Supplementary Information

Directed evolution of cytochrome P450DA hydroxylase activity for stereoselective biohydroxylation

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1. Materials and methods

General experimental materials and procedures

Column chromatography was performed on silica gel (200-400 mesh). ^{1}H NMR (400 MHz) chemical shifts were reported in ppm (δ) relative to tetramethylsilane (TMS) with the solvent resonance employed as the internal standard. Data were reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets), coupling constants (Hz) and integration. ^{13}C NMR (100 MHz) chemical shifts were reported in ppm (δ) from tetramethylsilane (TMS) with the solvent resonance as the internal standard. Melting point was uncorrected.

Isopropyl-β-D-thiogalactopyranoside (IPTG, >99%) and kanamycin sulfate (>99%) salt were purchased from Solarbio (Beijing, China). The following proteins were purchased: Taq polymerase (Biomad), KOD-Plus-Neo (Toyobo), *Dpn*I (Thermo scientific) and PrimerSTAR (Takara). Chemicals (2-chloroethyl)benzene **1a**, ethylbenzenes **1i-1m**, allylbenzenes **1n-1r**, β-haloalcohol (*R*,*S*)-**2a**, (*R*)-**2a**, racemic 1-phenylethan-1-ols **2i-2m**, racemic 1-phenylprop-2-en-1-ol (*R*,*S*)-**2n** and 2-phenyloxirane (*R*,*S*)-**3a** were purchased from commercial suppliers (J&K Chemical, Sigma Aldrich, Innochem). Halohydrocarbons **1b-1h** and racemic β-haloalcohols **2b-2h** were synthesized by chemical methods.[1-5] Other racemic 1-phenylprop-2-en-1-ols **2o-2r** were synthesized via chemical reduction of the corresponding benzaldehydes.[6] Unless otherwise noted, all the other chemical reagents and solvents were obtained from commercial suppliers and used without further purification.

Analytical chiral high-performance liquid chromatography (HPLC) was performed on Shimadzu LC-20AD equipped with Chiralcel OD-H column (4.6 mmΦ × 250 mmL, particle size 5 μm), Chiralcel OJ-H column (4.6 mmΦ × 250 mmL, particle size 5 μm), Chiralpak AD-H column (4.6 mmΦ × 250 mmL, particle size 5 μm), or Chiralpak OB-H column (4.6 mmΦ × 250 mmL, particle size 5 μm) purchased from Daicel Chemical Industries. Analytical chiral gas chromatography (GC) was performed on Agilent 7890B gas chromatograph equipped with a flame ionization detector (FID) using BGB-175 column (BGB Analytik, Switzerland, length: 30 m, inner diameter: 0.25 mm, film thickness: 0.25 μm) with nitrogen as the carrier gas. The HPLC and GC conditions for analyzing synthesized chiral alcohols **2a-2r** were described in chromatogram section.

Library creation

The recombinant strain *E. coli* (P450DA-WT) has been constructed in previous study.[7] Random mutagenesis was carried out at the region of 1-1575 bp containing the P450's heme domain and a small portion of cytochrome P450 reductase region by error-prone PCR using Taq polymerase and 0.2 mM $MnCl_2$. The resulting PCR products were purified by agarose gel electrophoresis and used as primers to perform MEGAWHOP PCR using whole P450DA-WT plasmid as the template.[8] Target genes were amplified using polymerase KOD-Plus-Neo, followed by digesting with *Dpn*I. After an additional DNA purification step, the PCR products were transformed into competent cells of strain *E. coli* BL21(DE3) by heat shock method. Site-saturation libraries were generated

by the "QuickChange" PCR method with PrimerSTAR DNA polymerase. The resulting PCR products were purified, digested with *Dpn*I and directly transformed into competent cells of strain *E. coli* BL21(DE3) by heat shock method. Primers used for random mutagenesis PCR and site-saturation mutagenesis PCR were listed in tables below.

Primers for random mutagenesis PCR

Primers	Primer sequence
T7-Forward	TAATACGACTCACTATAGGG
DA-Reverse	TCGCTTGGAACATTATTAAC

Primers for site-saturation mutagenesis PCR

Primers	Primer sequence
190-Forward	TCATACCATGACCATGNNKAGCCGTCCGCC
190-Reverse	CGGACGGCTMNNCATGGTCATGGTATGATGC
356-Forward	AGGTCAGCAGNNKGCACTTTTAATTCCG
356-Reverse	TAAAAGTGCMNNCTGCTGACCTTTACGG
486-Forward	ACCGGTGTGNNKCTGACAGTTGCCTATGG
486-Reverse	ATAGGCAACTGTCAGMNNCACACCGGTCCC

Expression of P450 libraries in 96-well plates

The expression of P450DA variants was performed in deep-96-well plate format (2.2-mL). Individual colonies from P450 libraries were cultivated in 1200 μL of Terrific Broth medium containing 50 μg mL^{-1} kanamycin (final concentration) and shaken at microplates thermo-shaker (37 ºC, 600 rpm) for 8 h. Then 200 μL culture was taken and transferred to another sterile deep-96-well plate, mixed with 200 μL sterile 30% glycerol, and stored in -80 ºC. To the 1000 μL remaining culture, 200 μL fresh Terrific Broth medium containing 1.2 mM IPTG was added (0.2 mM IPTG final concentration). Induced cells were shaken at microplates thermo-shaker (25 ºC, 600 rpm) for 14 h. The cells were harvested by centrifugation (2300×g, 12 min, 4 ºC).

Screening of variants by the colorimetric HTS assay

To each well, 220 μL PB buffer (Na_2HPO_4-KH_2PO_4) containing **1a** (2 mM final concentration) was added to resuspend the cell pellet. The plates were shaken on microplates thermo-shaker (30 ºC,1000 rpm) for 8 h, followed by centrifugation (2300×g, 12 min, 4 °C). Then 220 μL reaction supernatant was taken and transferred to another deep-96-well plate. To it, 100 μL cell-free extract of halohydrin dehalogenase HheD6 was added, and the deep-96-well plate was shaken on microplates thermo-shaker (30 °C,1000 rpm) for 4 h. Then 30 μL solution I was added to each well to denature the proteins. The 96-well plate was centrifugated for 20 min (2300×g, 4 °C). Then 100 μL supernatant was taken and added to a new 96-well plate (330-μL) that containing 50 μL solution I and 50 μL solution II. The 96-well plate was vibrated for few seconds and analyzed by measuring the absorbance at 460 nm. Enzyme: HheD6 cell-free extract. Microplate spectrophotometer: SpetraMax® Plus384 (Molecular

Devices, America). Microplates shaker: MB100-4A THERMO-SHAKER (Hangzhou Allsheng, China). Colorimetric reagents: solution I: 0.25M $NH_4Fe(SO_4)_2$ in 9 M HNO_3; solution II: saturated $Hg(SCN)_2$ in absolute ethanol.

Confirmation of positive variants by HPLC analysis

E. coli BL21(DE3) cells transformed with plasmid encoding P450DA positive mutants were grown overnight in 10 mL Terrific Broth medium containing 50 μg mL^{-1} kanamycin (final concentration) at 37 °C and 250 rpm. Expression cultures were inoculated with 2 mL of preculture into 100 mL Terrific Broth medium (50 μg mL^{-1} kanamycin, final concentration) in a 500 mL flask and incubated at 37 °C, 250 rpm for 2-4 h until the OD_{600} up to 0.6-0.8. Then the flask culture was induced with IPTG (0.2 mM final concentration) and shaken at 25 °C, 250 rpm for 12 h. The cells were harvested by centrifugation (7000×g, 5 min, 4 °C). PB buffer (Na_2HPO_4-KH_2PO_4) containing **1a** (2 mM, final concentration) was added to resuspend the cell pellet to a cell density of 10 g cdw/L. The reaction mixture (5 mL) was shaken at 25 °C, 250 rpm for 24 h. The reaction mixture was then extracted using 5 mL ethyl acetate. The organic phase was separated, dried on anhydrous sodium sulfate and analyzed by chiral HPLC.

Expression and purification of P450DA-WT

The P450DA-WT encode gene of heme region (1-1479 bp, 493 amino acids) was cloned and expressed in *E. coli* Rosetta (DE3) containing a C-terminal 6 x His-tag using pET-22b(+) with *Nde*I and *Xho*I, respectively. A single colony of the recombinant *E. coli* strain harboring plasmid pET-22-DAWT was picked to inoculate to 100 mL LB culture containing 50 μg/mL of ampicillin. The cells grew overnight at 37 °C, 220 rpm. For the expression and production of recombinant protein, 4000 mL LB supplemented with 50 μg/mL of ampicillin was inoculated with 10% (v/v) overnight culture, and the cells grew at 37 °C, 220 rpm until the OD_{600} around 0.8 (2-4 hours). Then IPTG was added to the culture with a final concentration of 0.5 mM, and the culture was incubated at 16°C, 220 rpm overnight to induce the expression of the recombinant P450 enzymes. Cells were harvested by centrifugation (5000×g, 15 min, 4 °C), and the cell pellet was stored at -20 °C for further use.

Cells were resuspended and disrupted by sonication, and the resulting solution was centrifuged (11000×g, 4 °C, 15 min) to obtain cell free extract (CFE). The CFE was filtered and loaded (flow rate 2.5 mL min^{-1}) on a 5 mL Hiscap Smart 6FF column (Smart-Lifesciences) pre-equilibrated with buffer A (25 mM Tris-HCl, 350 mM NaCl, pH 8.0) using an AKTA Pure system (GE Healthcare). To remove nonspecifically bound proteins, the column was washed with 20 column volumes of 5% buffer B (25 mM Tris-HCl, 350 mM NaCl, 200 mM imidazole, pH 8.0) at a flow rate of 2.5 mL min^{-1}. Then the target protein was eluted and collected with 50% buffer B. Fractions containing target protein were identified by SDS-PAGE and desalinized using Desalting column (GE Healthcare) with buffer C (20 mM Tris-HCl, 50 mM NaCl, pH 8.0). Then the collected fractions were loaded on an anion exchange column HiTrap Q HP (GE Healthcare) and pre-equilibrated with buffer C. Elution of target protein was achieved using buffer D (20 mM Tris-HCl, 500 mM NaCl, pH 8.0). The fractions containing

target protein were identified by SDS-PAGE, pooled and concentrated to 5 mL. Afterward, the concentrated purified protein was loaded on a 10/600 Superdex 200 column (GE Healthcare) and pre-equilibrated with buffer E (25 mM Tris-HCl, 200 mM NaCl, pH 8.0). Then the target protein was eluted with buffer F (10 mM Tris-HCl, pH 8.0, 200 mM NaCl, 1 mM DTT) at a flow rate of 1 ml/min. Protein concentration was measured by the Bradford method (4 mg/mL).

Crystallization and structures determination

Crystallization experiment of P450DA-WT was carried out at 18 °C using 96 well sitting drop vapor diffusion plates and commercial crystal screen kits. Drops consisting of 100 nL of protein sample and 100 nL of crystallization reagent were equilibrated against a 100 mL reservoir. Successful crystal growth of P450DA-WT could be observed in reservoir solutions [8 % (w/v) polyethylene glycol 8000, 0.1 M MES, pH 6.5]. Crystal was rapidly soaked in the corresponding reservoir solution supplemented with 20% glycerol as cryo-protectant, mounted on loops, and flash-cooled at 100 K in a nitrogen gas cryo-stream. Crystal diffraction data were collected from a single crystal at Shanghai Synchrotron Radiation Facility BL18U beamline (SSRF, Shanghai, China) with a wavelength of 0.9795 Å at 100 K. The diffraction data were processed and scaled with XDS.[9] The structure was solved by the molecular replacement method with starting model PDB entry 3NPL,[10] which shares 50 % sequence similarity with the P450DA. Initial model was build using PHENIX autobuild,[11] and manual adjustment of the model was carried out using the program COOT.[12] Afterward, the models were refined by PHENIX refinement[11] and Refmac5.[13] Stereochemical quality of the structure was checked by using PROCHECK.[14] All of residues locate in the favored and allowed region and none in the disallowed region. Final structure validation was done with Protein Data Bank ADIT Servers. Diffraction data and coordinates have been deposited in the Protein Data Bank under accession codes (7F3H, 2.50 Å). All structural illustrations were generated using the PyMOL software.[15]

Data collection and refinement statistics for P450DA-WT.

	P450DA-WT
PDB code	7F3H
Data collection	
Temperature (k)	100
Wavelength (Å)	0.9795
Space group	P 21 21 2
a, b, c (Å)	426.301 62.799 95.188
α, β, γ (°)	90.00 90.00 90.00
Resolution range (Å)	50.00- 2.66 (2.71-2.66)
No. of observations	852105
No. of unique reflections	74780 (3685)
Completeness (%)	100 (99.6)
<I>/δ(I)	10.9 (3.3)
Redundancy	11.4 (9.4)
R_{merge}	0.223 (0.865)
$R_{p.i.m}$	0.074 (0.273)
Structure refinement	
Resolution range (Å)	50.00- 2.66 (2.71-2.66)
R_{work}/R_{free}	0.20/ 0.25
No. atoms	
Protein	14900
Ligand/ion	168
Water	297
B-factors	
Protein	40.4
Ligand/ion	27.2
Water	31.1
Ramachandran	
Favored (%)	95.5
Allowed (%)	3.7
Outlier (%)	0.8
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.499

2. Supporting Fig. S1-S4

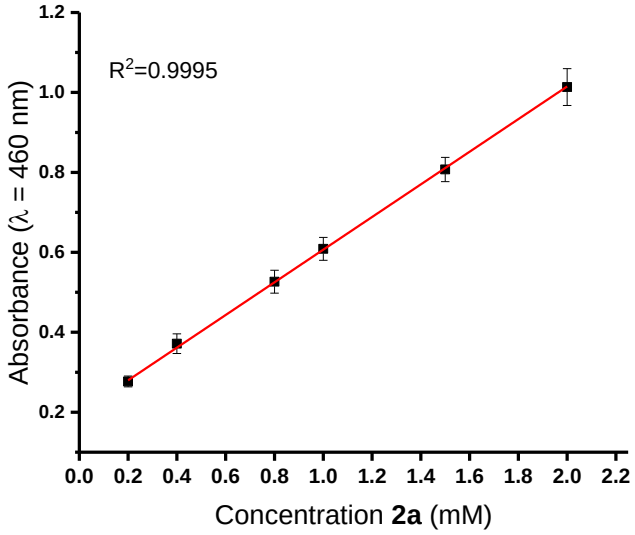


Fig. S1 Calibration curve for the concentration of **2a** determined by a dual-enzyme cascade based colorimetric assay.

(1) Preparation of cell-free extract. The HheD6 gene was optimized, synthesized and expressed in *E. coli* BL21(DE3) host using pET-28a(+) vector (*Nco*I/*Xho*I). The recombinant *E. coli* strain harboring recombinant plasmid pET-28-HheD6 was cultured in Terrific Broth medium (50 μg mL^{-1} kanamycin, final concentration) at 37 °C until the OD_{600} up to 0.6-0.8, followed by induction with 0.2 mM IPTG at 28 °C for 12-14 h. The cells were harvested (7000×g, 5 min, 4 °C) and washed twice with PB buffer (50 mM, pH 7.0). Then the resting cells were suspended to a concentration of 30 g cdw/L using PB buffer (50 mM, pH 7.0) and handled by ultrasonication. The crude cell-free extract of HheD6 was obtained by centrifugation (10000×g, 30 min, 4 °C) and stored at -20 °C.

(2) Determining the concentration of 2a by a dual-enzyme cascade based colorimetric assay. To a deep-96-well plate (2.2-mL) containing 200 μL PB buffer (50 mM, pH 7.0) containing 0.2-2 mM **2a**, 100 μL HheD6 cell-free extract was added. The 96-well plate was stirred at microplates thermo-shaker at 1000 rpm for 4 h. Then 30 μL solution I was added to the mixture to denature the proteins, and the 96-well plate was centrifugated for 20 min (2300×g, 4 °C). Then 100 μL supernatant was taken and added to a new 96-well plate (330-μL) containing 50 μL solution I and 50 μL solution II. The 96-well plate was vibrated for few seconds and analyzed on a microplate spectrophotometer at 460 nm.
Solution I: 0.25M $NH_4Fe(SO_4)_2$ in 9 M HNO_3.
Solution II: saturated $Hg(SCN)_2$ in absolute ethanol.

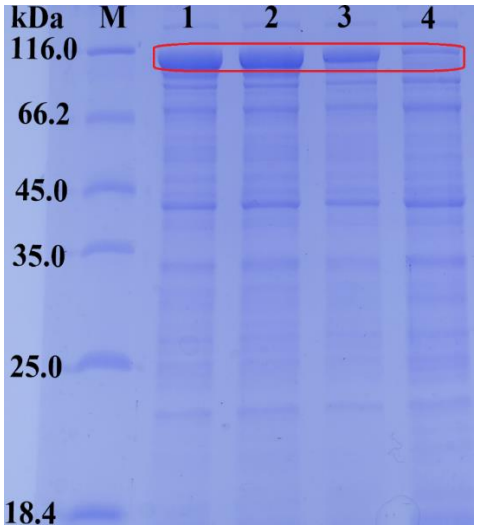


Fig. S2 SDS-PAGE analysis of the expressed P450DAs in *E. coli* BL21(DE3). Lane M, protein marker; Lane 1, P450DA-WT; Lane 2, P450DA-M2; Lane 3, P450DA-M5; Lane 4, P450DA-M3.

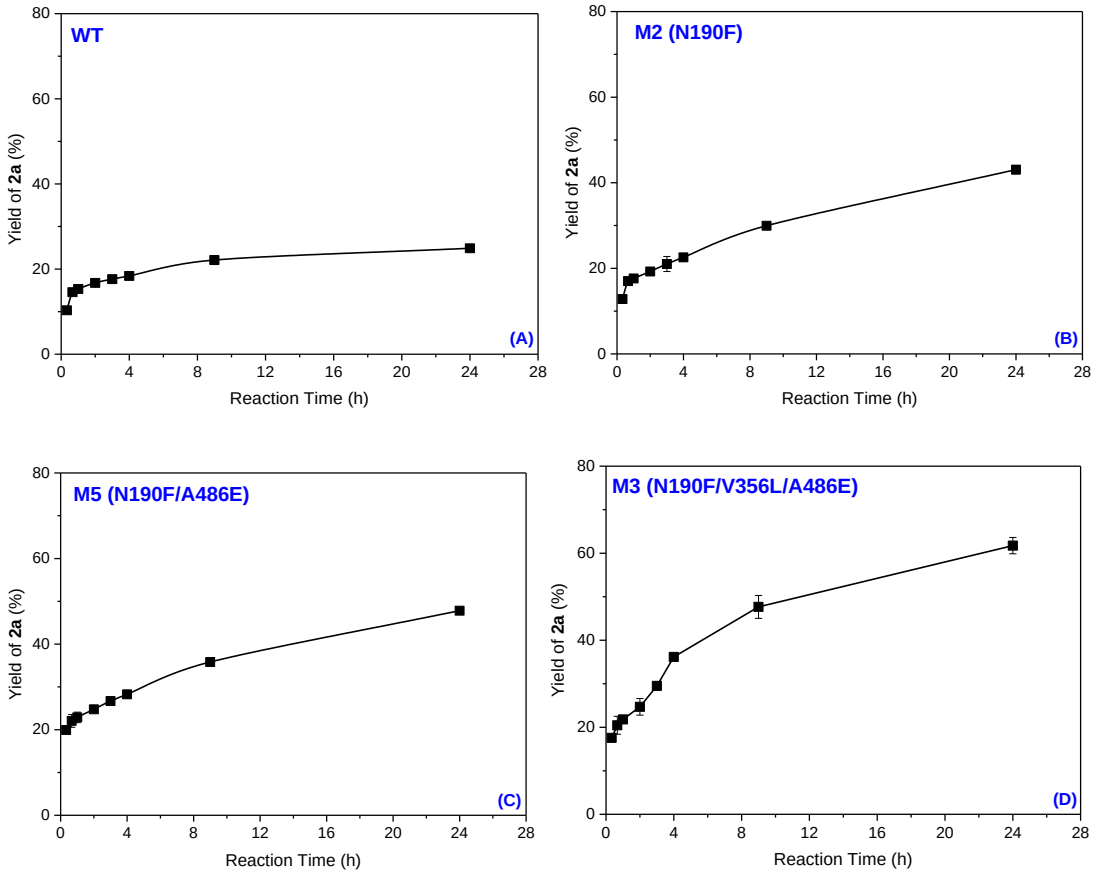


Fig. S3 Measurement of total turnover number (TTN).

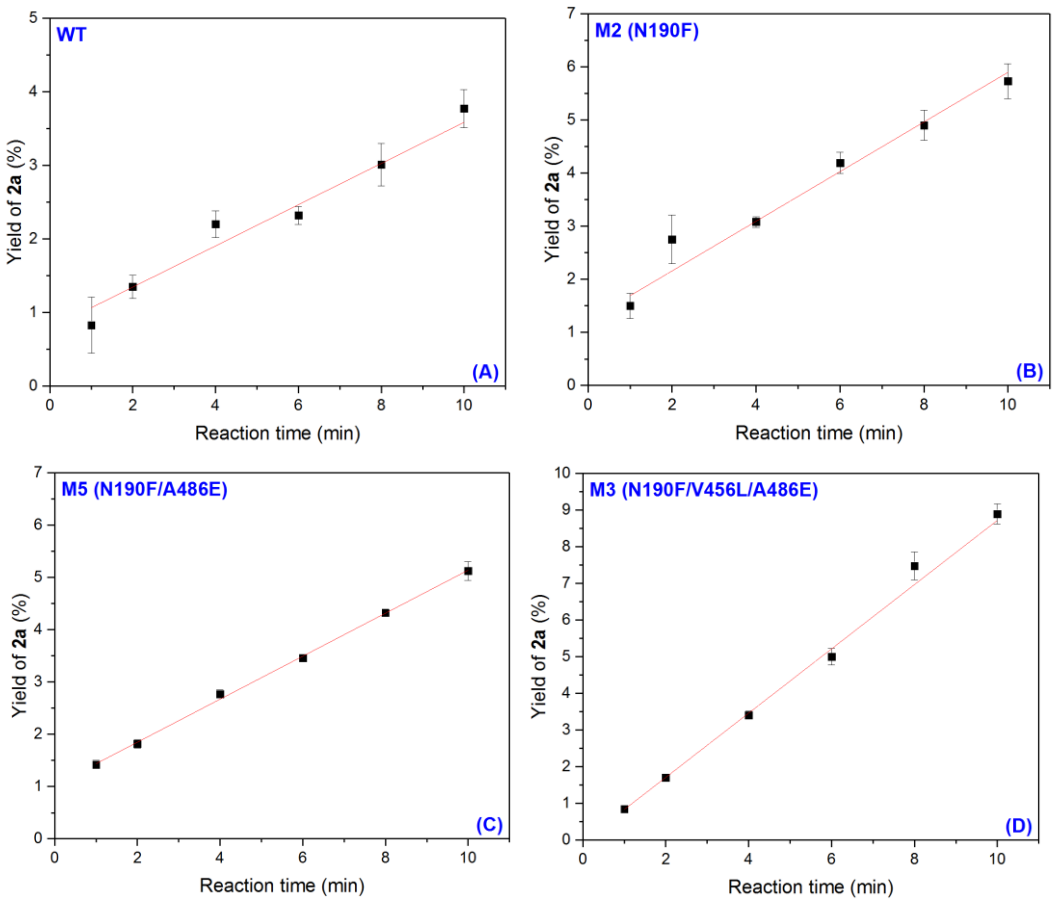
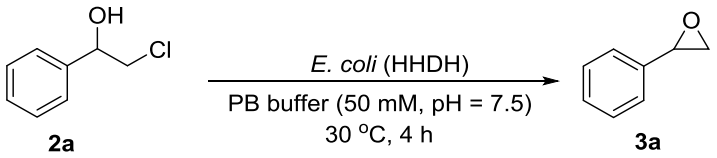


Fig. S4 Measurement of turnover frequency (TOF).

The *E. coli* BL21(DE3) cells containing pET-28-P450DA plasmids encoding for either WT or mutants (M2, M3, and M5) were cultured and harvested as described above. The resting cells were resuspended in 20 mL of PB buffer (50 mM, pH 7.5) to a final cell density of 10 g cdw/L. Biotransformation reactions were started in all cases by addition of substrate **1a** (8 mM initial concentration). Reactions were carried out in an Erlenmeyer flask (100 mL) for 24 h at 30°C and 250 rpm. Samples (1 mL) were taken from all biotransformation reactions at different time points (20, 40, 60,120, 180, 240, 540, and 1440 min) and subjected to HPLC analysis (Fig. S3). The TTN values were determined based on the product formation at 24 h. Additional biotransformation reactions were then carried to by taking samples at different time points (1, 2, 4, 6, 8, and 10 min) and subjected to HPLC analysis (Fig. S4). The TOF values were determined based on the product formation at initial 4 min. All reactions were done in parallel and in triplicate.

3. Supporting Tables S1 to S4

Table S1. Screening of HHDH biocatalysts for the dehalogenation of (*rac*)-**2a**.



Entry[a]	HHDH strain	Accession number	Yield **3a** [%][b]	*ee* **3a** [%][b]	Configuration[c]
1	HheA5	WP_014743557	74	18	*S*
2	HheA10	WP_019201195	29	92	*S*
3	HheA11	WP_020698933	17	76	*R*
4	HheD6	WP_011285856	61	0	ND
5	HheD8	WP_004302136	57	12	*R*
6	HheD15	WP_048670059	57	10	*S*
7	HheD20	AJP48347	60	16	*R*

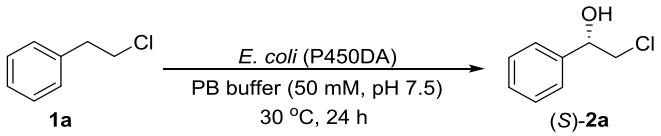
[a] Reactions were performed in 5 mL PB buffer (50 mM, pH = 7.5) containing 10 mM **2a** and 10 g cdw/L recombinant *E. coli* cells, and shaken at 30 °C, 250 rpm for 4 h.

[b] The yield and *ee* values of **3a** were determined by chiral GC analysis (Column: BGB-175. Injector temperature: 250 °C. Detector temperature: 250 °C. Oven temperature: 100 °C hold 10 min. Retention times: 8.36 min for (*S*)-**3a**; 8.62 min for (*R*)-**3a**).

[c] The absolute configuration was assigned by comparison with authentic samples. ND = not detected.

Halohydrin dehalogenase genes were optimized, synthesized and expressed in *E. coli* BL21(DE3) using the previously reported procedure.[16] *E. coli* BL21(DE3) cells transformed with plasmid encoding for HHDHs were grown overnight in 10 mL Terrific Broth medium (50 μg mL^{-1} kanamycin final concentration) at 37 °C, 250 rpm. Expression cultures were inoculated with 2 mL of preculture into 100 mL Terrific Broth medium (50 μg mL^{-1} kanamycin final concentration) in a 500 mL flask and incubated at 37 °C, 250 rpm until the OD_{600} up to 0.6-0.8. Then the flask culture was induced with IPTG (0.2 mM final concentration) and shaken at 28 °C, 250 rpm for 12-14 h. The cells were harvested by centrifugation (7000×g, 5 min, 4 °C). Dehalogenation reactions were carried out in 5 mL PB buffer (50 mM, pH = 7.5) containing 10 mM **2a** and 10 g cdw/L recombinant *E. coli* (HHDH) cells. The yield and ee were determined by chiral GC analysis after reaction at 30 °C, 250 rpm for 4 h.

Table S2. Catalytic characterization of P450DA-WT and several variants.



Entry[a]	Variant	P450 conc. (μmol/g cdw)[b]	TOF (min^{-1})[c]	TTN[d]
1	WT	1.53	29	1300
2	M2	1.39	44	2480
3	M5	0.80	69	4800
4	M3	0.65	105	7600

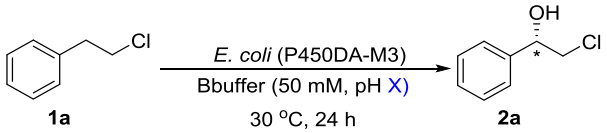
[a] Reactions were performed in 20 mL PB buffer (50 mM, pH = 7.5) containing 8 mM **1a** and 10 g cdw/L recombinant *E. coli* cells at 30 °C and 250 rpm for 24 h. All reactions were performed in triplicate.

[b] P450s concentrations were measured as previously described.[17]

[c] TOF values were calculated based on the product formation at initial 4 min.

[d] TTN values were calculated based on the product formation after reaction for 24 h.

Table S3. Investigation of reaction pH for asymmetric hydroxylation of **1a** to (*S*)-**2a**.

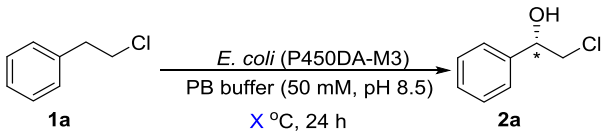


Entry[a]	pH	Buffer	Yield **2a** [%][b]	*ee* (*S*)-**2a** [%][b]
1	6.0	PB	10±1	82
2	7.0	PB	54±1	82
3	7.5	PB	64±1	82
4	8.0	PB	70±2	82
5	8.5	PB	74±1	81
6	9.0	PB	65±2	81
7	7.0	Tris-H_2SO_4	11±1	83
8	7.5	Tris-H_2SO_4	16±1	82
9	8.0	Tris-H_2SO_4	39±3	82
10	8.5	Tris-H_2SO_4	48±2	82
11	8.5	Gly-NaOH	33±2	82
12	9.0	Gly-NaOH	43±1	82
13	9.5	Gly-NaOH	25±1	82
14	10.0	Gly-NaOH	<5	82

[a] Reactions were performed in 5 mL buffer (50 mM, pH = X) containing 8 mM **1a** and 10 g cdw/L recombinant *E. coli* (P450DA-M3) cells, and stirred at 30 °C, 250 rpm for 24 h.

[b] The yield and *ee* values were determined by chiral HPLC analysis. All reactions were performed in parallel and in triplicate.

Table S4. Investigation of reaction pH for asymmetric hydroxylation of **1a** to (*S*)-**2a**.



Entry[a]	Temperature (°C)	Yield **2a** [%][b]	*ee* (*S*)-**2a** [%][b]
1	20	58±1	82
2	25	66±3	82
3	30	76±2	81
4	35	62±1	81
5	40	54±3	81

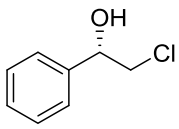
[a] Reactions were performed in 5 mL PB buffer (50 mM, pH = 8.5) containing 8 mM **1a** and 10 g cdw/L recombinant *E. coli* (P450DA-M3) cells, and shaken at X °C, 250 rpm for 24 h.

[b] The yield and *ee* values were determined by chiral HPLC analysis. All reactions were performed in parallel and in triplicate.

4. Synthesis of chiral alcohols via stereoselective biohydroxylation

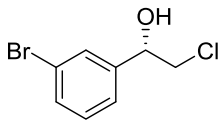
Representative procedure for the synthesis of chiral alcohols **2a-2r** from **1a-1r** (preparative scale): A 125 mL flask was charged with 20 mL of PB buffer (50 mM, pH = 8.5) containing 20 g cdw/L the resting cells of *E. coli* (P450DA-M3) and 10 mM (2-chloroethyl)benzene **1a** (28 mg, added by using 280 μL ethanol as co-solvent). The reaction was carried in quadruplicate (4×20 mL), at 30 ºC, 250 rpm for 24 h. After centrifugation of the reaction mixtures, the supernatants were separated, combined and extracted using ethyl acetate (3×80 mL). The extracts were then combined, dried on anhydrous sodium sulfate and concentrated in vacuo. The residue was purified by using flash column chromatography on silica gel (petroleum ether/ethyl acetate = 100:1-30:1), giving the desired chiral alcohol **2a** (light yellow liquid, 109.7 mg, 88% yield, 80% *ee*). Characterization of reaction products. Authentic standards corresponding to enzymatic reaction products were purchased from commercial suppliers or synthesized by chemical methods (determined by NMR).

(*S*)-2-chloro-1-phenylethan-1-ol (2a)



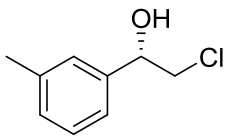
Light yellow oil (109.7 mg, 88% isolated yield, 80% *ee*), isolated yield was obtained from 80 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min, λ = 220 nm, $t_{(S)\text{-}\mathbf{2a}}$ = 12.3 min, $t_{(R)\text{-}\mathbf{2a}}$ = 13.5 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.26 (m, 5H), 4.90 (d, *J* = 8.7 Hz, 1H), 3.75 (d, *J* = 11.1 Hz, 1H), 3.65 (t, *J* = 10.0 Hz, 1H), 2.77 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.0, 128.8, 128.6, 126.2, 74.2, 51.0.

(*S*)-1-(3-bromophenyl)-2-chloroethan-1-ol (2b)



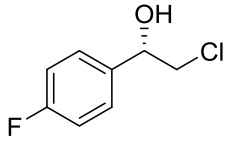
Light yellow oil (121.5 mg, 63% yield, 98% *ee*), isolated yield was obtained from 80 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min, λ = 220 nm, $t_{(R)\text{-}\mathbf{2b}}$ = 12.3 min, $t_{(S)\text{-}\mathbf{2b}}$ =14.6 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.44 (d, *J* = 7.7 Hz, 1H), 7.29 (d, *J* = 7.4 Hz, 1H), 7.26-7.20 (m, 1H), 4.85 (d, *J* = 8.6 Hz, 1H), 3.71 (d, *J* = 11.2 Hz, 1H), 3.59 (t, *J* = 10.0 Hz, 1H), 2.77 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.2, 131.6, 130.3, 129.3, 124.8, 122.9, 73.4, 50.8.

(*S*)-2-chloro-1-(*m*-tolyl)ethan-1-ol (2c)



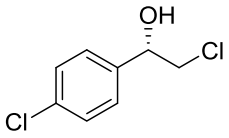
Light yellow oil (78.5 mg, 44% yield, 97% *ee*), isolated yield was obtained from 80 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min, λ = 220 nm, $t_{(S)\text{-}\mathbf{2c}}$ = 10.0 min, $t_{(R)\text{-}\mathbf{2c}}$ = 11.6 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26-7.23 (m, 1H), 7.18-7.10 (m, 3H), 4.84 (d, *J* = 8.6 Hz, 1H), 3.71 (d, *J* = 11.1 Hz, 1H), 3.62 (t, *J* = 9.9 Hz, 1H), 2.67 (s, 1H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.9, 138.6, 129.3, 128.7, 126.8, 123.3, 74.2, 51.1, 21.6.

(*S*)-2-chloro-1-(4-fluorophenyl)ethan-1-ol (2d)



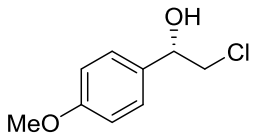
Light yellow oil (42.2 mg, 30% yield, 82% *ee*), isolated yield was obtained from 80 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OJ-H, hexane/*i*-PrOH = 90/10, flow rate = 0.8 mL/min, λ = 220 nm, $t_{(R)\text{-}\mathbf{2d}}$ = 16.0 min, $t_{(S)\text{-}\mathbf{2d}}$ =16.6 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (dd, *J* = 7.8, 5.8 Hz, 2H), 7.06 (t, *J* = 8.4 Hz, 2H), 4.87 (d, *J* = 8.5 Hz, 1H), 3.69 (dd, *J* = 5.9, 5.3 Hz, 1H), 3.64–3.57 (m, 1H), 2.85 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.8 (d, *J* = 245.5 Hz, 1C), 135.8 (d, *J* = 3.1 Hz, 1C), 127.9 (d, *J* = 8.2 Hz, 1C), 115.7 (d, *J* = 21.6 Hz, 1C), 73.5, 50.9.

(*S*)-2-chloro-1-(4-chlorophenyl)ethan-1-ol (2e)



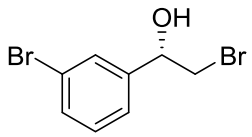
Light yellow oil (44.8 mg, 29% yield, 85% *ee*), isolated yield was obtained from 80 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OJ-H, hexane/*i*-PrOH = 90/10, flow rate = 0.8 mL/min, λ = 220 nm, $t_{(R)\text{-}\mathbf{2e}}$ = 14.5 min, $t_{(S)\text{-}\mathbf{2e}}$ =15.8 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.29 (m, 4H), 4.88 (d, *J* = 8.6 Hz, 1H), 3.72 (dd, *J* = 11.3, 3.5 Hz, 1H), 3.64–3.56 (m, 1H), 2.72 (d, *J* = 2.2 Hz,1H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 134.4, 129.0, 127.6, 73.5, 50.8.

(*S*)-2-chloro-1-(4-methoxyphenyl)ethan-1-ol (2f)



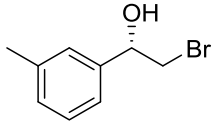
Light yellow oil (63.6 mg, 36% yield, 83% *ee*), isolated yield was obtained from 80 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min, λ = 220 nm, $t_{(S)\text{-}\mathbf{2f}}$ =15.3 min, $t_{(R)\text{-}\mathbf{2f}}$ = 19.1 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 7.8 Hz, 2H), 6.91 (d, *J* = 7.8 Hz, 2H), 4.86 (d, *J* = 8.2 Hz, 1H), 3.81 (s, 3H), 3.71 (d, *J* = 11.0 Hz, 1H), 3.63 (t, *J* = 10.0 Hz, 1H), 2.59 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.8, 132.2, 127.5, 114.2, 73.9, 55.5, 51.1.

(*S*)-2-bromo-1-(3-bromophenyl)ethan-1-ol (2g)



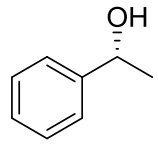
Light yellow oil (58.1 mg, 34% yield, 97% *ee*), isolated yield was obtained from 80 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min, λ = 220 nm, $t_{(R)\text{-}\mathbf{2g}}$ = 13.1 min, $t_{(S)\text{-}\mathbf{2g}}$ =15.4 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.44 (d, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 7.7 Hz, 1H), 7.23 (t, *J* = 7.7 Hz, 1H), 4.88 (dd, *J* = 8.6, 2.4 Hz, 1H), 3.61 (dd, *J* = 10.5, 3.3 Hz, 1H), 3.55-3.44 (m, 1H), 2.70 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.6, 131.7, 130.4, 129.3, 124.8, 122.9, 73.2, 40.0.

(*S*)-2-bromo-1-(*m*-tolyl)ethan-1-ol (2h)



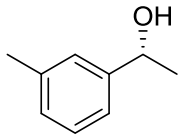
Light yellow oil (43.8 mg, 20% yield, 94% *ee*), isolated yield was obtained from 80 mL biotransformation mixture. The ee was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min, λ = 220 nm, $t_{(S)\text{-}\mathbf{2h}}$ = 10.4 min, $t_{(R)\text{-}\mathbf{2h}}$ = 12.2 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26-7.22 (m, 1H), 7.18-7.11 (m, 3H), 4.87 (d, *J* = 9.0 Hz, 1H), 3.61 (dd, *J* = 10.4, 3.2 Hz, 1H), 3.54-3.50 (m, 1H), 2.58 (s, 1H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.3, 138.6, 129.4, 128.7, 126.7, 123.2, 74.0, 40.5, 21.6.

(*R*)-1-phenylethan-1-ol (2i)



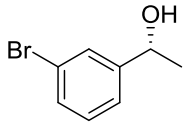
Viscous yellow oil (92.0 mg, 28% yield, 85% *ee*), isolated yield was obtained from 270 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min, λ = 220 nm, $t_{(R)\text{-}\mathbf{2i}}$ = 8.6 min, $t_{(S)\text{-}\mathbf{2i}}$ = 9.8 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 -7.30 (m, 4H), 7.30-7.23 (m, 1H), 4.87 (q, J = 6.5 Hz, 1H), 1.98 (s, 1H), 1.48 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.9, 128.6, 127.6, 125.6, 70.5, 25.3.

(*R*)-1-(*m*-tolyl)ethan-1-ol (2j)



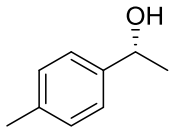
Viscous yellow oil (64.6 mg, 36% yield, 93% *ee*), isolated yield was obtained from 125 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 98/2, flow rate = 1.0 mL/min, λ = 220 nm, $t_{(R)\text{-}\mathbf{2j}}$ = 13.1 min, $t_{(S)\text{-}\mathbf{2j}}$ = 16.4 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24-7.18 (m, 1H), 7.16 (s, 1H), 7.13 (d, J = 7.5 Hz, 1H), 7.06 (d, J = 7.3 Hz, 1H), 4.84 (q, J = 6.4 Hz, 1H), 2.33 (s, 3H), 1.73 (s, 1H), 1.46 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.9, 138.3, 128.6, 128.4, 126.2, 122.5, 70.6, 25.3, 21.6.

(*R*)-1-(3-bromophenyl)ethan-1-ol (2k)



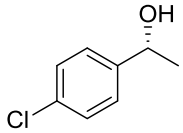
Viscous yellow oil (37.6 mg, 43% yield, 95% *ee*), isolated yield was obtained from 45 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/i-PrOH = 95/5, flow rate = 1.0 mL/min, λ = 220 nm, $t_{(S)\text{-}\mathbf{2k}}$ = 8.6 min, $t_{(R)\text{-}\mathbf{2k}}$ = 9.5 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.24 (t, J = 7.0 Hz, 1H), 7.18 (t, J = 7.7 Hz, 1H), 4.83 (q, J = 6.5 Hz, 1H), 1.83 (s, 1H), 1.44 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.2, 130.6, 130.2, 128.7, 124.1, 122.7, 69.9, 25.4.

(*R*)-1-(*p*-tolyl)ethan-1-ol (2l)



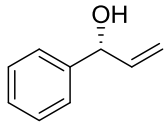
Viscous yellow oil (122.0 mg, 30% yield, 89% *ee*), isolated yield was obtained from 295 mL biotransformation mixture. The *ee* was determined by chiral GC (Agilent GC-7890B, FID, column BGB-175, 110 ^{o}C hold 20 min, $t_{(R)\text{-}\mathbf{2l}}$ = 14.3 min, $t_{(S)\text{-}\mathbf{2l}}$ = 15.6 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, J = 7.7 Hz, 2H), 7.13 (d, J = 7.8 Hz, 2H), 4.83 (q, J = 6.4 Hz, 1H), 2.32 (s, 3H), 1.88 (s, 1H), 1.45 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.0, 137.3, 129.3, 125.5, 70.4, 25.2, 21.2.

(*R*)-1-(4-chlorophenyl)ethan-1-ol (2m)



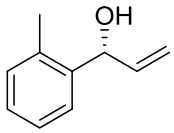
Viscous yellow oil (71.2 mg, 52% yield, 88% *ee*), isolated yield was obtained from 87 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OB-H, hexane/*i*-PrOH = 98/2, flow rate = 1.0 mL/min, λ = 220 nm, $t_{(S)\text{-}\mathbf{2m}}$ = 12.3 min, $t_{(R)\text{-}\mathbf{2m}}$ = 14.2 min). ^{1}H NMR (400 MHz, 400 MHz) δ 7.32-7.18 (m, 4H), 4.82 (q, J = 6.5 Hz, 1H), 2.04 (s, 1H), 1.42 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.3, 133.1, 128.7, 126.9, 69.8, 25.4.

(*R*)-1-phenylprop-2-en-1-ol (2n)



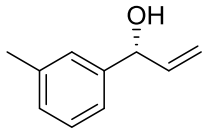
Viscous yellow oil (109.3 mg, 82% yield, 94% *ee*), isolated yield was obtained from 100 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 95/5, flow rate = 0.8 mL/min, λ = 220 nm, $t_{(R)\text{-}\mathbf{2n}}$ = 12.2 min, $t_{(S)\text{-}\mathbf{2n}}$ = 15.0 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, J = 4.7 Hz, 4H), 7.23 – 7.20 (m, 1H), 6.01 – 5.92 (m, 1H), 5.26 (dd, J = 17.1, 1.1 Hz, 1H), 5.11 (dd, J = 10.1, 1.2 Hz, 2H), 2.22 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.6, 140.3, 128.6, 127.8, 126.4, 115.2, 75.4.

(*R*)-1-(*o*-tolyl)prop-2-en-1-ol (2o)



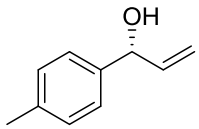
Viscous yellow oil (90.1 mg, 43% yield, 88% *ee*), isolated yield was obtained from 140 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 95/5, flow rate = 0.5 mL/min, λ = 220 nm, $t_{(R)\text{-}\mathbf{2o}}$ = 18.8 min, $t_{(S)\text{-}\mathbf{2o}}$ = 20.1 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.43 (m, 1H), 7.25 – 7.18 (m, 2H), 7.16 (t, J = 6.9 Hz, 1H), 6.05 – 5.97 (m, 1H), 5.36 (d, J = 5.7 Hz, 1H), 5.29 (dt, J = 17.2, 1.3 Hz, 1H), 5.19 (d, J = 10.3 Hz, 1H), 2.50 (s, 1H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.5, 139.4, 135.3, 130.5, 127.6, 126.3, 125.9, 115.2, 71.9, 19.2.

(*R*)-1-(*m*-tolyl)prop-2-en-1-ol (2p)



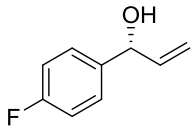
Viscous yellow oil (110.3 mg, 33% yield, 98% *ee*), isolated yield was obtained from 225 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OD-H, hexane/*i*-PrOH = 95/5, flow rate = 0.5 mL/min, λ = 220 nm, $t_{(R)\text{-}\mathbf{2p}}$ = 17.3 min, $t_{(S)\text{-}\mathbf{2p}}$ = 21.8 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (dd, J = 9.1, 5.9 Hz, 1H), 7.20 – 7.16 (m, 2H), 7.12 (d, J = 7.4 Hz, 1H), 6.10 – 6.01 (m, 1H), 5.38 – 5.34 (m, 1H), 5.22 – 5.19 (m, 1H), 5.15 (d, J = 5.9 Hz, 1H), 2.38 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.6, 140.3, 138.3, 128.6, 128.5, 127.1, 123.5, 115.0, 75.4, 21.5.

(*R*)-1-(*p*-tolyl)prop-2-en-1-ol (2q)



White solid (126.3 mg, 34% yield, 93% *ee*), mp 28.5-29.4 ºC, isolated yield was obtained from 250 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel AD-H, hexane/*i*-PrOH = 95/5, flow rate = 0.8 mL/min, λ = 220 nm, $t_{(R)\text{-}\mathbf{2q}}$ = 11.7 min, $t_{(S)\text{-}\mathbf{2q}}$ = 13.0 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 7.9 Hz, 2H), 6.07 – 5.99 (m, 1H), 5.33 (dt, J = 17.1, 1.3 Hz, 1H), 5.19 – 5.15 (m, 2H), 2.34 (s, 3H), 2.04 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.4, 139.8, 137.6, 129.4, 126.4, 115.0, 75.3, 21.3.

(*R*)-1-(4-fluorophenyl)prop-2-en-1-ol (2r)



Viscous yellow oil (108.4 mg, 49% yield, 92% *ee*), isolated yield was obtained from 145 mL biotransformation mixture. The *ee* was determined by chiral HPLC (Chiralcel OJ-H, hexane/*i*-PrOH = 95/5, flow rate = 0.8 mL/min, λ = 220 nm, $t_{(S)\text{-}\mathbf{2r}}$ = 17.8 min, $t_{(R)\text{-}\mathbf{2r}}$ = 19.1 min). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (dd, J = 8.1, 5.8 Hz, 2H), 7.00 (t, J = 8.5 Hz, 2H), 6.01 – 5.92 (m, 1H), 5.28 (dd, J = 17.1, 0.9 Hz, 1H), 5.16 (dd, J = 10.3, 1.0 Hz, 1H), 5.10 (d, J = 5.9 Hz, 1H), 2.76 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.3 (d, J = 245.6 Hz, 1C), 140.1, 138.4 (d, J = 3.1 Hz, 1C), 128.1 (d, J = 8.1 Hz, 1C), 115.36, 115.35 (d, J = 21.4 H, 1C), 74.6.

5. References

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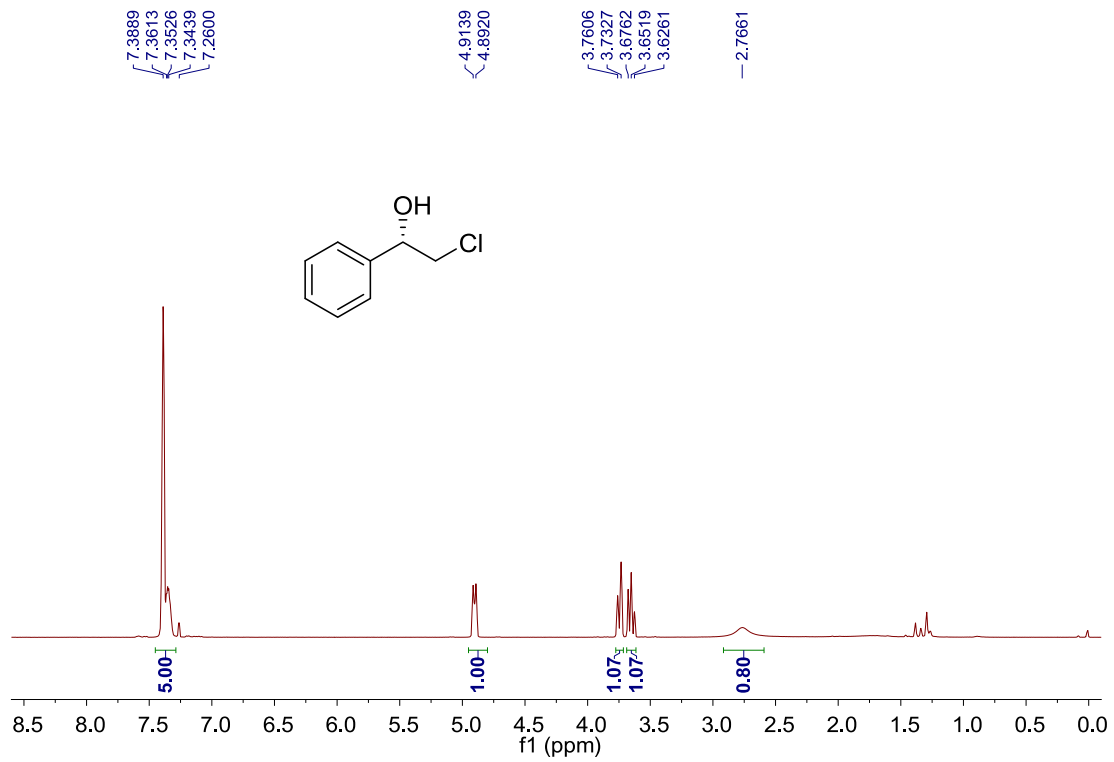
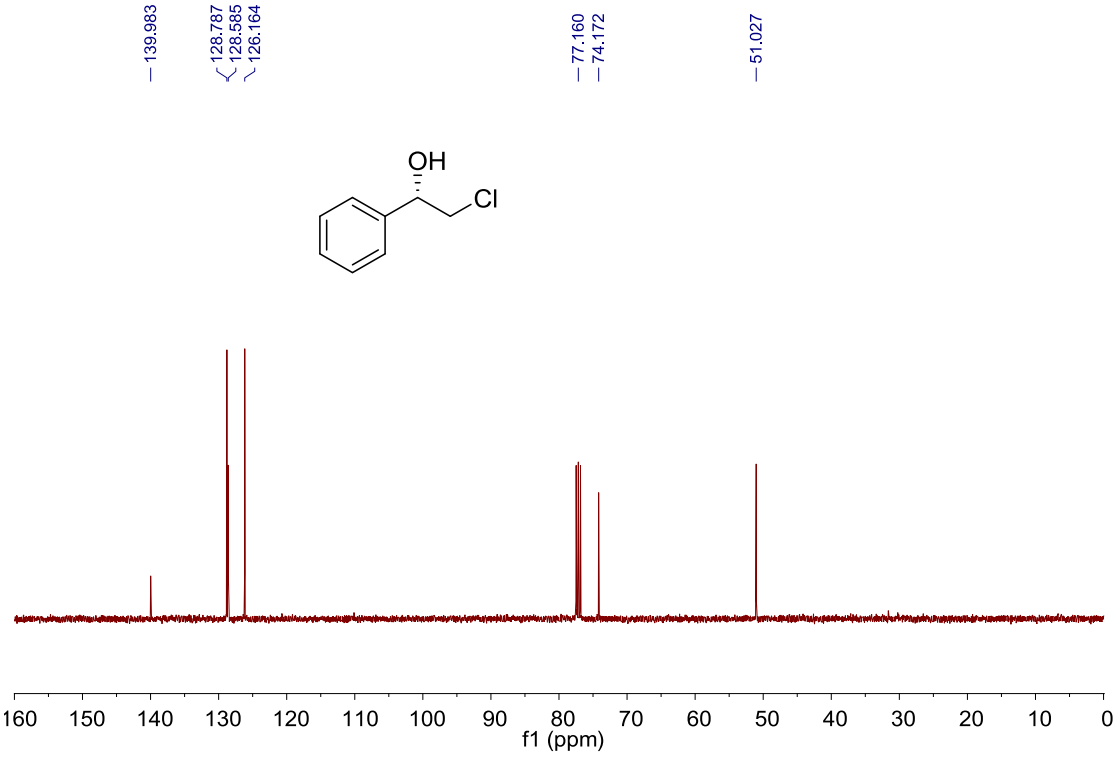
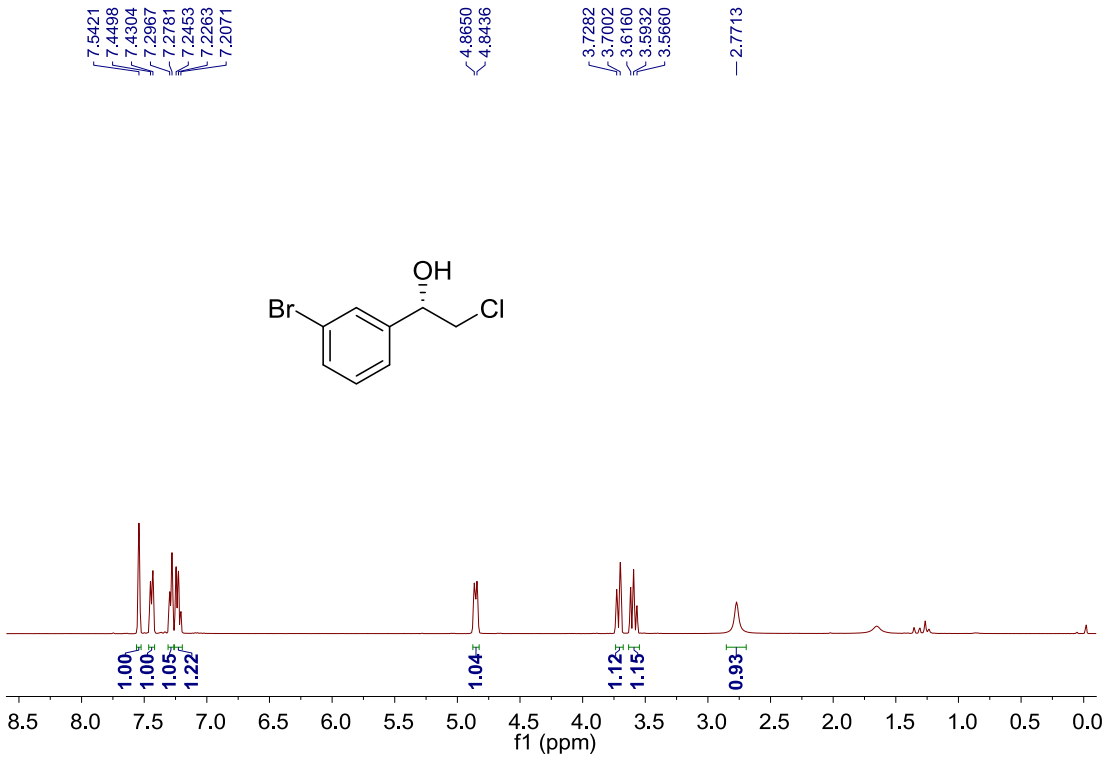
6. Copies of NMR spectra

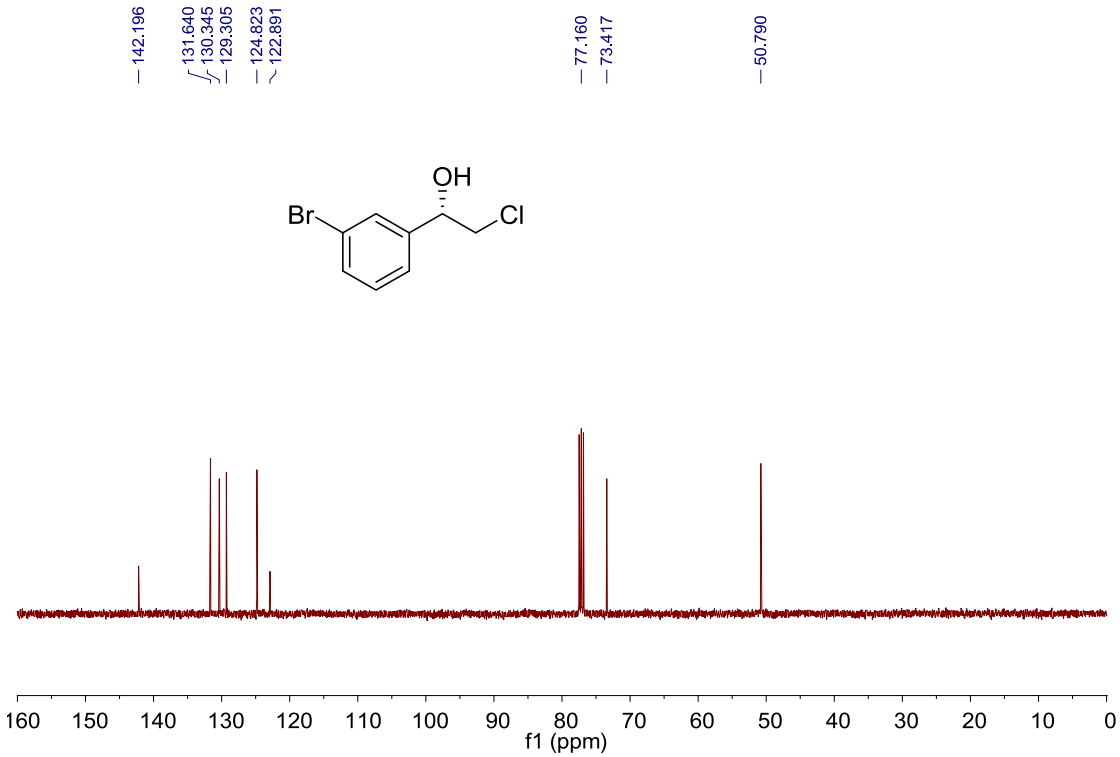
(*S*)-2-chloro-1-phenylethan-1-ol (2a)



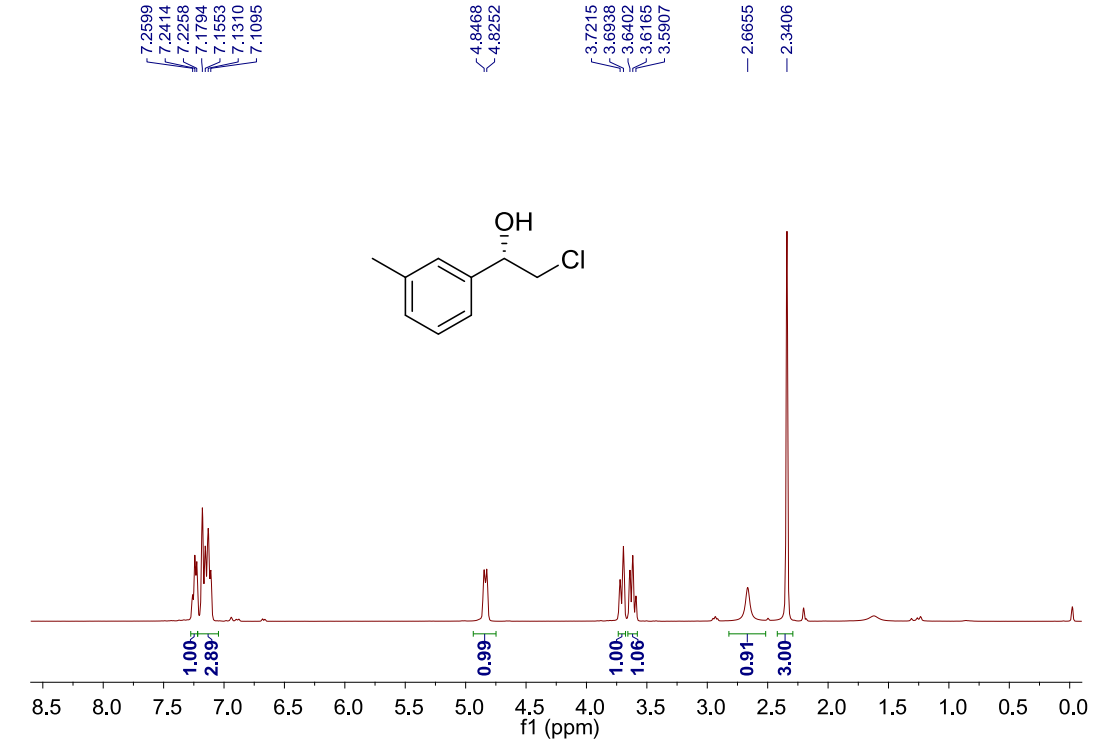


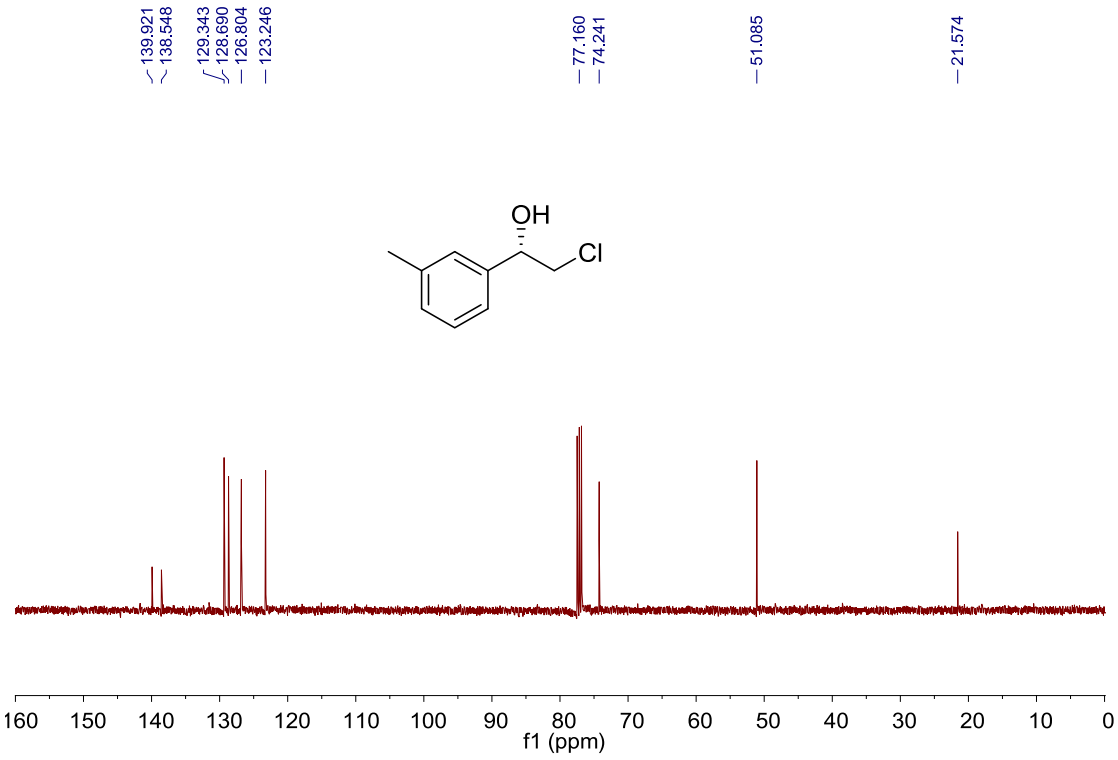
(*S*)-1-(3-bromophenyl)-2-chloroethan-1-ol (2b)



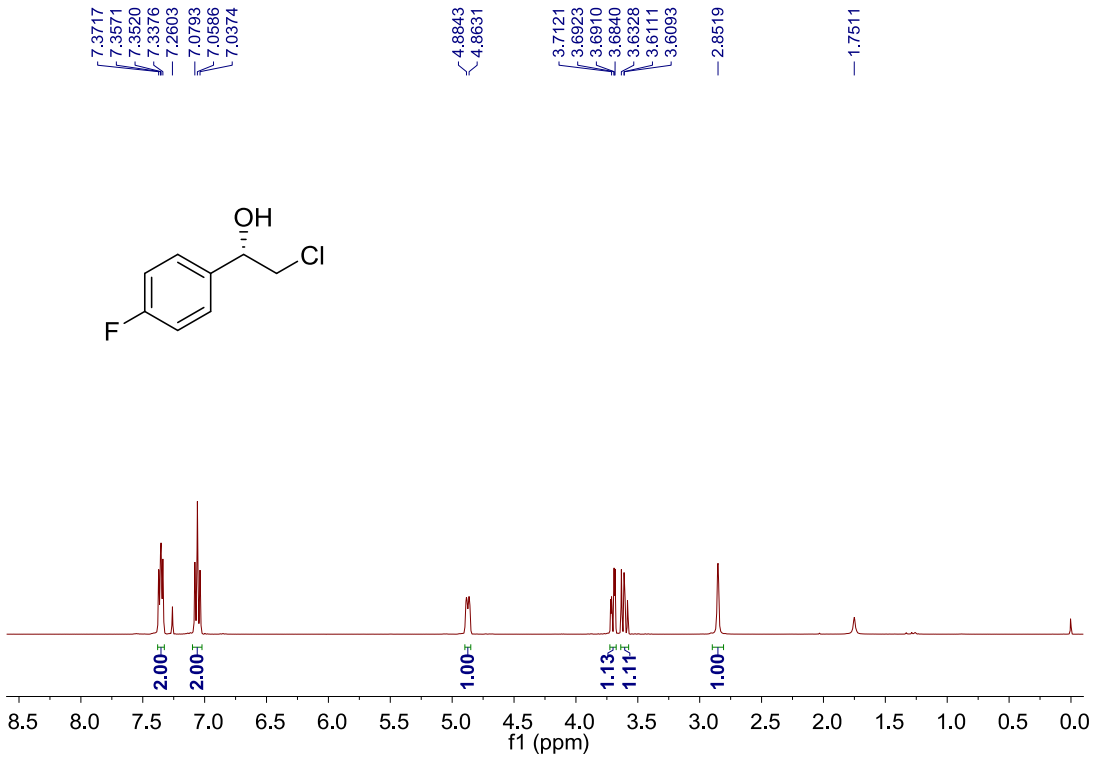


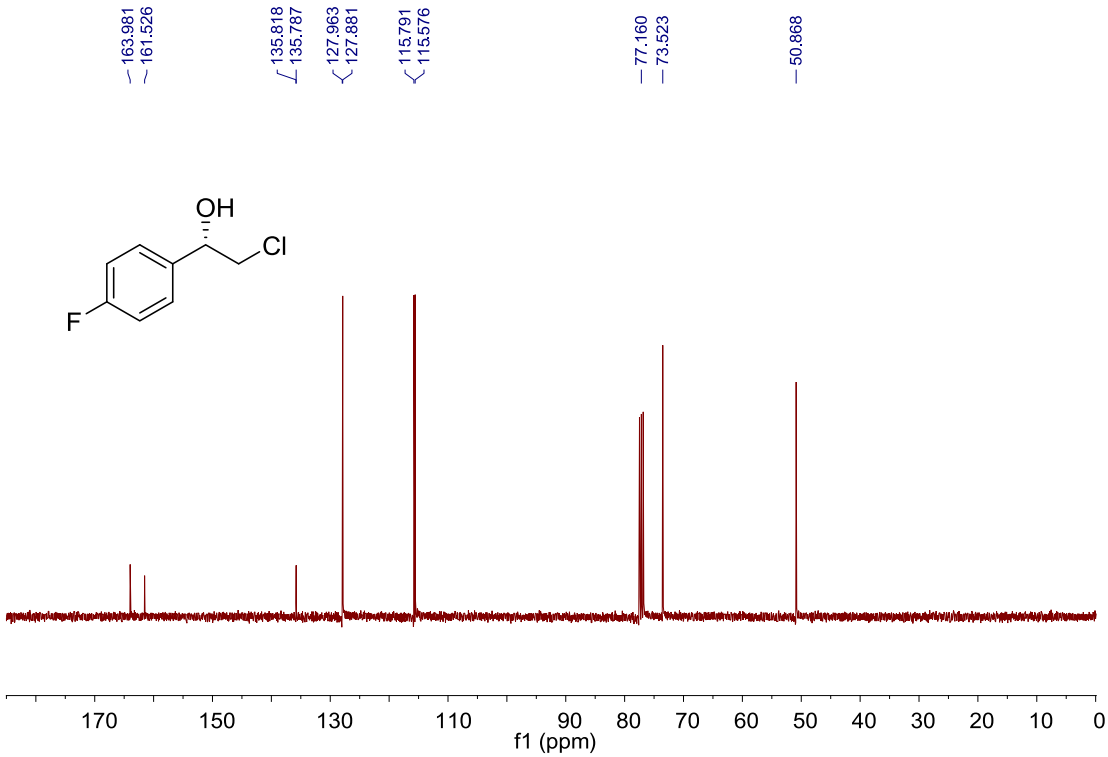
(*S*)-2-chloro-1-(*m*-tolyl)ethan-1-ol (2c)



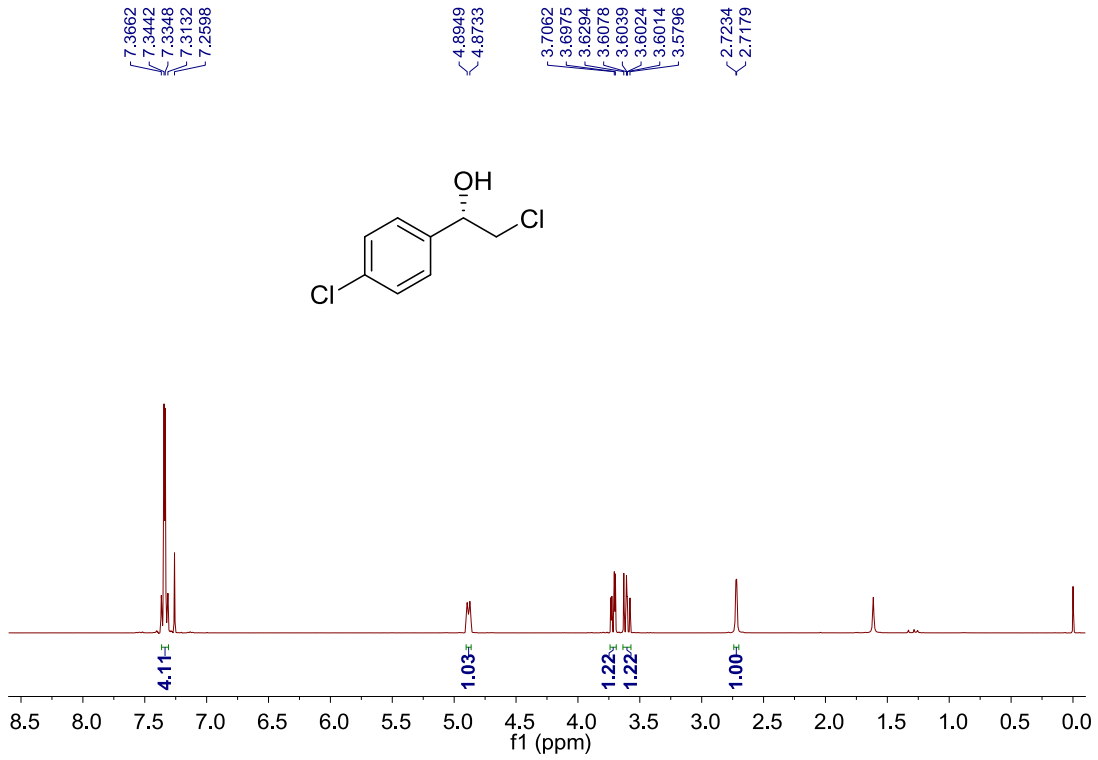


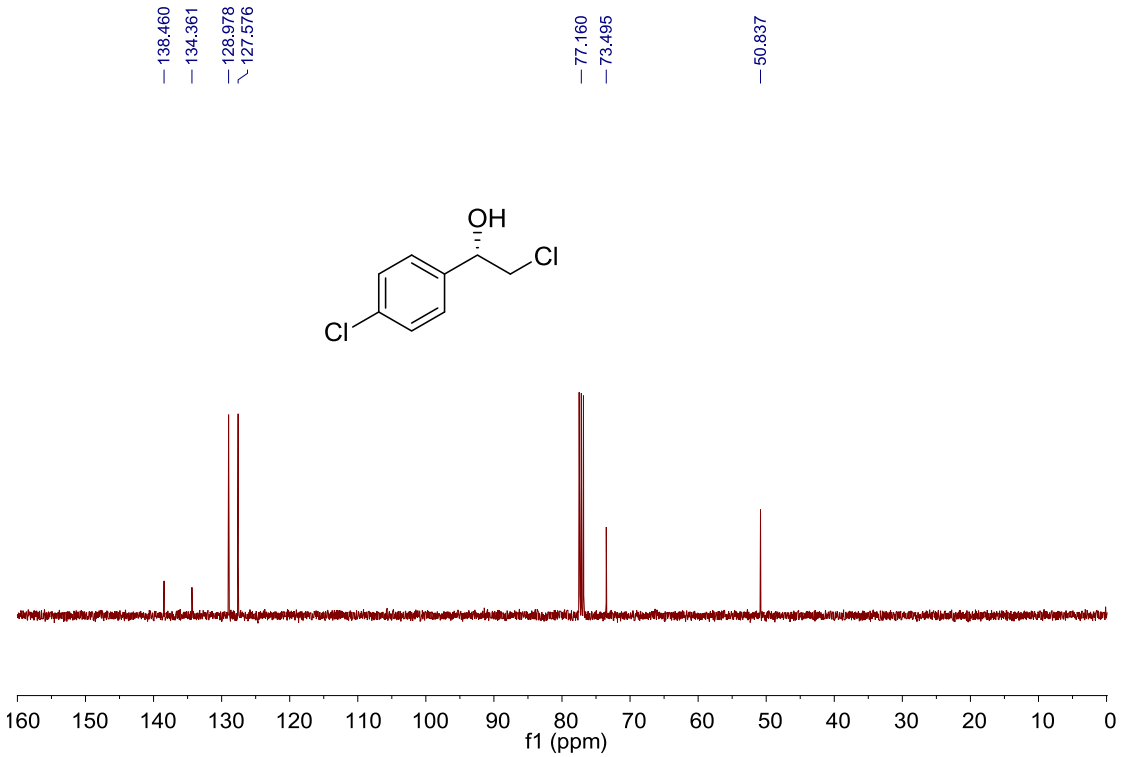
(*S*)-2-chloro-1-(4-fluorophenyl)ethan-1-ol (2d)



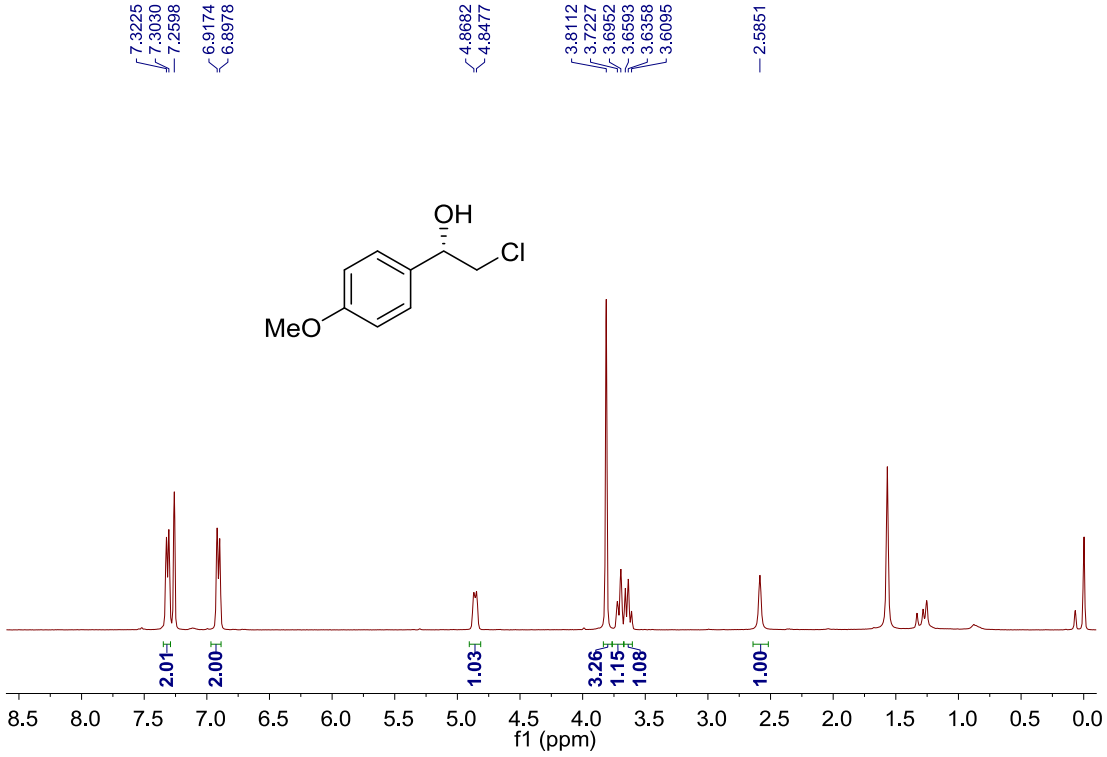


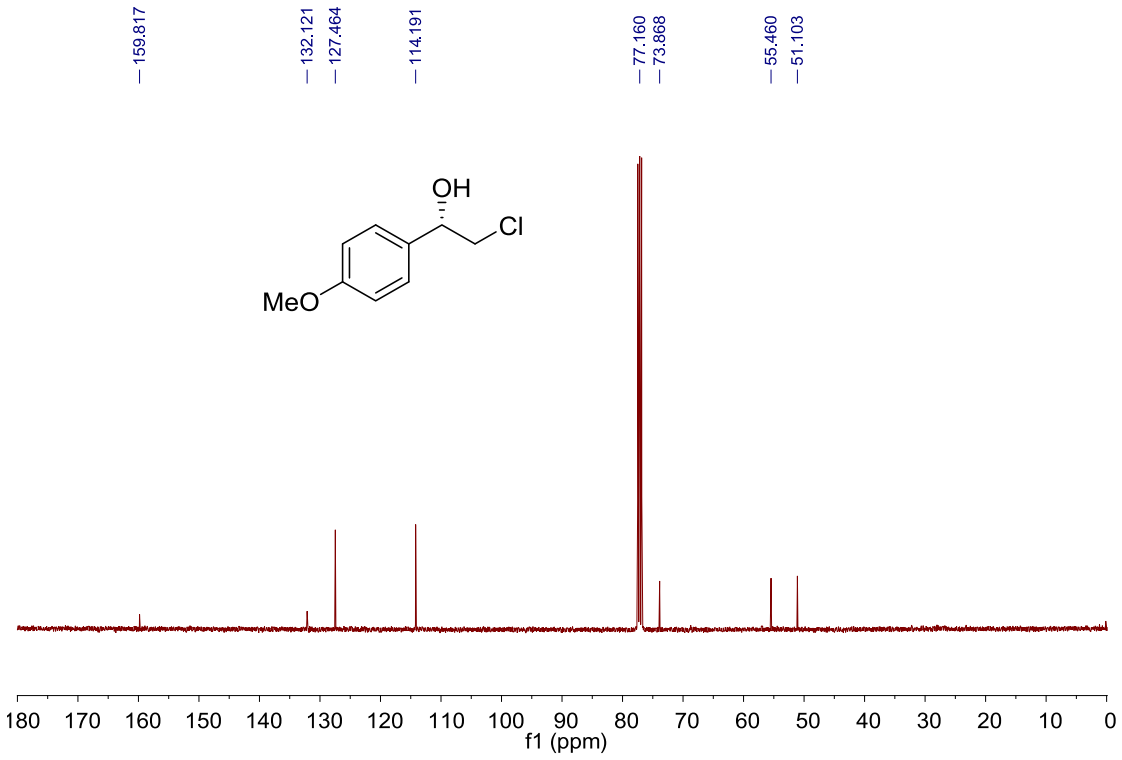
(*S*)-2-chloro-1-(4-chlorophenyl)ethan-1-ol (2e)



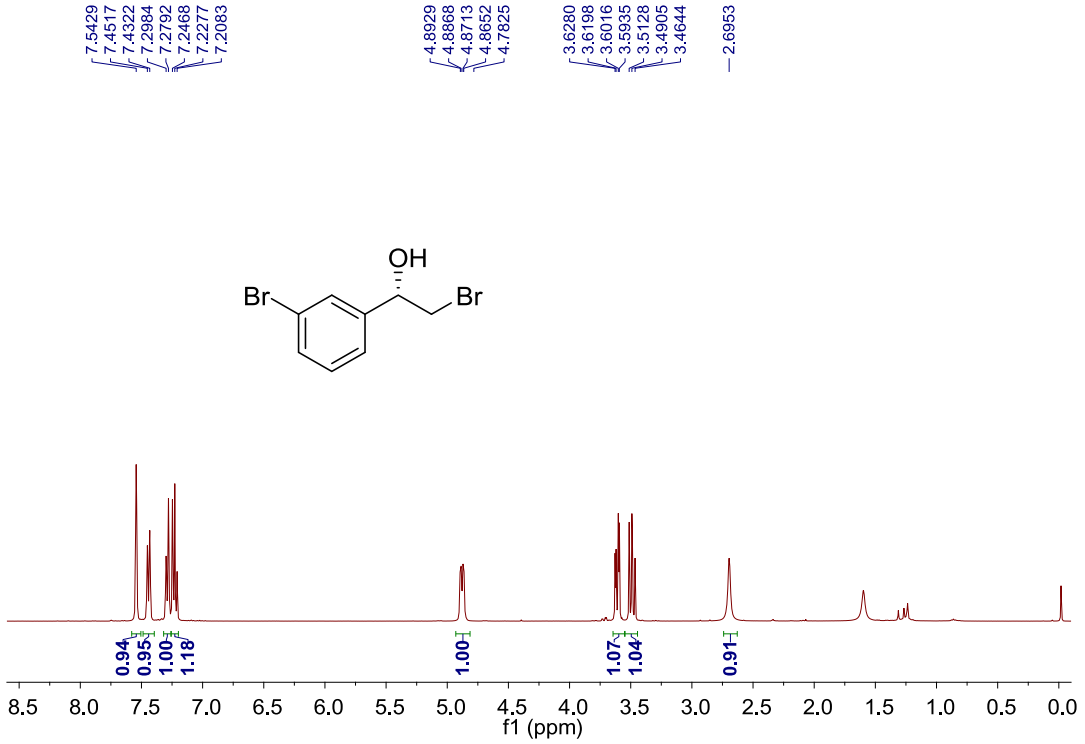


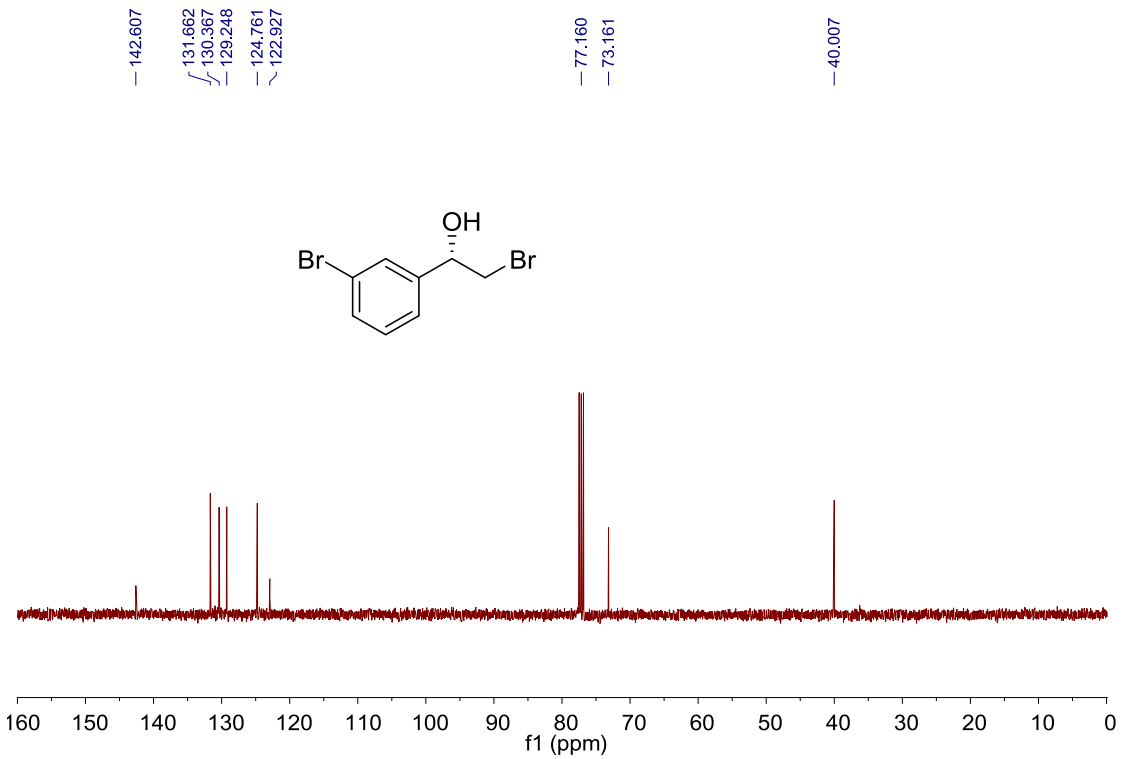
(*S*)-2-chloro-1-(4-methoxyphenyl)ethan-1-ol (2f)



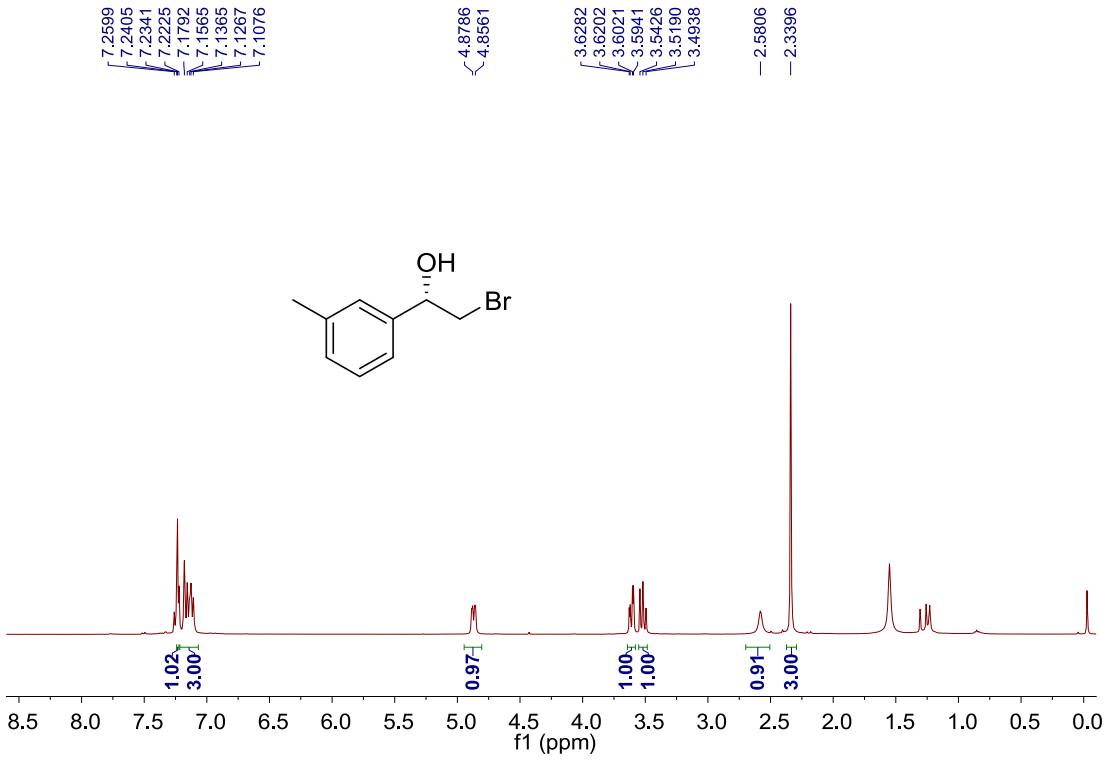


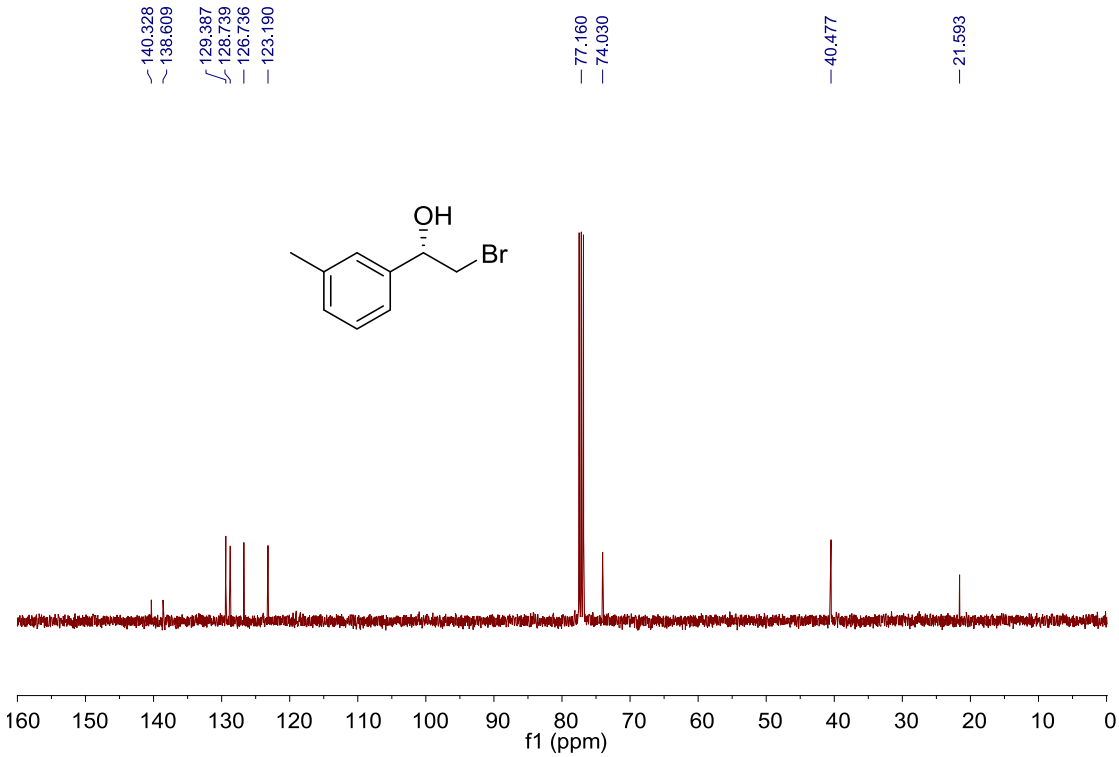
(*S*)-2-bromo-1-(3-bromophenyl)ethan-1-ol (2g)



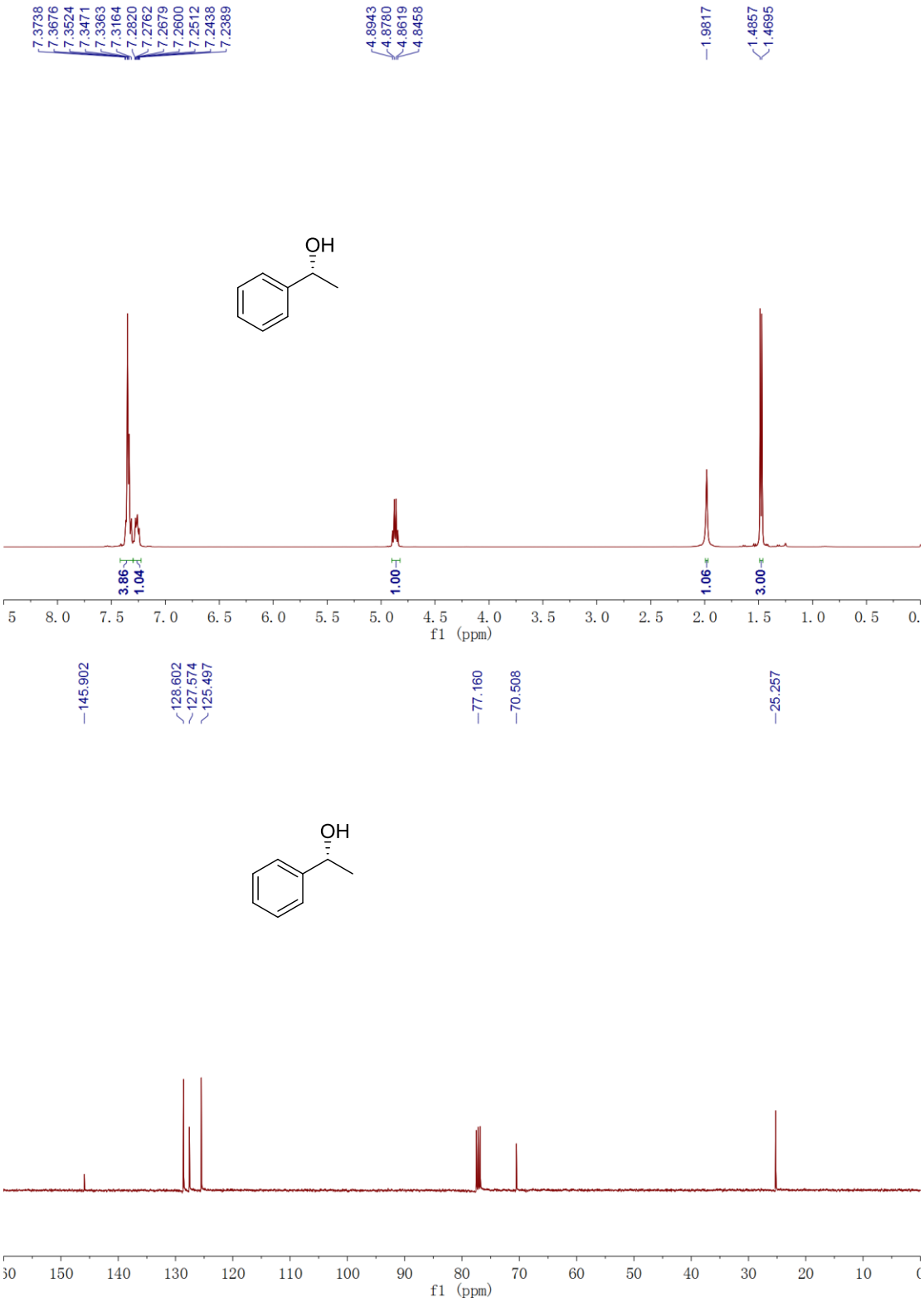


(*S*)-2-bromo-1-(*m*-tolyl)ethan-1-ol (2h)

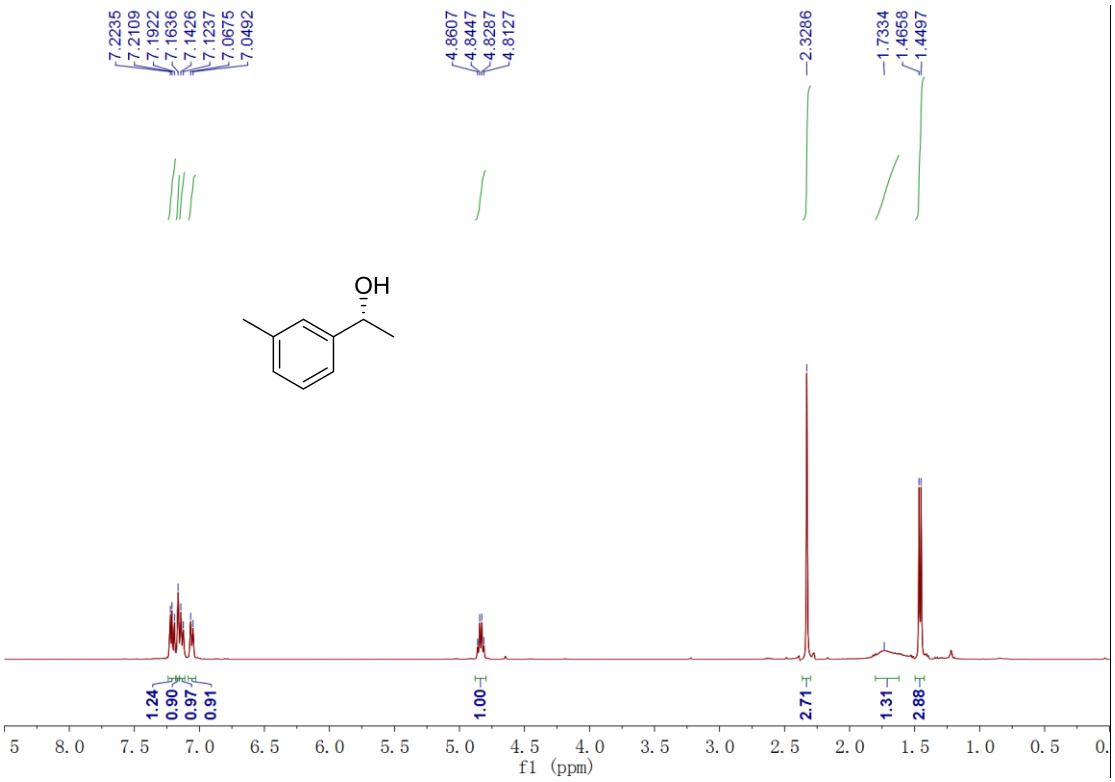


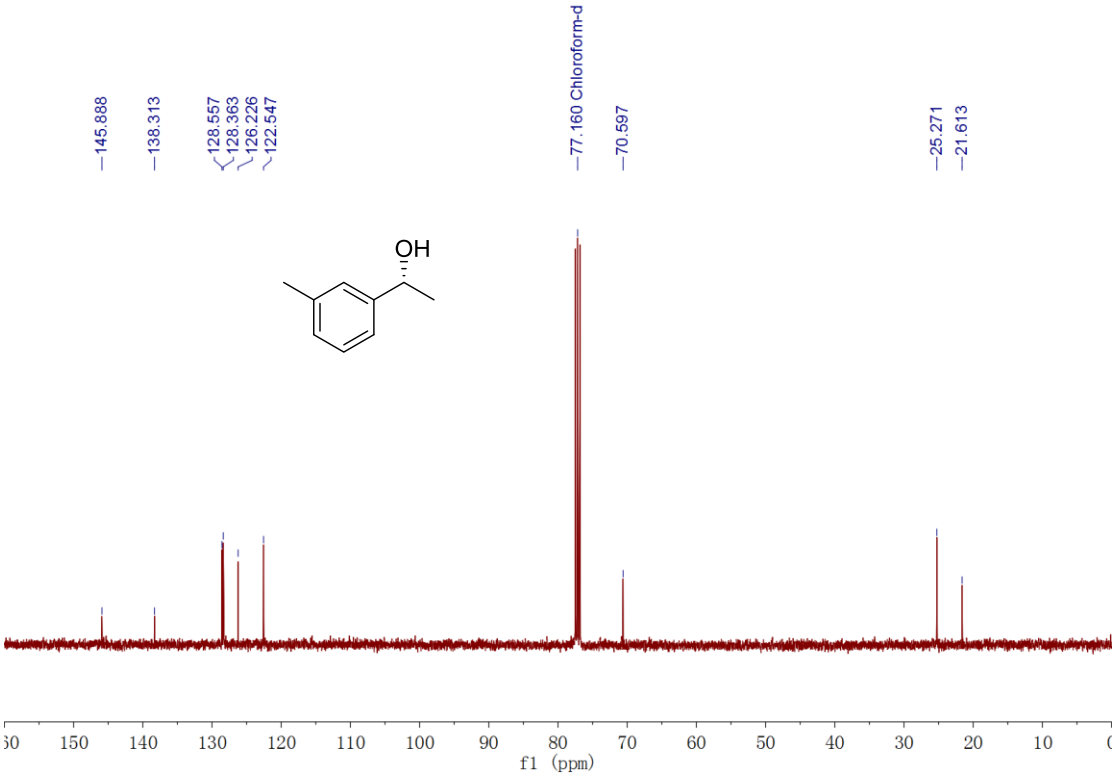


(*R*)-1-phenylethan-1-ol (2i)



(*R*)-1-(*m*-tolyl)ethan-1-ol (2j)





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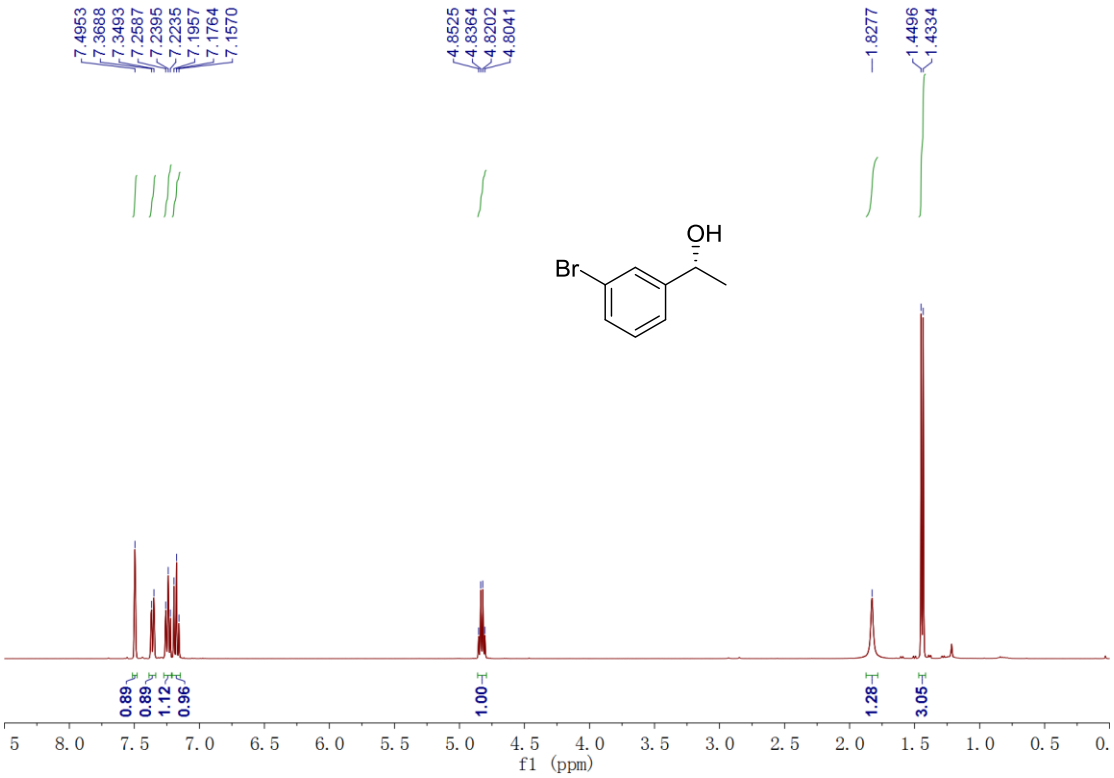
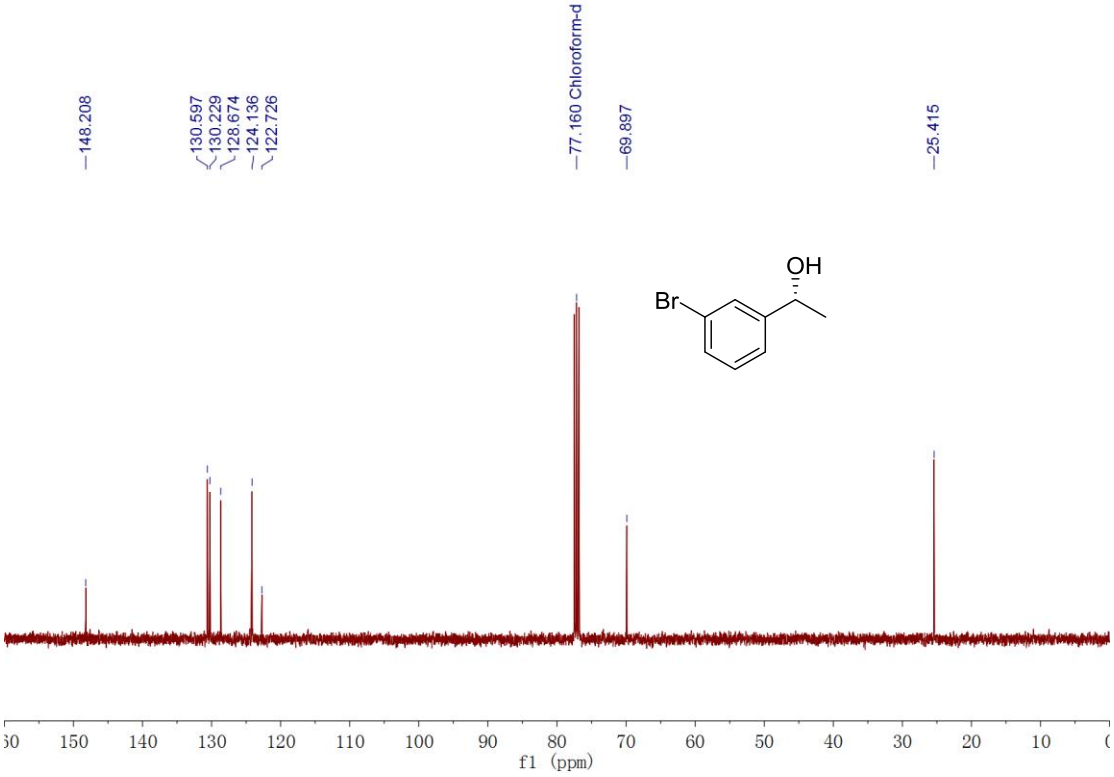
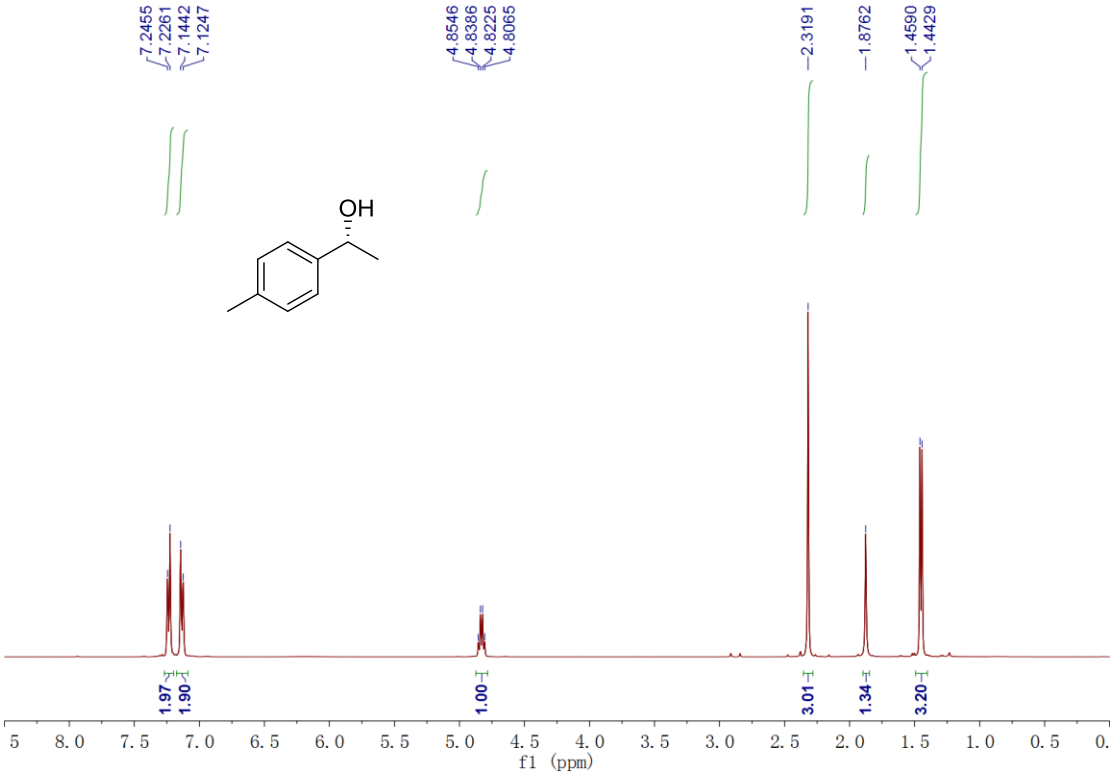
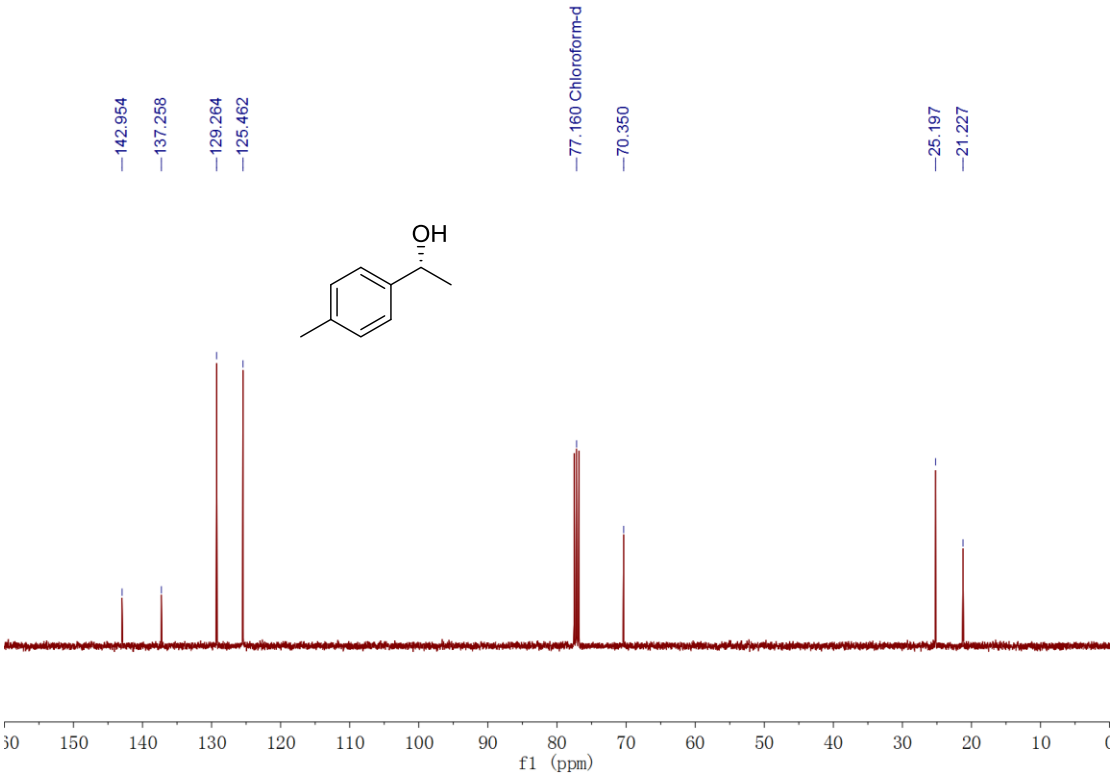


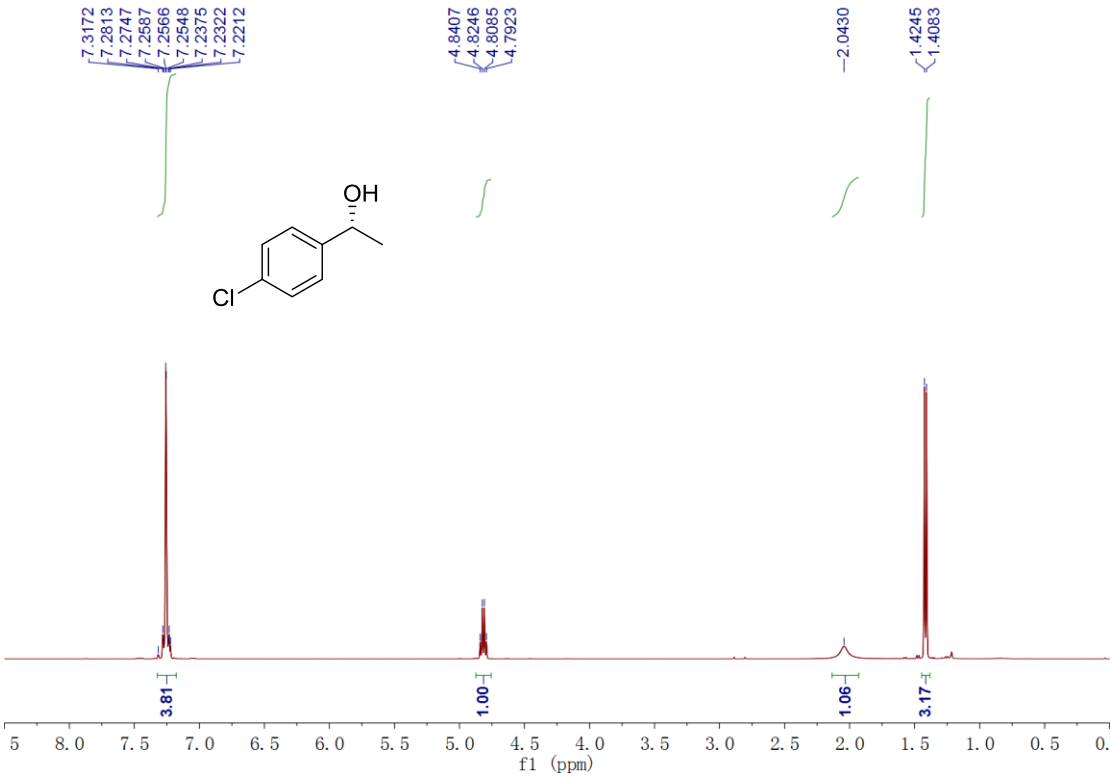


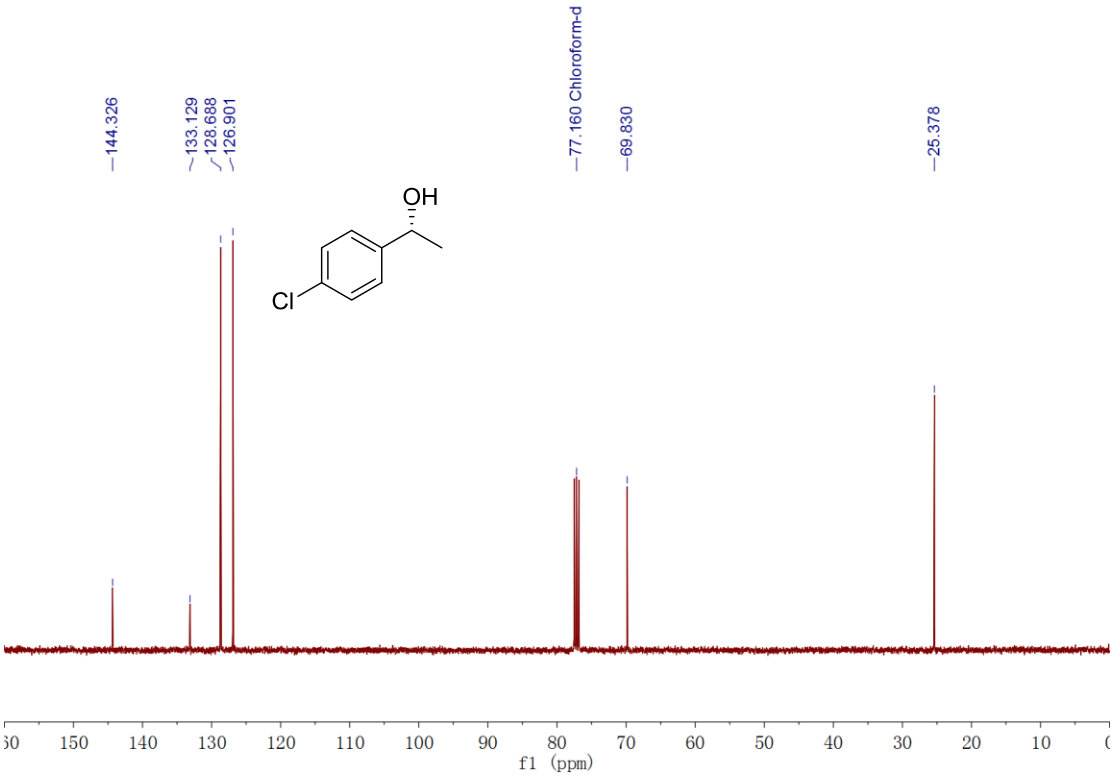
(*R*)-1-(*p*-tolyl)ethan-1-ol (2l)



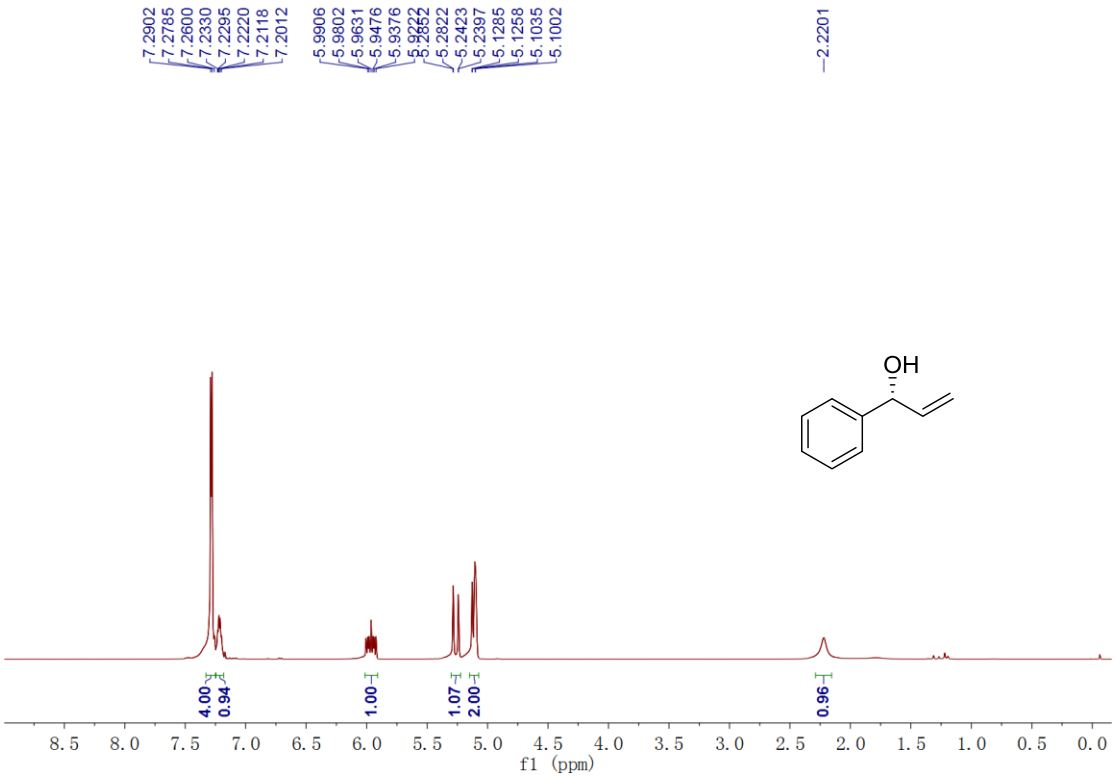


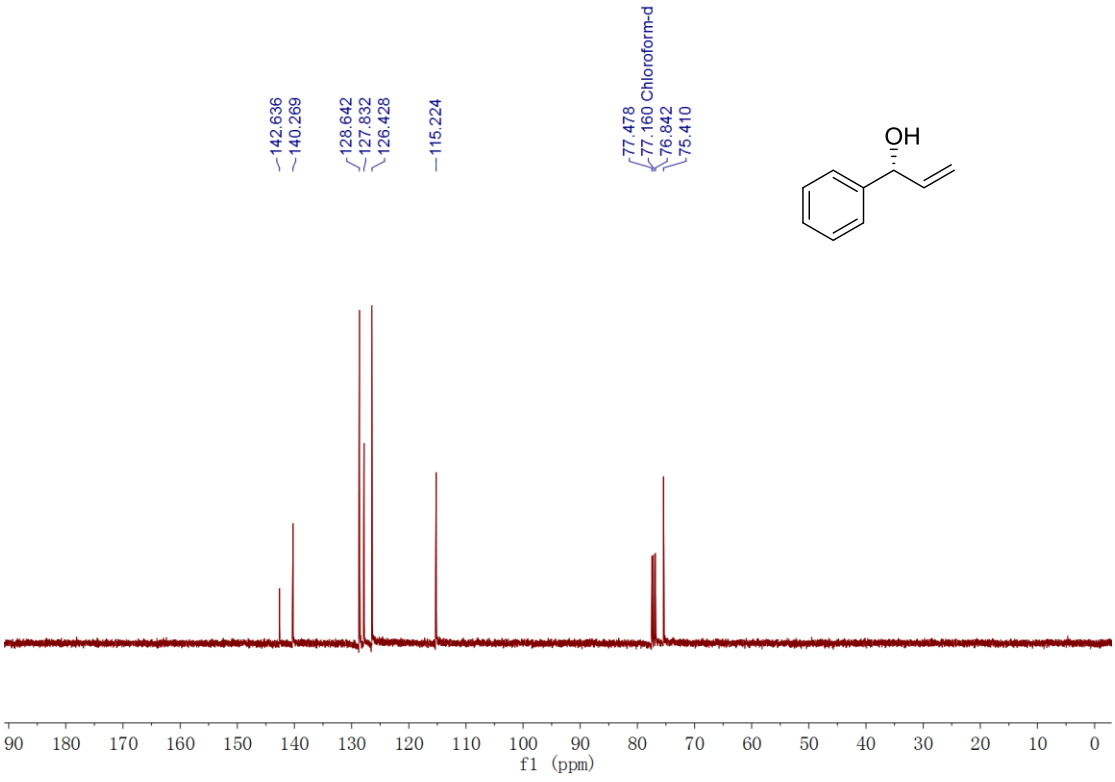
(*R*)-1-(4-chlorophenyl)ethan-1-ol (2m)



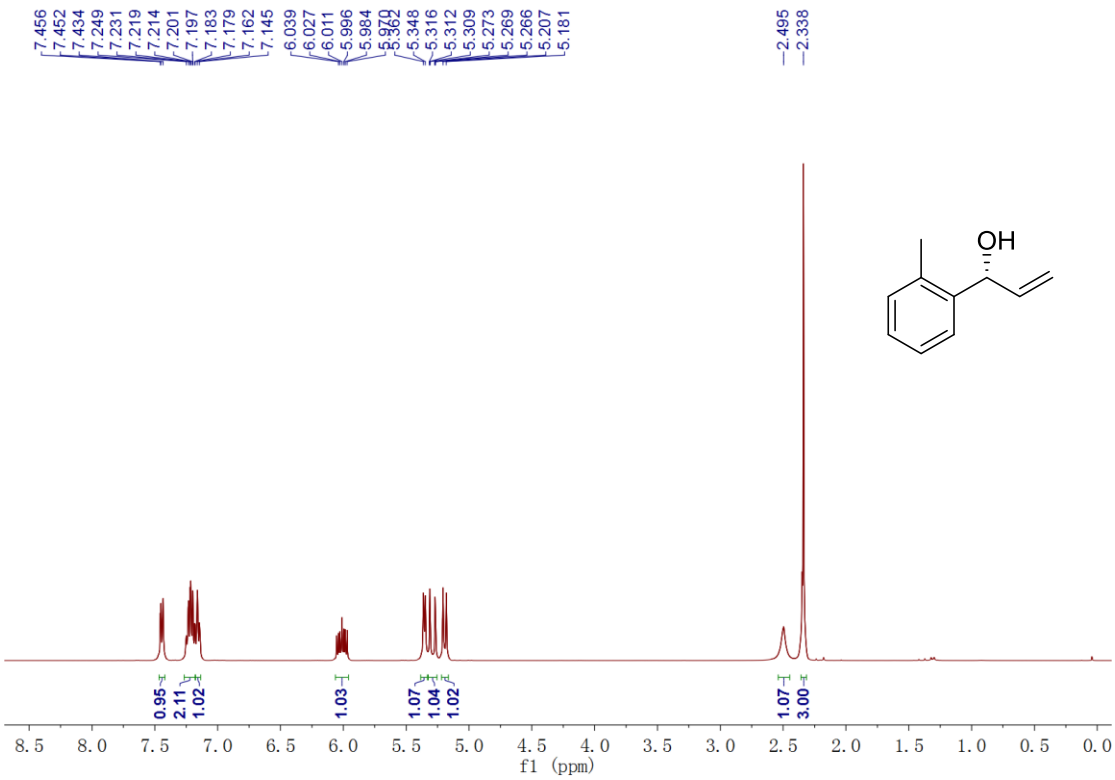


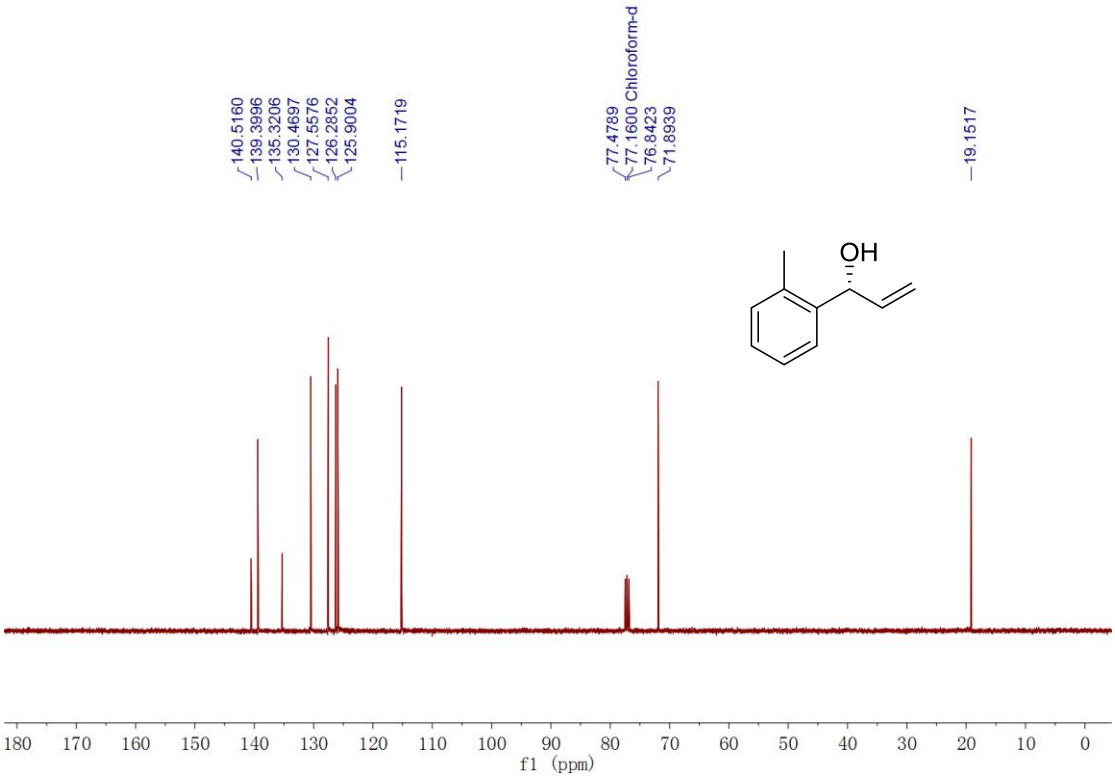
(*R*)-1-phenylprop-2-en-1-ol (2n)



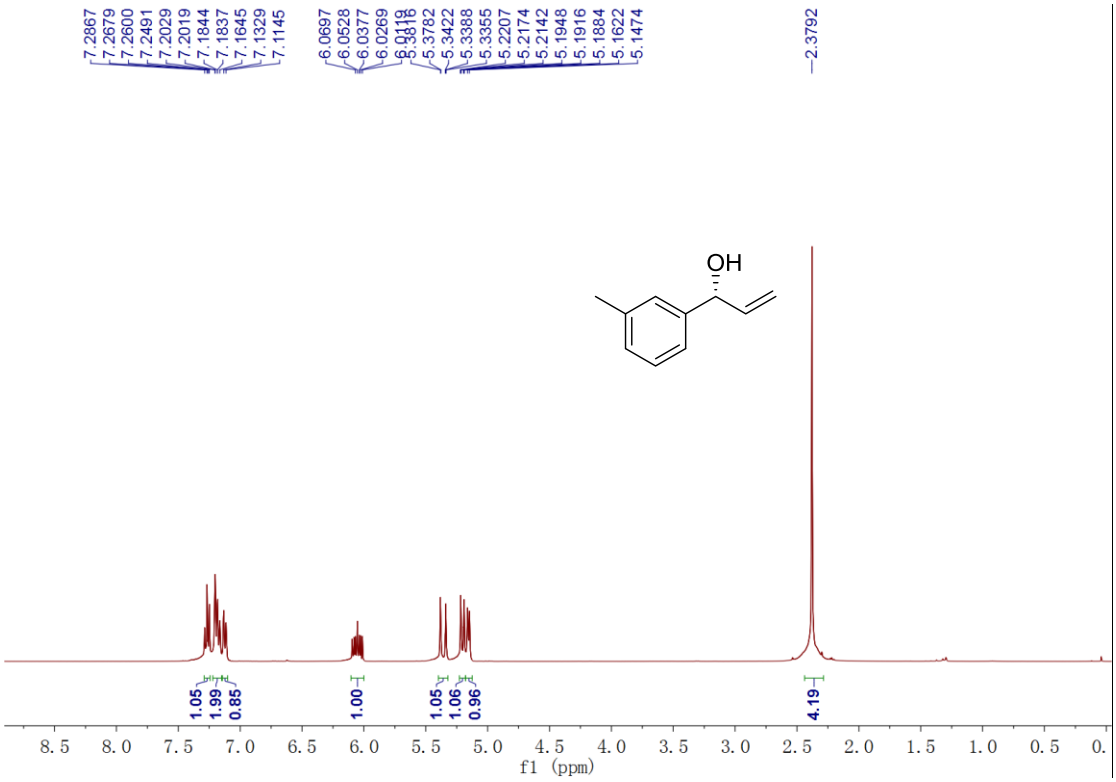


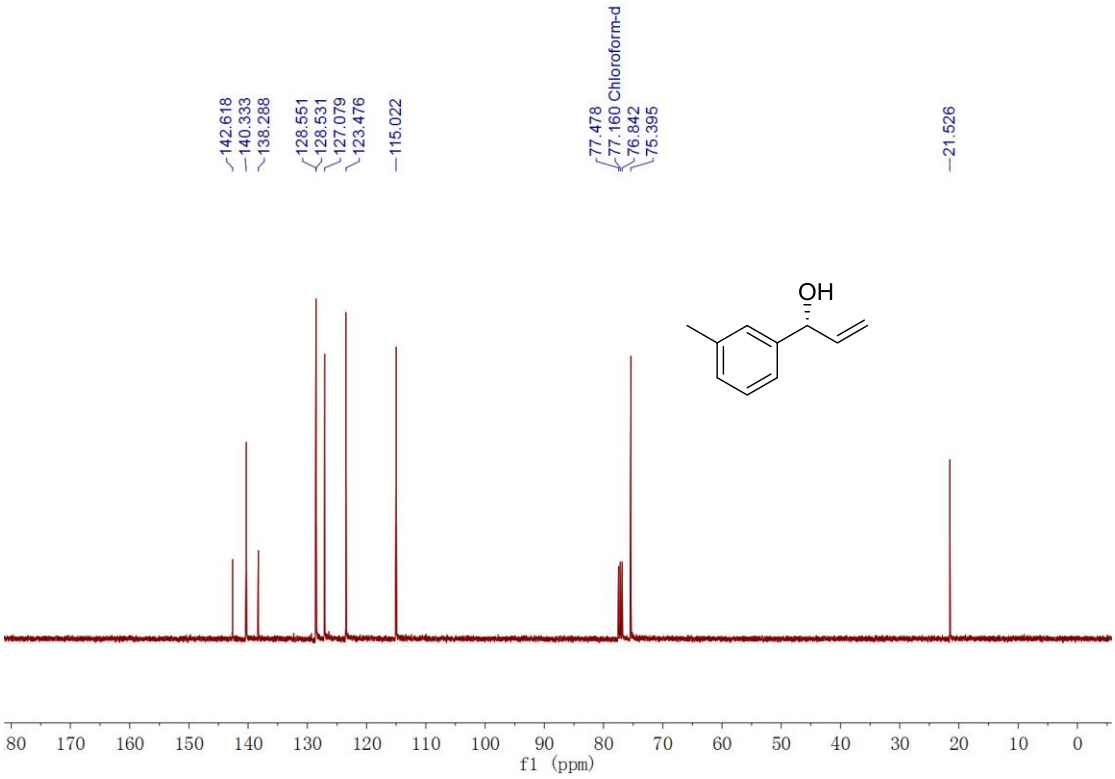
(*R*)-1-(*o*-tolyl)prop-2-en-1-ol (2o)



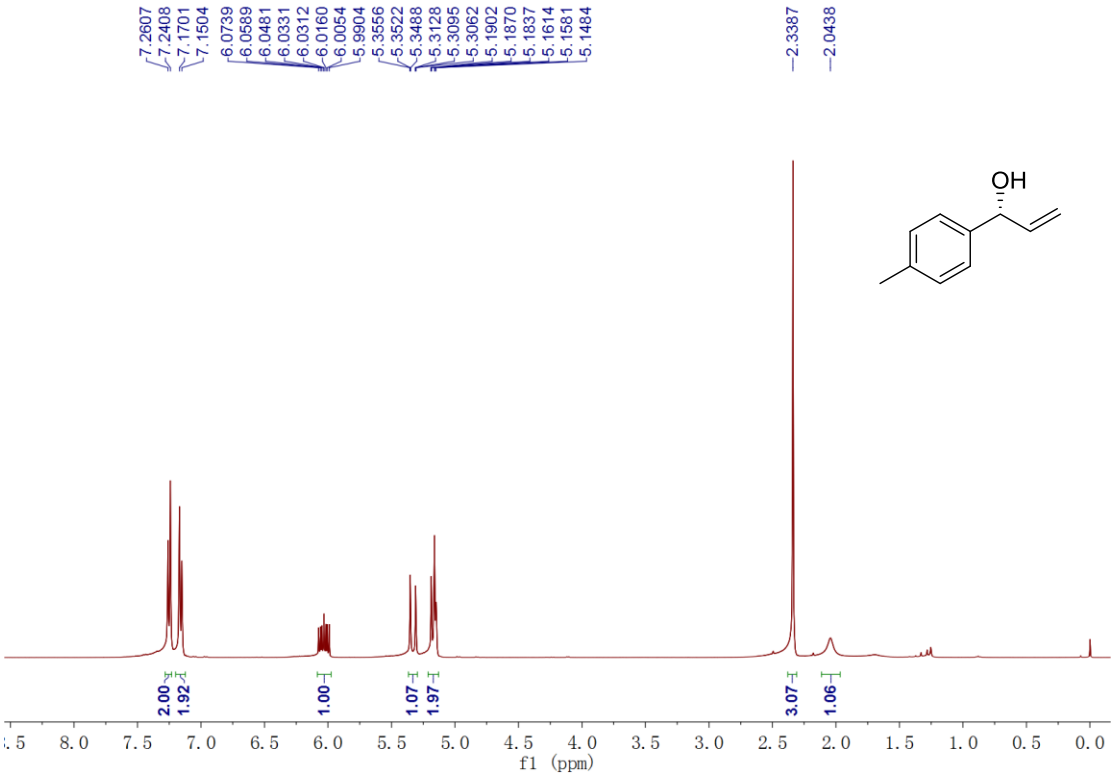


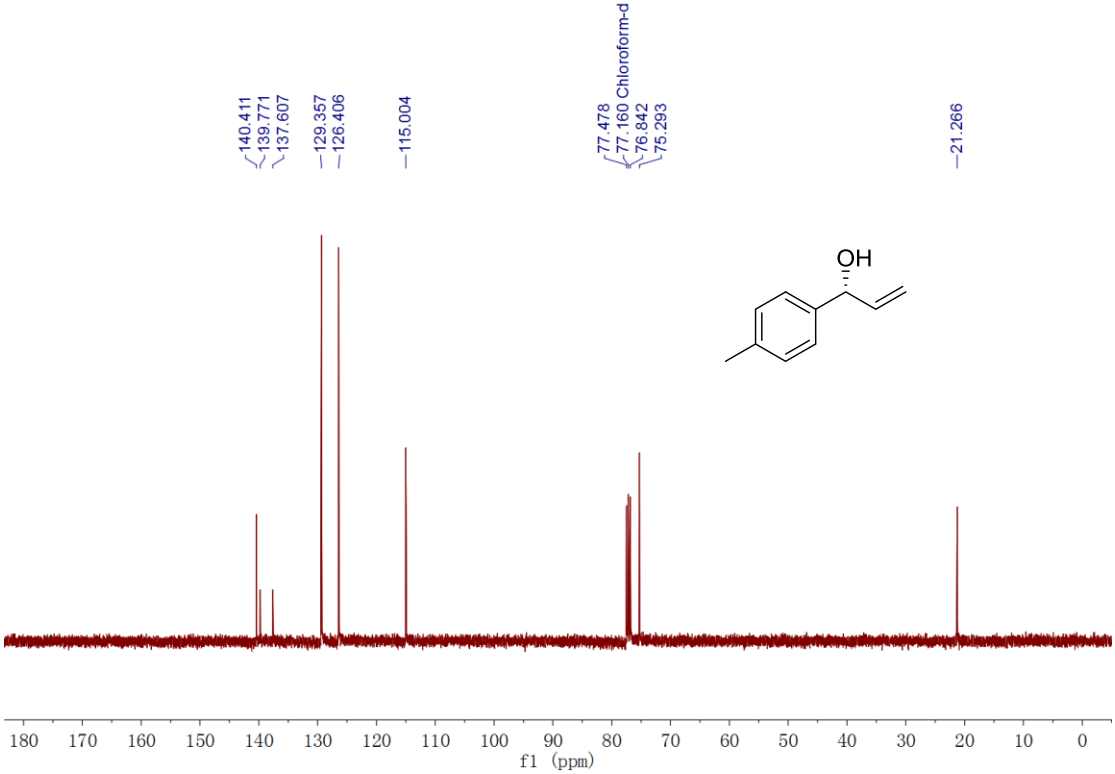
(*R*)-1-(*m*-tolyl)prop-2-en-1-ol (2p)



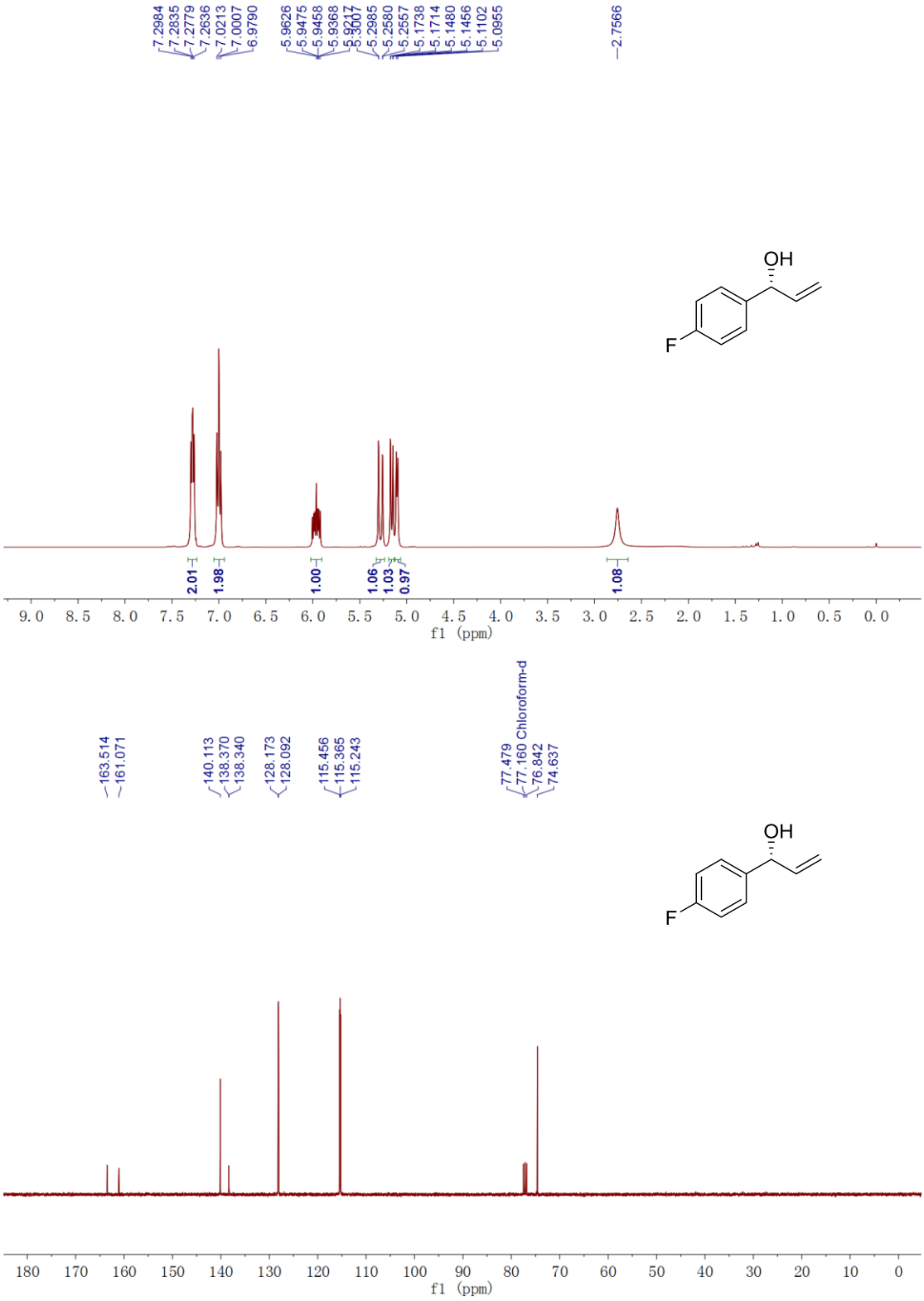


(*R*)-1-(*p*-tolyl)prop-2-en-1-ol (2q)



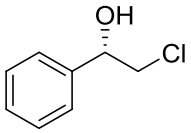


(*R*)-1-(4-fluorophenyl)prop-2-en-1-ol (2r)

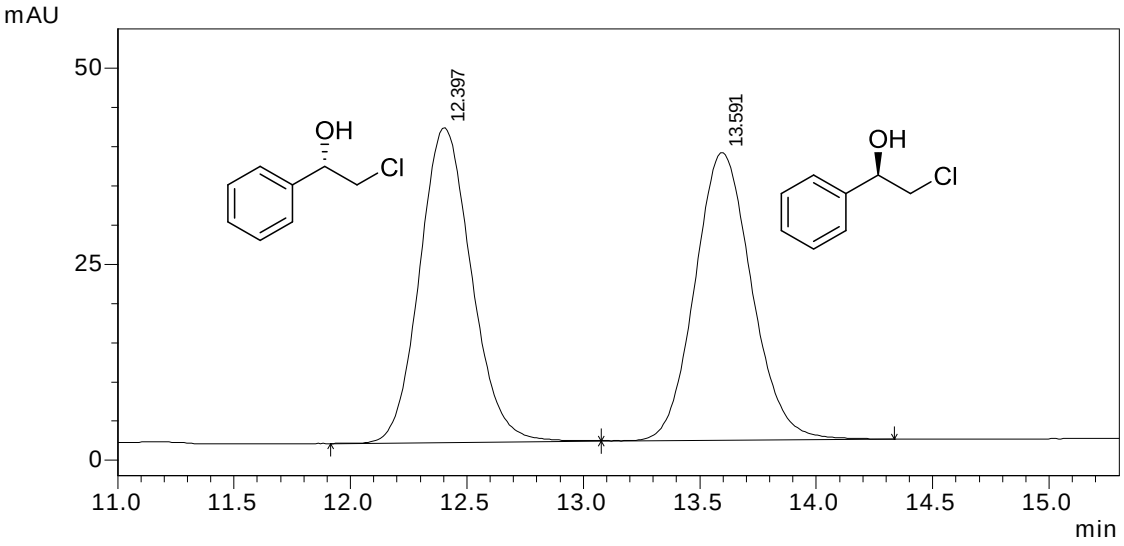


7. Copies of HPLC and GC chromatogram

(*S*)-2-chloro-1-phenylethan-1-ol (2a)



Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min)



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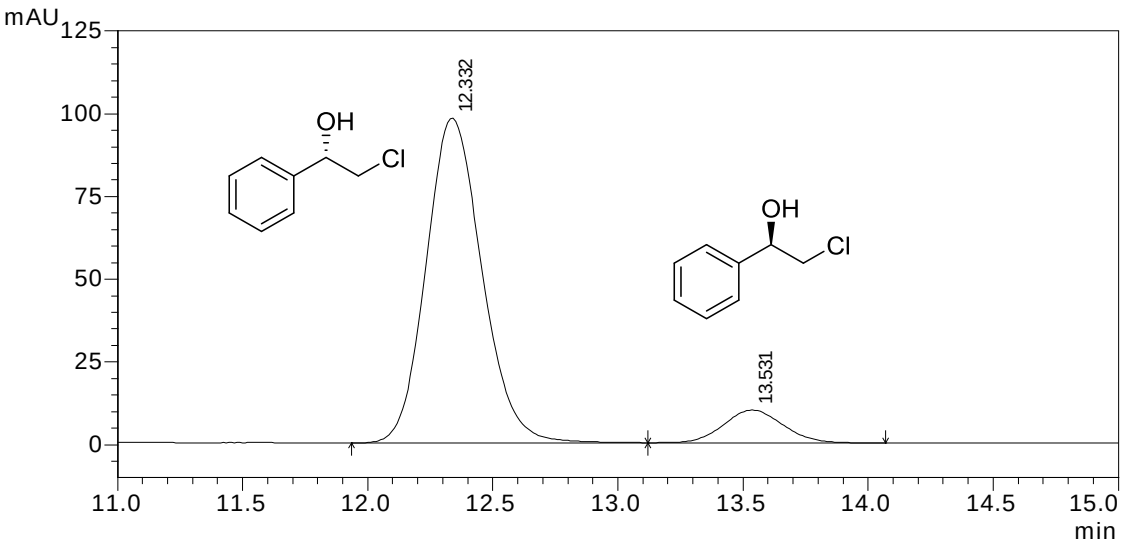

PDA

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1	12.332	1594008	102314	90.031
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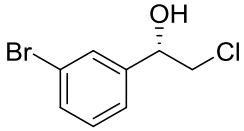
(*S*)-1-(3-bromophenyl)-2-chloroethan-1-ol (2b)



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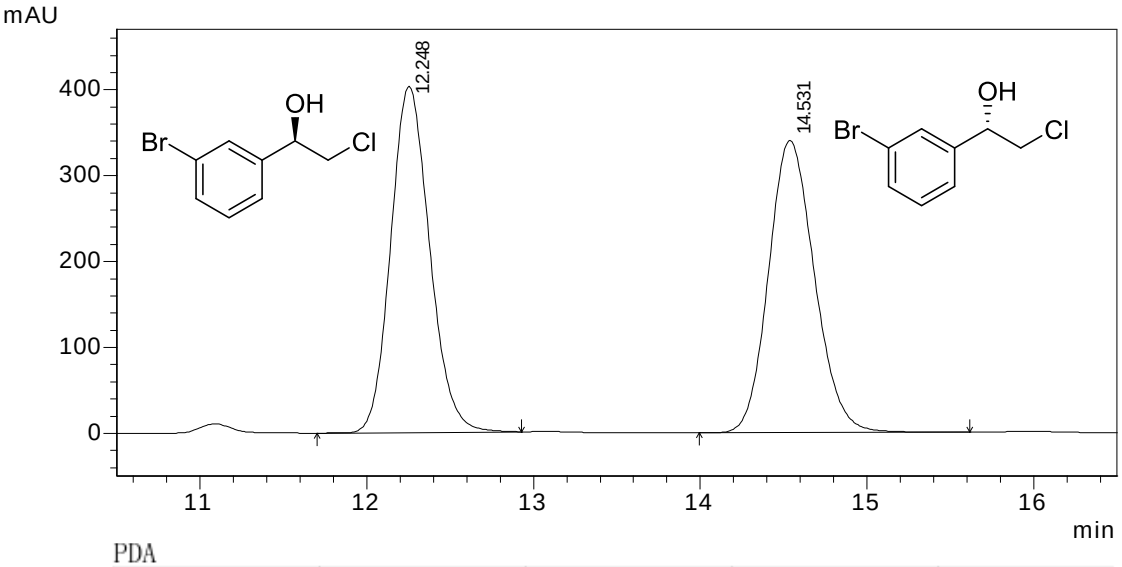

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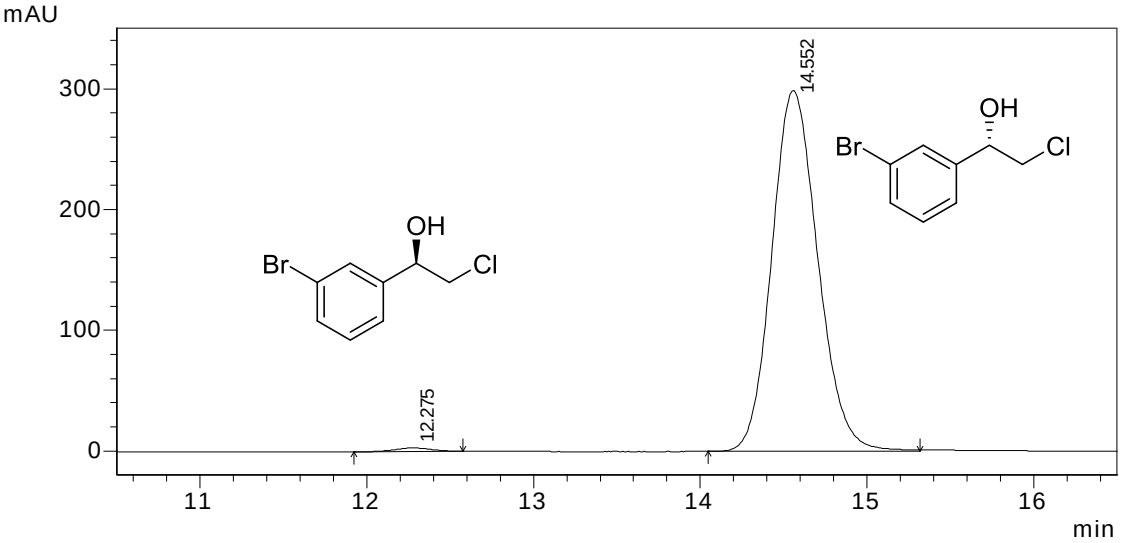

PDA

ID#	Rt. Time	Area	Height	Area %
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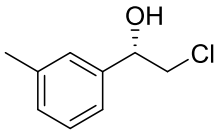
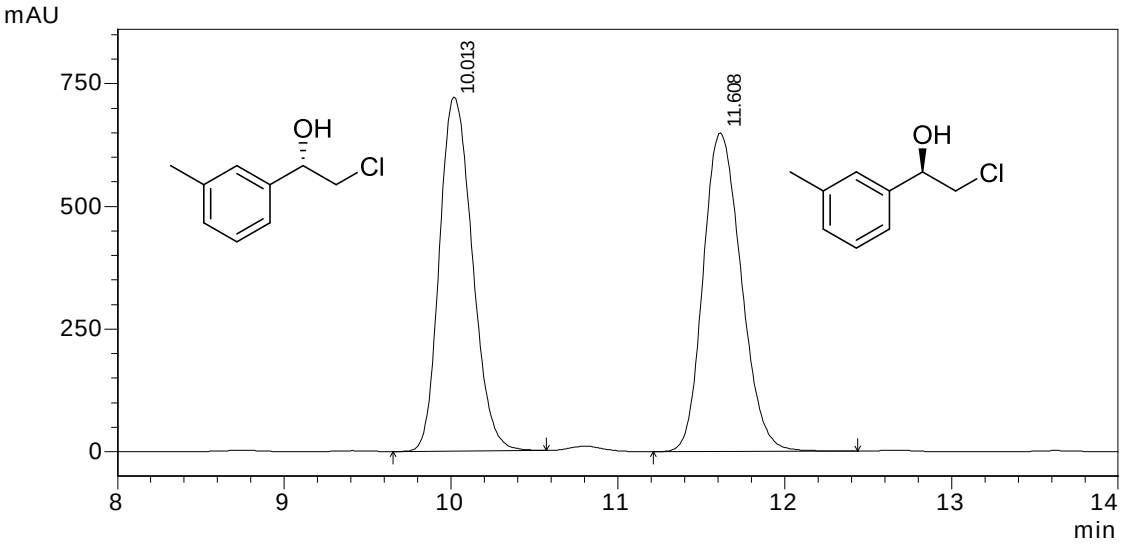
(*S*)-2-chloro-1-(*m*-tolyl)ethan-1-ol (2c)



Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min)



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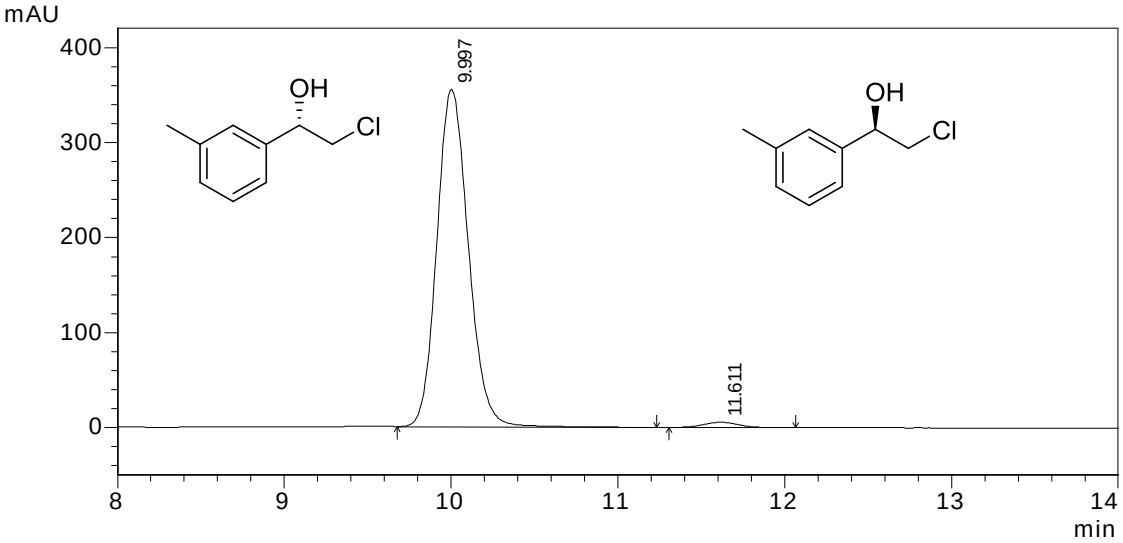

PDA

ID#	Rt. Time	Area	Height	Area %
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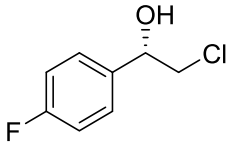
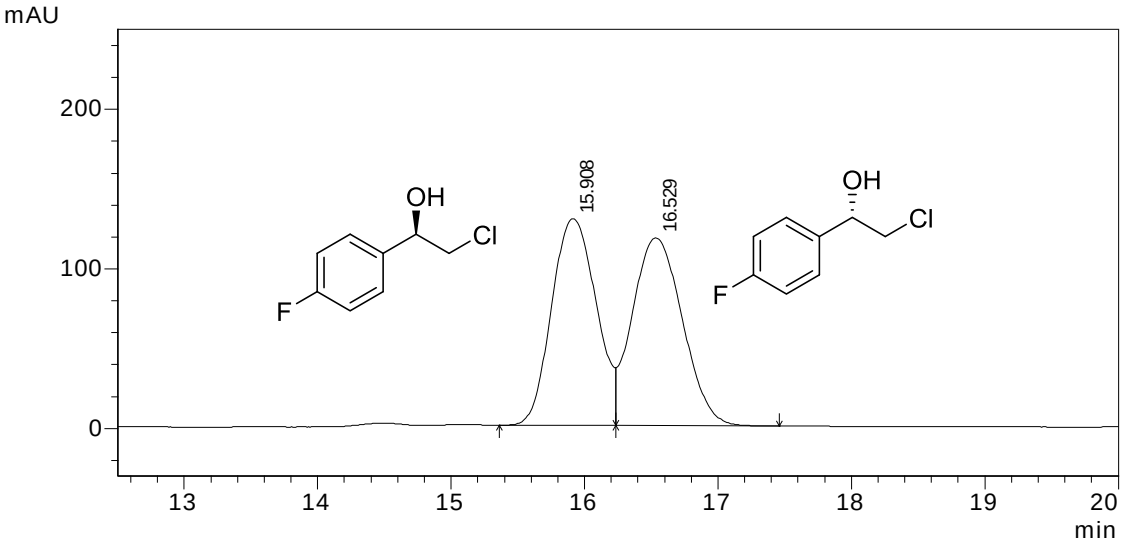
(*S*)-2-chloro-1-(4-fluorophenyl)ethan-1-ol (2d)



Chiral HPLC (Chiralcel OJ-H column, hexane/*i*-PrOH = 90/10, flow rate = 0.8 mL/min)



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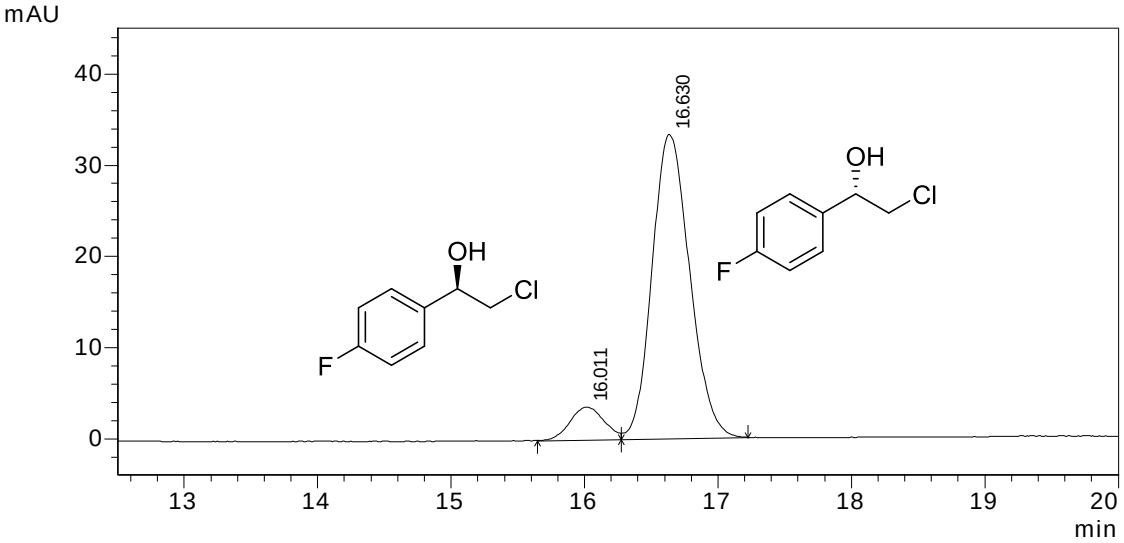

PDA

ID#	Rt. Time	Area	Height	Area %
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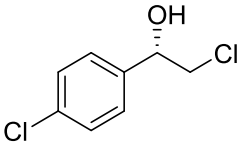
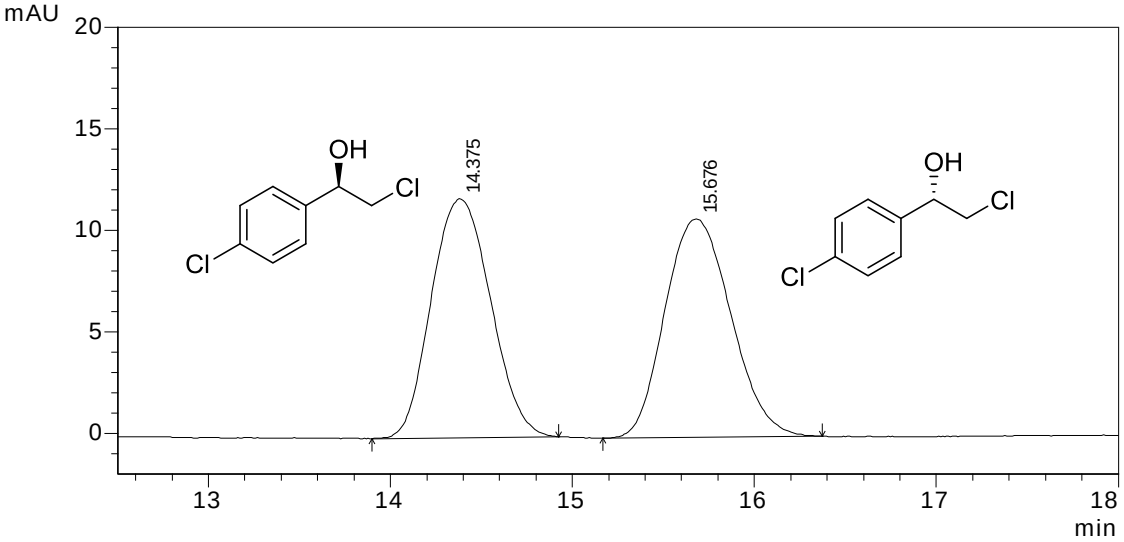
(*S*)-2-chloro-1-(4-chlorophenyl)ethan-1-ol (2e)



Chiral HPLC (Chiralcel OJ-H column, hexane/*i*-PrOH = 90/10, flow rate = 0.8 mL/min)



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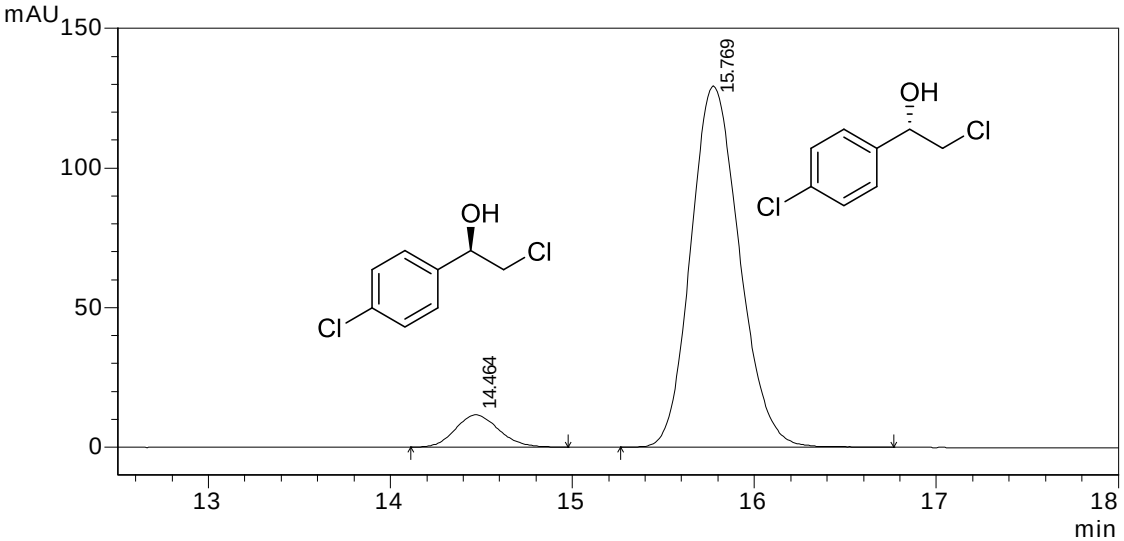

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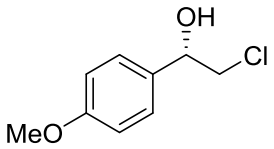
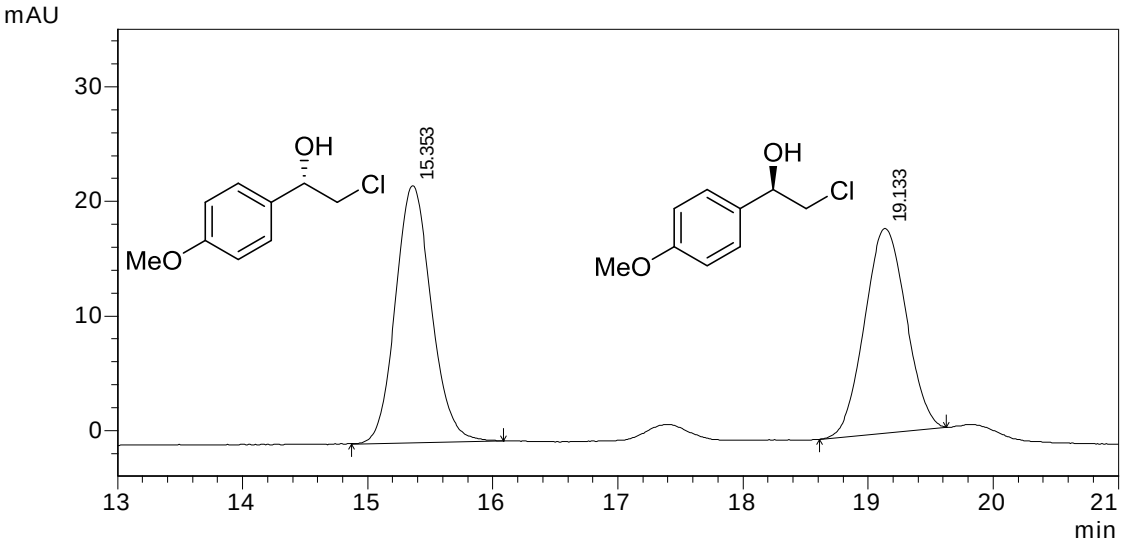
1	14.464	192564	11504	7.448
2	15.769	2392949	128834	92.552

(*S*)-2-chloro-1-(4-methoxyphenyl)ethan-1-ol (2f)

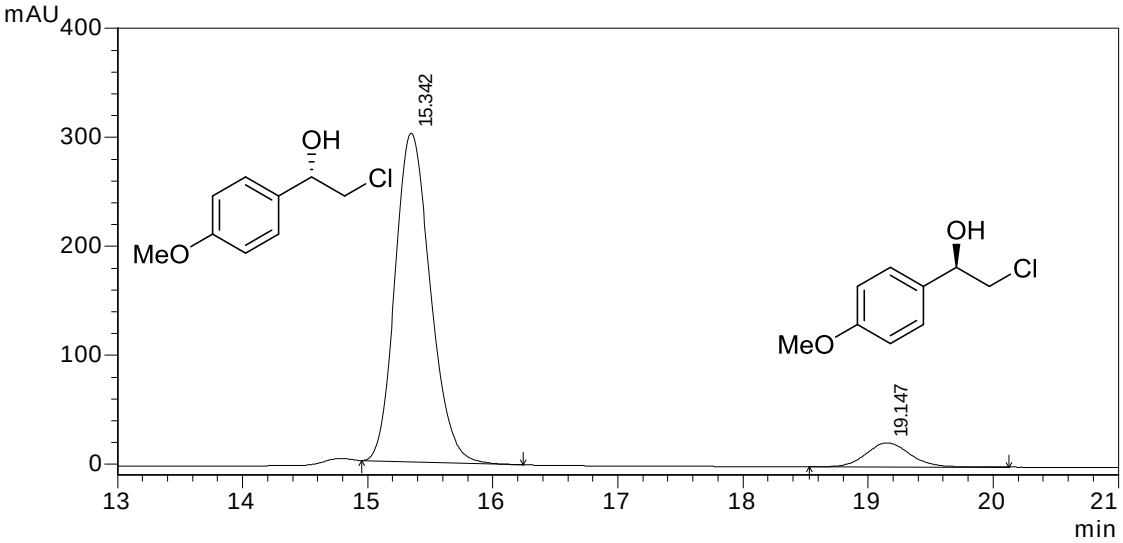


Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min)



PDA

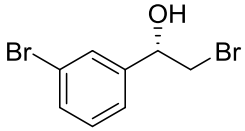
ID#	Rt. Time	Area	Height	Area %
1	15.353	445032	22299	52.098
2	19.133	409183	17703	47.902



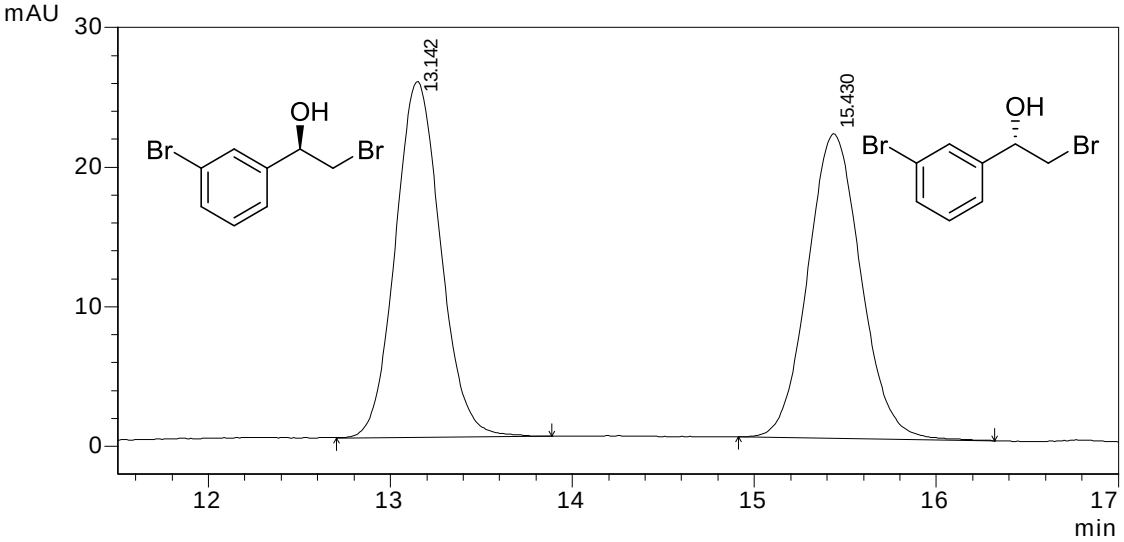
PDA

ID#	Rt. Time	Area	Height	Area %
1	15.342	5872632	299457	91.678
2	19.147	533118	21781	8.322

(*S*)-2-bromo-1-(3-bromophenyl)ethan-1-ol (2g)

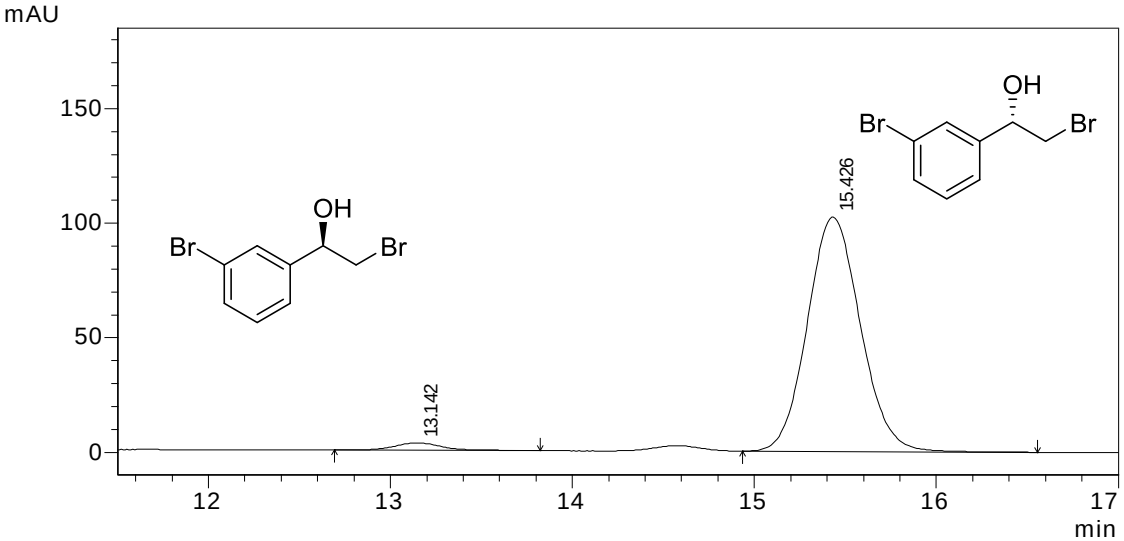


Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min)



PDA

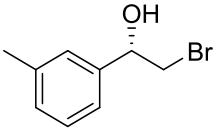
ID#	Rt. Time	Area	Height	Area %
1	13.142	444863	25654	49.907
2	15.430	446519	21979	50.093



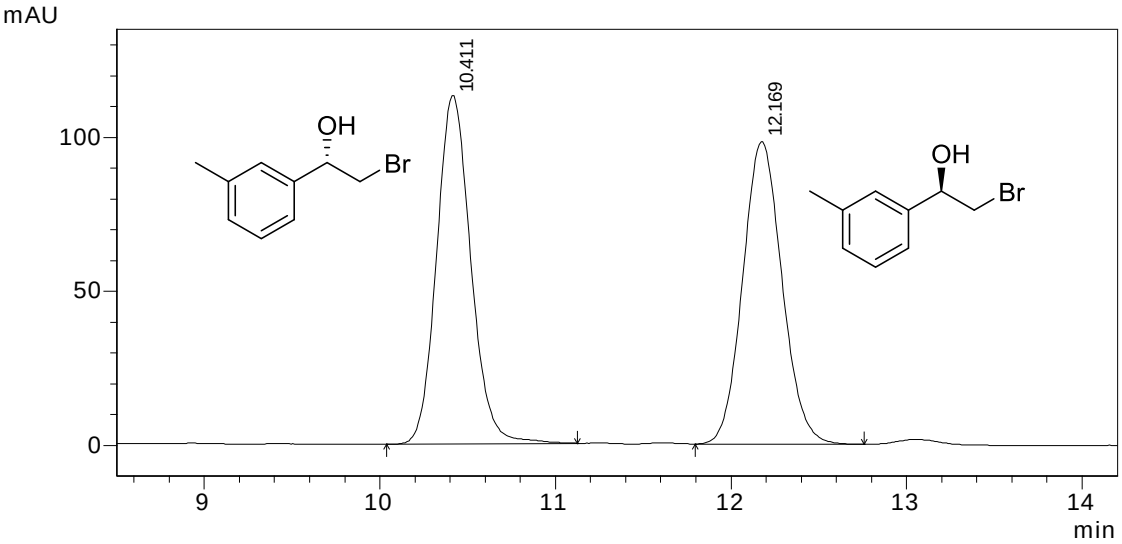
PDA

ID#	Rt. Time	Area	Height	Area %
1	13.142	61855	3331	2.878
2	15.426	2087326	103053	97.122

(*S*)-2-bromo-1-(*m*-tolyl)ethan-1-ol (2h)

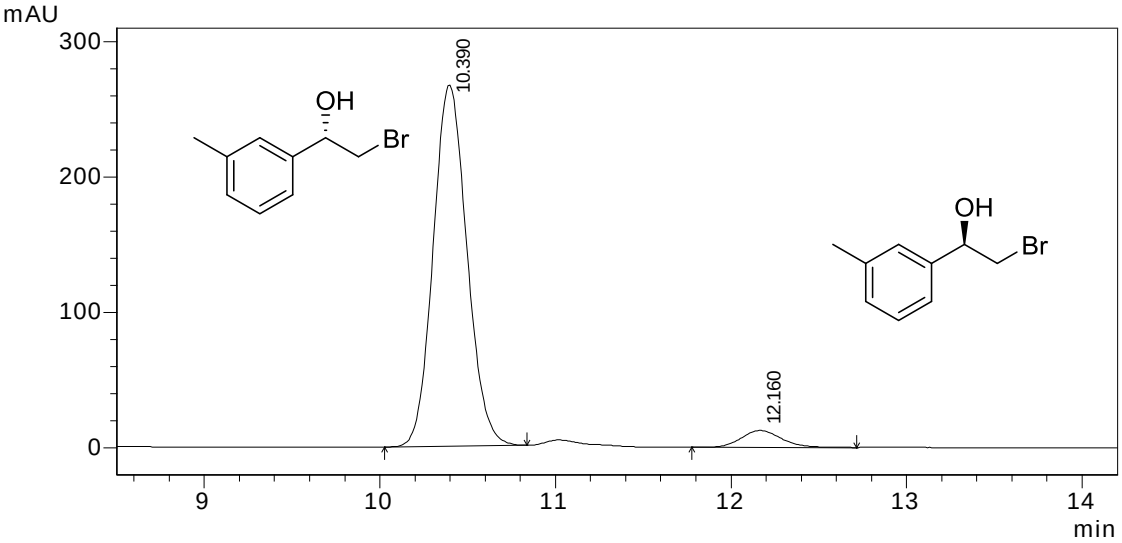


Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min)



PDA

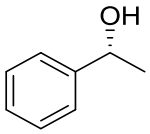
ID#	Rt. Time	Area	Height	Area %
1	10.411	1548889	114101	49.967
2	12.169	1550937	99266	50.033



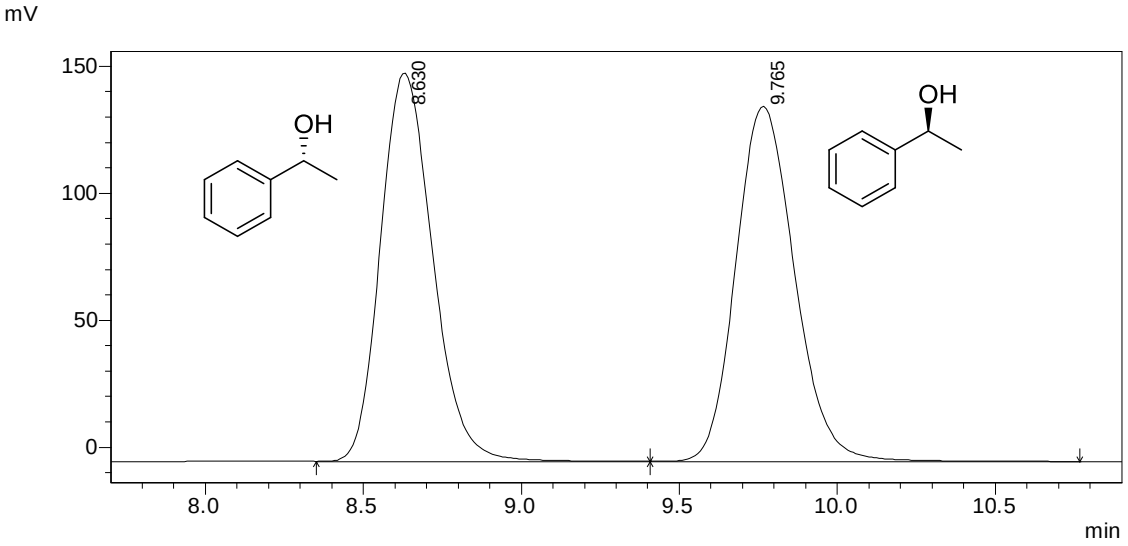
PDA

ID#	Rt. Time	Area	Height	Area %
1	10.390	3605187	269387	94.666
2	12.160	203135	12605	5.334

(*R*)-1-phenylethan-1-ol (2i)

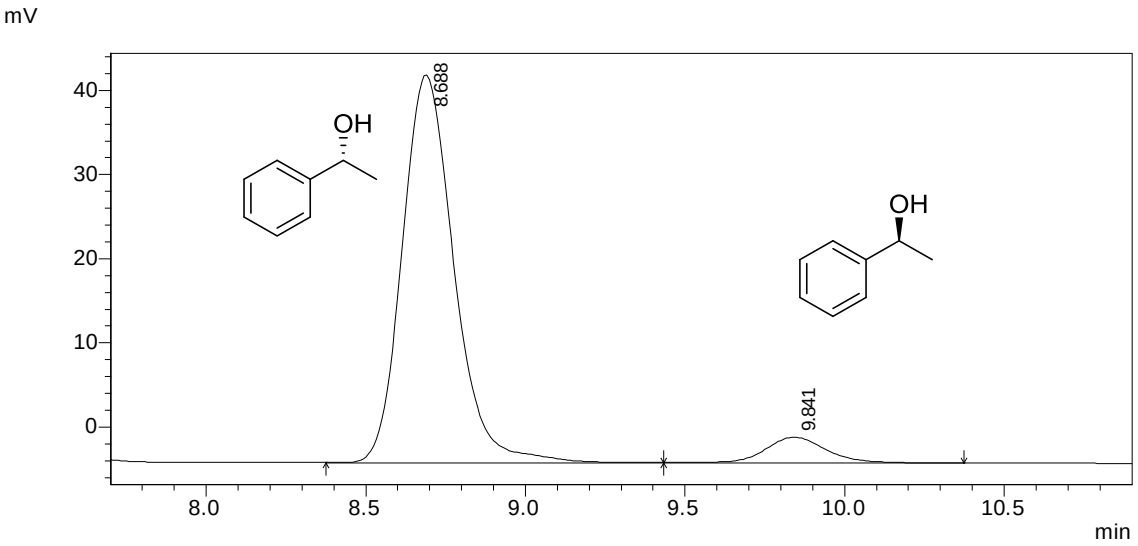


Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min)



PDA

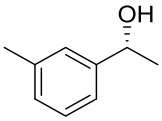
ID#	Ret. Time	Area	Height	Area %
1	8.630	1757205	152765	49.310
2	9.765	1806355	139738	50.690



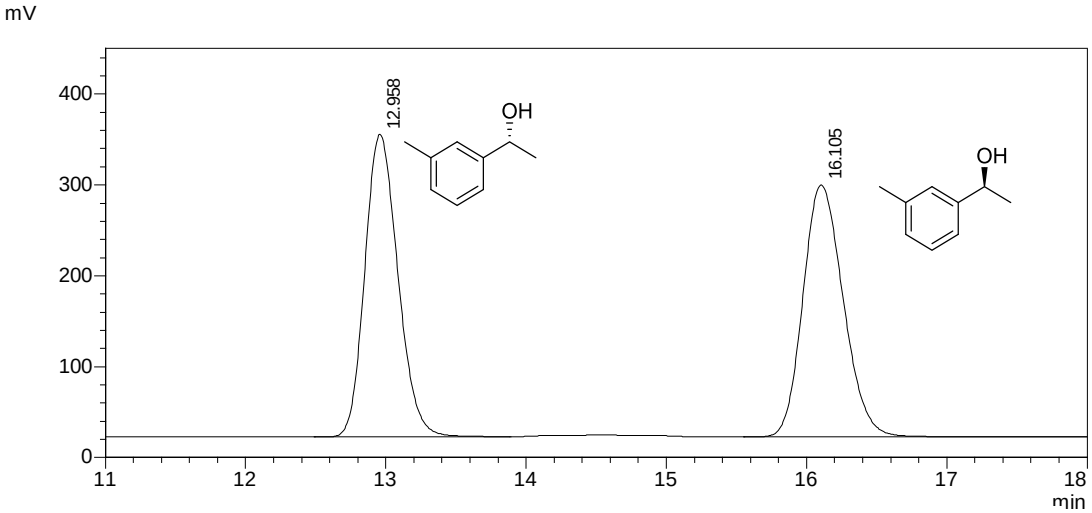
PDA

ID#	Ret. Time	Area	Height	Area %
1	8.688	518947	46042	92.723
2	9.841	40725	3074	7.277

(*R*)-1-(*m*-tolyl)ethan-1-ol (2i)

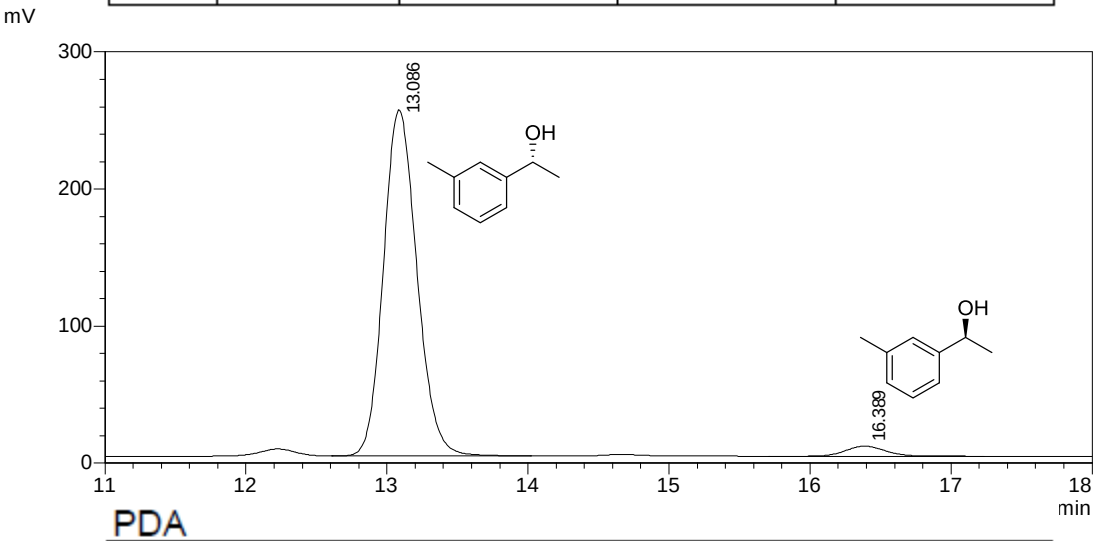


Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 98/2, flow rate = 1.0 mL/min)



PDA

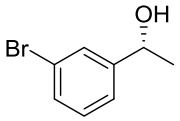
ID#	Rt. Time	Area	Height	Area %
1	12.958	5458235	332842	49.625
2	16.105	5540749	277103	50.375



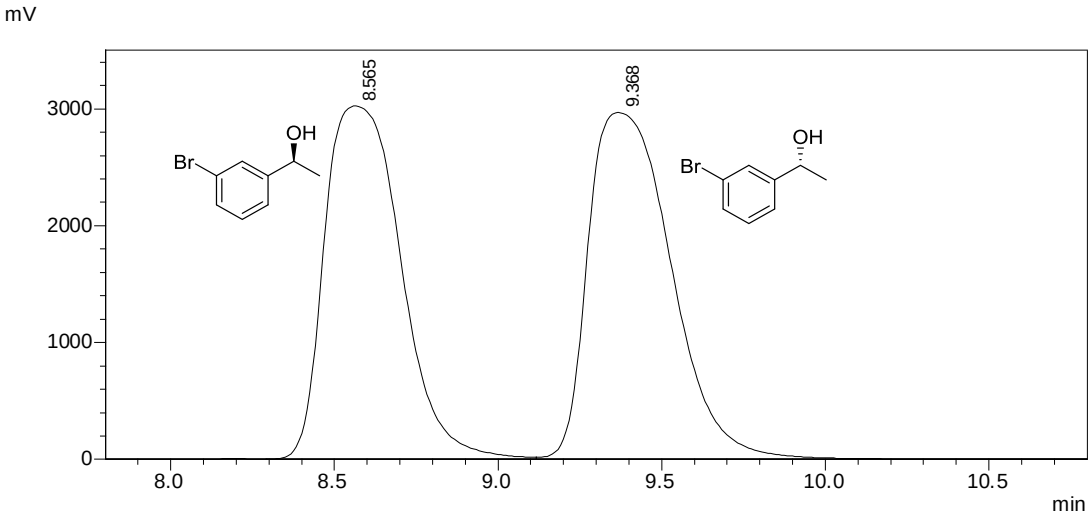
PDA

ID#	Rt. Time	Area	Height	Area %
1	13.086	4170588	252472	96.598
2	16.389	146885	7340	3.402

(*R*)-1-(3-bromophenyl)ethan-1-ol (2k)

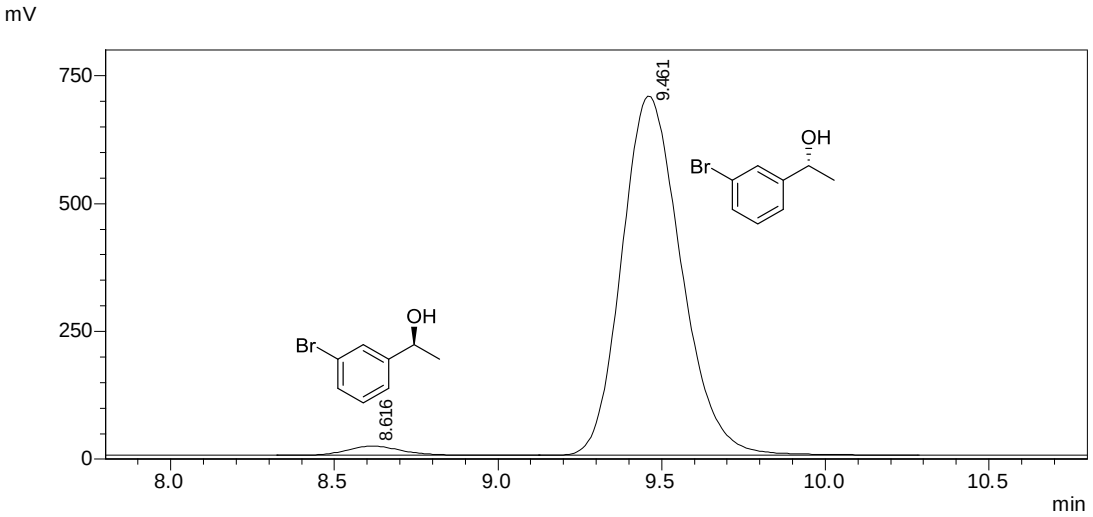


Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 95/5, flow rate = 1.0 mL/min)



PDA

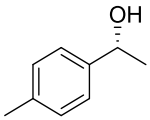
ID#	Rt. Time	Area	Height	Area %
1	8.565	49182016	3025315	48.343
2	9.368	52552664	2968428	51.657



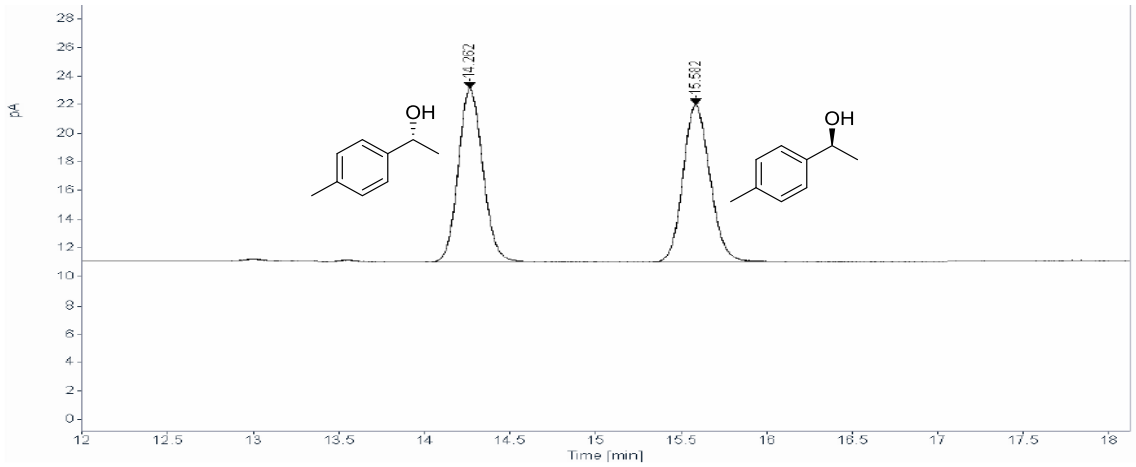
PDA

ID#	Rt. Time	Area	Height	Area %
1	8.616	216124	18208	2.376
2	9.461	8880736	702436	97.624

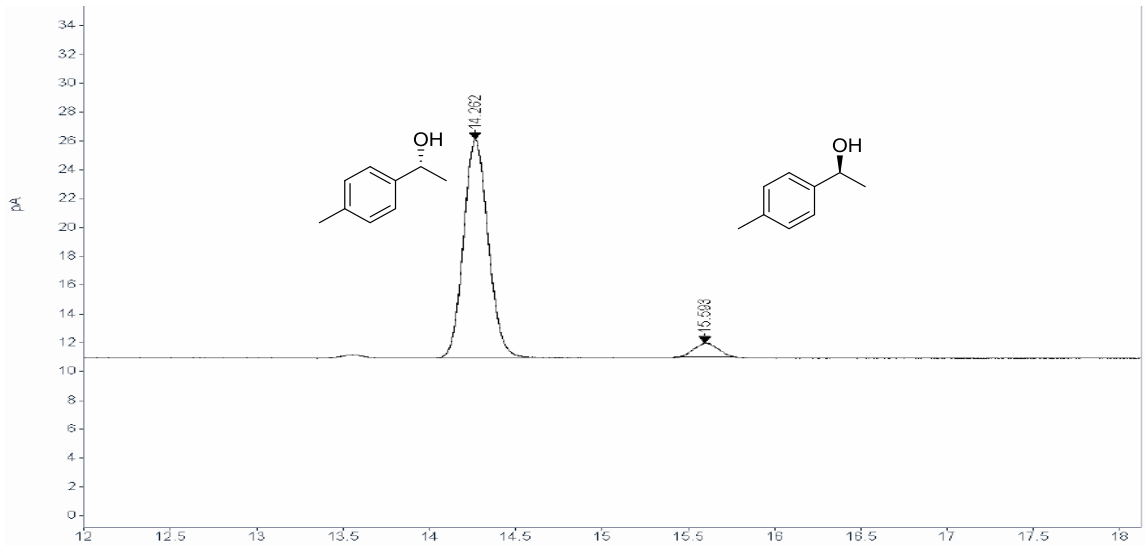
(*R*)-1-(*p*-tolyl)ethan-1-ol (2l)



Chiral GC (BGB-175 column, oven temperature: 110 °C hold 20 min)

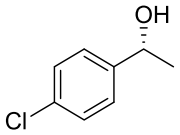


ID#	Rt. Time	Area	Height	Area %	Resolution
5	14.262	119.659	12.028	50.140	
6	15.582	118.990	10.870	49.860	4.840

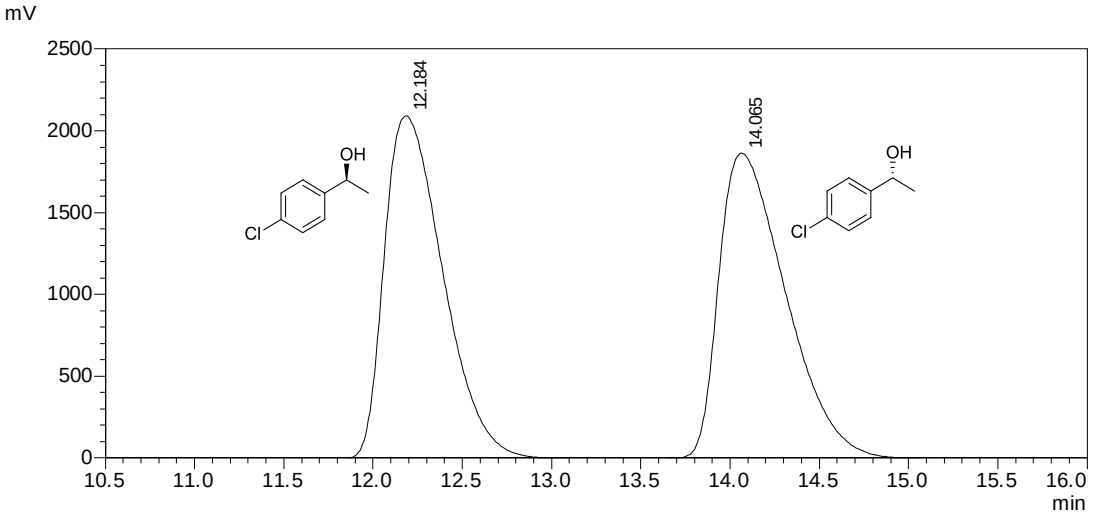


ID#	Rt. Time	Area	Height	Area %	Resolution
5	14.262	150.823	15.155	94.402	
6	15.593	8.943	0.931	5.598	5.061

(*R*)-1-(4-chlorophenyl)ethan-1-ol (2m)

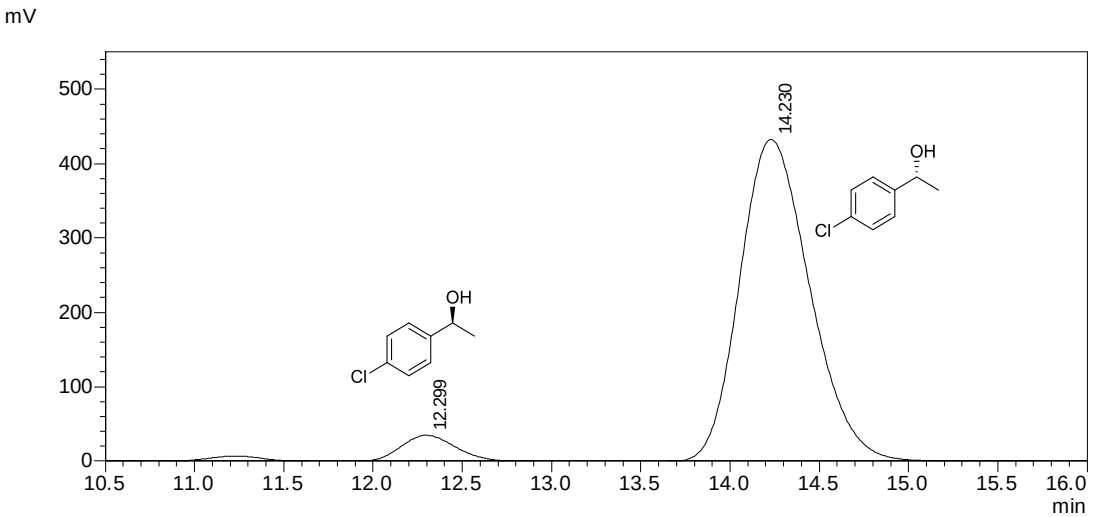


Chiral HPLC (Chiralcel OB-H column, hexane/*i*-PrOH = 98/2, flow rate = 1.0 mL/min)



PDA

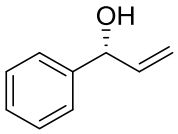
ID#	Rt. Time	Area	Height	Area %
1	12.184	48378821	2100141	49.507
2	14.065	49341513	1872589	50.493



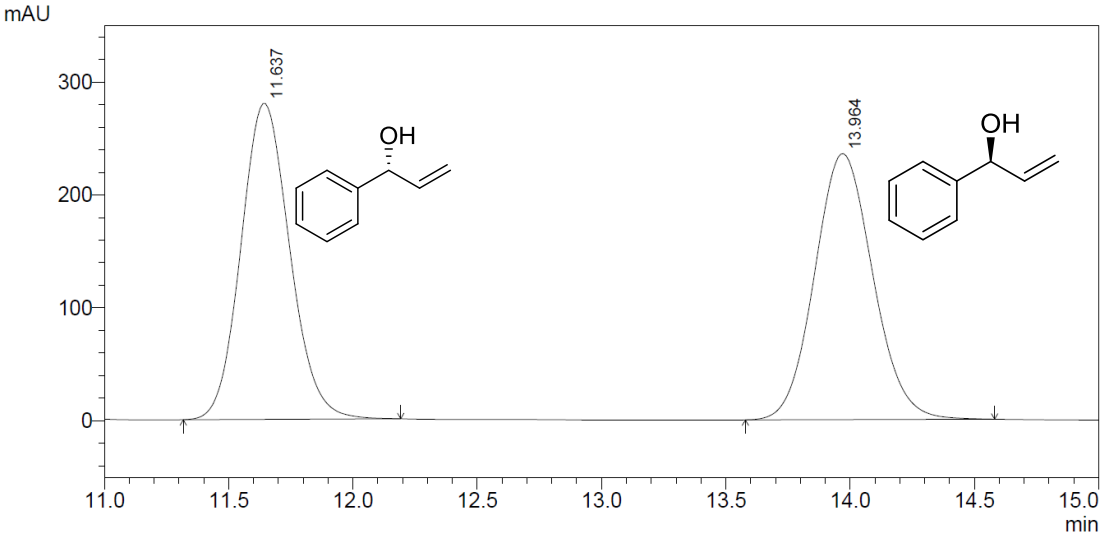
PDA

ID#	Rt. Time	Area	Height	Area %
1	12.299	789735	35823	6.219
2	14.230	11908499	433485	93.781

(*R*)-1-phenylprop-2-en-1-ol (2n)

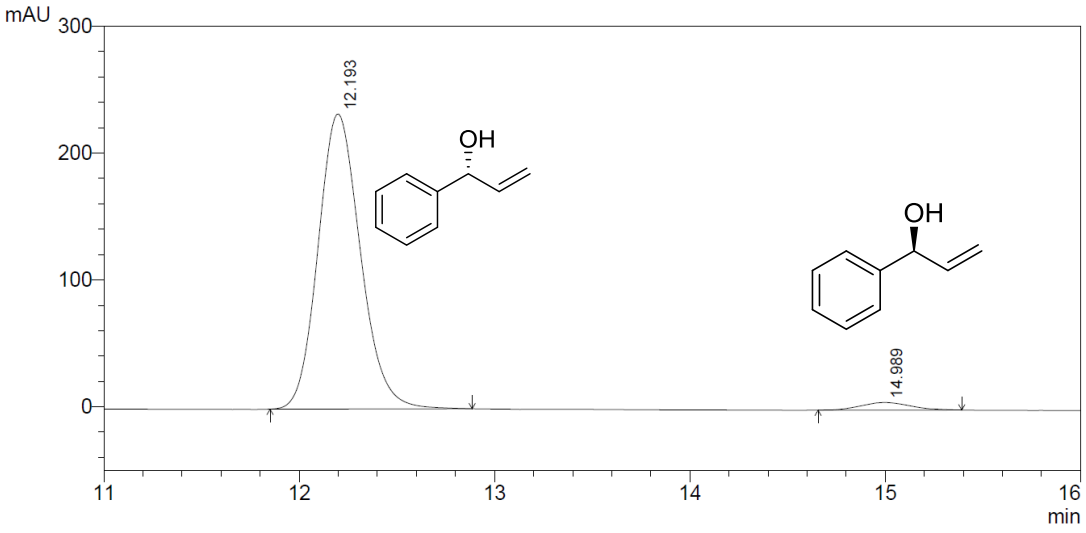


Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 95/5, flow rate = 0.8 mL/min)



PDA

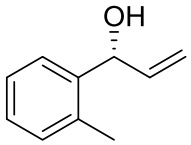
ID#	Rt. Time	Area	Height	Area %
1	11.637	3830711	279698	49.930
2	13.964	3841418	235506	50.070



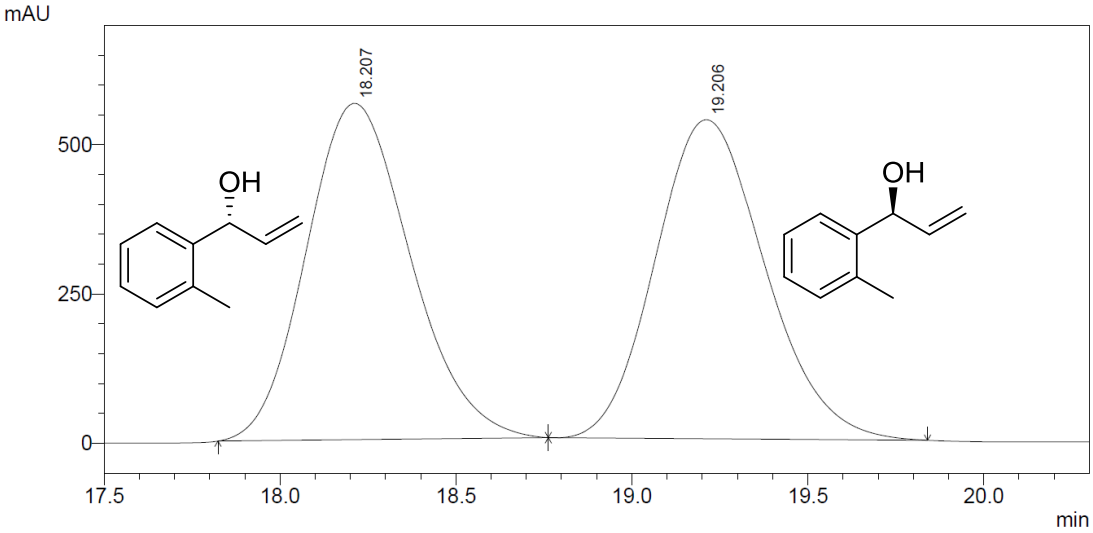
PDA

ID#	Rt. Time	Area	Height	Area %
1	12.193	3507275	232502	97.117
2	14.989	104110	5964	2.883

(*R*)-1-(*o*-tolyl)prop-2-en-1-ol (2o)

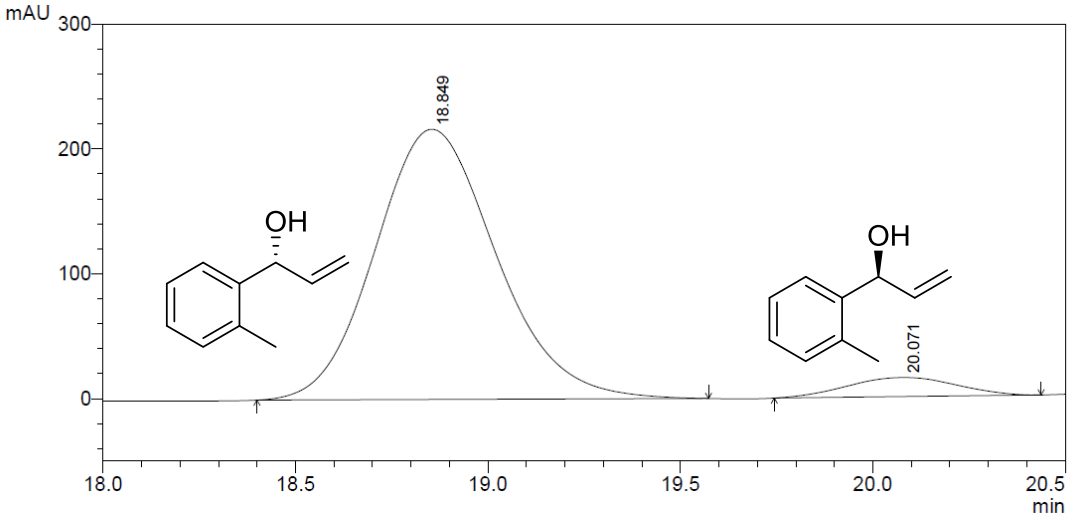


Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 95/5, flow rate = 0.5 mL/min)



PDA

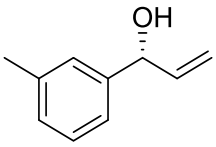
ID#	Rt. Time	Area	Height	Area %
1	18.207	11603534	563422	50.044
2	19.206	11583128	534664	49.956



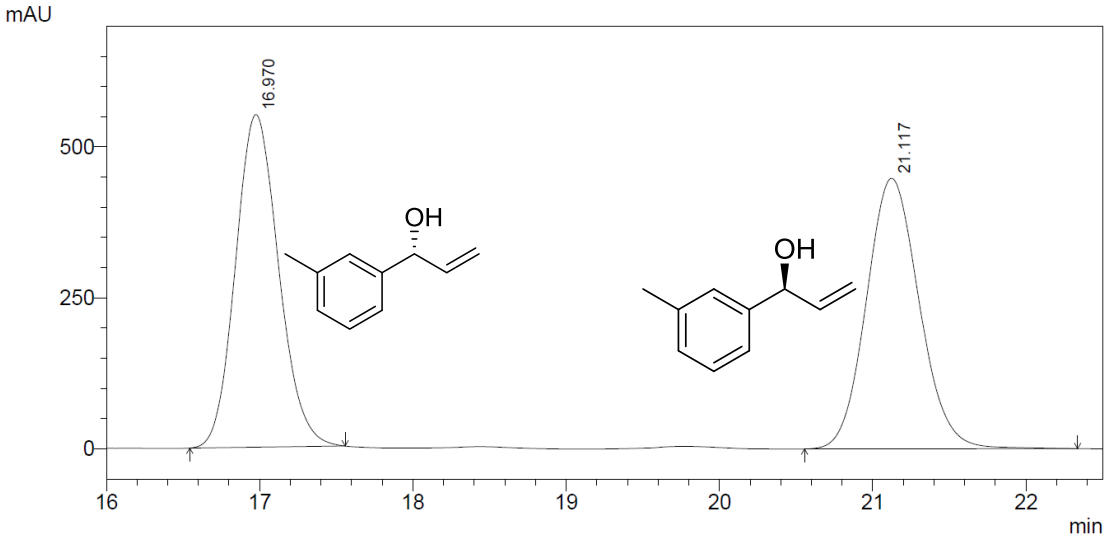
PDA

ID#	Rt. Time	Area	Height	Area %
1	18.849	4743539	216311	94.063
2	20.071	299405	15385	5.937

(*R*)-1-(*m*-tolyl)prop-2-en-1-ol (2p)

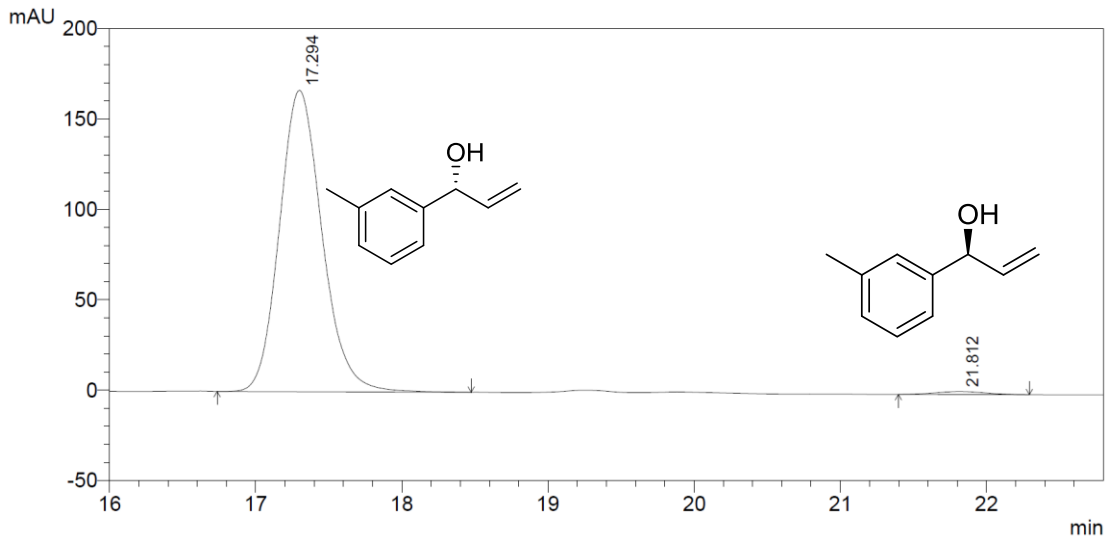


Chiral HPLC (Chiralcel OD-H column, hexane/*i*-PrOH = 95/5, flow rate = 0.5 mL/min)



PDA

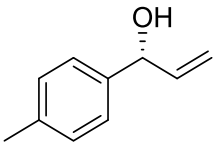
ID#	Rt. Time	Area	Height	Area %
1	16.970	10796595	550749	50.119
2	21.117	10745461	447987	49.881



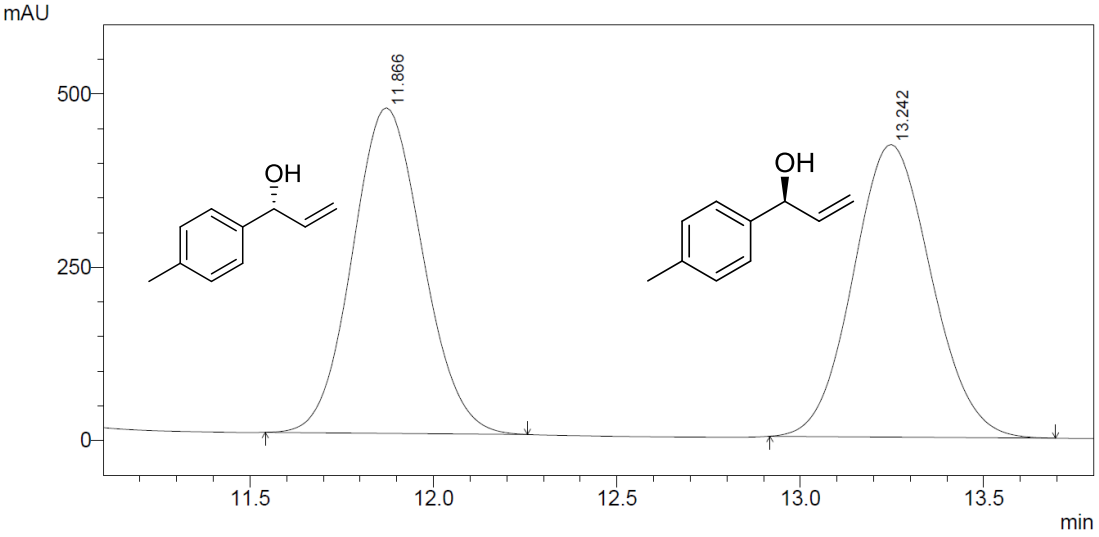
PDA

ID#	Rt. Time	Area	Height	Area %
1	17.294	3382394	166693	98.915
2	21.812	37113	1632	1.085

(*R*)-1-(*p*-tolyl)prop-2-en-1-ol (2q)

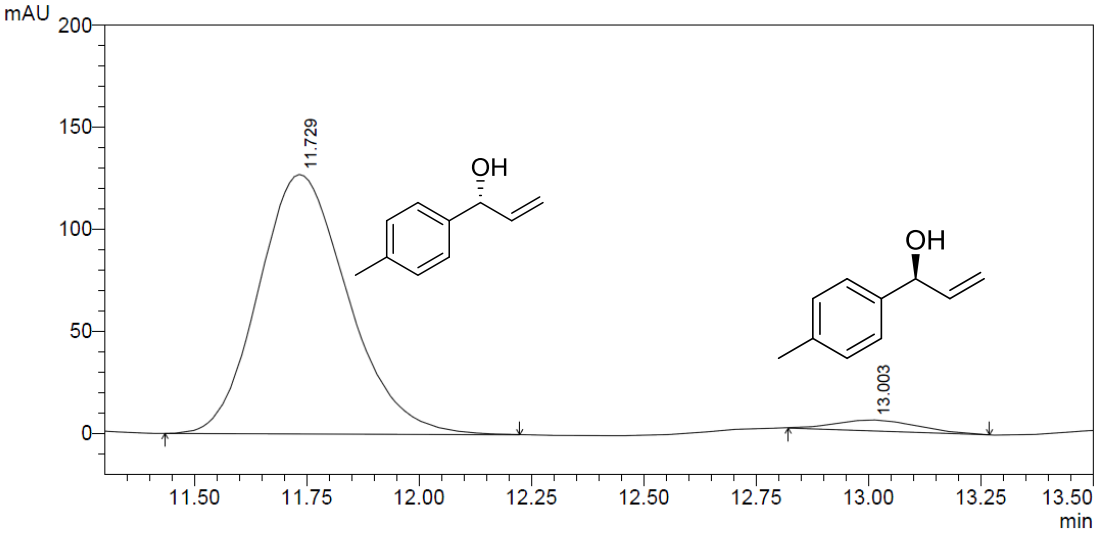


Chiral HPLC (Chiralcel AD-H column, hexane/*i*-PrOH = 95/5, flow rate = 0.8 mL/min)



PDA

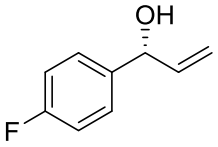
ID#	Rt. Time	Area	Height	Area %
1	11.866	6292774	469779	50.053
2	13.242	6279494	422175	49.947



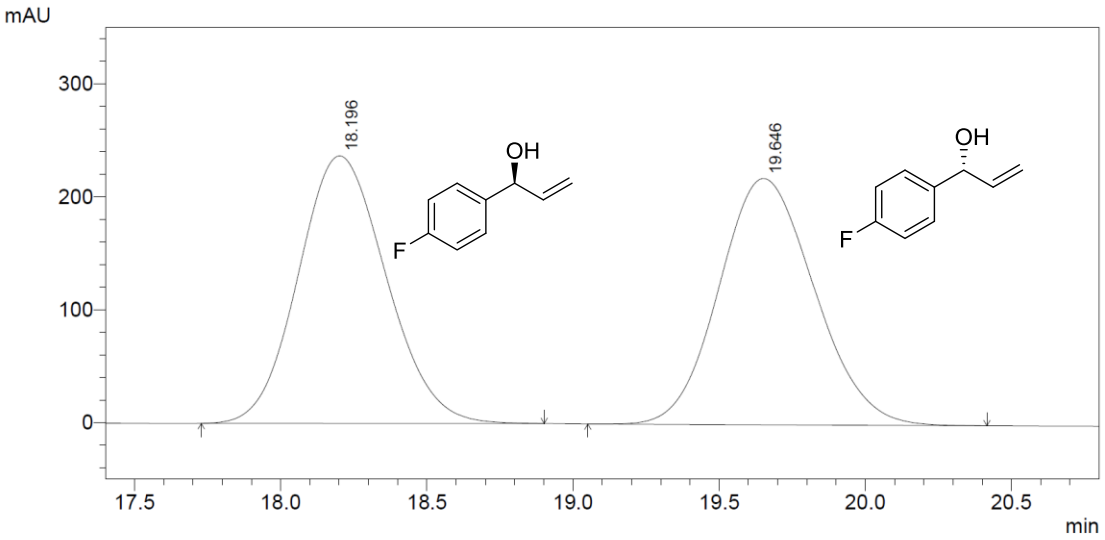
PDA

ID#	Rt.Time	Area	Height	Area %
1	11.729	1756501	127088	96.394
2	13.003	65701	5303	3.606

(*R*)-1-(4-fluorophenyl)prop-2-en-1-ol (2r)

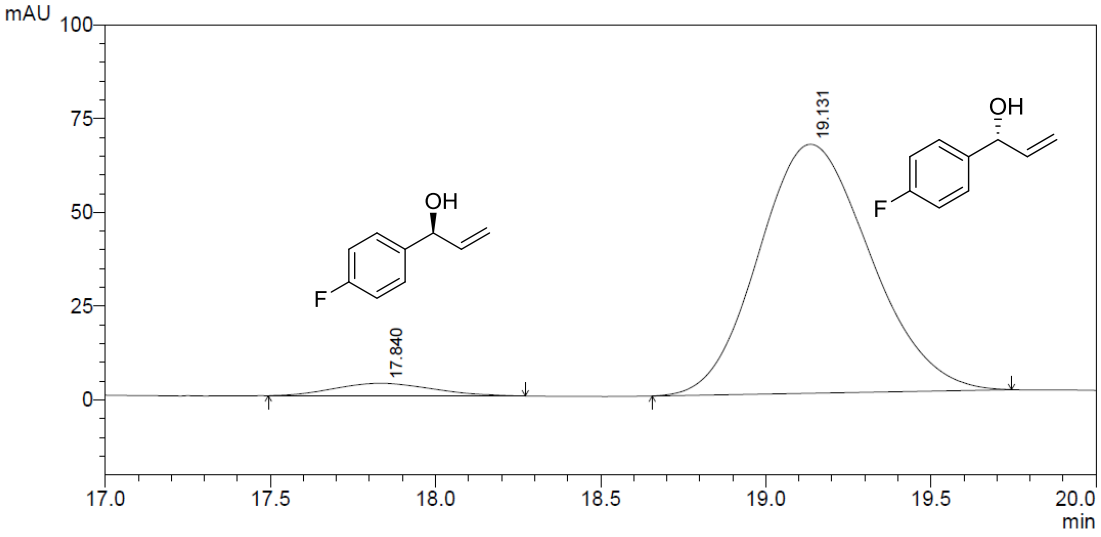


Chiral HPLC (Chiralcel OJ-H column, hexane/*i*-PrOH = 95/5, flow rate = 0.8 mL/min)



PDA

ID#	Rt. Time	Area	Height	Area %
1	18.196	4962860	236889	50.049
2	19.646	4953213	218159	49.951



PDA

ID#	Rt. Time	Area	Height	Area %
1	17.840	68916	3341	4.227
2	19.131	1561303	66316	95.773