Supporting Information

Enantioselective Reduction of α,β-Unsaturated Ketones and Aryl Ketones by Perakine Reductase

Sheng Cai,[†,¶] Nana Shao,[‡,¶] Yuanyuan Chen,[†,¶] Anbang Li,[§] Jie Pan,[†] Huajian Zhu,[⊥] Hongbin Zou,[‡] Su Zeng,[†] Lianli Sun,[*,†] and Jinhao Zhao[*,§]

[†]Institute of Drug Metabolism and Pharmaceutical Analysis, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, China.

[‡]Institute of Drug Discovery and Design, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, China.

[§]Institute of Pesticide and Environmental Toxicology, Ministry of Agriculture Key Lab of Molecular Biology of Crop Pathogens and Insects, Zhejiang University, Hangzhou, 310029, China.

[⊥]School of Medicine, Zhejiang University City College, Hangzhou, 310015, China.

¶S.C., N.S., and Y.C. contributed equally.

Corresponding Authors:
*E-mail: sunlianli@zju.edu.cn.

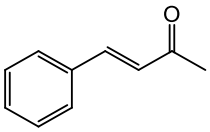
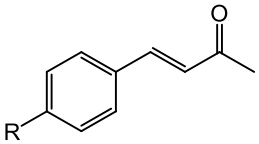
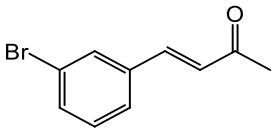
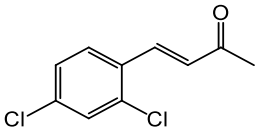
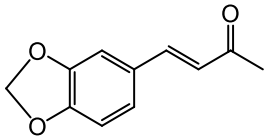
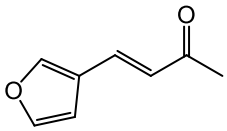
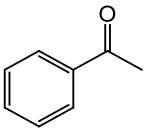
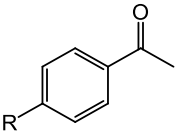
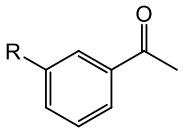
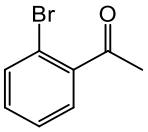
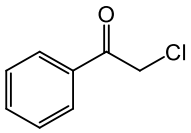
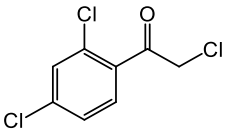
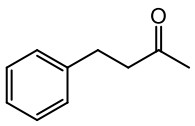
*E-mail: jinhaozhao@zju.edu.cn.

Table of Contents

1. General information

All chemical reagents were purchased from Sigma Aldrich (St. Louis, MO, USA), Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), Aladdin (Shanghai, China). Ampicillin, kanamycin, and RNAaseA were purchased from SangonBiotech Company (Shanghai, China). Substrates (**S1**), (**S9**) - (**S18**), standard (**9**) were purchased from Energy Chemical Company (Shanghai, China). Standard (**11**) and (**19**) was purchased from Sigma Aldrich (St. Louis, MO, USA). Standard (**12**) was purchased from Alfa aesar (USA). Substrates (**S4**) - (**S8**), standard (**7**) were kindly provided by Prof. Shaolin Zhu (Nanjing University, China). The chemical structures of substrates were listed in Table S1.

Table S1. Chemical structure of the substrates used in this study.

		
S1	**S2** R = NO_2 **S3** R = OCH_3 **S4** R = CN	**S5**
		
S6	**S7**	**S8**
		
S9	**S10** R = Cl **S11** R = Br **S12** R = NO_2 **S13** R = OCH_3	**S14** R = Br **S15** R = NH_2
		
S16	**S17**	**S18**
		
S19		

^{1}H NMR spectra were recorded at 500 MHz or 400 MHz. ^{13}C NMR spectra were recorded at 125 MHz or 100 MHz. The spectra data were reported as follows: chemical shift (δ ppm), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constant (Hz). High-pressure liquid chromatography (HPLC) was performed on Agilent 1290 Series chromatographs for reaction monitoring, and LC-2010AHT / 2010 CHT Series chromatographs with chiral columns for optical purity analysis.

2. Expression and purification of perakine reductase (PR)

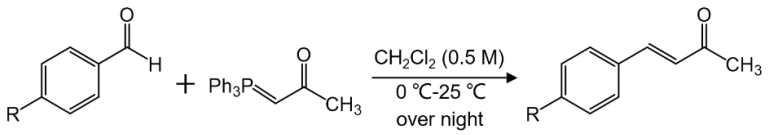
Expression and purification of recombinant His_6-PR was performed as previously reported with small modifications.[1] Briefly, *E. coli* M15 transformed with His_6-PR-pQE-2 plasmid were cultured in LB medium with 50 μg/mL ampicillin and 25 μg/mL kanamycin up to a cell density of OD_{600} 0.6-0.7. Subsequently, protein expression was induced with 0.3 mM isopropyl β-D-thiogalactopyranoside (IPTG) at 25 ℃ for 36 h. The bacteria were collected by centrifugation at the speed of 8000 rpm for 10 min at 4 ℃ and stored under -20 ℃.

In a typical PR purification, 3 g of bacteria were resuspended in 20 ml extract buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) containing 1 mg/mL lysozyme. After sonication (4 ℃) and centrifugation (22000 ×g 50 min, 4 ℃), the protein crude extract was loaded into a column with 2 ml Ni-nitrilo-tri-acetic acid (Ni-NTA) superflow (Qiagen, Hilden, Germany). The column was then washed with 20 ml extract buffer and 20 ml wash buffer containing 20 mM imidazole. PR enzyme was eluted with elution buffer containing 250 mM imidazole. The collected protein fraction was dialyzed against 5 L Kpi buffer (50 mM KH_2PO_4, 50 mM K_2HPO_4, 10 mM EDTA, pH 7.0) for 12 h for further enzymatic reaction.

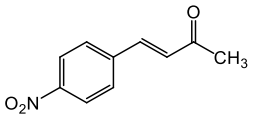
3. Expression and purification of glucose dehydrogenase from *Bacillus megaterium* (BmGDH)

The cDNA of BmGDH (GenBank: D10626.1) was synthesize and subcloned into pET-28a(+) vector by SangonBiotech Company (Shanghai, China). BmGDH was expressed as recombinant N-terminal His_6-tagged protein using *E. coli* BL21(DE3) as a host. His_6-BmGDH was expressed and purified under the same conditions that used for PR.

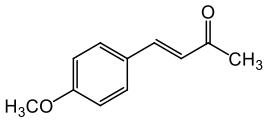
4. Preparation of substrates



Two substrates (*E*)-4-(4-nitrophenyl)but-3-en-2-one (**S2**) and (*E*)-4-(4-methoxyphenyl)but-3-en-2-one (**S3**) were synthesized according to the methodology described in literature.[2] The aldehyde (5.0 mmol) was dissolved in CH_2Cl_2 (0.5 M). Then 1-(triphenylphosphoranylidene)-2-propanones (6.0 mmol) was added to the aldehyde solution at 0 °C. The mixture was stirred until the starting aldehyde was completely reacted (12-24 h). By adding silica gel, the organic solvent was evaporated under reduced pressure. The desired product was isolated by silica gel column chromatography.



(*E*)-4-(4-nitrophenyl)but-3-en-2-one (**S2**) was prepared from 4-nitrobenzaldehyde (755.6 mg, 5.0 mmol) and 1-(triphenylphosphoranylidene)-2-propanones (1910 mg, 6.0 mmol) using above procedure. The product was isolated by silica gel column chromatography (PE: EtOAc = 10:1).



(*E*)-4-(4-methoxyphenyl)but-3-en-2-one (**S3**) was prepared from *p*-methoxybenzaldehyde (680.8 mg, 5.0 mmol) and 1-(triphenylphosphoranylidene)-2-propanones (1910 mg, 6.0 mmol) using above procedure. The product was isolated by silica gel column chromatography (PE: EtOAc = 8:1).

5. Preparation of racemic alcohols by $NaBH_4$

$NaBH_4$ was used for preparation of racemic allylic and aryl alcohols. One hundred mg ketones and 5 mL absolute methanol were added into round flask with nitrogen protection. After mixing 15 min on ice bath, $NaBH_4$ were injected with multiple times until the ketones were completely reduced. The reaction was terminated by adding 100-200 μl of saturated ammonium chloride solution. The products were purified by silica gel column chromatography.

6. Enzyme assay

The reaction system for enzyme assay consisted of 300 μg purified PR enzyme, 100 μg purified BmGDH, 0.8 mM substrate (ketone), 1.2 mM glucose, and 0.05 mM $NADP^+$ in a total volume of 200 μl Kpi buffer, pH 7.0. The reaction volumes for enantioselectivity analyses and NMR experiments were 5 ml and 25 ml, respectively. The mixture was incubated at 30℃ for 10 h. To accumulate enough product for NMR analysis, 4-12 times of 25 ml reaction mixtures were combined. Reaction was terminated by adding the equal volume of MeOH. Following adding MeOH and centrifugation at 14000×g for 5 min, 10 μl of the supernatant of the reaction mixture was subjected to HPLC analysis. HPLC analyses were performed using Agilent 1290 HPLC system. The methods used for analysis of PR catalyzed reactions are shown in as following:

Products	Chromatographic Column	Mobile Phase	Wavelength
1-3, 12	LiChroCART®250-4	ACN： H_2O=35:65	254 nm
15, 10, 16, 13, 9	HPLC-Cartidge C18,	ACN： H_2O=35:65	220 nm
11, 14	5 μm, 4.6×250 mm	ACN： H_2O=35:65	220 nm
5, 6, 8, 17, 18	Symmetry® C18，	ACN： H_2O =60:40	254 nm
4, 7	5 μm, 4.6×150 mm	ACN： H_2O =35:65	310 nm

7. Effects of temperature and pH

To evaluate the effects of temperature, the enzymatic reactions were carried out under different temperatures ranging from 4 to 70 ℃. To determine the optimal pH value, the reactions were incubated in reaction buffers with various pH values (4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0). All the determinations were used **S12** as the substrate with three parallel assays. The reactions were terminated with equal volume of MeOH. Following centrifugation at 15,000 for 30 min, the supernatants were subjected into HPLC for analysis. PR displayed the best activity under 30 to 50 ℃, and the optimal pH value was 7.5. (Figure S1)

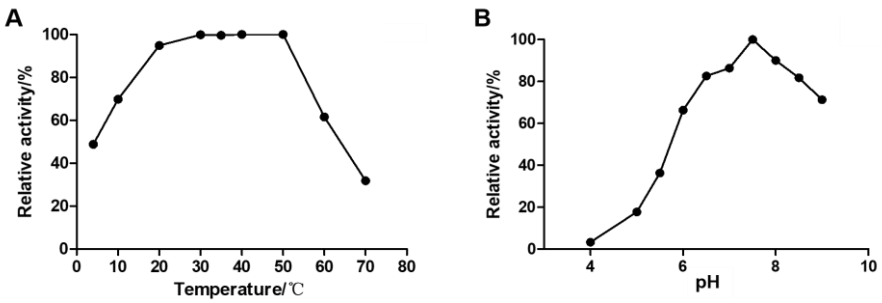


Figure S1. Determination of optimal temperature and pH on PR catalyzed reaction. S12 was used as substrate. The optimum temperature and pH for PR were 30~50 ℃ and 7.5, respectively.

8. Determination of kinetic parameters

Kinetic parameters were determined by monitoring product formation rates by HPLC. Varying concentrations of 4'-nitroacetophenone (**S12**, 1-12 mM) in the presence of excess NADPH (20 mM) were incubated under the same conditions as described above for 30 min. All experiments were performed in triplicate. *Km* value was calculated with the method of Lineweaver-Burk plot (Figure S2, Table S2).

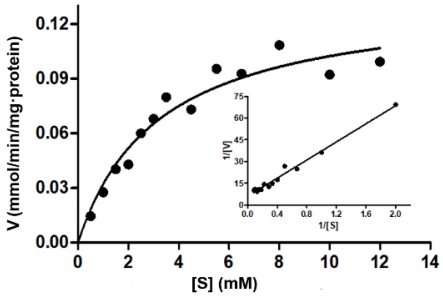
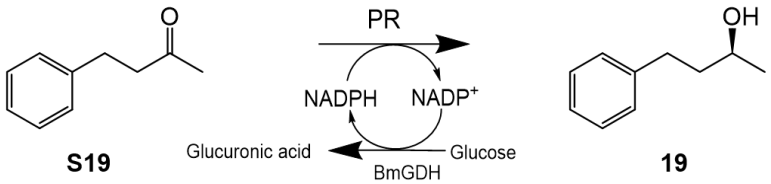


Figure S2. Kinetic parameters of 4'-nitroacetophenone (**S12**) were determined by Lineweaver-Burk plot of initial velocity (v) *versus* substrate.

Table S2. Kinetic parameters of 4'-nitroacetophenone (12) catalyzed by PR

Substrate	K_m [mM]	V_{max} [mmol/min/mg·protein]	k_{cat} [s^{-1}]	k_{cat}/K_m [s^{-1} mM^{-1}]
(S12)	3.22± 0.5	0.14±0.1	0.25 ± 0.1	0.08

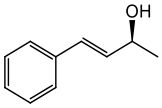
9. Conversion of 4-phenyl-2-butanone



The enzymatic activity assay of PR toward 4-phenyl-2-butanone (**S19**) was carried out under the same condition as described in the section "Enzyme assay" as other substrates. The yield and *ee* value of the corresponding product (**19**) were 73% and 95%, respectively. The yield is the analytical yield of product in the enzymatic reaction system (aqueous phase), determined by reverse phase HPLC.

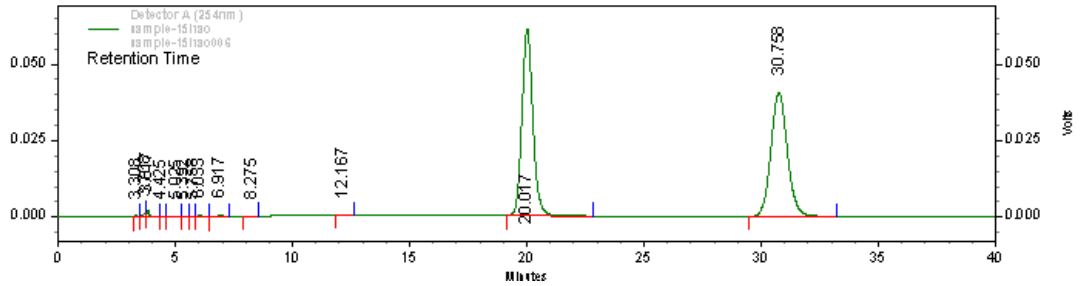
10. Enantioselectivity analysis of the enzymatic reaction

A volume of 5 ml enzymatic reaction mixture was extracted three times with the same volume of EtOAc. The organic parts were combined and evaporated under vacuum at 40 ℃. The residue was purified with a silica gel column (10 % EtOAc in PE). The fractions contained alcohol were combined and concentrated in vacuo for further optical purity analysis. The methods and chromatography for optical purity analysis are listed as following.

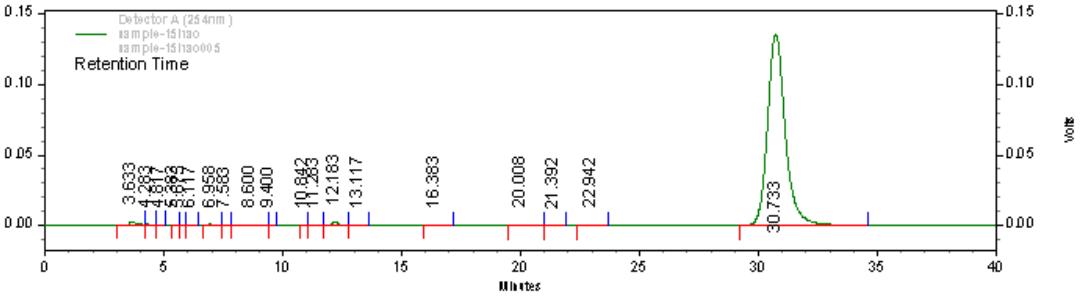


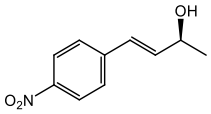
(*S*,*E*)-4-phenylbut-3-en-2-ol (1) Chiral separation (OD-H, 5 % IPA/hexanes, 0.5 mL/min, 25 ℃, 254 nm) indicated 99.2 % *ee*: t_R (minor) = 20.0 min, t_R (major) = 30.7 min.

$NaBH_4$ reduction product of **S1**



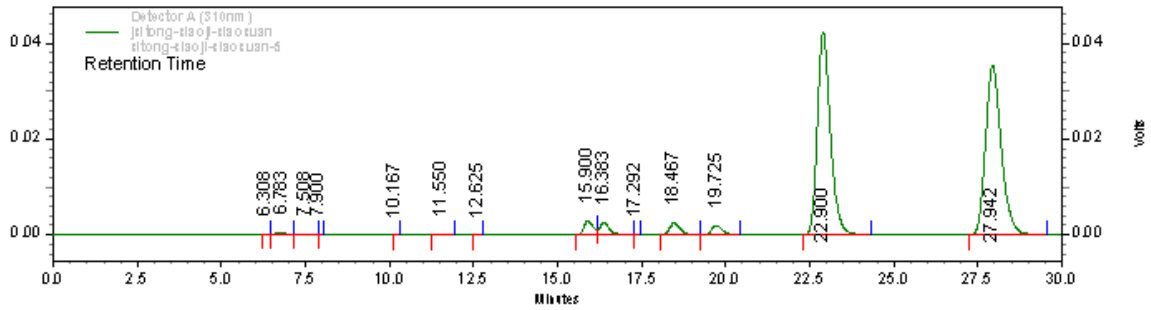
PR enzymatic reduction product of **S1 (1)**



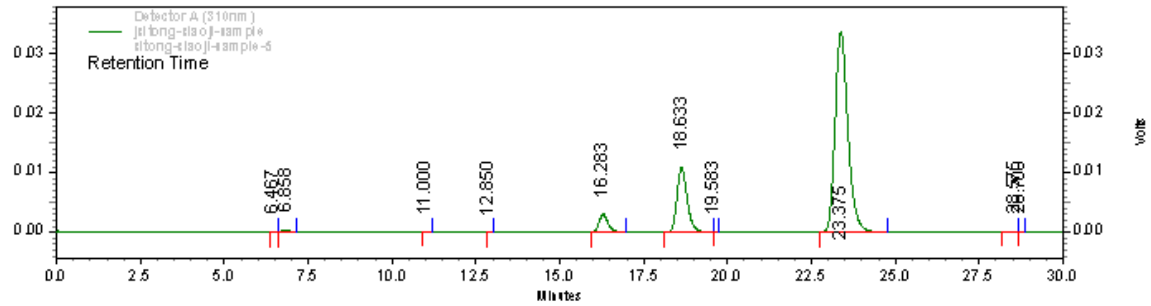


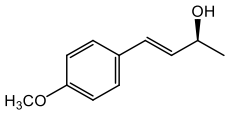
(*S*,*E*)-4-(4-nitrophenyl)but-3-en-2-ol (2) Chiral separation (AD-H, 15 % IPA/hexanes, 0.5 mL/min, 25 ℃, 310 nm) indicated 99.8 % *ee*: t_R (minor) = 22.9 min, t_R (major) = 27.9 min.

$NaBH_4$ reduction product of **S2**



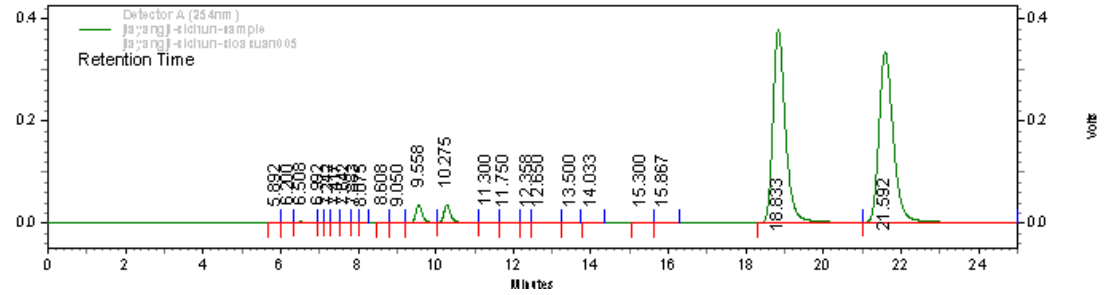
PR enzymatic reduction product of **S2 (2)**



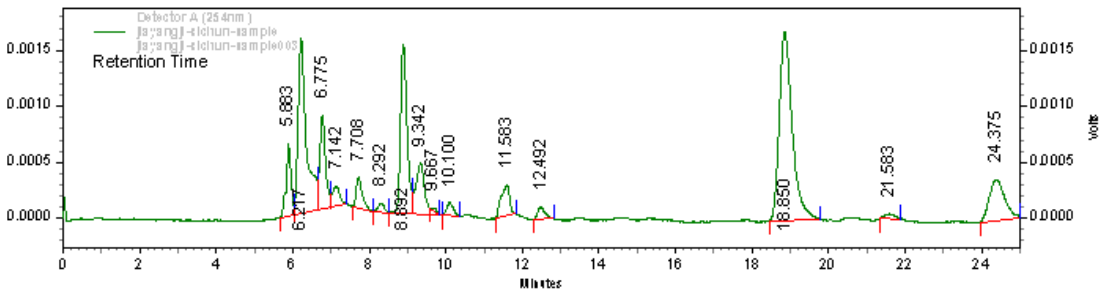


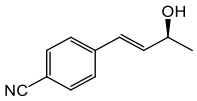
(*S,E*)-4-(4-methoxyphenyl)but-3-en-2-ol (3) Chiral separation (AD-H, 12 % IPA/hexanes, 0.5 mL/min, 25 ℃, 254 nm) indicated 96.3 % *ee*: t_R (mjor) = 18.8 min, t_R (minor) = 21.6 min.

$NaBH_4$ reduction product of **S3**



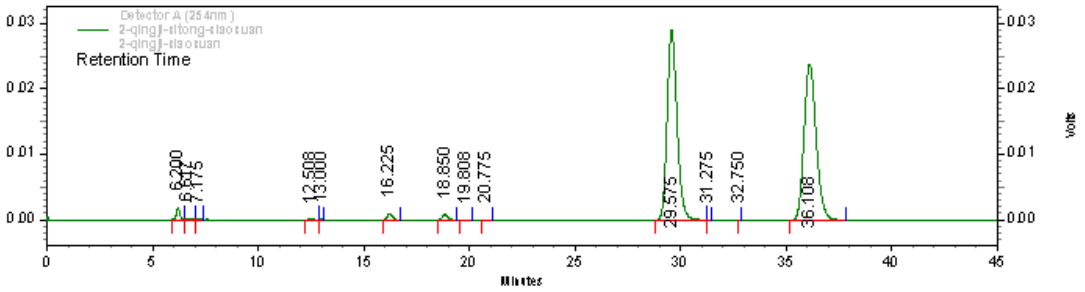
PR enzymatic reduction product of **S3 (3)**



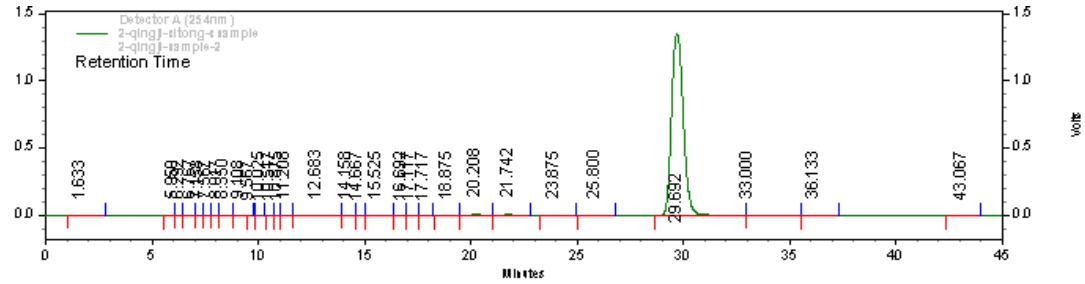


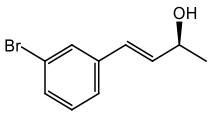
(*S*,*E*)-4-(3-hydroxybut-1-en-1-yl)benzonitrile (4) Chiral separation (AD-H, 8 % IPA/hexanes, 0.5 mL/min, 25 ℃, 254 nm) indicated 99.7 % *ee*: t_R (major) = 29.6 min, t_R (minor) = 36.1 min.

$NaBH_4$ reduction product of **S4**



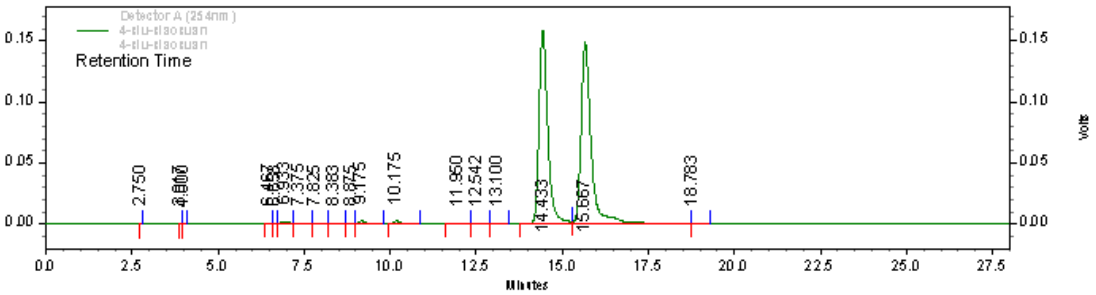
PR enzymatic reduction product of **S4 (4)**



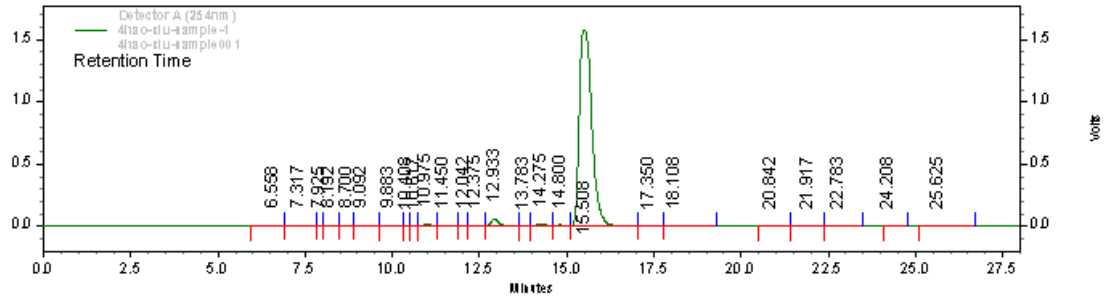


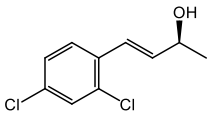
(*S*,*E*)-4-(3-bromophenyl)but-3-en-2-ol (5) Chiral separation (AD-H, 10 % IPA/hexanes, 0.5 mL/min, 25 ℃, 254 nm) indicated 99.1 % *ee*: t_R (minor) = 14.4 min, t_R (major) = 15.7 min.

$NaBH_4$ reduction product of **S5**



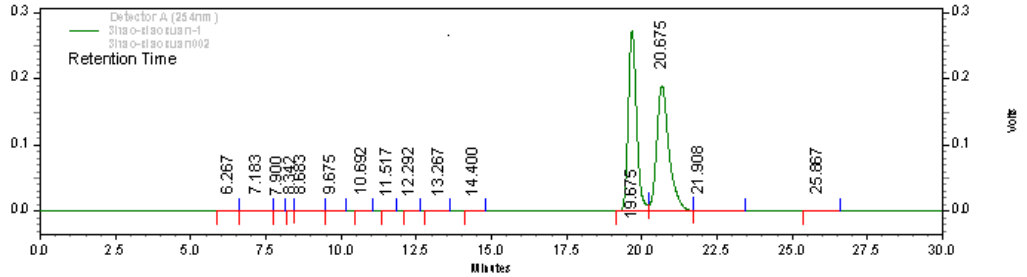
PR enzymatic reduction product of **S5 (5)**



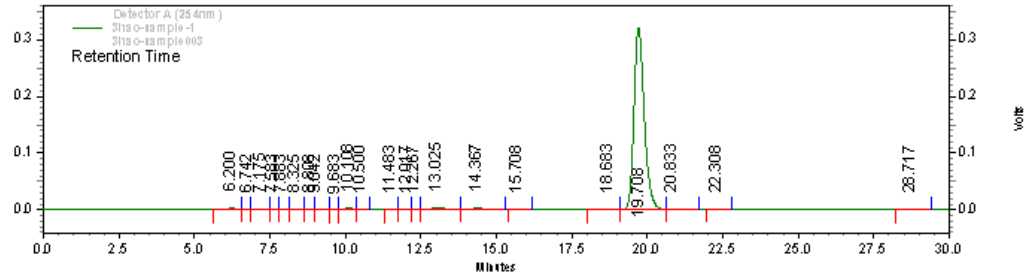


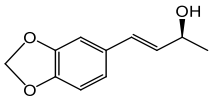
(*S,E*)-4-(2,4-dichlorophenyl)but-3-en-2-ol (6) Chiral separation (AD-H, 6 % IPA/hexanes, 0.5 mL/min, 25 ℃, 254 nm) indicated 98.9 % *ee*: t_R (major) = 19.7 min, t_R (minor) = 20.7 min.

$NaBH_4$ reduction product of **S6**



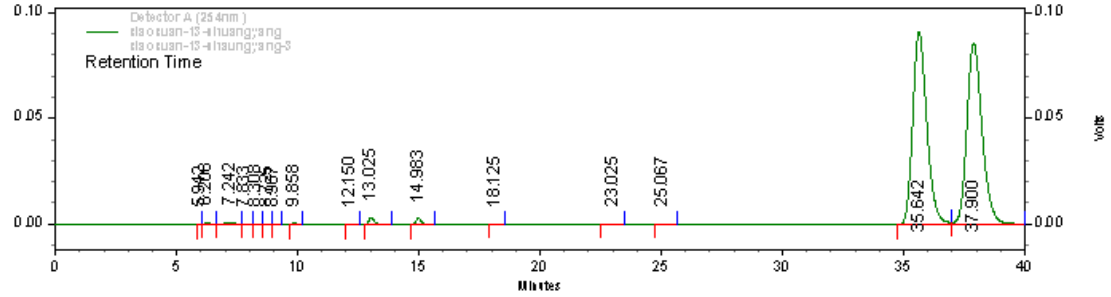
PR enzymatic reduction product of **S6 (6)**



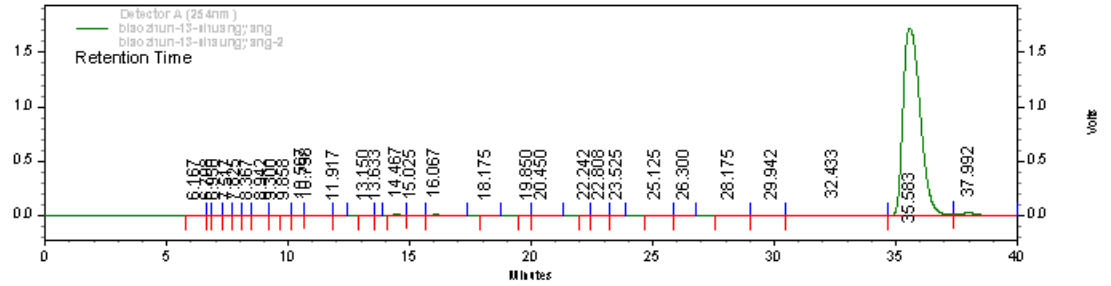


(*S,E*)-4-(benzo[d][1,3]dioxol-5-yl)but-3-en-2-ol (7) Chiral separation (AD-H, 6 % IPA/hexanes, 0.5 mL/min, 25 ℃, 310 nm) indicated 98.5 % *ee*: t_R (major) = 35.6 min, t_R (minor) = 37.9 min.

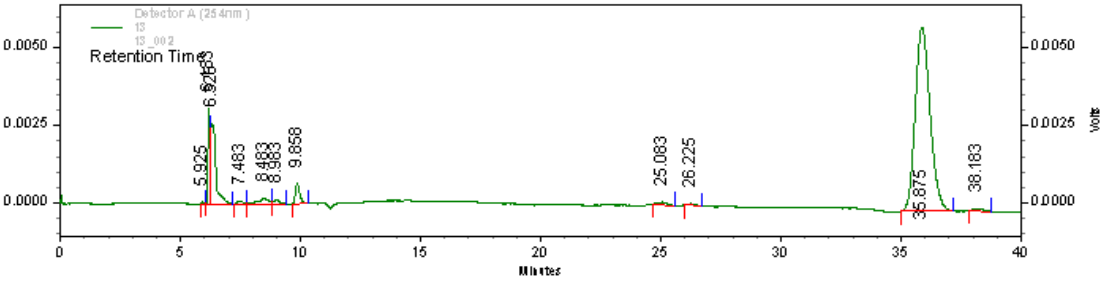
$NaBH_4$ reduction product of **S7**

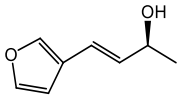


Standard compound of **(*S,E*)-4-(benzo[d][1,3]dioxol-5-yl)but-3-en-2-ol (7)**



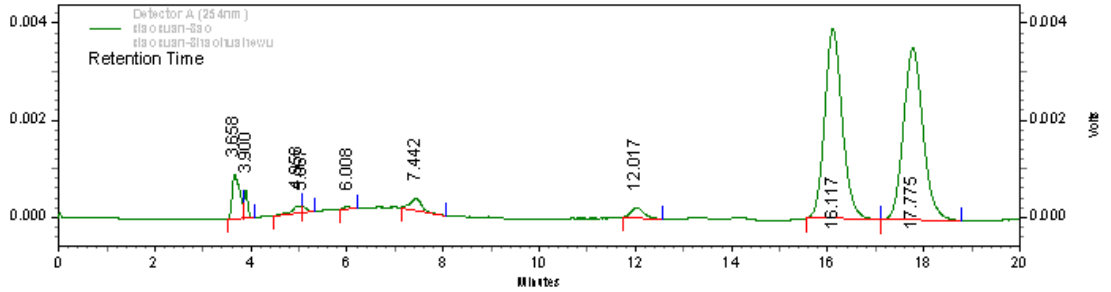
PR enzymatic reduction product of **S7 (7)**



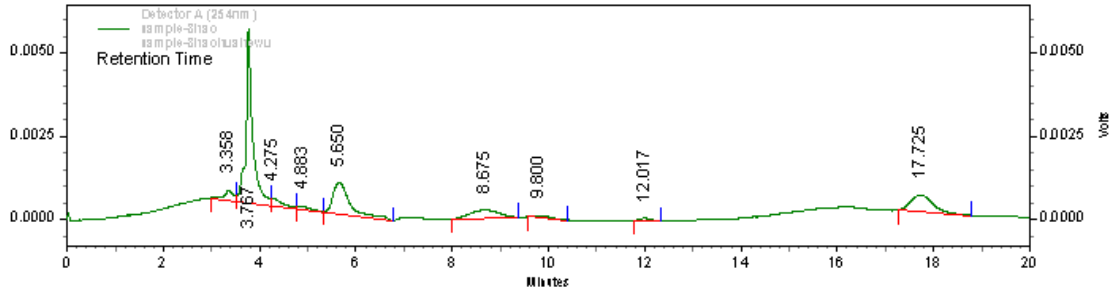


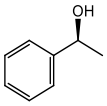
(*S,E*)-4-(furan-2-yl)but-3-en-2-ol (8) HPLC analysis (OD-H, 5 % IPA/hexanes, 0.5 mL/min, 20 ℃, 254 nm) indicated > 99.9 % *ee*: t_R (minor) = 16.1 min, t_R (major) = 17.8 min.

$NaBH_4$ reduction product of **S8**



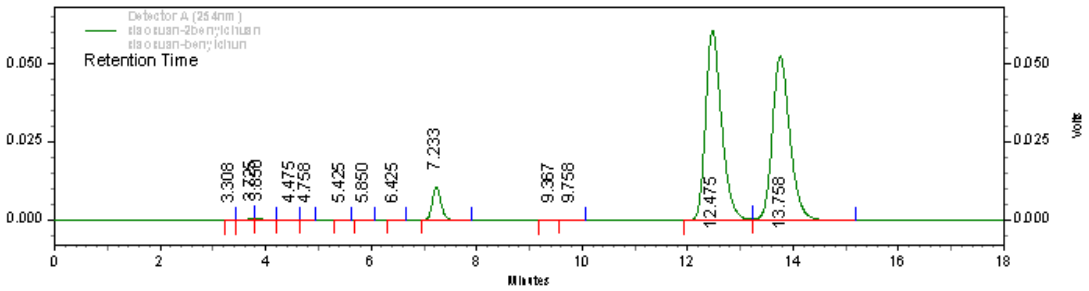
PR enzymatic reduction product of **S8 (8)**



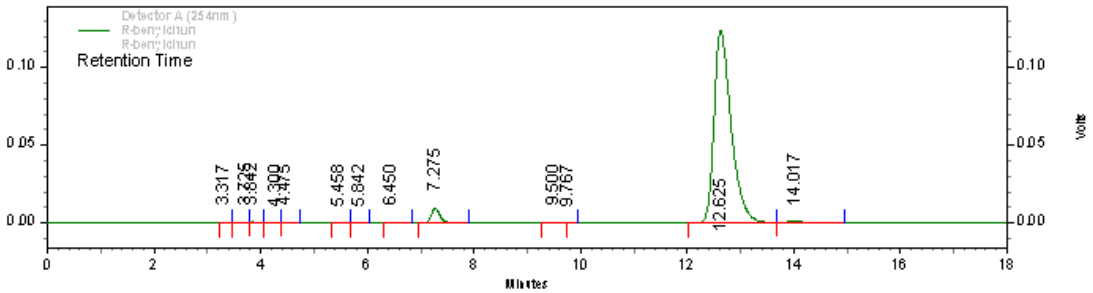


(*S*)-1-phenylethanol (9) Chiral separation (OD-H, 5 % IPA/hexanes, 0.5 mL/min, 20 ℃, 254 nm) indicated 99.2 % *ee*: t_R (minor) = 12.5 min, t_R (major) = 13.8 min.

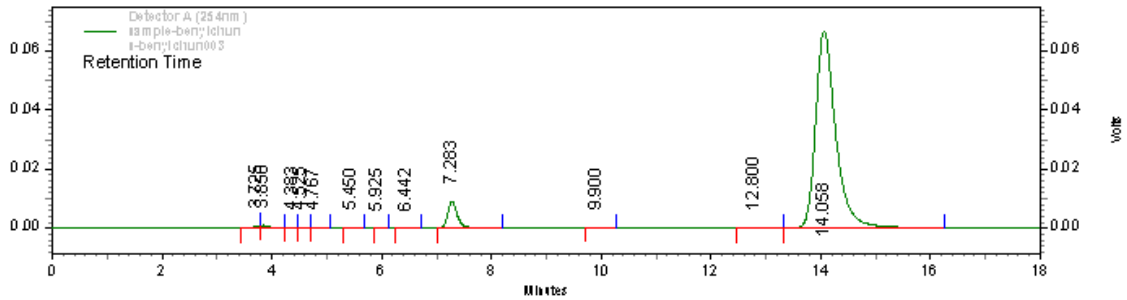
$NaBH_4$ reduction product of **S9**



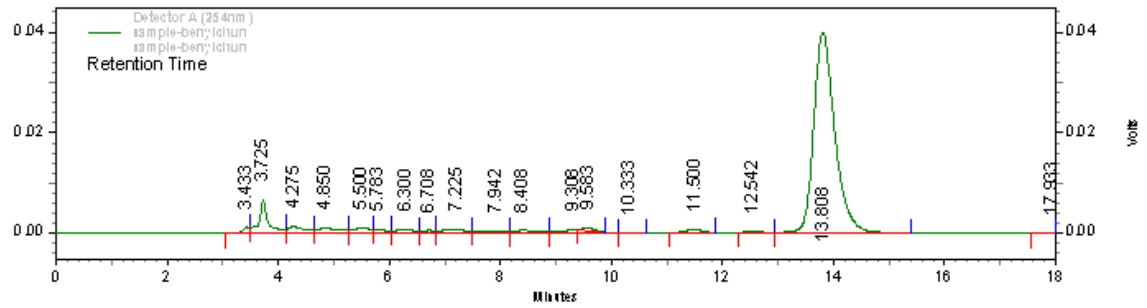
Standard compound of **(*R*)-1-phenylethanol**

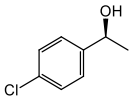


Standard compound of **(*S*)-1-phenylethanol (9)**



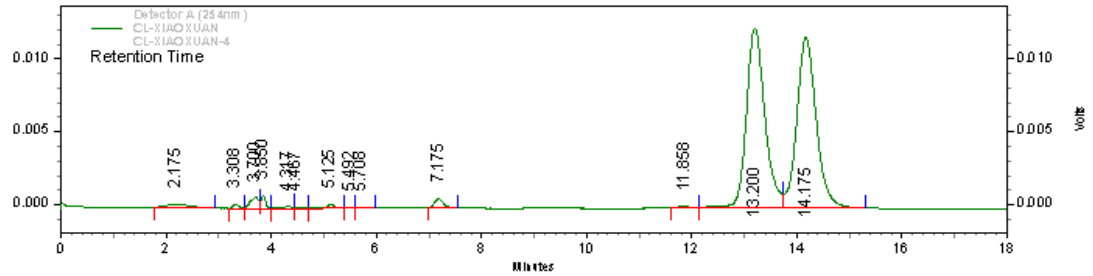
PR enzymatic reduction product of **S9 (9)**



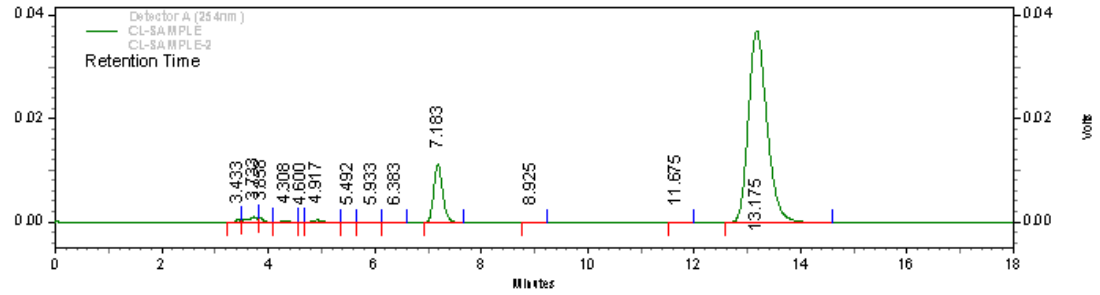


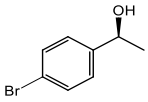
(*S*)-1-(4-chlorophenyl)ethanol (10). Chiral separation (OD-H, 5 % IPA/hexanes, 0.5 mL/min, 20 ℃, 254 nm) indicated > 99.9 % *ee*: t_R (major) = 13.2 min, t_R (minor) = 14.2 min.

$NaBH_4$ reduction product of **S10**



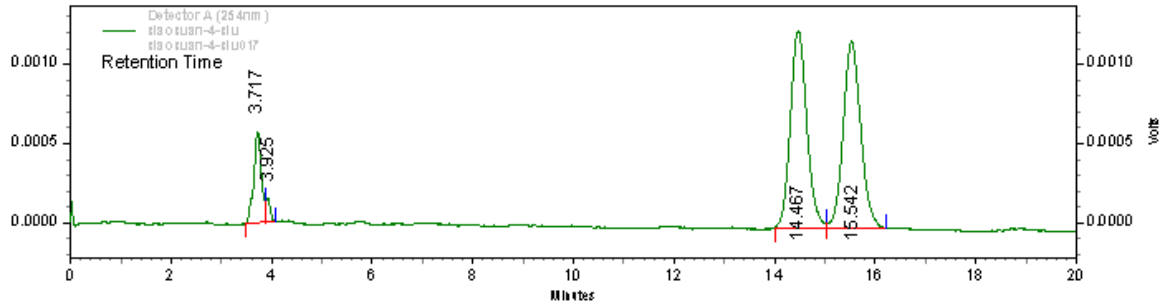
PR enzymatic reduction product of **S10 (10)**



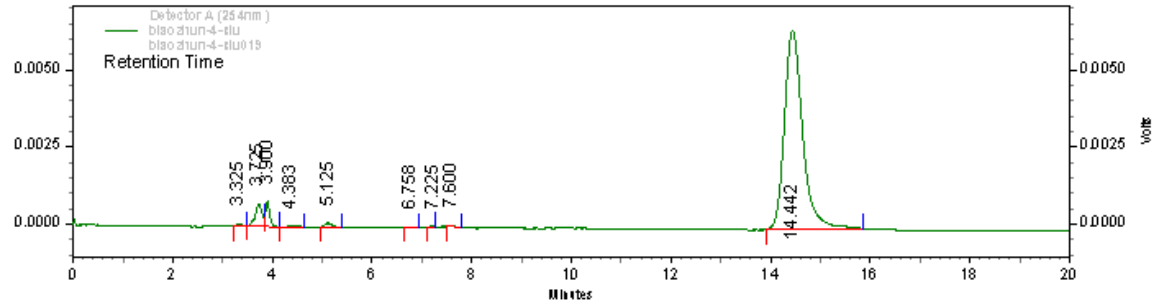


(*S*)-1-(4-bromophenyl)ethanol (11) Chiral separation (OD-H, 5 % IPA/hexanes, 0.5 mL/min, 20℃, 254 nm) indicated > 99.9 % *ee*: t_R (major) = 14.5 min, t_R (minor) = 15.5 min.

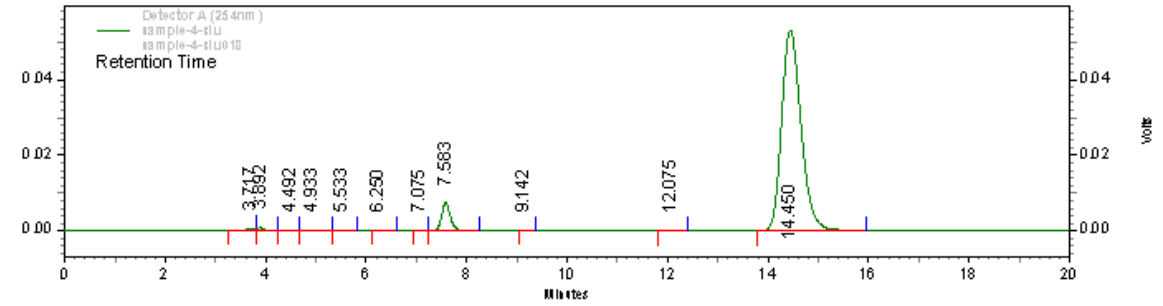
$NaBH_4$ reduction product of **S11**

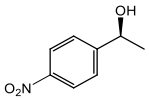


Standard compound of **(*S*)-1-(4-bromophenyl)ethanol (11)**



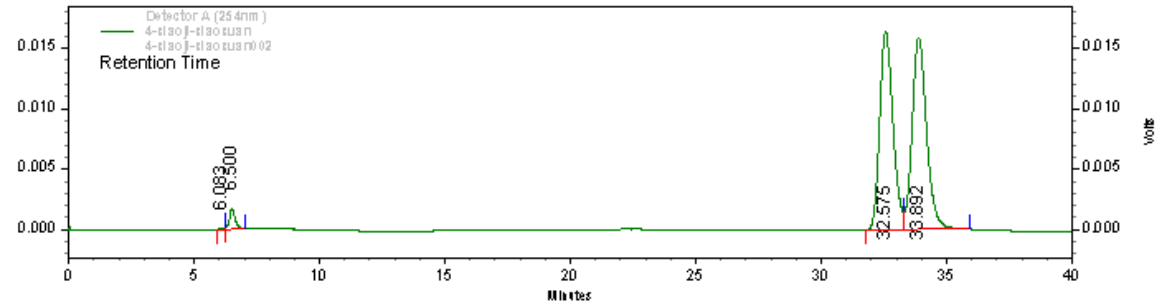
PR enzymatic reduction product of **S11 (11)**



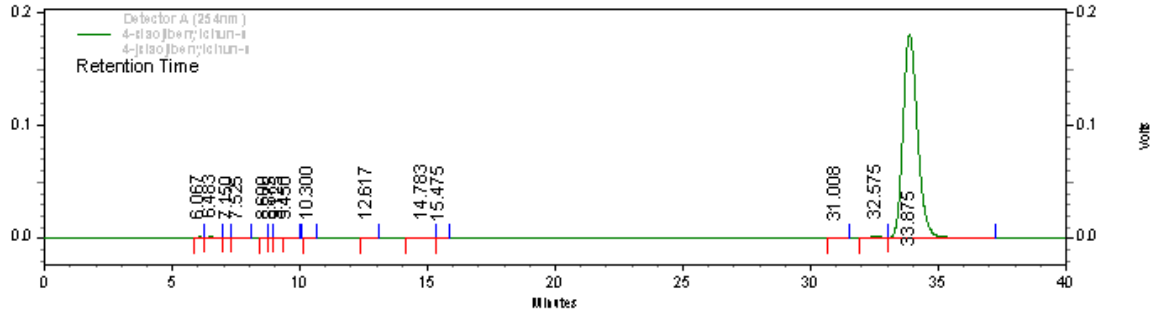


(*S*)-1-(4-nitrophenyl)ethanol (12) Chiral separation (AD-H, 5 % IPA/hexanes, 0.5 mL/min, 25℃, 254 nm) indicated > 99.9 % *ee*: t_R (minor) = 32.6 min, t_R (major) = 33.9 min.

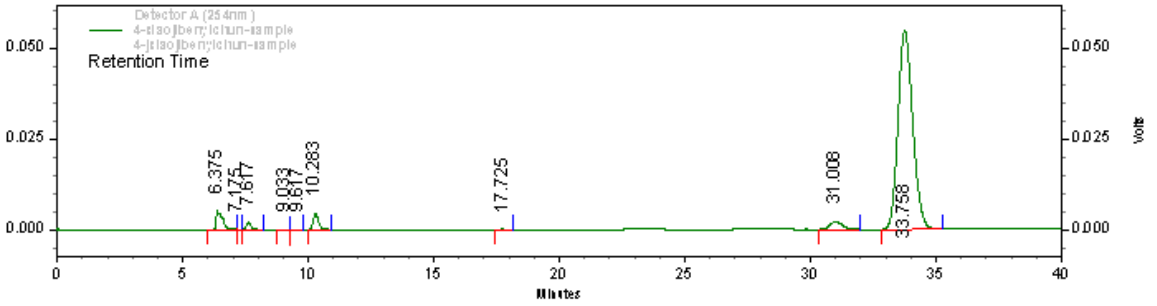
$NaBH_4$ reduction product of **S12**

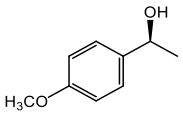


Standard compound of **(*S*)-1-(4-nitrophenyl)ethanol (12)**



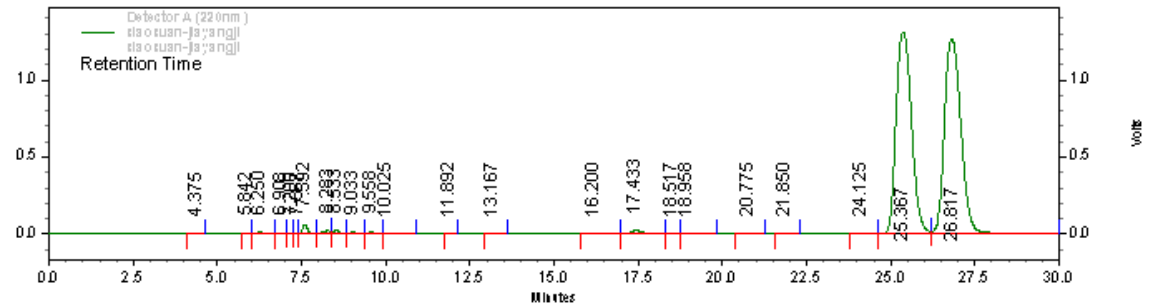
PR enzymatic reduction product of **S12 (12)**



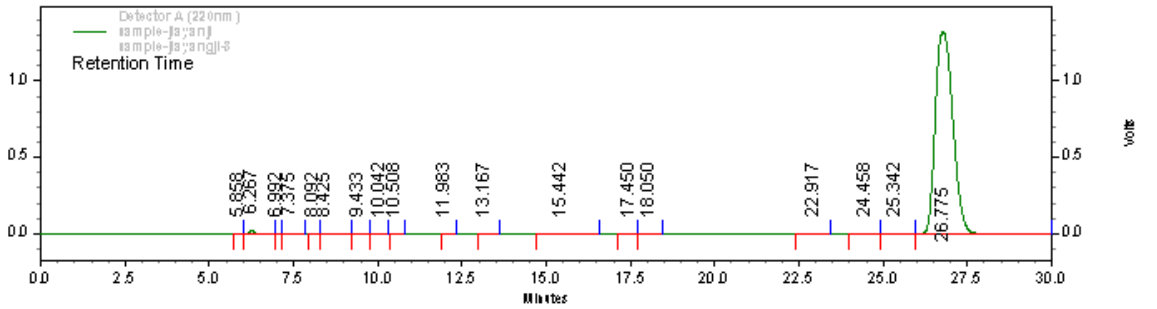


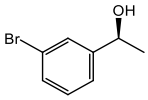
(*S*)-1-(4-methoxyphenyl)ethanol (13) Chiral separation (AD-H, 5 % IPA/hexanes, 0.5 mL/min, 25 ℃, 220 nm) indicated 99.9 % *ee*: t_R (minor) = 25.4 min, t_R (major) = 26.8 min.

$NaBH_4$ reduction product of **S13**



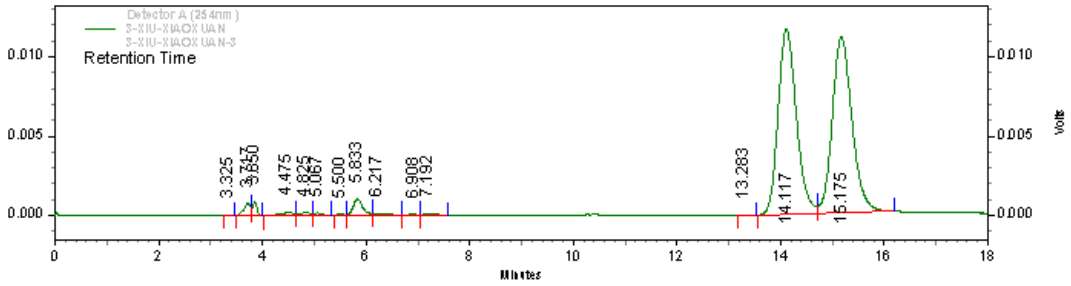
PR enzymatic reduction product of **S13 (13)**



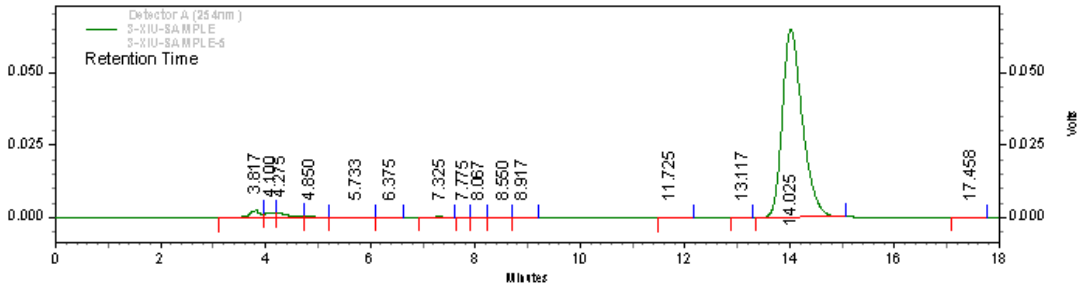


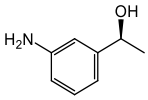
(*S*)-1-(3-bromophenyl)ethanol (14) Chiral separation (OD-H, 5 % IPA/hexanes, 0.5 mL/min, 20 ℃, 254 nm) indicated > 99.9 % *ee*: t_R (major) = 14.1 min, t_R (minor) = 15.2 min.

$NaBH_4$ reduction product of **S14**



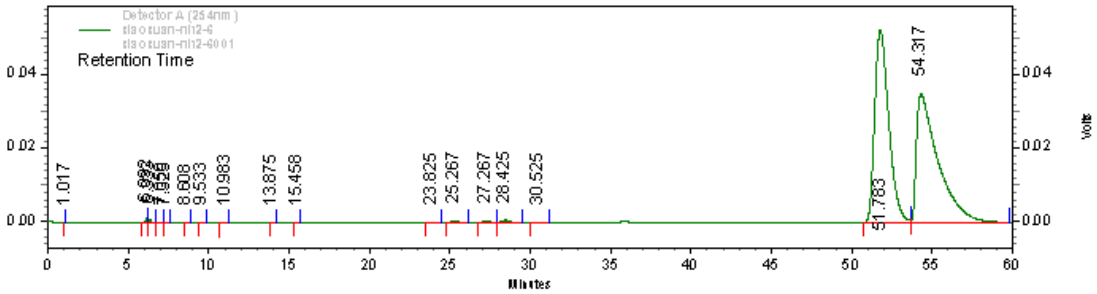
PR enzymatic reduction product of **S14 (14)**



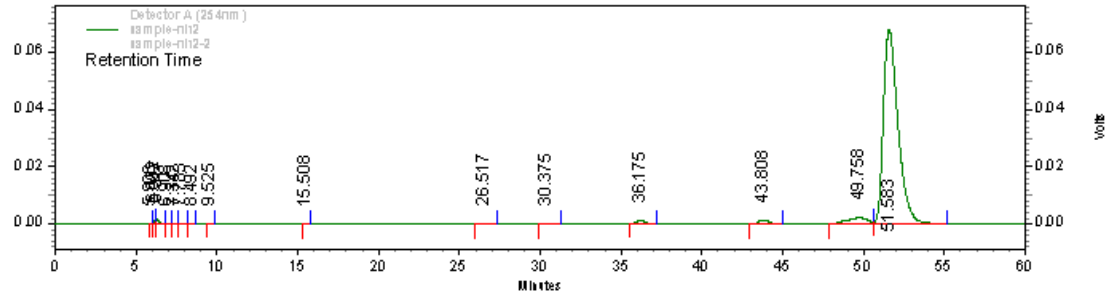


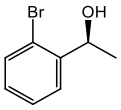
(*S*)-1-(3-aminophenyl)ethanol (15) Chiral separation (AD-H, 8 % IPA/hexanes, 0.5 mL/min, 24 ℃, 254 nm) indicated > 99.9 % *ee*: t_R (major) = 51.8 min, t_R (minor) = 54.3 min.

$NaBH_4$ reduction product of **S15**



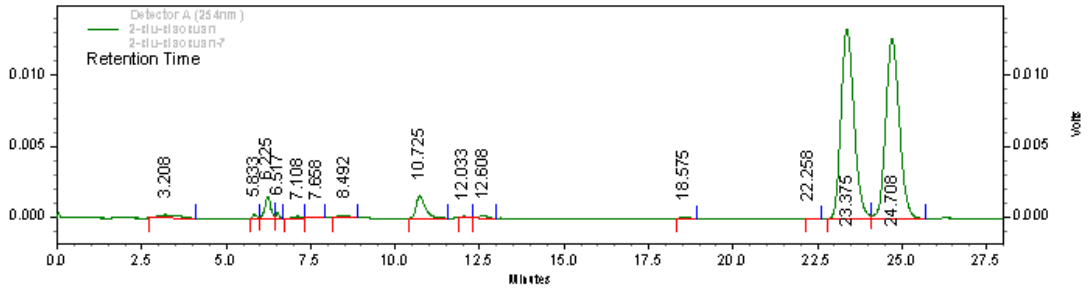
PR enzymatic reduction product of **S15 (15)**



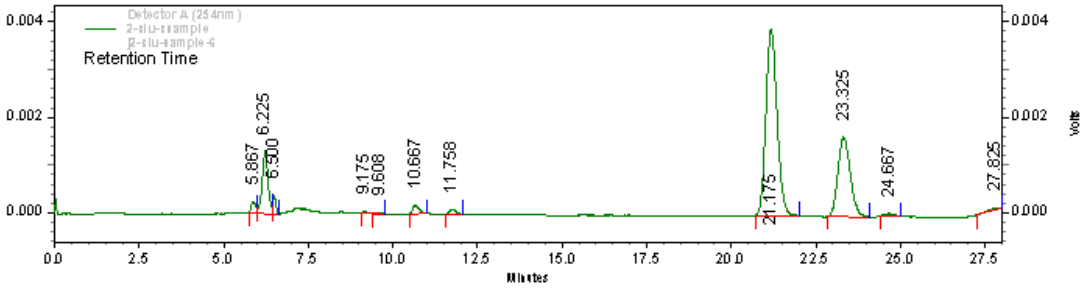


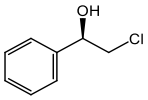
(*S*)-1-(2-bromophenyl)ethanol (16) Chiral separation (AD-H, 3 % IPA/hexanes, 0.5 mL/min, 25 ℃, 254 nm) indicated 95.2 % *ee*: t_R (major) = 23.4 min, t_R (minor) = 24.7 min.

$NaBH_4$ reduction product of **S16**



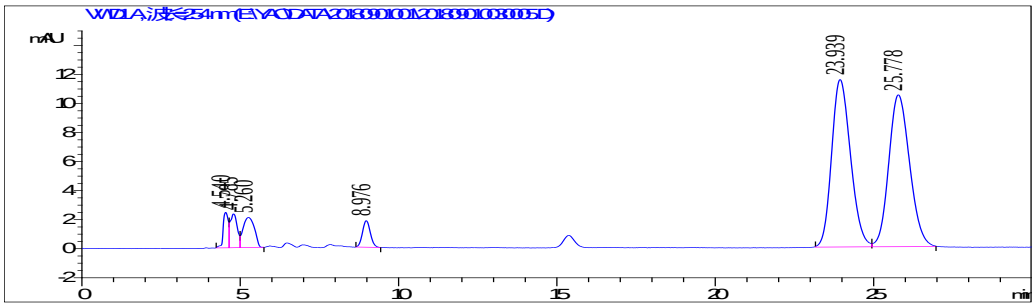
PR enzymatic reduction product of **S16 (16)**



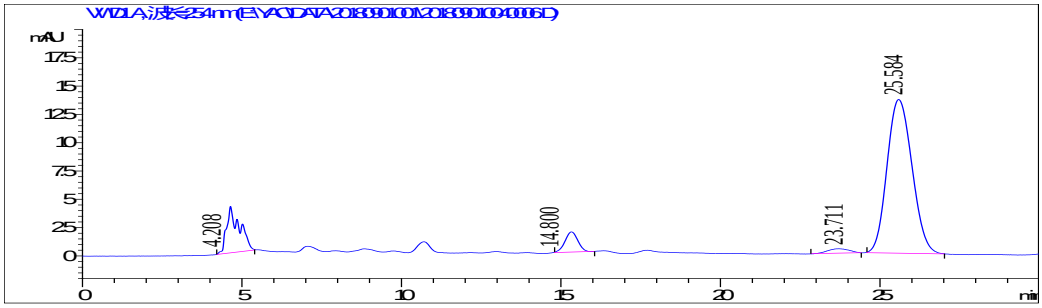


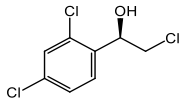
(*S*)-2-chloro-1-phenylethanol (17) Chiral separation (OD-H, 3 % IPA/hexanes, 0.5 mL/min, 16 ℃, 254 nm) indicated 95.1 % *ee*: t_R (minor) = 24.0 min, t_R (major) = 25.8 min.

$NaBH_4$ reduction product of **S17**



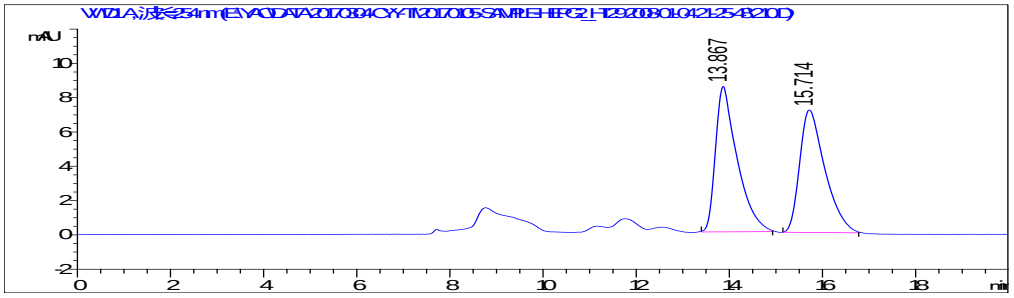
PR enzymatic reduction product of **S17 (17)**



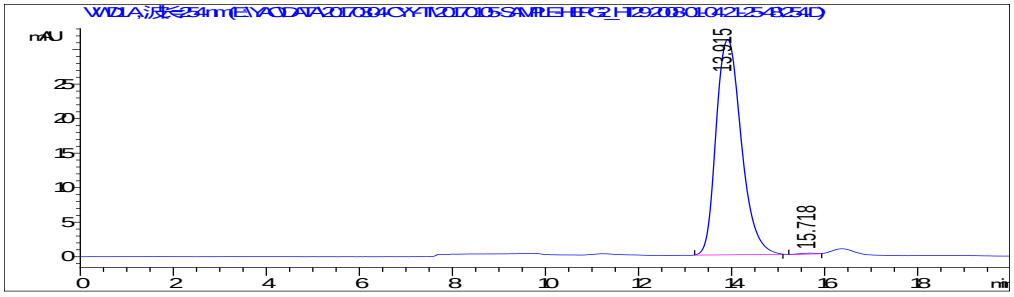


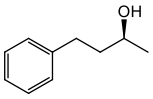
(*S*)-2-chloro-1-(2,4-dichlorophenyl)ethanol (18) Chiral separation (AD-H, 10 % IPA/hexanes, 0.5 mL/min, 25℃, 254 nm) indicated 99.4 % *ee*: t_R (major) = 13.9 min, t_R (minor) = 15.7 min.

$NaBH_4$ reduction product of **S18**



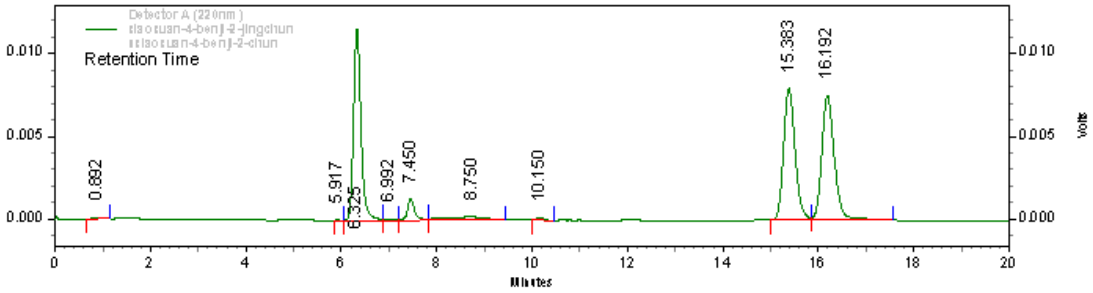
PR enzymatic reduction product of **S18 (18)**



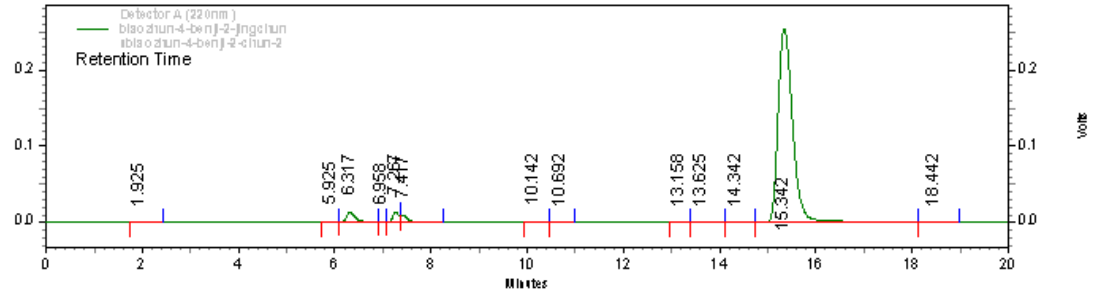


(*S*)-4-phenylbutan-2-ol (19) Chiral separation (AD-H, 5 % IPA/hexanes, 0.5 mL/min, 25℃, 220 nm) indicated 94.7 % *ee*: t_R (minor) = 15.4 min, t_R (major) = 16.2 min.

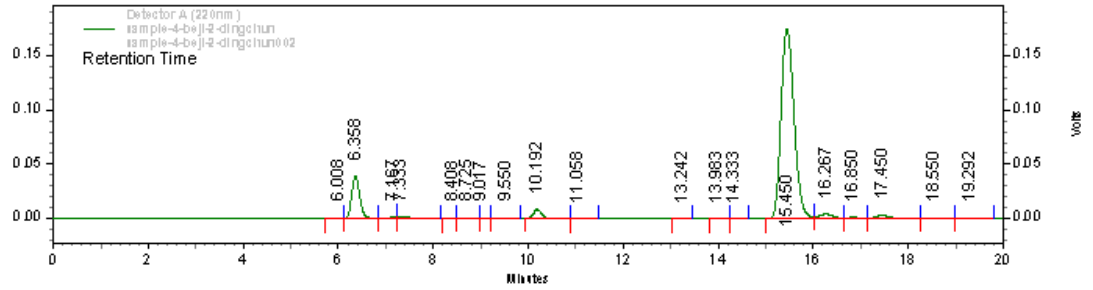
$NaBH_4$ reduction product of **S19**



Standard compound of **(*S*)-4-phenylbutan-2-ol (19)**



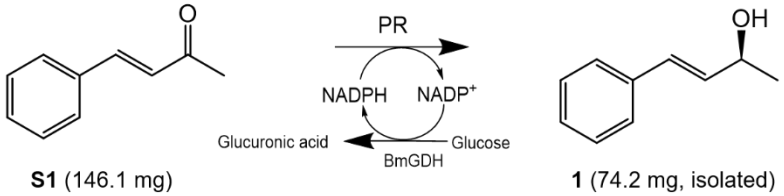
PR enzymatic reduction product of **S19 (19)**



11. 1 mmol scale reaction

To demonstrate the applicability of PR to large scale synthesis, the reaction system containing 1 mmol substrate (**S1** or **S12**), $NADP^+$ (49.2 mg, 0.0625 mmol), PR (7.5 mg/ml), BmGDH (2.5 mg/ml), glucose (271.5 mg, 1.5 mmol) in a total volume of 250 mL Kpi buffer (50 mM, pH = 7.0) in 1 L flask was incubated at 30 ℃ for 10 h with shaking speed 150 rpm. Subsequently, the reaction mixtures were extracted three times with the same volume of EtOAc. The organic parts were combined and evaporated under vacuum at 40 ℃. The residue was purified with

a silica gel column (10 % EtOAc in PE) to provide the desired product. The reaction yield and *ee* value of 1-mmol scale reaction were determined using the same procedure described in "Enzyme assay" and "Enantioselectivity analyses of the enzymatic reaction", respectively.

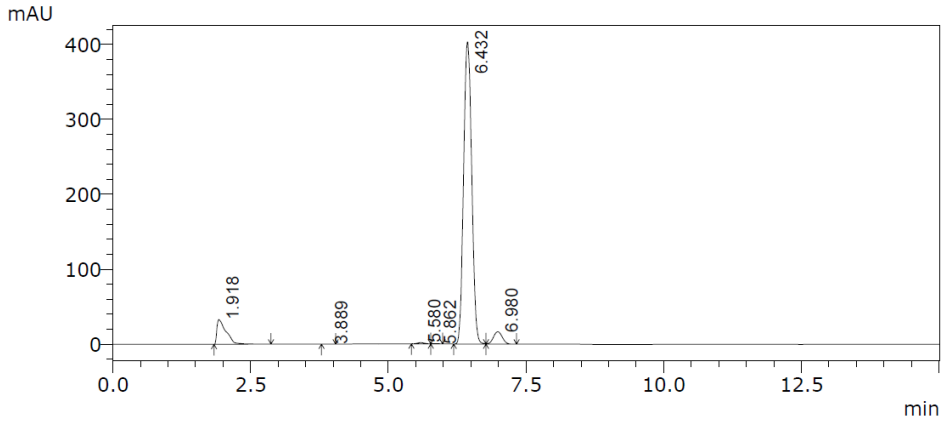


S1 (146.1 mg) **1** (74.2 mg, isolated)

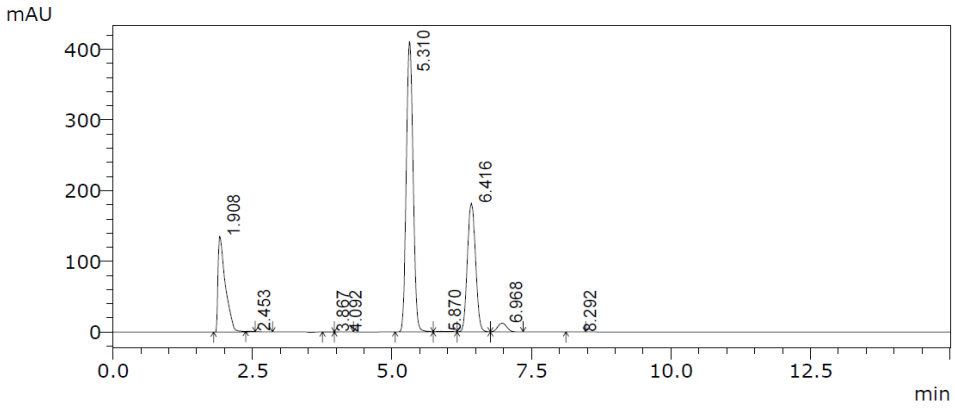
The 1-mmol scale reaction of (*S,E*)-4-phenylbut-3-en-2-ol **(1)** was carried out using substrate (**S1**) (146.1 mg, 1 mmol), providing the product (**1**) 74.2 mg (51% isolated yield), 59% reaction yield (HPLC), and >99% *ee*.

HPLC analysis of 1-mmol scale reaction of **S1**

Reaction for 0 h:

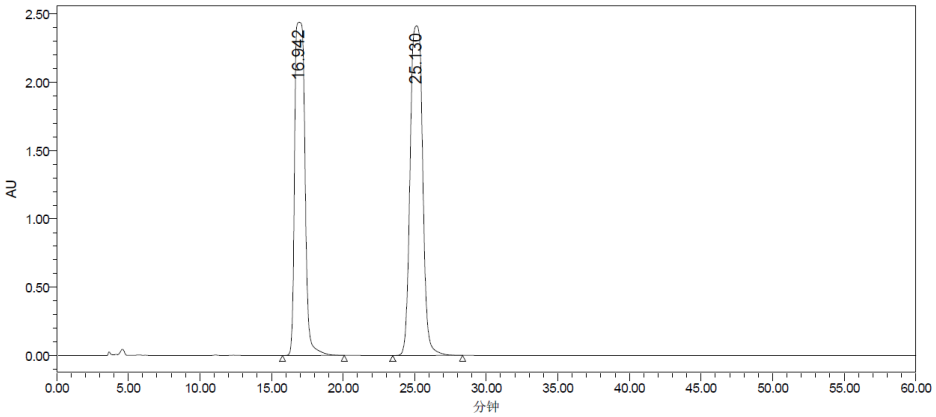


Reaction for 10 h:

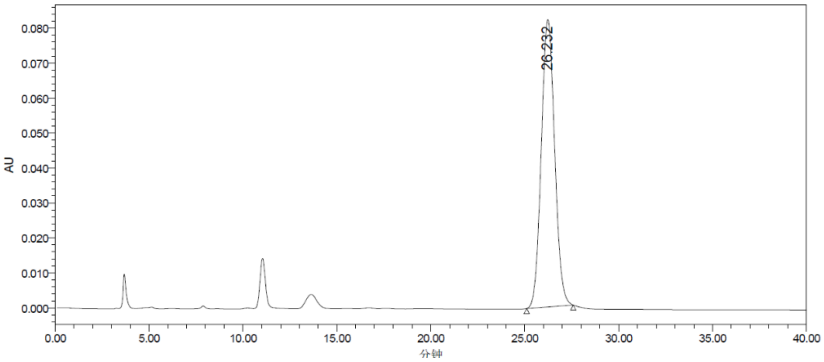


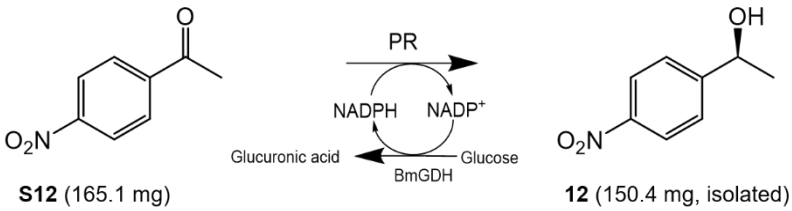
Chiral HPLC analysis of the product from 1-mmol scale reaction of **S1**

$NaBH_4$ reduction product of **S1**



PR enzymatic reduction product of **S1** (**1**) at 1-mmol scale



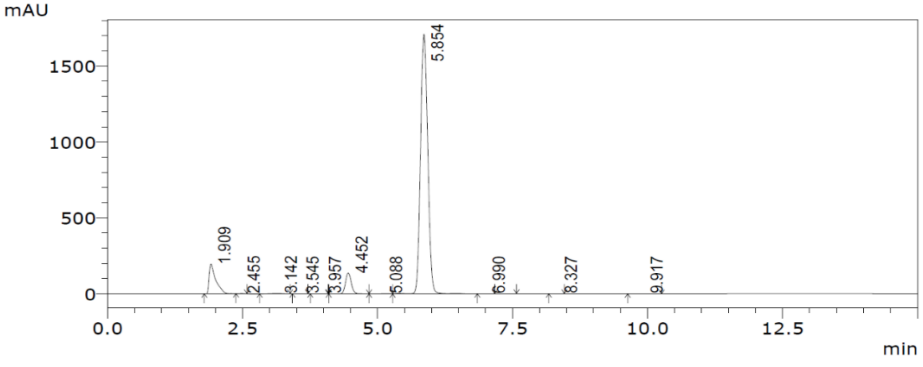


S12 (165.1 mg) **12** (150.4 mg, isolated)

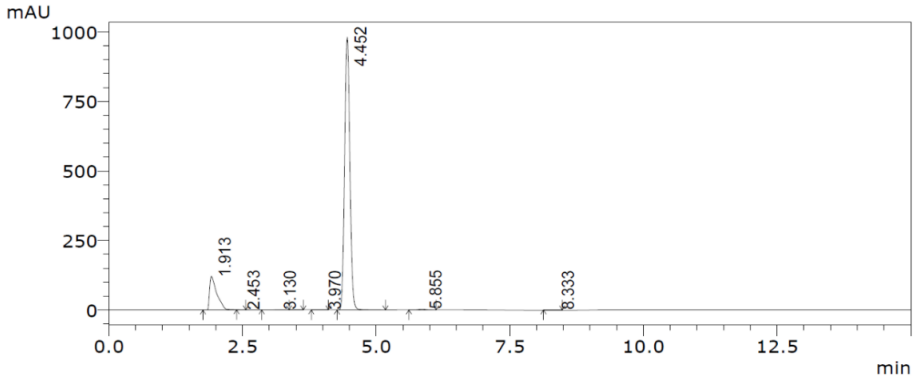
The 1-mmol scale reaction of **(*S*)-1-(4-nitrophenyl)ethanol (12)** was carried out using substrate (**S12**) (165.1 mg, 1 mmol), providing the product (**12**) 150.4 mg (91% isolated yield), >99% reaction yield (HPLC), and >99% *ee*.

HPLC analysis of 1-mmol scale reaction of **S12**

Reaction for 0 h:

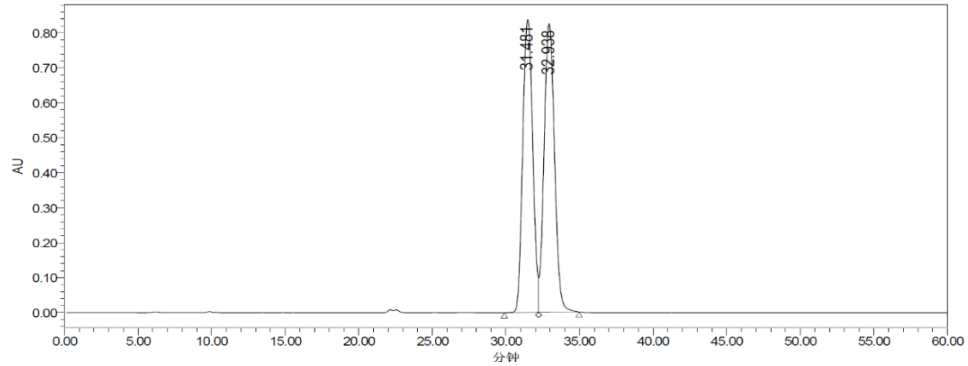


Reaction for 10 h:

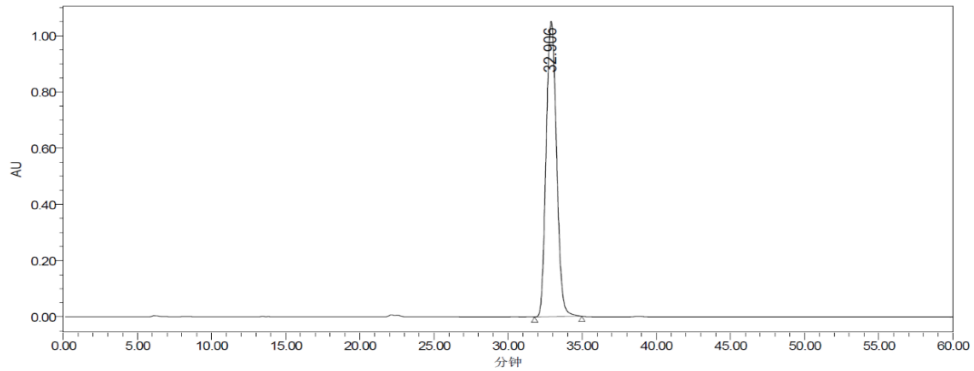


Chiral HPLC analysis of the product from 1-mmol scale reaction of **S12**

$NaBH_4$ reduction product of **S12**



PR enzymatic reduction product of **S12** (**12**) at 1-mmol scale



12. Molecular modeling

The structural file of PR-NADPH complex downloaded from Protein Data Bank (PDB ID 3V0S) was used for modelling. The software package Discovery Studio version 2.5 (DS2.5) was employed for all calculations and manipulations. The structures of NADPH, (*S*,E)-4-(benzo[d][1,3]dioxol-5-yl)but-3-en-2-ol (**7**) and (*S*)-1-(4-nitrophenyl)ethanol (**12**) were constructed by DS2.5. The structures of small molecules were optimized using the charmm22 force field to an energy gradient of 0.001 kcal/(mol*Å).

13. Interactions between nicotinamide riboside part of NADPH with PR

By transferring one proton to the substrate, the nicotinamide ring of NADPH is directly involved in the enzymatic reaction. AKR superfamily members follow bi-bi mechanism, in which cofactor binds prior to the substrate, thus, accommodation of cofactor is essential for binding of the substrates.[3] However, in our previously reported PR complex with NADPH, the nicotinamide riboside moiety of NADPH was disordered.[4] We firstly execute the modelling of the nicotinamide riboside part. The cofactor binding site is highly conserved across the whole AKR superfamily, especially for the nicotinamide ring.[3a, 3e] The nicotinamide riboside part was carefully rebuilt and optimized in its usual anti-conformation in the binding site as observed in other AKR members (Figure S3).[3b-d] In the model, the highly conserved residues Ser156, Gln176 and Tyr204 exhibited obvious interactions with this portion. The nicotinamide ring of $NADP^+$ formed π-π stacking with the aromatic ring of the residue Tyr204. Moreover, the oxygen atom and the hydrogen atom of the carboxamide group formed hydrogen-bond with the side chains of Ser156 (2.14 Å) and Gln176 (1.91 Å), respectively (Figure S3).

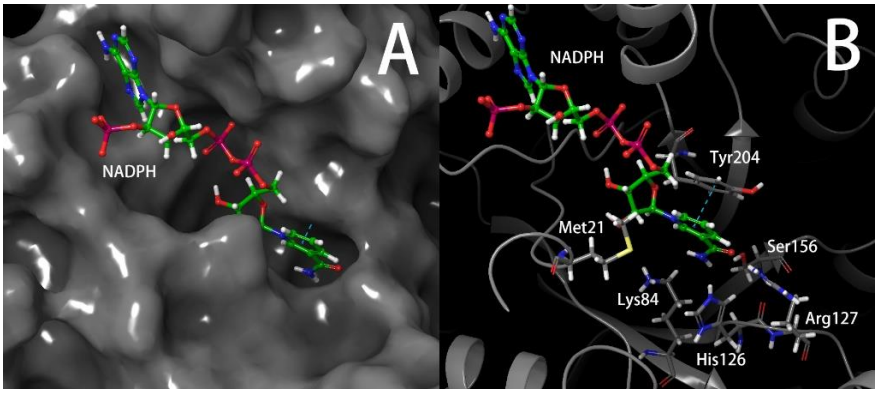
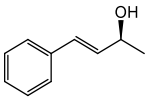


Figure S3. Accommodation of NADPH in PR. (**A**) PR is shown in surface mode; (**B**) PR is shown in cartoon mode. The residues interacted with nicotinamide ring of PR are shown in stick mode.

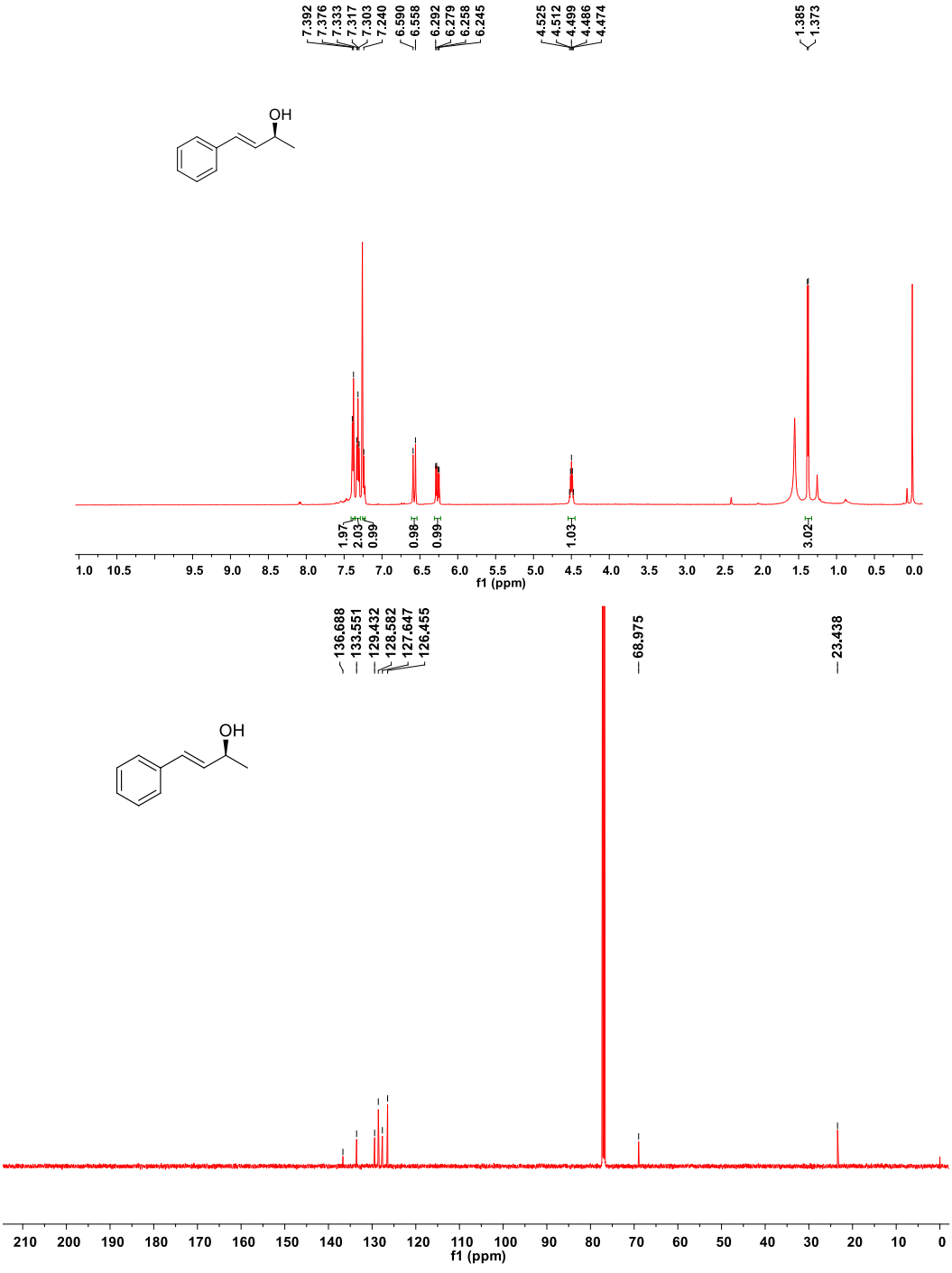
14. NMR Data

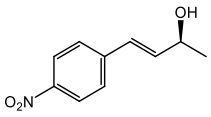


(*S,E*)-4-phenylbut-3-en-2-ol (1),[2] yellow oil (5.0 mg)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (2H, d, *J* = 8.0 Hz), 7.32 (2H, t, *J* = 8.0 Hz), 7.23 (1H, d, *J* = 6.5 Hz), 6.57 (1H, d, *J* = 16 Hz), 6.27 (1H, dd, *J* =6.5, 17 Hz), 4.50 (1H, m), 1.37 (3H, d, *J* = 6.0 Hz);

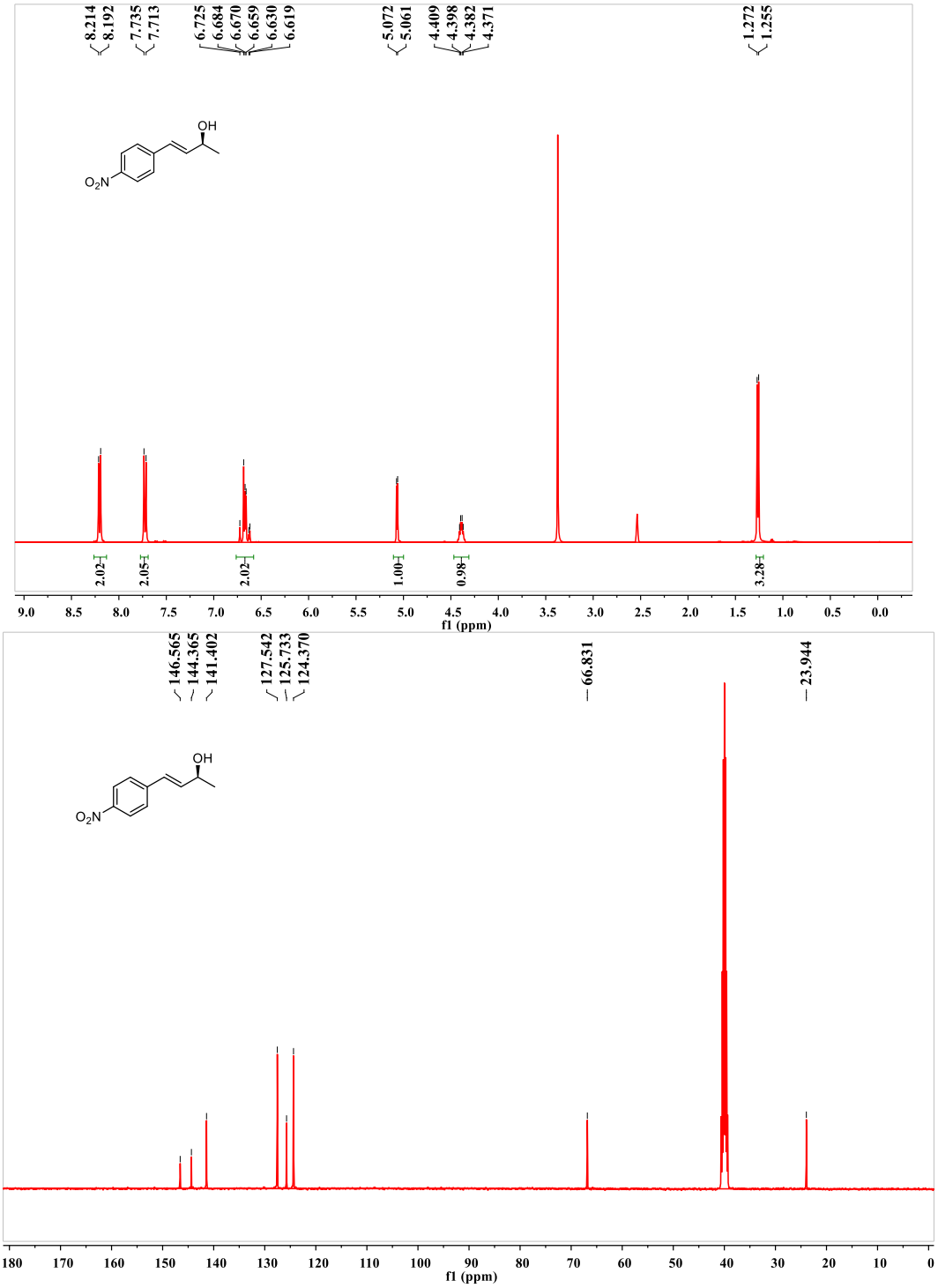
^{13}C NMR (125 MHz, $CDCl_3$): δ136.7, 133.6, 129.4, 128.6, 127.7, 126.5, 69.0, 23.4.

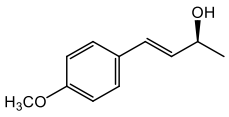




(*S*,*E*)-4-(4-nitrophenyl)but-3-en-2-ol (2),[5] yellow solid (6.5 mg)

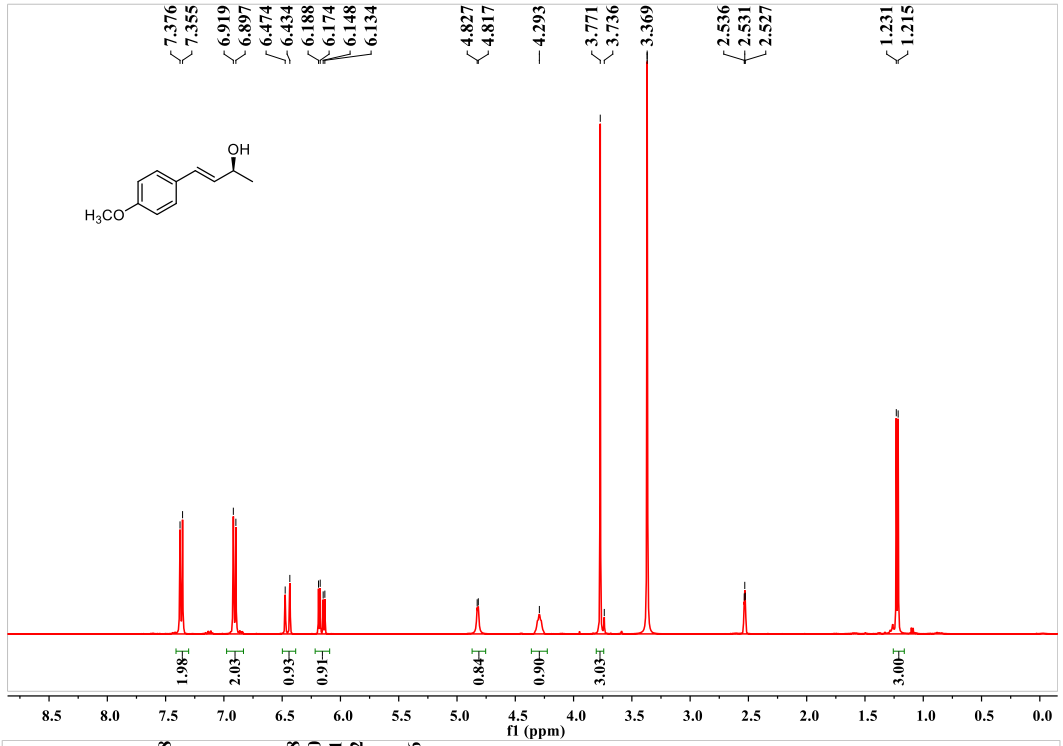
^{1}H NMR (400 MHz, d_6-DMSO): δ 8.21 (2H, d, J = 8.8 Hz), 7.73 (2H, d, J = 8.8 Hz), 6.67 (2H, m), 5.06 (1H, d, J = 4.4 Hz), 4.39 (1H, m), 1.27(3H, d, J =6.8 Hz); ^{13}C NMR (125 MHz, d_6-DMSO): δ 146.5, 144.4, 141.4, 127.5, 125.7, 124.3, 66.8, 23.9.

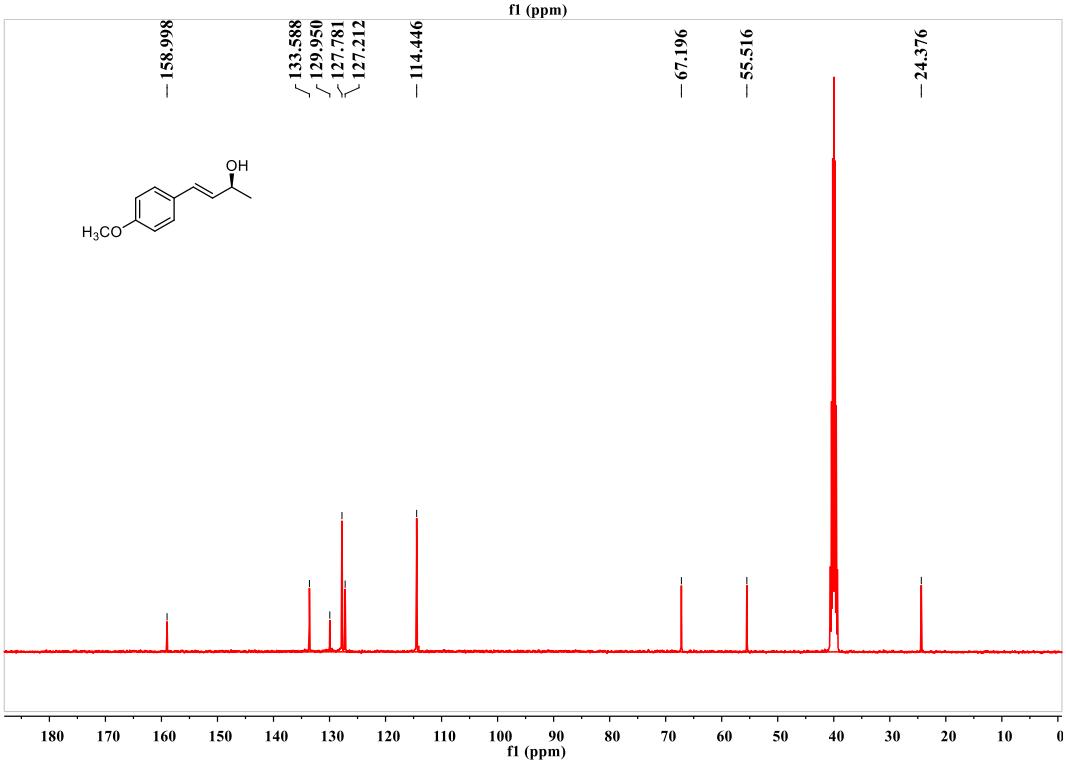


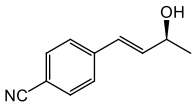


(*S*,*E*)-4-(4-methoxyphenyl)but-3-en-2-ol (3),[6] colorless oil (3.5 mg)

^{1}H NMR (400 MHz, d_6-DMSO): δ 7.37 (2H, d, J = 8.4 Hz), 6.91 (2H, d, J = 8.4 Hz), 6.47 (1H, d, J = 16.0 Hz), 6.17 (1H, m), 4.82 (1H, m), 4.29 (1H, m), 3.77 (3H, s), 1.23 (3H, d, J =6.4 Hz); ^{13}C NMR (125 MHz, d_6-DMSO): δ 158.9, 133.6, 129.9, 127.8, 127.2, 114.4, 67.2, 55.5, 24.4.

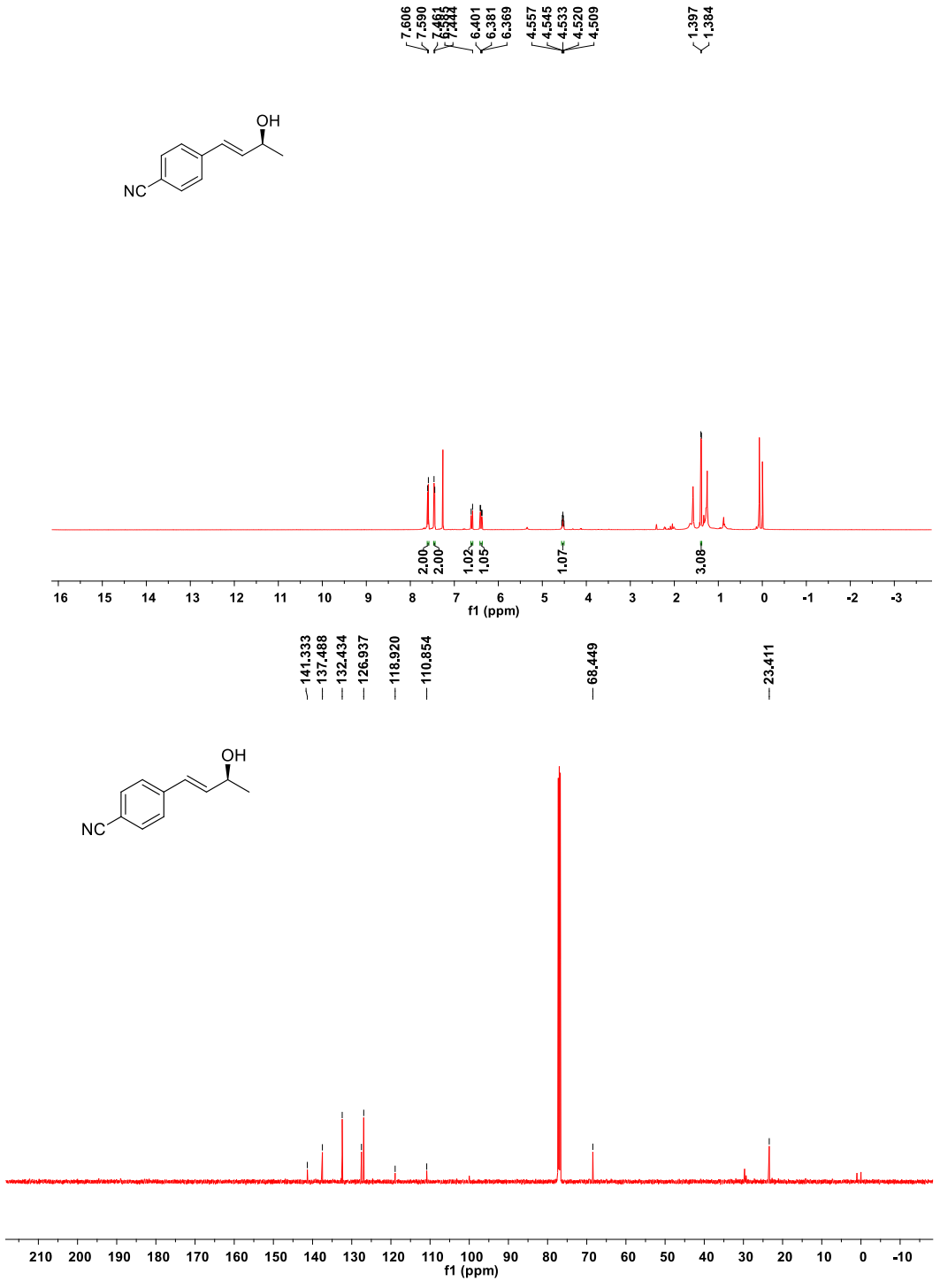


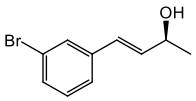




(*S*,*E*)-4-(3-hydroxybut-1-en-1-yl)benzonitrile (4),[2] colorless oil (6.7 mg)

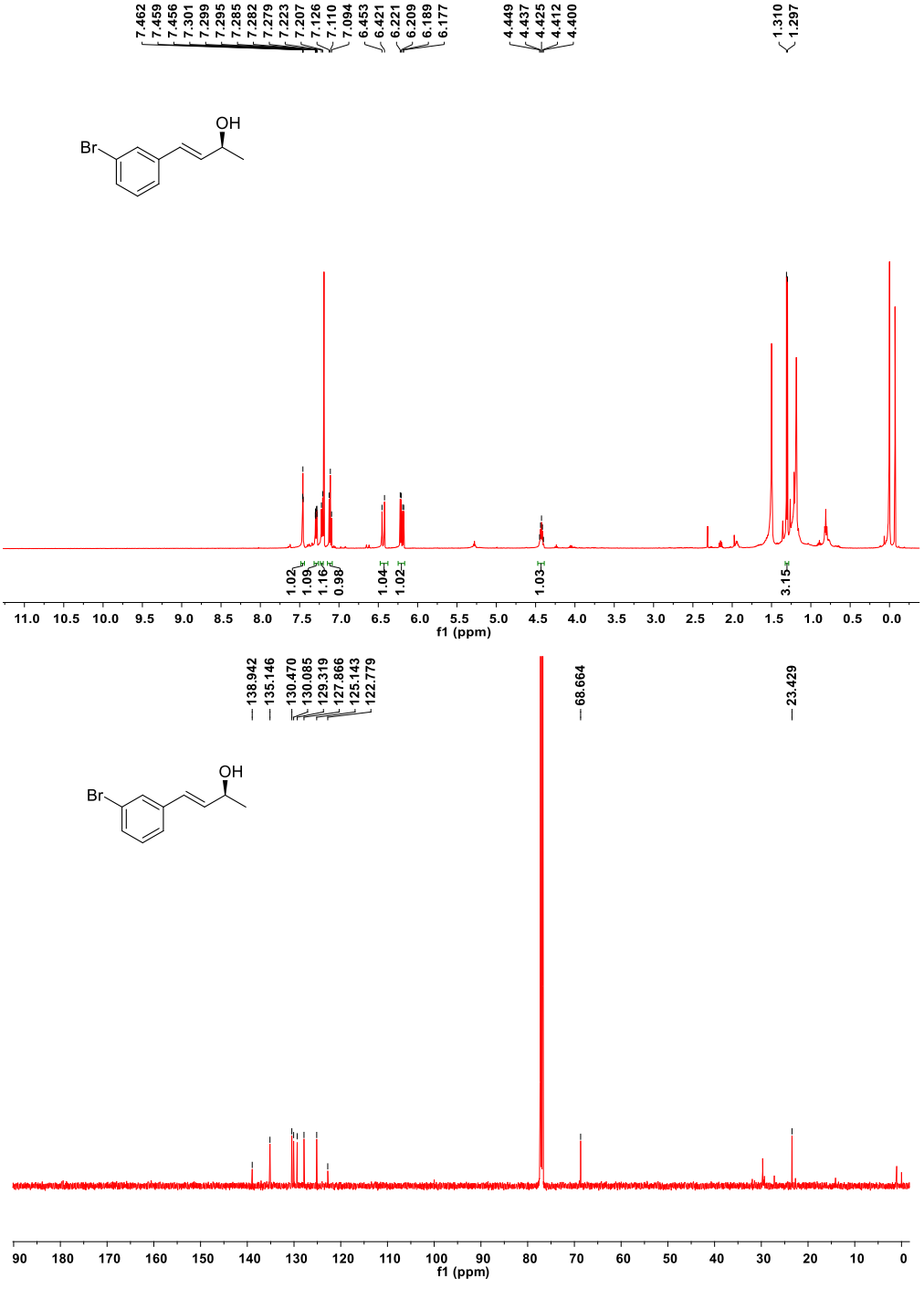
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.60 (2H, d, *J* = 8.0 Hz), 7.45 (2H, d, *J* = 8.5 Hz), 6.59 (1H, t, *J* = 16 Hz), 6.39 (1H, dd, *J* =16, 6.5 Hz), 4.53 (1H, m), 1.39 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ141.3, 137.5, 132.4, 127.5, 126.9, 118.9, 110.8, 68.4, 29.7, 23.4.

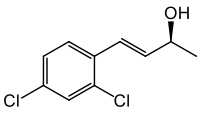




(*S,E*)-4-(3-bromophenyl)but-3-en-2-ol (5),[2] yellow oil (13.1 mg)

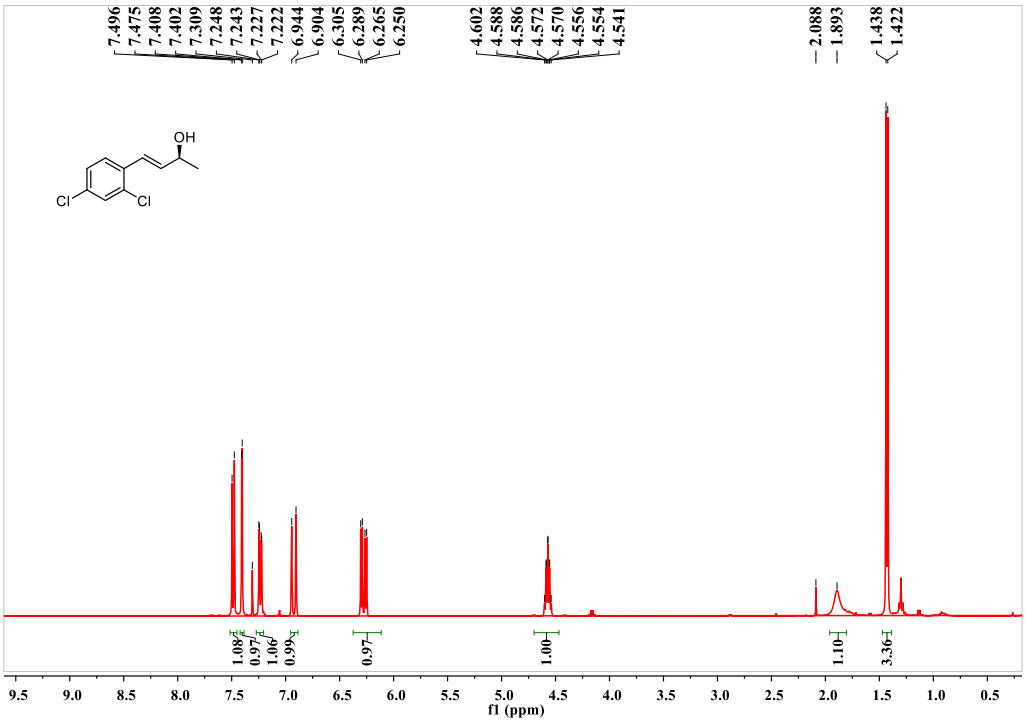
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46 (1H, t, *J* = 1.5 Hz), 7.29 (1H, dd, *J* = 8.0 ,8.5 Hz), 7.21 (1H, d, *J* = 8.0 Hz), 7.11 (1H, t, *J* = 8.0 Hz), 6.44 (1H, d, *J* = 16 Hz), 6.20 (1H, dd, *J* =6.0, 16 Hz), 4.43 (1H, m), 1.30 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ138.9, 135.1, 130.5, 130.1, 129.3, 127.9, 125.1, 122.8, 68.7, 23.4.

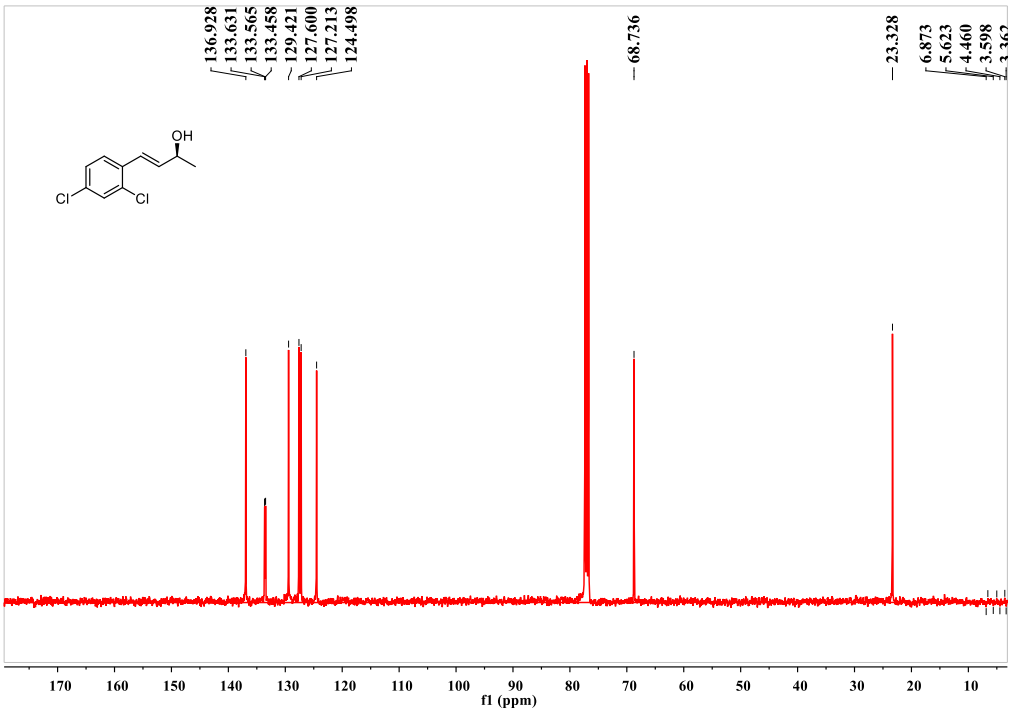


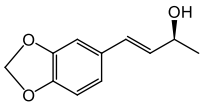


(*S*,*E*)-4-(2,4-dichlorophenyl)but-3-en-2-ol (6),[2] light yellow oil (10.1 mg)

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (1H, d, J = 8.4 Hz), 7.40 (1H, d, J = 2.4 Hz), 7.22 (1H, dd, J = 2.0, 8.4 Hz), 6.94 (1H, d, J = 16.0 Hz), 6.28 (1H, dd, J = 6.4, 16.0 Hz), 4.57 (1H, m), 1.89 (1H, s), 1.42 (3H, d, J =6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 136.9, 133.6, 133.5, 133.4, 129.4, 127.6, 127.2, 124.5, 68.7, 23.3.

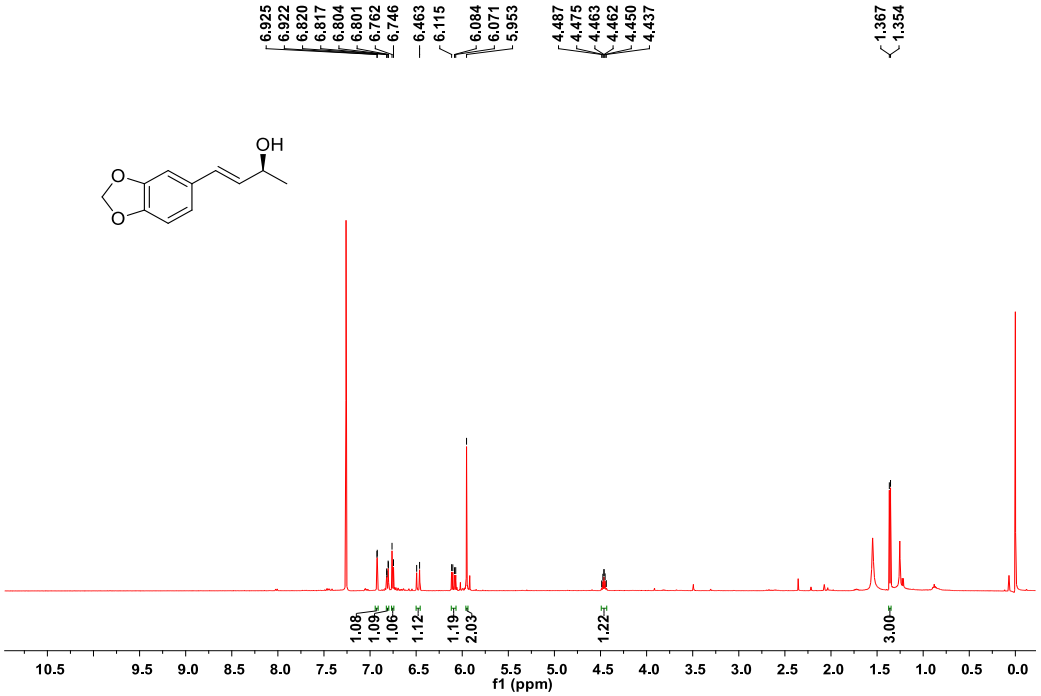


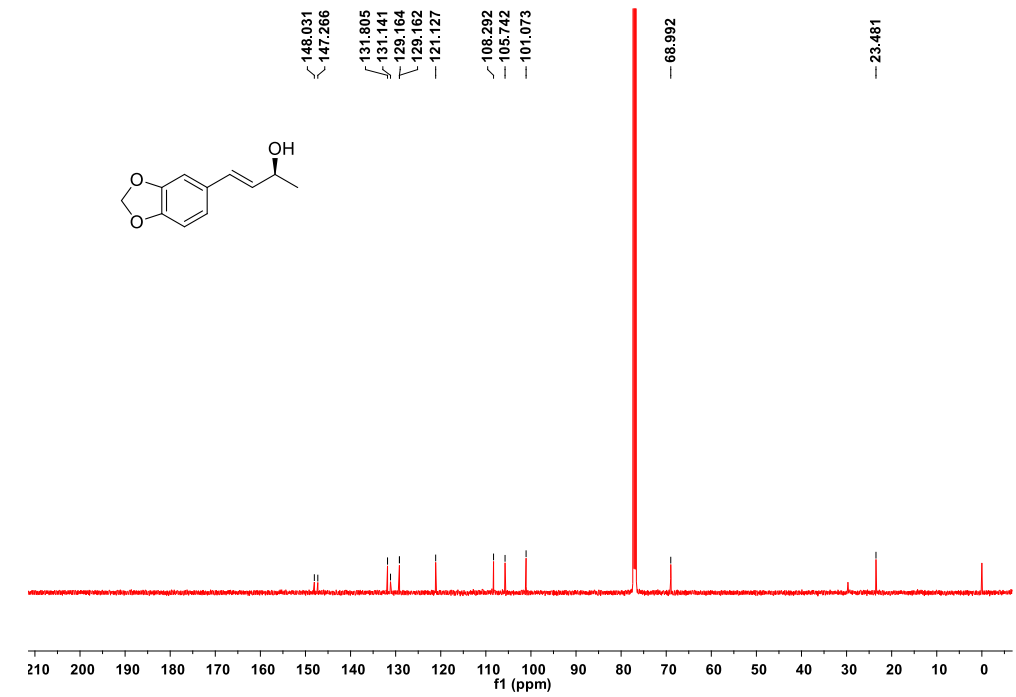


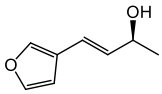


(*S*,*E*)-4-(benzo[d][1,3]dioxol-5-yl)but-3-en-2-ol (7),[2] yellow oil (4.2 mg)

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.92 (1H, d, *J* = 1.5 Hz), 6.81(1H, dd, *J* = 8.5, 8 Hz), 6.75 (1H, d, *J* = 8Hz), 6.48 (1H, d, *J* = 15.5 Hz), 6.09 (1H, dd, *J* =7.0, 16 Hz), 5.95 (2H, s), 4.45 (1H, m), 1.36 (3H, d, *J* = 6.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ148.0, 147.3, 131.8, 131.8, 131.1, 129.2, 129.2, 121.1, 108.3, 105.7, 101.1, 69.0, 23.5.

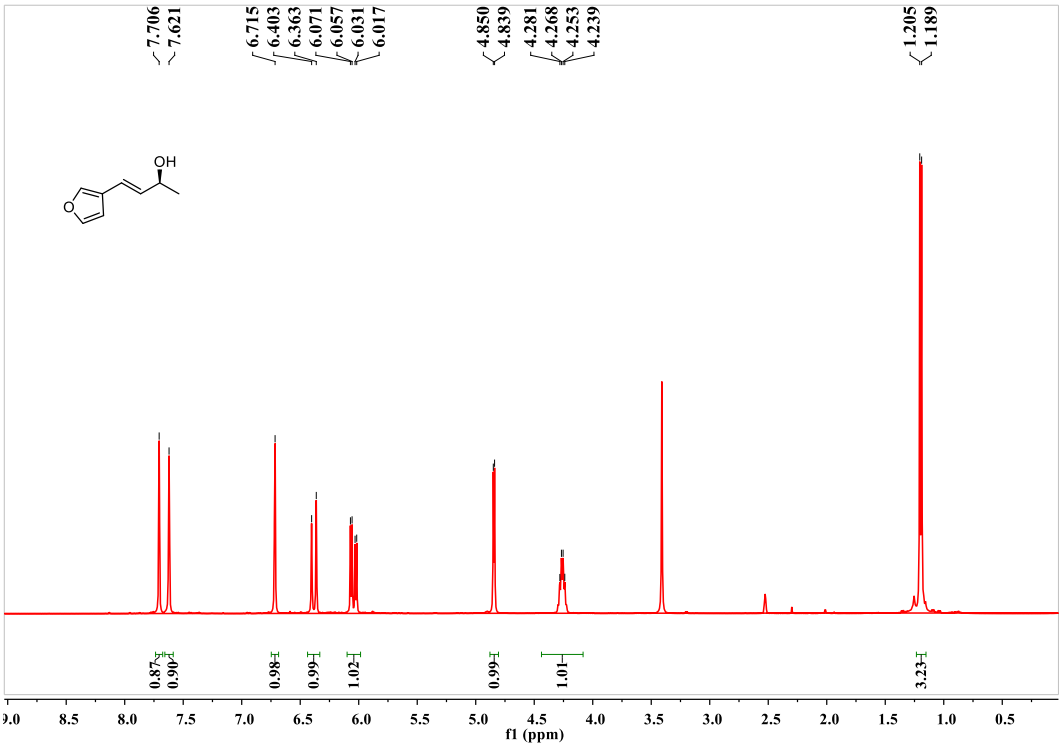


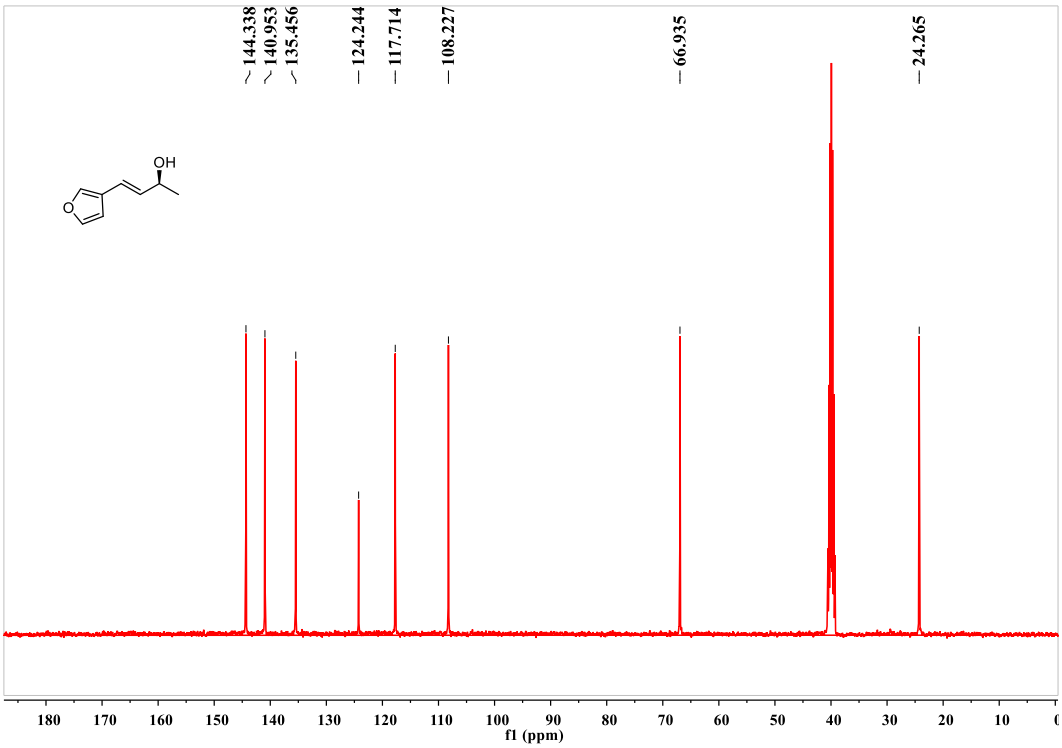


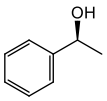


(*S*,*E*)-4-(furan-2-yl)but-3-en-2-ol (8),[2] yellow oil (5.5 mg)

^{1}H NMR (400 MHz, d_6-DMSO): δ 7.70 (1H, s), 7.62 (1H, s), 6.71 (1H,s), 6.40 (1H, d, J = 16 Hz), 6.05 (1H, dd, J =6.8, 16 Hz), 4.85 (1H, d, J =4.4 Hz),4.26, (1H, m), 1.20 (3H, d, J =6.4 Hz); ^{13}C NMR (125 MHz, d_6-DMSO): δ 144.3, 140.9, 135.4, 124.2, 117.7, 108.2, 66.9, 24.2.

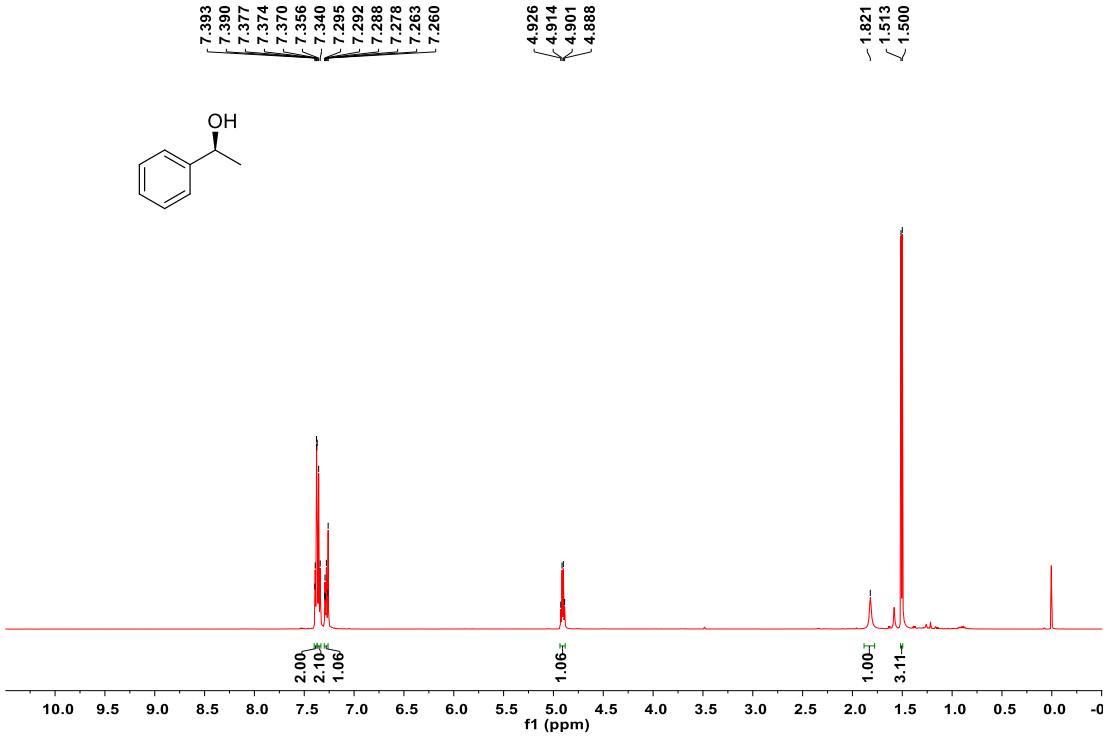


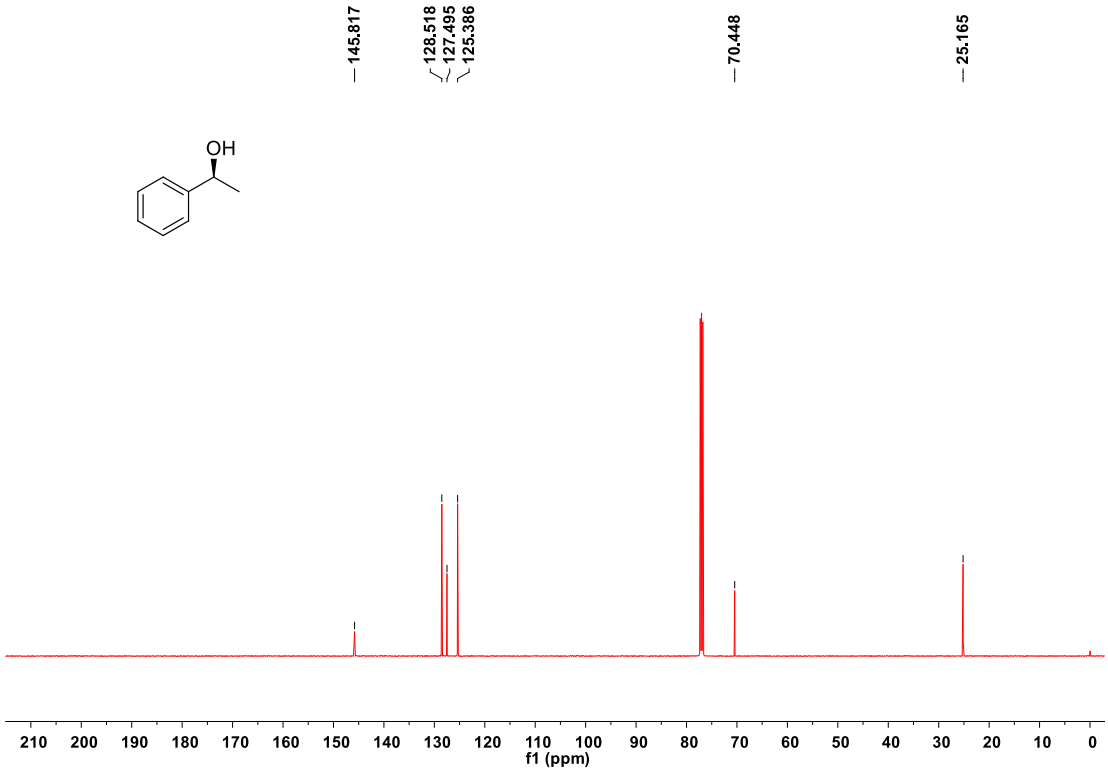


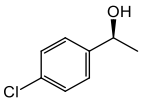


(*S*)-1-phenylethanol (9),[7] light yellow oil (13.8 mg)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (2H, m), 7.36 (2H, m), 7.28 (1H, m), 4.91 (1H, m), 1.51 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ145.8, 128.5, 127.5, 125.4, 70.5, 25.2.

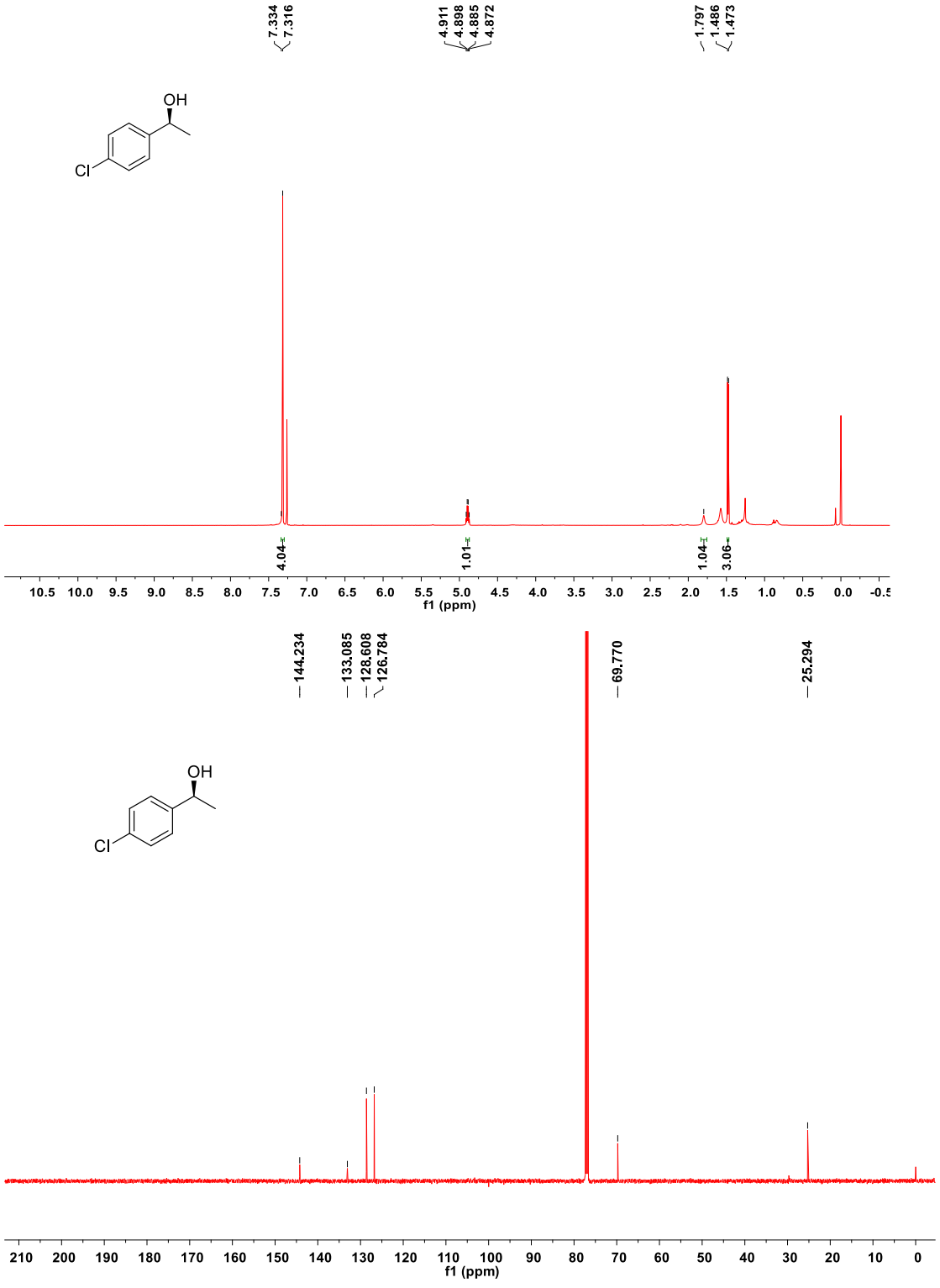


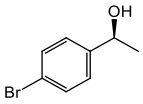




(*S*)-1-(4-chlorophenyl)ethanol (10),[7] light yellow oil (10.4 mg)

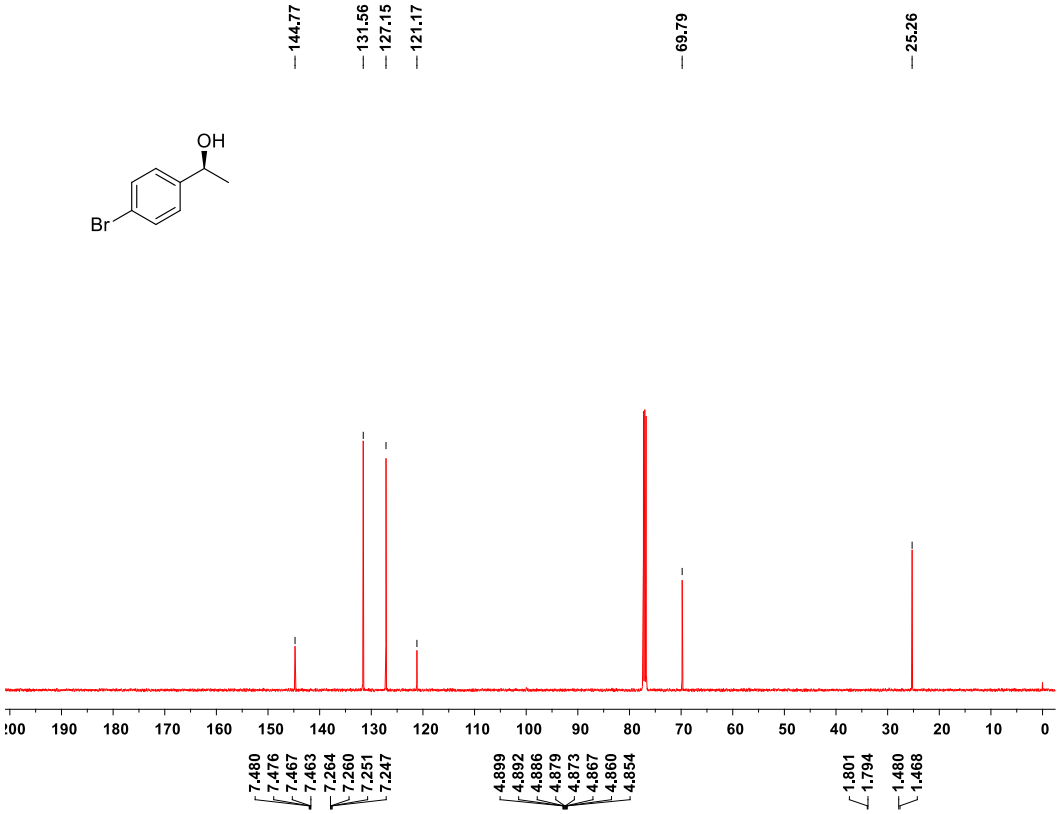
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33 (4H, d, *J* = 9.0 Hz), 4.89 (1H, q), 1.48 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ144.3, 133.1, 128.6, 126.8, 69.7, 25.3.

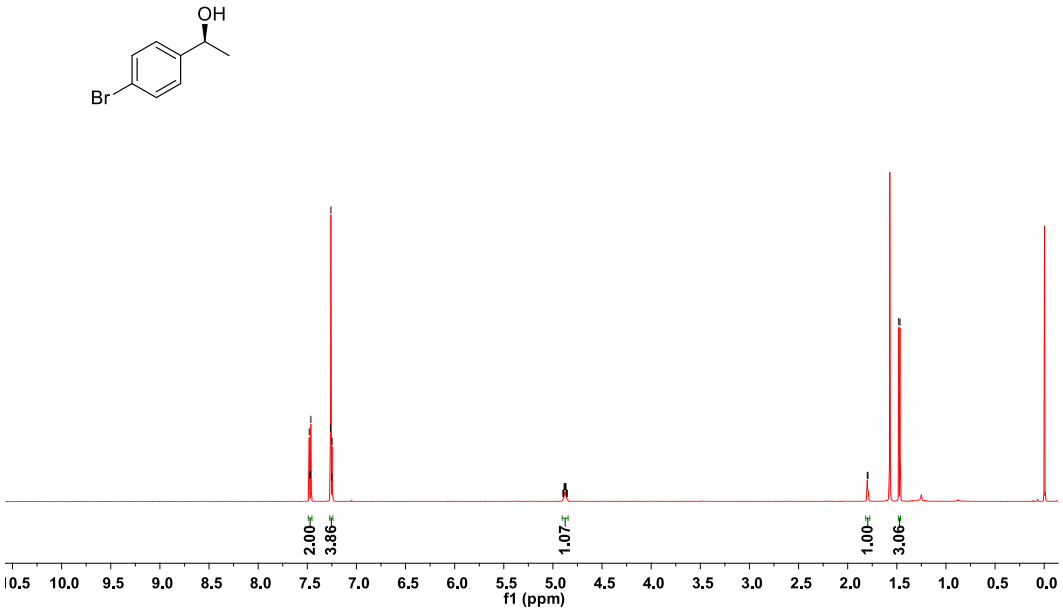


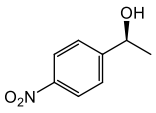


(*S*)-1-(4-bromophenyl)ethanol (11),[7] white solid (12.6 mg)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.47 (2H, dd, *J* = 2.0, 6.5 Hz), 7.26 (2H, dd, *J* = 2.0, 8.0 Hz), 4.87 (1H, m), 1.80 (1H, s), 1.47 (3H, d, *J* = 6.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ144.8, 131.6, 127.2, 121.2, 69.8, 25.3.

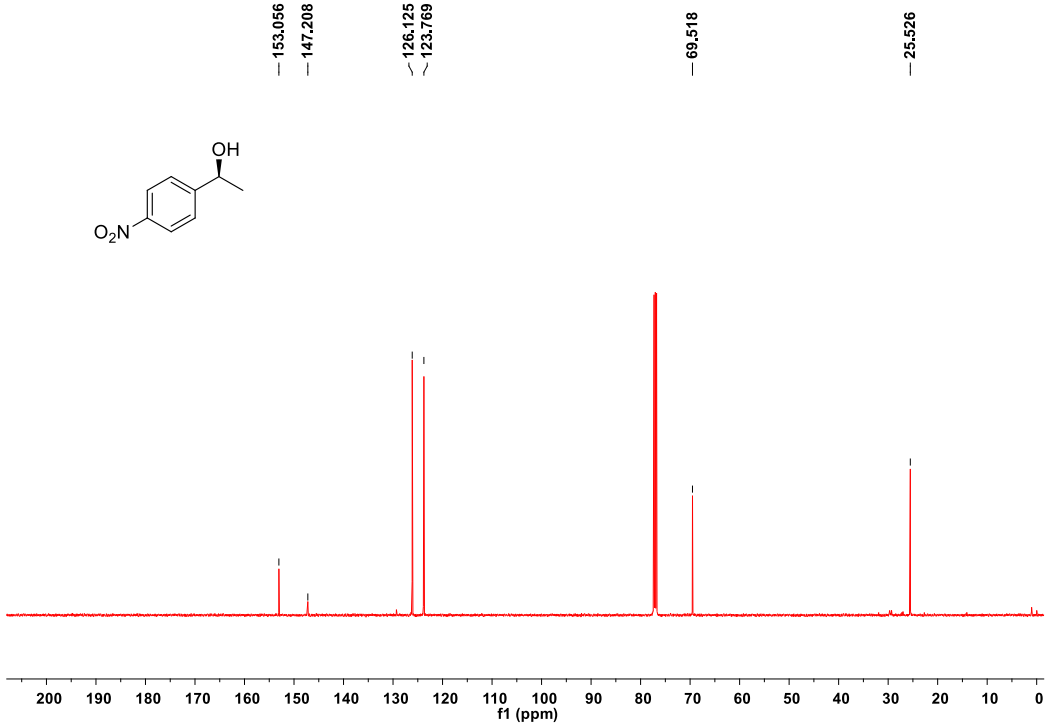


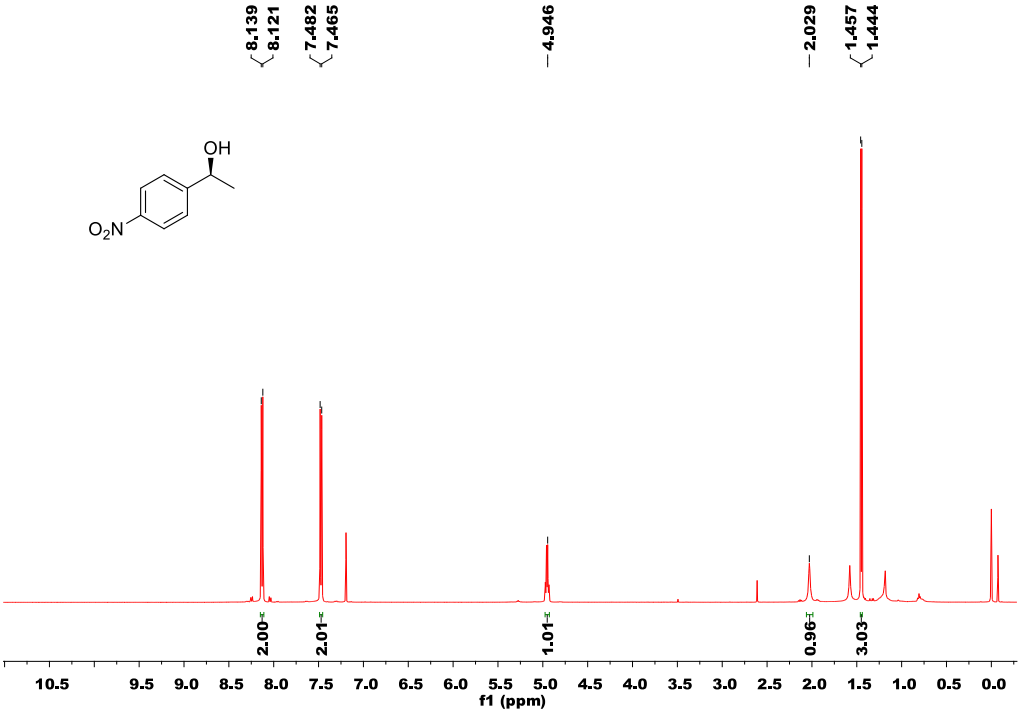


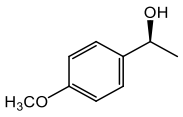


(*S*)-1-(4-nitrophenyl)ethanol (12),[7] light yellow oil (11.7 mg)

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13 (2H, d, *J* = 9.0 Hz), 7.47 (2H, d, *J* = 8.5 Hz), 4.95 (1H, m), 2.03 (1H, s), 1.45 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 153.1, 147.2, 126.1, 123.8, 69.5, 25.5.

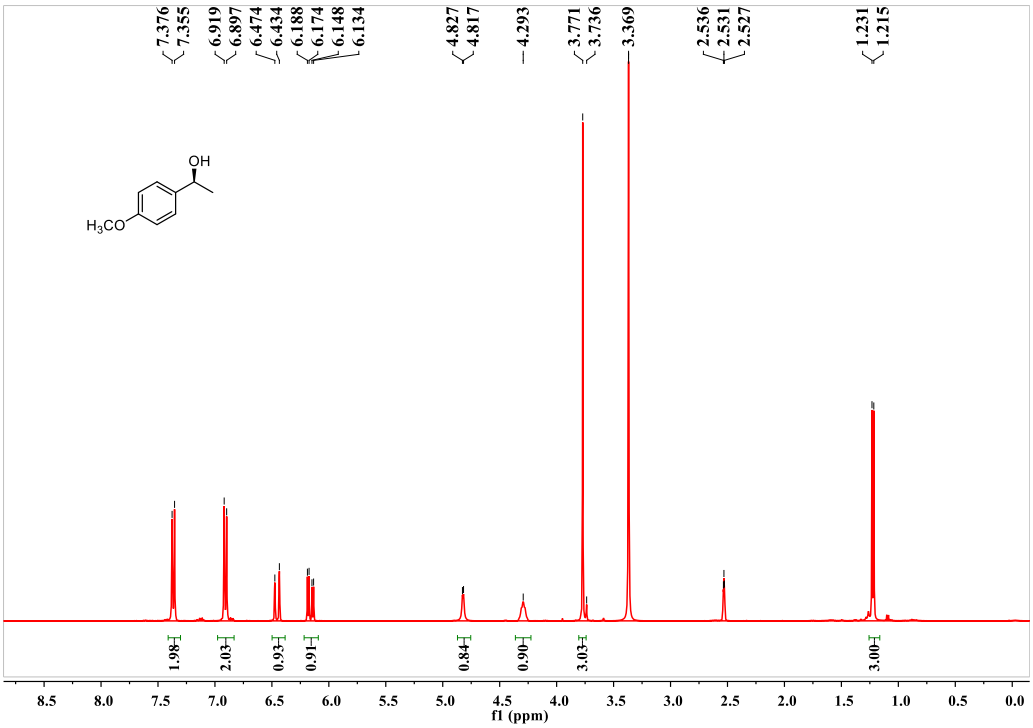


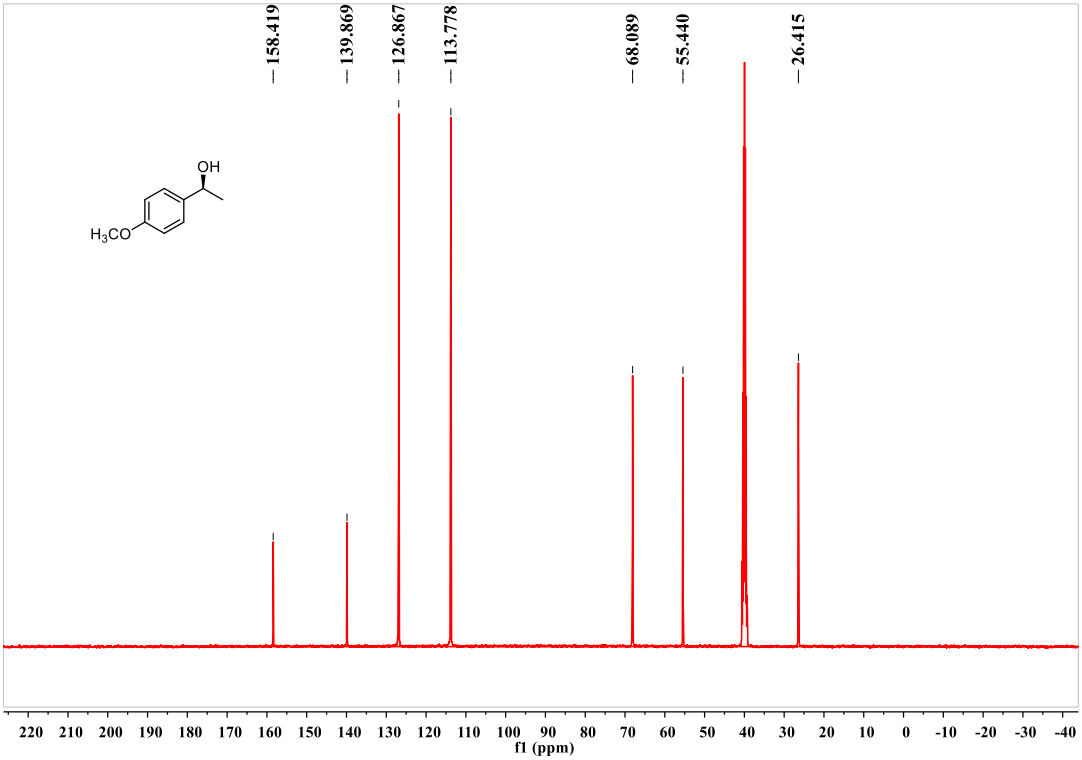


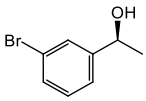


(*S*)-1-(4-methoxyphenyl)ethanol (13),[7] colorless oil (4.3 mg)

^{1}H NMR (400 MHz, d_6-DMSO): δ 7.28 (2H, d, J = 8.8 Hz), 6.89 (2H, d, J = 8.8 Hz), 5.04 (1H, d, J = 4.4 Hz), 4.68 (1H, m), 3.74 (3H, s), 1.32 (3H, d, J =6.4 Hz); ^{13}C NMR (125 MHz, d_6-DMSO): δ 158.4, 133.6, 139.8, 127.8, 126.8, 113.7, 68.1, 55.4, 26.4.

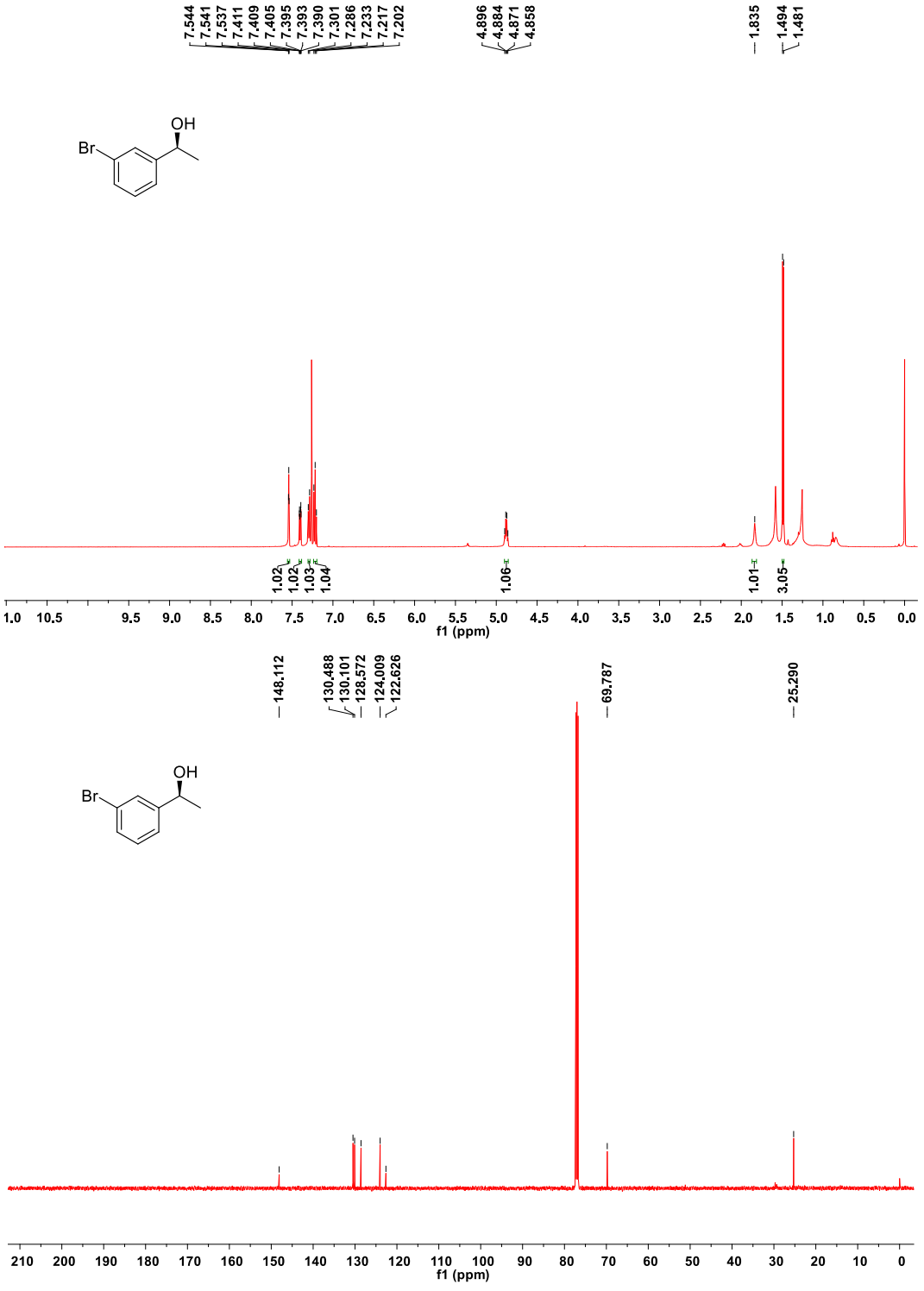


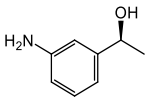




(*S*)-1-(3-bromophenyl)ethanol (14),[7] yellow oil (12.2 mg)

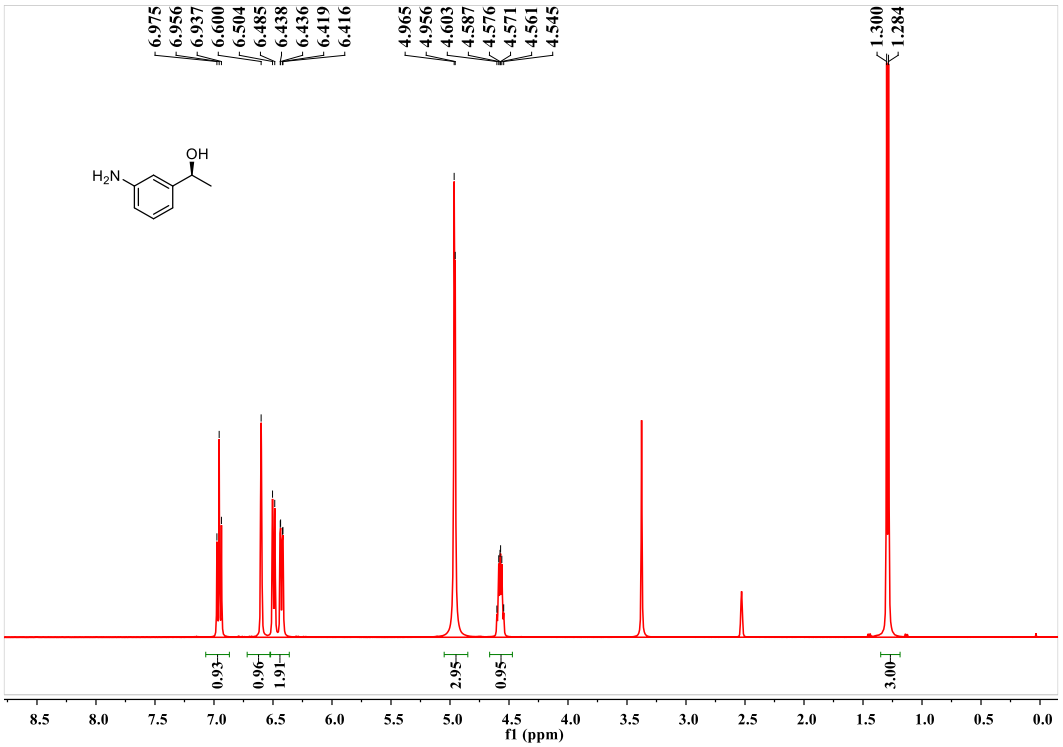
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51 (1H, d, *J* = 1.5 Hz), 7.40 (1H, m), 7.29 (1H, m), 7.22 (1H, m), 4.88 (1H, m), 1.49 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ148.1, 130.5, 130.1, 128.6, 124.0, 122.6, 69.8, 25.3.

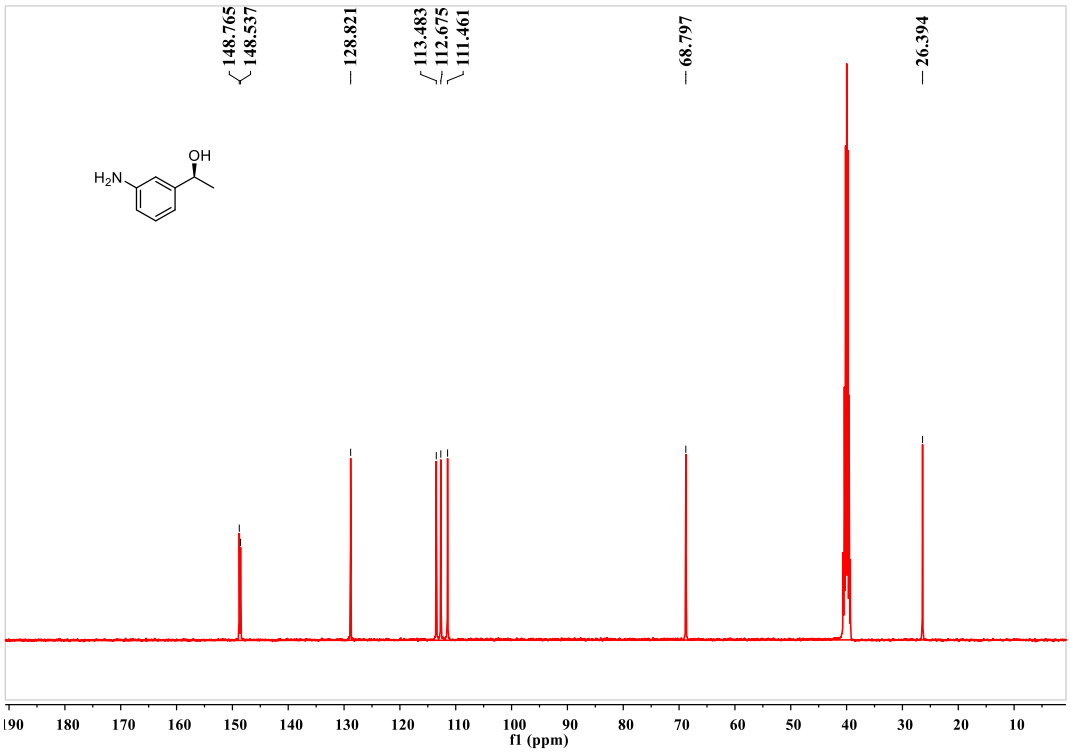


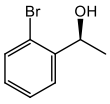


(*S*)-1-(3-aminophenyl)ethanol (15),[8] colorless oil (3.1 mg)

^{1}H NMR (400 MHz, d_6-DMSO): δ 6.97 (1H, t, J = 8.8 Hz), 6.60 (1H, s), 6.50 (1H, d, J = 7.6 Hz), 6.43 (1H, dd, J = 1.2, 8.0 Hz), 4.96 (2H, brs), 4.57 (1H, m), 1.30 (3H, d, J =6.4 Hz); ^{13}C NMR (125 MHz, d_6-DMSO): δ 148.7, 148.5, 128.8, 113.5, 112.6, 111.4, 68.8, 26.3.

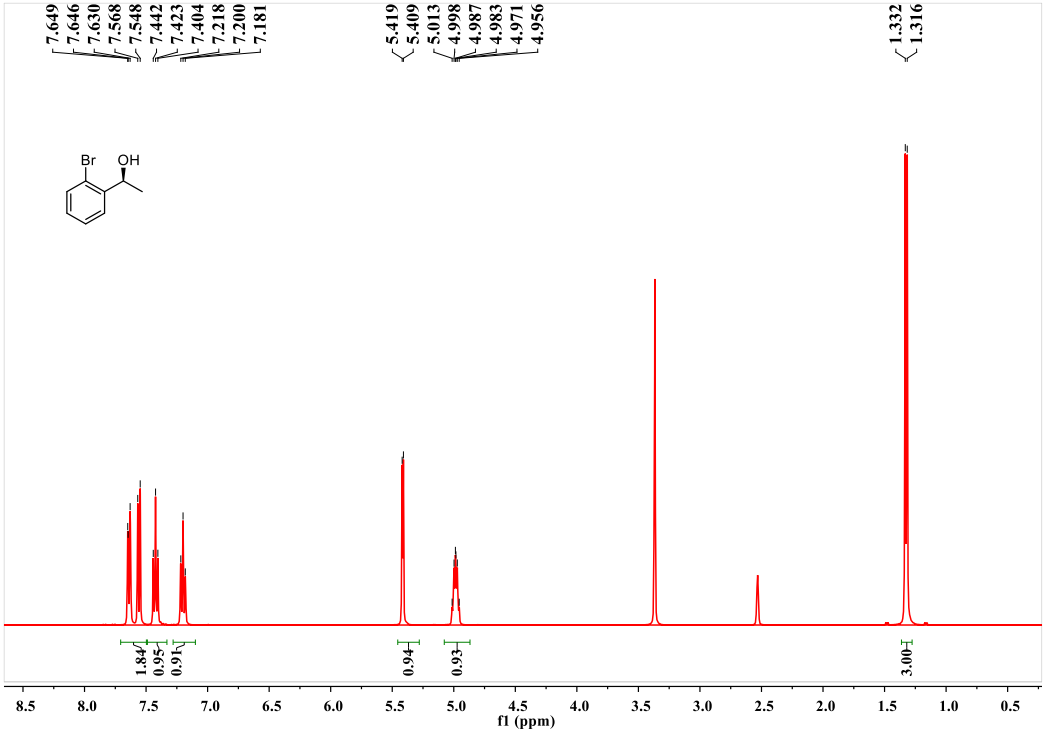


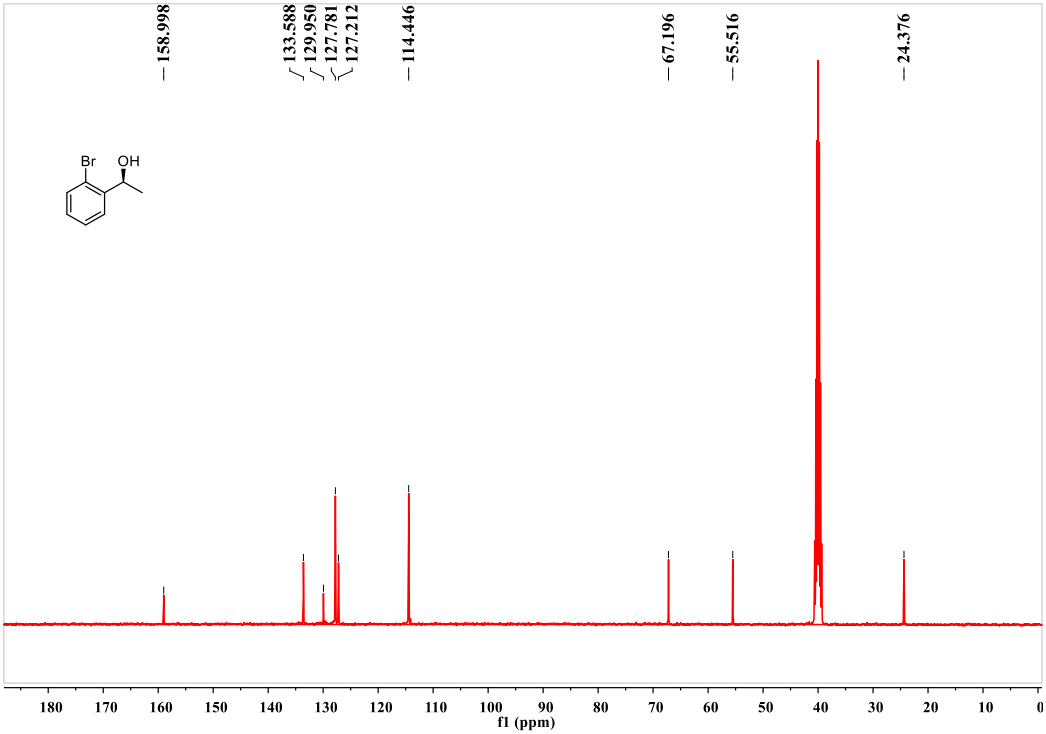


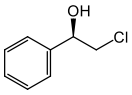


(*S*)-1-(2-bromophenyl)ethanol (16),[9] brown oil (11.8 mg)

^{1}H NMR (400 MHz, d_6-DMSO): δ 7.64 (1H,m), 7.56 (1H, d, J = 8.0 Hz), 7.42 (1H, t, J = 7.6 Hz), 7.20 (1H, t, J = 7.6 Hz), 5.41 (1H, d, J = 4.0 Hz), 4.98 (1H, m), 1.33 (3H, d, J =6.4 Hz); ^{13}C NMR (125 MHz, d_6-DMSO): δ 146.5, 132.5, 129.0, 128.3, 127.6, 121.1, 67.7, 24.8.

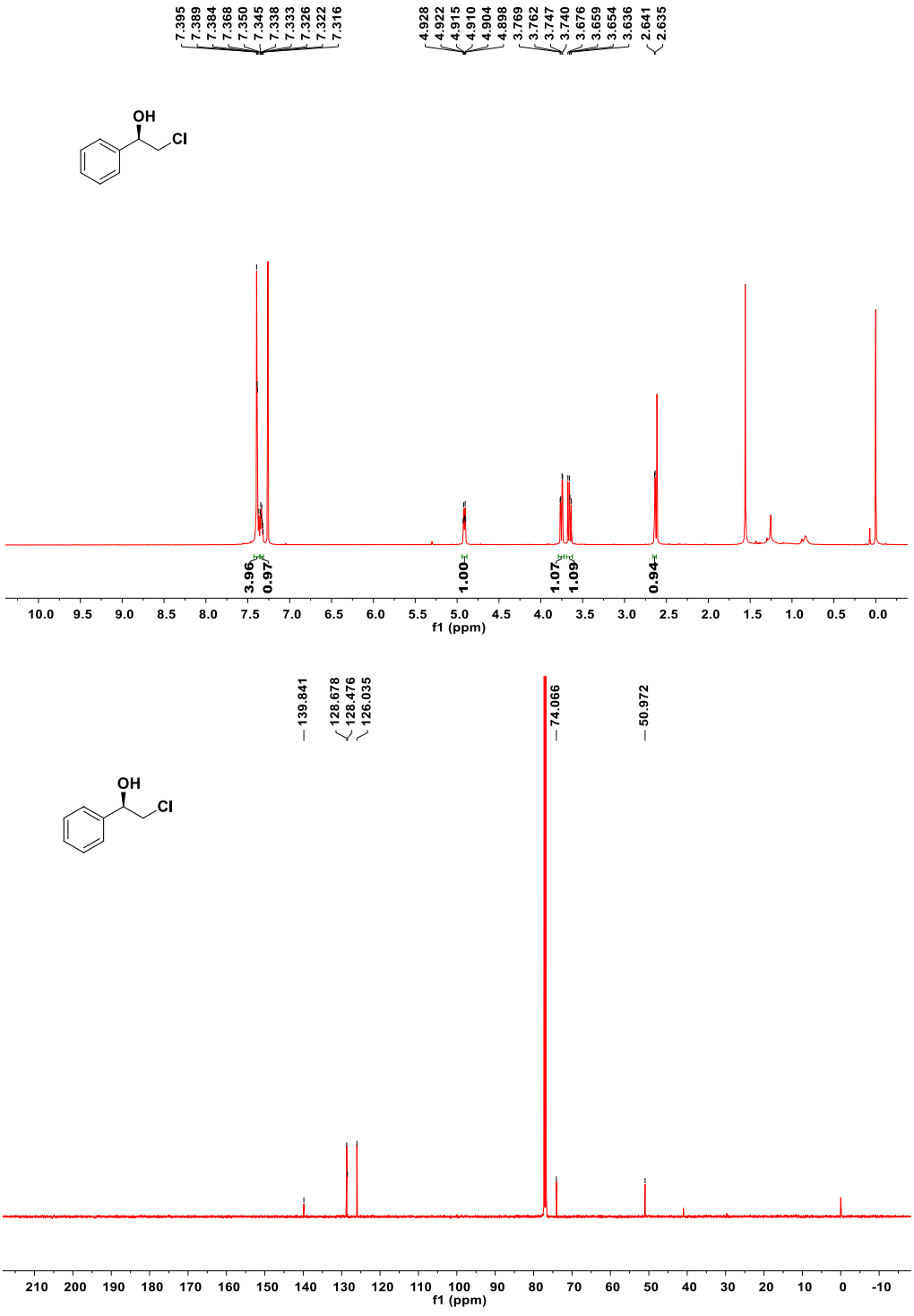


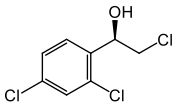




(*R*)-2-chloro-1-phenylethanol (17),[10] light yellow oil (9.6 mg)

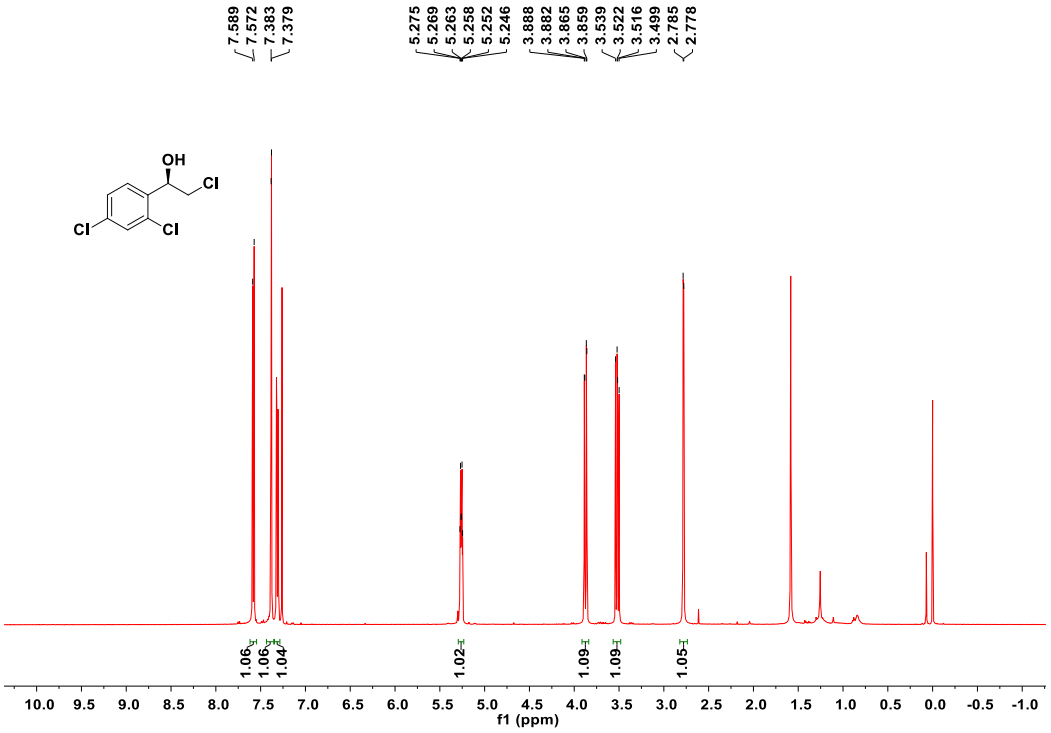
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38 (4H, m), 7.33 (1H, m), 4.91 (1H, m), 3.75 (1H, m), 3.66 (1H, dd, *J* =11, 8.5 Hz), 2.63 (1H, d, *J* =3.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 139.8, 128.7, 128.5, 126.0, 74.1, 51.0.

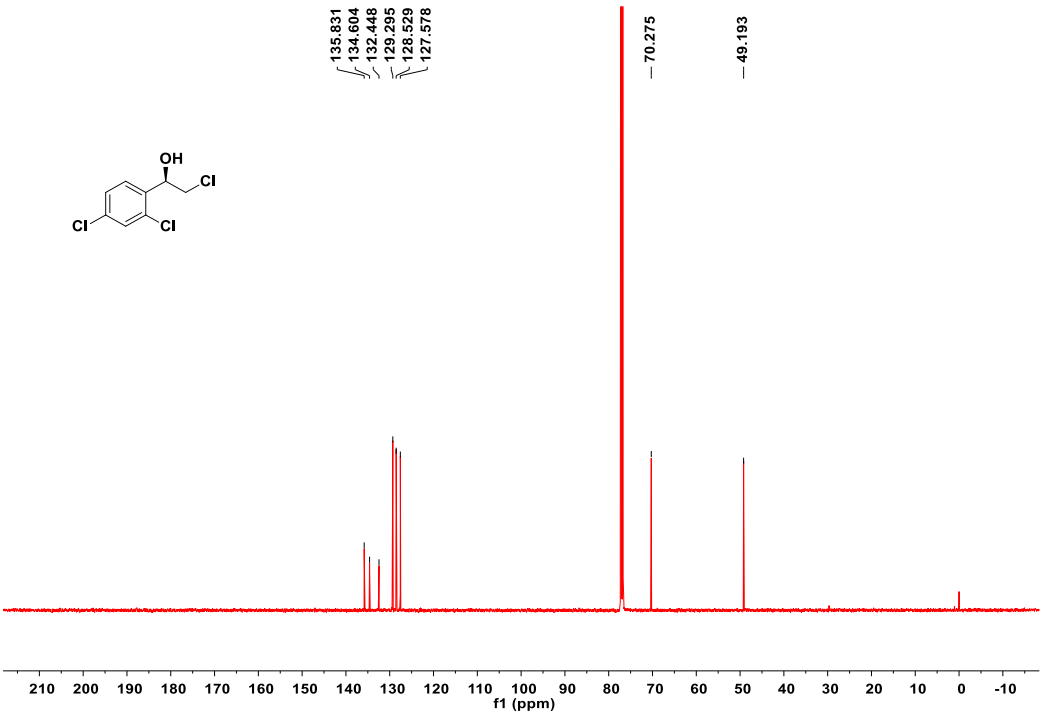


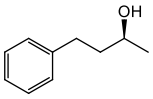


(*R*)-2-chloro-1-(2,4-dichlorophenyl)ethanol (18),[11] white solid (15.0 mg)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.58 (1H, d, *J* = 8.5 Hz), 7.38 (1H, d, *J* = 2.0 Hz), 7.31 (1H, dd, *J* = 8.5, 2.0Hz), 5.26 (1H, m), 3.87 (1H, dd, *J* =3.0, 11.5 Hz), 3.52 (1H, dd, *J* =11.5, 8.5 Hz), 2.78 (1H, d, *J* =3.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ135.8, 134.6, 132.5, 129.3, 128.5, 127.6, 70.3, 49.2.

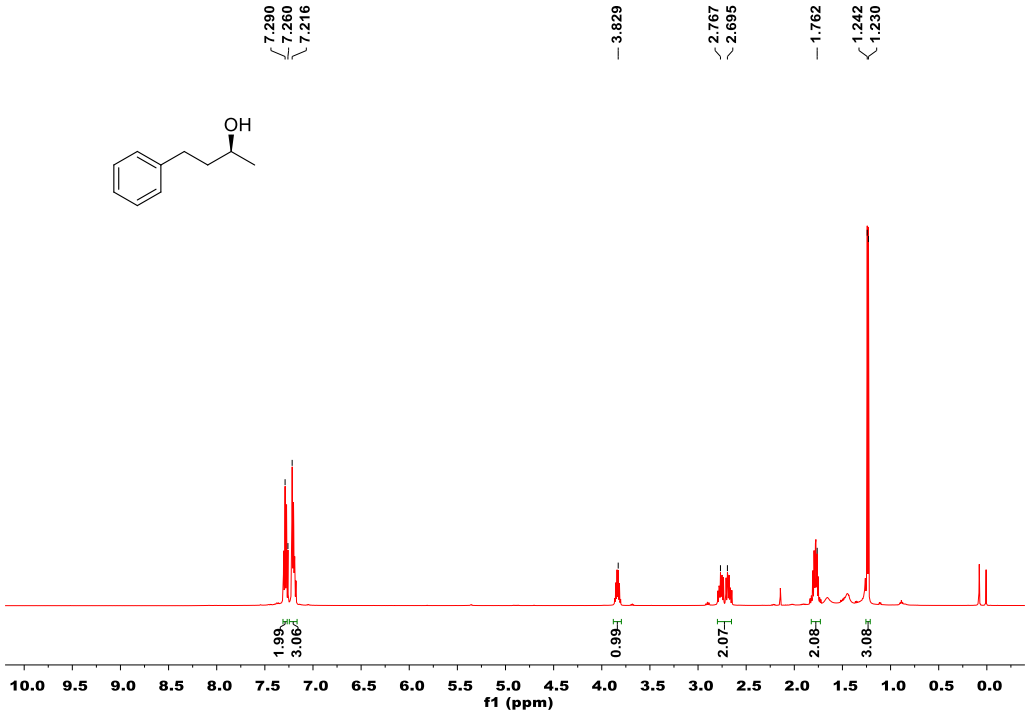


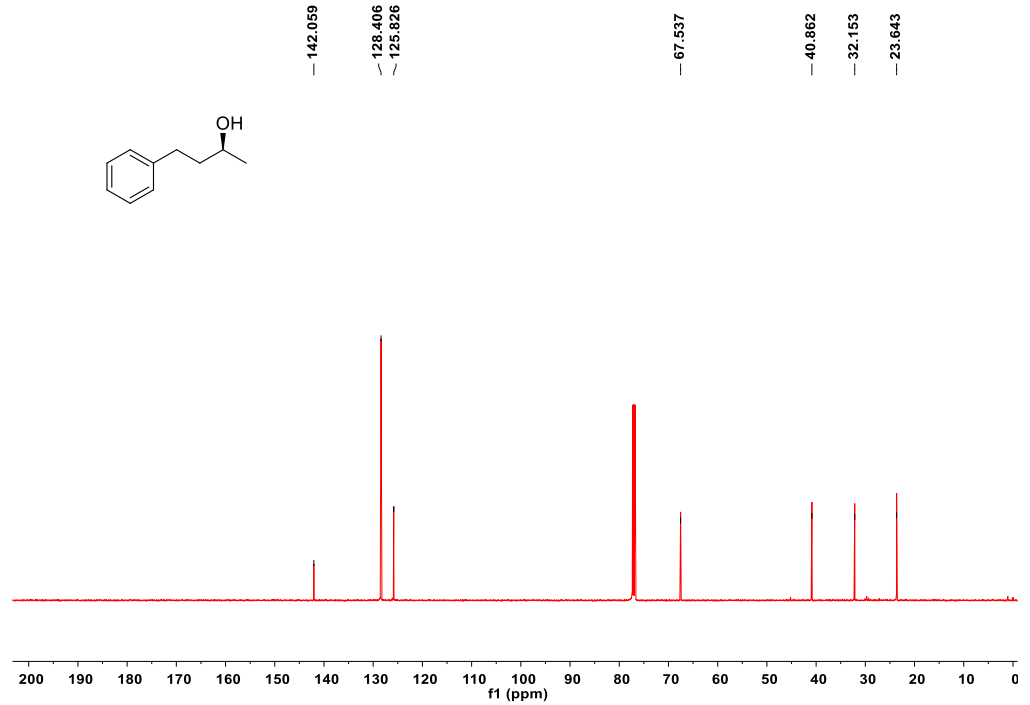


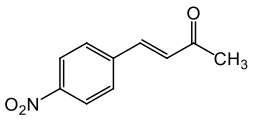


(*S*)-4-phenylbutan-2-ol (19),[12] colorless oil (6.7 mg)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29 (2H, d, J = 7.5 Hz), 7.21 (3H, m), 3.83 (1H, m), 2.76 (2H, m), 1.76 (2H, m), 1.24 (3H, d, J = 6.0 Hz); 13C NMR (125 MHz, $CDCl_3$): δ142.0, 128.4, 125.8, 67.5, 40.9, 32.1, 23.6.

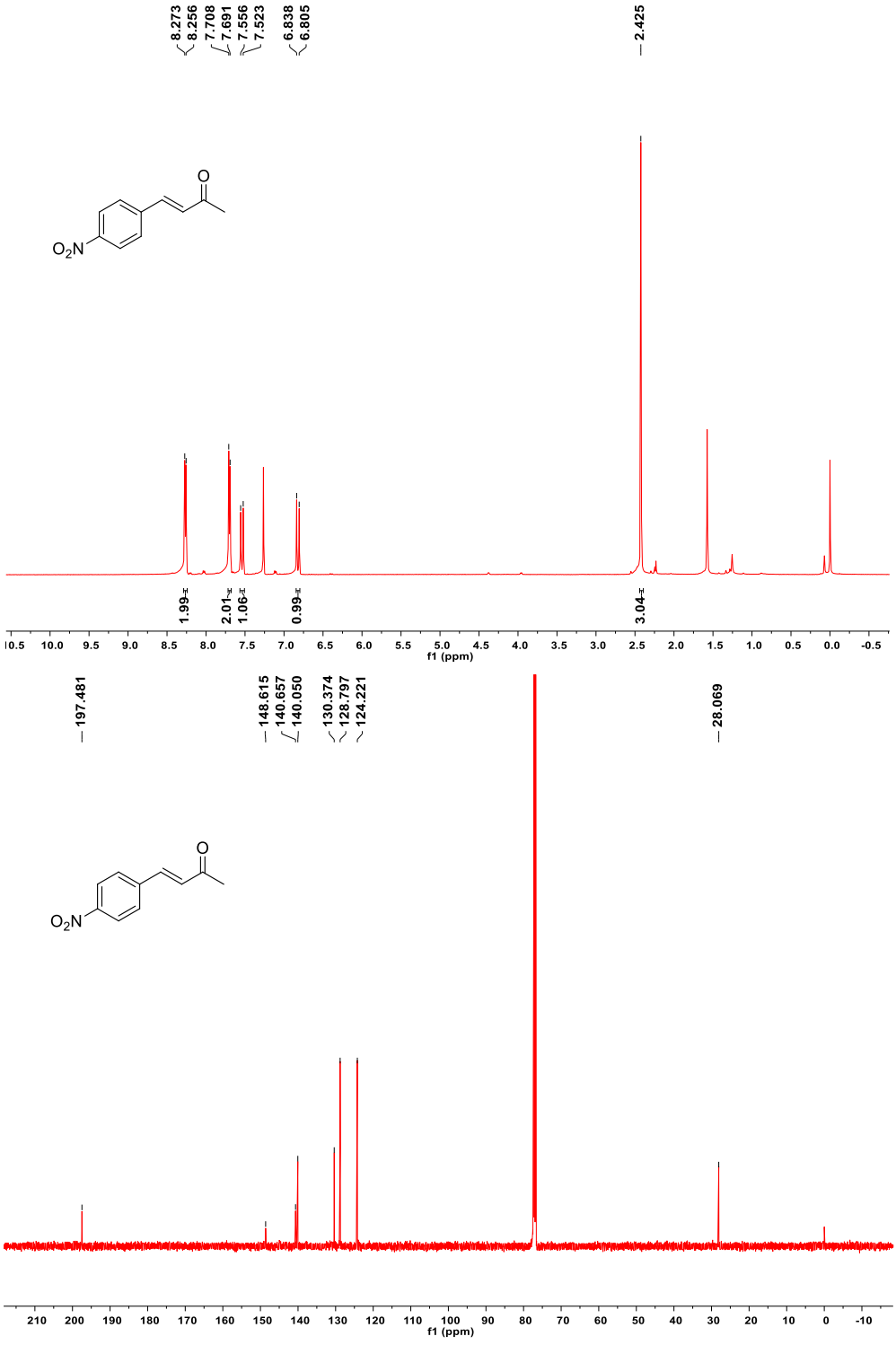


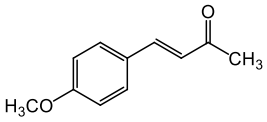




4-(4-nitrophenyl) -3-buten-2-one (S2),[13] yellow solid (15.7 mg)

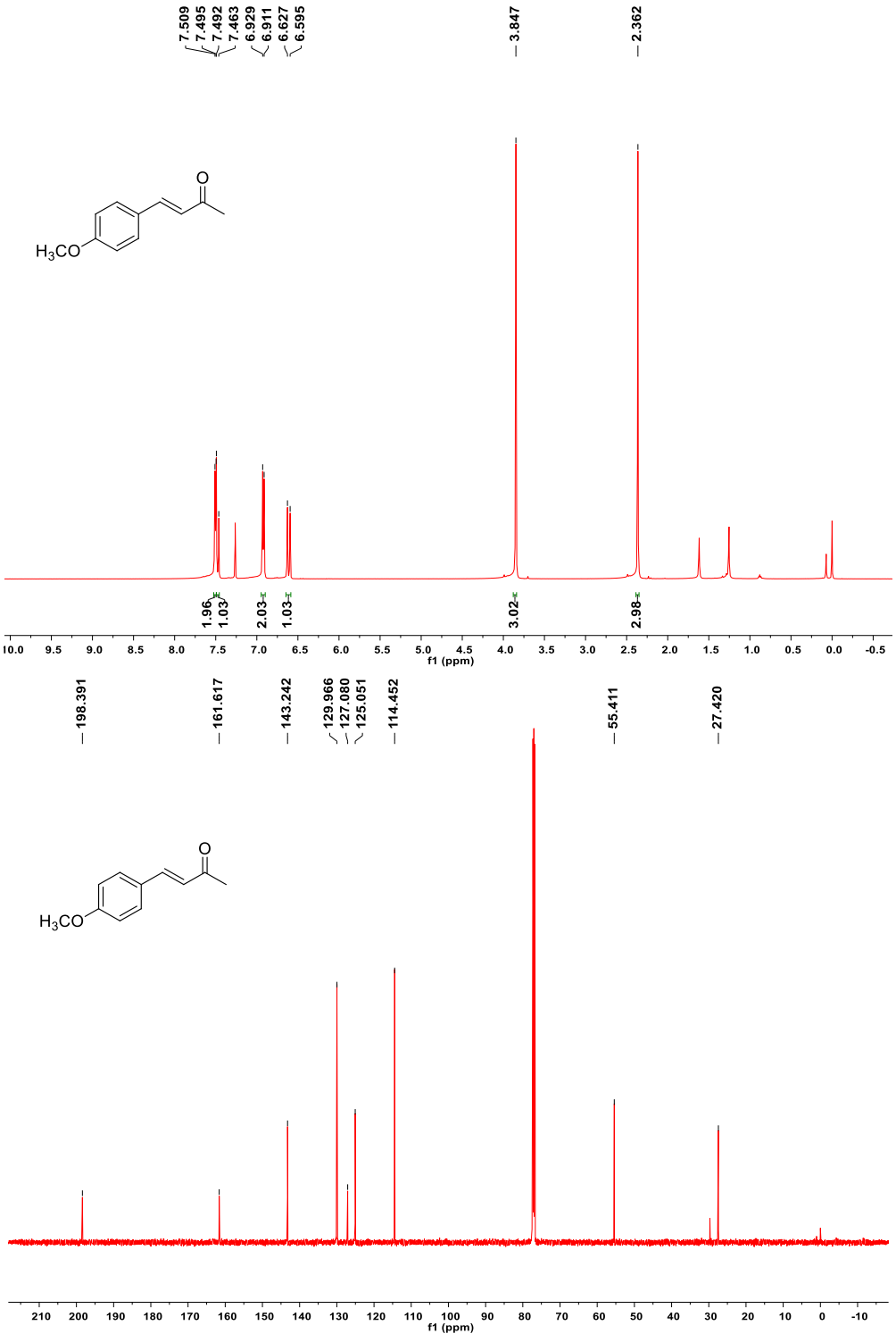
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.26 (2H, d, *J* = 8.5 Hz), 7.69 (2H, d, *J* = 8.5 Hz), 7.54 (1H, d, *J* = 16.5 Hz), 6.82 (1H, d, *J* = 16.5 Hz), 2.43 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ197.5, 148.6, 140.7, 140.1, 130.4, 128.8, 124.2, 28.1.





4-(4-methoxy-phenyl) -3-ene-2-butanone (S3),[14] white solid (16.8 mg)

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.50 (2H, d, *J* = 8.5 Hz), 7.47 (1H, d, *J* = 16.0 Hz), 6.92 (2H, d, *J* = 9.0 Hz), 6.61 (1H, d, *J* = 16.0 Hz), 3.85 (3H, s), 2.36 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ198.4, 161.6, 143.2, 130.0, 127.1, 125.1, 114.5, 55.4, 27.4.



References

1. Sun, L. L.; Ruppert, M.; Sheludko, Y.; Warzecha, H.; Zhao, Y.; Stockigt, J., Purification, cloning, functional expression and characterization of perakine reductase: the first example from the AKR enzyme family, extending the alkaloidal network of the plant Rauvolfia. *Plant Mol. Biol.* **2008,** 67, 455-467.

2. Chen, F. L.; Zhang, Y.; Yu, L.; Zhu, S. L., Enantioselective NiH/Pmrox-Catalyzed 1,2-Reduction of alpha,beta-Unsaturated Ketones. *Angew. Chem., Int. Ed.* **2017,** 56, 2022-2025.

3. (a) Jez, J. M.; Bennett, M. J.; Schlegel, B. P.; Lewis, M.; Penning, T. M., Comparative anatomy of the aldo-keto reductase superfamily. *Biochem. J.* **1997,** 326, 625-636. (b) Di Costanzo, L.; Drury, J. E.; Penning, T. M.; Christianson, D. W., Crystal structure of human liver Delta(4)-3-ketosteroid 5 beta-reductase (AKR1D1) and implications for substrate binding and catalysis. *J. Biol. Chem.* **2008,** 283, 16830-16839. (c) El-Kabbani, O.; Dhagat, U.; Soda, M.; Endo, S.; Matsunaga, T.; Hara, A., Probing the inhibitor selectivity pocket of human 20 alpha-hydroxysteroid dehydrogenase (AKR1C1) with X-ray crystallography and site-directed mutagenesis. *Bioorg. Med. Chem. Lett.* **2011,** 21, 2564-2567. (d) Zhang, L. P.; Zhang, H.; Zhao, Y. N.; Li, Z.; Chen, S. K.; Zhai, J.; Chen, Y. Y.; Xie, W.; Wang, Z.; Li, Q.; Zheng, X. H.; Hu, X. P., Inhibitor selectivity between aldo-keto reductase superfamily members AKR1B10 and AKR1B1: Role of Trp112 (Trp111). *FEBS Lett.* **2013,** 587, 3681-3686. (e) Penning, T. M., The aldo-keto reductases (AKRs): Overview. *Chem.-Biol. Interact.* **2015,** 234, 236-246.

4. Sun, L. L.; Chen, Y. X.; Rajendran, C.; Mueller, U.; Panjikar, S.; Wang, M. T.; Mindnich, R.; Rosenthal, C.; Penning, T. M.; Stoockigt, J., Crystal Structure of Perakine Reductase, Founding Member of a Novel Aldo-Keto Reductase (AKR) Subfamily That Undergoes Unique Conformational Changes during NADPH Binding. *J. Biol. Chem.* **2012,** 287, 11213-11221.

5. Martinez-Montero, L.; Gotor, V.; Gotor-Fernandez, V.; Lavandera, I., Mild Chemoenzymatic Oxidation of Allylic sec-Alcohols. Application to Biocatalytic Stereoselective Redox Isomerizations. *Acs Catal.* **2018,** 8, 2413-2419.

6. Chen, X. N.; Zhou, H.; Zhang, K. Y.; Li, J. W.; Huang, H. M., Highly Enantioselective Hydrogenation of Steric Hindrance Enones Catalyzed by Ru Complexes with Chiral Diamine and Achiral Phosphane. *Org. Lett.* **2014,** 16, 3912-3915.

7. Yadav, J. S.; Reddy, B. V. S.; Sreelakshmi, C.; Rao, A. B., Enantioselective Reduction of Prochiral Ketones Employing Sprouted Pisum sativa as Biocatalyst. *Synthesis* **2009**, 1881-1885.

8. Bhattacharya, P.; Krause, J. A.; Guan, H. R., Iron Hydride Complexes Bearing Phosphinite-Based Pincer Ligands: Synthesis, Reactivity, and Catalytic Application in Hydrosilylation Reactions. *Organometallics* **2011,** 30, 4720-4729.

9. Kantam, M. L.; Laha, S.; Yadav, J.; Likhar, P. R.; Sreedhar, B.; Jha, S.; Bhargava, S.; Udayakiran, M.; Jagadeesh, B., An efficient copper-aluminum hydrotalcite catalyst for asymmetric hydrosilylation of ketones at room temperature. *Org. Lett.* **2008,** 10, 2979-2982.

10. Ohkuma, T.; Tsutsumi, K.; Utsumi, N.; Arai, N.; Noyori, R.; Murata, K., Asymmetric hydrogenation of alpha-chloro aromatic ketones catalyzed by eta(6)-arene/TsDPEN-ruthenium(II) complexes. *Org. Lett.* **2007,** 9, 255-257.

11. Huang, X. H.; Ying, J. Y., Asymmetric transfer hydrogenation over Ru-TsDPEN catalysts supported on siliceous mesocellular foam. *Chem. Commun.* **2007**, 1825-1827.

12. Larouche-Gauthier, R.; Elford, T. G.; Aggarwal, V. K., Ate Complexes of Secondary Boronic Esters as Chiral Organometallic-Type Nucleophiles for Asymmetric Synthesis. *J. Am. Chem. Soc.* **2011,**

133, 16794-16797.
13. Leung, P. S. W.; Teng, Y.; Toy, P. H., Chromatography-Free Wittig Reactions Using a Bifunctional Polymeric Reagent. *Org. Lett.* **2010,** 12, 4996-4999.
14. Concellon, J. M.; Rodriguez-Solla, H.; Mejica, C., An efficient synthesis of (E)-alpha,beta-unsaturated ketones and esters with total stereoselectivity by using chromium dichloride. *Tetrahedron* **2006,** 62, 3292-3300.