Supporting Information

Directed evolution of an alcohol dehydrogenase for the desymmetric reduction of 2,2-disubstituted cyclopenta-1,3-diones by enzymatic hydrogen transfer

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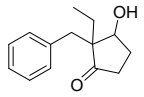
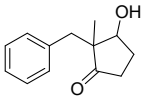
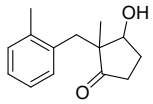
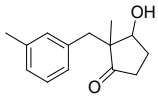
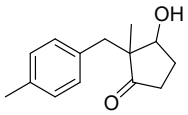
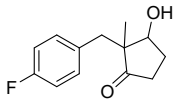
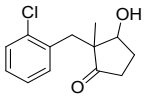
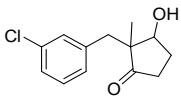
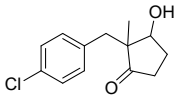
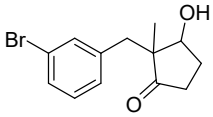
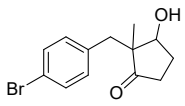
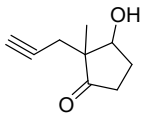
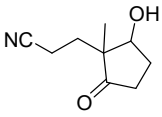
Table S1 List of Primers

Protein	Primers(5'-3')
I49-Forward	GTTTTTGAAGGCGCC**NNK**GGCGAAAGACATAACATGATACTCGG
I49-Reverse	GTATCATGTTATGTCTTTCGCC**MNN**GGCGCCTTCAAAAACGGTATG
L107-Forward	CTCCGGTGGAATG**NNK**GCAGGCTGGAAATTTTCGAATGTAAAAG
L107-Reverse	CGAAAATTTCCAGCCTGC**MNN**CATTCCACCGGAGTGCTGGTG
W110-Forward	GTGGAATGCTGGCAGGC**NNK**AAATTTTCGAATGTAAAAGATGGTG
W110-Reverse	CTTTTACATTCGAAAATTT**MNN**GCCTGCCAGCATTCCACCGGAGTG
N114-Forward	GGAAATTTTCG**NNK**GTAAAAGATGGTGTTTTTGGTGAATTTTTTC
N114-Reverse	CAAAAACACCATCTTTTAC**MNN**CGAAAATTTCCAGCCTGCCAGC
Y267-Forward	CACCATCGCTAATGTAAAT**NNK**TTTGGCGAAGGAGAGGTTTTGCC
Y267- Reverse	CCTCTCCTTCGCCAAA**MNN**ATTTACATTAGCGATGGTGCCACCAG G
M285-Forward	CTTGAATGGGGTTGCGGC**NNK**GCTCATAAAACTATAAAAGGCGGG
M285-Reverse	CTTTTATAGTTTTATGAGC**MNN**GCCGCAACCCCATTCAAGACGAG
L294-Forward	CTATAAAAGGCGGG**NNK**TGCCCCGGTGGACGTCTAAGAATGG
L294-Reverse	CTTAGACGTCCACCGGGGCA**MNN**CCCGCCTTTTATAGTTTTATG

Table S2. Chiral HPLC and GC methods of racemates

Racemate	Method
	Chiralcel OD-H, *n*-Hex/*i*-PrOH 97/3, 0.8 mL/min, 210 nm
	Chiralcel OD-H, *n*-Hex/*i*-PrOH 97/3, 0.8 mL/min, 210 nm
	Chiralcel OD-H, *n*-Hex/*i*-PrOH 97/3, 0.8 mL/min, 210 nm
	Chiralcel OD-H, *n*-Hex/*i*-PrOH 98/2, 0.8 mL/min, 210 nm
	Chiralcel OD-H, *n*-Hex/*i*-PrOH 98/2, 0.8 mL/min, 210 nm
	Chiralcel OD-H, *n*-Hex/*i*-PrOH 99/1, 1 mL/min, 210 nm
	Chiralcel OD-H, *n*-Hex/*i*-PrOH 97/3, 0.8 mL/min, 210 nm
	Chiralcel OD-H, *n*-Hex/*i*-PrOH 98/2, 0.8 mL/min, 210 nm
	Chiralcel OD-H, *n*-Hex/*i*-PrOH 99/1, 1 mL/min, 210 nm
	Chiralcel OD-H, *n*-Hex/*i*-PrOH 99/1, 1 mL/min, 210 nm

	Chiralcel OD-H, *n*-Hex/*i*-PrOH 99/1, 1 mL/min, 210 nm
	CP-Chirasil DEX CB 25×0.25, 60℃, maintain 1 min, 3℃/min to 120 ℃, then 40 ℃/min to 180 ℃, maintain 2 min
	CP-Chirasil DEX CB 25×0.25, 60℃, maintain 1 min, 3 ℃/min to 120 ℃, then 40 ℃/min to 180℃, maintain 2 min

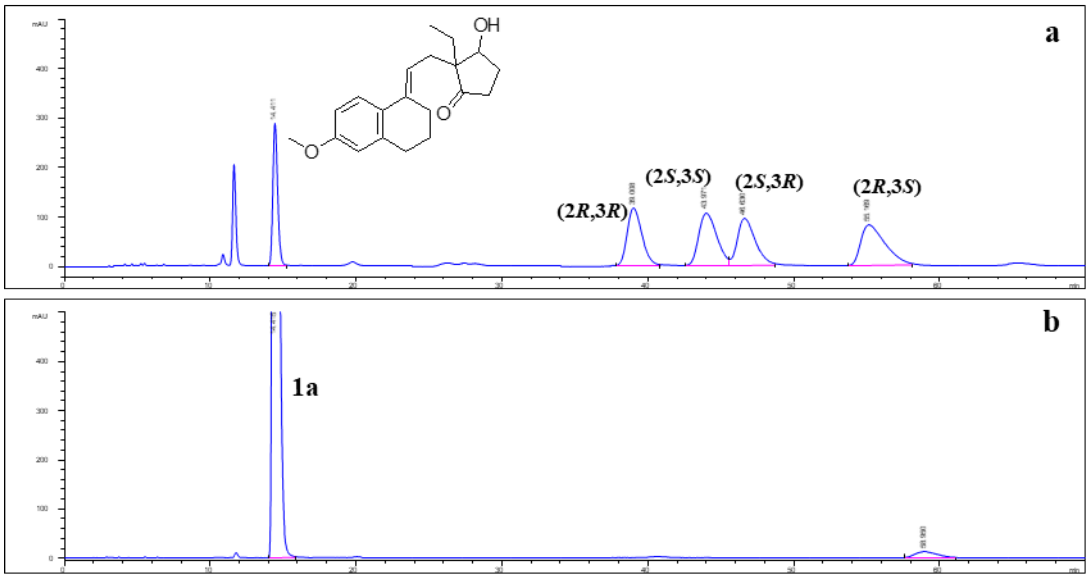


Figure S1 HPLC traces for **a**) the $NaBH_4$-reduction products of **1a** (racemic diastereomers); **b**) the WT enzyme reduction**.**

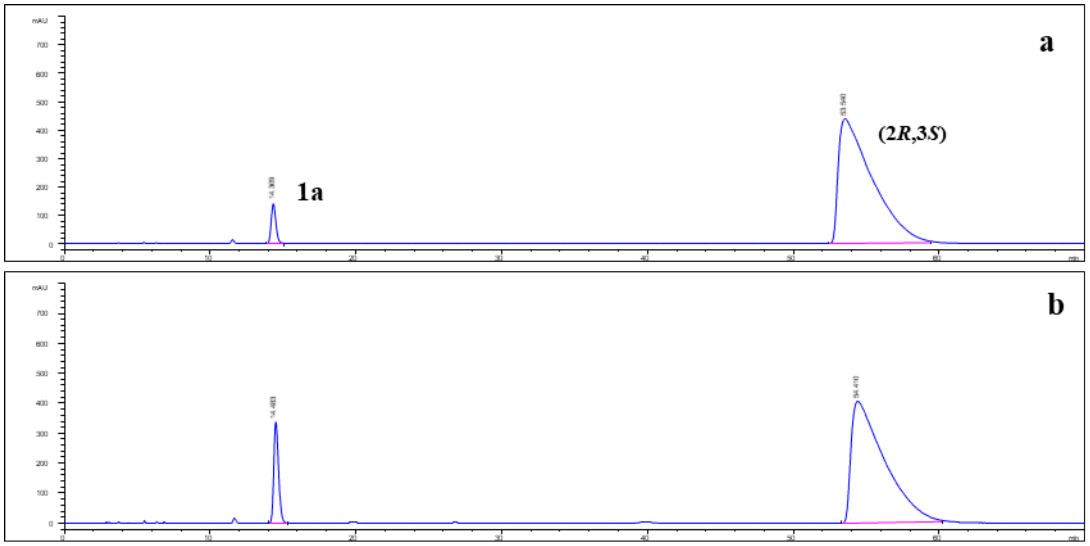


Figure S2 HPLC traces for **a**) the L294A enzyme reduction; **b**) the L294N enzyme reduction**.**

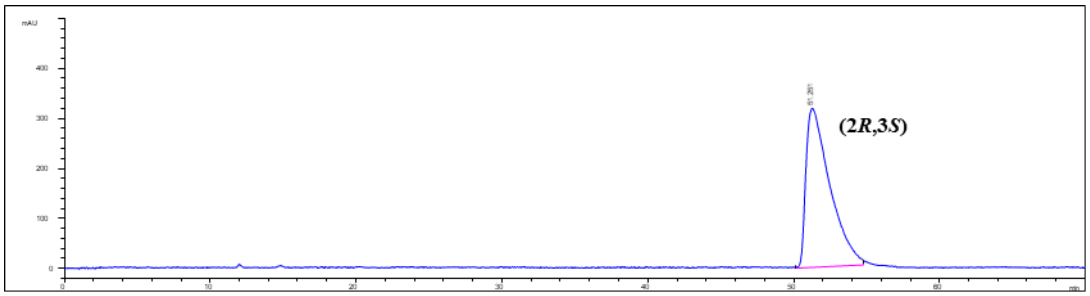


Figure S3 HPLC traces for the Tb1 (L294P) enzyme reduction**.**

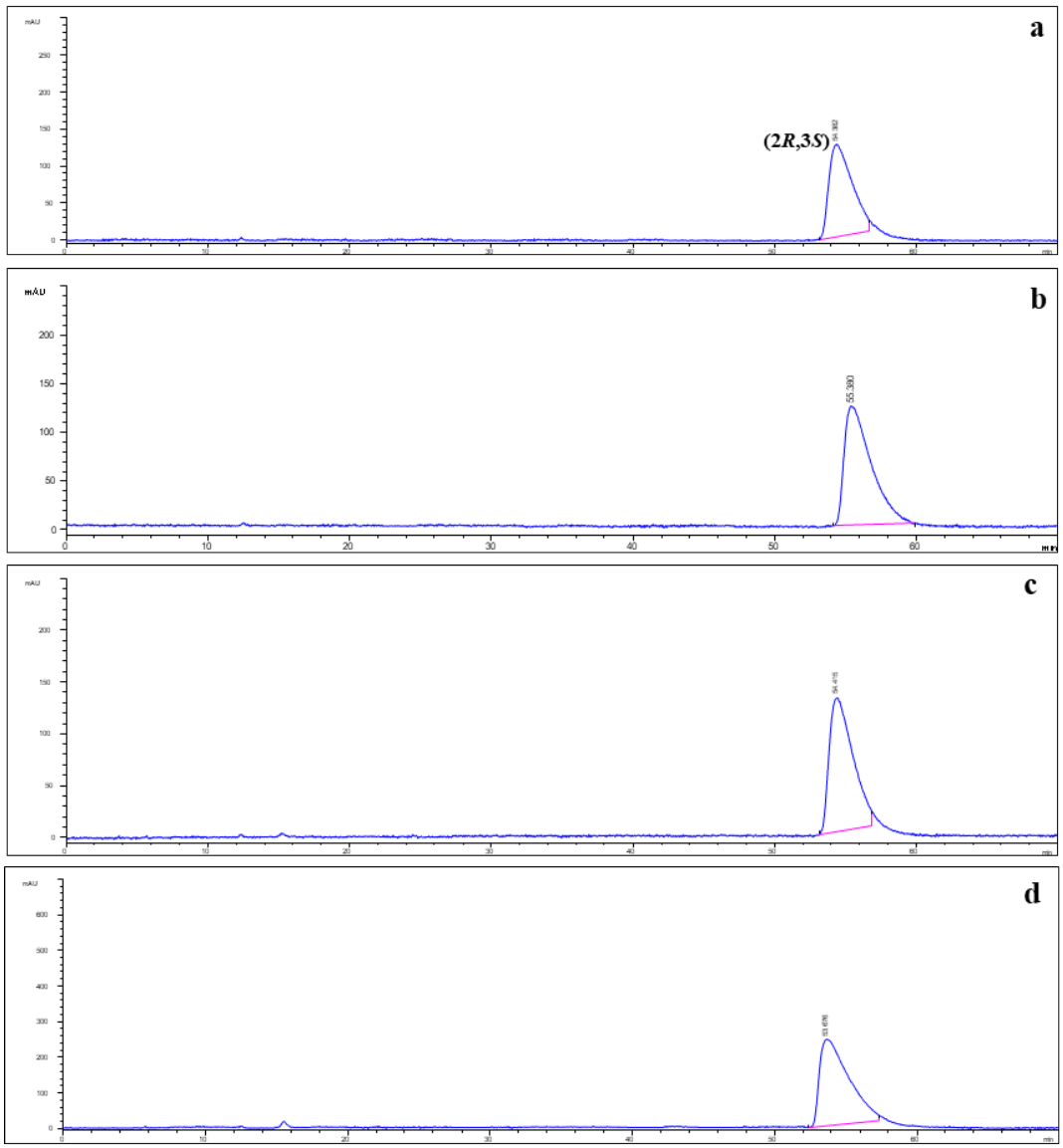


Figure S4 HPLC traces for **a**) the Tb2 (L294P/N114G) enzyme reduction; **b**) the L194P/W110V enzyme reduction; **c**) the L294P/M285L enzyme reduction; **c**) the Tb3(L294P/N114G/M285L) enzyme reduction**.**

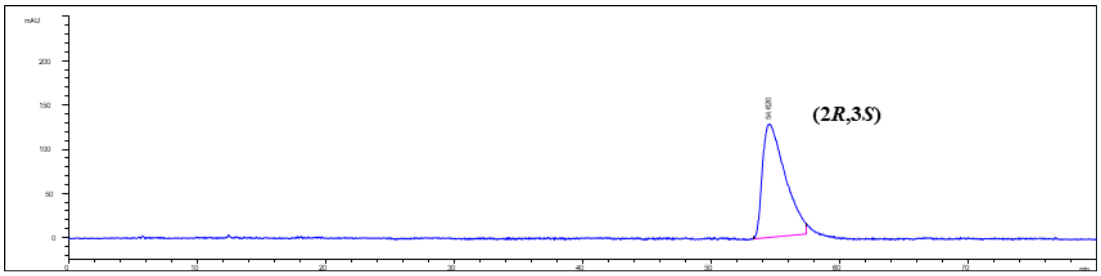


Figure S5 HPLC traces for **a**) the Tb4 (L294P/N114G/M285L/W110V) enzyme reduction.

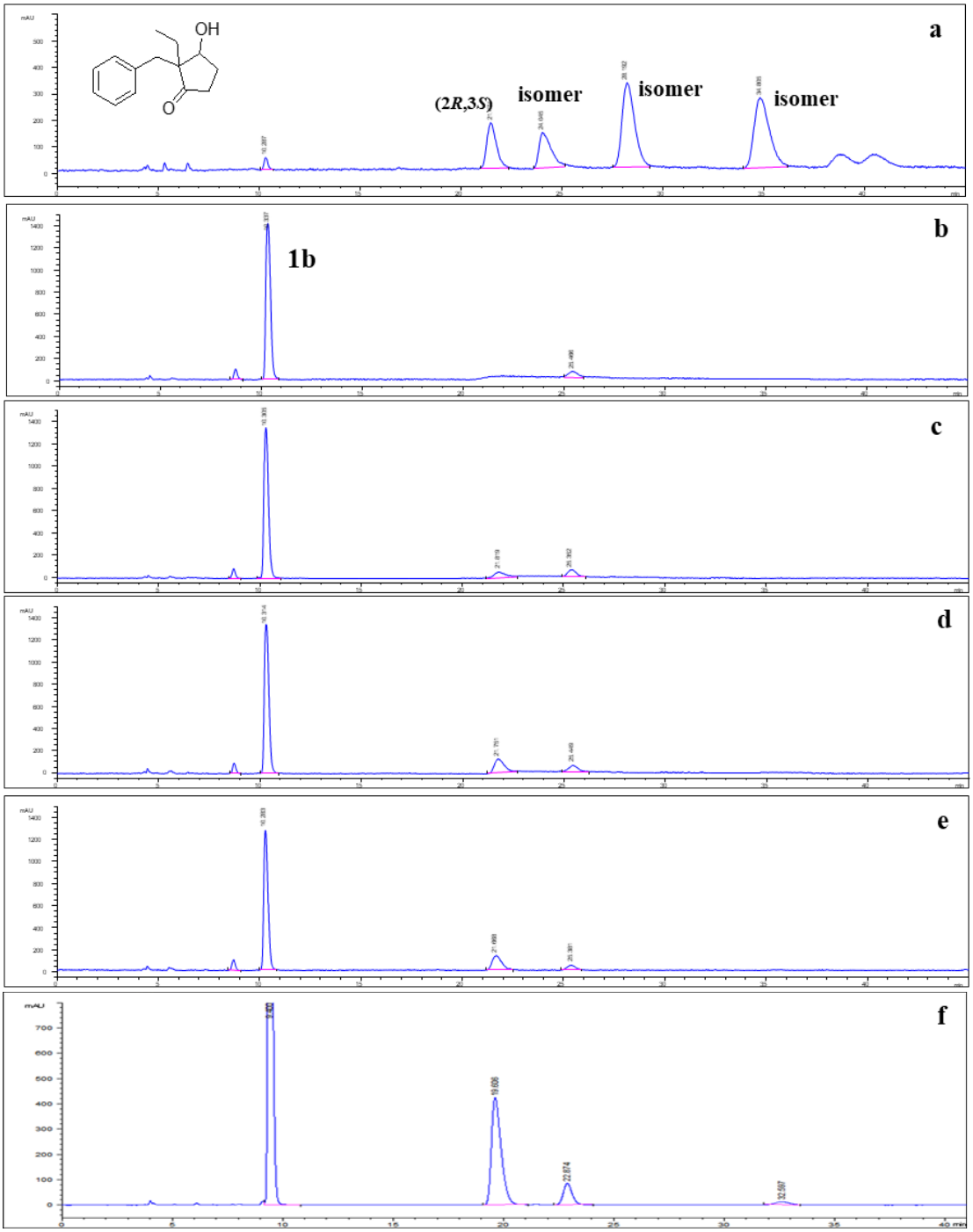


Figure S6 HPLC traces for **a**) the $NaBH_4$-reduction products of **1b** (racemic diastereomers) **b**) the WT enzyme reduction; **c**) the Tb1 enzyme reduction; **d**) the Tb2 enzyme reduction; **e**) the Tb3 enzyme reduction; **f)** the Tb4 enzyme reduction.

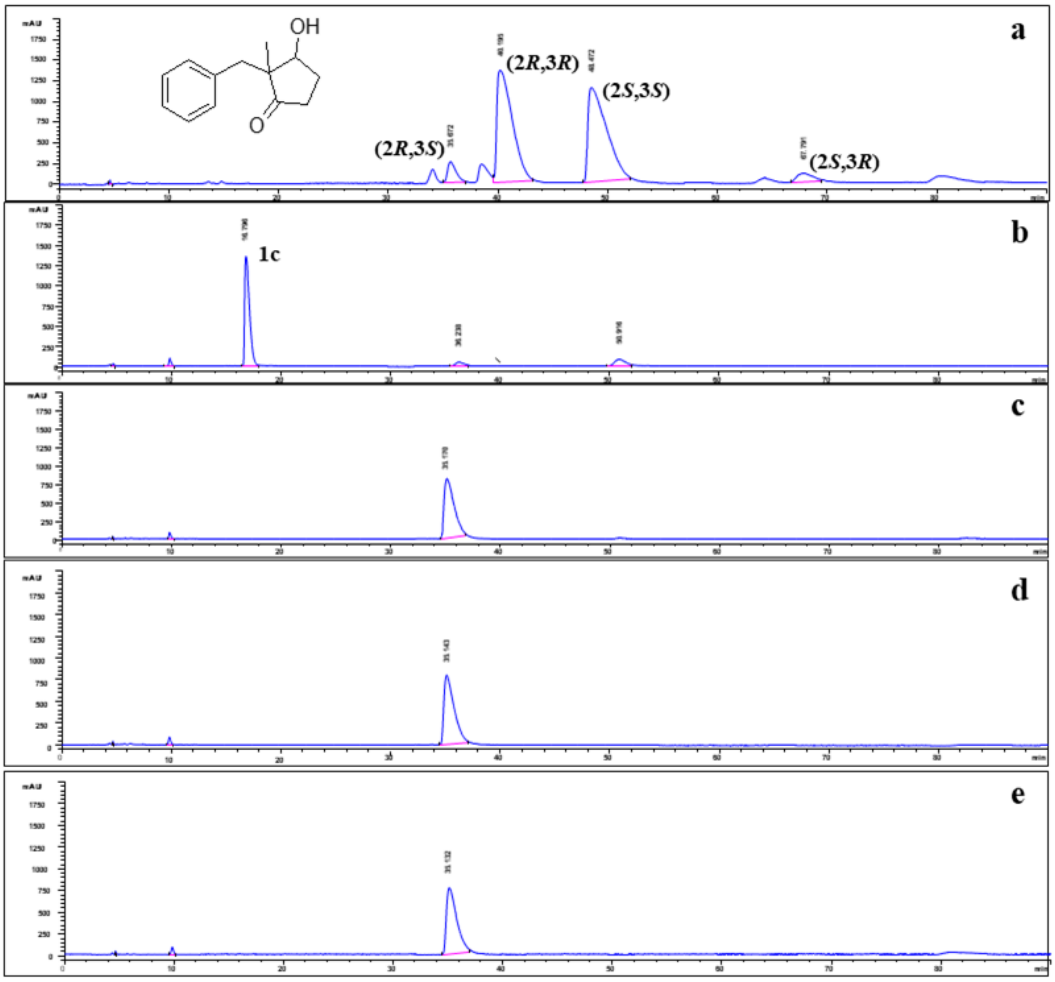


Figure S7 HPLC traces for **a**) the $NaBH_4$-reduction products of **1c** (racemic diastereomers); **b**) the WT enzyme reduction; **c**) the Tb1 enzyme reduction; **d**) the Tb2 enzyme reduction; **e**) the Tb3 enzyme reduction.

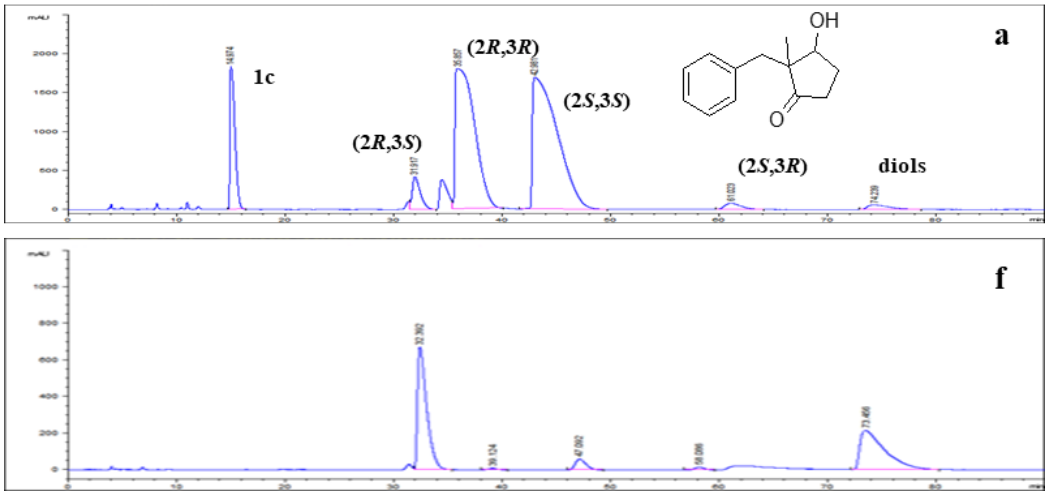


Figure S8 HPLC traces for a) the $NaBH_4$-reduction products of 1c (racemic diastereomers); f) the Tb4 enzyme reduction.

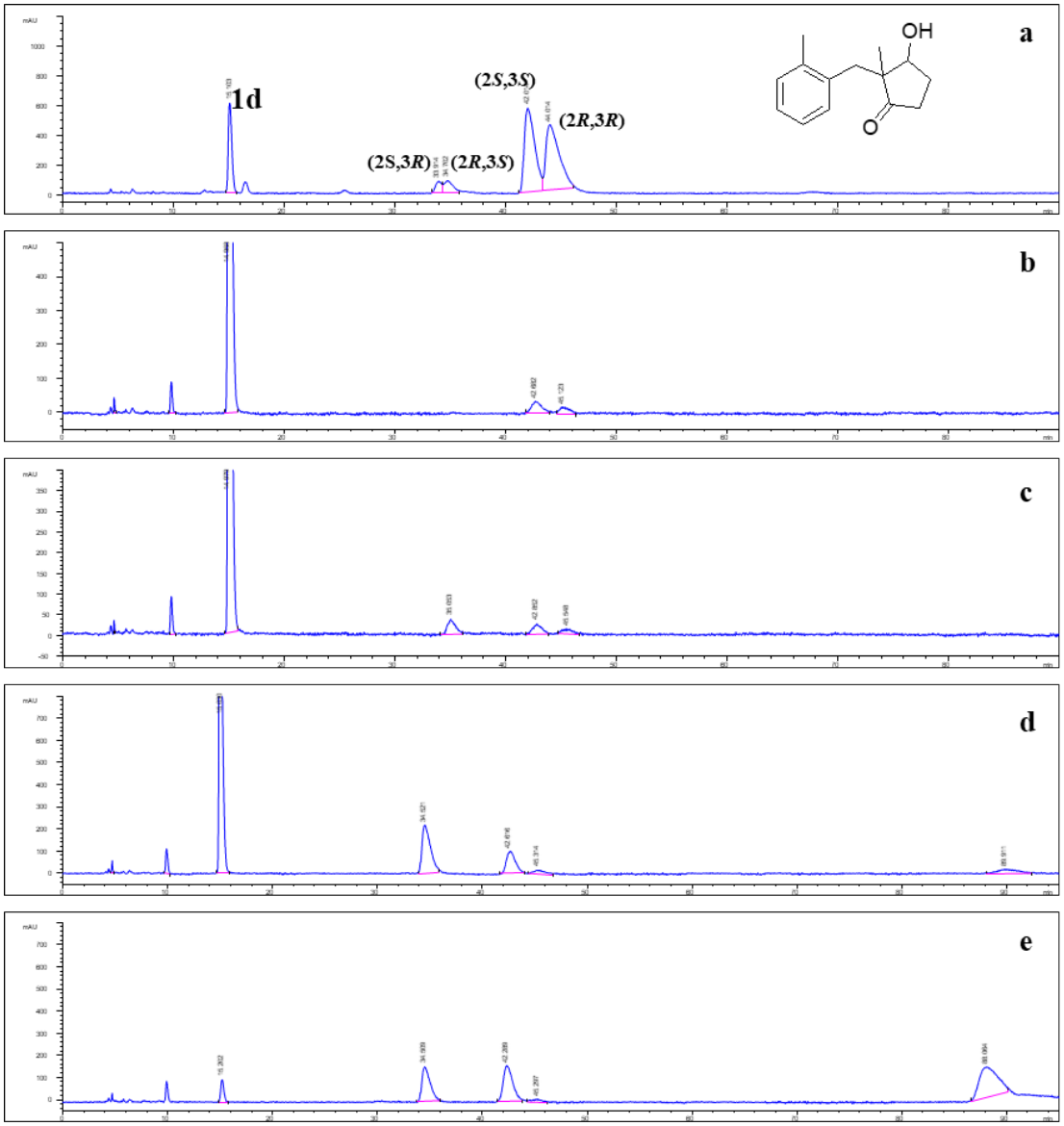


Figure S9 HPLC traces for a) the $NaBH_4$-reduction products of **1d** (racemic diastereomers); b) the WT enzyme reduction; c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction; e) the Tb3 enzyme reduction.

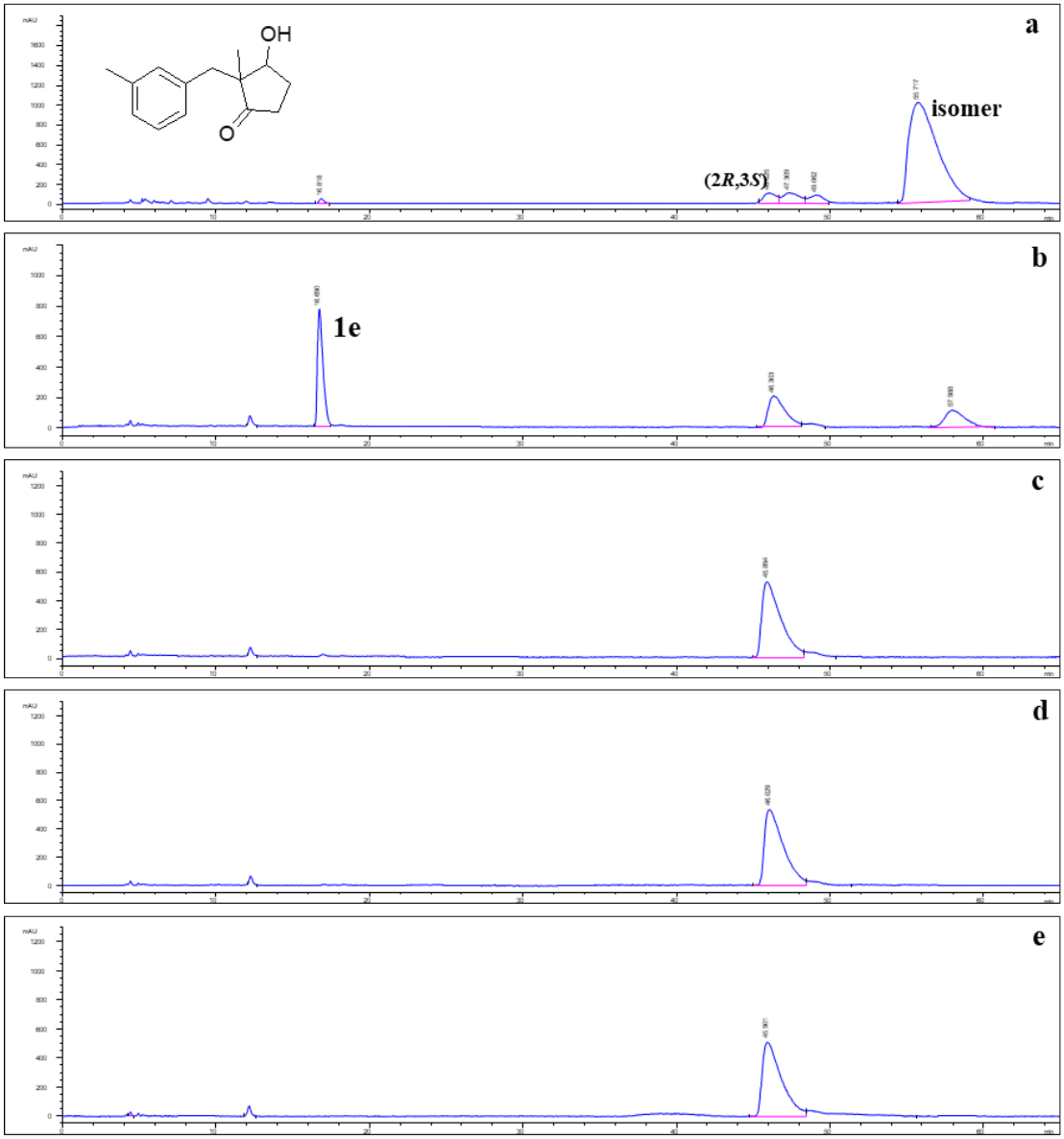


Figure S10 HPLC traces for a) the $NaBH_4$-reduction products of **1e** (racemic diastereomers); b) the WT enzyme reduction; c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction; e) the Tb3 enzyme reduction.

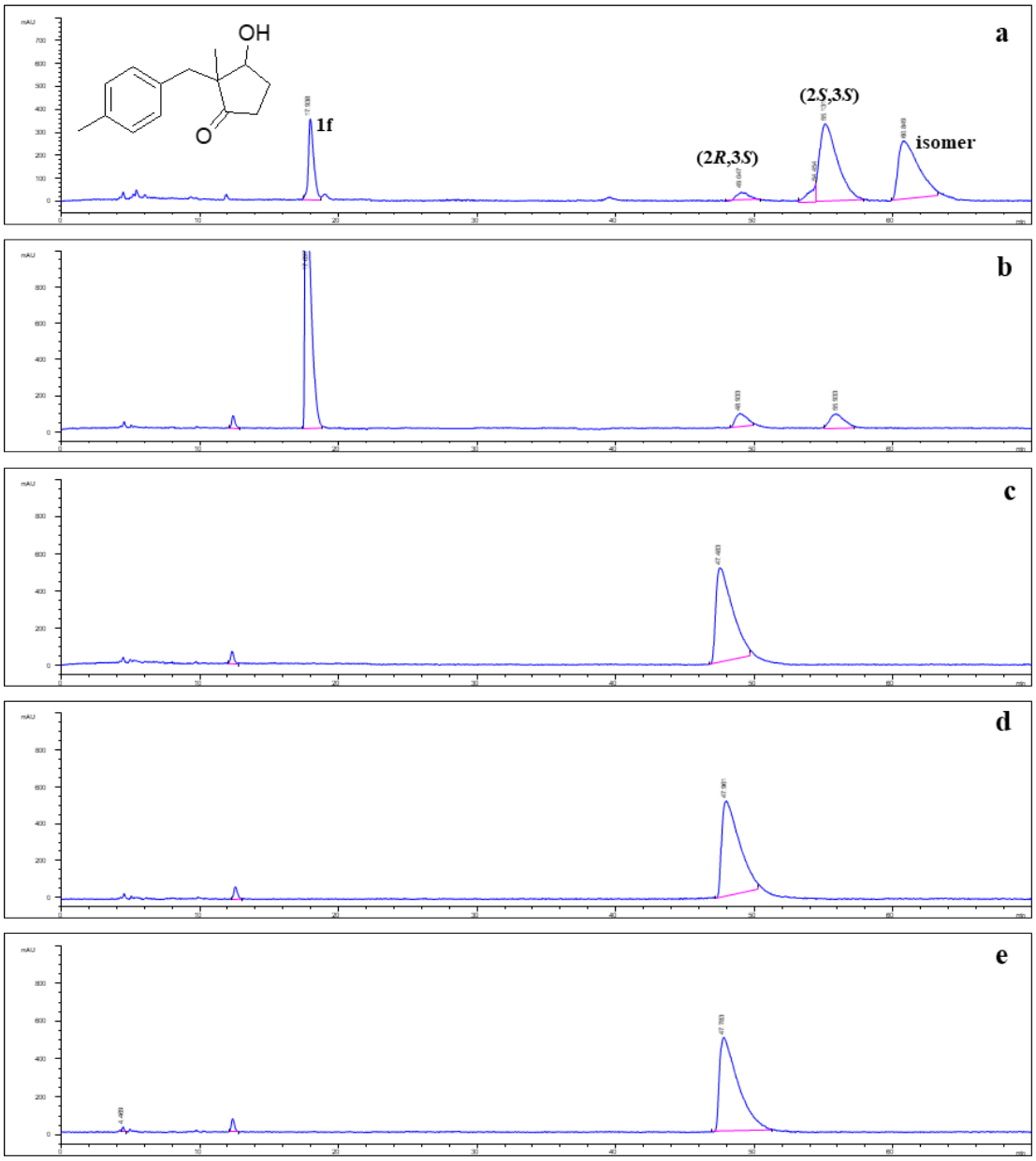


Figure S11 HPLC traces for a) the $NaBH_4$-reduction products of **1f** (racemic diastereomers); b) the WT enzyme reduction; c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction e) the Tb3 enzyme reduction.

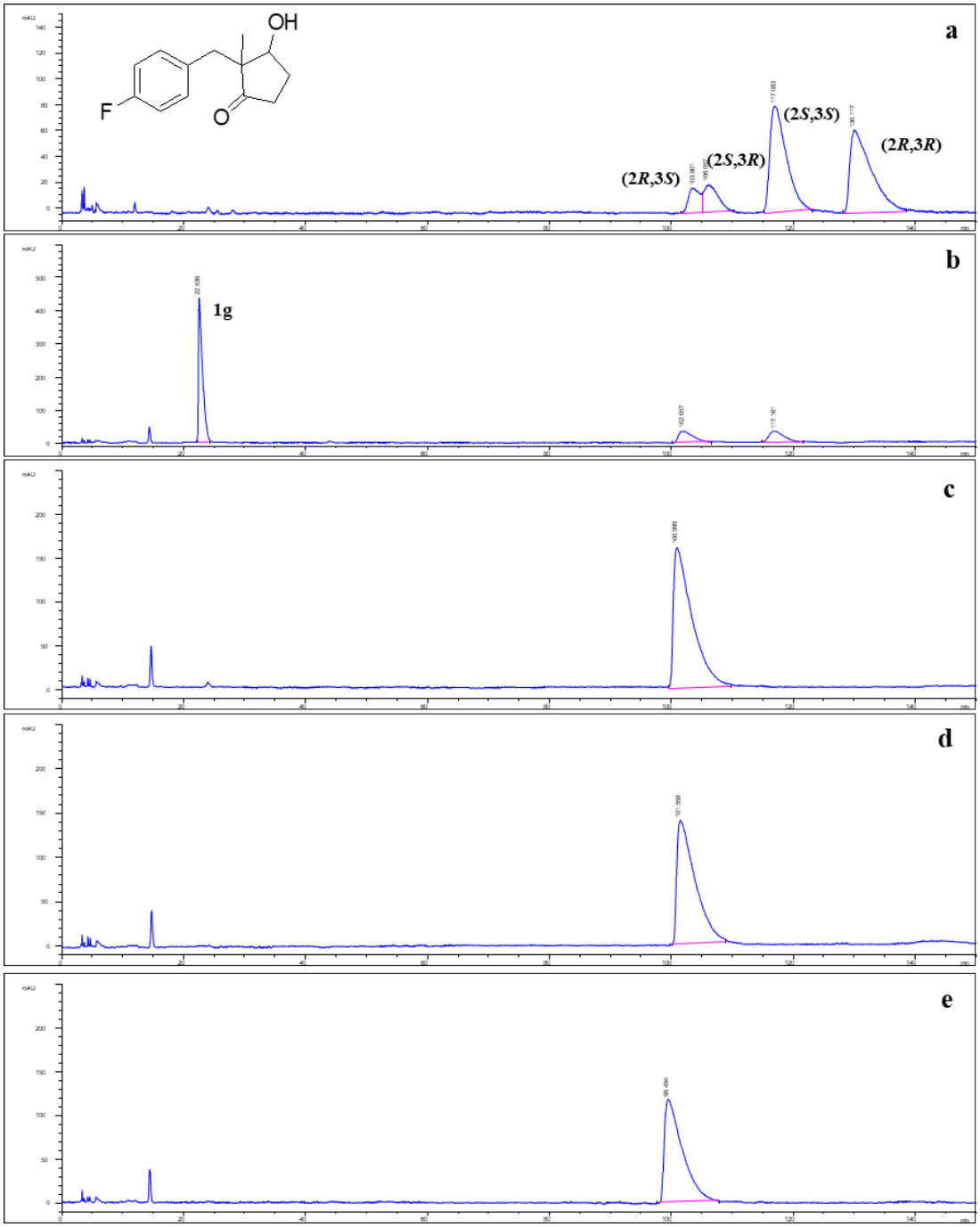


Figure S12 HPLC traces for a) the $NaBH_4$-reduction products of **1g** (racemic diastereomers) b) the WT enzyme reduction; c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction; e) the Tb3 enzyme reduction.

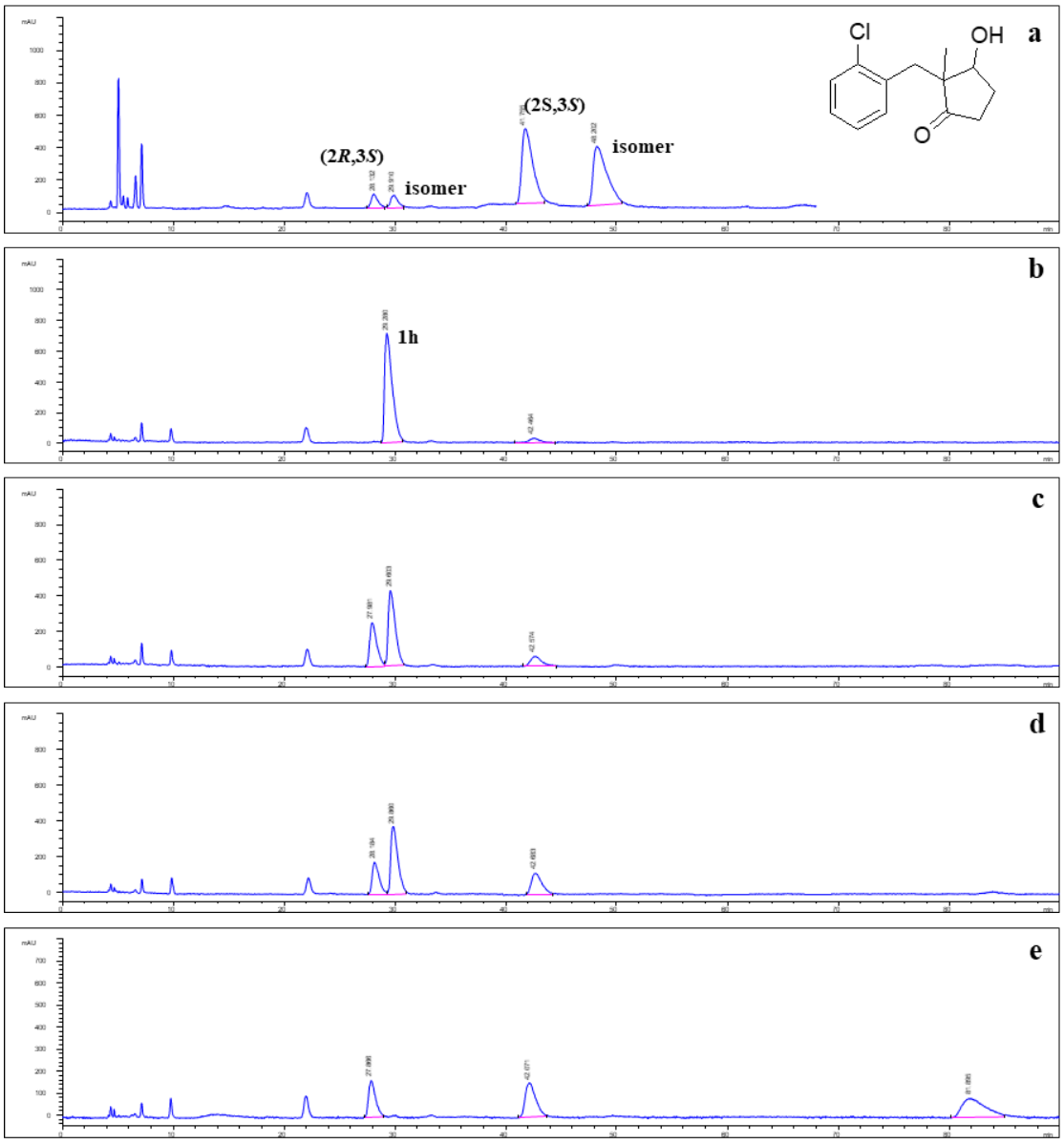


Figure S13 HPLC traces for a) the $NaBH_4$-reduction products of **1h** (racemic diastereomers) b) the WT enzyme reduction; c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction; e) the Tb3 enzyme reduction.

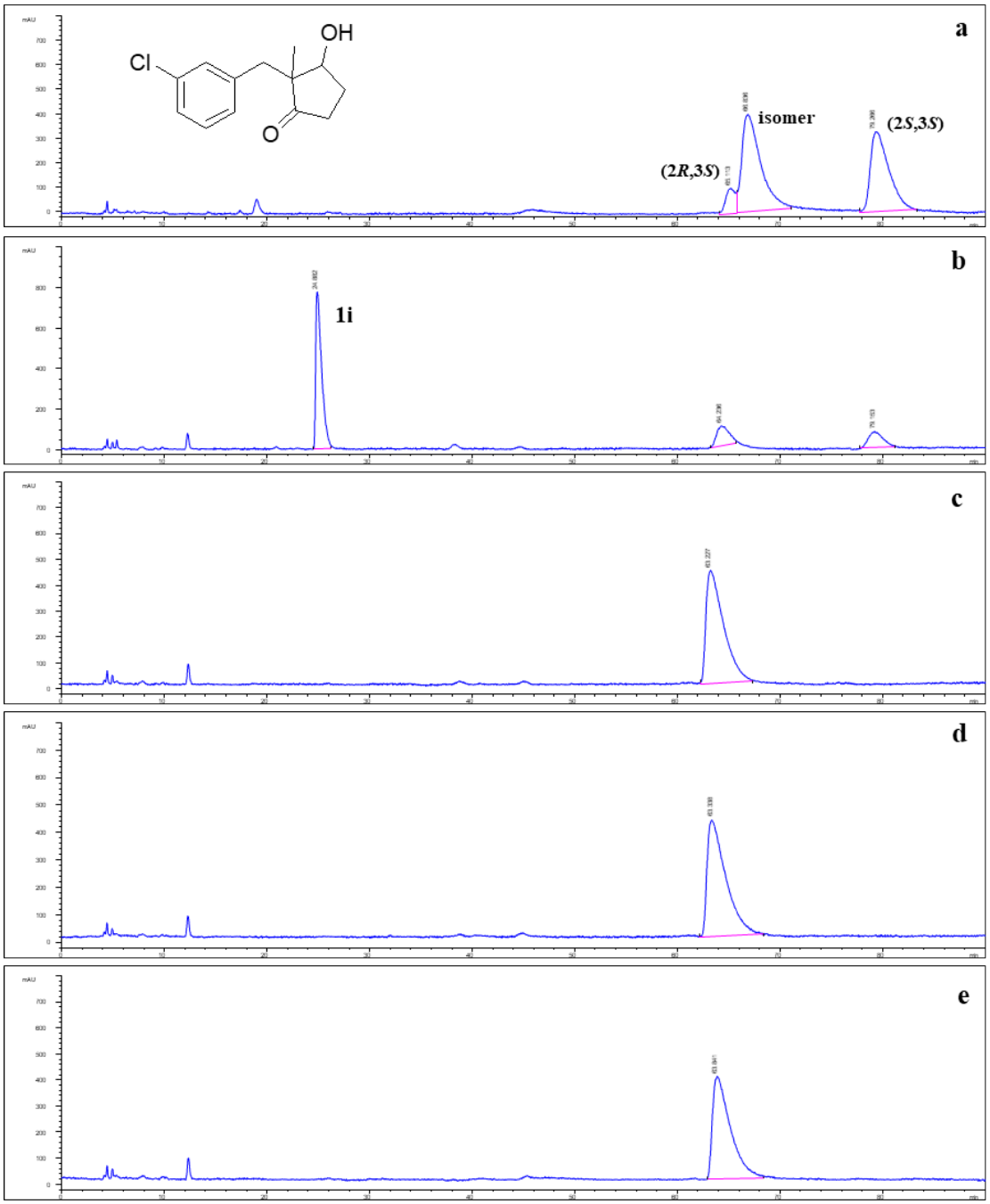


Figure S14 HPLC traces for a) the $NaBH_4$-reduction products of **1i** (racemic diastereomers) b) the WT enzyme reduction; c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction; e) the Tb3 enzyme reduction.

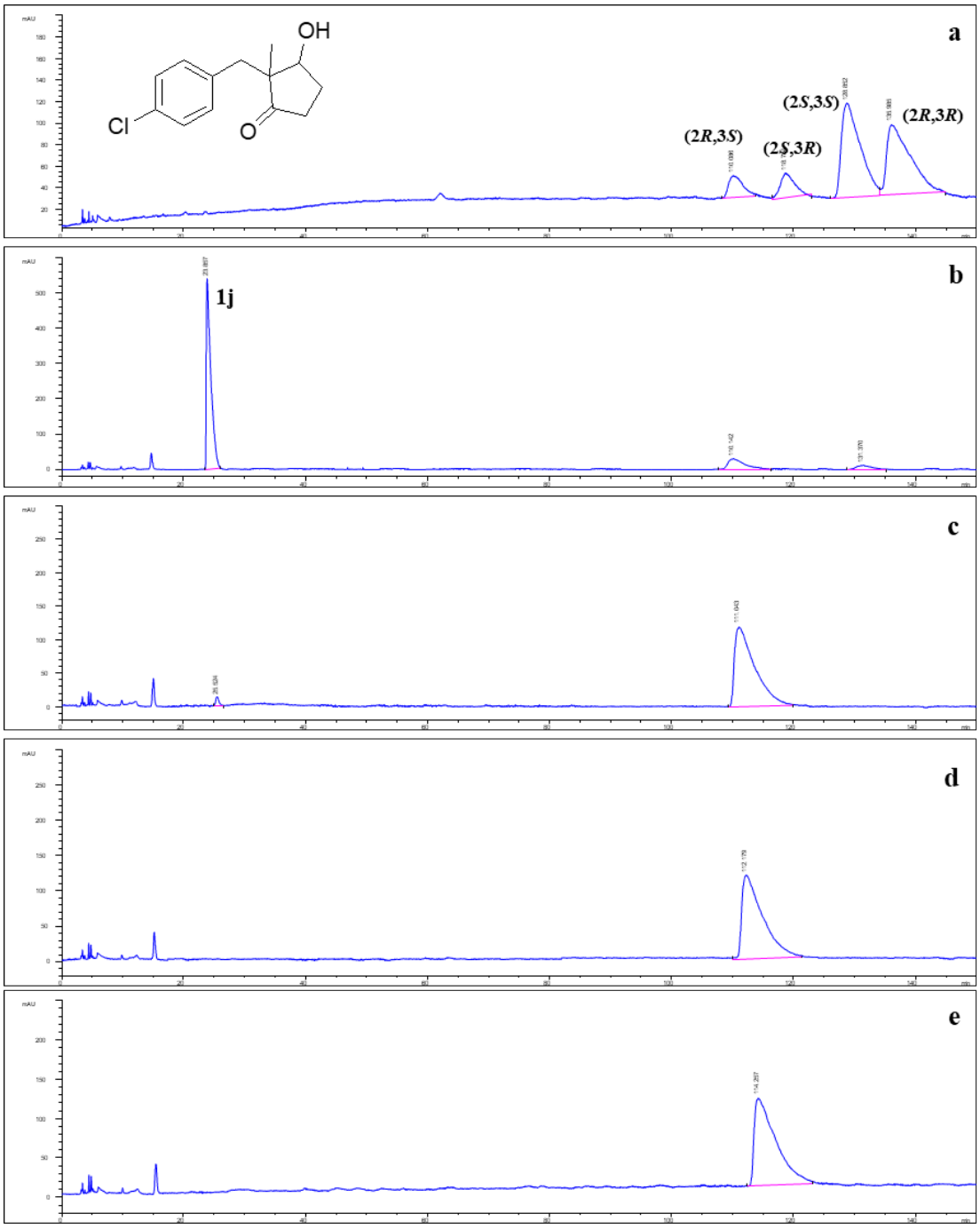


Figure S15 HPLC traces for a) the $NaBH_4$-reduction products of **1j** (racemic diastereomers) b) the WT enzyme reduction; c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction; e) the Tb3 enzyme reduction.

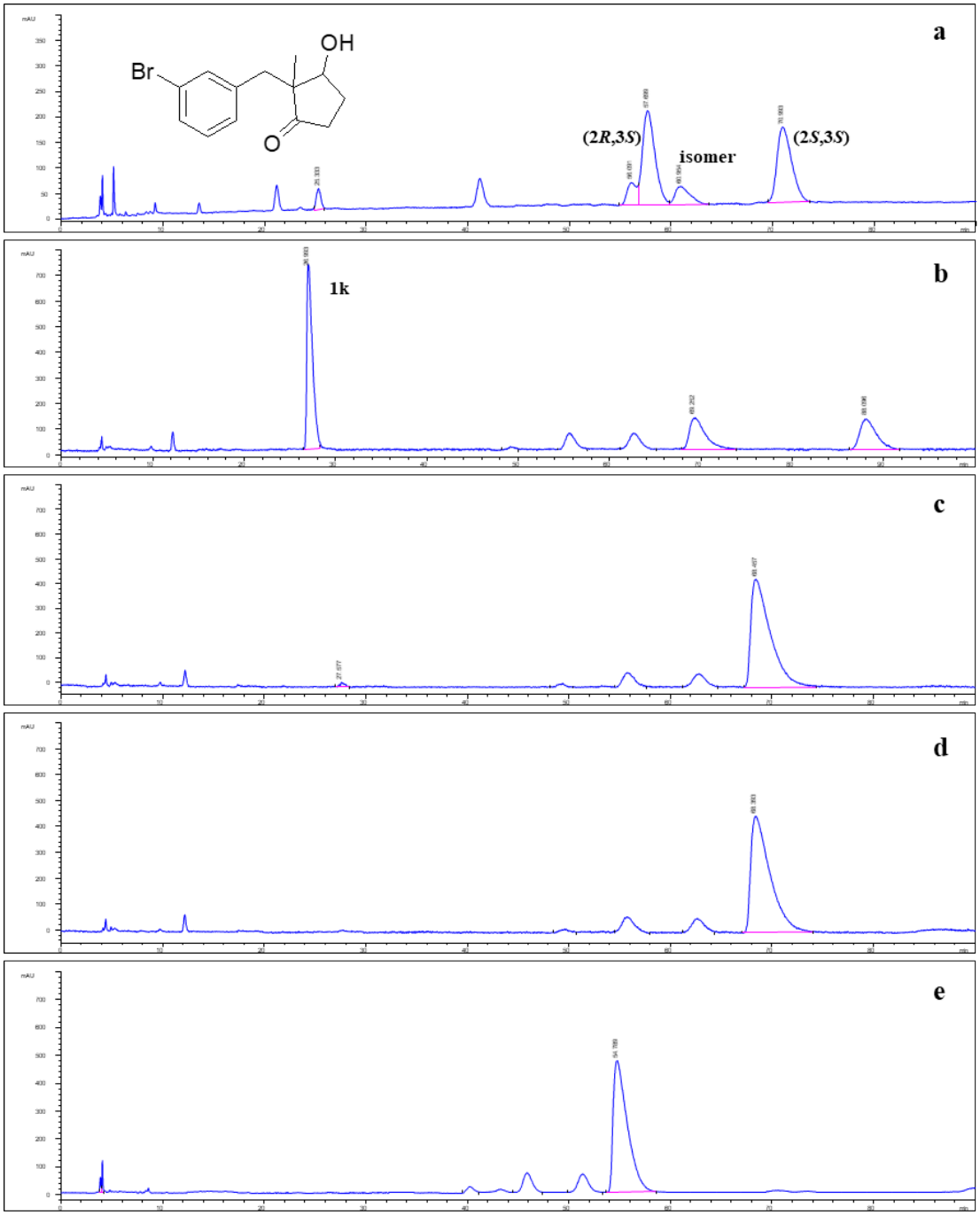


Figure S16 HPLC traces for a) the $NaBH_4$-reduction products of **1k** (racemic diastereomers) b) the WT enzyme c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction; e) the Tb3 enzyme reduction.

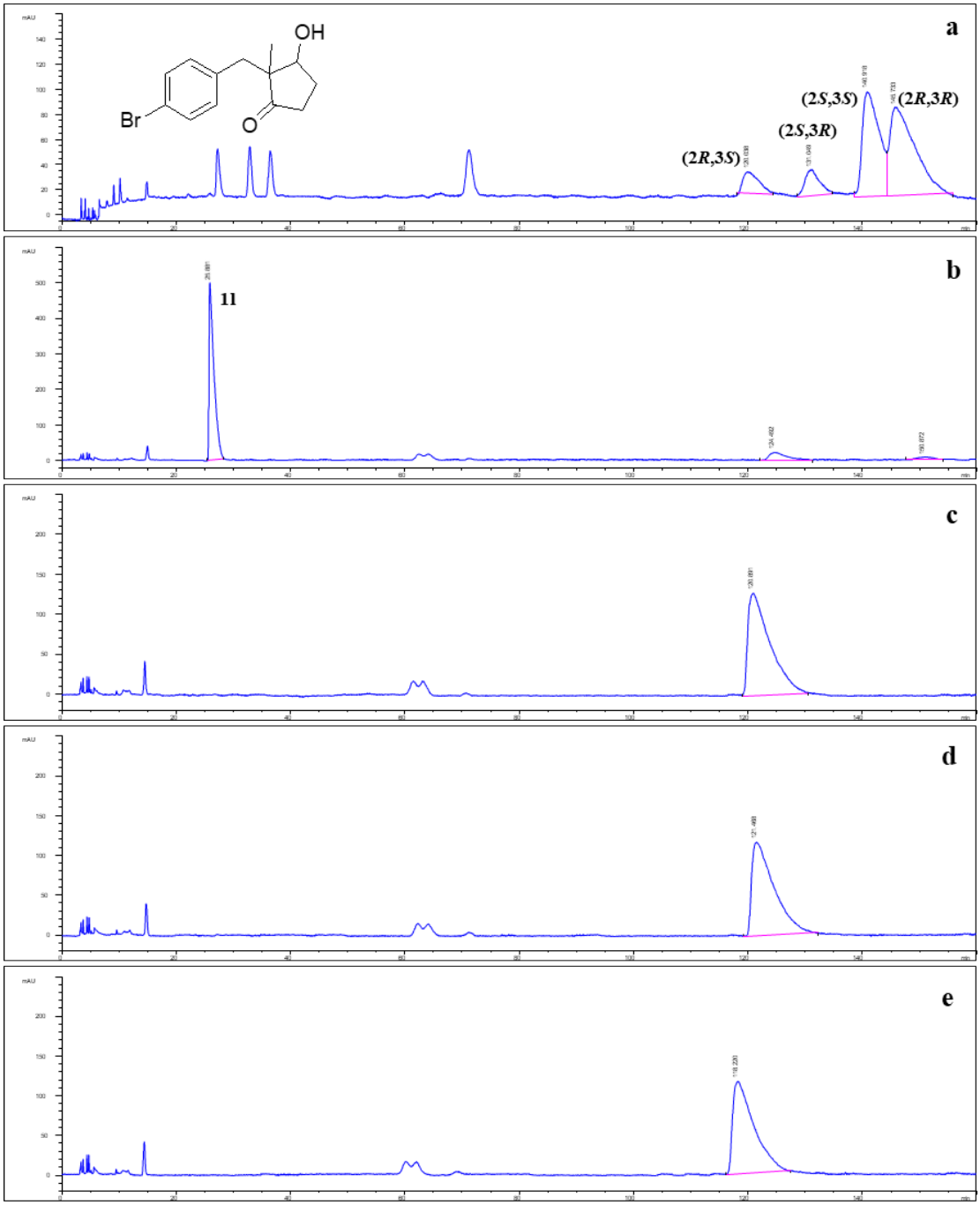


Figure S17 HPLC traces for a) the $NaBH_4$-reduction products of **1l** (racemic diastereomers) b) the WT enzyme reduction; c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction; e) the Tb3 enzyme reduction.

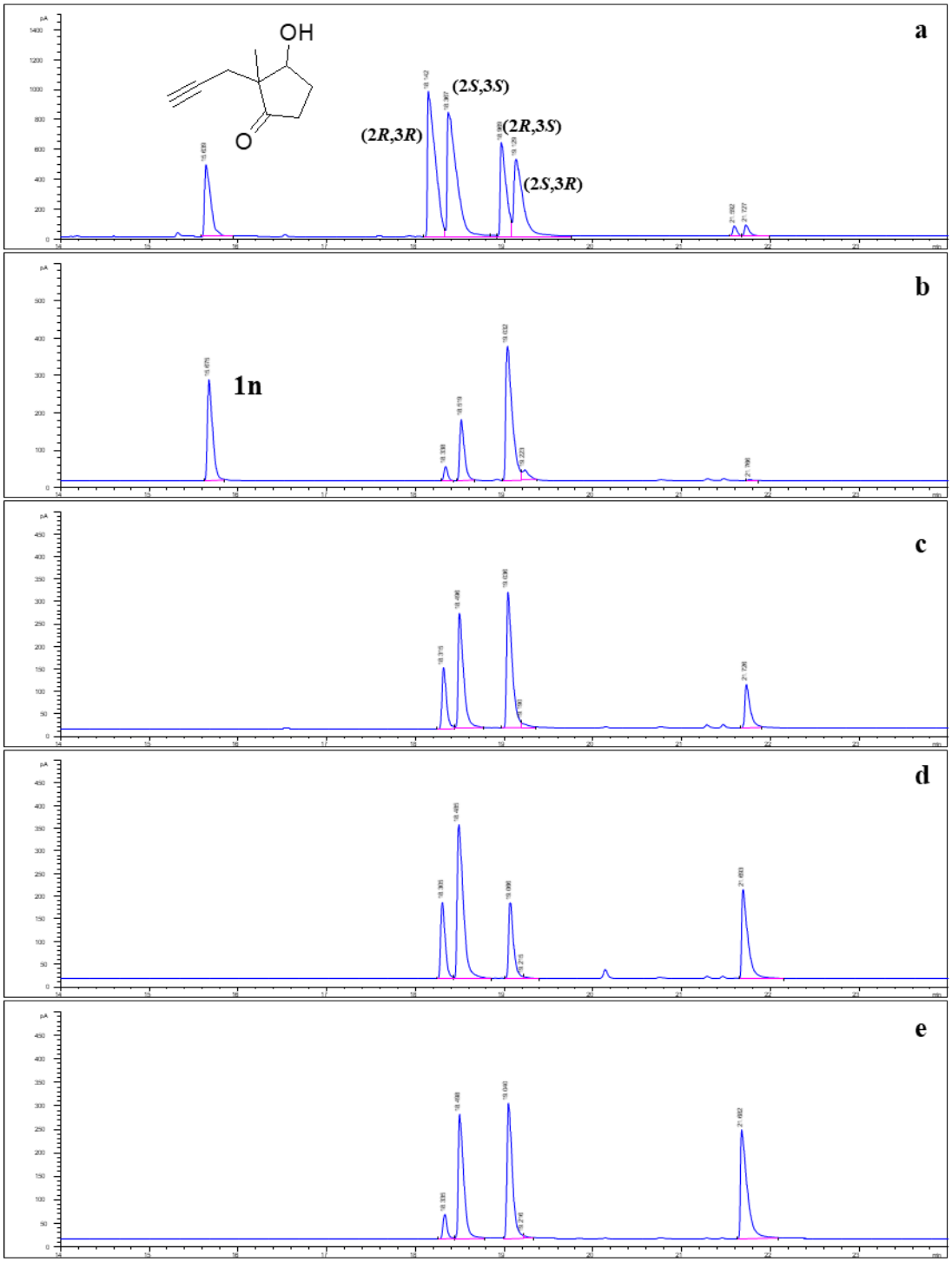


Figure S18 HPLC traces for a) the $NaBH_4$-reduction products of **1n** (racemic diastereomers) b) the WT enzyme reduction; c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction; e) the Tb3 enzyme reduction.

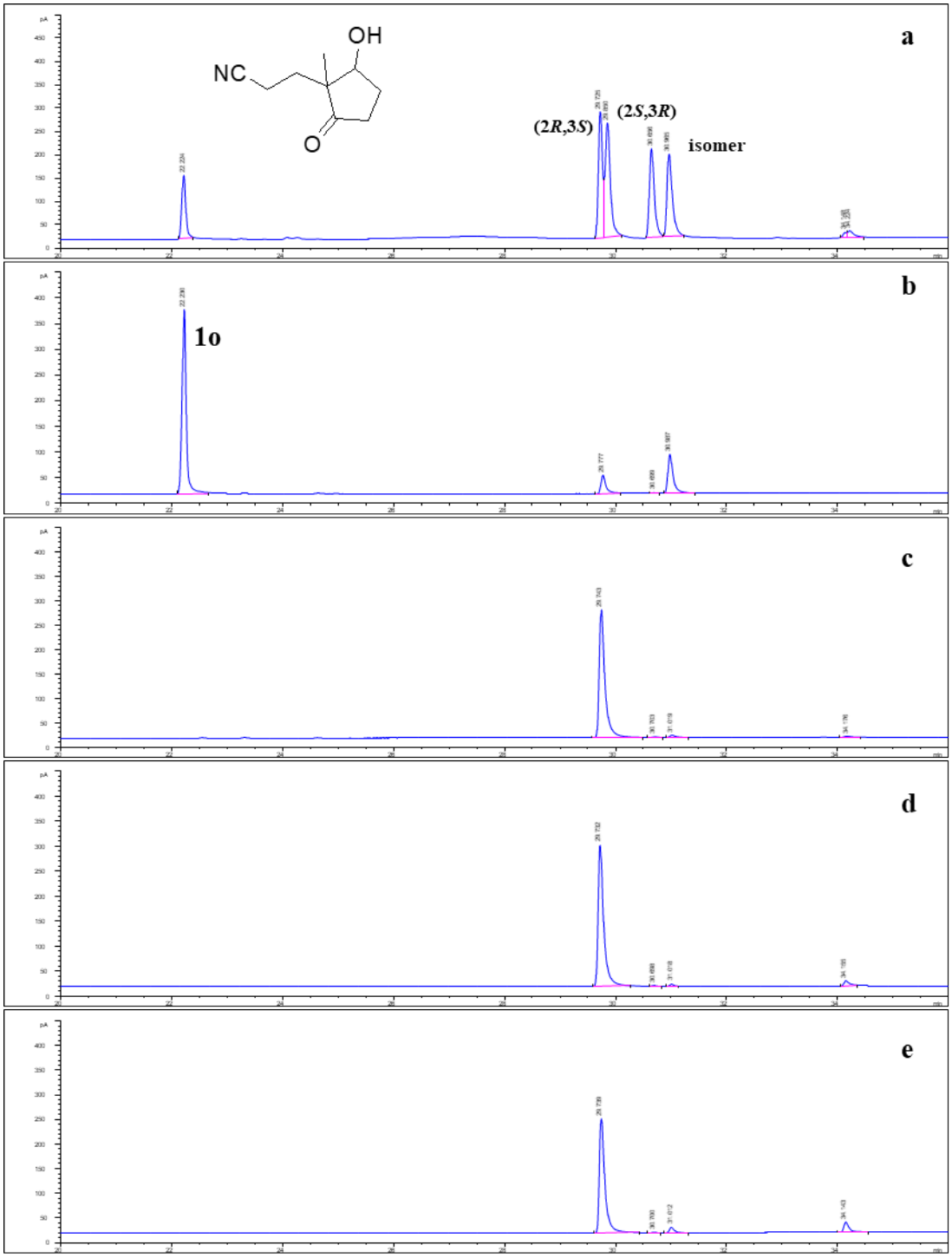


Figure S19 HPLC traces for a) the $NaBH_4$-reduction products of **1o** (racemic diastereomers) b) the WT enzyme reduction; c) the Tb1 enzyme reduction; d) the Tb2 enzyme reduction; e) the Tb3 enzyme reduction.

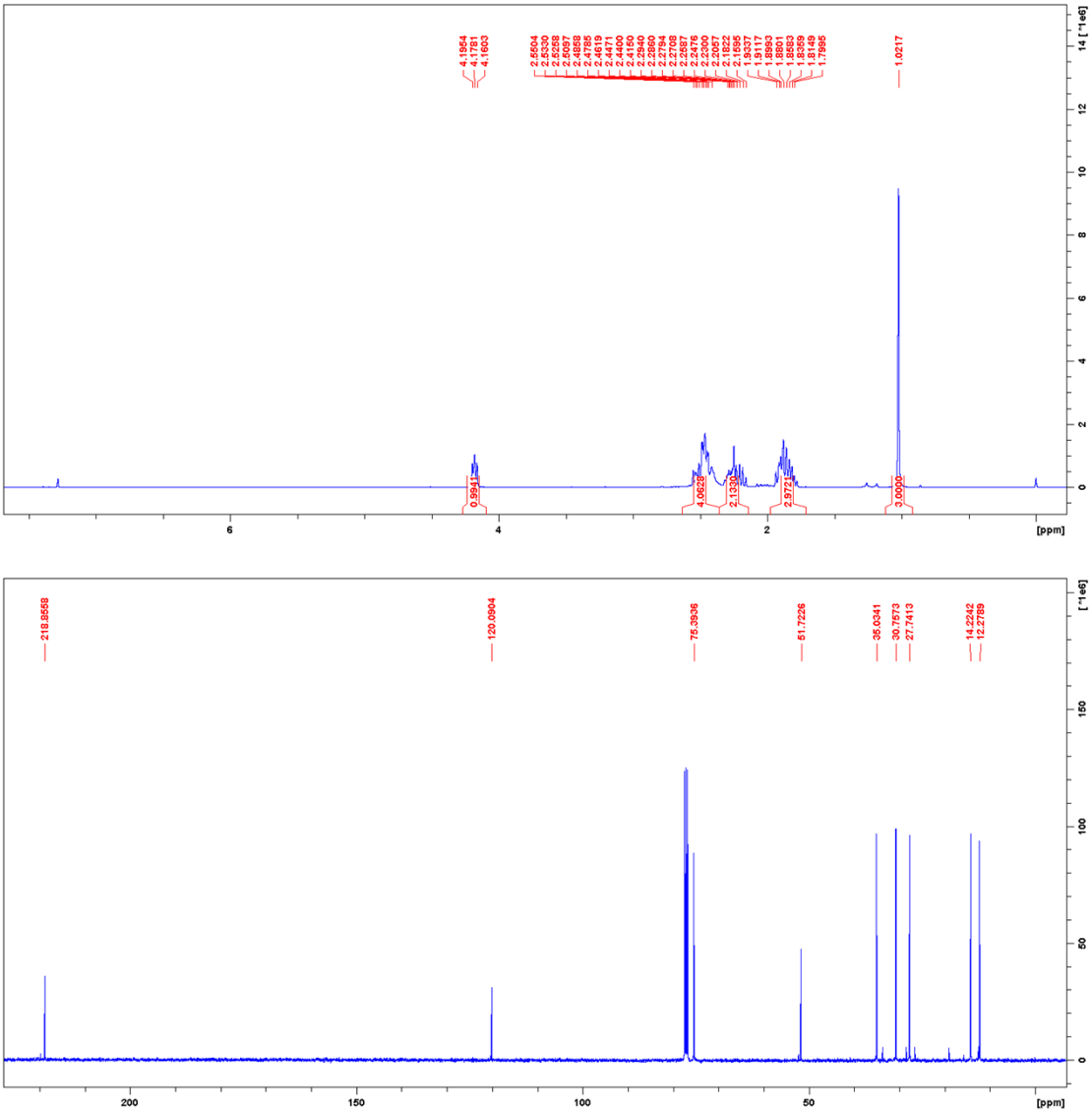


Figure S20 ^{1}H and ^{13}C NMR spectra of (2*R*,3*S*)-**2o**.

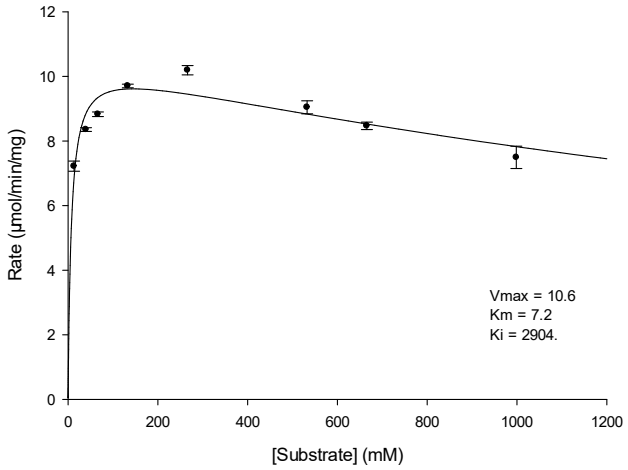


Figure S21 Michaelis-Menten equation curves of TbADH toward isopropanol.

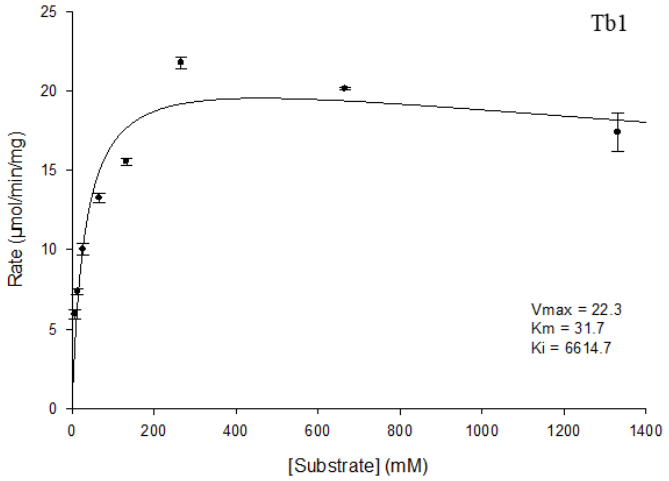


Figure S22 Michaelis-Menten equation curves of Tb1 toward isopropanol.

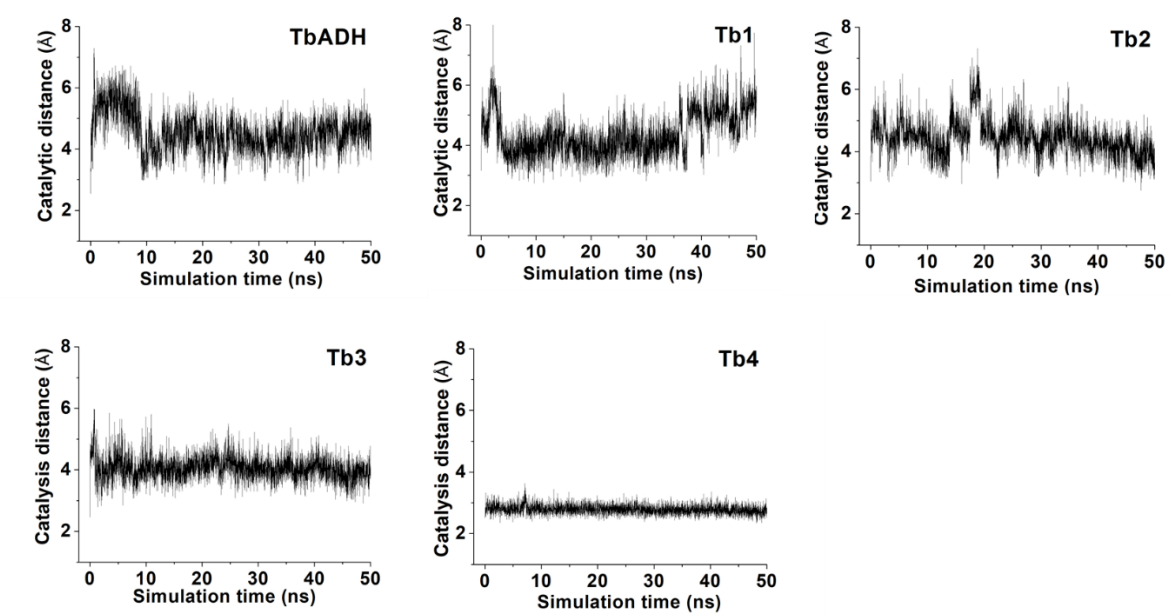


Figure S23 Catalytic distance of hydride transfer (NADPH-**1a**).