Supplementary Information (SI) for Organic & Biomolecular Chemistry.
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Supplementary Information

Total synthesis of linear lipodepsipeptide kavaratamide A and its C25-epimer

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Table of Contents

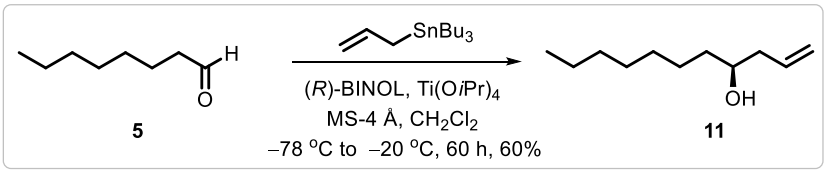
S. No	Contents	Page No
1)	General Information	S2
2)	Experimental Procedures and Analytical Data	S3-S19
3)	HPLC data	S20-S23
4)	Cytotoxicity study of C25-*epi*-Kavaratamide A and Kavaratamide A in HepG2 Cell Line	S24-S47
5)	Cytotoxicity study of C25-*epi*-Kavaratamide A and Kavaratamide A in PANC-1 Cell Line	S48-S70
6)	^{1}H, ^{13}C and DEPT spectra	S71-S110

1) ***General Information*****:**

All reactions were performed under argon atmosphere with oven (80 ºC) or flame-dried glassware with a septum seal. Tetrahydrofuran (THF) was distilled from sodium-benzophenone under the argon atmosphere immediately before use. Anhydrous dichloromethane, dichloroethane, methanol and fluorobenzene were purchased from commercial sources and used without any further treatment. Reaction temperatures are reported as the temperature of the bath surrounding the reaction vessel, and 30 °C corresponds to the room temperature (rt) of the laboratory when the experiments were carried out. Analytical thin-layer chromatography (TLC) was performed on TLC Silica gel 60 F254. Visualization was accomplished with short wave UV light, anisaldehyde or $KMnO_4$ staining solutions followed by heating. Chromatography was performed on silica gel (100-200 mesh) by standard techniques eluting with solvents as indicated. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV 200, 400, and 500 in solvents as indicated. Chemical shifts (δ) are given in ppm. The residual solvent signals were used as references and the chemical shifts converted to the TMS scale ($CDCl_3$: δ H = 7.26 ppm, δ C = 77.16 ppm), the following abbreviations were used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublet; td, triplet doublet; and br, broad. HRMS data were recorded on Q Exactive HybridTM Quadrupole-OrbitrapTM mass spectrometer (Thermo Scientific, TM Accela 1250 pump). Chiral HPLC separations were achieved using Shimadzu prominence-*i* LC-2030C 3D plus reverse phase HPLC unit and LabSolutions software with Kromasil C18 column (150 × 4.6 mm, 5 µm particle size). Experimental procedures for all new compounds and known compounds without published experimental procedures are described below. Compounds that are not presented in the main text of the manuscript are numbered starting from **S1**.

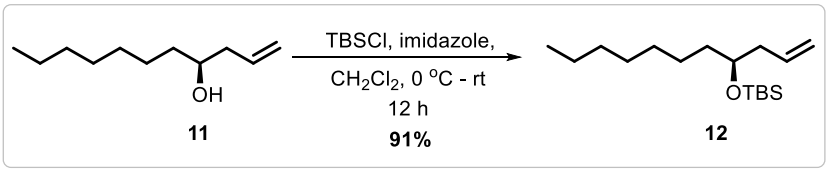
2. Experimental Procedures and Analytical Data:

(*S*)-Undec-1-en-4-ol (11):



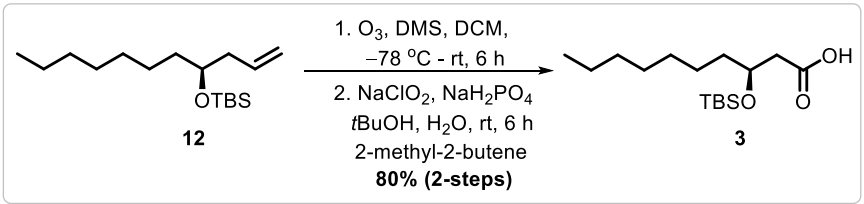
A 500 mL, round-bottom flask equipped with a stirring bar was charged with powdered 4 Å molecular sieves (100.0 g), (*R*)-BINOL (2.23 g, 7.79 mmol) and anhydrous dichloromethane (100 mL). To the resultant suspension was added titanium tetraisopropoxide (2.3 g, 1.13 mL, 7.79 mmol) by a syringe at room temperature. The resulting orange-red suspension was heated at reflux for 1 h. The red-brown mixture was cooled to ambient temperature and n-octanal (**5**) (5.0 g, 6.09 mL, 39.0 mmol) was added via syringe. The resulting mixture was stirred for 5 min at ambient temperature, then cooled to −78°C. To the reaction mixture slowly was added allyltributylstannane (15.49 g, 14.61 mL, 46.79 mmol) via syringe and continued to stir it at the same temperature for another 10 minutes. The resulting reaction mixture was then kept in a freezer at −20°C for 60 h without stirring. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (100 mL), diluted with dichloromethane (100 mL), and stirred at ambient temperature for 3 h. The molecular sieves were removed by filtration through a pad of Celite, the residue was extracted thrice with dichloromethane. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 4% EtOAc in hexanes) (100-200 mesh), to afford (*S*)-Undec-1-en-4-ol (**11**) (4.0 g, 60 %) as a colourless oil. TLC: R_f = 0.5 (SiO_2, 10% EtOAc/hexanes); $[\alpha]_D^{25}$= –7.6 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.87-5.77 (m, 1H), 5.15-5.07 (m, 2H), 3.62 (br. s., 1H), 2.33-2.23 (m, 1H), 2.18-2.07 (m, 1H), 1.69 (s, 1H), 1.44 (t, *J* = 5.6 Hz, 2H), 1.37-1.24 (m, 10H), 0.87 (t, *J* = 7.13 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): 135.1, 118.1, 70.8, 42.1, 36.9, 31.9, 29.7, 29.4, 25.8, 22.8, 14.2.

(*S*)-*tert*-Butyldimethyl(undec-1-en-4-yloxy)silane (12):



A 250 mL, round-bottom flask equipped with a stirring bar was charged (*S*)-Undec-1-en-4-ol (**11**) (4.0 g, 23.48 mmol) and anhydrous dichloromethane (50 mL) and cool it 0 °C. To the above-given solution was added imidazole (1.91 g, 28.18 mmol) in one portion at the same temperature. To the above cooled solution, TBSCl (4.2 g, 28.18 mmol) was added in small portions at 0 °C. After 10 minutes, temperature was increased to room temperature and continued the stirring for another 12 h. After the completion of the reaction, the reaction mixture was diluted with 100 ml of water and then the residue was extracted thrice with dichloromethane. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 100% hexanes) (100-200 mesh), to afford (*S*)-*tert*-Butyldimethyl(undec-1-en-4-yloxy)silane (**12**) (6.1 g, 91 %) as a colourless oil. TLC: R_f = 0.7 (SiO_2, 10% hexanes); $[\alpha]_D^{25}$ = –34.2 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.89-5.75 (m, 1H), 5.07-4.99 (m, 2H), 3.68 (t, *J* = 5.4 Hz, 1H), 2.26-2.16 (m, 2H), 1.42 (br. s., 2H), 1.38-1.25 (m, 10H), 0.89 (s, 12H), 0.05 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 135.7, 116.7, 72.2, 42.1, 37.0, 32.0, 29.9, 29.5, 26.1, 25.5, 22.8, 18.3, 14.3, -4.2, -4.4.

(*S*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoic acid (3):

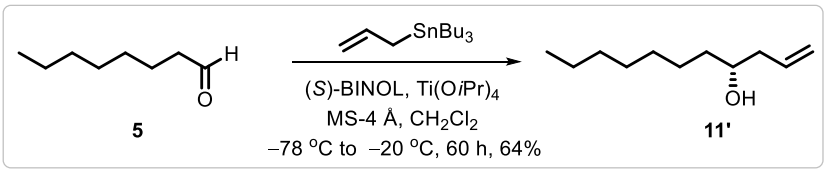


STEP-1: In a 250 mL, single necked round-bottom flask equipped with a stirring bar was charged with (*S*)-*tert*-Butyldimethyl(undec-1-en-4-yloxy)silane (**12**) (6.1 g, 21.43 mmol) and anhydrous dichloromethane (100 mL) and the given clear solution was cooled to −78°C. With the help of ozonizer machine first bubble O_2 gas into the solution for 5 minutes followed by continuous flow of O_3 until the reaction mixture turns blue. Once the solution becomes blue stop O_3 and replace residual O_3 with N_2 and quenched the reaction mixture with dimethyl sulphide. Slowly shift reaction mixture to ambient temperature and continue the stirring for another 3 h. On completion of the reaction dilute the residue with water (100 mL), the residue was extracted thrice with dichloromethane. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated and ultra-dried under reduced pressure. The resulting residue (aldehyde) was as it is forwarded for the next step without

further purification. TLC: R_f = 0.8 (SiO_2, 5% EtOAc/hexanes). (aldehyde = 5.8 g) as a colourless oil.

STEP-2: In a 250 mL, single necked round-bottomflask equipped with a stirring bar was charged with (*S*)-3-((*tert*-Butyldimethylsilyl)oxy)decanal (5.8 g, 20.24 mmol), tertiary butanol (10 mL), 2-methyl-2-butene (20 mL) and stir the given clear solution at ambient temperature. To the above solution was then added aqueous solution of $NaClO_2$ (18.30 g, 202.42 mmol) and NaH_2PO_4 (14.48 g, 121.45 mmol) and continued the stirring for another 6 h at the same temperature. After completion of the reaction dilute the residue with water (100 mL), the residue was extracted with ethyl acetate (3×40 mL). The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 4% EtOAc/hexanes) (100-200 mesh), to afford (*S*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoic acid (**3**) (5.2 g, 80 %) as a colourless oil (over 2 steps); TLC: R_f = 0.4 (SiO_2, 10 % hexanes); $[\alpha]_D^{25}$= –91 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.11 (quin, *J* = 6.0 Hz, 1H), 2.50-2.45 (m, 2H), 1.56-1.46 (m, 2H), 1.32-1.25 (m, 10H), 0.90-0.86 (m, 12H), 0.06 (d, *J* = 6.75 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 177.8, 69.5, 42.4, 37.6, 31.9, 29.7, 29.4, 25.9, 25.2, 22.8, 18.1, 14.2, -4.4, -4.7.

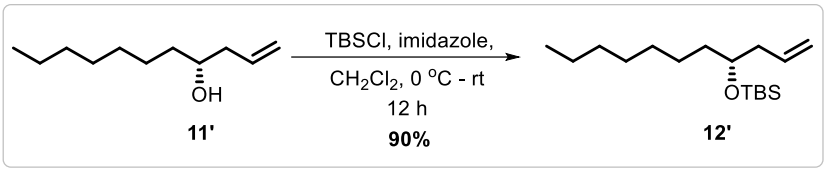
(*R*)-Undec-1-en-4-ol (11'):



A 500-mL, round-bottom flask equipped with a stirring bar was charged with powdered 4 Å molecular sieves (100.0 g), (*S*)-BINOL (2.23 g, 7.79 mmol) and anhydrous methylene chloride (100 mL). To the resultant suspension was added titanium tetraisopropoxide (2.3 g, 1.13 mL, 7.79 mmol) by syringe at ambient temperature. The resulting orange red suspension was heated at reflux for 1 h. The red-brown mixture was cooled to ambient temperature and n-octanal (**5**) (5.0 g, 6.09 mL, 39.0 mmol) was injected via syringe. The resulting mixture was stirred for 5 min at ambient temperature, then cooled to −78°C. To the reaction mixture slowly was added allyltributylstannane (15.49 g, 14.61 mL, 46.79 mmol) via syringe and continued to stir it at the same temperature for another 10 minutes. The resulting reaction mixture was then kept in a freezer at −20°C for 60 h without

stirring. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (100 mL), diluted with dichloromethane (100 mL), and stirred at ambient temperature for 3 h. The molecular sieves were removed by filtration through a pad of Celite, the residue was extracted thrice with dichloromethane. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 4% EtOAc in hexanes) (100-200 mesh), to afford (*R*)-Undec-1-en-4-ol (**11'**) (4.25 g, 64 %) as a colorless oil. TLC: R_f = 0.5 (SiO_2, 10% EtOAc/hexanes); $[\alpha]_D^{25}$ = +7.66 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.87-5.77 (m, 1H), 5.15-5.07 (m, 2H), 3.62 (br. s., 1H), 2.33-2.23 (m, 1H), 2.18-2.07 (m, 1H), 1.69 (s, 1H), 1.44 (t, *J* = 5.6 Hz, 2H), 1.37-1.24 (m, 10H), 0.87 (t, *J* = 7.13 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): 135.1, 118.1, 70.8, 42.1, 36.9, 31.9, 29.7, 29.4, 25.8, 22.8, 14.2.

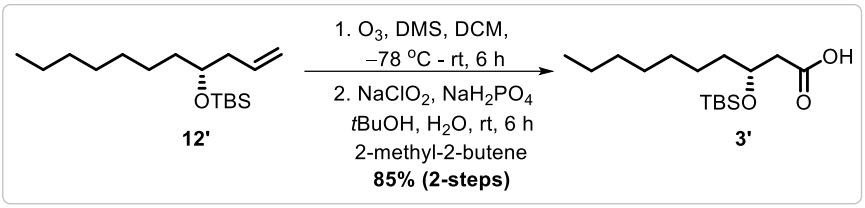
(*R*)-*tert*-Butyldimethyl(undec-1-en-4-yloxy)silane (12'):



A 250 mL, round-bottom flask equipped with a stirring bar was charged (*R*)-Undec-1-en-4-ol (**11'**) (4.0 g, 23.48 mmol) and anhydrous dichloromethane (50 mL) and cool it 0 °C. To the above given solution was added imidazole (1.91 g, 28.18 mmol) in one portion at same temperature. To the above cooled solution, TBSCl (4.2 g, 28.18 mmol) was then added in small portions at 0 °C and then after 10 minutes temperature was increased to room temperature and continued the stirring for another 12 h. After the completion of the reaction, the reaction mixture was diluted with 100 ml of water and then the residue was extracted thrice with dichloromethane. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 100% hexanes) (100-200 mesh), to afford (*R*)-*tert*-Butyldimethyl(undec-1-en-4-yloxy)silane (**12'**) (6.0 g, 90 %) as a colourless oil. TLC: R_f = 0.7 (SiO_2, 10o% hexanes); $[\alpha]_D^{25}$ = +37.98 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.88-5.75 (m, 1H), 5.07-4.98 (m, 2H), 3.68 (quin, *J* = 5.75 Hz, 1H), 2.24-2.09 (m, 2H), 1.47-1.39 (m, 2H), 1.35-1.23 (m, 10H), 0.92-0.86 (m, 12H), 0.05 (s, 6H); ^{13}C NMR (101

MHz, $CDCl_3$): δ 135.7, 116.7, 72.2, 42.1, 37.0, 32.0, 29.9, 29.5, 26.1, 25.5, 22.8, 18.3, 14.3, -4.2, -4.4.

(*R*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoic acid (3'):

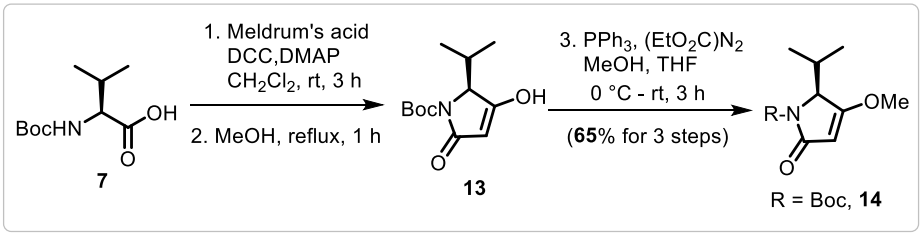


STEP-1: A 250 mL, single necked round-bottomflask equipped with a stirring bar was charged with (*R*)-*tert*-Butyldimethyl(undec-1-en-4-yloxy)silane (**12'**) (6.0 g, 21.08 mmol) and anhydrous dichloromethane (100 mL) and the given clear solution was cooled to −78°C. With the help of ozonizer machine first bubble O_2 gas in to the solution for 5 minutes followed by continuous flow of O_3 until the reaction mixture turns blue. Once the solution becomes blue stop O_3 and replace residual O_3 with N_2 and quenched the reaction mixture with dimethyl sulphide. Slowly shift reaction mixture to ambient temperature and continue stirring for another 3 h. On completion of the reaction dilute the residue with water (100 mL), the residue was extracted thrice with dichloromethane. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated and ultra dried under reduced pressure. The resulting residue (aldehyde) was as it is forwarded for the next step without further purification. TLC: R_f = 0.8 (SiO_2, 5% EtOAc/hexanes). (aldehyde = 5.9 g) as a colourless oil.

STEP-2: In a 250 mL, single necked round-bottomflask equipped with a stirring bar was charged with (*R*)-3-((*tert*-Butyldimethylsilyl)oxy)decanal (5.9 g, 20.58 mmol, 1 equiv.), tertiary butanol (5 mL), 2-methyl-2-butene (15 mL) and stir the given clear solution at ambient temperature. To the above solution was then added aqueous solution of $NaClO_2$ (18.62 g, 205.89 mmol, 10 equiv.) and NaH_2PO_4 (14.73 g, 123.54 mmol, 6 equiv.) and continued the stirring for another 6 h at the same temperature. On completion of the reaction dilute the residue with water (100 mL), the residue was extracted thrice with ethyl acetate. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 4% EtOAc/hexanes) (100-200 mesh), to afford (*R*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoic acid (**3'**) (5.42 g, 85 %) as a colourless oil (over 2 steps); TLC: R_f = 0.4 (SiO_2, 10 % hexanes); $[\alpha]_D^{25}$ = + 0.94 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400

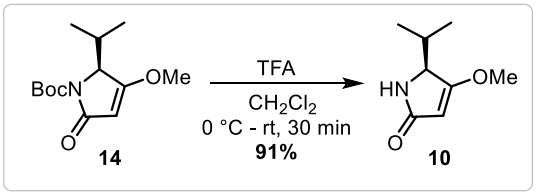
MHz, $CDCl_3$): δ 4.11 (quin, *J* = 6.0 Hz, 1H), 2.50-2.45 (m, 2H), 1.55-1.47 (m, 2H), 1.37-1.24 (m, 11H), 0.91-0.85 (m, 12H), 0.06 (d, *J* = 6.88 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 178.0, 69.5, 42.4, 37.6, 31.9, 29.7, 29.4, 25.9, 25.2, 22.8, 18.1, 14.2, -4.4, -4.7; HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{16}H_{34}O_3NaSiNa$, 325.2169; Found 325.2162.

***Tert*-butyl (*S*)-2-isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate (14):**



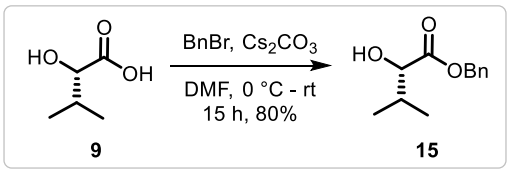
In a 250 mL two-necked round bottom flask, meldrum's acid (2.85 g, 19.8 mmol, 1.0 equiv.), DCC (4.9 g, 23.8 mmol, 1.2 equiv.), and DMAP (4.84 g, 39.6 mmol, 2.0 equiv.) were added to a solution of *N*-Boc-*L*-valine (**7**) (4.3 g, 19.8 mmol, 1.0 equiv.) in dry dichloromethane (44 mL). The mixture was stirred at room temperature for 3 h, and filtered. The filtrate was diluted with dichloromethane, transferred to a separatory funnel, and extracted. The combined organic phases were washed with 0.5 M HCl, H_2O and brine, dried over anhydrous sodium sulphate, and concentrated under reduced pressure on a rotary evaporator. The residual oil was then refluxed in methanol (300 mL) for 1 h. The reaction mixture was concentrated on a rotary evaporator to afford the crude product **13,** which was submitted to the next step without further purification. Crude **13** (4.2 g, 17.4 mmol, 1.0 equiv.) was dissolved in dry tetrahydrofuran (85 mL) and the solution was cooled to 0 °C. Triphenylphosphine (5.93 g, 22.62 mmol, 1.3 equiv.), diethyl diazocarboxylate in toluene (3 mL, 19.14 mmol, 1.1 equiv.), and dry methanol (3.87 mL, 95.7 mmol, 5.5 equiv.) was added at 0 °C. The mixture was stirred for 30 min at 0 °C and 3 h at room temperature. After completion of the reaction, the crude reaction mixture was concentrated under reduced pressure on a rotary evaporator, and roughly purified by column chromatography (using 40% EtOAc/hexanes) (100-200 mesh), to afford tert-butyl (*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate (**14**) (4.5 g, 90 %) over 3 steps as a yellow oil; TLC: R_f = 0.5 (SiO_2, 40% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.05 (s, 1H), 4.35 (br. s., 1H), 3.80 (s, 3H), 2.44 (td, *J* = 7.00, 13.76 Hz, 1H), 1.52 (s, 9H), 1.08 (d, *J* = 7.13 Hz, 3H), 0.78 (d, *J* = 6.88 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 178.0, 169.5, 149.7, 95.1, 82.6, 64.7, 58.4, 29.7, 28.3, 18.8, 15.6; HRMS (ESI) m/z $[M+Na]^+$ calcd for $C_{13}H_{21}NO_4Na$, 278.1354; found 278.1363.

(*S*)-5-Isopropyl-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (10):



In a 250 mL, two necked round-bottom flask charged with stirring bar was added tert-butyl (*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxylate (**14**) (4.5 g, 17.62 mmol, 1 equiv.) and dissolved it in anhydrous dichloromethane under the presence of nitrogen gas and stirred at 0 °C. To the above stirring solution was added TFA (40.19 g, 27.0 mL, 35.25 mmol, 20 equiv.) and after complete addition, the temperature was slowly increased to room temperature and continued for another 30 minutes. After completion of the reaction, the TFA was removed under reduced pressure and purified by column chromatography (5% MeOH/DCM) to afford (*S*)-5-Isopropyl-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (**10**) (2.5 g, 91%) as a white solid; TLC: R_f = 0.2 (SiO_2, 50% EtOAc/hexanes); $[\alpha]_D^{25}$= +8.47 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.52 (br. s., 1H), 5.01 (s, 1H), 3.98 (br. s., 1H), 3.77 (s, 3H), 2.07 (td, *J* = 3.25, 6.75 Hz, 1H), 1.01 (d, *J* = 6.88 Hz, 3H), 0.78 (d, *J* = 6.75 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 177.8, 175.1, 94.4, 62.8, 58.3, 29.4, 19.5, 15.3; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_8H_{14}NO_2$, 156.1019; found 156.1018.

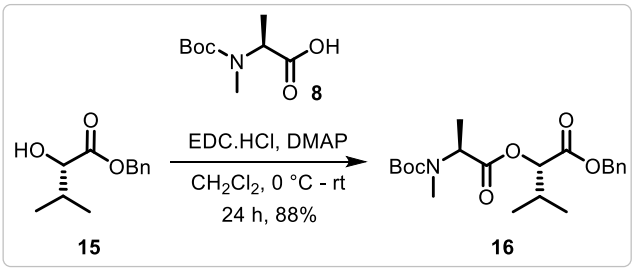
Benzyl (*S*)-2-hydroxy-3-methylbutanoate (15):



To a solution of (*S*)-2-Hydroxy-3-methylbutanoic acid (3.22 g, 27.3 mmol, 1 equiv.) in anhydrous *N*,*N*-dimethylformamide (20 mL) at 0 °C was added caesium carbonate (4.45 g, 13.7 mmol, 0.5 equiv.) and the resulting mixture was stirred for further 40 minutes at the same temperature. After that benzyl bromide (3.6 mL, 30.0 mmol, 1.1 equiv.) was added with a syringe to the above reaction mixture and then the stirring was further continued for another 15 h at room temperature. The reaction mixture was filtered and the filtrate was diluted with Hexane/ethyl acetate (4:1, 120 mL). The organic layer was washed with NH_4Cl solution (100 mL), saturated $NaHCO_3$ solution (100 mL) and saturated NaCl solution (100 mL) before being dried over sodium sulphate and the solvent being removed under reduced pressure. The

resulting residue was purified by silica gel column chromatography (using 4% EtOAc/hexanes) (100-200 mesh), to afford Benzyl (*S*)-2-Hydroxy-3-methylbutanoate (**15**) (4.56 g, 21.9 mmol, 80%) as a colourless oil; TLC: R_f = 0.6 (SiO_2, 10% EtOAc/hexanes); $[\alpha]_D^{25}$= –11.76 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.37 (br. s., 5H), 5.28-5.17 (m, 2H), 4.12-4.06 (m, 1H), 2.83 (d, *J* = 5.63 Hz, 1H), 2.16-2.05 (m, 1H), 1.02 (d, *J* = 6.88 Hz, 3H), 0.84 (d, *J* = 6.88 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 174.9, 135.3, 128.7, 128.6, 128.5, 75.1, 67.3, 32.3, 18.9, 16.0; HRMS (ESI) m/z $[M+Na]^+$ calcd for $C_{12}H_{16}O_3Na$, 231.0992; found 231.0990.

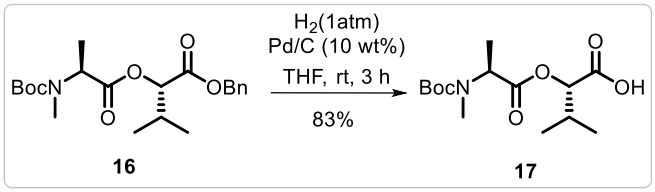
Benzyl (*S*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoate (16):



A 250 mL, two-necked round-bottom flask equipped with a stirring bar was charged with benzyl (*S*)-2-hydroxy-3-methyl butanoate (**15**) (4.56 g, 21.8 mmol, 1 equiv.), *N*-(*tert*-Butoxycarbonyl)-*N*-methyl-*L*-alanine (**8**) (6.23 g, 30.6 mmol, 1.4 equiv.), 4-DMAP (3.47 g, 28.4 mmol, 1.3 equiv.) and anhydrous dichloromethane (100 mL) and the given clear solution was cooled to 0 °C. To this above cooled clear solution was added slowly in portions EDC.HCl (6.29 g, 32.8 mmol, 1.5 equiv.) and then the stirring was continued at room temperature for another 24 h. After completion of the reaction, the crude reaction mixture was diluted with aqueous HCl (25 mL, 1M), and extracted with DCM (3×40 mL). The combined organic layers were washed with water, aqueous saturated $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 3% EtOAc/hexanes) (100-200 mesh), to afford Benzyl (*S*)-2-((*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoate (**16**) (7.58 g, 88%) as a colourless oil; TLC: R_f = 0.6 (SiO_2, 10 % hexanes); $[\alpha]_D^{25}$ = –41.18 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) (Mixture of rotamers) δ: 7.35 (br. s., 5H), 5.23 (d, *J* = 12.13 Hz, 1H), 5.12 (d, *J* = 11.63 Hz, 1H), 4.88 (br. s., 1.54H), 4.68-4.59 (d, *J* = 5.75 Hz, 0.47H), 2.89-2.76 (d, *J* = 26.14 Hz, 3H), 2.32-2.20 (m, 1H), 1.48-1.40 (m, 12H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.94 (d, *J* = 6.38 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 172.2, 169.4, 156.0, 155.3, 135.4, 128.7, 128.6, 128.5, 80.5, 80.1, 67.0, 54.8, 53.5, 31.0, 30.7, 30.2, 28.5,

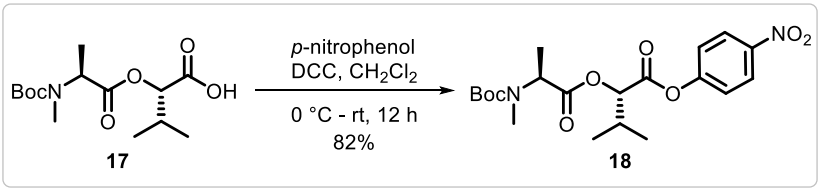
19.0, 17.2, 15.2, 14.8; HRMS (ESI) m/z $[M+Na]^+$ calcd for $C_{21}H_{31}NO_6Na$, 416.2044; found 416.2036.

(S)-2-((*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoic acid (17):



A 250 mL, single-necked round-bottom flask equipped with a stirring bar was charged with Benzyl (*S*)-2-((N-(*tert*-Butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoate (**16**) (7.58 g, 19.2 mmol, 1 equiv.) and anhydrous THF (80 mL). To this above clear solution was added 10% palladium on carbon (Pd/C) (~1.76 g) and then the reaction flask was purged with hydrogen gas for 20 minutes and further stirred at room temperature under a hydrogen atmosphere (1 atm.) for an additional 3 hr. After completion of the reaction, the crude reaction mixture was filtered through celite, washed with ethyl acetate (80 mL), and concentrated under reduced pressure, to afford (*S*)-2-((*N*-(*tert*-Butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoic acid (**17**) (4.9 g, 83%) as a colourless oil; TLC: R_f = 0.3 (SiO_2, 40 % EtOAc/hexane). The resulting residue was carried forward without further purification.

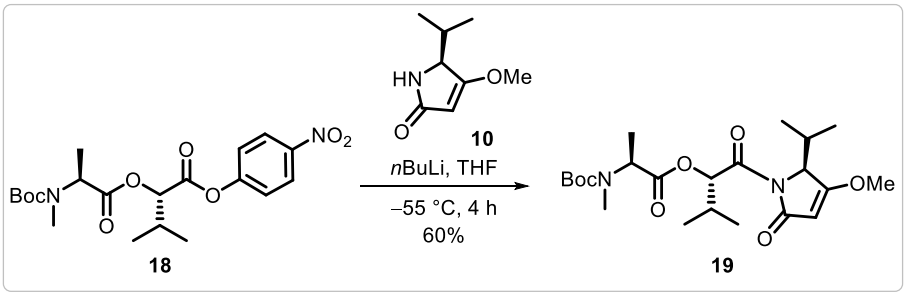
4-Nitrophenyl (*S*)-2-((*N*-(tert-butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoate (18):



A 250 mL, two necked round-bottom flask equipped with stirring bar was added (*S*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoic acid (**17**) (4.9 g, 16.1 mmol, 1 equiv.) and dissolved it in anhydrous dichloromethane under the presence of nitrogen gas and stirred at 0 °C. To this above stirring, solution was added *p*-nitrophenol (2.47 g, 17.7 mmol, 1.1 equiv.) followed by small portion-wise addition of DCC (6.6 g, 32.3 mmol, 2 equiv.). After complete addition, the temperature was slowly increased to room

temperature and continued for another 12 h. After completion of the reaction, the reaction mixture was filtered through celite, transferred to a separatory funnel and extracted with dichloromethane. The combined organic layers were washed with water, aqueous saturated $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 12% EtOAc/hexanes) (100-200 mesh), to afford 4-Nitrophenyl (*S*)-2-((*N*-(tert-butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoate (18) (5.6 g, 82%) as a colourless oil; TLC: R_f = 0.6 (SiO_2, 15 % hexanes); in $[\alpha]_D^{25}$ = –43.14 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$):(Mixture of rotamers) δ 8.26 (d, *J* = 8.25 Hz, 2H), 7.27 (d, *J* = 8.76 Hz, 2H), 4.96 (br. s., 1H), 4.87 (d, *J* = 6.13 Hz, 0.5H), 4.6 (d, J = 5.13 Hz, 0.5H), 2.86 (d, *J* = 20.14 Hz, 3H), 2.41-2.38 (m, 1H), 1.49-1.39 (m, 12H), 1.10 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.5, 167.6, 155.0, 145.8, 125.4, 122.5, 80.6, 80.4, 54.9, 53.8, 31.2, 31.1, 30.3, 28.5, 18.9, 17.5, 15.3, 14.8; HRMS (ESI) m/z [M+H]+ calcd for $C_{20}H_{29}N_2O_8$, 425.1918; found 425.1906.

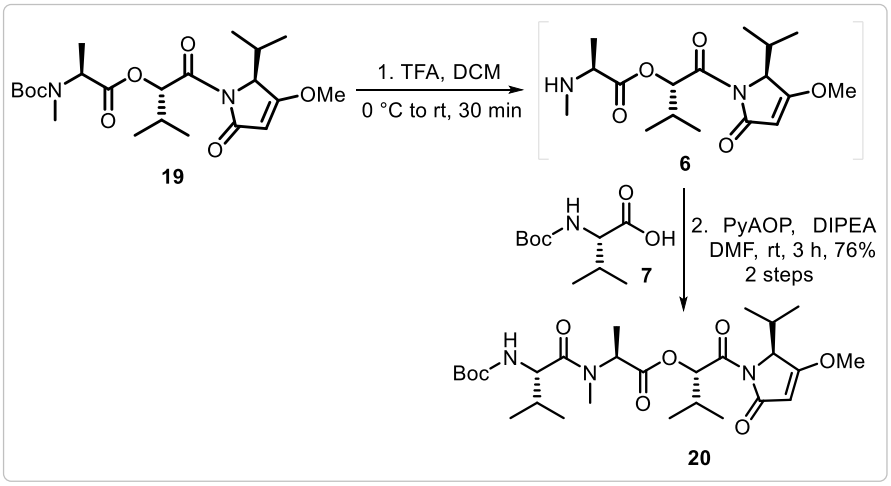
(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(tert-butoxycarbonyl)-*N*-methyl-*L*-alaninate (19):



In a 250 mL, two-necked round-bottom flask charged with a stirring bar was added *(S)*-5-Isopropyl-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (**10**) (1.7 g, 10.95 mmol, 1 equiv.) and dissolved it in anhydrous tetrahydrofuran under the presence of nitrogen gas and cooled it to –55 °C. To this above stirring solution was added slowly dropwise n-BuLi (10.2 mL, 1.6 M in hexanes, 16.4 mmol, 1.5 equiv.) over a time of 10 minutes, and continued the stirring for further 15 minutes. After 15 minutes of stirring to this resulting mixture was added a THF solution (10 mL) of 4-Nitrophenyl(*S*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoate (**18**) (5.56 g, 13.1 mmol, 1.2 equiv.) dropwise with a syringe over a time of 10 minutes and then continued the stirring for another 5 h. After completion of the reaction, the reaction mixture was quenched with an aqueous saturated NH_4Cl solution and the residue

was extracted with ethyl acetate (3×40 mL). The combined organic layers were washed with water, and brine solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 21% EtOAc/hexanes) (100-200 mesh), to afford (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl N-(tert-butoxycarbonyl)-*N*-methyl-*L*-alaninate (**19**) (2.9 g, 60%) as a colourless oil; TLC: R_f = 0.5 (SiO_2, 20% EtOAc/hexanes); in $[\alpha]_D^{25}$ = –2.4 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): (Mixture of rotamers) δ 5.80 (d, *J* = 3.25 Hz, 1H), 5.07 (s, 1H), 4.50 (d, *J* = 2.75 Hz, 1H), 3.84 (s, 3H), 2.89-2.75 (d, *J* = 30.14 Hz, 3H), 2.65-2.56 (m, 1H), 2.26-2.18 (m, 1H), 1.45 (s, 9H), 1.42 (d, *J* = 7.25 Hz, 3H), 1.08 (d, *J* = 7.25 Hz, 3H), 1.06 (d, *J* = 6.88 Hz, 3H), 0.93 (d, *J* = 6.75 Hz, 3H), 0.78 (d, *J* = 6.88 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 180.0, 172.1, 169.2, 94.7, 78.1, 4.4, 58.6, 29.0, 28.5, 19.9, 18.8, 16.1, 15.3; HRMS (ESI) m/z [M+Na] $^+$ calcd for $C_{22}H_{36}N_2O_7Na$, 463.2415; found 463.2401.

(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-((tert-butoxycarbonyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (20):

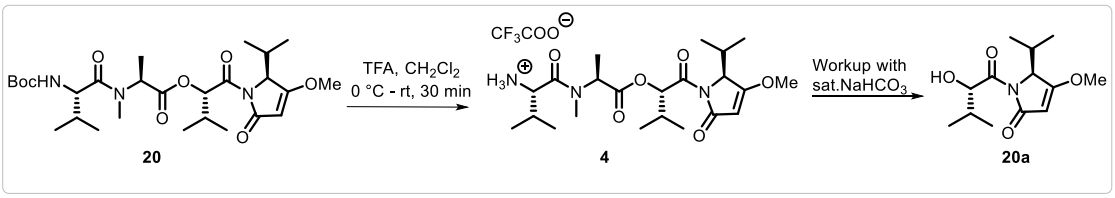


In a 100 mL, two necked round-bottom flask charged with stirring bar was added (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-alaninate (**19**) (2.9 g, 6.58 mmol, 1 equiv.) and dissolved it in anhydrous dichloromethane under the presence of nitrogen gas and stirred at 0 °C. To this above stirring solution was added TFA (7.5 g, 5.3 mL, 65.8 mmol, 15 equiv.) and after complete addition, the temperature was slowly increased to room temperature and continued for another 30 minutes. After completion of the reaction, the TFA was removed under reduced pressure to give crude (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-

1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl methyl-*L*-alaninate (**6**) which was submitted to the next step without work up and further purification.

In a 100 mL, two necked round-bottom flask charged with stirring bar was added *N*-Boc-*L*-valine (**7**) (1.85 g, 8.5 mmol, 1 equiv.), DIPEA (5.9 mL, 34.0 mmol, 4 equiv.), PyAOP (5.3 g, 10.2 mmol, 1.2 equiv.) and dissolved in anhydrous DMF (5.0 mL). The resultant mixture was stirred at rt for 2 minutes before the solution of crude (*S*)-1-((*S*)-2-isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl methyl—alaninate (**6**) (2.9 g, 8.5 mmol, 1 equiv.) in DCM (20 mL) was added dropwise with a syringe. The reaction mixture was allowed to stir at room temperature for 3 h. After completion of the reaction, crude reaction mixture was quenched with aqueous saturated $NaHCO_3$ solution, the residue was extracted with DCM (3×40 mL). The combined organic layers were washed with water, brine solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 40% EtOAc/hexanes) (100-200 mesh), to afford (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-*1H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl N-((*tert*-butoxycarbonyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (**20**) (2.7 g, 76%) as a white foam; TLC: R_f = 0.5 (SiO_2, 40% EtOAc/hexanes); in $[\alpha]_D^{25}$ = –6.66 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.80 (d, *J* = 3.13 Hz, 1H), 5.31-5.24 (m, 2H), 5.06 (s, 1H), 4.49 (d, *J* = 2.63 Hz, 1H), 3.84 (s, 3H), 3.02 (s, 3H), 2.62-2.53 (m, 1H), 2.27-2.17 (m, 1H), 2.04-1.94 (m, 1H), 1.45-1.40 (m, 12H), 1.08 (d, *J* = 7.25 Hz, 3H), 1.04 (d, *J* = 6.88 Hz, 3H), 0.99 (d, *J* = 6.88 Hz, 3H), 0.91 (d, *J* = 7.0 Hz, 3H), 0.88 (d, *J* = 6.75 Hz, 3H), 0.77 (d, *J* = 6.88 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 180.0, 172.6, 171.3, 170.1, 169.2, 156.0, 94.7, 79.5, 78.3, 64.4, 58.7, 55.2, 52.7, 31.9, 31.5, 28.9, 28.5, 19.8, 19.7, 18.8, 17.2, 16.1, 15.3, 14.2; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{27}H_{46}N_3O_8$, 540.3279; found 540.3278.

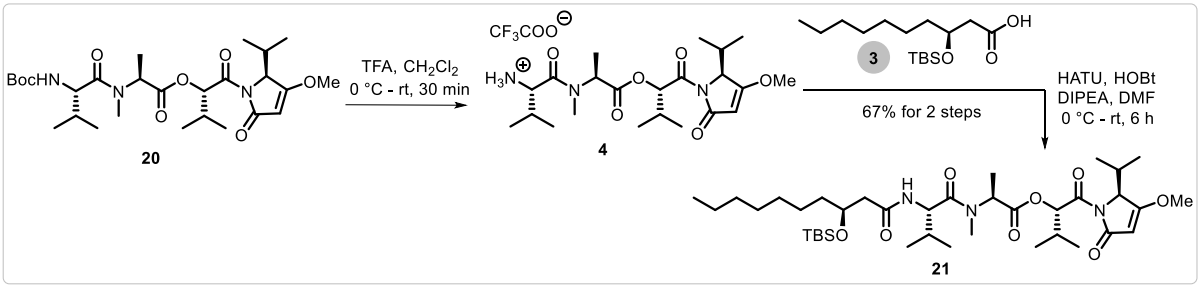
(*S*)-1-((*S*)-2-hydroxy-3-methylbutanoyl)-5-isopropyl-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (20a):



In a 25 mL, two necked round-bottom flask charged with stirring bar was added (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-

((tert-butoxycarbonyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (**20**) (0.2 g, 0.37 mmol, 1 equiv.) and dissolved it in anhydrous dichloromethane under the presence of nitrogen gas and stirred at 0 °C. To this above stirring solution was added TFA (1.0 mL) and after complete addition, the reaction mixture was stirred for another 30 minutes at room temperature. After completion of the reaction, the TFA was removed under reduced pressure to give crude TFA salt of (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(*L*-valyl)-*N*-methyl-*L*-alaninate (**4**) which was very unstable and after basic workup with $NaHCO_3$ afforded (*S*)-1-((*S*)-2-hydroxy-3-methylbutanoyl)-5-isopropyl-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (**20a**); TLC: R_f = 0.6 (SiO_2, 40% EtOAc/hexanes); in $[\alpha]_D^{25}$ = –20.4 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.09 (s, 1H), 4.74 (dd, *J* = 3.88, 8.25 Hz, 1H), 4.53 (d, *J* = 2.63 Hz, 1H), 3.86 (s, 3H), 3.77 (d, *J* = 8.5 Hz, 1H), 2.71-2.63 (m, 1H), 2.15-2.06 (m, 1H), 1.12 (d, *J* = 7.13 Hz, 3H), 1.05 (d, *J* = 6.88 Hz, 3H), 0.85 (d, *J* = 6.88 Hz, 3H), 0.74 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 180.4, 173.9, 170.8, 94.6, 76.2, 64.6, 58.8, 30.6, 28.8, 20.0, 18.9, 15.7, 15.4; HRMS (ESI) m/z $[M+Na]^+$ calcd for $C_{13}H_{20}NO_3$, 238.1438; found 238.1436.

(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*S*)-3-((*tert*-butyldimethylsilyl)oxy)decanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (21):

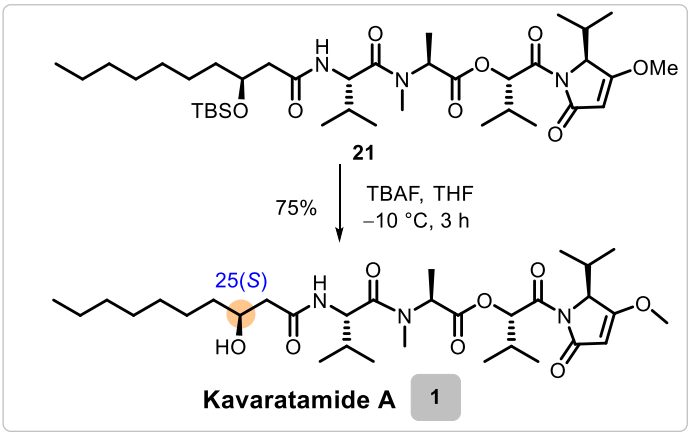


In a 25 mL, two necked round-bottom flask charged with stirring bar was added (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-((tert-butoxycarbonyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (**20**) (0.2 g, 0.37 mmol, 1 equiv.) and dissolved it in anhydrous dichloromethane under the presence of nitrogen gas and stirred at 0 °C. To this above stirring solution was added TFA (1.0 mL) and after complete addition, the reaction mixture was stirred for another 30 minutes at room temperature. After completion of the reaction, the TFA was removed under reduced pressure to give crude TFA salt of (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-

(*L*-valyl)-*N*-methyl-*L*-alaninate (**4**) which was very unstable and hence it was submitted to the next step without work up and further purification.

In a 25 mL, two necked round-bottom flask charged with a stirring bar were added (*S*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoic acid **(3)** (0.1 g, 0.33 mmol, 1 equiv.), HATU (0.15 g, 0.39 mmol, 1.2 equiv.), HOBt (0.53 g, 0.39 mmol, 1.2 equiv.), DIPEA (0.256 g, 0.34 mL, 1.9 mmol, 5 equiv.) in anhydrous DMF (1.0 mL). The resultant mixture was cooled to 0 °C and stirred for 10 minutes before the solution of crude TFA salt of (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(*L*-valyl)-*N*-methyl-*L*-alaninate (**4**) (0.174 g, 0.39 mmol, 1.2 equiv.) in DCM (4.0 mL) was added dropwise with a syringe. The reaction mixture was allowed to stir at room temperature for 6 h. After completion of the reaction, the crude reaction mixture was diluted with water, and aqueous saturated NH_4Cl solution and the residue was extracted with DCM (3×10 mL). The combined organic layers were washed with water, and brine solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 40% EtOAc/hexanes) (100-200 mesh), to afford (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*S*)-3-((*tert*-butyldimethylsilyl)oxy)decanoyl)-L-valyl)-*N*-methyl-*L*-alaninate (**21**) (0.18 g, 67%) as a colourless oil; TLC: R_f = 0.6 (SiO_2, 40% EtOAc/hexanes); in $[\alpha]_D^{25}$ = –7.78 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.68 (d, *J* = 8.76 Hz, 1H), 5.81 (d, *J* = 3.35 Hz, 1H), 5.39 (q, *J* = 7.25 Hz, 1H), 5.06 (s, 1H), 4.84 (dd, *J* = 6.25, 8.76 Hz, 1H), 4.49 (d, *J* = 2.75 Hz, 1H), 4.06 (quin, *J* = 5.75 Hz, 1H), 3.84 (s, 3H), 3.02 (s, 3H), 2.58 (dtd, *J* = 2.75, 7.0, 14.13 Hz, 1H), 2.41 (dd, *J* = 5.0, 14.51 Hz, 1H), 2.32 (dd, *J* = 5.63, 14.51 Hz, 1H), 2.26-2.16 (m, 1H), 2.03 (qd, *J* = 6.75, 13.38 Hz, 1H), 1.55-1.47 (m, 2H), 1.42 (d, *J* = 7.38 Hz, 3H), 1.30-1.22 (m, 10H), 1.08 (d, *J* = 7.25 Hz, 3H), 1.04 (d, *J* = 6.88 Hz, 3H), 0.99 (d, *J* = 6.75 Hz, 3H), 0.92 (dd, *J* = 1.63, 6.63 Hz, 6H), 0.89-0.85 (m, 12H), 0.77 (d, *J* = 6.88 Hz, 3H), 0.03 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ ^{13}C NMR (101MHz ,CHLOROFORM-d) d = 180.0, 172.2, 171.3, 170.9, 170.1, 169.2, 94.4417, 78.4, 69.9, 64.4, 58.7, 53.6, 52.1, 44.3, 37.0, 31.9, 31.8, 31.6, 29.8, 29.3, 28.9, 28.5, 26.0, 25.6, 22.8, 19.8, 19.7, 18.8, 18.2, 17.9, 16.1, 15.3, 14.3, 14.2, -4.6, -4.6; HRMS (ESI) m/z $[M+Na]^+$ calcd for $C_{38}H_{69}N_3O_8SiNa$, 746.4746; found 746.4737.

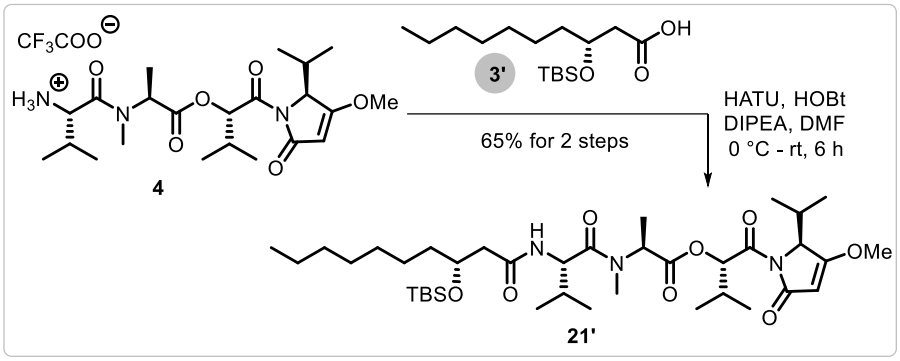
(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*S*)-3-hydroxydecanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (1) (Kavaratamide A):



In a 10 mL, two necked round-bottom flask charged with stirring bar was added (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*S*)-3-((*tert*-butyldimethylsilyl)oxy)decanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (**21**) (0.1 g, 0.13 mmol, 1 equiv.) in anhydrous tetrahydrofuran (1.0 mL) and the solution was cooled to -10 °C. At this temperature, TBAF (0.41 mL, 0.41 mmol, 1 M in THF, 3 equiv.) was added with a syringe and continued to stir for another 3 h at the same temperature. After completion of the reaction, the crude reaction mixture was diluted with water, aqueous saturated NH_4Cl solution, and the residue was extracted ethyl acetate (3×10 mL). The combined organic layers were washed with water, and brine solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 60% EtOAc/hexanes) (100-200 mesh), to afford Kavaratamide A (**1**) (0.063 g, 75%) as a colourless oil; TLC: R_f = 0.4 (SiO_2, 100% EtOAc); $[\alpha]_D^{25}$ = –25.46 (*c* = 1.0 in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.59 (d, *J* = 8.8 Hz, 1H), 5.80 (d, *J* = 3.3 Hz, 1H), 5.27 (q, *J* = 7.34 Hz, 1H), 5.07 (s, 1H), 4.82 (dd, *J* = 5.75, 8.8 Hz, 1H), 4.48 (d, *J* = 2.69 Hz, 1H), 3.94 (br. s., 1H), 3.84 (s, 3H), 3.03 (s, 3H), 2.57 (dtd, *J* = 2.69, 6.97, 14.06 Hz, 1H), 2.37 (dd, *J* = 2.69, 15.04 Hz, 1H), 2.31-2.24 (m, 1H), 2.24-2.17 (m, 1H), 2.10-1.99 (m, 1H), 1.56-1.47 (m, 1H), 1.44 (d, *J* = 7.21 Hz, 3H), 1.42-1.37 (m, 1H), 1.26 (m, 10H), 1.07 (d, *J* = 7.21 Hz, 3H), 1.03 (d, *J* = 6.85 Hz, 3H), 0.99 (d, *J* = 6.72 Hz, 3H), 0.91 (d, *J* = 6.85 Hz, 3H), 0.89 (d, *J* = 6.72 Hz, 3H), 0.86 (t, *J* = 6.97, 3H), 0.76 (d, *J* = 6.85 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 180.0, 172.9, 172.3, 171.1, 170.2, 169.1, 94.7, 78.4, 68.9, 64.3, 58.7, 53.9, 52.7, 42.9, 37.1, 31.9, 31.9, 31.2, 29.6, 29.4, 28.9, 28.4, 25.6, 22.8, 19.8, 19.7, 18.8, 17.5, 16.1, 15.3, 14.2, 14.2; IR (cm^{-1}): 3314, 2925, 2860, 1728, 1624, 1530, 1457, 1381, 1314, 1250, 1190, 1124,

1083, 996, 934, 813, 744, 644; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{32}H_{56}N_3O_8$, 610.4062; found 610.4055 and HRMS (ESI) *m/z* $[M+Na]^+$ calcd for $C_{32}H_{56}N_3O_8Na$, 632.3881; found 632.6866.

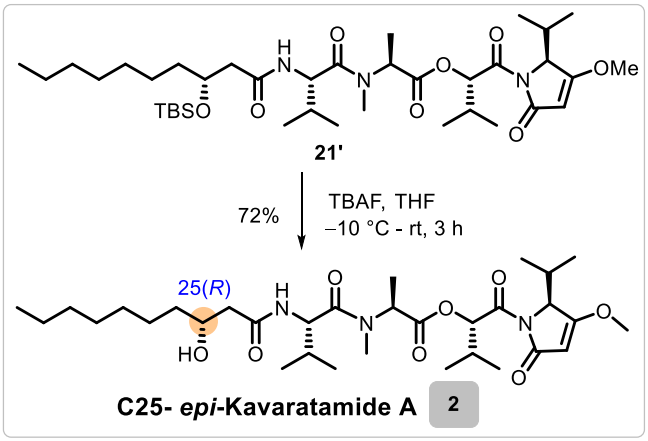
(*S*)-1-((*S*)-2-isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*R*)-3-((tert-butyldimethylsilyl)oxy)decanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (21'):



In a 25 mL, two necked round-bottom flask charged with a stirring bar were added (*R*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoic acid (**3'**) (0.1 g, 0.33 mmol, 1 equiv.), HATU (0.15 g, 0.39 mmol, 1.2 equiv.), HOBt (0.53 g, 0.39 mmol, 1.2 equiv.), DIPEA (0.256 g, 0.34 mL, 1.9 mmol, 5 equiv.) in anhydrous DMF (1.0 mL). The resultant mixture was cooled to 0 °C and stirred for 10 minutes before the solution of crude TFA salt of (*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(*L*-valyl)-*N*-methyl-*L*-alaninate (**4**) (0.174 g, 0.39 mmol, 1.2 equiv.) in DCM (2.0 mL) was added dropwise with a syringe. The reaction mixture was allowed to stir at room temperature for 6 h. After completion of the reaction, crude reaction mixture was diluted with water, quenched with aqueous saturated NH_4Cl solution, the residue was extracted with ethyl acetate (3×40 mL). The combined organic layers were washed with water, brine solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 15% EtOAc/hexanes) (100-200 mesh), to afford(*S*)-1-((*S*)-2-isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*R*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate: (**21'**) (0.175 g, 65%) as a colourless oil; TLC: R_f = 0.6 (SiO_2, 40% EtOAc/hexanes); $[\alpha]_D^{21}$ = –7.3 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.73 (d, *J* = 9.01 Hz, 1H), 5.81 (d, *J* = 3.25 Hz, 1H), 5.41 (q, *J* = 7.25 Hz, 1H), 5.06 (s, 1H), 4.78 (dd, *J* = 7.0, 9.01 Hz, 1H), 4.49 (d, *J* = 2.63 Hz, 1H), 4.04 (quin, *J* = 5.63 Hz, 1H), 3.84 (s, 3H), 3.03 (s, 3H), 2.63-2.52 (m, 1H), 2.47-2.39 (m, 1H), 2.37-2.28 (m, 1H), 2.28-2.15 (m, 1H), 2.07-1.97 (m, 1H), 1.51-1.44 (m,

2H), 1.41 (d, *J* = 7.25 Hz, 3H), 1.30-1.23 (m, 10H), 1.08 (d, *J* = 7.25 Hz, 3H), 1.04 (d, *J* = 6.88 Hz, 3H), 0.98 (d, *J* = 6.75 Hz, 3H), 0.92-0.86 (m, 18H), 0.77 (d, *J* = 6.88 Hz, 3H), 0.07 (d, *J* = 4.38 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 180.0, 172.3, 171.3, 171.1, 170.1, 169.1, 94.7, 78.4, 69.8, 64.4, 58.7, 53.8, 52.1, 44.0, 36.8, 31.9, 31.6, 31.4, 29.7, 29.3, 28.9, 28.5, 26.0, 25.7, 22.8, 19.8, 19.6, 18.8, 18.1, 18.1, 16.1, 15.3, 14.4, 14.2, -4.5, -4.5; HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{38}H_{70}N_3O_8Si$, 724.4927; found 724.4920.

(*S*)-1-((*S*)-2-isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*R*)-3-hydroxydecanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (2) (C25-*epi*-Kavaratamoide A):

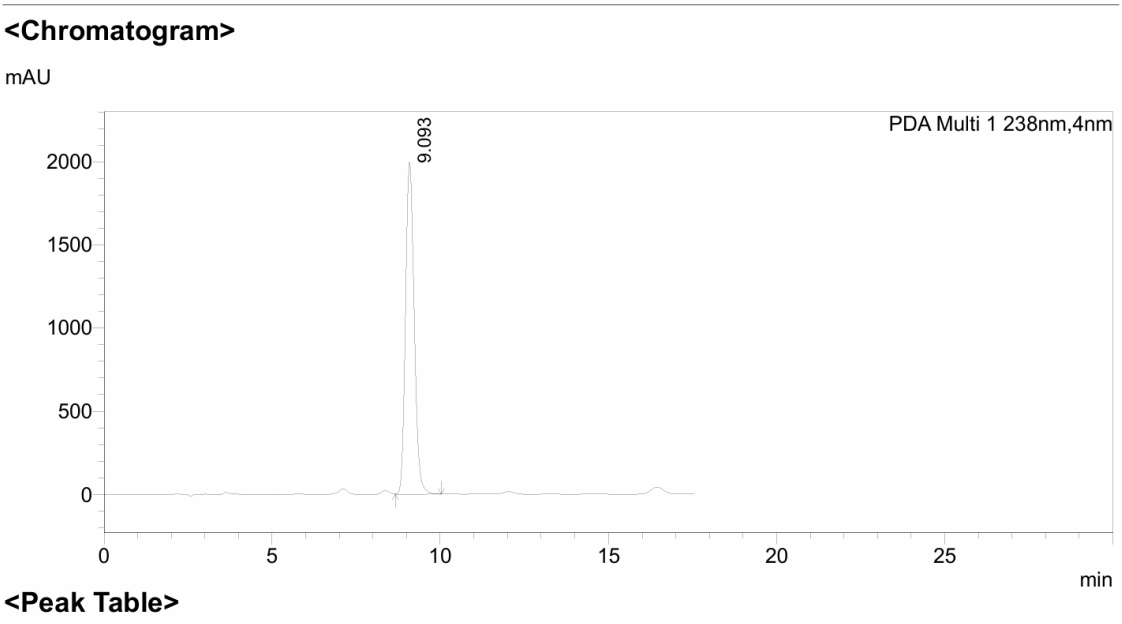


In a 50 mL, two necked round-bottom flask charged with stirring bar was added (*S*)-1-((*S*)-2-isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*R*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (**21'**) (0.1 g, 0.13 mmol, 1 equiv.), it dissolved in anhydrous tetrahydrofuran (1.0 mL) and the resultant solution was cooled to -10 °C. At this temperature TBAF (0.41 mL, 0.41 mmol, 3 equiv.) (1 M in THF) with a syringe and continued the stirring for another 3 h at the same temperature. After completion of the reaction, the crude reaction mixture was diluted with water, and quenched with aqueous saturated NH_4Cl solution. The residue was extracted with ethyl acetate (3×10 mL). The combined organic layers were washed with water, and brine solution, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (using 60% EtOAc/hexanes) (100-200 mesh), to afford C25-*epi*-Kavaratamide (**2**) (0.061 g, 72%) as a colourless oil; TLC: R_f = 0.4 (SiO_2, 100% EtOAc); in $[\alpha]_D^{25}$ = –30.74 (*c* = 1.0 in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.53 (d, *J* = 8.63 Hz, 1H), 5.81 (br. s., 1H), 5.25 (q, *J* = 7.25 Hz, 1H), 5.07 (s, 1H), 4.90-4.82 (m, 1H), 4.49 (br. s., 1H), 3.94 (br. s., 1H), 3.84 (s, 3H), 3.76 (br. s., 1H), 3.04 (s, 3H), 2.63-2.52

(m, 1H), 2.44-2.36 (m, 1H), 2.36-2.28 (m, 1H), 2.25-2.16 (m, 1H), 2.04 (dd, *J* = 6.1, 12.4 Hz, 1H), 1.56-1.48 (m, 1H), 1.44 (d, *J* = 7.13 Hz, 3H), 1.42-1.39 (m, 1H), 1.27 (br. s., 10H), 1.08 (d, *J* = 7.0 Hz, 3H), 1.04 (d, *J* = 6.38 Hz, 3H), 0.99 (d, *J* = 6.38 Hz, 3H), 0.92-0.85 (m, 9H), 0.77 (d, *J* = 6.63 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 180.0, 172.6, 172.1, 171.1, 170.2, 169.1, 94.7, 78.4, 68.7, 64.4, 58.7, 53.6, 52.8, 42.4, 36.9, 32.0, 31.9, 31.5, 29.7, 29.4, 28.9, 28.5, 25.7, 22.8, 19.8, 19.7, 18.8, 17.5, 16.1, 15.3, 14.2, 14.2; IR (cm^{-1}): 3423, 3313, 2962, 2928, 2874, 2855, 1726, 1695, 1619, 1532, 1463, 1385, 1339, 1317, 1298, 1248, 1202, 1184, 1121, 1088, 993, 938, 808, 750, 718, 644; HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{32}H_{56}N_3O_8$, 610.4062; found 610.4045

3. HPLC data:

Kavaratamide A (1):



<Peak Table>

PDA Ch1 238nm

Ret. Time	Area	Area%	Height%
9.093	35832322	100.000	100.000
	35832322		

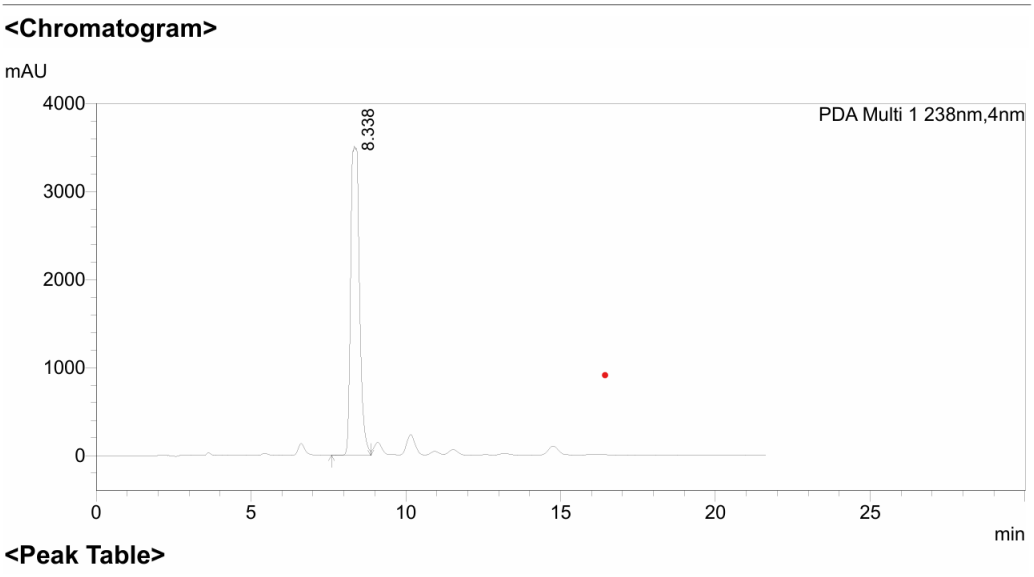
Column: Kromasil C18 column (150 × 4.6 mm, 5 µm particle size)

UV: 238 nm,

Mobile phase: with 78% MeCN in H_2O containing 0.1% formic acid

Flow rate: 0.6 mL/min

C25-*epi*-Kavaratamide A (2):



<Peak Table>

PDA Ch1 238nm

Ret. Time	Area	Area%	Height%
8.338	69868512	100.000	100.000
	69868512		

Column: Kromasil C18 column (150 × 4.6 mm, 5 µm particle size)

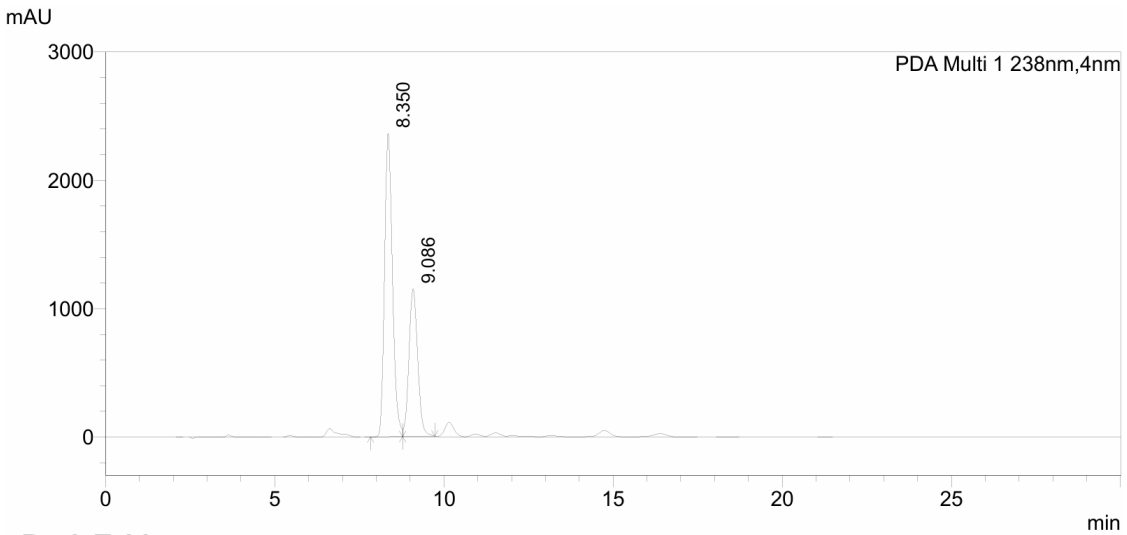
UV: 238 nm,

Mobile phase: with 78% MeCN in H_2O containing 0.1% formic acid

Flow rate: 0.6 mL/min

Co-injection of 1 and 2 (2:1 = 6.5:3.5):

<Chromatogram>



<Peak Table>

PDA Ch1 238nm

Ret. Time	Area	Area%	Height%
8.350	38314983	65.025	67.262
9.086	20608779	34.975	32.738
	58923762		

Column: Kromasil C18 column (150 × 4.6 mm, 5 µm particle size)

UV: 238 nm,

Mobile phase: with 78% MeCN in H_2O containing 0.1% formic acid

Flow rate: 0.6 mL/min

	ASTER ANALYTICS RESEARCH INSTITUTE, PUNE	
CELL LINE STUDY		
Report No.	AARI/IVCL/01/2024-25	
Version No.	01	**RESTRICTED DOCUMENTS**
Cell Line Study		

4. Report On

"Cytotoxicity study of C25 Epi-Kavaratamide A and Kavaratamide A in HepG2 Cell Line"

REPORT NUMBER

AARI/IVCL/01/2024-25

PREPARED & APPROVED BY

ASTER ANALYTICS RESEARCH INSTITUTE

227, SRP ROAD, PANDAVNAGAR,WADACHI WADI, PUNE-411060

SPONSORED BY

DR. RAVINDAR KONTHAM

PRINCIPAL SCIENTIST

CSIR-NATIONAL CHEMICAL LABORATORY

DATE OF ISSUE OF REPORT: 02.01.2025

DATE OF RELEASE OF REPORT: 02.01.2025

This report contains 24 pages.

	ASTER ANALYTICS RESEARCH INSTITUTE, PUNE	
CELL LINE STUDY		
Report No.	AARI/IVCL/01/2024-25	
Version No.	01	**RESTRICTED DOCUMENTS**
Cell Line Study		

CONTENTS

Sr. No	Description	Page Number
1.	Study Details	S26
2.	Compliance statement	S27
3.	Quality Assurance Statement	S28
4.	Document Control	S29
5.	Objective	S29
6.	Study Guidelines	S29
7.	Study Period	S29
8.	Materials	S29
9.	Experimental Procedures	S29
10.	MTT Assay	S30
11.	Observation and Results	S31
12.	Data Analysis and Report Preparation	S47
13.	Archives	S47
14.	Study Plan Amendment and Deviations	S47

STUDY DETAILS

Report Number	:	AARI/IVCL/01/2024-25
Study Title	:	Cytotoxicity study of C25 Epi-Kavaratamide A and Kavaratamide A in HepG2 Cell Line
Sponsor	:	Dr. Ravindar Kontham Principal Scientist CSIR-National Chemical Laboratory
Test Facility	:	Aster Analytics Research Institute 227, SRP Road, Pandavnagar, Wadachi Wadi, Pune-411060
Study Director	:	Dr. Amit Kasabe
Study personal		Ms. Karishma Markad
Period	:	30.12.2024 to 02.01.2025

COMPLIANCE STATEMENT

I, the Undersigned hereby state and declare that this, “Report No. AARI/IVCL/01/2024-25" was performed under my supervision in compliance with the ICH guidelines.

Characterization of the “Test Items/Samples” was performed by the sponsor. Test Laboratory is responsible for validity of the test procedure, interpretation, analysis, documentation and test reports.

Dr. Amit Kasabe
Director

Date: 02.01.2025

QUALITY ASSURANCE STATEMENT

This study report has been reviewed by the Quality Assurance department of **Aster Analytics Research Institute** for Study plan, Raw Data and Results.

Ms. Karishma Markad
Research Associate

Date: 02.01.2025

1. Document Control

Aster Analytics Research Institute, Pune

2. Objective

To determine the cytotoxicity of C25 Epi-Kavaratamide A *and* Kavaratamide A in the HepG2cell line using the MTT assay method.

3. Study Guidelines

The design and scope of the study are based on consideration of the study objectives.

Study Period

Sample Receiving Date	:	28/Dec/2024
Experiment Start Date	:	30/Dec/2024
Experiment Completion Date	:	02/Dec/2024
Study Completion Date	:	02/Dec/2024
Draft Report Date	:	02/Dec/2024
Final Report Date	:	02/Dec/2024

4. Materials

Test Item Details

Sr. No.	Name	Storage Conditions	Handling Precautions
1.	C25 Epi-Kavaratamide A	2-8˚C	Standard Laboratory Procedure
2.	Kavaratamide A		

**All data relating to the identity, purity and stability of the test materials are the responsibility of the sponsor and have not been verified by the test facility.*

5. Experimental Procedures

a. Preparation of Test Material

All Test Samples were filter sterilized using 0.22µ filters and diluted by double

dilution method in MEM with FBS.

b. Chemicals and Materials

Cell Culture Plates	:	96 well microtiter plates (Corning)
Cell culture flasks	:	T25 Flasks (Falcon)
Trypsin/EDTA	:	0.25% Trypsin and 0.02% EDTA in Dulbecco's Phosphate Buffered Saline (Gicbo Thermo Fisher)
DMSO	:	Dimethyl sulfoxide (Sigma)
ISOPROPANOL		Isopropanol
Cell culture Medium	:	D-Modified Eagle Medium (DMEM) containing 10% (v/v) Fetal Bovine Serum. P&S
Cell Line	:	HepG2
Culture Conditions	:	37^0C with 5% CO_2

6. MTT Assay

a. Preparation of Cells

HepG2 cells were cultured in D-Modified Eagle Medium (DMEM) with NEAA media supplemented with 10% (v/v) fetal bovine serum. Cells were cultured at 37^0C and 5% CO_2; the complete medium was changed every 2 to 3 days.

b. MTT Assay Procedure

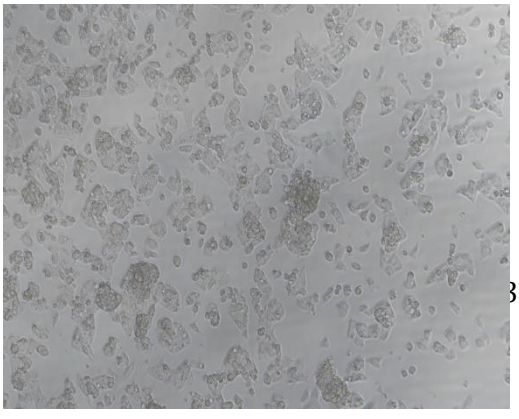
- Cells were seeded in 96-well plates at a concentration of 1,00,000 cells per well (100 µl). The plates were incubated at 37ºC and 5% CO_2 atmosphere for 24 hr.
- After the incubation period cells were observed for half confluent monolayer.
- Culture medium was removed, and cells were treated with 9 different concentrations of Test item.

- Cells in cell culture medium without any Test Item incubated for 24 hr under the same condition served as Control.
- Plates were incubated at 37ºC in 5% CO_2 incubator for 24 hr.
- After 48 hr, cells were observed under an inverted microscope for any changes in morphology or death.
- After observation, the culture medium was removed, and 100 µl of fresh medium was added along with 10 µl of MTT reagent in each well.
- Plates were incubated for 4 hr. at 37ºC in 5% CO_2 incubator.
- 100 µl of the Solubilization solution was added into each well.
- Plate was allowed to stand for 1 hr. at 37ºC in 5% CO2 incubator.
- After checking for complete solubilization of the purple formazan crystals, absorbance was measured at 570 nm using a microplate reader.
- IC50 values were calculated by plotting a log graph for the concentration of the test items vs %cell survival.
- Percentage Cell Survival was calculated using the formula:

$$Percent\ Cell\ Survival\ (\%) = \frac{\text{Absorbance of Test}}{Absorbance\ of\ Control}\ X\ 100$$

7. Observation and Results

1. C25 Epi-Kavaratamide A



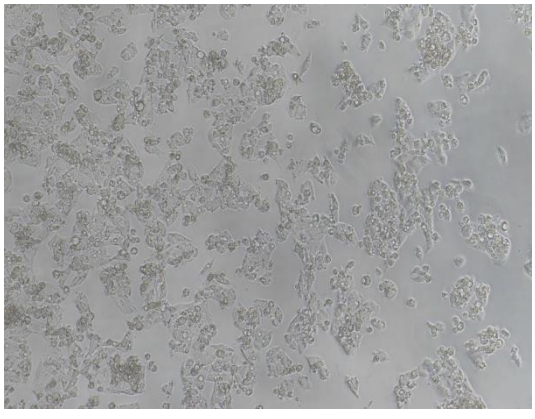
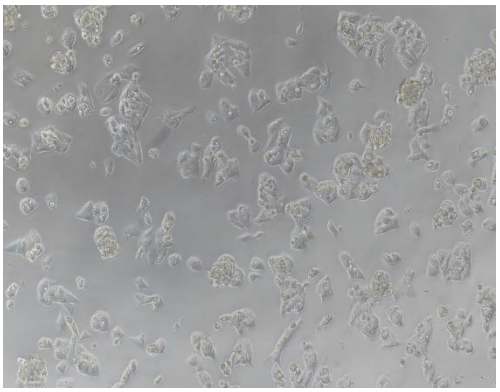
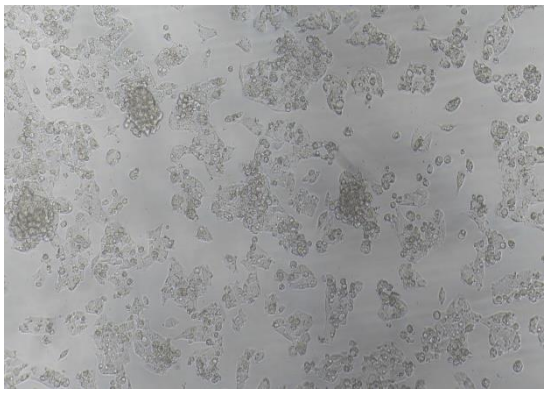
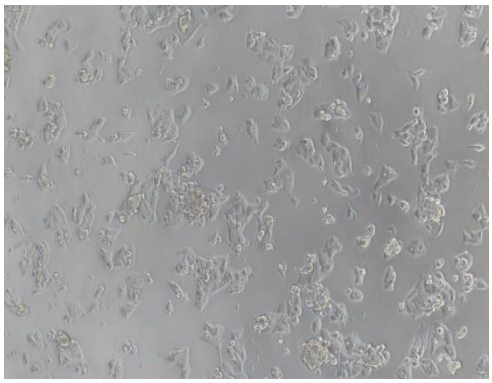
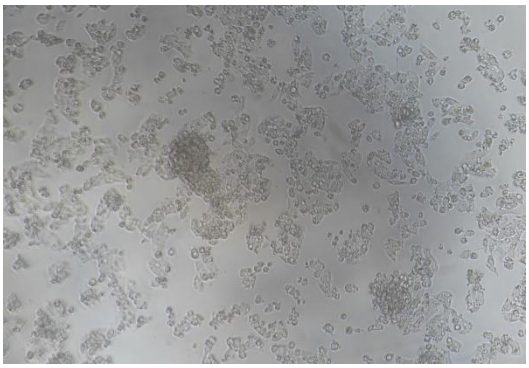
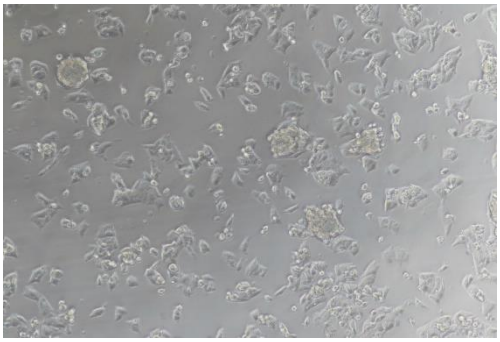
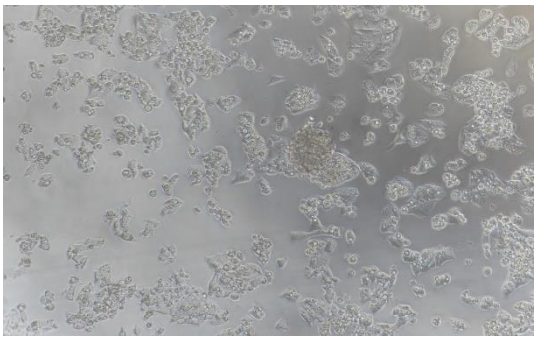
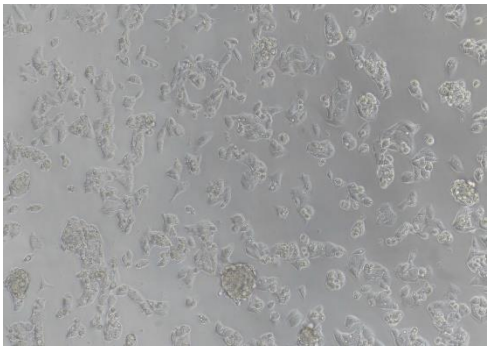
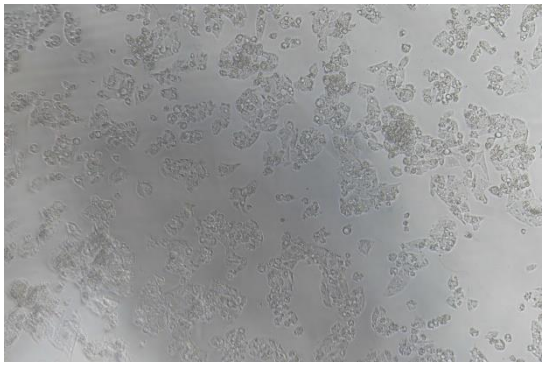
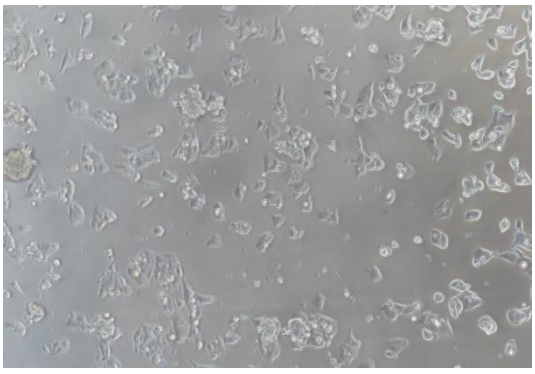
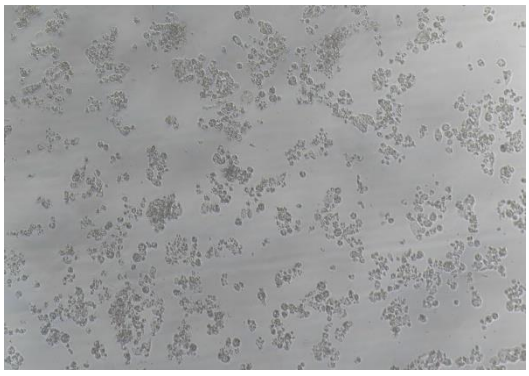
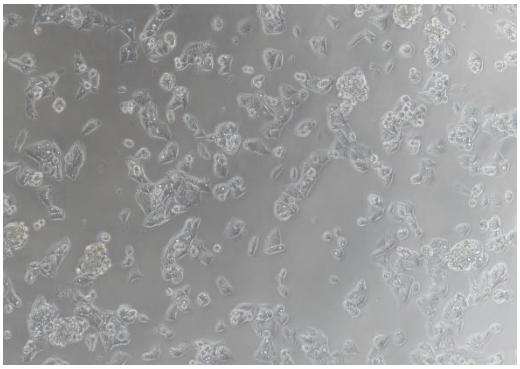
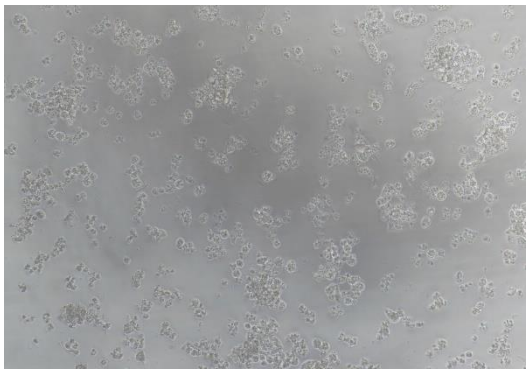
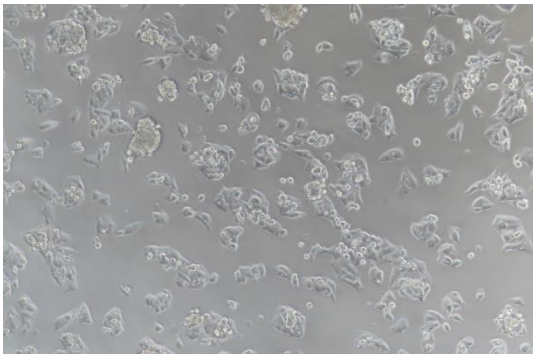
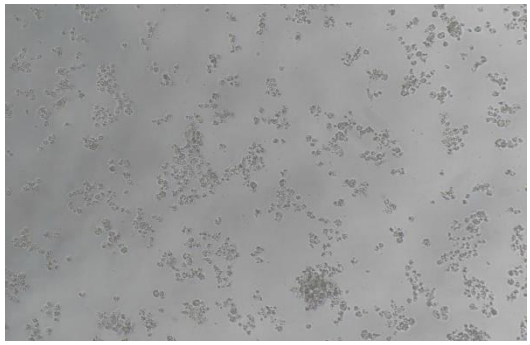
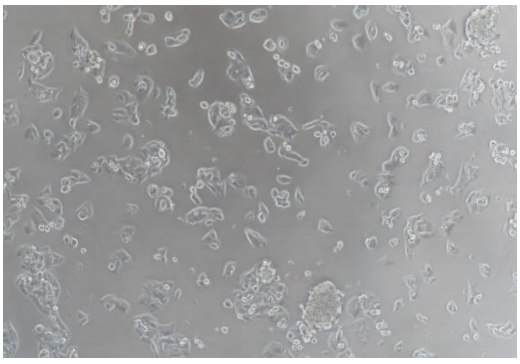
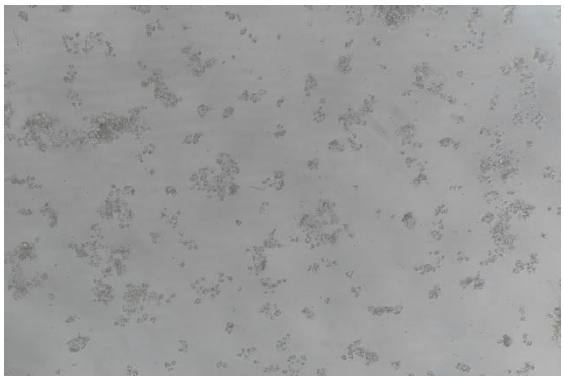


Figure 1. Morphological Observations of Control Well of C25 Epi-Kavaratamide A

Dilutions	**C25 Epi-Kavaratamide A (0 hrs)**	**C25 Epi-Kavaratamide A (24 hrs)**
0.01µM		
0.1 µM		

1 µM		
10 µM		
25 µM		

50 µM		
75 µM		
100 µM		

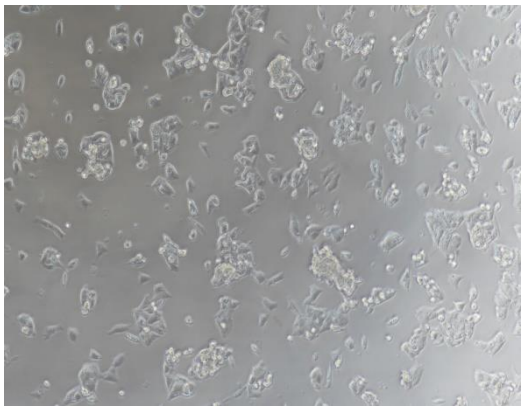
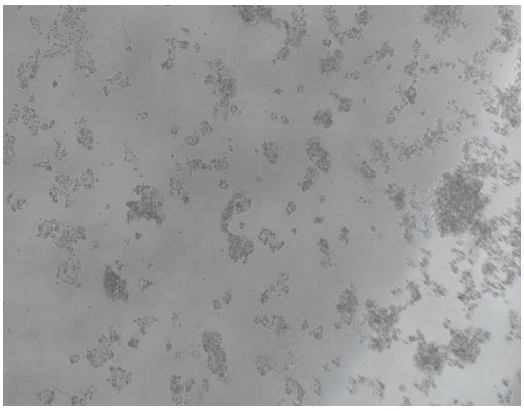
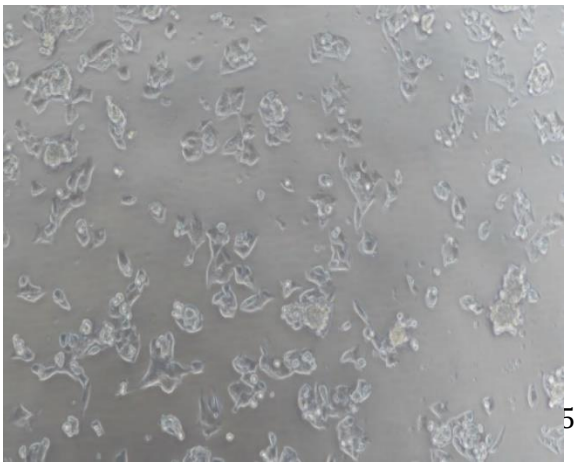
150 µM		

Figure 2. Morphological Observations of C25 Epi-Kavaratamide A Treated Wells

2. Kavaratamide A



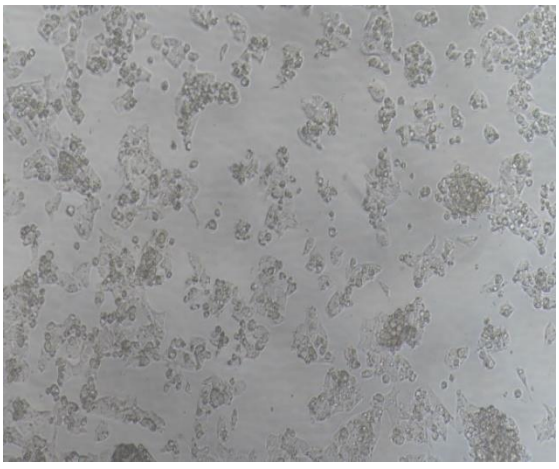
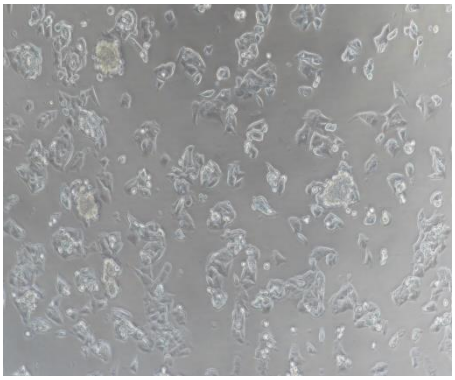
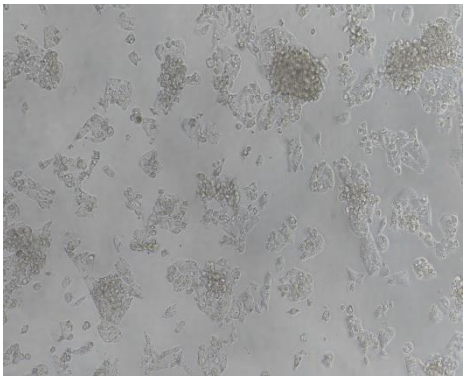
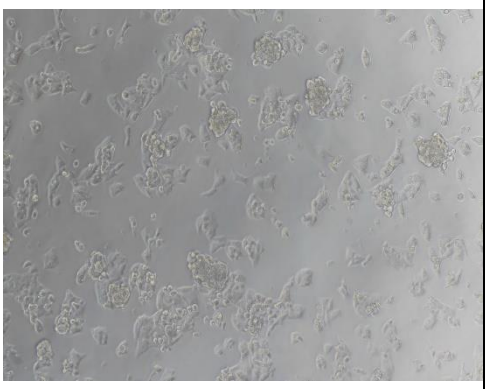
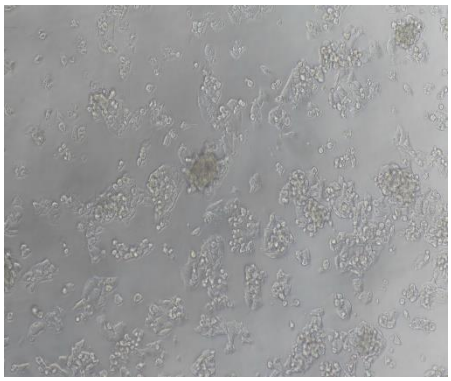
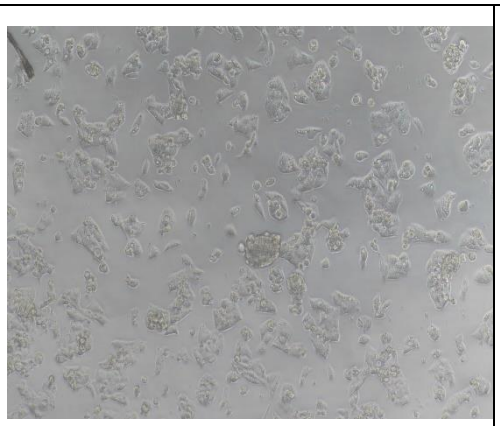
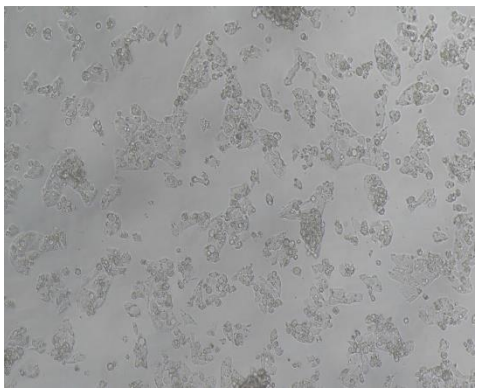
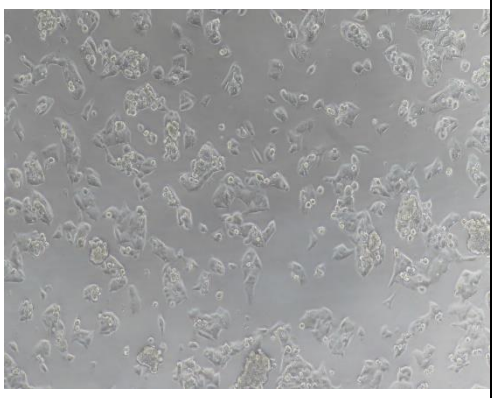
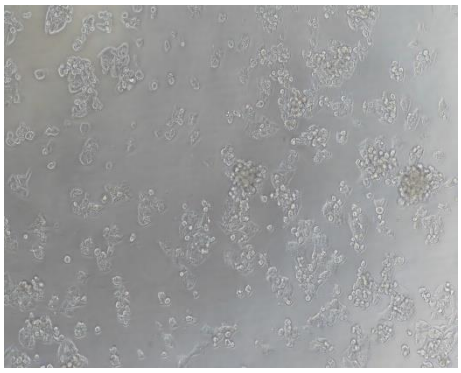
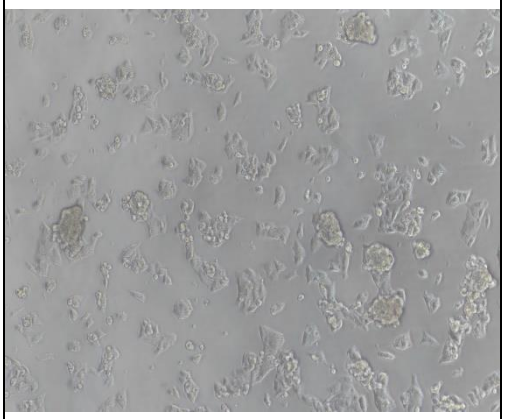
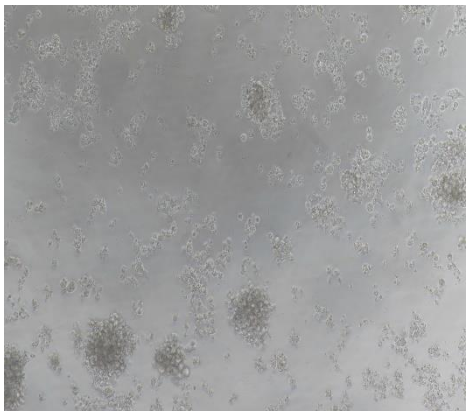
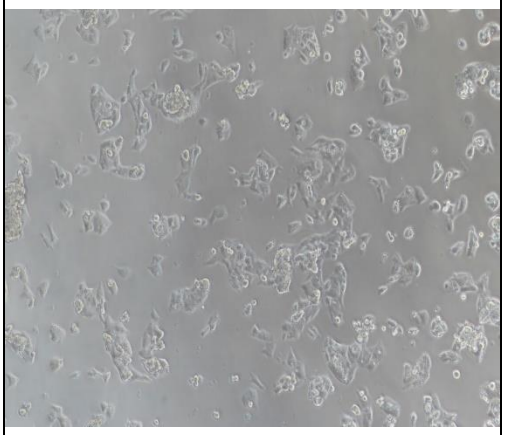
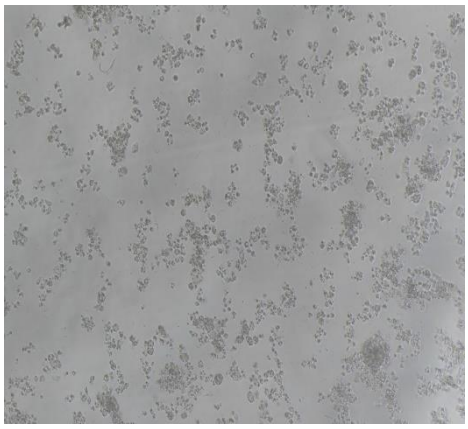
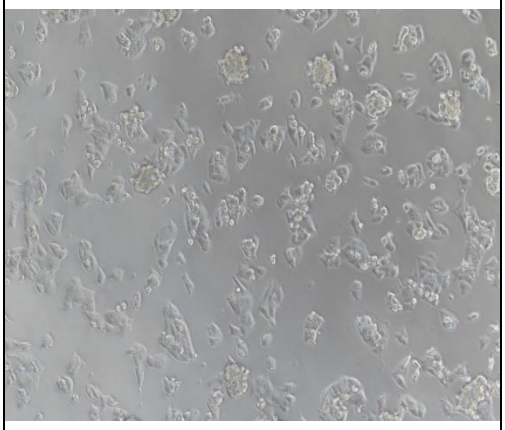
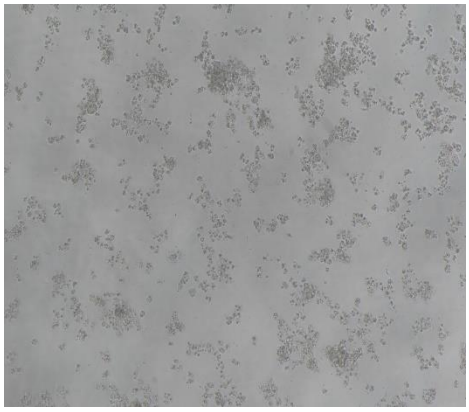
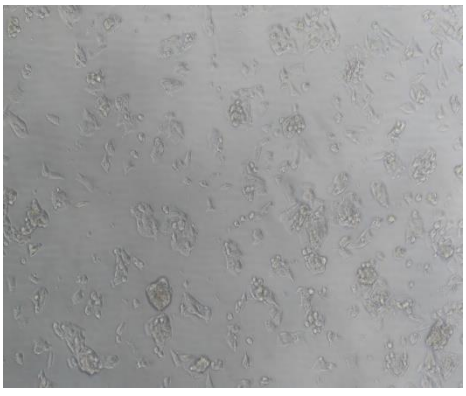
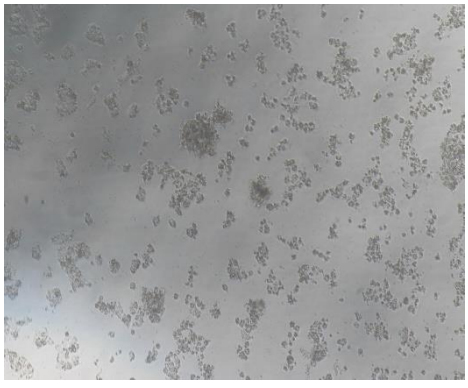


Figure 3. Morphological Observations of Control Well of Kavaratamide A

Dilutions	Kavaratamide A (0 hr)	Kavaratamide A (24 hrs)
0.01µM		
0.1 µM		

1 µM		
10 µM		
25 µM		

50 µM		
75 µM		
100 µM		

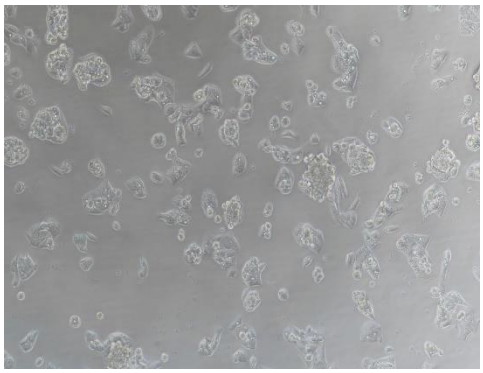
150 µM		

Figure 4. Morphological Observations of Kavaratamide A treated Wells

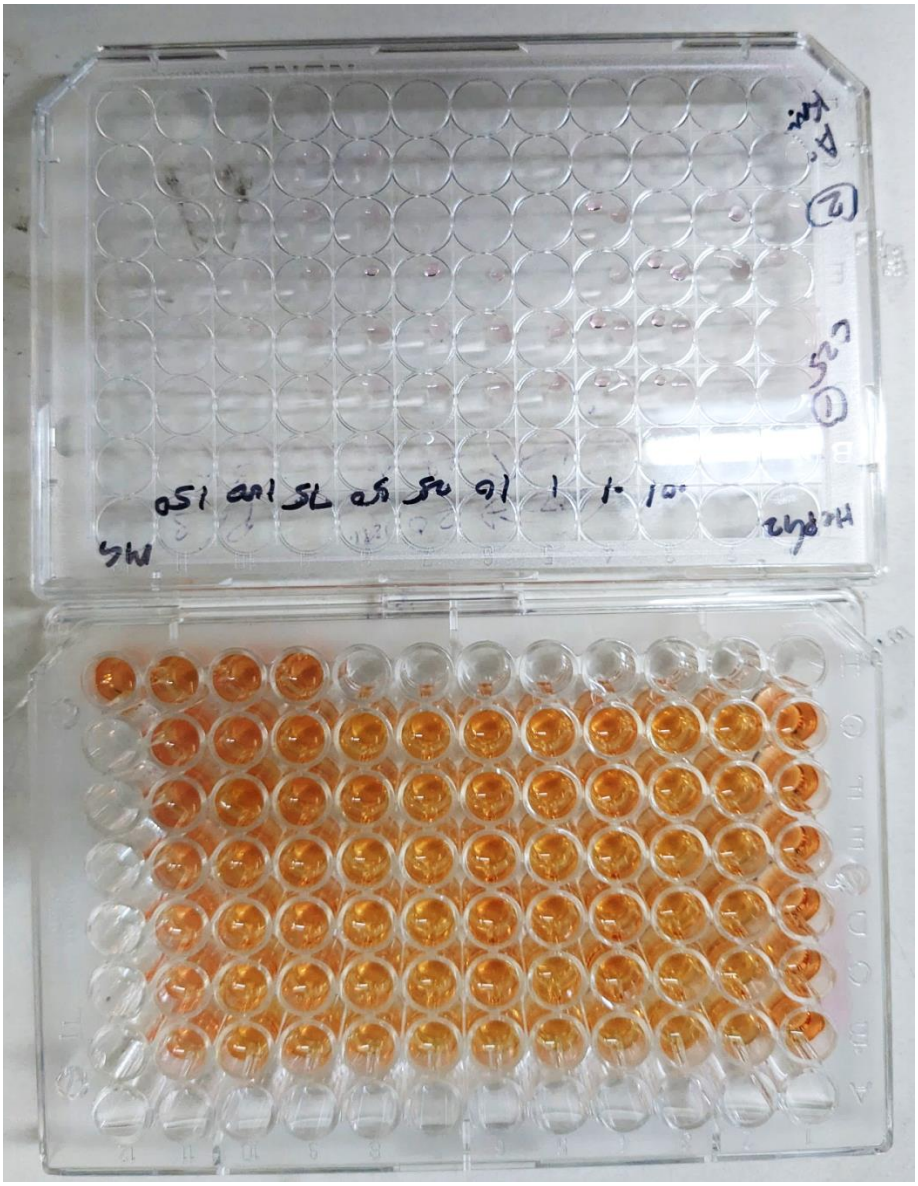


Figure 5: Microplate Photos of MTT added

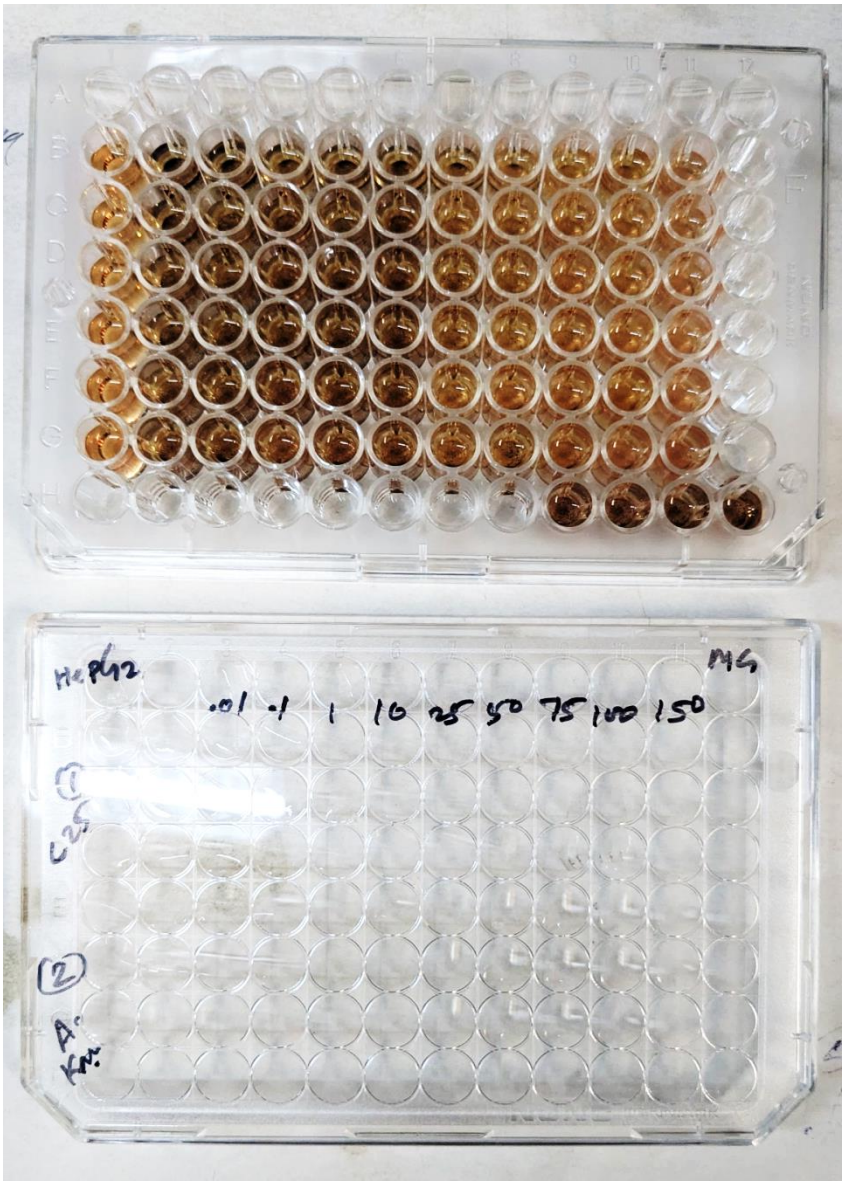


Figure 6: Microplate Photos of 3.5 hrs MTT added

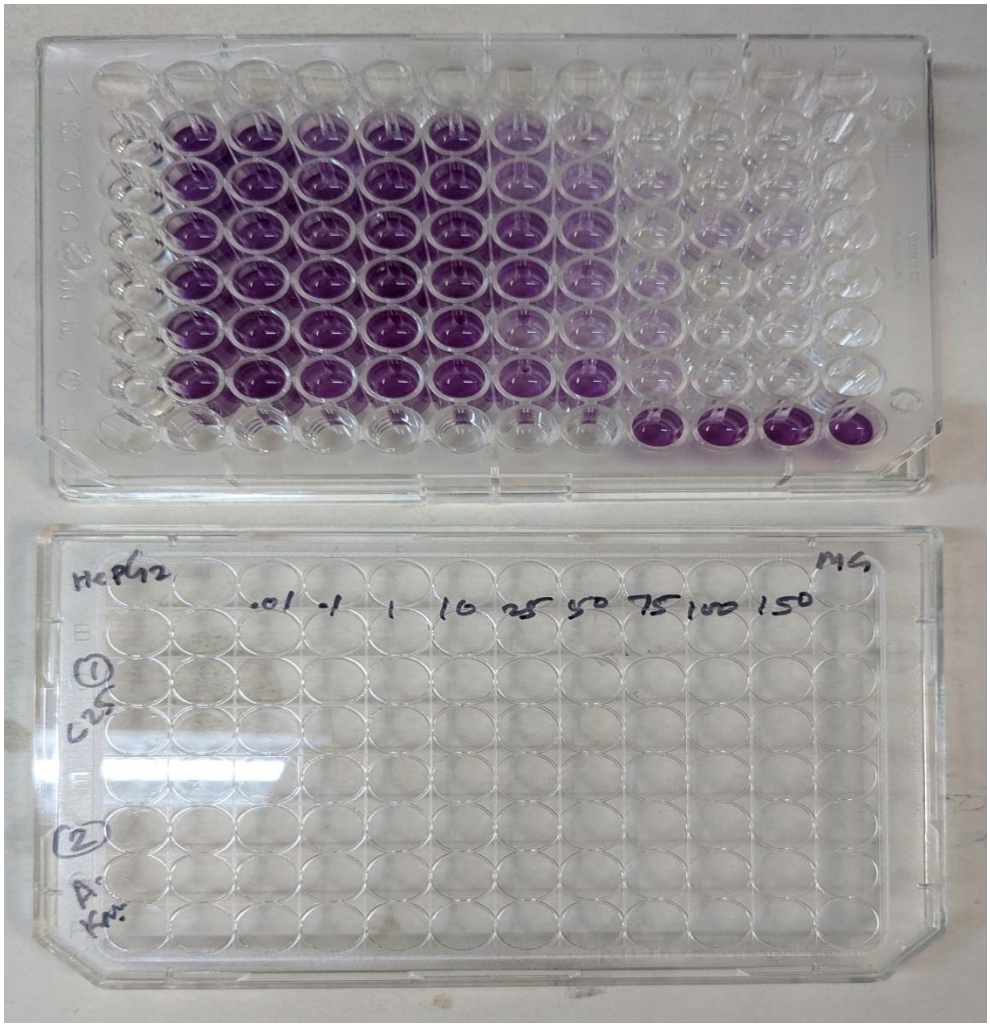


Figure 7. Microplate Photos of Before Reading, After Isopropanol added

Table 1. MTT Results: C25 Epi-Kavaratamide A

Concentration (µM)	Absorbance			Average	% Cell Survival			Average % Cell Survival
CTRL	1.407	1.325	1.446	1.393	101.03	95.14	103.83	100.00
0.01	1.065	1.175	1.184	1.141	76.47	84.37	85.02	81.95
0.1	0.748	1.24	1.173	1.054	53.71	89.04	84.23	75.66
1	1.013	1.074	1.118	1.068	72.74	77.12	80.28	76.71
10	0.991	0.995	1.259	1.082	71.16	71.45	90.40	77.67
25	0.404	0.522	0.736	0.554	29.01	37.48	52.85	39.78
50	0.236	0.391	0.518	0.382	16.95	28.08	37.19	27.41
75	0.084	0.21	0.121	0.138	6.03	15.08	8.69	9.93
100	0.103	0.128	0.228	0.153	7.40	9.19	16.37	10.99
150	0.089	0.123	0.168	0.127	6.39	8.83	12.06	9.10
Log IC50 Value	**1.581**							
IC50 Value	**38.10 µM**							

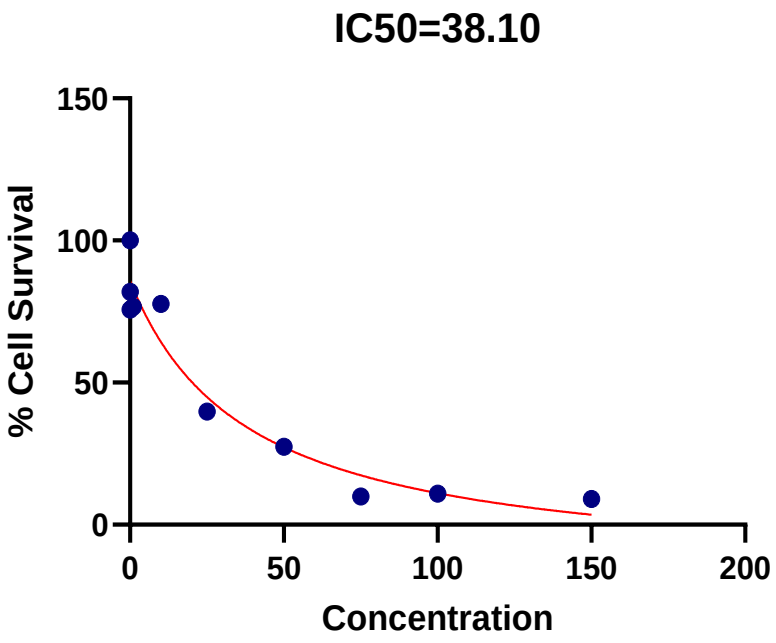


Figure 8. IC50 of C25 Epi-Kavaratamide A

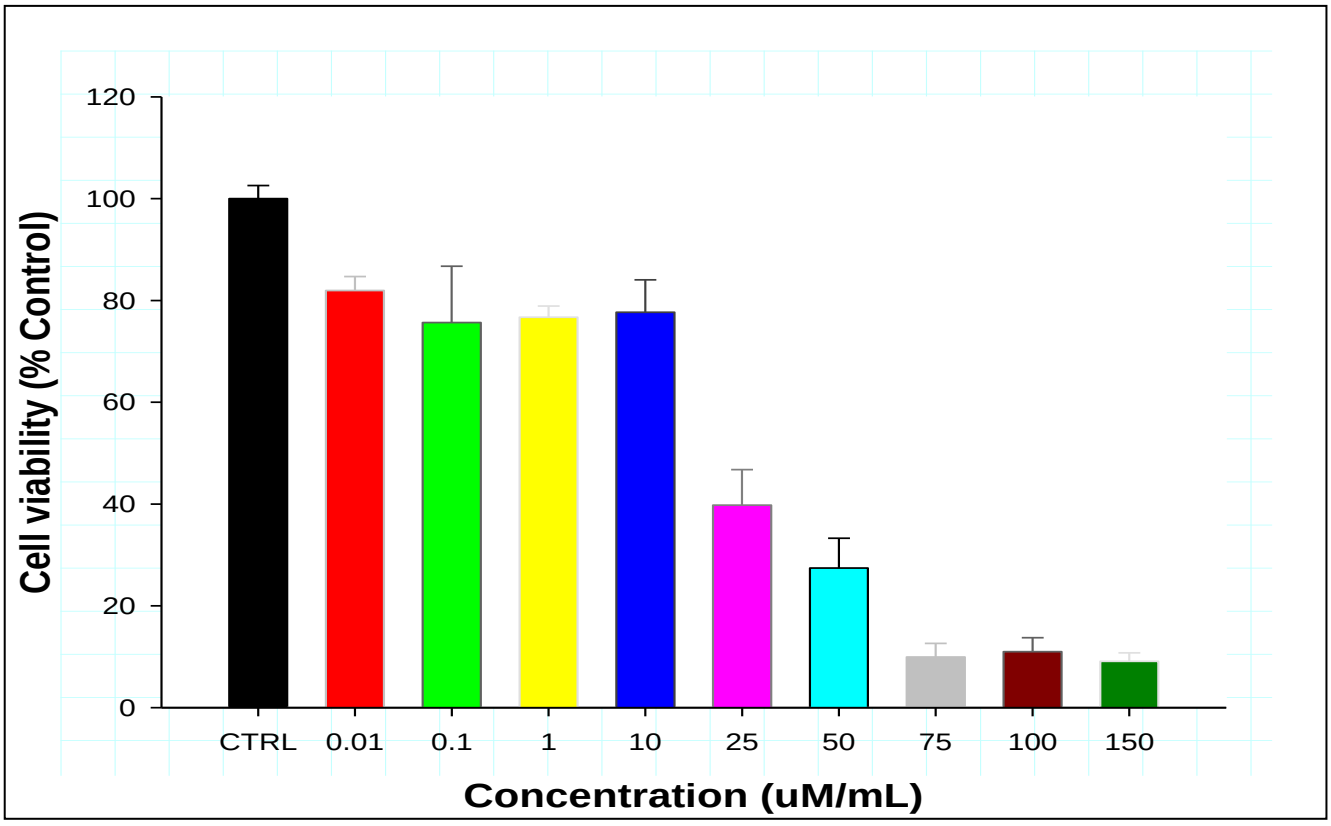


Figure 9. Graph of conc. vs % cell Viability

Table 2. MTT Results: Kavaratamide A

Concentration (µM)	Absorbance			Average	% Cell Survival			Average % Cell Survival
CTRL	1.507	1.193	1.293	1.331	113.22	89.63	97.15	100.00
0.01	1.199	1.173	1.353	1.242	90.08	88.13	101.65	93.29
0.1	1.062	1.039	0.983	1.028	79.79	78.06	73.85	77.24
1	1.14	1.157	0.976	1.091	85.65	86.93	73.33	81.97
10	0.808	0.962	0.951	0.907	60.71	72.28	71.45	68.14

25	0.733	0.475	0.644	0.617	55.07	35.69	48.38	46.38
50	0.353	0.307	0.724	0.461	26.52	23.07	54.40	34.66
75	0.238	0.225	0.198	0.220	17.88	16.90	14.88	16.55
100	0.12	0.119	0.112	0.117	9.02	8.94	8.41	8.79
150	0.111	0.079	0.16	0.117	8.34	5.94	12.02	8.77
Log IC50 Value	**1.579**							
IC50 Value	**37.90 µM**							

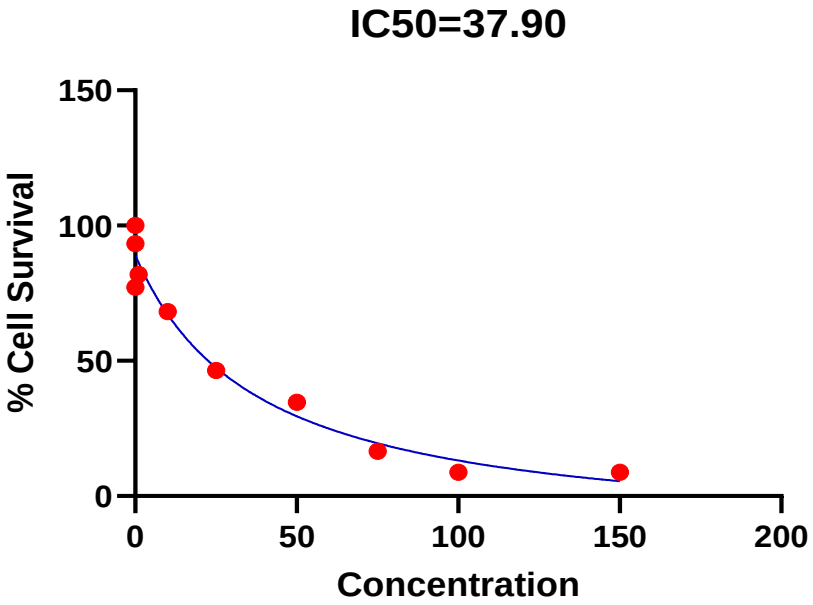


Figure 10. IC50 of Kavaratamide A

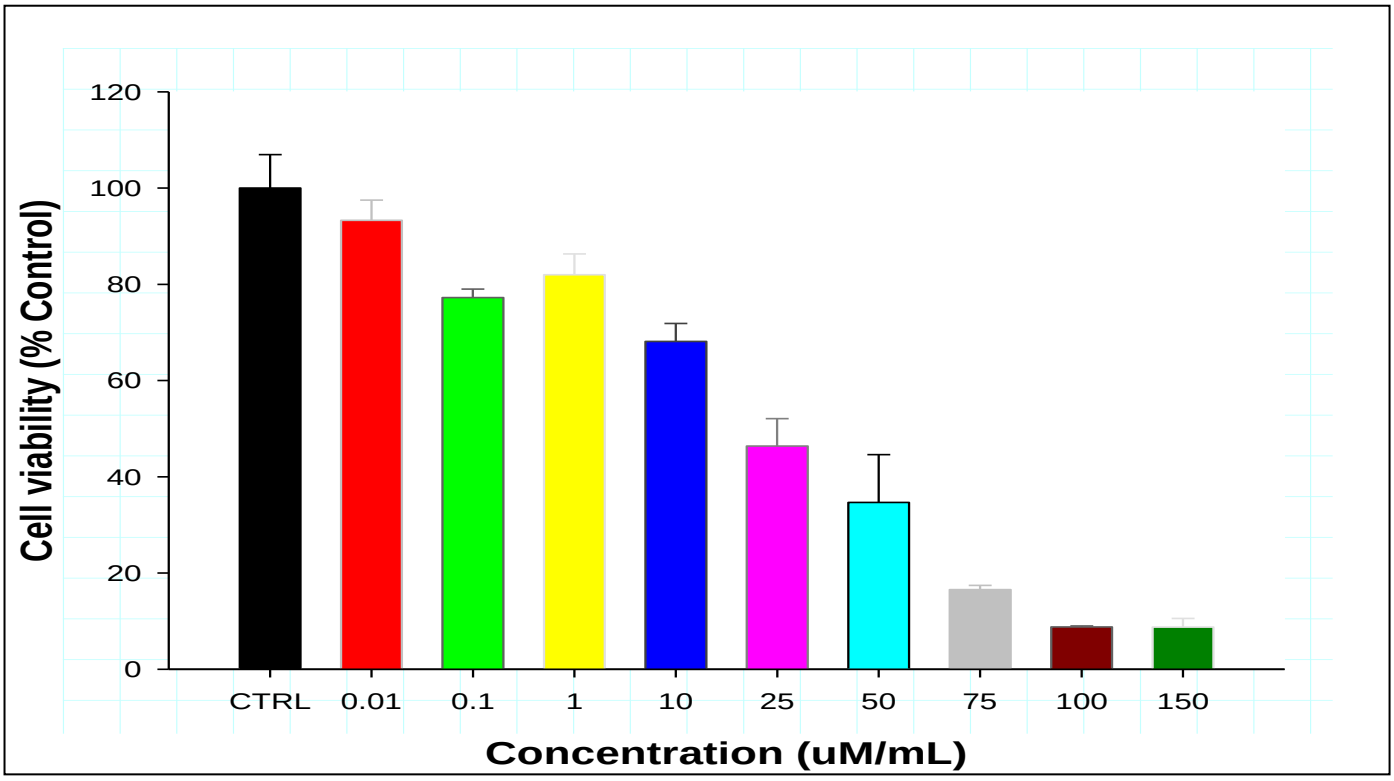


Figure 10. Graph of conc. vs % cell Viability

Table 3. IC50 values of MTT Assay

Sr. No.	Test Item	Log IC50 Value	IC50 Value
1.	C25 Epi-Kavaratamide A	1.581	38.10
2.	Kavaratamide A	1.579	37.90

8. Data Analysis and Report Preparation

GraphPad Prism Ver. 10.00 was used for the statistical analysis. The extracted sheets of statistical analysis are provided along with the report in text format.

Two final copies of final reports are generated. One copy is shared with the Sponsor, and the other copy is submitted to the archives.

Note - After sending a draft report, if no comments are received from the Sponsor within 10 days, the draft report and the raw data shall be archived as such.

9. Archives

All original raw data, final report and all electronic files generated will be retained in archives. Thereafter, the archived material will be either destroyed or stored for an extended period as per written consent from the sponsor.

10. Study Plan Amendment and Deviations

No study plan amendments or deviations occurred during the study.

	ASTER ANALYTICS RESEARCH INSTITUTE, PUNE	
CELL LINE STUDY		
Report No.	AARI/IVCL/02/2024-25	
Version No.	01	**RESTRICTED DOCUMENTS**
Cell Line Study		

5. Report On

"Cytotoxicity study of C25 Epi-Kavaratamide A and Kavaratamide A in PANC-1 Cell Line."

REPORT NUMBER

AARI/IVCL/02/2024-25

PREPARED & APPROVED BY

ASTER ANALYTICS RESEARCH INSTITUTE

227, SRP ROAD, PANDAVNAGAR,WADACHI WADI, PUNE-411060

SPONSORED BY

DR. RAVINDAR KONTHAM

PRINCIPAL SCIENTIST

CSIR-NATIONAL CHEMICAL LABORATORY

DATE OF ISSUE OF REPORT: 02.01.2025

DATE OF RELEASE OF REPORT: 02.01.2025

This report contains 23 pages.

CONTENTS

Sr. No	Description	Page Number
1.	Study Details	S50
2.	Compliance statement	S51
3.	Quality Assurance Statement	S52
4.	Document Control	S53
5.	Objective	S53
6.	Study Guidelines	S53
7.	Study Period	S53
8.	Materials	S53
9.	Experimental Procedures	S53
10.	MTT Assay	S54
11.	Observation and Results	S55
12.	Data Analysis and Report Preparation	S70
13.	Archives	S70
14.	Study Plan Amendment and Deviations	S70

STUDY DETAILS

Report Number	:	AARI/IVCL/02/2024-25
Study Title	:	Cytotoxicity study of C25 Epi-Kavaratamide A and Kavaratamide A in PANC-1 Cell Line.
Sponsor	:	Dr. Ravindar Kontham Principal Scientist CSIR-National Chemical Laboratory
Test Facility	:	Aster Analytics Research Institute 227, SRP Road, Pandavnagar, Wadachi Wadi, Pune-411060
Study Director	:	Dr. Amit Kasabe
Study personal		Ms. Karishma Markad
Period	:	30.12.2024 to 02.01.2025

COMPLIANCE STATEMENT

I, the Undersigned hereby state and declare that this, “Report No. AARI/IVCL/02/2024-25" was performed under my supervision in compliance with the ICH guidelines.

Characterization of the “Test Items/Samples” was performed by the sponsor. Test Laboratory is responsible for validity of the test procedure, interpretation, analysis, documentation and test reports.

Dr. Amit Kasabe
Director

Date: 02.01.2025

QUALITY ASSURANCE STATEMENT

This study report has been reviewed by the Quality Assurance department of **Aster Analytics Research Institute** for Study plan, Raw Data and Results.

Ms. Karishma Markad
Research Associate

Date: 02.01.2025

	ASTER ANALYTICS RESEARCH INSTITUTE, PUNE	
CELL LINE STUDY		
Report No.	AARI/IVCL/02/2024-25	
Version No.	01	**RESTRICTED DOCUMENTS**
Cell Line Study		

1. Document Control

Aster Analytics Research Institute, Pune

2. Objective

To determine the cytotoxicity of C25 Epi-Kavaratamide A and Kavaratamide A in the PANC-1 cell line using the MTT assay method.

3. Study Guidelines

The design and scope of the study are based on consideration of the study objectives.

Study Period

Sample Receiving Date	:	28/Dec/2024
Experiment Start Date	:	30/Dec/2024
Experiment Completion Date	:	02/Dec/2024
Study Completion Date	:	02/Dec/2024
Draft Report Date	:	02/Dec/2024
Final Report Date	:	02/Dec/2024

4. Materials

Test Item Details

Sr. No.	Name	Storage Conditions	Handling Precautions
1.	C25 Epi-Kavaratamide A	2-8˚C	Standard Laboratory Procedure
2.	Kavaratamide A		

**All data relating to the identity, purity and stability of the test materials are the responsibility of the sponsor and have not been verified by the test facility.*

5. Experimental Procedures

a. Preparation of Test Material

All Test Samples were filter sterilized using 0.22µ filters and diluted by double

dilution method in MEM with FBS.

b. Chemicals and Materials

Cell Culture Plates	:	96 well microtiter plates (Corning)
Cell culture flasks	:	T25 Flasks (Falcon)
Trypsin/EDTA	:	0.25% Trypsin and 0.02% EDTA in Dulbecco's Phosphate Buffered Saline (Gicbo Thermo Fisher)
DMSO	:	Dimethyl sulfoxide (Sigma)
ISOPROPANOL		Isopropanol
Cell culture Medium	:	D-Modified Eagle Medium (DMEM) containing 10% (v/v) Fetal Bovine Serum. P&S
Cell Line	:	PANC-1
Culture Conditions	:	370C with 5% CO2

6. MTT Assay

a. Preparation of Cells

PANC-1 cells were cultured in D-Modified Eagle Medium (DMEM) with NEAA media supplemented with 10% (v/v) fetal bovine serum. Cells were cultured at 370C and 5% CO2; the complete medium was changed every 2 to 3 days.

b. MTT Assay Procedure

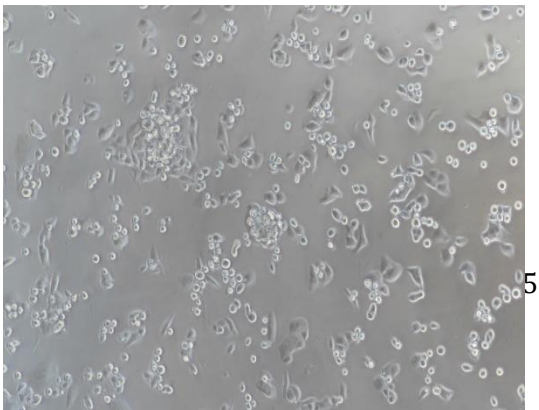
- Cells were seeded in 96-well plates at a concentration of 1,00,000 cells per well (100 µl). The plates were incubated at 37ºC and 5% CO_2 atmosphere for 24 hr.
- After the incubation period cells were observed for half confluent monolayer.
- Culture medium was removed, and cells were treated with 9 different concentrations of Test item.

- Cells in cell culture medium without any Test Item incubated for 24 hr. under the same condition served as Control.
- Plates were incubated at 37ºC in 5% CO_2 incubator for 24 hr.
- After 48 hr., cells were observed under an inverted microscope for any changes in morphology or death.
- After observation, the culture medium was removed, and 100 µl of fresh medium was added along with 10 µl of MTT reagent in each well.
- Plates were incubated for 4 hr. at 37ºC in 5% CO_2 incubator.
- 100 µl of the Solubilization solution was added into each well.
- Plate was allowed to stand for 1 hr. at 37ºC in 5% CO2 incubator.
- After checking for complete solubilization of the purple formazan crystals, absorbance was measured at 570 nm using a microplate reader.
- IC50 values were calculated by plotting a log graph for the concentration of the test items vs %cell survival.
- Percentage Cell Survival was calculated using the formula:

$$Percent\ Cell\ Survival\ (\%) = \frac{\text{Absorbance of Test}}{Absorbance\ of\ Control}\ X\ 100$$

7. Observation and Results

1. C25 Epi-Kavaratamide A



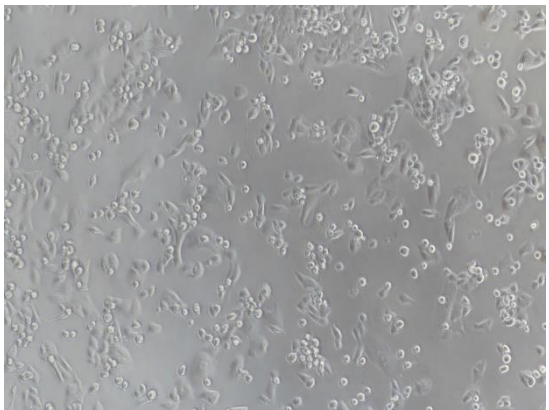
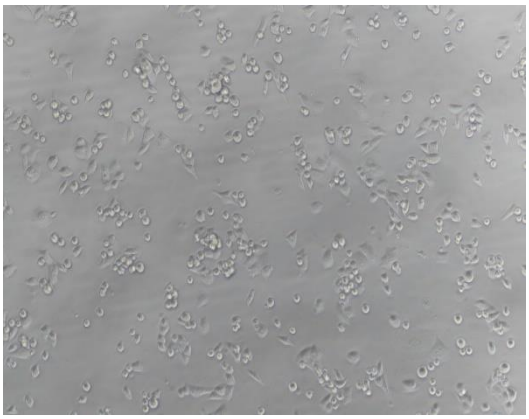
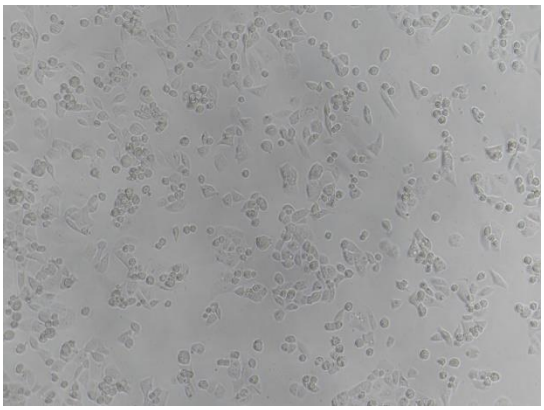
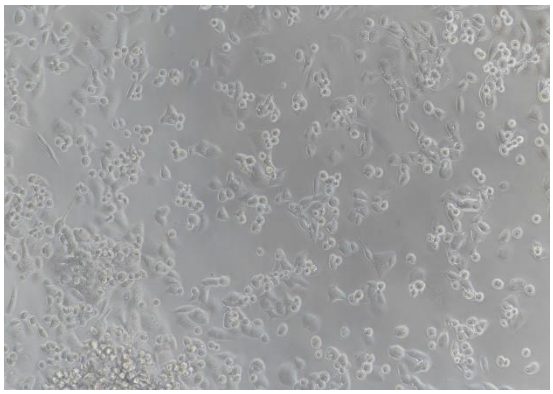
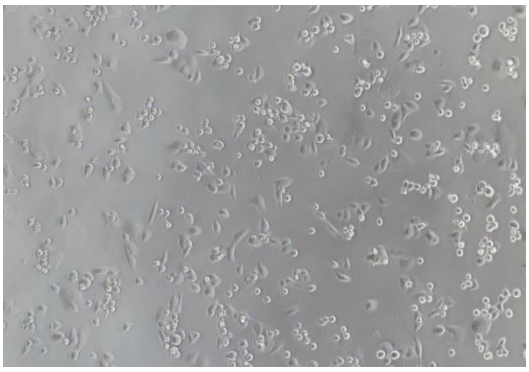
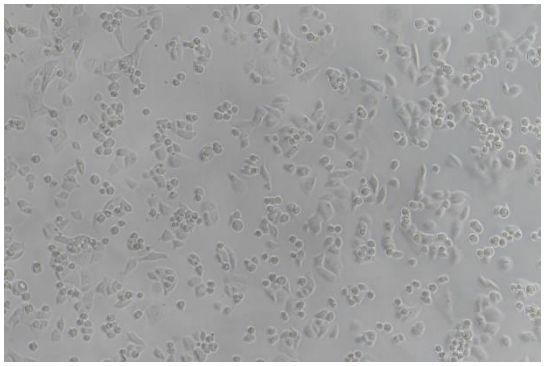
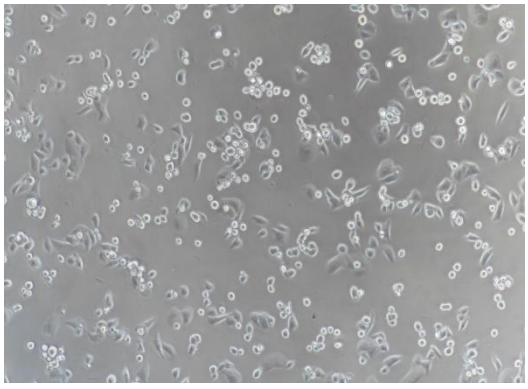
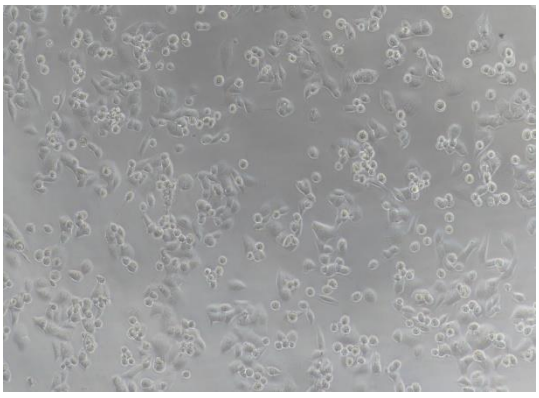
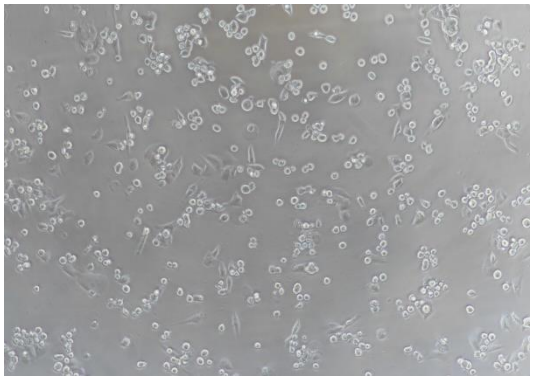
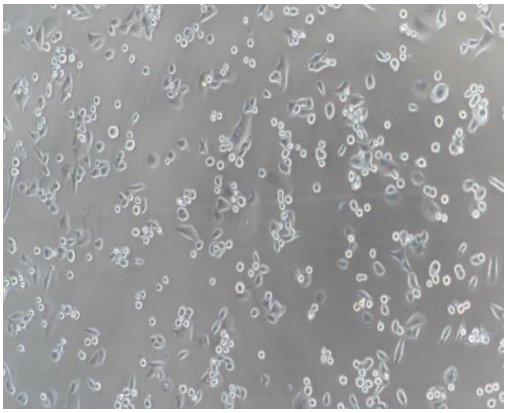
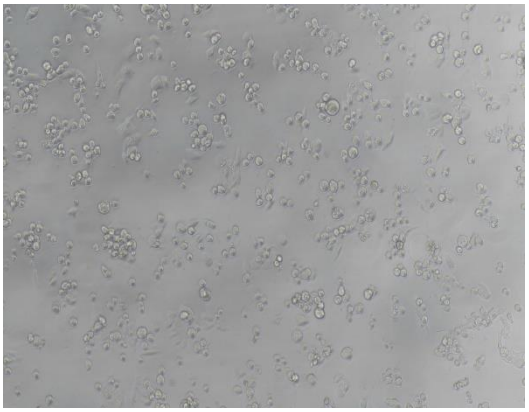
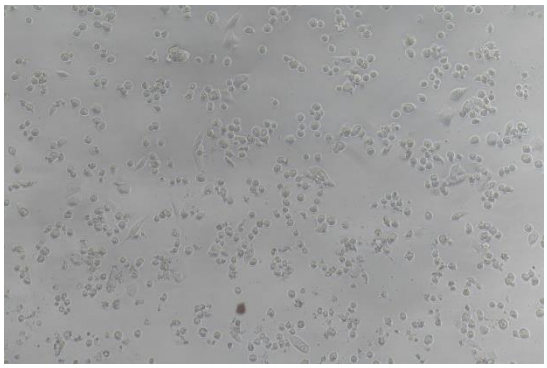


Figure 1. Morphological Observations of Control Well of C25 Epi-Kavaratamide A

Dilutions	**C25 Epi-Kavaratamide A (0 hr)**	**C25 Epi-Kavaratamide A (24 hrs)**
0.01µM		

0.1 µM		
1 µM		
10 µM		

25 µM		
50 µM		
75 µM		

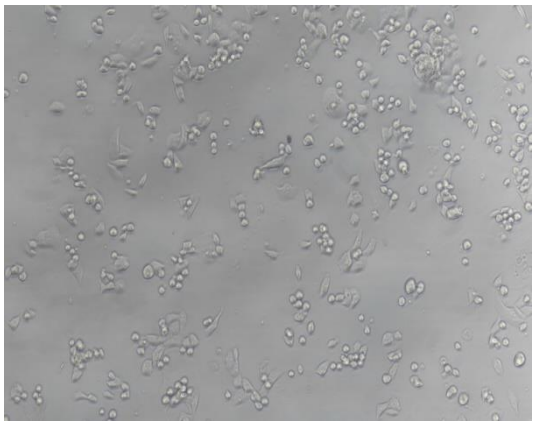
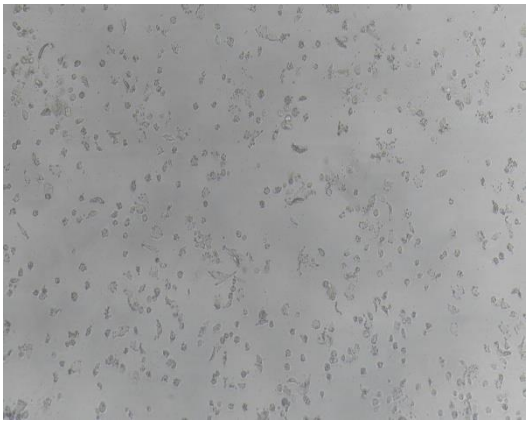
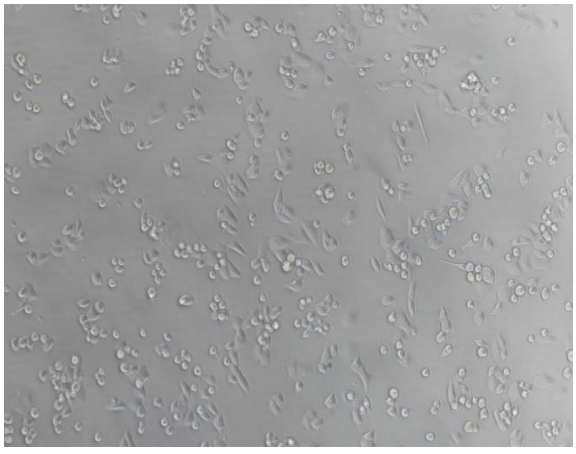
100 µM		

Figure 2. Morphological Observations of C25 Epi-Kavaratamide A Treated Wells

2. Kavaratamide A



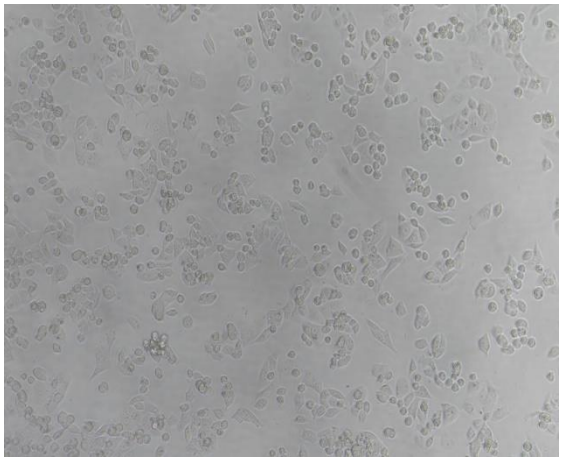
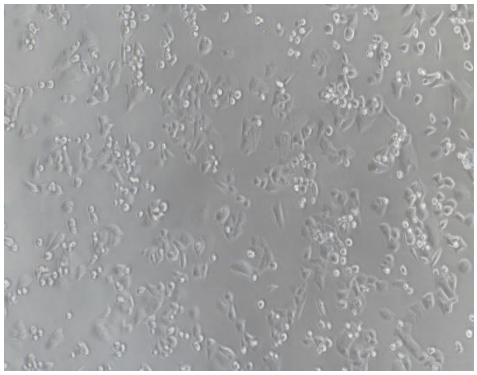
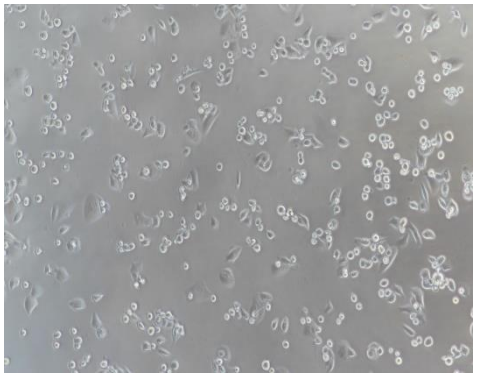
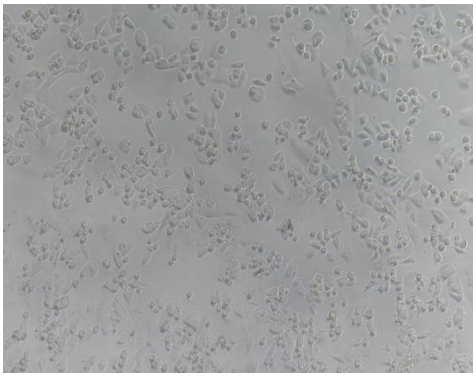
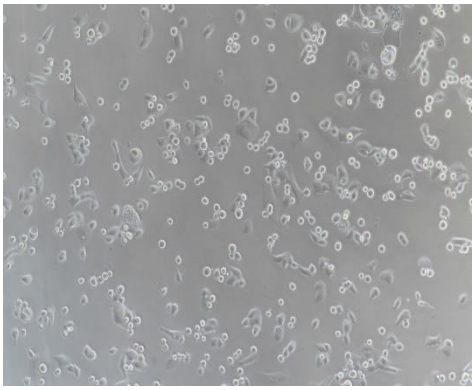
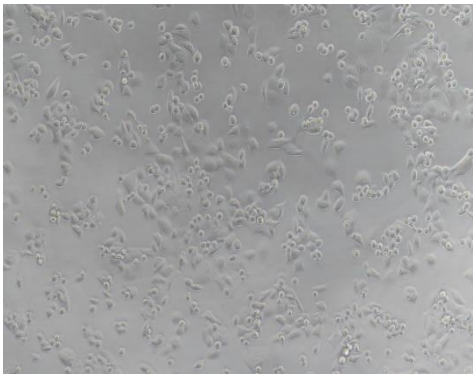
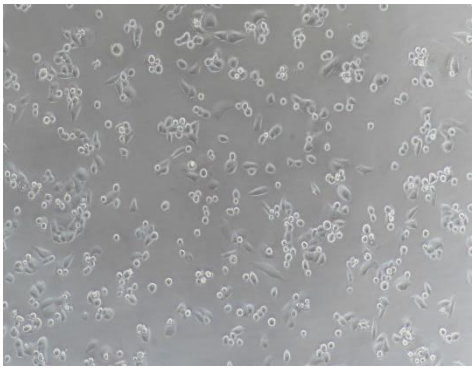
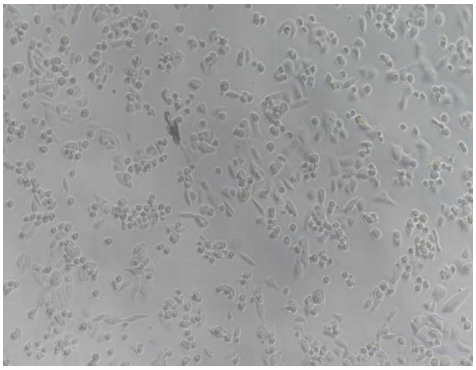
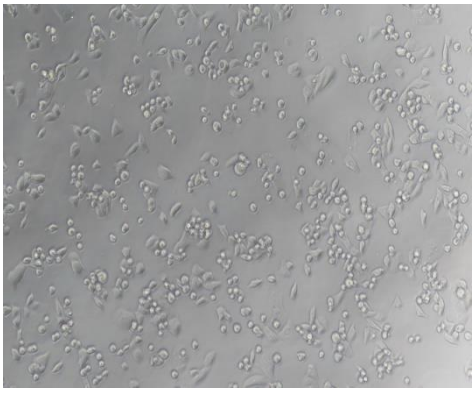
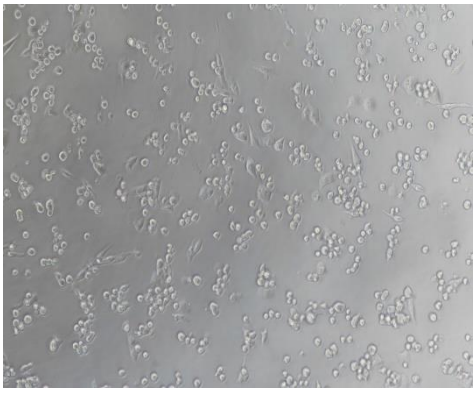


Figure 3. Morphological Observations of Control Well of Kavaratamide A

Dilutions	Kavaratamide A (0 hr)	Kavaratamide A (24 hrs)
0.01µM		
0.1 µM		

1 µM		
10 µM		
25 µM		

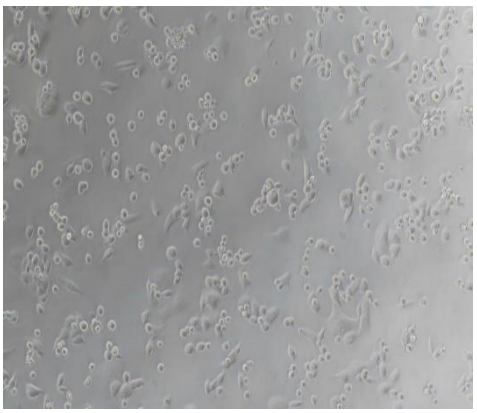
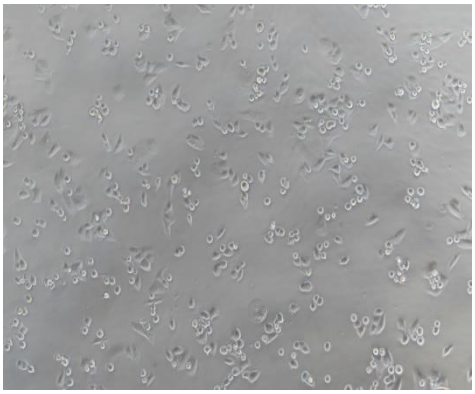
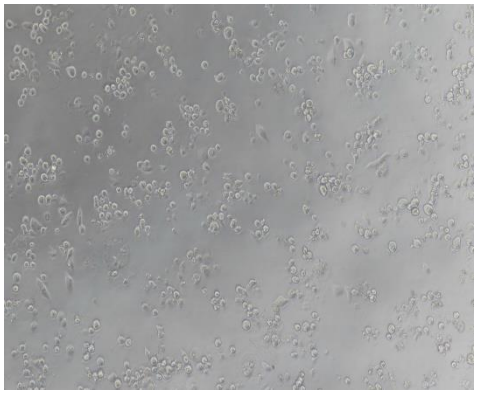
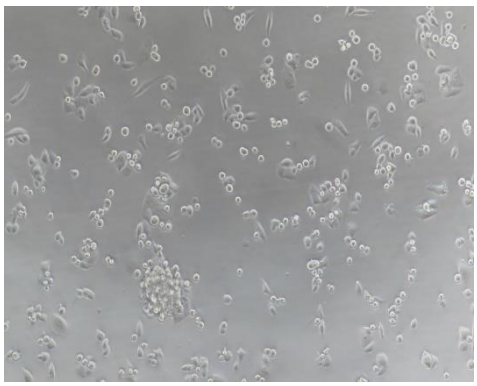
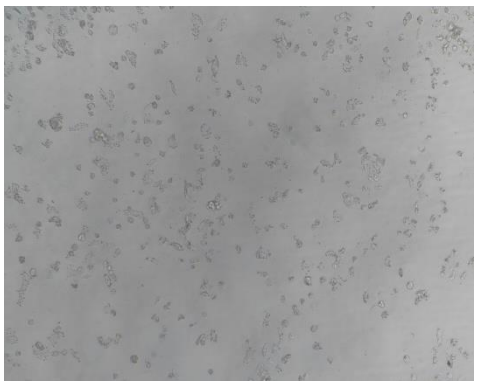
50 µM		
75 µM		
100 µM		

Figure 4. Morphological Observations of Kavaratamide A Treated Wells

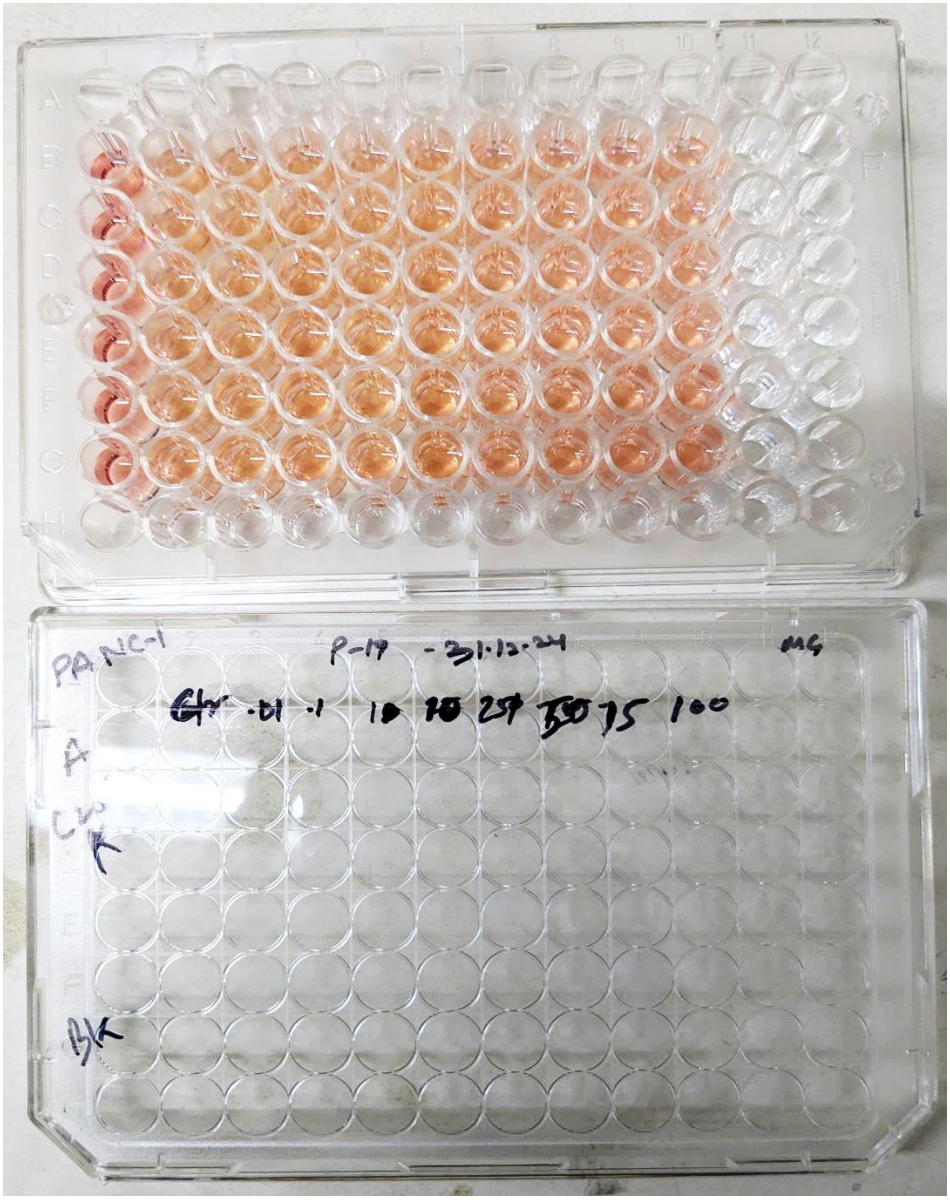


Figure 5: Microplate Photos of MTT added

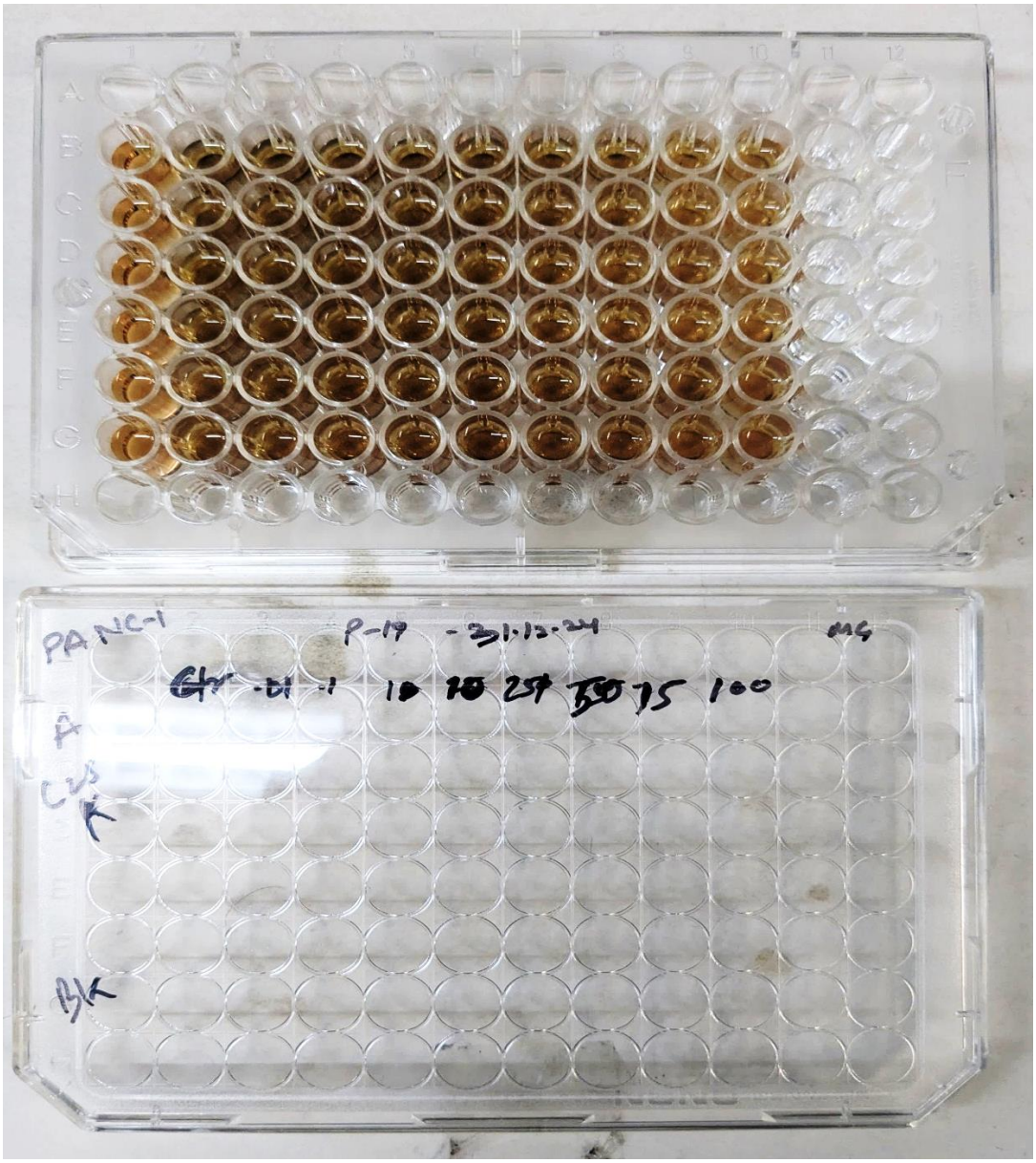


Figure 5: Microplate Photos of 3.5 hr. MTT added

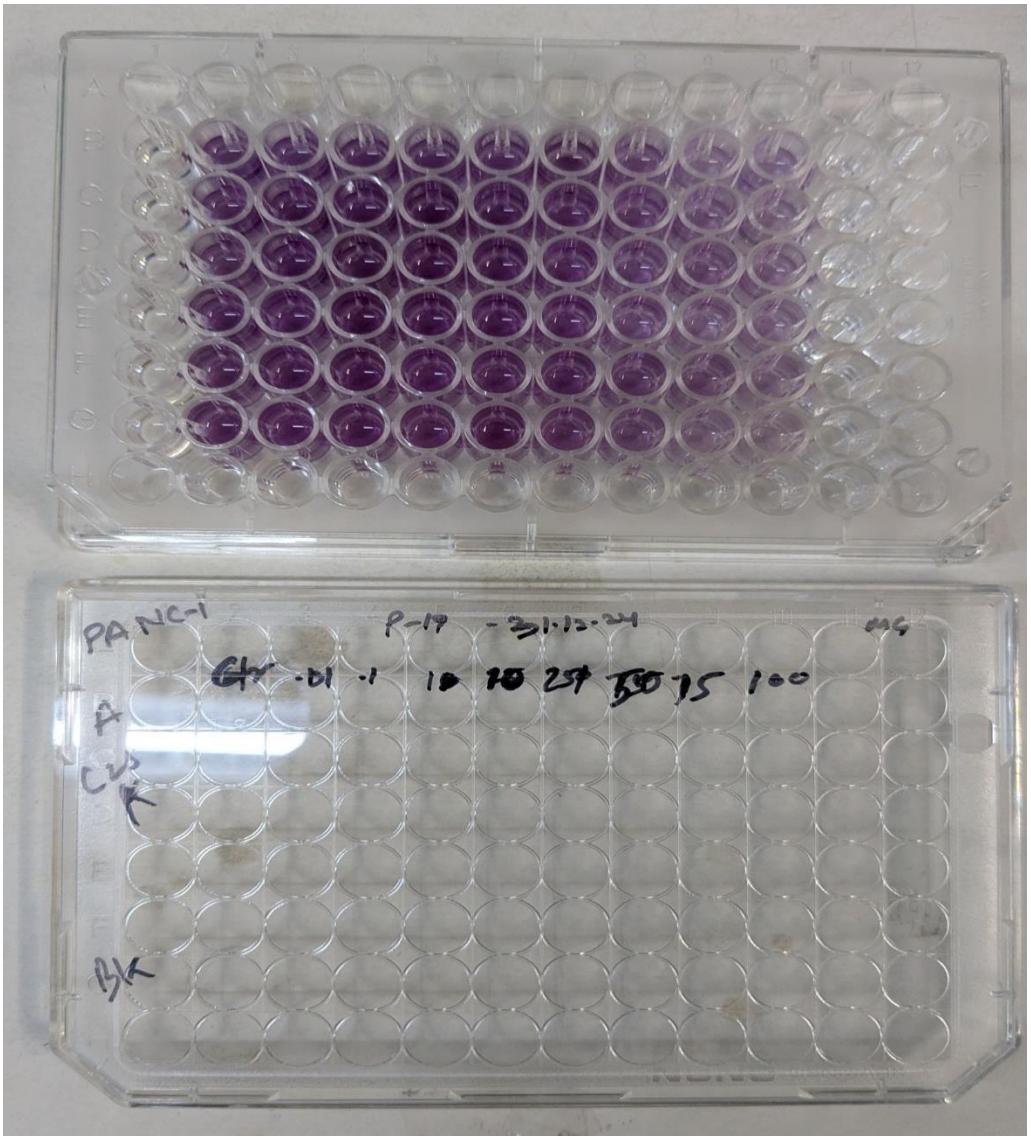


Figure 6. Microplate Photos of Before Reading, After Isopropanol added

Table 1. MTT Results: C25 Epi-Kavaratamide A

Concentration (µM)	Absorbance			Average	% Cell Survival			Average % Cell Survival
CTRL	0.789	0.758	0.807	0.785	100.55	96.60	102.85	100.00
0.01	0.78	0.82	0.662	0.754	99.41	104.50	84.37	96.09
0.1	0.813	0.788	0.936	0.846	103.61	100.42	119.29	107.77
1	0.798	0.935	0.796	0.843	101.70	119.16	101.44	107.43
10	0.756	0.753	0.757	0.755	96.35	95.96	96.47	96.26
25	0.834	0.671	0.624	0.710	106.29	85.51	79.52	90.44
50	0.592	0.555	0.474	0.540	75.45	70.73	60.41	68.86
75	0.341	0.417	0.442	0.400	43.46	53.14	56.33	50.98
100	0.352	0.34	0.383	0.358	44.86	43.33	48.81	45.67
Log IC50 Value	**2.619**							
IC50 Value	**415.5 µM**							

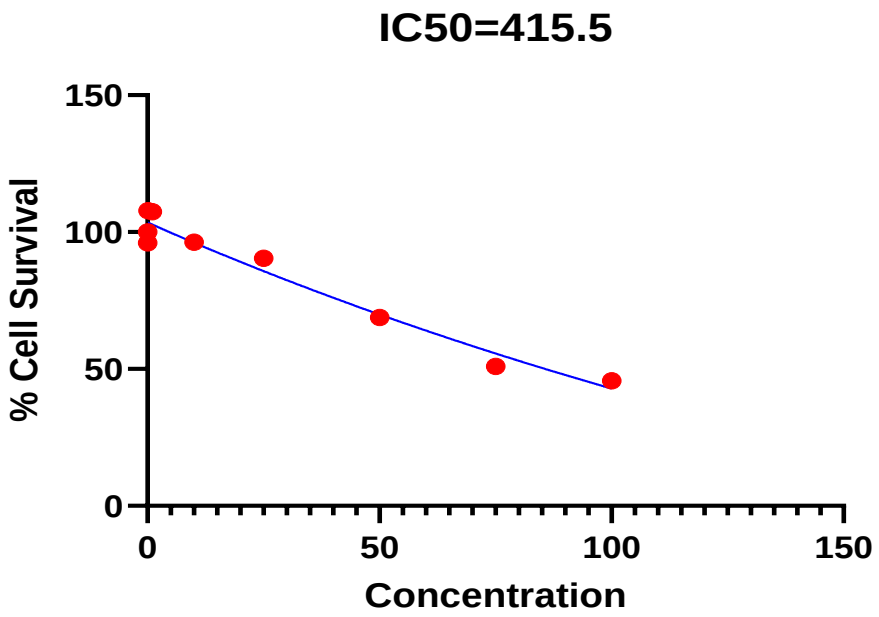


Figure 7. IC50 of C25 Epi-Kavaratamide A

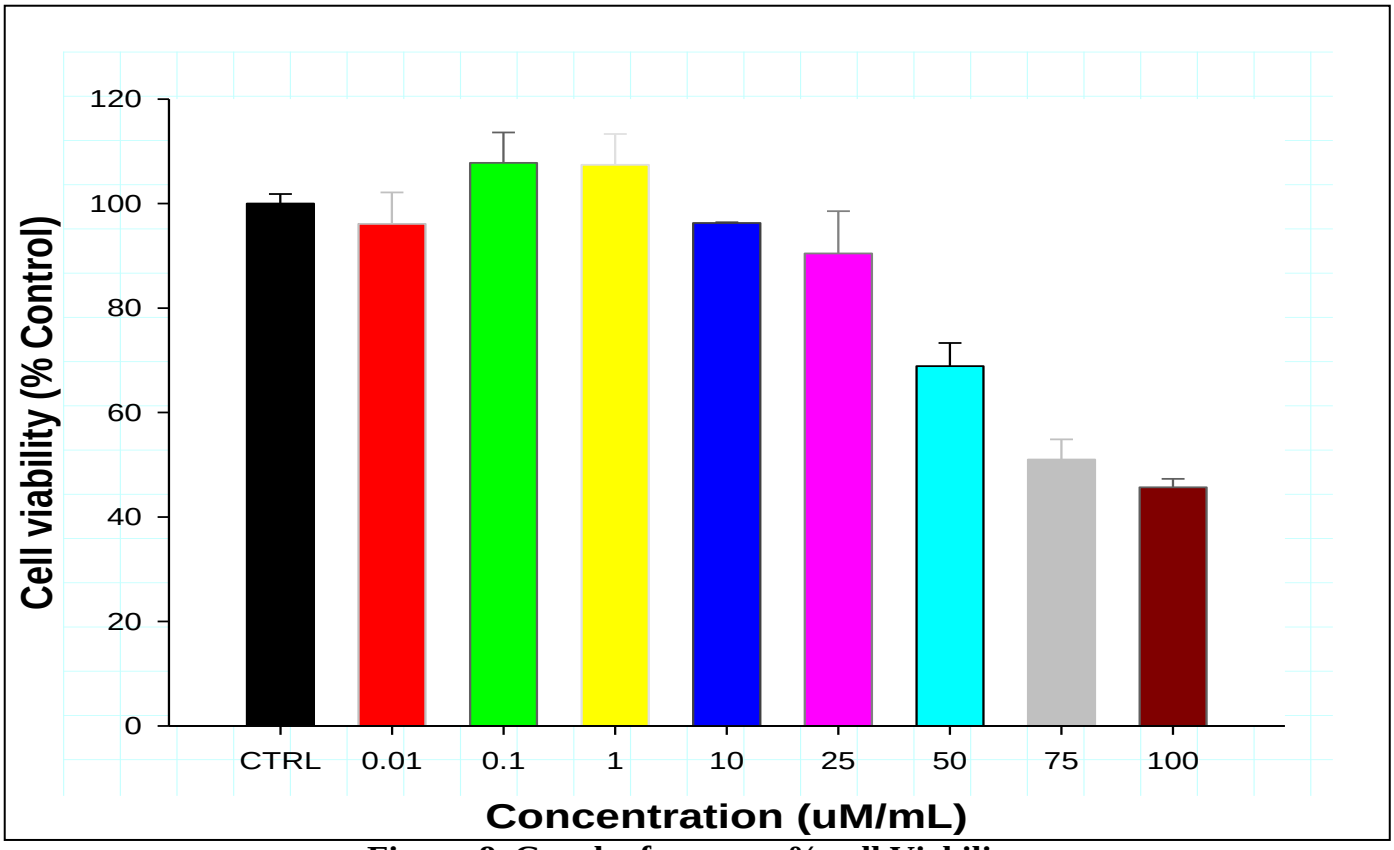


Figure 8. Graph of conc. vs % cell Viability

Table 2. MTT Results: Kavaratamide A

Concentration (µM)	Absorbance			Average	% Cell Survival			Average % Cell Survival
CTRL	0.815	0.757	0.805	0.792	102.86	95.54	101.60	100.00
0.01	0.7	0.775	0.753	0.743	88.35	97.81	95.04	93.73
0.1	0.902	0.825	0.775	0.834	113.84	104.12	97.81	105.26
1	0.757	0.668	0.692	0.706	95.54	84.31	87.34	89.06
10	0.707	0.622	0.877	0.735	89.23	78.50	110.69	92.81
25	0.592	0.633	0.573	0.599	74.72	79.89	72.32	75.64
50	0.421	0.5	0.442	0.454	53.13	63.10	55.78	57.34

75	0.325	0.405	0.296	0.342	41.02	51.11	37.36	43.16
100	0.346	0.286	0.271	0.301	43.67	36.10	34.20	37.99
Log IC50 Value	**2.124**							
IC50 Value	**133.1 µM**							

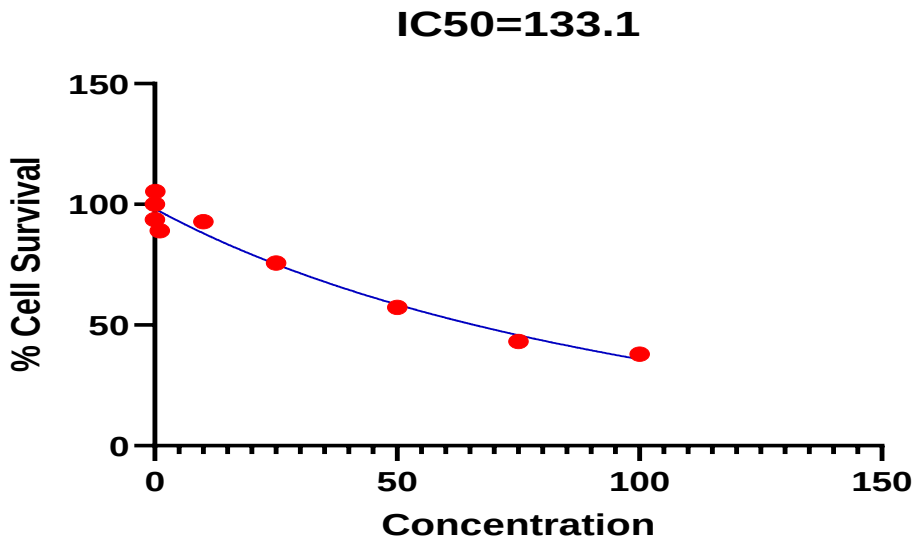


Figure 9. IC50 of Kavaratamide A

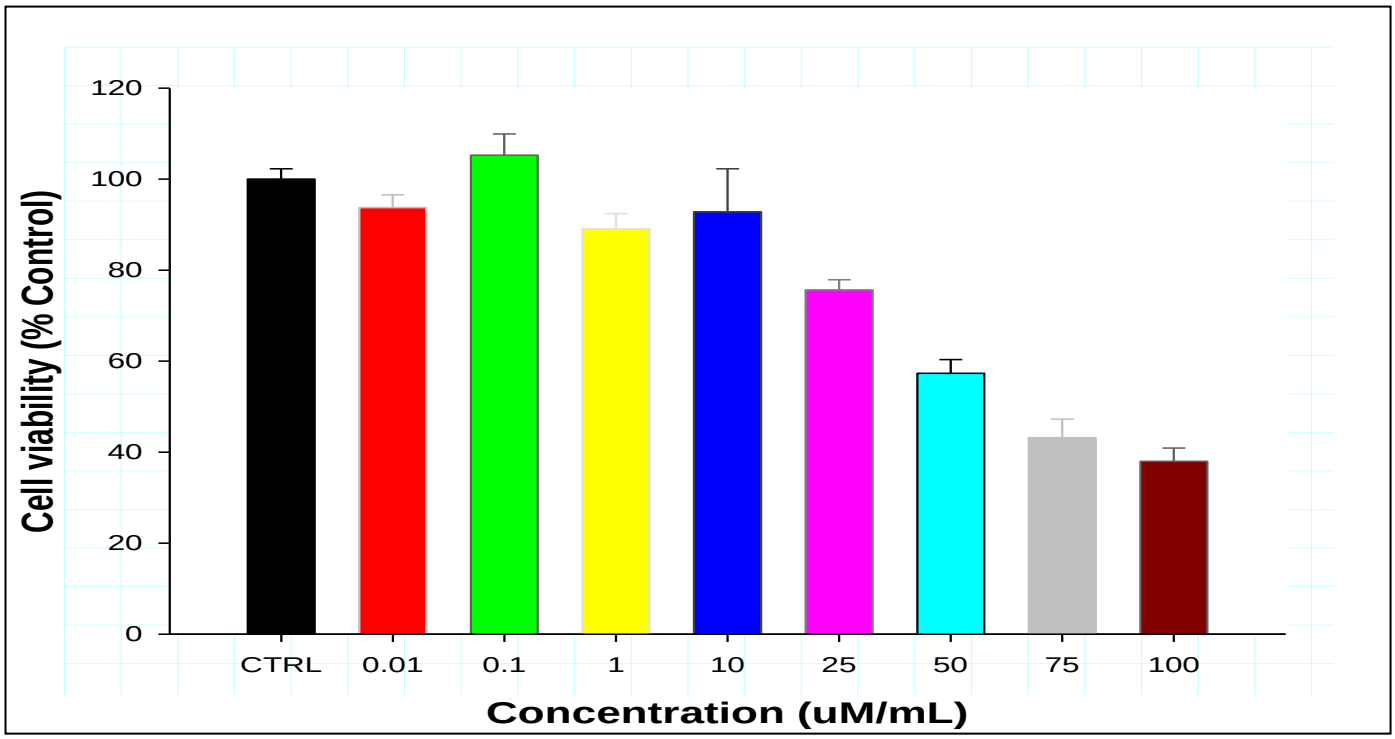


Figure 10. Graph of conc. vs % cell Viability

Table 3. IC50 values of MTT Assay

Sr. No.	Test Item	Log IC50 Value	IC50 Value
1.	C25 Epi-Kavaratamide A	2.619	415.5
2.	Kavaratamide A	2.124	133.1

8. Data Analysis and Report Preparation

GraphPad Prism Ver. 10.00 was used for the statistical analysis. The extracted sheets of statistical analysis are provided along with the report in text format.

Two final copies of final reports are generated. One copy is shared with the Sponsor, and the other copy is submitted to the archives.

Note - After sending a draft report, if no comments are received from the Sponsor within 10 days, the draft report and the raw data shall be archived as such.

9. Archives

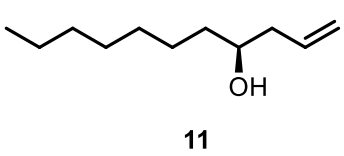
All original raw data, final report and all electronic files generated will be retained in archives. Thereafter, the archived material will be either destroyed or stored for an extended period as per written consent from the sponsor.

10. Study Plan Amendment and Deviations

No study plan amendments or deviations occurred during the study.

6. ^{1}H, ^{13}C and DEPT spectra

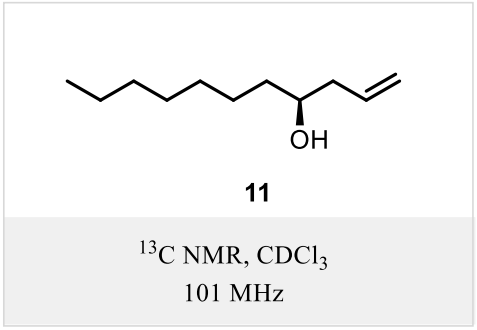
(*S*)-Undec-1-en-4-ol (11):



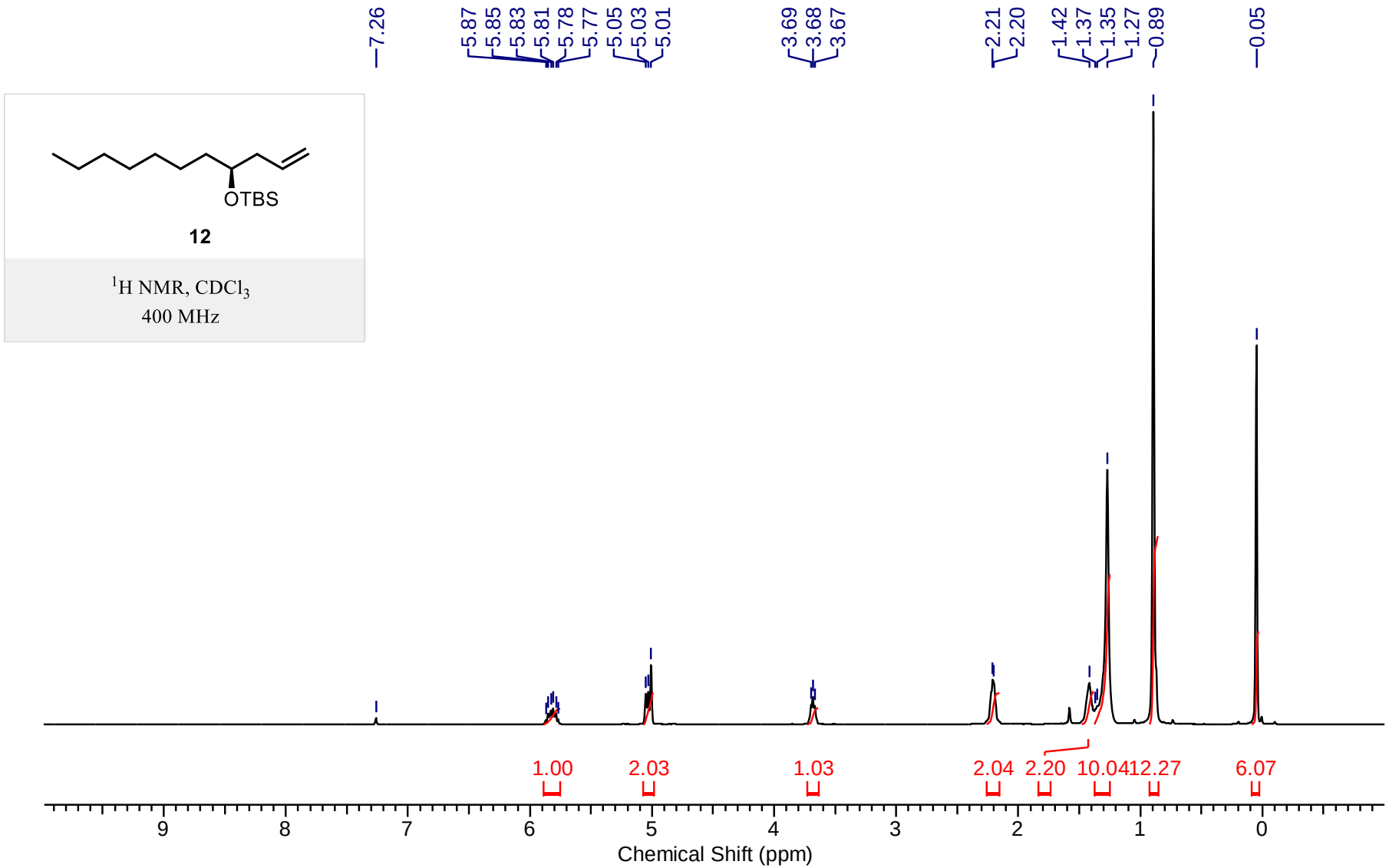
11

^{1}H NMR, $CDCl_3$
400 MHz

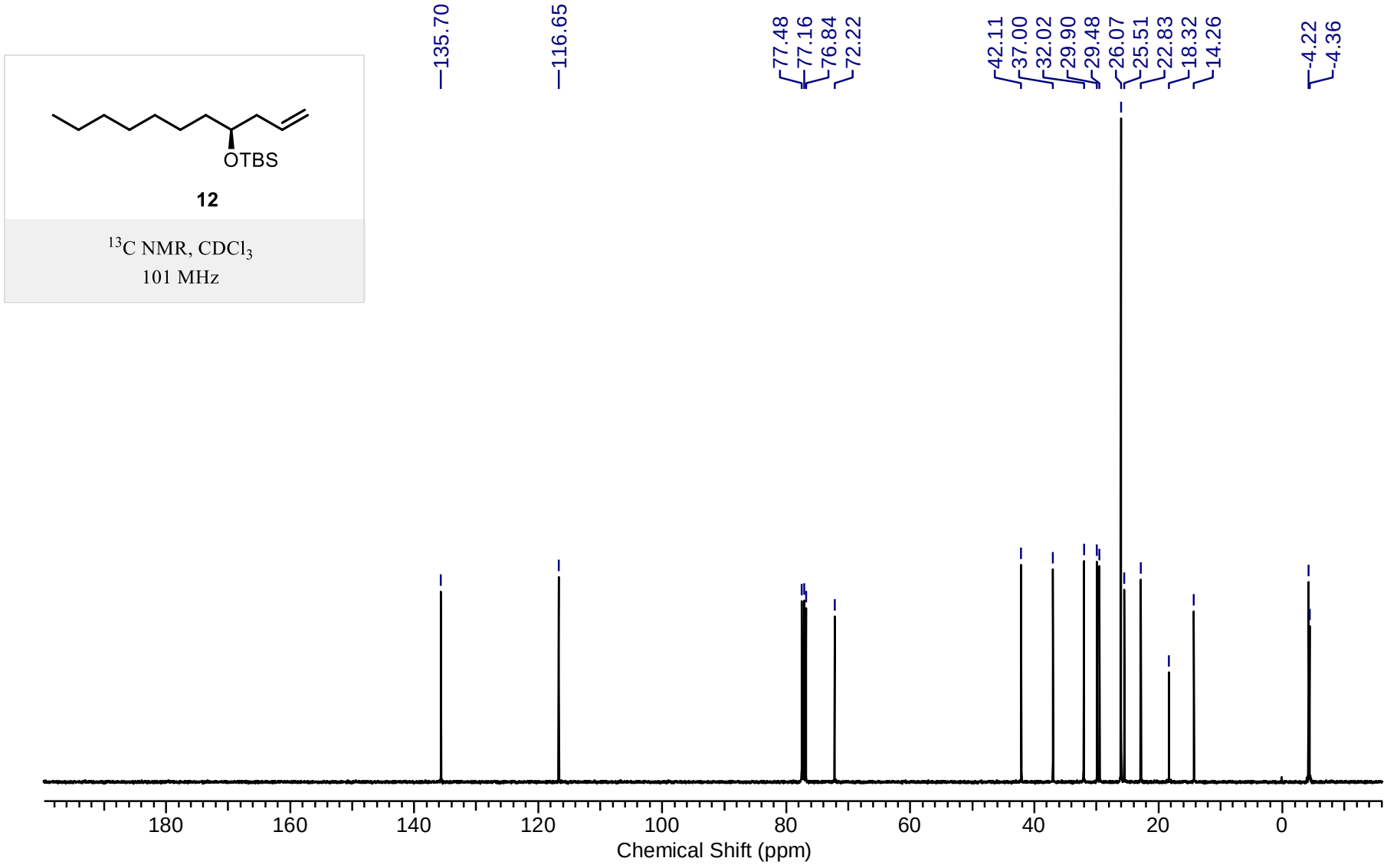
(*S*)-Undec-1-en-4-ol (11):



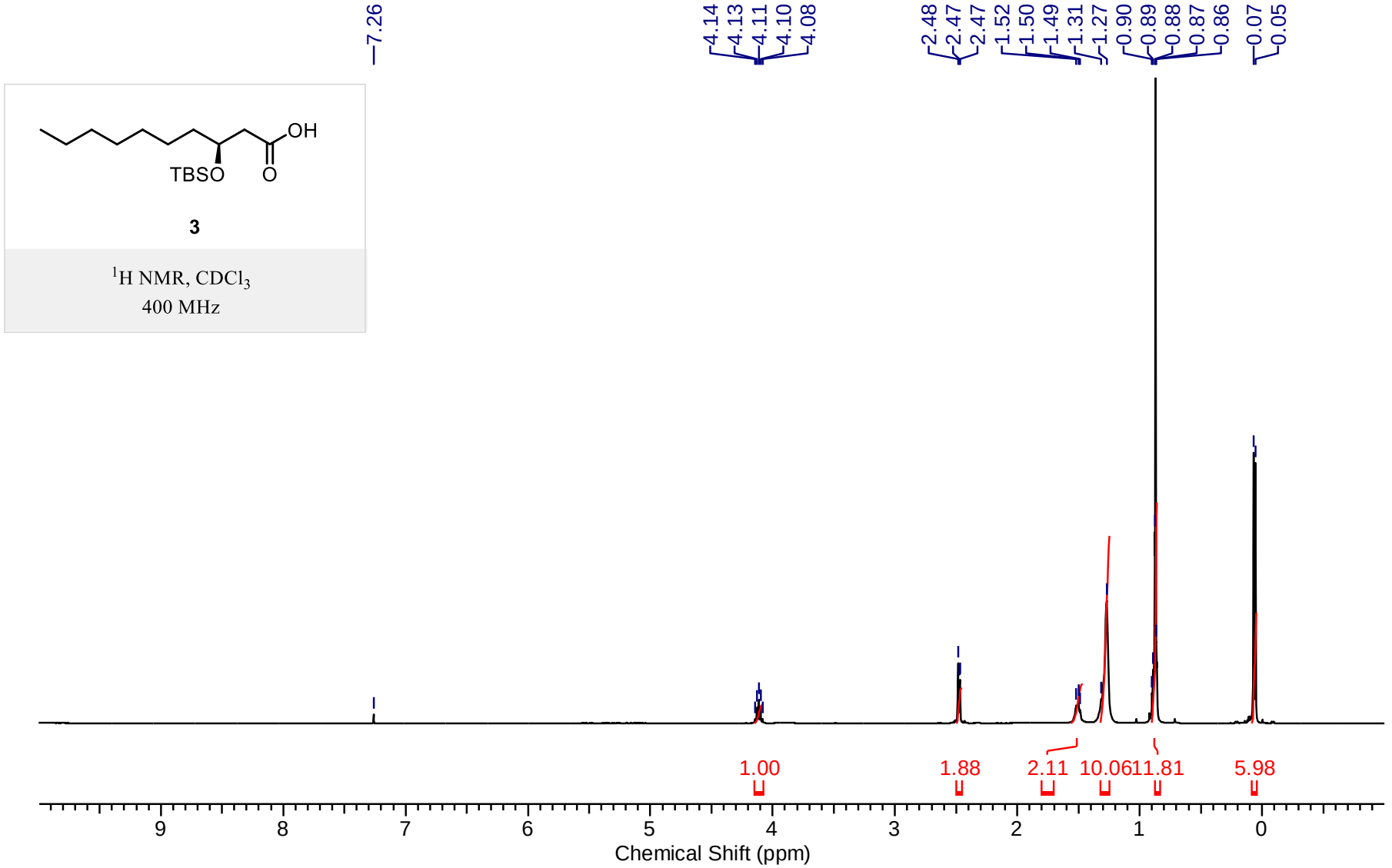
(*S*)-*tert*-Butyldimethyl(undec-1-en-4-yloxy)silane (12):



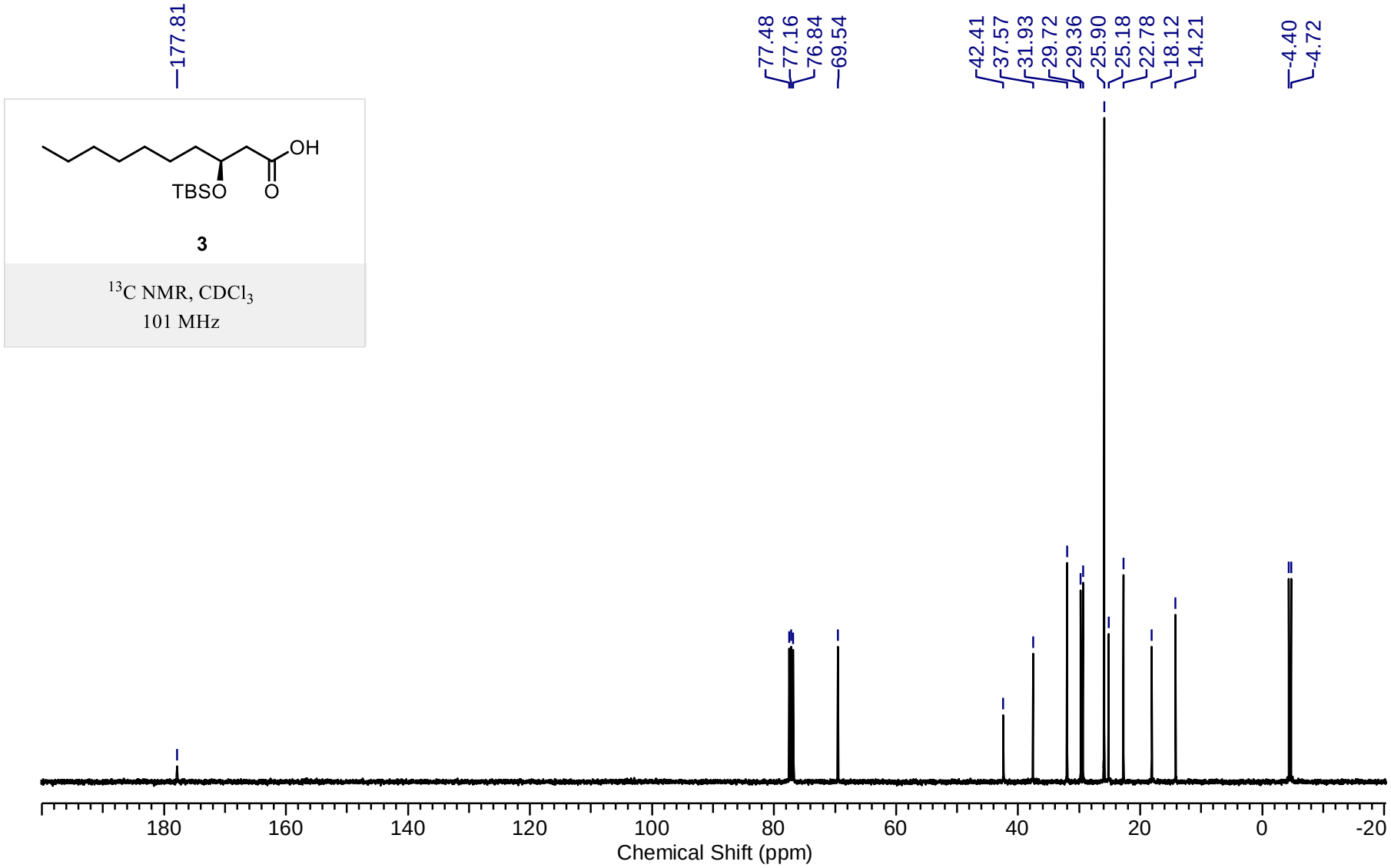
(*S*)-*tert*-Butyldimethyl(undec-1-en-4-yloxy)silane (12):



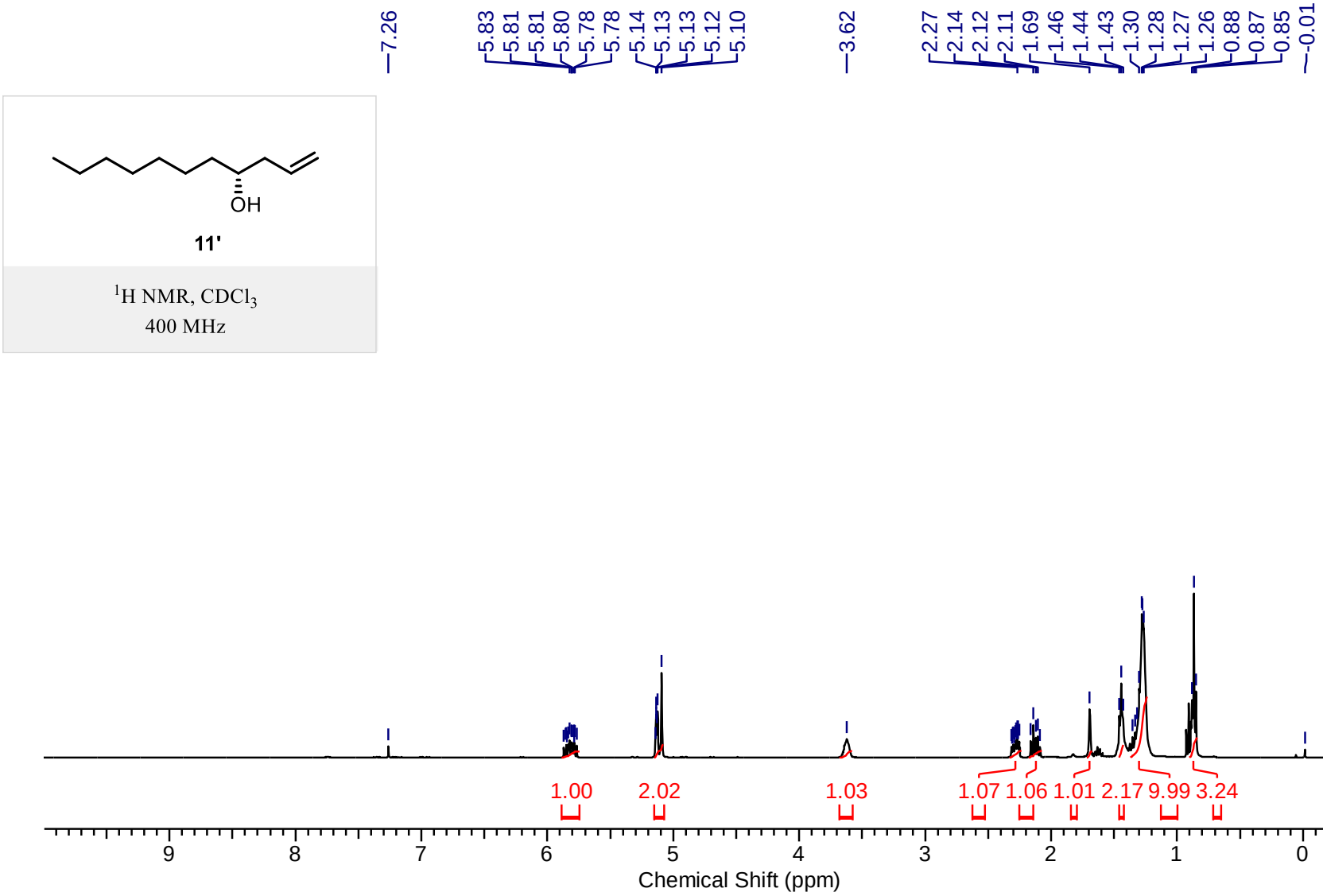
(*S*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoic acid (3):



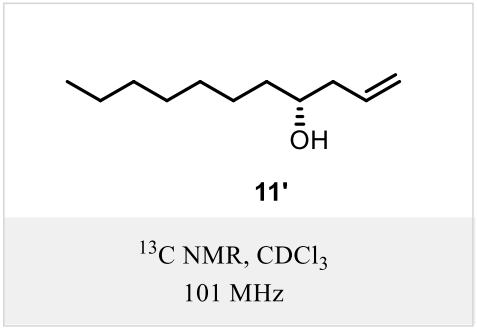
(*S*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoic acid (3):



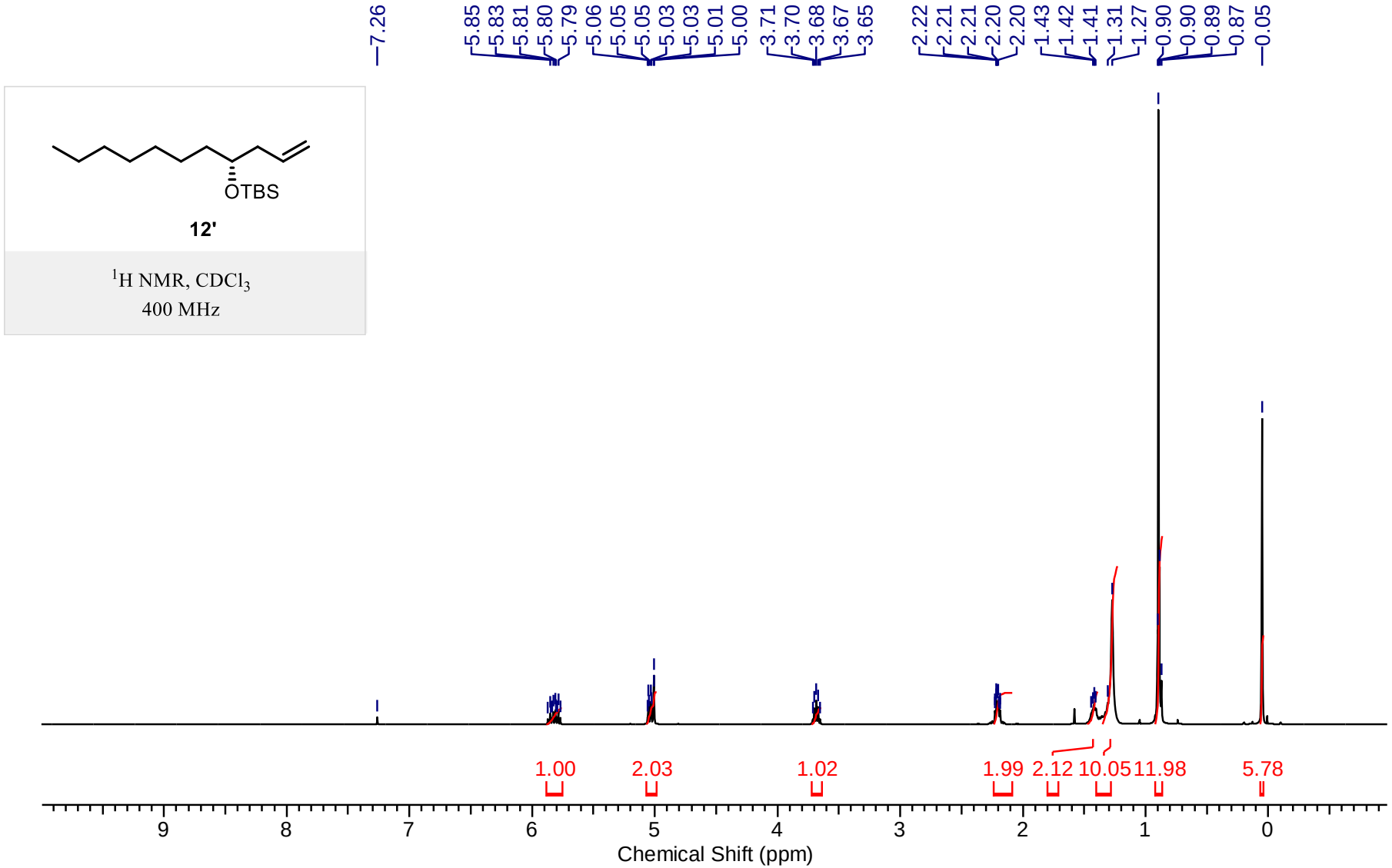
(*R*)-Undec-1-en-4-ol (11'):



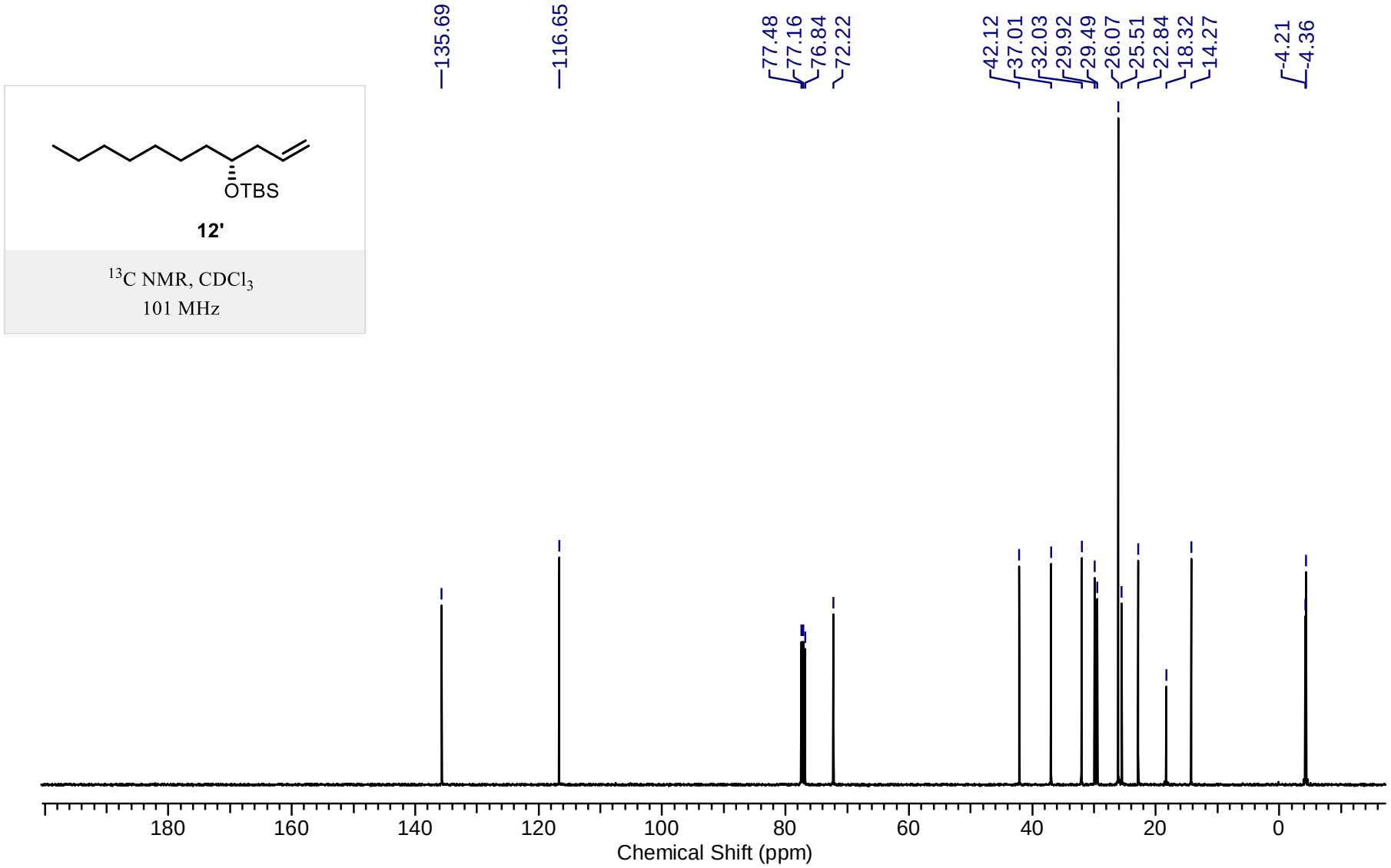
(*R*)-Undec-1-en-4-ol (11'):



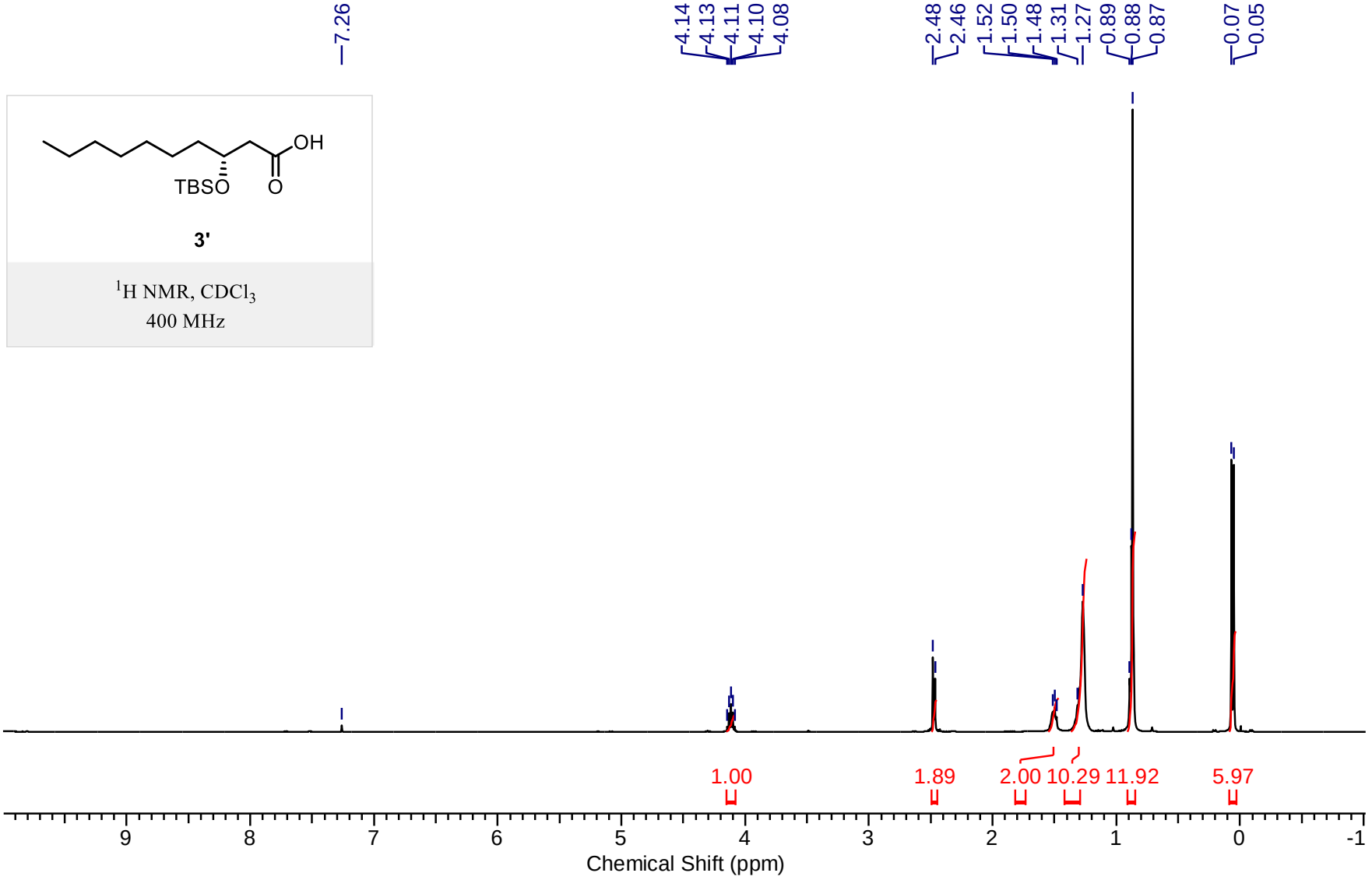
(*R*)-*tert*-Butyldimethyl(undec-1-en-4-yloxy)silane (12'):



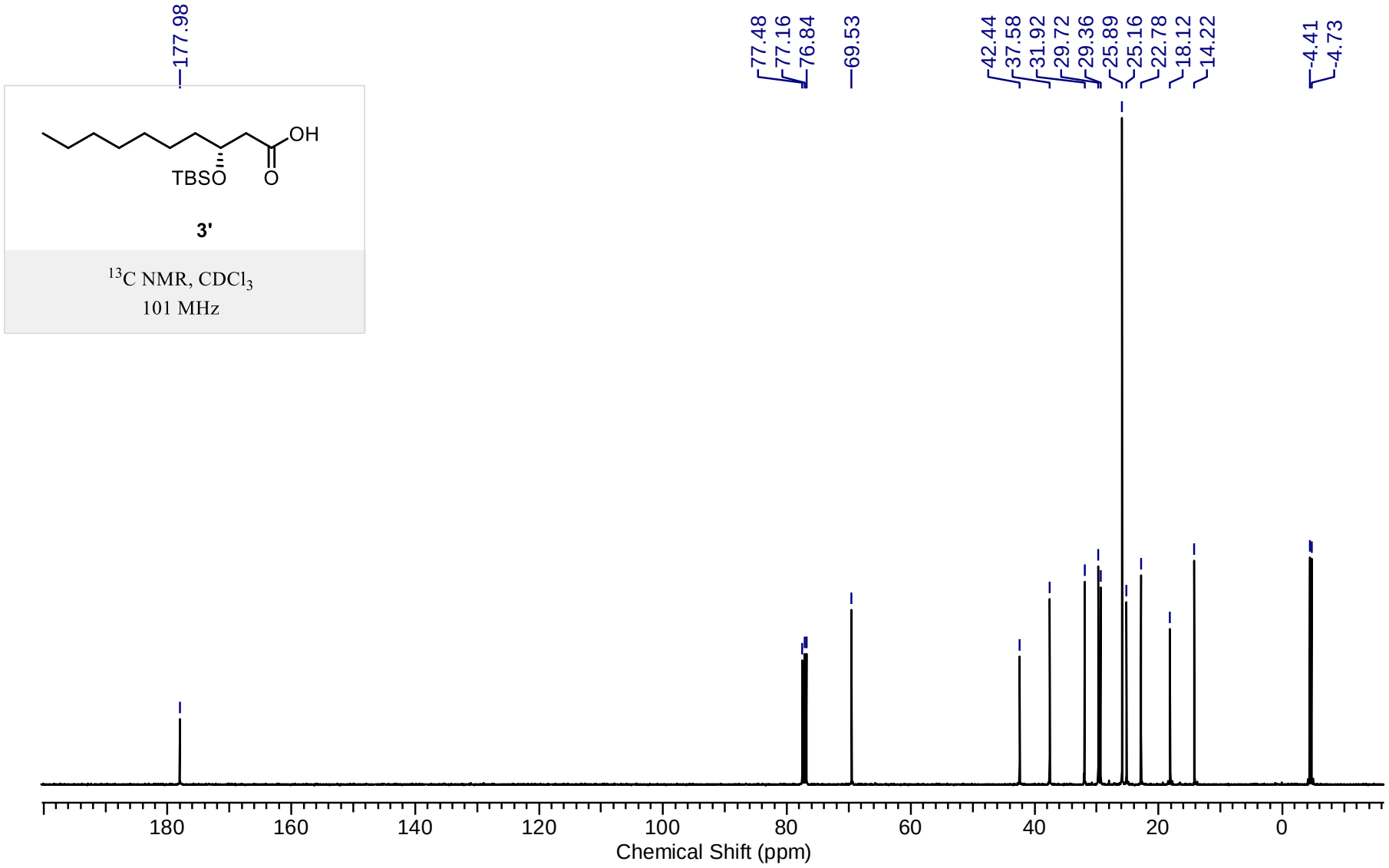
(*R*)-*tert*-Butyldimethyl(undec-1-en-4-yloxy)silane (12'):



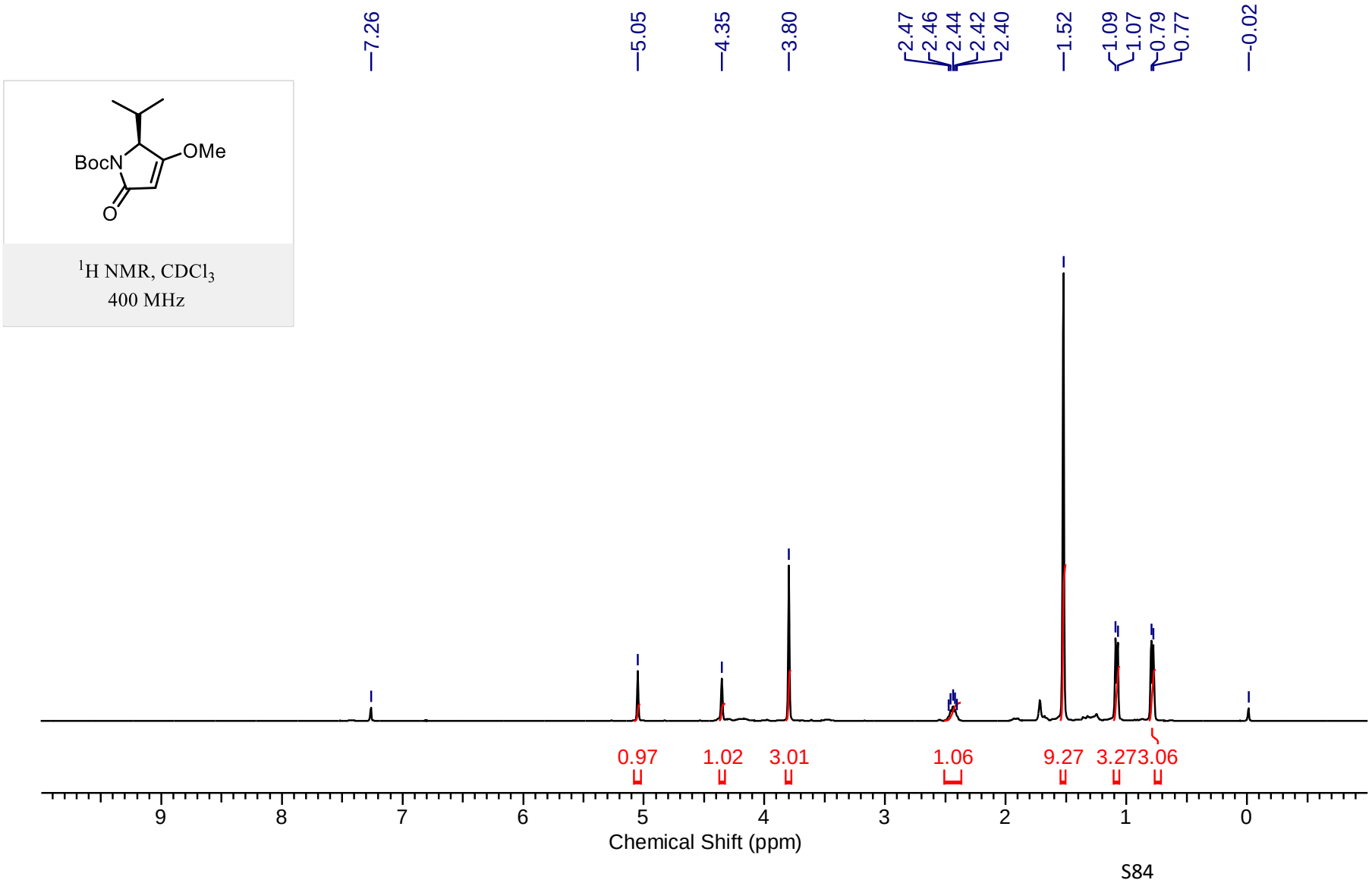
(*R*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoic acid (3'):



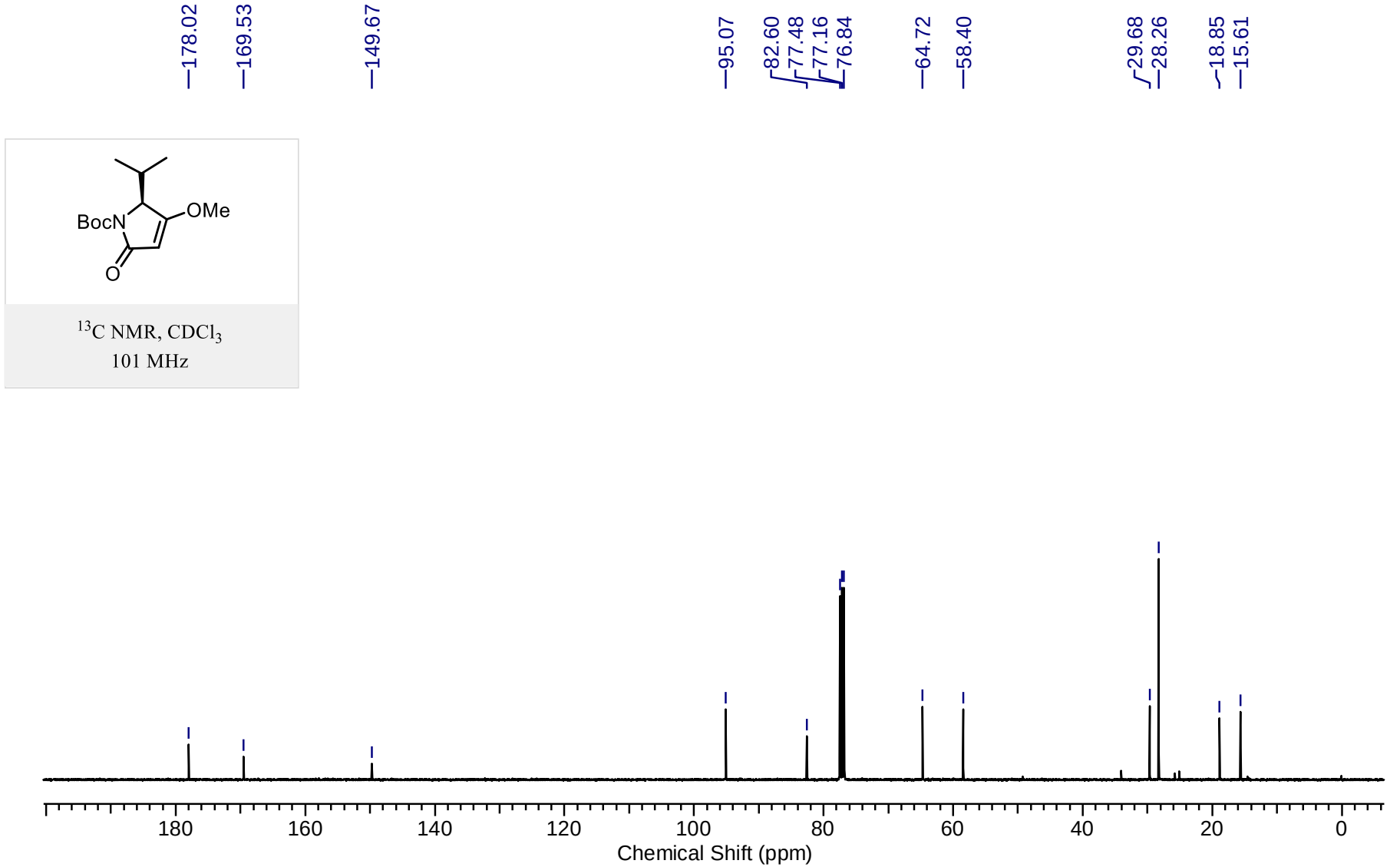
(*R*)-3-((*tert*-Butyldimethylsilyl)oxy)decanoic acid (3'):



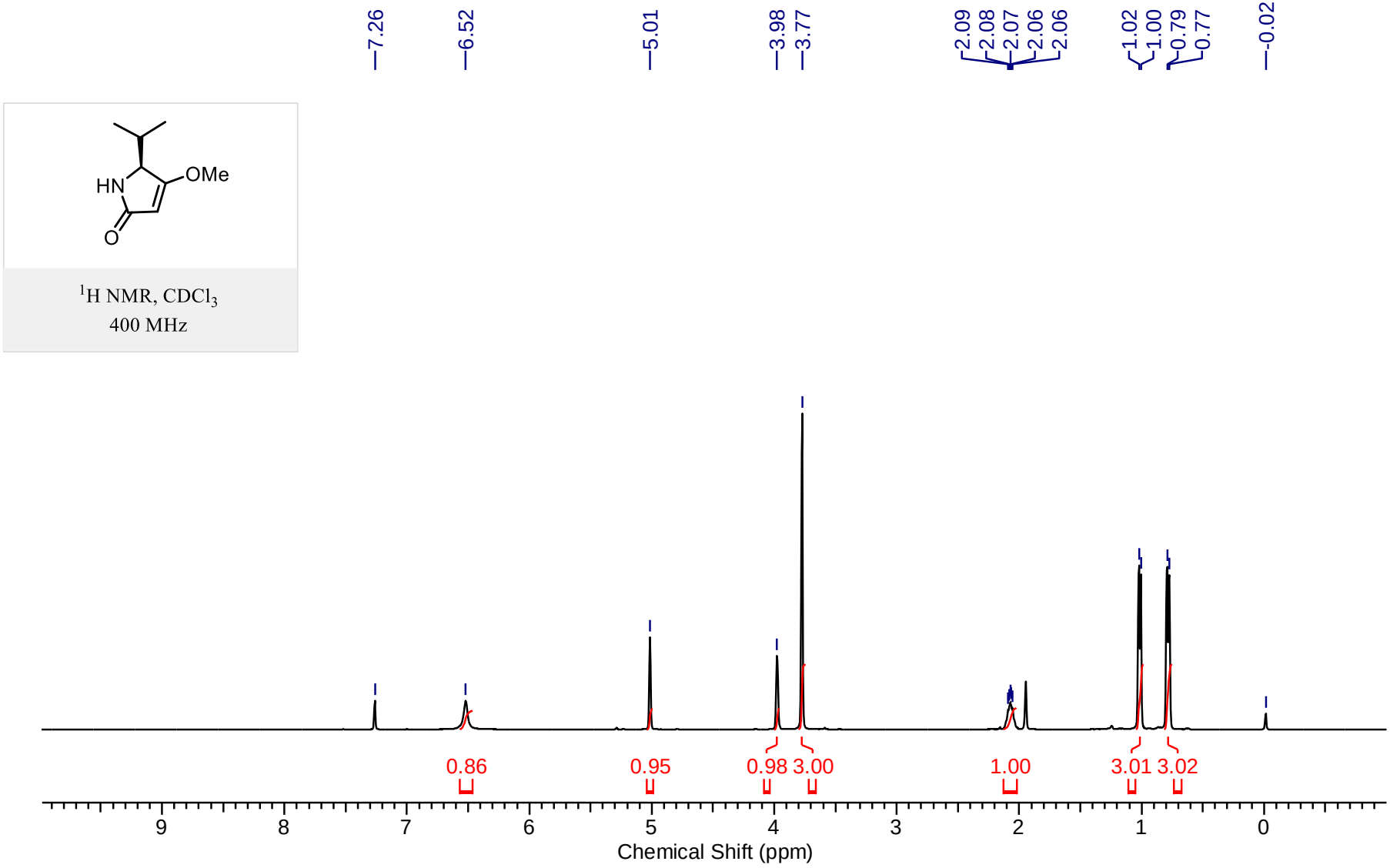
Tert-butyl (_S_)-2-isopropyl-3-methoxy-5-oxo-2,5-dihydro-1_H_-pyrrole-1-carboxylate (7):



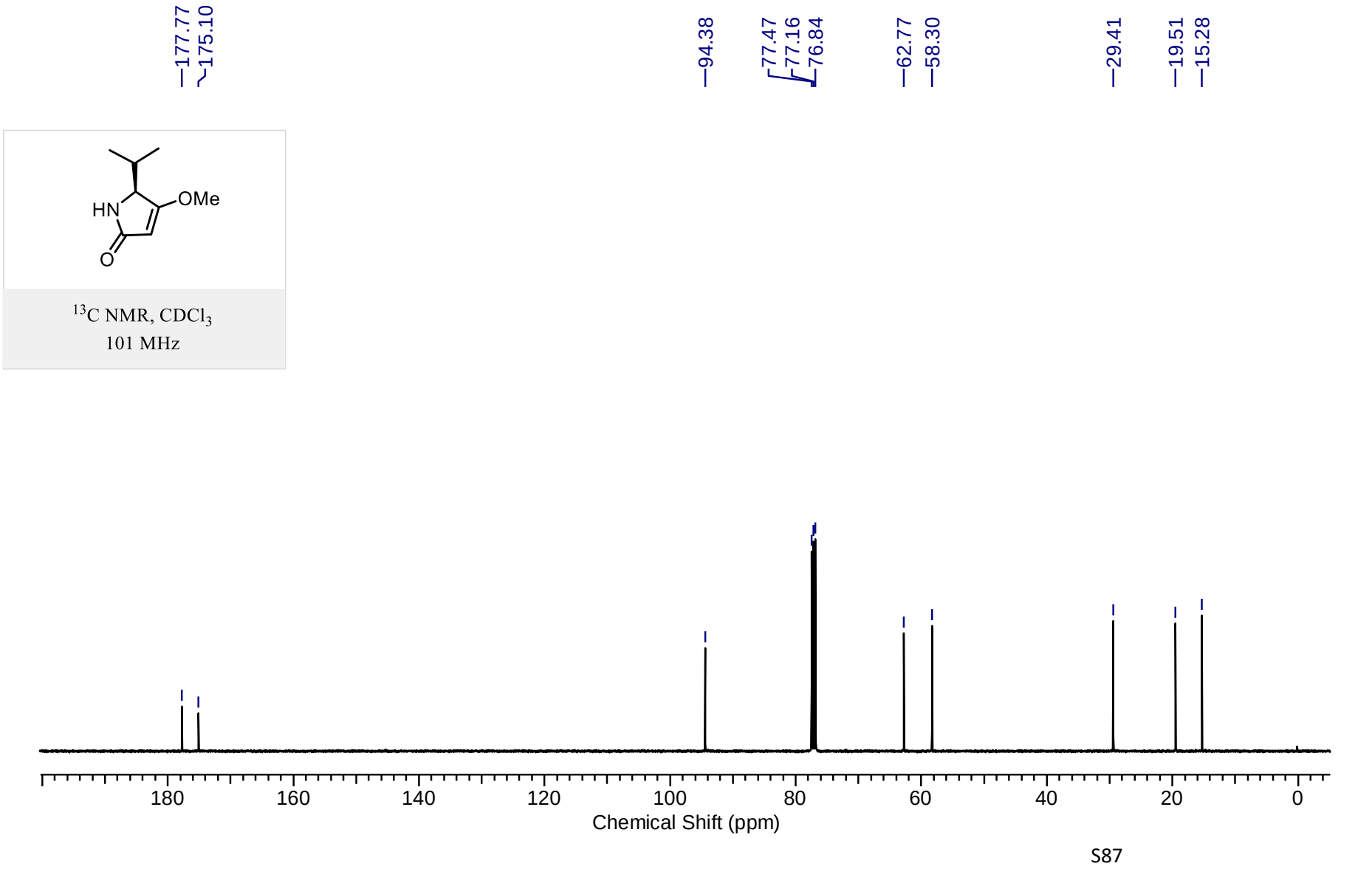
Tert-butyl (_S_)-2-isopropyl-3-methoxy-5-oxo-2,5-dihydro-1_H_-pyrrole-1-carboxylate (7):



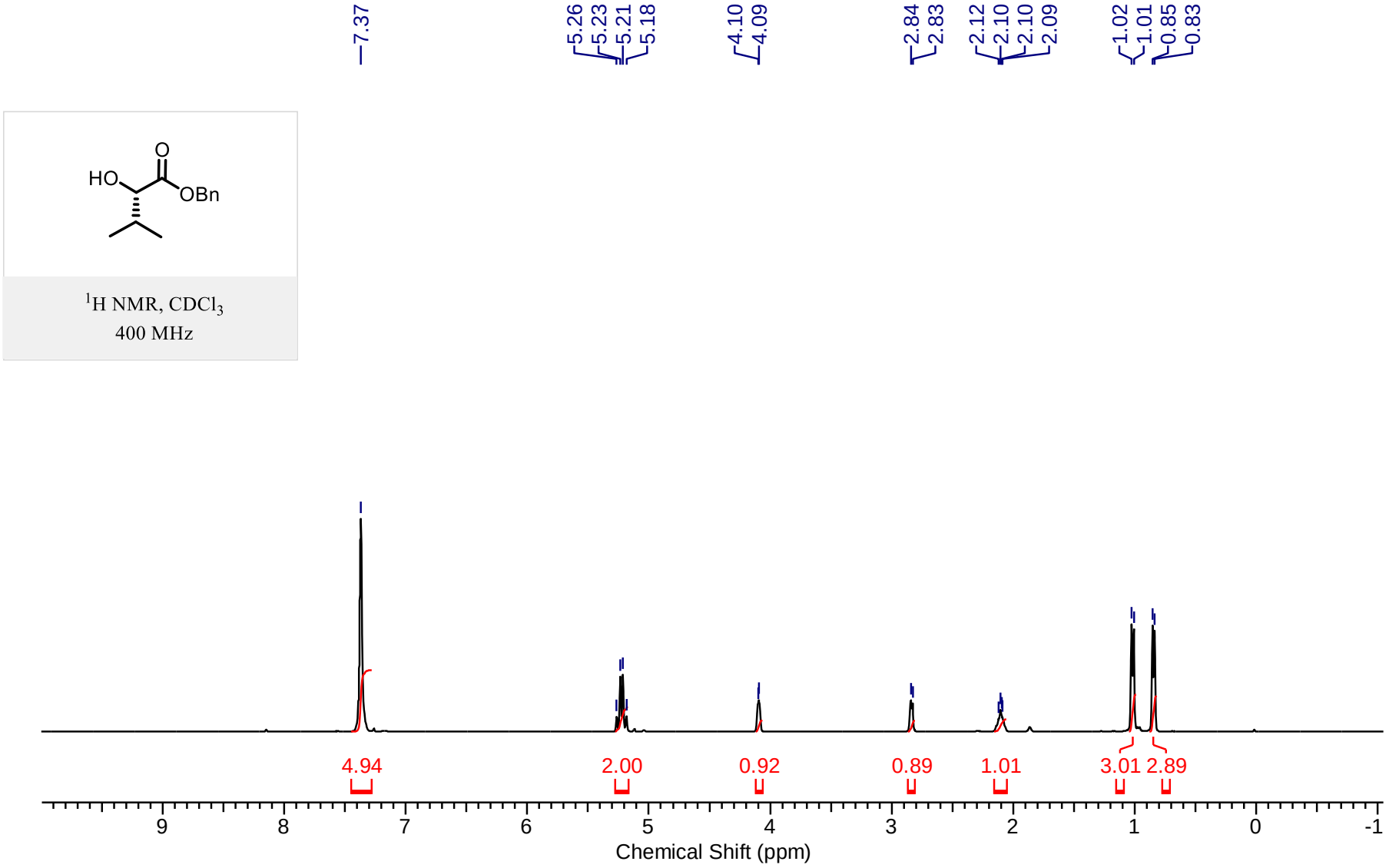
(*S*)-5-Isopropyl-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (10):



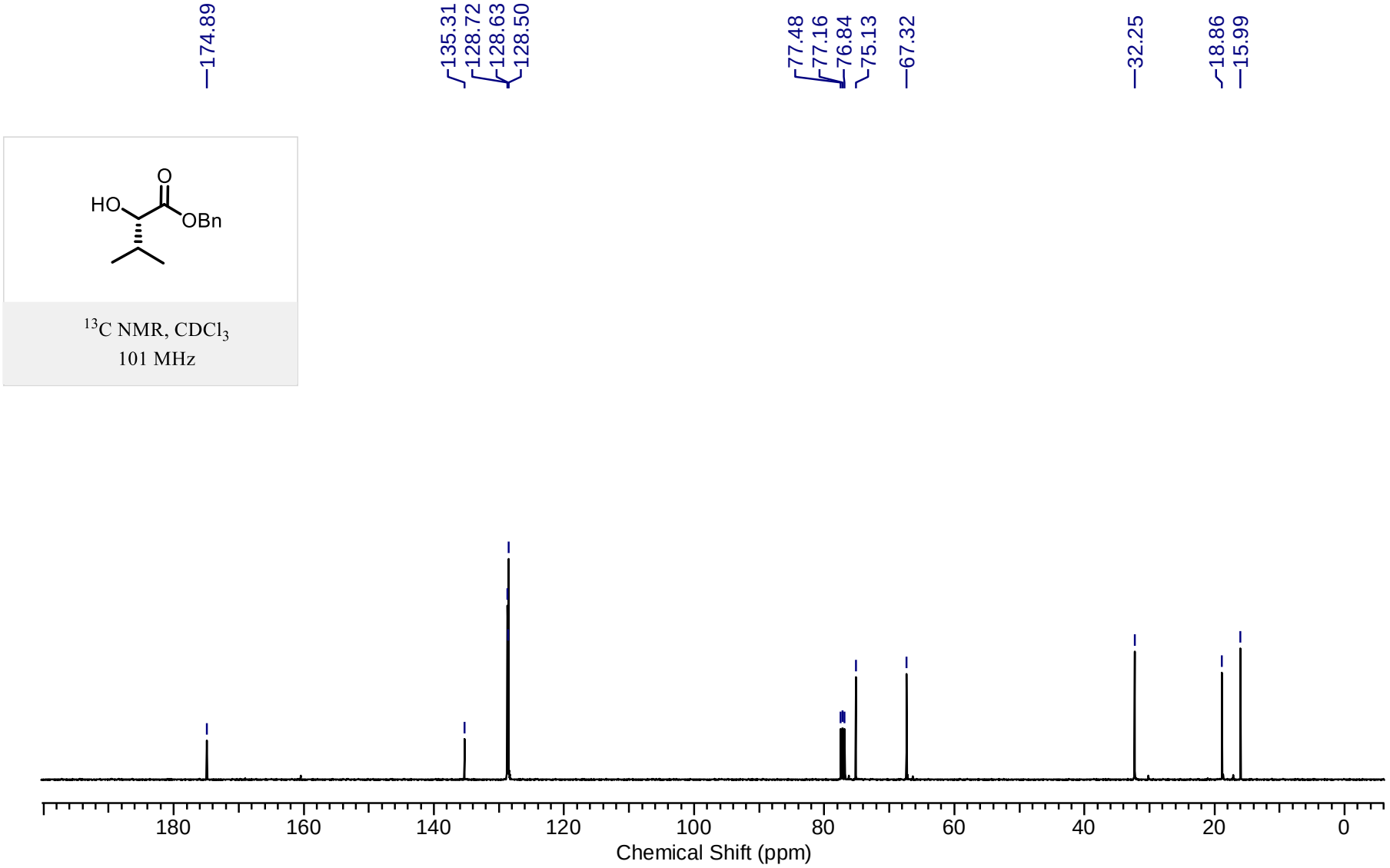
(*S*)-5-Isopropyl-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (10):



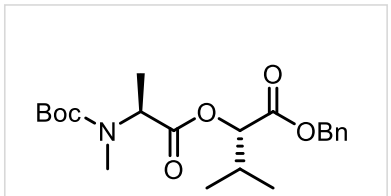
Benzyl (*S*)-2-hydroxy-3-methylbutanoate (15):



Benzyl (*S*)-2-hydroxy-3-methylbutanoate (15):

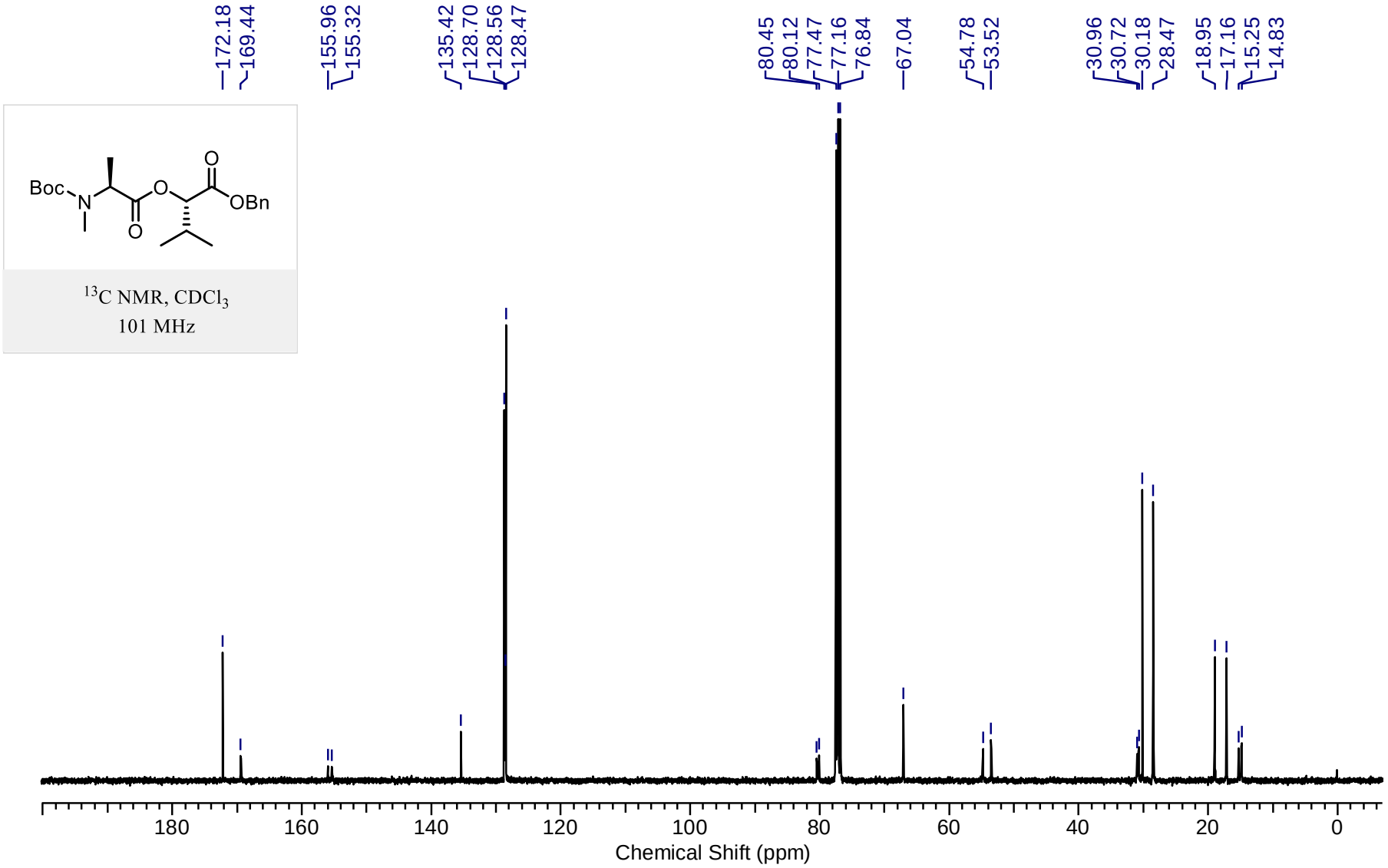


Benzyl (*S*)-2-((*N*-(tert-butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoate (16):

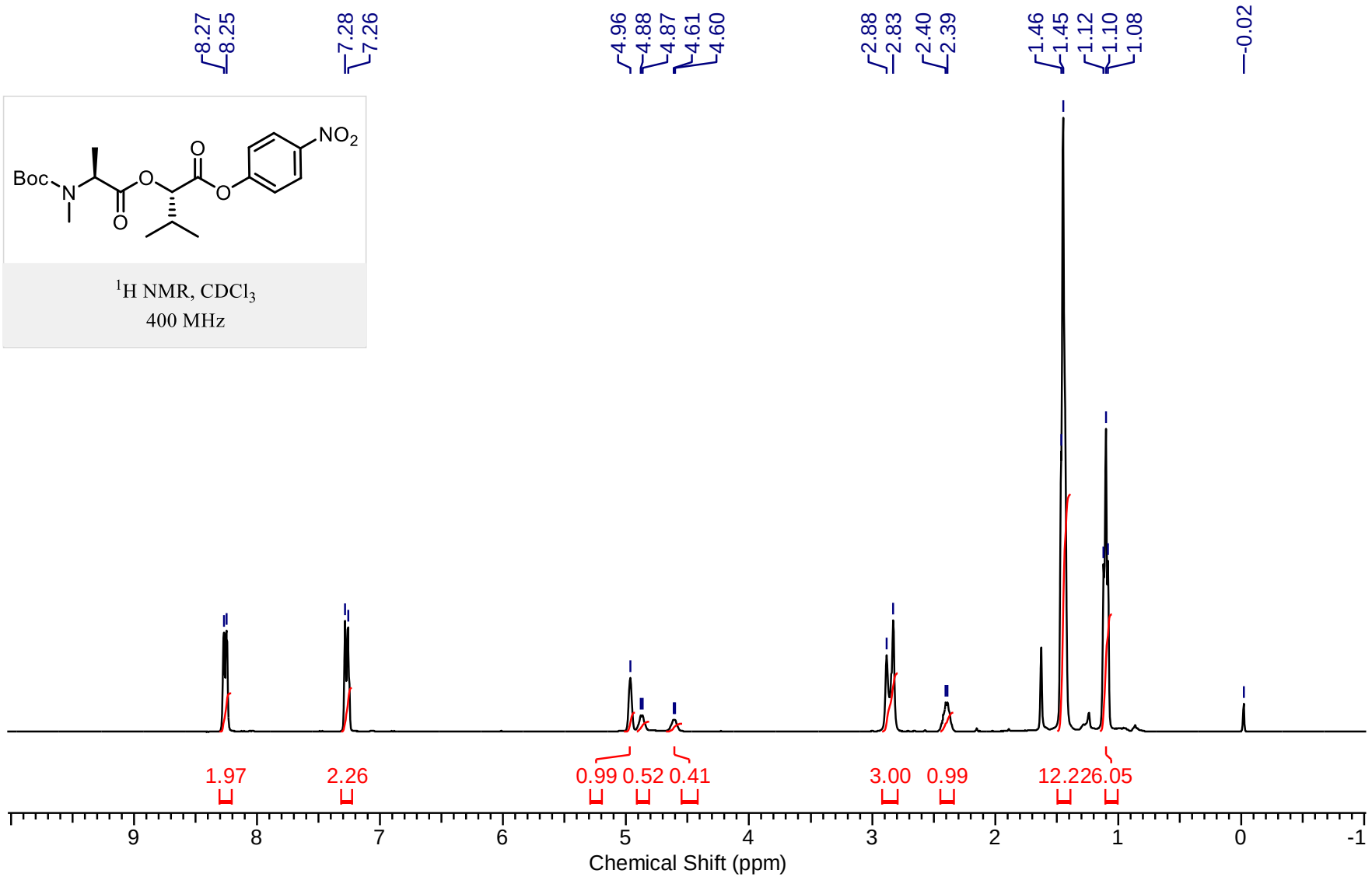


^{1}H NMR, $CDCl_3$
400 MHz

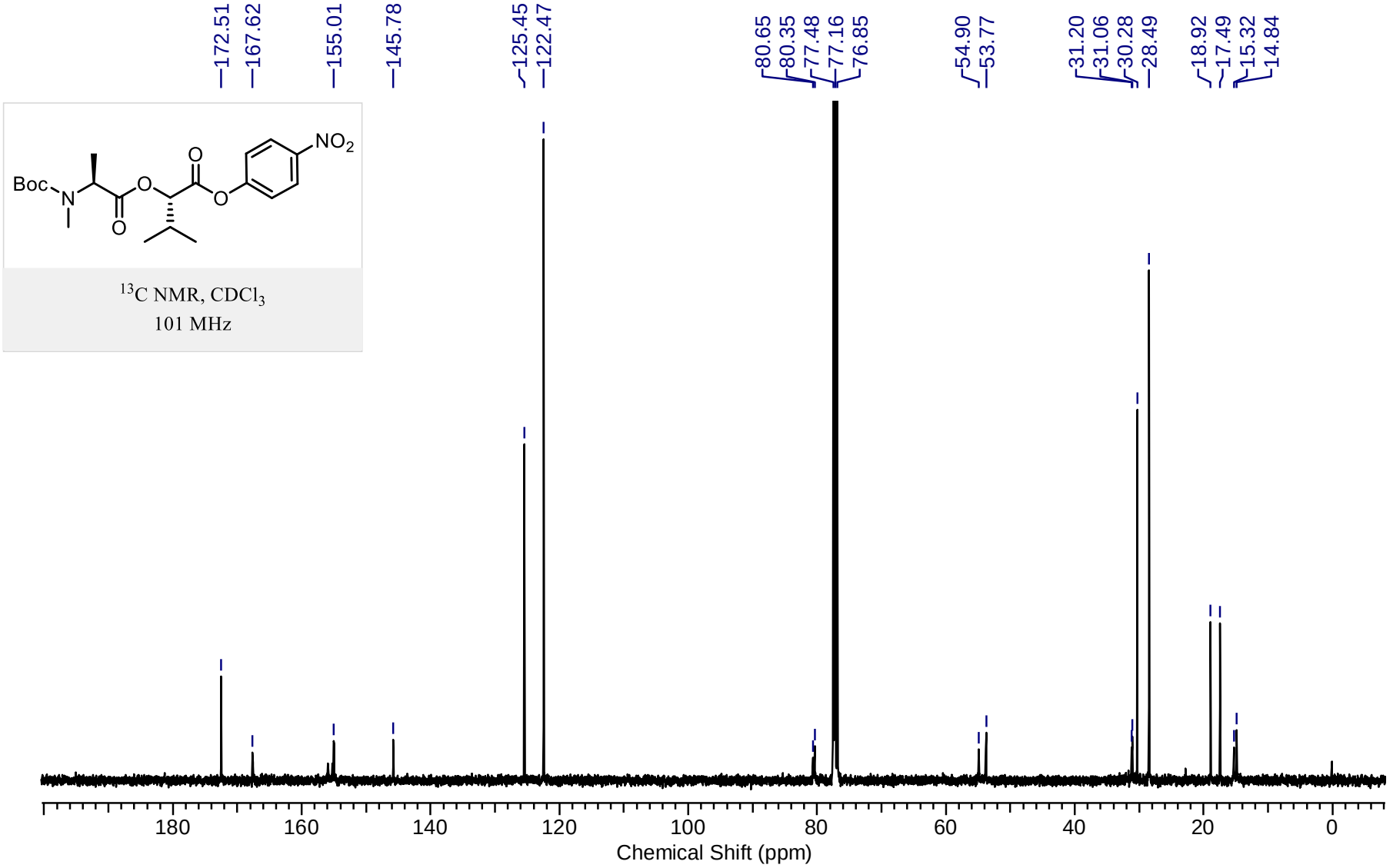
Benzyl (*S*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoate (16):



4-Nitrophenyl (*S*)-2-((*N*-(tert-butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoate (18):

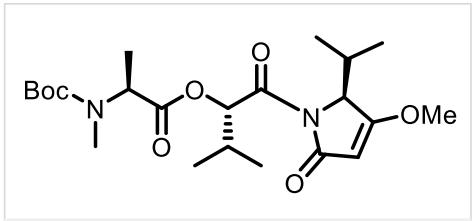


4-Nitrophenyl (*S*)-2-((*N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-alanyl)oxy)-3-methylbutanoate (18):



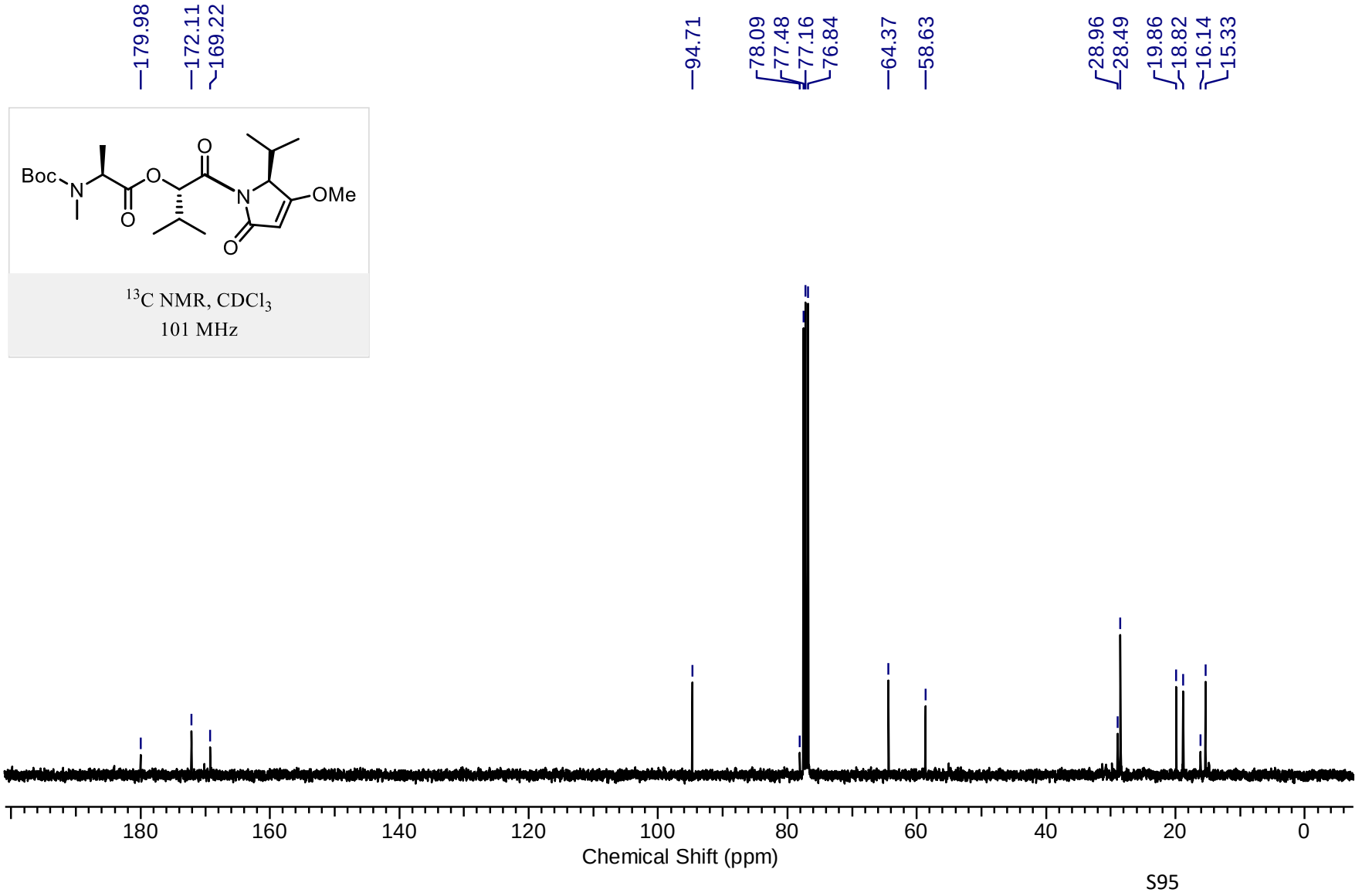
^{13}C NMR, $CDCl_3$
101 MHz

(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(tert-butoxycarbonyl)-*N*-methyl-*L*-alaninate (19):

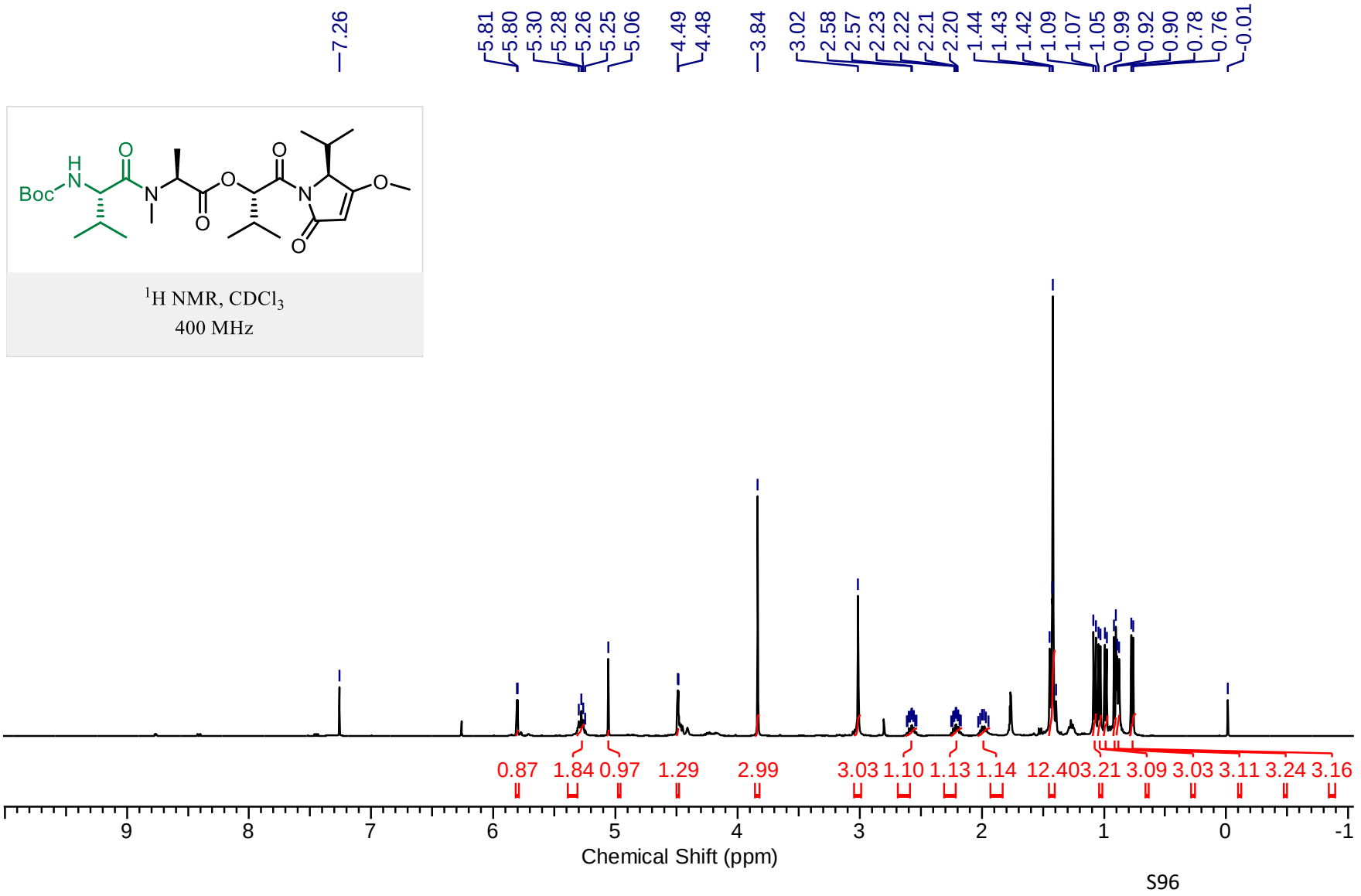


^{1}H NMR, $CDCl_3$
400 MHz

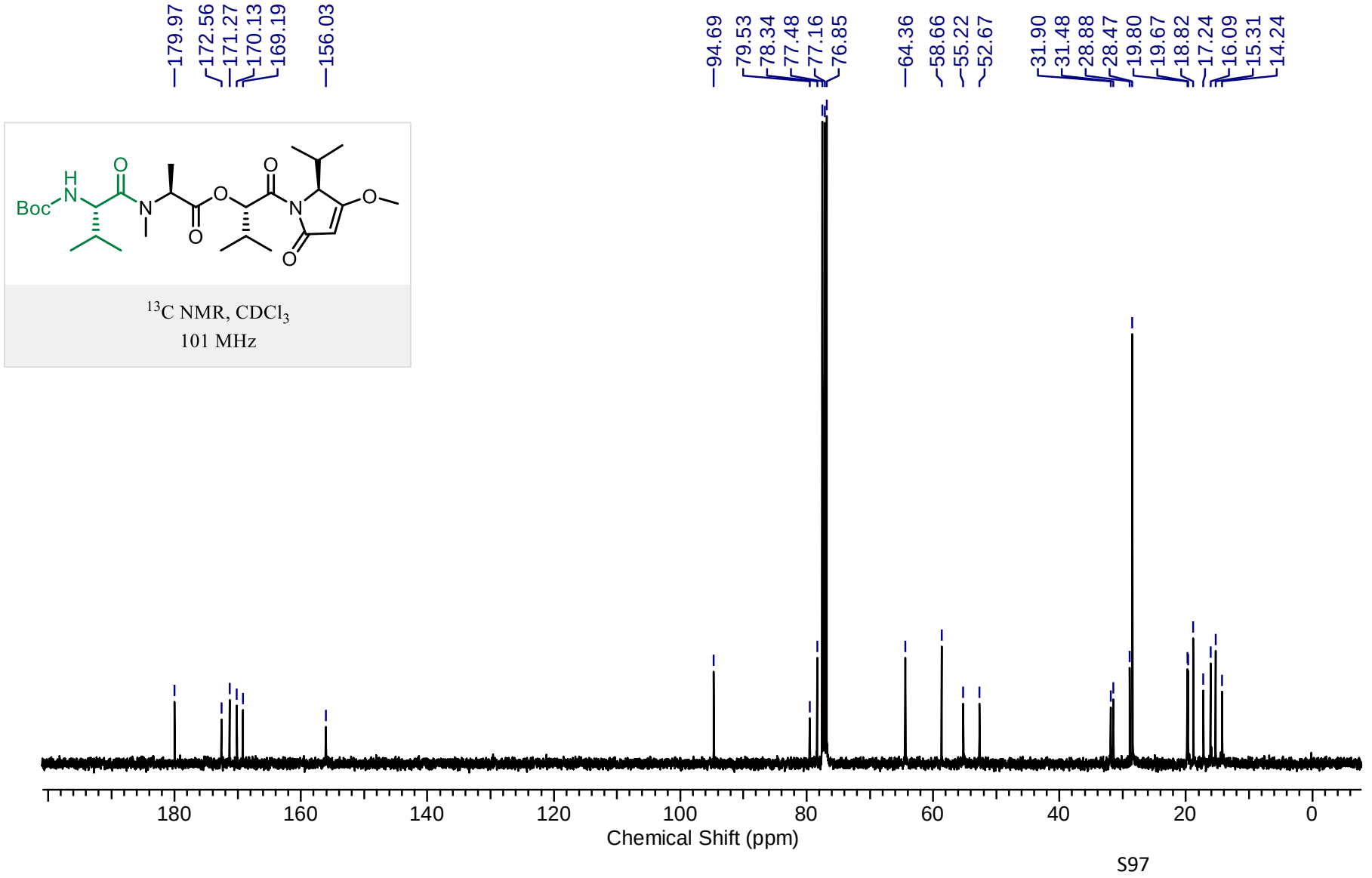
(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(tert-butoxycarbonyl)-*N*-methyl-*L*-alaninate (19):



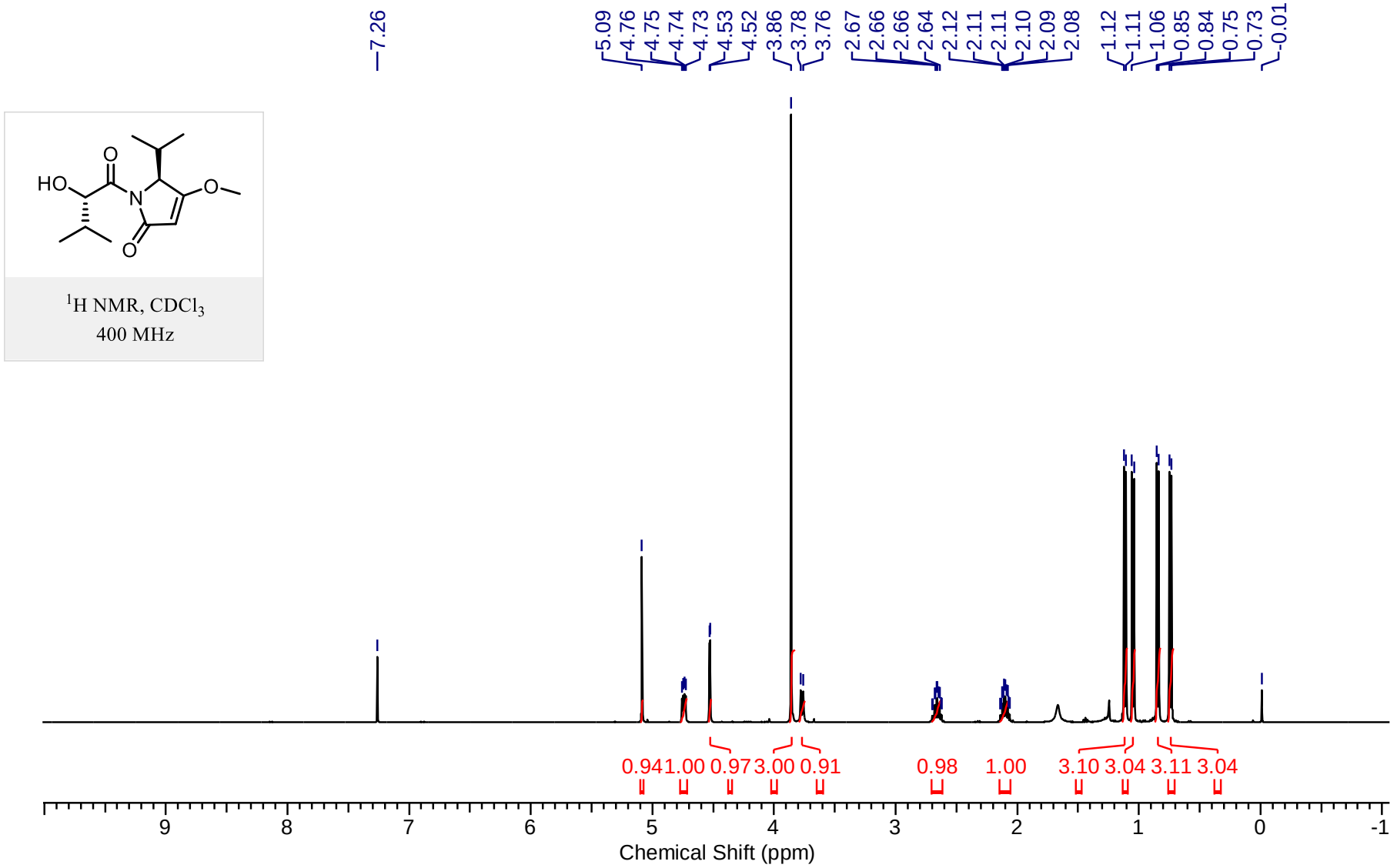
(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-((*tert*-butoxycarbonyl)-L-valyl)-*N*-methyl-*L*-alaninate (20):



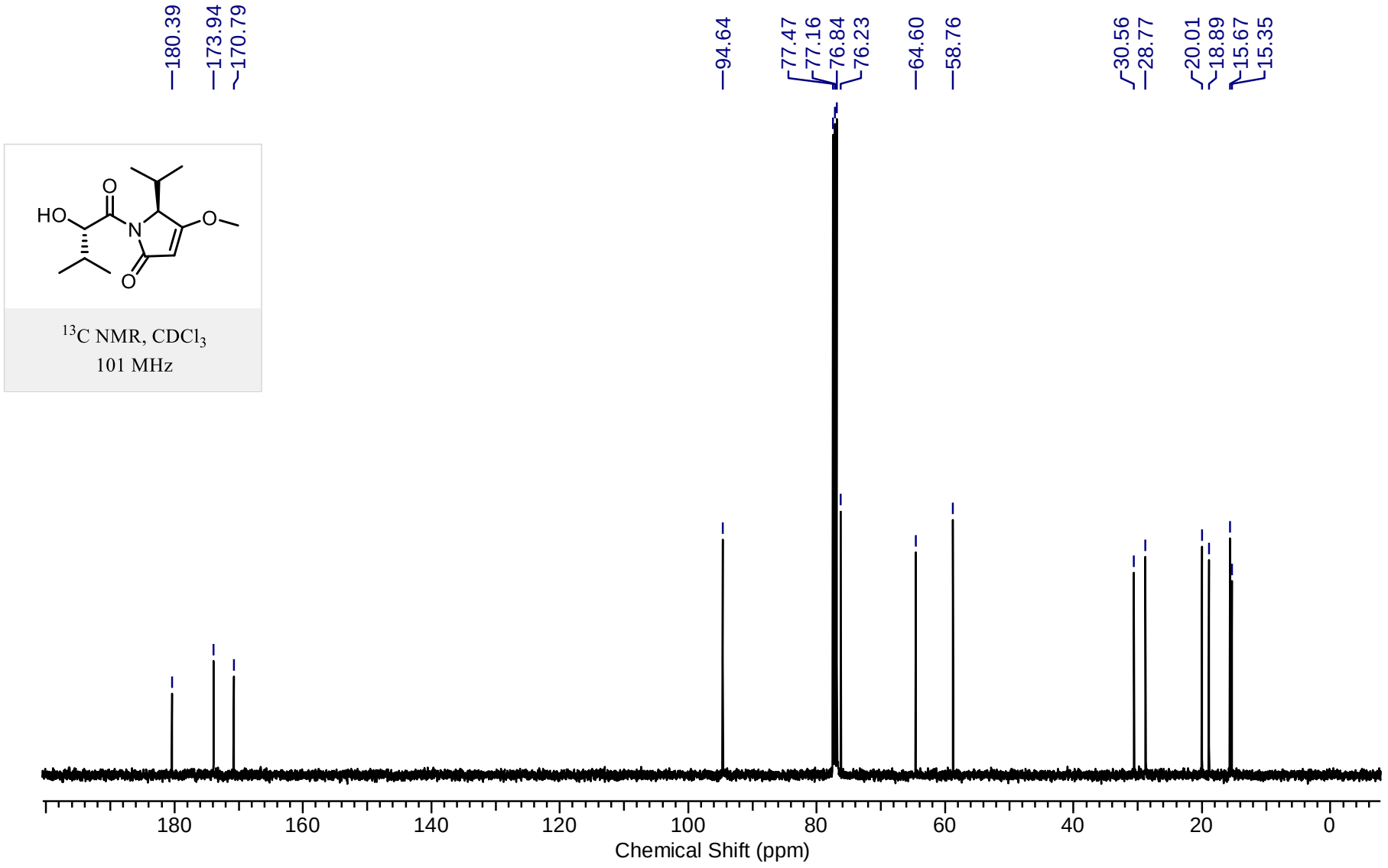
(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-((*tert*-butoxycarbonyl)-L-valyl)-*N*-methyl-*L*-alaninate (20):



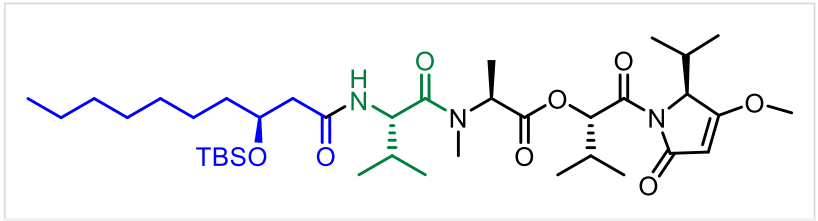
(*S*)-1-((*S*)-2-Hydroxy-3-methylbutanoyl)-5-isopropyl-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (20a):



(*S*)-1-((*S*)-2-Hydroxy-3-methylbutanoyl)-5-isopropyl-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (20a):

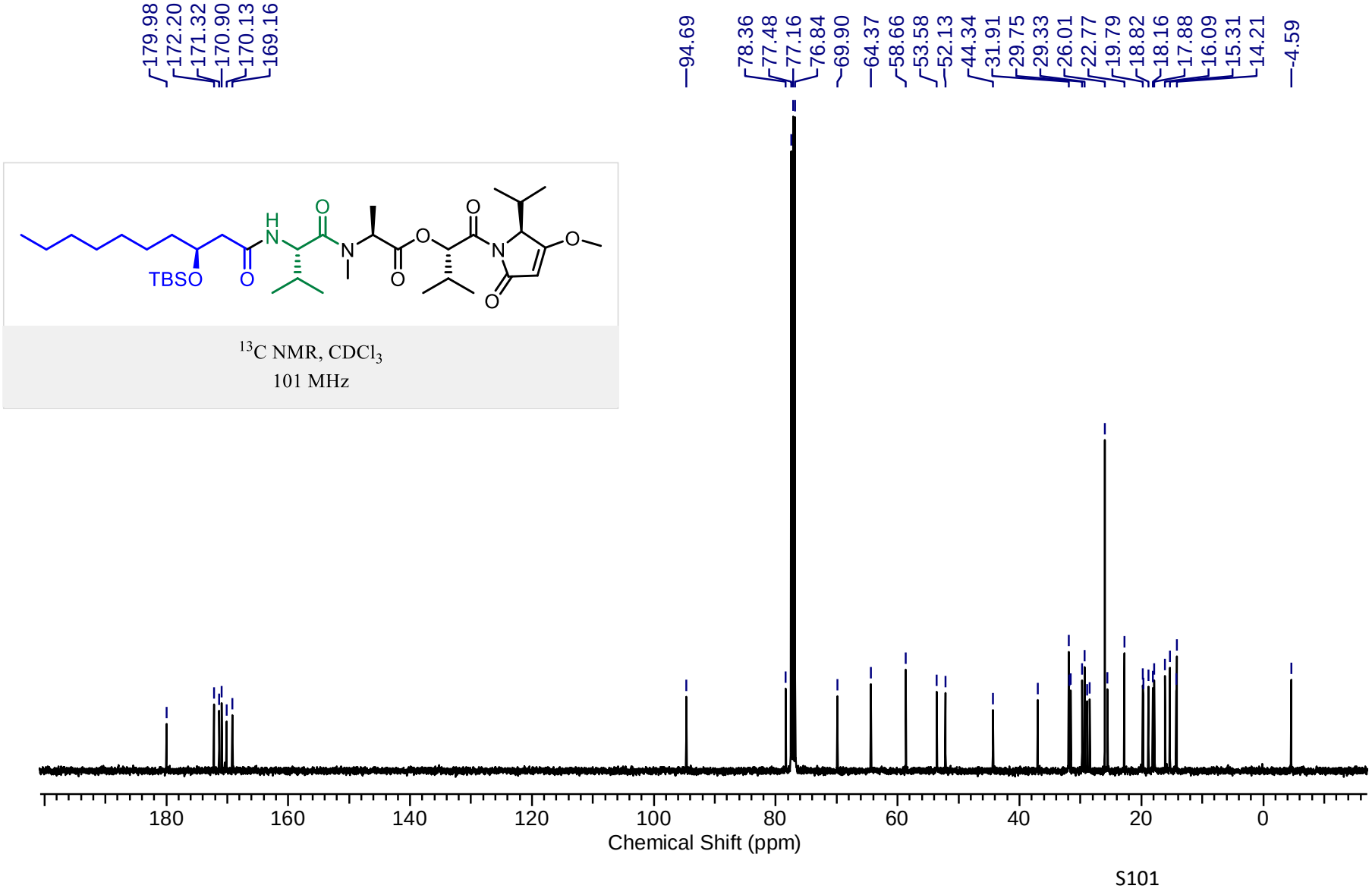


(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*S*)-3-((*tert*-butyldimethylsilyl)oxy)decanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (21):

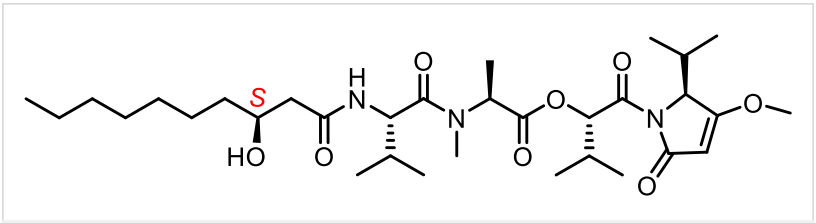


^{1}H NMR, $CDCl_3$
400 MHz

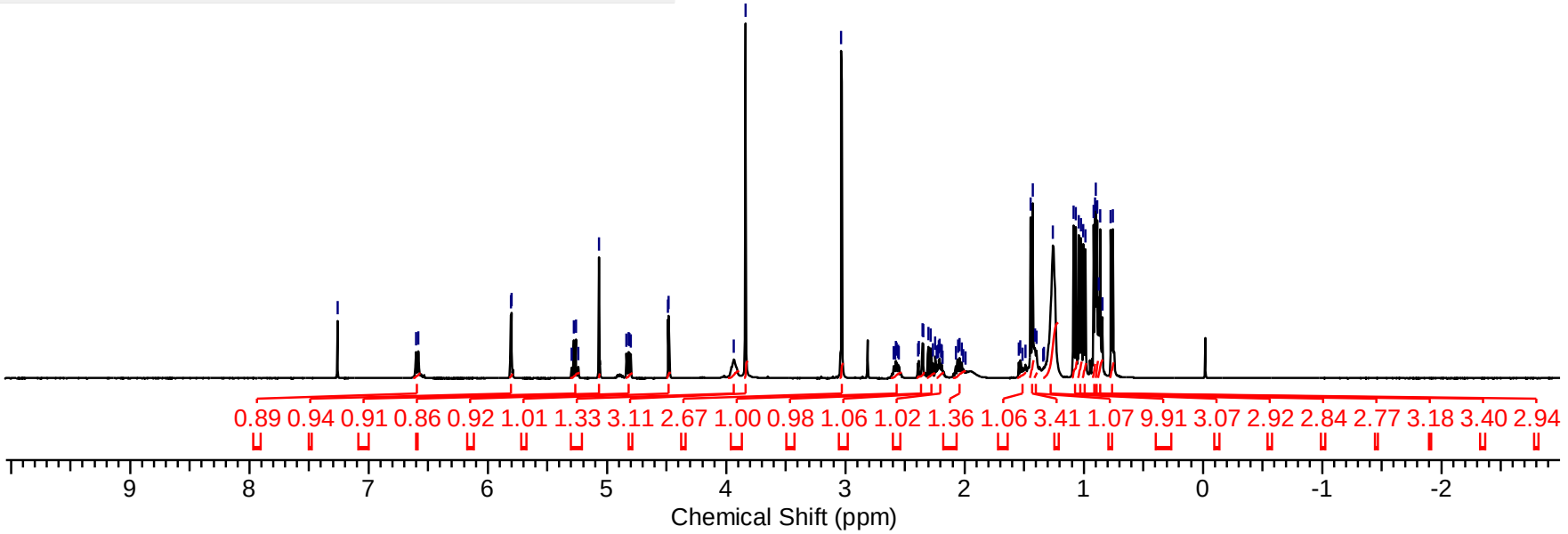
(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*S*)-3-((*tert*-butyldimethylsilyl)oxy)decanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (21):



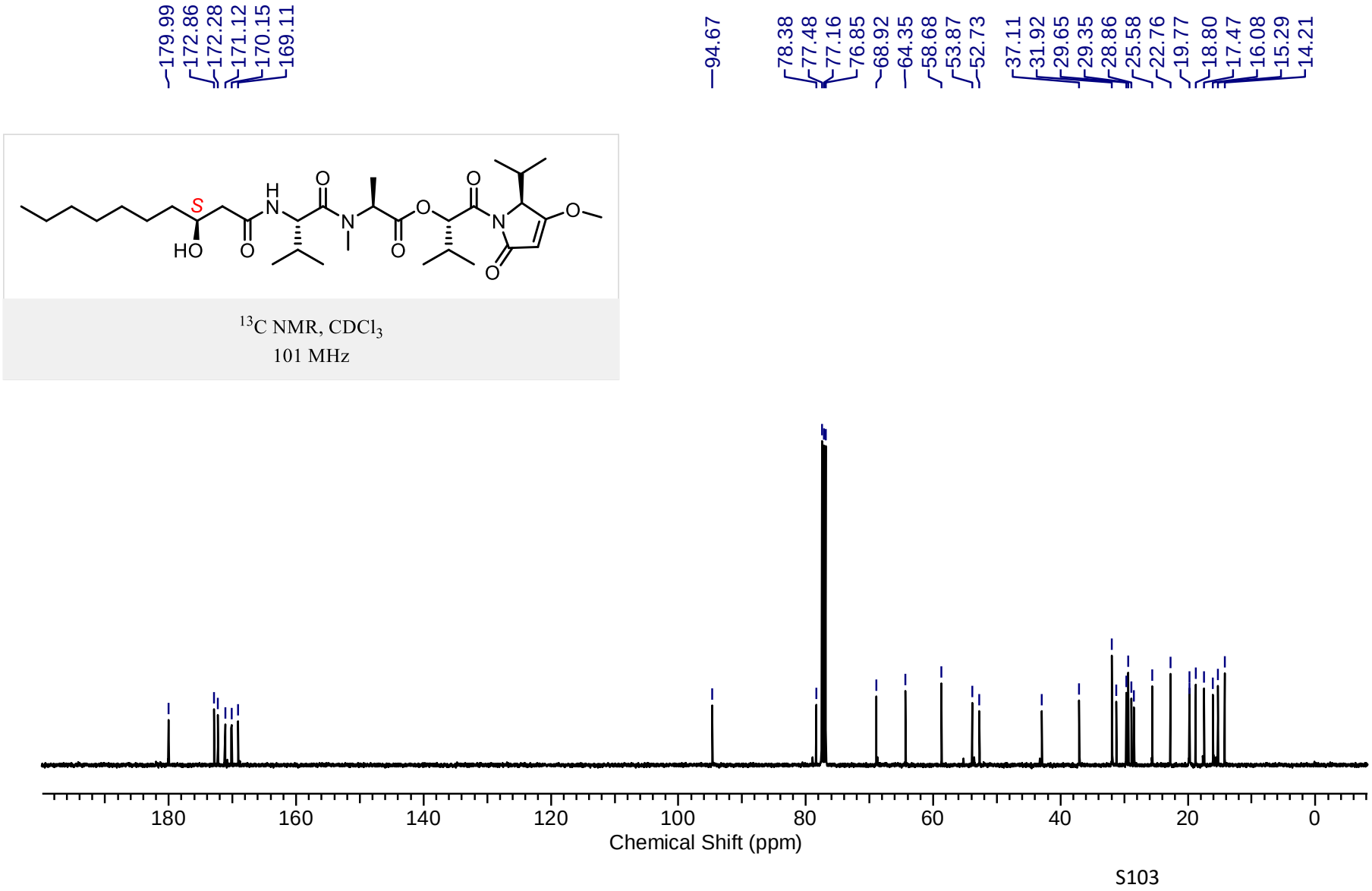
(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*S*)-3-hydroxydecanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (Kavaratamide A) (1):



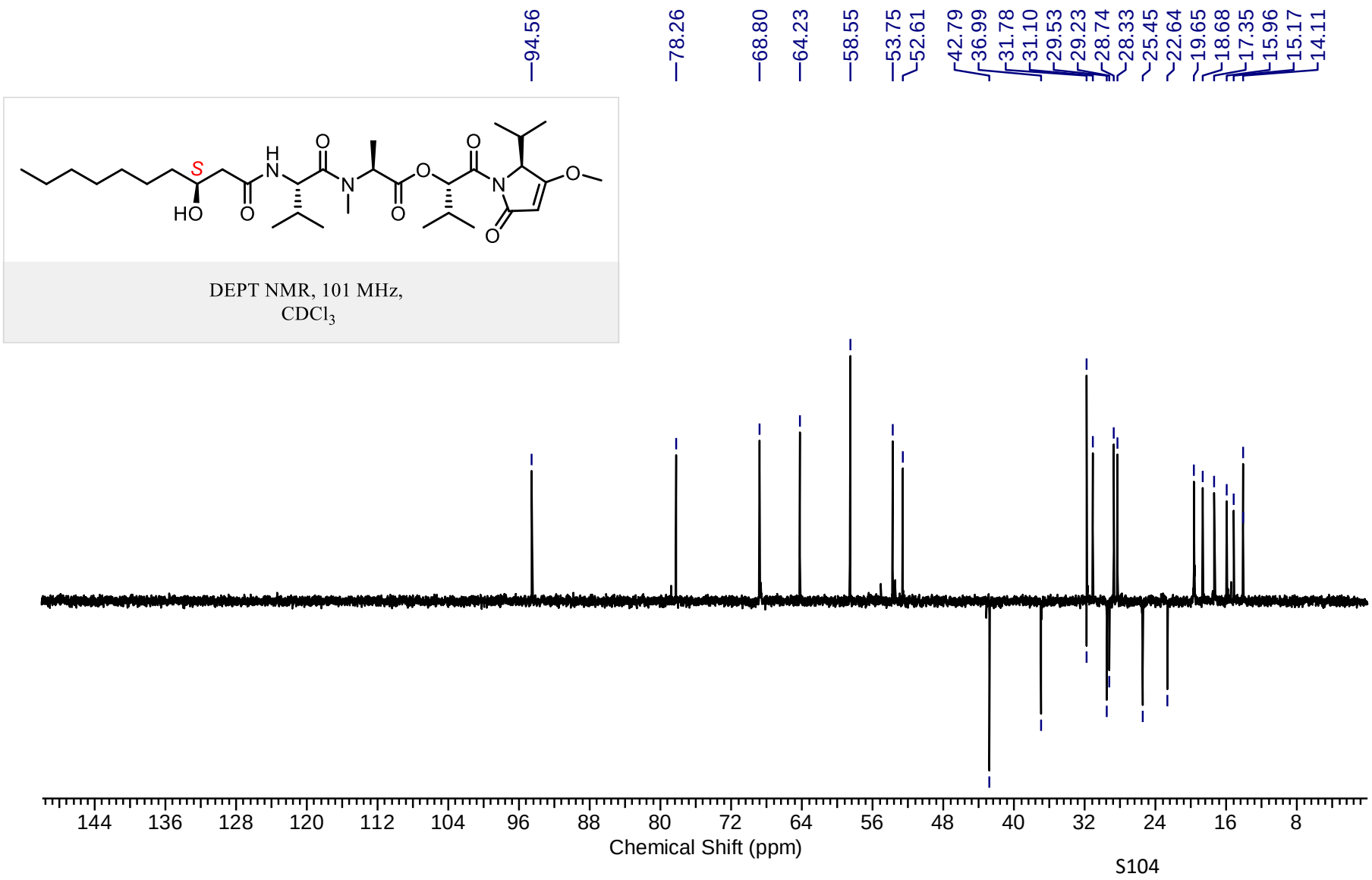
^{1}H NMR, $CDCl_3$
400 MHz



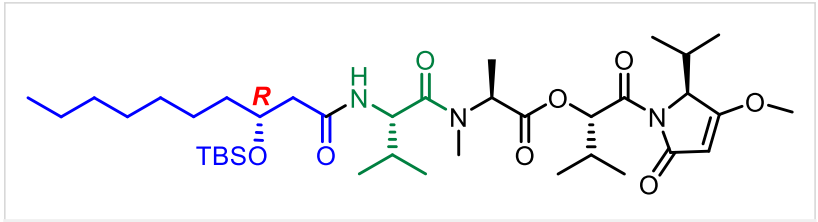
(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*S*)-3-hydroxydecanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (Kavaratamide A) (1):



(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*S*)-3-hydroxydecanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (Kavaratamide A) (1):

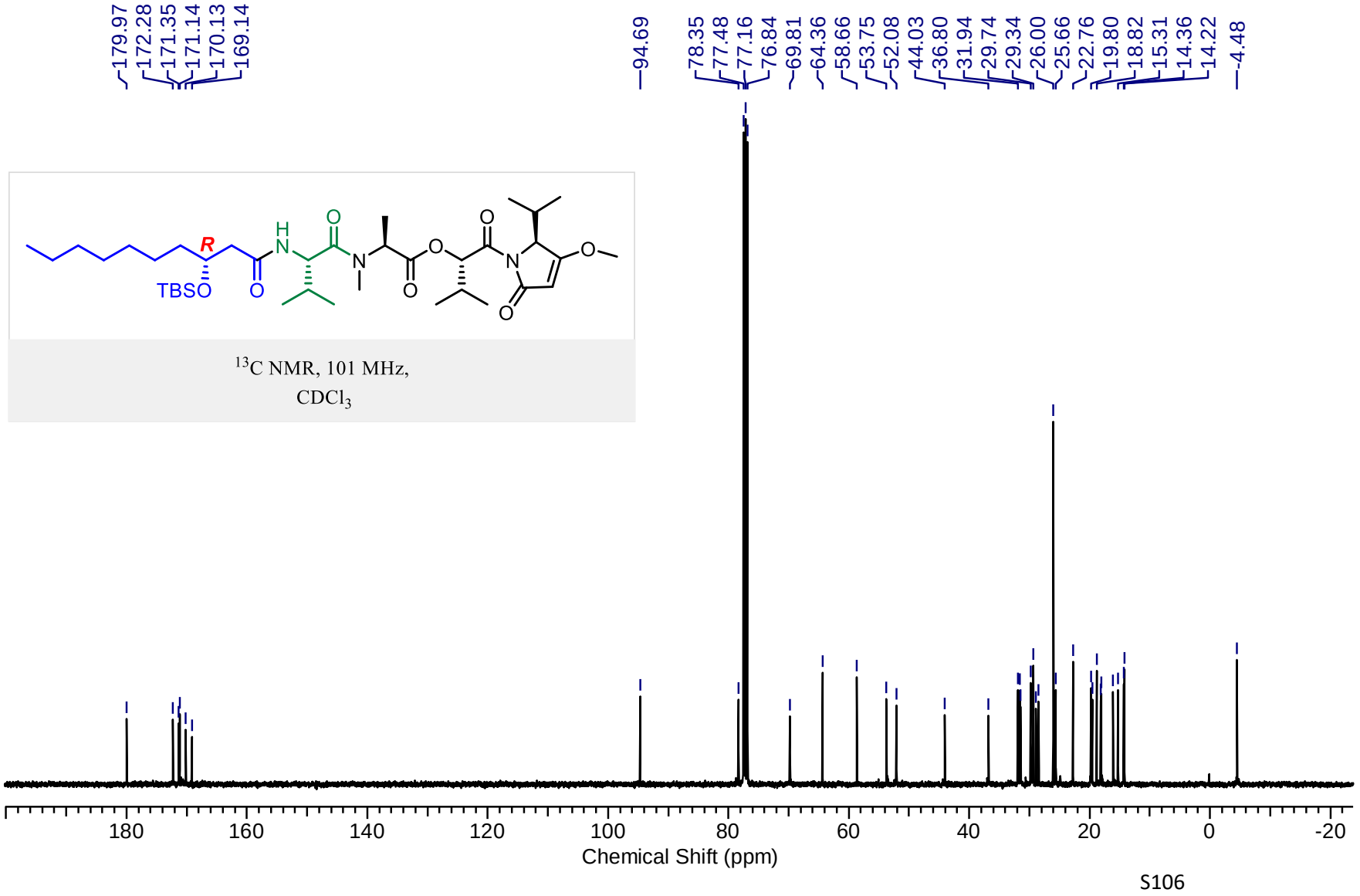


(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*R*)-3-((tert-butyldimethylsilyl)oxy)decanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (21'):



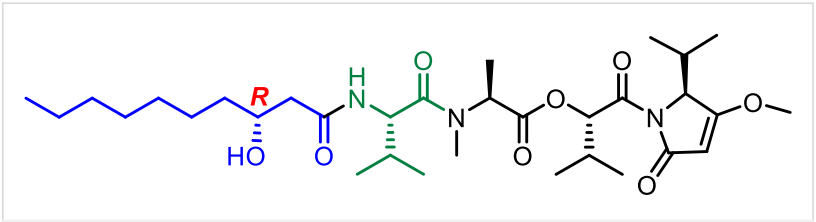
^{1}H NMR, 400 MHz,
$CDCl_3$

(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*R*)-3-((tert-butyldimethylsilyl)oxy)decanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate(21'):

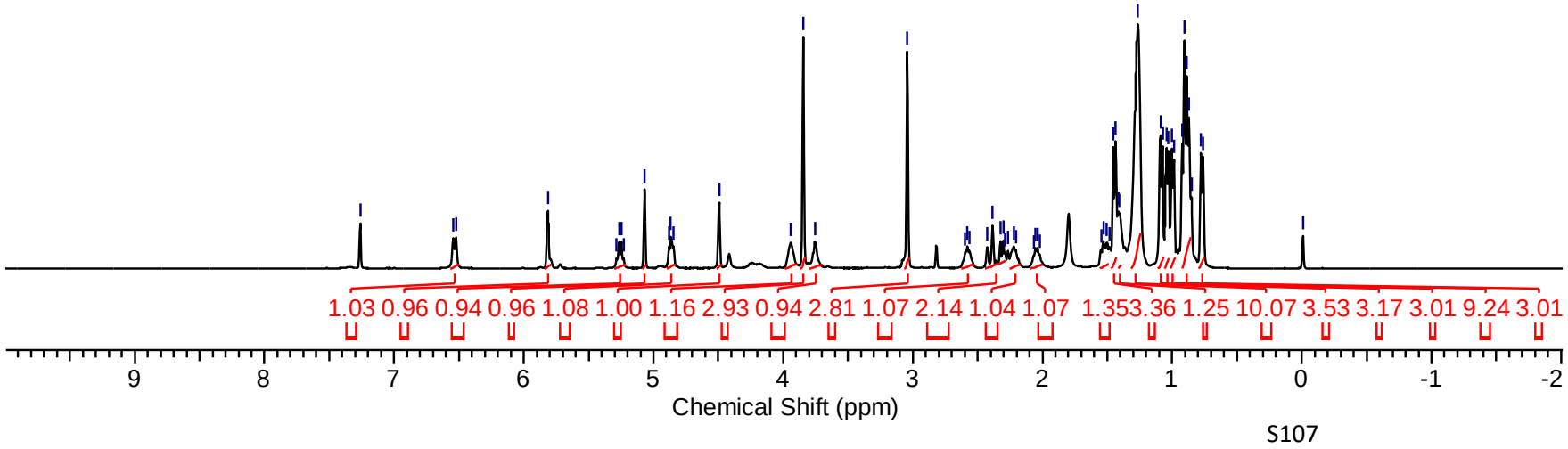


^{13}C NMR, 101 MHz, $CDCl_3$

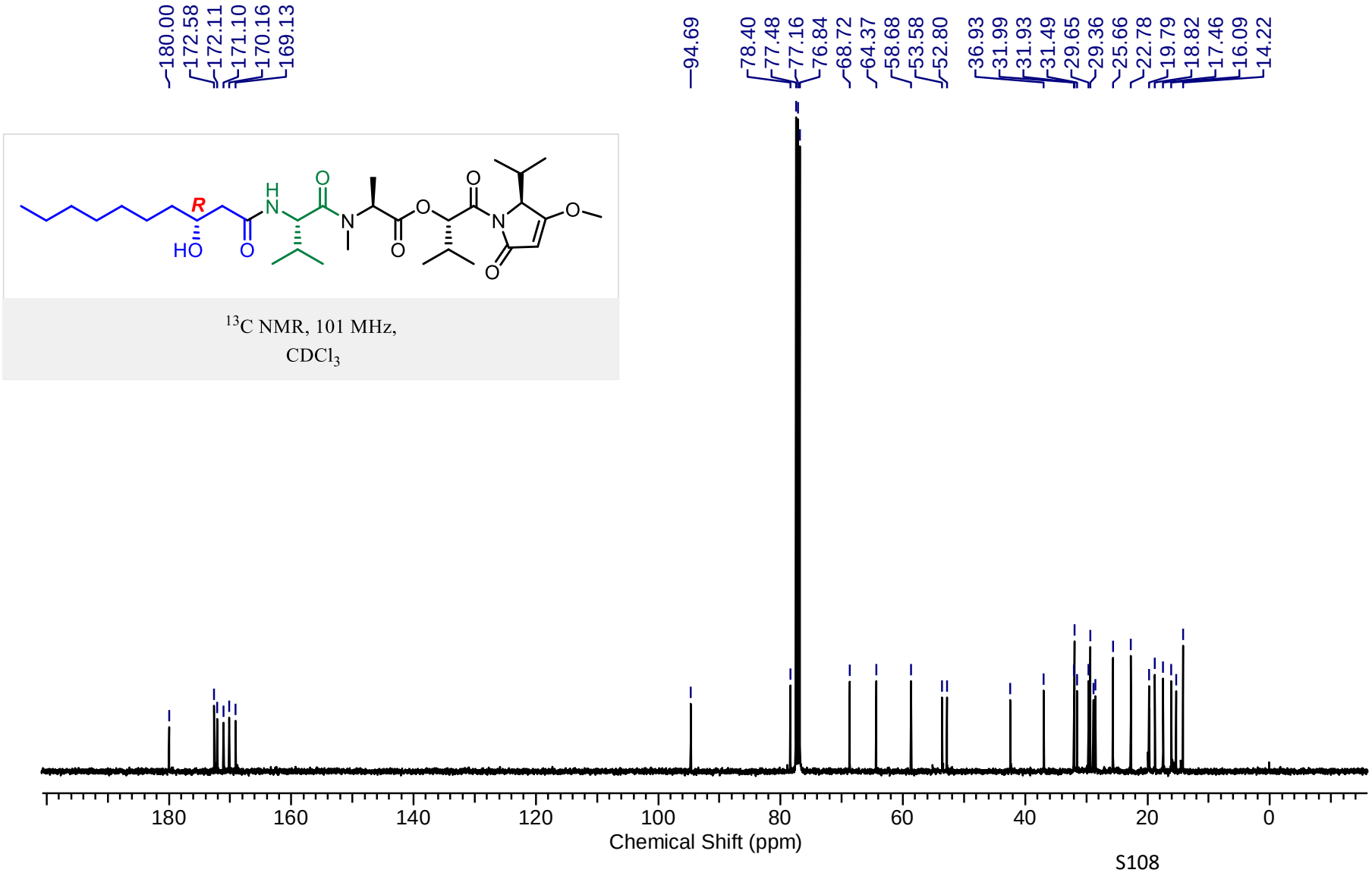
(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*R*)-3-hydroxydecanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (5-*epi*-Kavaratamide A) (2):



^{1}H NMR, 400 MHz,
$CDCl_3$



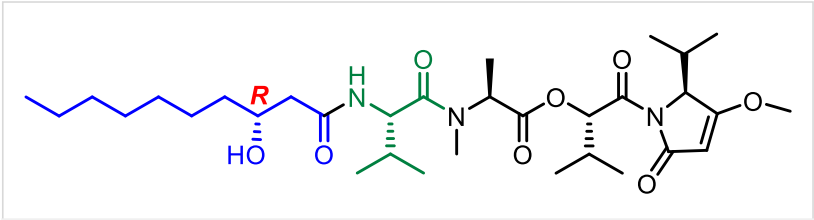
(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*R*)-3-hydroxydecanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (5-*epi*-Kavaratamide A) (2):



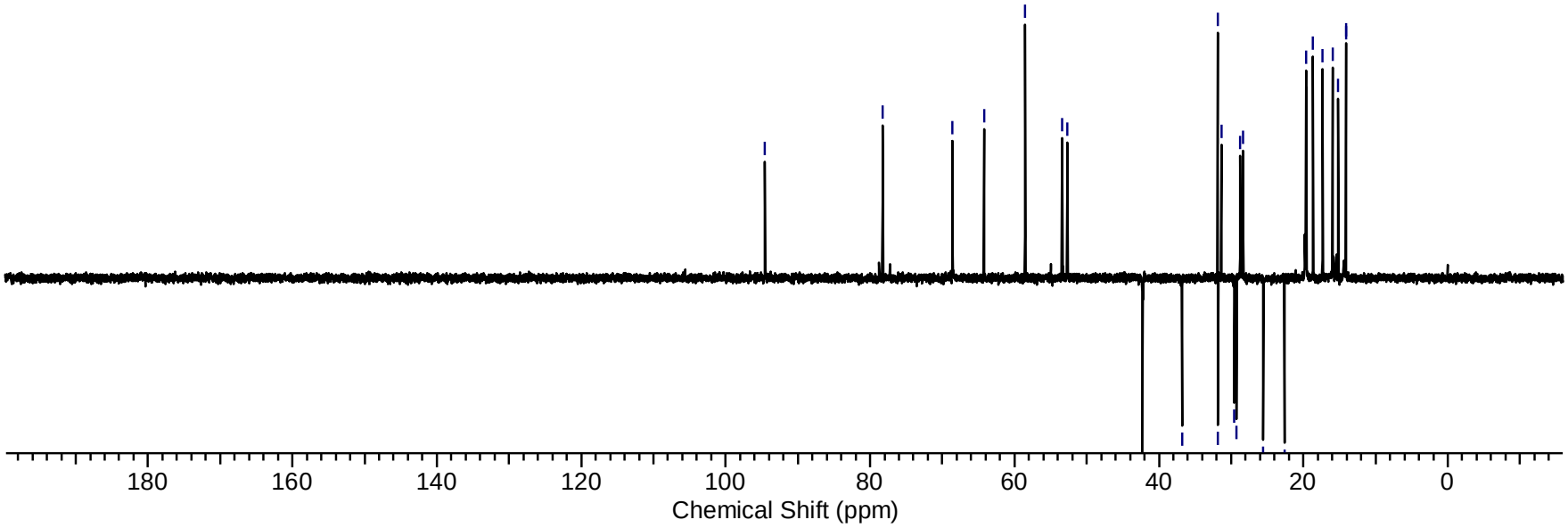
^{13}C NMR, 101 MHz,
$CDCl_3$

(*S*)-1-((*S*)-2-Isopropyl-3-methoxy-5-oxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3-methyl-1-oxobutan-2-yl *N*-(((*R*)-3-hydroxydecanoyl)-*L*-valyl)-*N*-methyl-*L*-alaninate (5-*epi*-Kavaratamide A) (2):

94.55 78.27 68.59 64.24 58.56 53.44 52.66 42.30 31.86 31.36 25.52 22.64 19.66 18.68 17.33 15.95 15.17 14.10 14.09



DEPT NMR, 101 MHz,
$CDCl_3$



THE END