Supporting Information

Total Synthesis of Lysobactin

Aikomari Guzman-Martinez, Ryan Lamer, and Michael S. VanNieuwenhze*

Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, CA 92093

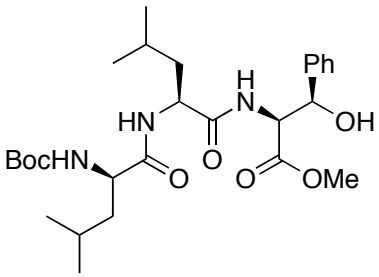
General. All reactions were carried out in flame-dried glassware under an atmosphere of dry nitrogen or argon. Unless otherwise mentioned, solvents were purified as follows. All solvents were dried by passing through a column of activated alumina (Seca Solvent System, Glass Contour). L-threo-hydroxyleucine was purchased from Acros Organics. All other commercially available reagents were used as received.

^{1}H NMR spectra were measured at 300 MHz on a Varian Mercury instrument, at 400 MHz on a Varian Gemini-400, or at 500 MHz on a Varian VXR-500 instrument. ^{13}C NMR spectra were measured at 100 MHz on a Varian Gemini spectrometer. Chemical shifts are reported relative to the central line of residual solvent. Infrared spectra were recorded using a Nicolet IR/42 spectrometer FT-IR (thin film, NaCl cells). High-resolution mass spectra were obtained via electrospray ionization on an Agilent ESI-TOF spectrometer. Optical rotations were measured on a Perkin–Elmer polarimeter (Model 241) using a 1 mL capacity quartz cell with a 10 cm path length.

Analytical thin layer chromatography (TLC) was performed using Whatman glass plates coated with a 0.25 mm thickness of silica gel containing PF 254 indicator, and compounds were visualized with UV light, cerium molybdate stain or ninhydrin stain. Analytical high performance liquid chromatography (HPLC) was performed on a Beckman-Coulter instrument (System Gold) with diode array detection. Analysis was carried out using Phenomenex Jupiter reverse-phase (C_{18}) column (10μ particle size, 300 Å pore size, 250 mm length x 4.6 mm diameter) with mobile phases consisting of either 1% trifluoroacetic acid in either water or acetonitrile. Preparatory HPLC purifications (Phenomenex Jupiter C_{18} reverse-phase column, 10μ particle size, 300 Å pore size, 250

mm length x 21.2 mm diameter) were performed with a Waters Millipore Model 510 System with an automated gradient collector and a Model 2487 Dual Absorbance Detector. Flash chromatography purifications were performed using Silicycle 60 Å, 35-75 μm silica gel or Biotage purification system (SP1 HPFC system). All compounds purified by chromatography were sufficiently pure for use in further experiments, unless otherwise noted.

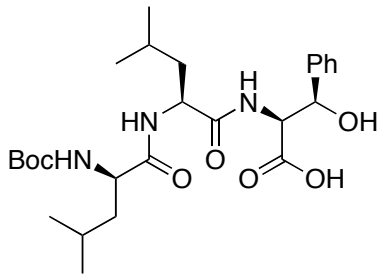
Experimental Section



Boc-D-Leu-L-Leu-L-HyPhe-OMe (10). TFA (2.35mL, 31.4 mmol) was added to a solution of Teoc-β-OH-Phe-OMe **8** (500 mg, 1.47 mmol) in DCM (2.35 mL) at 0ºC. The reaction was stirred at 0ºC for 1h and then it was concentrated. The residue was diluted with DCM (10 mL) and concentrated in vacuo. The product was used in the following reaction without further purification.

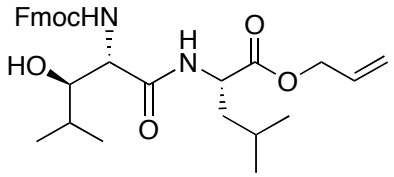
DEPBT (901 mg, 2.95 mmol) was added to a 0°C solution of H_2N-β-OH-Phe-OMe **9**, Boc-D-Leu-L-Leu-OH (610 mg, 1.77 mmol), and DIPEA (515 mL, 2.95 mmol) in THF (4.5 mL). The reaction was warmed slowly to room temperature stirred overnight. The reaction was diluted with EtOAc (15 mL), washed with 1M HCl sol. (2 x 25 mL), sat. $NaHCO_3$ (2 x 25 mL), and brine (1 x 25 mL). The organic layer was separated, dried with Na_2SO_4, filtered and concentrated. The product was purified by silica gel column with 10% - 50% EtOAc/hexanes. After concentration of the pure fractions, Boc-D-Leu-

L-Leu-L-HyPhe-OMe **10** (617 mg, 80% over two steps) was obtained as a clear foam. $[\alpha]^{23}_{D}$ -27 (*c* 1.00, $CHCl_3$); ^1H ($CDCl_3$, 400 MHz) δ 7.42-7.25 (m, 5H), 6.57 (d, J = 6, 1H), 5.27 (d, J = 4, 1H), 5.14 (d, J = 6, 1H), 4.93-4.86 (m, 1H), 4.60-4.51 (m, 1H), 4.06 (m, 1H), 3.70 (s, 3H), 1.73-1.54 (m, 4H), 1.47 (s, 11H), 1.00-0.87 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.4, 171.7, 170.2, 155.5, 139.6, 128.1, 127.7, 125.9, 80.3, 73.7, 58.6, 53.4, 52.5, 51.5, 41.3, 40.6, 28.3, 24.8, 24.7, 23.1, 22.9, 22.1, 22.0; IR (film) ν_{max} 3441, 3271, 3067, 2955, 2870, 1748, 1697, 1646, 1558, 1511, 1469, 1454, 1435, 1393, 1368, 1338, 1253, 1169, 1117, 1087, 1054, 1026, 756, 701, 669. HR-EI-MS m/z 522.3180 (MH^+, $C_{27}H_{43}O_7N_3$ requires 522.3174).



Boc-D-Leu-L-Leu-L-HyPhe-OH (11). A solution of LiOH (31 mg, 0.75 mmol) in H_2O (600 μL) was added dropwise to a solution of Boc-D-Leu-Leu-β-OH-Phe-OMe **10** (195 mg, 0.37 mmol) in THF (0.60 mL). The reaction was stirred at room temperature for 1h. The solution was diluted with EtOAc (2 mL), 1M HCl (5 mL) was added and the solution was extracted with EtOAc (2 x 2 mL). The organic layers where combined, dried over Na_2SO_4, filtered, and concentrated in vacuo to give Boc-D-Leu-L-Leu-L-HyPhe-OH **11** as a clear foamy solid (189 mg, 100%). $[\alpha]^{23}_{D}$ -13 (*c* 1.00, CH_3CH_2OH); ^1H (DMSO-d_6, 400 MHz) δ 7.91 (d, J = 9, 1H), 7.74 (d, J = 8.5, 1H), 7.35 (d, J = 7.5, 2H), 7.27 (t, J = 7, 7.5, 2H), 7.21-7.17 (m, 1H), 6.81 (d, J = 8.5, 1H), 5.10 (d, J = 3, 1H), 4.47-4.42 (m, 1H), 4.36-4.29 (m, 1H) 3.98-3.91 (m, 1H), 1.62-1.28 (m, 15H), 0.90-0.75 (m, 12H); ^{13}C NMR

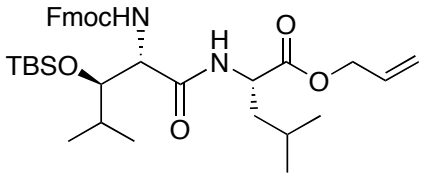
($CDCl_3$, 100 MHz) δ 172.2, 172.0, 171.5, 155.1, 142.0, 127.8, 127.1, 126.4, 78.0, 72.1, 58.1, 53.1, 50.6, 41.0, 40.9, 28.1, 24.3, 23.0, 21.6, 21.5; IR (film) ν_{max} 3297, 3065, 2958, 2934, 2873, 1721, 1699, 1652, 1538, 1470, 1456, 1393, 1368, 1251, 1168, 1117, 1059, 1028, 757, 700. HR-EI-MS m/z 508.3028 (MH^+, $C_{26}H_{41}O_7N_3$ requires 508.3017).



Fmoc-L-HyLeu-L-Leu-OAll. Fmoc-OSuc (230 mg, 0.68 mmol) was slowly added to a solution of β-OH-Leu (100 mg, 0.68 mmol) and Na_2CO_3 (72 mg, 0.68 mmol) in H_2O/acetone (1:1) (1.82 mL), at 0°C. The reaction was warmed to room temperature and stirred overnight. The solution was diluted in EtOAc (10 mL) and the mixture was acidified with 6M HCl (5 mL). The organic layer was separated, washed with H_2O (3 x 25 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was taken to the next reaction without further purification.

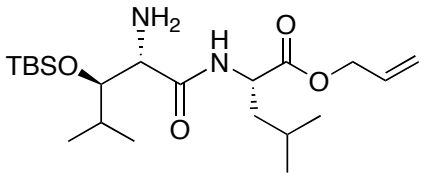
DEPBT (415 mg, 1.36mmol) was added to a at 0°C solution of Fmoc-β-OH-Leu-OH (251 mg, 0.68 mmol), TsOH•H_2N-Leu-OAl (280, 0.815 mmol), and DIPEA (237 μL, 1.36 mmol) in THF (2.06 mL). The reaction was stirred overnight at room temperature. The reaction was diluted with EtOAc (25 mL), washed with 1M HCl (2 x 50 mL), sat. $NaHCO_3$ (2 x 50 mL), and brine (1 x 50 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by silica gel column chromatography with 25% - 40% EtOAc/hexanes. After concentration of the pure fractions, Fmoc-L-HyLeu-Leu-OAll (285 mg, 80% over two steps) was obtained as

a clear foam. $[\alpha]^{23}_{D}$ -47 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.77 (d, J = 7, 2H), 7.58 (d, J = 6, 2H), 7.41 (t, J = 7, 7, 2H), 7.32 (t, J = 7, 7, 2H), 6.80 (d, J = 8, 1H), 5.94-5.86 (m, 1H), 5.74 (d, J = 9, 1H), 5.33 (d, J = 17, 1H), 5.25 (d, J = 10, 1H), 4.65-4.54 (m, 3H), 4.49-4.45 (m, 1H), 4.41-4.37 (m, 1H), 4.33 (d, J = 8, 1H), 4.22 (t, J = 7, 7, 1H), 3.77 (d, J = 9, 1H), 3.43 (s, 1H), 1.74-1.53 (m, 4H), 1.04-0.86 (m, 12H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1, 157.0, 143.6, 143.5, 141.3, 131.5, 127.8, 127.1, 125.0, 124.9, 120.1, 120.0, 118.9, 76.0, 67.3, 66.0, 55.0, 50.9, 47.1, 41.0, 29.9, 24.9, 22.7, 21.7, 19.2, 18.8; IR (film) ν_{max} 3323, 3065, 2958, 2930, 2875, 1736, 1709, 1660, 1533, 1468, 1450, 1387, 1368, 1272, 1246, 1150, 1116, 1053, 935, 758, 741. HR-EI-MS m/z 522.2722 (M, $C_{30}H_{38}O_6N_2$ requires 522.2724).



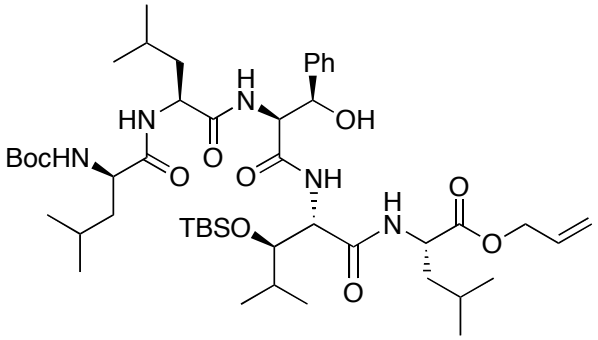
Fmoc-L-HyLeu(OTBS)-L-Leu-OAll. 2,6-lutidine (860 μL, 7.27 mmol) was added to a at 0°C solution of Fmoc-L-HyLeu-Leu-Leu-OAl (1.27 g, 2.42 mmol) in DCM (8.10mL) under argon. TBDMSOTf (970 μL, 4.12 mmol) was added dropwise to the reaction solution at 0°C. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was diluted with EtOAc (50 mL) and washed w/ 5% HCl (2 x 75 mL), water (2 x 75 mL), and brine (1 x 75 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by silica gel column chromatography, with 15% EtOAc/hexanes. After concentration of the pure

fractions, Fmoc-L-HyLeu(OTBS)-Leu-OAll (1.54g, 100%) was obtained as a clear oil. $[\alpha]^{23}_{D}$ -20 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.76 (d, J = 7.5, 2H), 7.59 (m, 2H), 7.40 (t, J = 7.5, 7, 2H), 7.31 (t, J = 7, 7.5, 2H), 7.04 (d, J = 8, 1H), 5.95-5.85 (m, 1H), 5.80 (d, J = 6.5, 1H), 5.32 (d, J = 17, 1H), 5.25 (d, J = 10, 1H), 4.62 (m, 3H), 4.51-4.45 (m, 1H), 4.38-4.33 (m, 1H), 4.29-4.21 (m, 2H), 4.03 (d, J = 6.5, 1H), 1.85 (m, 1H), 1.70-1.50 (m, 3H), 1.00-0.85 (m, 21H), 0.17 (s, 3H), 0.15 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.9, 170.3, 156.0, 143.8, 143.6, 141.3, 131.6, 127.7, 127.0, 125.0, 119.9, 118.7, 76.0, 67.0, 65.8, 57.1, 51.0, 47.0, 41.6, 31.8, 25.9, 25.6, 24.8, 22.6, 21.9, 19.4, 18.9, 18.0, -4.44, -4.71; IR (film) ν_{max} 3323, 2962, 2930, 2860, 1743, 1659, 1523, 1472, 1449, 1388, 1367, 1252, 1189, 1155, 1053,937, 837, 779, 760, 743. HR-EI-MS m/z 636.3605 (M, $C_{36}H_{52}O_6N_2Si$ requires 636.3589).



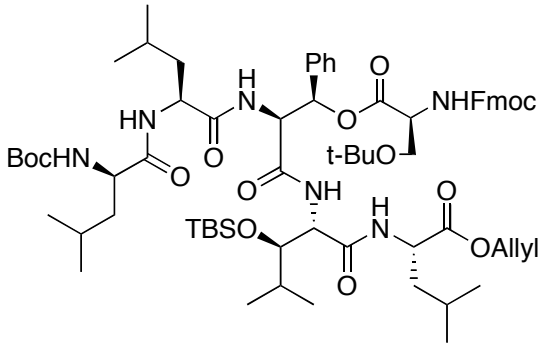
H-L-HyLeu-L-Leu-OAll. Fmoc-L-HyLeu(OTBS)-L-Leu-OAll (2.04 g, 3.20 mmol) was dissolved in a solution of 10% piperidine in DMF (12 mL) at room temperature. The reaction was stirred for 5 minutes then concentrated in vacuo. The crude product was purified by silica gel column chromatography with 10% - 25% EtOAc/hexanes. After concentration of the pure fractions, H-L-HyLeu-L-Leu-OAll (1.17g, 88%) was isolated as a white foam. $[\alpha]^{23}_{D}$ -66 (*c* 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (d, J = 8, 1H), 5.90 (m, 1H), 5.32 (d, J = 17, 1H), 5.23 (d, J = 10, 1H), 4.61 (d, J = 5, 2H), 4.46 (m, 1H), 4.13 (d, J = 7, 1H), 3.30 (s, 1H), 1.82-1.57 (m, 5H), 1.24 (m, 1H), 1.03-0.71 (m,

21H), 0.062 (s, 3H), 0.048 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.3, 172.5, 131.7, 118.3, 77.4, 65.7, 55.7, 51.0, 41.3, 33.1, 33.0, 26.1, 25.0, 22.8, 22.3, 19.5, 19.0, 18.3, -4.27; IR (film) ν_{max} 3195, 3061, 2959, 2857, 1673, 1661, 1462, 1256, 1076, 832. HR-EI-MS m/z 415.2992 (MH^+, $C_{21}H_{42}O_4N_2Si$ requires 415.2987).



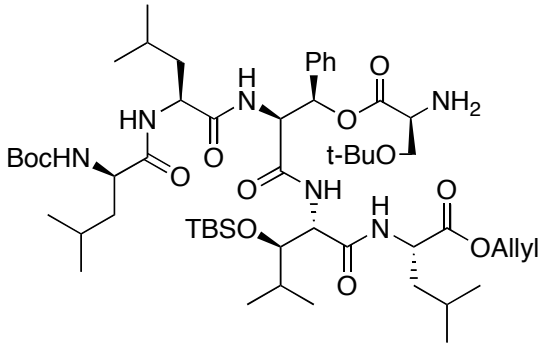
Boc-D-Leu-L-Leu-L-HyPhe-L-HyLeu(OTBS)-L-Leu-OAll (12). DEPBT (1.05 g, 3.42 mmol) was added to a 0°C solution of H-HyLeu(OTBS)-Leu-OAll (710 mg, 1.71 mmol), Boc-D-Leu-L-Leu-L-HyPhe-OH **7** (1.04 g, 2.05 mmol), and DIPEA (600 μL, 3.42 mmol) in THF (5.2 mL). The reaction was stirred for two days at room temperature. The reaction was diluted with EtOAc (30 mL) and washed with 1M HCl (2 x 50 mL), sat. $NaHCO_3$ (2 x 50 mL), and brine (1 x 50 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by silica gel column chromatography with 25% - 40% EtOAc/hexanes. After concentration of the pure fractions, Boc-D-Leu-L-Leu-L-HyPhe-L-HyLeu(OTBS)-L-Leu-OAll **12** (1.42g, 92%) was obtained as a foamy white solid. $[\alpha]^{23}_D$ -30 (*c* 1.00, $CHCl_3$); ^{1}H (DMSO-d_6, 500 MHz) δ 7.99 (d, J = 7.5, 1H), 7.82 (d, J = 8, 1H), 7.75 (d, J = 8, 1H), 7.51 (d, J = 9, 1H), 7.31 (d, J = 7.5, 2H), 7.24-7.16 (m, 3H), 6.77 (d, J = 8.5, 1H), 6.01 (d, J = 4, 1H), 5.94-5.85 (m, 1H), 5.32 (d, J = 17.5, 1H), 5.22 (d, J = 10.5, 1H), 5.03 (t, J = 4, 4, 1H), 4.65 (m,

1H), 4.57 (d, J = 5, 2H), 4.49-4.44 (m, 1H), 4.40-4.33 (m, 1H), 4.30 (q, J = 7.5, 7, 7.5, 1H), 4.00-3.95 (m, 1H), 3.90 (m, 1H), 1.78-1.69 (m, 1H), 1.67-1.47 (m, 5H), 1.40-1.35 (m, 11H), 0.91-0.76 (m, 33H), 0.037 (s, 3H), -0.028 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.6, 172.3, 170.1, 168.9, 155.6, 138.9, 131.6, 128.2, 127.8, 126.3, 118.6, 80.2, 76.0, 72.1, 65.7, 58.4, 55.8, 53.0, 51.3, 51.1, 41.7, 41.2, 40.5; IR (film) ν_{max} 3294, 3079, 2959, 2930, 2868, 1747, 1694, 1641, 1517, 1468, 1386, 1367, 1252, 1177, 1121, 1046, 837, 779, 701. ESI-TOF m/z 904.5799 (MH^+, $C_{47}H_{81}O_{10}N_5Si$ requires 904.5825).



Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OAll (13). EDCI (138 mg, 0.70 mmol) was slowly added to a 0°C solution of Boc-D-Leu-L-Leu-L-HyPhe-L-HyLeu(OTBS)-L-Leu-OAll **12** (212 mg, 0.236 mmol), Fmoc-Ser-(OtBu)-OH (181 mg, 0.471 mmol), and DMAP (9 mg, 0.07 mmol) in DCM (2 mL). The reaction was stirred overnight at room temperature. The reaction was diluted with EtOAc (10 mL). The solution washed with 1 M HCl (2 x 15 mL), sat. $NaHCO_3$ (2 x 15 mL), and brine (1 x 15 mL). The organic layer was separated and dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by silica gel column chromatography with 20% - 35% EtOAc in hexanes. After concentration of the pure fractions, Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-

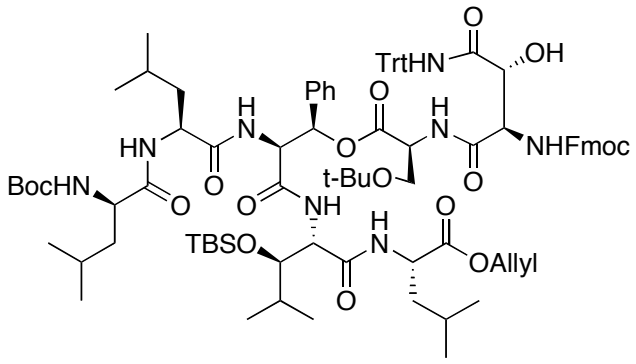
Leu-OAll **13** was isolated as a foamy solid (230 mg, 77%). $[\alpha]^{21}_{D}$ -37 (*c* 1.00, $CHCl_3$); ^1H ($CDCl_3$, 500 MHz) δ 8.26 (d, J = 8.5, 1H), 7.88 (d, J = 7.4, 3H), 7.84 (d, J = 6.8, 1H), 7.73 (d, J = 6.8, 2H), 7.60 (d, J = 9, 1H), 7.53 (d, J = 8, 1H), 7.41 (t, J = 7.4, 7.4, 2H), 7.33-7.29 (m, 4H), 7.23-7.20 (m, 3H), 6.66 (d, J = 8.5, 1H), 6.04 (d J = 5.8, 1H), 5.91-5.82 (m, 1H), 5.30 (d, J = 17, 1H), 5.20 (d, J = 10.5, 1H), 4.94-4.90 (m, 1H), 4.55-4.48 (m, 3H), 4.43-4.39 (m, 2H), 4.29-4.22 (m, 4H), 4.03 (q, J = 8, 7.4, 8, 1H), 3.78-3.74 (m, 2H), 3.56-3.52 (m, 1H), 1.72-1.46 (m, 7H), 1.40-1.29 (m, 12H), 1.05 (s, 9H), 0.91-0.76 (m, 33H), -0.016 (s, 3H), -0.076 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 172.5, 171.8, 169.5, 169.2, 167.4, 156.5, 155.6, 143.9, 143.6, 141.2, 135.8, 131.6, 128.8, 128.5, 127.7, 127.2, 127.1, 127.0, 125.2, 119.9, 118.6, 80.0, 75.5, 75.3, 73.5, 67.5, 65.7, 61.5, 57.3, 55.9, 54.5, 53.3, 51.3, 50.9, 46.9, 41.3, 40.7, 39.7, 31.7, 28.3, 27.1, 25.9, 24.7, 24.6, 23.1, 22.9, 22.7, 21.9, 21.5, 19.4, 18.9, 18.0, -4.38, -4.82; IR (film) ν_{max} 3337, 3072, 2959, 2930, 2871, 1747, 1702, 1670, 1661, 1515, 1472, 1451, 1390, 1364, 1342, 1252, 1194, 1104, 1063, 1044. ESI-TOF m/z 1269.7452 (MH^+, $C_{69}H_{104}O_{14}N_6Si$ requires 1269.7452).



Boc-D-Leu-L-Leu-L-*threo*-*O*-[L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OAll (14).

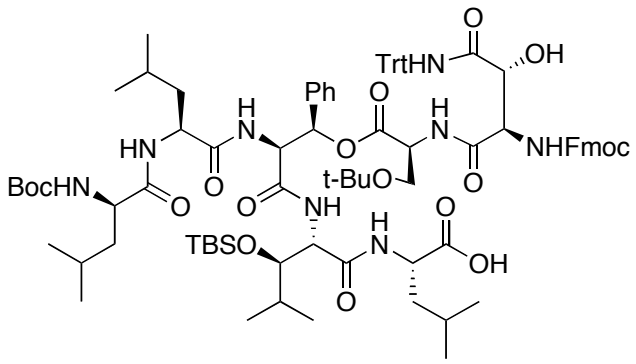
Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OAll

13 (1.00 g, 0.79 mmol) was dissolved in a solution of 10% piperidine in DMF (5 mL) at room temperature. The reaction was stirred for 5 minutes, concentrated in vacuo, and further dried under high vacuum. The product was purified by silica gel column chromatography with EtOAc/hexanes (2:3) and then with 10% MeOH in $CHCl_3$. After concentration of the pure fractions, Boc-D-Leu-L-Leu-L-*threo*-*O*-[L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OAll **14** was isolated (819 mg, 99%) as a clear foam. $[\alpha]^{23}_{D}$ -31 (*c* 1.00, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.24 (d, J = 8.5, 1H), 7.88 (m, 2H), 7.66 (d, J = 9, 1H), 7.33-7.19 (m, 5H), 6.81 (d, J = 8.5, 1H), 6.06 (d, J = 5.5, 1H), 5.93-5.82 (m, 1H), 5.31 (d, J = 17.6, 1H), 5.21 (d, J = 10, 1H), 4.94 (m, 1H), 4.54 (d, J = 4.5, 2H), 4.40 (m, 2H), 4.27-4.22 (m, 1H), 4.03-3.97 (m, 1H), 3.77 (m, 1H), 3.58-3.37 (m, 4H), 1.70-1.28 (m, 19H), 0.98 (s, 9H), 0.93-0.76 (m, 33H), 0.0047 (s, 3H), -0.079 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.2, 173.2, 172.2, 172.1, 171.6, 169.8, 167.6, 155.1, 136.8, 132.3, 127.7, 126.9, 117.9, 78.0, 76.9, 73.8, 72.3, 64.8, 63.7, 55.7, 55.0, 54.4, 53.0, 50.7, 50.5, 41.2, 40.6, 40.0, 31.0, 28.1, 27.2, 26.1, 24.3, 24.1, 23.9, 23.3, 22.9, 22.5, 21.8, 21.7, 21.2, 19.5, 18.0, 17.8, -4.16, -4.60; IR (film) ν_{max} 3321, 2958, 2928, 2873, 1745, 1652, 1651, 1514, 1472, 1387, 1367, 1252, 1071, 1048, 838, 779, 699. ESI-TOF m/z 1047.6765 (MH^+, $C_{54}H_{94}O_{12}N_6Si$ requires 1047.6771).



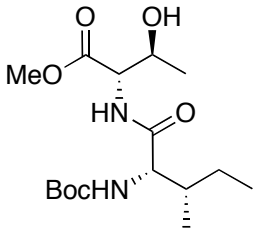
Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OAll (15). DEPBT (26mg, 0.09mmol) was added to a 0^{o}C solution of Boc-D-Leu-L-Leu-L-*threo*-*O*-[L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OAll **14** (45 mg, 0.04 mmol), Fmoc-HyAsn(Trt)-OH (26 mg, 0.04 mmol), and DIPEA (15 μL, 0.09 mmol) in THF (130 μL). The resulting solution was stirred overnight at room temperature. The reaction was diluted with EtOAc (5 mL), washed with 1M HCl (2 x 5 mL), sat. $NaHCO_3$ (2 x 5 mL), and brine (5 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by silica gel column chromatography with 10% - 35% EtOAc/hexanes. After concentration of the pure fractions, Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OAll **15** was isolated as a clear foamy solid (62 mg, 87%). $[\alpha]^{21}_{D}$ -44 (*c* 1.00, $CHCl_3$); ^{1}H (DMSO-d_6, 500 MHz) δ 8.51 (s, 1H), 7.93-7.87 (m, 3H), 7.82 (d, J = 7.4, 2H), 7.79 (d, J = 7.4, 1H), 7.66 (d, J = 9, 1H), 7.62 (d, J = 9.5, 1H), 7.42 (m, 2H), 7.34-7.29 (m, 4H), 7.27-7.13 (m, 20H), 6.76 (d, J = 8.45, 1H), 6.00 (d, J = 6.35, 1H), 5.93-5.85 (m, 1H), 5.78 (d, J = 6.85, 1H), 5.32 (d, J = 16.9, 1H), 5.22 (d, J = 10.5, 1H), 4.97 (t, J = 7.35, 7.4, 1H), 4.88-4,84 (m, 1H), 4.68 (d, J = 11, 1H), 4.57-4.53 (m, 3H), 4.44-4.36 (m, 3H), 4.29-4.19 (m, 3H), 4.05 (q, J = 7.4, 8, 7.4, 1H), 3.90 (d, J = 6.35, 1H), 3.77 (m, 1H), 3.54 (d, J = 8.45, 1H), 1.71-1.67 (m, 1H), 1.63-1.47 (m, 4H), 1.43-1.35 (m, 12H), 1.03 (s, 9H), 0.96-0.78 (m, 33H), 0.014 (s, 3H), -0.065 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 172.5, 172.1, 171.9, 169.4, 169.3, 167.7, 156.3, 156.2, 144.3, 143.8, 143.7, 141.2, 135.4, 131.6, 129.0, 128.6, 127.9, 127.6, 127.2, 127.1, 127.0, 125.2, 119.9, 118.6, 80.4, 77.2, 75.5, 73.6, 72.3, 70.2, 67.6, 65.8, 60.8, 57.3, 55.6, 53.2, 52.9, 51.3, 50.9, 47.1, 41.2, 40.7, 39.7, 31.8, 28.4, 28.1, 26.9, 25.9, 24.7, 23.1, 22.7, 22.3, 21.9; IR

(film) ν_{max} 3330, 3064, 3021, 2959, 2930, 2871, 1742, 1674, 1514, 1472, 1449, 1393, 1367, 1251, 1216, 1192, 1166, 1106, 1047. ESI-TOF m/z 1641.8930 (MH^+, $C_{92}H_{124}O_{17}N_8Si$ requires 1641.8926).



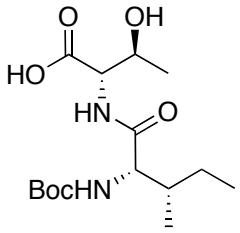
Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OH (2). $Cl_2Pd(PPh_3)_2$ (10mg, 0.014mmol) was dissolved in THF (1.71mL). PPh_3 (11mg, 0.42mmol) was added and the resulting solution was stirred for 30 minutes. Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OAll **15** (925 mg, 0.56 mmol) and phenylsilane (139 μL, 1.13 mmol) were added to the solution and the resulting mixture was stirred for 3 hours at room temperature under an argon atmosphere. The reaction was diluted with EtOAc (10 mL) and washed with sat. NH_4Cl (3 x 15 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. The product was purified by silica gel column chromatography with 0% - 3% MeOH/$CHCl_3$. After concentration of the pure fractions, Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OH **2** was isolated as a clear foamy solid **2** (890 mg, 99%). $[\alpha]^{23}_D$ -18 (*c* 1.00, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.50 (s, 1H), 8.38 (d, J = 8, 1H), 7.89 (m, 2H), 7.82-7.52 (m, 5H), 7.43-7.36 (m, 5H), 7.33-7.11 (m, 25H), 6.76 (d, J = 8.5, 1H),

5.99 (d, J = 6, 1H), 4.94 (t, J = 7, 7, 1H), 4.82 (m, 1H), 4.65 (d, J = 9.6, 1H), 4.52 (s, 1H), 4.46-4.34 (m, 3H), 4.21-4.12 (m, 3H), 4.06-3.99 (m, 1H), 3.88 (m, 1H), 3.78 (m, 1H), 3.52 (m, 1H), 1.73-1.32 (m, 18H), 1.01 (s, 9H), 0.95 (s, 1H), 0.91-0.76 (m, 33H), 0.001 (s, 3H), -0.059 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.6, 172.3, 172.1, 170.0, 169.2, 169.0, 167.4, 156.1, 155.1, 144.5, 143.7, 140.7, 140.6, 136.5, 128.4, 127.6, 127.1, 126.6, 125.6, 125.4, 120.0, 77.9, 76.8, 74.7, 72.7, 72.1, 69.2, 66.3, 61.6, 57.8, 55.9, 55.0, 52.9, 52.7, 50.6, 46.6, 41.4, 40.7, 40.6, 31.1, 28.1, 27.1, 26.0, 24.3, 24.2, 24.0, 23.3, 22.9, 22.6, 22.0, 21.7, 21.3, 19.3, 18.2, 18.0, -4.24, -4.67; IR (film) ν_{max} 3395, 3317, 3065, 2958, 2928, 2871, 1671, 1512, 1471, 1449, 1393, 1366, 1251, 1216, 1170, 1106, 1052, 1027, 1008, 837, 758, 699. ESI-TOF m/z 1601.8642 (MH^+, $C_{89}H_{120}O_{17}N_8Si$ requires 1601.8613).



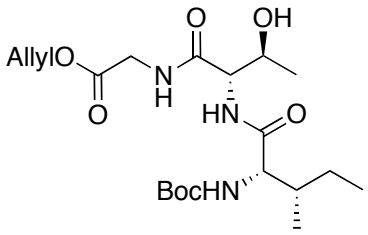
Boc-L-Ile-L-*a*Thr-OMe (17). Thionyl chloride (62 µL, 0.84 mmol) was slowly added to MeOH (420 µL). A solution of L-*allo*-Thr (100 mg, 0.84 mmol) in MeOH (420 µL) was added to the solution and the resulting mixture refluxed for 1.5 hours. After concentration in vacuo, this procedure was repeated and the mixture heated at reflux for an additional 2 h. The reaction mixture was concentrated in vacuo and evaporated from a mixture of DCM and hexanes (2:1) (2 x 6 mL) to remove any access of thionyl chloride. The residue (HCl • H_2N-*allo*-Thr-OMe) was taken to the next reaction without further purification.

HCl • H_2N-*allo*-Thr-OMe was dissolved in THF (4.2mL). This solution was cooled to 0°C, and then DEPBT (511mg 1.67mmol), DIPEA (300µL, 1.67mmol), and Boc-Ile-OH • ½ H_2O (264mg, 1.09mmol) were added. The reaction was stirred overnight at room temperature. The reaction was diluted with EtOAc (15 mL), washed with 1N HCl (2 x 20mL), sat. $NaHCO_3$ (2 x 20 mL), and brine (1 x 20 mL). The organic layer was separated, dried over sodium sulfate, and concentrated in vacuo. The product was purified by silica gel column chromatography with 0.5% - 2% MeOH/ $CHCl_3$. After concentration of the pure fractions, Boc-L-Ile-L-*a*Thr-OMe **17** (267mg, 92% over two steps) was isolated as a clear solid. $[\alpha]^{23}_D$ +12 (*c* 1.00, $CHCl_3$); ^1H ($CDCl_3$, 400 MHz) δ 7.14 (m, 1H), 5.21 (m, 1H), 4.65 (dd, J = 3.5, 4.5, 3.5, 1H), 4.17-4.11 (m, 1H), 3.98 (t, J = 8, 8, 1H), 3.76 (s, 3H), 1.83 (m, 1H), 1.57-1.48 (m, 2H), 1.41 (s, 9H), 1.19 (d, J = 6.5, 3H), 1.15-1.06 (m, 1H), 0.97-0.85 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5, 170.4, 156.0, 80.1, 68.6, 59.3, 58.1, 52.5, 36.9, 28.2, 24.8, 18.8, 15.4, 11.2; IR (film) ν_{max} 33316, 1083, 2970, 2933, 2879, 1747, 1683, 1658, 1530, 1455, 1437, 1392, 1367, 1316, 1294, 1248, 1172, 1046, 1022, 867, 761. HR-EI-MS m/z 347.2174 (MH^+, $C_{16}H_{30}O_6N_2$ requires 347.2177).



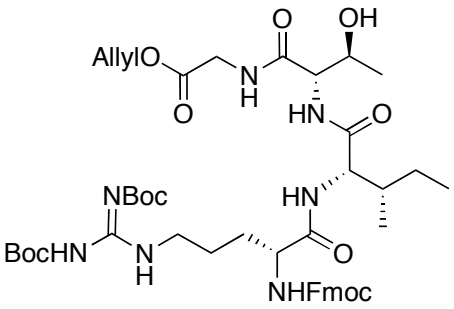
Boc-L-Ile-L-*a*Thr-OH (18). A solution of LiOH (38 mg, 0.90 mmol) in H_2O (1.21 mL) was added dropwise to a solution of Boc-L-Ile-L-*a*Thr-OMe **17** (260 mg, 0.75 mmol) in THF (1.21 mL). The reaction was stirred at room temperature for 1 h. The solution was

diluted with EtOAc (5 mL) and 1M HCl (10mL). The mixture was extracted with EtOAc (3 x 5 mL). The organic layers where combined, dried over Na_2SO_4, filtered and concentrated in vacuo to give Boc-L-Ile-L-*a*Thr-OH **18** (249mg, 100%) as a foamy white solid. $[\alpha]^{23}_D$ -12 (*c* 1.00, CH_3CH_2OH) ^{1}H NMR (CD_3OD, 400 MHz) δ 4.45 (d, J = 5.5, 1H), 4.07 (quint, J = 6,6,6,6, 1H), 3.96 (d, J = 7.5, 1H), 1.85-1.76 (m, 1H), 1.60-1.40 (m, 10H), 1.25 (d, J = 6.5, 3H), 1.18-1.12 (m, 1H), 1.00-0.87 (m, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.6, 173.0, 158.0, 80.6, 69.0, 60.6, 59.4, 38.2, 28.7, 25.8, 19.6, 15.9, 11.5; IR (film) ν_{max} 3323, 3091, 2973, 2933, 2879, 2615, 1698, 1666, 1537, 1454, 1393, 1368, 1294, 1217, 1165, 1092, 1048, 1022, 945, 865, 755. HR-EI-MS m/z 333.2023 (MH^+, $C_{15}H_{28}O_6N_2$ requires 333.2020).



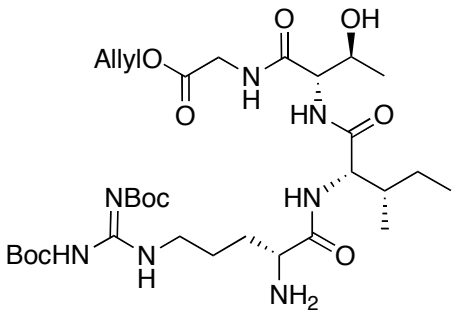
Boc-L-Ile-L-*a*Thr-Gly-OAll (19). Boc-Gly-OAll (1.30 g, 6.04 mmol) was dissolved in a solution of 4M HCl in dioxane (15 mL) and was stirred for 30 min at room temperature. The reaction solution was concentrated in vacuo and the residue was dissolved in THF (20 mL). This solution was cooled to 0^{o}C, and then DEPBT (1.84g, 6.04 mmol), DIPEA (1.06 mL, 6.04 mmol), and Boc-L-Ile-L-*a*Thr-OH **18** (1.01 g, 3.02 mmol) were added. The reaction was stirred for 3 days at room temperature. The reaction was diluted with EtOAc (25 mL), washed with 1N HCl (2 x 50 mL), sat. $NaHCO_3$ (2 x 50 mL), and brine (1 x 50 mL). The organic layer was separated, dried over sodium sulfate, filtered, and concentrated in vacuo. The product was purified by silica gel column chromatography

with 35% - 60% EtOAc/hexanes. After concentration of the purified fractions, Boc-L-Ile-L-*a*Thr-Gly-OAll **19** (1.24g, 95% over the two steps) was isolated as a clear foam. $[\alpha]^{23}_{D}$ -34 (*c* 1.00, $CHCl_3$); ^1H ($CDCl_3$, 400 MHz) δ 7.36 (t, J = 5.5, 5.5, 1H), 7.17 (d, J = 8, 1H), 5.94-5.84 (m, 1H), 5.35-5.29 (m, 1H), 5.27-5.24 (, 1H), 5.17 (d, J = 7.5, 1H), 4.62 (d, J = 5.5, 2H), 4.47-4.44 (m, 1H), 4.06-3.97 (m, 4H), 1.93 (s, 1H), 1.90-1.81 (m, 1H), 1.55-1.39 (m, 10H), 1.27 (d, J = 6, 3H), 1.21-1.06 (m, 1H), 0.96-0.87 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.3, 170.7, 169.5, 156.1, 131.3, 119.1, 80.3, 68.9, 66.1, 59.6, 57.6, 41.2, 36.9, 28.2, 24.8, 19.5, 15.6, 11.4; IR (film) ν_{max} 33323, 3294, 3090, 2970, 2933, 2879, 1750, 1692, 1642, 1557, 1526, 1393, 1367, 1346, 1316, 1290, 1242, 1205, 1176, 1048, 1020, 987, 758. HR-EI-MS m/z 430.2544 (MH^+, $C_{20}H_{35}O_7N_3$ requires 430.2548).



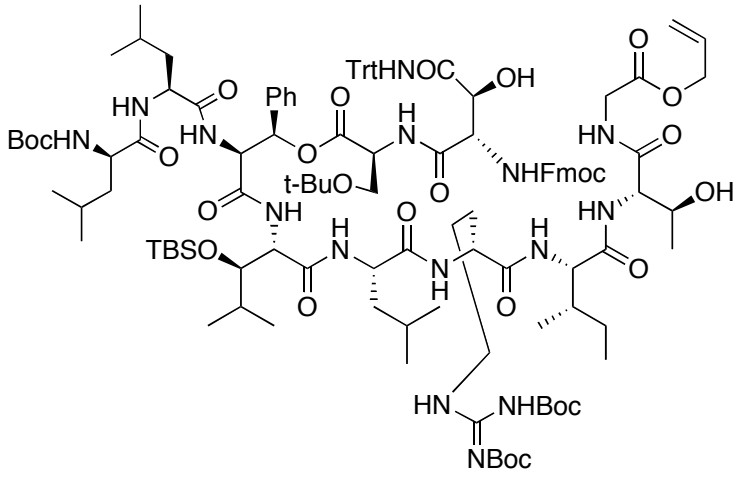
Fmoc-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OAll (20). Boc-L-Ile-L-*a*Thr-Gly-OAll **19** (700 mg, 1.63 mmol) was dissolved in a solution of 4M HCl in dioxane (4.07 mL) and was stirred for 30 min at room temperature. The reaction was concentrated in vacuo and the residue was dissolved in THF (8.15 mL). This solution was cooled to 0^{o}C, and then DEPBT (995 mg, 3.26 mmol), DIPEA (574 µL, 3.26 mmol), and Fmoc-D-Arg(Boc_2)-OH (1.08 g, 1.79 mmol) were added. The reaction was stirred overnight at room temperature. The reaction was diluted with EtOAc (25 mL), washed with 1N HCl (2 x 50 mL), sat.

$NaHCO_3$ (2 x 50 mL), and brine (50 mL). The organic layer was separated, dried over sodium sulfate, and concentrated in vacuo. The crude product was purified by silica gel column chromatography with 35% - 60% EtOAc/hexanes. After concentration of the pure fractions, Fmoc-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OAll **20** (1.42g, 96% over the two steps) was isolated as a clear yellow foam. $[\alpha]^{23}_D$ -16 (*c* 1.00, MeOH/$CHCl_3$, 1:1); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.5 (s, 1H), 8.32 (m, 1H), 8.21 (m, 1H), 8.03 (d, J = 8.5, 1H), 7.88 (d, J = 7.5, 3H), 7.72 (t, J = 7.5, 7.5, 2H), 7.58-7.53 (m, 1H), 7.41 (t, J = 7.5, 7.5, 2H), 7.32 (t, J = 7.5, 7.5, 2H), 5.92-5.81 (m, 1H), 5.29 (d, J = 17, 1H), 5.19 (d, J = 10, 1H), 4.77 (d, J = 5, 1H), 4.53 (d, J = 5, 2H), 4.37-4.12 (m, 6H), 3.90-3.83 (m, 3H), 3.32-3.26 (m, 2H), 1.79-1.44 (m, 15H), 1.38 (s, 9H), 1.05 (d, J = 6, 3H), 0.82-0.73 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1, 171.5, 171.1, 169.5, 163.3, 156.6, 156.2, 153.2, 143.8, 143.5, 141.2, 131.3, 127.7, 127.1, 125.1, 125.0, 119.9, 118.9, 83.2, 79.4, 76.4, 68.6, 67.2, 66.0, 58.0, 57.2, 54.5, 47.0, 41.2, 40.1, 36.9, 31.2, 29.7, 28.2, 28.0, 25.3, 24.9, 19.5, 15.5, 11.2; IR (film) ν_{max} 3284, 3081, 2971, 2932, 1721, 1695, 1639, 1550, 1453, 1415, 1368, 1330, 1254, 1228, 1157, 1135, 1052, 1025, 987, 932, 808, 758. ESI-TOF m/z 908.4752 (MH^+, $C_{46}H_{65}O_{12}N_7$ requires 908.4764).



H-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OAll (3). Fmoc-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OAll **20** (550 mg, 0.606 mmol) was dissolved in a solution of 10% piperidine in DMF

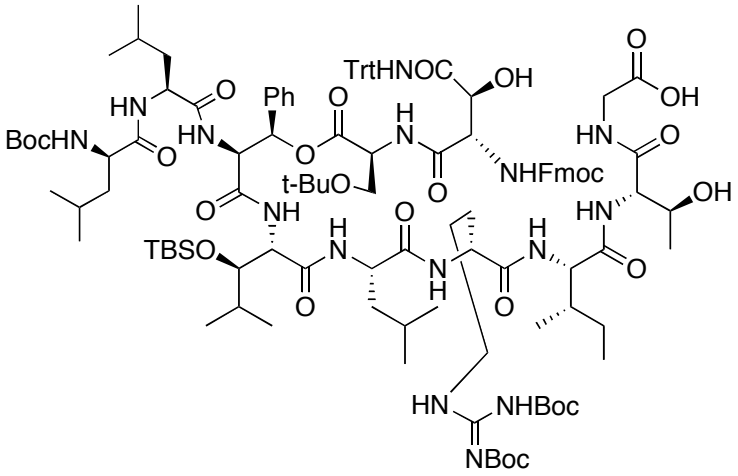
(3.5 mL) at room temperature. The resulting mixture was stirred for 10 minutes. The reaction solution was concentrated in vacuo. The crude product was purified by silica gel column chromatography with 0% - 8% MeOH/$CHCl_3$. After concentration of the pure fractions, H-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OAll **3** (407 mg, 98%) was isolated as a clear foam. $[\alpha]^{23}_D$ -32 (*c* 1.00, CH_3CH_2OH); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.32-8.26 (m, 2H), 8.00 (m, 1H), 7.92 (d J = 8.5, 1H), 5.93-5.83 (m, 1H), 5.31 (d, J = 17, 1H), 5.20 (d, J = 10.5, 1H), 4.81 (m, 1H), 4.56 (d, J = 5.5, 2H), 4.31-4.24 (m, 2H), 3.89-3.82 (m, 3H), 3.39-3.21 (m, 4H), 1.79-1.69 (m, 1H), 1.60-1.29 (m, 23H), 1.10 (s, 2H), 1.08-1.02 (m, 3H), 0.85-0.76 (m, 6H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 1724.8, 171.0, 170.4, 169.4, 163.2, 155.2, 152.1, 132.4, 117.9, 82.9, 78.1, 66.8, 64.8, 58.0, 56.4, 53.9, 40.7, 40.0, 36.8, 32.3, 31.3, 28.3, 28.0, 27.6, 25.0, 24.2, 19.4, 15.4, 10.9; IR (film) ν_{max} 3277, 3081, 2974, 2934, 2875, 1753, 1722, 1676, 1639, 1561, 1415, 1368, 1334, 1134, 1054, 1027, 809, 757. ESI-TOF m/z 686.4079 (MH^+, $C_{31}H_{55}O_{10}N_7$ requires 686.4083).



Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OAll (21). DEPBT (64 mg,

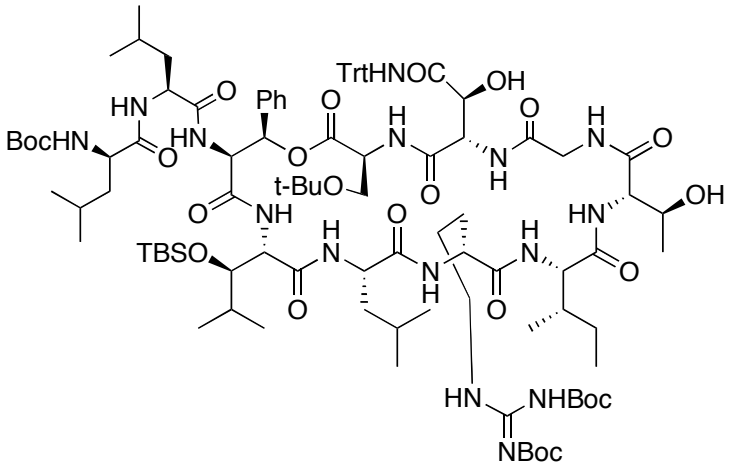
0.208 mmol) was added to a 0°C solution of Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-OH **2** (167 mg, 0.104 mmol), H-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OAll **3** (79 mg, 0.115 mmol), and DIPEA (36 μL, 0.208 mmol) in DMF (316 μL). The reaction was stirred for 3 days at room temperature. The reaction was diluted with ethyl acetate (5 mL) and washed with 1M aq. HCl (10 mL x 2), a saturated solution of aq. $NaHCO_3$ (10 mL x 2), and brine (10 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with 0%-10% methanol in chloroform. After concentration of the pure fractions, Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OAll **21** (223 mg, 94%) was isolated as a clear solid. $[\alpha]^{23}_D$ -32 (*c* 1.00, $CHCl_3$); ^{1}H (DMSO-d_6, 500 MHz) δ 8.51 (s, 1H), 8.46 (m, 1H), 8.30 - 8.23 (m, 2H), 8.06 (s, 1H), 7.98 (d, J = 6.35, 1H), 7.91 (d, J = 7.4, 4H), 7.82 (d, J = 7.4, 1H), 7.79 (d, J = 9.4, 1H), 7.73 (m, 1H), 7.65 (d, J = 7.9, 1H), 7.52 (m, 1H), 7.49 - 7.38 (m, 3H), 7.37 - 7.29 (m, 3H), 7.27 - 7.13 (m, 20H), 6.76 (d, J = 7, 1H), 6.02 (m, 1H), 5.94-5.86 (m, 1H), 5.78 (s, 1H), 5.32 (d, J = 17, 1H), 5.22 (d, J = 10, 1H), 4.95 (m, 1H), 4.85 (m, 1H), 4.80 (m, 1H), 4.68 (d, J = 9, 1H), 4.60-4.51 (m, 3H), 4.47-4.25 (m, 6H), 4.20 (s, 2H), 4.05 (d, J = 7.4, 1H), 3.89 (s, 4H), 3.79 (s, 1H), 3.53 (m, 1H), 3.28 (s, 2H), 1.82 - 1.28 (m, 46H), 1.25 - 0.91 (m, 12H), 0.90 - 0.70 (m, 36H), 0.005 (s, 3H), -0.053 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 173.8, 173.0, 172.8, 172.5, 171.6, 171.1, 171.0, 170.3, 170.0, 169.6, 168.7, 163.2, 156.4, 156.2, 156.1, 156.0, 155.9, 155.8, 152.9, 144.3, 144.1, 143.4, 141.0, 134.4, 131.2, 129.1, 128.4, 127.8, 127.5, 127.0, 125.0, 120.8, 119.8, 118.7, 82.9, 80.2, 79.2, 76.5, 75.8, 75.0, 74.9, 73.6, 72.3, 70.2, 68.5, 67.9, 67.6, 66.0, 61.1, 59.2, 57.2, 56.6, 56.3,

55.7, 53.9, 53.1, 52.9, 51.2, 47.1, 41.2, 40.5, 40.1, 39.9, 39.3, 35.7, 35.5, 32.7, 29.8, 28.5, 28.4, 28.1, 26.1, 25.8, 25.1, 24.9, 24.6, 23.4, 23.1, 22.8, 22.3, 22.0, 21.4, 19.7, 18.8, 18.2, 15.7, 11.3, -4.22, -4.36. IR (film) ν_{max} 3289, 3068, 2961, 2926, 2874, 2854, 2357, 1717, 1639, 1560, 1540, 1522, 1508, 1474, 1458, 1417, 1392, 1367, 1332, 1250, 1229, 1162, 1134, 1050, 1025 ESI-TOF m/z 2269.2497 (MH^+, $C_{120}H_{173}O_{26}N_{15}Si$ requires 2269.2518).



Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OH (22). Triphenylphosphine (1.5 mg, 6 μmol) was added to the solution of $Cl_2Pd(PPh_3)_2$ (1.3 mg, 1.9 μmol) in DMF(237μL), and stirred for 30 minutes. Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OAll **21** (174 mg, 77 μmol) and phenylsilane (19 μL, 153 μmol) were added to the solution and the resulting mixture was stirred for 3 h under argon, at room temperature. The reaction was diluted with ethyl acetate and washed with a saturated solution of aq. NH_4Cl. The organic layer was separated, dried with Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting from 2% -

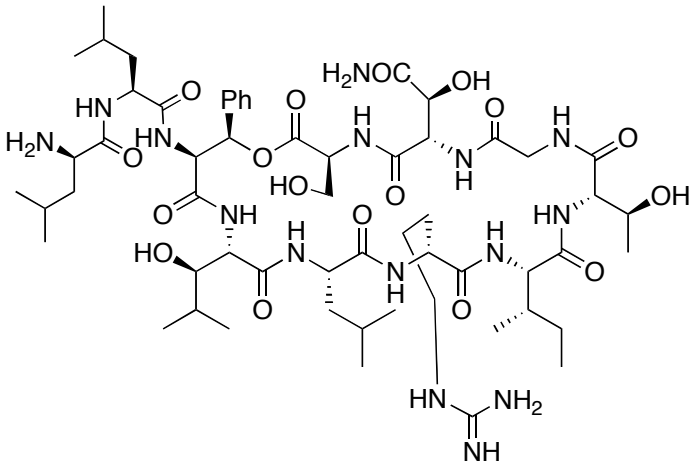
20% methanol in chloroform. Concentration of the pure fractions provided Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OH **22** (158 mg, 93%) as a clear solid. $[\alpha]^{23}_{D}$ -34 (*c* 1.00, $CHCl_3$); ^{1}H (DMSO-d_6, 500 MHz) δ 8.51 (s, 1H), 8.45 (d, J = 5.3, 1H), 8.29 (m, 1H), 8.11 (m, 1H), 7.99-7.86 (m, 4H), 7.84-7.77 (m, 2H), 7.73 (m, 1H) 7.67 (d, J = 9, 1H), 7.54 (m, 1H), 7.45 - 7.11 (m, 28H), 6.77 (d, J = 8, 1H), 6.01 (d, J = 5.8, 1H), 5.88 (m, 1H), 4.94 (m, 1H), 4.84 (m, 1H), 4.67 (d, J = 9.5, 1H), 4.54 (s, 1H), 4.46 - 4.26 (m, 6H), 4.23 - 4.12 (m, 3H), 4.07 - 3.99 (m, 1H), 3.89 (d, J = 5.6, 1H), 3.79 - 3.76 (m, 2H), 1.80 - 1.26 (m, 46H), 1.23 (s, 2H), 1.11 - 0.91 (m, 12H), 0.90 - 0.66 (m, 36H), -0.004 (s, 3H), -0.058 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 172.4, 172.1, 171.9, 171.2, 171.1, 170.2, 170.1, 169.3, 169.0, 167.7, 163.2, 156.2, 155.2, 155.1, 152.1, 144.5, 143.7, 140.7, 136.6, 128.4, 127.8, 127.7, 127.3, 127.2, 127.1, 126.7, 125.6, 125.4, 120.1, 82.9, 79.2, 78.1, 78.0, 76.7, 74.7, 72.7, 72.1, 69.2, 67.4, 66.3, 58.7, 57.8, 56.5, 56.1, 55.1, 52.9, 51.8, 50.6, 46.6, 42.8, 41.5, 36.5, 31.3, 31.2, 30.0, 28.1, 28.0, 27.6, 27.1, 26.1, 25.0, 24.4, 24.2, 24.0, 23.3, 22.9, 22.7, 22.3, 21.8, 19.9, 19.3, 18.4, 17.9, 10.8, -4.25, -4.70. IR (film) ν_{max} 3295, 3065, 2961, 2926, 2872, 2359, 2339, 1717, 1646, 1575, 1561, 1544, 1522, 1506 ESI-TOF m/z 2229.2192 (MH^+, $C_{117}H_{169}O_{23}N_{15}Si$ requires 2229.2205).



Cyclo-Boc-D-Leu-L-Leu-L-[*O*-[L-HyLeu-L-Leu--D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe] (23). Boc-D-Leu-L-Leu-L-*threo*-*O*-[Fmoc-L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe-L-Leu(OTBS)-L-Leu-D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly-OH **22** (98 mg, 44 μmol) was dissolved in a solution of 10% piperidine in DMF (150 μL) at room temperature. The reaction was stirred for 5 minutes, and then concentrated in vacuo and further dried under high vacuum. The residue was titrated with hexanes, centrifuged to remove the solvents, and then dried in vacuum to afford a white solid that was used directly in the next reaction.

The white solid (88 mg, 44 μmol), DEPBT (80 mg, 0.263 mmol), and DIPEA (31 μL, 0.175 mmol) were dissolved in DMF (44 mL), at 0^{o}C. The reaction was stirred for 3 days at room temperature. The reaction solution was diluted with ethyl acetate (5 mL) and washed with 1M aq. HCl (10 mL x 2), a saturated solution of aq. $NaHCO_3$ (10 mL x 2), and brine (10 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel, eluting with 0%-10% methanol in chloroform. Concentration of the purified fractions provided cyclo-Boc-D-Leu-L-Leu-L-[*O*-[L-HyLeu-L-Leu--D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe] **23** (73mg, 83% in two steps) as a clear solid.

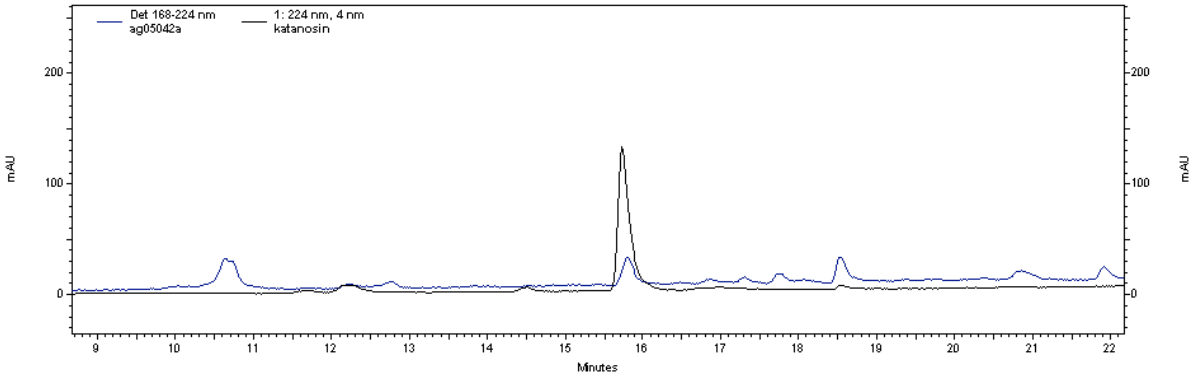
$[\alpha]^{23}_{D}$ -25 (*c* 1.00, $CHCl_3$); ^1H (DMSO-d_6, 500 MHz, temp. 75ºC) δ 8.29 (s, 1H), 8.20 (m, 2H), 8.13 (d, *J* = 7.4, 1H), 7.92-7.83 (m, 2H), 7.76 (m, 1H), 7.65 (d, J = 8, 1H), 7.59 (d, J = 8.45, 1H), 7.54 (d, J = 8, 1H), 7.48 (d, J = 7, 1H), 7.35-7.14 (m, 20H), 6.11 (d, J= 3.7, 2H), 4.92-4.89 (m, 2H), 4.75-4.72 (m, 1H), 4.64 (d, J = 5.3, 1H), 4.58-4.51 (m, 2H), 4.46-4.21 (m, 5H), 4.06-3.74 (m, 5H), 3.64-3.56 (m, 1H), 3.35-3.28 (m, 2H), 1.93 (m, 1H), 1.80 (m, 2H), 1.67-1.35 (m, 43H), 1.27 (s, 2H), 1.17-1.10 (m, 12H), 1.04-0.97 (m, 12H), 0.92-0.80 (m, 24H), 0.073 (s, 3H), 0.045 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 172.2, 171.9, 171.7, 170.9, 170.3, 169.9, 169.6, 169.2, 168.8, 168.7, 168.6, 163.2, 155.2, 152.1, 144.5, 128.3, 127.8, 127.1, 126.9, 83.0, 79.2, 78.1, 76.2, 72.7, 69.2, 61.8, 58.6, 55.6, 53.0, 41.6, 28.1, 28.0, 27.6, 27.0, 26.1, 26.0, 24.4, 23.9, 23.4, 23.2, 23.0, 21.9, 17.9, 15.5, 11.3, -3.89, -4.67; IR (film) ν_{max} 3314, 3063, 2958, 2932, 2871, 1717, 1671, 1647, 1558, 1540, 1522, 1506, 1497, 1472, 1457, 1418, 1390, 1368, 1333, 1255, 1229, 1157, 1134; ESI-TOF m/z 1989.1400 (MH^+, $C_{102}H_{157}O_{23}N_{15}Si$ requires 1989.1418).



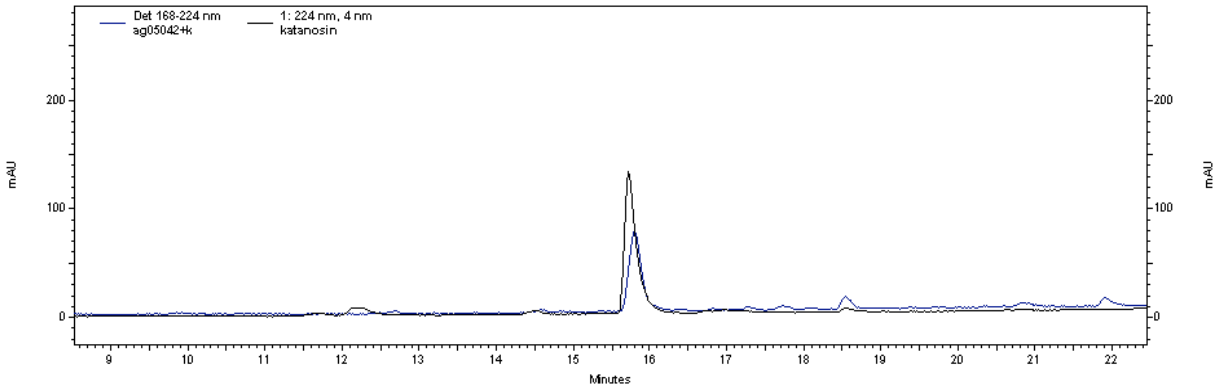
Lysobactin (1). Cyclo-Boc-D-Leu-L-Leu-L-[*O*-[L-HyLeu-L-Leu--D-Arg(Boc)$_2$-L-Ile-L-*a*Thr-Gly L-HyAsn(Trt)-L-Ser(OtBu)]-HyPhe] **23** (24.5 mg, 0.0123 μmol) was dissolved with a solution of TFA/H_2O (95:5, 25.8 mL). The reaction was stirred for 5 hours at room temperature and then concentrated in vacuo. The residue was purified by reverse-phase HPLC with 10-90% H_2O in acetonitrile. Lyophilization of the pure fractions provided lysobactin (5 mg, 33% after TFA salt neutralization) was a white solid. $[\alpha]^{21}_D$ -63 [*c* 0.82, CH_3CN/H_2O (1:1)]; ^{1}H (DMSO-d_6, 500 MHz) δ 9.70 (s, 1H), 9.24 (s, 2H), 9.14 (s, 1H), 8.97 (s, 1H), 8.23 (s, 3H), 7.64-7.49 (m, 2H), 7.44-7.13 (m, 6H), 7.10-6.92 (m, 4H), 6.79-6.55 (m, 6H), 5.78-5.55 (m, 4H), 5.36 (s, 1H), 5.21 (s, 1H), 4.79 (d, J = 10, 1H), 4.55-4.49 (m, 1H), 4.36 (m, 1H), 4.16 (m, 2H), 4.09-3.95 (m, 2H), 3.79-3.54 (m, 8H), 2.84-2.75 (m, 2H), 2.02-1.75 (m, 4H), 1.63-1.31 (m, 14H), 1.02-0.57 (m, 27H); ^{13}C [D_3C-CN/D_2O/TFA (500:500:1) 100 MHz] δ 177.2, 176.2, 175.2, 174.9, 174.8, 174.7, 173.7, 173.4, 172.9, 172.5, 171.4, 170.8, 169.5, 157.2, 135.6, 130.7, 129.8, 128.5, 76.2, 75.7, 71.5, 70.9, 62.3, 61.5, 60.8, 60.3, 58.4, 56.9, 56.6, 56.3, 55.8, 53.3, 52.8, 44.0, 41.8, 41.2, 39.9, 36.8, 31.4, 28.7, 26.8, 26.4, 25.2, 25.0, 24.1, 23.9, 22.4, 22.2, 20.8, 20.6, 20.5, 19.6, 19.5 15.9, 11.1; IR (KBr) ν_{max} 3352, 2959, 2925, 2872, 1702, 1686, 1665, 1654, 1647, 1577, 1570, 1560, 1541, 1534, 1527, 1475, 1467, 1458, 1436, 1419, 1290, 1203, 1181, 1120; ESI-TOF m/z 1276.7235 (MH^+, $C_{58}H_{97}O_{17}N_{15}$ requires 1276.7259).

HPLC Comparison of Synthetic and Natural Lysobactin

HPLC Chromatogram Comparison
Synthetic lysobactin vs. natural lysobactin (injected separately)

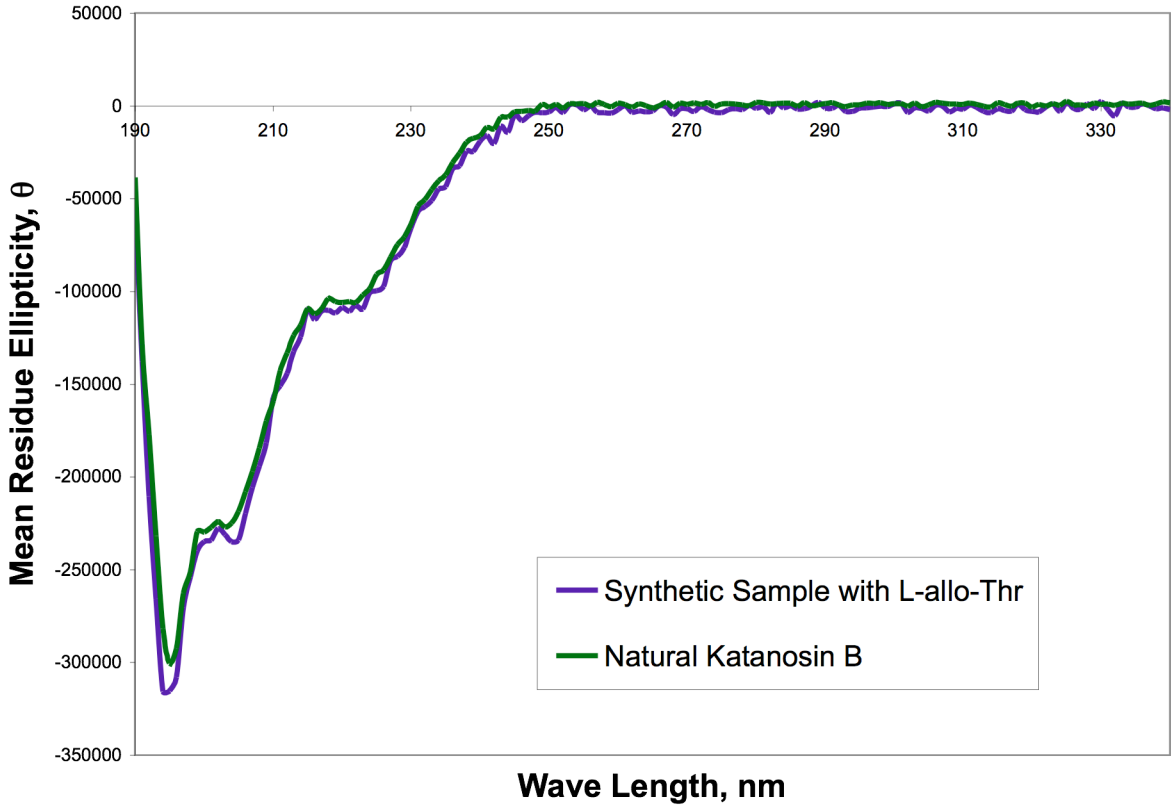


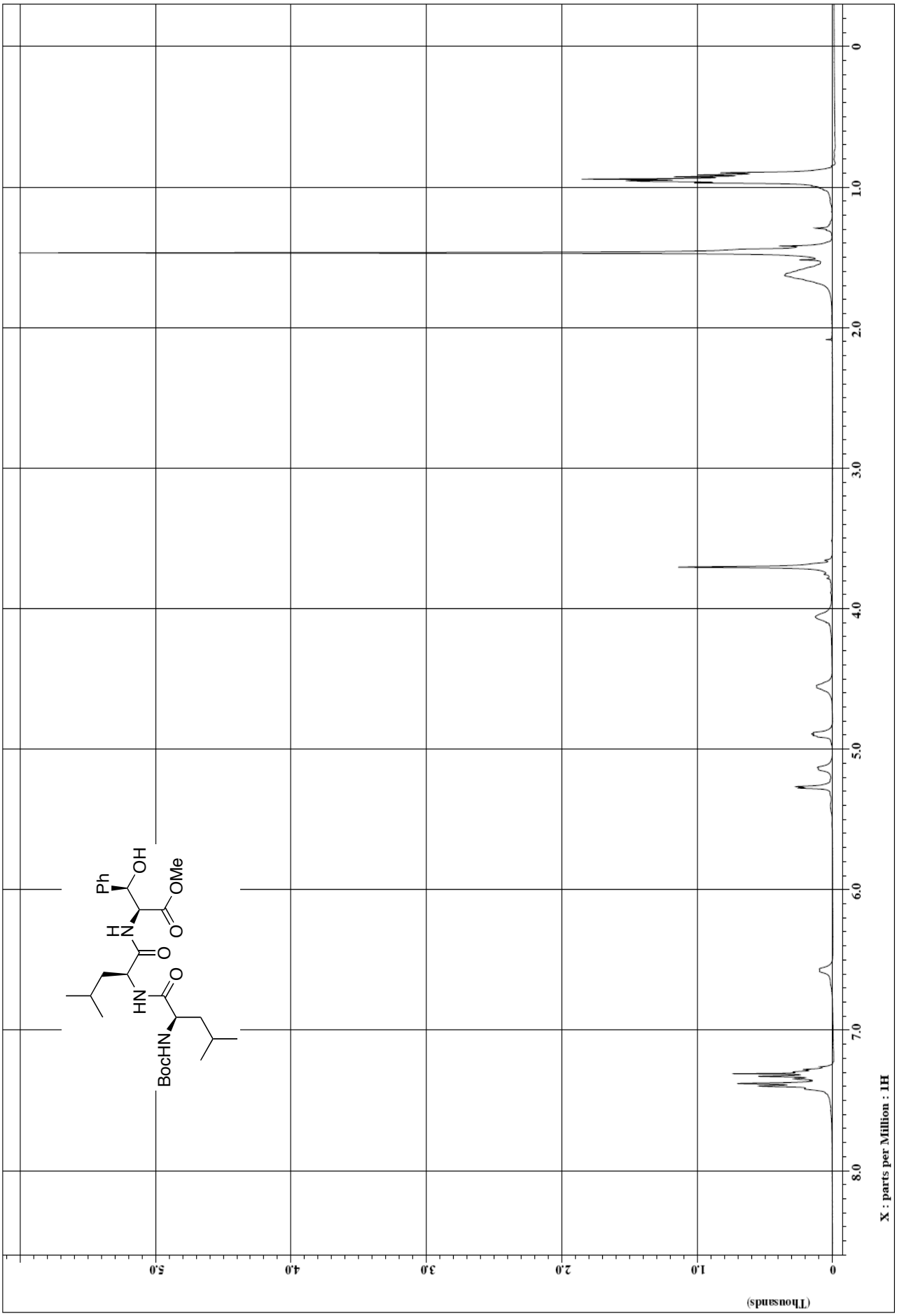
HPLC Chromatogram Comparison
Co-injection of synthetic and natural lysobactin

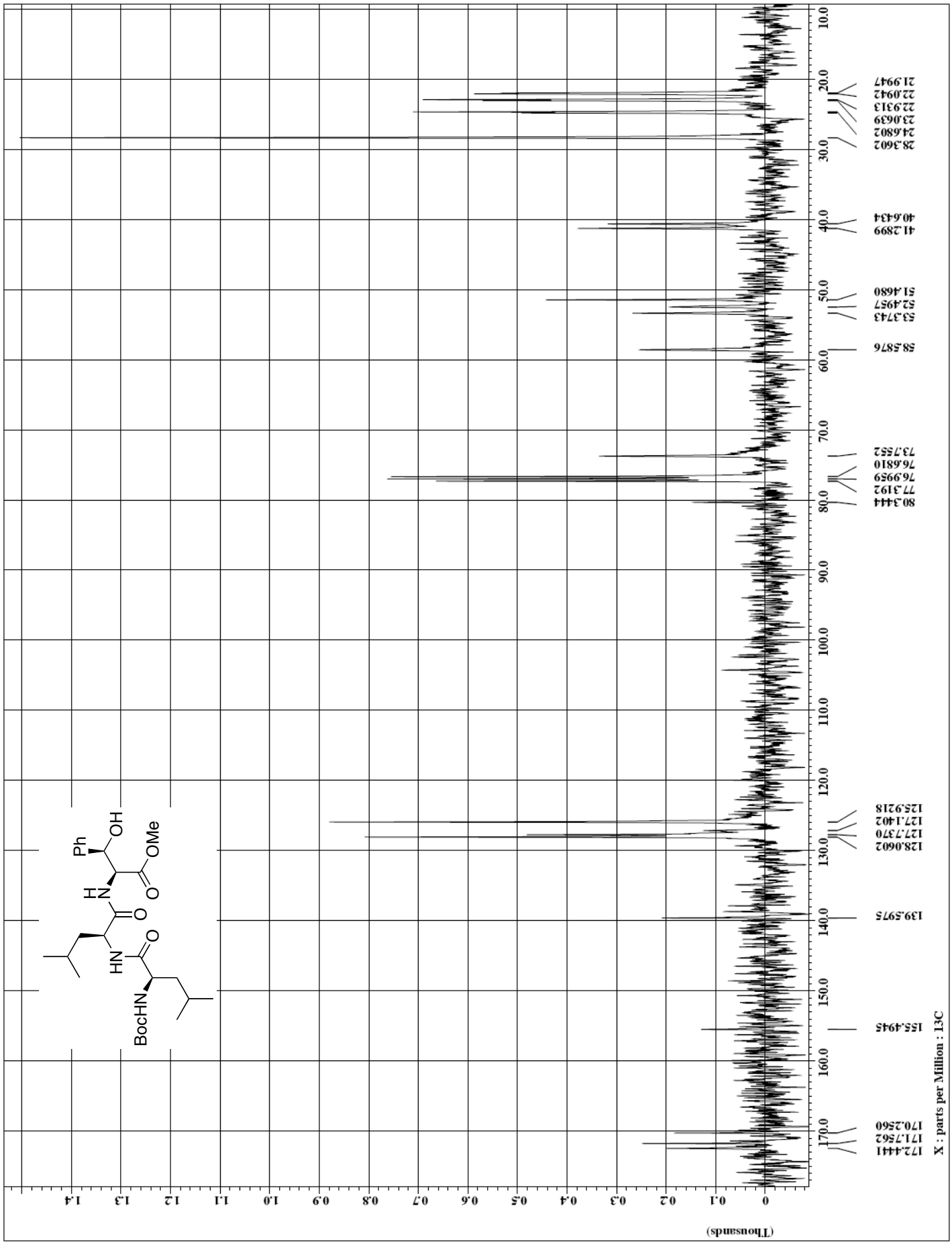


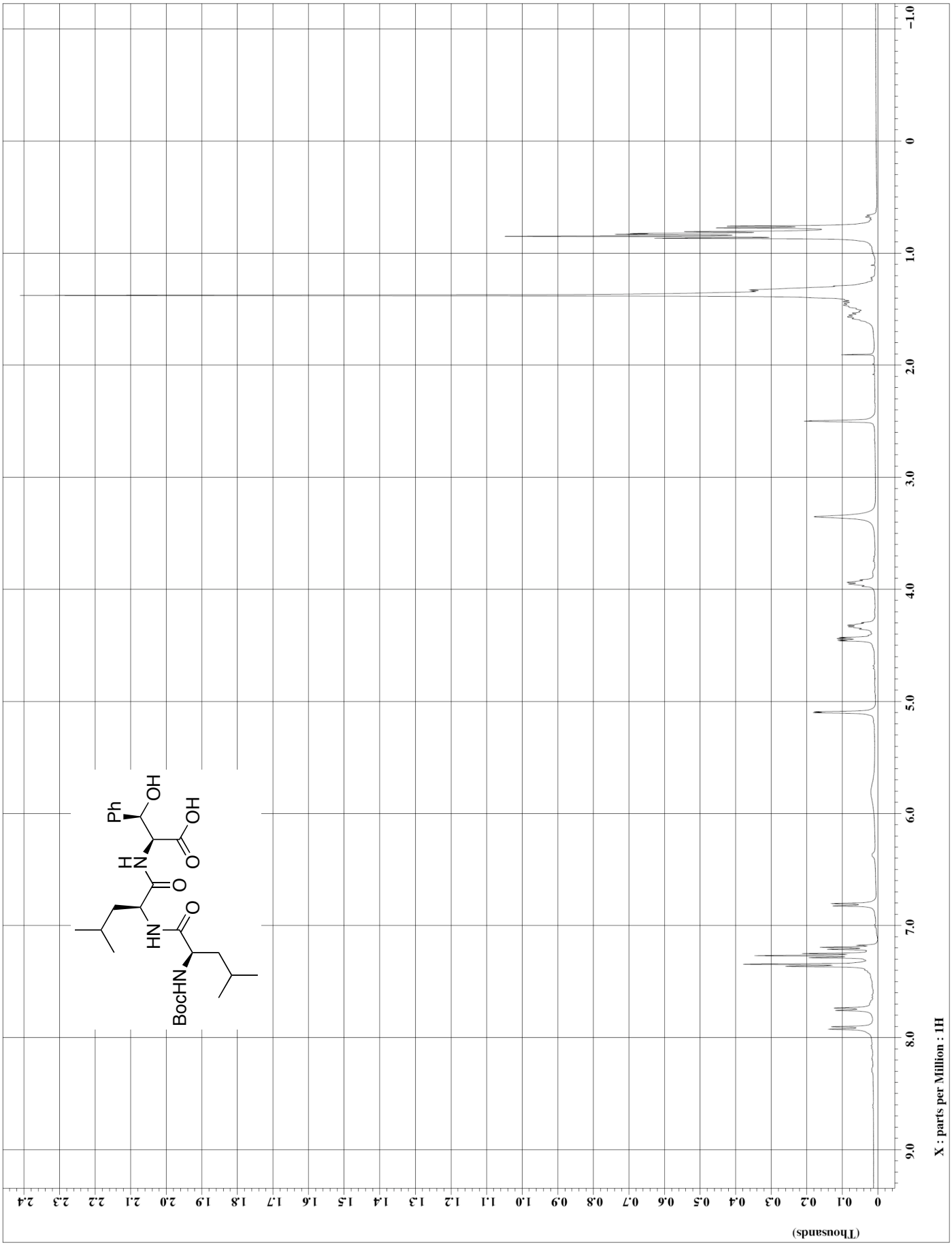
CD Curves for Synthetic Lysobactin and Natural Katanosin B (Lysobactin)

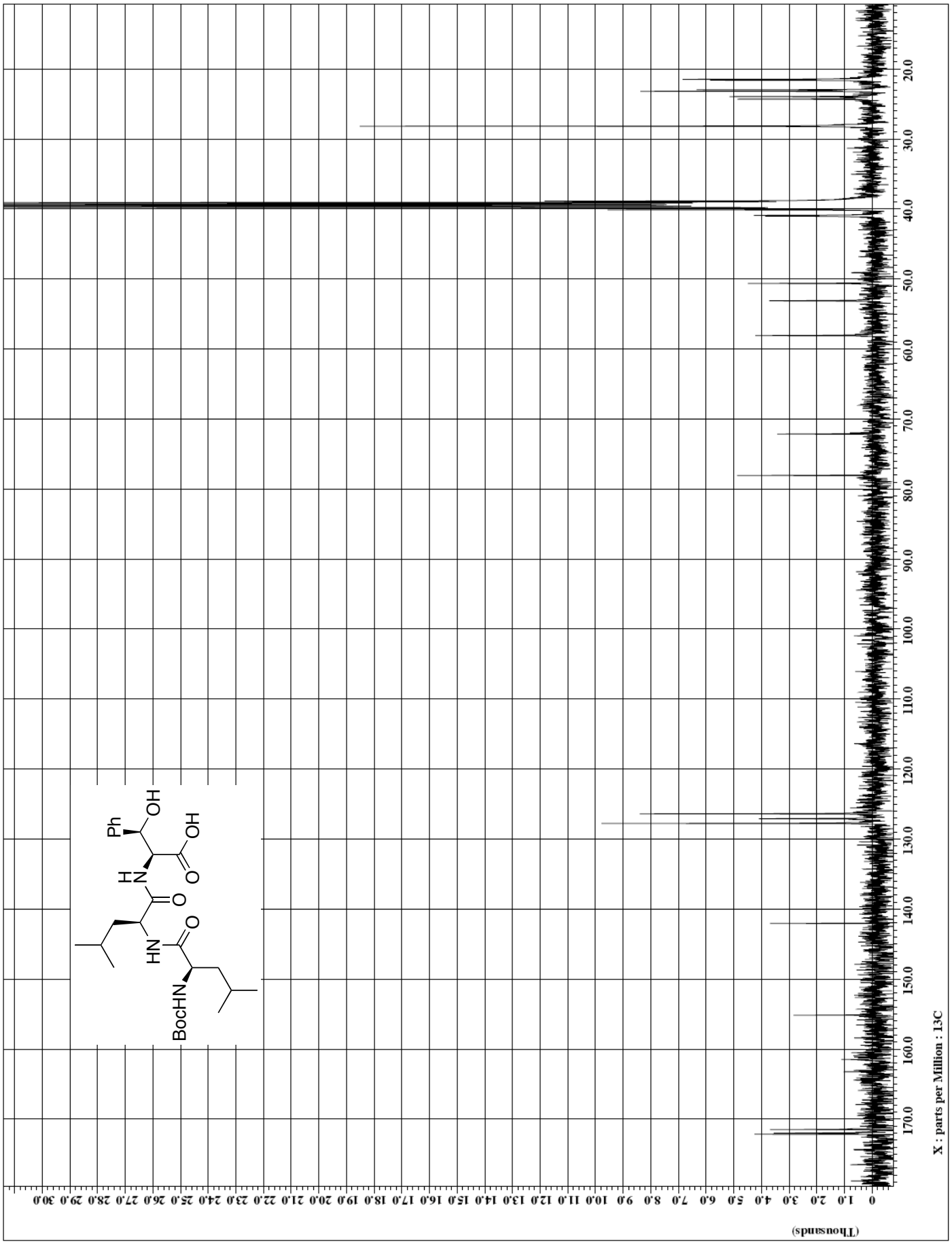
Synthetic Lysobactin vs. authentic natural lysobactin

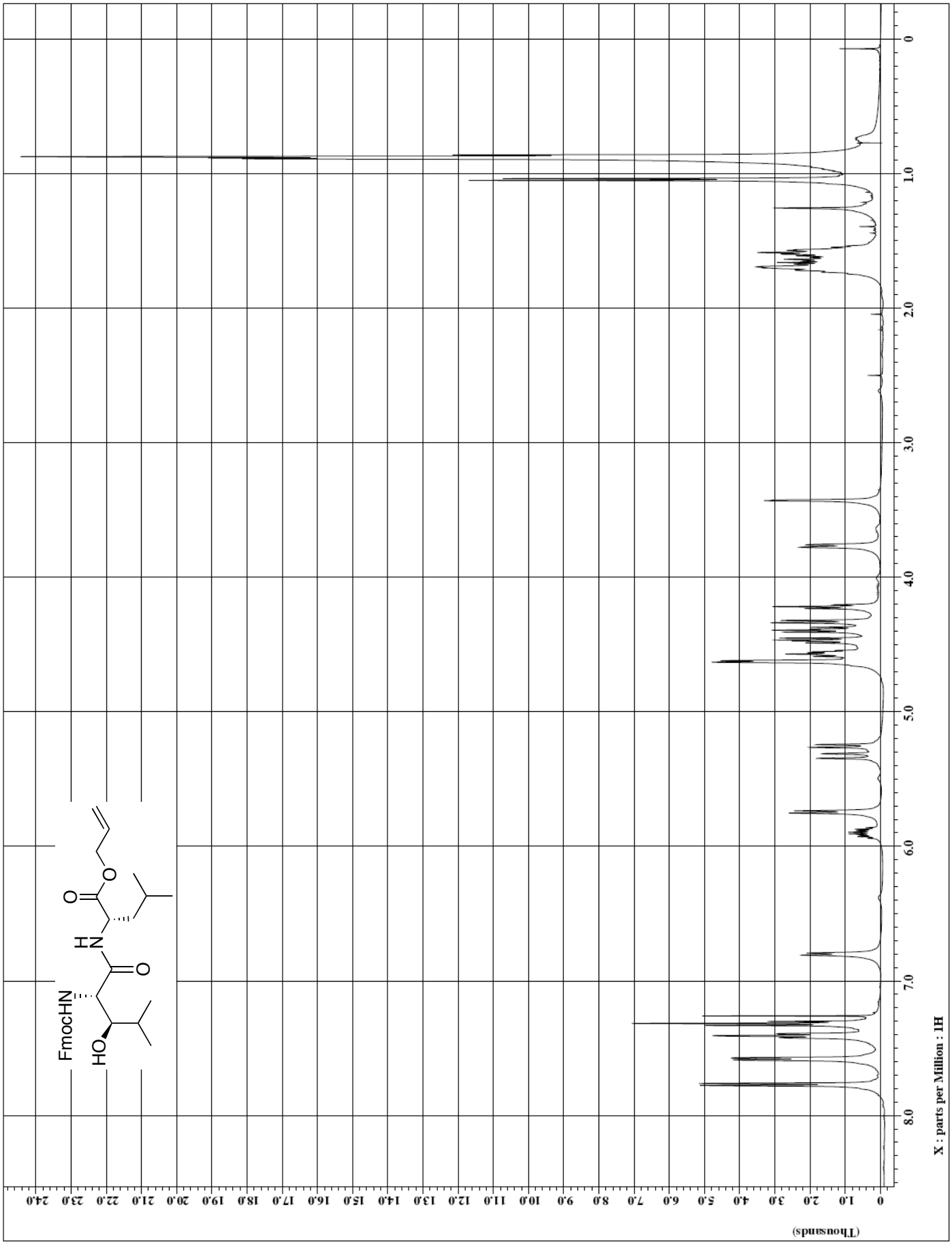


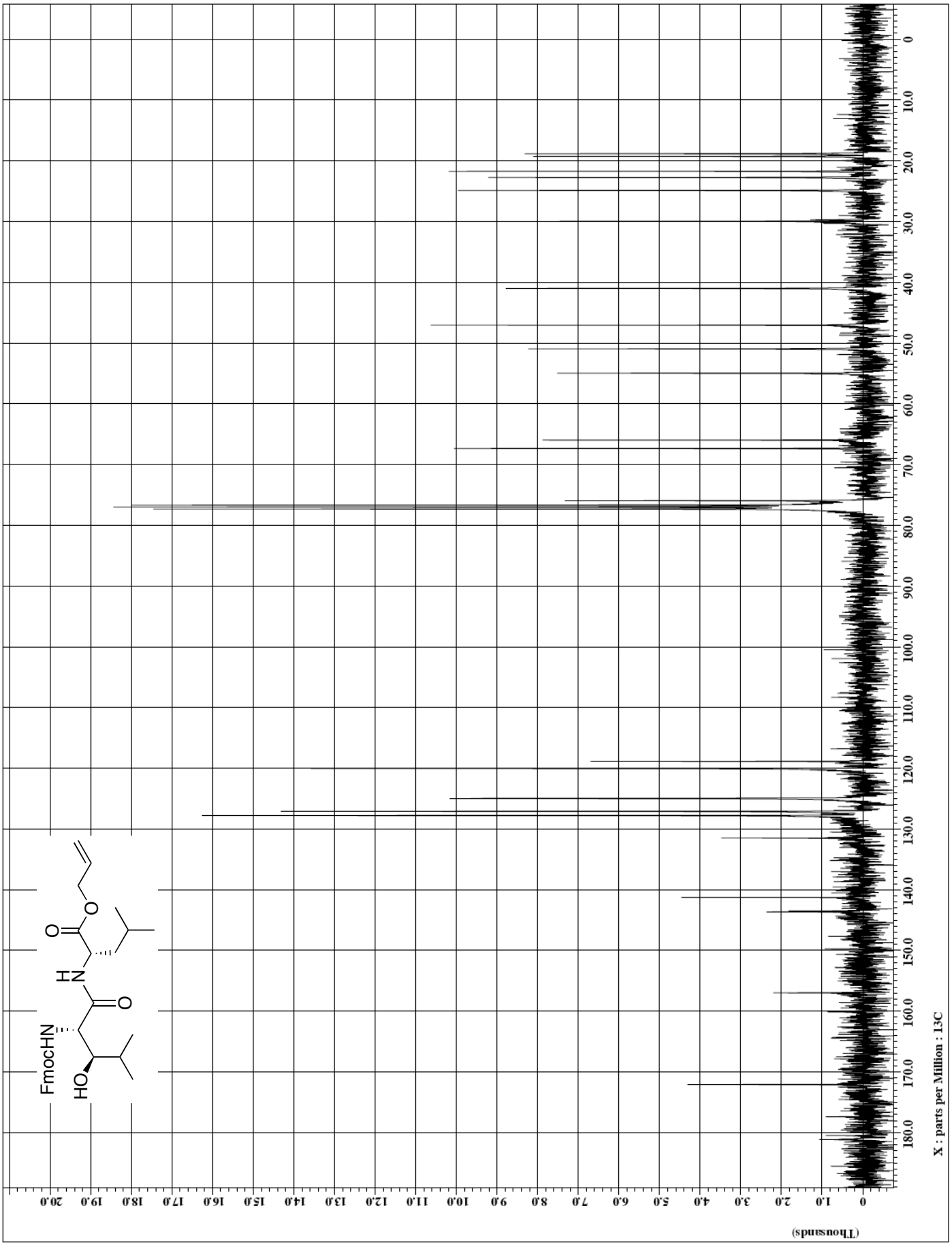

(Thousands)
X : parts per Million : 1H
BocHN
HN
O
NH
O
O
OMe
Ph
OH

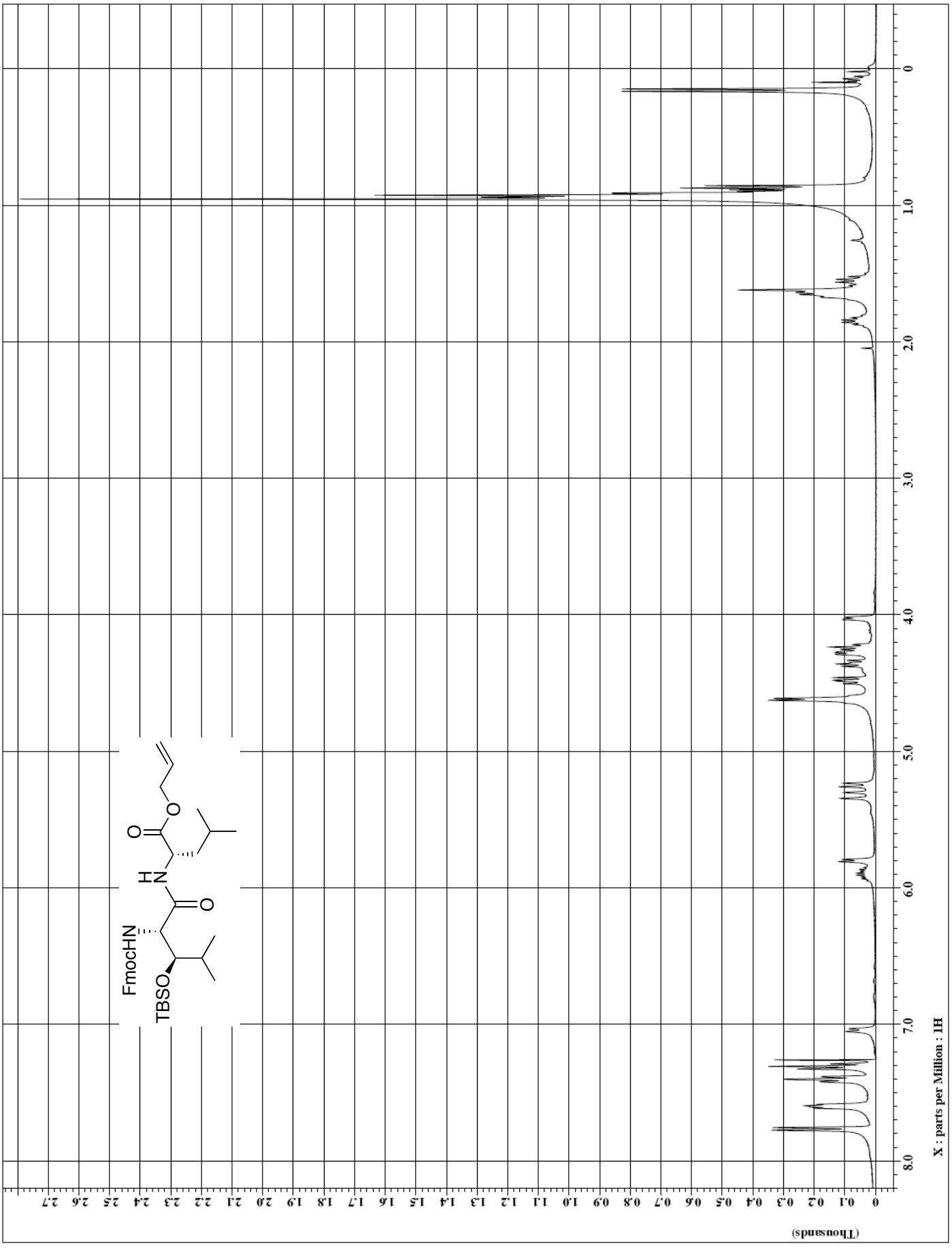

(Thousands)
X : parts per Million : 13C
172.4441
171.7562
170.2560
155.4945
139.5975
128.0602
127.7370
127.1402
125.9218
80.3444
77.3192
76.9959
76.6810
73.7552
58.5876
53.3743
52.4957
51.4680
41.2899
40.6434
28.3602
24.6802
23.0639
22.9313
22.0942
21.9947
BocHN
HN
O
H
N
O
O
OMe
Ph
OH

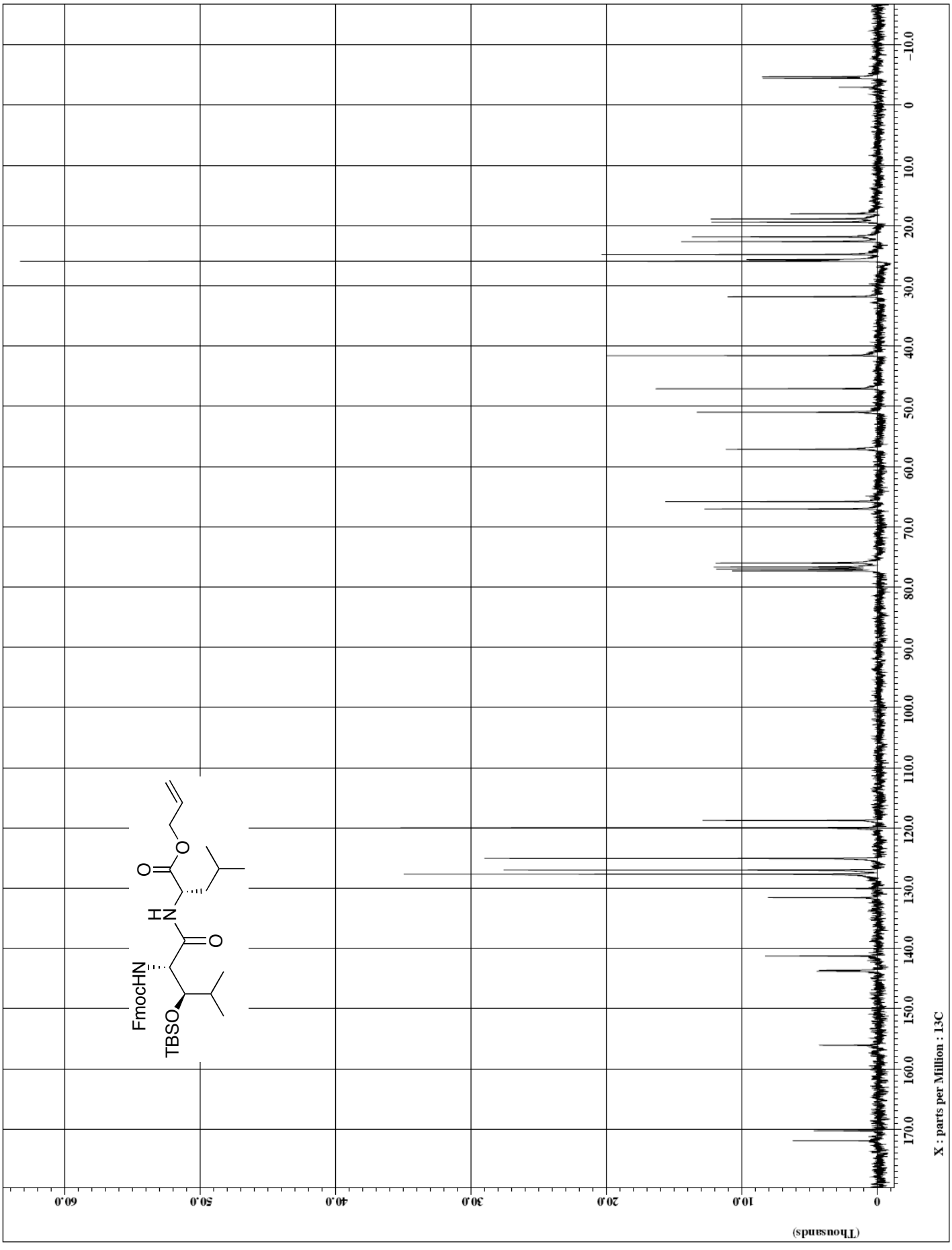

(Thousands)
X : parts per Million : 1H
BocHN
HN
H
N
O
O
OH
OH
Ph

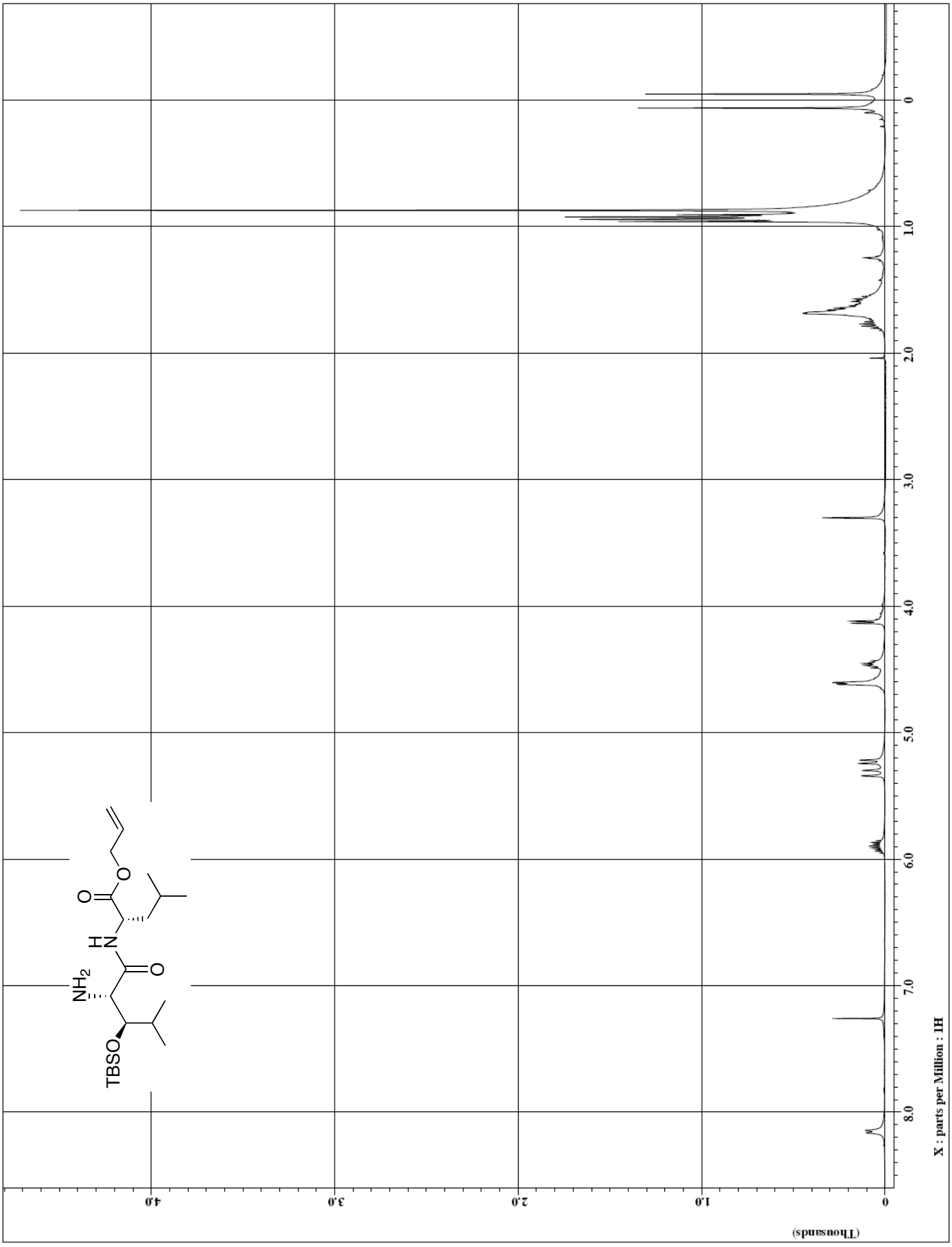

(Thousands)
X : parts per Million : 13C
BocHN
HN
O
O
H
N
O
OH
Ph
OH

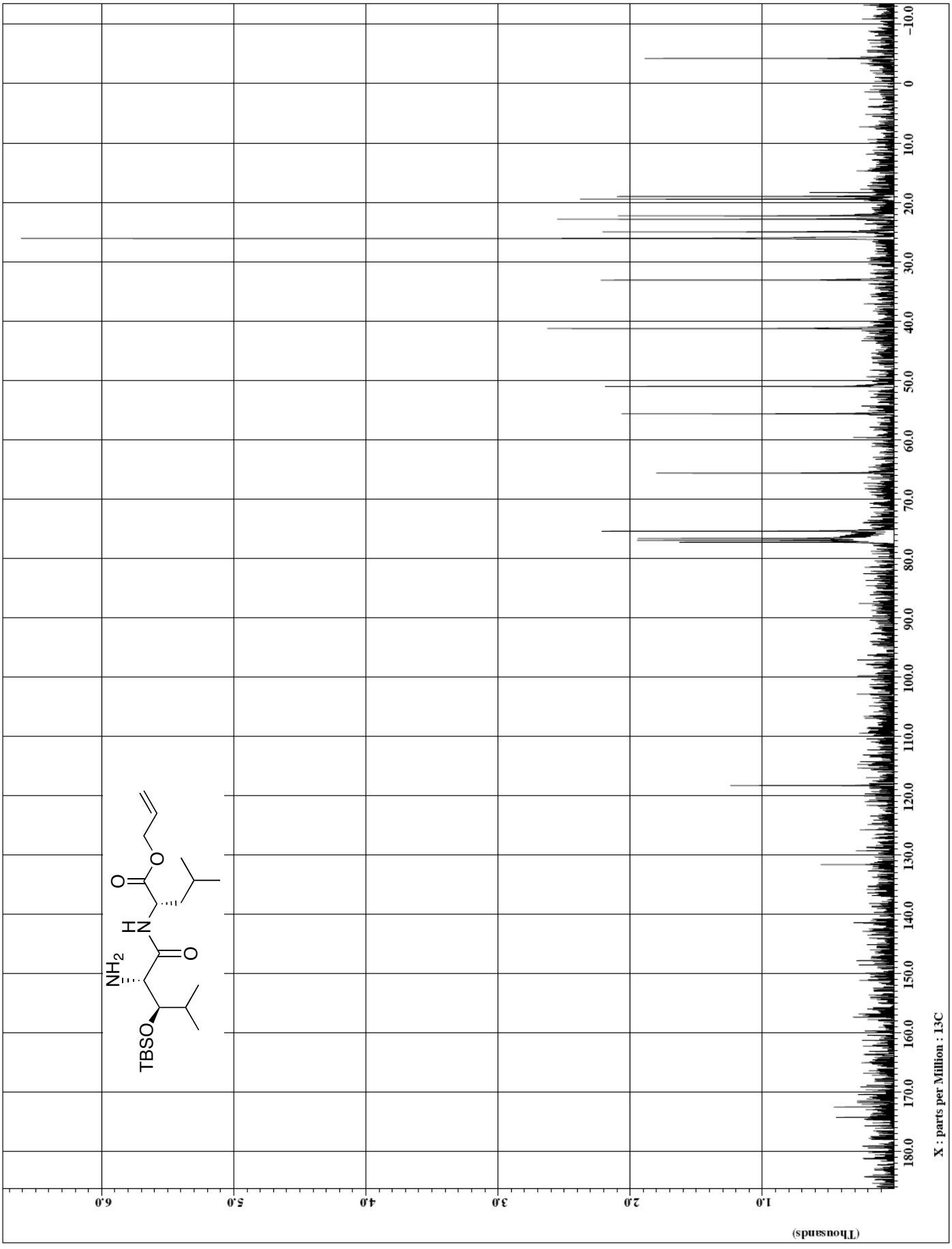

FmocHN
HO
O
H
N
O
O
O
(Thousands)
X : parts per Million : 1H

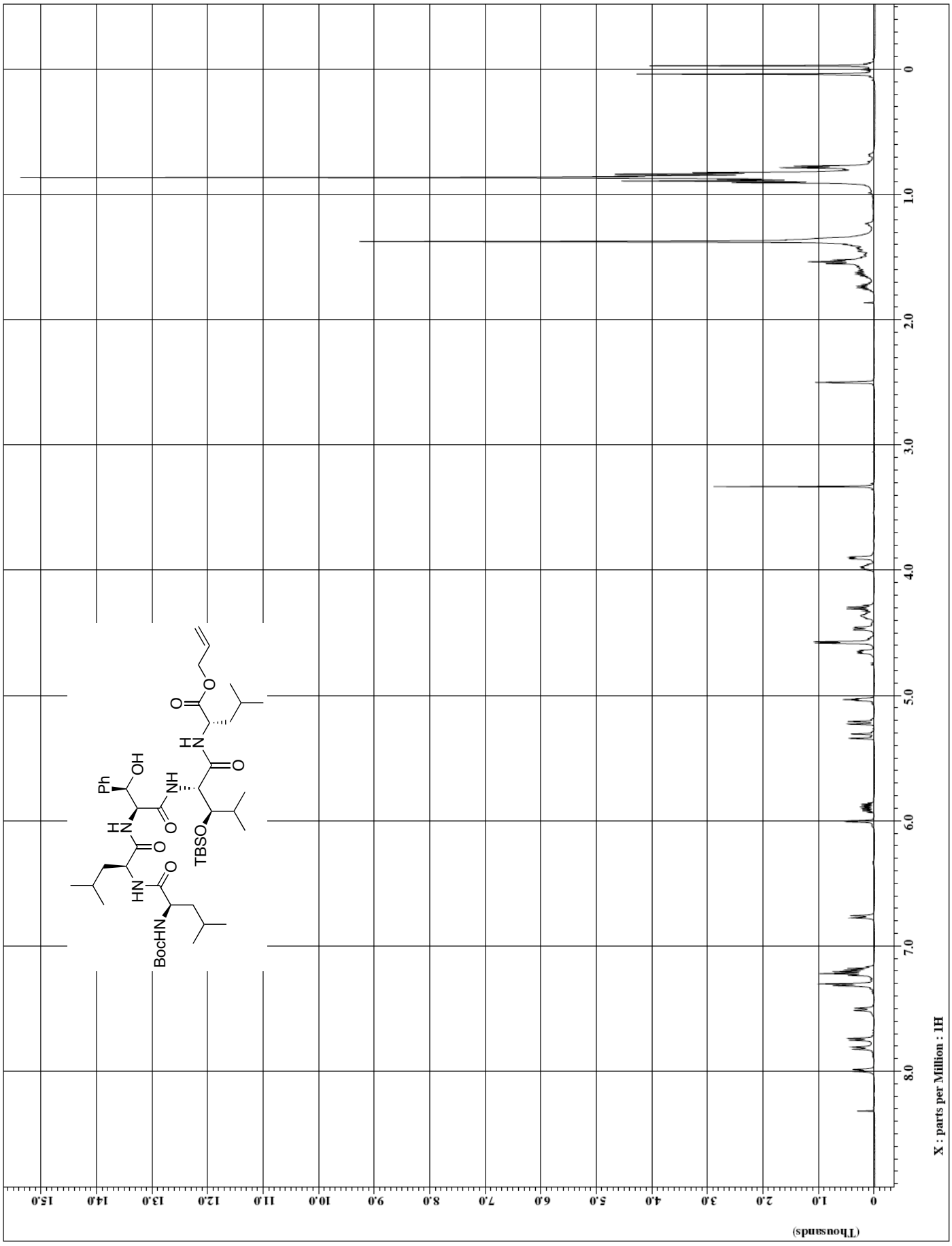

FmocHN
HO
O
H
N
O
O
(Thousands)
X : parts per Million : 13C

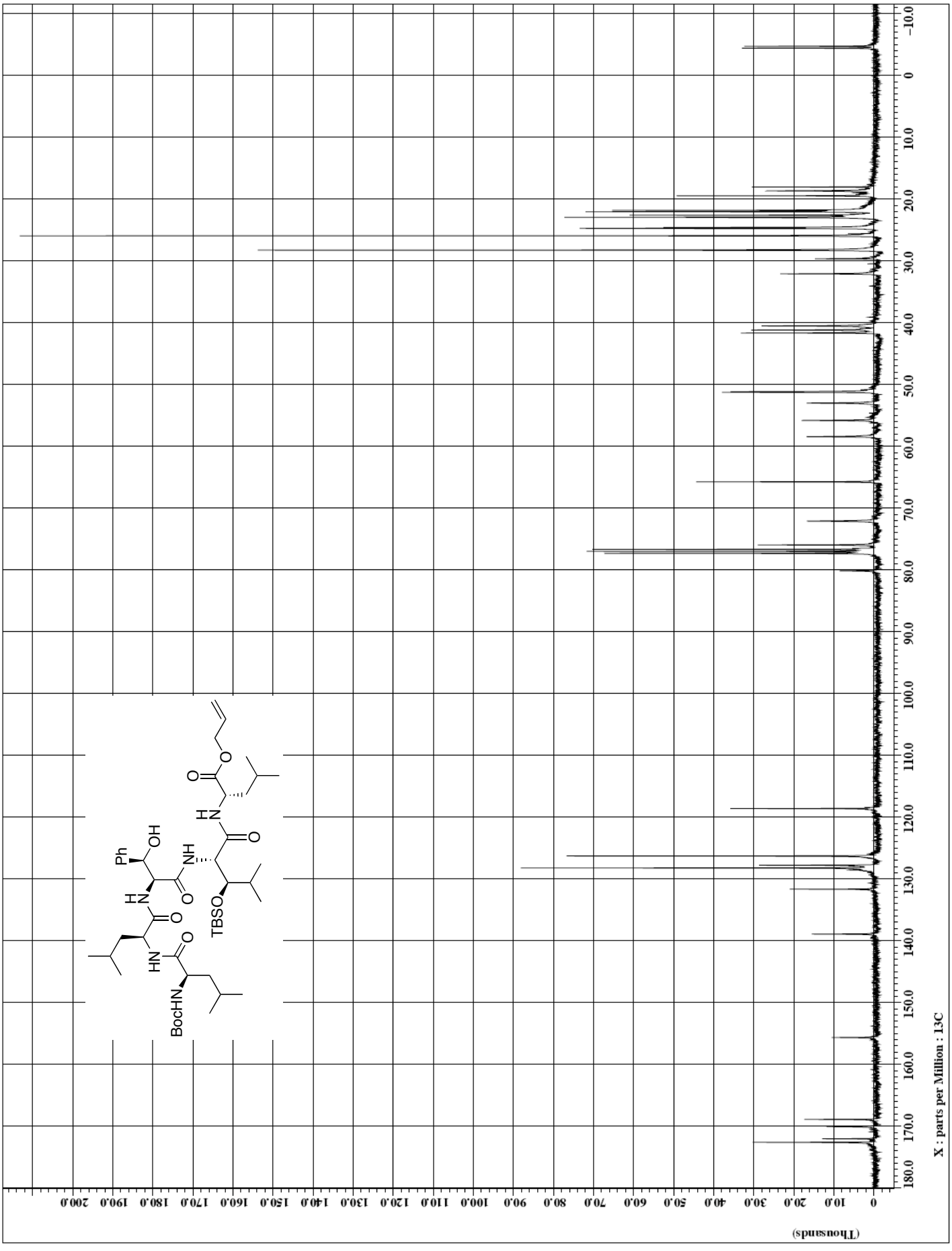

FmocHN
TBSO
O
O
O
H
N
O
(Thousands)
X : parts per Million : 1H

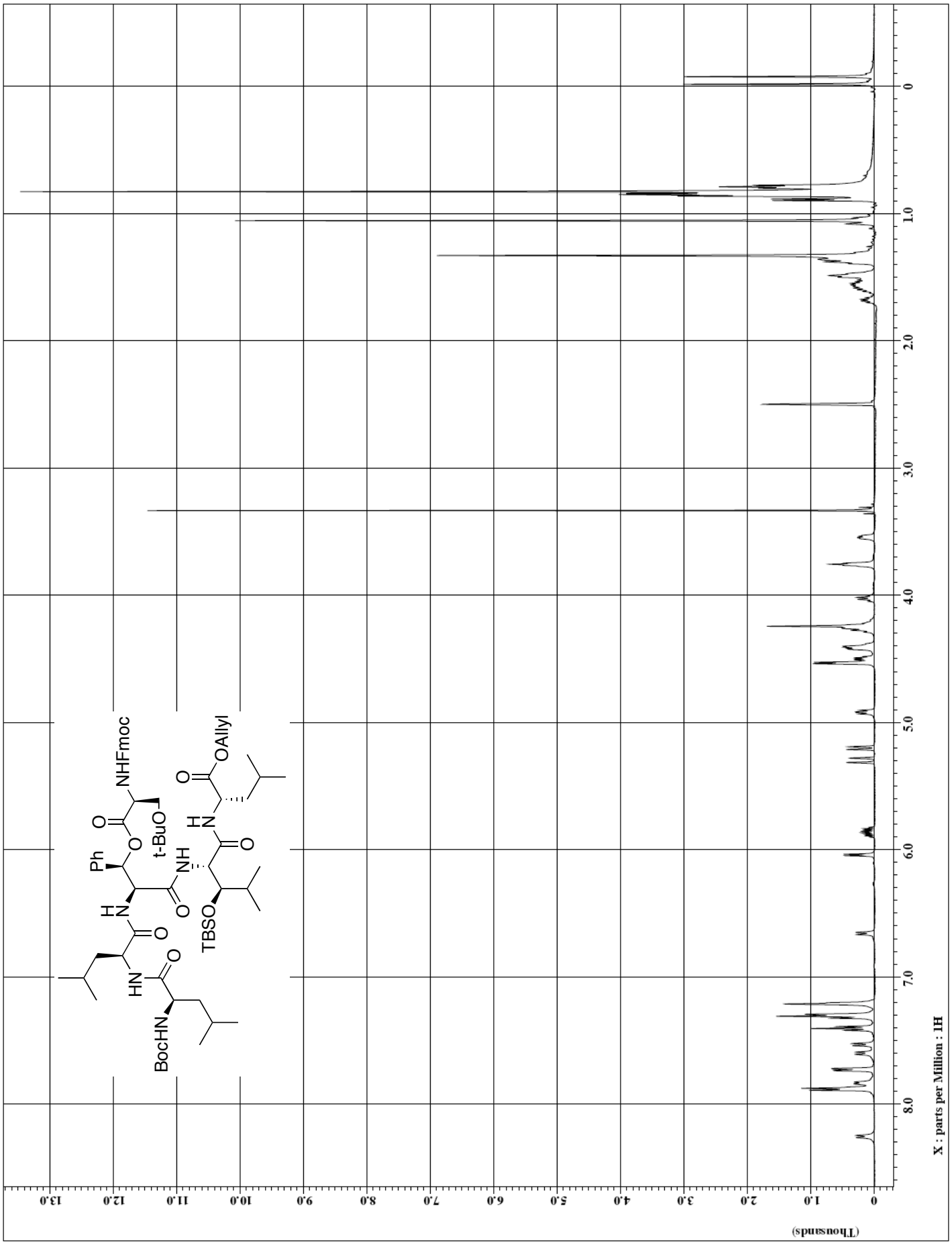

FmocHN
TBSO
H
N
O
O
O
O
(Thousands)
X : parts per Million : 13C

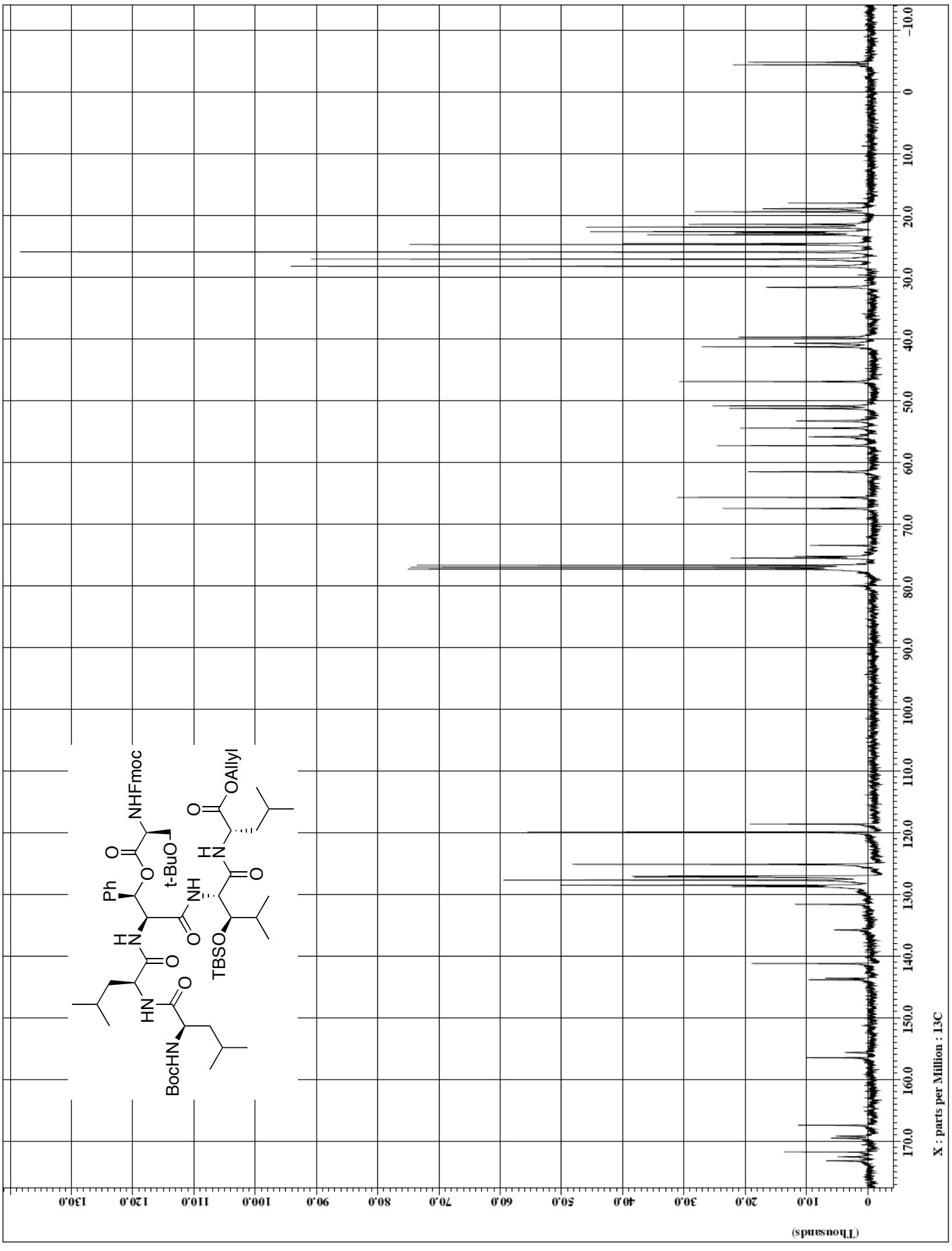

(Thousands)
4.0
3.0
2.0
1.0
0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
X : parts per Million : 1H
TBSO
NH2
H
N
O
O
O

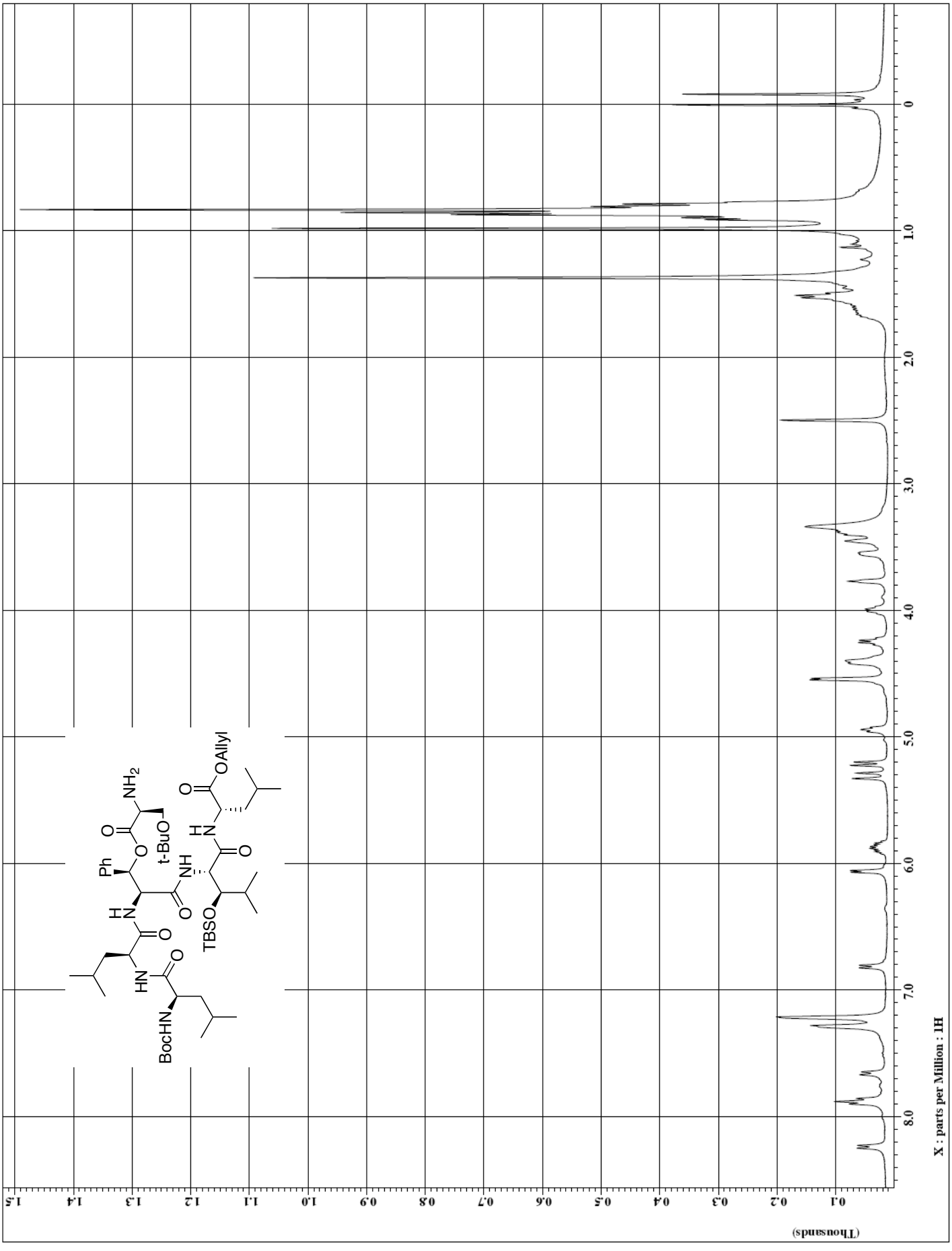

(Thousands)
X : parts per Million : 13C
TBSO
NH2
H
N
O
O
O
O

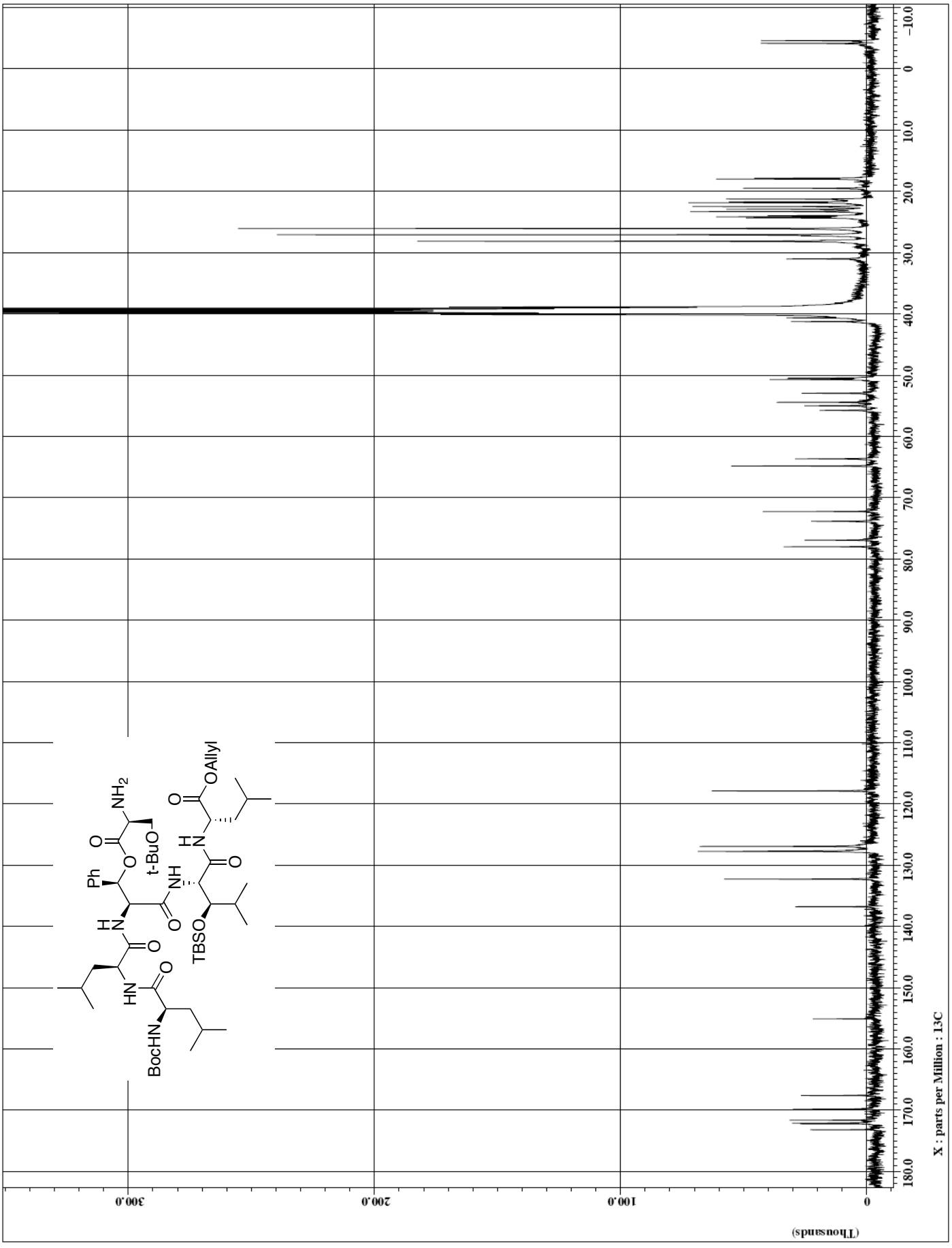

(Thousands)
X : parts per Million : 1H
BocHN
TBSO
Ph
OH

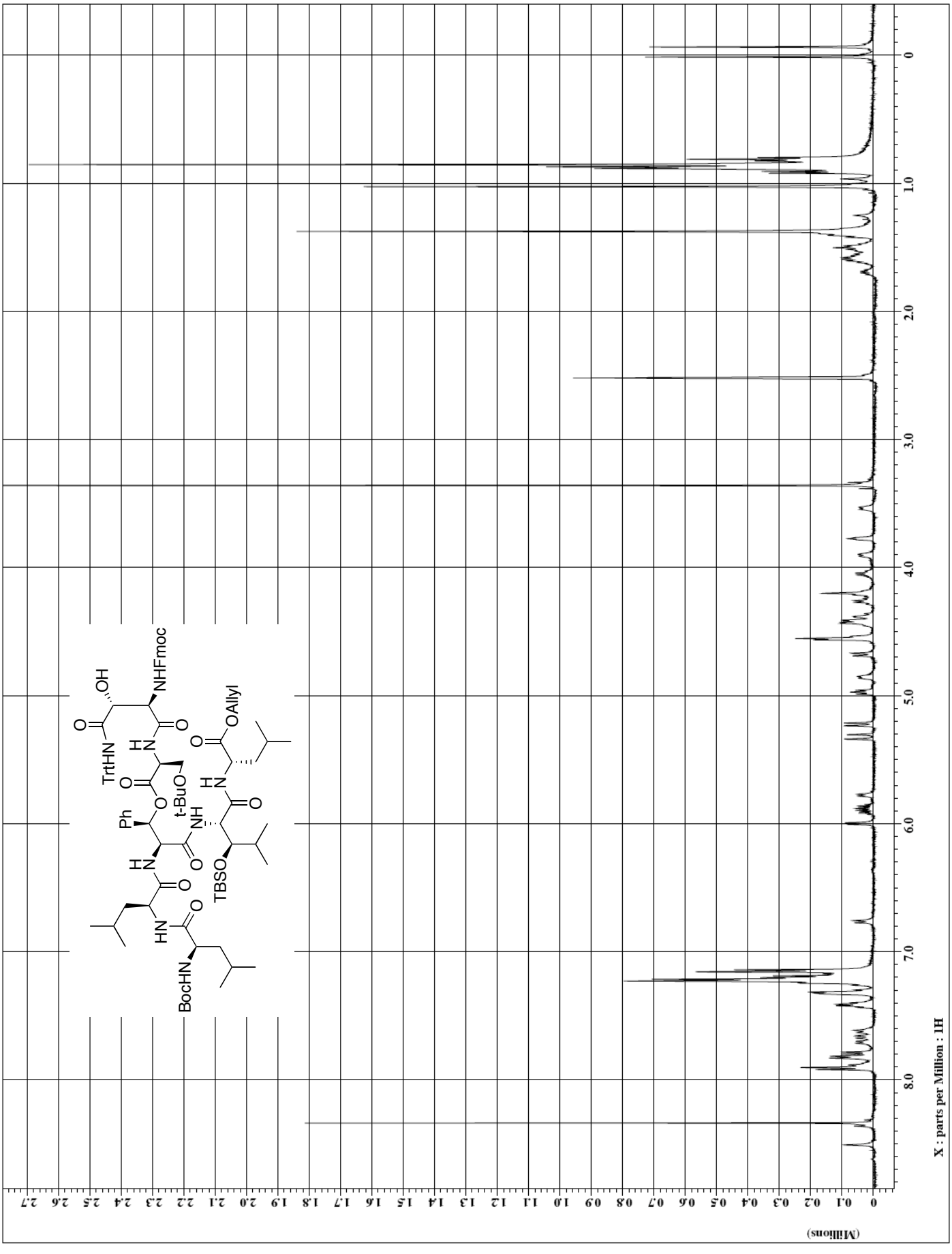

(Thousands)
X : parts per Million : 13C
BocHN
HN
O
N
H
Ph
OH
NH
TBSO
O

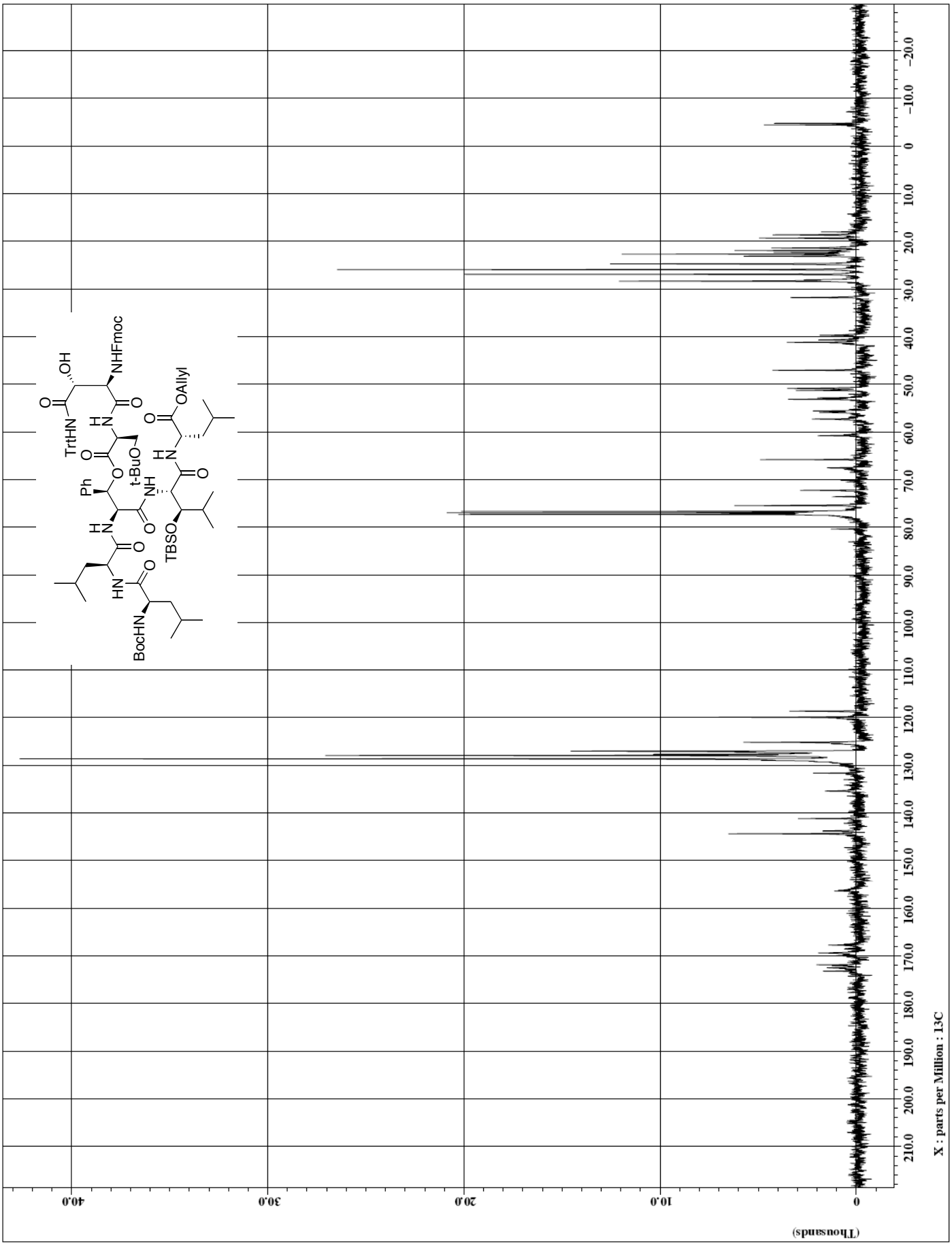

(Thousands)
X : parts per Million : 1H
BocHN
HN
H
N
Ph
O
O
NHFmoc
t-BuO
NH
TBSO
H
N
OAllyl

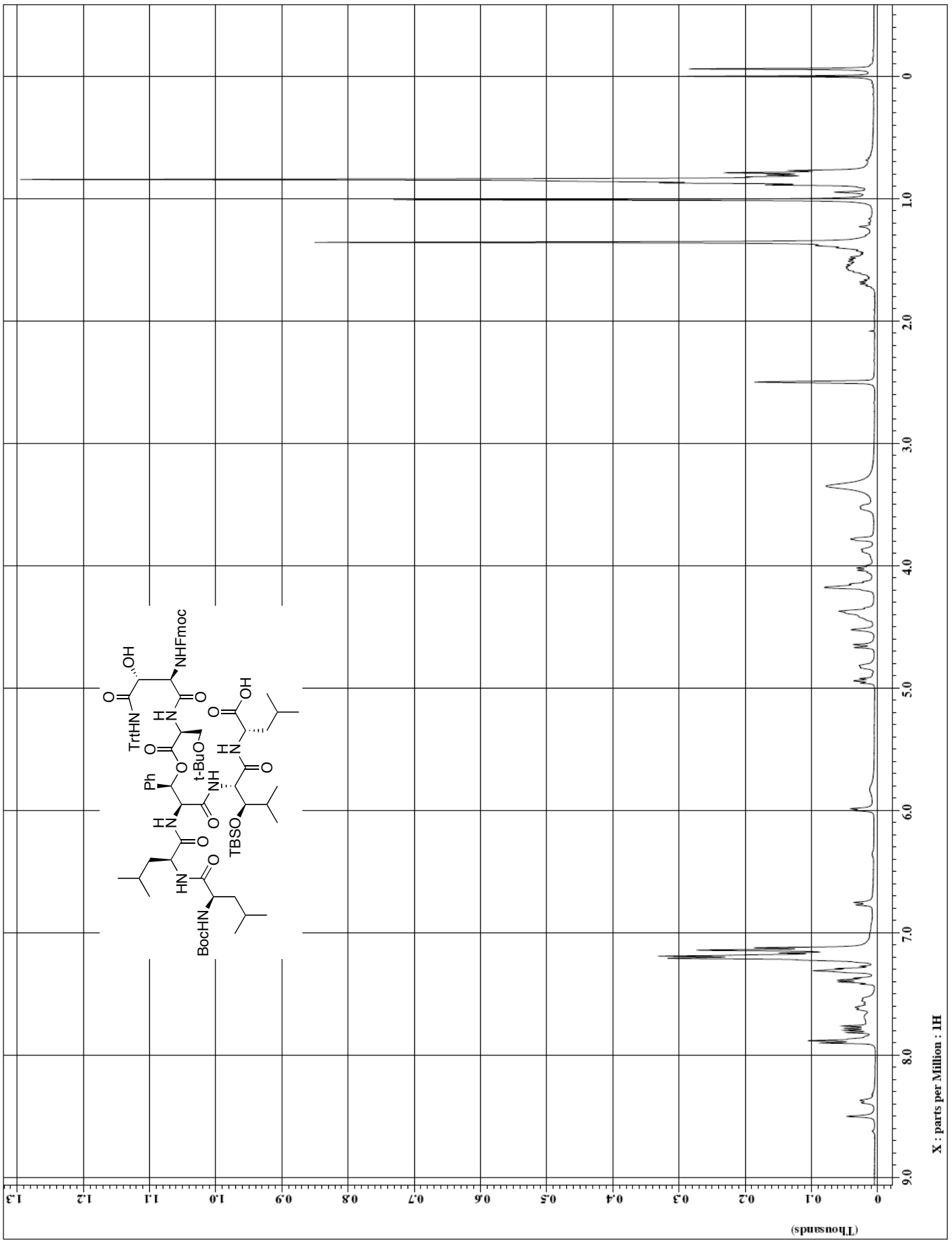

BocHN
NHFmoc
Ph
t-BuO
TBSO
OAllyl
X : parts per Million : 13C
(Thousands)

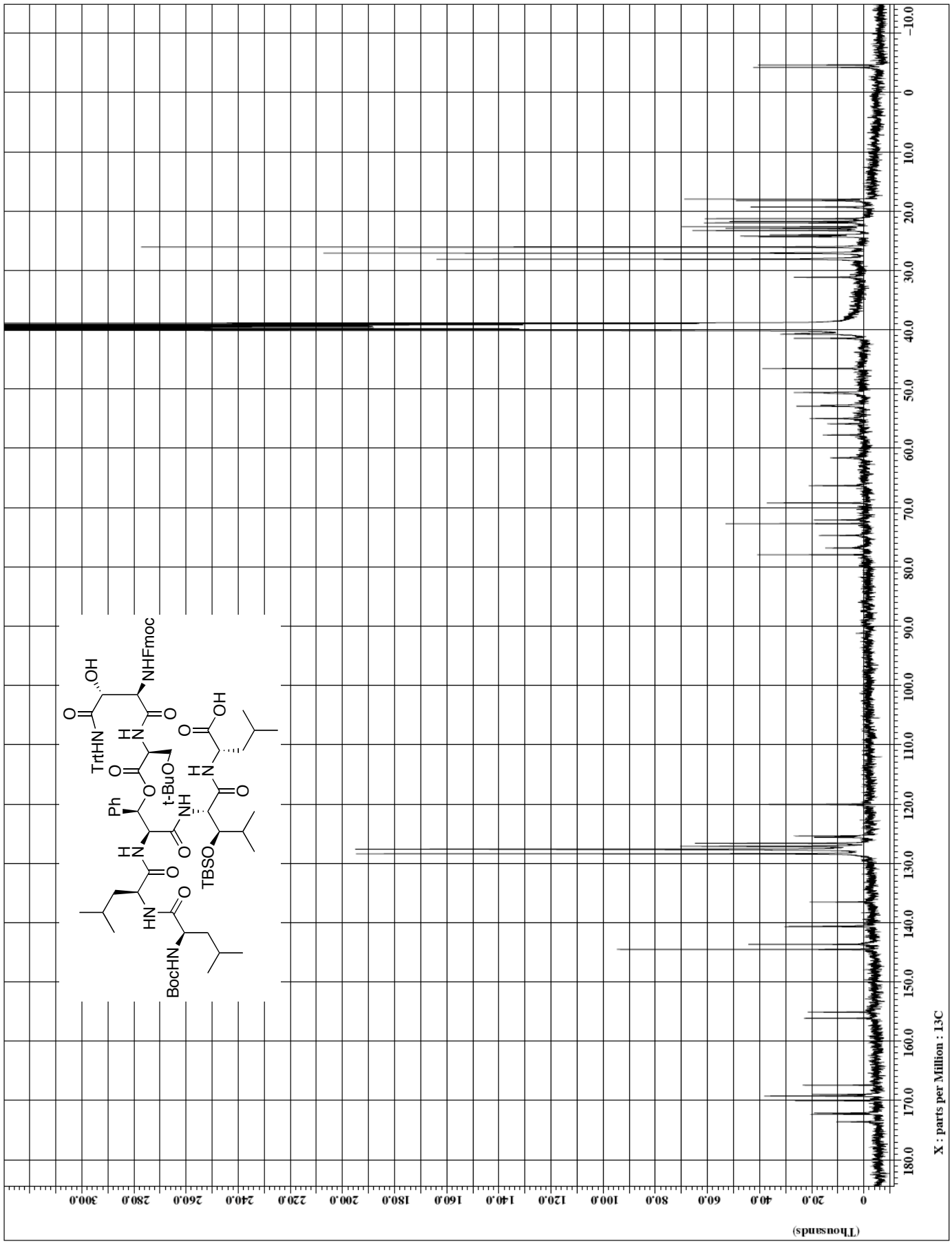

(Thousands)
X : parts per Million : 1H
BocHN
HN
H
N
Ph
O
O
NH2
t-BuO
NH
TBSO
OAllyl

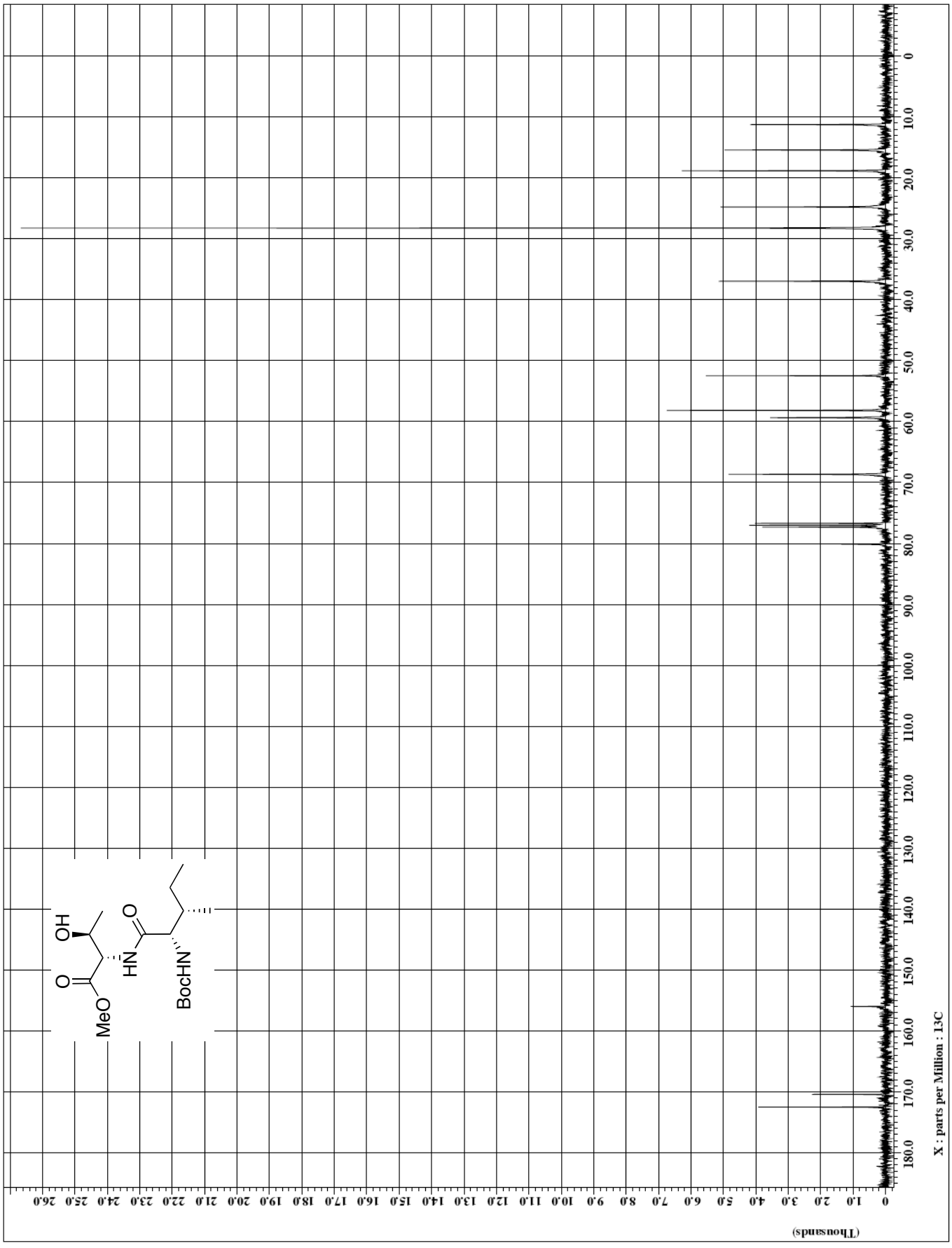

BocHN
HN
O
H
N
O
Ph
O
O
t-BuO
NH2
O
H
N
O
NH
O
TBSO
O
OAllyl
(Thousands)
X : parts per Million : 13C

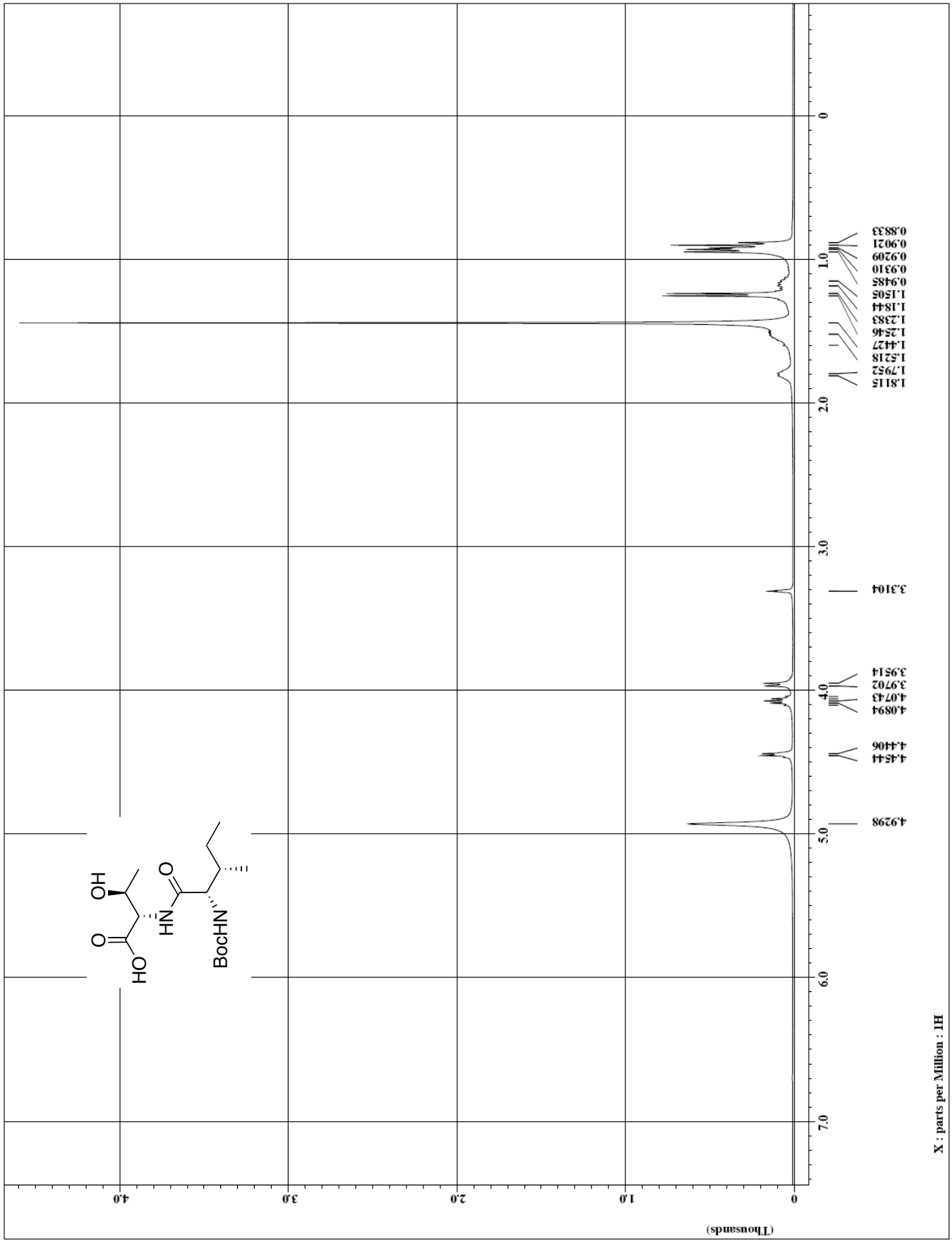

(Millions)
X : parts per Million : 1H
BocHN
TBSO
t-BuO
Ph
TrtHN
OH
NHFmoc
OAllyl

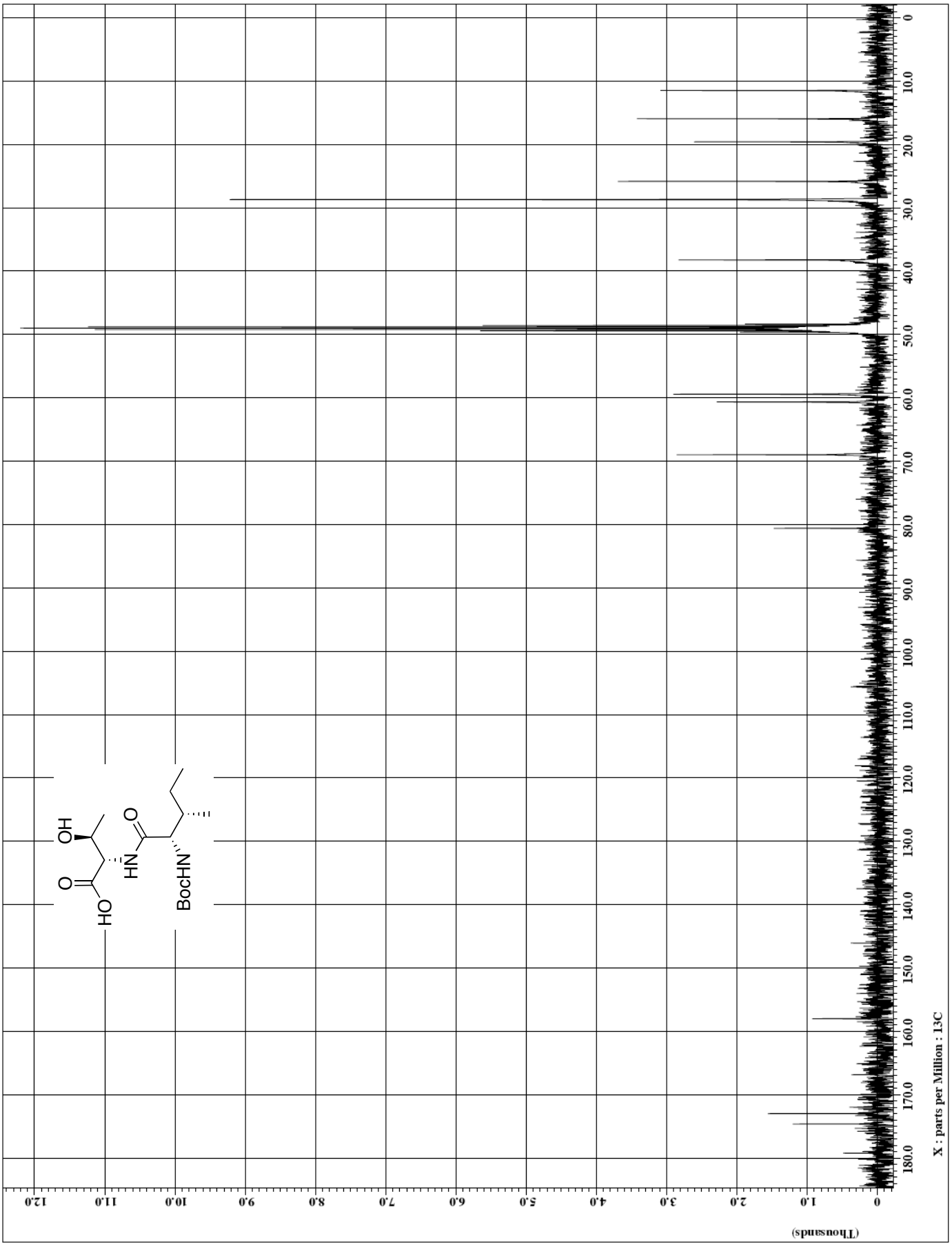

(Thousands)
X : parts per Million : 13C
OAllyl
NHFmoc
TrtHN
t-BuO
Ph
TBSO
BocHN

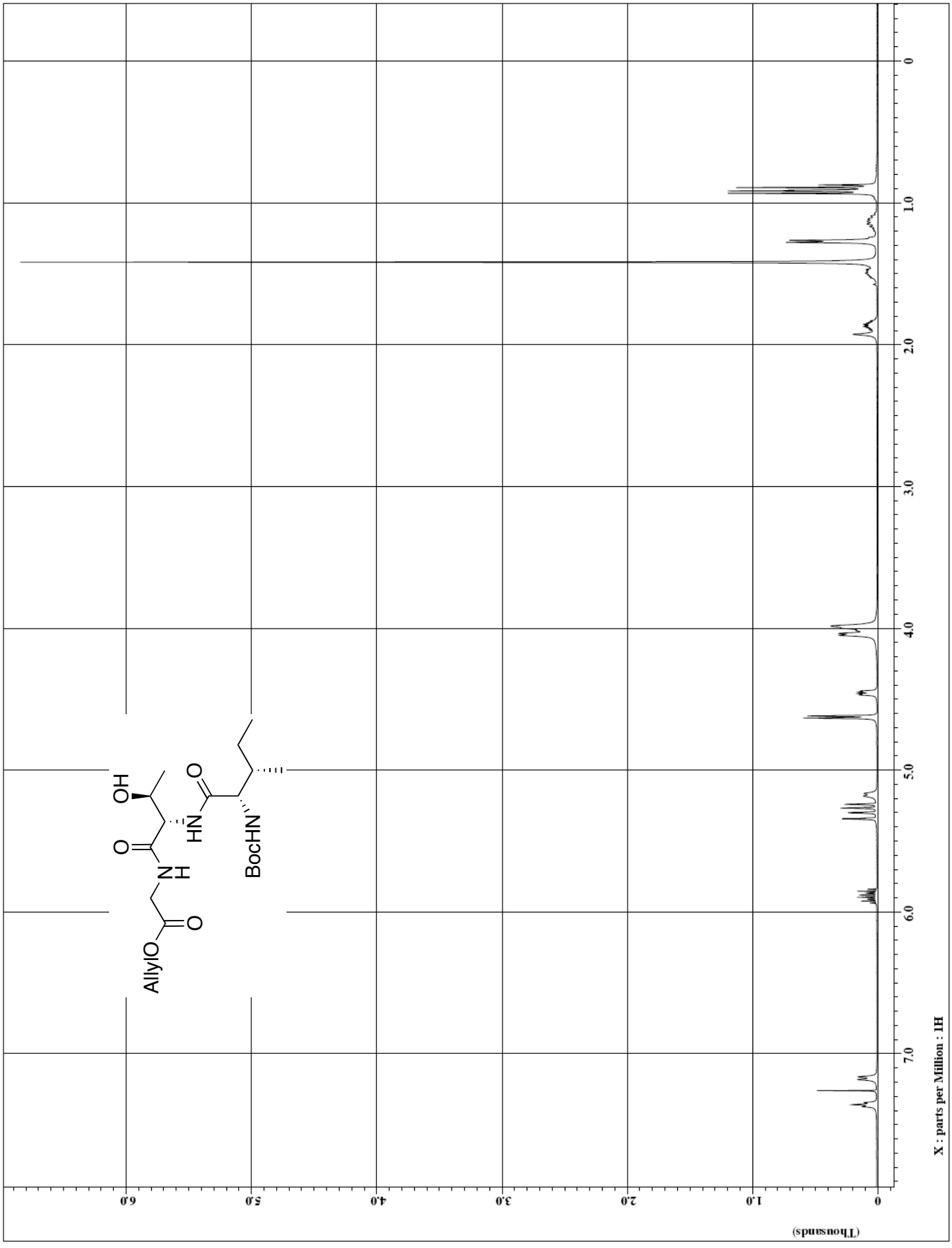

(Thousands)
X : parts per Million : 1H
NHFmoc
OH
TrtHN
t-BuO
Ph
TBSO
BocHN

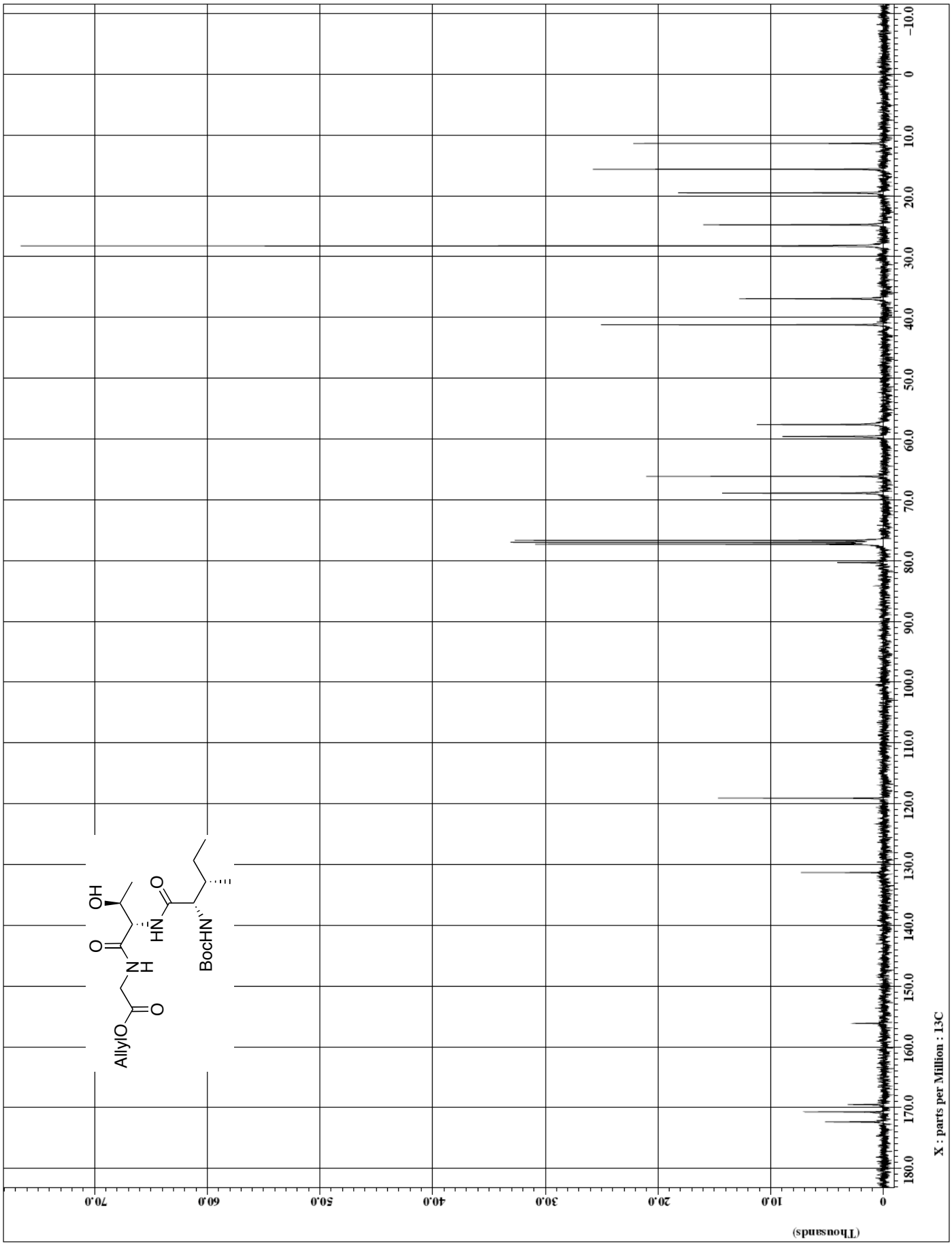

(Thousands)
X : parts per Million : 13C
BocHN
HN
TBSO
Ph
t-BuO
TrtHN
NHFmoc
OH

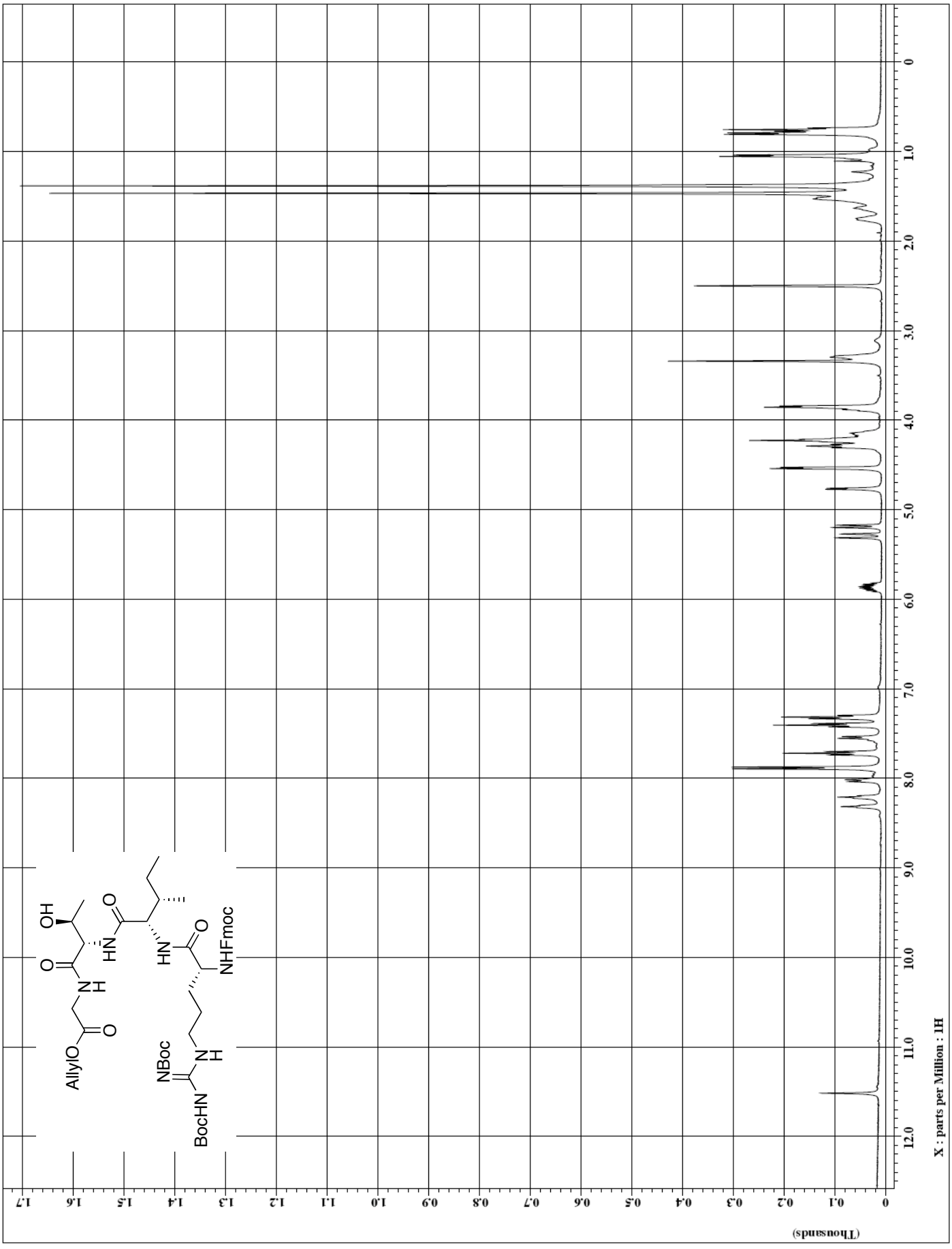

(Thousands)
X : parts per Million : 13C
MeO
O
OH
HN
O
BocHN

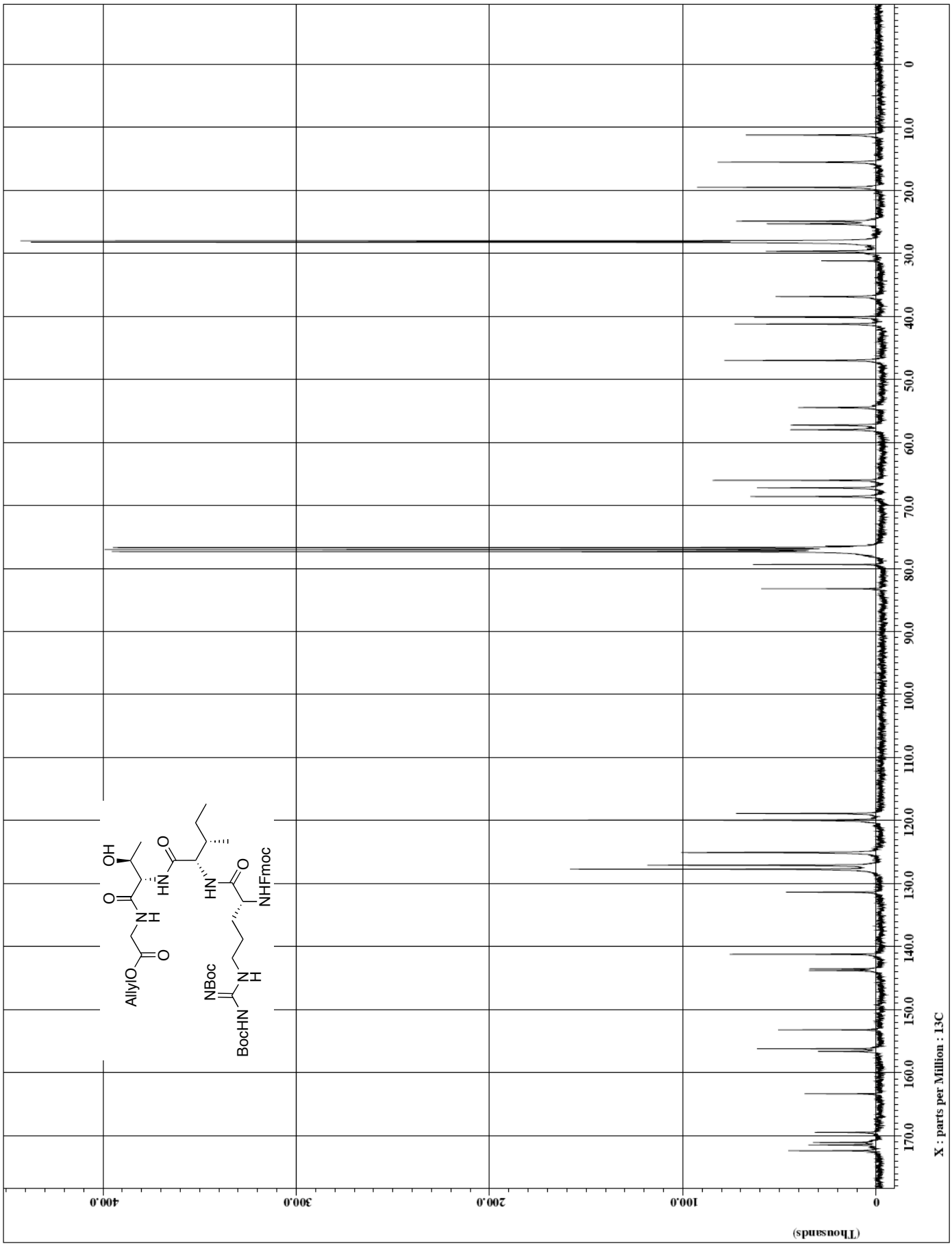

(Thousands)
X : parts per Million : 1H
4.9298
4.4544
4.4406
4.0894
4.0743
3.9702
3.9514
3.3104
1.8115
1.7952
1.5218
1.4427
1.2546
1.2383
1.1844
1.1505
0.9485
0.9310
0.9209
0.9021
0.8833
BocHN
NH
O
OH
HO

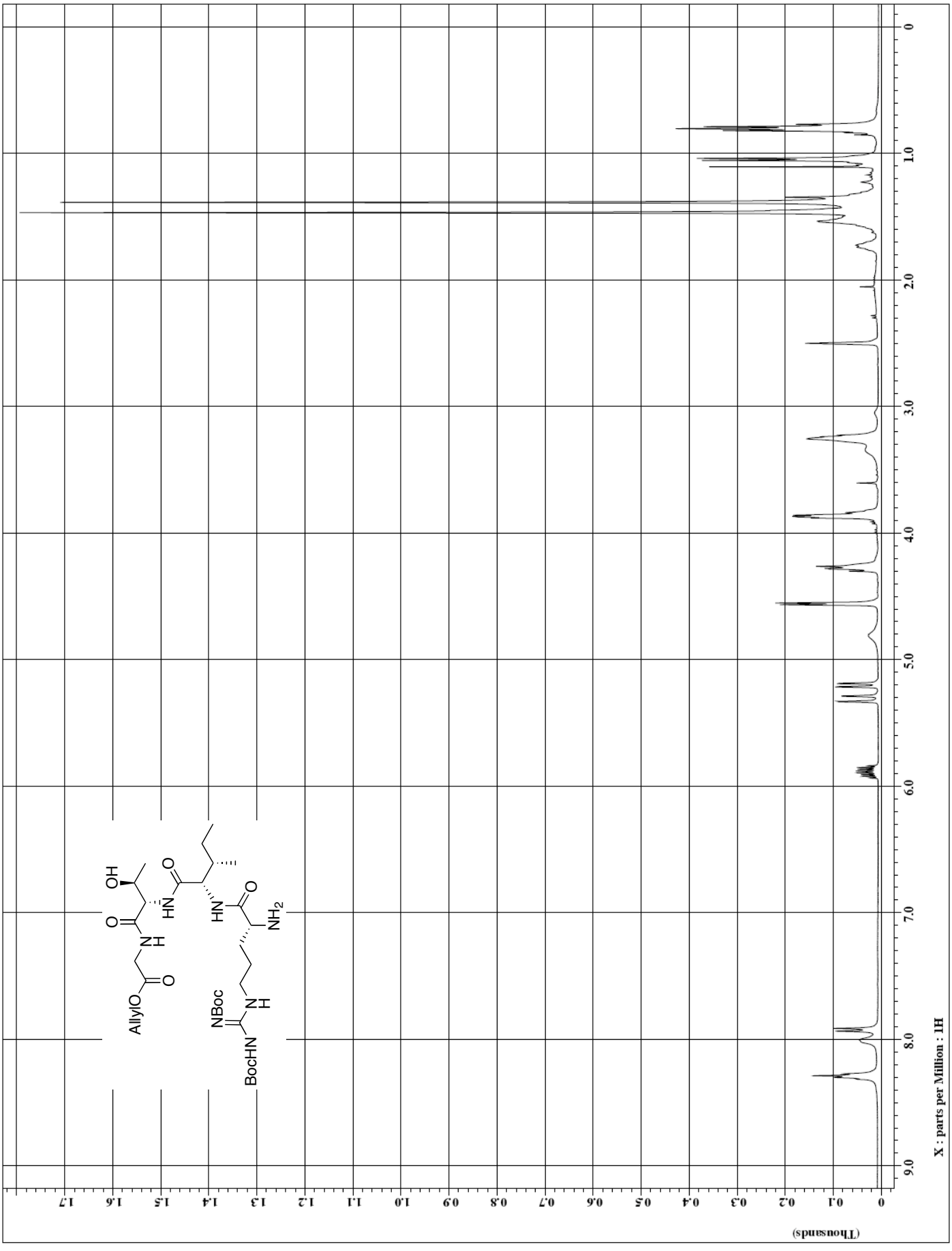

(Thousands)
X : parts per Million : 13C
BocHN
NH
O
OH
O
HO

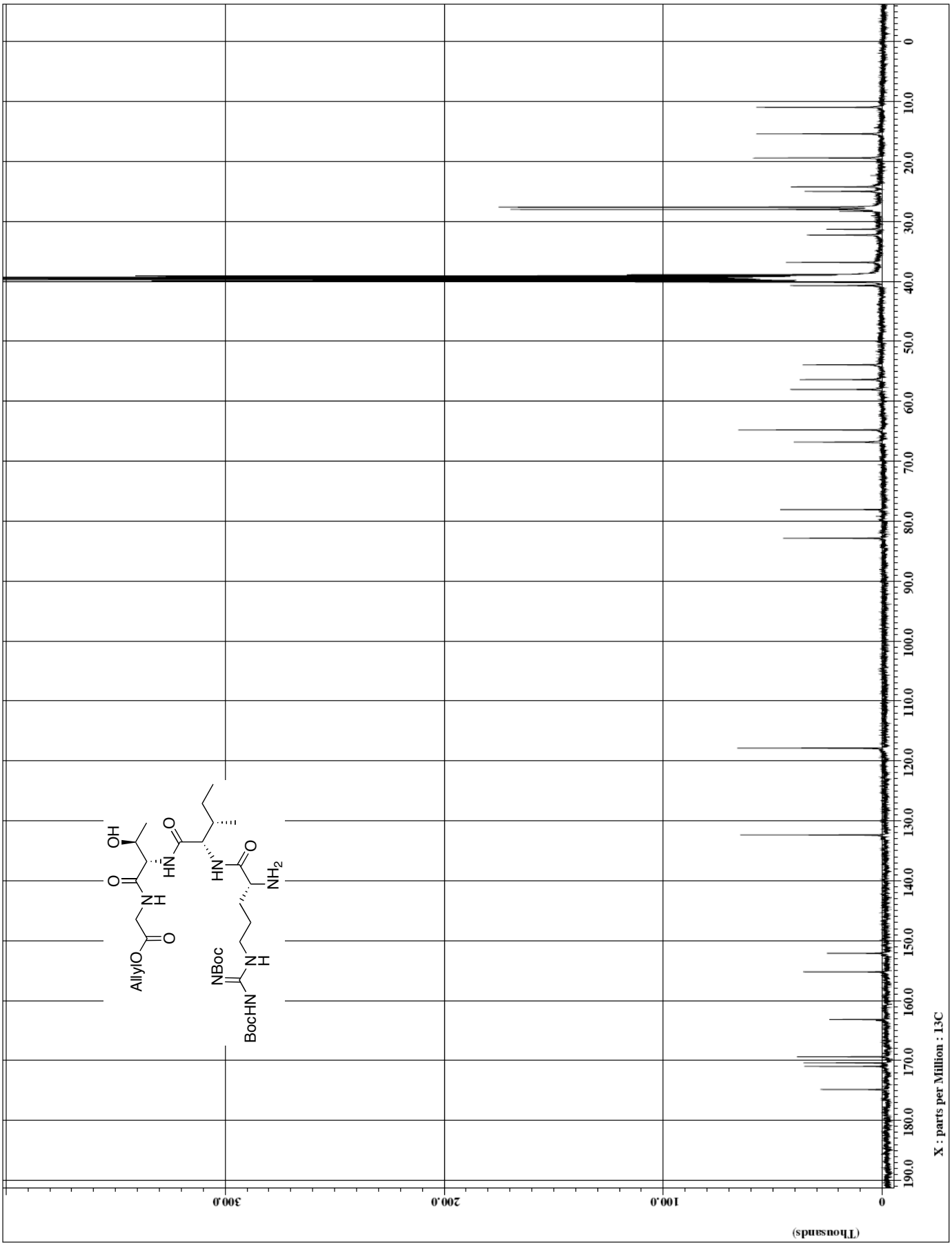

(Thousands)
6.0
5.0
4.0
3.0
2.0
1.0
0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
X : parts per Million : 1H
AllylO
O
O
N
H
O
OH
HN
O
BocHN

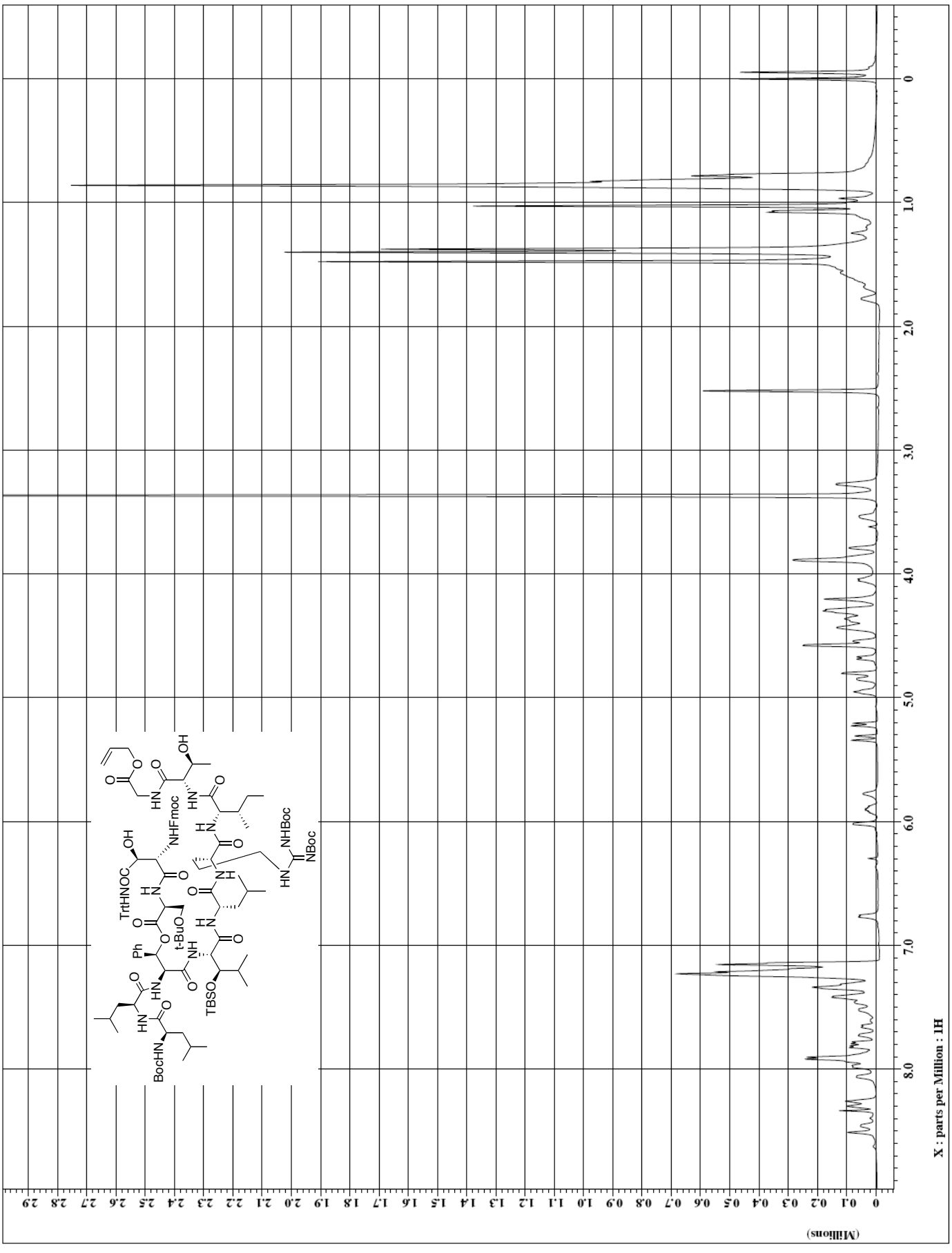

AllylO
O
O
H
N
OH
O
HN
O
BocHN
X : parts per Million : 13C
(Thousands)

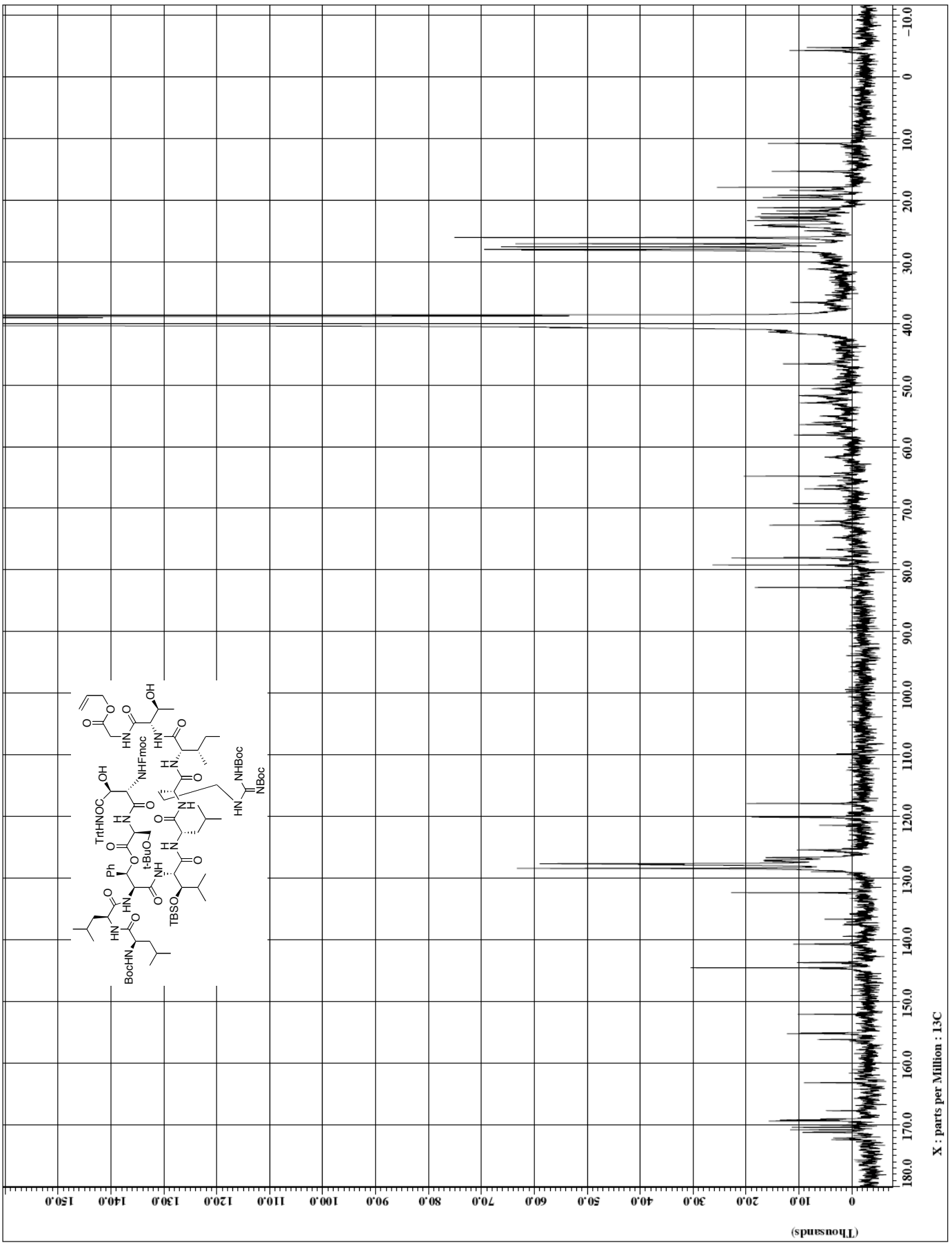

(Thousands)
X : parts per Million : 1H
AllylO
NBoc
BocHN
NHFmoc
OH

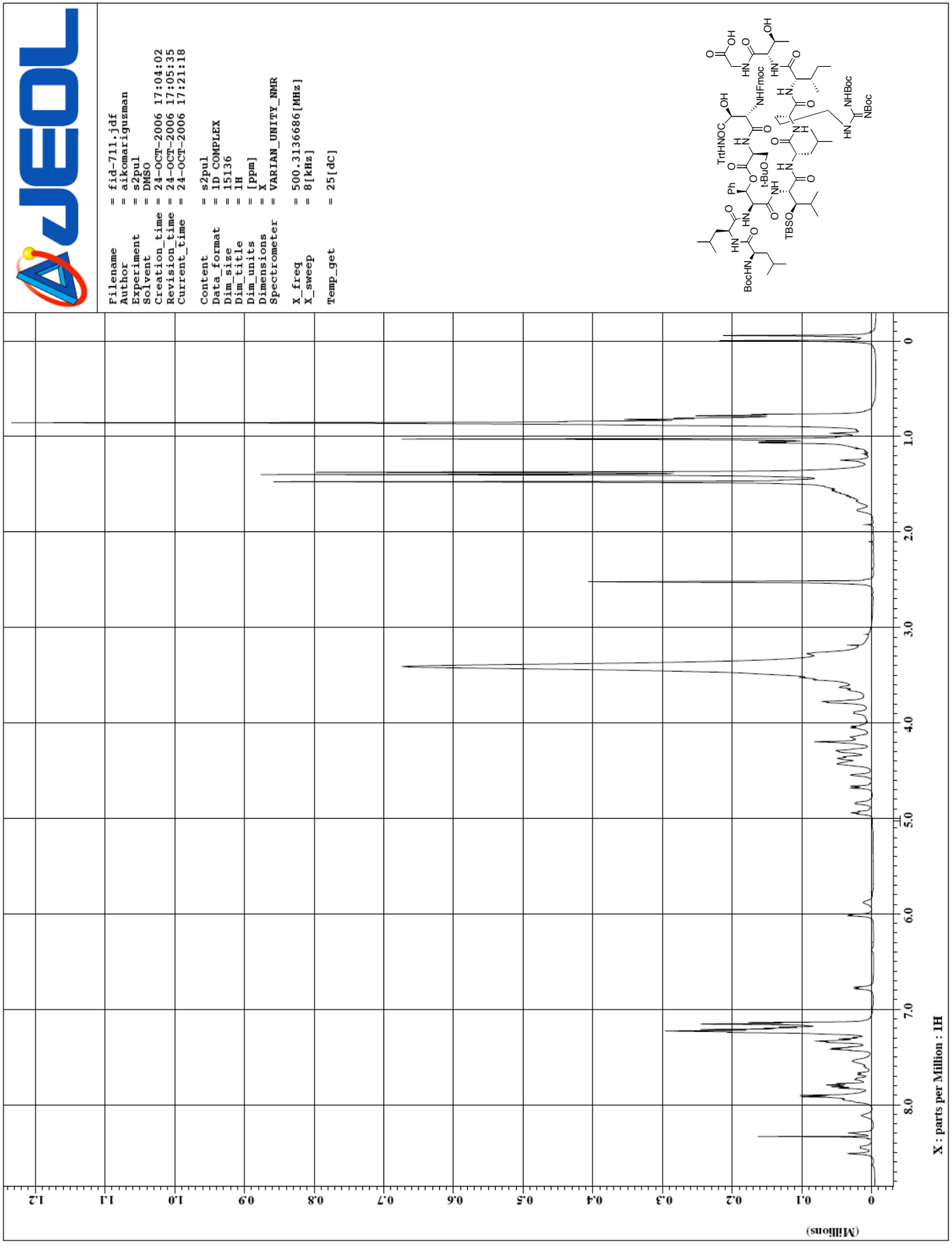

(Thousands)
400.0
300.0
200.0
100.0
0
X : parts per Million : 13C
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
AllylO
O
O
H
N
OH
O
HN
O
HN
O
NHFmoc
NBoc
BocHN
H
N

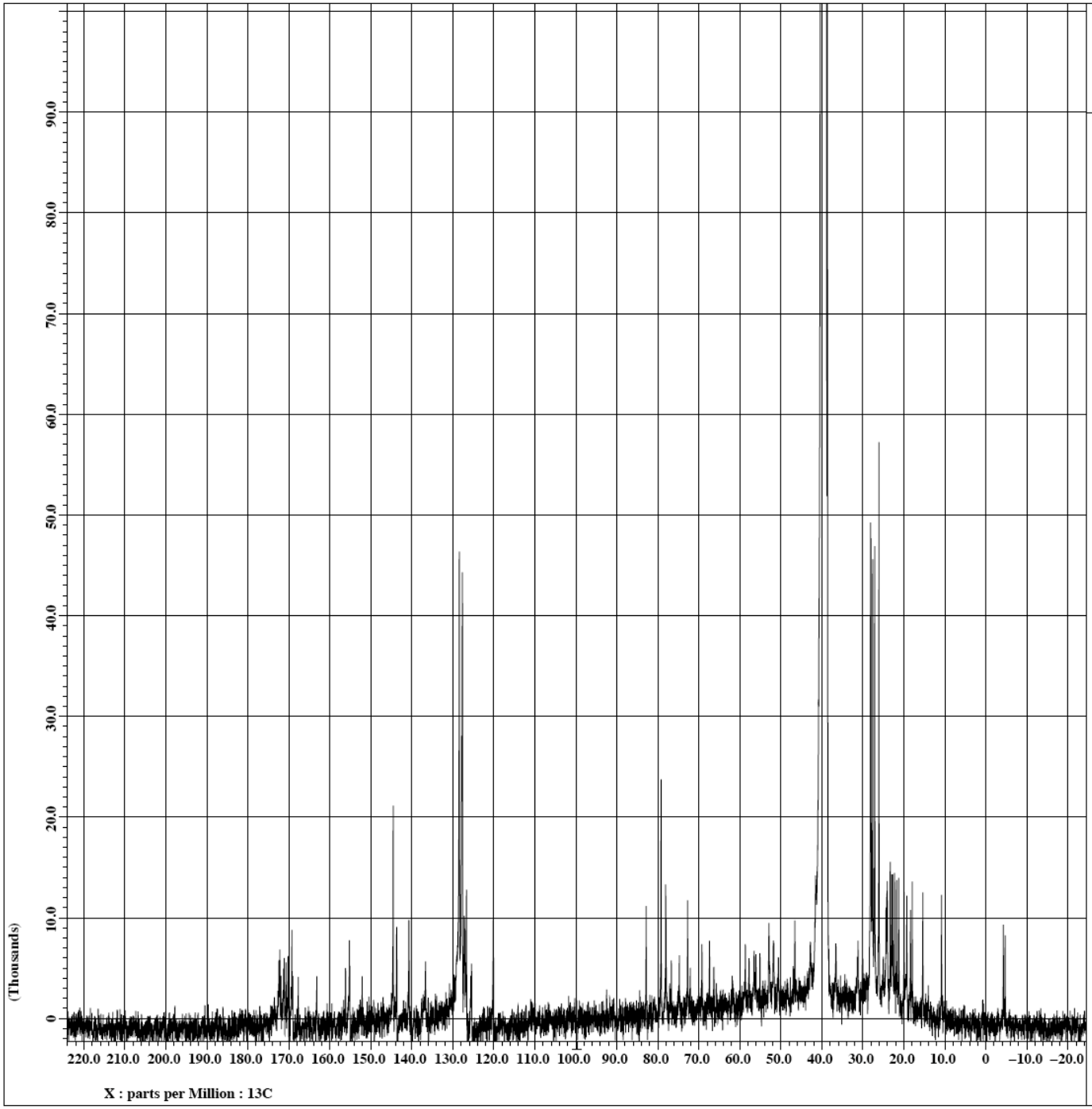

(Thousands)
X : parts per Million : 1H
AllylO
NBoc
BocHN
OH
NH2


(Thousands)
300.0
200.0
100.0
0
X : parts per Million : 13C
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
AllylO
O
O
H
N
OH
O
HN
O
HN
O
NH2
NBoc
BocHN
H
N


(Millions)
X : parts per Million : 1H

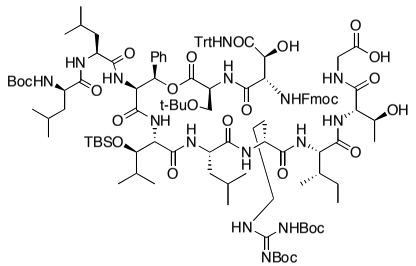

X : parts per Million : 13C
(Thousands)
BocHN
TrtHNOC
NHFmoc
t-BuO
TBSO
NHBoc
NBoc

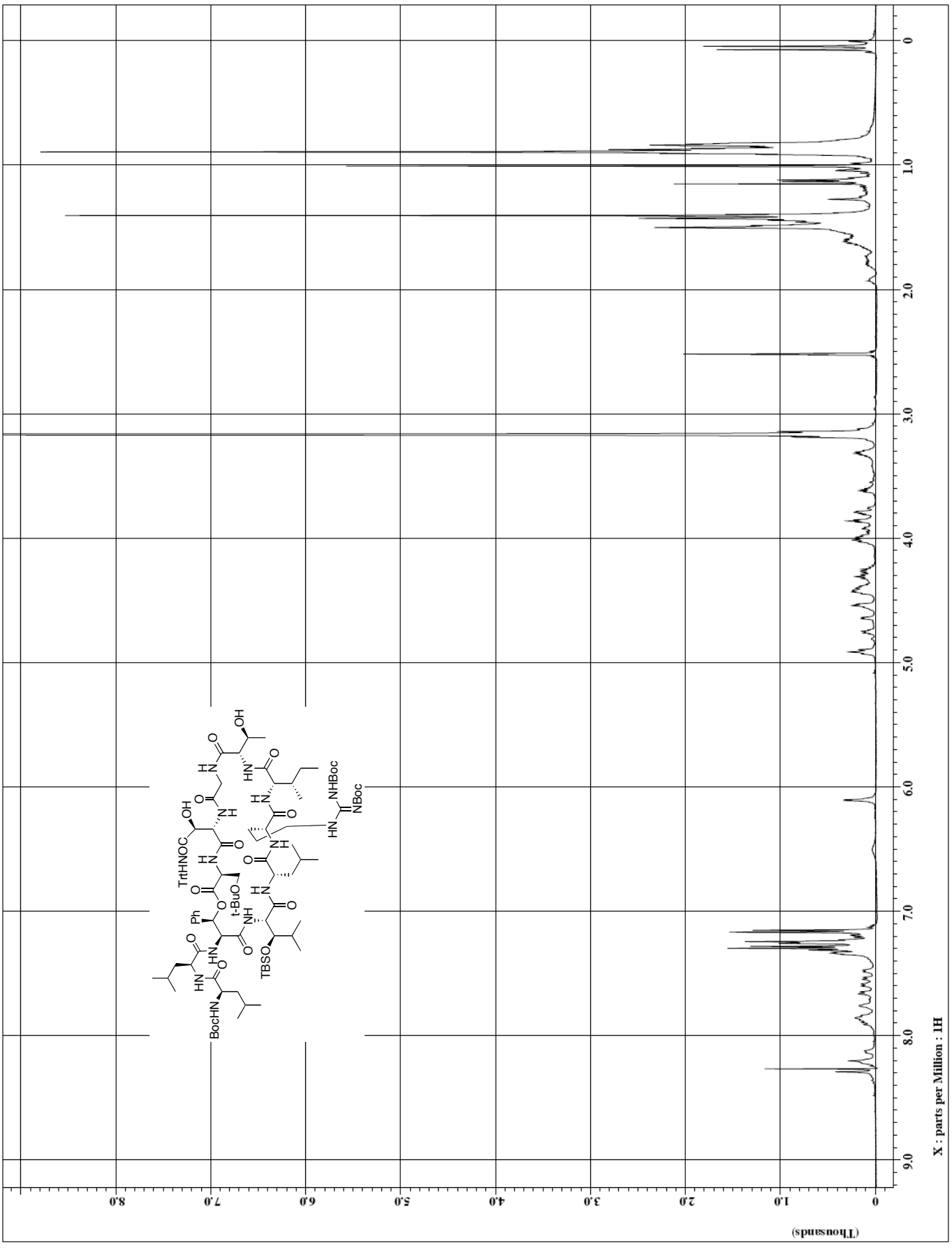

JEOL
Filename = fid-711.jdf
Author = aikomariguzman
Experiment = s2pul
Solvent = DMSO
Creation_time = 24-OCT-2006 17:04:02
Revision_time = 24-OCT-2006 17:05:35
Current_time = 24-OCT-2006 17:21:18
Content = s2pul
Data_format = 1D COMPLEX
Dim_size = 15136
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Spectrometer = VARIAN_UNITY_NMR
X_freq = 500.3136686[MHz]
X_sweep = 8[kHz]
Temp_get = 25[dC]
(Millions)
X : parts per Million : 1H

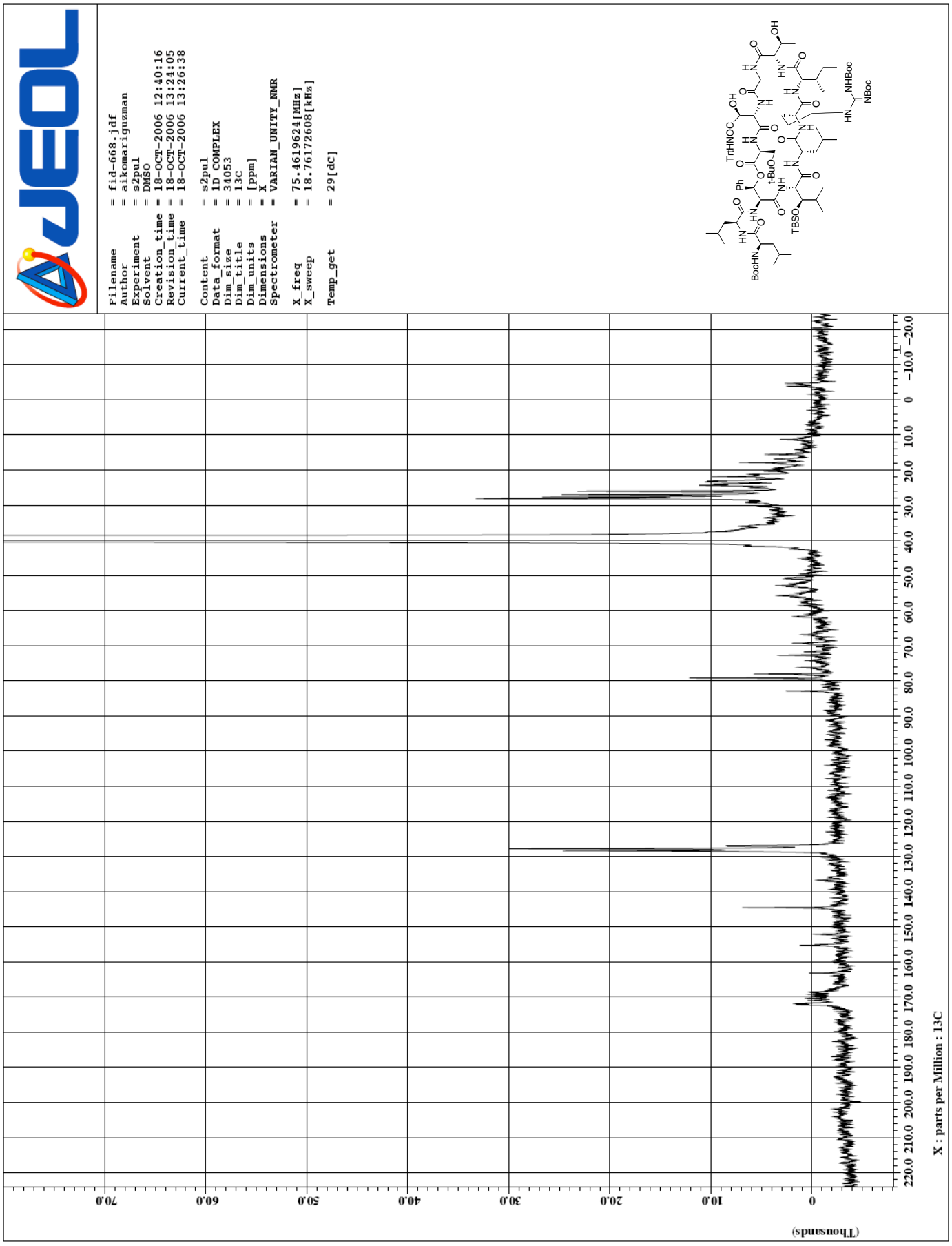


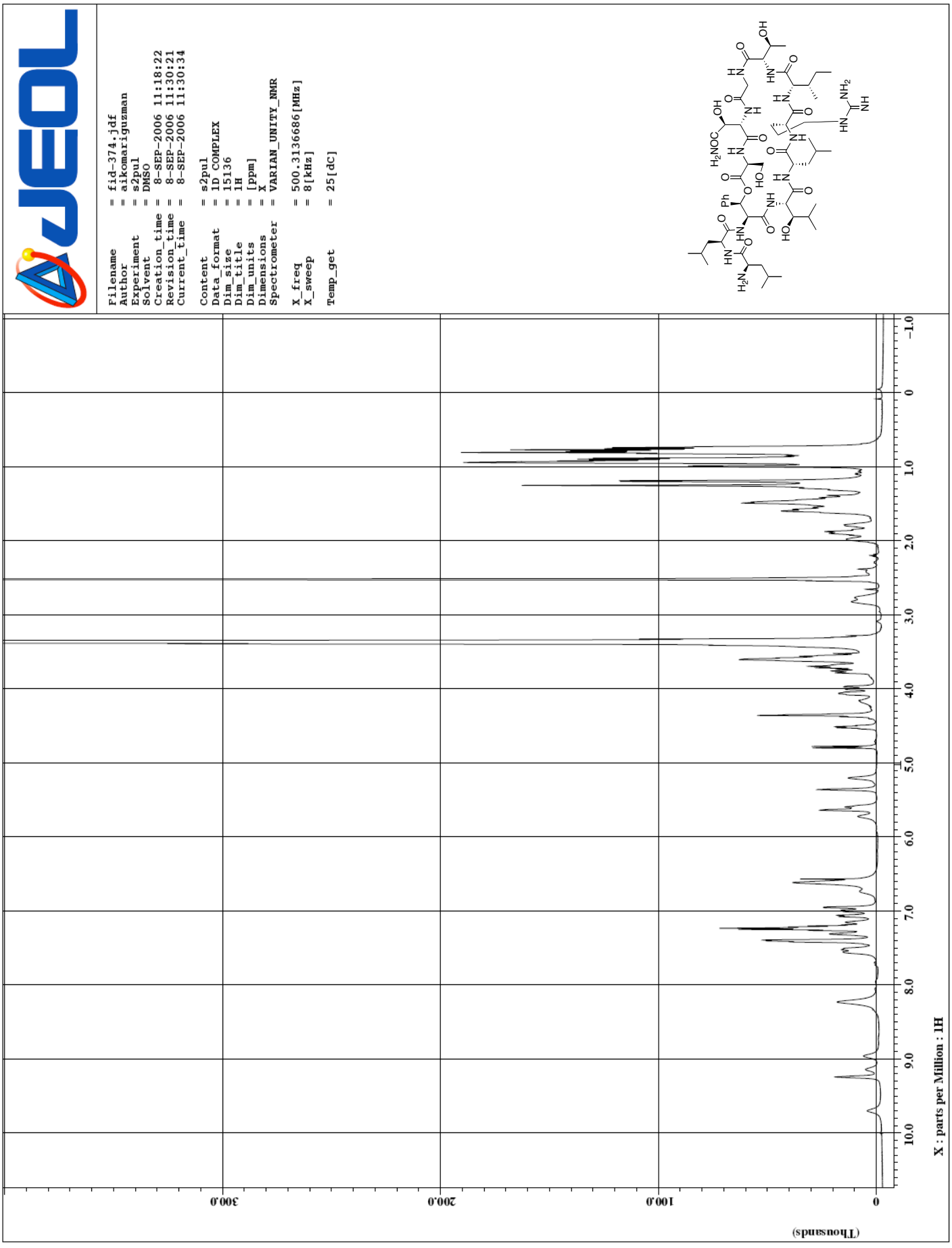
JEOL

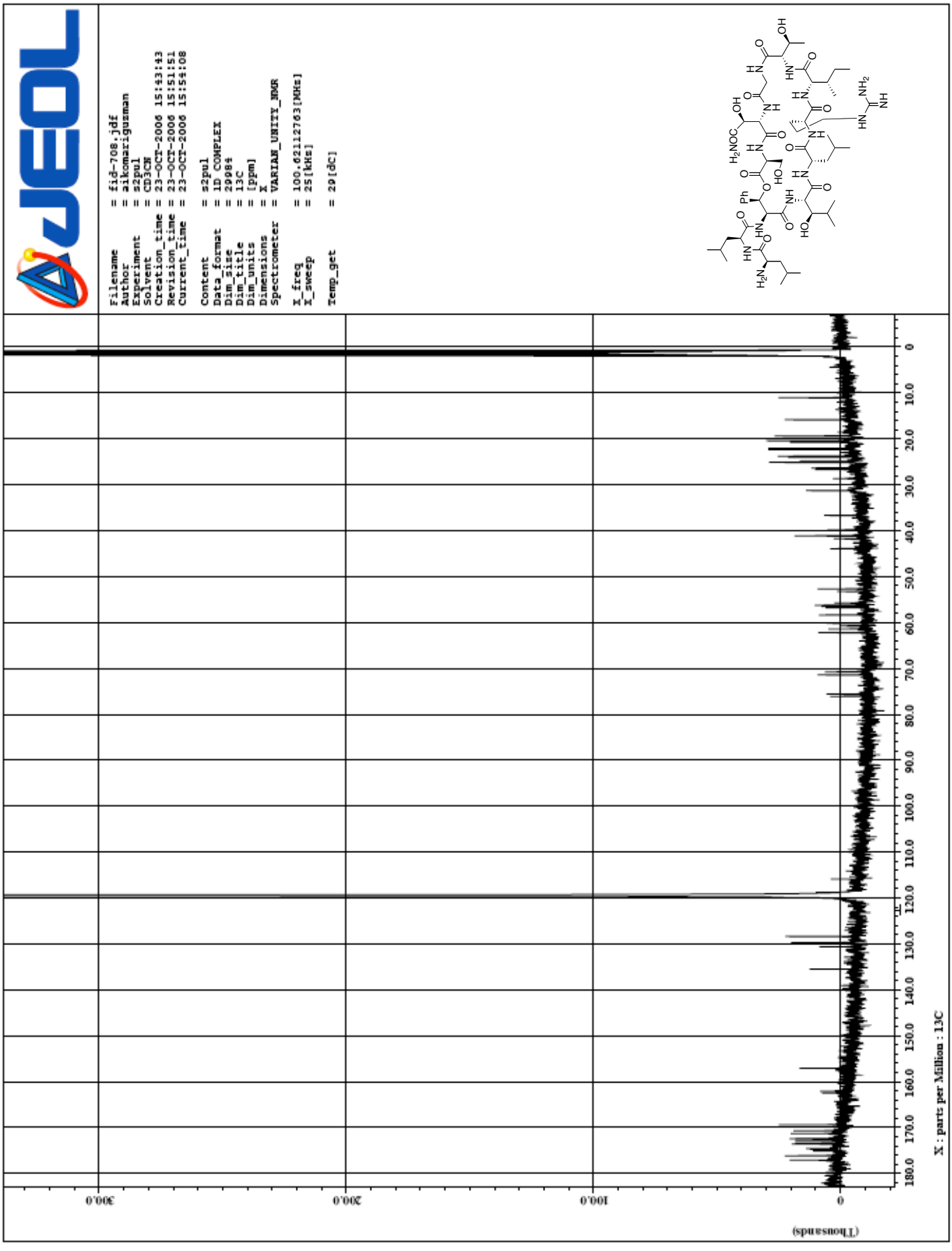
```
Filename       = fid-303.jdf
Author         = aikomariguzman
Experiment     = s2pul
Solvent        = DMSO
Creation_time  = 31-AUG-2006 09:32:46
Revision_time  = 31-AUG-2006 10:30:11
Current_time   = 31-AUG-2006 10:30:30

Content        = s2pul
Data_format    = 1D COMPLEX
Dim_size       = 34053
Dim_title      = 13C
Dim_units      = [ppm]
Dimensions     = X
Spectrometer   = VARIAN_UNITY_NMR

X_freq         = 75.4619624[MHz]
X_sweep        = 18.76172608[kHz]

Temp_get       = 29[dC]
```




(Thousands)
X : parts per Million : 1H


Filename = fid-668.jdf
Author = aikomariguzman
Experiment = s2pul
Solvent = DMSO
Creation_time = 18-OCT-2006 12:40:16
Revision_time = 18-OCT-2006 13:24:05
Current_time = 18-OCT-2006 13:26:38
Content = s2pul
Data_format = 1D COMPLEX
Dim_size = 34053
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Spectrometer = VARIAN_UNITY_NMR
X_freq = 75.4619624[MHz]
X_sweep = 18.76172608[kHz]
Temp_get = 29[dC]
BocHN
TBSO
tBuO
Ph
TrtHNOC
NHBoc
NBoc
(Thousands)
X : parts per Million : 13C


Filename = fid-374.jdf
Author = aikomariguzman
Experiment = s2pul
Solvent = DMSO
Creation_time = 8-SEP-2006 11:18:22
Revision_time = 8-SEP-2006 11:30:21
Current_time = 8-SEP-2006 11:30:34
Content = s2pul
Data_format = 1D COMPLEX
Dim_size = 15136
Dim_title = 1H
Dim_units = [ppm]
Dimensions = X
Spectrometer = VARIAN_UNITY_NMR
X_freq = 500.3136686[MHz]
X_sweep = 8[kHz]
Temp_get = 25[dC]
(Thousands)
300.0
200.0
100.0
0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
X : parts per Million : 1H


JEOL
Filename = fid-708.jdf
Author = aikomarigusman
Experiment = s2pul
Solvent = CD3CN
Creation_time = 23-OCT-2006 15:43:43
Revision_time = 23-OCT-2006 15:51:51
Current_time = 23-OCT-2006 15:54:08
Content = s2pul
Data_format = 1D COMPLEX
Dim_Size = 29984
Dim_title = 13C
Dim_units = [ppm]
Dimensions = X
Spectrometer = VARIAN_UNITY_NMR
X_freq = 100.62112763[MHz]
X_sweep = 25[kHz]
Temp_get = 29[dC]
(Thousands)
300.0
200.0
100.0
0
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
110.0
120.0
130.0
140.0
150.0
160.0
170.0
180.0
X : parts per Million : 13C