Supplementary Information (SI) for RSC Advances.

α-Alkylated α-Amino Acids Reduce the Aggregation of Unfolded Peptides

Haoliang Zheng and Robert W. Newberry*

Supplementary Information

Page	Contents
S1	Table of Contents
S2	**Table S1.** Optical Rotation of Valine and Lysine Derivatives
S3	Analytical Procedures
S3	General Synthetic Procedures
S4	Peptide Synthetic Procedures
S5-S9	Small Molecules Synthesis
S10-S20	^{1}H and ^{13}C NMR spectra of small molecules
S21-S24	Analytical LC–MS traces for purified peptides
S25	Far-UV circular dichroism spectra of TrpZipE peptides
S25	References

Table S1. Optical Rotation of Valine and Lysine Derivatives

Product	**[α]**
Fmoc-L-Val-OH	–27.5°
Fmoc-α-Me-L-Val-OH	–12.5°
Fmoc-α-Et-L-Val-OH	–17.5°
Fmoc-L-Val-OH	–12.5°
Fmoc-α-Me-L-Lys-OH	–22.5°
Fmoc-α-Et-L-Lys-OH	–20.50°

NMR Spectroscopy. Nuclear magnetic resonance (NMR) spectra were acquired with Prodigy-500MHz, or Varian 600MHz NMR spectrometers. Spectra were analyzed using MestReNova x64. The chemical substances were dissolved in methanol-d_4 or DMSO-d_6.

Optical rotation spectroscopy. The optical rotation was measured using an automatic polarimeter from Azzota (Claymont, DE) at 20 °C with the sodium D line (λ = 589 nm). The sample was dissolved in chloroform to obtain a concentration of 0.1 g/mL. Measurements were performed using a 1 dm quartz cell.

Circular Dichroism Spectroscopy. CD data were acquired with a J-1500 Circular Dichroism Spectrophotometer from JASCO (Easton, MD). Far-UV CD spectra of TrpZipE peptides were collected at 20 µM in 20 mM potassium phosphate buffer, pH 7.0. TrpZipE peptides concentration was determined spectrophotometrically.[1] Far-UV CD spectra of VW19 peptides were collected at 200 µM in 10 mM tris/HCl buffer, pH 7.4. VW19 peptides concentration was determined spectrophotometrically.[2] Thermal denaturation for TrpZipE peptide was monitored at 228 nm by averaging over 16 s with a 1-nm bandwidth. The temperature was raised in 1 °C increments from 4 to 96 °C, with a 5-min equilibration period at each temperature. Far-UV CD spectra were collected with different temperature in the 190–260 nm region by recording ellipticity in 0.5-nm increments with 1-nm bandwidth and 2 s averaging.

Turbidity assay. After CD heating, samples were cooled to room temperature and loaded in triplicate into half-area 96-well plates. Data points were collected with wavelength of 600 nm. The plates were read using a Synergy H1 microplate reader (BioTek, USA).

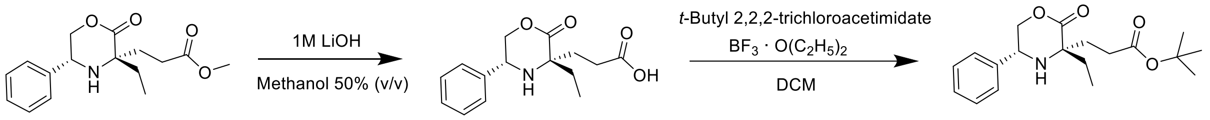
General Synthesis. Commercial chemicals were of reagent quality or better and were used without additional purification. Amino acids and resins were obtained from Chem-Impex International (Wood Dale, IL) and Ambeed, Inc (Arlington Heights, IL). α-methylated amino acids were obtained from Ambeed, Inc (Arlington Heights, IL). All other reagents and solvents were obtained from Fisher Scientific and Sigma–Aldrich. Reactions were monitored by thin-layer chromatography with visualization by UV light or staining with $KMnO_4$. Chromatography was performed by Teledyne Isco Combiflash RF200i UV/VIS Flash Chromatography System with RediSep Gold Silica Disposable column (Thousand Oaks, CA). Solvents and other volatile compounds are removed "under reduced pressure" using a rotating evaporator at water-aspirator pressure (<20 torr) and a 40°C water bath. Mass spectrometry was performed on either Agilent 6125 LC/MS or Agilent 6530 and 6546 Q-TOF.

Peptide Synthesis. Peptides were synthesized on a Liberty Blue 2.0 Microwave Peptide Synthesizer from CEM Corporation (Matthews, NC) using standard Fmoc-based methods starting from Rink Amide ProTide Resin (LL) obtained from CEM Corporation (Matthews, NC) (for TrpZipE peptides) or TentaGel S RAM obtained from Chem-Impex International (Wood Dale, IL) (for VW19 peptides). Amide bond formation was achieved using 5 eq. of amino acid (0.2 M), 10 eq. of DIC (1 M), and 5 eq. of ethyl cyanohydroxyiminoacetate (1M) and heating to 90°C for 120 s. Fmoc deprotection was achieved with 20% *N*-methylpiperidine in DMF at a temperature of 75°C for 15 s, followed by heating to 90 °C for 50 s. For modified peptides: α-Methylated or α-ethylated amino acids (1 eq.) were coupled to the corresponding positions of the presynthesized peptide segment using HATU (1 eq.), HoAT (1 eq.), and DIPEA (2 eq.) in DMF at room temperature for 1 h. Fmoc deprotection was carried out with 20% *N*-methylpiperidine in DMF at room temperature for 30 min. The adjacent amino acid was subsequently coupled using 2.0 eq. of the amino acid, HATU (2 eq.), HoAT (2 eq.), and DIPEA (4 eq.). The resin was then transferred back to the peptide synthesizer for further peptide elongation.

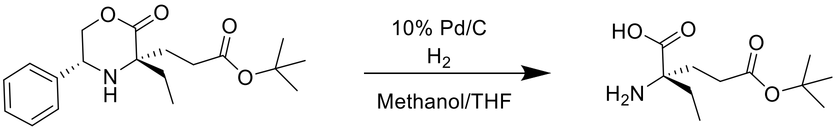
N-terminal acetylation was achieved using 4 eq. acetic anhydride and 8 eq. DIPEA at RT for 2 h. Cleavage and global deprotection was achieved by a 3-hour treatment with a solution of 90% v/v TFA, 5% v/v phenol, 2.5% v/v TIPS, and 2.5% v/v H_2O.

After precipitation in anhydrous diethyl ether, peptides were isolated by centrifugation and dissolved in 25% v/v MeCN in water. Peptides were purified by reversed-phase HPLC using an UltiMate™ 3000 Basic HPLC System from Thermo Fisher Scientific (Waltham, MA) on a Vydac 214TP 10µm C18 column from HiChrom (Wilmington, DE) using a linear gradient of 5–65% v/v B over 30 min (A: 0.1% v/v TFA in H_2O; B: 0.1% v/v TFA in MeCN) Subsequent lyophilization yielded white powders. Purified peptides were analyzed by LC/MS using Agilent ZORBAX Eclipse Plus C18 narrow bore column; 2.1 mm internal diameter; 50 mm length; 5-micron particle size; P.N. 959746-902 with a linear gradient of 5–95% v/v B over 15 min (A: 0.1% v/v formic acid in H_2O; B: 0.1% v/v formic acid in MeCN).

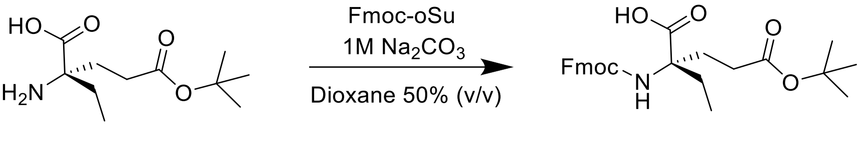
Small molecules synthesis. (3*R*,5*S*)-3-Ethyl-3-[2-(methoxycarbonyl)ethyl]-5-phenyl-3,4,5,6-tetrahydro-2*H*-1,4-oxazin-2-one was synthesized by the method of Harwood *et al.*[3]



Synthesis of tert-butyl 3-((3*S*,5*R*)-3-ethyl-2-oxo-5-phenylmorpholin-3-yl)propanoate. (3*R*,5*S*)-3-Ethyl-3-[2-(methoxycarbonyl)ethyl]-5-phenyl-3,4,5,6-tetrahydro-2H-1,4-oxazin-2-one (0.50 g, 1.71mmol) was stirred in methanol (50 mL), followed by the addition of 1.0 M lithium hydroxide solution (50 mL). The reaction mixture was stirred at room temperature for 3 h, then acidified with 1.0 M HCl and extracted with ethyl acetate. The organic phase was dried with sodium sulfate and concentrated in *vacuo*. Without purification, a solution of previous compound in dichloromethane (100 mL) was treated with tert-Butyl 2,2,2-trichloroacetimidate (1.75 g, 8.0 mmol), followed by the addition of a catalytic amount of boron trifluoride etherate (40 µL, 0.32µmol). The reaction mixture was stirred at room temperature for 24 h, after which solid $NaHCO_3$ (0.84 g, 10 mmol) was added. The resulting precipitate was removed by filtration, and the filtrate was concentrated in *vacuo* and purified by flash chromatography on silica gel (ethyl acetate/hexane). Yield was 21% (120 mg, 0.36 mmol). ESI–MS: $[M + H]^+$ calculated 334.2013, found 334.2013. ^{1}H NMR (500 MHz, methanol-*d*4, δ) 7.45 (m, 5H), 4.68 (dd, 1H), 4.44 (m, 2H), 2.68 (dd, 1H), 2.39 (dd, 1H), 2.09 (m, 4H) 1.49 (s, 9H), 1.05 (t, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, δ) 173.58, 171.43, 129.03, 128.94, 127.28, 81.11, 74.23, 63.69, 53.06, 33.19, 31.23, 31.03, 28.10, 7.84.



Synthesis of H-α-Et-L-Glu(OtBu)-OH. tert-butyl 3-((3*S*,5*R*)-3-ethyl-2-oxo-5-phenylmorpholin-3-yl)propanoate (120 mg, 0.36 mmol) and 10% Pd/C (21 mg, 0.21 mmol) in a mixture of methanol (30 mL) and tetrahydrofuran (30 mL) under hydrogen for overnight. After completion, the reaction mixture was diluted with methanol and filtered through Celite. The filtrate was concentrated in *vacuo*. The crude product was purified by flash chromatography on silica gel (methanol/dichloromethane). Yield was 42% (36 mg, 0.15 mmol). ESI–MS: $[M + H]^+$ calculated 232.1543, found 232.1550. ^{1}H NMR (500 MHz, methanol-*d*4, δ) 2.47 (m, 1H), 2.30 (m, 1H), 2.15 (m, 2H), 2.00 (m, 1H), 1.86 (m, 1H) 1.45 (s, 9H), 1.01 (t, 3H). ^{13}C NMR (126 MHz, methanol-*d*4, δ) 171.48, 171.28, 80.98, 30.53, 29.15, 28.64, 26.86, 6.58.

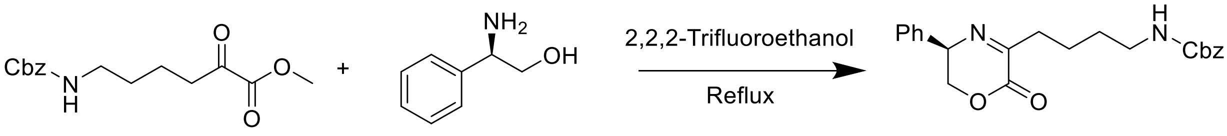


Synthesis of Fmoc-α-Et-L-Glu(OtBu)-OH. H-α-Et-L-Glu(OtBu)-OH (36 mg, 0.15 mmol) was dissolved in a mixture of 1,4-dioxane and 1M aqueous sodium bicarbonate (1:1, 50 mL).

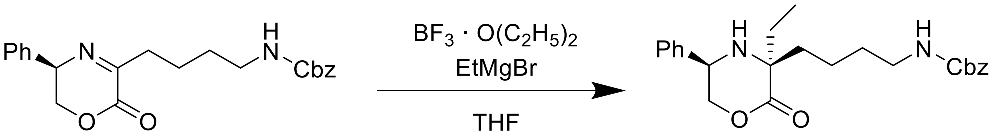
N-(9-Fluorenylmethoxycarbonyloxy)succinimide (51 mg, 0.15 mmol) was added to the solution, and the reaction mixture was stirred at room temperature overnight. The reaction was then acidified with 1 M HCl, and was extracted with ethyl acetate. The organic phase was dried with sodium sulfate and concentrated in *vacuo*. The crude product was purified by flash chromatography on silica gel (ethyl acetate/hexane). Yield was 100% (72 mg, 0.15 mmol). ESI–MS: $[M + Na]^+$ calculated 476.2044, found 476.2045. ^{1}H NMR (500 MHz, methanol-*d*4, δ) 7.81 (d, 2H), 7.67 (d, 2H), 7.40 (t, 2H), 7.33 (t, 2H), 4.39 (d, 2H), 4.25 (t, 1H), 2.36 (m, 1H), 2.11 (m, 4H), 1.83 (m, 1H) 1.45 (s, 9H), 0.80 (t, 3H). ^{13}C NMR (500 MHz, methanol-*d*4, δ) ^{13}C NMR (126 MHz, methanol-*d*4) δ 174.62, 172.75, 154.95, 143.91, 141.23, 127.38, 126.75, 124.78, 119.53, 80.28, 65.93, 62.66, 30.00, 29.21, 27.45, 26.91, 26.31, 7.01.

According to the same stereoselective method[3], we synthesized Fmoc-α-Et-L-Lys(Boc)-OH and Fmoc-α-Et-L-Val-OH.

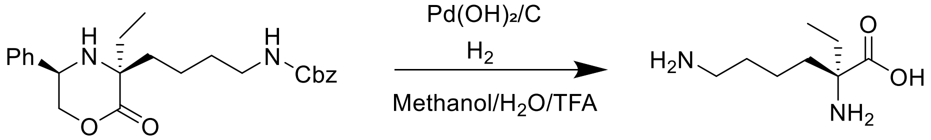
Methyl 6-(((benzyloxy)carbonyl)amino)-2-oxohexanoate was synthesized by method of Thorsett.[4]



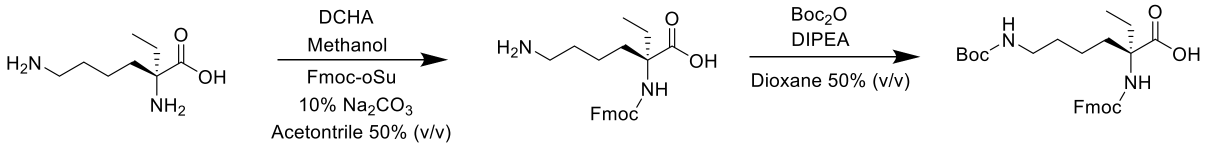
Synthesis of benzyl (*R*)-(4-(2-oxo-5-phenyl-5,6-dihydro-2H-1,4-oxazin-3-yl)butyl)carbamate. A solution of 2-(*S*)-phenylglycinol (1.93 g, 14.08 mmol) in anhydrous 2,2,2-trifluoroethanol (100 mL) was prepared in a flask containing activated 3 Å molecular sieves (15 g) under nitrogen. Methyl 6-(((benzyloxy)carbonyl)amino)-2-oxohexanoate (4.13 g, 14.08 mmol) was added, and the reaction mixture was heated at reflux for 24 h. After cooling to room temperature, the mixture was filtered through Celite, and the solvent was concentrated in *vacuo*. The crude product was purified by flash chromatography on silica gel (ethyl acetate/hexane). Yield was 12% (1.69 g, 4.44 mmol). ESI–MS: $[M + H]^+$ calculated 381.1809, found 381.1809. ^{1}H NMR (500 MHz, methanol-*d*4, δ) 7.39 (m, 10H), 6.27 (s, 2H), 5.12 (m, 2H), 4.54 (d, 1H), 3.67 (m, 4H) 2.32 (dd, 2H), 1.85 (dd, 2H). ^{13}C NMR (126 MHz, methanol-*d*4, δ) 163.89, 154.45, 133.23, 131.26, 129.54, 129.13, 128.27, 128.09, 127.74, 127.04, 125.48, 67.85, 64.63, 43.54, 22.49, 22.23.



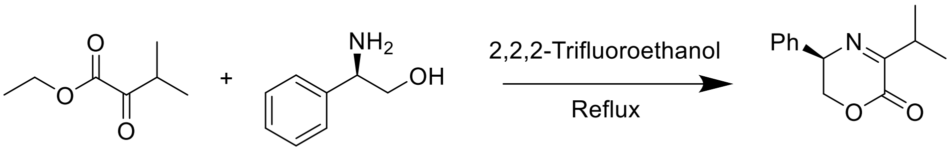
Synthesis of benzyl (4-((3*S*,5*R*)-3-ethyl-2-oxo-5-phenylmorpholin-3-yl)butyl)carbamate. A solution of benzyl (*R*)-(4-(2-oxo-5-phenyl-5,6-dihydro-2H-1,4-oxazin-3-yl)butyl)carbamate (1.69 g, 4.44 mmol) in anhydrous tetrahydrofuran (100 mL) was cooled to −80 °C under nitrogen, and boron trifluoride etherate (1.68 mL, 13.32 mmol) was added in one portion. The reaction mixture was stirred at −80 °C for 2 h. 1 M Ethylmagnesium bromide in tetrahydrofuran solution (6.66 mL, 6.66 mmol) was added dropwise via syringe over 30 min. Stirring was continued for an additional 5 h, and the reaction was quenched at −80 °C by addition of saturated aqueous ammonium chloride (100 mL). The organic layer was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, and concentrated in *vacuo*. The crude product was purified by flash chromatography on silica gel (ethyl acetate/hexane). Yield was 41% (0.75 g, 1.82 mmol). ESI–MS: $[M + H]^+$ calculated 411.2278, found 411.2282. ^{1}H NMR (600 MHz, DMSO-*d*6, δ) 7.33 (m, 10H), 4.98 (t, 3H), 3.64 (m, 2H), 2.97 (m, 4H), 1.70 (m, 3H) 1.38 (m, 3H), 0.94 (m, 3H). ^{13}C NMR (151 MHz, DMSO-*d*6, δ) 170.73, 156.55, 137.75, 137.68, 128.79, 128.17, 128.15, 128.13, 72.26, 65.53, 52.88, 52.50, 40.51, 30.62, 30.24, 29.31, 20.80, 8.81.



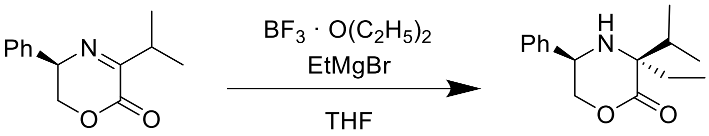
Synthesis of H-α-Et-L-Lys-OH. A solution of the benzyl (4-((3*S*,5*R*)-3-ethyl-2-oxo-5-phenylmorpholin-3-yl)butyl)carbamate (0.75 g, 1.82 mmol) in methanol (20 mL) was treated with Pd(OH)$_2$/C (0.26 g, 1.82 mmol), trifluoroacetic acid (0.4 mL), and water (2 mL) under hydrogen at room temperature for 24 h. After release of the hydrogen, the reaction mixture was filtered through Celite, and the solvent was concentrated in in *vacuo*. The crude product was purified by flash chromatography on silica gel (methanol/dichloromethane). Yield was 81% (0.26 g, 1.47 mmol). ESI–MS: $[M + H]^+$ calculated 175.1441, found 175.1441. ^{1}H NMR (500 MHz, methanol-*d*4, δ) 2.97 (t, 2H), 1.97 (m, 4H), 1.72 (m, 2H), 1.59 (m, 1H), 1.40 (m, 1H) 1.04 (t, 3H). ^{13}C NMR (126 MHz, methanol-*d*4, δ) 171.66, 63.53, 38.89, 34.93, 28.88, 27.04, 20.24, 6.66.



Synthesis of Fmoc-α-Et-L-Lys(Boc)-OH. According to the reported N$^\alpha$ Fmoc protection method[5], H-α-Et-L-Lys-OH (0.26 g, 1.47 mmol) was dissolved in acetone (50 mL), and dicyclohexylamine (DCHA, 0.32 g, 1.75 mmol) was added. The mixture was stirred at room temperature for 5 h. The solution was concentrated in in *vacuo*. The H-α-Et-L-Lys-OH DCHA salt was dissolved in water (50 mL), and the pH was adjusted to 8 using 10% aqueous Na$_2$CO$_3$. A solution of *N*-(9-Fluorenylmethoxycarbonyloxy)succinimide (0.50 g, 1.47 mmol) in acetonitrile (50 mL) was added to the solution at 0 °C. After completion of the addition, the reaction mixture was allowed to warm to room temperature and stirred overnight. The mixture was then acidified with 5% KHSO$_4$ to pH 2–3 and extracted with ethyl acetate. The organic layer was washed with brine and water, dried over anhydrous sodium sulfate and concentrated in *vacuo*. After flash chromatography on silica gel (methanol/dichloromethane), the intermediate was dissolved in water (20 mL), and NaHCO$_3$ (0.13 g, 1.56 mmol) was added with stirring. The solution was cooled to 0 °C, and a solution of di-*tert*-butyl dicarbonate (0.34 g, 1.56 mmol) in 1,4-dioxane (15 mL) was added dropwise over 20 min. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 12 h. The mixture was diluted with water. The aqueous phase was acidified to pH 4 with 2 M HCl and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated in *vacuo*. The crude product was purified by flash chromatography on silica gel (ethyl acetate/hexane). Yield was 17% (124 mg, 0.25 mmol). ESI–MS: $[M + Na]^+$ calculated 519.2466, found 519.2470. ^{1}H NMR (500 MHz, methanol-*d*4, δ) 7.82 (m, 2H), 7.67 (m, 2H), 7.40 (m, 2H), 7.32 (m, 2H), 4.33 (d, 2H), 4.20 (t, 1H), 3.10 (t, 2H), 2.14 (m, 2H), 1.84 (m, 2H), 1.50 (m, 2H), 1.44 (s, 9H), 1.32 (m, 1H), 1.21 (m, 1H), 0.81 (t, 3H). ^{13}C NMR (126 MHz, methanol-*d*4, δ) 175.45, 157.49, 143.96, 141.20, 127.36, 126.75, 124.78, 119.52, 78.71, 66.25, 40.27, 34.05, 29.54, 27.58, 27.34, 25.09, 21.00, 7.06.

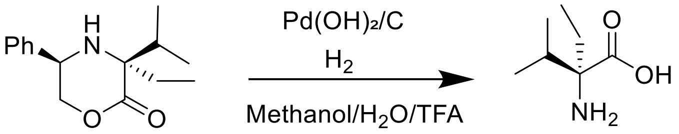


Synthesis of (*R*)-3-isopropyl-5-phenyl-5,6-dihydro-2H-1,4-oxazin-2-one. A solution of 2-(*S*)-phenylglycinol (7.04 g, 51.33 mmol) in anhydrous 2,2,2-trifluoroethanol (200 mL) was prepared in a flask containing activated 3 Å molecular sieves (30 g) under nitrogen. Ethyl 3-methyl-2-oxobutanoate (7.40 g, 51.33 mmol) was added, and the reaction mixture was heated at reflux for 24 h. After cooling to room temperature, the mixture was filtered through Celite, and the solvent was concentrated in *vacuo*. The crude product was purified by flash chromatography on silica gel (ethyl acetate/hexane). Yield was 24% (2.68 g, 12.32 mmol). ESI–MS: $[M + H]^+$ calculated 218.1176, found 218.1172. ^{1}H NMR (500 MHz, methanol-*d*4, δ) 7.41 (m, 5H), 4.55 (m, 1H), 4.21 (m, 1H), 3.25 (t, 1H), 2.06 (m, 1H), 1.24 (d, 6H). ^{13}C NMR (126 MHz, methanol-*d*4, δ) 167.10, 155.70, 137.55, 128.43, 127.74, 126.99, 70.95, 59.10, 32.02, 19.17, 18.82.

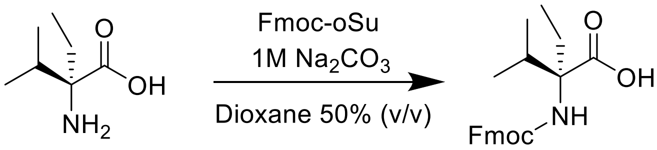


Synthesis of (3*S*,5*R*)-3-ethyl-3-isopropyl-5-phenylmorpholin-2-one. A solution of (*R*)-3-isopropyl-5-phenyl-5,6-dihydro-2H-1,4-oxazin-2-one (2.68 g, 12.32 mmol) in anhydrous tetrahydrofuran (150 mL) was cooled to −80 °C under nitrogen, and boron trifluoride etherate (4.67 mL, 36.96 mmol) was added in one portion. The reaction mixture was stirred at −80 °C for 2 h. 1 M Ethylmagnesium bromide in tetrahydrofuran solution (18.48 mL, 18.48 mmol) was added dropwise via syringe over 30 min. Stirring was continued for an additional 5 h, and the reaction was quenched at −80 °C by addition of saturated aqueous ammonium chloride (150 mL). The organic layer was separated, and the aqueous phase was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over anhydrous sodium sulfate, and concentrated in *vacuo*. The crude product was purified by flash chromatography on silica gel (ethyl acetate/hexane). Yield was 22% (0.67 g, 2.71 mmol). ESI–MS: $[M + H]^+$ calculated 248.1645, found 248.1641. ^{1}H NMR (500 MHz, methanol-*d*4, δ) 7.55 (m, 2H), 7.39 (m, 3H), 4.61 (t, 1H), 4.36 (m, 2H), 2.14 (m, 1H), 1.95 (m, 2H), 1.12 (m, 6H), 1.04 (m, 3H).

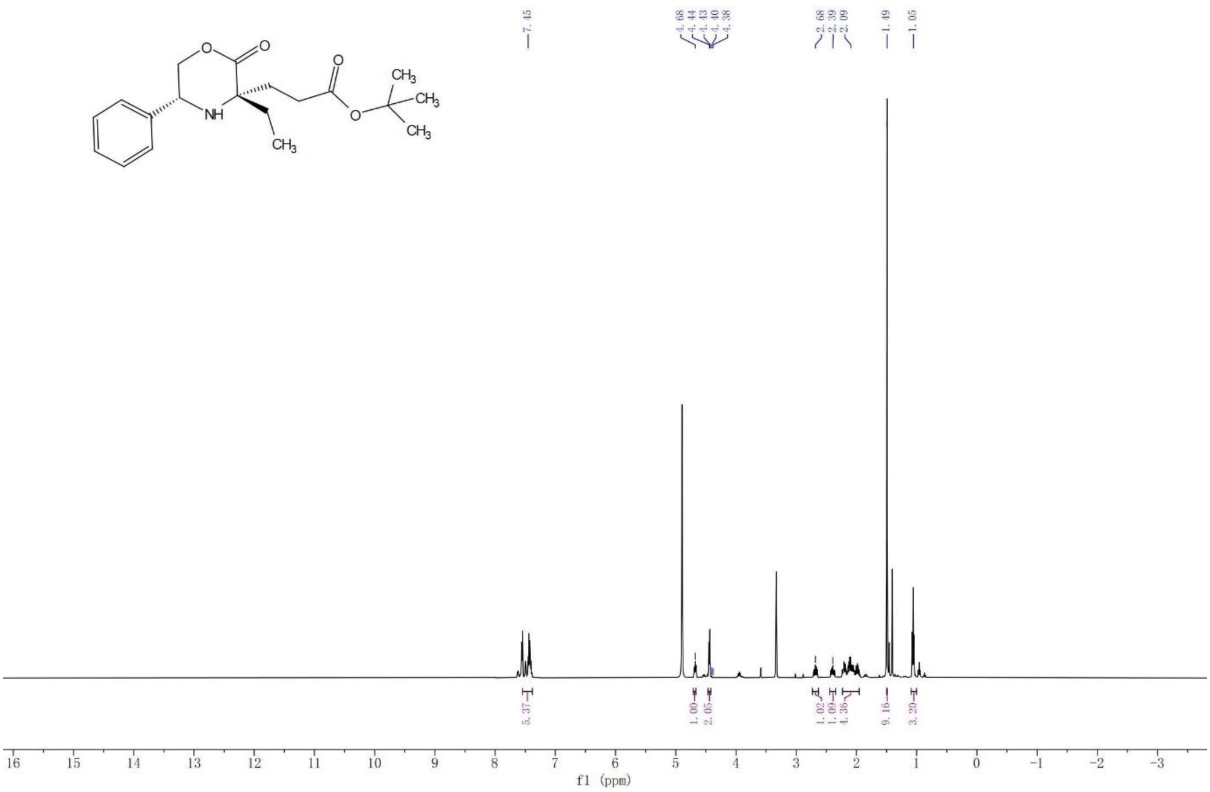
^{13}C NMR (126 MHz, methanol-*d*4, δ) 173.25, 137.24, 128.43, 127.35, 73.37, 67.44, 54.67, 36.81, 31.56, 17.14, 14.98, 8.57.

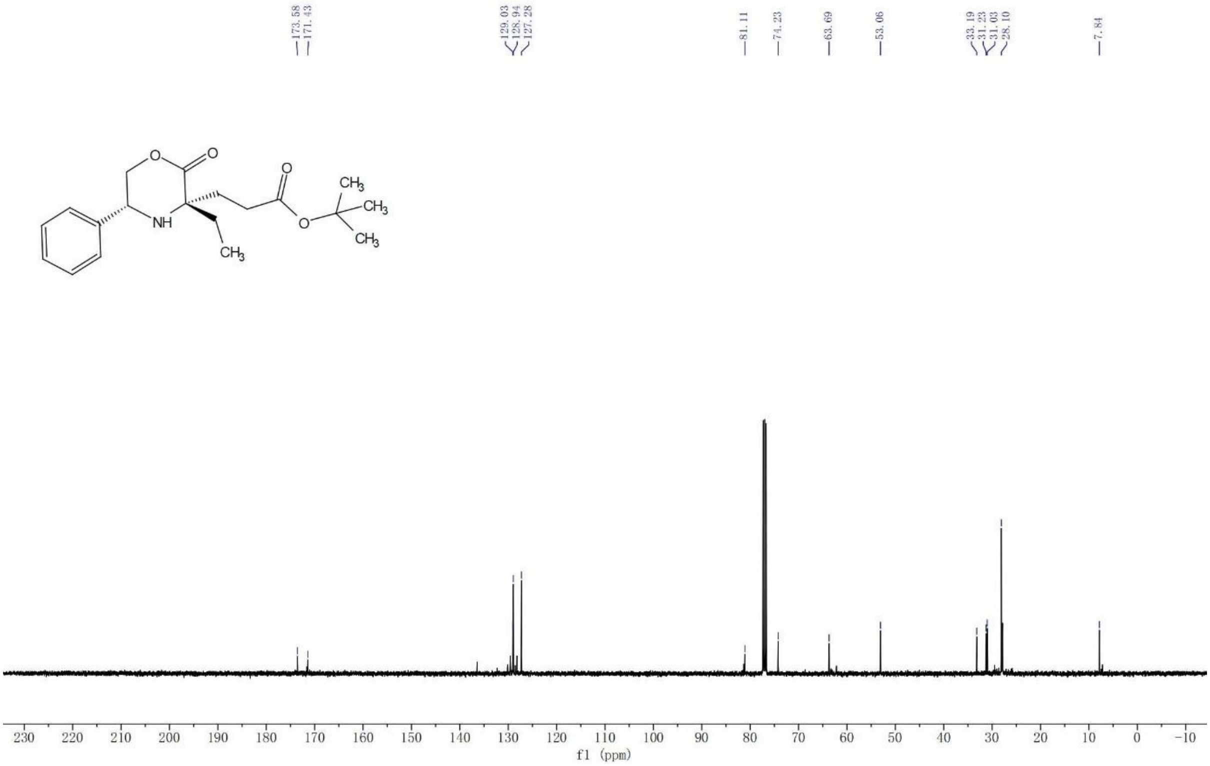


Synthesis of H-α-Et-L-Val-OH. A solution of the (3*S*,5*R*)-3-ethyl-3-isopropyl-5-phenylmorpholin-2-one (0.67 g, 2.71 mmol) in methanol (40 mL) was treated with $Pd(OH)_2/C$ (0.38 g, 2.71 mmol), trifluoroacetic acid (0.8 mL), and water (4 mL) under hydrogen at room temperature for 24 h. After release of the hydrogen, the reaction mixture was filtered through Celite, and the solvent was concentrated in in *vacuo*. The crude product was purified by flash chromatography on silica gel (methanol/dichloromethane). Yield was 90% (0.36 g, 2.44 mmol). ESI–MS: $[M + H]^+$ calculated 146.1176, found 146.1173. ^1H NMR (500 MHz, methanol-*d*4, δ) 2.25 (m, 1H), 1.98 (m, 2H), 1.05 (m, 9H). ^{13}C NMR (126 MHz, methanol-*d*4, δ) 171.67, 67.40, 33.04, 26.79, 16.21, 14.92, 6.83.

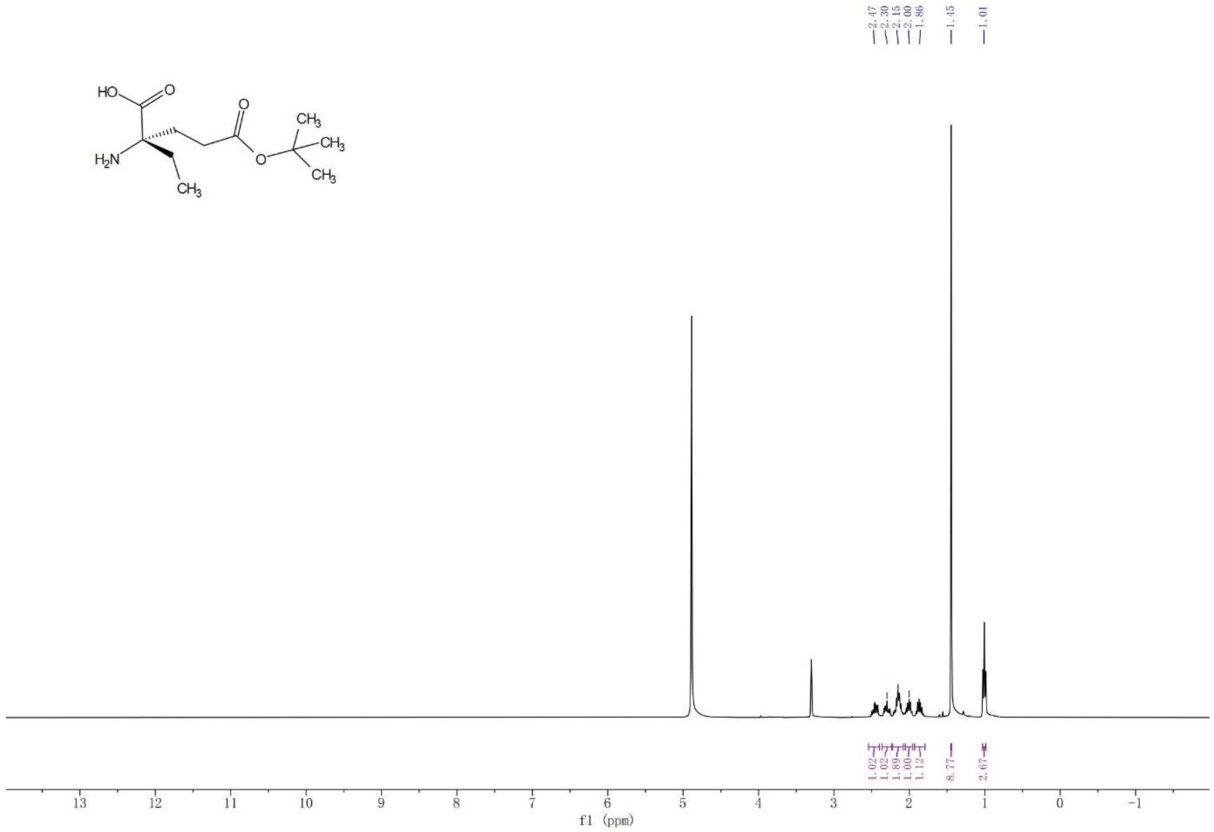


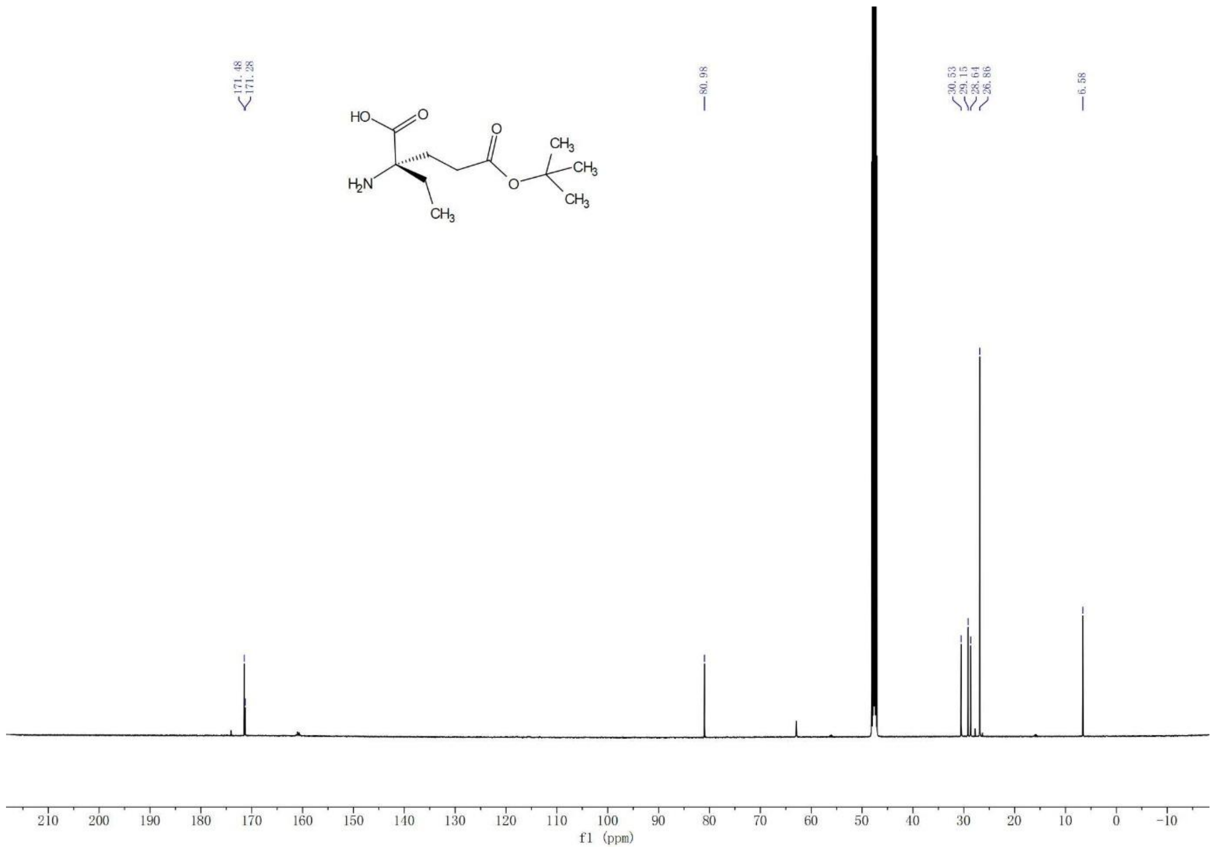
Synthesis of Fmoc-α-Et-L-Val-OH. H-α-Et-L-Val-OH (0.36 g, 2.44 mmol) was dissolved in a mixture of 1,4-dioxane and 1M aqueous sodium bicarbonate (1:1, 100 mL). *N*-(9-Fluorenylmethoxycarbonyloxy)succinimide (0.82 g, 2.44 mmol) was added to the solution, and the reaction mixture was stirred at room temperature overnight. The reaction was then acidified with 1 M HCl, and was extracted with ethyl acetate. The organic phase was dried with sodium sulfate and concentrated in *vacuo*. The crude product was purified by flash chromatography on silica gel (ethyl acetate/hexane). Yield was 25% (224 mg, 0.61 mmol). ESI–MS: $[M + H]^+$ calculated 368.1856, found 368.1858. ^1H NMR (500 MHz, methanol-*d*4, δ) 7.83 (m, 2H), 7.67 (m, 2H), 7.41 (m, 2H), 7.33 (m, 2H), 4.36 (d, 2H), 4.23 (t, 1H), 2.33 (m, 2H), 2.02 (m, 1H), 0.97 (m, 6H), 0.77 (m, 3H). ^{13}C NMR (126 MHz, methanol-*d*4, δ) 174.25, 154.86, 144.00, 141.22, 127.38, 126.74, 124.76, 119.54, 65.81, 33.06, 24.52, 16.85, 16.64, 7.44.



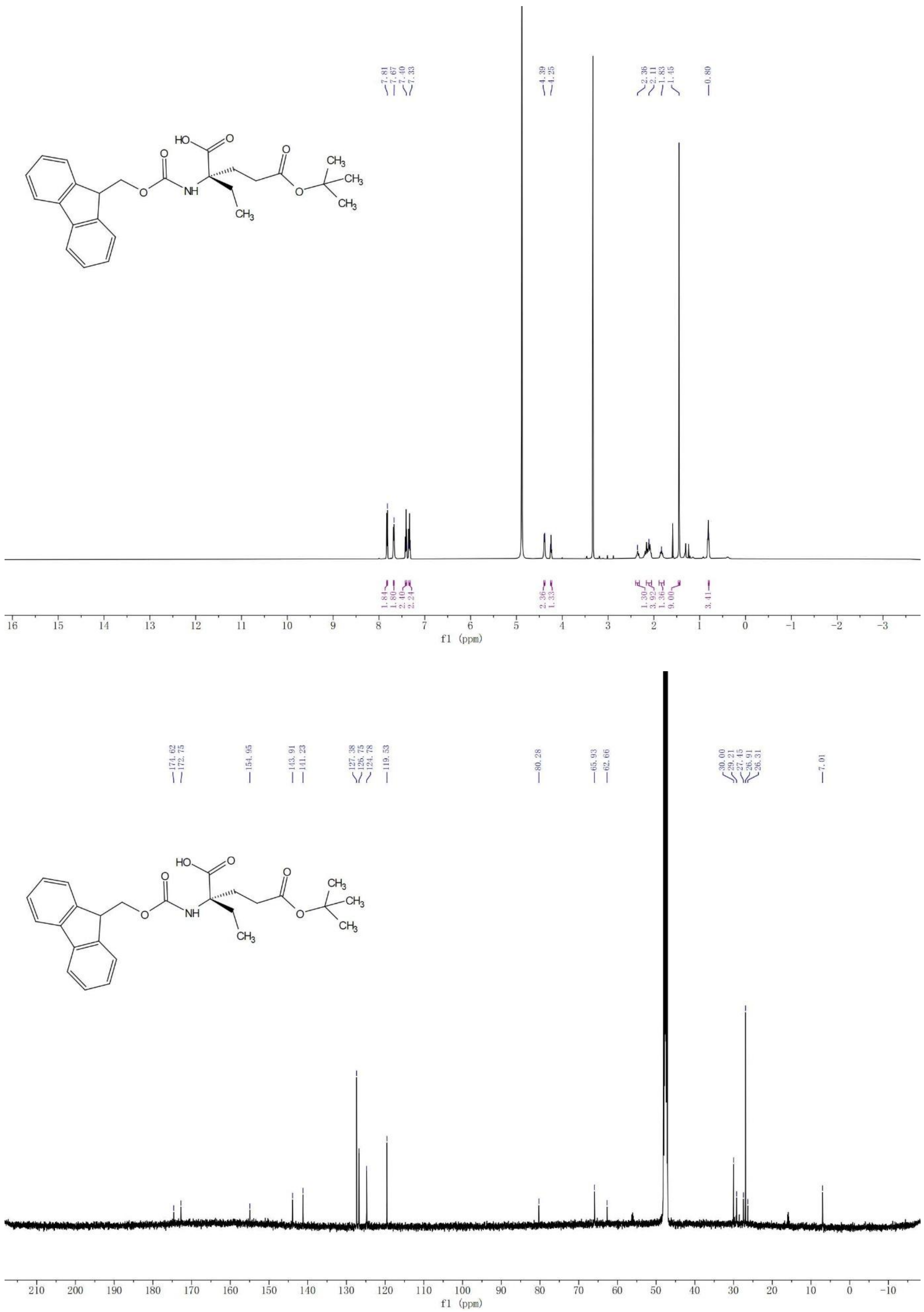


^{1}H and ^{13}C{^{1}H} NMR spectra of tert-butyl 3-((3*S*,5*R*)-3-ethyl-2-oxo-5-phenylmorpholin-3-yl)propanoate in methanol-*d*4 (CDCl$_3$ ^{13}C) at 500 MHz ^{1}H (100 MHz ^{13}C)

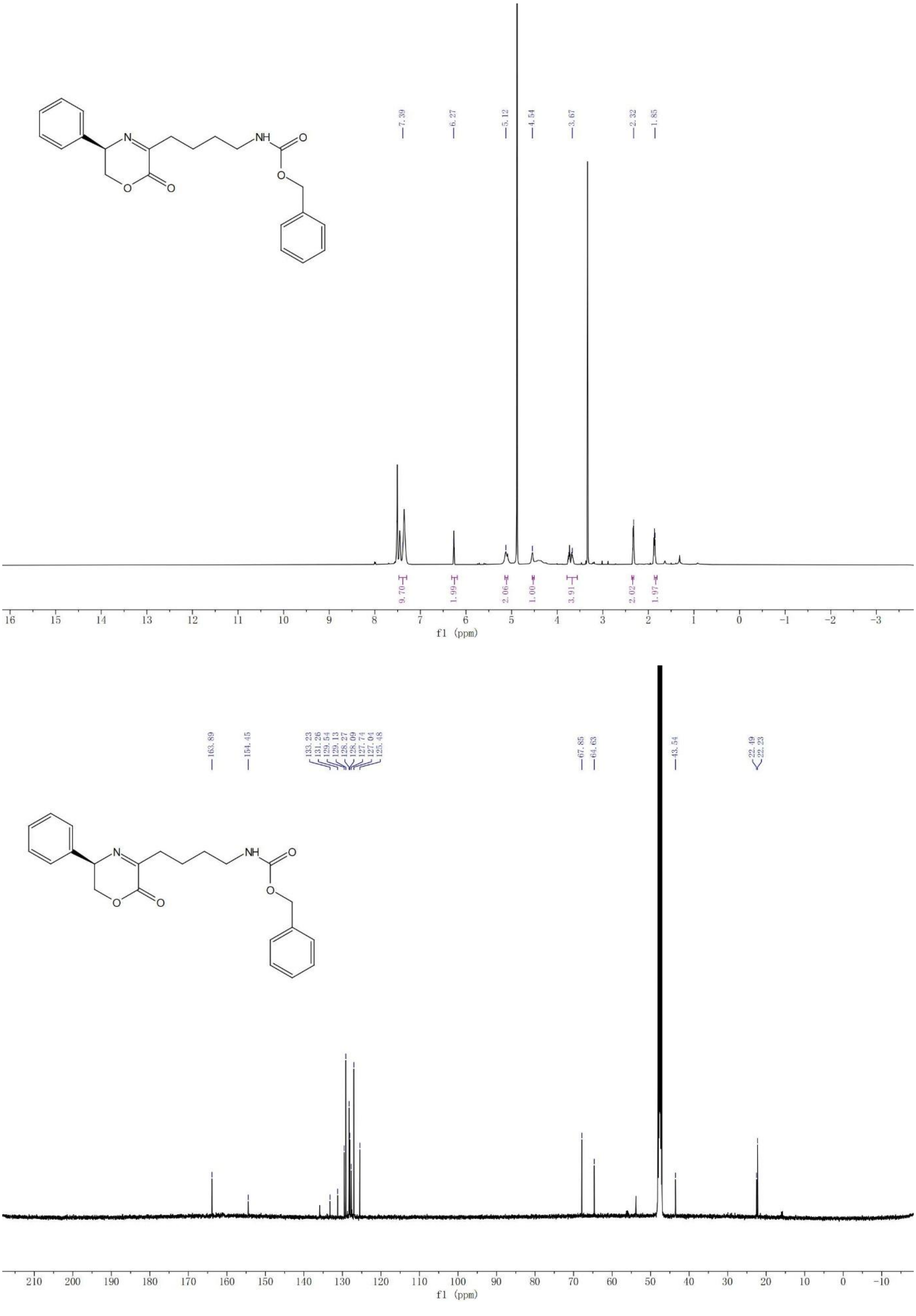




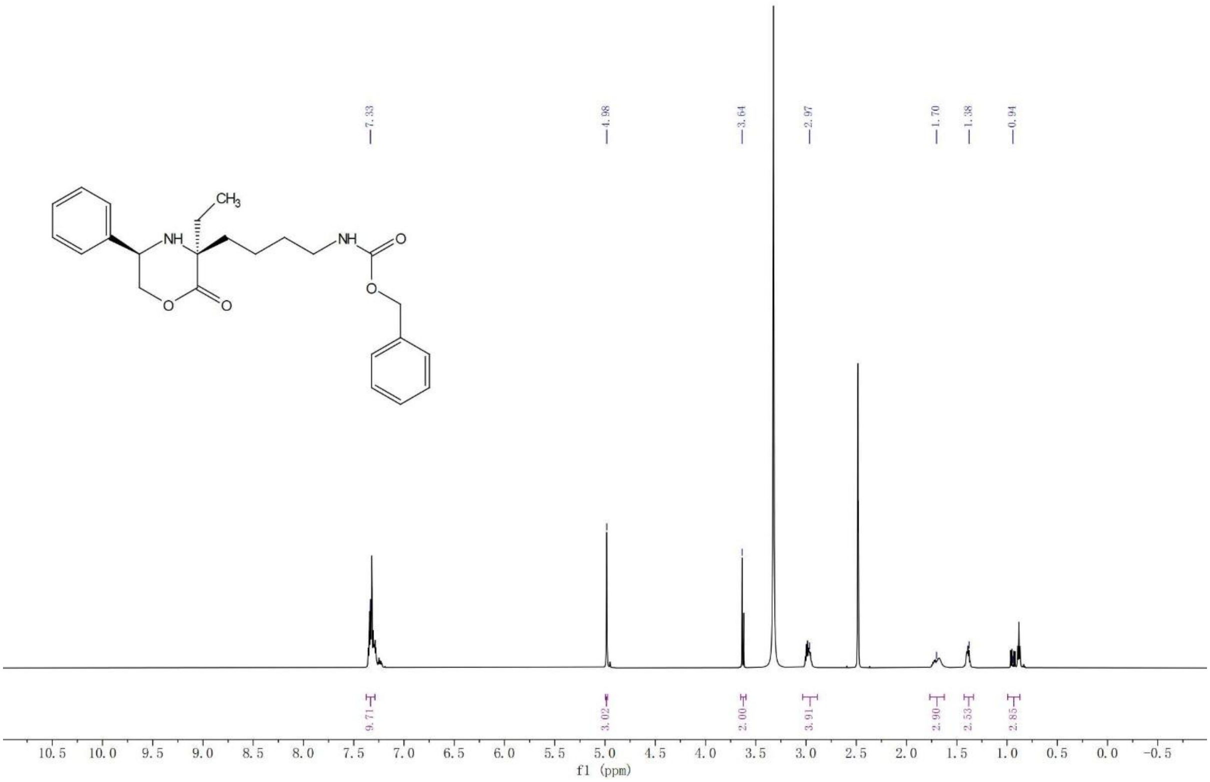
^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of H-α-Et-L-Glu(OtBu)-OH in methanol-*d*4 at 500 MHz ^{1}H (126 MHz ^{13}C)

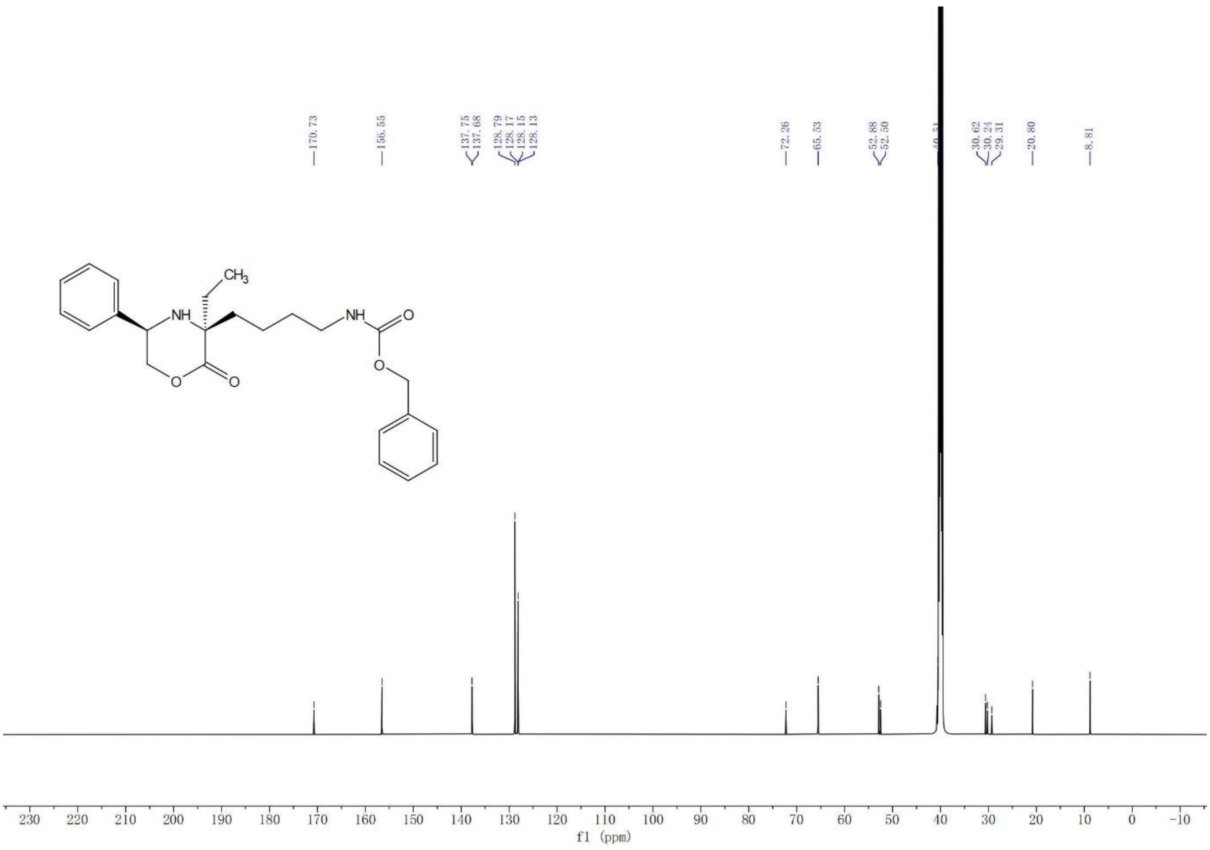


^{1}H and ^{13}C{^{1}H} NMR spectra of Fmoc-α-Et-L-Glu(OtBu)-OH in methanol-*d*4 at 500 MHz ^{1}H (126 MHz ^{13}C)

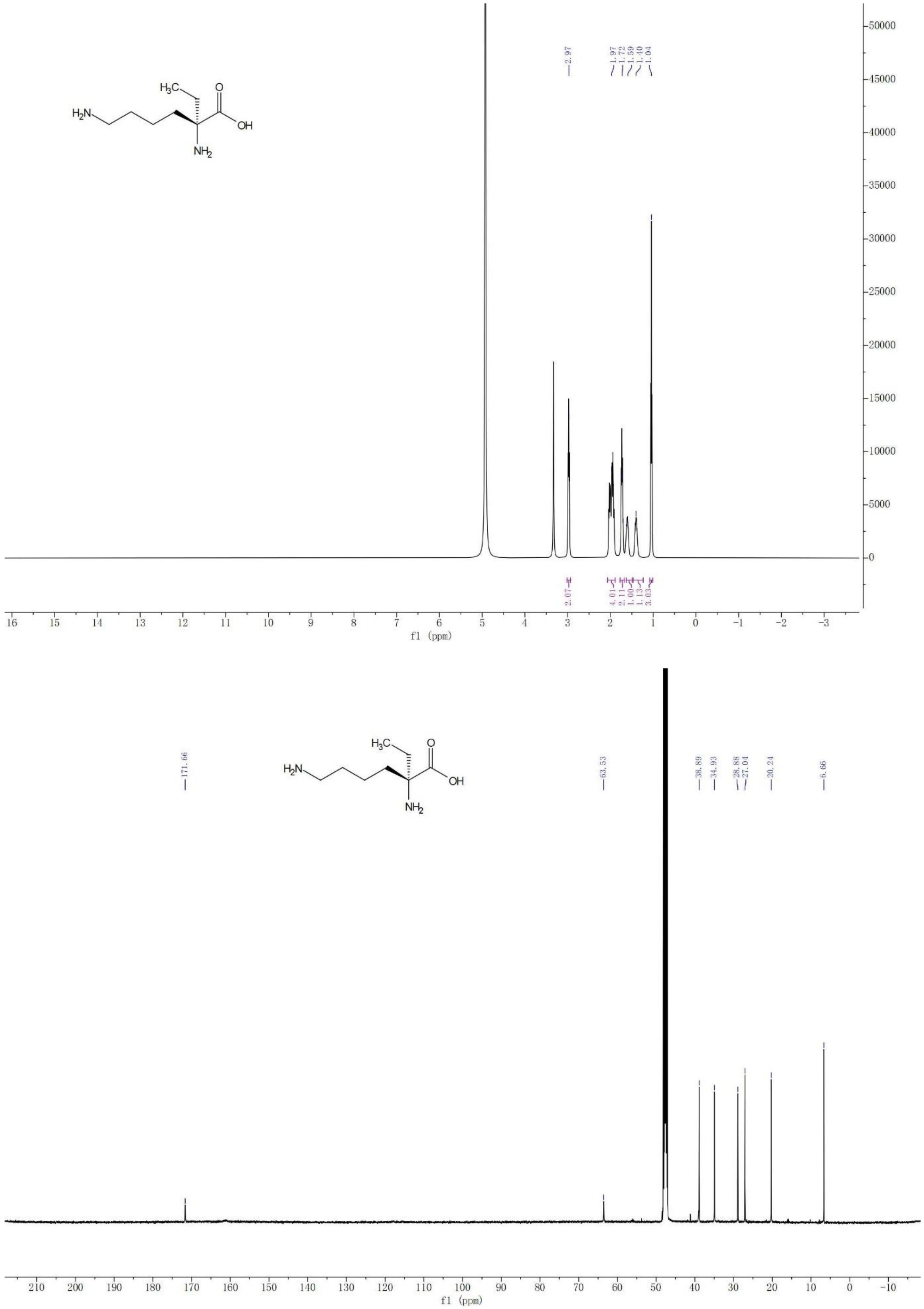


^{1}H and ^{13}C{^{1}H} NMR spectra of benzyl (*R*)-(4-(2-oxo-5-phenyl-5,6-dihydro-2H-1,4-oxazin-3-yl)butyl)carbamate in methanol-*d*4 at 500 MHz ^{1}H (126 MHz ^{13}C)

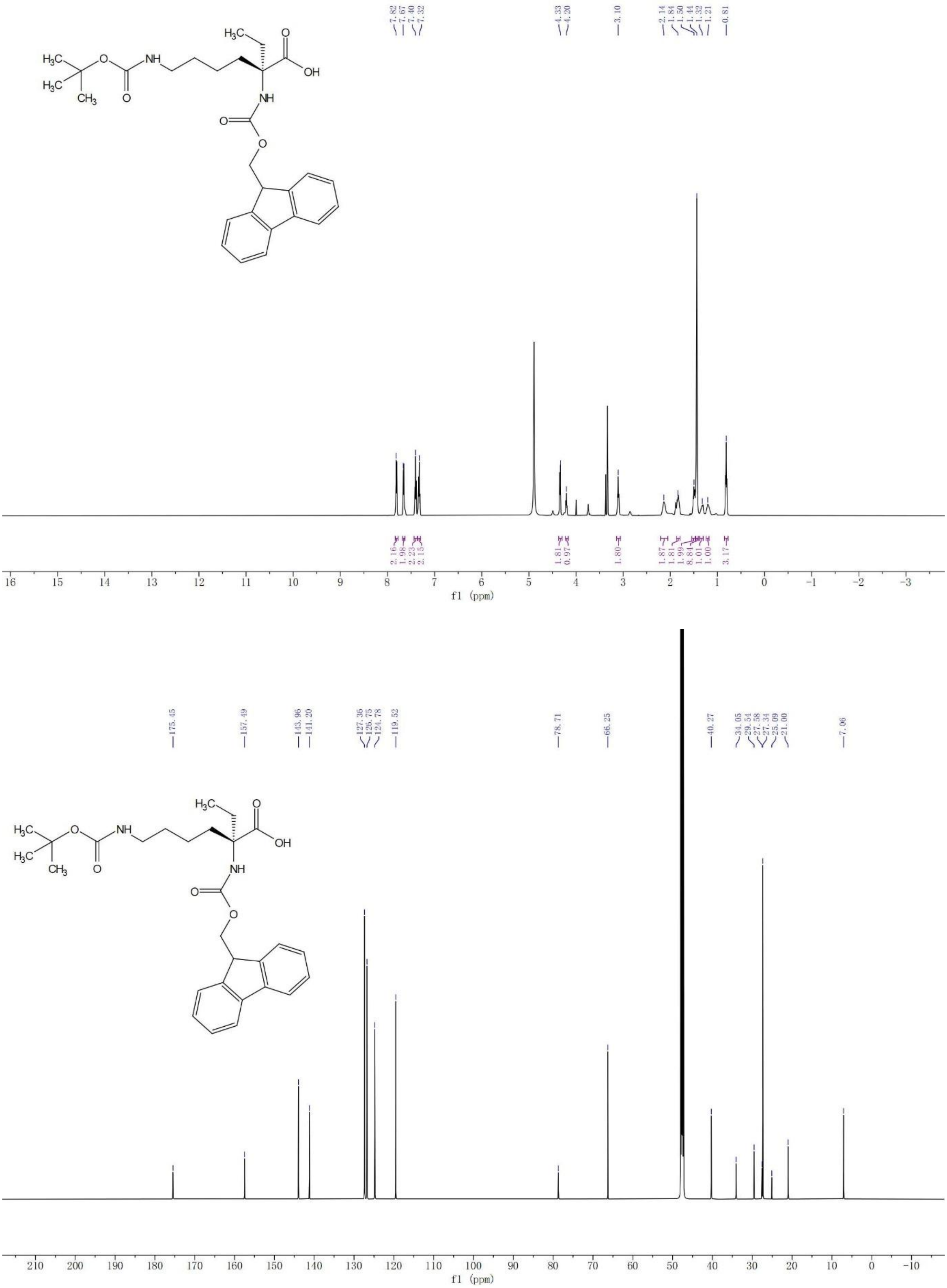




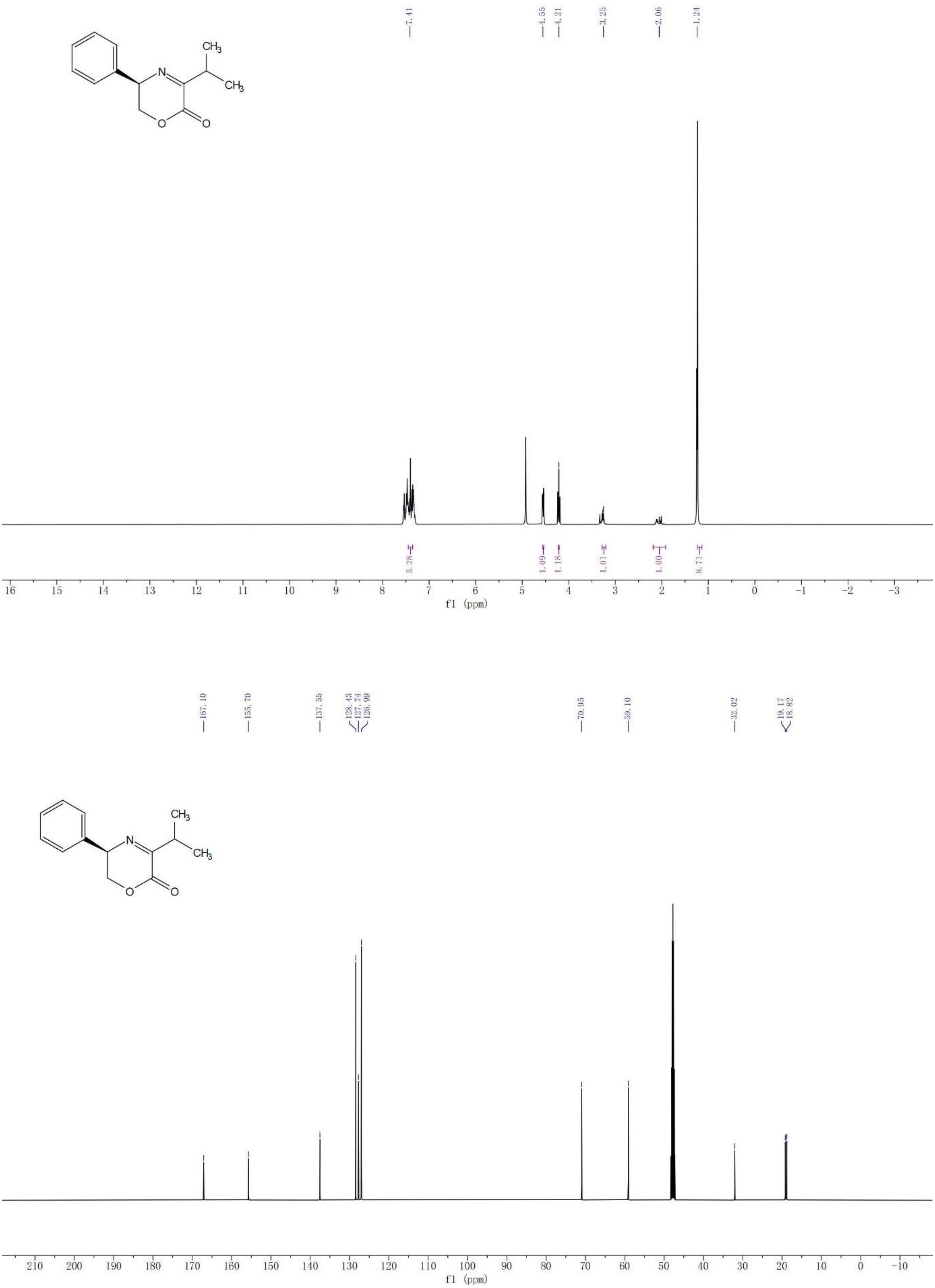
^{1}H and ^{13}C{^{1}H} NMR spectra of benzyl (4-((3*S*,5*R*)-3-ethyl-2-oxo-5-phenylmorpholin-3-yl)butyl)carbamate in DMSO-*d*6 at 600 MHz ^{1}H (151 MHz ^{13}C)



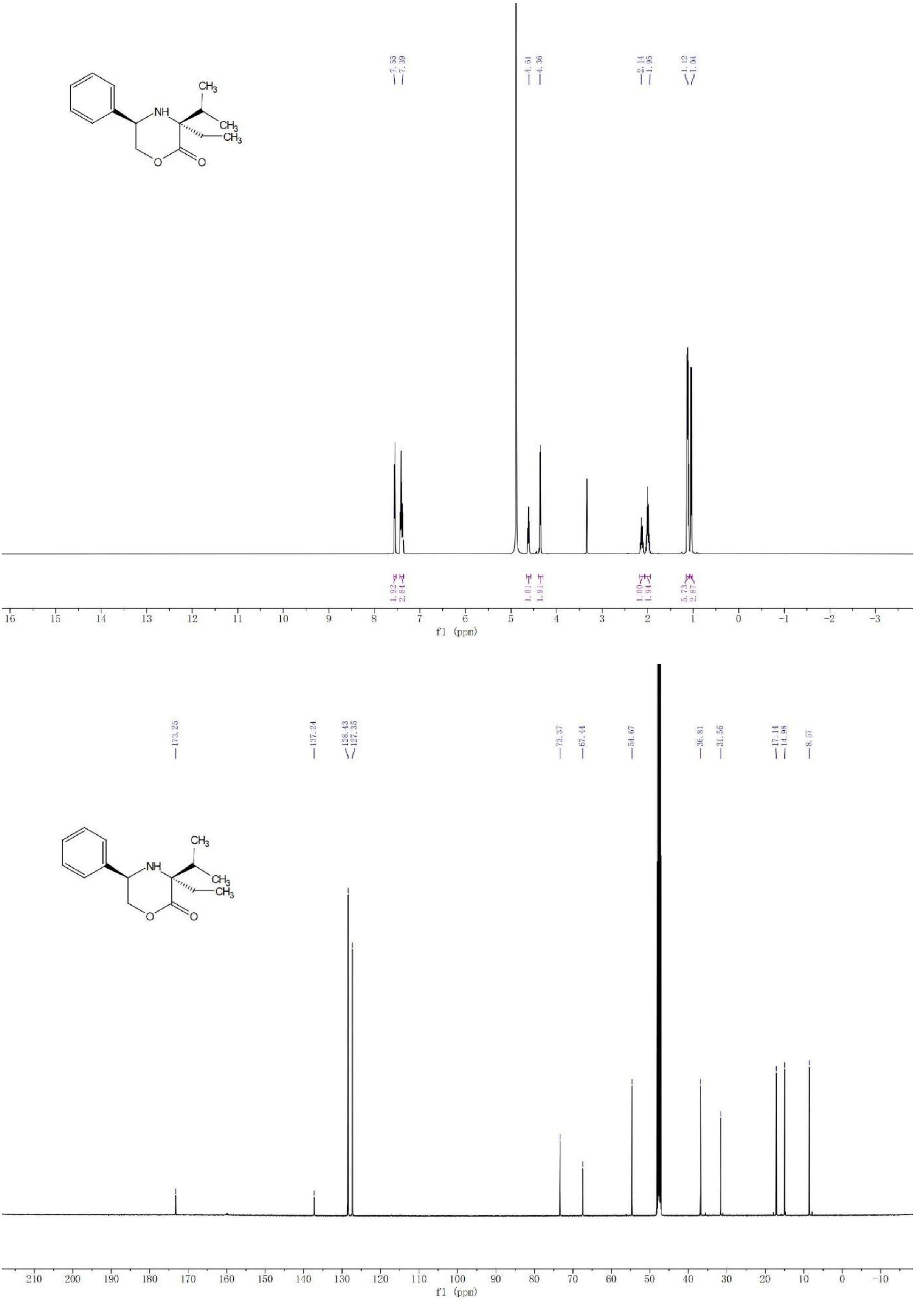
^{1}H and ^{13}C{^{1}H} NMR spectra of H-α-Et-L-Lys-OH in methanol-*d*4 at 500 MHz ^{1}H (126 MHz ^{13}C)



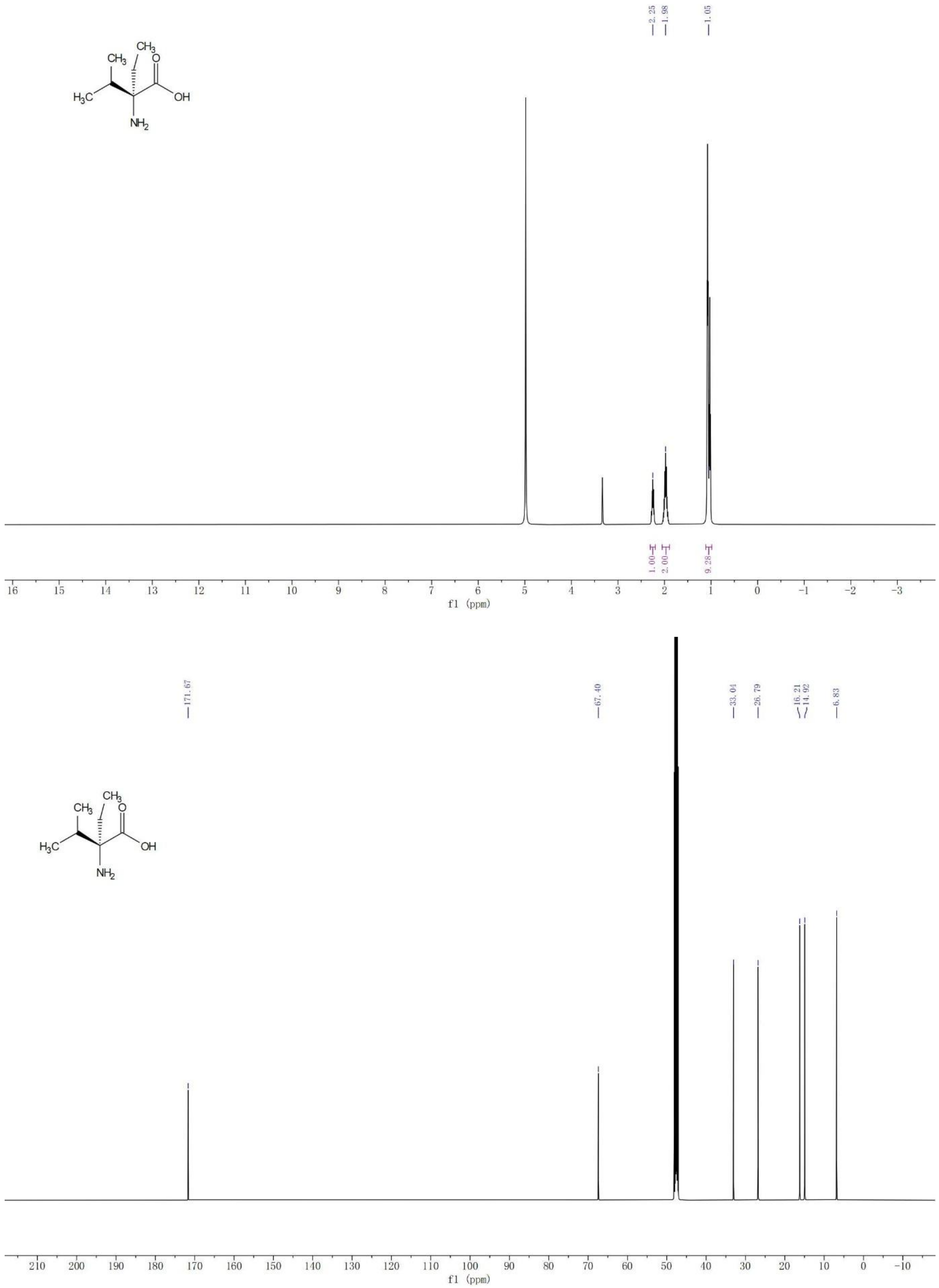
^{1}H and ^{13}C{^{1}H} NMR spectra of Fmoc-α-Et-L-Lys(Boc)-OH in methanol-*d*4 at 500 MHz ^{1}H (126 MHz ^{13}C)



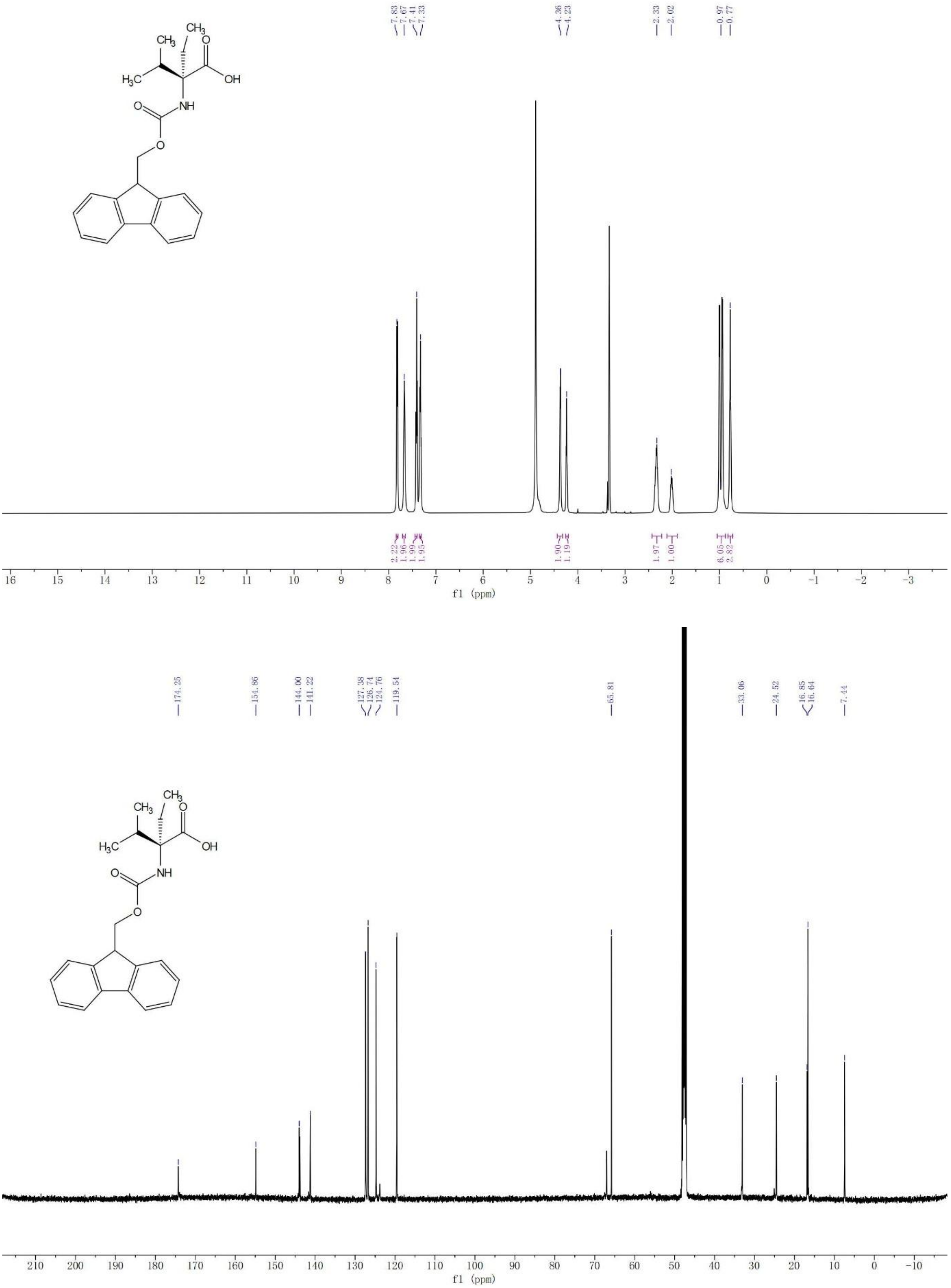
^{1}H and ^{13}C{^{1}H} NMR spectra of (*R*)-3-isopropyl-5-phenyl-5,6-dihydro-2H-1,4-oxazin-2-one in methanol-*d*4 at 500 MHz ^{1}H (126 MHz ^{13}C)



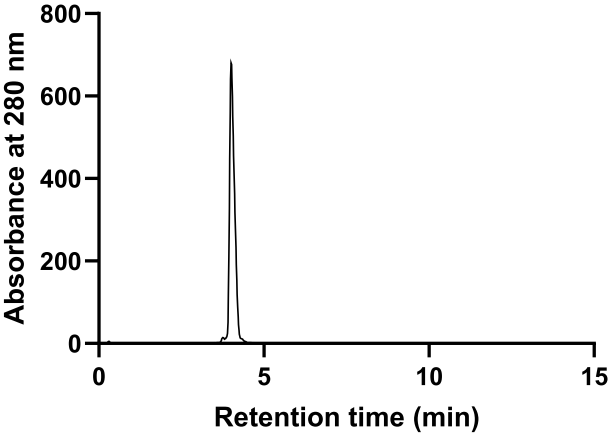
^{1}H and ^{13}C{^{1}H} NMR spectra of (3*S*,5*R*)-3-ethyl-3-isopropyl-5-phenylmorpholin-2-one in methanol-*d*4 at 500 MHz ^{1}H (126 MHz ^{13}C)



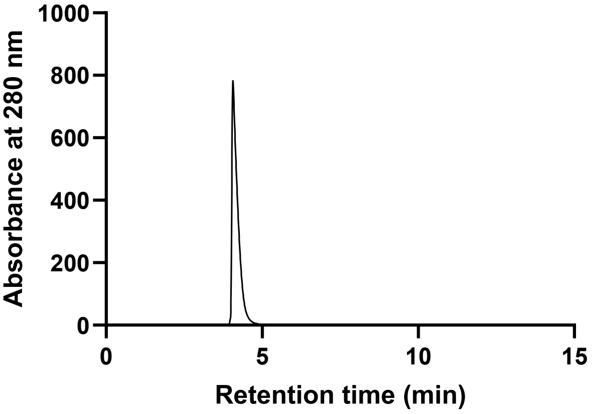
^{1}H and ^{13}C{^{1}H} NMR spectra of H-α-Et-L-Val-OH in methanol-*d*4 at 500 MHz ^{1}H (126 MHz ^{13}C)



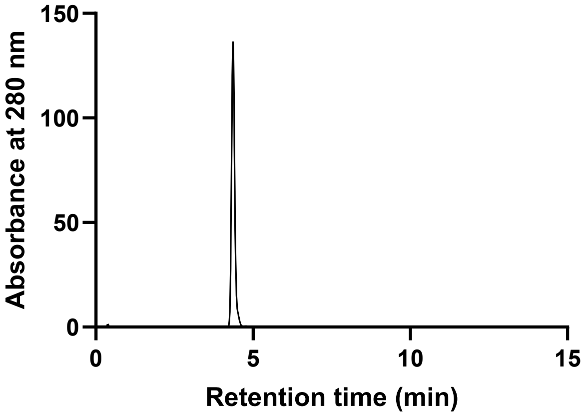
^{1}H and ^{13}C{^{1}H} NMR spectra of Fmoc-α-Et-L-Val-OH in methanol-*d*4 at 500 MHz ^{1}H (126 MHz ^{13}C)



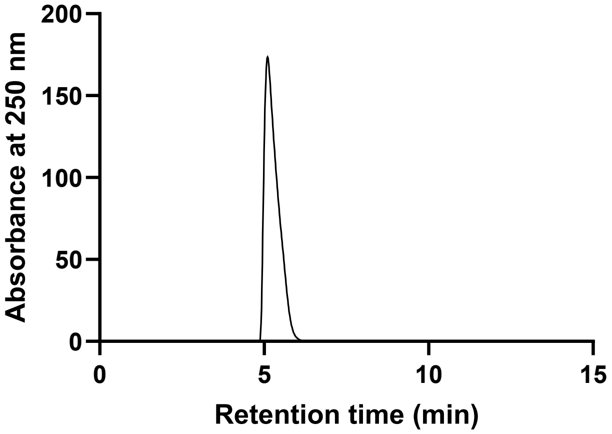
Analytical LC–MS trace of purified TrpZipE-WT. MS (ESI) m/z: $[M+H]^+$ calculated for $C_{82}H_{104}N_{20}O_{21}$ 1706.8; found 1707.7.



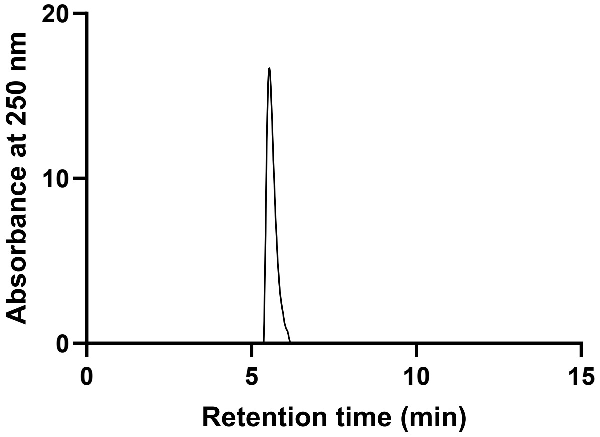
Analytical LC–MS trace of purified TrpZipE-αMe-E. MS (ESI) m/z: $[M+H]^+$ calculated for $C_{83}H_{106}N_{20}O_{21}$ 1720.9; found 1719.7.



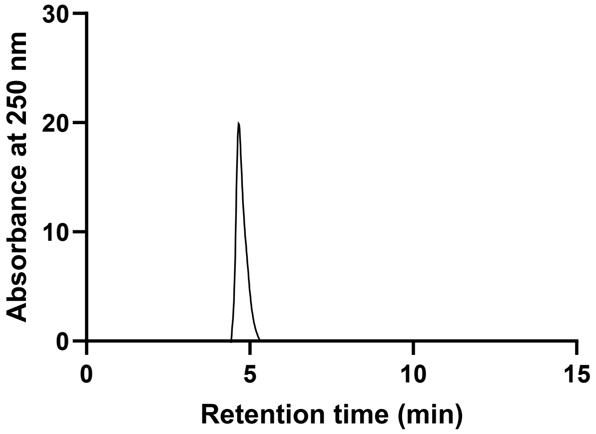
Analytical LC–MS trace of purified TrpZipE-αEt-E. MS (ESI) m/z: $[M+H]^+$ calculated for $C_{84}H_{108}N_{20}O_{21}$ 1734.9; found 1733.8.



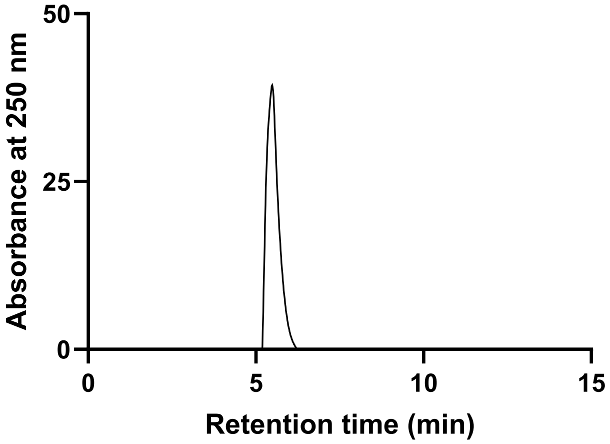
Analytical LC–MS trace of purified VW19-WT. MS (ESI) m/z: [M+3H]$^+$ calculated for $C_{151}H_{266}N_{36}O_{40}$ 1706.3; found 1706.2.



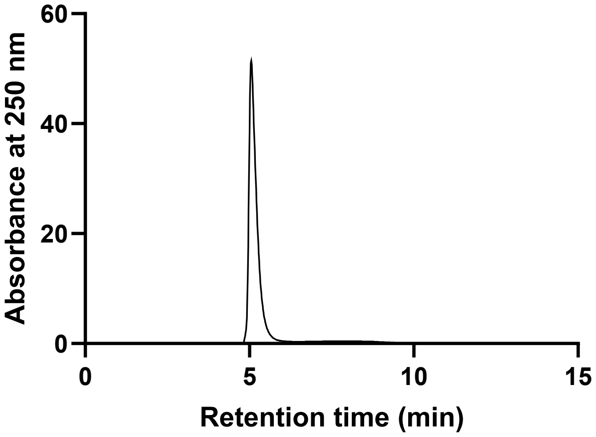
Analytical LC–MS trace of purified VW19-αMe-Val^c. MS (ESI) m/z: [M+3H]$^+$ calculated for $C_{152}H_{268}N_{36}O_{40}$ 1081.0; found 1080.7.



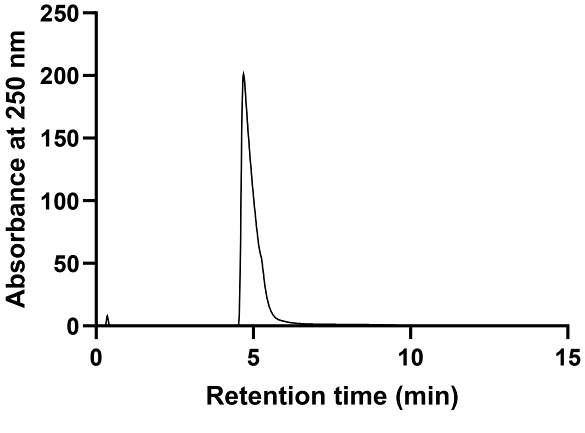
Analytical LC–MS trace of purified VW19-αEt-Val^c. MS (ESI) m/z: [M+3H]$^+$ calculated for $C_{153}H_{270}N_{36}O_{40}$ 1085.7; found 1085.3.



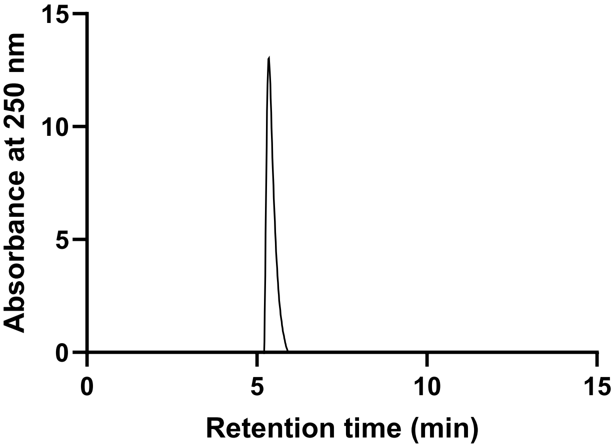
Analytical LC–MS trace of purified VW19-αMe-Lys[e]. MS (ESI) m/z: [M+3H]$^+$ calculated for $C_{152}H_{268}N_{36}O_{40}$ 1081.0; found 1080.7.



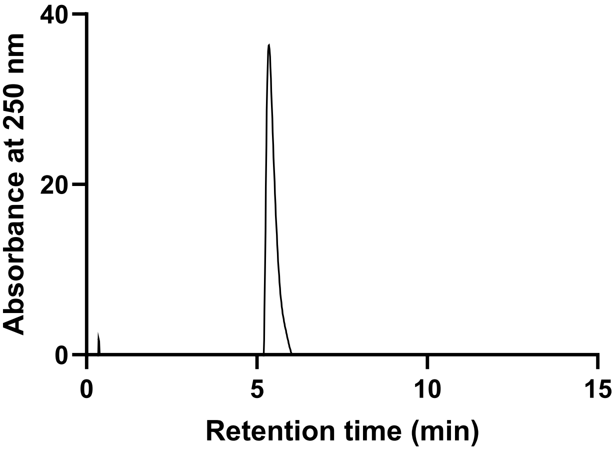
Analytical LC–MS trace of purified VW19-αEt-Lys[e]. MS (ESI) m/z: [M+3H]$^+$ calculated for $C_{153}H_{270}N_{36}O_{40}$ 1085.7; found 1085.4.



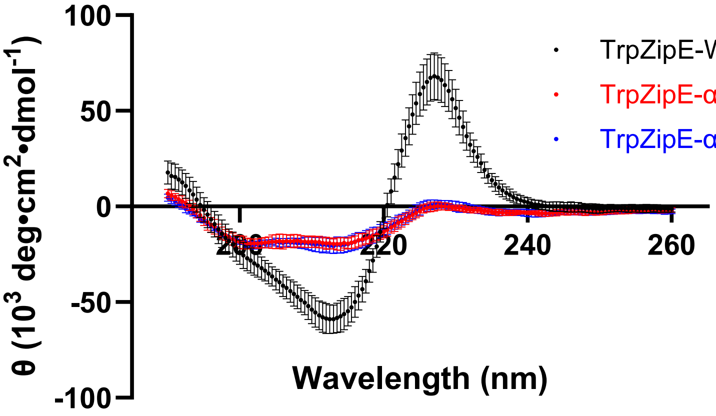
Analytical LC–MS trace of purified VW19-αMe-Glu[g]. MS (ESI) m/z: [M+3H]$^+$ calculated for $C_{152}H_{268}N_{36}O_{40}$ 1081.0; found 1080.8.



Analytical LC–MS trace of purified VW19-αEt-Glu[g]. MS (ESI) m/z: $[M+3H]^+$ calculated for $C_{153}H_{270}N_{36}O_{40}$ 1085.7; found 1085.2.



Analytical LC–MS trace of purified VW19-αMe-Glu[c]. MS (ESI) m/z: $[M+3H]^+$ calculated for $C_{152}H_{268}N_{36}O_{40}$ 1081.0; found 1080.8.



Far-UV circular dichroism spectra of TrpZipE peptides at 4°C

References:

1. H. Zheng and R. W. Newberry, *J. Org. Chem.*, 2025, **90**, 13984–13988.
2. K. Pagel, S. C. Wagner, K. Samedov, H. von Berlepsch, C. Böttcher and B. Koksch, *J. Am. Chem. Soc.*, 2006, **128**, 2196–2197.
3. L. M. Harwood, S. N. Tyler, M. G. Drew, A. Jahans and I. D. MacGilp, *Arkivoc*, 2000, **820**, 831.
4. E. D. Thorsett, *Tetrahedron Lett.*, 1982, **23**, 1875–1876.
5. S. Nowshuddin, M. Rao and A. R. Reddy, *Synth. Commun.*, 2009, **39**, 2022–2031.