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. ^{1}H NMR spectra were recorded on a Brucker-600 (600/150 MHz) spectrometer in $CDCl_3$.

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, $CHCl_3$) {$[\alpha]_D^{25}$ = +21.1，(83 mM, $CHCl_3$) for 99% ee, (*S*)}[2]; ^{1}H NMR (600 MHz, $CDCl_3$): 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); ^{1}H NMR (600 MHz, $CDCl_3$): 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); ^{1}H NMR (600

MHz, $CDCl_3$): 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); ^{1}H NMR (600 MHz, $CDCl_3$): 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); ^{1}H NMR (600 MHz, $CDCl_3$): 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); ^{1}H NMR (600 MHz, $CDCl_3$): 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); ^{1}H NMR (600 MHz, $CDCl_3$): 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); ^{1}H NMR (400 MHz, $CDCl_3$): 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); ^{1}H 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_R(*R*) 5.5 min, t_R(*S*) 5.7 min, AS-H, *n*-Hexane: 2-propanol = 90: 10, 1 mL/min), ^{1}H 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_{R1}(*R*) 10.1 min, t_{R1}(*S*) 10.4 min (Chiralpak AS-H, *n*-hexane: 2-propanol 99: 1, 1 mL/min); t_{R2}(*R*) 26.8 min, t_{R2}(*S*) 27.0 min (Chiral GC, CHIRASIL-DEX CB column); ^{1}H 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).

(1*R*,2*S*)-1,2-dihydronaphthalene oxide(13b): white solid; retention time: t_R(1*R*,2*S*) 10.9 min, t_R(1*S*,2*R*) 11.9 min (Chiralpak AS-H, *n*-hexane: 2-propanol 90: 10, 0.5 mL/min); ^{1}H 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'-TATACTAGTTCACTCCTCAATGGATTCAG-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'-TATACTAGTTTAACGGTATTCAGACAGGAAC-3'
WP 067345360.1	5'-TAATTACATATGACCAGCGTGGGC-3' 5'-TATACTAGTTTAACGTTGACGCGGACC-3'
WP_073449381.1	5'-TAATTACATATGACCAGCGTGGGC-3' 5'-TATACTAGTTTAACCTTGACGCGGACC-3'
WP_102925532.1	5'-TAATTACATATGACCAGCGTGGGC-3' 5'-TATACTAGTTTAACCTTGACGCGGACC-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'-TATACTAGTTTAACCCGCCTCGGTGAT-3'
WP_141293187.1	5'-TAATTACATATGAGCGACATCGCGATTG-3' 5'-TATACTAGTTTAACCCGCCTCGGTGAT-3'

Table S2. Primers for site-directed mutagenesis.

Mutant	Nucleotide sequence
*Aa*StyA F46T	5'-CGGCCGCGGAACACCCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGGGTGTTCCGCGGCCG-3'
*Pb*StyA F46T	5'-GTGGGCCACGCAACACCCCGGTCCGATTCGG-3' 5'-CCGAATCGGACCGGGGTGTTGCGTGGCCCAC-3'
*Se*StyA F56T	5'-CGGCCGCGGAACACCCCCGCCCGGTTC-3' 5'-GAACCGGGCGGGGGTGTTCCGCGGCCG-3'
*Ps*StyA T47F	5'-GCCTTCGGCTGCTGAACTTTGTGGCTCATCACGCGG-3' 5'-CCGCGTGATGAGCCACAAAGTTCAGCAGCCGAAGGC-3'
*Aa*StyA F46H	5'-CGGCCGCGGAACCATCCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGATGGTTCCGCGGCCG-3'
*Aa*StyA F46R	5'-CGGCCGCGGAACCGTCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGACGGTTCCGCGGCCG-3'
*Aa*StyA F46K	5'-GCGGCCGCGGAACAAACCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGTTTGTTCCGCGGCCGC-3'
*Aa*StyA F46I	5'-GCGGCCGCGGAACATTCCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGAATGTTCCGCGGCCGC-3'
*Aa*StyA F46L	5'-CGGCCGCGGAACCTGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCAGGTTCCGCGGCCG-3'
*Aa*StyA F46W	5'-GCCGCGGAACTGGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCCAGTTCCGCGGC-3'
*Aa*StyA F46A	5'-GGCCGCGGAACGCGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCGCGTTCCGCGGCC-3'
*Aa*StyA F46M	5'-CGGCCGCGGAACATGCCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGCATGTTCCGCGGCCG-3'
*Aa*StyA F46P	5'-GGCCGCGGAACCCGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCGGGTTCCGCGGCC-3'
*Aa*StyA F46C	5'-GGCCGCGGAACTGCCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGGCAGTTCCGCGGCC-3'
*Aa*StyA F46N	5'-GCGGCCGCGGAACAATCCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGATTGTTCCGCGGCCGC-3'
*Aa*StyA F46V	5'-CGGCCGCGGAACGTGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCACGTTCCGCGGCCG-3'
*Aa*StyA F46G	5'-CGGCCGCGGAACGGCCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGGCCGTTCCGCGGCCG-3'
*Aa*StyA F46S	5'-CGGCCGCGGAACAGCCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGGCTGTTCCGCGGCCG-3'
*Aa*StyA F46Q	5'-CGGCCGCGGAACCAGCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGCTGGTTCCGCGGCCG-3'
*Aa*StyA F46Y	5'-CGGCCGCGGAACTATCCGGCGCGGTTC-3' 5'-GAACCGCGCCGGATAGTTCCGCGGCCG-3'
*Aa*StyA F46D	5'-CGGCCGCGGAACGATCCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGATCGTTCCGCGGCCG-3'
*Aa*StyA F46E	5'-CGGCCGCGGAACGAACCGGCGCGGTTCG-3' 5'-CGAACCGCGCCGGTTCGTTCCGCGGCCG-3'

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

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

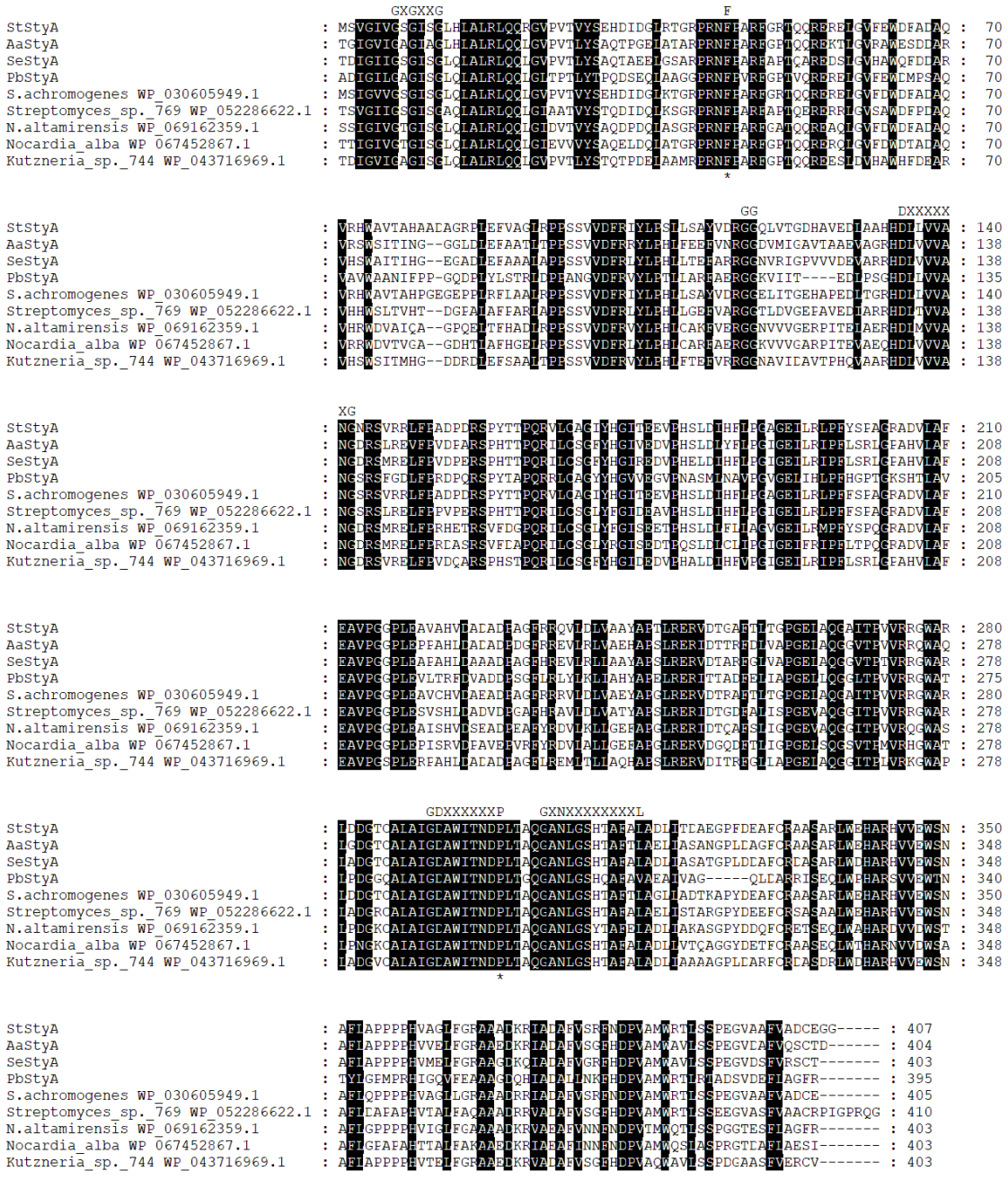


Figure S1. Multiple sequence alignment of (*R*)-selective SMOs.

Conserved domains are showed 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]

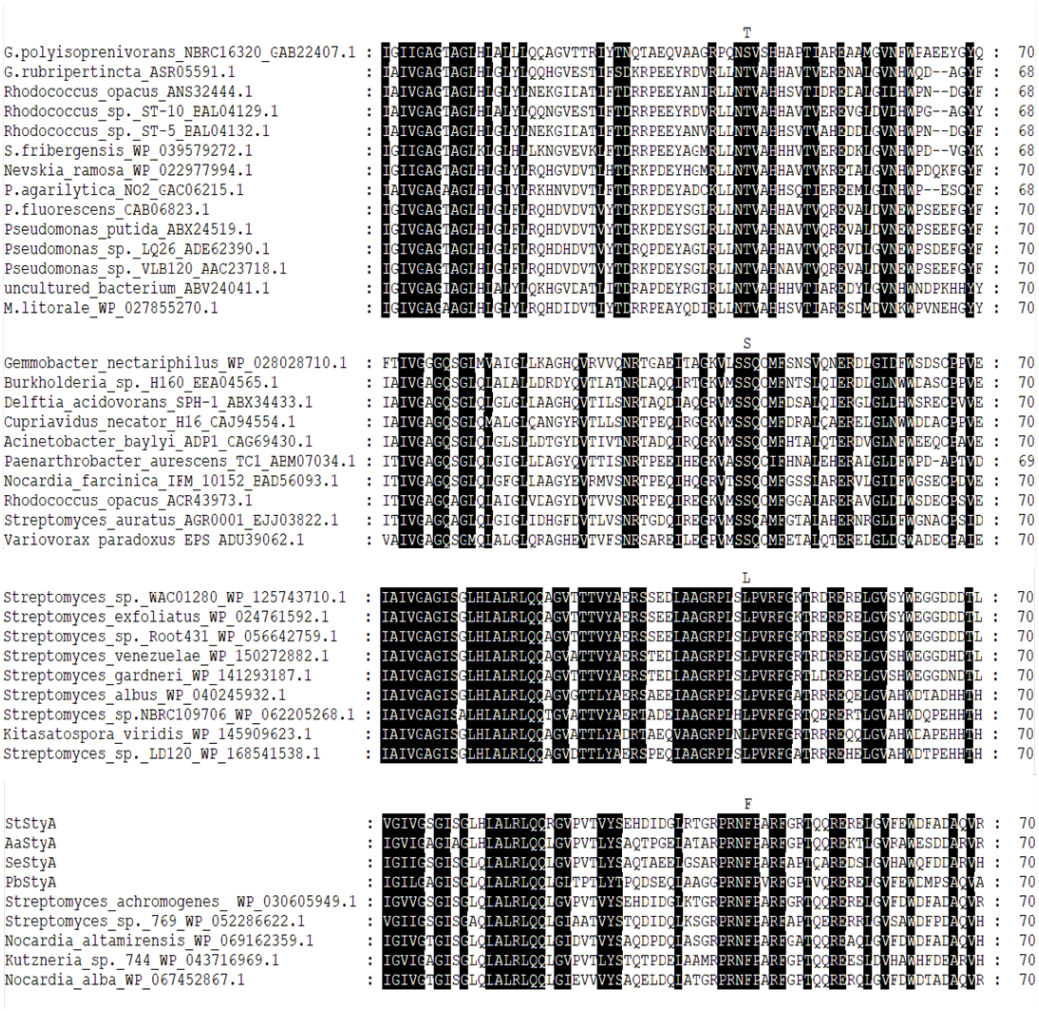


Figure S2. Sequence alignment of Groups I to IV SMOs at position 46.

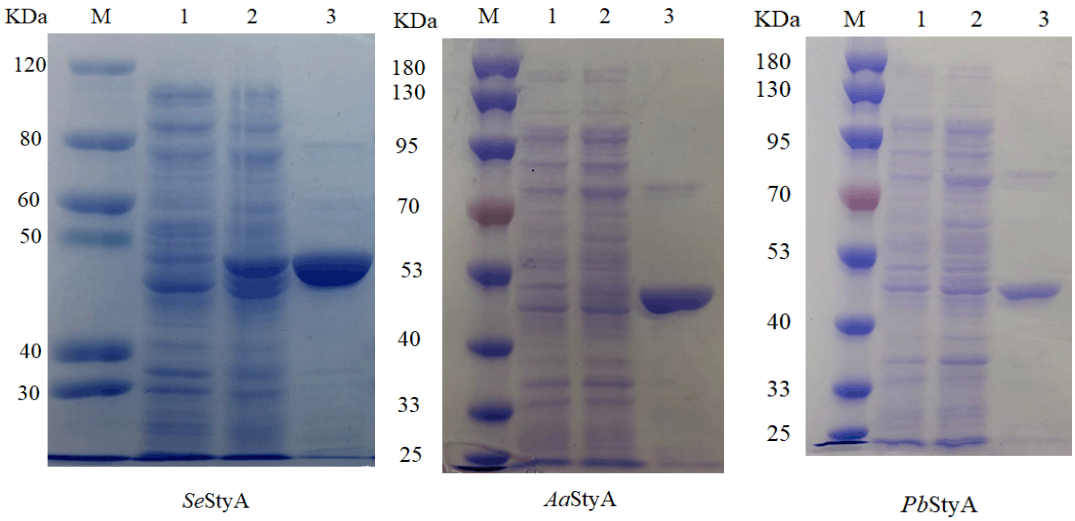


Figure S3. SDS-PAGE analysis of *Se*StyA, *Aa*StyA and *Pb*StyA.

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

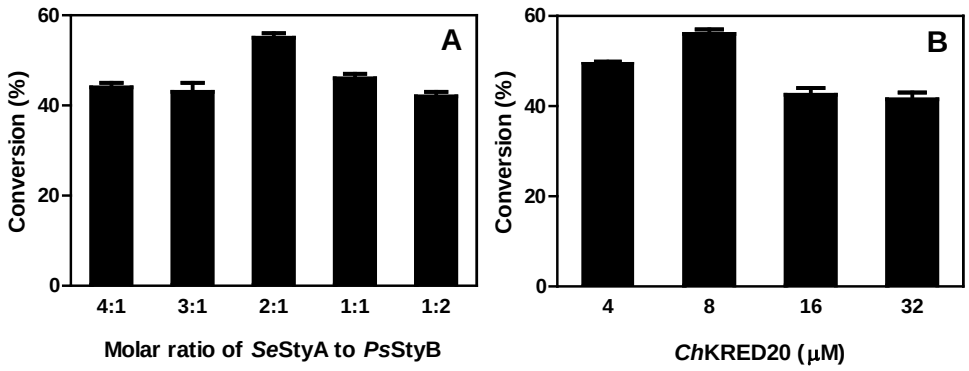


Figure S4. Optimizing reaction conditions for kinetic analysis.

A) The reaction mixture contained 5-40 μM of purified *St*StyA, 10 μM of purified *Ps*StyB, 0.5 mM NADH, 50 μM FAD and 650 U catalase B) The reaction mixture contained 20 μM of purified *St*StyA, 10 μM of purified *Ps*StyB, 4-32 μM of purified *Ch*KRED20, 0.1 mM NAD^+, 50 μM FAD and 650 U catalase.

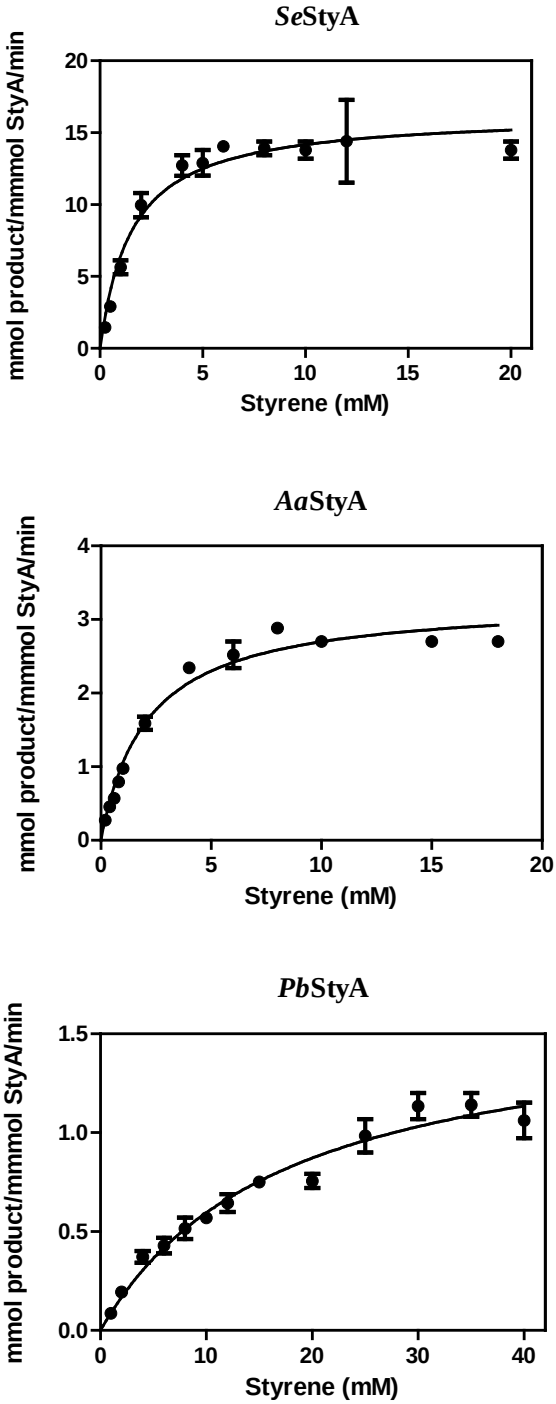
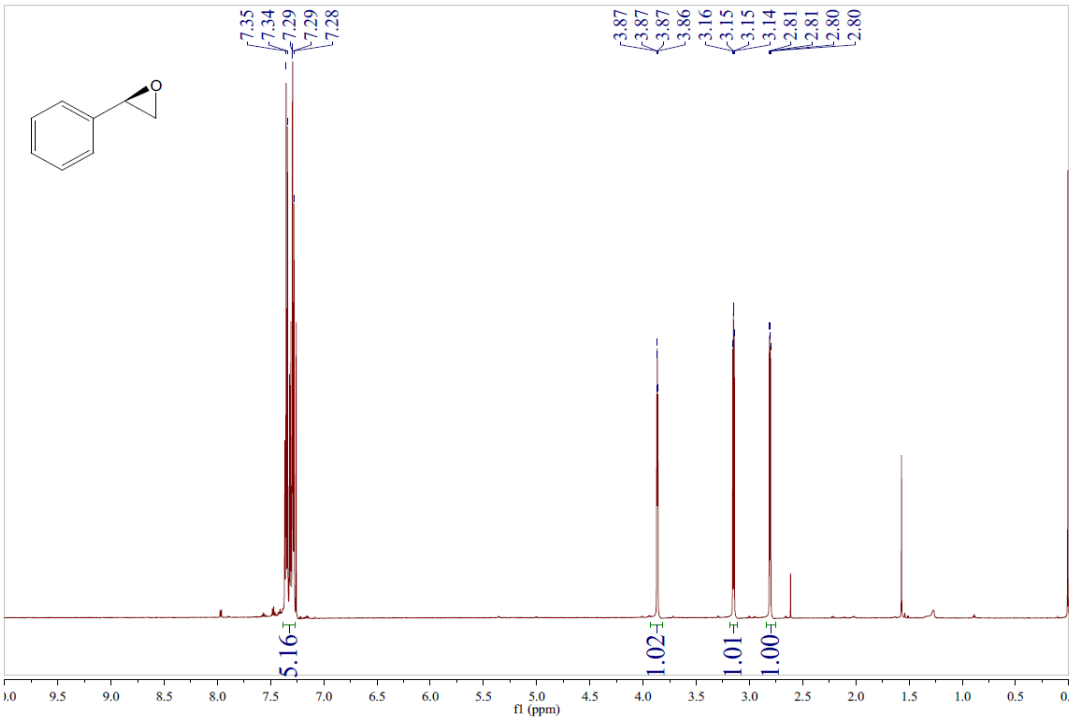
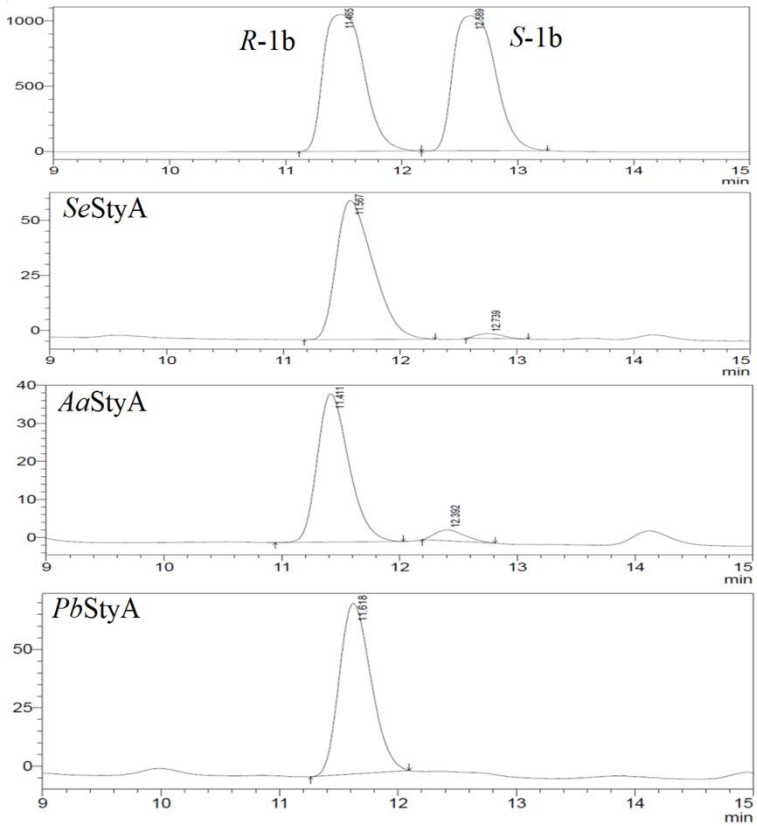


Figure S5. Steady-state kinetics of StyAs in the epoxidation of styrene.

^{1}H NMR spectra and HPLC chromatograms

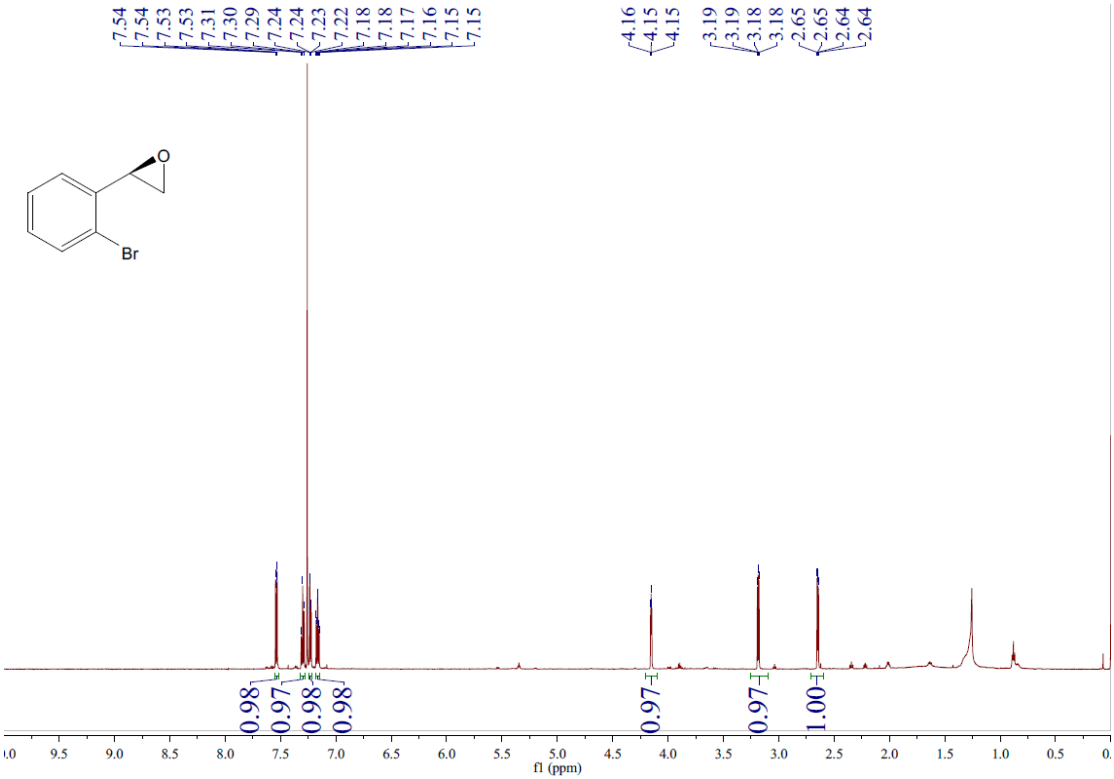
(*R*)-Styrene epoxide (1b)

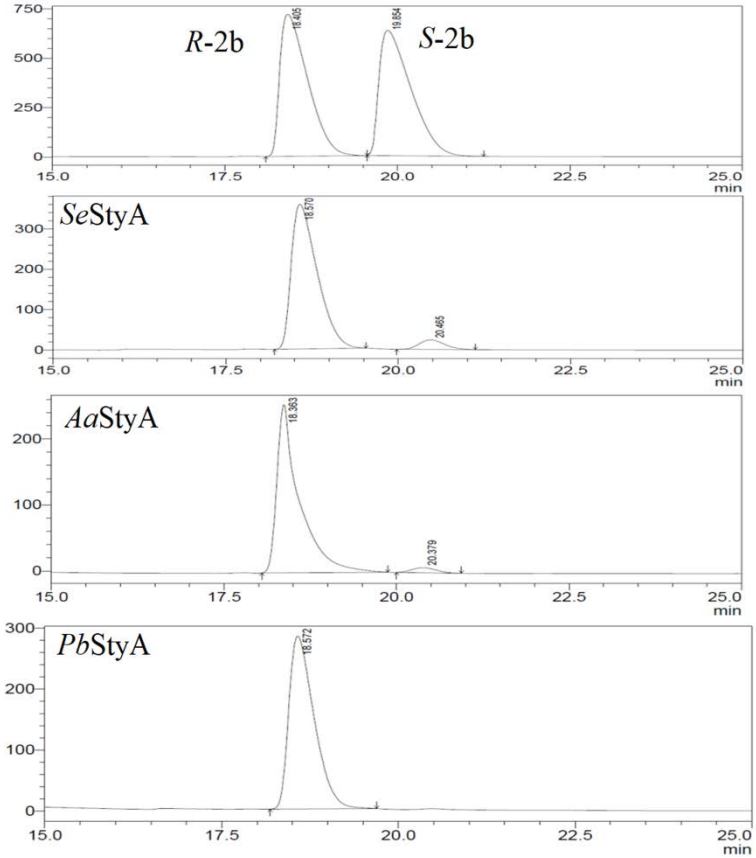




Chiralpak IC-H, *n*-Hexane: 2-propanol 98: 2, 0.5 mL/min

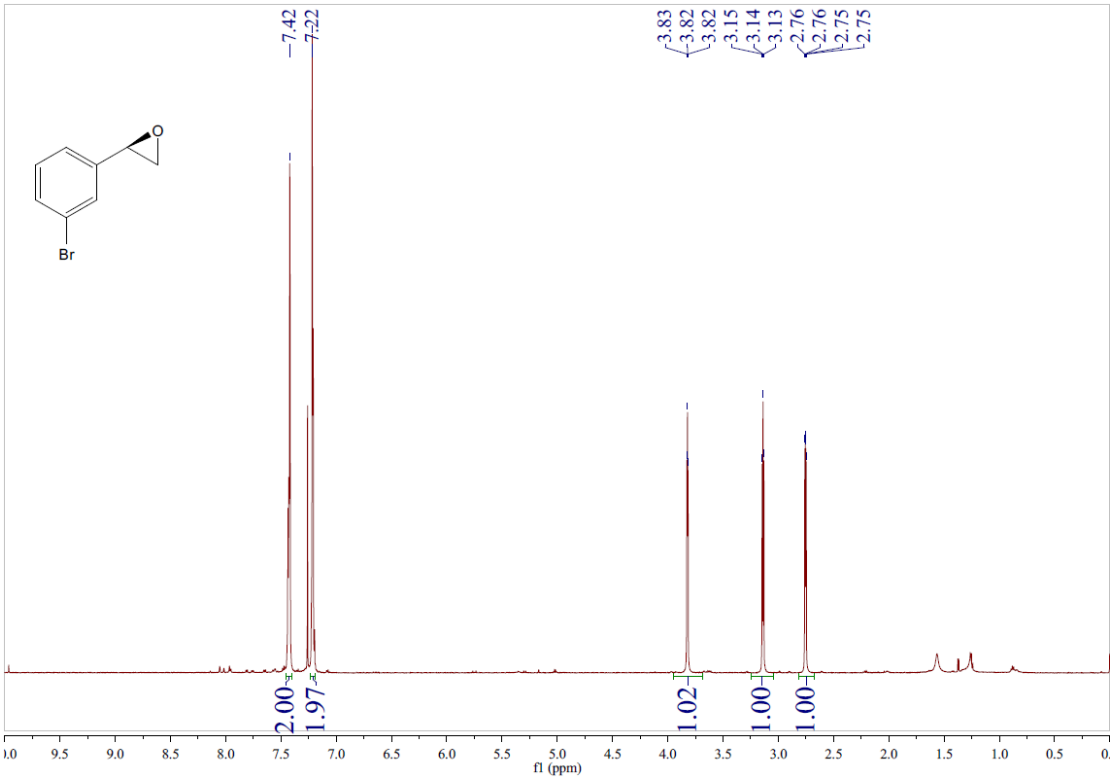
(*R*)-2-(2-bromophenyl)oxirane (2b)

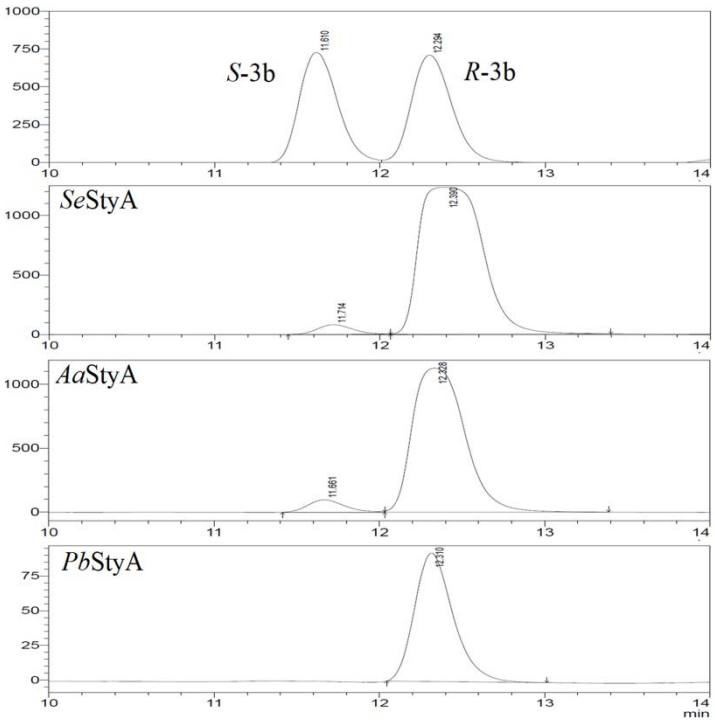




Chiralpak AS-H, *n*-Hexane: 2-propanol 90: 10, 0.5 mL/min

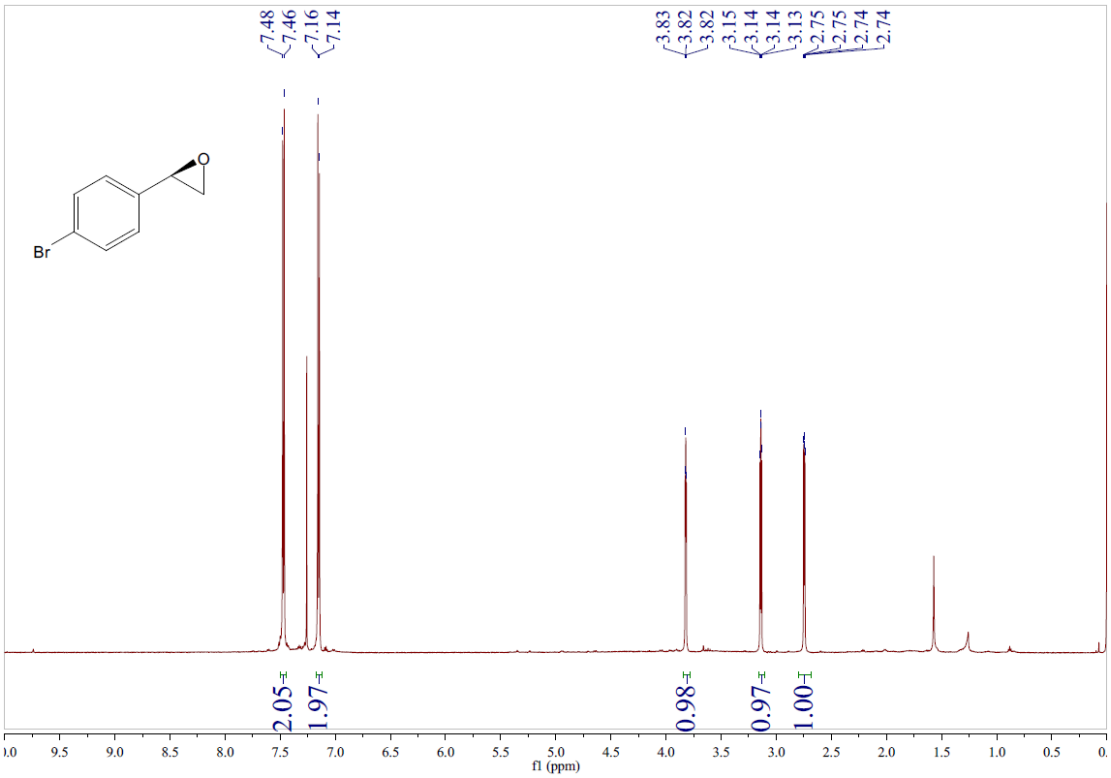
(*R*)-2-(3-bromophenyl)oxirane (3b)

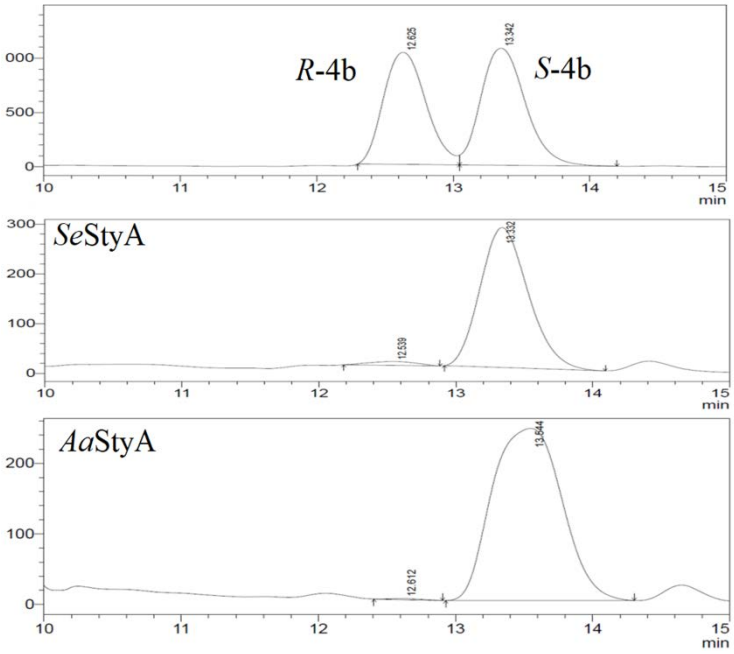




Chiralpak AD-H, *n*-hexane: 2-propanol 98: 2, 0.5 mL/min

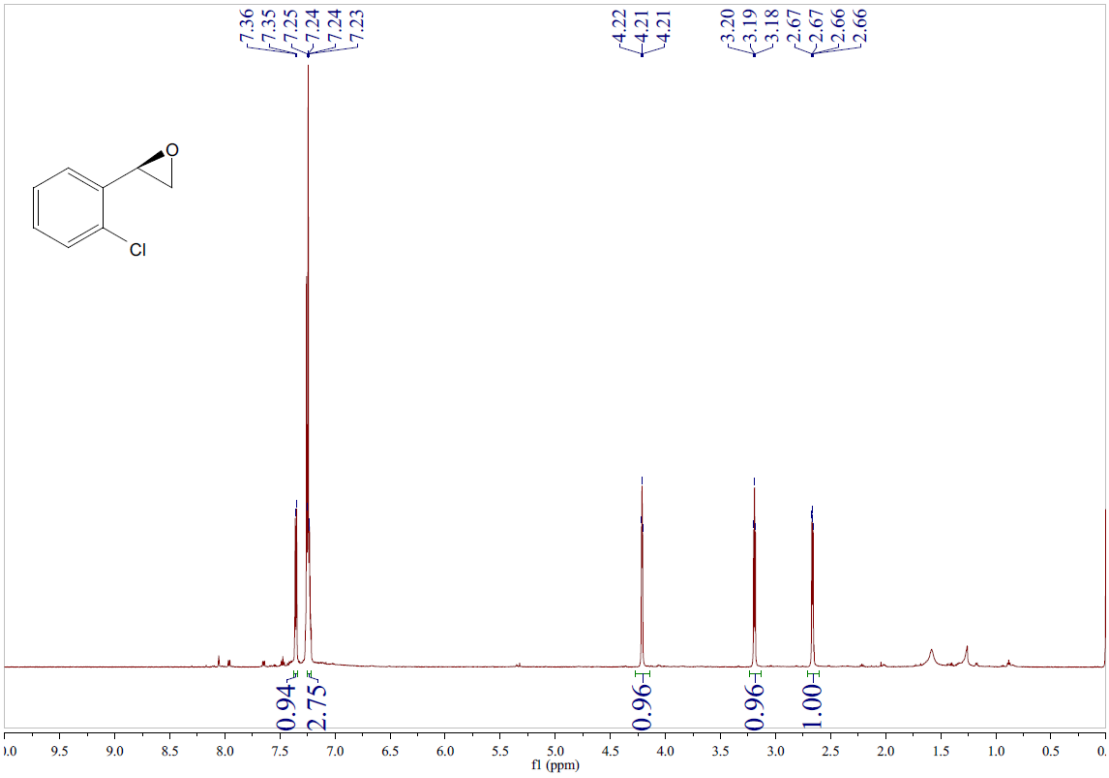
(*S*)-2-(4-bromophenyl)oxirane (4b)

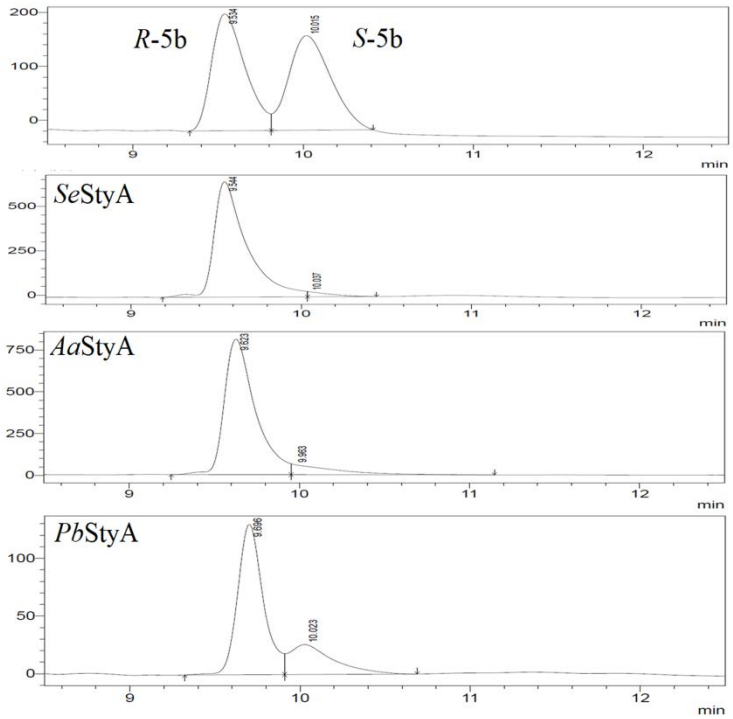




Chiralpak AD-H, *n*-hexane: 2-propanol 99: 1, 0.5 mL/min

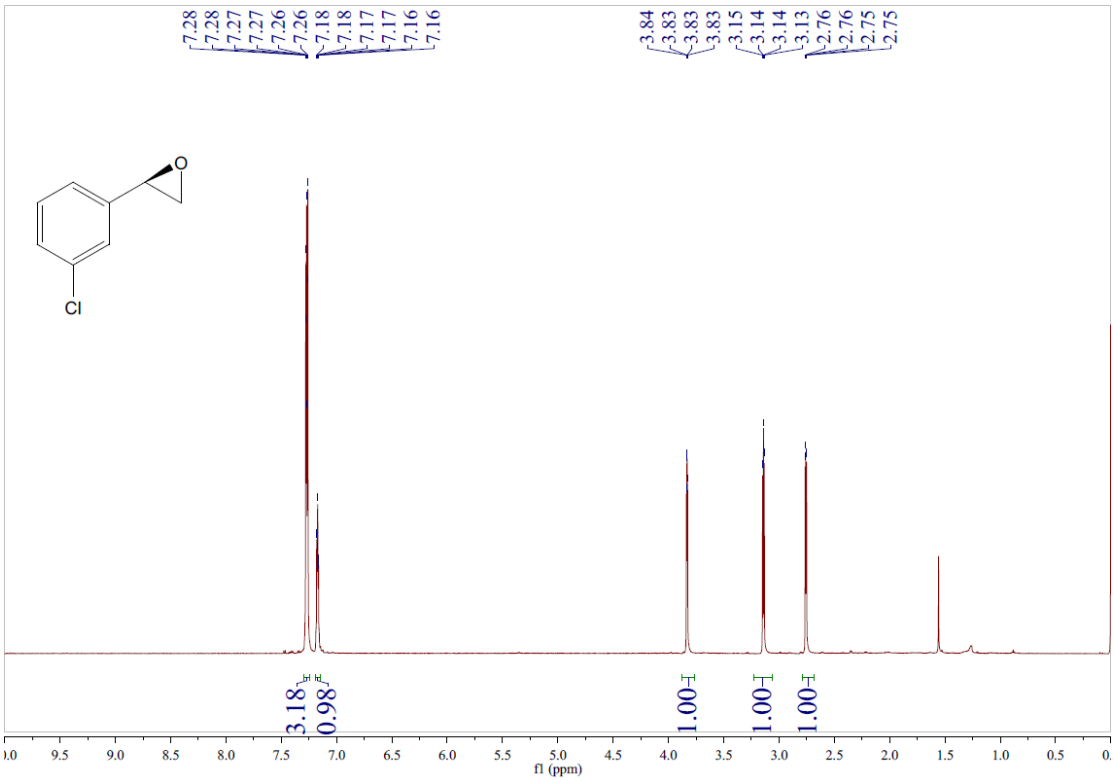
(*R*)-2-(2-Chlorophenyl)oxirane (5b)

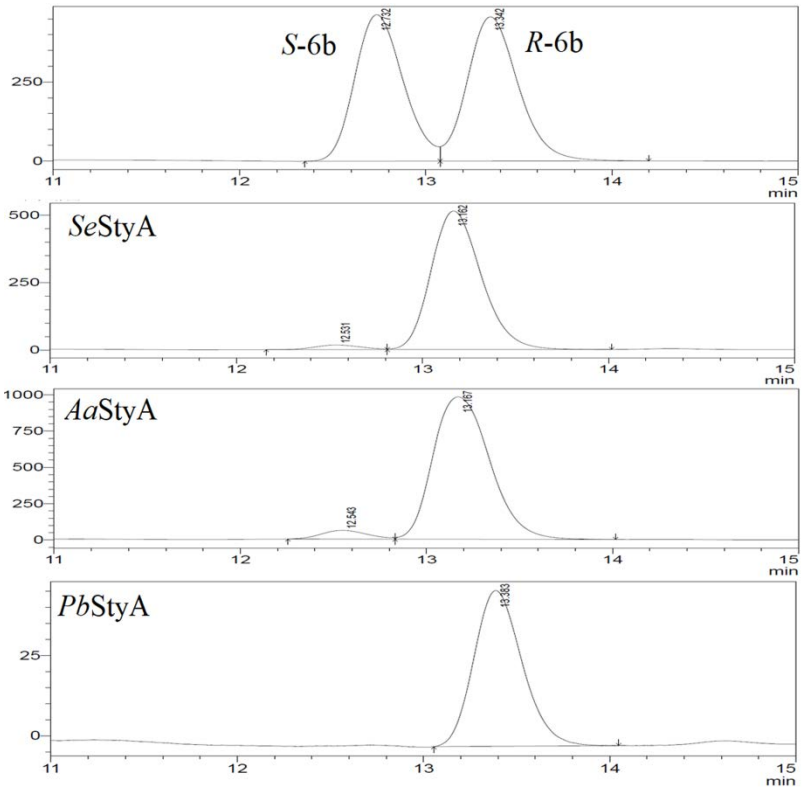




Chiralpak IC-H, *n*-hexane: 2-propanol 99: 1, 0.5 mL/min

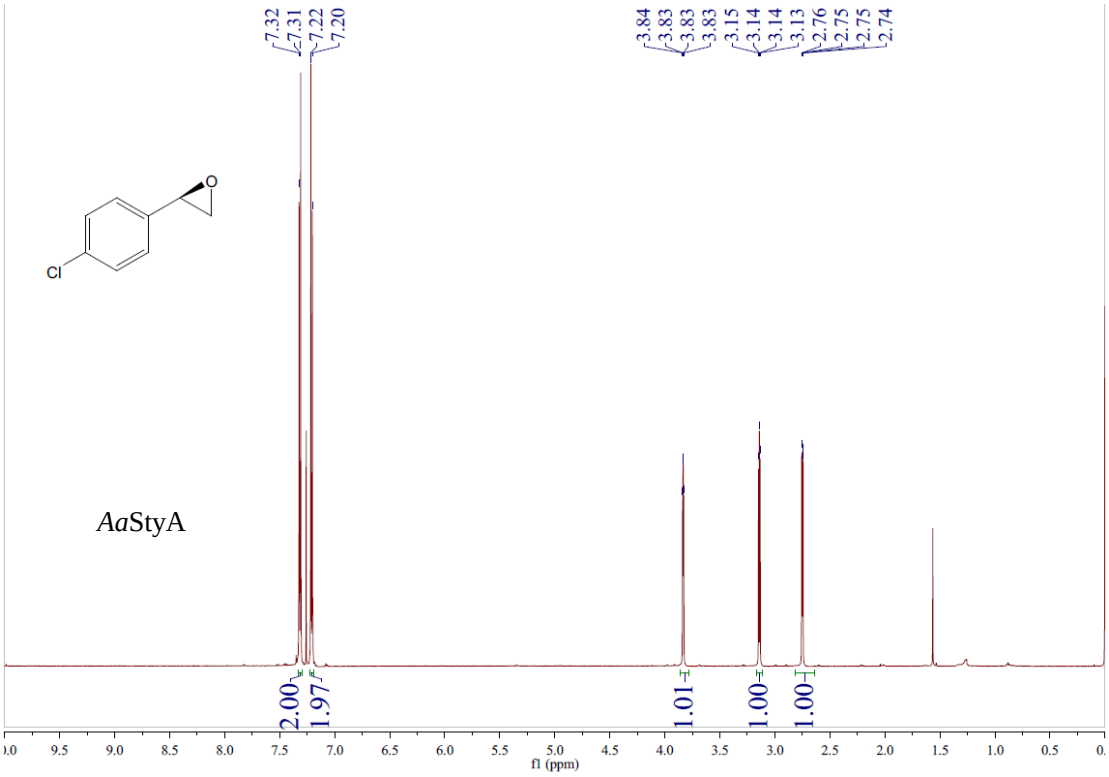
(*R*)-2-(3-Chlorophenyl)oxirane (6b)

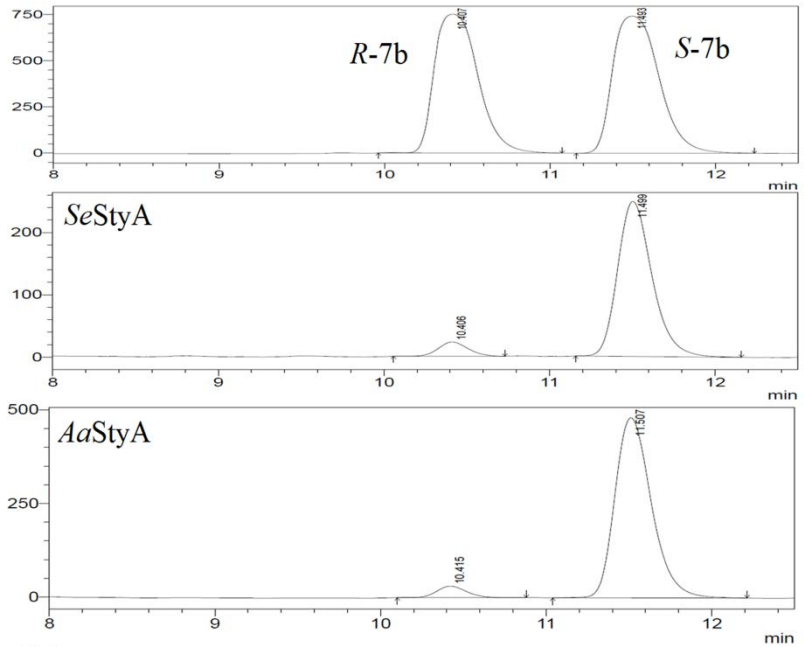




Chiralpak AD-H, *n*-hexane: 2-propanol 99: 1, 0.5 mL/min

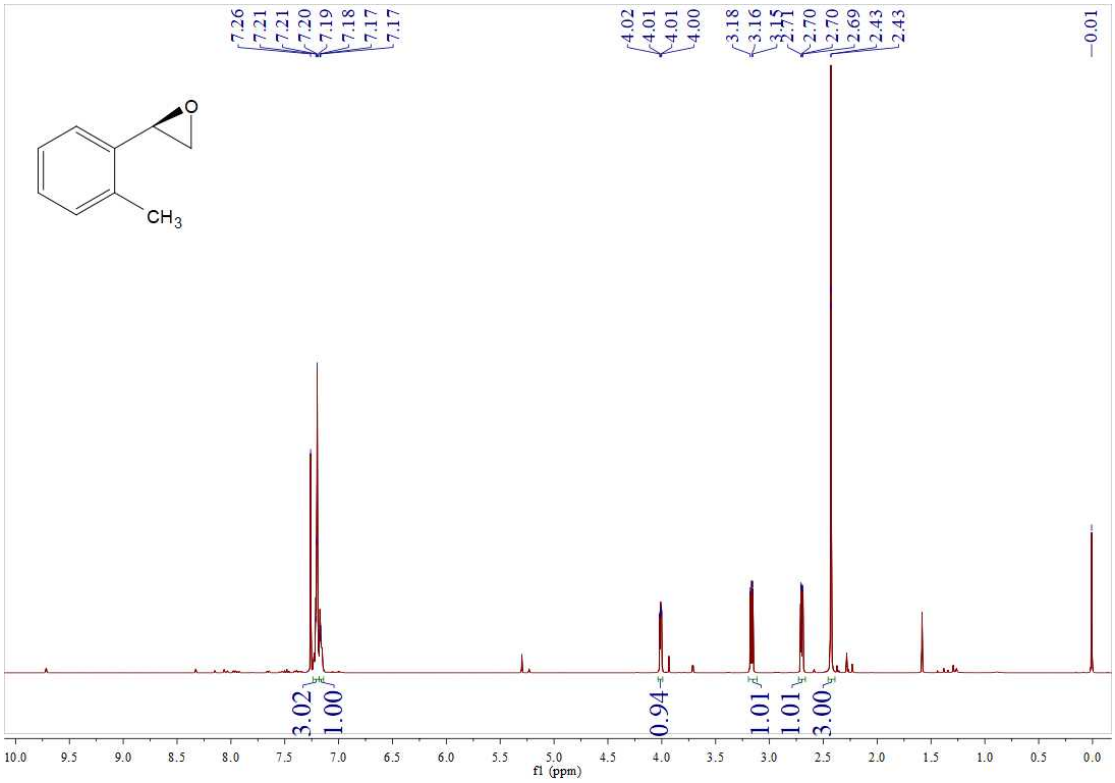
(*S*)-2-(4-Chlorophenyl)oxirane (7b)

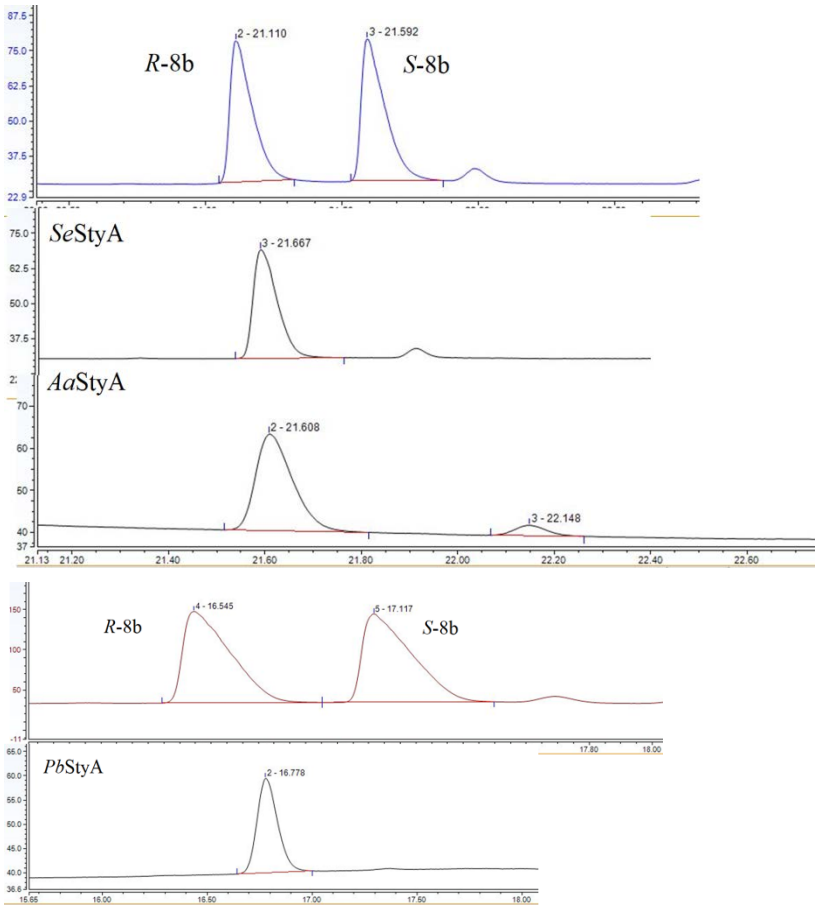




Chiralpak AS-H, *n*-hexane: 2-propanol 90: 10, 0.5 mL/min

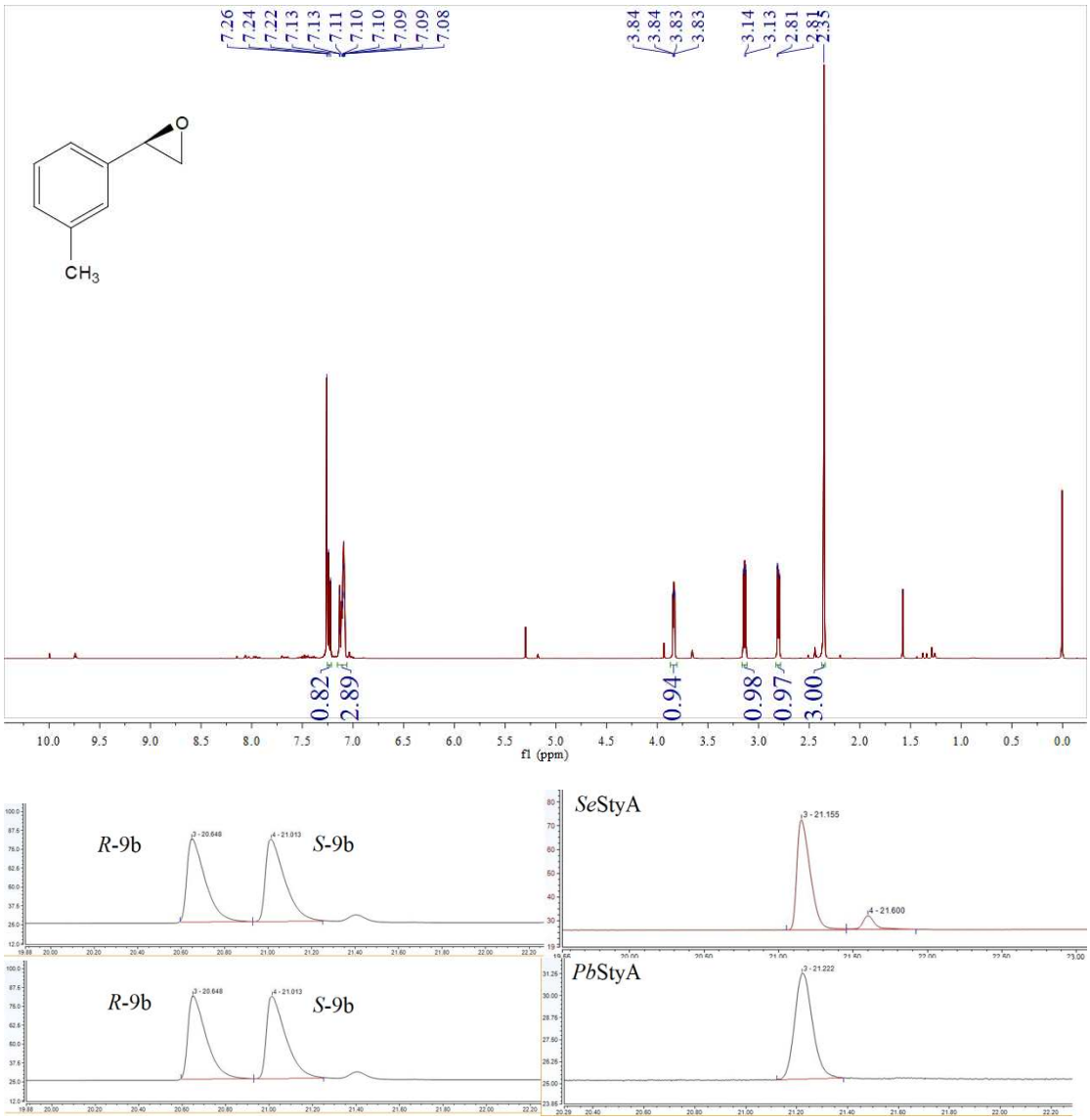
(*R*)-2-(2-tolyl)oxirane (8b)



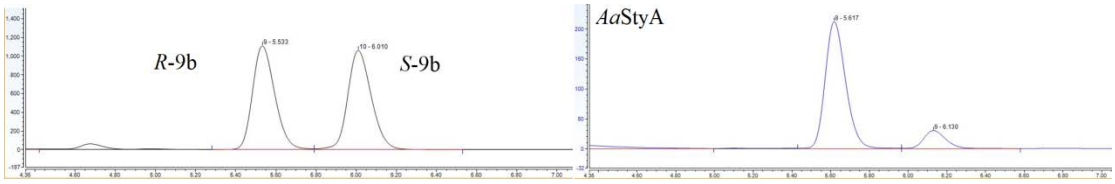


Chiral GC, CHIRASIL-DEX CB column

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

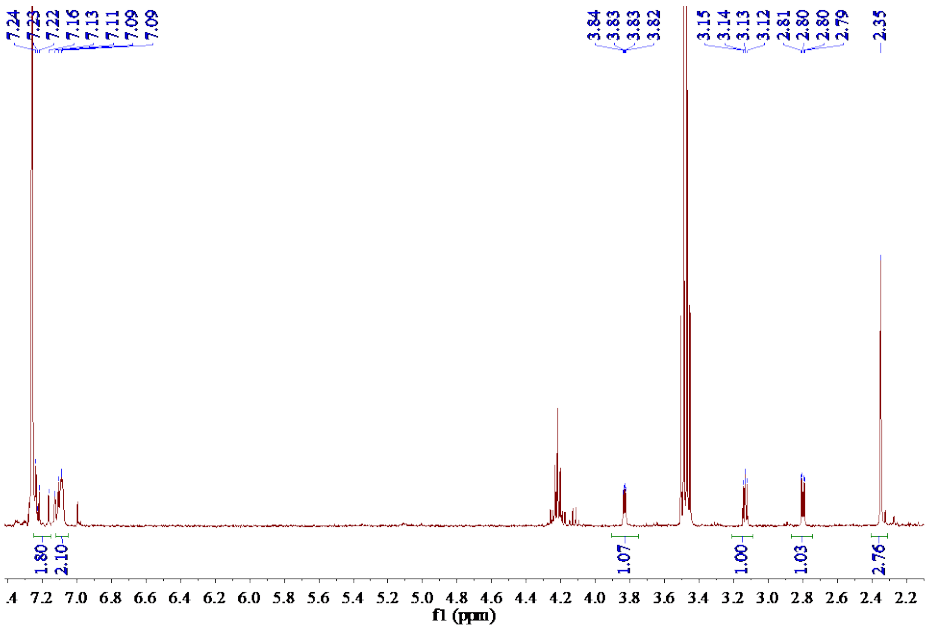


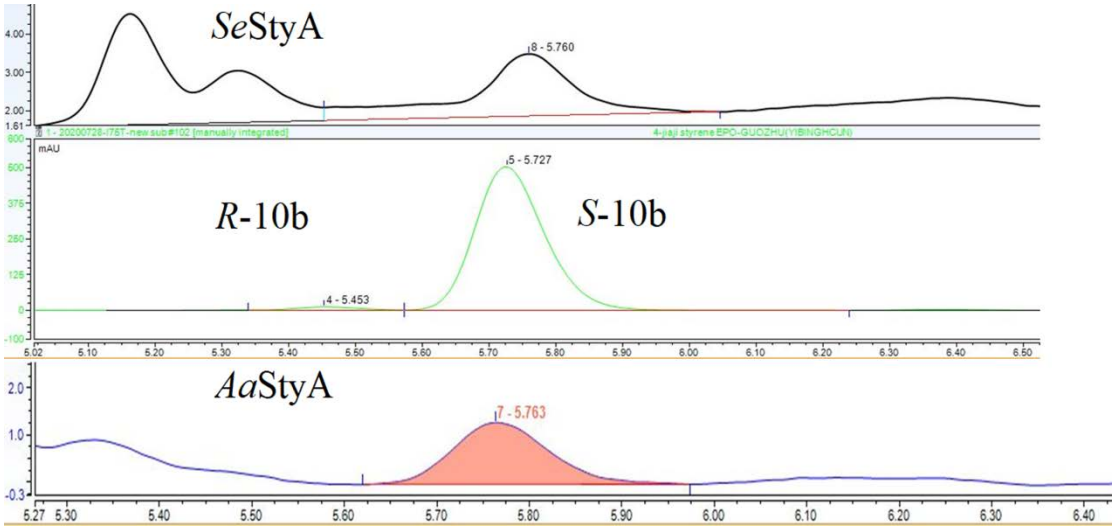
Chiral GC, CHIRASIL-DEX CB column



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

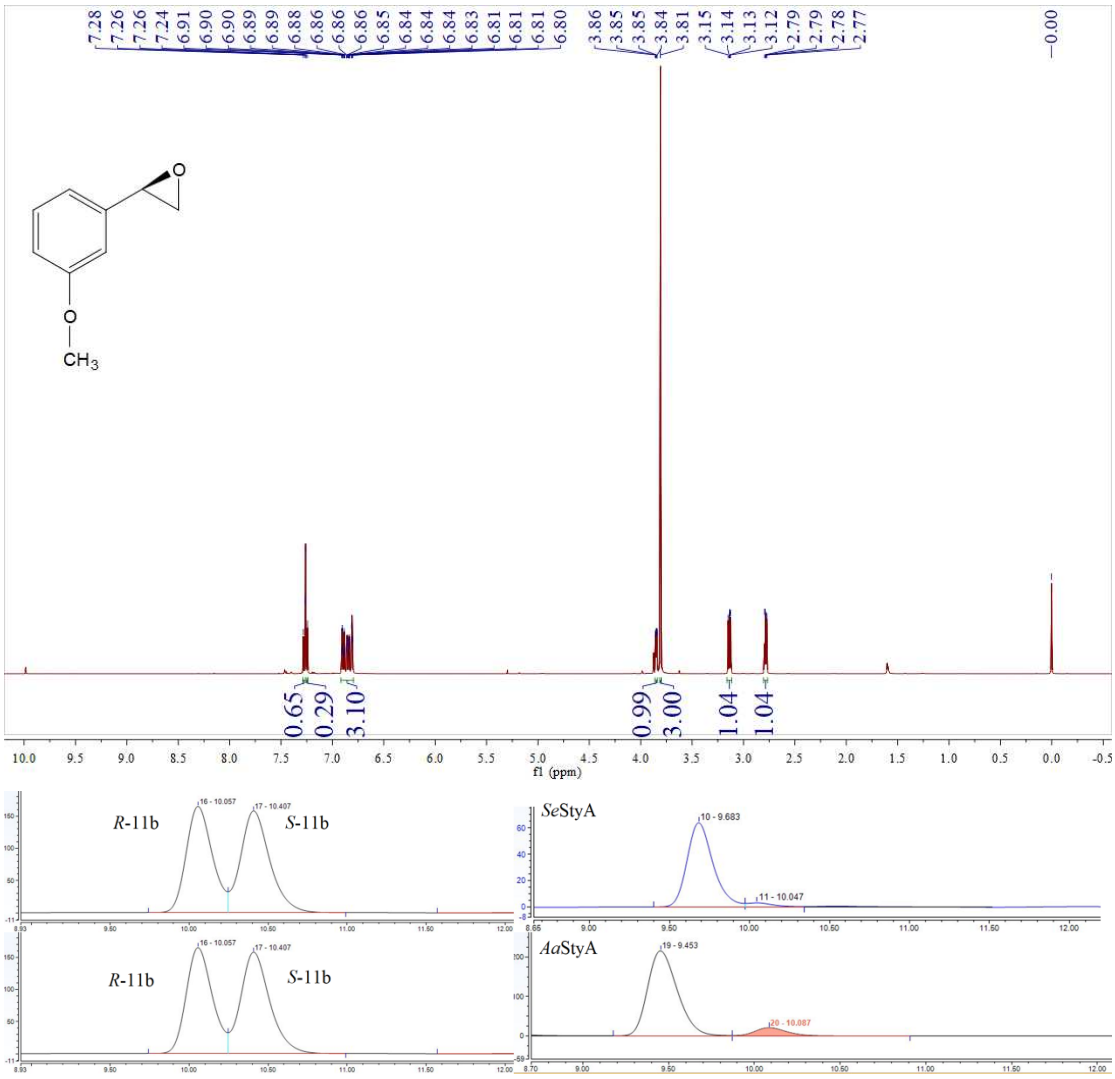
(*S*)-2-(4-tolyl)oxirane (10b)



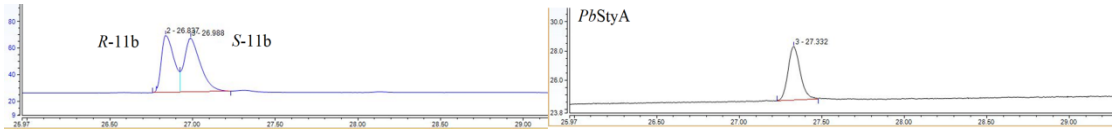


Chiralpak AS-H, *n*-hexane: 2-propanol 90: 10, 1 mL/min

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

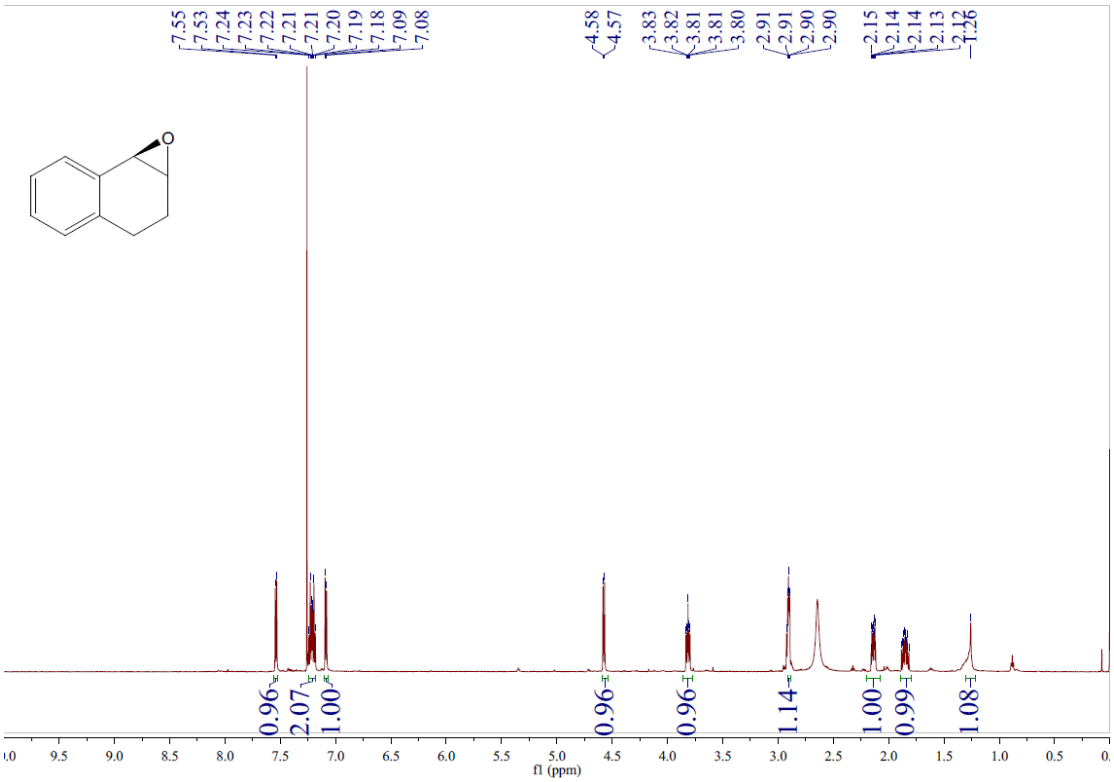


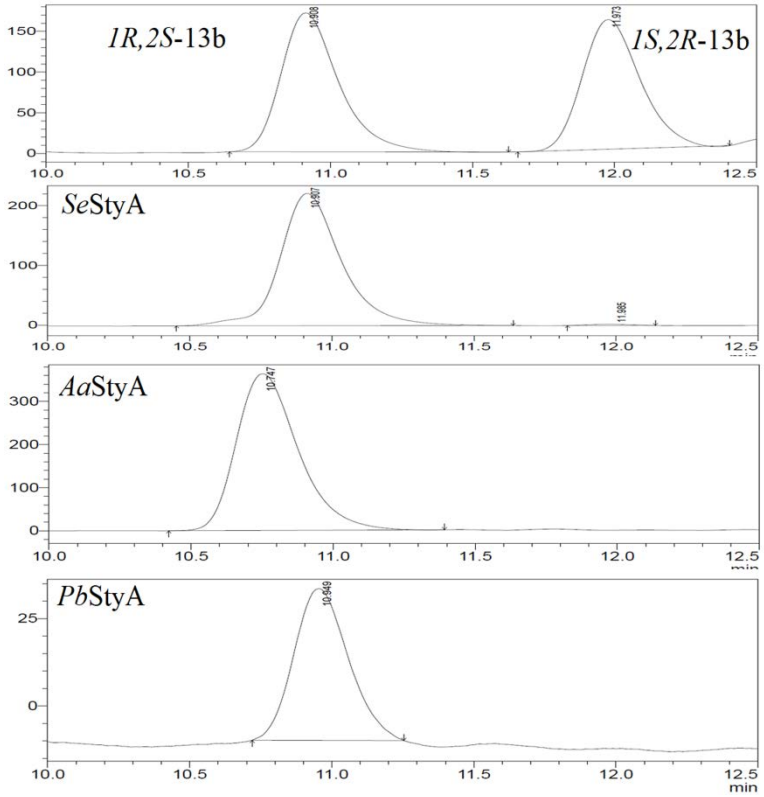
Chiralpak AS-H, *n*-hexane: 2-propanol 99: 1, 1 mL/min;



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.