Supplementary Information

A highly efficient one-enzyme protocol using ω-transaminase and an amino donor enabling equilibrium displacement assisted by molecular oxygen

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1. General information

All pro-chiral ketones and candidate amino donors were commercially available and purchased from Sigma-Aldrich (China Mainland) and were used as received without further

purification. All chemicals used are of analytical grade. ^{1}H- and ^{13}C -NMR were recorded on a Bruker 500 MHz spectrometer at 500 MHz and 125.8 MHz, respectively. All chemical shifts (δ) were quoted in parts per million (ppm) and reported relative to an internal tetramethylsilane (TMS, δ 0.00) standard. The following abbreviations were used to define the multiplicities: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; p, pentet and m, multiplet. The conversions of prochiral ketones were measured by HPLC analysis equipped with a C18 column, and the ee values of the desired chiral amines were measured by HPLC analysis equipped with a coating Silica-gel Crownpak CR(+) (0.4 cm x 15 cm) column, both the conversions and ees were recorded with a Shimadzu Essentia Prep LC-16P HPLC instrument. Absolute configuration of product was determined by comparing with the standard pure products. Kinetic constants were recorded on a dual beam spectrophotometer (Cary 300 UV-Vis, Varian Inc.) with appropriate blank correction.

2. General procedure for the cloning, protein expression and purification of transaminase

The gene for Chromobacterium violaceum ω-transaminasewas inserted in the plasmid pET28a(+) with an N-terminal His6-tag. After digestion (NheI, HindIII) and sequence verification the construct was transformed into *E. coli BL21 (DE)*. Expression was done by mixing a 20 ml overnight culture with 180 ml of Luria-Bertoni (LB) medium with 50 mg/L Kanamycin and 0.4 mM IPTG and incubating for 24 h at 25 °C (150 rpm, baffled flask). The cells were thereafter separated from the medium by centrifugation and resuspended in IMAC binding buffer (20 mM sodium phosphate, 500 mM sodium chloride, pH 8.2), disrupted by addition of BugBuster® 10X (Merck) and applied to a column with Chelating Sepharose FastFlow (GE Healthcare) resin treated with a saturated water solution of Cobalt(II) chloride. After washing with the binding buffer, the His6-tagged enzyme was eluted with IMAC elution buffer (20 mM sodium phosphate, 500 mM sodium chloride, 500 mM Imidazole, pH 8.2, (for

Chromobacterium violaceum ω-transaminase W60C pH 7.0). An excess of cofactor (PLP) was added before desalting on a PD10 column (GE Healthcare). The simple procedure of adding PLP before buffer change ensures that the amount of cofactor is balanced with the enzyme concentration, assuming that a negligible amount of enzyme is in apo form during the desalting.

3. Enzyme reactions

The reaction was performed in 50 mM HEPES buffer solution on a 1 ml scale, the pH of buffer is 7.0 for Chromobacterium violaceum ω-transaminase W60C and 8.2 for other ω-transaminases. All components were dissolved separately in the buffer and pH corrected. These solutions were then combined to final concentration of 5 mM acetophenone or other substituted-acetophenones (4-methylacetophenone, 4-methoxylacetophenone, 4-nitroacetophenone, 4-chloroacetophenone), benzylamine or benzylmethylamine (SigmaAldrich), 15 mM, and transaminase (purified ω-transaminases, $[E]_{395}$ = 0.104, approximately 0.8 U, ATA 117 from Codexis**, 0.8 U).**

4. General procedures oxidation of aldehydes using O_2

A closed reaction vessel, charged with 5 mL HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid) buffer at 50 mM, aldehyde was introduced to form a final concentration of 1.0mM (benzaldehyde (n = 1)) or 0.8 mM (phenylacetaldehyde(n = 2)). Oxygen was flushed into the vessel and sealed with a balloon filled with oxygen. After that, the reaction mixture was heated to room temperature and kept in a shaker incubator (160rpm/min). After reaction completed, samples were taken for further HPLC analysis using a SHIMADZU LC-16 instrument equipped with a C18-WR column at the wavelength of 254 nm.

5. Determination of yields of products

The reactions were performed as described in Chapter 3 Enzyme reactions. When reactions were completed, perchloric acid of 10 uL at the concentration of 70% was added into the

reaction solution, and the mixed solution was centrifuged at 3000 r/min for 5 min. After that, 300 uL solution of the supernatant fluid was taken and subjected to High Performance Liquid Chromatography (HPLC) equipped with a C18 column. Before calculating the conversions, we prepared standard curves with authentic standards (Figure S1).

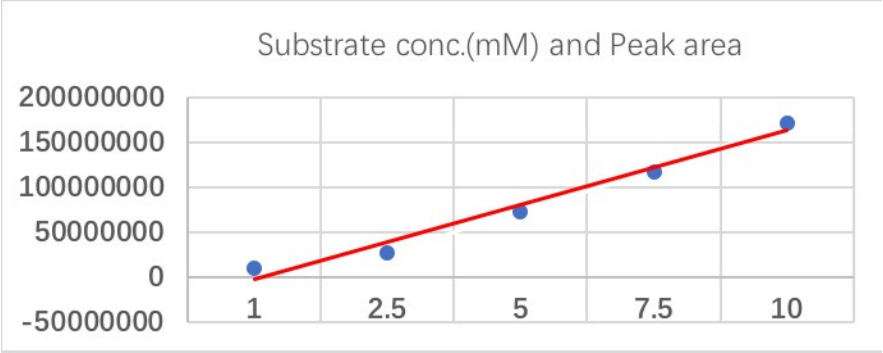


Figure S1 Standard curves

6. Determination of enantiomeric excess of chiral amines

The samples were subjected to high pressure liquid chromatography (HPLC) with a CrownpakCR(+) column (Daicel), after filtration (0.22 μm) and acidification by perchloric acid. Ultrapure water with addition of perchloric acid to pH 1.6 was used as mobile phase at the flow rate of 0.5 ml/min.

$$ee_S = \frac{[S]-[R]}{[S]+[R]} \quad or \quad ee_R = \frac{[R]-[S]}{[R]+[S]} \qquad \text{(Equ.1)}$$

7. General procedure for isolating pure products

To obtain the desired pure chiral amines, reactions were performed on a 10-mL scale, reaction parameters were identical with which described in the footnote of Table 1 and Table 2 in the main text of the manuscript. When reactions were completed, 100 uL perchloric (70%) was added into the reaction solution to acidate the solution, and the reaction solution was centrifuged for 5 min to remove the precipitate.

The supernatant was added into with 5 mL ethyl acetate, mixed and extracted three times. The organic solution was isolated and combined together. The combined organic solution was then distilled under evacuation to remove the organic solvent, and the residues were chromatographed to afford the desired pure amines.

8. ^{1}H NMR and ^{13}C NMR spectrum of chiral amines

7.1 (*S*)- or (*R*)- 1-phenylethanamine

Colorless liquid, ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25 (d, J = 2.5 Hz, 3H), 4.12 (q, J = 10.0 Hz, 1H), 7.22-7.25 (m, 2H), 7.32-7.36 (m, 3H). ^{13}C NMR (125.8 MHz, CDCl3) δ 25.7, 51.3, 75.8, 125.7, 126.8, 128.5.

7.2 (*S*)- or (*R*)- 1-(4-chlorophenyl)ethanamine

Colorless oil, ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.36 (d, J = 2.5 Hz, 3H), 4.11 (q, J = 7.5 Hz, 1H), 7.29-7.38 (m, 4H). ^{13}C NMR (125.8 MHz, CDCl3) δ 25.8, 50.7, 127.1, 128.5, 132.3, 146.2.

7.3 (*S*)- or (*R*)- 1-(4-flurophenyl)ethylamine (C-F decoupled)

Colorless oil, ^{1}H NMR (400MHz, DMSO) δ 1.23 (d, J = 4 Hz, 3H), 4.01 (q, J = 8 Hz , 1H), 7.08-7.14 (m, 2H), 7.38-7.43 (m, 2H). ^{13}C NMR (400 MHz, DMSO) δ 26.8, 50.5, 115.2, 128.1, 145.4, 160.0.

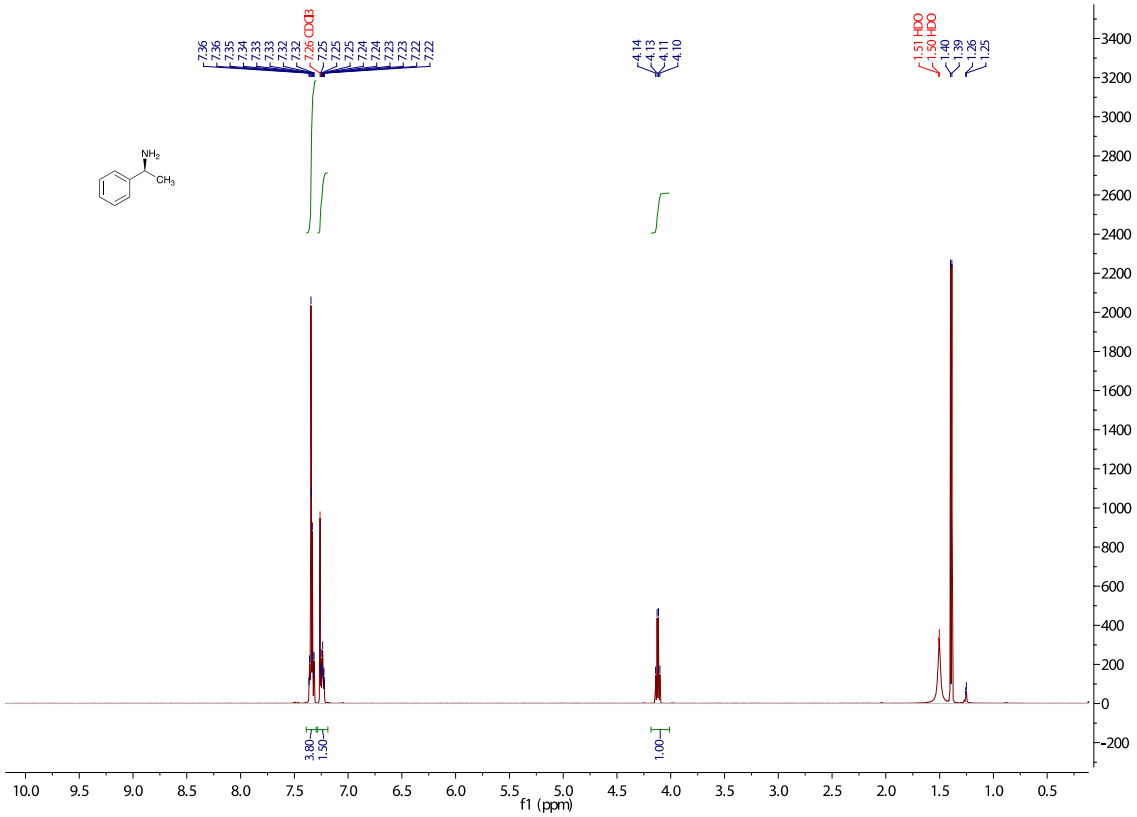
7.4 (*S*)- or (*R*)- 1-(p-tolyl)ethanamine

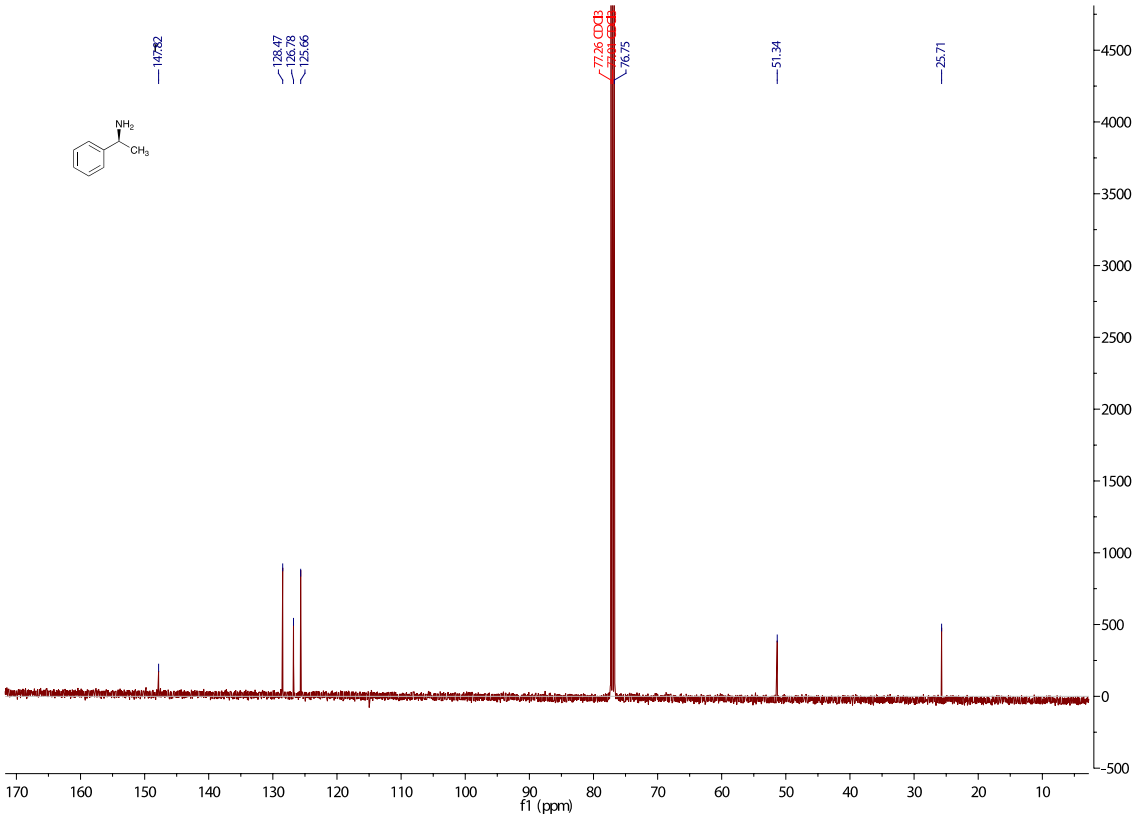
Colorless oil, ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (d, J = 2.5 Hz, 3H), 2.34 (s, 3H), 4.08 (q, J = 7.5 Hz, 1H), 7.14-7.15 (m, 2H), 7.23-7.25 (m, 2H). ^{13}C NMR (125.8 MHz, CDCl3) δ 21.0, 25.7, 51.0, 76.8, 125.6, 129.1, 136.4, 144.9.

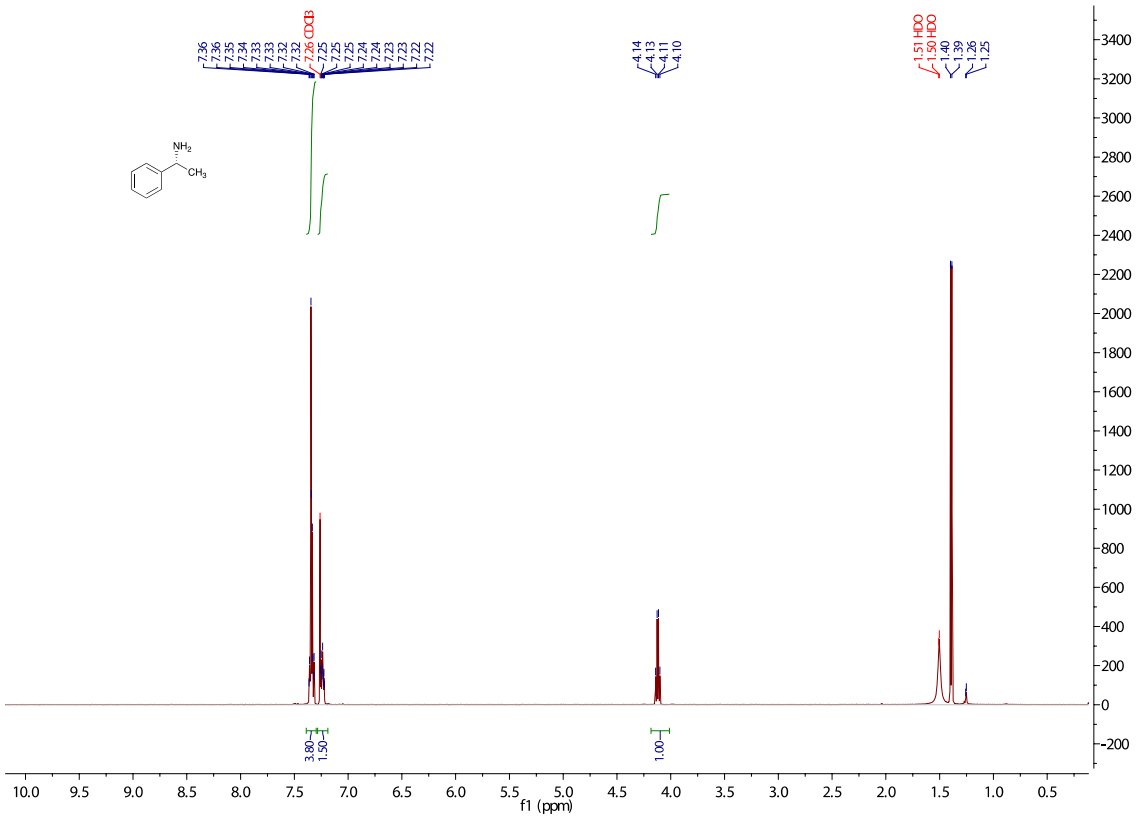
7.5 (*S*)- or (*R*)- 1-(3-methylphenyl)ethylamine

Colorless oil, ^{1}H NMR (400 MHz, DMSO) δ 1.24 (d, J = 4 Hz, 3H), 2.30 (s, 3H), 3.94 (q, J = 8 Hz, 1H), 6.99-7.02 (m, 2H), 7.13-7.20 (m, 2H). ^{13}C NMR (400 MHz, CDCl3) δ 21.6, 26.7, 51.1, 123.3, 127.1, 128.4, 137.4, 149.2.

9. ^{1}H NMR and ^{13}C NMR spectra of chiral amines



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7.35
7.34
7.33
7.33
7.32
7.32
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7.25
7.25
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7.24
7.24
7.23
7.23
7.22
7.22
4.14
4.13
4.11
4.10
1.51 H2O
1.50 H2O
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1.39
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1.25
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3.80
1.50
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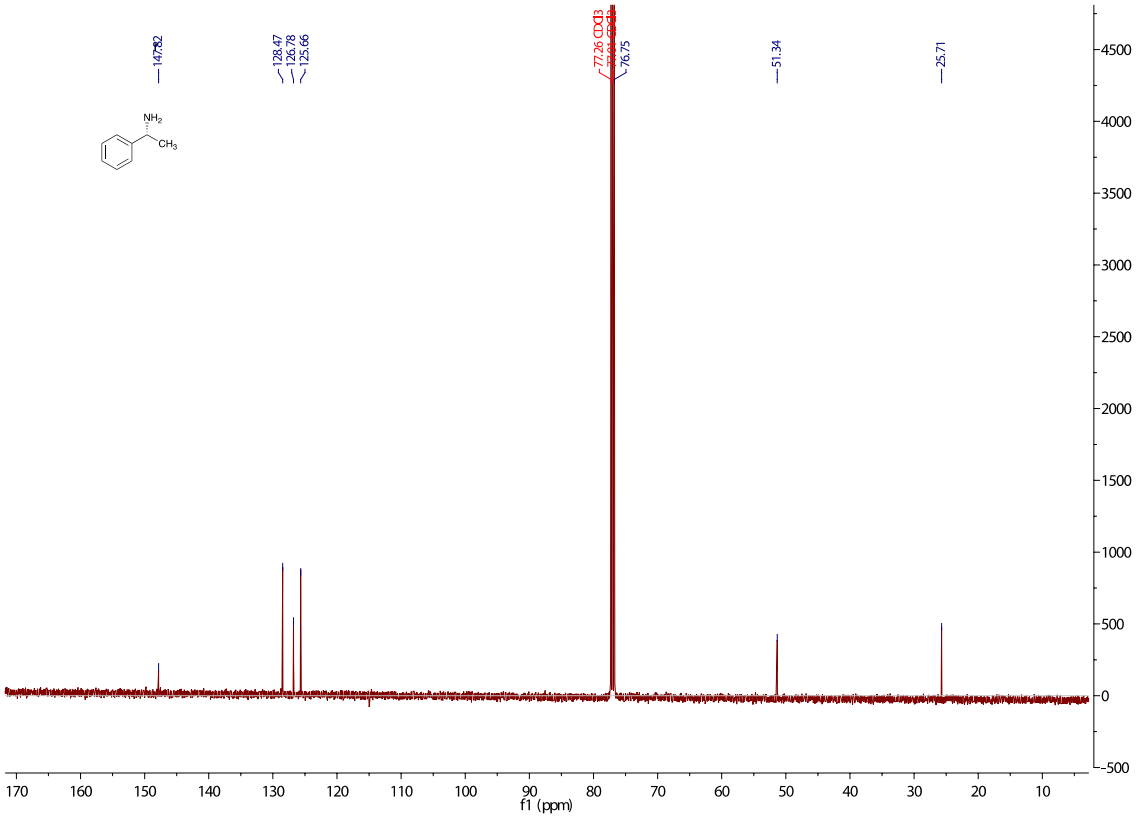
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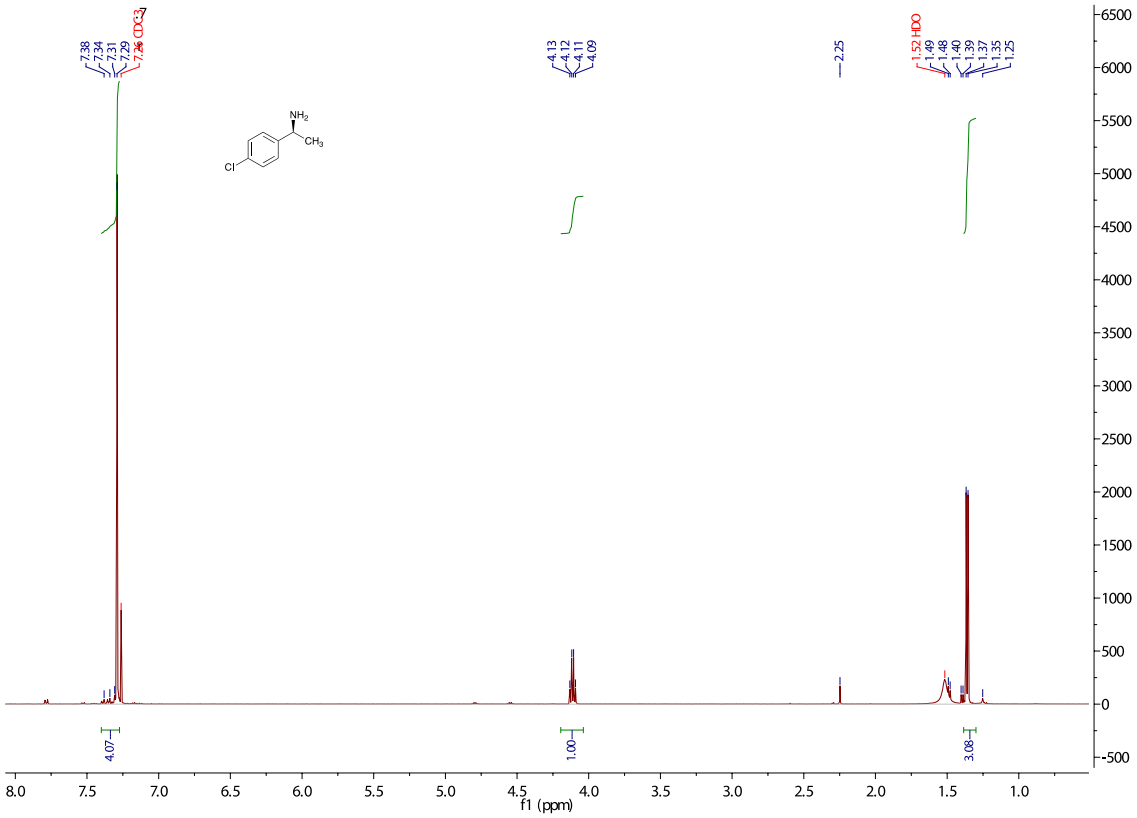
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7.34
7.31
7.29
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4.13
4.12
4.11
4.09
2.25
1.52 HDO
1.49
1.48
1.40
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1.37
1.35
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CH3
Cl
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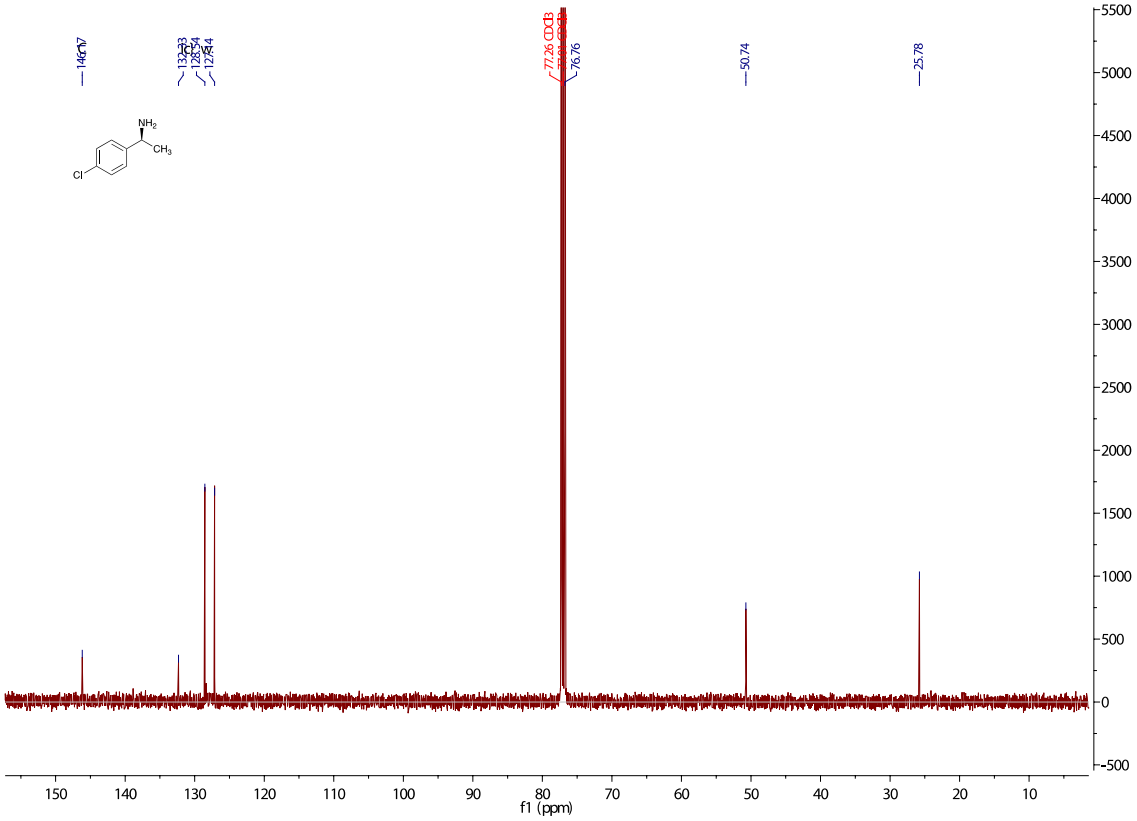
3.08
f1 (ppm)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CH3
Cl
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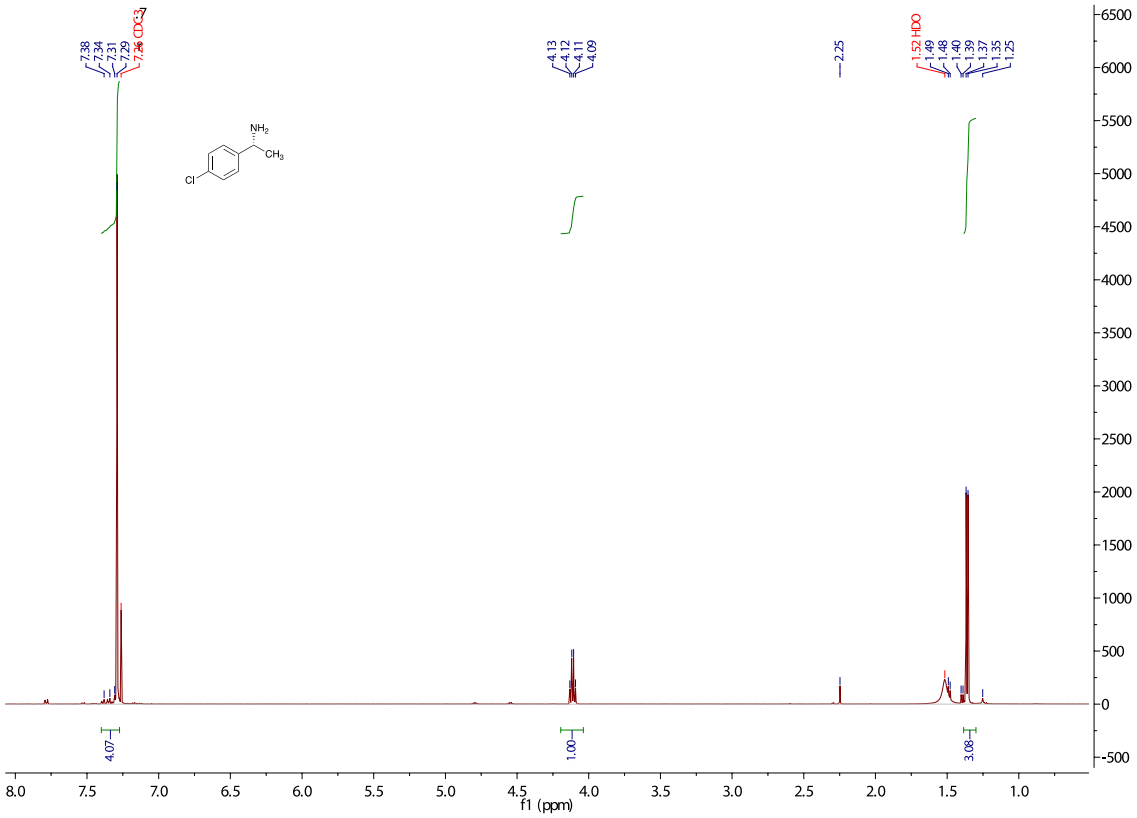
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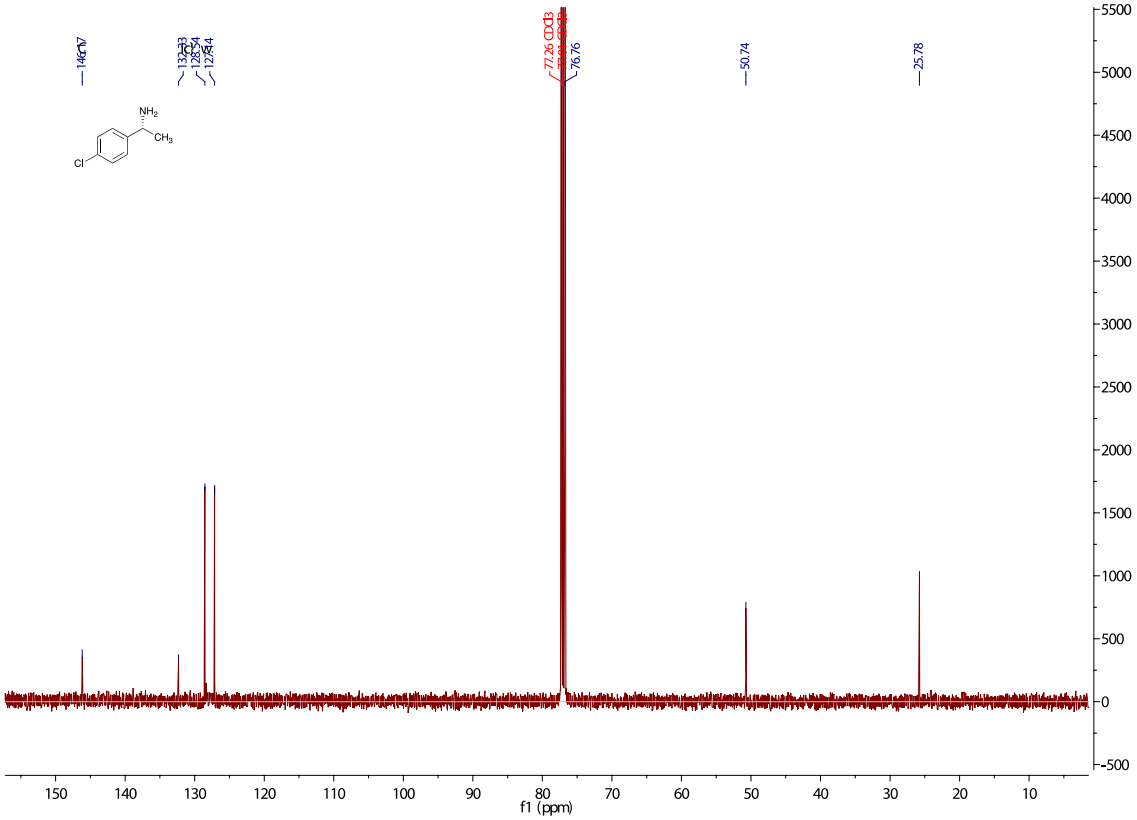
f1 (ppm)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Cl
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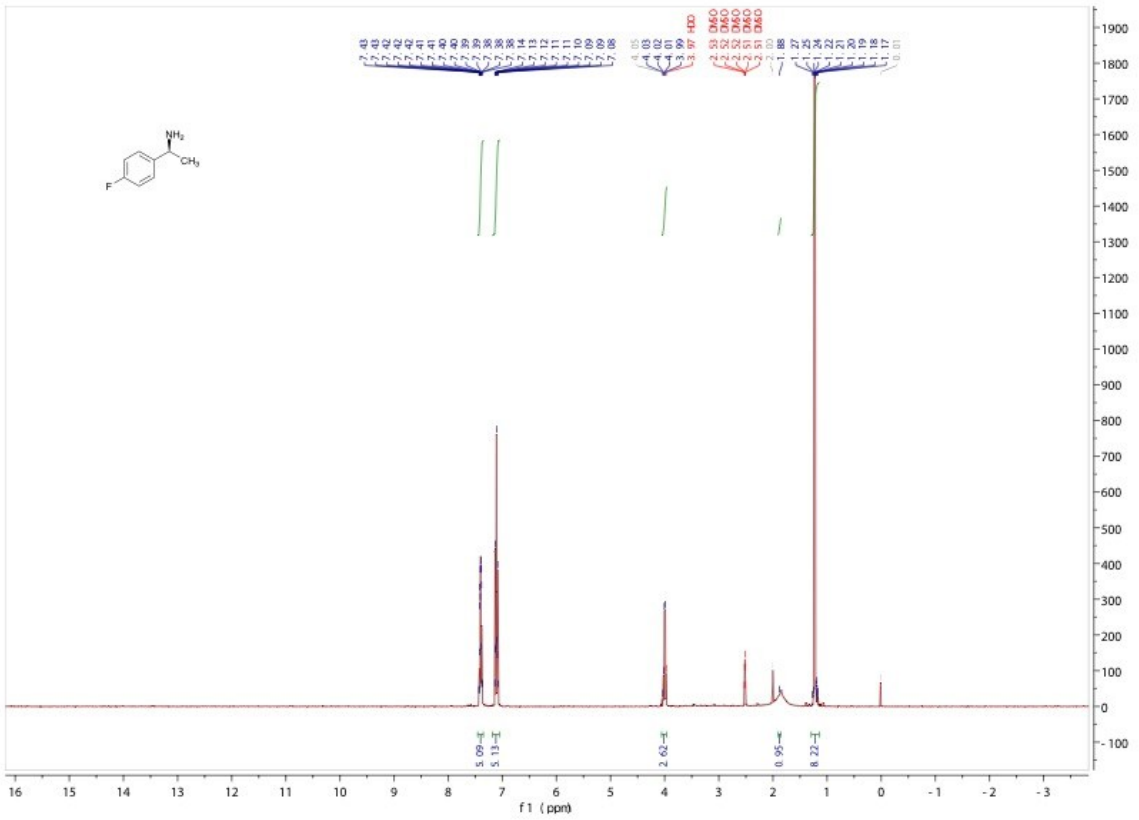
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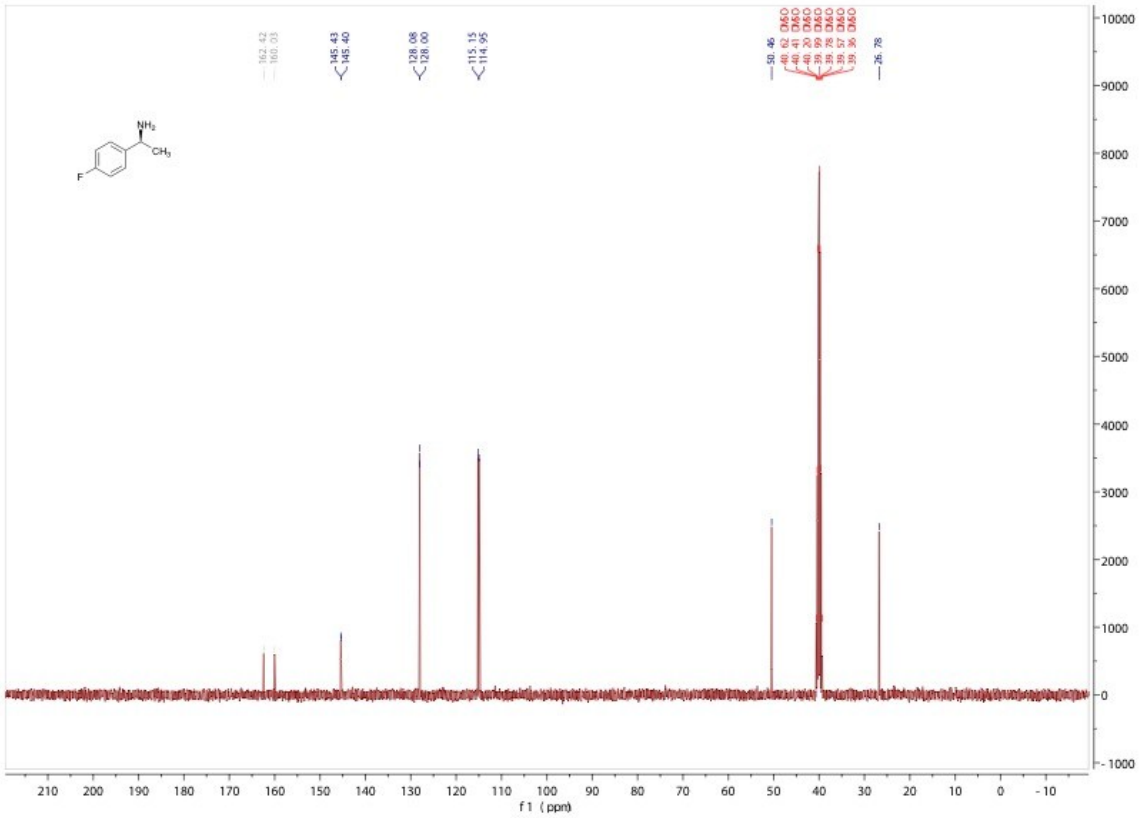
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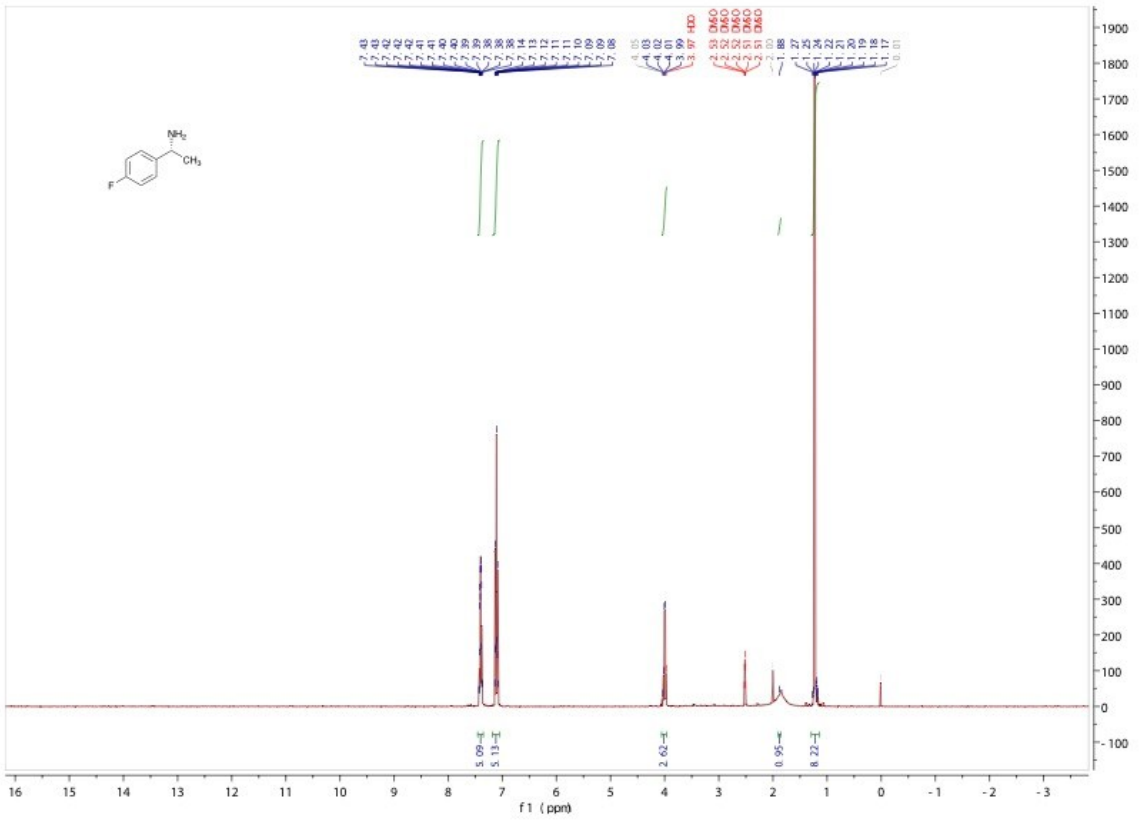
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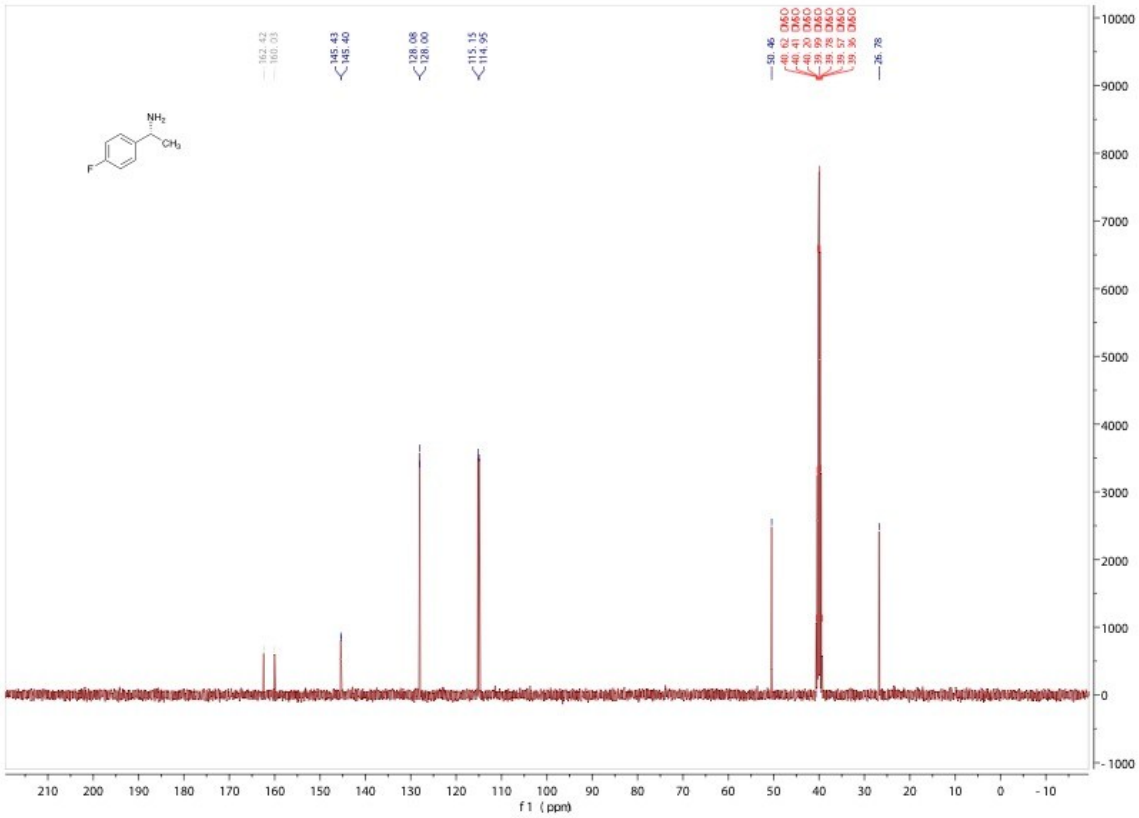
CH3
F
f1 (ppm)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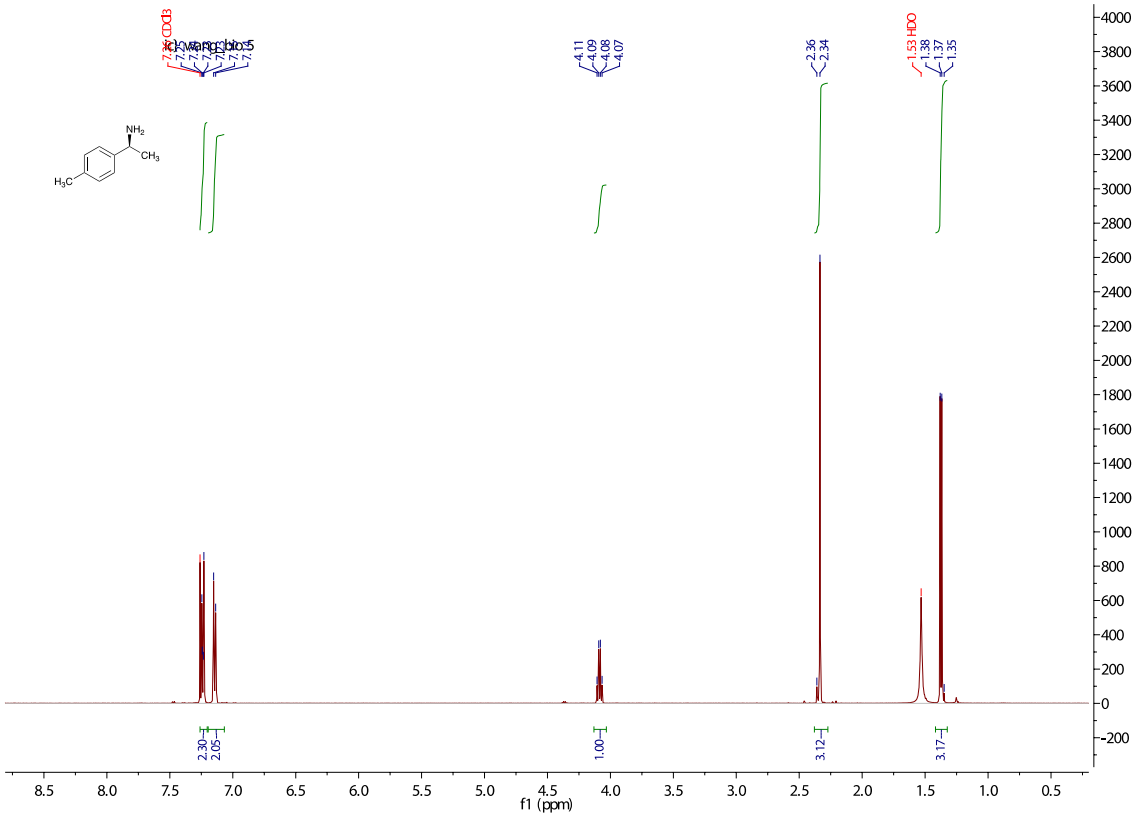
CH3
F
f1 (ppm)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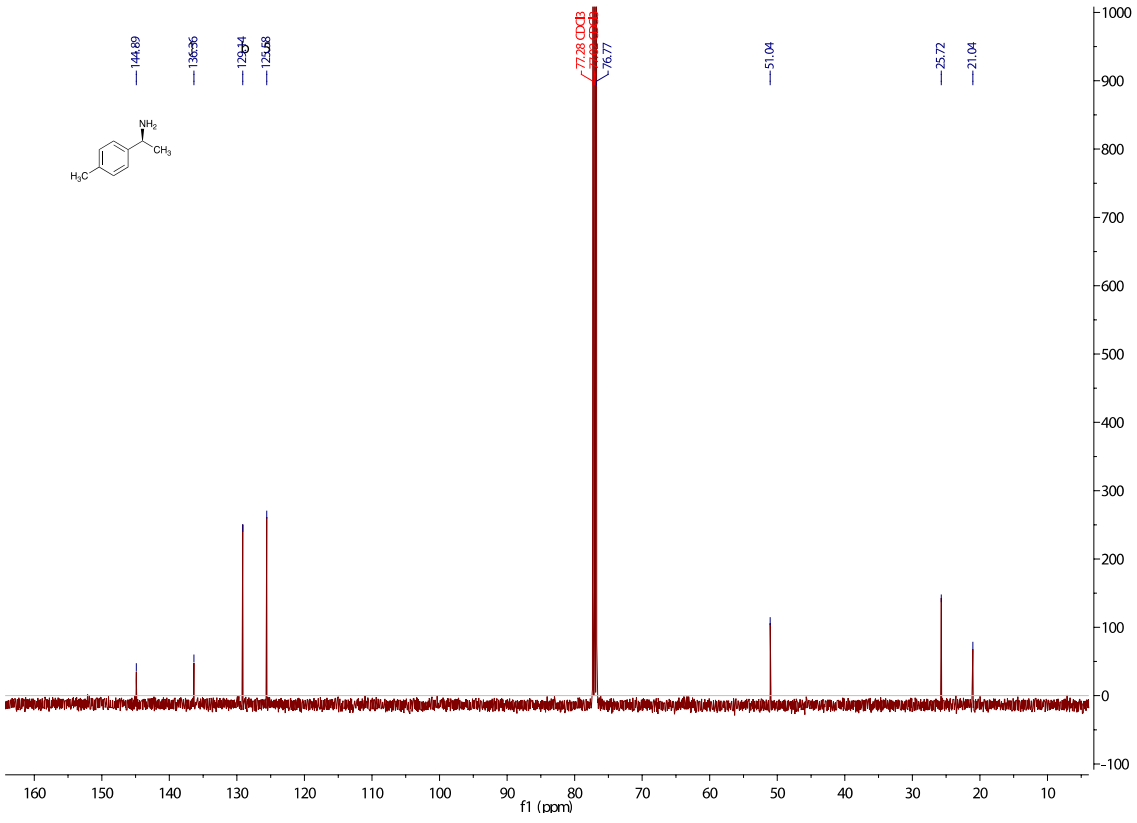
1.00
3.12
3.17
f1 (ppm)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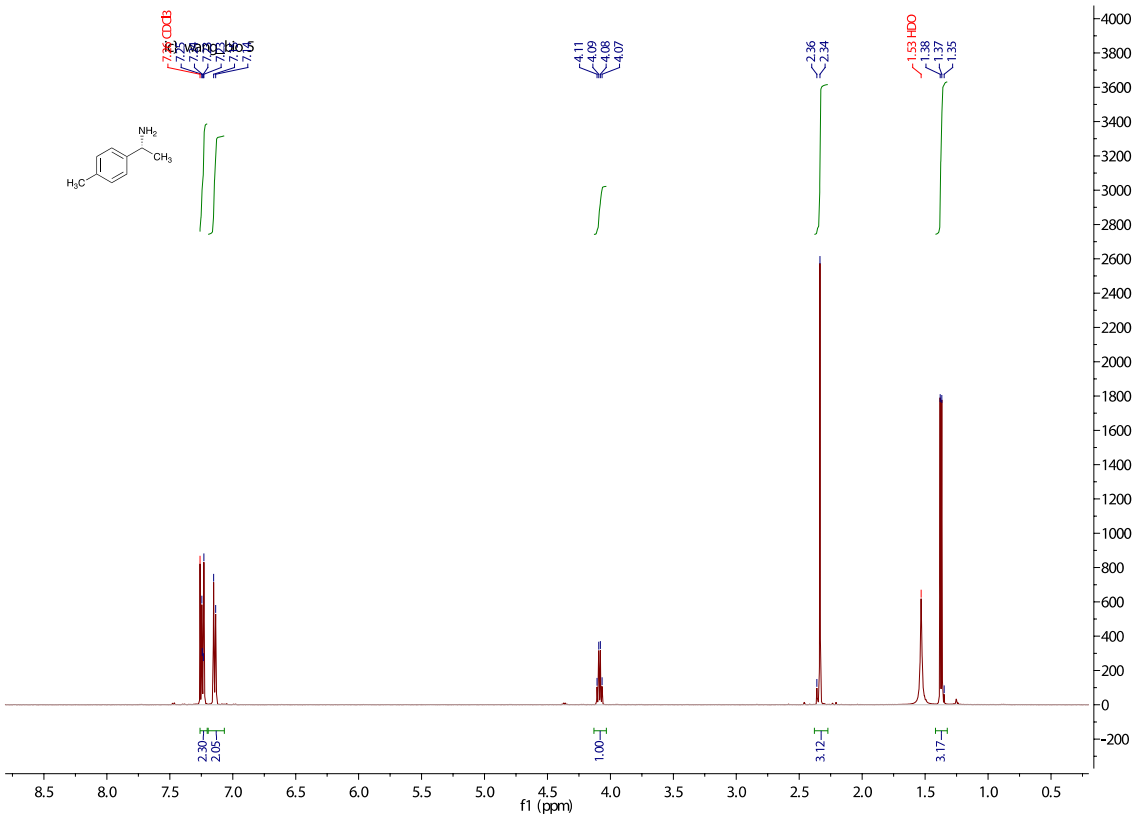
f1 (ppm)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4.07
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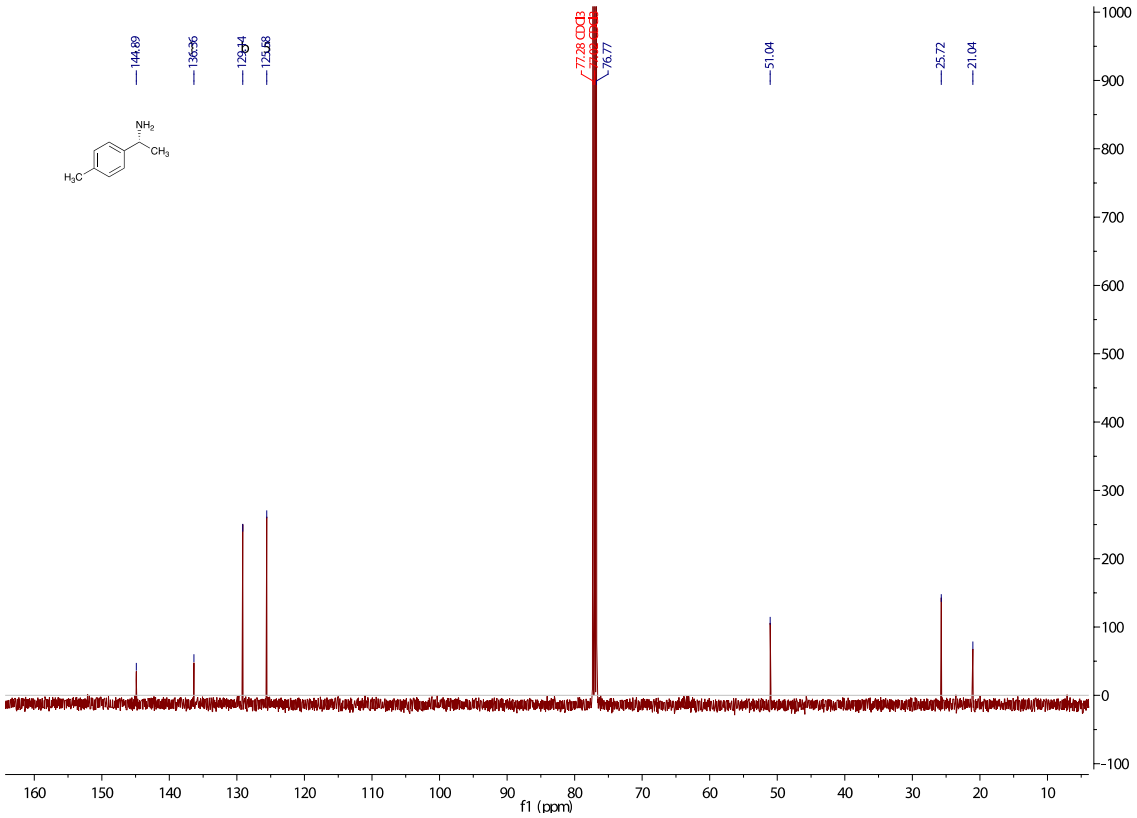
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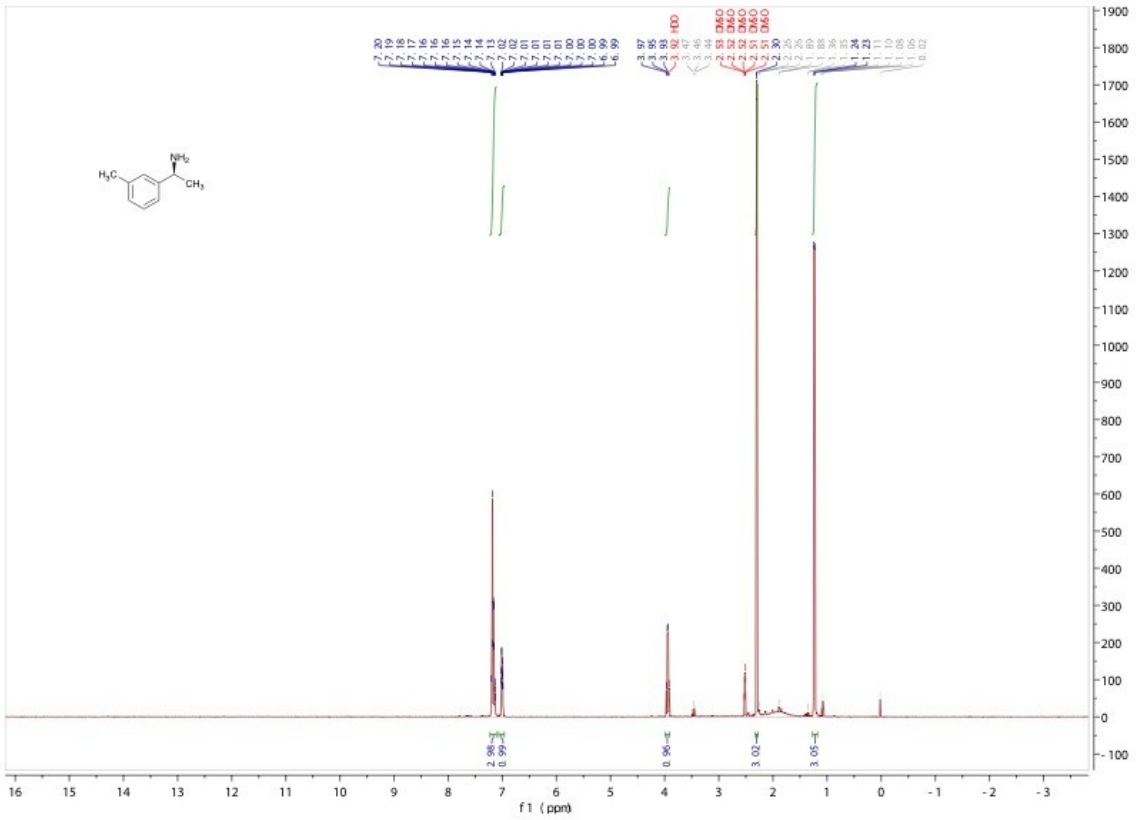
H3C
f1 (ppm)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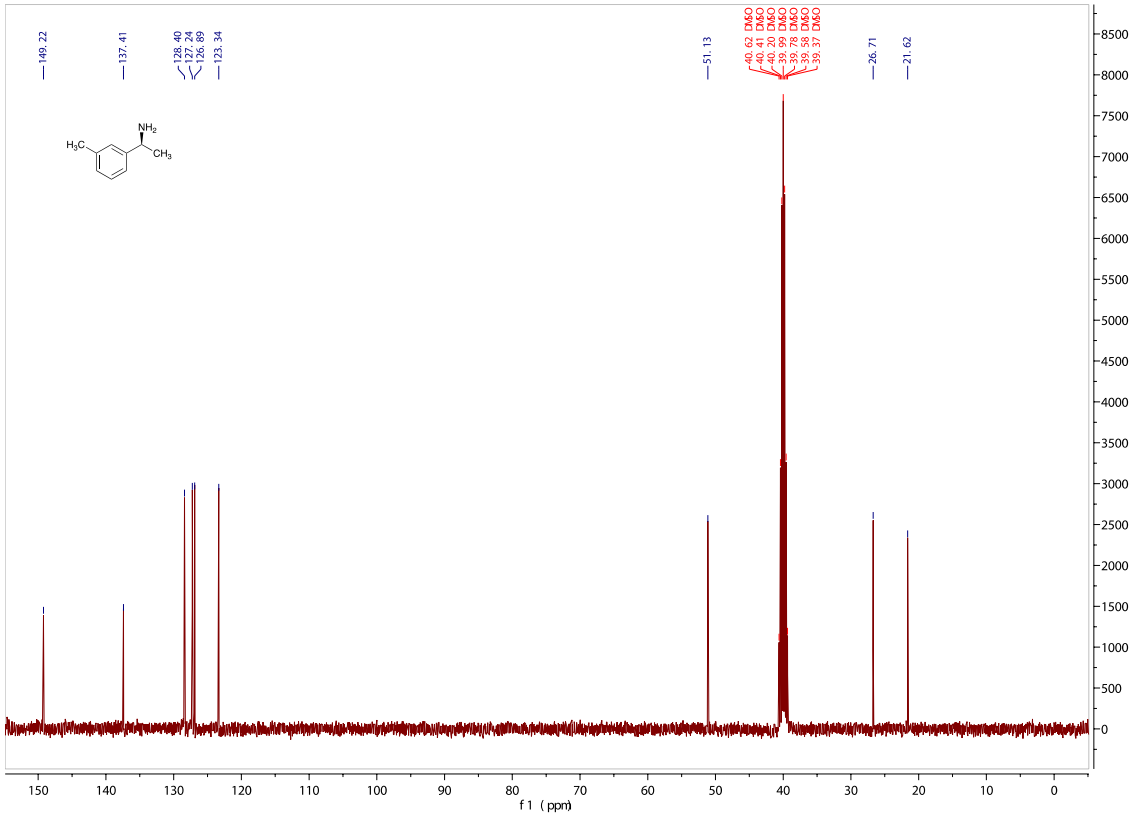
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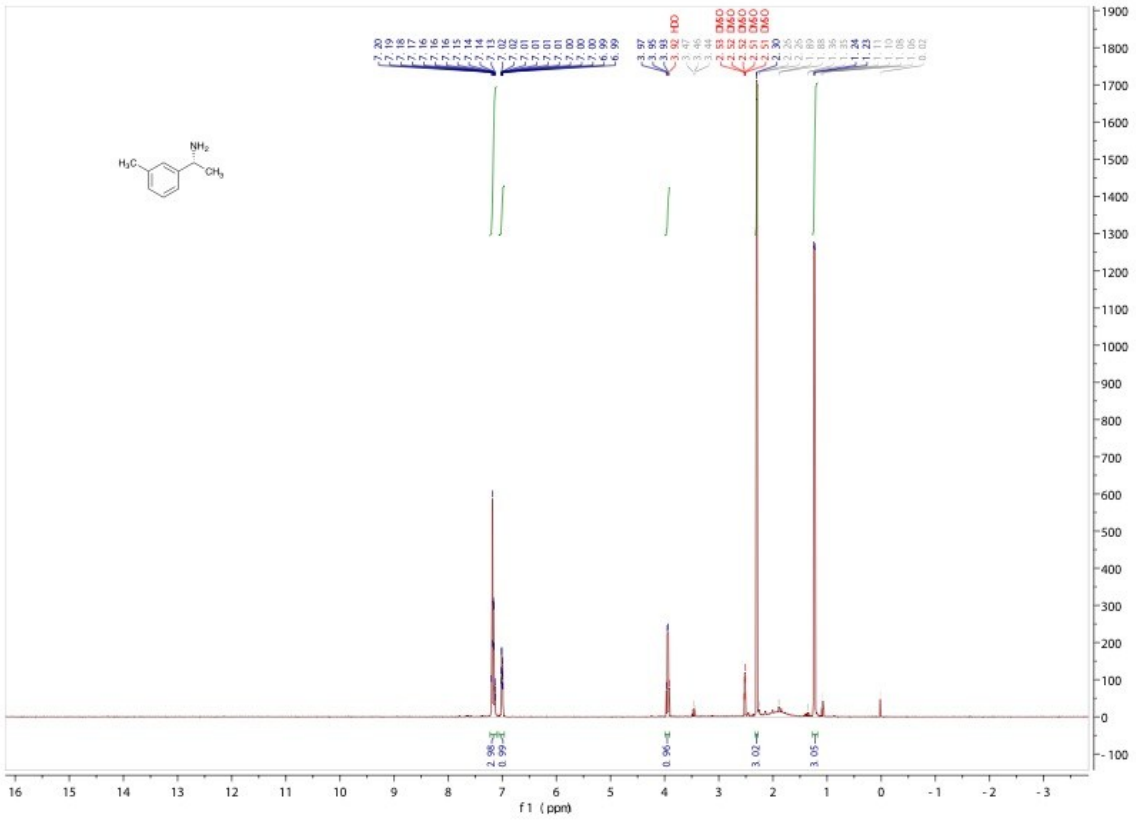
39.58 DMSO
39.37 DMSO
26.71
21.62
f1 (ppm)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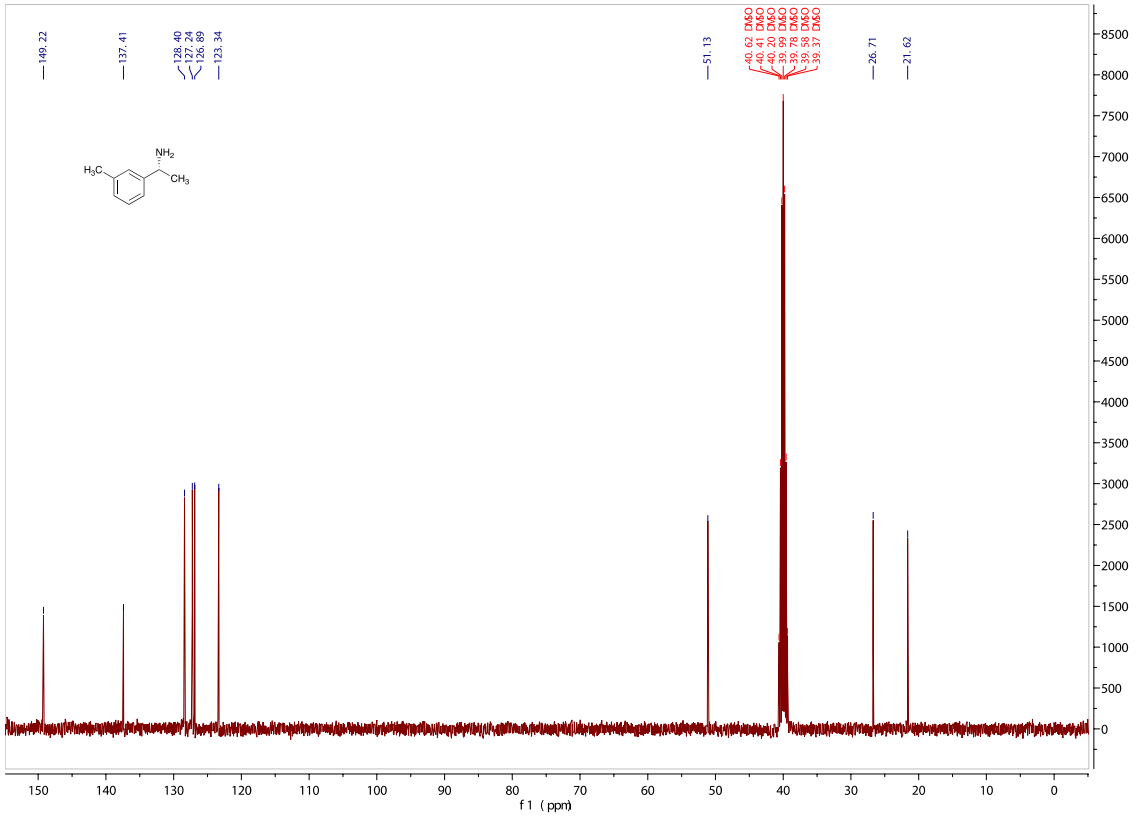
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127.24
126.89
123.34
51.13
40.62 DMSO
40.41 DMSO
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39.58 DMSO
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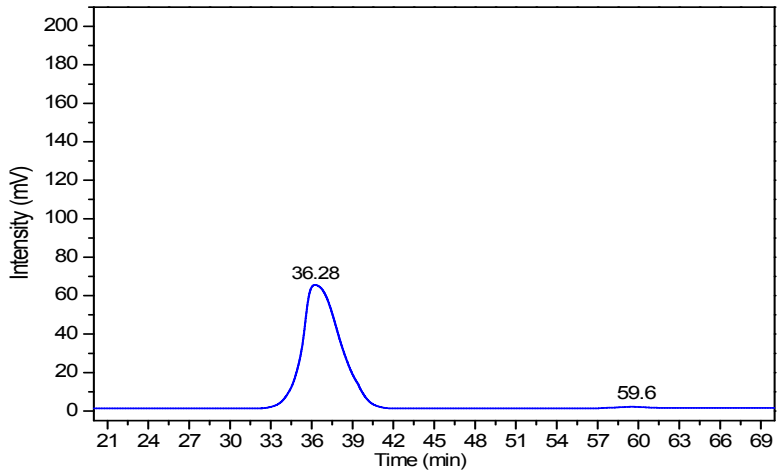
26.71
21.62
f1 (ppm)

10. HPLC chromatogram of products



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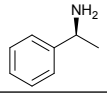
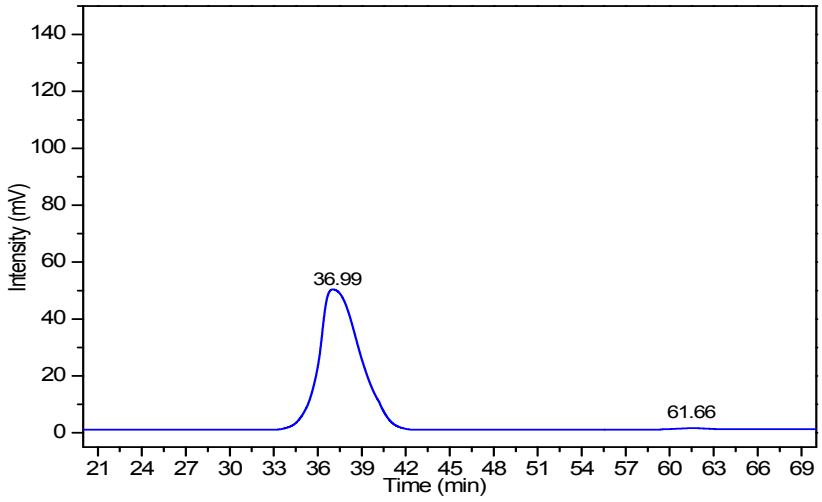
1		*S*	71968	*S*	36.28	99.7
		R	102	*R*	59.6	



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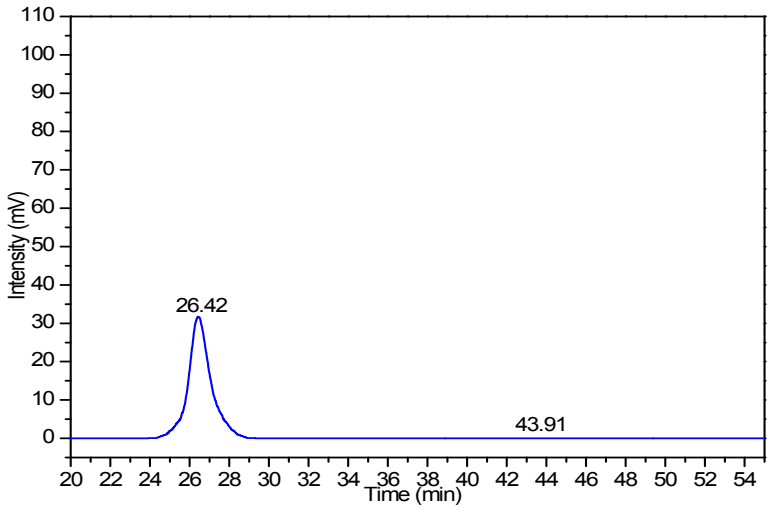
		R	106	*R*	61.66	



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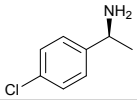
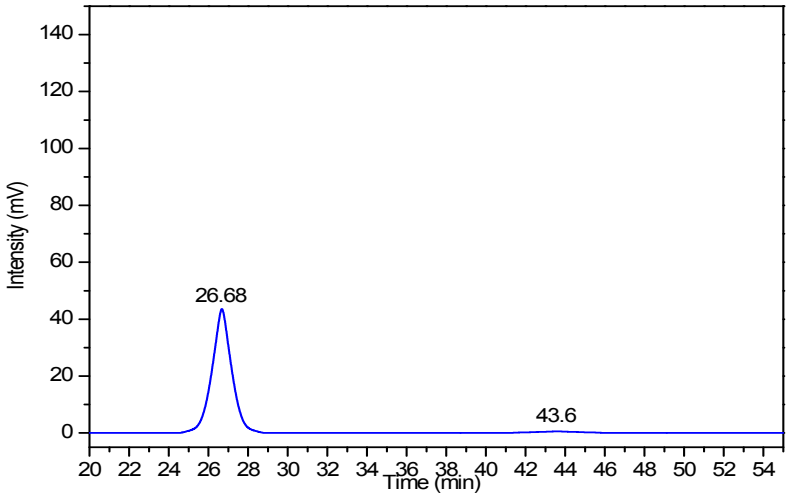
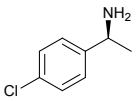
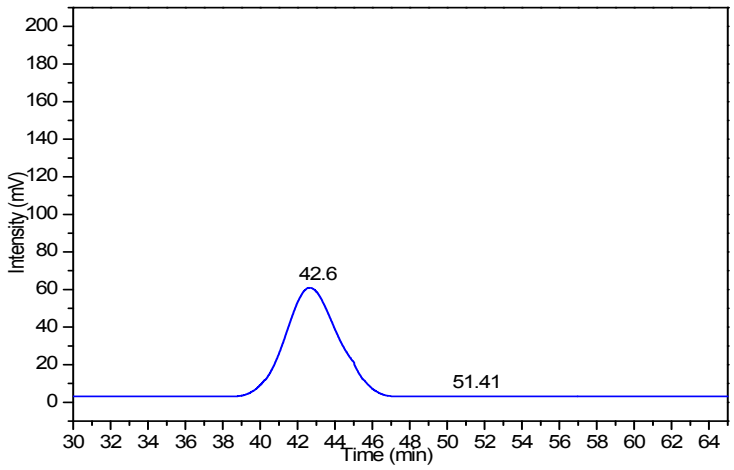
2		*S*	15189	*S*	26.42	99.9
		R	7	*R*	43.91	



Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
2		*S*	62155	*S*	26.68	99.7
		R	102	*R*	43.6	



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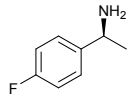
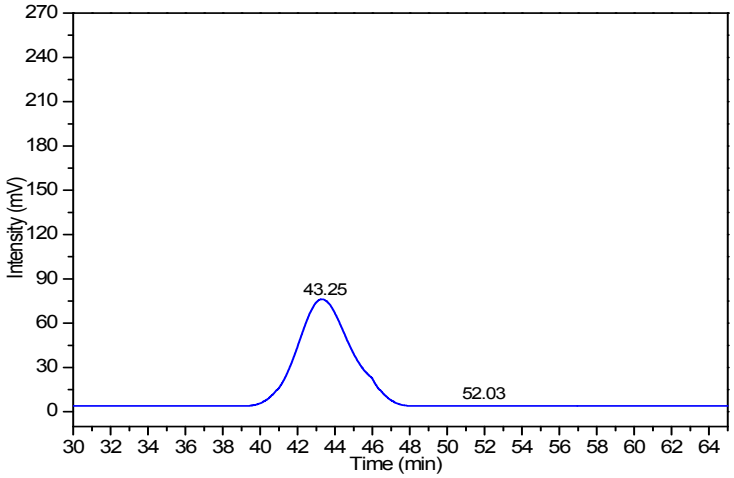
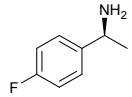
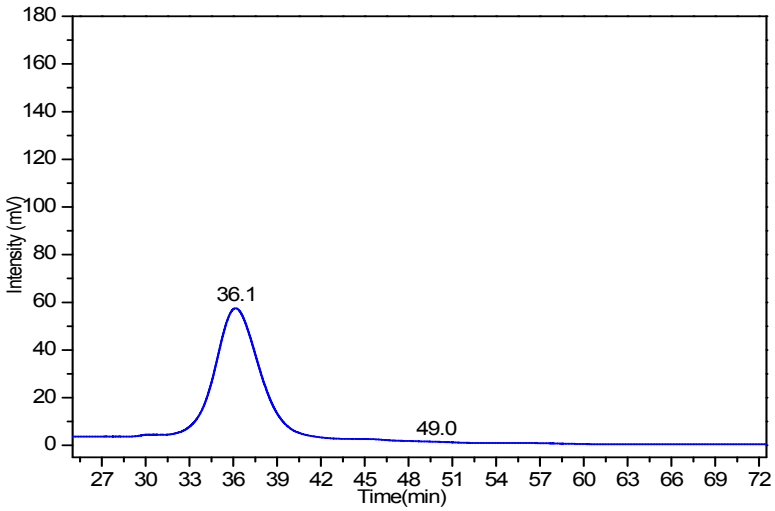
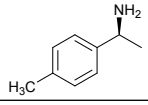
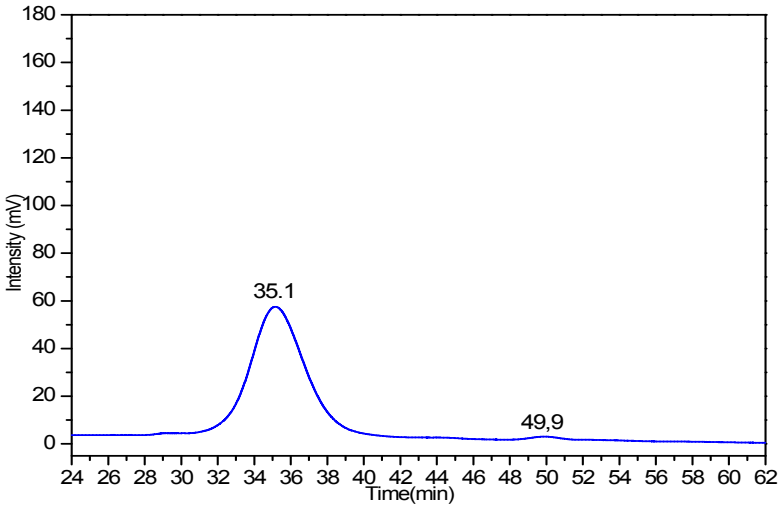
3		*S*	27867	*S*	42.6	99.9
		R	13	*R*	51.41	

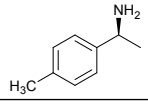


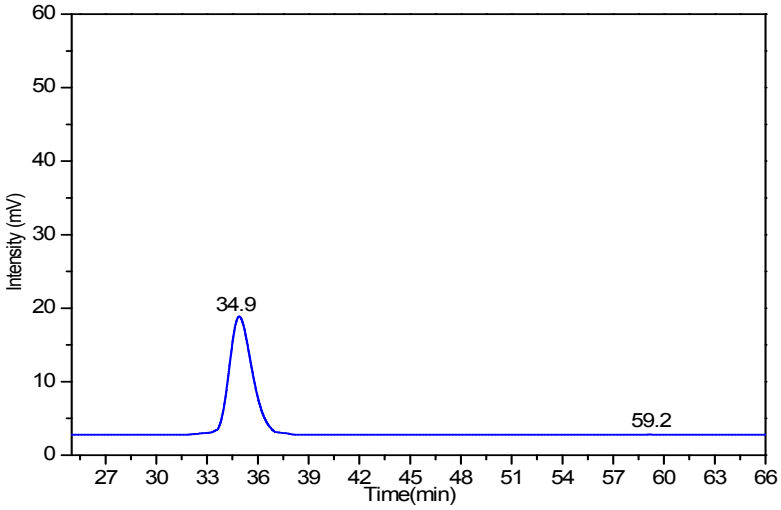
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
3		*S*	94799	*S*	43.25	99.9
		R	25	*R*	52.03	

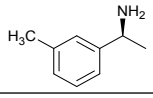


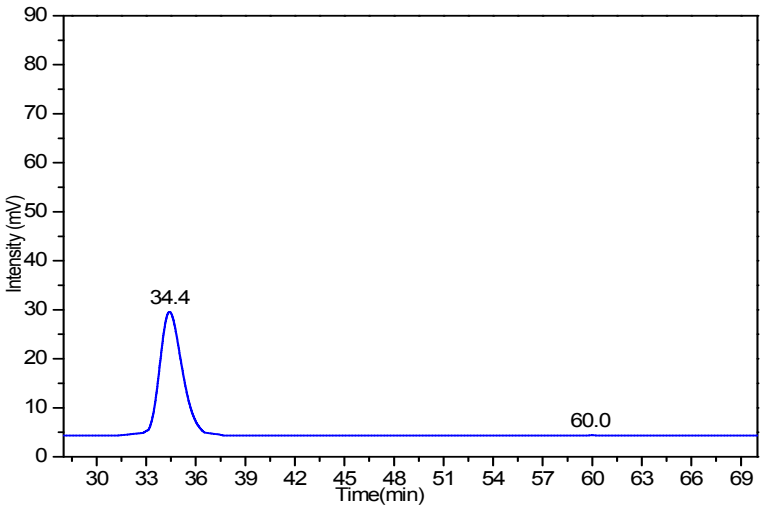
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
4		*S*	31064	*S*	36.1	99.4
		R	99	*R*	49.0	

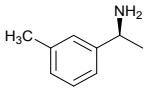


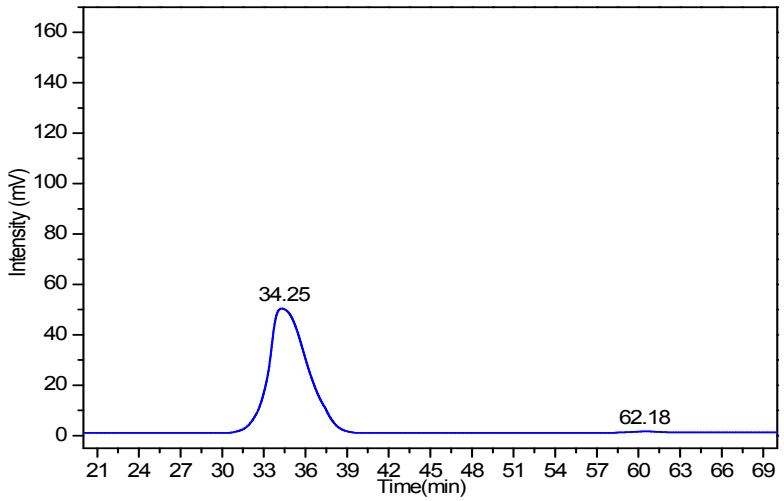
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
4		*S*	54402	*S*	35.1	99.4
		R	168	*R*	49.9	

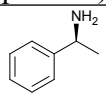


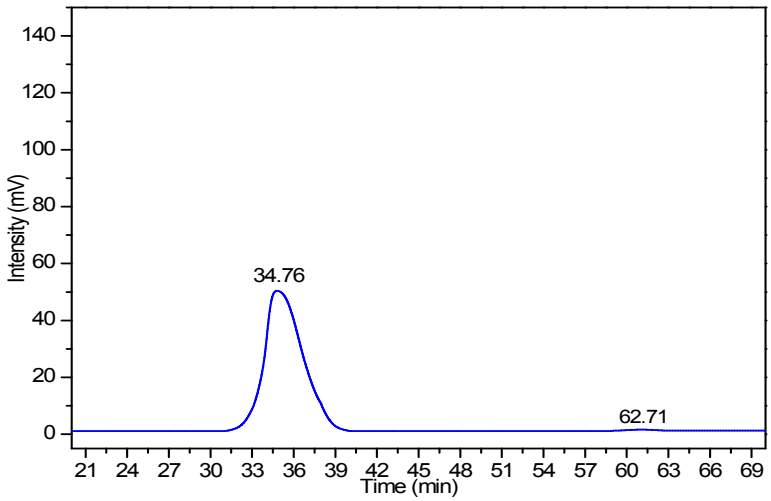
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
5		*S*	4520	*S*	34.9	99.5
		R	12	*R*	59.2	

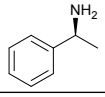


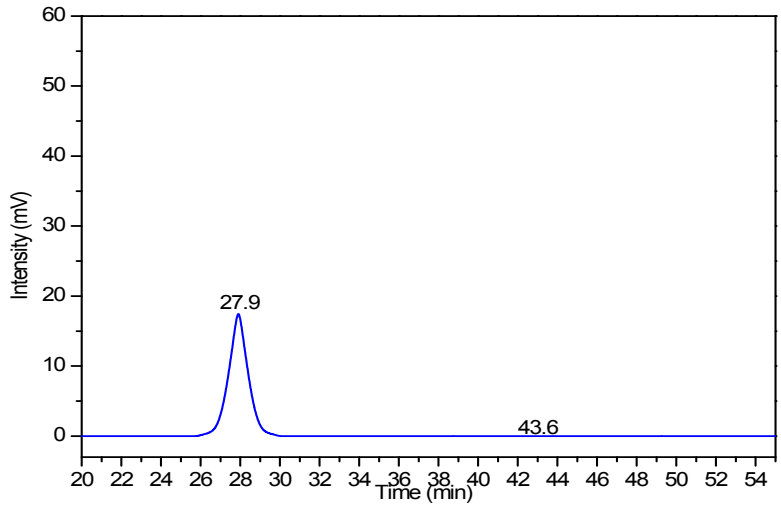
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
5		*S*	8711	*S*	34.4	99.7
		R	13	*R*	60.0	

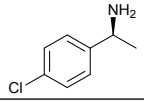


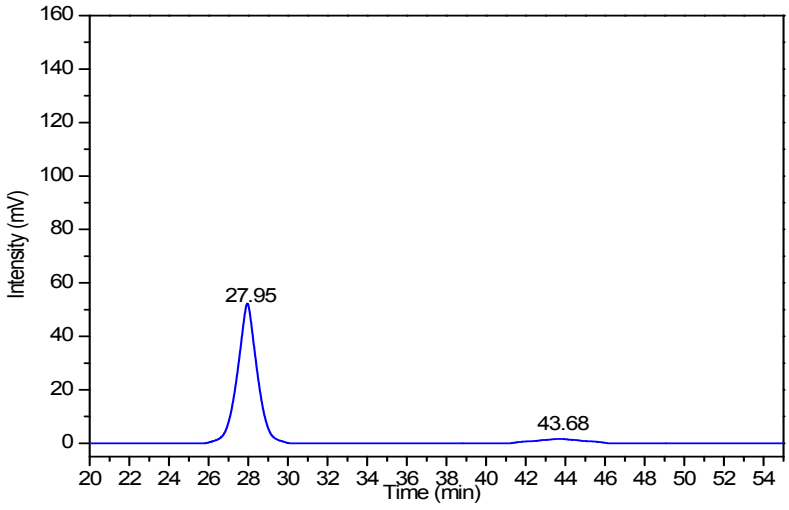
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
6		*S*	13067	*S*	34.25	99.1
		R	60	*R*	62.18	

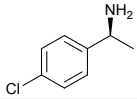


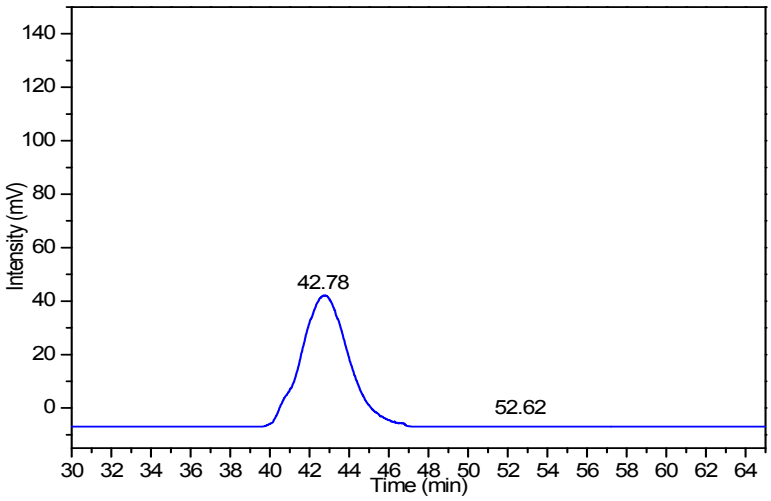
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
6		*S*	11755	*S*	34.76	99.9
		R	15	*R*	62.71	

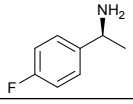


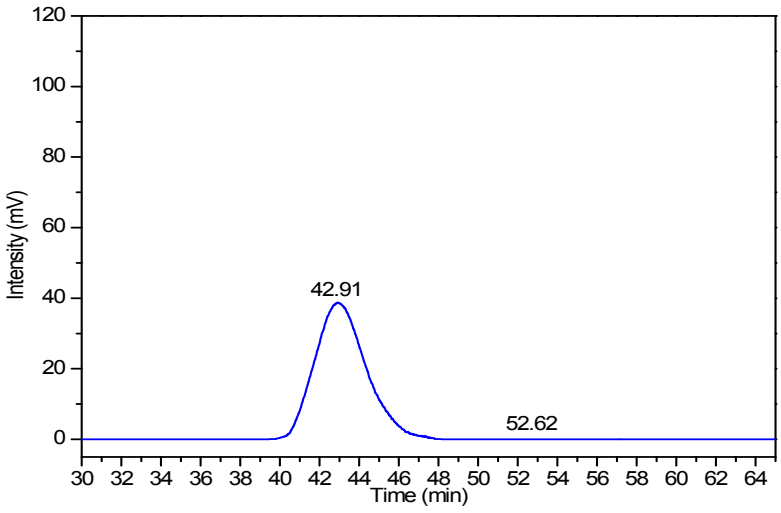
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
7		*S*	30940	*S*	27.9	99.4
		R	91	*R*	43.6	

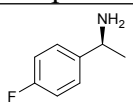


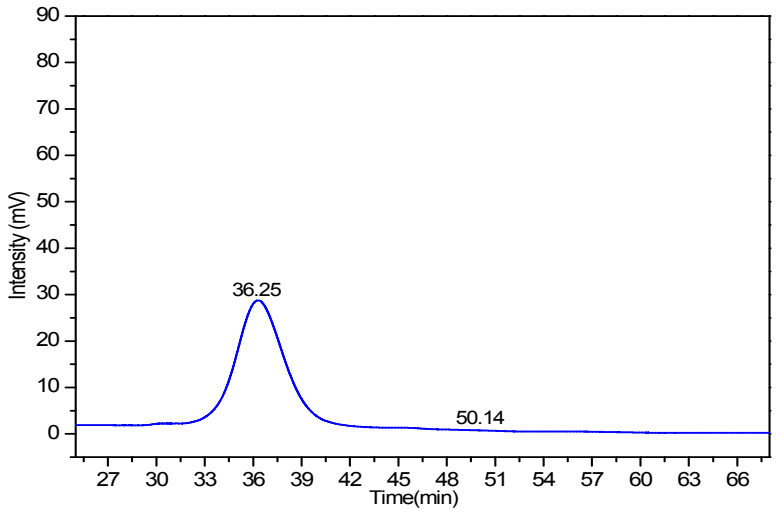
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
7		*S*	105573	*S*	27.95	99.1
		R	462	*R*	43.68	

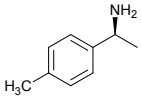


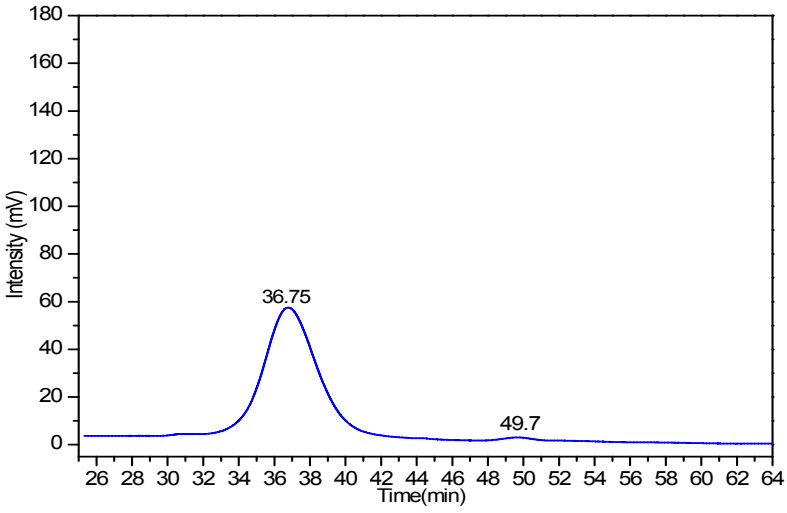
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
8		*S*	69435	*S*	42.78	99.7
		R	91	*R*	52.62	

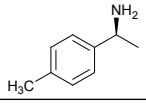


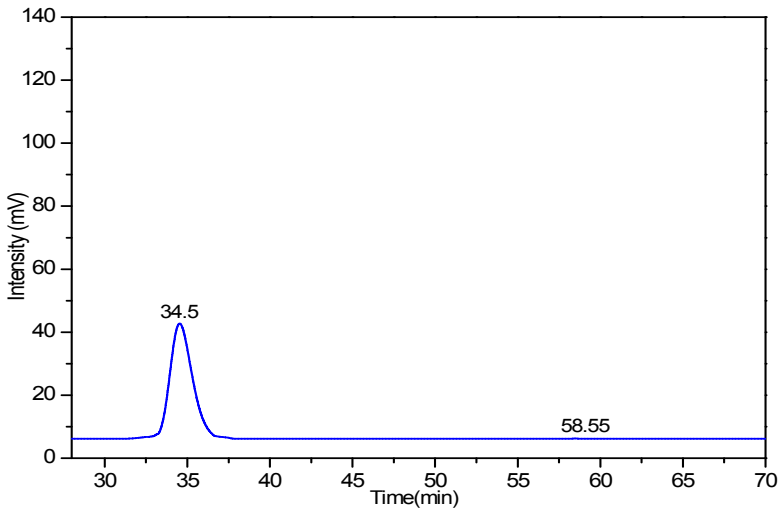
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
8		*S*	66826	*S*	42.91	99.7
		R	85	*R*	52.62	

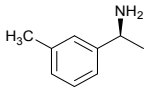


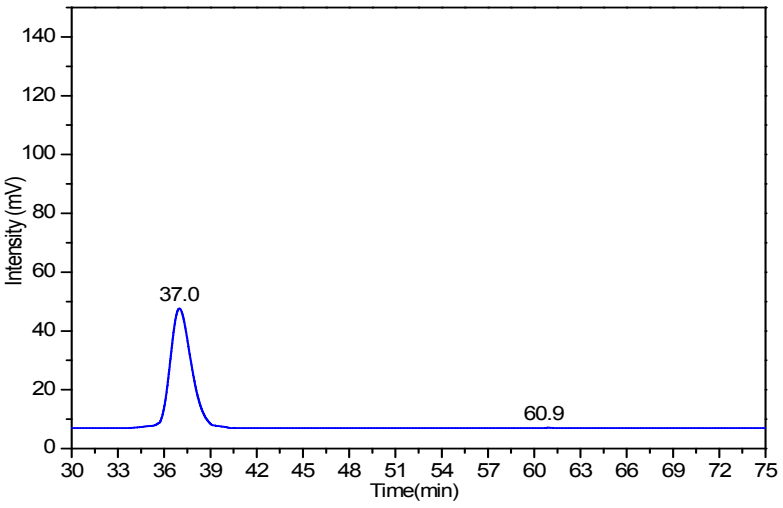
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
9		*S*	45414	*S*	36.25	99.3
		R	160	*R*	50.14	

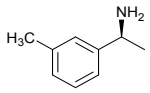


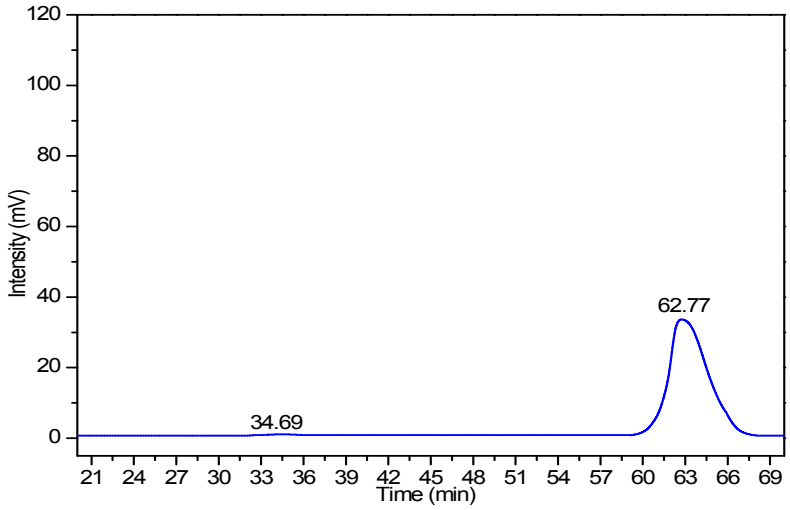
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
9		*S*	83146	*S*	36.75	99.5
		R	206	*R*	49.7	



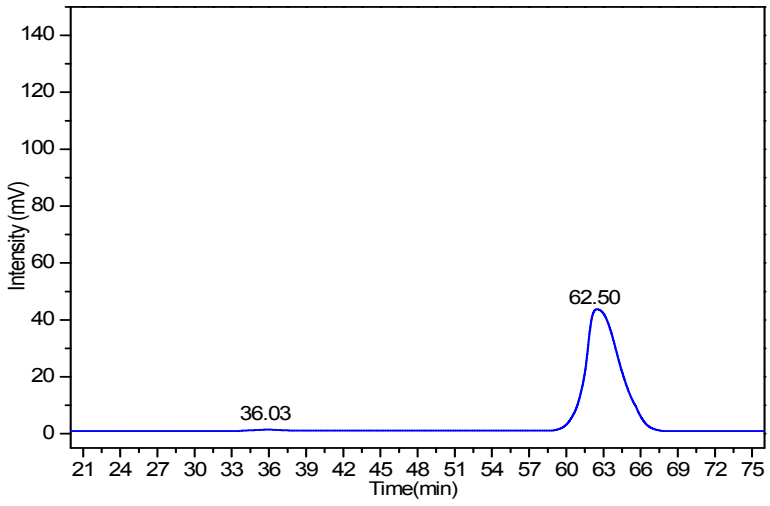
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
10		*S*	13617	*S*	34.5	99.0
		R	66	*R*	58.55	



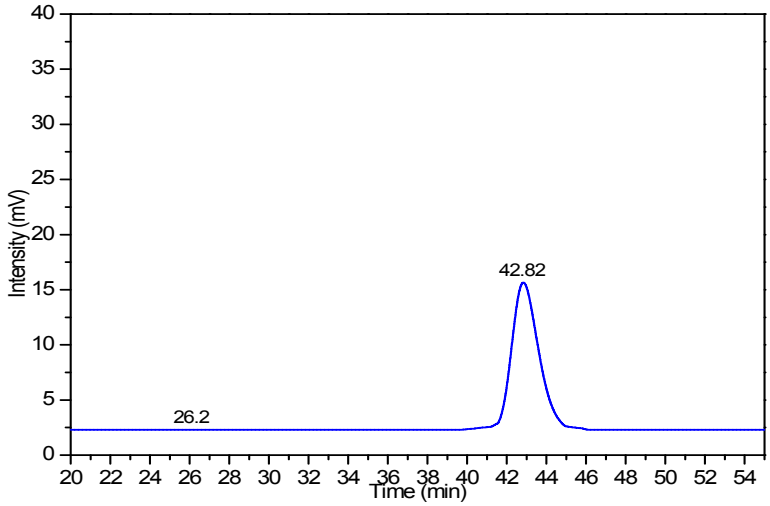
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
10		*S*	19103	*S*	37.0	99.6
		R	35	*R*	60.9	

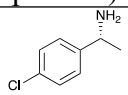


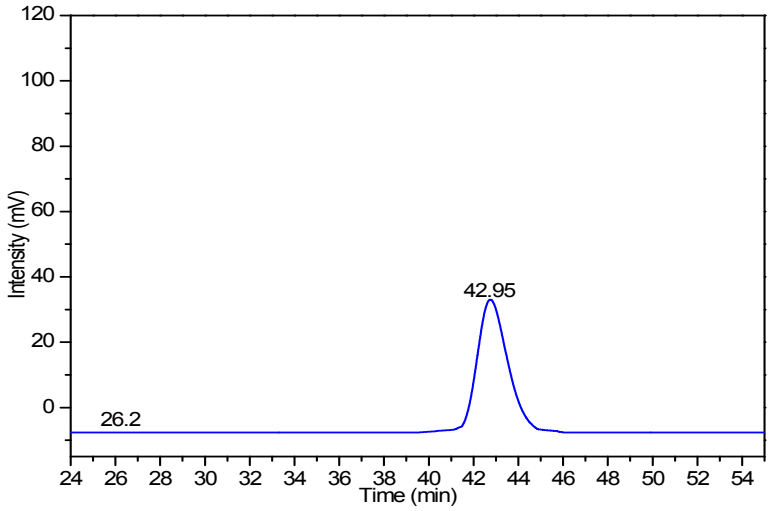
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
11		*S*	108	*S*	34.69	99.1
		R	22922	*R*	62.77	

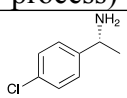


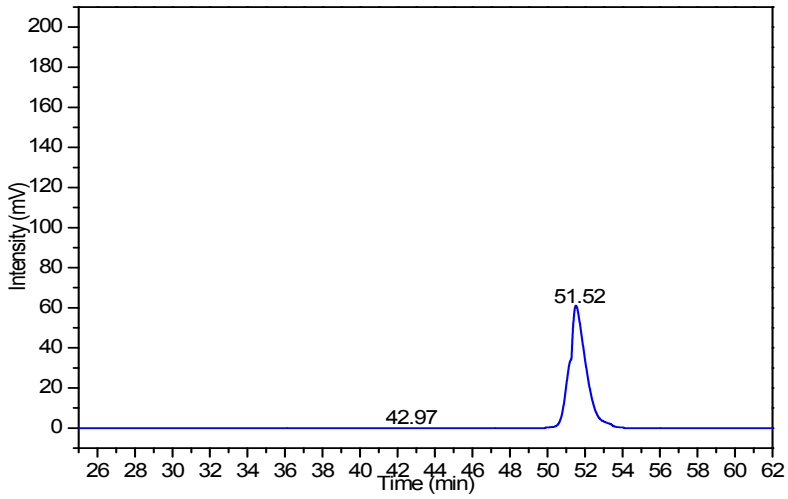
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
11		*S*	120	*S*	36.03	99.7
		R	72484	*R*	62.5	

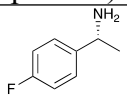


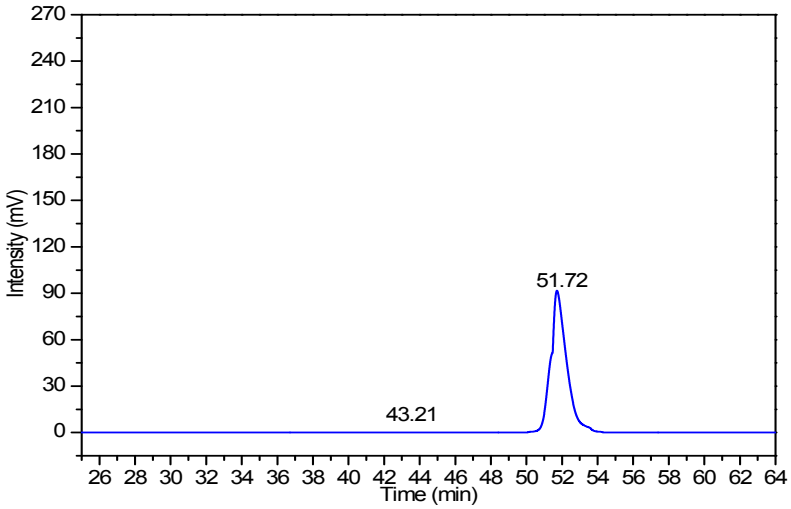
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
12		*S*	24	*S*	26.2	99.7
		R	18962	*R*	42.82	

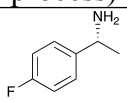


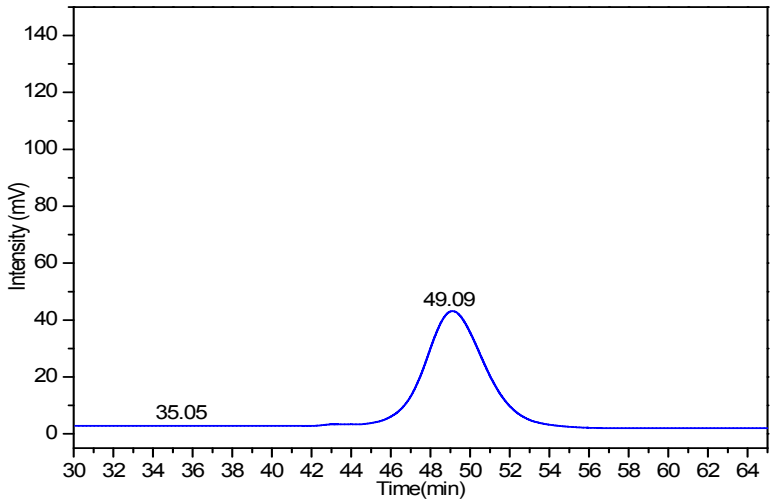
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
12		*S*	67	*S*	26.2	99.8
		R	77985	*R*	42.95	

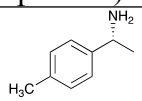


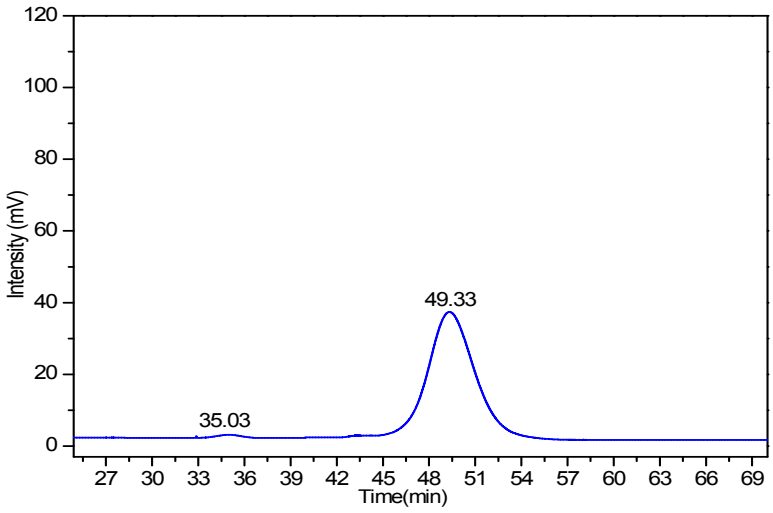
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
13		*S*	27	*S*	42.97	99.9
		R	62260	*R*	51.52	

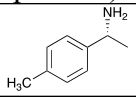


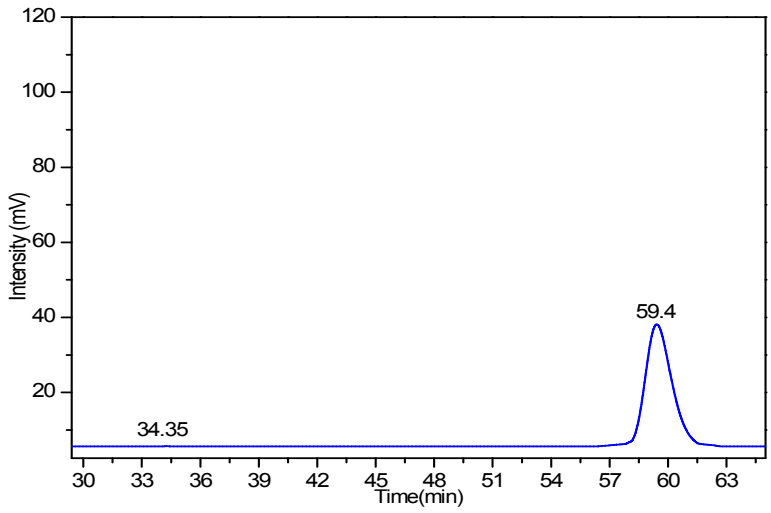
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
13		*S*	57	*S*	43.21	99.9
		R	86633	*R*	51.72	

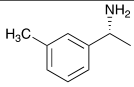


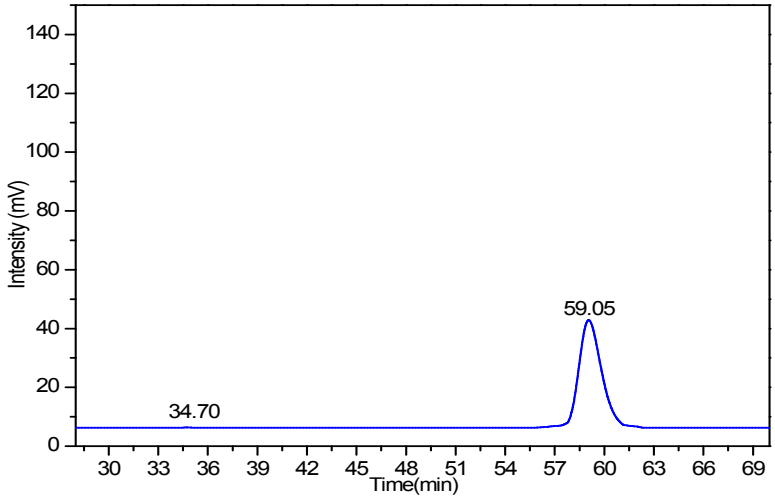
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
14		*S*	83	*S*	35.05	99.1
		R	17799	*R*	49.09	

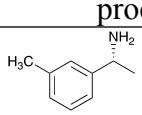


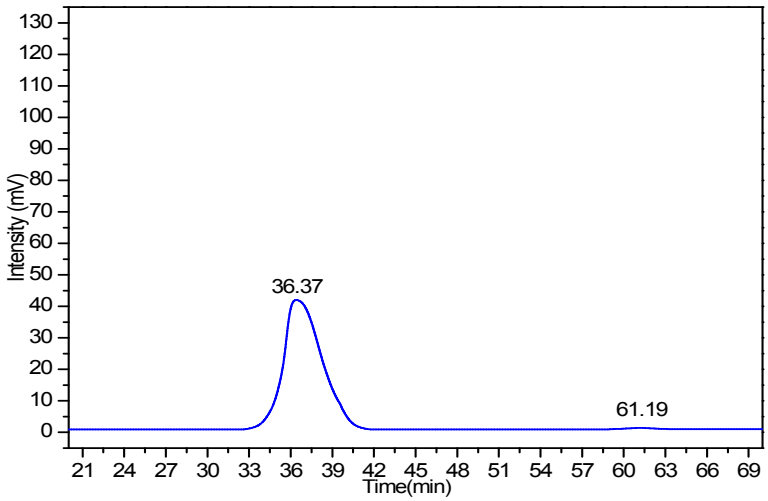
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
14		*S*	103	*S*	35.03	99.1
		R	22520	*R*	49.33	



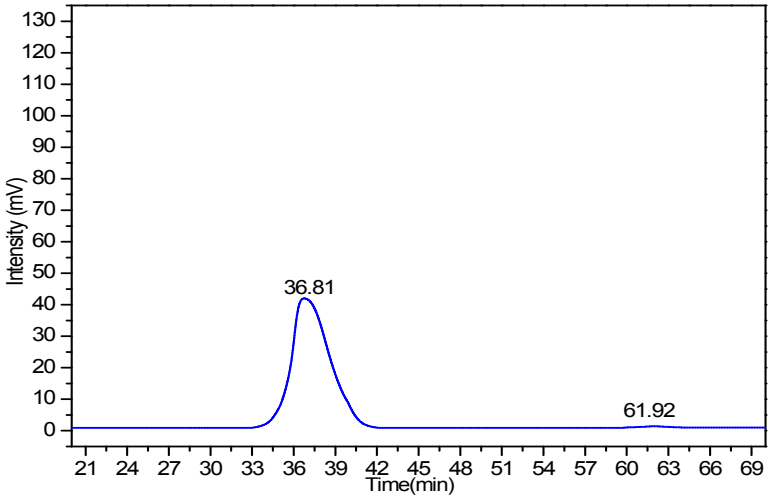
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
15		*S*	17	*S*	34.35	99.8
		R	17148	*R*	59.4	

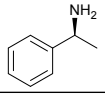


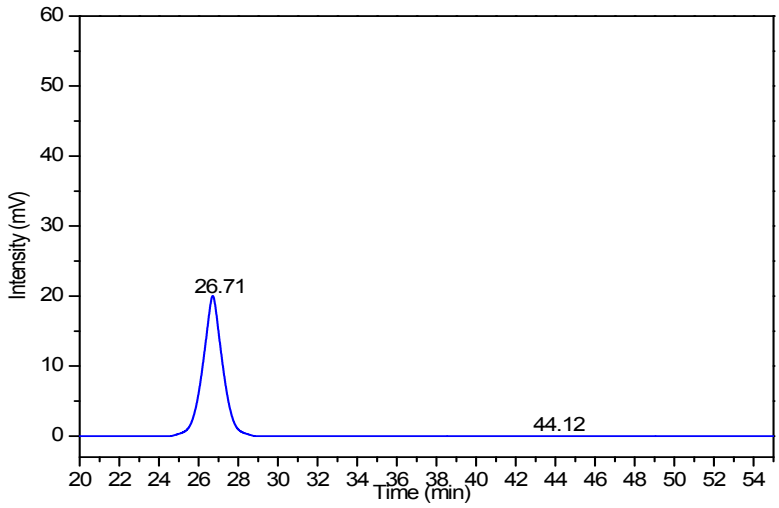
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
15		*S*	46	*S*	34.7	99.6
		R	22640	*R*	59.05	

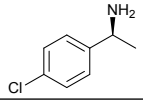


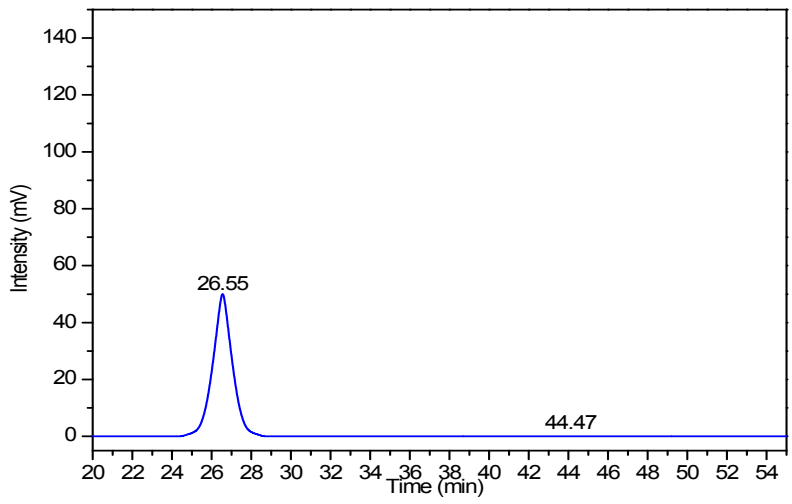
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
16		*S*	17026	*S*	36.37	99.4
		R	54	*R*	61.19	

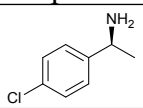


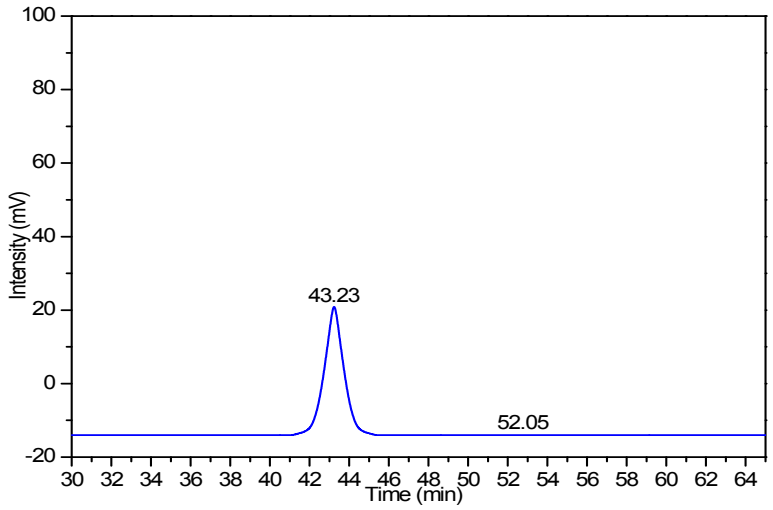
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
16		*S*	14057	*S*	36.81	99.4
		R	43	*R*	61.92	

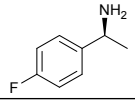


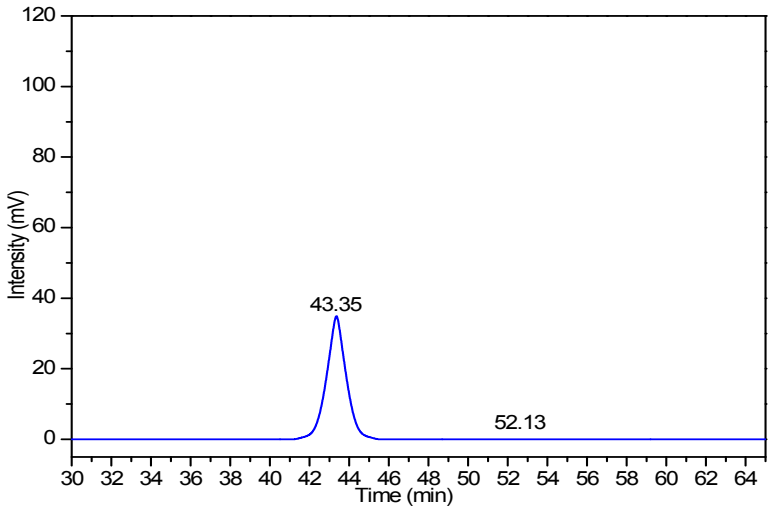
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
17		*S*	6828	*S*	26.71	99.6
		R	12	*R*	44.12	

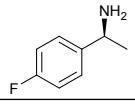


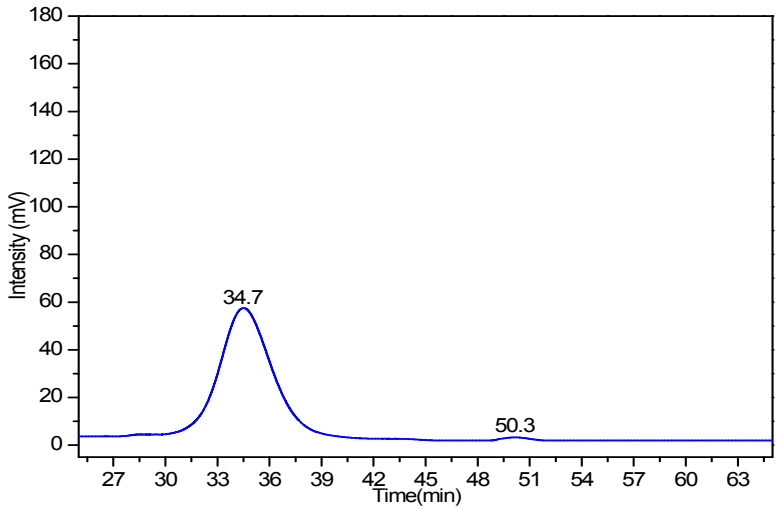
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
17		*S*	13214	*S*	26.55	99.4
		R	43	*R*	44.47	

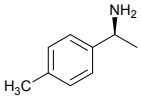


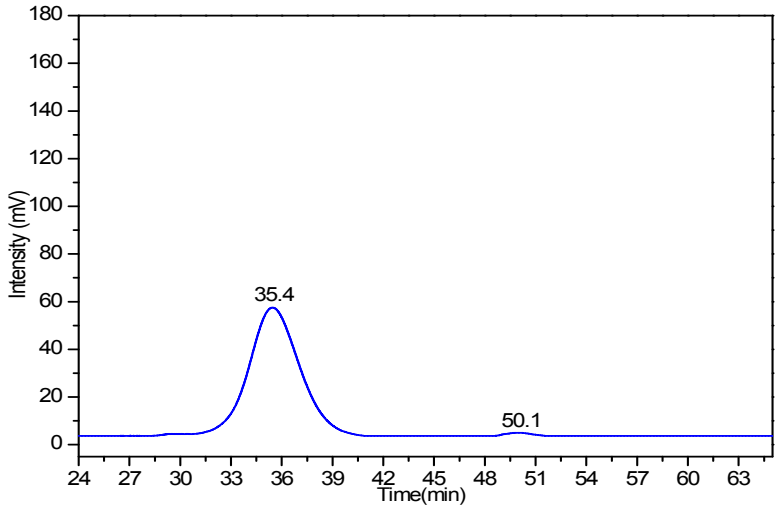
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
18		*S*	9894	*S*	43.23	99.1
		R	43	*R*	52.05	

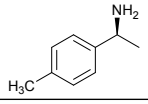


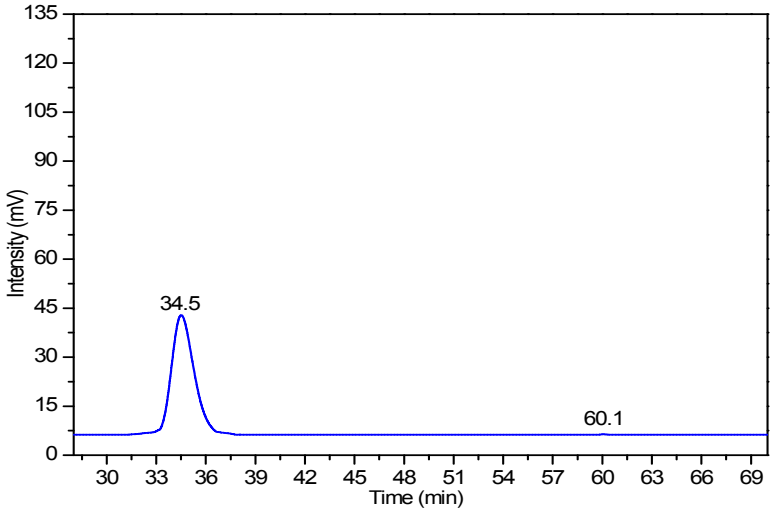
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
18		*S*	21471	*S*	43.35	99.1
		R	95	*R*	52.13	

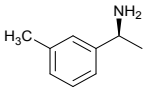


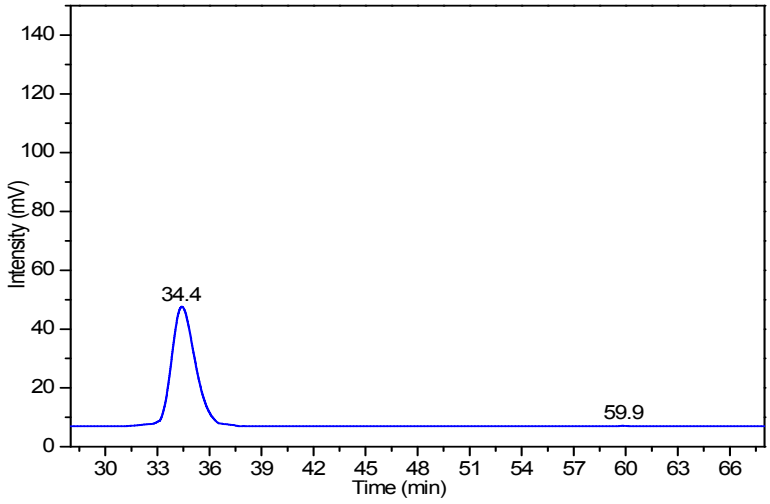
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
19		*S*	66448	*S*	34.7	98.1
		R	648	*R*	50.3	

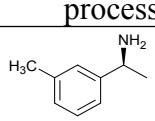


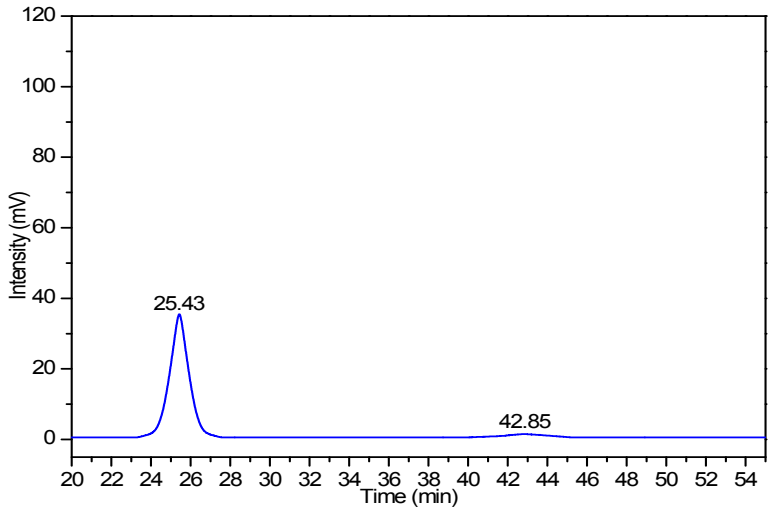
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
19		*S*	91341	*S*	35.4	98.8
		R	532	*R*	50.1	

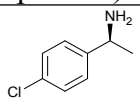


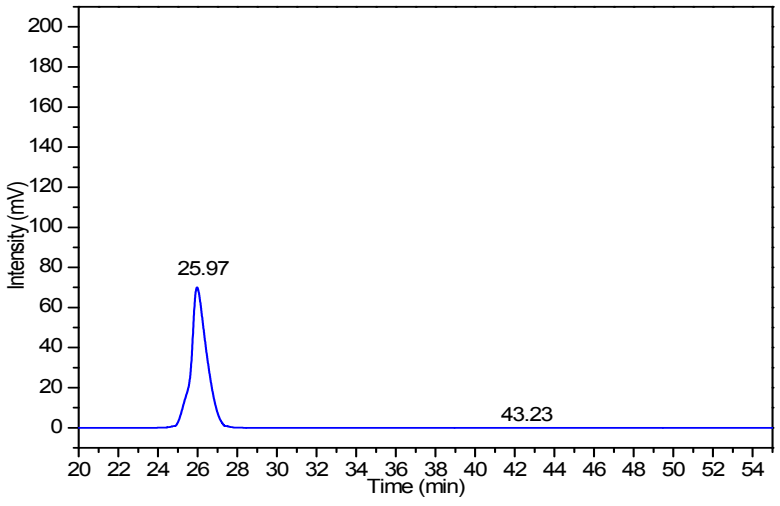
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
20		*S*	15188	*S*	34.5	99.2
		R	58	*R*	60.1	

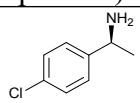


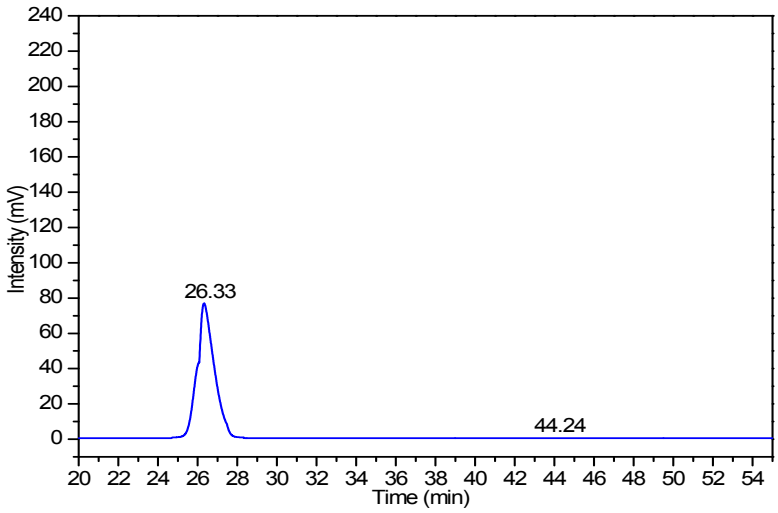
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
20		*S*	25663	*S*	34.4	99.5
		R	64	*R*	59.9	

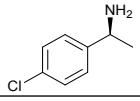


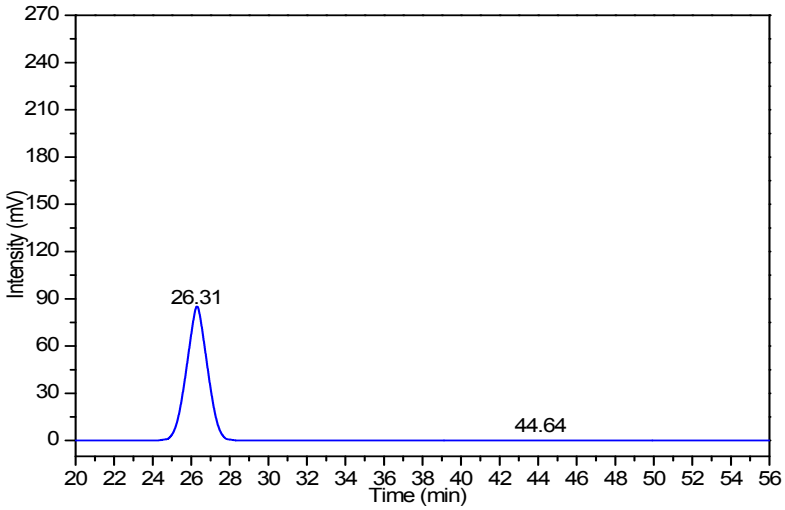
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
21		*S*	75080	*S*	25.43	99.7
		R	125	*R*	42.85	

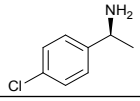


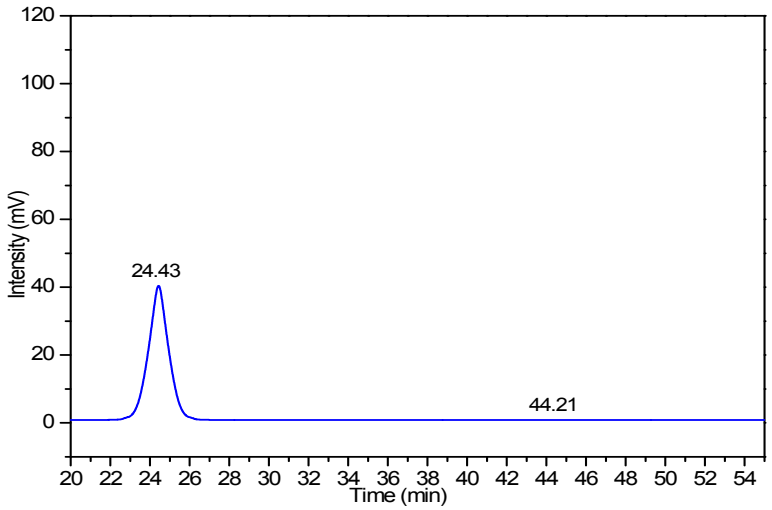
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
21		*S*	97332	*S*	25.97	99.6
		R	167	*R*	43.23	

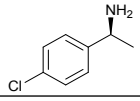


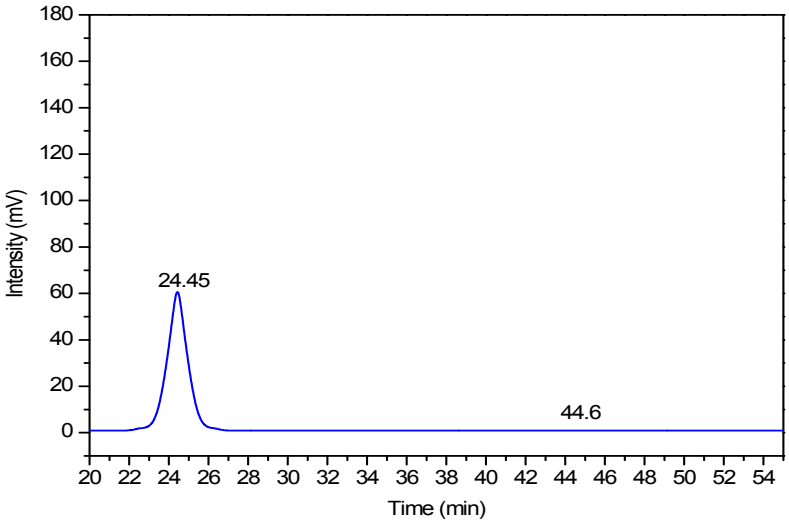
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
22		*S*	92210	*S*	26.33	99.8
		R	112	*R*	44.24	

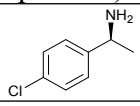


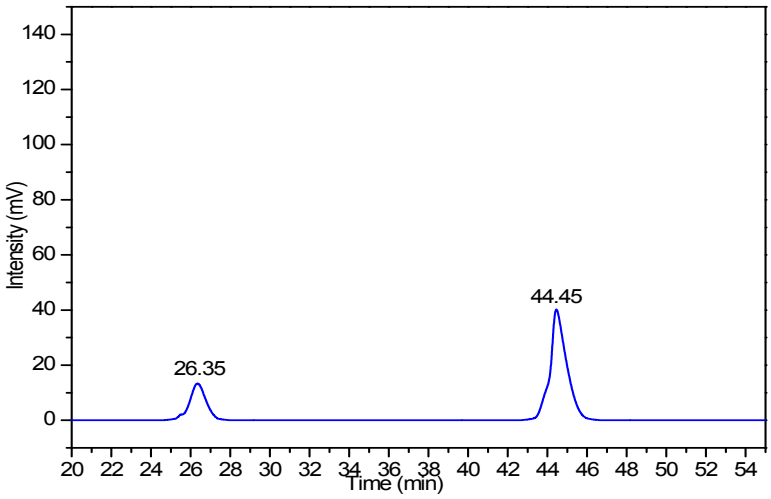
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
22		*S*	107391	*S*	26.31	99.7
		R	167	*R*	44.64	

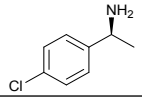


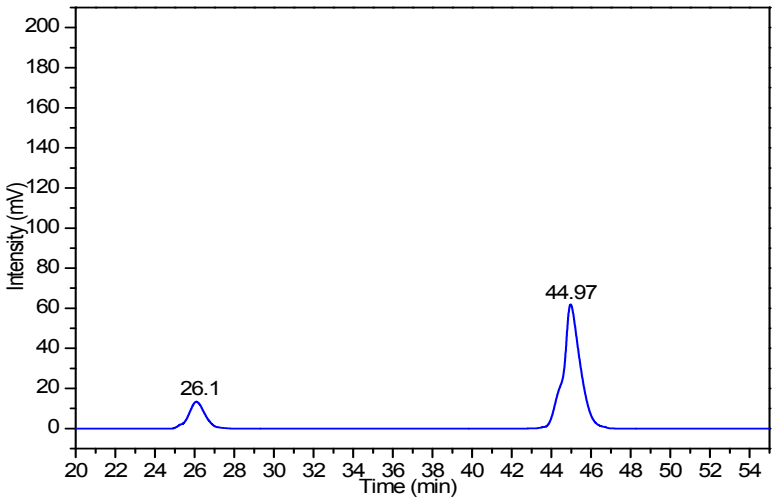
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
23		*S*	39790	*S*	24.43	99.7
		R	67	*R*	44.21	

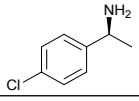


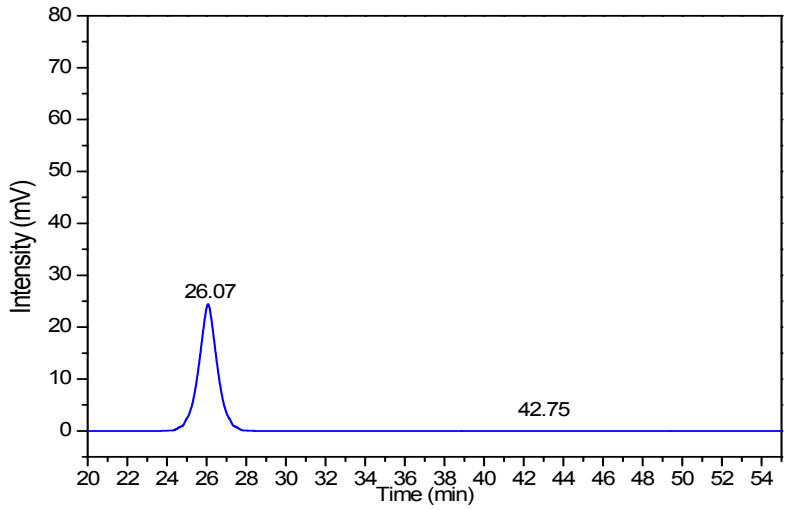
Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
23		*S*	97618	*S*	24.45	99.7
		R	131	*R*	44.6	

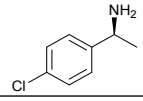


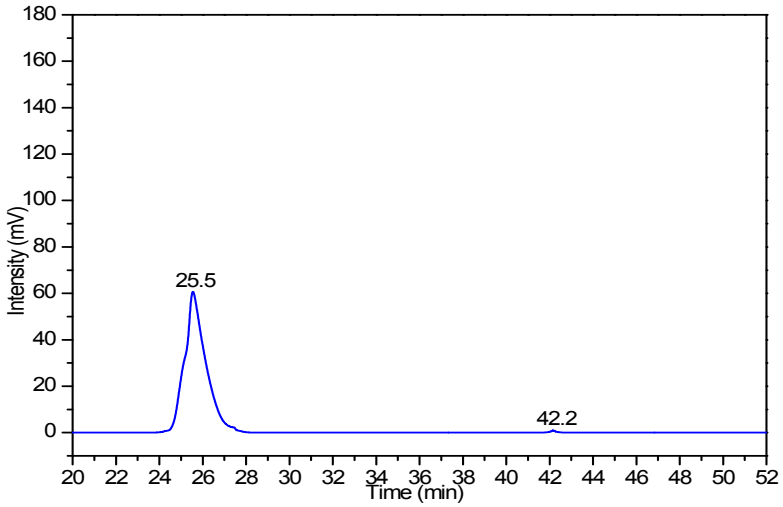
Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
24		*S*	11428	*S*	26.35	67.1
		R	58141	*R*	44.45	

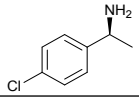


Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
24		*S*	11240	*S*	26.1	72.4
		R	70180	*R*	44.97	



Entry	Product (without oxidation process)	Area		Retention time (min)		ee (%)
25		*S*	32885	*S*	26.07	99.4
		R	97	*R*	42.75	



Entry	Product (with oxidation process)	Area		Retention time (min)		ee (%)
25		*S*	87731	*S*	25.5	99.7
		R	110	*R*	42.2	