A General Strategy for the Preparation of C-Terminal Peptide α-Ketoacids by Solid Phase Peptide Synthesis

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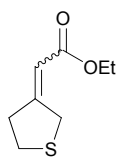
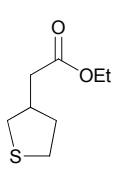
Supporting Information

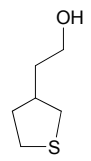
General Methods. All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of dry Ar. CH_2Cl_2 was distilled over CaH_2. DMF were dried by passage over molecular sieves under an Ar atmosphere. CH_3CN, $PhCH_3$ were dried by passage over activated alumina under an Ar atmosphere. Diisopropylethylamine (DIPEA) was disd from KOH. Other reagents were used without further purification. Oxone® was purchased from Alfa Aesar. Rink amide MBHA (0.54–0.72 mmol/g loading, 100–200 mesh) and Wang resin (1.3 mmol/g loading) were purchased from Novabiochem®. Hydroxylamines were prepared from the corresponding primary amines by the method of Fukuyama.[1] Thin layer chromatography (TLC) was performed on EMD precoated plates (silica gel 60 F_{254}, Art 5715, 0.25 mm) and compounds visualized by fluorescence under UV light or by staining with phosphomolybdic acid or potassium permanganate. Silica-gel preparative thin-layer chromatography (PTLC) was performed using plates prepared from Merck Kieselgel 60 PF254 (Art 7747). Column chromatography was performed on EMD Silica Gel 60 (230-400 Mesh) using a forced flow of 0.5–1.0 bar. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were measured on a Bruker Avance AVII-500 spectrometer. Chemical shifts are expressed in parts per million (ppm) and are referenced to the internal solvent signals. Coupling constants are reported in Hertz (Hz). Splitting patterns are indicated as follows: a, apparent; br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared (IR) spectra were recorded on a JASCO FT/IR-430 spectrophotometer and are reported as wavenumbers (cm^{-1}). HPLC analyses were performed with a JASCO HPLC system composed of a degasser DG-2080-54, HPLC pump PU-2087, dynamic mixer MX-2080-32, and a UV detector UV-2077. HPLC analyses were performed using a Shishedo CapcellPak C18 or YMC R-ODS-10-A column (250 x 4.6 mm for analytical and 250 x 20 mm for preparative column) using a gradient of CH_3CN or isopronanol (IPA) and millipure H_2O containing 0.1 % TFA. SFC analyses were performed using an AS-H or OJ-H column and a gradient of isopropanol (IPA) and super critical CO_2.



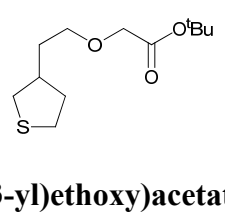
(*E*) and (*Z*)-ethyl 2-(dihydrothiophen-3(2H)-ylidene)acetate (**1**) Triethyl phosphonoacetate (2.38 mL, 10.95 mmol) was added dropwise at room temperature to a suspension of NaH (57-63% in mineral oil, 0.292 g, 7.30 mmol) and dry THF (15 mL). The resulting solution was stirred for 0.5 h and cooled to -78 ^{o}C, and tetrahydrothiphen-3-one (0.626 g, 7.30 mmol) was then added. The solution was maintained at -78 ^{o}C for 1 h, then warmed to room temperature, diluted with ethyl ether, washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography over silica gel (12:1 hexanes:EtOAc) to provide the product as a clear oil (1.219 g, 97% yield). ν_{max} (film)/cm^{-1} 2916.3, 2848.8, 1712.9, 1652.2, 1370.2, 1347.0, 1213.5, 1141.2; δ_H (400 MHz, $CDCl_3$) 5.91–5.87 (m, 1H, vinyl-CH), 4.17 (q, 2H, *J*=7.0 Hz, OCH_2), 4.00 (s, 1H, CH), 3.61 (s, 1H, CH), 3.20 (t, 1H, *J*=4.0 Hz, CH), 2.97 (t, 1H, *J*=6.8 Hz, CH), 2.87 (t, 2H, *J*=4.0 Hz, CH_2), 1.29 (t, 3H, *J*=7.0 Hz, CH_3); δ_C (100 MHz, $CDCl_3$) 162.7, 113.4, 111.3, 60.1, 40.2, 38.9, 33.9, 34.7, 31.8, 29.5, 14.4; HRMS (ESI) $C_8H_{12}O_2S$ calcd. 172.0558, found 172.0551 M^+.



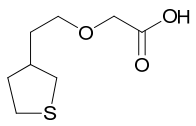
Ethyl 2-(tetrahydrothiophen-3-yl)acetate (**2**)[2] A 100 mL round bottom flask equipped with a magnetic stir bar was charged with (*E*) and (*Z*)-ethyl 2-(dihydrothiophen-3(2H)-ylidene)- acetate **1** (1.184 g, 6.88 mmol), nickel (II) chloride (0.893 g, 6.89 mmol) and 15 mL of dry EtOH. Sodium borohydride (1.041 g, 27.52 mmol) was added in four portions at 0 ^{o}C. Precipitation of a finely divided black solid was observed immediately upon the addition of $NaBH_4$. The reaction was allowed to stir for an additional 10 min at 0 ^{o}C and warmed to room temperature. The progress of the reaction was monitored by TLC. After the complete disappearance of the olefin, the reaction mixture was filtered through a plug of Celite, the black nickel boride precipitate was washed repetitively with ethanol (200 mL). The filtrate was concentrated (20 mL) under reduced pressure without heating, diluted with (100 mL) and extracted twice with Et_2O (100 mL). The organic layer was extracted with H_2O, washed with brine, and dried over Na_2SO_4. The crude product was obtained as dark brown oil and purified by column chromatography (12:1 hexanes:EtOAc) to give the product as a clear oil (0.766 g–0.816 g, 64–68% yield). ν_{max} (film)/cm^{-1} 2922.1, 2848.8, 1735.1, 1468.1, 1384.6, 1181.7; δ_H (500 MHz, $CDCl_3$) δ 4.15 (q, 2H, *J*=7.0 Hz, OCH_2), 3.01 (dd, 1H, *J*=6.0 Hz, 4.0 Hz, SCH), 2.90–2.87 (m, 2H, SCH), 2.64–2.60 (m, 1H, SCH), 2.57–2.53 (m, 1H, CH), 2.43 (d, 2H, *J*=7.0 Hz, CH_2), 2.19–2.16 (m, 1H, CH), 1.17–1.65 (m, 1H, CH), 1.27 (t, 3H, *J*=7.2 Hz, CH_3); δ_C (125 MHz, $CDCl_3$) 172.3, 60.4, 40.5, 37.8, 36.4, 36.3, 30.3, 14.2; HRMS (ESI) $C_8H_{14}O_2S$ calcd. 174.0715, found 175.0773 $[M+H]^+$.



2-(tetrahydrothiophen-3-yl)ethanol (**3**) In a 100 mL flame dried round bottom flask, ethyl 2-(tetrahydrothiophen-3-yl)acetate **2** (0.749 g, 4.30 mmol) was added cautiously over 5 min to a stirring solution of $LiAlH_4$ (95%, 0.710 g, 17.22 mmol) in 40 mL of dry Et_2O at 0°C under N_2. The reaction was stirred for 1.5 h and quenched by dropwise addition of 0.7 mL H_2O, 0.7 mL of 15% NaOH solution, and 2 mL of H_2O. The mixture was stirred vigorously for 30 min and the resulting granular precipitate was filtered and rinsed with Et_2O. The filtrate was added water, the layers were separated and the combined organic layer was washed with brine, dried over Na_2SO_4. After removal of solvent, the product was purified by column chromatography (7:3 hexanes:EtOAc) to give the product as a clear oil (0.596 g, 89% yield). ν_{max} (film)/cm^{-1} 2924.5, 2848.8, 2327.7, 1447.3, 1405.4, 1004.3; δ_H (500 MHz, $CDCl_3$) 3.72 (t, 2H, *J*=6.75 Hz, OCH_2), 2.97 (dd, 1H, *J*=6.5 Hz, 4 Hz, SCH), 2.88–2.83 (m, 2H, SCH_2), 2.52 (t, 1H, *J*=9.2 Hz, SCH), 2.32–2.25 (m, 1H, CH), 2.21–2.15 (m, 1H, CH), 1.77–1.66 (m, 2H, CH_2), 1.64–1.57 (m, 1H, CH); δ_C (125 MHz, $CDCl_3$) 61.9, 41.3, 36.8, 36.8, 36.3, 30.8; HRMS (ESI) $C_6H_{12}OS$ calcd. 132.0609, found 131.0569 $[M\text{-}H]^+$.



***tert*-Butyl 2-(2-(tetrahydrothiophen-3-yl)ethoxy)acetate** (**4**) To a solution of 2-(tetrahydrothiophen-3-yl)ethanol **3** (0.500 g, 3.78 mmol) in 2 mL of benzene, was added 2 mL of 12 N NaOH, tetrabutylammonium sulfate (0.128 g, 0.38 mmol), followed by *tert*-butyl bromoacetate (1.11 mL, 7.56 mmol). The biphasic reaction was allowed to stir vigorously at room temperature for 3 h, then was neutralized with sat. NH_4Cl. The mixture was extracted with Et_2O, and the organic layers were combined, washed with brine, dried over Na_2SO_4. The solution was concentrated *in vacuo*. to afford a yellow crude oil, which was purified by silica column chromatography (15:1 hexanes:EtOAc) to give the product as a clear oil (0.596 g, 64% yield). ν_{max} (film)/cm^{-1} 2979.0, 2931.8, 1745.7, 1734.7, 1369.2, 1228.4, 1136.8; δ_H (500 MHz, $CDCl_3$) 3.94 (s, 2H, $COCH_2$), 3.57 (t, 2H, *J*=7.5 Hz, OCH_2), 2.97 (dd, 1H, *J*=6.5 Hz, 4.0 Hz, SCH), 2.86–2.83 (m, 2H, SCH_2), 2.50 (t, 1H, *J*=9.2 Hz, CH), 2.32–2.29 (m, 1H, CH), 2.19–2.16 (m, 1H, CH), 1.78–1.72 (m, 2H, CH_2), 1.61–1.57 (m, 1H, CH), 1.48 (s, 9H, $C(CH_3)_3$); δ_C (125 MHz, $CDCl_3$) 169.8, 81.7, 70.6, 69.0, 41.6, 36.8, 33.4, 30.8, 28.3; HRMS (ESI) $C_{12}H_{22}O_3S$ calcd. 246.1290, found 246.1302 $[M]^+$.



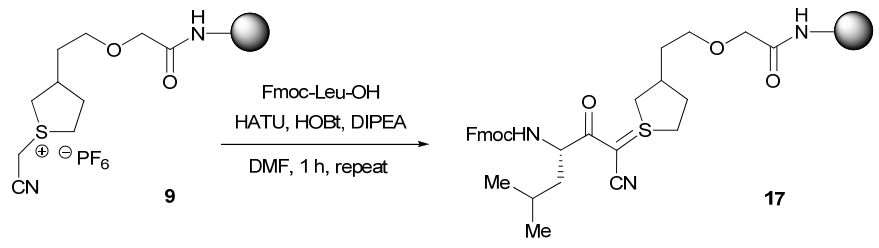
2-(2-(tetrahydrothiophen-3-yl)ethoxy)acetic acid (5). *tert*-butyl 2-(2-(tetrahydrothiophen-3-yl)-ethoxy)acetate **4** (0.522 g, 2.12 mmol) was dissolved in dry CH_2Cl_2 (2 mL), to which was added trifluoroacetate (2 mL) and triisopropylsilane (0.1 mL). Upon disappearance of the *tert*-butyl ester, the reaction was concentrated and was purified over silica gel by column chromatography (9:1 CH_2Cl_2:CH_3OH) to afford a clear oil (0.352 g, 87% yield). ν_{max} (film)/cm^{-1} 2933.7, 2864.3, 1734.2, 1641.6, 1436.7, 1240.5, 1133.9; δ_H (500 MHz, $CDCl_3$) racemic mixture 4.13 (s, 2H, CH_2), 3.62 (t, 2H, *J*=6.3 Hz, OCH_2), 2.96 (dd, 1H, *J*= 6.5 Hz, 4.0 Hz, SCH), 2.87–2.84 (m, 2H, SCH_2), 2.50 (t, 1H, *J*=9.5 Hz, SCH), 2.31–2.28 (m, 1H, CH), 2.18–2.15 (m, 1H, CH), 1.80–1.74 (m, 2H, CH_2), 1.62–1.58 (m, 1H, CH); δ_C (125 MHz, $CDCl_3$) 175.0, 71.1, 68.0, 41.5, 36.8, 33.3, 30.8; HRMS (ESI) $C_8H_{14}O_3S$ calcd. 190.0664, found 191.0742 $[M+H]^+$.

Loading and Cyanomethylation of Linker 9 onto Rink Amide MBHA resin.

Loading of linker **5** onto Rink amide MBHA resin followed the standard HBTU coupling protocol. Fmoc protected Rink amide MBHA resin (0.72 mmol/g loading, 0.112 mmol) was pre-swollen with CH_2Cl_2 for 1 h. The resin was drained, washed thoroughly with DMF, and treated with a solution of piperidine/DMF (1:4 v/v, 2 mL) for 20 min. Carboxylic acid **5** (0.107 mg, 0.56 mmol) and HBTU (0.212 mg, 0.56 mmol) were dissolved in DMF, followed by addition of DIPEA (195 µL, 1.12 mmol). The mixture was added immediately to the resin, and left for 5 h. The resin was drained and washed with DMF, CH_2Cl_2 (3 times each).

A 4 mL oven dried clear glass vial equipped with a magnetic stirring bar was charged with $AgPF_6$ (141.59 mg, 0.560 mmol) and MeCN (0.2 mL) under N_2. The vial was covered with aluminum foil to exclude light. Iodoacetonitrile (40.3 µL, 0.560 mmol) was added to the stirring slurry via syringe. After stirring for 30 min, the light orange slurry was filtered through a plug of Celite to remove the precipitated AgI, which was washed with CH_3CN (0.4 mL). The resulting clear supernatant was mixed with 0.8 mL of toluene and added to pre-swollen (0.5 h in CH_2Cl_2) resin in a vial. Cyanomethylation was generally completed after agitation at 60 ^oC for 8–12 h. The resin was drained and washed sequentially with DMF, CH_2Cl_2, CH_3OH (3 times each).

General Procedure for Coupling of the First Amino Acid:



Sulfonium resin **9** was swollen in dry DMF for 1 h and subjected to coupling with Fmoc-proteced amino acids. Fmoc-Leu-OH (198 mg, 0.560 mmol), HATU (213 mg, 0.560 mmol), HOBt (76 mg, 0.560 mmol) were dissolved in dry DMF (2 mL) in an oven dried glass vial, followed by addition of DIPEA (195 μL, 1.120 mmol). The mixture was allowed to react for 10 min, and added to the cyanomethylated resin with an additional 195 μL DIPEA. The resin was agitated gently for 1 h and the process was repeated once. After washing sequentially with DMF, CH_2Cl_2 and CH_3OH, the resin was air dried for 10 min and the last traces of solvent was removed *in vacuo*.

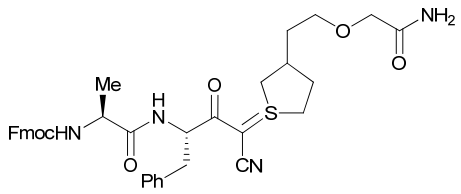
To determine the level of first residue attachment: A small portion of the beads **17** (20 mg) was weighed and transferred to a small vial. The beads were agitated in a mixture of TFA/H_2O/TIS (95:2.5:2.5) for 1 h and the filtrate collected and concentrated under reduced pressure. The ratio between desired sulfur ylide **11** and unalkylated sulfide **7** was determined from ^{1}H NMR analysis. The coupling process was repeated if a notable amount of sulfide was observed. Alternatively, yield of the amino acid loading was examined by spectrophotometric Fmoc-determination (0.50–0.55 mmol/g from 0.72 mmol Rink amide MBHA resin).

General Procedure for Peptide Elongation from Solid Supported Sulfur Ylide:

Derivatized Rink amide MBHA resin (0.112 mmol) was swollen in DCM for 30 min, drained and treated with a solution of piperidine/DMF (1:4 v/v, 2 mL) for 7 min. The resin was drained and washed with DMF (5 times), the process was repeated 1–2 times. Kaiser test was performed after each Fmoc-deprotection. The subsequent peptide coupling followed the standard HBTU coupling protocol. Fmoc-protected amino acid (5 equiv), HBTU (5 equiv), HOBt (5 equiv) were dissolved in DMF, followed by addition of DIPEA (10 equiv). After agitation at room temperature for 10 min, the mixture was added to the resin, agitated for 2–3 hours and the coupling was monitored by Kaiser test. The resin was then drained, washed sequentially with DMF, CH_2Cl_2, CH_3OH (3 times each). At the end of the last coupling, the resin was dried under reduced pressure (1 torr).

General Procedure for TFA Cleavage of Peptide Sulfur Ylide:

The dry beads were placed in a small glass vial and were agitated gently in a mixture of TFA/TIS/EDT/H_2O (95:1:2.5:2.5) for 1 h. The resin was removed by filtrate, washed with TFA/CH_2Cl_2. The combined filtrate was concentrated to minimal volumn, and added 10 fold volume of cold ether. The crude tetrapeptide sulfur ylide was obtained by collecting the precipitated solid.



Fmoc-Ala-Phe-SY (**13**) 80% PTLC yield; ν_{max} (film)/cm^{-1} 3297, 2930, 2246, 2172, 1677, 1592, 1530, 1451, 1246, 1116; δ_H (500 MHz, $CDCl_3$) 7.76 (d, 2H, *J*=7.0 Hz, Ar-H), 7.60 (t, 2H, *J*=5.5 Hz, Ar-H), 7.40 (t, 2H, J=7.5 Hz, Fmoc Ar-H), 7.20-7.14 (m, 5H, Phe-Ar-H), 6.94–6.87 (m, 1H, NH), 6.47–6.37 (m, 1H, NH), 5.88–5.67 (m, 1H, NH), 5.48–5.41 (m, 1H, NH), 5.09 (t, 1H, *J*=6 Hz, Phe-αCH), 4.37 (q, 2H, *J*=7.0 Hz, Fmoc-OCH_2), 4.26–4.22 (m, 2H, CH, Ala-αCH), 3.93 (d, 2H, *J*=6.5 Hz, $COCH_2$), 3.53-3.49 (m, 2H, OCH_2), 3.27–2.11 (m, 2H, SCH, SCH), 3.10–3.01 (m, 3H, SCH, Phe-CH_2), 2.88–2.85 (m, 1H, SCH) 2.53–2.50 (m, 1H, CH), 2.29–2.23 (m, 1H, CH), 1.92–1.79 (m, 1H, CH), 1.63–1.51 (m, 2H, CH_2), 1.35 (d, 3H, J=6.5 Hz, Ala-CH_3) ; δ_C (125 MHz, $CDCl_3$) 171.8, 171.3, 156.0, 143.9, 141.4, 136.6, 129.8, 129.7, 128.8, 128.4, 127.9, 127.3, 126.9, 125.3, 120.1, 119.3, 70.6, 69.4, 67.3, 55.6, 50.6, 47.3, 40.6, 39.5, 39.4, 39.3, 39.2, 34.8, 32.7, 19.1; HRMS (ESI) $C_{37}H_{40}N_4O_6S$ calcd. 668.2669, found 691.2589 $[M+Na]^+$.

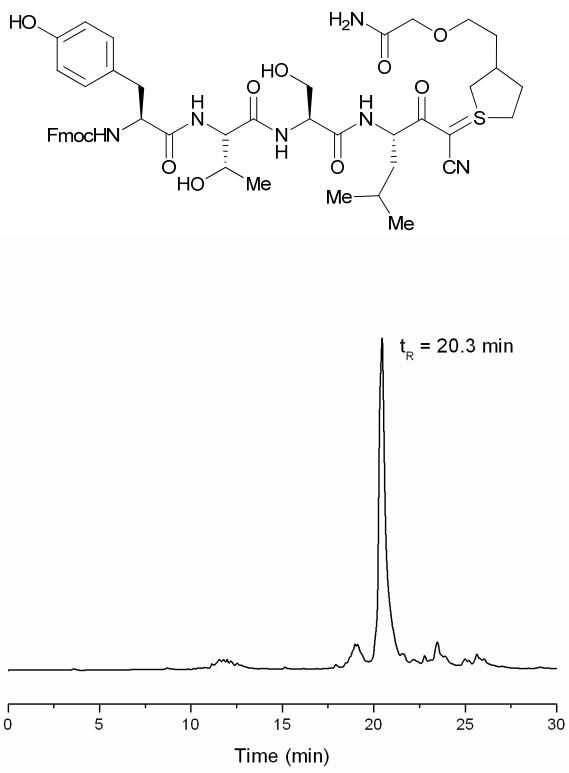


Fig. 1 HPLC chromatogram of crude Fmoc-Tyr-Thr-Ser-Leu-SY **21** (300 nm).

Fmoc-Tyr-Thr-Ser-Leu-SY (**21**) Cleavage from solid support provided the crude sulfur ylide in 78% yield (by weight) after washing with cold either and 72% purity as determined by RP-HPLC (Shiseido CapcellPak C18 column, 300 nm. Gradient: 0 min, 0% CH_3CN in H_2O; 30 min, 100% CH_3CN), t_r (sulfur ylide) = 20.3 min. Purification by PTLC (7:1 CH_2Cl_2:CH_3OH) provided the product in 33% isolated yield (39.0 mg). ν_{max} (film)/cm^{-1} 3304 (br), 2954, 2174, 1674, 1516, 1450, 1256, 1107, 1081, 1026; δ_H (500 MHz, CD_3OD) 7.80 (d, 2H, *J*=7.5 Hz, Ar-H), 7.57 (d, 2H, *J*=7.0 Hz, Ar-H), 7.37 (t, 2H, *J*=7.5 Hz, Ar-H), 7.29 (q, 2H, *J*=7.0 Hz, Ar-H), 7.09 (d, 2H, *J*=8.0 Hz, Tyr-Ar-H), 6.70 (d, 2H, *J*=8.0 Hz, Tyr-Ar-H), 4.83–4.78 (m, 1H, αCH), 4.48 (t, 1H, *J*=5.0 Hz, αCH), 4.43–4.39 (m, 2H, Fmoc-OCH_2), 4.35–4.32 (m, 1H, αCH), 4.24–4.20 (m, 1H, αCH), 4.16 (t, 1H, *J*=6.8 Hz, CH), 3.94–3.92 (m, 2H, $COCH_2$), 3.87–3.69 (m, 3H, Tyr-CH_2, CH), 3.58 (q, 2H, *J*=5.2 Hz, OCH_2), 3.19–3.11 (m, 1H, SCH), 3.08–2.99 (m, 1H, SCH), 2.86–2.80 (m, 1H, SCH), 2.61–2.18 (m, 2H, SCH), 1.96–1.92 (m, 1H, CH), 1.85–1.79 (m, 1H, CH), 1.74–1.70 (m, 2H, Leu-CH_2), 1.57–1.51 (m, 2H, CH_2), 1.22 (d, 3H, J=6.5 Hz, Thr-CH_3), 0.94 (d, 6H, J=7.0 Hz, Leu-$C(CH_3)_2$) ; δ_C (125 MHz, CD_3OD) 191.8, 175.6, 174.7, 172.2, 172.0, 158.6, 157.4, 145.4, 145.3, 142.7, 131.5, 129.4, 128.9, 128.4, 126.5, 126.4, 121.0, 120.9, 116.4, 71.0 (x3) 68.5, 68.3, 63.2, 59.9, 58.5, 58.2, 56.9, 55.2, 51.5, 50.8, 49.8, 47.4, 46.7, 46.3, 45.7, 42.9, 41.9, 41.2, 38.1, 35.4, 35.2, 33.8, 33.7, 27.4, 24.1, 22.1, 20.2; HRMS (ESI) $C_{47}H_{58}N_6O_{11}S$ calcd. 914.3884, found 915.3950 $[M+H]^+$, 937.3762 $[M+ Na]^+$.

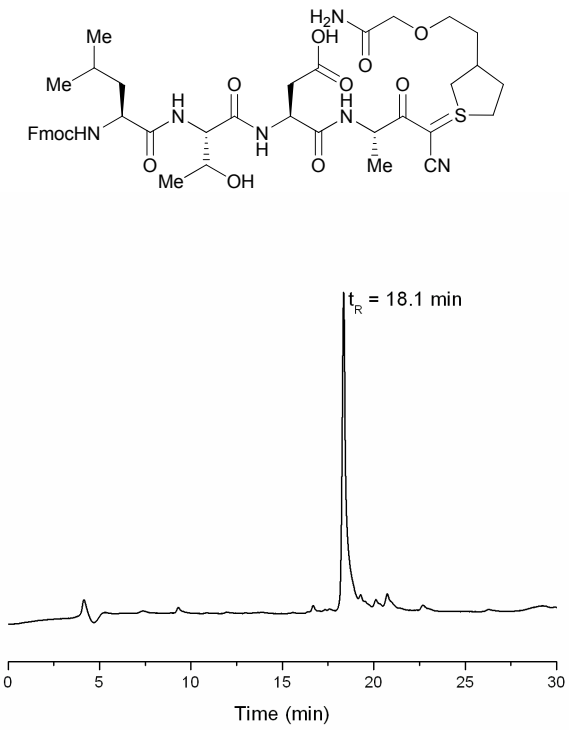
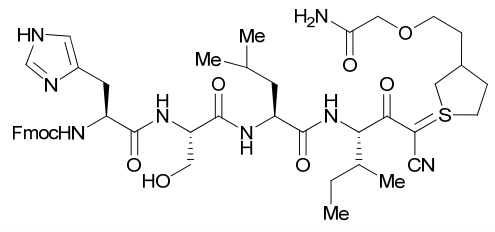


Fig. 2 HPLC chromatogram of crude Fmoc-Leu-Thr-Asp-Ala-SY **22** (300 nm).

Fmoc-Leu-Thr-Asp-Ala-SY (**22**) Cleavage from solid support provided the crude sulfur ylide (Fig. 2) in 81% yield (by weight) after washing with cold either and 72% purity as determined by RP-HPLC (Shiseido CapcellPak C18 column, 300 nm. Gradient: 0 min, 5% IPA in H_2O; 30min, 90% IPA; 35 min, 90% IPA), t_r (sulfur ylide) = 18.1 min. Purification by preparative RP-HPLC provided the product in 52% isolated yield (43.2 mg). ν_{max} (film)/cm^{-1} 3301 (br), 2955, 2477, 2172, 1658, 1588, 1450, 1258, 1123; δ_H (500 MHz, CD_3OD) 7.78 (d, 2H, *J*=7.5 Hz, Ar-H), 7.67(q, 2H, *J*=6.5 Hz, Ar-H), 7.38 (t, 2H, *J*=7.5 Hz, Ar-H), 7.31 (t, 2H, *J*=7.3 Hz, Ar-H), 4.80–4.75 (m, 1H, αCH), 4.66–4.63 (m, 1H, αCH), 4.46–4.35 (m, 3H, Fmoc-OCH_2, αCH), 4.23–4.17 (m, 3H, CH_2, αCH), 3.93–3.91 (m, 2H, $COCH_2$), 3.83-3.70 (m, 1H, CH), 3.62-3.56 (m, 2H, OCH_2), 3.45-3.37 (m, 1H, CH), 3.19–3.06 (m, 1H, CH), 2.88–2.74 (m, 2H, 2xSCH), 2.61–2.34 (m, 1H, SCH), 2.26–2.23 (m, 1H, CH), 1.95–1.81 (m, 2H, CH_2), 1.74–1.68 (m, 2H, Leu-CH_2), 1.59 (t, 2H, *J*=7.3 Hz, CH_2), 1.37 (t, 3H, *J*=5.3 Hz, Ala-CH_3), 1.17–1.14 (m, 4H, CH), 0.96–0.92 (dd, 6H, *J*=10, 6 Hz, Leu-$C(CH_3)_2$) ; δ_C (125 MHz, CD_3OD) 175.0, 174.9 (x2), 172.9, 170.9, 157.5, 155.7, 144.1, 143.8, 141.3, 127.5 (x2), 127.0, 126.9, 125.0, 119.7, 80.9, 78.2, 69.6, 67.0, 66.7, 58.5, 54.0, 51.1, 49.9, 40.4, 39.8, 35.2 (x2), 35.0, 34.1 (x2), 32.3, 24.6, 24.0, 22.2, 20.5 (x2), 18.5, 17.1 (x2); HRMS (ESI) $C_{47}H_{58}N_6O_{11}S$ calcd. 850.3571 found 873.3478 $[M+Na]^+$.



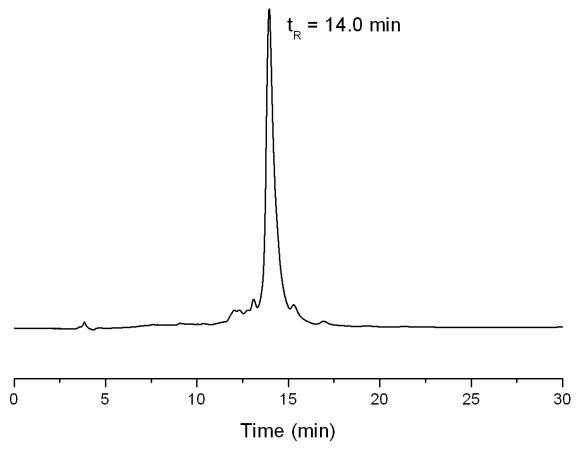
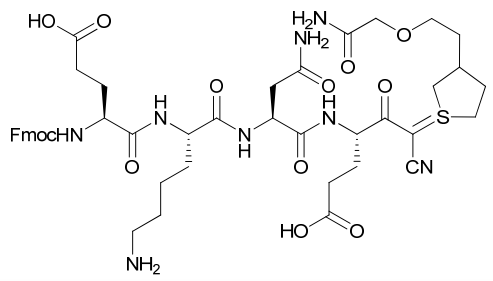


Fig. 3 HPLC chromatogram of crude Fmoc-His-Ser-Leu-Ile-SY **23** (300 nm).

Fmoc-His-Ser-Leu-Ile-SY (**23**) Acid cleavage from solid support yielded the crude sulfur ylide (Fig. 3) in 67% yield (by weight) after washing with cold either and 93% purity as determined by RP-HPLC (Shiseido CapcellPak C18 column, 300 nm. Gradient: 0 min, 5% CH_3CN in H_2O; 5 min, 40% CH_3CN; 30min, 60% CH_3CN; 35 min, 90% CH_3CN), t_r (sulfur ylide) = 14.0 min. Purification by prep RP-HPLC yielded the product in 32% isolated yield (20.5 mg). ν_{max} (film)/cm^{-1} 3267 (br), 2961, 2173, 1671, 1536, 1202, 1133; δ_H (500 MHz, CD_3OD) 8.79 (s, 1H, His-iminyl), 7.79 (d, 2H, *J*=7.5 Hz, AR-H), 7.62 (t, 2H, *J*=7.0 Hz, Ar-H), 7.39 (t, 2H, *J*=7.2 Hz), 7.35–7.27 (m, 3H, *J*=7.2 Hz, His-vinyl, Ar-H), 4.65–4.62 (m, 1H, αCH), 4.54–4.49 (m, 1H, αCH), 4.44–4.38 (m, 3H, Fmoc-OCH_2, αCH), 4.19 (t, 1H, *J*=6.25 Hz, Ser-CH), 3.98 (s, 1H, Ser-CH), 3.96–3.91 (m, 2H, $COCH_2$), 3.89–3.95 (m, 1H, Ser-CH), 3.82–3.71 (m, 2H, $COCH_2$), 3.64–3.55 (m, 2H, OCH_2), 3.33–3.20 (m, 1H, CH), 3.17–3.08 (m, 1H, His-CH), 3.06–2.92 (m, 1H, SCH), 2.63–2.52 (m, 1H, SCH), 2.47–2.15 (m, 1H, SCH), 1.92–1.37 (m, 7H, SCH, CH, CH_2, Ile-CH_3), 1.19–1.12 (m, 1H, CH), 1.05–0.98 (m, 1H, CH), 0.93 (t, 3H, J=6.0 Hz, Ile-CH_3), 0.87 (dd, 6H, *J*=6.5 Hz, 6.0 Hz, Leu-C$(CH_3)_2$) ; δ_C (125 MHz, CD_3OD) 189.4, 174.2, 173.0, 172.9, 171.5, 171.1, 156.9, 143.9, 141.4, 133.7, 129.6, 127.6, 127.6, 126.9, 124.9, 119.7, 117.4, 69.6 (x2), 69.5, 66.8, 61.6, 58.7, 58.6, 55.5, 53.9, 53.8, 52.5, 50.0, 49.4, 48.7, 48.6, 48.4, 48.2, 45.8, 45.3, 44.7, 44.4, 40.6, 40.5, 40.3, 40.0, 39.9, 37.5, 37.4, 33.9, 33.8, 32.4, 27.5, 25.8, 22.2, 21.5, 14.3, 10.1; HRMS (ESI) $C_{46}H_{60}N_8O_9S$ calcd. 900.4204, found 901.4272 $[M+H]^+$.



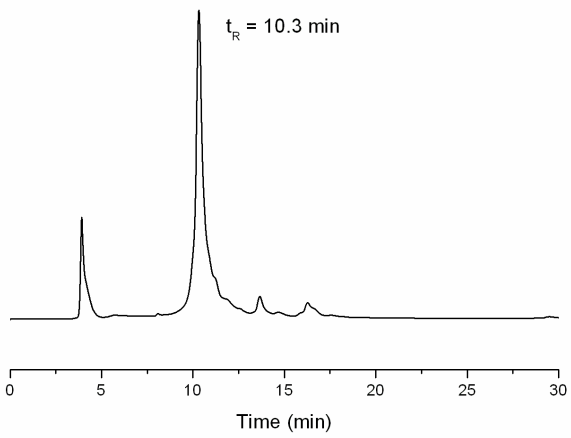


Fig. 4 HPLC chromatogram of crude Fmoc-Glu-Lys-Asn-Glu-SY **24** (300 nm).

Fmoc-Glu-Lys-Asn-Glu-SY (**24**) Acid cleavage from solid support yielded the crude sulfur ylide (Fig 4) in 70% yield (by weight) after washing with cold either and 78% purity as determined by RP-HPLC (R-ODS-10-A column, 300 nm. Gradient: 0 min, 5% IPA in H_2O; 5 min, 40% IPA; 30min, 60% IPA; 35 min, 90% IPA), t_r (sulfur ylide) = 10.3 min. Purification by prep RP-HPLC yielded the product in 49% isolated yield (61.2 mg). ν_{max} (film)/cm^{-1} 3264 (br), 2896, 2170, 1666, 1632, 1530, 1408, 1200, 1128; δ_H (500 MHz, CD_3OD) 7.80 (d, 2H, *J*=7.5 Hz, Ar-H), 7.67 (d, 2H, *J*=7.5 Hz, Ar-H), 7.39 (t, 2H, *J*=7.5 Hz, Ar-H) 7.32 (t, 2H, *J*=7.5 Hz, Ar-H), 4.75–4.70 (m, 2H, Fmoc-OCH_2), 4.42–4.31 (m, 3H, α-CH, Lys-NCH_2), 4.23, (t, 1H, *J*=7.5 Hz, Ser-CH), 4.14 (q, 1H, *J*=5.0 Hz, α-CH), 3.97–3.93 (m, 3H, α-CH, $COCH_2$), 3.89–3.72 (m, 1H, CH), 3.67–3.57 (m, 3H, OCH_2), 3.29–3.01 (m, 2H, Lys-NH_2), 3.12–3.01 (m, 1H, CH), 2.98 (t, 2H, *J*=7.0 Hz, Glu-CH_2), 2.80–2.68 (m, 2H, Glu-CH_2), 2.62–2.53 (m, 1H, SCH), 2.46–2.33 (m, 5H, SCH, Asn-CH_2), 2.15–2.08 (m, 2H, Glu-CH_2), 1.98–1.86 (m, 5H, Glu-CH_2), 1.76–1.62 (m, 4H, Lys-CH_2) 1.51–1.42 (m, 2H, CH_2, Lys-CH_2); δ_C (125 MHz, CD_3OD) 187.4, 187.3, 174.6, 174.5, 172.2, 172.1 (x2), 172.0, 171.0, 162.9, 159.1, 158.9, 158.6, 158.4, 156.5, 144.4 (x2), 141.3 (x2), 128.3, 127.7, 126.0, 125.9, 120.7 (x2), 120.6, 120.3, 70.3, 69.8, 69.7, 66.3, 56.4, 54.5, 54.1, 53.5, 52.5, 50.3, 50.2, 47.2, 37.3, 36.4, 34.1, 32.9, 32.6, 32.2, 31.4, 30.9, 30.3, 28.7, 27.9, 27.2, 22.6, 18.6, 17.3; HRMS (ESI) $C_{45}H_{56}N_8O_{13}S$ calcd. 950.3844, found 973.3732 $[M+Na]^+$.

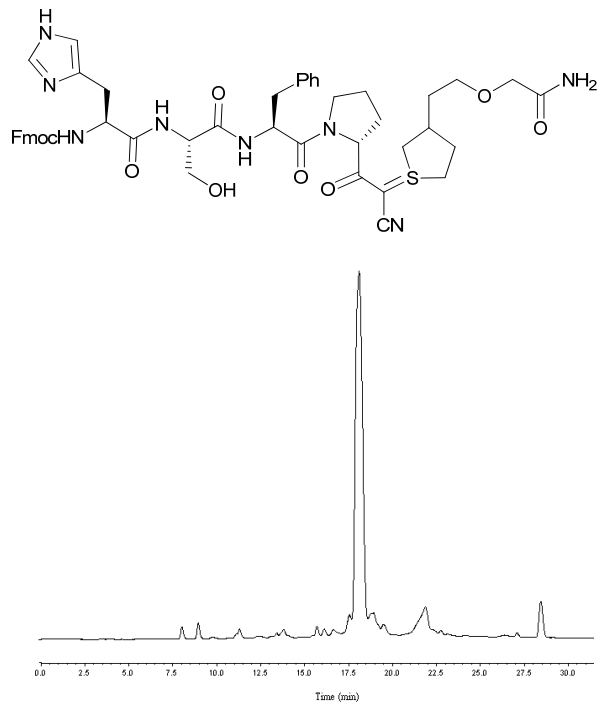


Fig. 5 HPLC chromatogram of crude Fmoc-His-Ser-Phe-Pro-SY **25** (300 nm).

Fmoc-His-Ser-Phe-Pro-SY (**25**) Acid cleavage from solid support yielded the crude sulfur ylide (Fig. 5) in 67% yield (by weight) after washing with cold either and 93% purity as determined by RP-HPLC (R-ODS-10-A column, 300 nm. Gradient: 0 min, 5% CH_3CN in H_2O; 30min, 90% CH_3CN; 35 min, 90% CH_3CN), t_r (sulfur ylide) = 17.8 min. Purification by prep RP-HPLC yielded the product in 33% isolated yield (34.9 mg). ν_{max} (film)/cm^{-1} 3307 (br), 2944, 2359, 2341, 2171, 1676, 1627, 1451, 1201, 1028; δ_H (500 MHz, CD_3OD) 8.79–8.75 (m, 1H, His-iminyl), 7.80 (t, 2H, *J*=5.8 Hz, Ar-H), 7.62 (t, 2H, *J*=7.5 Hz, Ar-H), 7.40 (q, 2H, *J*=7.0 Hz, Ar-H), 7.34–7.18 (m, 8H, Ar-H, His-vinyl, NH), 4.74–4.62 (m, 1H, α-CH), 4.59–4.45 (m, 1H, α-CH), 4.45–4.38 (m, 3H, α-CH, Fmoc-OCH_2), 4.20 (q, 1H, *J*=6.0 Hz, Ser-CH), 3.94–3.89 (m, 3H, Ser-CH, $COCH_2$), 3.87–3.83 (m, 1H, CH), 3.83–3.71 (m, 2H, OCH_2), 3.63–3.40 (m, 4H, Pro-CH_2), 3.22–2.18 (m, 1H, His-CH), 3.12–3.03 (m, 2H, Phe-CH_2), 2.93–2.88 (m, 2H, His-CH, CH), 2.67–2.39 (m, 1H, SCH), 2.32–2.19 (m, 1H, SCH), 2.03–1.87 (m, 2H, SCH), 1.79–1.70 (m, 3H, CH, Pro-CH_2), 1.58–1.52 (m, 1H, CH); δ_C (125 MHz, CD_3OD) 173.1, 172.6, 171.8, 159.1, 146.0, 145.9, 143.5, 138.3, 135.8, 135.7, 131.9, 131.7, 131.5, 131.3, 130.6, 130.3, 130.0, 129.7, 129.1, 129.0, 128.6, 127.0, 121.9, 121.8, 119.5, 71.9, 71.7, 68.9, 68.8, 64.4 (x2), 64.0, 63.6, 57.8, 57.7, 57.6, 55.9, 55.2, 55.1, 55.0, 54.9, 50.8, 49.2, 49.0, 42.6, 42.1, 42.0 (x3), 38.9, 36.0, 34.5, 34.4, 34.3, 33.7, 31.7, 29.2, 29.0, 26.8, 24.4; HRMS (ESI) $C_{48}H_{54}N_8O_9S$ calcd. 918.3734, found 919.3831 $[M+H]^+$.

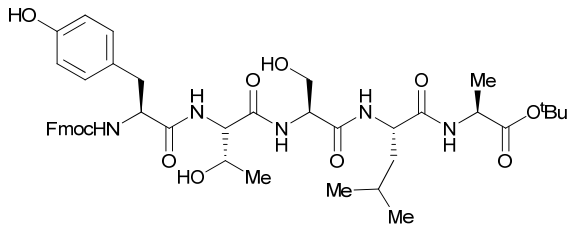
General Procedure for Oxidation of Peptide Sulfur Ylides to α-Ketoacids:

The peptide sulfur ylide (0.01-0.02 mmol) was dissolved in 2:1 DMF/H_2O (0.02-0.05 M). Oxone (2 equiv) was added in one portion and the slurry was stirred at room temperatue. The oxidation was monitored by LCMS or by reverse phase HPLC and were generally complete within 30 minutes. Upon observing of the disappearance of the sulfur ylide, the reaction mixture was filtered through cotton and

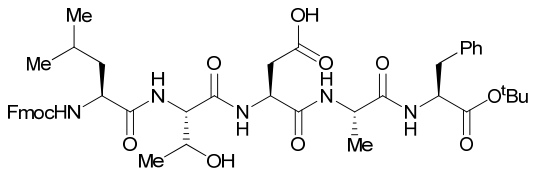
washed with DMF (1 mL). Dimethylsulfide (0.25mL) was added to quench oxone instead of an aqueous work up. Following the addition of DMF to the solution, water and dimethylsulfide were removed *in vacuo*. The crude α-keto acid was obtained as an approximately 0.05 M solution in DMF.

General Procedure for Ligation Reaction:

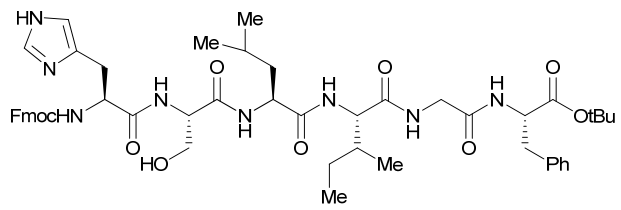
The hydroxylamine oxalate salt (1.5 equiv) was added to a solution of the ketoacid in DMF (0.01-0.02 mmol). The reaction was allowed to stir at 40-60 ^{o}C, and was monitored on LCMS and HPLC. After the reaction was completed, the reaction mixture was concentrated *in vacuo* and purified by preparative RP-HPLC to afford the expected product.



Fmoc-Tyr-Thr-Ser-Leu-Ala-O^{t}Bu (**26**) Oxidation of Fmoc-Tyr-Thr-Ser-Leu-SY **21** (19.0 mg, 0.021 mmol) yielded the α-ketoacid (71%) as determined by RP-HPLC (R-ODS-10-A column, 300 nm. Gradient: 0 min, 0% CH_3CN in H_2O; 30 min, 100% CH_3CN, flow rate = 1 mL/min), t_r (α-ketoacid) = 20.4 min. Ligation with HONH-Ala-O^{t}Bu (50 ^{o}C, 25 h) provided the pentapeptide (56%) as determined by HPLC. t_r (pentapeptide) = 23.6 min. Purification on preparative HPLC (flow rate = 20 mL/min) yielded the desired product (7.2 mg, 42% yield). ν_{max} (film)/cm^{-1} 3307 (br), 2925, 2362, 2340, 1675, 1451, 1301, 1151, 1124; δ_H (500 MHz, CD_3OD) 7.78 (d, 2H, *J*=7.5 Hz, Ar-H), 7.73 (d, 2H, *J*=7.5 Hz, Ar-H), 7.37 (t, 2H, *J*=7.5 Hz, Ar-H), 7.30 (t, 2H, *J*=8.0 Hz, Ar-H), 7.0 (d, 2H, *J*=8.5 Hz, Tyr-Ar-H), 6.73 (d, 2H, *J*=8.5 Hz, Tyr-Ar-H), 4.46–4.43 (m, 2H, Fmoc-OCH_2), 4.37 (d, 1H, *J*=7.5 Hz, αCH), 4.25–4.17 (m, 3H, αCH), 3.80–3.76 (m, 1H, Tyr-CH), 3.63 (q, 1H, J=5.0 Hz, Tyr-CH), 3.59 (t, 1H, *J*=6.2 Hz, Thr-CH), 3.19–3.16 (m, 1H, CH), 3.08–3.00 (m, 2H, CH_2), 2.80–2.72 (m, 2H, CH_2), 2.64–2.55 (m, 1H, CH), 1.87–1.80 (m, 1H, CH), 1.75–1.67 (m, 1H, CH), 1.67–1.58 (m, 2H, Leu-CH_2), 1.45 (s, 9H, $C(CH_3)_3$), 1.36 (d, 3H, *J*=7.0 Hz, Thr-CH_3), 1.13 (d, 3H, *J*=6.5 Hz, Ala-CH_3), 0.92 (dd, 6H, *J*=15 Hz, 6.5 Hz, Leu-$C(CH_3)_2$); δ_C (125 MHz, CD_3OD) 172.1, 171.9, 156.2, 143.8, 143.7, 141.1, 130.2, 127.7, 127.1, 125.2, 119.9, 115.6, 70.3, 69.1, 66.7, 62.0, 58.5, 56.9, 52.0, 51.9, 48.8, 47.1, 45.3, 37.1, 34.1 (x2), 29.6, 29.1, 27.9, 24.6, 24.3, 23.3, 21.5, 19.0, 17.7; HRMS m/z (ESI) $C_{44}H_{57}N_5O_{11}$ calcd. 831.4055, found 854.3956 $[M+Na]^+$.

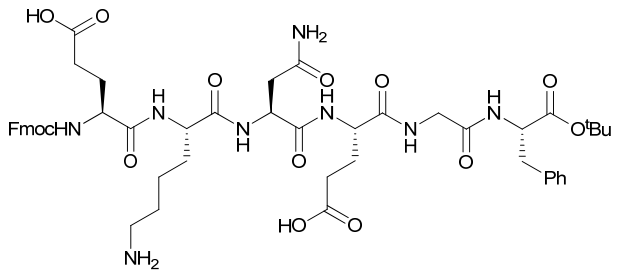


Fmoc-Leu-Thr-Asp-Ala-Phe-O^{t}Bu (**27**) Oxidation of Fmoc-Leu-Thr-Asp-Ala-SY **22** (19.7 mg, 0.023 mmol) yielded the α-ketoacid (90%) as determined by RP-HPLC (R-ODS-10-A column, 300 nm. Gradient: 0 min, 5% IPA in H_2O; 30 min, 95% IPA, flow rate = 1 mL/min), t_r (α-ketoacid) = 20.4 min. Ligation with HONH-Phe-O^{t}Bu (50 ^{o}C, 18 h) yielded the pentapeptide (36.2%) as determined by HPLC. t_r (pentapeptide) = 22.7 min. Purification on preparative HPLC (flow rate = 9 mL/min) yielded the isolated product in 36% yield (7.0 mg). ν_{max} (film)/cm^{-1} 3030 (br), 2965, 2877, 2358, 2342, 1700, 1635, 1388, 1205, 1132; δ_H (500 MHz, DMF-d7) 8.29 (d, 1H, *J*=5 Hz, NH), 7.98 (d, 2H, *J*=7.5 Hz, Ar-H), 7.91 (d, 1H, *J*=5.0 Hz, Ar-H), 7.80 (q, 2H, *J*=6.3 Hz, Ar-H), 7.76 (d, 1H, *J*=5.5 Hz, Ar-H), 7.49 (t, 2H, *J*=7.3 Hz, Ar-H), 7.41–7.38 (m, 2H, Ar-H), 7.35–7.7.33 (m, 4H, Ar-H, NH), 7.27–7.24 (m, 1H, NH), 4.82 (q, 1H, *J*=6.5 Hz, αCH), 4.51–4.44 (m, 3H, Fmoc-OCH_2, αCH), 4.38–4.27 (d, 5H, αCH, CH), 3.11–3.06 (m, 2H, Phe-CH_2), 2.83 (d, 1H, *J*=6.5 Hz, CH), 1.87–1.81 (m, 1H, Leu-CH), 1.74–1.71 (m, 1H, Leu-CH), 1.42 (s, 9H, $C(CH_3)_3$), 1.33 (d, 3H, *J*=7.0 Hz, Ala-CH_3), 1.19 (d, 3H, *J*=6.0 Hz, Thr-CH_3), 0.97 (d, 6H, *J*=6.8 Hz, Leu-$C(CH_3)_2$); δ_C (125 MHz, DMF-d7) 174.0, 173.7, 173.5, 171.9, 157.8, 143.2, 139.5, 131.5, 130.2, 129.7, 129.2, 129.1, 128.5, 127.5, 127.4, 122.1, 82.8, 68.7, 68.3, 56.7, 52.2, 51.4, 49.1, 43.0, 39.3, 29.3, 26.6, 24.8, 23.0, 20.9, 19.6; HRMS (ESI) $C_{45}H_{57}N_5O_{11}$ calcd 843.4055, found 865.4023 $[M+Na]^+$.

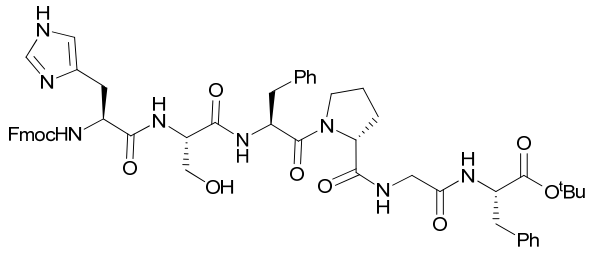


Fmoc-His-Ser-Leu-Ile-Gly-Phe-O^{t}Bu (**28**) Oxidation of Fmoc-His-Ser-Leu-Ile-SY **23** (9.1 mg, 0.010 mmol) yielded the α-ketoacid (70.8%) and carboxylic acid (25.1%) as determined by RP-HPLC (R-ODS-10-A column, 300 nm. Gradient: 0 min, 10% CH_3CN in H_2O; 30 min, 90% CH_3CN, flow rate = 1 mL/min), t_r (α-ketoacid) = 11.6 min. Ligation with HONH-Gly-Phe-O^{t}Bu (60 ^{o}C, 22.5 h) yielded the hexapeptide (54.8%) as determined by HPLC. t_r (hexapeptide) = 17.2 min. Purification on preparative HPLC (flow rate = 20 mL/min) yielded the isolated product in 45% yield (4.3 mg). ν_{max} (film)/cm^{-1} 3302 (br), 2959, 2177, 1643, 1537, 1452, 1370, 1253, 1203, 1154, 1027; δ_H (500 MHz, CD_3OD) 8.67 (s, 1H, His-iminyl), 7.80 (d, 2H, *J*=7.5 Hz, Ar-H), 7.61 (t, 2H, *J*=7.5 Hz, Ar-H), 7.39 (t, 2H, *J*=5.5 Hz, Ar-H), 7.31 (t, 2H, *J*=7.0 Hz, Ar-H), 7.27–7.17 (m, 6H, His-vinyl, Phe-Ar-H), 4.51 (t, 1H, *J*=7.5 Hz, αCH), 4.46 (t, 1H, *J*=7.0 Hz, αCH), 4.44–4.35 (m, 4H, 2xαCH, Fmoc-OCH_2), 4.19 (d, 2H, *J*=7.5 Hz, Ser-CH), 3.79–3.76 (m, 2H, Gly-CH_2), 3.22–3.16 (m, 1H, His-CH), 3.10–3.00 (m, 3H, Phe-CH_2, CH), 1.90–1.84 (m, 1H, CH), 1.74–1.61 (m, 5H, Leu-CH_2, Ile-CH_3), 1.55–1.48 (m, 1H, CH), 1.34 (s, 9H,

$C(CH_3)_3$)1.30–1.27 (m, 1H, CH), 1.22–1.14 (m, 2H, Ile-CH_2), 0.94–0.84 (m, 12H, Ile-CH_3, Leu-$C(CH_3)_2$); δ_C (125 MHz, CD_3OD) 175.1, 174.1, 172.2, 171.2, 145.3 (x2), 142.8, 138.3, 130.6, 129.6, 129.0, 128.3, 128.0, 126.3, 121.1, 118.8, 83.2, 68.2, 63.0, 59.9, 56.2, 55.5, 54.1, 49.8, 49.6, 41.5, 38.9, 37.8, 30.9, 28.3, 26.2, 26.0, 23.7, 21.8, 16.0, 11.5; HRMS (ESI) $C_{51}H_{66}N_8O_{10}$ calcd. 950.4902, found 951.4982 $[M+H]^+$.



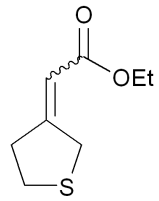
Fmoc-Glu-Lys-Asn-Glu-Gly-Phe-O^{t}Bu (**29**) Oxidation of Fmoc-Glu-Lys-Asn-Glu-SY **24** (15.4 mg, 0.016 mmol) yielded the α-ketoacid (87%) as determined by RP-HPLC (R-ODS-10-A column, 300 nm. Gradient: 0 min, 5% IPA in H_2O; 30 min, 60% IPA, 35 min, 95% IPA, flow rate = 1 mL/min), t_r (α-ketoacid) = 21.1 min. Ligation with HONH-Gly-Phe-O^{t}Bu (50 ^oC, 17 h) yielded the hexapeptide (34%) as determined by HPLC. t_r (pentapeptide) = 26.8 min. Purification on preparative HPLC (flow rate = 20 mL/min) yielded the isolated product in 31% yield (5.1 mg). ν_{max} (film)/cm^{-1} 2991 (br), 2927, 2360, 2338, 2071, 1623, 1457, 1154, 1116; δ_H (500 MHz, CD_3OD) 7.80 (d, 2H, *J*=7.5 Hz, Ar-H), 7.67 (d, 2H, *J*=7.0 Hz, Ar-H), 7.39 (t, 2H, *J*=7.5 Hz), 7.31 (t, 2H, *J*=7.5 Hz), 7.29–7.26 (m, 5 H, NH), 7.22–7.19 (m, 6H, Phe-Ar-H), 4.82 (d, 1H, *J*=7.0 Hz, NH), 4.64 (t, 2H, *J*=7.0 Hz, Fmoc-OCH_2), 4.59 (q, 1H, *J*=7.0 Hz, α-CH), 4.53 (t, 1H, *J*=7.3 Hz, α-CH), 4.43–4.29 (m, 3H, α-CH, Lys-NCH_2), 4.22 (t, 1H, *J*=6.8 Hz, α-CH), 4.11 (q, 1H, *J*=5.0 Hz, α-CH), 3.92–3.78 (m, 2H, Gly-CH_2), 3.17–3.00 (m, 5H, CH, Lys-NH_2, Phe-CH_2), 2.88 (t, 2H, *J*=7.0 Hz, Glu-$COCH_2$), 2.82–2.76 (m, 2H, 2xCH), 2.45–2.42 (m, 4H, Asn-CH_2, Glu-CH_2), 2.25–2.23 (m, 1H, Glu-CH), 2.11–2.06 (m, 1H, Glu-CH), 2.01–1.92 (m, 2H, Glu-CH_2), 1.87–1.83 (m, 1H, Glu-CH), 1.75–1.71 (m, 1H, Glu-CH), 1.66–1.61 (m, 2H, Lys-CH_2), 1.41–1.39 (m, 4H, Lys), 1.37 (s, 9H, $C(CH_3)_3$); δ_C (125 MHz, CD_3OD) 175.3, 173.2 (x3), 173.1, 172.2, 170.1, 169.5, 168.0, 141.3, 129.2 (x3), 128.2, 128.1, 127.6, 126.9, 126.7, 124.9, 119.7, 92.9, 82.1, 81.8, 66.7, 54.7, 54.2, 54.1 (x2), 50.4, 41.5, 39.2, 37.4, 37.2, 30.0, 29.9, 26.9, 26.8, 26.7, 22.2; HRMS (ESI) $C_{50}H_{64}N_8O_{14}$ calcd. 1000.4542, found 1001.3005 $[M+H]^+$.



Fmoc-His-Ser-Phe-Pro-Gly-Phe-O^{t}Bu (30) Oxidation of Fmoc-His-Ser-Phe-Pro-SY **25** (29.9 mg, 0.033 mmol) yielded the α-ketoacid as determined by RP-HPLC (R-ODS-10-A column, 300 nm. Gradient: 0 min, 40% CH_3CN in H_2O; 30 min, 90% CH_3CN, flow rate = 1 mL/min), t_r (α-ketoacid) = 8.9 min. Ligation with HONH-Gly-Phe-O^{t}Bu (50 ^oC, 11 h) yielded the hexapeptide (30%) as determined by HPLC. t_r (pentapeptide) = 15.9 min. Purification on preparative HPLC (flow rate = 20 mL/min) yielded the isolated product in 36% yield (11.5 mg). ν_{max} (film)/cm^{-1} 3297 (br), 3062, 2924, 2362, 1673, 1531, 1451, 1369, 1252, 1202, 1134, 1079; δ_H (500 MHz, CD_3OD) 8.65 (d, 1H, *J*=7.5 Hz, His-iminyl), 7.61 (q, 2H, *J*=6.5 Hz, Ar-H), 7.39 (t, 2H, *J*=7.5 Hz, Ar-H), 7.41–7.15 (m, 10H, Ar-H, His-vinyl, Phe-Ar-H), 4.55–4.37 (m, 7H, α-CH, Fmoc-OCH_2), 4.19 (t, 2H, *J*=6.5 Hz, Ser-CH), 3.97–3.94 (m, 1H, Ser-CH), 3.85–3.65 (m, 4H, CH, Pro-CH), 3.14–2.92 (m, 7H, Phe-CH_2), 2.18–2.09 (m, 2H, Pro-CH_2), 1.98–1.01 (m, 4H, Pro-CH_2), 1.64–1.61 (m, 1H, CH), 1.35 (s, 9H, $C(CH_3)_3$); δ_C (125 MHz, CD_3OD) 174.66, 172.49, 172.39, 172.33, 172.21, 171.86, 171.37, 171.09, 158.34, 145.40, 145.26, 143.84, 138.40, 138.22, 138.09, 137.42, 135.32, 135.20, 130.99, 130.79, 130.72, 130.66, 130.66, 130.06, 129.72, 129.62, 129.03, 128.61, 128.36, 128.07, 128.02, 127.96, 126.44, 126.28, 121.17, 118.99, 118.81, 112.13, 83.39, 83.21, 83.16, 68.31, 68.16, 63.13, 62.95, 62.37, 62.22, 62.01, 56.78, 56.25, 56.20, 55.89, 55.83, 55.20, 54.98, 54.70, 54.51, 54.40, 43.93, 43.52, 43.42, 38.84, 38.48, 39.36, 30.94, 30.41, 28.45, 28.31, 26.18, 26.11, 23.16; HRMS (ESI) $C_{53}H_{60}N_8O_{10}$ calcd. 968.4432, found 969.4526 $[M+H]^+$, 991.4317 $[M+Na]^+$.

Reference:

1 H. Tokuyama, T. Kuboyama, T. Fukuyama, *Org. Synth*. 2003, **80**, 207-212.
2 M. J. Fisher, W. J. Hehre, S. D. Kahn, L. E. Overman, *J. Am. Chem. Soc.* 1988, **110**, 4625-4633.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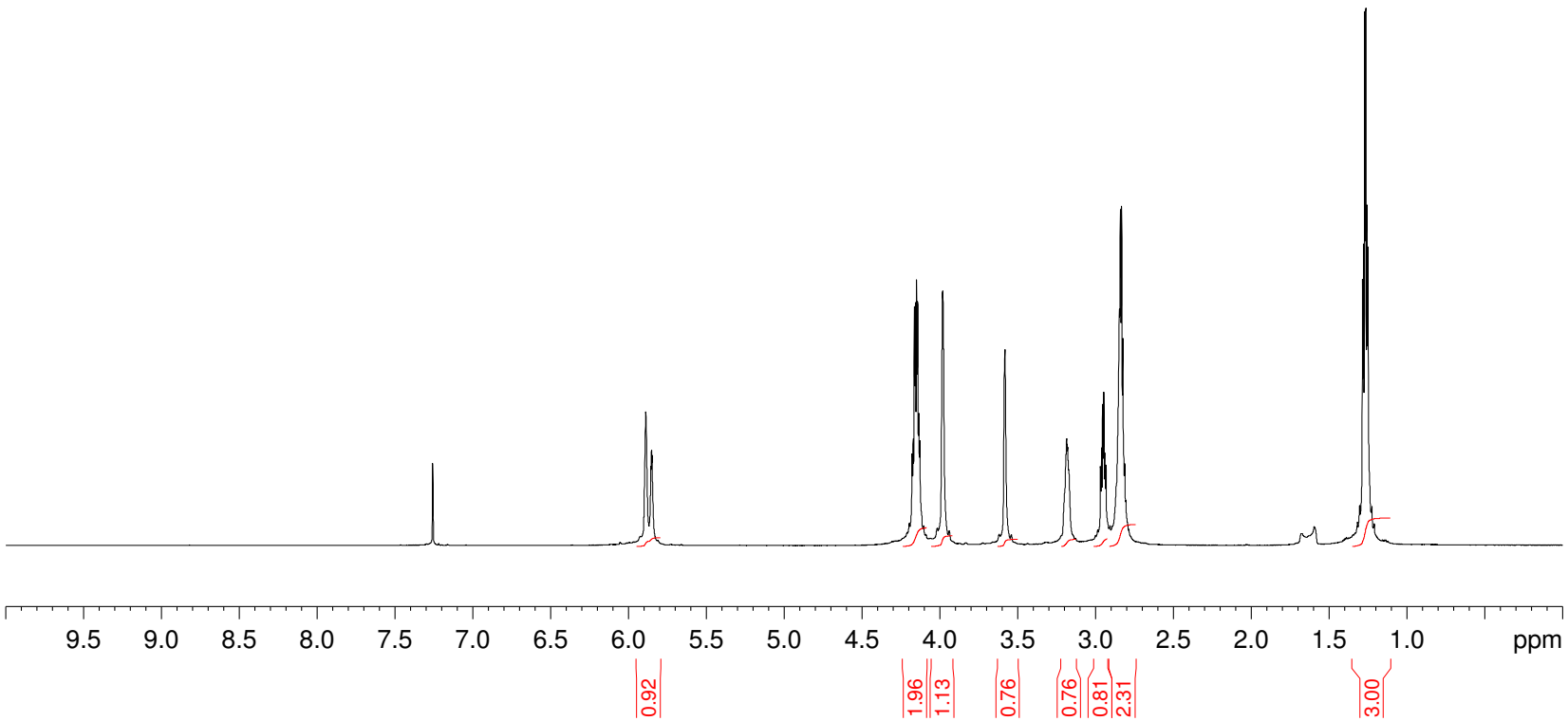
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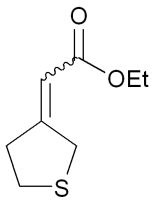
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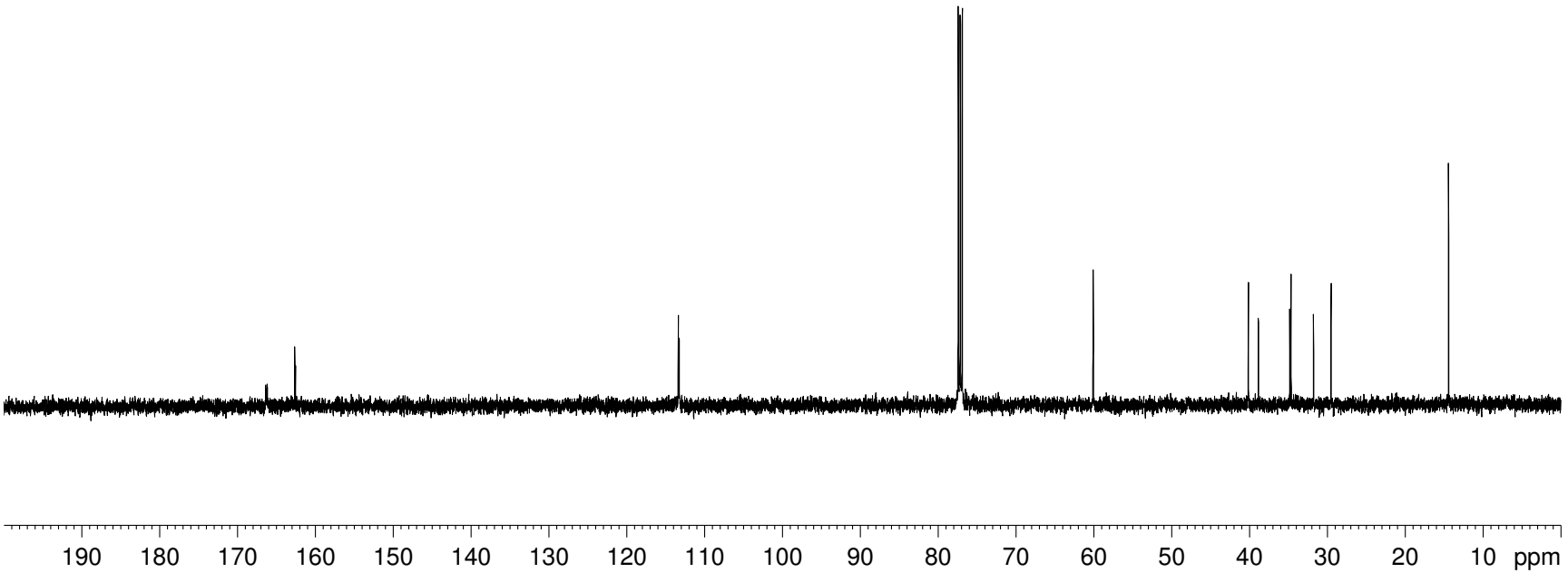
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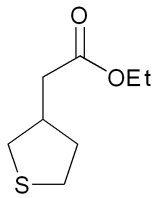
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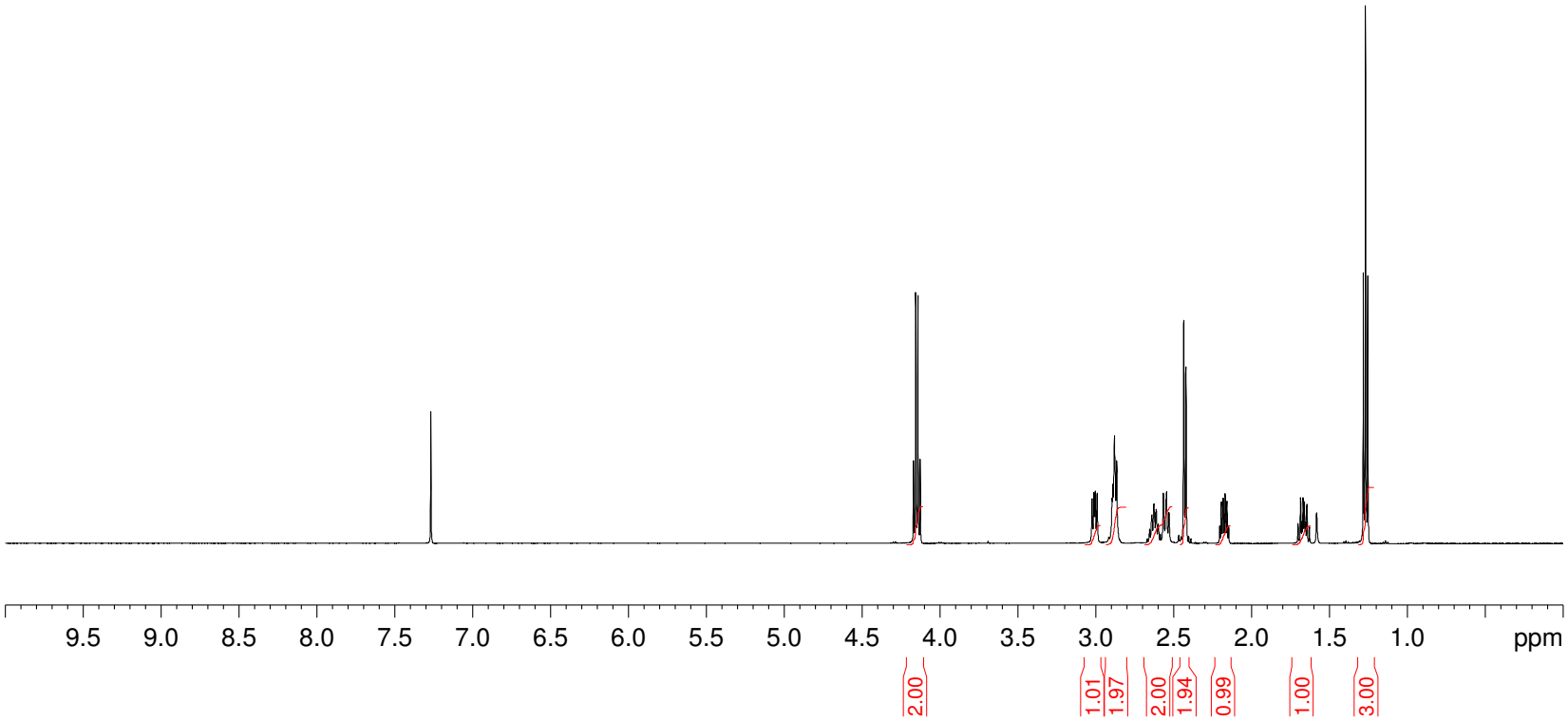
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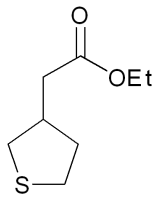
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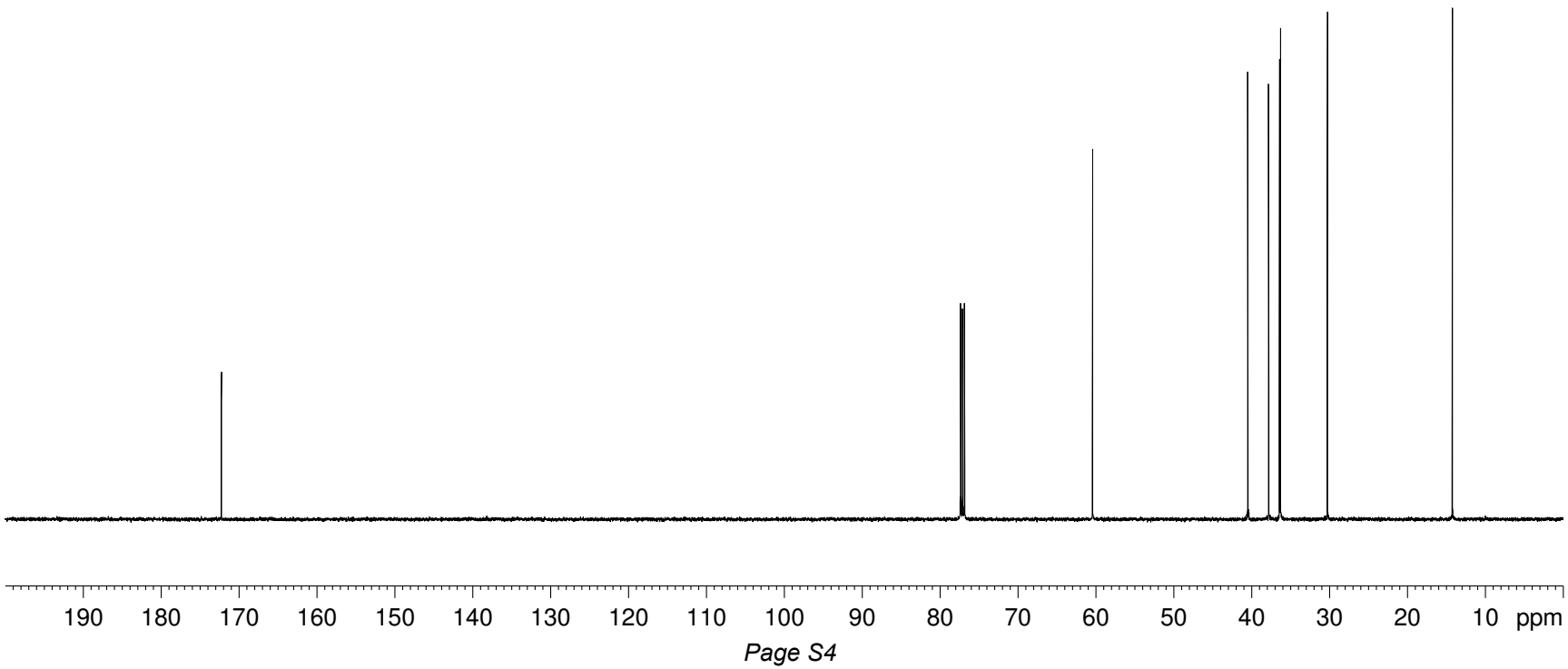
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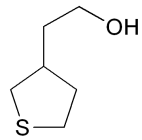
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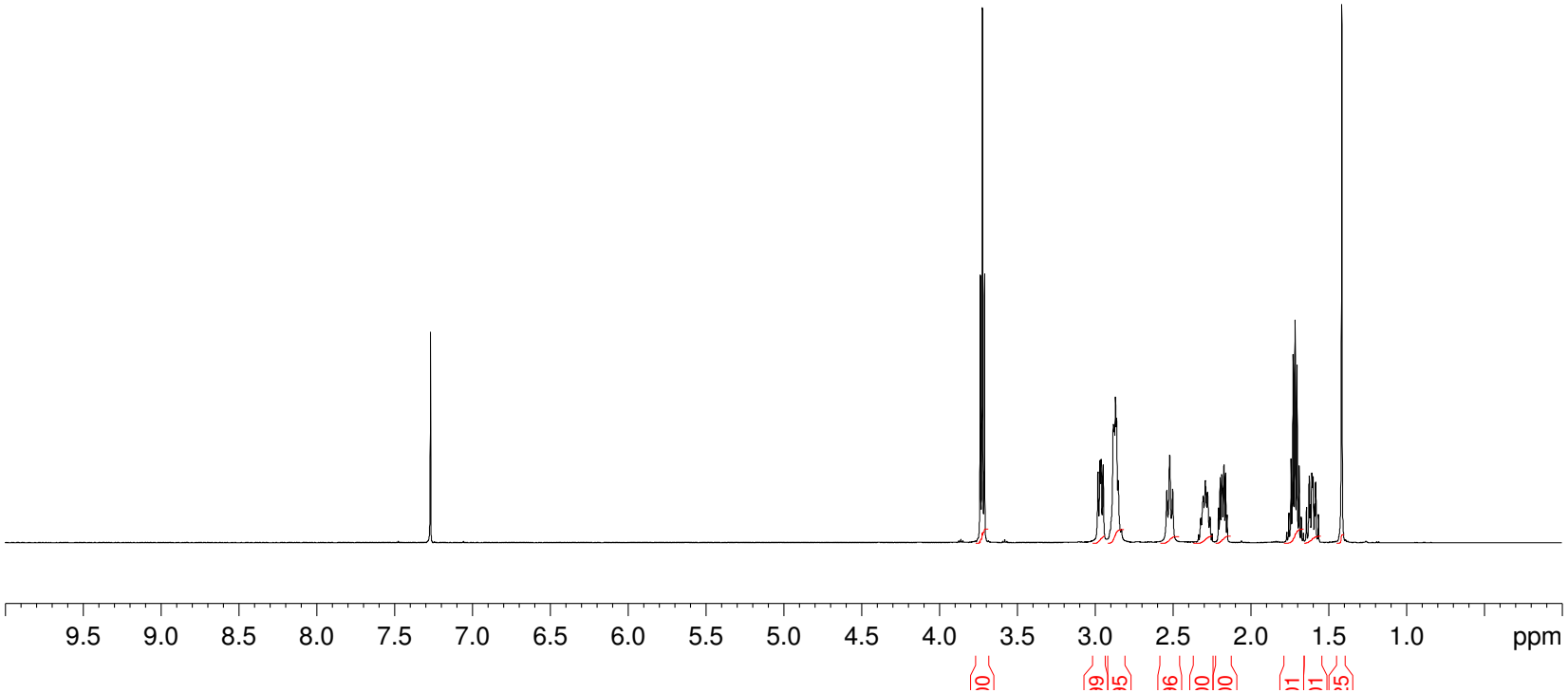
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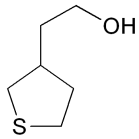
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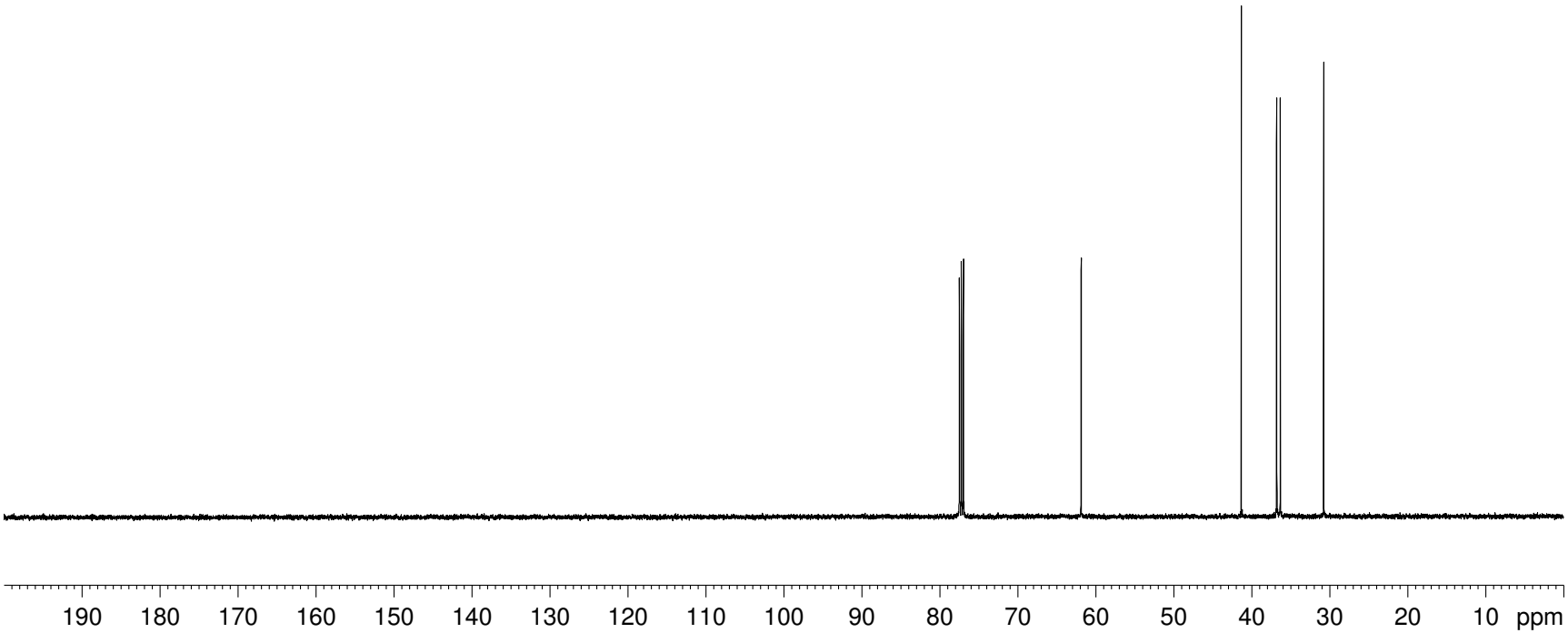
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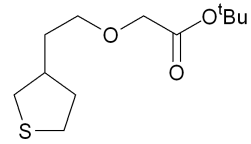
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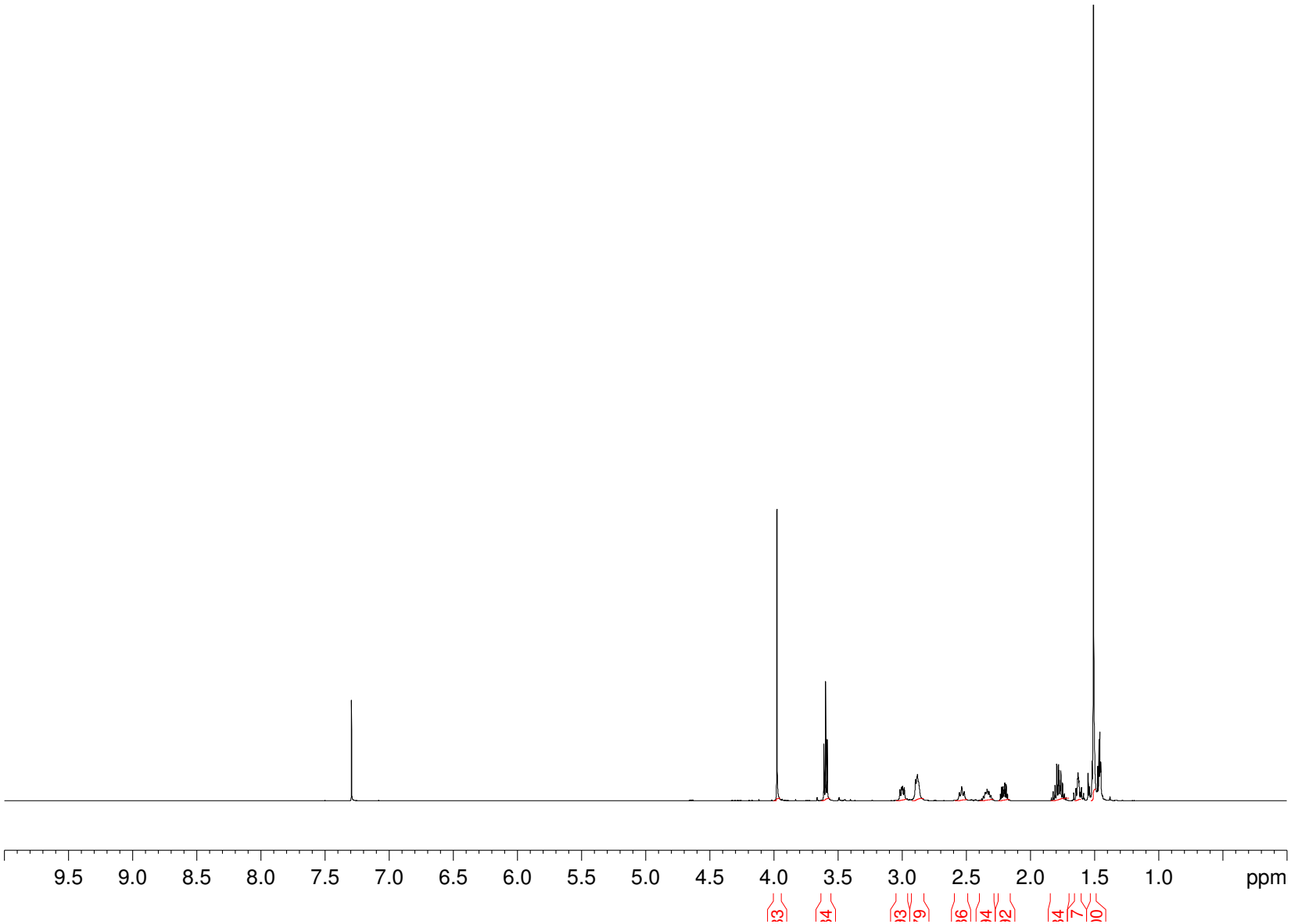
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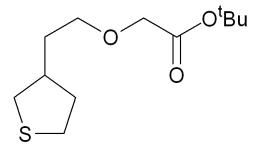
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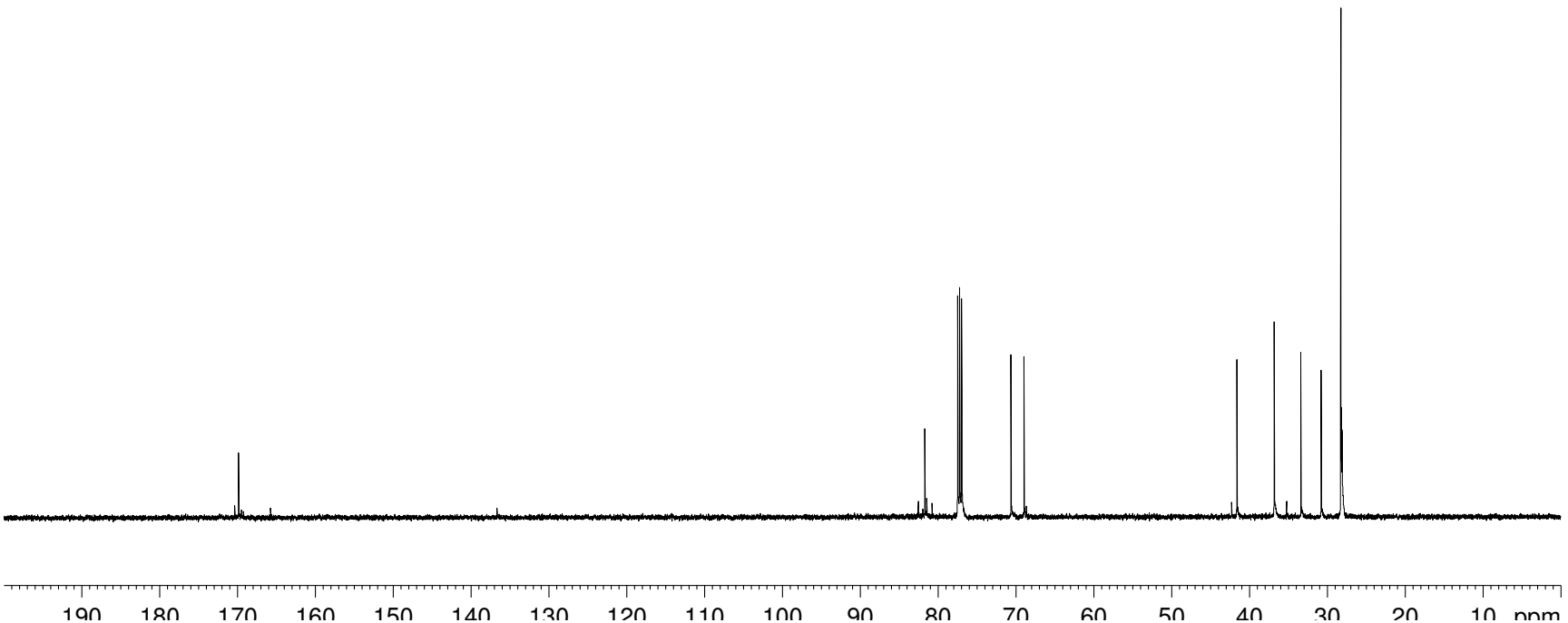
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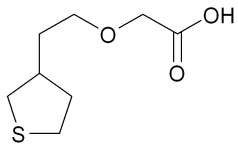
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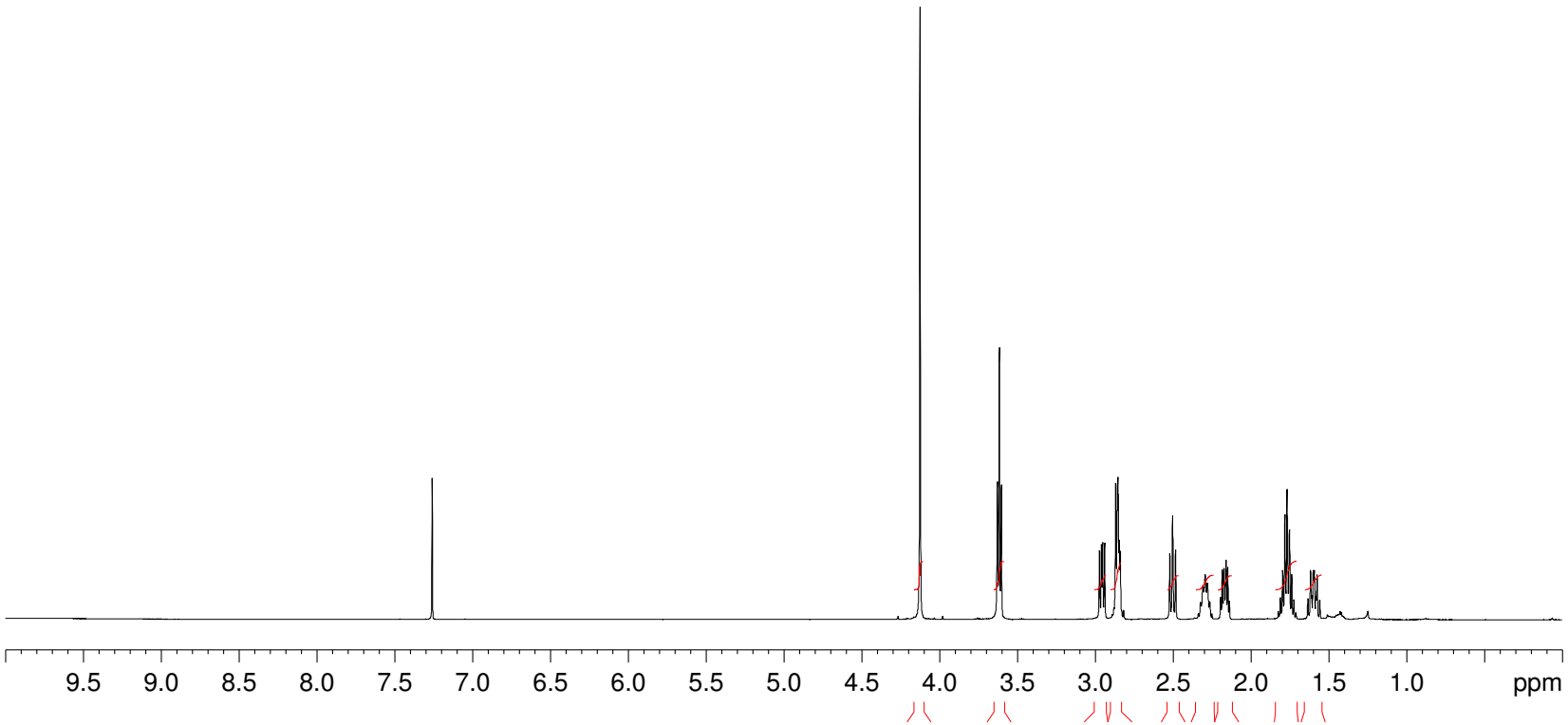
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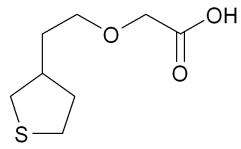
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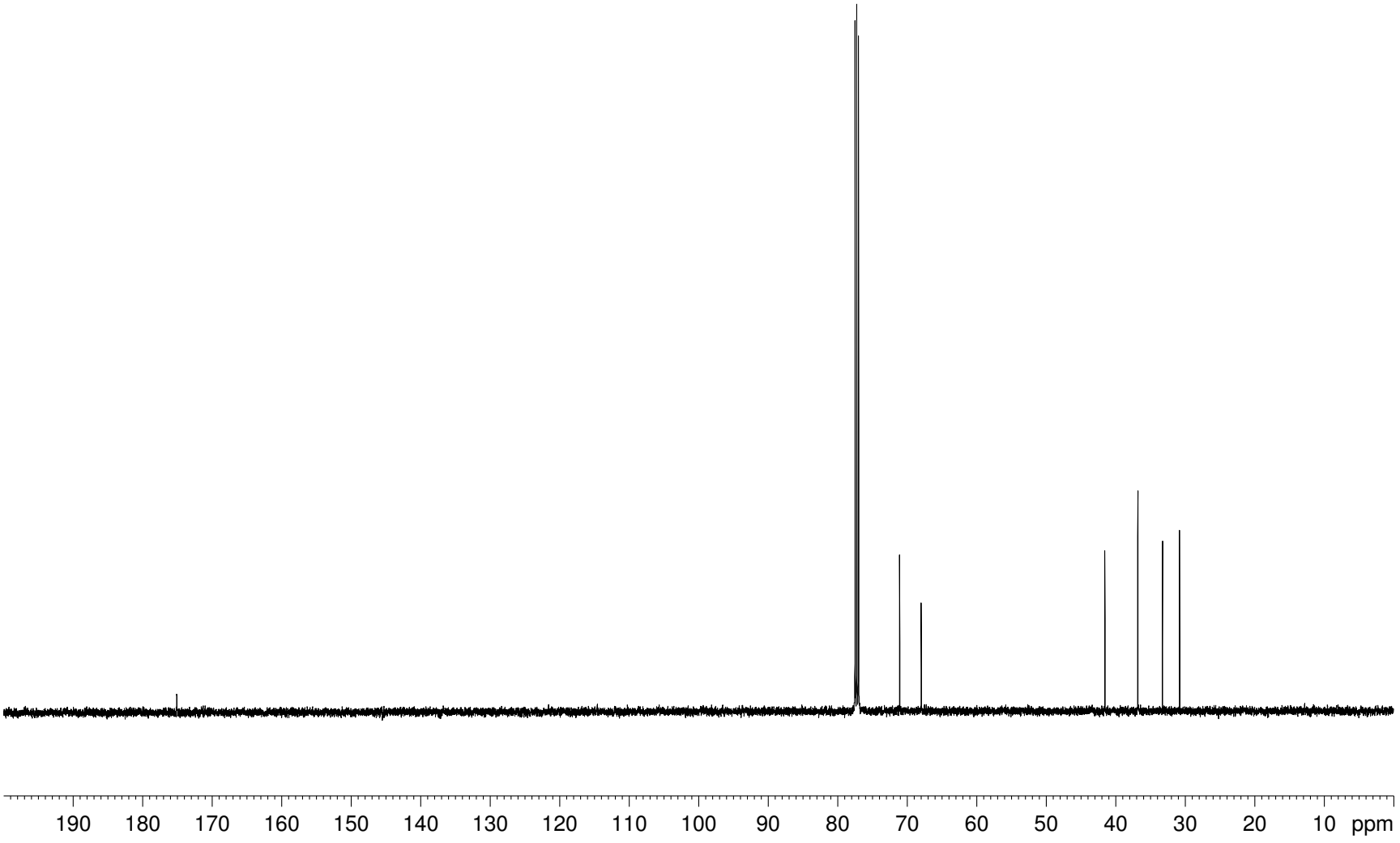
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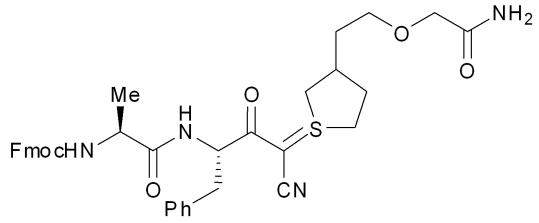
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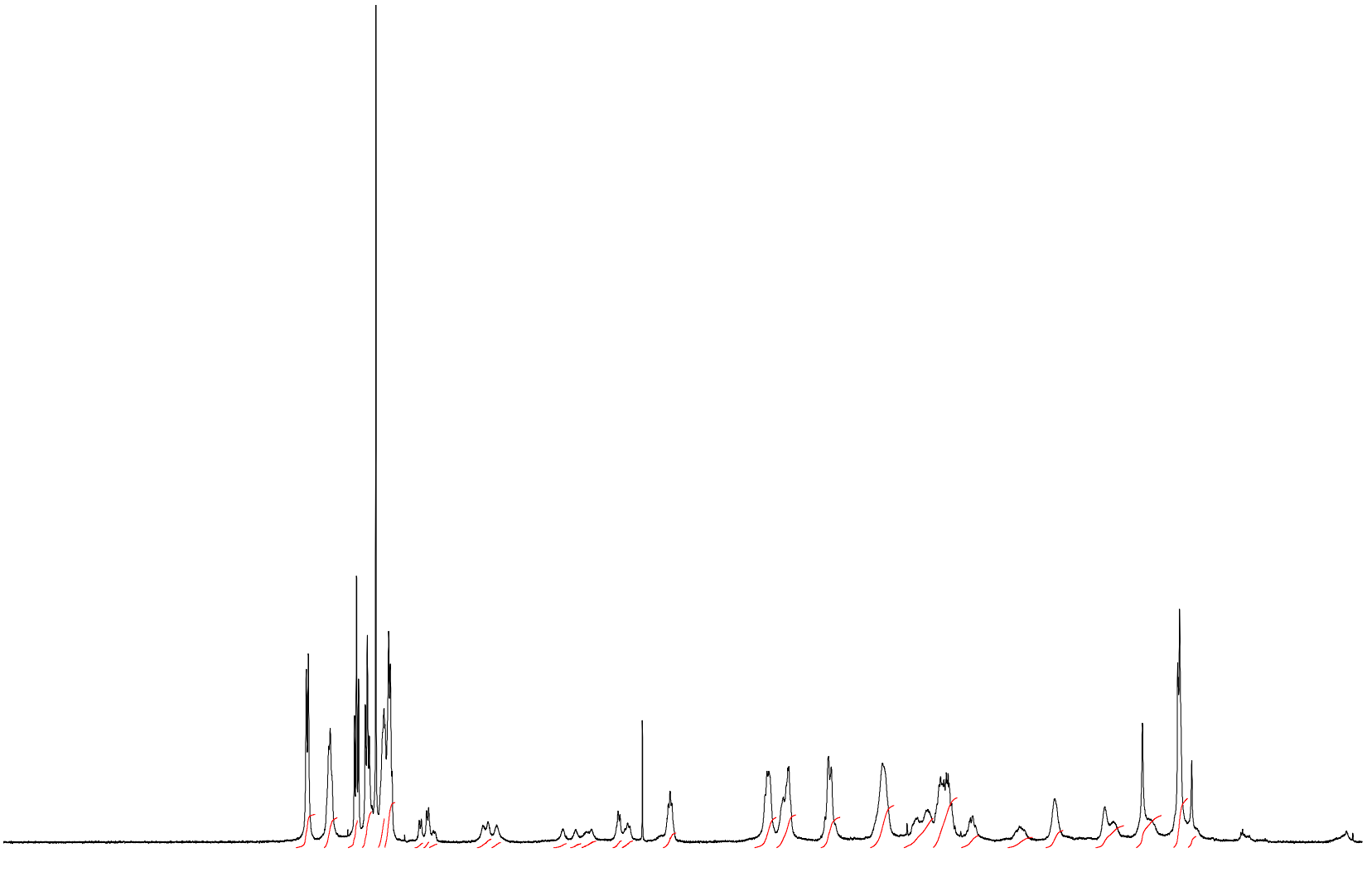
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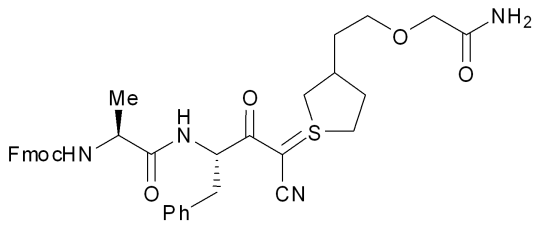
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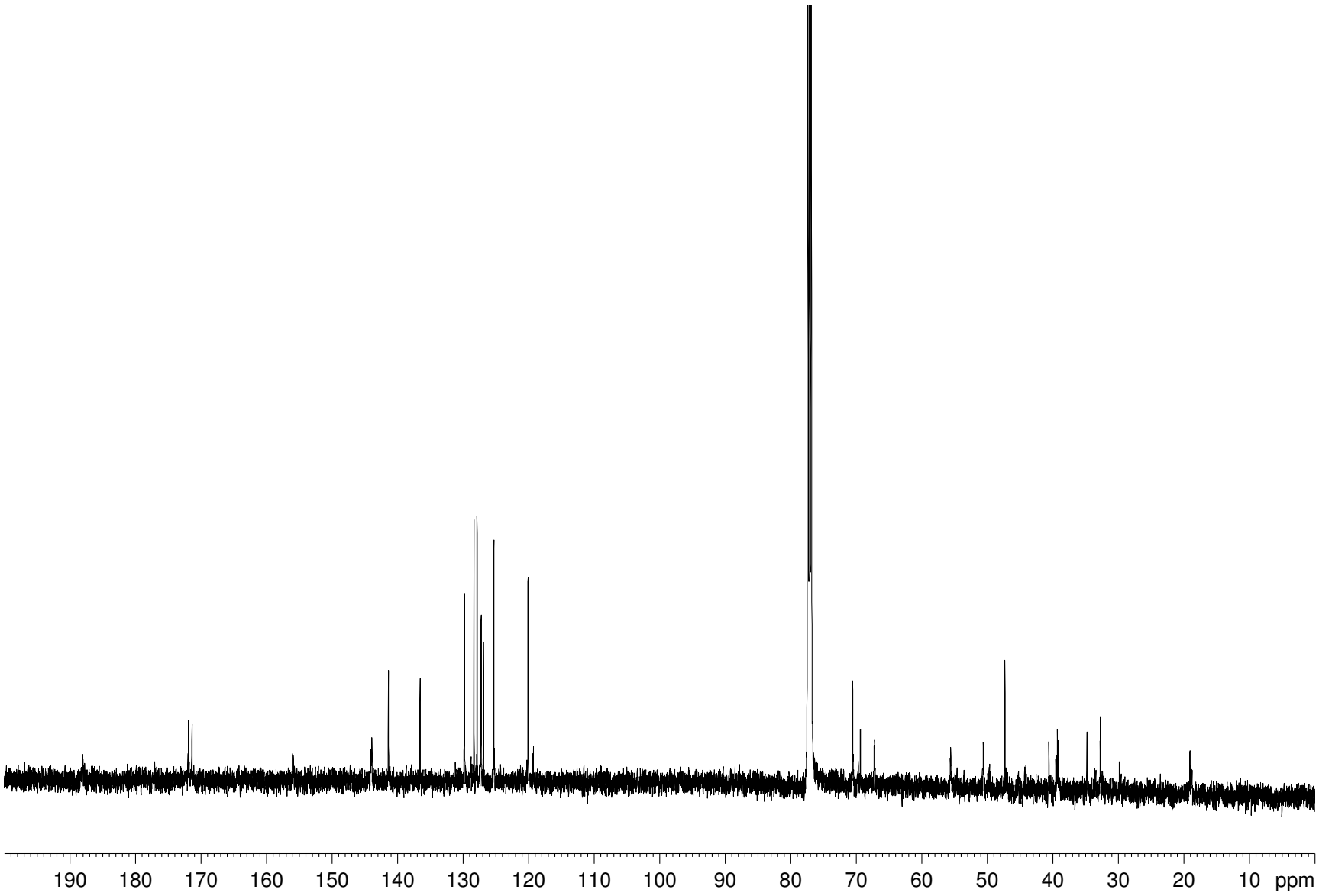
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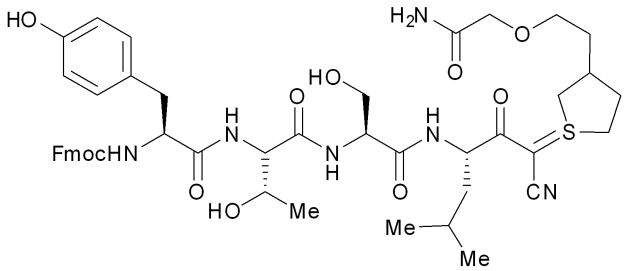
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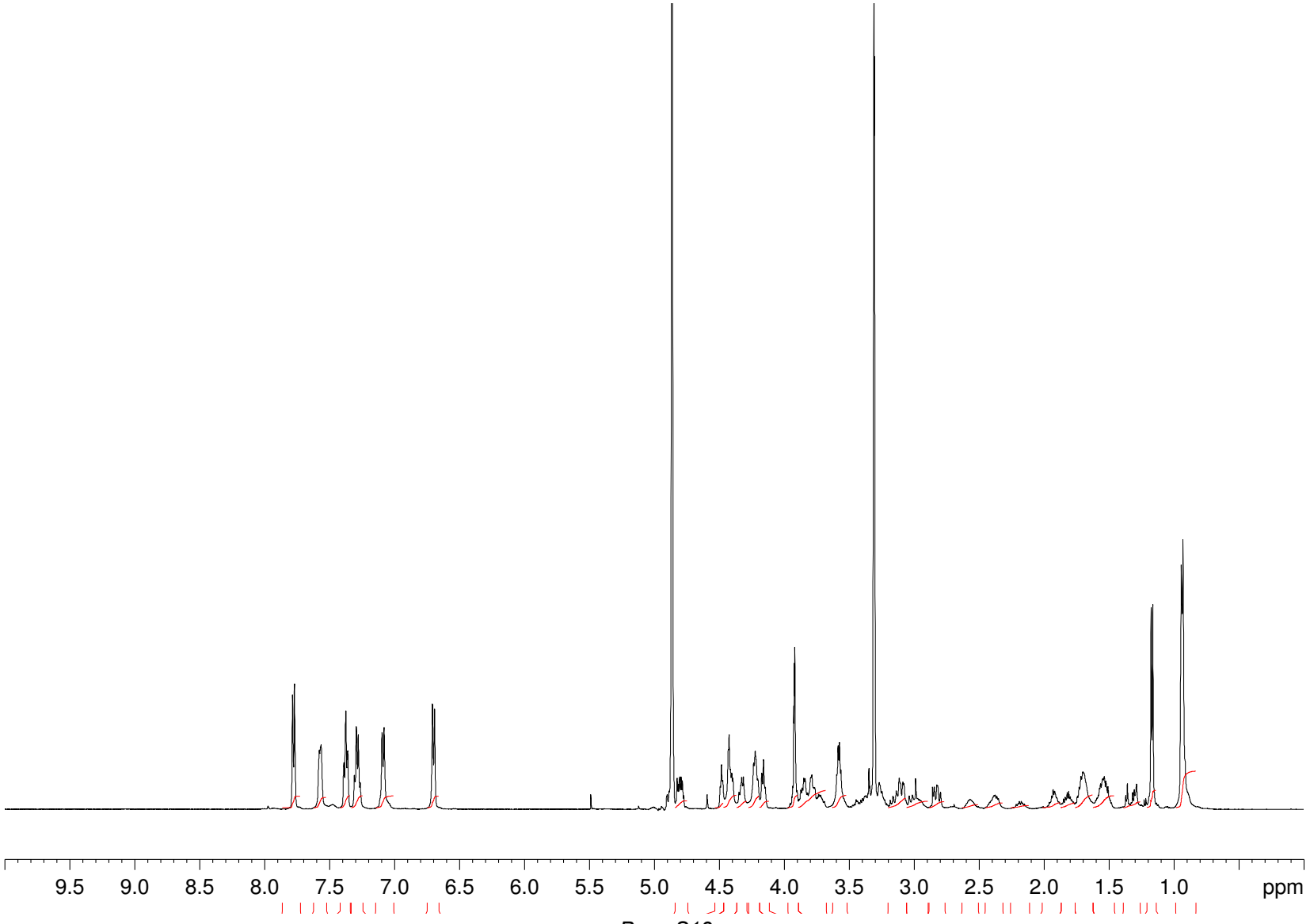
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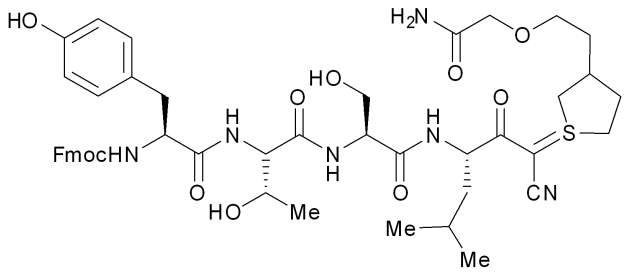
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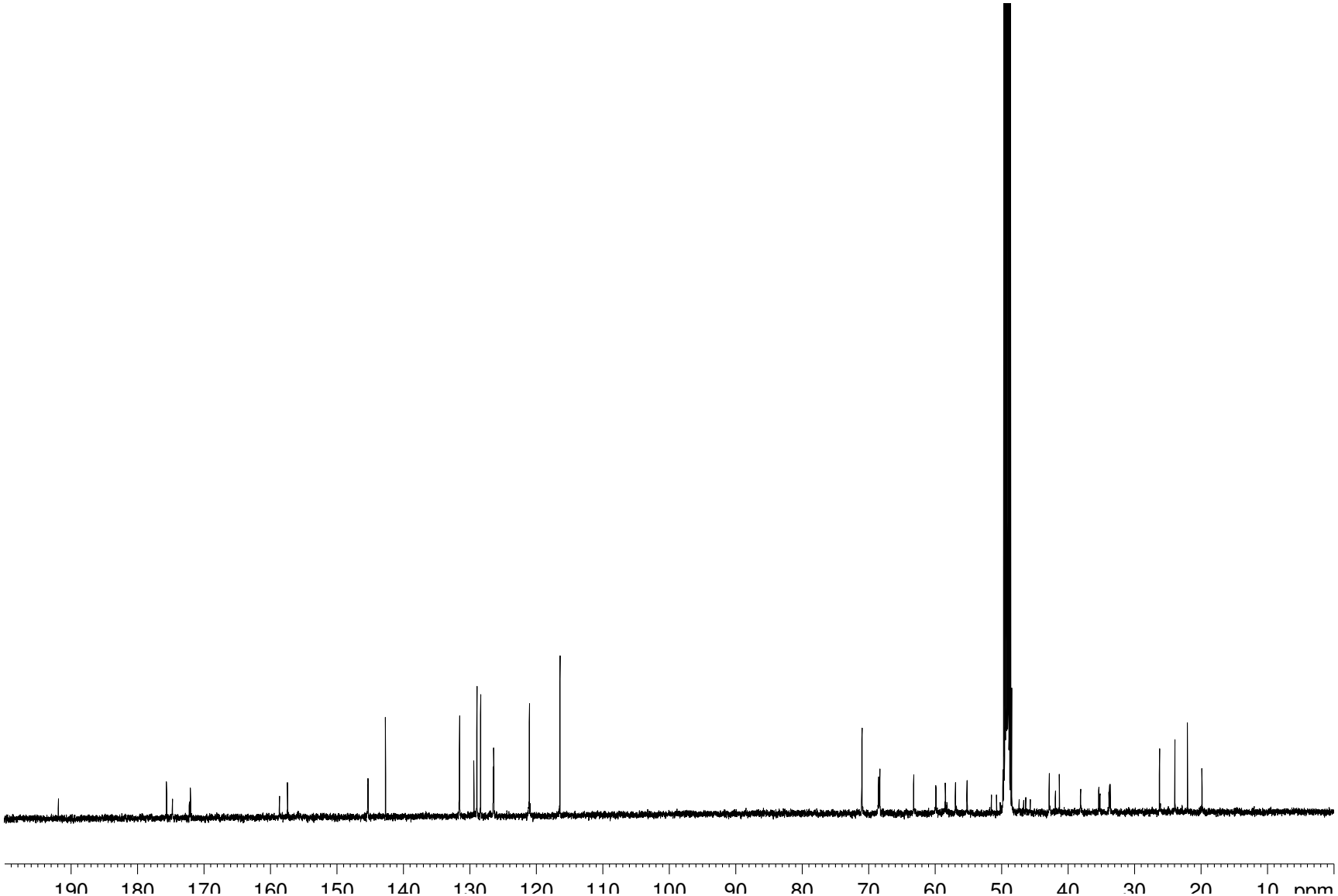
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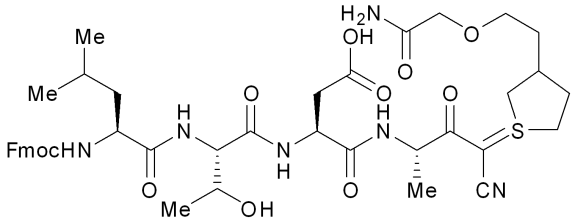
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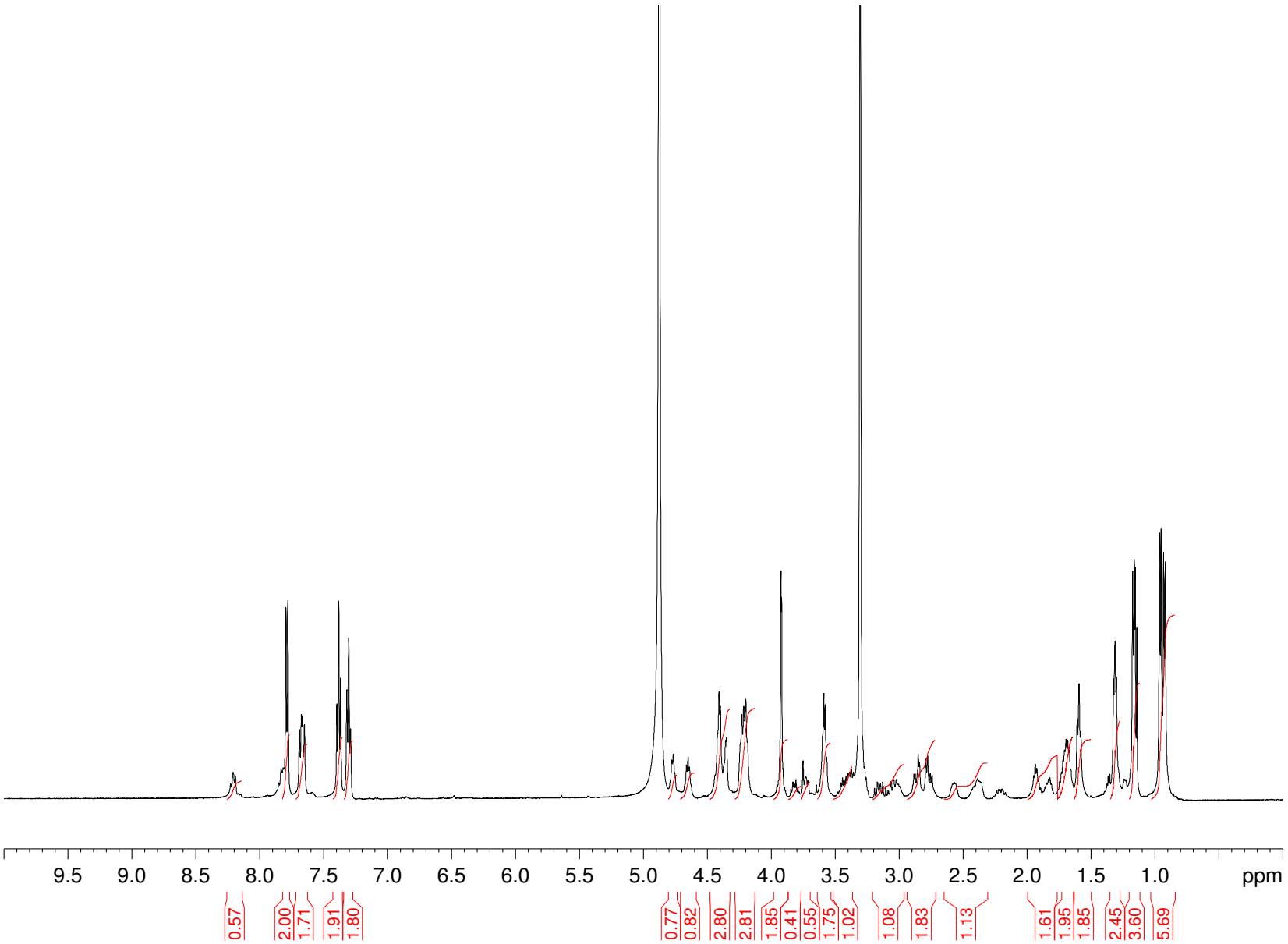
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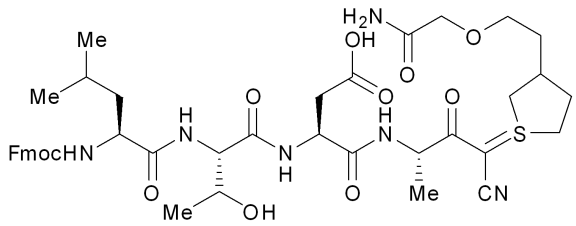
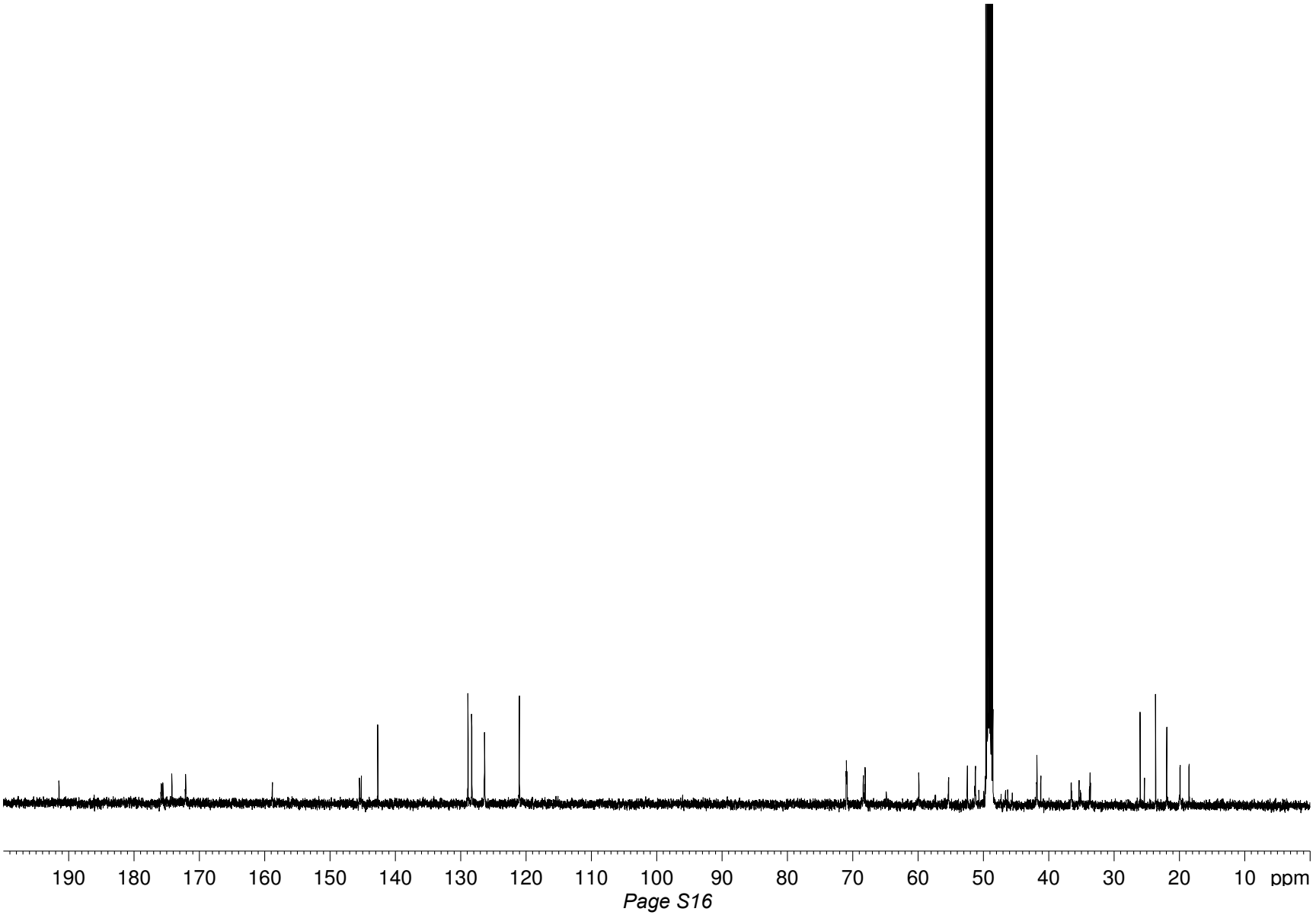
3.60
5.69


Me
Me
FmocHN
H
N
O
Me
OH
N
H
O
OH
O
O
H2N
O
O
N
O
Me
CN
S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180
170
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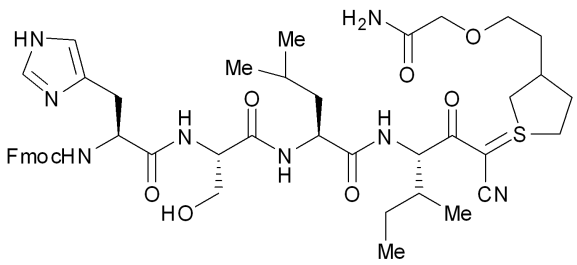
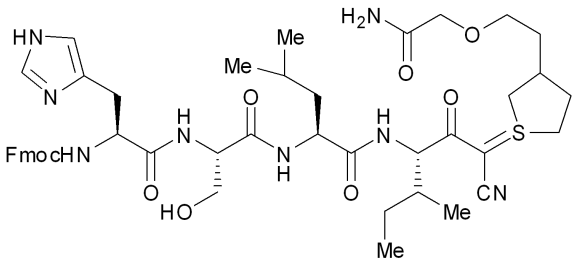
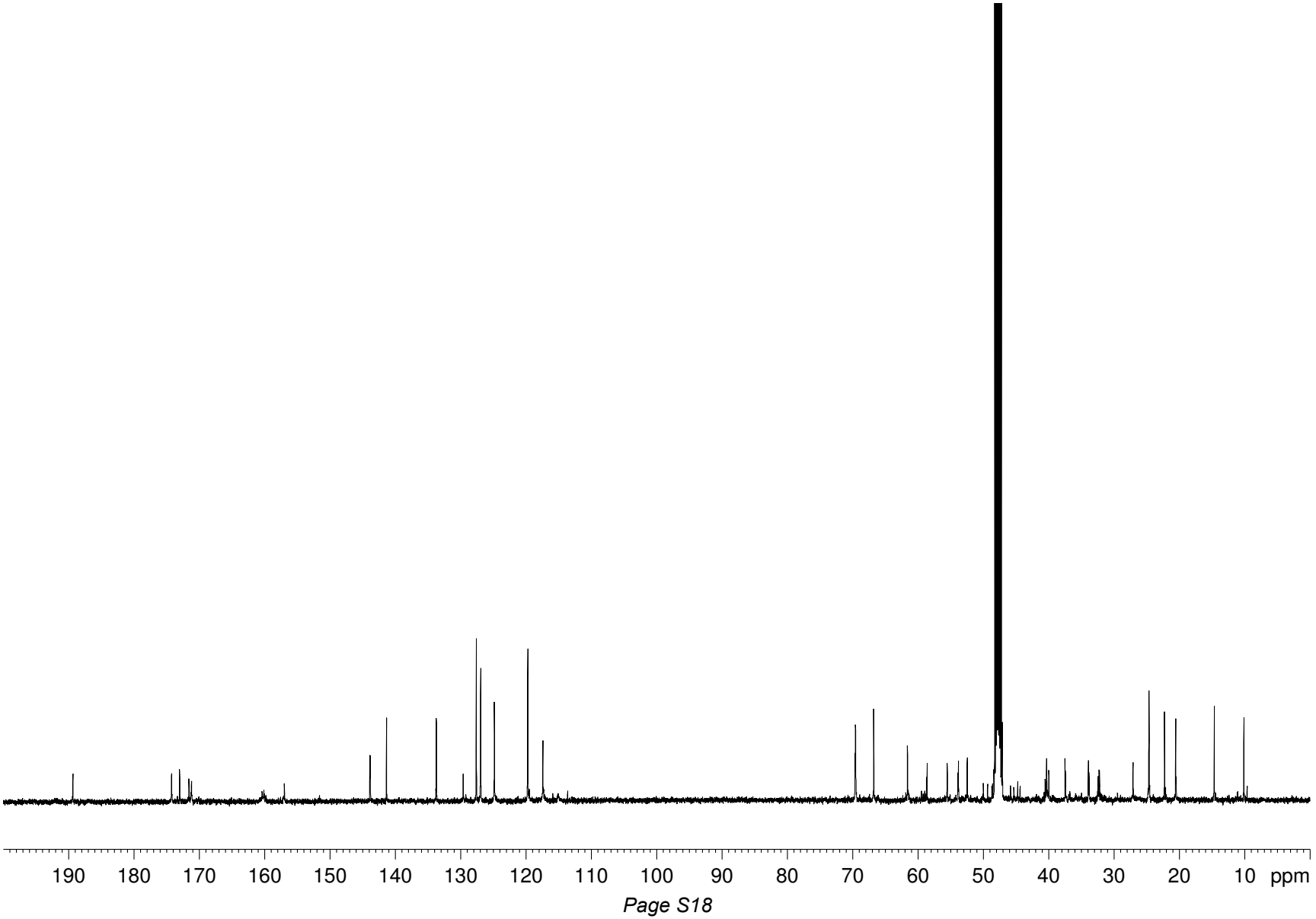
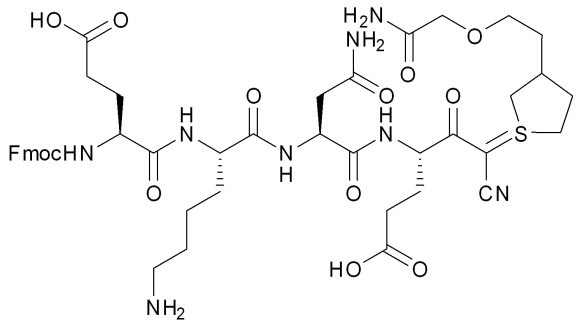
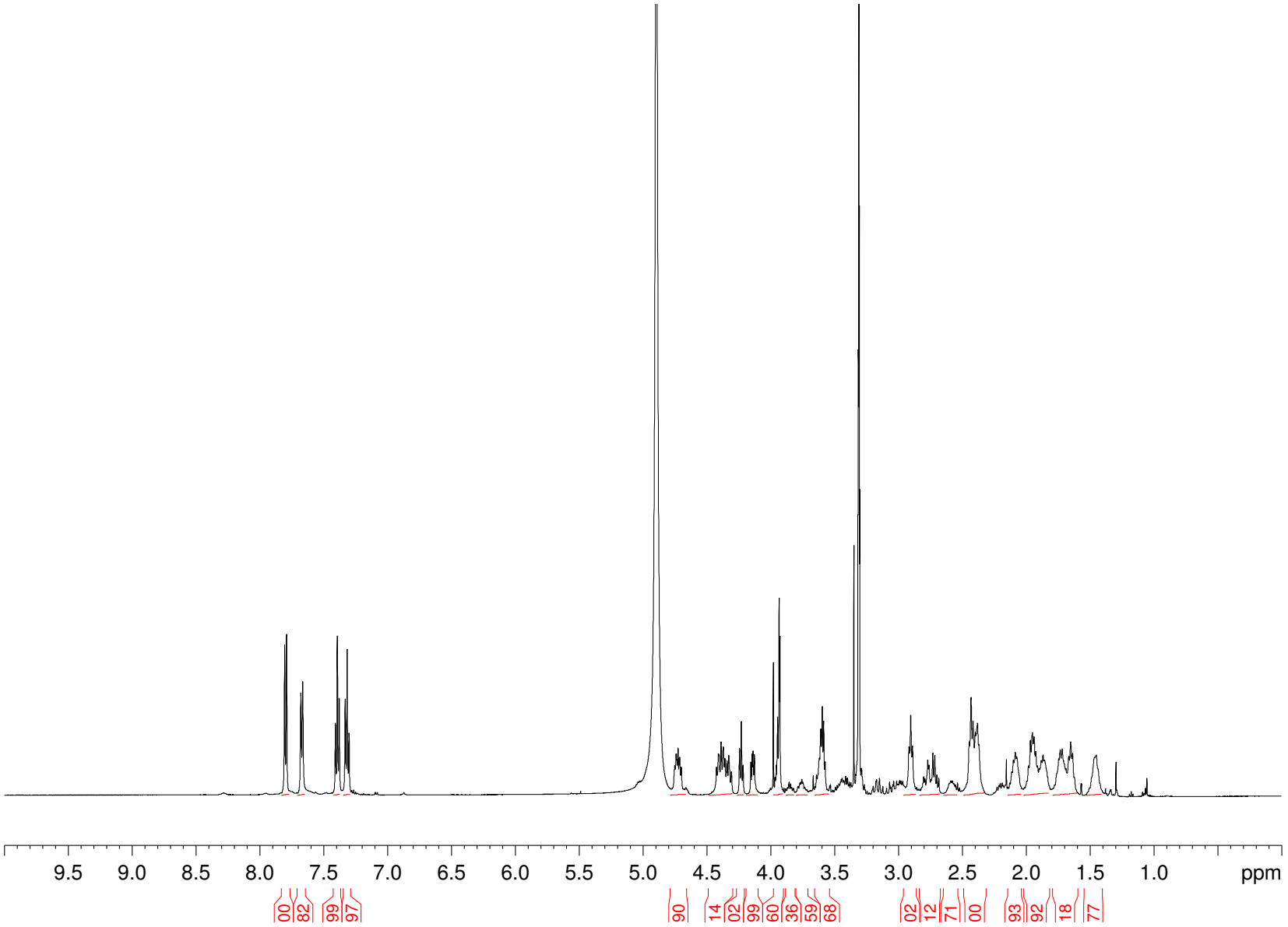
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


HN
N
FmocHN
H
N
O
HO
Me
Me
N
H
O
H
N
O
O
H_2N
O
O
S
CN
Me
Me


HN
N
FmocHN
H
N
O
HO
O
N
H
Me
Me
O
H
N
O
O
S
CN
Me
Me
H_2N
O
O


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


HO
O
FmocHN
H
N
O
N
H
O
H2N
NH2
O
O
H
N
O
O
S
CN
HO
O
NH2


9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
00
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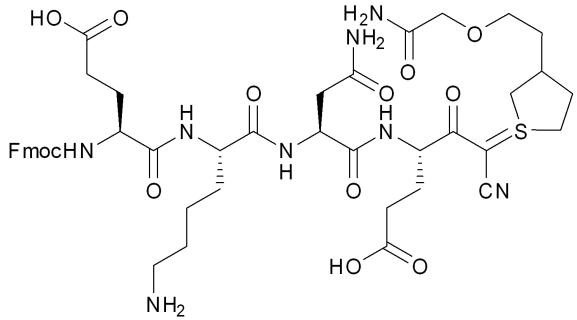
90
14
02
99
60
36
59
68
02
12
71
00
93
92
18
77


HO
O
FmocHN
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N
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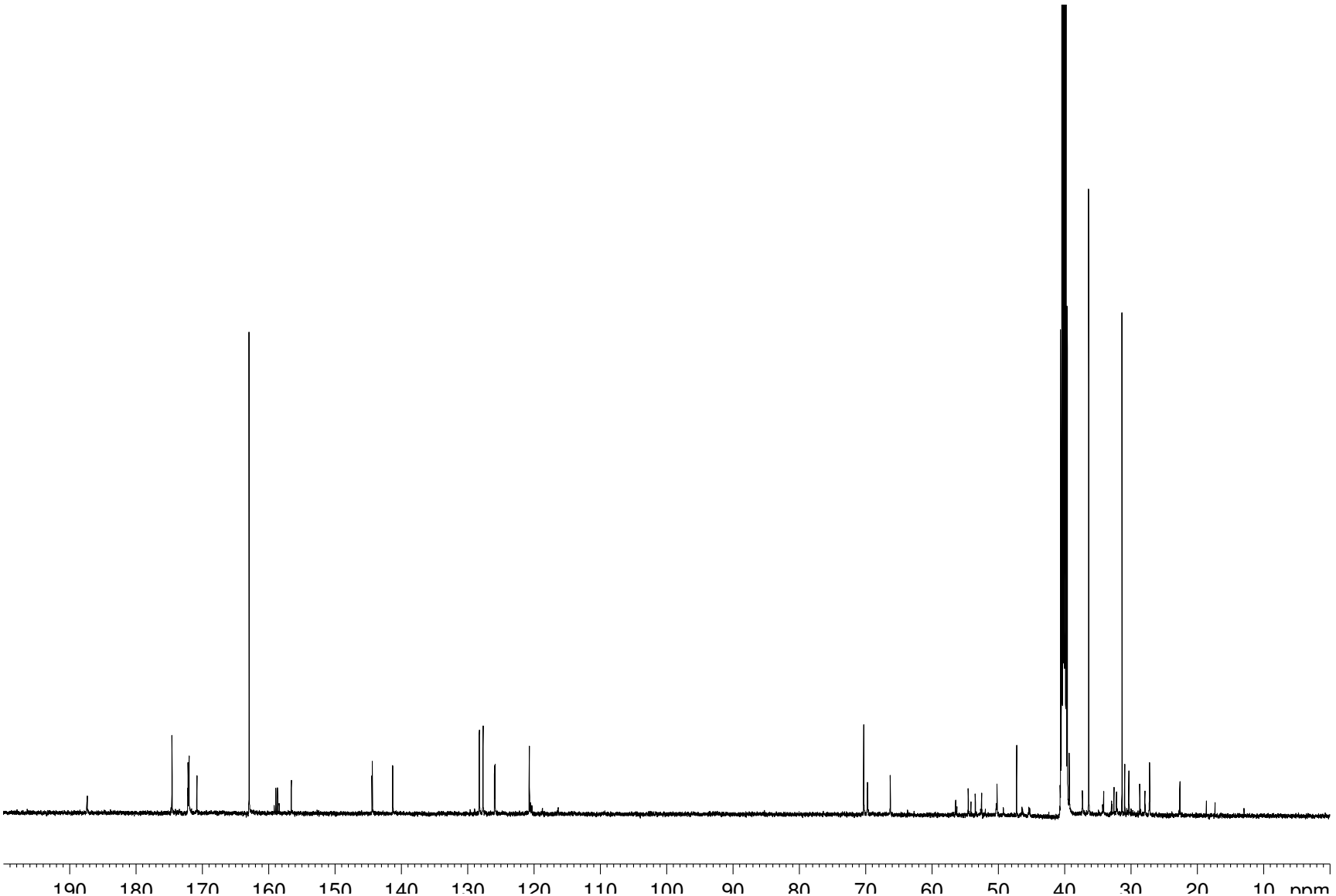
N
H
O
H2N
NH2
O
O
H
N
O
S
CN
HO
O
NH2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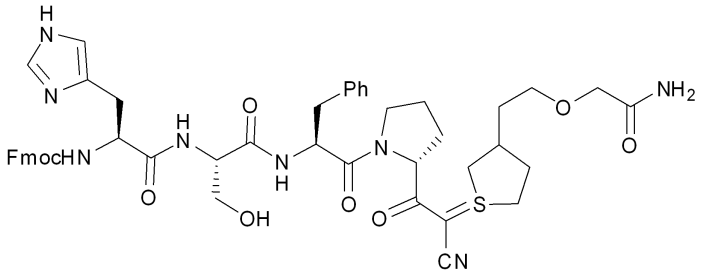
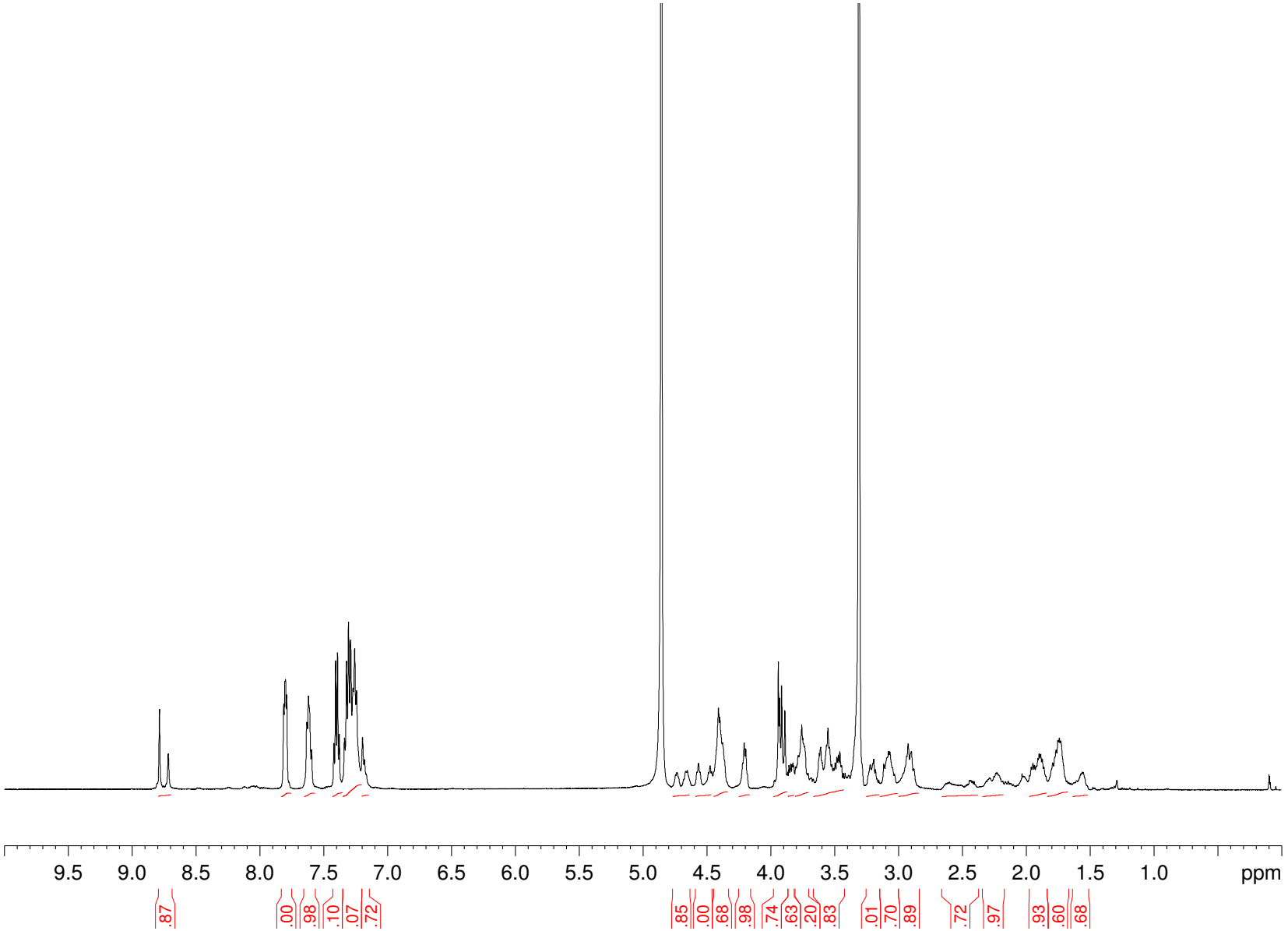
10
ppm


H
N
N
FmocHN
O
H
N
O
OH
N
H
Ph
O
N
O
S
CN
O
O
NH2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9.0
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8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
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1.5
1.0
ppm
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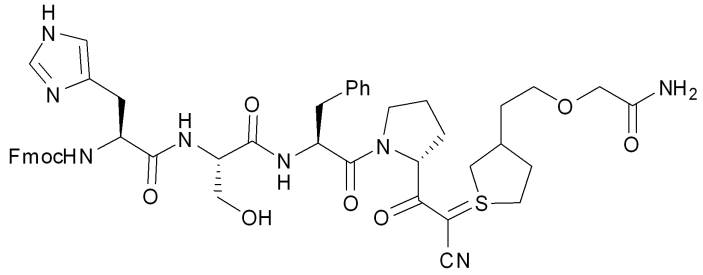
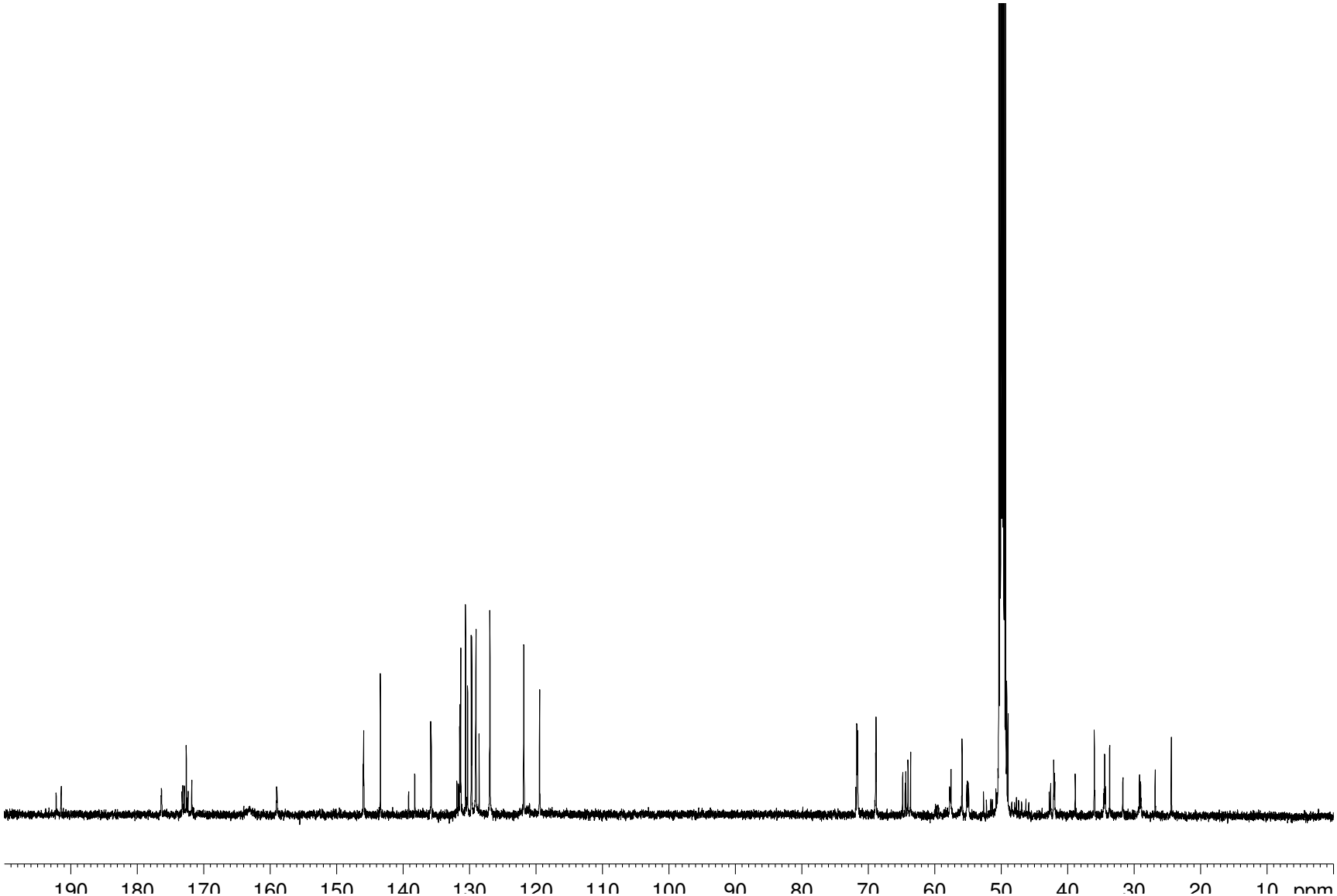
.00
.98
.10
.07
.72
.85
.00
.68
.98
.74
.63
.20
.83
.01
.70
.89
.72
.97
.93
.60
.68


H
N
N
FmocHN
O
H
N
O
OH
N
H
Ph
O
N
O
S
CN
O
O
NH2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180
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150
140
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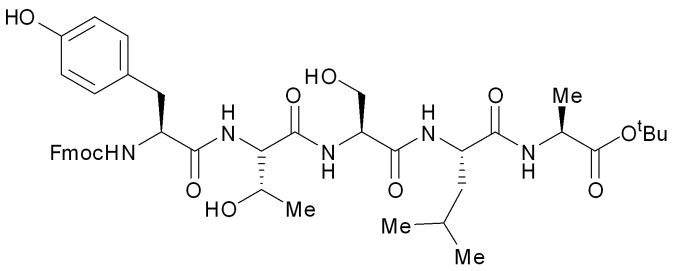
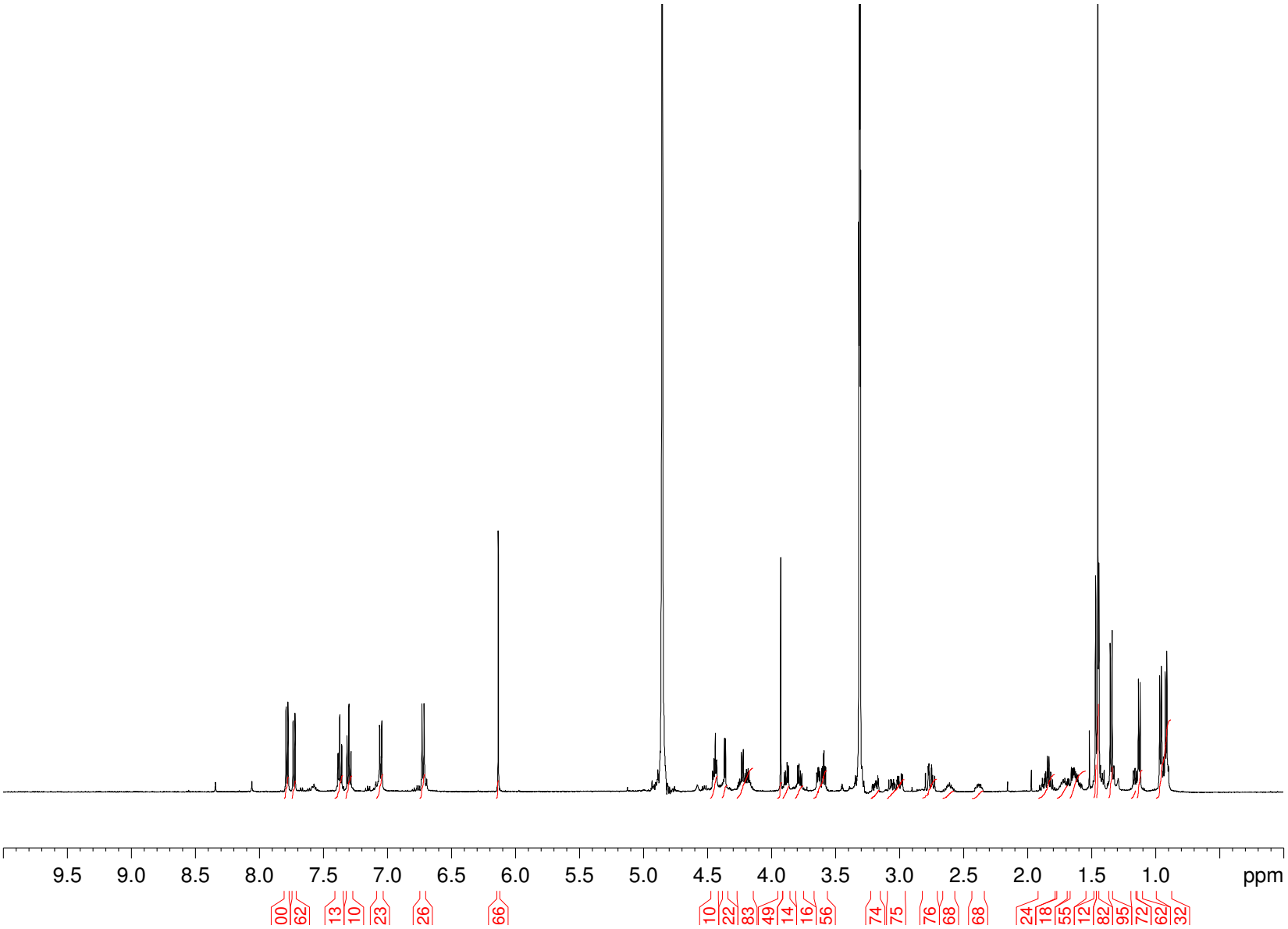
40
30
20
10
ppm


HO
HO
Me
FmocHN
H
N
O
H
N
H
N
O
O
O
N
H
O
O
HO
Me
Me
Me
O^{t}Bu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9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
00
62
13
10
23
26
66
10
22
83
49
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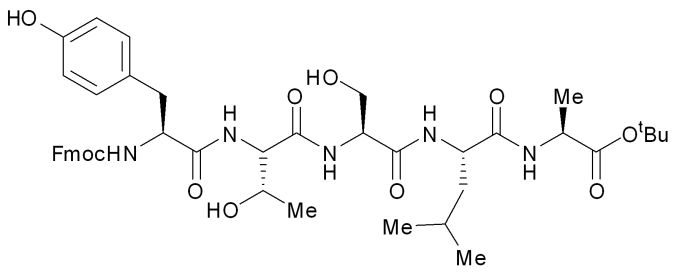
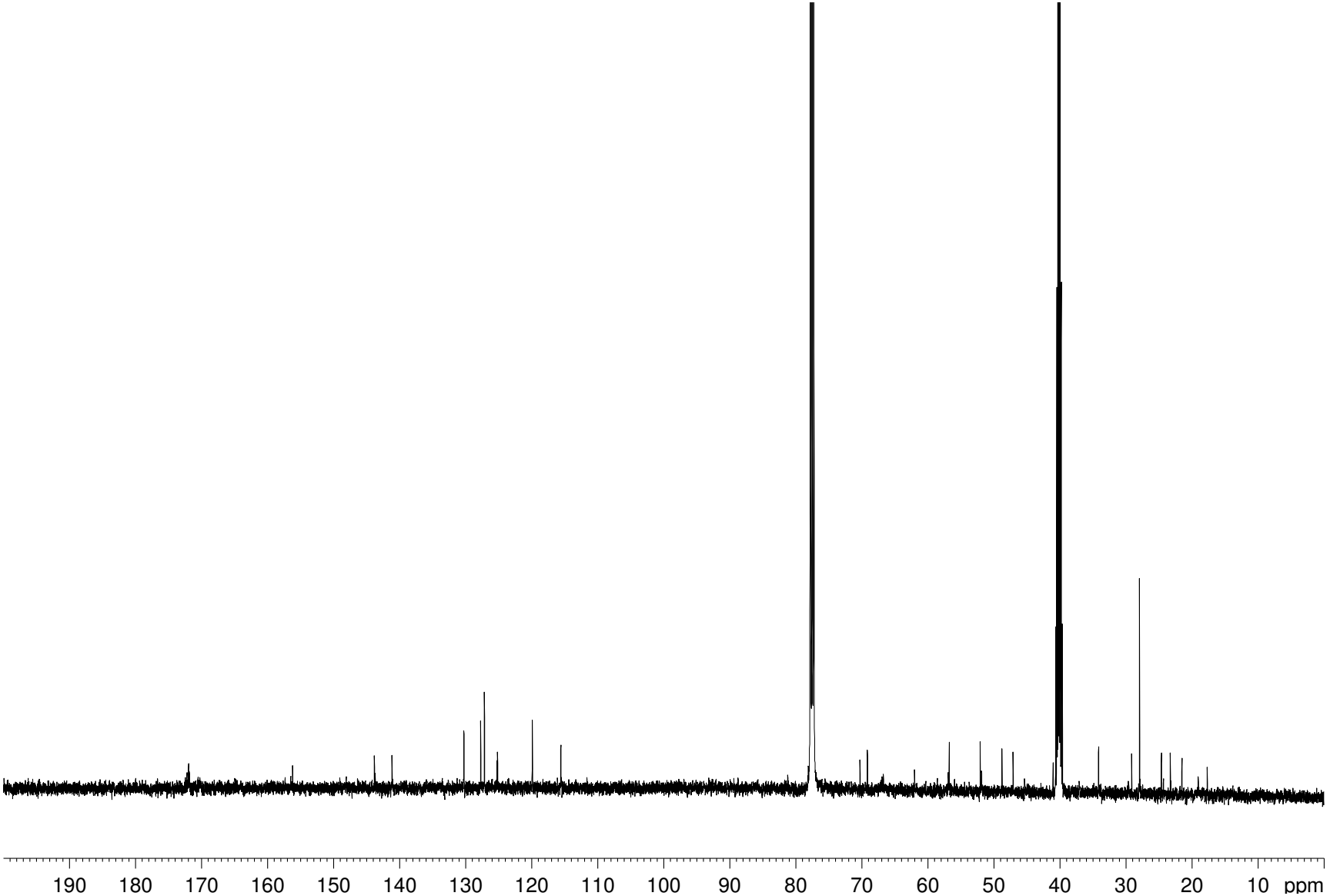
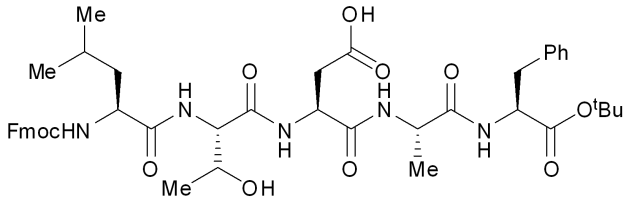
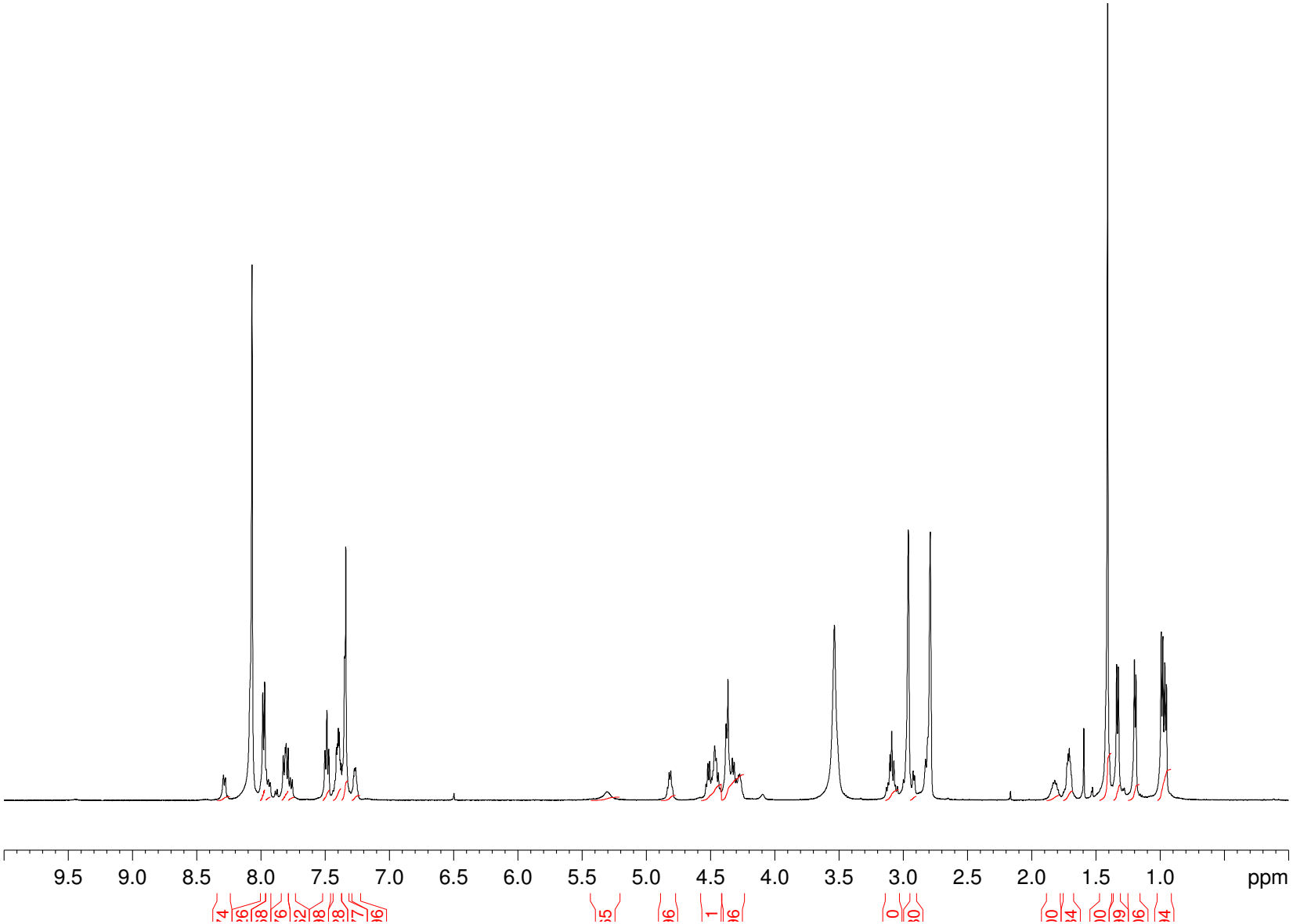
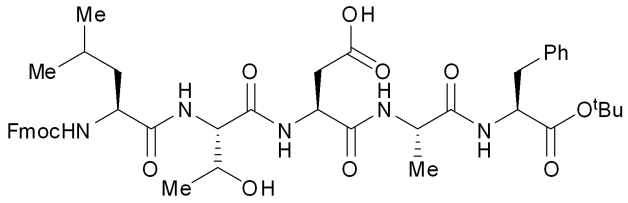
16
56
74
75
76
68
68
24
18
55
12
82
95
72
62
32

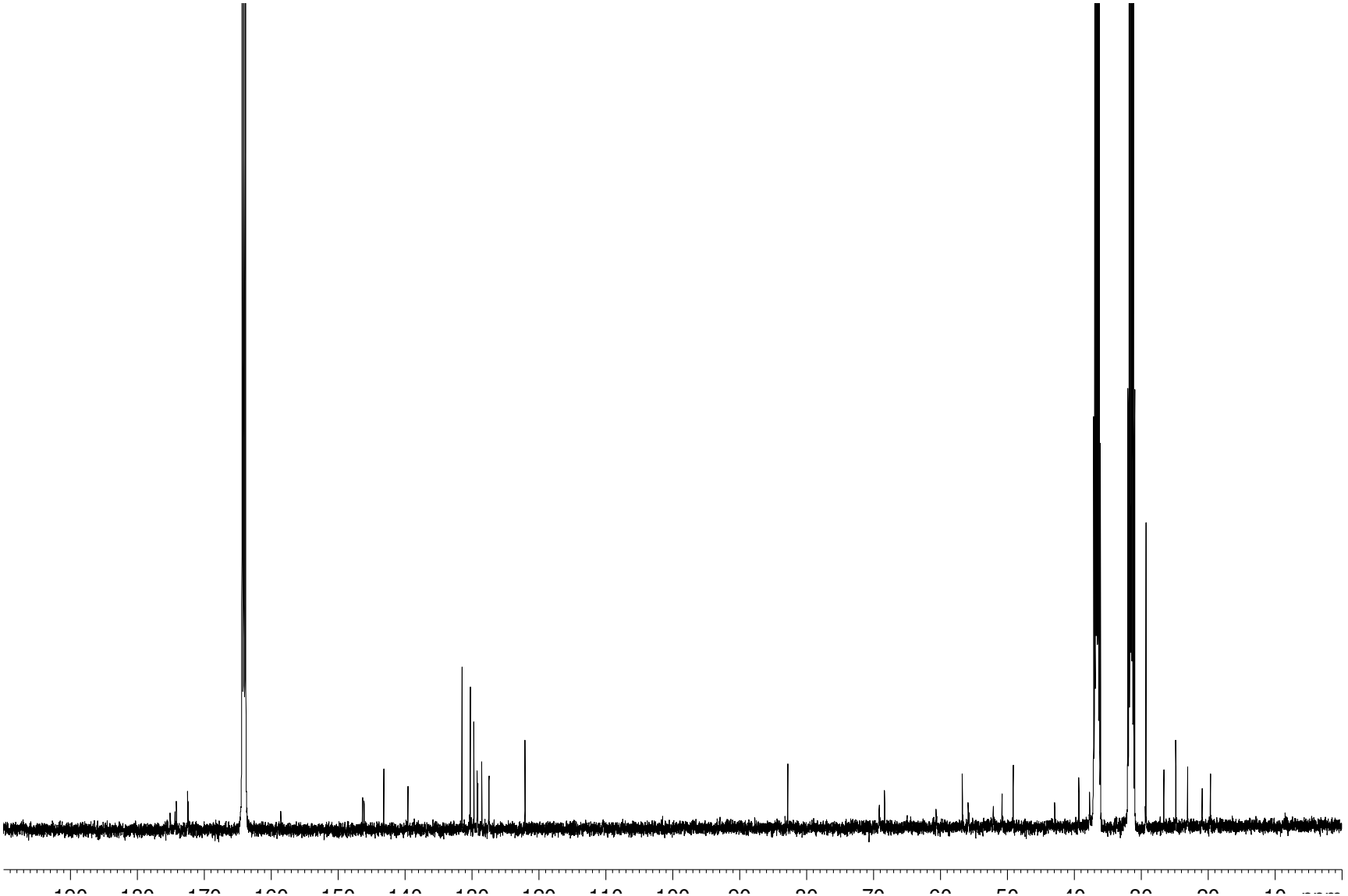

HO
HO
O
H
N
Me
FmocHN
O
N
H
O
N
H
O
N
H
O
O^{t}Bu
HO
Me
Me
Me

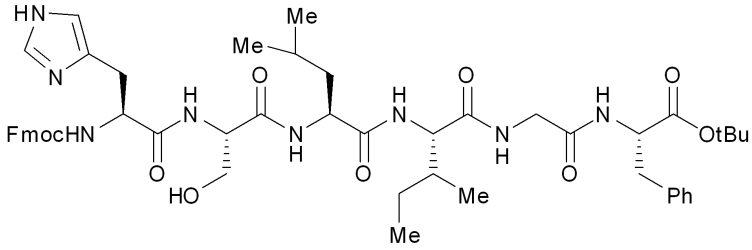

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

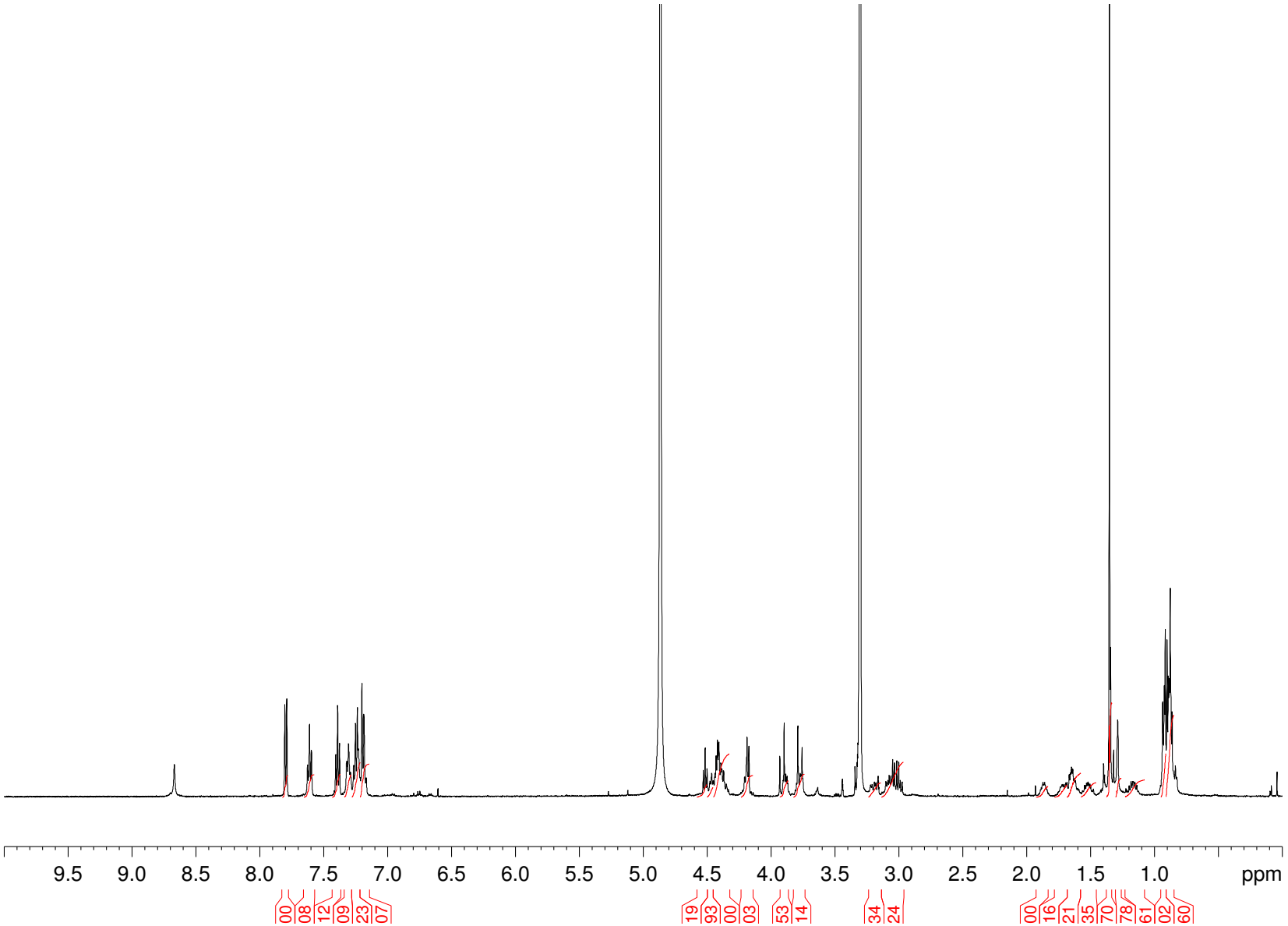

Me
Me
FmocHN
O
H
N
O
N
H
OH
O
O
H
N
O
Me
OH
O
Me
N
H
Ph
O
O
tBu

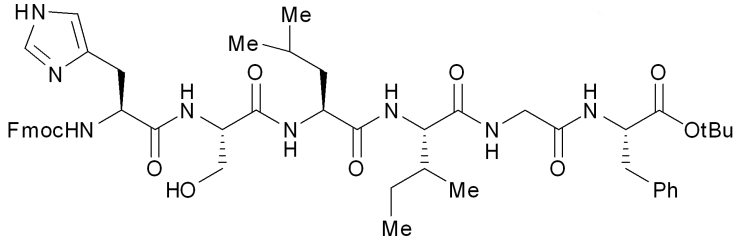

9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


Me
Me
FmocHN
H
N
O
O
Me
OH
N
H
OH
O
O
H
N
O
Me
N
H
Ph
O
O^{t}Bu




HN
N
FmocHN
H
N
O
HO
N
H
Me
Me
O
H
N
O
N
H
Me
Me
O
H
N
O
N
H
O
OtBu
Ph


9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
00
08
12
09
23
07
19
93
00
03
53
14
34
24
00
16
21
35
70
78
61
02
60


HN
N
FmocHN
H
N
O
HO
N
H
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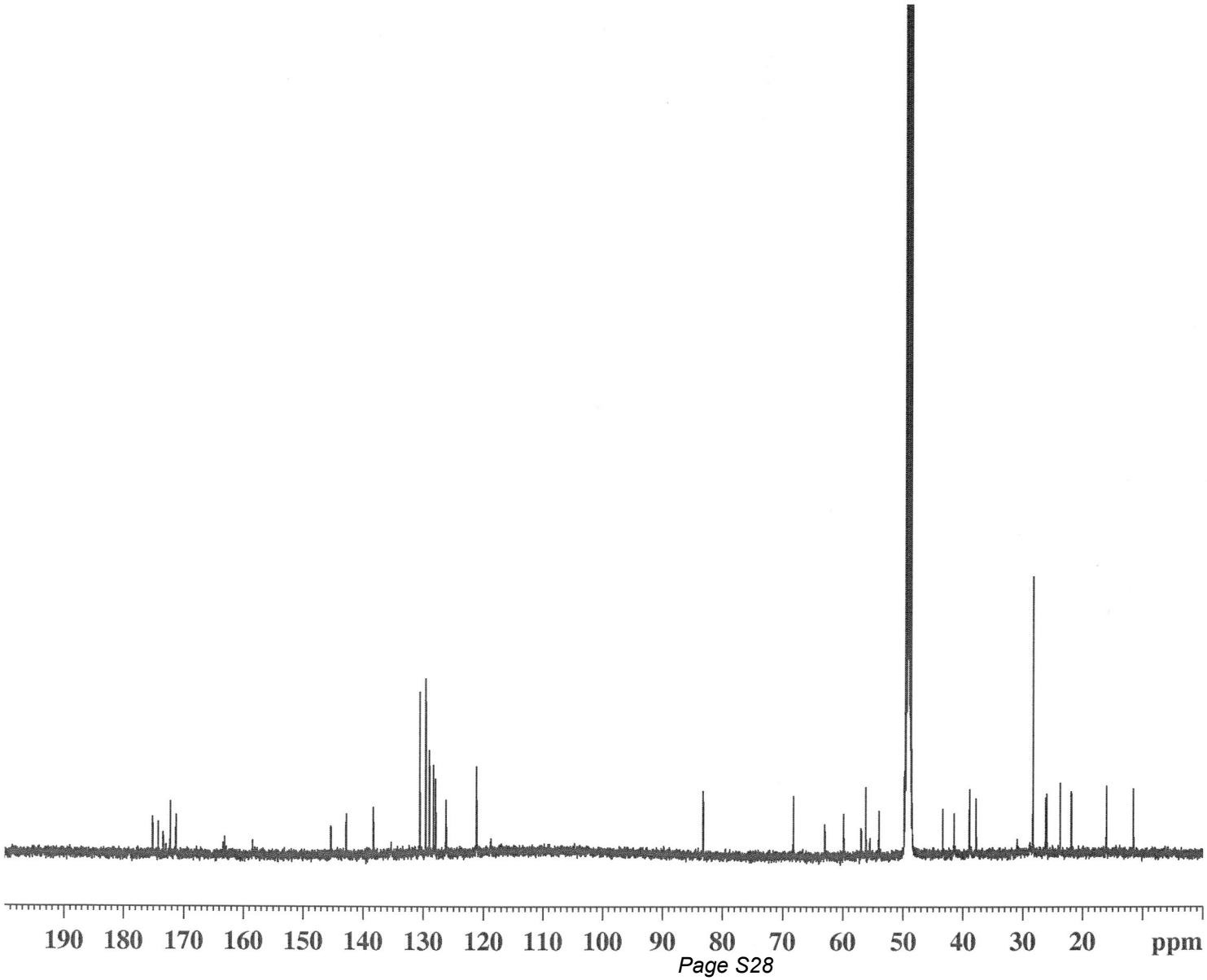
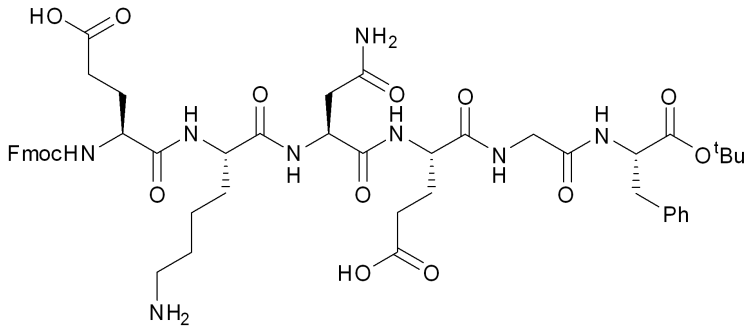
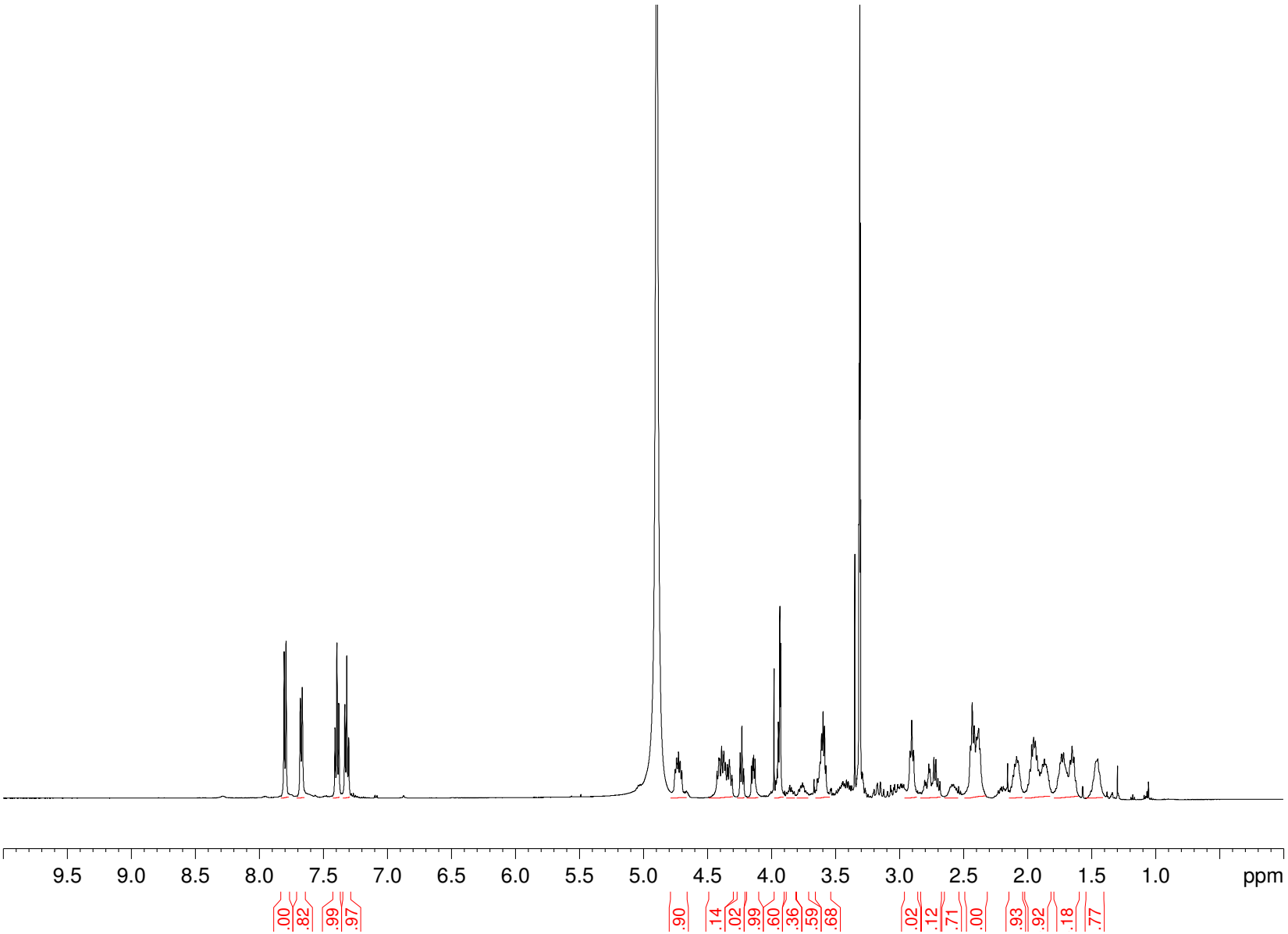
Me
Me
H
N
O
N
H
O
Me
Me
H
N
O
N
H
O
Ph
OtBu


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


HO
O
NH2
O
FmocHN
H
N
O
N
H
O
H
N
O
N
H
O
H
N
O
O
tBu
Ph
NH2
HO
O


9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
.00
.82
.99
.97
.90
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.02
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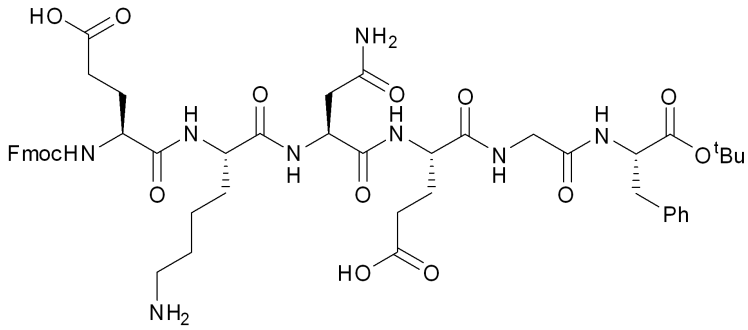
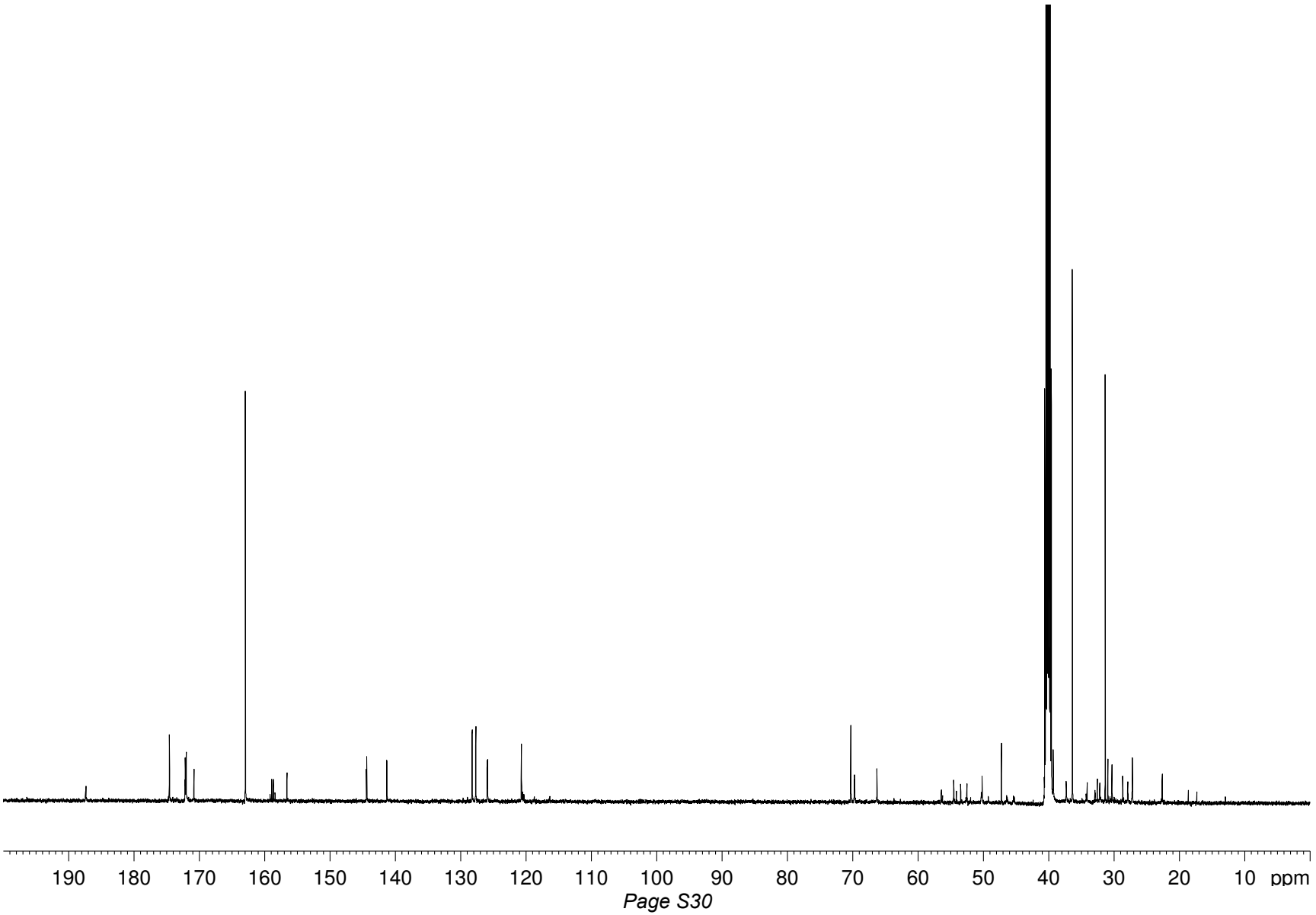
.60
.36
.59
.68
.02
.12
.71
.00
.93
.92
.18
.77


HO
O
NH2
FmocHN
H
N
O
N
H
O
H
N
O
N
H
O
H
N
O
O
tBu
Ph
HO
O
NH2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150
140
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120
110
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70
60
50
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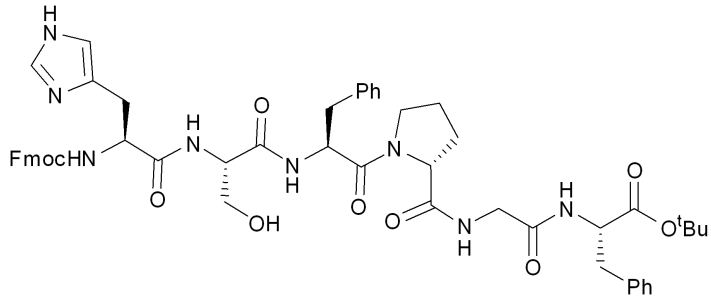
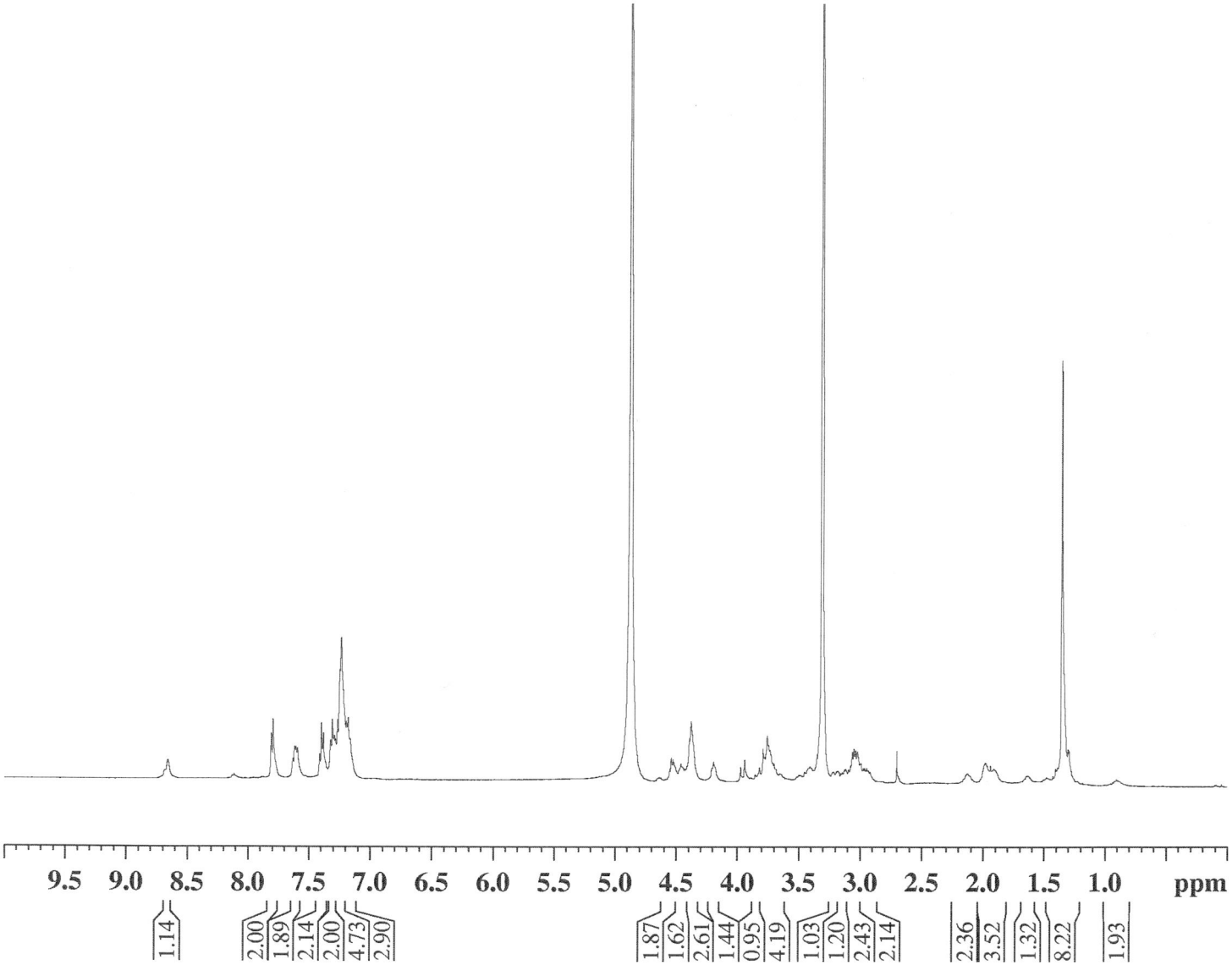
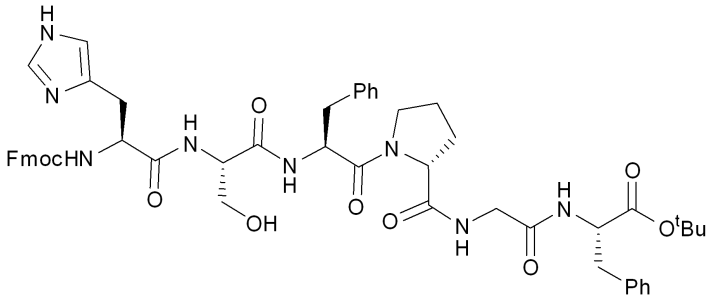
30
20
10
ppm


H
N
N
FmocHN
O
H
N
OH
O
N
H
O
Ph
N
O
O
N
H
H
N
O
O
O^{t}Bu
Ph


9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
1.14
2.00
1.89
2.14
2.00
4.73
2.90
1.87
1.62
2.61
1.44
0.95
4.19
1.03
1.20
2.43
2.14
2.36
3.52
1.32
8.22
1.93


H
N
N
FmocHN
O
H
N
OH
O
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H
O
N
Ph
O
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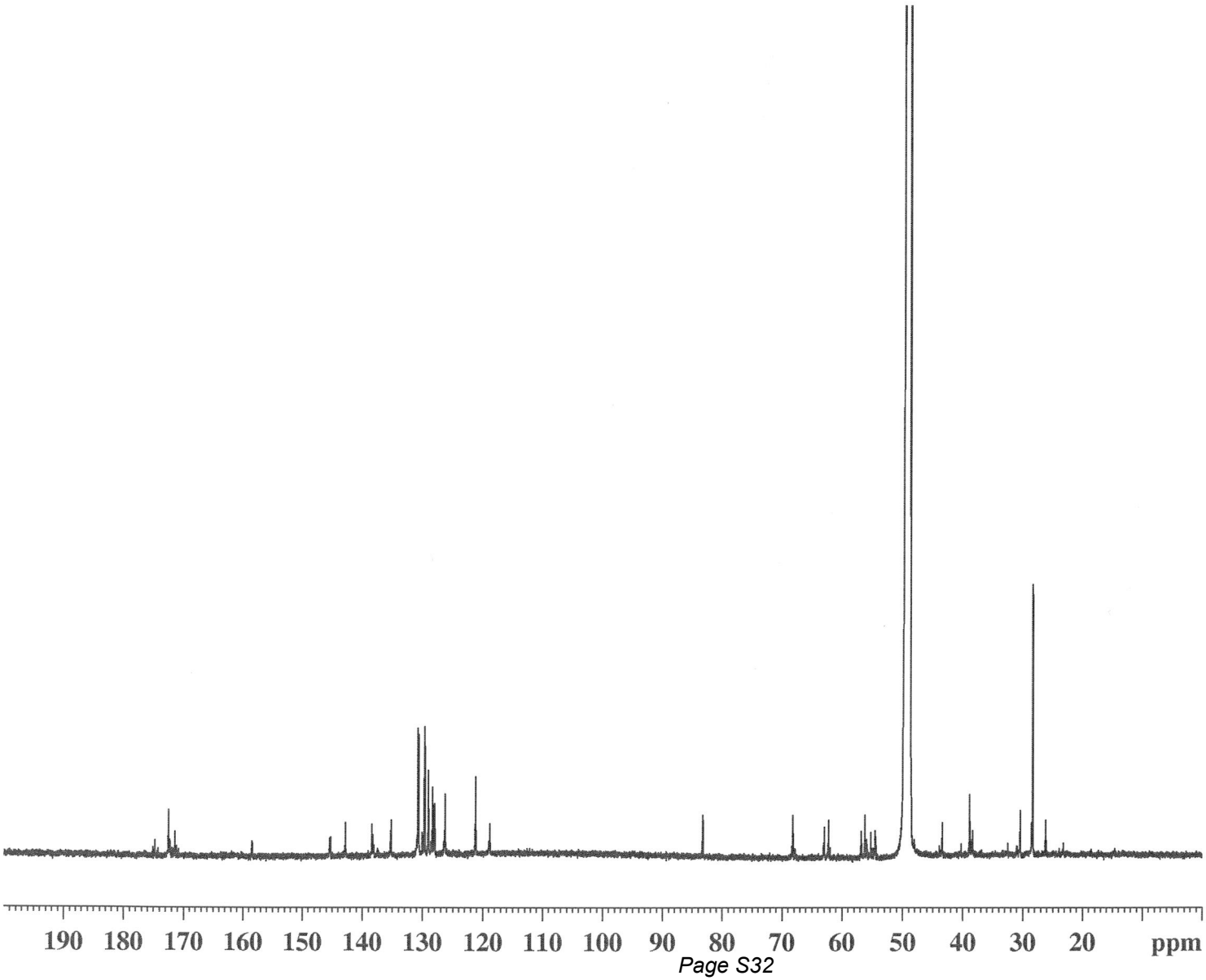
H
O
H
N
O
O
tBu
Ph


190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm