Support information for

Enantioselective Synthesis of α-Functionalized Phenylpyrrolidine *via* Photo-enzymatic Cascade Multi-Component Reaction

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Materials and methods

Materials.

All the chemicals and reagents were purchased from commercial suppliers (Sigma- Aldrich, Bide Pharmatech Ltd., Aladdin, Energy Chemical, TCI, Shanghai Chemical Reagent Company) and used without any further purification, unless otherwise stated. All photocatalytic reactions were carried out in flame-dried sealed borosilicate glass tubes with magnetic stirring. The light sources are a green LED (30 W, 530-540 nm) a blue LED (30 W, 450 - 455 nm) and a White CFL purchased from Jia Deng, model: CT, made in China. The material of the irradiation vessel: borosilicate glass. Test tubes are placed in the test tube rack as shown in the photos below. Horseradish peroxidase, Myoglobin from Equine skeletal muscle, Hemoglobin from bovine, rabbit and human blood, Cytochrome C from horse, porcine and bovine heart was purchased from Shanghai Yuan Ye Biological Technology Company. Ni-NTA Superflow resin obtained from Beijing Solarbio Science & Technology Co., Ltd. *E. coli* BL21(DE3) Competent Cell, Spin Miniprep, and Gel Extraction Kits were all obtained from Tiangen. Silica gel chromatography purifications were carried out using AMD Silica Gel 60 230-400 mesh. Thin Layer Chromatography (TLC) and preparative TLC were carried out using Merck Millipore TLC silica gel 60 F254 glass plates. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a 400 MHz spectrometer in $CDCl_3$. Chemical shifts for protons are reported in parts per million downfield from tetramethylsilane (TMS) and are referenced to residual protium in the NMR solvent ($CHCl_3$ = δ 7.26 ppm). Chemical shifts for carbon are reported in parts per million downfield from tetramethylsilane (TMS). NMR data are presented as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant in Hertz (Hz), integration. The experiments were performed triplicate, and all data were obtained based on the average values.

Cloning and mutagenesis.

Plasmid pET20b was used as a cloning and expression vector for all constructs described in this study. FRISM libraries were performed using primers containing degenerate codons, PCR was performed using *Transtart@ FastPfu* DNA polymerase (TransGen Biotech) and the resulting PCR products were digested with DpnI (NEB). Then gel was purified (Omega), repaired (Vazyme) and the product was directly transformed to *E. coli* BL21(DE3) by incubating at 42℃. Following transformation, cells were recovered for 45 min at 37℃ in Luria-Bertani (LB) medium, aliquots were plated on LB agar plates supplemented with 100 μg/mL ampicillin (LB-Amp plates), and plates were incubated at 37℃ overnight. For FRISM libraries, 20 colonies were picked to be cultured and screened.

Protein expression and purification.

Single colonies were used to inoculate 5 mL of LB-Amp medium, followed by incubation at 37°C with shaking (180 rpm) for 10 to 15 hours. For expression of VHb, the overnight cultures were transferred to 1 L LB-Amp medium, followed by incubation at 37°C with shaking (180 rpm). At an OD_{600} of 1.5, cells were induced by its anaerobic promoter in an anaerobic environment and incubated at 25°C with shaking (110 rpm) for 30 hours. Cell cultures were harvested by centrifugation at 5000 rpm. The overall pelleted bacteria were dissolved in 50 mL of 20 mM

phosphate buffer (pH 7.4, 20 mM). After sonication for 30 min on ice, the cell lysates were centrifuged at 12,000 rpm for 30 min. For purification, the lysate was transferred to a Ni-NTA column equilibrated with Ni-NTA Lysis Buffer. The resin was washed with 50 mL of Ni-NTA Lysis Buffer and then 50 mL of Ni-NTA Wash Buffer (20 mM phosphate buffer, 20 mM imidazole, pH = 7.4). Proteins were eluted with Ni-NTA Elution Buffer (20 mM phosphate buffer, 250 mM imidazole, pH = 7.4). After elution from the Ni-NTA column, the protein was loaded into a 5ml G-25 desalting column to remove imidazole. The concentration of the protein was tested by NanoDrop.

Small-scale reaction screening of FRISM library based on SD-VHb using whole cell catalysts.

Small-scale photo-biocatalytic were set up in a quartz test tube containing a stirring bar with bromobenzene (**1**, 0.005 mmol), aliphatic secondary amines (**2**, 0.0075 mmol), $NiBr_2$ (5 mol%), DABCO (0.0075 mmol, 1.5 equiv.) and Ir $(ppy)_3$ (3 mol% in DMSO (100 μL). Next, the test tube was sealed with septa, and an N_2-atmosphere was incorporated through an N_2-filled balloon via syringe. The resulting mixture was stirred under blue LED irradiation (450 nm) for 12 h at ambient temperature (ca. 25 - 30 °C). Afterward, the reaction conditions were changed to the biocatalytic mode by diluting the residue with 50 mM PBS buffer (the total volume 900 μL.) containing **3a** (0.010 mmol), $Na_2S_2O_4$ (2 equiv., 0.010 mmol), SD-VHb variants expressing cells at OD_{600} = 20 (10μM) was stirred (500 rpm) for 12 h under positive argon pressure at room temperature. Typically, the reaction solution final concentrations, depending on the substrates tested, typically ranged from 0.5 to 2 equiv. **3a**. Following addition of substrates, the tubes were shaken at room temperature, 500 rpm for 12 – 24 h. For sample work-up for HPLC analysis the reactions were quenched by adding extractive solvent (*n*-hexane: ethyl acetate = 4:1, 400 μL/tube) supplemented. The sample solutions were incubated for 30 min, transferred to 1.5 mL tubes and centrifuged at 13,000×g for 10 minutes. The cleared supernatant was transferred to clean 2 mL vials and analyzed by HPLC.

Preparative scale procedure for enzymatic synthesis of products 4 (0.1 mmol scale, Procedure A):

A quartz test tube containing a stirring bar was charged with a solution of racemic bromobenzene (**1**, 0.1 mmol), aliphatic secondary amines (**2**, 0.15 mmol), $NiBr_2$ (5 mol%), DABCO (0.15 mmol, 1.5 equiv.) and Ir $(ppy)_3$ (3 mol%) in DMSO (200 μL). Next, the test tube was sealed with septa, and an N_2-atmosphere was incorporated through an N_2-filled balloon via syringe. The resulting mixture was stirred under blue LED irradiation (450 nm) for 12 h at ambient temperature (ca. 25–30 °C). Afterward, the reaction conditions were changed to the biocatalytic mode by diluting the residue with 50 mM PBS buffer (1500 μL, pH 7.4), diazo ester (0.15 mmol). The resulting mixture (1700 μL) containing the **Int 1** was added to SD-VHb_{CH} preincubated in 50 mM PBS buffer (300 μL, pH 7.4, OD_{600} = 20, 10μM). The thus composed reaction mixture was shaken (500 rpm) at 25 °C for 12 h and then stopped by extracting the content of the vial with EtOAc (3 × 5 mL). The organic layers were combined and dried over sodium sulfate, evaporated under reduced pressure, and the residue was purified by column chromatography (hexanes/ethyl ether, 50:1 to 20:1) to afford the product.

Large-scale procedure for enzymatic synthesis of products 4 (2 mmol scale, Procedure B):

A quartz test tube containing a stirring bar was charged with a solution of racemic bromobenzene (**1**, 2 mmol), aliphatic secondary amines (**2**, 3 mmol), $NiBr_2$ (5 mol%), DABCO (3 mmol, 1.5 equiv.) and Ir $(ppy)_3$ (3 mol%) in DMSO (2 mL). Next, the test tube was sealed with septa, and an N_2-atmosphere was incorporated through an N_2-filled balloon via syringe. The resulting mixture was stirred under blue LED irradiation (450 nm) for 12 h at ambient temperature (ca. 25–30 °C). Afterward, the reaction conditions were changed to the biocatalytic mode by diluting the residue with 50 mM PBS buffer (15 mL, pH 7.4), diazo ester (3 mmol). The resulting mixture (17 mL) containing the **Int 1** was added to SD-VHb_{CH} preincubated in 50 mM PBS buffer (3 mL, pH 7.4, OD_{600} = 20, 20μM). The thus composed reaction mixture was shaken (500 rpm) at 25 °C for 12 h and then stopped by extracting the content of the vial with EtOAc (3 × 5 mL). The organic layers were combined and dried over sodium sulfate, evaporated under reduced pressure, and the residue was purified by column chromatography (hexanes/ethyl ether, 50:1 to 20:1) to afford the product.

Molecular Docking Analysis.

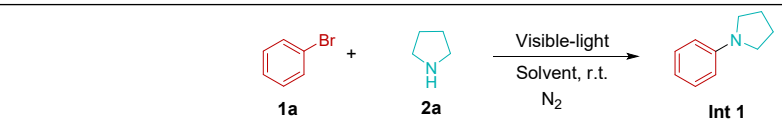
The initial structure of VHb was taken from PDB code of 3tm3, structures of SD-VHb_{CH} was obtained by SWISS-MODEL[1] using homology modeling strategy. Benzofuran was docked into the active site of WT-VHb and SD-VHb_{Tric} using AutoDock Vina tool in Chimera respectively [2, 3].

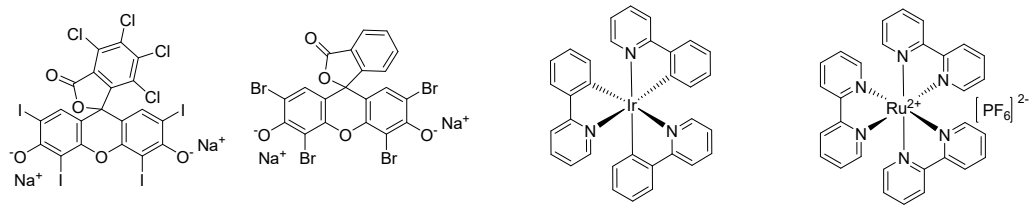
Molecular Dynamics simulation

To further investigate the interaction and stability of the complex, molecular dynamics (MD) simulations were performed using GROMACS software to simulate 100 ns of the protein-ligand complex. The AMBER99SB-ILDN force field was used to generate the protein topology file, and the Amber20 software with the GAFF force field was utilized to generate the small molecule ligand topology file. A truncated octahedral TIP3P water box was added with a cutoff distance of 10 nm, and Na/Cl ions were introduced to neutralize the system's charge. Energy minimization was performed using 2500 steps of steepest descent and 2500 steps of conjugate gradient methods. The system was then subjected to a 100 ps NVT ensemble simulation followed by a 100 ps NPT equilibration simulation at a temperature of 368.15 K. Finally, a 100 ns MD ensemble simulation was conducted under periodic boundary conditions. Long-range electrostatic interactions were calculated using the PME method, with a non-bonded cutoff distance of 1 nm, a collision frequency of 2 ps, a pressure of 101.325 kPa, and an integration step of 2 ps, with trajectories saved every 10 ps.

Supporting Experimental Tables and Figures

Table S1. Optimization of the photocatalysis reaction conditions [a]





Entry	Photocatalysts (mol%)	Co-Catalysts	Light source	Solvent	Yield (%)
1	PC-1 (3)	$NiBr_2$ (5 mol%)	Blue LED [b]	DMSO	trace
2	PC-2 (3)	$NiBr_2$ (5 mol%)	Blue LED [b]	DMSO	trace
3	PC-3 (3)	$NiBr_2$ (5 mol%)	Blue LED [b]	DMSO	94
4	PC-4 (3)	$NiBr_2$ (5 mol%)	Blue LED [b]	DMSO	32
5	PC-3 (3)	-	Blue LED [b]	DMSO	ND [c]
6	PC-3 (3)	$NiBr_2$ (5 mol%)	Blue LED [b]	CH_3CN	67
7	PC-3 (3)	$NiBr_2$ (5 mol%)	Blue LED [b]	H_2O	trace
8	PC-3 (3)	$NiBr_2$ (5 mol%)	-	DMSO	ND
9	PC-3 (3)	$NiCl_2$ (5 mol%)	Blue LED [b]	DMSO	68
10	PC-3 (3)	$NiBr_2$ (5 mol%)	Green LED [b]	DMSO	71
11	PC-3 (3)	$NiBr_2$ (5 mol%)	White CFL [b]	DMSO	54
12	PC-3 (3)	$NiBr_2$ (5 mol%) [d]	Blue LED [b]	DMSO	49

a. Reaction condition: **1a** (0.005 mmol), **2a** (0.0075 mmol), solvents (100 μL), PC, Co-Catalysts, DACBO (1.5 equiv.), room temperature, 12 h, stir; b. 30 W green LED, 530–540 nm or 30 W blue LED, 450 – 455 nm or 30 W white CFL; c. No detected. d. without DACBO.

Table S2. Screening of FRISM library based on SD-VHb.

Mutations	Yield of **4a** (%)	e.r. of **4a**	Mutations	Yield of **4a** (%)	e.r. of **4a**
VHb_{WT}	21	64:36	VHb (F28T, P54S)	58	86:14
VHb (F28A)	19	71:29	VHb (F28T, P54F)	47	66:34
VHb (Y29A)	27	52:48	VHb (F28T, L57A)	71	76:24
VHb (F43A)	16	60:40	VHb (F28T, L57V)	54	95:5
VHb (P54A)	29	57:43	VHb (F28T, L57S)	57	69:31
VHb(L57A)	24	68:32	VHb (F28T, L57F)	63	80:20
VHb (F28V)	31	81:19	VHbTV (P54I)	58	84:16
VHb (F28S)	28	84:16	VHbTV (P54V)	67	88:12

VHb (F28T)	23	88:12	VHbTV (P54S)	85	92:8
VHb (F28R)	11	77:23	VHbTV (P54C)	56	85:15
VHb (F28N)	35	68:32	VHbTV (P54D)	82	99:1
VHb (F28D)	36	62:38	VHbTV (P54T)	71	94:6
VHb (F28T, P54A)	37	77:23	VHbTV (P54N)	69	90:10
VHb (F28T, P54V)	51	71:29	VHbTV (P54R)	58	93:7

Table S3. Optimization of the biocatalytic reaction conditions [a]

Entry	Catalysts (μM/OD_{600})	**3a** (equiv.)	Reaction time (h)	Yield of **4a** /e.r.
1	Whole (10/20)	2	12	82%/99:1
2	Whole (10/20)	1	12	67%/99:1
3	Whole (10/20)	1.5	12	79%/99:1
4	Whole (10/20)	2.5	12	83%/99:1
5	Whole (5/10)	1.5	12	59%/99:1
6	Whole (2/4)	1.5	24	39%/99:1
7	Whole (1/2)	1.5	24	29%/99:1
8	Whole (20/40)	1.5	12	86%/99:1

Table S4. Energy contribution of amino acid residues.

WT		**MU**	
No.	ΔG(Kcal/mol)	No.	ΔG(Kcal/mol)
Phe28	-0.01074	Thr28	-0.8182
Tyr29	0.003903	Tyr29	-0.88295
Leu32	-0.18701	Leu32	-0.34362
Phe43	0.001742	Phe43	-0.43995
Leu57	-0.00048	Val57	-1.10702
Ala58	-0.02445	Ala58	-0.52679
Val98	-0.08383	Val98	-1.32123

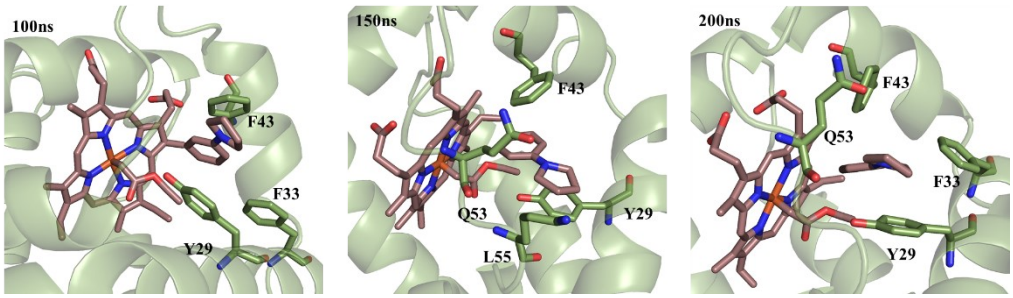


Figure S1. Snapshots of VHb_{WT} molecular dynamics simulation in different time periods.

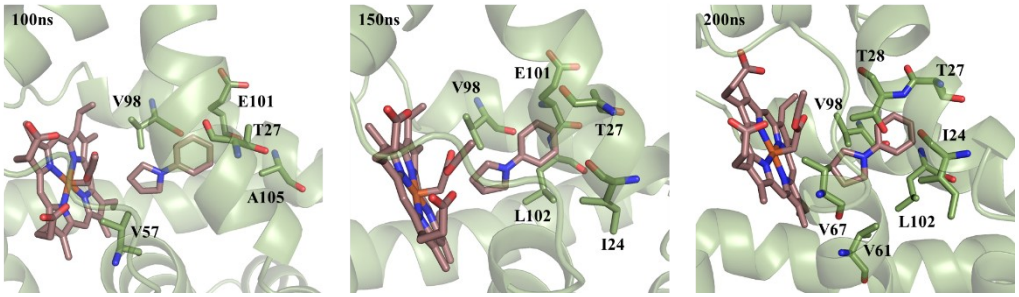


Figure S2. Snapshots of VHb_{CH} molecular dynamics simulation in different time periods.

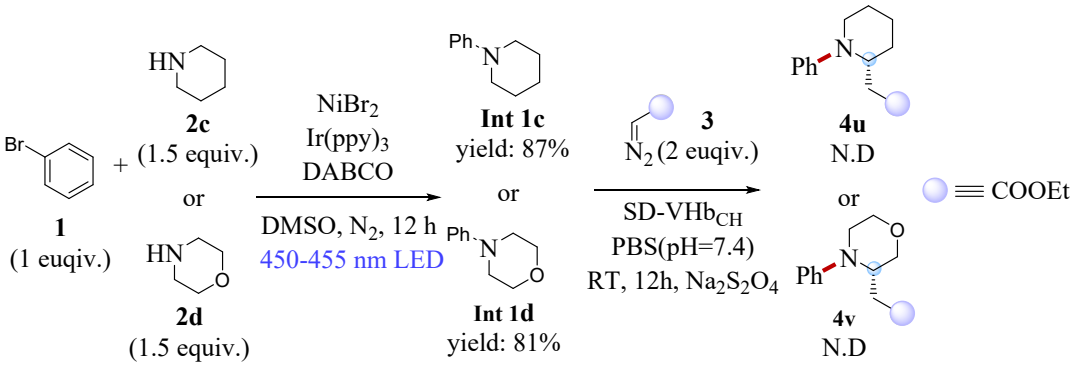


Figure S3. Unsuccessful substrates.

Sequence of primers and variants:

Table S5. Primers of PCR reactions

Primer	Sequence
28AVSF-F	CCACGACTKYCTATAAAAACTTGTTTGCCA
28AVSF-R	TTTATAAGRMGTCGTGGTAATGGTAACGCCAT
28N/D-F	CCACGACTRACTATAAAAACTTGTTTGCCAAACACC
28N/D-R	TTTATAAGTYGTCGTGGTAATGGTAACGCCATG
28T/R-F	CCACGACTASATATAAAAACTTGTTTGCCAAACACCC
28T/R-R	GCATGGCGTTACCATTACCACGACTSTTTATAAA
29AVSF-F	CGACTTTTKYCAAAAACTTGTTTGCCAAACACCC
29AVSF-R	AGTTTTTGRMAAAAGTCGTGGTAATGGTAACGCC
43AVSF-F	TCCTTTGKYCGATATGGGTCGCCAAGAATC
43AVSF-R	CCATATCGRMCAAAGGACGTACTTCAGGGT
54AVSF-F	GAGCAGKYCAAGGCTTTGGCGATGACGGTA
54AVSF-R	AGCCTTGRMCTGCTCCAAAGATTCTTGGCG
57AVSF-F	TAAGGCTKYCGCGATGACGGTATTGGCGGCA
57AVSF-R	CATCGCGRMAGCCTTAGGCTGCTCCAAAGATTC
57I/L-F	CTAAGGCTMTAGCGATGACGGTATTGGCGGCA
57I/L-R	CATCGCTAKAGCCTTAGGCTGCTCCAAAGATTC
57C-F	CTAAGGCTTGCGCGATGACGGTATTGG
57C-R	CATCGCGCAAGCCTTAGGCTGCTCCAAAGATT
54N/D-F	GAGCAGRACAAGGCTTTGGCGATGACGGTA

54N/D-R	AGCCTTGTYCTGCTCCAAAGATTCTTGGCG
54T/R-F	GAGCAGASAAAGGCTTTGGCGATGACGGTA
54T/R-R	AGCCTTTSTCTGCTCCAAAGATTCTTGGCG

Nucleotide Sequence of SD-VHb$_{CH}$

ATGAAAGCGACCAAACTGGTGCTGGGCGCGGTGATTCTGGGCAGCACCCTGCTGGCG
GGCTGCAGCAGCAACGCGAAAATTGATCAGAACAACAACGGCCCGACCCATGAAAA
CCAGCTGGGCGCGGGCGCGTTTGGCGGCTATCAGGTGAACCCGTATGTGGGCTTTGA
AATGGGCTATGATTGGCTGGGCCGCATGCCGTATAAAGGCAGCGTGGAAAACGGCGC
GTATAAAGCGCAGGGCGTGCAGCTGACCGCGAAACTGGGCTATCCGATTACCGATGA
TCTGGATATTTATACCCGCCTGGGCGGCATGGTGTGGCGCGCGGATACCAAAAGCAA
CGTGTATGGCAAAAACCATGATACCGGCGTGAGCCCGGTGTTTGCGGGCGGCGTGGA
ATATGCGATTACCCCGGAAATTGCGACCGGATCCGGCGGAGGCGGAGGCATGTTAGA
CCAGCAAACCATTAACATCATCAAAGCCACTGTTCCTGTATTGAAGGAGCATGGCGTT
ACCATTACCACGACTACATATAAAAACTTGTTTGCCAAACACCCTGAAGTACGTCCTT
TGTTTGATATGGGTCGCCAAGAATCTTTGGAGCAGGATAAGGCTGTTGCGATGACGG
TATTGGCGCAGGATGCAGCGCAAAACATTGAAAATTTGCCAGCTATTTTGCCTGCGGT
CAAAAAAATTGCAGTCAAACATTGTCAAGCAGGCGTGGCAGCAGCGCATTATCCGAT
TGTCGGTCAAGAATTGTTGGGTGCGATTAAAGAAGTATTGGGCGATGCCGCAACCGA
TGACATTTTGGACGCGTGGGGCAAGGCTTATGGCGTGATTGCAGATGTGTTTATTCAA
GTGGAAGCAGATTTGTACGCTCAAGCGGTTGAACATCATCATCATCATCATTAA

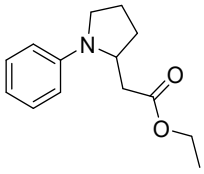
Green: Lpp; yellow: OpmA; grey: 5xGly link; blue: SD-VHb$_{CH}$ (red: mutations)

Amino Acid Sequence of SD-VHb$_{Tric}$

MKATKLVLGAVILGSTLLAGCSSNAKIDQNNNGPTHENQLGAGAFGGYQVNPYVGFEM
GYDWLGRMPYKGSVENGAYKAQGVQLTAKLGYPITDDLDIYTRLGGMVWRADTKSNV
YGKNHDTGVSPVFAGGVEYAITPEIATGSGGGGGMLDQQTINIIKATVPVLKEHGVTITTT
TYKNLFAKHPEVRPLFDMGRQESLEQDKAVAMTVLAAAQNIENLPAILPAVKKIAVKHC
QAGVAAAHYPIVGQELLGAIKEVLGDAATDDILDAWGKAYGVIADVFIQVEADLYAQAV
EHHHHHH*

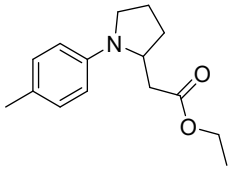
Green: Lpp; yellow: OpmA; grey: 5xGly link; blue: SD-VHb$_{CH}$ (red: mutations)

^{1}H-NMR and HPLC Data [6-7]



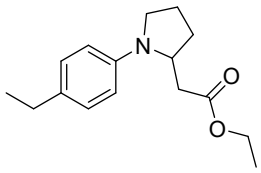
4a

Follow the procedure A, colorless oil, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.32 – 7.22 (m, 2H), 6.73 (t, *J* = 7.3 Hz, 1H), 6.66 (d, *J* = 8.1 Hz, 2H), 4.21 (qd, *J* = 7.1, 1.2 Hz, 3H), 3.54 – 3.36 (m, 1H), 3.22 (q, *J* = 8.6 Hz, 1H), 2.83 (dd, *J* = 15.0, 2.9 Hz, 1H), 2.26 (dd, *J* = 15.0, 10.5 Hz, 1H), 2.14 – 2.04 (m, 3H), 1.97 – 1.89 (m, 1H), 1.33 (td, *J* = 7.2, 1.2 Hz, 3H). HPLC analysis: Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 6.68 min and 10.62 min.



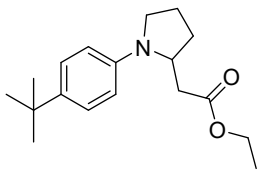
4b

Follow the procedure A, colorless oil, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.14 – 7.04 (m, 2H), 6.61 – 6.55 (m, 2H), 4.25 – 4.12 (m, 3H), 3.48 – 3.38 (m, 1H), 3.19 (q, *J* = 8.6 Hz, 1H), 2.82 (dd, *J* = 14.9, 2.9 Hz, 1H), 2.29 (s, 3H), 2.24 (dd, *J* = 14.9, 10.5 Hz, 1H), 2.13 – 2.02 (m, 3H), 1.95 – 1.89 (m, 1H), 1.32 (t, *J* = 7.2 Hz, 3H). HPLC analysis: Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 5.28 min and 6.85 min.



4c

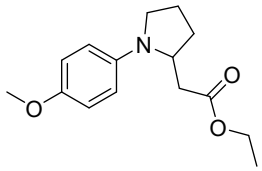
Follow the procedure A, colorless oil, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.21 – 7.04 (m, 2H), 6.70 – 6.56 (m, 2H), 4.21 (q, *J* = 7.2 Hz, 3H), 3.51 – 3.39 (m, 1H), 3.26 – 3.10 (m, 1H), 2.84 (dd, *J* = 15.0, 3.0 Hz, 1H), 2.60 (q, *J* = 7.6 Hz, 2H), 2.25 (dd, *J* = 15.0, 10.5 Hz, 1H), 2.15 – 2.02 (m, 3H), 1.97 – 1.88 (m, 1H), 1.33 (t, *J* = 7.2 Hz, 3H), 1.24 (t, *J* = 7.6 Hz, 3H). HPLC analysis: Superchiral S-OJ (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 9.90 min and 10.59 min.



4d

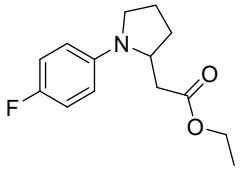
Follow the procedure A, colorless oil, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.33 – 7.30 (m, 2H), 6.69 – 6.56 (m, 2H), 4.27 – 4.14 (m, 4H), 3.48 – 3.42 (m, 1H), 3.25 – 3.14 (m, 1H), 2.84 (dd, *J* = 14.9, 3.0 Hz, 1H), 2.24 (dd, *J* = 15.0, 10.5 Hz, 1H), 2.13 – 2.01 (m, 3H), 1.97 – 1.86 (m, 1H), 1.35 – 1.30 (m,

12H). HPLC analysis: Superchiral S-OJ (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 12.10 min and 13.12 min.



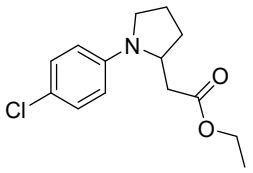
4e

Follow the procedure A, colorless oil, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.95 – 6.85 (m, 2H), 6.62 (d, *J* = 8.4 Hz, 2H), 4.24 – 4.09 (m, 3H), 3.79 (s, 3H), 3.42 (d, *J* = 6.5 Hz, 1H), 3.17 (q, *J* = 8.1 Hz, 1H), 2.80 (dd, *J* = 15.0, 3.0 Hz, 1H), 2.24 (dd, *J* = 14.9, 10.4 Hz, 1H), 2.12 – 2.00 (m, 3H), 1.90 (d, *J* = 6.0 Hz, 1H), 1.31 (t, *J* = 7.1 Hz, 3H). HPLC analysis: Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 8.87 min and 10.46 min.



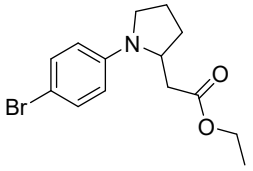
4f

Follow the procedure A, colorless oil, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.04 – 6.96 (m, 2H), 6.56 (dd, *J* = 9.1, 4.3 Hz, 2H), 4.24 – 4.11 (m, 2H), 3.46 – 3.36 (m, 1H), 3.24 – 3.11 (m, 1H), 2.77 (dd, *J* = 15.0, 3.0 Hz, 1H), 2.25 (dd, *J* = 14.9, 10.4 Hz, 1H), 2.13 – 1.95 (m, 2H), 1.95 – 1.85 (m, 1H), 1.30 – 1.25 (m, 3H). HPLC analysis: Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 5.24 min and 6.33 min.



4g

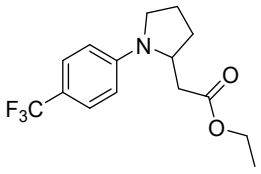
Follow the procedure A, colorless oil, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.23 – 7.16 (m, 2H), 6.58 – 6.48 (m, 2H), 4.25 – 4.13 (m, 2H), 3.47 – 3.38 (m, 1H), 3.24 – 3.13 (m, 1H), 2.76 (dd, *J* = 15.0, 3.0 Hz, 1H), 2.32 – 2.22 (m, 1H), 2.14 – 2.02 (m, 3H), 1.94 (dt, *J* = 10.8, 2.7 Hz, 1H), 1.32 (t, *J* = 7.1 Hz, 3H). HPLC analysis: Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 5.26 min and 5.77 min.



4h

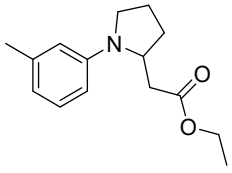
Follow the procedure A, colorless oil, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 – 7.31 (m, 2H), 6.54 – 6.49 (m, 2H), 4.25 – 4.12 (m, 3H), 3.46 – 3.39 (m, 1H), 3.23 – 3.13 (m, 1H), 2.75 (dd, *J* = 15.1, 3.0 Hz, 1H), 2.25 (dd, *J* = 15.0, 10.4 Hz, 1H), 2.13 – 2.05 (m, 3H), 1.98 – 1.91 (m, 1H), 1.32 (t, *J* = 7.1 Hz, 3H). HPLC analysis: Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9

mL/min, λ = 254 nm, retention time: 6.42 min and 7.18 min.



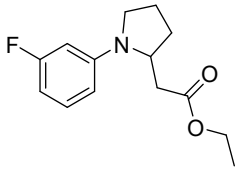
4i

Follow the procedure A, colorless oil, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 (d, *J* = 8.4 Hz, 2H), 6.64 (d, *J* = 8.4 Hz, 2H), 4.30 – 4.15 (m, 3H), 3.55 – 3.44 (m, 1H), 3.30 – 3.18 (m, 1H), 2.77 (dd, *J* = 15.2, 2.9 Hz, 1H), 2.28 (dd, *J* = 15.2, 10.4 Hz, 1H), 2.15 – 2.04 (m, 3H), 2.00-1.91 (m, 1H), 1.32 (td, *J* = 7.2, 1.0 Hz, 3H). Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 4.98 min and 5.37 min.



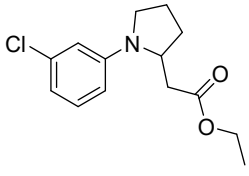
4j

Follow the procedure A, colorless oil, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.22 – 7.13 (m, 1H), 6.56 (d, *J* = 7.5 Hz, 1H), 6.48 (d, *J* = 6.6 Hz, 2H), 4.25 – 4.14 (m, 3H), 3.52 – 3.40 (m, 1H), 3.29 – 3.18 (m, 1H), 2.83 (dd, *J* = 15.0, 3.0 Hz, 1H), 2.36 (s, 3H), 2.25 (dd, *J* = 15.0, 10.5 Hz, 1H), 2.12 – 1.95 (m, 3H), 1.95 – 1.84 (m, 1H), 1.33 (t, *J* = 7.1 Hz, 3H). Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 5.81 min and 9.98 min.



4k

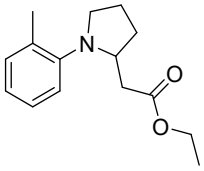
Follow the procedure A, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.19 (td, *J* = 8.4, 7.2 Hz, 1H), 6.40 (dt, *J* = 8.4, 2.0 Hz, 2H), 6.32 (dt, *J* = 12.4, 2.4 Hz, 1H), 4.21 (q, *J* = 7.2 Hz, 3H), 3.45 – 3.40 (m, 1H), 3.25 – 3.14 (m, 1H), 2.79 (dd, *J* = 15.2, 3.2 Hz, 1H), 2.26 (dd, *J* = 15.2, 10.4 Hz, 1H), 2.14 – 2.03 (m, 3H), 1.95 - 1.92 (m, 1H), 1.32 (t, *J* = 7.2 Hz, 3H). Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 6.11 min and 7.27 min.



4l

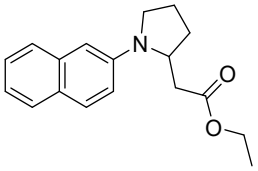
Follow the procedure A, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.16 (t, *J* = 8.1 Hz, 1H), 6.70 – 6.65 (m, 1H), 6.60 (d, *J* = 2.3 Hz, 1H), 6.51 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.21 (q, *J* = 7.2 Hz, 3H), 3.43 (dt, *J* = 9.2, 5.4 Hz, 1H), 3.20 (q, *J* = 8.2 Hz, 1H), 2.77 (dd, *J* = 15.1, 3.1 Hz, 1H), 2.26 (dd, *J* = 15.0, 10.4 Hz, 1H), 2.16 – 2.04 (m, 3H), 1.97 - 1.90 (m, 1H), 1.32 (td, *J* = 7.5, 6.6 Hz, 3H). Superchiral S-OD (150×4.6 mm),

n-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 6.51 min and 7.77 min.



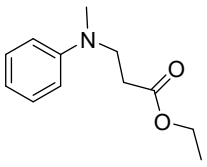
4m

Follow the procedure A, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.17 (t, *J* = 7.5 Hz, 2H), 7.06 (d, *J* = 7.9 Hz, 1H), 6.95 (td, *J* = 7.3, 1.3 Hz, 1H), 4.13 – 3.98 (m, 3H), 3.61 – 3.46 (m, 1H), 2.89 – 2.78 (m, 1H), 2.56 (dd, *J* = 15.0, 4.1 Hz, 1H), 2.38 – 2.26 (m, 4H), 2.16 (dd, *J* = 14.9, 9.3 Hz, 1H), 2.02 – 1.93 (m, 1H), 1.92 – 1.82 (m, 1H), 1.77 – 1.68 (m, 1H), 1.23 (t, *J* = 7.2 Hz, 3H). Superchiral S-OJ (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 5.54 min and 10.61 min.



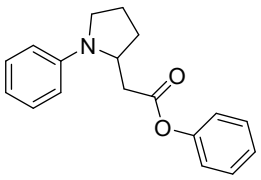
4n

Follow the procedure A, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.76 – 7.65 (m, 3H), 7.39 (ddd, *J* = 8.4, 4.8, 1.2 Hz, 1H), 7.21 (t, *J* = 7.6 Hz, 1H), 7.08 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.89 – 6.78 (m, 1H), 4.42 – 4.34 (m, 1H), 4.23 (q, *J* = 7.2 Hz, 2H), 3.63 – 3.53 (m, 1H), 3.36 (dd, *J* = 9.6, 6.6 Hz, 1H), 2.89 (dd, *J* = 15.2, 3.2 Hz, 1H), 2.31 (dd, *J* = 15.2, 10.4 Hz, 1H), 2.21 – 2.07 (m, 3H), 2.02 – 1.95 (m, 1H), 1.34 (t, *J* = 7.2 Hz, 3H). Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 7.22 min and 9.34 min.



4o

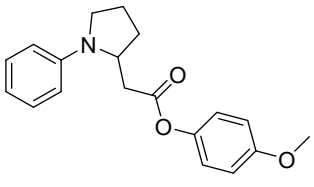
Follow the procedure A, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.30 – 7.25 (m, 2H), 6.81 – 6.73 (m, 3H), 4.17 (q, *J* = 7.2 Hz, 2H), 3.72 (t, *J* = 7.2 Hz, 2H), 2.98 (s, 3H), 2.61 (t, *J* = 7.2 Hz, 2H), 1.29 (t, *J* = 7.2 Hz, 3H).



4p

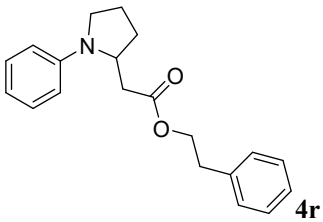
Follow the procedure A, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.48 – 7.40 (m, 2H), 7.34 – 7.24 (m, 3H), 7.16 – 7.11 (m, 2H), 6.80 – 6.68 (m, 3H), 4.43 – 4.31 (m, 1H), 3.52 (ddd, *J* = 9.6, 6.8, 2.8 Hz, 1H), 3.31 – 3.24 (m, 1H), 3.10 (dd, *J* = 15.2, 3.2 Hz, 1H), 2.52 (dd, *J* = 15.2, 10.4 Hz, 1H), 2.23 – 2.02 (m, 4H). Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254

nm, retention time: 11.64 min and 16.49 min.



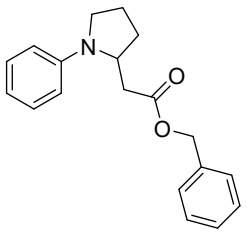
4q

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.32 (td, *J* = 7.3, 1.6 Hz, 2H), 7.09 – 7.01 (m, 2H), 6.93 (dd, *J* = 6.0, 1.8 Hz, 2H), 6.75 (dd, *J* = 21.8, 7.7 Hz, 3H), 4.42 - 4.28 (m, 1H), 3.85 (s, 3H), 3.56 - 3.49 (m, 1H), 3.32 - 3.23 (m, 1H), 3.08 (dd, *J* = 15.0, 3.1 Hz, 1H), 2.50 (dd, *J* = 15.0, 10.3 Hz, 1H), 2.25 – 2.03 (m, 4H). Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 95:5, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 9.83 min and 18.47 min.f



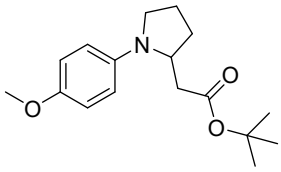
4r

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.35 (d, *J* = 6.4 Hz, 2H), 7.31 – 7.23 (m, 7H), 6.73 (t, *J* = 7.3 Hz, 1H), 6.63 (d, *J* = 8.1 Hz, 2H), 4.39 (td, *J* = 7.0, 3.1 Hz, 2H), 4.15 (ddd, *J* = 12.0, 5.7, 2.4 Hz, 1H), 3.44 (td, *J* = 7.4, 5.9, 3.8 Hz, 1H), 3.21 (q, *J* = 8.7, 8.2 Hz, 1H), 3.03 – 2.98 (m, 2H), 2.82 (dd, *J* = 14.9, 3.0 Hz, 1H), 2.24 (dd, *J* = 14.9, 10.5 Hz, 1H), 2.07 – 2.00 (m, 3H), 1.86 - 1.81 (m, 1H). Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 8.64 min and 14.23 min.



4s

Follow the procedure A, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 (s, 5H), 7.28 (t, *J* = 7.6 Hz, 2H), 6.74 (t, *J* = 7.2 Hz, 1H), 6.66 (d, *J* = 8.0 Hz, 2H), 5.20 (s, 2H), 4.31 – 4.20 (m, 1H), 3.45 (qd, *J* = 6.0, 4.0, 2.8 Hz, 1H), 3.22 (q, *J* = 8.4 Hz, 1H), 2.90 (dd, *J* = 15.2, 3.2 Hz, 1H), 2.40 – 2.29 (m, 1H), 2.12 – 2.03 (m, 3H), 1.97 – 1.89 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 171.92, 146.46, 135.94, 129.44, 128.67, 128.35, 128.27, 116.01, 111.94, 66.41, 55.39, 47.94, 37.76, 31.04, 23.07. Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 10.37 min and 19.17 min.



4t

Follow the procedure A, ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.93 – 6.84 (m, 2H), 6.61 (d, *J* = 8.4 Hz, 2H), 4.09 (s, 1H), 3.80 (s, 3H), 3.41 (s, 1H), 3.20 – 3.11 (m, 1H), 2.73 (d, *J* = 14.8 Hz, 1H), 2.22 – 2.00 (m, 3H), 1.91 (s, 1H), 1.51 (s, 9H). HPLC analysis: Superchiral S-OD (150×4.6 mm), *n*-hexane/iso-propanol= 99:1, flow rate = 0.9 mL/min, λ = 254 nm, retention time: 5.35 min and 5. 95 min.

Reference

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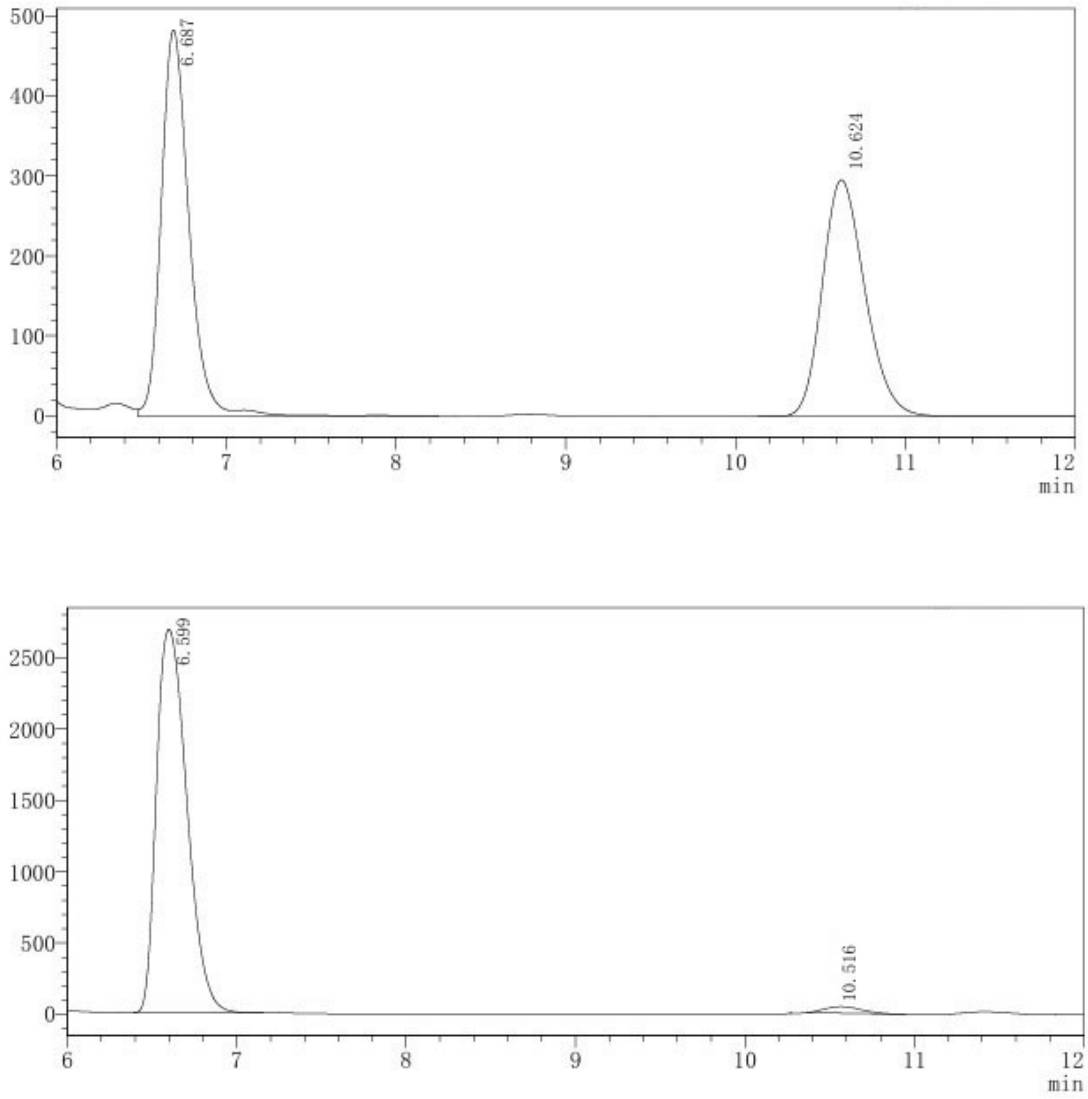
HPLC analysis

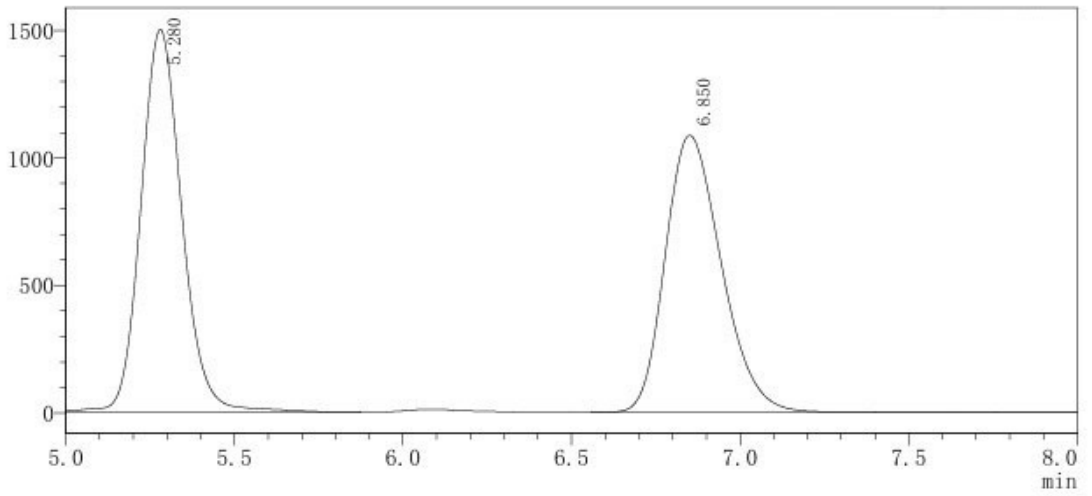
Chiral HPLC

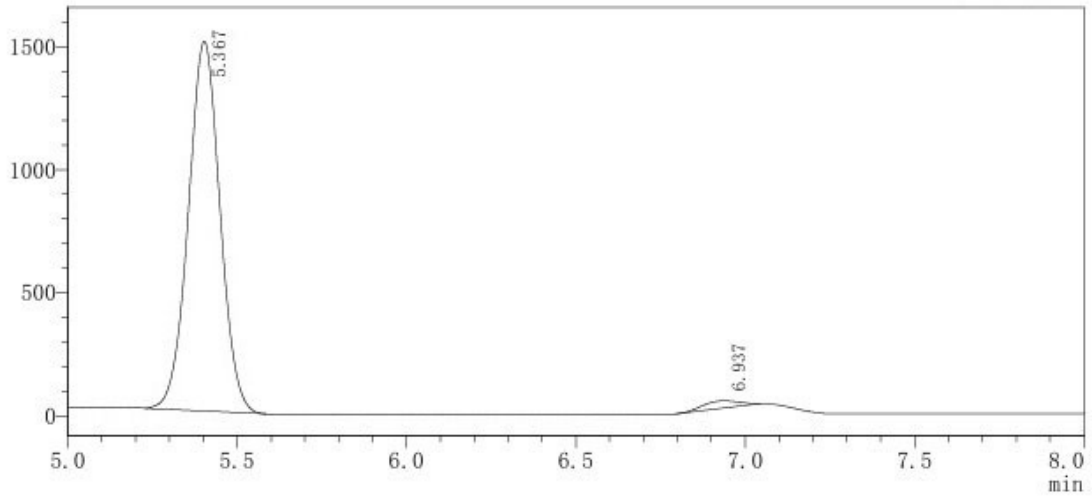
Analytical normal-phase high-performance liquid chromatography (HPLC) was carried out using a Shimadzu LC-20AT series instrument. The column purchased by Shanghai Chiralway Biotech. Co., Ltd. The optical purity of products was determined with an HPLC equipped with a Superchiral S-OD, S-OJ (150×4.6 mm). The analytical conditions were as follows: flow rate: 0.9 mL/min, mobile phase: hexane/2- propanol (99/1), oven temperature: 25 °C, UV-detector: 254 nm.

Chiral HPLC analysis of racemic **4a** (top), SD-VHb$_{CH}$ produced **4a** product(bottom):

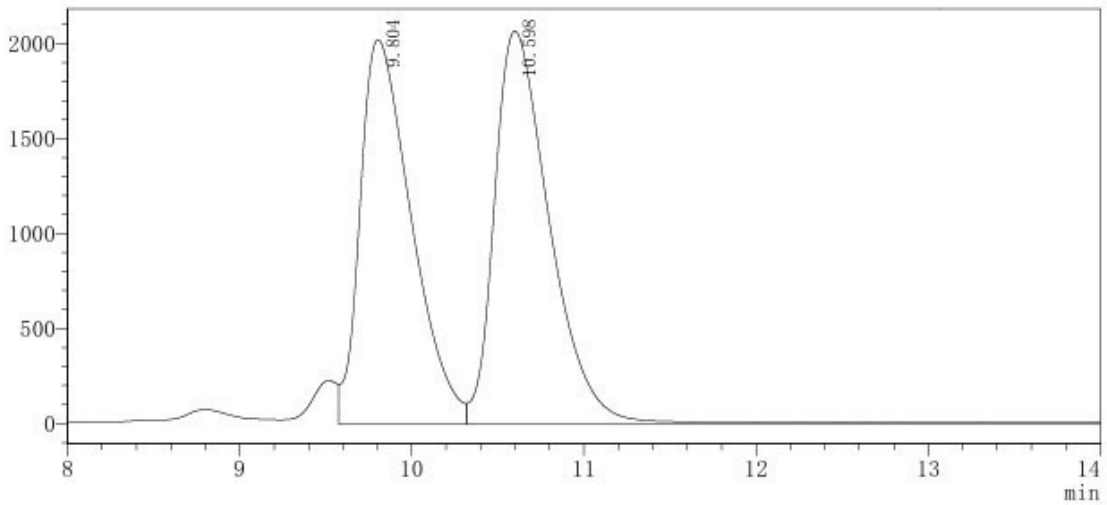


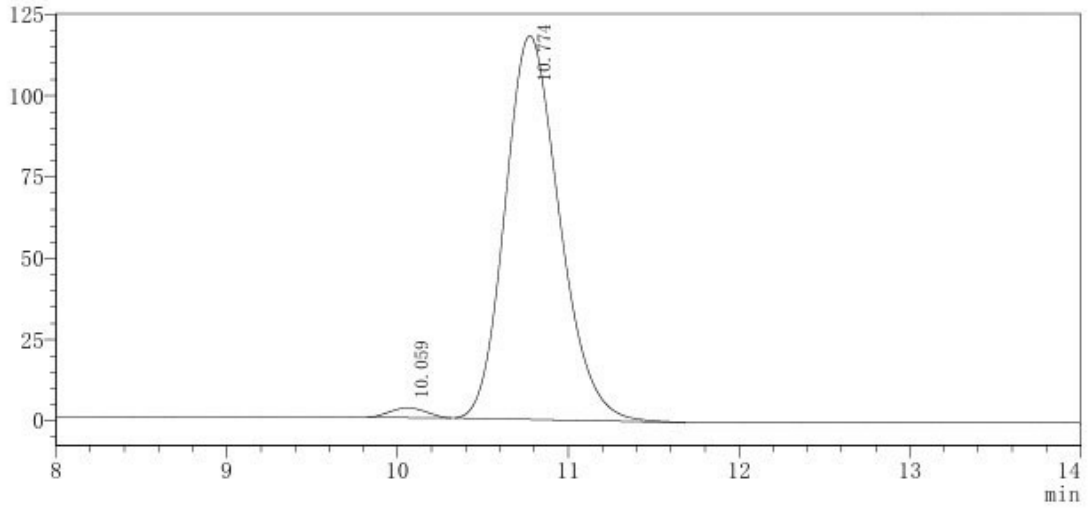
Chiral HPLC analysis of racemic **4b** (top), SD-VHb$_{CH}$ produced **4b** product(bottom):



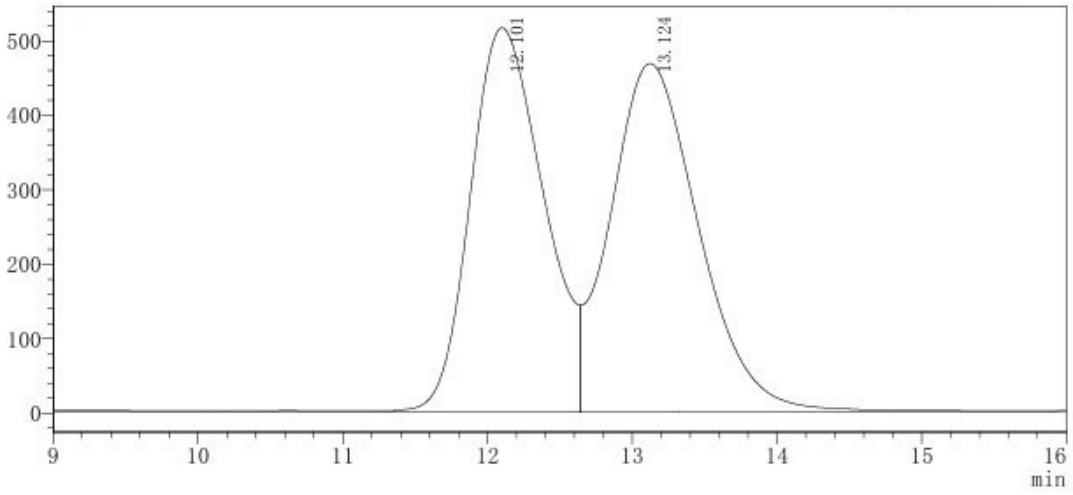


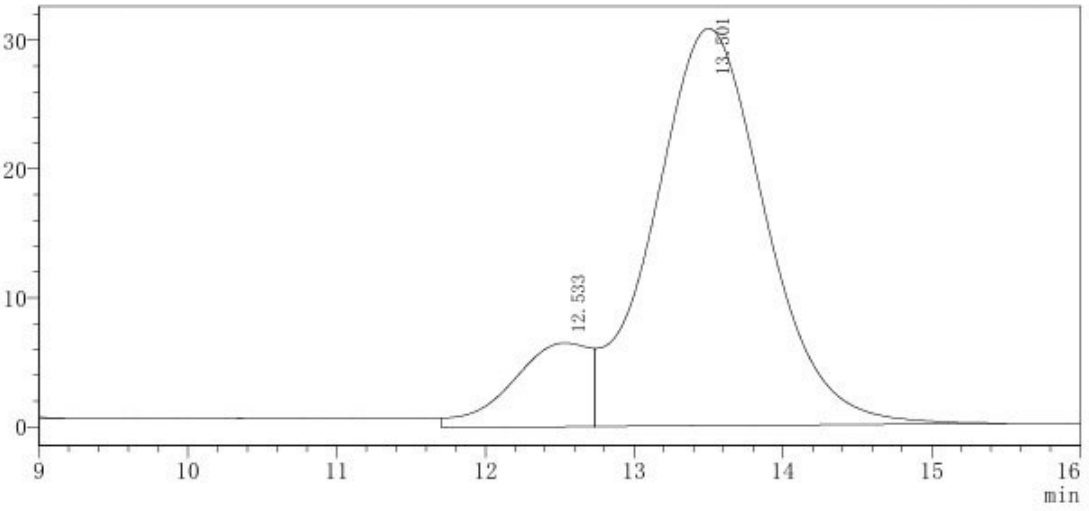
Chiral HPLC analysis of racemic **4c** (top), SD-VHb$_{CH}$ produced **4c** product(bottom):



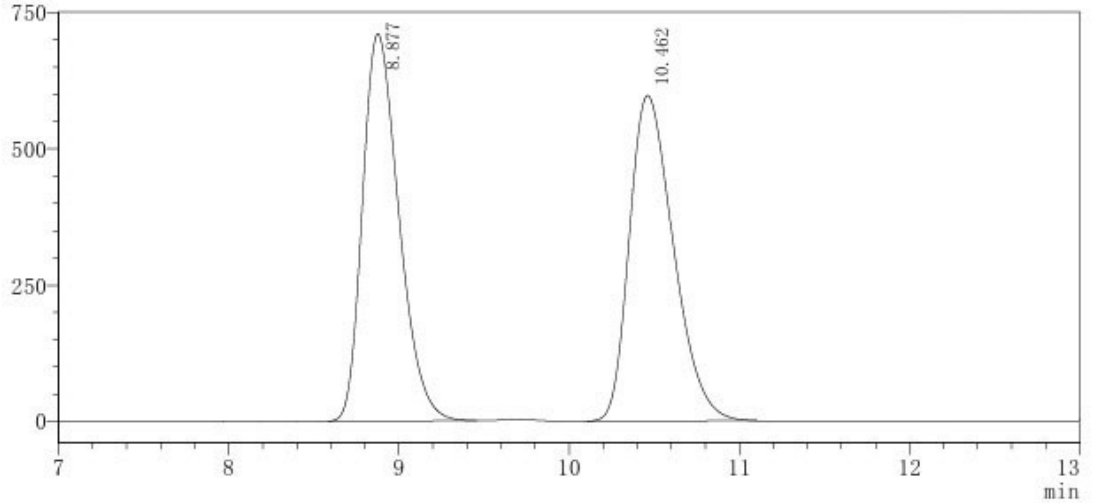


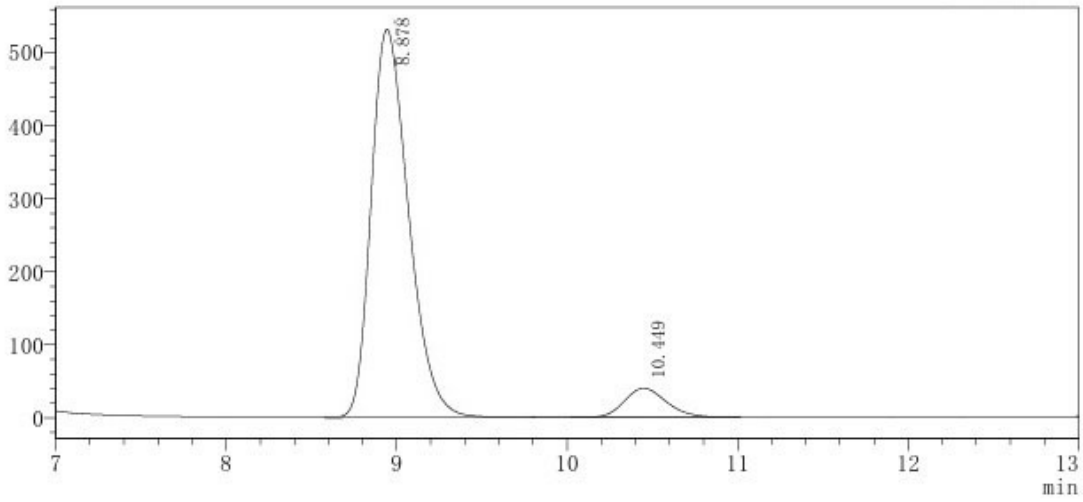
Chiral HPLC analysis of racemic **4d** (top), SD-VHb$_{CH}$ produced **4d** product(bottom):



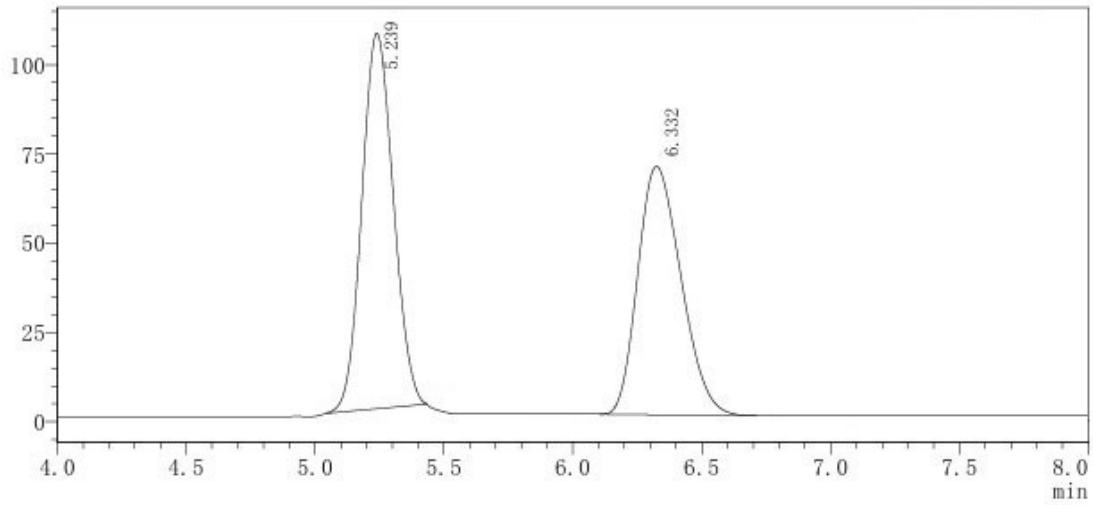


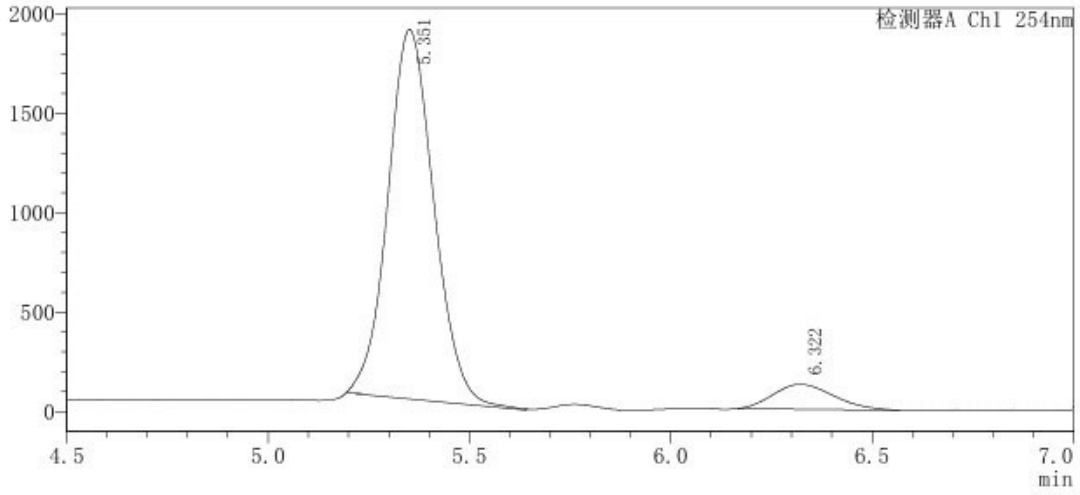
Chiral HPLC analysis of racemic **4e** (top), SD-VHb$_{CH}$ produced **4e** product(bottom):



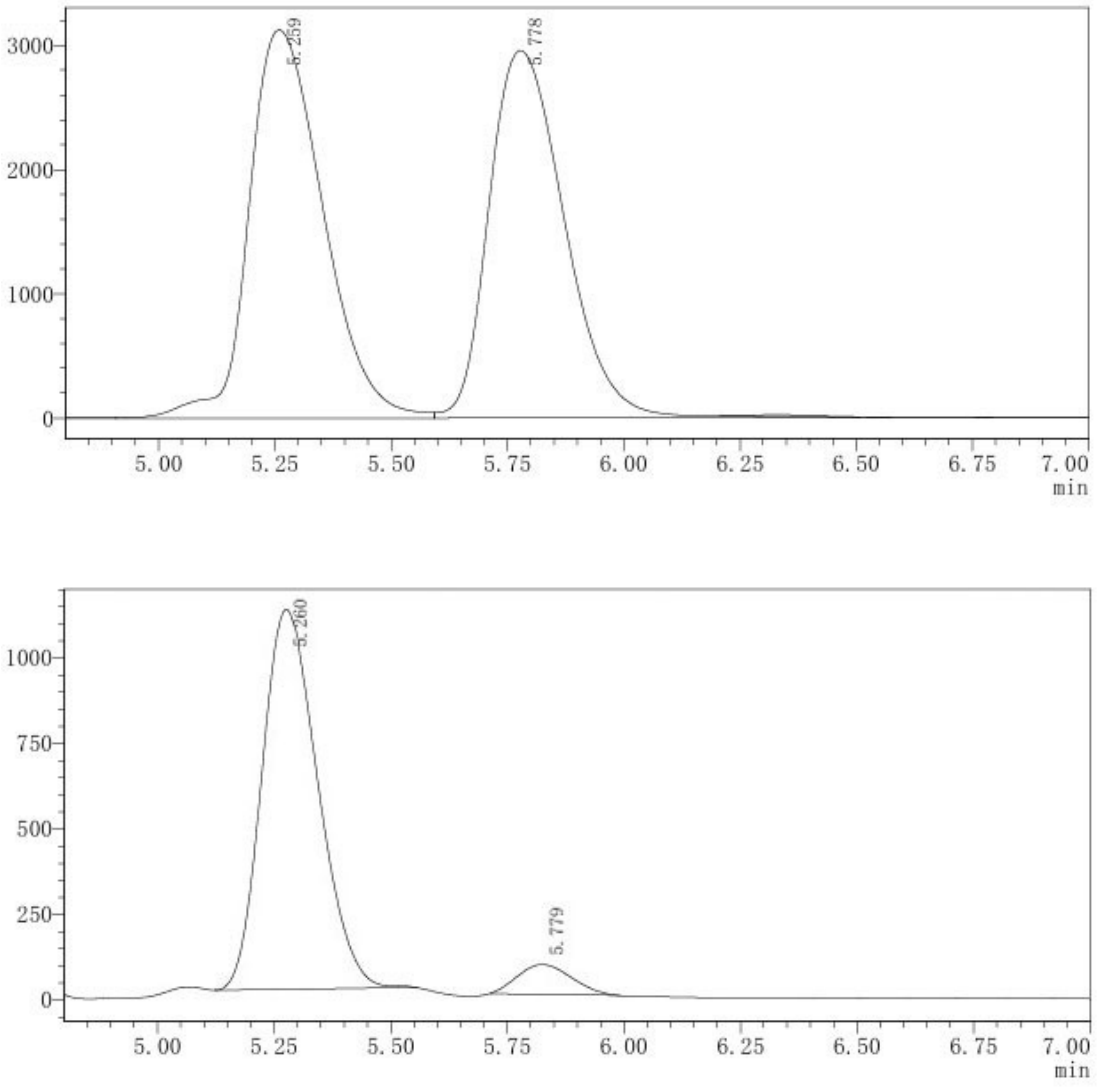


Chiral HPLC analysis of racemic **4f** (top), SD-VHb$_{CH}$ produced **4f** product(bottom):

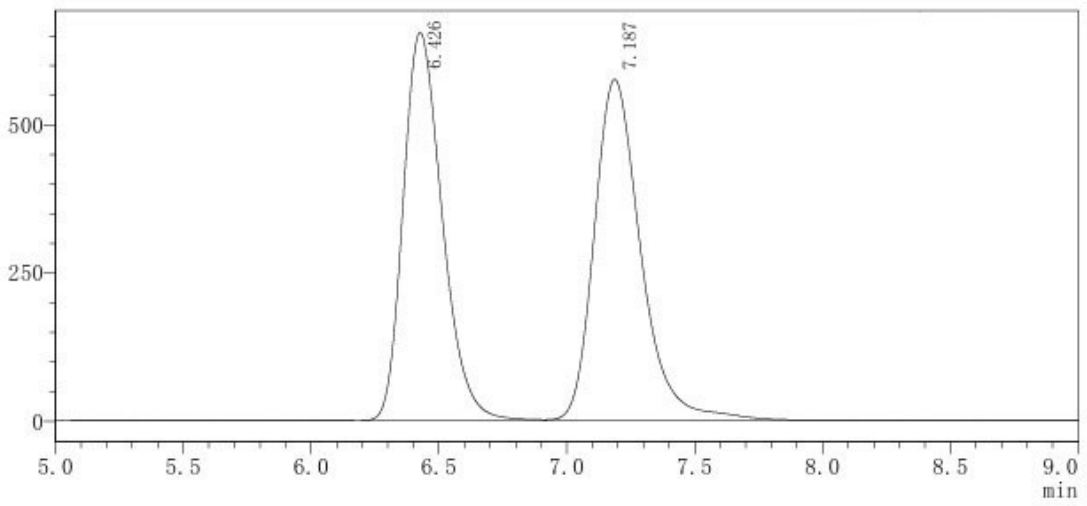


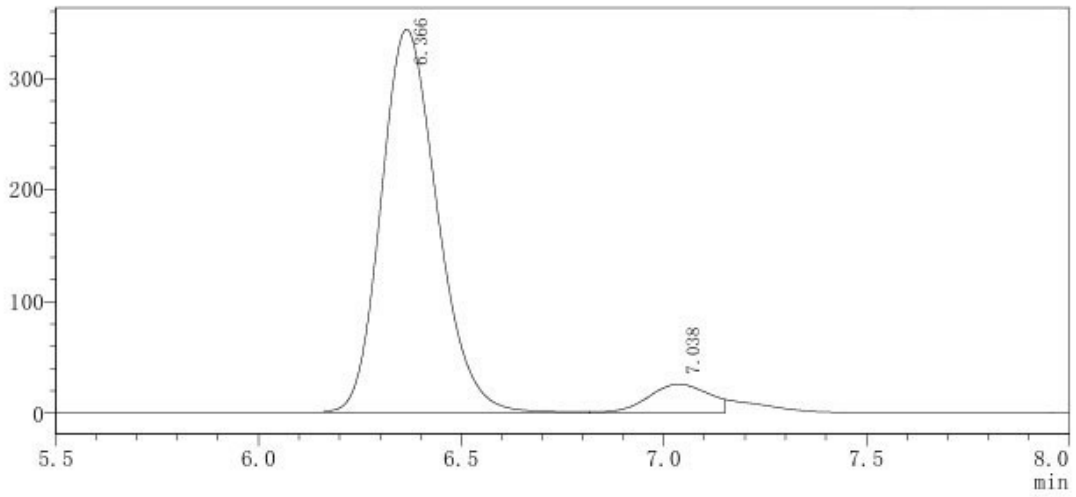


Chiral HPLC analysis of racemic **4g** (top), SD-VHb$_{CH}$ produced **4g** product(bottom):

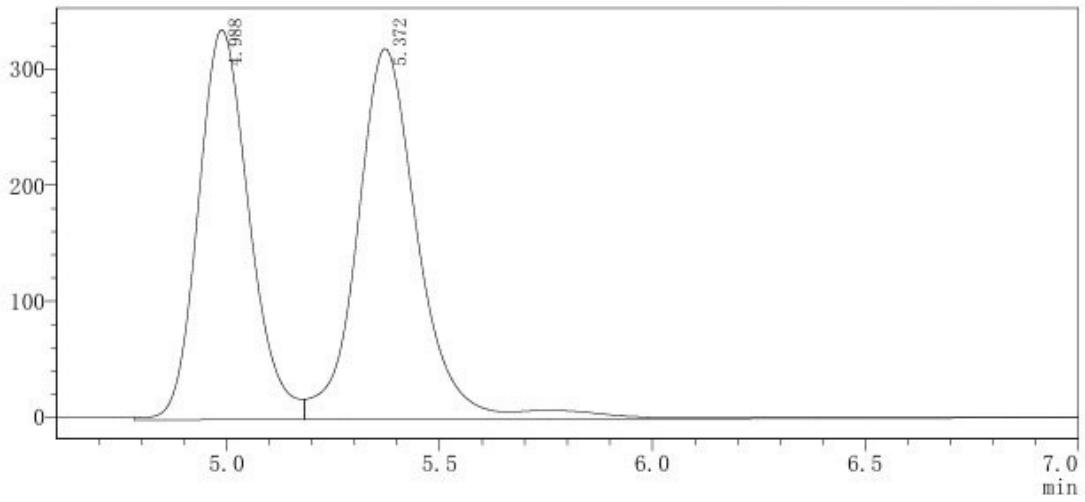


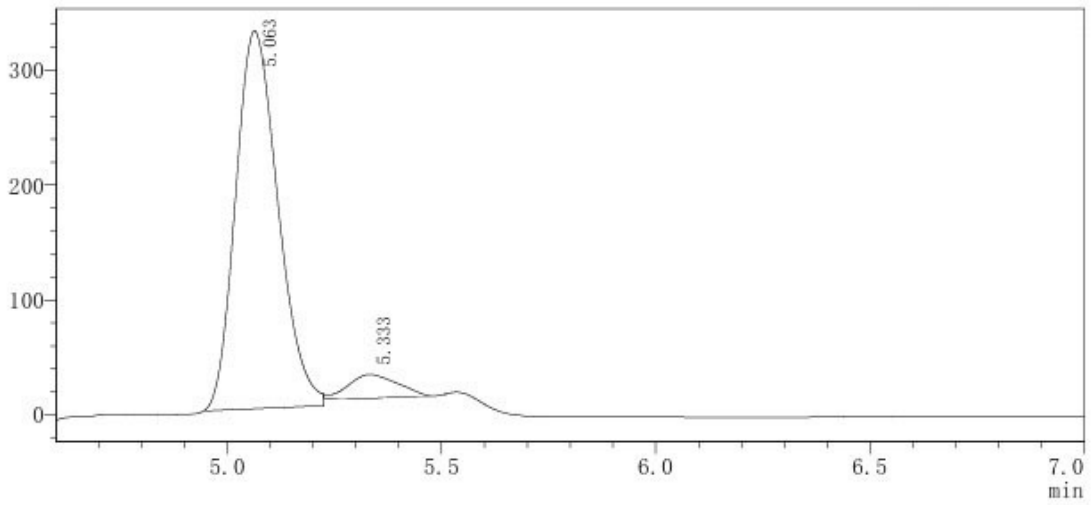
Chiral HPLC analysis of racemic **4h** (top), SD-VHb$_{CH}$ produced **4h** product(bottom):



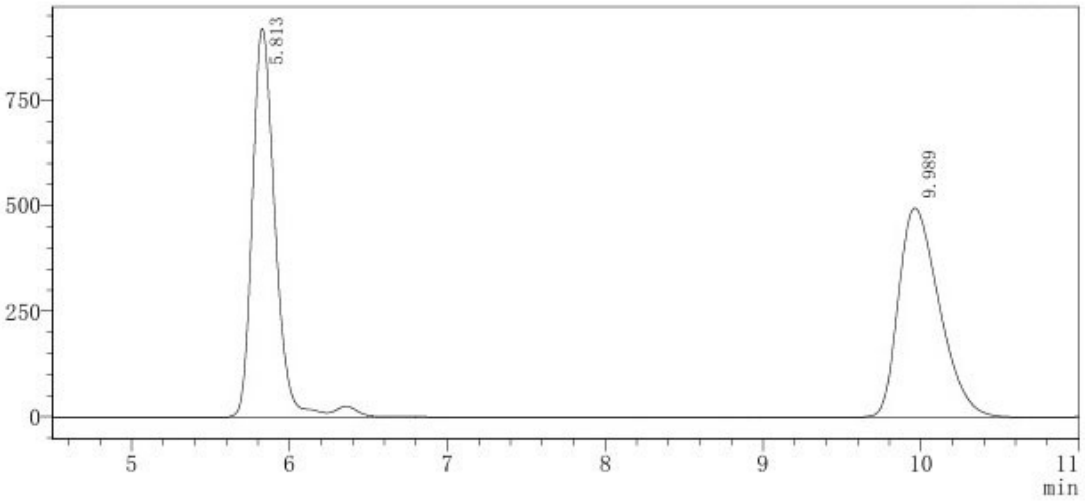


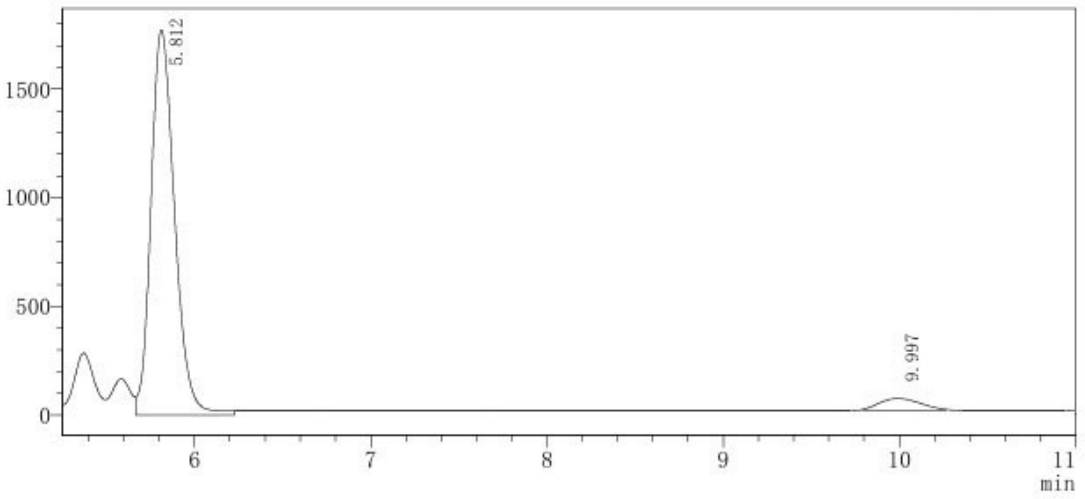
Chiral HPLC analysis of racemic **4i** (top), SD-VHb$_{CH}$ produced **4i** product(bottom):



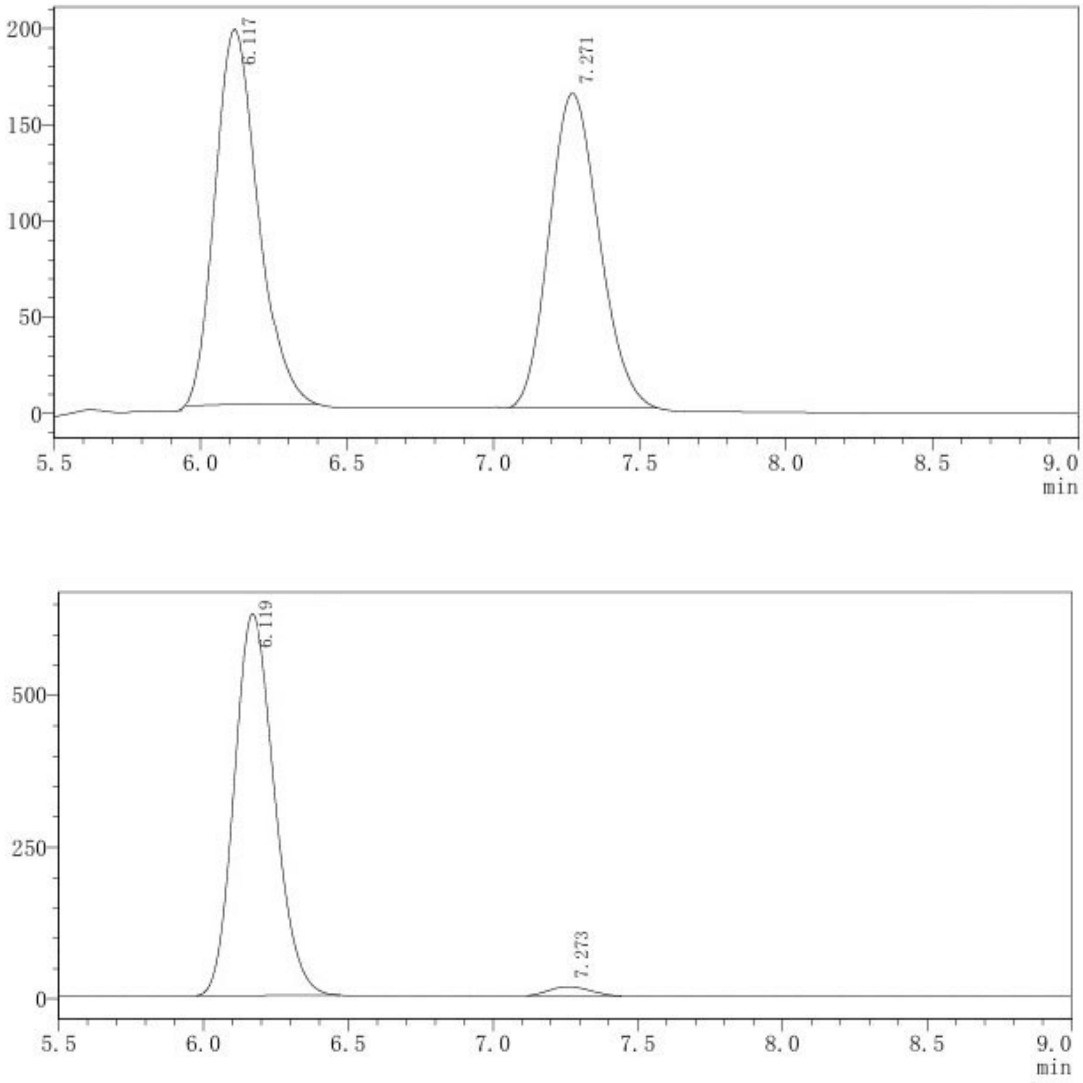


Chiral HPLC analysis of racemic **4j** (top), SD-VHb$_{CH}$ produced **4j** product(bottom):

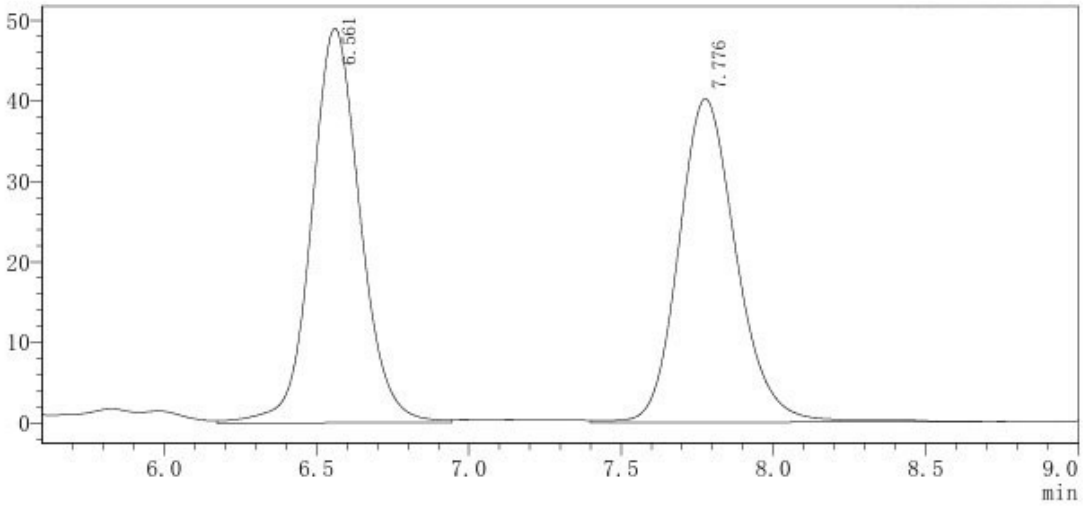


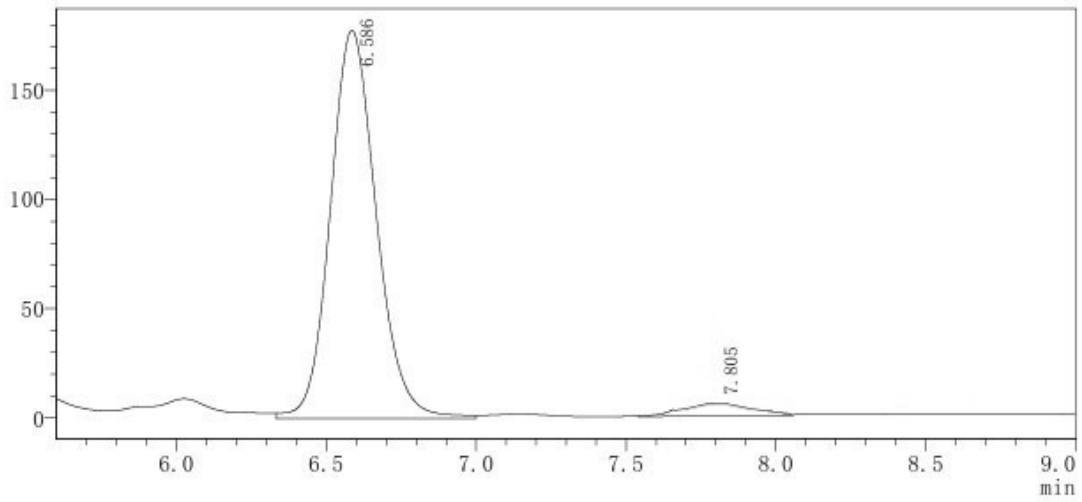


Chiral HPLC analysis of racemic **4k** (top), SD-VHb$_{CH}$ produced **4k** product(bottom):

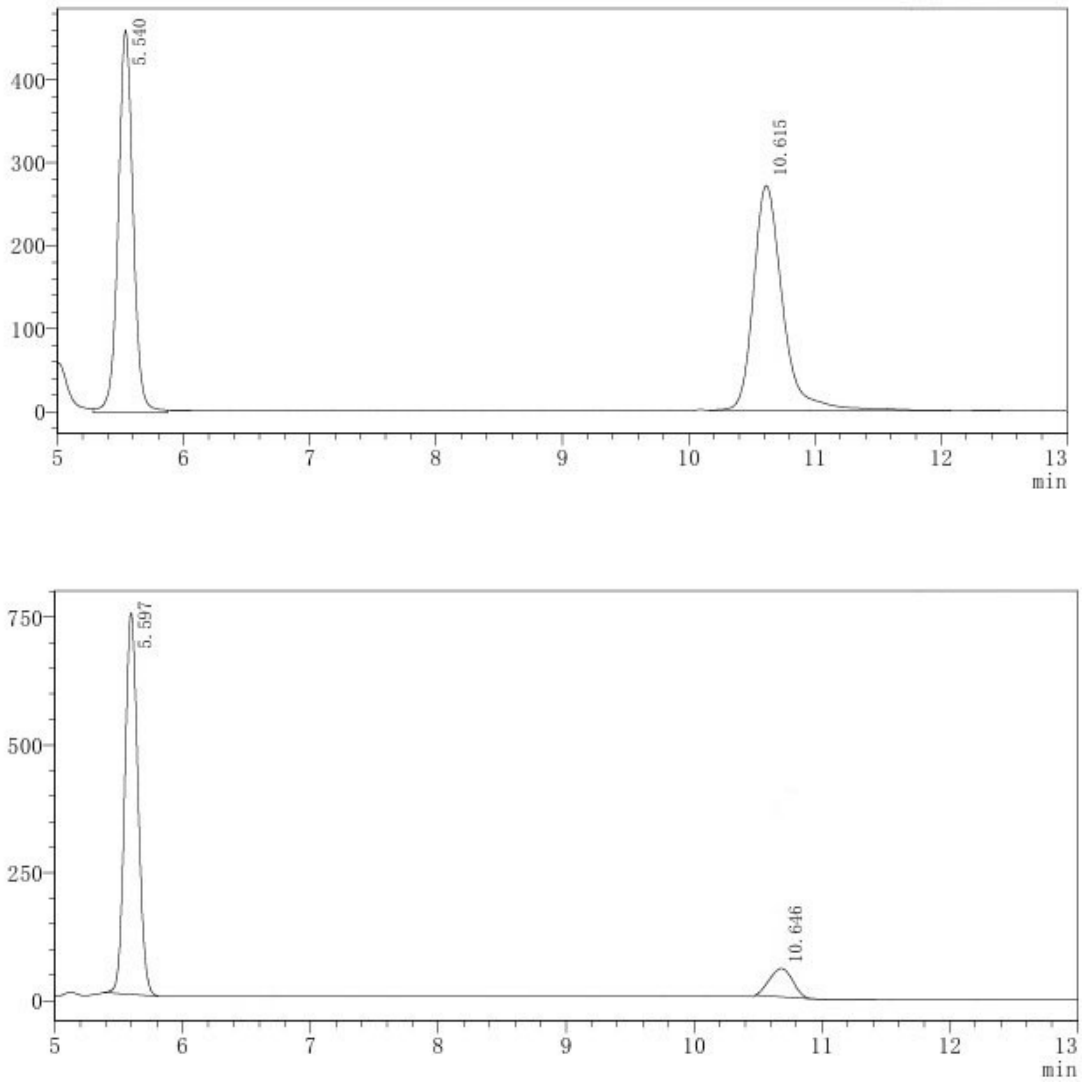


Chiral HPLC analysis of racemic **4l** (top), SD-VHb$_{CH}$ produced **4l** product(bottom):

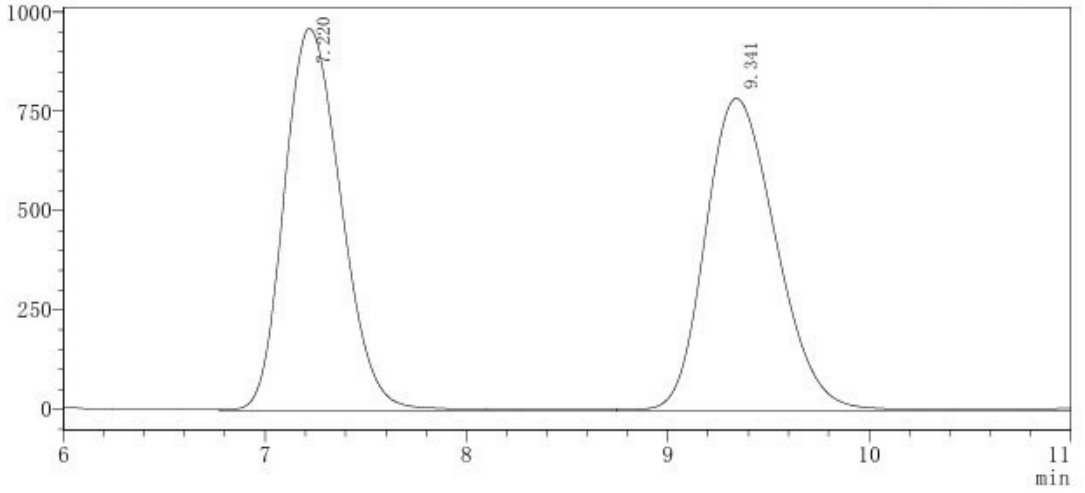


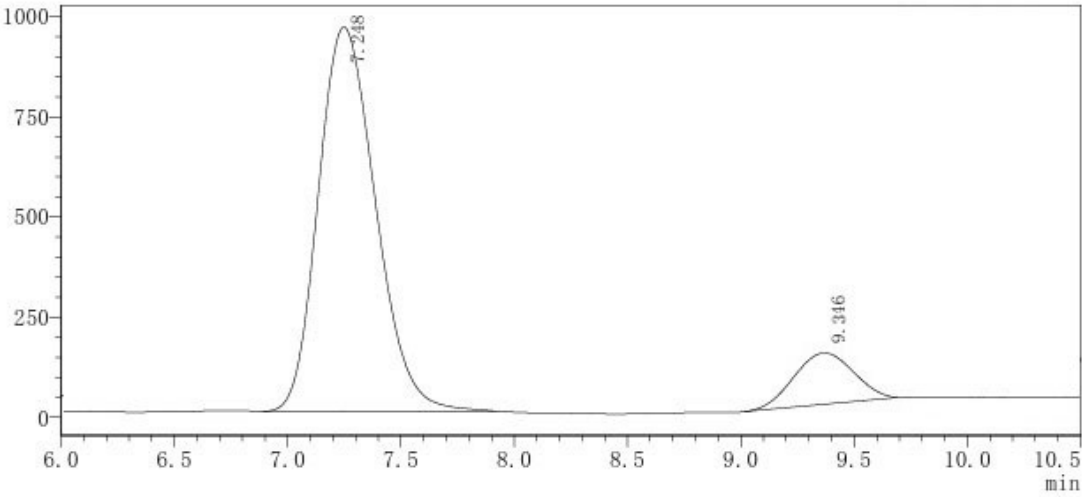


Chiral HPLC analysis of racemic **4m** (top), SD-VHb$_{CH}$ produced **4m** product(bottom):

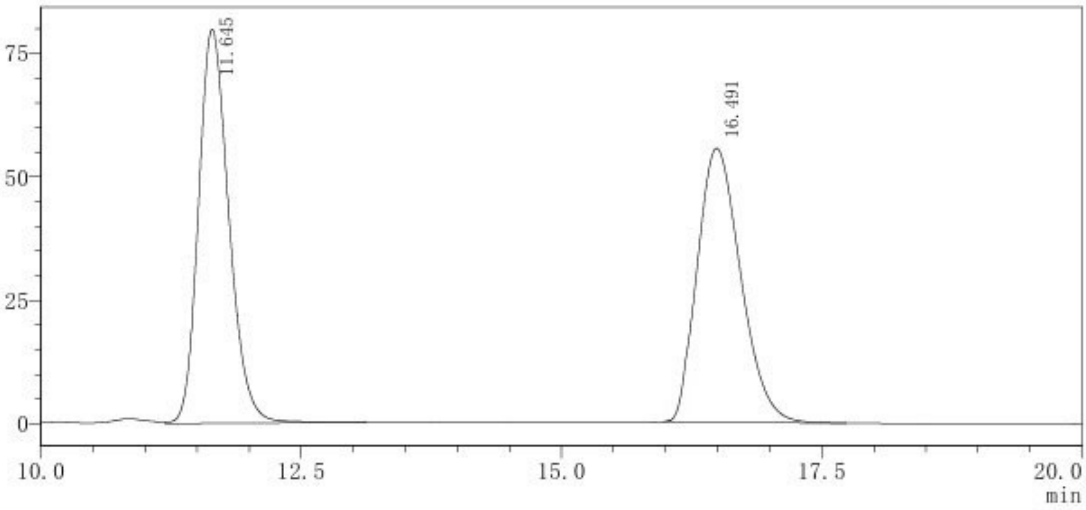


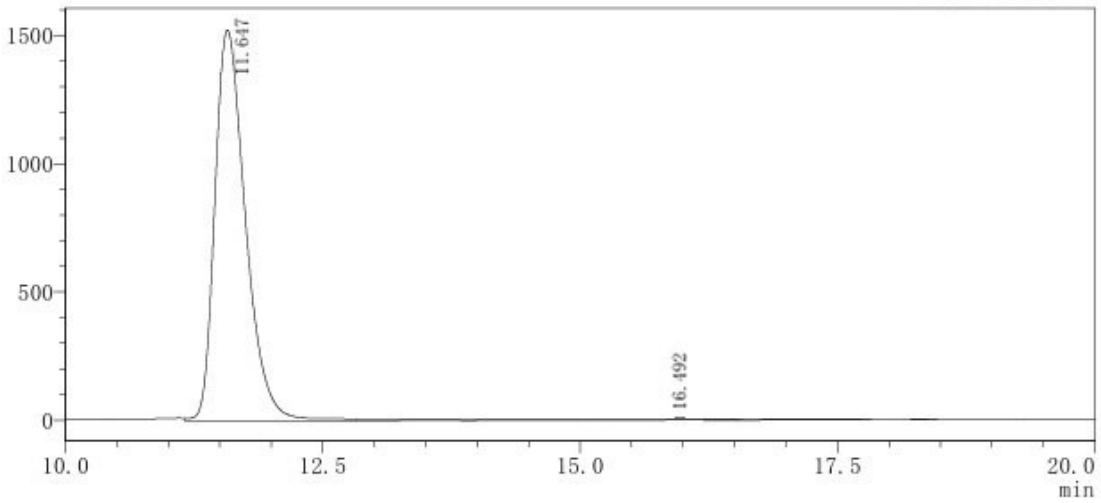
Chiral HPLC analysis of racemic **4n** (top), SD-VHb$_{CH}$ produced **4n** product(bottom):



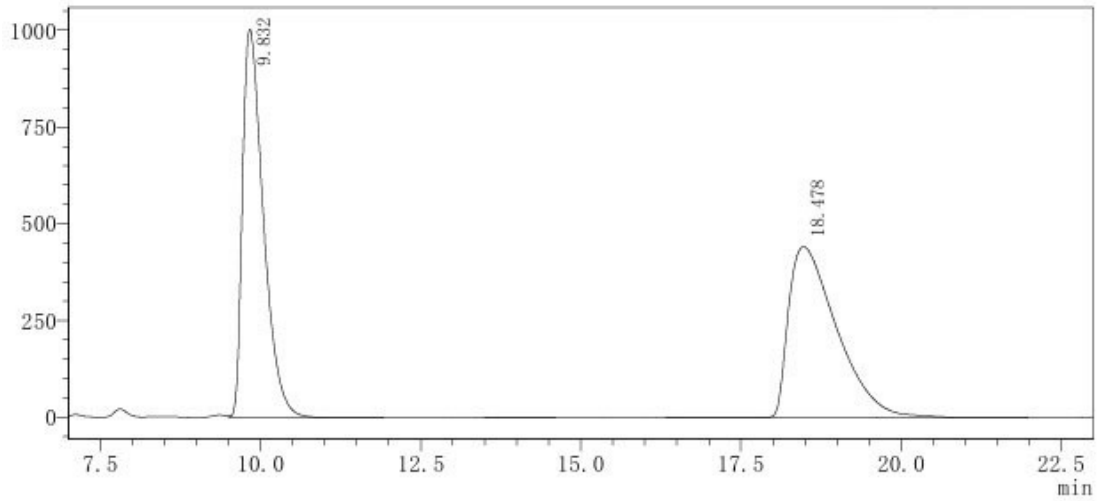


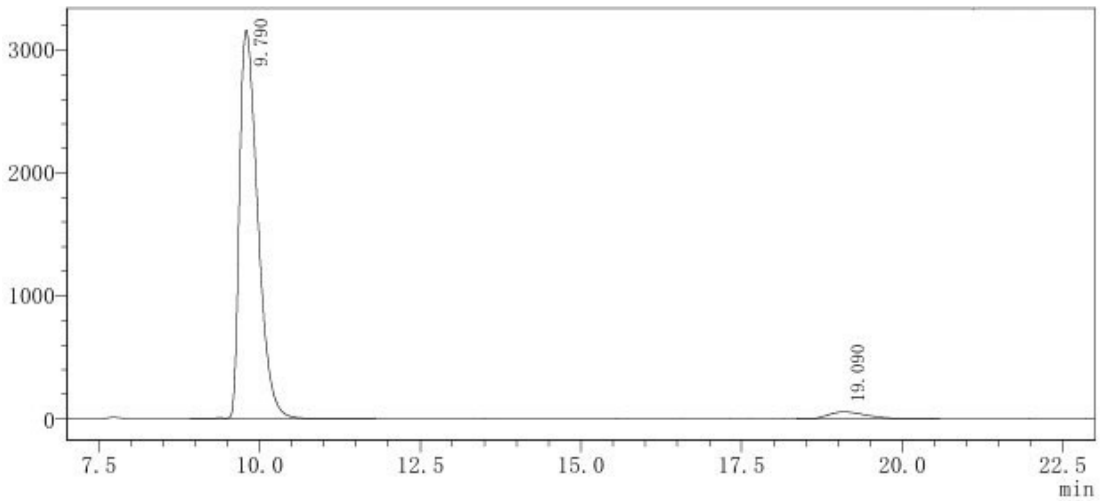
Chiral HPLC analysis of racemic **4p** (top), SD-VHb$_{CH}$ produced **4p** product(bottom):



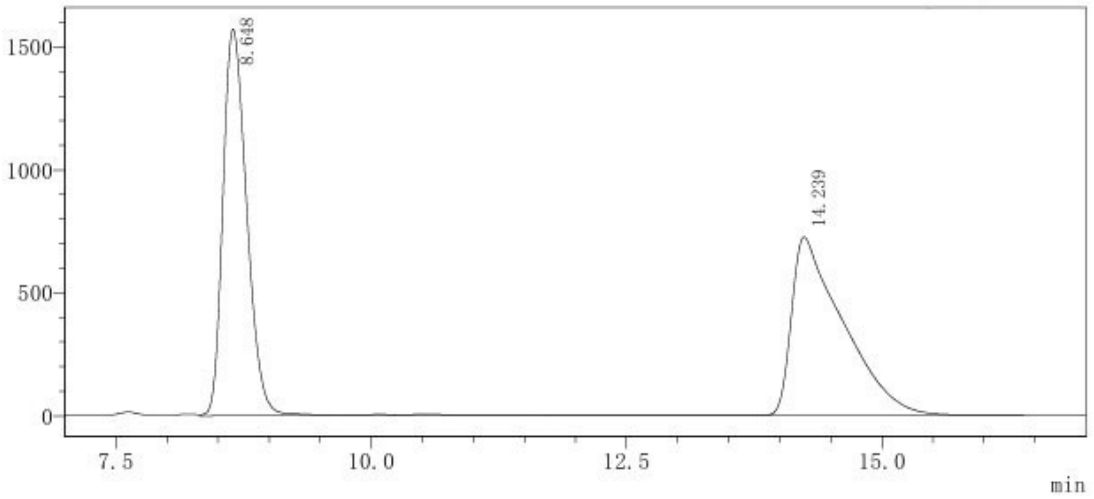


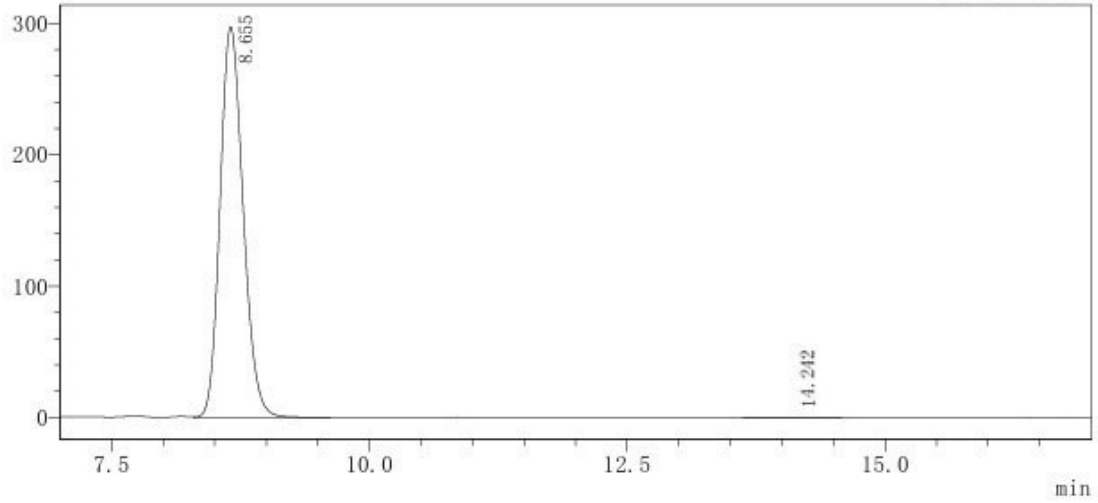
Chiral HPLC analysis of racemic **4q** (top), SD-VHb$_{CH}$ produced **4q** product(bottom):



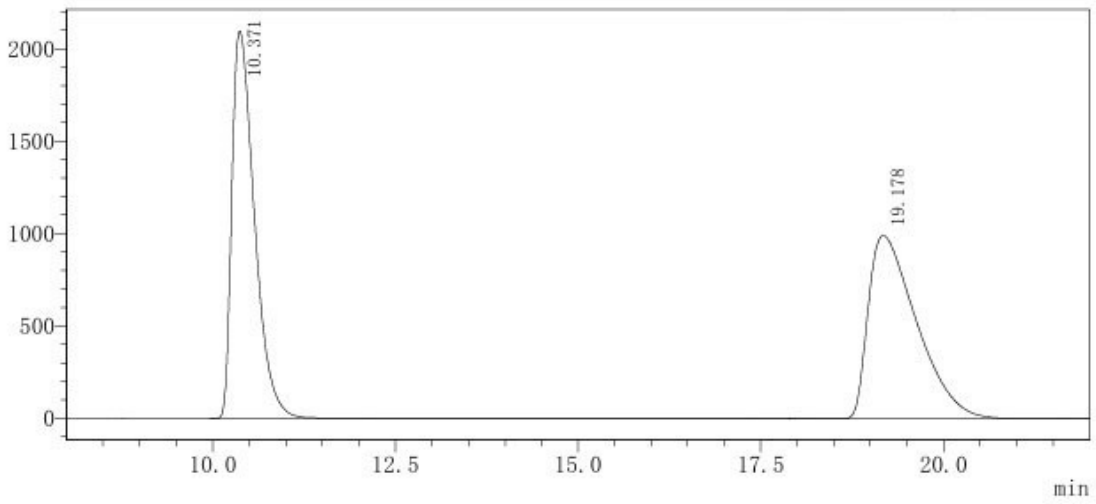


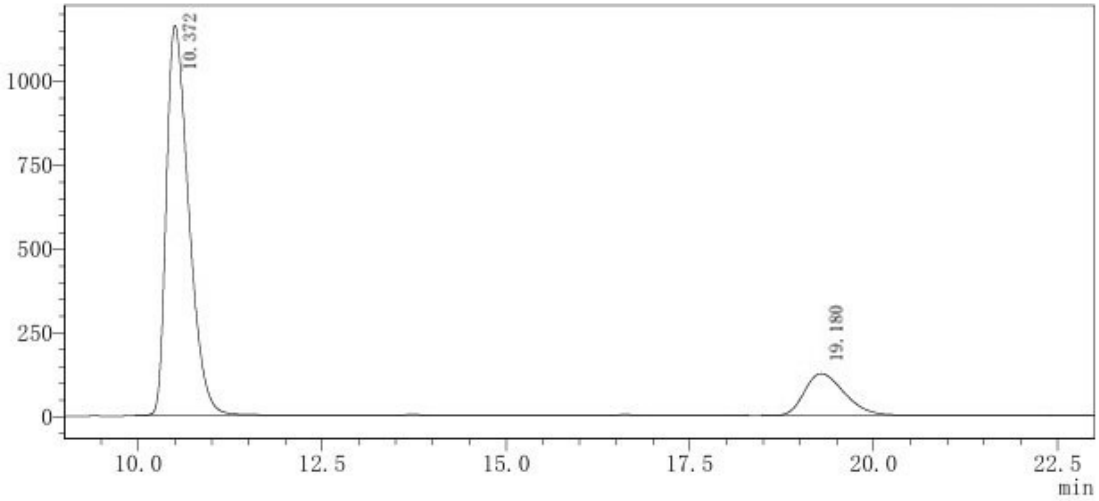
Chiral HPLC analysis of racemic **4r** (top), SD-VHb$_{CH}$ produced **4r** product(bottom):



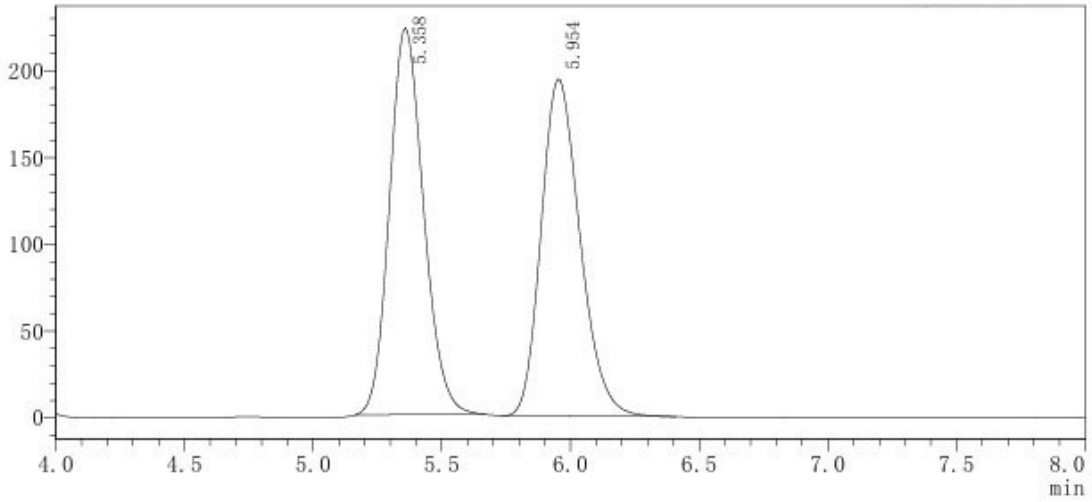


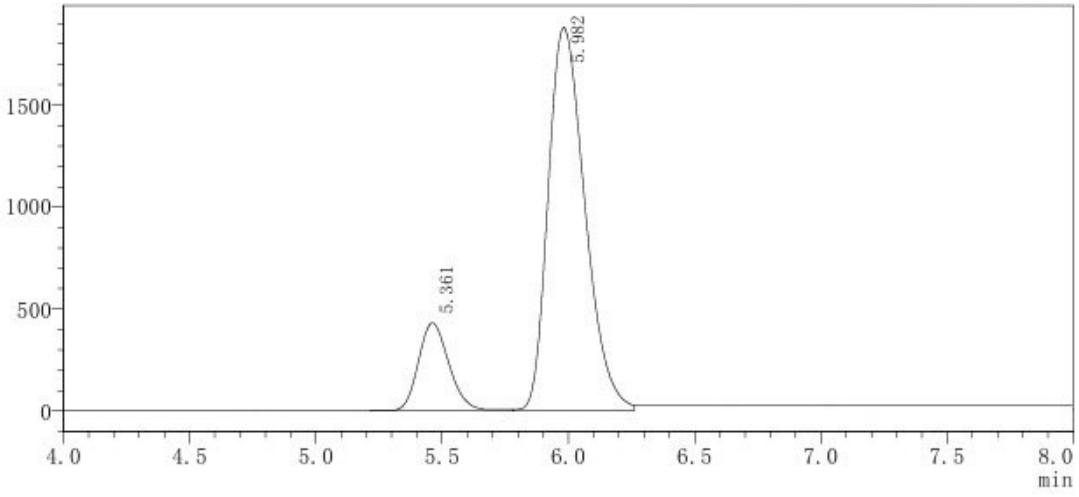
Chiral HPLC analysis of racemic **4s** (top), SD-VHb$_{CH}$ produced **4s** product(bottom):



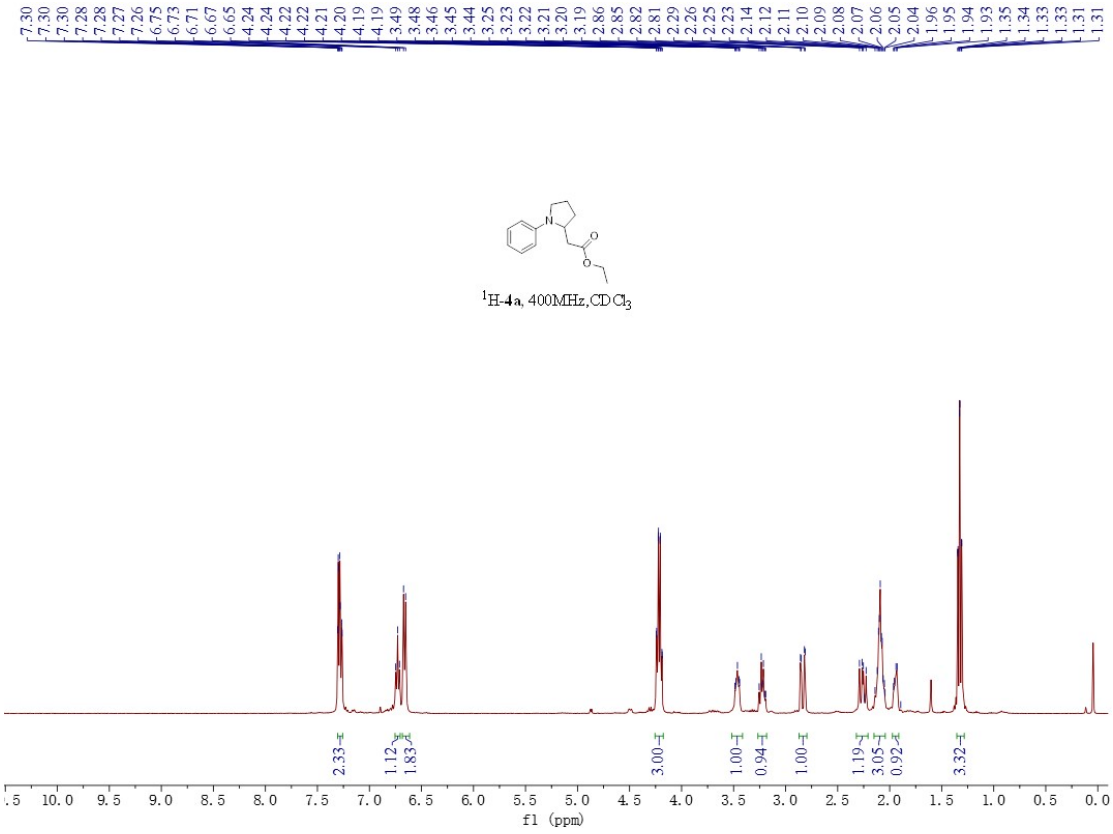


Chiral HPLC analysis of racemic **4t** (top), SD-VHb$_{CH}$ produced **4t** product(bottom):

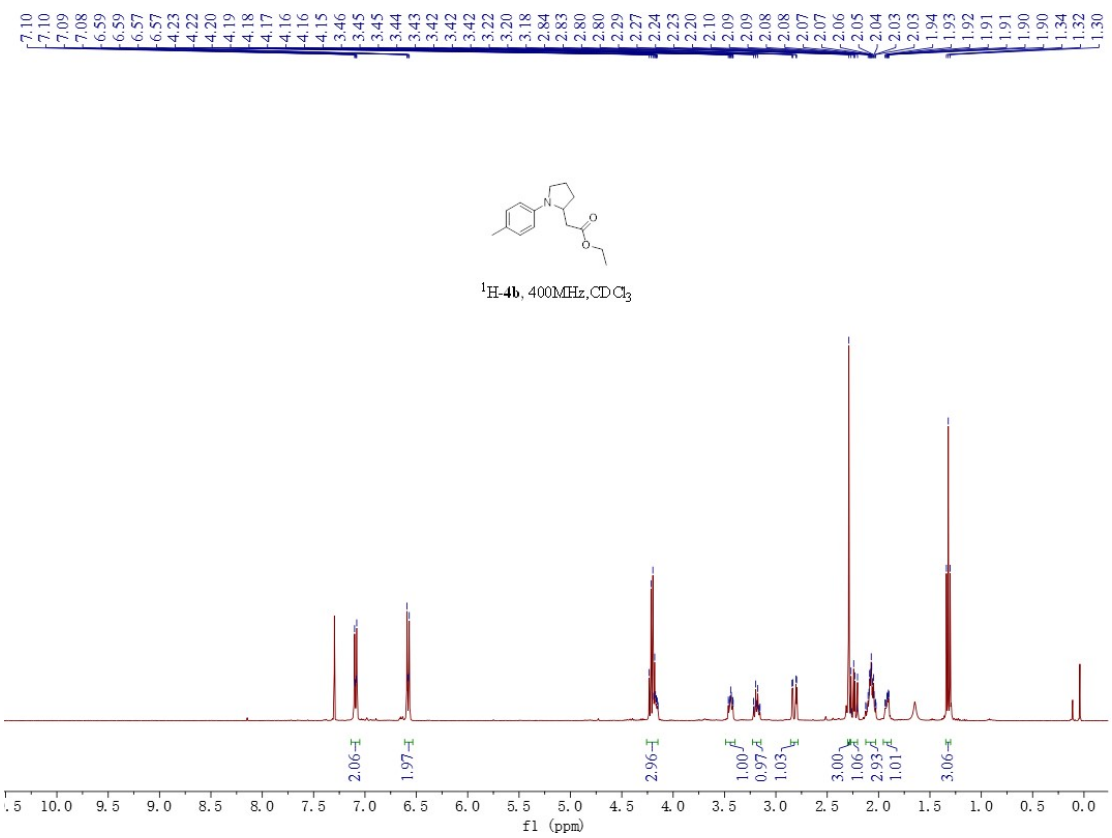




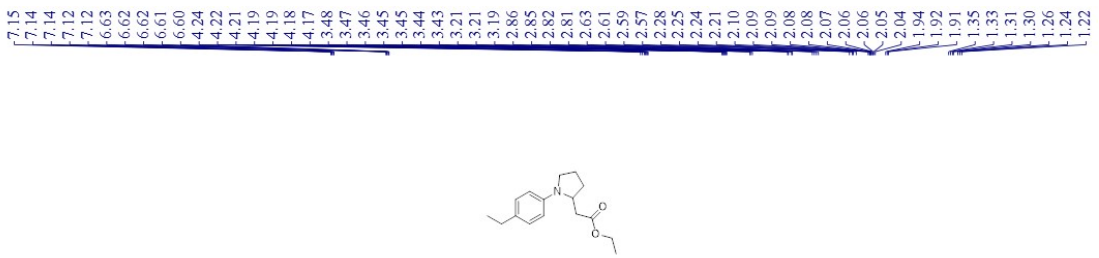
NMR Spectra



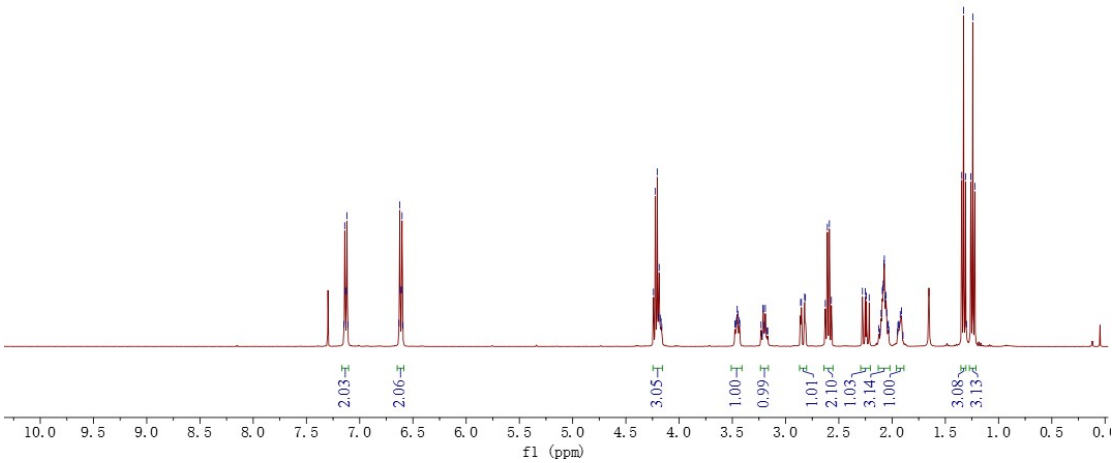
^{1}H-**4a**, 400MHz, $CDCl_3$

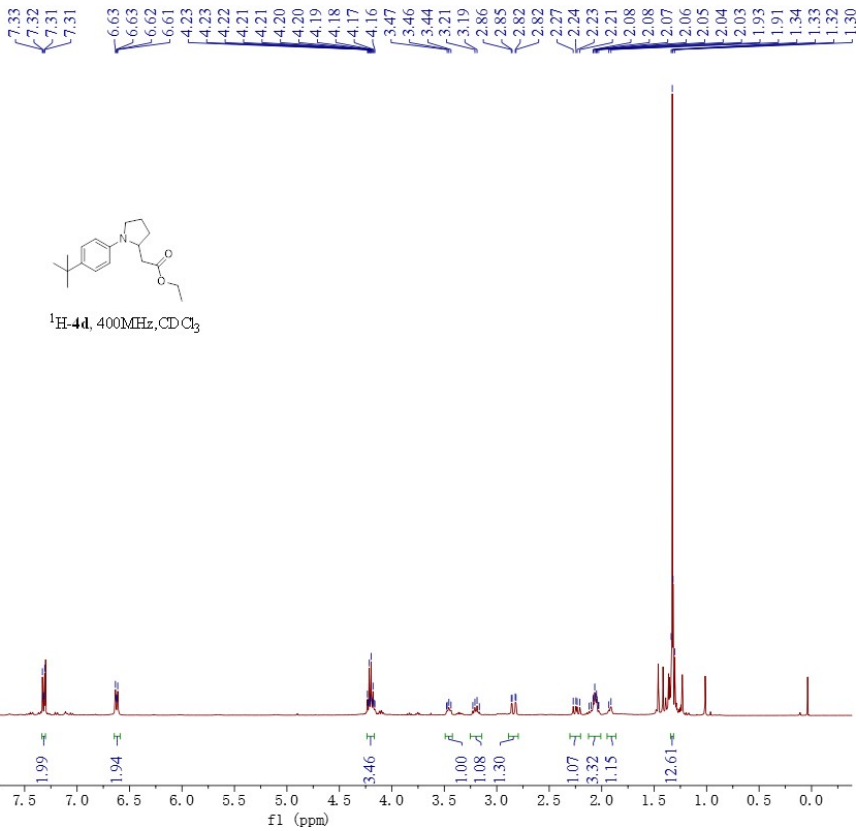


^{1}H-**4b**, 400MHz, $CDCl_3$

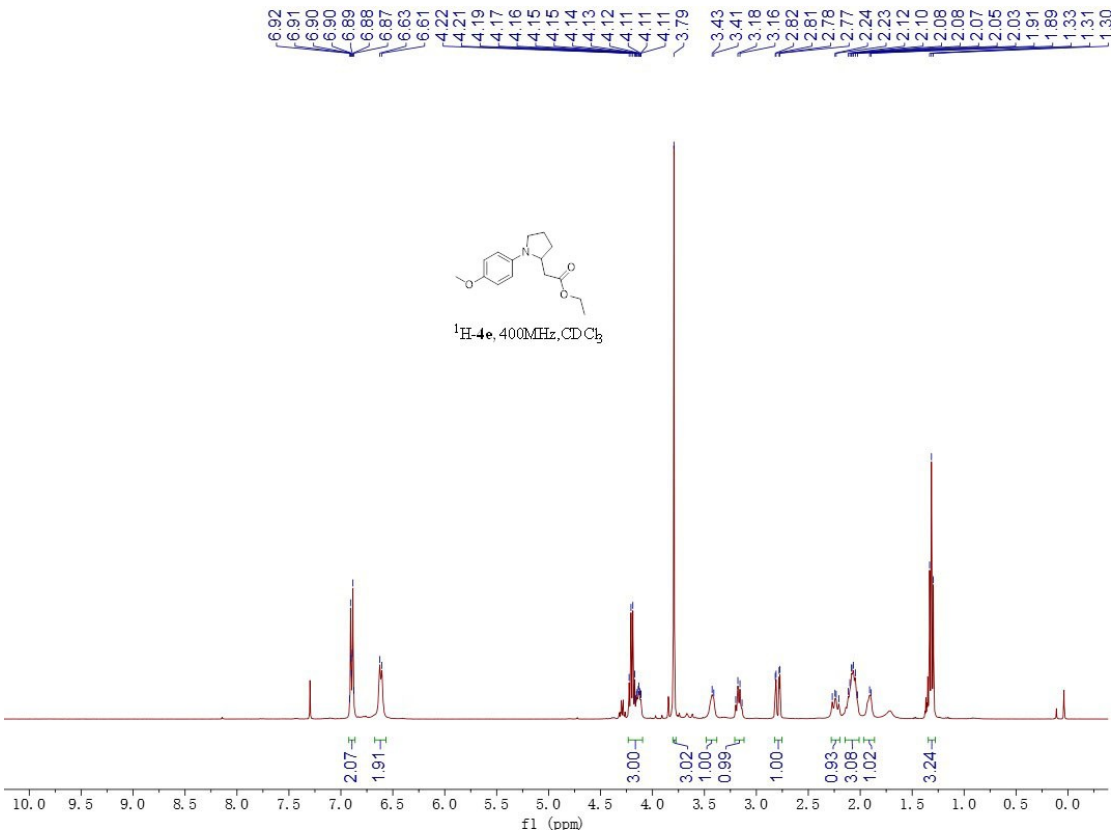


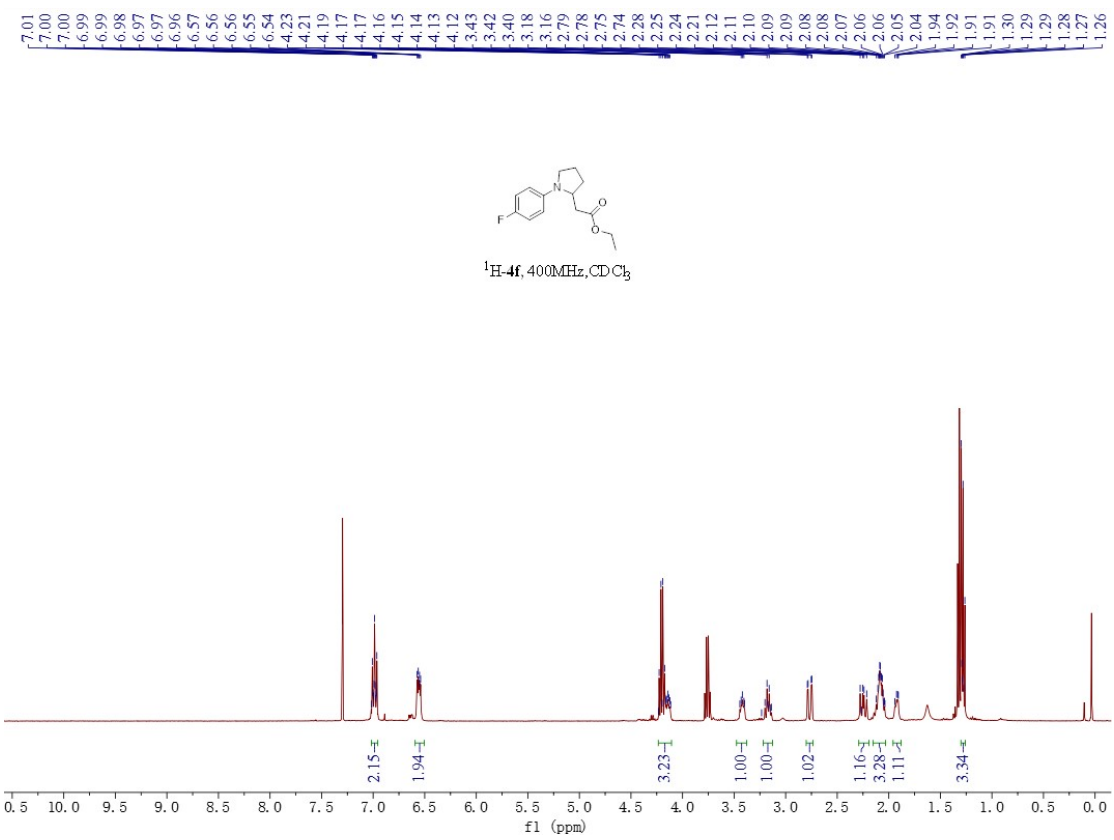
^{1}H-**4c**, 400MHz, $CDCl_3$

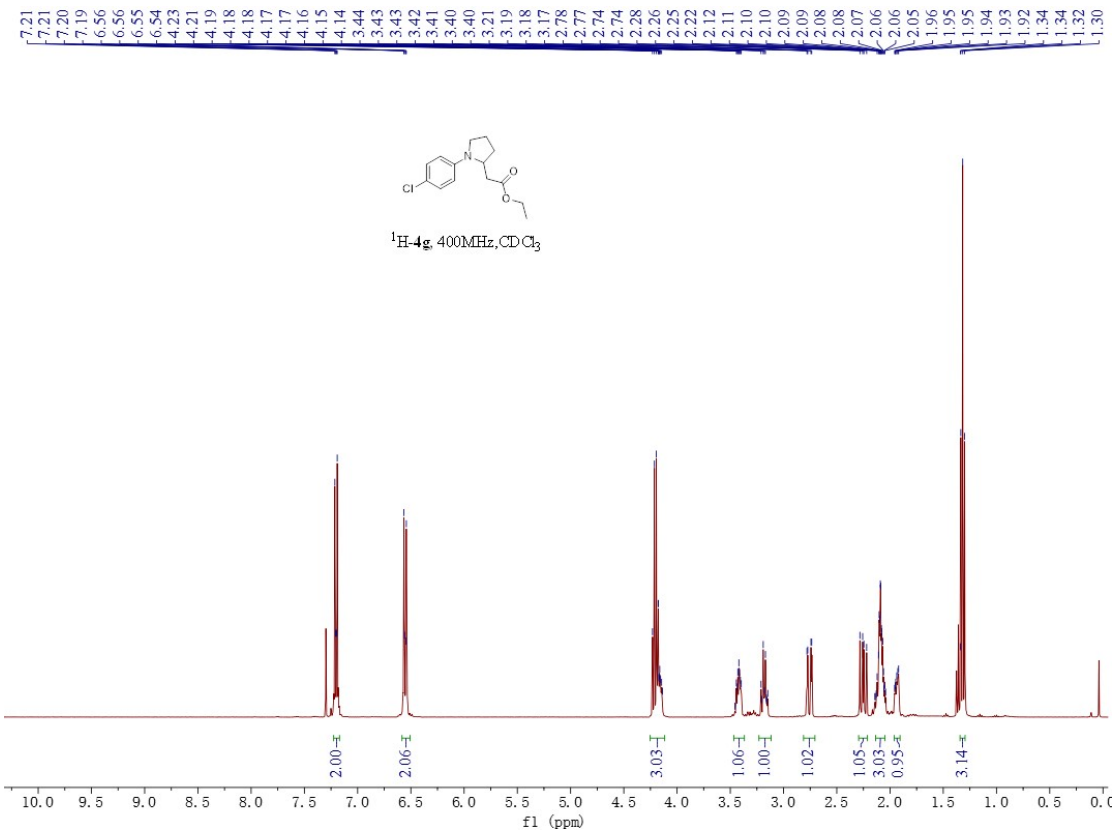




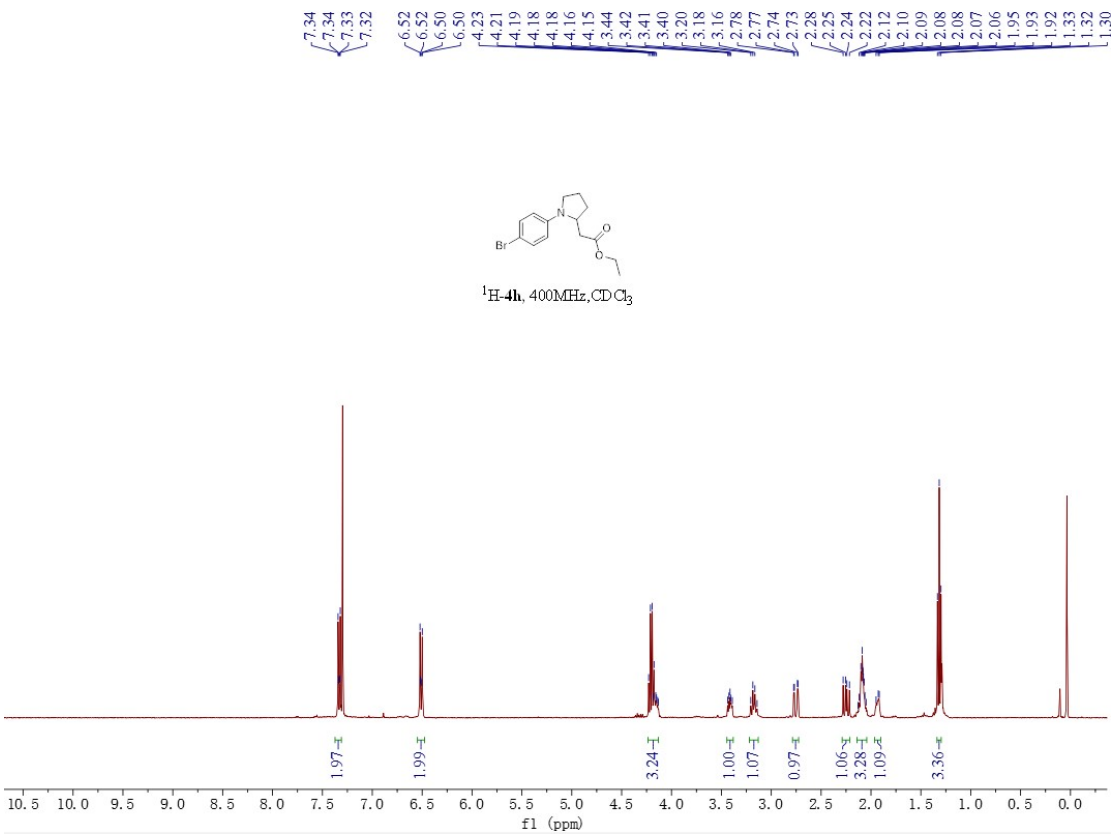
^{1}H-**4d**, 400MHz, $CDCl_3$


6.92 6.91 6.90 6.90 6.89 6.88 6.87 6.63 6.61 4.22 4.21 4.19 4.17 4.16 4.15 4.15 4.14 4.13 4.12 4.11 4.11 4.11 3.79 3.43 3.41 3.18 3.16 2.82 2.81 2.78 2.77 2.24 2.23 2.12 2.10 2.08 2.08 2.07 2.05 2.03 1.91 1.89 1.33 1.31 1.30
1H-4e, 400MHz, CDCl3
2.07 1.91 3.00 3.02 1.00 0.99 1.00 0.93 3.08 1.02 3.24
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

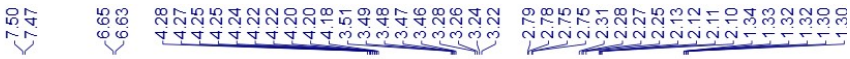

7.01 7.00 7.00 6.99 6.99 6.98 6.97 6.97 6.96 6.57 6.56 6.56 6.55 6.54 4.23 4.21 4.19 4.17 4.17 4.16 4.15 4.14 4.13 4.12 3.43 3.42 3.40 3.18 3.16 2.79 2.78 2.75 2.74 2.28 2.25 2.24 2.21 2.12 2.11 2.10 2.09 2.09 2.08 2.08 2.07 2.06 2.06 2.05 2.04 1.94 1.92 1.91 1.91 1.30 1.29 1.29 1.28 1.27 1.26
1H-4f, 400MHz, CDCl3
2.15 1.94 3.23 1.00 1.00 1.02 1.16 3.28 1.11 3.34
0.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

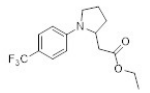


^{1}H-**4g**, 400MHz, $CDCl_3$

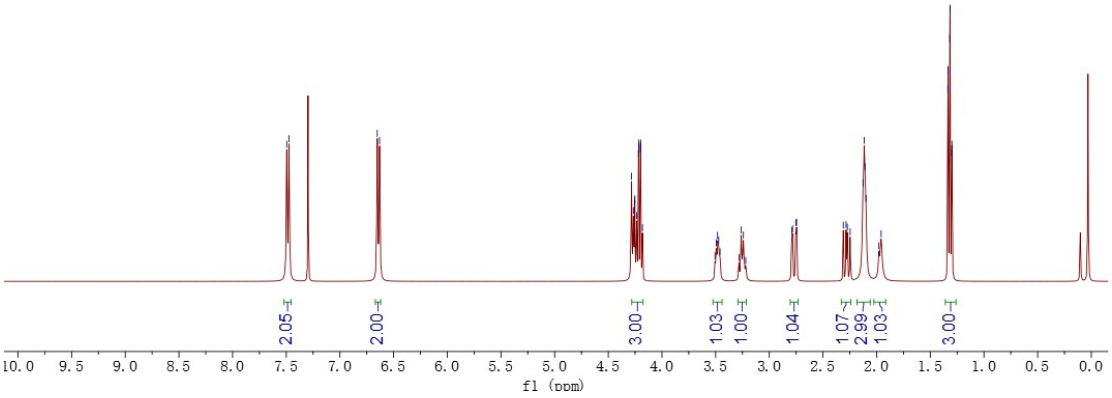


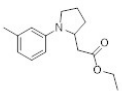
^{1}H-**4h**, 400MHz, $CDCl_3$



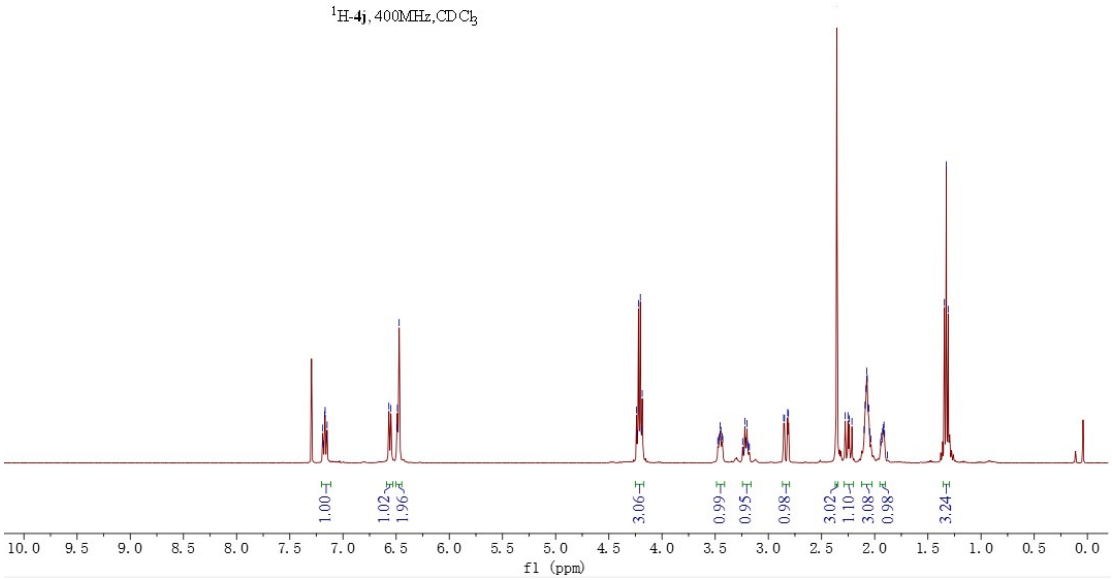


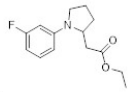
^{1}H-**4i**, 400MHz, $CDCl_3$



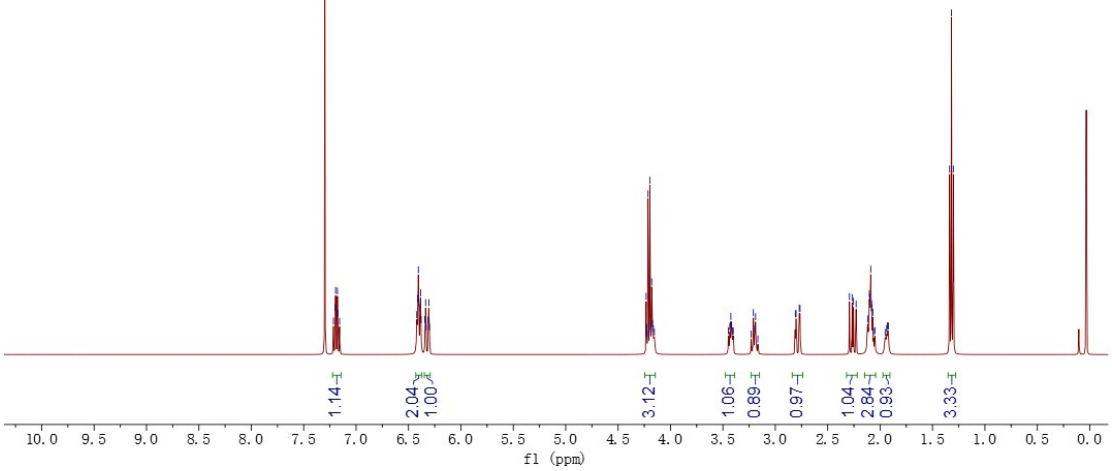


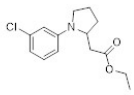
^{1}H-**4j**, 400MHz, $CDCl_3$



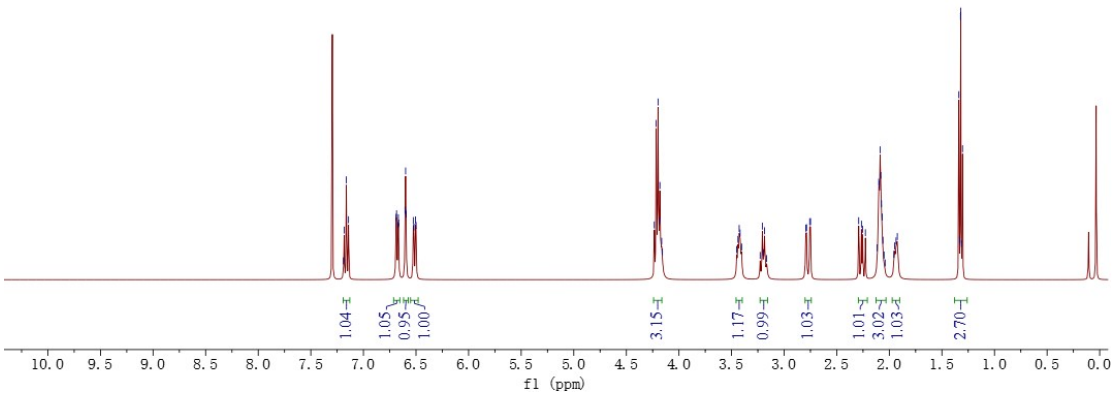


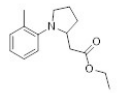
^{1}H-**4j**, 400MHz, $CDCl_3$



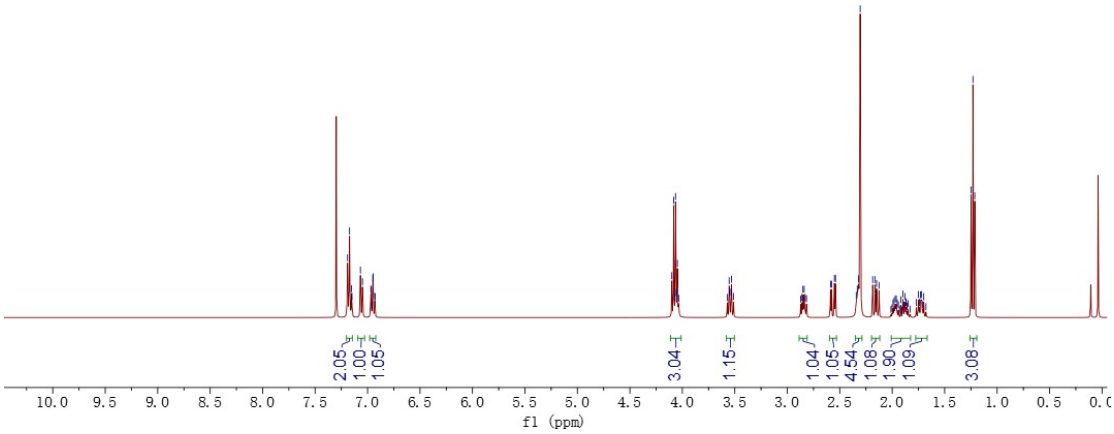


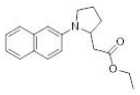
^{1}H-**4k**, 400MHz, $CDCl_3$



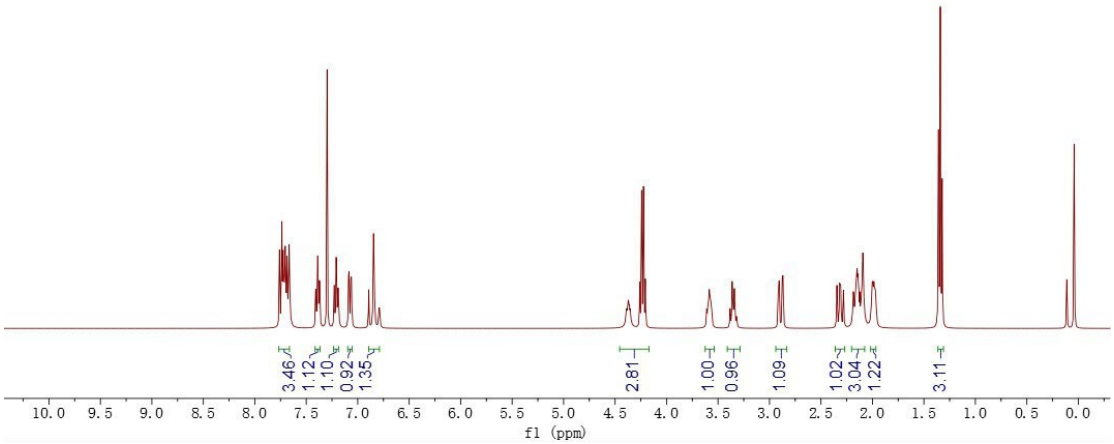


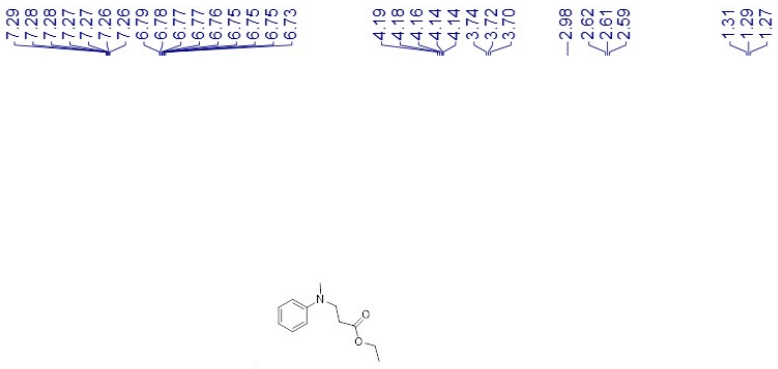
^{1}H-**4l**, 400MHz, $CDCl_3$



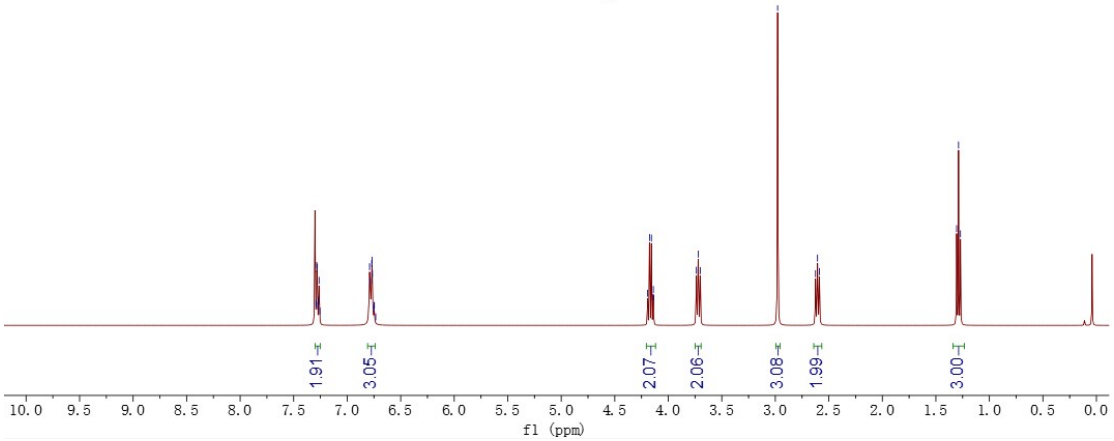


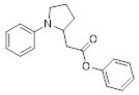
^{1}H-**4n**, 400MHz, $CDCl_3$



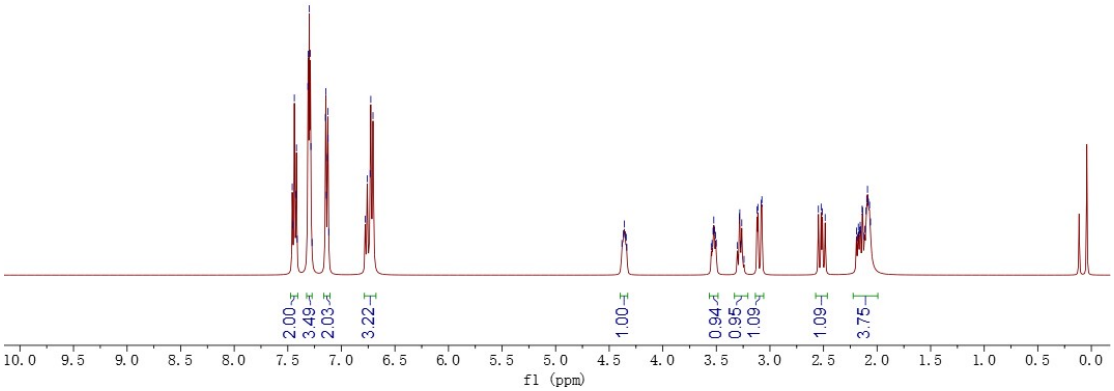


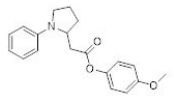
^{1}H-**4o**, 400MHz, $CDCl_3$



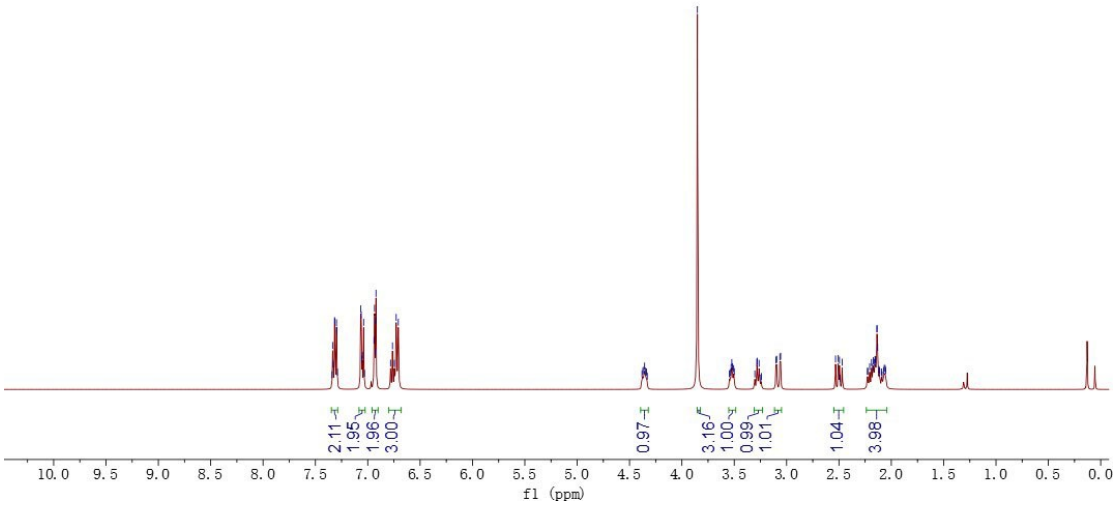


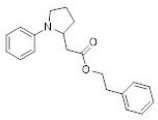
^{1}H-**4p**, 400MHz, $CDCl_3$



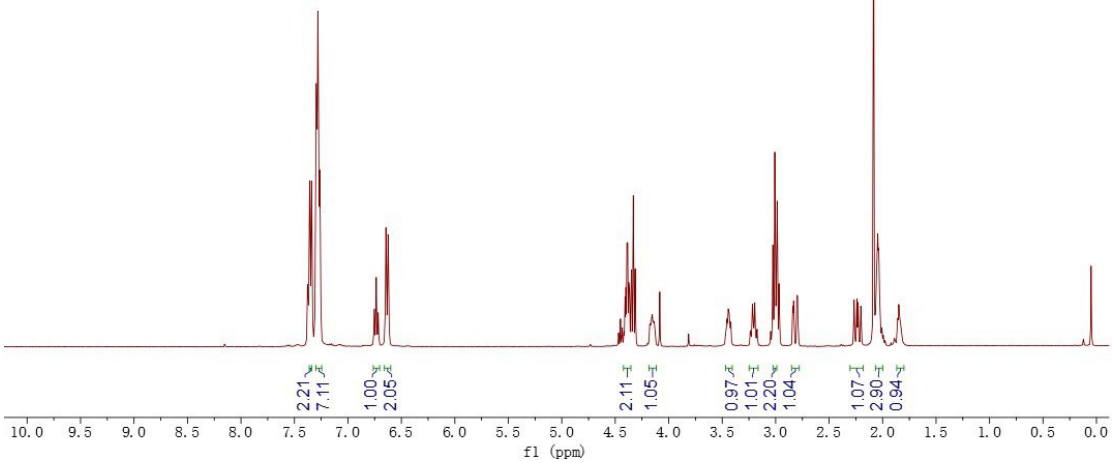


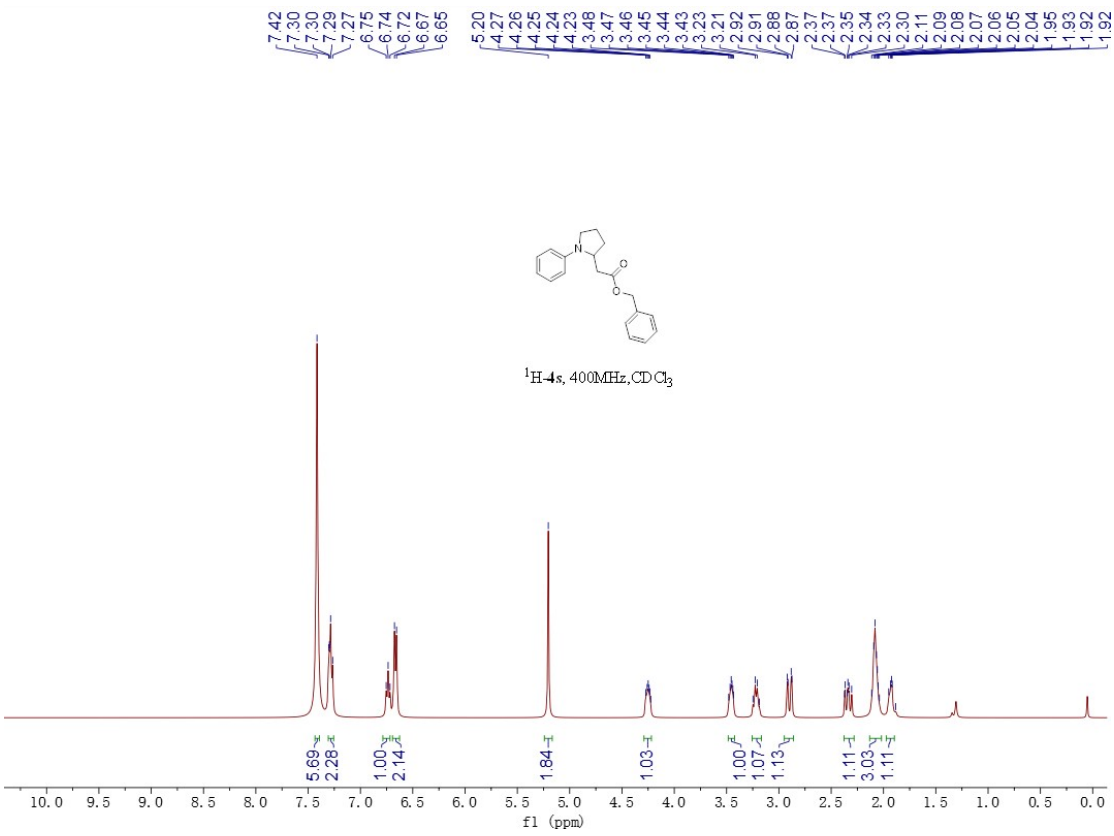
^{1}H-**4q**, 400MHz, $CDCl_3$



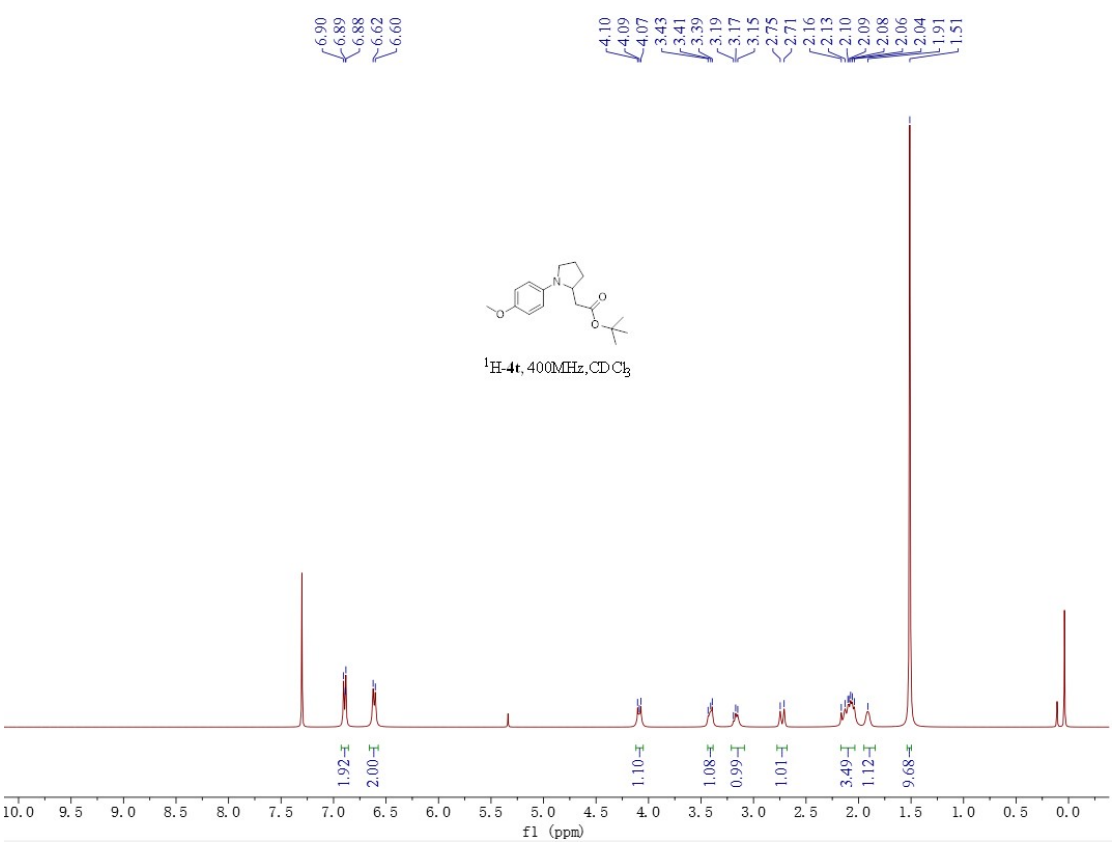


^{1}H-**4r**, 400MHz, $CDCl_3$





^{1}H-**4s**, 400MHz, $CDCl_3$



^{1}H-**4t**, 400MHz, $CDCl_3$