Exploring the Potential of the β-Thiolactones in Bioorganic Chemistry

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Supplementary Information

Chemistry

General methods. Analytical thin layer chromatography (TLC) was performed using Merck TLC 60F-254 plates (0.25 mm), and visualization was accomplished with a PAA stain, which was a mixture of *p*-anisaldehyde (6.5 mL), AcOH (2.5 mL), 95% EtOH (300 mL) and H_2SO_4 (8.5 mL) or a ninhydrin stain which was a mixture of ninhydrin (0.3 g), AcOH (3.0 mL) and 95% EtOH (97.0 mL). Optical rotations were measured on a JASCO P-1010. Melting points were determined on a BÜCHI B-540. IR-spectra were recorded on a PERKIN ELMER Spectrum 100, and ^1H and ^{13}C NMR spectra were recorded on a BRUKER UltraShield 300 MHz or 500 MHz. ^1H NMR data are reported as follows: chemical shift in parts per million (ppm) downfield or upfield from $CDCl_3$ (7.26), CD_3CN (1.94), D_2O (4.79) or DMF-d_7 (8.01), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet), coupling constants (Hz) and integration. ^{13}C chemical shifts are reported in ppm downfield or upfield from $CDCl_3$ (77.16), CD_3CN (118.26), or DMF-d_7 (162.70). High resolution mass spectra were recorded on a WATERS LCT Premier XE.

General procedure A for peptide chain elongation of β-thiolactones. TFA (0.33 M) was added to a flask containing β-thiolactone (1.0 equiv.) and PTSA (1.2 equiv., dried over P_2O_5 in a desiccator) at 0 °C and stirred for 15 minutes. Then, TFA was removed under reduced pressure, and the residual TFA coevaporated with toluene. To a stirred solution of β-thiolactone amine salt in CH_2Cl_2 (0.12 M) at -20°C was added *N*-protected amino-acid (2.0 equiv.), PyBop (2.0 equiv.) followed by *i*-Pr_2NEt (3.0 equiv.). The reaction mixture was then stirred for 1 h at -20°C and then diluted with CH_2Cl_2, washed with a 1M HCl solution, saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4. Evaporation of the solvent followed by column chromatography provided the products as described below.

General procedure B for peptide chain elongation of β-thiolactones, β-lactones or β-lactams. TFA (0.33 M) was added to a flask containing β-thiolactone, β-lactone or β-lactam (1.0 equiv.) and PTSA (1.2 equiv, dried over P_2O_5 in a desiccator) at 0 °C and stirred for 15 minutes. Then, TFA was removed under reduced pressure, and the residual TFA co-evaporated with toluene. Separately, *N*-protected amino-acid (1.5 equiv.), EDCI (1.6 equiv.) and HOBt.H_2O (1.7 equiv.) were stirred in CH_2Cl_2 (0.24 M) for 20 minutes at room temperature. This mixture was cooled to -20 °C followed by the addition of *i*-Pr_2NEt (3.0 equiv.) and the resulting mixture added to a stirred solution of β-thiolactone, β-lactone or β-lactam amine salt in CH_2Cl_2 (0.24 M) at -20 °C. The reaction mixture was then stirred for 1 h

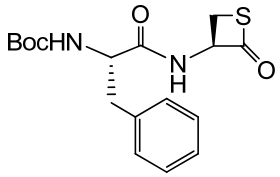
at -20 °C and then diluted with CH_2Cl_2, washed with a 1M HCl solution, saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4. Evaporation of the solvent followed by column chromatography provided the products as described below.

General procedure C for peptide chain elongation of β-thiolactones. TFA (0.33 M) was added to a flask containing β-thiolactone (1.0 equiv.) and PTSA (1.2 equiv, dried over P_2O_5 in a desiccator) at 0 °C and stirred for 15 minutes. Then, TFA was removed under reduced pressure, and the residual TFA coevaporated with toluene. Separately, pyrazine-2-carboxylic acid (4.4 equiv.), EDCI (4.4 equiv.) and HOBt.H_2O (4.4 equiv.) were stirred in a 1:1 mixture of THF/CH_2Cl_2 (0.12 M) for 20 minutes at room temperature and then solvents were removed under reduced pressure. The resulting activated ester was dissolved in CH_2Cl_2 (0.24 M) and *i*-Pr_2NEt (3.0 equiv.) was added. This mixture was cooled at -20 °C and added to a stirred solution of β-thiolactone amine salt in CH_2Cl_2 (0.24 M) at -20 °C. After 10 minutes, a 0.5 M HCl solution was added at -20 °C, diluted with CH_2Cl_2, washed with brine, and dried over Na_2SO_4. Evaporation of the solvent followed by column chromatography Evaporation of the solvent followed by column chromatography provided the products as described below.

General procedure D for peptide chain elongation of β-lactones. TFA (0.10 M) was added to a flask containing β-lactone (1.0 equiv.) and Et_3SiH (4.2 equiv.) in CH_2Cl_2 (0.10 M) at 0 °C and stirred for 30 minutes. Then, volatiles were removed under reduced pressure, and the residue co-evaporated with toluene. Separately, *N*-protected amino-acid (1.5 equiv.), EDCI (1.6 equiv.) and HOBt.H_2O (1.7 equiv.) were stirred in CH_2Cl_2 (0.24 M) for 20 minutes at room temperature. This mixture was cooled at -20 °C followed by the addition of *i*-Pr_2NEt (3.0 equiv.) and the resulting mixture added to a stirred solution of β-lactone amine salt in CH_2Cl_2 (0.24 M) at -20 °C. The reaction mixture was then stirred for 1 h at -20 °C and then diluted with CH_2Cl_2, washed with a 1M HCl solution, saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4. Evaporation of the solvent followed by column chromatography provided the products as described below.

General procedure E for peptide chain elongation of β-lactams. DBU (1.5 equiv.) was added to a flask containing β-lactam (1.0 equiv.) in CH_2Cl_2 (0.24 M) at room temperature and stirred for 15 minutes. Separately, *N*-protected amino-acid (1.5 equiv), EDCI (1.6 equiv) and HOBt.H_2O (1.7 equiv) were stirred in CH_2Cl_2 (0.1 M) for 20 minutes at room temperature. This mixture was cooled at -20 °C followed by the addition of *i*-Pr_2NEt (2.0 equiv) and the above mixture of deprotected amine contained in CH_2Cl_2 (0.1 M) at -20 °C. The reaction mixture was then stirred for 1 h at -20 °C and then diluted with CH_2Cl_2, washed with a 1M HCl solution, saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4. Evaporation of the solvent followed by column chromatography provided the products as described below.

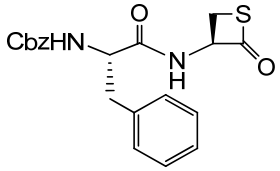
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)oxetan-2-one (5)



Following the general procedure A, using **1**[1], Boc-Phe-OH as amino acid and eluting with heptane/EtOAc mixture (90:10 to 80:20), **5** was obtained in 67% yield as a white solid. Mp 134-135 °C. $[\alpha]^{24}_D$ -28.6 (*c* 0.50, $CHCl_3$). IR (thin film): 3295, 2978, 1755, 1659, 1496, 1366, 1265, 1246, 1161 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 2H), 7.28 (d, *J* 6.5 Hz, 1H), 7.22 (d, *J* 7.5 Hz, 2H), 6.92 (br d, *J* 6.5 Hz, 1H), 5.51 (br s, 1H), 5.04 (m, 1H), 4.39 (m, 1H),

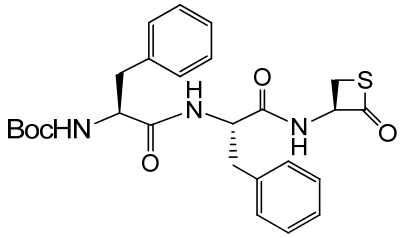
3.37 (m, 1H), 3.26 (m, 1H), 3.10 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.8, 171.6, 155.8, 136.2, 129.5 (2C), 128.9 (2C), 127.3, 80.9, 71.1, 55.6, 38.2, 28.4 (3C), 26.8. HRMS (ESI): *m/z* calcd. for $C_{17}H_{22}N_2O_4SNa$ $[M + Na]^+$ 373.1198, found 373.1193.

(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanylamino)oxetan-2-one (6)



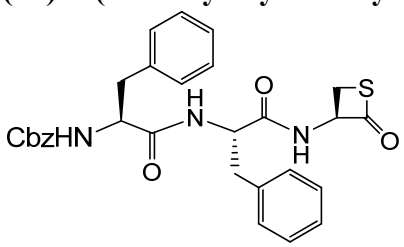
Following the general procedure A, using **1**[1], Cbz-Phe-OH as amino acid and eluting with CH_2Cl_2/EtOAc mixture (90:10 to 80:20), **6** was obtained in 65% yield as a white solid. Mp 165-166 °C. $[\alpha]^{24}_D$ -37.0 (*c* 0.50, $CHCl_3$). IR (thin film): 3300, 2927, 1753, 1688, 1664, 1529, 1258, 1243, 1042 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32-7.14 (m, 8H), 7.10 (d, *J* 7.5 Hz, 2H), 6.84 (br s, 1H), 5.36 (dd, *J* 7.5, 13.0 Hz, 1H), 5.30 (br s, 1H), 4.99 (s, 2H), 4.38 (m, 1H), 3.23 (m, 1H), 3.12 (m, 1H), 3.00 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.9, 171.2, 156.3, 136.0, 135.9, 129.5 (4C), 129.0 (2C), 128.7, 128.5, 128.1, 127.4, 71.0, 67.5, 56.0, 38.3, 26.7. HRMS (ESI): *m/z* calcd. for $C_{20}H_{20}N_2O_4SNa$ $[M + Na]^+$ 407.1041, found 407.1042.

(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (9)



Following the general procedure A, using **5**, Boc-Phe-OH as amino acid and eluting with heptane/EtOAc mixture (80:20 to 60:40), **9** was obtained in 61% yield as a white solid. Mp 148-149 °C. $[\alpha]^{24}_D$ -75.8 (*c* 0.50, $CHCl_3$). IR (thin film): 3280, 2977, 1758, 1688, 1647, 1521, 1366, 1250, 1169 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.12 (m, 7H), 7.09 (d, *J* 7.0 Hz, 2H), 6.87 (m, 2H), 6.02 (br d, *J* 5.5 Hz, 1H), 5.09 (br d, *J* 4.5 Hz, 1H), 4.68 (m, 2H), 4.10 (dd, *J* 5.5, 11.0 Hz, 1H), 3.30-3.16 (m, 2H), 2.99-2.85 (m, 2H), 2.81-2.71 (dd, *J* 5.0, 13.5 Hz, 1H), 1.18 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.7, 171.3, 171.2, 156.3, 135.9, 135.6, 129.5 (2C), 129.3 (2C), 129.1 (2C), 128.9 (2C), 127.5, 127.3, 81.3, 71.5, 56.5, 52.9, 37.4, 36.9, 28.3 (3C), 26.2. HRMS (ESI): *m/z* calcd. for $C_{26}H_{31}N_3O_5SNa$ $[M + Na]^+$ 520.1882, found 520.1894.

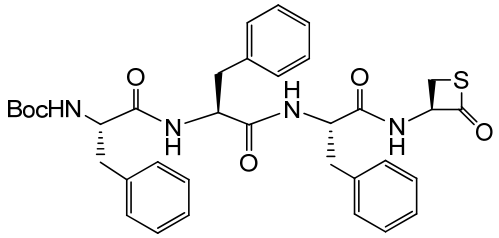
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (10)



Following the general procedure A, using **5**, Cbz-Phe-OH as amino acid and eluting with CH_2Cl_2/EtOAc mixture (75:25), **10** was obtained in 59% yield as a white solid. Mp 190-191 °C. $[\alpha]^{24}_D$ -48.5 (*c* 0.35, DMF). IR (thin film): 3282, 2924, 1764, 1693, 1649, 1530, 1260, 1029 cm^{-1}. ^{1}H NMR (500 MHz, DMF-d_7) δ 8.87 (br d, *J* 7.5 Hz, 1H), 8.23 (br d, *J* 7.5 Hz, 1H), 7.40-7.15 (m, 16H), 5.73 (m, 1H), 5.02 (d, *J* 13.0 Hz, 1H), 4.99 (d, *J* 13.5 Hz, 1H), 4.70

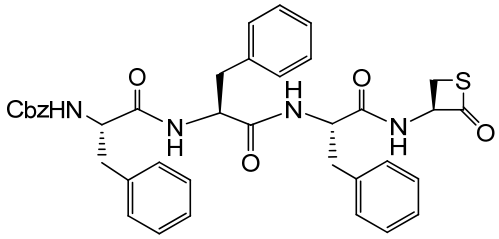
(m, 1H), 4.43 (m, 1H), 3.40 (m, 1H), 3.33 (m, 1H), 3.18-3.06 (m, 2H), 3.00 (dd, *J* 8.0, 13.5 Hz, 1H), 3.33 (dd, *J* 11.0, 13.5 Hz, 1H). ^{13}C NMR (125 MHz, DMF-d_7) δ 193.6, 172.1, 171.9, 156.9, 138.9, 138.1, 138.0, 130.2 (2C), 130.0 (2C), 129.0, 128.8 (3C), 128.8 (3C), 128.3, 128.1, 127.1, 127.0, 72.4, 66.3, 57.4, 54.8, 38.4, 38.3, 26.5. HRMS (ESI): *m/z* calcd. for $C_{29}H_{29}N_3O_5SNa$ $[M + Na]^+$ 554.1726, found 554.1737.

(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (16)



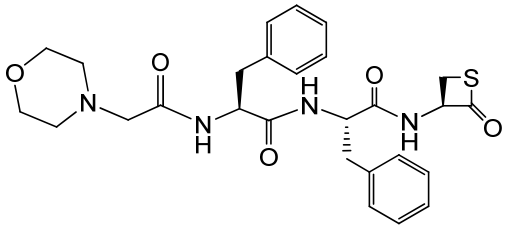
Following the general procedure A, using **9**, Boc-Phe-OH as amino acid and eluting with heptane/EtOAc mixture (80:20 to 60:40), **16** was obtained in 62% yield as a white solid. Mp 219-220 °C. $[\alpha]^{24}_D$ -43.1 (*c* 0.50, DMF). IR (thin film): 3276, 2961, 1730, 1691, 1643, 1519, 1246, 1168 cm^{-1}. ^{1}H NMR (300 MHz, CD_3CN) δ 7.42-7.05 (m, 16H), 6.95 (m, 2H), 5.47 (m, 2H), 4.54 (m, 1H), 4.39 (m, 1H), 4.18 (m, 1H), 3.41-3.22 (m, 2H), 3.22-3.08 (dd, *J* 4.5, 14.0 Hz, 1H), 3.04-2.79 (m, 1H), 2.71 (m, 1H), 1.31 (s, 9H). ^{13}C NMR (125 MHz, CD_3CN) δ 193.8, 173.4, 171.9, 171.6, 156.7, 138.4, 138.1, 137.9, 130.3 (4C), 130.2 (2C), 129.4 (2C), 129.4 (2C), 129.4 (2C), 127.7, 127.7, 127.6, 80.4, 72.2, 57.1, 56.0, 55.0, 38.2, 37.9, 37.9, 28.5 (3C), 26.7. HRMS (ESI): *m/z* calcd. for $C_{35}H_{40}N_4O_6SNa$ $[M + Na]^+$ 667.2566, found 667.2588.

(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (17)



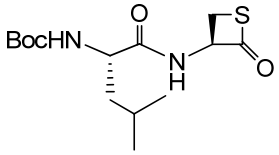
Following the general procedure A, using **9**, Cbz-Phe-OH as amino acid and eluting with $CHCl_3$/MeOH mixture (98:2), **17** was obtained in 63% yield as a white solid. Mp 234-235 °C. $[\alpha]^{26}_D$ -38.1 (*c* 0.20, DMF). IR (thin film): 3278, 2955, 1771, 1695, 1642, 1533, 1258, 1226, 1044 cm^{-1}. ^{1}H NMR (500 MHz, DMF-d_7) δ 8.79 (br d, *J* 7.5 Hz, 1H), 8.26 (br d, *J* 8.0 Hz, 1H), 8.16 (br d, *J* 7.0 Hz, 1H), 7.41-7.11 (m, 21H), 5.07 (m, 1H), 5.00 (d, *J* 12.5 Hz, 1H), 4.95 (d, *J* 12.5 Hz, 1H), 4.66 (m, 1H), 4.41 (m, 1H), 3.47-3.40 (m, 1H), 3.34 (m, 1H), 3.17-3.05 (m, 3H), 3.04-2.93 (m, 2H), 2.82 (m, 1H). ^{13}C NMR (125 MHz, DMF-d_7) δ 193.5, 172.5, 171.9, 171.6, 156.9, 139.0, 138.3, 138.2, 138.0, 130.2 (2C), 130.1 (2C), 130.0 (2C), 129.0, 128.9 (2C), 128.8 (5C), 128.3, 128.1, 127.1, 126.9 (3C), 72.4, 66.2, 57.3, 55.2, 54.9, 38.4, 38.3, 38.2, 26.4. HRMS (ESI): *m/z* calcd. for $C_{38}H_{38}N_4O_6SNa$ $[M + Na]^+$ 701.2410, found 701.2416.

(3*S*)-3-(4-morpholinylacetyl-L-phenylalanyl- L-phenylalanylamino)thietan-2-one (26)



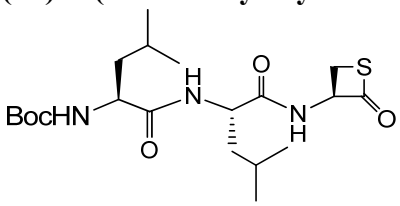
TFA (125 μL) was added to a flask containing **9** (20 mg, 40.2 μmol) and PTSA (8.3 mg, 48.2 μmol) at 0 °C and stirred for 15 minutes. Then, TFA was removed under reduced pressure, and the residual TFA coevaporated with toluene. To a mixture of 2-morpholinoacetic acid (11.7 mg, 40.2 μmol) and DMF (14.4 μL) in CH_2Cl_2 (0.4 mL) was added oxalyl chloride (6.9 μl, 0.1 mL). The mixture was stirred at room temperature for 0.5 h, followed by the addition of pyridine (8 μl, 101 μmol) and β-thiolactone amine salt in CH_2Cl_2 (0.3 mL) at -20°C. The mixture was stirred for 1 h at -20°C, and then diluted with CH_2Cl_2 (5 mL), washed H_2O (3 mL), saturated aqueous $NaHCO_3$ (3 mL) and brine (3 mL), dried over Na_2SO_4. Evaporation of the solvent followed by column chromatography, eluting with $CHCl_3$/MeOH mixture (95:5), provided **26** in 47% yield. For stability issue, this compound was readily transformed in 2,2,2-trifluoroacetate salt by dilution with a $CHCl_3$/TFA (95:5) mixture and evaporation to give a white solid. Mp 101-102 °C. IR (thin film): 3283, 3031, 2924, 2855, 1761, 1660, 1518, 1454, 1201, 1177, 1115 cm^{-1}. $[\alpha]^{26}_D$ -18.6 (*c* 0.40, $CHCl_3$). ^{1}H NMR (300 MHz, DMF-d_7) δ 8.94 (br d, *J* 8.1 Hz, 1H), 8.31 (br d, *J* 8.4 Hz, 1H), 7.76 (br d, *J* 7.8 Hz, 1H), 7.33-7.16 (m, 10H), 5.71 (m, 1H), 4.77-4.61(m, 2H), 6.02 (br d, *J* 5.5 Hz, 1H), 4.68 (m, 2H), 4.10 (dd, *J* 5.5, 11.0 Hz, 1H), 3.30-3.16 (m, 2H), 3.49 (m, 4H), 3.35 (m, 1H), 3.19-3.06 (m, 2H), 3.03-2.92 (m, 3H), 2.80-2.74 (m, 1H), 2.33-2.13 (m, 4H). ^{13}C NMR (2,2,2-trifluoroacetate salt, 125 MHz, DMF-d_7) δ 193.7, 172.0 (2C), 171.3, 138.4, 138.2, 130.1 (2C), 130.1 (2C), 128.3 (4C), 127.2, 127.1, 72.4, 65.2 (2C), 59.4 (2C), 55.0, 54.9, 53.3, 38.5, 38.4, 26.4. HRMS (ESI): *m/z* calcd. for $C_{27}H_{33}N_4O_5S$ $[M + H]^+$ 525.2172, found 525.2162.

(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-leucylamino)thietan-2-one (7)



Following the general procedure A, using **1**[1], Boc-Leu-OH as amino acid and eluting with heptane/EtOAc mixture (90:10 to 80:20), **7** was obtained in 53% yield as colorless oil. $[\alpha]^{26}_D$ -72.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (br s, 1H), 5.62 (m, 1H), 4.90 (br s, 1H), 4.12 (m, 1H), 3.41 (t, *J* 8.0 Hz, 1H), 3.27 (dd, *J* 5.0, 8.0 Hz, 1H), 1.72-1.62 (m, 2H), 1.49 (m, 1H), 1.45 (s, 9H), 0.94 (dd, *J* 6.5 Hz, 1H), 0.92 (dd, *J* 6.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.6, 173.1, 156.3, 80.8, 71.1, 52.8, 41.1, 28.4 (3C), 26.9, 24.8, 23.1, 22.0. HRMS (ESI): *m/z* calcd. for $C_{14}H_{24}N_2O_4SNa$ $[M + Na]^+$ 339.1354, found 339.1351.

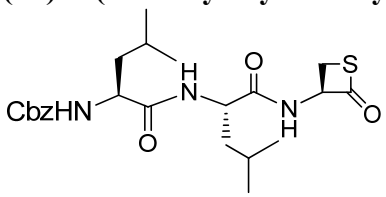
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-leucyl-L-leucylamino)thietan-2-one (11)



Following the general procedure A, using **7**, Boc-Leu-OH as amino acid and eluting with heptane/EtOAc mixture (80:20 to 60:40), **11** was obtained in 56% yield as a white solid. Mp

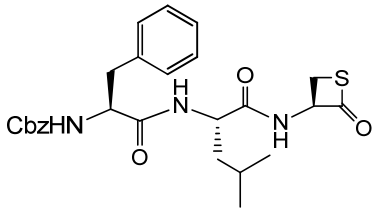
153-154 °C. $[\alpha]^{26}_{D}$ -81.2 (*c* 0.20, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (br s, 1H), 6.39 (br d, *J* 7.0 Hz, 1H), 5.39 (m, 1H), 4.85 (br s, 1H), 4.48 (m, 1H), 4.01 (m, 1H), 3.39 (m, 1H), 3.35 (m, 1H), 1.80 (m, 1H), 1.75-1.59 (m, 3H), 1.58-1.50 (m, 1H), 1.49-1.45 (m, 1H), 1.45 (s, 9H), 0.97 (d, *J* 6.0 Hz, 3H), 0.95-0.92 (m, 6H), 0.89 (d, *J* 6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.4, 173.2, 172.2, 156.3, 80.9, 71.3, 54.0, 51.5, 40.8, 40.3, 28.4 (3C), 26.7, 25.0, 24.9, 23.1 (2C), 22.0. HRMS (ESI): *m/z* calcd. for $C_{20}H_{35}N_3O_5SNa$ $[M + Na]^+$ 452.2195, found 452.2201.

(3*S*)-3-(*N*-benzyloxycarbonyl-L-leucyl-L-leucylamino)thietan-2-one (12)



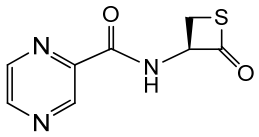
Following the general procedure A, using **7**, Cbz-Leu-OH as amino acid and eluting with heptane/EtOAc mixture (80:20 to 60:40), **12** was obtained in 35% yield as a white solid. Mp 140-141 °C. $[\alpha]^{26}_{D}$ -69.5 (*c* 0.50, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (br s, 1H), 7.40-7.28 (m, 5H), 6.64 (br d, *J* 5.5 Hz, 1H), 5.49 (m, 1H), 5.38 (br d, *J* 5.5 Hz, 1H), 5.09 (s, 2H), 4.47 (m, 1H), 4.15 (m, 1H), 3.36-3.20 (m, 2H), 1.80-1.45 (m, 6H), 0.93 (d, *J* 6.5 Hz, 3H), 0.92 (d, *J* 6.5 Hz, 3H), 0.90 (d, *J* 6.5 Hz, 3H), 0.87 (d, *J* 6.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.5, 172.8, 171.9, 156.8, 136.0, 128.8 (3C), 128.6, 128.2, 71.3, 67.6, 54.3, 51.6, 41.0, 40.1, 26.8, 25.0, 24.9, 23.0, 23.0, 22.0. HRMS (ESI): *m/z* calcd. for $C_{23}H_{33}N_3O_5SNa$ $[M + Na]^+$ 486.2039, found 486.2030.

(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-leucylamino)thietan-2-one (13)



Following the general procedure A, using **7**, Cbz-Phe-OH as amino acid and eluting with CH_2Cl_2/EtOAc mixture (80:20 to 60:40), **13** was obtained in 52% yield as a white solid. Mp 135-136 °C. $[\alpha]^{26}_{D}$ -14.1 (*c* 0.20, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41-7.23 (m, 8H), 7.21-7.12 (m, 3H), 6.05 (br d, *J* 8.0 Hz, 1H), 5.34 (m, 1H), 5.15 (br s, 1H), 5.11 (d, *J* 12.0 Hz, 1H), 5.06 (d, *J* 12.0 Hz, 1H), 4.43 (m, 1H), 4.36 (m, 1H), 3.32 (m, 2H), 3.18-3.03 (m, 2H), 1.75 (m, 1H), 1.51-1.35 (m, 2H), 0.88 (d, *J* 6.0 Hz, 3H), 0.87 (d, *J* 6.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.2, 171.8, 171.4, 156.8, 135.7 (2C), 129.3 (3C), 129.2 (3C), 128.9, 128.8, 128.4, 127.7, 71.3, 67.9, 56.9, 51.6, 39.8, 37.6, 26.6, 24.8, 23.1, 21.6. HRMS (ESI): *m/z* calcd. for $C_{26}H_{31}N_3O_5SNa$ $[M + Na]^+$ 520.1882, found 520.1878.

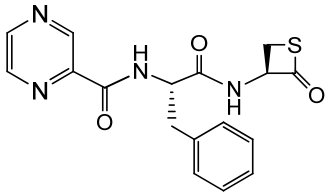
(3*S*)-3-(pyrazin-2-carbonylamino)thietan-2-one (21)



Following the general procedure C, using **1**[1], and eluting with EtOAc/heptanes mixture (60:40), **20** was obtained in 76% yield as a white solid. Mp 166-167 °C. $[\alpha]^{24}_{D}$ -64.7 (*c* 0.20, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.39 (s, 1H), 8.82 (s, 1H), 8.54 (s, 1H), 8.46 (br d, *J* 6.0 Hz, 1H), 5.04 (ddd, *J* 5.5, 8.0, 13.0 Hz, 1H), 3.55 (m, 1H), 3.44 (m, 1H). ^{13}C NMR (125

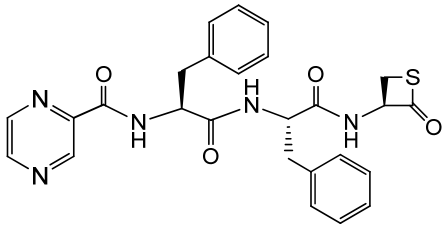
MHz, $CDCl_3$) δ 191.9, 162.8, 148.2, 144.8, 143.3, 142.8, 70.8, 27.2. HRMS (ESI): *m/z* calcd. for $C_8H_7N_3O_2SNa$ $[M + Na]^+$ 232.0157, found 232.0147.

(3*S*)-3-(pyrazin-2-carbonyl-L-phenylalanylamino)thietan-2-one (**24**)



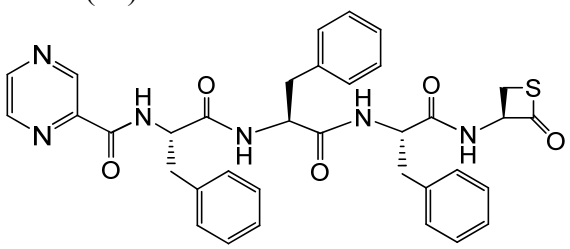
Following the general procedure A at -30°C, using **1**[1], pyrazin-2-carbonyl-L-phenylalanine[2] and eluting with EtOAc/heptanes mixture (80:20), **24** was obtained in 67% yield as a white solid. Mp 151-152 °C. $[\alpha]^{24}_D$ -21.8 (*c* 0.60, $CHCl_3$). ^{1}H NMR (300 MHz, CD_3CN) δ 9.21 (s, 1H), 8.77 (s, 1H), 8.59 (s, 1H), 8.23 (br s, 1H), 7.46 (br s, 1H), 7.32-7.15 (m, 5H), 4.48 (m, 1H), 4.82 (m, 1H), 3.42-3.20 (m, 3H), 3.12 (dd, *J* 7.5, 13.5 Hz, 1H). ^{13}C NMR (125 MHz, CD_3CN) δ 193.8, 171.7, 163.8, 148.9, 145.0, 144.7, 144.2, 137.7, 130.4 (2C), 129.4 (2C), 127.9, 72.0, 54.8, 38.4, 26.7. HRMS (ESI): *m/z* calcd. for $C_8H_7N_3O_2SNa$ $[M + Na]^+$ 232.0157, found 232.0147.

(3*S*)-3-(pyrazin-2-carbonyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (**22**)



Following the general procedure C, using **9**, and eluting with EtOAc **24** was obtained in 75% yield as a white solid. Mp 147-148 °C. $[\alpha]^{24}_D$ -43.1 (*c* 0.30, $CHCl_3$). IR (thin film): 3292, 2923, 1769, 1645, 1518, 1020 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.23 (s, 1H), 8.81 (s, 1H), 8.55 (s, 1H), 8.17 (br d, *J* 5.5 Hz, 1H), 7.41-7.19 (m, 5H), 7.10-6.87 (m, 6H), 6.26 (br d, *J* 7.0 Hz, 1H), 5.39 (m, 1H), 4.67 (m, 2H), 3.38-3.26 (m, 2H), 3.25-3.09 (m, 3H), 2.98 (dd, *J* 6.0, 14.0 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.9, 170.7, 170.3, 163.9, 148.2, 144.5, 143.3, 142.9, 135.9, 135.7, 129.4 (2C), 129.3 (2C), 129.2 (2C), 128.7 (2C), 127.6, 127.0, 71.2, 55.3, 53.6, 37.4, 36.8, 26.5. HRMS (ESI): *m/z* calcd. for $C_{26}H_{25}N_5O_4SNa$ $[M + Na]^+$ 526.1525, found 526.1528.

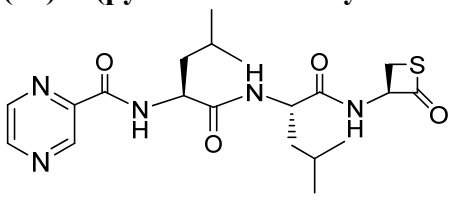
(3*S*)-3-(pyrazin-2-carbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (**25**)



Following the general procedure A at -30°C, using **9**, pyrazin-2-carbonyl-L-phenylalanine[2] and eluting with $CHCl_3$/MeOH mixture (98:2), **25** was obtained in 38% yield as a white solid. Mp 215-216 °C. $[\alpha]^{26}_D$ -18.8 (*c* 0.20, CH_3CN). ^{1}H NMR (500 MHz, CD_3CN) δ 9.15 (s, 1H), 8.79 (s, 1H), 8.58 (s, 1H), 8.17 (br d, *J* 6.0 Hz, 1H), 7.39 (br d, *J* 7.5 Hz, 1H), 7.32-7.03 (m, 13H), 7.02-6.94 (m, 2H), 5.48 (m, 1H), 4.68 (m, 1H), 4.56 (m, 1H), 4.42 (m, 1H), 3.34 (m, 1H), 3.28 (m, 1H), 3.22-3.09 (m, 2H), 3.04-2.91 (m, 3H), 2.80 (dd, *J* 9.0, 14.5 Hz, 1H). ^{13}C

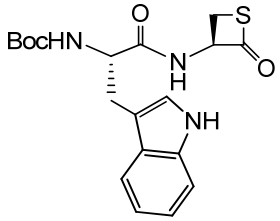
NMR (125 MHz, CD_3CN) δ 193.8, 172.3, 171.9, 171.6, 164.4, 149.0, 144.8, 144.7, 144.2, 138.2, 137.8, 137.8, 130.3 (2C), 130.3 (2C), 130.1 (2C), 129.5 (2C), 129.4 (2C), 129.3 (2C), 127.8, 127.7, 127.6, 72.2, 56.0, 55.7, 55.1, 38.0, 37.9, 37.7, 26.7. HRMS (ESI): *m/z* calcd. for $C_{35}H_{34}N_6O_5SNa$ $[M + Na]^+$ 673.2209, found 673.2192.

(3*S*)-3-(pyrazin-2-carbonyl-L-leucyl-L-leucylamino)thietan-2-one (23)



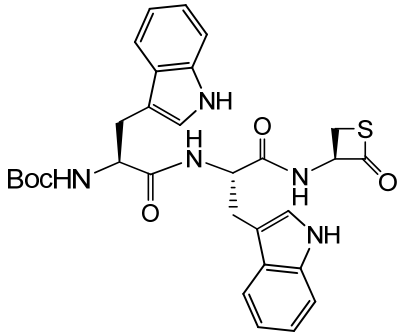
Following the general procedure C, using **11**, and eluting with EtOAc, **23** was obtained in 32% yield as a white solid. Mp 99-101 °C. $[\alpha]^{26}_D$ -46.1 (*c* 0.20, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.80 (s, 1H), 8.63 (s, 1H), 8.24 (br d, *J* 6.0 Hz, 1H), 7.53 (br d, *J* 7.0 Hz, 1H), 6.96 (br d, *J* 7.5 Hz, 1H), 5.55 (ddd, *J* 5.0, 8.0, 13.0 Hz, 1H), 4.50 (m, 1H), 4.32 (m, 1H), 3.38 (m, 1H), 3.30 (m, 1H), 1.79-1.65 (m, 3H), 1.64-1.50 (m, 3H), 1.00-0.92 (m, 6H), 0.89 (d, *J* 6.0 Hz, 3H), 0.85 (d, *J* 5.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 194.0, 173.1, 172.8, 164.7, 148.9, 145.2, 144.8, 144.2, 72.2, 53.4, 52.4, 41.7, 40.9, 26.9, 25.7, 25.5, 23.3, 22.0, 21.6. HRMS (ESI): *m/z* calcd. for $C_{20}H_{29}N_5O_4SNa$ $[M + Na]^+$ 458.1838, found 458.1844.

(3*S*)-3-(*N*- *tert*-butyloxycarbonyl-L-tryptophanylamino)thietan-2-one (8)



Following the general procedure B, using **1**[1], Boc-Trp-OH as amino acid and eluting with heptane/EtOAc mixture (30:70), **8** was obtained in 62% yield as a white solid. Mp 159-160 °C. $[\alpha]^{24}_D$ -32.3 (*c* 0.50, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (br s, 1H), 7.62 (br d, *J* 7.0 Hz, 1H), 7.36 (d, *J* 8.0 Hz, 1H), 7.21 (t, *J* 7.5 Hz, 1H), 7.13 (t, *J* 7.5 Hz, 1H), 7.07 (br s, 1H), 6.72 (br d, *J* 8.0 Hz, 1H), 5.44 (m, 1H), 5.15 (m, 1H), 4.48 (m, 1H), 3.40-3.04 (m, 4H), 1.42 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.3, 172.0, 155.7, 136.4, 127.5, 123.9, 122.5, 120.0, 118.8, 111.5, 109.9, 80.7, 70.9, 55.2, 28.4 (3C), 26.8, 28.1, 26.8. HRMS (ESI): *m/z* calcd. for $C_{19}H_{23}N_3O_4SNa$ $[M + Na]^+$ 412.1307, found 421.1318.

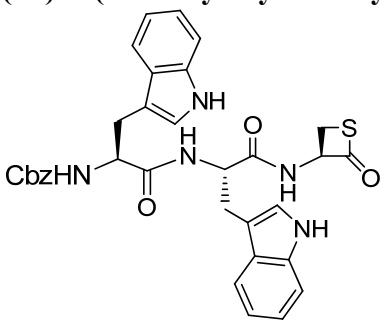
(3*S*)-3-(*N*- *tert*-butyloxycarbonyl-L-tryptophanyl-L-tryptophanylamino)thietan-2-one (14)



Following the general procedure B, using **8**, Boc-Trp-OH as amino acid and eluting with CH_2Cl_2/EtOAc mixture (20:80), **14** was obtained in 65% yield as a white solid. Mp 175-176

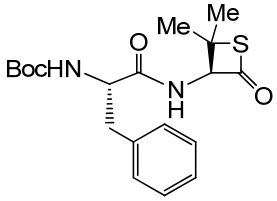
°C. $[\alpha]^{24}_D$ -75.2 (*c* 0.30, $CHCl_3$). ^{1}H NMR (500 MHz, CD_3CN) δ 9.25 (br s, 1H), 9.16 (br s, 1H), 7.53 (d, *J* 8.0 Hz, 1H), 7.44 (d, *J* 8.0 Hz, 1H), 7.35 (d, *J* 8.0 Hz, 2H), 7.17 (t, *J* 8.0 Hz, 1H), 7.13-7.08 (m, 3H), 6.97-6.85 (m, 3H), 6.56 (br s, 1H), 5.38 (m, 1H), 5.23 (br s, 1H), 4.51 (m, 1H), 4.11 (m, 1H), 3.24 (t, *J* 8.0 Hz, 1H), 3.18-3.00 (m, 4H), 2.79-2.68 (m, 1H), 1.16 (s, 9H). ^{13}C NMR (125 MHz, CD_3CN) δ 193.7, 172.7, 172.6, 157.2, 137.6, 137.3, 128.5, 128.4, 125.0, 124.9, 122.8, 122.6, 120.2, 120.1, 119.6, 119.0, 112.5, 112.4, 110.5, 109.7, 80.7, 72.2, 56.7, 54.4, 28.3 (3C), 27.8, 27.1, 26.7. HRMS (ESI): *m/z* calcd. for $C_{30}H_{34}N_5O_5S$ $[M + H]^+$ 576.2281, found 576.2305.

(3*S*)-3-(*N*-benzyloxycarbonyl-L-tryptophanyl-L-tryptophanylamino)thietan-2-one (15)



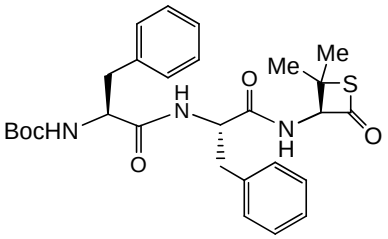
Following the general procedure B, using **8**, Cbz-Trp-OH as amino acid and eluting with $CHCl_3$/MeOH mixture (95:5), **15** was obtained in 64% yield as a white solid. Mp 189-190 °C. $[\alpha]^{24}_D$ -69.2 (*c* 0.20, $CHCl_3$). IR (thin film): 3302, 2925, 1748, 1649, 1503, 1519, 1262, 1229 cm^{-1}. ^{1}H NMR (500 MHz, CD_3CN) δ 9.13 (br s, 2H), 7.51 (d, *J* 8.0 Hz, 1H), 7.41 (d, *J* 8.5 Hz, 1H), 7.38-7.27 (m, 5H), 7.23 (m, *J* 7.0 Hz, 1H), 7.17-7.08 (m, 2H), 7.07-6.94 (m, 4H), 6.81 (br d, *J* 7.5 Hz, 1H), 5.72 (br d, *J* 5.05 Hz, 1H), 5.40 (m, 1H), 4.97 (d, *J* 12.5 Hz, 1H), 4.86 (d, *J* 12.5 Hz, 1H), 4.55 (m, 1H), 4.27 (m, 1H), 3.22 (t, *J* 8.0 Hz, 1H), 3.16-2.97 (m, 5H),. ^{13}C NMR (125 MHz, CD_3CN) δ 194.0, 172.4, 172.4, 157.5, 137.8, 137.4, 137.3, 129.4 (2C), 128.9, 128.6 (2C), 128.4 (2C), 124.9, 124.8, 122.6, 122.5, 120.1, 120.0, 119.4, 119.3, 112.4, 112.3, 110.6, 110.4, 72.1, 67.3, 57.0, 54.5, 28.1, 27.7, 26.7. HRMS (ESI): *m/z* calcd. for $C_{33}H_{31}N_5O_5SNa$ $[M + Na]^+$ 632.1944, found 632.1963.

(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)-4,4-dimethyl-thietan-2-one (18)



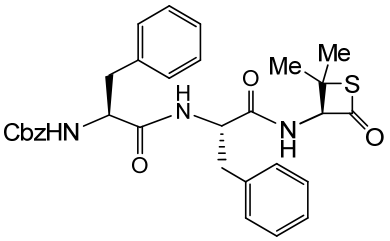
Following the general procedure B, using **2**[3], Boc-Phe-OH as amino acid and eluting with heptane/EtOAc mixture (90:10 to 80:20), **18** was obtained in 75% yield as a white solid. Mp 129-130 °C. $[\alpha]^{24}_D$ +7.6 (*c* 0.52, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36-7.31 (m, 2H), 7.28 (m, 1H), 7.24-7.19 (d, *J* 7.0 Hz, 2H), 6.98 (br d, *J* 7.5 Hz, 1H), 5.62 (br d, *J* 8.0 Hz, 1H), 5.00 (m, 1H), 4.36 (m, 1H), 3.37 (dd, *J* 6.5, 14.0 Hz, 1H), 3.26 (dd, *J* 7.5, 13.5 Hz, 1H), 1.84 (s, 3H), 1.53 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.7, 171.1, 155.7, 136.3, 129.4 (2C), 129.0 (2C), 127.3, 80.8, 76.3, 55.7, 50.8, 37.6, 30.4, 28.4 (3C), 26.3. HRMS (ESI): *m/z* calcd. for $C_{19}H_{26}N_2O_4SNa$ $[M + Na]^+$ 401.1511, found 401.1508.

(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)-4,4-dimethyl-thietan-2-one (19)



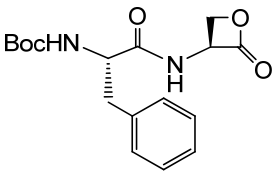
Following the general procedure B, using **18**, Boc-Phe-OH as amino acid and eluting with CH_2Cl_2/EtOAc mixture (80:20), **19** was obtained in 60% yield as a white solid. Mp 109-110 °C. $[\alpha]^{24}_D$ -12.4 (*c* 0.53, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33-7.20 (m, 7H), 7.15 (d, *J* 7.5 Hz, 2H), 7.06 (d, *J* 7.0 Hz, 2H), 6.53 (br d, *J* 7.5 Hz, 1H), 5.48 (d, *J* 8.0 Hz, 1H), 4.86 (br s, 1H), 4.66 (m, 1H), 4.26 (m, 1H), 3.13-2.89 (m, 4H), 1.79 (s, 3H), 1.47 (s, 3H), 1.35 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.2, 171.6, 170.3, 155.7, 136.3, 136.1, 129.4 (2C), 129.3 (2C), 129.0 (2C), 129.0(2C), 127.4 (2C), 80.9, 76.6, 56.2, 54.2, 50.6, 37.7, 37.5, 30.5, 28.4 (3C), 26.2. HRMS (ESI): *m/z* calcd. for $C_{28}H_{35}N_3O_5SNa$ $[M + Na]^+$ 548.2195, found 548.2195.

(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)-4,4-dimethyl-thietan-2-one (20)



Following the general procedure B, using **18**, Cbz-Phe-OH as amino acid and eluting with CH_2Cl_2/EtOAc mixture (80:20), **20** was obtained in 66% yield as a white solid. Mp 102-103 °C. $[\alpha]^{26}_D$ -17.2 (*c* 0.48, $CHCl_3$). IR (thin film): 3286, 2925, 1754, 1696, 1644, 1528, 1246, 1230, 1028 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (br s, 1H), 7.38-7.31 (m, 3H), 7.31-7.24 (m, 5H), 7.24-7.18 (m, 3H), 7.14 (d, *J* 7.0 Hz, 2H), 7.02 (d, *J* 7.0 Hz, 2H), 6.79 (br s, 1H), 5.51 (d, *J* 8.0 Hz, 1H), 5.45 (br s, 1H), 5.03 (d, *J* 12.0 Hz, 1H), 4.98 (d, *J* 12.5 Hz, 1H), 4.69 (m, 1H), 4.42 (m, 1H), 3.09-2.90 (m, 4H), 1.79 (s, 3H), 1.44 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.5, 171.4, 170.2, 156.4, 136.1, 136.0, 136.0, 129.3 (6C), 129.0 (2C), 128.9 (2C), 128.7, 128.5, 128.2, 127.4, 127.3, 76.5, 67.5, 56.5, 54.4, 50.7, 37.8, 37.6, 30.5, 26.2. HRMS (ESI): *m/z* calcd. for $C_{31}H_{33}N_3O_5SNa$ $[M + Na]^+$ 582.2039, found 582.2037.

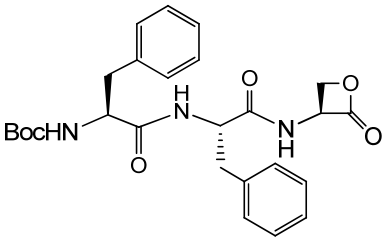
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)oxetan-2-one (27)



Following the general procedure A, using **3**[4], Boc-Phe-OH as amino acid and eluting with heptane/EtOAc mixture (80:20 to 70:30), **27** was obtained in 89% yield as a white solid. Mp 139-140 °C. $[\alpha]^{24}_D$ -18.2 (*c* 0.33, $CHCl_3$). IR (thin film): 3314, 2979, 1831, 1662, 1521, 1367, 1249, 1166 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (m, 2H), 7.19 (d, *J* 6.5 Hz, 1H), 7.13 (d, *J* 7.5 Hz, 2H), 6.90 (br d, *J* 7.0 Hz, 1H), 5.08-4.90 (m, 2H), 4.42-4.25 (m, 3H), 3.08-2.95 (m, 2H), 1.33 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 168.2, 155.9, 136.2, 129.5 (2C),

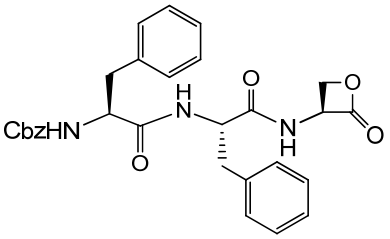
129.0 (2C), 127.4, 81.0, 65.6, 58.5, 55.6, 38.0, 28.4 (3C). HRMS (ESI): *m/z* calcd. for $C_{17}H_{22}N_2O_5Na$ $[M + Na]^+$ 357.1426, found 357.1427.

(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (28)



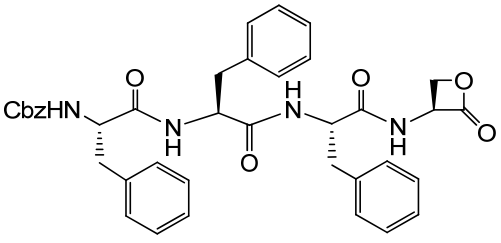
Following the general procedure D, using **27**, Boc-Phe-OH as amino acid and eluting with heptane/EtOAc mixture (80:20 to 60:40), **28** was obtained in 48% yield as a white solid. Mp 118-119 °C. $[\alpha]^{26}_D$ -79.6 (*c* 0.23, $CHCl_3$). IR (thin film): 3284, 2979, 1836, 1689, 1647, 1520, 1367, 1250, 1168 cm^{-1}. ^1H NMR (500 MHz, CD_3CN) δ 7.34-7.18 (m, 9H), 7.13 (d, *J* 7.0 Hz, 2H), 6.92 (br d, *J* 7.0 Hz, 1H), 5.50 (br s, 1H), 4.62-4.55 (dd, *J* 6.0, 11.0 Hz, 1H), 4.58 (m, 1H), 4.34 (d, *J* 5.5 Hz, 2H), 4.14 (br d, *J* 6.0 Hz, 1H), 3.17-3.09 (dd, *J* 5.5, 14.0 Hz, 1H), 3.03-2.92 (m, 2H), 2.74 (dd, *J* 9.5, 13.5 Hz, 1H), 1.32 (s, 9H). ^{13}C NMR (125 MHz, CD_3CN) δ 172.6, 172.4, 170.0, 157.0, 138.2, 138.0, 130.4 (2C), 130.2 (2C), 129.5 (2C), 129.4 (2C), 127.8, 127.6, 80.5, 65.8, 59.3, 57.3, 54.7, 38.1, 37.7, 28.5 (3C). HRMS (ESI): *m/z* calcd. for $C_{26}H_{31}N_3O_6Na$ $[M + Na]^+$ 504.2111, found 504.2097.

(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (29)



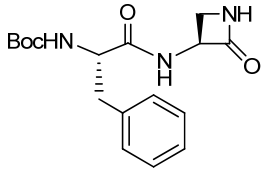
Following the general procedure D, using **27**, Cbz-Phe-OH as amino acid and eluting with $CHCl_3$/MeOH mixture (98:2), **29** was obtained in 72% yield as a white solid. Mp 167-168 °C. $[\alpha]^{26}_D$ -33.8 (*c* 0.21, DMF). IR (thin film): 3288, 2928, 1834, 1714, 1651, 1530, 1387, 1255, 1168 cm^{-1}. ^1H NMR (500 MHz, DMF-d_7) δ 8.78 (br d, *J* 7.5 Hz, 1H), 8.27 (br d, *J* 8.0 Hz, 1H), 7.40-7.15 (m, 16H), 5.37 (m, 1H), 5.02 (d, *J* 13.0 Hz, 1H), 4.98 (d, *J* 12.5 Hz, 1H), 4.53-4.32 (m, 3H), 3.15 (dd, *J* 5.5, 12.5 Hz, 1H), 3.10 (dd, *J* 4.5, 14.0 Hz, 1H), 3.01 (dd, *J* 8.0, 14.0 Hz, 1H), 2.86 (dd, *J* 10.0, 14.0 Hz, 1H). ^{13}C NMR (125 MHz, DMF-d_7) δ 172.4, 172.2, 170.2, 157.0, 138.9, 138.2, 138.1, 130.2 (2C), 130.0 (2C), 129.0, 128.9 (3C), 128.8 (3C), 128.4, 128.2, 127.2, 127.0, 65.3, 65.7, 59.1, 57.4, 54.8, 38.3 (2C). HRMS (ESI): *m/z* calcd. for $C_{29}H_{29}N_3O_6Na$ $[M + Na]^+$ 538.1954, found 538.1965.

(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (30)



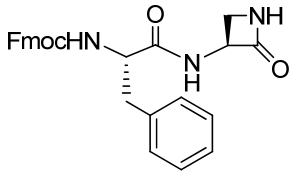
Following the general procedure D, using **28**, Cbz-Phe-OH as amino acid and eluting with $CHCl_3$/MeOH mixture (97:3), **30** was obtained in 53% yield as a white solid. Mp 169-170 °C. $[\alpha]^{26}_D$ -38.8 (*c* 0.22, DMF). IR (thin film): 3285, 2926, 1835, 1695, 1642, 1532, 1387, 1256, 1202, 1045 cm^{-1}. ^1H NMR (500 MHz, DMF-d_7) δ 8.71 (br d, *J* 7.5 Hz, 1H), 8.31 (br d, *J* 8.0 Hz, 1H), 8.15 (br d, *J* 7.5 Hz, 1H), 7.44-7.13 (m, 21H), 5.36 (m, 1H), 5.00 (d, *J* 13.0 Hz, 1H), 4.95 (d, *J* 12.5 Hz, 1H), 4.74-4.63 (m, 2H), 4.50-4.34 (m, 3H), 3.20-3.05 (m, 3H), 3.01 (dd, *J* 8.0, 13.5 Hz, 1H), 2.95 (dd, *J* 8.5, 14.0 Hz, 1H), 2.82 (dd, *J* 11.0, 14.0 Hz, 1H). ^{13}C NMR (125 MHz, DMF-d_7) δ 172.5 (2C), 171.7, 170.2, 156.9, 139.0, 138.3, 138.2, 138.1, 130.2 (2C), 130.1 (2C), 130.0 (2C), 129.0, 128.9 (2C), 128.8 (5C), 128.3, 128.1, 127.2, 127.0 (3C), 66.2, 65.6, 59.1, 57.4, 55.2, 55.0, 38.4, 38.3, 38.2. HRMS (ESI): *m/z* calcd. for $C_{38}H_{38}N_4O_7Na$ $[M + Na]^+$ 685.2638, found 685.2641.

(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)azetidin-2-one (31)



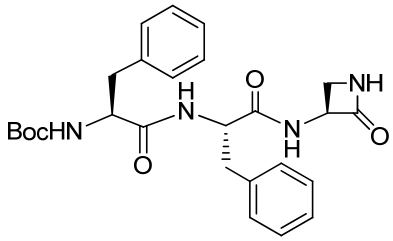
Following the general procedure B, using **4**[5], Boc-Phe-OH as amino acid and eluting with CH_2Cl_2/acetone (70:30), **31** was obtained in 27% yield as a white solid. Mp 147-148 °C. $[\alpha]^{24}_D$ +2.6 (*c* 0.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) δ 7.32-7.15 (m, 5H), 6.44 (br s, 1H), 5.51 (br s, 1H), 5.26 (m, 1H), 4.83 (br s, 1H), 4.42 (m, 1H), 3.55 (m, 1H), 3.28 (m, 1H), 3.10 (m, 1H), 2.98 (m, 2H), 1.36 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 168.2, 155.7, 136.7, 129.5 (2C), 128.7 (2C), 127.0, 80.4, 57.9, 55.6, 44.1, 38.6, 28.4 (3C). HRMS (ESI): *m/z* calcd. for $C_{17}H_{23}N_3O_4Na$ $[M + Na]^+$ 356.1586, found 356.1601.

(3*S*)-3-(*N*-fluorenyloxycarbonyl-L-phenylalanylamino)azetidin-2-one (32)



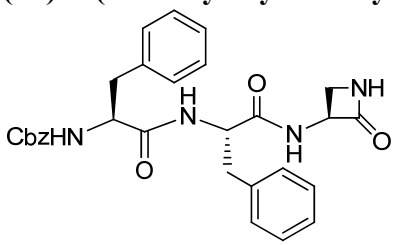
Following the general procedure B, using **4**[5], Fmoc-Phe-OH as amino acid and eluting with CH_2Cl_2/EtOAc (70:30 to 40:60), **32** was obtained in 43% yield as a white solid. Mp 228-230 °C. $[\alpha]^{26}_D$ -26.6 (*c* 0.50, DMF). ^1H NMR (500 MHz, DMF-d_7) δ 8.78 (d, *J* 8.0 Hz, 1H), 7.91 (m, 3H), 7.68 (d, *J* 7.0 Hz, 2H), 7.56 (d, *J* 9.0 Hz, 1H), 7.38-7.23 (m, 6H), 7.19 (m, 1H), 5.03 (m, 1H), 4.44 (m, 1H), 4.26-4.10 (m, 3H), 3.52 (m, 1H), 3.24-3.14 (m, 2H), 2.96 (dd, *J* 10.0, 13.0 Hz, 1H). ^{13}C NMR (125 MHz, DMF-d_7) δ 172.4, 168.6, 156.8, 144.8, 141.8, 141.7, 138.9, 130.1 (2C), 128.8 (2C), 128.3 (2C), 127.8 (2C), 127.0, 126.2, 126.1, 120.5 (2C), 67.0, 58.3, 57.3, 47.7, 43.6, 38.7. HRMS (ESI): *m/z* calcd. for $C_{27}H_{29}N_4O_4$ $[M + NH_4]^+$ 473.2189, found 473.2204.

(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)azetidin-2-one (33)



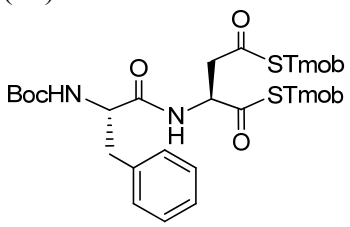
Following the general procedure E, using **32**, Boc-Phe-OH as amino acid and eluting with $CHCl_3$/MeOH (97:3), **33** was obtained in 46% yield as a white solid. Mp 159-160 °C. $[\alpha]^{24}_D$ -31.0 (*c* 0.20, $(CH_3)_2CO$). IR (thin film): 3278, 2975, 1767, 11693, 1647, 1531, 1391, 1260, 1169 cm^{-1}. ^1H NMR (500 MHz, $(CD_3)_2CO$) δ 7.82 (br d, *J* 6.0 Hz, 1H), 7.39 (br d, *J* 7.5 Hz, 1H), 7.30-7.16 (m, 10H), 7.10 (br s, 1H), 6.08 (br d, *J* 7.5 Hz, 1H), 5.83 (br s, 1H), 4.93 (m, 1H), 4.68 (m, 1H), 4.32 (m, 1H), 3.52 (m, 1H), 3.22 (m, 1H), 3.17-3.06 (m, 2H), 3.02 (dd, *J* 7.5, 14.0 Hz, 1H), 2.87 (dd, *J* 9.0, 12.5 Hz, 1H), 1.33 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 171.7, 168.1, 156.6, 138.9, 138.3, 130.6 (2C), 130.4 (2C), 129.3 (2C), 129.2 (2C), 127.5, 127.4, 79.8, 58.9, 57.2, 55.1, 44.0, 38.8, 38.6, 28.7 (3C). HRMS (ESI): *m/z* calcd. for $C_{26}H_{32}N_4O_5Na$ $[M + Na]^+$ 503.2270, found 503.2285.

(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)azetidin-2-one (34)



Following the general procedure E, using **32**, Cbz-Phe-OH as amino acid and eluting with $CHCl_3$/MeOH mixture (98:2 to 95:5), **34** was obtained in 33% yield as a white solid. Mp 238-239 °C. $[\alpha]^{26}_D$ -32.3 (*c* 0.20, DMF). IR (thin film): 3275, 2926, 1768, 1693, 1648, 1533, 1390, 1261, 1044 cm^{-1}. ^1H NMR (500 MHz, DMF-d_7) δ 8.62 (br d, *J* 8.5 Hz, 1H), 8.20 (br d, *J* 8.0 Hz, 1H), 7.92 (br s, 1H), 7.40-7.15 (m, 16H), 5.37 (m, 1H), 5.02 (d, *J* 13.0 Hz, 1H), 4.97 (d, *J* 13.0 Hz, 1H), 4.68 (m, 1H), 4.40 (m, 1H), 3.35 (m, 1H), 3.23-3.06 (m, 3H), 3.00 (dd, *J* 8.0, 13.0 Hz, 1H), 2.85 (m, 1H),. ^{13}C NMR (125 MHz, DMF-d_7) δ 172.1, 171.7, 168.4, 156.9, 138.9, 138.4, 138.0, 130.2 (2C), 130.0 (2C), 129.0, 128.8 (6C), 128.3, 128.1, 127.0, 126.9, 66.2, 58.3, 57.5, 55.0, 43.5, 38.6, 38.3. HRMS (ESI): *m/z* calcd. for $C_{29}H_{30}N_4O_5Na$ $[M + Na]^+$ 537.2114, found 537.2122.

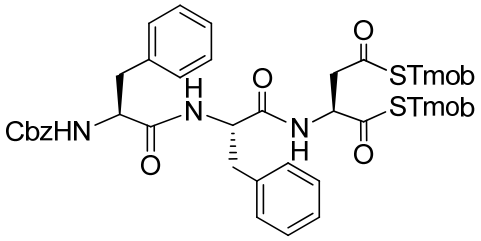
***S*,*S*'-bis(2,4,6-trimethoxybenzyl) *N*-*tert*-butoxycarbonyl-L-phenylalanyl-L-thioaspartate (36)**



Compound **35**[6] (300 mg, 479 µmol) was dissolved in 40% TFA/CH_2Cl_2 (6.2 mL), and the solution was stirred for 5 min. Then, volatiles were removed under reduced pressure, and the residue coevaporated with toluene. Separately, Boc-Phe-OH (152.6 mg, 575 µmol), EDCI (110.3 mg, 575 µmol) and HOBt.H_2O (97.2 mg, 719 µmol) were stirred in CH_2Cl_2 (2.5 mL) and stirred for 20 min. This mixture was cooled at -20°C followed by the addition of *i*-Pr_2NEt (100 µL, 575 µmol) and the resulting mixture added to a stirred solution of the above amine salt in CH_2Cl_2 (2 mL) at -20°C. The reaction mixture was then stirred for 1 h at -20°C and then diluted with CH_2Cl_2, washed with a 1M HCl solution, saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4. Evaporation of the solvent followed by column chromatography eluting with heptane/EtOAc mixture (90:10 to 60:40), **36** was obtained in 63% yield (232 mg) as a white solid. Mp 81-82 °C. $[\alpha]^{26}_D$ +1.3 (*c* 0.50, $CHCl_3$). ^1H NMR (500 MHz, CD_3CN) δ 7.30 (br d, *J* 8.5 Hz, 1H), 7.28-7.14 (m, 5H), 6.18 (s, 4H), 5.48 (br s,

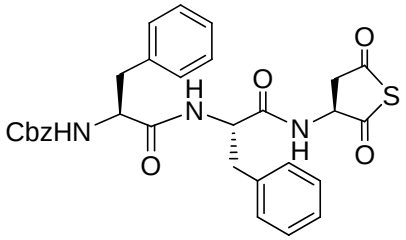
1H), 4.91 (m, 1H), 4.27 (m, 1H), 4.16 (s, 2H), 4.08 (s, 2H), 3.78 (s, 18H), 3.17 -3.08 (m, 2H), 3.01 (dd, *J* 4.0, 15.0 Hz, 1H), 2.79 (m, 1H), 1.29 (s, 9H). ^{13}C NMR (125 MHz, CD_3CN) δ 200.7, 197.7, 172.6, 162.2 (2C), 160.2 (2C), 160.1 (2C), 156.3, 138.5, 130.2 (2C), 129.3 (2C), 127.5, 104.8, 104.7, 91.8 (2C), 91.8 (2C), 80.1, 56.8, 56.6 (2C), 56.6 (2C), 56.6, 56.1 (2C), 45.3, 38.1, 28.4 (3C), 23.1, 22.9. HRMS (ESI): *m/z* calcd. for $C_{38}H_{50}N_2O_{11}S_2$ $[M + H]^+$ 773.2778, found 773.2771.

S,S'-bis(2,4,6-trimethoxybenzyl) *N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-thioaspartate (37)



Dipeptide **36** (70 mg, 90.5 μmol) was dissolved in 40% TFA/CH_2Cl_2 (0.91 mL), and the solution was stirred for 5 min. Then, volatiles were removed under reduced pressure, and the residue coevaporated with toluene. Separately, Cbz-Phe-OH (32.5 mg, 108.6 μmol), EDCI (20.8 mg, 108.6 μmol) and HOBt.H_2O (18.4 mg, 136.2 μmol) were stirred in CH_2Cl_2 (0.4 mL). This mixture was cooled at -20°C followed by the addition of *i*-Pr_2NEt (19 μL, 108.6 μmol) and the resulting mixture added to a stirred solution of the above amine salt in CH_2Cl_2 (0.3 mL) at -20 °C. The reaction mixture was then stirred for 1 h at -20 °C and then diluted with CH_2Cl_2, washed with a 1M HCl solution, saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4. Evaporation of the solvent followed by column chromatography eluting with heptane/EtOAc mixture (80:20 to 40:60), **37** was obtained in 50% yield (43 mg) as a white solid. Mp 159-160 °C. $[\alpha]^{24}_D$ -10.9 (*c* 0.50, CH_2Cl_2). ^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.37-7.16 (m, 10H), 7.15-7.06 (m, 5H), 6.91 (br d, *J* 7.0 Hz, 1H), 6.33 (br d, *J* 5.0 Hz, 1H), 6.12 (s, 2H), 6.11 (s, 2H), 5.15 (br s, 1H), 5.05 (d, *J* 12.5 Hz, 1H), 4.99 (d, *J* 12.5 Hz, 1H), 4.91 (m, 1H), 4.62 (m, 1H), 4.29 (m, 1H), 4.25-4.11 (m, 4H), 3.79 (s, 12H), 3.77 (s, 6H), 3.20-3.08 (m, 2H), 3.07-2.84 (m, 4H). ^{13}C NMR (125 MHz, CD_2Cl_2) δ 199.4, 197.6, 171.0, 170.6, 161.5, 161.5, 159.7 (2C), 159.6 (2C), 156.5, 136.9, 136.8, 136.7, 129.8 (2C), 129.7 (2C), 129.1 (3C), 128.9 (3C), 128.9, 128.5, 128.3, 127.4, 127.3, 104.5, 104.5, 90.6 (4C), 67.5, 56.7 (4C), 56.2, 56.2 (2C), 55.7, 54.5, 44.9, 38.1, 37.8, 22.9, 22.8. HRMS (ESI): *m/z* calcd. for $C_{50}H_{56}N_3O_{12}S_2$ $[M + H]^+$ 954.3305, found 954.3305.

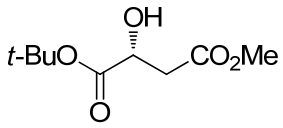
***N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-thioaspartic anhydride (38)**



Tripeptide **37** (20 mg, 21.0 μmol) was dissolved in a 4:1:5mixture of TFA/Et_3SiH/CH_2Cl_2 (200 μL), and the solution was stirred for 2 h. Then, volatiles were removed under reduced pressure, and the residue coevaporated with benzene. The residue was then washed three times with benzene to give **38** in 60% yield (7.0 mg) as a white solid. Mp 144-145 °C. $[\alpha]^{24}_D$ -57.5 (*c* 0.30, CH_3CN). IR (thin film): 3287, 2926, 1714, 1648, 1534, 1259, 1053 cm^{-1}. ^{1}H NMR (500 MHz, CD_3CN) δ 7.41-7.19 (m, 14H), 7.18-7.12 (m, 2H), 7.02 (br d, *J* 7.5 Hz, 1H),

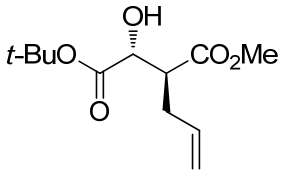
5.88 (br d, *J* 6.5 Hz, 1H), 5.02 (d, *J* 12.5 Hz, 2H), 4.96 (d, *J* 12.5 Hz, 1H), 4.84 (m, 1H), 4.59 (m, 1H), 4.24 (m, 1H), 3.22 (dd, *J* 9.0, 17.5 Hz, 1H), 3.15 (dd, *J* 5.0, 14.0 Hz, 1H), 3.08 (dd, *J* 8.0, 17.5 Hz, 1H), 3.01 (dd, *J* 5.5, 14.0 Hz, 1H), 2.93 (dd, *J* 8.5, 14.0 Hz, 1H), 2.75 (dd, *J* 9.5, 14.0 Hz, 1H). ^{13}C NMR (125 MHz, CD_3CN) δ 199.7, 197.2, 172.2, 172.1, 157.3, 138.3, 138.1, 137.9, 130.4 (2C), 130.2 (2C), 129.5 (2C), 129.4 (2C), 129.4 (2C), 128.9, 128.6 (2C), 127.7, 127.6, 67.3, 60.1, 57.5, 54.9, 45.8, 38.2, 38.0. HRMS (ESI): *m/z* calcd. for $C_{30}H_{29}N_3O_6SNa$ $[M + Na]^+$ 582.1667, found 582.1675.

1-*O*-*tert*-butyl 4-*O*-methyl (2*R*)-2-hydroxysuccinate (39)



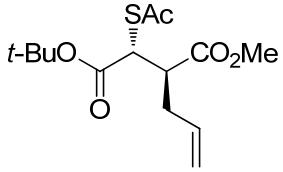
To a solution of 1-*O*-methyl (3*R*)-3-hydroxysuccinate[7] (2.0 g, 14 mmol) in CH_2Cl_2 (14 mL) was added *O*-tert-butyl *N*,*N*'-diisopropyl isourea[8] (8.1 g, 41 mmol) at 0 °C. The mixture was allowed to warm up to 25 °C, and stirred for 16 h, which gave a white precipitate. The precipitate was removed by filtration, and the filtrate was evaporated under reduced pressure. Purification of the residue by column chromatography eluting with heptane/EtOAc mixture (9:1 to 4:1) gave **39** in 73% yield (2.0 g) as a colorless syrup. $[\alpha]^{26}_D$ +14.0 (*c* 2.23, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.36 (dd, *J* 6.0, 4.8 Hz, 1H), 3.70 (s, 3H), 3.23 (br s, 1H), 2.80 (dd, *J* 15.9, 4.8 Hz, 1H), 2.73 (dd, *J* 15.9, 6.0 Hz), 1.48 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 170.7, 82.9, 67.5, 51.8, 38.8, 27.9. HRMS (ESI): *m/z* calcd. for $C_9H_{16}O_5Na$ $[M + Na]^+$ 227.0895, found 227.0873.

1-*O*-*tert*-butyl 4-*O*-methyl (2*R*,3*S*)-3-allyl-2-hydroxysuccinate (40)



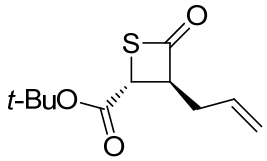
To a solution of *i*-Pr_2NH (1.8 mL, 13 mmol) in THF (13 mL) was added *n*-BuLi (1.16M in hexane, 11 mL, 12 mmol) at -78 °C, then the solution was stirred for 10 min at 0 °C to obtain an LDA solution. To a solution of **39** (1.2 g, 5.9 mmol) in THF (5.9 mL) was added the LDA solution at -78 °C, and the mixture was stirred for 10 min. Allyl bromide (2.0 mL, 24 mmol) was added to the reaction mixture, which was then stirred for 2 h. The reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with CH_2Cl_2, and the combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. Purification of the residue by column chromatography eluting with heptane/EtOAc mixture (9:1 to 4:1) gave **40** in 51% yield (730 mg) as a colorless syrup. $[\alpha]^{26}_D$ -0.9 (*c* 0.84, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.84 (dddd, *J* 17.1, 10.5, 7.5, 6.0 Hz, 1H), 5.17 (dd *J* 17,1, 1.2 Hz, 1H), 5.11 (d *J* 10.5 Hz, 1H), 4.21 (br s, 1H), 3.69 (s, 3H), 3.12 (br d, *J* = 5.4 Hz, 1H), 2.95 (ddd, *J* 7.5, 6.0 Hz, 1H), 2.62 (ddd, *J* 14.4, 6.0, 6.0 Hz, 1H), 2.43 (ddd, *J* 14.4, 7.5, 7.5 Hz), 1.51 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 172.2, 135.1, 117.6, 82.9, 70.5, 51.6, 48.4, 31.9, 27.9. HRMS (ESI): *m/z* calcd. for $C_{12}H_{20}O_5Na$ $[M + Na]^+$ 267.1208, found 267.1186.

1-*O*-*tert*-butyl 4-*O*-methyl (2*R*,3*S*)-2-acetylsulfanyl-3-allyl-succinate (**41**)



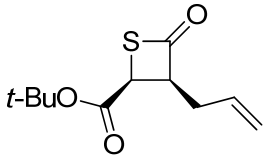
To a solution of **40** (750 mg, 3.1 mmol) in CCl_4 (24 mL) and MeCN (7.1 mL) was added PPh_3 (3.2 g, 12 mmol), and the mixture was heated to reflux for 3 h. The mixture was then concentrated *in vacuo*, and the residue was partially purified by quick column chromatography eluting with heptane/EtOAc mixture (20:1). The crude product was dissolved in DMF (2.7 mL), and CsSAc (610 mg, 2.9 mmol) was added. The mixture was stirred for 1 h, and diluted with water. The mixture was extracted with CH_2Cl_2, and the combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. Purification of the residue by column chromatography eluting with heptane/EtOAc mixture (20:1) gave **41** in 56% yield (480 mg) as a yellowish syrup. $[\alpha]^{24}_D$ +23.5 (*c* 1.03, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.84 (dddd, *J* 17.7, 9.9, 6.9, 6.9 Hz, 1H), 5.09 (d *J* 17.7 Hz, 1H), 5.08 (d *J* 9.9 Hz, 1H), 4.49 (d *J* 5.4 Hz, 1H), 3.70 (s, 3H), 3.18 (ddd *J* 7.5, 7.5, 5.4 Hz, 1H), 2.52 (ddd *J* 13.5, 7.5, 6.9 Hz, 1H), 2.31 (ddd *J* 13.5, 7.5, 6.9 Hz, 1H), 2.39 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.5, 172.7, 168.9, 134.3, 118.0, 82.6, 51.9, 47.6, 46.4, 34.0, 30.1, 27.8. HRMS (ESI): *m/z* calcd. for $C_{14}H_{22}O_5SNa$ $[M + Na]^+$ 325.1086, found 325.1074.

(3*S*,4*R*)-3-allyl-4-*tert*-butylcarbonylthietan-2-one (*trans*-**42**)



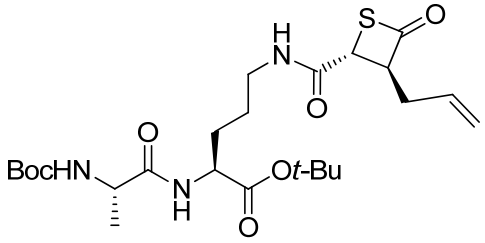
To a solution of **41** (100 mg, 0.330 mmol) in THF (3.3 mL) was added a 1M aqueous solution of LiOH. Under Ar atmosphere, and the mixture was stirred for 16 h. The mixture was neutralized with Amberlyst 15, and the resin was removed by filtration. The filtrate was concentrated *in vacuo*, and the residue was dried under high vacuum. A suspension of the residue (65 mg), C_6F_5OH (150 mg, 0.79 mmol), and MS4A (130 mg) in CH_2Cl_2 was stirred for 15 min, and EDCI was added at 0 °C. The reaction mixture was stirred for 1 h at the temperature, and filtrated through celite pad. The filtrate was washed with water and brine. The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. Purification of the residue by preparative TLC developed with a mixture of heptanes/EtOAc (9:1) gave the less polar component, *trans*-**42** (20 mg, 27%) as a colorless syrup accompanied by *cis*-**42** (10 mg, 13%) as a colorless syrup. $[\alpha]^{26}_D$ -92.6 (*c* 1.18, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.79 (dddd *J* 16.8, 10.2, 6.9, 6.9 Hz, 1H), 5.20 (ddd *J* 16.8, 1.5, 1.2 Hz, 1H), 5.19 (ddd *J* 10.2, 1.2, 1.2 Hz, 1H), 4.52 (ddd *J* 7.5, 6.9, 3.9 Hz, 1H), 3.75 (d *J* 3.9 Hz, 1H), 2.55 (ddd *J* 6.9, 1.5, 1.2 Hz, 1H), 2.53 (ddd *J* 7.5, 1.5, 1.2 Hz, 1H), 1.51 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.1, 169.9, 132.2, 118.8, 83.0, 70.9, 37.0, 34.0, 27.9. HRMS (ESI): *m/z* calcd. for $C_{11}H_{16}O_sSNa$ $[M + Na]^+$ 251.0718, found 251.0713.

(3*S*,4*S*)-3-allyl-4-*tert*-butylcarbonylthietan-2-one (*cis*-**42**)



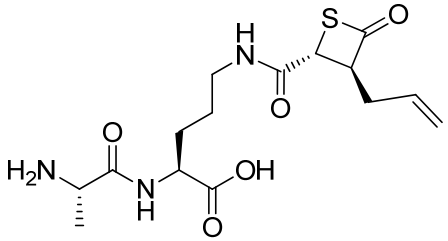
$[\alpha]^{24}_{D}$ +67.9 (*c* 0.54, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.69 (dddd *J* 17.0, 10.5, 6.5, 6.5 Hz, 1H), 5.03 (d *J* 17.0 Hz, 1H), 5.02 (d *J* 10.5 Hz, 1H), 4.37 (ddd *J* 8.0, 8.0, 8.0 Hz, 1H), 4.05 (d *J* 7.5 Hz, 1H), 2.56-2.47 (m, 2H), 1.44 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.0, 168.7, 133.1, 117.8, 83.3, 70.8, 37.8, 31.4, 28.0. HRMS (ESI): *m/z* calcd. for $C_{11}H_{16}O_sSNa$ $[M + Na]^+$ 251.0718, found 251.0703.

tert-butyl (3*S*,4*R*)-*N*-_tert_-butylcarbonyl-L-alanyl-N^{δ}-(3-allyl-2-oxothietan-4-yl)-L-ornithinate (43)



trans-**42** (15 mg, 0.066 mmol) was dissolved in a 1:1 mixture of TFA and CH_2Cl_2 (660 μL), and the mixture was stirred for 1 h. The volatiles were removed *in vacuo*, and the residue was dissolved in CH_2Cl_2 (660 μL). To the solution were added HOBt.H_2O (12 mg, 0.078 mmol) and EDCI (14 mg, 0.072 mmol), followed by Boc-Ala-Orn-O*t*-Bu (24 mg, 0.066 mmol). The reaction mixture was stirred for 1 h, and the reaction was quenched with water. The mixture was extracted with CH_2Cl_2, and the organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. Purification of the residue by preparative TLC developed with a mixture of CH_2Cl_2/MeOH (19:1) gave **43** (28 mg, 85%) as a colorless syrup. $[\alpha]^{22}_{D}$ -39.3 (*c* 0.85, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.00 (br t *J* 5.1 Hz, 1H), 6.82 (br d *J* 7.5 Hz, 1H), 5.81 (dddd *J* 17.1, 10.2, 7.2, 7.2 Hz, 1H), 5.22 (dd *J* 17.1, 1.2 Hz, 1H), 5.19 (d *J* 10.2 Hz, 1H), 5.07 (br s, 1H), 4.56 (ddd *J* 7.2, 7.2, 3.6 Hz, 1H), 4.47-4.36 (m, 1H), 4.16 (t *J* 7.2 Hz, 1H), 3.79 (d *J* 3.9 Hz, 1H), 3.49-3.23 (m, 2H), 2.57 (t *J* 7.2 Hz, 2H), 1.48 & 1.45 (s, 9H each), 1.39 (d *J* 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.3 172.8, 170.9, 169.4, 132.4, 118.9, 82.7, 72.3, 52.1, 50.4, 39.4, 37.7, 34.3, 30.3, 28.3, 28.0, 24.6, 18.1. HRMS (ESI): *m/z* calcd. for $C_{24}H_{40}N_3O_7S$ $[M + H]^+$ 514.2587, found 514.2593.

(3*S*,4*R*)-L-alanyl-N^{δ}-(3-allyl-2-oxothietan-4-yl)-L-ornithine (44)



Compound **43** (5.0 mg, 9.7 μmol) was dissolved in 40% TFA in CH_2Cl_2, and the reaction mixture was stirred for 15 h at 0 °C. The volatiles were removed under reduced pressure, which gave **44** as colorless syrup (3.5 mg, quant). $[\alpha]^{23}_{D}$ -33.8 (*c* 0.42, MeOH). ^{1}H NMR (300 MHz, D_2O) δ 5.71 (dddd *J* 17.1, 10.2, 6.6, 6.6 Hz, 1H), 5.14 (d *J* 17.1 Hz, 1H), 5.09 (d *J* 10.2 Hz, 1H), 4.40-4.28 (m, 2H), 4.03 (q *J* 6.9 Hz, 1H), 3.92 (d *J* 3.6 Hz, 1H), 3.31-3.09 (m, 2H), 2.47 (t *J* 6.9 Hz, 1H), 1.91-1.76 (m, 1H), 1.76-1.61 (m, 1H), 1.60-1.49 (m, 2H), 1.47 (d *J* 6.9 Hz, 3H). ^{13}C NMR (125 MHz, D_2O) δ 196.6, 175.1, 172.2, 170.9, 132.6, 118.6, 71.0, 52.6, 48.9, 39.1, 33.5, 27.5, 24.6, 16.5, 2.3 172.8, 170.9, 169.4, 132.4, 118.9, 82.7, 72.3, 52.1, 50.4, 39.4, 37.7, 34.3, 30.3, 28.3, 28.0, 24.6, 18.1. HRMS (ESI): *m/z* calcd. for $C_{15}H_{24}N_3O_5S$ $[M + H]^+$ 358.1437, found 358.1433.

Biology

Cell culture and cell proliferation assay. The human cell lines KB3-1 (mouth epidermoid carcinoma), originating from the NCI, and U87 (human glioblastoma), kindly provided by Dr Beclin (Marseille, France), were grown in D-MEM medium supplemented with 10% fetal calf serum, in the presence of penicillin, streptomycin and fungizone in a 75 cm² flask under 5% CO_2. HL60 cells (acute promyelocytic leukaemia), MDA231 (human breast adenocarcinoma), HCT116 (colorectal carcinoma) and PC3 prostate carcinoma obtained from ATCC were grown in RPMI medium supplemented with 10% fetal calf serum, in the presence of penicillin, streptomycin and fungizone in a 75 cm² flask under 5% CO_2. For cell proliferation assays, cells were plated in 96-well tissue culture plates in 200 µl medium and treated 24 h later with 2 µl stock solution of compounds dissolved in DMSO using a Biomek 3000 (Beckman-Coulter). Controls received the same volume of DMSO (1% final volume). After 72 h exposure, MTS reagent (Celltiter 96AQeous One, Promega) was added and incubated for 3 h at 37 °C: the absorbance was monitored at 490 nm and results expressed as the inhibition of cell proliferation calculated as the ratio [(1-(OD490 treated/OD490 control))×100] in triplicate experiments. For IC_{50} determination [50% inhibition of cell proliferation], cells were incubated for 72 h following the same protocol with compound concentrations ranging from 5 nM to 100 µM in separate duplicate experiments.

Necrosis. Necrosis was estimated through the release of LDH in the culture medium. 20,000 HL60 cells were incubated for 24 and 48 h in the presence of **17** in 96-well microplates containing 100 µl RPMI medium. After centrifugation at 300 G for 1 min, 25 µl of culture medium was added with 25 µl Cytotox-ONE reagent (Promega) and kept in the dark at room temperature for 20 min. Fluorescence was recorded (exc 560nm, em 590 nm): results are expressed as the residual activity in the presence of chemical **17** compared to activity in the presence of vehicle alone. 50 µM menadione was used as positive control.

Flow cytometric detection of apoptosis. Apoptotic and necrotic cells were analysed in FACS using HL60 cells (5000 cells/well in 100 µl complete RPMI) exposed for 24 and 48 h in 96-well microplates. Double-staining for phosphatidyl serine and DNA was performed by addition of 50 µl staining solution consisting of 10 mM Hepes pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$ containing annexin V-PE (Bender MedSystems) 6.5 µl/cm^3 and 1.7 µM 7-AAD from Enzo Life Sciences (Farmingdale, NY). The mixture was incubated for 20 min in the dark at room temperature, and fluorescence was measured by FACS with a Guava EasyCyte plus cytometer (Millipore).

Caspase activity assay. Caspase activities were assayed in HL60 cell after a 48 h treatment with **17**. HL60 cells (20,000 cells per well in 180 µl RPMI medium) were plated in black 96-well culture microplates and treated with chemicals dissolved in DMSO (1% v/v, final concentration). Plates were kept under 5% CO_2 for 48 h. Lysis buffer (20 µl of a 10x stock solution) consisting in 250 mM Hepes (pH 7.5) 5 mM EDTA, 0.5% NP40, 0.1% SDS and 50 mM dithiothreitol, was added before the caspase substrate (DEVD-AMC) dissolved in water at a final concentration of 50 µM. Plates were incubated at 37 °C and fluorescence was recorded (exc 360 nm, em 435 nm) after 0, 30, 60, 120, and 180 min. Reaction rates were calculated from the slope of the linear time-dependent reaction and are expressed as the fold-activation relative to the control (HL60 with DMSO alone). Doxorubicin 10^{-6} M was used as a positive control.

DNA strand breaks by the TUNEL assay. 15,000 HL60 cells were incubated in 100 µl RPMI for 24 and 48 h with **17**. Cells were washed in PBS and fixed in 200 µl 1% paraformaldehyde for 30 min at 4 °C. Cells were washed twice in PBS, permeabilized by addition of ice-cold ethanol (70%) and kept overnight at -20 °C. Cells were washed in PBS and resuspended in 100 µl TdT reaction solution containing 0.25 nmoles BrdUTP, 10U TdT in 200 mM cacodylate buffer, pH 7.4, 10 mM $CoCl_2$. After 1 h at 37 °C, cells were washed and incubated with anti-BrdU-FITC antibody for 30 min at 37 °C in the presence of 25 µg RNase A. Cells were washed twice in PBS and resuspended in 150 µl PBS solution containing 30 µg propidium iodide for 30 min in the dark at room temperature. FACS analyze was performed for FITC/PI fluorescence with a Guava Easycyte cytometer (Millipore). Cell populations were quantified using Modfit LT (Verity Software House).

Mitochondrial transmembrane potential. 60,000 HL60 cells were incubated for 24 and 48 h in 100 µl RPMI with **17** before the addition of 0.5 µg JC-10 (Enzo Life Sciences) dissolved in 100 µl RPMI. After a 10 min incubation at 37 °C, cells were gently centrifuged, washed and resuspended in 200 µl PBS before being analyzed by FACS with a Guava Easycyte cytometer (Millipore). Cell populations were quantified using Modfit LT (Verity Software House).

Cell cycle analysis. KB or HL60 cells (25,000 cells/well in 96-well microplates) were exposed to **17** for 24 and 48 h at 37 °C under 5% CO_2 in 100 µl complete D-MEM or RPMI medium. Controls received the same volume of DMSO (1% final volume). Culture media were carefully collected and gently centrifuged to collect floating cells, adherent cells harvested after addition of trypsin, mixed with the pellet of floating cells, washed with PBS and fixed in ice-cold 70% ethanol. After 2 h at 4 °C, cells were spun down by centrifugation, washed with 2% FCS in PBS and stained with 50 µg/ml propidium iodide in hypotonic buffer (3.4 mM Na citrate, pH 7.4, 00.2% Triton X-100) in the presence of RNase A (50 µg/ml) for 30 min at room temperature shielded from light, before being analyzed by FACS with a Guava Easycyte cytometer (Millipore). Cell populations were quantified using Modfit LT (Verity Software House).

Proteasome inhibition. HL60 cells were suspended in a buffer consisting of 25 mM Hepes (pH 7.5), 0.5 mM EDTA, 0.05% NP40, 0.01% SDS and 0.2 mM ATP. Cells at a concentration of 15,000 cells in 180 µl lysis buffer were added to each well of black 96-well microplates and kept on ice for 20 min: proteosomal activity was assayed after addition of 50 µM LLVY-AMC or RLR-AMC. Microplates were incubated at 37 °C in the dark and fluorescence monitored every hour over a 3 h incubation period (exc 360 nm, em 465 nm). Compounds were added to the incubation mixture at concentrations ranging from 10^{-6} to 10^{-9} M with a fixed volume of DMSO. Controls were performed in the same conditions with vehicle only and 100 nM MG132 was used as control in chymotrypsin-like samples and elicited more than 95% inhibition. Results are expressed as the residual activity in the presence of chemical **17** compared to activity in the presence of vehicle alone.

In vitro recombinant cathepsin inhibition. Recombinant cathepsin B and recombinant cathepsin L were purchased from Enzo Life Sciences (Farmingdale, NY). Pure enzymes were diluted respectively 1/20 and 1/10 in assay buffer consisting of 100 mM Na acetate, pH 5.5, NaCl 120 mM and 5 mM DTT in 96-well black microplates. Chemicals and E-64 dissolved in DMSO were introduced before the substrate (FR-AMC) dissolved in assay buffer to a final concentration 100 µM. The total volume was 50 µl. Incubations were carried out at 37 °C in the dark and fluorescence monitored every 10 min over a 30 min period (exc 360 nm, em 465

nm). Results are expressed as the residual activity in the presence of chemical **17** compared to activity in the presence of vehicle only.

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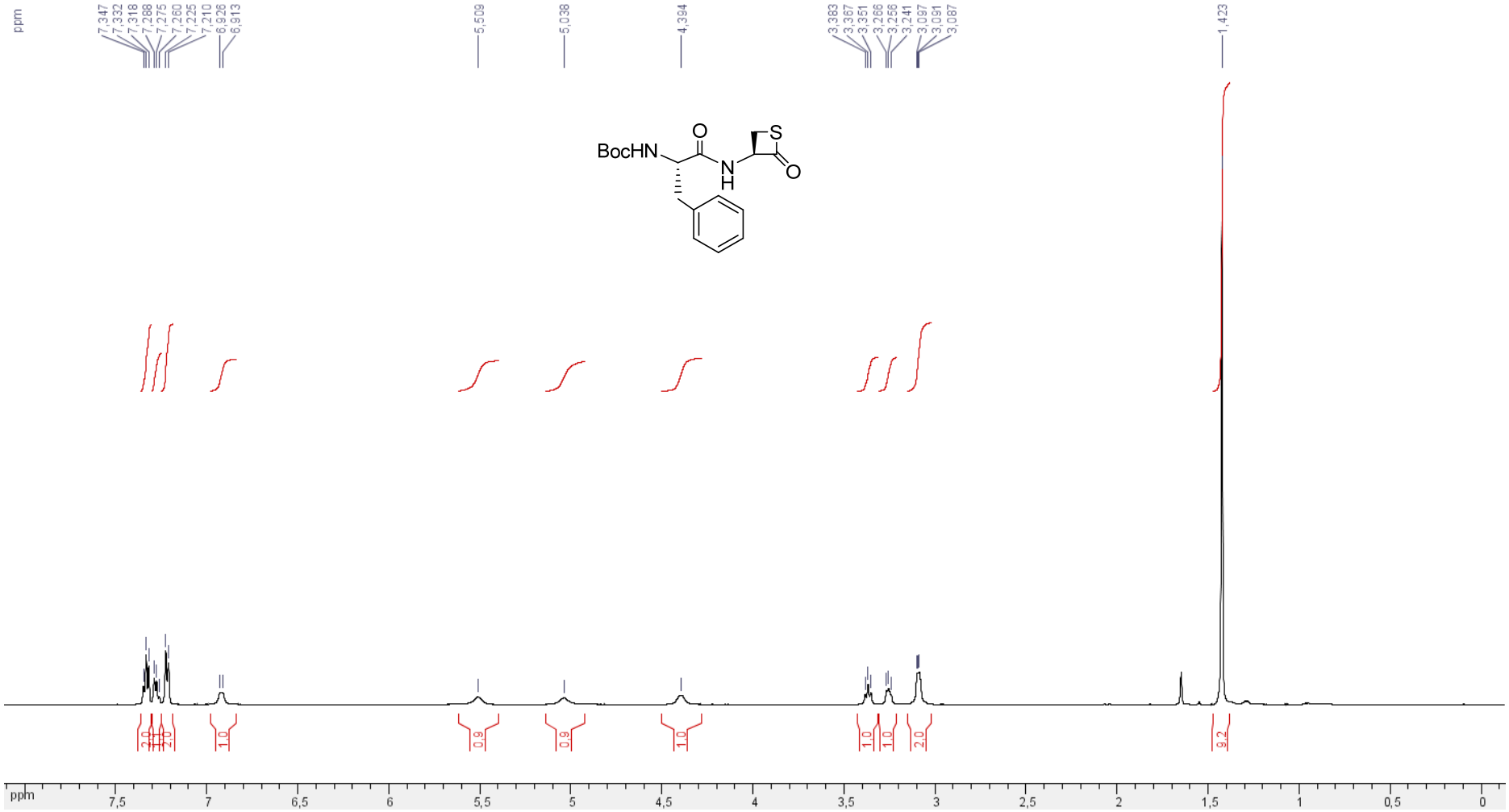
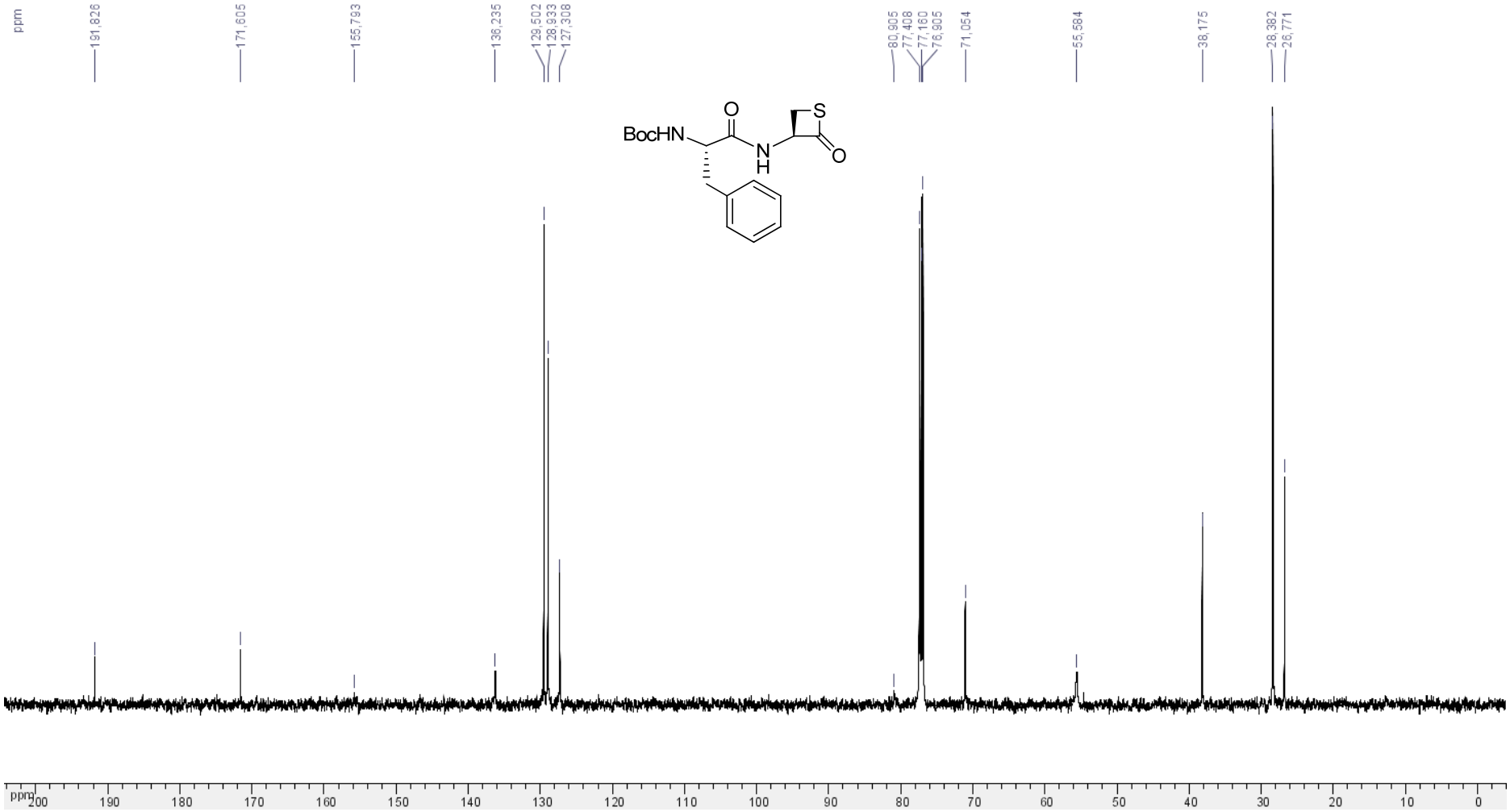
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)oxetan-2-one (5) ^{1}H NMR (500 MHz, $CDCl_3$)



(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)oxetan-2-one (5) ^{13}C NMR (125 MHz, $CDCl_3$)



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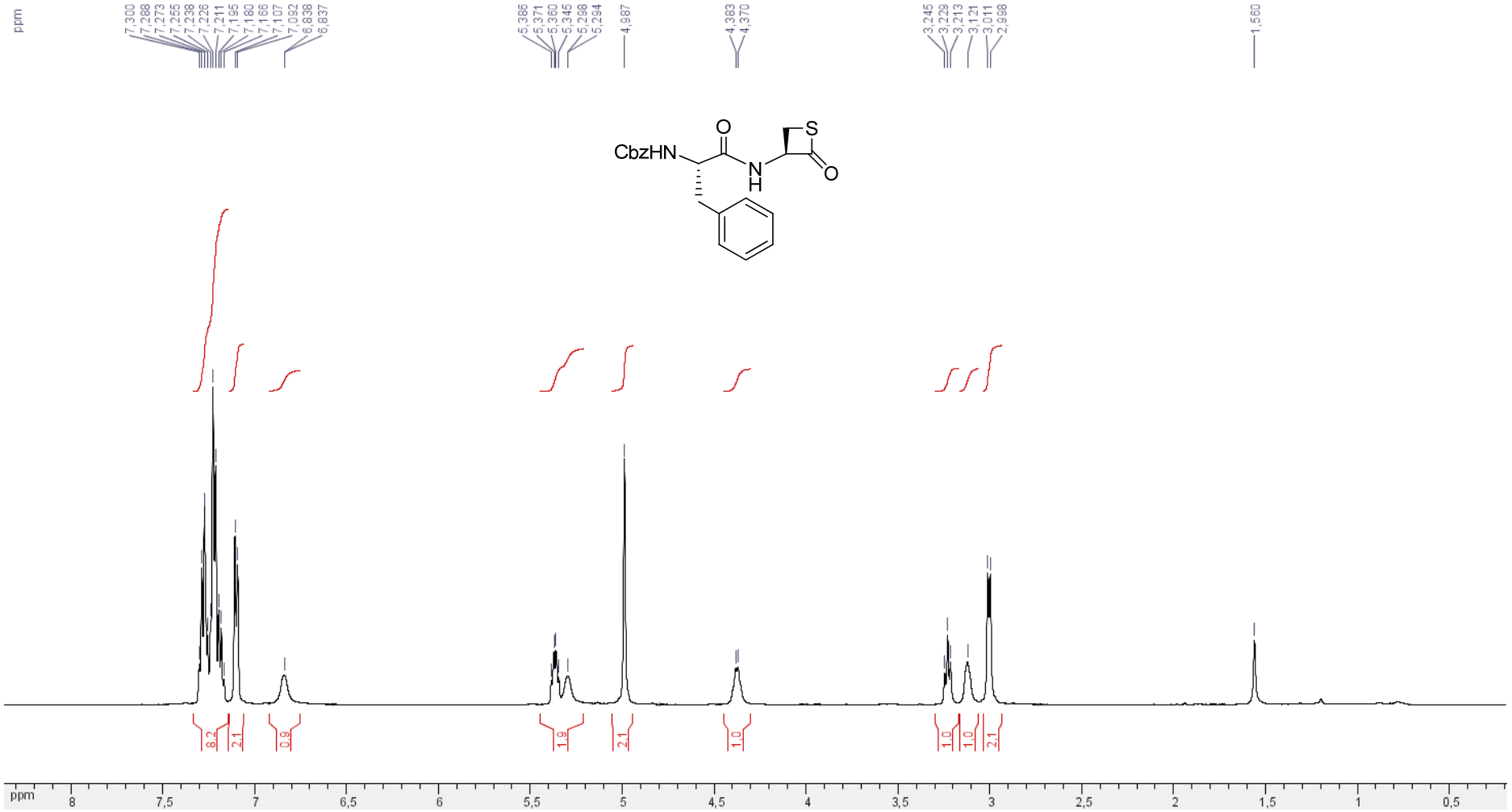

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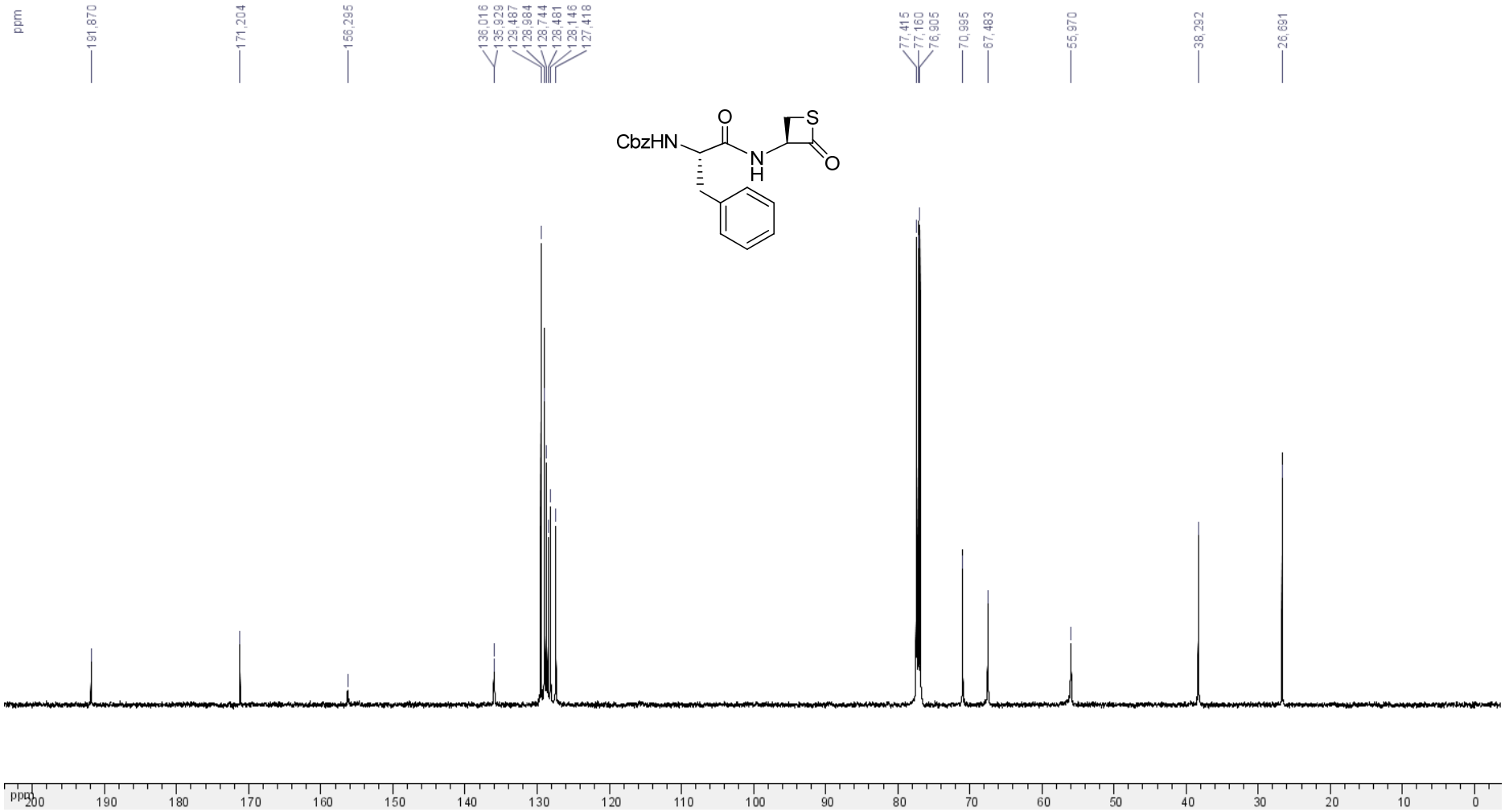

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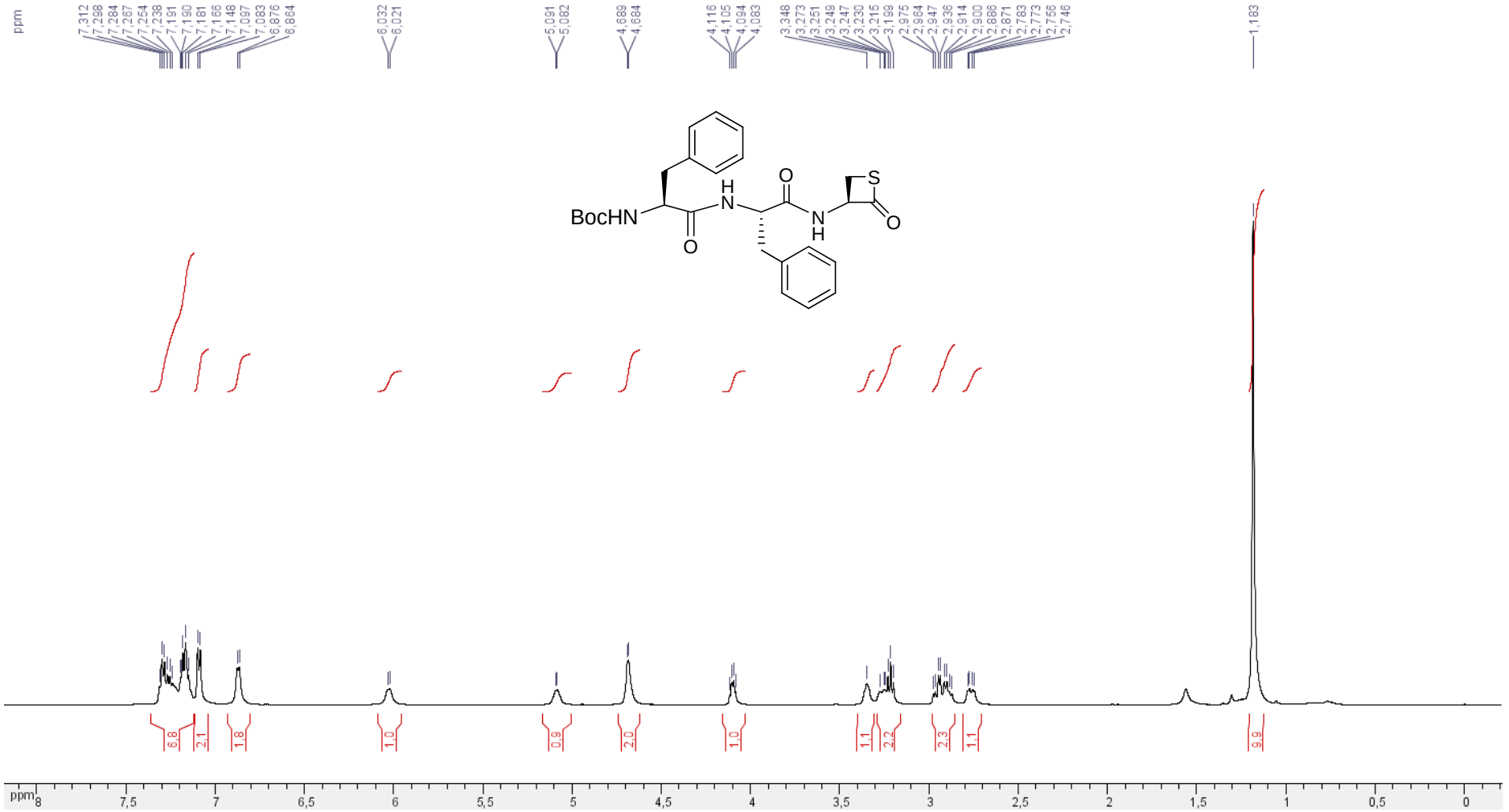
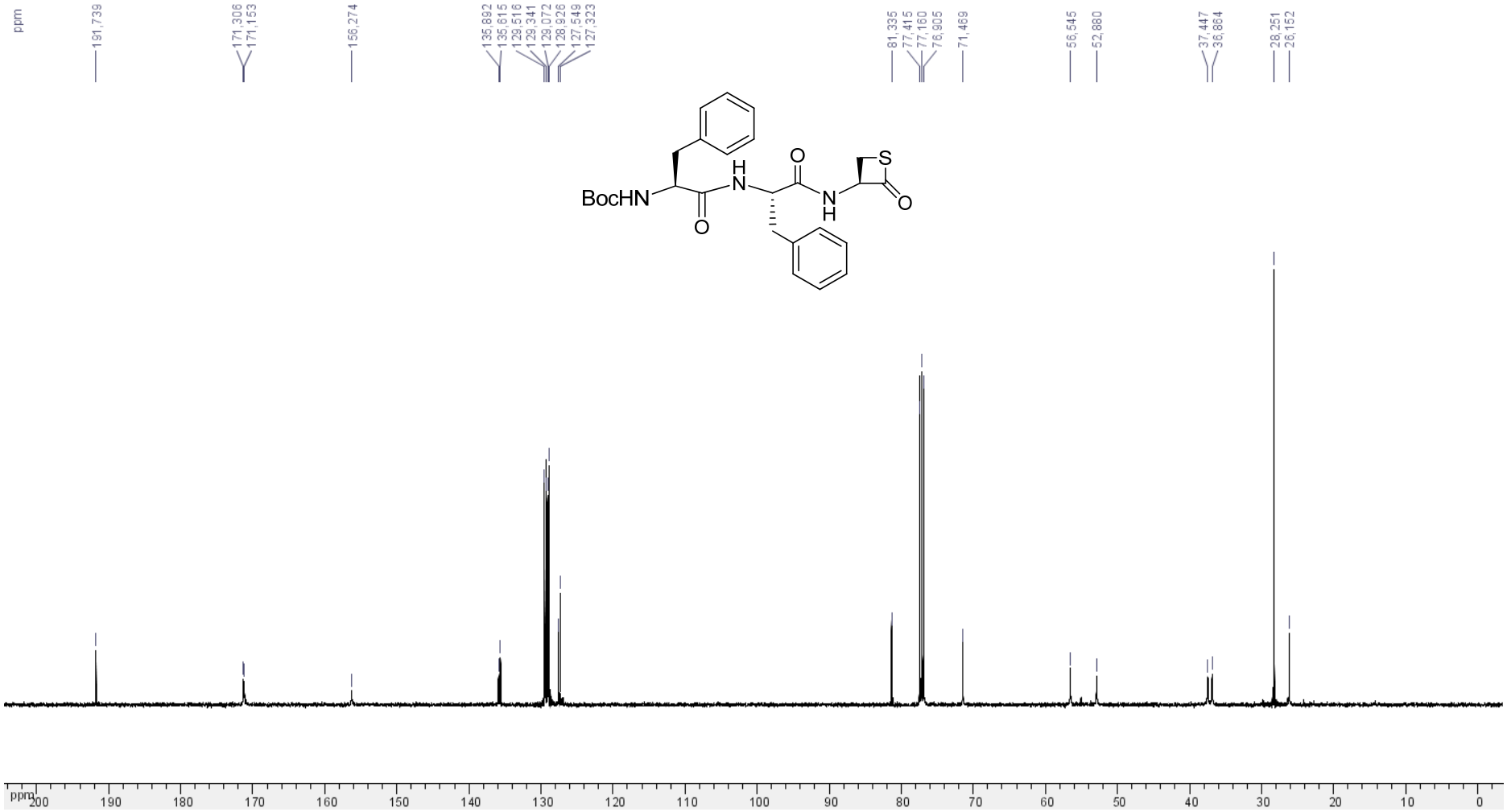
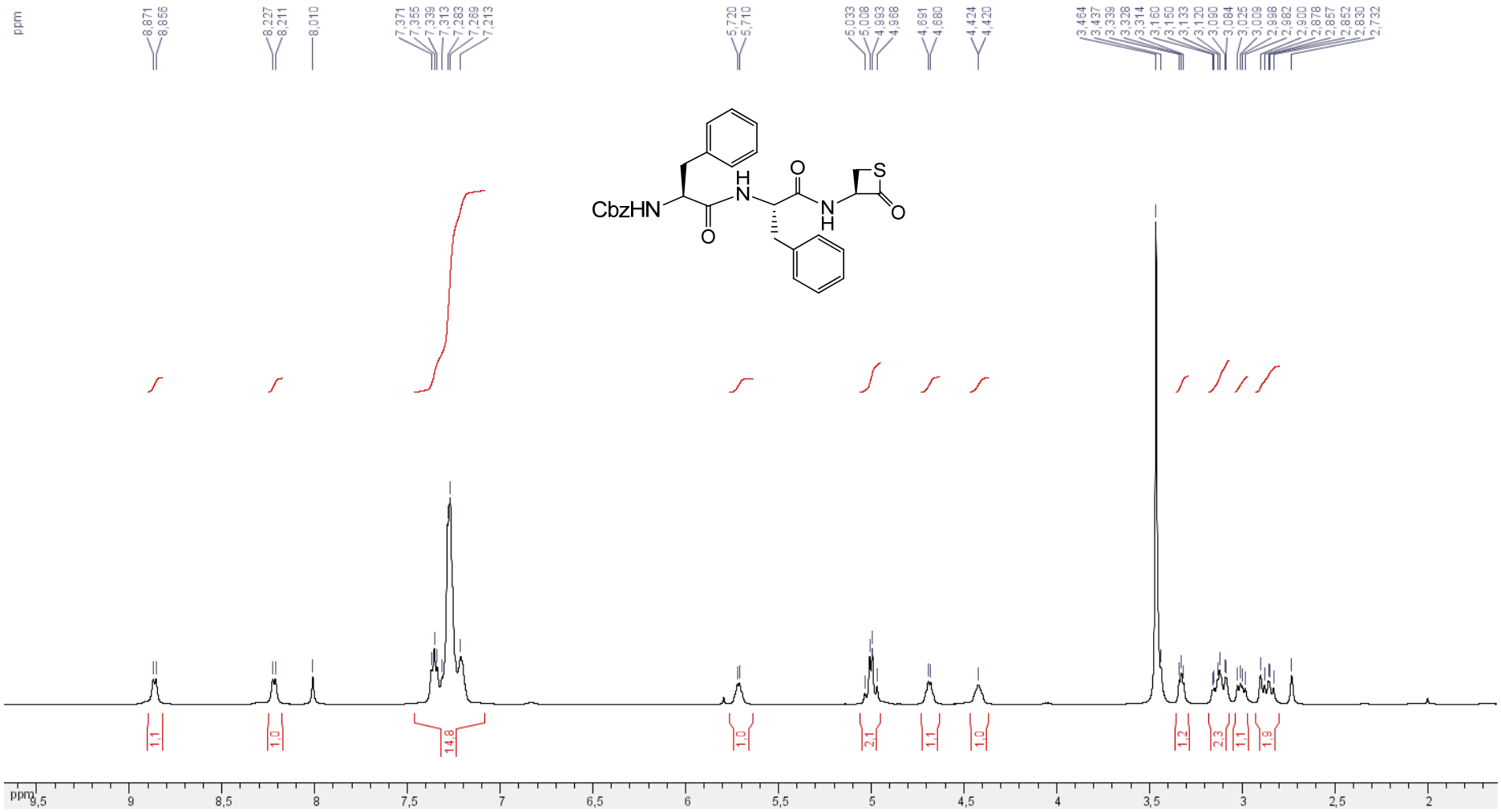


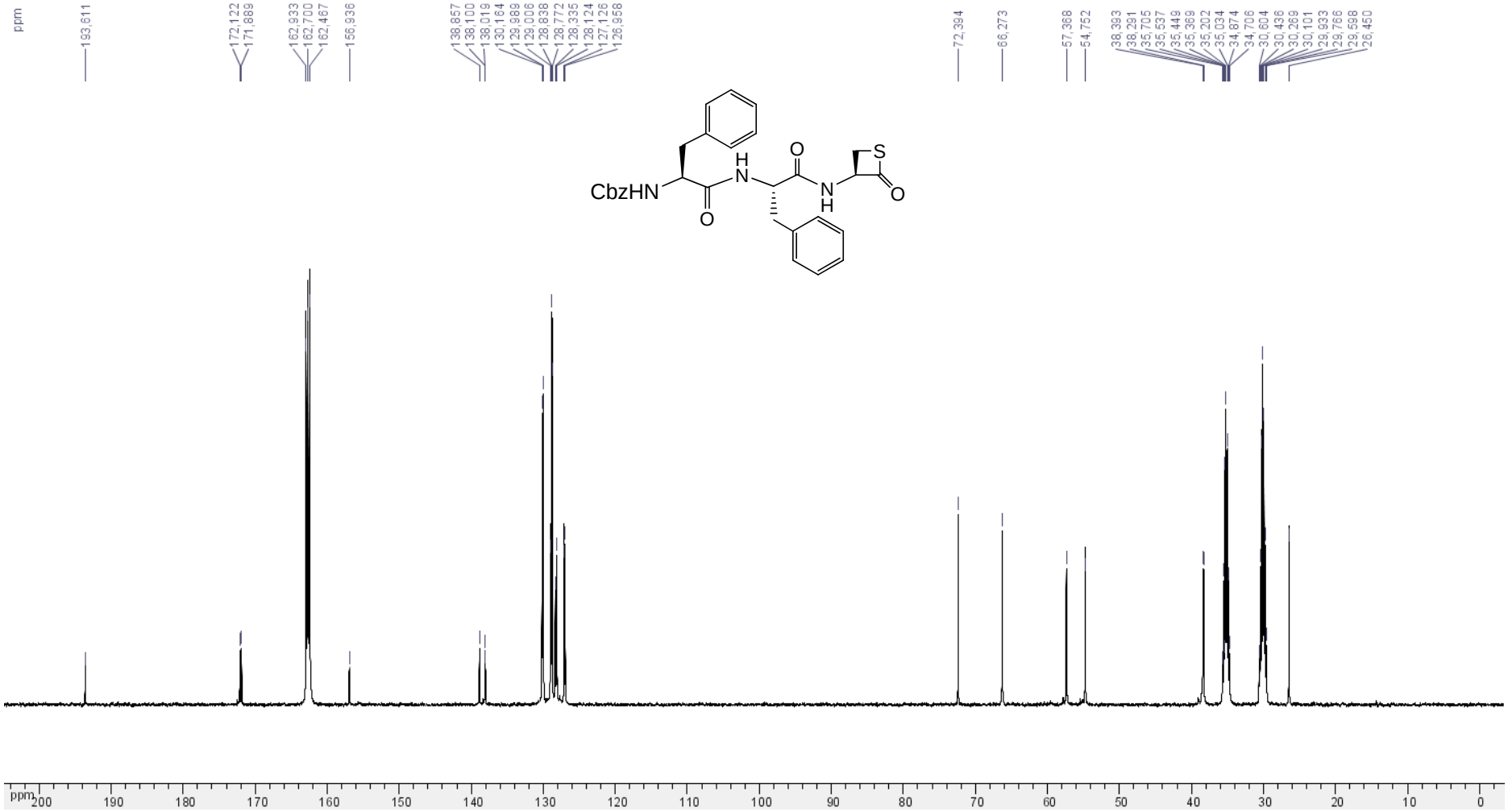
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (9) ^{13}C NMR (125 MHz, $CDCl_3$)



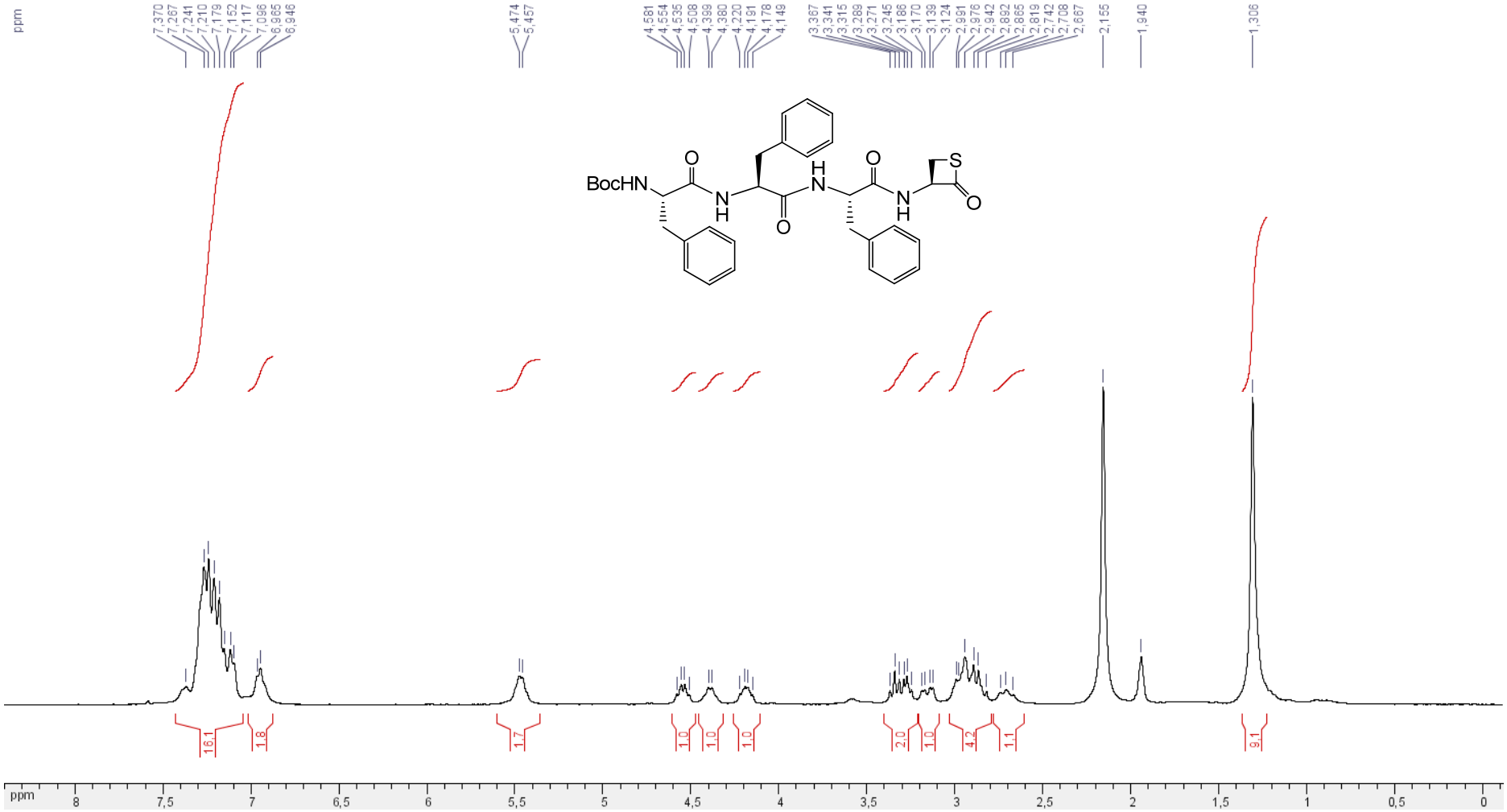
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (10) ^{1}H NMR (500 MHz, DMF-d_7)



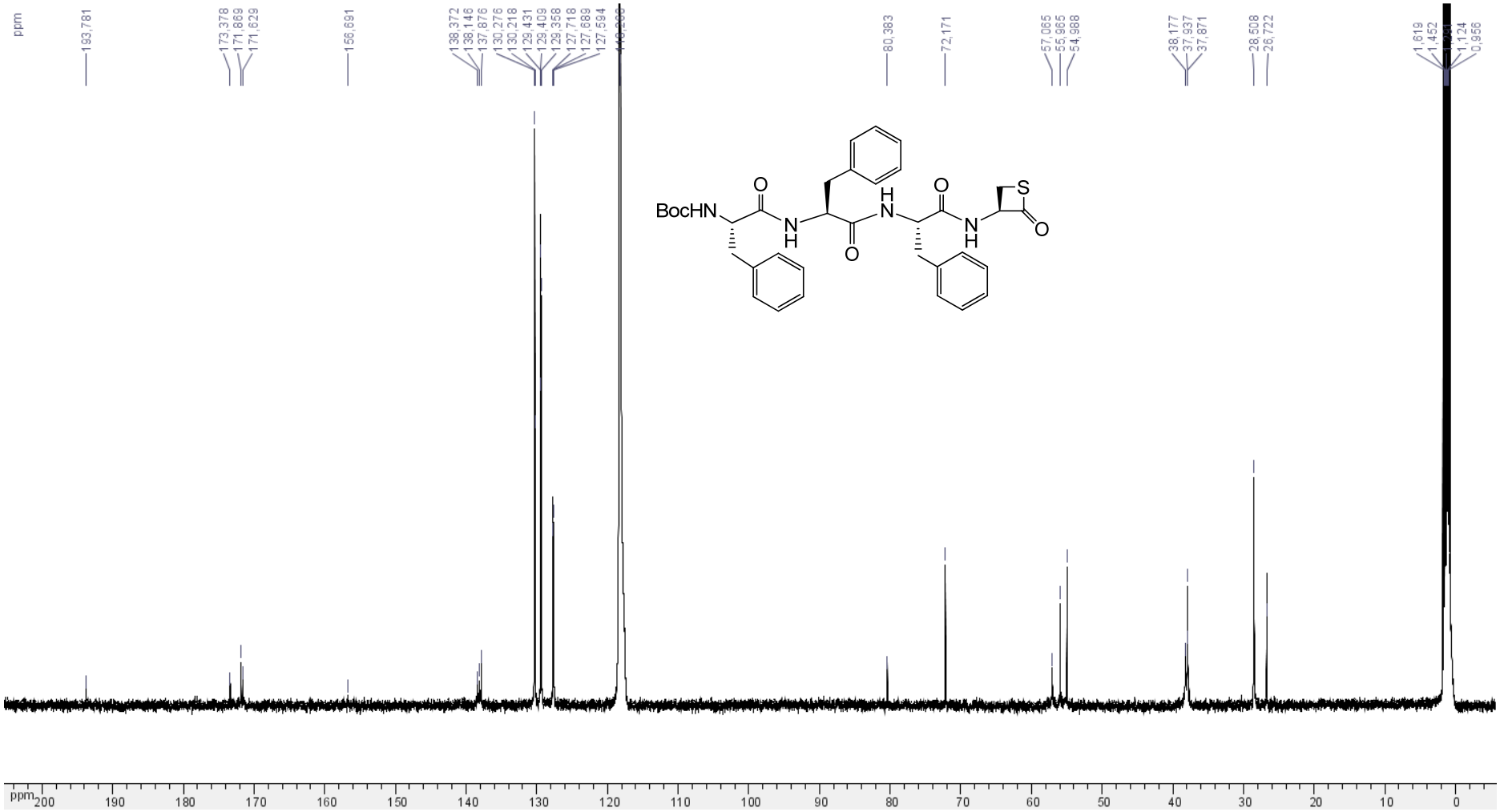
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (10) ^{13}C NMR (125 MHz, DMF-d_7)



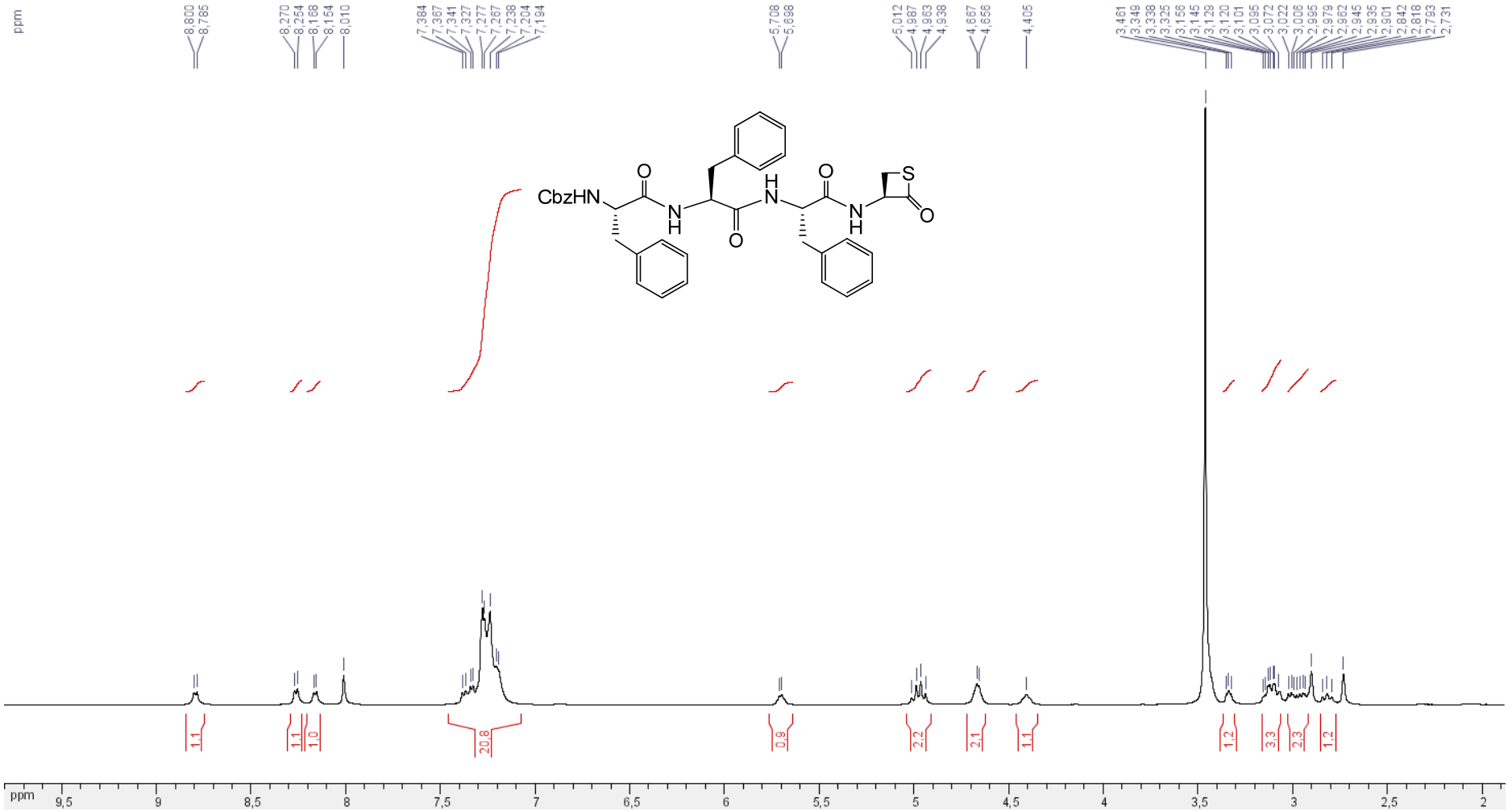
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (16) ^{1}H NMR (500 MHz, CD_3CN)



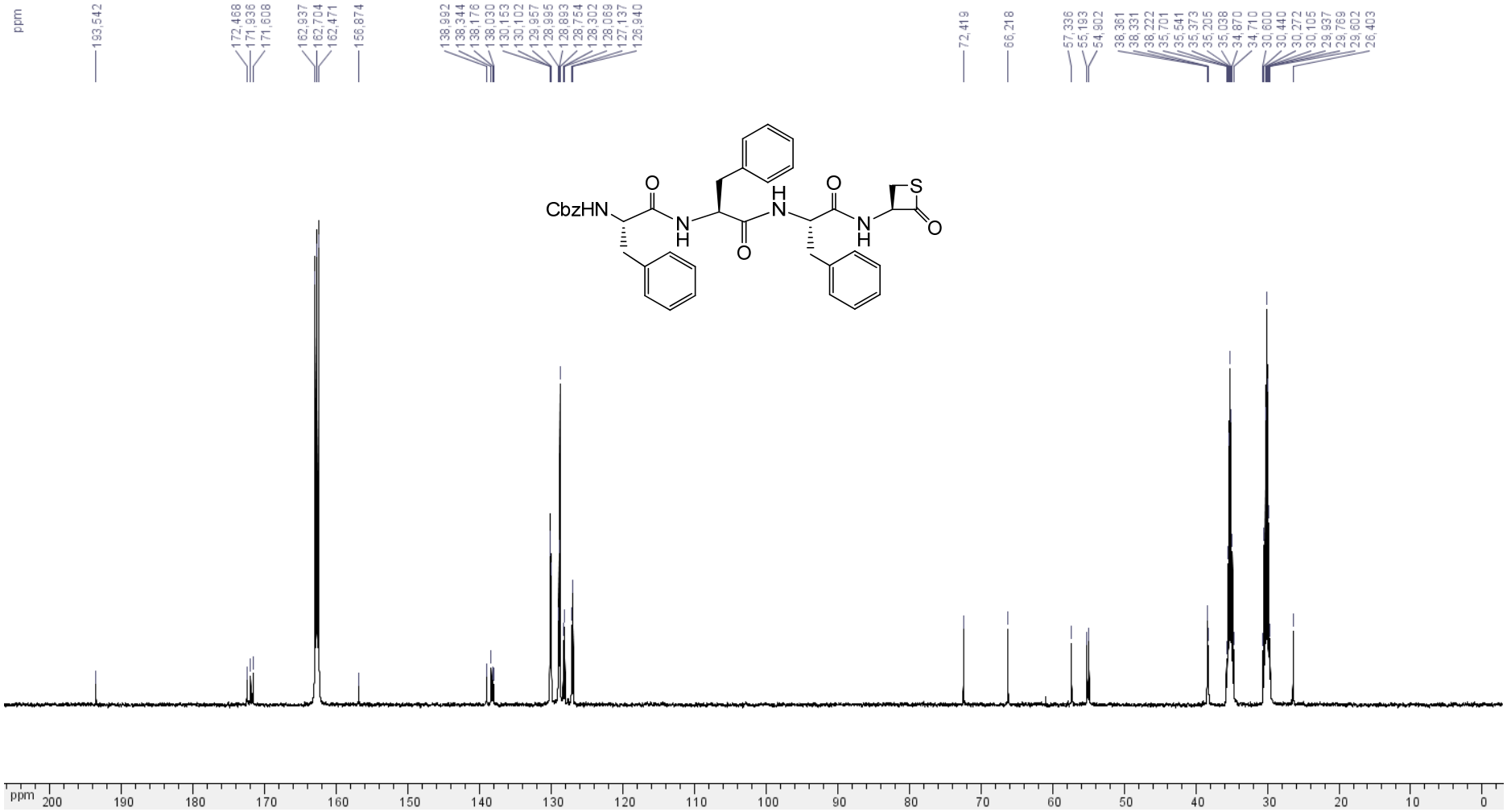
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (16) ^{13}C NMR (125 MHz, CD_3CN)



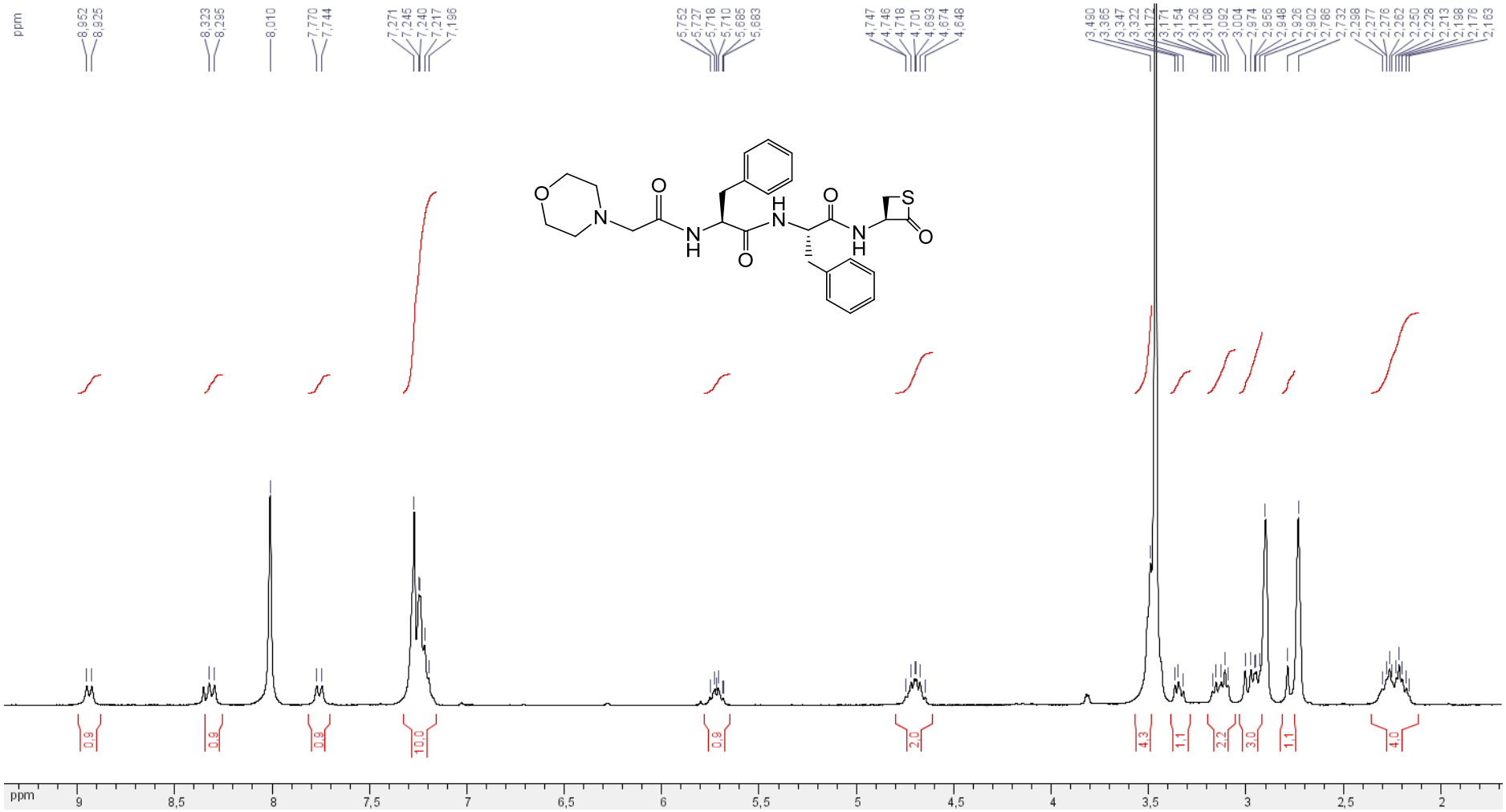
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (17) ^{1}H NMR (500 MHz, DMF-d_7)



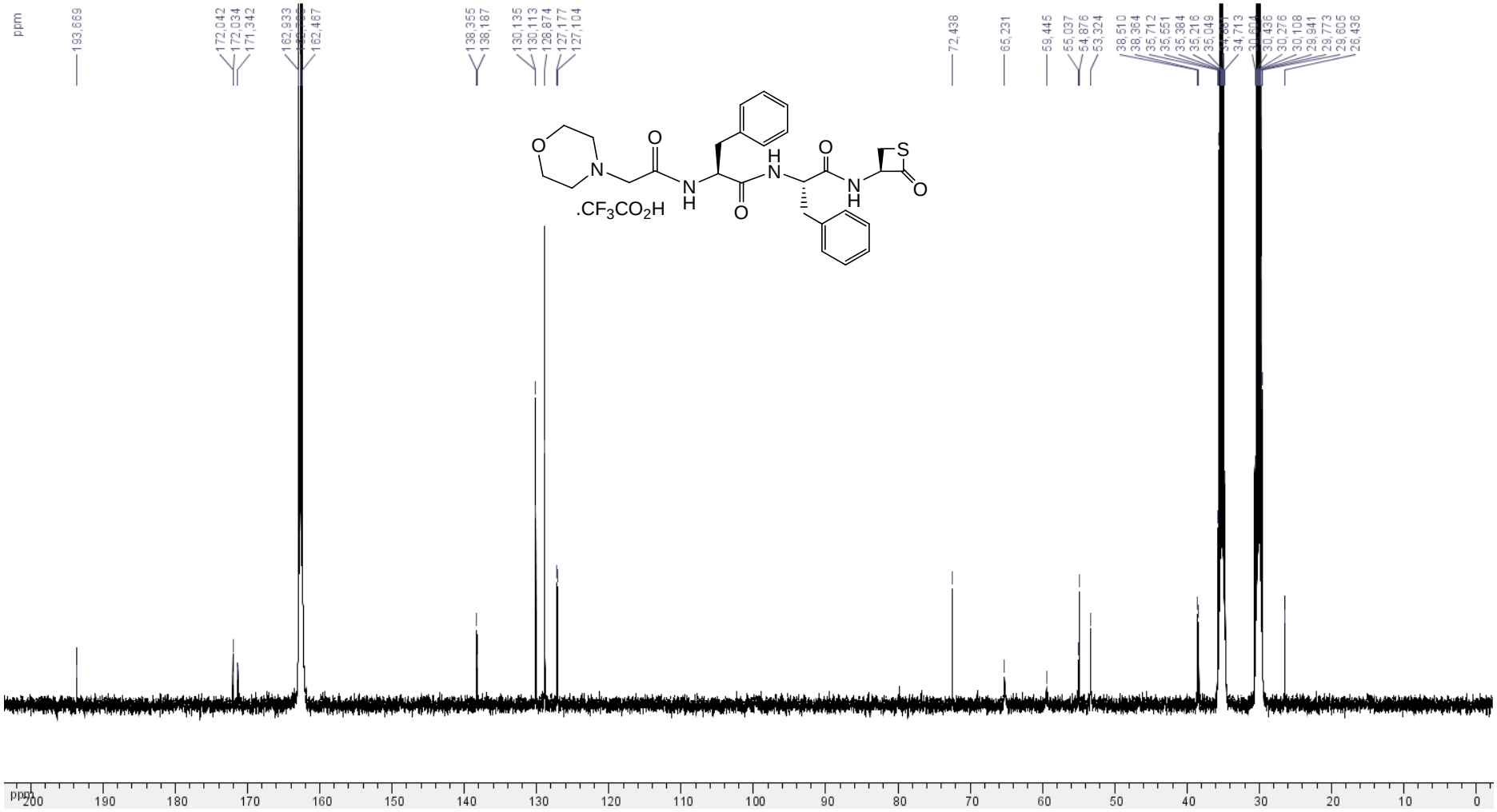
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (17) ^{13}C NMR (125 MHz, DMF-d_7)



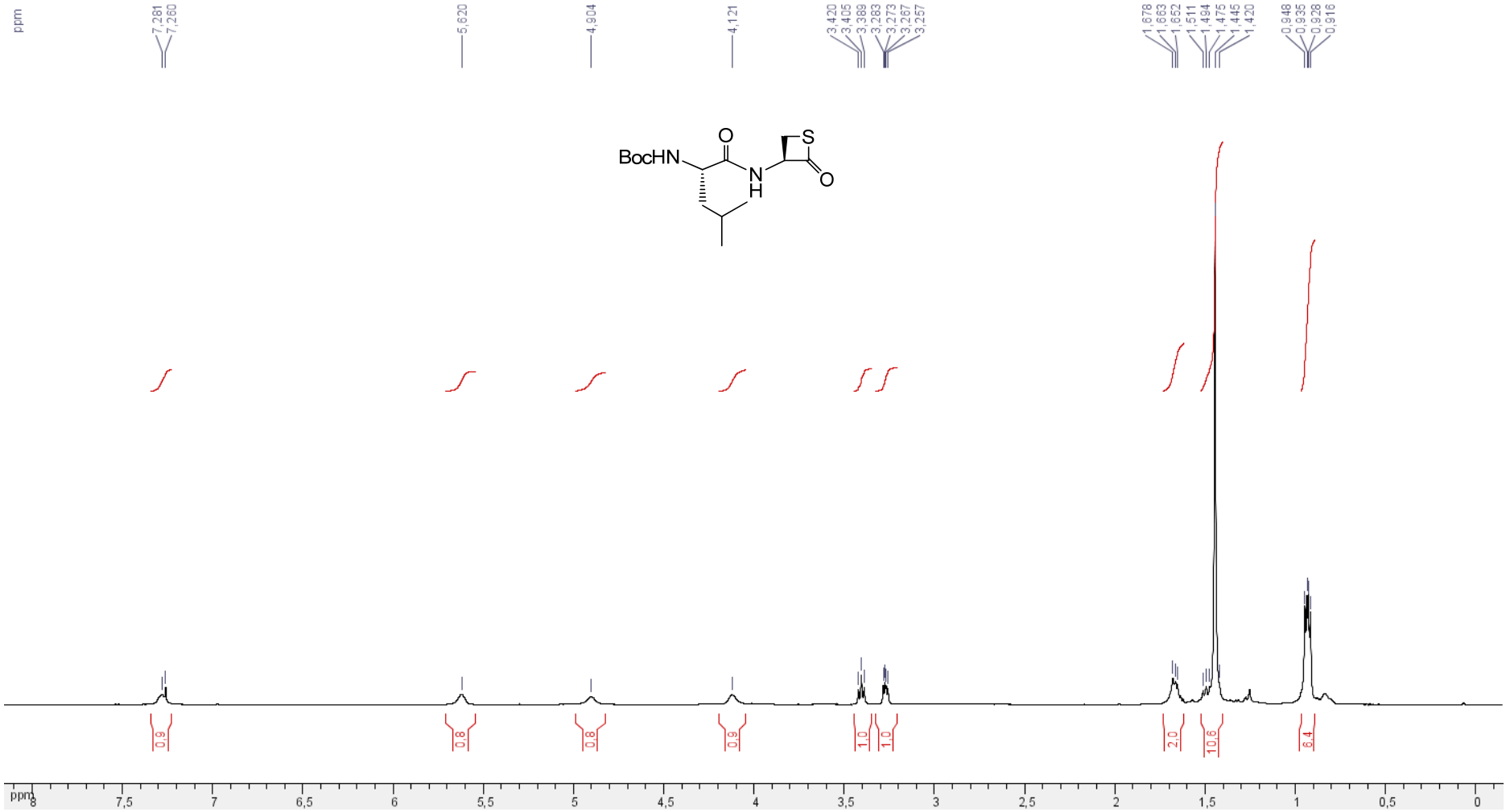
(3*S*)-3-(4-morpholinylacetyl-L-phenylalanyl- L-phenylalanylamino)thietan-2-one (26) ^{1}H NMR (300 MHz, DMF-d_7)



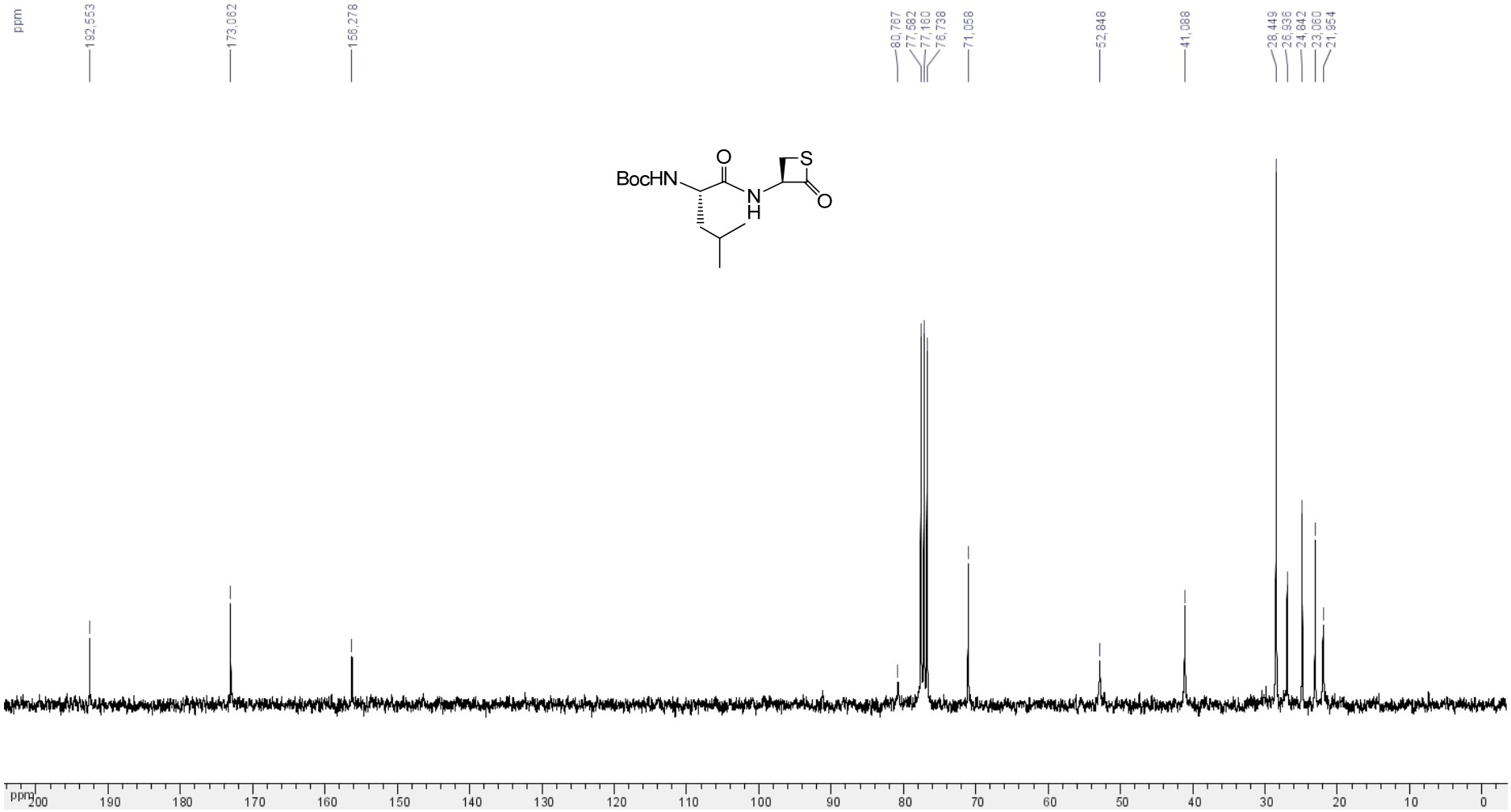
(3*S*)-3-(4-morpholinylacetyl-L-phenylalanyl- L-phenylalanylamino)thietan-2-one (26) ^{13}C NMR (125 MHz, DMF-d_7)



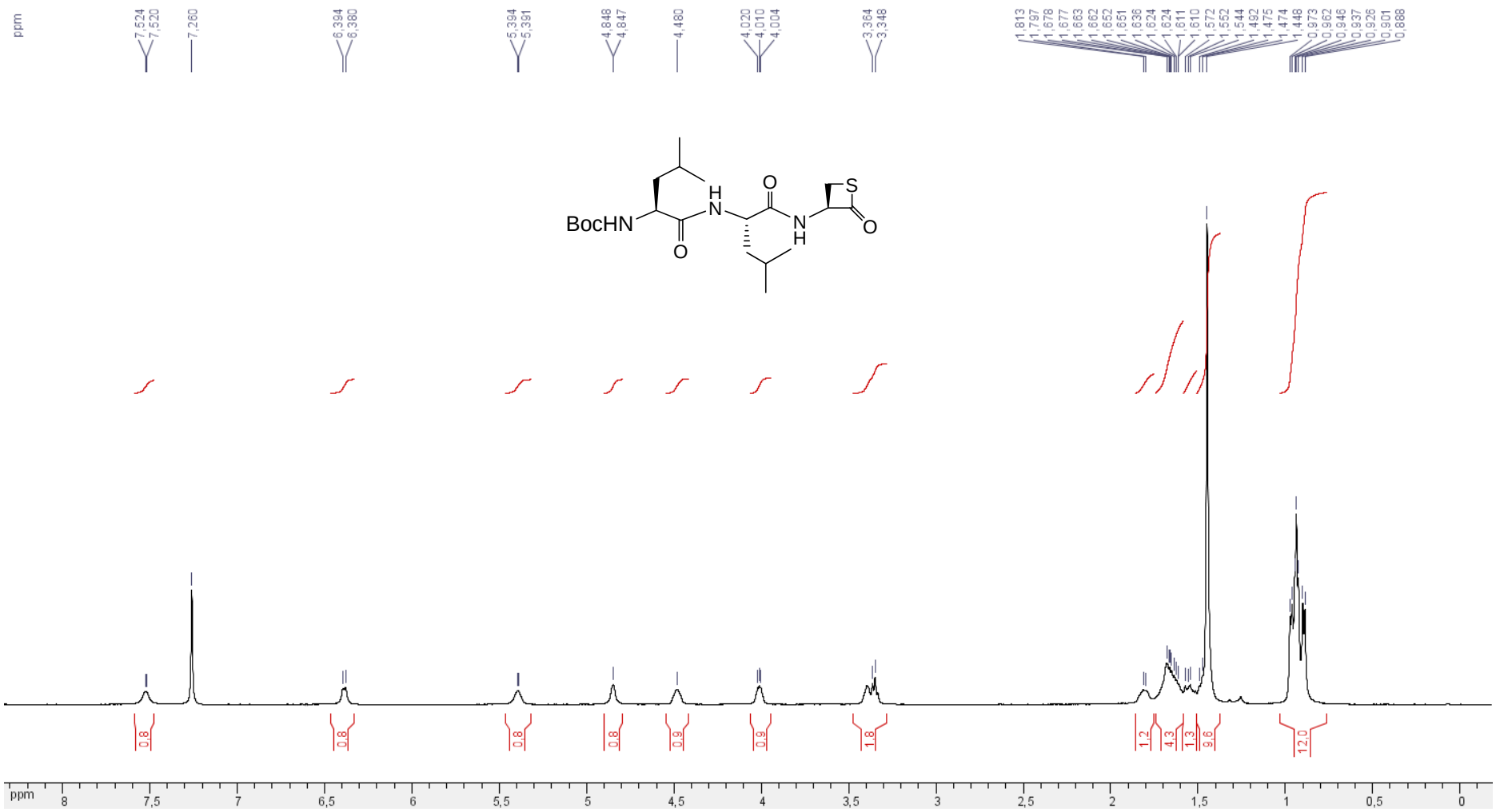
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-leucylamino)thietan-2-one (**7**) ^{1}H NMR (500 MHz, $CDCl_3$)



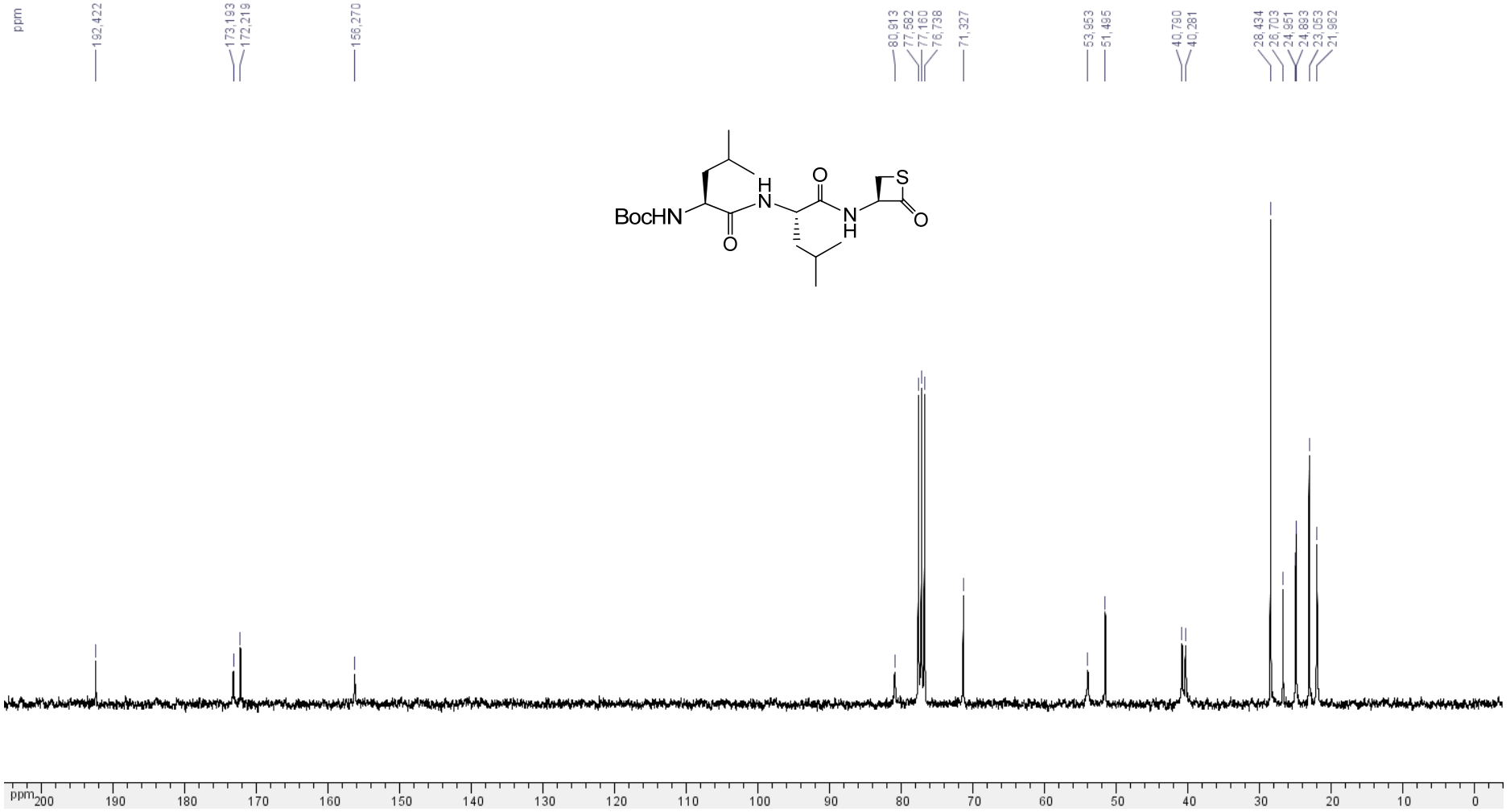
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-leucylamino)thietan-2-one (**7**) ^{13}C NMR (75 MHz, $CDCl_3$)



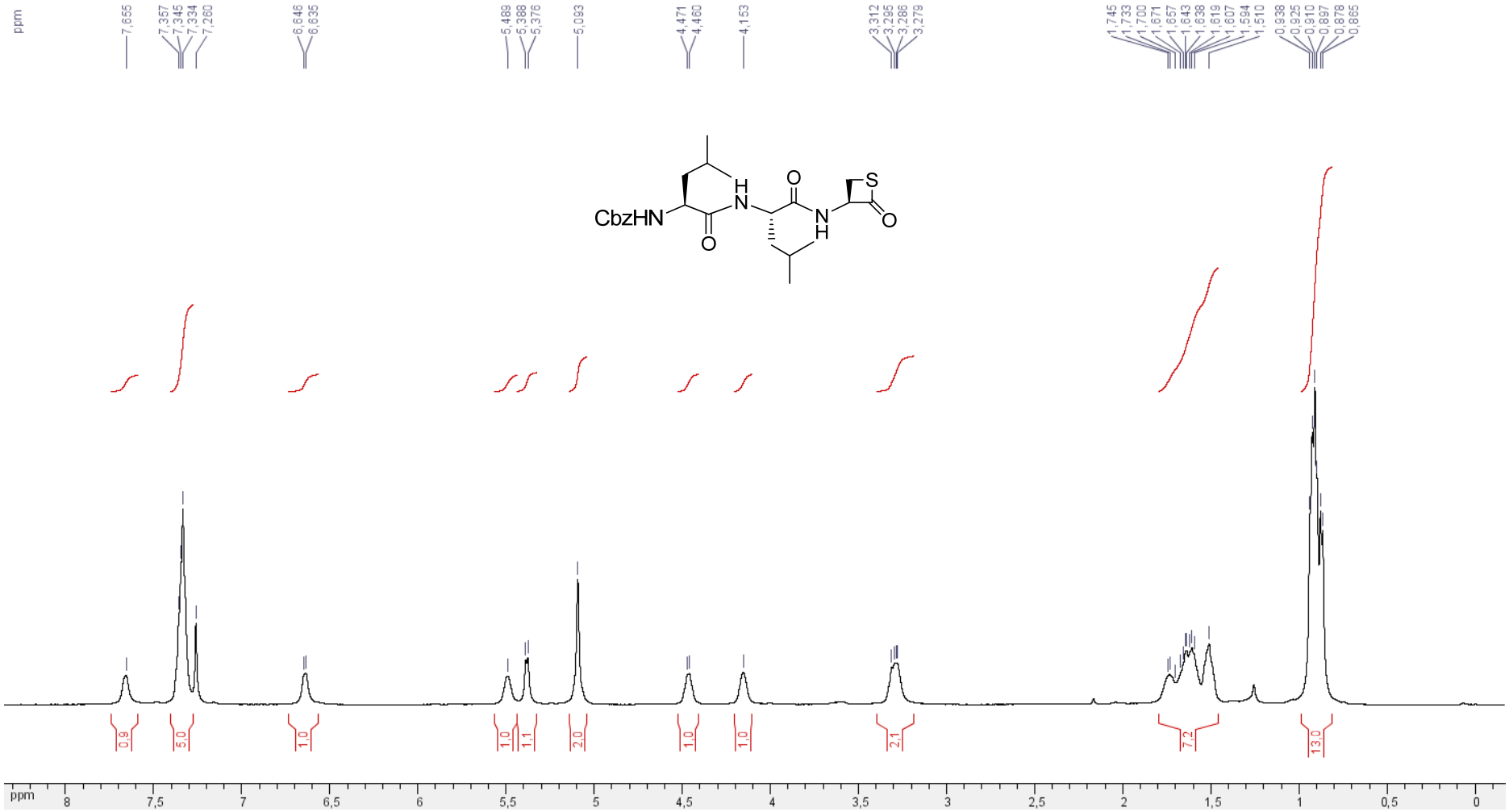
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-leucyl-L-leucylamino)thietan-2-one (11) ^{1}H NMR (500 MHz, $CDCl_3$)



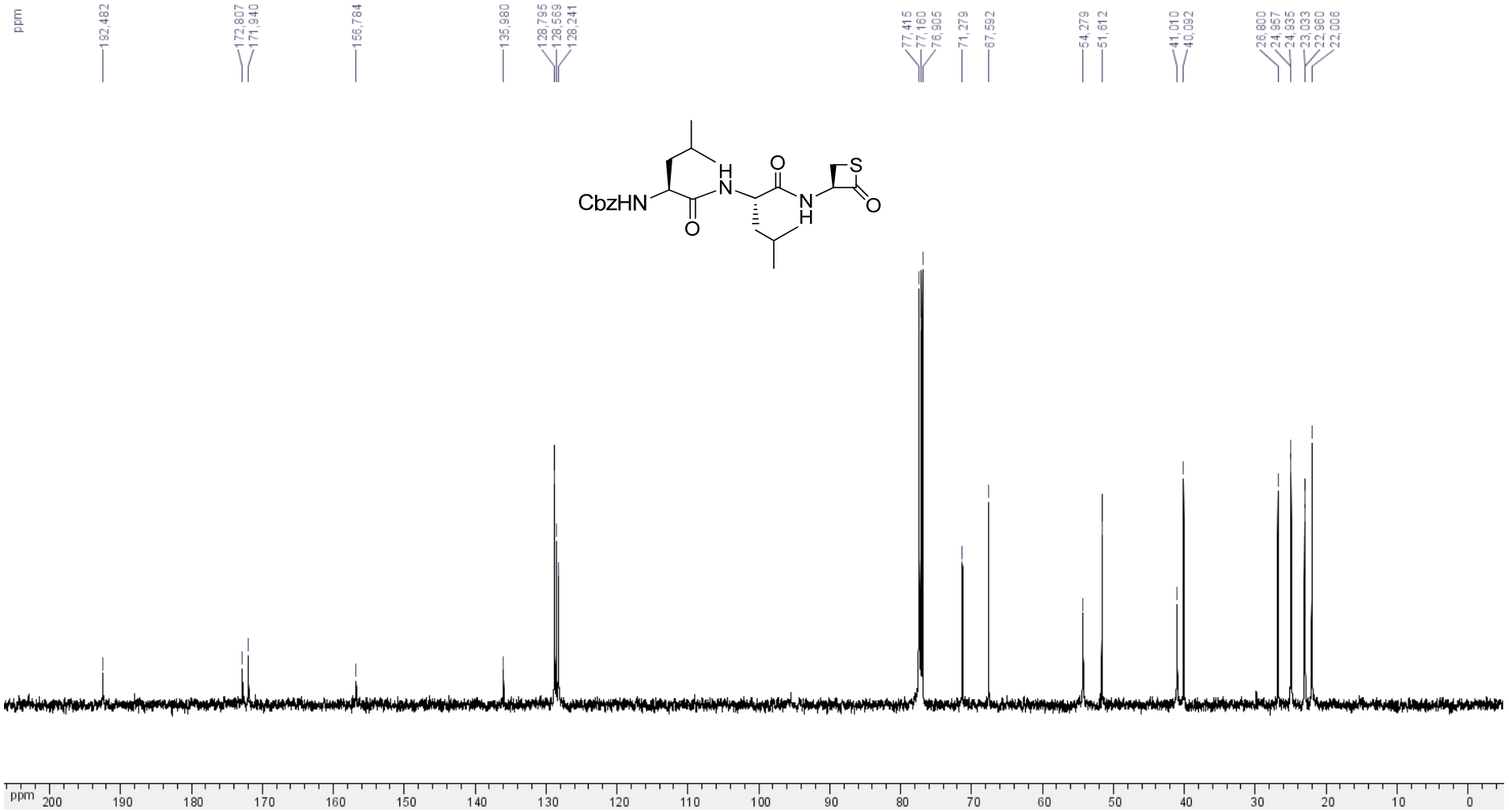
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-leucyl-L-leucylamino)thietan-2-one (11) ^{13}C NMR (75 MHz, $CDCl_3$)



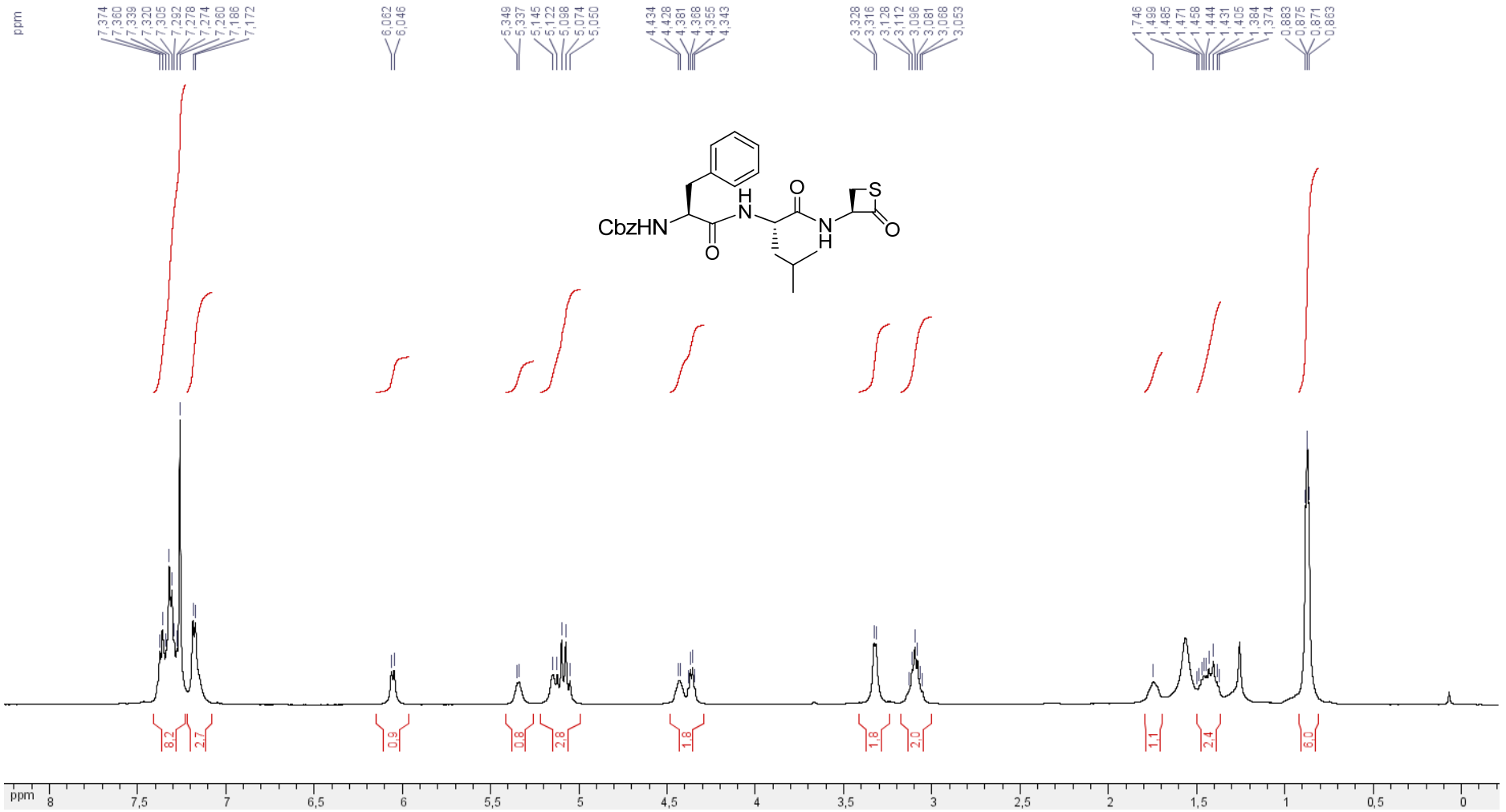
(3*S*)-3-(*N*-benzyloxycarbonyl-L-leucyl-L-leucylamino)thietan-2-one (12) ^{1}H NMR (500 MHz, $CDCl_3$)



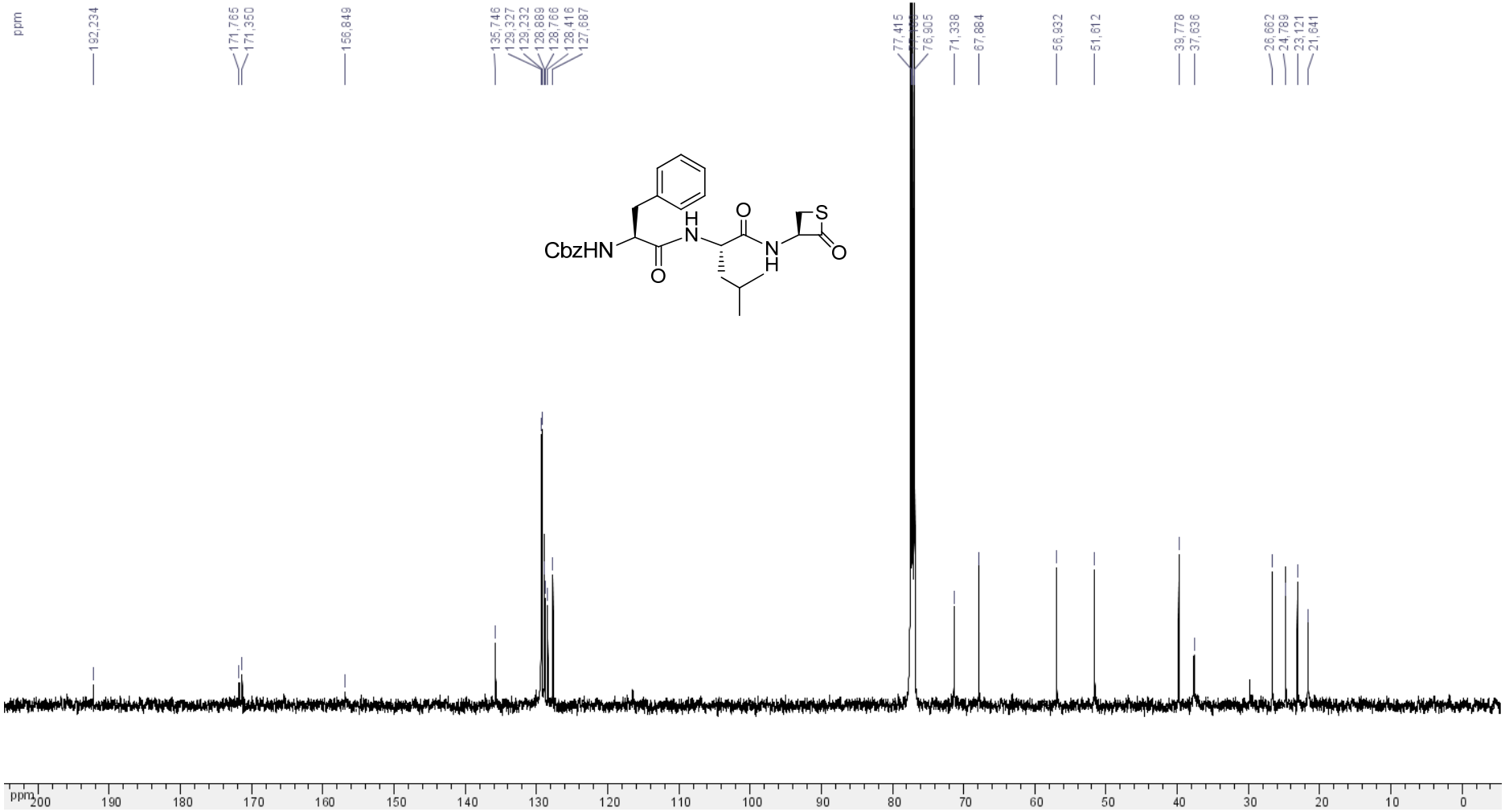
(3*S*)-3-(*N*-benzyloxycarbonyl-L-leucyl-L-leucylamino)thietan-2-one (12) ^{13}C NMR (125 MHz, $CDCl_3$)



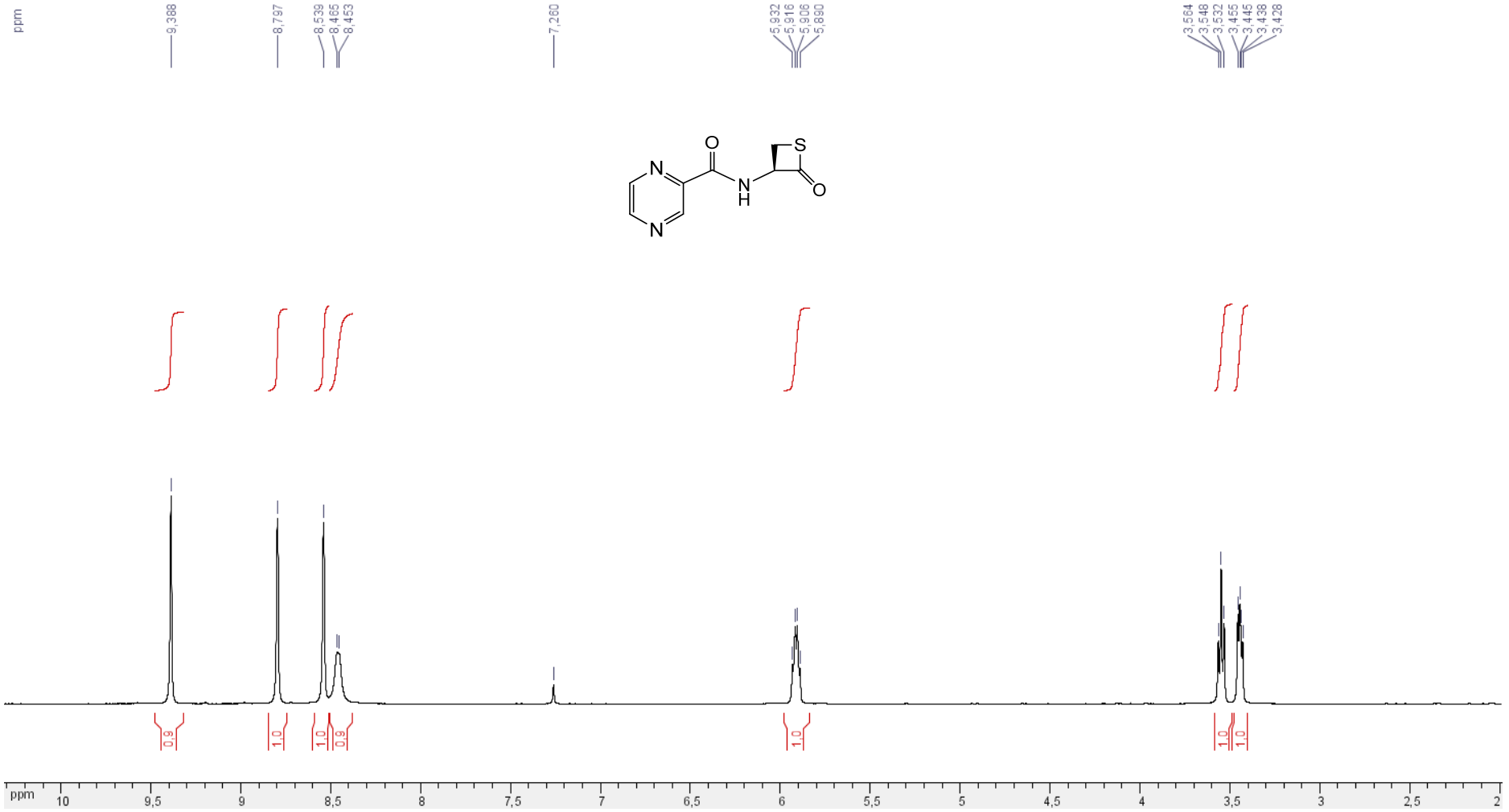
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-leucylamino)thietan-2-one (13) ^{1}H NMR (500 MHz, $CDCl_3$)



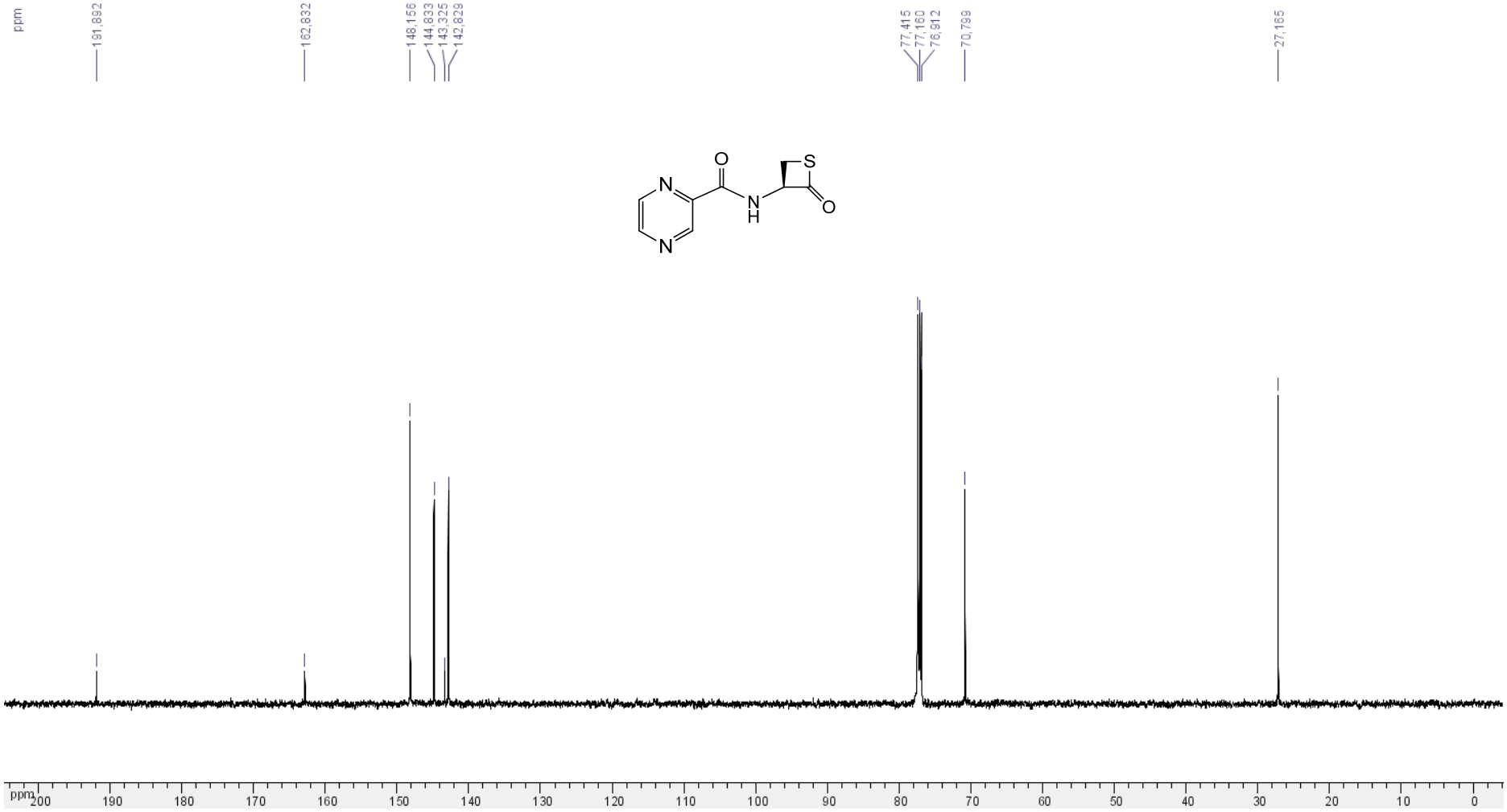
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-leucylamino)thietan-2-one (13) ^{13}C NMR (125 MHz, $CDCl_3$)



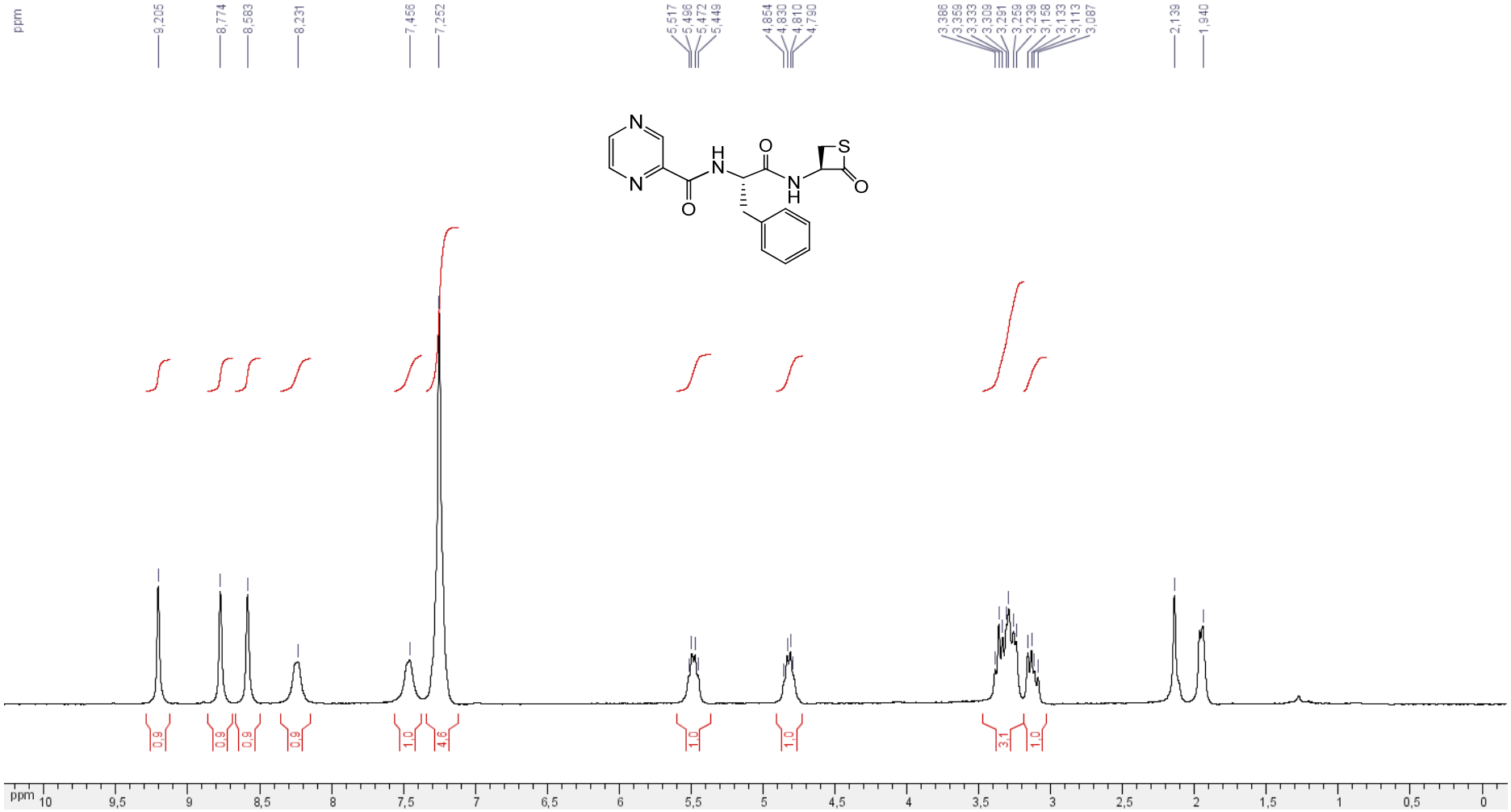
(3*S*)-3-(pyrazin-2-carbonylamino)thietan-2-one (21) ^{1}H NMR (500 MHz, $CDCl_3$)



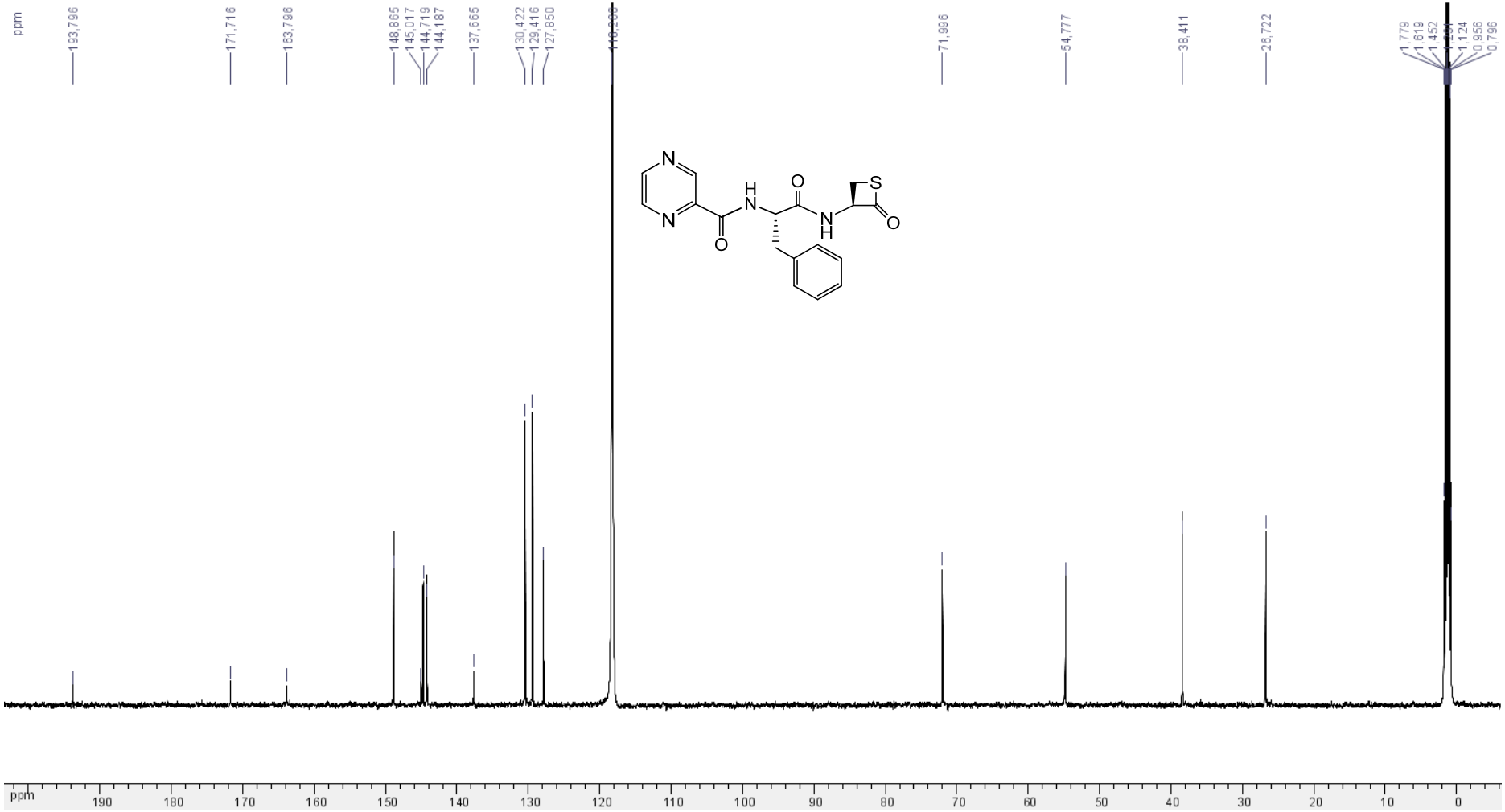
(3*S*)-3-(pyrazin-2-carbonylamino)thietan-2-one (21) ^{13}C NMR (125 MHz, $CDCl_3$)



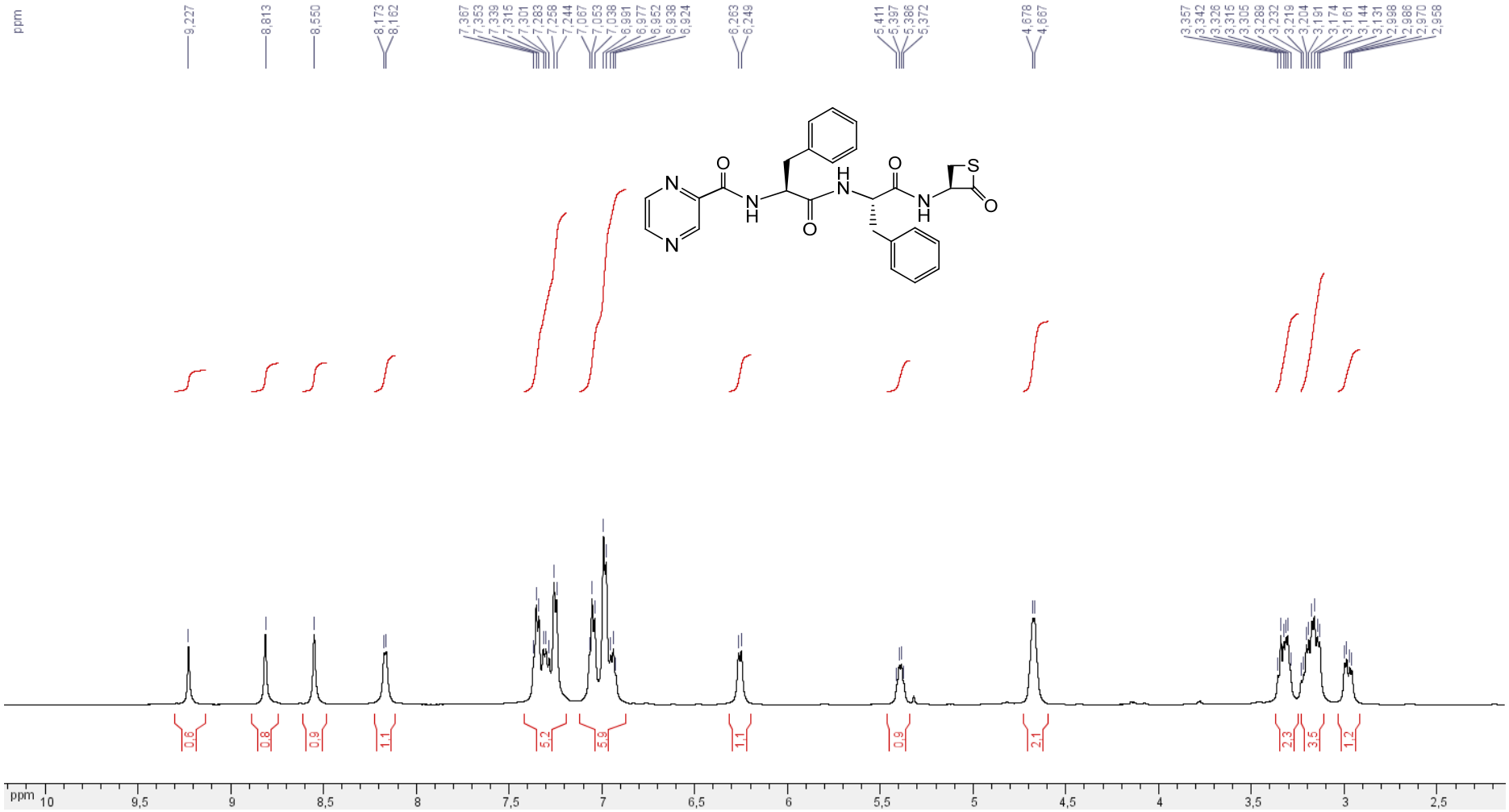
(3*S*)-3-(pyrazin-2-carbonyl-L-phenylalanylamino)thietan-2-one (24) ^{1}H NMR (300 MHz, CD_3CN)



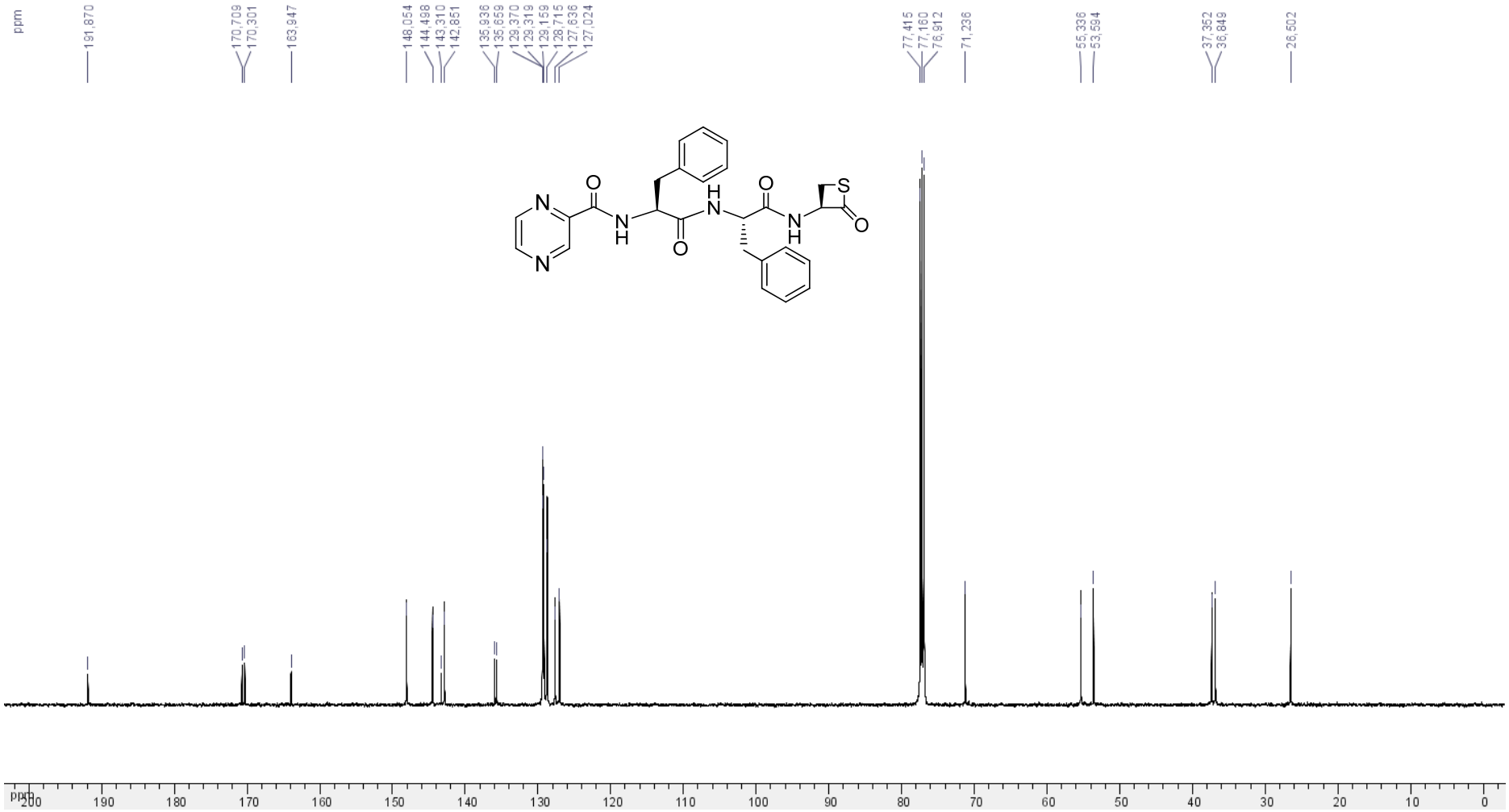
(3*S*)-3-(pyrazin-2-carbonyl-L-phenylalanylamino)thietan-2-one (24) ^{13}C NMR (125 MHz, CD_3CN)



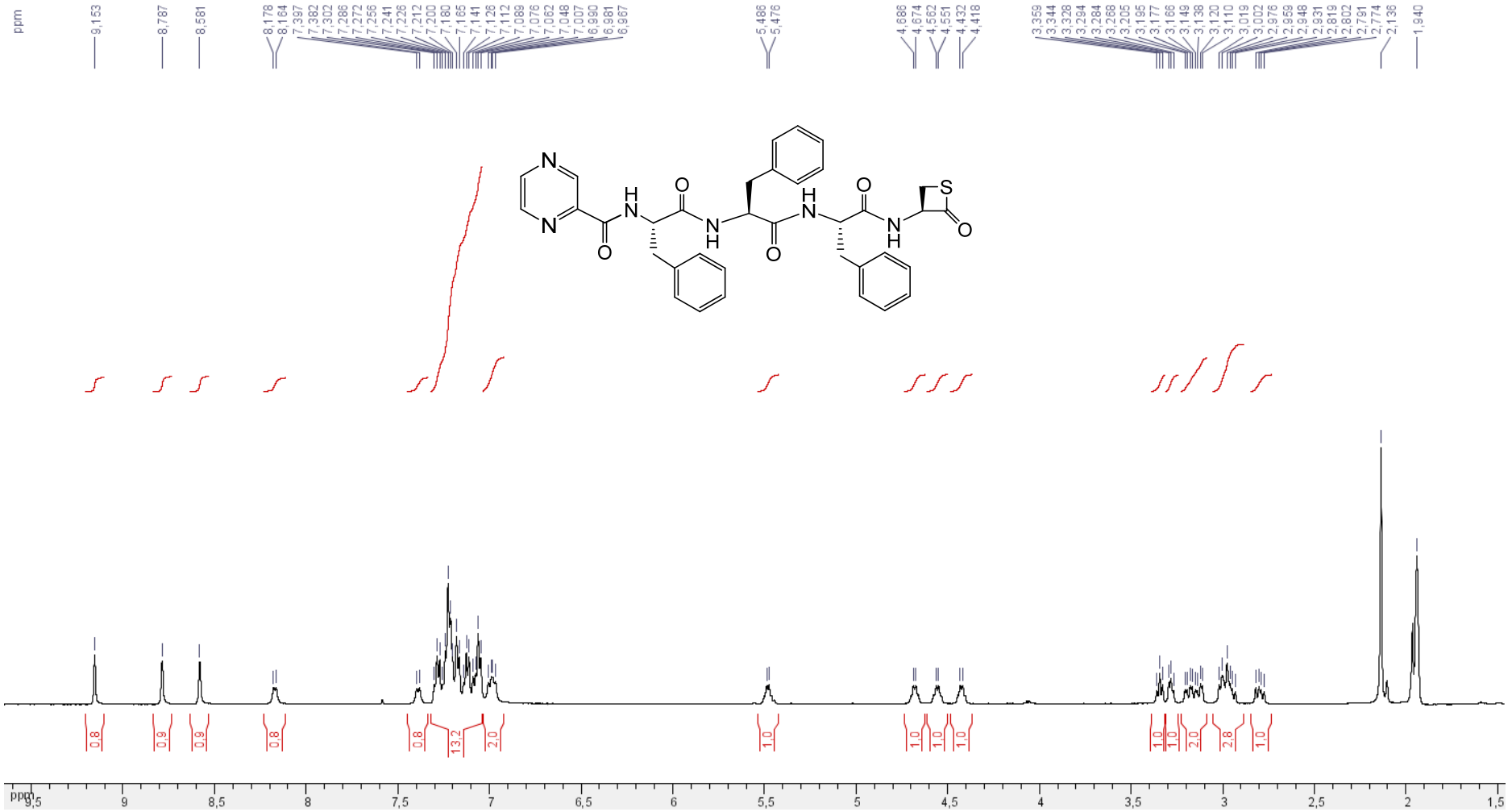
(3*S*)-3-(pyrazin-2-carbonyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (**22**) ^{1}H NMR (500 MHz, $CDCl_3$)



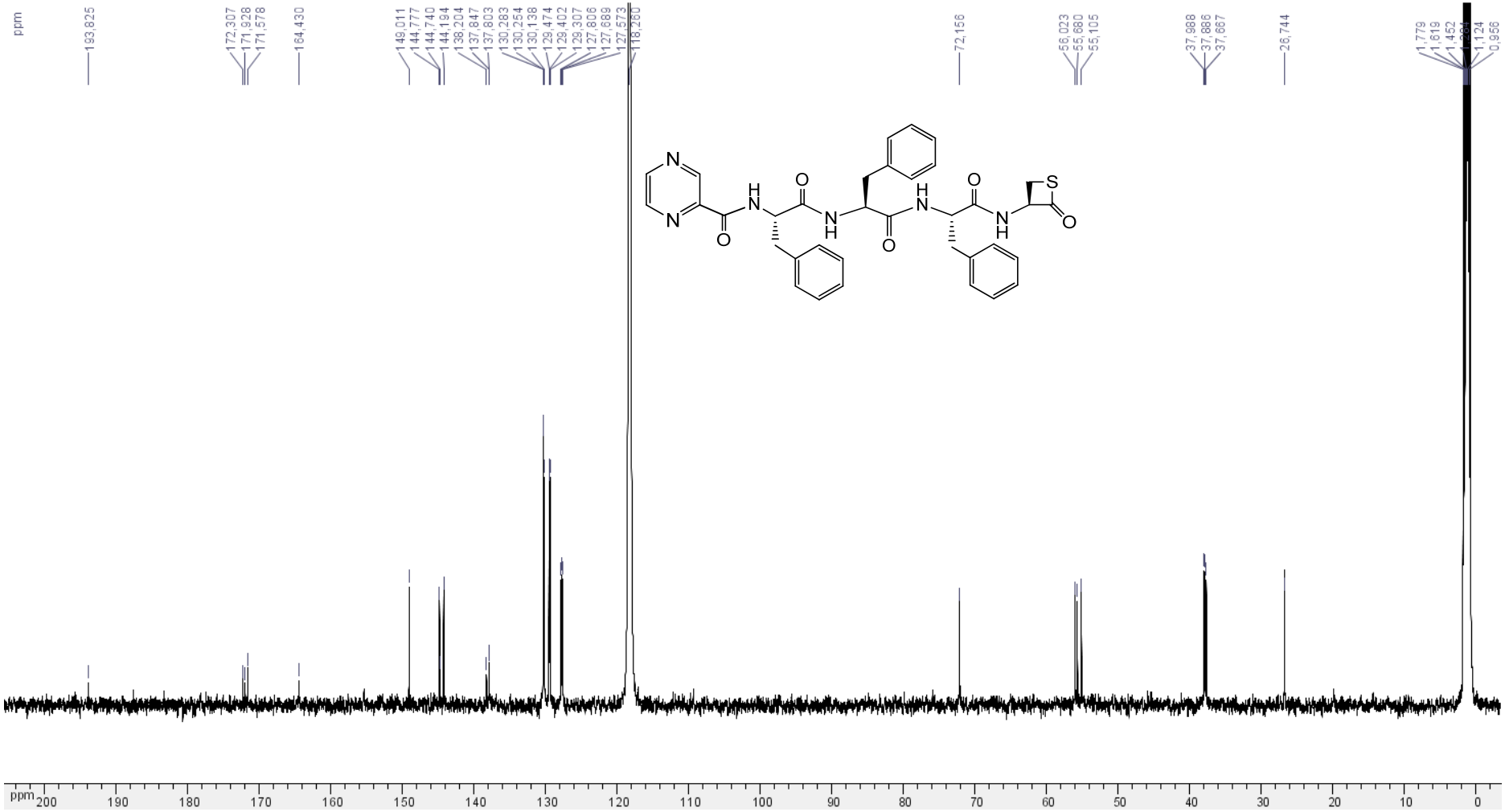
(3*S*)-3-(pyrazin-2-carbonyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (**22**) ^{13}C NMR (125 MHz, $CDCl_3$)



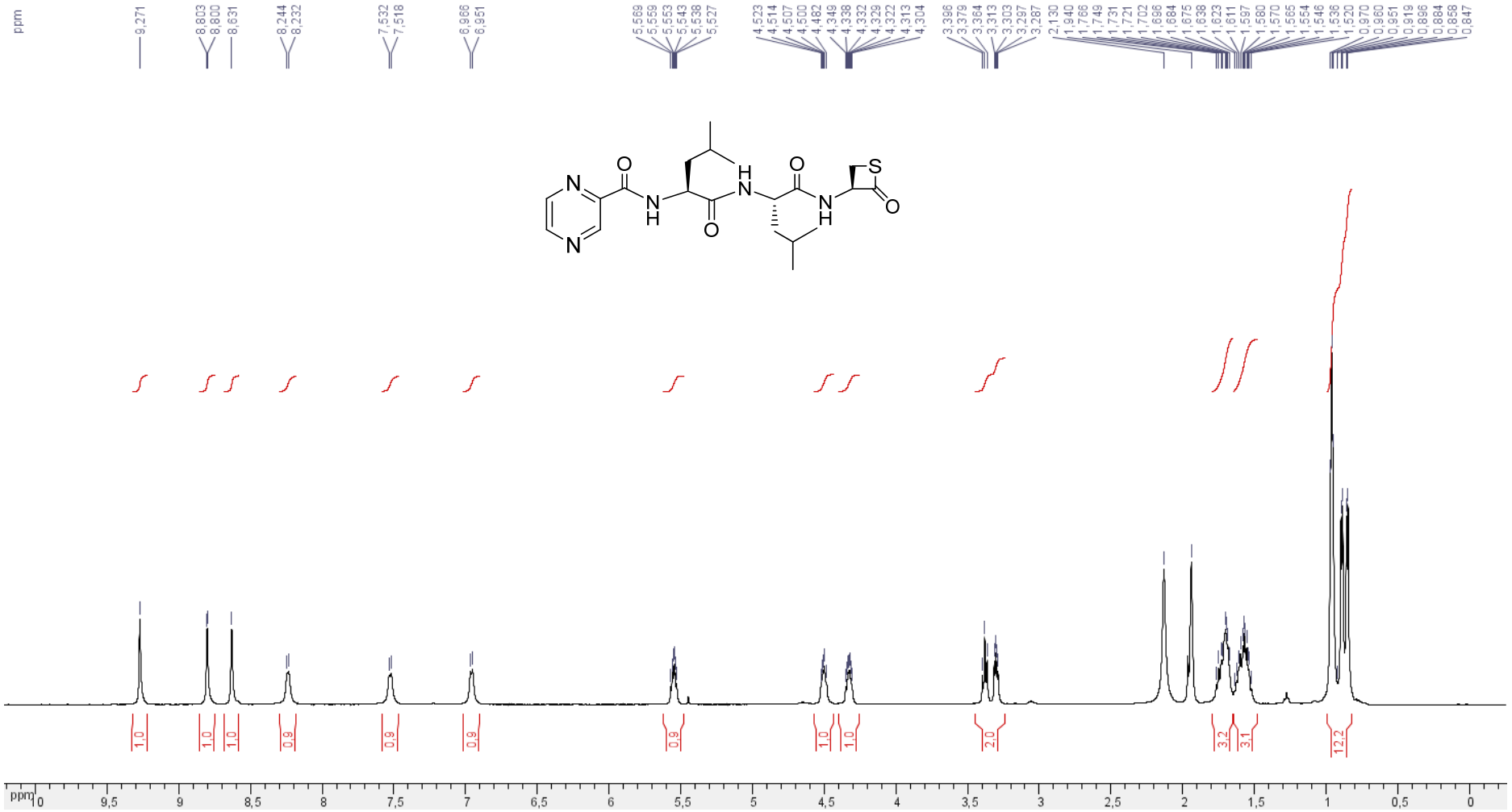
(3*S*)-3-(pyrazin-2-carbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (25) ^{1}H NMR (500 MHz, CD_3CN)



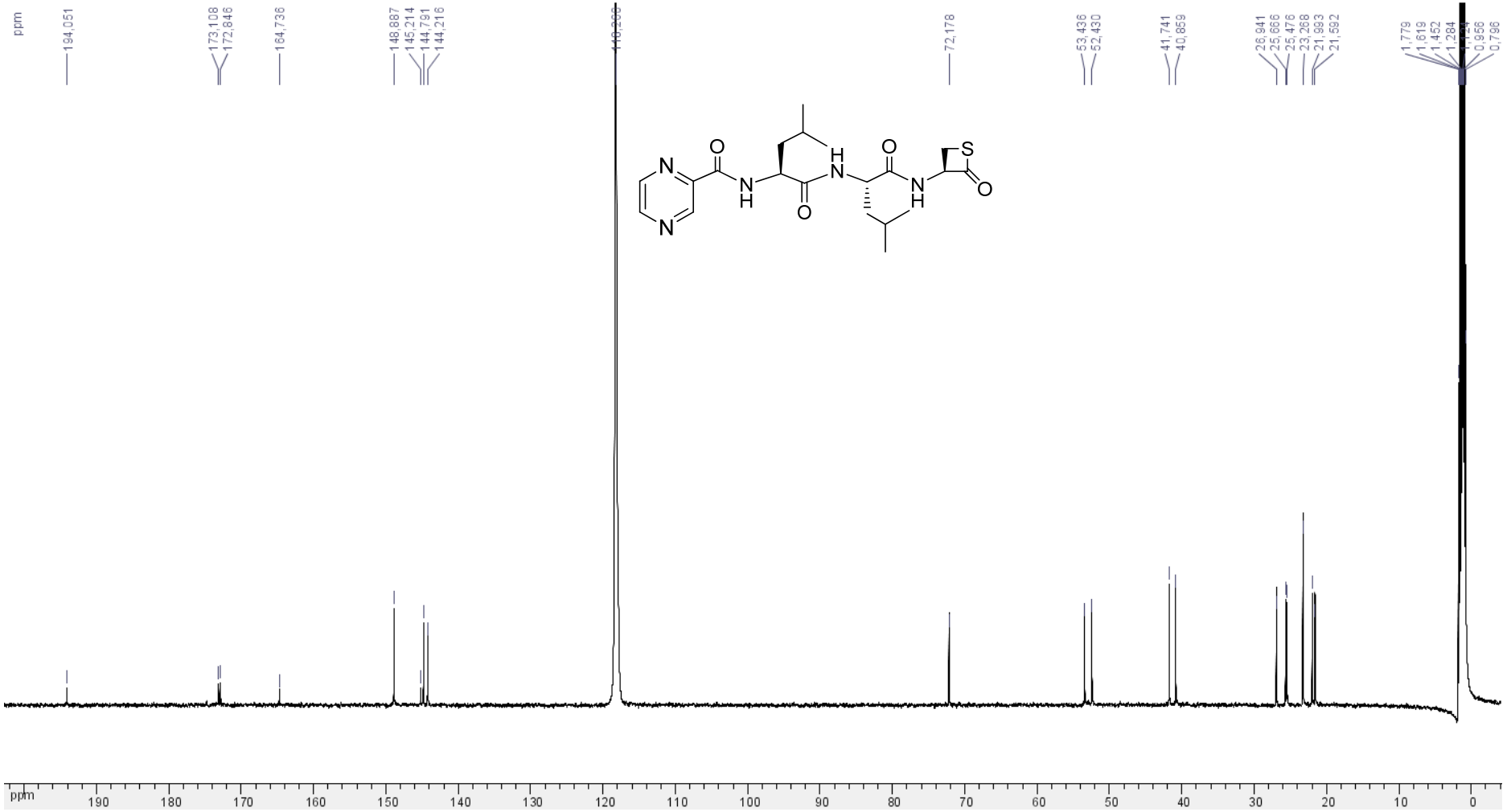
(3*S*)-3-(pyrazin-2-carbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)thietan-2-one (25) ^{13}C NMR (125 MHz, CD_3CN)



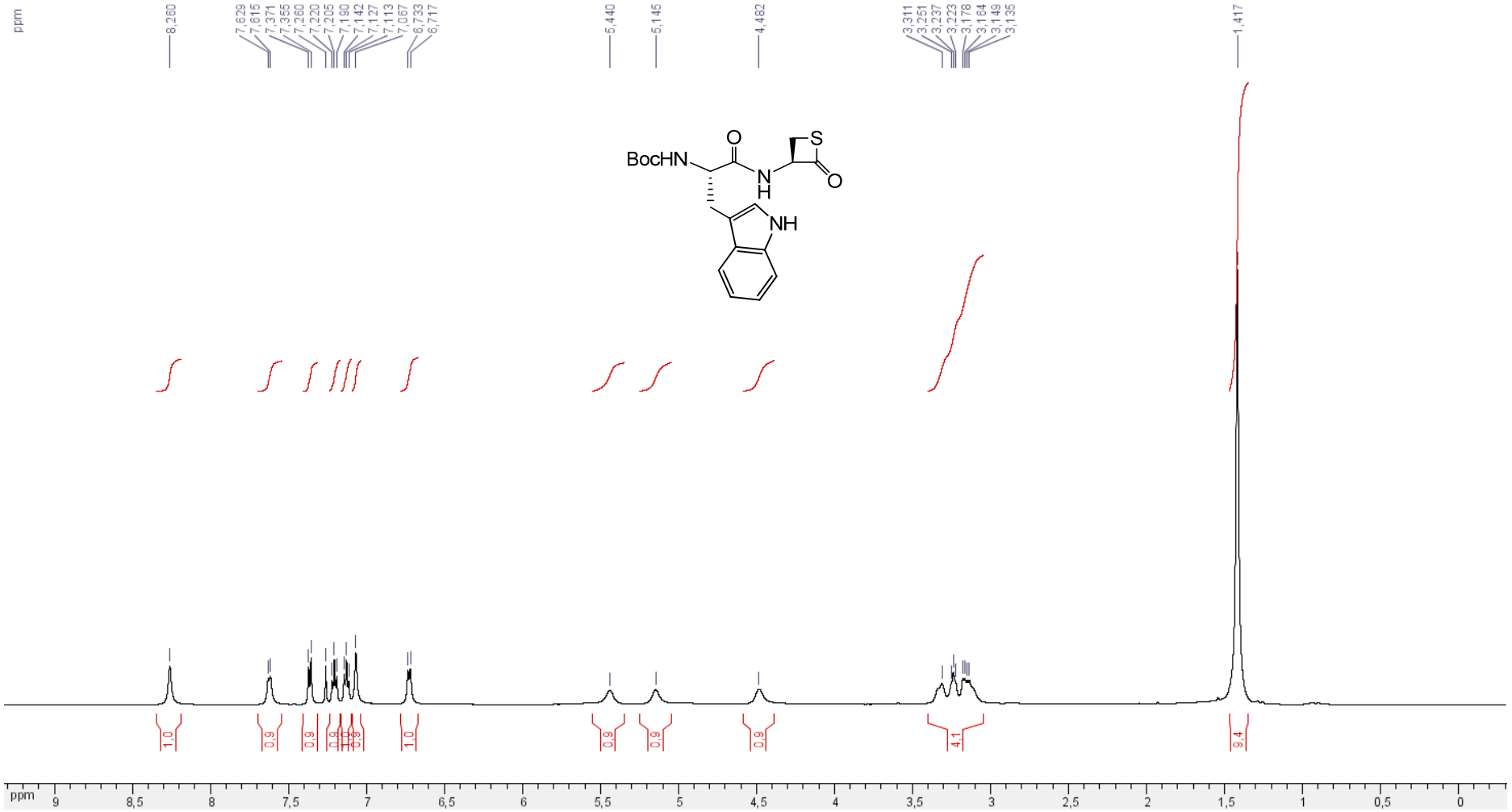
(3*S*)-3-(pyrazin-2-carbonyl-L-leucyl-L-leucylamino)thietan-2-one (23) ^{1}H NMR (500 MHz, CD_3CN)



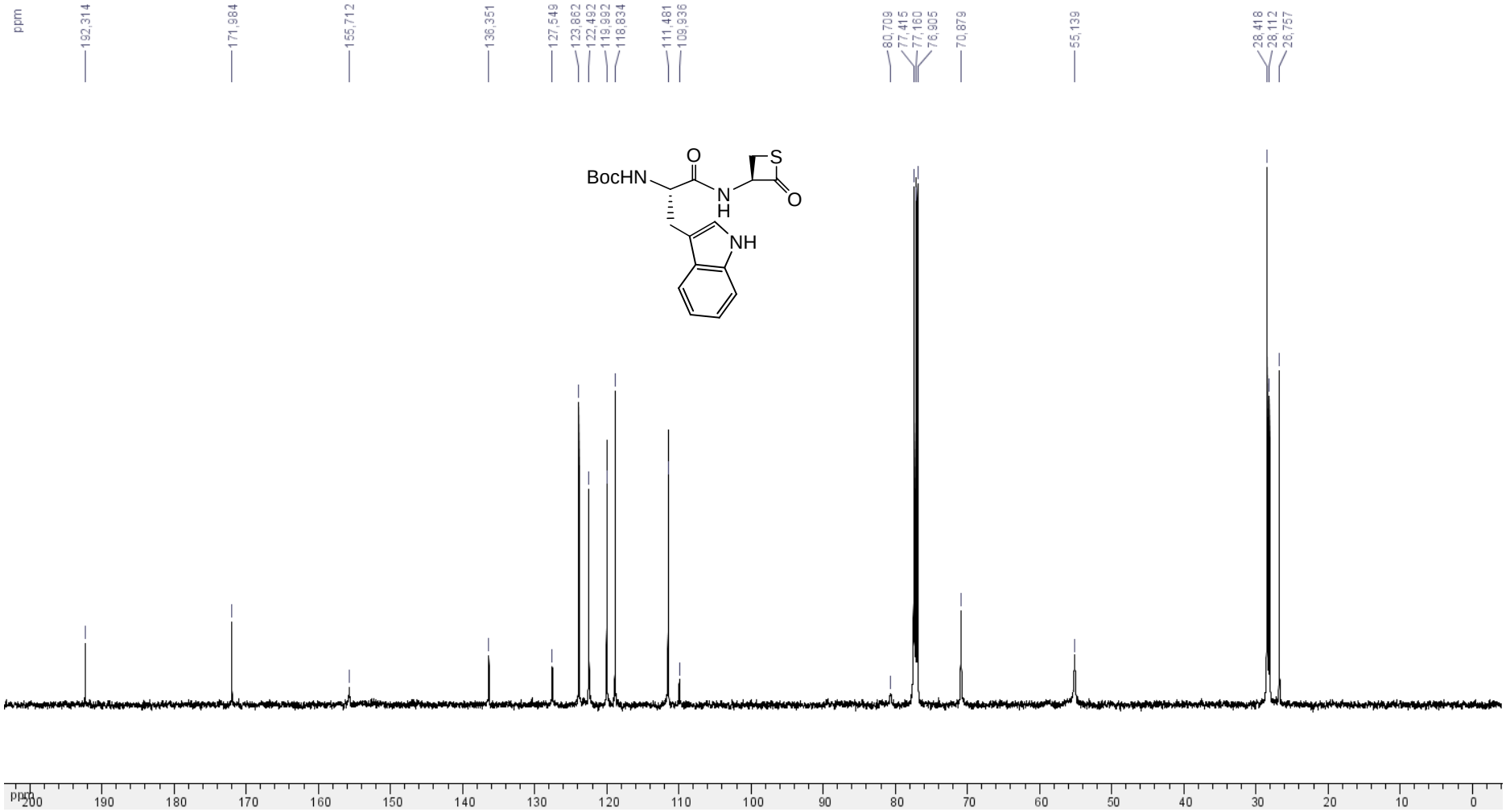
(3*S*)-3-(pyrazin-2-carbonyl-L-leucyl-L-leucylamino)thietan-2-one (23) ^{13}C NMR (125 MHz, CD_3CN)



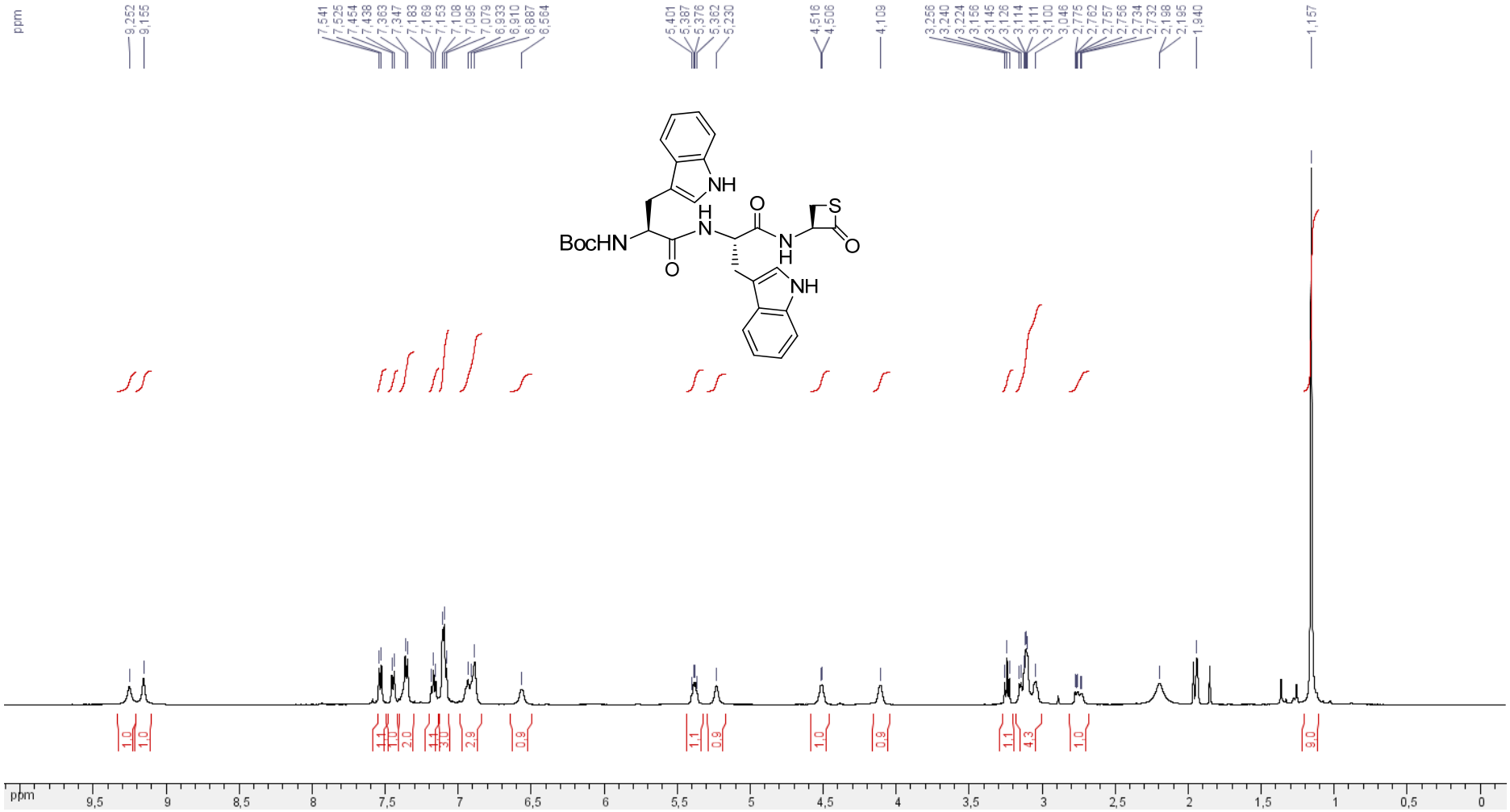
(3*S*)-3-(*N*- *tert*-butyloxycarbonyl-L-tryptophanylamino)thietan-2-one (8) ^{1}H NMR (500 MHz, $CDCl_3$)



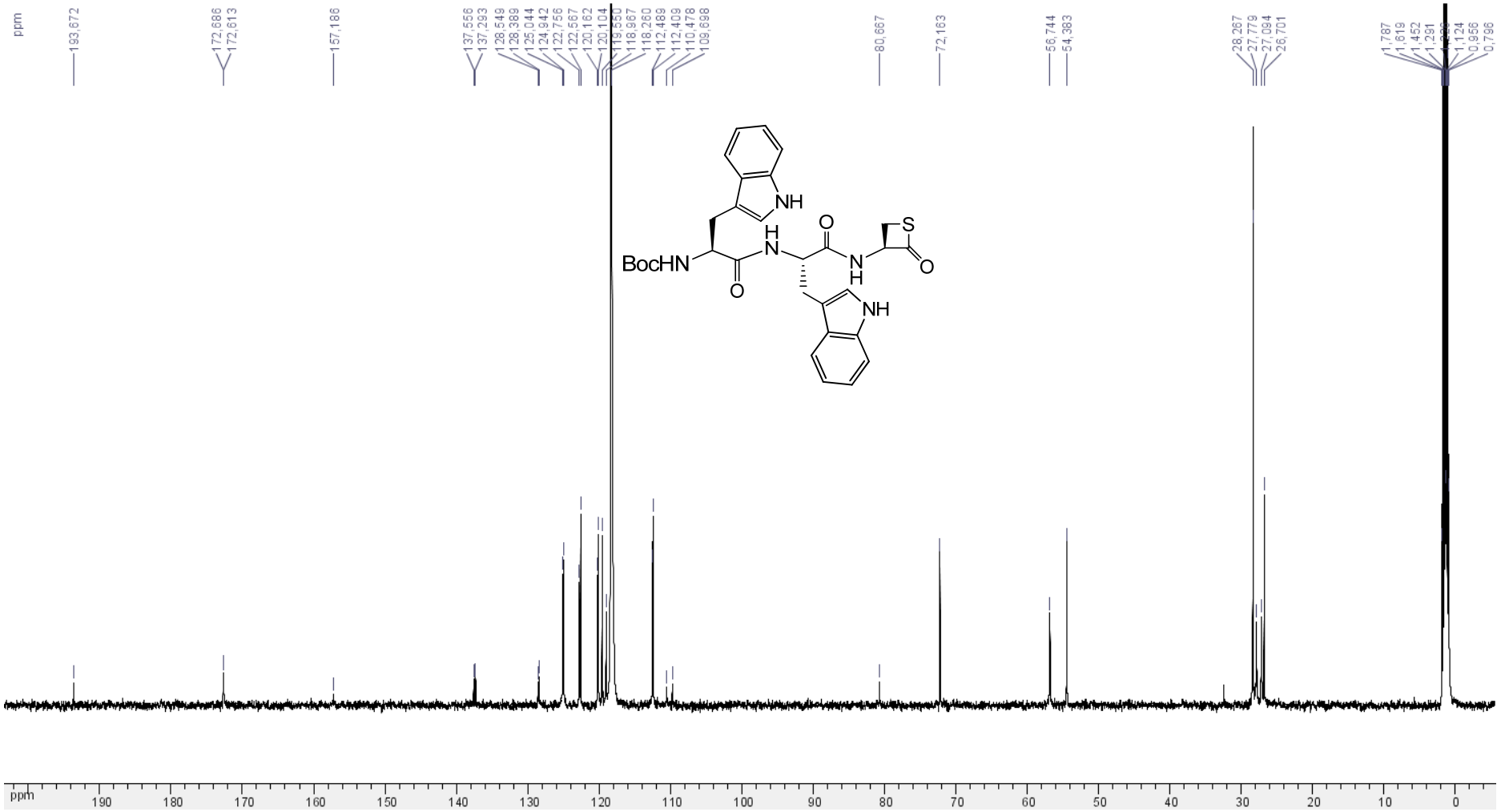
(3*S*)-3-(*N*- *tert*-butyloxycarbonyl-L-tryptophanylamino)thietan-2-one (8) ^{13}C NMR (125 MHz, $CDCl_3$)



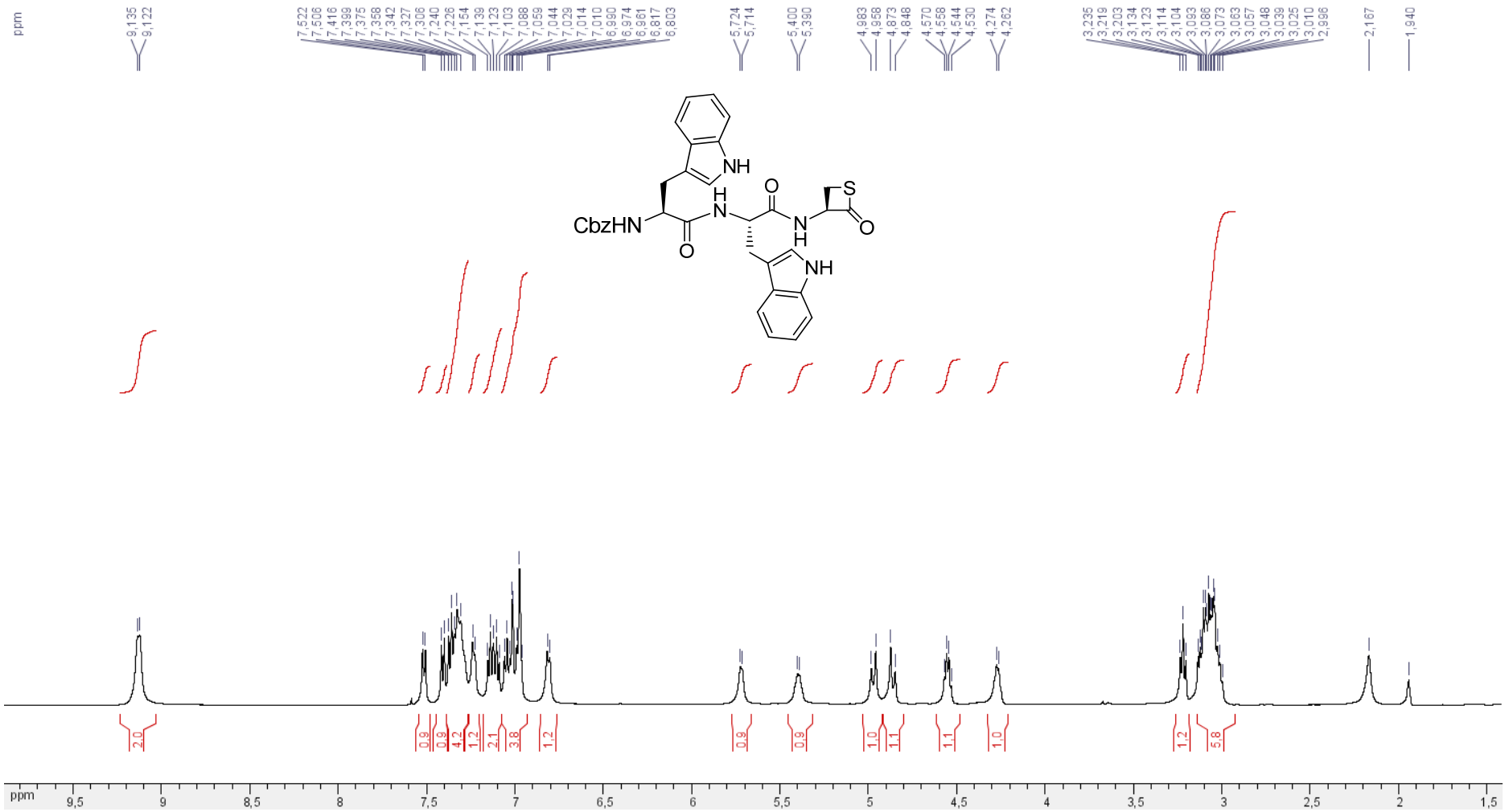
(3*S*)-3-(*N*- *tert*-butyloxycarbonyl-L-tryptophanyl-L-tryptophanylamino)thietan-2-one (14) ^{1}H NMR (500 MHz, CD_3CN)



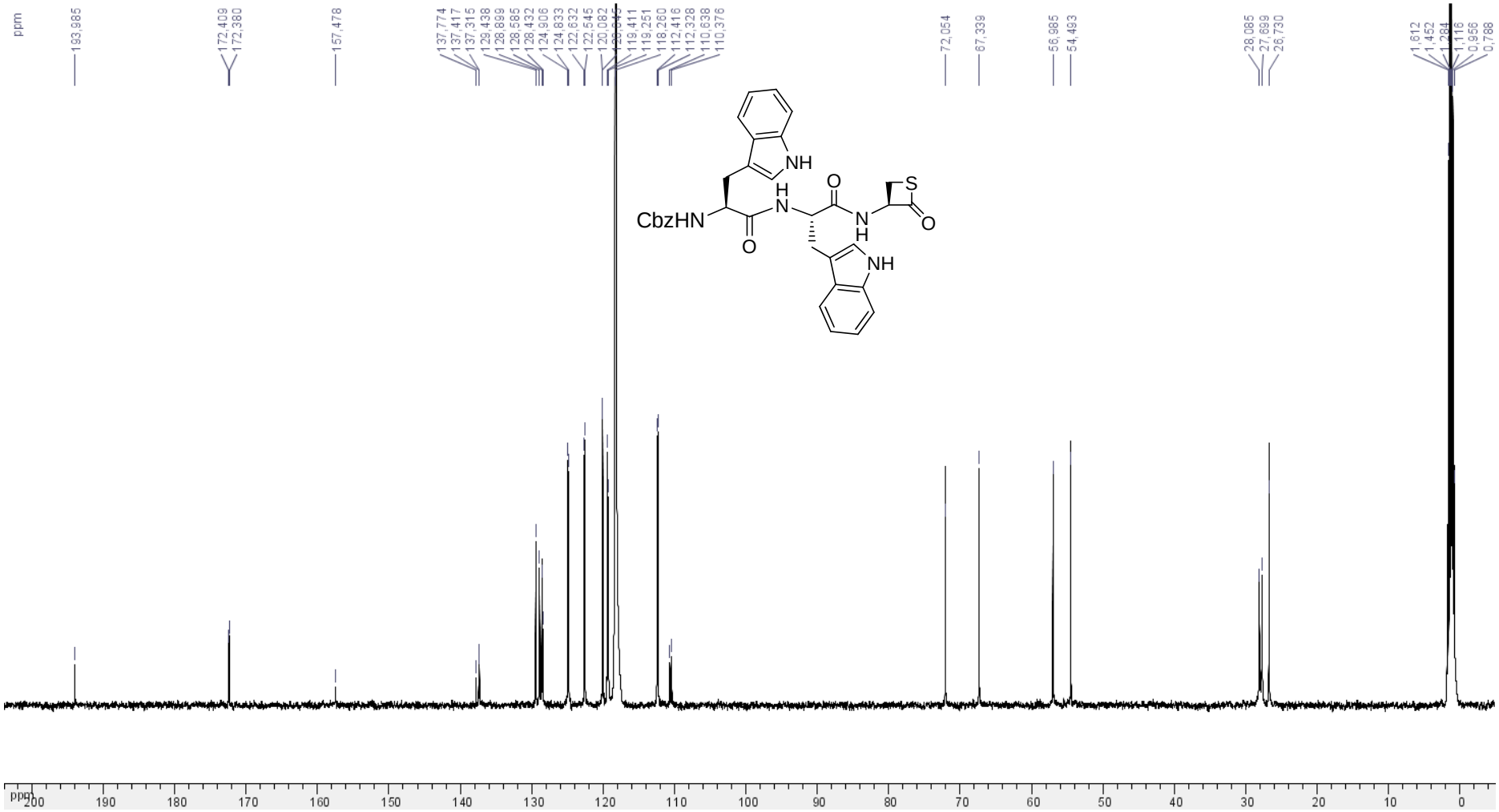
(3*S*)-3-(*N*- *tert*-butyloxycarbonyl-L-tryptophanyl-L-tryptophanylamino)thietan-2-one (14) ^{13}C NMR (125 MHz, CD_3CN)



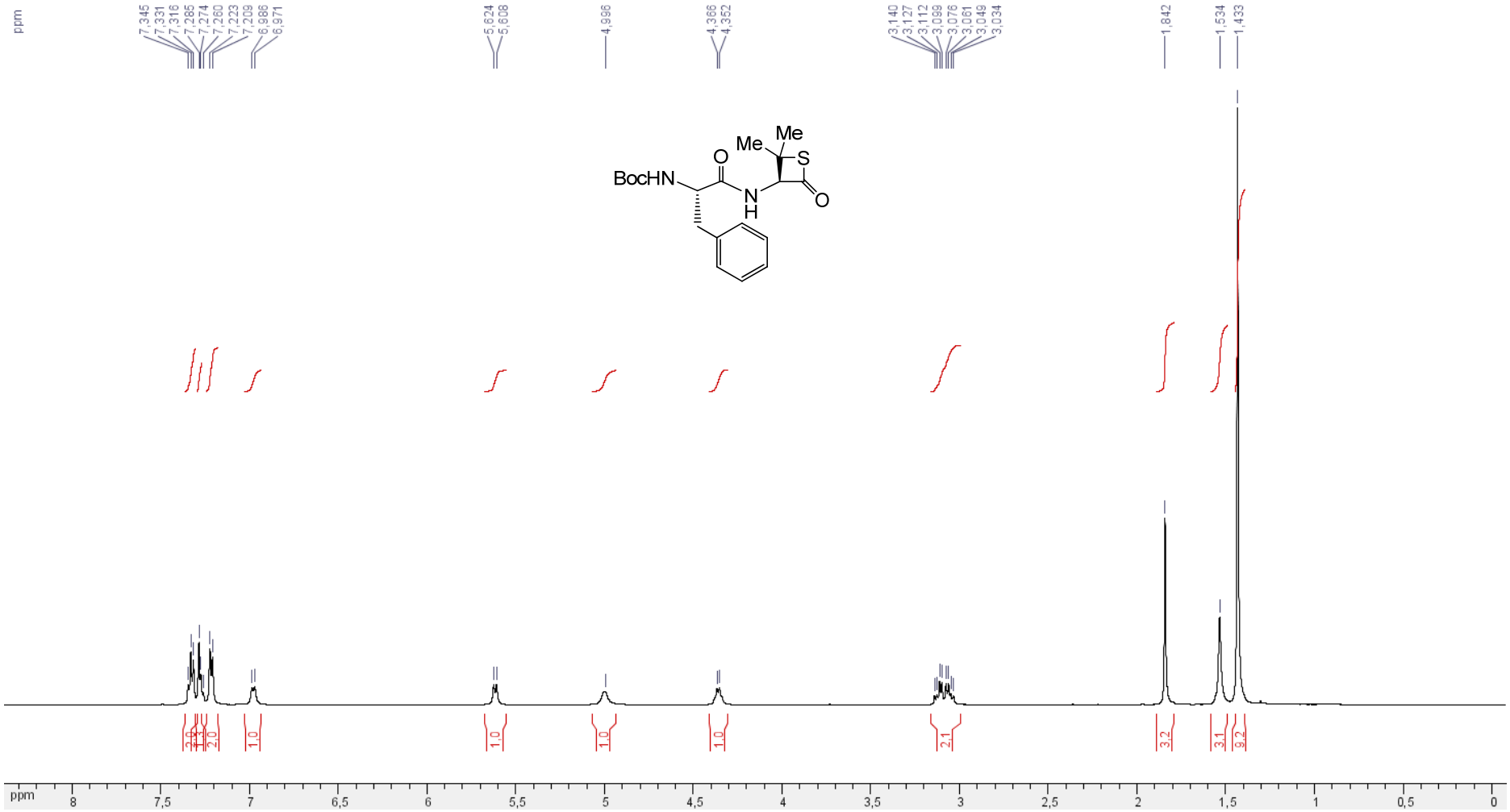
(3*S*)-3-(*N*-benzyloxycarbonyl-L-tryptophanyl-L-tryptophanylamino)thietan-2-one (15) ^{1}H NMR (500 MHz, CD_3CN)



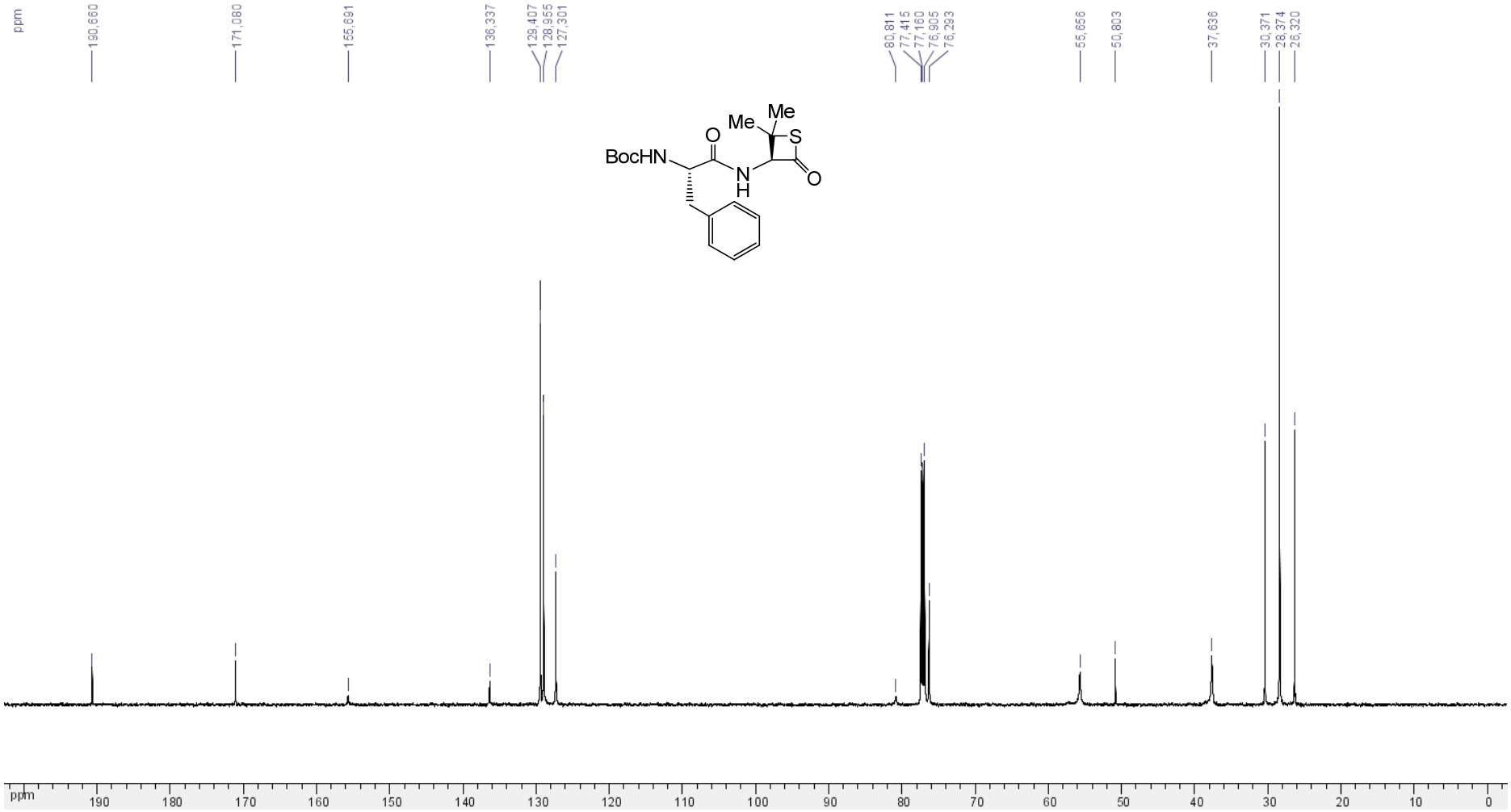
(3*S*)-3-(*N*-benzyloxycarbonyl-L-tryptophanyl-L-tryptophanylamino)thietan-2-one (15) ^{13}C NMR (125 MHz, CD_3CN)



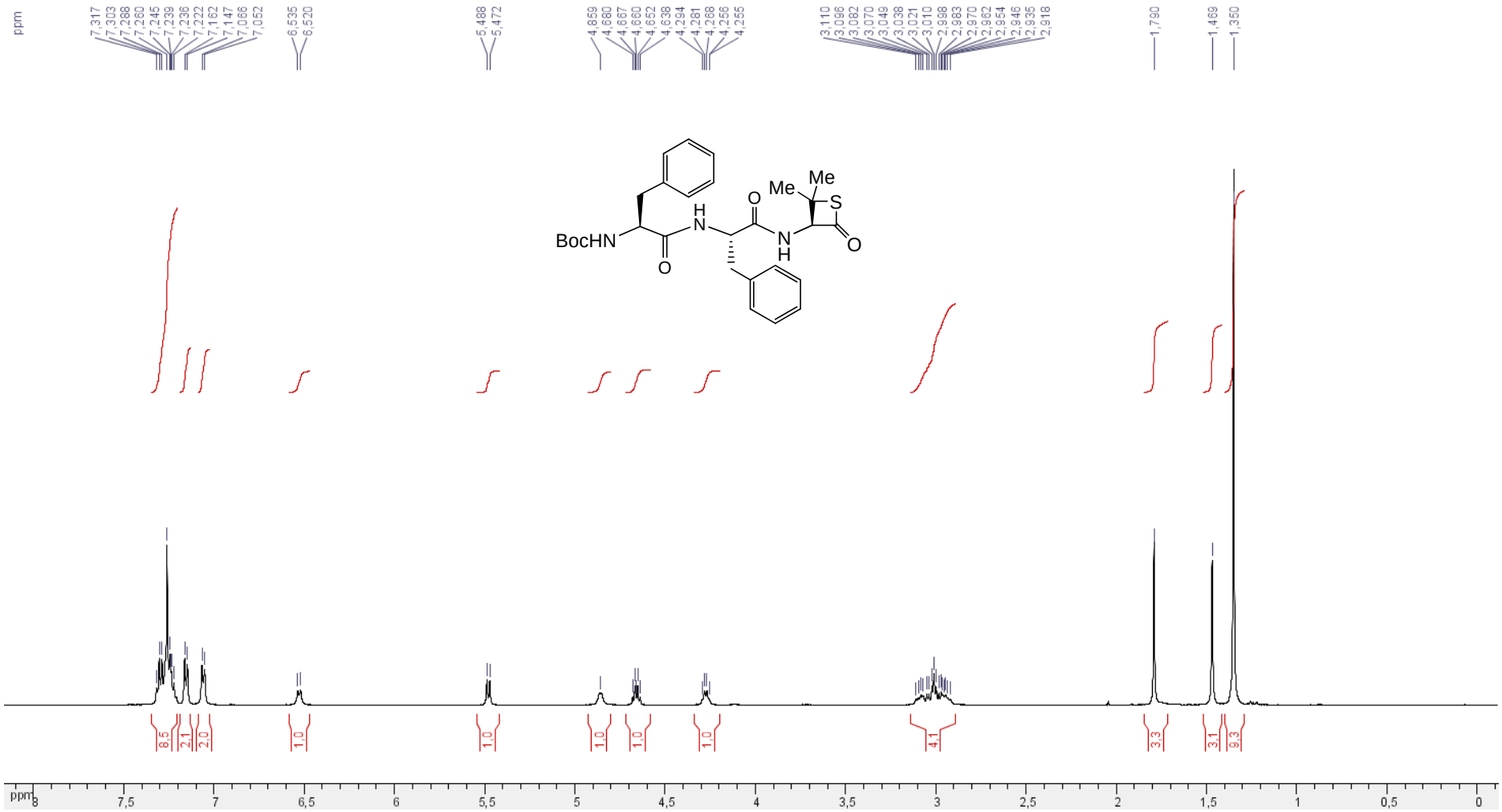
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)-4,4-dimethyl-thietan-2-one (18) ^{1}H NMR (500 MHz, $CDCl_3$)



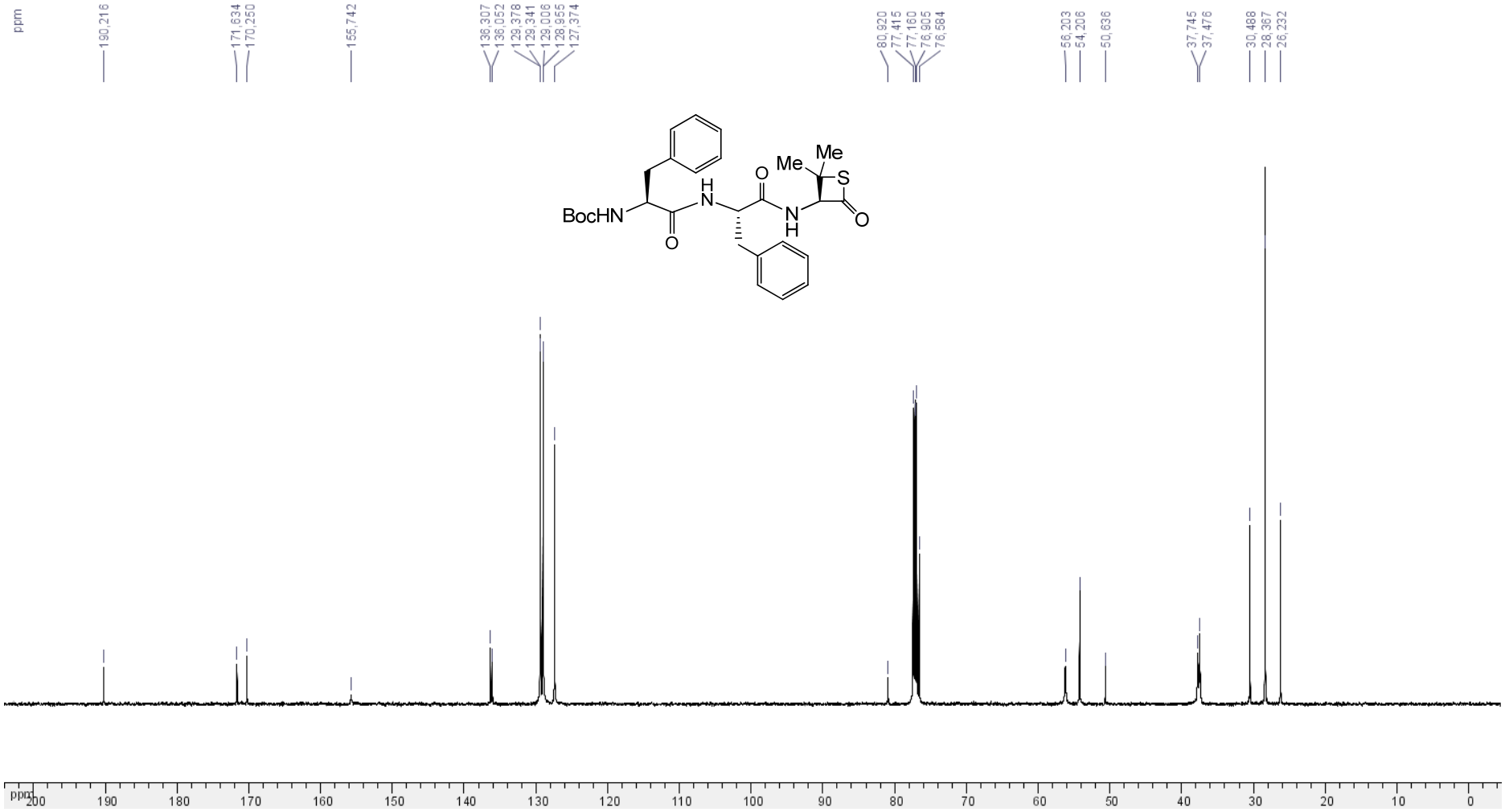
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)-4,4-dimethyl-thietan-2-one (18) ^{13}C NMR (125 MHz, $CDCl_3$)



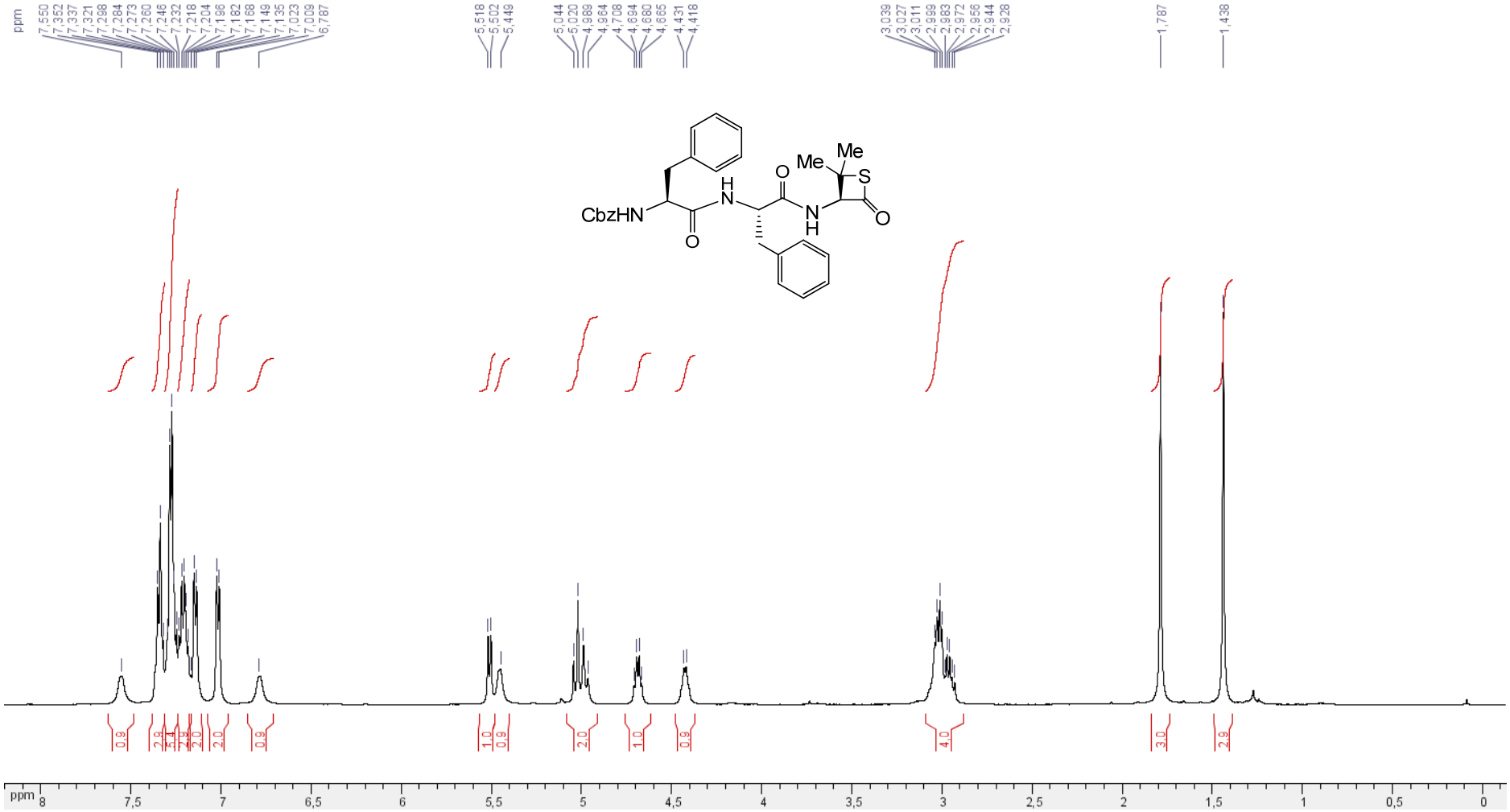
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)-4,4-dimethyl-thietan-2-one (19) ^{1}H NMR (500 MHz, $CDCl_3$)



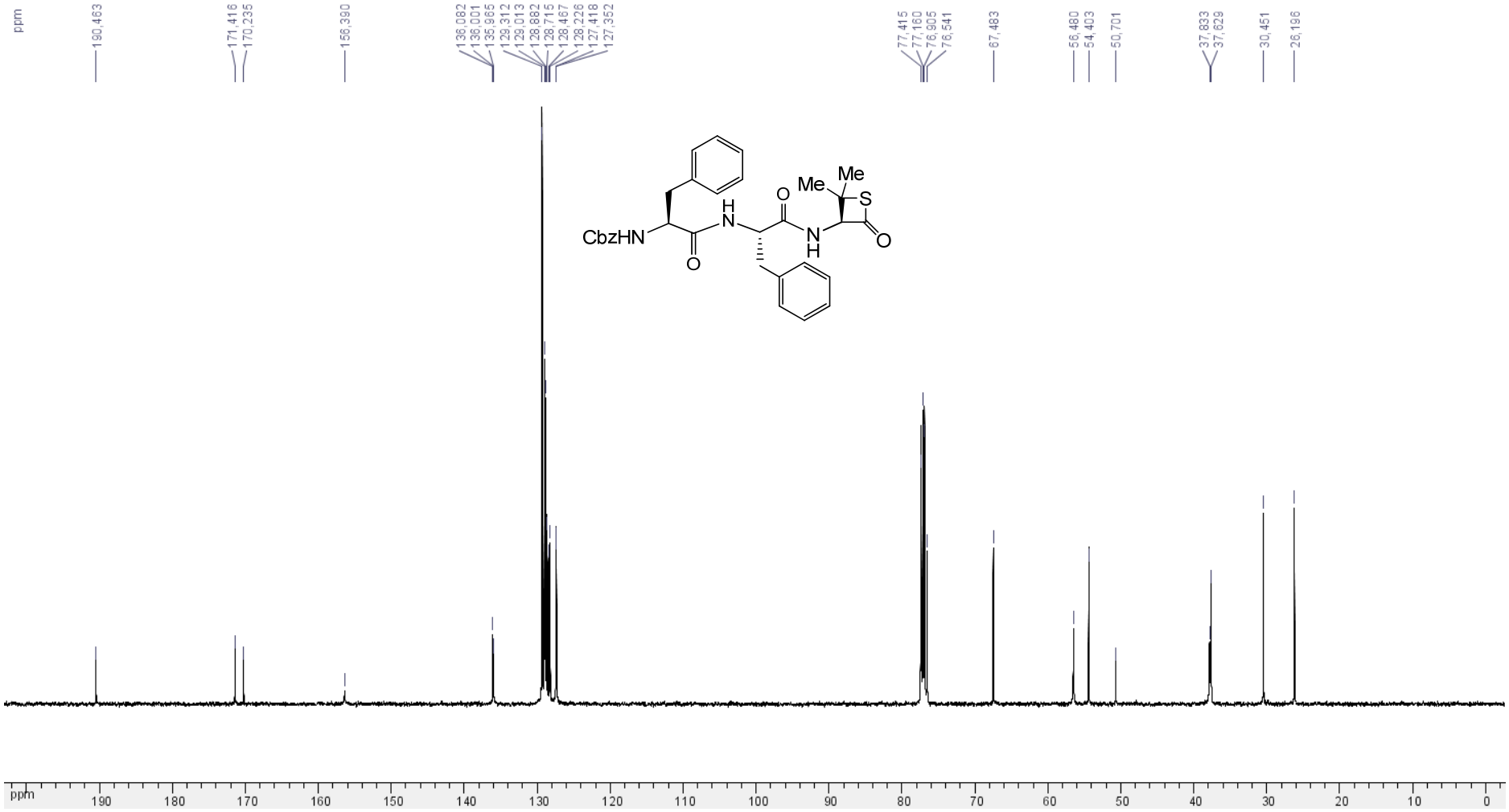
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)-4,4-dimethyl-thietan-2-one (19) ^{13}C NMR (125 MHz, $CDCl_3$)



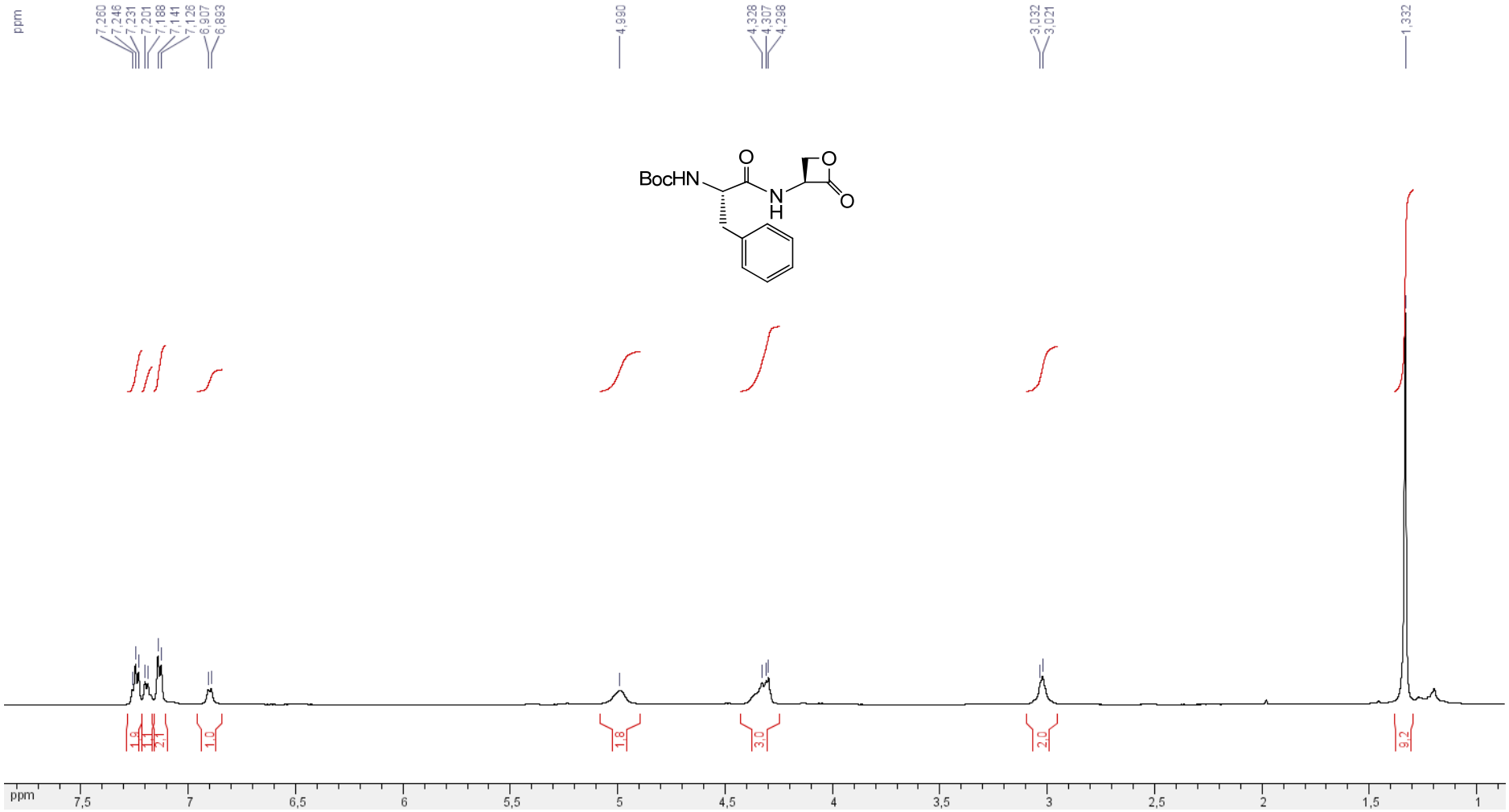
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)-4,4-dimethyl-thietan-2-one (20) ^{1}H NMR (500 MHz, CD_3CN)



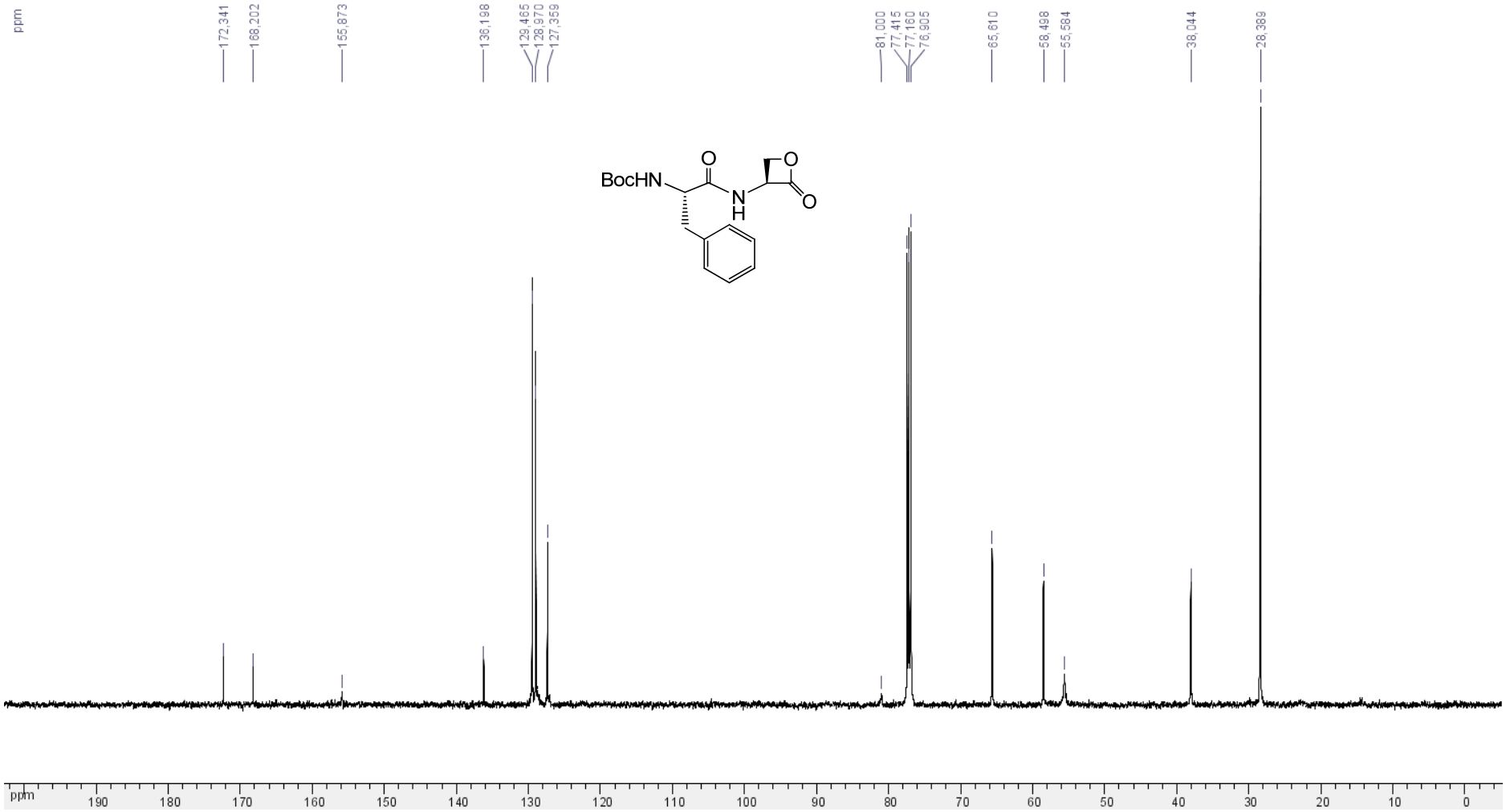
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)-4,4-dimethyl-thietan-2-one (20) ^{13}C NMR (125 MHz, CD_3CN)



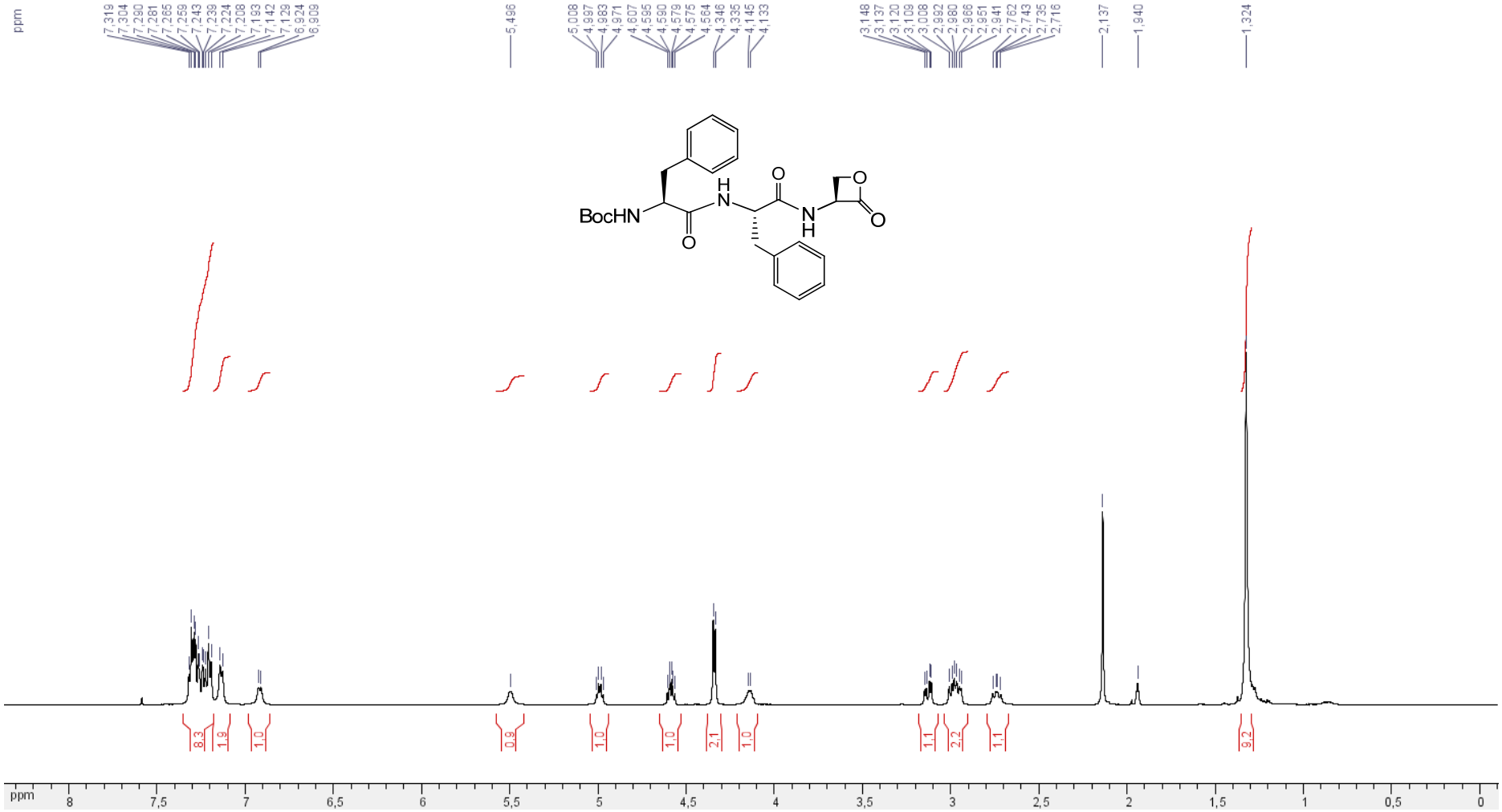
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)oxetan-2-one (27) ^{1}H NMR (500 MHz, $CDCl_3$)



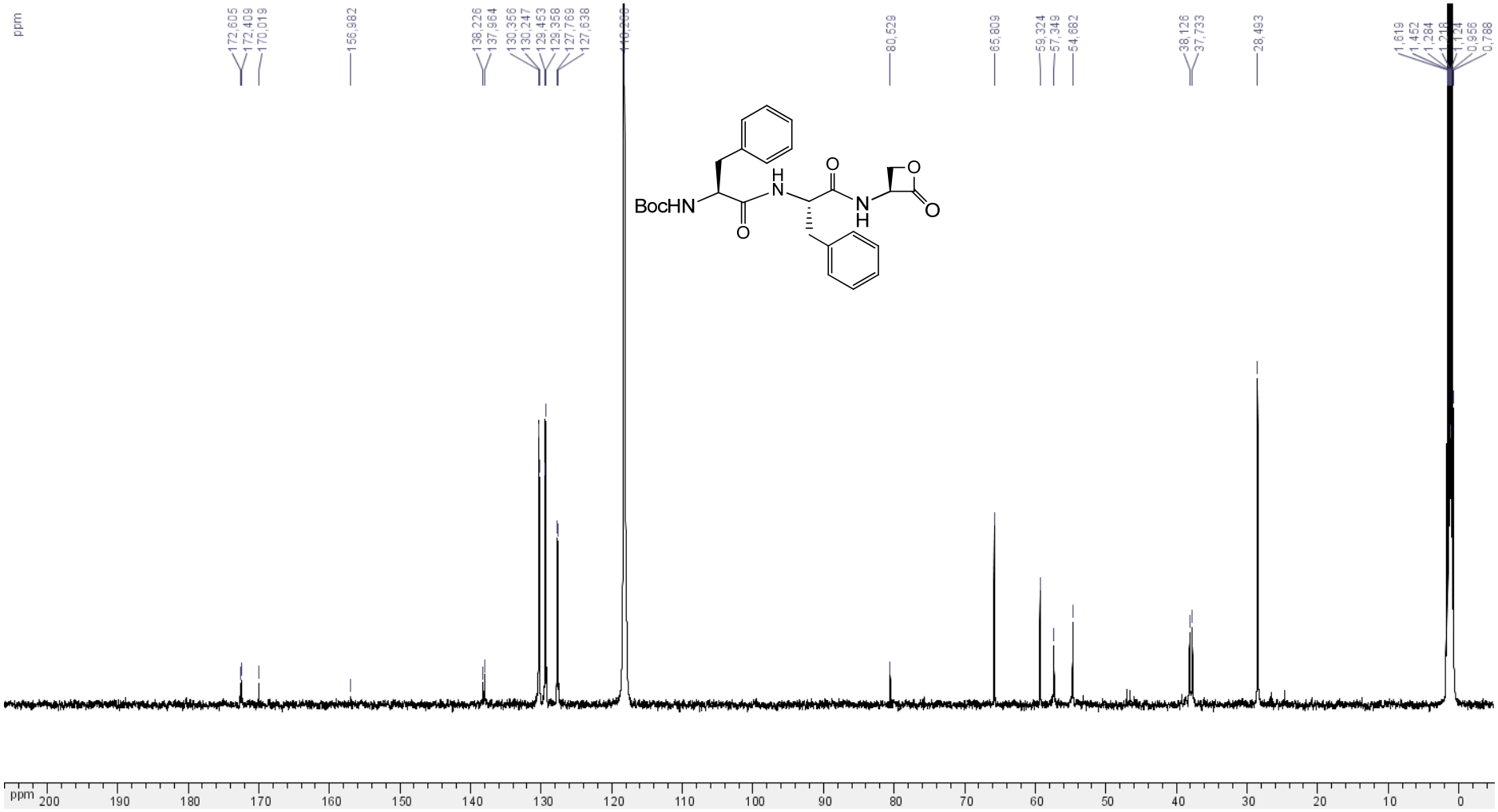
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)oxetan-2-one (27) ^{13}C NMR (125 MHz, $CDCl_3$)



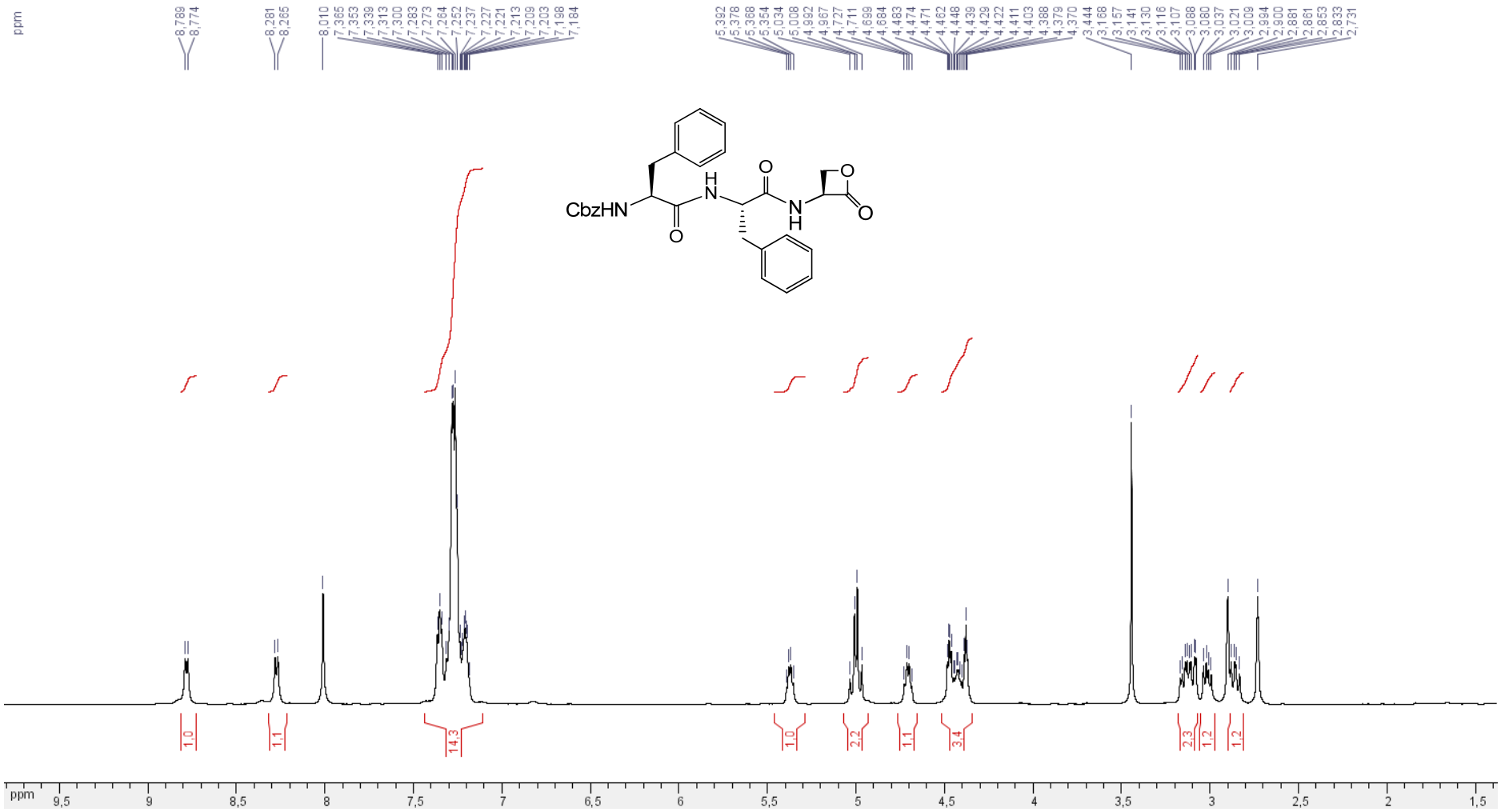
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (28) ^{1}H NMR (500 MHz, CD_3CN)



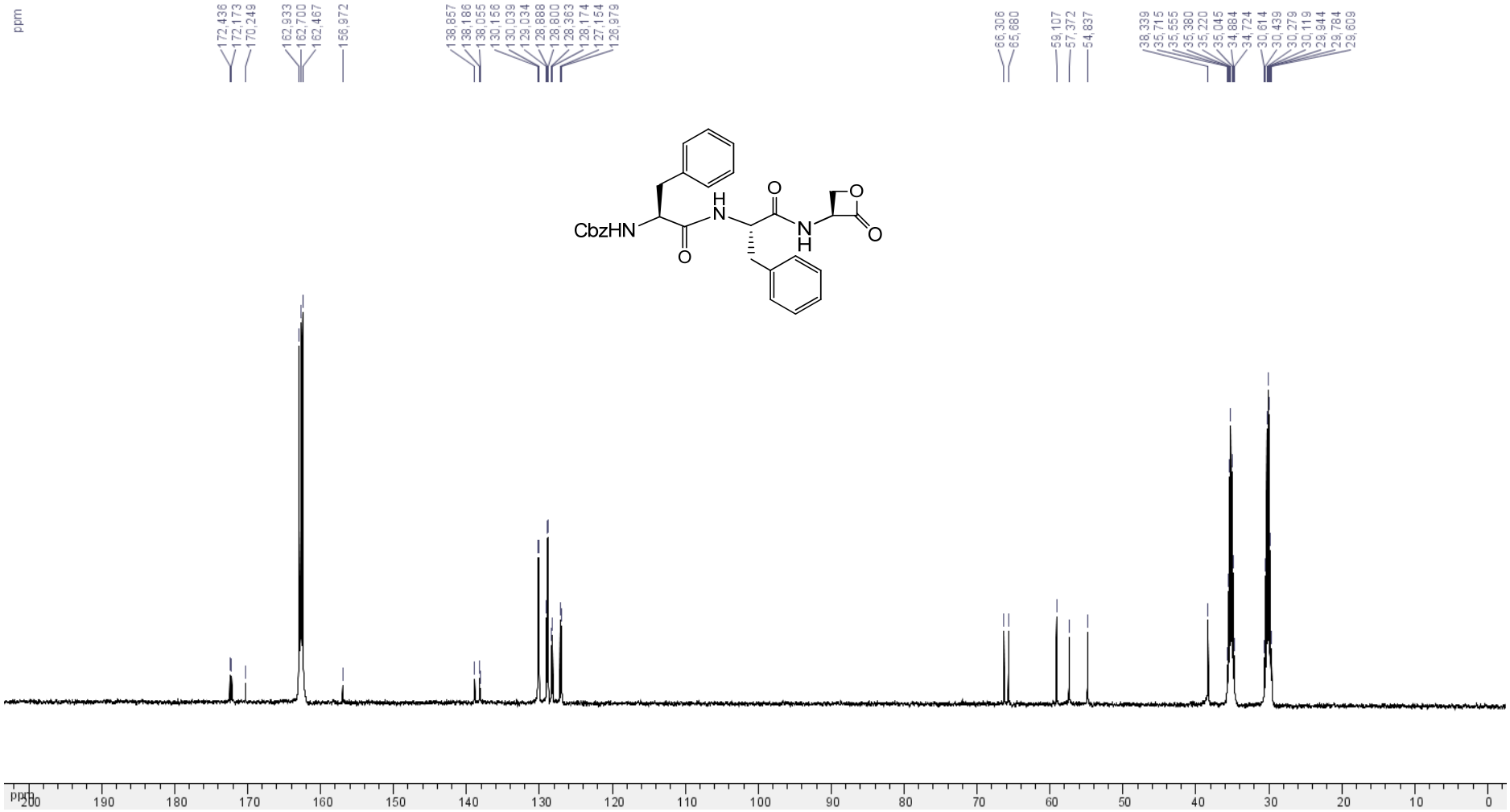
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (28) ^{13}C NMR (125 MHz, CD_3CN)



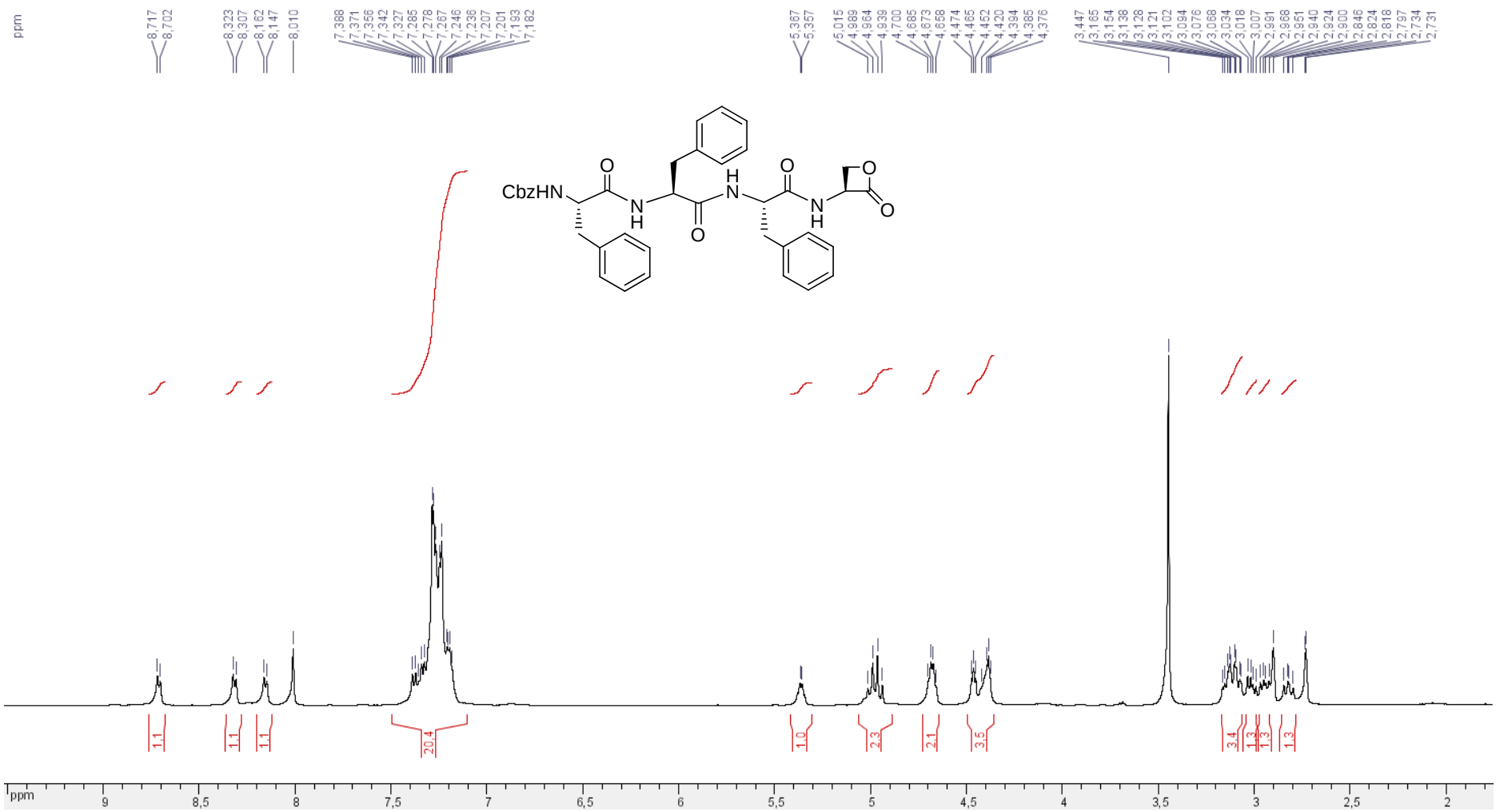
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (29) ^{1}H NMR (500 MHz, DMF-d_7)



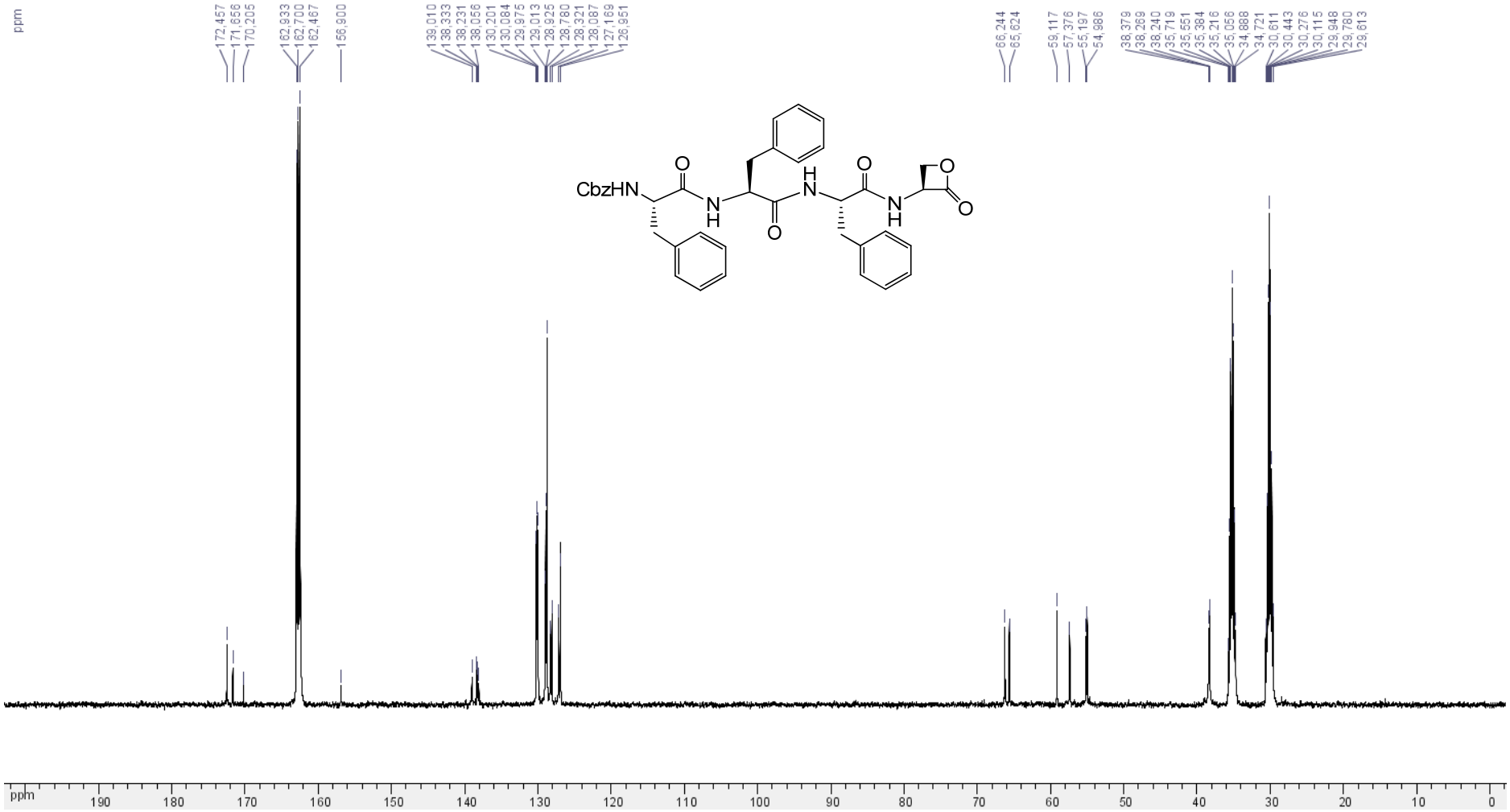
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (29) ^{13}C NMR (125 MHz, DMF-d_7)



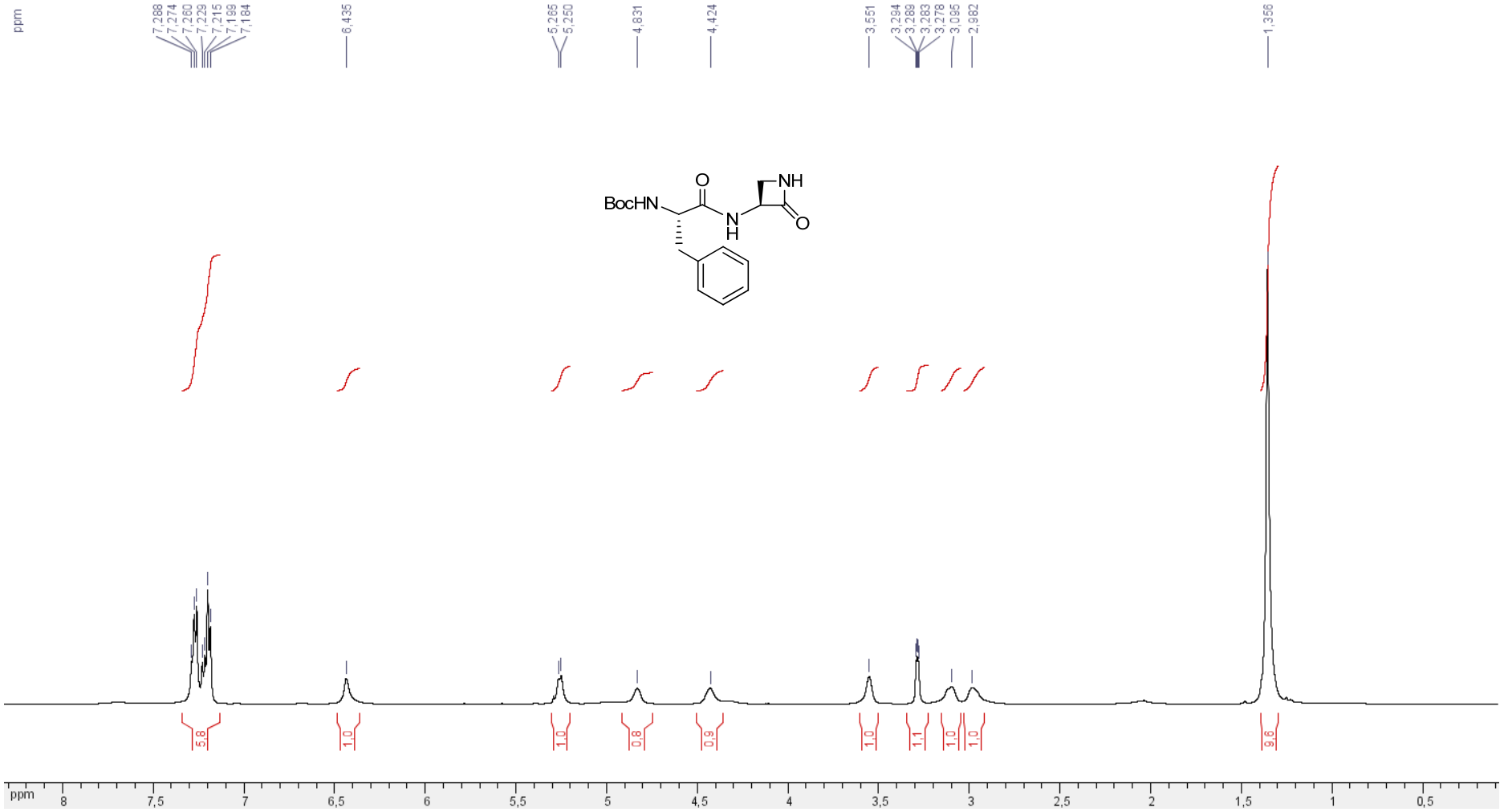
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (30) ^{1}H NMR (500 MHz, DMF-d_7)



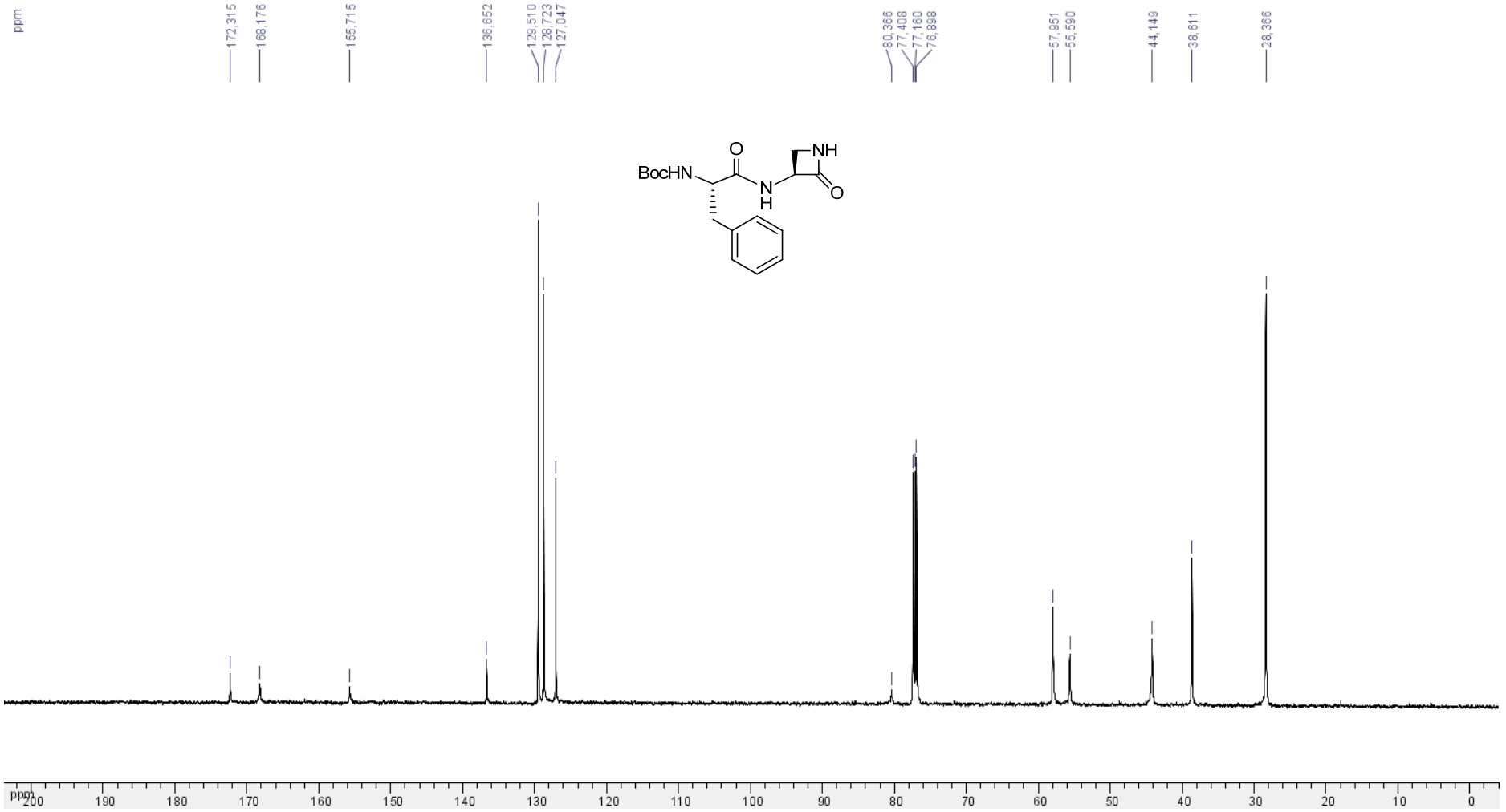
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-phenylalanylamino)oxetan-2-one (30) ^{13}C NMR (125 MHz, DMF-d_7)



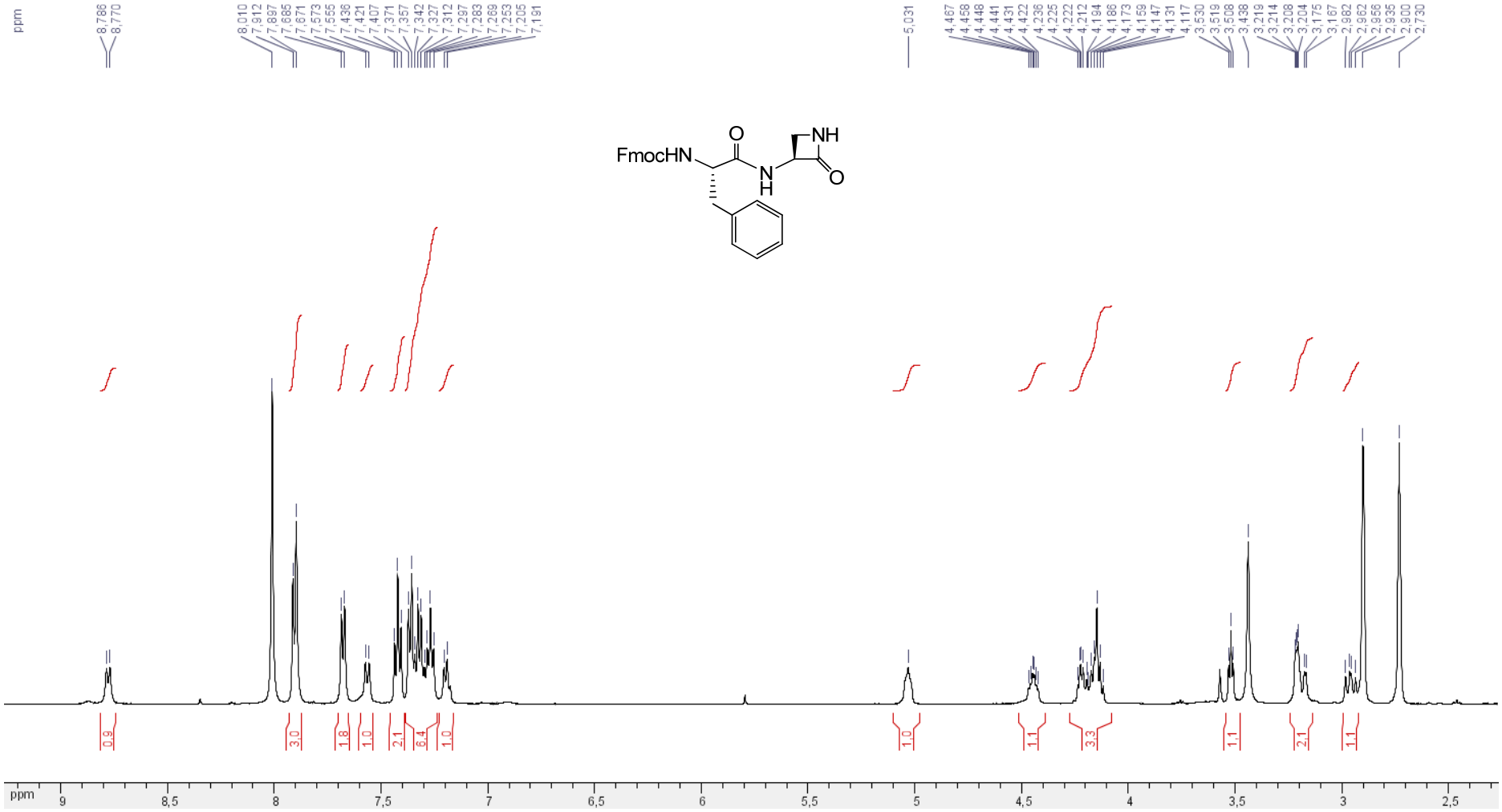
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)azetidin-2-one (31) ^{1}H NMR (500 MHz, $CDCl_3$)



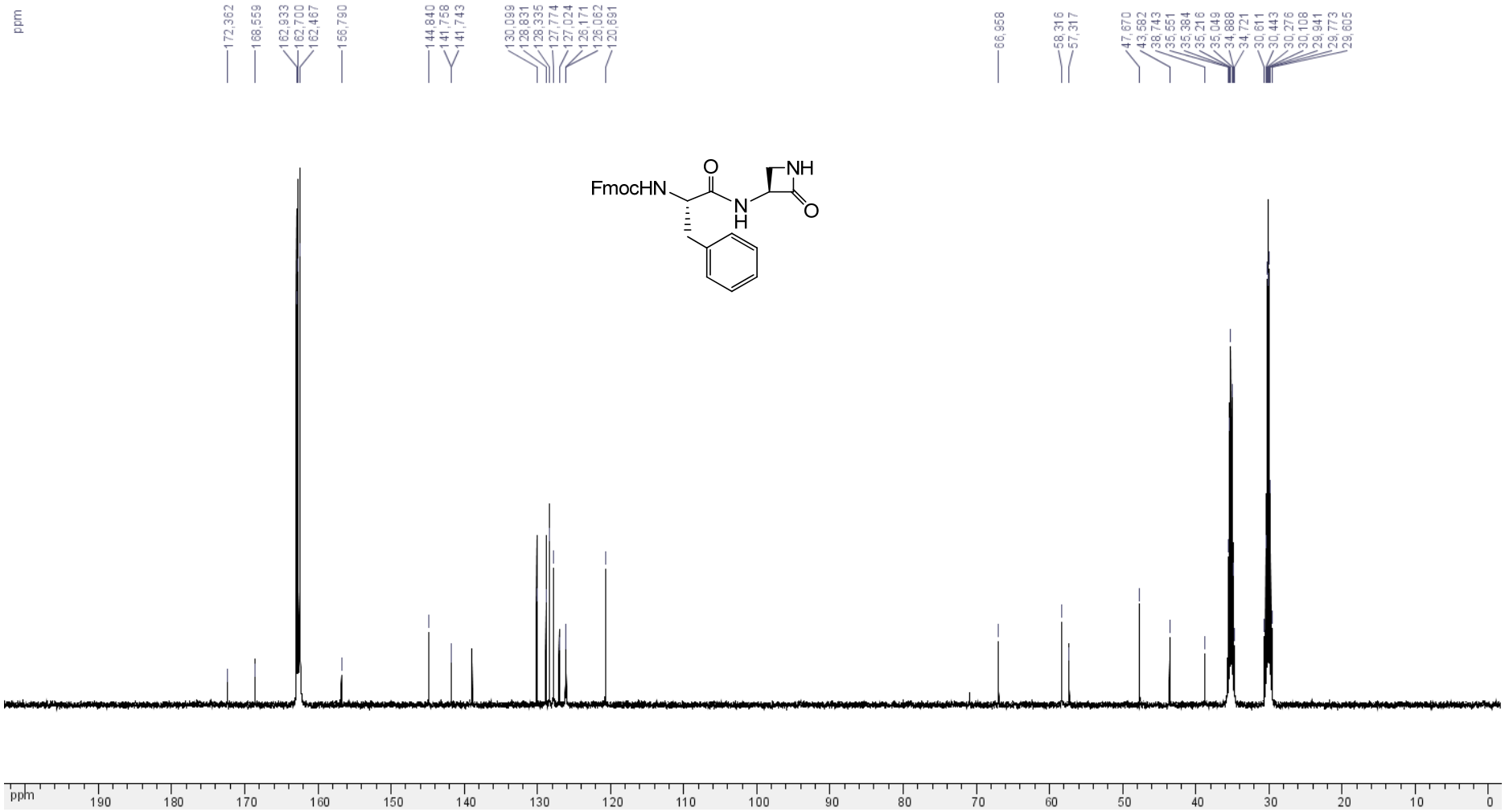
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanylamino)azetidin-2-one (31) ^{13}C NMR (125 MHz, $CDCl_3$)



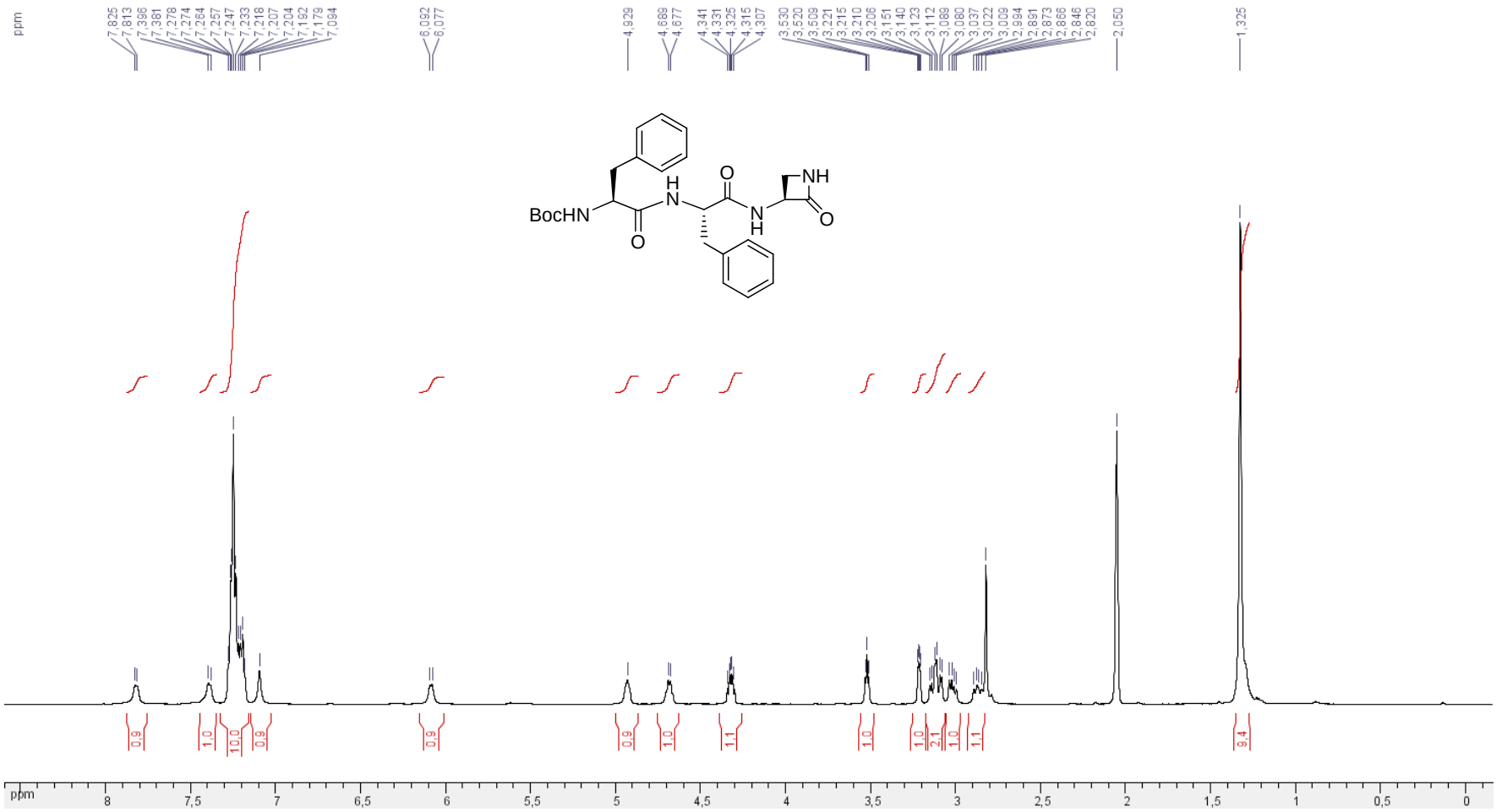
(3*S*)-3-(*N*-fluorenyloxycarbonyl-L-phenylalanylamino)azetidin-2-one (32) ^{1}H NMR (500 MHz, DMF-d_7)



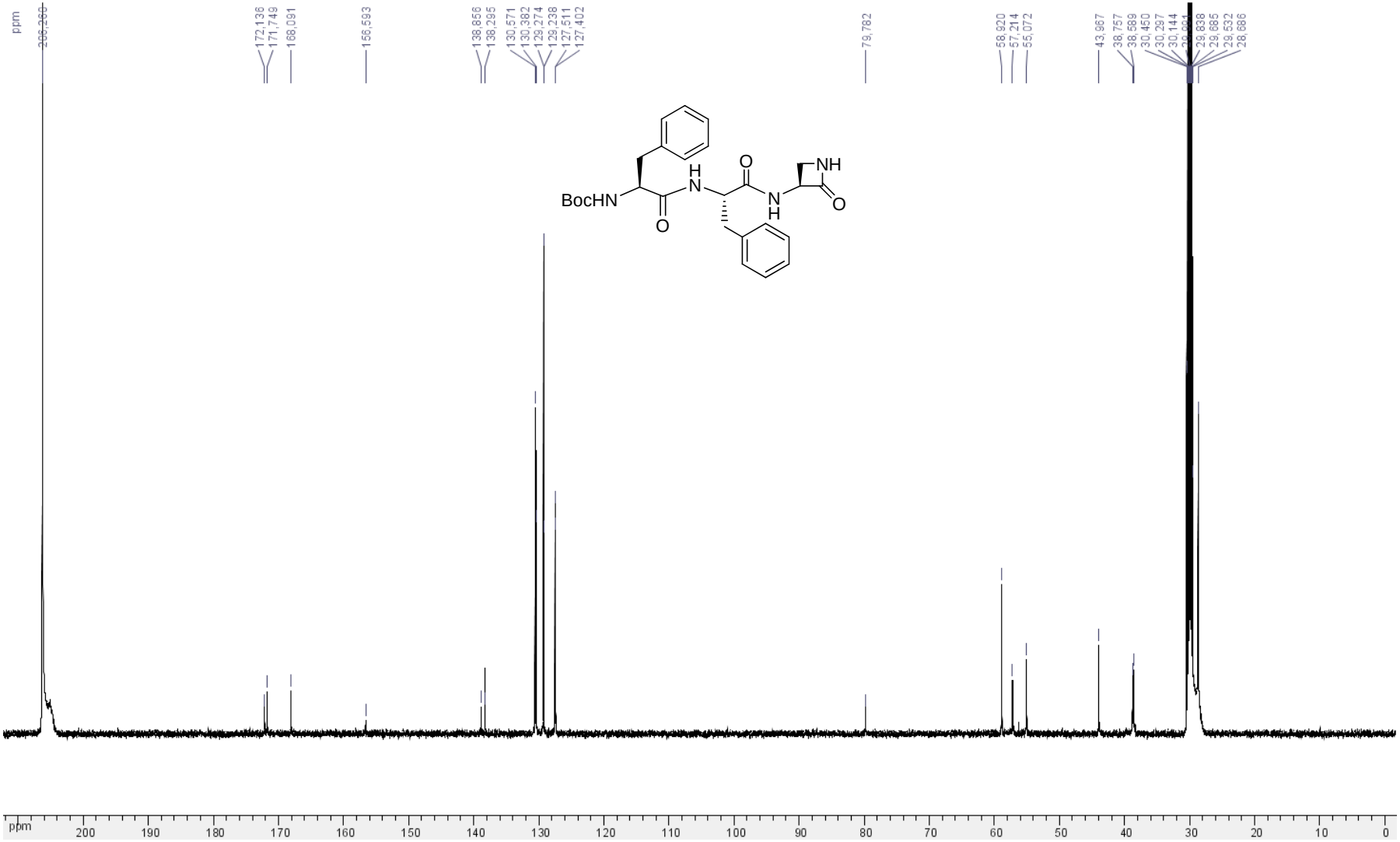
(3*S*)-3-(*N*-fluorenyloxycarbonyl-L-phenylalanylamino)azetidin-2-one (32) ^{13}C NMR (125 MHz, DMF-d_7)



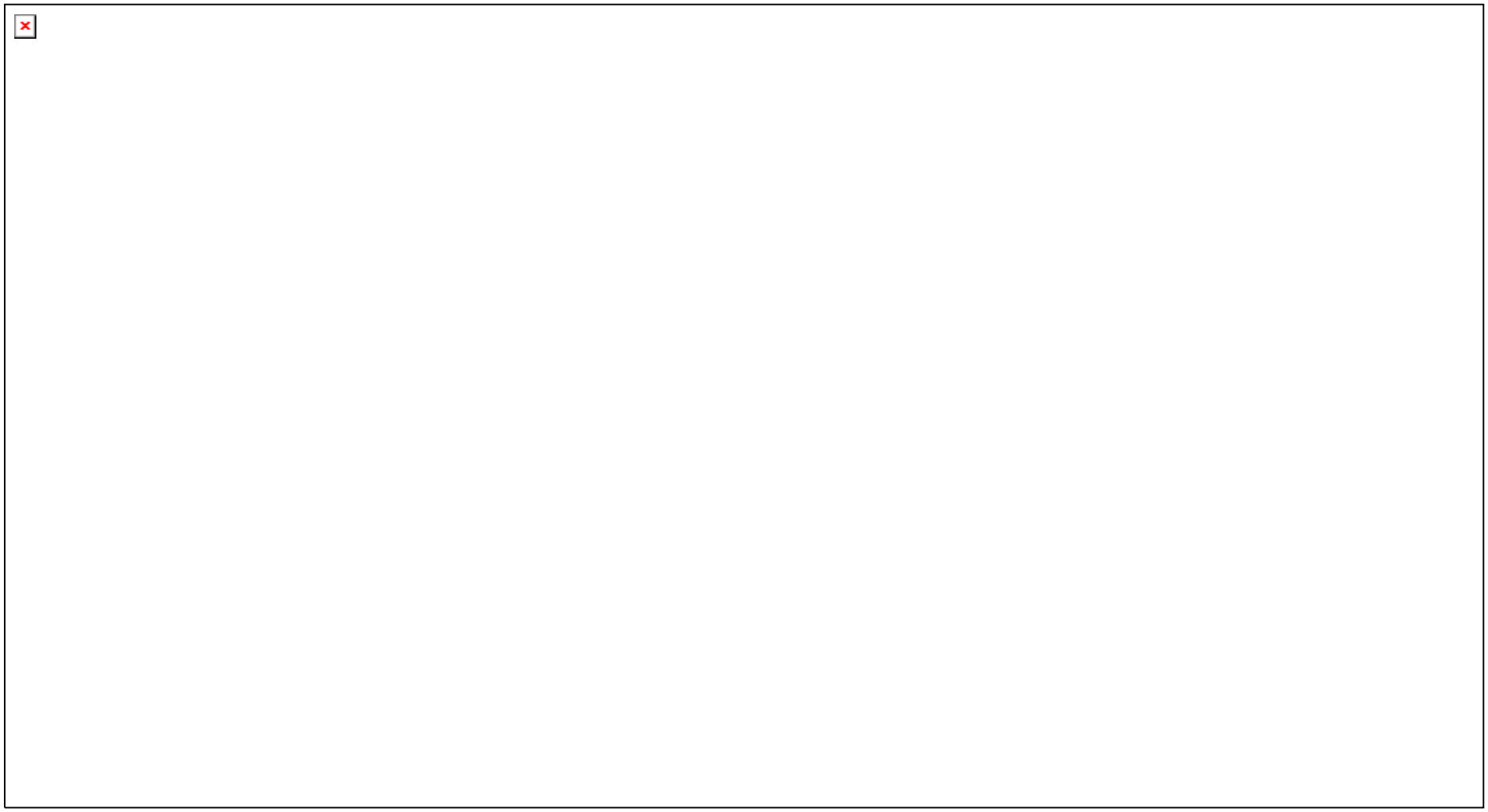
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)azetidin-2-one (33) ^{1}H NMR (500 MHz, $(CD_3)_2CO$)



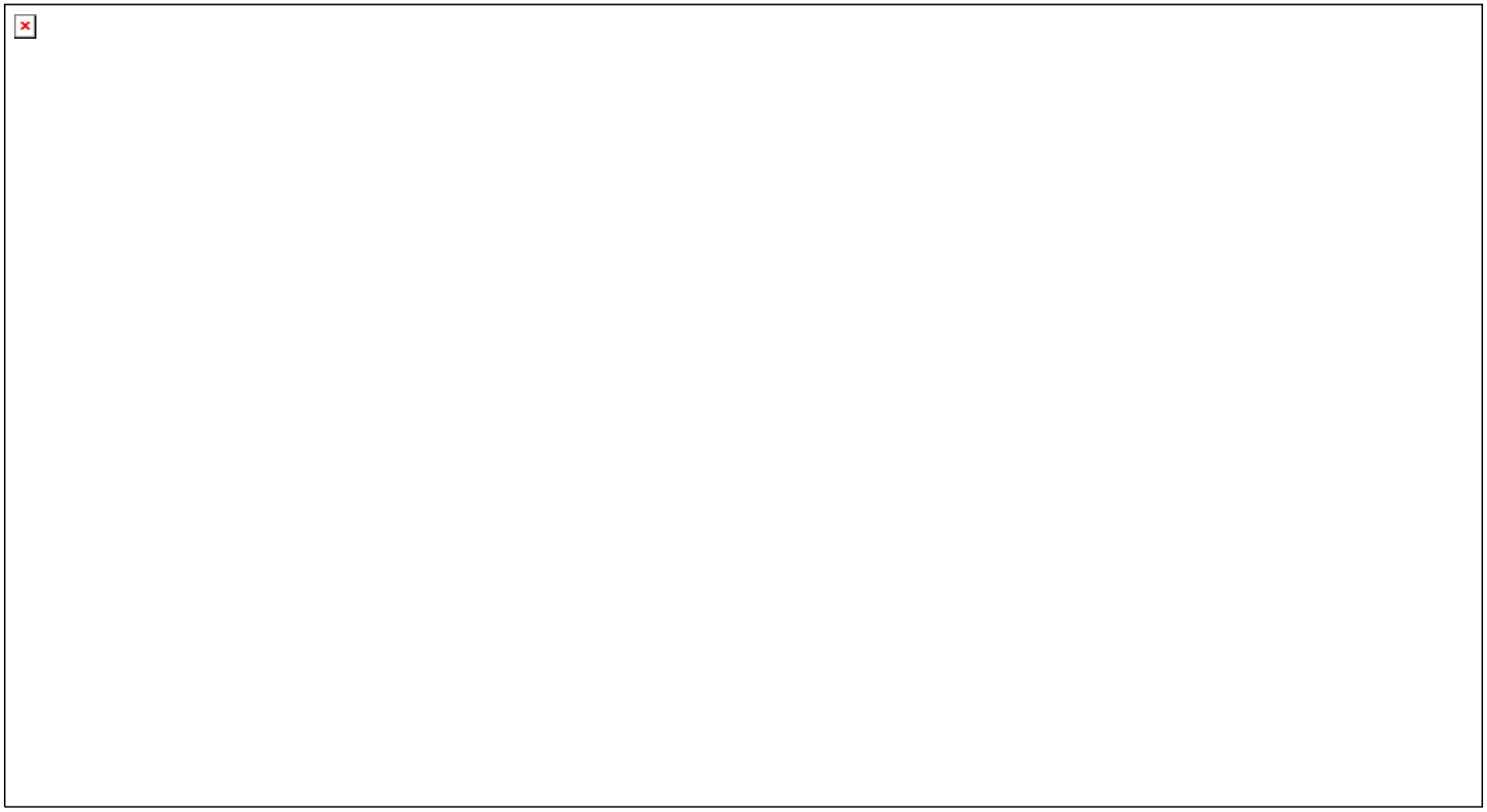
(3*S*)-3-(*N*-*tert*-butyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)azetidin-2-one (33) ^{13}C NMR (125 MHz, $(CD_3)_2CO$)



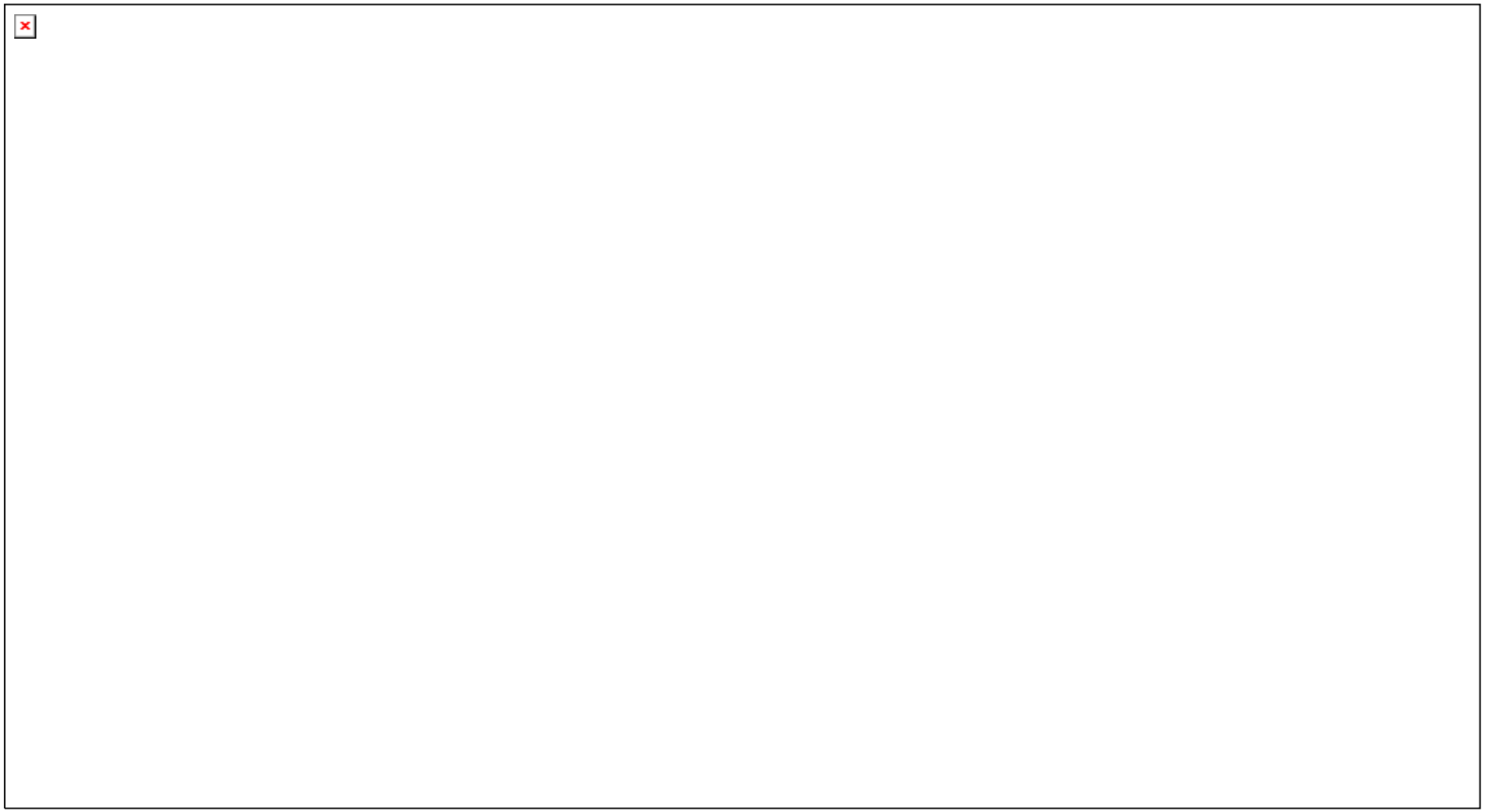
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)azetidin-2-one (**34**) ^{1}H NMR (500 MHz, DMF-d_7)



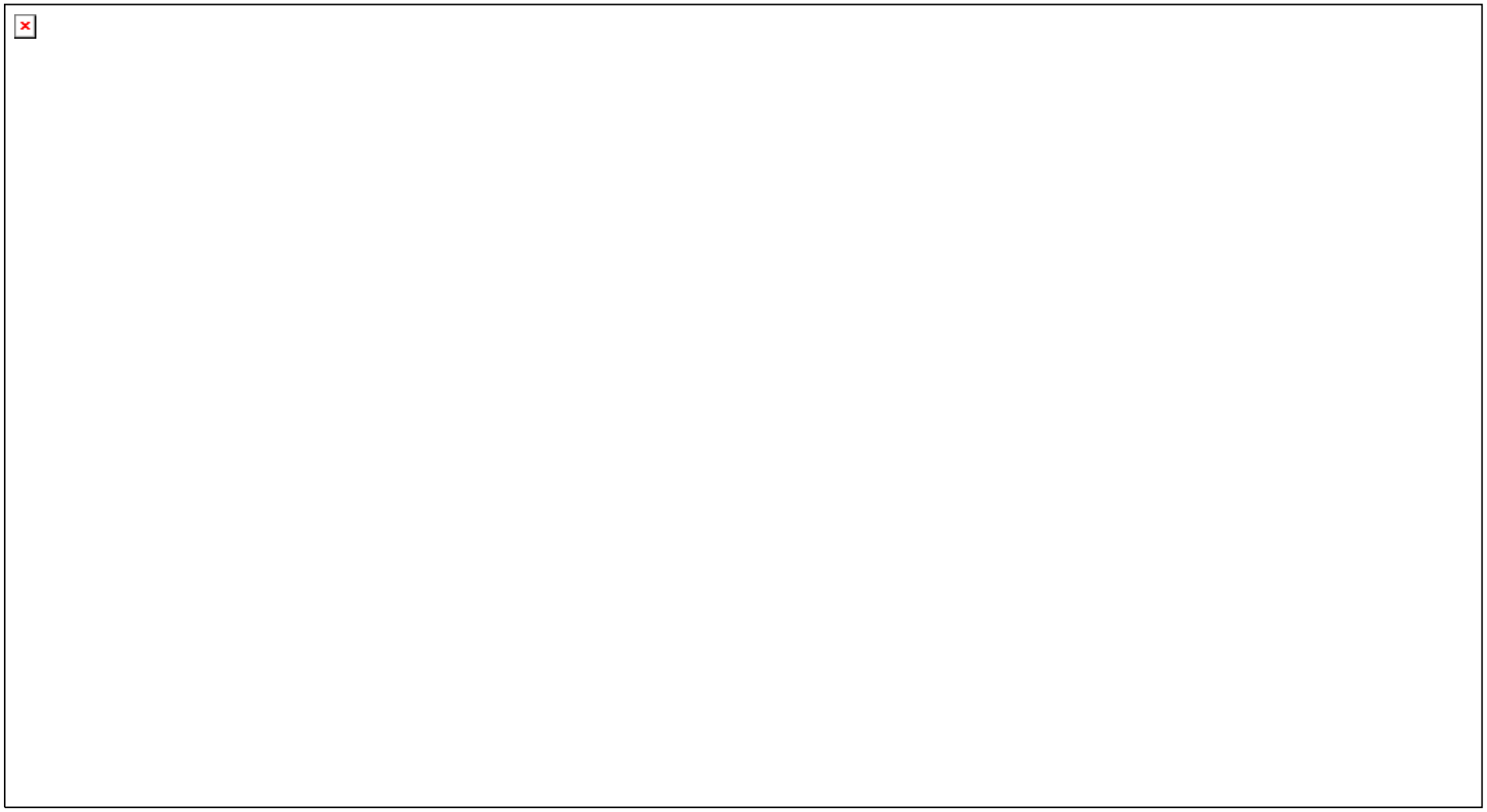
(3*S*)-3-(*N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanylamino)azetidin-2-one (**34**) ^{13}C NMR (125 MHz, DMF-d_7)



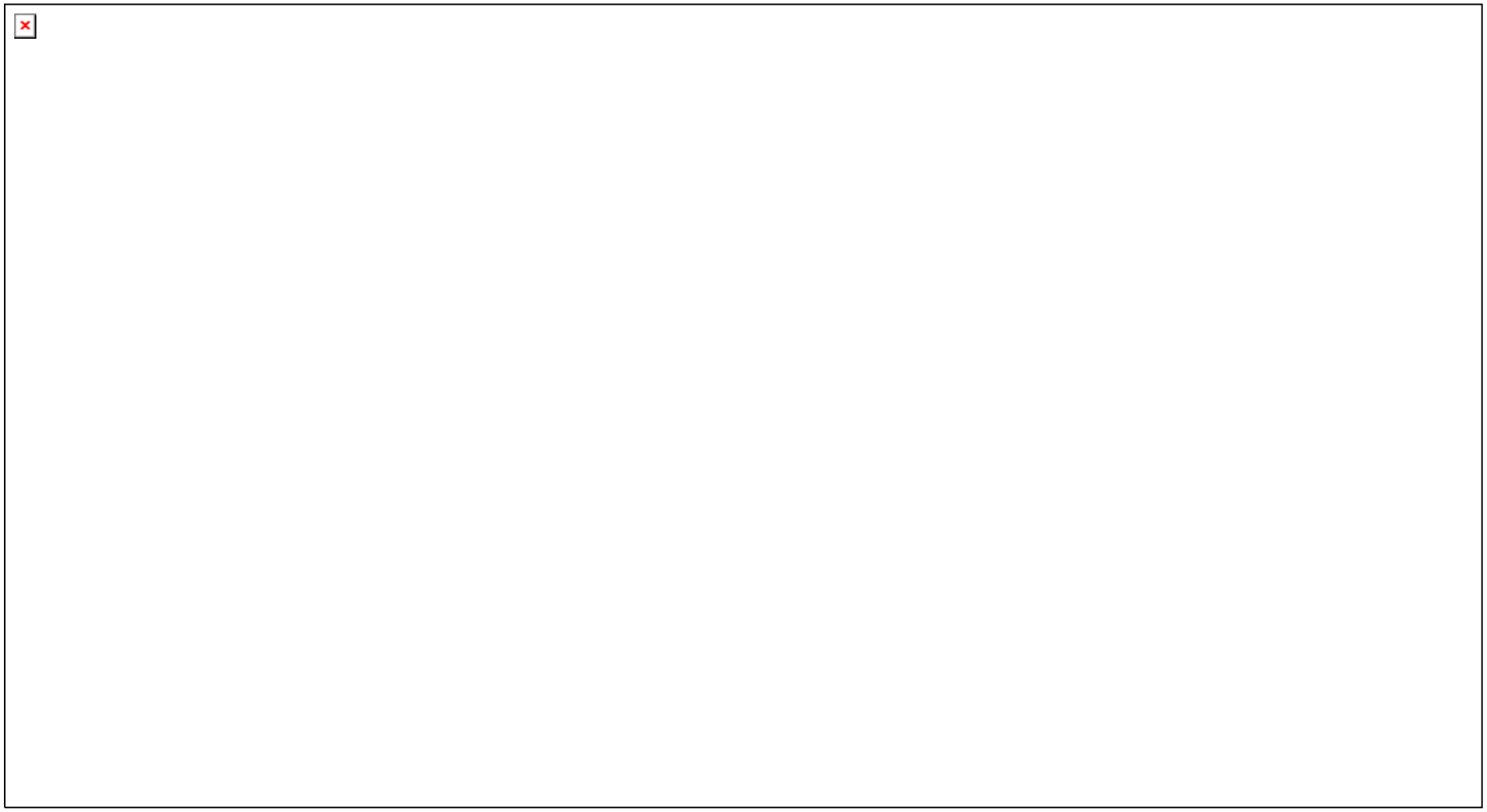
_S_,_S_'-bis(2,4,6-trimethoxybenzyl) _N_-_tert_-butoxycarbonyl-L-phenylalanyl-L-thioaspartate (36) ^{1}H NMR (500 MHz, CD_3CN)



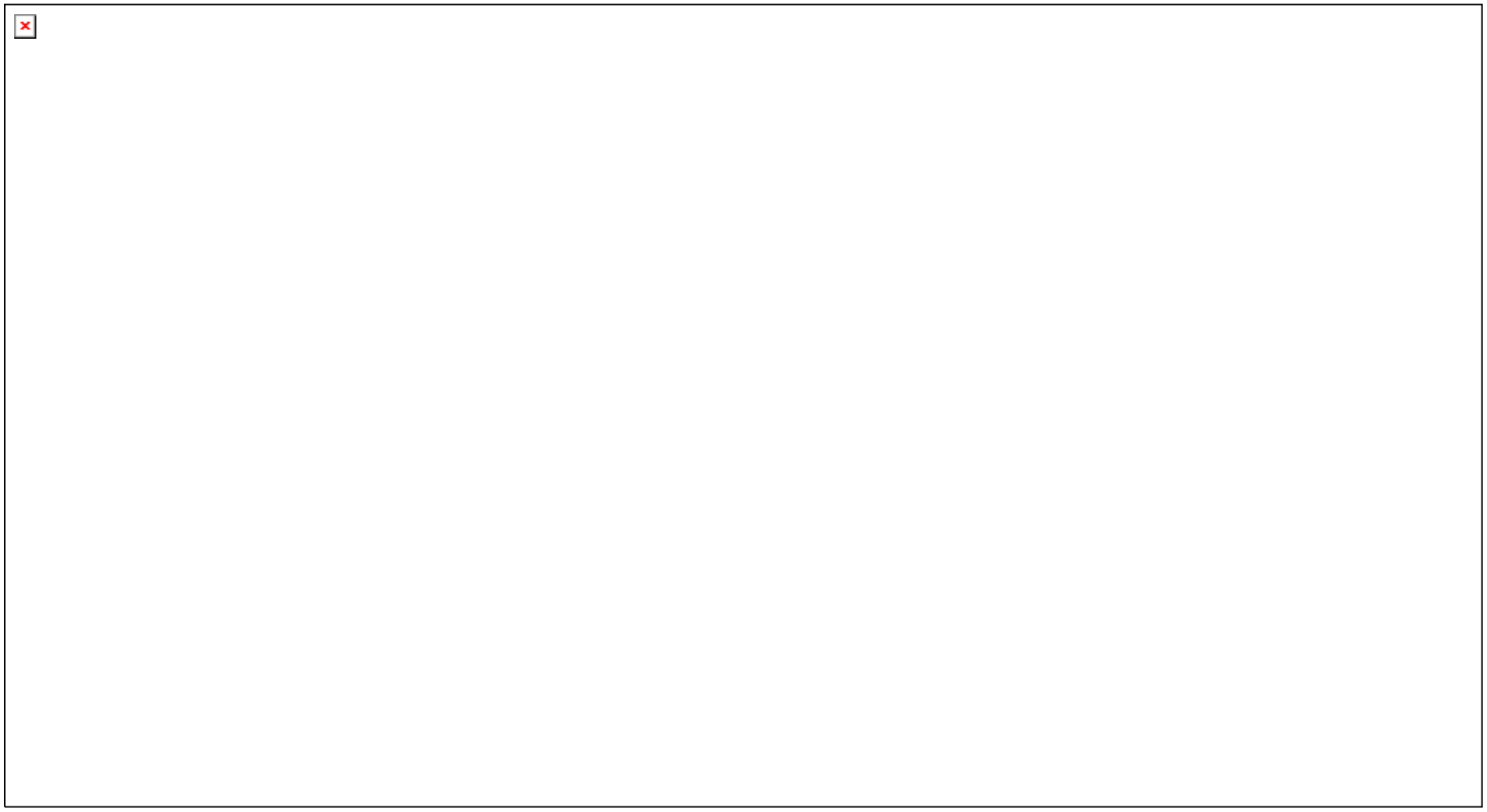
_S_,_S_'-bis(2,4,6-trimethoxybenzyl) _N_-_tert_-butoxycarbonyl-L-phenylalanyl-L-thioaspartate (36) ^{13}C NMR (125 MHz, CD_3CN)



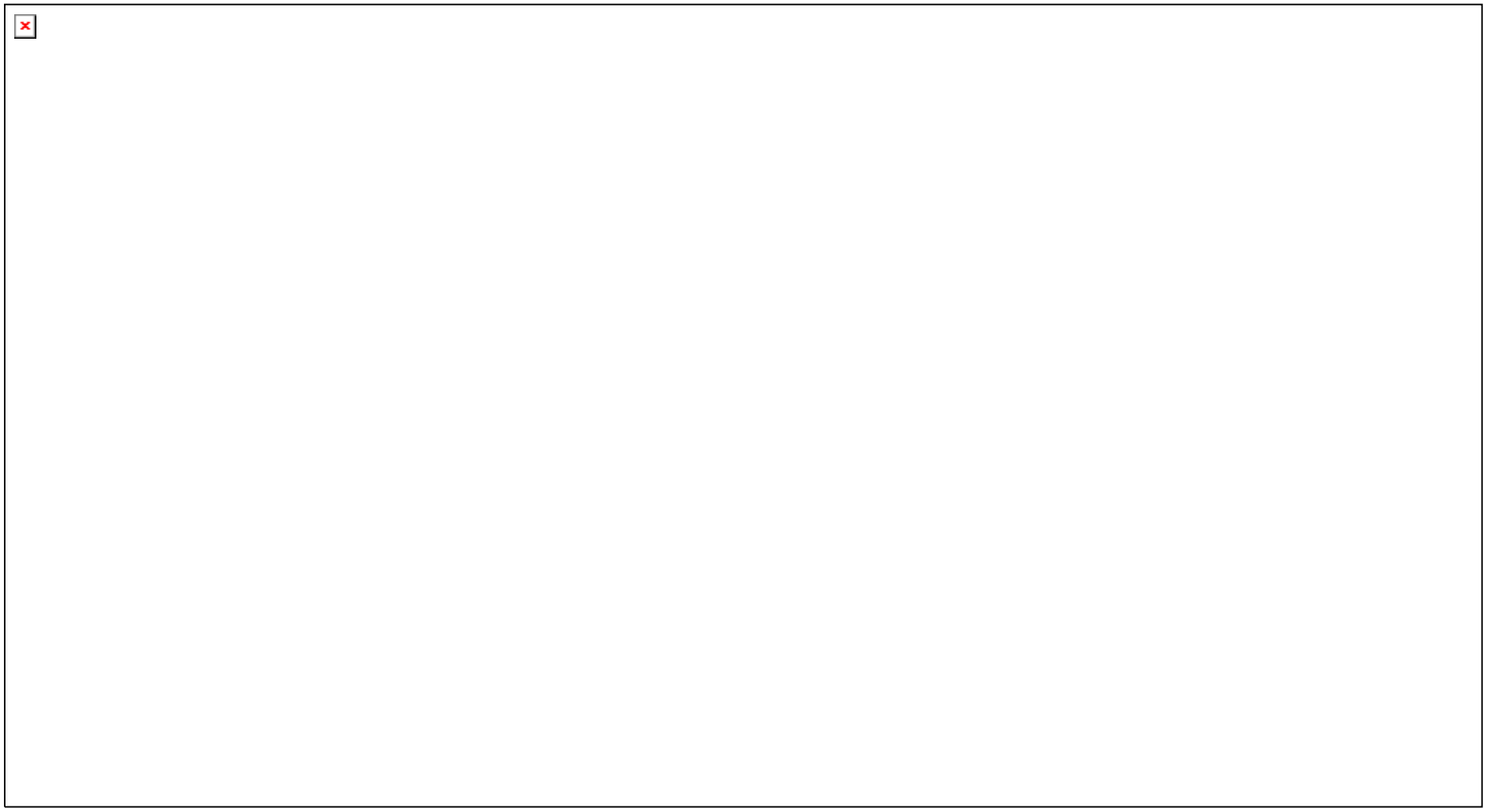
***S,S'*-bis(2,4,6-trimethoxybenzyl) *N*-benzyloxycarbonyl-L-phenylalanyl- L-phenylalanyl-L-thioaspartate (37)** ^{1}H NMR (500 MHz, CD_2Cl_2)



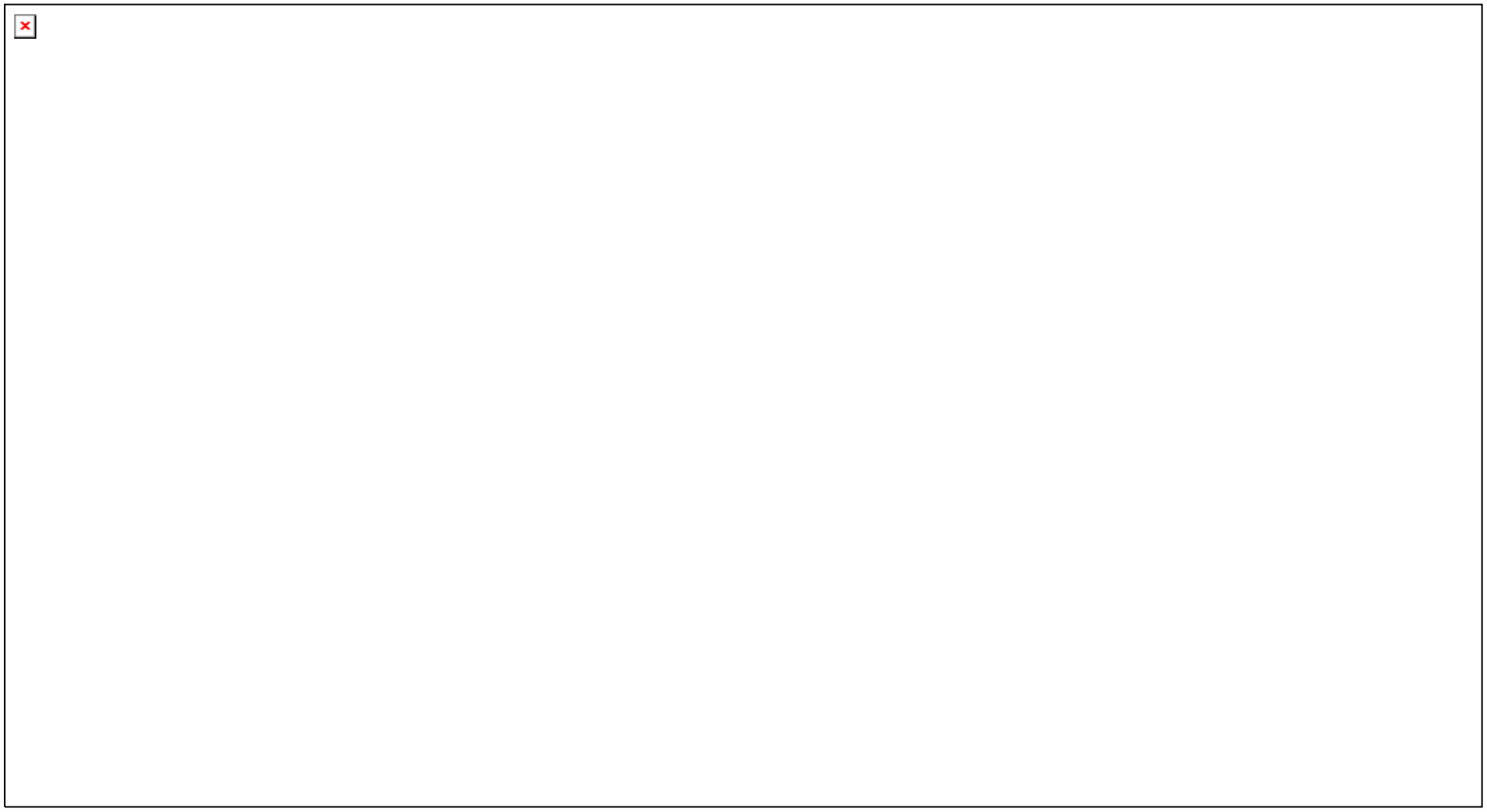
_S_,_S_'-bis(2,4,6-trimethoxybenzyl) _N_-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-thioaspartate (**37**) ^{13}C NMR (125 MHz, CD_2Cl_2)



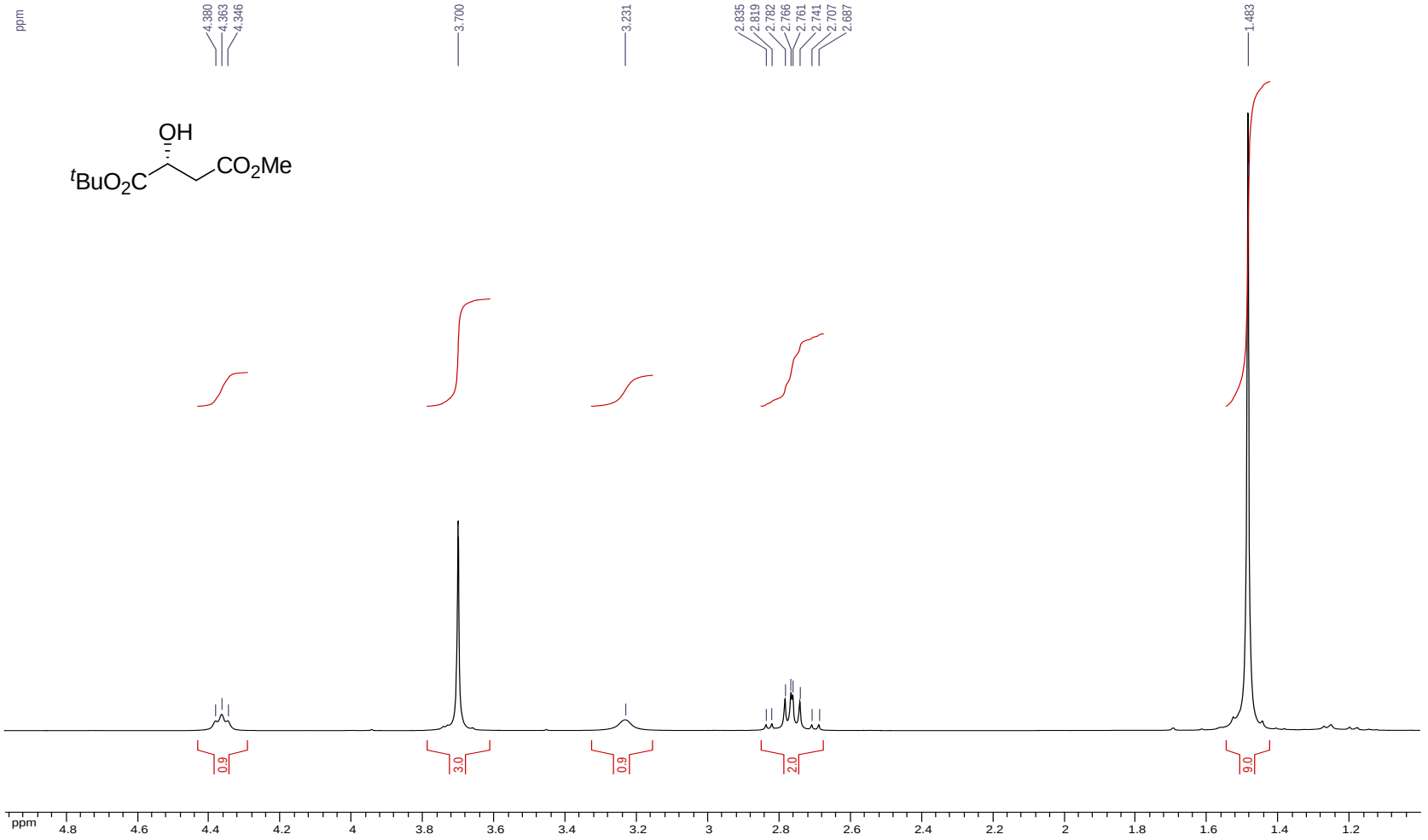
***N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-thioaspartic anhydride (38)** ^{1}H NMR (500 MHz, CD_3CN)



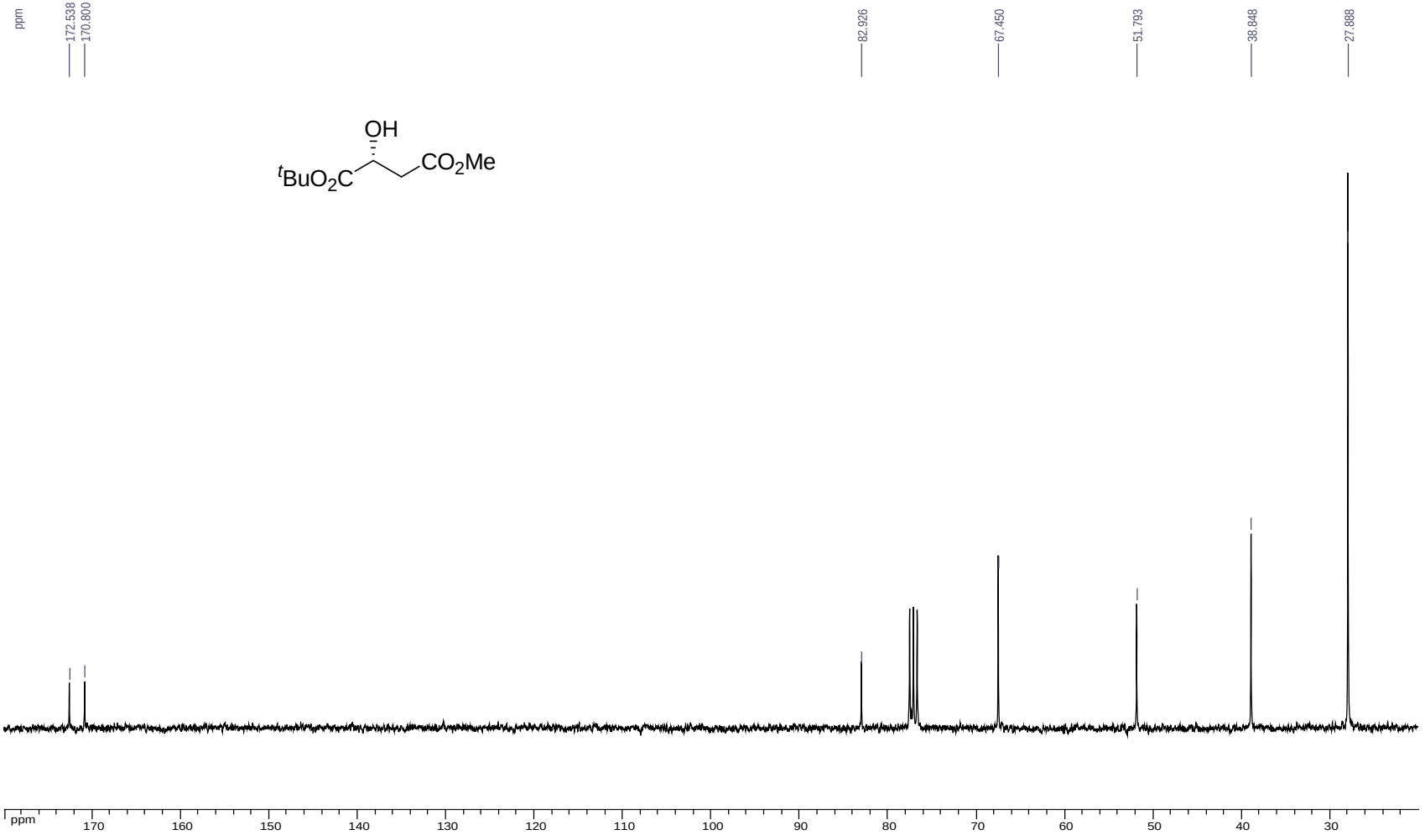
***N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanyl-L-thioaspartic anhydride (38)** ^{13}C NMR (125 MHz, CD_3CN)



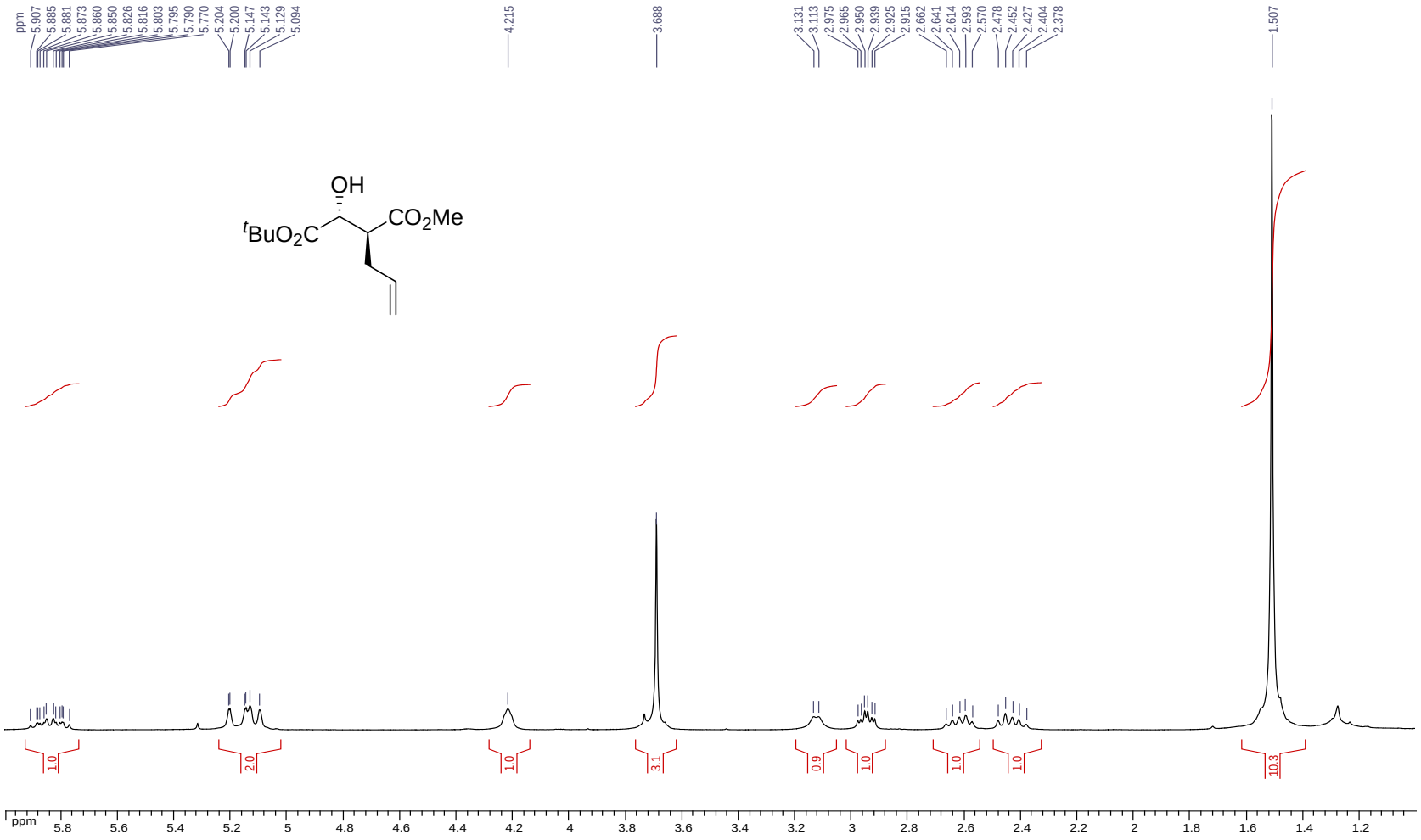
1-*O*-*tert*-butyl 4-*O*-methyl (2*R*)-2-hydroxysuccinate (39) ^{1}H NMR (300 MHz, $CDCl_3$)



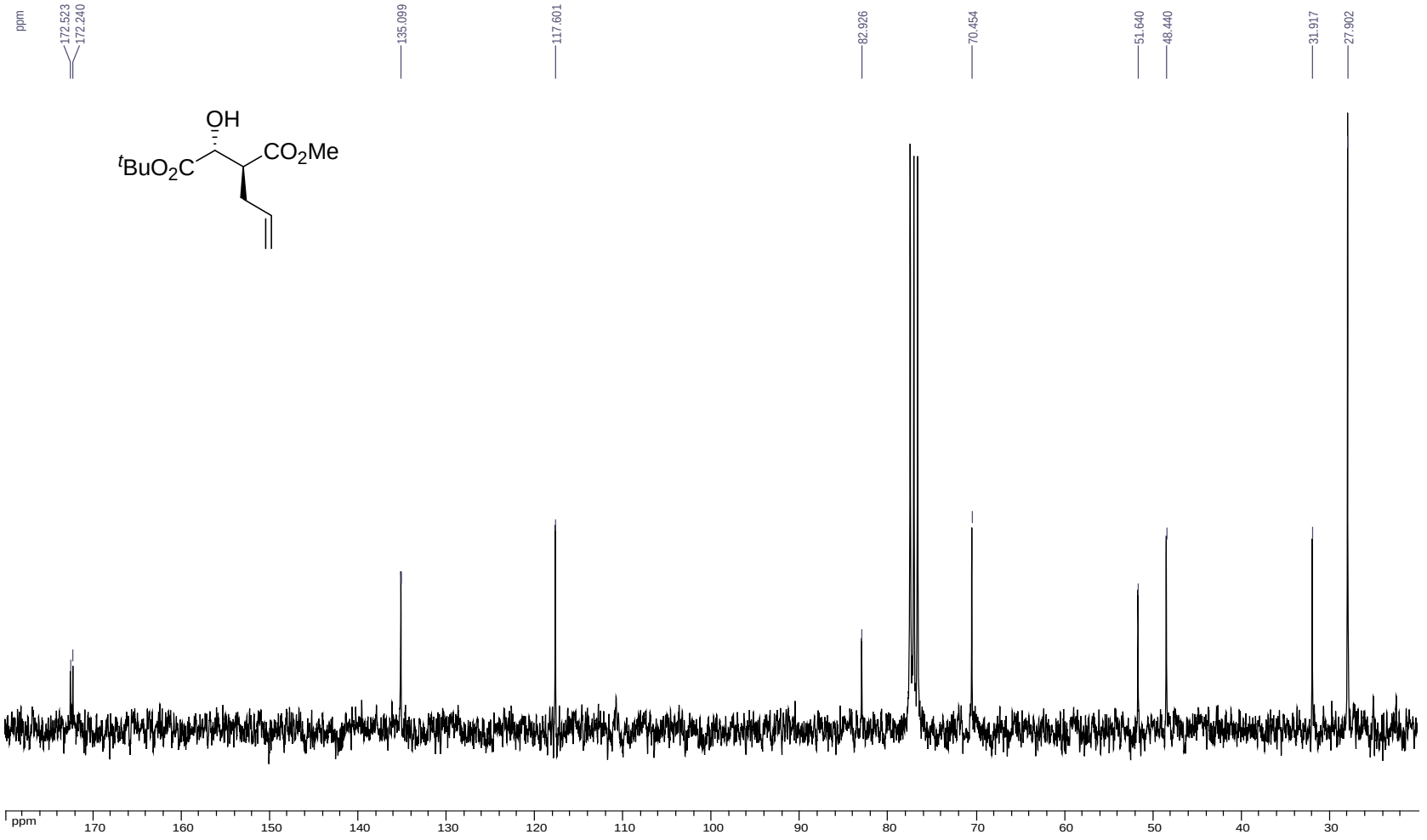
1-*O*-*tert*-butyl 4-*O*-methyl (2*R*)-2-hydroxysuccinate (39) ^{13}C NMR (75 MHz, $CDCl_3$)



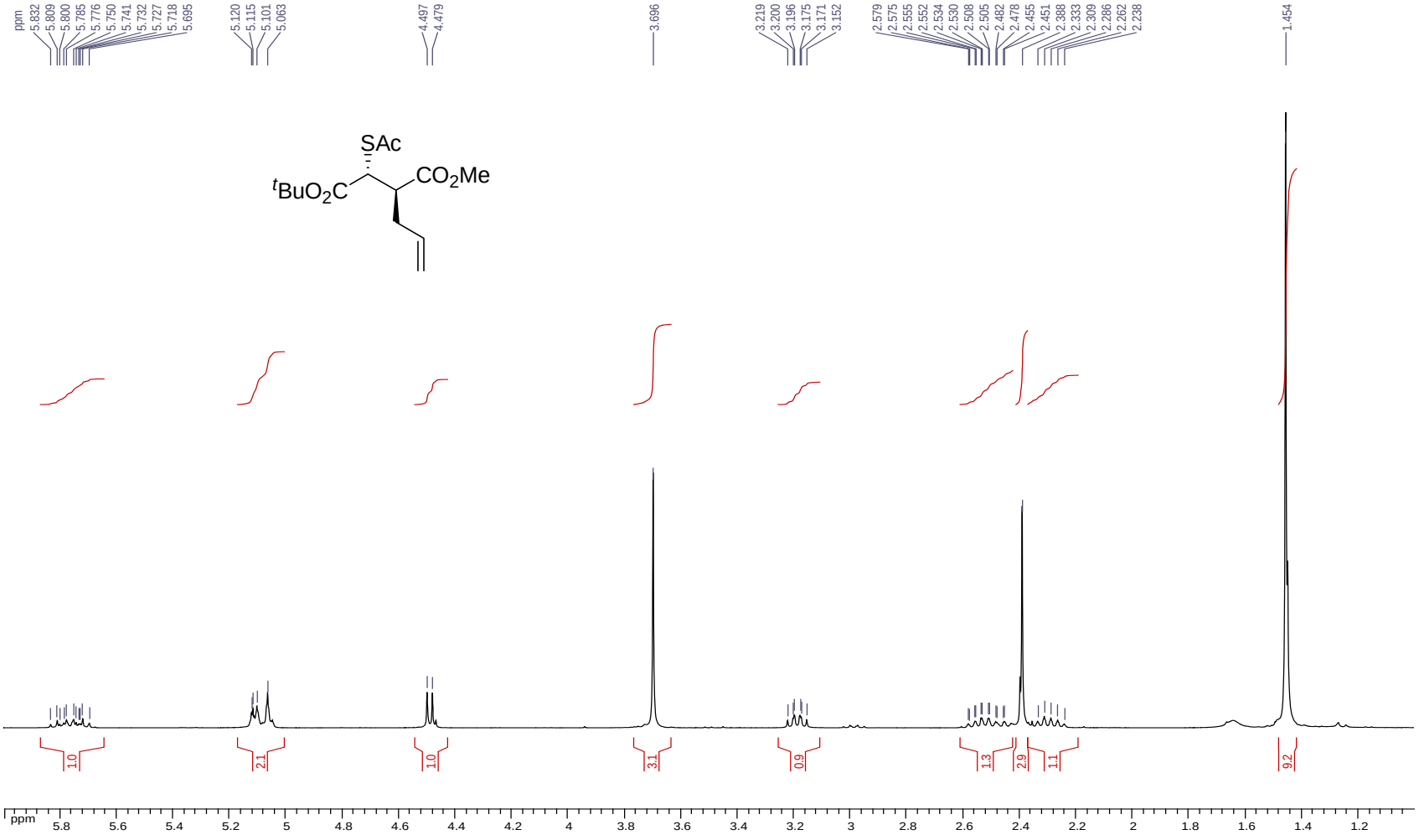
1-*O*-*tert*-butyl 4-*O*-methyl (2*R*,3*S*)-3-allyl-2-hydroxysuccinate (40) ^{1}H NMR (300 MHz, $CDCl_3$)



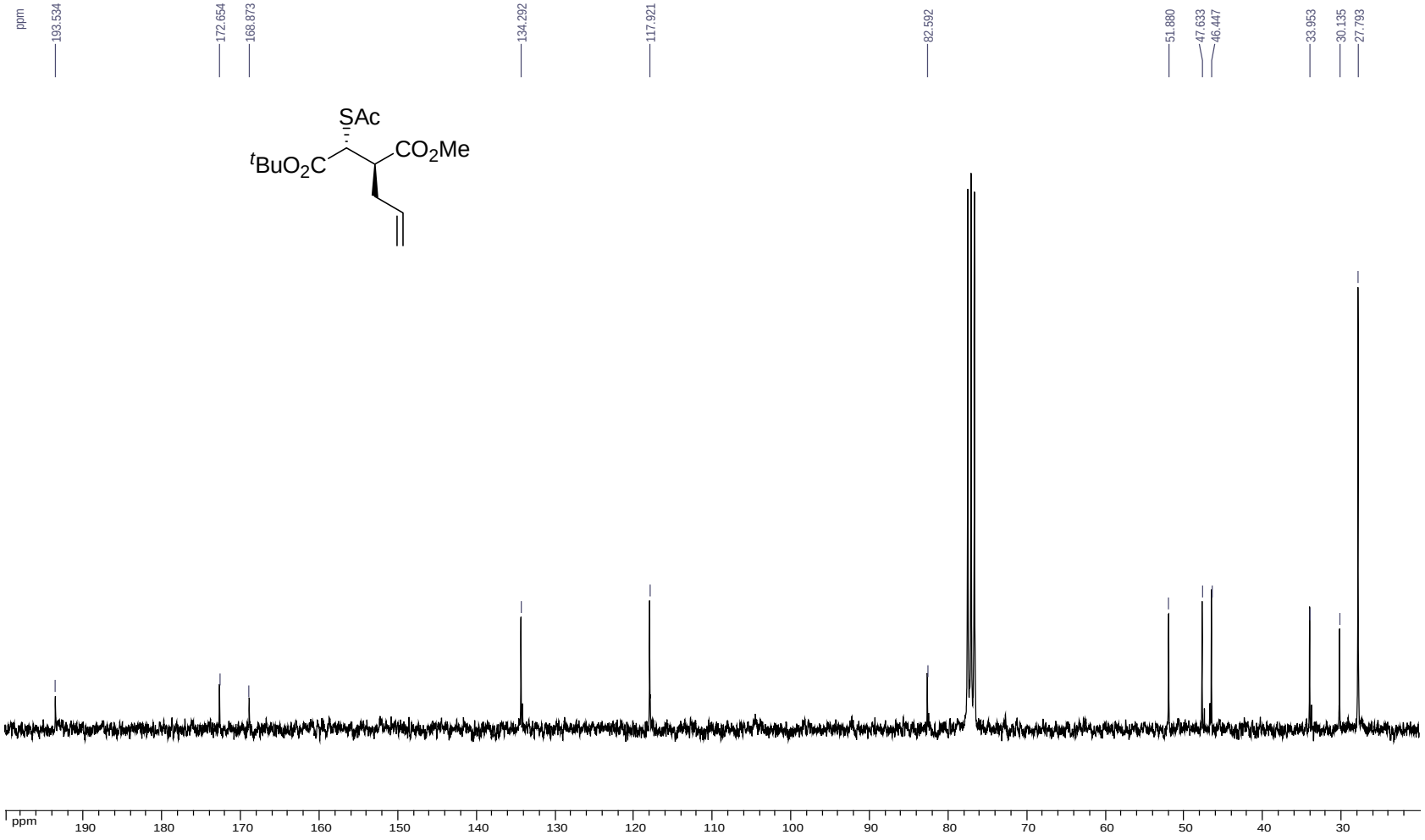
1-*O*-*tert*-butyl 4-*O*-methyl (2*R*,3*S*)-3-allyl-2-hydroxysuccinate (40) ^{13}C NMR (75 MHz, $CDCl_3$)



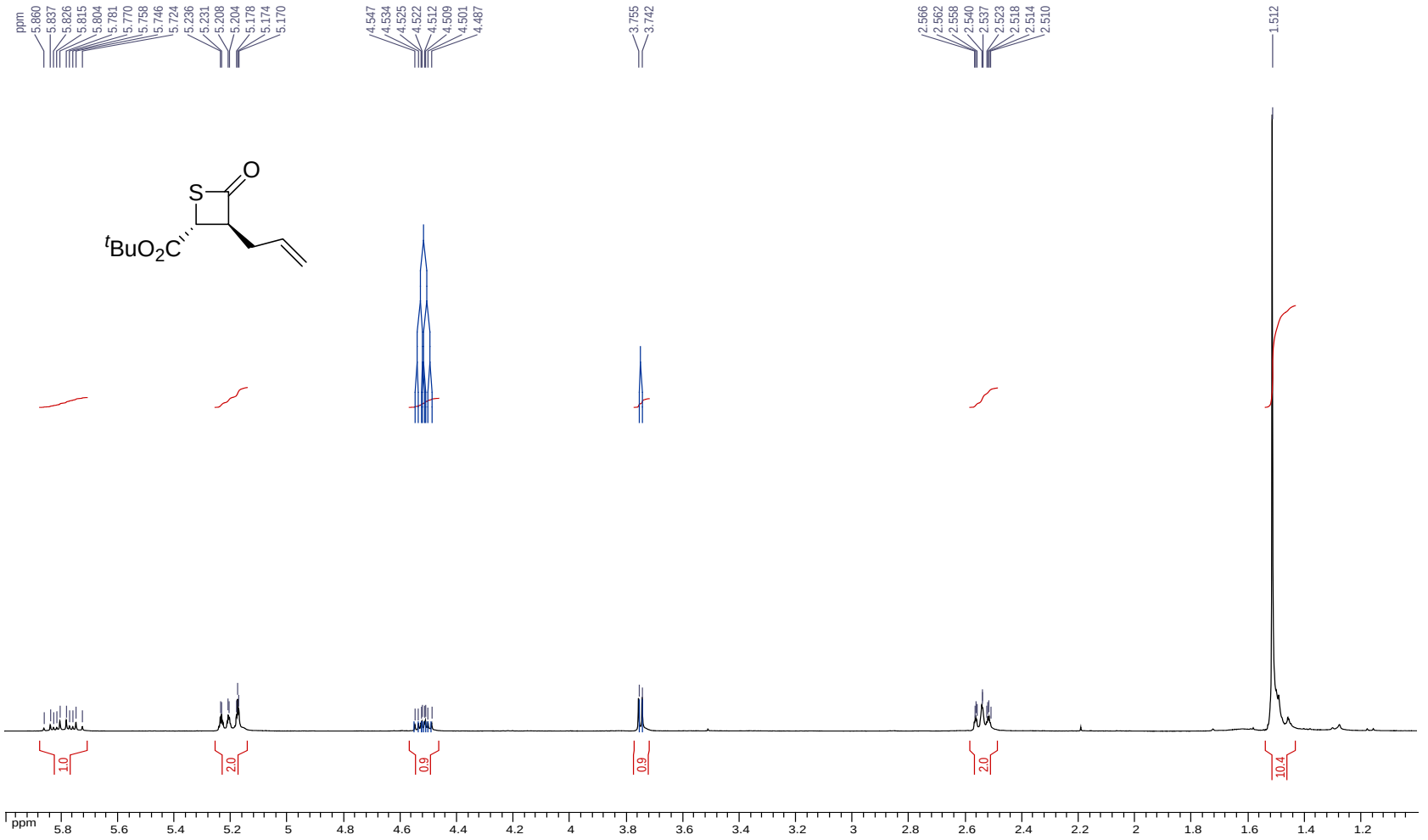
1-*O*-*tert*-butyl 4-*O*-methyl (2*R*,3*S*)-2-acetylsulfanyl-3-allyl-succinate (41) ^{1}H NMR (300 MHz, $CDCl_3$)



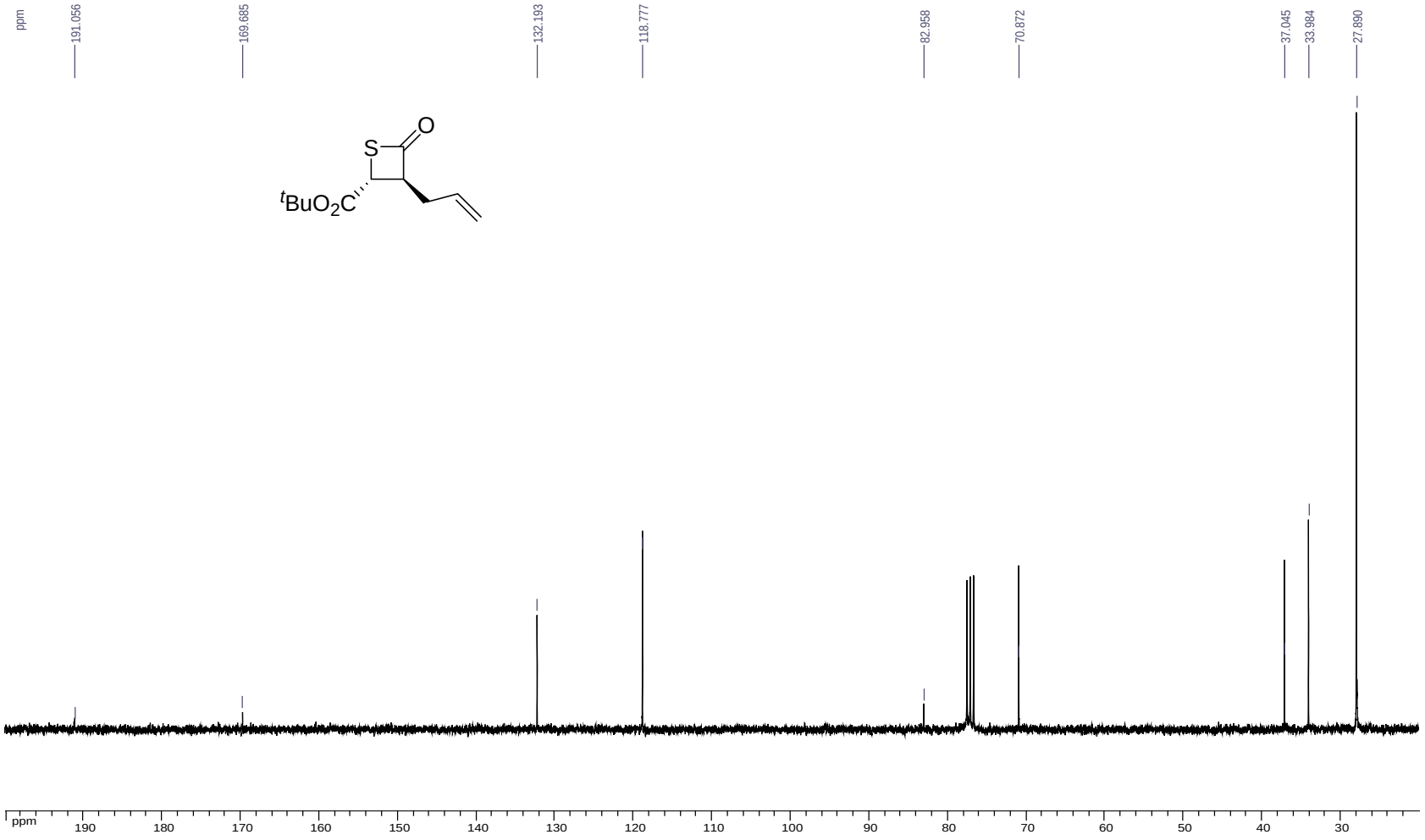
1-*O*-*tert*-butyl 4-*O*-methyl (2*R*,3*S*)-2-acetylsulfanyl-3-allyl-succinate (41) ^{13}C NMR (75 MHz, $CDCl_3$)



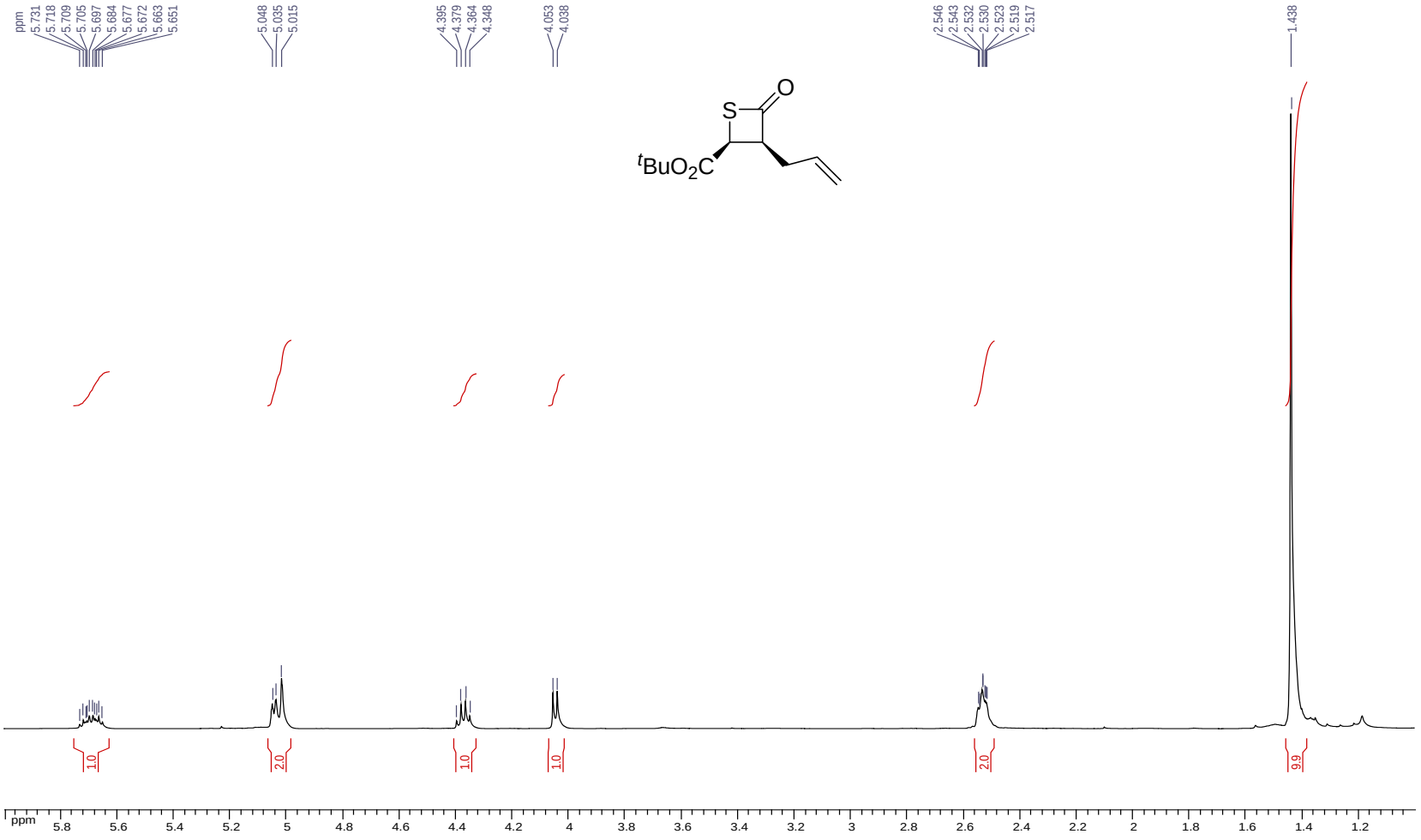
(3*S*,4*R*)-3-allyl-4-*tert*-butylcarbonylthietan-2-one (*trans*-**42**) ^{1}H NMR (300 MHz, $CDCl_3$)



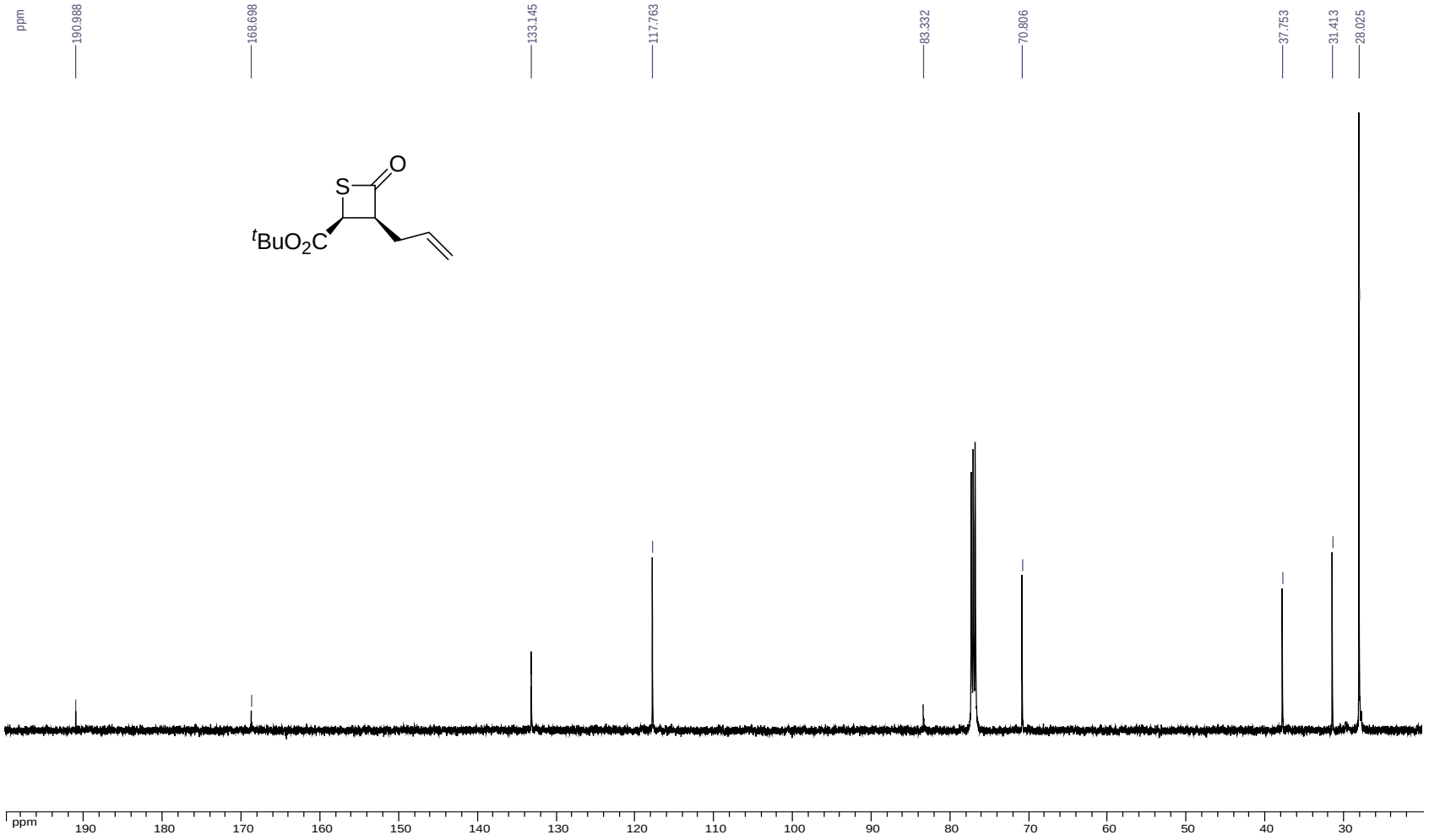
(3*S*,4*R*)-3-allyl-4-*tert*-butylcarbonylthietan-2-one (*trans*-**42**) ^{13}C NMR (75 MHz, $CDCl_3$)



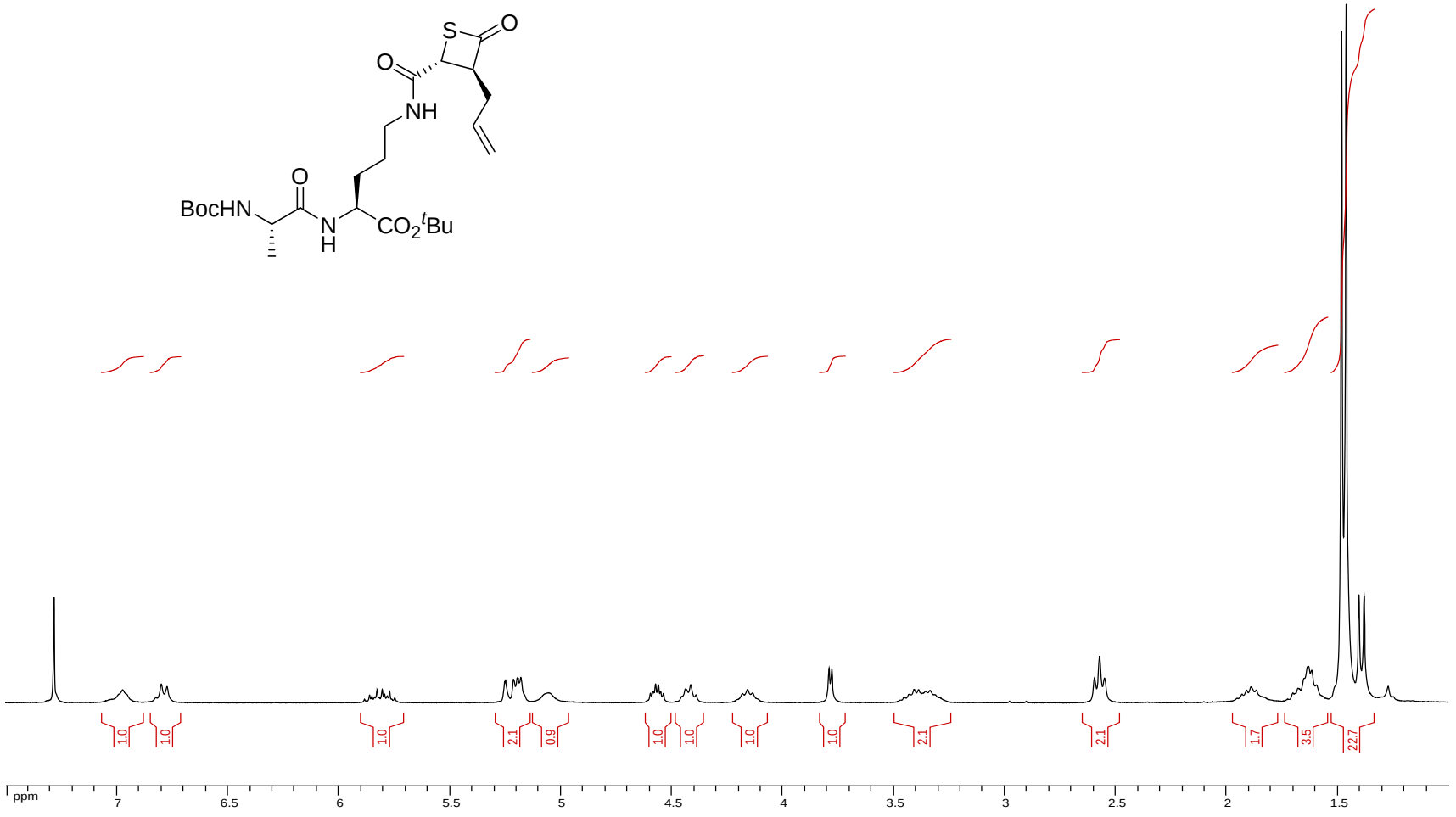
(3*S*,4*S*)-3-allyl-4-*tert*-butylcarbonylthietan-2-one (*cis*-**42**). ^{1}H NMR (500 MHz, $CDCl_3$)



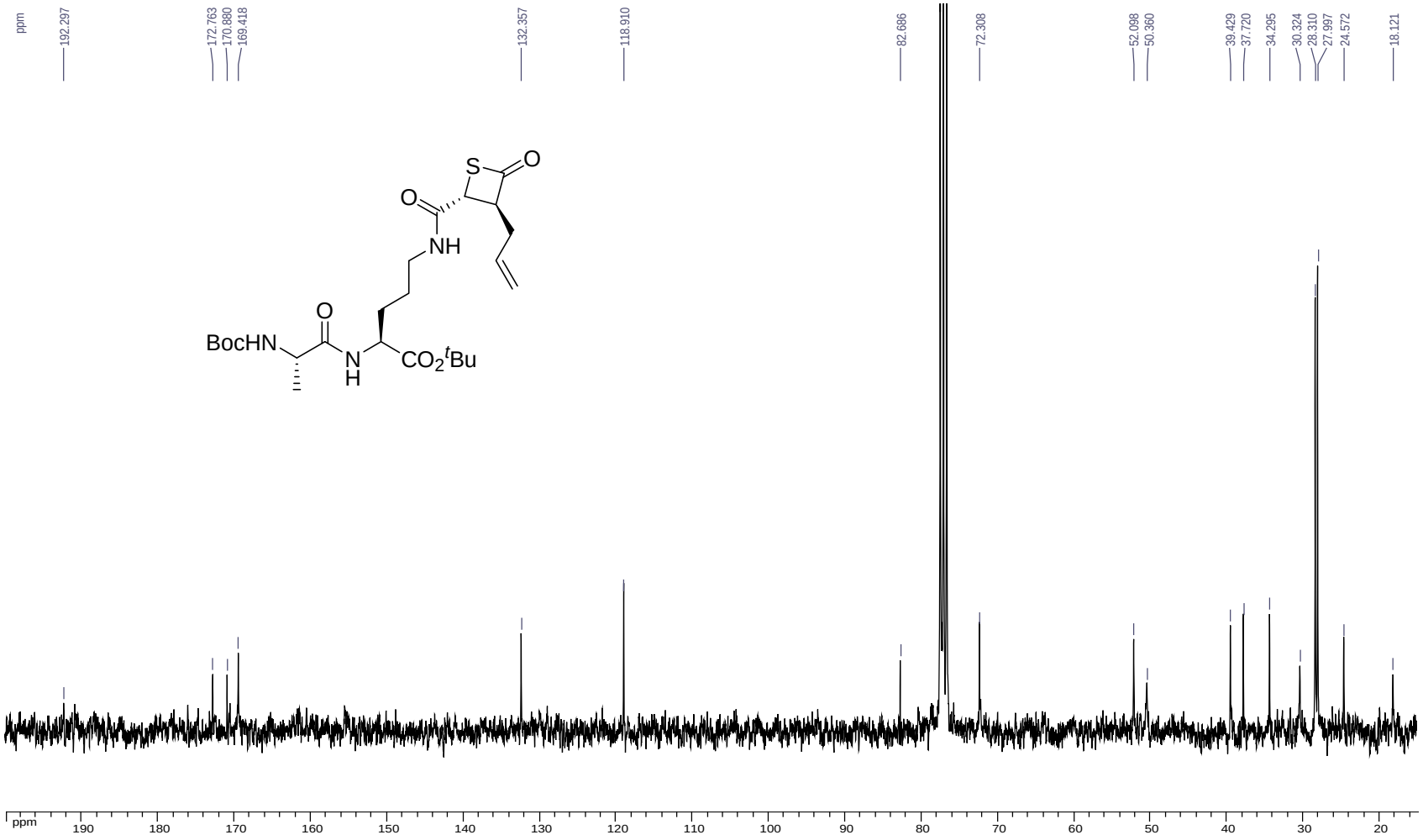
(3*S*,4*S*)-3-allyl-4-*tert*-butylcarbonylthietan-2-one (*cis*-**42**). ^{13}C NMR (125 MHz, $CDCl_3$)



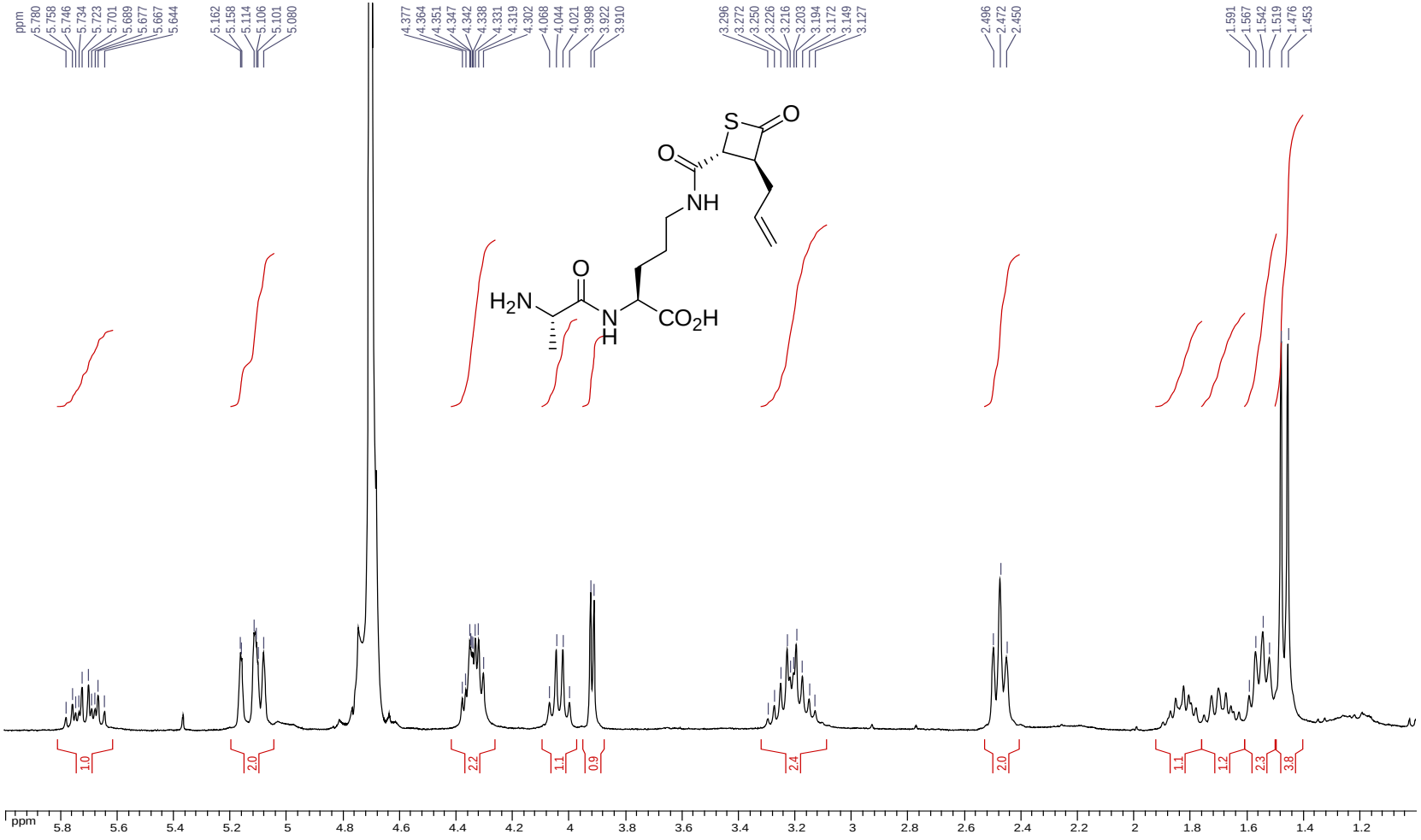
***tert*-butyl (3*S*,4*R*)-*N*-*tert*-butylcarbonyl-L-alanyl-N^{δ}-(3-allyl-2-oxothietan-4-yl)-L-ornithinate** (**43**) ^{1}H NMR (300 MHz, $CDCl_3$)



tert-butyl (3_S_,4_R_)-_N_-_tert_-butylcarbonyl-L-alanyl-N^{δ}-(3-allyl-2-oxothietan-4-yl)-L-ornithinate (43) ^{13}C NMR (75 MHz, $CDCl_3$)



(3*S*,4*R*)-L-alanyl-N^{δ}-(3-allyl-2-oxothietan-4-yl)-L-ornithine (44) ^{1}H NMR (300 MHz, D_2O)



(3*S*,4*R*)-L-alanyl-N^{δ}-(3-allyl-2-oxothietan-4-yl)-L-ornithine (44) ^{13}C NMR (125 MHz, D_2O)