Supporting Information

Enzymatic Synthesis of Optical Pure β-Nitroalcohols by Combining D-aminoacylase-catalyzed Nitroaldol Reaction and Immobilized Lipase PS-catalyzed Kinetic Resolution

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1. Experimental Section

1.1. Material.

Lipase immobilized on acrylic resin from *Candida antarctica* (≥10,000 U g^{-1}, recombinant, expressed in *Aspergillus oryzae*), Amano lipase PS-C I (immobilized on ceramic), Amano lipase PS-IM (immobilized on diatomaceous earth) and Amano lipase M from *M. javanicus* (≥10,000 U g^{-1} enzyme activity, pH 7.0, 40 °C) were purchased from Sigma-Aldrich (Steinheim, Germany), Lipase from *hog pancreas* (2.4 U mg^{-1}, 1 U is the amount of immobilized enzyme which forms 1% octyl laurate from 0.5 mmol lauric acid and 1.0 mmol 1-octanol in 10 ml water-saturated isooctane in 1 h at 20 °C) was purchased from Fluka (Switzerland)., Lipozyme®, immobilized from *Mucor miehei* (42 U g^{-1}, 1 U corresponds to the amount of enzyme which liberates 1 mol oleic acid at pH 8.0 and 40 °C per minute), D-Aminoacylase from *Escherichia. coli* (10,000 U mg^{-1}, 1 U is defined as enzyme quantity which produces 1 μmol of D-Amino acid per 30 min). All solvents were analytical grade and were dried by storing over activated 3 Å molecular sieves for 24 h prior to use. All other reagents were analytical grade and used as received.

1.2. General Procedure.

The ^{1}H NMR spectra were recorded with TMS as internal standard using a Bruker AMX-400 MHz spectrometer. Chiral HPLC was performed using an OJ-H column or an AD-H column and a UV detector (210 nm or 260 nm). All the known products were characterized by comparing the ^{1}H NMR with those reported in the literature.

1.3. D-aminoacylase-catalyzed Nitroaldol Reaction.

Benzaldehyde (1 mmol) and nitroalkane (2 mmol) were dissolved in 1 ml DMSO and added into a 10 mL conical flask. After D-aminoacylase was added, the mixture was shaken at 200 rpm at 50^{o}C for a period of time. The reaction was terminated by filtering off the enzyme, and then the mixture was washed by water and extracted with ethyl acetate. After the evaporation, the crude product was isolated by silica gel column chromatography with an eluent consisting of petrol ether/ethyl acetate (6/1 or 9/1 v/v).

The results of nitroaldol reactions were listed in Table 1.

Table 1 The Results of Nitroaldol Reaction catalyzed by D-aminoacylase

Entry	Compound	R	Reaction time (h)	Yield (%)[b]
1	**2a**	*p*-NO_2	0.5	81
2	**2b**	*m*-NO_2	0.5	83
3	**2c**	*o*-NO_2	0.5	85
4	**2d**	*p*-Cl	2	62
5	**2e**	*m*-Cl	5	60
6	**2f**	*o*-Cl	5	51
7	**2g**	H	6	33
8	**2h**	*p*-CH_3	6	21
9	**2i**	*p*-OCH_3	3.5	12

[a] Reaction condition: 1 mmol aldehyde, 2 mmol nitroalkane, 1 ml DMSO, 40 mg DA, 50 ^{o}C.
[b] Isolated yield.

1.4. Procedure for the Preparation of Racemic Acylation β-nitroalcohols Standards.

Racemic β-nitroalcohol (200 mg) and acetyl chloride (5 mL) were added to a 25 mL balloon flask. For β-nitroalcohols bearing nitro groups, the mixture was stirred at reflux temperature and reacted overnight. For other β-nitroalcohols, the mixture was stirred at ambient temperature with TLC monitoring for 2 or more hours. The products were obtained by evaporation of the acetyl chloride, without further purification.

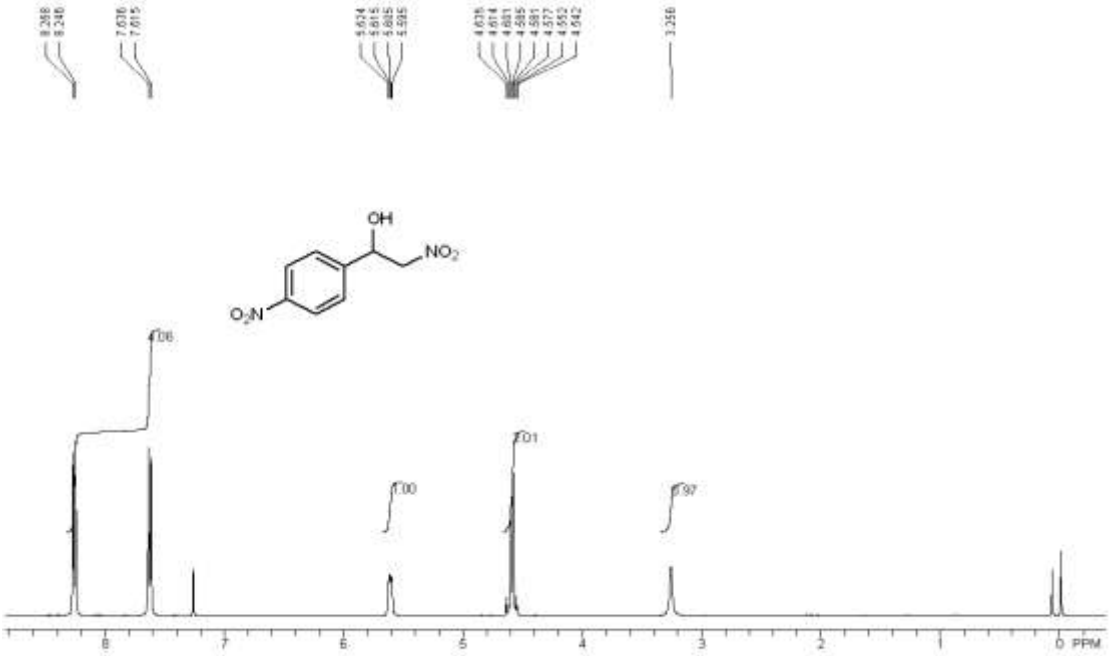
1.5. Lipase PS-IM-catalyzed Kinetic Resolution

To a solution of racemic β-nitroalcohols (rac-**2a–i**, 0.1 mmol), in 0.5 mL toluene, 50 mg lipase PS-IM was added. The reaction mixture was shaken at 200 rpm at 25^{o}C for 12 h. For chiral LC analysis, after 1 mL ethyl acetate and 2 mL ethanol were added into the mixture, 100 μL diluted mixture was sampled and then diluted to 1 mL with ethanol.

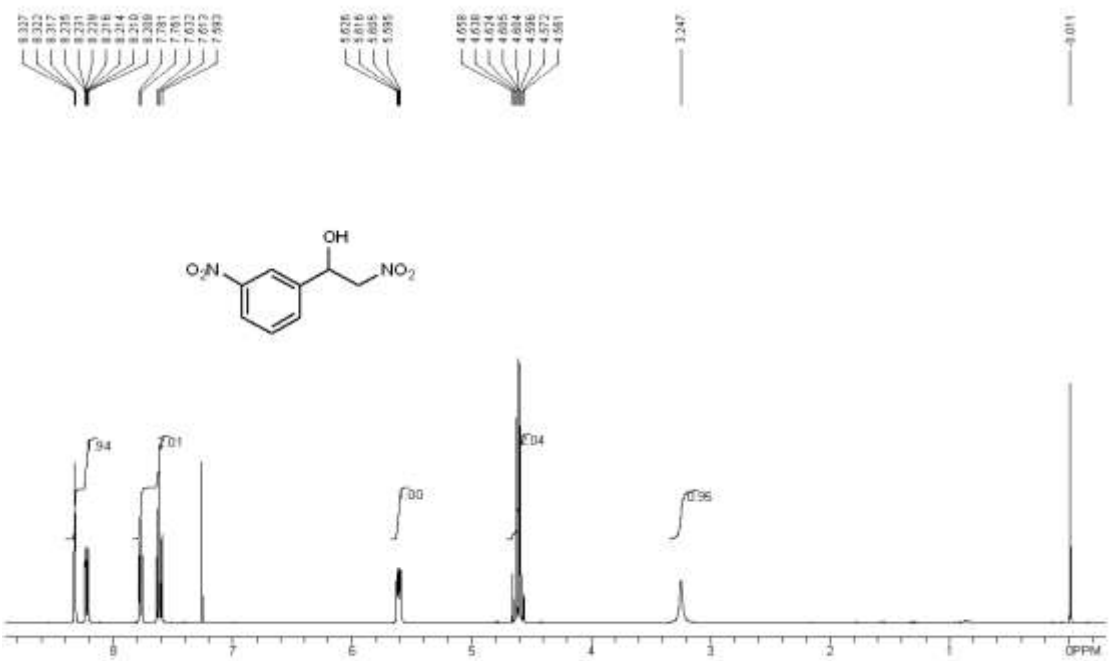
2. Data of ^{1}H NMR ($CDCl_3$, δ , ppm, 400 MHz)

2.1. ^{1}H NMR of β-Nitroalcohols

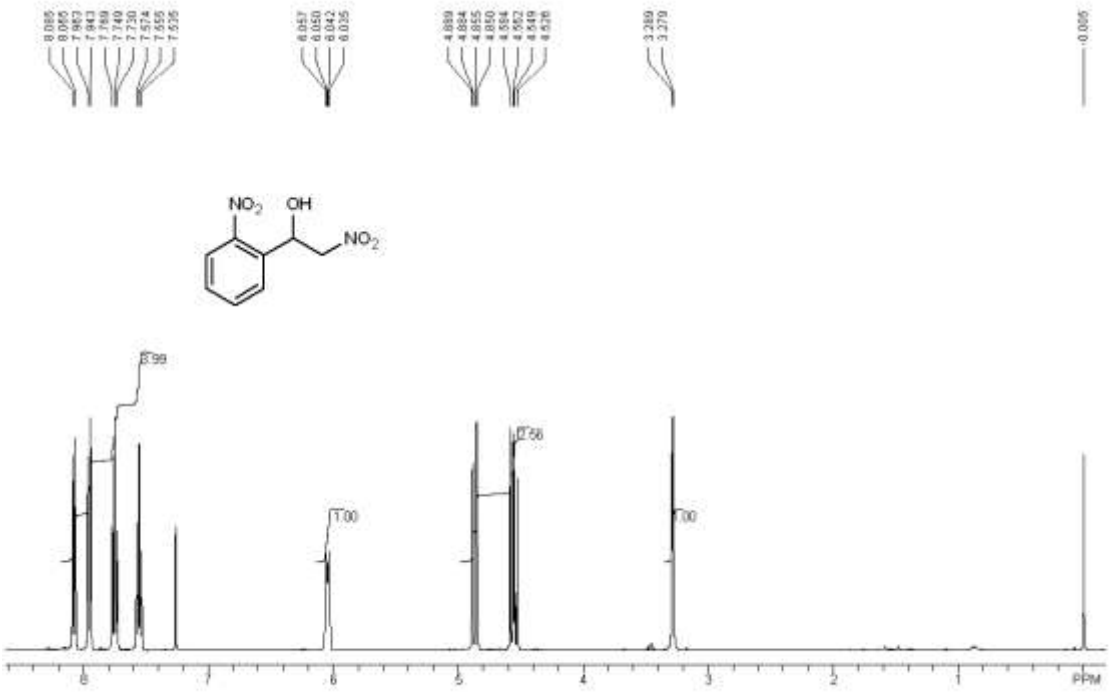
2-nitro-1-(4-nitrophenyl)ethanol (**2a**)



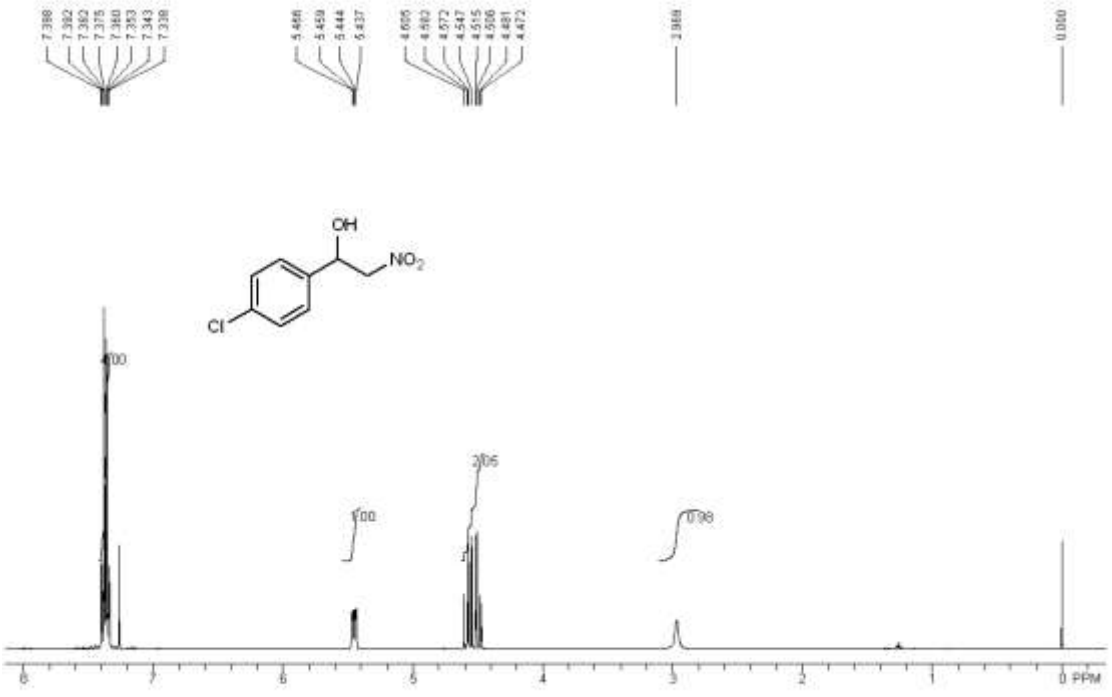
2-nitro-1-(3-nitrophenyl)ethanol (**2b**)



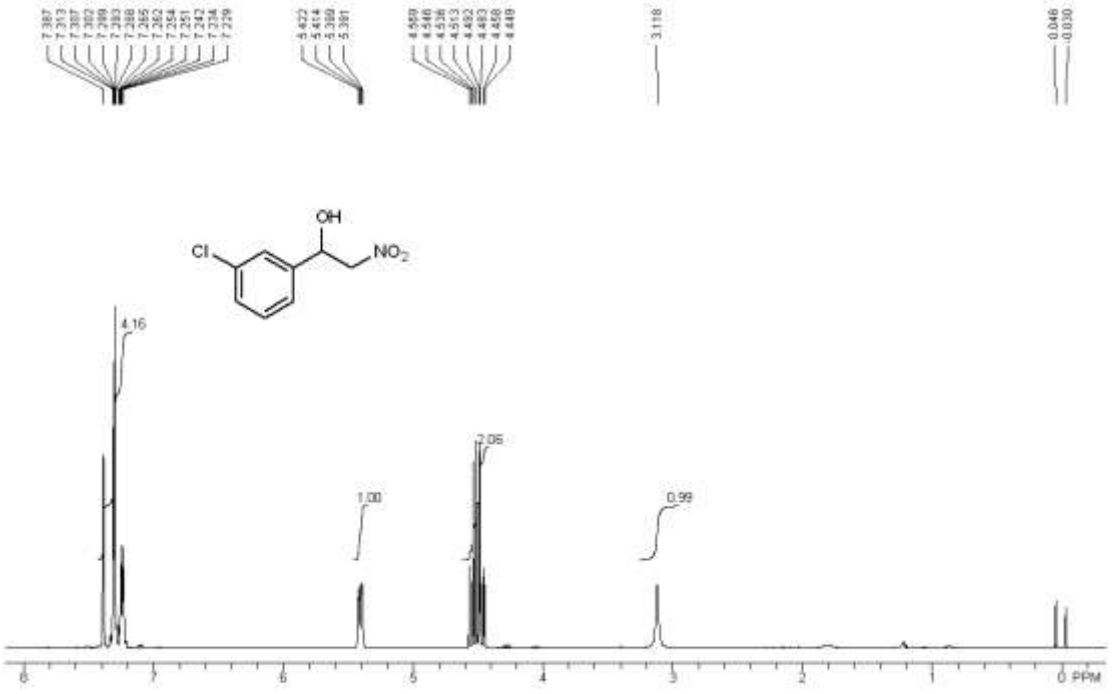
2-nitro-1-(2-nitrophenyl)ethanol (**2c**)



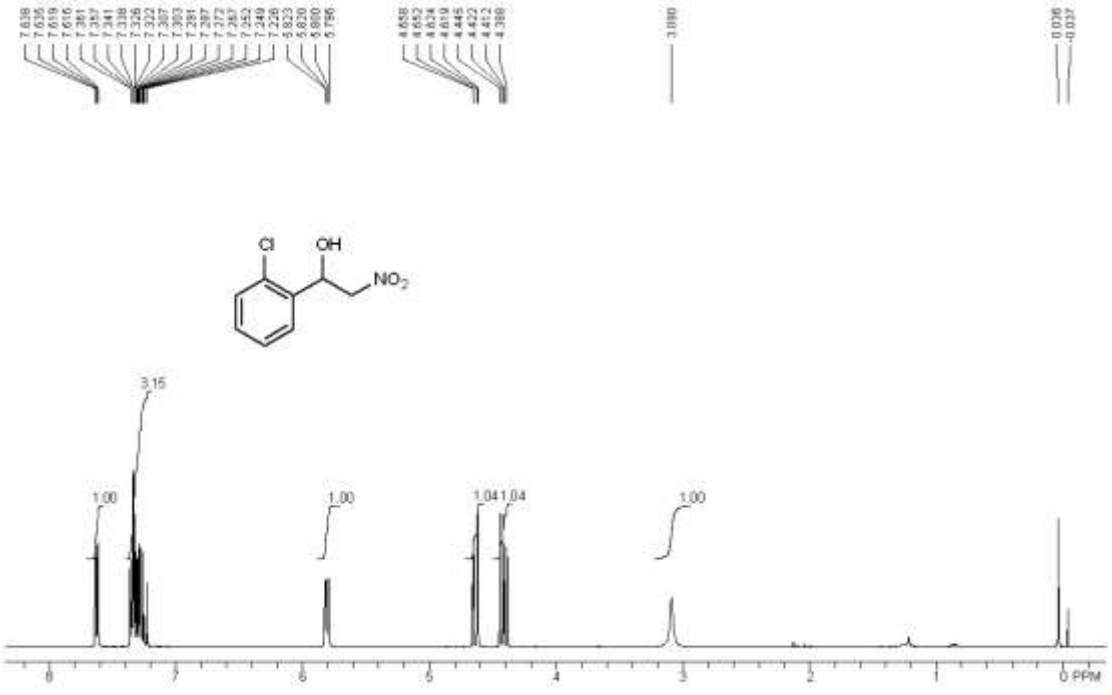
1-(4-chlorophenyl)-2-nitroethanol (**2d**)



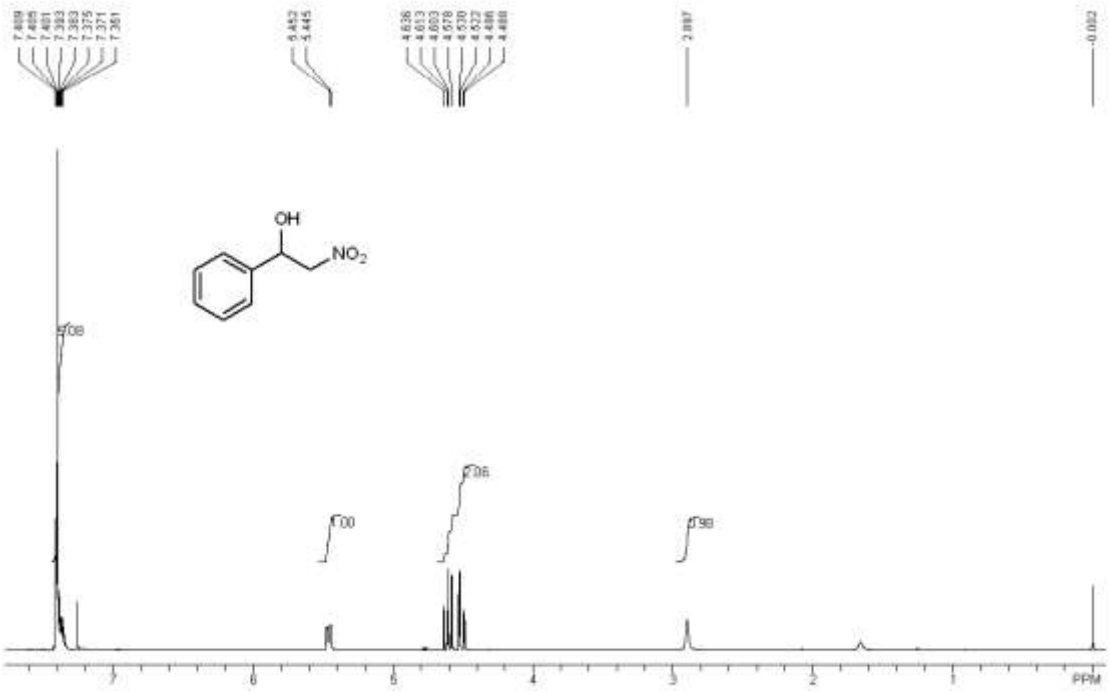
1-(3-chlorophenyl)-2-nitroethanol (**2e**)



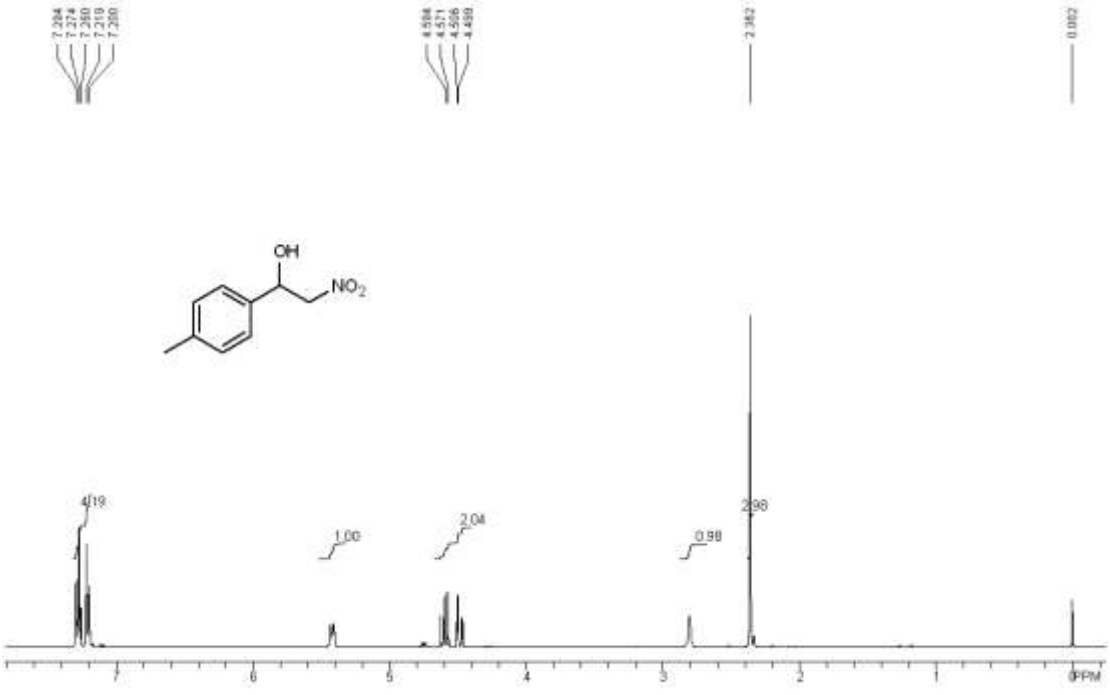
1-(2-chlorophenyl)-2-nitroethanol (**2f**)



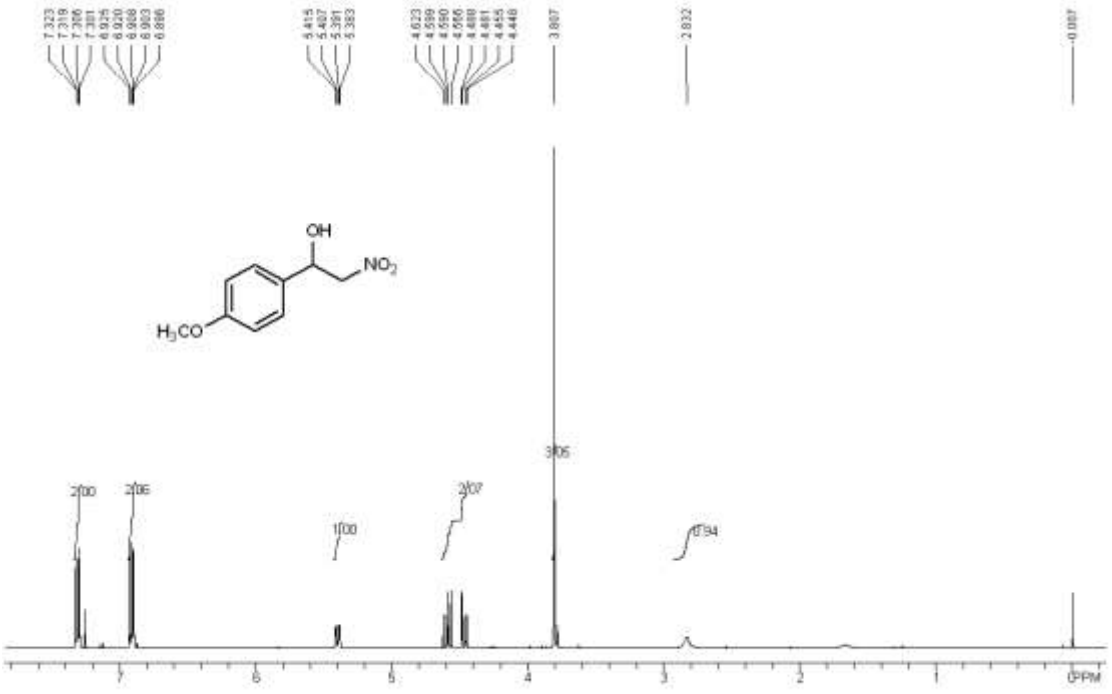
2-nitro-1-phenylethanol (**2g**)



2-nitro-1-(p-tolyl)ethanol (**2h**)

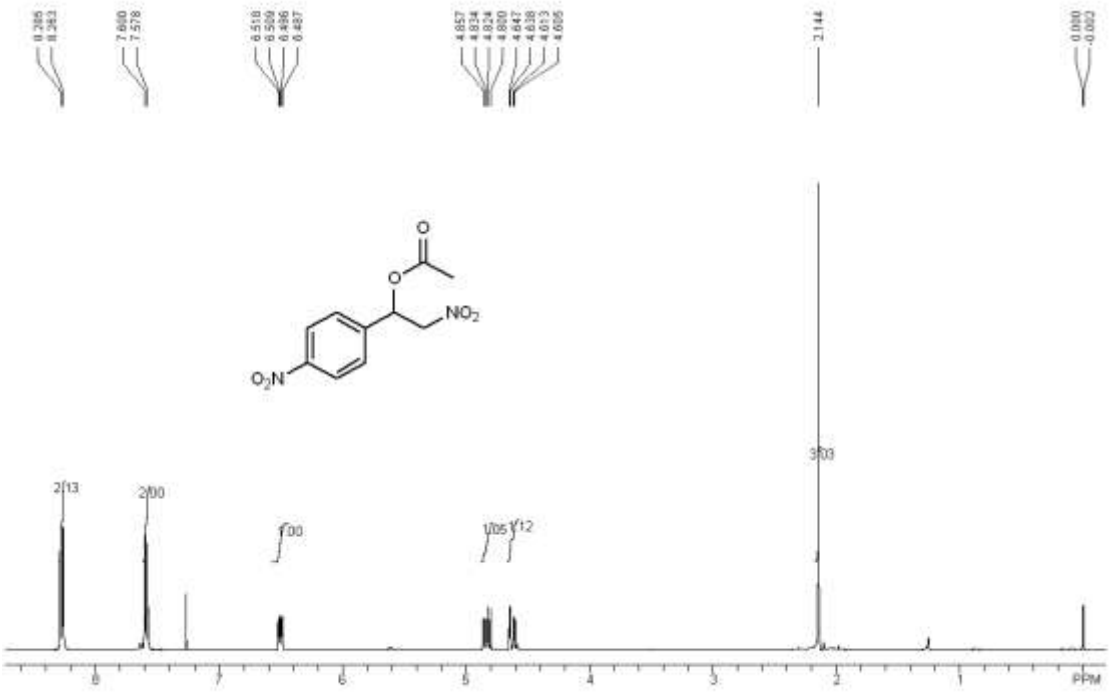


1-(4-methoxyphenyl)-2-nitroethanol (**2i**)

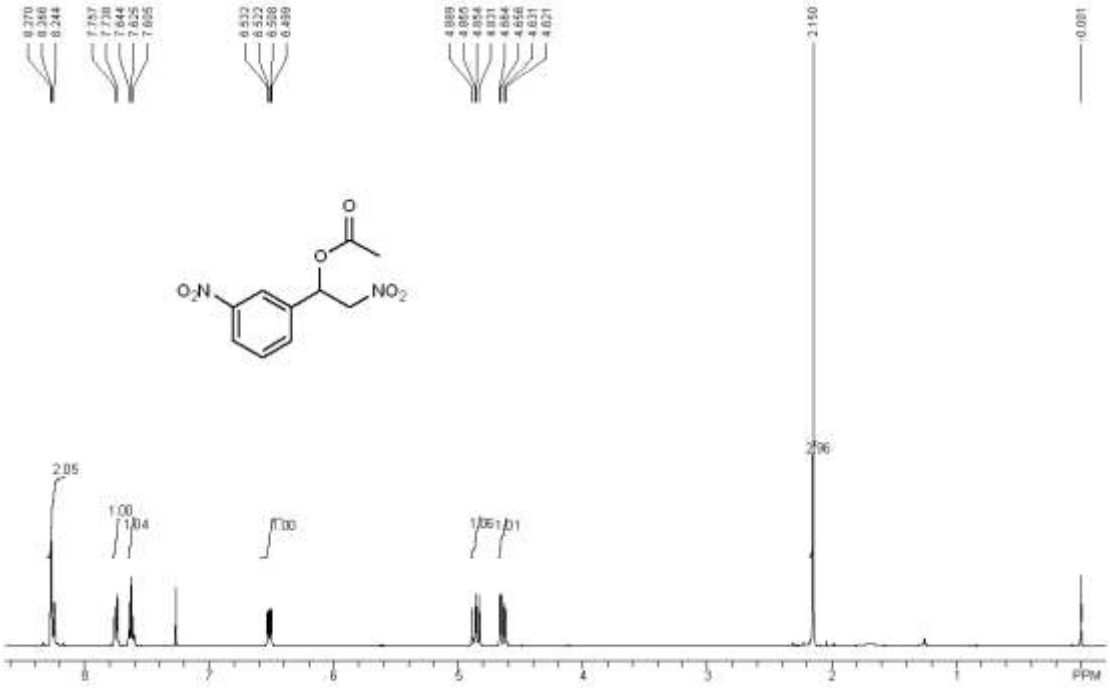


2.2 NMR of acylated β-Nitroalcohols

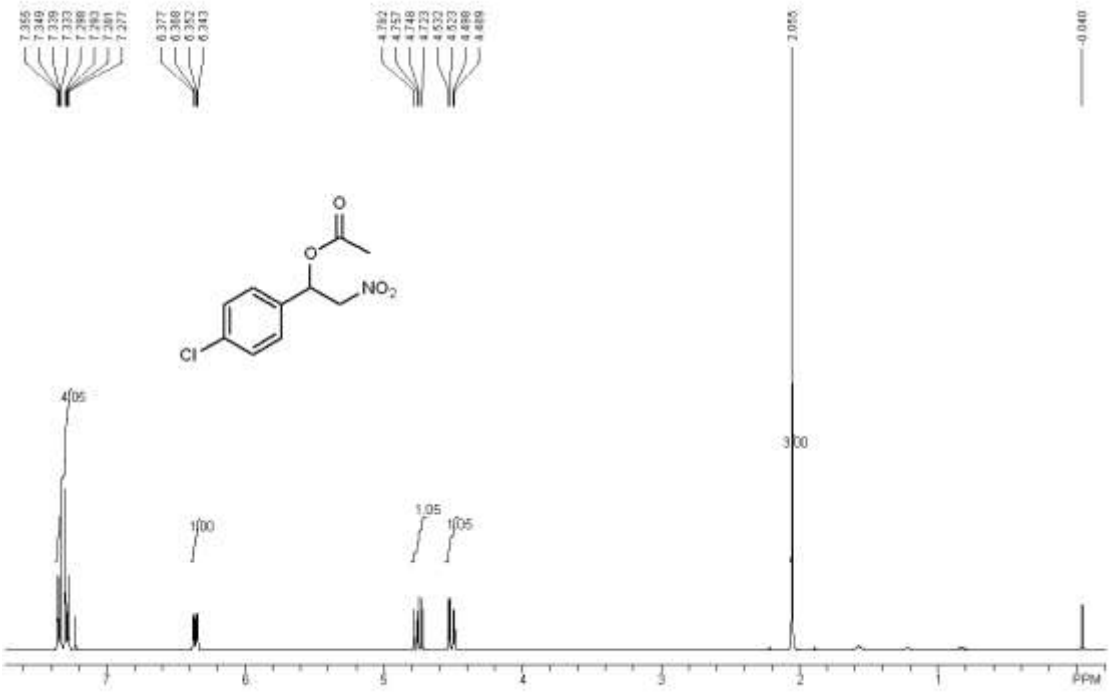
2-nitro-1-(4-nitrophenyl)ethyl acetate (**3a**)



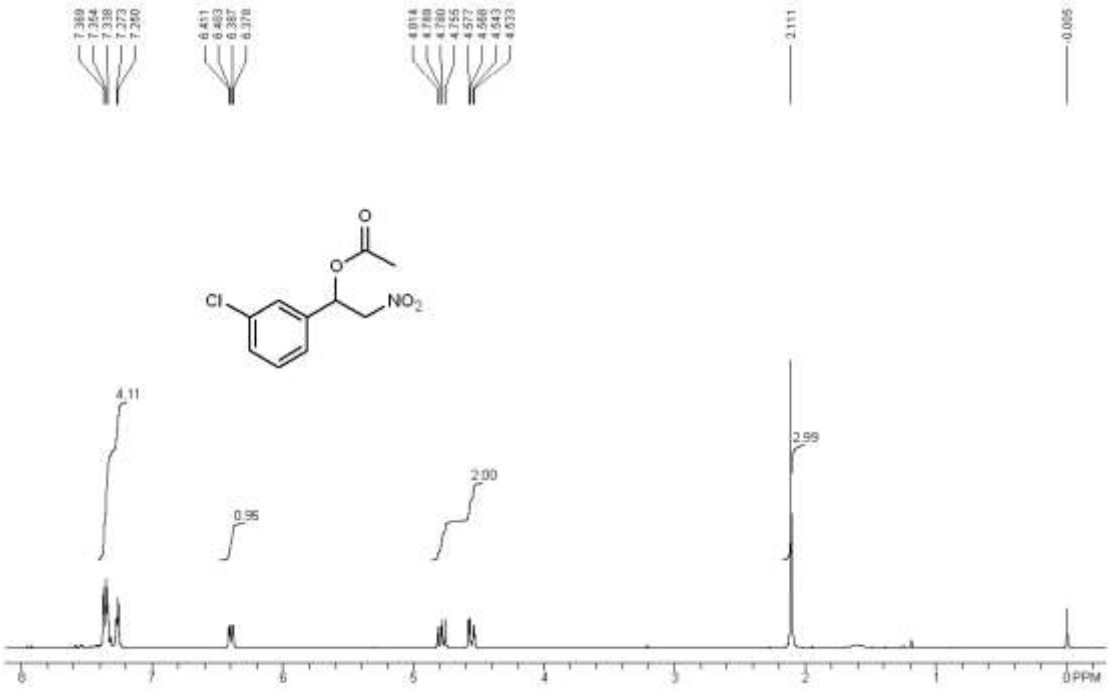
2-nitro-1-(3-nitrophenyl)ethyl acetate (**3b**)



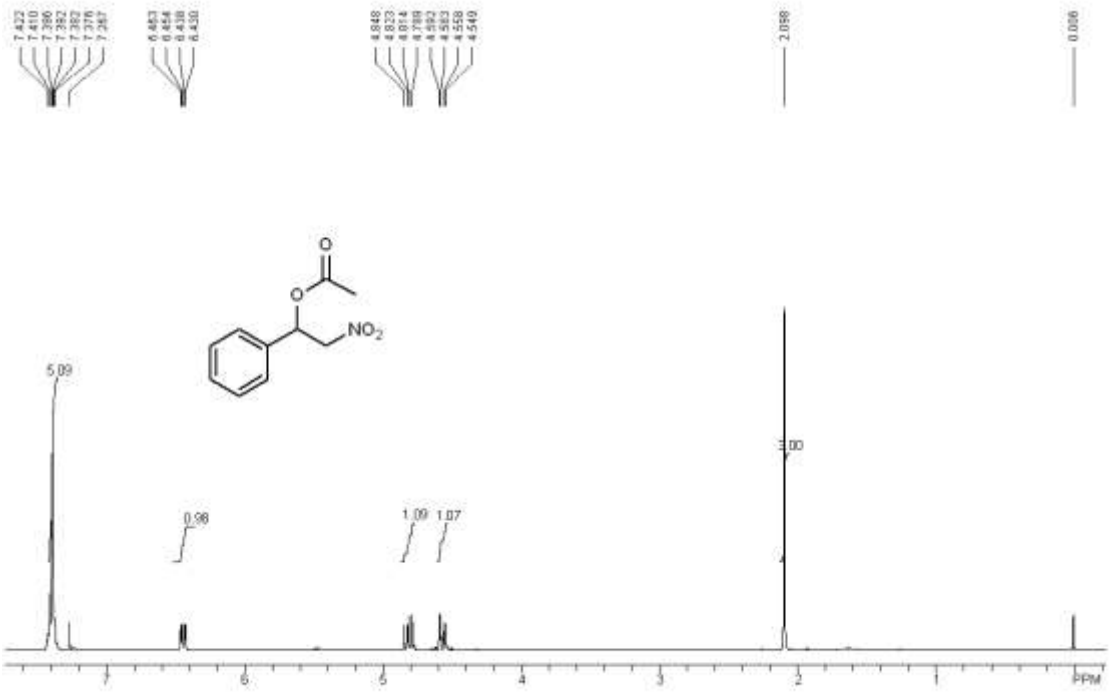
1-(4-chlorophenyl)-2-nitroethyl acetate (**3d**)



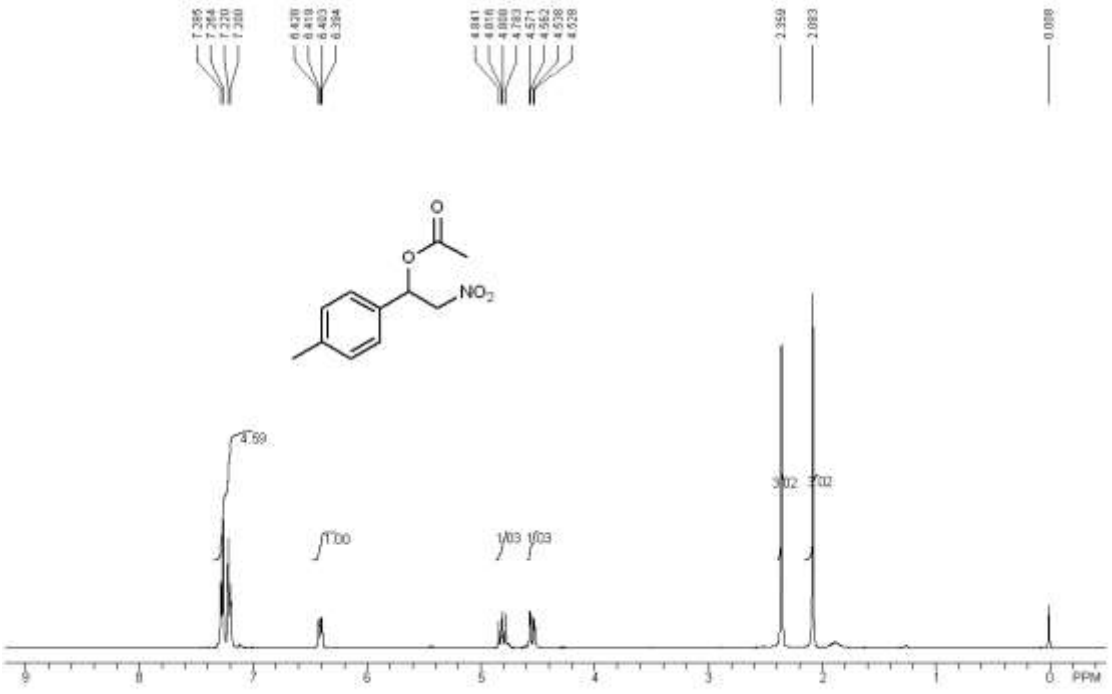
1-(3-chlorophenyl)-2-nitroethyl acetate (**3e**)



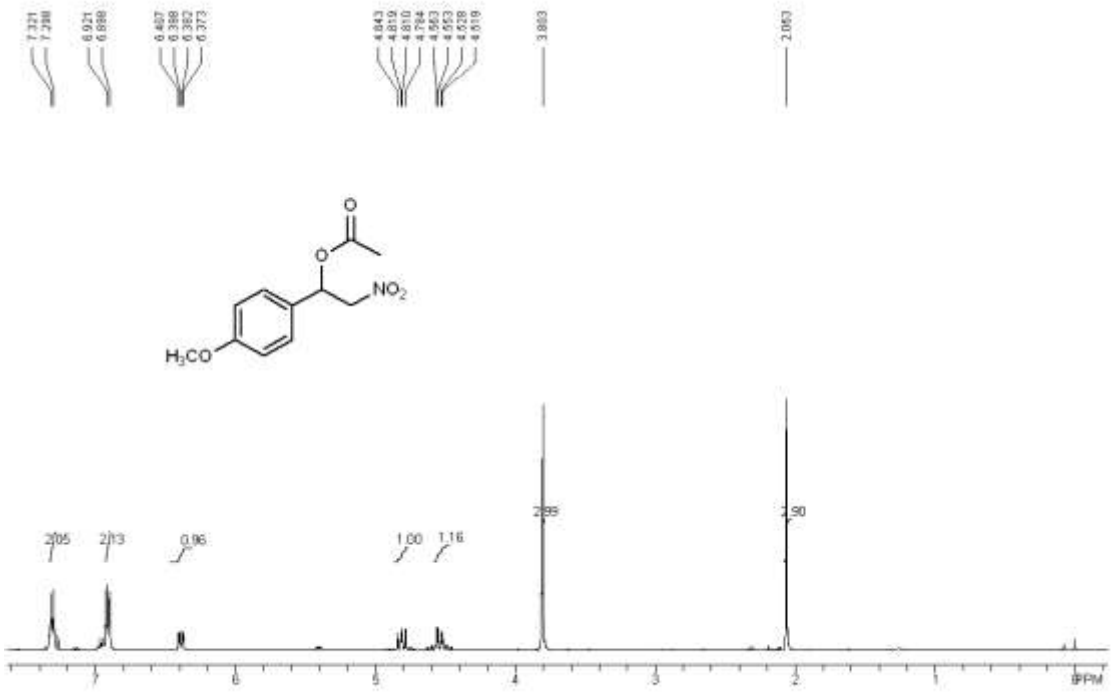
2-nitro-1-phenylethyl acetate (**3g**)



2-nitro-1-(p-tolyl)ethyl acetate (**3h**)



1-(4-methoxyphenyl)-2-nitroethyl acetate (**3i**)



3. Data of Chiral-LC.

Kinetic Resolution of **2a**

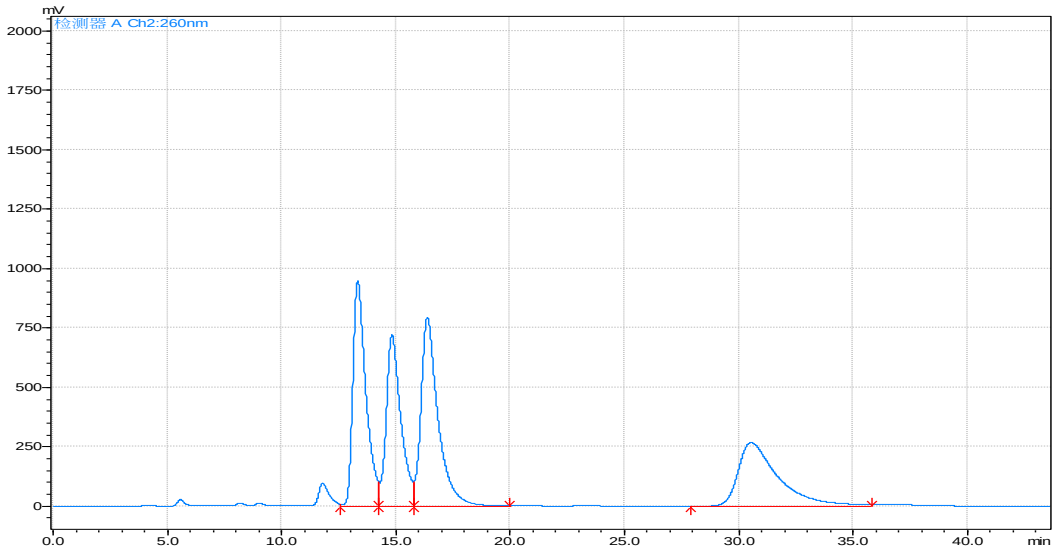
Column: AD-H; Eluant: Hexane/Ethanol=71/29, 0.8 ml/min; UV detector: 260 nm

RT:

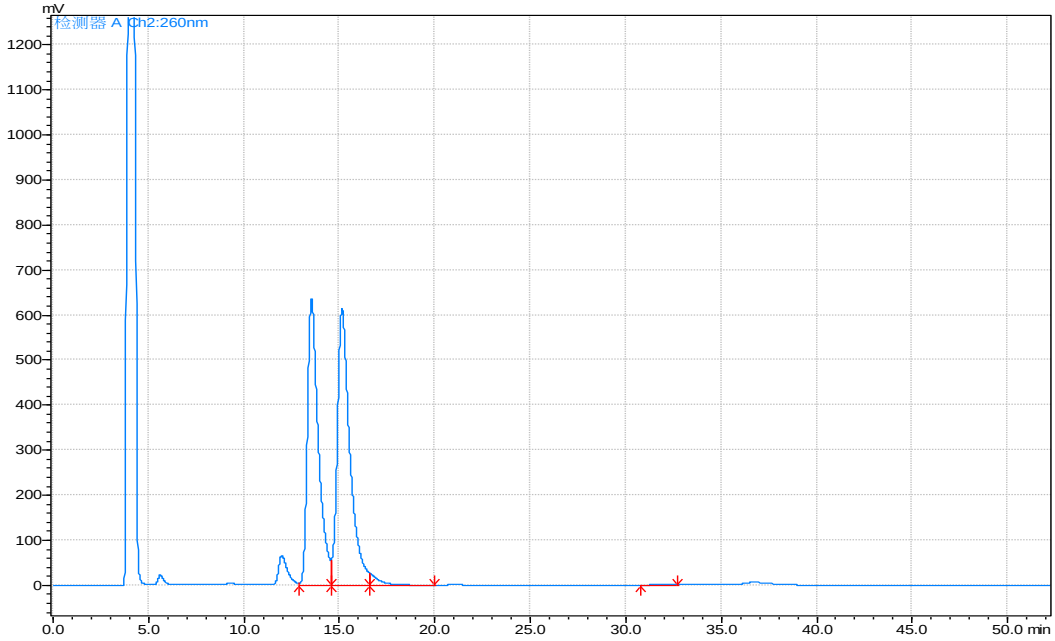
(S)-Substrate: 13.33 min; (R)-Substrate: 16.37 min

(S)-Product: 30.54 min; (R)-Product: 14.82 min

Standard:



Reaction mixture:



Kinetic Resolution of **2b**

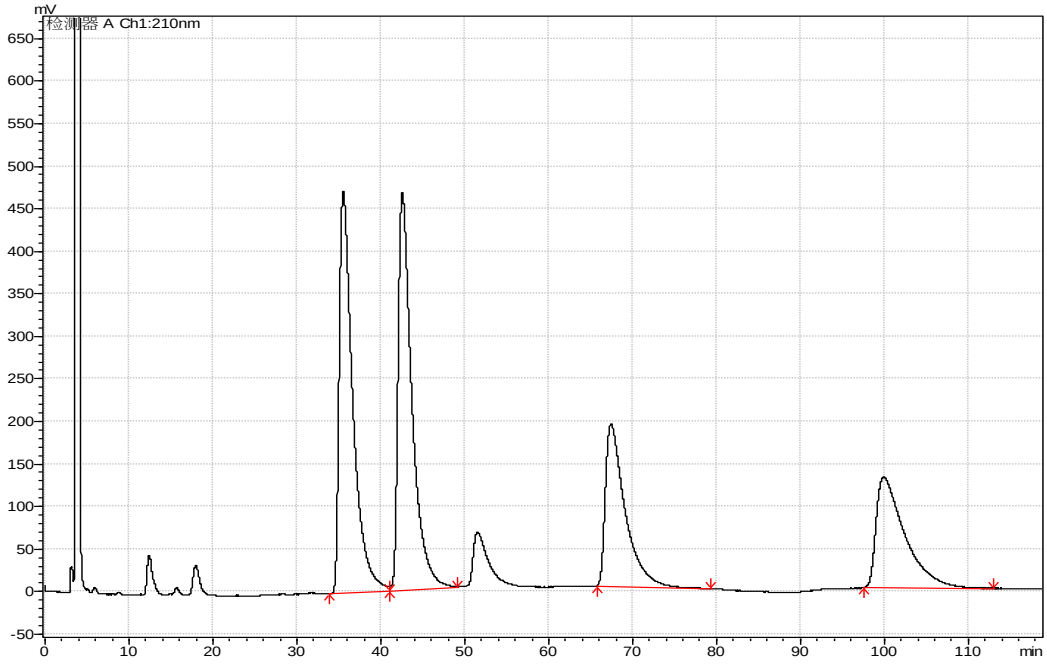
Column: OJ-H; Eluant: Hexane/Ethanol=81/19, 1.0 ml/min; UV detector: 210 nm

RT:

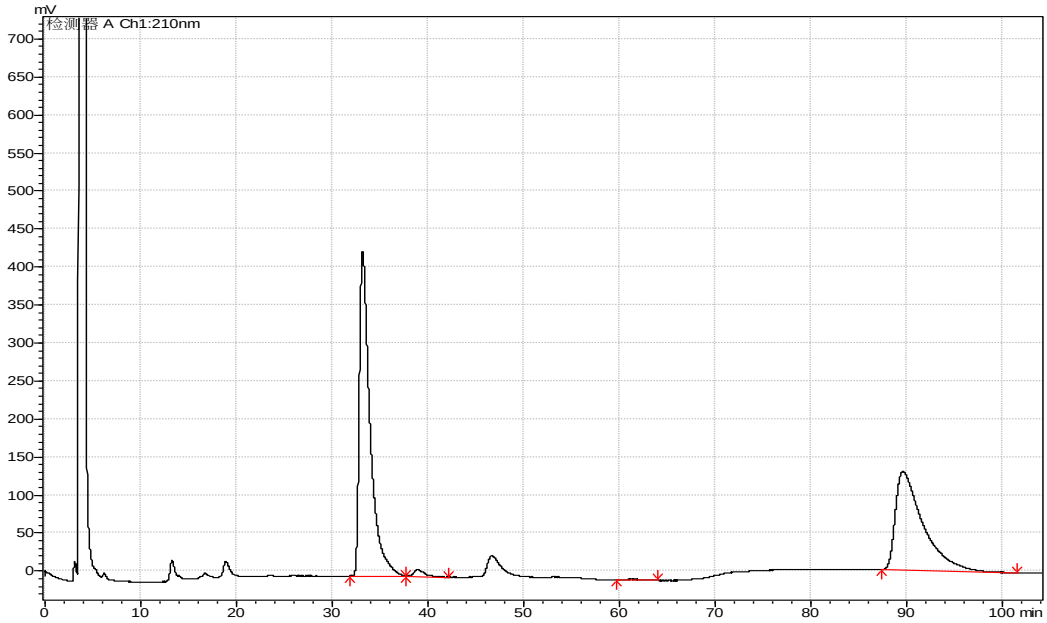
(S)-Substrate: 35.52 min; (R)-Substrate: 42.56 min

(S)-Product: 67.42 min; (R)-Product: 99.89 min

Standard:



Reaction mixture:



Kinetic Resolution of **2d**

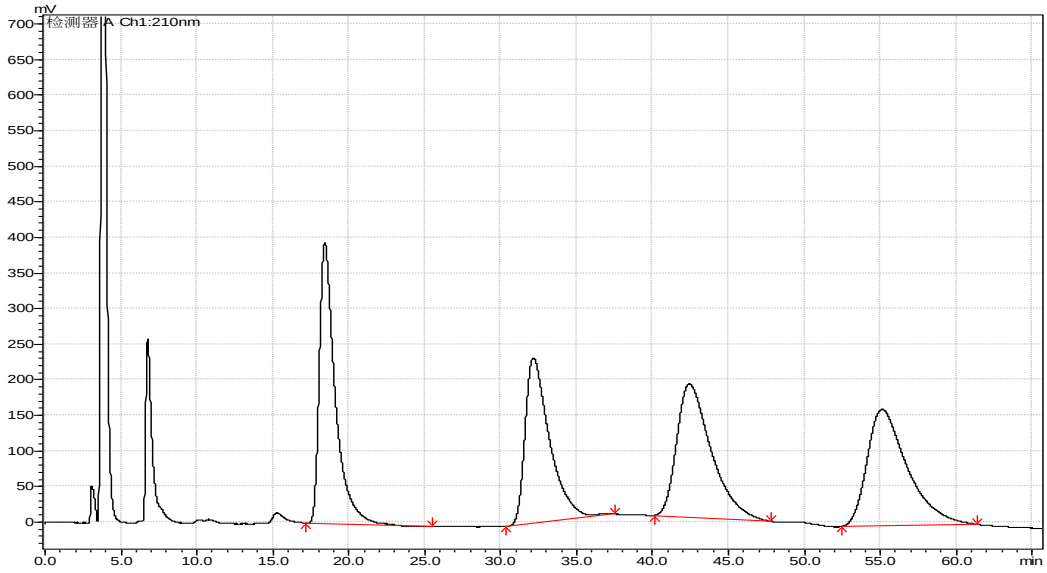
Column: AD-H; Eluant: Hexane/Ethanol=95/5, 1.0 ml/min; UV detector: 210 nm

RT:

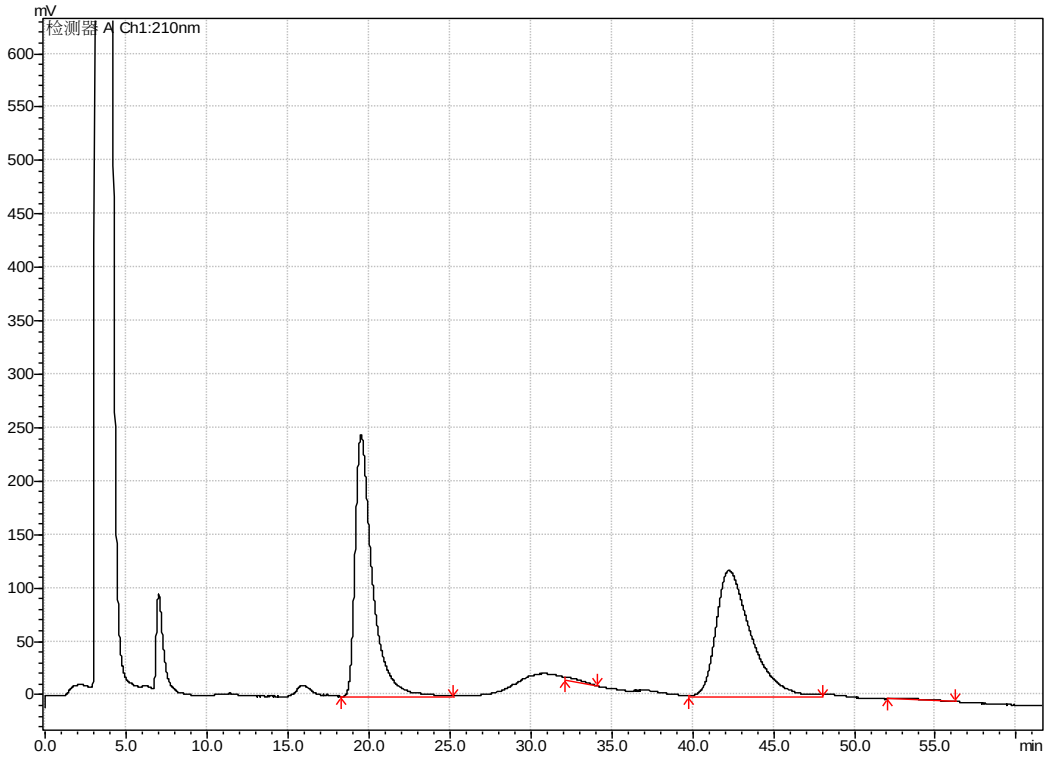
(S)-Substrate: 18.41 min; (R)-Substrate: 32.13 min

(S)-Product: 55.08 min; (R)-Product: 42.41 min

Standard:



Reaction Mixture:



Kinetic Resolution of **2e**

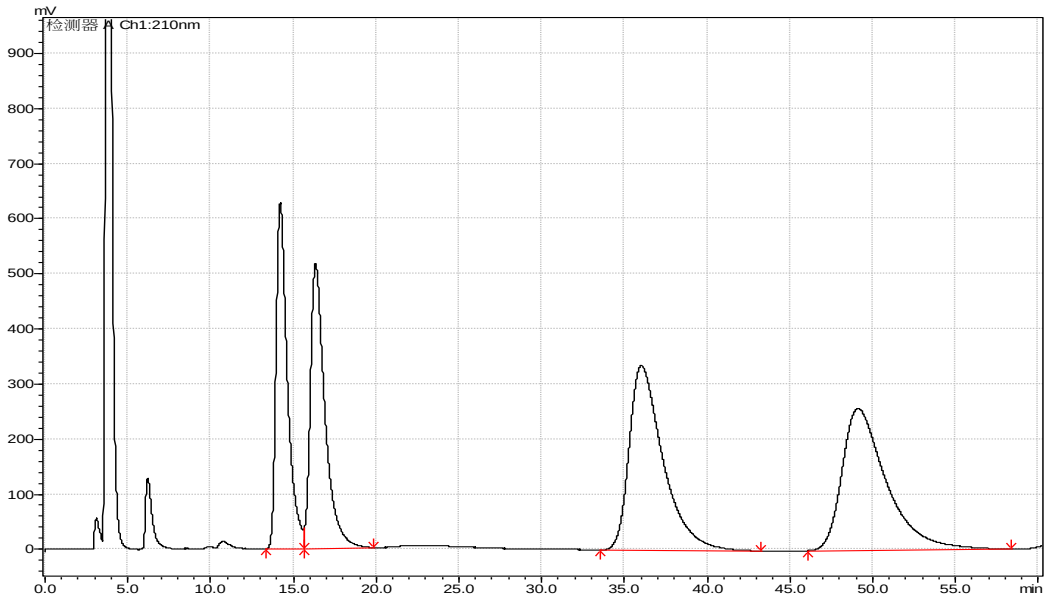
Column: AD-H; Eluant: Hexane/Ethanol=95/5, 1.0 ml/min; UV detector: 210 nm

RT:

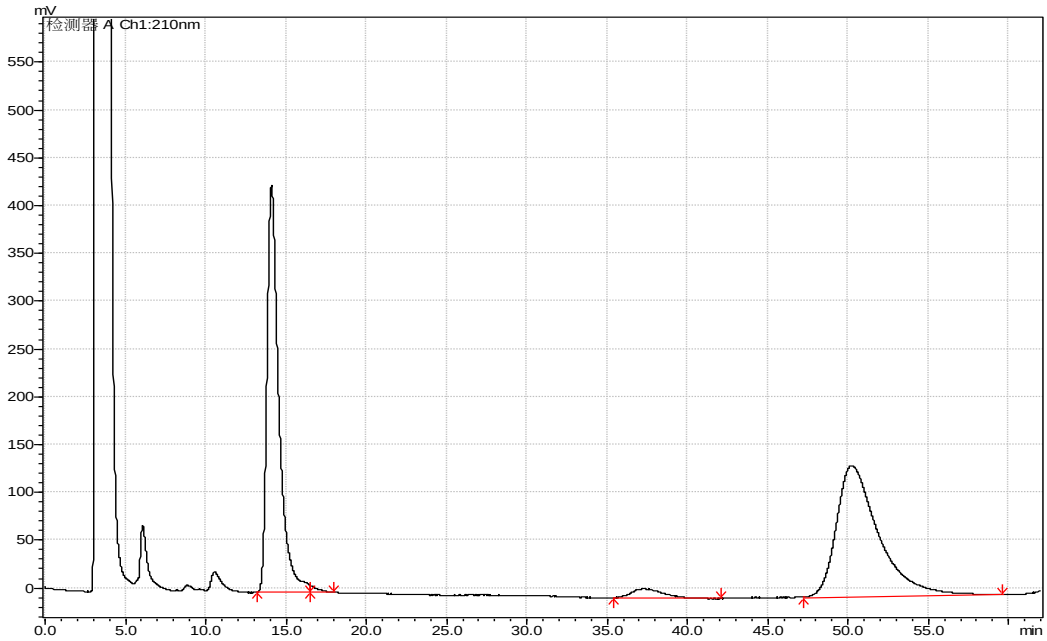
(S)-Substrate: 14.20 min; (R)-Substrate: 16.34 min

(S)-Product: 36.01 min; (R)-Product: 49.11 min

Standard:



Reaction Mixture:



Kinetic Resolution of **2g**

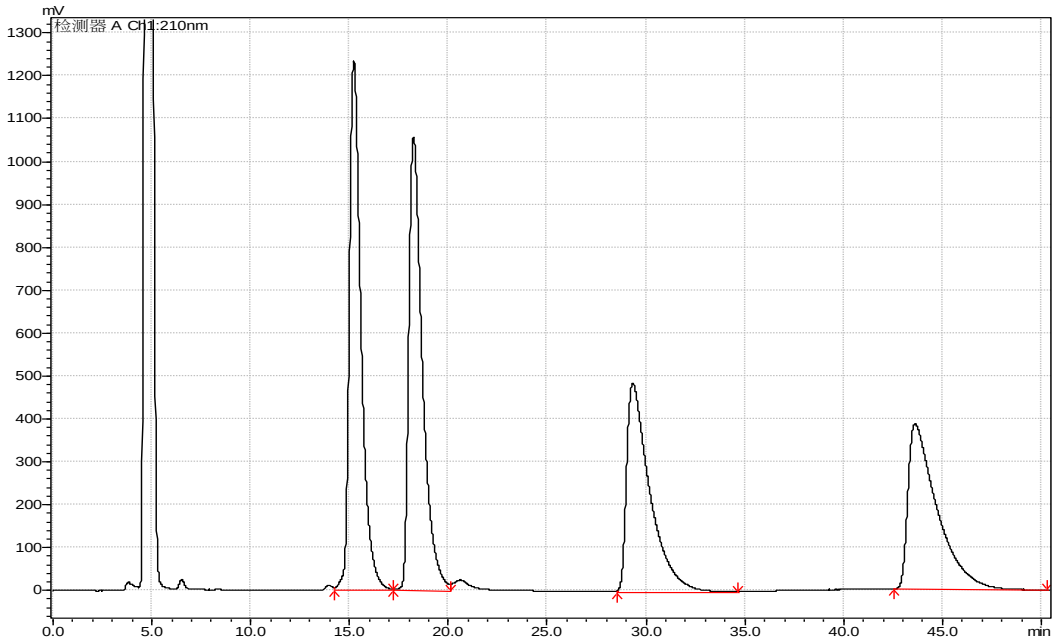
Column: OJ-H; Eluant: Hexane/Ethanol=80/20, 0.8 ml/min; UV detector: 210 nm

RT:

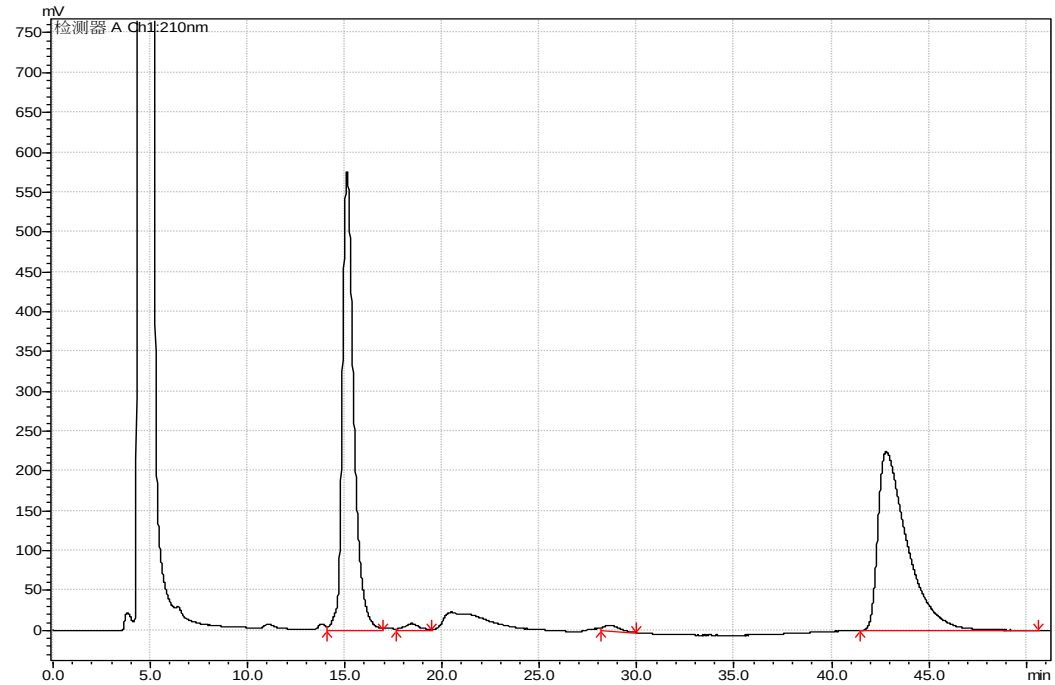
(S)-Substrate: 15.20 min; (R)-Substrate: 18.23 min

(S)-Product: 29.32 min; (R)-Product: 43.60 min

Standard:



Reaction Mixture:



Kinetic Resolution of **2h**

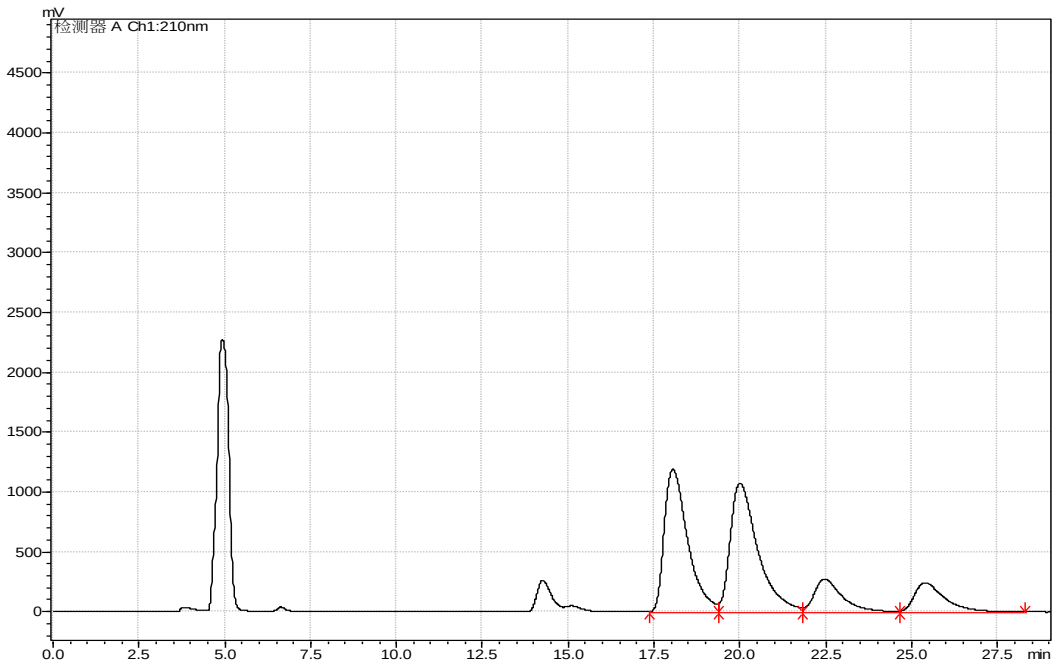
Column: OJ-H; Eluant: Hexane/Ethanol=87/13, 1.0 ml/min; UV detector: 210 nm

RT:

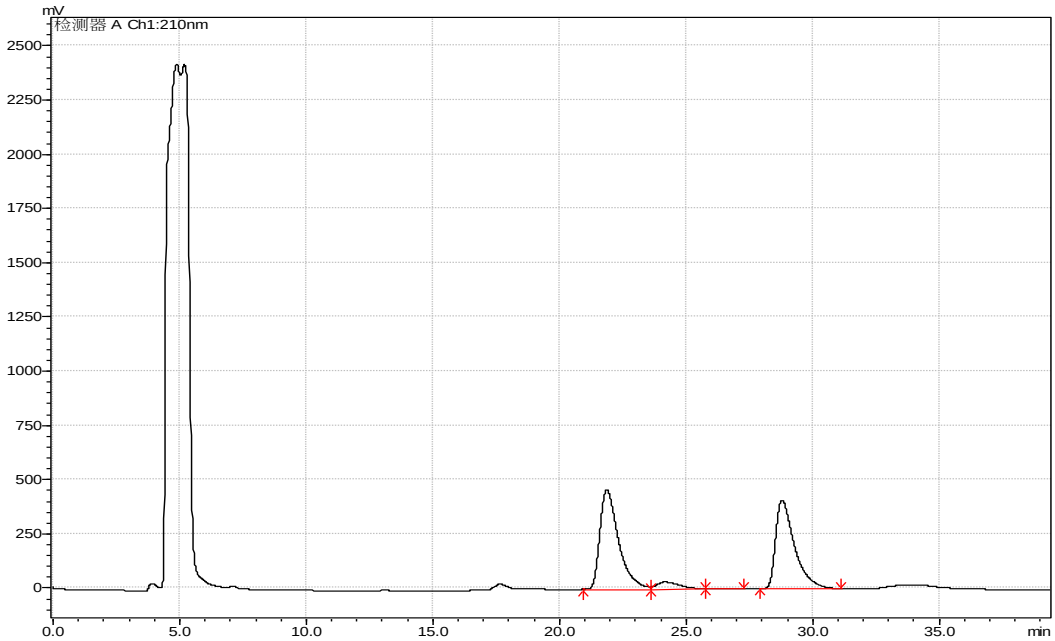
(S)-Substrate: 18.11 min; (R)-Substrate: 20.23 min

(S)-Product: 22.50 min; (R)-Product: 25.48 min

Standard:



Reaction Mixture:



Kinetic Resolution of **2i**

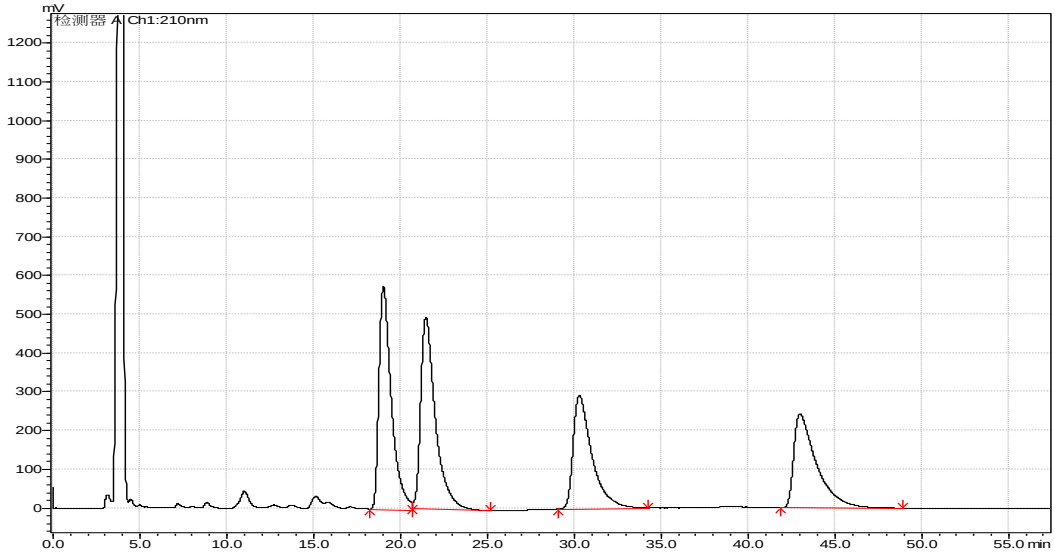
Column: OJ-H; Eluant: Hexane/Ethanol=80/20, 1.0 ml/min; UV detector: 210 nm

RT:

(S)-Substrate: 19.00 min; (R)-Substrate: 21.45 min

(S)-Product: 30.28 min; (R)-Product: 42.99 min

Standard:



Reaction Mixture: