Efficient Access to β-Branched Noncanonical Amino Acids via Transaminase-Catalyzed Dynamic Kinetic Resolutions driven by a Lysine Amine Donor

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SUPPORTING INFORMATION

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General information

Unless otherwise noted, all reagents and solvents were purchased from commercial suppliers (Acros, Arch Bioscience, Merck Millipore Sigma, TCI America or Fisher Scientific) and were used as received. NMR solvents and $^{15}N_{\alpha}$-L-lysine were purchased from Cambridge Isotope Laboratories. Branched keto-acid substrates were prepared using previously disclosed methods.[1-4] ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500 spectrometer at 500 and 125 MHz, respectively with chemical shifts given in ppm relative to TMS at δ = 0.0 ppm. Silica gel chromatography was performed on a Teledyne ISCO Combiflash Rf. Reactions were analyzed on an Agilent 1290 UPLC system with detection on a 1290 DAD and/or an InfinityLab LC/MSD XT mass spectrometer. HRMS analysis was performed on an Agilent 1290 Infinity II UPLC system with detection on a 6230 LC/TOF. For the initial testing, engineered and wild-type transaminases were obtained from: Johnson Matthey, UK; Prozomix, UK; and Codexis, USA. The panel of 384 wild-type enzymes and fermentation powders of PRO-TRANS(248) were obtained from Prozomix, UK. Transaminase screening reactions and mechanistic experiments were performed on a Chemglass Life Sciences Incu-Shaker at 300 rpm.

Determination of Apparent Equilibrium Constants (K_{eq}^{app}) of Amine Donors

UPLC detection method. Chromatographic separation was achieved using an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm particle size, PN: 186002350) heated to 55 ºC. The flow-rate was set to 0.7 mL/min and the column was equilibrated to 90% solvent A (0.1% phosphoric acid in water) and 10% B (MeCN) before injection. Elution of the sample and preparation for the next injection were achieved by flowing 10–50% B from 0–1.5 min, 50–95% B from 1.5–2.5 min, 95–10% B from 2.5–2.6 min, and 10% B from 2.6–4 min. Detection and quantification of 3-(naphthalen-1-yl)-2-oxobutanoic acid (**3**) and (2*S*,3*S*)-2-amino-3-(naphthalen-1-yl)-butanoic acid (**3**) was achieved by UV/Vis absorption at 280 nm.

*Product **3** standard preparation.* An authentic Fmoc-derivatized **3** standard was deprotected in a solution of 20 vol% piperidine in 80 vol% DMF, and then diluted in a 1:1 MeCN/H_2O solution to the following concentrations: 0.071 mM, 0.36 mM, 0.71 mM, 2.1 mM, 3.6 mM, and 7.1 mM. Standard solutions were prepared in triplicate and analyzed by UPLC to generate the standard curve shown in **Figure S1**.

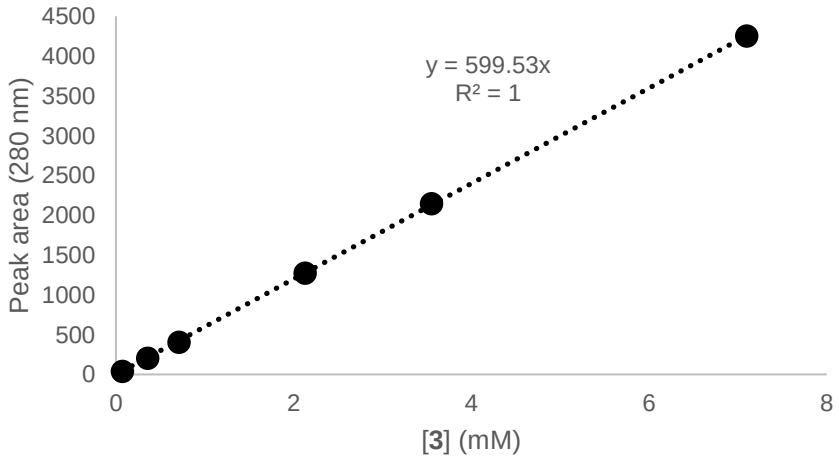
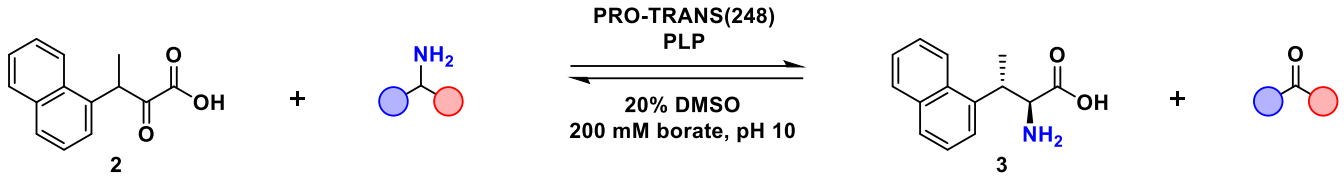


Figure S1. Product **3** calibration curve measured at 280 nm.



Reaction preparation. Reactions were prepared in 96-well plates to a final volume of 200 µL and temperature of 30 ºC to the following final concentrations: 33 mM **2**, 40 mM (1.2 equivalents) amine donor (**Figure 2**), 4 mM pyridoxal 5'-phosphate, and 24 g/L PRO-TRANS(248) fermentation powder in a solvent mixture of 20 vol% DMSO and 80 vol% 200 mM sodium borate, pH 10. The formation of **3** was monitored to determine when the thermodynamic equilibria were reached. All reaction mixtures showed a clean conversion of substrate to product with no significant byproduct peaks. For each time point, 20 µL of a given reaction mixture was diluted into 180 µL of a 1:1 MeCN/H_2O solution, mixed, filtered, and then analyzed by UPLC. The K_{eq}^{app} for each reaction was calculated using the following equation:

$$K_{eq}^{app} = \frac{[\mathbf{3}]^2}{(33.1-[\mathbf{3}]) \times (40-[\mathbf{3}])} \qquad \text{(Equation S1)}$$

Each K_{eq}^{app} value reported in **Figure 2** is the average of three replicates.

Differentiating the Transferred Amine with $^{15}N_{\alpha}$-L-Lysine

UPLC-MS detection method. Chromatographic separation was achieved using an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.7 µm particle size, PN: 186002350) heated to 55 ºC. The flow-rate was set to 0.75 mL/min and the column was equilibrated to 85% solvent A (5 mM ammonium formate in water) and 15% B (MeCN) before injection. Elution of the sample and preparation for the next injection were achieved by flowing 15–68% B from 0–2 min, 68–95% B from 2–2.3 min, 95% B from 2.3–2.7 min, 95–15% B from 2.7–2.8 min, and 15% B from 2.8–3.2 min. Detection of **3** was achieved by ESI in negative mode.

Reaction preparation. Reactions were prepared in 2 mL glass vials to a final volume of 200 µL and temperature of 30 ºC to the following final concentrations: 5 mM **2**, 1 g/L pyridoxal 5'-phosphate, 5 mM L-lysine or $^{15}N_{\alpha}$-L-lysine, and 4 g/L PRO-TRANS(248) fermentation powder in a solvent mixture of 20 vol% DMSO and 80 vol% 100 mM borate, pH 10.0. The reactions were allowed to proceed for ~23 h, whereupon 20 µL of each reaction mixture was diluted into 180 µL of a 1:1 MeCN/H_2O, mixed, filtered, and then analyzed by UPLC-MS.

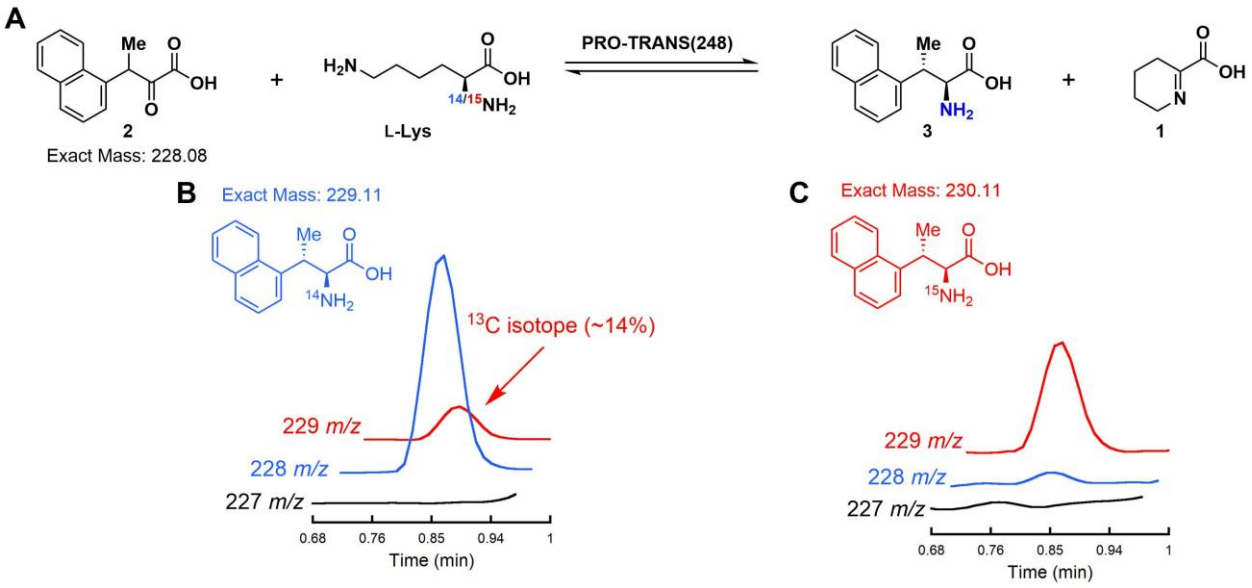
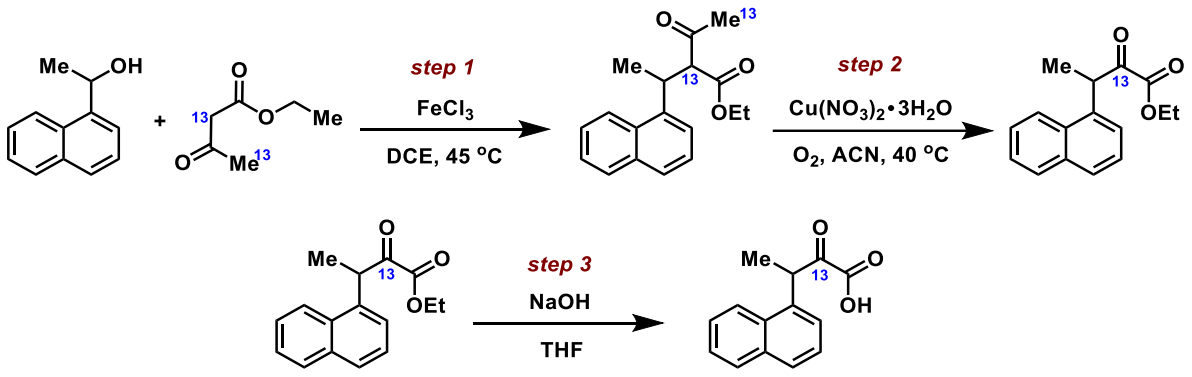


Figure S2. Reactions were prepared with unlabeled L-lysine and $^{15}N_{\alpha}$-L-lysine to differentiate which amine is transferred by PRO-TRANS(248) (**A**). In unlabeled L-lysine reactions (**B**), the product is observed at 228 *m/z* in negative mode. The peak at 229 *m/z* is the ^{13}C isotope, which originates from the 1.1% natural abundance of ^{13}C in the C14 product. In $^{15}N_{\alpha}$-L-lysine reactions (**C**), the product is observed at 229 *m/z*, consistent with the transfer of the α-amine.

Synthesis and Characterization of ^{13}C Labeled 3-(Naphthalen-1-yl)-2-oxobutanoic acid

General. ^{13}C labeled 3-(naphthalen-1-yl)-2-oxobutanoic acid (^{13}C-**2**) was prepared according to literature procedure via iron chloride-catalyzed alkylation of ^{13}C labeled active methylene compounds with benzylic alcohols and subsequent aerobic deacylation[1-3] to access β-aryl-α-keto esters, followed by hydrolysis to obtain the acid product in the final step.



Step 1. To a solution of 1-(naphthalen-1-yl)ethan-1-ol (1.25 g, 7.5 mmol) in DCE (10 ml) was added ethyl acetoacetate-2,4-$^{13}C_2$ (1 g, 7.6 mmol), followed by ferric chloride (142 mg, 0.875 mmol). The solution was stirred at 45 °C for 2 h, cooled to room temperature and stirred overnight. The mixture was concentrated and then diluted with 1:1 MTBE:hexanes (200 mL), followed by filtration through a funnel containing ~3 inches of silica gel, and subsequently washed with 1:1 MTBE:hexanes (200 mL). The crude intermediate was concentrated to give a yellow oil. The subsequent step was carried without further oxidation.

Step 2. To a solution of ethyl 2-acetyl-3-(naphthalen-1-yl)butanoate-2,4-$^{13}C2$ (1.7 g, 6 mmol) in acetonitrile (25 ml) was added copper(II) nitrate trihydrate (1.4 g, 6 mmol). The mixture was heated to 40 °C and oxygen was bubbled through it (using a balloon) for 15 min, and was stirred for 5 h, at which time it appeared to have reached full conversion. At the end of the reaction, the mixture was cooled to room temperature, diluted with MTBE (200 mL) and water (200 mL). The aqueous layer was removed followed by washing with brine. The organic phase was dried over anhydrous sodium sulfate and

concentrated. The compound was purified by flash chromatography on silica gel (5-25% MTBE in hexanes) to obtain the product as a pale-yellow oil.

Step 3. To a solution of ethyl 3-(naphthalen-1-yl)-2-oxobutanoate-2-^{13}C (0.975 g, 3.8 mmol) in THF (25 ml) was added sodium hydroxide (7.64 ml, 15.3 mmol). The pale brown solution was stirred for 15 min, during which time it darkened. The mixture was quenched with hydrochloric acid (1 M, 30.6 ml, 30.6 mmol), and ethyl acetate (200 mL) was added. The aqueous layer was removed, and the mixture washed with saturated $NaHCO_3$ and brine. The aqueous phase was retreated with 1N HCl to attain a neutral pH, and ethyl acetate (200 mL) was added. The organic phase was dried over sodium sulfate, filtered, and concentrated to obtain the pure ^{13}C-**2** in 55% yield.

^{1}H NMR (500 MHz, D_2O) δ 7.76 (d, J = 8.5 Hz, 1H), 7.40 (dd, J = 33.6, 8.2 Hz, 2H), 7.22 (t, J = 7.6 Hz, 1H), 7.11 (q, J = 7.0 Hz, 2H), 6.99 (d, J = 7.1 Hz, 1H), 4.84 (q, J = 7.0 Hz, 3H), 1.20 (d, J = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, D_2O) δ 207.05, 170.32, 135.34, 133.62, 130.92, 128.75, 127.78, 126.52, 125.86, 125.59, 125.23, 123.04, 44.19, 16.92, 15.54.

HRMS: (ESI) *m/z*: [M - H] Calculated for $C_{13}{}^{13}CH_{11}O_3^-$: 228.0747; Found: 228.0039.

Reaction Progress Curves Monitored by NMR Spectroscopy

Sample preparation. Reactions were prepared to the following final concentrations and then transferred to a 5 mm NMR tube for analysis at 25 ºC: 55 mM ^{13}C-**2**, 66 mM ^{13}C-L-lysine, 6.3 g/L PRO-TRANS(248) fermentation powder (50 wt%), 4 mM pyridoxal 5'-phosphate (6 mol%), 10 vol% DMSO-d_6, and 200 mM borate, pH 10.

Reaction progress by ^{13}C NMR. ^{13}C NMR data were collected on Bruker 600MHz spectrometer with a TCI cryoprobe. Each spectrum was acquired with inverse-gated decoupling and a 30-degree flip angle (pulse program "zgig30" from the Bruker library), AQ of 0.77 s, relaxation delay of 52 s and 32 scans for a total experiment time of 29 min. Between each ^{13}C NMR experiment a ^{1}H NMR spectrum was collected using presaturation water suppression "zgpr" pulse program with a 30-degree flip angle, AQ of 1.7 s, relaxation delay of 8 s and 4 scans for a total experiment time of 1 min.

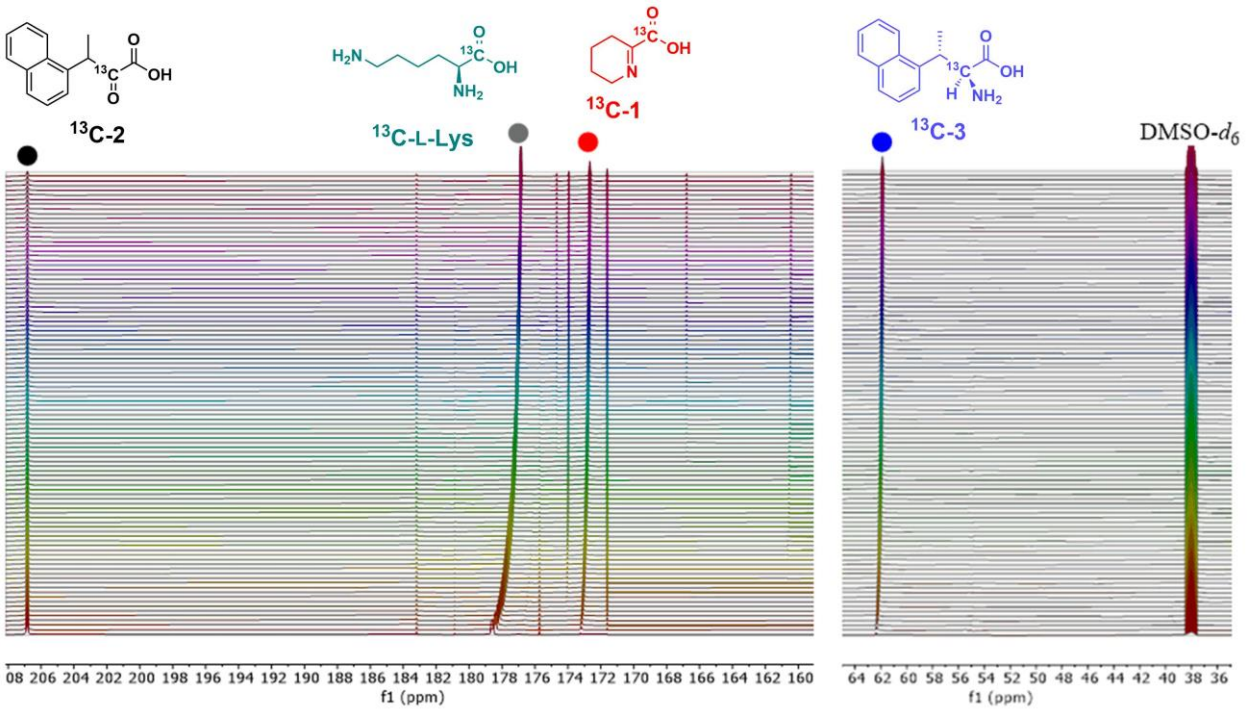


Figure S3. Stacked plot showing the ^{13}C NMR spectra collected over time.

Imine characterization by ^{1}H–^{15}N HMBC. At the end of the reaction, 2D ^{1}H–^{15}N HMBC spectrum was collected utilizing natural abundance ^{15}N. The experiment was acquired with the following parameters: “hmbcgpndqf” pulse program, AQ 0.13s, D1 1 s, 0ppm to 340 ppm ^{15}N spectral region, 160 increments in the indirect (^{15}N) dimension, 512 scans for each indirect increment for a total acquisition time of 28 h and 12 min.

In **Figure S4** the observed ^{15}N resonances at 293.9 ppm (to 2.26 ppm and 2.19 ppm in ^{1}H dimension) are in good agreement with the imine correlations shown in the embedded scheme and ^{15}N chemical shift is consistent with that structure. Another observed peak at 2.25 ppm in ^{1}H and 237.1 ppm in ^{15}N corresponds to the PLP enzyme cofactor and represents a correlation between its aromatic methyl protons and a pyridine nitrogen.

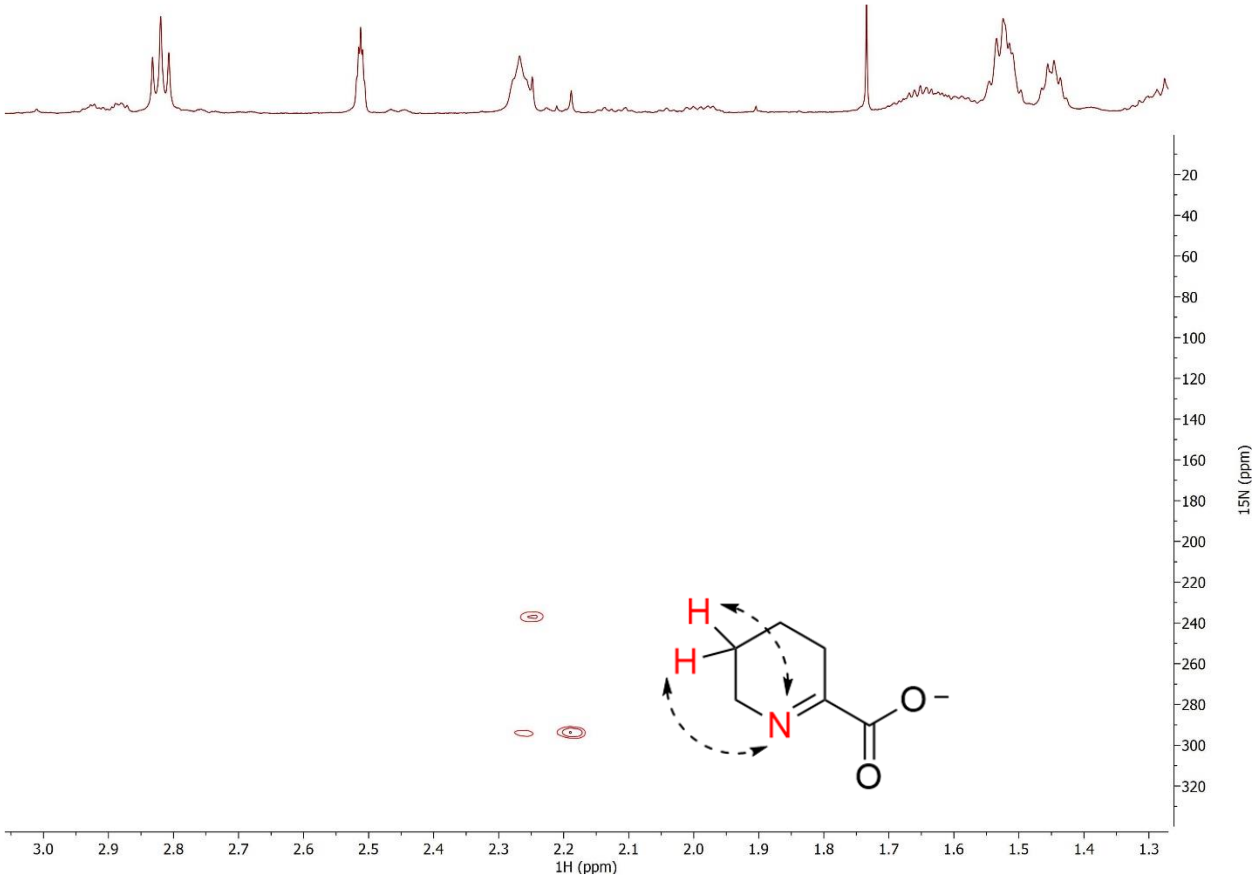


Figure S4. ^{1}H-^{15}N HMBC spectrum with a corresponding ^{1}H NMR spectrum overlaid.

Determination of L-lysine, L-ornithine, and L-alanine Michaelis constant (K_M)

Reaction preparation. Reactions were prepared in 96-well plates at a volume of 200 µL and a temperature of 30 ºC to the following final concentrations: 20 mM **2**, 1 mM pyridoxal 5'-phosphate, 4.6 g/L PRO-TRANS(248) fermentation powder, and 1 mM, 5 mM, 10 mM, 25 mM, 50 mM, 100 mM, 200 mM, or 500 mM L-lysine, L-ornithine, or L-alanine in a solvent mixture of 20 vol% DMSO and 80 vol% 200 mM sodium borate, pH 10. The pH of all stock solutions was adjusted to 10 as needed prior to initiation of the reaction. The formation of **3** was monitored over the course of 0.15–23 h. For each time point, 20 µL of a given reaction mixture was diluted into 180 µL of a 1:1 MeCN/H_2O solution, mixed, filtered, and then analyzed by the UPLC method described previously. The [**3**] was calculated from the standard curve shown in **Figure S1**.

K_M *determination.* The formation of **3** as a function of time in the reaction progress curves shown in **Figure S4** was described by the following equation, wherein ΔA represents the change in amplitude and *k* represents the observed rate constant:

$$\Delta[\mathbf{3}](t) = (\Delta A)(1 - e^{(-kt)}) \quad \text{(Equation S2)}$$

The initial rate (V_0) of each reaction was determined by the following equation:

$$V_0 = k(\Delta A) \quad \text{(Equation S3)}$$

The change in V_0 as a function of [amine donor] shown in **Figure 4** was described and the K_M determined using the Michaelis-Menten equation:

$$V_0 = \frac{V_{max} \times [amine\ donor]}{K_M + [amine\ donor]} \quad \text{(Equation S4)}$$

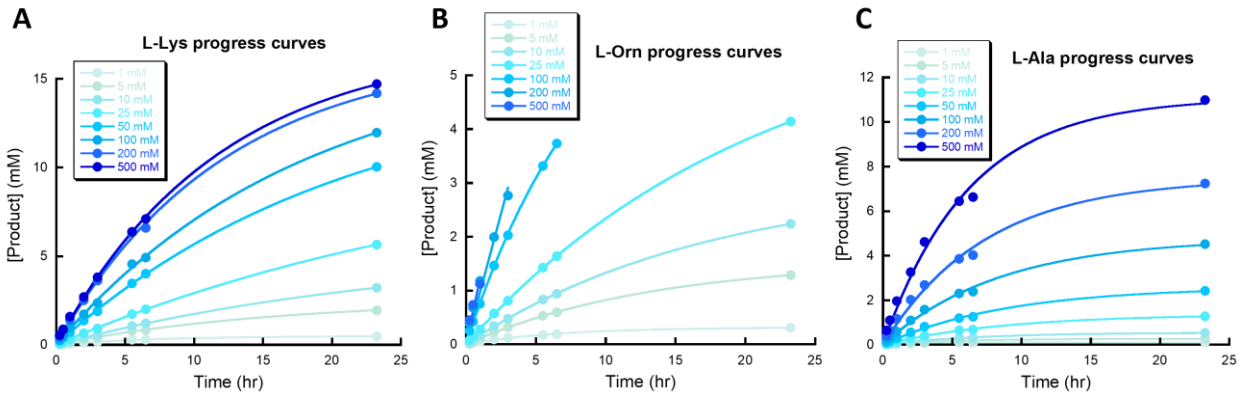


Figure S4. Reaction progress curves for L-lysine (**A**), L-ornithine (**B**), and L-alanine (**C**). Non-linear regression analyses are described by Equation S2. In the L-ornithine graph (**B**), the later time points in the 100 mM, 200 mM, and 500 mM reactions were truncated because degradation of the starting material was observed.

Lysine Compatibility with Wild-type Transaminases

UPLC-MS detection method. Chromatographic separation was achieved using an Acquity UPLC BEH Amide column (2.1 x 50 mm, 1.7 µm particle size, PN: 186004800) heated to 50 ºC. The flow-rate was set to 0.6 mL/min and the column was equilibrated to 0.1% mobile phase A (10 mM ammonium acetate in 50:50 (v/v) MeCN/water, pH 9) and 99.9% mobile phase B (10 mM ammonium acetate in 95:5 (v/v) MeCN/water, pH 9) before injection. Elution of the sample and preparation for the next injection were achieved by flowing 99.9% B from 0–0.2 min, 99.9–60% B from 0.2–0.25 min, 60–30% B from 0.25–2.5 min, 30–99.9% B from 2.5–2.6 min, and 99.9% B from 2.6–3 min. Detection of L-alanine was achieved by ESI in positive mode.

L-alanine standard preparation. An authentic L-alanine standard was prepared in a solution of water, and then diluted in a 1:1 MeCN/H_2O solution to the following concentrations: 5 µM, 25 µM, 50 µM, 100 µM, 250 µM, 500 µM, 750 µM, and 1000 µM. Solutions were sampled in triplicate and analyzed by UPLC-MS to generate the standard curve shown in **Figure S5.**

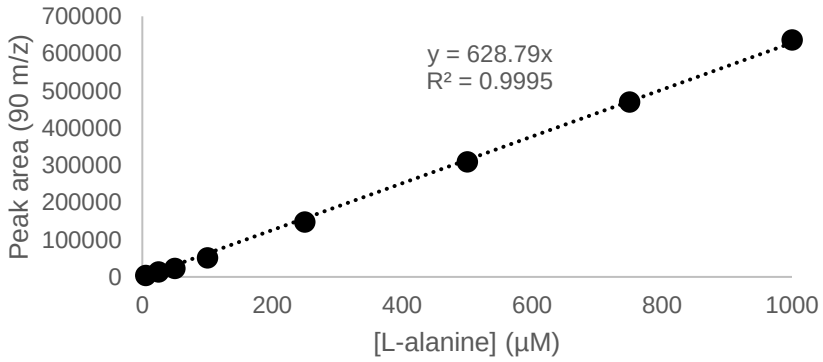


Figure S5. L-alanine calibration curve measured at 90 *m/z* in positive mode.

Reaction preparation. Reactions with the PRO-TRANS(1–384) panel was prepared directly in the provided 384-well plates to a final volume of 50 µL and temperature of 30 ºC to the following final concentrations: 20 mM sodium pyruvate, 4 mM pyridoxal 5'-phosphate (20 mol%), 100 mM L- or D-lysine, and 20 g/L PRO-TRANS fermentation powder (1 mg deposited into each well) in a solvent mixture of 20 vol% DMSO and 80 vol% 100 mM potassium phosphate, pH 7.0. The formation of alanine was monitored over the course of 24–72 h. For each time point, 2.5 µL of a given reaction mixture was diluted into 47.5 µL of a 1:1 MeCN/100 mM potassium phosphate, pH 7.0, mixed, filtered, and then analyzed by UPLC-MS. The results are shown in **Figures S6**–**S8**.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	15.6	24.3	11.2	2.2	5.3	1.7	1.8	1.1	9.4	3.2	20.1	30.4	2.2	3.5	3.1	4.5	2.5	3.9	10.5	7.5	12.2	3.5	5.0	3.4
B	3.1	3.1	1.9	2.9	11.2	6.7	8.3	3.0	2.5	6.0	2.3	5.2	2.7	12.8	45.2	8.4	8.9	2.9	3.5	10.2	4.4	2.6	8.4	9.5
C	8.6	10.3	2.7	2.5	19.4	2.4	28.4	2.0	2.1	2.1	6.2	2.1	1.6	13.7	12.4	2.3	1.7	4.4	21.6	2.4	1.9	10.9	10.0	2.0
D	3.4	0.7	2.3	2.3	9.1	15.0	20.9	10.7	19.7	5.6	2.8	31.4	3.6	1.8	2.6	33.7	11.4	3.1	3.8	2.4	6.6	31.8	35.5	13.8
E	4.2	3.5	2.9	25.4	21.4	3.4	16.1	20.9	3.4	2.9	35.8	4.3	2.8	3.0	2.2	2.7	3.5	6.8	2.4	7.2	3.5	2.3	2.1	4.0
F	6.0	11.2	2.7	4.4	7.5	3.3	3.4	3.7	3.3	8.9	3.3	12.0	8.4	38.2	3.0	3.4	9.1	16.6	16.3	4.7	19.5	5.5	23.9	28.3
G	28.0	4.0	8.2	25.7	12.0	25.4	7.3	3.6	2.9	5.8	9.4	14.2	5.0	4.8	4.2	3.9	25.9	2.2	2.6	5.0	2.7	6.0	4.7	9.5
H	4.5	11.8	3.1	9.8	8.8	4.6	10.6	4.5	11.1	4.0	3.9	6.2	11.3	3.4	22.7	6.4	5.5	2.9	3.6	3.9	4.8	3.3	11.5	4.5
I	6.9	7.9	2.1	1.6	4.6	5.2	5.1	3.4	4.8	2.4	31.2	2.6	2.6	3.0	7.0	1.9	3.1	12.5	6.4	8.4	4.3	7.2	3.7	2.8
J	11.3	7.9	4.6	15.7	26.6	13.4	3.8	41.5	1.7	2.1	2.2	2.6	1.5	10.5	0.8	1.2	1.3	2.1	1.7	1.7	2.7	1.0	1.6	1.5
K	1.6	1.0	4.8	2.6	2.3	1.5	5.2	22.5	1.9	3.6	1.4	0.6	6.2	3.3	4.8	6.4	20.7	0.9	0.9	2.7	0.9	2.0	3.8	2.9
L	16.5	9.4	1.6	3.1	1.7	8.4	4.4	1.4	2.3	3.6	2.7	1.0	1.4	2.5	6.1	0.9	3.3	4.5	3.1	1.5	1.5	2.6	5.0	3.1
M	4.6	4.6	4.9	5.9	1.8	3.2	4.2	2.9	2.4	2.0	1.7	1.7	1.8	1.8	2.1	2.1	1.9	2.1	2.5	2.2	2.0	1.9	2.2	2.6
N	1.4	2.3	2.4	4.6	2.0	9.8	3.0	3.0	3.8	2.0	1.9	1.6	2.0	2.2	9.2	2.3	3.8	6.8	2.4	2.9	1.9	2.1	1.2	6.2
O	38.2	2.1	6.0	4.1	2.8	4.4	2.9	3.4	5.3	11.2	23.4	6.0	1.1	1.3	1.5	1.0	6.1	12.2	8.1	7.1	4.9	9.8	12.9	5.8
P	11.7	6.4	2.4	3.7	4.2	10.5	6.1	3.2	2.6	8.9	4.9	7.8	3.4	4.9	1.6	0.9	1.6	1.6	1.2	14.1	0.7	5.6	1.1	2.7

< 5% AY 5 – 22% AY > 22% AY

Figure S6. Heat map depicting the assay yields (AYs) of the PRO-TRANS(1–384) panel with L-lysine after 24 h.

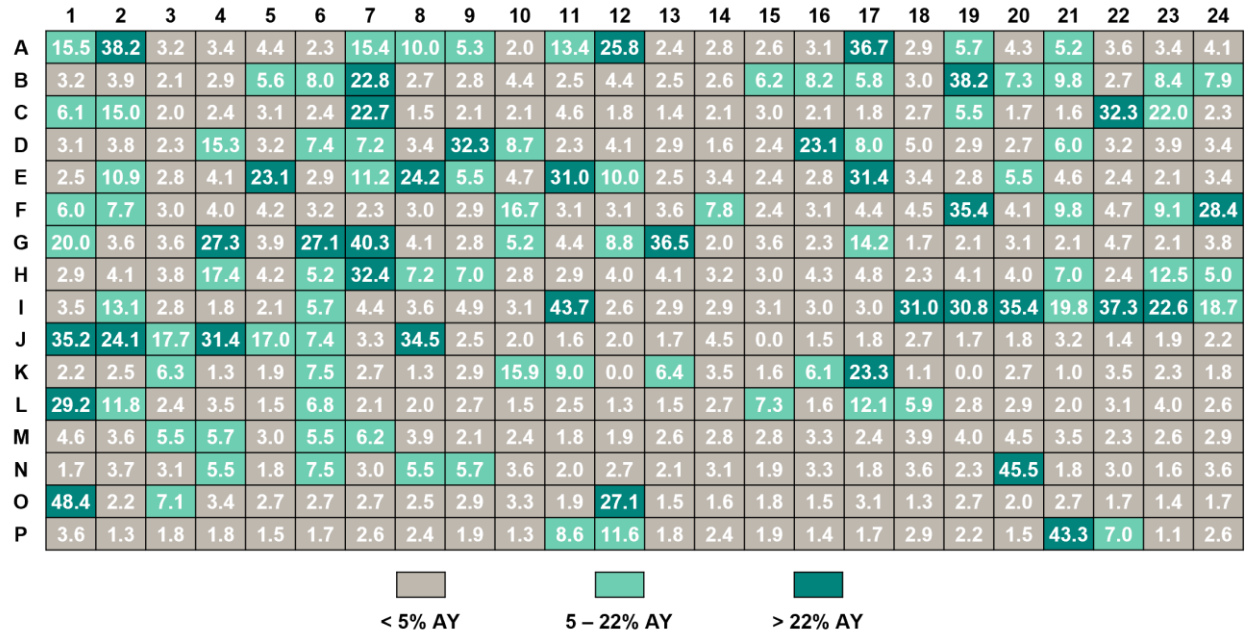


Figure S7. Heat map depicting the assay yields (AYs) of the PRO-TRANS(1–384) panel with D-lysine after 24 h.

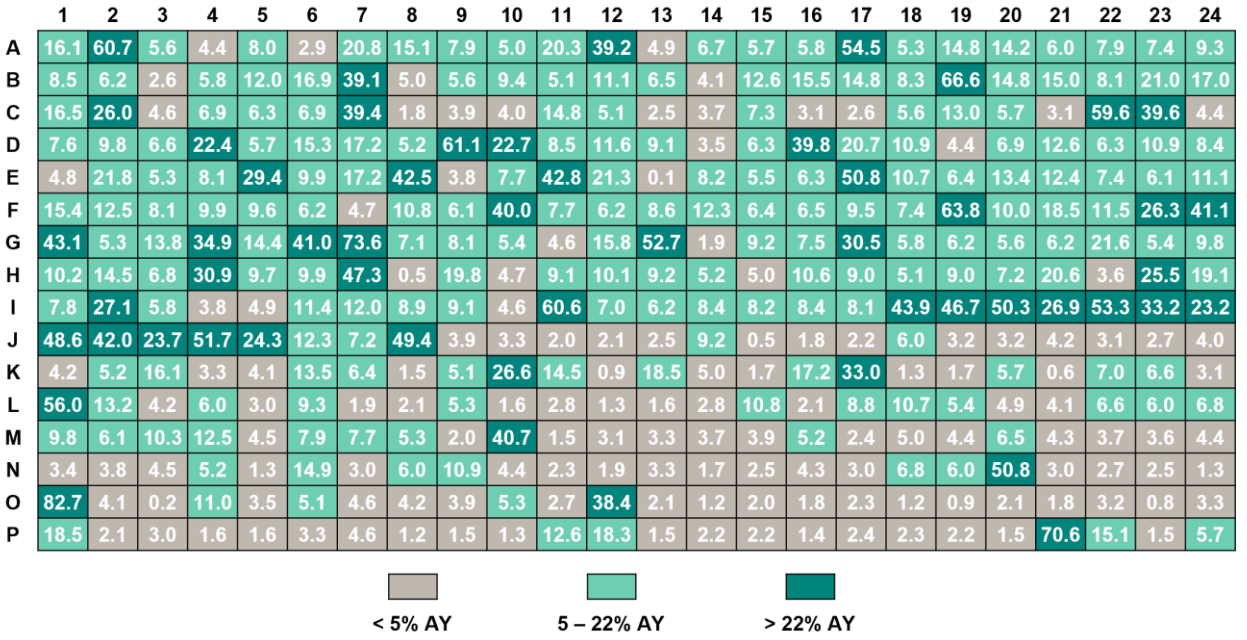
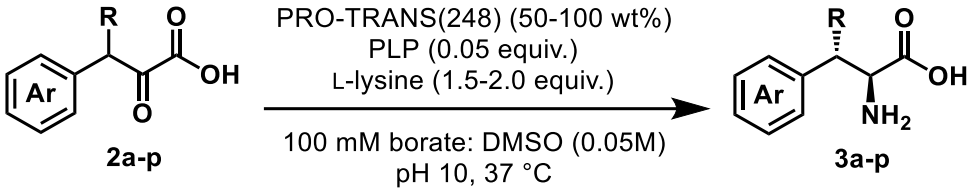


Figure S8. Heat map depicting the assay yields (AYs) of the PRO-TRANS(1–384) panel with D-lysine after 72 h.

Substrate Scope



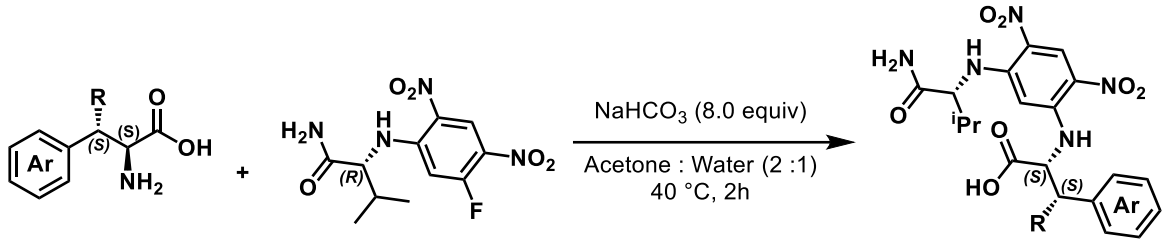
General procedure for mmol scale transaminase-catalyzed reactions. A 40 mL scintillation vial was charged with β-aryl-α-keto acid **2** (1.0 equiv.), pyridoxal 5'-phosphate (0.05 equiv.), L-lysine monohydrate (2 equiv.), and PRO-TRANS(248) fermentation powder (50-100 wt%) in a solvent mixture of 100 mM borate at pH 10.0 containing up to 10 vol% DMSO if necessary for substrate solubility. The final concentration was maintained at approximately 0.05 M. The reactions were allowed to proceed for 48 h, at which time, >90% conversion was observed by LCMS. In cases where conversion >90% conversion was not achieved (sterically encumbered substrates), additional L-Lysine (0.5 equiv.) and PRO-TRANS(248) (50 wt%) was added and the mixture stirred for an additional 24 h. Upon reaching conversion, the solution was adjusted to pH = 2 using 3M HCl. The crude mixture was then loaded onto a C18 column (150 g) and was purified by reverse phase chromatography using 5-75% MeCN:H_2O with no additional modifier. The fractions were concentrated or lyophilized to give the desired products as off-white or pale yellow solids.

Confirmation of selectivity. NMR, achiral UPLC, and chiral UPLC analyses of both the product and Valinamide derivatives were obtained where standards were not available. These data, coupled with the highly (2*S*)-selective nature of PRO-TRANS(248), provide confidence that no isomeric impurities are formed beyond what is reported.

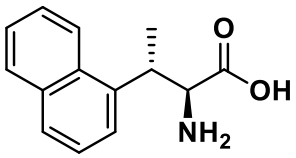
Selectivity analysis by chiral UPLC. Chromatographic separation of isomers was achieved using an Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST) heated to 35 ºC. The

flow-rate was set to 0.5 mL/min and the sample was eluted by flowing an isocratic composition of 10% 10 mM ammonium formate in water and 90% MeOH. Detection of **3** was achieved by UV/Vis absorption at 210 or 280 nm and conducted on the crude reaction mixture.

General procedure for the formation of valinamide derivatives to confirm selectivity.

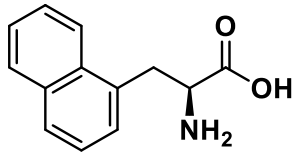


To a solution of the respective (2*S*,3*S*)-amino acid (**3**) (0.05 mmol) in acetone (2 ml) and water (1 ml) was added N_α-(2,4-dinitro-5-fluorophenyl)-D-valinamide (0.065 mmol). This was followed by the addition of sodium bicarbonate (0.40 mmol) and the resulting mixture was heated at 40 °C for 2 h after which complete consumption of the amino acid was observed. Monitored using an Agilent Technologies 1290 Infinity II UPLC-MS using a 1.8 µm, 2.1 mm X 50 mm Agilent Zorbax EclipsePlus column at 40 °C: 10 mM ammonium formate in water /acetonitrile = 95/5 to 5/95 over 6 min, flow rate = 1.0 mL/min, λ = 254 nm). The trace was analyzed for peaks showing the mass of the adduct, and in all cases, analysis confirms the NMR, achiral UPLC and chiral UPLC product ratios observed.

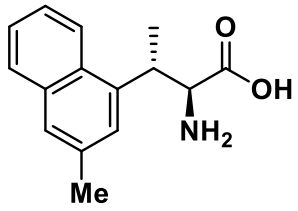


(2*S*,3*S*)-2-amino-3-(naphthalen-1-yl)butanoic acid (**3**): Reaction performed on 2.0 mmol scale to give the desired product as a white solid (375 mg, 82%). ^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 8.23 (d, *J* = 8.5 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 7.5 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.55 (m, 3H), 4.36 (d, *J* = 6.1 Hz, 1H), 4.30 (d, *J* = 6.3 Hz, 1H), 1.57 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR

(126 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 170.87, 137.00, 135.64, 132.58, 130.27, 129.43, 127.78, 126.93, 126.65, 125.77, 123.51, 58.66, 36.33, 17.72; Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 4.18 (2S, 3S), 4.85 (2R, 3R), 5.09 (2S, 3R), 7.62 (2R, 3S). HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{14}H_{16}NO_2$+: 230.1176; Found: 230.1185.

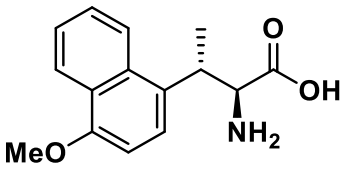


(*S*)-2-amino-3-(naphthalen-1-yl)propanoic acid (**3a**): Reaction performed on 1.0 mmol scale to give the desired product as a white solid (173 mg, 80%) with NMR spectral data matching commercially available material. ^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 8.13 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.85 (d, *J* = 7.1 Hz, 1H), 7.65 – 7.57 (m, 1H), 7.53 (t, *J* = 7.0 Hz, 1H), 7.47 (m,, 2H), 4.32 (t, *J* = 7.1 Hz, 1H), 3.85 (dd, *J* = 14.6, 5.8 Hz, 1H), 3.57 (dd, *J* = 14.5, 8.6 Hz, 1H). Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 5.30 (2S), 6.99 (2R). HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{13}H_{14}NO_2$+: 216.1019; Found: 216.1027.

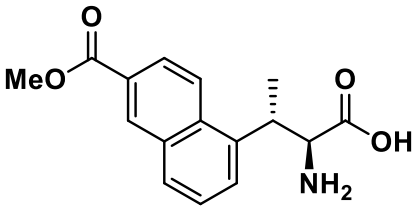


(2*S*,3*S*)-2-amino-3-(3-methylnaphthalen-1-yl)butanoic acid (**3b**): Reaction performed on 1.5 mmol scale to give the desired product as a white solid (280 mg, 77%). ^{1}H NMR (500 MHz, MeOD with 5 uL

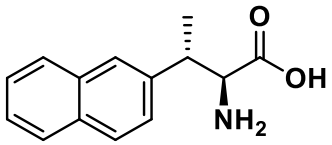
35 wt% DCl in D_2O) δ 8.15 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 7.8 Hz, 1H), 7.62 (s, 1H), 7.57 – 7.46 (m, 2H), 7.41 (s, 1H), 4.34 (d, *J* = 6.6 Hz, 1H), 4.29 – 4.22 (m, 1H), 2.52 (s, 3H), 1.56 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ 169.57, 136.62, 134.48, 133.81, 129.25, 128.16, 127.12, 126.30, 125.59, 125.35, 122.84, 57.06, 34.43, 21.43, 16.27. Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 3.85 (2S, 3S), 4.71 (2S, 3R). Achiral UPLC-MS (valinamide derivative): t_R = 2.52 (minor), 2.56 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{26}H_{29}N_5O_7$: 524.2; Found: 524.2. UPLC trace indicates >100:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{15}H_{18}NO_2$+: 244.1332; Found: 244.1348.



(2*S*,3*S*)-2-amino-3-(4-methoxynaphthalen-1-yl)butanoic acid (**3c**): Reaction performed on 1.0 mmol scale to give the desired product as a white solid (190 mg, 73%). ^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 8.33 – 8.27 (m, 1H), 8.15 (d, *J* = 8.6 Hz, 1H), 7.61 (ddd, *J* = 8.4, 6.8, 1.3 Hz, 1H), 7.50 (ddd, *J* = 8.0, 6.9, 0.8 Hz, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 4.30 (d, *J* = 6.4 Hz, 1H), 4.19 (s, 1H), 4.02 (s, 3H), 1.55 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (126 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 170.99, 156.46, 133.46, 128.54, 128.16, 127.38, 126.23, 126.13, 123.81, 123.39, 104.73, 58.72, 56.17, 35.97, 17.74; Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 4.25 (2S, 3S), 5.18 (2S, 3R). Achiral UPLC-MS (valinamide derivative): t_R = 2.44 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{26}H_{29}N_5O_8$: 540.2; Found: 540.2. UPLC trace indicates >100:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{15}H_{18}NO_3$+: 243.1281; Found: 260.1300.

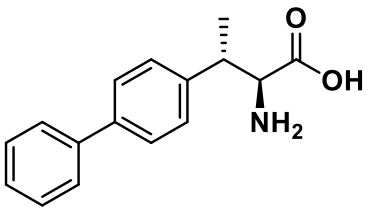


(2*S*,3*S*)-2-amino-3-(6-(methoxycarbonyl)naphthalen-1-yl)butanoic acid (**3d**): Reaction performed on 1.0 mmol scale to give the desired product as a white solid (268 mg, 93%).^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 8.65 (s, 1H), 8.36 (d, *J* = 9.0 Hz, 1H), 8.14 (d, *J* = 8.9 Hz, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 7.72 (d, *J* = 7.1 Hz, 1H), 7.64 (t, *J* = 7.7 Hz, 1H), 4.37-4.35 (m, 2H), 3.98 (s, 3H), 1.60 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (126 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 170.65, 168.74, 137.35, 134.71, 134.66, 133.06, 130.81, 128.65, 128.36, 127.80, 126.72, 124.39, 58.53, 53.03, 40.16, 17.19; Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 4.60 (2S, 3S). Achiral UPLC-MS (valinamide derivative): t_R = 2.32 (major), 2.37 (minor). MS (ESI) *m/z* [M + H] Calculated for $C_{27}H_{25}N_5O_9$: 568.2; Found: 568.2. UPLC trace indicates >100:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{16}H_{18}NO_4$+: 288.1230; Found: 288.1250.

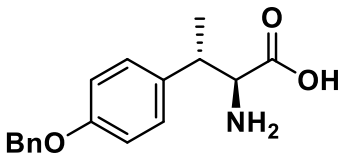


(2*S*,3*S*)-2-amino-3-(naphthalen-2-yl)butanoic acid (**3e**): Reaction performed on 0.5 mmol scale to give the desired product as a white solid (101 mg, 88%). ^{1}H NMR (400 MHz, MeOD + 5 uL 35 wt% DCl in D2O) δ 7.91 (d, *J* = 8.6 Hz, 1H), 7.90 – 7.85 (m, 2H), 7.83 (br s, 1H), 7.52 – 7.46 (m, 3H), 4.21 (d, J = 7.5 Hz, 1H), 3.53 (p, J = 7.2 Hz, 1H), 1.56 (d, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, MeOD + 5 uL 35 wt% DCl in D2O) δ 171.03, 138.24, 135.06, 134.54, 130.13, 128.94, 128.70, 128.14, 127.50, 127.30, 126.32, 59.54, 42.28, 18.32; Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280

nm: t_R = 4.18 (2S, 3S), 4.89 (2S, 3R). Achiral UPLC-MS (valinamide derivative): t_R = 2.43 (minor), 2.46 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{28}H_{28}N_5O_7$: 510.2; Found: 510.2. UPLC trace indicates 9.8:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{14}H_{16}NO_2$+: 230.1176; Found: 230.1194.

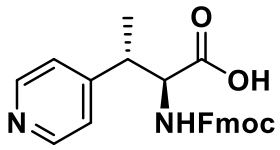


(2*S*,3*S*)-3-([1,1'-biphenyl]-4-yl)-2-aminobutanoic acid (**3f**): Reaction performed on 1.0 mmol scale to give the desired product as a white solid (210 mg, 82%). ^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 7.64 – 7.59 (m, 4H), 7.45 – 7.39 (m, 4H), 7.33 (t, *J* = 7.4 Hz, 1H), 3.65 (d, *J* = 7.7 Hz, 1H), 3.29-3.27 (m, 1H), 1.47 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (126 MHz, 500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 173.51, 142.01, 141.78, 141.22, 129.88, 129.48, 128.61, 128.40, 127.90, 62.26, 42.33, 19.34; Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 4.04 (2S, 3S), 4.69 (2S, 3R). Trace indicates 68:1 ratio. Achiral UPLC-MS (valinamide derivative): t_R = 2.60 (minor), 2.63 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{27}H_{29}N_5O_7$: 536.2; Found: 536.2. UPLC trace indicates >100:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{16}H_{18}NO_2$+: 256.1332; Found: 256.1343.



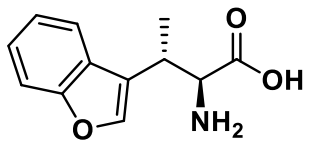
(2*S*,3*S*)-2-amino-3-(4-(benzyloxy)phenyl)butanoic acid (**3g**): Reaction performed on 1.0 mmol scale using 2.0 equivalents L-Lysine and 150 wt% PRO-TRANS(248) to give the desired product as a white

solid (205 mg, 72%). ^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 7.44 (d, J = 7.4 Hz, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.30 (dd, J = 19.5, 8.0 Hz, 3H), 7.02 (d, J = 8.7 Hz, 2H), 5.10 (s, 2H), 4.08 (d, J = 6.8 Hz, 1H), 3.38 (p, J = 7.1 Hz, 1H), 1.44 (d, J = 7.2 Hz, 3H); ^{13}C NMR (126 MHz, 500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 1 δ 169.66, 158.42, 137.25, 131.51, 128.75, 128.68, 128.18, 127.56, 127.20, 115.26, 115.21, 69.65, 58.44, 48.19, 48.01, 47.84, 47.67, 47.50, 47.33, 47.16, 39.90, 39.73, 16.88, 16.56; Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 210 nm: t_R = 3.78 (2S, 3S), 4.34 (2S, 3R). Trace indicates a 34:1 ratio; Achiral UPLC-MS (valinamide derivative): t_R = 2.63 (minor), 2.67 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{28}H_{31}N_5O_8$: 566.2; Found: 566.4. UPLC trace indicates 32:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{17}H_{20}NO_3$+: 286.1438; Found: 286.1447.

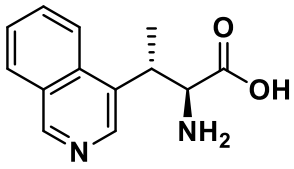


(2*S*,3*S*)-2-amino-3-(pyridin-4-yl)butanoic acid (**3h**): Reaction was performed on 1.0 mmol scale. However, reverse phase chromatography failed to fully separate the desired product from residual lysine, so the Fmoc-protected amino acid was directly prepared from the reaction mixture. Upon completion of the reaction as determined by UPLC, THF (1.8 ml) was added to the reaction, followed by sodium carbonate (530 mg, 5.00 mmol) and Fmoc-OSu (675 mg, 2.000 mmol). The mixture was stirred for 4h, at which time UPLC indicated remaining Fmoc-OSu, bis-Fmoc Lysine, and desired product were present. HCl (5 M, 4 ml, 20.00 mmol) was added and the mixture directly purified by RP isco (5-90% MeCN/water). The product eluted cleanly at 25-35% MeCN. Concentrated fractions containing the product to give 299mg of a tan solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.90 (s, 1H), 8.46 (s, 2H), 7.86 (d, *J* = 7.2 Hz, 2H), 7.73 (d, *J* = 8.7 Hz, 1H), 7.62 – 7.54 (m, 2H), 7.40 (t, *J* = 6.8 Hz, 2H), 7.37 – 7.24

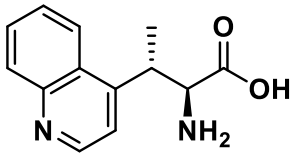
(m, 4H), 4.31 – 4.16 (m, 2H), 4.10 (s, 2H), 3.15 (d, *J* = 7.0 Hz, 1H), 1.24 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (126 MHz, DMSO) δ 172.69, 155.74, 151.93, 149.34, 143.67, 140.66, 127.62, 127.05, 125.33, 125.15, 123.29, 120.07, 65.74, 58.99, 46.50, 40.18, 17.92; Chiral HPLC (reaction at 48h, before Fmoc protection): Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 210 nm: t_R = 8.16 (2S, 3S), 10.29 (2S, 3R). Trace indicates 32:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{24}H_{23}N_2O_4$+: 403.1652; Found: 403.1662.



(2*S*,3*S*)-2-amino-3-(benzofuran-3-yl)butanoic acid (**3i**): Reaction performed on 1.0 mmol scale to give the desired product HCl salt as a tan solid (225 mg, 88%). ^{1}H NMR (400 MHz, MeOD + 5 uL 35 wt% DCl in D_2O) δ 7.77 (s, 1H), 7.75 – 7.66 (m, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.33 (td, J = 7.8, 1.3 Hz, 1H), 7.27 (td, J = 7.6, 1.0 Hz, 1H), 4.29 (d, J = 5.4 Hz, 1H), 3.90 – 3.65 (m, 1H), 1.58 (d, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD + 5 uL 35 wt% DCl in D_2O) δ 170.7, 157.0, 144.6, 127.7, 125.9, 123.9, 120.9, 120.3, 112.6, 57.9, 32.7, 17.1.Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 210 nm: t_R = 4.13 (2S, 3S). Achiral UPLC-MS (valinamide derivative): t_R = 2.27 (minor), 2.29 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{23}H_{25}N_5O_8$: 500.2; Found: 500.2. UPLC trace indicates >100:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{12}H_{14}NO_3$+: 220.0968; Found: 220.0976.

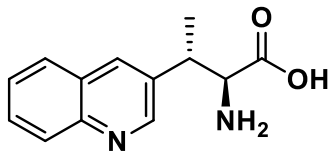


(2*S*,3*S*)-2-amino-3-(isoquinolin-4-yl)butanoic acid (**3j**): Reaction performed on 1.0 mmol scale to give the desired product as a tan solid (224 mg, 97%). ^{1}H NMR (500 MHz, MeOD) δ 9.82 (s, 1H), 8.75 – 8.66 (m, 2H), 8.64 (d, *J* = 8.2 Hz, 1H), 8.39 (t, *J* = 7.5 Hz, 1H), 8.13 (t, *J* = 7.6 Hz, 1H), 4.59 – 4.51 (m, 1H), 4.49 (d, *J* = 4.9 Hz, 1H), 1.72 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, MeOD) δ 169.53, 148.02, 139.07, 138.50, 137.95, 133.25, 132.27, 131.74, 128.96, 124.61, 57.70, 35.06, 15.22. Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 6.69 (2S, 3S). Achiral UPLC-MS (valinamide derivative): t_R = 1.67 (major), 1.72 (minor). MS (ESI) *m/z* [M + H] Calculated for $C_{24}H_{26}N_6O_7$: 511.2; Found: 511.2. UPLC trace indicates 85:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{13}H_{15}N_2O_2$+: 231.1128; Found: 231.1136.

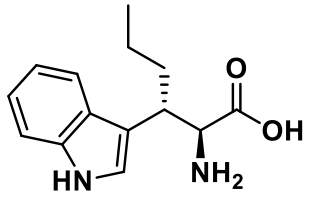


(2*S*,3*S*)-2-amino-3-(quinolin-4-yl)butanoic acid (**3k**): Reaction performed on 1.0 mmol scale to give the desired product HCl salt as a tan solid (240 mg, 90%). ^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 9.25 (d, *J* = 5.7 Hz, 1H), 8.78 (d, *J* = 8.6 Hz, 1H), 8.41 (d, *J* = 8.5 Hz, 1H), 8.26 (t, *J* = 7.6 Hz, 2H), 8.13 (t, *J* = 7.7 Hz, 1H), 4.74 – 4.66 (m, 1H), 4.55 (d, *J* = 5.0 Hz, 1H), 1.72 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (126 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 169.35, 160.99, 145.06, 138.99, 136.30, 132.09, 128.80, 125.97, 122.63, 122.54, 57.87, 37.04, 15.57. Chiral HPLC: Astec Chirobiotic T column

(4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 6.47 (2S, 3S). Achiral UPLC-MS (valinamide derivative): t_R = 1.65 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{24}H_{26}N_6O_7$: 511.2; Found: 511.2. UPLC trace indicates a single isomer. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{13}H_{15}N_2O_2$+: 231.1128; Found: 231.1136.

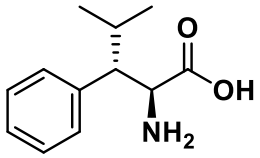


(2*S*,3*S*)-2-amino-3-(quinolin-3-yl)butanoic acid (**3l**): Reaction performed on 1.0 mmol scale to give the desired product as a white solid (188 mg, 82%). ^{1}H NMR (500 MHz, D_2O) δ 8.78 (d, *J* = 1.9 Hz, 1H), 8.33 (s, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.97 (d, *J* = 8.2 Hz, 1H), 7.81 (t, *J* = 7.7 Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 3.97 (d, *J* = 6.6 Hz, 1H), 3.63 – 3.56 (m, 1H), 1.53 (d, *J* = 7.2 Hz, 3H).^{13}C NMR (126 MHz, MeOD) δ 169.88, 147.90, 146.03, 138.27, 136.58, 136.46, 131.69, 130.62, 130.33, 121.25, 58.72, 38.93, 15.42. Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 6.68 (2S, 3S). Achiral UPLC-MS (valinamide derivative): t_R = 1.88 (minor), 1.92 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{24}H_{26}N_6O_7$: 511.2; Found: 511.2. UPLC trace indicates >100:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{13}H_{15}N_2O_2$+: 231.1128; Found: 231.1139.

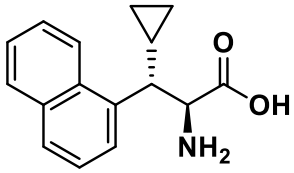


(2*S*,3*S*)-2-amino-3-(1H-indol-3-yl)hexanoic acid (**3m**): Reaction performed on 1.0 mmol scale using 2.0 equivalents L-Lysine and 150 wt% PRO-TRANS(248) to give the desired product as a white solid (79

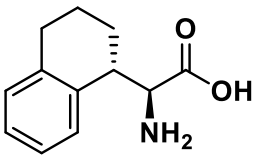
mg, 32%). ^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O): δ 7.63 (d, J = 8.0 Hz, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.27 (s, 1H), 7.14 (t, J = 7.5 Hz, 1H), 7.05 (t, J = 7.5 Hz, 1H), 4.20 (d, J = 6.0 Hz, 1H), 3.65 (dt, J = 10.8, 5.4 Hz, 1H), 2.09 – 1.79 (m, 2H), 1.31 (d, J = 15.5 Hz, 2H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 170.11, 137.03, 126.53, 123.79, 121.50, 118.84, 118.11, 111.35, 109.85, 56.92, 48.13, 48.08, 47.96, 47.91, 47.79, 47.62, 47.45, 47.28, 47.11, 38.65, 37.89, 33.87, 24.29, 20.23, 12.62. Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 3.70 (2S, 3S). Achiral UPLC-MS (valinamide derivative): t_R = 2.51 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{25}H_{30}N_6O_7$: 527.2; Found: 527.4. UPLC trace indicates >100:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{14}H_{19}N_2O_2$+: 247.1441; Found: 247.1447.



(2*S*,3*S*)-2-amino-4-methyl-3-phenylpentanoic acid (**3n**): Reaction performed on 1.0 mmol scale using 2.0 equivalents L-Lysine and 100 wt% PRO-TRANS(248) to give the desired product as a white solid (155 mg, 75%). ^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O): δ 7.38 (dt, J = 28.2, 7.1 Hz, 3H), 7.26 (d, J = 7.8 Hz, 2H), 4.43 (d, J = 5.4 Hz, 1H), 3.07 (dd, J = 9.0, 5.7 Hz, 1H), 2.41 – 2.23 (m, 1H), 1.08 (d, J = 6.5 Hz, 3H), 0.84 (d, J = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 169.86, 135.46, 129.06, 128.69, 127.77, 54.85, 53.09, 48.14, 48.09, 47.97, 47.92, 47.80, 47.63, 47.46, 47.29, 47.12, 27.96, 20.08, 19.15. Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 210 nm: t_R = 3.56 (2S, 3S). Achiral UPLC-MS (valinamide derivative): t_R = 2.65 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{25}H_{29}N_5O_7$: 488.2; Found: 488.2. UPLC trace indicates >100:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{12}H_{18}NO_2$+: 208.1332; Found: 208.1337.



(2*S*,3*S*)-2-amino-3-cyclopropyl-3-(naphthalen-1-yl)propanoic acid (**3o**)**:** Reaction performed on 1.0 mmol scale using 2.0 equivalents L-Lysine and 100 wt% PRO-TRANS(248) to give the desired product as a white solid (181 mg, 71%). ^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O): δ 8.15 (d, J = 8.4 Hz, 1H), 7.91 (dd, J = 30.7, 8.1 Hz, 2H), 7.69 (d, J = 7.0 Hz, 1H), 7.56 (dq, J = 21.0, 7.3 Hz, 3H), 4.47 (d, J = 6.3 Hz, 1H), 3.39 (s, 1H), 1.55 (s, 1H), 0.88 (s, 1H), 0.68 – 0.46 (m, 2H), 0.09 (dd, J = 9.3, 4.9 Hz, 1H). ^{13}C NMR (126 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ 169.47, 134.68, 134.21, 131.65, 128.84, 128.07, 126.30, 125.79, 125.47, 125.26, 122.33, 57.33, 48.14, 47.97, 47.80, 47.63, 47.46, 47.29, 47.12, 12.69, 6.61, 2.91. Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 280 nm: t_R = 3.94 (2S, 3S). Achiral UPLC-MS (valinamide derivative): t_R = 2.59 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{27}H_{29}N_5O_7$: 536.2; Found: 536.2. UPLC trace indicates >100:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{16}H_{18}NO_2$+: 256.1332; Found: 256.1339.



(*S*)-2-amino-2-((*S*)-1,2,3,4-tetrahydronaphthalen-1-yl)acetic acid (**3p**): Reaction performed on 1.0 mmol scale to give the desired product as a white solid (154 mg, 75%). ^{1}H NMR (500 MHz, MeOD with 5 uL 35 wt% DCl in D_2O) δ7.33 (d, *J* = 7.0 Hz, 1H), 7.19 – 7.13 (m, 2H), 7.10 (d, *J* = 6.7 Hz, 1H), 4.43 (d, *J* = 4.1 Hz, 1H), 3.59 – 3.53 (m, 1H), 2.74 (p, *J* = 10.7, 10.1 Hz, 2H), 2.09 (dd, *J* = 6.7, 3.4 Hz, 1H), 2.03 – 1.92 (m, 1H), 1.81 (dd, *J* = 6.8, 3.0 Hz, 1H), 1.69 (dq, *J* = 8.6, 4.3 Hz, 1H). ^{13}C NMR (126 MHz,

MeOD with 5 uL 35 wt% DCl in D_2O) δ 171.25, 139.48, 134.81, 130.61, 129.26, 127.94, 127.20, 58.38, 40.23, 30.32, 25.84, 21.72. Chiral HPLC: Astec Chirobiotic T column (4.6 x 100 mm, 5 µm particle size, PN: 12022AST), 35 ˚C, 10% 10 mM aqueous ammonium formate in MeOH, 0.5 mL/min, λ = 210 nm: t_R = 4.68 (2S, 3S). Achiral UPLC-MS (valinamide derivative): t_R = 2.36 (minor), 2.40 (major). MS (ESI) *m/z* [M + H] Calculated for $C_{23}H_{27}N_6O_7$: 486.2; Found: 486.2. UPLC trace indicates 23:1 ratio. HRMS: (ESI) *m/z*: [M + H] Calculated for $C_{12}H_{16}NO_2$+: 206.1176. Found: 206.1182.

Synthesis of Compound 3 on Scale. A reactor under N_2 flow was charged with an aqueous solution of 3-(naphthalen-1-yl)-2-oxobutanoic acid (assayed 100 g = 1449.4 g x 6.9 wt%, 438 mmol, 1.0 equiv.), DMSO (500 mL), followed by 0.1 M borate buffer (1059.5 g), and L-lysine (224 g, 1533 mmol, 3.5 equiv.). The resulting mixture was adjusted to pH=10 with sodium tetraborate decahydrate (52.9 g). PRO-TRANS(248) (32 g, 32 wt%) and pyridoxal-5-phosphate (5 g, 0.05 equiv.) was charged into the batch. The resulting mixture was agitated for 66 h at 42 °C. After the reaction was finished, the batch was adjusted to pH=5 with 5N HCl and cooled down to 0 °C, the resulting mixture was filtered and washed with water (2 vol.). The wet cake was slurried in water (10 vol.), filtered, and washed with water (2.5 vol. x 2). 167 g of wet **5** (Purity: 96.8%, assay: 48.1%, 80.3% isolated yield) was obtained as a light yellow solid. The wet cake was used directly in a subsequent Fmoc protection and not fully dried. ^{1}H NMR (CD_3CN, 400 MHz): δ 8.133 (d, 1H), 7.865 (d, 1 H), 7.802-7.763 (m, 1H), 7.552−7.466 (m, 4H), 3.977-3.902 (m, 2H), 1.381-1.360 (m, 3H) ppm. ^{13}C NMR (CD_3CN, 125 MHz): δ 175.20, 130.65, 128.65, 126.03, 123.71, 122.73, 121.16, 120.41, 120.25, 119.32, 117.75, 35.71, 12.50 ppm. HRMS *m/z*: [M + H]+ calculated for ($C_{14}H_{16}NO_2$+): 230.1181; Found: 230.1276.

Crystal Data and Structure Refinement for Compound 3 (CCDC 2345091)

A single crystal grown from cooling a saturated solution of the molecule in water at ~100C to room temperature was selected for single crystal X-ray data analysis. The crystal was a plate with dimensions of 0.33 mm x 0.20 mm x 0.03mm. Data collection was performed on a XtaLAB Synergy, Dualflex, HyPix diffractometer at 100K using CuKα radiation. The unit cell was determined to be trigonal in space group $P3_121$. The structure is a dihydrate and contained one molecule in the crystallographic asymmetric unit.

Absolute configuration was established by anomalous-dispersion effects in diffraction measurements on the crystal and confirmed that the stereochemistry was as shown. Crystallographic data is summarized in **Table S1**. **Figure S9** shows a thermal ellipsoid representation of **3** with thermal ellipsoids set at the 50% probability level. Coordinates, refinement details and structure factors have been deposited with the Cambridge Crystallographic Data Centre (CCDC 2345091).

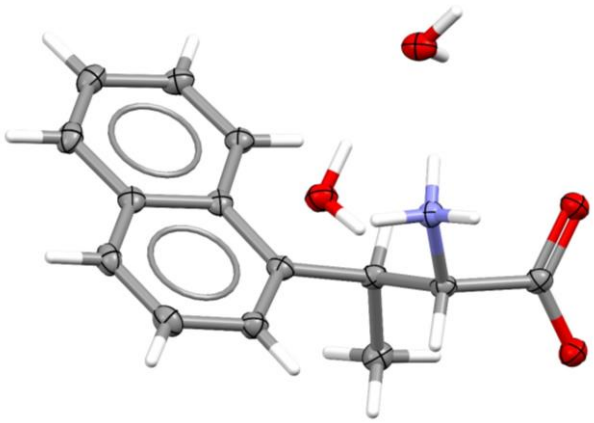


Figure S9. Thermal ellipsoid representation of **3** with thermal ellipsoids set at the 50% probability level.

Table S1. Crystal data and structure refinement for **2** [CCDC 2345091]

Empirical formula	$C_{14}H_{19}NO_4$
Formula weight	265.30
Temperature/K	100.00(10)
Crystal system	trigonal
Space group	$P3_121$
a/Å	6.96690(10)
b/Å	6.96690(10)
c/Å	47.1708(8)
α/°	90
β/°	90
γ/°	120
Volume/Å^3	1982.82(7)
Z	6
ρ_{calc}g/cm^3	1.333
μ/mm^{-1}	0.805
F(000)	852.0
Crystal size/mm^3	0.325 × 0.199 × 0.034
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	5.62 to 142.602
Index ranges	$-8 \le h \le 8$, $-8 \le k \le 8$, $-56 \le l \le 58$
Reflections collected	24220
Independent reflections	2575 [R_{int} = 0.0512, R_{sigma} = 0.0246]
Data/restraints/parameters	2575/0/186
Goodness-of-fit on F^2	1.052
Final R indexes [I>=2σ (I)]	R_1 = 0.0302, wR_2 = 0.0765
Final R indexes [all data]	R_1 = 0.0315, wR_2 = 0.0773
Largest diff. peak/hole / e Å^{-3}	0.17/-0.16
Flack parameter	0.11(9)

Crystal Data and Structure Refinement for Compound 3p (CCDC 2345090)

A single crystal, grown from cooling a room temperature saturated solution in water to 5˚C, was selected for single crystal X-ray data analysis. The crystal was a plate with dimensions of 0.13 mm x 0.11 mm x 0.05mm. Data collection was performed on a XtaLAB Synergy, Dualflex, HyPix diffractometer at 100K using CuKα radiation. The unit cell was determined to be orthorhombic in space group $P2_12_12_1$. The structure is a mono-HCl salt with one water of hydration in the crystallographic asymmetric unit. Disorder is observed in the conformation of the cyclohexyl ring and is removed from **Figure S10** for clarity.

Absolute configuration was established by anomalous-dispersion effects in diffraction measurements on the crystal and confirmed that the stereochemistry was as shown. Crystallographic data is summarized in **Table S2**. **Figure S10** shows a thermal ellipsoid representation of **3p** with thermal ellipsoids set at the 50% probability level. Coordinates, refinement details and structure factors have been deposited with the Cambridge Crystallographic Data Centre (CCDC 2345090).

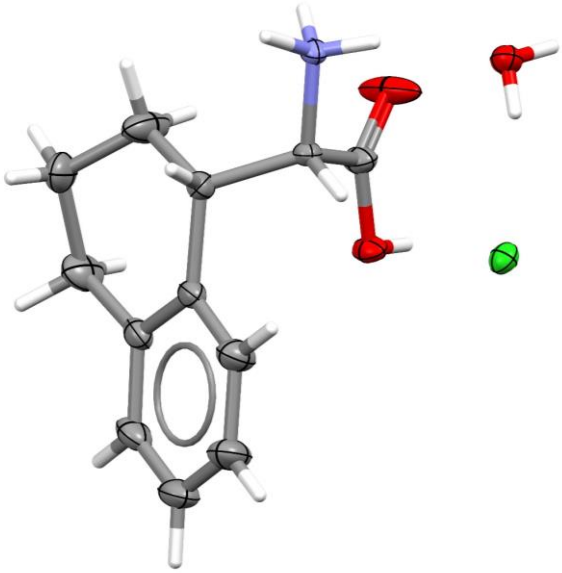


Figure S10. Thermal ellipsoid representation of **3p** with thermal ellipsoids set at the 50% probability level.

Table S2. Crystal data and structure refinement for **3p** [CCDC 2345090]

Empirical formula	$C_{12}H_{18}ClNO_3$
Formula weight	259.72
Temperature/K	100.00(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	5.87110(10)
b/Å	6.75650(10)
c/Å	32.6786(5)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	1296.30(4)
Z	4
ρ_{calc}g/cm^3	1.331
μ/mm^{-1}	2.597
F(000)	552.0
Crystal size/mm^3	0.131 × 0.113 × 0.047
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	5.408 to 150.23
Index ranges	$-7 \leq h \leq 7$, $-5 \leq k \leq 8$, $-40 \leq l \leq 39$
Reflections collected	12132
Independent reflections	2550 [R_{int} = 0.0407, R_{sigma} = 0.0272]
Data/restraints/parameters	2550/2/167
Goodness-of-fit on F^2	1.051
Final R indexes [I>=2σ (I)]	R_1 = 0.0285, wR_2 = 0.0732
Final R indexes [all data]	R_1 = 0.0301, wR_2 = 0.0745
Largest diff. peak/hole / e Å^{-3}	0.29/-0.28
Flack parameter	0.002(8)

References

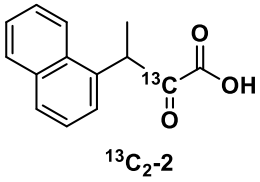
(1) Jana,U.; Biswas, S.; Maiti, S. A simple and efficient $FeCl_3$-catalyzed direct alkylation of active methylene compounds with benzylic and allylic alcohols under mild conditions. *Tetrahedron Lett.* **2007**, *48*, 4065-4069.

(2) Steward, K.M.; Johnson, J. S. Asymmetric Synthesis of α-Keto Esters via Cu(II)-Catalyzed Aerobic Deacylation of Acetoacetate Alkylation Products: An Unusually Simple Synthetic Equivalent to the Glyoxylate Anion Synthon. *Org. Lett.* **2011**, *13*, 2426–2429.

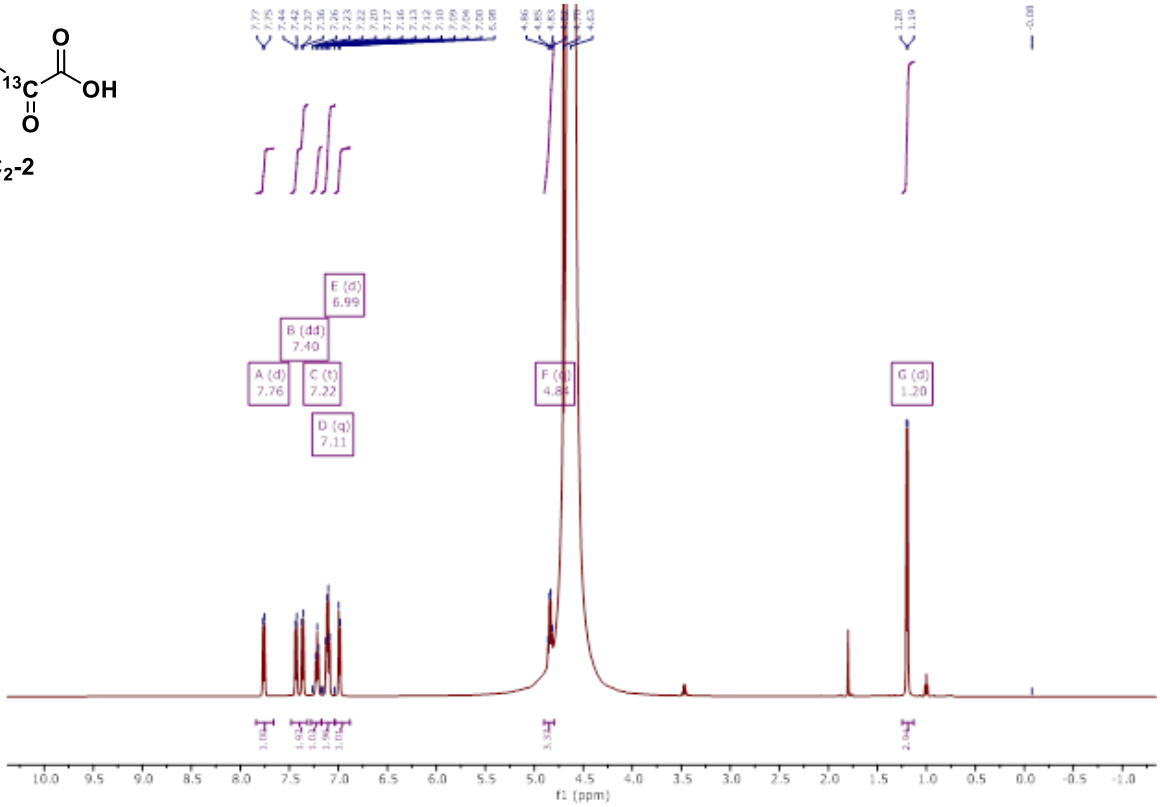
(3) Zavesky B. P.; Johnson, J. S. Direct Zinc(II)-Catalyzed Enantioconvergent Additions of Terminal Alkynes to α-Keto Esters. *Angew. Chem. Int. Ed.* **2017**, *56*, 8805–8808.

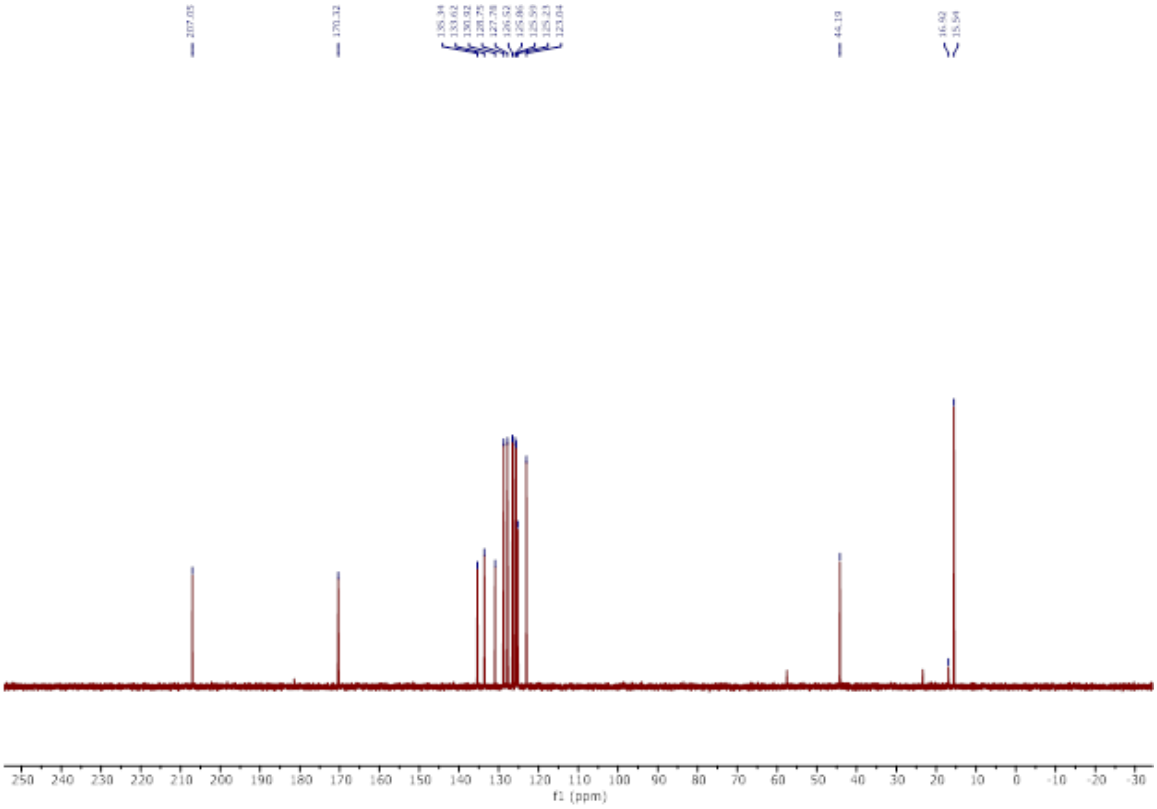
(4) Zavesky B. P.; Bartless, S. L.; Johnson, J. S. Palladium-Catalyzed β-Arylation of α-Keto Esters. *Org. Lett.* **2017**, *19*, 2126–2129.

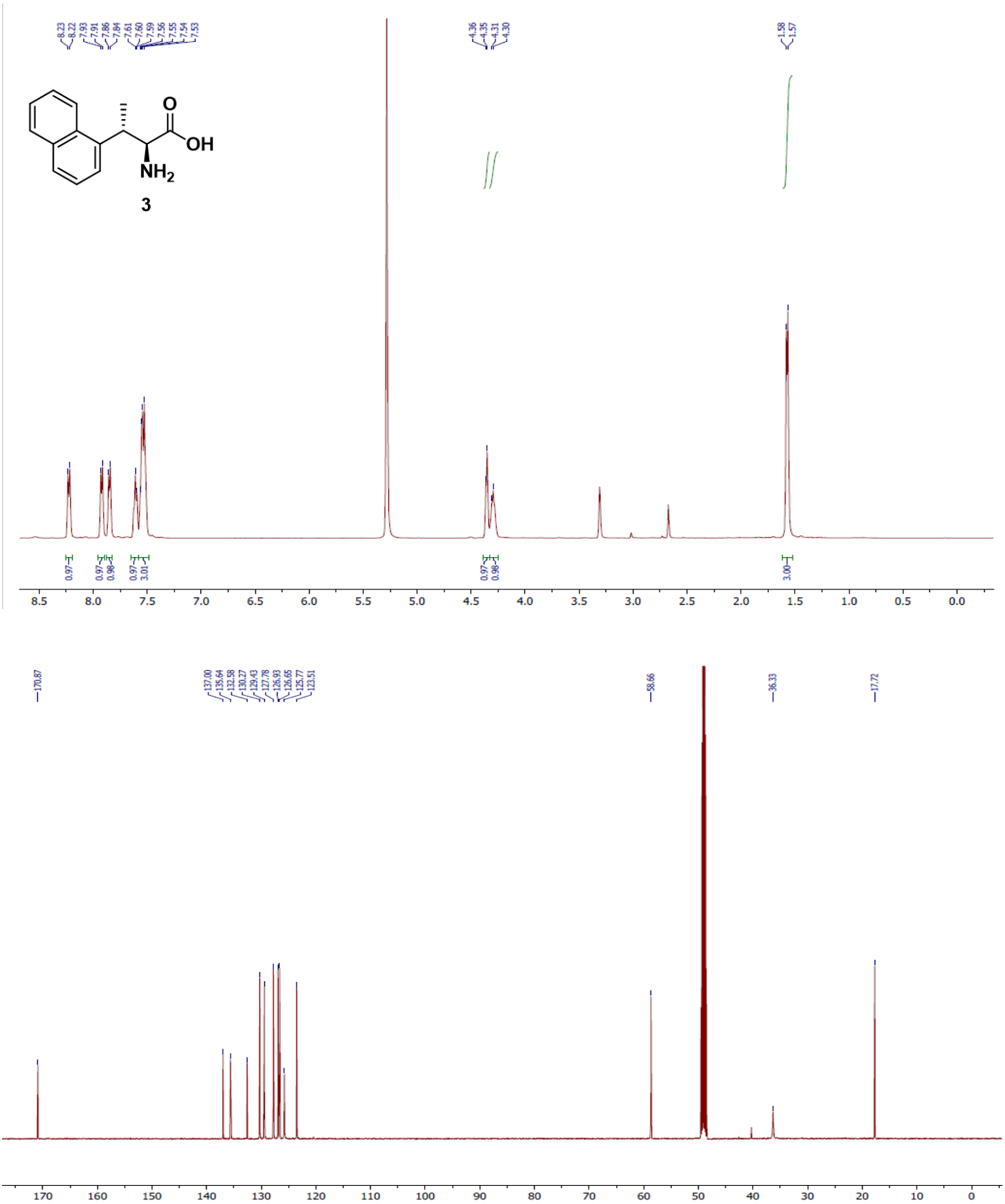
NMR Spectra and UPLC/HPLC Chromatograms



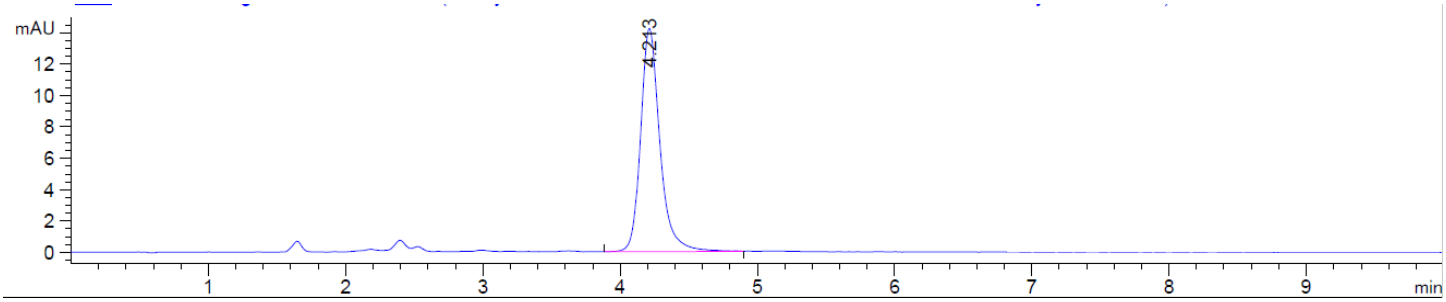
$^{13}C_2$-2



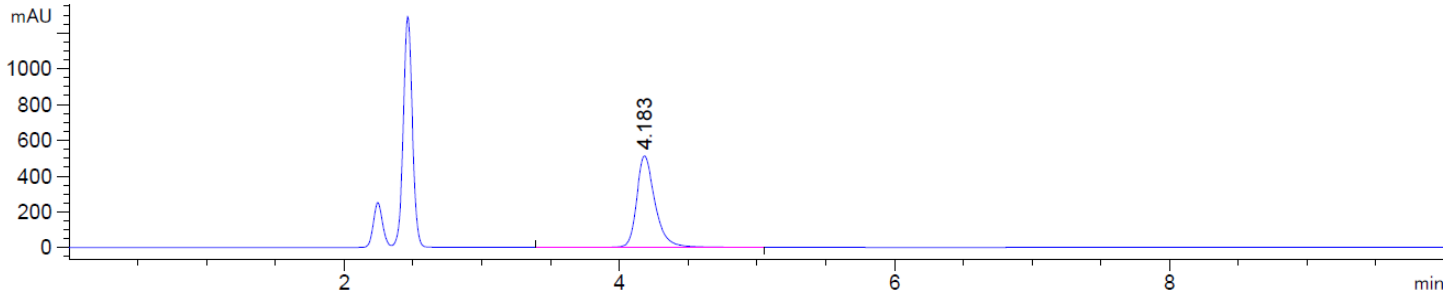




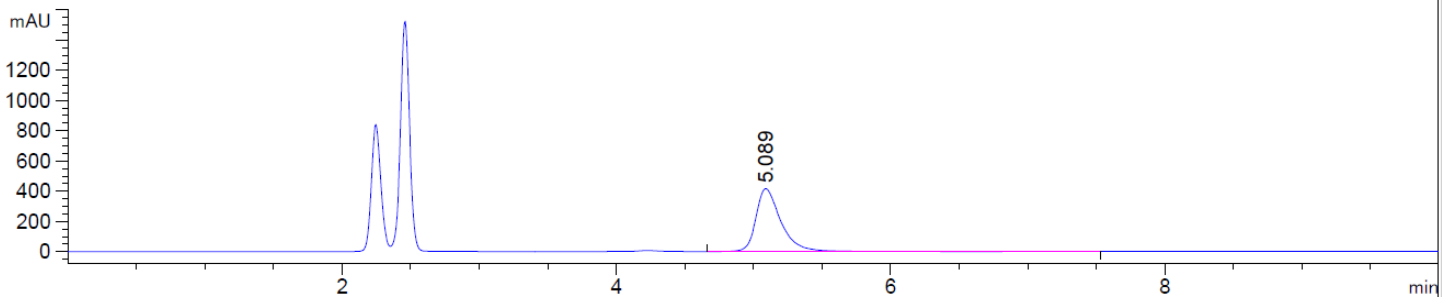
Chiral HPLC (48h):



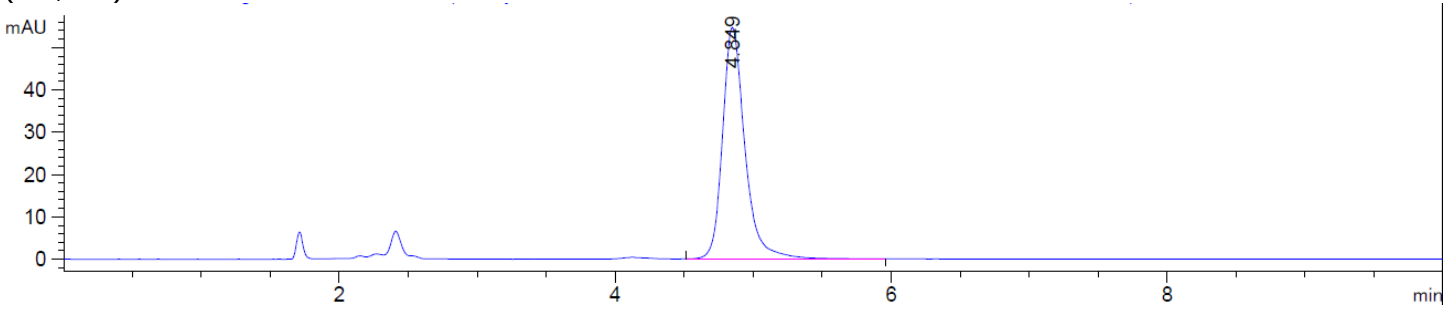
(2S, 3S) Standard:



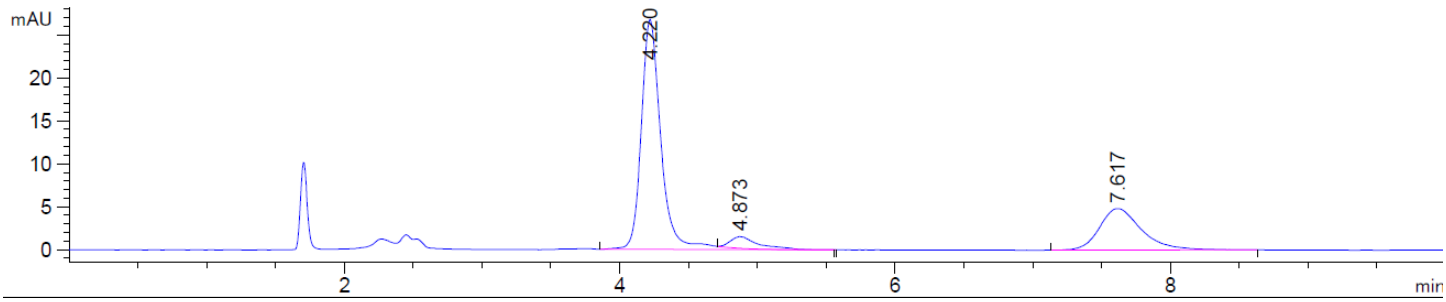
(2S, 3R) Standard:

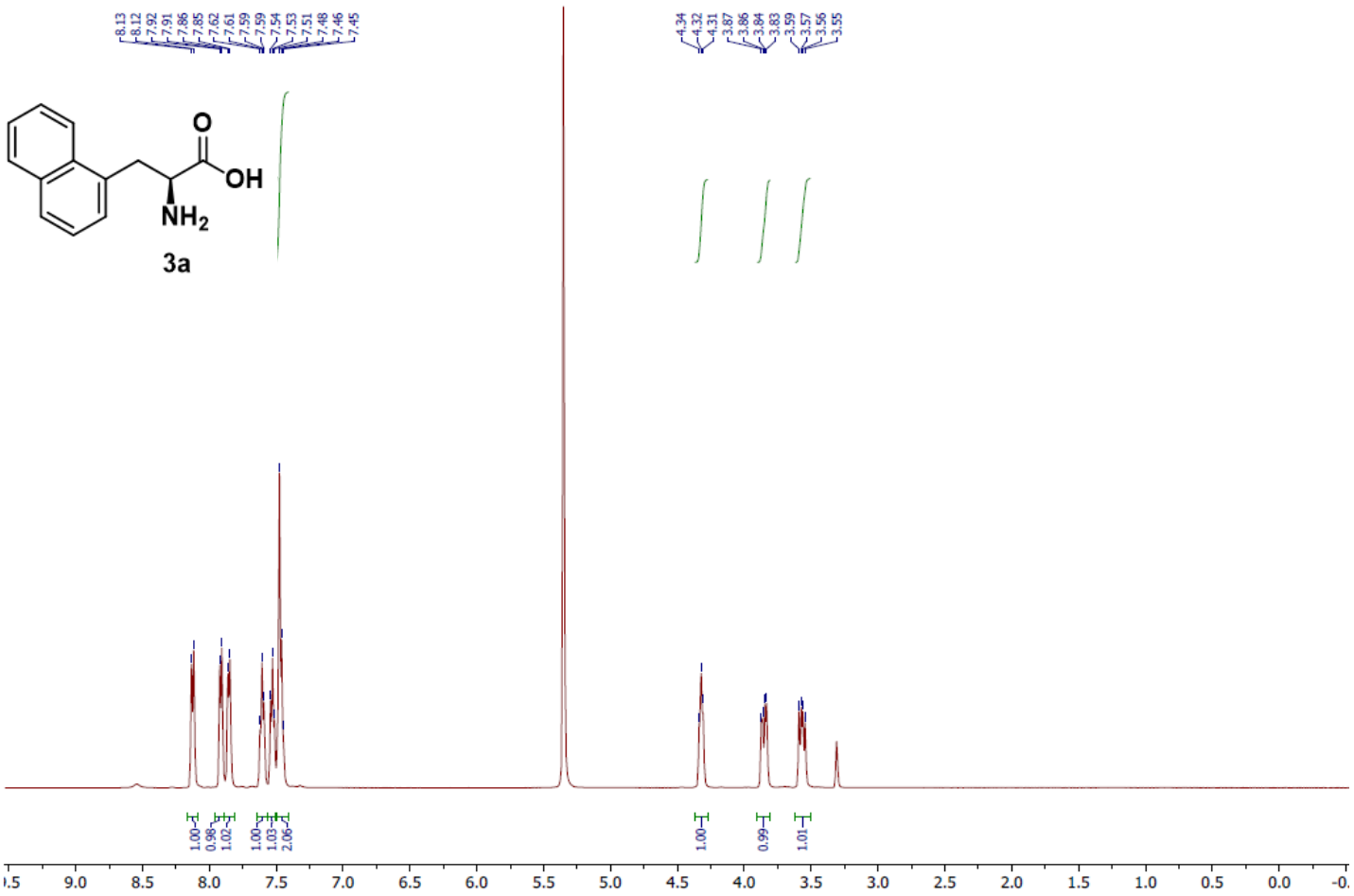


(2R, 3R) Standard:

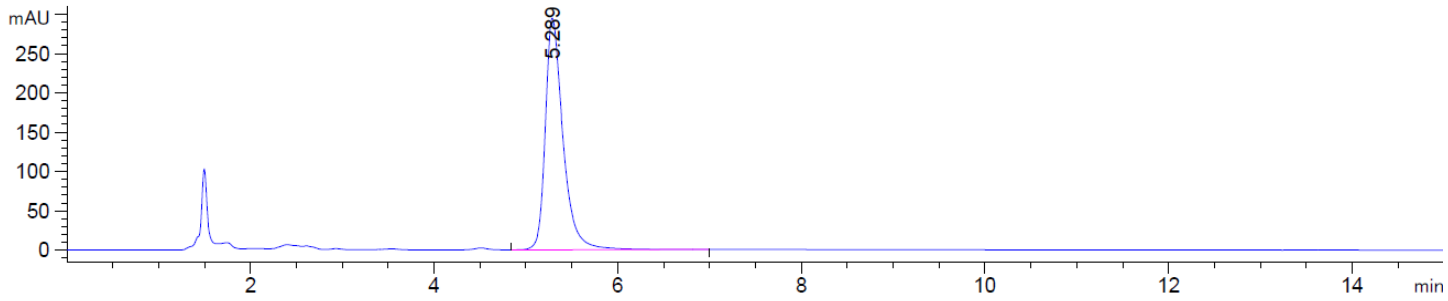


(2R, 3S) Standard (combined with 2S, 3S):

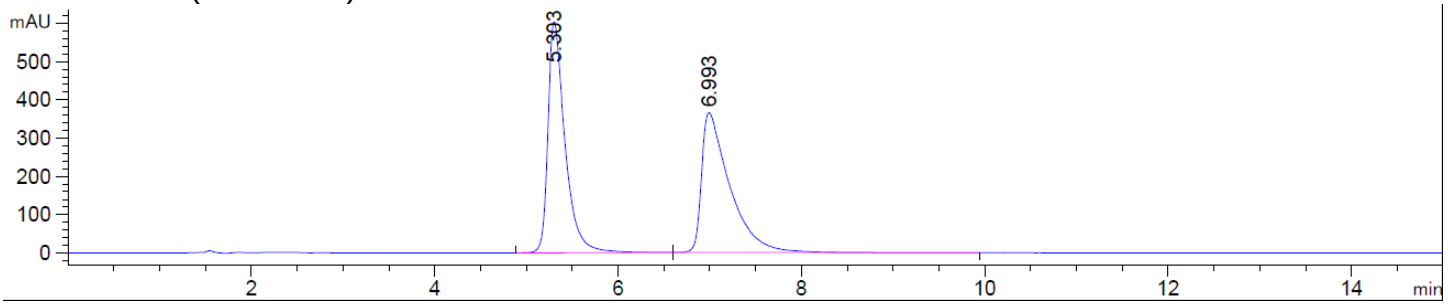


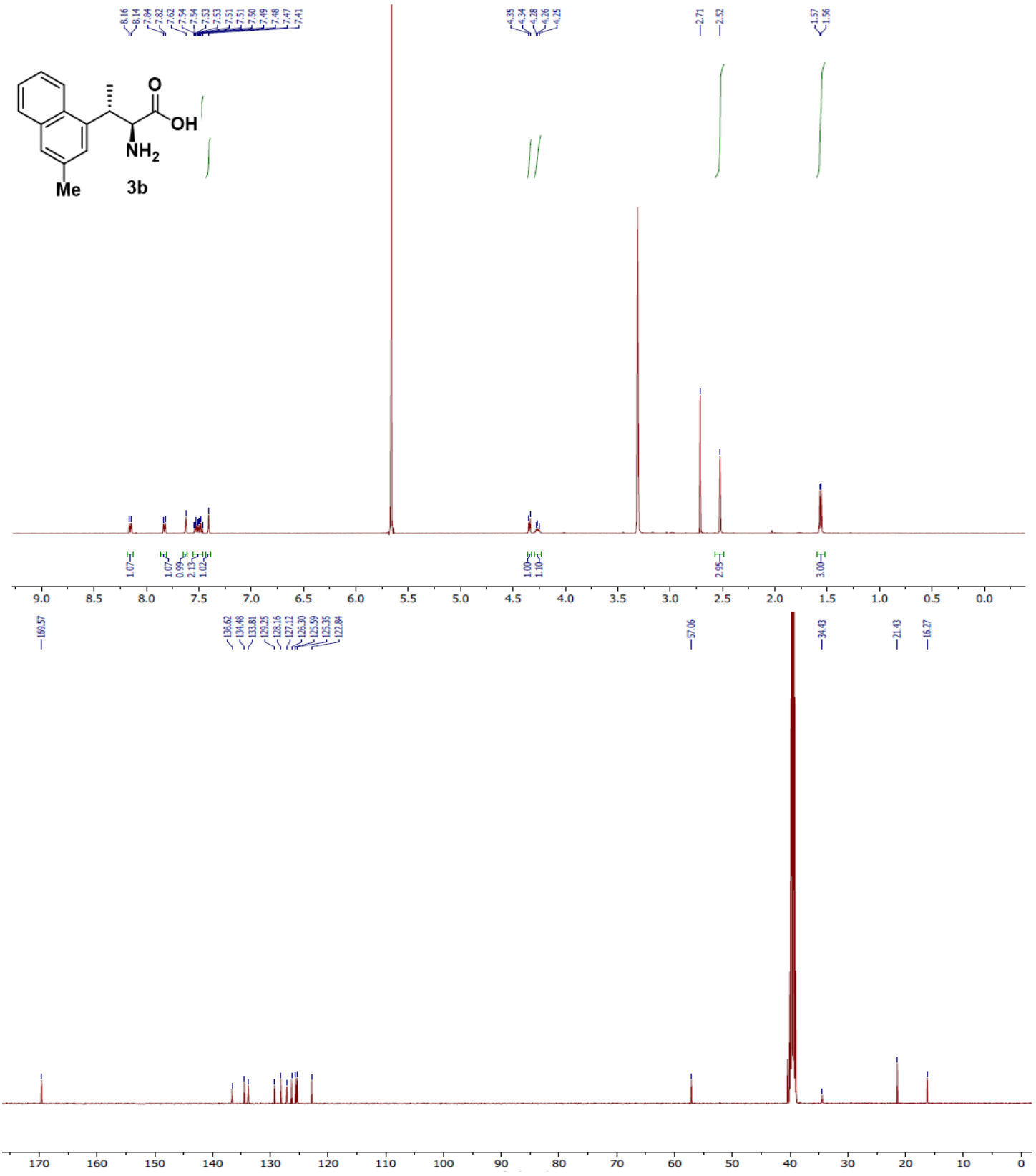


Chiral HPLC (48h):

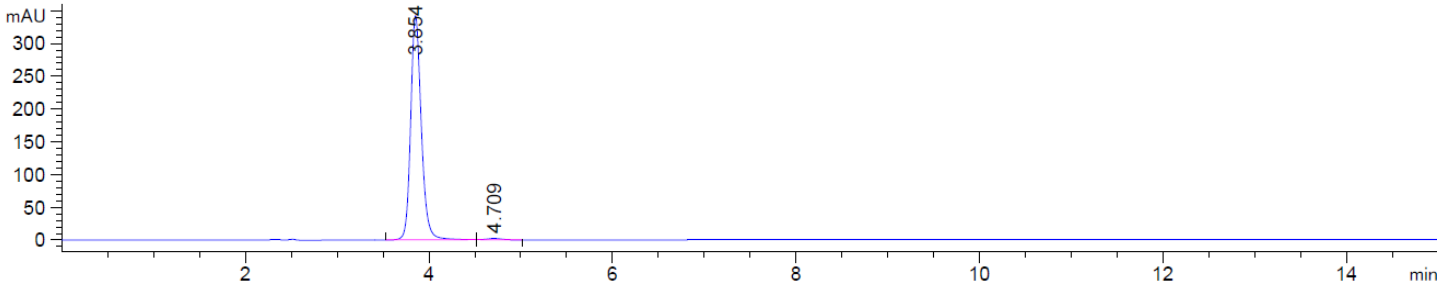


Chiral HPLC (Racemate):

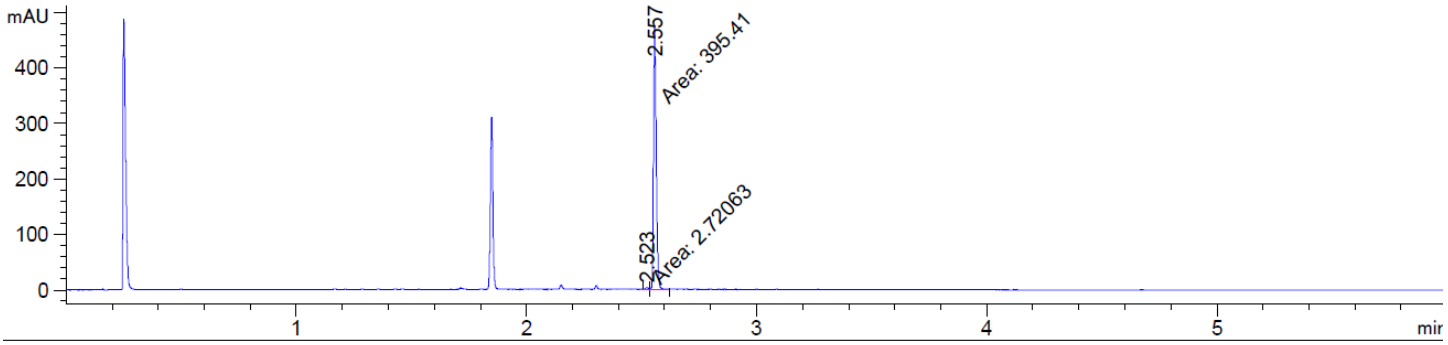


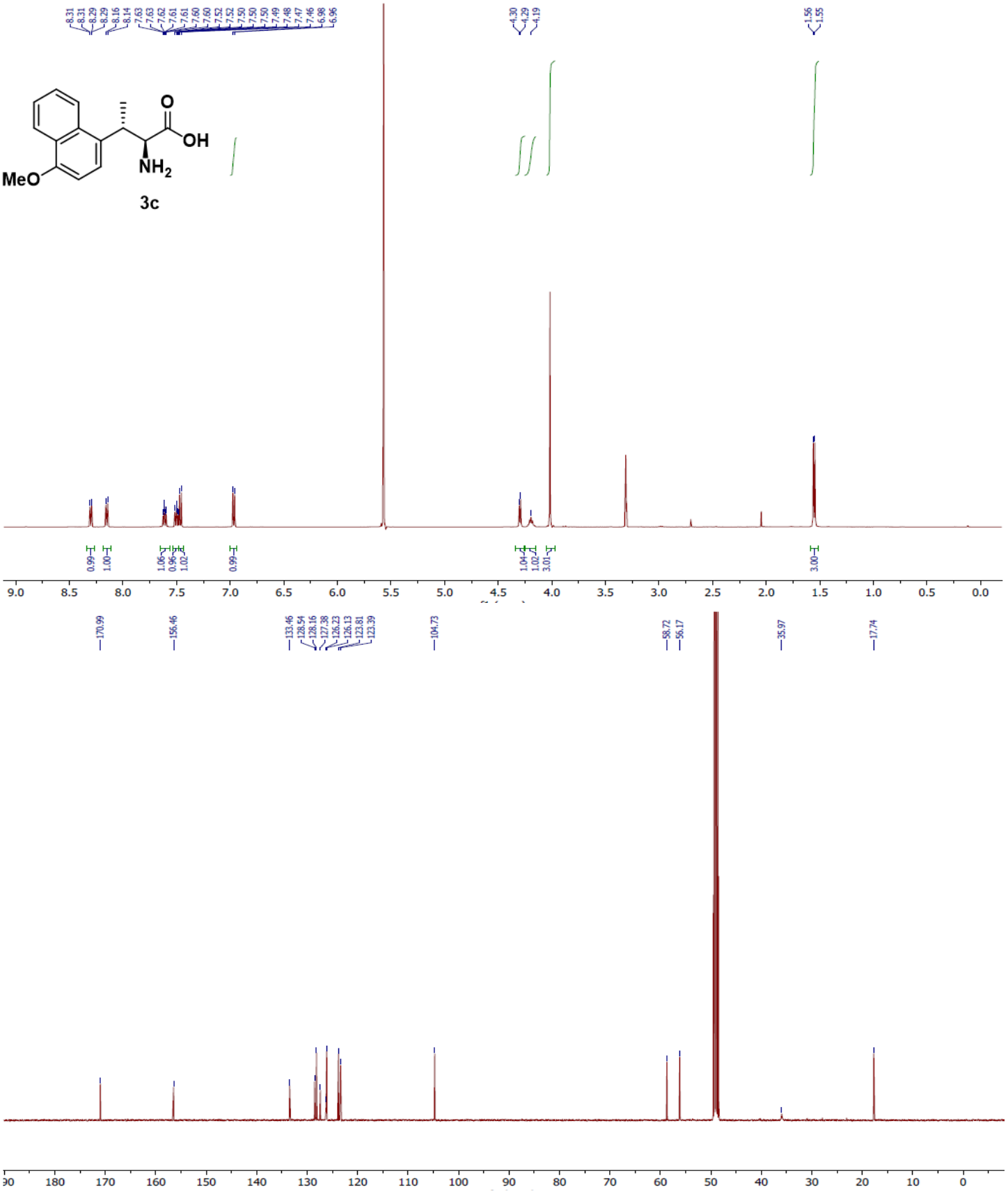


Chiral HPLC (48h):

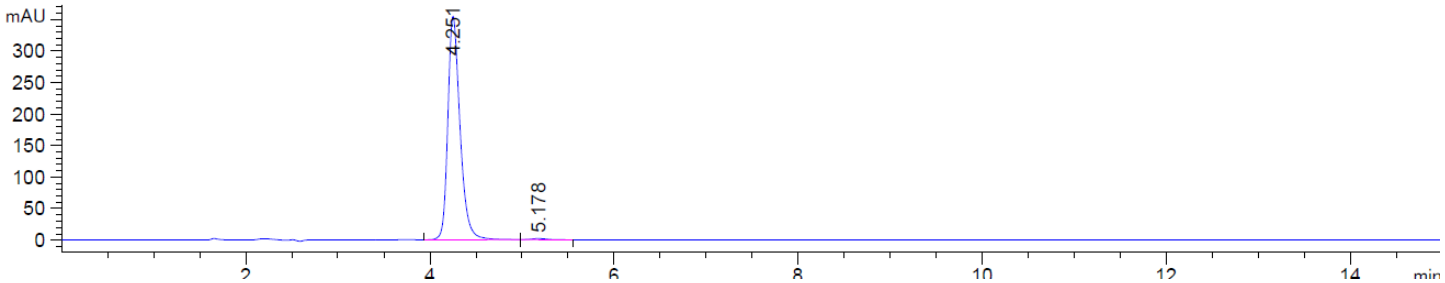


Achiral UPLC (valinamide derivative):

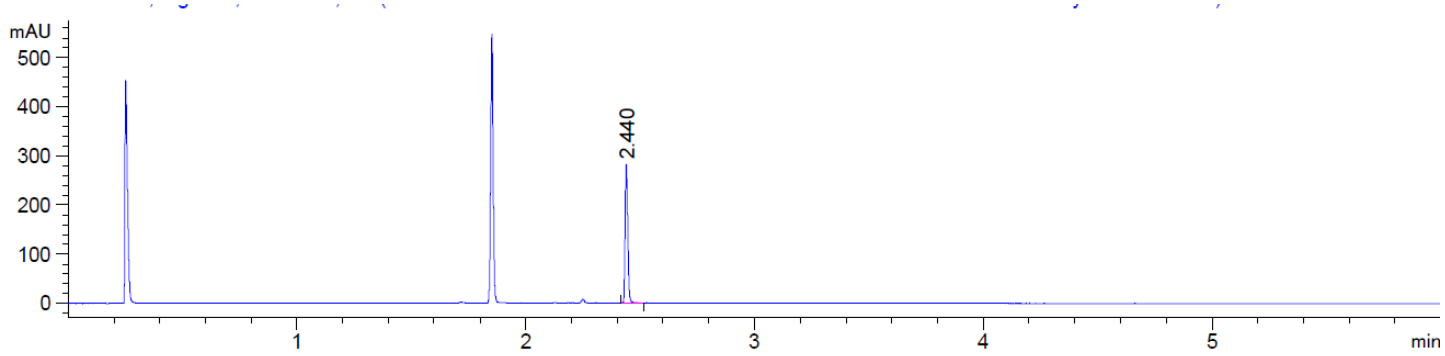


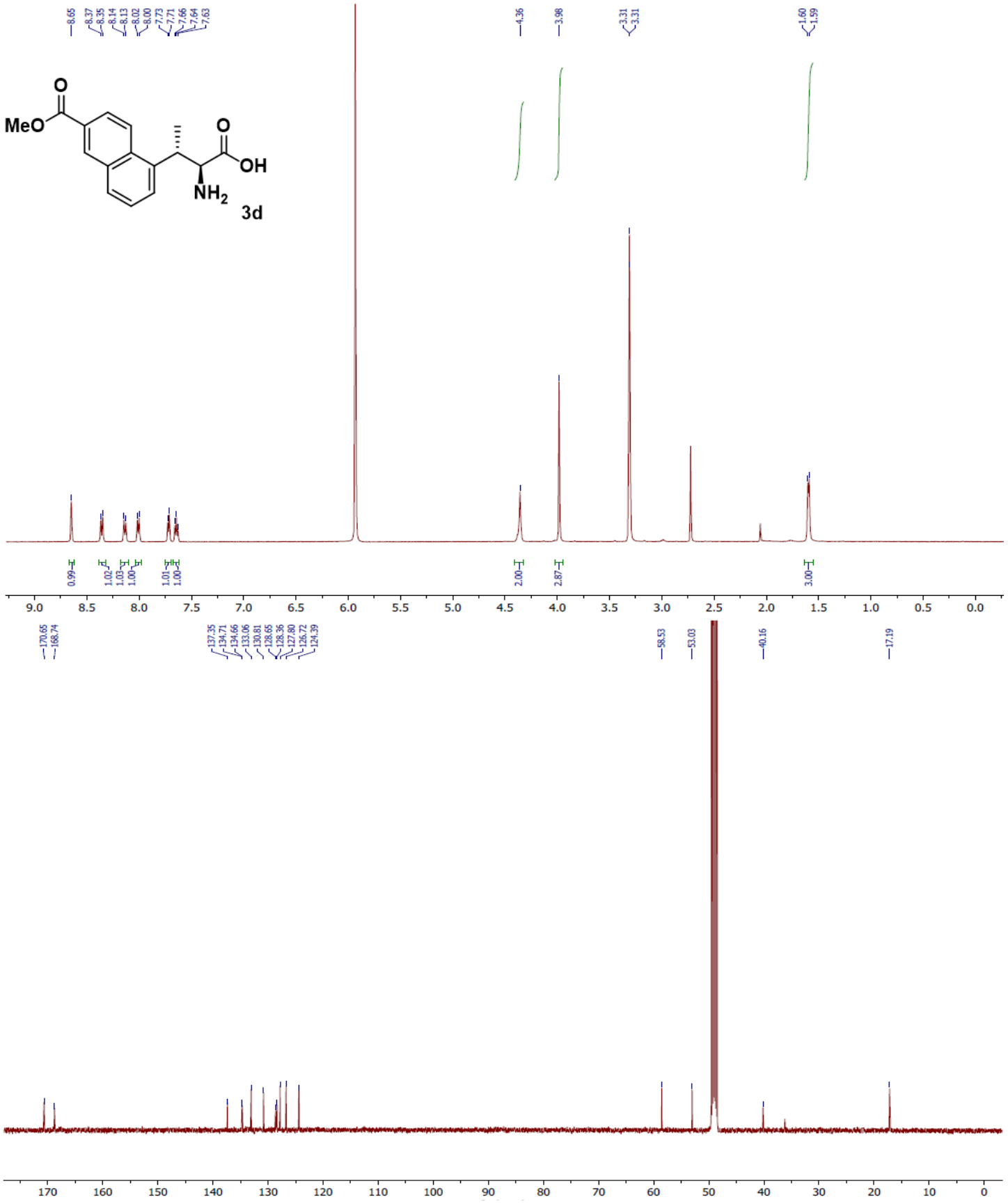


Chiral HPLC (48h):

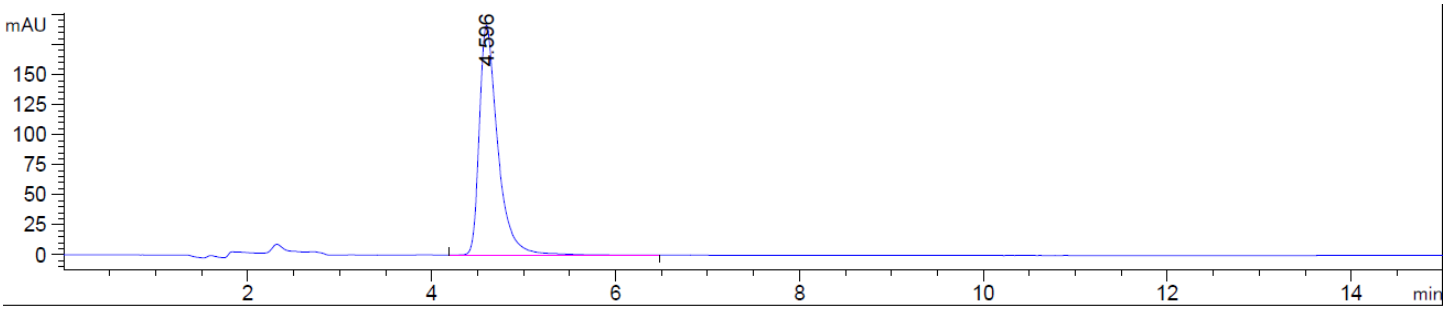


Achiral UPLC (valinamide derivative):

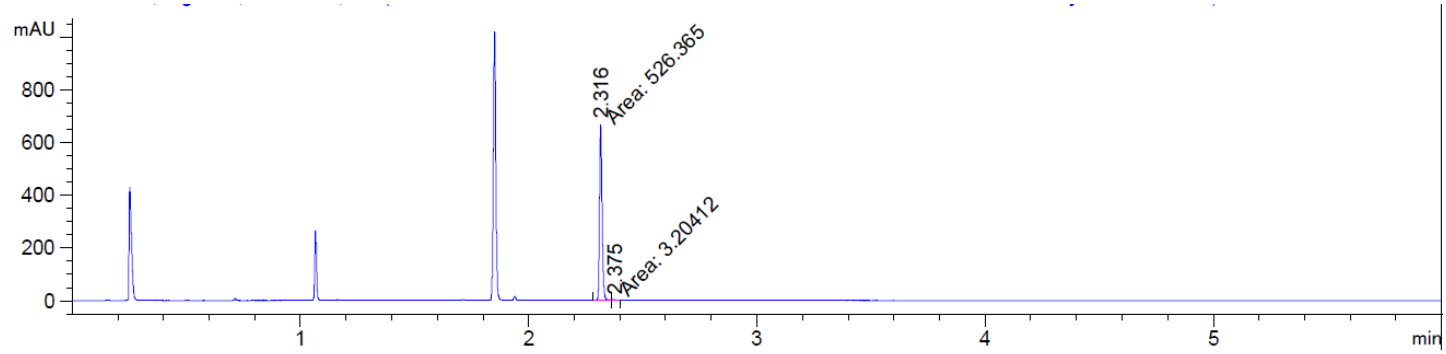


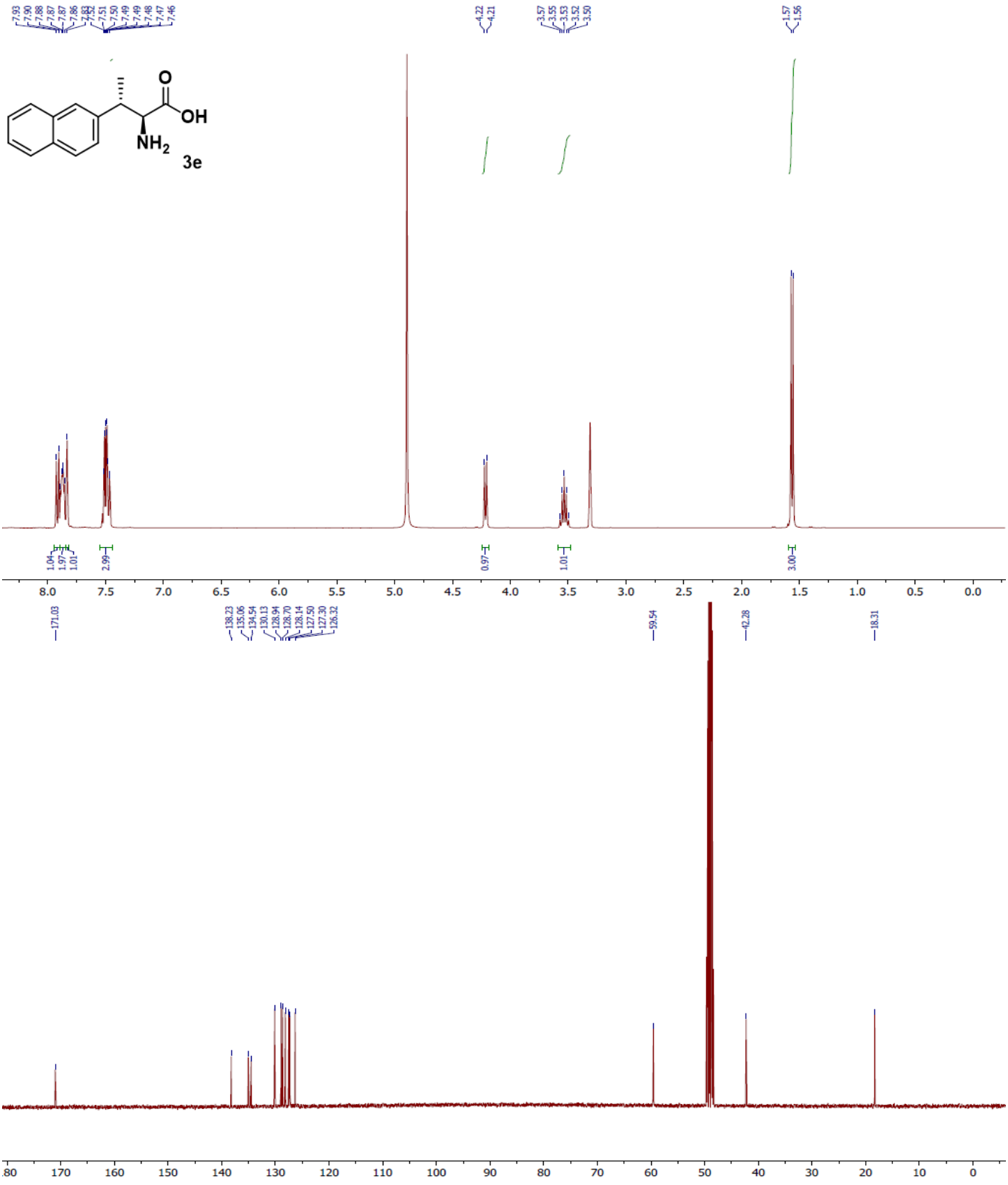


Chiral HPLC (48h):



Achiral UPLC (valinamide derivative):





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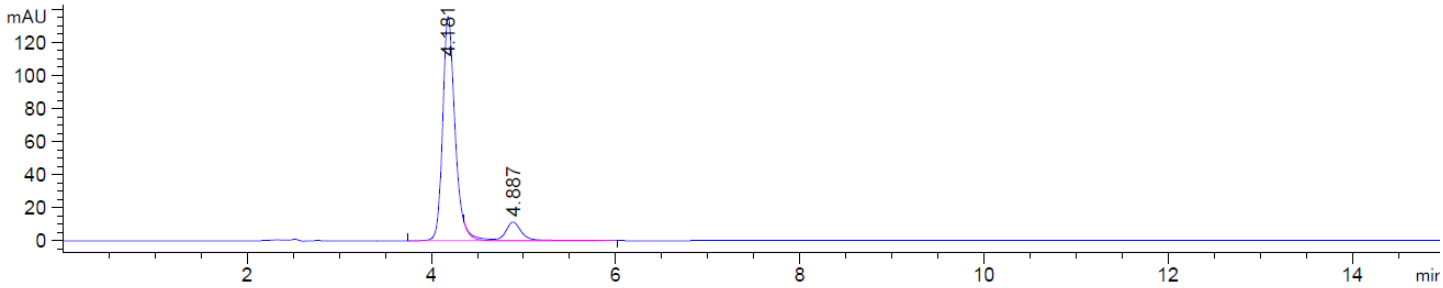
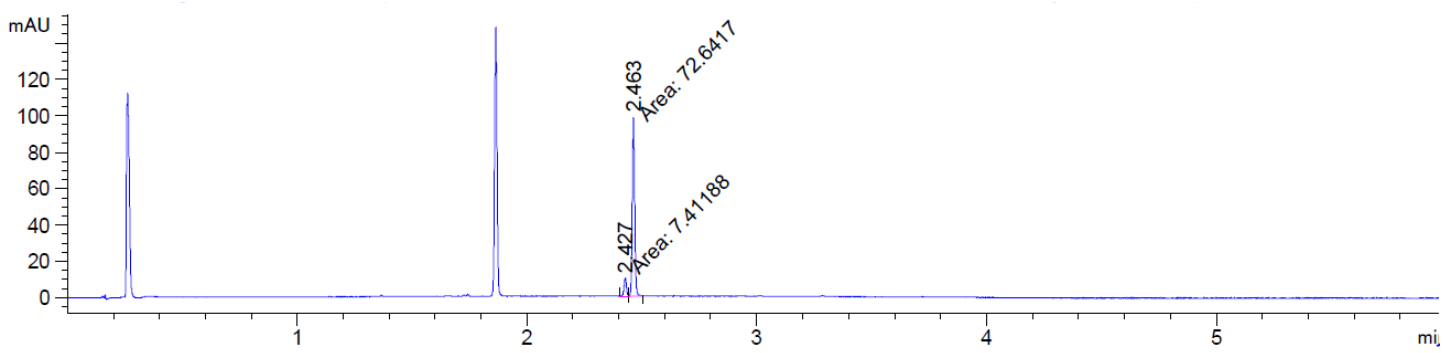
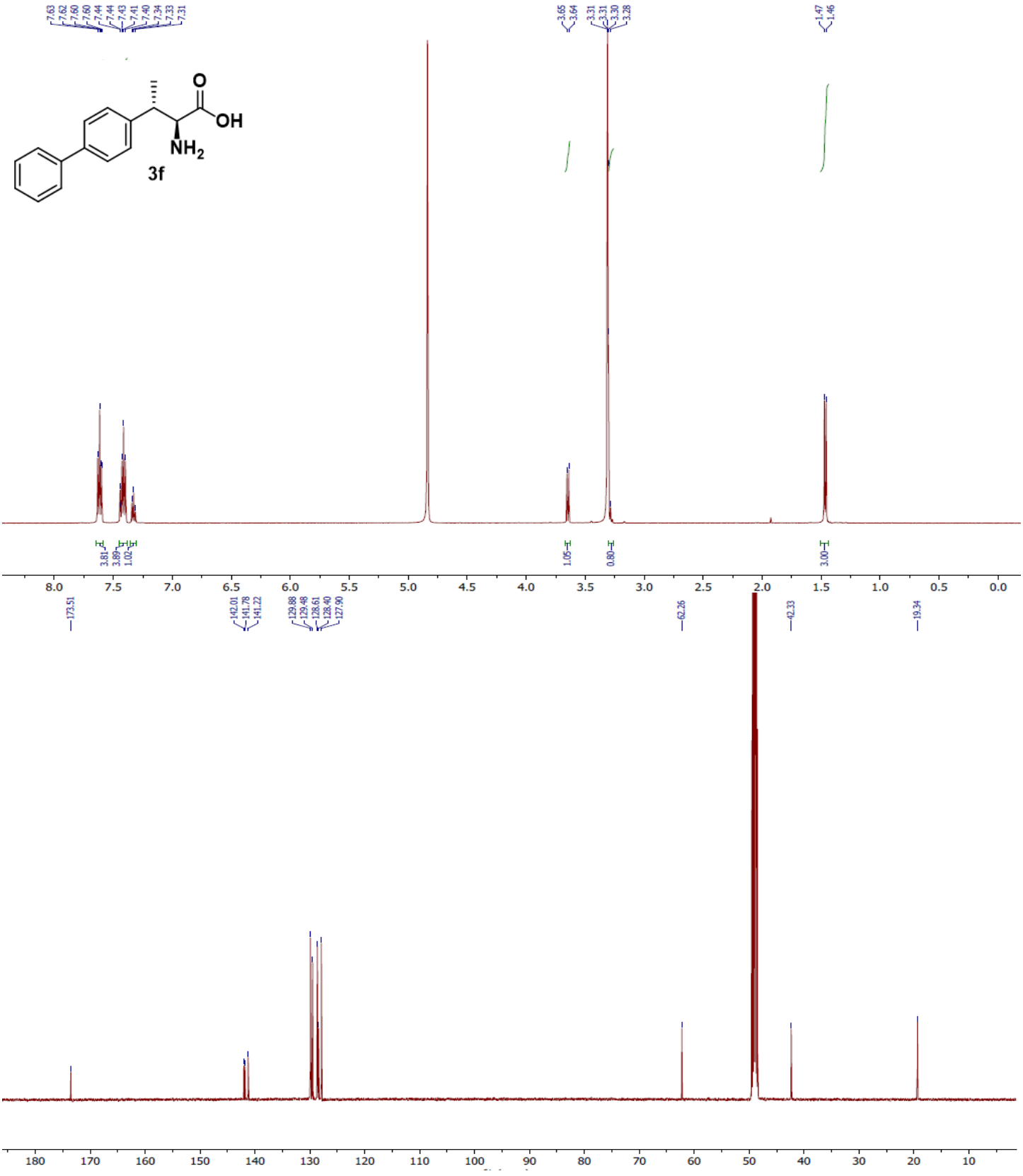


Achiral UPLC (valinamide derivative):





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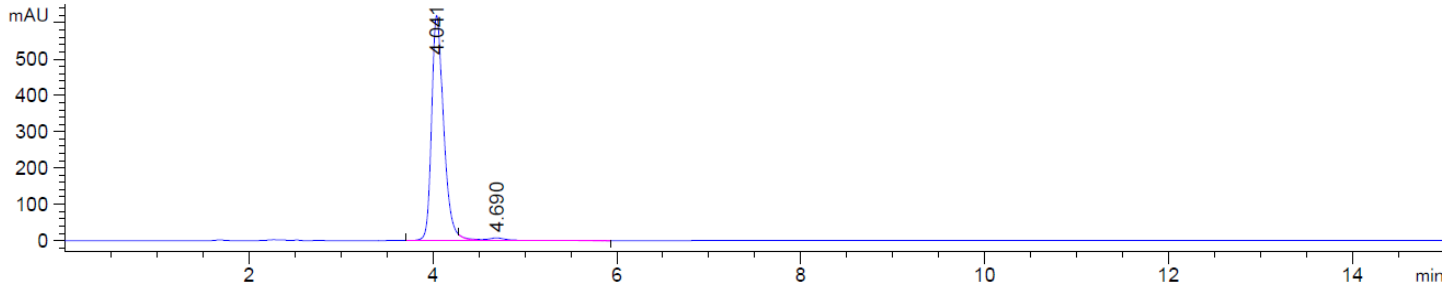
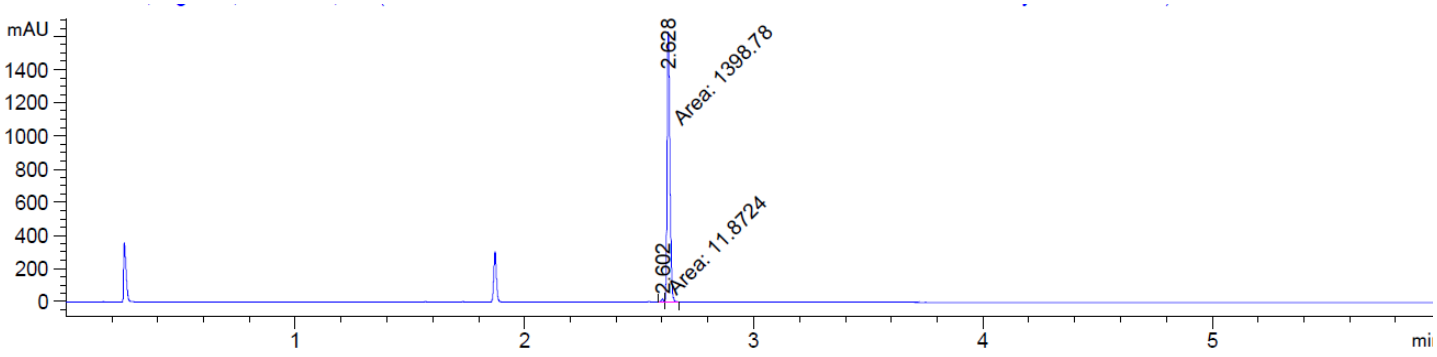
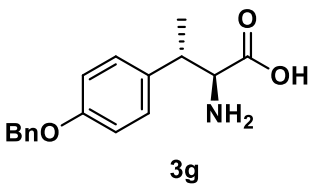


Achiral UPLC (valinamide derivative):





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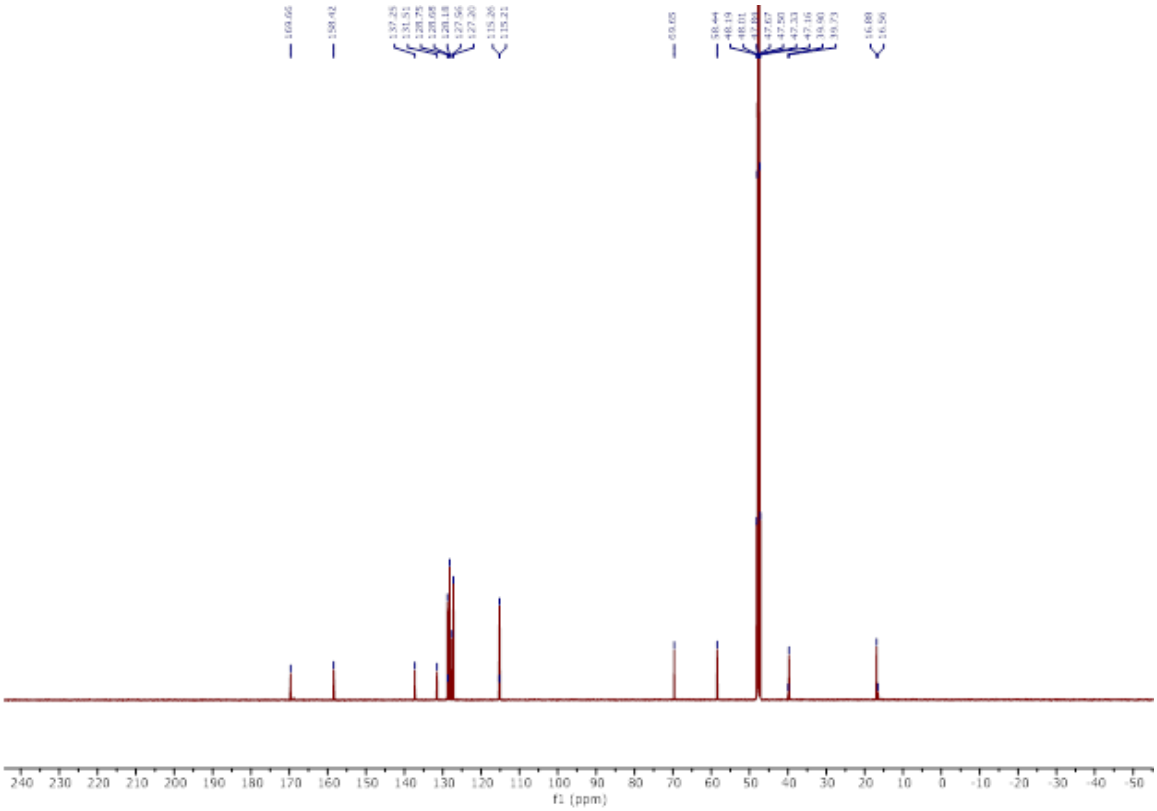
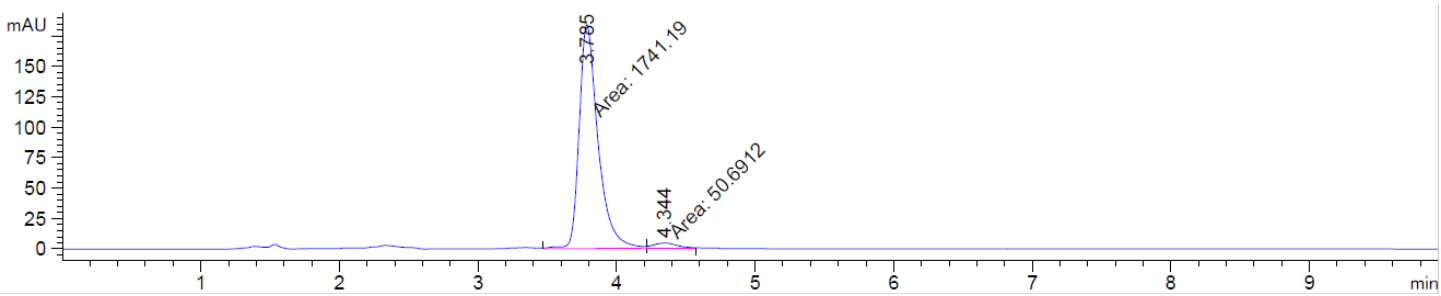


Chiral HPLC (72h):



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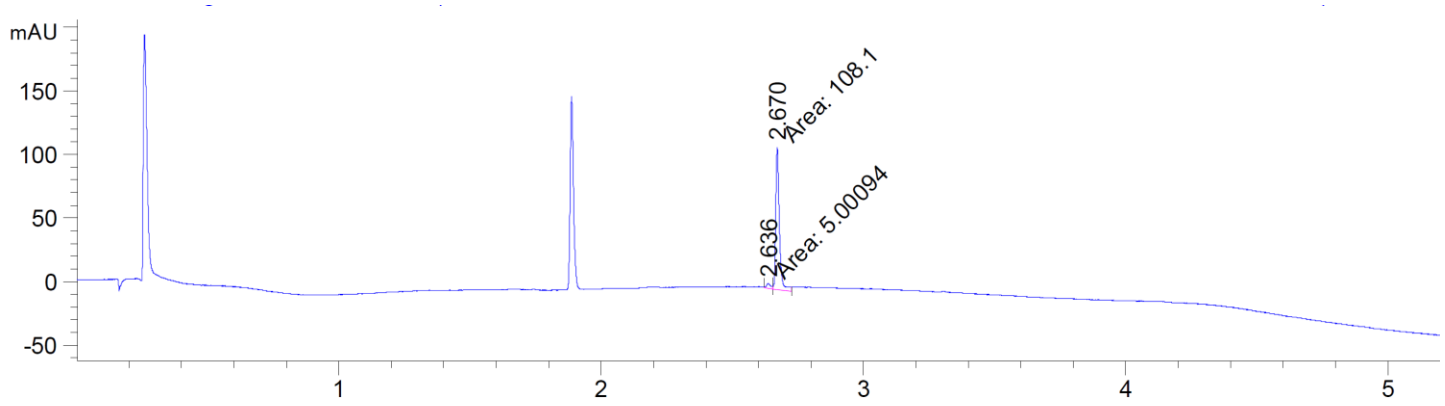

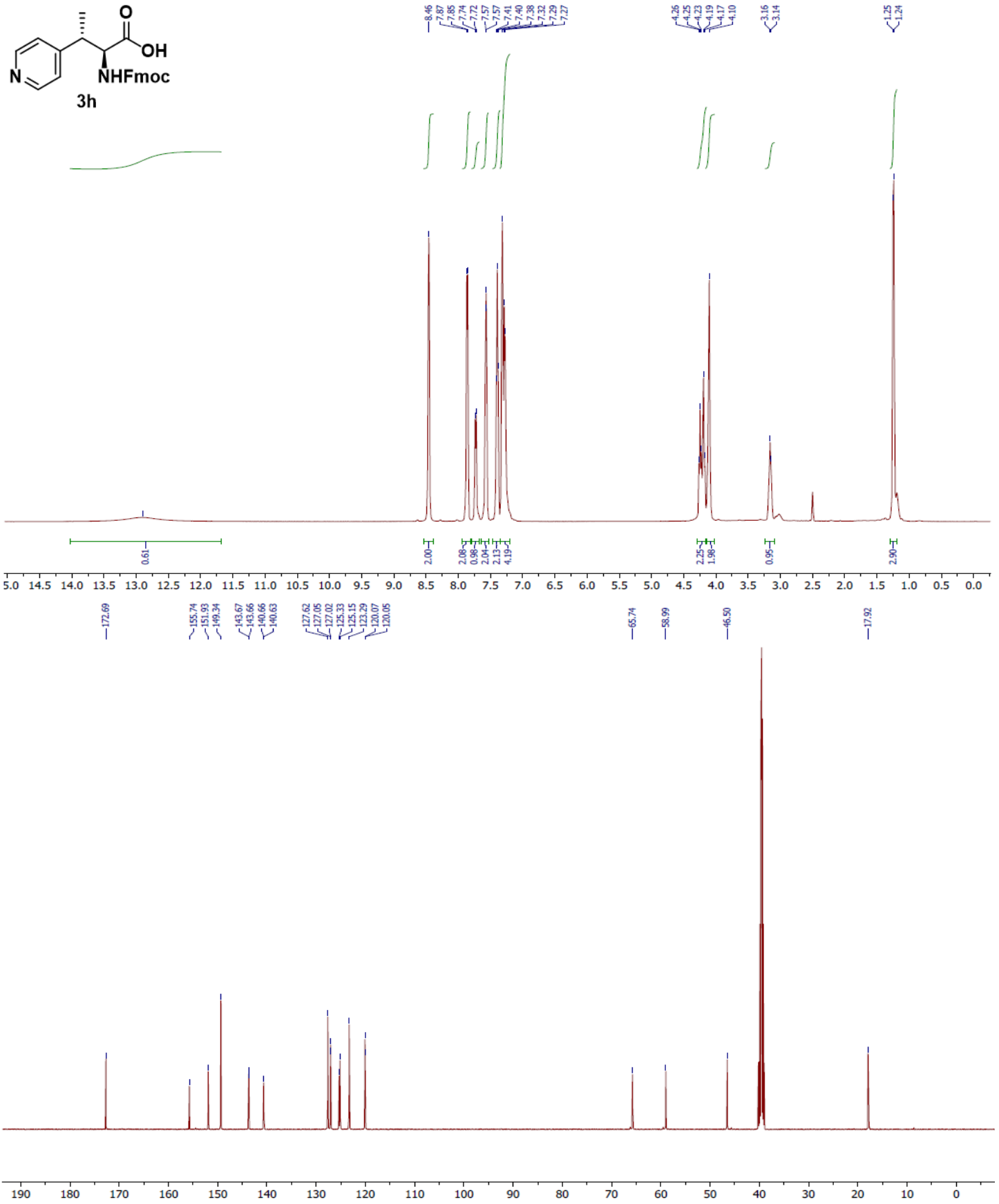

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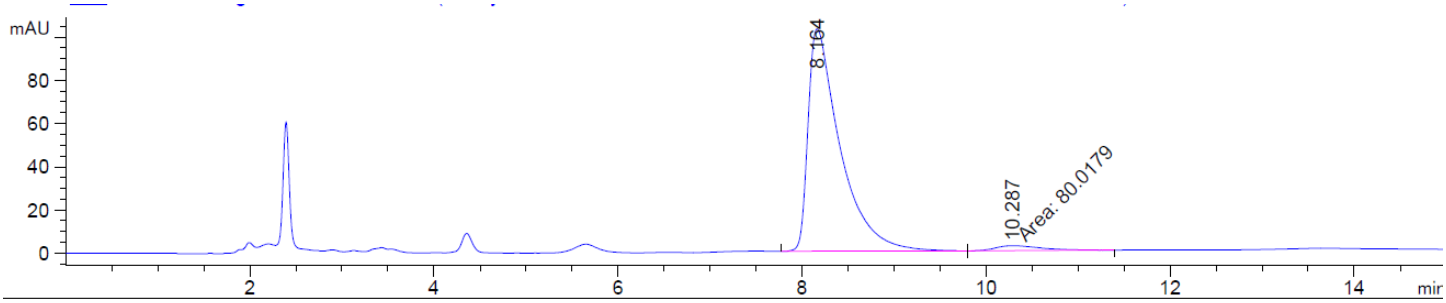
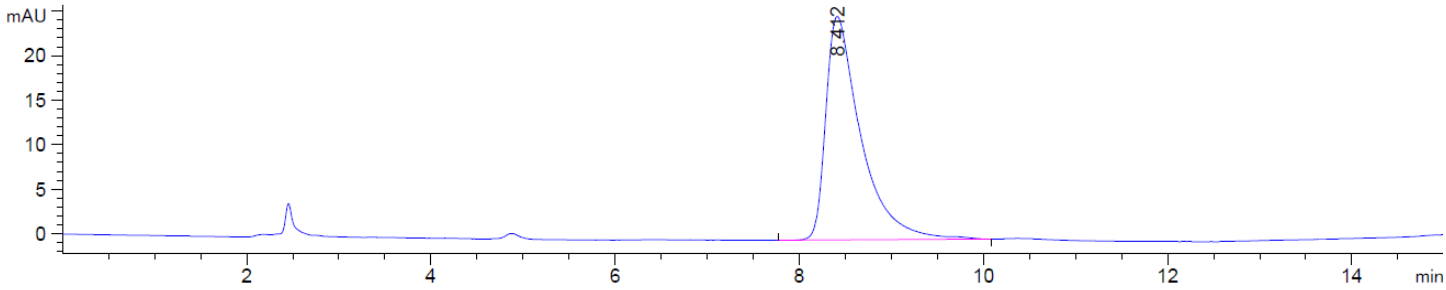


(2S, 3S) Standard:



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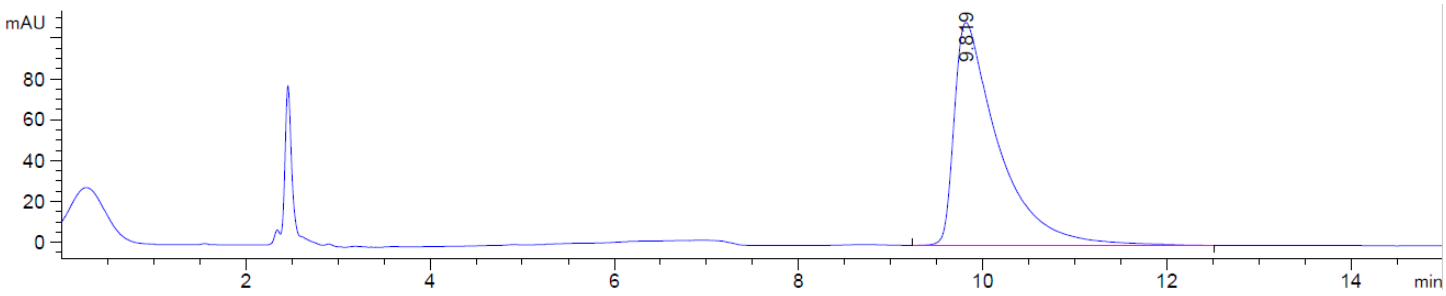

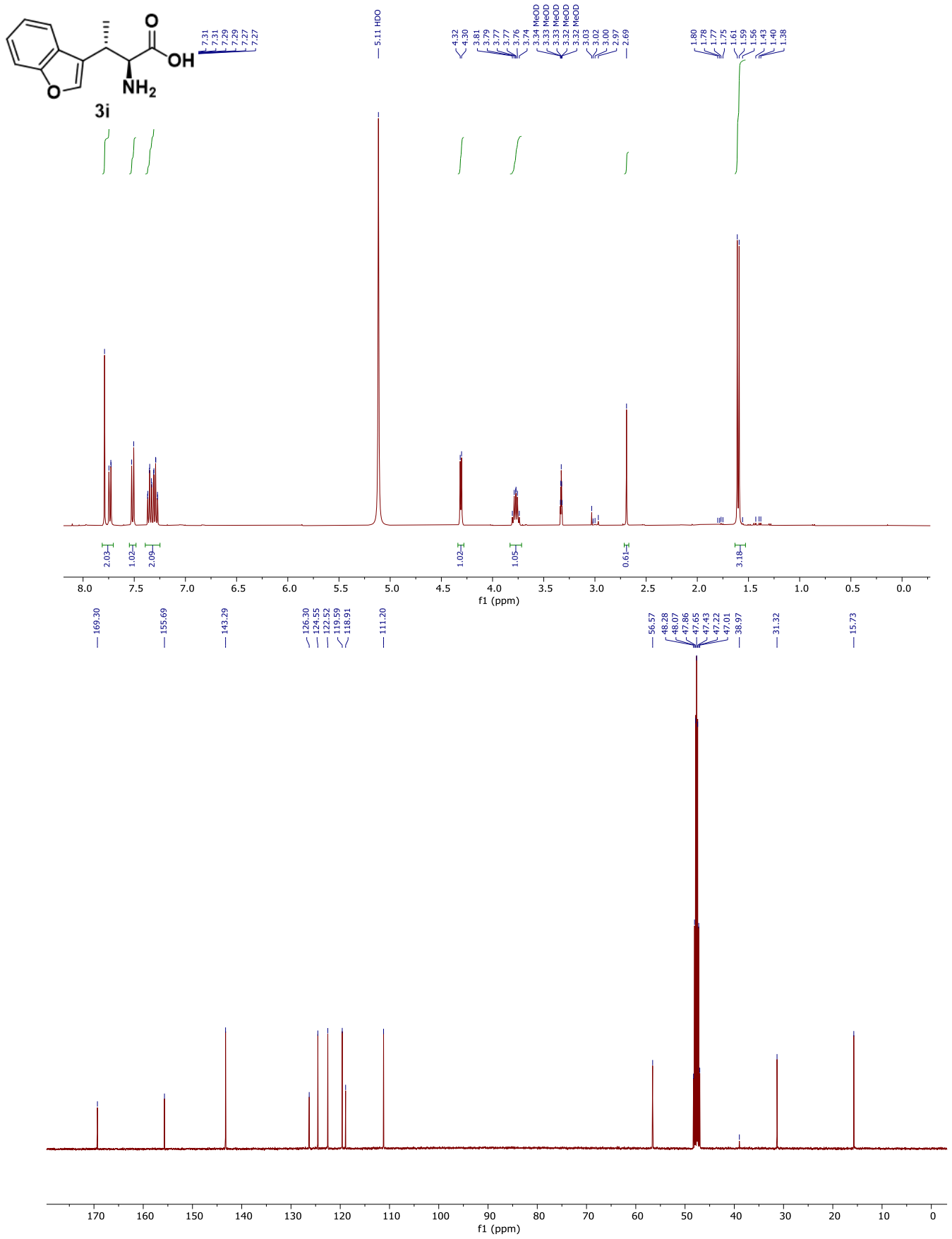

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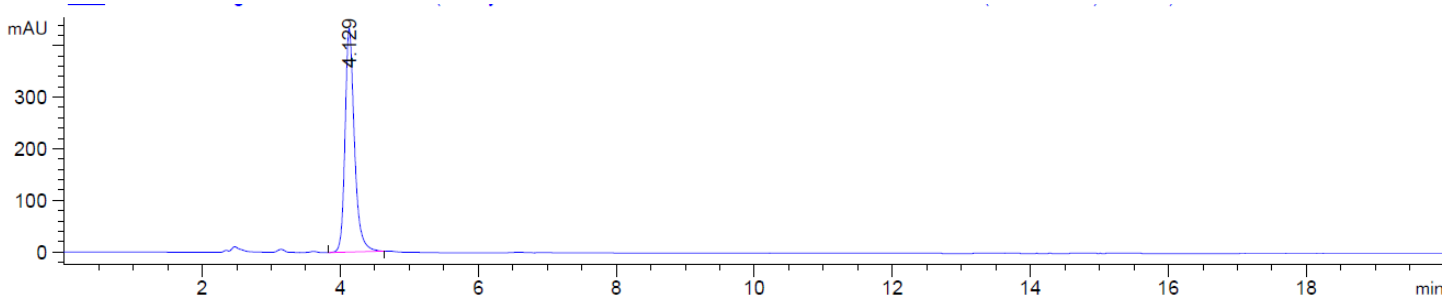
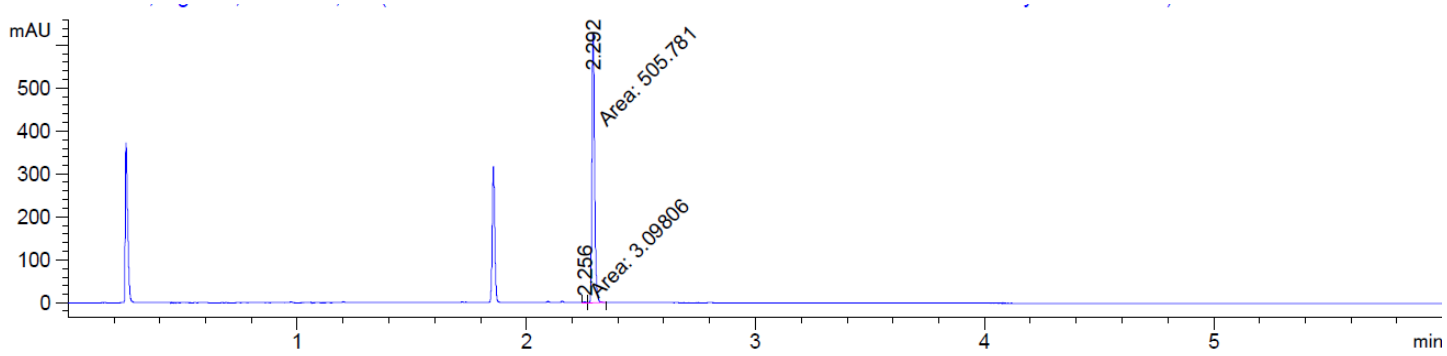
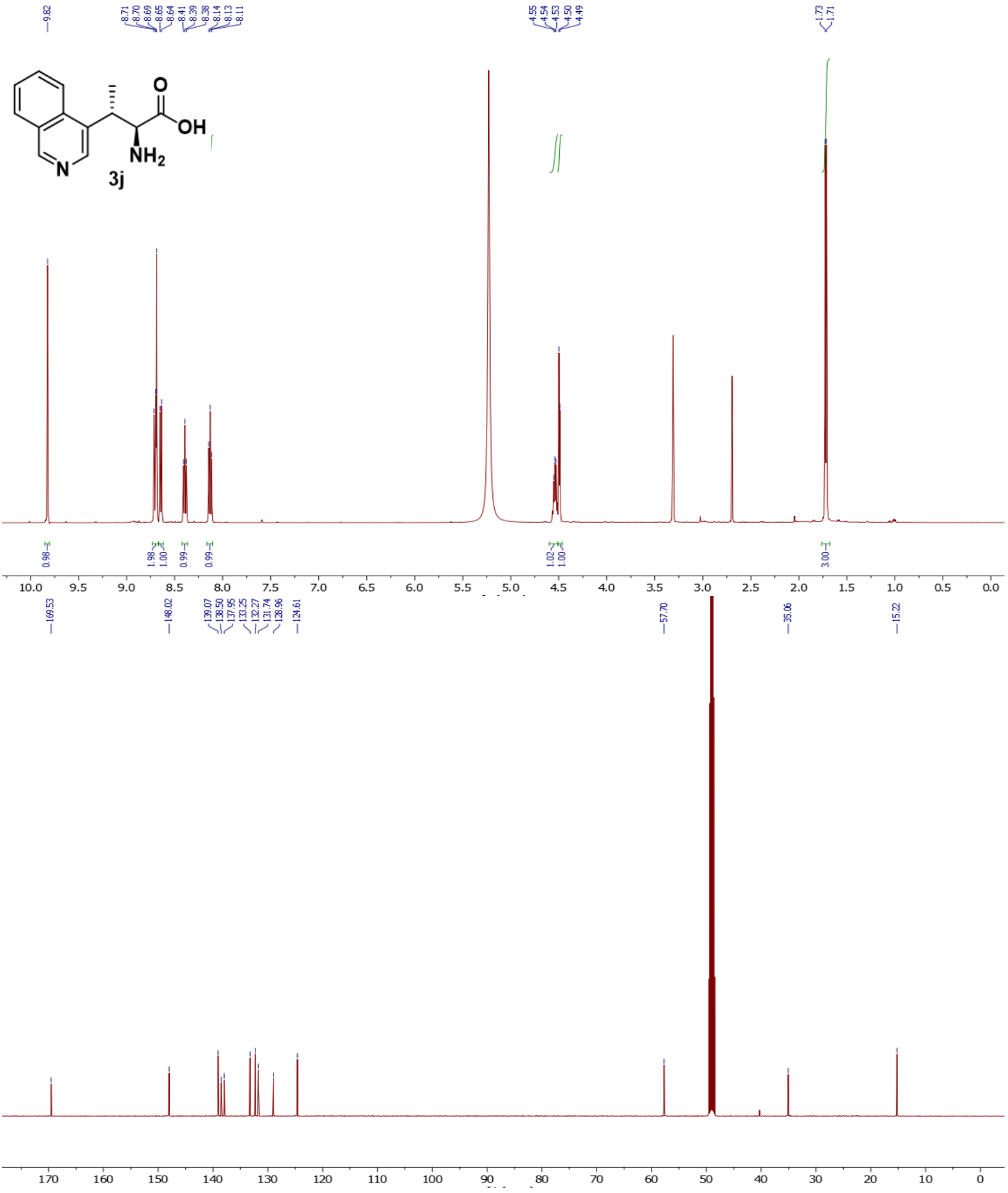


Achiral UPLC (valinamide derivative):





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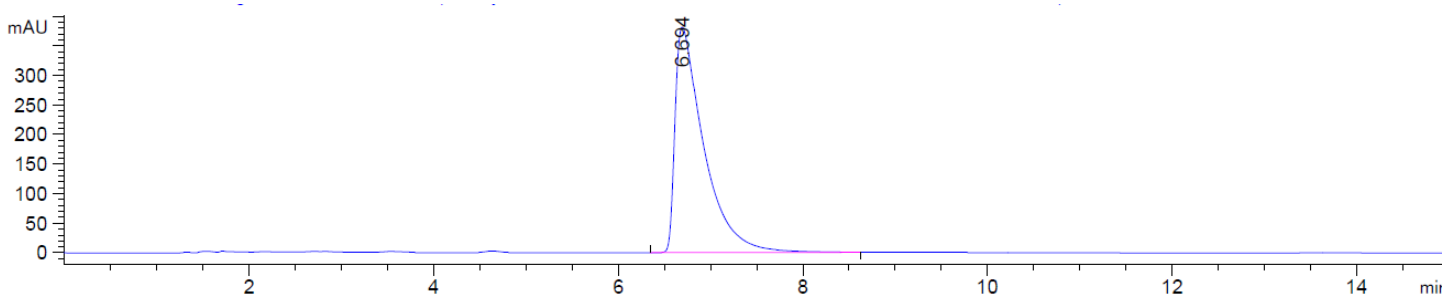

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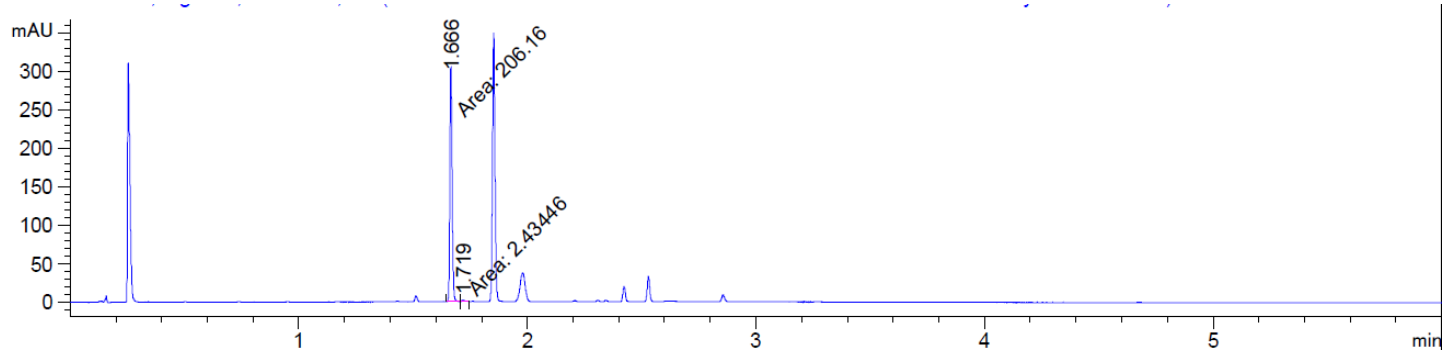

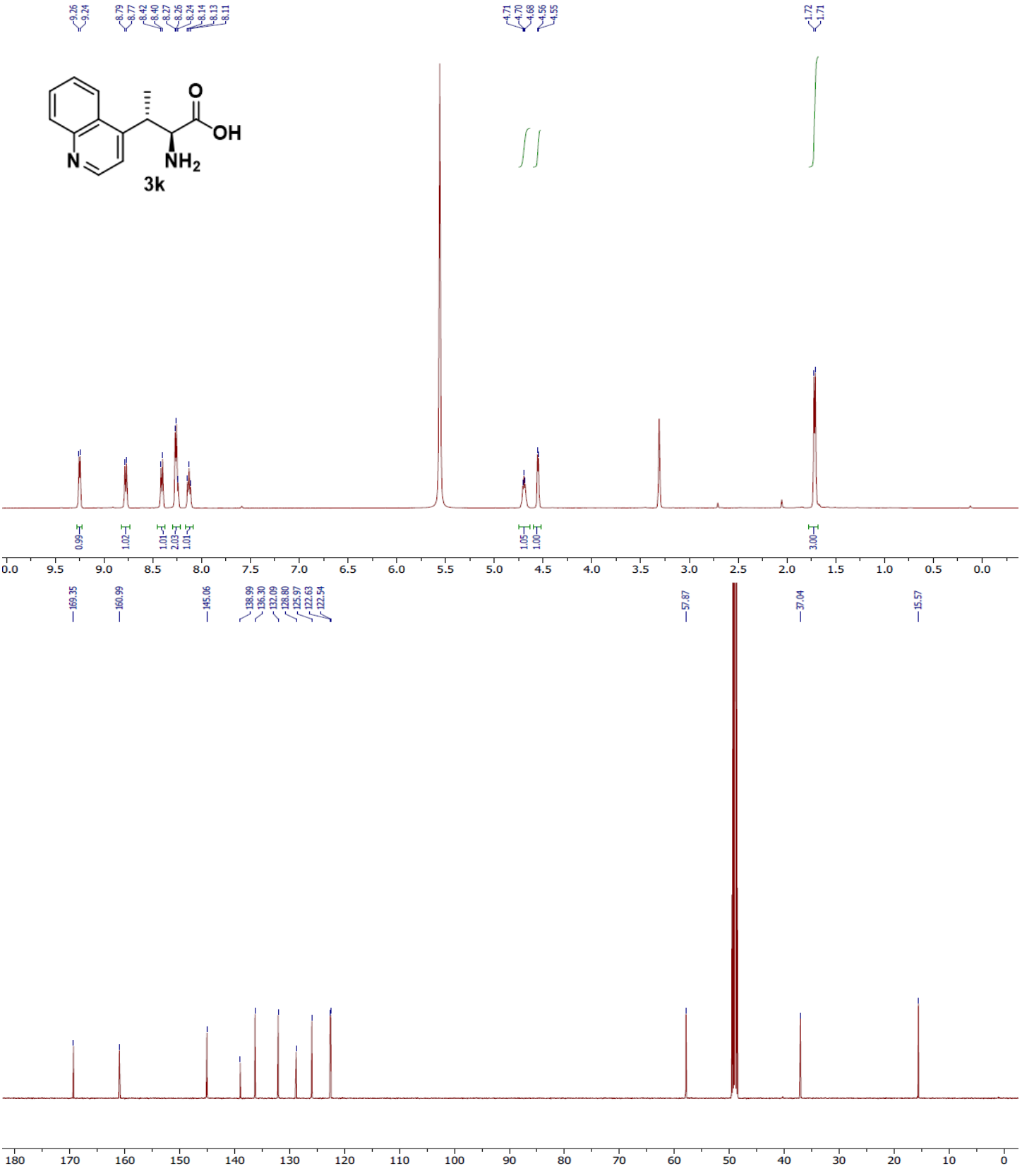

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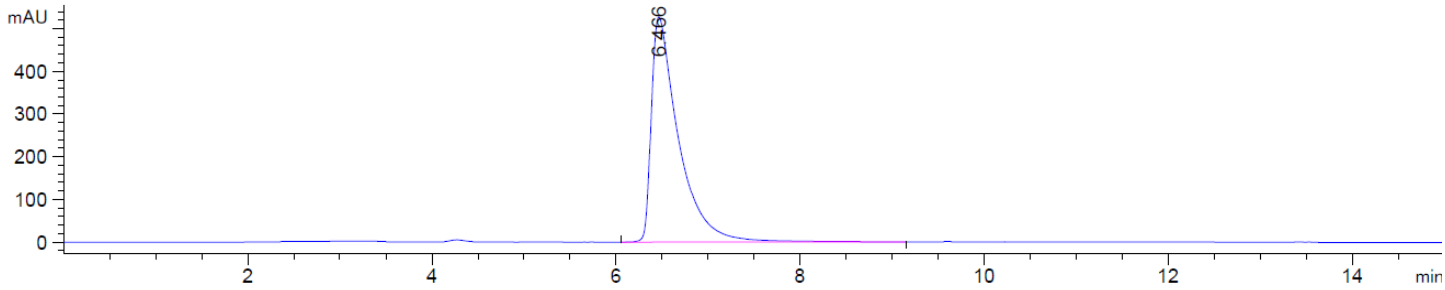
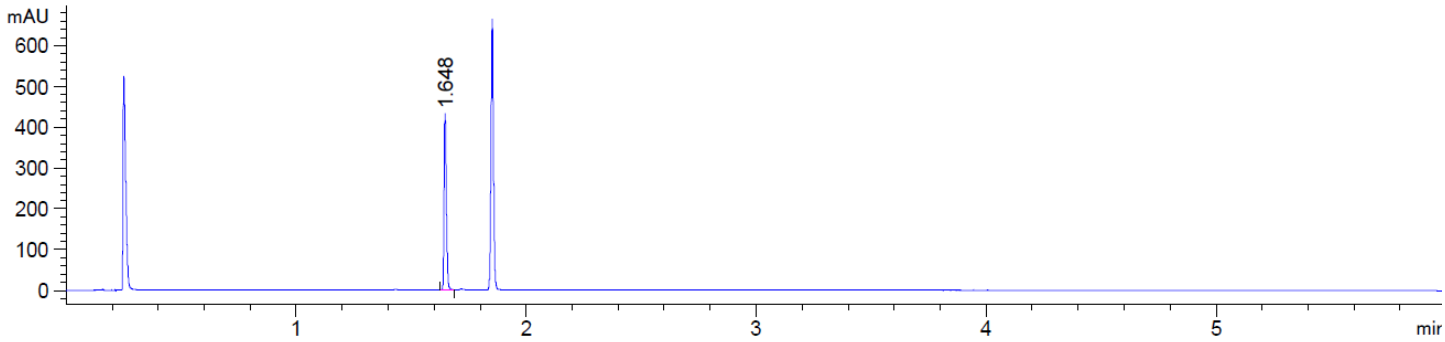
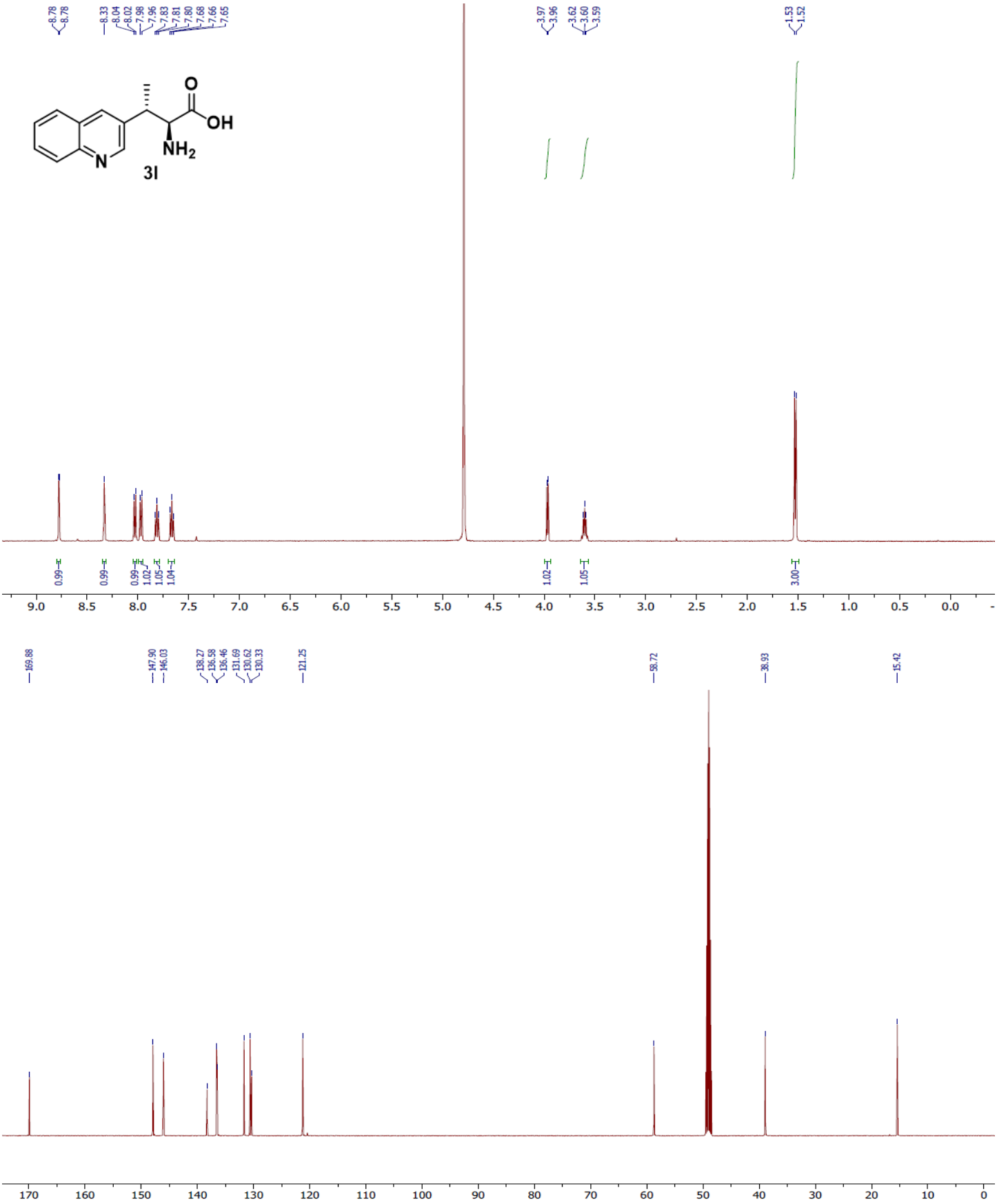


Achiral UPLC (valinamide derivative):




8.78
8.78
8.33
8.04
8.02
7.98
7.96
7.83
7.81
7.80
7.68
7.66
7.65
3.97
3.96
3.62
3.60
3.59
1.53
1.52
O
OH
NH2
N
3l
0.99
0.99
0.99
1.02
1.05
1.04
1.02
1.05
3.00
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
169.88
147.90
146.03
138.27
136.58
136.46
131.69
130.62
130.33
121.25
58.72
38.93
15.42
170
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150
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110
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50
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30
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0

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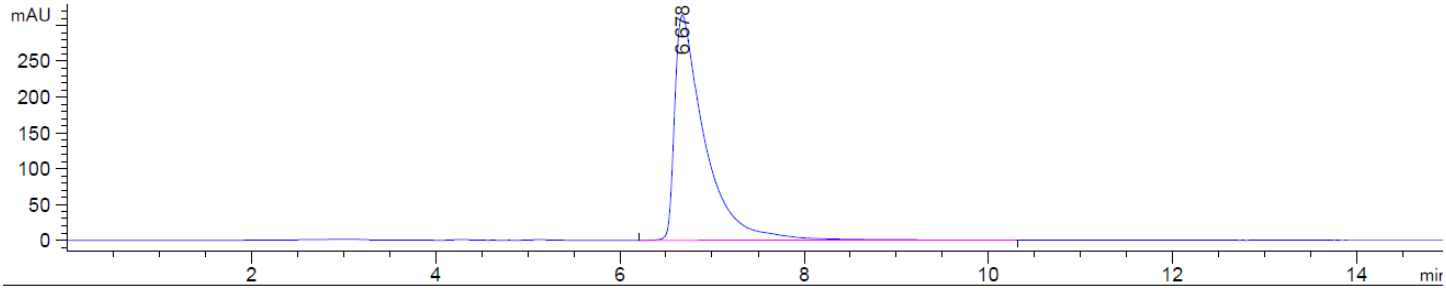
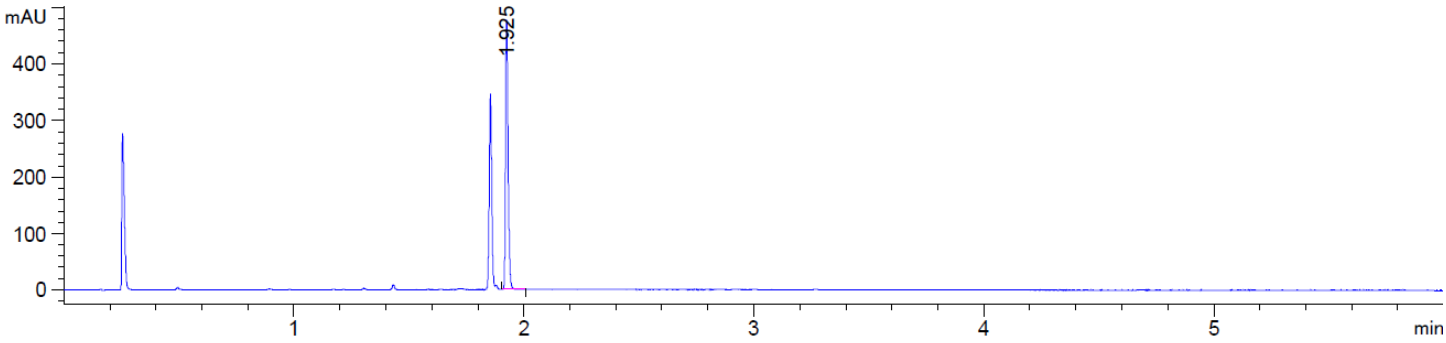
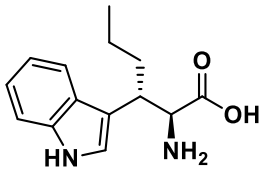


Achiral UPLC (valinamide derivative):





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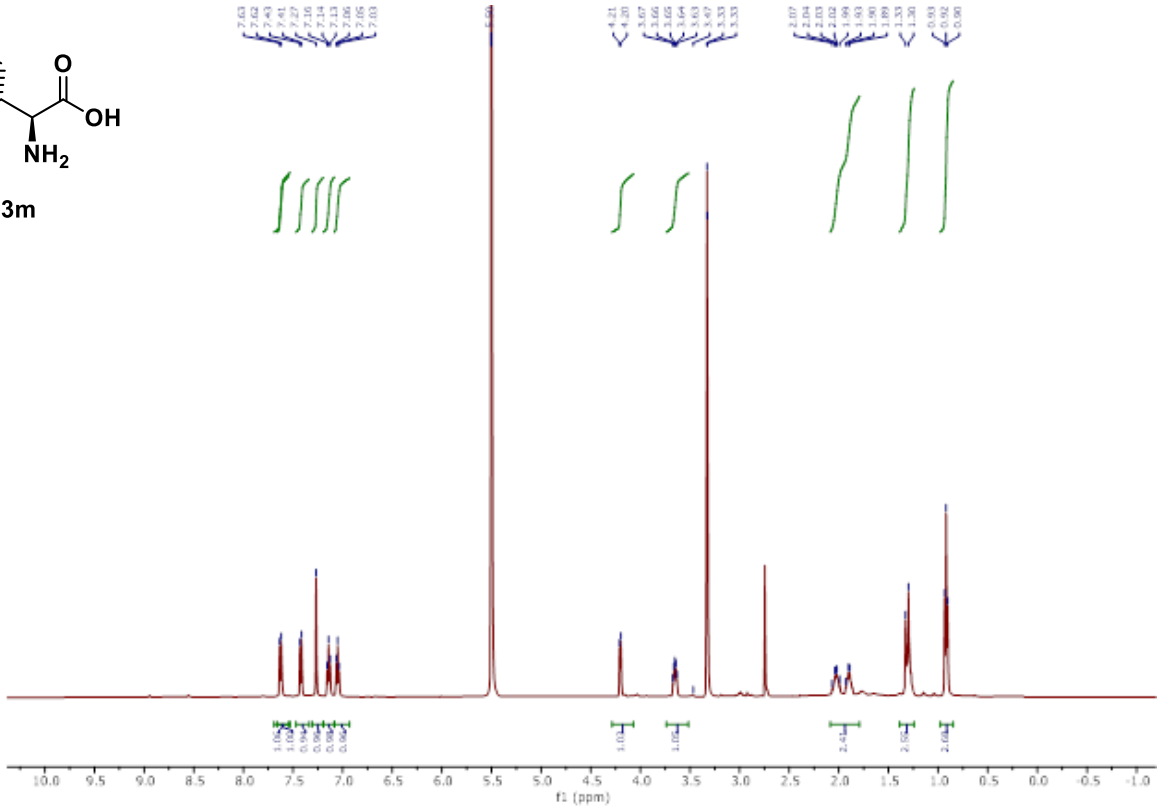
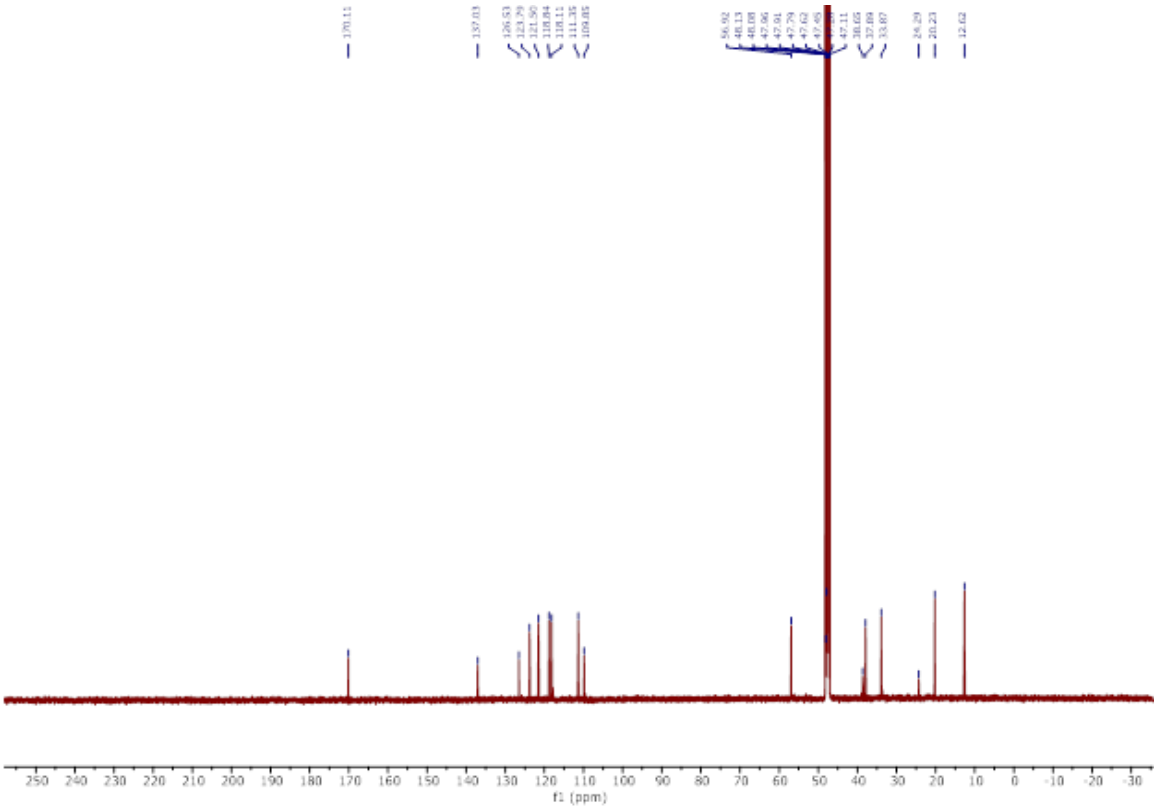
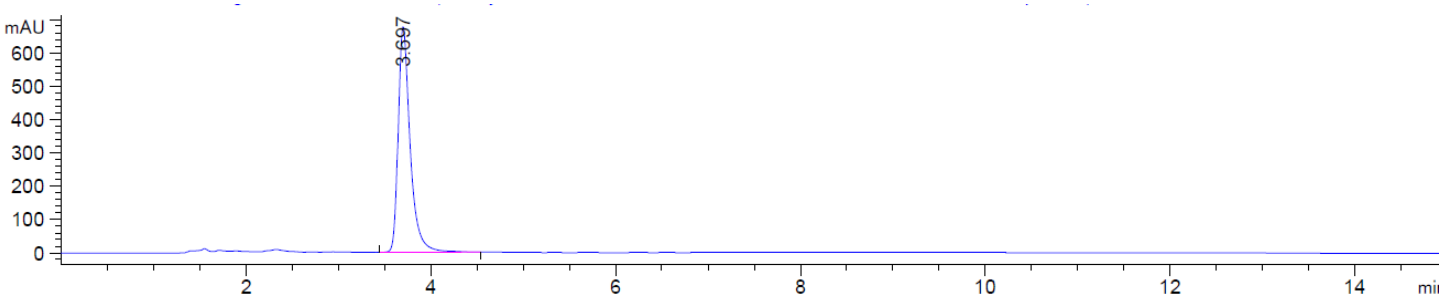
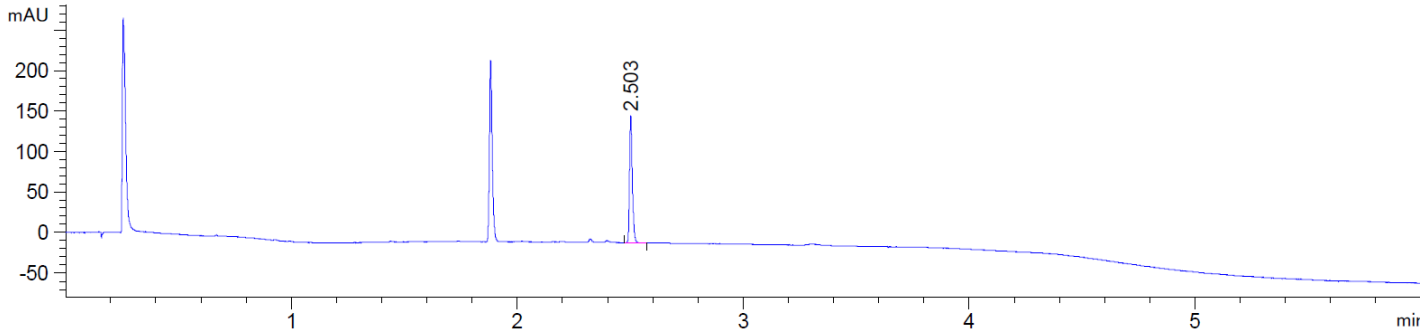


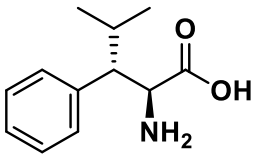


Chiral HPLC (72h):



Achiral UPLC (valinamide derivative):





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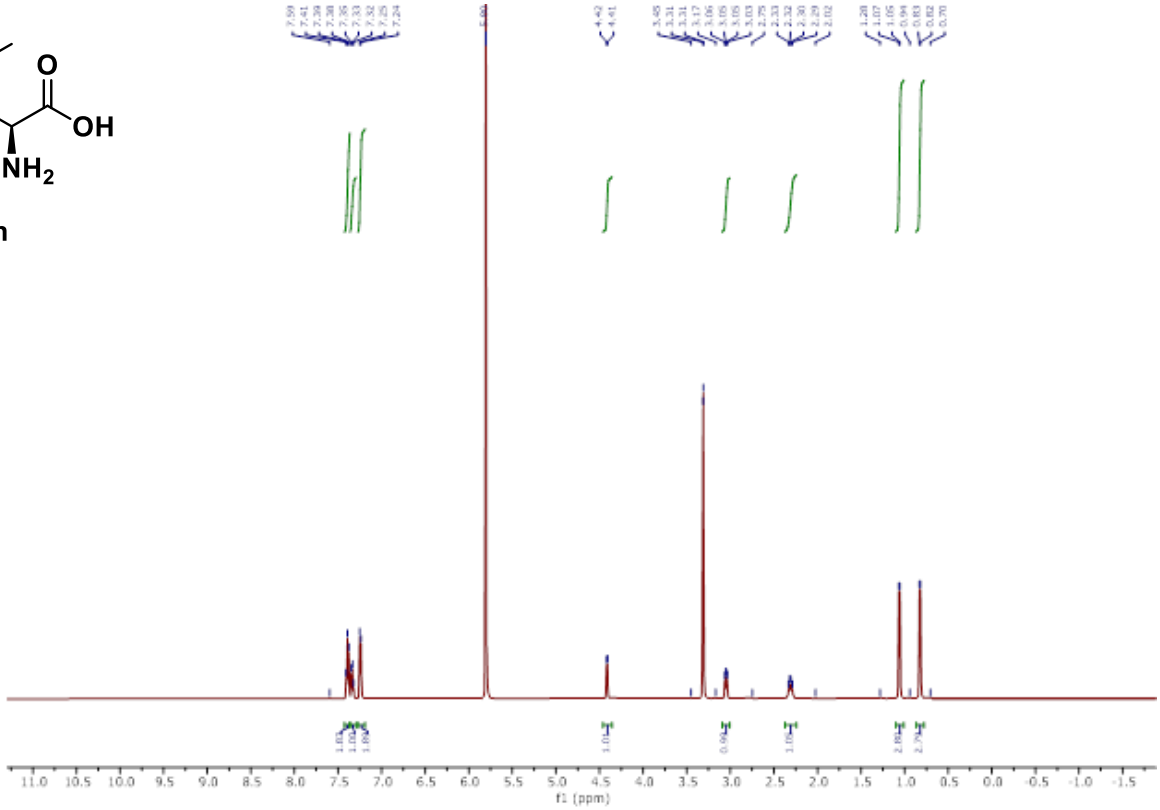

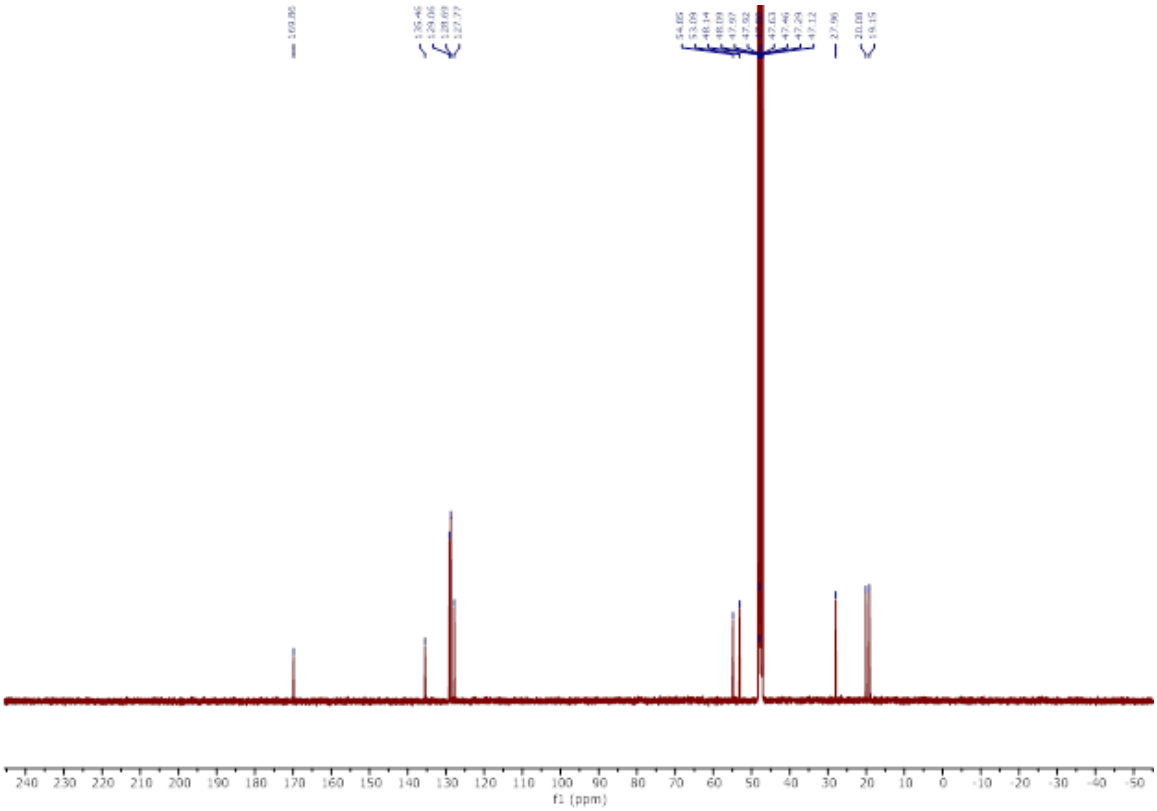

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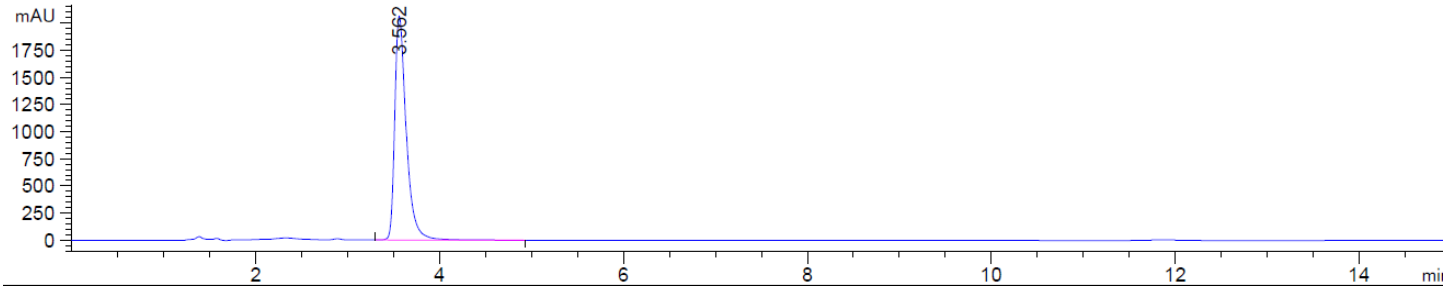
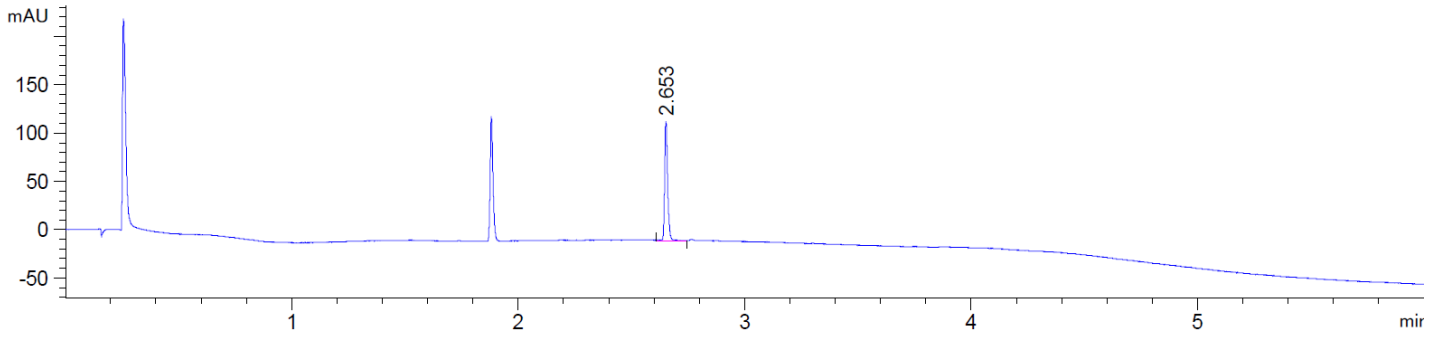
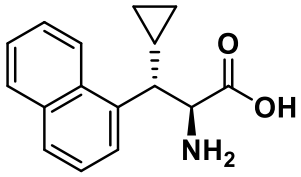


Achiral UPLC (valinamide derivative):





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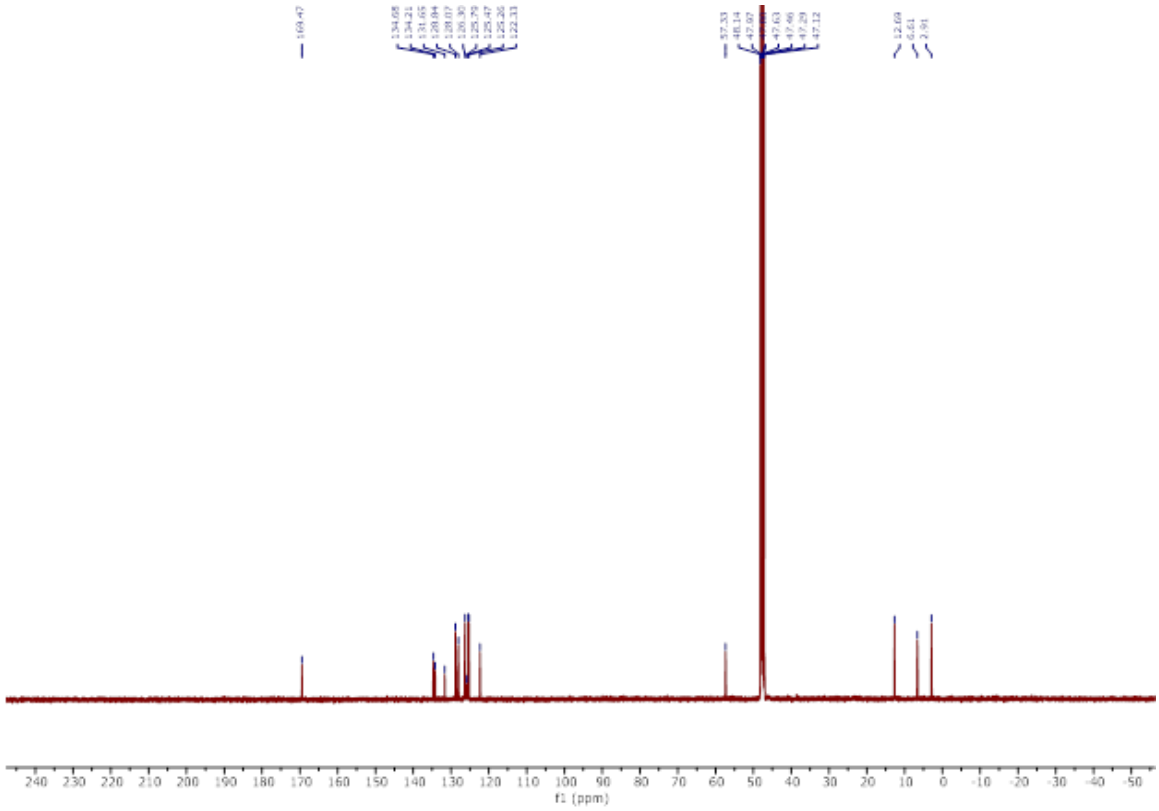
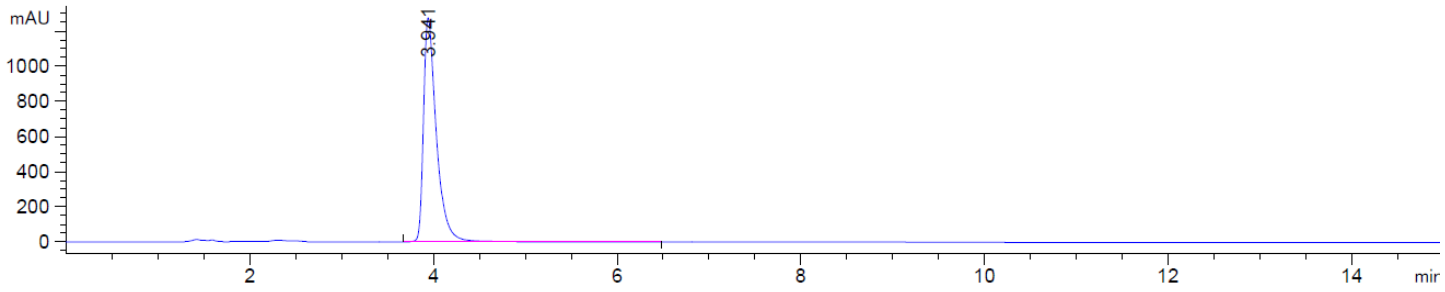
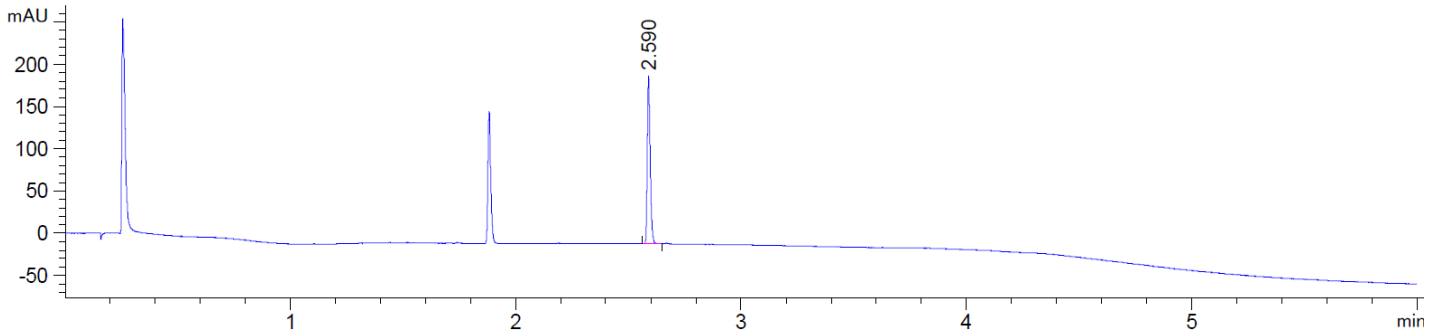
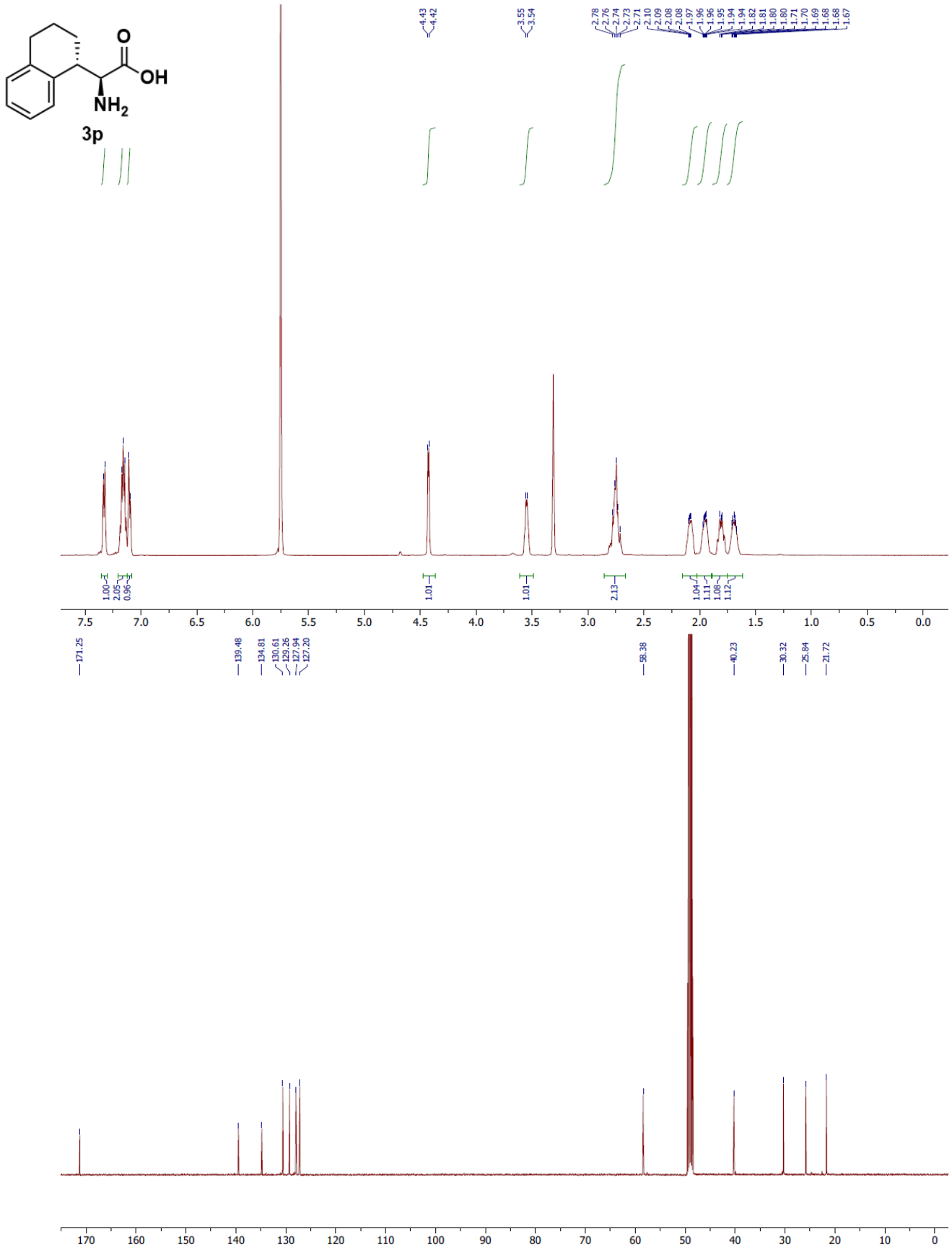


Chiral HPLC (48h):

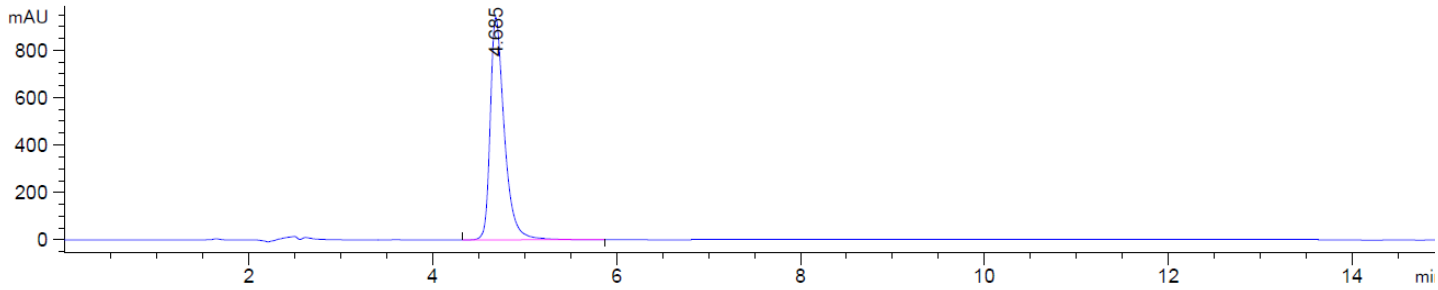


Achiral UPLC (valinamide derivative):





Chiral HPLC (48h):



Achiral UPLC (valinamide derivative):