Supplementary Information

Solution-Phase Synthesis and Biological Evaluation of Triostin A and its Analogues

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General experimental conditions

All commercially available reagents and solvents were used without further purification. These reactions were carried out under the nitrogen atmosphere. Normal-phase TLC was carried out on Silica gel 60 F_{254} (Merck, 1.05715.0009) using reagent grade solvents. TLC was detected by the absorption of UV light (254 nm) or visualization reagent (molybdophosphoric acid). Column chromatography was performed on silica gel (AP-300S Taiko-shoji) with mixed solvents as described. ^{1}H and ^{13}C NMR spectra were obtained for samples in indicated solution at 25 ˚C on a JEOL JNM-ECA500 spectrometer at 500 MHz frequency or JNM-AL400 spectrometer at 400 MHz frequency in $CDCl_3$ with tetramethylsilane as an internal standard. ^{1}H NMR chemical shifts are reported in terms of chemical shift (δ, ppm) relative to the singlet at 0 ppm for tetramethylsilane. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; dd, doubledoublet; td, tripledoublet; q, quartet; ddd, doubledoubledoublet; ddt, doubledoubletriplet; m, multiplet; br, broad. Coupling constants are reported in Hz. ^{13}C NMR spectra were fully decoupled and are reported in terms of chemical shift (δ, ppm) relative to the triplet at δ = 77.0 ppm for $CDCl_3$. Melting points were obtained on cover glasses and were uncorrected. The direct analysis in real time ESI-MS or DART-MS measurements were carried out on a JEOL JMS-T100TD. Optical rotations were measured on JASCO P-1020. Infrared Spectroscopy (IR) was recorded on a JASCO FT/IR-230 spectrometer.

***N*-Cbz-*D*-Ser-OAll (7).** To a solution of *N*-Cbz-*D*-Ser-OH 10 g (41.8 mmol) and imidazole 8.51 g (125 mmol, 3 equiv.) in THF (150 mL) was added a solution of TBDMSCl 7.57 g (50.2 mmol, 1.2 equiv.) in THF (50 mL) at 0 ˚C. The mixture was warmed to room temperature, and stirred at the temperature for overnight (16 h). The mixture was then filtrated and the filtrate was extracted with Et_2O (200 mL) and 1 M $KHSO_4$ (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. Then a solution of the residue (colorless oil, 17.37 g), allyl bromide 5.3 mL (62.7 mmol, 1.5 equiv.) and K_2CO_3 6.94 g (50.2 mmol, 1.2 equiv.) in DMF (140) mL was stirred at room temperature for overnight (20 h). After the filtration of an insoluble solid, the filtrate was extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue (pale yellow oil, 15.00 g) was dissolved in the mixture of AcOH (80 mL), THF (40 mL), and water (40 mL). The mixture was stirred at 40 ˚C for overnight (22 h), and

extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 9:1, 8:2, 6:4) to afford the target compound **7** 10.41 g (37.3 mmol, 89% yield on 3 steps) as colorless oil: R_f = 0.5 (*n*-hexane:AcOEt = 5:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50-7.28 (m, 5H, Cbz-Ar), 5.91 (ddt, *J* = 22.7, 10.9 Hz, 1H, All-CH), 5.72 (d, *J* = 7.2 Hz, 1H, NH), 5.34 (d, *J* = 17.4 Hz, 1H, All-CH_2(*E*)), 5.27 (d, *J* = 10.6 Hz, 1H, All-CH_2(*Z*)), 5.13 (s, 2H, Cbz-CH_2), 4.68 (d, *J* = 5.8 Hz, 2H, All-OCH_2), 4.48 (t, *J* = 3.9 Hz, 1H, α-CH), 3.99 (dd, *J* = 31.4, 11.1 Hz, 2H, β-CH_2), 2.21 (br, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2 (Ser-CO), 156.2 (Cbz-CO), 136.0 (Cbz-Ar), 131.3 (All-CH), 128.5, 128.2, 128.1 (Cbz-Ar), 119.0 (All-CH_2), 67.2 (All-OCH_2), 66.3 (Cbz-CH_2), 63.3 (β-CH_2), 56.1 (α-CH); HRMS (DART) calcd for $C_{14}H_{18}NO_5^+$ $[M+H]^+$ 280.1180, found: 280.1159; $[\alpha]_D^{27.5}$ -47.2° (c 0.1, $CHCl_3$).

***N*-Boc-*N*-Me-*L*-Val-OH (8) [45170-31-8].**[1] To a solution of *N*-Boc-*L*-Val-OH 20 g (92.1 mmol) in dry THF (300 mL) was added NaH 9.20 g (60% in oil, 230 mmol, 2.5 equiv.) at 0 ˚C, and the reaction mixture was stirred at 0 ˚C for 30 min. Then methyl iodide 28.7 mL (461 mmol, 5 equiv.) was added to the reaction mixture and the resulting solution was warmed to room temperature and stirred for overnight (17 h). The mixture was then quenched with 10% citric acid aq. (500 mL), and extracted with AcOEt (500 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (300 g) column chromatography eluted with CH_2Cl_2/MeOH (10:0, 50:1, 20:1) to afford the target compound **8** 20.19 g (87.3 mmol, 95% yield) as yellow oil: R_f = 0.55 (CH_2Cl_2:MeOH = 9:1); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 4.11 (d, *J* = 10.3 Hz, 0.43H, α-CH), 4.04 (d, *J* = 9.7 Hz, 0.57H, α-CH), 2.88 (s, 3H, *N*-Me), 2.42-2.28 (m, 0.58H, β-CH), 2.27-2.13 (m, 0.42H, β-CH), 1.48 (s, 5H, Boc), 1.46 (s, 4H, Boc), 1.03 (d, *J* = 6.3 Hz, 3H, γ-CH_3), 0.92 (d, *J* = 6.3 Hz, 3H, γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 176.2, 175.3 (Val-CO), 156.9, 155.7 (Boc-CO), 80.9, 80.6 (Boc-C), 65.3, 65.1 (Val-α-CH), 32.2, 31.1 (*N*-Me), 28.3 (Boc-Me), 27.8, 27.4 (Val-β-CH), 20.1, 19.7, 19.1, 18.9 (Val-γ-CH_3); HRMS (DART) calcd for $C_{11}H_{22}NO_4^+$ $[M+H]^+$ 232.1543, found: 232.1547; $[\alpha]_D^{26.9}$ -98.5° (c 0.1, $CHCl_3$).

***N*-Boc-*N*-Me-*L*-Cys(Bam)-OH (10) [64840-24-0].** To a solution of *N*-Boc-*N*-Me-*L*-Cys(Trt)-OH[2] 30 g (62.8 mmol) and triisopropylsilane 64.6 mL (314 mmol, 5 equiv.) in CH_2Cl_2 (160 mL) was added TFA 46.8 mL (628 mmol, 10 equiv.) at 0 ˚C. Then

the resulting solution was warmed to 26 ˚C, and stirred at the temperature for overnight (23 h). The mixture was then concentrated under the reduced pressure. Then the residue (a mixture of colorless oil and solid, 39.25 g) was washed with MeOH (100 mL), and the elution was removed under the reduced pressure. A solution of the residue (a mixture of pale yellow oil and colorless solid, 24.35 g) and *N*-(hydroxymethyl)benzamide (Bam-OH)[3] 11.40 g (75.4 mmol, 1.2 equiv.) in TFA (210 mL) was stirred at 26 ˚C for overnight (21 h). Then solvent was removed under the reduced pressure. The residue (colorless solid, 51.94 g) was then triturated with Et_2O (100 mL) to give the colorless solid (25.35 g). Then the obtained solid was dissolved in a mixture of 1,4-dioxane (110 mL) and 2 M NaOH aq. (160 mL). A solution of Boc_2O 19.18 g (87.9 mmol, 1.4 equiv.) in 1,4-dioxane (50 mL) was added to the solution, and the resulting solution was stirred at 26 ˚C for overnight (21 h). The mixture was then quenched with 10% citric acid aq. (300 mL), and extracted with AcOEt (300 mL × 4). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (200 g) column chromatography eluted with *n*-hexane/CH_2Cl_2/MeOH (10:0:0, 5:5:0, 0:10:0, 0:50:1) to afford the target compound **10** 13.07 g (35.5 mmol, 57% yield on 3 steps) as colorless amorphous solid: R_f = 0.4 (CH_2Cl_2:MeOH = 10:1); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 7.90-7.40 (m, 6H, Bam-Ar and NH), 4.99 (dd, *J* = 10.6, 4.9 Hz, 1H, Bam-CH_2), 4.88 (dd, *J* = 14.3, 6.9 Hz, 1H, Bam-CH_2), 4.48-4.33 (m, 1H, α-CH), 3.25 (dd, *J* = 14.6, 4.9 Hz, 1H, β-CH_2), 3.12-2.70 (m, 4H, *N*-Me and β-CH_2), 1.43 (s, 9H, Boc); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 173.3, 172.8 (Bam-CO), 168.3, 168.0 (Cys-CO), 157.0, 156.4 (Boc-CO), 133.4, 133.2, 131.9, 131.7, 128.6, 128.4, 127.4, 127.2 (Bam-Ar), 81.0 (Boc-C), 59.7, 58.1 (Cys-α-CH), 42.7, 41.6 (*N*-Me), 32.7, 31.5 (Bam-CH_2), 31.3, 31.0 (Cys-β-CH_2), 28.2 (Boc-Me); HRMS (ESI) calcd for $C_{17}H_{23}N_2O_5S^-$ [M-H]$^-$ 367.1333, found: 367.1331; $[\alpha]_D^{26.9}$ -39.3° (c 0.1, $CHCl_3$).

***N*-Boc-*N*-Me-*L*-Val-OAll (13) [914648-32-1].**[4] To a solution of *N*-Boc-*N*-Me-*L*-Val-OH **8** 10.65 g (46.0 mmol) and allyl bromide 5.9 mL (69.0 mmol, 1.5 equiv.) in DMF (150 mL) was added K_2CO_3 7.63 g (55.2 mmol, 1.2 equiv.) at 0 ˚C. The resulting solution was warmed to room temperature, and stirred at the temperature for overnight (16 h). The mixture was then filtrated and the filtrate was extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 10:1) to afford the target compound **13** 9.79 g (36.1 mmol, 78% yield) as colorless oil: R_f =

0.5 (*n*-hexane:AcOEt = 9:1); ^{1}H NMR (mixture of rotamers, 400 MHz, $CDCl_3$) δ 5.91 (ddt, *J* = 22.6, 10.5 Hz, 1H, All-CH), 5.32 (d, *J* = 16.4 Hz, 1H, All-CH_2(*E*)), 5.23 (dd, *J* = 9.6 Hz, 1H, All-CH_2(*Z*)), 4.62 (d, *J* = 4.8 Hz, 2H, All-OCH_2), 4.47 (d, *J* = 10.6 Hz, 0.44H, α-CH), 4.24-3.98 (m, 0.56H, α-CH), 2.86 (s, 1.49H, *N*-Me), 2.82 (s, 1.51H, *N*-Me), 2.28-2.18 (m, 1H, β-CH), 1.46 (s, 9H, Boc), 0.98 (d, *J* = 6.3 Hz, 3H, γ-CH_3), 0.90 (d, *J* = 5.8 Hz, 3H, γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 171.1, 170.6 (Val-CO), 156.1, 155.5 (Boc-CO), 131.9, 131.8 (All-CH), 118.2, 118.0 (All-CH_2), 80.1, 79.8 (Boc-C), 65.0 (All-OCH_2), 63.2 (Val-α-CH), 30.5, 30.4 (*N*-Me), 28.3 (Boc-Me), 27.7, 27.6 (Val-β-CH), 19.9, 19.7, 18.9, 18.7 (Val-γ-CH_3); HRMS (ESI) calcd for $C_{14}H_{25}NNaO_4^+$ $[M+Na]^+$ 294.1676, found: 294.1661; $[\alpha]_D^{24.8}$ -85.0° (c 1.1, $CHCl_3$).

***N*-Me-*L*-Val-OAll·HCl (14).** To a solution of *N*-Boc-*N*-Me-*L*-Val-OAll **13** 9.79 g (36.1 mmol) in AcOEt (70 mL) was added 4 M HCl in AcOEt 50 mL (200 mmol) at 0 ˚C. Then the resulting solution was warmed to room temperature, and stirred at the temperature for overnight (20 h). The mixture was concentrated under the reduced pressure, and the residue was washed with Et_2O (50 mL) to afford the target compound **14** 7.35 g (35.4 mmol, 98% yield) as colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (br, 1H, NH), 5.96 (ddt, *J* = 22.8, 11.0 Hz, 1H, All-CH), 5.42 (d, *J* = 17.4 Hz, 1H, All-CH_2(*E*)), 5.33 (d, *J* = 10.6 Hz, 1H All-CH_2(*Z*)), 4.75 (ddd, *J* = 25.4, 12.8, 6.3 Hz, 2H, All-OCH_2), 3.63 (d, *J* = 4.3 Hz, 1H, α-CH), 2.78 (s, 3H, *N*-Me), 2.71-2.57 (m, 1H, β-CH), 1.19 (d, *J* = 6.8 Hz, 3H, γ-CH_3), 1.15 (d, *J* = 7.2 Hz, 3H, γ-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6 (Val-CO), 130.7 (All-CH), 120.4 (All-CH_2), 67.1 (All-OCH_2), 66.9 (α-CH), 32.8 (*N*-Me), 29.5 (β-CH), 19.7 (γ-CH_3), 17.6 (γ-CH_3); HRMS (DART) calcd for $C_9H_{18}NO_2^+$ $[M\text{-}HCl+H]^+$ 172.1332, found: 172.1309.

***N*-Boc-*N*-Me-*D*-Val-OH (18) [89536-85-6].[1]** To a solution of *N*-Boc-*D*-Val-OH 5 g (23.0 mmol) in dry THF (80 mL) was added NaH 2.30 g (60% in oil, 57.5 mmol, 2.5 equiv.) at 0 ˚C, and the reaction mixture was stirred at 0 ˚C for 30 min. Then methyl iodide 7.2 mL (115 mmol, 5 equiv.) was added to the reaction mixture and the resulting solution was warmed to room temperature and stirred for overnight (16 h). Judging from monitoring TLC analysis, the starting material was still remained, then additional NaH 2.30 g (60% in oil, 57.5 mmol, 2.5 equiv.) and methyl iodide 7.2 mL (115 mmol, 5 equiv.) were added. The mixture was stirred at room temperature for overnight (20 h). The mixture was then quenched with 10% citric acid aq. (300 mL), and extracted with AcOEt (300 mL × 2). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by

silica gel (200 g) column chromatography eluted with *n*-hexane/CH_2Cl_2/MeOH (10:0:0, 5:5:0, 0:10:0, 0:50:1) to afford the target compound **18** 5.26 g (22.7 mmol, quantitative yield) as dark green oil: R_f = 0.4 (CH_2Cl_2:MeOH = 10:1); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$): δ 4.11 (d, *J* = 10.3 Hz, 0.39H, α-CH), 4.06 (d, *J* = 9.7 Hz, 0.61H, α-CH), 2.88 (s, 3H, *N*-Me), 2.41-2.28 (m, 0.70H, β-CH), 2.26-2.14 (m, 0.30H, β-CH), 1.48 (s, 6.1H, Boc), 1.46 (s, 2.9H, Boc), 1.03 (d, *J* = 6.3 Hz, 3H, γ-CH_3), 0.92 (d, *J* = 6.9 Hz, 3H, γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$): δ 175.8, 175.6 (Val-CO), 156.6, 155.7 (Boc-CO), 80.6 (Boc-C), 65.0, 64.3 (Val-α-CH), 31.5, 31.0 (*N*-Me), 28.2 (Boc-Me), 27.7, 27.4 (Val-β-CH), 20.0, 19.6, 19.0, 18.8 (Val-γ-CH_3); HRMS (DART) calcd for $C_{11}H_{22}NO_4^+$ $[M+H]^+$ 232.1543, found: 232.1553; $[\alpha]_D^{23.5}$ +66.2° (c 0.1, $CHCl_3$).

***N*-Boc-*N*-Me-*D*-Val-OAll (19) [914648-32-1].**[5] To a solution of *N*-Boc-*N*-Me-*D*-Val-OH **18** 1.5 g (6.5 mmol) and allyl bromide 0.83 mL (9.7 mmol, 1.5 equiv.) in DMF (15 mL) was added K_2CO_3 1.08 g (7.8 mmol, 1.2 equiv.). The resulting solution was stirred at room temperature for overnight (15 h). The mixture was then filtrated and the filtrate was extracted with AcOEt (200 mL) and water (200 mL). The organic layer was washed with sat. NaCl aq. (200 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 50:1, 20:1, 10:1) to afford the target compound **19** 1.40 g (5.2 mmol, 79% yield) as colorless oil: R_f = 0.4 (*n*-hexane:AcOEt = 9:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 (ddt, *J* = 22.5, 10.9 Hz, 1H, All-CH), 5.32 (d, *J* = 17.4 Hz, 1H, All-CH_2(*E*)), 5.23 (dd, *J* = 9.7 Hz, 1H, All-CH_2(*Z*)), 4.62 (d, *J* = 4.8 Hz, 2H, All-OCH_2), 4.48 (d, *J* = 10.6 Hz, 0.5H, α-CH), 4.13 (d, *J* = 10.1 Hz, 0.5H, α-CH), 2.86 (s, 1.47H, *N*-Me), 2.82 (s, 1.53H, *N*-Me), 2.31-2.12 (m, 1H, β-CH), 1.46 (s, 9H, Boc), 0.98 (d, *J* = 4.8 Hz, 3H, γ-CH_3), 0.90 (d, *J* = 6.3 Hz, 3H, γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$): δ 171.1, 170.6 (Val-CO), 156.1, 155.5 (Boc-CO), 131.9, 131.8 (All-CH), 118.2, 118.0 (All-CH_2), 80.1, 79.8 (Boc-C), 65.2, 63.2 (Val-α-CH), 30.5, 30.4 (*N*-Me), 28.2 (Boc-Me), 27.7, 27.6 (Val-β-CH), 19.9, 19.6, 18.9, 18.7 (Val-γ-CH_3); HRMS (DART) calcd for $C_{14}H_{26}NO_4^+$ $[M+H]^+$ 272.1856, found: 272.1848; $[\alpha]_D^{23.5}$ +92.7° (c 0.1, $CHCl_3$).

***N*-Me-*D*-Val-OAll·HCl (20).** To a solution of *N*-Boc-*N*-Me-*D*-Val-OAll **19** 1.40 g (5.2 mmol) in AcOEt (5 mL) was added 4 M HCl in AcOEt 5 mL (20 mmol) at 0 ˚C. Then the resulting solution was warmed to room temperature, and stirred at the temperature for overnight (12 h). The mixture was concentrated under the reduced pressure, and the residue was washed with Et_2O (50 mL) to afford the target compound **20** 976.7 mg (4.7 mmol, 90% yield) as colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (ddt, *J* = 21.5 Hz,

1H, All-CH), 5.43 (dd, *J* = 17.2, 1.1 Hz, 1H, All-CH_2(*E*)), 5.33 (d, *J* = 10.3 Hz, 1H All-CH_2(*Z*)), 4.75 (ddd, *J* = 30.6, 12.9, 6.0 Hz, 2H, All-OCH_2), 3.59 (d, *J* = 4.0 Hz, 1H, α-CH), 2.77 (s, 3H, *N*-Me), 2.70-2.55 (m, 1H, β-CH), 1.19 (d, *J* = 7.4 Hz, 3H, γ-CH_3), 1.16 (d, *J* = 6.9 Hz, 3H, γ-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.3 (Val-CO), 130.4 (All-CH), 119.8 (All-CH_2), 66.6, 66.4 (All-OCH_2, Val-α-CH), 32.4 (*N*-Me), 29.0 (Val-β-CH), 19.4, 17.2 (Val-γ-CH_3); HRMS (DART) calcd for $C_9H_{18}NO_2^+$ $[M\text{-}HCl+H]^+$ 172.1332, found: 172.1337.

_N_-Cbz-_D_-Ser[_N_-Boc-_L_-Ala-_N_-Me-_L_-Cys(Bam)-_N_-Me-_D_-Val]-OH (30). To a solution of *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OAll **26** 1.6 g (2.0 mmol), PPh_3 20.6 mg (0.079 mmol, 4 mol%), *N*-methylaniline 0.26 mL (2.4 mmol, 1.2 equiv.) in THF (10 mL) was added $Pd_2(dba)_3$ 18.0 mg (0.020 mmol, 1 mol%). The resulting solution was stirred at room temperature for overnight (8 h) in the dark. Then the mixture extracted with AcOEt (300 mL × 2) and sat. NH_4Cl aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with CH_2Cl_2/MeOH (10:0, 50:1, 20:1) to afford the target compound **30** 1.43 g (1.8 mmol, 92% yield) as pale brown amorphous solid: R_f = 0.2 (CH_2Cl_2:MeOH = 9:1); ^{1}H NMR (mixture of rotamers, 500 MHz, $CDCl_3$) δ 7.93-7.75, 7.57-7.28 (m, 11H, Bam-Ar, NH, Cbz-Ar), 6.24 (d, *J* = 7.4 Hz, 0.26H, Ser-NH), 5.76 (m, 0.74H+0.26H, Ser-NH, Cys-α-CH), 5.61 (t, *J* = 7.2 Hz, 0.74H, Cys-α-CH), 5.43 (d, *J* = 8.0 Hz, 0.68H, Ala-NH), 5.36 (d, *J* = 8.6 Hz, 0.32H, Ala-NH), 5.20-4.94 (m, 2H, Cbz-CH_2), 4.87 (dd, *J* = 14.3, 6.9 Hz, 1H, Bam-CH_2), 4.78-4.23 (m, 5H, Ban-CH_2, Ala-α-CH, Ser-α-CH, β-CH_2), 4.54-4.23 (m, 4H), 4.12 (d, *J* = 10.3 Hz, 0.28H, Val-α-CH), 4.08-3.93 (m, 0.60H, Val-α-CH), 3.72 (d, *J* = 10.9 Hz, 0.12H, Val-α-CH), 3.28-3.08 (m, 1H, Cys-β-CH_2), 3.06-2.60 (m, 7H, Cys-β-CH_2, *N*-Me, Val-*N*-Me), 2.43-2.14 (m, 1H, Val-β-CH), 1.44 (s, 6.06H, Boc-CH_3), 1.43 (s, 2.94H, Boc-CH_3), 1.30 (t, *J* = 3.2 Hz, 3H), 0.98 (d, *J* = 6.3 Hz, 1.90H, Val-γ-CH_3), 0.86 (d, *J* = 6.3 Hz, 1.10H, Val-γ-CH_3), 0.77 (d, *J* = 6.9 Hz, 1.95H, Val-γ-CH_3), 0.71 (d, *J* = 6.9 Hz, 1.05H, Val-γ-CH_3); ^{13}C NMR (mixture of rotamers, 125 MHz, $CDCl_3$) δ 174.2, 173.7, 171.0, 170.6, 170.0, 169.6, 169.2, 167.94, 167.87 (Ser-CO, Ala-CO, Cys-CO, Val-CO, Bam-CO), 156.1, 155.7, 155.1, 155.0 (Cbz-CO, Boc-CO), 135.9, 133.2, 131.9, 131.8, 128.5, 128.4, 128.3, 128.1, 128.03, 127.95, 127.3, 127.2 (Cbz-Ar, Bam-Ar), 79.9 (Boc-C), 67.0, 64.7 (Cbz-CH_2, Ser-β-CH_2), 64.2, 53.6, 53.3, 53.0, 52.9, 51.2, 46.8, 46.4 (Ser-α-CH, Ala-α-CH, Cys-α-CH, Val-α-CH), 42.4, 41.9 (Bam-CH_2), 32.9, 30.0, 28.9 (Cys-*N*-Me, Val-*N*-Me), 30.4 (Cys-β-CH_2), 28.2 (Boc-CH_3), 27.0, 26.8 (Val-β-CH), 20.0, 19.2, 18.7, 18.4, 18.0 (Val-γ-CH_3, Ala-β-CH_3); HRMS (ESI)

calcd for $C_{37}H_{50}N_5O_{11}S^-$ $[M-H]^-$ 772.3233, found: 772.3207.

***N*-Cbz-*D*-Ser[*N*-Cbz-*D*-Ser(*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val)-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OAll (31).** To a solution of *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OAll **26** 1.26 g (1.6 mmol, 1.2 equiv.) in AcOEt (10 mL) was added 4 M HCl in AcOEt 5 mL (20 mmol) at 0 ˚C. Then the resulting solution was warmed to room temperature, and stirred at the temperature for overnight (14 h). The mixture was concentrated under the reduced pressure, and the residue was extracted with AcOEt (300 mL × 2) and sat. $NaHCO_3$ aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. Then a solution of the residue (colorless amorphous solid, 1.04 g), *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OH **30** 1 g (1.3 mmol, 1.2 equiv.) and DMT-MM 0.43 g (1.6 mmol, 1.2 equiv.) in AcOEt (10 mL) was stirred at room temperature for overnight (18 h). The mixture was then extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 8:2, 6:4, 4:6, 3:7) to afford the target compound **31** 1.24 g (0.84 mmol, 65% yield on 2 steps) as pale yellow amorphous solid: R_f = 0.55 (*n*-hexane:AcOEt = 2:8); HRMS (ESI) calcd for $C_{72}H_{96}N_{10}NaO_{19}S_2^+$ $[M+Na]^+$ 1491.6187, found: 1491.6161; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) chart were shown in page S80, 81 in Supplementary Information.

***N*-Cbz-*D*-Ser[*N*-Cbz-*D*-Ser(*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val)-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OH (32).** To a solution of Boc-*D, D*-octadepsipeptide-OAll **31** 1.2 g (0.82 mmol), PPh_3 17.1 mg (0.065 mmol, 8 mol%), *N*-methylaniline 0.11 mL (0.98 mmol, 1.2 equiv.) in THF (10 mL) was added $Pd_2(dba)_3$ 17.1 mg (0.016 mmol, 2 mol%). The resulting solution was stirred at room temperature for overnight (14 h) in the dark. Then the mixture extracted with AcOEt (300 mL × 2) and sat. NH_4Cl aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with CH_2Cl_2/MeOH (10:0, 100:1, 50:1, 20:1) to afford the target compound **32** 1.04 g (0.73 mmol, 88% yield) as pale brown amorphous solid: R_f = 0.4 (CH_2Cl_2:MeOH = 9:1); HRMS (ESI) calcd for $C_{69}H_{91}N_{10}O_{19}S_2^-$ $[M-H]^-$ 1427.5909, found: 1427.5898; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) chart were shown in page S82, 83 in Supplementary Information.

(*N*-Cbz-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*D*-Val)$_2$ (Serine-hydroxy)-Dilactone Disulfide (33). The reaction condition was almost the same of the method, the synthesis of macrolide **1**. Boc-*D, D*-octadepsipeptide-OH **32** 500 mg (0.35 mmol) was used instead of Boc-octadepsipeptide-OH **29**. The target compound **33** was obtained 219.7 mg (0.21 mmol, 60% yield on 2 steps) as colorless solid: R_f = 0.5 (*n*-hexane:AcOEt = 2:8); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.6 Hz, 2H, Ser-NH), 7.49-7.30 (m, 12H, Ala-NH, Cbz-Ar), 5.40 (d, *J* = 11.5 Hz, 2H, Cbz-CH_2), 5.29 (d, *J* = 9.2 Hz, 2H, Ser-β-CH_2), 4.97 (d, *J* = 8.6 Hz, 2H, Val-α-CH), 4.92 (d, *J* = 11.5 Hz, 2H, Val-α-CH), 4.90-4.78 (m, 4H, Ala-α-CH, Ser-α-CH), 4.46 (dd, *J* = 12.6, 2.3 Hz, 2H, Cys-α-CH), 3.84 (dd, *J* = 11.2, 2.6 Hz, 2H, Ser-β-CH_2), 3.11 (s, 6H, Cys-*N*-Me), 2.98 (dd, *J* = 16.3, 12.9 Hz, 2H, Cys-β-CH_2), 2.77 (dd, *J* = 16.6, 2.3 Hz, 2H, Cys-β-CH_2), 2.70 (s, 6H, Val-*N*-Me), 2.31-2.13 (m, 2H, Val-β-CH), 1.07 (d, *J* = 6.3 Hz, 6H, Val-γ-CH_3), 0.78 (d, *J* = 6.9 Hz, 6H, Ala-β-CH_3), 0.77 (d, *J* = 6.5 Hz, 6H, Val-γ-CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.8 (Ala-CO), 170.6 (Cys-CO), 169.2 (Val-CO), 167.2 (Ser-CO), 156.2 (Cbz-CO), 135.2, 129.8, 128.7, 128.5 (Cbz-Ar), 68.7 (Cbz-CH_2), 64.5 (Ser-β-CH_2), 60.9 (Val-α-CH), 54.2 (Cys-α-CH), 53.8 (Ser-α-CH), 45.5 (Ala-α-CH), 36.3 (Cys-β-CH_2), 31.8 (Cys-*N*-Me), 31.6 (Val-*N*-Me), 27.8 (Val-β-CH), 20.8 (Val-γ-CH_3), 18.9 (Val-γ-CH_3), 16.9 (Ala-β-CH_3); HRMS (ESI) calcd for $C_{48}H_{66}N_8NaO_{14}S_2^+$ $[M+Na]^+$ 1065.4032, found: 1065.4032; needles; m.p. 184.2-187.8 °C (recrystallization from AcOEt); $[\alpha]_D^{27.8}$ +88.3 ° (c 0.1, $CHCl_3$). Crystallographic data for the structure of **33** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 1441091.

***N*-Cbz-*D*-Ser[*N*-Cbz-*D*-Ser(*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val)-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OAll (34).** To a solution of *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OAll **26** 1.26 g (1.6 mmol, 1.2 equiv.) in AcOEt (10 mL) was added 4 M HCl in AcOEt 5 mL (20 mmol) at 0 ˚C. Then the resulting solution was warmed to room temperature, and stirred at the temperature for overnight (8 h). The mixture was concentrated under the reduced pressure, and the residue was extracted with AcOEt (300 mL × 2) and sat. $NaHCO_3$ aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. Then a solution of the residue (colorless amorphous solid, 1.04 g), *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OH **27** 1 g (1.3 mmol, 1.2 equiv.) and DMT-MM 0.43 g (1.6 mmol, 1.2 equiv.) in AcOEt (10 mL) was stirred at room temperature for overnight (14 h). The mixture was then extracted with AcOEt (300 mL × 2) and water (300 mL). The organic layer was washed

with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with *n*-hexane/AcOEt (10:0, 8:2, 6:4, 4:6, 3:7) to afford the target compound **34** 1.26 g (0.86 mmol, 67% yield on 2 steps) as yellow amorphous solid: R_f = 0.4 (*n*-hexane:AcOEt = 2:8); HRMS (ESI) calcd for $C_{72}H_{96}N_{10}NaO_{19}S_2^+$ $[M+Na]^+$ 1491.6187, found: 1491.6161; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) chart were shown in page S86, 87 in Supplementary Information.

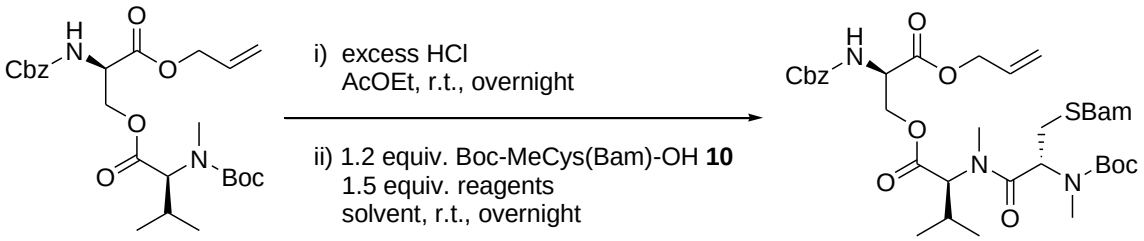
***N*-Cbz-*D*-Ser[*N*-Cbz-*D*-Ser(*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val)-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OH (35).** To a solution of Boc-*D, L*-octadepsipeptide-OAll **34** 1.26 g (0.86 mmol), PPh_3 18.0 mg (0.069 mmol, 8 mol%), *N*-methylaniline 0.11 mL (1.0 mmol, 1.2 equiv.) in THF (10 mL) was added $Pd_2(dba)_3$ 15.7 mg (0.017 mmol, 2 mol%). The resulting solution was stirred at room temperature for overnight (14 h) in the dark. Then the mixture extracted with AcOEt (300 mL × 2) and sat. NH_4Cl aq. (300 mL). The organic layer was washed with sat. NaCl aq. (300 mL), and dried with $MgSO_4$, solvent was removed under the reduced pressure. The residue was purified by silica gel (100 g) column chromatography eluted with CH_2Cl_2/MeOH (10:0, 100:1, 50:1, 20:1) to afford the target compound **35** 1.16 g (0.81 mmol, 94% yield) as pale brown amorphous solid: R_f = 0.3 (CH_2Cl_2:MeOH = 9:1); HRMS (ESI) calcd for $C_{69}H_{91}N_{10}O_{19}S_2^-$ $[M-H]^-$ 1427.5909, found: 1427.5924; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) chart were shown in page S88, 89 in Supplementary Information.

(*N*-Cbz-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*D*-Val)-(*N*-Cbz-*D*-Ser-*L*-Ala-*N*-Me-*L*-Cys-*N*-Me-*L*-Val) (Serine-hydroxy)-Dilactone Disulfide (36). The reaction condition was almost the same of the method, the synthesis of macrolide **1**. Boc-*D, L*-octadepsipeptide-OH **35** 500 mg (0.35 mmol) was used instead of Boc-octadepsipeptide-OH **29**. The target compound **36** was obtained 197.5 mg (0.19 mmol, 54% yield on 2 steps) as colorless solid: R_f = 0.3 (*n*-hexane:AcOEt = 2:8); ^1H NMR (mixture of conformers, 500 MHz, $CDCl_3$) δ 7.69-7.13 (m, 14H, Ser-NH, Ala-NH, Cbz-Ar), 5.46-5.34 (m, 3.5H, Cbz-CH_2, Cys-α-CH), 5.36 (dd, *J* = 10.9, 8.6 Hz, 0.5H, Ser-β-CH_2), 5.26 (d, *J* = 10.9 Hz, 0.5H, Ser-β-CH_2), 5.23 (d, *J* = 10.9 Hz, 0.5H), 5.09 (dd, *J* = 10.9, 2.3 Hz, 0.5H), 5.05-4.74 (m, 7.5H, Val-α-CH, Cbz-CH_2, Ser-α-CH, Ala- α-CH), 4.64 (dd, *J* = 12.9, 2.0 Hz, 0.5H, Cys-α-CH), 4.55 (d, *J* = 10.9 Hz, 0.5H, Ser-β-CH_2), 4.50 (dd, *J* = 11.5, 2.9 Hz, 0.5H, Ser-β-CH_2), 4.43 (dd, *J* = 12.6, 2.3 Hz, 1H, 0.5H, Cys-α-CH), 4.39 (dd, *J* = 12.6, 2.9 Hz, 0.5H, Cys-α-CH), 4.04 (d, *J* = 9.7 Hz, 0.5H, Ser-β-CH_2), 3.91 (d, *J* = 10.3 Hz, 0.5H, Val-α-CH), 3.86 (dd, *J* = 10.9, 2.3 Hz, 1H, Ser-β-CH_2), 3.32-2.51 (m, 16H, Cys-*N*-Me, Cys-β-CH_2, Val-*N*-Me), 2.36 (d, *J* = 6.9 Hz,

1H), 2.42-2.14 (m, 2H, Val-β-CH), 1.13 (d, J= 6.3 Hz, 2H, Val-γ-CH_3), 1.08 (d, J= 6.9 Hz, 2H, Val-γ-CH_3), 1.07 (d, J = 6.0 Hz, 1.5H, Val-γ-CH_3), 0.98 (d, J = 6.3 Hz, 1.5H, Val-γ-CH_3), 0.91 (d, J = 6.9 Hz, 1.5H, Val-γ-CH_3), 0.83-0.74 (m, 7.5H, Ala-β-CH_3, Val-γ-CH_3), 0.72 (d, J = 6.0 Hz, 2H, Val-γ-CH_3); ^{13}C NMR (mixture of conformers, 125 MHz, $CDCl_3$) δ 175.2, 174.6, 174.5, 173.9 (Ala-CO), 171.4 (Val-CO), 170.5, 170.4 (Cys-CO), 169.5 (Val-CO), 169.3 (Ser-CO), 169.2, 168.6 (Val-CO), 168.1, 167.9 (Cys-CO), 167.4, 167.3, 167.1 (Ser-CO), 156.3, 156.2, 156.1 (Cbz-CO), 135.3, 135.23, 135.17, 135.09, 132.1, 132.0, 131.9, 129.9, 129.80, 129.76, 129.5, 128.7, 128.6, 128.5, 128.4 (Cbz-Ar), 68.7, 66.7 (Ser-β-CH_2), 65.5 (Val-α-CH), 64.9, 64.3 (Ser-β-CH_2), 60.9 (Val-α-CH), 56.0, 54.8, 54.3 (Cys-α-CH), 54.0, 53.9, 53.6 (Ser-α-CH), 50.0 (Cys-α-CH), 46.1, 45.7, 45.6 (Ala-α-CH), 39.7 (Cys-*N*-Me), 36.7, 36.3, 35.7, 35.5 (Cys-β-CH_2), 32.1, 31.9 (Val-*N*-Me), 31.5 (Cys-*N*-Me), 29.6 (Val-*N*-Me), 28.1, 27.8, 27.6 (Val-β-CH), 22.5, 20.8, 20.1, 20.0, 19.8, 19.0, 18.9 (Val-γ-CH_3), 17.3, 17.0, 16.7 (Ala-β-CH_3); HRMS (ESI) calcd for $C_{48}H_{66}N_8NaO_{14}S_2^+$ $[M+Na]^+$ 1065.4032, found: 1065.4005; m.p. 148.3-150.7 °C; $[\alpha]_D^{27.6}$ +50.3 ° (c 0.1, $CHCl_3$).

References

1. A. V. Malkov, K. Vrankova, M. Cerny and P. Kocovsky, *J. Org. Chem.*, 2009, **74**, 8425-8427.
2. E. Marcucci, N. Bayó-Puxan, J. Tulla-Puche, J. Spengler and F. Albericio, *J. Comb. Chem.*, 2008, **10**, 69-78.
3. B. R. Nair and J. D. Francis, *J. Chromatogr. A*, 1980, **195**, 158-161.
4. S. Yu, X. Pan and D. Ma, *Chem. Eur. J.*, 2006, **12**, 6572-6584.
5. D. Wang, X. Jia and A. Zhang, *Org. Biomol. Chem.*, 2012, **10**, 7027-7030.



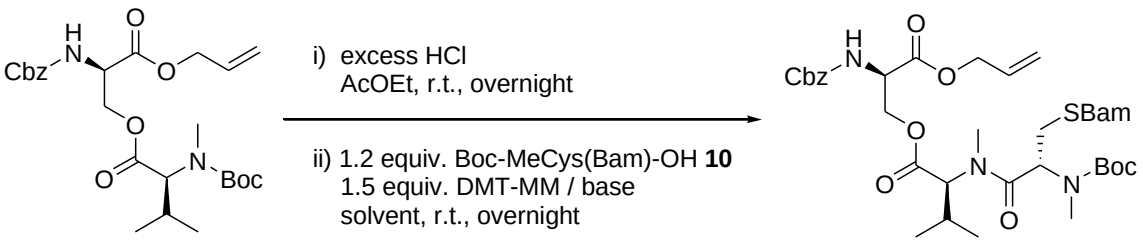
9
50 mg, 0.10 mmol

11

entry	reagents	solvent	tridepsipeptide **11**
1	DMT-MM	AcOEt	62.5 mg, 0.084 mmol, 84% yield on 2 steps
2	EDCI·HCl HOAt DIPEA	CH_2Cl_2	44.8 mg, 0.060 mmol, 60% yield on 2 steps
3	HATU HOAt DIPEA	DMF	25.9 mg, 0.035 mmol, 35% yield on 2 steps
4[a]	PyBOP HOAt DIPEA	DMF	19.9 mg, 0.027 mmol, 27% yield on 2 steps
5[a]	DEPBT DIPEA	DMF	42.5 mg, 0.057 mmol, 57% yield on 2 steps

[a]The isomerization of the target tridepsipeptide was observed.

Table S1. Optimization about condensation reagent on the acylation of *N*-Cbz-*D*-Ser(*N*-Boc-*N*-Me-*L*-Val)-OH **9** and *N*-Boc-*N*-Me-*L*-Cys(Bam)-OH **10**.

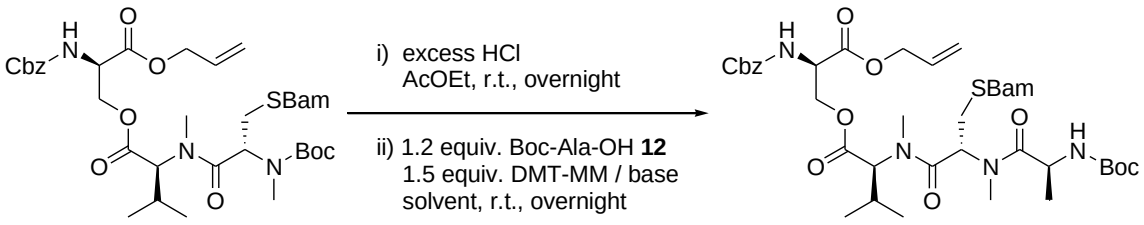


9
50 mg, 0.10 mmol

11

entry	base	solvent	tridepsipeptide **11**
1	NMM	AcOEt	54.9 mg, 0.074 mmol, 74% yield on 2 steps
2		DMF	trace (complex mixture)
3		CH_3CN	trace (complex mixture)
4		THF	46.1 mg, 0.062 mmol, 62% yield on 2 steps
5	none	AcOEt	62.5 mg, 0.084 mmol, 84% yield on 2 steps
6		DMF	trace (complex mixture)
7		CH_3CN	16.7 mg, 0.022 mmol, 22% yield on 2 steps
8		THF	56.4 mg, 0.076 mmol, 76% yield on 2 steps

Table S2. Optimization of the reaction solvent and base on the acylation of *N*-Cbz-*D*-Ser(*N*-Boc-*N*-Me-*L*-Val)-OAll **9** and *N*-Boc-*N*-Me-*L*-Cys(Bam)-OH **10**. In the case of *N*-methylmorpholine (NMM) addition, the quench method of the de-Boc step was just concentration to remove the reaction solvent. On the other hand, the extraction of the reaction solvent with sat. $NaHCO_3$ aq. for neutralization was performed in the case of none base condition.

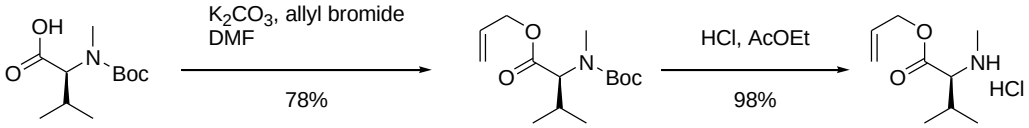


11
50 mg, 0.067 mmol

2

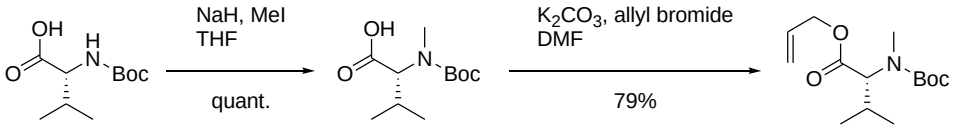
entry	base	solvent	tetradepsipeptide **2**
1	NMM	AcOEt	8.9 mg, 0.011 mmol, 16% yield on 2 steps
2		DMF	26.8 mg, 0.033 mmol, 49% yield on 2 steps
3		CH_3CN	15.3 mg, 0.019 mmol, 28% yield on 2 steps
4		THF	11.9 mg, 0.015 mmol, 22% yield on 2 steps
5	none	AcOEt	5.5 mg, 0.0068 mmol, 10% yield on 2 steps
6		DMF	23.8 mg, 0.029 mmol, 43% yield on 2 steps
7		CH_3CN	21.3 mg, 0.026 mmol, 39% yield on 2 steps
8		THF	16.4 mg, 0.020 mmol, 30% yield on 2 steps

Table S3. Optimization of the reaction solvent and base on the acylation of *N*-Cbz-*D*-Ser[*N*-Boc-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll **11** and *N*-Boc-*L*-Ala-OH **12**. In the case of *N*-methylmorpholine (NMM) addition, the quench method of the de-Boc step was just concentration to remove the reaction solvent. On the other hand, the extraction of the reaction solvent with sat. $NaHCO_3$ aq. for neutralization was performed in the case of none base condition.

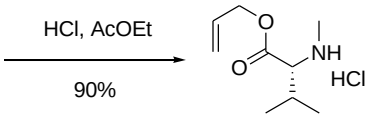


8 **13** **14**

Scheme S1. Synthesis of *N*-Me-*L*-Val-OAll·HCl **14**.



18 **19**



20

Scheme S2. Synthesis of *N*-Me-*D*-Val-OAll·HCl **20**.

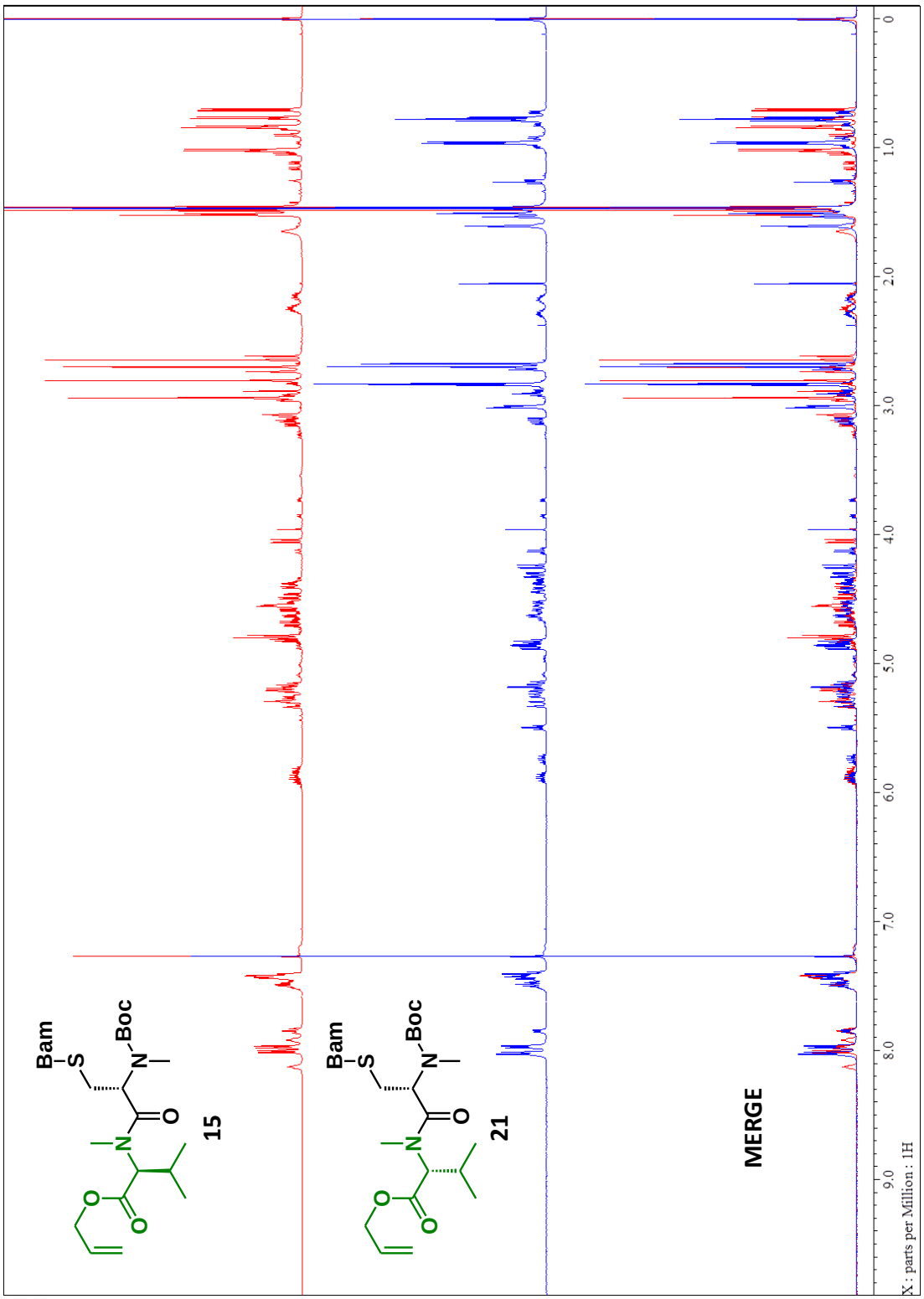


Figure S1. ^{1}H NMR charts of *N*-Boc-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val-OAll **15** (upper), *N*-Boc-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val-OAll **21** (middle). The bottom chart is merging of them.

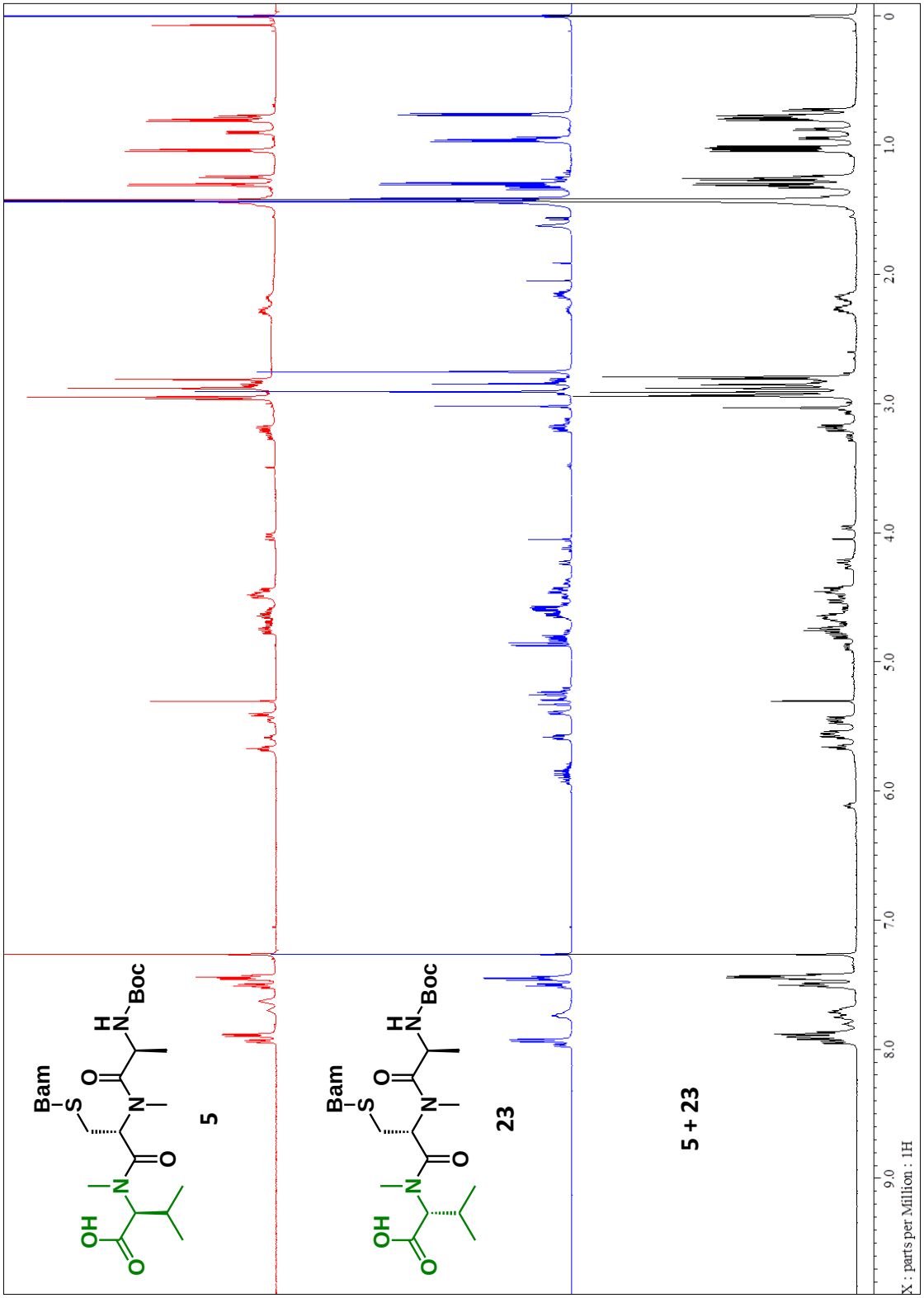


Figure S2. ^{1}H NMR charts of *N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val-OH **5** (upper), *N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val-OH **23** (middle) and the mixture of them (lower).

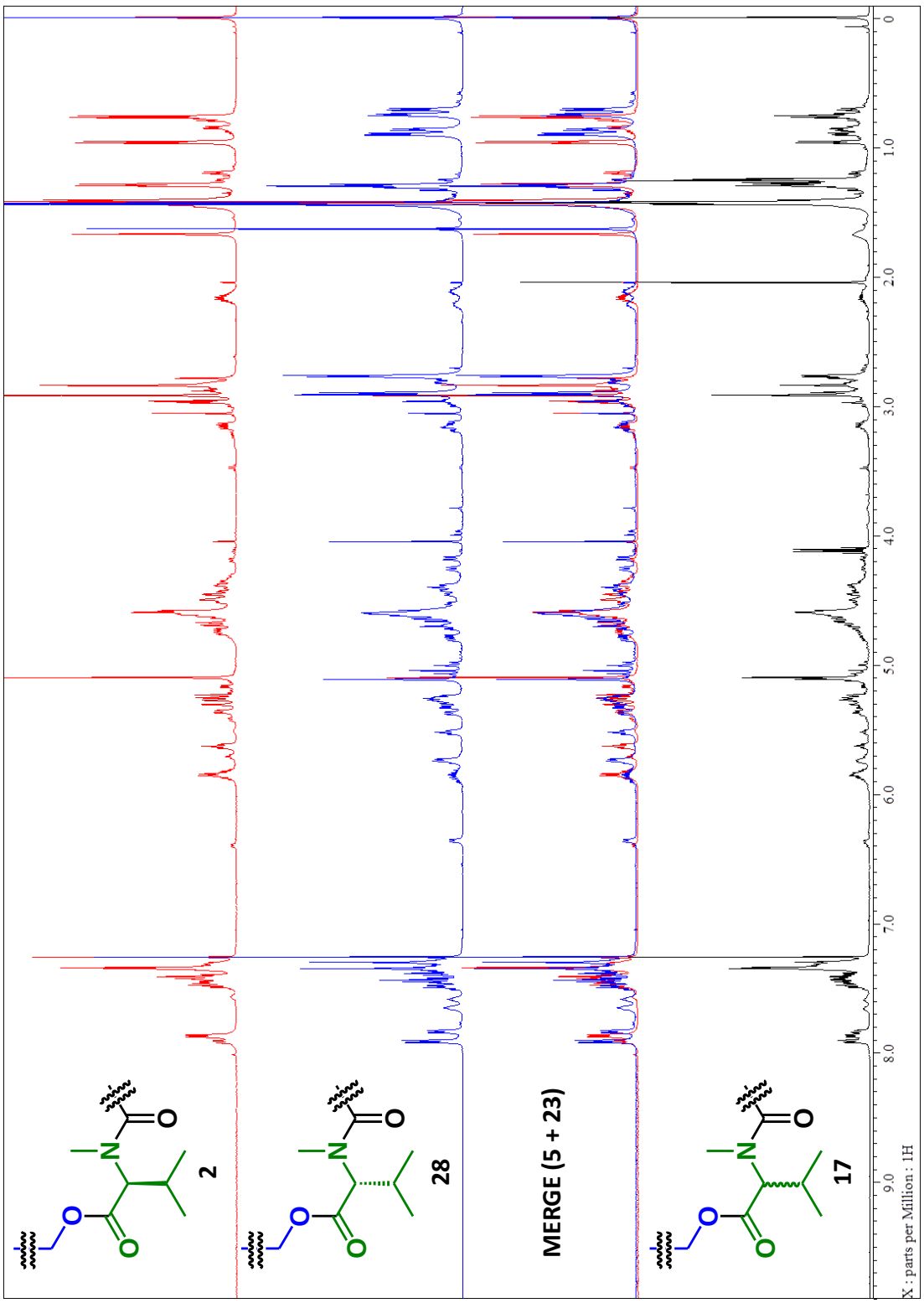


Figure S3. ^{1}H NMR charts of *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll **2** (red), *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OAll **28** (blue) and *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D, L*-Val]-OAll **17** (black).

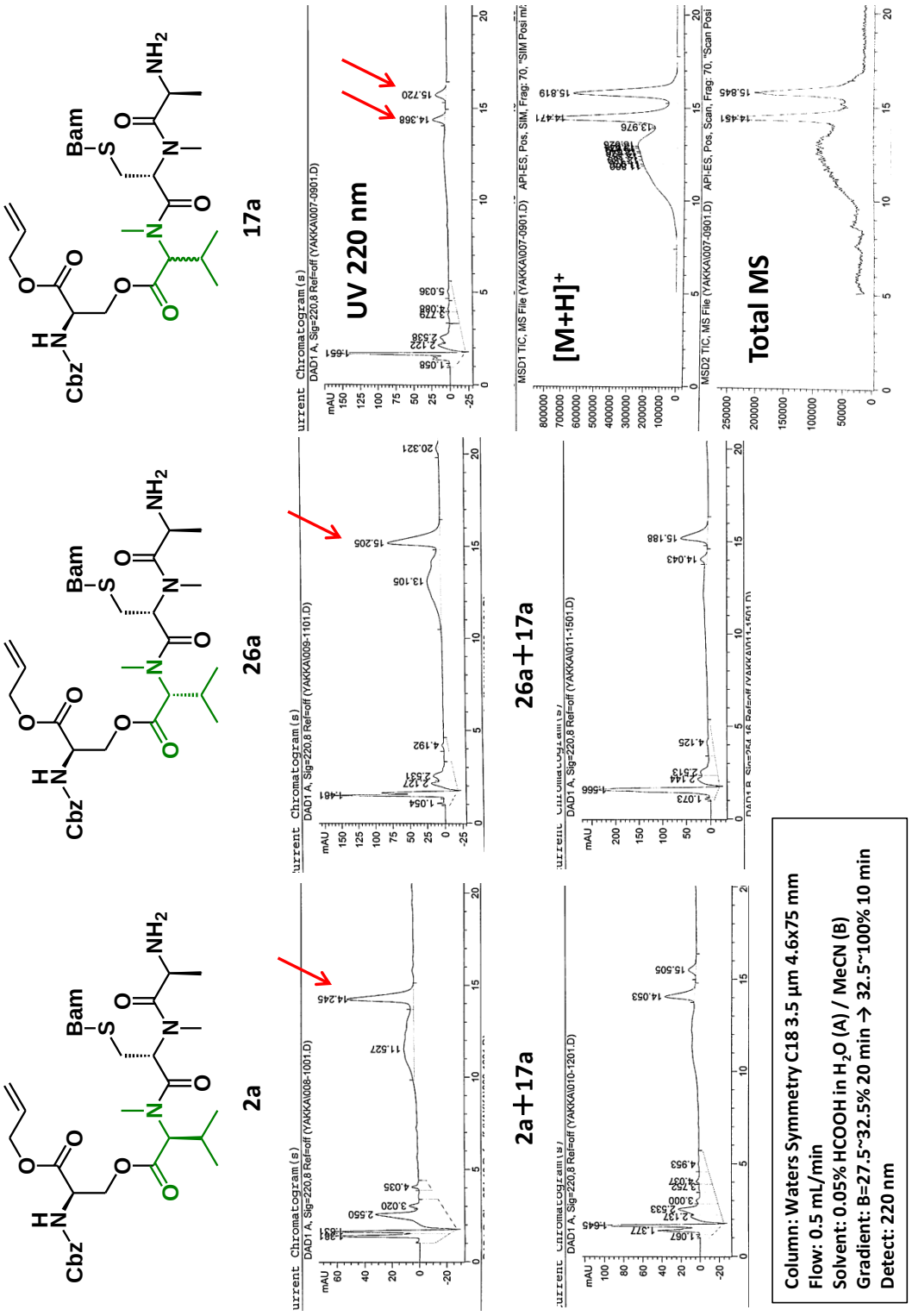
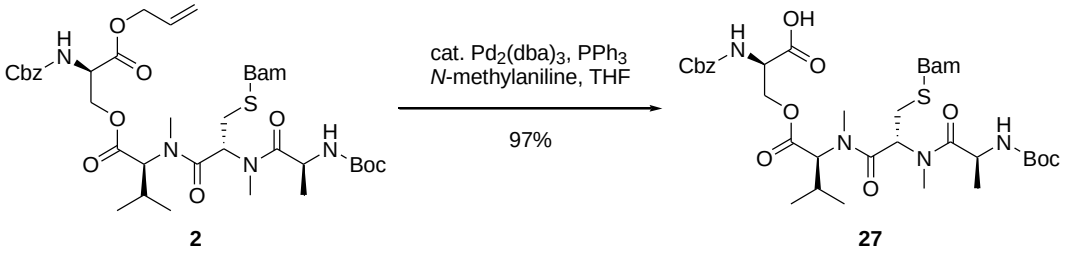
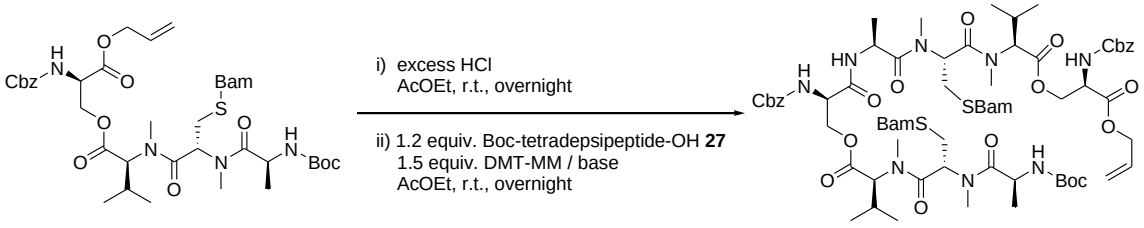


Figure S4. LC/MS analysis of corresponding amine derivatives of *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll **2a**, *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D*-Val]-OAll **26a** and *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*D, L*-Val]-OAll **17a**.



2 **27**

Scheme S3. Synthesis of *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OH **27**.



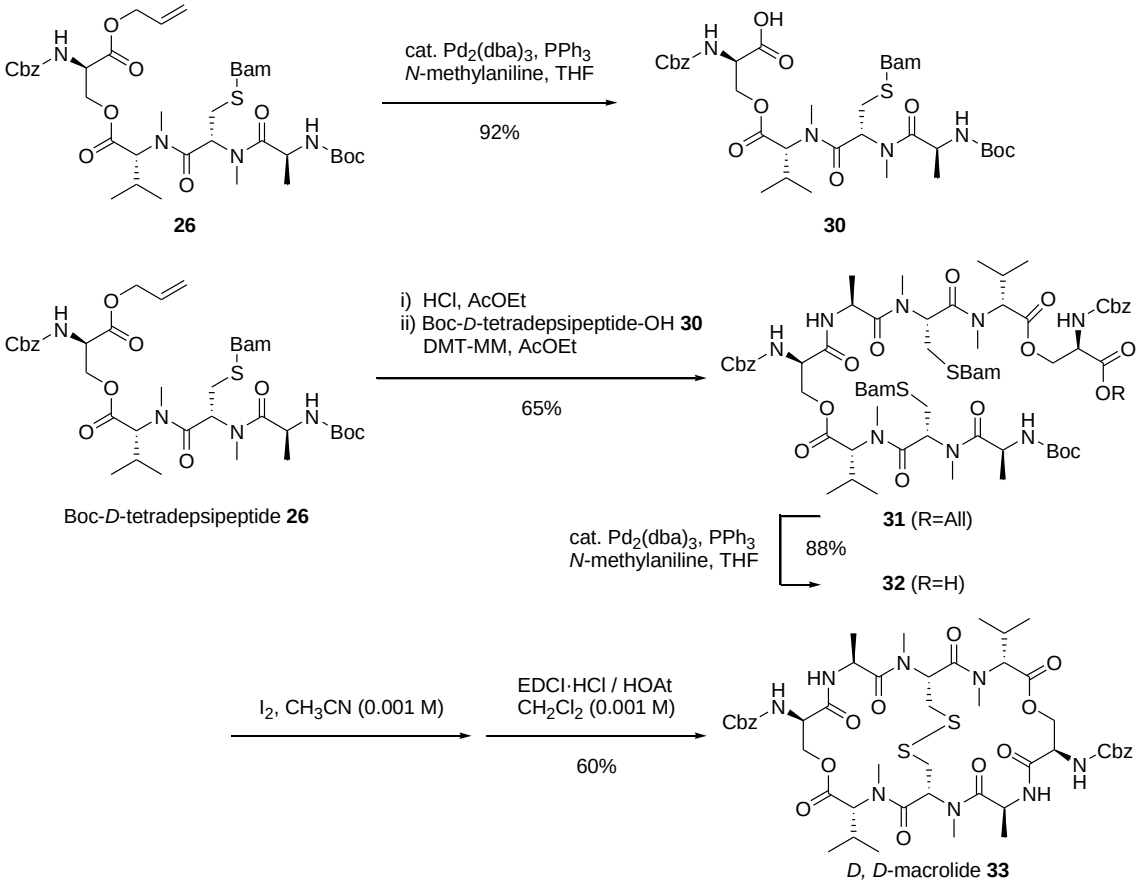
2
50 mg, 0.061 mmol

28

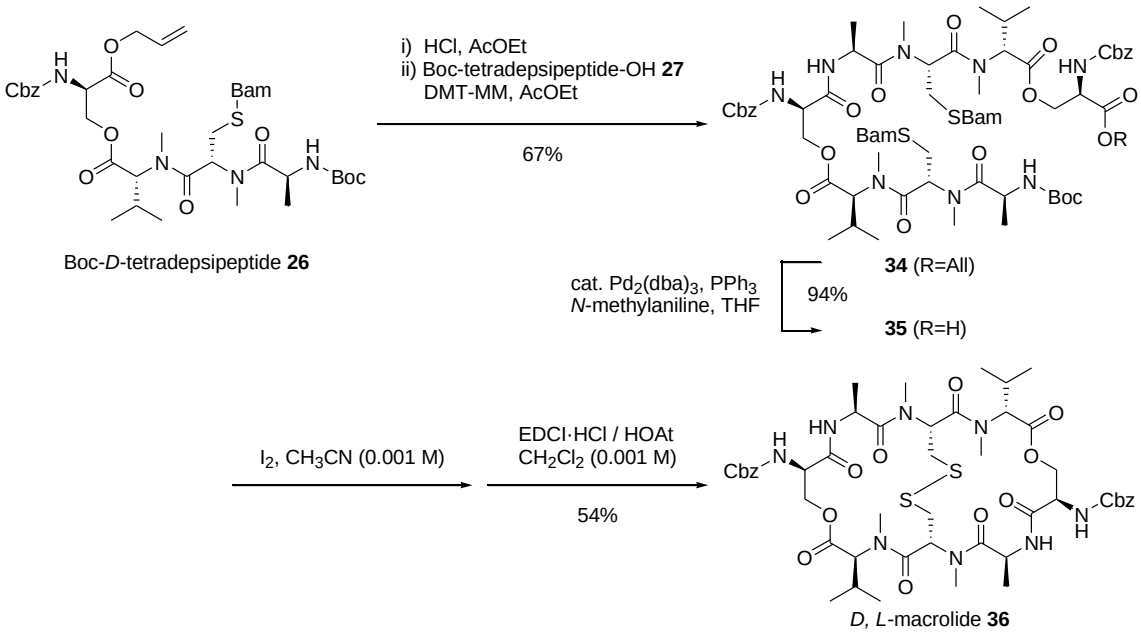
entry	base	solvent	Boc-octadepsipeptide-OAll **28**
1	NMM	AcOEt	46.4 mg, 0.032 mmol, 52% yield on 2 steps
2		DMF	36.1 mg, 0.025 mmol, 41% yield on 2 steps
3		CH_3CN	25.4 mg, 0.017 mmol, 28% yield on 2 steps
4		THF	42.8 mg, 0.029 mmol, 48% yield on 2 steps
5	none	AcOEt	43.8 mg, 0.030 mmol, 49% yield on 2 steps
6		DMF	46.2 mg, 0.031 mmol, 51% yield on 2 steps
7		CH_3CN	47.9 mg, 0.033 mmol, 54% yield on 2 steps
8		THF	41.5 mg, 0.028 mmol, 46% yield on 2 steps
9[a]		AcOEt	56.6 mg, 0.039 mmol, 60% yield on 2 steps

[a]s.m. **27** = 50 mg (0.065 mmol, 1 equiv.), Boc-tetradepsipeptide-OAll **2** = 1.2 equiv.

Table S4. Optimization of the reaction solvent and base on the acylation of *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OAll **2** and *N*-Cbz-*D*-Ser[*N*-Boc-*L*-Ala-*N*-Me-*L*-Cys(Bam)-*N*-Me-*L*-Val]-OH **27**. In the case of *N*-methylmorpholine (NMM) addition, the quench method of the de-Boc step was just concentration to remove the reaction solvent. On the other hand, the extraction of the reaction solvent with sat. $NaHCO_3$ aq. for neutralization was performed in the case of none base condition.



Scheme S4. Synthesis of *D, D*-macrolide **33**.



Scheme S5. Synthesis of *D, L*-macrolide **36**.

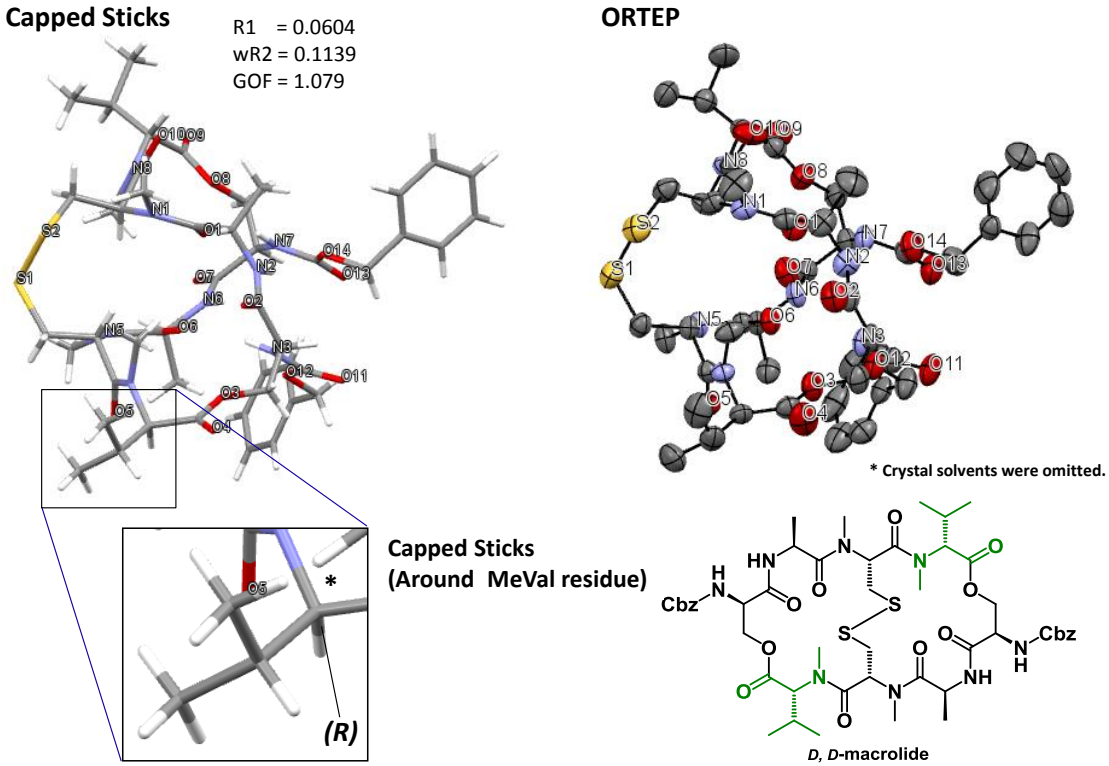


Figure S5. X ray crystal structural analysis of **33** (*D*, *D*-macrolide).

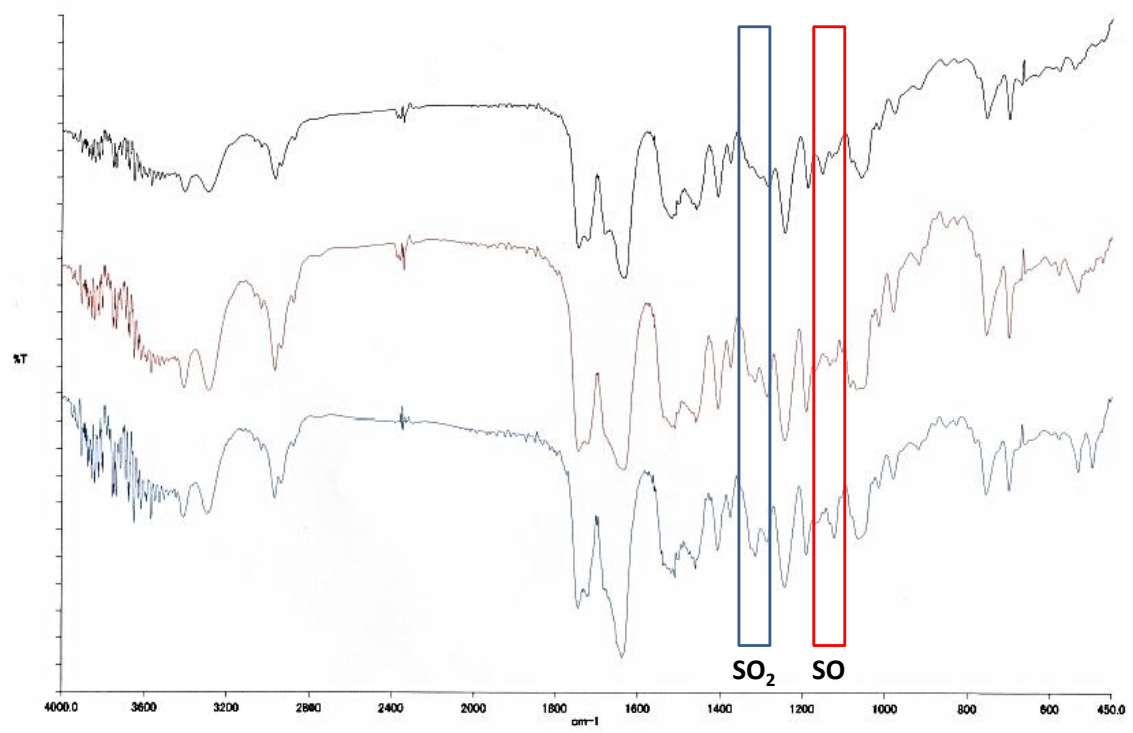


Figure S6. IR spectra (KBr). **1** : black, **44** (sulfoxide) : red, **45** (sulfone) : blue.

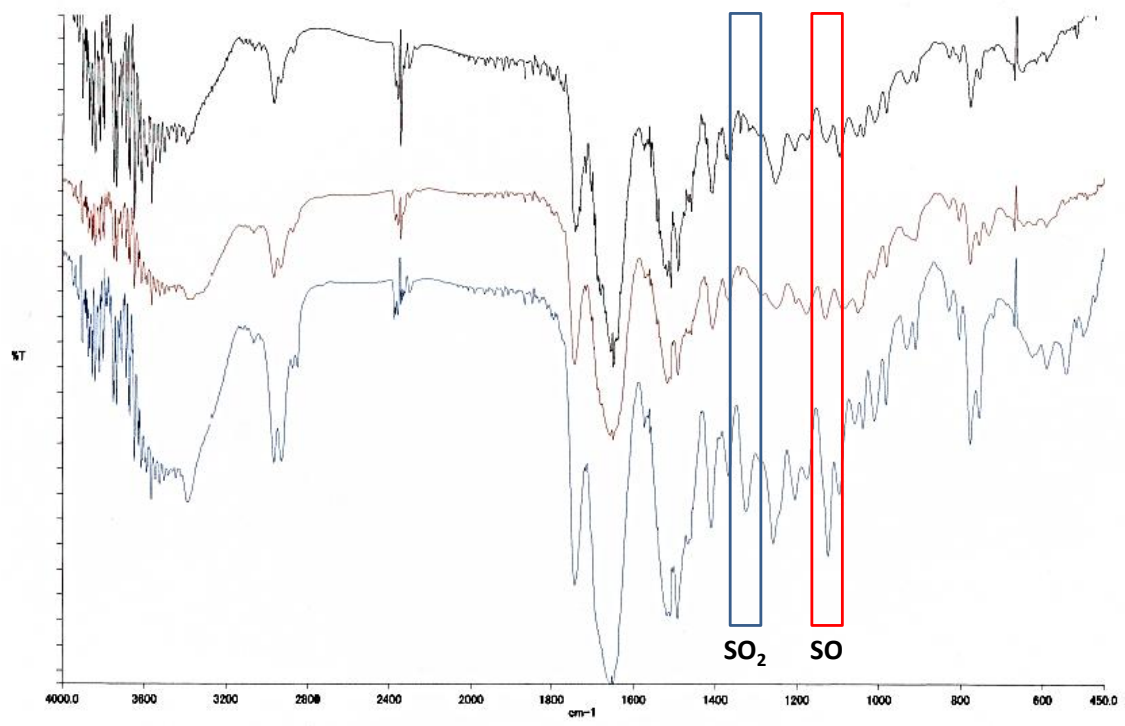
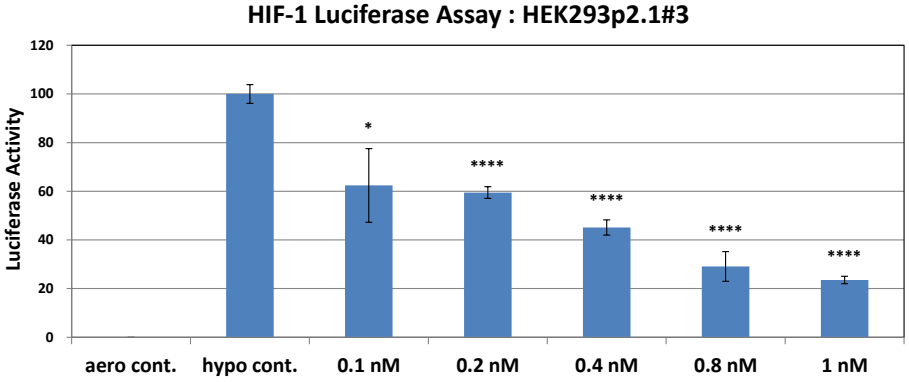
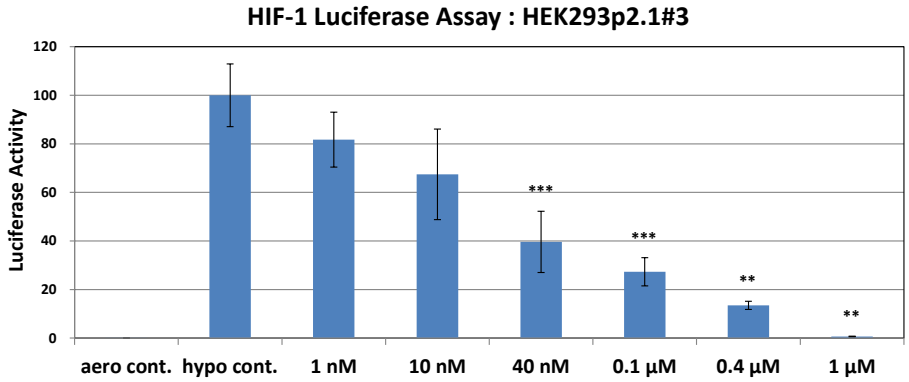


Figure S7. IR spectra (KBr). TA : black, **46** (SO) : red, **47** (SO_2) : blue.

A) Echinomycin (EC)

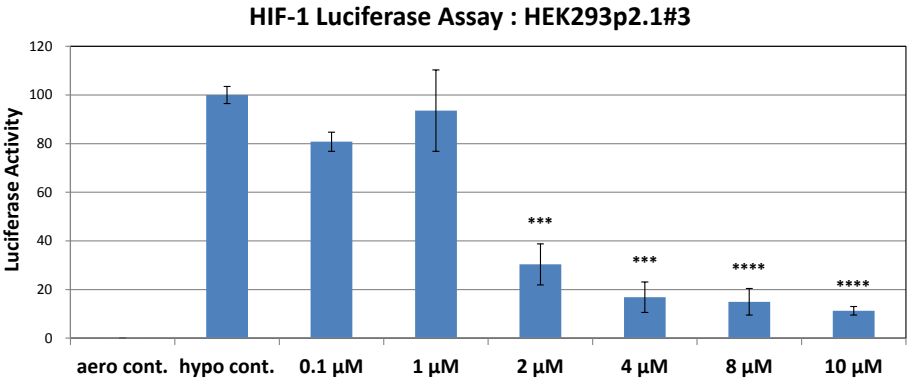


B) Triostin A (TA)



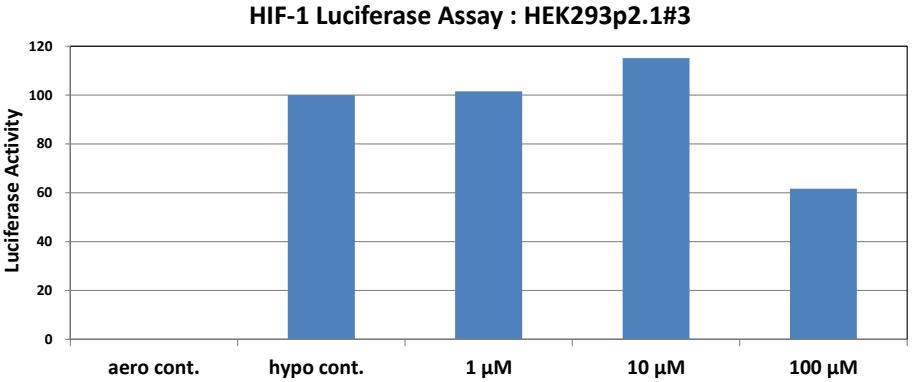
p < 0.05: *
p < 0.01: **
p < 0.005: ***
p < 0.0005: ****

C) 40 (Qn)

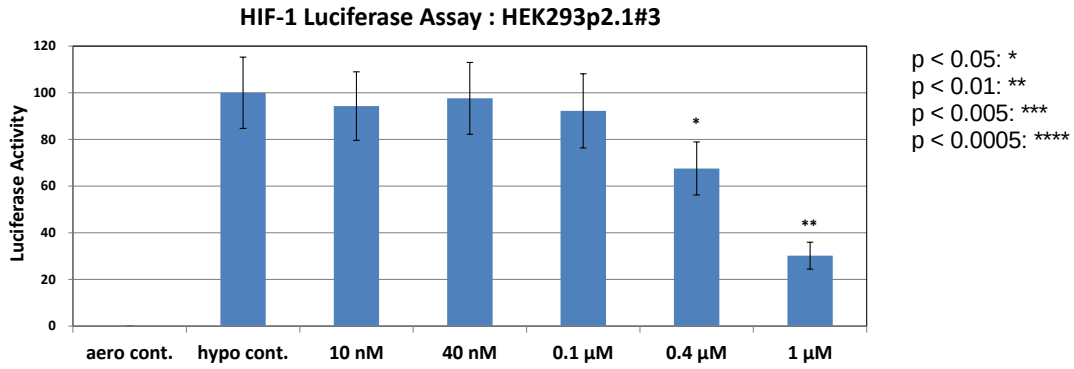


p < 0.05: *
p < 0.01: **
p < 0.005: ***
p < 0.0005: ****

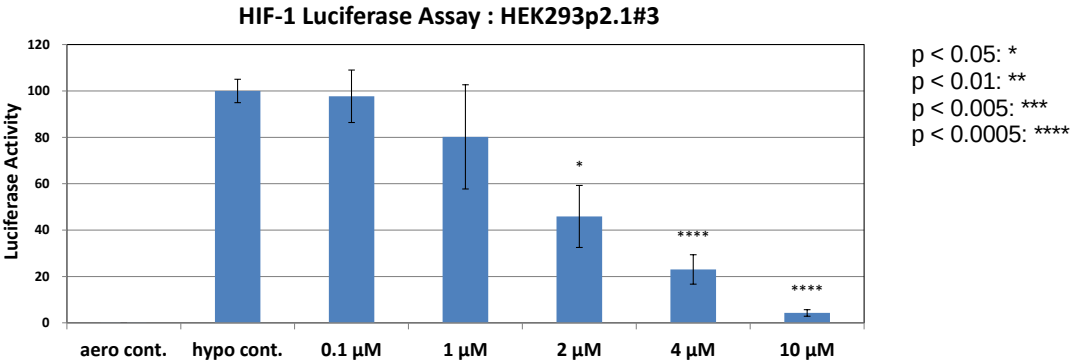
D) 41 (Np)



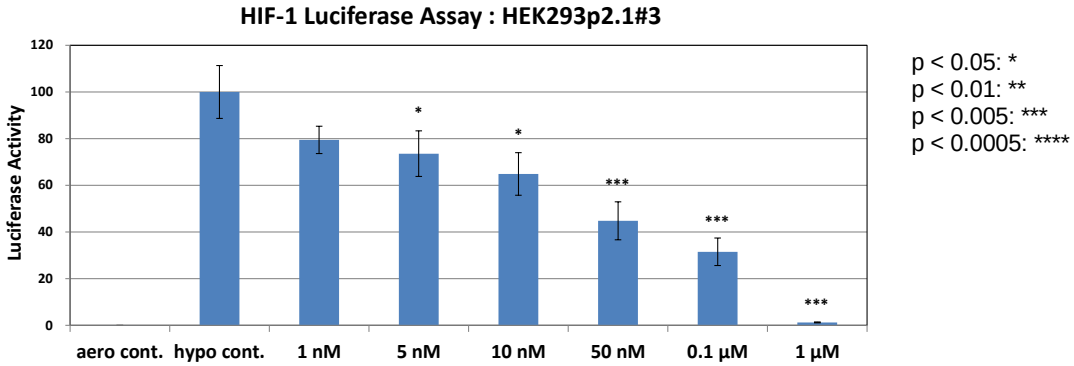
E) 42 (D,D)



F) 43 (D,L)



G) 46 (SO)



H) 47 (SO_2)

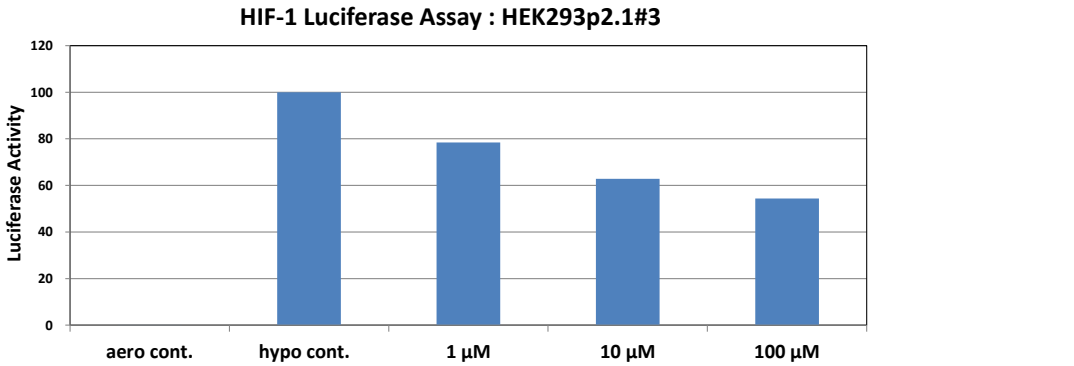
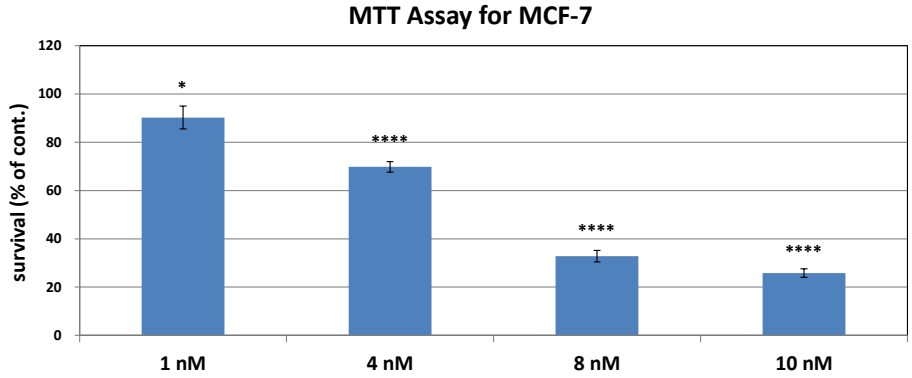


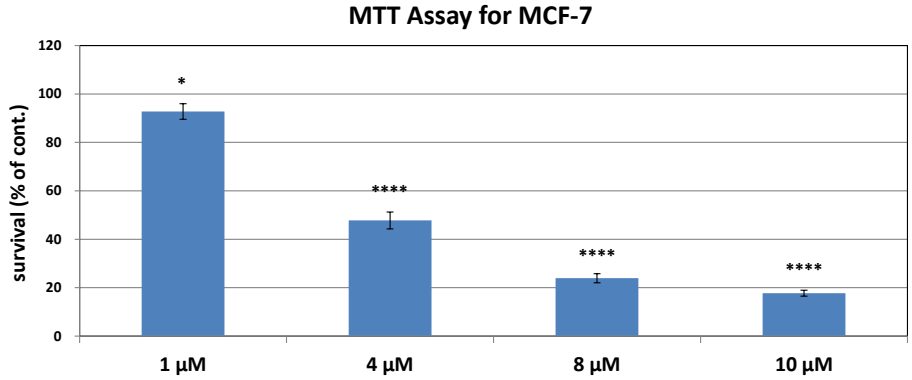
Figure S8. HIF-1 Dependent Luciferase Assay.

A) Echinomycin (EC)



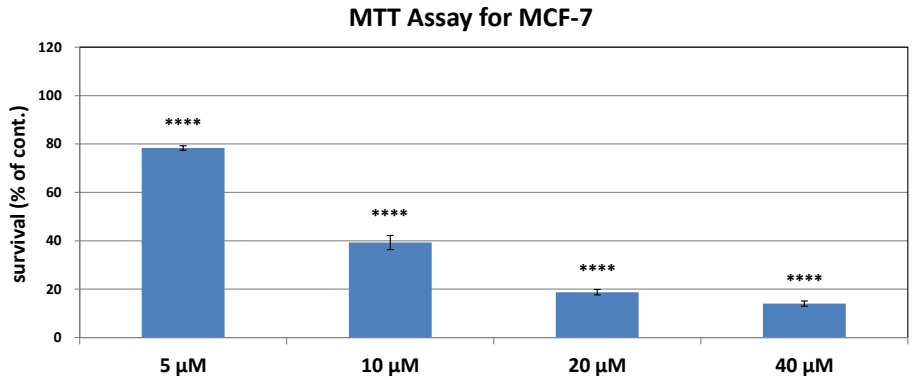
p < 0.05: *
p < 0.01: **
p < 0.005: ***
p < 0.0005: ****

B) Triostin A (TA)



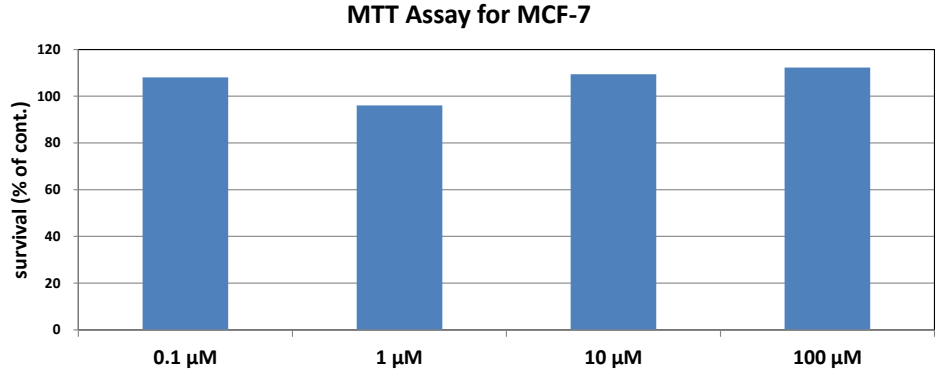
p < 0.05: *
p < 0.01: **
p < 0.005: ***
p < 0.0005: ****

C) 40 (Qn)

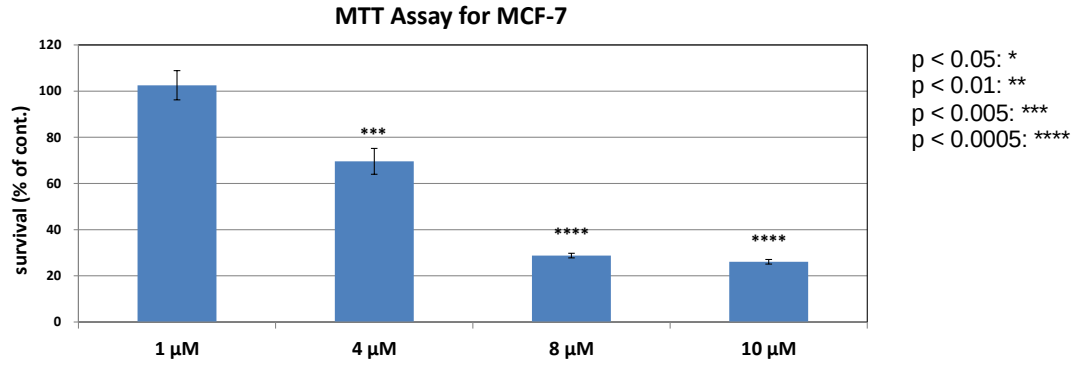


p < 0.05: *
p < 0.01: **
p < 0.005: ***
p < 0.0005: ****

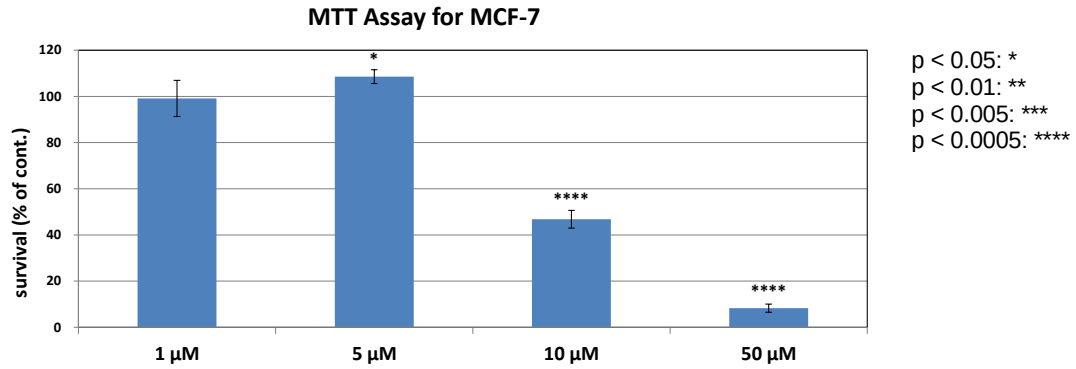
D) 41 (Np)



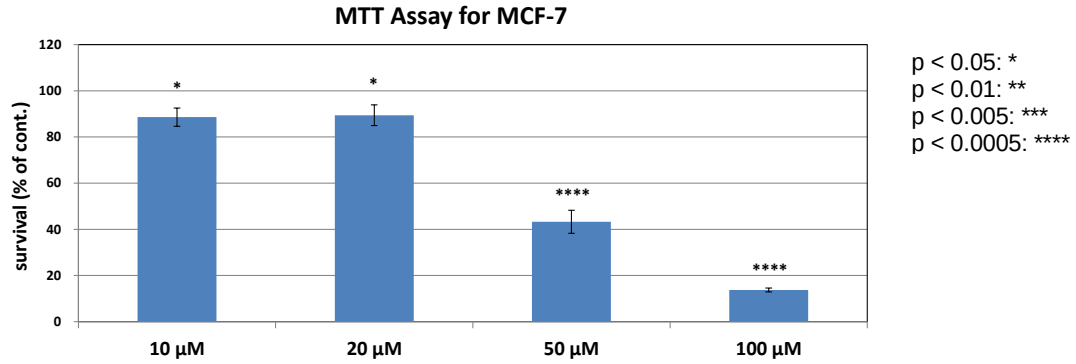
E) 42 (D,D)



F) 43 (D,L)



G) 46 (SO)



H) 47 (SO$_2$)

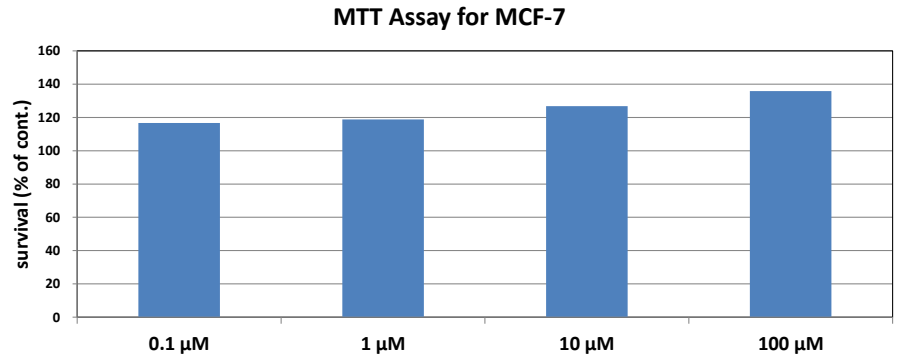


Figure S9. MTT Assay on MCF-7.

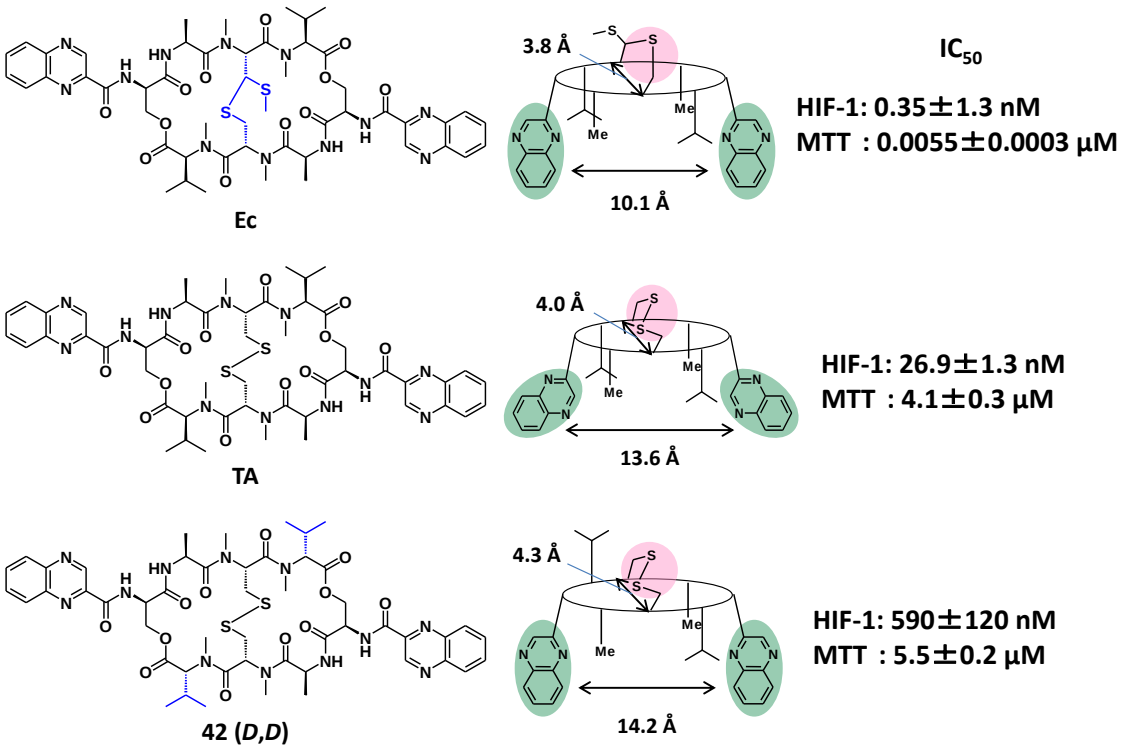
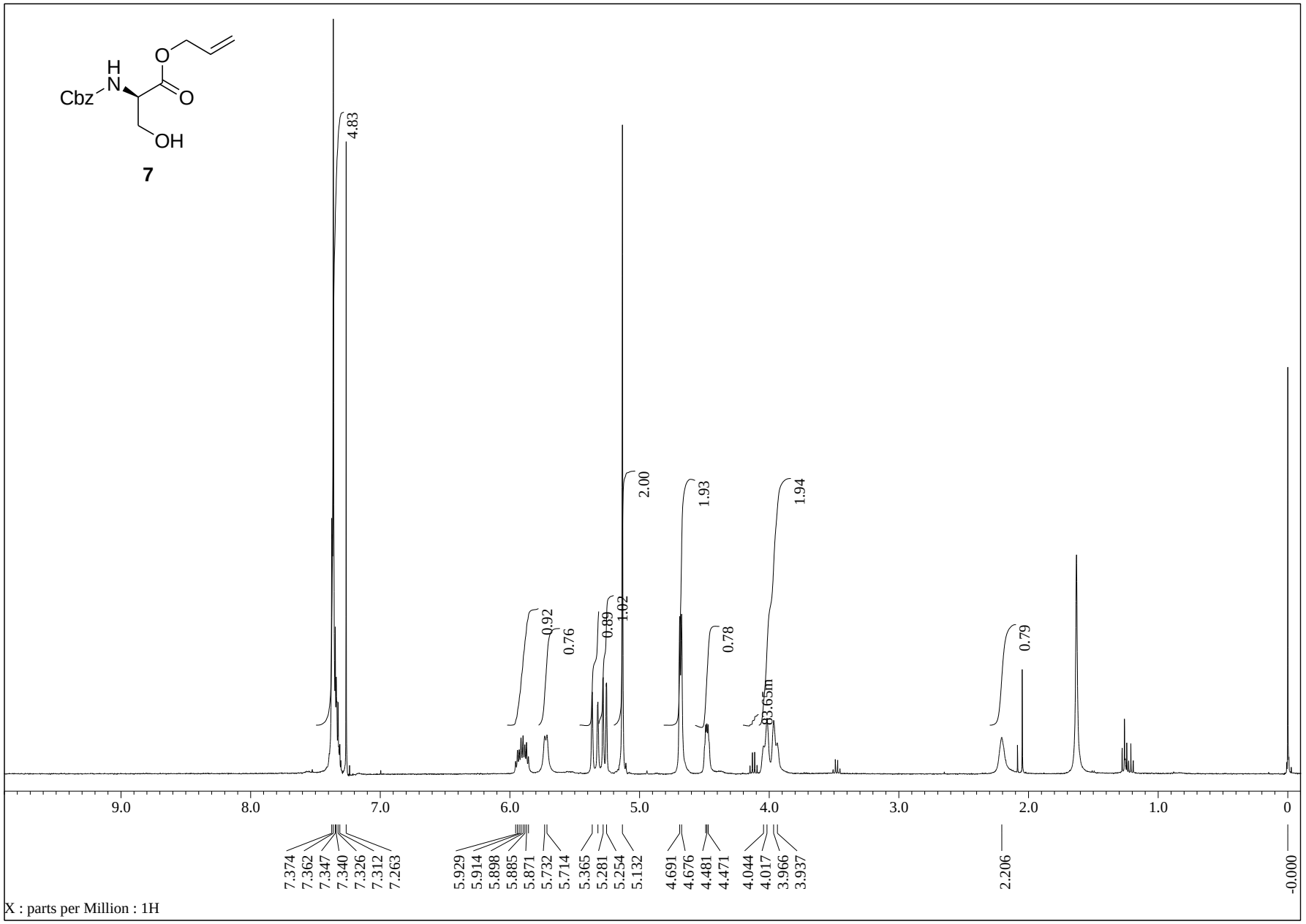


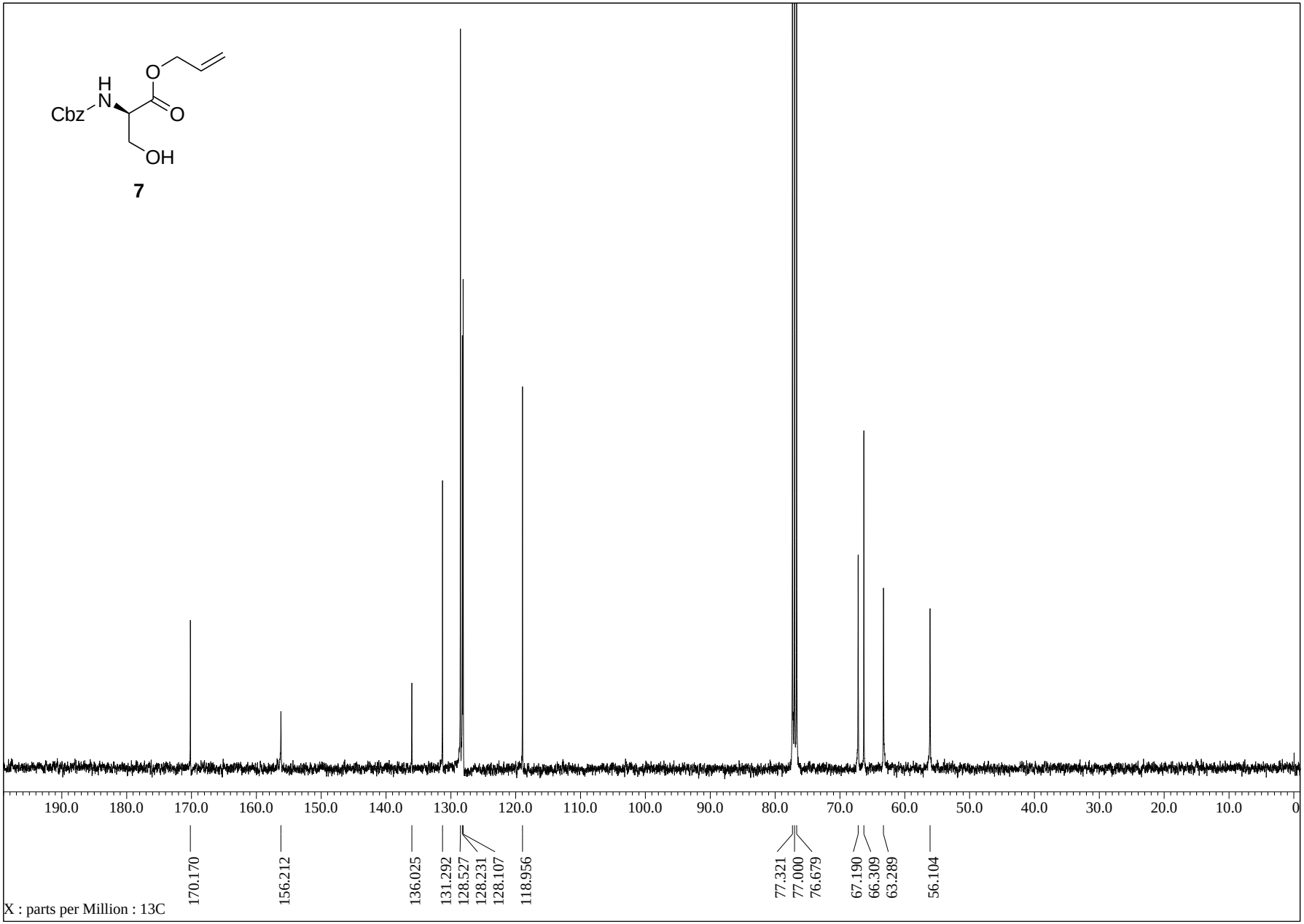
Figure S10. Comparison of echinomycin (Ec), triostin A (TA) and **42** (*D*,*D*). The distance between the pair of carbonyl carbon atoms of the two quinoxaline-2-carboxamide moieties and α-carbon atoms of the two cysteine residues are shown.

^{1}H NMR and ^{13}C NMR Charts

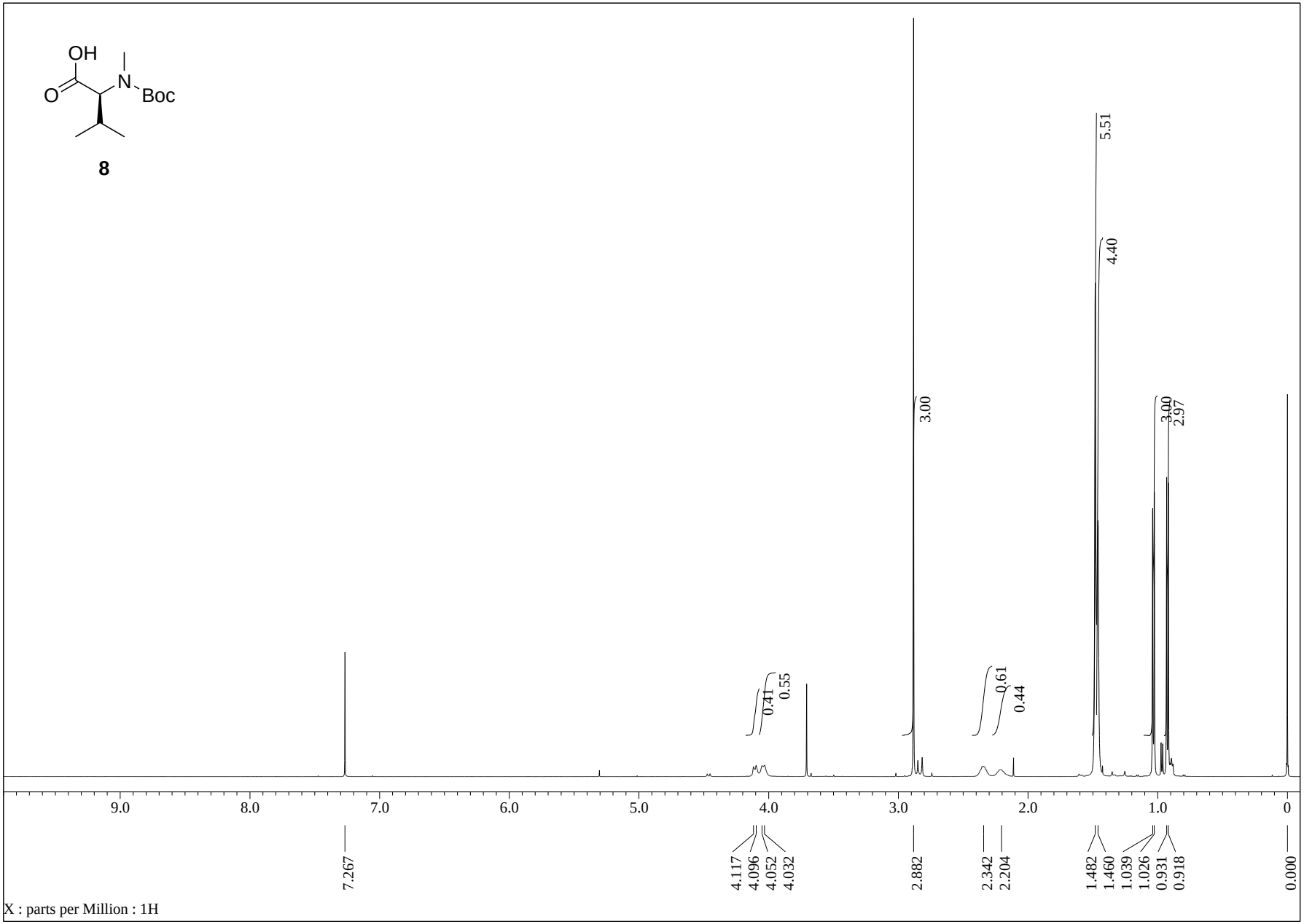
^{1}H NMR (400 MHz, $CDCl_3$)



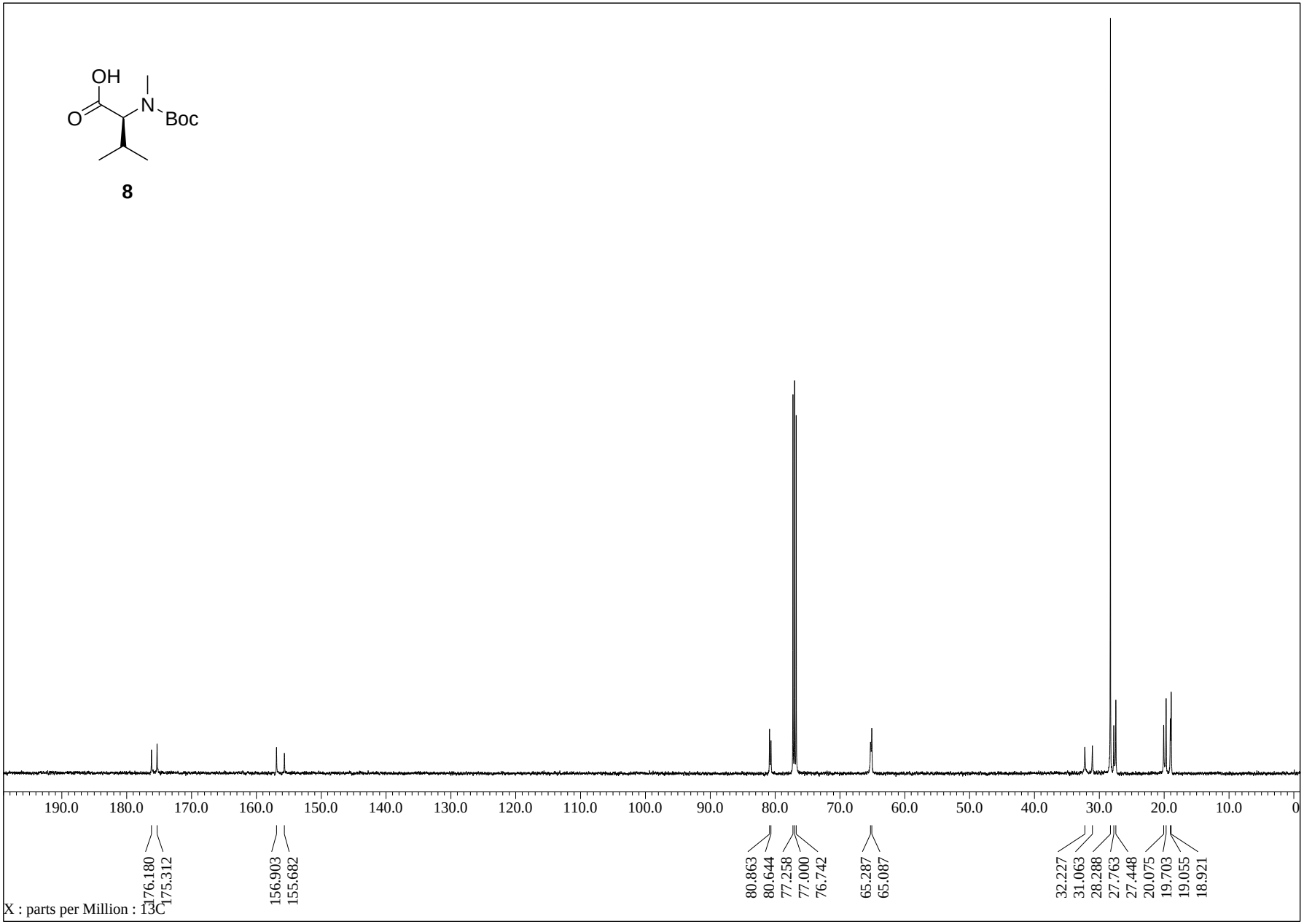
^{13}C NMR (100 MHz, $CDCl_3$)



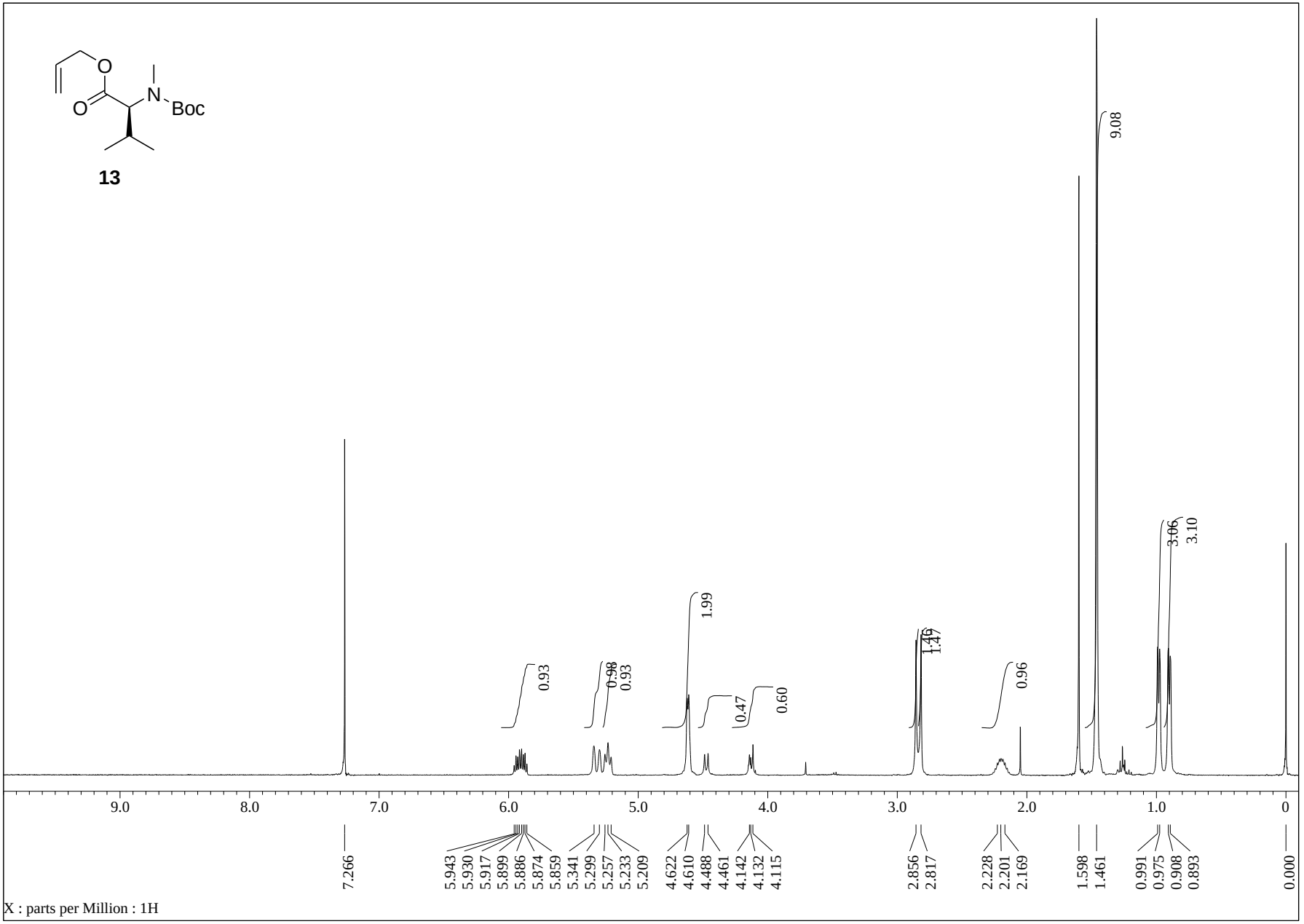
^{1}H NMR (500 MHz, $CDCl_3$)



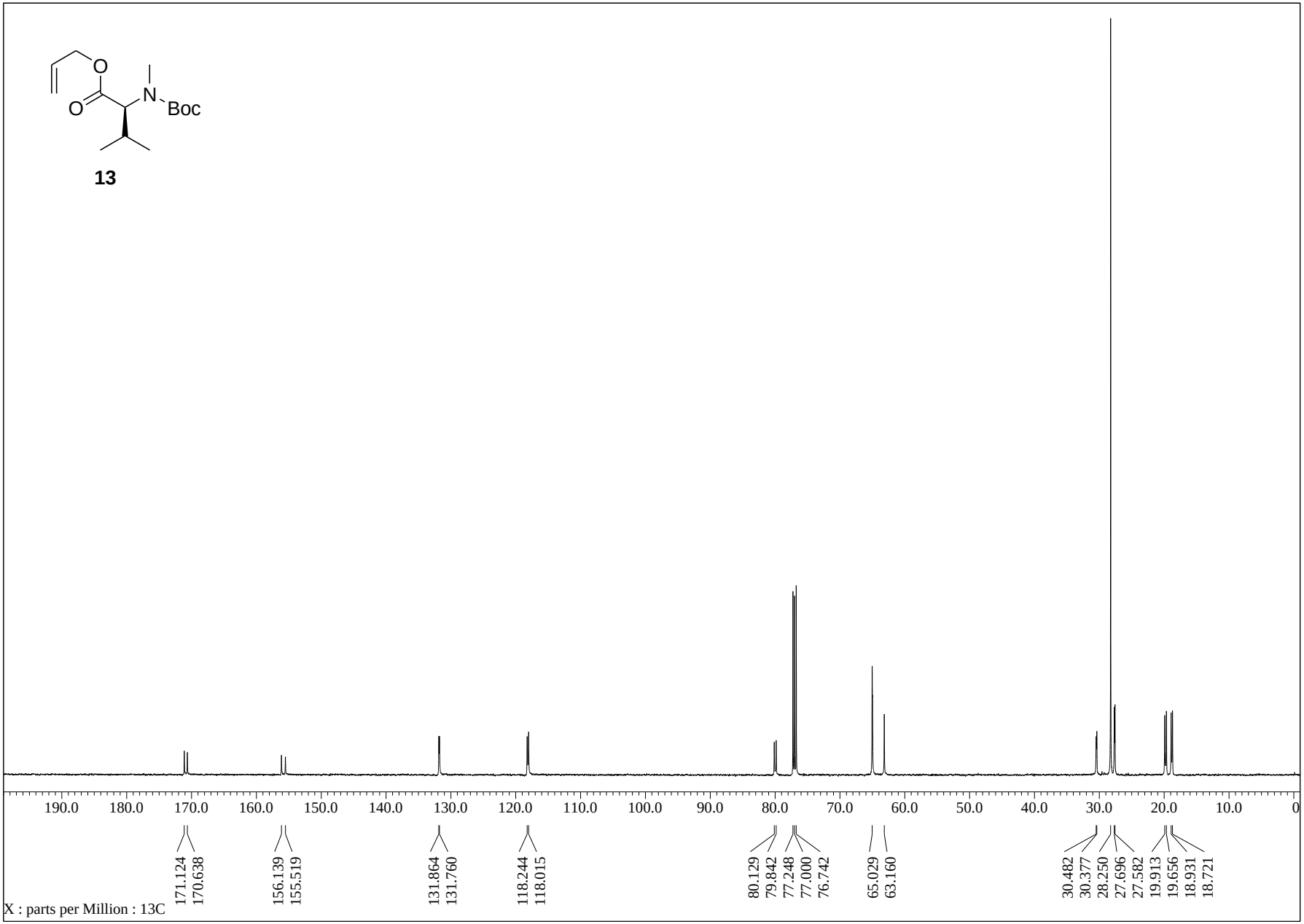
^{13}C NMR (125 MHz, $CDCl_3$)



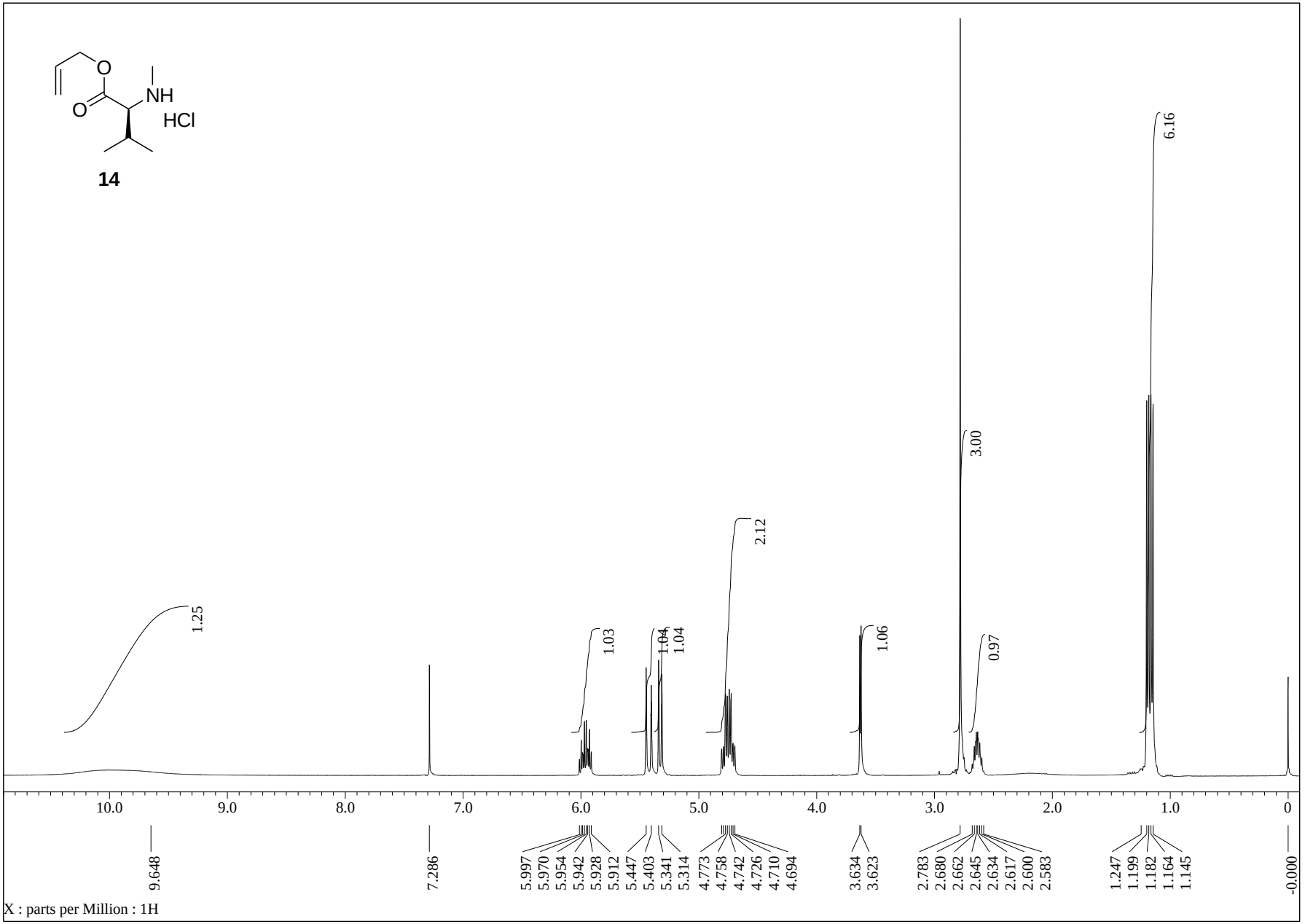
^{1}H NMR (400 MHz, $CDCl_3$)



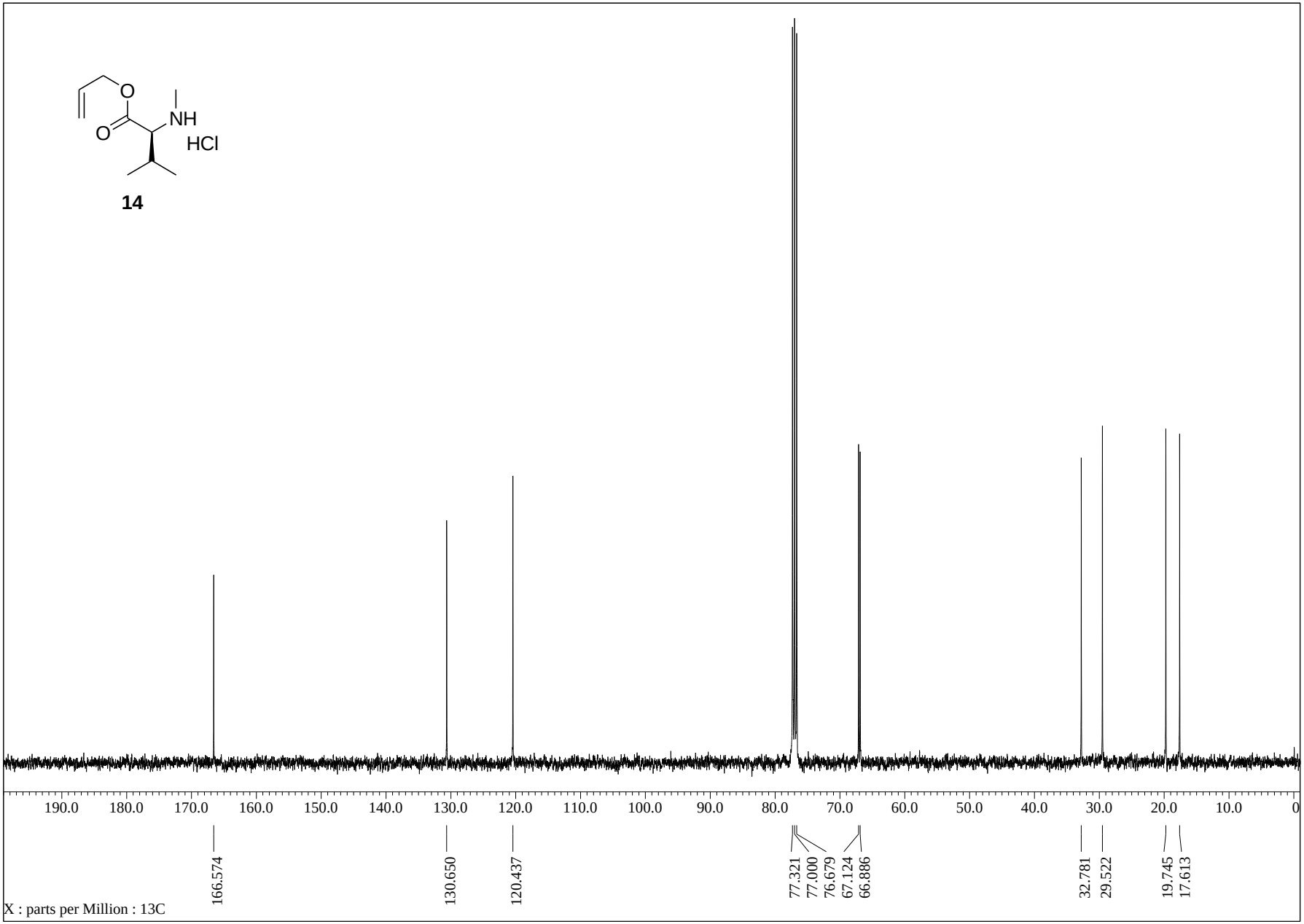
^{13}C NMR (125 MHz, $CDCl_3$)



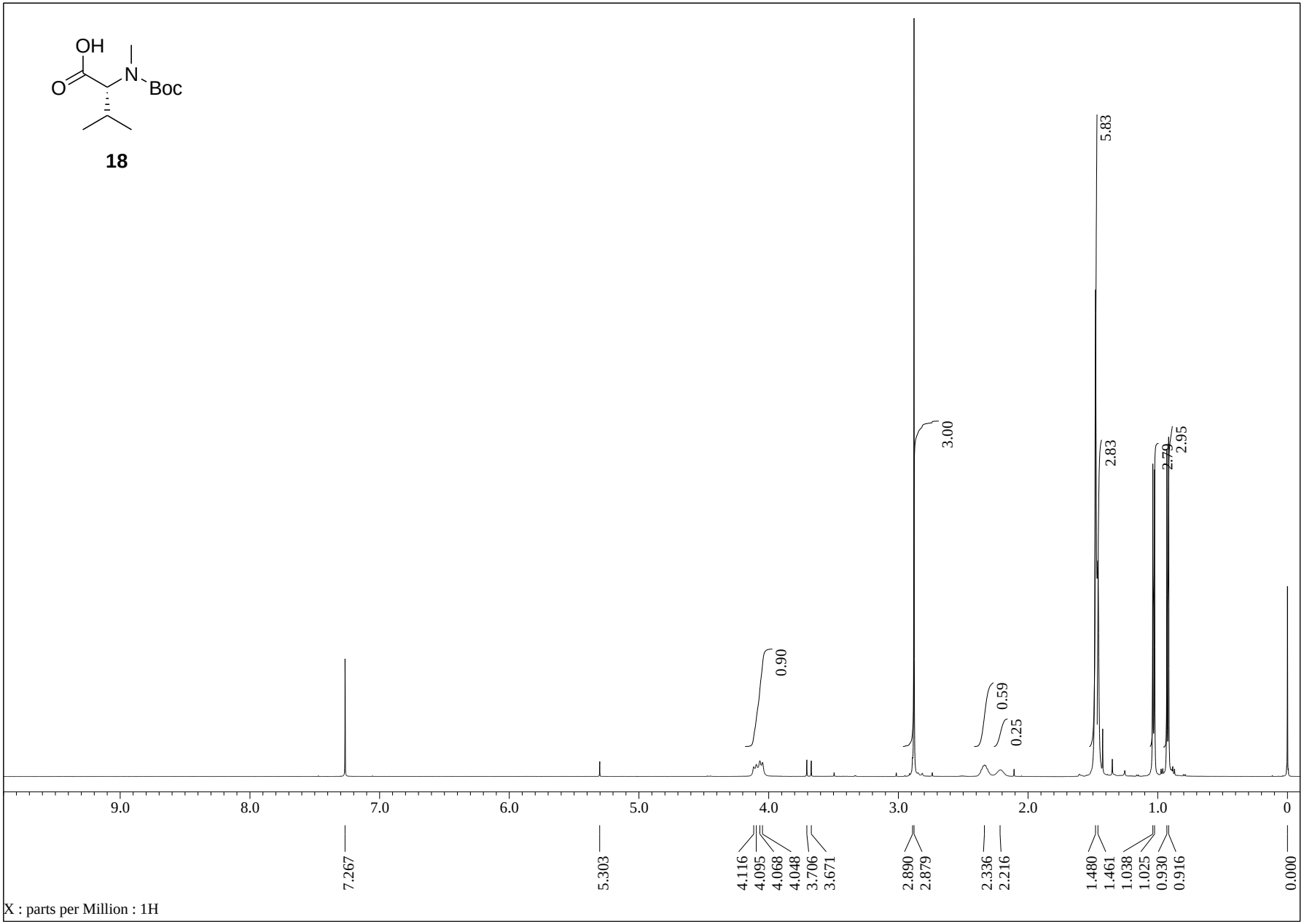
^{1}H NMR (400 MHz, $CDCl_3$)



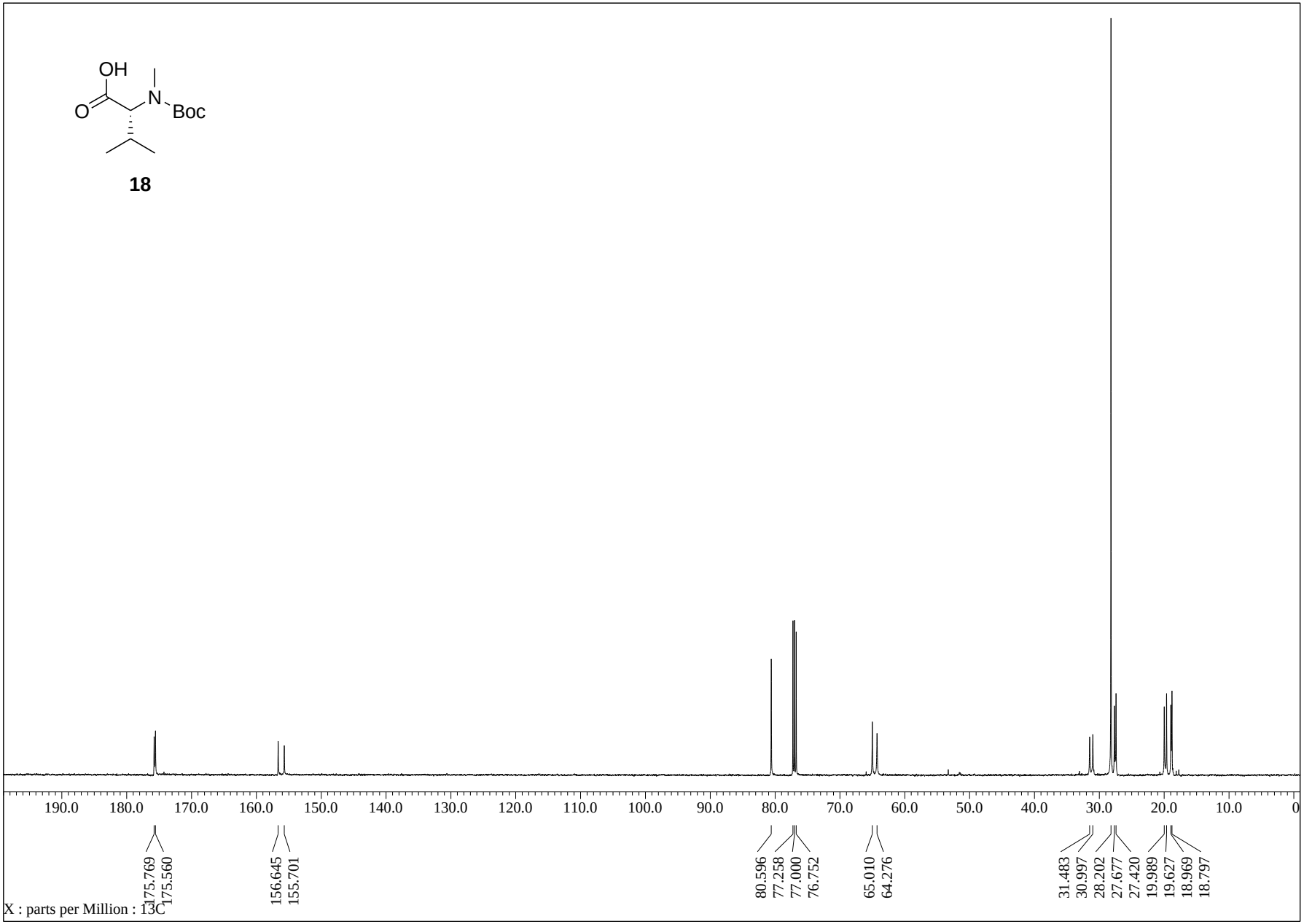
^{13}C NMR (100 MHz, $CDCl_3$)



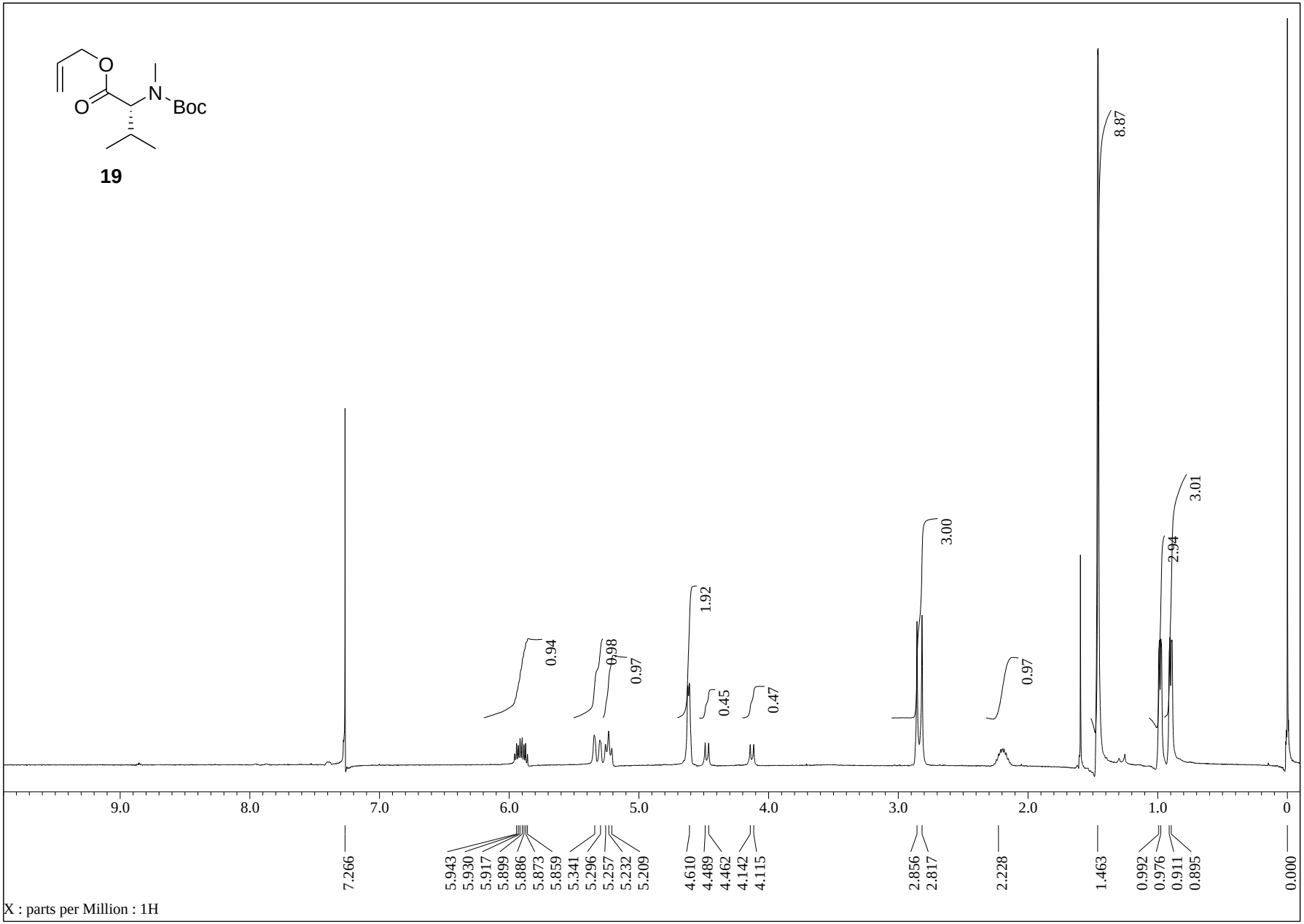
^{1}H NMR (500 MHz, $CDCl_3$)



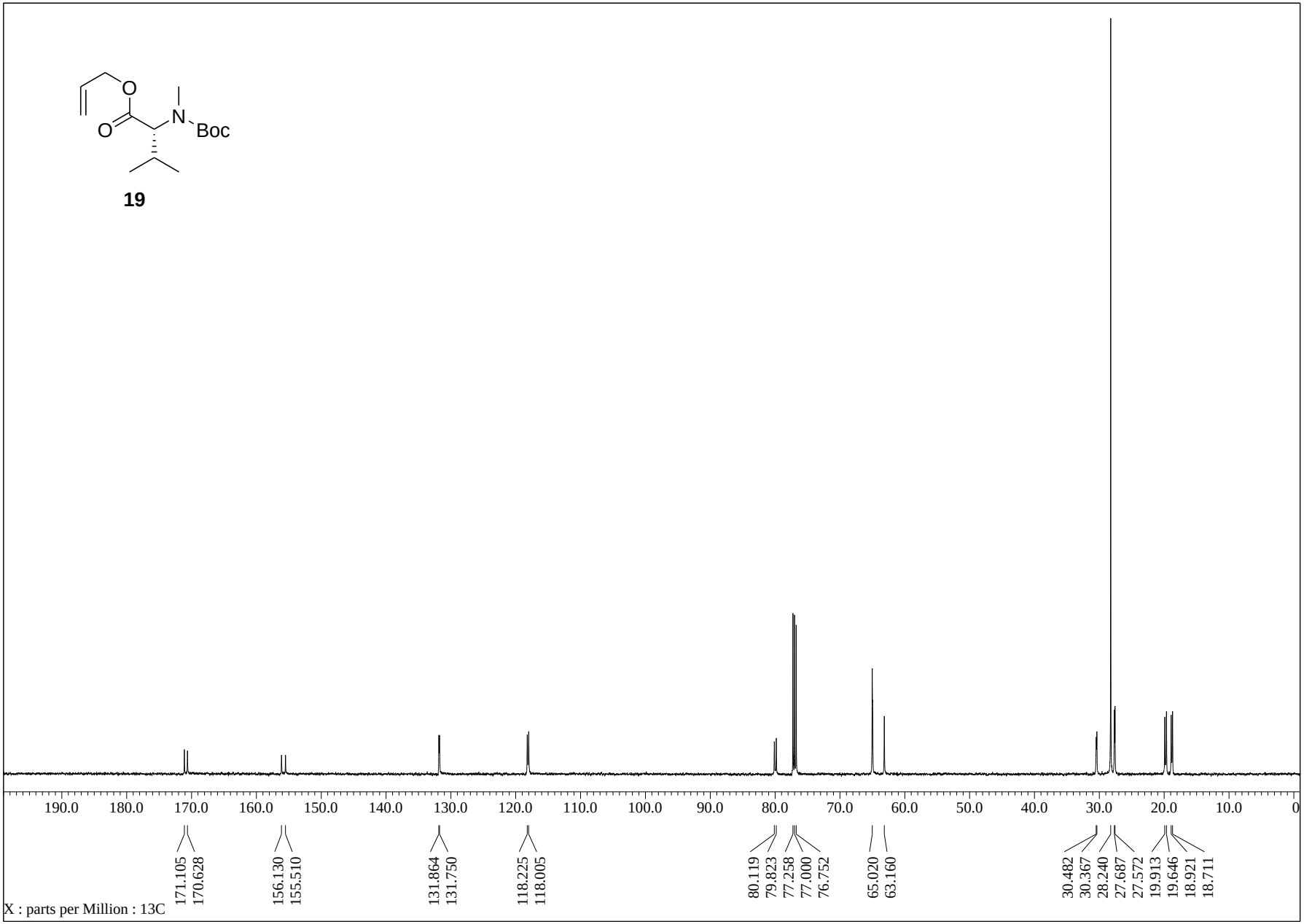
^{13}C NMR (125 MHz, $CDCl_3$)



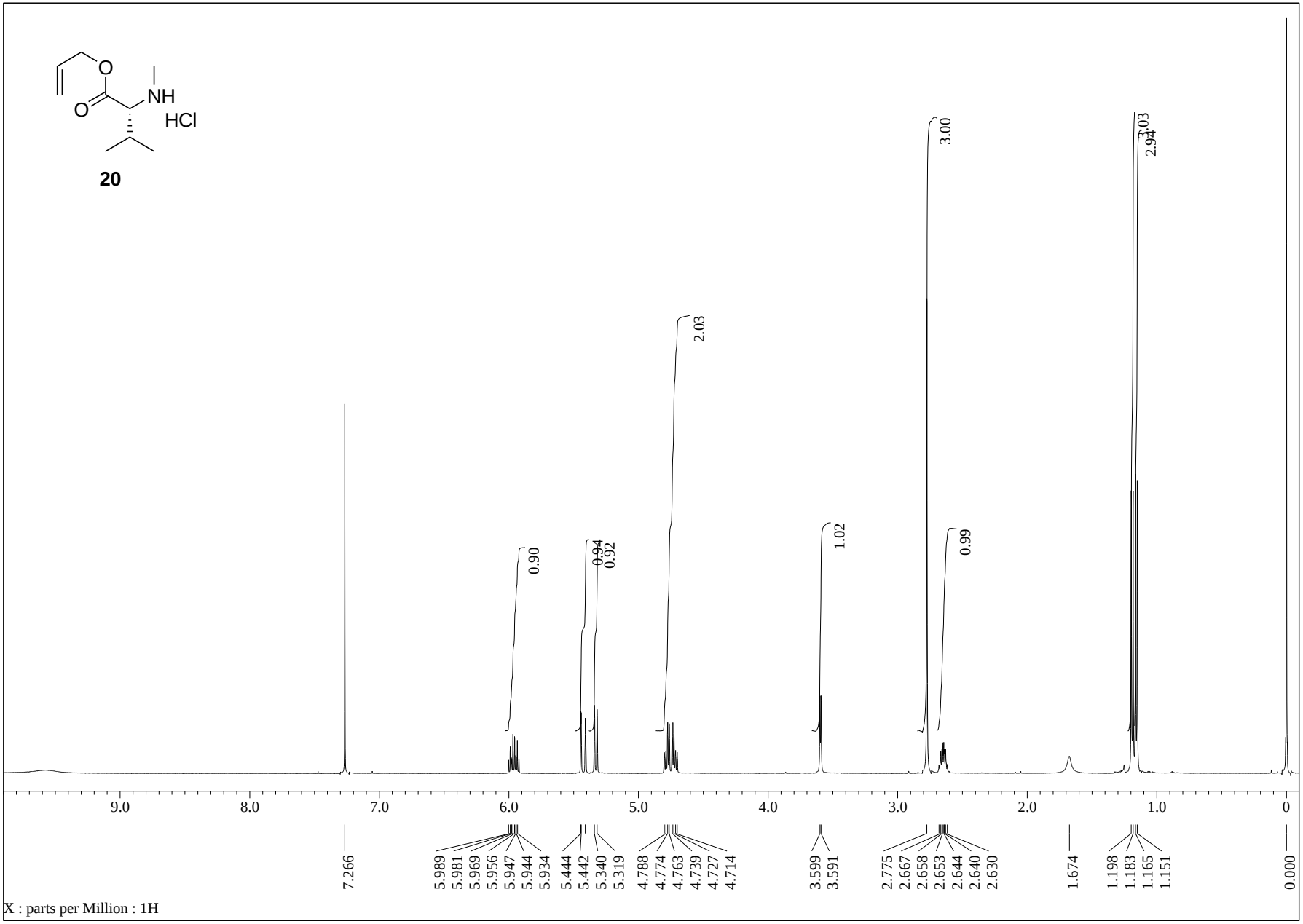
^{1}H NMR (400 MHz, $CDCl_3$)



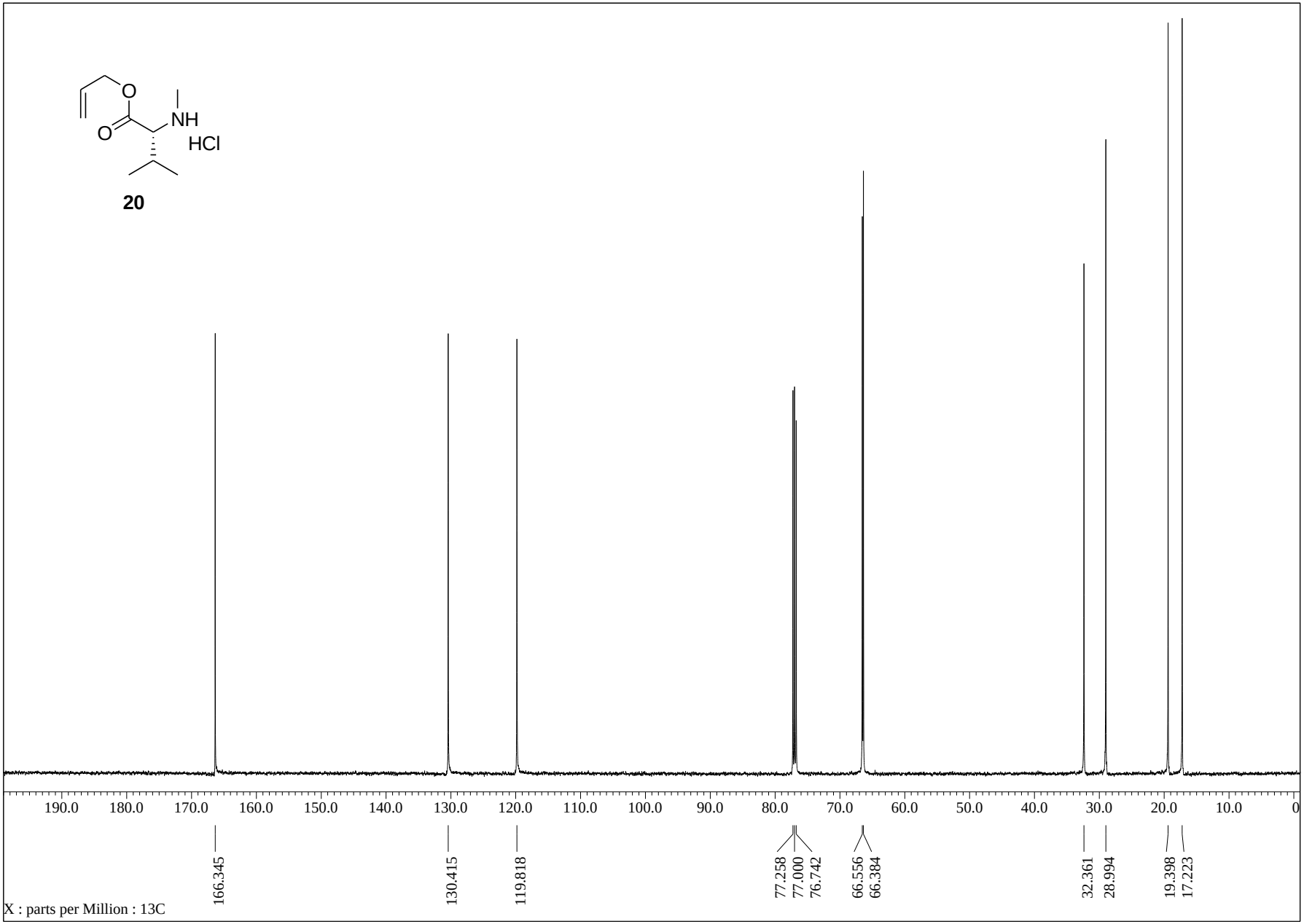
^{13}C NMR (125 MHz, $CDCl_3$)



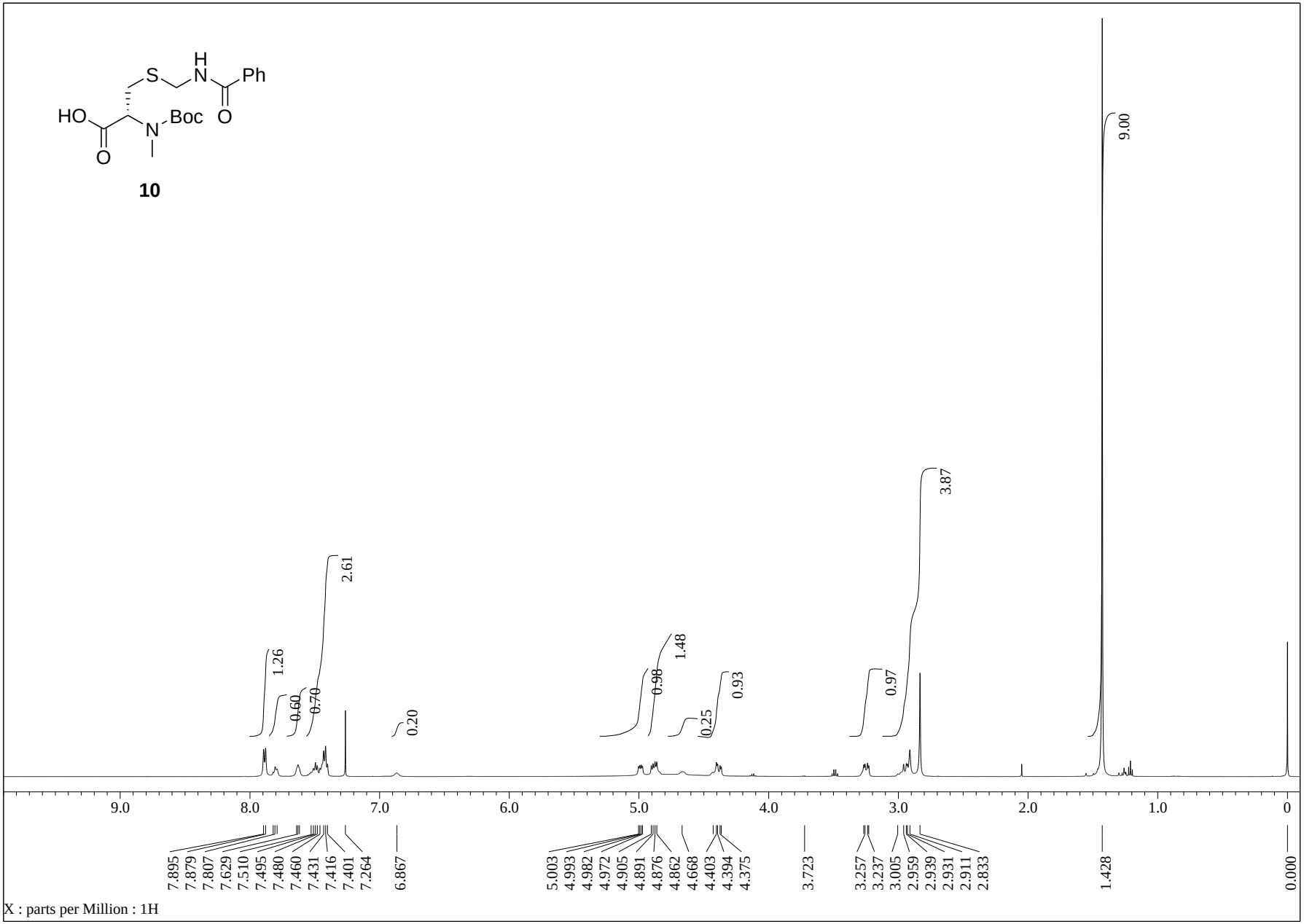
^{1}H NMR (500 MHz, $CDCl_3$)



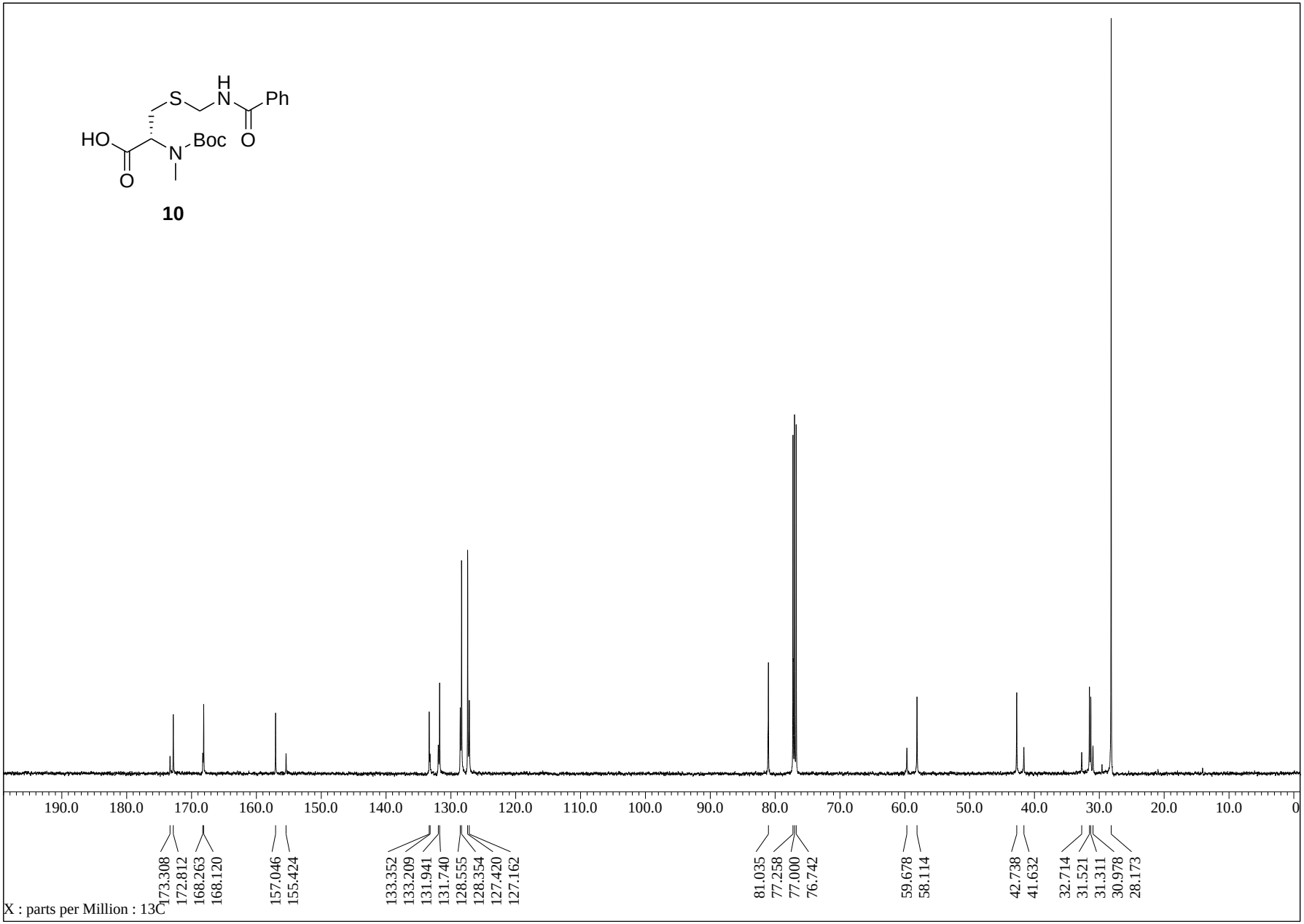
^{13}C NMR (125 MHz, $CDCl_3$)



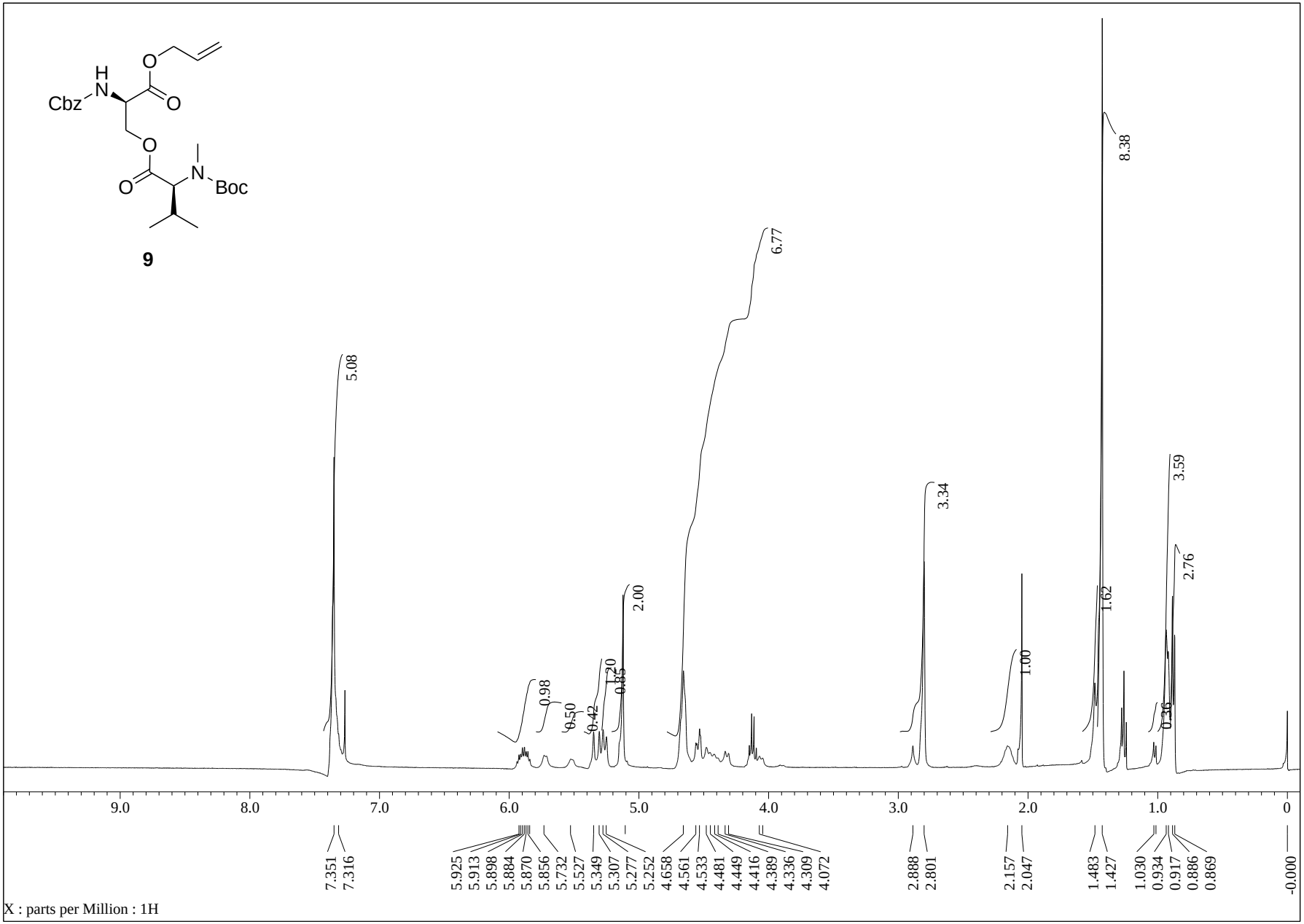
^{1}H NMR (500 MHz, $CDCl_3$)



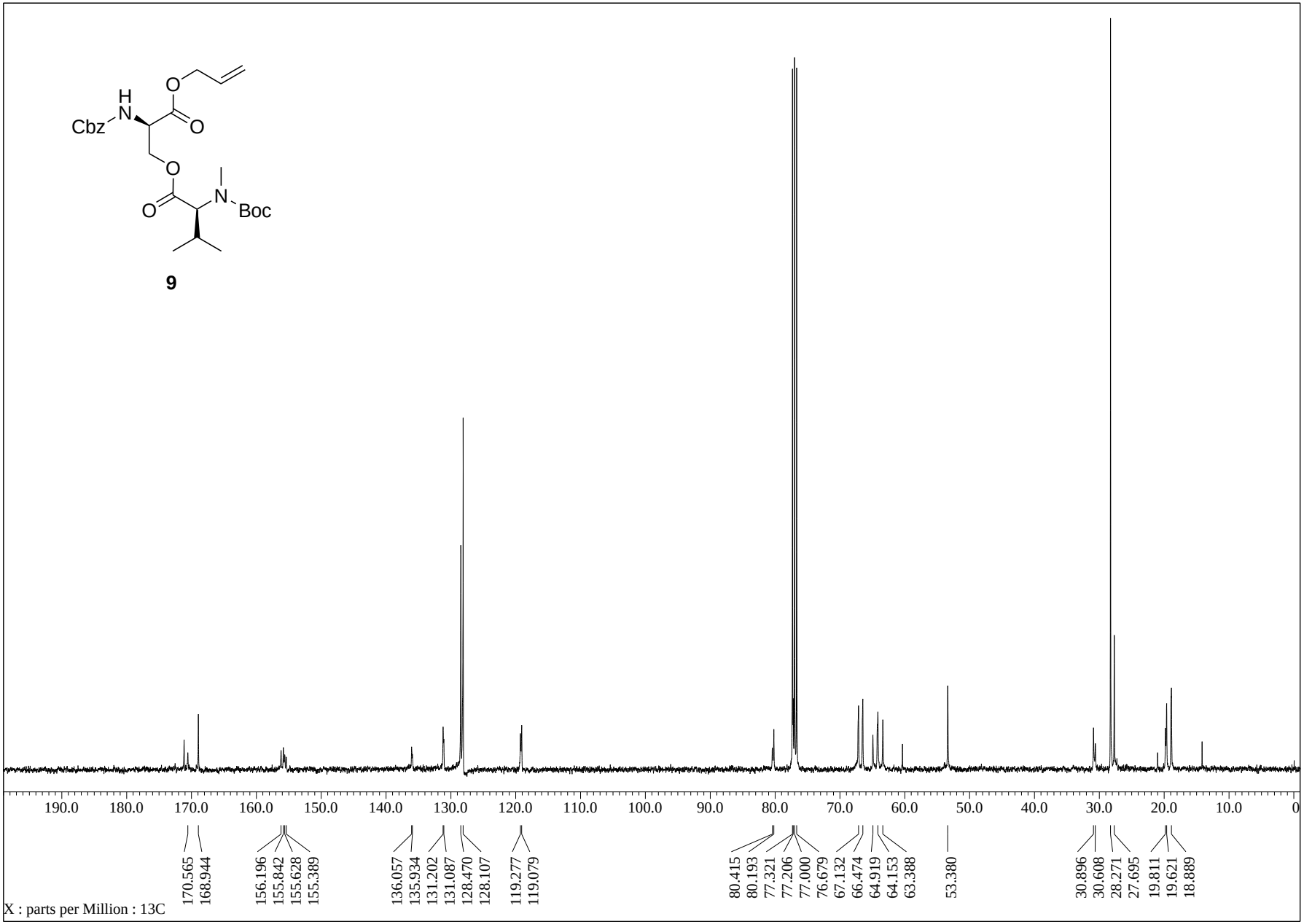
^{13}C NMR (125 MHz, $CDCl_3$)



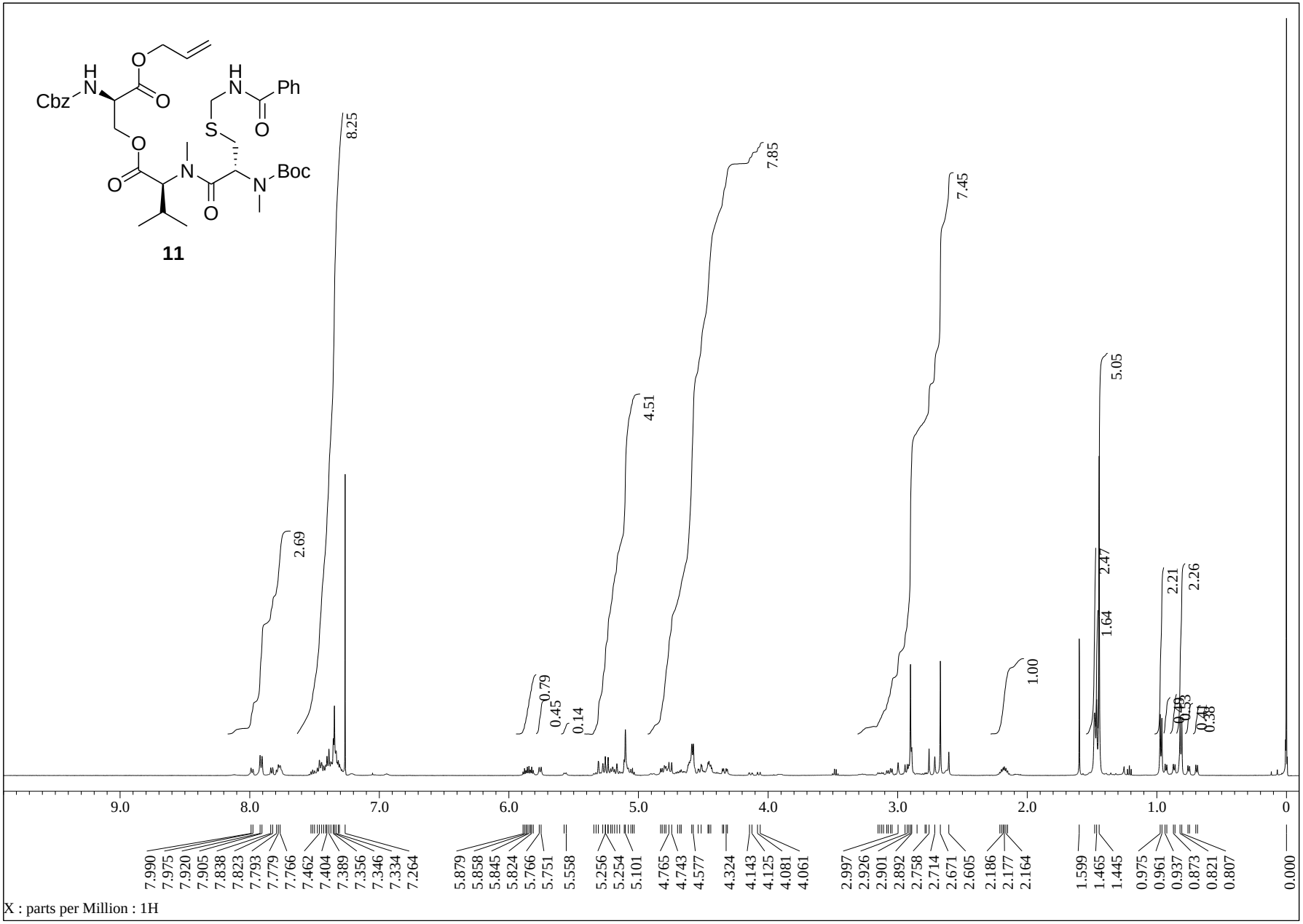
^{1}H NMR (400 MHz, $CDCl_3$)



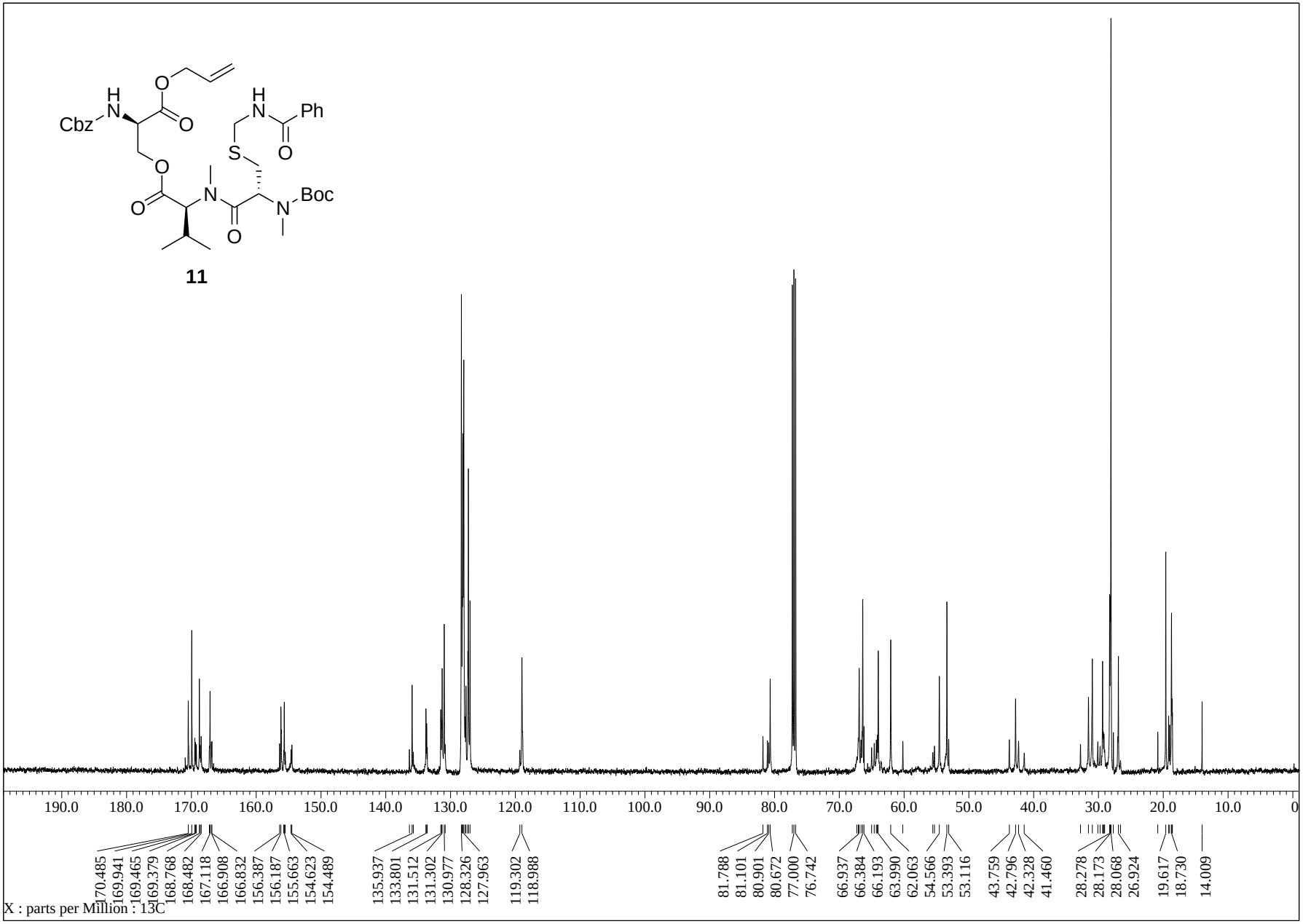
^{13}C NMR (100 MHz, $CDCl_3$)



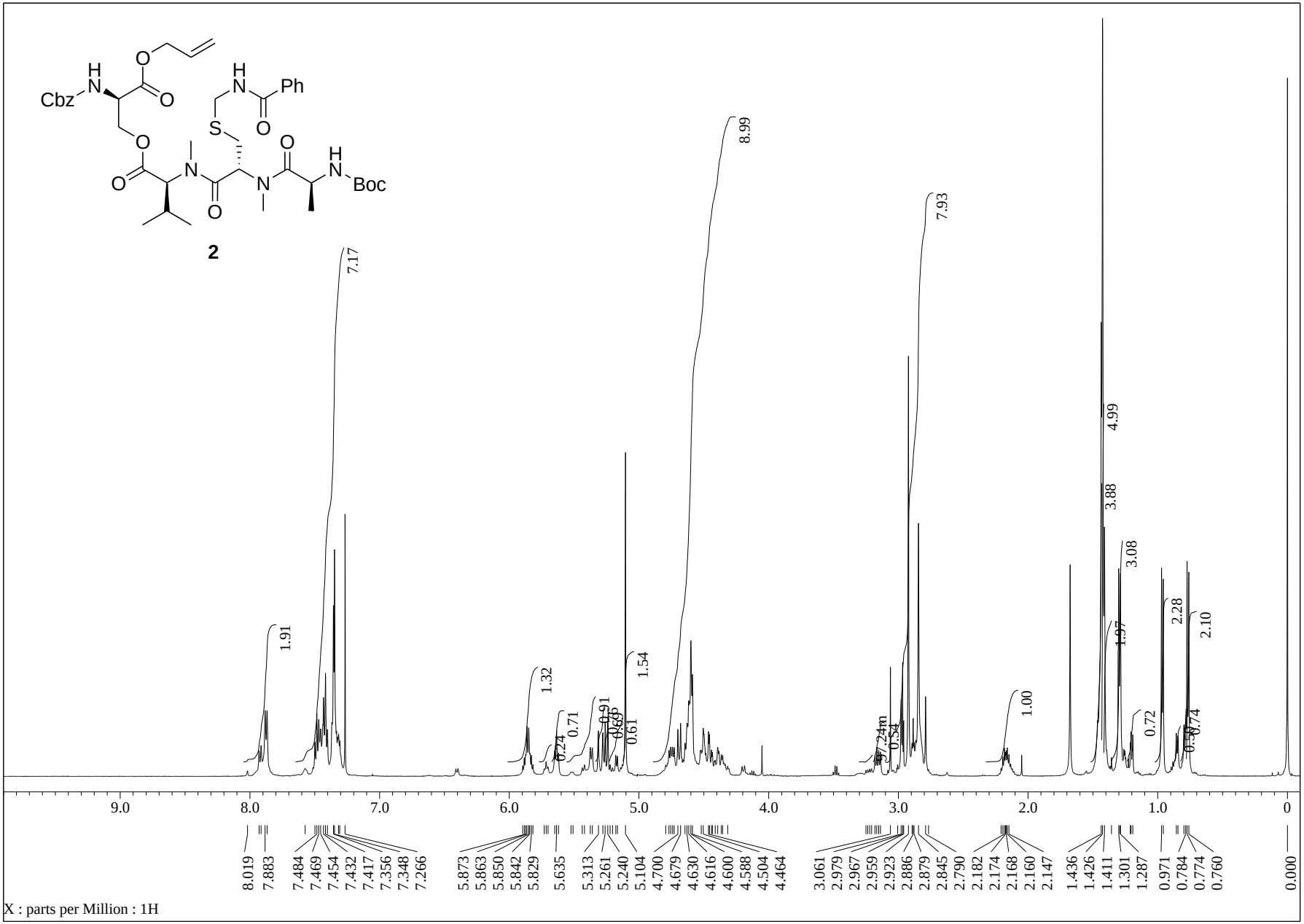
^{1}H NMR (500 MHz, $CDCl_3$)



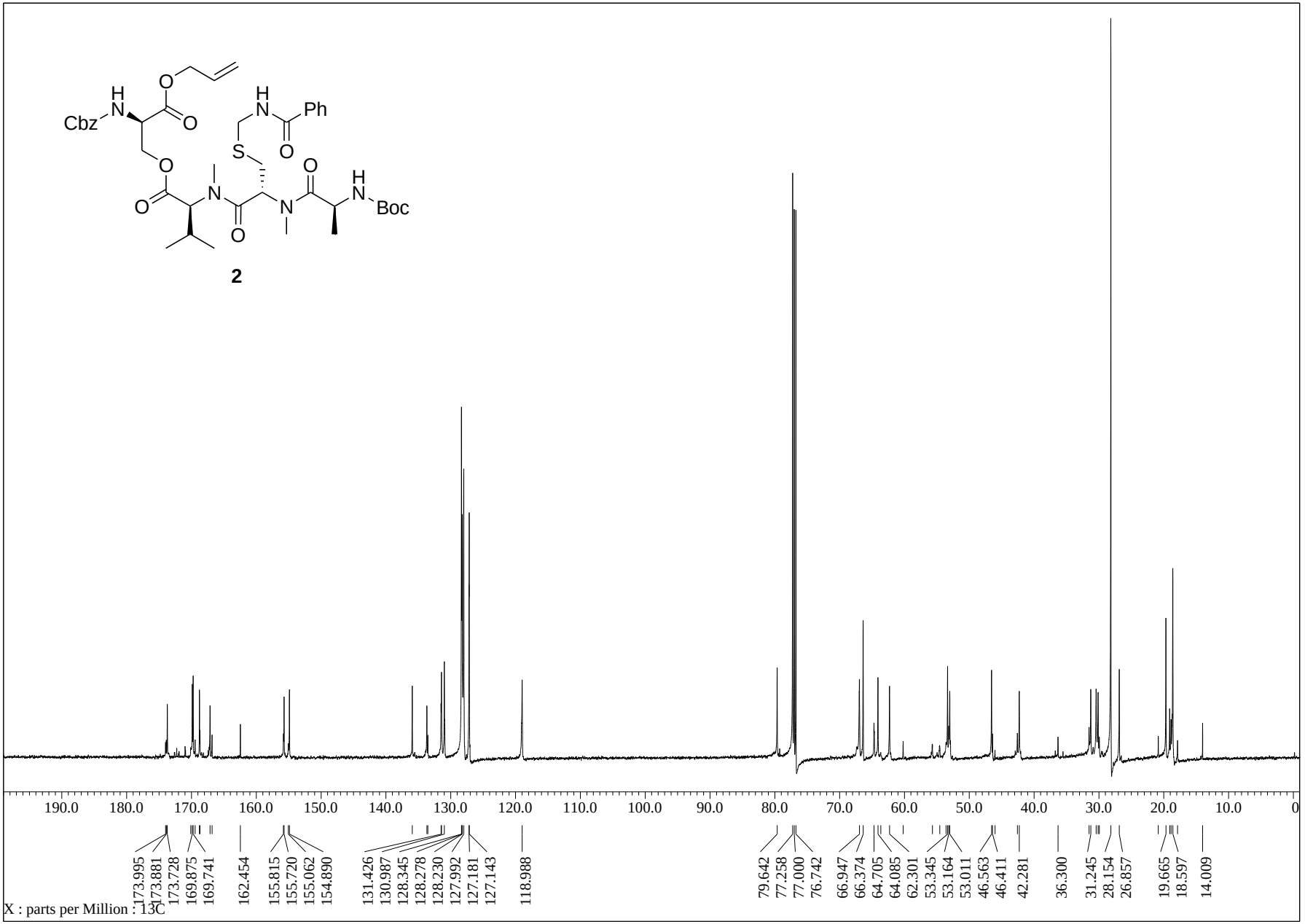
^{13}C NMR (125 MHz, $CDCl_3$)



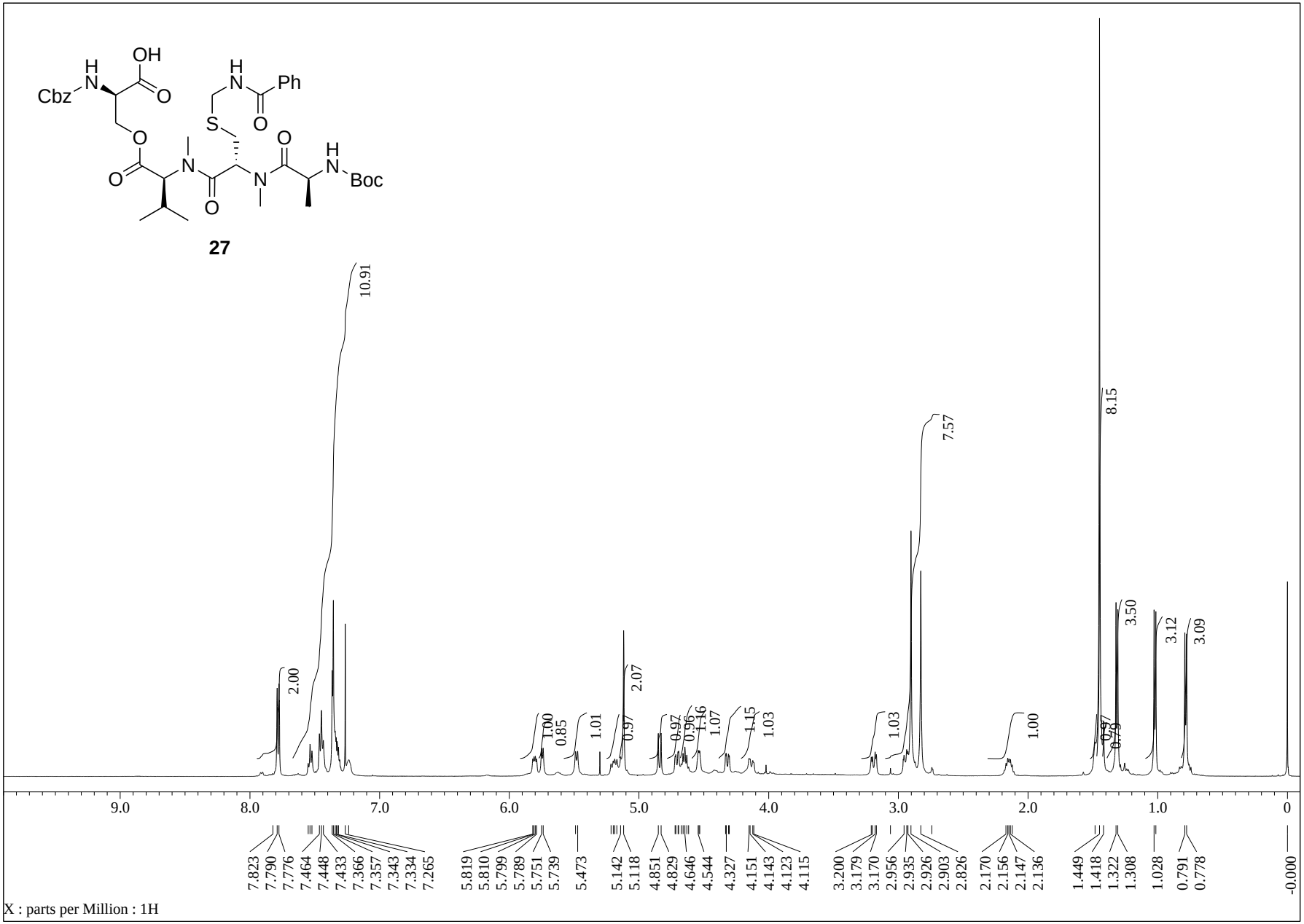
^{1}H NMR (500 MHz, $CDCl_3$)



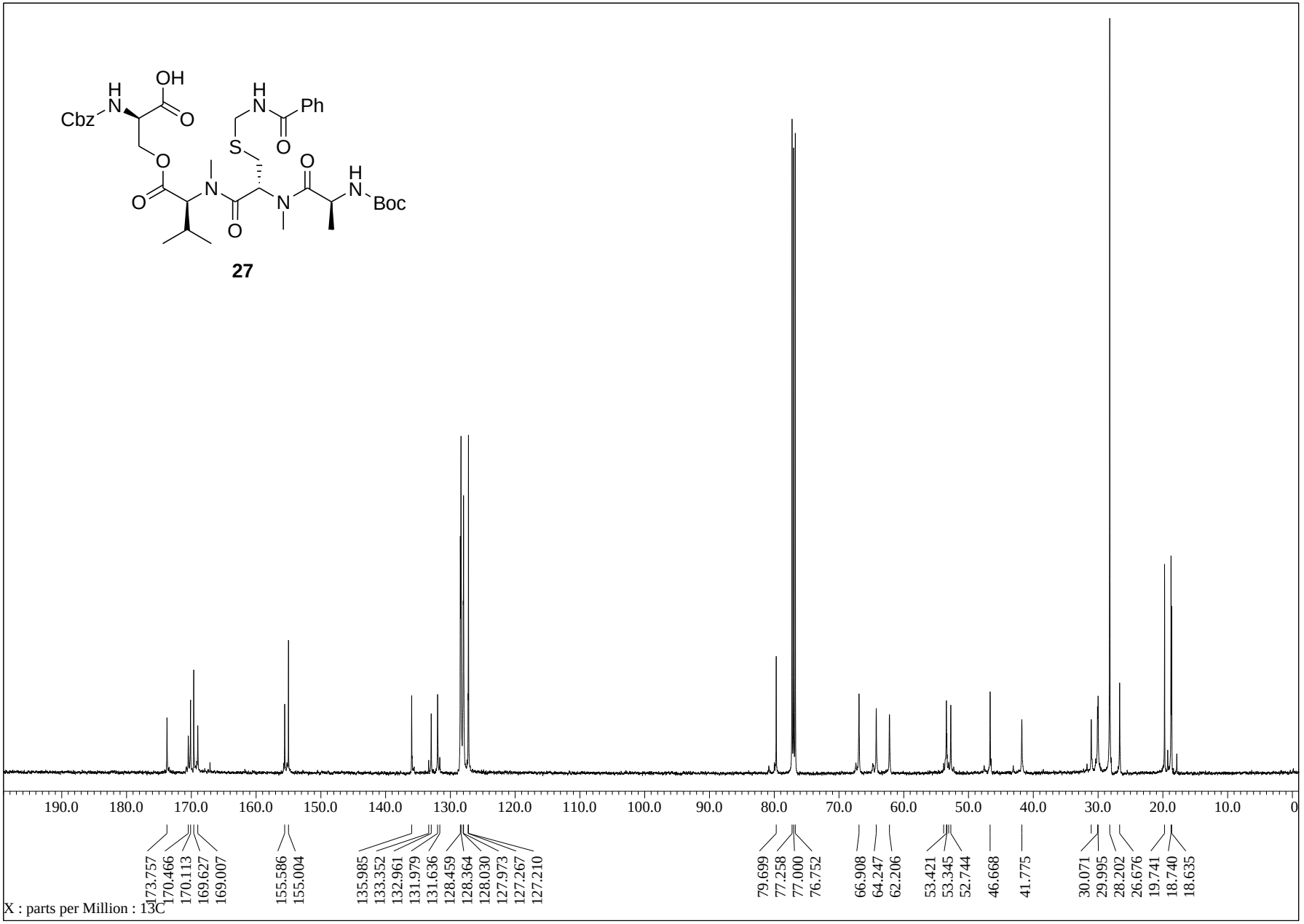
^{13}C NMR (125 MHz, $CDCl_3$)



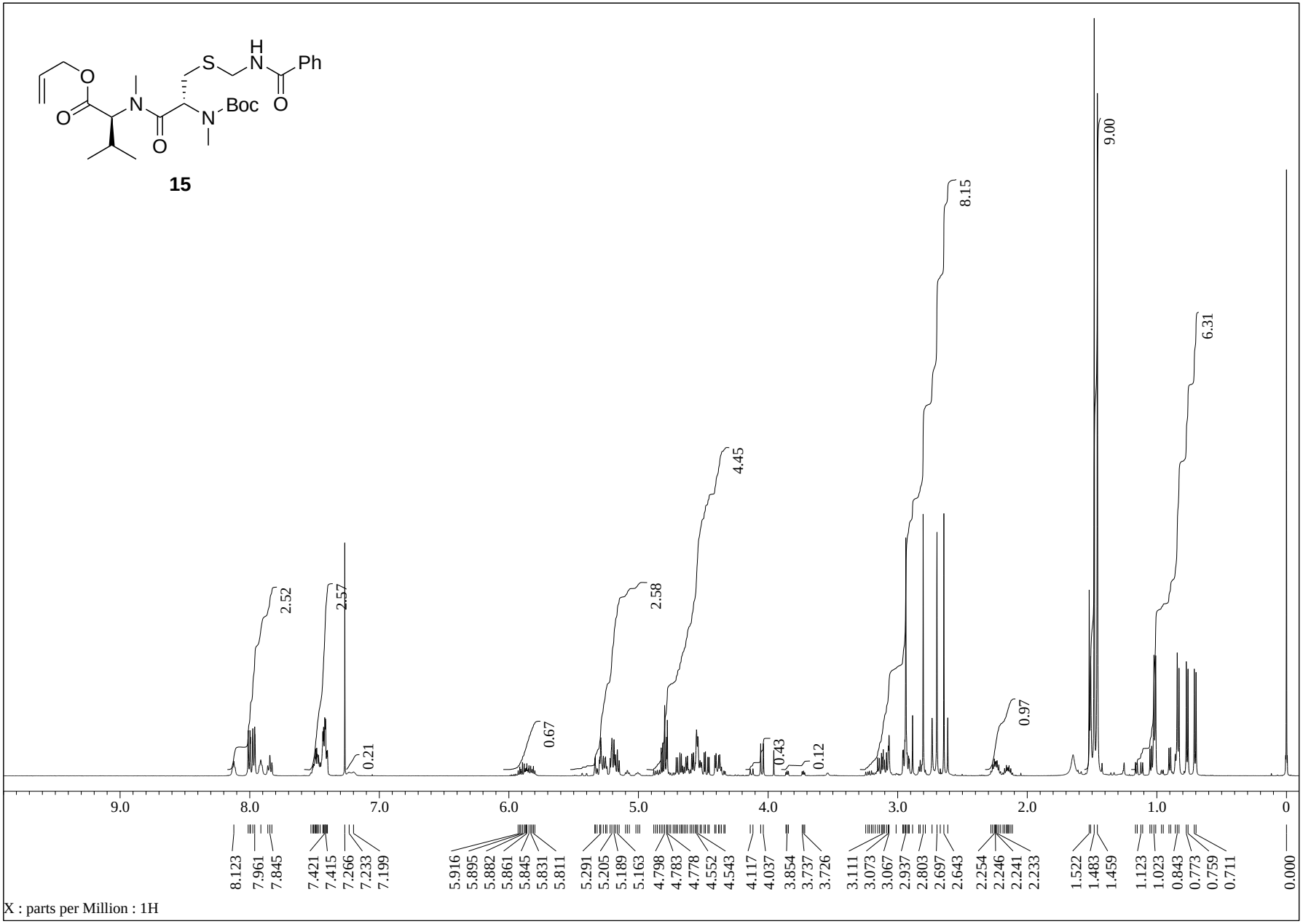
^{1}H NMR (500 MHz, $CDCl_3$)



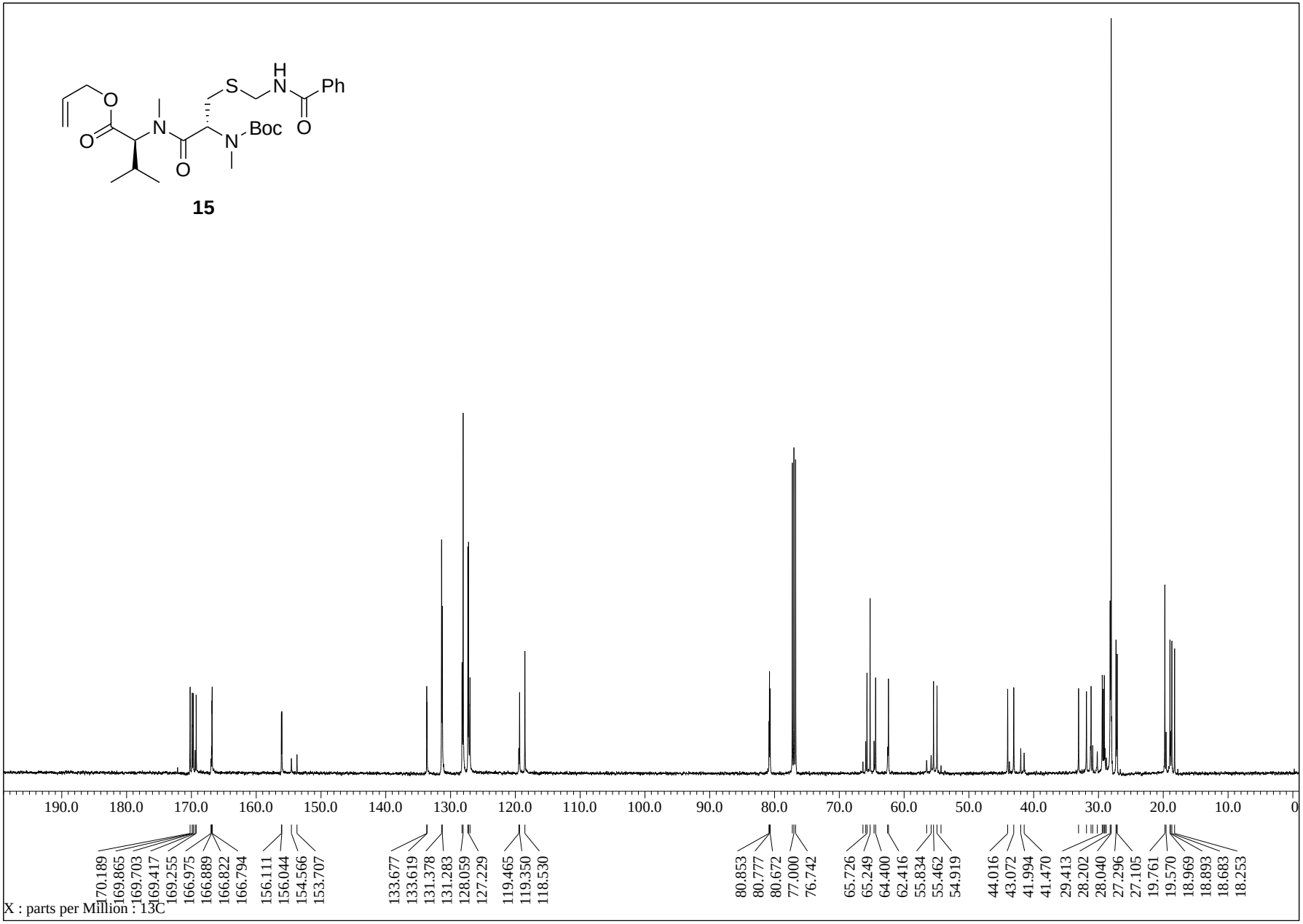
^{13}C NMR (125 MHz, $CDCl_3$)



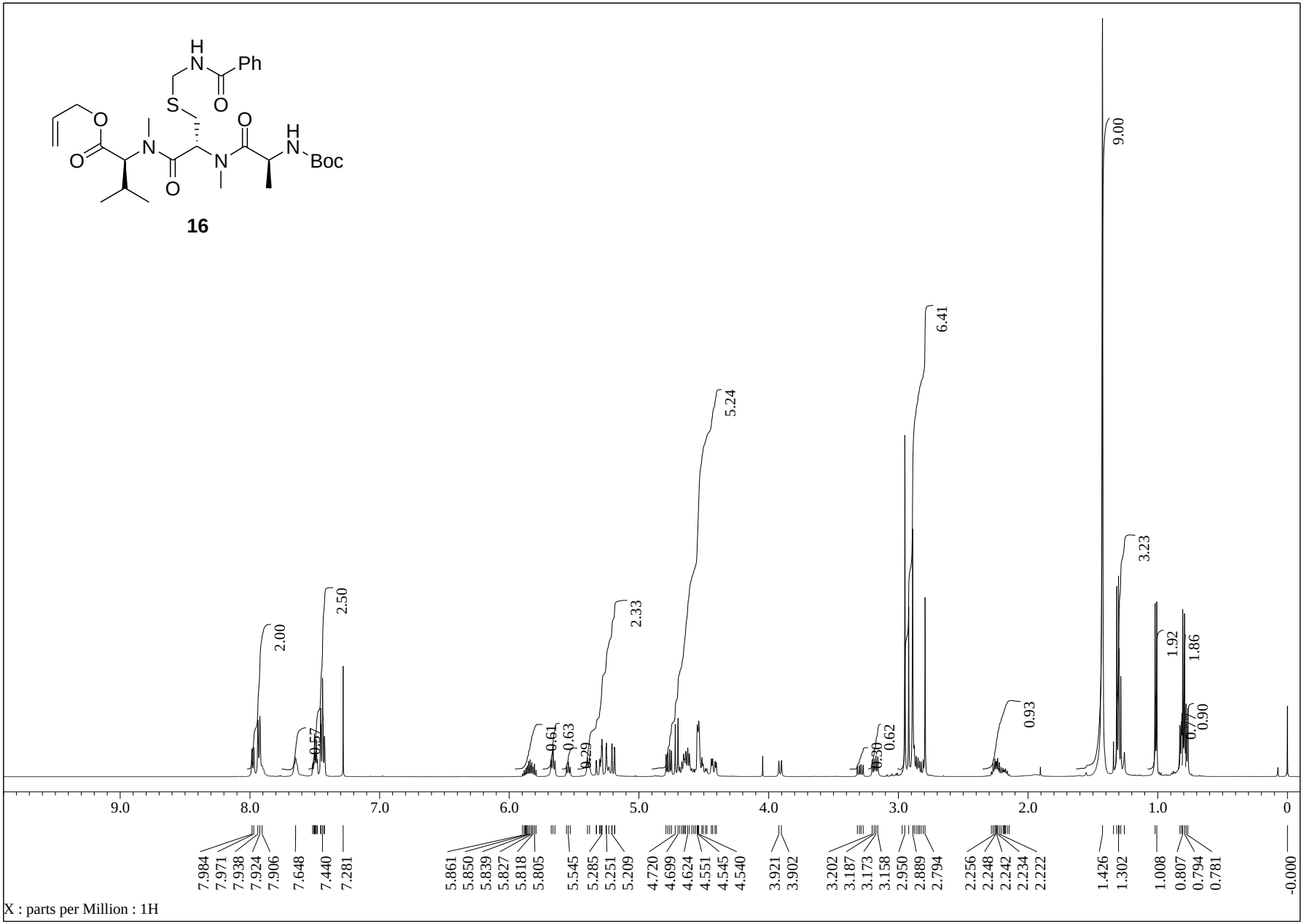
^{1}H NMR (500 MHz, $CDCl_3$)



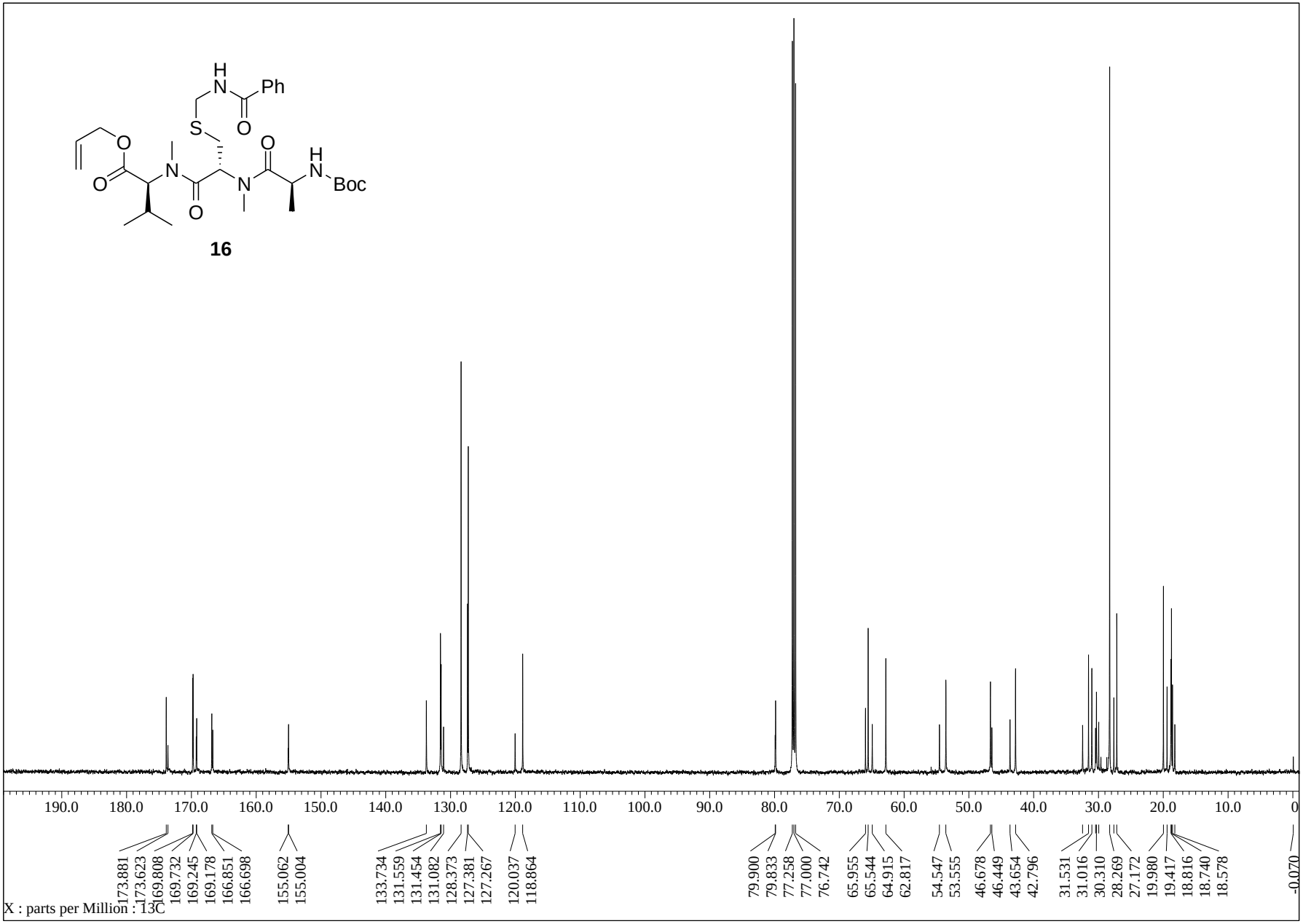
^{13}C NMR (125 MHz, $CDCl_3$)



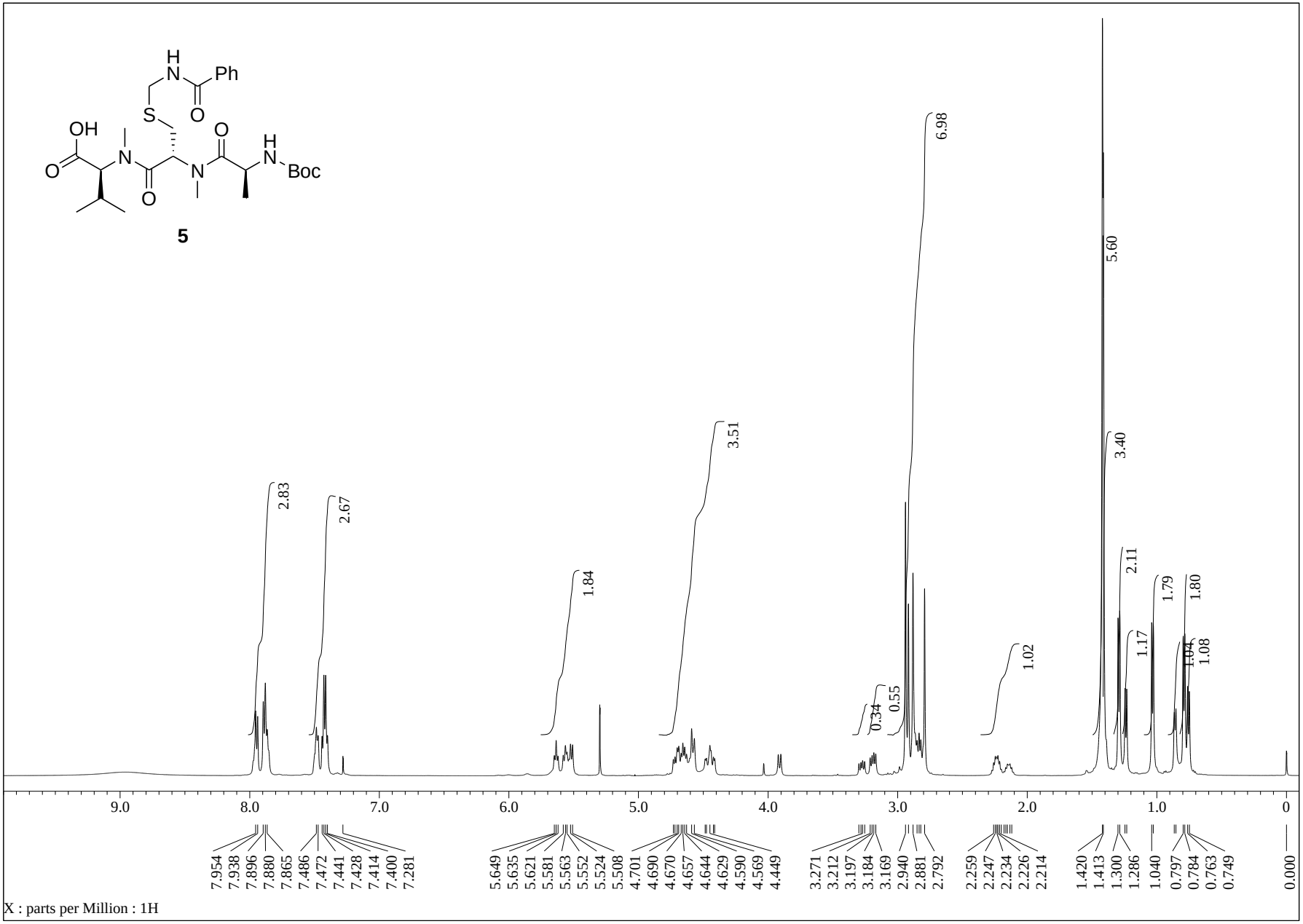
^{1}H NMR (500 MHz, $CDCl_3$)



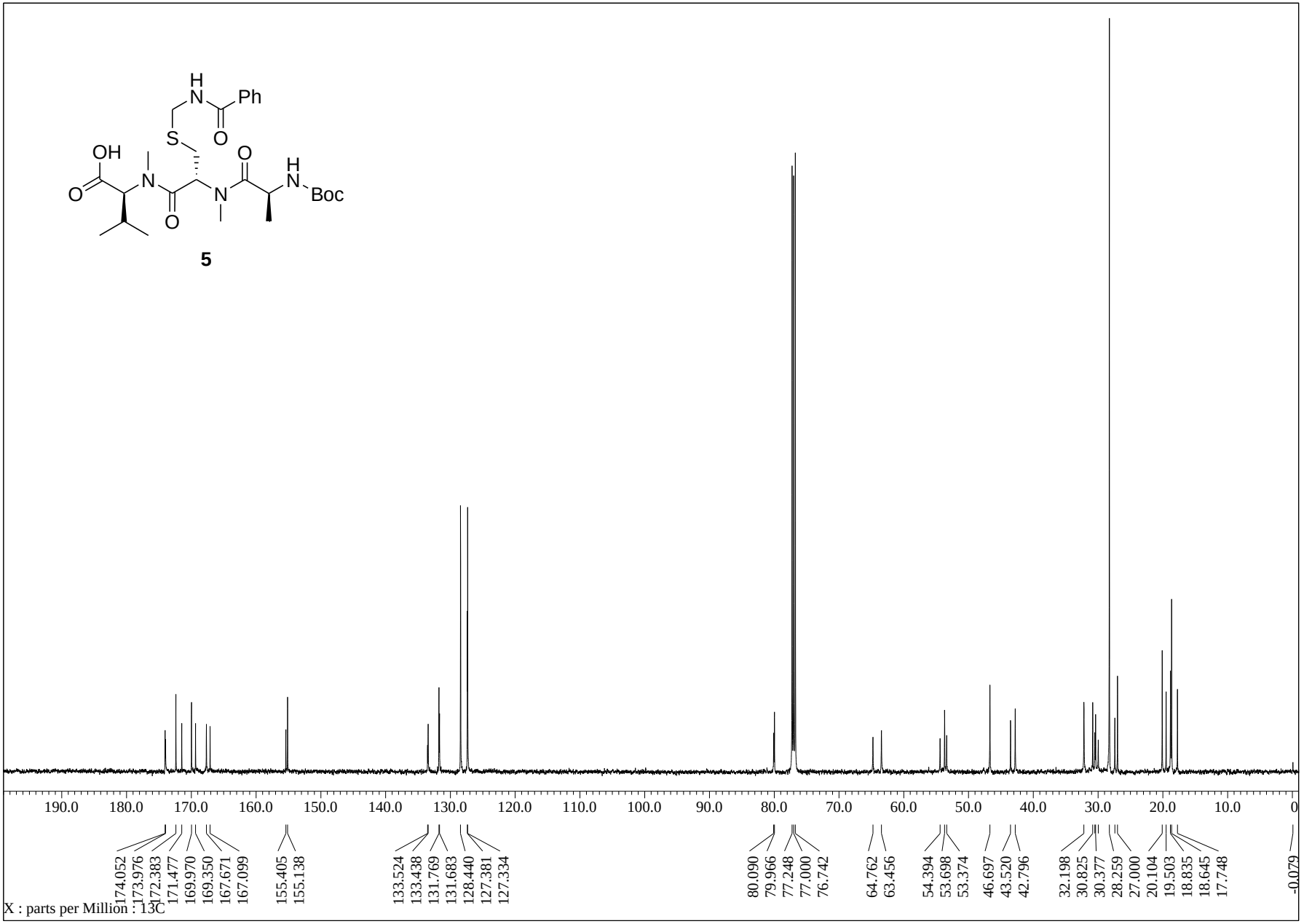
^{13}C NMR (125 MHz, $CDCl_3$)



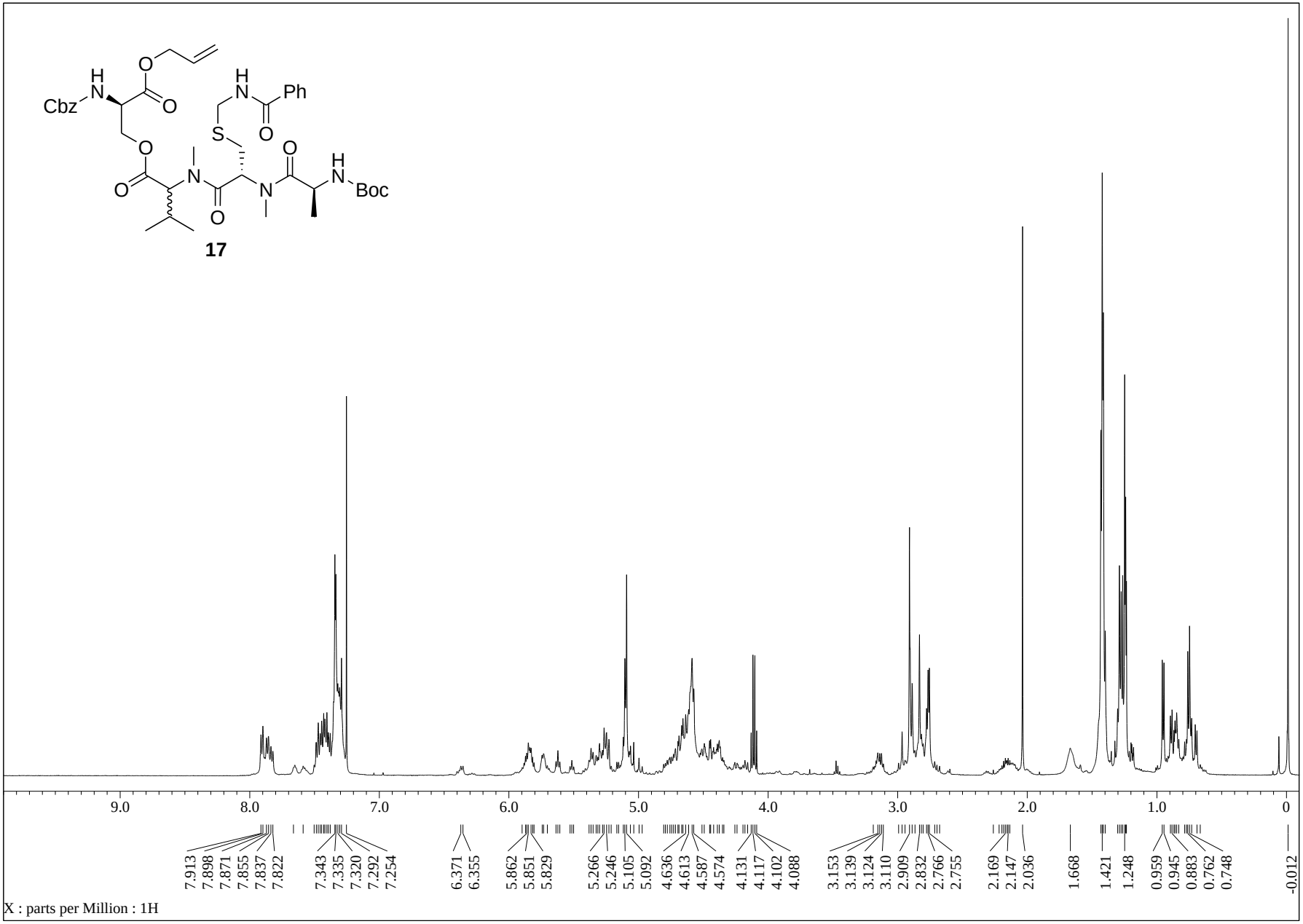
^{1}H NMR (500 MHz, $CDCl_3$)



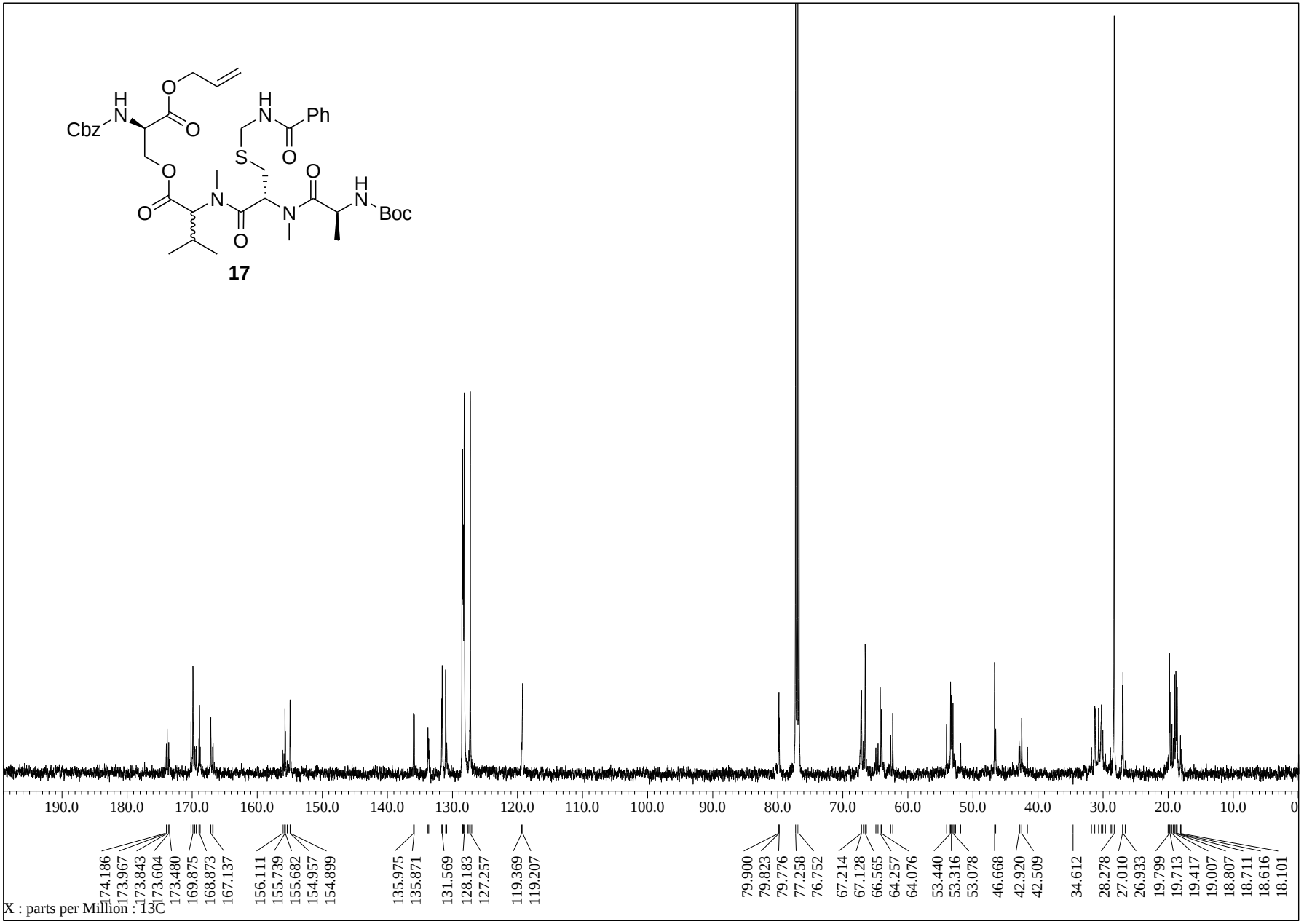
^{13}C NMR (125 MHz, $CDCl_3$)



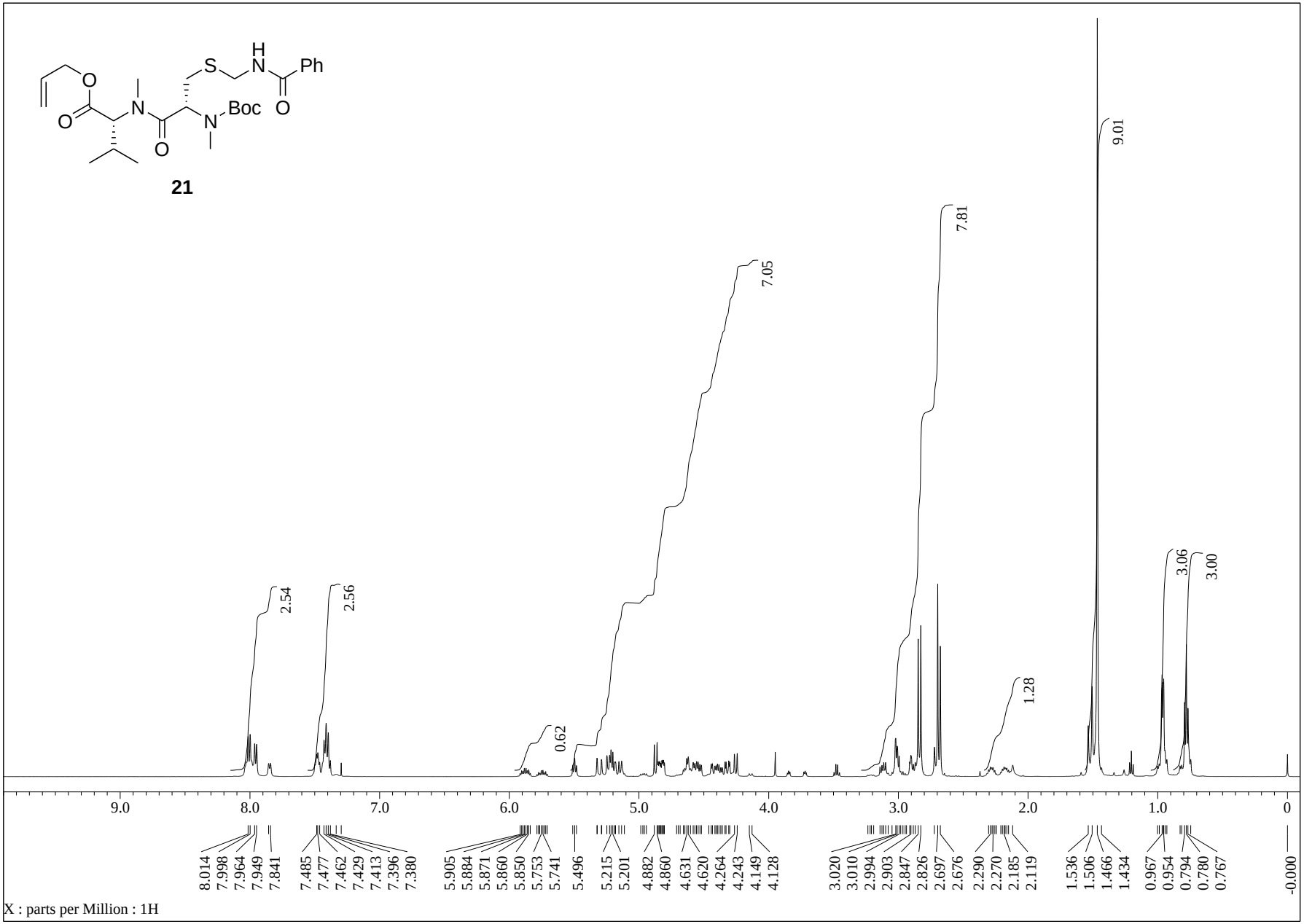
^{1}H NMR (500 MHz, $CDCl_3$)



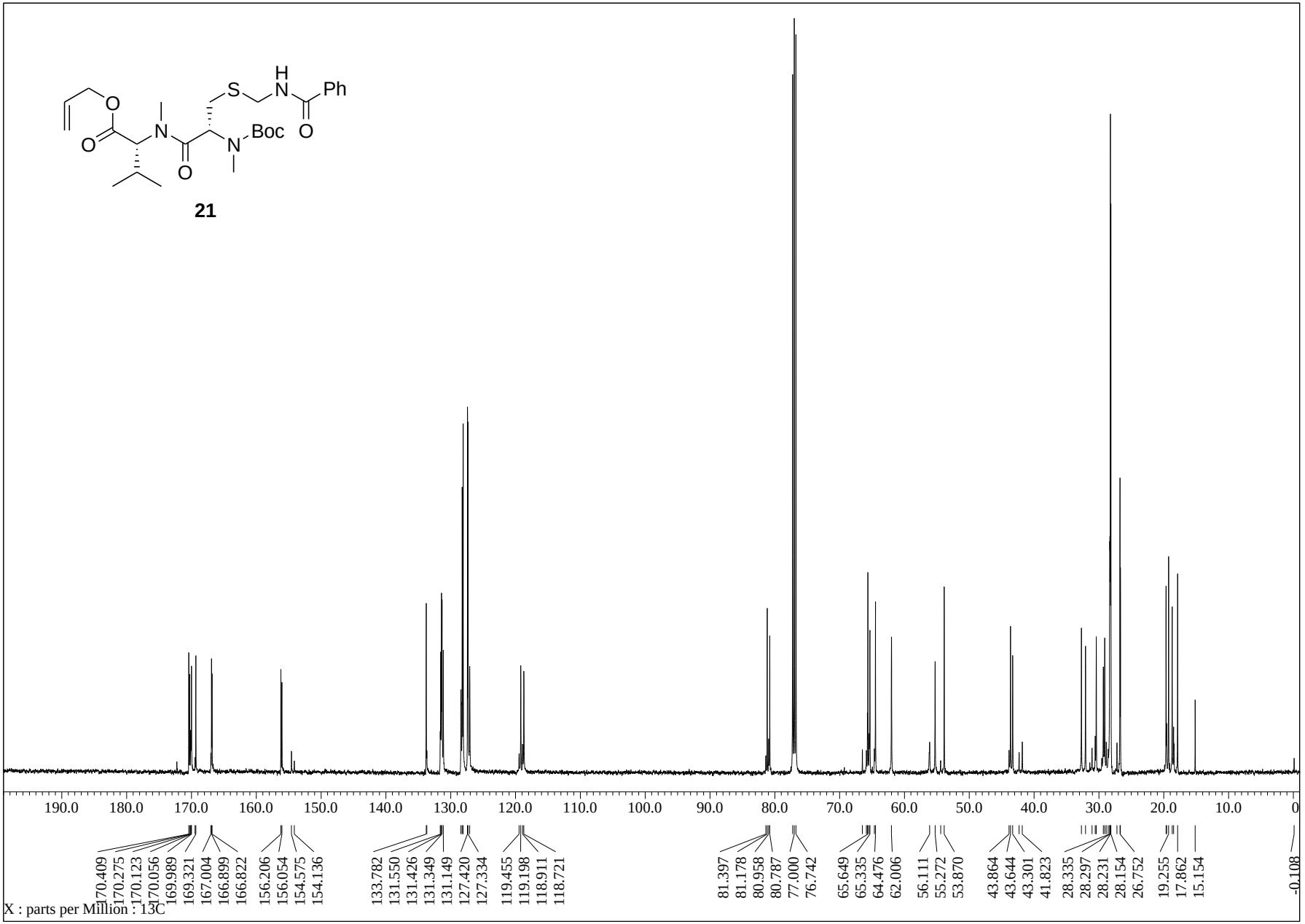
^{13}C NMR (125 MHz, $CDCl_3$)



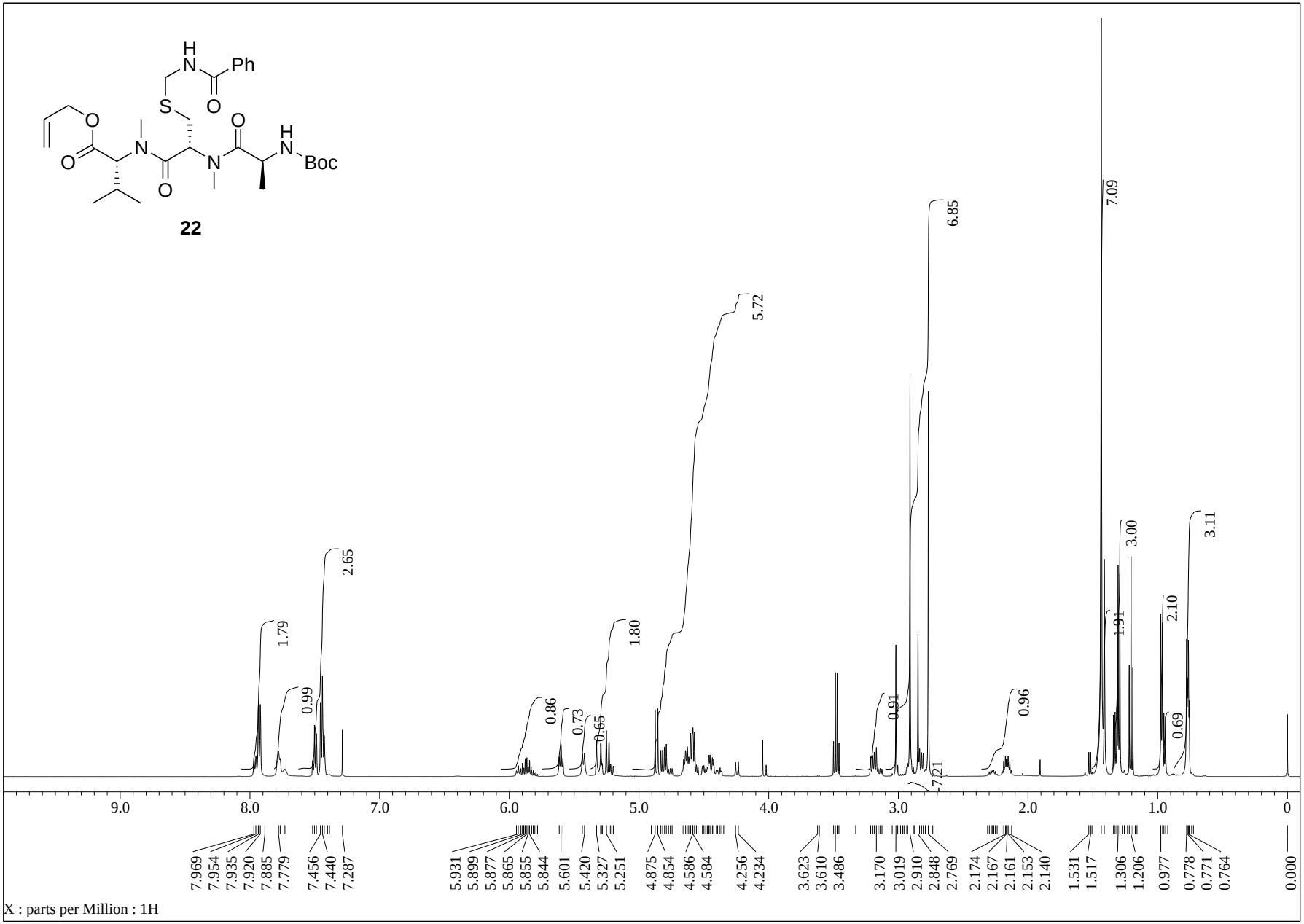
^{1}H NMR (500 MHz, $CDCl_3$)



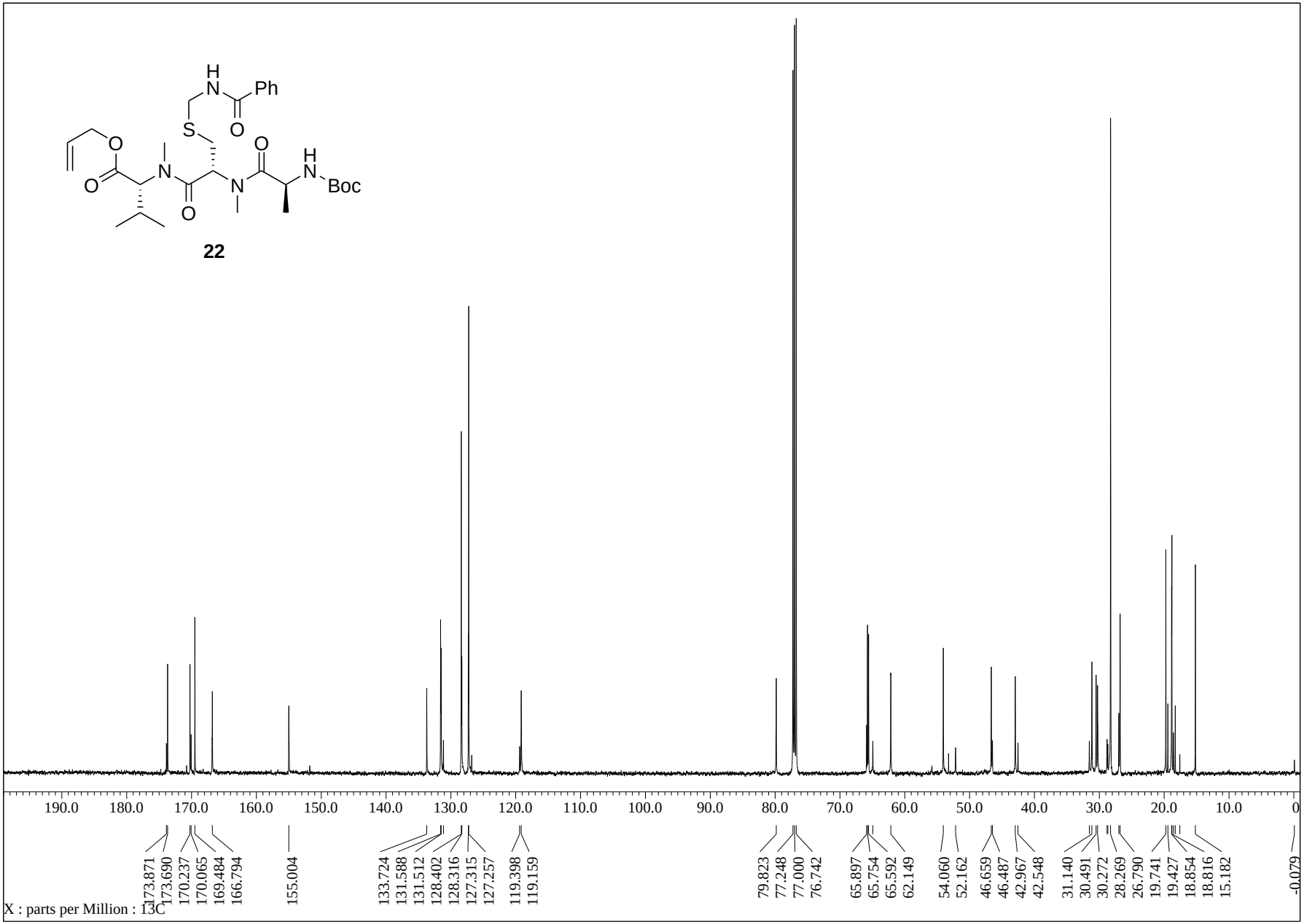
^{13}C NMR (125 MHz, $CDCl_3$)



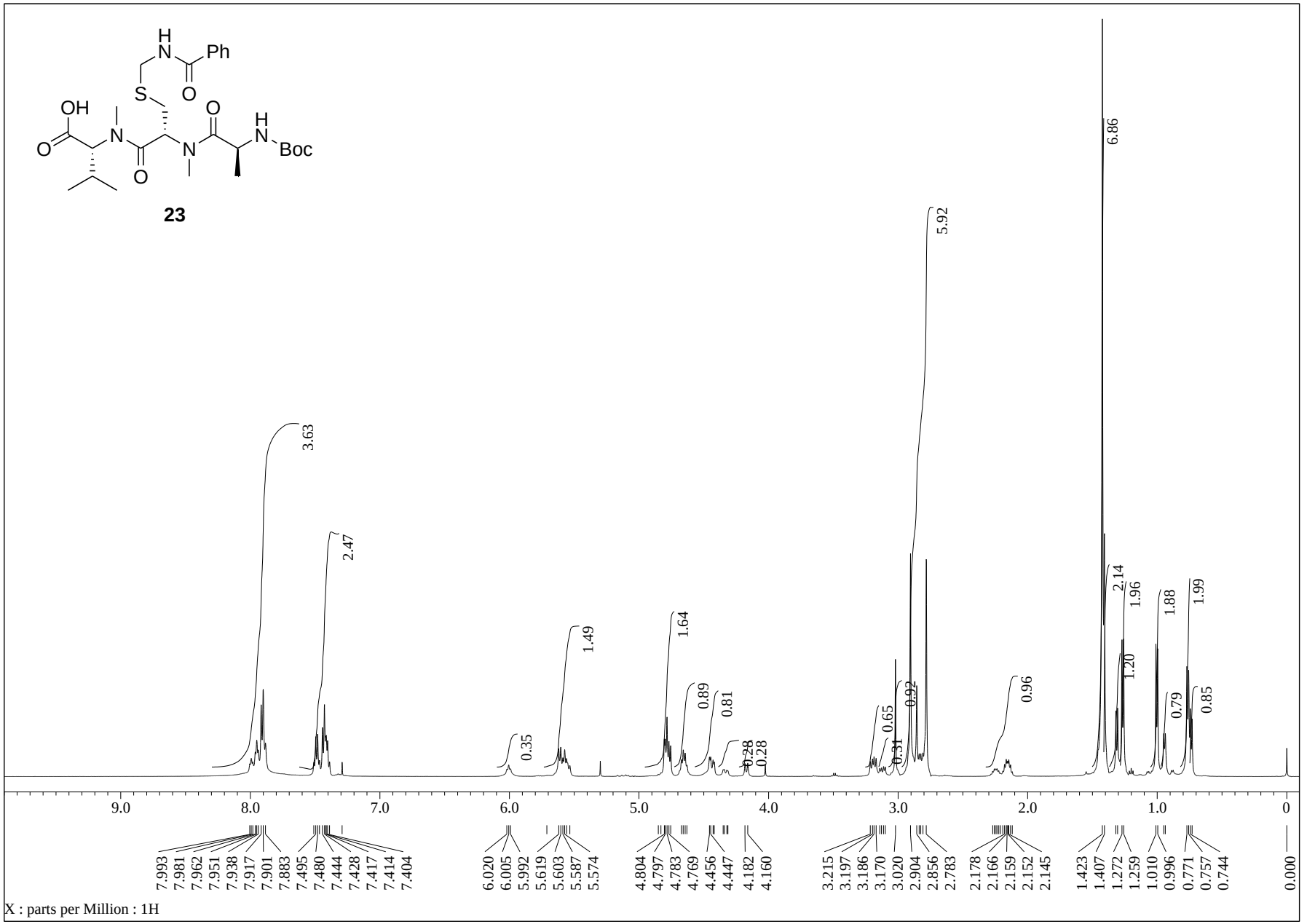
^{1}H NMR (500 MHz, $CDCl_3$)



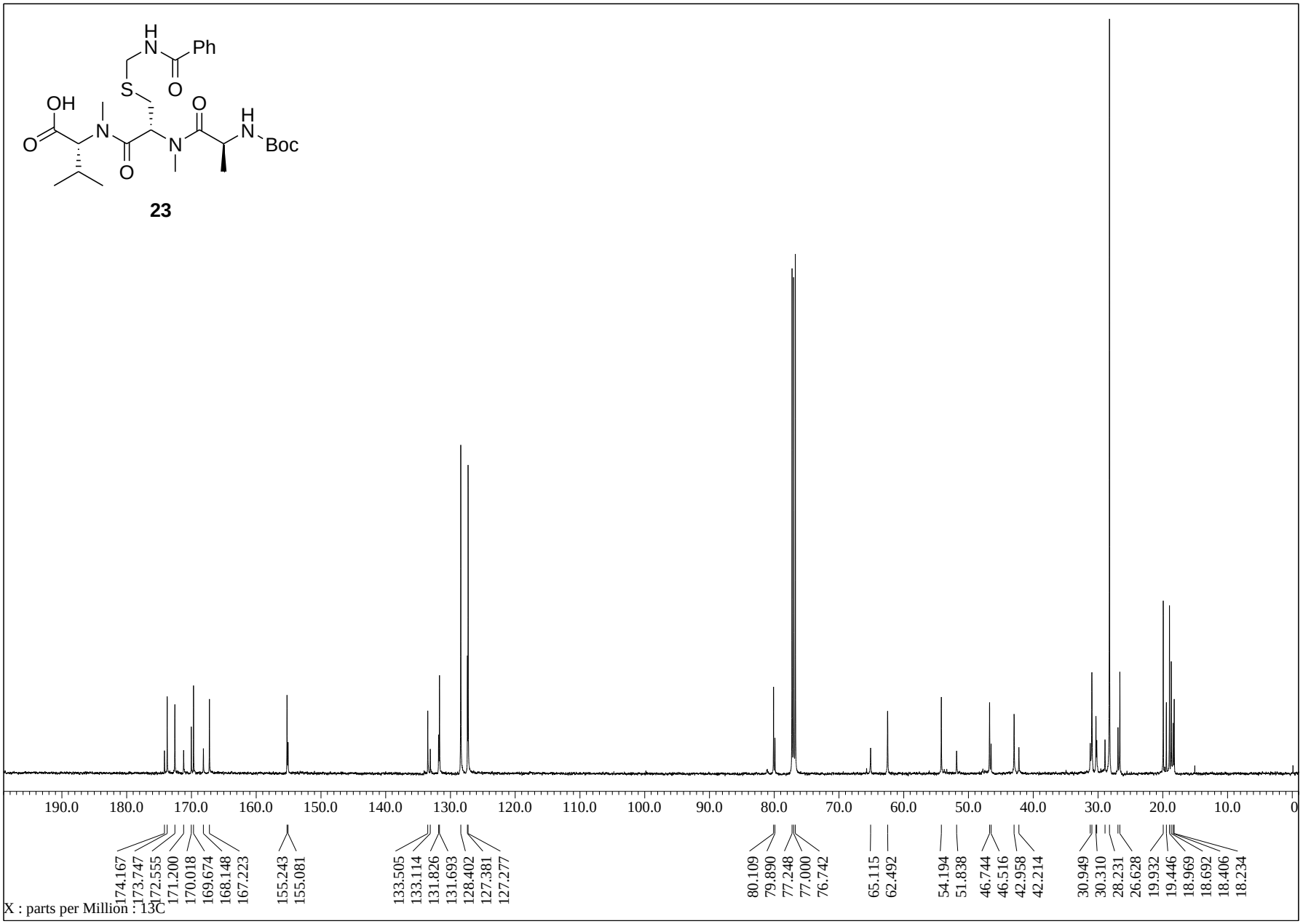
^{13}C NMR (125 MHz, $CDCl_3$)



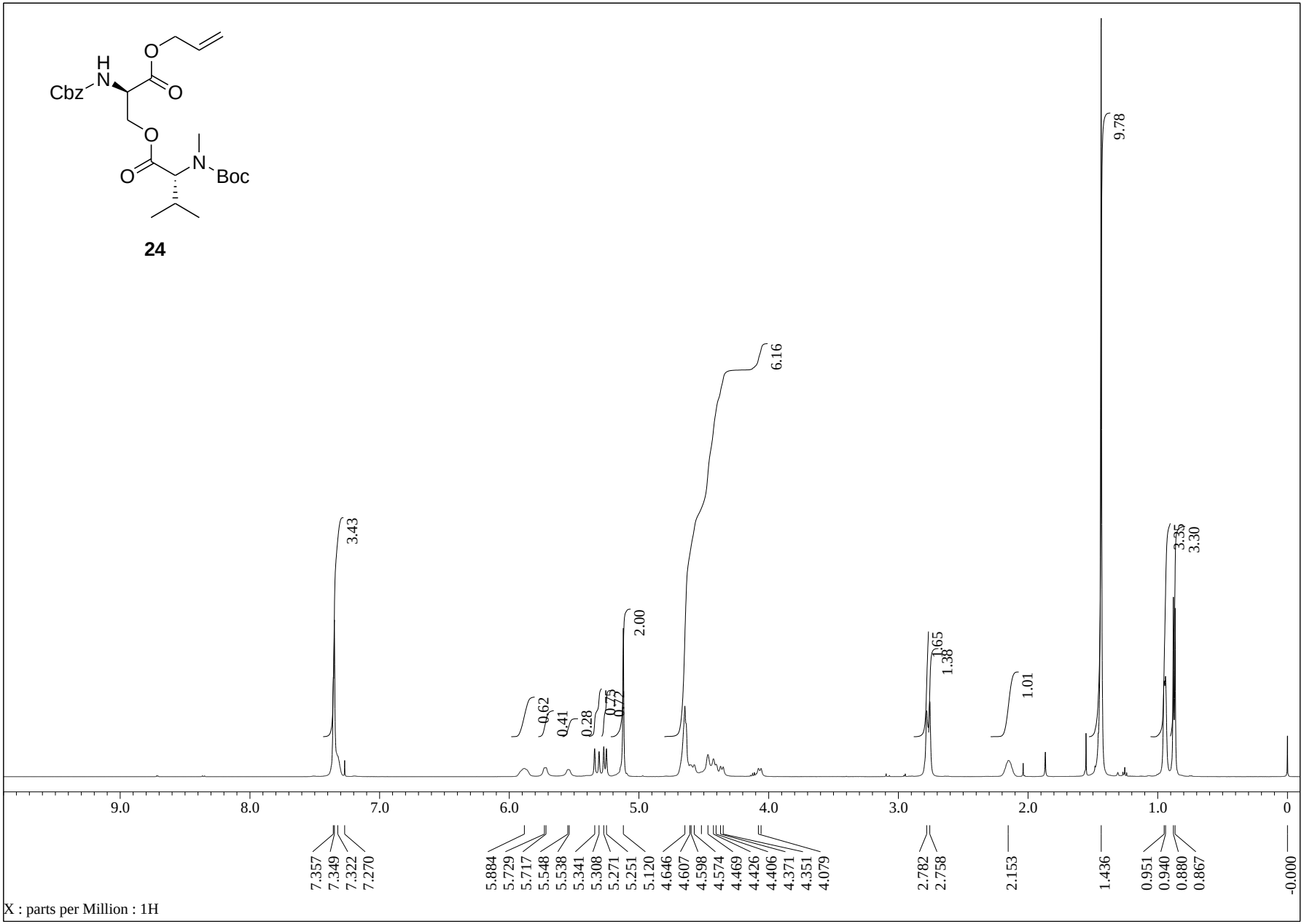
^{1}H NMR (500 MHz, $CDCl_3$)



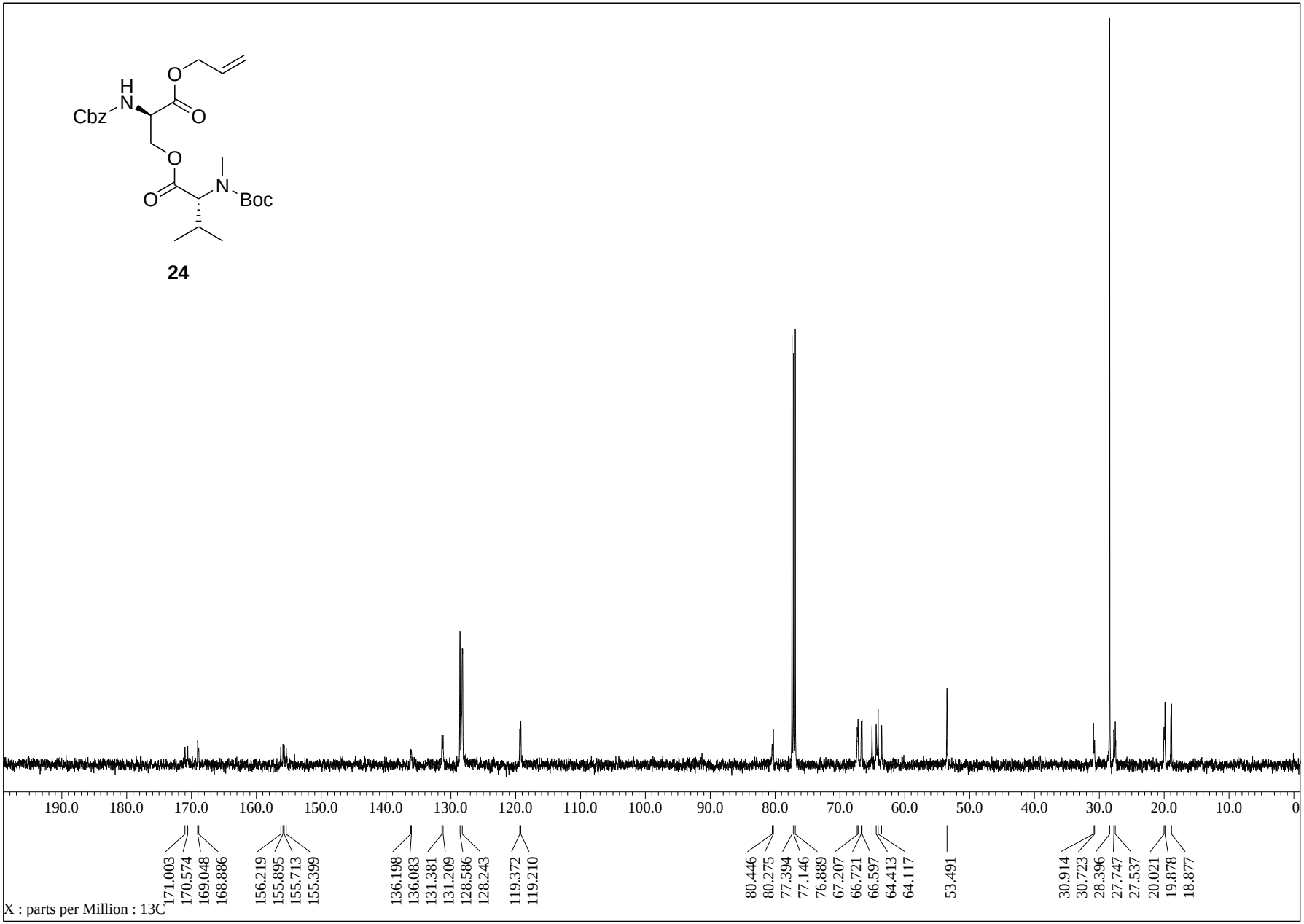
^{13}C NMR (125 MHz, $CDCl_3$)



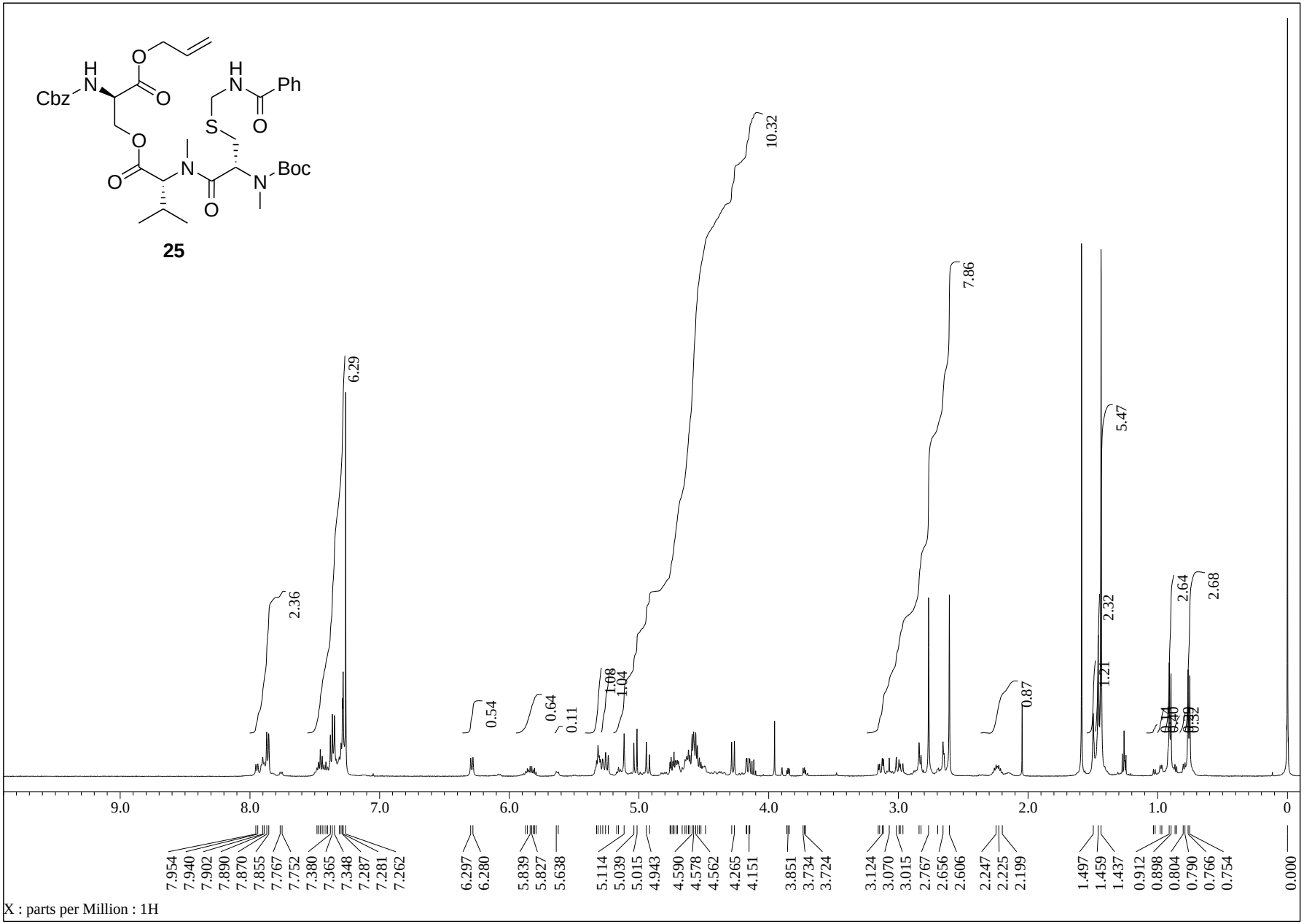
^{1}H NMR (500 MHz, $CDCl_3$)



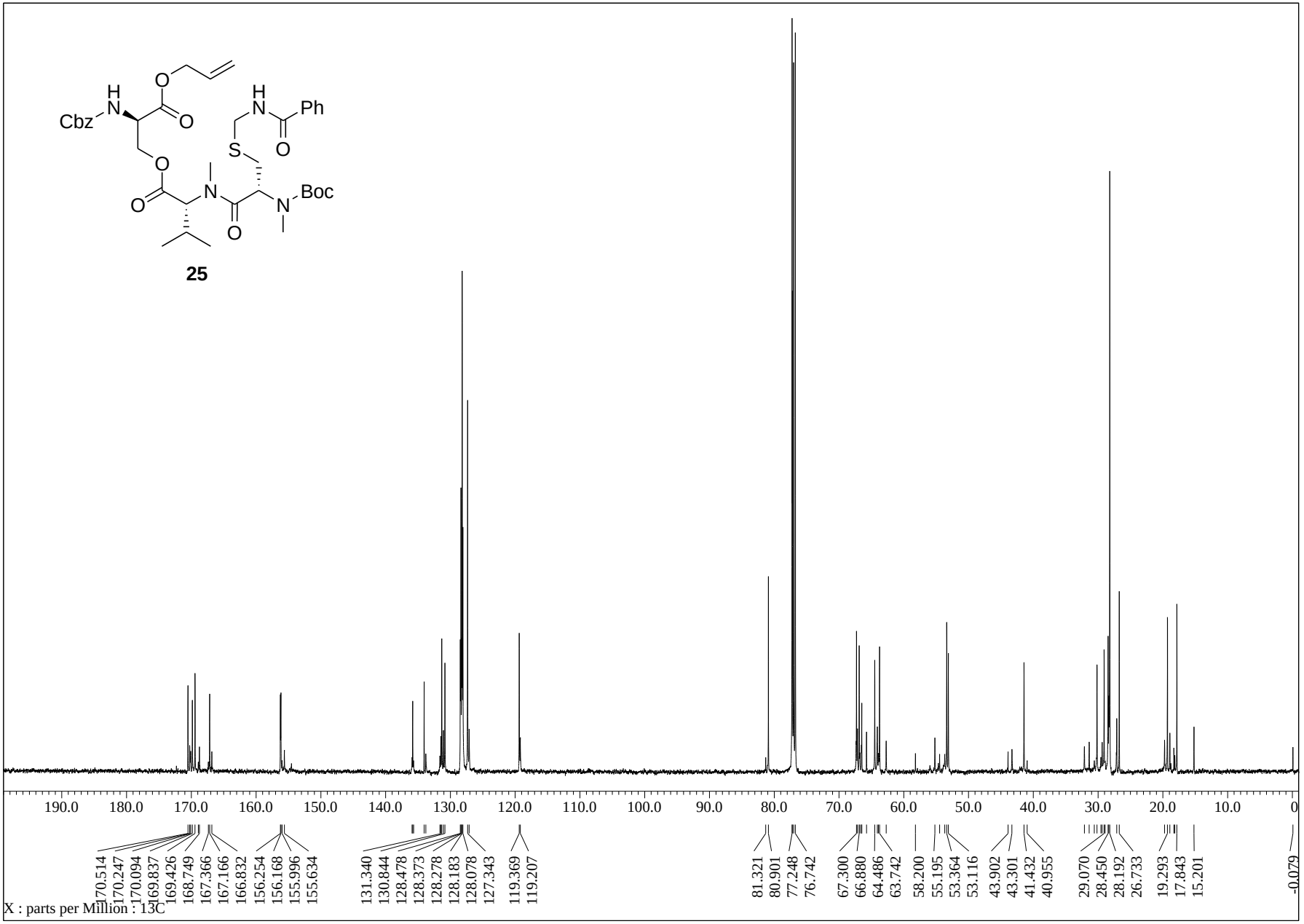
^{13}C NMR (125 MHz, $CDCl_3$)



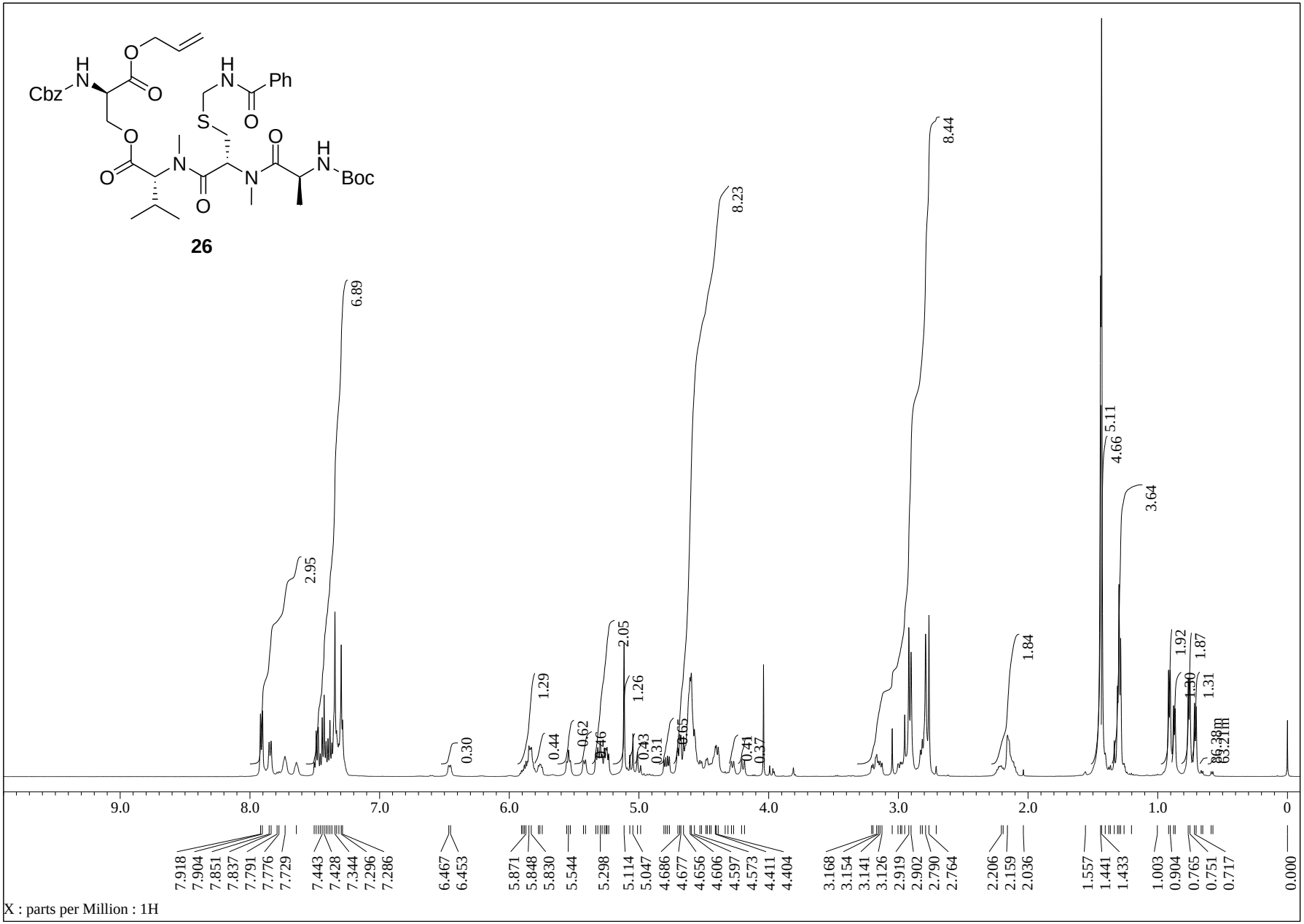
^{1}H NMR (500 MHz, $CDCl_3$)



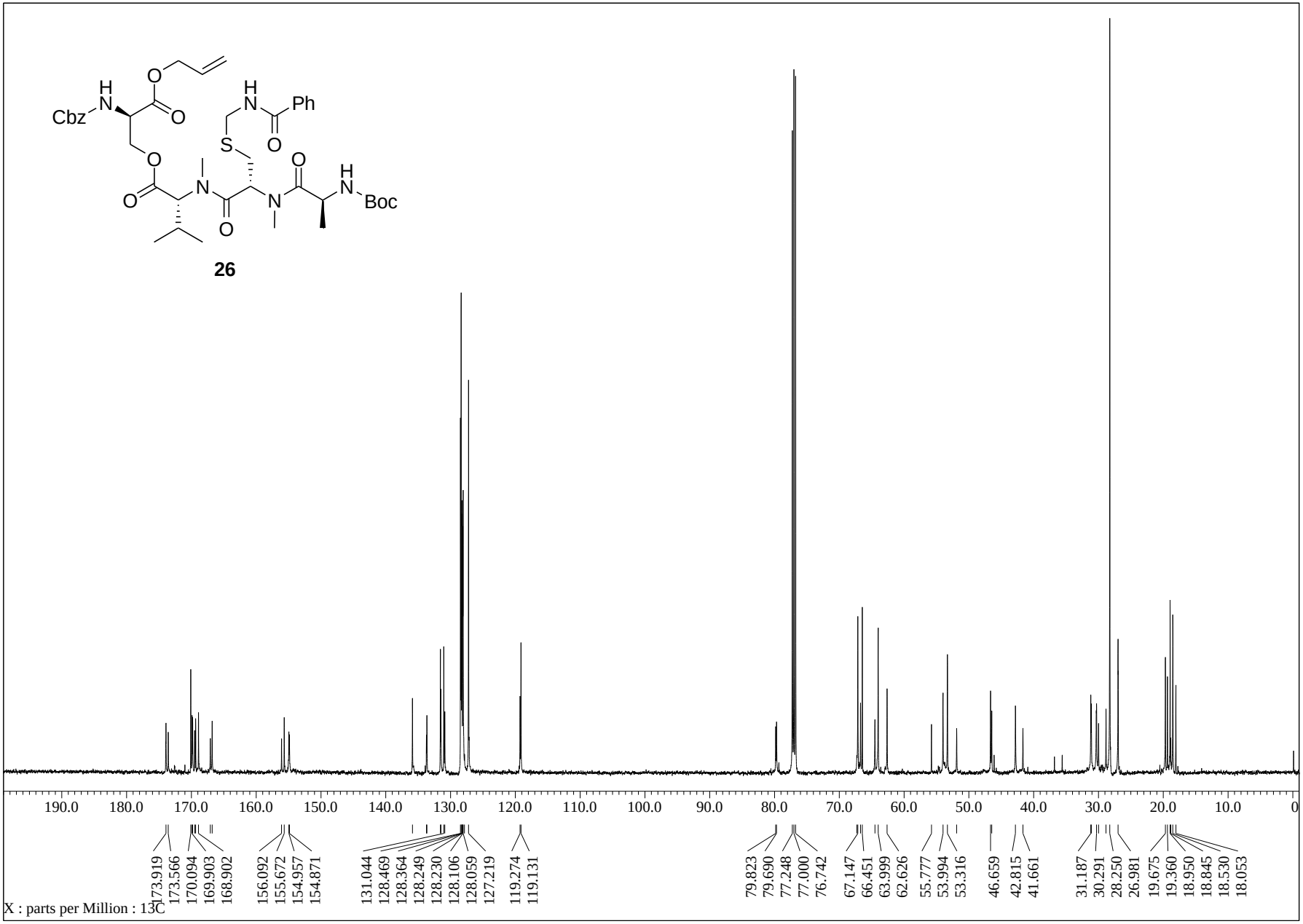
^{13}C NMR (125 MHz, $CDCl_3$)



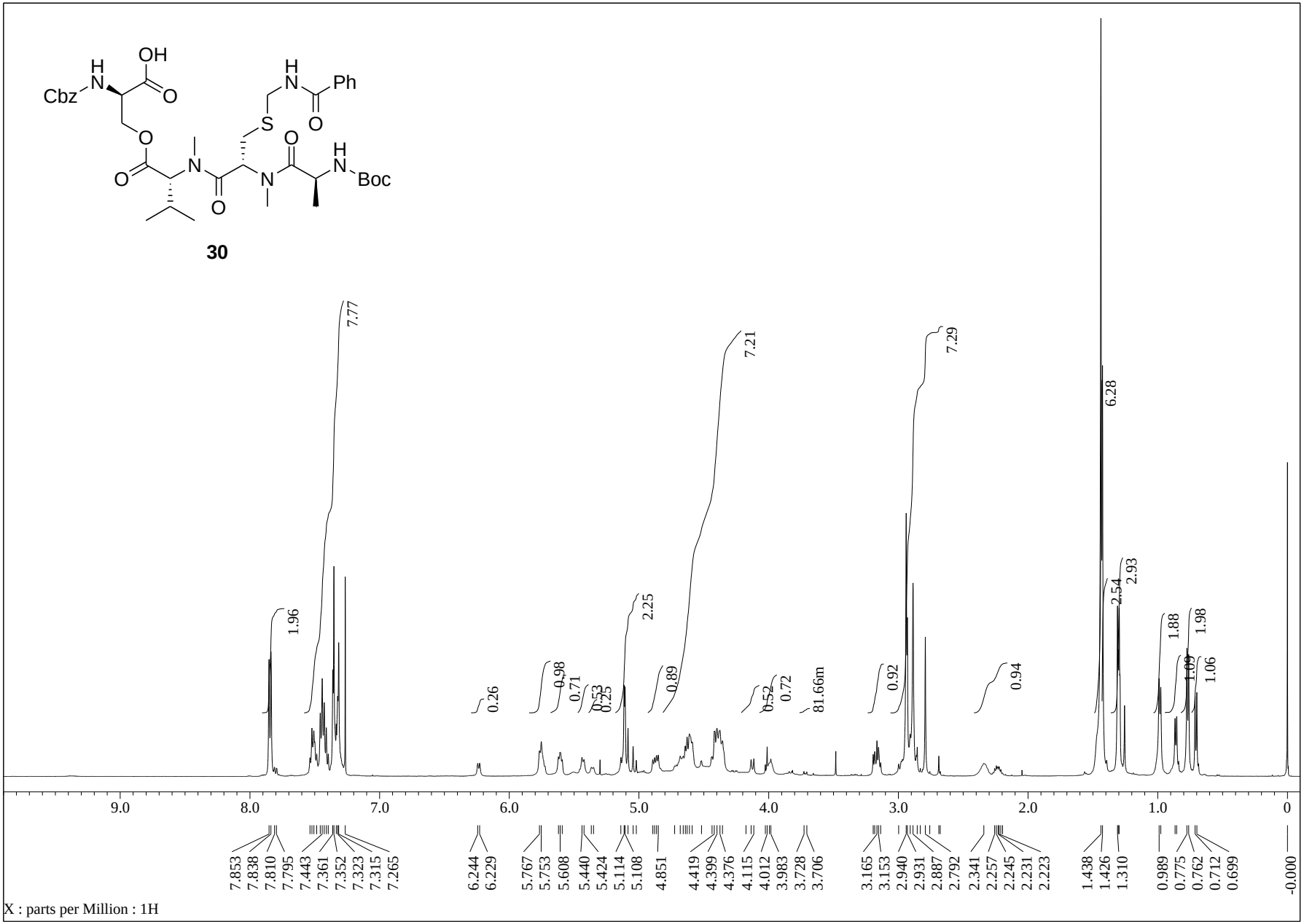
^{1}H NMR (500 MHz, $CDCl_3$)



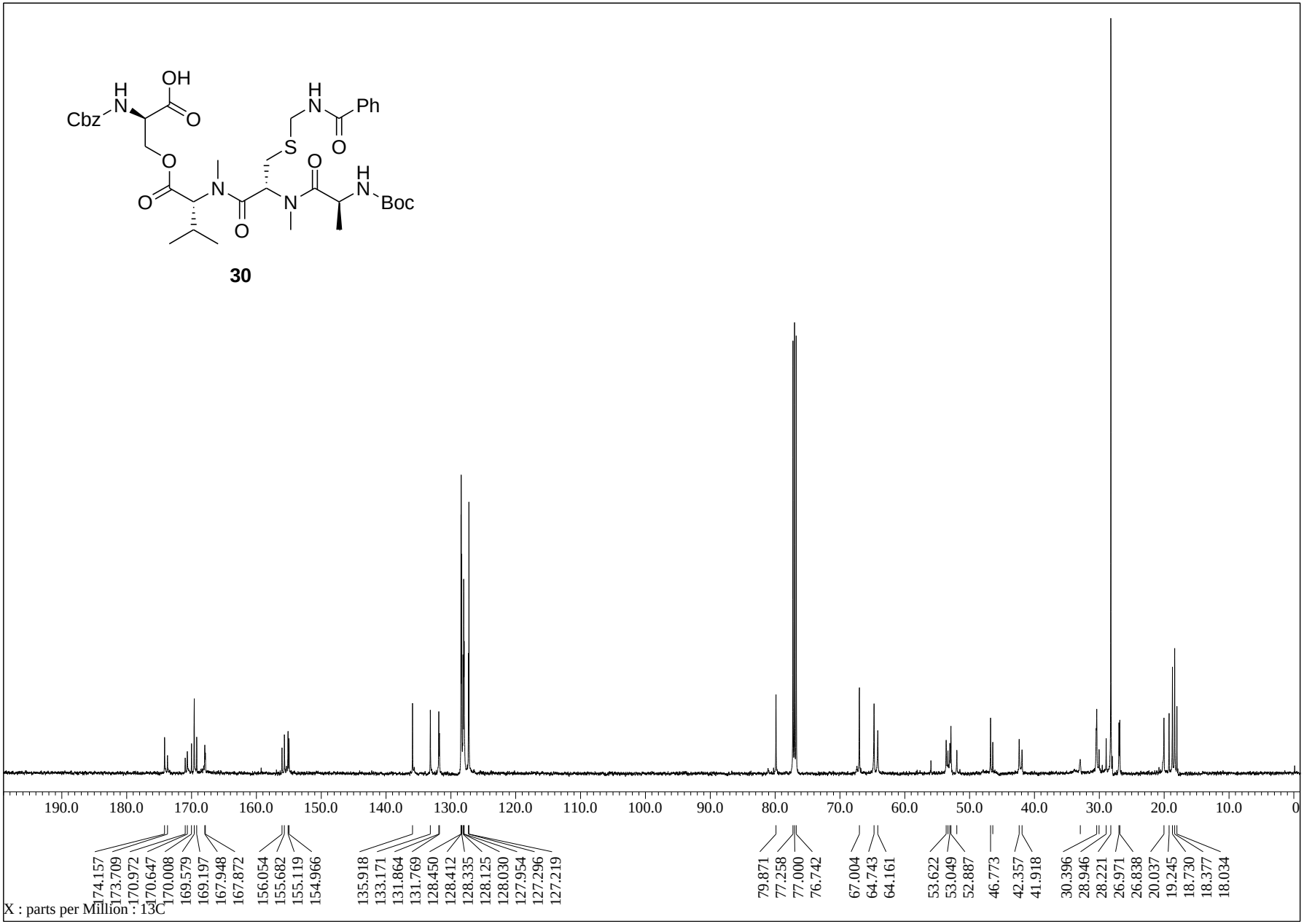
^{13}C NMR (125 MHz, $CDCl_3$)



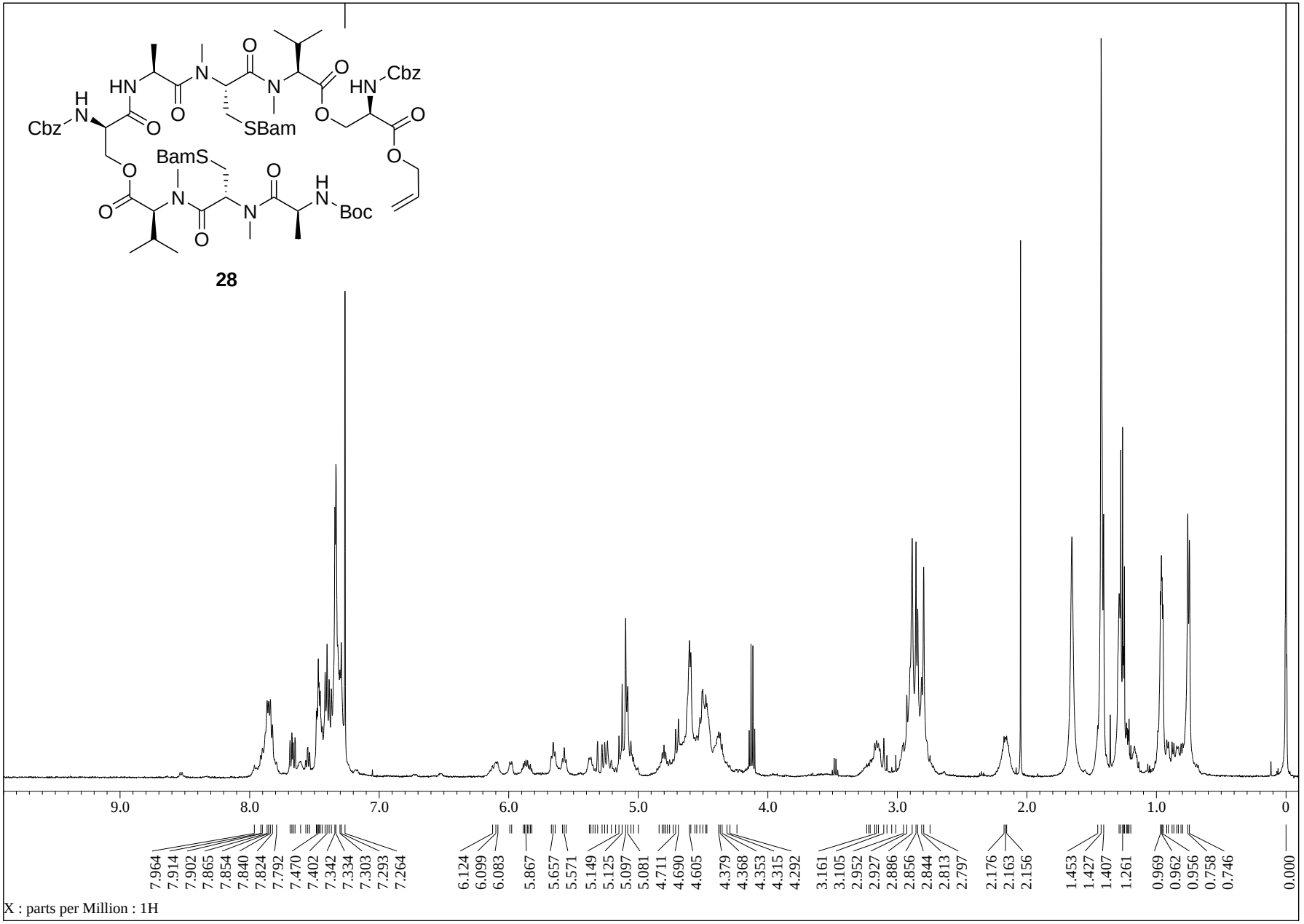
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (125 MHz, $CDCl_3$)

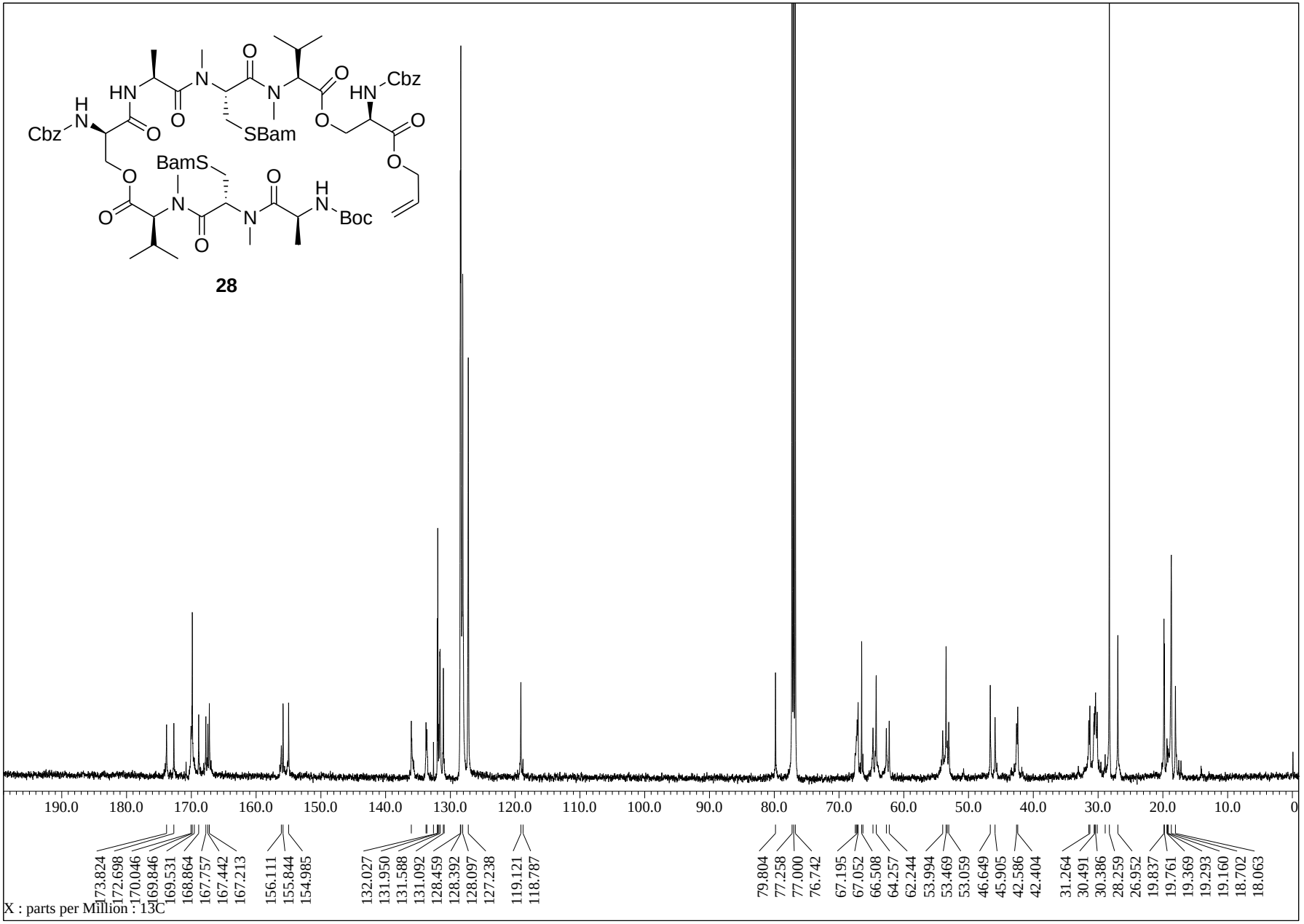


^{1}H NMR (500 MHz, $CDCl_3$)

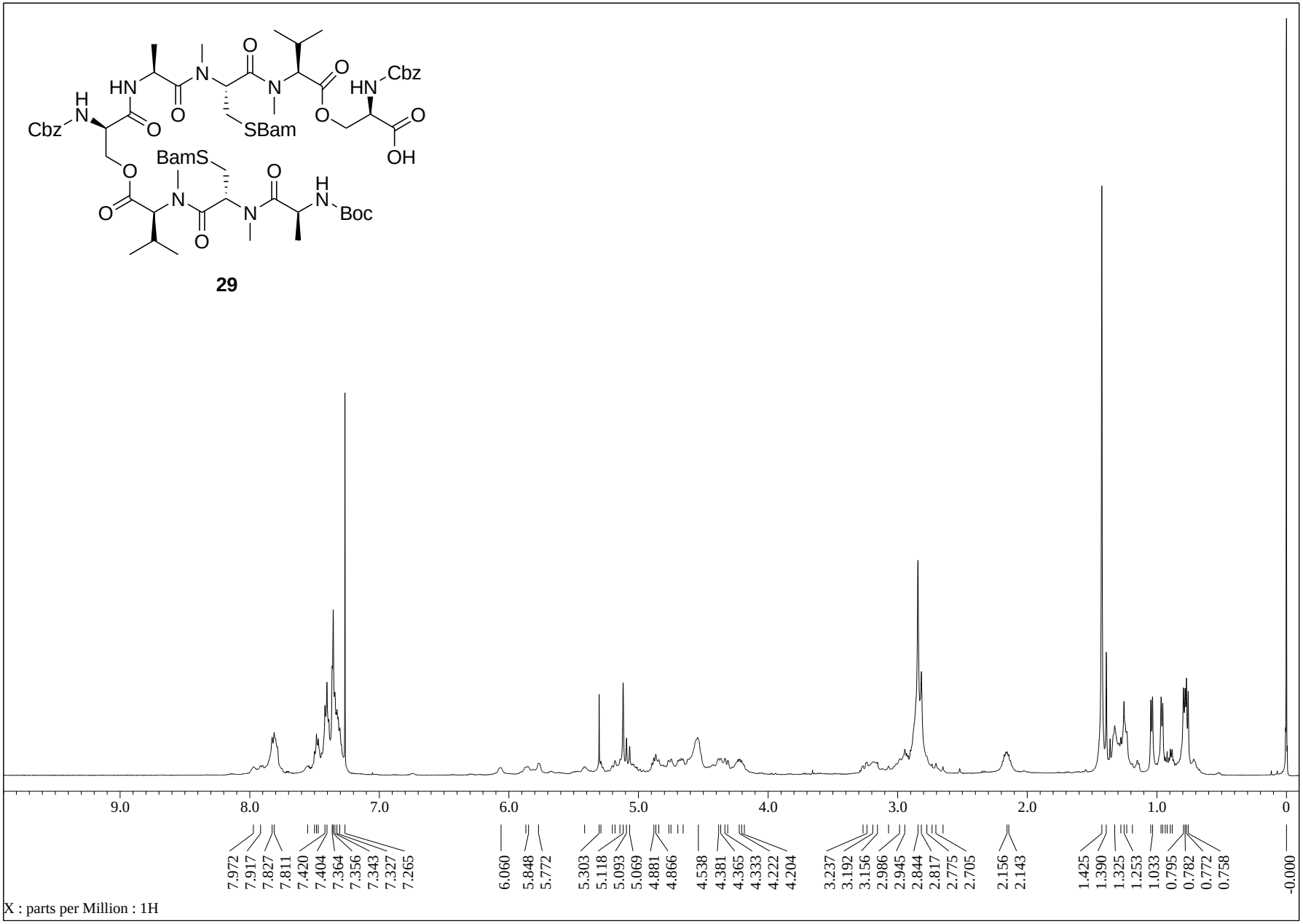


28

^{13}C NMR (125 MHz, $CDCl_3$)

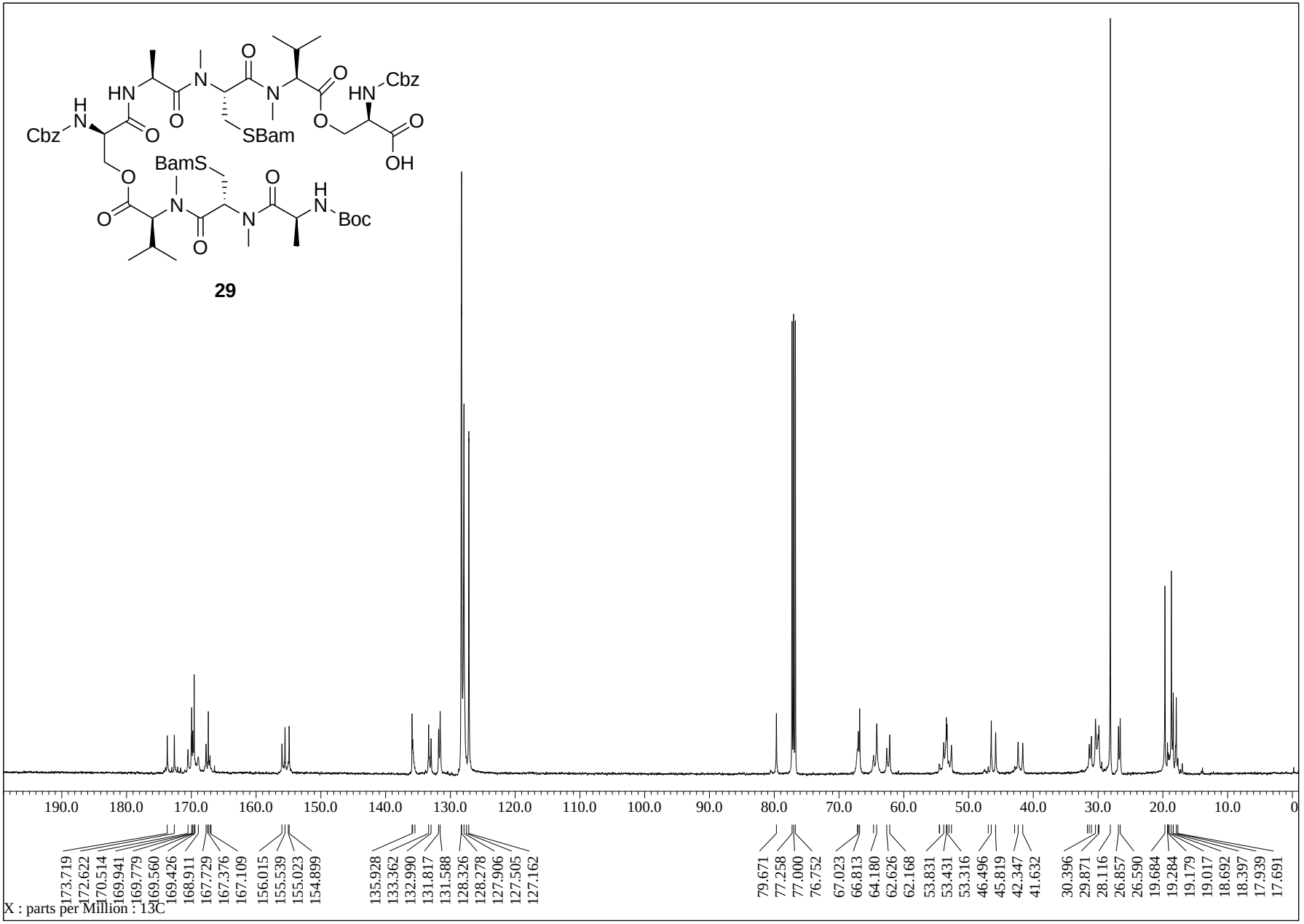


^{1}H NMR (500 MHz, $CDCl_3$)

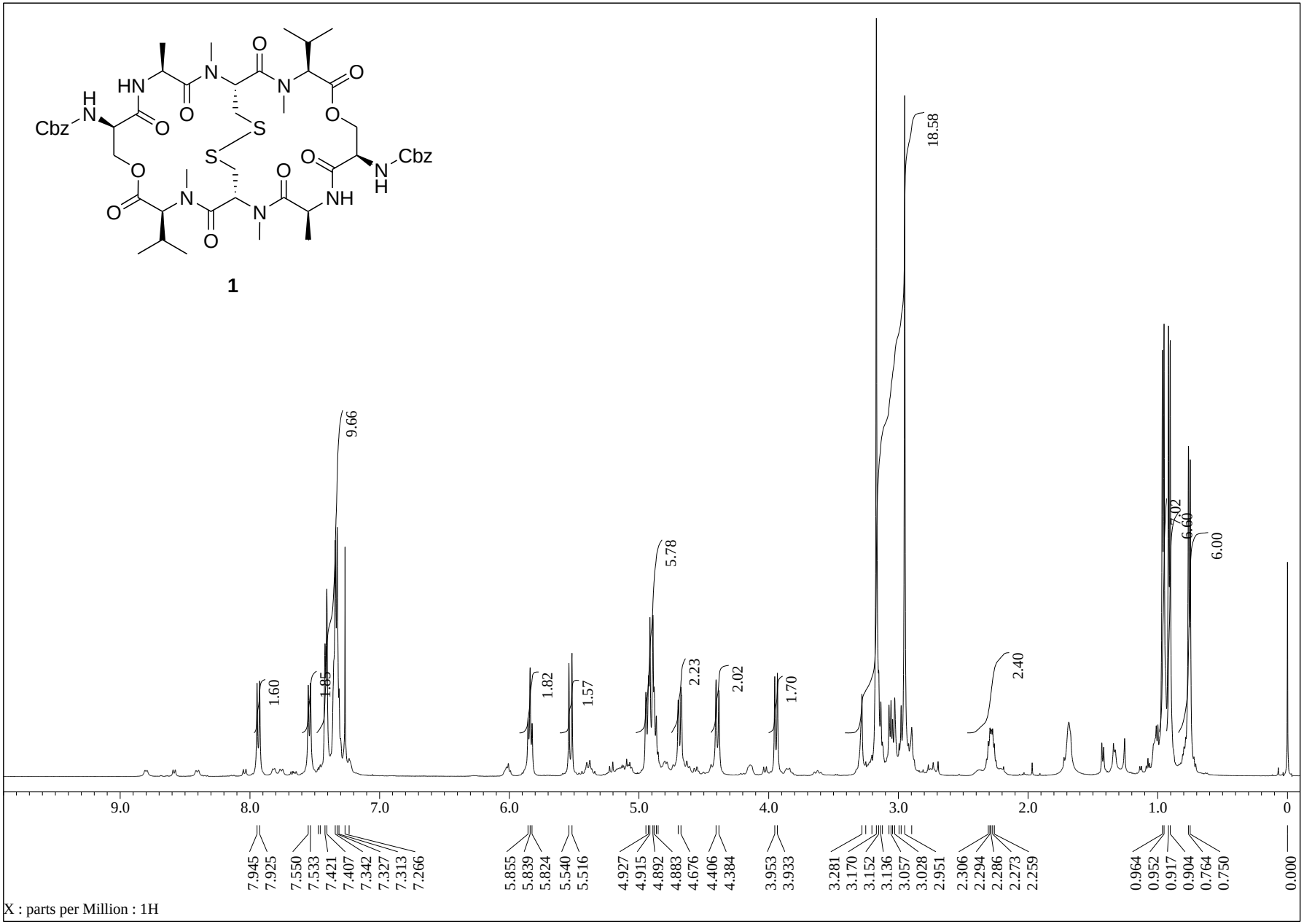


29

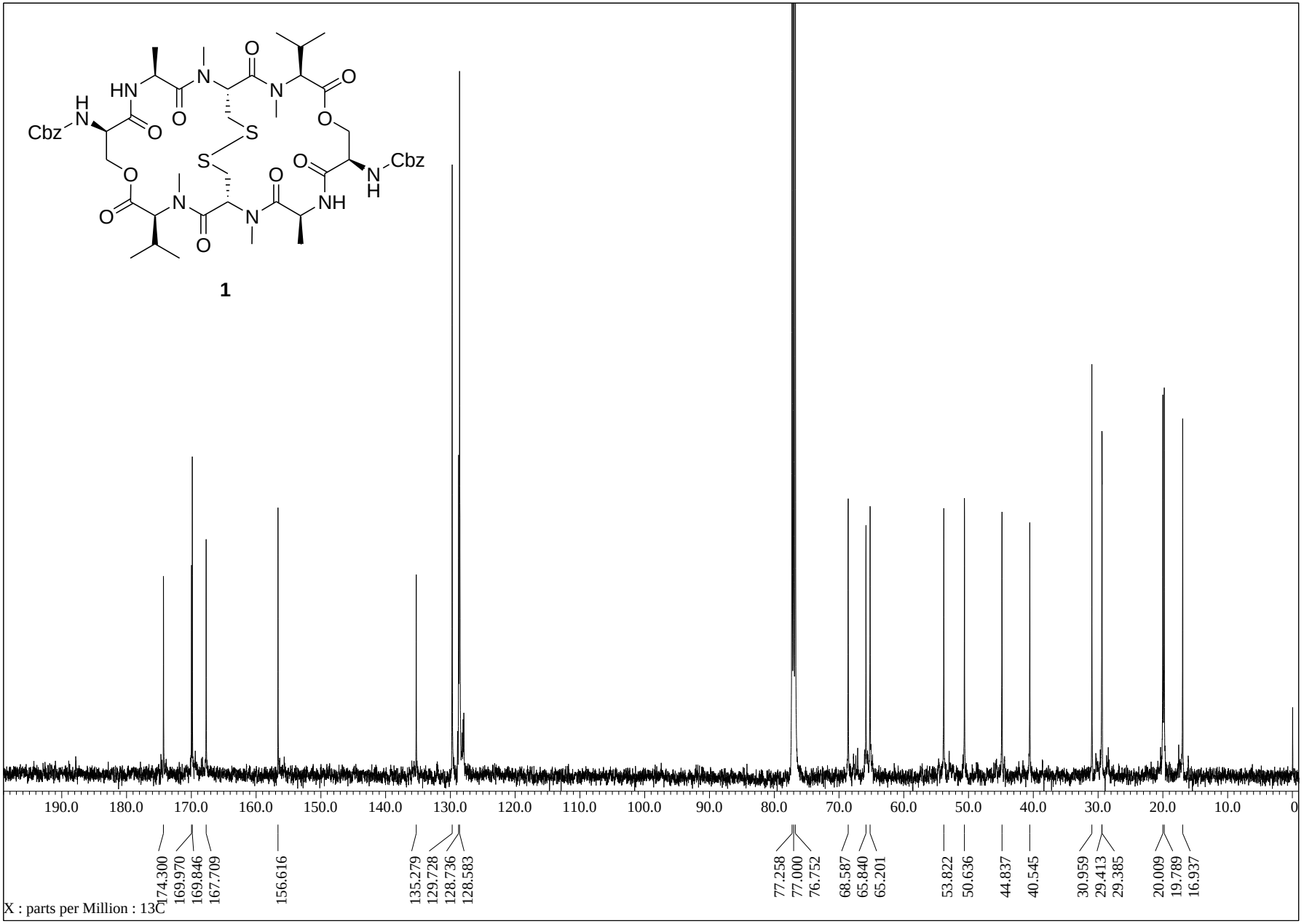
^{13}C NMR (125 MHz, $CDCl_3$)



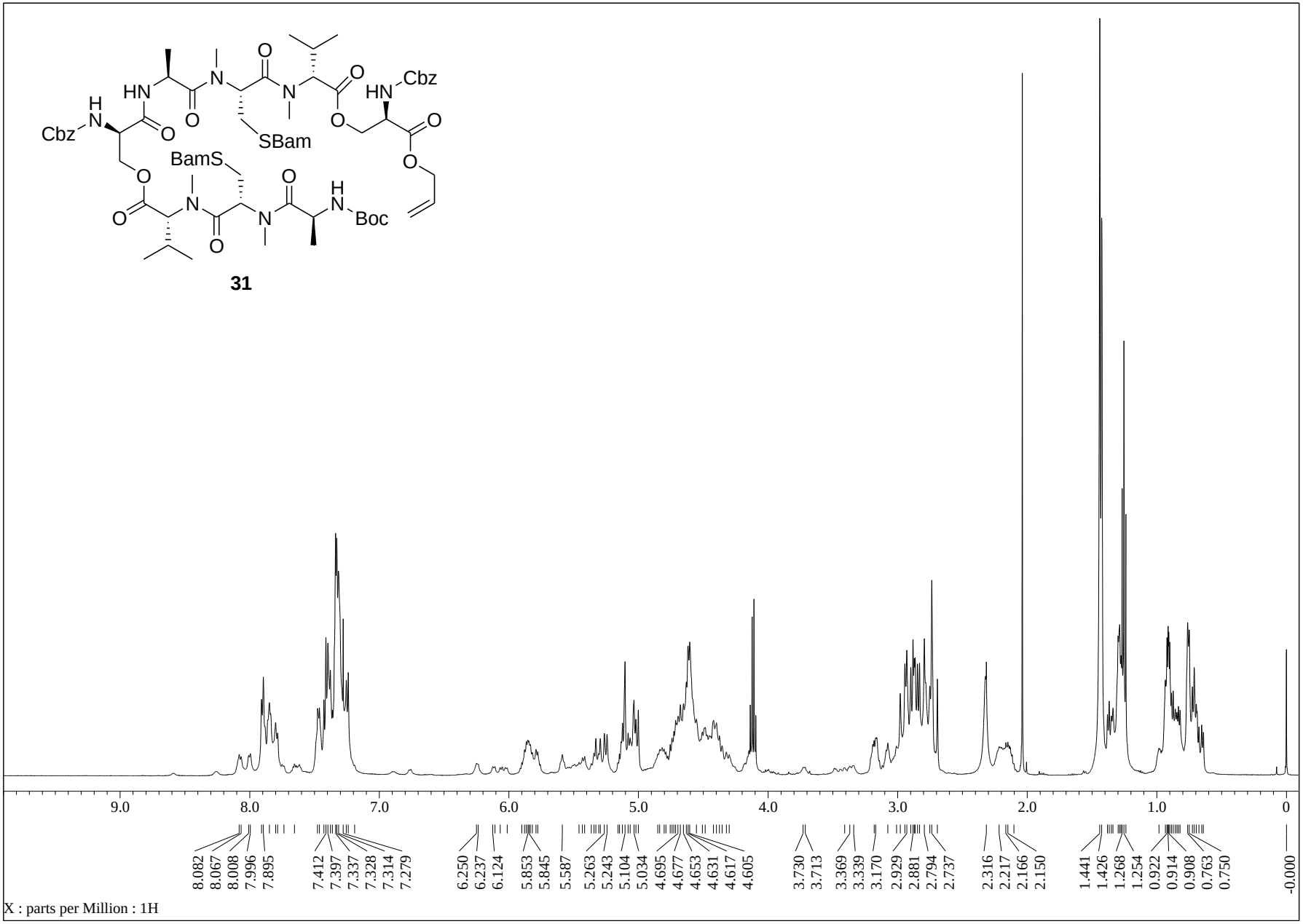
^{1}H NMR (500 MHz, $CDCl_3$)



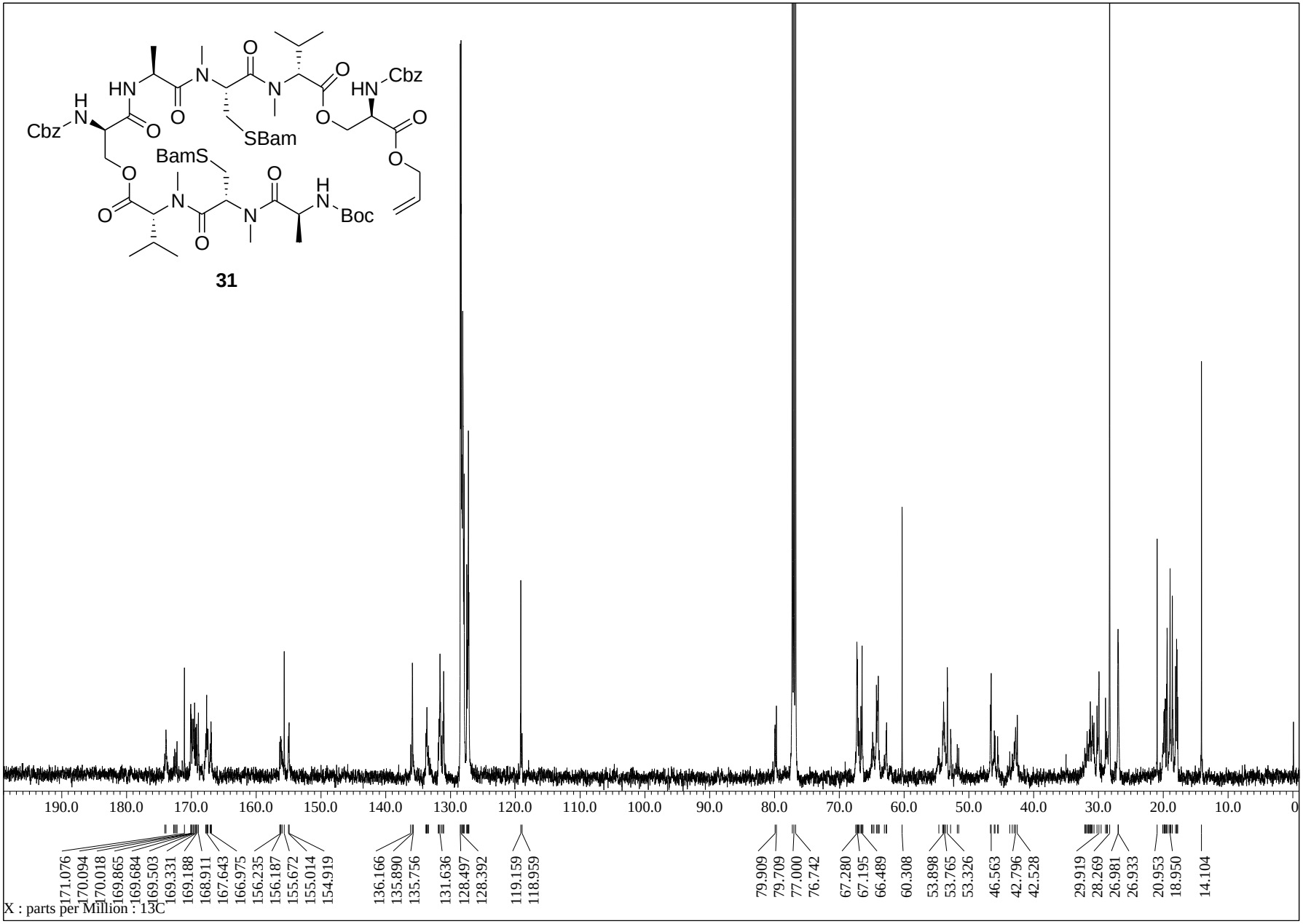
^{13}C NMR (125 MHz, $CDCl_3$)



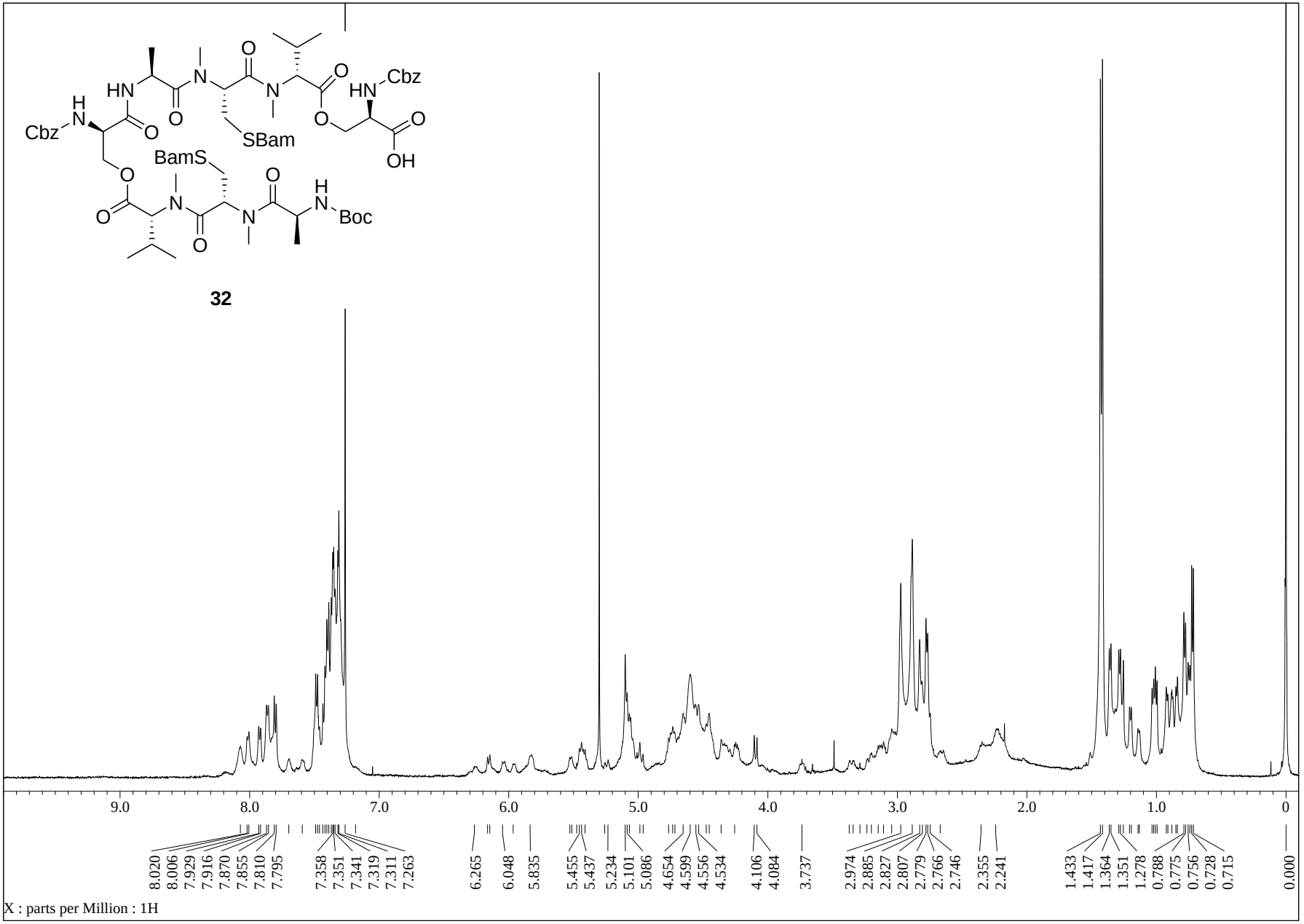
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (125 MHz, $CDCl_3$)

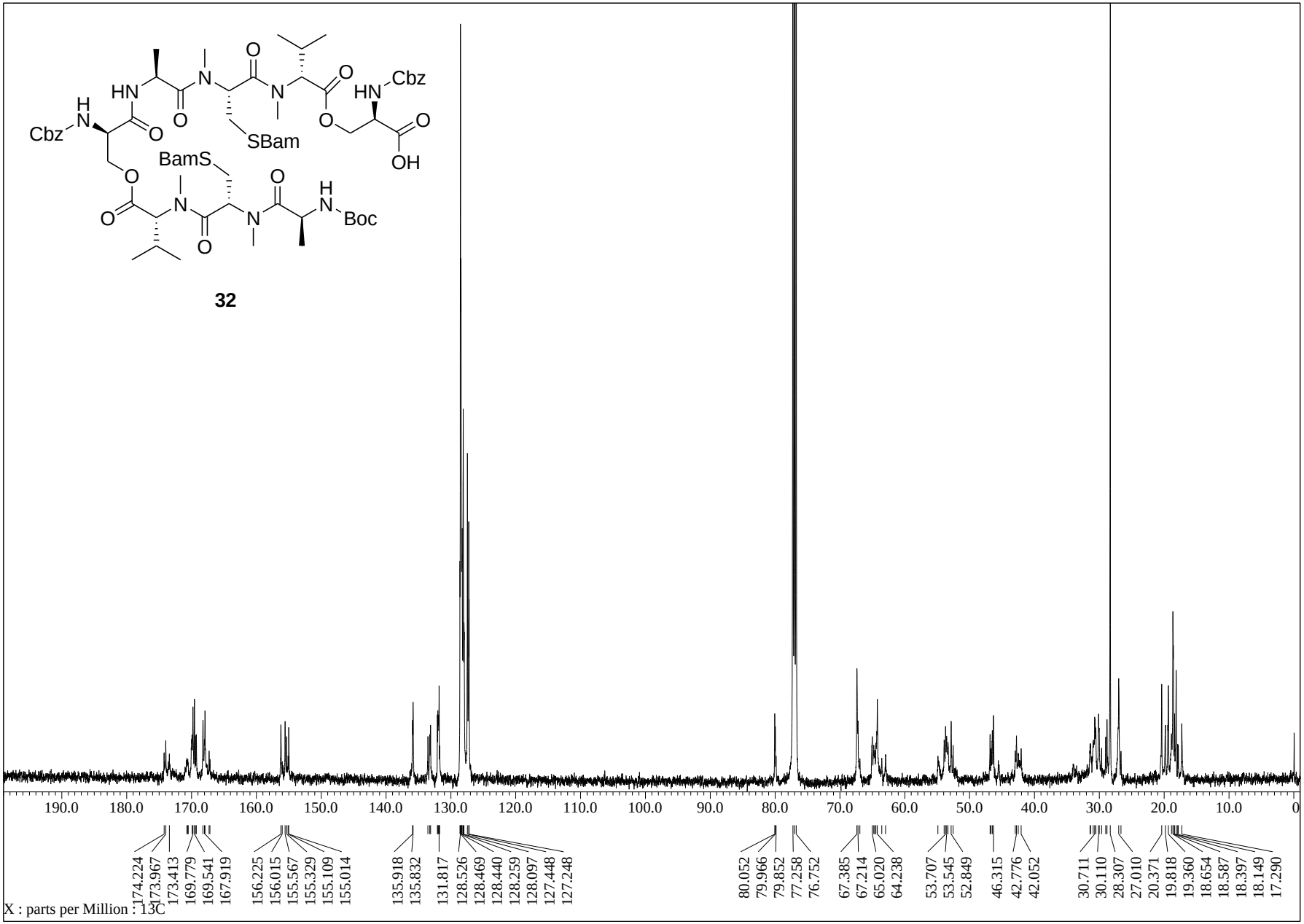


^{1}H NMR (500 MHz, $CDCl_3$)



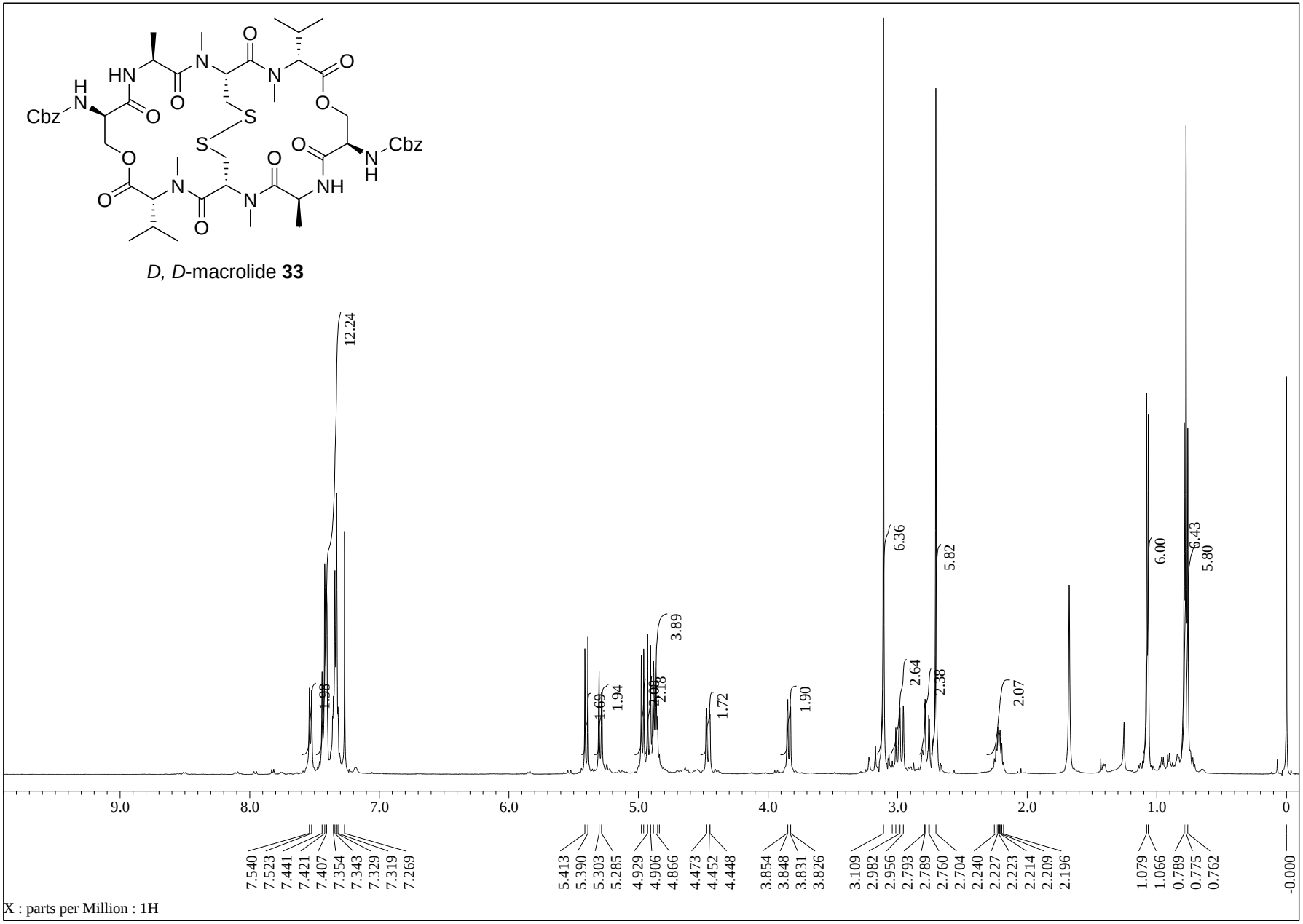
32

^{13}C NMR (125 MHz, $CDCl_3$)

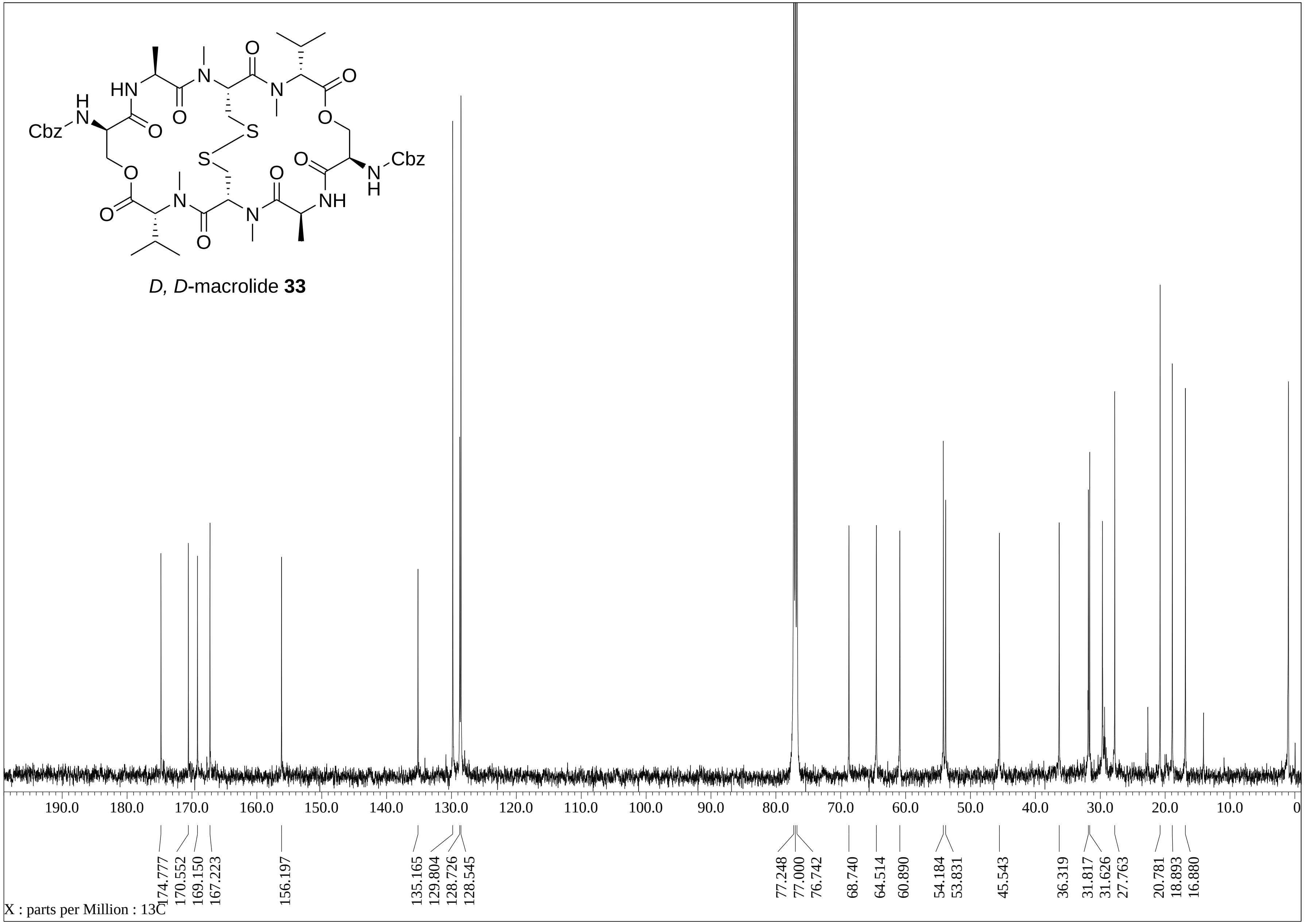


32

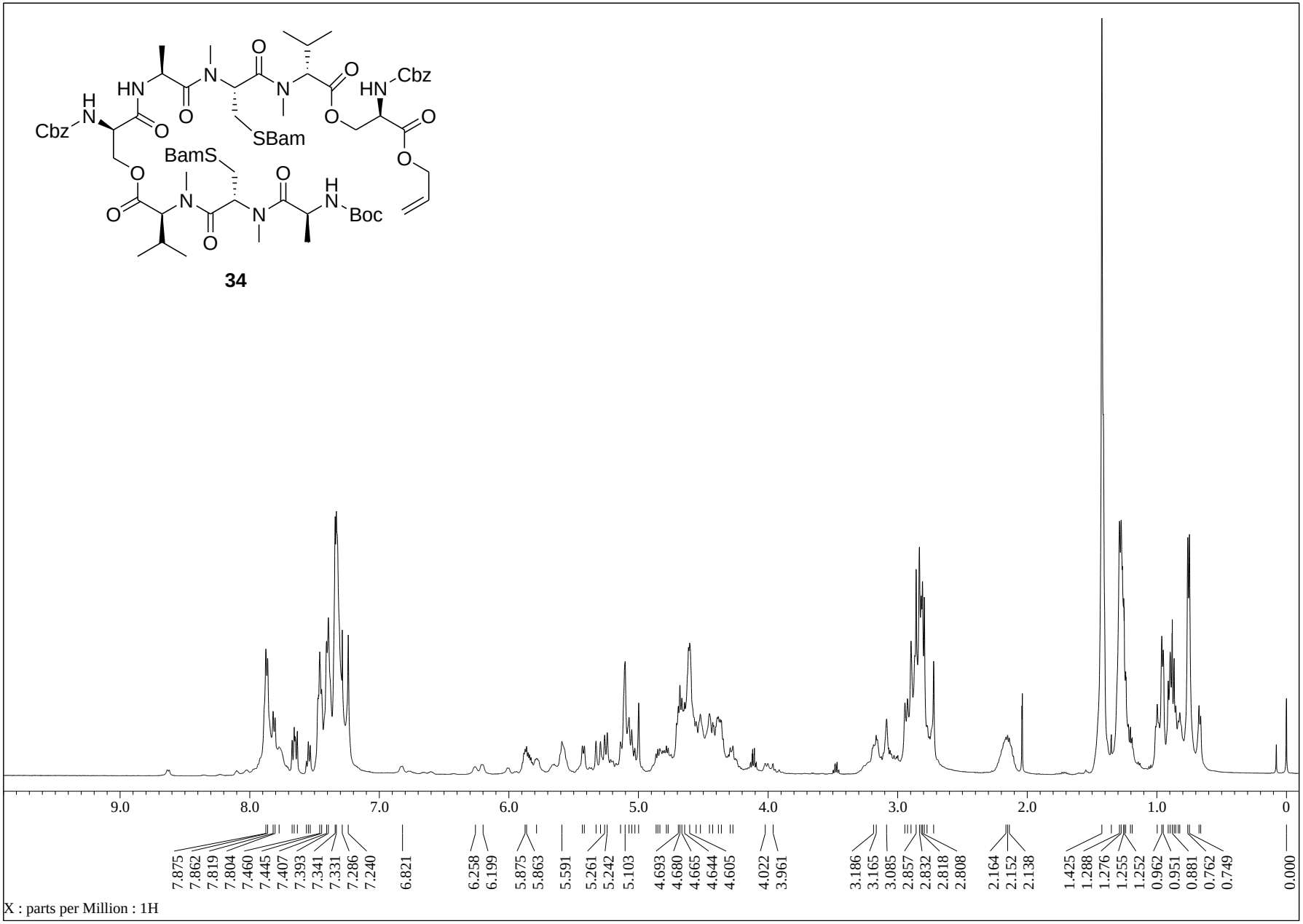
^{1}H NMR (500 MHz, $CDCl_3$)



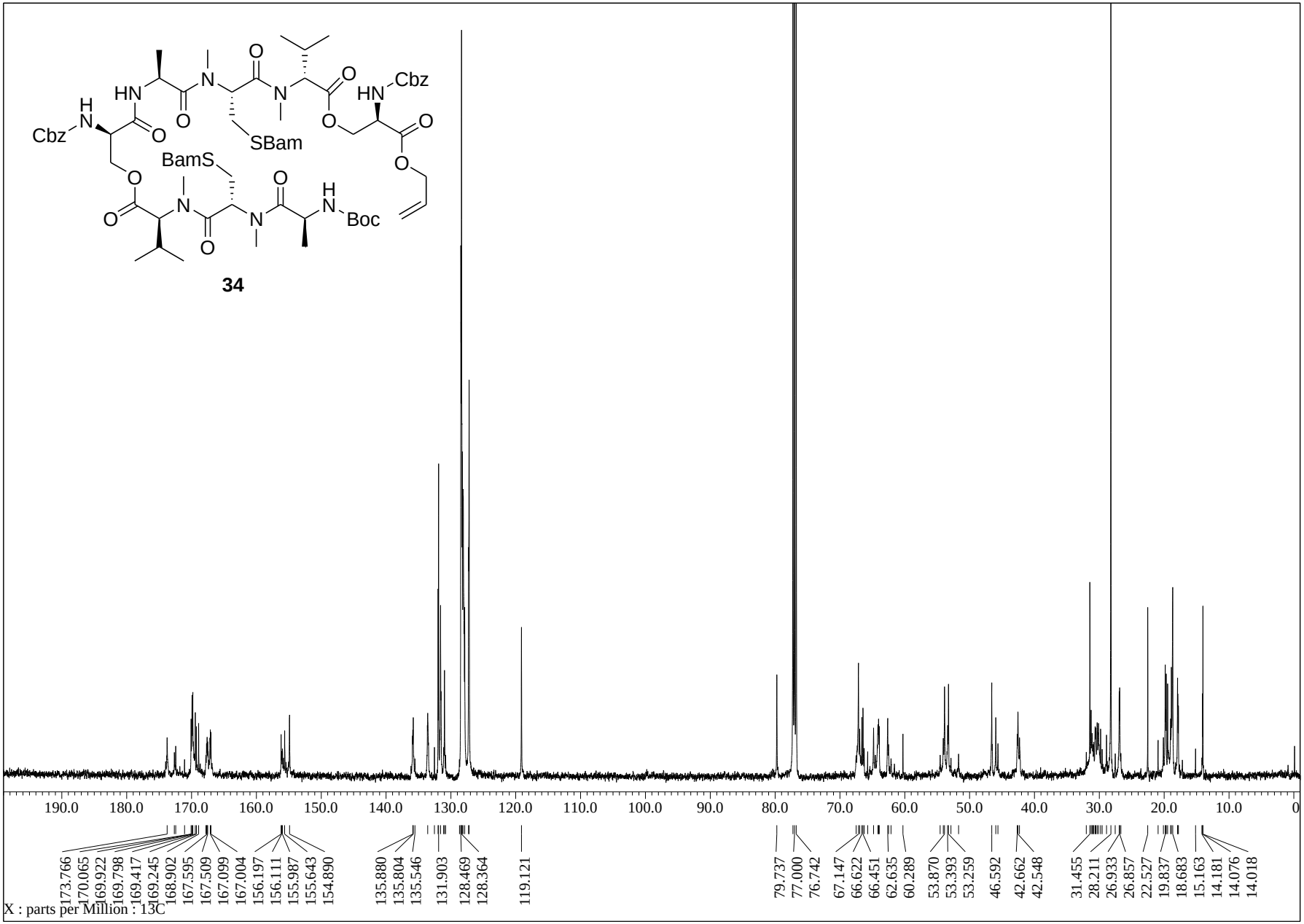
^{13}C NMR (125 MHz, $CDCl_3$)



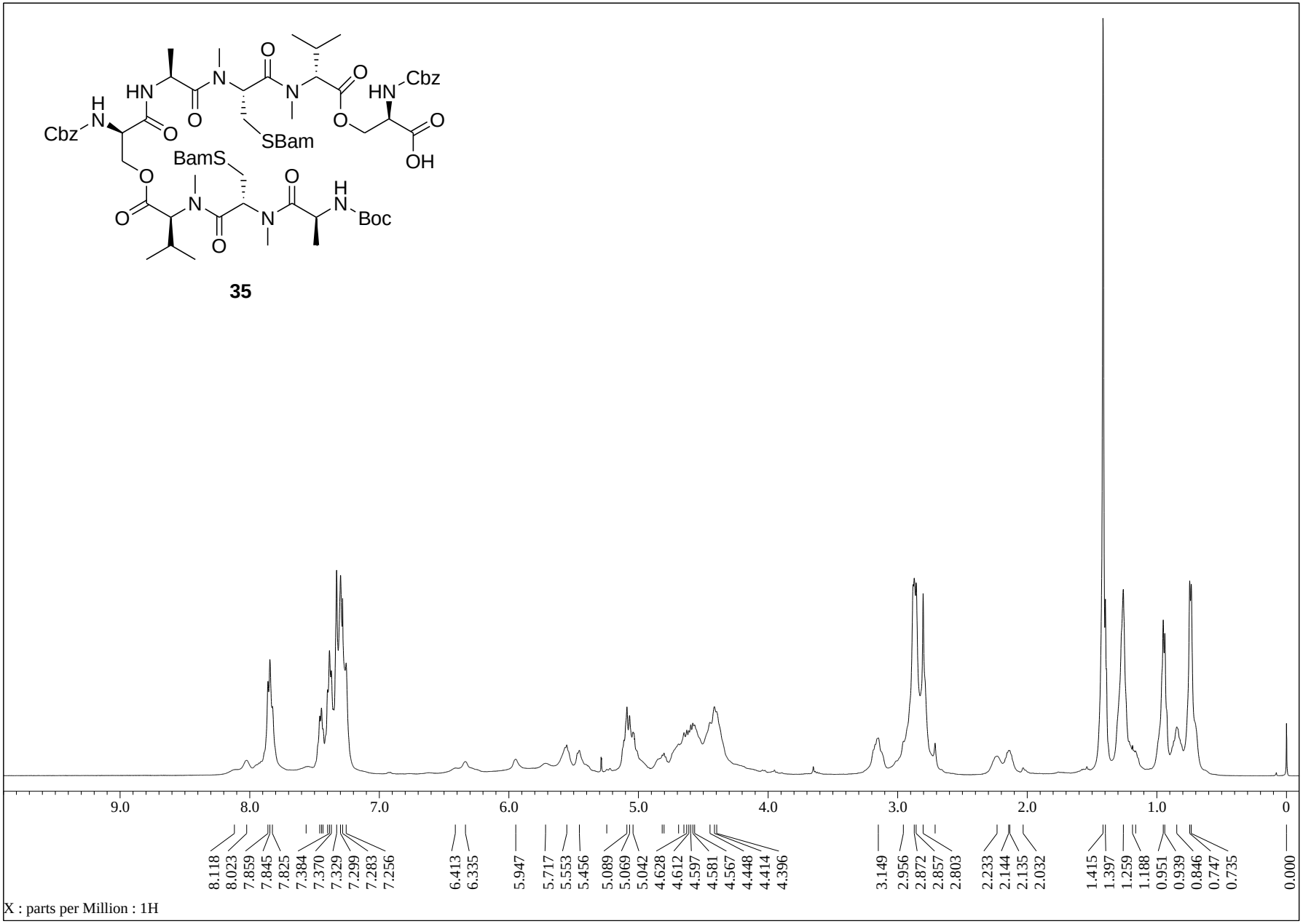
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (125 MHz, $CDCl_3$)

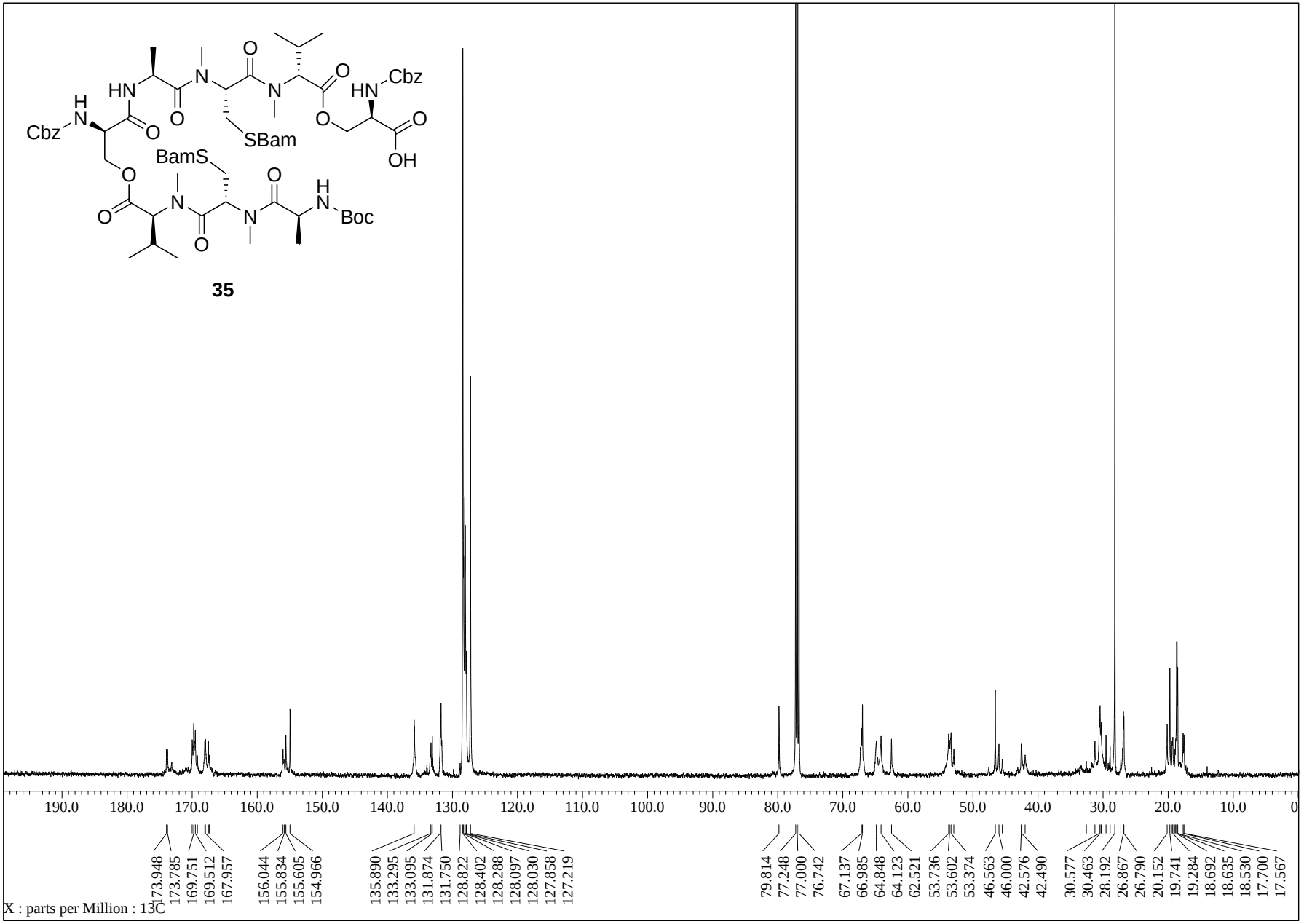


^{1}H NMR (500 MHz, $CDCl_3$)

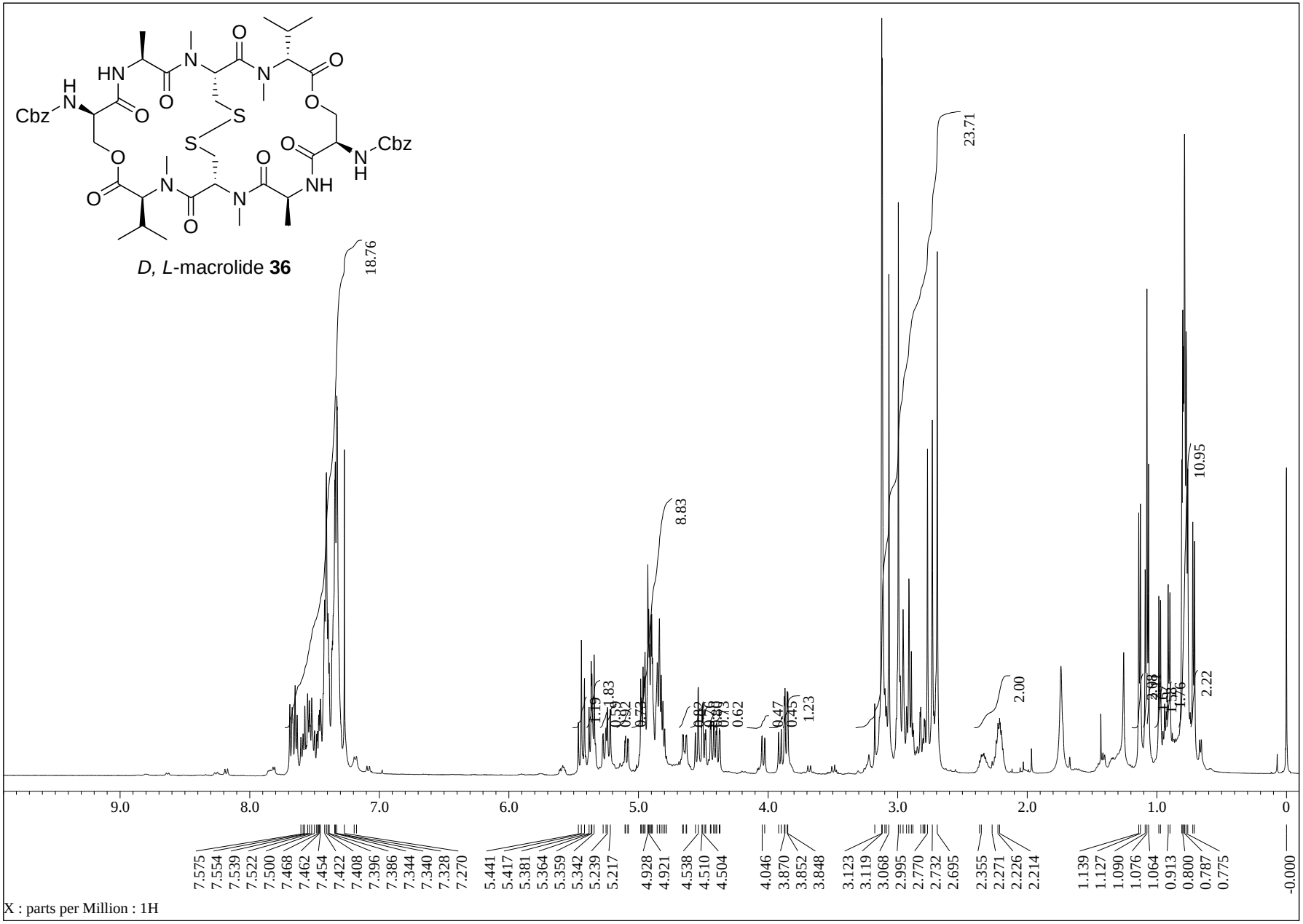


35

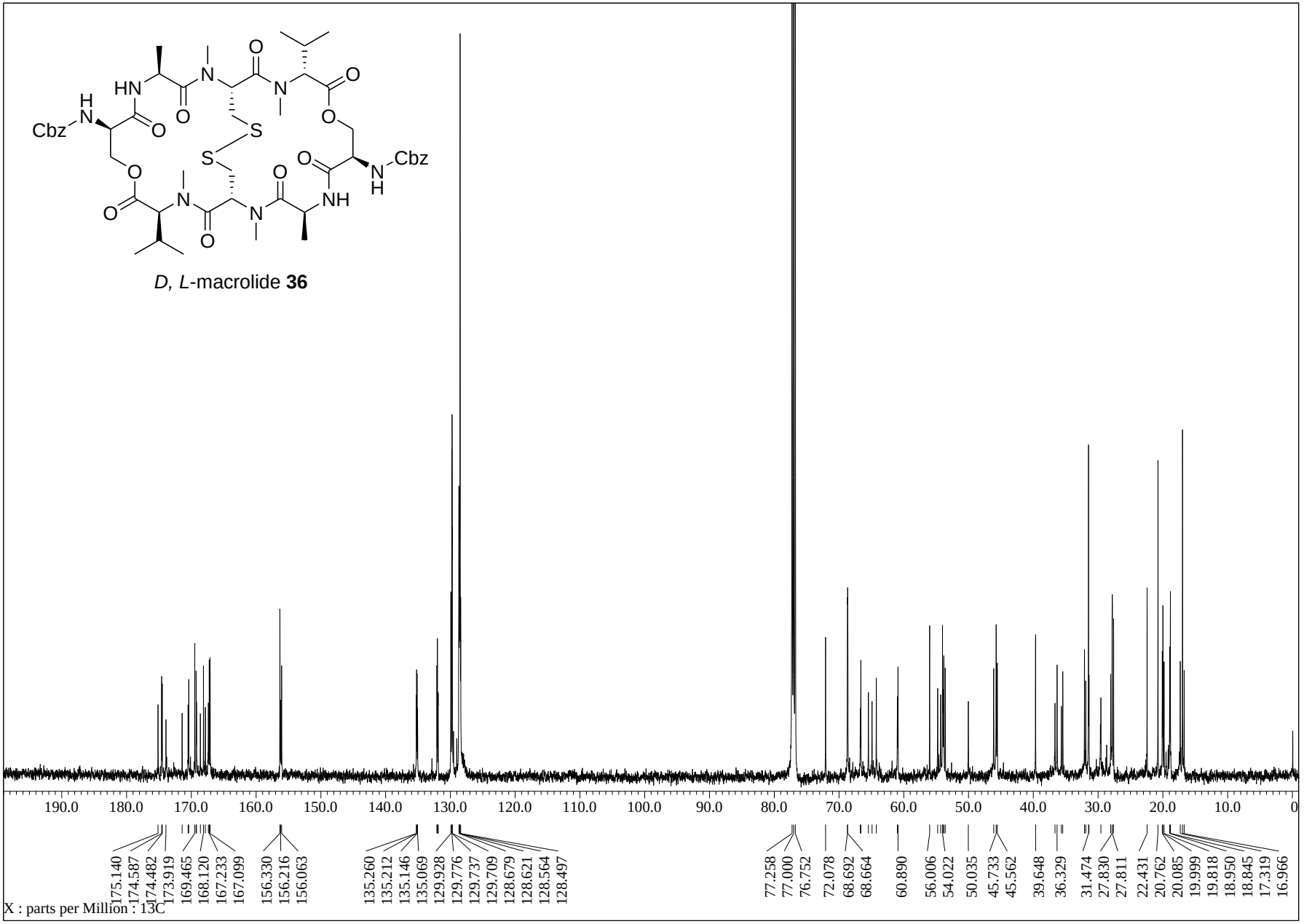
^{13}C NMR (125 MHz, $CDCl_3$)



^{1}H NMR (500 MHz, $CDCl_3$)

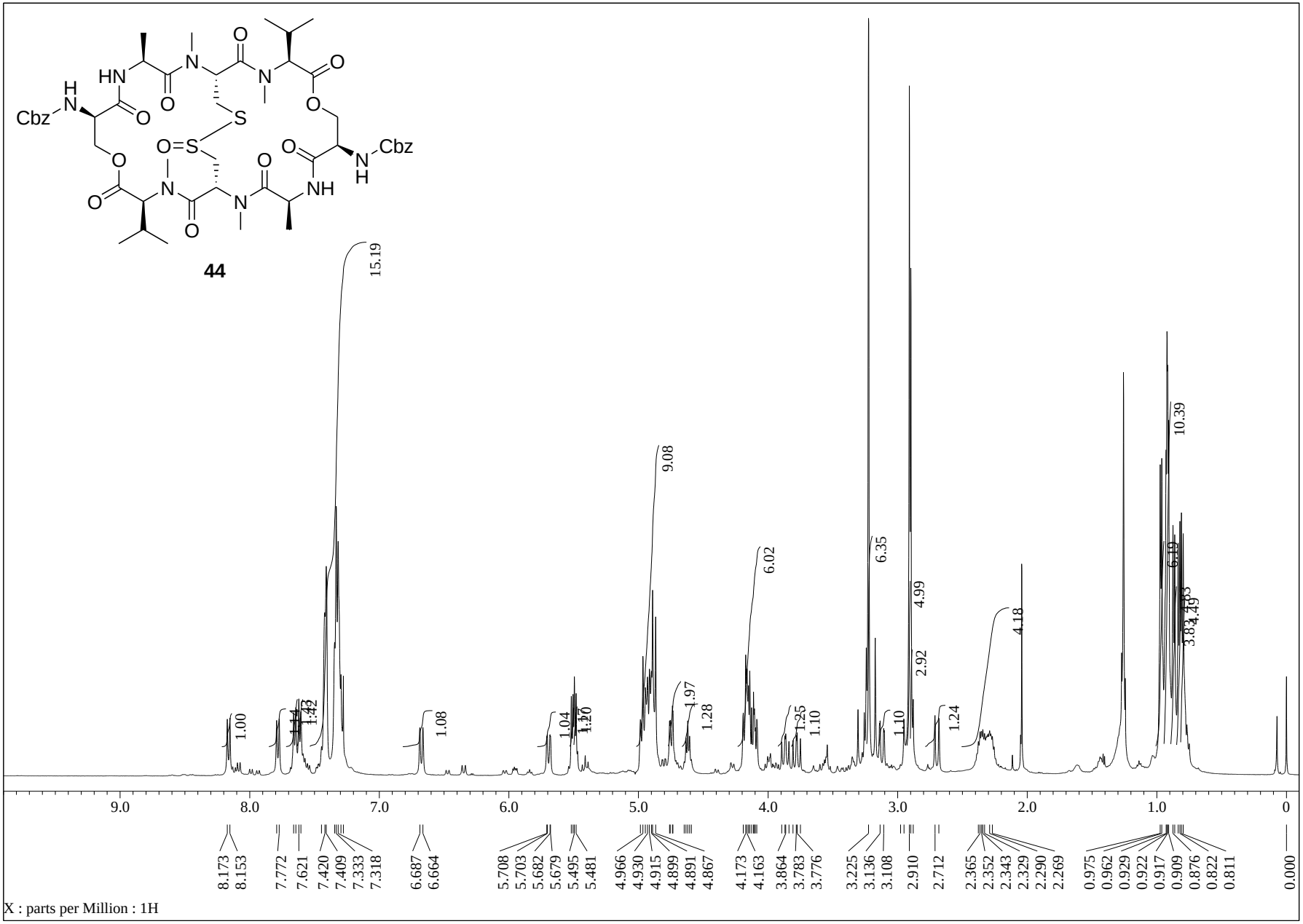


^{13}C NMR (125 MHz, $CDCl_3$)

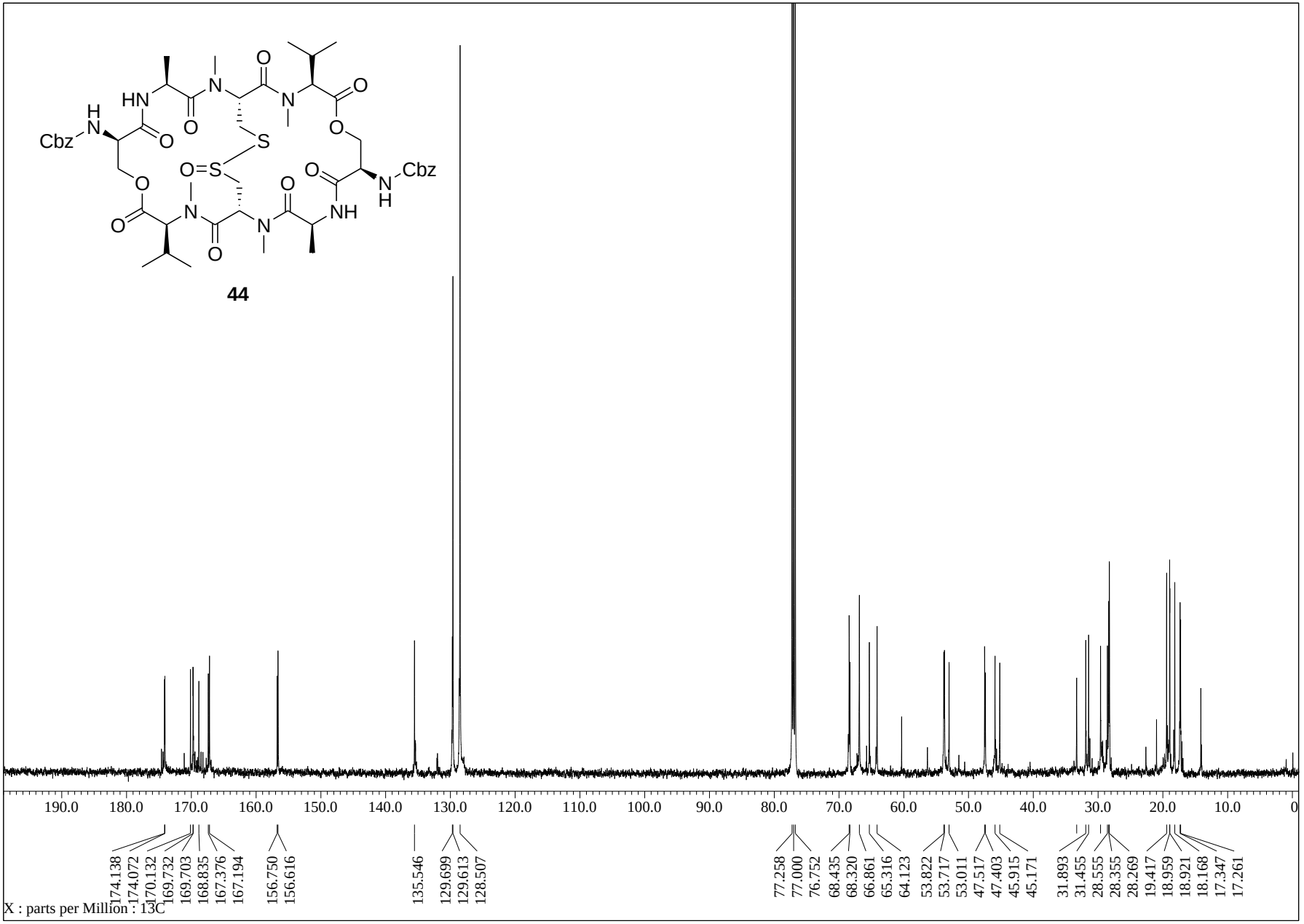


D, L-macrolide **36**

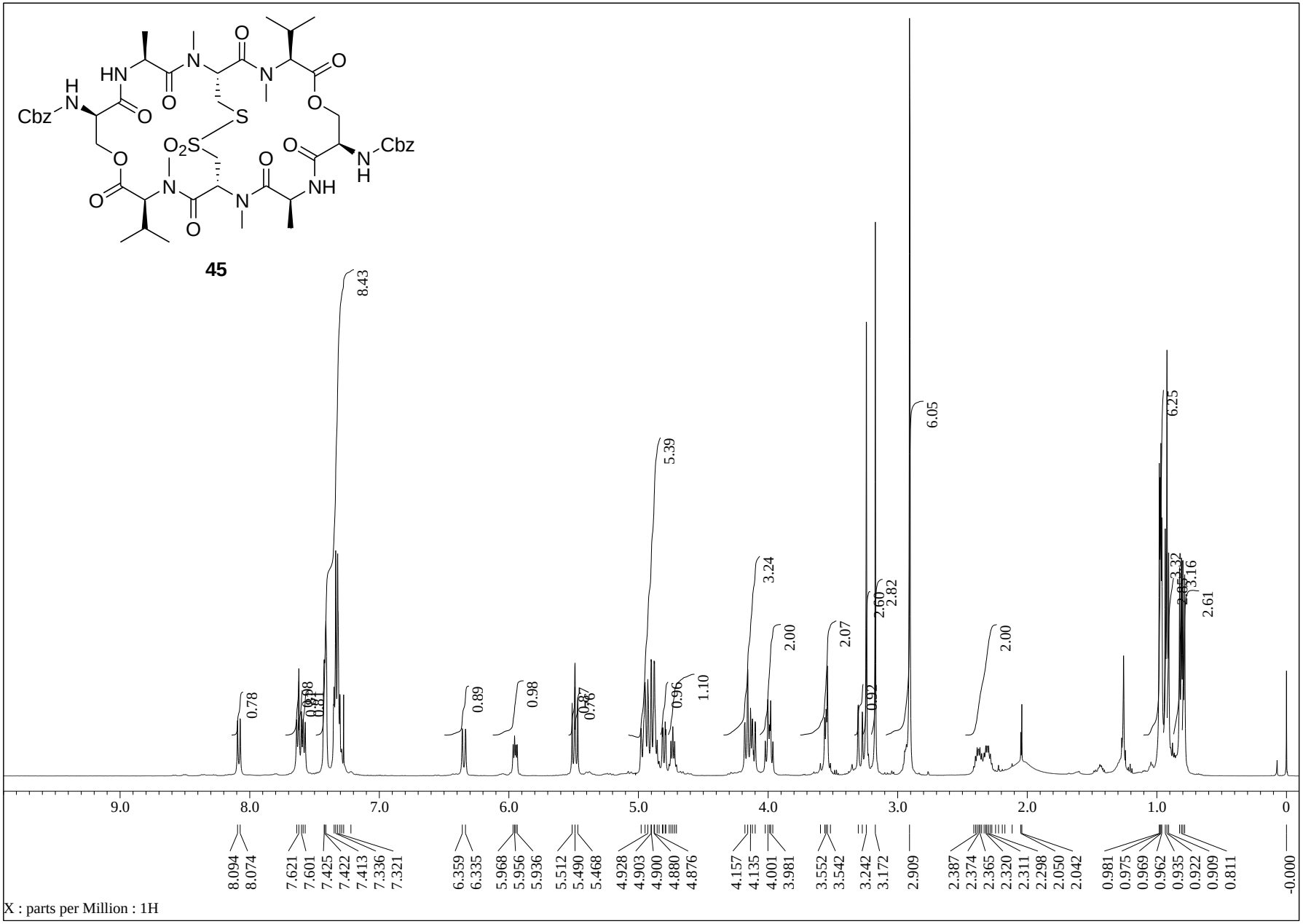
^{1}H NMR (500 MHz, $CDCl_3$)



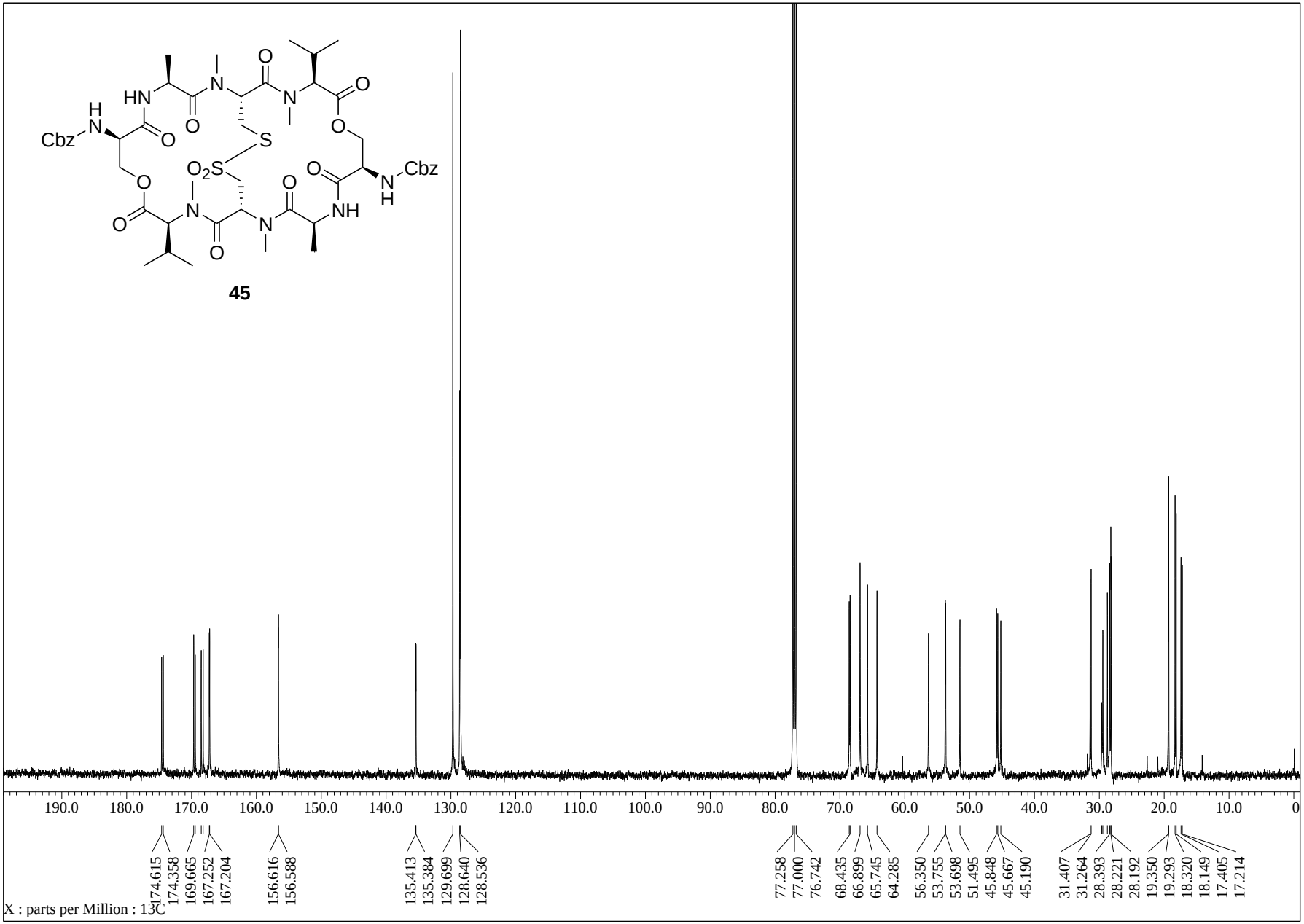
^{13}C NMR (125 MHz, $CDCl_3$)



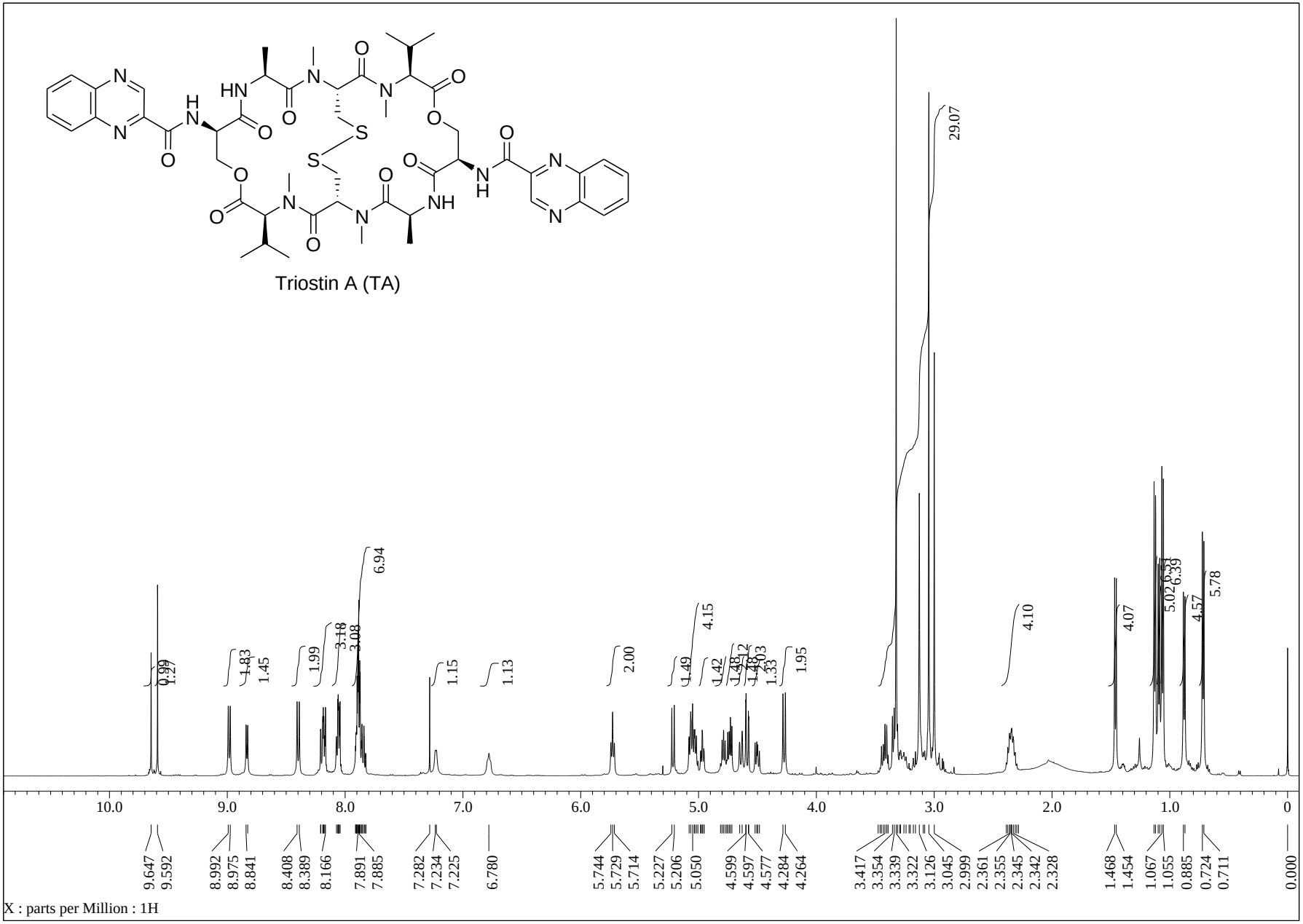
^{1}H NMR (500 MHz, $CDCl_3$)



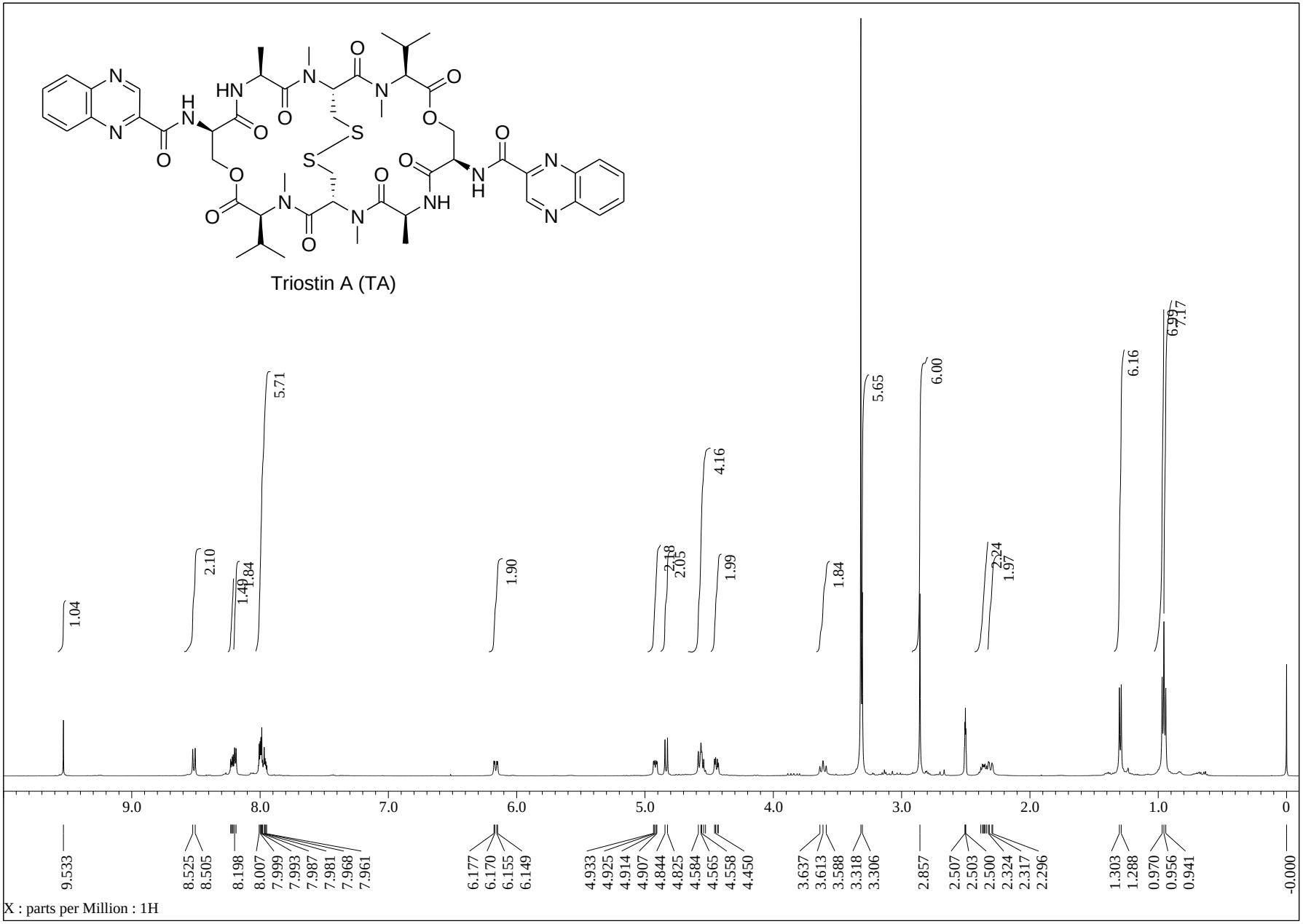
^{13}C NMR (125 MHz, $CDCl_3$)



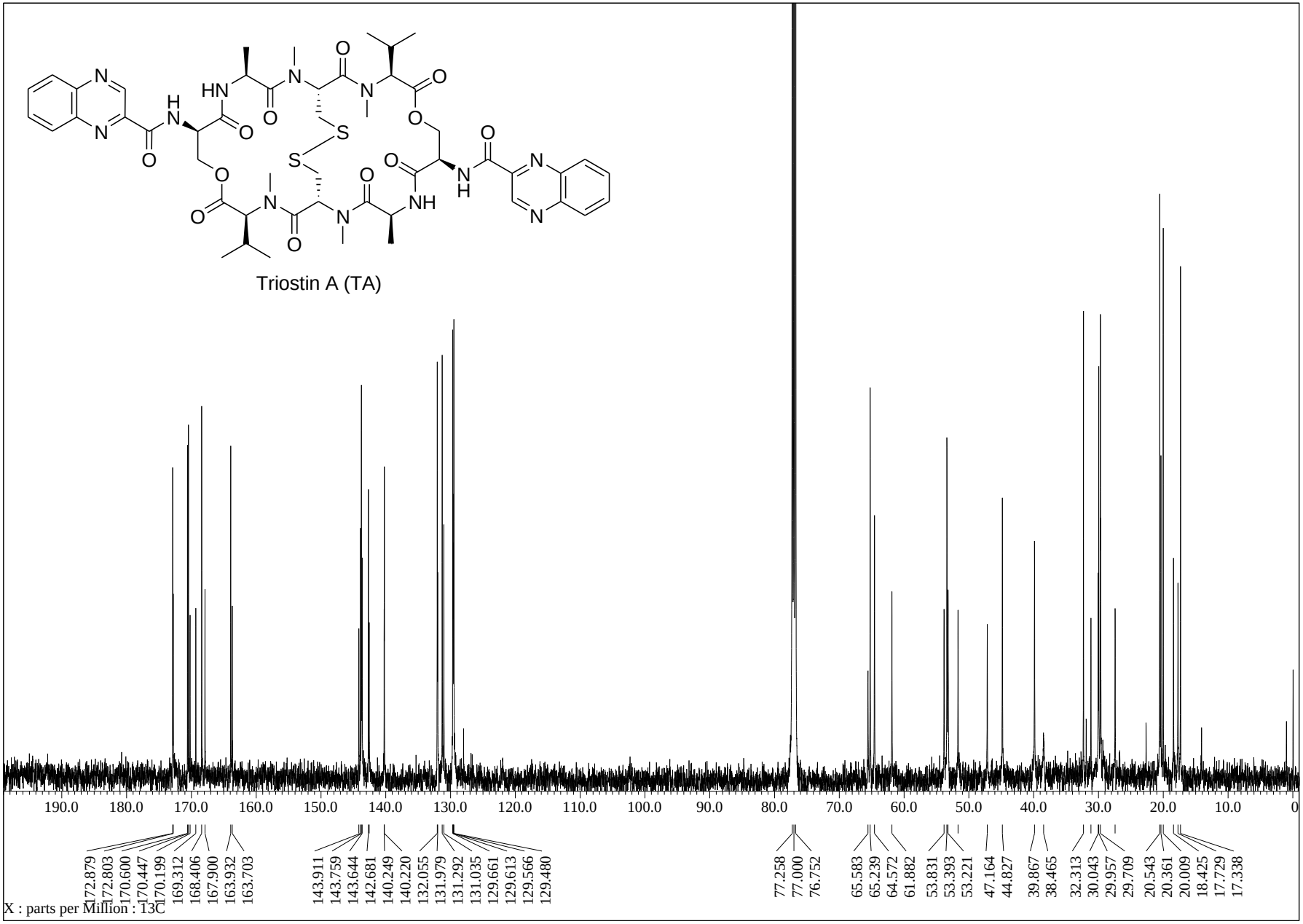
^{1}H NMR (500 MHz, $CDCl_3$)



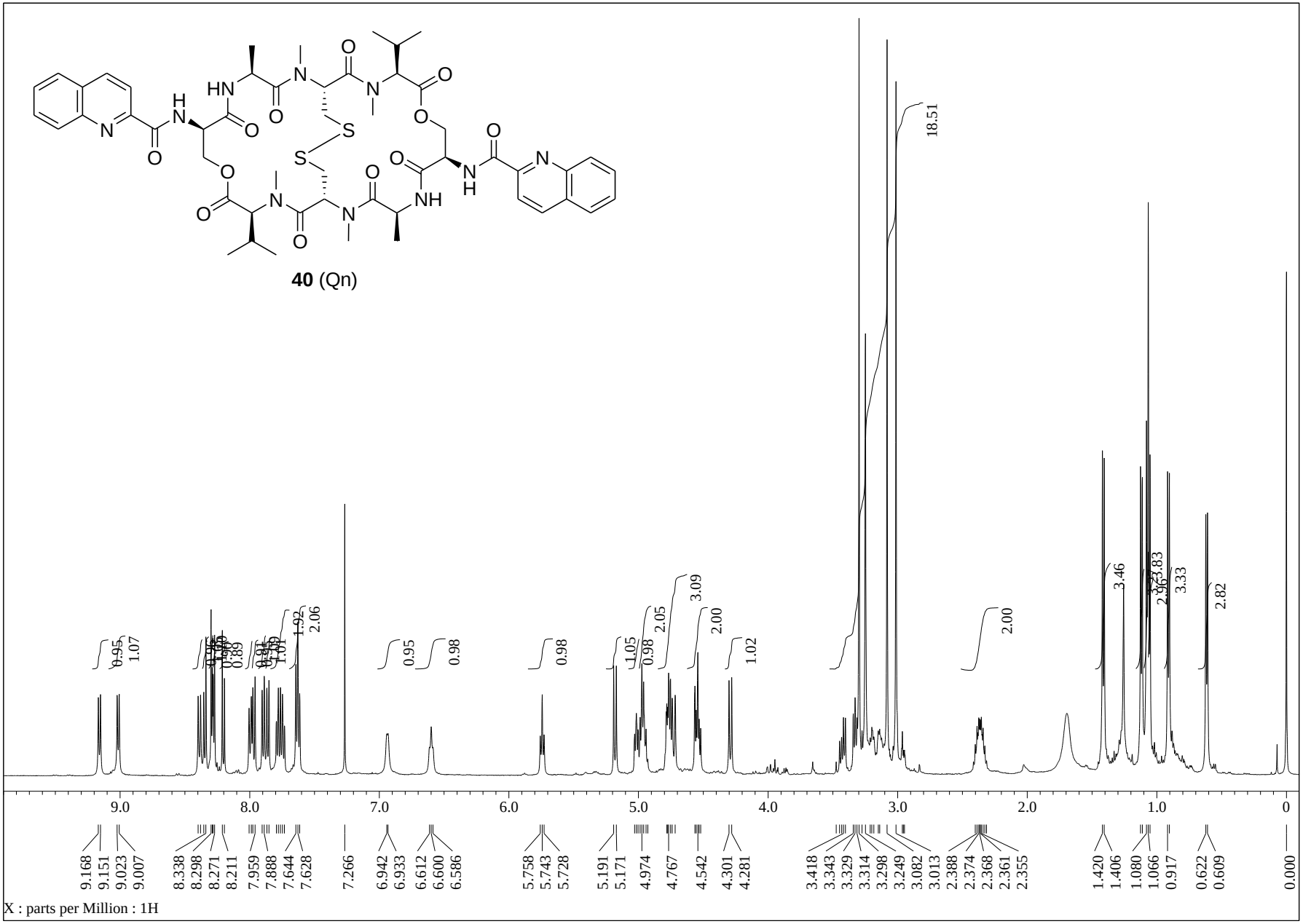
^{1}H NMR (500 MHz, DMSO-d_6)



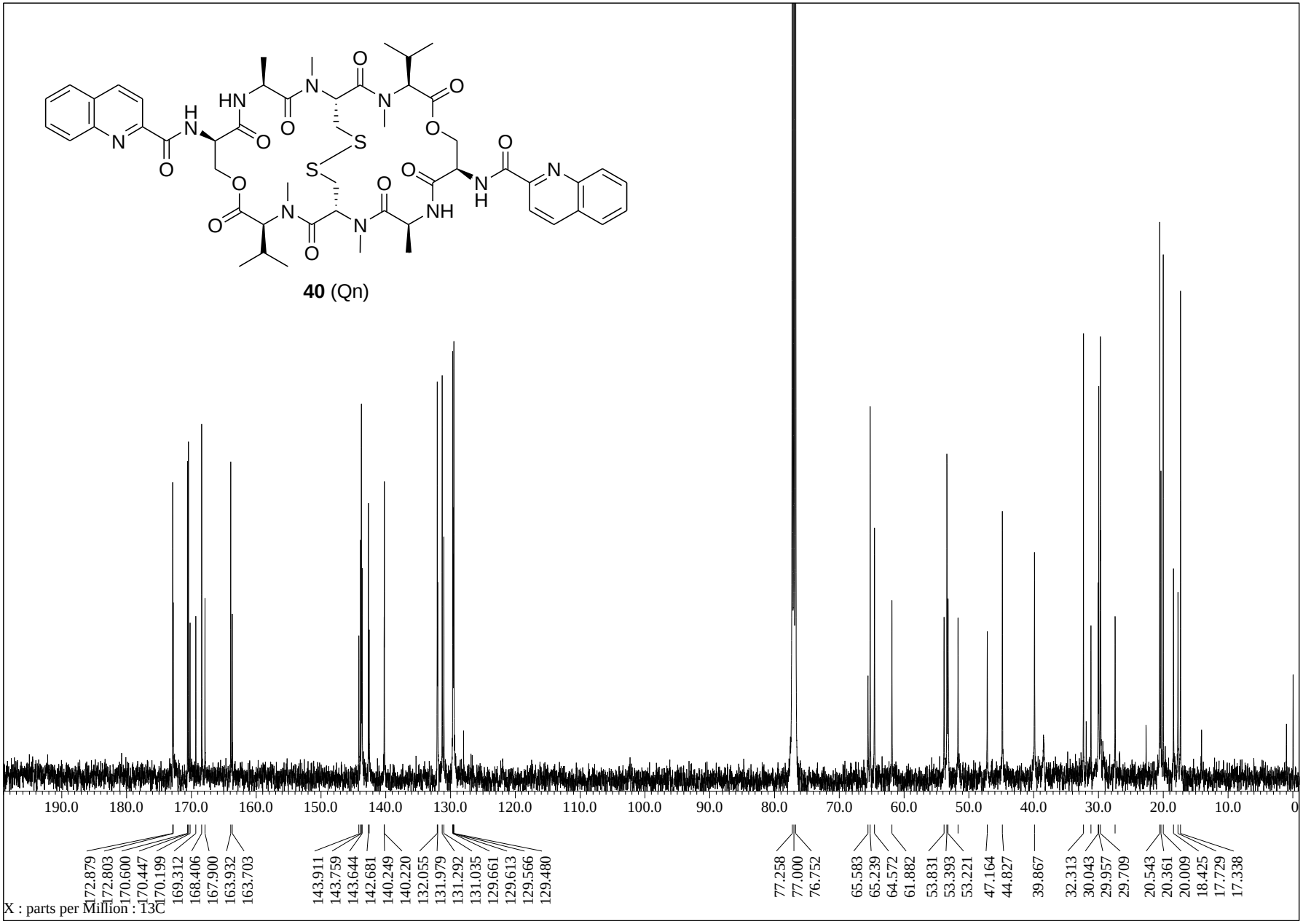
^{13}C NMR (125 MHz, $CDCl_3$)



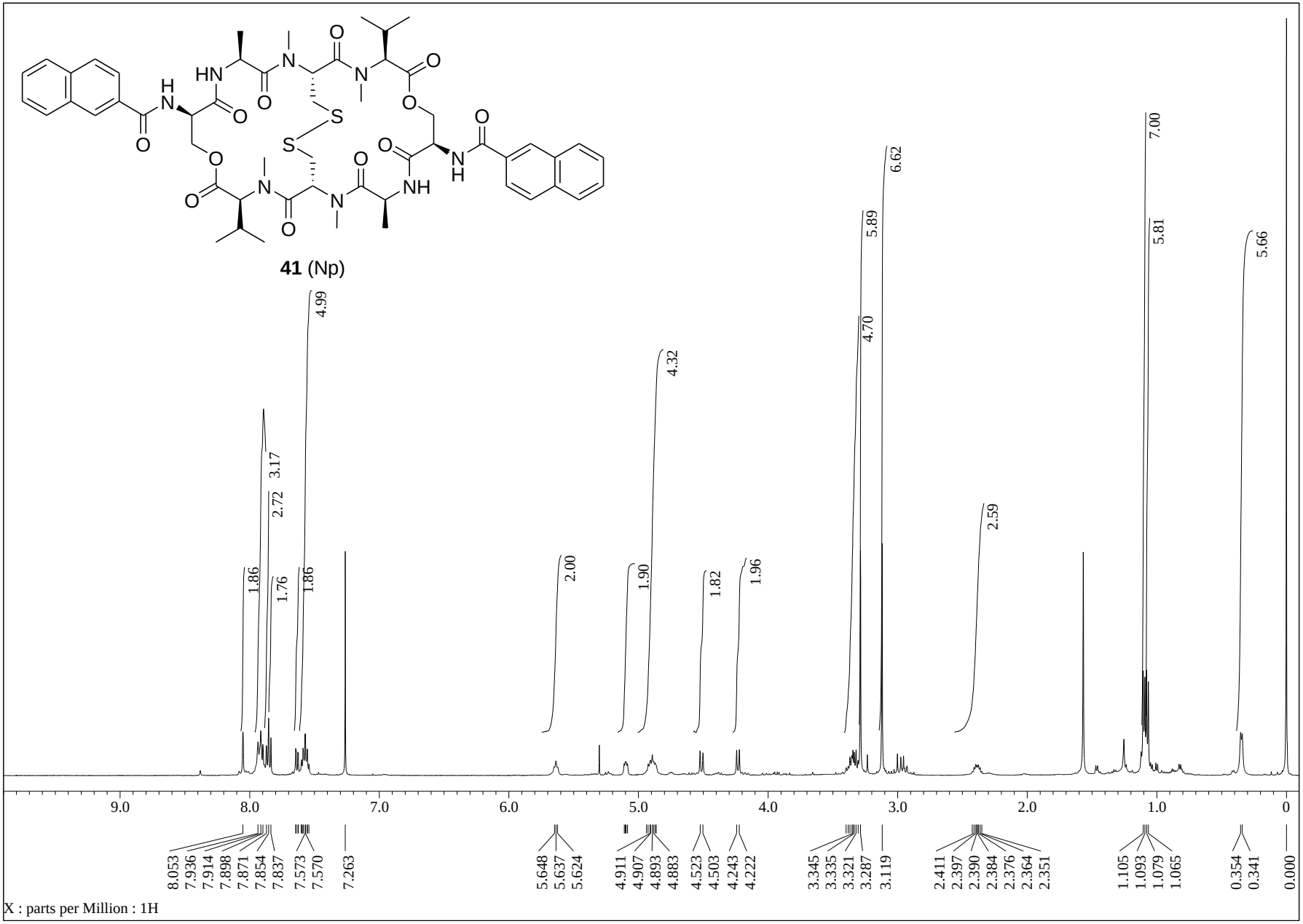
^{1}H NMR (500 MHz, $CDCl_3$)



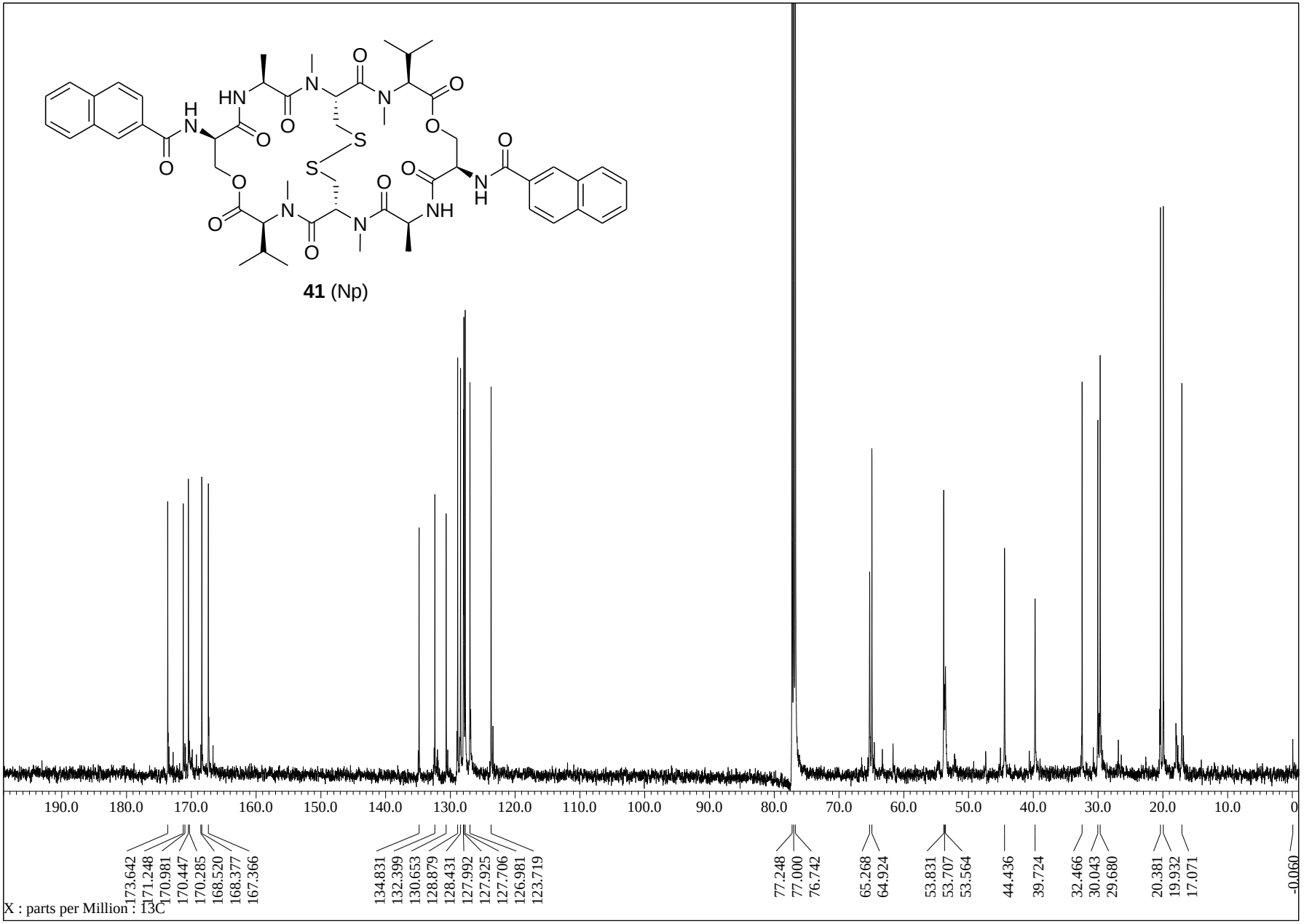
^{13}C NMR (125 MHz, $CDCl_3$)



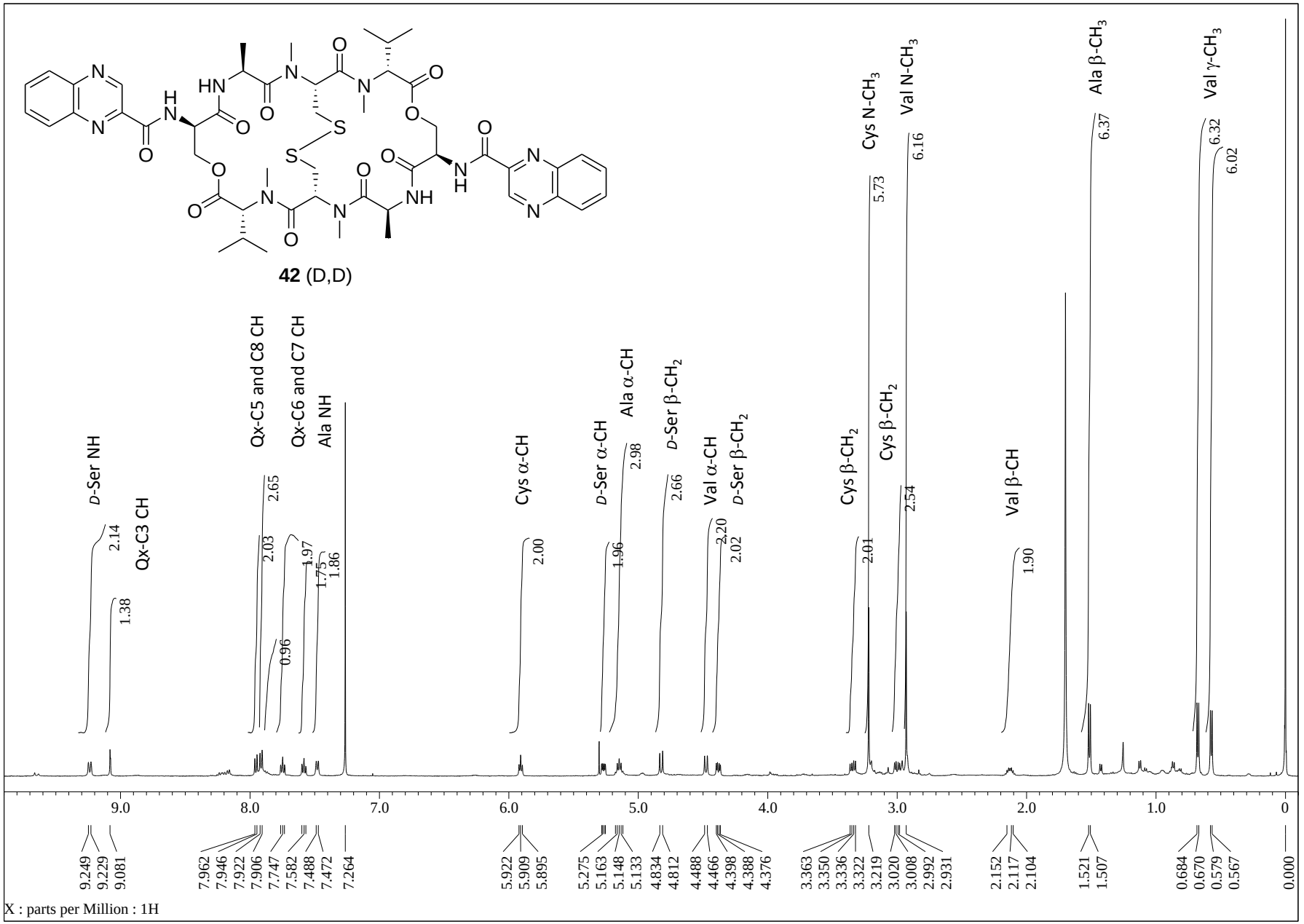
^{1}H NMR (500 MHz, $CDCl_3$)



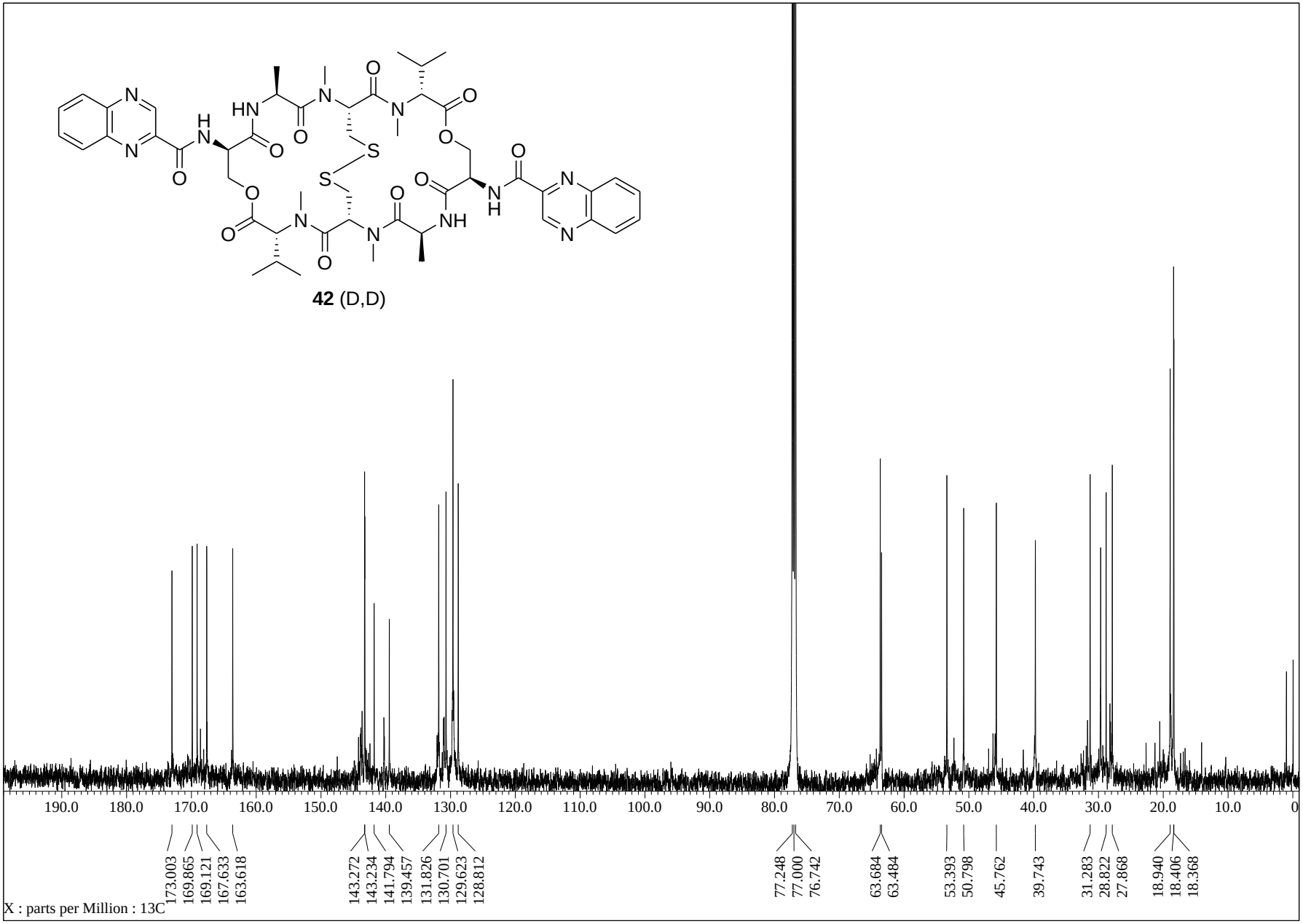
^{13}C NMR (125 MHz, $CDCl_3$)



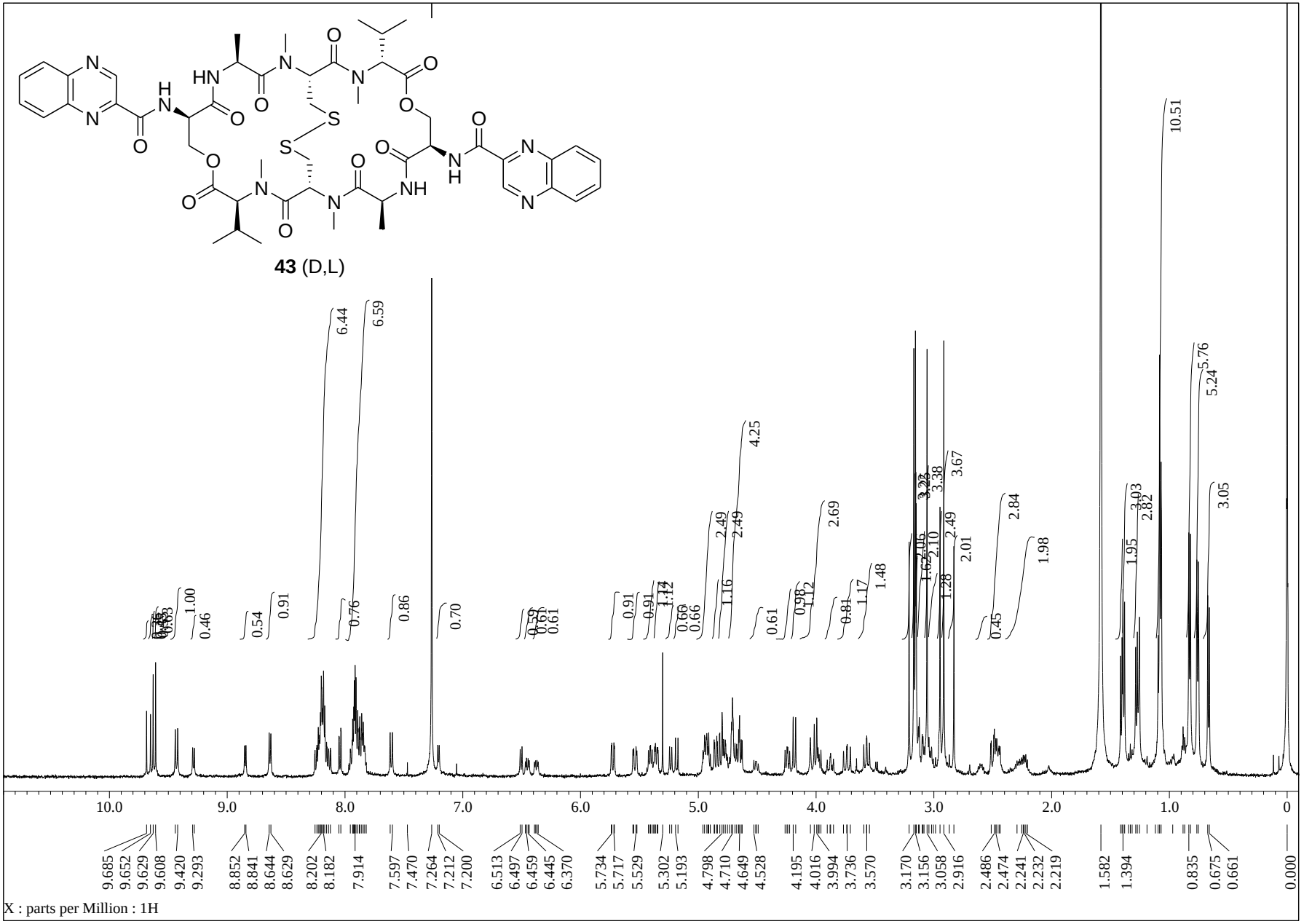
^{1}H NMR (500 MHz, $CDCl_3$)



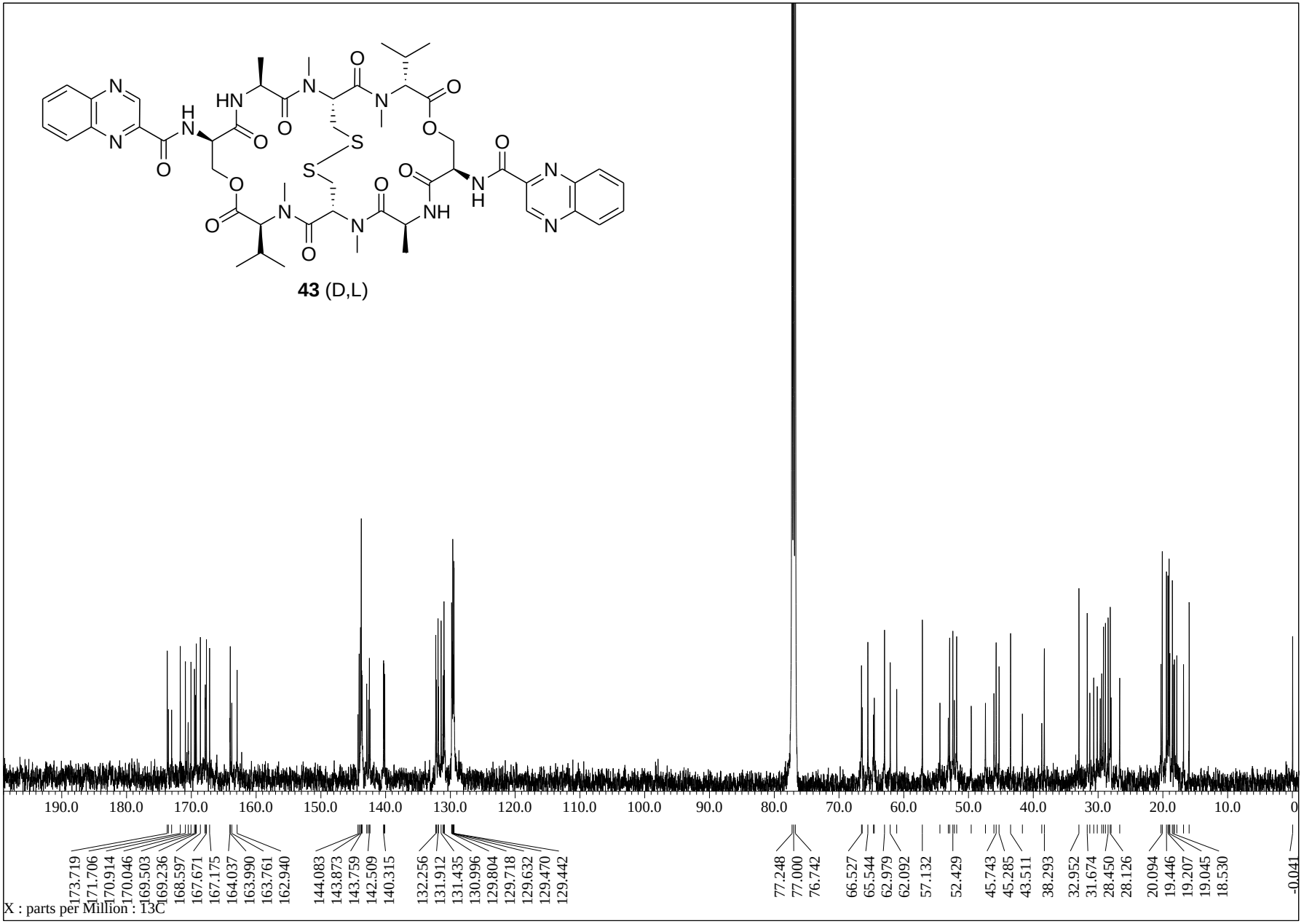
^{13}C NMR (125 MHz, $CDCl_3$)



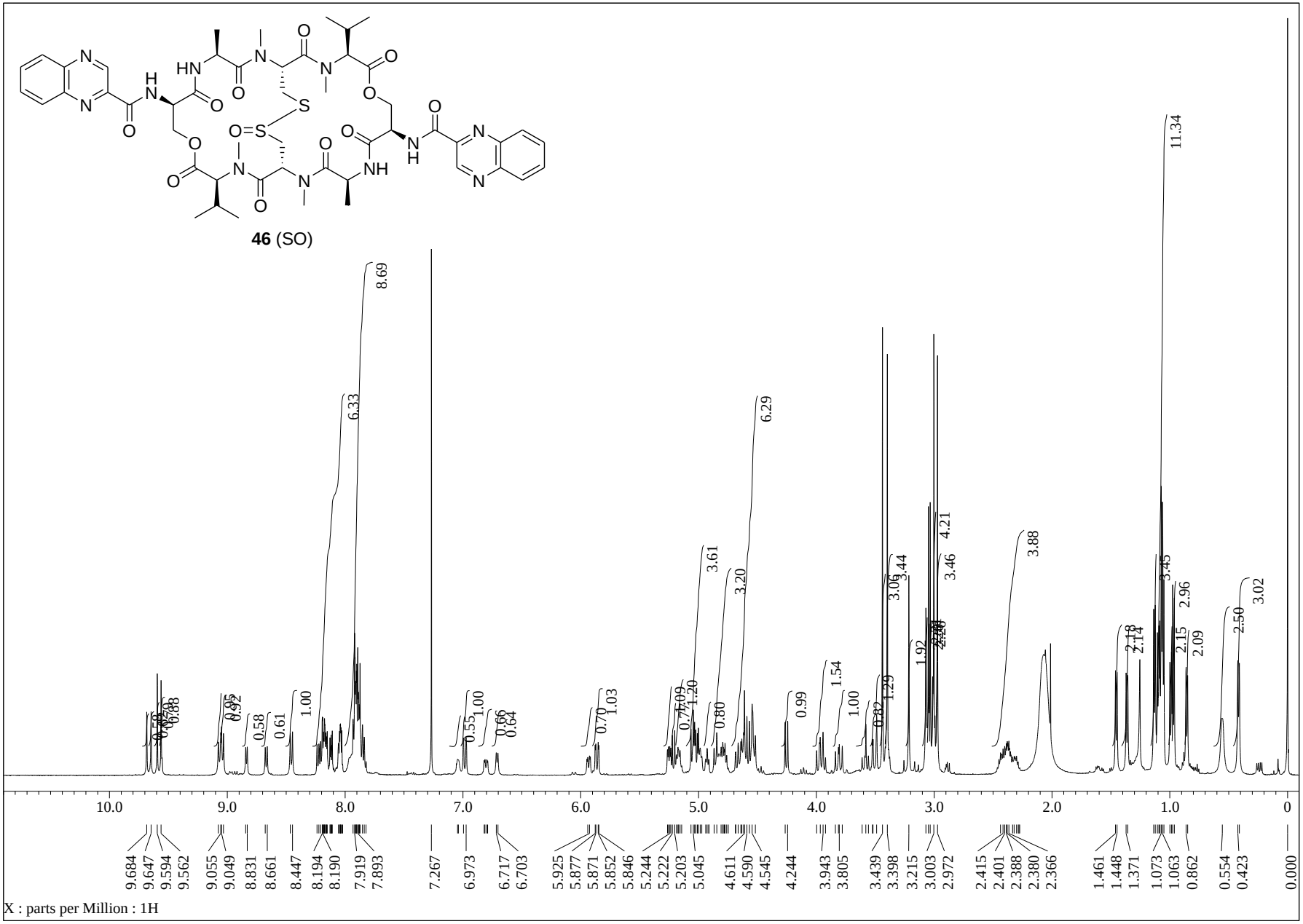
^{1}H NMR (500 MHz, $CDCl_3$)



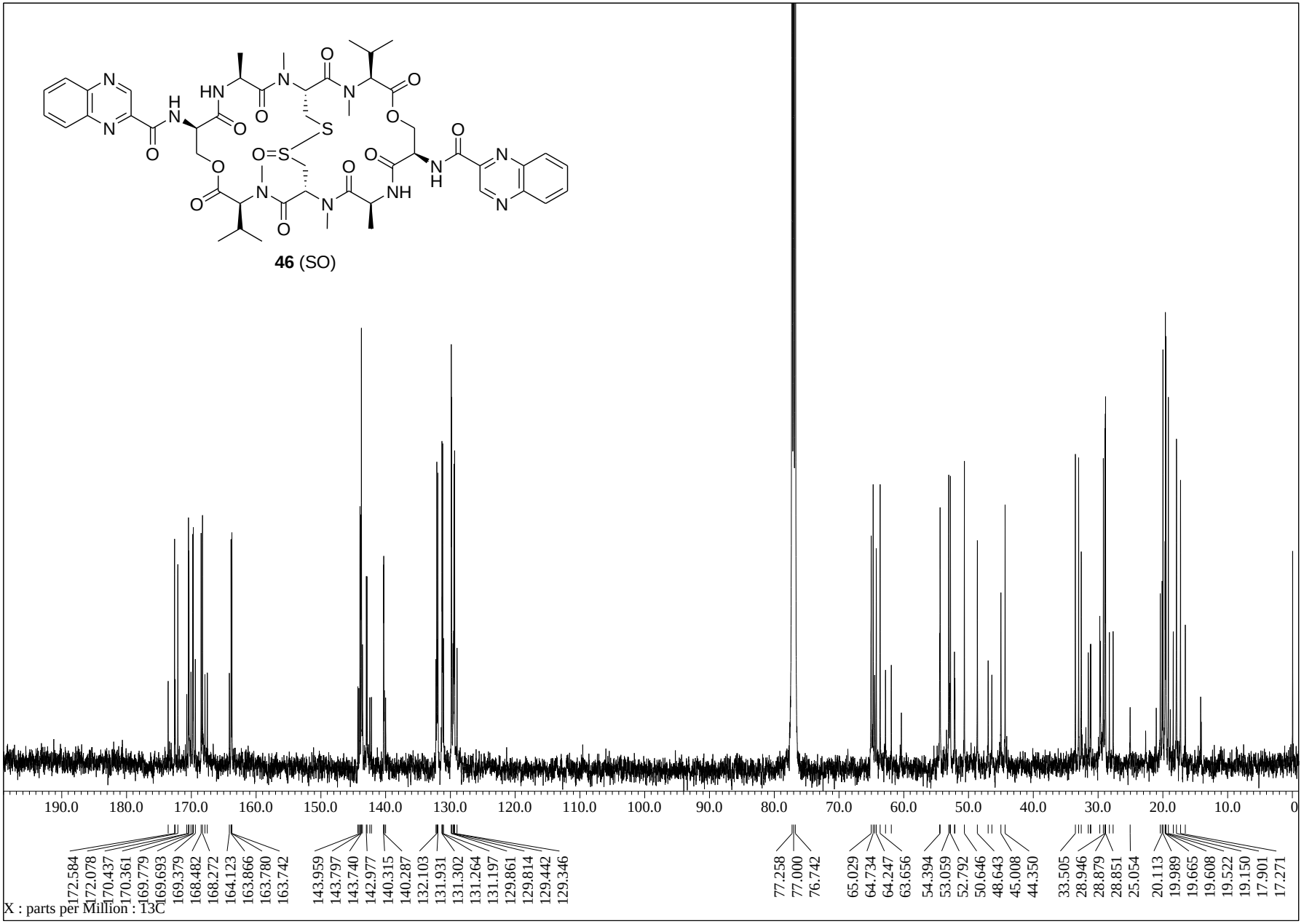
^{13}C NMR (125 MHz, $CDCl_3$)



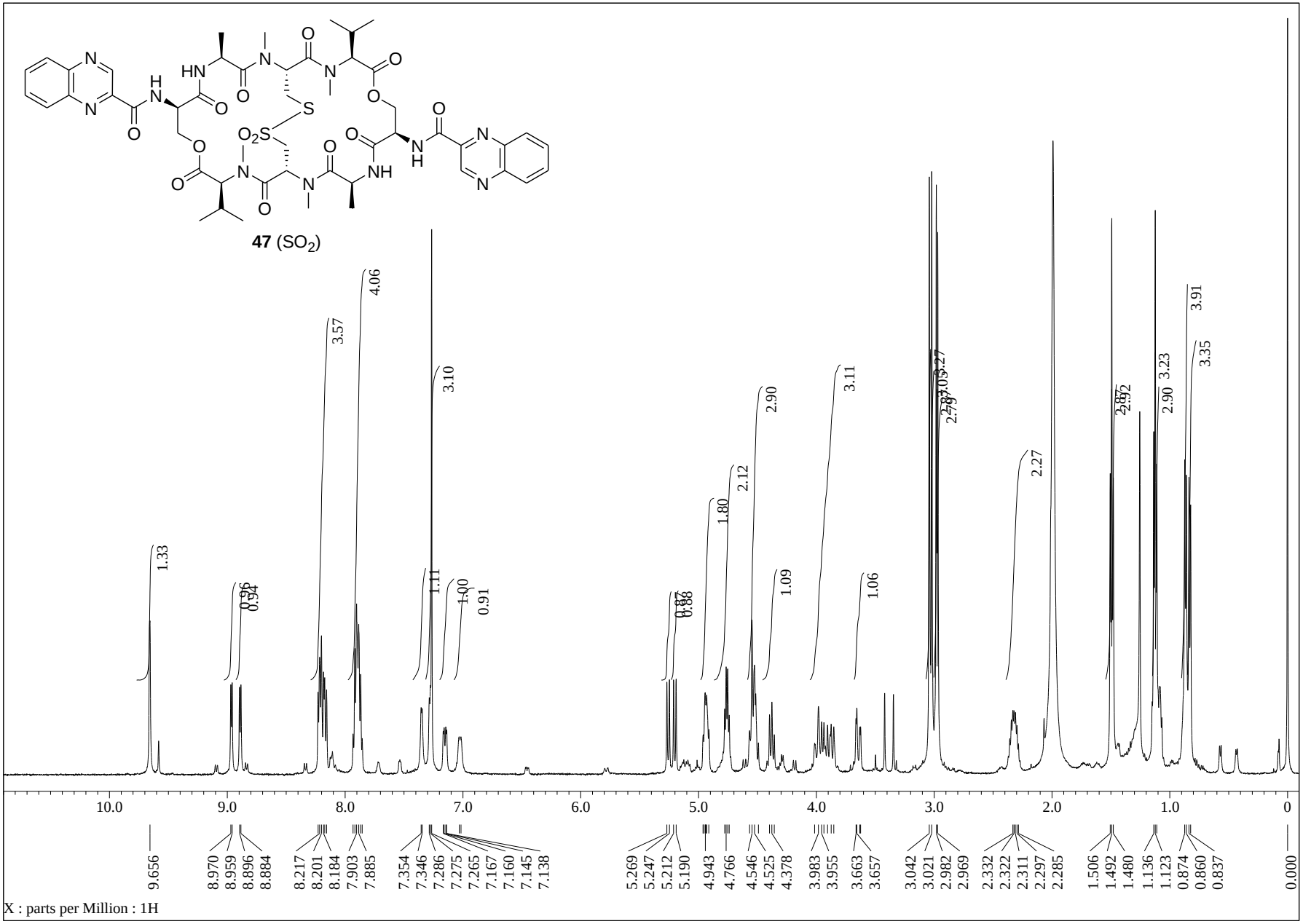
^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (125 MHz, $CDCl_3$)



^{1}H NMR (500 MHz, $CDCl_3$)



^{13}C NMR (125 MHz, $CDCl_3$)