased from New England Biolabs (Beijing, China). All other chemicals were purchased from commercial supplies and used without further purification. Gas chromatography (GC) was performed on an Agilent Technologies 7890B GC system connected to an FID detector using a Cyclodex-B column (30m × 0.25mm × 0.25 μm, USA) or CHIRASIL-DEX CB column (Agilent Technologies, USA). High performance liquid chromatography (HPLC) was performed using Shimadzu Prominence LC-20CE system (Shimadzu, Japan) connected to a PDA-detector. ¹H NMR spectra were recorded on a Bruker-600 (600/150 MHz) spectrometer in CDCl₃.

Spectral data of epoxide products

(R)-Styrene epoxide (1b): colorless oil; retention time: $t_R(R)$ 11.5 min, $t_R(S)$ 12.7 min (Chiralpak IC-H, *n*-hexane:2-propanol 98: 2, 0.5 mL/min); $[\alpha]_D^{25} = -20.2$ (48 mM, CHCl₃) $\{[\alpha]_D^{25} = +21.1$, (83 mM, CHCl₃) for 99% ee, (*S*) $\}^2$; ¹H NMR (600 MHz, CDCl₃): δ 7.31 (m, 5H), 3.87 (dd, 1H, $J=2.62$, 3.96 Hz), 3.15 (dd, 1H, $J= 3.96$, 5.52 Hz), 2.81 (dd, 1H, $J=2.62$, 5.52 Hz).

(R)-2-(2-bromophenyl)oxirane (2b): colorless oil; retention time: $t_R(R)$ 18.4 min, $t_R(S)$ 20.4 min (Chiralpak AS-H, *n*-hexane: 2-propanol 90: 10, 0.5 mL/min); ¹H NMR (600 MHz, CDCl₃): δ 7.54 (m, 1H), 7.24-7.30 (m, 2H), 7.16 (m, 1H), 4.15 (dd, 1H, $J=2.68$, 4.46 Hz), 3.19 (dd, 1H, $J=4.46$, 5.84 Hz), 2.65 (dd, 1H, $J=2.68$, 5.84 Hz).

(R)-2-(3-bromophenyl)oxirane (3b): yellow oil; retention time: $t_R(R)$ 12.3 min, $t_R(S)$ 11.6 min (Chiralpak AD-H, *n*-hexane: 2-propanol 98: 2, 0.5 mL/min); ¹H NMR (600

MHz, CDCl₃): δ 7.42 (m, 2H), 7.22 (m, 2H), 3.82 (dd, 1H, $J= 2.68, 4.00$ Hz), 3.14 (dd, 1H, $J= 4.00, 5.54$ Hz), 2.75 (dd, 1H, $J= 2.68, 5.54$ Hz).

(S)-2-(4-bromophenyl)oxirane (4b): colorless oil; retention time: $t_R(R)$ 12.5 min, $t_R(S)$ 13.3 min (Chiralpak AD-H, *n*-hexane: 2-propanol 99: 1, 0.5 mL/min); ¹H NMR (600 MHz, CDCl₃): δ 7.47 (d, 2H, $J= 8.44$ Hz), 7.15 (d, 2H, $J= 8.44$ Hz), 3.82 (dd, 1H, $J= 2.34, 4.00$ Hz), 3.14 (dd, 1H, $J= 4.00, 5.48$ Hz), 2.75 (dd, 1H, $J= 2.34, 5.48$ Hz).

(R)-2-(2-Chlorophenyl)oxirane (5b): yellow oil; retention time: $t_R(R)$ 9.5 min, $t_R(S)$ 10.0 min (Chiralpak IC-H, *n*-hexane: 2-propanol 99: 1, 0.5 mL/min); ¹H NMR (600 MHz, CDCl₃): δ 7.36 (d, 1H, $J= 7.21$ Hz), 7.24 (m, 3H), 4.21 (dd, 1H, $J= 2.84, 4.06$ Hz), 3.19 (dd, 1H, $J= 4.06, 6.00$ Hz), 2.66 (dd, 1H, $J= 2.84, 6.00$ Hz).

(R)-2-(3-Chlorophenyl)oxirane (6b): yellow oil; retention time: $t_R(R)$ 13.3 min, $t_R(S)$ 12.7 min (Chiralpak AD-H, *n*-hexane: 2-propanol 99: 1, 0.5 mL/min); ¹H NMR (600 MHz, CDCl₃): δ 7.27 (m, 3H), 7.17 (m, 1H), 3.83 (dd, 1H, $J= 2.54, 4.32$ Hz), 3.14 (dd, 1H, $J= 4.32, 5.56$ Hz), 2.76 (dd, 1H, $J= 2.54, 5.56$ Hz).

(S)-2-(4-Chlorophenyl)oxirane (7b): yellow oil; retention time: $t_R(R)$ 10.4 min, $t_R(S)$ 11.5 min (Chiralpak AS-H, *n*-hexane: 2-propanol 90: 10, 0.5 mL/min); ¹H NMR (600 MHz, CDCl₃): δ 7.32 (d, 2H, $J= 8.80$ Hz), 7.21 (d, 2H, $J= 8.80$ Hz), 3.83 (dd, 1H, $J= 2.70, 3.98$ Hz), 3.14 (dd, 1H, $J= 3.98, 5.44$ Hz), 2.75 (dd, 1H, $J= 2.70, 5.44$ Hz).

(R)-2-(2-tolyl)oxirane (8b): colorless oil; retention time: $t_R(R)$ 21.5 min, $t_R(S)$ 22.0 min (Chiral GC, CHIRASIL-DEX CB column); ¹H NMR (400 MHz, CDCl₃): δ 7.20 (m, 3H), 7.17 (m, 1H), 4.01 (dd, 1H, $J= 2.78, 4.04$ Hz), 3.17 (dd, 1H, $J= 4.04, 5.78$ Hz), 2.70 (dd, 1H, $J= 2.78, 5.78$ Hz), 2.43 (s, 3H).

(R)-2-(3-tolyl)oxirane (9b): colorless oil; retention time: $t_{R1}(R)$ 5.5 min, $t_{R1}(S)$ 6.0 min (Chiralpak AS-H, *n*-hexane: 2-propanol 99: 1, 1 mL/min); $t_{R2}(R)$ 20.6 min, $t_{R2}(S)$ 21.0

min (Chiral GC, CHIRASIL-DEX CB column); ^1H NMR (400 MHz, CDCl_3): δ 7.23 (d, 1H, $J=7.80$ Hz), 7.10 (m, 3H), 3.84 (dd, 1H, $J=2.64, 4.10$ Hz), 3.14 (dd, 1H, $J=4.10, 5.60$ Hz), 2.80 (dd, 1H, $J=2.64, 5.60$ Hz), 2.35 (s, 3H).

(S)-2-(4-tolyl)oxirane (10b): colorless oil, (retention time: $t_{\text{R}}(R)$ 5.5 min, $t_{\text{R}}(S)$ 5.7 min, AS-H, *n*-Hexane: 2-propanol = 90: 10, 1 mL/min), ^1H NMR (400 MHz, CDCl_3): δ 7.23 (m, 2H), 7.13 (m, 2H), 3.83 (dd, 1H, $J= 2.48, 4.04$ Hz), 3.13 (dd, 1H, $J= 4.04, 5.64$ Hz), 2.80 (dd, 1H, $J= 2.48, 5.64$ Hz), 2.35 (s, 3H).

(R)-2-(3-methoxyphenyl)oxirane (11b): colorless oil; retention time: $t_{\text{R}1}(R)$ 10.1 min, $t_{\text{R}1}(S)$ 10.4 min (Chiralpak AS-H, *n*-hexane: 2-propanol 99: 1, 1 mL/min); $t_{\text{R}2}(R)$ 26.8 min, $t_{\text{R}2}(S)$ 27.0 min (Chiral GC, CHIRASIL-DEX CB column); ^1H NMR (400 MHz, CDCl_3): δ 7.26 (dd, 1H, $J=4.04, 5.78$ Hz), 6.86 (m, 3H), 3.85 (dd, 1H, $J=2.84, 4.02$ Hz), 3.81 (s, 3H), 3.14 (dd, 1H, $J=4.02, 5.56$ Hz), 2.79 (dd, 1H, $J=2.84, 5.56$ Hz).

(1R,2S)-1,2-dihydronaphthalene oxide(13b): white solid; retention time: $t_{\text{R}}(1R,2S)$ 10.9 min, $t_{\text{R}}(1S,2R)$ 11.9 min (Chiralpak AS-H, *n*-hexane: 2-propanol 90: 10, 0.5 mL/min); ^1H NMR (600 MHz, CDCl_3): δ 7.54 (d, 1H, $J= 7.62$ Hz), 7.21 (m, 2H), 7.09 (d, 1H, $J= 7.32$ Hz), 4.58 (d, 1H, $J= 8.38$ Hz), 3.81 (m, 1H), 2.91 (m, 1H), 2.14 (m, 1H), 1.85 (m, 1H), 1.26(m, 1H).

Table S1. Primers for plasmid construction

Accession No. of targeted protein	Nucleotide sequence
WP_030605949.1	5'-TAATTACATATGAGCATCGGCGTCGTC-3' 5'-TATACTAGTTCAGCCGGCCGGTGC-3'
WP_112440363.1	5'-TAATTACATATGAGCCGCGTGGGTGT-3' 5'-TATACTAGTTCAGCCGTGCGGTGC-3'
WP_052286622.1	5'-TAATTACATATGACGAGCGTCGGC-3' 5'-TATACTAGTTCAGCCCTGCCGTGG-3'
WP_113694022.1	5'-TAATTACATATGACCGGCATCGGCGT-3' 5'-TATACTAGTTCAATCGGTTTTGCCGGC-3'
WP_137992763.1	5'-TAATTACATATGACCGACACCGGCA-3' 5'-TATACTAGTTCATCCATGCGCCACGT-3'
WP_069162359.1	5'-TAATTACATATGAGCAGTATTGGCATTG-3' 5'-TATACTAGTTCACTCCGTACGGAAGC-3'
WP_067993035.1	5'-TAATTACATATGACCAGCATCGGCAT-3' 5'-TATACTAGTTCACTCCGCACGGAAG-3'
WP_067452867.1	5'-TAATTACATATGACCACCATCGGC-3' 5'-TATACTAGTTCACTCCTCAATGGATTTCAG-3'
WP_043716969.1	5'-TAATTACATATGACCGACATCGGCGTC-3' 5'-TATACTAGTTCAGCCACCGGCGACG-3'
WP_120086312.1	5'-TAATTACATATGGCCGATATCGGC-3' 5'-TATACTAGTCTAGACCCTG AATCCGGC-3'
WP_120086314.1	5'-TAATTACATATGGGAGGAATAGGCATTC-3' 5'-TATACTAGTTTAAACGGTATTCAGACAGGAAC-3'
WP_067345360.1	5'-TAATTACATATGACCAGCGTGGGC-3' 5'-TATACTAGTTTAAACGTTGACGCGGACC-3'
WP_073449381.1	5'-TAATTACATATGACCAGCGTGGGC-3' 5'-TATACTAGTTTAAACCTTGACGCGGACC-3'
WP_102925532.1	5'-TAATTACATATGACCAGCGTGGGC-3' 5'-TATACTAGTTTAAACCTTGACGCGGACC-3'
WP_121802755.1	5'-TAATTACATATGCGTATCGGTATTATGG-3' 5'-TATACTAGTTTAGGTTTGACCACGG-3'
WP_051753842.1	5'-TAATTACATATGAGCATCGGTATTGTT-3' 5'-TATACTAGTTTAAACCGCGCTACG-3'
WP_102918856.1	5'-TAATTACATATGCGTATCGCGATCATTG-3' 5'-TATACTAGTTTAGCTCGGACGCGCG-3'
WP_067345356.1	5'-TAATTACATATGAGCATCGGCATTGTTG-3' 5'-TATACTAGTTTAAACACCGCTACGCACC-3'
WP_073449384.1	5'-TAATTACATATGAGCATGGGCATCGTT-3' 5'-TATACTAGTTTAAACACCGCTACGCACC-3'
WP_125743710.1	5'-TAATTACATATGAGCGATATCGCGATTG-3' 5'-TATACTAGTTTAAACCCGCCTCGGTGAT-3'
WP_141293187.1	5'-TAATTACATATGAGCGACATCGCGATTG-3' 5'-TATACTAGTTTAAACCCGCCTCGGTGAT-3'

Table S2. Primers for site-directed mutagenesis.

Mutant	Nucleotide sequence
<i>AaStyA</i> F46T	5'-CGGCCGCGGAACACCCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGGGTGTTCGCGGCCG-3'
<i>PbStyA</i> F46T	5'-GTGGGCCACGCAACACCCCGGTCCGATTCGG-3' 5'-CCGAATCGGACCGGGGTGTTGCGTGGCCCCAC-3'
<i>SeStyA</i> F56T	5'-CGGCCGCGGAACACCCCGCCCGGTTC-3' 5'-GAACCGGGCGGGGGTGTTCGCGGCCG-3'
<i>PsStyA</i> T47F	5'-GCCTTCGGCTGCTGAACTTTGTGGCTCATCACGCGG-3' 5'-CCGCGTGATGAGCCACAAGTTCAGCAGCCGAAGGC-3'
<i>AaStyA</i> F46H	5'-CGGCCGCGGAACCATCCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGATGGTTCCGCGGCCG-3'
<i>AaStyA</i> F46R	5'-CGGCCGCGGAACCGTCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGACGGTTCGCGGCCG-3'
<i>AaStyA</i> F46K	5'-GCGGCCGCGGAACAAACCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGTTTGTTCGCGGCCG-3'
<i>AaStyA</i> F46I	5'-GCGGCCGCGGAACATTCCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGAATGTTCCGCGGCCG-3'
<i>AaStyA</i> F46L	5'-CGGCCGCGGAACCTGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCAGGTTCCGCGGCCG-3'
<i>AaStyA</i> F46W	5'-GCCGCGGAACCTGGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCCAGTTCGCGGC-3'
<i>AaStyA</i> F46A	5'-GGCCGCGGAACGCGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCGCGTTCGCGGCC-3'
<i>AaStyA</i> F46M	5'-CGGCCGCGGAACATGCCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGCATGTTCCGCGGCCG-3'
<i>AaStyA</i> F46P	5'-GGCCGCGGAACCCGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCGGGTTCGCGGCC-3'
<i>AaStyA</i> F46C	5'-GGCCGCGGAACCTGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGGCAGTTCGCGGCC-3'
<i>AaStyA</i> F46N	5'-GCGGCCGCGGAACAATCCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGATTGTTCCGCGGCCG-3'
<i>AaStyA</i> F46V	5'-CGGCCGCGGAACGTGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCACGTTCCGCGGCCG-3'
<i>AaStyA</i> F46G	5'-CGGCCGCGGAACGGCCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGGCCGTTCCGCGGCCG-3'
<i>AaStyA</i> F46S	5'-CGGCCGCGGAACAGCCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGGCTGTTCGCGGCCG-3'
<i>AaStyA</i> F46Q	5'-CGGCCGCGGAACAGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCTGGTTCGCGGCCG-3'
<i>AaStyA</i> F46Y	5'-CGGCCGCGGAACATCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGATAGTTCGCGGCCG-3'
<i>AaStyA</i> F46D	5'-CGGCCGCGGAACGATCCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGATCGTTCGCGGCCG-3'
<i>AaStyA</i> F46E	5'-CGGCCGCGGAACGAACCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGTTCGTTCCGCGGCCG-3'

Table S3. Biotransformation of styrene by wild-type *AaStyA* and mutants.

Enzyme	Yield (%)	ee (%)
wild type	66%	94(<i>R</i>)
F46E	0	--
F46G	2	78(<i>R</i>)
F46H	33	65(<i>S</i>)
F46I	17	88(<i>R</i>)
F46K	0	--
F46L	32	84(<i>R</i>)
F46M	31	86(<i>R</i>)
F46P	39	5(<i>S</i>)
F46R	0	--
F46W	18	71(<i>R</i>)
F46S	18	58(<i>R</i>)
F46C	37	36(<i>R</i>)
F46D	0	--
F46Y	39	47(<i>R</i>)
F46A	21	54(<i>R</i>)
F46N	22	23(<i>S</i>)
F46T	32	22(<i>S</i>)
F46Q	3	82(<i>R</i>)
F46V	34	24(<i>R</i>)

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                                GXGXXG                                F
StStyA      : MSVGVIGSGISGLALRLRQQLGVPVTVYSEHDIDGLTGRPRNFHARHGFTQQCRRE  CVFEDDFALAQ : 70
AaStyA      : TGIQVIGAGIAGLHLALRLRQQLGVPVTVYSAQTTPGELATARPRNFHARHGFTQQCRRE  CVFAMESDDAR : 70
SeStyA      : TIIGIIGSGISGLALRLRQQLGVPVTVYSAQTAEELGASRPRNFHARHAPTQCRREDS  CVFHAMQDFDAR : 70
PbStyA      : AIIGILGAGISGLALRLRQQLGVPVTVYTPDQSEQLAAGCPNRFVRRGFTVQRREEL  CVFELMPSAQ : 70
S.achromogenes WP_030605949.1 : MSIGVVSGISGLALRLRQQLGVPVTVYSEHDIDGLTGRPRNFHARHGFTQQCRRE  CVFEDDFALAQ : 70
Streptomyces sp. 769 WP_052286622.1 : TSVGIIGSGISGLALRLRQQLGTAATVYSTQDIDQLKSGRPRNFHARHAPTQCRRE  CVFSADEFDPAQ : 70
N.altamirensis WP_069162359.1 : SSIGIVGTGISGLALRLRQQLGTDVTVYSAQDPDQLASGRPRNFHARHGFTQQCRRE  CVFEDDFALAQ : 70
Nocardia_alba WP_067452867.1 : TTIGIVGTGISGLALRLRQQLGIEVVVYSAQELDQLATGRPRNFHARHGFTQQCRRE  CVFEDDFALAQ : 70
Kutzneria_sp._744 WP_043716969.1 : TLIGVIGAGISGLALRLRQQLGVPVTVYSTQTPDELAAAMRPRNFHARHGFTQQCRRE  CVFEDDFALAQ : 70
                                *
                                GG                                DXXXXX
StStyA      : VRHWAVTAHAALAGRHEFVAGLTPPSSVVDFFRYLLELSAVVDRGGQLVTGDHAVEDLA  HDLHVVVA : 140
AaStyA      : VRSWSITING--GGLLEFAAALTPSSVVDFFRYLLELFEFVNRGGQVMIGAVTAADV  AGRHDLHVVVA : 138
SeStyA      : VHSWAIITHG--EGADLEFAAALTPSSVVDFFRYLLELFEFARGGQVNRIGPVVD  EVARRHDLHVVVA : 138
PbStyA      : VAVVAANIIFPP--GQDPLYLSTRIDPPANGVDFRVYLLIARHAEERGKVIIT---  ELLPSGHDLHVVVA : 135
S.achromogenes WP_030605949.1 : VRHWAVTAHPGEGEPPRFLAALTPPSSVVDFFRYLLELSAVVDRGGQLITG  GHAFELITGRHDLHVVVA : 140
Streptomyces sp. 769 WP_052286622.1 : VHHWSTLVHT--DGFAIAFFARHAPSSVVDFFRYLLELHLLGEEVARGGLD  VGEFAVEDIARRHDLHVVVA : 138
N.altamirensis WP_069162359.1 : VHRWDVAIQA--GPQETFFHADTPPSSVVDFFRYLLELCAKGVARGGQV  VVGERPITIAERHDLHVVVA : 138
Nocardia_alba WP_067452867.1 : VRRWDVTVGA--GDHTAIFHGBLTPPSSVVDFFRYLLELCAKHAERG  CKVVVGARPIITVAEQHDLHVVVA : 138
Kutzneria_sp._744 WP_043716969.1 : VHSWSITMHG--DDRDLEFSAAALTPSSVVDFFRYLLELFEFVNRGG  NAVILAVTFHQVAARHDLHVVVA : 138
                                *
                                XG
StStyA      : NGRSRVRLFFHADPPRSYPTTPQRFVLCAGIYHGITTEVPHSLDTHL  FBGAGEILRLFFFSFACRADVLAF : 210
AaStyA      : NGRSRLREFFVDFARSPHHTPQRFVLCGEGYHGIVDVEHSLDTHL  FBGAGEILRLFFFSRIGFAHVLAF : 208
SeStyA      : NGRSRLREFFVDFARSPHHTPQRFVLCGEGYHGIVDVEHSLDTHL  FBGAGEILRLFFFSRIGFAHVLAF : 208
PbStyA      : NGRSRVRLFFHADPPRSYPTTPQRFVLCGEGYHGIVDVEHSLDTHL  FBGAGEILRLFFFSFACRADVLAF : 210
S.achromogenes WP_030605949.1 : NGRSRVRLFFHADPPRSYPTTPQRFVLCAGIYHGITTEVPHSLDTHL  FBGAGEILRLFFFSFACRADVLAF : 210
Streptomyces sp. 769 WP_052286622.1 : NGRSRLREFFVDFARSPHHTPQRFVLCGEGYHGIVDVEHSLDTHL  FBGAGEILRLFFFSRIGFAHVLAF : 208
N.altamirensis WP_069162359.1 : NGRSRLREFFVDFARSPHHTPQRFVLCGEGYHGIVDVEHSLDTHL  FBGAGEILRLFFFSRIGFAHVLAF : 208
Nocardia_alba WP_067452867.1 : NGRSRLREFFVDFARSPHHTPQRFVLCGEGYHGIVDVEHSLDTHL  FBGAGEILRLFFFSRIGFAHVLAF : 208
Kutzneria_sp._744 WP_043716969.1 : NGRSRLREFFVDFARSPHHTPQRFVLCGEGYHGIVDVEHSLDTHL  FBGAGEILRLFFFSRIGFAHVLAF : 208
                                *
StStyA      : EAVPGGPLEFAVAHVLAALADAGLFRROVLDLVAAYAEFLRERV  DTCATHTCGGELAQCAITFVVRGQWAR : 280
AaStyA      : EAVPGGPLEFAHLLAALADAGLFRREVRLVAEHAFLRERIDT  TRFDLVAPGELAQGVVTFVVRGQWAR : 278
SeStyA      : EAVPGGPLEFAHLLAALADAGLFRREVRLVAAYAEFLRERV  DTCATHTCGGELAQGVVTFVVRGQWAR : 278
PbStyA      : EAVPGGPLEVLTFRFVADDSGLRLDLYKLAHYAEFLRERIT  TAFELIAPGELAQGVVTFVVRGQWAR : 275
S.achromogenes WP_030605949.1 : EAVPGGPLEAVCHVLAALADAGLFRROVLDLVAAYAEFLRERV  DTCATHTCGGELAQCAITFVVRGQWAR : 280
Streptomyces sp. 769 WP_052286622.1 : EAVPGGPLEVSHLADVDVGGABHRAVDLVAIYAEFLRERID  TGFELISPGVVAQCAITFVVRGQWAR : 278
N.altamirensis WP_069162359.1 : EAVPGGPLEAISHVLEADLEAAYRVDVYKLLGEFAFLRER  IDTCAFTIICPGVVAQCAITFVVRGQWAR : 278
Nocardia_alba WP_067452867.1 : EAVPGGPLEPISRVDFAVEVRYRDNVALLGEFAFLRERV  DQCFETIICPGELAQGVVTFVVRGQWAR : 278
Kutzneria_sp._744 WP_043716969.1 : EAVPGSPLERFAHLLAALADAGLFRSEMLLVAQHAFLRERV  DITREGLIAPGELAQCAITFVVRGQWAR : 278
                                *
                                GDXXXXXXP    GXNXXXXXXXXL
StStyA      : LDDGTCALAIICLAWITNDPLTAQCANLGSHTAHALADLIT  LAEGPFDEAFGRASARLWEHARVVEWNS : 350
AaStyA      : LDDGTCALAIICLAWITNDPLTAQCANLGSHTAHALADLIT  LAASANGPLLAGFGRASARLWEHARVVEWNS : 348
SeStyA      : LDDGTCALAIICLAWITNDPLTAQCANLGSHTAHALADLIT  ASATGPLDDAFGRDLASARLWEHARVVEWNS : 348
PbStyA      : LDDGTCALAIICLAWITNDPLTAQCANLGSHTAHALADLIT  ARRISEQLWEHARVVEWNS : 340
S.achromogenes WP_030605949.1 : LDDGTCALAIICLAWITNDPLTAQCANLGSHTAHALADLIT  ADTRAPYDEAFGRASARLWEHARVVEWNS : 350
Streptomyces sp. 769 WP_052286622.1 : LDDGTCALAIICLAWITNDPLTAQCANLGSHTAHALADLIT  STARGPYDEEFGRSASALWEHARVVEWNS : 348
N.altamirensis WP_069162359.1 : LDDGTCALAIICLAWITNDPLTAQCANLGSHTAHALADLIT  AKASGPDYDQCFRETSQCLWAHARVVEWNS : 348
Nocardia_alba WP_067452867.1 : LDDGTCALAIICLAWITNDPLTAQCANLGSHTAHALADLIT  VTAGGYDETFGRASARLWEHARVVEWNS : 348
Kutzneria_sp._744 WP_043716969.1 : LDDGTCALAIICLAWITNDPLTAQCANLGSHTAHALADLIT  AAAGPLARFCRLASDRLWEHARVVEWNS : 348
                                *
StStyA      : AFLAPPPHVAQIFGFAAEKRTALAFVSRNDPVMAMRTISS  PEGVAAFVADCEGG----- : 407
AaStyA      : AFLAPPPHVVLEIFGFAAEKRTALAFVSRNDPVMAMRTISS  PEGVIAFVQSCDT----- : 404
SeStyA      : AFLAPPPHVMPIFGFAAGKRTALAFVSRNDPVMAMRTISS  PEGVDSFVRSCT----- : 403
PbStyA      : TYLGMPPRHGCVFFFAAGKRTALAFVSRNDPVMAMRTISS  PADSVDFTAGFR----- : 395
S.achromogenes WP_030605949.1 : AFLQPPPHVACILGFAAEKRTALAFVSRNDPVMAMRTISS  PEGVAAFVADCE----- : 405
Streptomyces sp. 769 WP_052286622.1 : AFLLAFAHVTALFAAARARRVALAFVSRNDPVMAMRTISS  EGVASFVAACRPIGPRQG : 410
N.altamirensis WP_069162359.1 : AFLGPPPHVVICIFGFAAEKRTALAFVSRNDPVMAMRTISS  PGGTESFTAGFR----- : 403
Nocardia_alba WP_067452867.1 : AFLGFAAHTTALFAAARARRVALAFVSRNDPVMAMRTISS  PGTAFVAESI----- : 403
Kutzneria_sp._744 WP_043716969.1 : AFLAPPPHVTDFGFAAEKRTALAFVSRNDPVMAMRTISS  PDGAASFVERCV----- : 403

```

Figure S1. Multiple sequence alignment of (R)-selective SMOs.

Conserved domains are shown on top of the sequences, including the FAD-binding motifs GxGxxG, GG, and Dx6G, the putative dual function motif GDx6P related to FAD and/or NAD(P)H interaction, and the conserved GxNx8L motif.^{3, 4}

	T	
G.polyisoprenivorans_NBRC16320_GAB22407.1	: TCIIVGAGT	AGLHGLHLLIQCGVTTT
G.rubripertincta_ASR05591.1	: TAIIVGAGT	AGLHGLHLLIQGHVEST
Rhodococcus_opacus_ANS32444.1	: TAIIVGAGT	AGLHGLHLLNEKGILAT
Rhodococcus_sp._ST-10_BAL04129.1	: TAIIVGAGT	AGLHGLHLLIQGHVEST
Rhodococcus_sp._ST-5_BAL04132.1	: TAIIVGAGT	AGLHGLHLLNEKGILAT
S.fribergensis_WP_039579272.1	: TCIIVGAGT	AGLHGLHLLKNGVEVRL
Nevskia_amosa_WP_022977994.1	: TCIIVGAGT	AGLHGLHLLIQGHVEST
P.agarilytica_NO2_GAC06215.1	: TAIIVGAGT	AGLHGLHLLYRKHNDVTL
P.fluorescens_CAB06823.1	: TCIIVGAGT	AGLHGLHLLRQHDVDVT
Pseudomonas_putida_ABX24519.1	: TCIIVGAGT	AGLHGLHLLRQHDVDVT
Pseudomonas_sp._LQ26_ADE62390.1	: TCIIVGAGT	AGLHGLHLLRQHDVDVT
Pseudomonas_sp._VLB120_AAC23718.1	: TCIIVGAGT	AGLHGLHLLRQHDVDVT
uncultured bacterium_ABV24041.1	: TCIIVGAGT	AGLHGLHLLYRKHNDVTL
M.litorale_WP_027855270.1	: TCIIVGAGT	AGLHGLHLLRQHDVDVT
		S
Gemmobacter_nectarophilus_WP_028028710.1	: FTIIVGCGS	GLHVAIGL
Burkholderia_sp._H160_EEA04565.1	: TAIIVGCGS	GLHVAIGLDRYVVT
Delftia_acidovorans_SPH-1_ABX34433.1	: TAIIVGCGS	GLHVAIGLAAAGHVT
Cupriavidus_necator_H16_CAJ94554.1	: TAIIVGCGS	GLHVAIGLCAAGYVVT
Acinetobacter_baylyi_ADP1_CAG69430.1	: TAIIVGCGS	GLHVAIGLGLSLLDT
Paenarthrobacter_aurescens_TC1_AEM07034.1	: ITIIVGCGS	GLHVAIGLGLLQAGYV
Nocardia_farcinica_IFM_10152_EAD56093.1	: ITIIVGCGS	GLHVAIGLGLFGLA
Rhodococcus_opacus_ACR43973.1	: ITIIVGCGS	GLHVAIGLGLAIGL
Streptomyces_auratus_AGR0001_EJJ03822.1	: ITIIVGCGS	GLHVAIGLGLGIDH
Variovorax_paradoxus_EPS_ADU39062.1	: VAIIVGCGS	EMLALGIRAGHVT
		L
Streptomyces_sp._WAC01280_WP_125743710.1	: TAIIVGAGIS	LHLALRLOQAGV
Streptomyces_exfoliatus_WP_024761592.1	: TAIIVGAGIS	LHLALRLOQAGV
Streptomyces_sp._Root43l_WP_056642759.1	: TAIIVGAGIS	LHLALRLOQAGV
Streptomyces_venezuelae_WP_150272882.1	: TAIIVGAGIS	LHLALRLOQAGV
Streptomyces_gardneri_WP_141293187.1	: TAIIVGAGIS	LHLALRLOQAGV
Streptomyces_albus_WP_040245932.1	: TAIIVGAGIS	LHLALRLOQAGV
Streptomyces_sp._NBRC109706_WP_062205268.1	: TAIIVGAGIS	LHLALRLOQAGV
Kitasatospora_viridis_WP_145909623.1	: TAIIVGAGIS	LHLALRLOQAGV
Streptomyces_sp._LD120_WP_168541538.1	: TAIIVGAGIS	LHLALRLOQAGV
		E
StStyA	: VGIIVGSGIS	LHLALRLOQGV
AaStyA	: IGVIVGAGT	LHLALRLOQLGVP
SeStyA	: IGIIVGSGIS	LHLALRLOQLGVP
PbStyA	: IGIIVGSGIS	LHLALRLOQLGVP
Streptomyces_achromogenes_WP_030605949.1	: IGVIVGSGIS	LHLALRLOQLGVP
Streptomyces_sp._769_WP_052286622.1	: VGIIVGSGIS	LHLALRLOQLGVP
Nocardia_altamirensis_WP_069162359.1	: IGIIVGSGIS	LHLALRLOQLGVP
Kutzneria_sp._744_WP_043716969.1	: IGVIVGSGIS	LHLALRLOQLGVP
Nocardia_alba_WP_067452867.1	: IGIIVGSGIS	LHLALRLOQLGVP

Group I

Group II

Group III

Group IV

Figure S2. Sequence alignment of Groups I to IV SMOs at position 46.

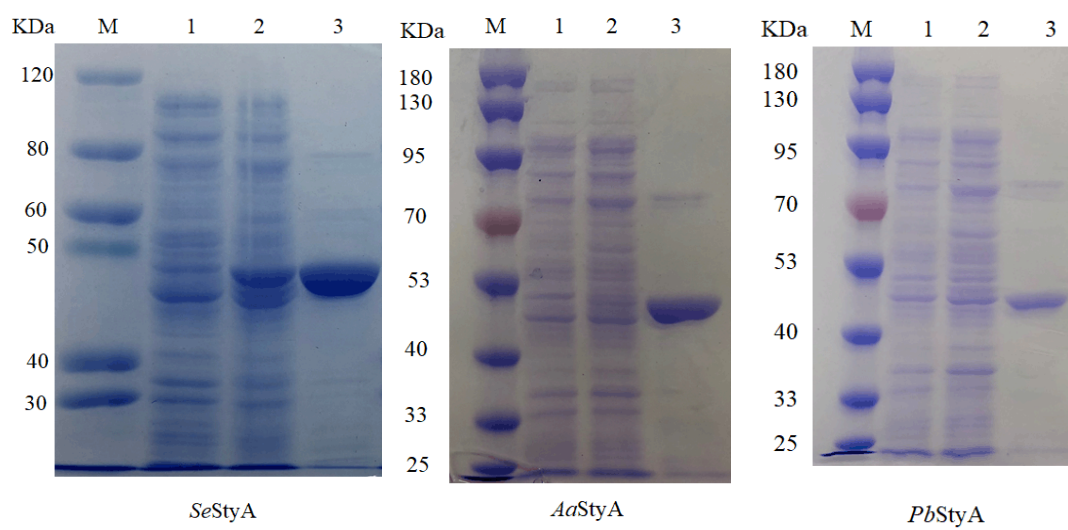


Figure S3. SDS-PAGE analysis of *SeStyA*, *AaStyA* and *PbStyA*.

M: Protein Marker; lane 1: cell-free extract of pET28a(+); lane 2: cell-free extract of StyA; lane 3: purified StyA.

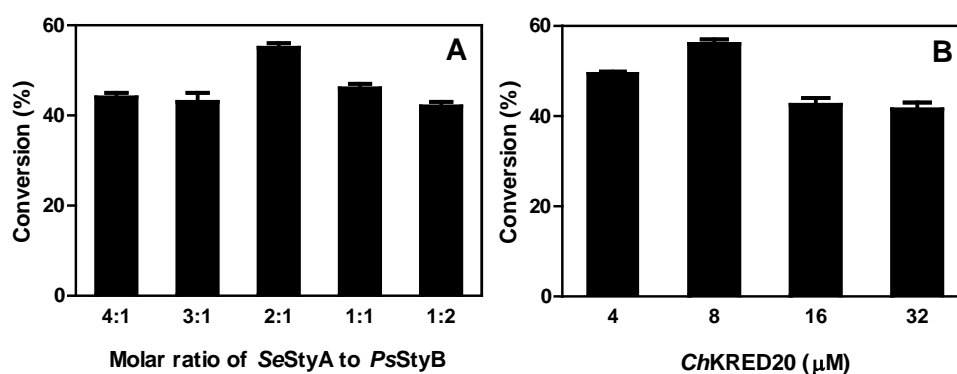


Figure S4. Optimizing reaction conditions for kinetic analysis.

A) The reaction mixture contained 5-40 μM of purified *StStyA*, 10 μM of purified *PsStyB*, 0.5 mM NADH, 50 μM FAD and 650 U catalase B) The reaction mixture contained 20 μM of purified *StStyA*, 10 μM of purified *PsStyB*, 4-32 μM of purified *ChKRED20*, 0.1 mM NAD^+ , 50 μM FAD and 650 U catalase.

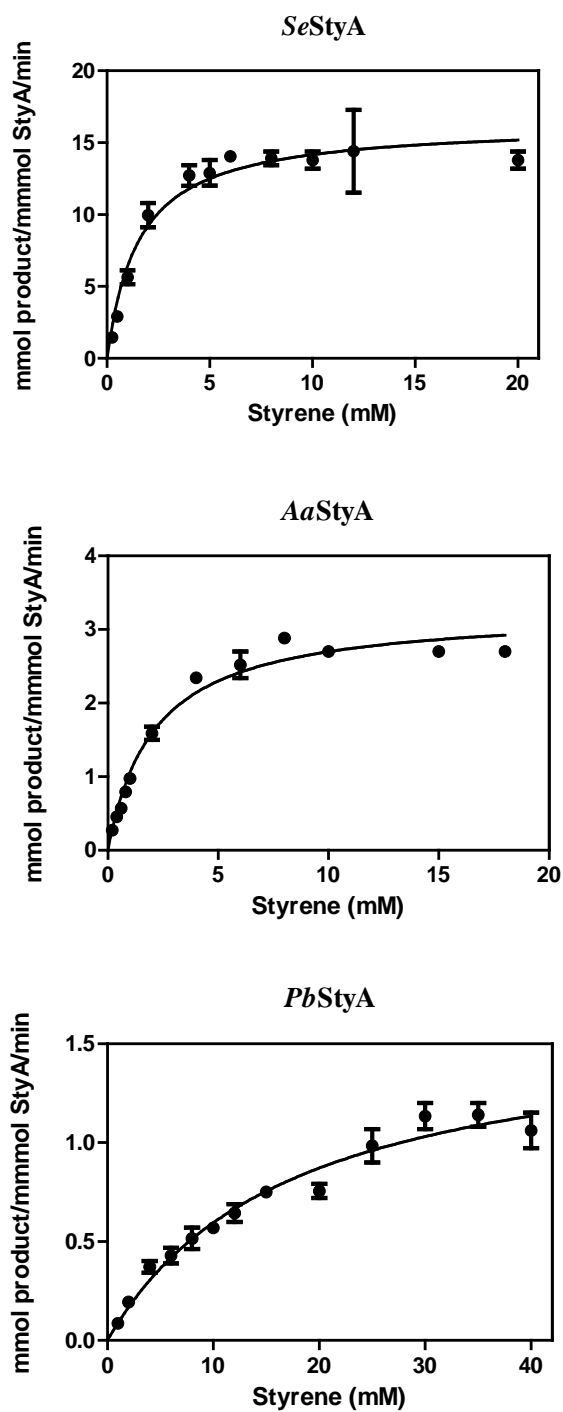
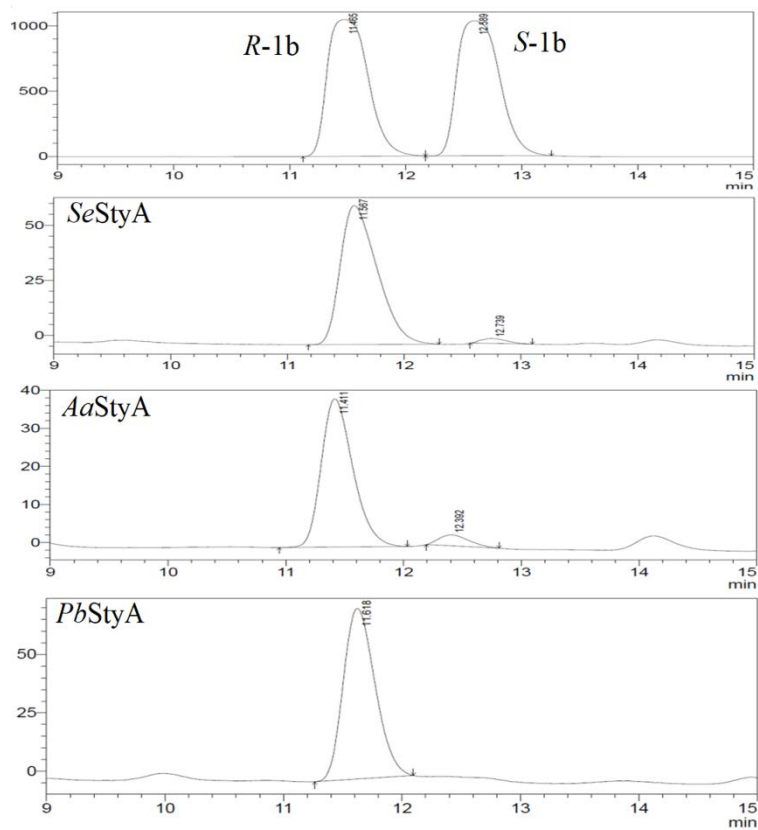
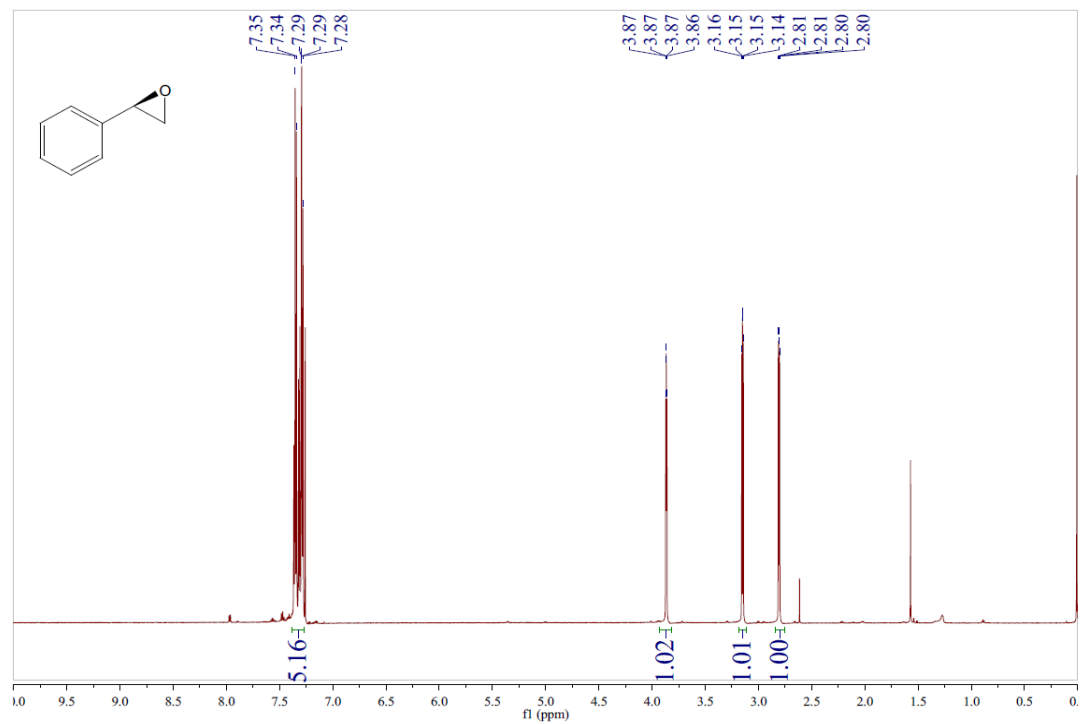


Figure S5. Steady-state kinetics of StyAs in the epoxidation of styrene.

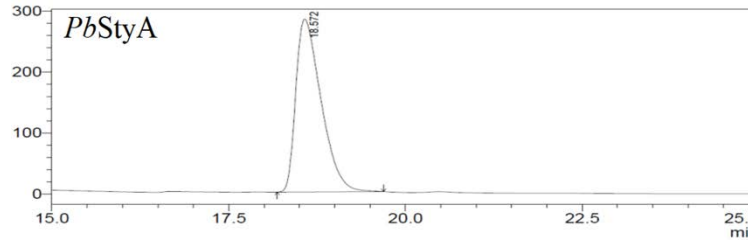
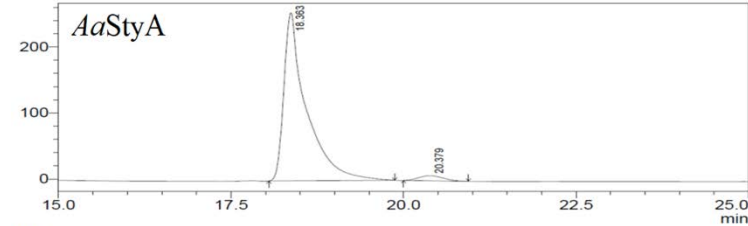
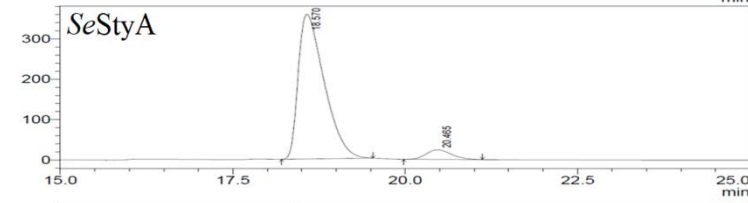
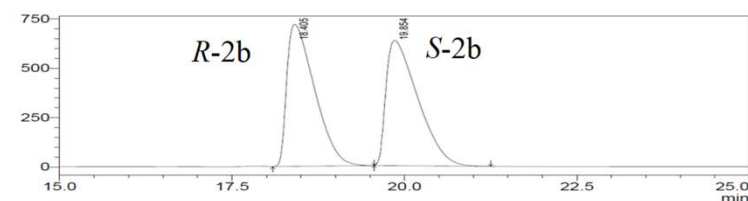
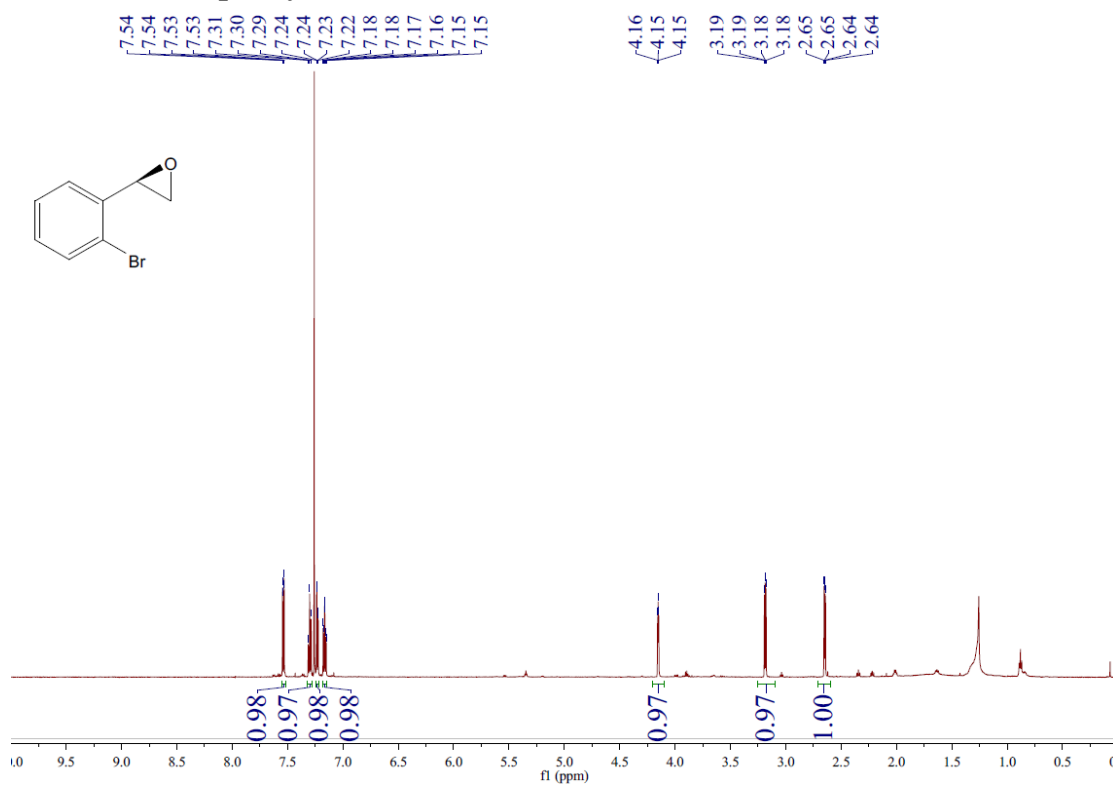
^1H NMR spectra and HPLC chromatograms

(*R*)-Styrene epoxide (**1b**)



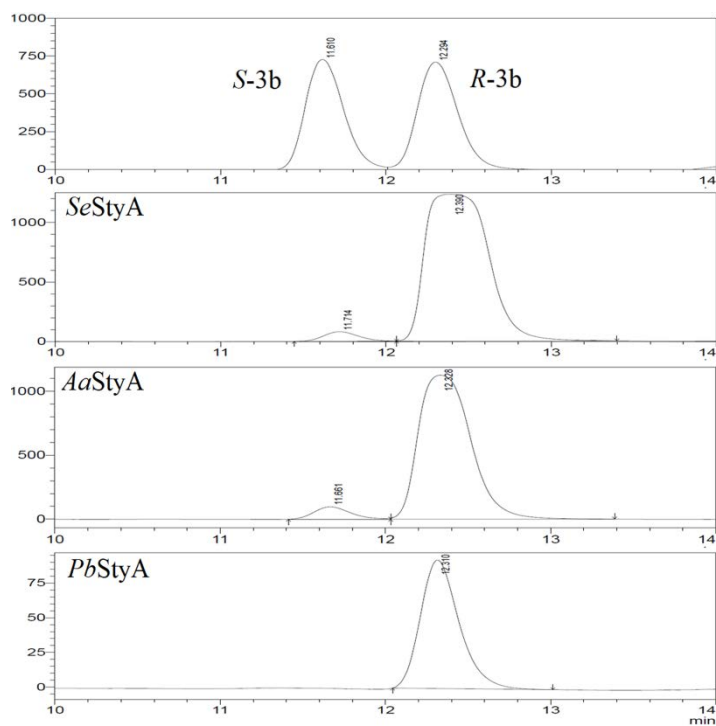
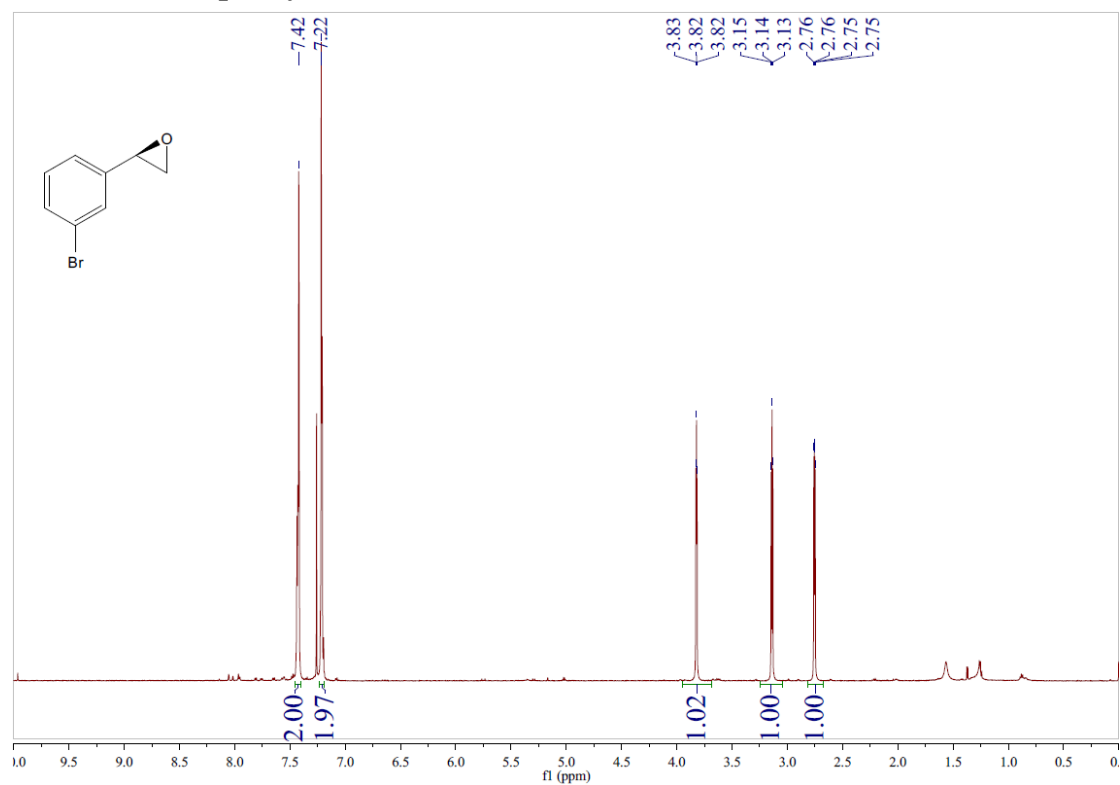
Chiralpak IC-H, *n*-Hexane: 2-propanol 98: 2, 0.5 mL/min

(R)-2-(2-bromophenyl)oxirane (2b)



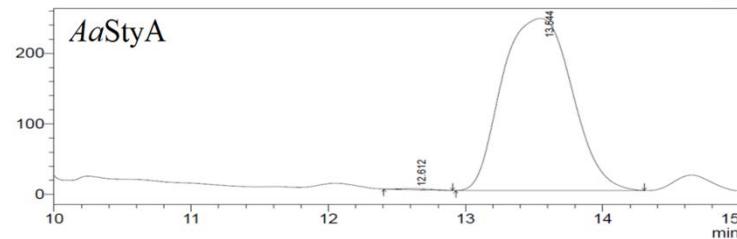
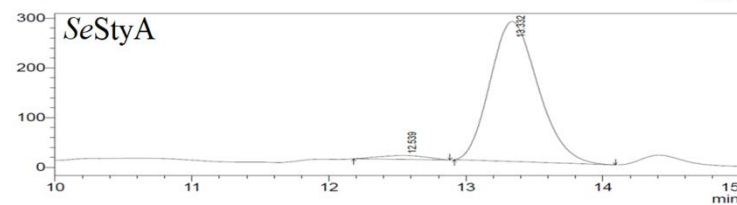
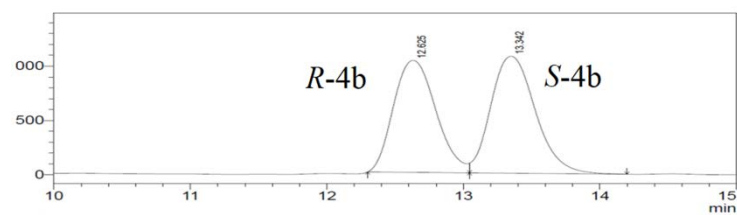
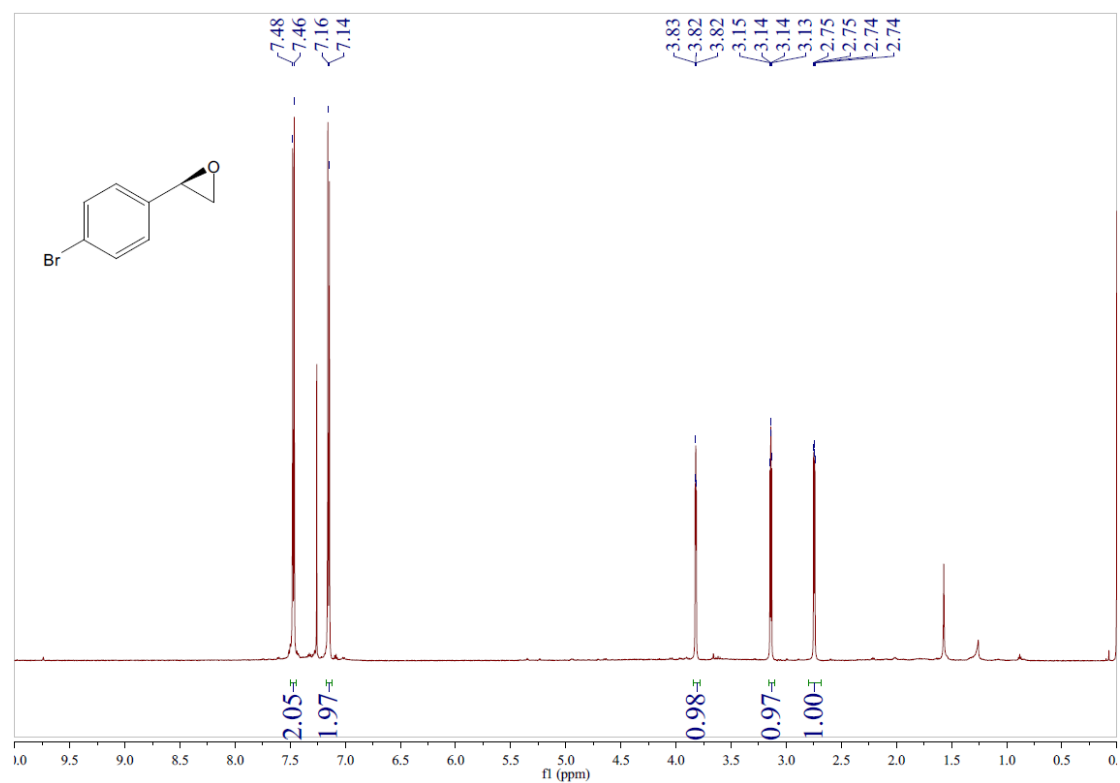
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(R)-2-(3-bromophenyl)oxirane (3b)



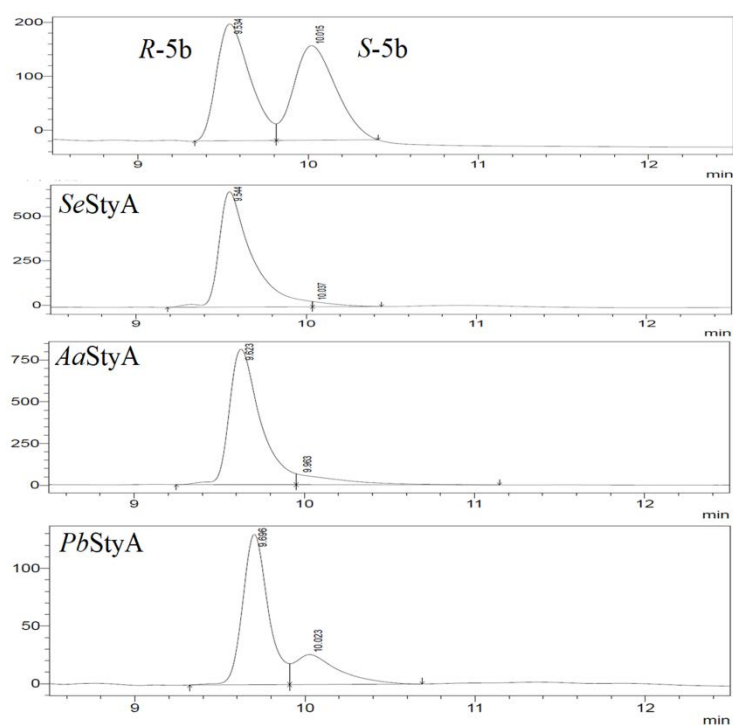
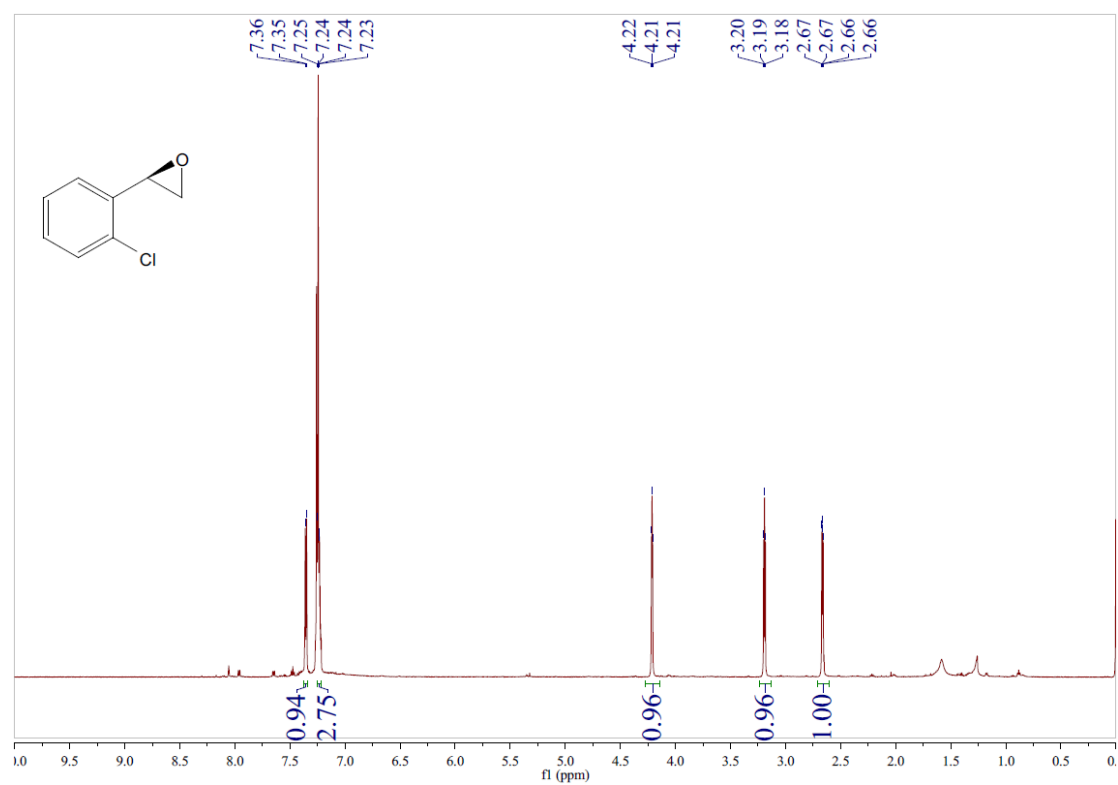
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(S)-2-(4-bromophenyl)oxirane (4b)



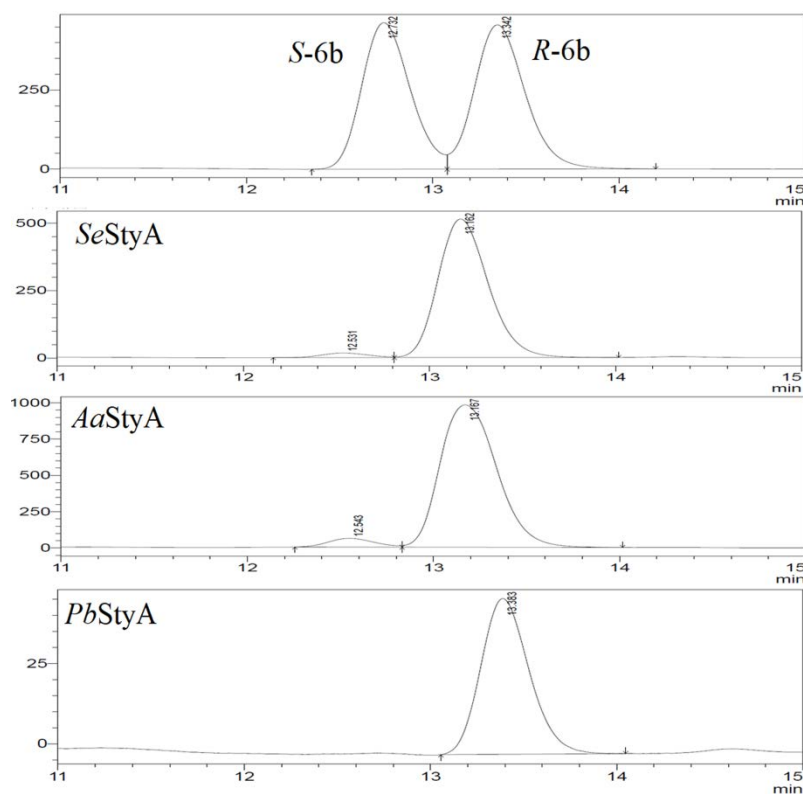
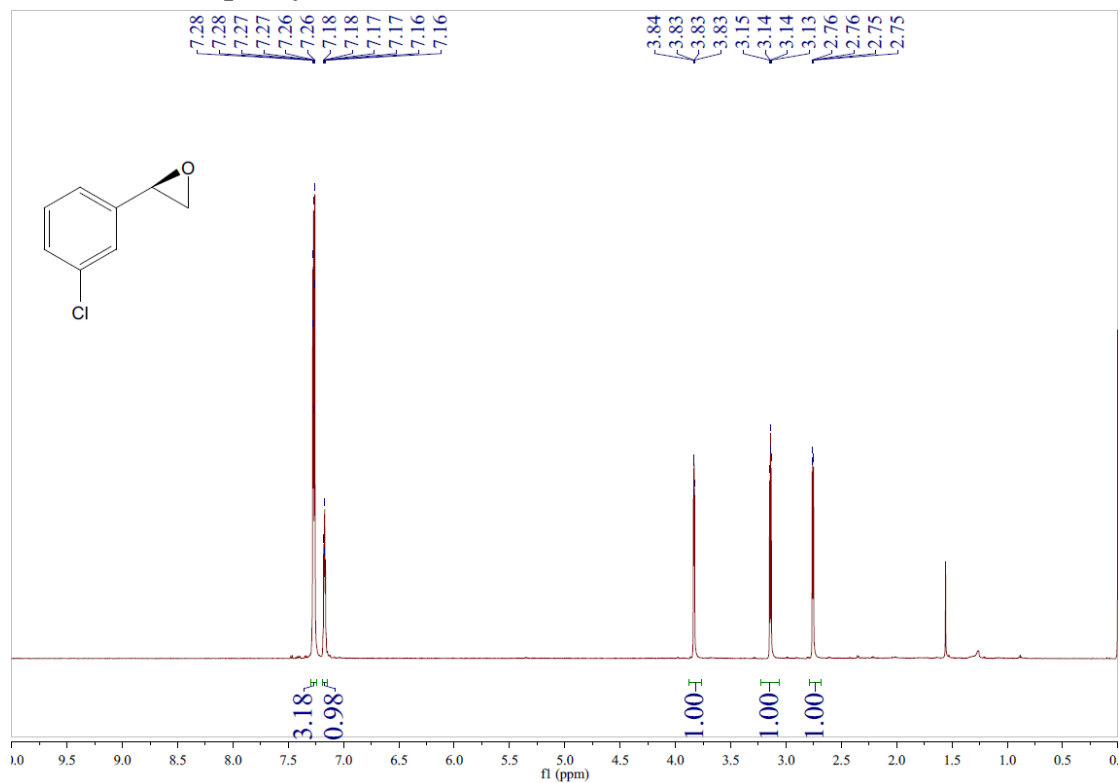
Chiralpak AD-H, *n*-hexane: 2-propanol 99: 1, 0.5 mL/min

(R)-2-(2-Chlorophenyl)oxirane (5b)



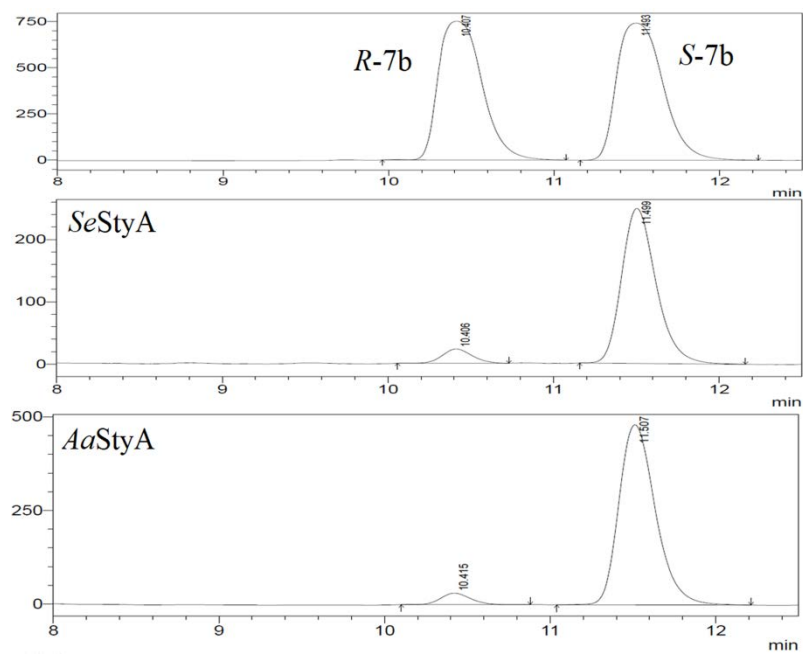
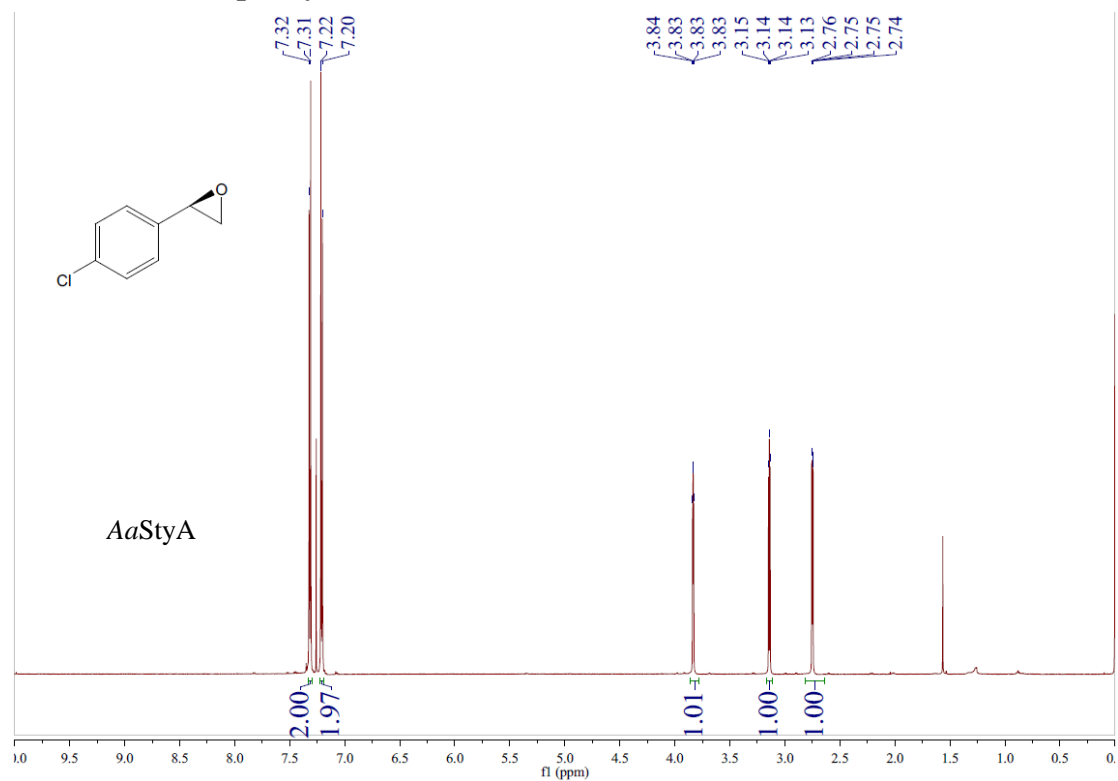
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(R)-2-(3-Chlorophenyl)oxirane (6b)



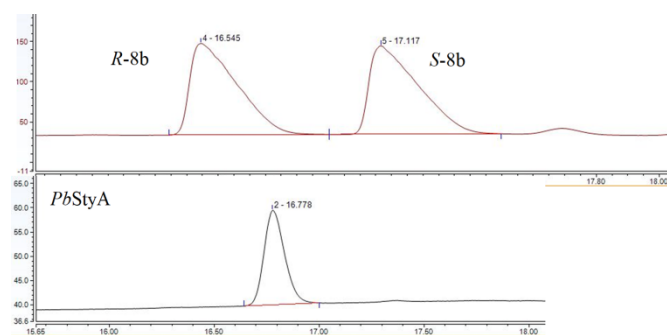
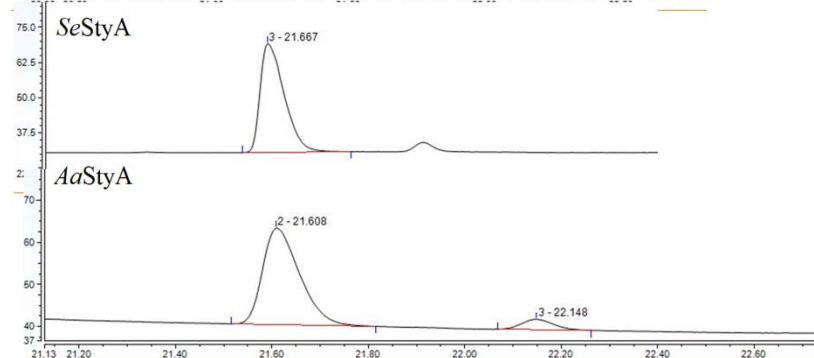
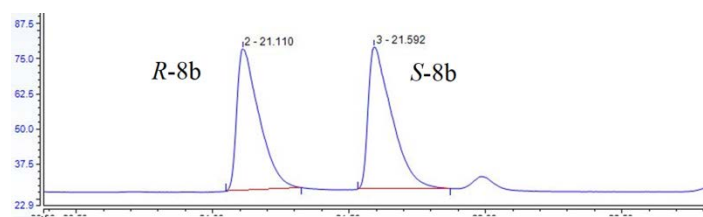
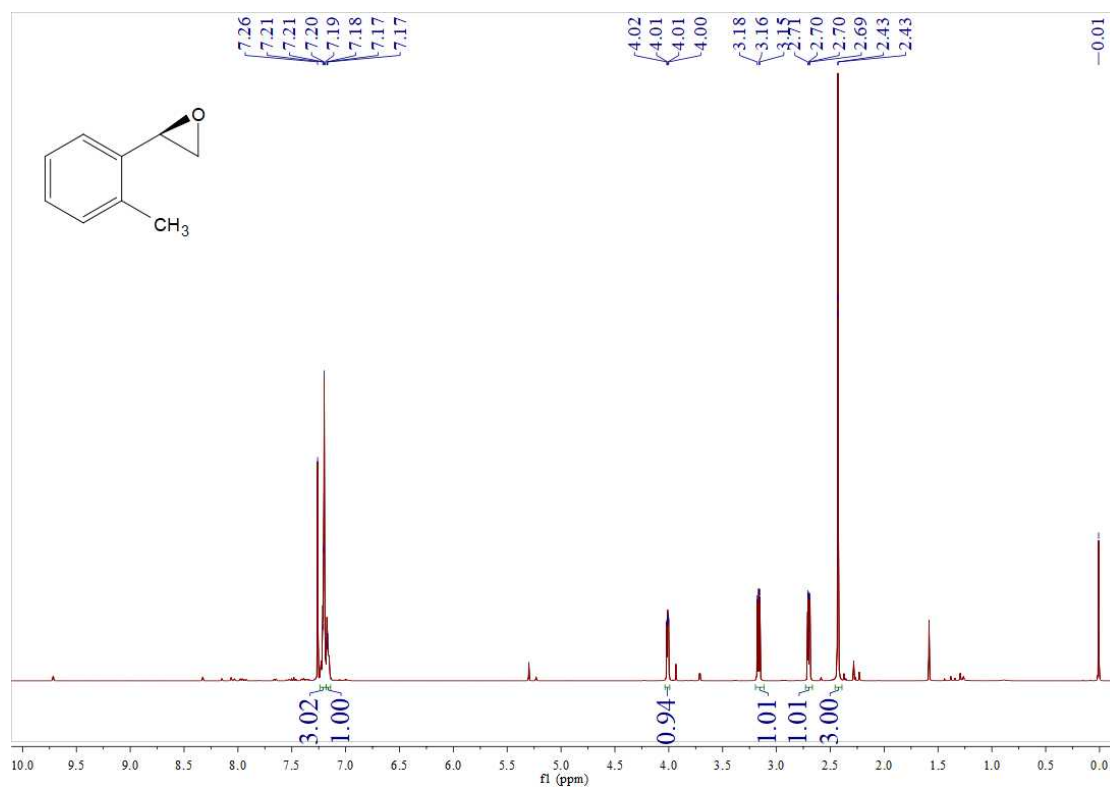
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(S)-2-(4-Chlorophenyl)oxirane (7b)



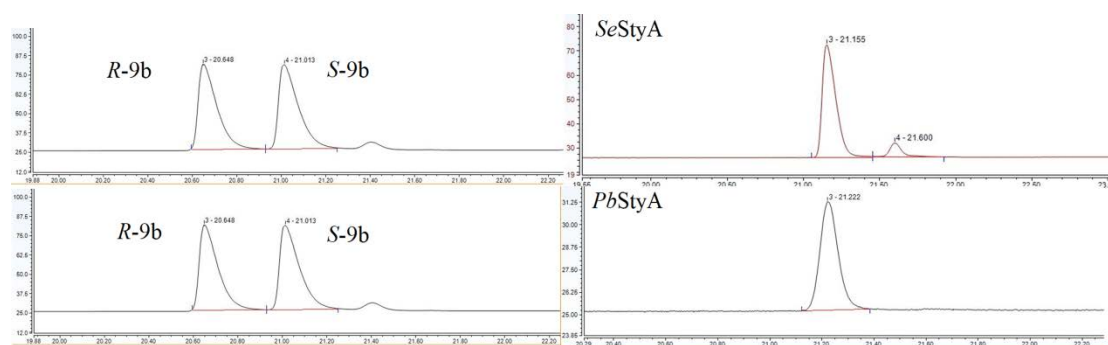
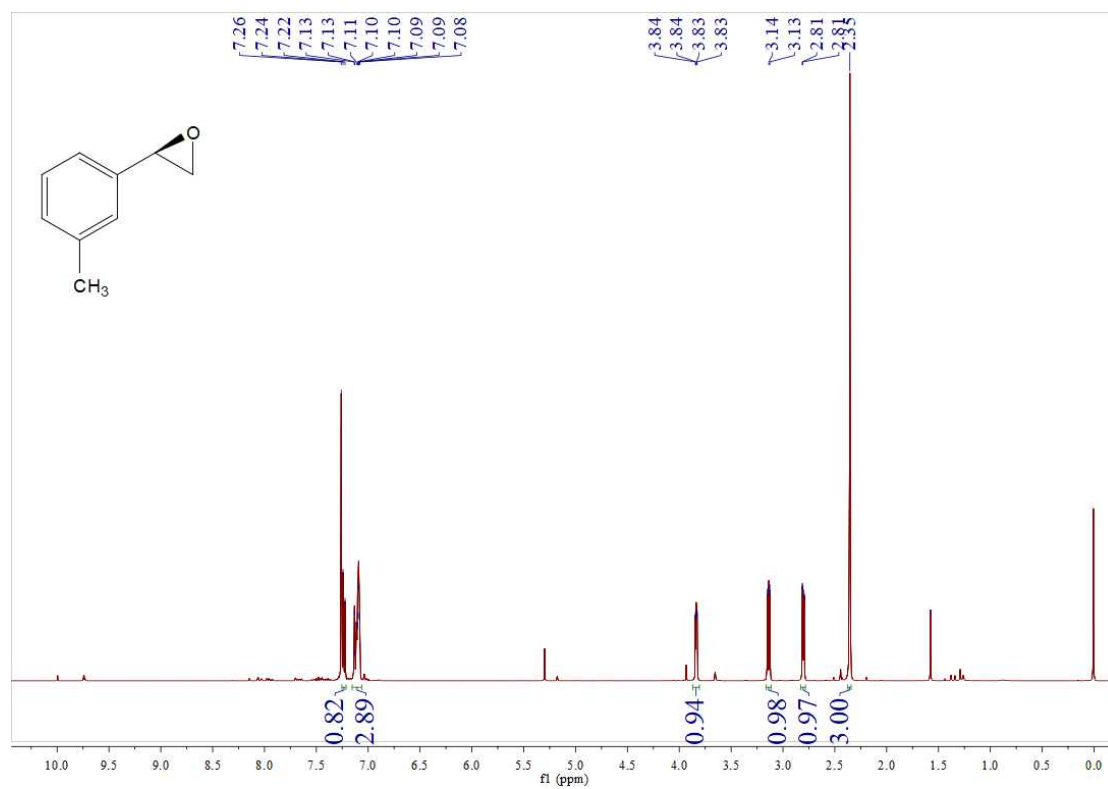
Chiralpak AS-H, *n*-hexane: 2-propanol 90: 10, 0.5 mL/min

(R)-2-(2-tolyl)oxirane (8b)

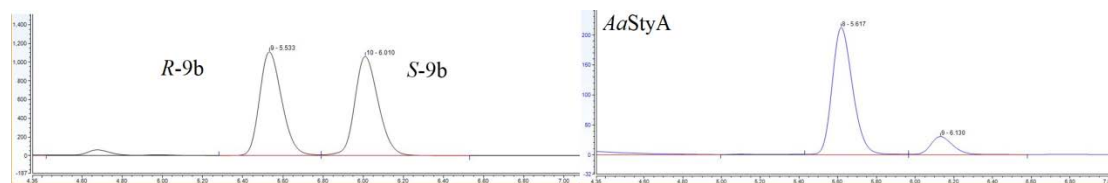


Chiral GC, CHIRASIL-DEX CB column

(R)-2-(3-tolyl)oxirane (9b)

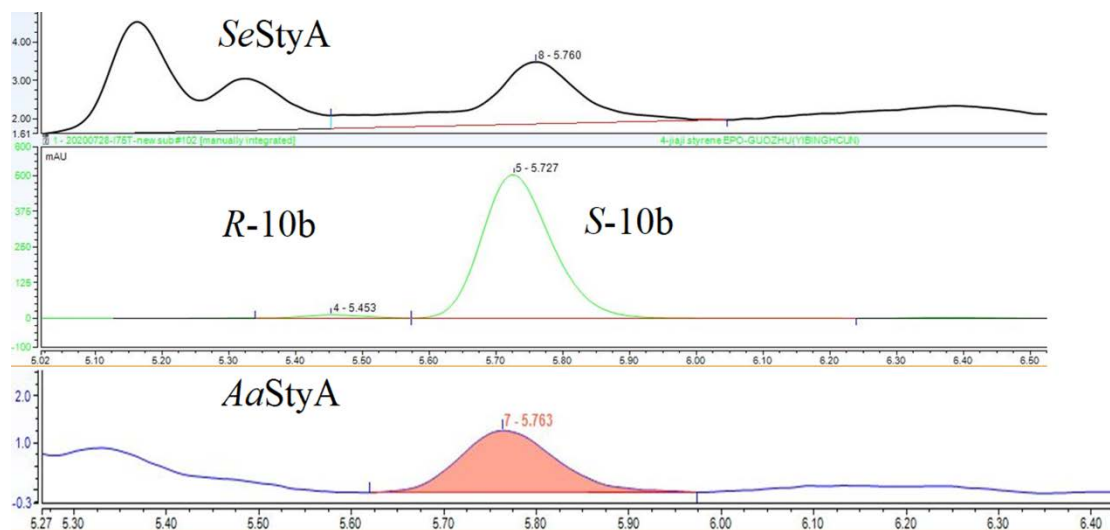
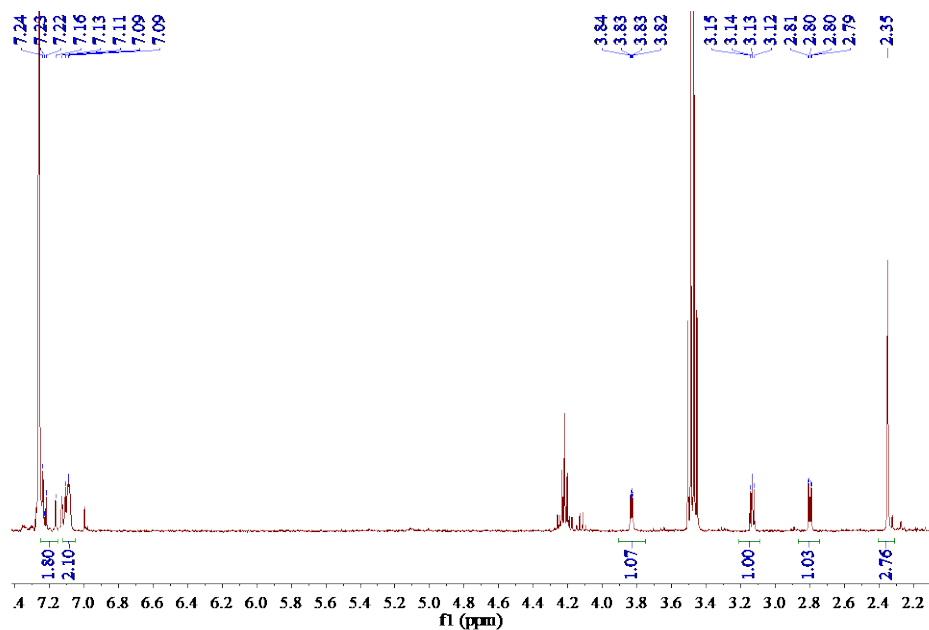


Chiral GC, CHIRASIL-DEX CB column



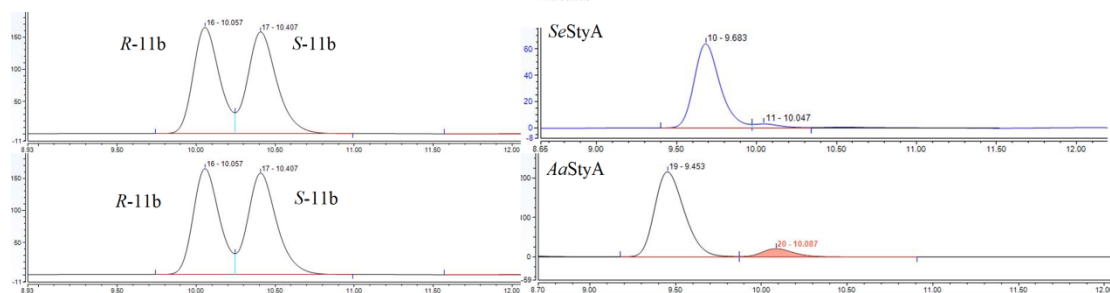
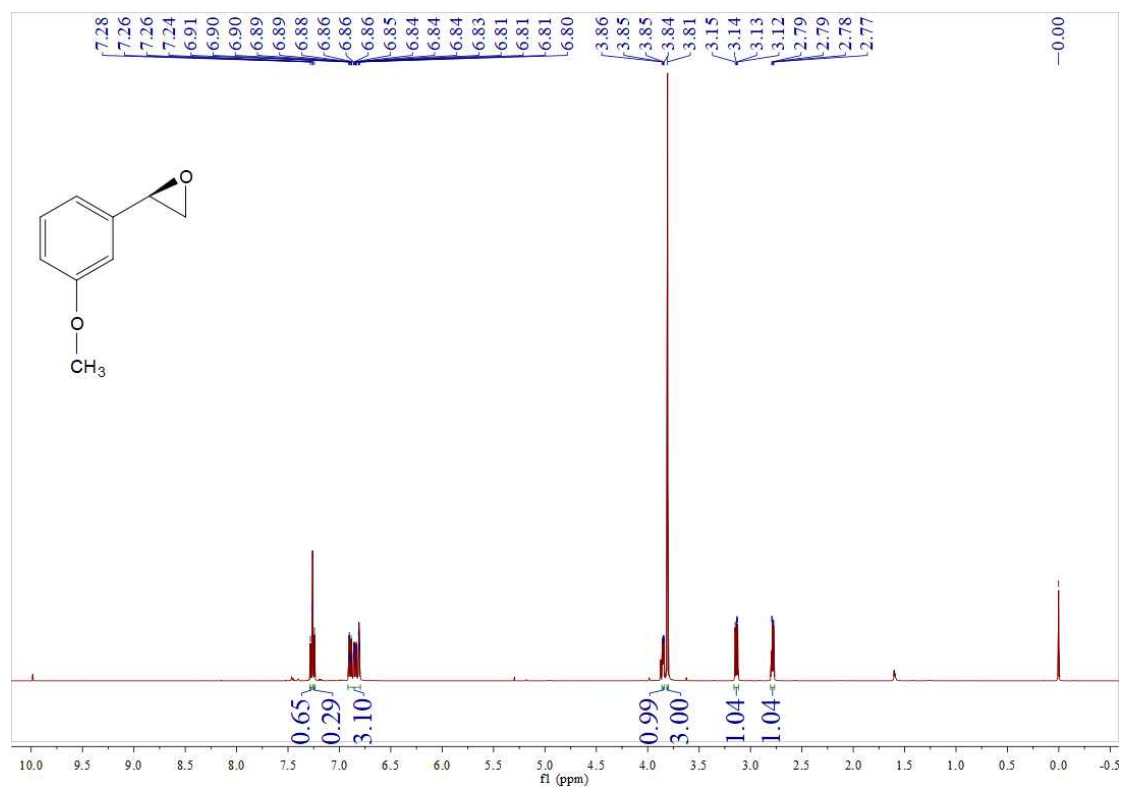
Chiralpak AS-H, *n*-hexane: 2-propanol 99: 1, 1 mL/min

(S)-2-(4-tolyl)oxirane (10b)

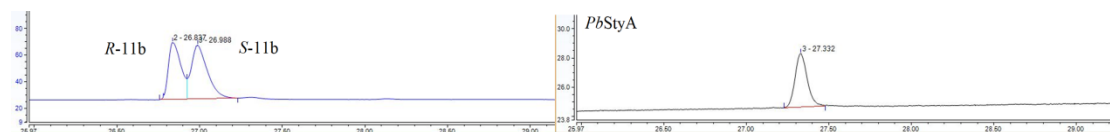


Chiralpak AS-H, *n*-hexane: 2-propanol 90: 10, 1 mL/min

(R)-2-(3-methoxyphenyl)oxirane (11b)

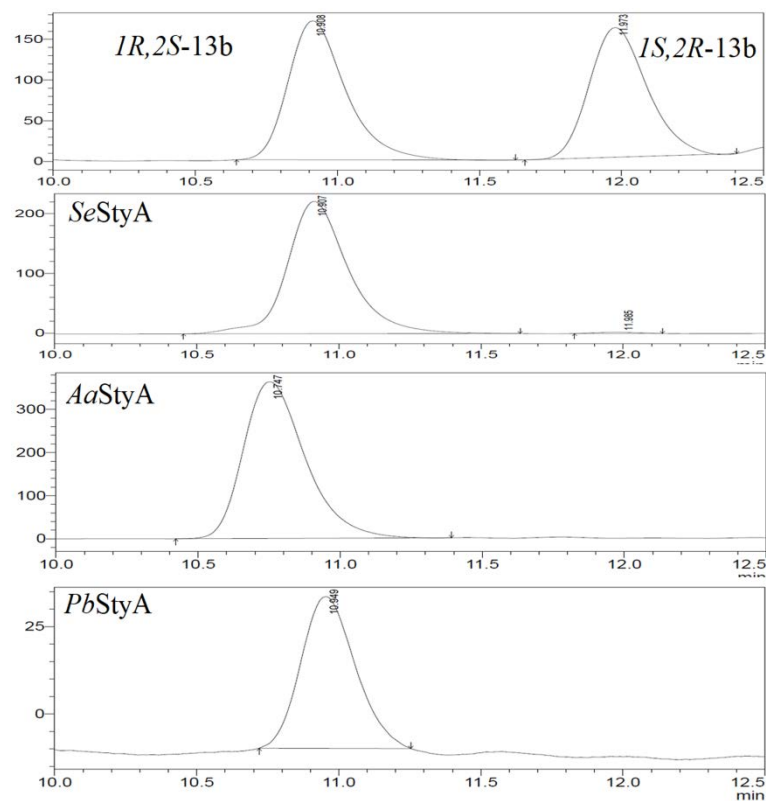
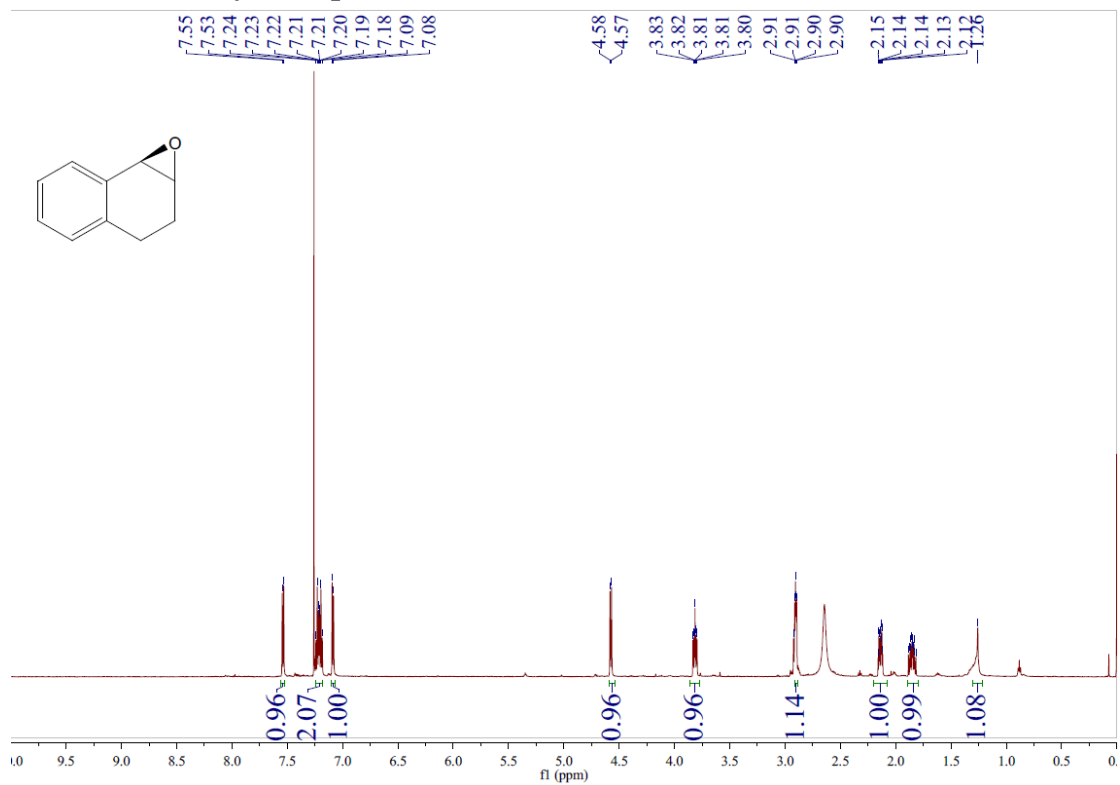


Chiralpak AS-H, *n*-hexane: 2-propanol 99: 1, 1 mL/min;



Chiral GC, CHIRASIL-DEX CB column

(1*R*,2*S*)-1,2-dihydronaphthalene oxide (13b)



Chiralpak AS-H, *n*-hexane: 2-propanol 90: 10, 0.5 mL/min

1. H. Lin, J. Qiao, Y. Liu and Z.-L. Wu, *Journal of Molecular Catalysis B: Enzymatic*, 2010, **67**, 236-241.
2. J. B. Park, B. Bühler, T. Habicher, B. Hauer, S. Panke, B. Witholt and A. Schmid, *Biotechnol. Bioeng.*, 2006.
3. M. H. Eppink, W. J. V. Berkel and H. A. Schreuder, *Protein Sci.*, 1997, **6**, 2454-2458.
4. U. E. Ukaegbu, A. Kantz, M. Beaton, G. T. Gassner and A. C. Rosenzweig, *Biochemistry*, 2010, **49**, 1678-1688.