Supplementary information

Title：

Fine-tuning of the substrate binding mode to enhance the catalytic efficiency of an *ortho*-haloacetophenones-specific carbonyl reductase

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Table S1 PCR primers used for mutants construction

Mutants	Primers[a]
Q139S	TCGTAGCTGGCAAA **AGC** CAGGCAGTAGAAGATATTGCTG
	TCTTCTACTGCCTG **GCT** TTTGCCAGCTACGAGCGCTAGA
Q139A	TCGTAGCTGGCAAA **GCA** CAGGCAGTAGAAGATATTGCTG
	TCTTCTACTGCCTG **TGC** TTTGCCAGCTACGAGCGCTAGA
Q139D	TCGTAGCTGGCAAA **GAC** CAGGCAGTAGAAGATATTGCTG
	TCTTCTACTGCCTG **GTC** TTTGCCAGCTACGAGCGCTAGA
Q139L	TCGTAGCTGGCAAA **CTC** CAGGCAGTAGAAGATATTGCTG
	TCTTCTACTGCCTG **GAG** TTTGCCAGCTACGAGCGCTAGA
Q139N	TCGTAGCTGGCAAA **AAT** CAGGCAGTAGAAGATATTGCTG
	TCTTCTACTGCCTG **ATT** TTTGCCAGCTACGAGCGCTAGA
D253Y	CAGGCTTTGGACAA **TAT** ACACCTTTGCAGCGTGCTGGTC
	CGCTGCAAAGGTGT **ATA** TTGTCCAAAGCCTGGAATAGCG
D253E	CAGGCTTTGGACAA **GAA** ACACCTTTGCAGCGTGCTGGTC
	CGCTGCAAAGGTGT **TTC** TTGTCCAAAGCCTGGAATAGCG
D253Q	CAGGCTTTGGACAA **CAA** ACACCTTTGCAGCGTGCTGGTC
	CGCTGCAAAGGTGT **TTG** TTGTCCAAAGCCTGGAATAGCG
D253L	CAGGCTTTGGACAA **CTC** ACACCTTTGCAGCGTGCTGGTC
	CGCTGCAAAGGTGT **GAG** TTGTCCAAAGCCTGGAATAGCG
D253F	CAGGCTTTGGACAA **TTT** ACACCTTTGCAGCGTGCTGGTC
	CGCTGCAAAGGTGT **AAA** TTGTCCAAAGCCTGGAATAGCG
Q188Y	CCACAAGTTCTGTA **TAT** GGCTATAGCCCAAGTCCTAATT
	CTTGGGCTATAGCC **ATA** TACAGAACTTGTGGTAATAATA
Q188L	CCACAAGTTCTGTA **CTC** GGCTATAGCCCAAGTCCTAATT
	CTTGGGCTATAGCC **GAG** TACAGAACTTGTGGTAATAATA
Q188E	CCACAAGTTCTGTA **GAA** GGCTATAGCCCAAGTCCTAATT
	CTTGGGCTATAGCC **TCC** TACAGAACTTGTGGTAATAATA
L196A	GCCCAAGTCCTAAT **GCA** TTAGACTATGCAGCTACAAAGT
	GCTGCATAGTCTAA **TGC** ATTAGGACTTGGGCTATAGCCT
L196F	GCCCAAGTCCTAAT **TTT** TTAGACTATGCAGCTACAAAGT
	GCTGCATAGTCTAA **AAA** ATTAGGACTTGGGCTATAGCCT
L196E	GCCCAAGTCCTAAT **GAA** TTAGACTATGCAGCTACAAAGT
	GCTGCATAGTCTAA **TTC** ATTAGGACTTGGGCTATAGCCT
V187A	TTACCACAAGTTCT **GCA** CAAGGCTATAGCCCAAGTCCTA
	GGGCTATAGCCTTG **TGC** AGAACTTGTGGTAATAATAGAA
V187S	TTACCACAAGTTCT **TCT** CAAGGCTATAGCCCAAGTCCTA
	GGGCTATAGCCTTG **AGA** AGAACTTGTGGTAATAATAGAA
V187D	TTACCACAAGTTCT **GAT** CAAGGCTATAGCCCAAGTCCTA
	GGGCTATAGCCTTG **ATC** AGAACTTGTGGTAATAATAGAA
P229A	TCAACTCCGTTGCT **GCA** GGACCTATCTGGACGCCGCTGC
	GTCCAGATAGGTCC **TGC** AGCAACGGAGTTGACGCGAATA
P229S	TCAACTCCGTTGCT **AGC** GGACCTATCTGGACGCCGCTGC
	GTCCAGATAGGTCC **GCT** AGCAACGGAGTTGACGCGAATA
P229H	TCAACTCCGTTGCT **CAT** GGACCTATCTGGACGCCGCTGC
	GTCCAGATAGGTCC **ATG** AGCAACGGAGTTGACGCGAATA

[a]The mutated sites are underlined.

Table S2 Specific activities of BaSDR1 and its variants toward substrate **2a**.

Enzyme	Specific activity (U/mg)	ee (%)
WT	0.34±0.04	99, *S*
Q139S	1.40±0.13	99, *S*
Q139A	1.25±0.12	99, *S*
Q139D	0.52±0.17	99, *S*
Q139L	0.62±0.09	99, *S*
Q139N	1.03±0.16	99, *S*
D253Y	1.13±0.10	99, *S*
D253E	0.28±0.08	99, *S*
D253Q	0.21±0.06	99, *S*
D253L	0.23±0.12	99, *S*
D253F	0.33±0.14	99, *S*
Q188Y	0.11±0.02	99, *S*
Q188L	0.09±0.04	99, *S*
Q188E	0.17±0.10	99, *S*
L196A	0.06±0.01	99, *S*
L196F	0.16±0.03	99, *S*
L196E	0.06±0.04	99, *S*
V187A	0.27±0.06	99, *S*
V187S	0.14±0.05	99, *S*
V187D	0.10±0.08	99, *S*
P229A	0.22±0.05	99, *S*
P229S	0.13±0.07	99, *S*
P229H	0.12±0.07	99, *S*

Table S3 Parameters for interactions between protein and substrate **9a**

Figure	Interactions (Protein residue→Substrate atom)	Parameters		
		Distance (Å)	Angle (°)	Angle (°)
Fig. 1a and Fig. 2c	Hydrogen bond (Y199→Sub O)	2.87[a]	140.67	/
	Hydrogen bond (S186→Sub O)	3.03[b]	164.76	/
	Halogen bond (P229→Sub 2'-F)	3.45[c]	158.66[d]	117.71[e]
	Hydrophobic interaction (Q188→Sub C1)	3.67[f]	/	/
	Hydrophobic interaction (L196→Sub C6)	3.61	/	/
Fig. 2d	Hydrogen bond (Y199→Sub O)	2.76	110.91	/
	Hydrogen bond (S186→Sub O)	2.78	160.76	/
	Halogen bond (P229→Sub 2'-F)	3.39	160.47	120.94
	Hydrophobic interaction (Q188→Sub C1)	3.62	/	/
	Hydrophobic interaction (L196→Sub C6)	3.66	/	/

[a]The distance between the oxygen atoms of Tyr^{199} OH and the substrate carbonyl group.

[b]The distance between the oxygen atoms of Ser^{186} OH and the substrate carbonyl group.

[c]The distance between the oxygen atoms of related residues and the halogen atom of substrate.

[d]The angle between the carbon atoms, halogen atoms of substrate and the oxygen atoms of related residues.

[e]The angle between the oxygen atoms, carbon atoms of related residues and the halogen atoms of substrate.

[f]The distance between the carbon atoms of related residues and the labeled carbon atoms of substrate.

Table S4 Parameters for interactions between protein and substrate **10a**

Figure	Interactions (Protein residue→Substrate atom)	Parameters		
		Distance (Å)	Angle (°)	Angle (°)
Fig. 1c and Fig. 4c	Hydrogen bond (Y199→Sub O)	3.65[a]	142.51	/
	Hydrogen bond (S186→Sub O)	2.81[b]	149.44	/
	Halogen bond (P229→Sub 2'-F)	3.25[c]	164.61[d]	119.40[e]
	Halogen bond (D253→Sub 4'-Cl)	3.67	158.76	129.29
Fig. 4d	Hydrogen bond (Y199→Sub O)	3.10	137.56	/
	Hydrogen bond (S186→Sub O)	3.16	165.82	/
	Halogen bond (Q242→Sub 2'-F)	3.17	142.88	149.16
	Hydrophobic interaction (V187→Sub C5)	3.26[f]	/	/
	Hydrophobic interaction (Q188→Sub C1)	3.75	/	/

[a]The distance between the oxygen atoms of Tyr^{199} OH and the substrate carbonyl group.

[b]The distance between the oxygen atoms of Ser^{186} OH and the substrate carbonyl group.

[c]The distance between the oxygen atoms of related residues and the halogen atom of substrate.

[d]The angle between the carbon atoms, halogen atoms of substrate and the oxygen atoms of related residues.

[e]The angle between the oxygen atoms, carbon atoms of related residues and the halogen atoms of substrate.

[f]The distance between the carbon atoms of related residues and the labeled carbon atoms of substrate.

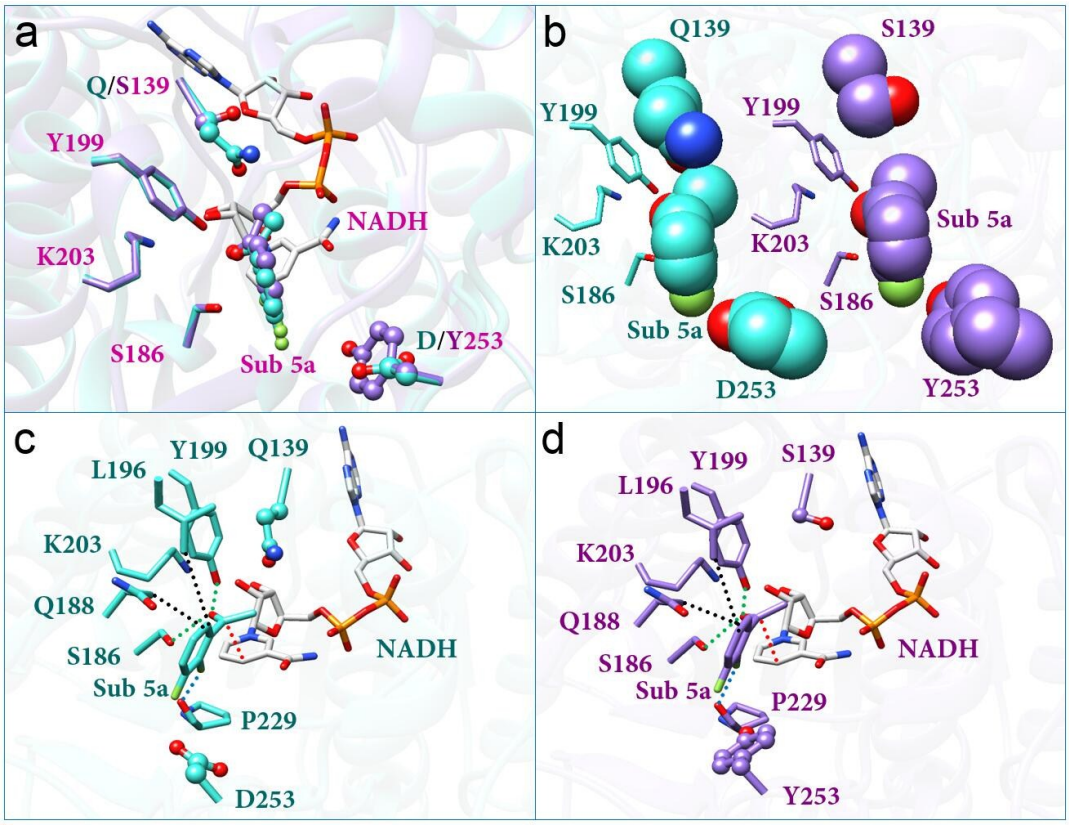


Fig. S1 The complex models of substrate **5a** and wild-type BaSDR1 (turquoise) or variant Q139S/D253Y (purple). (a) and (b): Superimposition of wild-type BaSDR1 and variant Q139S/D253Y complex models; (c): The wild type; (d): Q139S/D253Y. Green dash lines, hydrogen bonds; Blue dash lines, halogen bonds; Black dash line, hydrophobic interactions. Red dash lines, the distance between the carbonyl carbon atom of substrate and the C_4 atom in the nicotinamide ring of NADH. The specific residues are indicated by labels.

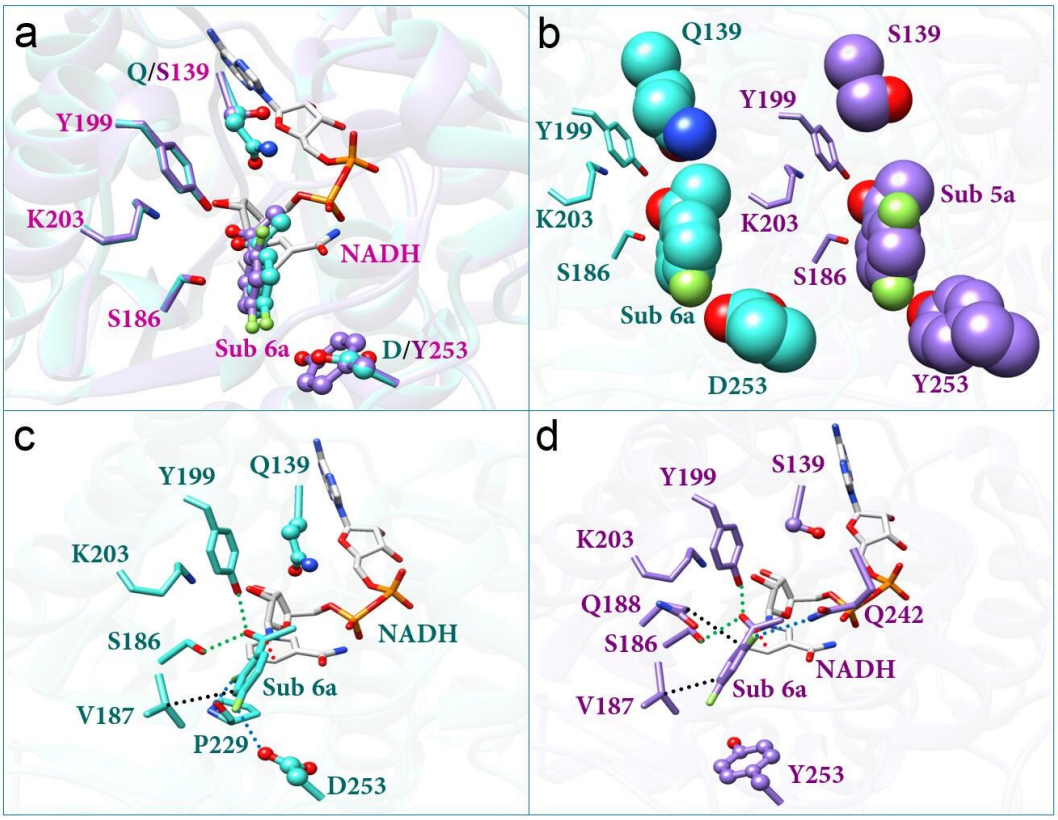


Fig. S2 The complex models of substrate **6a** and wild-type BaSDR1 (turquoise) or variant Q139S/D253Y (purple). (a) and (b): Superimposition of wild-type BaSDR1 and variant Q139S/D253Y complex models; (c): The wild type; (d): Q139S/D253Y. Green dash lines, hydrogen bonds; Blue dash lines, halogen bonds; Black dash line, hydrophobic interactions. Red dash lines, the distance between the carbonyl carbon atom of substrate and the C_4 atom in the nicotinamide ring of NADH. The specific residues are indicated by labels.

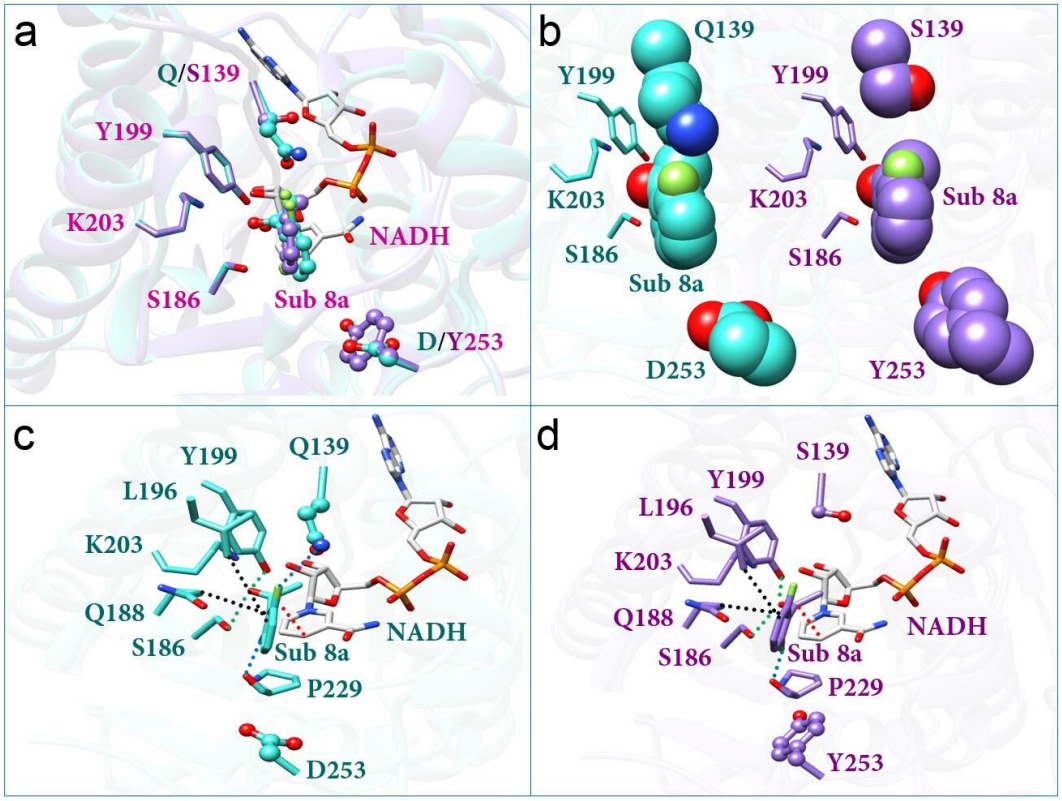


Fig. S3 The complex models of substrate **8a** and wild-type BaSDR1 (turquoise) or variant Q139S/D253Y (purple). (a) and (b): Superimposition of wild-type BaSDR1 and variant Q139S/D253Y complex models; (c): The wild type; (d): Q139S/D253Y. Green dash lines, hydrogen bonds; Blue dash lines, halogen bonds; Black dash line, hydrophobic interactions. Red dash lines, the distance between the carbonyl carbon atom of substrate and the C_4 atom in the nicotinamide ring of NADH. The specific residues are indicated by labels.

Table S5 Parameters for interactions between protein and substrate **5a**

Figure	Interactions (Protein residue→Substrate atom)	Parameters		
		Distance (Å)	Angle (°)	Angle (°)
Fig. S1c	Hydrogen bond (Y199→Sub O)	3.02[a]	138.88	/
	Hydrogen bond (S186→Sub O)	2.85[b]	154.22	/
	Halogen bond (P229→Sub 2'-F)	3.14[c]	153.98[d]	116.82[e]
	Hydrophobic interaction (Q188→Sub C1)	3.65[f]	/	/
	Hydrophobic interaction (L196→Sub C6)	3.92	/	/
Fig. S1d	Hydrogen bond (Y199→Sub O)	2.70	139.74	/
	Hydrogen bond (S186→Sub O)	2.75	161.92	/
	Halogen bond (P229→Sub 2'-F)	3.36	159.84	119.94
	Hydrophobic interaction (Q188→Sub C1)	3.62	/	/
	Hydrophobic interaction (L196→Sub C6)	3.67	/	/

[a]The distance between the oxygen atoms of Tyr^{199} OH and the substrate carbonyl group.

[b]The distance between the oxygen atoms of Ser^{186} OH and the substrate carbonyl group.

[c]The distance between the oxygen atoms of related residues and the halogen atom of substrate.

[d]The angle between the carbon atoms, halogen atoms of substrate and the oxygen atoms of related residues.

[e]The angle between the oxygen atoms, carbon atoms of related residues and the halogen atoms of substrate.

[f]The distance between the carbon atoms of related residues and the labeled carbon atoms of substrate.

Table S6 Parameters for interactions between protein and substrate **6a**

Figure	Interactions (Protein residue→Substrate atom)	Parameters		
		Distance (Å)	Angle (°)	Angle (°)
Fig. S2c	Hydrogen bond (Y199→Sub O)	3.90[a]	142.34	/
	Hydrogen bond (S186→Sub O)	2.94[b]	143.54	/
	Halogen bond (P229→Sub 2'-F)	3.14[c]	162.10[d]	118.71[e]
	Halogen bond (D253→Sub 4'-F)	3.82	144.79	133.25
	Hydrophobic interaction (V187→Sub C3)	3.77[f]	/	/
Fig. S2d	Hydrogen bond (Y199→Sub O)	3.10	118.59	/
	Hydrogen bond (S186→Sub O)	3.03	108.06	/
	Halogen bond (Q242→Sub 2'-F)	3.17	141.74	148.92
	Hydrophobic interaction (V187→Sub C5)	3.30	/	/
	Hydrophobic interaction (Q188→Sub C1)	3.74	/	/

[a]The distance between the oxygen atoms of Tyr^{199} OH and the substrate carbonyl group.

[b]The distance between the oxygen atoms of Ser^{186} OH and the substrate carbonyl group.

[c]The distance between the oxygen atoms of related residues and the halogen atom of substrate.

[d]The angle between the carbon atoms, halogen atoms of substrate and the oxygen atoms of related residues.

[e]The angle between the oxygen atoms, carbon atoms of related residues and the halogen atoms of substrate.

[f]The distance between the carbon atoms of related residues and the labeled carbon atoms of substrate.

Table S7 Parameters for interactions between protein and substrate **8a**

Figure	Interactions (Protein residue→Substrate atom)	Parameters		
		Distance (Å)	Angle (°)	Angle (°)
Fig. S3c	Hydrogen bond (Y199→Sub O)	2.87[a]	115.70	/
	Hydrogen bond (S186→Sub O)	2.72[b]	161.94	/
	Halogen bond (Q139→Sub 6'-F)	3.05[c]	142.25[d]	111.87[e]
	Halogen bond (P229→Sub 2'-F)	3.49	166.22	118.18
	Hydrophobic interaction (Q188→Sub C1)	3.56[f]	/	/
	Hydrophobic interaction (L196→Sub C5)	3.62	/	/
Fig. S3d	Hydrogen bond (Y199→Sub O)	2.52	115.32	/
	Hydrogen bond (S186→Sub O)	2.78	161.58	/
	Halogen bond (P229→Sub 2'-F)	3.61	171.26	120.62
	Hydrophobic interaction (Q188→Sub C1)	3.60	/	/
	Hydrophobic interaction (L196→Sub C5)	3.59	/	/

[a]The distance between the oxygen atoms of Tyr^{199} OH and the substrate carbonyl group.

[b]The distance between the oxygen atoms of Ser^{186} OH and the substrate carbonyl group.

[c]The distance between the oxygen atoms of related residues and the halogen atom of substrate.

[d]The angle between the carbon atoms, halogen atoms of substrate and the oxygen atoms of related residues.

[e]The angle between the oxygen atoms, carbon atoms of related residues and the halogen atoms of substrate.

[f]The distance between the carbon atoms of related residues and the labeled carbon atoms of substrate.

Table S8 The distance between the carbonyl carbon atom of substrate and the C_4 atom in the nicotinamide ring of NADH.

Substrate	Figure	Enzyme	Distance (Å)
5a	Fig. S1c	WT	3.50
	Fig. S1d	Q139S/D253Y	3.48
6a	Fig. S2c	WT	3.80
	Fig. S2d	Q139S/D253Y	3.61
8a	Fig. S3c	WT	3.80
	Fig. S3d	Q139S/D253Y	3.42
9a	Fig. 2c	WT	3.50
	Fig. 2d	Q139S/D253Y	3.21
10a	Fig. 3c	WT	3.69
	Fig. 3d	Q139S/D253Y	3.60

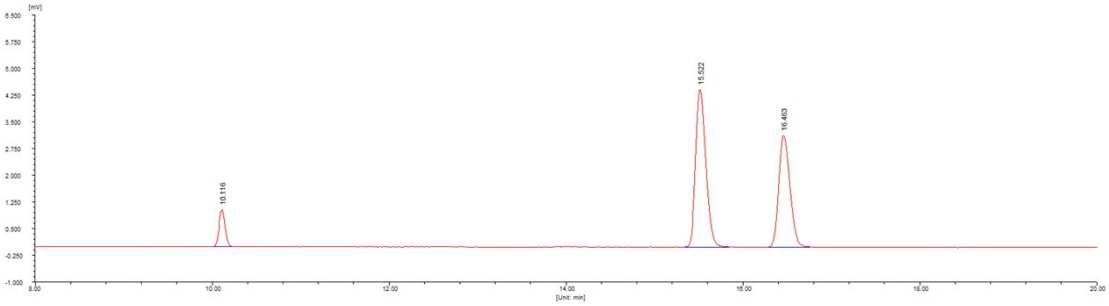
Table S9 Binding free energy of enzyme-substrate complexes (kcal/mol)

Substrate	Enzyme	Delta G binding	Binding energy contribution	
			Residue 139	Residue 253
5a	WT	-4.0647±2.4413	0.059±0.334	0.084±0.419
	Q139S/D253Y	-9.5561±2.8024	-0.363±0.278	-0.114±0.167
6a	WT	-3.8313±2.9372	0.217±0.536	0.663±0.875
	Q139S/D253Y	-8.4552±2.6118	-0.007±0.066	-0.184±0.454
8a	WT	-7.0181±2.7916	0.286±0.421	0.123±0.057
	Q139S/D253Y	-8.5020±2.0416	-0.036±0.288	-0.358±0.227
9a	WT	-6.8673±1.9743	-0.073±0.323	0.141±0.567
	Q139S/D253Y	-11.2142±2.1698	-0.461±0.343	-0.048±0.249
10a	WT	-5.5447±1.8397	0.412±0.472	-0.062±0.051
	Q139S/D253Y	-8.9495±2.3985	0.079±0.478	-0.108±0.550

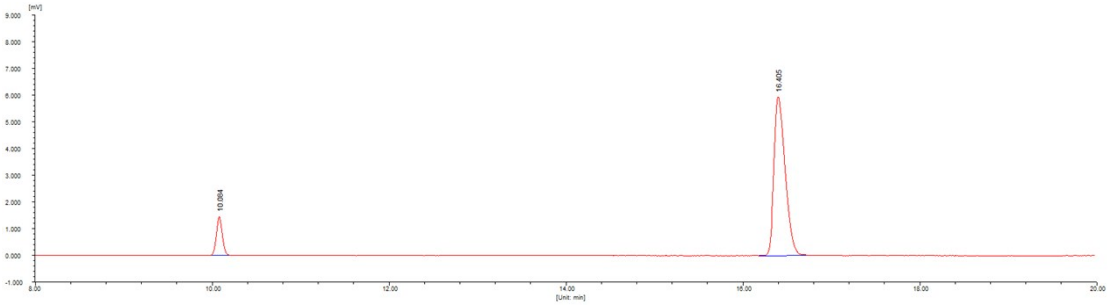
GC chromatograms

Substrate 1a Column temperature: 120°C. Retention time: substrate, 10.1 min; R-product, 15.5 min; S-product, 16.4 min.

(A) Substrate and corresponding alcohol

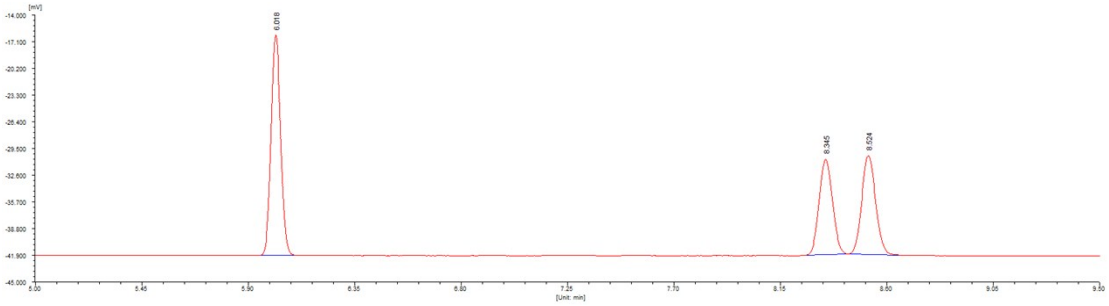


(B) Mutant Q139S/D253Y-catalysed asymmetric reduction

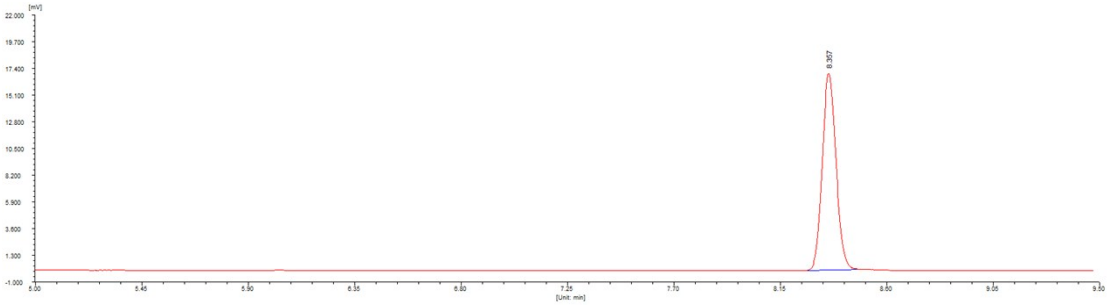


Substrate 2a Column temperature: 130°C. Retention time: substrate, 6.0 min; S-product, 8.3 min; R-product, 8.5 min.

(A) Substrate and corresponding alcohol

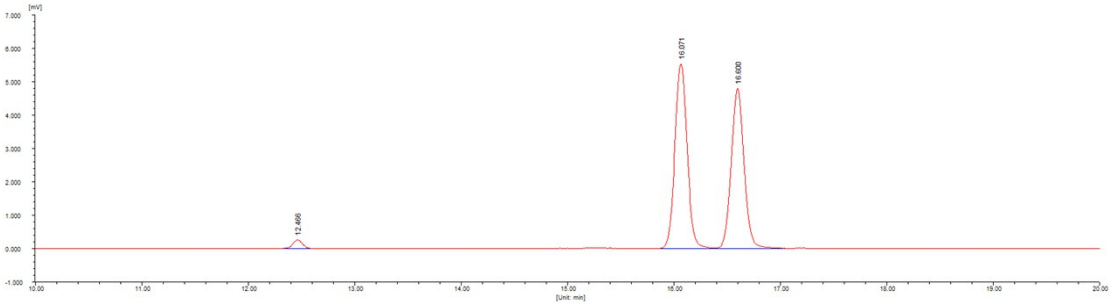


(B) Mutant Q139S-catalysed asymmetric reduction

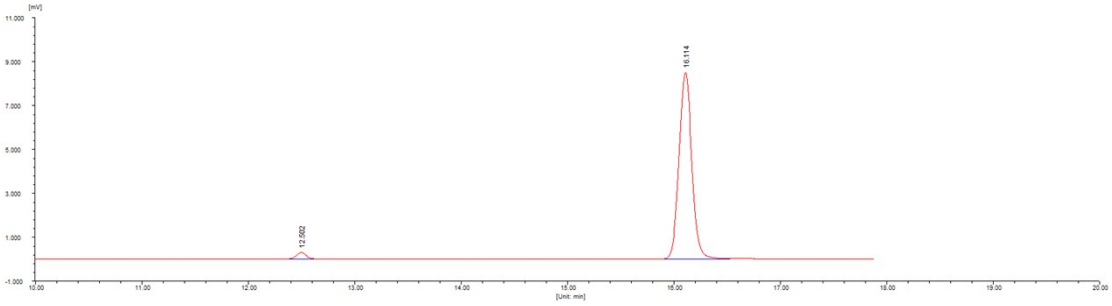


Substrate 3a Column temperature: 130°C. Retention time: substrate, 12.4 min; S-product, 16.1 min; R-product, 16.6 min.

(A) Substrate and corresponding alcohol

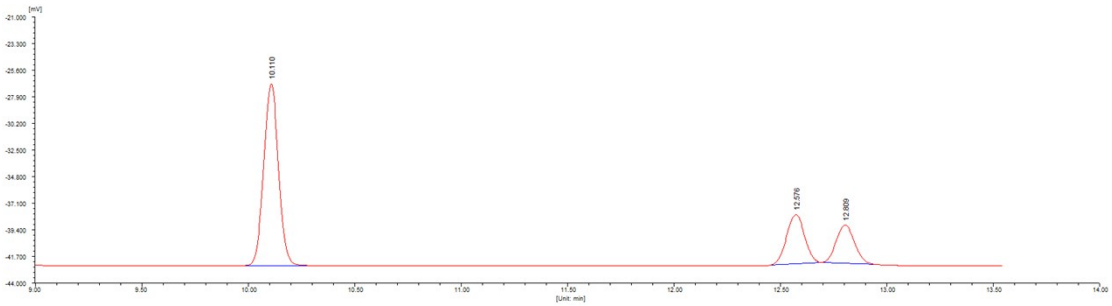


(B) Mutant Q139S/D253Y-catalysed asymmetric reduction

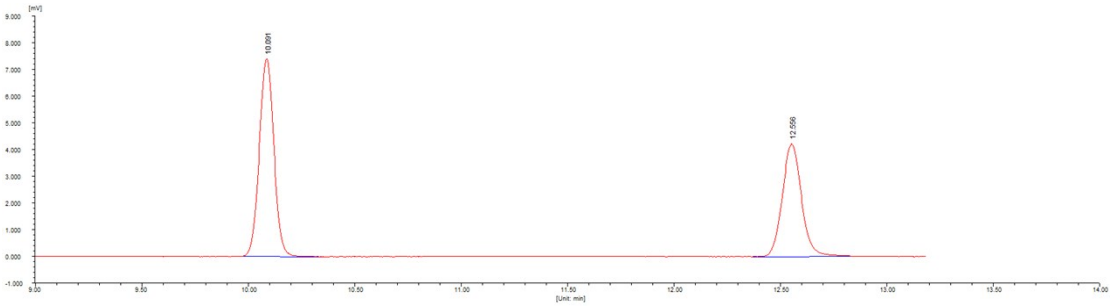


Substrate 4a Column temperature: 150°C. Retention time: substrate, 10.1 min; S-product, 12.5 min; R-product, 12.8 min.

(A) Substrate and corresponding alcohol

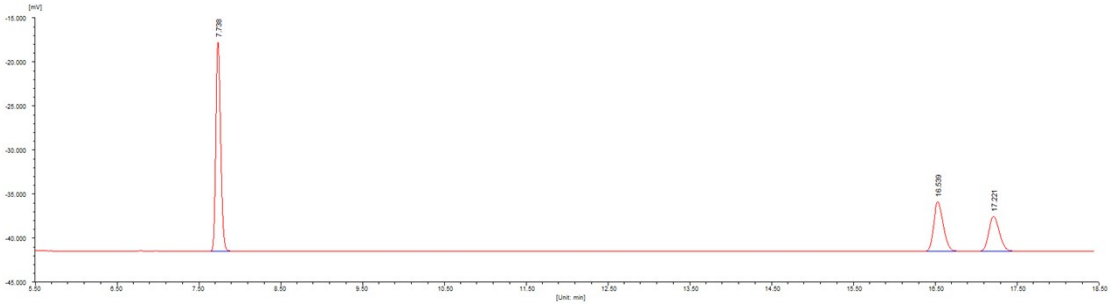


(B) Mutant Q139S/D253Y-catalysed asymmetric reduction

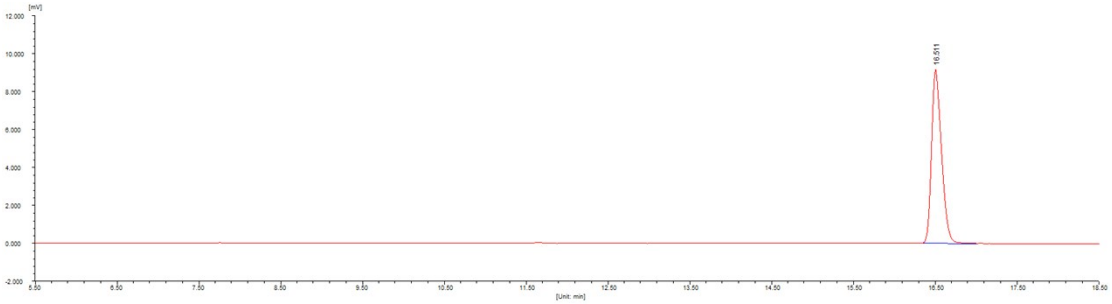


Substrate 5a Column temperature: 120°C. Retention time: substrate, 7.7 min; S-product, 16.5 min; R-product, 17.2 min.

(A) Substrate and corresponding alcohol

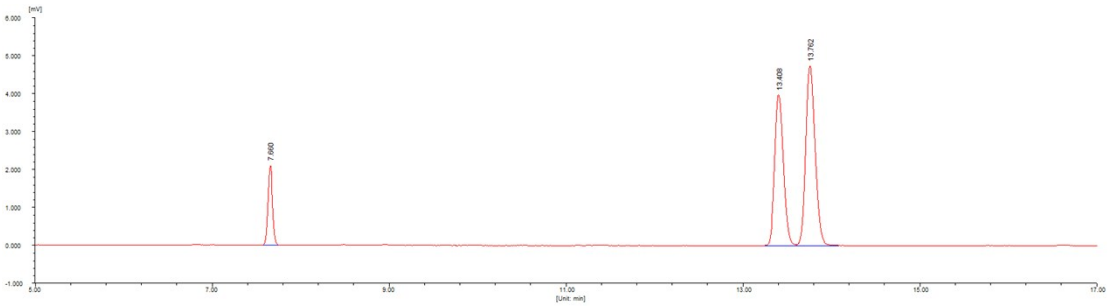


(B) Mutant Q139S-catalysed asymmetric reduction

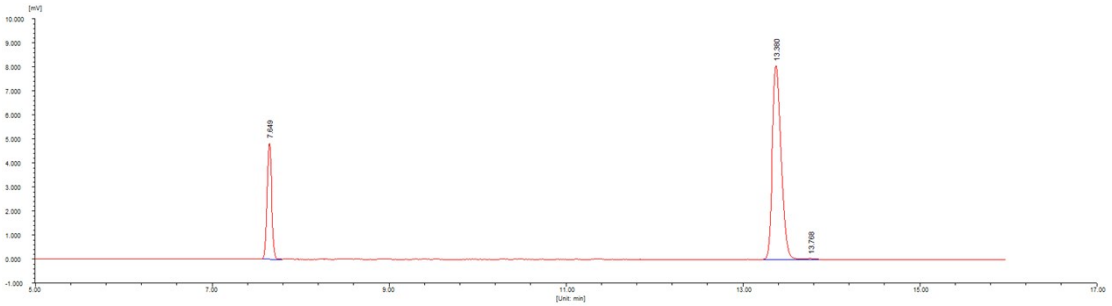


Substrate 6a Column temperature: 120°C. Retention time: substrate, 7.6 min; S-product, 13.4 min; R-product, 13.7 min.

(A) Substrate and corresponding alcohol

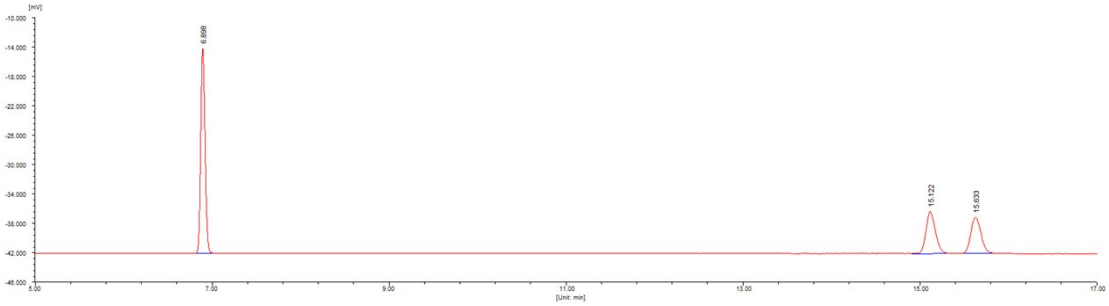


(B) Mutant Q139S-catalysed asymmetric reduction

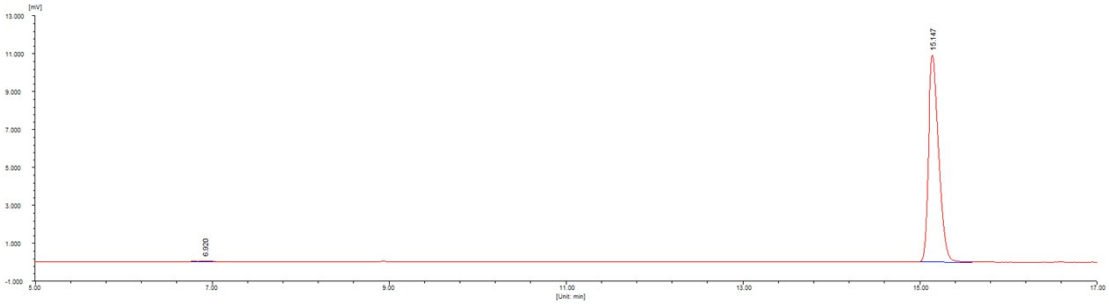


Substrate 7a Column temperature: 120°C. Retention time: substrate, 6.8 min; S-product, 15.1 min; R-product, 15.6 min.

(A) Substrate and corresponding alcohol

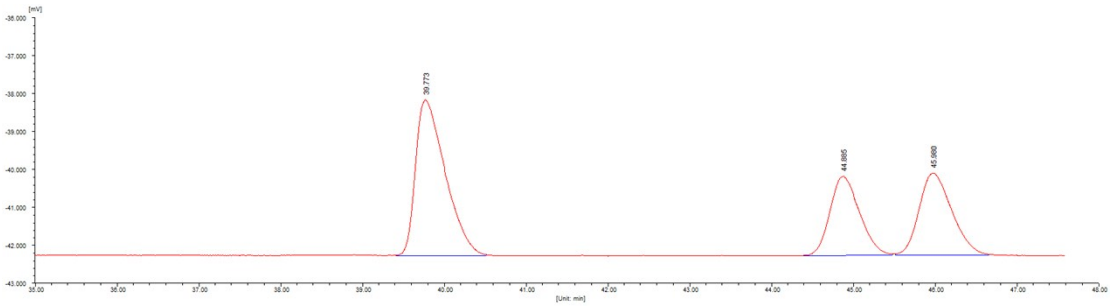


(B) Mutant Q139S/D253Y-catalysed asymmetric reduction

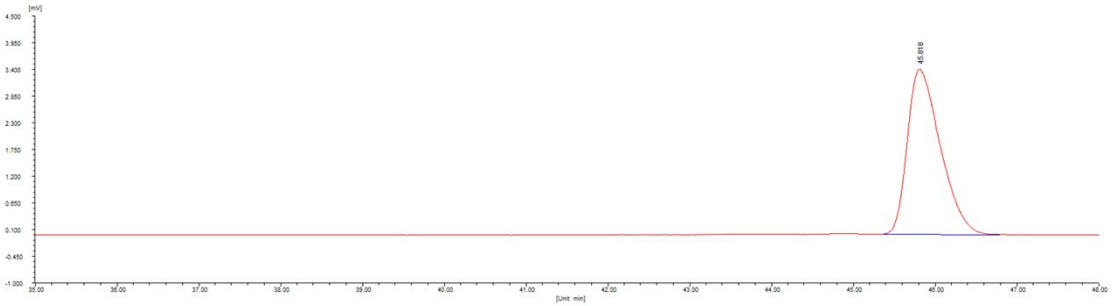


Substrate 8a Column temperature: 80°C. Retention time: substrate, 39.7 min; R-product, 44.8 min; S-product, 45.9 min.

(A) Substrate and corresponding alcohol

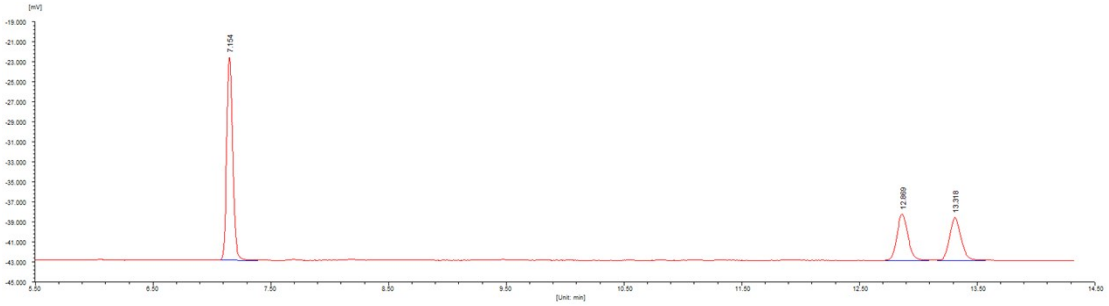


(B) Mutant D253Y-catalysed asymmetric reduction

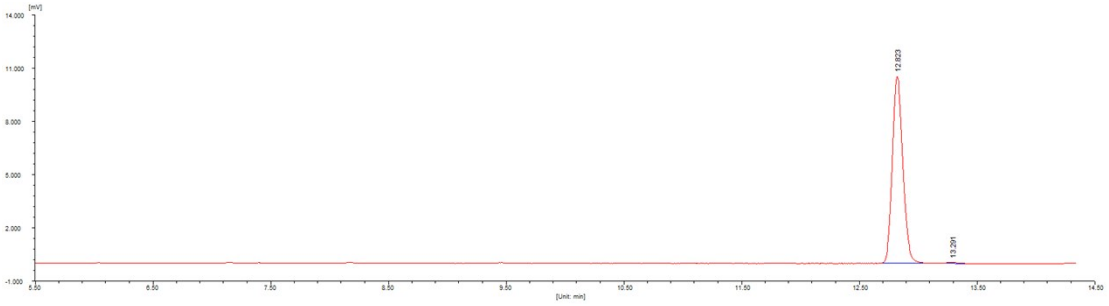


Substrate 9a Column temperature: 150°C. Retention time: substrate, 7.1 min; R-product, 12.8 min; S-product, 13.3 min.

(A) Substrate and corresponding alcohol

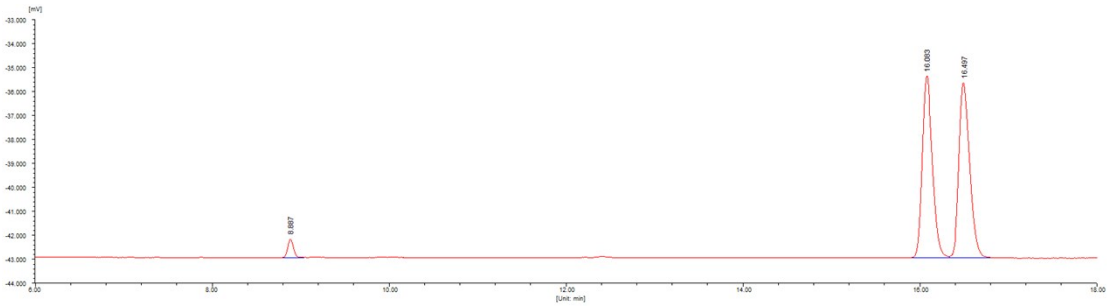


(B) Mutant Q139S-catalysed asymmetric reduction

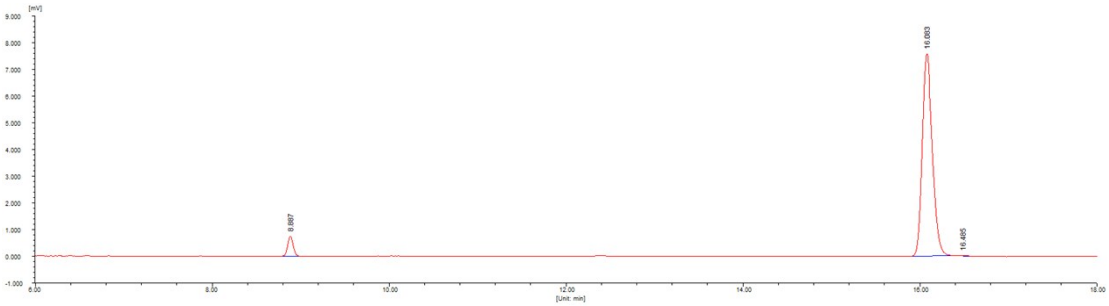


Substrate 10a Column temperature: 140°C. Retention time: substrate, 8.8 min; S-product, 16.0 min; R-product, 16.4 min.

(A) Substrate and corresponding alcohol

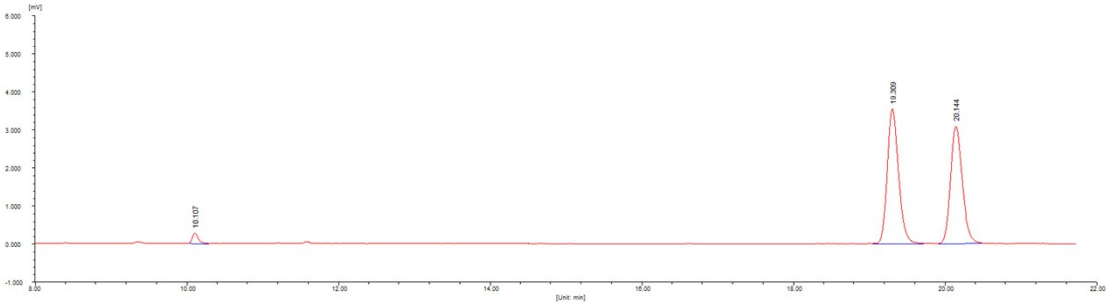


(B) Mutant Q139S/D253Y-catalysed asymmetric reduction

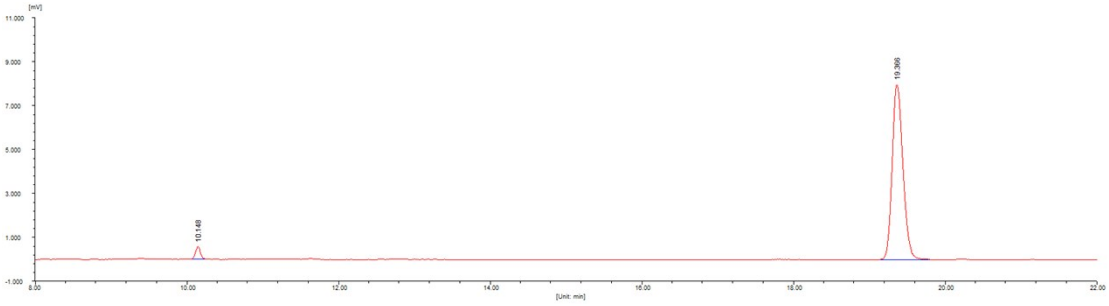


Substrate 11a Column temperature: 150°C. Retention time: substrate, 10.1 min; S-product, 19.3 min; R-product, 20.1 min.

(A) Substrate and corresponding alcohol

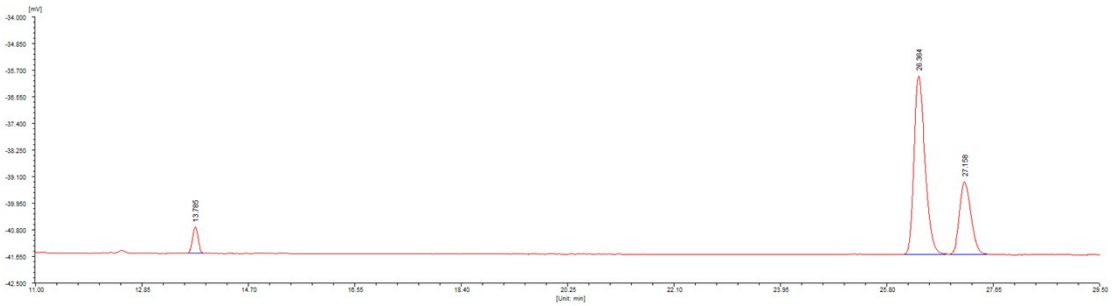


(B) Mutant Q139S/D253Y-catalysed asymmetric reduction



Substrate 12a Column temperature: 140°C. Retention time: substrate, 13.7 min; S-product, 26.3 min; R-product, 27.1 min.

(A) Substrate and corresponding alcohol



(B) Mutant Q139S-catalysed asymmetric reduction