

Quillaja Saponin Variants with Central Glycosidic Linkage Modifications Exhibit Distinct Conformations and Adjuvant Activities

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[†]*Deceased March 22, 2011*

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A. SUPPLEMENTARY FIGURES S1–S10 AND TABLE S1

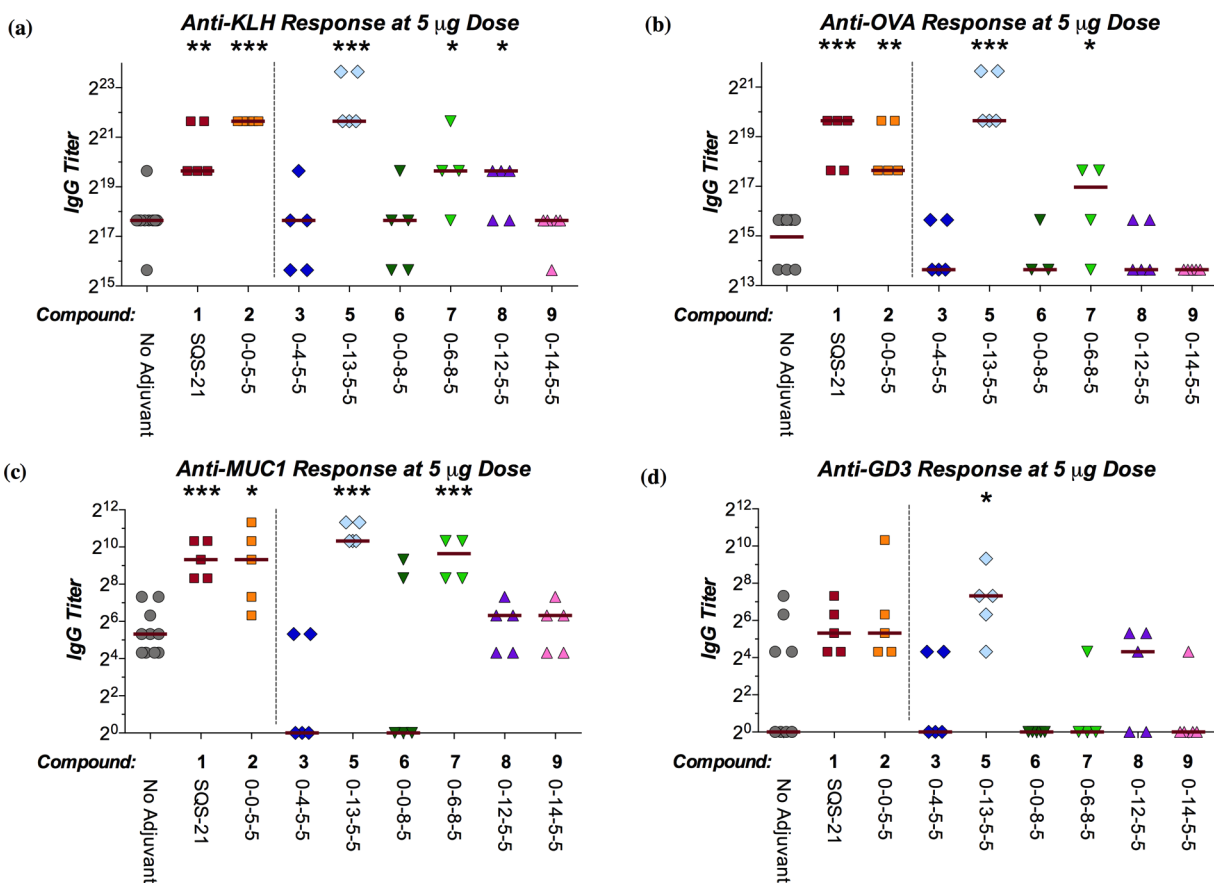


Figure S1. Adjuvant activity of central linkage variants in a preclinical mouse vaccination model. (a) Anti-KLH (IgG), (b) anti-OVA (IgG), (c) anti-MUC1 (IgG), and (d) Anti-GD3 (IgG) antibody titers at 5 µg dose of each saponin variant. Median titer values represented as black horizontal bars. Statistical significance compared to the no-adjuvant negative control assessed using unpaired Student's *t*-test with CI = 95%: * = 0.01 ≤ *p* ≤ 0.05, ** = 0.001 < *p* < 0.01, *** = *p* < 0.001.

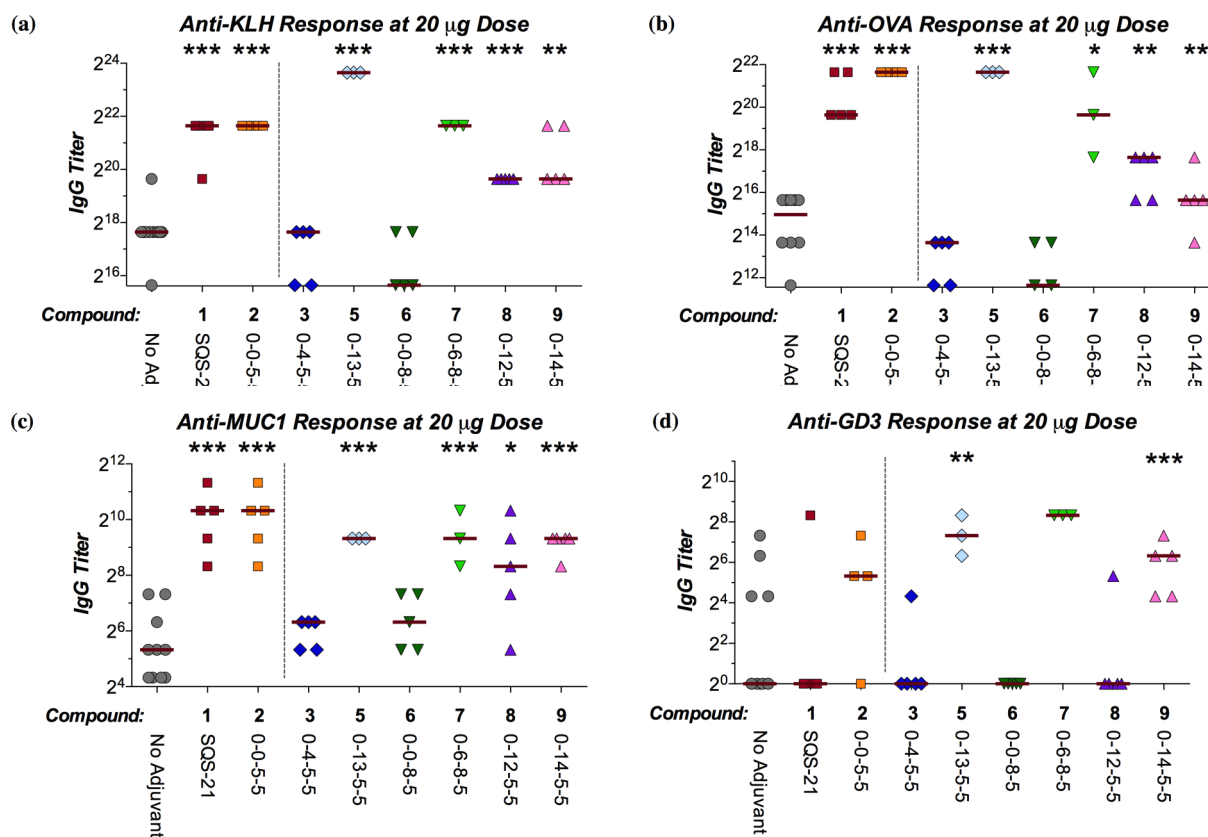


Figure S2. Adjuvant activity of central linkage variants in a preclinical mouse vaccination model. (a) Anti-KLH (IgG), (b) anti-OVA (IgG), (c) anti-MUC1 (IgG), and (d) Anti-GD3 (IgG) antibody titers at 20 µg dose of each saponin variant. Median titer values represented as black horizontal bars. Statistical significance compared to the no-adjuvant negative control assessed using unpaired Student's *t*-test with CI = 95%: * = 0.01 ≤ *p* ≤ 0.05, ** = 0.001 < *p* < 0.01, *** = *p* < 0.001.

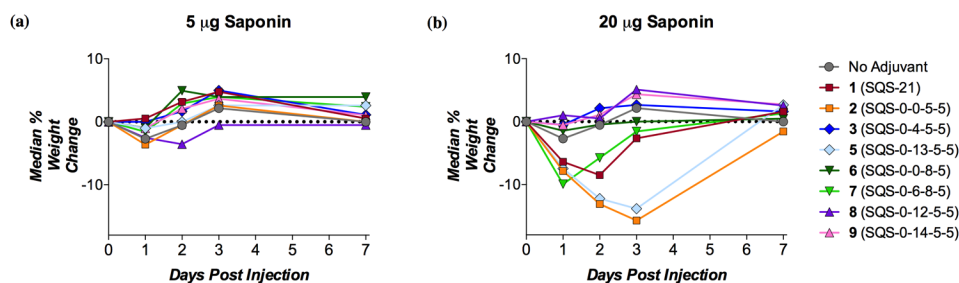


Figure S3. Toxicity assessment based on median percent weight loss over one week after the first vaccine injection. Assessments at (a) 5 µg saponin and (b) 20 µg saponin indicate minimal toxicity for all saponin analogues at 5 µg, and considerable toxicity for 1 (SQS-21), lead compound 2 (SQS-0-0-5-5), β-thioester 5 (SQS-0-13-5-5), and α-amide 7 (SQS-0-0-8-5) at the 20 µg dose.

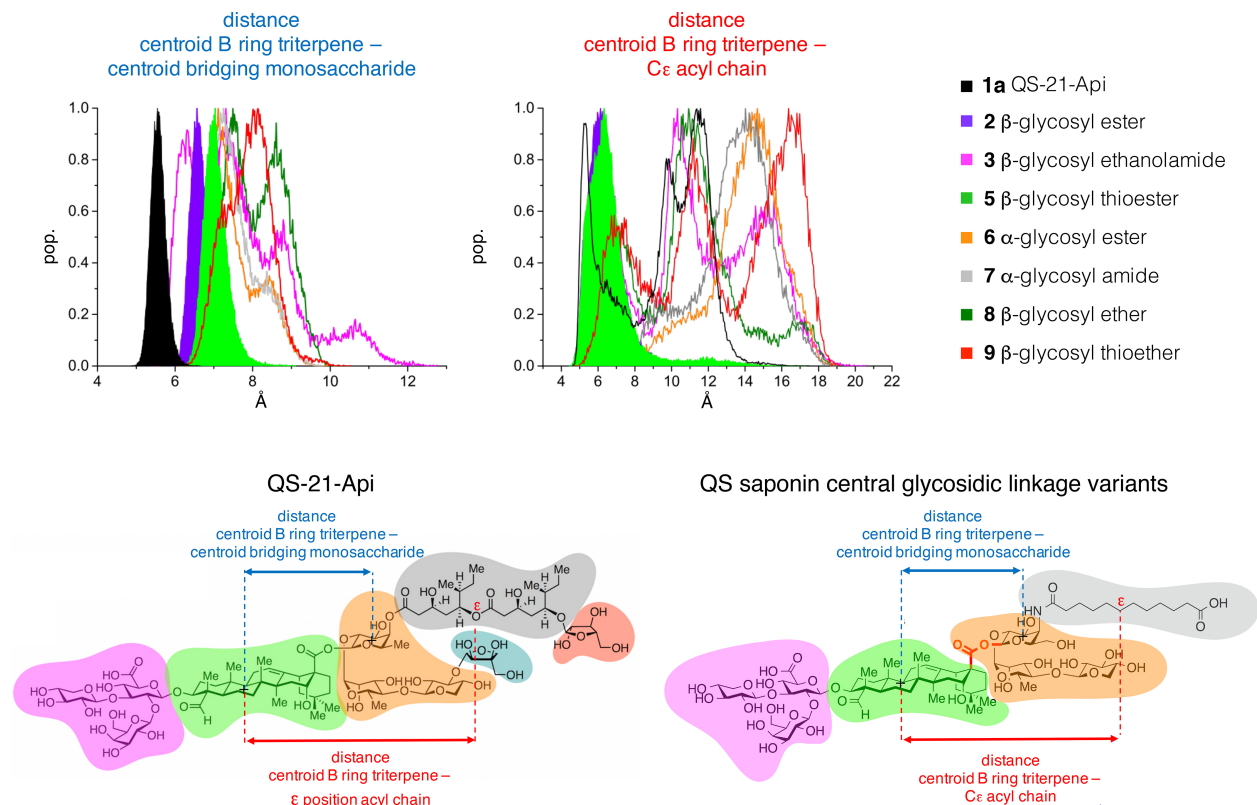


Figure S4. Ensemble measurement of diagnostic distances in saponin adjuvants. Populations from the 200 ns molecular dynamics simulations in explicit water with Na⁺ counterions shown for QS-21-Api (**1a**), β -glycosyl ester **2** (active), β -glycosyl ethanolamide **3** (inactive), β -glycosyl thioester **5** (active), α -glycosyl ester **6** (inactive), α -glycosyl amide **7** (attenuated), β -glycosyl ether **8** (attenuated), and β -glycosyl thioether **9** (attenuated).

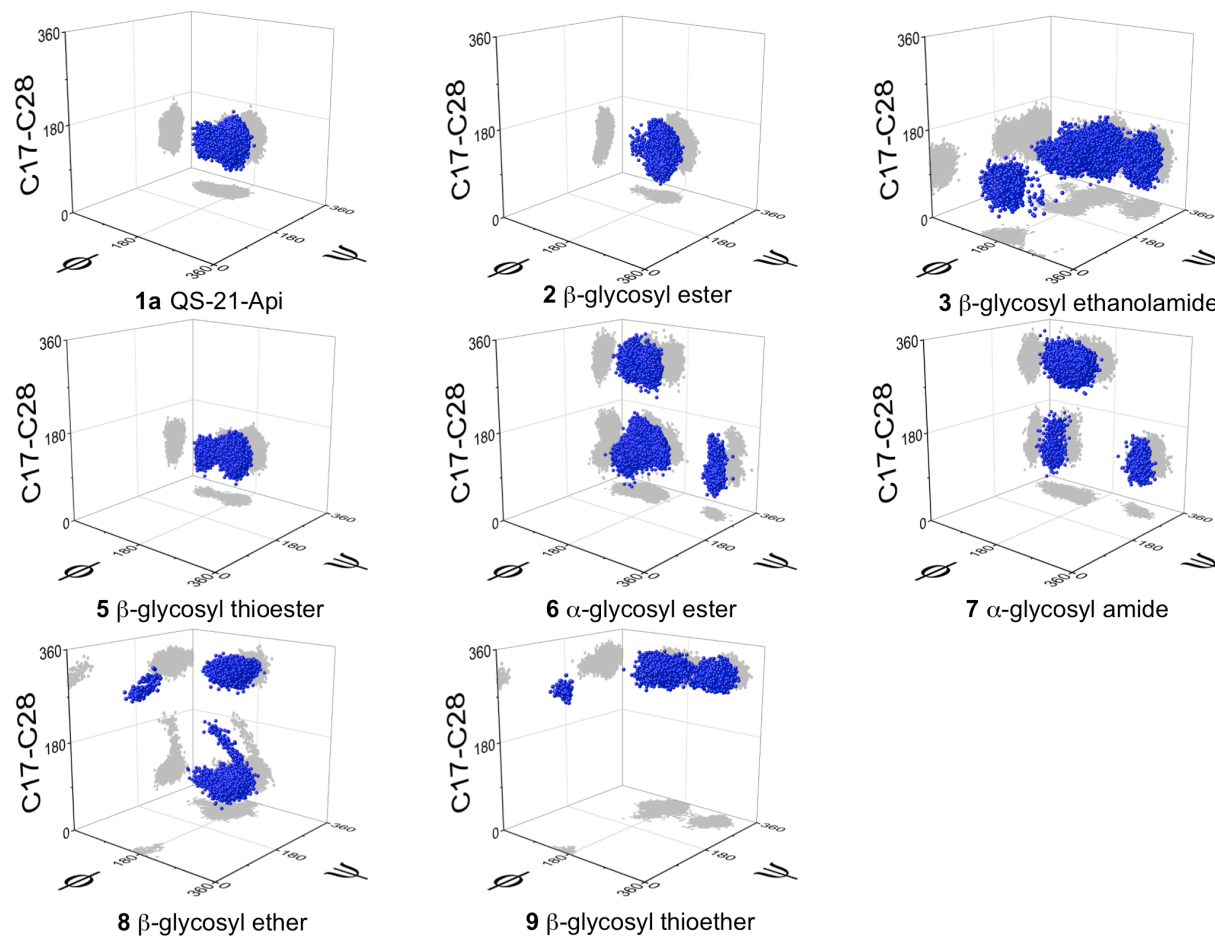


Figure S5. Torsional angle distributions of the central glycosidic linkage for QS-21-Api (1a) and saponin variants 2, 3, 5–9. The dihedral angles, ϕ , ψ , and C17–C28, which define the connection from C17 of the triterpene core to C1' of the bridging monosaccharide in the linear oligosaccharide domain, were obtained from the unrestrained molecular dynamics simulations in explicit water with Na^+ counterions. For clarity of presentation, the axes have been shifted to minimize the number of datapoints appearing on the $360^\circ \rightarrow 0^\circ$ radial transition (see Section E for details).

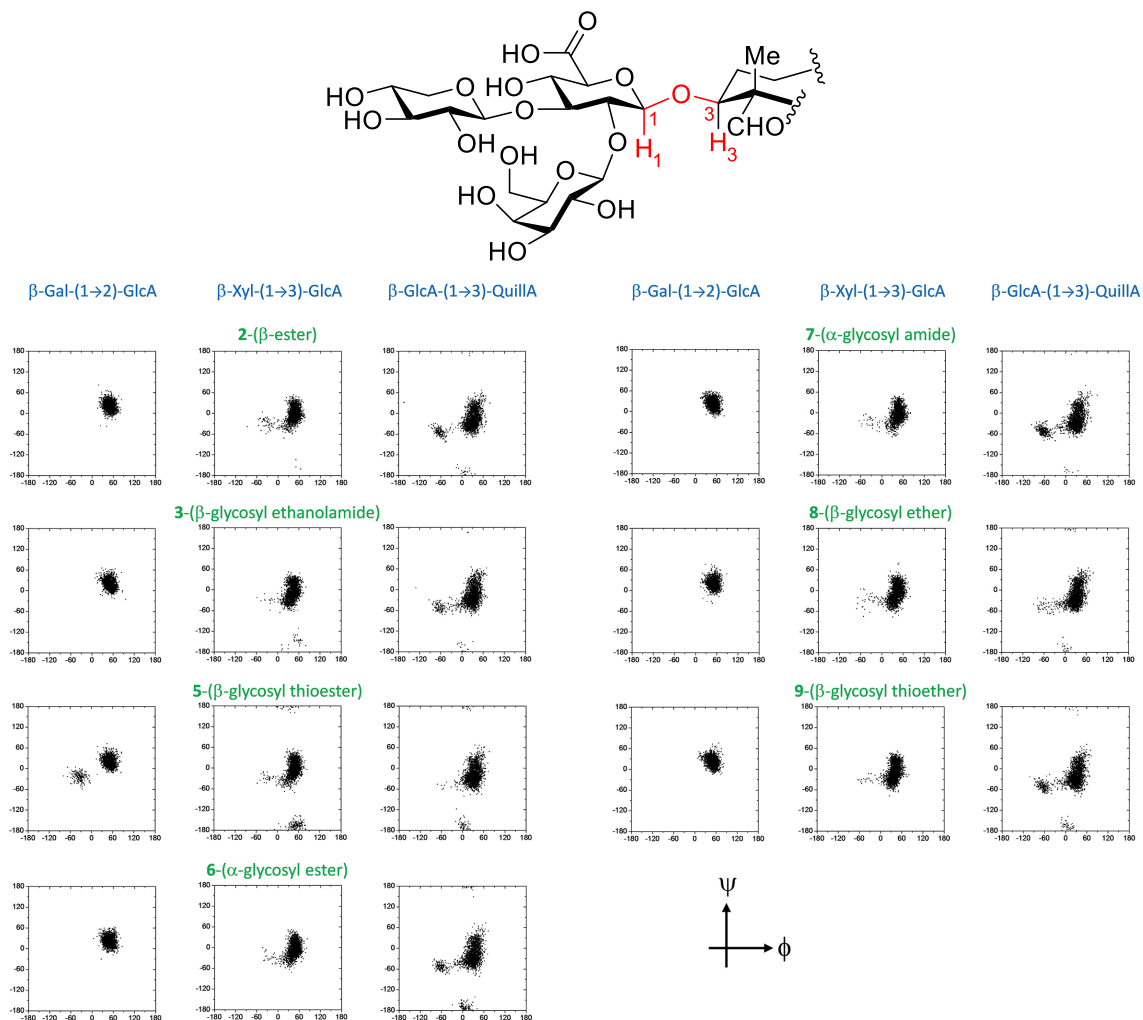


Figure S6. Torsional angle distributions of glycosidic linkages in the branched trisaccharide domain for saponin variants 2, 3, 5–9. The dihedral angles, ϕ and ψ , were obtained from the unrestrained molecular dynamics simulations in explicit water with Na^+ counterions. The dihedral angles were defined as exemplified for the β -GlcA-(1 \rightarrow 3)-QuillA linkage shown in red: $\phi = \text{H1-C1-O-C3}$, $\psi = \text{C1-O-C3-H3}$ (see Section E for details).

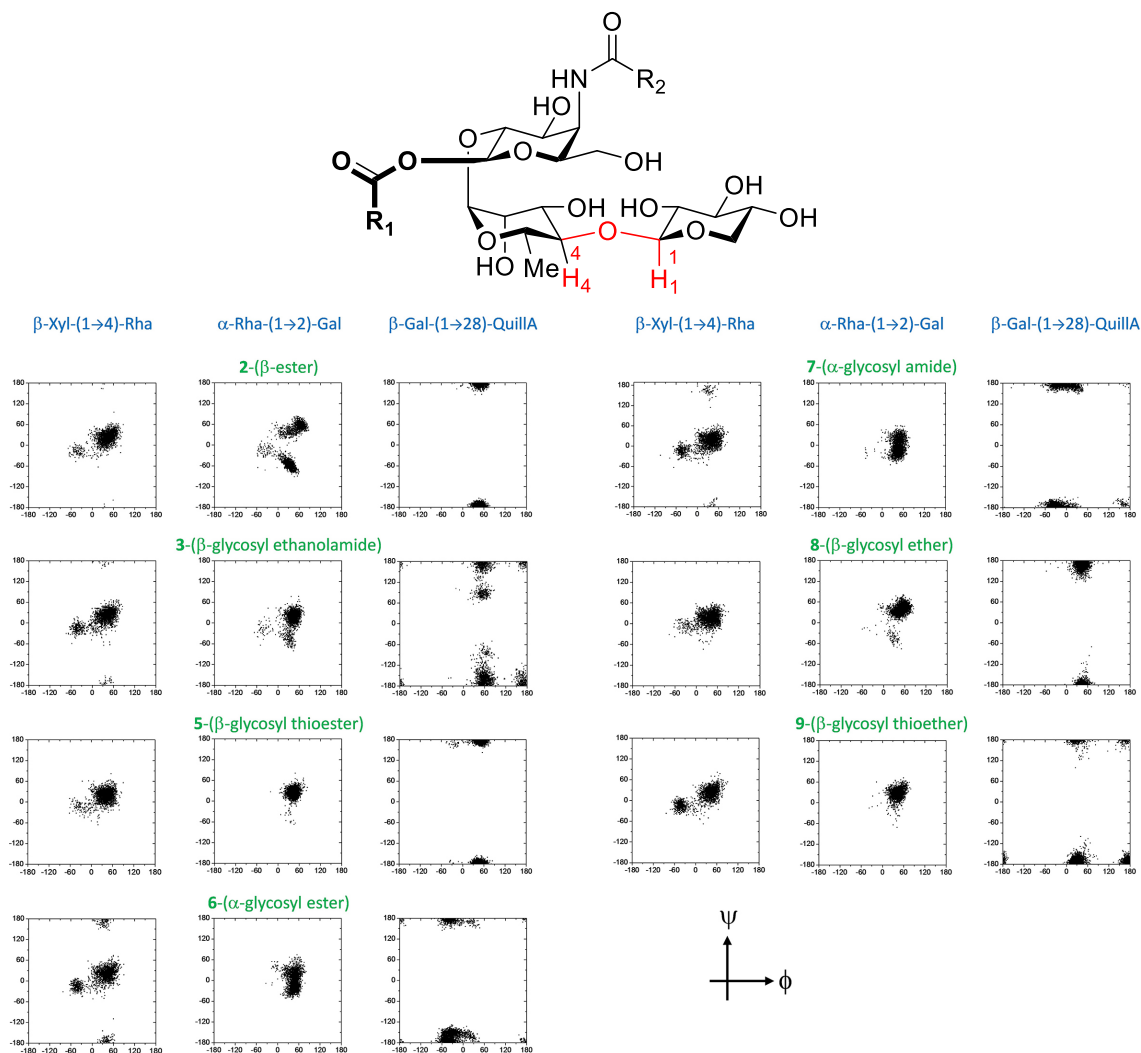


Figure S7. Torsional angle distributions of glycosidic linkages in the linear oligosaccharide domain for saponin variants 2, 3, 5–9. The dihedral angles, ϕ and ψ , were obtained from the unrestrained molecular dynamics simulations in explicit water with Na⁺ counterions. The dihedral angles were defined as exemplified for the β -Xyl-(1→4)-Rha linkage shown in red: $\phi = \text{H1-C1-O-C4}$, $\psi = \text{C1-O-C4-H4}$ (see Section E for details).

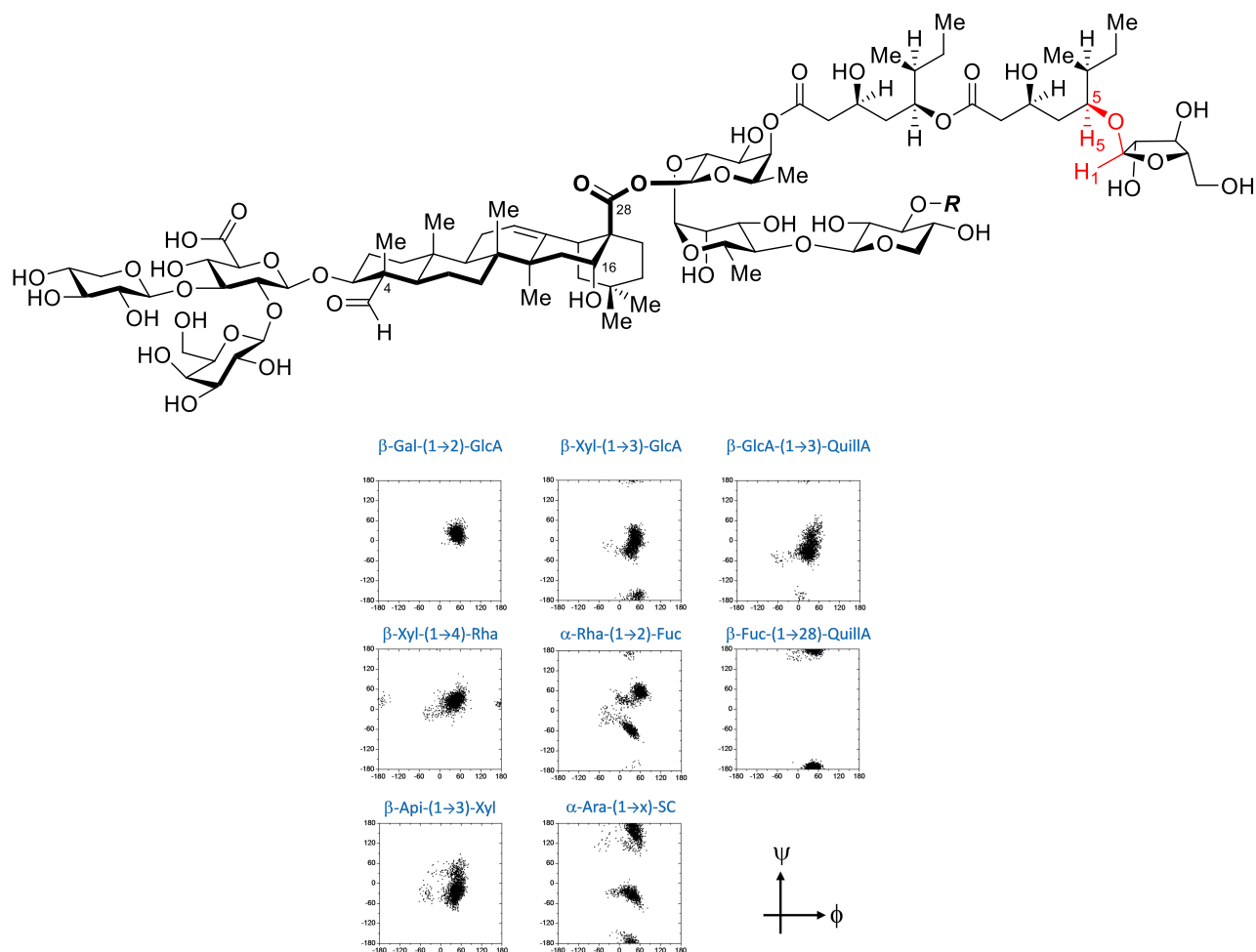


Figure S8. Torsional angle distributions of glycosidic linkages in the branched trisaccharide and linear tetrasaccharide domain for QS-21-Api (1a). The dihedral angles, ϕ and ψ , were obtained from the unrestrained molecular dynamics simulations in explicit water with Na^+ counterions. The dihedral angles were defined as exemplified for the α -Ara-(1→C5)-acyl-chain linkage shown in red: $\phi = \text{H1-C1-O-C5}$, $\psi = \text{C1-O-C5-H5}$ (see Section E for details).

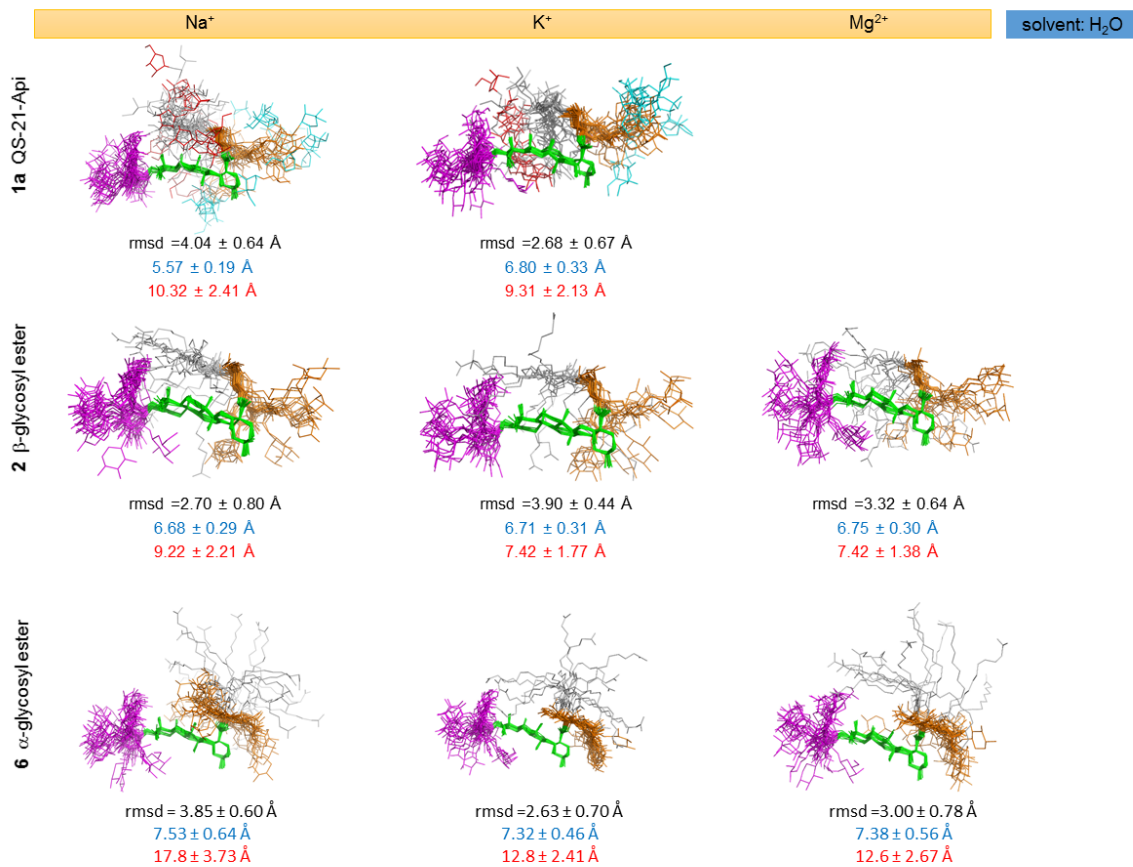


Figure S9. Molecular dynamics simulations with alternative cations. Ensembles were obtained from unrestrained 200 ns molecular dynamics simulations of QS-21-Api (**1a**), active β-glycosyl ester **2**, and inactive α-glycosyl ester **6** in explicit water with Na⁺, K⁺, or Mg²⁺ counterions.

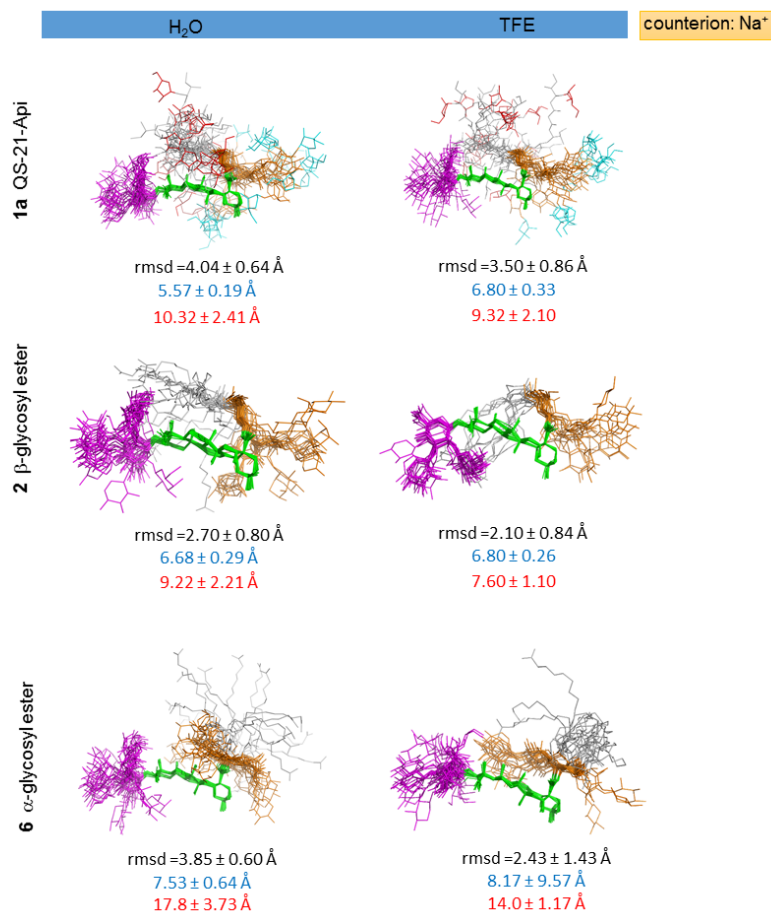


Figure S10. Molecular dynamics simulations in alternative solvent. Ensembles were obtained from unrestrained 200 ns molecular dynamics simulations of QS-21-Api (**1a**), active β-glycosyl ester **2**, and inactive α-glycosyl ester **6** in explicit water or trifluoroethanol with Na⁺ counterions.

Table S1. Survival of individual mice over 72-day vaccination schedule.

QS Saponin	Number surviving to Day 72	
	5 μg	20 μg
No adjuvant	5	5
QS-21 (1a/1b)	5	5
β-ester variant 2 (0-0-5-5)	5	5
β-ethanolamide variant 3 (0-4-5-5)	5	5
β-thioester variant 5 (0-13-5-5)	5	3*
α-ester variant 6 (0-0-8-5)	4 [†]	5
α-amide variant 7 (0-6-8-5)	5	5
β-ether variant 8 (0-12-5-5)	5	5
β-thioether variant 9 (0-14-5-5)	5	5

* Both mice found dead on Day 7.

[†] Found dead on Day 2.

B. MATERIALS AND METHODS

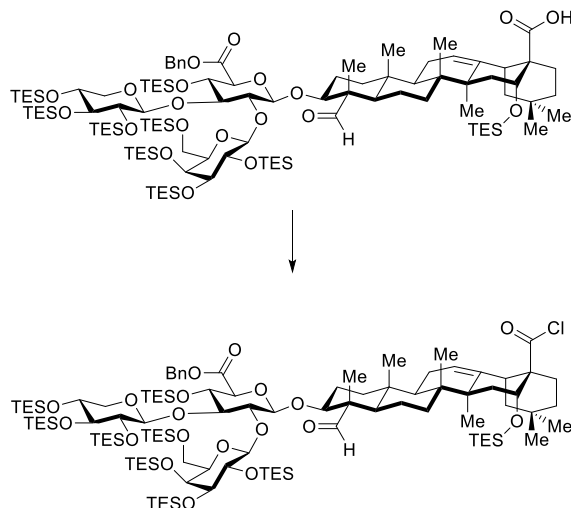
General Synthetic Procedures. Reactions were performed in flame-dried sealed-tubes or modified Schlenk (Kjeldahl shape) flasks fitted with a glass stopper under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe. The appropriate carbohydrate and sulfoxide reagents were dried via azeotropic removal of water with toluene. Molecular sieves were activated at 350 °C and were crushed immediately prior to use, then flame-dried under vacuum. Organic solutions were concentrated by rotary evaporation below 30 °C. Flash column chromatography was performed employing 230–400 mesh silica gel. Thin-layer chromatography was performed using glass plates pre-coated to a depth of 0.25 mm with 230–400 mesh silica gel impregnated with a fluorescent indicator (254 nm).

Materials. Dichloromethane, tetrahydrofuran, diethyl ether, and toluene were purified by passage through two packed columns of neutral alumina under an argon atmosphere.¹ Methanol was distilled from magnesium at 760 Torr. Trifluoromethanesulfonic anhydride was distilled from phosphorus pentoxide at 760 Torr. Boron trifluoride diethyl etherate was distilled from calcium hydride at 760 Torr. All other chemicals were obtained from commercial vendors and were used without further purification unless noted otherwise.

Instrumentation. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum BX spectrophotometer or a Bruker Tensor 27. Data are presented as the frequency of absorption (cm^{-1}). Proton and carbon-13 nuclear magnetic resonance (^1H NMR and ^{13}C NMR) spectra were recorded on a Bruker Avance III instrument; chemical shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane and are referenced to residual proton in the NMR solvent (d-chloroform: δ 7.26 for ^1H NMR, δ 77.16 for ^{13}C NMR; d6-benzene: δ 7.16 for ^1H NMR, δ 128.06 for ^{13}C NMR; d4-methanol: δ 3.31 for ^1H NMR, δ 49.00 for ^{13}C NMR; d3-acetonitrile: δ 1.94 for ^1H NMR, δ 1.32 for ^{13}C NMR; deuterium oxide: δ 4.79 for ^1H NMR). Data are presented as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration, assignment. RP-HPLC purification and analyses were carried out on a Waters 2545 binary gradient HPLC system equipped with a Waters 2996 photodiode array detector, and absorbances were monitored at wavelengths of 210–600 nm.

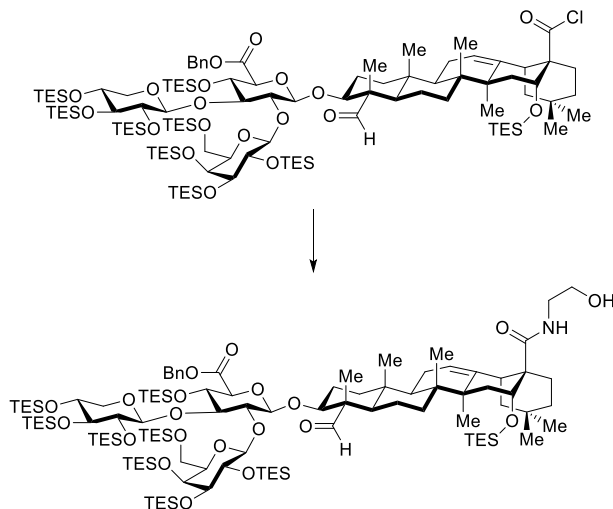
C. SYNTHESIS OF QS SAPONIN CENTRAL LINKAGE VARIANTS

1. SYNTHESIS OF β -ETHANOLAMIDE VARIANT 3 (SQS-0-4-5-5)



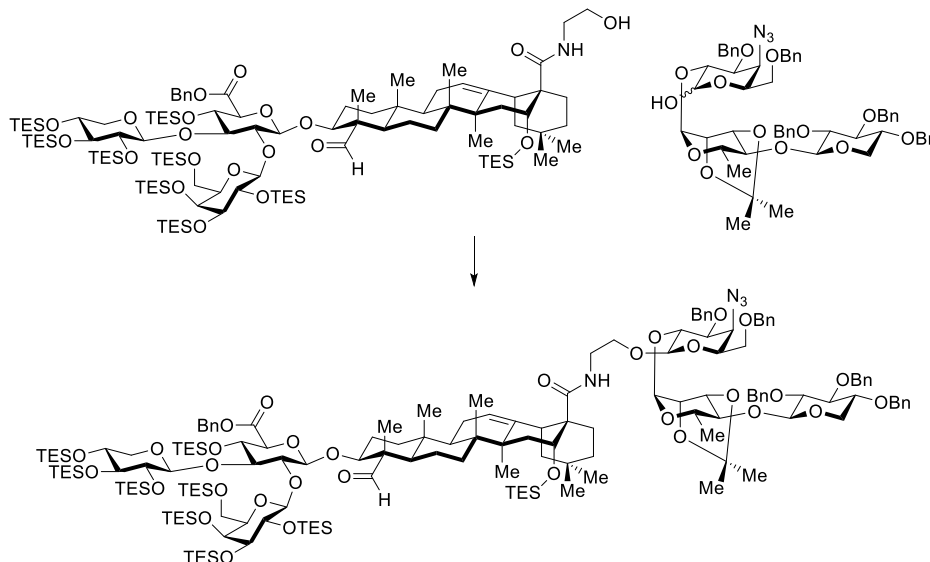
Protected prosapogenin acid chloride 12. Thionyl chloride (31 μ l, 0.425 mmol, 2 equiv) was added, drop-wise, to an ice-cooled solution of **10** (ref. 2) and pyridine (170 μ l, 2.13 mmol, 10 equiv) in dichloromethane (6 ml). After two hours, a majority of the volatiles were removed under a stream of nitrogen, then high-vacuum. Residual solids were suspended in anhydrous benzene and filtered through celite. Solvent removal *in vacuo* furnished **12** (441 mg, 99 % yield) as a white foam.

TLC R_f 0.36 (20:1 hexanes/ethyl acetate); **FTIR** (NaCl, film): 2953, 2911, 2877, 1792 (O–CCl st), 1756, 1725, 14.58, 1414, 1378, 1240, 1171, 1102, 1007, 739 cm^{-1} ; **$^1\text{H-NMR}$** (600 MHz, C_6D_6) δ 9.75 (s, 1H), 7.20 – 7.17 (m, 2H), 7.10 – 6.97 (m, 3H), 5.42 (t, $J = 3.5$ Hz, 1H), 5.18 (d, $J = 12.4$ Hz, 1H), 4.99 (dd, $J = 22.3, 9.9$ Hz, 2H), 4.79 – 4.71 (m, 2H), 4.59 (d, $J = 7.0$ Hz, 1H), 4.47 (t, $J = 8.7$ Hz, 1H), 4.37 (t, $J = 9.1$ Hz, 1H), 4.25 – 4.18 (m, 2H), 4.15 (d, $J = 9.4$ Hz, 1H), 4.13 – 4.08 (m, 1H), 4.05 (dd, $J = 11.2, 5.0$ Hz, 1H), 4.00 (dd, $J = 9.3, 7.4$ Hz, 1H), 3.95 (dd, $J = 9.5, 5.5$ Hz, 1H), 3.84 – 3.76 (m, 2H), 3.73 – 3.66 (m, 3H), 3.65 – 3.61 (m, 1H), 3.49 (t, $J = 10.8$ Hz, 1H), 3.16 (dd, $J = 14.1, 4.1$ Hz, 1H), 2.39 (t, $J = 13.6$ Hz, 1H), 1.97 – 1.67 (m, 8H), 1.58 (dd, $J = 10.2, 7.4$ Hz, 1H), 1.46 (m, 139H), 0.81 – 0.70 (m, 18H), 0.62 (dd, $J = 8.0, 3.0$ Hz, 7H). **$^{13}\text{C-NMR}$** (151 MHz, C_6D_6) δ 209.67, 177.78, 168.61, 141.76, 135.75, 128.54, 128.38, 128.24, 128.19, 123.96, 102.72, 101.57, 101.40, 83.84, 79.58, 79.26, 77.85, 77.00, 76.78, 76.32, 75.76, 75.32, 73.16, 73.01, 72.28, 71.69, 66.90, 65.97, 61.41, 59.13, 54.48, 49.01, 46.61, 46.53, 42.64, 41.61, 39.92, 37.92, 35.97, 35.25, 34.90, 32.58, 32.34, 30.61, 30.40, 26.59, 25.40, 24.13, 23.46, 20.43, 16.88, 15.66, 11.87, 7.82, 7.64, 7.51, 7.48, 7.45, 7.38, 7.37, 7.35, 7.27, 7.19, 7.07, 7.06, 6.29, 6.15, 6.08, 6.00, 5.95, 5.86, 5.81, 5.76, 5.68, 5.62, 5.48, 5.42, 5.33, 5.14, 4.96, 4.94, 4.77, 4.57; **HRMS** m/z (ESI): For methyl ester derivative, calcd for $\text{C}_{102}\text{H}_{210}\text{O}_{25}\text{NaSi}_9$ $[\text{M}+\text{Na}]^+$ 2110.2982, found 2110.2986.



Protected prosapogenin ethanolamide 13. Ethanolamine (29 μL , 0.478 mmol, 10 equiv) was added to an ice-cooled solution of acyl chloride **12** (100 mg, 0.0478 mmol), 1 equiv) in dichloromethane (2 mL). After 10 minutes, reaction was warmed to room temperature, concentrated, and purified by silica gel chromatography (hexanes:ethyl acetate, 4:1 to 2:1) to give ethanolamide **13** (89 mg, 88% yield).

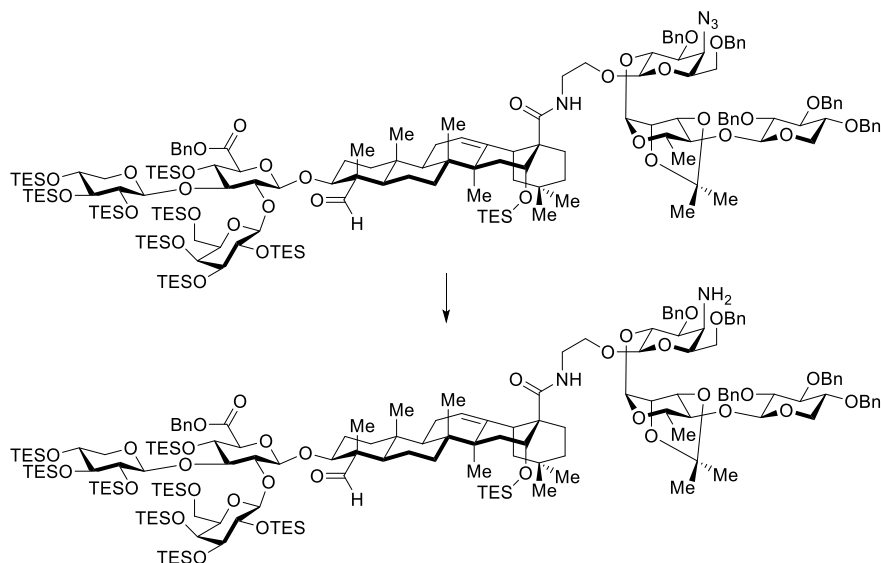
TLC R_f 0.32 (2:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 3407 (br), 2953, 2911, 2877, 1754, 1725, 1656, 1632, 1518, 1459, 1414, 1378, 1239, 1171, 1103, 1005, 971, 899, 864, 825, 799, 738, 695, 668 cm^{-1} ; **$^1\text{H-NMR}$** (600 MHz, CDCl_3) δ 9.72 (s, 1H), 7.40 – 7.29 (m, 5H), 6.54 (t, $J = 5.5$ Hz, 1H), 5.51 – 5.46 (m, 1H), 5.28 (d, $J = 12.4$ Hz, 1H), 5.10 (d, $J = 12.4$ Hz, 1H), 4.56 (d, $J = 7.4$ Hz, 1H), 4.53 – 4.50 (m, 1H), 4.43 (d, $J = 7.3$ Hz, 1H), 4.17 (d, $J = 7.4$ Hz, 1H), 3.95 – 3.89 (m, 2H), 3.88 – 3.77 (m, 4H), 3.75 (t, $J = 9.3$ Hz, 1H), 3.70 – 3.65 (m, 2H), 3.62 – 3.53 (m, 3H), 3.50 – 3.32 (m, 5H), 3.27 – 3.18 (m, 2H), 3.13 (t, $J = 10.9$ Hz, 1H), 3.03 (t, $J = 4.9$ Hz, 1H), 2.56 (dd, $J = 13.5, 4.1$ Hz, 1H), 2.37 (t, $J = 13.0$ Hz, 1H), 2.11 – 2.03 (m, 1H), 1.92 (dd, $J = 8.9, 3.6$ Hz, 2H), 1.88 – 1.76 (m, 2H), 1.74 – 1.66 (m, 2H), 1.66 – 1.49 (m, 5H), 1.46 – 1.36 (m, 6H), 1.31 (s, 3H), 1.29 – 1.24 (m, 2H), 1.17 – 1.07 (m, 5H), 1.07 – 0.88 (m, 97H), 0.81 – 0.54 (m, 61H); **$^{13}\text{C-NMR}$** (151 MHz, CDCl_3) δ 212.72, 179.86, 168.37, 144.93, 135.26, 128.45, 128.25, 128.12, 122.65, 103.68, 101.39, 100.83, 86.42, 78.79, 78.71, 76.44, 75.93, 75.89, 75.82, 75.80, 75.07, 72.60, 72.52, 71.37, 71.09, 66.84, 65.33, 62.93, 60.23, 53.79, 49.33, 49.08, 47.30, 45.95, 43.01, 41.88, 41.84, 39.65, 37.93, 36.02, 35.38, 34.07, 32.53, 31.81, 31.40, 30.53, 26.31, 25.36, 24.25, 23.42, 20.16, 16.72, 15.84, 12.26, 7.56, 7.47, 7.25, 7.16, 7.15, 7.13, 6.98, 6.85, 6.78, 5.92, 5.65, 5.44, 5.37, 5.34, 5.26, 5.23, 5.01, 4.42; **HRMS** (ESI) m/z : Calcd for $\text{C}_{110}\text{H}_{209}\text{NO}_{20}\text{NaSi}_9$ ($\text{M}+\text{Na}$) $^+$ 2139.3189, found 2139.3206.



Protected prosapogenin ethanamide trisaccharide azide 18. Trifluoromethanesulfonic anhydride (5.2 μL , 0.041, 1.5 equiv) was added to a solution of trisaccharide **17** (ref. 3) (20 mg, 0.021 mmol, 1.00 equiv), phenyl sulfoxide (12.5 mg, 0.061 mmol, 3.0 equiv) and 2,4,6-tri-*t*-butylpyridine (18 mg, 0.074 mmol, 3.6 equiv) in dichloromethane (1 mL) at $-78\text{ }^\circ\text{C}$. The reaction stirred in a cold bath at $-78\text{ }^\circ\text{C}$ for 5 min and then was transferred to a bath between $-40\text{ }^\circ\text{C}$ for 60 min. A solution of ethanamide **13** (84 mg, 0.040 mmol, 1.95 equiv) was added in dichloromethane (1.0 ml) via syringe. After 30 min, flask was transferred to an ice-bath and stirred for 15 min. Triethylamine was added, concentrated and purified via silica gel chromatography (hexanes:ethyl acetate, 10:1 to 2:1) furnishing β -glycoside **18** (55 mg, 87% yield) as a colorless film.

TLC R_f 0.67 (5:1 benzene/ethyl acetate); **FTIR** (NaCl, film) 3430, 2953, 2911, 2877, 2105, 1751, 1724, 1653, 1497, 1457, 1414, 1379, 1240, 1172, 1098, 1006, 910, 864, 826, 799, 737, 697, 666 cm^{-1} ; **$^1\text{H-NMR}$** (600 MHz, CDCl_3) δ 9.71 (s, 1H), 7.39 – 7.22 (m, 30H), 6.36 (t, $J = 5.5$ Hz, 1H), 5.43 (t, $J = 3.8$ Hz, 1H), 5.38 (s, 1H), 5.27 (d, $J = 12.3$ Hz, 1H), 5.10 (d, $J = 12.4$ Hz, 1H), 4.91 – 4.87 (m, 2H), 4.87 – 4.80 (m, 2H), 4.76 – 4.69 (m, 2H), 4.68 (d, $J = 11.1$ Hz, 1H), 4.61 (d, $J = 11.7$ Hz, 1H), 4.58 – 4.52 (m, 4H), 4.51 (d, $J = 3.0$ Hz, 1H), 4.43 (d, $J = 7.2$ Hz, 1H), 4.20 (d, $J = 7.5$ Hz, 2H), 4.15 (dd, $J = 7.4, 5.5$ Hz, 1H), 4.07 (d, $J = 3.5$ Hz, 1H), 4.04 (d, $J = 5.6$ Hz, 1H), 3.96 – 3.91 (m, 3H), 3.89 – 3.79 (m, 6H), 3.77 (t, $J = 9.2$ Hz, 1H), 3.69 (ddd, $J = 10.8, 6.8, 4.7$ Hz, 1H), 3.65 – 3.53 (m, 11H), 3.49 (ddd, $J = 10.4, 8.4, 5.1$ Hz, 1H), 3.40 (dd, $J = 9.4, 2.5$ Hz, 1H), 3.38 – 3.17 (m, 7H), 3.14 (t, $J = 11.0$ Hz, 1H), 2.52 (dd, $J = 13.7, 4.5$ Hz, 1H), 2.34 (t, $J = 13.0$ Hz, 1H), 2.02 – 1.51 (m, 12H), 1.42 – 1.38 (m, 1H), 1.37 (s, 3H), 1.34 (s, 3H), 1.31 (s, 3H), 1.22 (d, $J = 6.0$ Hz, 3H), 1.13 – 1.03 (m, 4H), 1.03 – 0.90 (m, 82H), 0.89 (s, 3H), 0.87 (s, 3H), 0.81 – 0.56 (m, 56H); **$^{13}\text{C-NMR}$** (151 MHz, CDCl_3) δ 212.60, 177.85, 168.36, 144.49, 138.81, 138.73, 138.23, 137.41, 136.89, 135.20, 128.58, 128.55, 128.47, 128.41, 128.32, 128.26, 128.24, 128.21, 128.18, 128.16, 128.07, 127.95, 127.88, 127.82, 127.77, 127.48, 122.56, 109.12, 103.64, 102.86, 102.06, 101.38, 100.82, 98.24, 86.43, 83.87, 81.87, 81.39, 78.81, 78.71, 78.25, 78.12, 77.95, 76.45, 75.99, 75.97, 75.92, 75.81, 75.49, 75.07, 74.87, 74.62, 73.71, 73.21, 72.61, 72.51, 71.83, 71.38, 71.09, 68.76, 68.44, 66.87, 65.33, 64.56, 63.81, 60.24, 58.36, 53.82, 49.25, 49.12, 47.22, 46.06, 41.85, 41.73, 39.94, 39.57, 37.97, 36.06, 35.95, 35.39, 34.66, 34.53, 34.07,

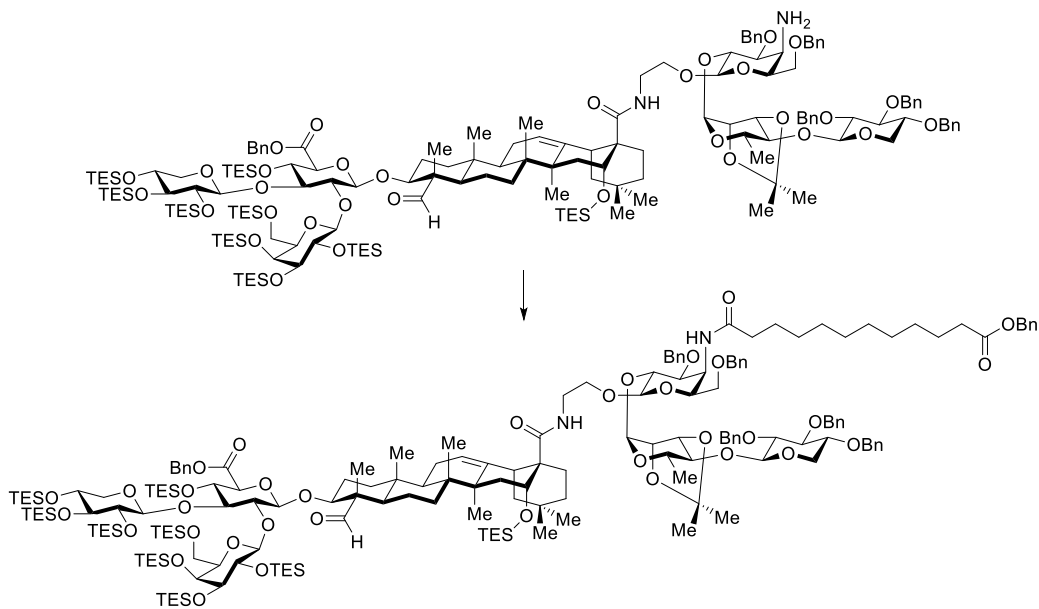
32.59, 31.98, 31.59, 31.42, 30.48, 29.06, 27.80, 26.91, 26.49, 26.30, 25.35, 25.27, 24.38, 23.35, 22.66, 20.70, 20.21, 18.77, 17.62, 16.94, 15.75, 14.14, 12.23, 11.45, 7.56, 7.46, 7.25, 7.17, 7.16, 7.13, 7.09, 6.99, 6.89, 6.85, 6.78, 5.92, 5.64, 5.44, 5.37, 5.34, 5.26, 5.23, 5.20, 5.18, 5.14, 5.06, 5.01, 4.41; **HRMS** (ESI) m/z : Calcd for $C_{165}H_{270}N_4O_{32}NaSi_9$ ($M+Na$)⁺ 3094.7445, found 3094.7344.



Protected prosapogenin ethanolamide trisaccharide amine S1. An excess of hydrogen sulfide was bubbled through an ice-cooled solution of azide **18** (35 mg, 0.011, 1 equiv) in pyridine and triethylamine (3:1, 2 mL) for two min via steel needle, then needle removed from septum. After stirring for 2 min, ice-bath was removed and warmed to ambient temperature. After 4.5 h, the dark green solution was purged of excess hydrogen sulfide, then volatiles removed with a stream of nitrogen. The resulting light-orange solid was purified by silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 8:1 to 1:1) to give amine **S1** (27 mg, 78% yield).

TLC R_f 0.44 (3% methanol/dichloromethane); **FTIR** (NaCl film) 3422, 3031, 2953, 2910, 2876, 1751, 1734, 1719, 1653, 1648, 1507, 1496, 1465, 1457, 1454, 1419, 1413, 1379, 1240, 1097, 1008, 908, 863, 825, 734, 697, 668 cm^{-1} ; **¹H-NMR** (600 MHz, $CDCl_3$) δ 9.70 (s, 1H), 7.39 – 7.22 (m, 30H), 6.31 (t, $J = 5.5$ Hz, 1H), 5.42 – 5.36 (m, 2H), 5.27 (d, $J = 12.4$ Hz, 1H), 5.09 (d, $J = 12.4$ Hz, 1H), 4.91 – 4.87 (m, 2H), 4.83 (q, $J = 11.1$ Hz, 2H), 4.73 – 4.64 (m, 3H), 4.61 (d, $J = 11.7$ Hz, 1H), 4.58 – 4.54 (m, 3H), 4.54 – 4.51 (m, 1H), 4.49 (d, $J = 11.6$ Hz, 1H), 4.42 (d, $J = 7.2$ Hz, 1H), 4.23 (d, $J = 7.7$ Hz, 1H), 4.20 – 4.14 (m, 2H), 4.07 (d, $J = 5.6$ Hz, 1H), 3.96 – 3.90 (m, 3H), 3.88 – 3.78 (m, 5H), 3.78 – 3.67 (m, 4H), 3.67 – 3.63 (m, 1H), 3.63 – 3.53 (m, 8H), 3.51 – 3.43 (m, 2H), 3.43 – 3.29 (m, 6H), 3.28 – 3.23 (m, 2H), 3.22 – 3.17 (m, 1H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.52 (dd, $J = 13.3, 2.9$ Hz, 1H), 2.33 (t, $J = 13.0$ Hz, 1H), 2.02 – 1.94 (m, 1H), 1.89 – 1.76 (m, 4H), 1.72 – 1.54 (m, 3H), 1.48 – 1.38 (m, 2H), 1.38 – 1.32 (m, 8H), 1.29 (s, 3H), 1.21 (d, $J = 6.0$ Hz, 3H), 1.10 – 1.02 (m, 4H), 1.02 – 0.90 (m, 83H), 0.89 – 0.86 (m, 9H), 0.81 – 0.55 (m, 58H); **¹³C-NMR** (151 MHz, $CDCl_3$) δ 212.57, 177.70, 168.37, 144.75, 138.81, 138.70, 138.23, 137.78, 137.34, 135.20, 131.04, 129.31, 128.53, 128.48, 128.46, 128.41, 128.34, 128.30, 128.26, 128.25, 128.18, 128.15, 128.13, 128.05, 128.03, 128.00, 127.97, 127.94, 127.88, 127.86, 127.84, 127.81, 127.76, 127.70, 127.51, 127.48, 124.77, 122.23, 109.10, 103.65, 103.09, 102.06, 101.37, 100.82, 98.07, 86.39, 83.86, 82.07, 81.86, 78.78, 78.70, 78.25, 78.07, 77.96, 76.44,

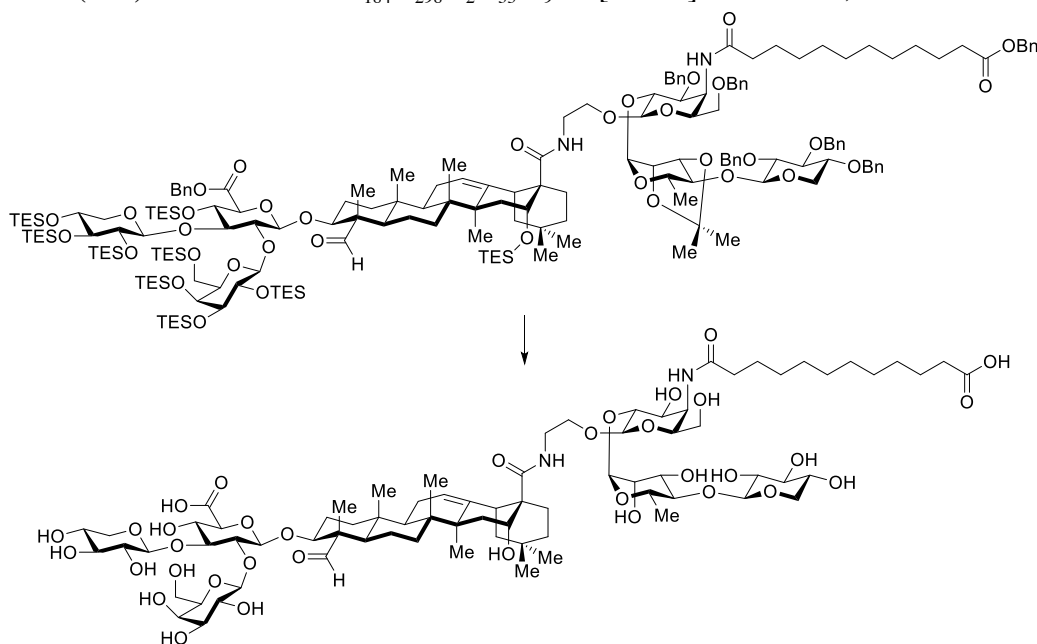
76.06, 75.93, 75.88, 75.82, 75.80, 75.49, 75.06, 74.65, 74.58, 73.59, 73.35, 73.21, 72.60, 72.51, 71.36, 71.10, 71.01, 69.35, 68.65, 66.88, 65.32, 64.51, 63.80, 60.23, 53.79, 49.27, 49.00, 48.74, 47.18, 45.97, 39.89, 39.56, 37.94, 35.95, 35.35, 34.03, 32.58, 31.91, 31.49, 30.49, 27.82, 26.50, 26.29, 25.34, 24.34, 23.44, 20.18, 17.53, 16.81, 15.92, 12.23, 7.55, 7.46, 7.25, 7.17, 7.16, 7.13, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.37, 5.33, 5.25, 5.22, 5.01, 4.41; **HRMS** (ESI) m/z : Calcd for $C_{165}H_{273}N_2O_{32}Si_9$ (M+H) 3046.7720, found 3046.7788.



Protected ethanolamide variant S3. Isobutyl chloroformate (6.1 μ L, 0.047 mmol, 3.0 equiv) was added to an ice-cooled solution of 12-(benzyloxy)-12-oxododecanoic acid³ **S2** (30 mg, 0.094 mmol, 6 equiv) and triethylamine (22 μ L, 0.156 mmol, 10 equiv) in tetrahydrofuran (3.0 mL) and stirred for 4 hours, then transferred via cannula to an ice-cooled solution of amine **S1** (42 mg, 0.013 mmol, 1 equiv) in tetrahydrofuran (2.0 mL). After 24 h, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 \times 25 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give fully protected ethanolamide analogue **S2** (44 mg, 84 % yield) as a colorless film.

TLC R_f 0.37 (3:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3582, 3417, 3090, 3063, 3030, 2952, 2911, 2876, 1738, 1727, 1657, 1547, 1512, 1498, 1454, 1413, 1379, 1240, 1166, 1094, 1069, 1007, 910, 863, 823, 799, 731, 696 cm^{-1} ; **¹H-NMR** (600 MHz, $CDCl_3$) δ 9.70 (s, 1H), 7.39 – 7.24 (m, 35H), 6.16 (t, J = 5.6 Hz, 1H), 5.45 (d, J = 10.0 Hz, 1H), 5.40 (s, 1H), 5.31 (t, J = 3.7 Hz, 1H), 5.25 (d, J = 12.4 Hz, 1H), 5.14 – 5.07 (m, 3H), 4.91 – 4.79 (m, 5H), 4.74 (d, J = 11.4 Hz, 1H), 4.71 (d, J = 11.7 Hz, 1H), 4.67 (d, J = 11.0 Hz, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.56 (d, J = 7.4 Hz, 1H), 4.54 – 4.46 (m, 3H), 4.45 – 4.39 (m, 2H), 4.25 (d, J = 7.5 Hz, 1H), 4.20 – 4.14 (m, 2H), 4.07 (d, J = 5.6 Hz, 1H), 3.95 – 3.89 (m, 3H), 3.88 – 3.78 (m, 5H), 3.75 (t, J = 9.3 Hz, 1H), 3.72 – 3.44 (m, 15H), 3.43 – 3.28 (m, 5H), 3.25 (t, J = 8.0 Hz, 1H), 3.23 – 3.16 (m, 2H), 3.13 (t, J = 10.9 Hz, 1H), 2.49 (dd, J = 13.1, 4.6 Hz, 1H), 2.37 – 2.29 (m, 3H), 2.17 (tt, J = 11.2, 5.7 Hz, 2H), 1.95 (dt, J = 14.5, 3.5 Hz, 1H), 1.85 – 1.75 (m, 4H), 1.69 – 1.52 (m, 8H), 1.48 – 1.42 (m, 3H), 1.38 – 1.30 (m, 8H), 1.29 (s, 3H), 1.28 – 1.23 (m, 6H), 1.21 (d, J = 6.2 Hz, 7H), 1.19 – 1.15

(m, 2H), 1.10 – 1.01 (m, 4H), 1.01 – 0.89 (m, 85H), 0.89 – 0.84 (m, 9H), 0.81 – 0.54 (m, 60H); $^{13}\text{C-NMR}$ (151 MHz, CDCl_3) δ 212.60, 177.74, 173.67, 173.33, 168.38, 144.94, 138.80, 138.65, 138.21, 137.58, 137.40, 136.12, 135.19, 128.52, 128.48, 128.43, 128.41, 128.31, 128.27, 128.25, 128.15, 128.13, 128.10, 128.03, 127.87, 127.84, 127.81, 127.77, 127.76, 127.73, 127.56, 127.48, 122.05, 109.09, 103.63, 102.64, 102.07, 101.37, 100.82, 98.08, 86.37, 83.85, 81.85, 79.51, 78.78, 78.70, 78.17, 78.04, 77.97, 76.43, 75.94, 75.92, 75.85, 75.80, 75.50, 75.06, 74.83, 74.67, 73.66, 73.21, 72.97, 72.59, 72.50, 71.36, 71.12, 70.85, 68.98, 68.15, 66.88, 66.04, 65.32, 64.56, 63.82, 60.22, 53.76, 49.25, 48.93, 47.13, 46.13, 45.94, 41.83, 41.76, 39.53, 39.49, 37.91, 36.96, 36.62, 35.96, 35.26, 34.33, 34.04, 32.56, 31.83, 31.49, 30.49, 29.44, 29.40, 29.36, 29.23, 29.14, 27.79, 26.48, 26.31, 25.92, 25.32, 24.96, 24.68, 24.41, 23.39, 23.35, 20.15, 17.42, 16.75, 15.85, 12.22, 7.56, 7.46, 7.24, 7.16, 7.16, 7.13, 6.98, 6.85, 6.79, 5.91, 5.63, 5.43, 5.37, 5.33, 5.25, 5.22, 5.01, 4.41; **HRMS** (ESI) m/z : Calcd for $\text{C}_{184}\text{H}_{298}\text{N}_2\text{O}_{35}\text{Si}_9\text{Na}$ $[\text{M}+23]$ 3379.9421, found 3370.9590.

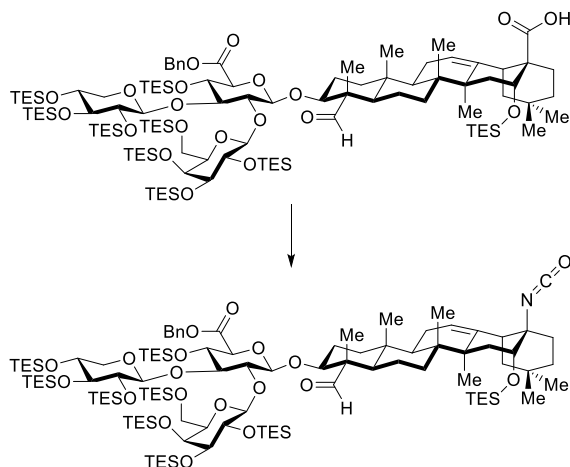


Ethanolamide variant 3 (SQS-0-4-5-5). A solution of fully protected β -ethanolamide analogue (**S2**) (25.0 mg, 0.008 mmol, 1.0 equiv) in tetrahydrofuran (5 mL) and ethanol (5 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (17 mg, 0.016 mmol, 2.2 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 $^\circ\text{C}$) solution of trifluoroacetic acid (5.0 mL, TFA/ H_2O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 $^\circ\text{C}$ to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 water:acetonitrile) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 μm , 10 \times 250 mm) using a linear gradient of 10 \rightarrow 49% acetonitrile (0.05% TFA) in water (0.05% TFA) over 18 min at a flow rate of 5 mL/min. The fraction containing the major peak (t_{R} = 16.42 min) was collected and lyophilized to dryness to afford SQS-0-4-5-5 (**3**) (5.5 mg, 45% yield) as a white solid.

$^1\text{H NMR}$ (600 MHz, $\text{D}_2\text{O}/\text{CD}_3\text{CN}$) δ 9.30 (s, 1H), 6.98 (d, J = 9.6 Hz, 1H), 6.80 (t, J = 5.2 Hz, 1H), 5.41 (t, J = 3.8 Hz, 1H), 4.86 (d, J = 1.8 Hz, 1H), 4.60 (d, J = 7.8 Hz, 1H), 4.47 (d, J = 7.8

Hz, 1H), 4.44 (d, $J = 7.8$ Hz, 1H), 4.35 (d, $J = 7.8$ Hz, 1H), 4.26 (d, $J = 7.8$ Hz, 1H), 3.86 – 3.67 (m, 10H), 3.65 (dd, $J = 11.1, 7.8$ Hz, 2H), 3.61 – 3.50 (m, 4H), 3.50 – 3.37 (m, 8H), 3.34 – 3.18 (m, 5H), 3.18 – 3.07 (m, 5H), 2.69 (dd, $J = 13.1, 2.6$ Hz, 1H), 2.29 – 2.12 (m, 5H), 1.86 – 1.74 (m, 4H), 1.72 – 1.56 (m, 4H), 1.53 – 1.35 (m, 9H), 1.24 (s, 4H), 1.12 (d, $J = 6.2$ Hz, 3H), 1.03 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.80 (s, 3H), 0.65 (s, 3H); **HRMS** (ESI) m/z : Calcd for $C_{78}H_{126}N_2O_{35}Si_9Na$ [$M+23$] 1673.8039, found 1673.8019.

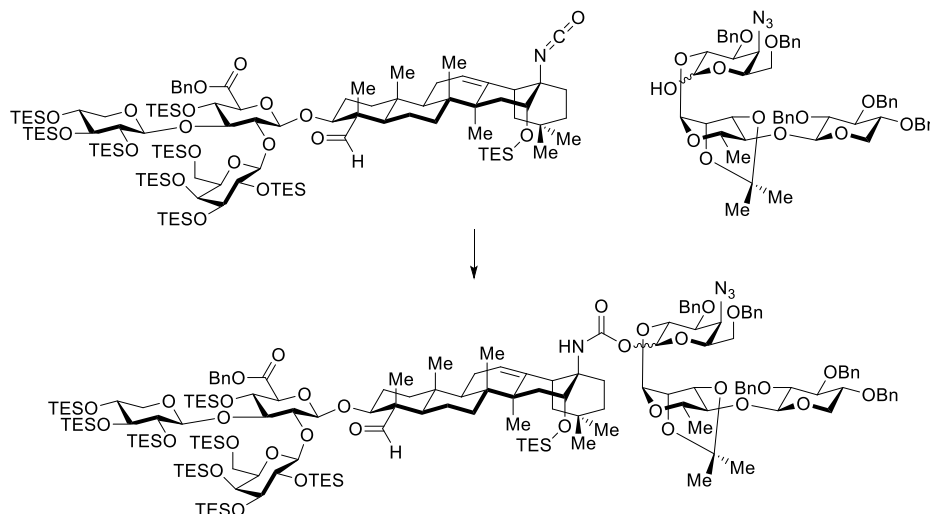
2. SYNTHESIS OF α/β -CARBAMATE VARIANTS α/β -4 (SQS-0-5-8-5 / SQS-0-5-5-5)



Protected prosapogenin isocyanate 11. Diphenylphosphoryl azide (24 μ l, 0.116 mmol, 1.5 eq) was added to a solution of **10** (161 mg, 0.0776 mmol, 1 equiv) and triethylamine (19 μ l, 0.136 mmol, 1.75 eq) in benzene (8 ml) in a vessel fitted with a water-cooled condenser, then submerged in a 90 C oil bath. After 30 min, additional portions of triethylamine (86 μ l, 0.62 mmol, 8 equiv) and diphenylphosphoryl azide (80 μ l, 0.387 mmol, 5 equiv) were added sequentially. After 20 min, reaction was cooled to room temperature, concentrated, and purified by silica gel chromatography (hexanes/ethyl acetate, 40:1 to 10:1) to give isocyanate **11** (127 mg, 79% yield).

TLC R_f 0.41 (20:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 2953, 2912, 2876, 2248 (NCO st), 1754, 1724, 1458, 1413, 1377, 1239, 1171, 1101, 1006, 971, 908, 864, 825, 801, 736, 695 cm^{-1} ; **1H -NMR** (500 MHz, $CDCl_3$) δ 9.71 (s, 1H), 7.38 – 7.28 (m, 5H), 5.37 (s, 1H), 5.28 (d, $J = 12.4$ Hz, 1H), 5.10 (d, $J = 12.4$ Hz, 1H), 4.55 (d, $J = 7.4$ Hz, 1H), 4.42 (d, $J = 7.3$ Hz, 1H), 4.18 (d, $J = 7.3$ Hz, 1H), 3.96 – 3.72 (m, 8H), 3.63 – 3.54 (m, 3H), 3.51 – 3.45 (m, 1H), 3.42 – 3.31 (m, 3H), 3.28 – 3.22 (m, 1H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.51 – 2.43 (m, 1H), 2.22 (t, $J = 13.6$ Hz, 1H), 2.07 (m, 1H), 1.99 – 1.55 (m, 9H), 1.53 (s, 2H), 1.52 – 1.35 (m, 3H), 1.33 (s, 3H), 1.32 (s, 3H), 1.23 (m, 6H), 0.93 (m, 103H), 0.81 – 0.54 (m, 60H); **^{13}C -NMR** (151 MHz, $CDCl_3$) δ 212.92, 168.33, 142.29, 135.26, 128.52, 128.49, 128.46, 128.34, 128.28, 128.25, 128.23, 128.21, 128.19, 128.19, 128.13, 123.76, 122.06, 103.68, 101.40, 100.83, 86.53, 78.81, 78.71, 77.45, 76.44, 75.91, 75.81, 75.08, 72.61, 72.52, 71.38, 71.08, 66.87, 66.84, 65.33, 62.11, 62.08, 60.22, 53.84, 49.44, 48.29, 47.09, 46.16, 41.34, 41.32, 39.77, 39.63, 37.91, 37.18, 37.14, 36.38, 36.11, 33.70, 32.45, 32.40, 32.38, 32.36, 30.63, 30.61, 26.51, 26.44, 25.37, 24.30, 24.27, 23.42, 20.26, 17.04, 17.01, 15.78, 12.30, 7.57, 7.47, 7.38, 7.28, 7.25, 7.23, 7.22, 7.16, 7.14, 7.11, 7.08, 7.05, 7.04, 7.03, 6.99,

6.95, 6.94, 6.91, 6.85, 6.83, 6.81, 6.79, 6.77, 5.93, 5.65, 5.48, 5.44, 5.41, 5.37, 5.35, 5.30, 5.28, 5.26, 5.23, 5.19, 4.94, 4.88, 4.86, 4.42, 4.38; **HRMS** (ESI) m/z : Calcd for $C_{108}H_{203}NO_{19}NaSi_9$ ($M+Na$)⁺ 2093.2771, found 2093.2708.

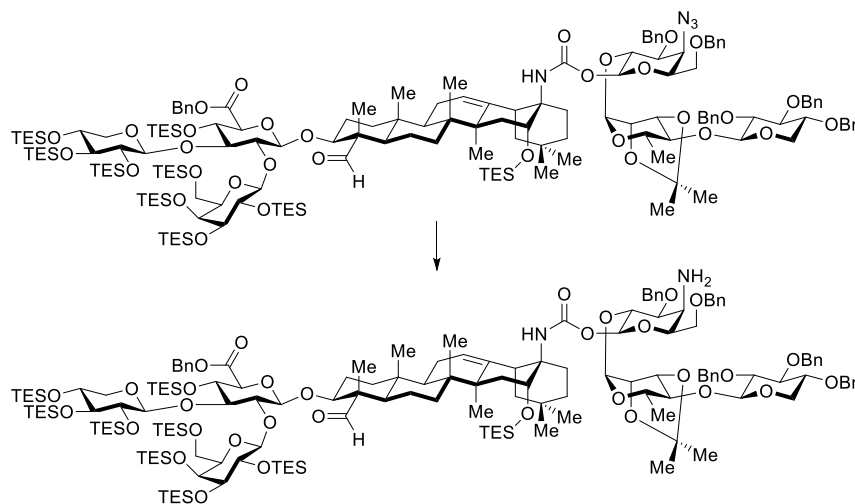


Protected prosapogenin carbamate azides α/β -23. Sodium hydride (60% dispersion in mineral oil, 4.3 mg, 0.108 mmol, 3 equiv) was added to a solution of hemiacetal (35 mg, 0.036 mmol, 1 equiv) in tetrahydrofuran (0.5 mL). After 80 min, isocyanate was added in 0.5 mL tetrahydrofuran. After three hours, suspension was diluted with concentrated ammonium chloride, and extracted with ethyl acetate (3 \times 25 mL). Combined organics were washed with brine and dried over sodium sulfate, decanted, concentrated, and purified via silica gel chromatography (hexanes/ethyl acetate, 20:1 to 4:1) to give easily separable glycosyl carbamates (57 mg **β -23** and 29 mg **α -23**, total yield 79%).

β -23: TLC R_f 0.42 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 3422, 2953, 2877, 2107, 1745, 1497, 1456, 1378, 1240, 1096, 1007, 909, 863, 825, 730, 697, 666 cm^{-1} ; **1H NMR** (500 MHz, $CDCl_3$) δ 9.70 (s, 1H), 7.40 – 7.27 (m, 30H), 5.35 (s, 1H), 5.34 – 5.32 (m, 1H), 5.29 (d, J = 2.0 Hz, 1H), 5.27 (d, J = 6.3 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 4.90 – 4.81 (m, 4H), 4.77 – 4.69 (m, 3H), 4.65 – 4.60 (m, 3H), 4.57 – 4.48 (m, 4H), 4.42 (d, J = 7.2 Hz, 1H), 4.18 (d, J = 7.3 Hz, 1H), 4.11 – 4.06 (m, 2H), 4.04 (d, J = 5.7 Hz, 1H), 3.96 – 3.72 (m, 9H), 3.71 – 3.52 (m, 11H), 3.52 – 3.43 (m, 1H), 3.42 – 3.31 (m, 3H), 3.26 (q, J = 7.6 Hz, 2H), 3.22 – 3.16 (m, 1H), 3.13 (t, J = 11.0 Hz, 1H), 2.52 – 2.45 (m, 1H), 2.33 (t, J = 13.7 Hz, 1H), 2.02 (d, J = 14.2 Hz, 1H), 1.93 – 1.73 (m, 4H), 1.74 – 1.65 (m, 2H), 1.64 – 1.56 (m, 1H), 1.52 – 1.51 (m, 1H), 1.49 (s, 3H), 1.47 – 1.33 (m, 3H), 1.31 (d, J = 2.5 Hz, 6H), 1.28 – 1.23 (m, 4H), 1.10 (d, J = 13.8 Hz, 2H), 1.05 – 0.87 (m, 89H), 0.85 (s, 3H), 0.79 (s, 3H), 0.76 – 0.52 (m, 53H); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 212.76, 168.32, 151.95, 141.94, 138.80, 138.58, 138.23, 137.62, 136.81, 135.24, 128.55, 128.49, 128.45, 128.42, 128.29, 128.26, 128.19, 128.13, 127.98, 127.96, 127.93, 127.91, 127.77, 127.74, 127.53, 127.49, 124.99, 109.02, 103.65, 102.34, 101.39, 100.82, 98.50, 93.20, 86.46, 83.96, 82.45, 81.63, 78.80, 78.71, 78.14, 78.12, 78.00, 76.43, 75.99, 75.90, 75.80, 75.63, 75.06, 74.93, 74.09, 73.72, 73.21, 72.60, 72.50, 72.05, 71.92, 71.89, 71.37, 71.07, 68.10, 66.83, 65.32, 64.44, 63.83, 60.21, 58.27, 55.96, 53.83, 49.33, 47.32, 46.59, 46.06, 41.20, 39.63, 37.91, 36.12, 36.01, 33.17, 32.48, 32.24, 32.08, 30.54, 29.70, 27.79, 26.45, 26.41, 25.34, 24.56, 23.50,

20.20, 17.97, 16.90, 15.80, 12.26, 7.56, 7.46, 7.24, 7.16, 7.13, 7.06, 6.98, 6.85, 6.79, 5.92, 5.64, 5.43, 5.36, 5.33, 5.25, 5.22, 4.92, 4.87, 4.41; **HRMS** (ESI) m/z : Calcd for $C_{163}H_{266}N_4O_{32}NaSi_9$ $[M+Na]$ 3066.7132, found 3066.7073.

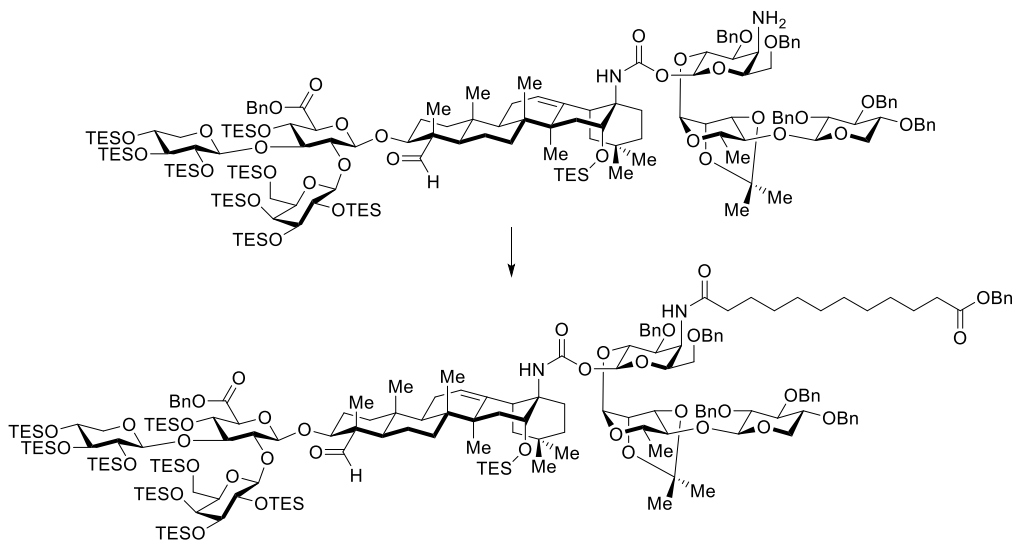
α -23: **TLC** R_f 0.60 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 2953, 2877, 2108, 1745, 1456, 1379, 1240, 1096, 1008, 733, 665 cm^{-1} ; **1H -NMR** (600 MHz, $CDCl_3$) δ 9.69 (s, 1H), 7.41 – 7.26 (m, 31H), 5.97 (d, $J = 3.8$ Hz, 1H), 5.37 (t, $J = 3.6$ Hz, 1H), 5.31 – 5.26 (m, 2H), 5.10 (d, $J = 12.4$ Hz, 1H), 4.88 (d, $J = 11.0$ Hz, 1H), 4.85 – 4.76 (m, 3H), 4.74 – 4.67 (m, 2H), 4.66 – 4.58 (m, 3H), 4.43 (d, $J = 7.2$ Hz, 1H), 4.36 (s, 1H), 4.28 – 4.24 (m, 1H), 4.21 – 4.15 (m, 2H), 4.15 – 4.09 (m, 2H), 4.07 (d, $J = 5.8$ Hz, 1H), 3.99 – 3.89 (m, 4H), 3.89 – 3.73 (m, 6H), 3.65 – 3.51 (m, 9H), 3.50 – 3.44 (m, 1H), 3.40 (dd, $J = 9.4, 2.5$ Hz, 1H), 3.38 – 3.33 (m, 2H), 3.30 (t, $J = 7.9$ Hz, 1H), 3.25 (dd, $J = 8.6, 7.4$ Hz, 1H), 3.19 (dd, $J = 11.7, 8.9$ Hz, 1H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.53 (dd, $J = 14.4, 4.4$ Hz, 1H), 2.28 (t, $J = 13.4$ Hz, 1H), 2.21 – 2.14 (m, 1H), 1.94 – 1.48 (m, 13H), 1.39 – 1.19 (m, 15H), 1.15 – 0.82 (m, 101H), 0.82 – 0.51 (m, 61H); **^{13}C -NMR** (151 MHz, $CDCl_3$) δ 212.17, 168.37, 151.39, 142.42, 138.80, 138.46, 138.23, 137.57, 137.47, 135.23, 128.48, 128.45, 128.42, 128.40, 128.35, 128.29, 128.26, 128.25, 128.23, 128.19, 128.15, 128.12, 128.09, 128.01, 128.00, 127.91, 127.89, 127.86, 127.74, 127.61, 127.59, 127.53, 127.49, 127.46, 124.22, 109.09, 103.52, 102.47, 101.36, 100.82, 99.01, 91.59, 86.14, 83.76, 81.63, 78.77, 78.71, 78.33, 78.22, 77.92, 77.61, 76.44, 76.33, 75.91, 75.82, 75.79, 75.48, 75.05, 74.52, 73.92, 73.83, 73.10, 72.59, 72.48, 72.18, 71.39, 71.10, 68.85, 68.29, 66.85, 65.62, 65.33, 63.63, 60.28, 60.14, 56.04, 53.74, 49.08, 46.67, 45.96, 44.57, 41.06, 39.67, 37.76, 36.31, 35.94, 33.26, 32.49, 32.14, 31.93, 31.92, 30.65, 29.70, 27.66, 26.56, 26.15, 25.28, 24.40, 23.38, 20.16, 17.45, 16.76, 15.72, 12.04, 7.56, 7.46, 7.25, 7.19, 7.16, 7.13, 7.10, 7.07, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.42, 5.36, 5.33, 5.30, 5.28, 5.25, 5.22, 4.88, 4.41; **HRMS** (ESI) m/z : Calcd for $C_{163}H_{266}N_4O_{32}NaSi_9$ $[M+Na]$ 3066.7132, found 3066.6929.



Protected prosapogenin β -carbamate amine β -S4. An excess of hydrogen sulfide was bubbled through an ice-cooled solution of azide **β -23** (17 mg, 0.006 mmol, 1 eq) in pyridine and triethylamine (3.5:1, 4.5 mL) for two min via steel needle, then needle removed from septum. After stirring for 2 min, ice-bath was removed and warmed to ambient temperature. After 7 h, the dark green solution was purged of excess hydrogen sulfide, then volatiles removed with a

stream of nitrogen. The resulting light-orange solid was purified by silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 8:1 to 1:1) to give amine **β -S4** (14 mg, 83% yield).

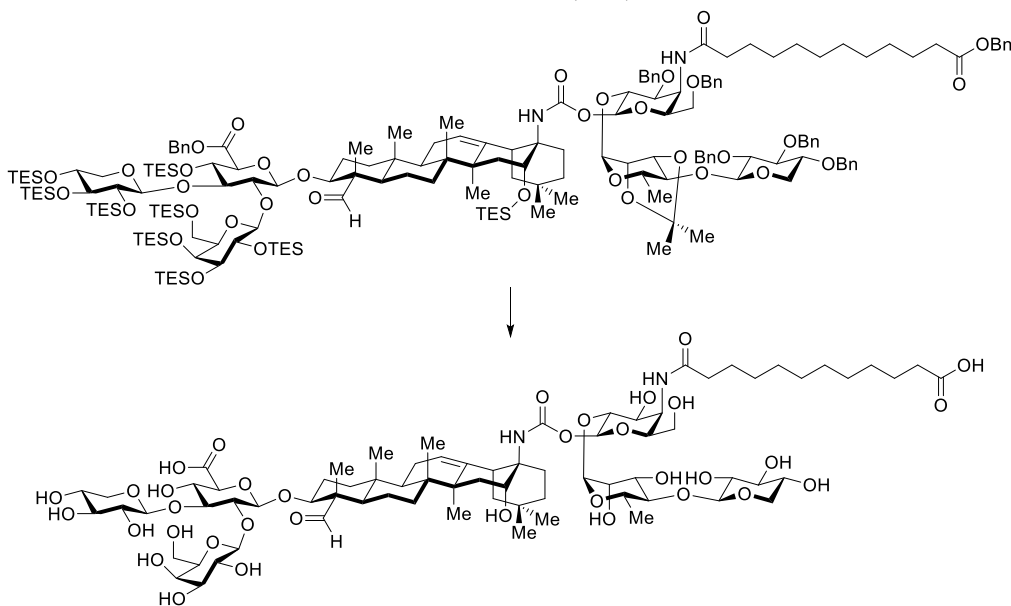
TLC R_f 0.42 (hexanes:ethyl acetate, 2:1 + 0.5% triethylamine); **FTIR** (NaCl, film) 3425, 3066, 3033, 2955, 2913, 2878, 1741, 1498, 1458, 1415, 1382, 1314, 1242, 1098, 904, 865, 827, 735, 699, 667 cm^{-1} ; **$^1\text{H NMR}$** (500 MHz, CDCl_3) δ 9.70 (s, 1H), 7.41 – 7.27 (m, 30H), 5.39 – 5.30 (m, 3H), 5.28 (d, $J = 12.4$ Hz, 1H), 5.10 (d, $J = 12.4$ Hz, 1H), 4.92 – 4.80 (m, 4H), 4.77 (s, 1H), 4.72 (d, $J = 11.7$ Hz, 1H), 4.68 – 4.59 (m, 5H), 4.59 – 4.46 (m, 4H), 4.43 (d, $J = 7.2$ Hz, 1H), 4.18 (d, $J = 7.3$ Hz, 1H), 4.13 – 4.06 (m, 2H), 3.97 – 3.87 (m, 4H), 3.87 – 3.45 (m, 20H), 3.42 – 3.31 (m, 4H), 3.26 (dt, $J = 11.1, 8.0$ Hz, 2H), 3.22 – 3.16 (m, 1H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.54 – 2.46 (m, 1H), 2.33 (dd, $J = 15.2, 11.4$ Hz, 1H), 2.02 (dd, $J = 13.6, 2.4$ Hz, 1H), 1.94 – 1.75 (m, 4H), 1.75 – 1.53 (m, 5H), 1.48 – 1.34 (m, 6H), 1.28 – 1.24 (m, 4H), 1.12 – 0.86 (m, 104H), 0.85 (s, 4H), 0.79 (s, 4H), 0.78 – 0.53 (m, 60H); **$^{13}\text{C NMR}$** (151 MHz, CDCl_3) δ 212.78, 168.32, 152.02, 142.07, 138.80, 138.55, 138.24, 138.01, 137.35, 135.24, 128.49, 128.45, 128.42, 128.41, 128.30, 128.27, 128.12, 128.02, 127.98, 127.94, 127.93, 127.82, 127.76, 127.74, 127.53, 127.50, 124.90, 109.00, 103.66, 102.31, 101.38, 100.81, 98.37, 93.52, 86.48, 83.96, 82.45, 82.37, 78.80, 78.70, 78.11, 77.99, 76.43, 76.07, 75.89, 75.79, 75.63, 75.06, 74.95, 74.15, 73.99, 73.69, 73.21, 72.59, 72.50, 72.02, 71.37, 71.07, 71.03, 68.82, 66.83, 65.31, 64.37, 63.83, 60.21, 55.86, 53.81, 49.33, 48.50, 47.33, 46.58, 46.03, 41.19, 39.81, 39.62, 37.89, 36.11, 36.00, 33.16, 32.66, 32.48, 32.27, 32.02, 31.93, 30.90, 30.55, 29.70, 29.37, 27.82, 26.64, 26.46, 26.41, 25.34, 24.54, 24.45, 23.50, 22.70, 20.18, 17.97, 17.01, 16.88, 15.78, 14.14, 12.25, 7.56, 7.46, 7.24, 7.16, 7.13, 7.05, 6.98, 6.85, 6.79, 5.91, 5.63, 5.43, 5.36, 5.33, 5.25, 5.22, 4.95, 4.86, 4.40; **HRMS** (ESI) m/z : Calcd for $\text{C}_{163}\text{H}_{269}\text{N}_2\text{O}_{32}\text{Si}_9$ $[\text{M}+\text{H}]^+$ 3018.7407, found 3018.7476.



Protected β -carbamate variant S5. Isobutyl chloroformate (1.2 μL , 0.009 mmol, 4 equiv) was added to an ice-cooled solution of carboxylic acid **S2** (4.8 mg, 0.015 mmol, 6 equiv) and triethylamine (2.8 μL , 0.020 mmol, 8 equiv) in tetrahydrofuran (2.5 mL) and stirred for 3 hours, then transferred via cannula to an ice-cooled solution of amine **β -S4** (7.5 mg, 0.002 mmol, 1 equiv) in tetrahydrofuran (1.5 mL). After 1 h, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 \times 25 ml). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography

(hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give amide **β -S5** (6.0 mg, 71 % yield) as a colorless film.

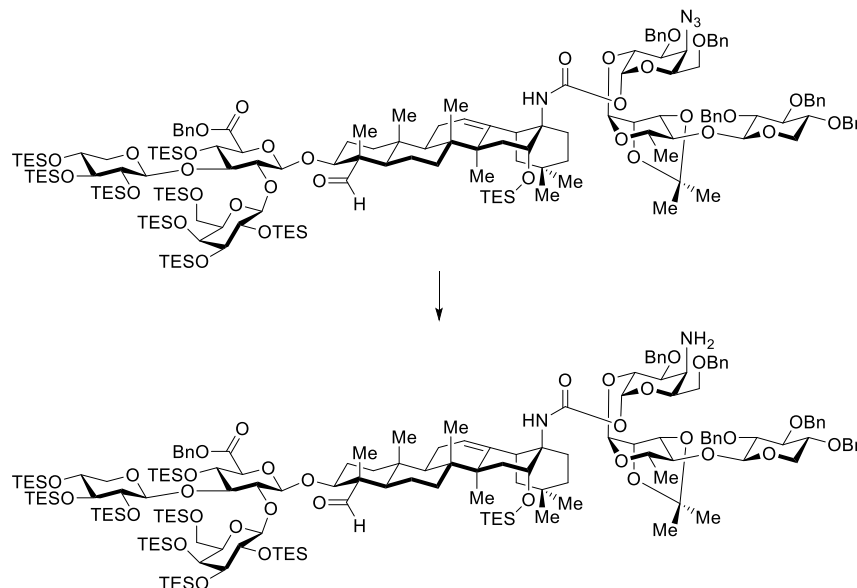
TLC R_f 0.66 (hexanes:ethyl acetate, 2:1 + 0.5% triethylamine); **FTIR** (NaCl, film) 3424, 2952, 2876, 1744, 1679, 1496, 1454, 1379, 1240, 1096, 1008, 825, 733, 696, 665 cm^{-1} ; **$^1\text{H NMR}$** (500 MHz, CDCl_3) δ 9.70 (s, 1H), 7.38 – 7.27 (m, 35H), 5.43 – 5.32 (m, 4H), 5.28 (d, $J = 12.4$ Hz, 1H), 5.13 – 5.07 (m, 4H), 4.92 – 4.86 (m, 3H), 4.86 – 4.81 (m, 3H), 4.76 (s, 2H), 4.72 (d, $J = 11.8$ Hz, 1H), 4.64 – 4.59 (m, 3H), 4.57 – 4.52 (m, 2H), 4.47 – 4.39 (m, 3H), 4.18 (d, $J = 7.3$ Hz, 1H), 4.12 – 4.06 (m, 2H), 3.96 – 3.90 (m, 3H), 3.89 – 3.72 (m, 7H), 3.68 – 3.44 (m, 14H), 3.39 (dd, $J = 9.5, 2.5$ Hz, 1H), 3.38 – 3.32 (m, 2H), 3.26 (dt, $J = 8.8, 7.3$ Hz, 2H), 3.19 (dd, $J = 11.6, 9.0$ Hz, 1H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.56 – 2.49 (m, 1H), 2.39 – 2.29 (m, 4H), 2.22 – 2.08 (m, 2H), 2.08 – 1.99 (m, 1H), 1.92 – 1.75 (m, 4H), 1.74 – 1.57 (m, 8H), 1.55 (s, 5H), 1.35 (s, 5H), 1.33 (s, 3H), 1.31 – 1.17 (m, 22H), 1.14 – 1.03 (m, 4H), 1.02 – 0.88 (m, 106H), 0.85 (s, 3H), 0.80 (s, 3H), 0.74 (s, 65H); **$^{13}\text{C NMR}$** (151 MHz, CDCl_3) δ 212.63, 173.67, 173.21, 168.33, 151.68, 142.11, 138.78, 138.51, 138.24, 137.83, 137.38, 136.11, 135.24, 128.52, 128.45, 128.42, 128.39, 128.30, 128.29, 128.27, 128.15, 128.14, 128.12, 127.98, 127.94, 127.83, 127.77, 127.73, 127.54, 124.83, 109.02, 103.67, 102.43, 101.38, 100.82, 98.46, 93.36, 83.97, 82.49, 79.92, 78.78, 78.70, 78.13, 78.00, 76.43, 75.91, 75.81, 75.65, 75.04, 74.97, 74.58, 73.72, 73.23, 72.59, 72.50, 71.96, 71.37, 71.08, 68.46, 66.84, 66.05, 65.32, 64.43, 63.86, 60.20, 56.05, 53.77, 49.21, 47.34, 46.65, 46.10, 45.99, 41.23, 39.63, 37.86, 36.92, 36.11, 35.99, 34.33, 33.15, 32.47, 32.20, 31.98, 30.55, 29.70, 29.44, 29.38, 29.22, 29.14, 27.81, 26.45, 26.39, 25.85, 25.32, 24.96, 24.59, 23.50, 20.18, 18.08, 17.00, 15.79, 14.14, 12.24, 7.56, 7.46, 7.24, 7.15, 7.13, 7.05, 6.98, 6.85, 6.78, 5.92, 5.63, 5.43, 5.36, 5.33, 5.25, 5.22, 4.87, 4.40; **HRMS** (ESI) m/z : Calcd for $[\text{M}+\text{H}]^+$, found.



β -Carbamate variant β -4 (SQS-0-5-5-5). A solution of fully protected β -carbamate analogue (**β -S5**) (9 mg, 0.003 mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (13 mg, 0.011 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-

cooled (0 °C) solution of trifluoroacetic acid (2.0 mL, TFA/H₂O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 °C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 water:acetonitrile) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 15→51% acetonitrile (0.05% TFA) in over 18 min at a flow rate of 5 mL/min. The fraction containing the major peak (*t_R* = 17.35 min) was collected and lyophilized to dryness to afford SQS-0-5-5-5 (**β-4**) (3.3 mg, 77 % yield) as a fluffy white solid.

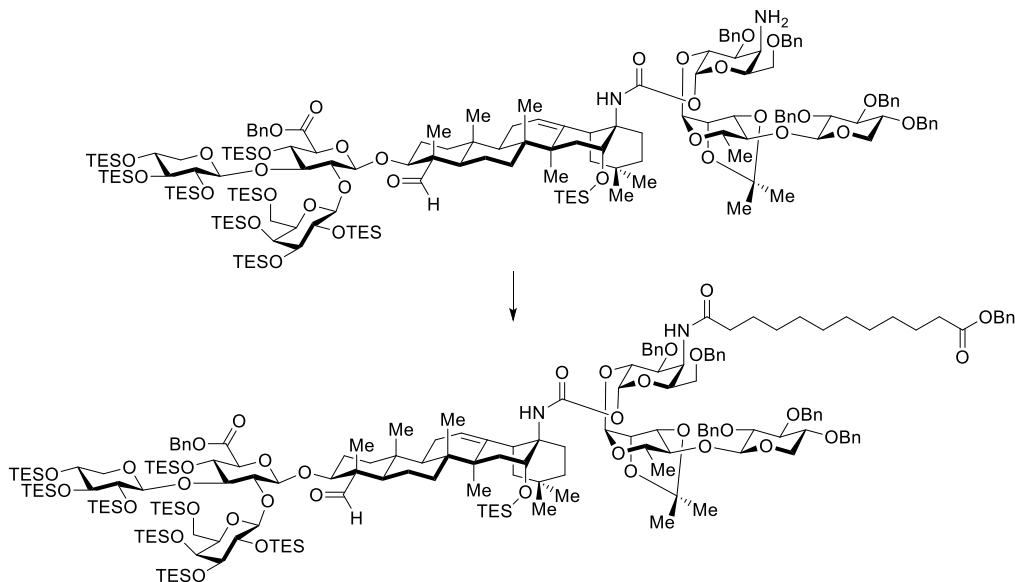
¹H NMR (600 MHz, D₂O/CD₃CN) δ 9.90 (s, 1H), 5.98 – 5.94 (m, 1H), 5.87 (s, 1H), 5.73 (d, *J* = 8.0 Hz, 1H), 5.44 (s, 1H), 5.21 (d, *J* = 7.8 Hz, 1H), 5.10 (d, *J* = 7.8 Hz, 1H), 5.00 (d, *J* = 7.8 Hz, 2H), 4.92 (d, *J* = 7.9 Hz, 1H), 4.44 – 4.37 (m, 4H), 4.34 – 4.19 (m, 6H), 4.17 – 4.10 (m, 5H), 4.08 – 3.93 (m, 8H), 3.93 – 3.82 (m, 4H), 3.77 – 3.66 (m, 4H), 3.63 (q, *J* = 7.2 Hz, 1H), 3.00 – 2.92 (m, 1H), 2.83 – 2.71 (m, 7H), 2.65 – 2.61 (m, 1H), 2.31 – 2.14 (m, 5H), 2.12 – 2.02 (m, 5H), 2.01 – 1.94 (m, 1H), 1.85 (s, 3H), 1.62 (s, 3H), 1.51 – 1.45 (m, 6H), 1.42 – 1.33 (m, 9H); **HRMS** (ESI) *m/z*: Calcd for C₇₆H₁₂₂N₂O₃₅Na [M+Na]⁺ 1645.7726, found 1645.7681.



Protected prosapogenin α -carbamate amine α -S6. An excess of hydrogen sulfide was bubbled through an ice-cooled solution of azide **α -23** (29 mg, 0.010 mmol, 1 eq) in pyridine and triethylamine (3.5:1, 4.5 mL) for two min via steel needle, then needle removed from septum. After stirring for 2 min, ice-bath was removed and warmed to ambient temperature. After 6 h, the dark green solution was purged of excess hydrogen sulfide, then volatiles removed with a stream of nitrogen. The resulting light-orange solid was purified by silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 8:1 to 1:1) to give amine (**α -S6**) (22.5 mg, 78% yield).

TLC *R_f* 0.11 (hexanes:ethyl acetate, 2:1 + 0.5% triethylamine); **FTIR** (NaCl, film) 3426, 3066, 3033, 2955, 2913, 2878, 1741, 1498, 1458, 1415, 1382, 1314, 1242, 1098, 1009, 904, 865, 827, 735, 699, 667 cm⁻¹; **¹H NMR** (600 MHz, CDCl₃) δ 9.68 (s, 1H), 7.40 – 7.27 (m, 33H), 6.00 (d, *J* = 3.6 Hz, 1H), 5.37 (s, 1H), 5.33 – 5.25 (m, 2H), 5.09 (d, *J* = 12.4 Hz, 1H), 4.89 (d, *J* = 11.1 Hz,

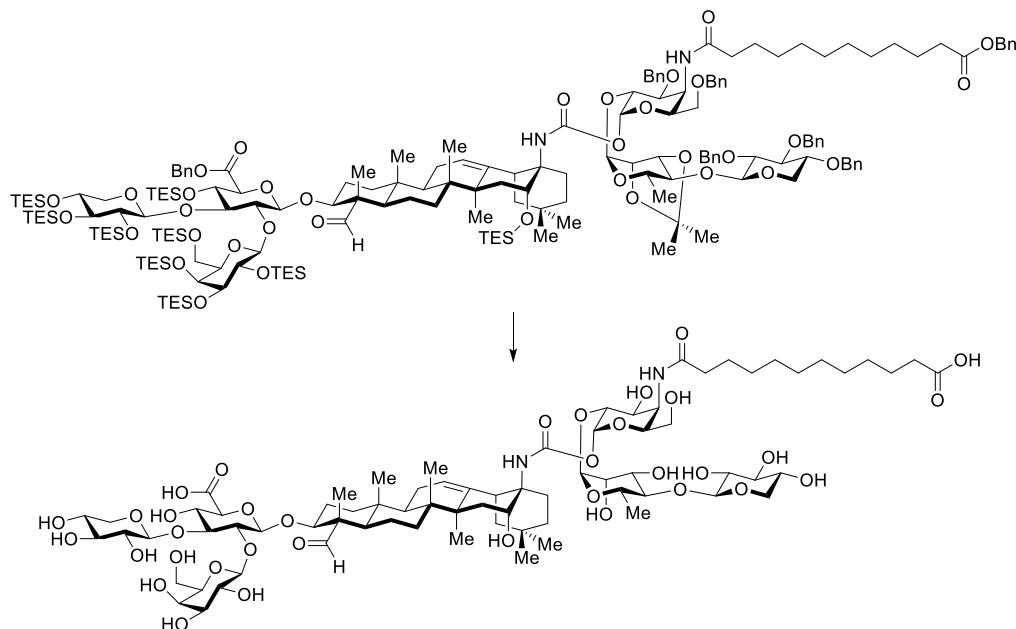
1H), 4.85 (d, $J = 11.0$ Hz, 1H), 4.82 (d, $J = 7.3$ Hz, 1H), 4.79 (d, $J = 11.0$ Hz, 1H), 4.70 (d, $J = 11.7$ Hz, 1H), 4.68 – 4.49 (m, 7H), 4.42 (d, $J = 7.3$ Hz, 1H), 4.40 (s, 1H), 4.34 (s, 1H), 4.19 – 4.07 (m, 4H), 4.02 (t, $J = 6.2$ Hz, 1H), 3.95 – 3.89 (m, 3H), 3.88 – 3.77 (m, 4H), 3.75 (t, $J = 9.2$ Hz, 1H), 3.71 (dd, $J = 10.0, 3.6$ Hz, 1H), 3.67 – 3.51 (m, 9H), 3.50 – 3.44 (m, 2H), 3.42 – 3.38 (m, 1H), 3.35 (t, $J = 8.5$ Hz, 2H), 3.31 (t, $J = 7.7$ Hz, 1H), 3.27 – 3.23 (m, 1H), 3.19 (dd, $J = 11.5, 9.1$ Hz, 1H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.48 (dd, $J = 13.4, 2.7$ Hz, 0H), 2.32 – 2.20 (m, 2H), 1.94 – 1.76 (m, 5H), 1.75 – 1.67 (m, 1H), 1.68 – 1.57 (m, 2H), 1.53 – 1.47 (m, 3H), 1.35 – 1.32 (m, 2H), 1.31 (s, 3H), 1.30 (s, 3H), 1.25 (s, 3H), 1.22 (d, $J = 6.0$ Hz, 3H), 1.13 – 1.07 (m, 2H), 1.03 – 0.88 (m, 105H), 0.86 (s, 4H), 0.85 (s, 4H), 0.80 (s, 3H), 0.79 – 0.53 (m, 66H); ^{13}C NMR (151 MHz, CDCl_3) δ 212.18, 168.37, 151.64, 142.45, 138.81, 138.46, 138.24, 138.00, 137.81, 135.24, 128.45, 128.44, 128.39, 128.26, 128.25, 128.18, 128.12, 127.90, 127.88, 127.83, 127.74, 127.58, 127.46, 127.36, 124.25, 109.02, 103.49, 102.43, 101.35, 100.82, 98.69, 91.74, 83.75, 81.55, 78.71, 78.21, 77.91, 76.44, 75.81, 75.47, 75.05, 74.49, 73.77, 73.70, 73.10, 72.59, 72.48, 71.39, 71.20, 71.10, 70.42, 68.82, 66.85, 65.40, 65.32, 63.61, 60.29, 55.89, 53.75, 49.28, 49.10, 46.71, 45.98, 44.92, 41.08, 39.67, 37.77, 36.31, 35.94, 33.27, 32.51, 32.16, 31.99, 30.65, 29.70, 27.70, 26.57, 26.22, 25.28, 24.42, 23.40, 20.17, 17.45, 16.74, 15.70, 12.04, 7.56, 7.46, 7.25, 7.18, 7.13, 7.12, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.36, 5.33, 5.25, 5.22, 4.89, 4.41.



Protected α -carbamate variant α -S7. Isobutyl chloroformate (4.5 μL , 0.037 mmol, 5 equiv) was added to an ice-cooled solution of carboxylic acid **S2** (14 mg, 0.045 mmol, 6 equiv) and triethylamine (8.3 μL , 0.060 mmol, 8 equiv) in tetrahydrofuran (2 mL) and stirred for 3 hours, then transferred via cannula to an ice-cooled solution of amine α -S6 (22.5 mg, 0.008 mmol, 1 equiv) in tetrahydrofuran (0.6 mL). After 2.5 h, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 \times 25 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give amide α -S7 (23 mg, 93 % yield) as a colorless film.

TLC R_f 0.73 (hexanes:ethyl acetate, 2:1 + 0.5% triethylamine); **FTIR** (NaCl, film) 3421, 3089, 3064, 3031, 2953, 2913, 2876, 1740, 1678, 1655, 1607, 1587, 1456, 1413, 1380, 1312, 1240, 1165, 1097, 1008, 908, 863, 825, 735, 697, 666 cm^{-1} ; ^1H NMR (600 MHz, CDCl_3) δ 9.69 (s,

1H), 7.38 – 7.27 (m, 35H), 5.99 (d, $J = 3.6$ Hz, 1H), 5.50 (d, $J = 10.1$ Hz, 1H), 5.36 (s, 1H), 5.31 (s, 1H), 5.28 (d, $J = 12.4$ Hz, 1H), 5.10 (d, $J = 14.9$ Hz, 3H), 4.91 – 4.87 (m, 2H), 4.86 – 4.77 (m, 4H), 4.70 (d, $J = 11.7$ Hz, 1H), 4.63 (d, $J = 11.1$ Hz, 1H), 4.61 (d, $J = 11.7$ Hz, 1H), 4.56 (d, $J = 7.4$ Hz, 1H), 4.51 (d, $J = 11.6$ Hz, 1H), 4.46 – 4.40 (m, 4H), 4.33 (s, 1H), 4.18 (d, $J = 7.3$ Hz, 1H), 4.15 – 4.09 (m, 3H), 3.95 – 3.89 (m, 3H), 3.88 – 3.73 (m, 7H), 3.65 – 3.52 (m, 8H), 3.51 – 3.45 (m, 3H), 3.40 (d, $J = 9.4$ Hz, 1H), 3.37 – 3.33 (m, 2H), 3.30 (t, $J = 7.6$ Hz, 1H), 3.25 (t, $J = 8.0$ Hz, 1H), 3.19 (dd, $J = 13.6, 6.8$ Hz, 1H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.47 (dd, $J = 13.6, 2.4$ Hz, 1H), 2.34 (t, $J = 7.6$ Hz, 2H), 2.30 – 2.15 (m, 4H), 1.90 – 1.57 (m, 13H), 1.52 – 1.47 (m, 2H), 1.46 (s, 3H), 1.33 (s, 4H), 1.30 – 1.20 (m, 24H), 1.13 – 1.02 (m, 6H), 1.02 – 0.90 (m, 95H), 0.89 (s, 3H), 0.87 (s, 3H), 0.85 (s, 6H), 0.80 – 0.51 (m, 66H); ^{13}C NMR (151 MHz, CDCl_3) δ 212.23, 173.69, 173.41, 168.36, 151.40, 142.24, 138.78, 138.40, 138.24, 138.05, 137.56, 136.11, 135.24, 128.52, 128.45, 128.42, 128.39, 128.25, 128.19, 128.15, 128.14, 128.12, 128.00, 127.90, 127.80, 127.73, 127.63, 127.47, 124.43, 109.00, 103.51, 102.53, 101.36, 100.82, 98.90, 91.22, 86.13, 83.77, 81.49, 78.78, 78.71, 78.14, 77.90, 76.44, 76.26, 76.10, 75.92, 75.82, 75.50, 75.06, 74.48, 73.83, 73.75, 73.09, 72.59, 72.48, 71.68, 71.40, 71.10, 70.85, 69.88, 68.76, 66.84, 66.05, 65.40, 65.32, 63.62, 60.29, 56.00, 53.76, 49.12, 46.88, 46.68, 45.97, 44.85, 41.05, 39.64, 37.78, 36.96, 36.28, 35.93, 34.33, 33.25, 32.49, 32.14, 31.97, 30.63, 29.70, 29.40, 29.37, 29.31, 29.19, 29.12, 27.69, 26.57, 26.20, 25.87, 25.29, 24.94, 24.40, 23.38, 22.70, 20.15, 17.48, 16.63, 15.74, 14.14, 12.05, 7.56, 7.46, 7.25, 7.19, 7.13, 7.11, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.36, 5.33, 5.25, 5.22, 4.89, 4.41; **HRMS** (ESI) m/z : Calcd for $\text{C}_{182}\text{H}_{294}\text{N}_2\text{O}_{35}\text{NaSi}_9$ $[\text{M}+\text{Na}]$ 3342.9108, found 3342.9001.

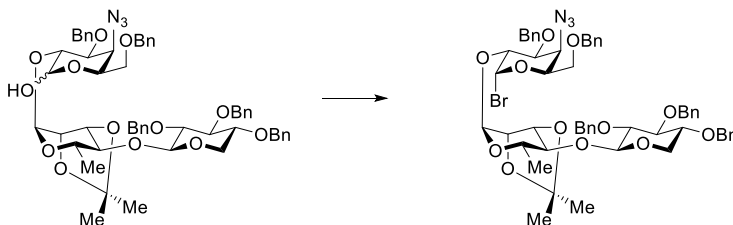


α -Carbamate variant α -4 (SQS-0-5-8-5). A solution of fully protected β -carbamate analogue (α -S7) (5 mg, 0.0015 mmol, 1.0 equiv) in tetrahydrofuran (1 mL) and ethanol (1 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (4 mg, 0.004 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled

(0 °C) solution of trifluoroacetic acid (1.0 mL, TFA/H₂O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 °C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 water:acetonitrile) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 μm, 10 × 250 mm) using a linear gradient of 15→51% acetonitrile (0.05% TFA) in over 18 min at a flow rate of 5 mL/min. The fraction containing the major peak (*t_R* = 17.2 min) was collected and lyophilized to dryness to afford SQS-0-5-8-5 (**α-4**) (2.0 mg, 82 % yield) as a fluffy white solid.

¹H NMR (600 MHz, D₂O/CD₃CN) δ 9.90 (s, 1H), 6.45 (d, *J* = 3.7 Hz, 1H), 6.11 (s, 1H), 5.89 (s, 1H), 5.31 (s, 1H), 5.19 (d, *J* = 7.8 Hz, 1H), 5.07 (d, *J* = 7.8 Hz, 1H), 4.97 (d, *J* = 7.8 Hz, 1H), 4.93 (d, *J* = 7.8 Hz, 1H), 4.86 (s, 1H), 4.47 (t, *J* = 6.3 Hz, 1H), 4.46 – 4.36 (m, 3H), 4.35 – 4.20 (m, 5H), 4.18 – 4.11 (m, 2H), 4.11 – 3.95 (m, 5H), 3.93 – 3.82 (m, 4H), 3.77 – 3.66 (m, 3H), 2.98 (dd, *J* = 13.5, 1.7 Hz, 1H), 2.77 – 2.72 (m, 1H), 2.46 – 2.35 (m, 3H), 2.31 – 2.14 (m, 4H), 2.01 – 1.98 (m, 1H), 1.82 (s, 3H), 1.72 (d, *J* = 6.1 Hz, 3H), 1.63 (s, 2H), 1.60 – 1.53 (m, 1H), 1.48 (s, 3H), 1.37 (s, 3H), 1.36 (s, 3H); **HRMS** (ESI) *m/z*: Calcd for C₇₆H₁₂₂N₂O₃₅Na [M+Na]⁺ 1645.7726, found 1645.7754.

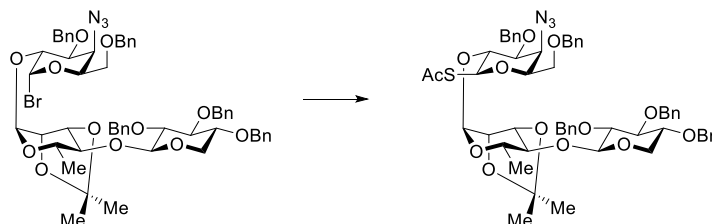
3. SYNTHESIS OF β-THIOESTER VARIANT 5 (SQS-0-13-5-5)



Trisaccharide glycosyl bromide 20. Oxalyl bromide was added to an ice-cooled solution of hemiacetal **17** (125 mg, 0.128 mmol, 1.0 equiv), 2,4,6-tri-*t*-butylpyridine (127 mg, 0.513 mmol, 4.0 equiv), and diethylformamide (150 μL, 1.925 mmol, 15 equiv) in dichloromethane (2 mL) with immediate evolution of CO and CO₂. After five min, ice-bath was removed and warmed to ambient temperature. After three hours, solvent was removed with a stream of nitrogen and crude mixture was purified directly via silica gel chromatography (hexanes:ethyl acetate, 10:1 to 4:1) to give glycosyl bromide as a colorless, thin, and flaky film **20** (98 mg, 74% yield).

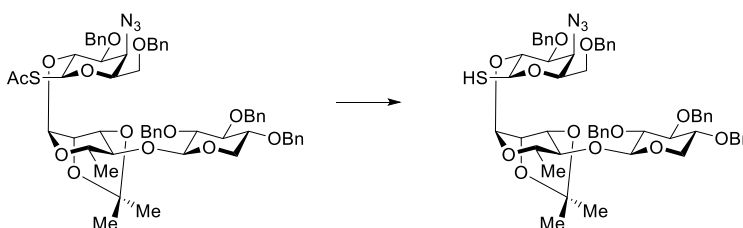
TLC *R_f* 0.43 (hexanes:ethyl acetate, 4:1); **FTIR** (NaCl film) 3583, 3063, 3030, 2983, 2932, 2109, 1496, 1453, 1370, 1242, 1219, 1100, 1027, 992, 862, 792, 735, 697, 666 cm⁻¹; **¹H-NMR** (600 MHz, C₆D₆-*d*₆) δ 7.49 – 7.06 (m, 25H), 6.62 (d, *J* = 3.7 Hz, 1H), 5.34 (s, 1H), 5.16 (d, *J* = 7.5 Hz, 1H), 5.02 (d, *J* = 11.3 Hz, 1H), 4.93 (d, *J* = 11.5 Hz, 1H), 4.86 (d, *J* = 11.5 Hz, 1H), 4.77 (d, *J* = 11.3 Hz, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.40 – 4.32 (m, 3H), 4.33 – 4.25 (m, 3H), 4.24 (d, *J* = 5.8 Hz, 1H), 4.20 (d, *J* = 11.7 Hz, 1H), 4.12 (dq, *J* = 9.9, 6.2 Hz, 1H), 4.07 (dd, *J* = 9.6, 3.8 Hz, 1H), 3.97 (dd, *J* = 9.9, 7.4 Hz, 1H), 3.92 (dd, *J* = 9.7, 3.6 Hz, 1H), 3.86 (dd, *J* = 3.6, 1.6 Hz, 1H), 3.82 (dd, *J* = 11.5, 5.3 Hz, 1H), 3.65 – 3.46 (m, 5H), 3.18 (dd, *J* = 11.6, 9.8 Hz, 1H), 1.45 – 1.40 (m, 6H), 1.20 (s, 3H); **¹³C-NMR** (151 MHz, C₆D₆-*d*₆) δ 139.63, 139.44, 139.01, 138.08, 137.99, 128.63, 128.44, 128.39, 128.34, 128.32, 128.23, 127.68, 127.67, 127.65, 127.55, 127.43, 109.18, 102.71, 100.72, 93.84, 84.15, 82.33, 78.60, 78.32, 78.10, 77.92, 76.85, 76.45,

75.43, 74.81, 73.55, 72.84, 72.76, 72.36, 67.98, 67.71, 66.32, 63.97, 60.24, 27.74, 26.18, 25.69, 17.73; **HRMS** (ESI) m/z : Calcd for $C_{55}H_{62}N_3O_{12}NaBr$ $[M+Na]$ 1058.3415, found 1058.3418.



Trisaccharide glycosyl thioacetate S8. Cesium carbonate (77 mg, 0.237 mmol, 5 equiv) was added to an ice-cooled solution of thioacetic acid (67 μ L, 0.946 mmol, 20 equiv) and bromide **20** (49 mg, 0.047 mmol, 1 equiv) and in tetrahydrofuran/dimethylformamide (2 mL, 1:1). After one hour, reaction was diluted with ethyl acetate, washed with a saturated sodium bicarbonate and brine, dried over sodium sulfate, concentrated and purified with silica gel chromatography (hexanes/ethyl acetate, 10:1 to 2:1) to give thioacetate as a colorless oil **S8** (42 mg, 87% yield).

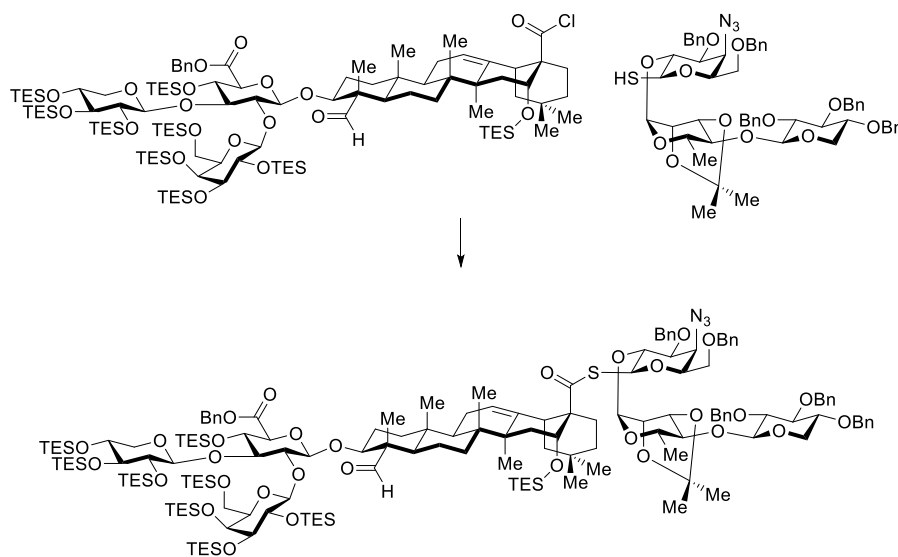
TLC R_f 0.55 (hexanes:ethyl acetate, 2:1); **FTIR** (NaCl film) 3088, 3063, 3030, 2983, 2904, 2872, 2162, 2109, 1706, 1704, 1700, 1496, 1453, 1419, 1381, 1363, 1310, 1274, 1241, 1221, 1091, 1021, 989, 952, 912, 864, 862, 814, 790, 736, 697, 668, 625 cm^{-1} ; **1H -NMR** (600 MHz, $CDCl_3-d$) δ 7.36 – 7.20 (m, 25H), 5.44 (s, 1H), 4.98 (d, $J = 10.1$ Hz, 1H), 4.85 (d, $J = 7.6$ Hz, 1H), 4.84 – 4.75 (m, 3H), 4.70 (d, $J = 11.2$ Hz, 1H), 4.66 (d, $J = 11.7$ Hz, 1H), 4.62 (d, $J = 11.0$ Hz, 1H), 4.57 (d, $J = 11.7$ Hz, 1H), 4.55 – 4.45 (m, 3H), 4.11 (d, $J = 2.8$ Hz, 1H), 4.07 (dd, $J = 7.4, 5.7$ Hz, 1H), 4.02 (d, $J = 5.7$ Hz, 1H), 3.98 (t, $J = 9.5$ Hz, 1H), 3.89 (dd, $J = 11.8, 4.1$ Hz, 1H), 3.76 – 3.66 (m, 3H), 3.60 – 3.48 (m, 5H), 3.26 (t, $J = 8.1$ Hz, 1H), 3.19 – 3.11 (m, 1H), 2.21 (s, 3H), 1.44 (s, 3H), 1.25 (s, 3H), 1.23 (d, $J = 6.2$ Hz, 3H); **^{13}C -NMR** (151 MHz, $CDCl_3$) δ 192.87, 138.78, 138.59, 138.23, 137.47, 136.58, 128.62, 128.56, 128.53, 128.45, 128.39, 128.36, 128.31, 128.29, 128.26, 128.11, 128.08, 128.04, 127.96, 127.94, 127.81, 127.78, 127.62, 127.56, 109.04, 101.99, 98.83, 83.86, 83.16, 81.94, 81.47, 78.09, 77.94, 77.72, 76.17, 75.70, 75.58, 74.70, 73.70, 73.21, 73.05, 71.80, 67.89, 65.32, 63.81, 58.58, 30.77, 27.75, 26.42, 17.17; **HRMS** (ESI) m/z : Calcd for $C_{57}H_{65}N_3O_{13}SNa$ $[M+Na]^+$ 1054.4136, found 1054.4182.



Trisaccharide thiohemiacetal 25. Hydrazine (6.1 μ L, 0.194 mmol, 5.0 equiv) was added to a solution of thioacetate **S8** (40 mg, 0.039 mmol, 1 equiv) and dithiothreitol (18 mg, 0.116 mmol, 3 equiv) in tetrahydrofuran/methanol (2 mL, 1:1). After 1 h, reaction contents was diluted with ethyl acetate, washed with water and brine, dried over sodium sulfated, concentrated, and purified with silica gel chromatography to give thiohemiacetal as a clear oil **25** (36 mg, 94%).

TLC R_f 0.51 (hexanes:ethyl acetate, 2:1); **FTIR** (NaCl film) 3583, 3063, 3031, 2983, 2870, 2106, 1496, 1453, 1369, 1274, 1241, 1220, 1091, 1021, 990, 912, 862, 793, 736, 697, 665 cm^{-1} ;

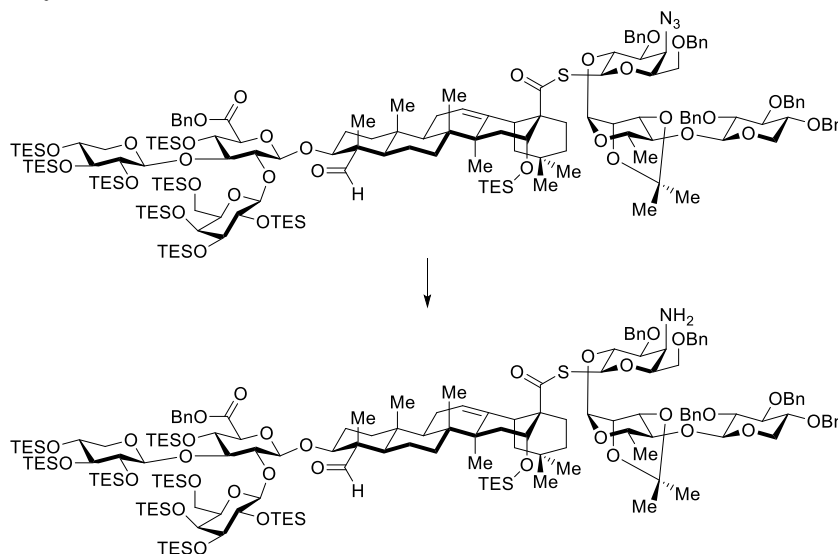
$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 7.40 – 7.26 (m, 25H), 5.52 (s, 1H), 4.93 – 4.80 (m, 4H), 4.71 (q, $J = 11.2, 10.7$ Hz, 3H), 4.62 (d, $J = 11.7$ Hz, 1H), 4.59 – 4.51 (m, 3H), 4.35 (t, $J = 8.9$ Hz, 1H), 4.17 (dd, $J = 7.5, 5.7$ Hz, 1H), 4.11 (d, $J = 3.4$ Hz, 1H), 4.08 (d, $J = 5.7$ Hz, 1H), 4.04 (dd, $J = 9.9, 6.6$ Hz, 1H), 3.94 (dd, $J = 11.9, 4.1$ Hz, 1H), 3.86 (t, $J = 9.2$ Hz, 1H), 3.66 – 3.57 (m, 7H), 3.31 (dd, $J = 9.5, 8.7$ Hz, 1H), 3.24 – 3.14 (m, 1H), 2.31 (d, $J = 8.4$ Hz, 1H), 1.49 (s, 3H), 1.31 (s, 3H), 1.23 (d, $J = 6.2$ Hz, 3H); **$^{13}\text{C-NMR}$** (151 MHz, CDCl_3) δ 138.75, 138.71, 138.20, 137.39, 136.55, 128.57, 128.51, 128.40, 128.32, 128.27, 128.25, 128.22, 128.06, 128.05, 128.04, 127.91, 127.76, 127.75, 127.50, 127.48, 108.97, 101.96, 98.80, 83.86, 82.88, 81.90, 79.70, 78.14, 77.92, 77.69, 77.60, 76.27, 75.71, 75.53, 74.71, 73.73, 73.17, 71.66, 68.25, 65.38, 63.76, 58.53, 27.74, 26.42, 17.24; **HRMS** (ESI) m/z : Calcd for $\text{C}_{55}\text{H}_{63}\text{N}_3\text{O}_{12}\text{SNa}$ $[\text{M}+\text{Na}]^+$ 1012.4030, found 1012.4025.



Protected prosapogenin β -thioester azide **26.** Sodium hydride (60% dispersion in mineral oil, 4.7 mg, 0.115 mmol, 3 equiv) was added to an ice-cooled solution of thiohemiacetal **25** (38 mg, 0.038 mmol, 1.0 equiv) and acyl chloride **12** (88 mg, 0.042 mmol, 1.1 equiv) in tetrahydrofuran (5 mL). After two hours, saturated ammonium chloride (1 mL) was added and the mixture diluted with dichloromethane and washed with water and brine, then dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes/ethyl acetate, 20:1 to 4:1) to give glycosyl thioester **26** (102 mg, 87% yield) as a flaky white film. Characteristic chemical shift of newly formed thioester anomeric proton at 4.84 ppm, $J = 10.0$ Hz and carbon at 81.6 ppm.

TLC R_f 0.80 (hexanes:ethyl acetate, 2:1) **FTIR** (NaCl film) 2953, 2876, 2109, 1751, 1685, 1456, 1380, 1240, 1096, 1006, 900, 808, 733, 696, 665; **$^1\text{H-NMR}$** (500 MHz, CDCl_3 -*d*) δ 9.68 (s, 1H), 7.39 – 7.27 (m, 30H), 5.55 (s, 1H), 5.32 (t, $J = 3.8$ Hz, 1H), 5.29 (d, $J = 12.3$ Hz, 1H), 5.09 (d, $J = 12.4$ Hz, 1H), 4.91 (d, $J = 7.6$ Hz, 1H), 4.86 – 4.80 (m, 3H), 4.76 – 4.70 (m, 2H), 4.63 (d, $J = 9.3$ Hz, 1H), 4.61 (d, $J = 8.3$ Hz, 1H), 4.58 – 4.51 (m, 4H), 4.49 (s, 1H), 4.42 (d, $J = 7.2$ Hz, 1H), 4.19 (d, $J = 7.3$ Hz, 1H), 4.15 (dd, $J = 3.4, 1.4$ Hz, 1H), 4.13 – 4.04 (m, 3H), 3.99 – 3.66 (m, 13H), 3.65 – 3.53 (m, 7H), 3.51 – 3.32 (m, 7H), 3.30 – 3.23 (m, 2H), 3.19 (dd, $J = 11.5, 9.2$ Hz, 1H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.84 (dd, $J = 13.3, 3.3$ Hz, 1H), 2.22 (t, $J = 13.3$ Hz, 1H), 1.95 – 1.75 (m, 4H), 1.74 – 1.57 (m, 5H), 1.53 – 1.46 (m, 5H), 1.45 – 1.23 (m, 17H), 1.19 – 1.05 (m,

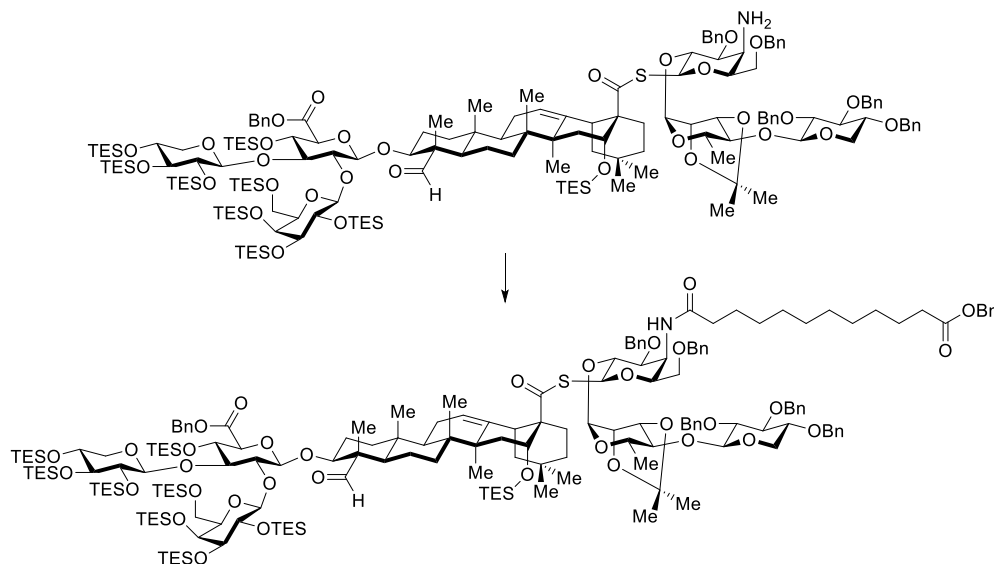
2H), 1.03 – 0.85 (m, 114H), 0.84 – 0.52 (m, 77H); $^{13}\text{C-NMR}$ (151 MHz, CDCl_3) δ 212.51, 204.16, 168.32, 142.00, 138.68, 138.48, 138.21, 137.60, 136.58, 135.23, 128.55, 128.46, 128.43, 128.42, 128.34, 128.32, 128.29, 128.24, 128.21, 128.14, 128.12, 128.11, 128.03, 128.01, 128.00, 127.88, 127.85, 127.79, 127.75, 127.60, 127.59, 123.87, 108.91, 103.51, 102.28, 101.37, 100.82, 98.50, 86.26, 83.92, 83.61, 82.46, 81.62, 78.78, 78.70, 78.23, 78.01, 77.80, 76.43, 76.16, 75.94, 75.80, 75.76, 75.57, 75.53, 75.05, 75.03, 73.60, 73.20, 72.59, 72.50, 71.64, 71.60, 71.38, 71.05, 67.93, 66.84, 65.38, 65.32, 63.84, 60.25, 58.80, 56.19, 53.84, 53.43, 49.30, 46.67, 46.09, 41.81, 41.21, 39.84, 37.85, 36.04, 35.01, 33.94, 32.56, 32.20, 32.04, 30.32, 27.74, 26.46, 26.39, 25.31, 24.64, 23.40, 20.21, 17.57, 17.10, 15.75, 12.22, 7.55, 7.46, 7.25, 7.16, 7.13, 7.09, 6.98, 6.85, 6.78, 5.91, 5.63, 5.43, 5.36, 5.33, 5.31, 5.25, 5.22, 4.88, 4.40; **HRMS** (ESI) m/z : Calcd for $\text{C}_{163}\text{H}_{265}\text{N}_3\text{O}_{31}\text{NaSi}_9\text{S}$ $[\text{M}+\text{Na}]^+$ 3067.6794, found 3067.6711.



Protected prosapogenin β -thioester amine **S9.** An excess of hydrogen sulfide was bubbled via cannula through an ice-cooled solution of azide **26** (80 mg, 0.026 mmol, 1.0 equiv) in pyridine/triethylamine (3.5:1, 4.5 mL) for two min. Vent needle and cannula were removed, and septum sealed with Teflon tape and parafilm, then warmed to ambient temperature and stirred overnight. Hydrogen sulfide was removed with a stream of nitrogen, then resulting orange solution was concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 5:1 to 2:1) furnishing amine **S9** (71 mg, 90 % yield).

TLC R_f 0.50 (hexanes:ethyl acetate, 2:1 +0.5% triethylamine); **FTIR** (NaCl film) 3583, 2951, 2876, 1751, 1724, 1685, 1496, 1457, 1380, 1240, 1097, 1006, 900, 807, 731 cm^{-1} ; **$^1\text{H-NMR}$** (600 MHz, CDCl_3 - d) δ 9.69 (s, 1H), 7.37 – 7.26 (m, 30H), 5.57 (s, 1H), 5.32 (t, J = 3.8 Hz, 1H), 5.29 (d, J = 12.3 Hz, 1H), 5.09 (d, J = 12.4 Hz, 1H), 4.92 (d, J = 7.6 Hz, 1H), 4.88 – 4.81 (m, 4H), 4.74 – 4.68 (m, 2H), 4.64 (d, J = 8.3 Hz, 1H), 4.62 (d, J = 7.4 Hz, 1H), 4.58 (d, J = 11.8 Hz, 1H), 4.56 (d, J = 7.5 Hz, 1H), 4.53 – 4.49 (m, 3H), 4.42 (d, J = 7.3 Hz, 1H), 4.18 (d, J = 7.4 Hz, 1H), 4.13 (dd, J = 7.4, 5.6 Hz, 1H), 4.10 (d, J = 5.7 Hz, 1H), 4.00 (dd, J = 10.3, 8.5 Hz, 1H), 3.97 – 3.90 (m, 4H), 3.87 (d, J = 9.2 Hz, 1H), 3.85 – 3.72 (m, 6H), 3.68 – 3.53 (m, 10H), 3.48 (ddd, J = 10.5, 8.4, 5.1 Hz, 1H), 3.39 (dd, J = 9.4, 2.5 Hz, 1H), 3.37 – 3.32 (m, 2H), 3.28 (dd, J = 8.7, 7.5 Hz, 1H), 3.25 (t, J = 8.0 Hz, 1H), 3.20 (dd, J = 11.7, 9.3 Hz, 1H), 3.13 (t, J = 11.0 Hz, 1H), 2.84 (dd, J = 13.4, 4.6 Hz, 1H), 2.22 (t, J = 13.2 Hz, 1H), 1.95 – 1.74 (m, 5H), 1.73 – 1.53 (m, 7H), 1.51 (s, 4H), 1.45 – 1.38 (m, 2H), 1.34 (s, 4H), 1.33 – 1.26 (m, 11H), 1.16 – 0.89 (m, 97H), 0.88

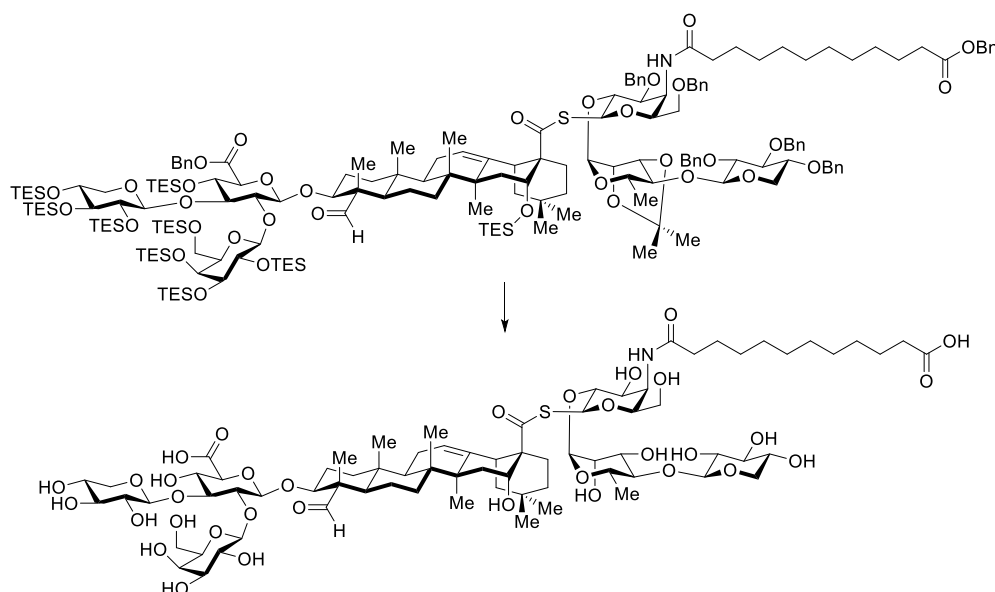
(s, 3H), 0.82 (s, 3H), 0.80 – 0.51 (m, 61H); $^{13}\text{C-NMR}$ (151 MHz, CDCl_3) δ 212.59, 204.03, 168.36, 142.34, 138.70, 138.50, 138.24, 137.93, 136.88, 135.26, 128.55, 128.52, 128.49, 128.46, 128.44, 128.38, 128.35, 128.33, 128.31, 128.24, 128.18, 128.16, 128.15, 128.07, 128.05, 127.82, 127.78, 127.72, 127.65, 127.63, 123.70, 108.96, 103.55, 102.32, 101.39, 100.85, 98.36, 86.28, 83.95, 82.49, 81.76, 78.81, 78.73, 78.22, 78.03, 77.85, 76.45, 76.24, 75.97, 75.83, 75.79, 75.68, 75.09, 75.07, 73.59, 73.23, 72.62, 72.53, 71.41, 71.08, 70.80, 69.05, 66.86, 65.35, 63.87, 60.28, 56.29, 53.86, 49.36, 49.04, 46.84, 46.10, 41.68, 41.28, 39.86, 37.88, 36.08, 35.06, 33.95, 32.58, 32.19, 32.10, 30.35, 29.73, 27.81, 26.46, 26.44, 25.34, 24.66, 23.46, 20.24, 17.62, 17.21, 15.80, 12.24, 7.58, 7.49, 7.27, 7.19, 7.16, 7.12, 7.01, 6.88, 6.81, 5.94, 5.66, 5.46, 5.39, 5.36, 5.28, 5.25, 4.92, 4.43; **HRMS** (ESI) m/z : Calcd for $\text{C}_{163}\text{H}_{268}\text{NO}_{31}\text{Si}_9\text{S}$ $[\text{M}+\text{H}]^+$ 3019.7070, found 3019.7112.



Protected β -thioester variant S10. Isobutyl chloroformate (3.5 μL , 0.0264 mmol, 4 equiv) was added to an ice-cooled solution of carboxylic acid **S2** (11 mg, 0.033 mmol, 5 equiv) and triethylamine (9 μL , 0.066 mmol, 10 equiv) in tetrahydrofuran (3 mL) and stirred for 2 hours, then transferred via cannula to an ice-cooled solution of amine **S9** (20 mg, 0.007 mmol, 1 equiv) in tetrahydrofuran (1 mL). After 2 h, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 \times 25 ml). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give fully protected thioester analogue **S10** (19 mg, 87 % yield) as a colorless film.

TLC R_f 0.57 (hexanes:dichloromethane:ethyl acetate, 4:2:1) **FTIR** (NaCl film) 3583, 3381, 2954, 2876, 1751, 1738, 1682, 1497, 1455, 1414, 1380, 1240, 1099, 1005, 901, 863, 806, 732, 696, 665 cm^{-1} ; $^1\text{H-NMR}$ (600 MHz, CDCl_3 -*d*) δ 9.70 (s, 1H), 7.39 – 7.25 (m, 35H), 5.89 (s, 1H), 5.52 (s, 1H), 5.31 (d, J = 3.7 Hz, 1H), 5.28 (d, J = 12.4 Hz, 1H), 5.11 (s, 2H), 5.09 (d, J = 12.4 Hz, 1H), 4.93 – 4.87 (m, 2H), 4.89 – 4.74 (m, 6H), 4.72 (d, J = 11.7 Hz, 1H), 4.64 (d, J = 3.4 Hz, 1H), 4.62 (d, J = 4.4 Hz, 1H), 4.55 (d, J = 7.4 Hz, 1H), 4.52 (d, J = 11.8 Hz, 2H), 4.47 – 4.40 (m, 3H), 4.17 (d, J = 7.4 Hz, 1H), 4.12 (dd, J = 7.4, 5.6 Hz, 1H), 4.09 (d, J = 5.6 Hz, 1H), 3.97 – 3.72 (m, 12H), 3.65 – 3.51 (m, 8H), 3.48 (ddd, J = 10.4, 8.4, 5.1 Hz, 1H), 3.44 – 3.31 (m, 6H), 3.28 (dd, J = 8.7, 7.4 Hz, 1H), 3.25 (t, J = 8.0 Hz, 1H), 3.19 (dd, J = 11.6, 9.1 Hz, 1H), 3.13 (t, J =

11.0 Hz, 1H), 2.83 (dd, $J = 13.6, 4.5$ Hz, 1H), 2.34 (t, $J = 7.6$ Hz, 2H), 2.25 – 2.14 (m, 3H), 1.92 – 1.81 (m, 2H), 1.83 – 1.74 (m, 1H), 1.41 (ddd, $J = 13.5, 9.3, 3.8$ Hz, 1H), 1.71 – 1.53 (m, 12H) 1.50 (s, 3H) 1.35 (s, 3H), 1.33 – 1.03 (m, 31H), 1.03 – 0.87 (m, 99H), 0.87 (s, 3H), 0.83 (s, 4H), 0.81 – 0.50 (m, 70H); $^{13}\text{C-NMR}$ (151 MHz, CDCl_3) δ 212.56, 203.39, 173.67, 173.31, 168.34, 142.85, 138.68, 138.49, 138.22, 137.85, 137.19, 136.12, 135.24, 128.72, 128.52, 128.44, 128.43, 128.39, 128.32, 128.31, 128.26, 128.25, 128.21, 128.15, 128.13, 128.08, 128.05, 128.02, 127.90, 127.79, 127.74, 127.63, 127.61, 127.59, 127.53, 122.89, 108.98, 103.57, 102.26, 101.37, 100.82, 98.39, 86.32, 83.91, 82.38, 81.95, 81.74, 78.79, 78.70, 78.06, 77.98, 77.92, 76.43, 76.12, 75.92, 75.84, 75.80, 75.74, 75.06, 74.99, 73.53, 73.20, 72.59, 72.50, 71.38, 71.07, 70.75, 68.76, 66.84, 66.03, 65.32, 65.26, 63.84, 60.25, 56.54, 53.80, 49.29, 46.86, 46.19, 45.99, 41.33, 41.28, 39.81, 37.83, 36.93, 36.03, 35.10, 34.33, 33.90, 32.53, 32.11, 32.08, 30.30, 29.70, 29.45, 29.40, 29.38, 29.24, 29.22, 29.14, 27.79, 26.42, 26.41, 25.87, 25.32, 24.97, 24.51, 23.40, 20.18, 17.67, 17.07, 15.79, 12.24, 7.55, 7.46, 7.24, 7.15, 7.13, 7.09, 6.98, 6.85, 6.78, 5.91, 5.63, 5.44, 5.36, 5.33, 5.25, 5.22, 4.90, 4.40; **HRMS** (ESI) m/z : Calcd for $\text{C}_{182}\text{H}_{293}\text{NO}_{34}\text{NaSi}_9\text{S}$ $[\text{M}+\text{Na}]^+$ 3343.8771, found 3343.8735.

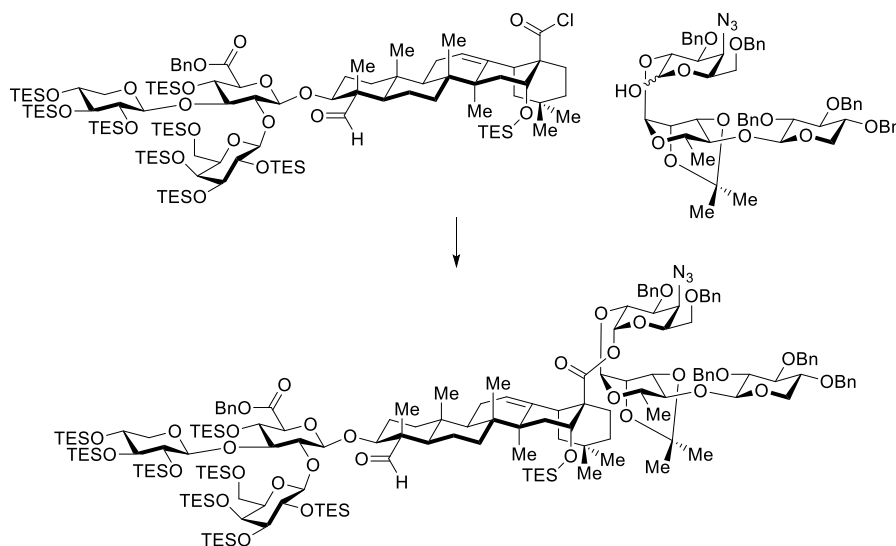


β -Thioester variant 5 (SQS-0-13-5-5). A solution of fully protected β -thioester analogue (**S10**) (18 mg, mmol, 1.0 equiv) in tetrahydrofuran (4 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (13 mg, 0.011 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) for 11 h, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. Care must be taken to avoid quantitative reduction of the thioester to the corresponding aldehyde, which will occur if the reaction hydrogenation is allowed to proceed overnight. At the stated reaction time, a crude NMR in methanol- d_4 showed 2.5 aromatic protons (relative to the C27 aldehyde, integrated to 1.0) Further hydrogenation results in diminished yields. To the hydrogenation product was added a pre-cooled (0 $^\circ\text{C}$) solution of trifluoroacetic acid (2.0 mL, TFA/ H_2O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 $^\circ\text{C}$ to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (4:1 water:acetonitrile) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 μm , 10 \times 250 mm) using a linear

gradient of 20→66% acetonitrile (0.05% TFA) in over 16 min at a flow rate of 5 mL/min. The fraction containing the major peak ($t_R = 13.2$ min) was collected and lyophilized to dryness to afford SQS-0-13-5-5 (**5**) (3.1 mg, 33 % yield) as a fluffy white solid.

¹H NMR (600 MHz, D₂O/CD₃CN, 1:1) δ 9.91 (s, 1H), 5.87 (t, $J = 4.1$ Hz, 1H), 5.55 (d, $J = 2.0$ Hz, 1H), 5.38 (d, $J = 10.0$ Hz, 1H), 5.22 (d, $J = 7.8$ Hz, 1H), 5.10 (d, $J = 7.8$ Hz, 1H), 5.00 – 4.95 (m, 2H), 4.92 (d, $J = 7.8$ Hz, 1H), 4.46 – 4.36 (m, 5H), 4.35 – 4.25 (m, 4H), 4.24 – 4.12 (m, 6H), 4.09 – 3.95 (m, 6H), 3.93 – 3.84 (m, 4H), 3.82 (dd, $J = 11.5, 6.3$ Hz, 1H), 3.78 – 3.70 (m, 3H), 3.67 (dd, $J = 9.3, 7.7$ Hz, 1H), 3.45 (dd, $J = 13.6, 2.9$ Hz, 1H), 2.81 (t, $J = 7.6$ Hz, 3H), 2.79 – 2.67 (m, 3H), 2.47 – 2.32 (m, 5H), 2.32 – 2.14 (m, 6H), 2.07 (q, $J = 6.9$ Hz, 6H), 1.97 (t, $J = 9.7$ Hz, 2H), 1.89 (d, $J = 15.4$ Hz, 2H), 1.85 (s, 3H), 1.76 – 1.69 (m, 2H), 1.64 (s, 3H), 1.63 – 1.58 (m, 2H), 1.48 (s, 3H), 1.44 (s, 4H), 1.40 (s, 3H), 1.38 (d, $J = 6.7$ Hz, 2H), 1.19 (s, 3H); **HRMS** (ESI) m/z : Calcd for C₇₆H₁₂₁NO₃₄NaS [M+Na]⁺ 1646.7388, found 1646.7373.

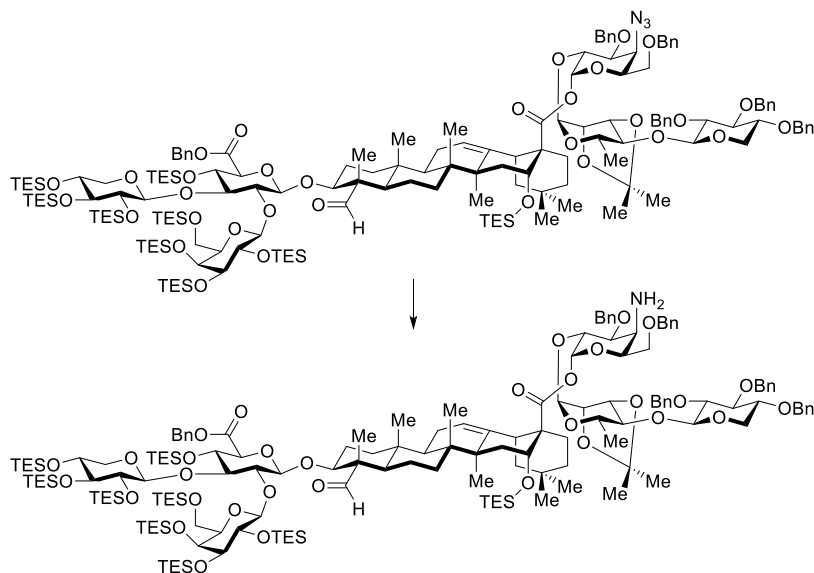
4. SYNTHESIS OF α -ESTER VARIANT 6 (SQS-0-0-8-5)



Protected prosapogenin α -ester azide **24**. Sodium hydride was added to a solution of hemiacetal **17** (28 mg, 0.029 mmol, 1.5 equiv) in tetrahydrofuran/dimethylformamide (2.0 mL, 1:1) at -20 C. After 5 min, a solution acyl chloride **12** (40 mg, 0.019 mmol, 1.0 equiv) in tetrahydrofuran (1.5 mL) was added over 1 min. After 10 min, concentrated aqueous ammonium chloride (0.5 mL) was added. Suspension was diluted with water and extracted with benzene (3 \times 25 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes/ethyl acetate, 20:1 to 4:1) furnishing separable esters (α -35 mg (**α -24**), β -6 mg **β -24**, 70% total yield).

TLC R_f 0.55 (benzene:ethyl acetate, 20:1); **FTIR** (NaCl film) 2953, 2876, 2106, 1752, 1736, 1455, 1380, 1240, 1098, 1005, 825, 732, 696 cm⁻¹; **¹H-NMR** (600 MHz, CDCl₃-*d*) δ 9.74 (s, 1H), 7.47 – 7.26 (m, 30H), 6.12 (d, $J = 3.7$ Hz, 1H), 5.35 – 5.28 (m, 2H), 5.27 (t, $J = 3.8$ Hz, 1H), 5.12 (d, $J = 12.4$ Hz, 1H), 4.93 (d, $J = 11.1$ Hz, 1H), 4.89 (d, $J = 7.4$ Hz, 1H), 4.89 – 4.80 (m, 2H), 4.77 (d, $J = 11.7$ Hz, 1H), 4.73 (d, $J = 11.7$ Hz, 1H), 4.70 (d, $J = 11.1$ Hz, 1H), 4.64 (dd,

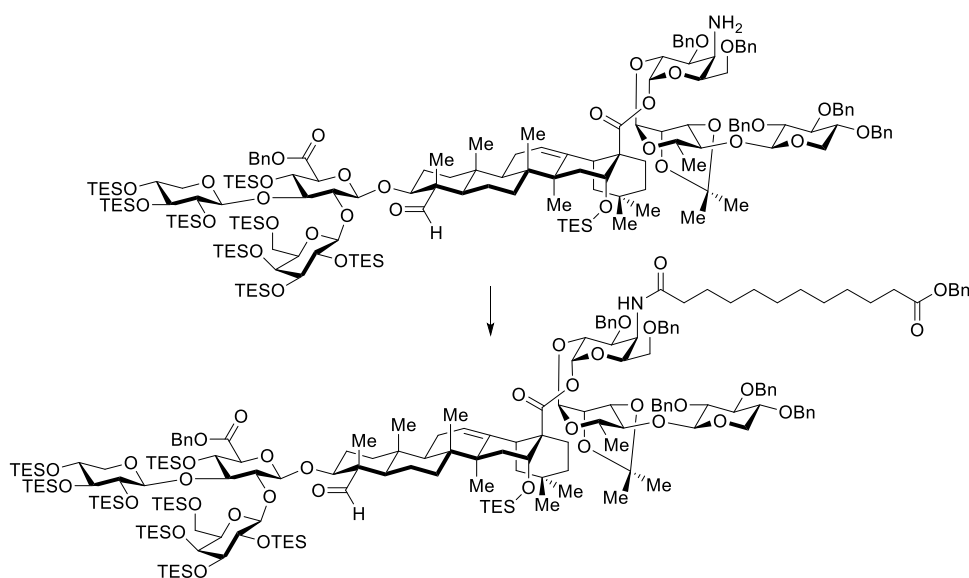
$J = 11.7, 4.1$ Hz, 2H), 4.59 (d, $J = 7.5$ Hz, 1H), 4.57 – 4.49 (m, 3H), 4.46 (d, $J = 7.2$ Hz, 1H), 4.23 (dd, $J = 9.9, 3.8$ Hz, 1H), 4.20 (d, $J = 7.4$ Hz, 1H), 4.17 (dd, $J = 3.5, 1.6$ Hz, 1H), 4.13 (dd, $J = 7.1, 5.6$ Hz, 1H), 4.05 (d, $J = 5.6$ Hz, 1H), 3.99 – 3.79 (m, 10H), 3.78 (t, $J = 9.2$ Hz, 1H), 3.68 – 3.54 (m, 9H), 3.55 – 3.45 (m, 2H), 3.42 (dd, $J = 9.4, 2.5$ Hz, 1H), 3.41 – 3.33 (m, 2H), 3.35 – 3.29 (m, 1H), 3.28 (t, $J = 8.0$ Hz, 1H), 3.26 – 3.19 (m, 1H), 3.16 (t, $J = 11.0$ Hz, 1H), 2.94 (dd, $J = 14.4, 4.4$ Hz, 1H), 2.15 (t, $J = 13.6$ Hz, 1H), 1.86 – 1.75 (m, 4H), 1.77 – 1.55 (m, 7H), 1.52 – 1.47 (m, 2H), 1.38 (s, 4H), 1.33 (s, 6H), 1.24 (d, $J = 5.6$ Hz, 3H), 1.14 – 0.86 (m, 101H), 0.87 – 0.56 (m, 78H); $^{13}\text{C-NMR}$ (151 MHz, CDCl_3) δ 212.86, 174.19, 168.33, 142.70, 138.80, 138.41, 138.25, 137.45, 137.28, 135.26, 128.56, 128.52, 128.50, 128.48, 128.44, 128.40, 128.31, 128.28, 128.26, 128.23, 128.16, 128.13, 128.00, 127.96, 127.94, 127.90, 127.81, 127.78, 127.58, 127.50, 122.32, 109.19, 103.63, 102.83, 101.41, 100.85, 99.33, 91.33, 86.48, 83.78, 81.31, 78.83, 78.73, 78.32, 78.21, 77.98, 77.68, 76.65, 76.46, 76.09, 75.93, 75.82, 75.56, 75.08, 74.95, 74.51, 73.62, 73.15, 72.92, 72.62, 72.54, 72.41, 71.40, 71.09, 70.05, 68.30, 66.87, 65.35, 65.08, 63.70, 60.24, 59.95, 53.85, 49.60, 49.37, 46.37, 46.05, 41.50, 40.22, 39.53, 37.86, 36.09, 35.08, 34.69, 34.59, 34.55, 32.48, 32.40, 31.41, 30.36, 29.09, 27.83, 26.37, 26.32, 25.34, 25.30, 24.22, 23.21, 20.73, 20.27, 17.37, 17.13, 15.78, 12.27, 11.48, 7.59, 7.49, 7.27, 7.24, 7.22, 7.16, 7.01, 6.88, 6.81, 5.94, 5.66, 5.46, 5.39, 5.36, 5.33, 5.28, 5.25, 5.21, 5.04, 4.43; **HRMS** (ESI) m/z : Calcd for $\text{C}_{163}\text{H}_{265}\text{N}_3\text{O}_{32}\text{NaSi}_9$ 3051.7023 [M+Na], found 3051.7041.



Protected prosapogenin α -ester amine S11. Hydrogen sulfide was bubbled via cannula through an ice-cooled solution of azide **24** (44 mg, 0.015 mmol, 1.0 equiv) in pyridine/triethylamine (3.5:1, 4.5 mL) for two min. Vent needle and cannula were removed, and septum sealed with Teflon tape and parafilm, then warmed to ambient temperature and stirred overnight. Hydrogen sulfide was removed with a stream of nitrogen, then resulting orange solution was concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 5:1 to 2:1) furnishing amine **S11** (36 mg, 83 % yield) as a colorless oil.

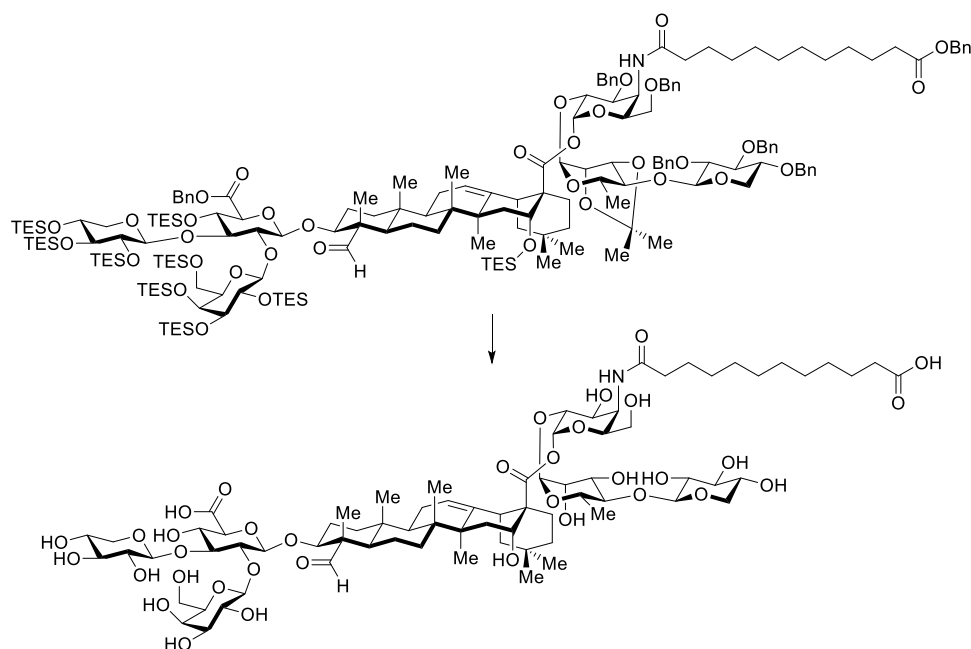
TLC R_f 0.33 (hexanes:ethyl acetate, 2:1+0.5% triethylamine; **FTIR** (NaCl film) 2951, 2876, 1753, 1726 cm^{-1} ; $^1\text{H-NMR}$ (600 MHz, CDCl_3 -*d*) δ 9.70 (s, 1H), 7.42 – 7.25 (m, 33H), 6.15 (d, $J = 3.8$ Hz, 1H), 5.30 – 5.26 (m, 2H), 5.24 (t, $J = 3.8$ Hz, 1H), 5.09 (d, $J = 12.4$ Hz, 1H), 4.91 (d, J

= 11.1 Hz, 1H), 4.87 (d, $J = 7.4$ Hz, 1H), 4.85 – 4.77 (m, 2H), 4.72 – 4.65 (m, 3H), 4.61 (d, $J = 11.7$ Hz, 1H), 4.58 – 4.52 (m, 4H), 4.47 (d, $J = 12.1$ Hz, 1H), 4.42 (d, $J = 7.3$ Hz, 1H), 4.19 – 4.13 (m, 2H), 4.11 (dd, $J = 7.6, 5.5$ Hz, 1H), 4.06 (d, $J = 5.5$ Hz, 1H), 3.98 – 3.89 (m, 4H), 3.88 – 3.71 (m, 6H), 3.67 – 3.51 (m, 10H), 3.51 – 3.45 (m, 2H), 3.41 – 3.28 (m, 5H), 3.25 (t, $J = 8.0$ Hz, 1H), 3.20 (ddd, $J = 11.4, 7.6, 3.5$ Hz, 1H), 3.13 (t, $J = 11.0$ Hz, 1H), 2.92 (dd, $J = 14.4, 4.4$ Hz, 1H), 2.12 (t, $J = 13.6$ Hz, 1H), 1.87 – 1.73 (m, 4H), 1.73 – 1.53 (m, 6H), 1.52 (s, 3H), 1.50 – 1.43 (m, 4H), 1.36 (s, 3H), 1.35 – 1.24 (m, 10H), 1.21 (d, $J = 6.1$ Hz, 3H), 1.08 – 0.89 (m, 92H), 0.88 (s, 3H), 0.80 (s, 3H), 0.78 (d, $J = 7.8$ Hz, 2H), 0.76 (s, 3H), 0.75 – 0.56 (m, 60H); $^{13}\text{C-NMR}$ (151 MHz, CDCl_3) δ 212.88, 174.27, 168.33, 142.87, 138.82, 138.40, 138.26, 137.75, 137.70, 135.26, 128.48, 128.47, 128.43, 128.39, 128.38, 128.36, 128.31, 128.27, 128.25, 128.18, 128.15, 127.95, 127.93, 127.81, 127.78, 127.77, 127.72, 127.68, 127.64, 127.59, 127.56, 127.49, 122.20, 109.15, 103.63, 102.88, 101.40, 100.85, 99.05, 91.41, 86.48, 83.76, 81.20, 78.83, 78.73, 78.33, 78.29, 78.13, 77.99, 76.46, 76.19, 75.94, 75.82, 75.54, 75.08, 74.99, 74.47, 73.41, 73.14, 72.62, 72.53, 71.97, 71.58, 71.46, 71.39, 71.09, 68.86, 66.86, 65.34, 64.96, 63.68, 60.23, 53.85, 49.59, 49.58, 49.43, 46.43, 46.07, 42.86, 41.48, 40.17, 39.99, 39.54, 37.86, 36.09, 35.12, 34.56, 32.41, 31.46, 30.37, 29.73, 27.87, 26.40, 26.37, 26.34, 25.35, 24.25, 23.21, 21.48, 20.28, 17.78, 17.77, 17.39, 17.06, 15.77, 14.20, 13.13, 12.28, 12.17, 7.59, 7.49, 7.27, 7.25, 7.16, 7.14, 7.01, 6.88, 6.81, 5.94, 5.66, 5.46, 5.39, 5.32, 5.28, 5.25, 5.04, 4.43; **HRMS** (ESI) m/z : Calcd for $\text{C}_{182}\text{H}_{293}\text{NO}_{35}\text{Si}_9\text{Na}$ $[\text{M}+\text{Na}]^+$ 3327.8999, found 3327.9016.



Protected α -ester variant S12. Isobutyl chloroformate (6.3 μL , 0.048 mmol, 4 equiv) was added to an ice-cooled solution of carboxylic acid **S2** (23 mg, 0.072 mmol, 6 equiv) and triethylamine (17 μL , 0.122 mmol, 10 equiv) in tetrahydrofuran (3 mL) and stirred for 3 hours, then transferred via cannula to an ice-cooled solution of amine **S11** (36 mg, 0.012 mmol, 1 equiv) in tetrahydrofuran (1 mL). After 16 h, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 \times 25 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give fully protected α -ester analogue **S12** (30 mg, 76 % yield) as a colorless film.

TLC R_f 0.60 (hexanes:ethyl acetate, 2:1+0.5% triethylamine; **$^1\text{H-NMR}$** (600 MHz, CDCl_3-d) δ 9.69 (s, 1H), 7.40 – 7.21 (m, 35H), 6.15 (d, $J = 3.7$ Hz, 1H), 5.50 (d, $J = 10.0$ Hz, 1H), 5.30 – 5.26 (m, 2H), 5.19 (t, $J = 3.8$ Hz, 1H), 5.11 (s, 2H), 5.09 (d, $J = 12.4$ Hz, 1H), 4.91 (d, $J = 11.2$ Hz, 1H), 4.89 – 4.85 (m, 2H), 4.84 (d, $J = 11.0$ Hz, 1H), 4.79 (d, $J = 8.0$ Hz, 1H), 4.77 (d, $J = 8.7$ Hz, 1H), 4.70 (d, $J = 9.6$ Hz, 1H), 4.68 (d, $J = 9.2$ Hz, 1H), 4.61 (d, 1H), 4.55 (d, $J = 7.5$ Hz, 1H), 4.53 (d, $J = 3.2$ Hz, 1H), 4.49 (d, $J = 12.2$ Hz, 1H), 4.44 – 4.39 (m, 3H), 4.16 (d, $J = 7.4$ Hz, 1H), 4.10 (dd, $J = 7.6, 5.5$ Hz, 1H), 4.08 – 4.04 (m, 2H), 3.94 – 3.89 (m, 3H), 3.87 – 3.76 (m, 6H), 3.74 (t, $J = 9.2$ Hz, 1H), 3.67 – 3.62 (m, 1H), 3.62 – 3.51 (m, 6H), 3.50 – 3.43 (m, 2H), 3.43 – 3.28 (m, 5H), 3.25 (t, $J = 8.1$ Hz, 1H), 3.22 – 3.17 (m, 1H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.89 (dd, $J = 14.4, 4.4$ Hz, 1H), 2.35 (t, $J = 7.5$ Hz, 2H), 2.18 (t, $J = 7.6$ Hz, 2H), 2.11 (t, $J = 13.6$ Hz, 1H), 1.81 – 1.71 (m, 4H), 1.71 – 1.53 (m, 10H), 1.51 (s, 4H), 1.49 – 1.41 (m, 2H), 1.38 (s, 3H), 1.34 – 1.16 (m, 25H), 1.03 – 0.88 (m, 86H), 0.87 (s, 3H), 0.79 – 0.78 (m, 3H), 0.75 (s, 3H), 0.74 – 0.55 (m, 55H); **$^{13}\text{C-NMR}$** (151 MHz, CDCl_3) δ 212.77, 174.17, 173.72, 173.57, 168.34, 142.80, 138.80, 138.34, 138.26, 137.68, 137.58, 136.13, 135.25, 128.55, 128.50, 128.48, 128.43, 128.38, 128.35, 128.30, 128.27, 128.25, 128.25, 128.21, 128.18, 128.17, 128.15, 128.02, 127.93, 127.77, 127.72, 127.70, 127.57, 127.50, 122.23, 109.16, 103.62, 102.88, 101.39, 100.84, 99.01, 90.84, 86.44, 83.75, 81.03, 78.81, 78.72, 78.23, 78.18, 77.97, 76.45, 76.04, 75.93, 75.85, 75.82, 75.55, 75.07, 74.92, 74.40, 73.35, 73.15, 72.62, 72.53, 72.12, 71.39, 71.27, 71.08, 70.92, 68.93, 66.86, 66.08, 65.34, 65.03, 63.69, 60.23, 53.83, 49.62, 49.40, 47.03, 46.38, 46.04, 41.47, 40.18, 39.52, 37.85, 36.99, 36.08, 35.09, 34.54, 34.35, 32.37, 32.32, 31.45, 30.33, 29.42, 29.38, 29.31, 29.27, 29.21, 29.18, 29.14, 27.85, 26.38, 26.33, 25.90, 25.33, 24.97, 24.21, 23.17, 20.24, 17.44, 17.00, 15.77, 12.27, 7.58, 7.48, 7.27, 7.22, 7.15, 7.14, 7.01, 6.87, 6.81, 5.94, 5.66, 5.46, 5.39, 5.32, 5.28, 5.24, 5.01, 4.43; **HRMS** (ESI) m/z : Calcd for $\text{C}_{182}\text{H}_{293}\text{NO}_{35}\text{Si}_9\text{Na}$ $[\text{M}+\text{Na}]^+$ 3327.8999, found 3327.9016.

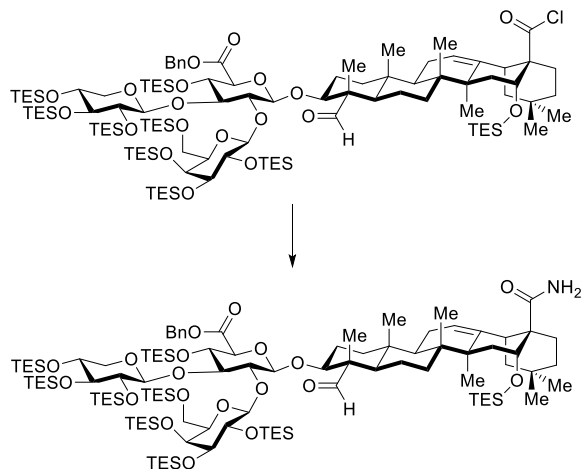


α -Ester variant 6 (SQS-0-0-8-5). A solution of fully protected α -ester analogue (**S12**) (9 mg, 0.003 mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (13

mg, 0.011 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 $^{\circ}\text{C}$) solution of trifluoroacetic acid (2.0 mL, TFA/ H_2O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 $^{\circ}\text{C}$ to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 water:acetonitrile) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 μm , 10 \times 250 mm) using a linear gradient of 20 \rightarrow 75% acetonitrile (0.05% TFA) in over 19 min at a flow rate of 5 mL/min. The fraction containing the major peak (t_{R} = 10.10 min) was collected and lyophilized to dryness to afford SQS-0-0-8-5 (**6**) (3.3 mg, 77 % yield) as a fluffy white solid

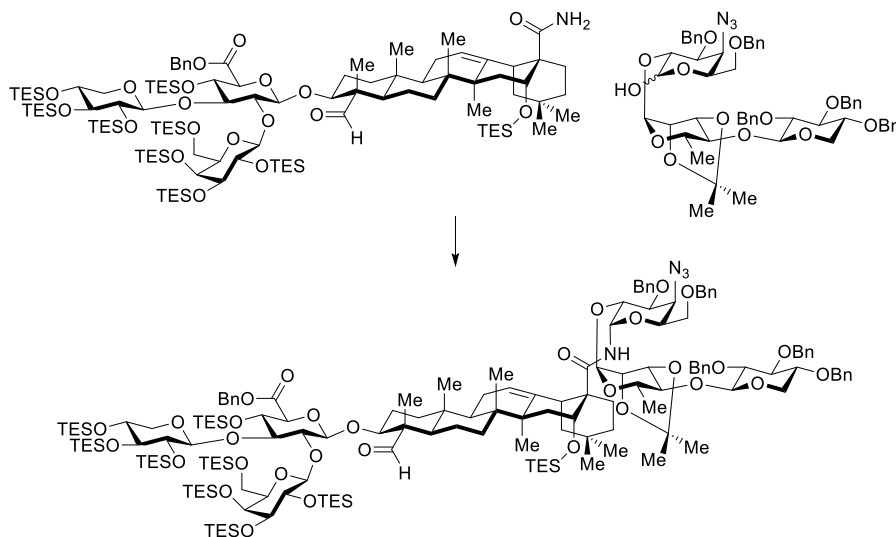
^1H NMR (600 MHz, D_2O , CD_3CN , 1:1) δ 9.29 (s, 1H), 5.97 (d, J = 4.0 Hz, 1H), 5.26 (t, J = 3.5 Hz, 1H), 4.72 (d, J = 1.7 Hz, 1H), 4.60 (d, J = 7.7 Hz, 1H), 4.48 (d, J = 7.8 Hz, 1H), 4.39 (t, J = 4.2 Hz, 1H), 4.36 (d, J = 7.8 Hz, 1H), 4.34 (d, J = 4.6 Hz, 1H), 4.32 (d, J = 8.0 Hz, 1H), 3.97 (dd, J = 10.5, 4.6 Hz, 1H), 3.88 – 3.78 (m, 5H), 3.78 – 3.59 (m, 8H), 3.59 – 3.51 (m, 2H), 3.50 – 3.33 (m, 9H), 3.33 – 3.21 (m, 6H), 3.20 – 3.04 (m, 5H), 2.77 (dd, J = 14.1, 4.5 Hz, 1H), 2.20 (t, J = 7.5 Hz, 3H), 2.18 – 2.00 (m, 4H), 1.88 – 1.76 (m, 4H), 1.71 – 1.57 (m, 5H), 1.55 – 1.41 (m, 8H), 1.41 – 1.30 (m, 4H), 1.23 (s, 3H), 1.12 (d, J = 5.6 Hz, 3H), 1.01 (s, 3H), 0.86 (s, 3H), 0.82 (s, 3H), 0.80 (s, 3H), 0.78 – 0.73 (m, 1H), 0.62 (s, 3H); **HRMS** (ESI) m/z : Calcd for $\text{C}_{76}\text{H}_{121}\text{NO}_{35}\text{Na}$ $[\text{M}+\text{Na}]^+$ 1630.7617, found 1630.7596.

5. SYNTHESIS OF α -AMIDE VARIANT 7 (SQS-0-6-8-5)



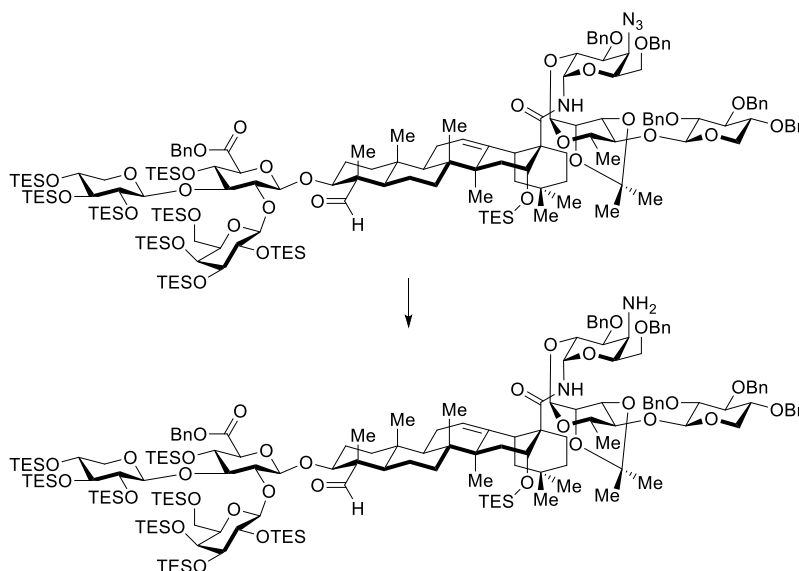
Protected prosapogenin primary amide 14. A large excess of freshly condensed ammonia (\sim 1 mL, \sim 900 equiv) in dichloromethane (2 mL) was added to an ice-cooled solution of **12** (110 mg, 0.525 mmol, 1 equiv) in dichloromethane (5 mL). After 20 min, reaction mixture was warmed to room temperature allowing excess ammonia to evaporate. Mixture was diluted with water and layers separated. After extraction with dichloromethane (2 \times 10 mL), organic fractions combined and washed with brine, then dried over sodium sulfate, and concentrated and purified by silica gel chromatography (hexanes:EtOAc + 0.5% triethylamine 10:1 to 2:1) to afford **14** (100 mg, 92 % yield) as a white foam.

TLC R_f 0.26 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 3454, 2953, 2911, 2877, 1753, 1725, 1674, 1602, 1456, 1414, 1377, 1239, 1104, 1005, 913, 864, 826, 740 cm^{-1} ; **$^1\text{H-NMR}$** (600 MHz, CDCl_3) δ 9.72 (s, 1H), 7.39 – 7.29 (m, 5H), 6.06 (s, 1H), 5.46 (t, $J = 3.6$ Hz, 1H), 5.36 (s, 1H), 5.28 (d, $J = 12.4$ Hz, 1H), 5.10 (d, $J = 12.4$ Hz, 1H), 4.56 (d, $J = 7.4$ Hz, 1H), 4.49 (s, 1H), 4.43 (d, $J = 7.3$ Hz, 1H), 4.18 (d, $J = 7.4$ Hz, 1H), 3.95 – 3.90 (m, 2H), 3.88 – 3.82 (m, 2H), 3.82 – 3.77 (m, 2H), 3.75 (t, $J = 9.2$ Hz, 1H), 3.62 – 3.53 (m, 3H), 3.48 (ddd, $J = 10.5, 8.4, 5.1$ Hz, 1H), 3.39 (dd, $J = 9.4, 2.5$ Hz, 1H), 3.35 (t, $J = 8.7$ Hz, 2H), 3.25 (dd, $J = 8.7, 7.4$ Hz, 1H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.57 (dd, $J = 13.7, 4.2$ Hz, 1H), 2.36 (t, $J = 13.1$ Hz, 1H), 2.03 (dt, $J = 14.6, 4.0$ Hz, 1H), 1.92 (dd, $J = 8.9, 3.6$ Hz, 2H), 1.90 – 1.42 (m, 12H), 1.32 (s, 3H), 1.30 – 1.25 (m, 2H), 1.19 – 0.84 (m, 96H), 0.79 (s, 3H), 0.78 – 0.55 (m, 53H); **$^{13}\text{C-NMR}$** (151 MHz, CDCl_3) δ 212.78, 180.71, 168.38, 145.17, 135.29, 128.47, 128.27, 128.14, 122.50, 103.71, 101.41, 100.85, 86.45, 78.81, 78.73, 76.46, 75.95, 75.91, 75.83, 75.09, 72.62, 72.53, 71.38, 71.11, 66.85, 65.34, 60.25, 53.81, 49.39, 49.19, 47.24, 45.99, 42.27, 41.95, 39.56, 37.95, 36.05, 35.39, 34.67, 34.54, 34.19, 32.57, 31.98, 31.61, 31.27, 30.54, 29.07, 26.32, 25.38, 25.29, 24.22, 23.40, 22.68, 20.71, 20.21, 16.90, 15.86, 14.14, 12.26, 11.45, 7.57, 7.47, 7.25, 7.16, 7.15, 7.14, 6.99, 6.85, 6.79, 5.93, 5.65, 5.45, 5.38, 5.34, 5.27, 5.23, 5.18, 5.01, 4.42; **HRMS** m/z (ESI): Calcd for $\text{C}_{108}\text{H}_{205}\text{NO}_{19}\text{Si}_9\text{Na}$ [M+Na] 2095.2927, found 2095.3020.



Protected prosapogenin α -amide azide 19. Trifluoromethanesulfonic anhydride (22 μL , 0.13 mmol, 3.0 equiv) was added to a solution of trisaccharide **17** (85 mg, 0.087 mmol, 2.00 equiv), phenyl sulfoxide (53 mg, 0.260 mmol, 6.0 equiv) and 2,4,6-tri-*t*-butylpyridine (65 mg, 0.261 mmol, 6.0 equiv) in dichloromethane (5 mL) at -78 $^{\circ}\text{C}$. The reaction stirred in a cold bath at -78 $^{\circ}\text{C}$ for 8 min and then was transferred to a bath between -55 and -50 $^{\circ}\text{C}$ for 65 min. A solution of amide **14** (90 mg, 0.043 mmol, 1 equiv) was added in dichloromethane (2 mL) via syringe. Bath temperature was warmed to -45 $^{\circ}\text{C}$ for 45 min then 0 $^{\circ}\text{C}$ for 15 min. Triethylamine was added, concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 10:1 to 2:1) furnishing readily separable disaccharides **α -19** (80 mg) and **β -19** (13 mg) as a flaky white film (6.1:1, α : β , 71% total).

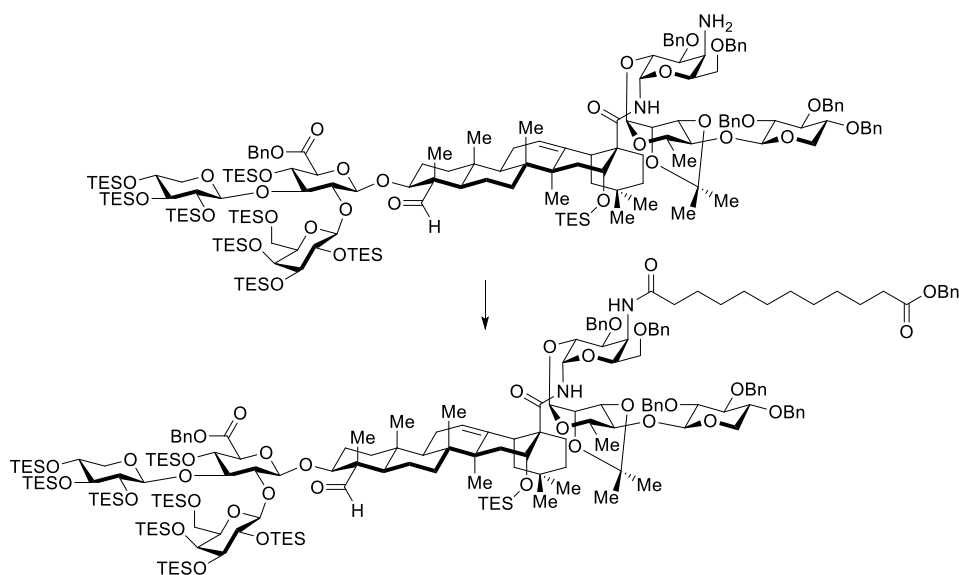
TLC R_f 0.64 (2:1 hexanes/ethyl acetate); **FTIR** (NaCl, film) 3420, 2953, 2911, 2876, 2105, 1751, 1675, 1496, 1457, 1413, 1375, 1240, 1160, 1098, 1005, 898, 865, 825, 732, 697 cm^{-1} ; **$^1\text{H-NMR}$** (600 MHz, CDCl_3) δ 9.71 (s, 1H), 7.98 – 7.92 (m, 1H), 7.59 – 7.54 (m, 1H), 7.53 – 7.48 (m, 1H), 7.42 – 7.26 (m, 27H), 6.64 (d, $J = 8.5$ Hz, 1H), 5.43 (t, $J = 3.6$ Hz, 1H), 5.29 (d, $J = 12.4$ Hz, 1H), 5.19 (d, $J = 4.7$ Hz, 1H), 5.10 (d, $J = 12.4$ Hz, 1H), 4.92 (d, $J = 11.0$ Hz, 1H), 4.89 – 4.81 (m, 4H), 4.73 (dd, $J = 11.5, 3.0$ Hz, 2H), 4.68 (d, $J = 11.0$ Hz, 1H), 4.63 (dd, $J = 11.5, 5.3$ Hz, 2H), 4.56 (d, $J = 7.5$ Hz, 1H), 4.52 (s, 2H), 4.48 (s, 1H), 4.43 (d, $J = 7.3$ Hz, 1H), 4.21 – 4.15 (m, 2H), 4.09 (dd, $J = 7.1, 4.7$ Hz, 1H), 4.00 (dd, $J = 3.0, 1.5$ Hz, 1H), 3.96 – 3.90 (m, 3H), 3.88 – 3.70 (m, 8H), 3.67 – 3.51 (m, 8H), 3.51 – 3.45 (m, 2H), 3.39 (dd, $J = 9.4, 2.5$ Hz, 1H), 3.38 – 3.29 (m, 3H), 3.25 (dd, $J = 8.6, 7.4$ Hz, 1H), 3.22 – 3.16 (m, 1H), 3.13 (t, $J = 11.0$ Hz, 1H), 2.62 (dd, $J = 13.4, 4.2$ Hz, 1H), 2.33 (t, $J = 13.2$ Hz, 1H), 1.98 – 1.63 (m, 7H), 1.62 – 1.45 (m, 5H), 1.44 (s, 3H), 1.43 – 1.30 (m, 10H), 1.29 (s, 3H), 1.28 – 1.17 (m, 3H), 1.12 – 1.03 (m, 4H), 0.89 (s, 81H), 0.84 (s, 3H), 0.81 – 0.56 (m, 51H); **$^{13}\text{C-NMR}$** (151 MHz, CDCl_3) δ 212.68, 178.34, 168.32, 144.58, 141.57, 138.70, 138.57, 138.19, 137.70, 137.13, 135.25, 133.16, 129.26, 128.52, 128.49, 128.46, 128.44, 128.38, 128.35, 128.31, 128.29, 128.27, 128.22, 128.14, 128.08, 128.05, 128.04, 127.99, 127.97, 127.95, 127.94, 127.91, 127.88, 127.86, 127.85, 127.81, 127.76, 127.65, 127.57, 122.60, 110.03, 103.64, 102.61, 101.39, 100.82, 97.64, 86.47, 83.82, 82.01, 81.63, 79.21, 78.99, 78.79, 78.71, 78.32, 77.92, 76.76, 76.64, 76.44, 76.15, 75.90, 75.80, 75.65, 75.06, 74.75, 73.97, 73.53, 73.25, 72.76, 72.60, 72.50, 72.06, 71.38, 71.08, 69.00, 67.78, 66.83, 65.32, 63.77, 60.24, 59.21, 53.84, 49.36, 49.20, 47.17, 46.06, 41.87, 41.25, 39.70, 37.98, 36.02, 35.39, 34.66, 34.52, 34.09, 32.56, 32.21, 31.59, 31.46, 30.52, 29.06, 27.32, 26.25, 25.39, 25.31, 25.27, 24.16, 23.39, 22.66, 20.70, 20.26, 18.77, 17.91, 17.22, 15.95, 14.14, 12.27, 11.45, 7.56, 7.46, 7.25, 7.17, 7.14, 7.13, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.37, 5.33, 5.25, 5.22, 4.95, 4.41; **HRMS** m/z (ESI): Calcd for $\text{C}_{163}\text{H}_{266}\text{N}_4\text{O}_{31}\text{NaSi}_9$ 3050.7182, found 3050.7034.



Protected prosapogenin α -amide amine S13. Hydrogen sulfide was bubbled via cannula through an ice-cooled solution of azide **α -19** (45 mg, 0.0148 mmol, equiv) in pyridine/triethylamine (3.5:1, 4.5 mL) in a 50 mL conical vial. After two min, vent needle and cannula were removed, and septum sealed with Teflon tape and parafilm, then warmed to RT and stirred overnight. Hydrogen sulfide was removed with a stream of nitrogen, then resulting

orange solution was concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 5:1 to 2:1) furnishing amine **S13** (36 mg, 81 % yield).

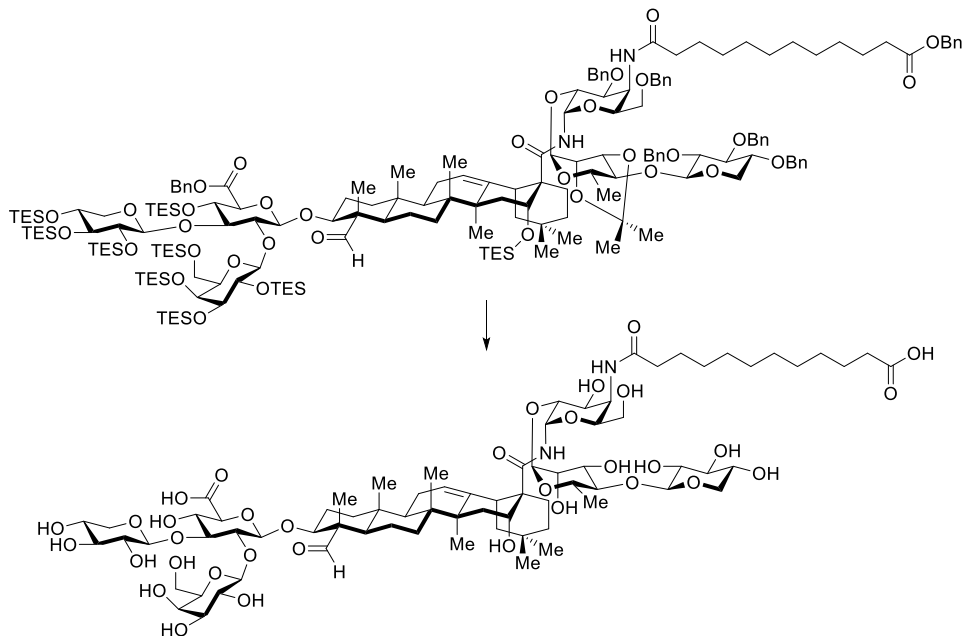
TLC R_f 0.35 (5:1 benzene:ethyl acetate); **FTIR** (NaCl film) 3393, 3031, 2953, 2911, 2876, 1752, 1724, 1676, 1497, 1457, 1414, 1380, 1240, 1169, 1097, 1006, 909, 864, 826, 737, 697, 666, 602 cm^{-1} ; **$^1\text{H-NMR}$** (500 MHz, CDCl_3) δ 9.70 (s, 1H), 7.31 (s, 30H), 6.60 (d, $J = 8.5$ Hz, 1H), 5.49 – 5.44 (m, 0H), 5.44 – 5.39 (m, 1H), 5.33 – 5.25 (m, 2H), 5.10 (d, $J = 12.4$ Hz, 1H), 4.94 – 4.79 (m, 4H), 4.75 – 4.59 (m, 5H), 4.60 – 4.51 (m, 4H), 4.48 (s, 1H), 4.42 (d, $J = 7.3$ Hz, 1H), 4.18 (dt, $J = 7.6, 3.7$ Hz, 2H), 4.10 (dd, $J = 6.7, 3.7$ Hz, 1H), 3.96 – 3.70 (m, 10H), 3.71 – 3.51 (m, 11H), 3.48 (td, $J = 9.8, 9.3, 5.0$ Hz, 1H), 3.44 – 3.27 (m, 5H), 3.25 (t, $J = 8.0$ Hz, 1H), 3.19 (t, $J = 10.3$ Hz, 1H), 3.13 (t, $J = 11.0$ Hz, 1H), 2.62 (dd, $J = 14.5, 3.9$ Hz, 1H), 2.32 (t, $J = 13.1$ Hz, 1H), 1.99 – 1.92 (m, 1H), 1.92 – 1.85 (m, 1H), 1.83 – 1.74 (m, 2H), 1.73 – 1.47 (m, 7H), 1.40 (d, $J = 11.7$ Hz, 1H), 1.37 (s, 6H), 1.35 (s, 2H), 1.30 (s, 6H), 1.29 (s, 6H), 1.28 – 1.15 (m, 2H), 1.13 – 1.02 (m, 3H), 1.03 – 0.85 (m, 98H), 0.84 (s, 3H), 0.83 – 0.52 (m, 61H); **$^{13}\text{C NMR}$** (151 MHz, CDCl_3) δ 212.70, 178.33, 168.33, 144.82, 138.70, 138.55, 138.20, 138.08, 137.47, 135.24, 128.46, 128.43, 128.38, 128.31, 128.28, 128.13, 128.02, 127.96, 127.93, 127.80, 127.75, 127.70, 127.67, 127.58, 122.45, 109.77, 103.64, 102.58, 101.38, 100.82, 97.47, 86.42, 83.83, 82.56, 82.04, 79.38, 78.97, 78.79, 78.71, 78.15, 77.94, 76.59, 76.44, 76.21, 75.87, 75.80, 75.65, 75.07, 74.78, 74.46, 73.98, 73.39, 73.23, 72.60, 72.51, 71.38, 71.20, 71.08, 68.24, 68.10, 66.83, 65.32, 63.76, 60.25, 53.81, 49.31, 49.17, 48.77, 47.35, 45.93, 45.74, 41.88, 41.22, 39.73, 37.93, 36.00, 35.41, 33.97, 32.57, 32.06, 31.43, 30.50, 29.70, 27.46, 26.25, 25.57, 25.37, 24.34, 23.42, 20.22, 18.18, 17.16, 15.94, 12.27, 7.56, 7.46, 7.25, 7.17, 7.14, 7.13, 6.98, 6.85, 6.79, 5.92, 5.63, 5.44, 5.37, 5.33, 5.25, 5.22, 4.95, 4.41; **HRMS** (ESI) m/z : Calcd for $\text{C}_{163}\text{H}_{269}\text{N}_2\text{O}_{31}\text{Si}_9$ 3002.7458 [M+H], found 3002.7354.



Protected α -amide variant S14. Isobutyl chloroformate was added to an ice-cooled solution of carboxylic acid **S2** (21 mg, 0.064 mmol, 6 equiv) and triethylamine (15 μL , 0.107 mmol, 10 equiv) in tetrahydrofuran (2 mL) and stirred for 4 hours, then transferred via cannula to an ice-cooled solution of amine **S13** (32 mg, 0.011 mmol, 1.0 equiv) in tetrahydrofuran (1 mL). After 5 h, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl

acetate (3 × 25 ml). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give amide **S14** (26 mg, 74 % yield).

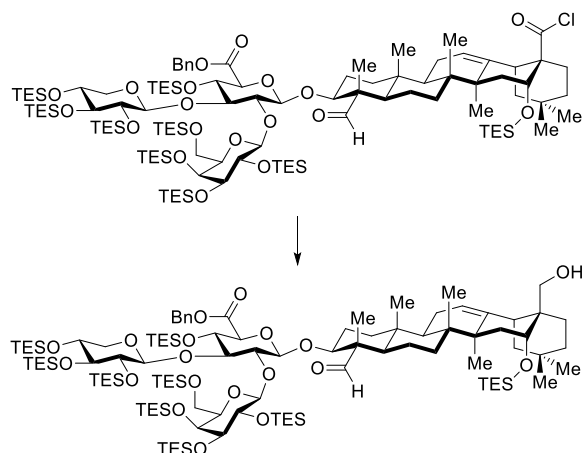
TLC R_f 0.62 (2:1 hexanes:ethyl acetate); **FTIR** (NaCl film) 3610, 3584, 3032, 2954, 2878, 1745, 1725, 1680, 1549, 1499, 1457, 1415, 1381, 1242, 1168, 1099, 1009, 911, 865, 825, 733 cm^{-1} ; **$^1\text{H-NMR}$** (600 MHz, CDCl_3 -*d*) δ 9.71 (s, 1H), 7.43 – 7.20 (m, 35H), 6.54 (d, $J = 8.1$ Hz, 1H), 5.45 (s, 1H), 5.32 (d, $J = 3.4$ Hz, 1H), 5.28 (d, $J = 12.4$ Hz, 1H), 5.12 – 5.06 (m, 3H), 4.90 (d, $J = 11.0$ Hz, 1H), 4.88 – 4.80 (m, 6H), 4.72 (d, $J = 11.7$ Hz, 1H), 4.67 (d, $J = 11.0$ Hz, 1H), 4.62 (d, $J = 11.8$ Hz, 1H), 4.56 (d, $J = 7.4$ Hz, 1H), 4.52 (d, $J = 11.9$ Hz, 1H), 4.46 – 4.39 (m, 4H), 4.20 – 4.13 (m, 2H), 4.08 (dd, $J = 6.5, 3.3$ Hz, 1H), 3.95 – 3.88 (m, 4H), 3.87 – 3.69 (m, 8H), 3.65 (dd, $J = 9.1, 4.1$ Hz, 1H), 3.63 – 3.52 (m, 7H), 3.51 – 3.45 (m, 3H), 3.43 – 3.31 (m, 5H), 3.31 – 3.27 (m, 1H), 3.25 (t, $J = 8.1$ Hz, 1H), 3.22 – 3.16 (m, 1H), 3.13 (t, $J = 11.0$ Hz, 1H), 2.66 (dd, $J = 13.6, 4.4$ Hz, 1H), 2.36 – 2.26 (m, 3H), 2.14 (t, $J = 7.4$ Hz, 2H), 1.98 – 1.85 (m, 3H), 1.85 – 1.75 (m, 3H), 1.74 – 1.50 (m, 14H), 1.49 (s, 4H), 1.42 – 1.33 (m, 6H), 1.12 – 1.04 (m, 4H), 1.04 – 0.81 (m, 110H), 0.81 – 0.54 (m, 69H); **$^{13}\text{C-NMR}$** (151 MHz, CDCl_3) δ 212.49, 178.25, 173.61, 172.87, 168.34, 145.03, 138.68, 138.57, 138.20, 137.81, 137.44, 136.11, 135.23, 128.65, 128.55, 128.51, 128.48, 128.44, 128.43, 128.40, 128.37, 128.35, 128.31, 128.28, 128.27, 128.23, 128.16, 128.15, 128.13, 128.09, 127.97, 127.94, 127.89, 127.85, 127.82, 127.80, 127.76, 127.73, 127.67, 127.65, 127.60, 127.58, 122.15, 109.74, 103.60, 102.51, 101.38, 100.83, 97.51, 86.38, 83.84, 82.08, 80.58, 78.79, 78.71, 78.02, 77.92, 76.68, 76.46, 76.43, 76.34, 75.87, 75.82, 75.79, 75.67, 75.06, 74.79, 74.74, 73.80, 73.48, 73.23, 72.59, 72.50, 71.38, 71.10, 71.05, 68.20, 67.65, 66.85, 66.05, 66.03, 65.33, 63.78, 60.25, 53.75, 49.23, 49.10, 47.39, 46.27, 45.87, 41.94, 41.05, 39.99, 39.81, 37.89, 37.01, 36.98, 36.62, 35.97, 35.39, 34.33, 34.31, 33.92, 33.17, 32.54, 31.98, 31.30, 30.47, 29.70, 29.54, 29.52, 29.48, 29.46, 29.43, 29.39, 29.38, 29.34, 29.28, 29.25, 29.22, 29.20, 29.16, 29.14, 29.11, 28.40, 27.53, 27.49, 26.26, 25.86, 25.80, 25.75, 25.66, 25.50, 25.31, 24.97, 24.94, 24.68, 24.38, 23.46, 23.35, 20.57, 20.20, 18.17, 17.19, 15.93, 14.41, 13.13, 12.16, 7.56, 7.46, 7.42, 7.26, 7.25, 7.22, 7.16, 7.14, 7.13, 7.10, 7.08, 7.06, 7.05, 6.98, 6.94, 6.90, 6.88, 6.85, 6.78, 5.92, 5.63, 5.43, 5.36, 5.32, 5.25, 5.23, 5.18, 5.17, 4.95, 4.92, 4.43, 4.41; **HRMS** (ESI) m/z : Calcd for $\text{C}_{182}\text{H}_{294}\text{N}_2\text{O}_{34}\text{NaSi}_9$ $[\text{M}+\text{Na}]^+$ 3326.9159, found 3326.9211.



α -Amide variant 7 (SQS-0-6-8-5). A solution of fully protected amide analogue (**S14**) (10.2 mg, 0.003 mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (8 mg, 0.0042 mmol, 2.5 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μ m polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 $^{\circ}$ C) solution of trifluoroacetic acid (3.0 mL, TFA/H₂O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 $^{\circ}$ C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (5:1 water:acetonitrile) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 μ m, 10 \times 250 mm) using a linear gradient of 15 \rightarrow 46% acetonitrile (0.05% TFA) in water (0.05% TFA) to 14 min followed by another linear gradient from 46% to 90 % acetonitrile (0.05% TFA) in water (0.05% TFA) to 16 min at a flow rate of 5 mL/min. The fraction containing the major peak (t_R = 14.72 min) was collected and lyophilized to dryness to afford SQS-0-6-8-5 (**7**) (2.5 mg, 50% yield) as a white solid.

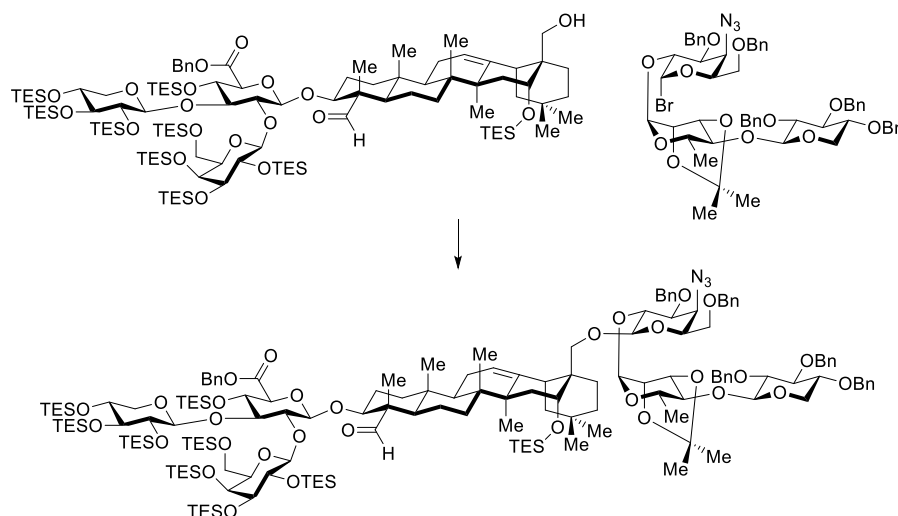
1 H NMR (600 MHz, MeOD) δ 9.35 (s, 1H), 7.47 (d, J = 8.3 Hz, 1H), 7.44 (d, J = 8.9 Hz, 1H), 5.27 (s, 1H), 5.14 (s, 1H), 4.73 – 4.68 (m, 2H), 4.54 – 4.50 (m, 1H), 4.48 (d, J = 7.7 Hz, 1H), 4.34 (t, J = 8.2 Hz, 2H), 4.23 – 4.20 (dd, J = 8.3, 3.8 Hz, 1H), 4.19 – 4.15 (m, 1H), 3.85 (s, 1H), 3.83 – 3.60 (m, 11H), 3.60 – 3.53 (m, 2H), 3.51 (t, J = 6.3 Hz, 1H), 3.45 (t, J = 9.1 Hz, 1H), 3.43 – 3.35 (m, 5H), 3.33 (s, 3H), 3.27 (dd, J = 12.2, 7.3 Hz, 1H), 3.10 (d, J = 15.3 Hz, 7H), 2.92 (dd, J = 14.2, 3.0 Hz, 0H), 2.25 – 2.12 (m, 5H), 1.90 – 1.73 (m, 5H), 1.71 – 1.58 (m, 4H), 1.58 – 1.52 (m, 3H), 1.52 – 1.46 (m, 3H), 1.44 – 1.37 (m, 2H), 1.27 (s, 3H), 1.08 (s, 3H), 1.06 – 0.94 (m, 4H), 0.92 (s, 3H), 0.85 (s, 3H), 0.79 (s, 3H), 0.72 (s, 3H); **HRMS** (ESI) m/z : Calcd for C₇₆H₁₂₂N₂O₃₄Na (M+Na)⁺ 1629.7777, found 1629.7731.

6. SYNTHESIS OF β -ETHER VARIANT 8 (SQS-0-12-5-5)



Protected prosapogenin neopentyl alcohol 15. Solid tetrabutylammonium borohydride (32 mg, 0.124 mmol, 2 equiv) was added to an ice-cooled solution of acyl chloride **12** (130 mg, 0.062 mmol, 1 equiv) in dichloromethane (4 mL) for 4 h then diluted with a saturated solution of sodium bicarbonate (50 mL). Aqueous mixture was extracted with dichloromethane (3x25 mL), organic fractions combined, washed with brine, dried over sodium sulfate, filtered, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate, 30:1 to 10:1) to give neopentyl alcohol **15** as a white foam (99 mg, 77%).

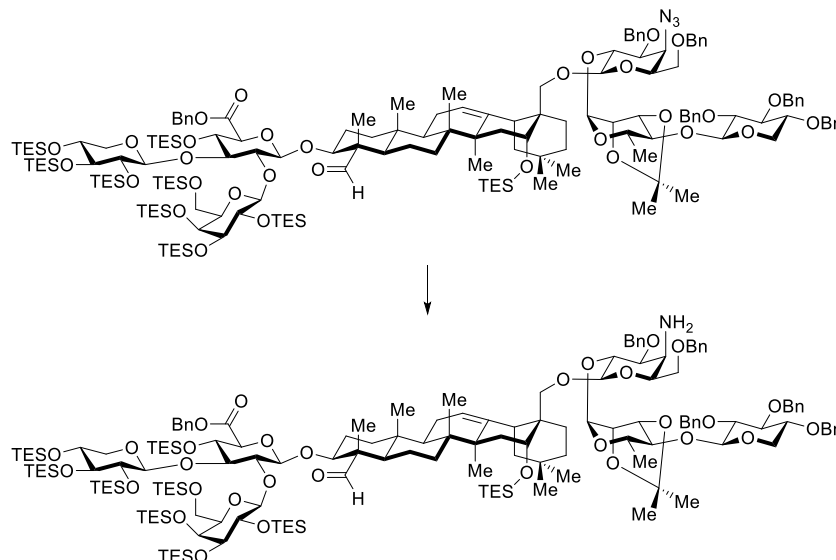
TLC R_f 0.38 (4:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 3538 (OH st), 2952, 2877, 1754, 1722, 1459, 1413, 1377, 1239, 1171, 1103, 1005, 908, 863, 825, 774, 728, 695 cm^{-1} ; **$^1\text{H-NMR}$** (600 MHz, CDCl_3) δ 9.60 (s, 1H), 7.31 – 7.17 (m, 5H), 5.17 (d, $J = 12.4$ Hz, 1H), 5.14 (t, $J = 3.7$ Hz, 1H), 4.97 (d, $J = 12.4$ Hz, 1H), 4.44 (d, $J = 7.4$ Hz, 1H), 4.31 (d, $J = 7.2$ Hz, 1H), 4.07 (d, $J = 7.4$ Hz, 1H), 3.96 (t, $J = 3.3$ Hz, 1H), 3.82 (s, 1H), 3.80 (d, $J = 8.9$ Hz, 1H), 3.77 – 3.66 (m, 4H), 3.63 (t, $J = 9.2$ Hz, 1H), 3.52 – 3.42 (m, 3H), 3.36 (ddd, $J = 10.5, 8.4, 5.1$ Hz, 1H), 3.28 (dd, $J = 9.4, 2.5$ Hz, 1H), 3.26 – 3.18 (m, 3H), 3.13 (t, $J = 8.0$ Hz, 1H), 3.01 (dt, $J = 10.9, 5.4$ Hz, 2H), 2.08 (t, $J = 13.2$ Hz, 1H), 1.87 (dd, $J = 13.9, 4.3$ Hz, 1H), 1.80 – 1.50 (m, 9H), 1.50 – 1.28 (m, 5H), 1.26 (s, 4H), 1.19 (s, 3H), 1.16 – 0.93 (m, 5H), 0.92 – 0.71 (m, 100H), 0.71 – 0.38 (m, 58H); **$^{13}\text{C-NMR}$** (151 MHz, CDCl_3) δ 212.67, 168.37, 143.98, 135.27, 128.47, 128.45, 128.28, 128.14, 121.89, 103.62, 101.40, 100.84, 86.32, 78.81, 78.73, 76.45, 75.96, 75.82, 75.08, 74.40, 72.62, 72.53, 71.39, 71.31, 71.09, 66.84, 65.34, 60.26, 53.89, 49.42, 47.44, 46.13, 42.10, 41.58, 40.33, 39.90, 38.02, 36.03, 35.96, 33.34, 32.85, 32.19, 30.78, 29.60, 26.76, 25.39, 24.37, 23.39, 20.27, 16.83, 15.95, 12.24, 7.57, 7.50, 7.48, 7.26, 7.21, 7.17, 7.15, 7.08, 7.02, 7.00, 6.96, 6.86, 6.80, 5.93, 5.66, 5.46, 5.42, 5.38, 5.35, 5.32, 5.30, 5.27, 5.24, 5.21, 5.07, 4.43; **HRMS** (ESI) m/z : Calcd for $\text{C}_{108}\text{H}_{206}\text{O}_{19}\text{NaSi}_9$ $[\text{M}+\text{Na}]^+$ 2082.2975, found 2082.2942.



Protected prosapogenin β -ether azide 21. To a suspension of primary alcohol acceptor **15** (58 mg, 0.0280 mmol, 1.0 equiv), bromide donor **20** (29 mg, 0.0280 mmol, 1.0 equiv), 2,4,5-tritertbutylpyridine (20.8 mg, 0.084 mmol, 3.0 equiv), and ~25 mg 4 Å MS in 1 mL dichloromethane, cooled to -40 °C, was added solid AgOTf (15 mg, 0.058 mmol, 2.1 equiv). After 45 min, reaction was warmed to 0 °C, stirred for 15 min, then diluted with 5 mL dichloromethane. Crude suspension was sonicated for two min, filtered through a pad of celite, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate, 15:1 to 4:1) to give β -glycoside **21** (58 mg, 0.0192 mmol, 69% yield).

TLC R_f 0.45 (hexanes:ethyl acetate, 5:1); **FTIR** (NaCl film) 2953, 2911, 2876, 2106, 1753, 1725, 1456, 1413, 1379, 1240, 1170, 1097, 1006, 910, 863, 825, 800, 735 cm^{-1} ; **¹H-NMR** (600 MHz, CDCl_3 -*d*) δ 9.69 (s, 1H), 7.42 – 7.16 (m, 30H), 5.37 (s, 1H), 5.28 (d, $J = 12.4$ Hz, 1H), 5.15 (t, $J = 3.6$ Hz, 1H), 5.09 (d, $J = 12.4$ Hz, 1H), 4.89 – 4.80 (m, 4H), 4.72 – 4.66 (m, 2H), 4.64 – 4.60 (m, 2H), 4.57 – 4.51 (m, 4H), 4.42 (d, $J = 7.3$ Hz, 1H), 4.19 (d, $J = 7.4$ Hz, 1H), 4.14 – 4.11 (m, 2H), 4.09 (d, $J = 7.6$ Hz, 1H), 4.00 – 3.97 (m, 2H), 3.97 – 3.89 (m, 4H), 3.87 – 3.72 (m, 8H), 3.64 – 3.53 (m, 10H), 3.52 – 3.45 (m, 2H), 3.39 (dd, $J = 9.5, 2.5$ Hz, 1H), 3.37 – 3.27 (m, 4H), 3.27 – 3.16 (m, 4H), 3.13 (t, $J = 10.9$ Hz, 1H), 2.17 (t, $J = 13.2$ Hz, 1H), 1.98 (dd, $J = 14.1, 4.5$ Hz, 1H), 1.85 – 1.75 (m, 3H), 1.75 – 1.60 (m, 6H), 1.45 (s, 3H), 1.43 – 1.32 (m, 8H), 1.30 (s, 4H), 1.28 (s, 3H), 1.26 – 1.24 (m, 1H), 1.22 (d, $J = 6.1$ Hz, 3H), 1.19 – 1.03 (m, 4H), 1.02 – 0.88 (m, 102H), 0.88 – 0.81 (m, 11H), 0.81 – 0.52 (m, 66H); **¹³C-NMR** (151 MHz, CDCl_3) δ 212.35, 168.38, 144.17, 138.79, 138.60, 138.22, 137.52, 137.08, 135.24, 128.53, 128.52, 128.49, 128.45, 128.44, 128.41, 128.37, 128.32, 128.30, 128.27, 128.26, 128.24, 128.21, 128.14, 128.12, 128.08, 128.04, 128.01, 128.00, 127.99, 127.91, 127.90, 127.86, 127.82, 127.80, 127.76, 127.75, 127.72, 127.65, 127.51, 127.49, 121.68, 109.17, 103.48, 102.78, 102.07, 101.37, 100.83, 97.66, 86.06, 83.81, 82.06, 80.79, 78.78, 78.71, 78.25, 78.11, 78.01, 77.47, 76.42, 76.00, 75.81, 75.53, 75.49, 75.06, 74.69, 74.61, 74.36, 73.72, 73.18, 72.59, 72.51, 72.09, 71.38, 71.28, 71.06, 68.26, 66.83, 65.72, 65.33, 63.75, 60.25, 58.96, 53.90, 49.29, 47.41, 46.05, 41.98, 41.37, 39.90, 39.50, 37.96, 37.67, 35.99, 33.03, 32.85, 32.06, 31.59, 30.86, 30.79, 30.77, 30.31, 30.27, 29.70, 29.05, 27.78, 27.73, 26.63, 26.52, 26.41, 26.33, 26.27, 25.32, 25.27, 24.48, 23.36, 22.66, 20.21, 18.16, 18.13, 17.45, 16.85, 15.94, 14.14, 12.17, 11.45, 7.56, 7.48, 7.46, 7.25, 7.22, 7.20, 7.17, 7.13, 7.07, 7.00, 6.98, 6.95, 6.94, 6.91, 6.85, 6.79, 6.78, 5.91, 5.64, 5.44, 5.40, 5.36, 5.34, 5.30, 5.29, 5.27, 5.25,

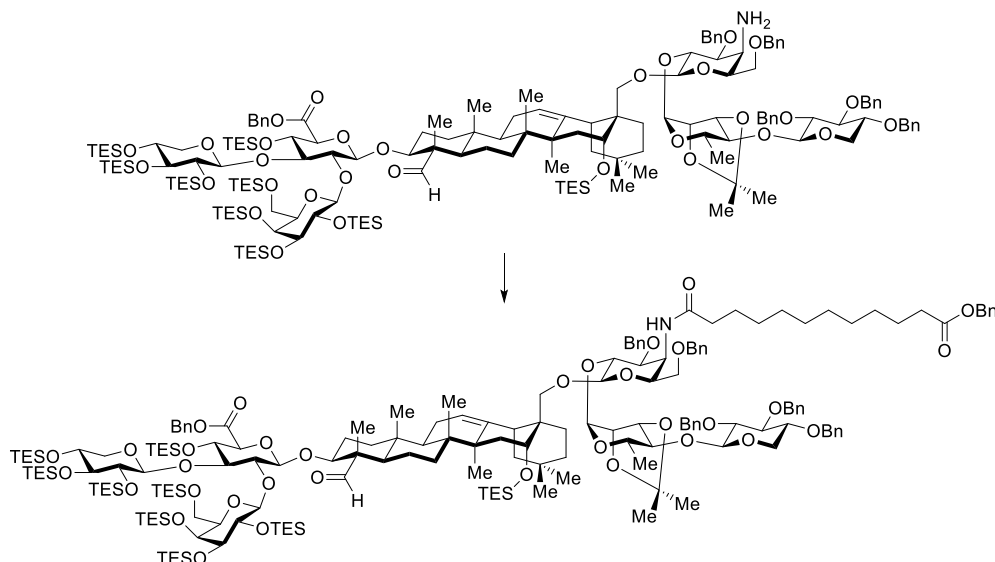
5.23, 4.90, 4.41, 4.40; **HRMS** (ESI) m/z : Calcd for $C_{163}H_{267}N_3O_{31}Si_9Na$ $[M+Na]$ 3037.7230, found 3037.7188.



Protected prosapogenin β -ether amine S15. Hydrogen sulfide was bubbled via cannula through an ice-cooled solution of azide **21** (45 mg, 0.015 mmol, 1 equiv) in pyridine/triethylamine (3.5:1, 4.5 mL) for two min. Vent needle and cannula were removed, septum sealed with Teflon tape and parafilm, then warmed to RT and stirred overnight. Hydrogen sulfide was removed with a stream of nitrogen. The resulting orange solution was concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 5:1 to 2:1) furnishing amine **S15** (40 mg, 88 % yield).

TLC R_f 0.41 (hexanes:ethyl acetate, 2:1 + 0.5% triethylamine); **FTIR** (NaCl film) 3608, 3583, 3028, 2954, 2910, 2876, 1753, 1725, 1631, 1497, 1454, 1413, 1380, 1240, 1168, 1095, 1006, 900, 862, 825, 799, 730, 695, 665 cm^{-1} ; **1H -NMR** (600 MHz, $CDCl_3-d$) δ 9.70 (s, 1H), 7.38 – 7.22 (m, 31H), 5.40 (s, 1H), 5.29 (d, $J = 12.3$ Hz, 1H), 5.17 (d, $J = 3.8$ Hz, 1H), 5.10 (d, $J = 12.3$ Hz, 1H), 4.91 (d, $J = 7.6$ Hz, 1H), 4.89 – 4.81 (m, 3H), 4.71 (d, $J = 11.7$ Hz, 1H), 4.65 – 4.62 (m, 2H), 4.61 (d, $J = 4.9$ Hz, 1H), 4.58 – 4.55 (m, 3H), 4.49 (d, $J = 11.5$ Hz, 1H), 4.43 (d, $J = 7.3$ Hz, 1H), 4.20 (d, $J = 7.3$ Hz, 1H), 4.19 – 4.13 (m, 3H), 4.03 – 3.98 (m, 1H), 3.98 – 3.90 (m, 3H), 3.91 – 3.69 (m, 7H), 3.68 – 3.53 (m, 9H), 3.48 (ddd, $J = 10.5, 8.5, 5.1$ Hz, 1H), 3.42 – 3.19 (m, 10H), 3.14 (t, $J = 10.9$ Hz, 1H), 2.19 (t, $J = 13.2$ Hz, 1H), 2.02 (dd, $J = 14.0, 4.2$ Hz, 1H), 1.86 – 1.50 (m, 13H), 1.47 (s, 3H), 1.35 (s, 6H), 1.31 (s, 3H), 1.29 (s, 3H), 1.23 (d, $J = 6.2$ Hz, 3H), 1.20 – 1.12 (m, 3H), 1.11 – 1.05 (m, 2H), 1.04 – 0.90 (m, 89H), 0.89 – 0.82 (m, 10H), 0.81 – 0.55 (m, 56H). **^{13}C -NMR** (151 MHz, $CDCl_3$) 212.40, 168.40, 144.16, 138.82, 138.60, 138.25, 138.00, 137.61, 135.27, 128.51, 128.49, 128.47, 128.44, 128.31, 128.29, 128.25, 128.15, 128.09, 127.92, 127.90, 127.81, 127.79, 127.77, 127.76, 127.55, 127.52, 121.77, 109.16, 103.50, 102.96, 102.10, 101.40, 100.86, 97.56, 86.07, 83.84, 82.12, 81.65, 78.82, 78.74, 78.27, 78.19, 78.04, 76.45, 76.04, 75.84, 75.67, 75.56, 75.09, 74.77, 74.53, 73.61, 73.28, 73.22, 72.62, 72.54, 71.42, 71.20, 71.09, 69.16, 66.86, 65.56, 65.36, 63.79, 60.29, 53.94, 49.35, 48.97, 47.44, 46.09, 42.01, 41.42, 39.96, 39.59, 38.01, 36.08, 36.02, 33.08, 32.89, 32.11, 30.82, 30.32, 27.80, 26.67, 26.36, 25.35, 24.53, 23.39, 20.24, 18.19, 16.96, 15.97, 12.19, 7.59, 7.49, 7.28, 7.26, 7.24, 7.20, 7.16,

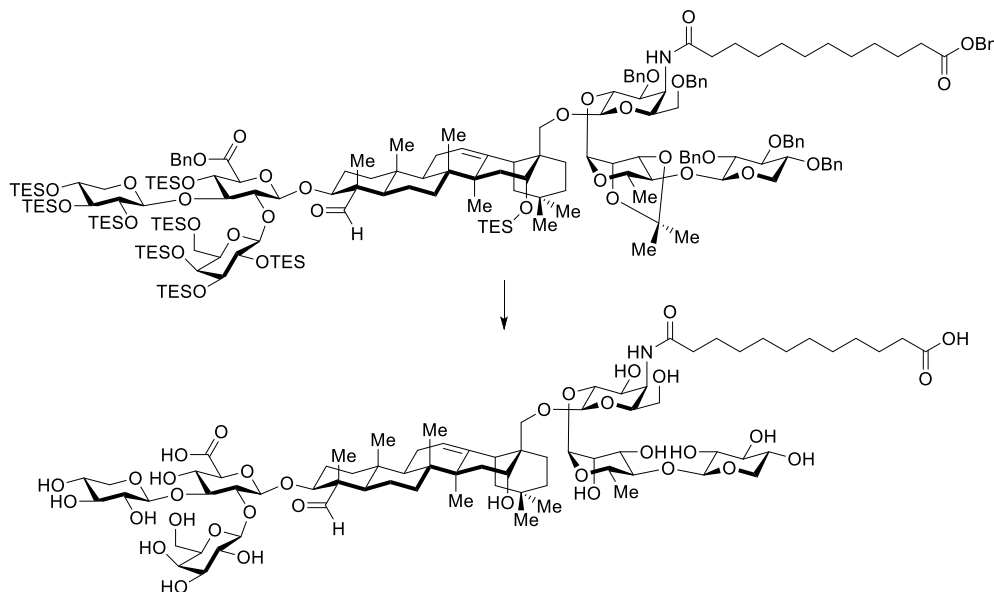
7.03, 7.01, 6.88, 6.82, 5.94, 5.67, 5.47, 5.39, 5.37, 5.31, 5.28, 5.26, 4.95, 4.45; **HRMS** (ESI) m/z : Calcd for $C_{163}H_{270}NO_{31}Si_9$ $[M+H]^+$ 2989.7505, found 2989.7542.



Protected β -ether variant S16. Isobutyl chloroformate (7.0 μ L, 0.053 mmol, 4 equiv) was added to an ice-cooled solution of carboxylic acid **S2** (26 mg, 0.081 mmol, 6 equiv) and triethylamine (37 μ L, 0.268 mmol, 20 equiv) in tetrahydrofuran (2 mL) and stirred for 2 hours, then transferred via cannula to an ice-cooled solution of amine **S15** (40 mg, 0.0134 mmol, 1 equiv) in tetrahydrofuran (1.5 mL). After 4 h, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 \times 25 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give fully protected glycosyl ether analogue **S16** (25 mg, 57 % yield) as a colorless film.

TLC R_f 0.24 (hexanes:ethyl acetate, 4:1); **1H -NMR** (600 MHz, $CDCl_3$) δ 9.70 (s, 1H), 7.40 – 7.16 (m, 35H), 5.52 (d, J = 10.1 Hz, 1H), 5.38 (s, 1H), 5.28 (d, J = 12.3 Hz, 1H), 5.15 (t, J = 3.6 Hz, 1H), 5.13 – 5.07 (m, 3H), 4.92 – 4.78 (m, 5H), 4.76 (d, J = 11.1 Hz, 1H), 4.71 (d, J = 11.6 Hz, 1H), 4.63 (t, J = 10.9 Hz, 2H), 4.56 (d, J = 7.4 Hz, 1H), 4.55 – 4.47 (m, 2H), 4.43 (d, J = 7.2 Hz, 1H), 4.39 (d, J = 11.0 Hz, 1H), 4.21 – 4.14 (m, 3H), 4.12 (d, J = 5.9 Hz, 1H), 3.99 (s, 1H), 3.97 – 3.90 (m, 3H), 3.89 – 3.71 (m, 6H), 3.65 – 3.42 (m, 13H), 3.40 (dd, J = 9.4, 2.5 Hz, 1H), 3.38 – 3.17 (m, 7H), 3.13 (t, J = 10.9 Hz, 1H), 2.35 (t, J = 7.5 Hz, 2H), 2.24 – 2.14 (m, 3H), 2.02 (dd, J = 14.0, 4.3 Hz, 1H), 1.86 – 1.75 (m, 3H), 1.75 – 1.49 (m, 15H), 1.44 – 1.05 (m, 33H), 1.04 – 0.82 (m, 104H), 0.82 – 0.50 (m, 60H); **^{13}C -NMR** (151 MHz, $CDCl_3$) δ 212.46, 173.70, 173.26, 168.40, 144.02, 138.82, 138.57, 138.24, 137.73, 137.59, 136.13, 135.27, 128.55, 128.47, 128.43, 128.35, 128.31, 128.29, 128.28, 128.21, 128.18, 128.17, 128.14, 128.09, 127.91, 127.86, 127.83, 127.79, 127.77, 127.74, 127.69, 127.61, 127.52, 121.90, 109.20, 103.53, 103.16, 102.13, 101.40, 100.86, 97.60, 86.15, 83.84, 82.15, 79.25, 78.82, 78.73, 78.30, 78.15, 78.13, 78.03, 76.45, 76.01, 75.85, 75.83, 75.76, 75.57, 75.12, 75.09, 74.82, 74.51, 73.72, 73.23, 72.70, 72.62, 72.54, 71.41, 71.09, 71.00, 68.86, 66.86, 66.08, 65.74, 65.36, 63.80, 60.28, 53.90, 49.32, 47.35, 46.18, 46.06, 41.92, 41.41, 39.94, 39.66, 38.00, 36.98, 36.08, 36.00, 34.35, 33.08, 32.86, 32.09, 30.81, 30.36, 29.73, 29.46, 29.41, 29.38, 29.28, 29.25, 29.16, 27.73, 26.66, 26.23, 25.90, 25.34, 24.98, 24.47,

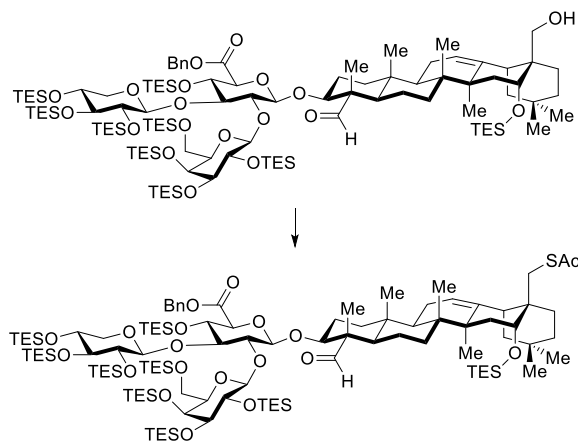
23.39, 20.23, 18.07, 17.00, 15.96, 12.20, 7.59, 7.51, 7.49, 7.32, 7.28, 7.25, 7.23, 7.20, 7.16, 7.09, 7.05, 7.03, 7.01, 6.97, 6.92, 6.88, 6.82, 5.94, 5.67, 5.59, 5.56, 5.46, 5.43, 5.39, 5.37, 5.33, 5.30, 5.28, 5.26, 5.19, 5.17, 5.14, 5.08, 4.99, 4.95, 4.44; **HRMS** (ESI) m/z : Calcd for $C_{182}H_{295}N_3O_{34}Si_9Na$ $[M+Na]^+$ 3133.9207, found 3133.9258.



β -Ether variant 8 (SQS-0-12-5-5). A solution of fully protected ether analogue (**S16**) (25 mg, 0.008 mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (25 mg, 0.023 mmol, 3 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μ m polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 $^{\circ}$ C) solution of trifluoroacetic acid (3.0 mL, TFA/ H_2O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 $^{\circ}$ C to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (4:1 water:acetonitrile) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 μ m, 10 \times 250 mm) using a linear gradient of 20 \rightarrow 66% acetonitrile (0.05% TFA) in water (0.05% TFA) over 16 min at a flow rate of 5 mL/min. The fraction containing the major peak (t_R = 12.55 min) was collected and lyophilized to dryness to afford SQS-0-12-5-5 (**8**) (7.5 mg, 62 % yield) as a white solid.

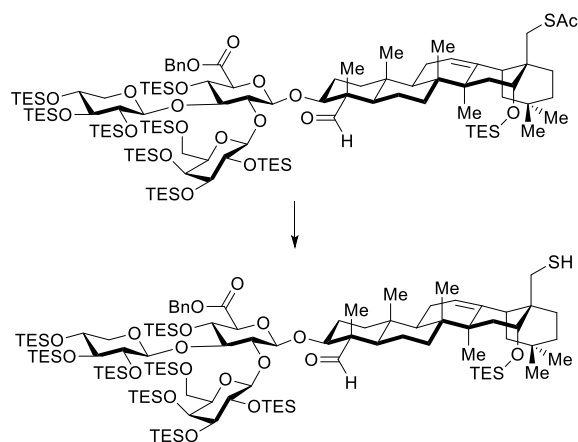
1H -NMR (600 MHz, D_2O/CD_3CN , 1:1) δ 9.97 (s, 1H), 7.73 (d, J = 9.7 Hz, 1H), 5.82 (t, J = 3.6 Hz, 1H), 5.74 (d, J = 1.9 Hz, 1H), 5.26 (d, J = 7.8 Hz, 1H), 5.13 (d, J = 7.8 Hz, 1H), 5.08 (d, J = 7.8 Hz, 1H), 5.01 (d, J = 7.8 Hz, 1H), 4.78 (s, 1H), 4.71 (d, J = 7.6 Hz, 1H), 4.61 (d, J = 4.0 Hz, 1H), 4.51 – 4.43 (m, 4H), 4.43 – 4.33 (m, 5H), 4.30 (t, J = 8.9 Hz, 2H), 4.25 – 4.18 (m, 2H), 4.17 – 4.00 (m, 9H), 3.93 (p, J = 8.9, 8.5 Hz, 6H), 3.86 – 3.77 (m, 4H), 3.78 – 3.72 (m, 1H), 2.90–2.77 (m, J = 7.5 Hz, 5H), 2.75 – 2.65 (m, 2H), 2.48 – 2.41 (m, 3H), 2.41 – 2.01 (m, 17H), 1.90 (s, 3H), 1.83 (d, J = 6.3 Hz, 5H), 1.74 (d, J = 12.2 Hz, 1H), 1.69 (s, 3H), 1.64 (t, J = 12.9 Hz, 1H), 1.55 (s, 3H), 1.47 (s, 3H), 1.46 (s, 3H), 2.83 – 2.77 (m, 1H), 1.43 (s, 3H); **HRMS** (ESI) m/z : Calcd for $C_{76}H_{123}NO_{34}Na$ $[M+Na]^+$ 1616.7824, found 1616.7848

7. SYNTHESIS OF β -THIOETHER VARIANT 9 (SQS-0-14-5-5)



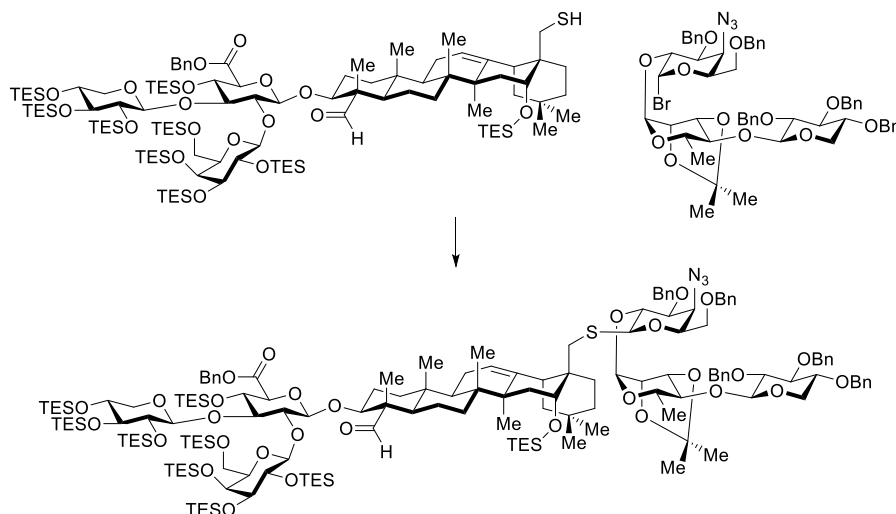
Protected prosapogenin neopentyl thioacetate S17. Triflic anhydride (12.6 μ L, 0.075 mmol, 1.5 equiv) was added to an ice-cooled solution of neopentyl alcohol **15** (103 mg, 0.05 mmol, 1 equiv) and pyridine (80 μ L, 1.0 mmol, 20 equiv) and dichloromethane (4 mL). After 15 min, dichloromethane was removed with a stream of argon. Residual volatiles were removed under reduced pressure. Resulting oil was taken up in tetrahydrofuran (2 mL), cooled to 0 $^{\circ}$ C, then treated with 4 Å MS (~100 mg), and dimethylformamide (2 mL). Suspension was treated with potassium thioacetate (57 mg, 0.5 mmol, 10 equiv). After 2.5 h, suspension was decanted into a saturated solution of sodium bicarbonate and extracted with ethyl acetate (3 \times 25 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated and purified with silica gel chromatography (hexanes: ethyl acetate, 50:1 to 25:1) to give thioacetate **S17** (98 mg, 92%).

TLC R_f 0.60 (10:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 2953, 2911, 2876, 1754, 1723, 1700, 1696, 1653, 1635, 1576, 1560, 1539, 1457, 1414, 1375, 1239, 1171, 1103, 1005, 970, 898, 864, 826, 799, 736, 695, 668, 628 cm^{-1} ; **$^1\text{H-NMR}$** (600 MHz, CDCl_3) δ 9.65 (s, 1H), 7.31 – 7.22 (m, 5H), 5.25 – 5.19 (m, 2H), 5.02 (d, J = 12.4 Hz, 1H), 4.48 (d, J = 7.4 Hz, 1H), 4.36 (d, J = 7.3 Hz, 1H), 4.11 (d, J = 7.4 Hz, 1H), 3.88 – 3.81 (m, 3H), 3.81 – 3.70 (m, 4H), 3.68 (t, J = 9.2 Hz, 1H), 3.56 – 3.47 (m, 3H), 3.41 (ddd, J = 10.5, 8.4, 5.1 Hz, 1H), 3.32 (dd, J = 9.4, 2.5 Hz, 1H), 3.30 – 3.24 (m, 2H), 3.18 (dd, J = 8.7, 7.4 Hz, 1H), 3.06 (t, J = 11.0 Hz, 1H), 2.86 (d, J = 13.4 Hz, 1H), 2.57 (d, J = 13.5 Hz, 1H), 2.25 (s, 3H), 2.16 (dd, J = 13.9, 12.4 Hz, 1H), 2.07 (dd, J = 14.0, 4.0 Hz, 1H), 1.88 – 1.51 (m, 10H), 1.49 – 1.44 (m, 2H), 1.43 – 1.30 (m, 3H), 1.29 (s, 3H), 1.24 (s, 3H), 1.16 – 1.10 (m, 2H), 1.07 – 0.99 (m, 2H), 0.97 – 0.81 (m, 93H), 0.79 (s, 3H), 0.75 – 0.47 (m, 56H); **$^{13}\text{C-NMR}$** (151 MHz, CDCl_3) δ 212.83, 195.72, 168.36, 143.29, 135.26, 128.48, 128.46, 128.30, 128.15, 128.11, 122.68, 103.65, 101.42, 100.84, 86.43, 78.82, 78.73, 76.45, 76.30, 75.95, 75.83, 75.81, 75.09, 72.62, 72.53, 71.38, 71.08, 66.85, 65.34, 60.22, 53.89, 49.43, 47.57, 46.13, 45.72, 41.44, 41.02, 39.89, 38.86, 38.02, 36.34, 36.03, 33.41, 32.80, 32.13, 31.71, 30.83, 30.81, 26.82, 25.40, 24.29, 23.48, 20.24, 16.73, 15.94, 12.30, 7.58, 7.48, 7.27, 7.18, 7.17, 7.15, 7.00, 6.87, 6.81, 6.80, 5.93, 5.66, 5.46, 5.38, 5.36, 5.29, 5.27, 5.24, 5.01, 4.43; **HRMS** (ESI) m/z : Calcd for $\text{C}_{110}\text{H}_{208}\text{O}_{19}\text{SSi}_9\text{Na}$ [M+Na] $^{+}$ 2140.2883, found 2140.2852.



Protected prosapogenin neopentyl thiol 16. Hydrazine (10 μ L, 0.323 mmol, 7 equiv) was added to a solution of thioacetate **S17** (98 mg, 0.046 mmol, 1 equiv) and dithiothreitol (21 mg, 0.139 mmol, 3 equiv) in tetrahydrofuran/dimethylformamide (4 mL, 1:1) for 6 h, concentrated and purified with silica gel chromatography (hexanes: ethyl acetate) to give thiol **16** as a colorless film (86 mg, 90%).

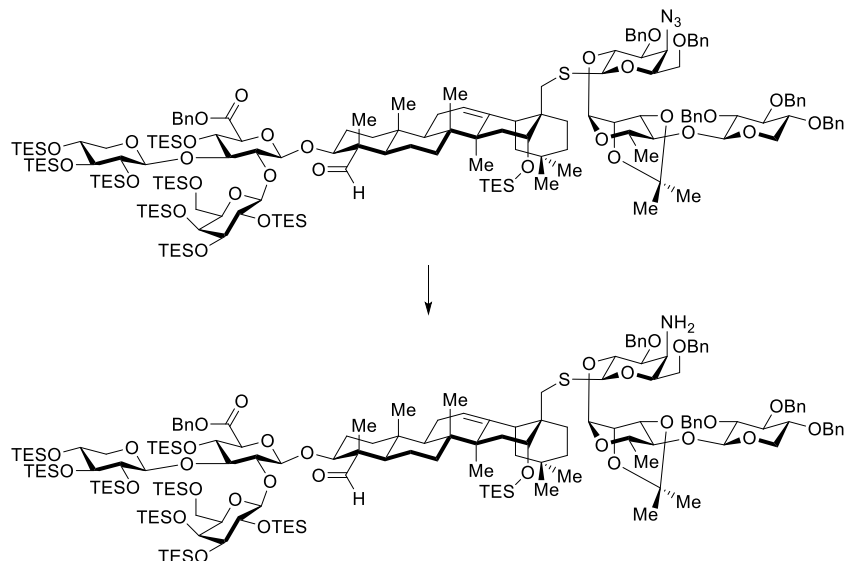
TLC R_f 0.71 (10:1 hexanes/ethyl acetate); **FTIR** (NaCl film) 2953, 2911, 2877, 1756, 1726, 1653, 1458, 1414, 1375, 1240, 1172, 1104, 1006, 971, 899, 865, 827, 801, 739, 695, 679, 668; **¹H-NMR** (600 MHz, CDCl₃) δ 9.72 (s, 1H), 7.34 (m, 5H), 5.29 – 5.25 (m, 2H), 5.09 (d, J = 12.4 Hz, 1H), 4.56 (d, J = 7.5 Hz, 1H), 4.43 (d, J = 7.3 Hz, 1H), 4.18 (d, J = 7.4 Hz, 1H), 4.14 – 4.09 (m, 1H), 3.94 – 3.89 (m, 2H), 3.88 – 3.77 (m, 4H), 3.75 (t, J = 9.3 Hz, 1H), 3.63 – 3.54 (m, 3H), 3.51 – 3.45 (m, 1H), 3.39 (dd, J = 9.3, 2.5 Hz, 1H), 3.37 – 3.32 (m, 2H), 3.25 (t, J = 8.0 Hz, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.38 – 2.31 (m, 1H), 2.31 – 2.22 (m, 2H), 2.15 (dd, J = 14.0, 4.1 Hz, 1H), 1.87 (dt, J = 11.1, 4.1 Hz, 2H), 1.82 – 1.73 (m, 2H), 1.72 – 1.38 (m, 10H), 1.37 (s, 3H), 1.31 (s, 2H), 1.25 (s, 1H), 1.23 – 1.15 (m, 2H), 1.16 – 1.05 (m, 3H), 1.04 – 0.89 (m, 92H), 0.87 (s, 3H), 0.87 (s, 3H), 0.81 – 0.55 (m, 57H); **¹³C-NMR** (151 MHz, CDCl₃) δ 212.71, 168.34, 143.60, 135.25, 128.50, 128.46, 128.44, 128.27, 128.15, 128.13, 128.09, 122.34, 103.63, 101.40, 100.83, 78.80, 78.72, 76.44, 75.94, 75.81, 75.71, 75.08, 72.61, 72.51, 71.38, 71.08, 66.83, 65.33, 60.23, 41.39, 39.82, 38.41, 37.99, 36.71, 36.43, 36.42, 36.01, 33.12, 32.84, 32.09, 30.96, 30.84, 26.76, 25.38, 24.23, 23.43, 20.23, 16.71, 15.92, 12.25, 7.57, 7.49, 7.47, 7.25, 7.19, 7.15, 7.14, 7.01, 6.99, 6.85, 6.79, 6.78, 5.92, 5.65, 5.45, 5.41, 5.37, 5.35, 5.30, 5.28, 5.26, 5.23, 5.04, 4.42, 4.40; **HRMS** (ESI) m/z : Calcd for C₁₀₈H₂₀₆O₁₈NaSi₉S [M+Na]⁺ 2098.2746, found 2098.2778.



Protected prosapogenin β -thioether azide **22.** A solution of bromide **20** (32 mg, 0.031 mmol, 1.5 equiv) in tetrahydrofuran (1.5 mL) was added dropwise to a suspension of thiol **16** (43 mg, 0.021 mmol, 1.0 equiv) and sodium hydride (60% dispersion in mineral oil, 2.5 mg, 0.062, 3.0 equiv) in tetrahydrofuran/dimethylformamide (2 mL, 1:1) over four min. After 20 min, a saturated solution of ammonium chloride was added, diluted with water, and extracted with (3 \times 25 mL). Combined extracts were washed with brine, dried over sodium sulfate, and concentrated. Before loading onto silica column, silver triflate (2 mg) was added to crude solution in DCM to destroy excess glycosyl bromide. Mixture was purified with silica gel chromatography (benzene:ethyl acetate, 1:0 to 30:1) to give glycosyl thioether **22** as a colorless film (43 mg, 69% yield). Note: Extended reaction times gave lower yields, due to formation of the trisaccharide glycal, through base-promoted elimination of the thiolate.

TLC R_f 0.52 (benzene:ethyl acetate, 20:1); **FTIR** (NaCl film) 3032, 2953, 2912, 2876, 2107, 1752, 1724, 1701, 1497, 1457, 1413, 1380, 1240, 1169, 1094, 1006, 899, 864, 826, 736, 697, 668, 610 cm^{-1} ; **$^1\text{H-NMR}$** (600 MHz, CDCl_3 -*d*) δ 9.69 (s, 1H), 7.40 – 7.24 (m, 30H), 5.53 (s, 1H), 5.28 (d, $J = 12.4$ Hz, 1H), 5.19 (t, $J = 3.7$ Hz, 1H), 5.09 (d, $J = 12.4$ Hz, 1H), 4.91 (d, $J = 5.3$ Hz, 1H), 4.89 (d, $J = 8.5$ Hz, 1H), 4.87 – 4.79 (m, 2H), 4.72 (t, $J = 11.1$ Hz, 2H), 4.64 (d, $J = 10.8$ Hz, 1H), 4.61 (d, $J = 11.7$ Hz, 1H), 4.57 – 4.52 (m, 4H), 4.42 (d, $J = 7.2$ Hz, 1H), 4.20 – 4.16 (m, 2H), 4.14 (d, $J = 9.6$ Hz, 1H), 4.10 (d, $J = 3.4$ Hz, 1H), 4.08 – 4.01 (m, 2H), 4.00 – 3.97 (m, 1H), 3.96 – 3.73 (m, 9H), 3.64 – 3.52 (m, 9H), 3.48 (ddd, $J = 10.5, 8.4, 5.1$ Hz, 1H), 3.39 (dd, $J = 9.4, 2.5$ Hz, 1H), 3.37 – 3.32 (m, 2H), 3.30 (dd, $J = 8.9, 7.5$ Hz, 1H), 3.25 (dd, $J = 8.7, 7.4$ Hz, 1H), 3.22 – 3.16 (m, 1H), 3.13 (t, $J = 11.0$ Hz, 1H), 2.49 – 2.37 (m, 2H), 2.21 – 2.05 (m, 2H), 1.84 – 1.73 (m, 3H), 1.55 – 1.46 (m, 7H), 1.39 – 1.22 (m, 16H), 1.11 – 0.83 (m, 99H), 0.83 – 0.50 (m, 63H); **$^{13}\text{C-NMR}$** (151 MHz, CDCl_3) δ 212.40, 168.35, 143.40, 138.78, 138.60, 138.23, 137.48, 137.46, 136.69, 135.23, 128.55, 128.51, 128.46, 128.44, 128.41, 128.32, 128.29, 128.27, 128.26, 128.24, 128.20, 128.18, 128.13, 128.12, 128.09, 128.02, 127.93, 127.91, 127.87, 127.76, 127.75, 127.54, 127.51, 127.49, 122.30, 108.91, 103.49, 102.27, 102.24, 101.37, 100.83, 98.65, 86.14, 84.66, 83.87, 83.20, 82.12, 78.78, 78.70, 78.25, 78.07, 77.99, 77.98, 76.42, 76.32, 76.10, 75.96, 75.80, 75.58, 75.43, 75.05, 74.81, 74.03, 73.75, 73.73, 73.19, 72.59, 72.51, 71.58, 71.38, 71.05, 68.23, 66.84, 65.44, 65.32, 63.80, 60.25, 58.59, 53.87, 49.24, 47.56, 46.05, 45.40, 45.35, 42.86, 41.53, 41.27, 41.20, 39.78, 39.54, 39.02, 39.01, 38.70, 37.94, 36.40, 36.38, 35.98, 35.95, 34.00, 33.03, 32.77, 32.04, 31.93, 31.65, 30.74, 30.73, 30.38, 29.73, 29.70, 28.91, 27.77, 26.76, 26.73,

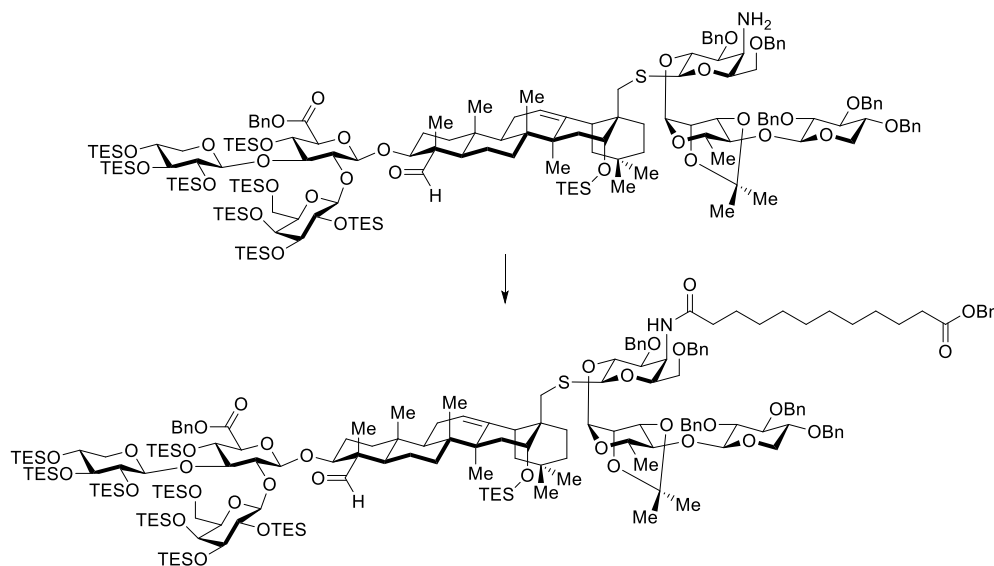
26.61, 26.40, 25.31, 24.47, 24.35, 24.34, 23.76, 23.72, 23.35, 22.99, 22.97, 22.70, 20.22, 17.56, 17.54, 16.81, 16.09, 15.88, 14.14, 14.07, 14.06, 13.15, 12.19, 10.98, 10.96, 7.56, 7.46, 7.25, 7.19, 7.16, 7.13, 6.98, 6.85, 6.79, 6.78, 5.91, 5.64, 5.43, 5.36, 5.33, 5.27, 5.25, 5.22, 4.94, 4.41; **HRMS** (ESI) m/z : Calcd for $C_{163}H_{267}N_3O_{30}NaSi_9S$ 3053.7001 $[M+Na]^+$, found 3053.7014.



Protected prosapogenin β -thioether amine S18. Hydrogen sulfide was bubbled via cannula through an ice-cooled solution of azide **22** (41 mg, 0.014 mmol, 1.0 equiv) in pyridine/triethylamine (3.5:1, 4.5 mL) for two min. Vent needle and cannula were removed, and septum sealed with Teflon tape and parafilm, then warmed to RT and stirred overnight. Hydrogen sulfide was removed with a stream of nitrogen, then resulting orange solution was concentrated and purified via silica gel chromatography (hexanes:[ethyl acetate + 1% triethylamine], 5:1 to 2:1) furnishing amine **S18** (38 mg, 94 % yield).

TLC R_f 0.47 (hexanes:ethyl acetate, 2:1+0.5% triethylamine); **FTIR** (NaCl film) 3608, 2953, 2911, 2876, 1754, 1725, 1692, 1530, 1497, 1454, 1413, 1380, 1240, 1094, 1005, 825, 734, 696 cm^{-1} ; **1H NMR** (500 MHz, $CDCl_3$) δ 9.68 (s, 1H), 7.42 – 7.27 (m, 32H), 5.55 (s, 1H), 5.28 (d, J = 12.4 Hz, 1H), 5.23 – 5.17 (m, 1H), 5.10 (d, J = 12.4 Hz, 1H), 4.94 – 4.89 (m, 2H), 4.88 – 4.79 (m, 2H), 4.71 (d, J = 11.7 Hz, 1H), 4.68 – 4.52 (m, 7H), 4.49 (d, J = 11.4 Hz, 1H), 4.42 (d, J = 7.3 Hz, 1H), 4.23 – 4.16 (m, 3H), 4.12 (d, J = 5.7 Hz, 1H), 4.11 – 4.04 (m, 1H), 4.02 – 3.98 (m, 1H), 3.98 – 3.91 (m, 3H), 3.90 – 3.73 (m, 6H), 3.73 – 3.66 (m, 1H), 3.67 – 3.54 (m, 9H), 3.53 – 3.44 (m, 2H), 3.42 – 3.29 (m, 5H), 3.26 (t, J = 8.0 Hz, 1H), 3.22 – 3.18 (m, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.52 (d, J = 12.3 Hz, 1H), 2.43 (d, J = 12.2 Hz, 1H), 2.23 – 2.07 (m, 2H), 1.84 – 1.76 (m, 3H), 1.76 – 1.65 (m, 3H), 1.63 – 1.53 (m, 4H), 1.39 – 1.24 (m, 17H), 1.17 – 1.07 (m, 2H), 1.08 – 0.88 (m, 100H), 0.87 (s, 4H), 0.81 (s, 4H), 0.80 (s, 3H), 0.77 – 0.52 (m, 61H); **^{13}C -NMR** (151 MHz, $CDCl_3$) δ 212.39, 168.36, 143.46, 138.81, 138.78, 138.57, 138.24, 137.97, 137.18, 135.23, 128.49, 128.46, 128.44, 128.42, 128.41, 128.37, 128.33, 128.28, 128.25, 128.13, 128.09, 128.00, 127.96, 127.93, 127.91, 127.87, 127.85, 127.83, 127.78, 127.75, 127.70, 127.66, 127.57, 127.52, 127.50, 122.28, 108.89, 103.50, 102.32, 102.29, 101.36, 100.83, 98.44, 86.11, 84.76, 83.97, 83.89, 82.18, 78.78, 78.71, 78.25, 78.10, 78.00, 77.98, 77.92, 76.54, 76.43, 76.40, 76.24, 75.97, 75.80, 75.60, 75.05, 74.86, 73.93, 73.65, 73.63, 73.20, 73.17, 72.59, 72.51, 71.39, 71.06,

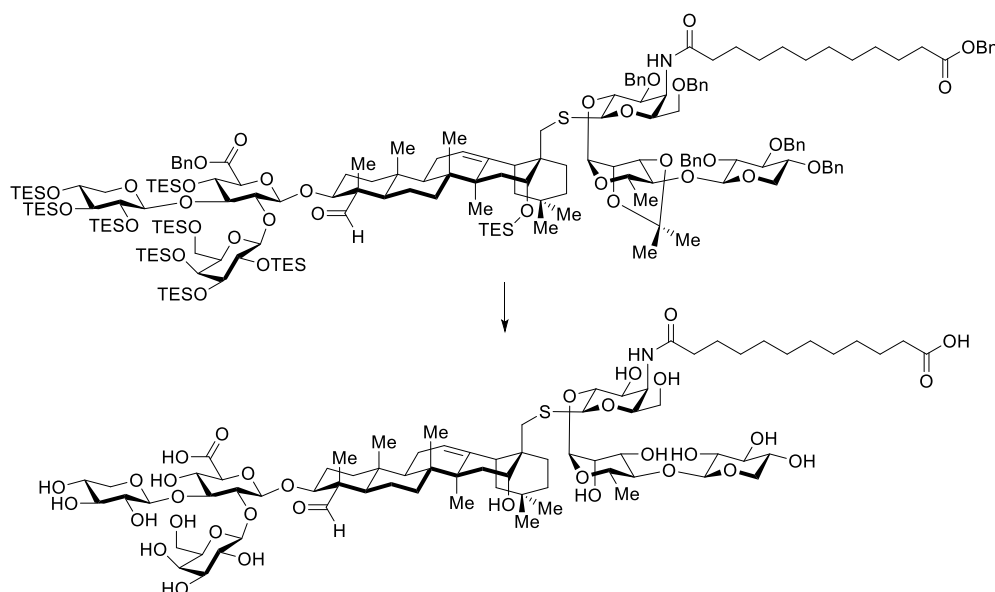
70.64, 69.42, 66.84, 66.81, 65.34, 65.32, 63.81, 60.27, 53.87, 49.26, 49.02, 47.52, 46.03, 45.36, 42.86, 41.71, 41.31, 41.29, 41.22, 39.80, 39.56, 39.09, 39.03, 37.95, 36.61, 36.40, 36.37, 35.98, 35.96, 35.95, 33.07, 32.78, 32.01, 31.93, 31.71, 30.78, 30.75, 29.70, 29.66, 29.37, 28.40, 27.81, 27.78, 26.73, 26.71, 26.61, 26.42, 26.35, 25.31, 24.68, 24.40, 24.37, 23.34, 22.70, 22.39, 20.20, 17.56, 17.07, 16.92, 16.91, 16.09, 15.90, 15.88, 14.14, 13.13, 12.16, 7.56, 7.48, 7.46, 7.25, 7.21, 7.19, 7.17, 7.13, 7.00, 6.98, 6.95, 6.93, 6.88, 6.85, 6.79, 6.78, 5.91, 5.75, 5.64, 5.44, 5.40, 5.36, 5.33, 5.28, 5.27, 5.25, 5.23, 4.96, 4.94, 4.41, 4.40; **HRMS** (ESI) m/z : Calcd for $C_{163}H_{270}NO_{30}Si_9S$ 3005.7277 $[M+H]^+$, found 3005.7317.



Protected β -thioether variant S19. Isobutyl chloroformate (6.4 μ L, 0.049 mmol, 4 equiv) was added to an ice-cooled solution of carboxylic acid **S2** (23.5 mg, 0.073 mmol, 6 equiv) and triethylamine (17 μ L, 0.122 mmol, 10 equiv) in tetrahydrofuran (3 mL) and stirred for 3 hours, then transferred via cannula to an ice-cooled solution of amine **S18** (37 mg, 0.012 mmol, 1 equiv) in tetrahydrofuran (1 mL). After 16 h, suspension was diluted with saturated sodium bicarbonate and then extracted with ethyl acetate (3 \times 25 mL). Combined organics were washed with brine, dried over sodium sulfate, concentrated, and purified with silica gel chromatography (hexanes:ethyl acetate + 0.5% triethylamine, 10:1 to 1:1) to give glycosyl thioether **S19** (27 mg, 67% yield) as a colorless film.

TLC R_f (hexanes:ethyl acetate, 2:1+0.5% triethylamine); **FTIR** (NaCl film) 2952, 2876, 1752, 1741, 1732, 1886, 1681, 1497, 1455, 1380, 1240, 1100, 1006, 826, 734, 697 cm^{-1} ; **1H -NMR** (600 MHz, $CDCl_3$) δ 9.69 (s, 1H), 7.38 – 7.26 (m, 35H), 5.54 (s, 1H), 5.49 (d, J = 10.2 Hz, 1H), 5.28 (d, J = 12.4 Hz, 1H), 5.18 (s, 1H), 5.13 – 5.06 (m, 3H), 4.94 – 4.80 (m, 5H), 4.76 (d, J = 11.0 Hz, 1H), 4.71 (d, J = 11.7 Hz, 1H), 4.63 (dd, J = 13.9, 11.2 Hz, 2H), 4.56 (d, J = 7.4 Hz, 1H), 4.52 – 4.47 (m, 2H), 4.44 – 4.39 (m, 2H), 4.23 (d, J = 8.6 Hz, 1H), 4.20 – 4.16 (m, 2H), 4.11 (d, J = 5.7 Hz, 1H), 4.05 (dd, J = 10.1, 6.1 Hz, 1H), 3.96 – 3.90 (m, 4H), 3.88 – 3.72 (m, 5H), 3.67 – 3.53 (m, 9H), 3.52 – 3.45 (m, 3H), 3.40 (dd, J = 9.4, 2.5 Hz, 1H), 3.31 (dd, J = 9.0, 7.5 Hz, 3H), 3.25 (dd, J = 8.7, 7.4 Hz, 1H), 3.20 (dd, J = 12.1, 10.2 Hz, 1H), 3.13 (t, J = 10.9 Hz, 1H), 2.56 (d, J = 12.2 Hz, 1H), 2.39 (d, J = 12.2 Hz, 1H), 2.35 (t, J = 7.6 Hz, 2H), 2.15 (dtt, J = 18.0, 14.0, 8.5 Hz, 4H), 1.82 – 1.75 (m, 3H), 1.74 – 1.52 (m, 13H), 1.36 (s, 3H), 1.34 – 1.17 (m, 25H), 1.05 – 0.83

(m, 99H), 0.81 – 0.53 (m, 65H); $^{13}\text{C-NMR}$ (151 MHz, CDCl_3) δ 212.41, 173.72, 173.38, 168.38, 143.46, 138.79, 138.58, 138.56, 138.26, 137.76, 137.19, 137.17, 136.14, 135.26, 128.74, 128.55, 128.53, 128.48, 128.46, 128.45, 128.43, 128.35, 128.32, 128.31, 128.29, 128.25, 128.18, 128.16, 128.15, 128.11, 127.96, 127.94, 127.83, 127.81, 127.79, 127.77, 127.74, 127.73, 127.70, 127.68, 127.63, 127.56, 127.53, 122.34, 108.90, 103.53, 102.44, 101.39, 100.86, 98.66, 86.14, 85.30, 83.91, 82.26, 81.10, 78.81, 78.73, 78.25, 78.18, 78.01, 77.46, 76.45, 76.30, 75.98, 75.84, 75.82, 75.63, 75.09, 74.91, 74.76, 73.73, 73.71, 73.22, 72.62, 72.54, 71.41, 71.09, 70.71, 69.10, 66.86, 66.84, 66.07, 65.46, 65.35, 63.85, 60.29, 53.86, 49.25, 47.40, 46.47, 46.03, 45.22, 42.86, 42.37, 41.30, 41.22, 39.81, 39.58, 39.10, 37.96, 36.92, 36.40, 36.37, 36.09, 35.95, 34.68, 34.55, 34.36, 33.14, 32.78, 31.96, 31.83, 31.62, 30.78, 29.73, 29.51, 29.46, 29.42, 29.40, 29.37, 29.29, 29.24, 29.21, 29.16, 29.08, 27.83, 26.76, 26.64, 26.49, 26.45, 25.90, 25.34, 25.30, 24.99, 24.39, 23.37, 22.69, 20.73, 20.20, 18.79, 17.51, 17.48, 16.86, 16.12, 15.91, 14.17, 13.15, 12.20, 11.48, 7.59, 7.51, 7.49, 7.30, 7.28, 7.22, 7.20, 7.19, 7.16, 7.03, 7.01, 6.88, 6.82, 6.81, 5.94, 5.66, 5.46, 5.42, 5.39, 5.36, 5.34, 5.31, 5.30, 5.28, 5.25, 4.96, 4.44, 4.42; **HRMS** (ESI) m/z : Calcd for $\text{C}_{182}\text{H}_{295}\text{NO}_{33}\text{NaSi}_9\text{S}$ 3329.8978 $[\text{M}+\text{Na}]^+$, found 3329.9033.



β -Thioether variant 9 (SQS-0-14-5-5). A solution of fully protected thioether analogue (**S19**) (26 mg, 0.008 mmol, 1.0 equiv) in tetrahydrofuran (2 mL) and ethanol (2 mL) in a 25 mL round bottom flask was charged with 10% (dry basis) palladium on carbon, wet, Degussa type E101 NE/W (33 mg, 0.031 mmol, 4 equiv). Reaction mixture was stirred under hydrogen pressure (50 psi) overnight, then filtered through a 0.45 μm polyvinylidene fluoride filter disk, washed with methanol (5 mL), and concentrated. To the hydrogenation product was added a pre-cooled (0 $^\circ\text{C}$) solution of trifluoroacetic acid (4.0 mL, TFA/ H_2O 3:1). After vigorous stirring for 60 min, the solution was concentrated *in vacuo* at 0 $^\circ\text{C}$ to give white solid residue. This crude product was partially dissolved in a solution of aqueous acetonitrile (4:1 water:acetonitrile) and purified by RP-HPLC on an XBridge Prep BEH300 C18 column (5 μm , 10 \times 250 mm) using a linear gradient of 20 \rightarrow 75% acetonitrile (0.05% TFA) in water (0.05% TFA) over 19 min at a flow rate of 5 mL/min. The fraction containing the major peak (t_{R} = 12.60 min) was collected and lyophilized to dryness to afford SQS-0-14-5-5 (**9**) (5.8 mg, 46 % yield) as a white solid.

¹H-NMR (600 MHz, D₂O/CD₃CN, 1:1) δ 9.99 (s, 1H), 7.64 (d, *J* = 9.6 Hz, 1H), 5.89 (t, *J* = 3.7 Hz, 1H), 5.60 (d, *J* = 1.8 Hz, 1H), 5.28 (d, *J* = 7.8 Hz, 1H), 5.15 (d, *J* = 7.8 Hz, 1H), 5.08 (d, *J* = 7.8 Hz, 1H), 5.02 (d, *J* = 7.8 Hz, 1H), 4.80 (d, *J* = 9.7 Hz, 1H), 4.72 (dq, *J* = 9.7, 6.2 Hz, 1H), 4.58 – 4.54 (m, 1H), 4.52 – 4.50 (m, 1H), 4.50 – 4.45 (m, 1H), 4.42 – 4.29 (m, 7H), 4.25 – 4.21 (m, 2H), 4.16 – 4.05 (m, 8H), 4.05 – 4.00 (m, 1H), 3.98 – 3.88 (m, 4H), 3.85 – 3.79 (m, 3H), 3.79 – 3.74 (m, 1H), 3.20 (d, *J* = 11.8 Hz, 1H), 3.13 (d, *J* = 11.8 Hz, 1H), 2.88 (t, *J* = 7.5 Hz, 2H), 2.86 – 2.80 (m, 2H), 2.80 – 2.75 (m, 2H), 2.71 – 2.68 (m, 0H), 2.52 – 2.41 (m, 5H), 2.35 – 2.23 (m, 5H), 2.18 – 2.06 (m, 7H), 1.94 (s, 3H), 1.84 (d, *J* = 6.1 Hz, 3H), 1.77 – 1.73 (m, 1H), 1.72 (s, 3H), 1.57 (s, 3H), 1.50 (s, 3H), 1.48 (s, 3H), 1.45 (s, 3H); **HRMS** (ESI) *m/z*: Calcd for C₇₆H₁₂₃NO₃₃NaS 1632.7596 [M+Na]⁺, found 1632.7648.

D. PRECLINICAL EVALUATION OF SAPONIN ADJUVANTS

Preparation of GD3 KLH conjugate. GD3 was extracted from bovine buttermilk and conjugated to keyhole limpet hemocyanin (KLH) as described previously.^{4,5} The double bond of GD3–ceramide was converted to aldehyde by ozonolysis and the aldehyde group was conjugated to ϵ -amino groups of lysine on KLH by reductive amination. Briefly, conjugation method is as follows, 50 mg of GD3 was dissolved in 5mL methanol and cooled in an ethanol-dry ice bath. Ozone was generated by an ozone generator (Del Industries, San Luis Obispo, CA) and passed through the sample for 20 min. Methyl sulfide (1mL) was added, and the sample was stirred at room temperature for 60 min. After this time the sample was dried under reduced pressure. The free fatty aldehydes were removed by treating sample with *n*-hexane. 100 mg KLH and 20 mg sodium cyanoborohydride were added to GD3-aldehyde and incubated at 37 °C for 48 h. Unreacted GD3 was removed by a molecular cut-off filter Centriprep 30 (MW 30,000, Millipore, Billerica, MA). KLH content was determined using the Bio-Rad dye-binding method according to the manufacturers instructions and GD3 content by estimating sialic acid as described by Svennerholm.⁶ The epitope ratio of GD3-KLH was found to be 849/1.

Preparation of MUC1 KLH conjugate. MUC1 peptide containing 33 amino acids: CHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPA–OH, (synthesized at MSKCC's Microchemistry Core facility) was covalently conjugated to KLH (Sigma Chemical Co., St Louis, MO) using an MBS linker as previously described.^{4,5} Briefly, 5 mg MBS in 70 μ l dimethylformamide (Sigma Chemical Co., St Louis, MO) was added to 9 mg KLH in 1ml 0.01M phosphate buffer, pH 7.0. After an hour incubation at room temperature, the MBS activated KLH was separated using a Sephadex G 15 column equilibrated with 0.1 M phosphate buffer (pH 6.0), and stirred with 5 mg MUC1 peptide for 2 h at room temperature. The unconjugated peptide was separated using a Centriprep 30. The epitope ratio of MUC1:KLH was 1367:1 (calculated based on the initial amount of peptide and KLH, the amount of unconjugated peptide in the filtrate, and a KLH molecular weight of 8.6×10^6 Da).

Vaccination of mice. All experiments involving laboratory animals were performed in accordance with the Memorial Sloan Kettering Institutional Animal Care and Use Committee (IACUC) approved protocol # 97-11-051. Groups of five mice (C57BL/6J, female, 6-8 weeks old) were vaccinated three times with indicated combinations of GD3-KLH conjugate (5 μ g equivalent of GD3), MUC1-KLH (2.5 μ g equivalent of MUC1), and/or OVA (20 μ g, Sigma Chemical Co., St Louis, MO) in 100 μ L phosphate buffered saline either alone (without adjuvant), with synthetic QS-21 (SQS-21), or other saponins at indicated doses. Vaccines were administered subcutaneously to each mouse on days 0, 7, 14, and 65. Mice were bled 7 days after the third and fourth vaccinations.

Measurement of immunological response. The presence of antibodies was tested by an enzyme-linked immunosorbent assay (ELISA). ELISAs were performed to determine antibody response against GD3, MUC1, OVA, and/or KLH as described previously.^{4,5} The ELISA plates were coated with either GD3 antigen at 0.2 μ g/well in ethanol, MUC1 antigen at 0.1 μ g/well in carbonate buffer (pH 10), or KLH at 0.1 μ g/well in carbonate buffer (pH 10). The GD3-coated plates were kept overnight at room temperature to evaporate ethanol, and while MUC1 or KLH coated plates were incubated at 4 °C overnight. ELISA plates were washed, blocked with 1% human serum albumin (HSA) in phosphate-buffered saline containing 0.05% Tween 20. Serially

diluted pre- and post-vaccination sera in PBS with 1% HSA were added to wells of the coated plate with appropriate controls and incubated for 1 h at room temperature. After wash, goat anti-mouse IgM or IgG conjugated with alkaline phosphatase (Southern Biotechnology Associates, Inc., Birmingham, AL) was added to each well. Absorbance was measured at 405 nm. The titer was defined as the highest serum dilution that showed an absorbance of 0.1 or greater over that of the pre-sera.

E. CONFORMATIONAL ANALYSIS BY MOLECULAR DYNAMICS SIMULATIONS

Molecular Dynamics Simulations (200 ns in explicit water). Parameters for the substrates were generated with the *antechamber* module of Amber12⁷ using a combination of GLYCAM06⁸ parameters for the sugar units and the general Amber force field (GAFF)⁹ for the rest of the molecule, with partial charges set to fit the electrostatic potential generated with HF/6-31G(d) by RESP.¹⁰ The charges are calculated according to the Merz-Singh-Kollman scheme using Gaussian 09 (ref. 11). Each saponin molecule was immersed in a water box with a 10 Å buffer of TIP3P¹² water molecules. The systems were neutralized by adding explicit counterions (Na⁺). All subsequent simulations were performed using the *ff14SB* force field, which is an evolution of the Stony Brook modification of the Amber 99 force field force field (*ff99SB*).¹³ A two-stage geometry optimization approach was performed. The first stage minimizes only the positions of solvent molecules and ions, and the second stage is an unrestrained minimization of all the atoms in the simulation cell. The systems were then heated by incrementing the temperature from 0 to 300 K under a constant pressure of 1 atm and periodic boundary conditions. Harmonic restraints of 30 kcal/mol were applied to the solute, and the Andersen temperature coupling scheme¹⁴ was used to control and equalize the temperature. The time step was kept at 1 fs during the heating stages, allowing potential inhomogeneities to self-adjust. Water molecules were treated with the SHAKE algorithm such that the angle between the hydrogen atoms is kept fixed. Long-range electrostatic effects were modeled using the particle-mesh-Ewald method.¹⁵ An 8 Å cutoff was applied to Lennard-Jones and electrostatic interactions. Each system was equilibrated for 2 ns with a 2 fs timestep at a constant pressure (1 atm) and temperature of 300 K. Production trajectories were then run for additional 200 ns under the same simulation conditions.

The same approach was used for simulations with alternative counterions (K⁺ or Mg²⁺, Figure S9) and in alternative solvent (trifluoroethanol, Figure S10).

Torsional angle definitions. For each (C1–O1–Cⁿ) glycosidic linkage in the branched trisaccharide and linear oligosaccharide domains, ϕ and ψ dihedral angles were defined as:

$$\begin{aligned}\phi &= \text{H1-C1-O1-C}^n \\ \psi &= \text{C1-O1-C}^n\text{-H}^n\end{aligned}$$

For the central glycosidic linkages connecting the bridging monosaccharide to the triterpene core, the ψ dihedral angles were defined based on heavy atoms as follows:

$$\psi = \text{C1-X1-A-B}$$

where X is a heteroatom, A is the first heavy atom attached to X (*e.g.*, C28 in QS-21), and B is the next heavy atom attached to A excluding carbonyl oxygens (*e.g.*, C17 in QS-21).

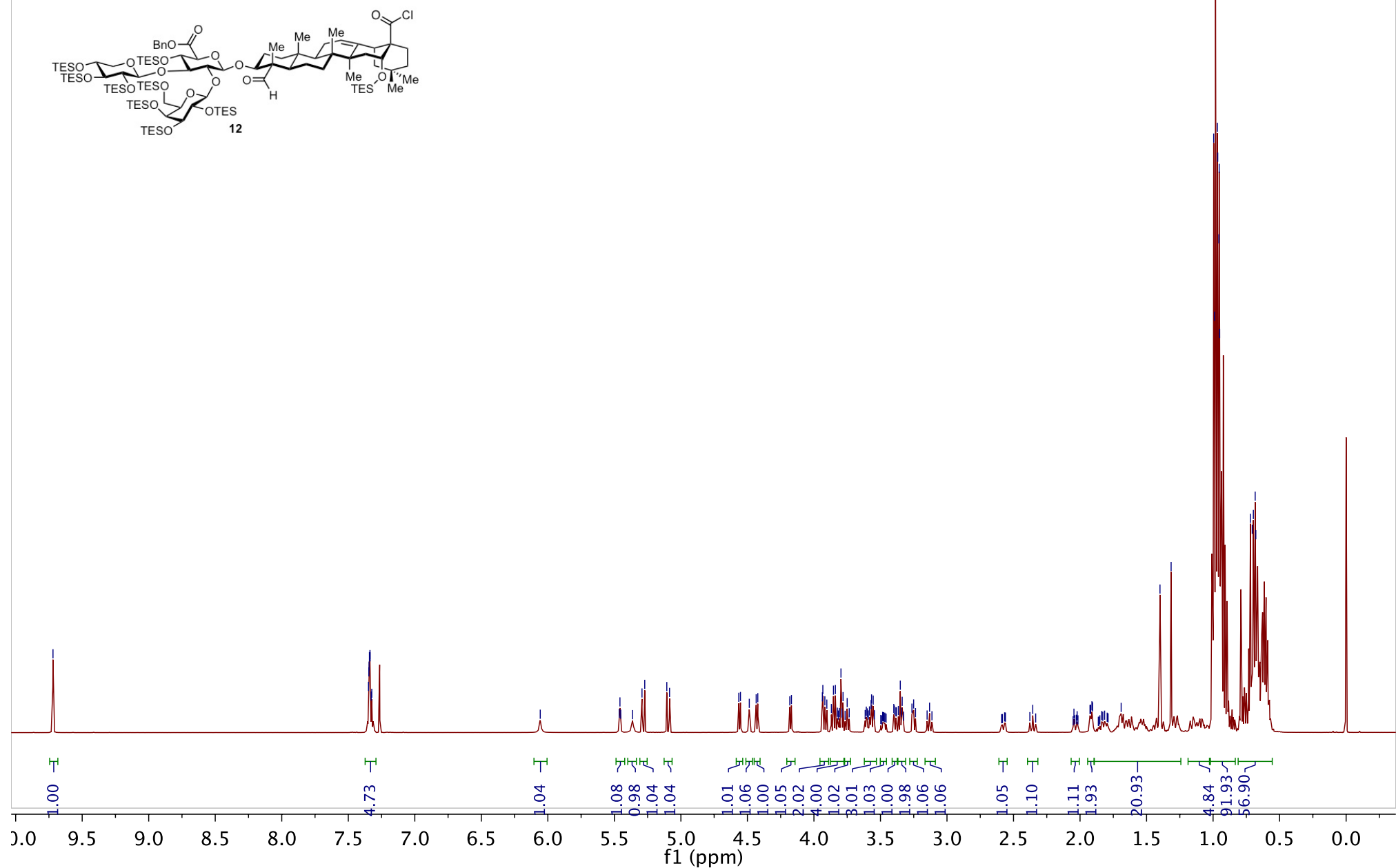
For the C17–C28 bond, the dihedral angle was defined as follows:

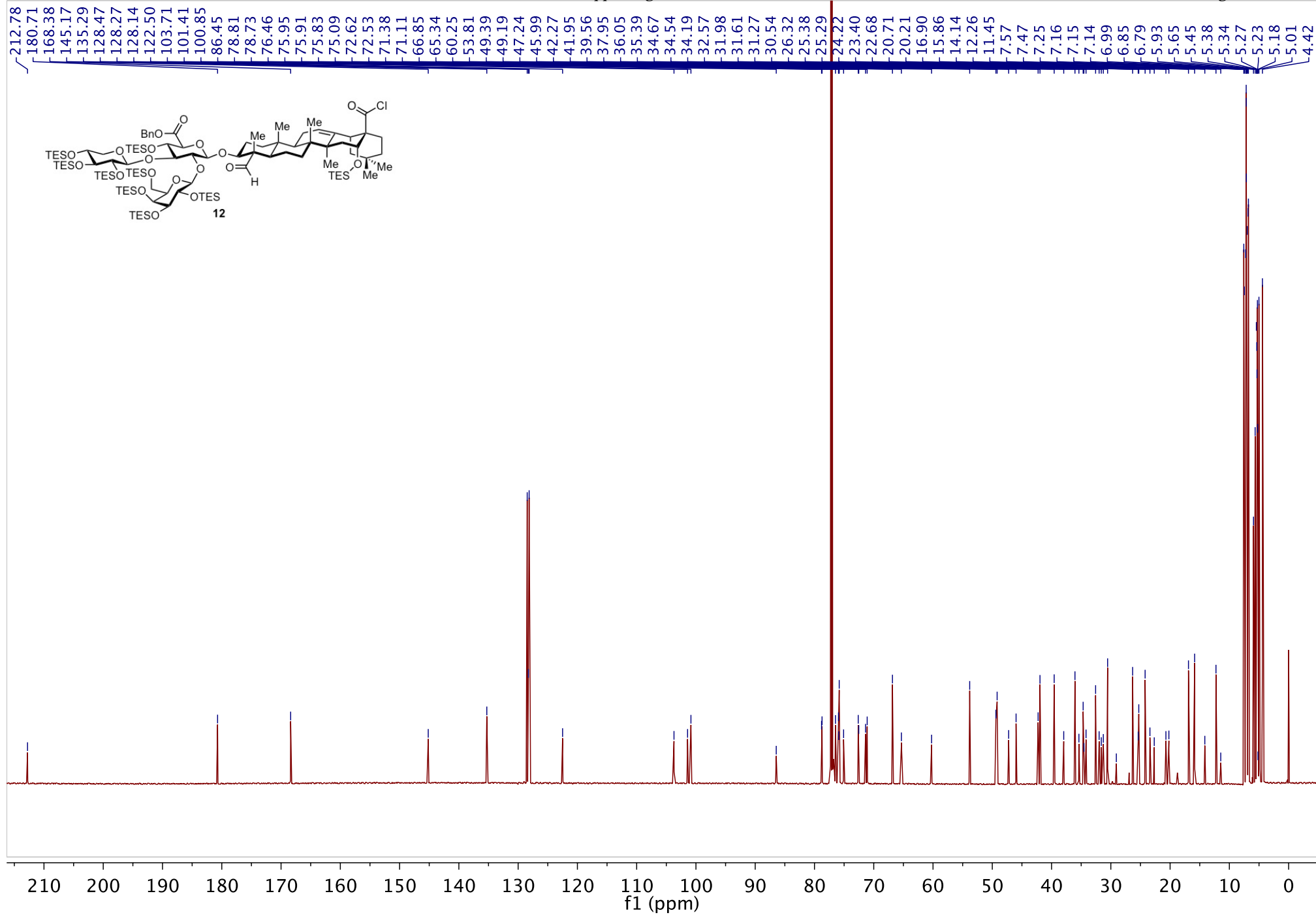
$$\text{C17-C28} = \text{C16-C17-C28-X}$$

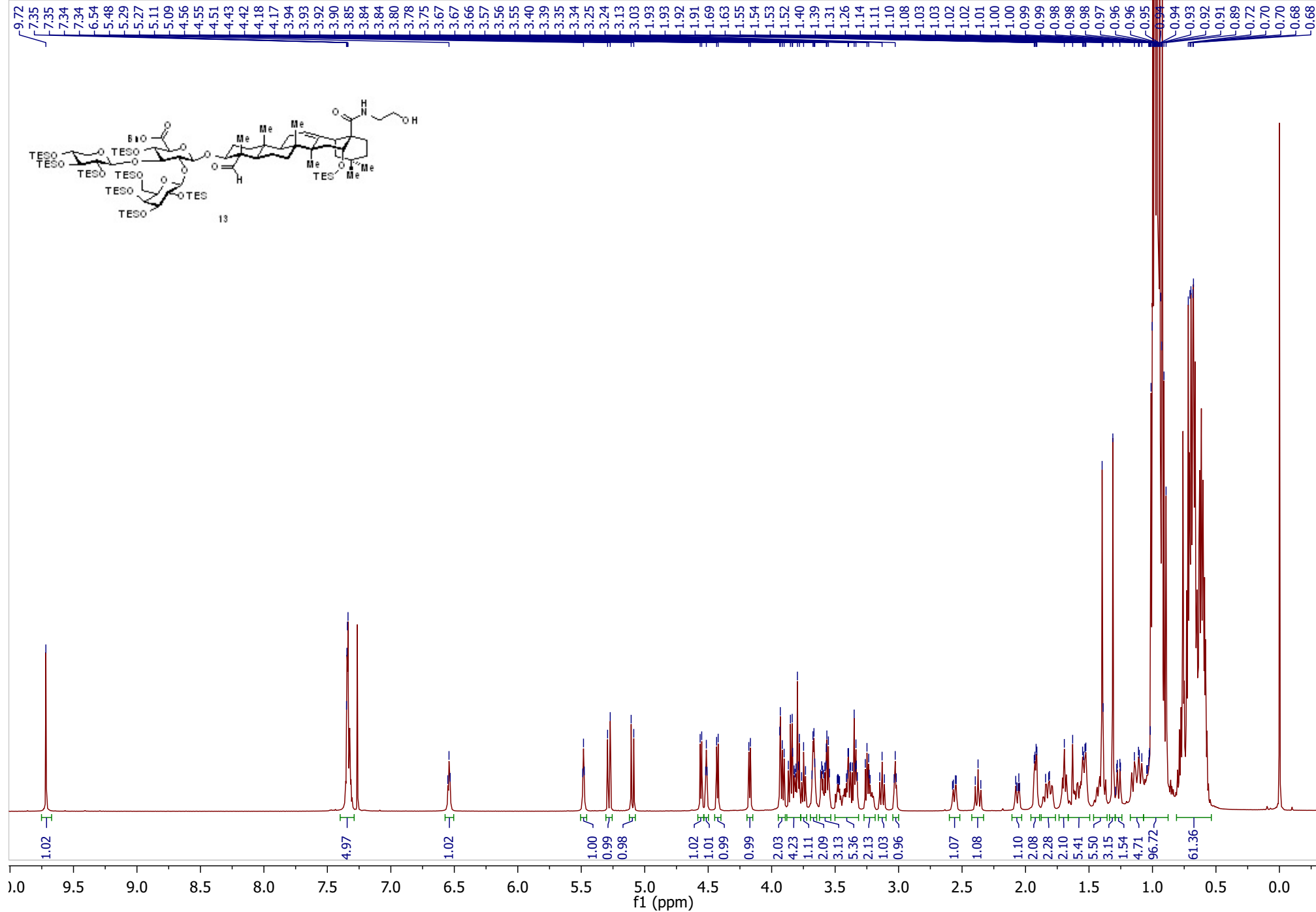
where X is a heteroatom.

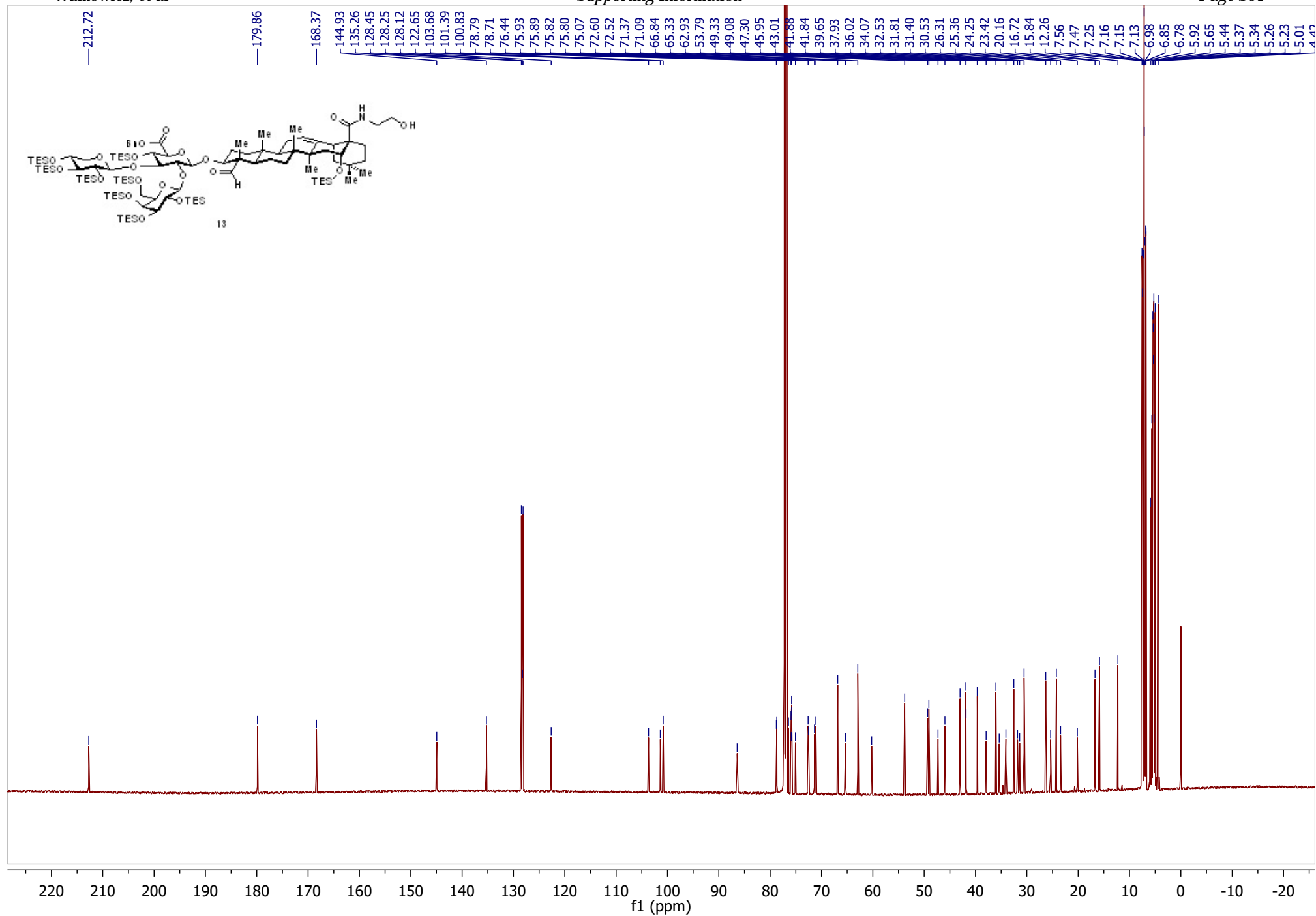
To generate three-dimensional plots of torsional angle distributions (ϕ , ψ , C17–C28) about the central glycosidic linkage (Figures 3 and S5), each axis was shifted by a fixed value to minimize the number of datapoints appearing along the $360^\circ \rightarrow 0^\circ$ radial transition point, for clarity of presentation. Thus, the raw data (-180° to $+180^\circ$) were shifted in Excel as follows:

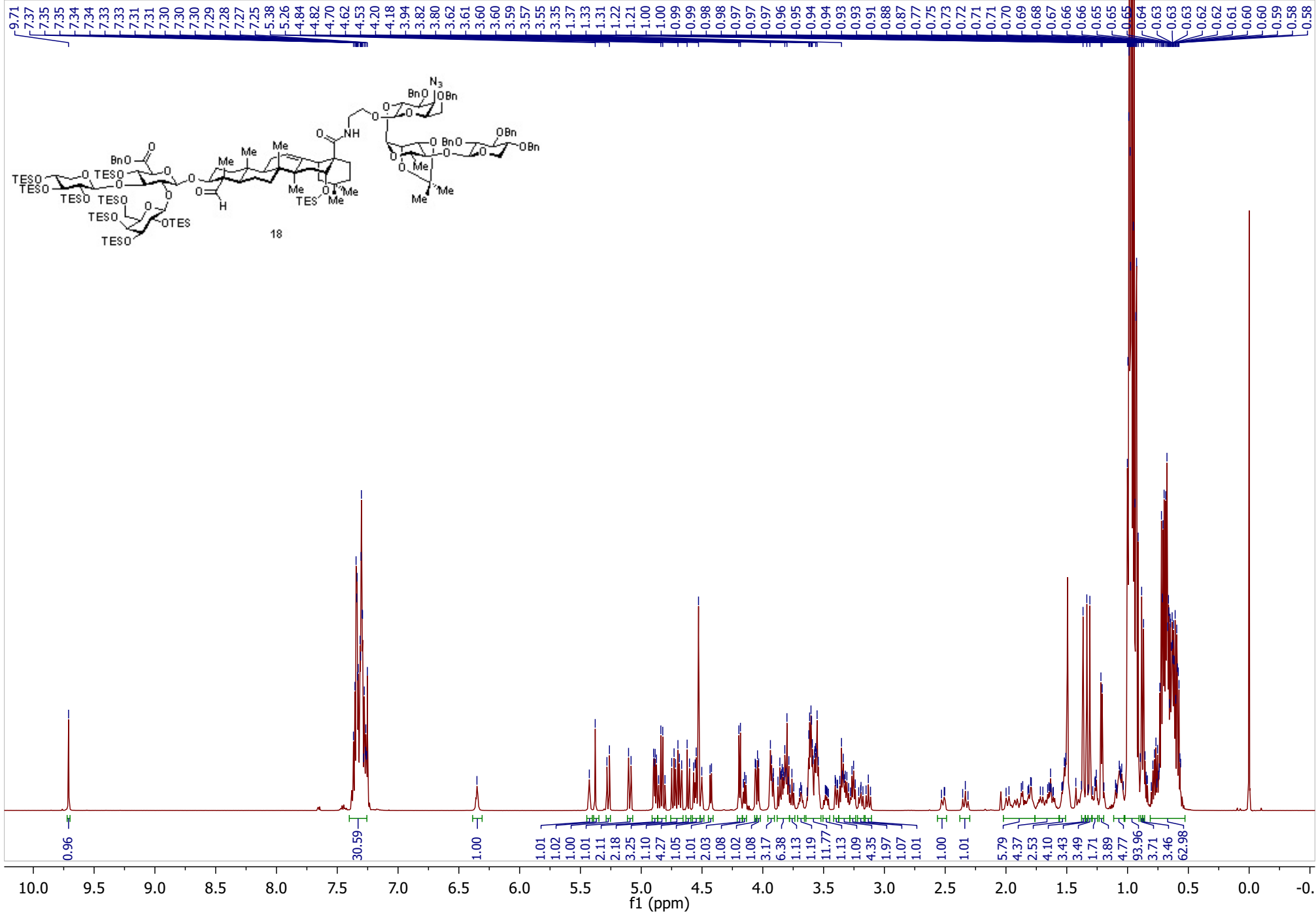
$$\begin{aligned}\phi_{\text{shift}} &= \text{MOD}(\phi + 480, 360) \\ \psi_{\text{shift}} &= \text{MOD}(\psi + 480, 360) \\ \text{C17–C28}_{\text{shift}} &= \text{MOD}(\text{C17–C28} + 240, 360)\end{aligned}$$

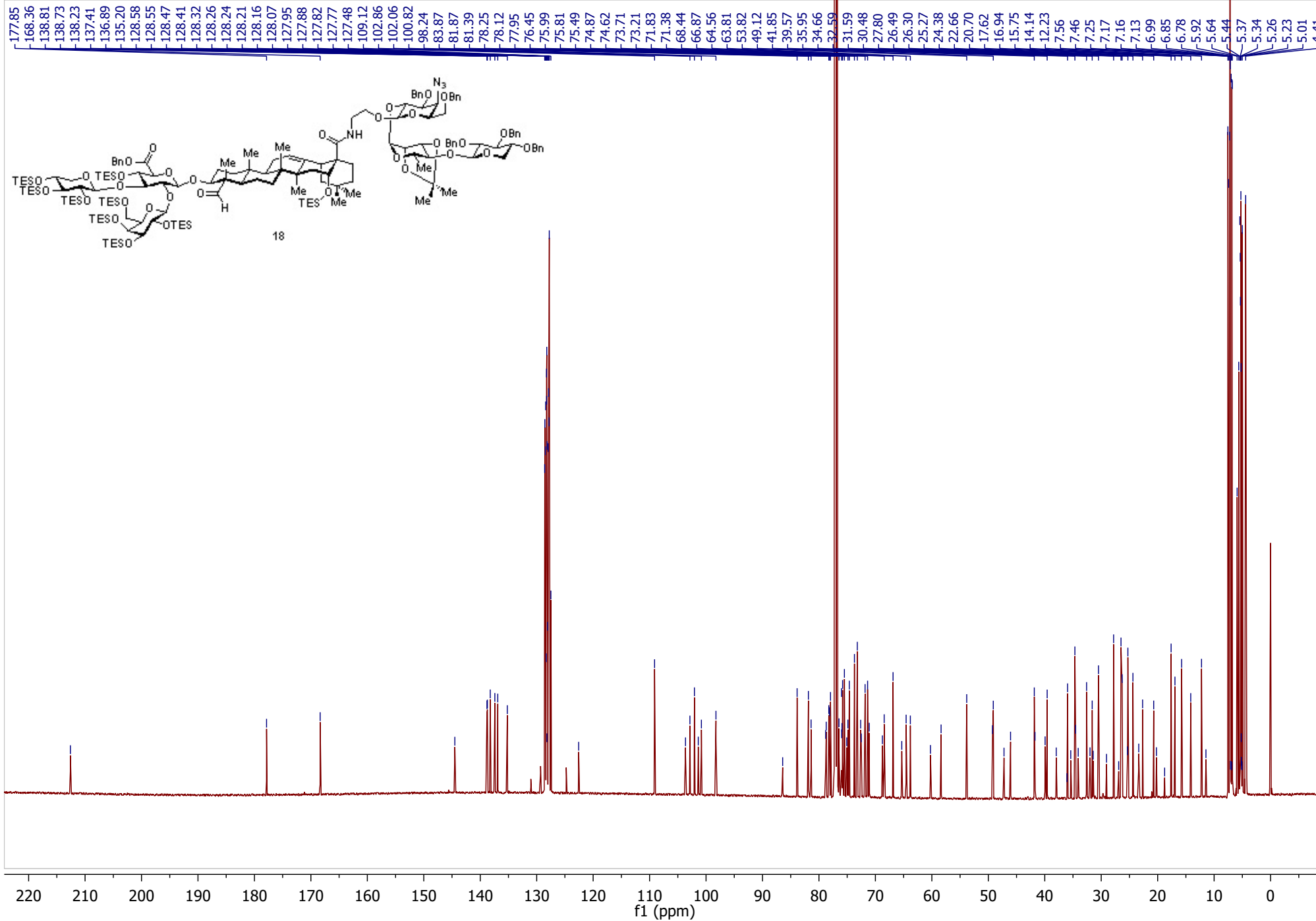
F. ^1H -NMR and ^{13}C -NMR spectra

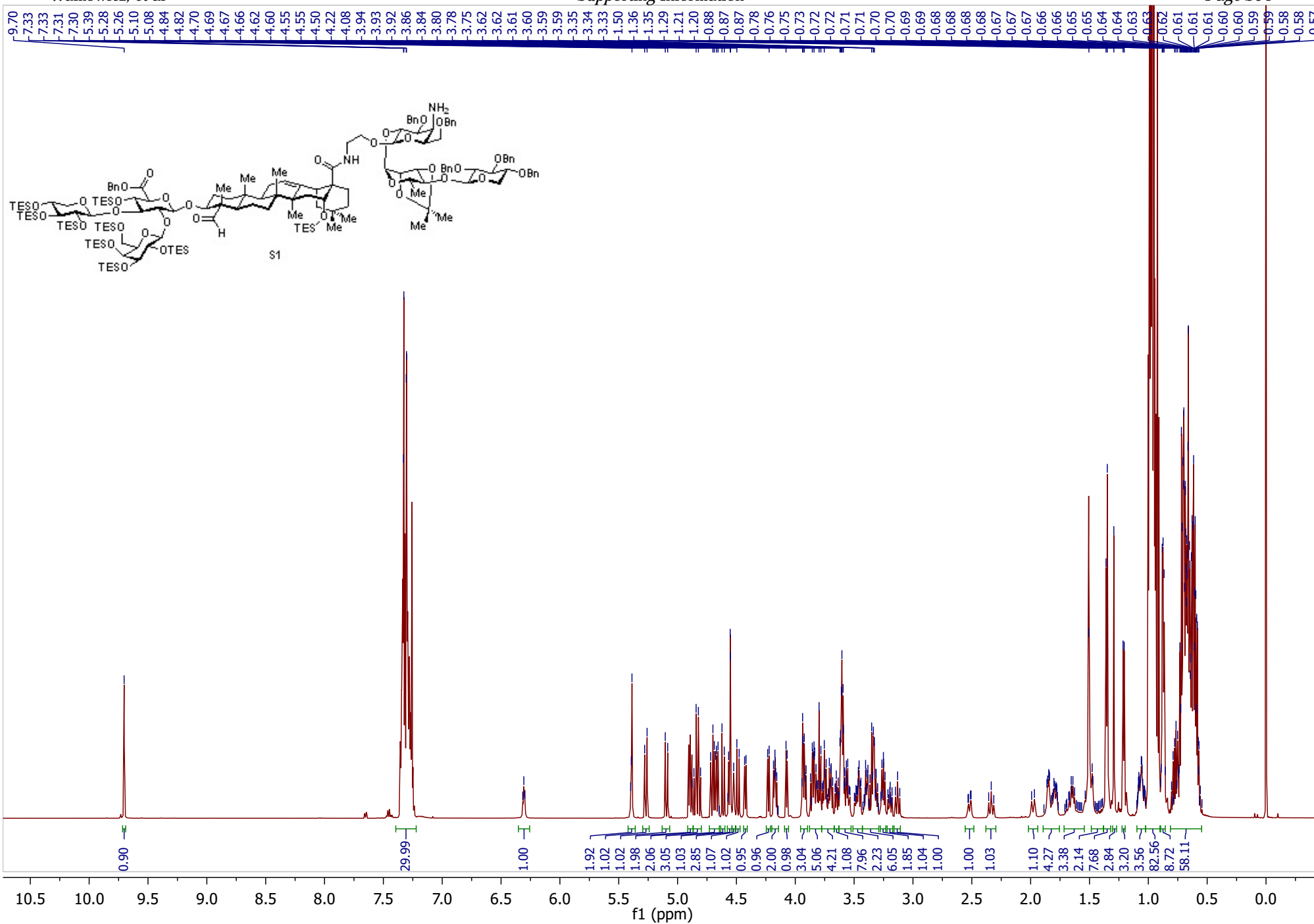


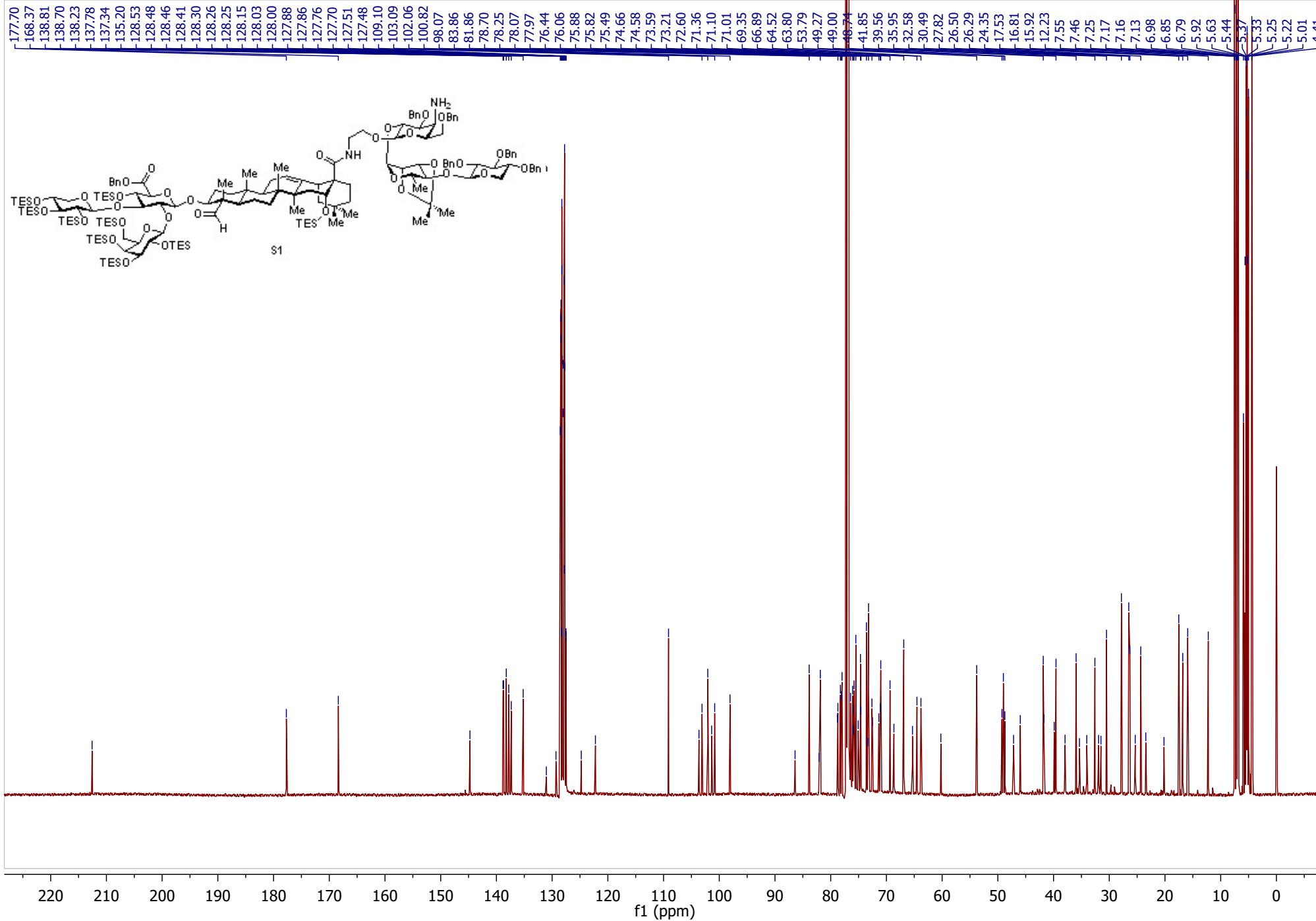


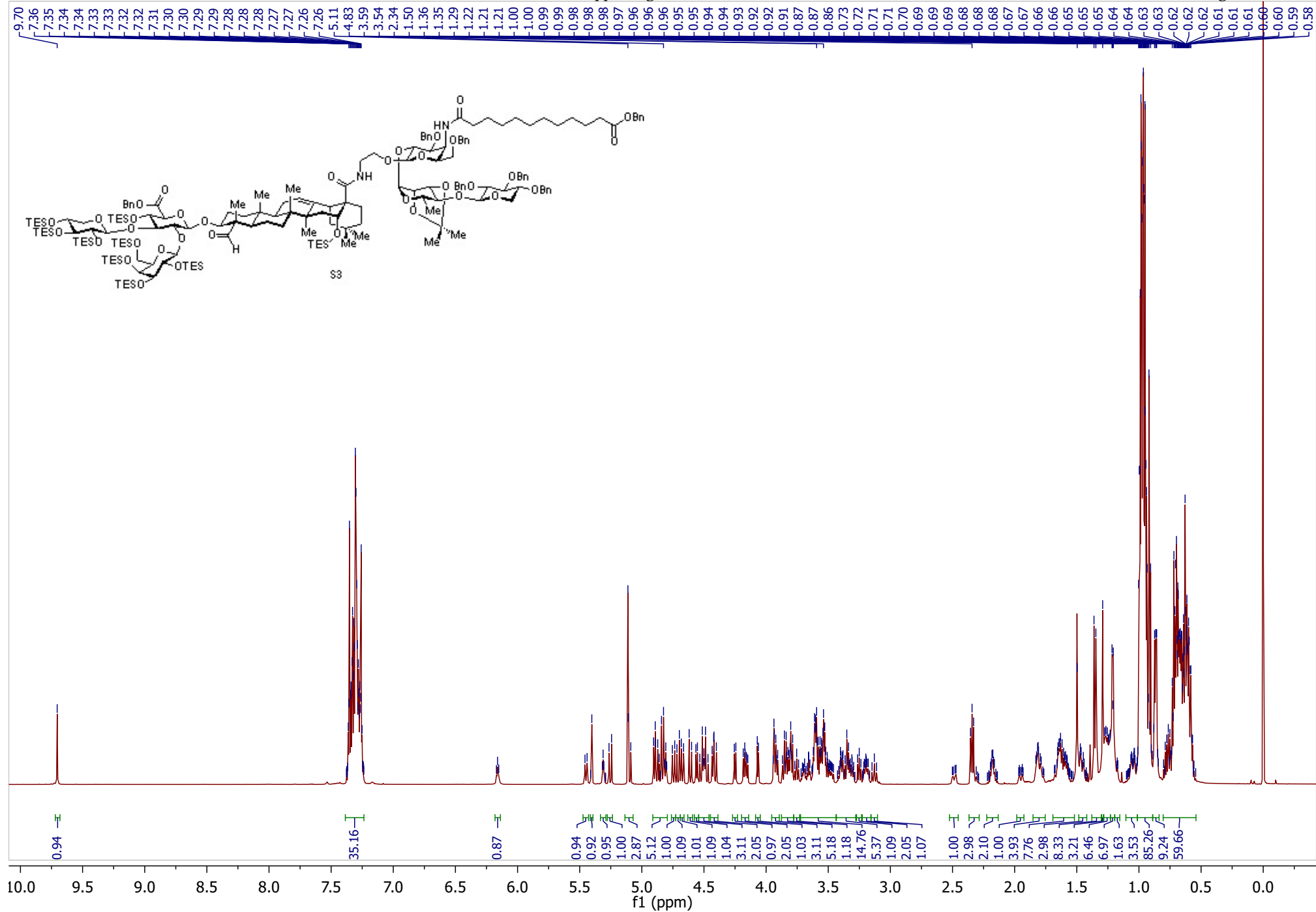


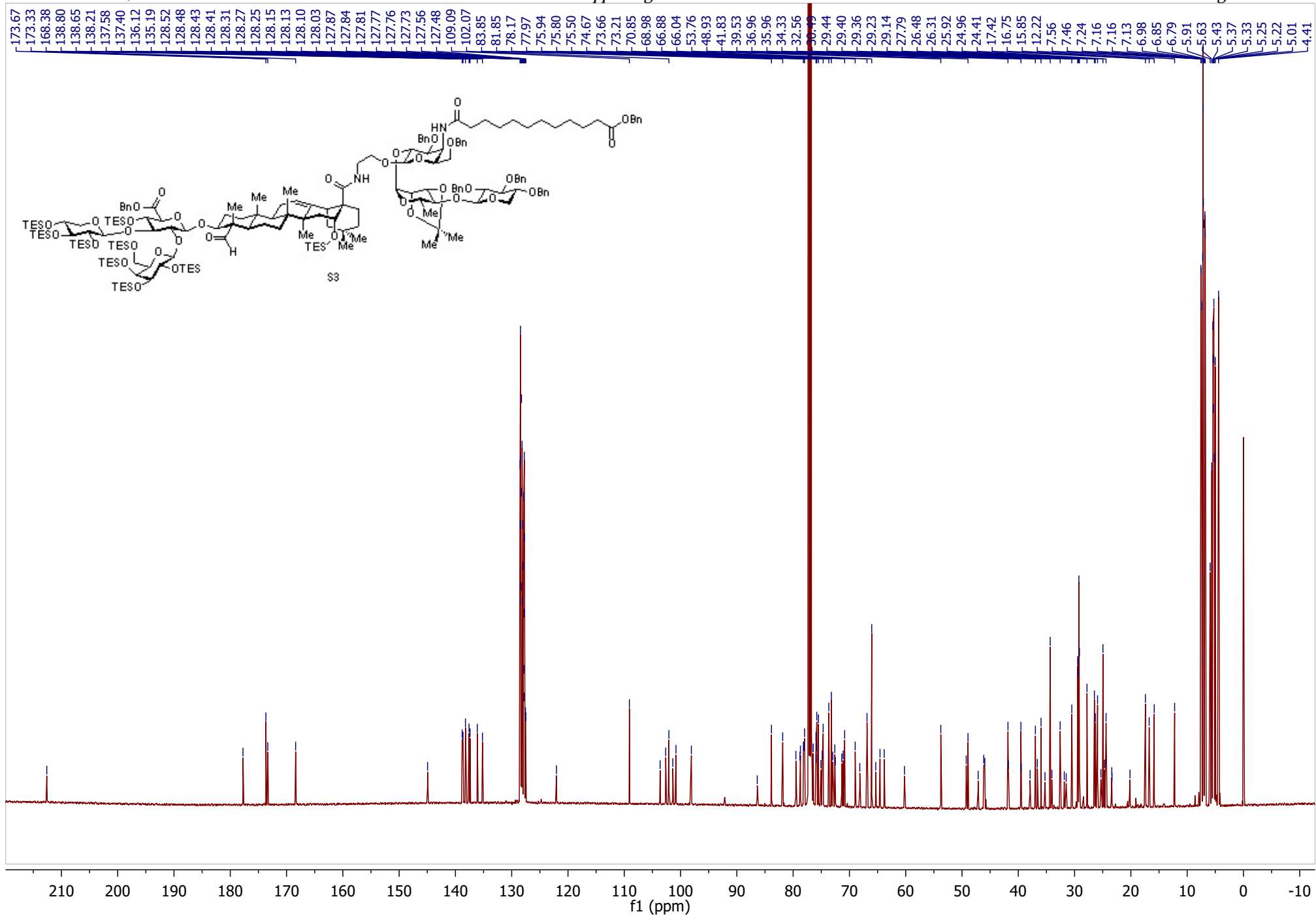


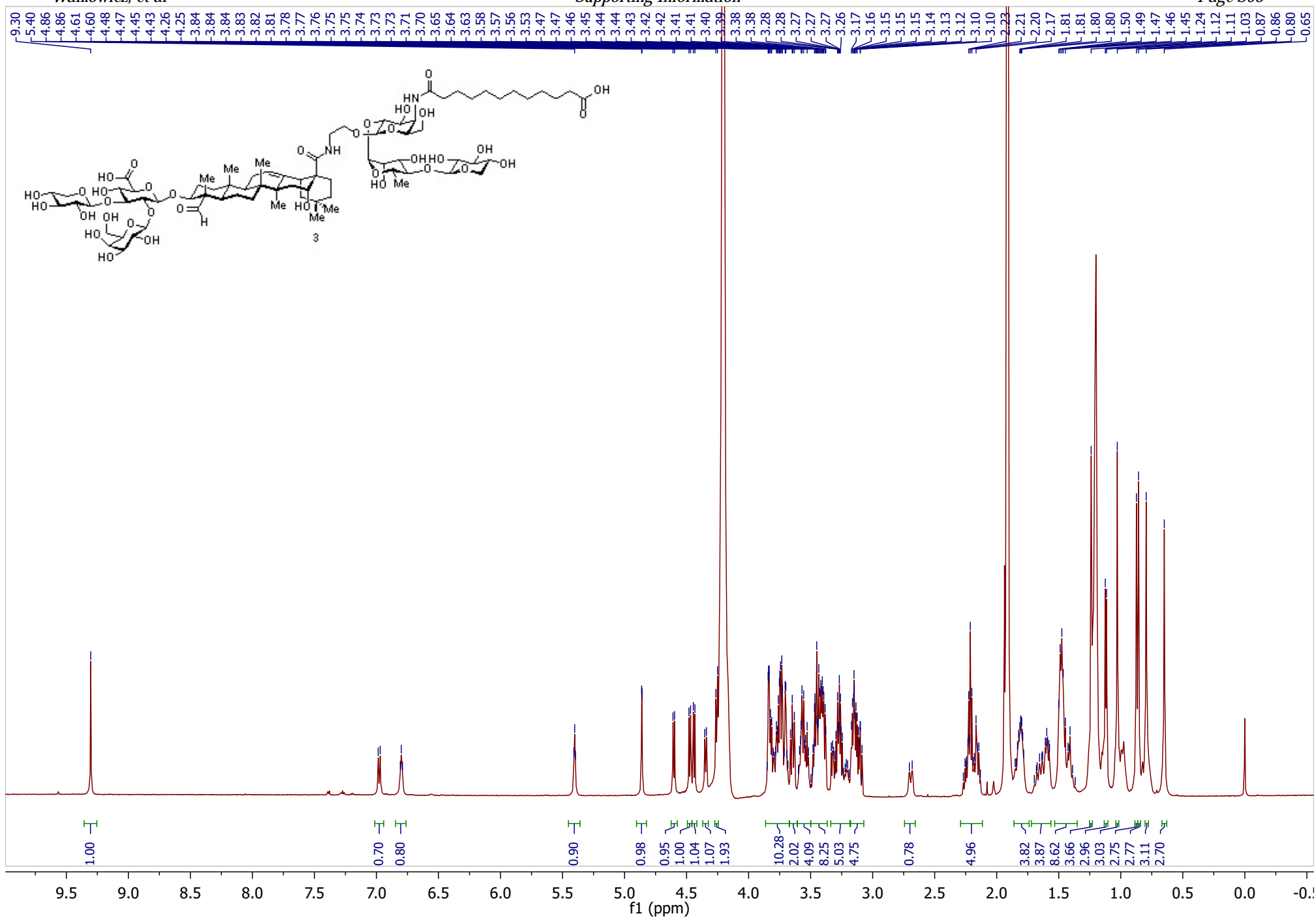


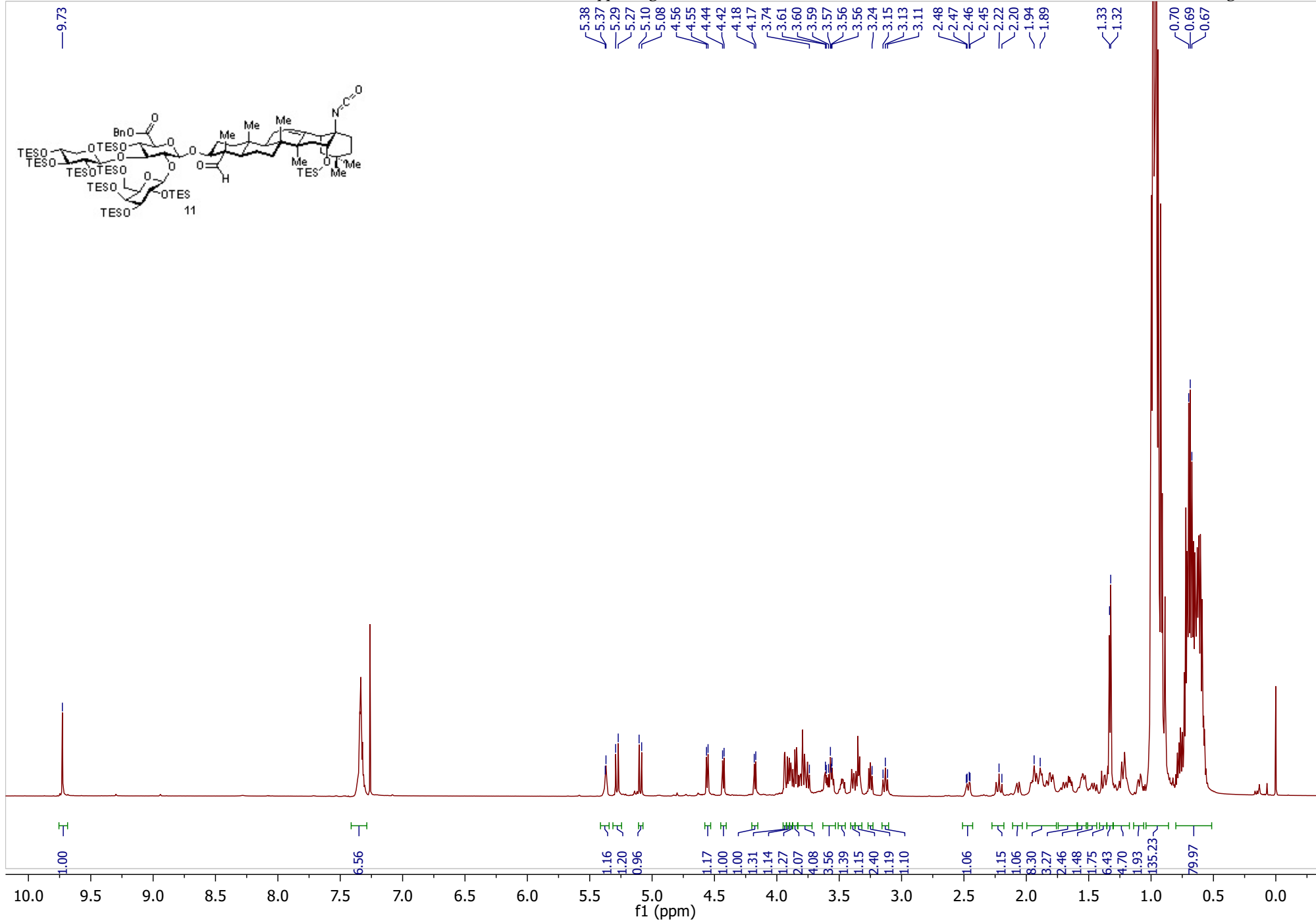


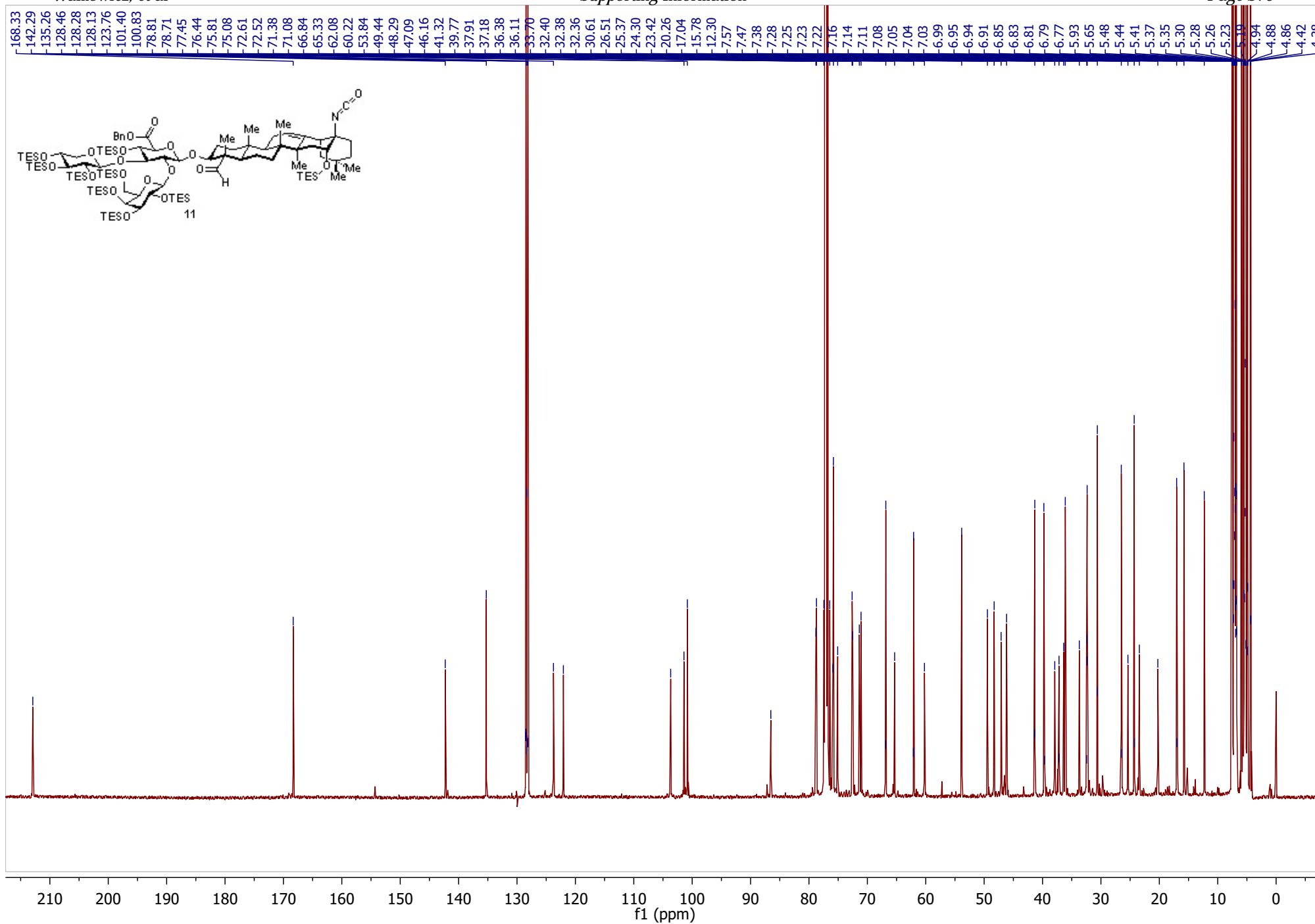


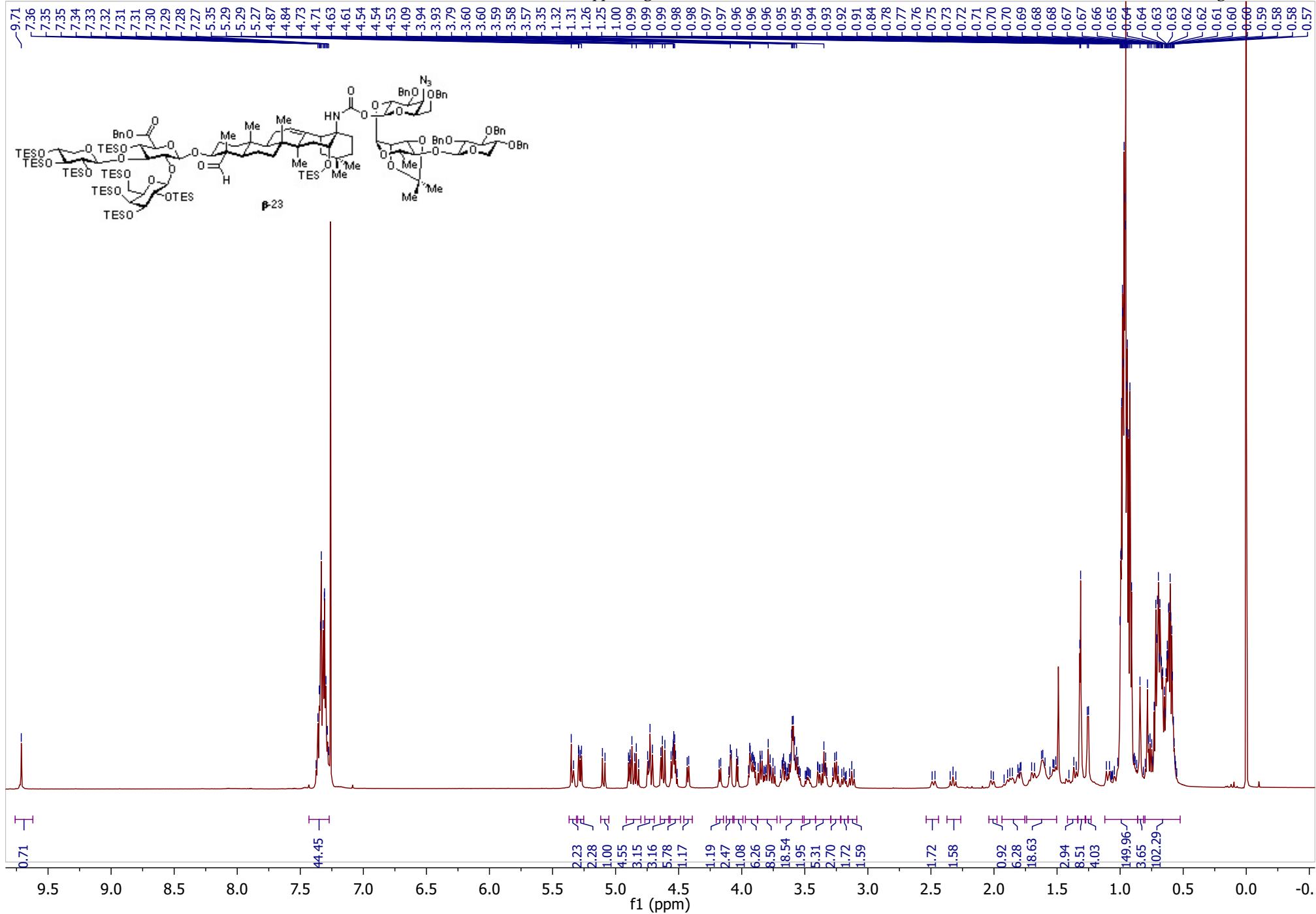


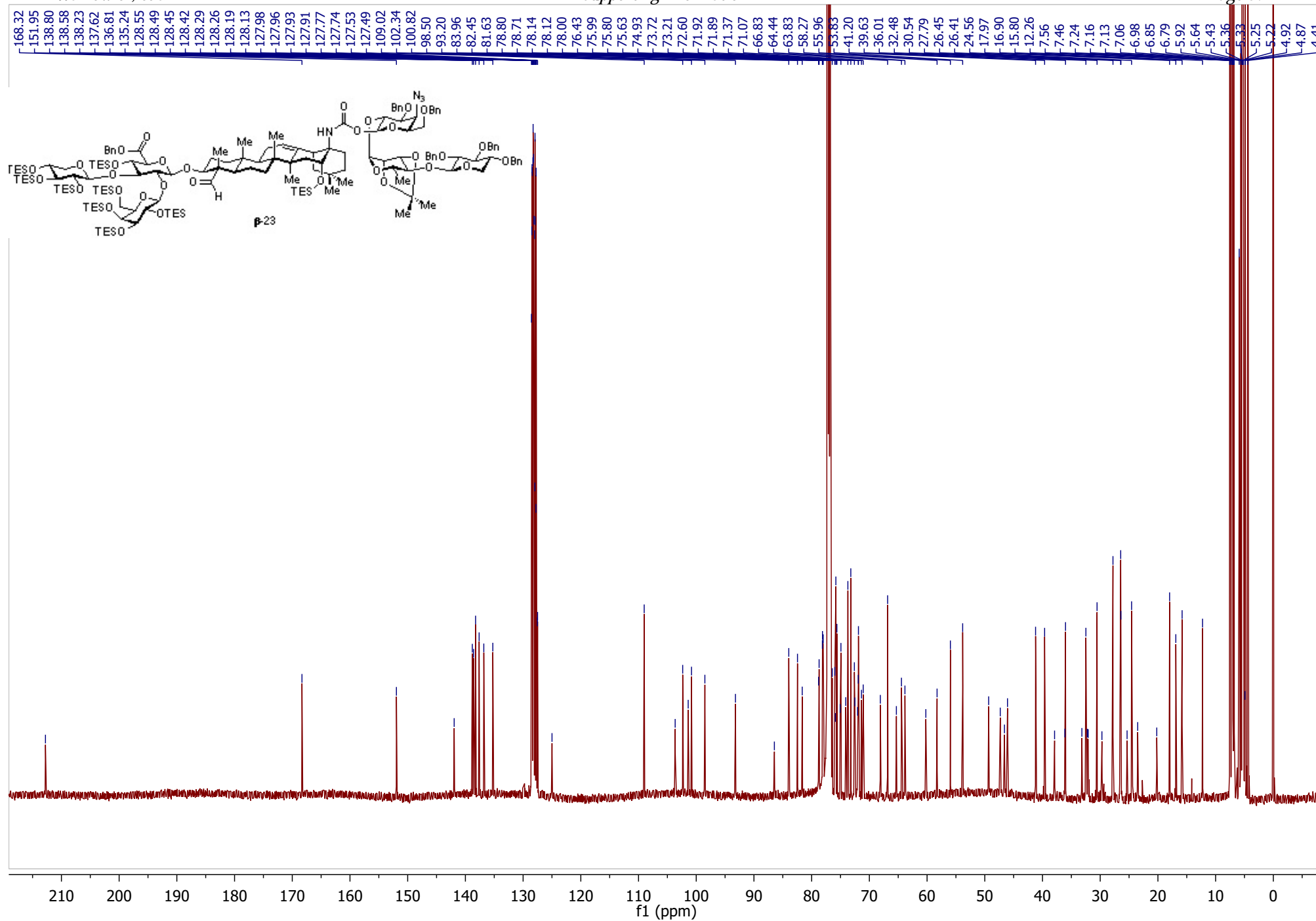


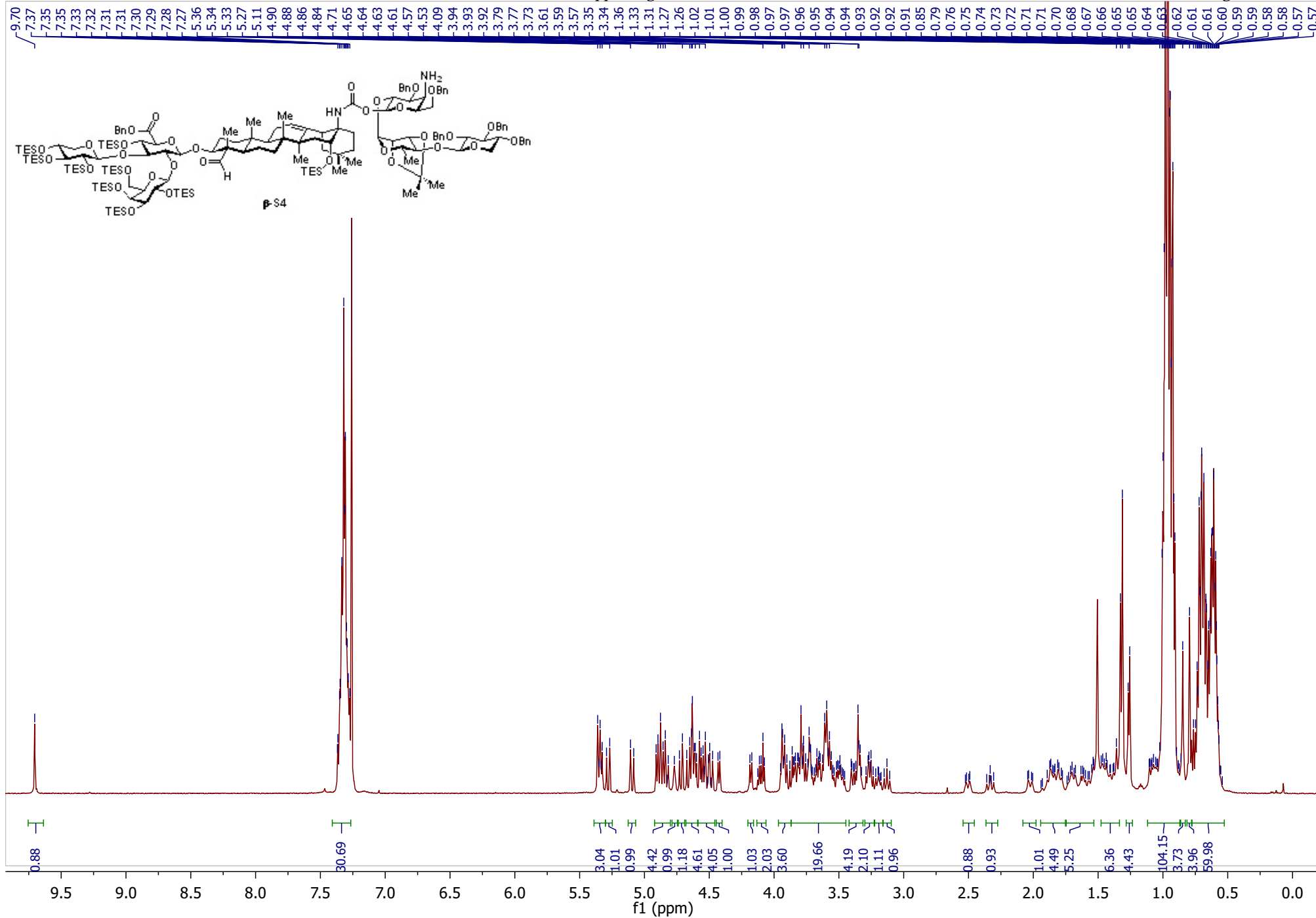


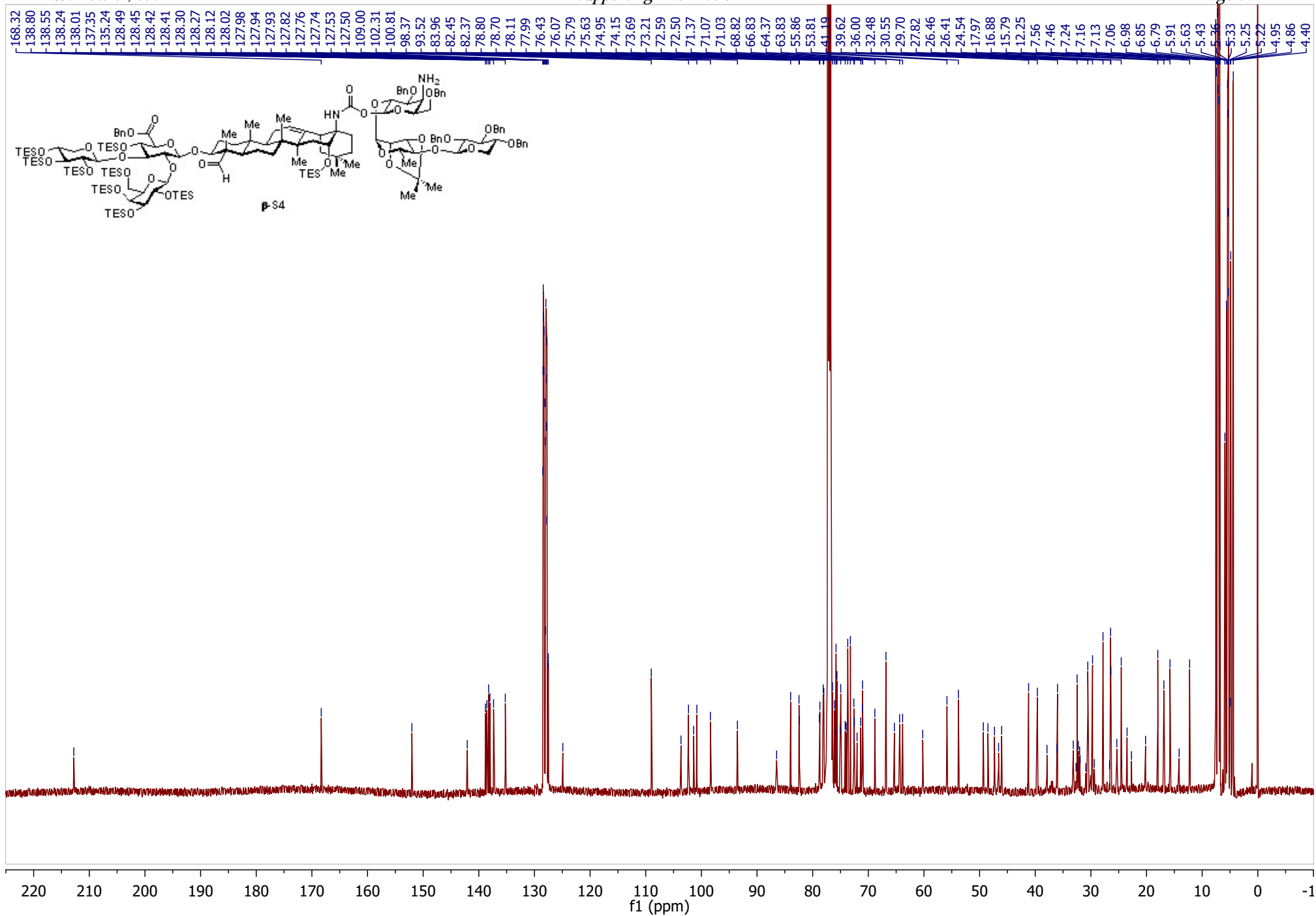


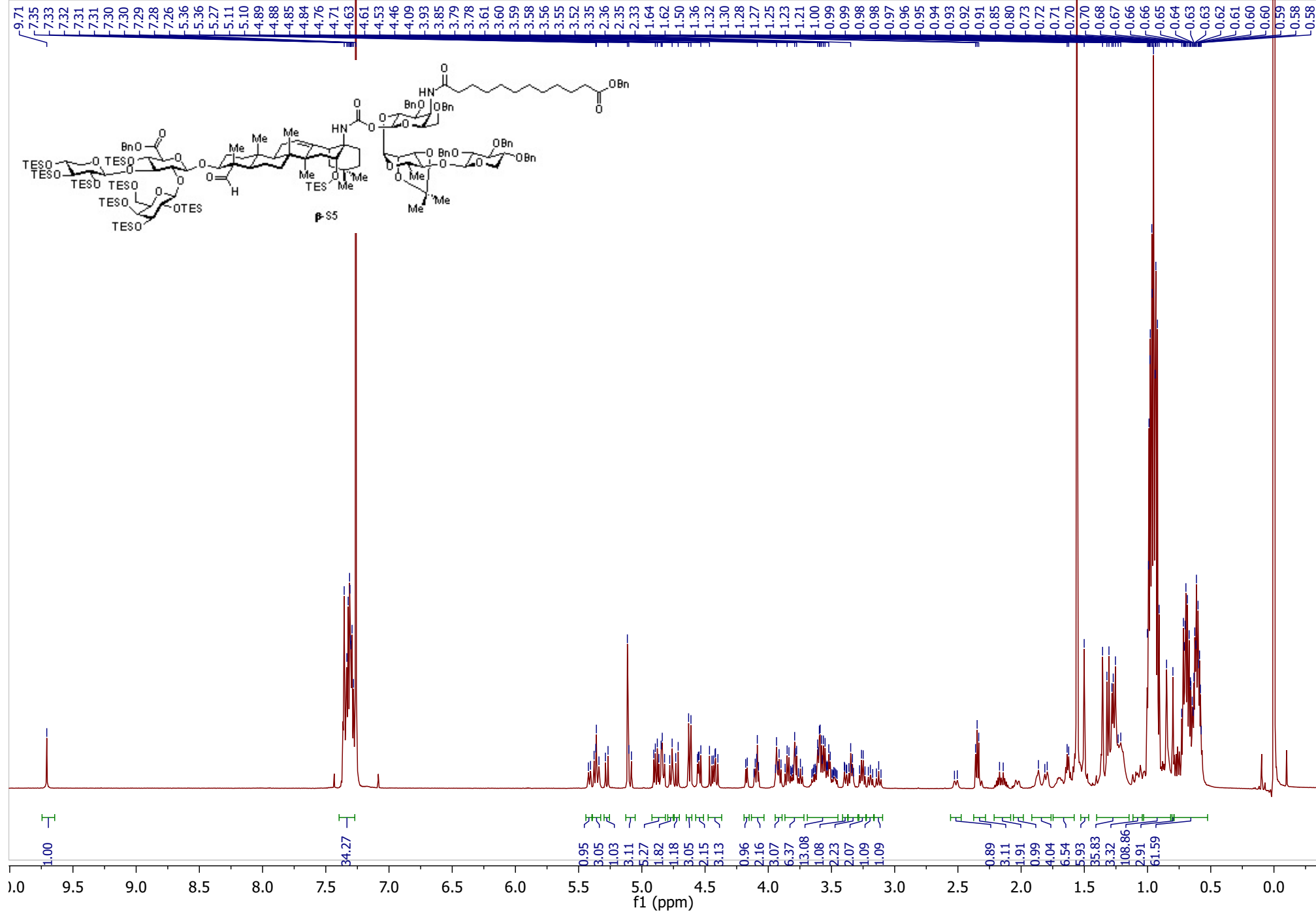


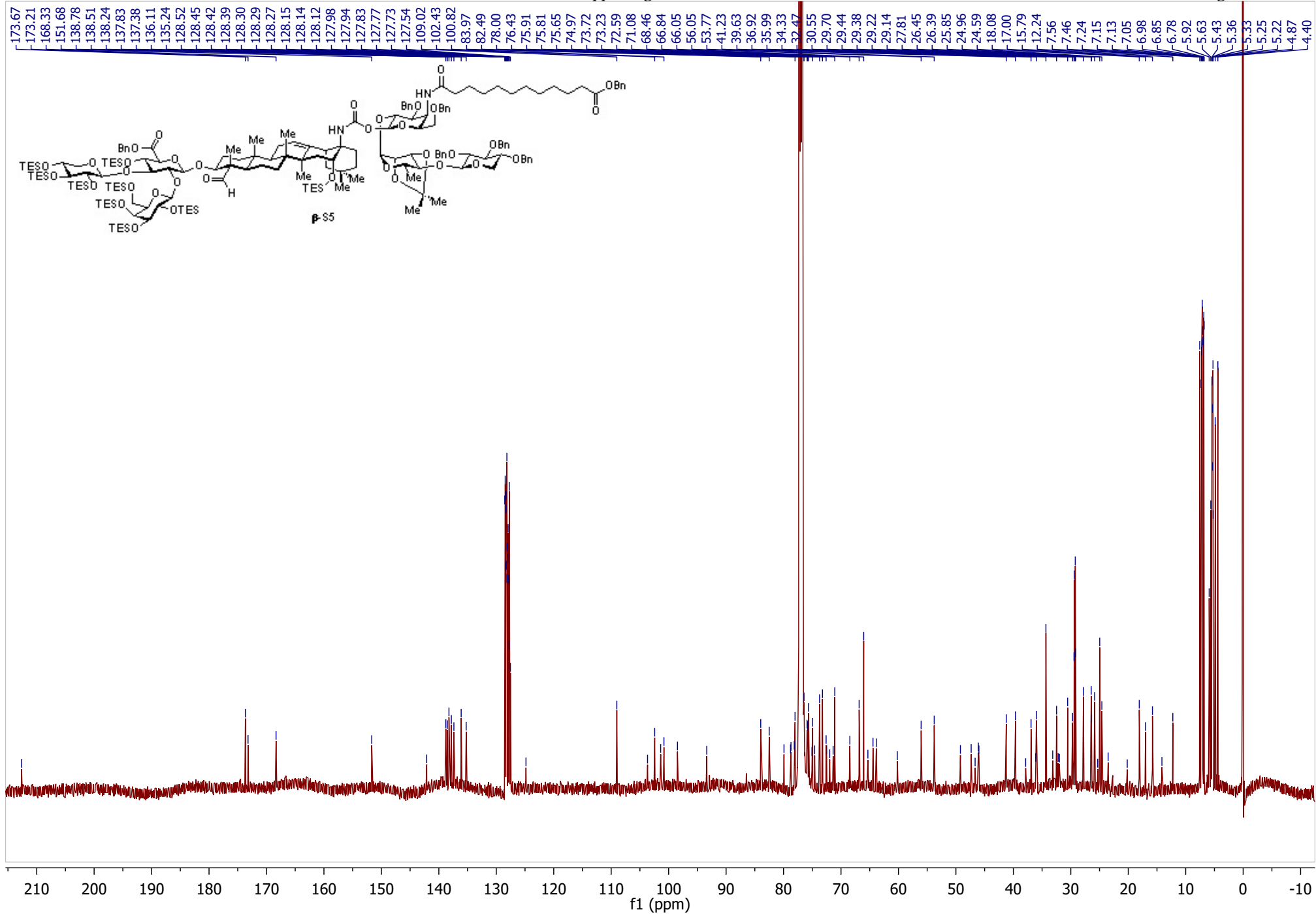


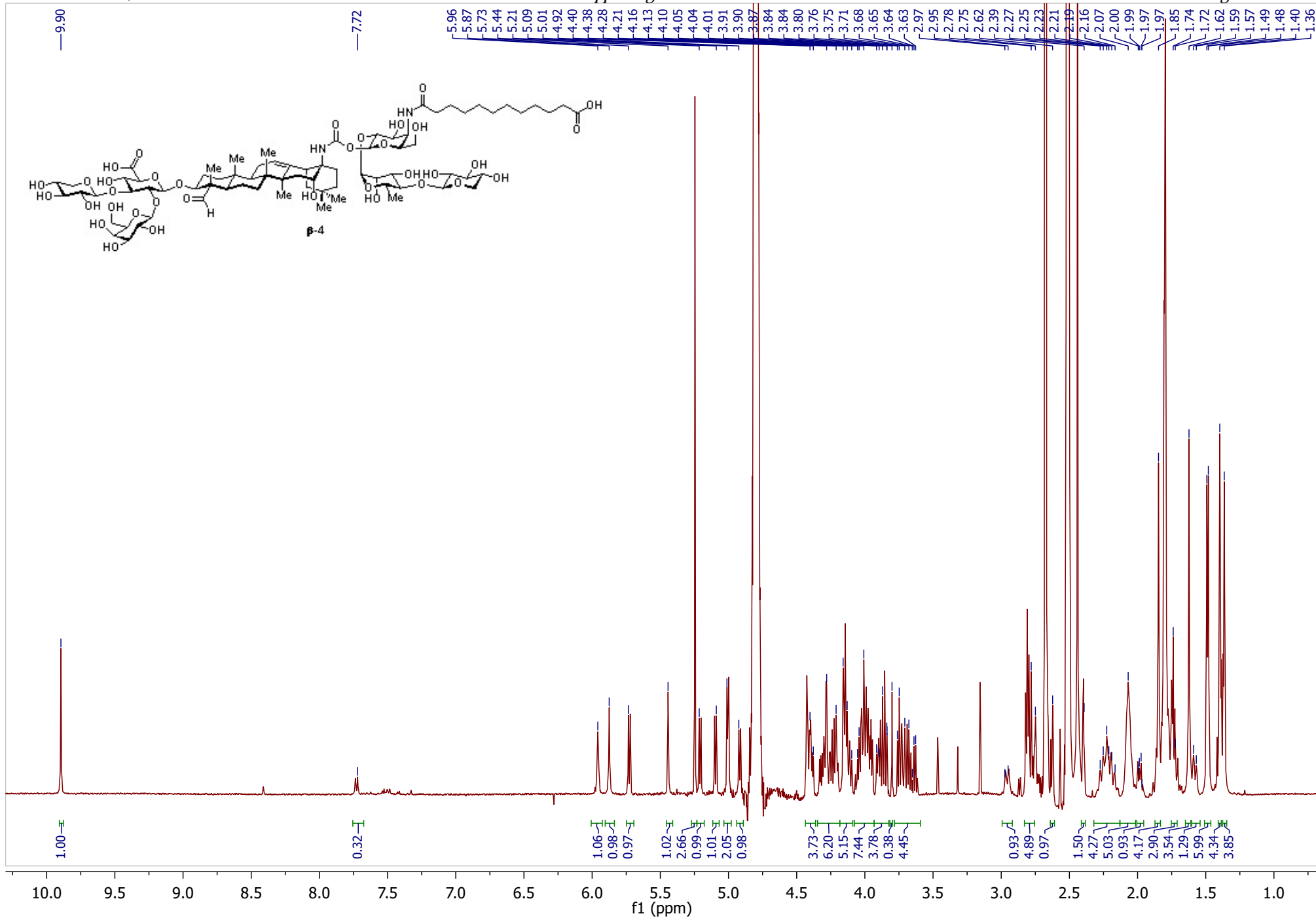


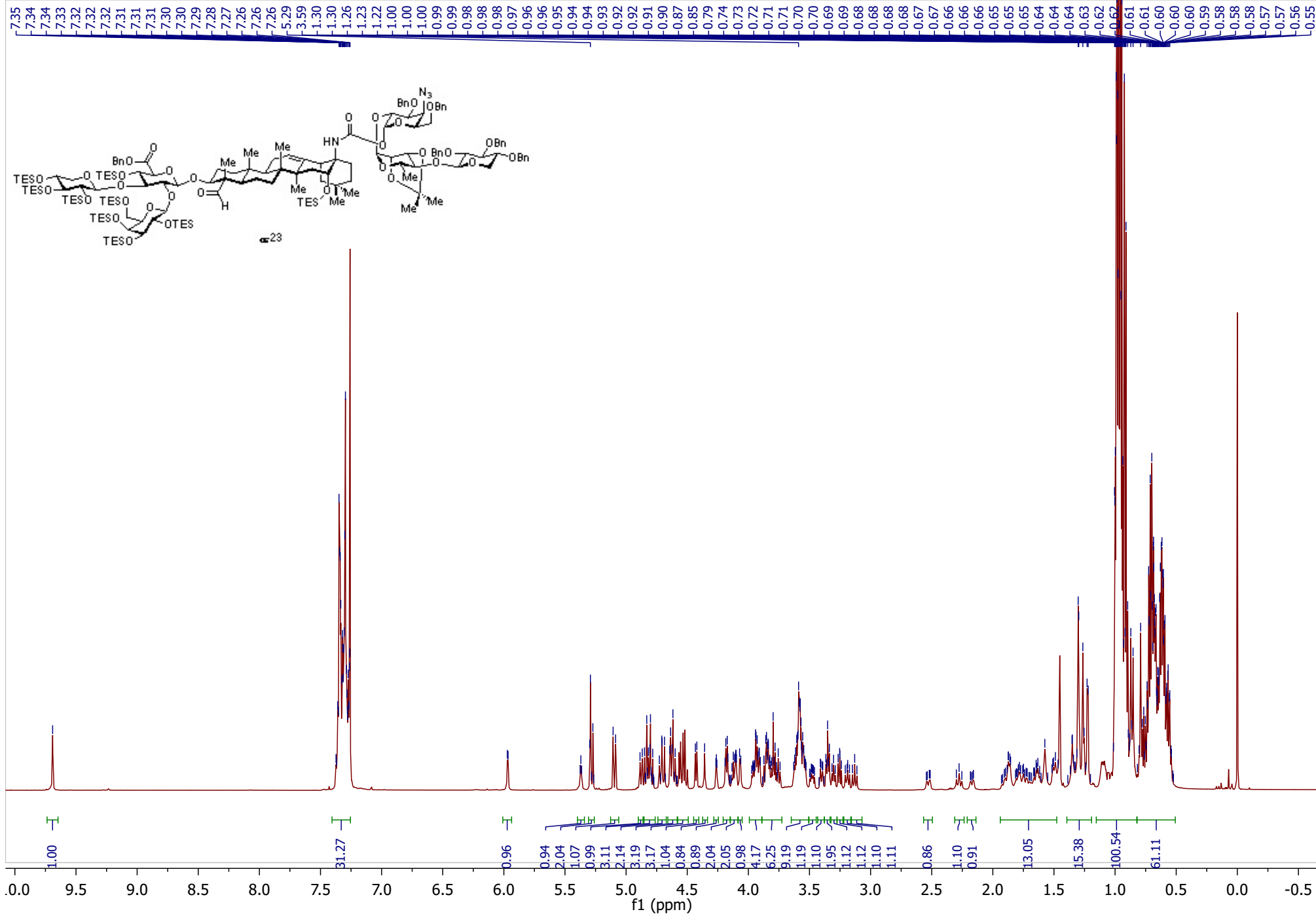


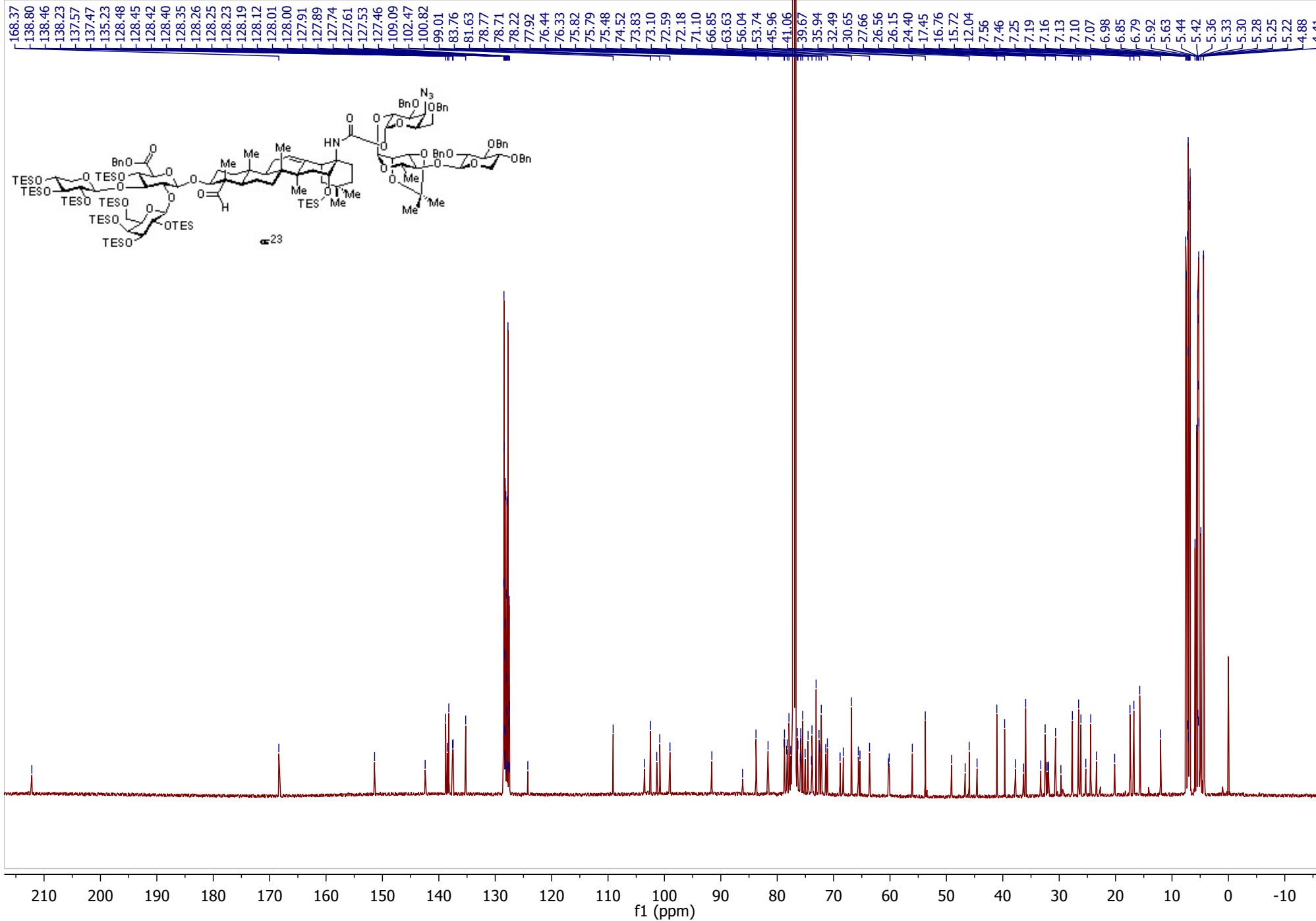


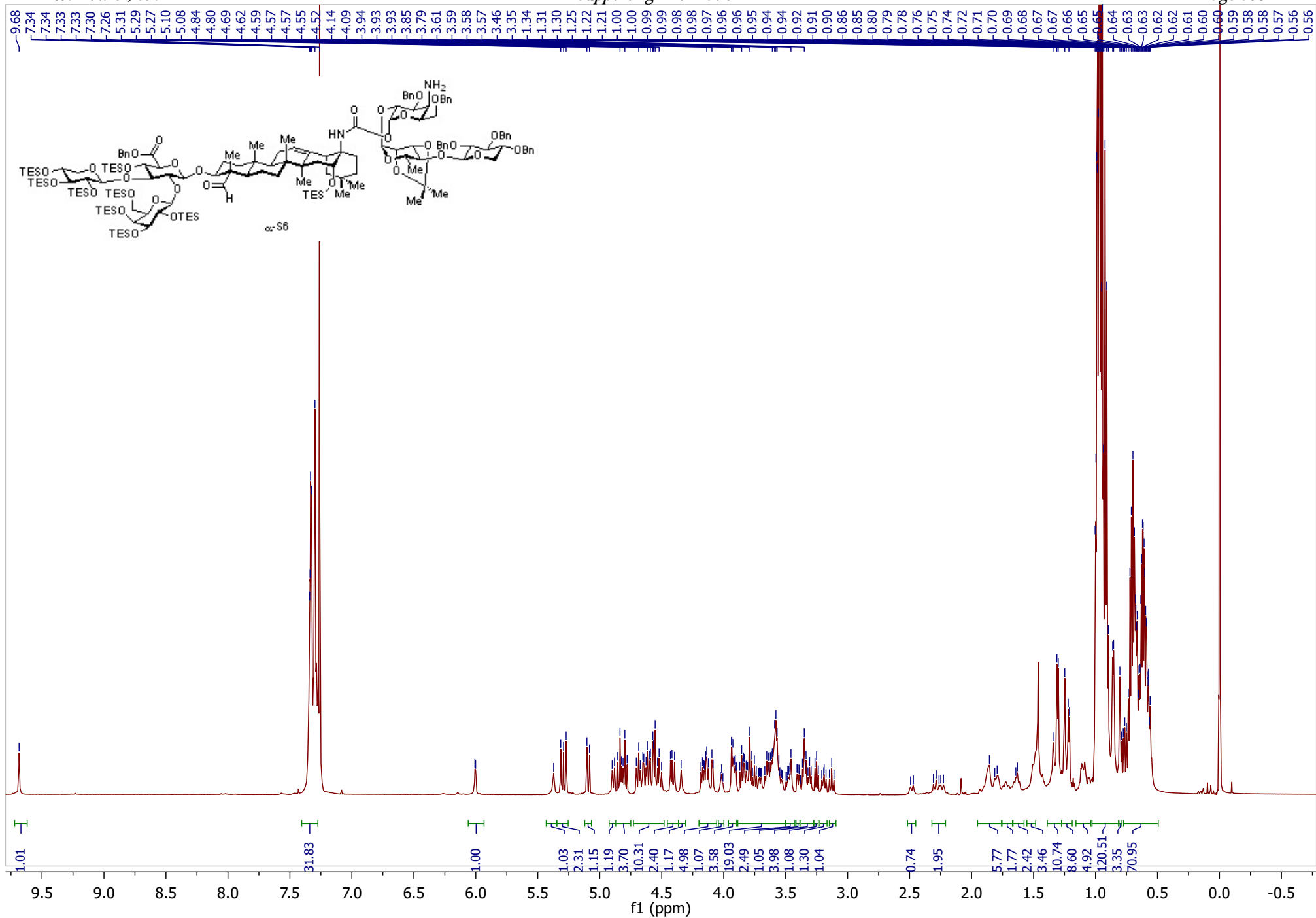


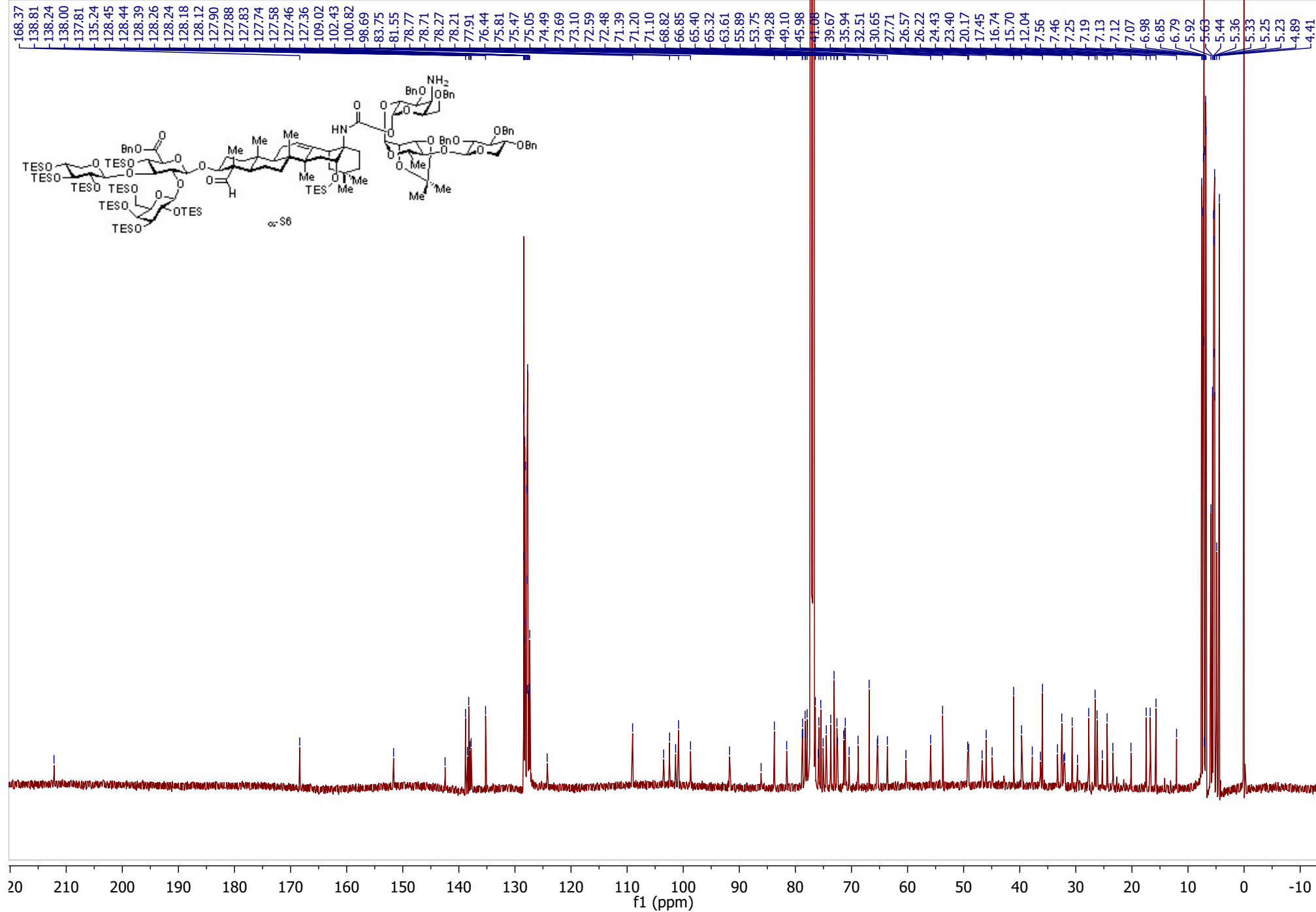


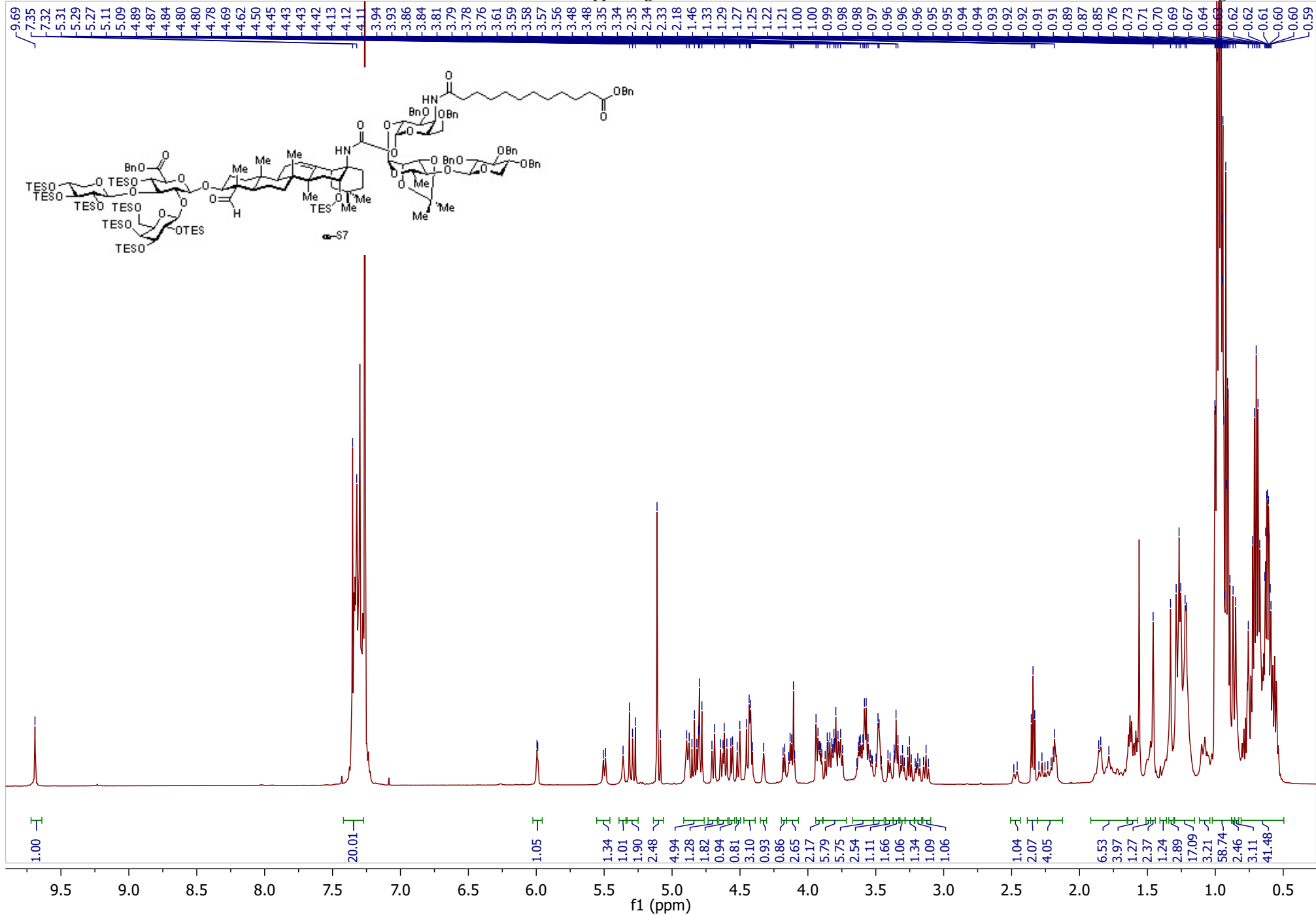


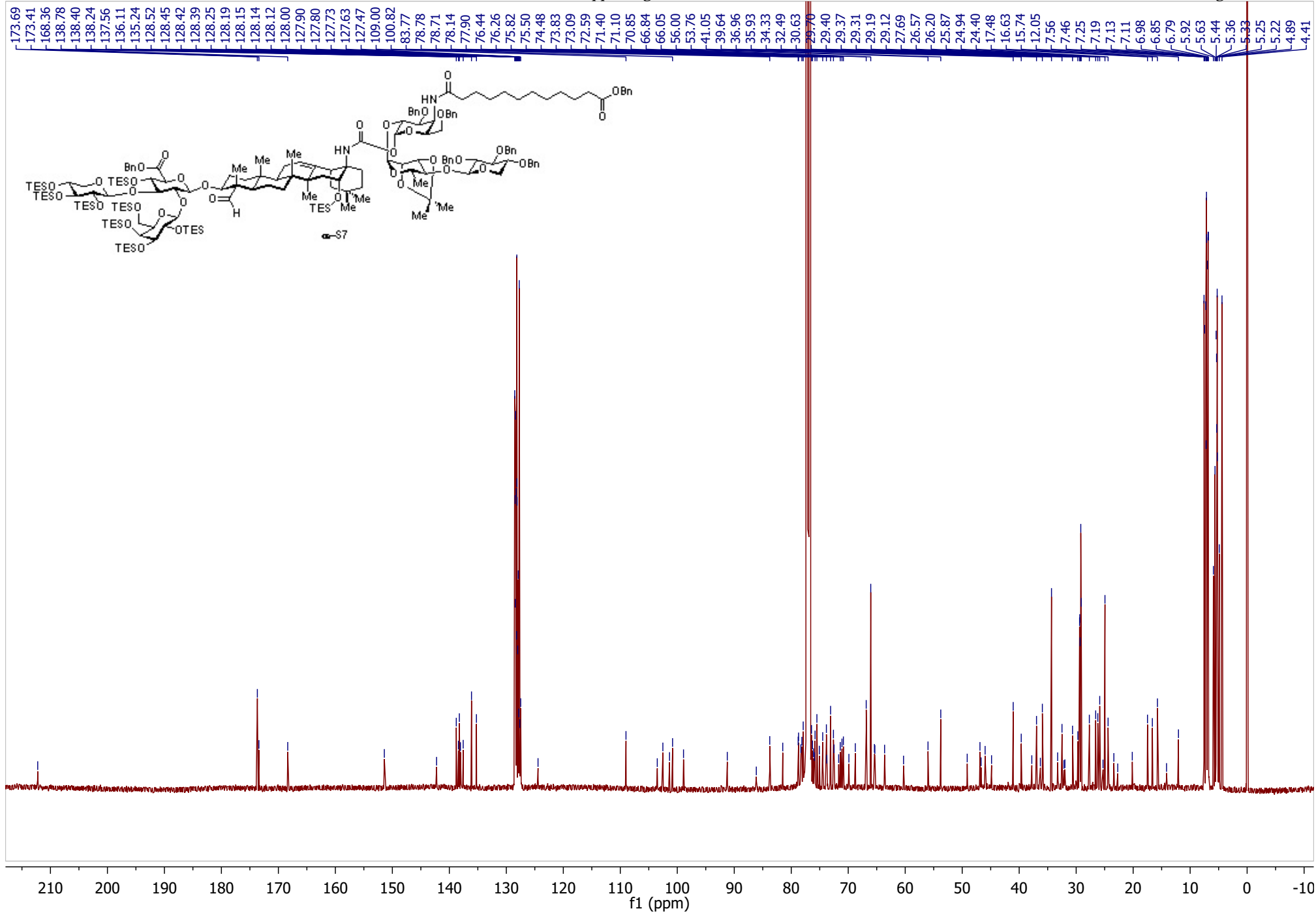


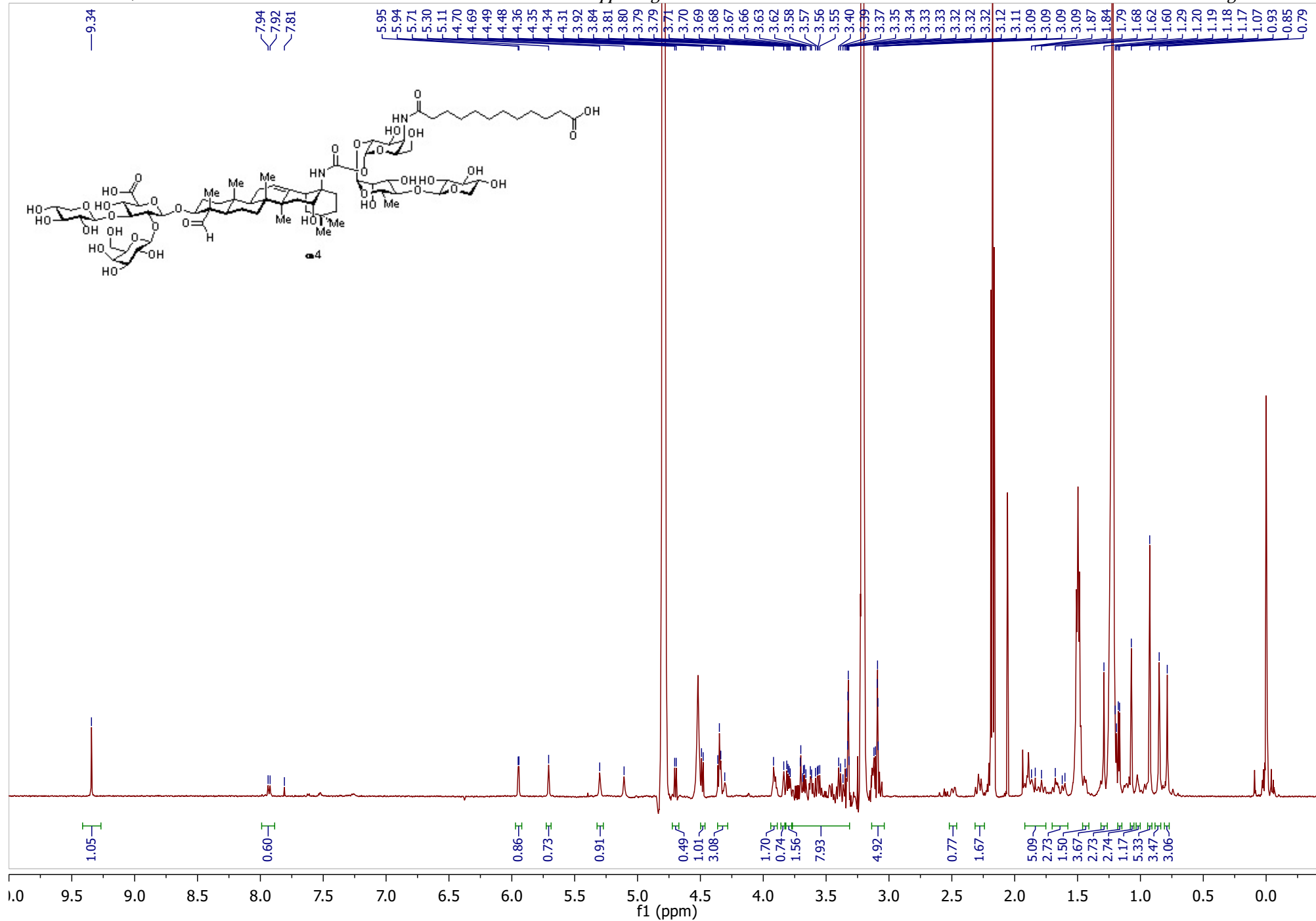


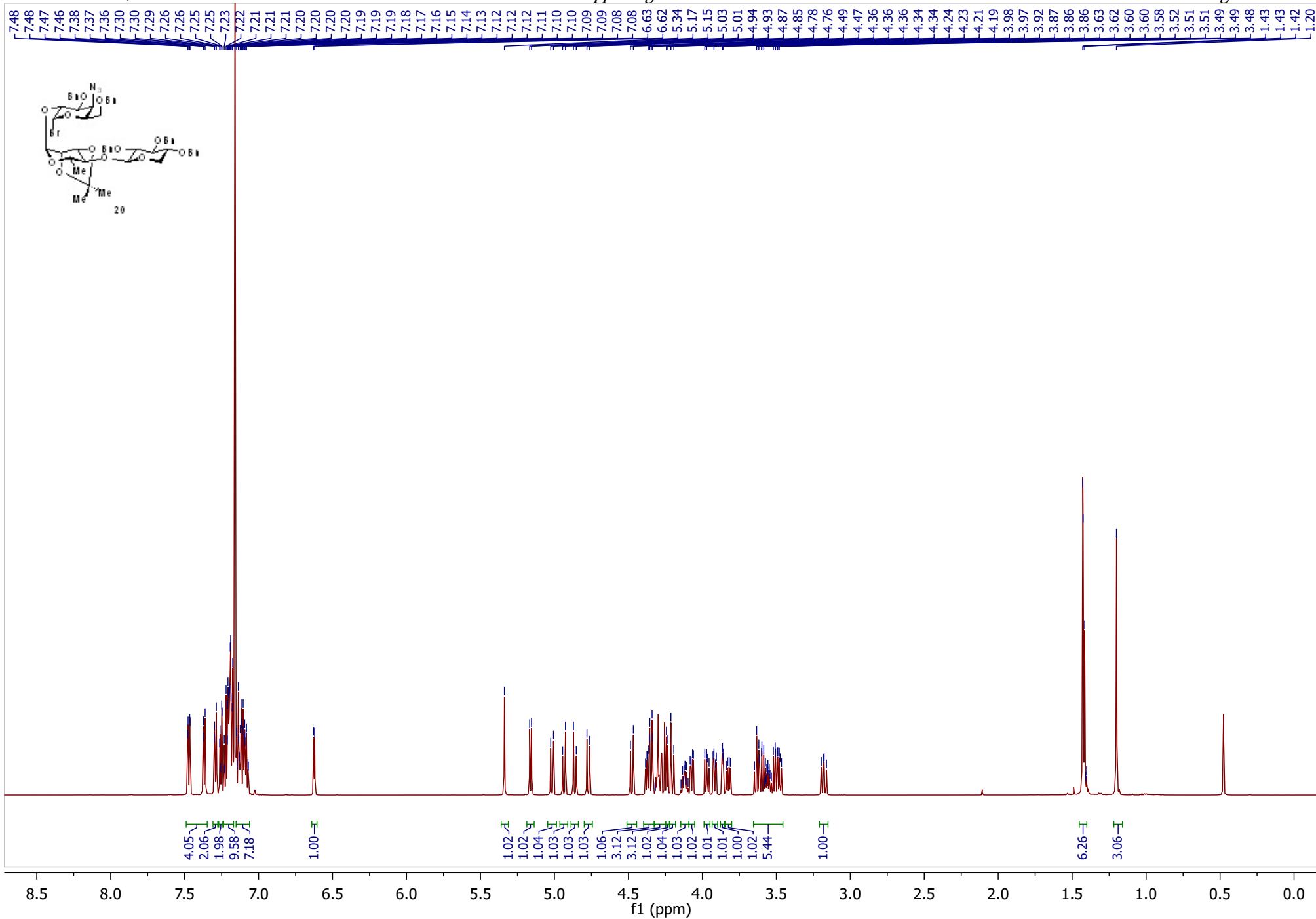


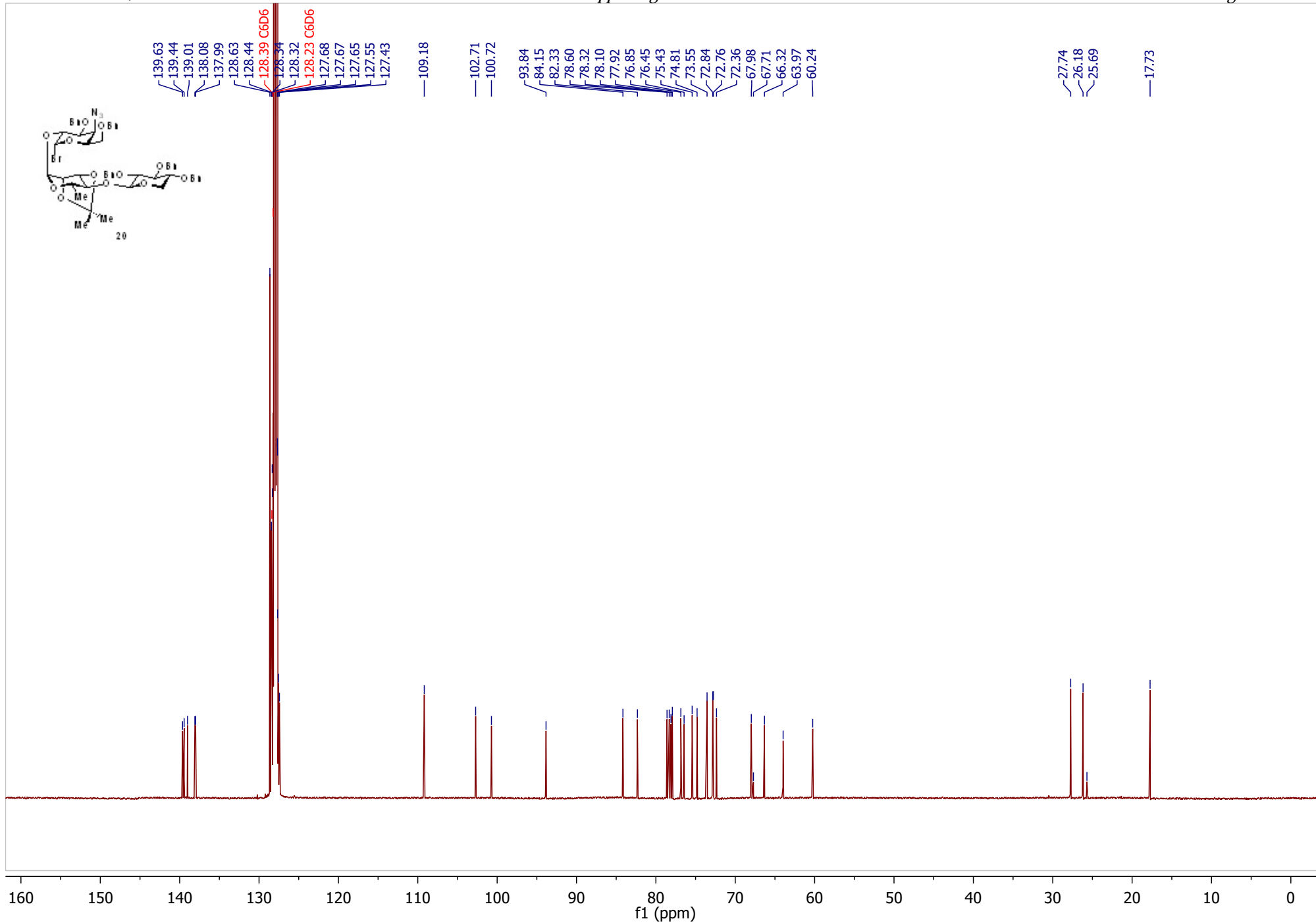


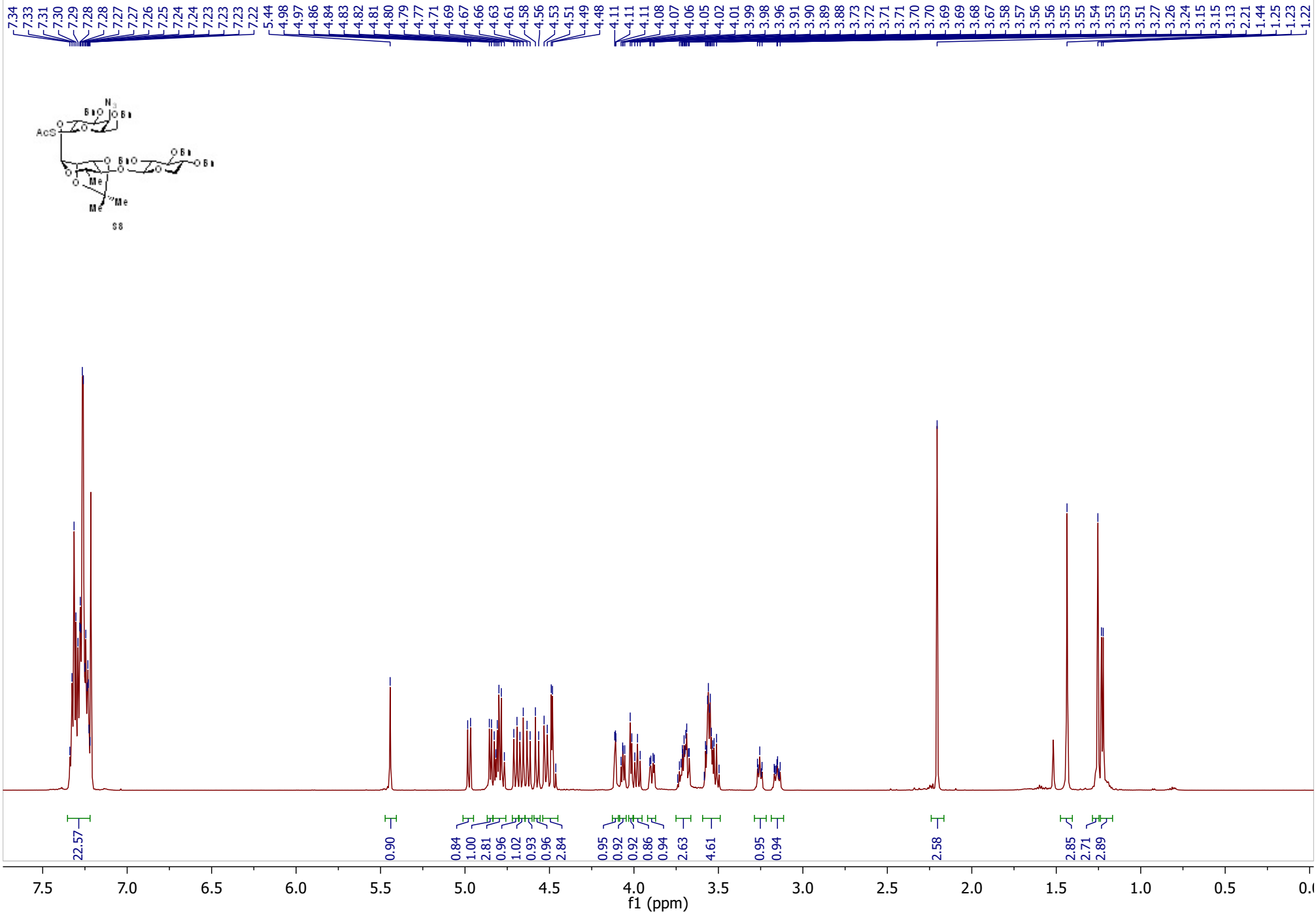


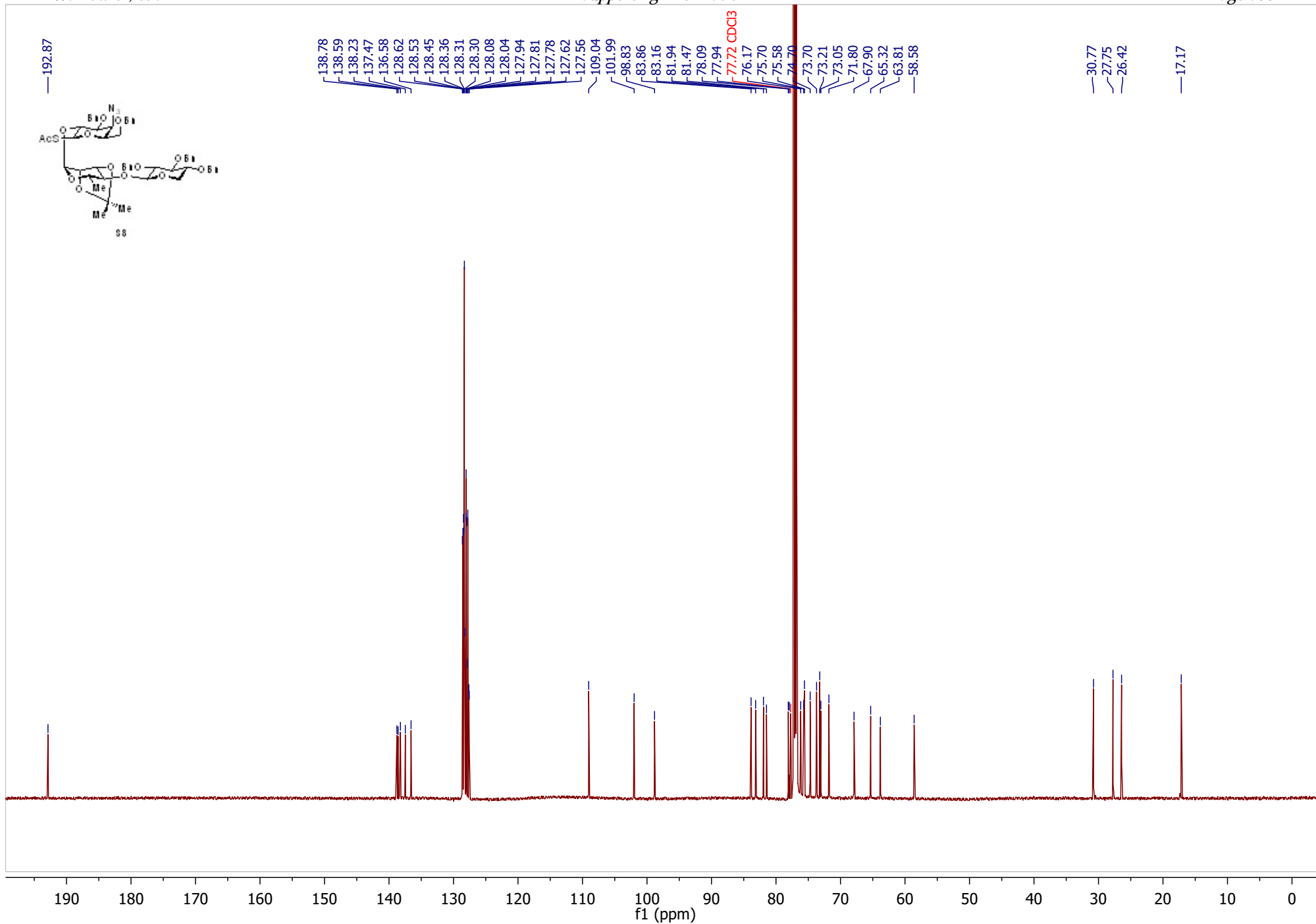


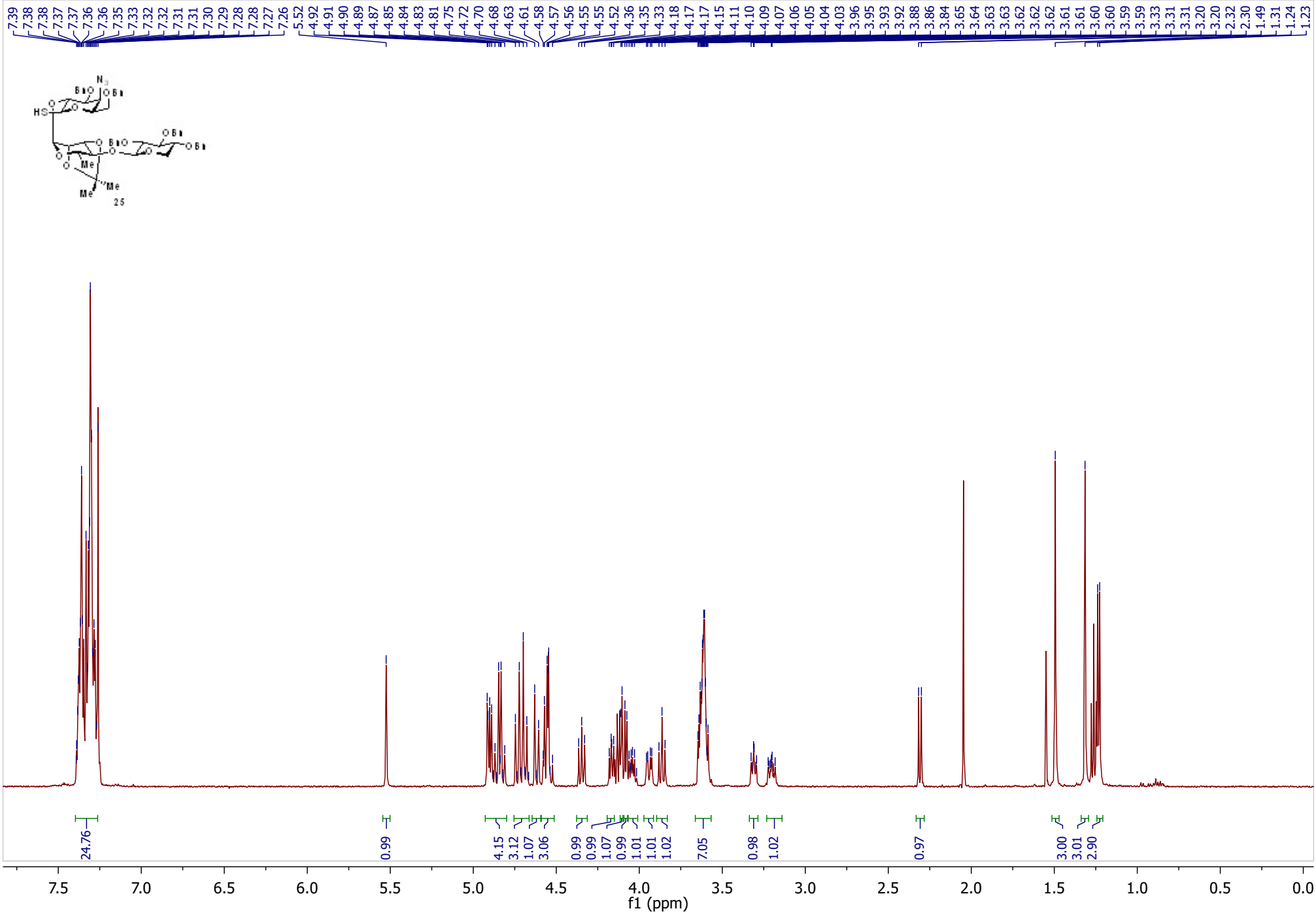


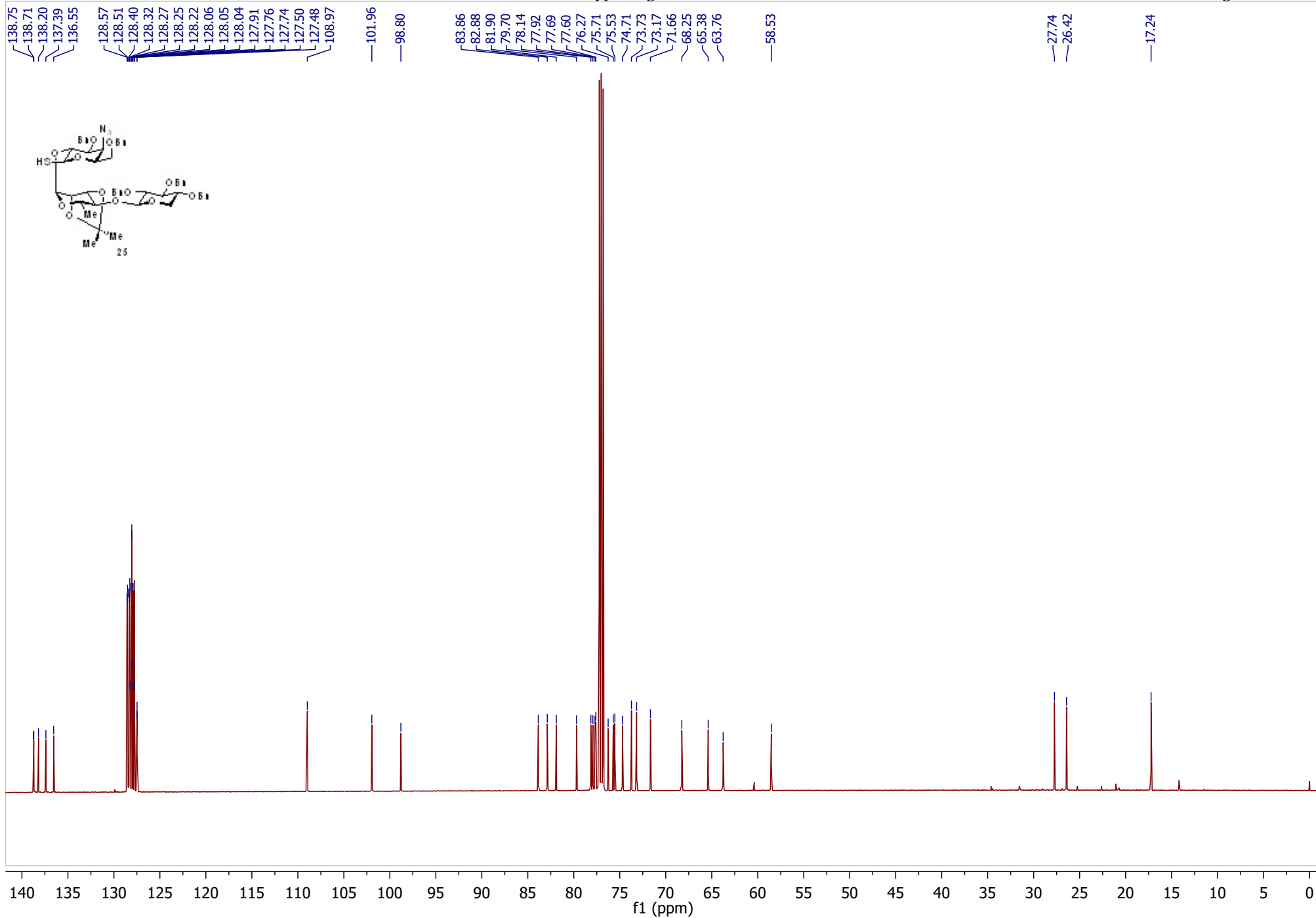


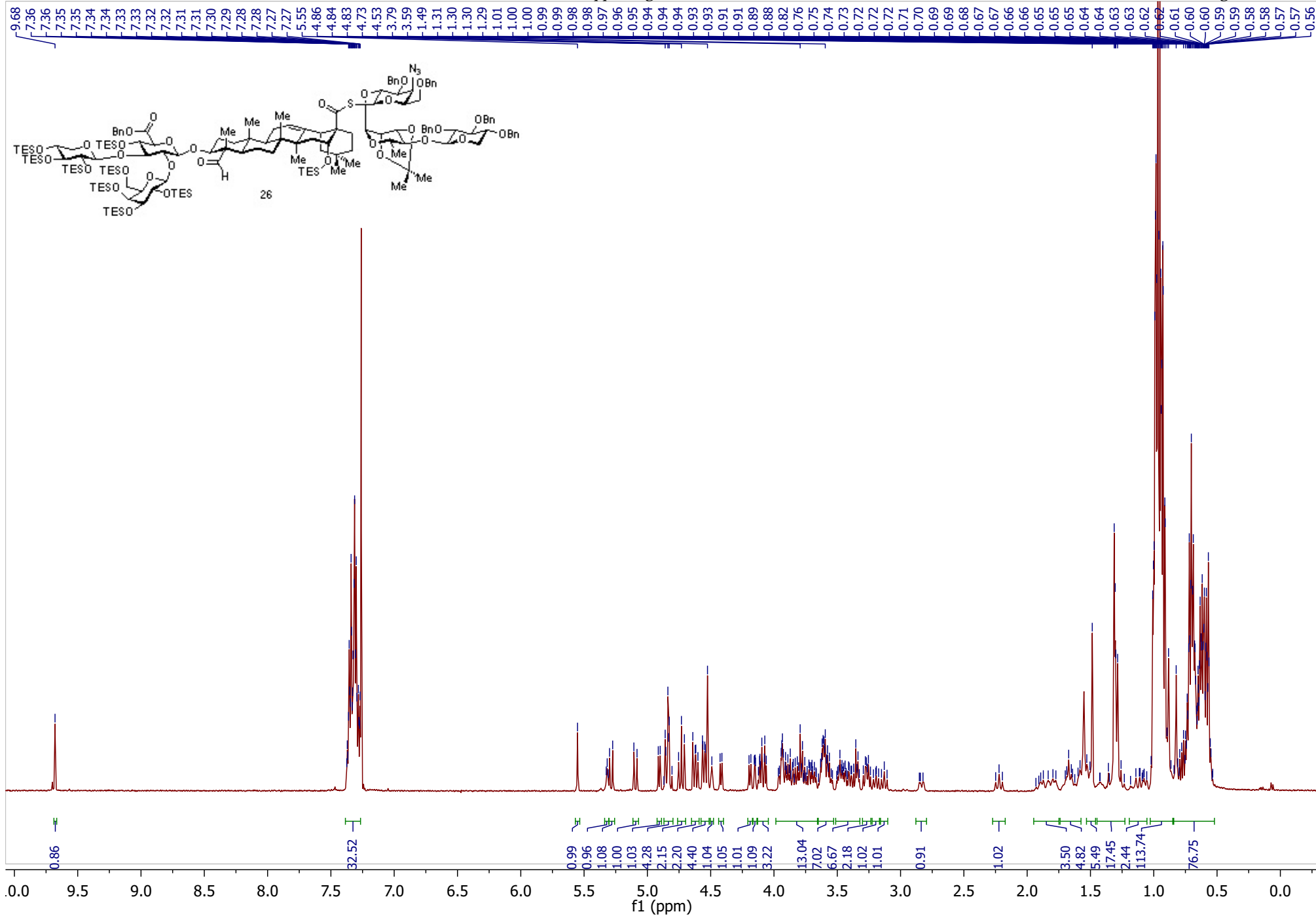


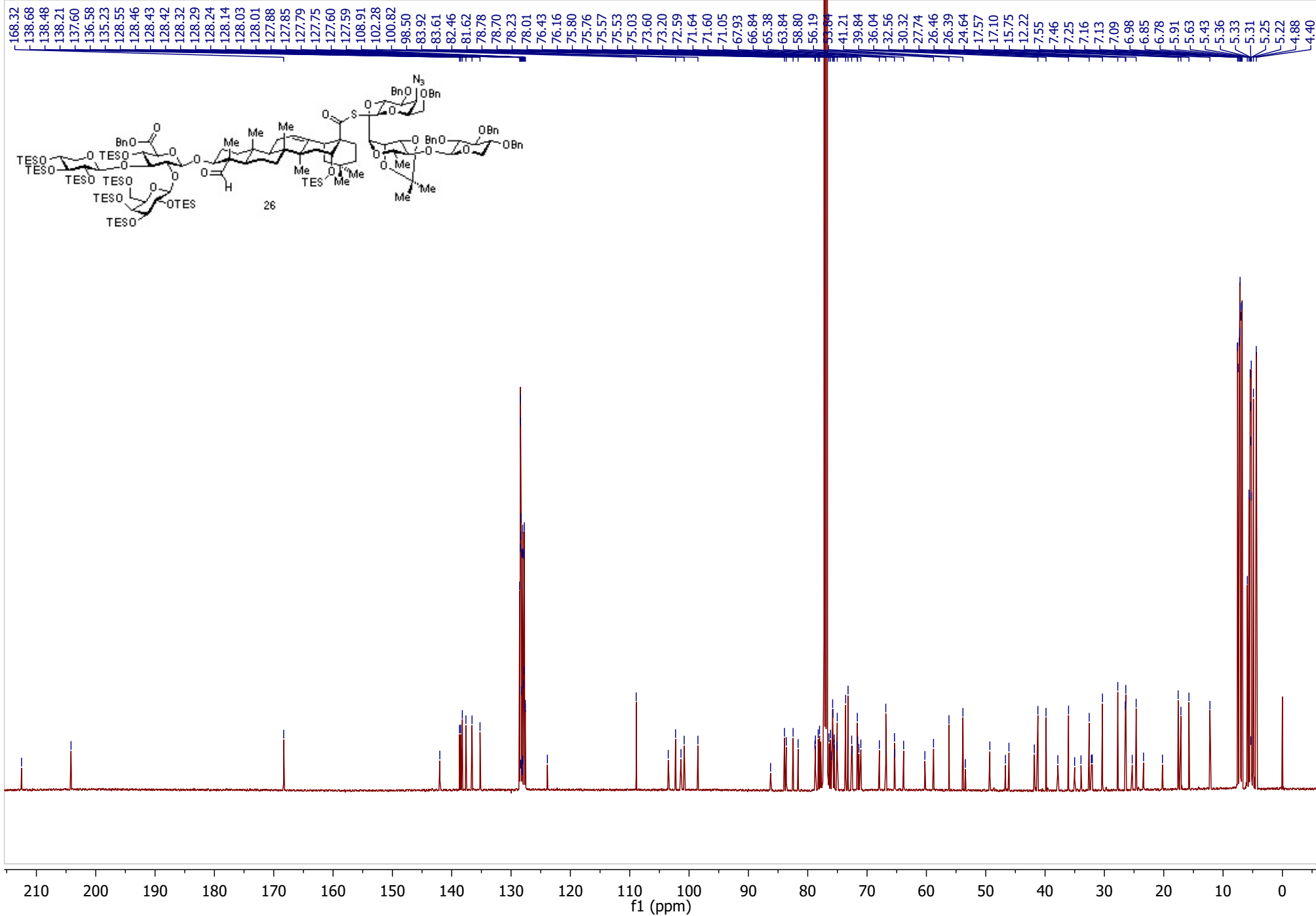


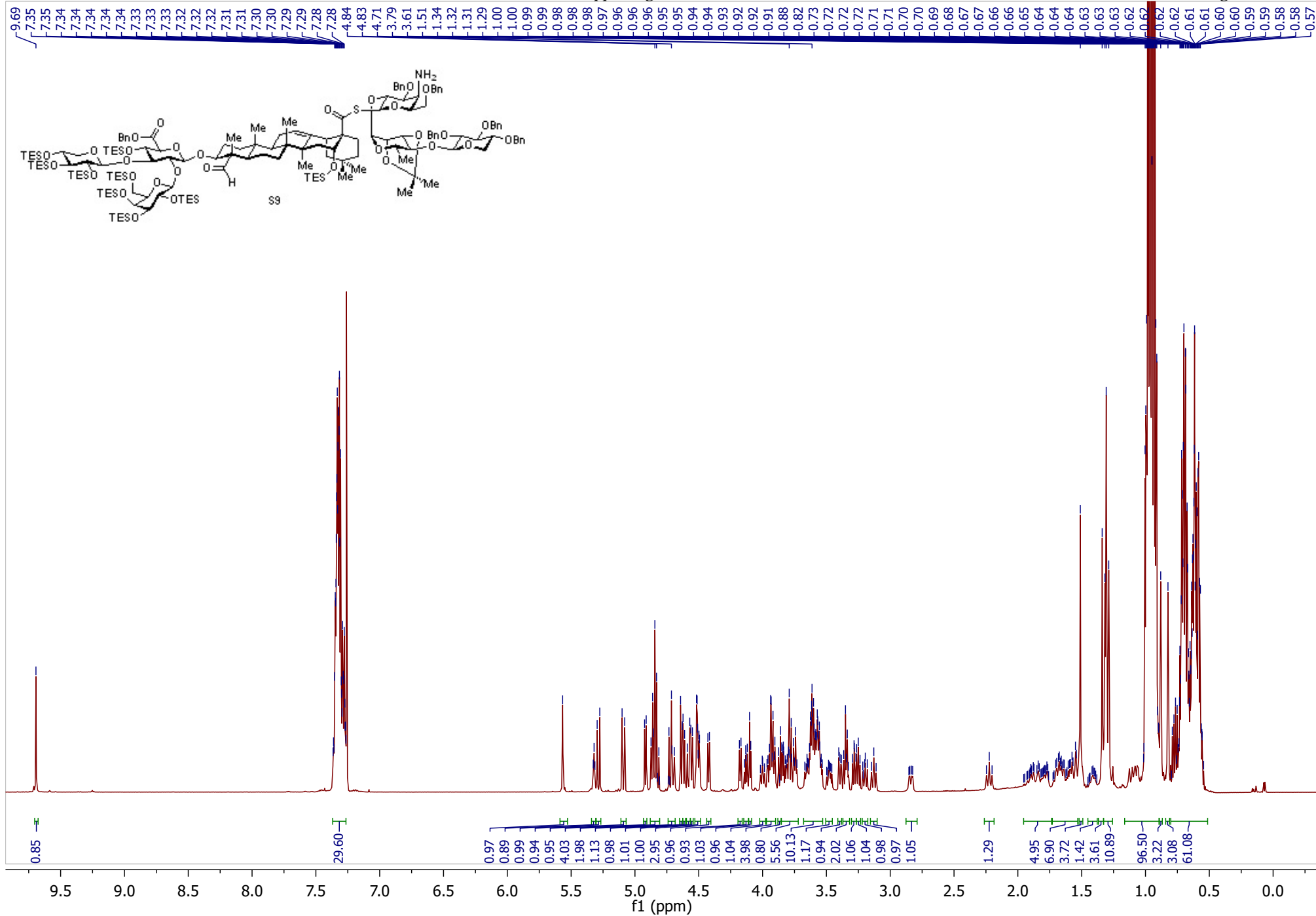


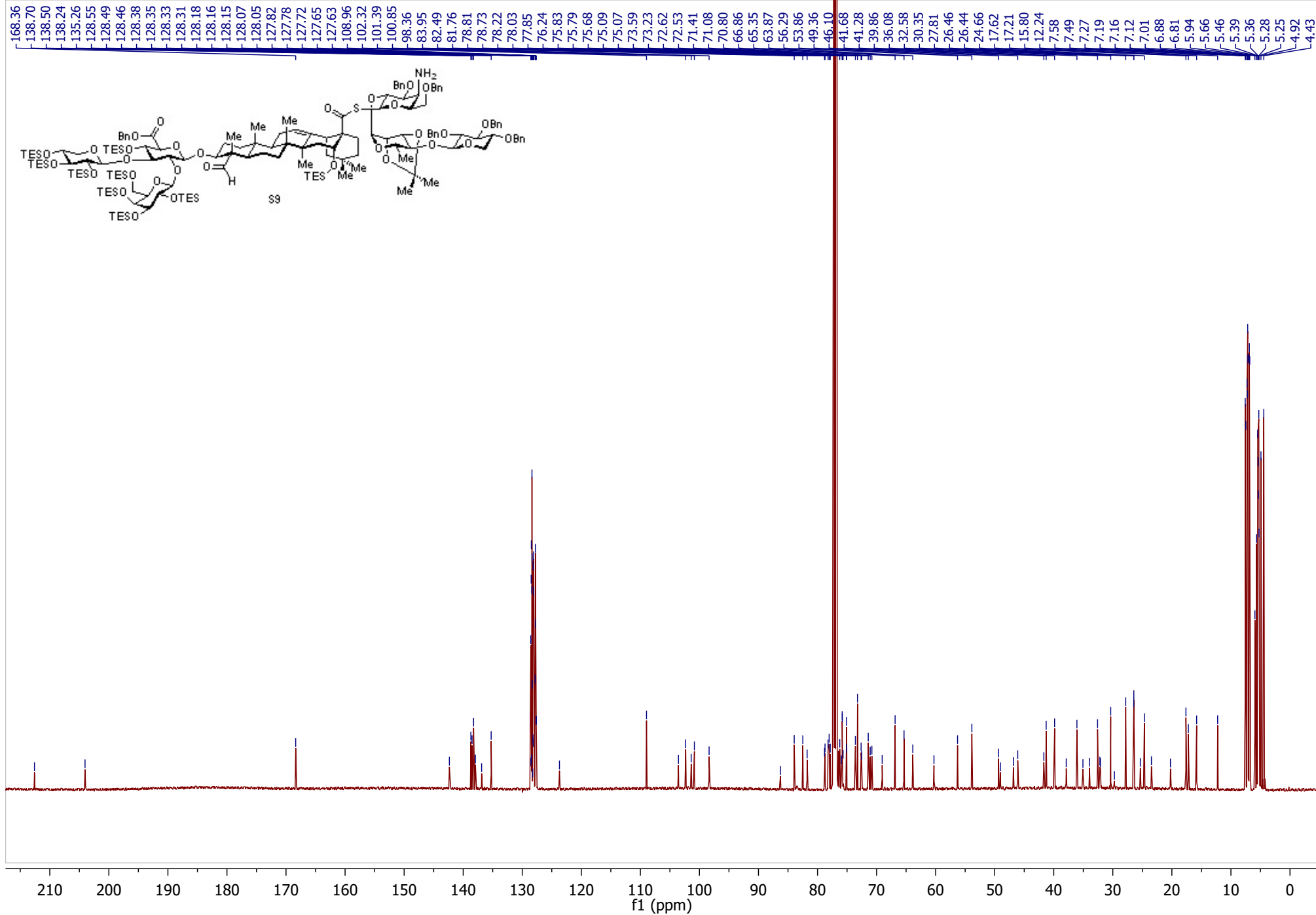


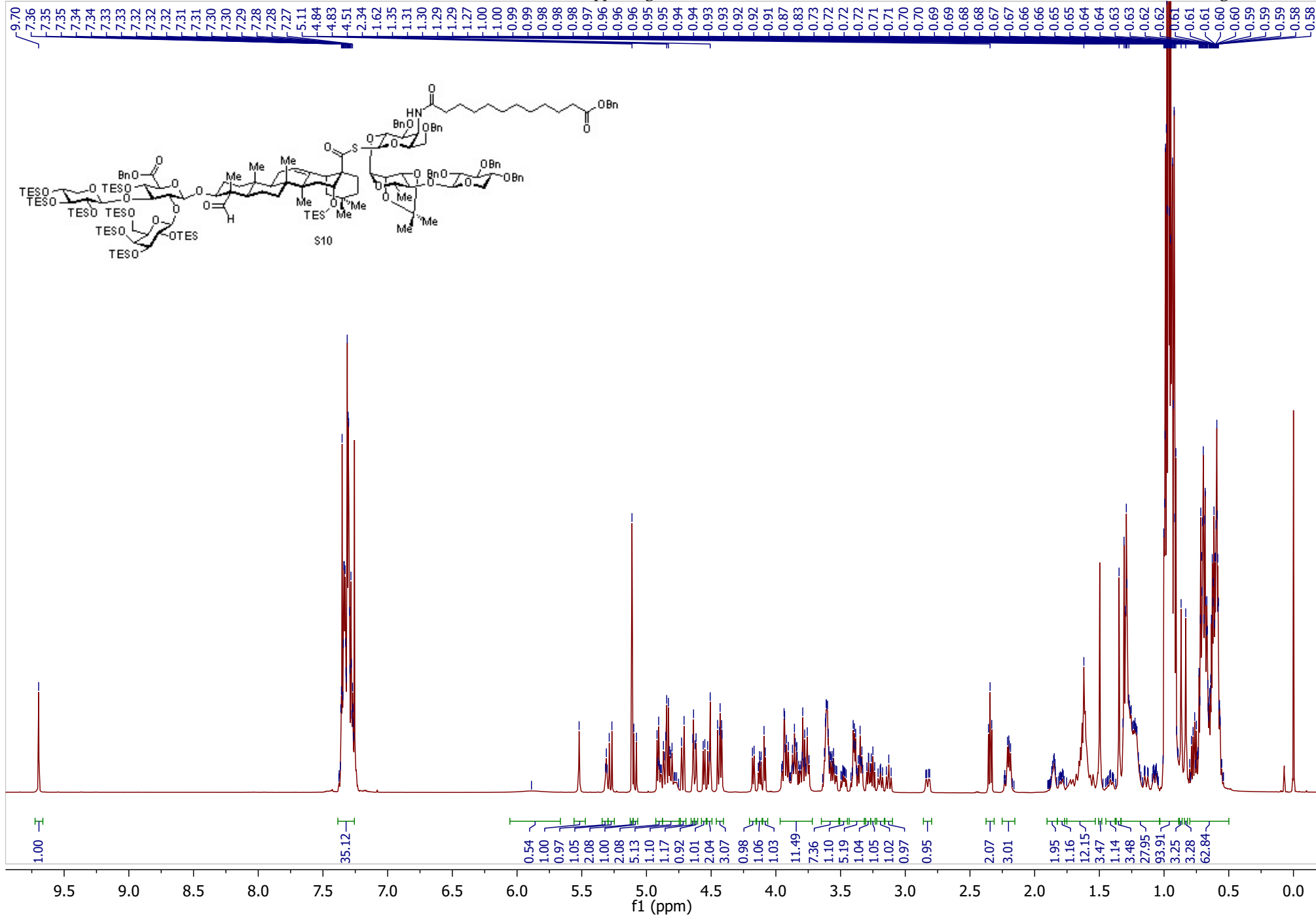


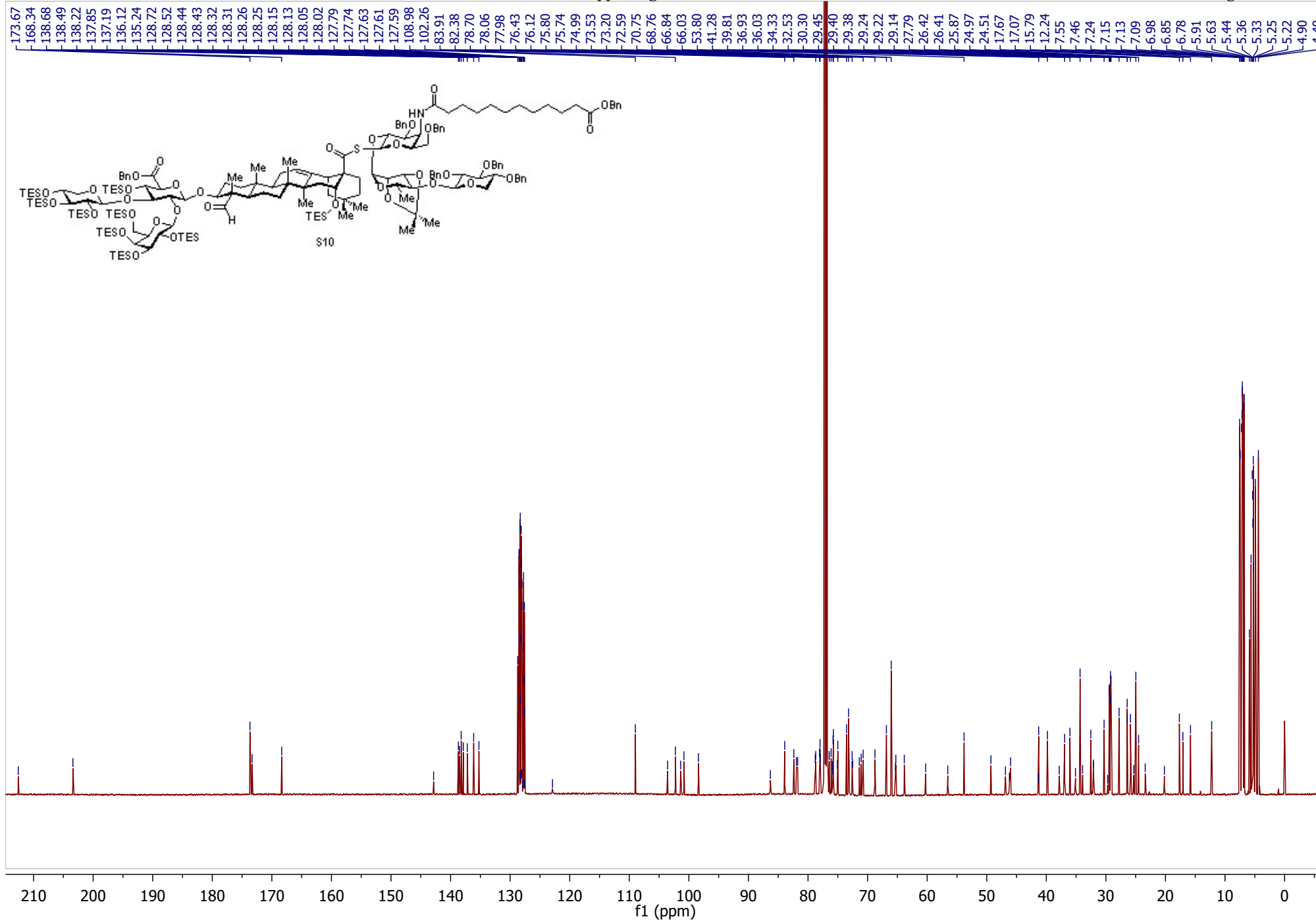


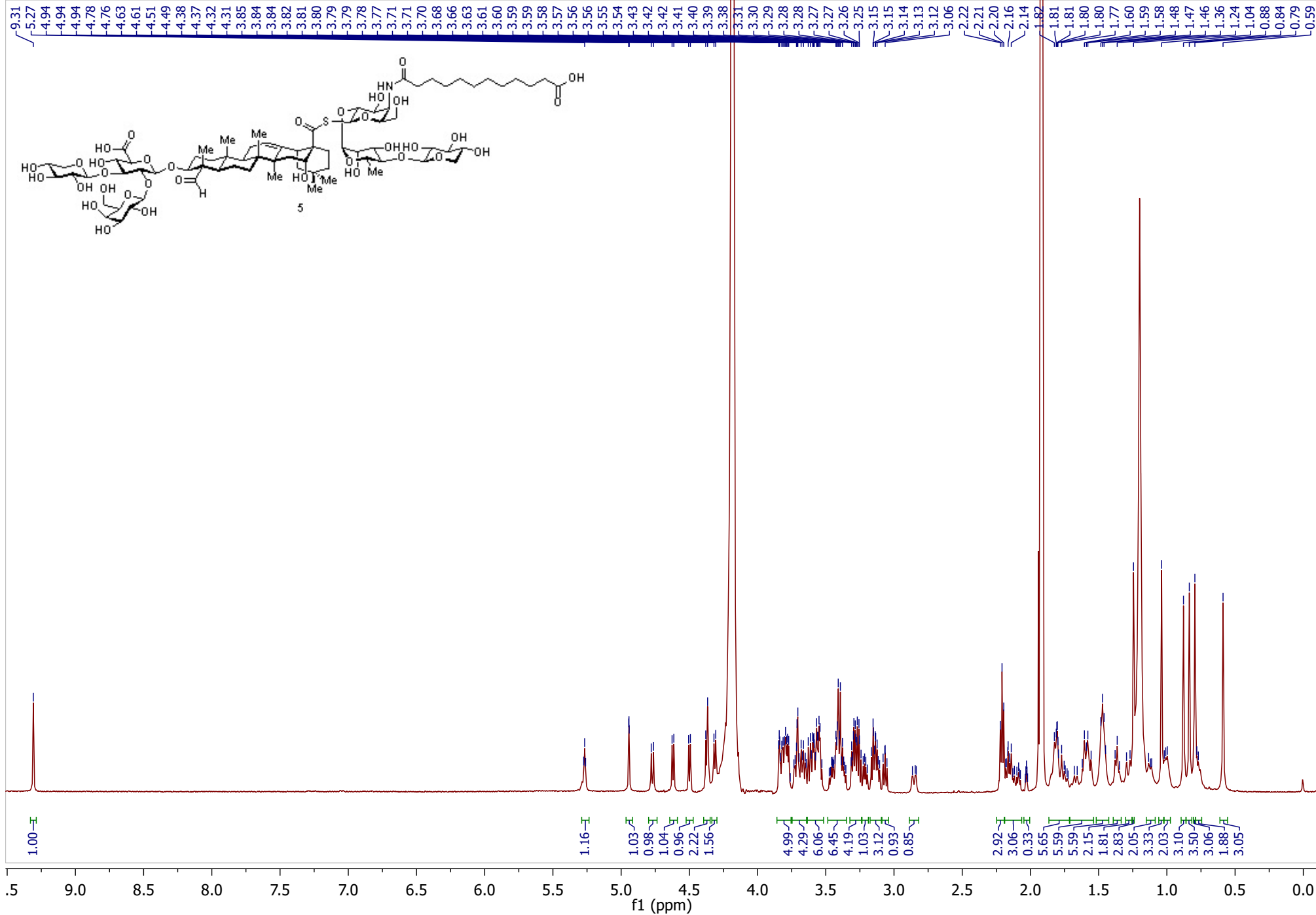


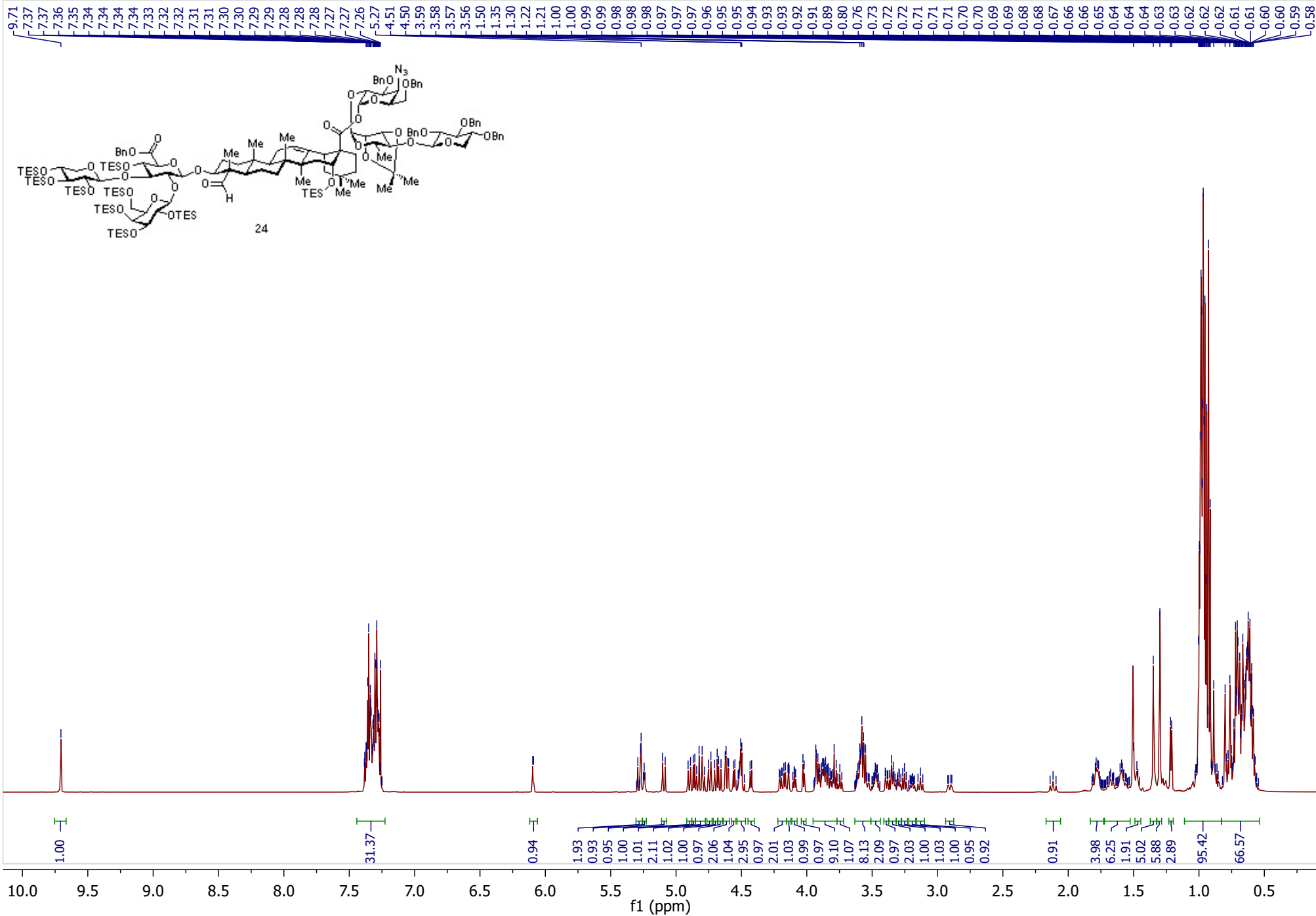


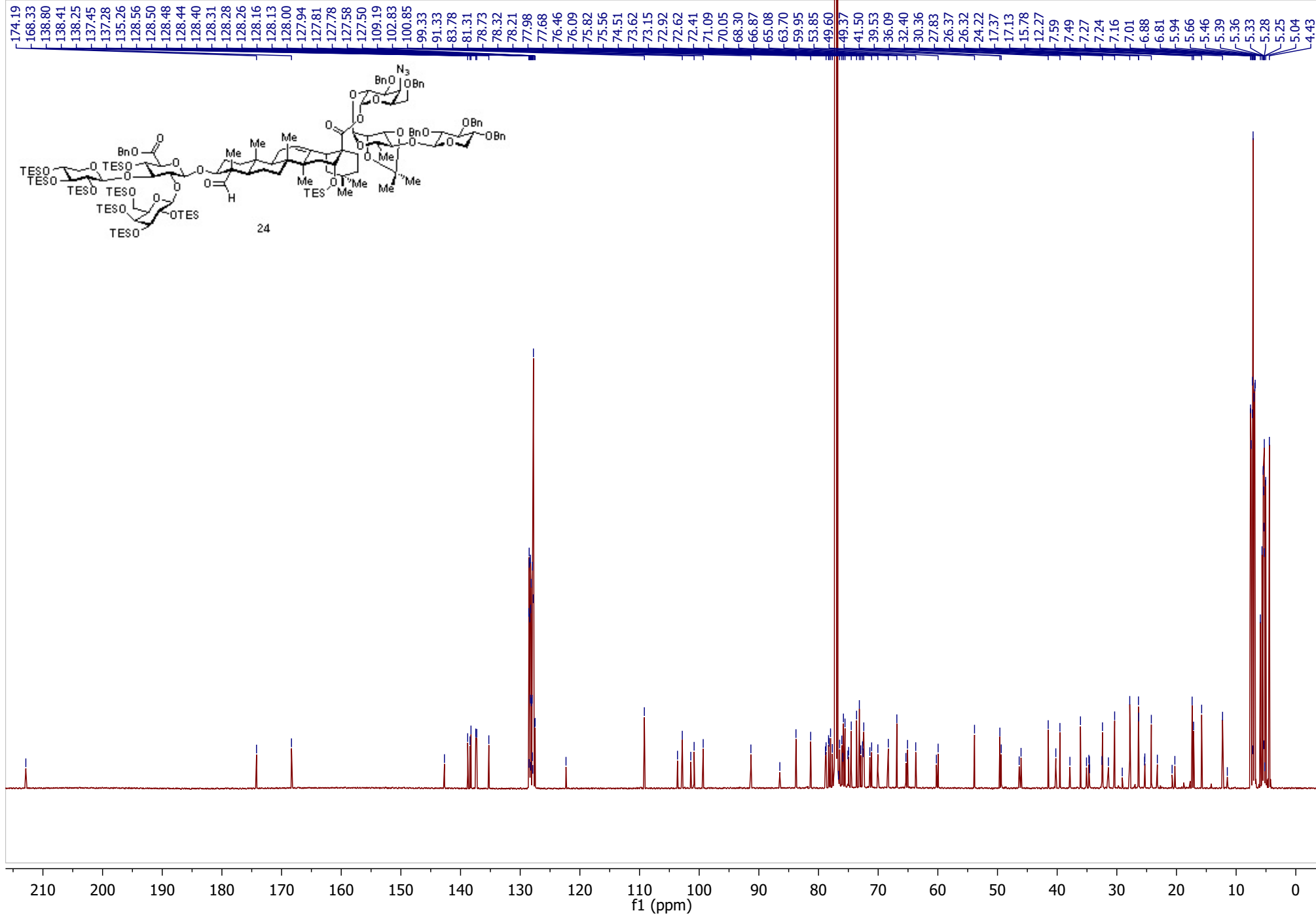


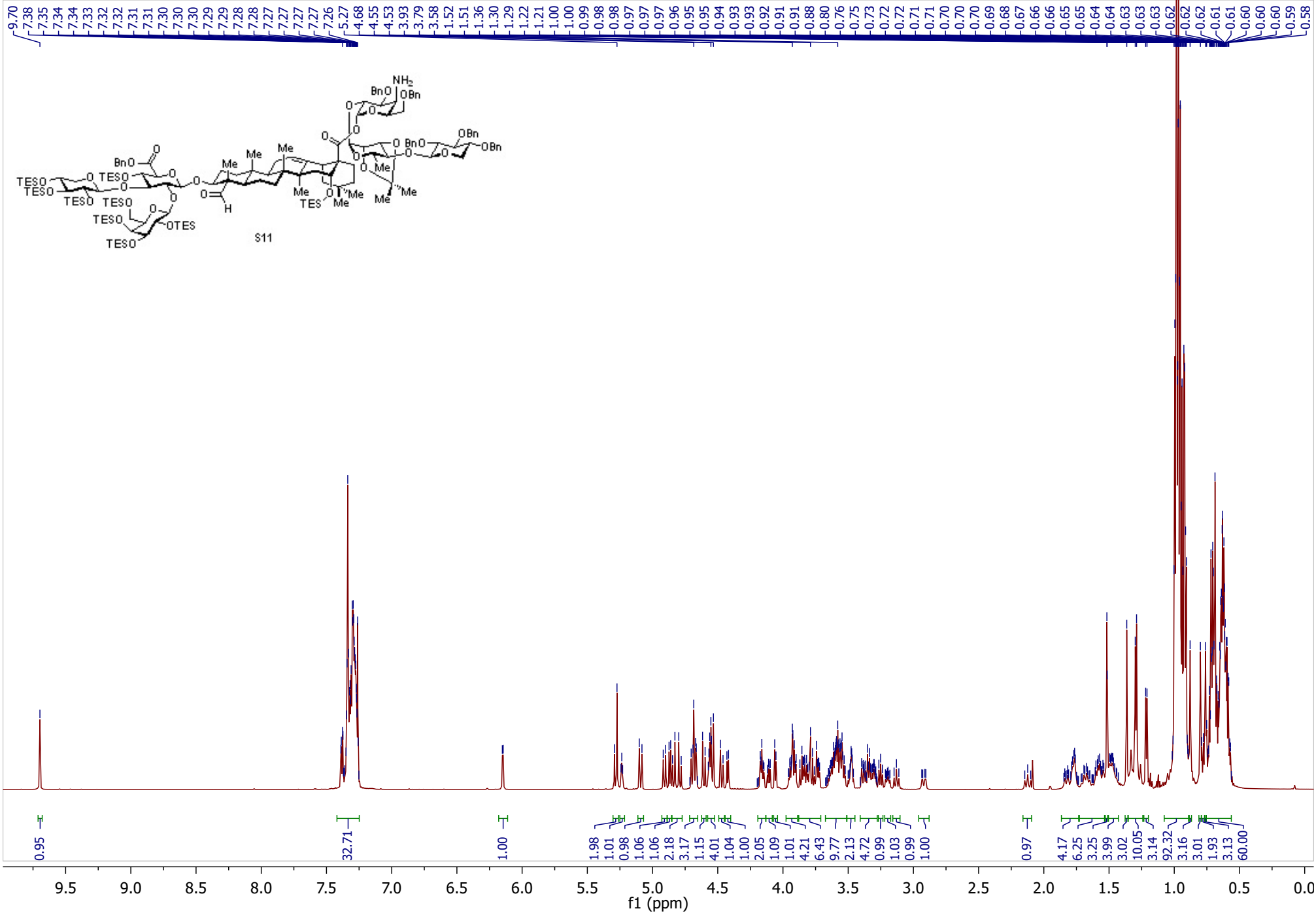


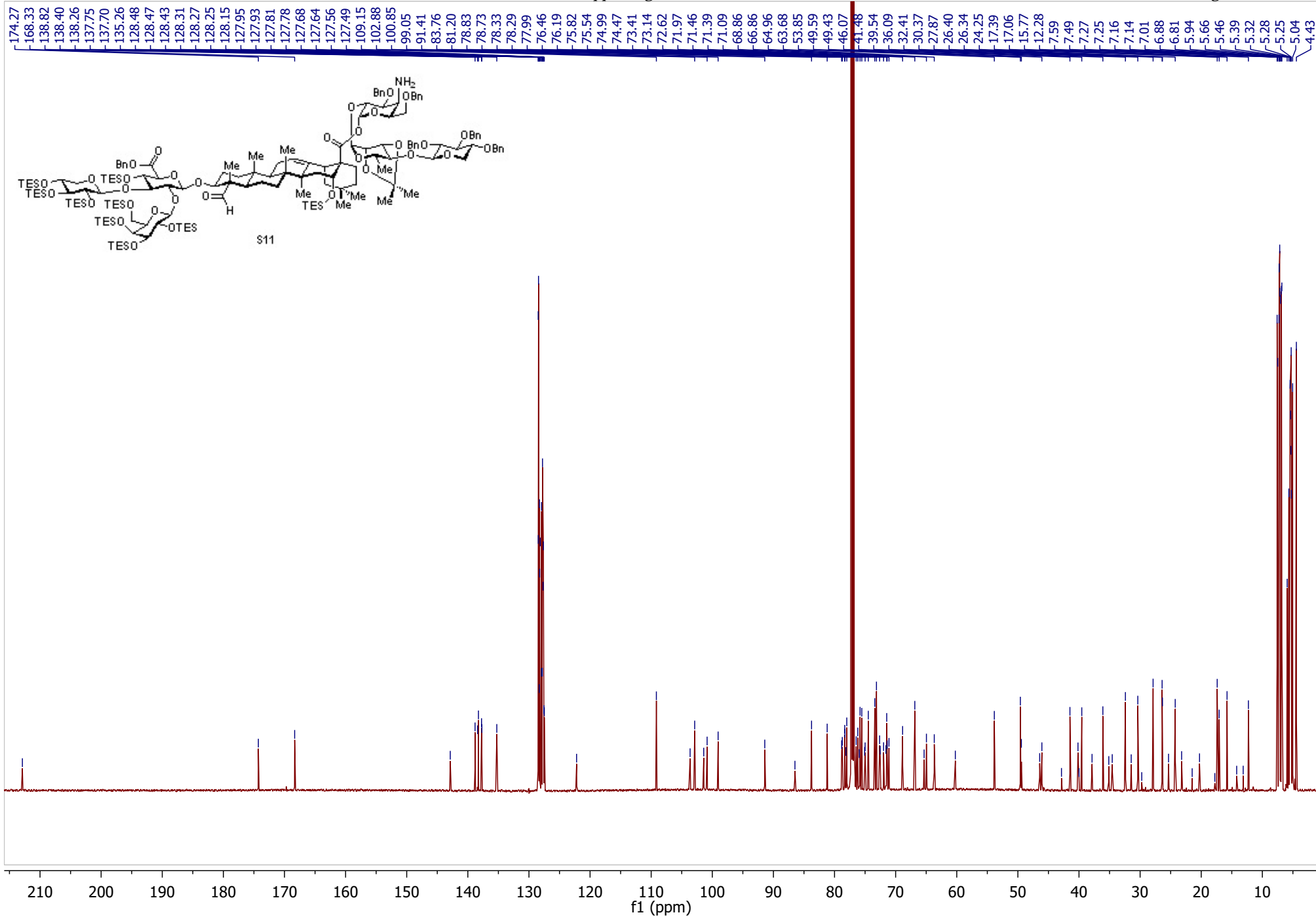


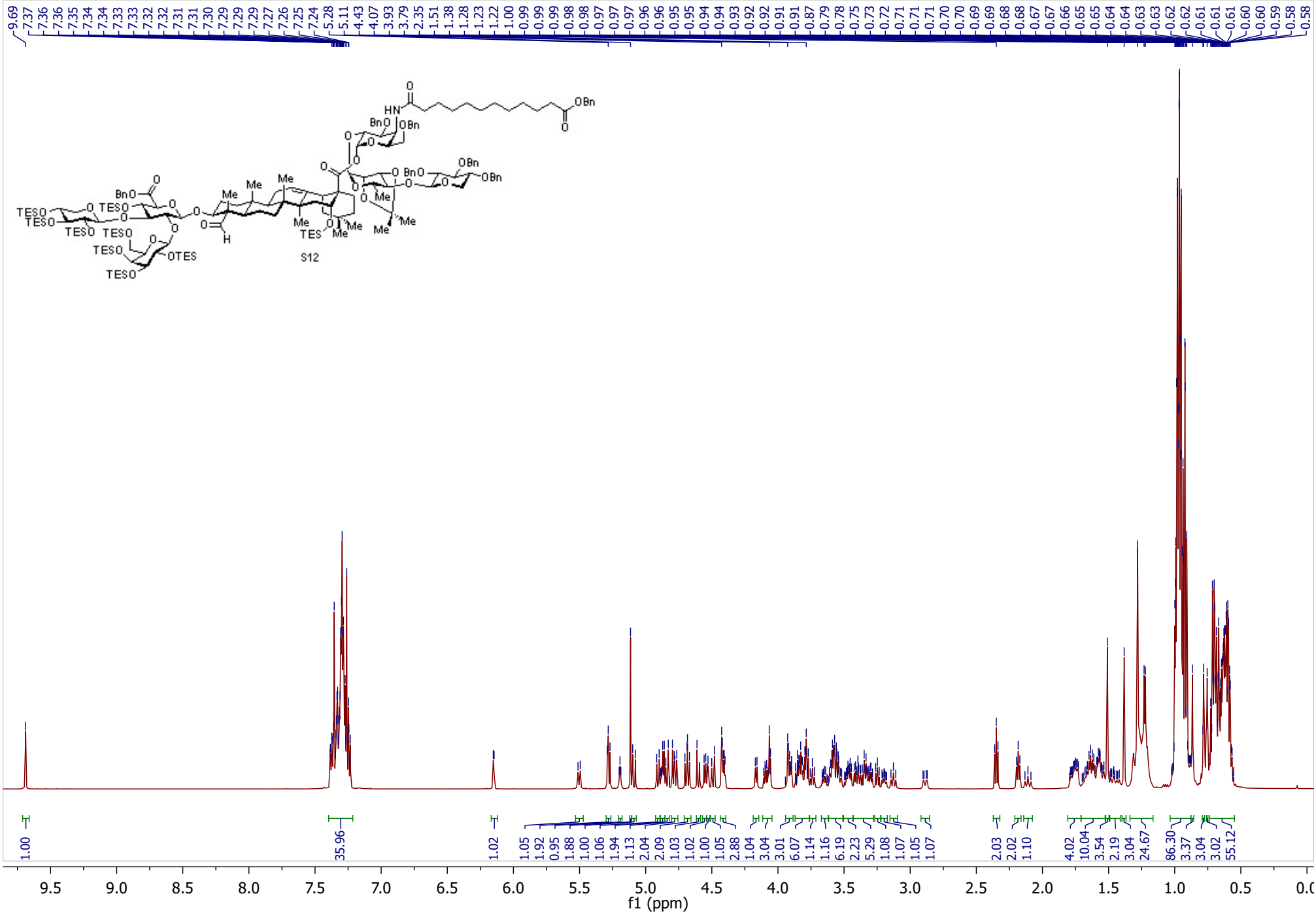


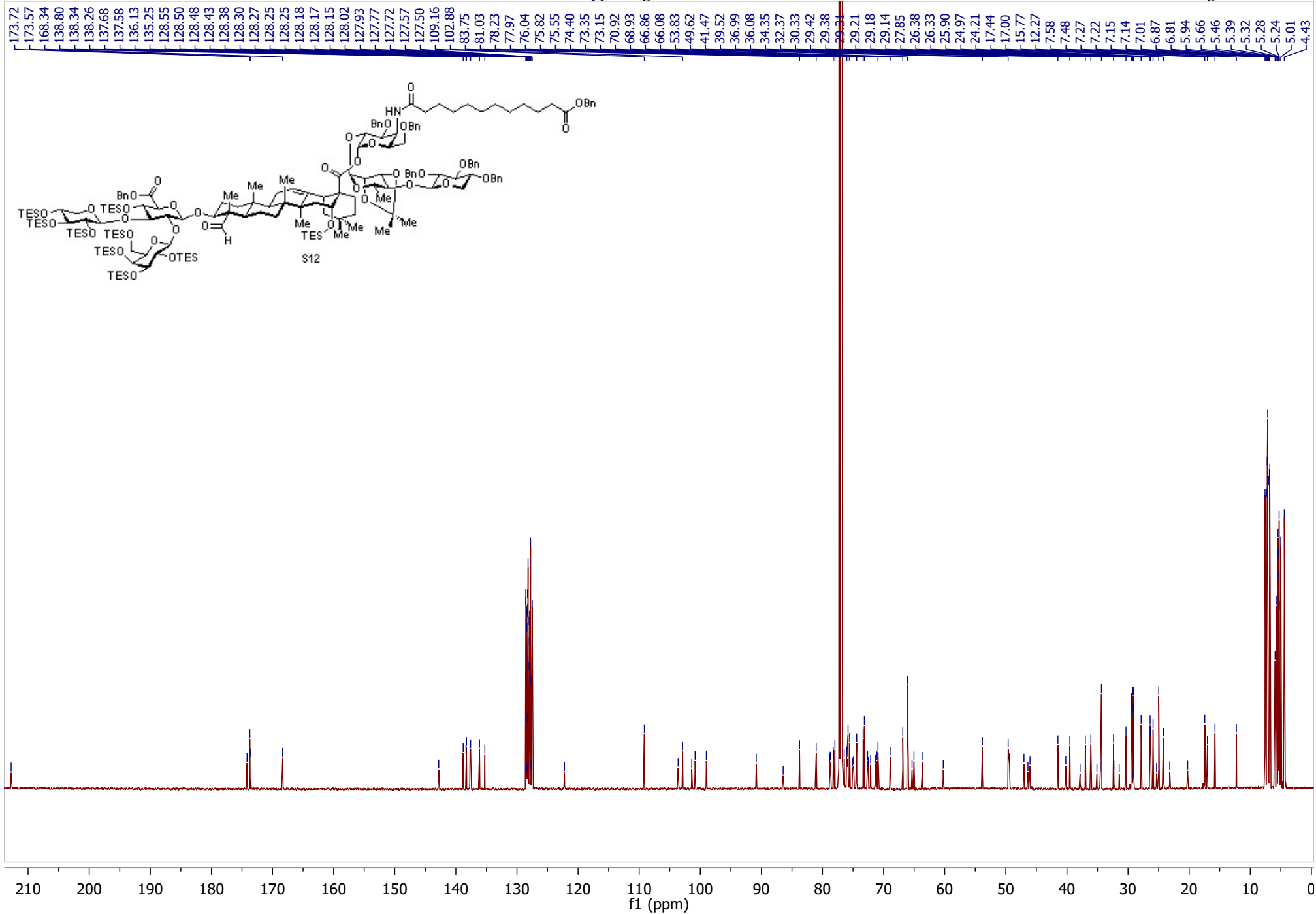


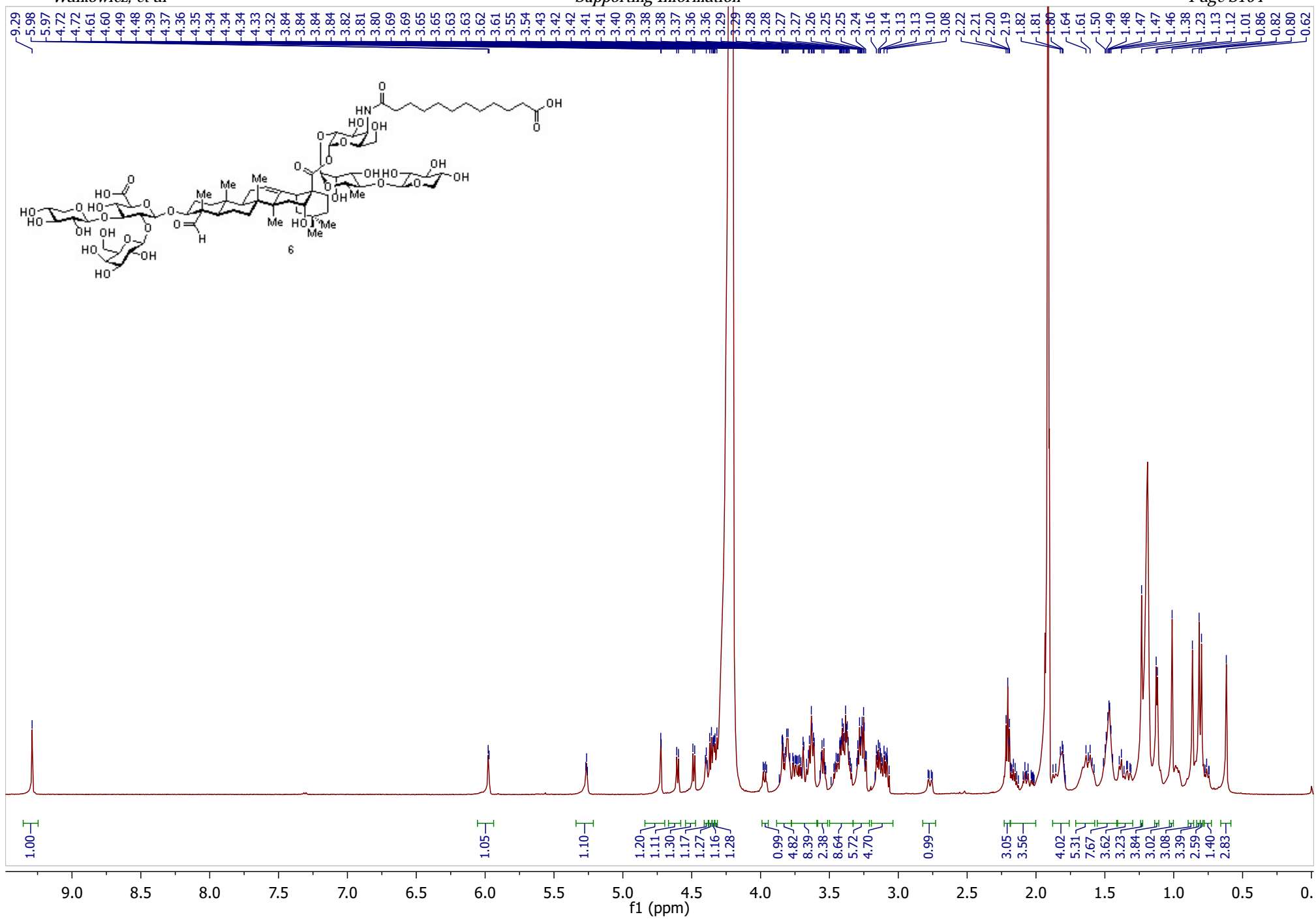


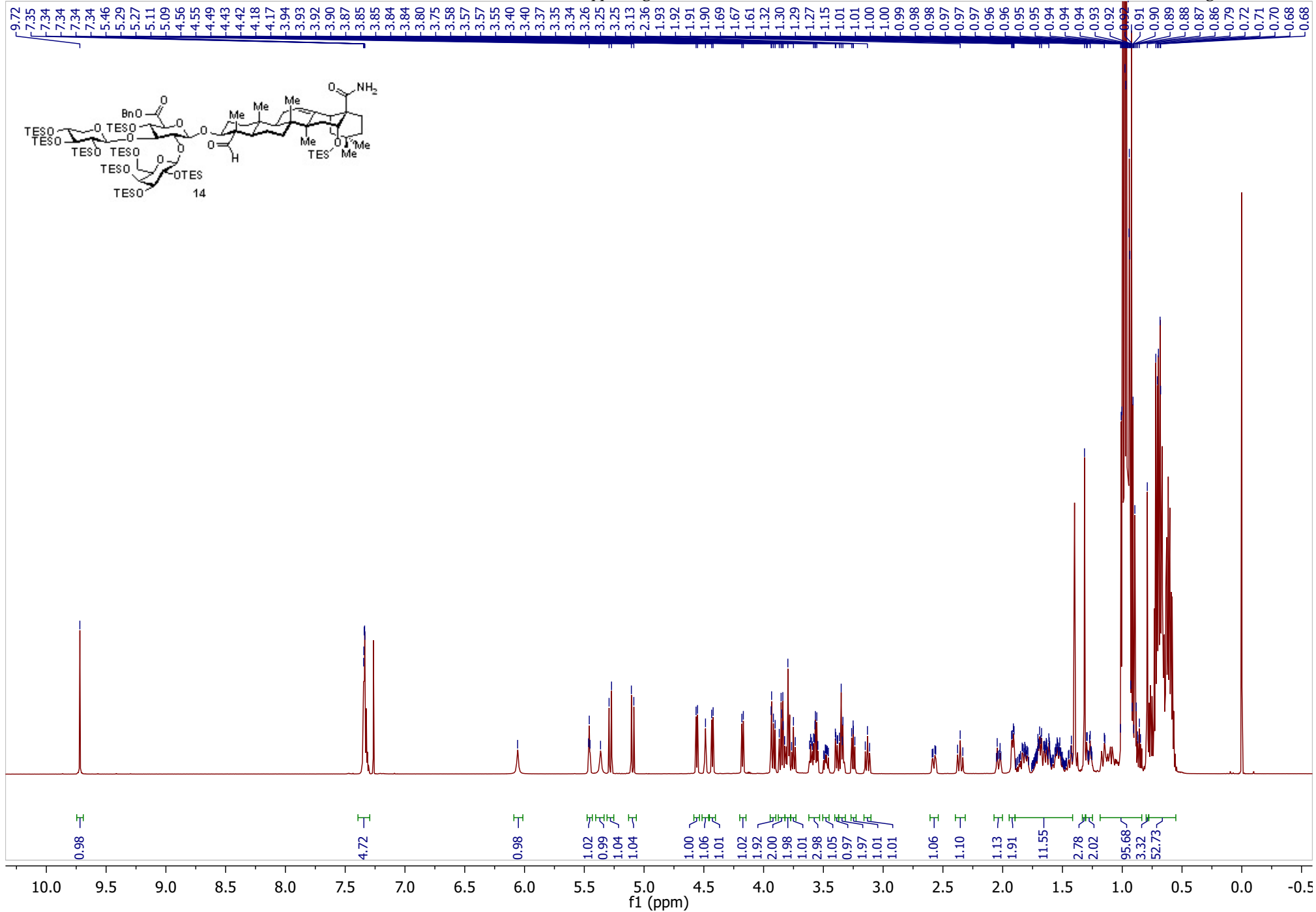


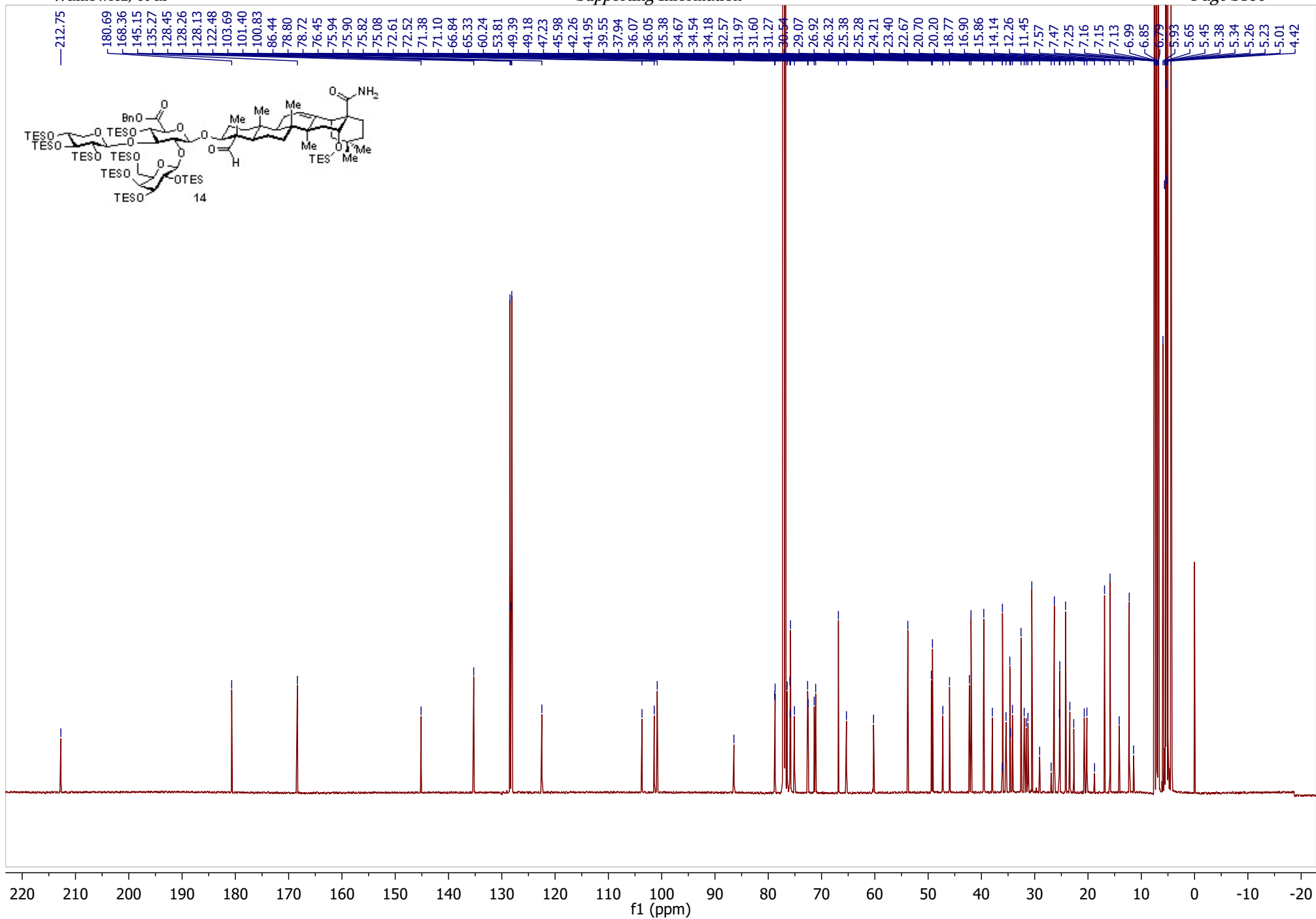


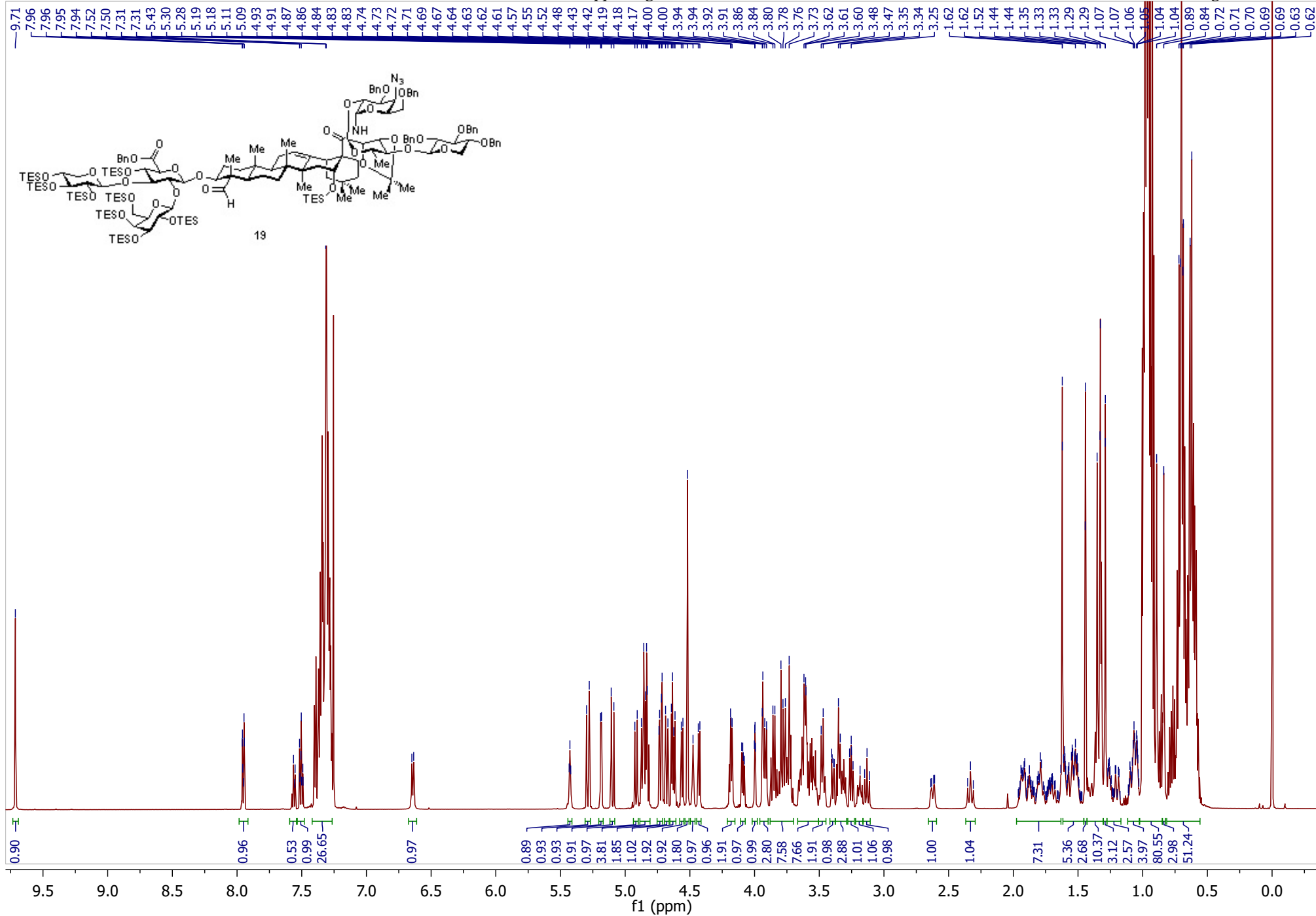


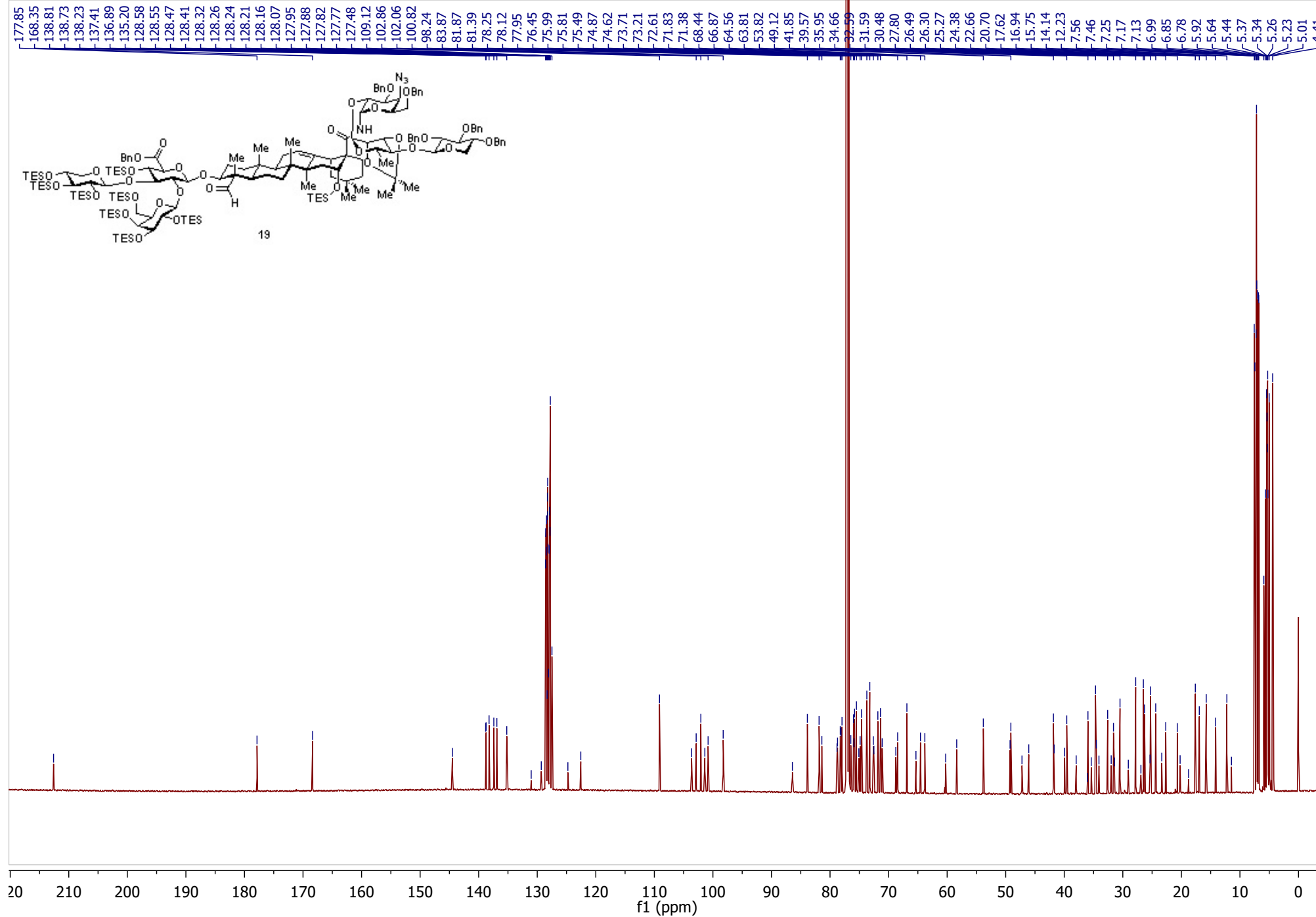


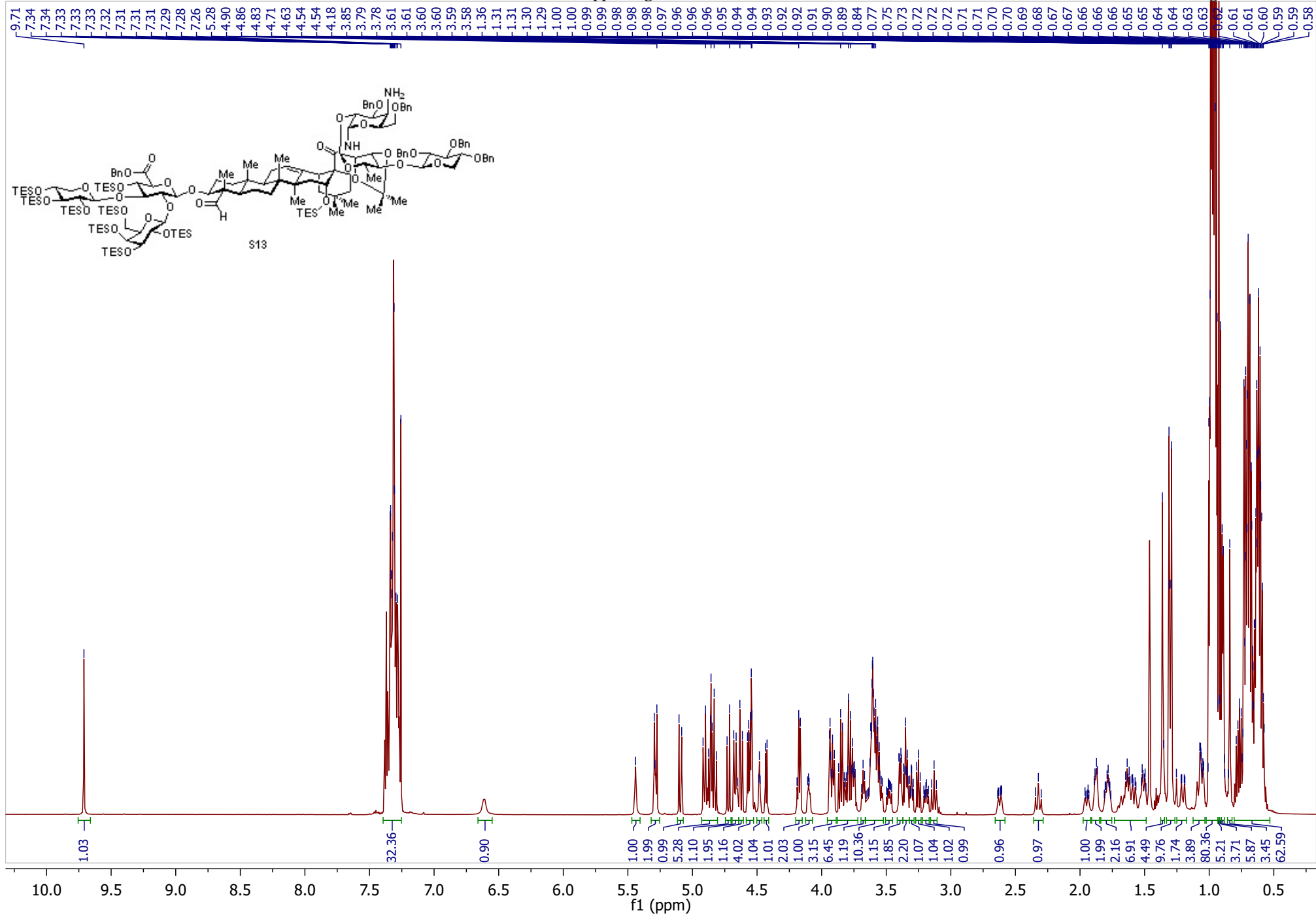


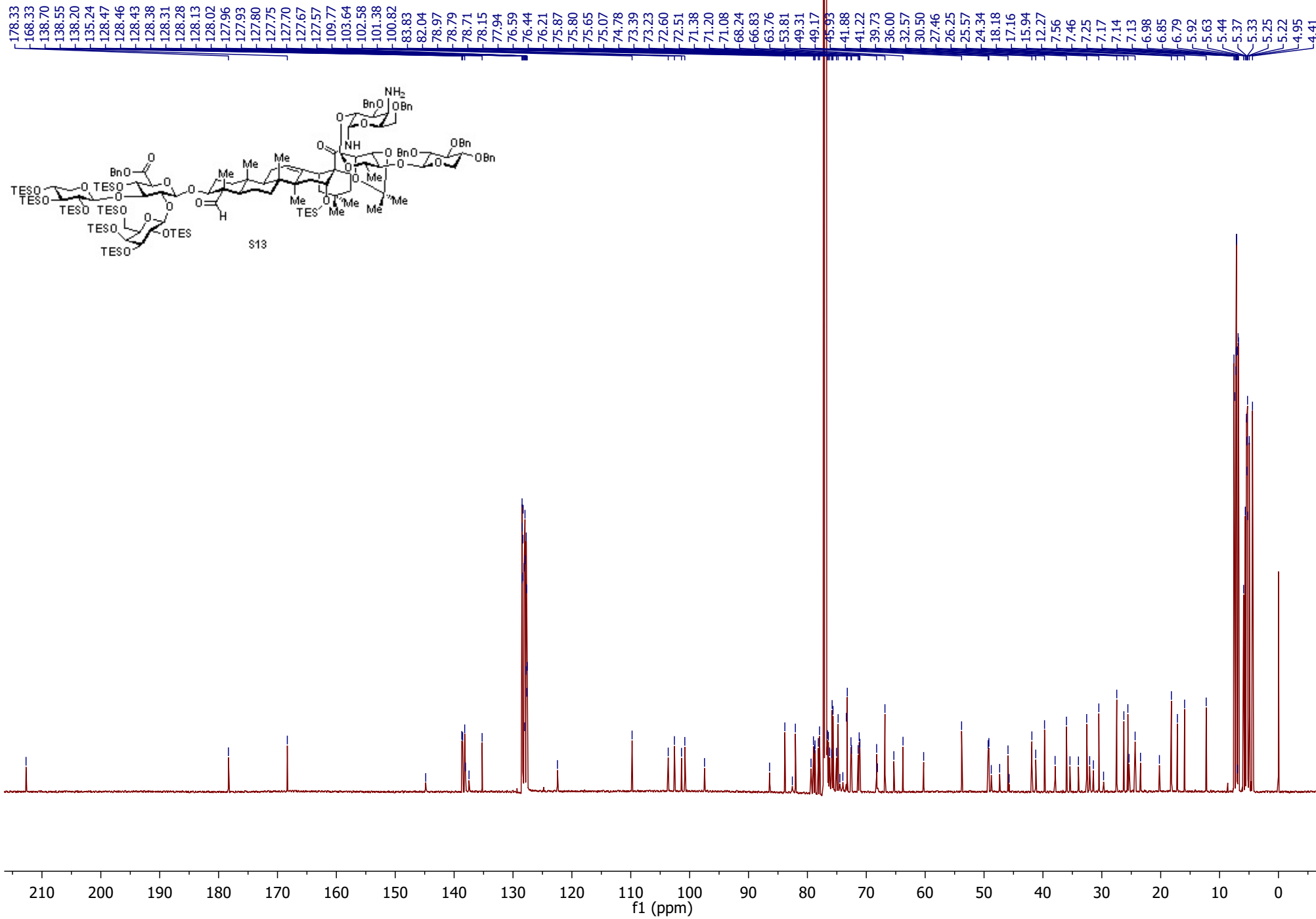


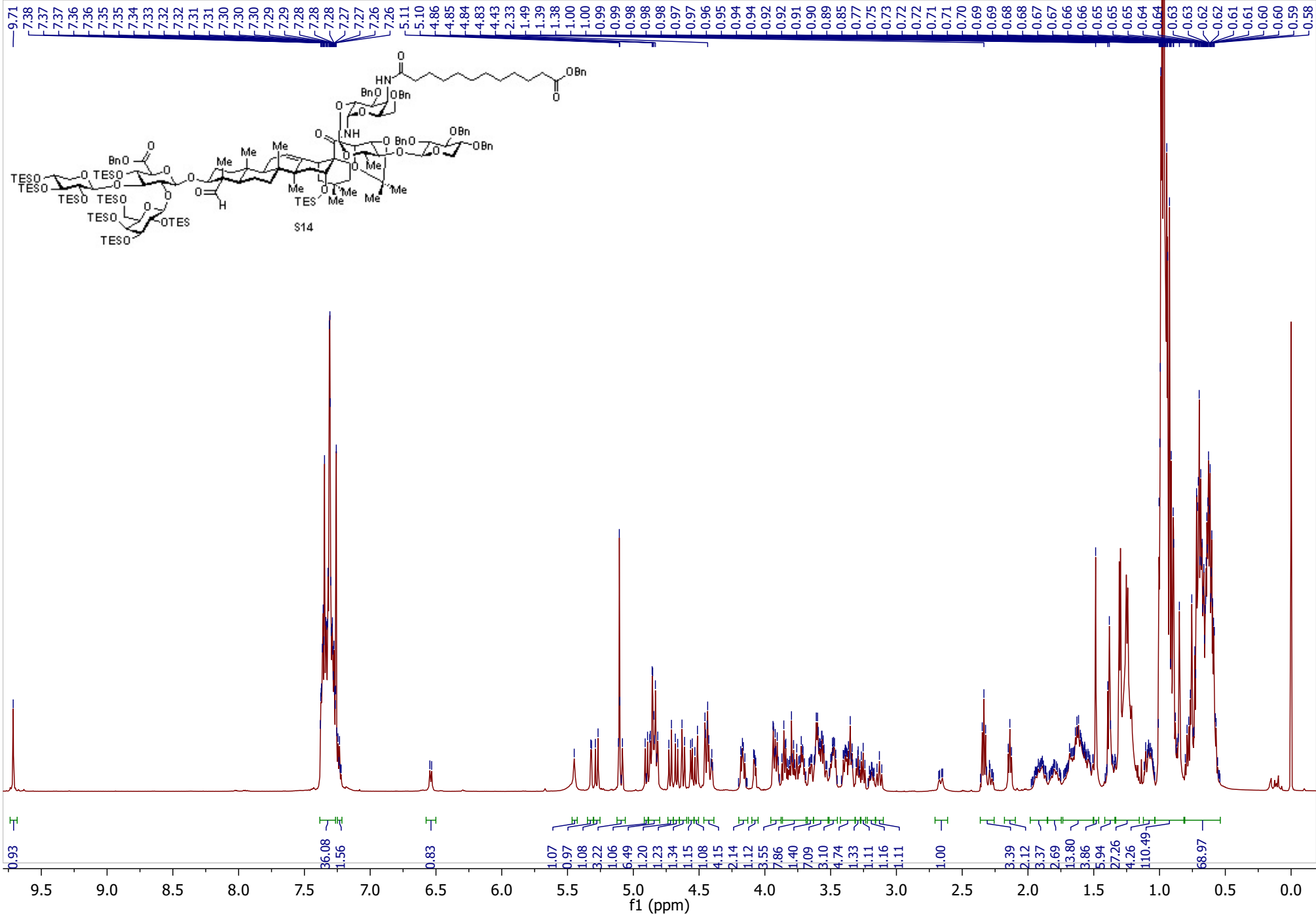


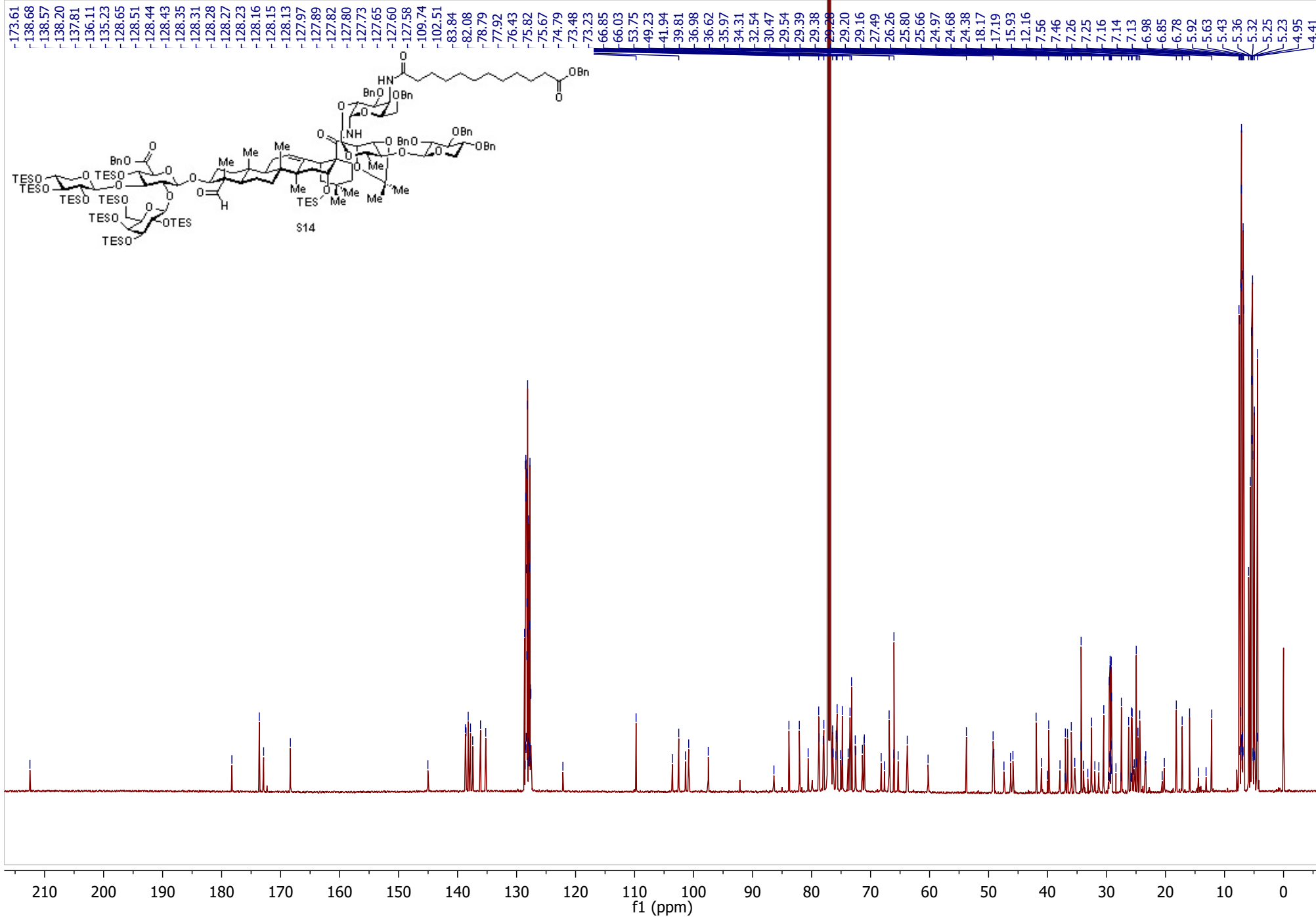


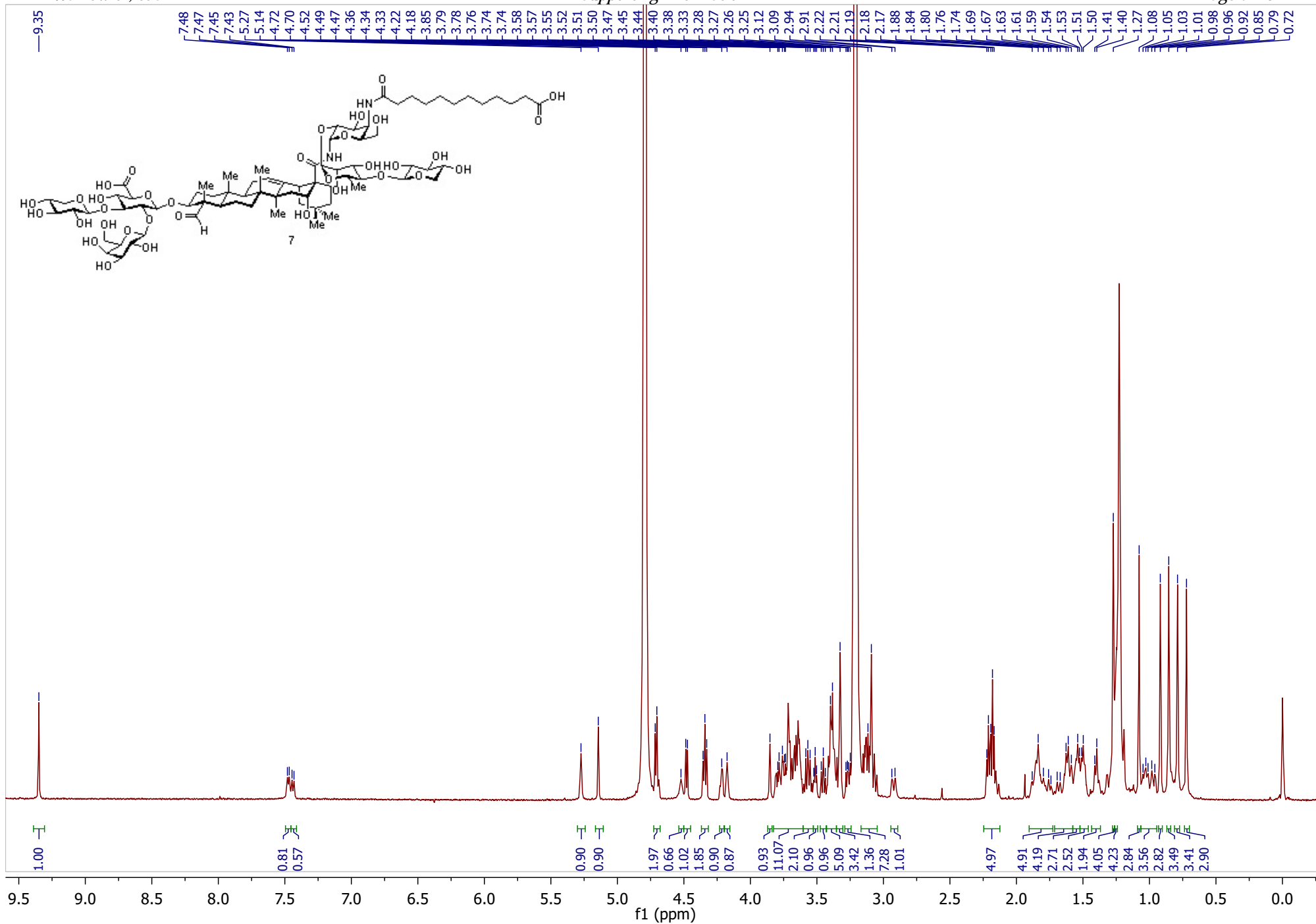


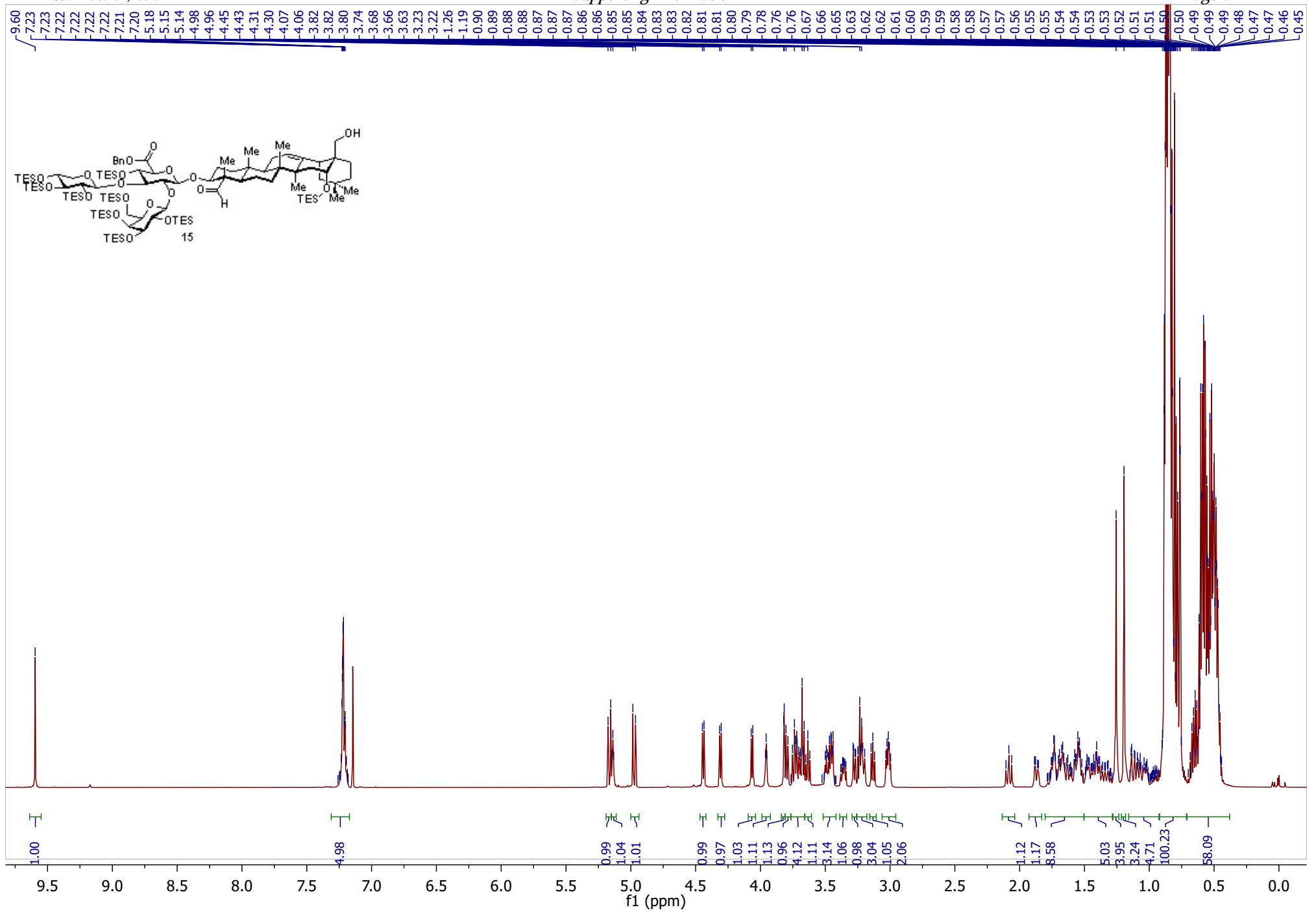


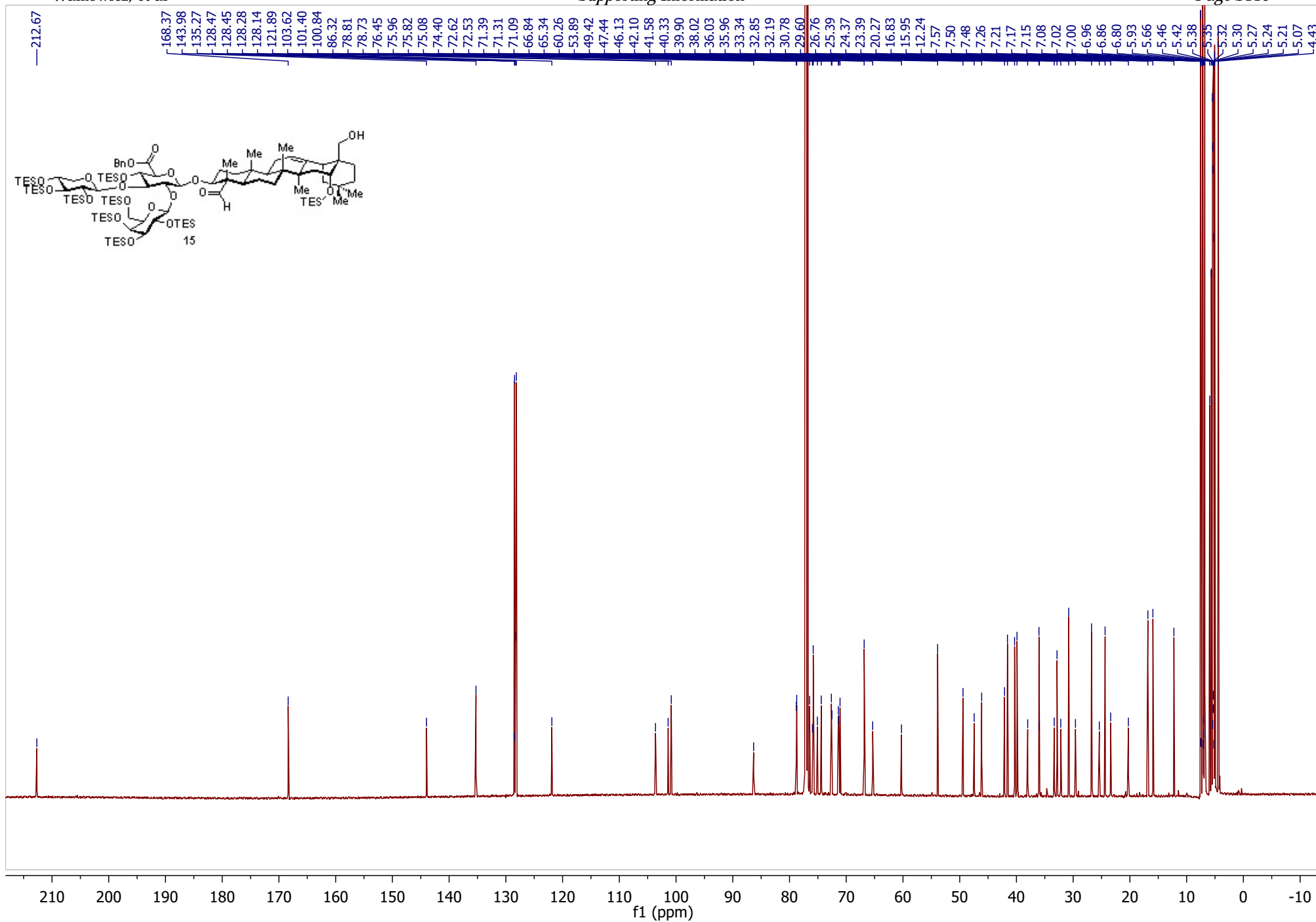


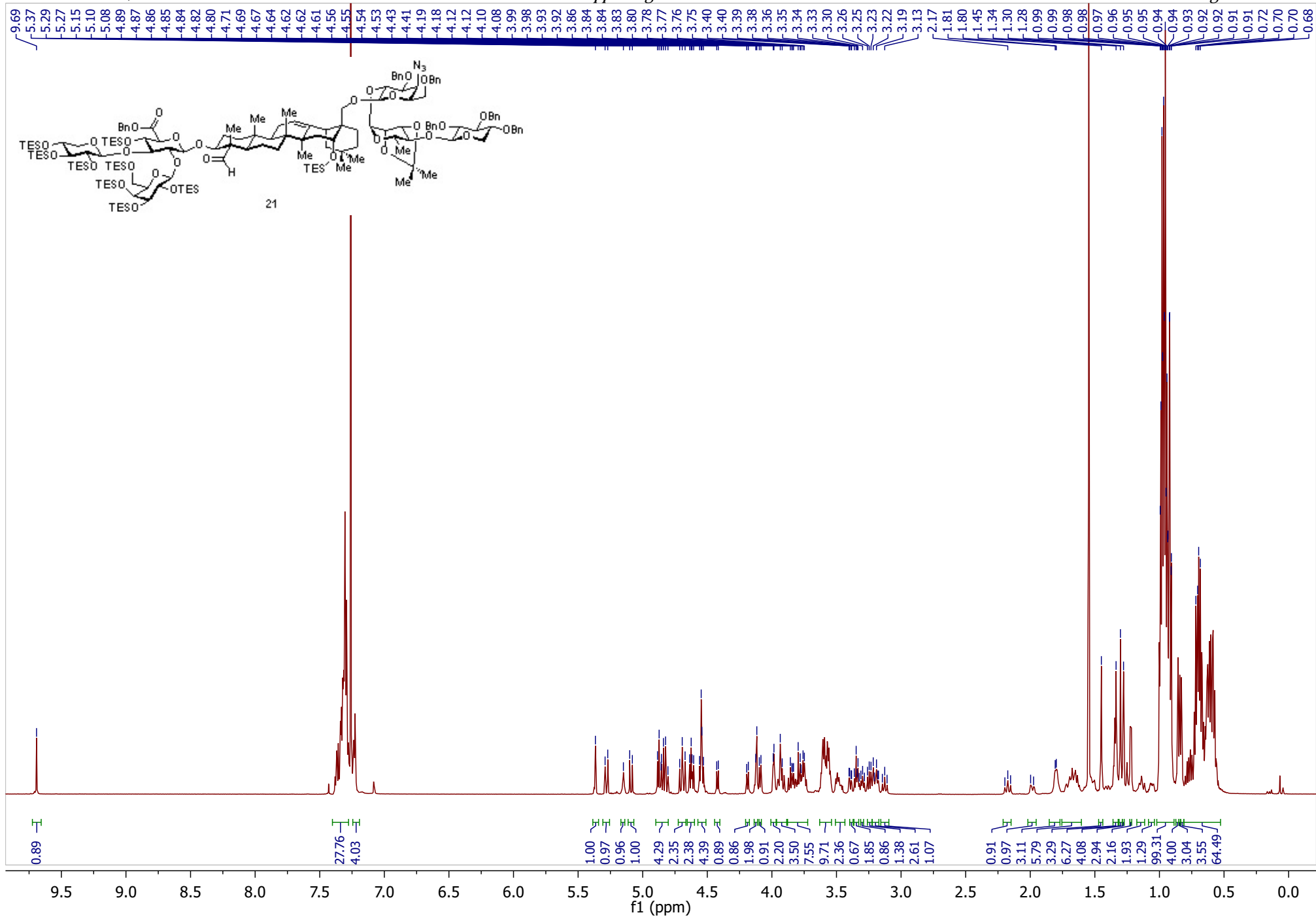


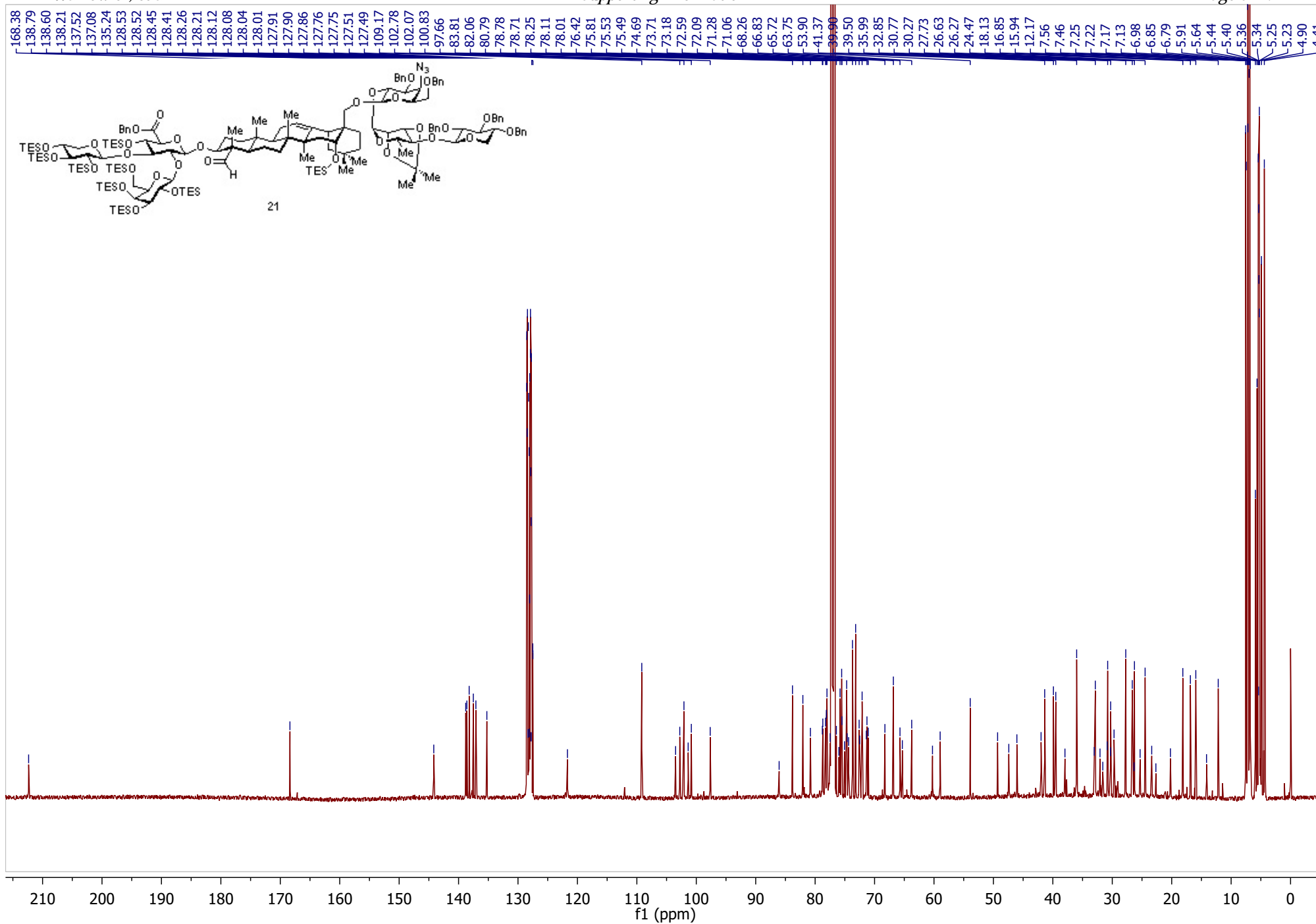


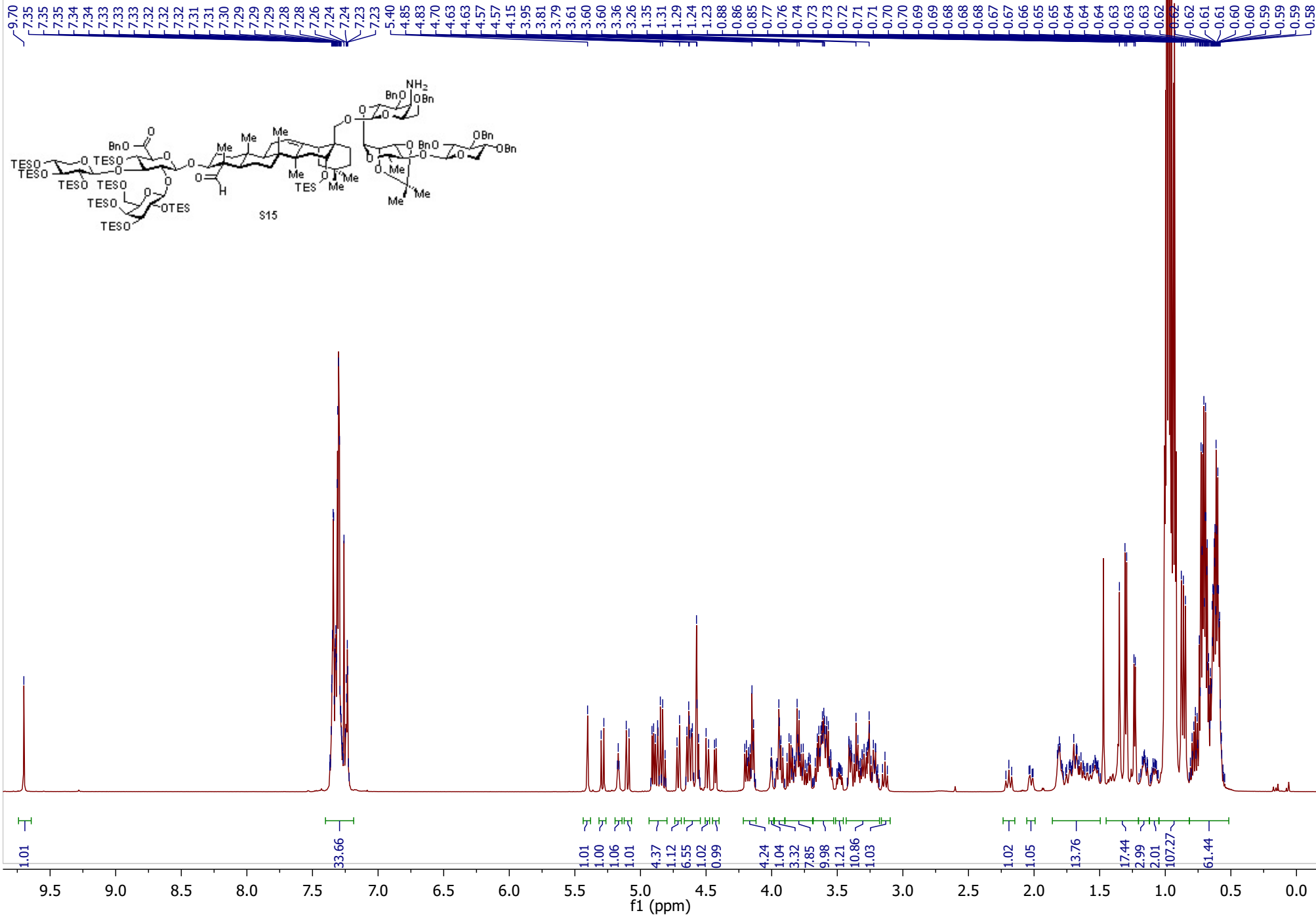


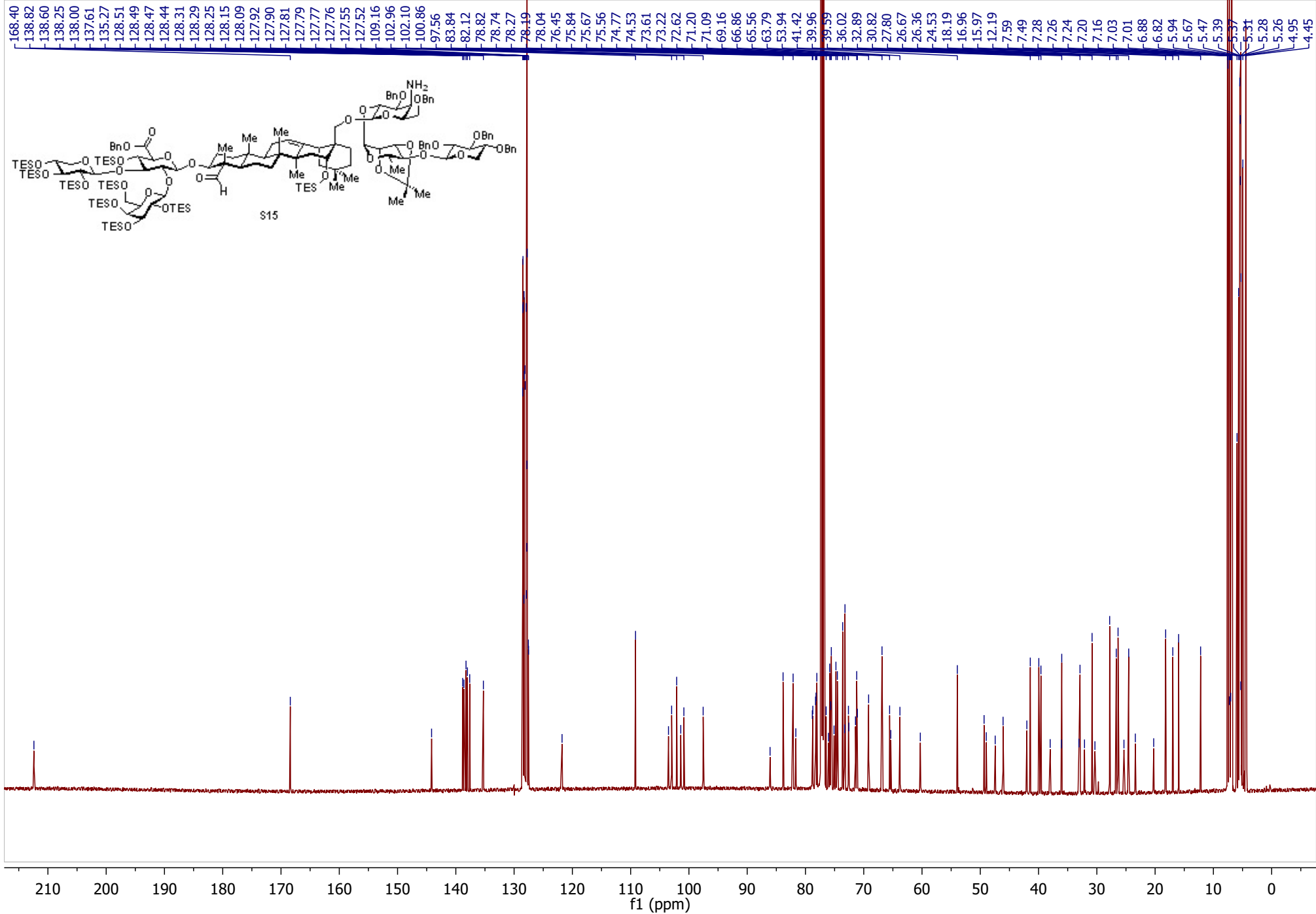


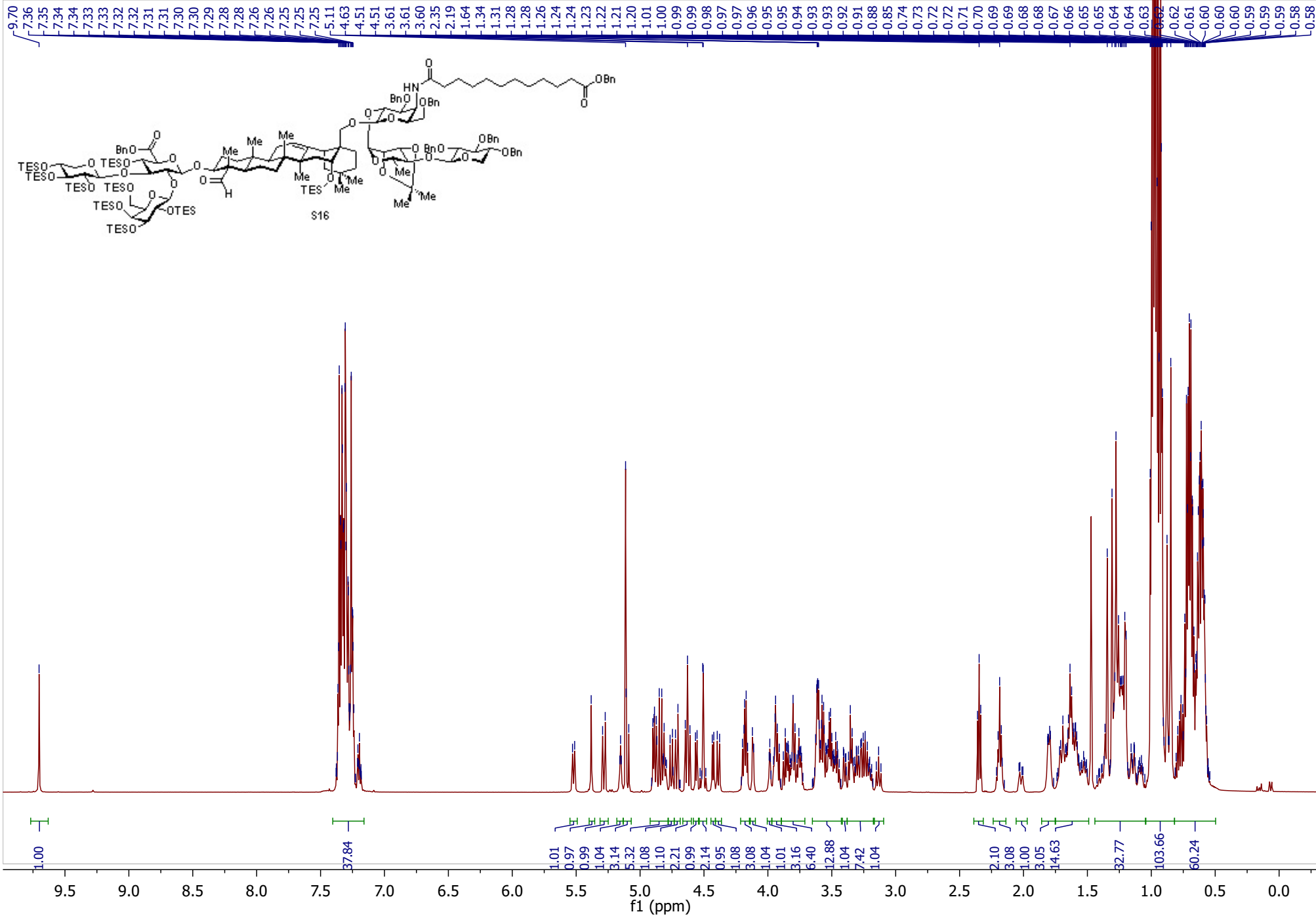


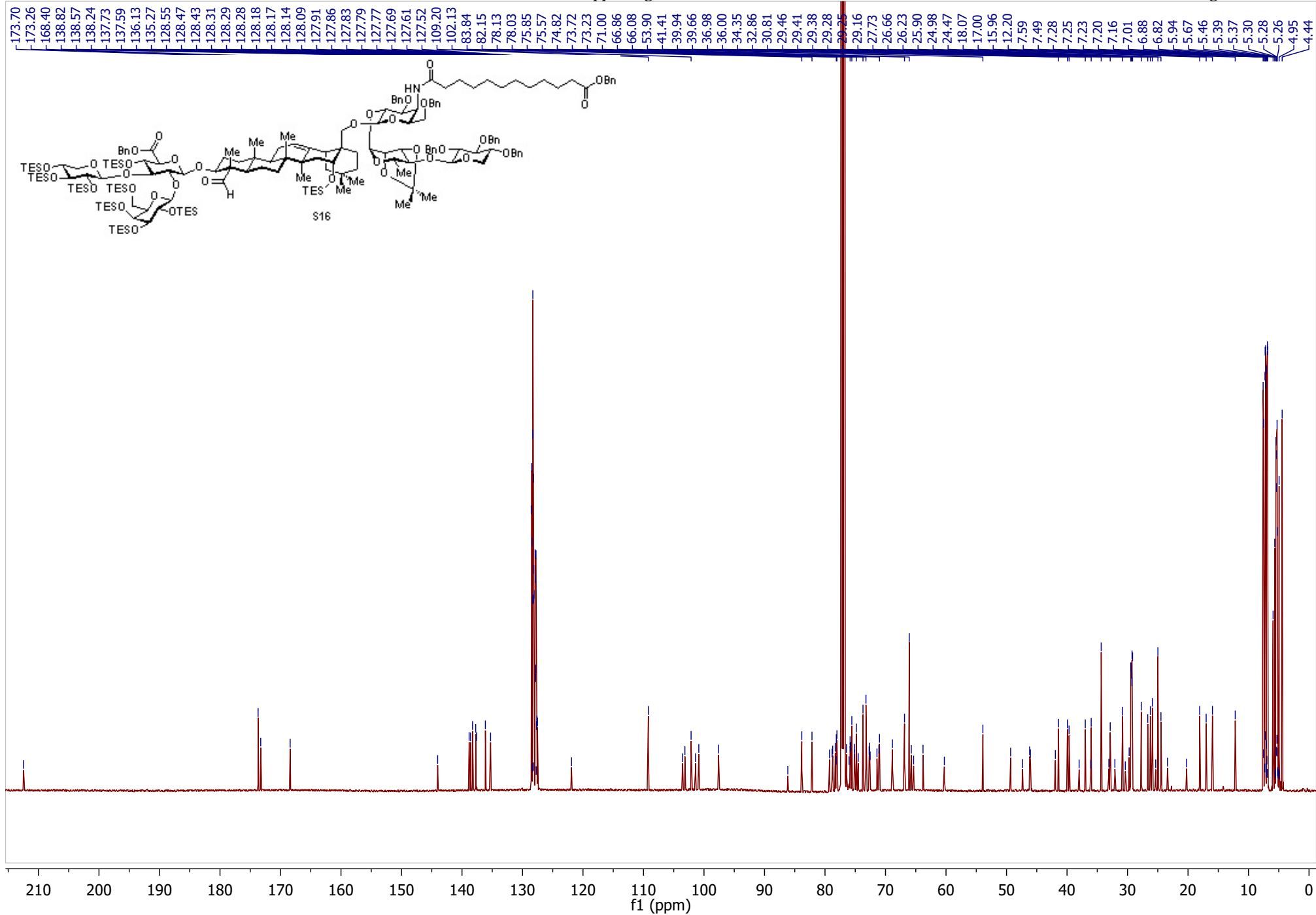


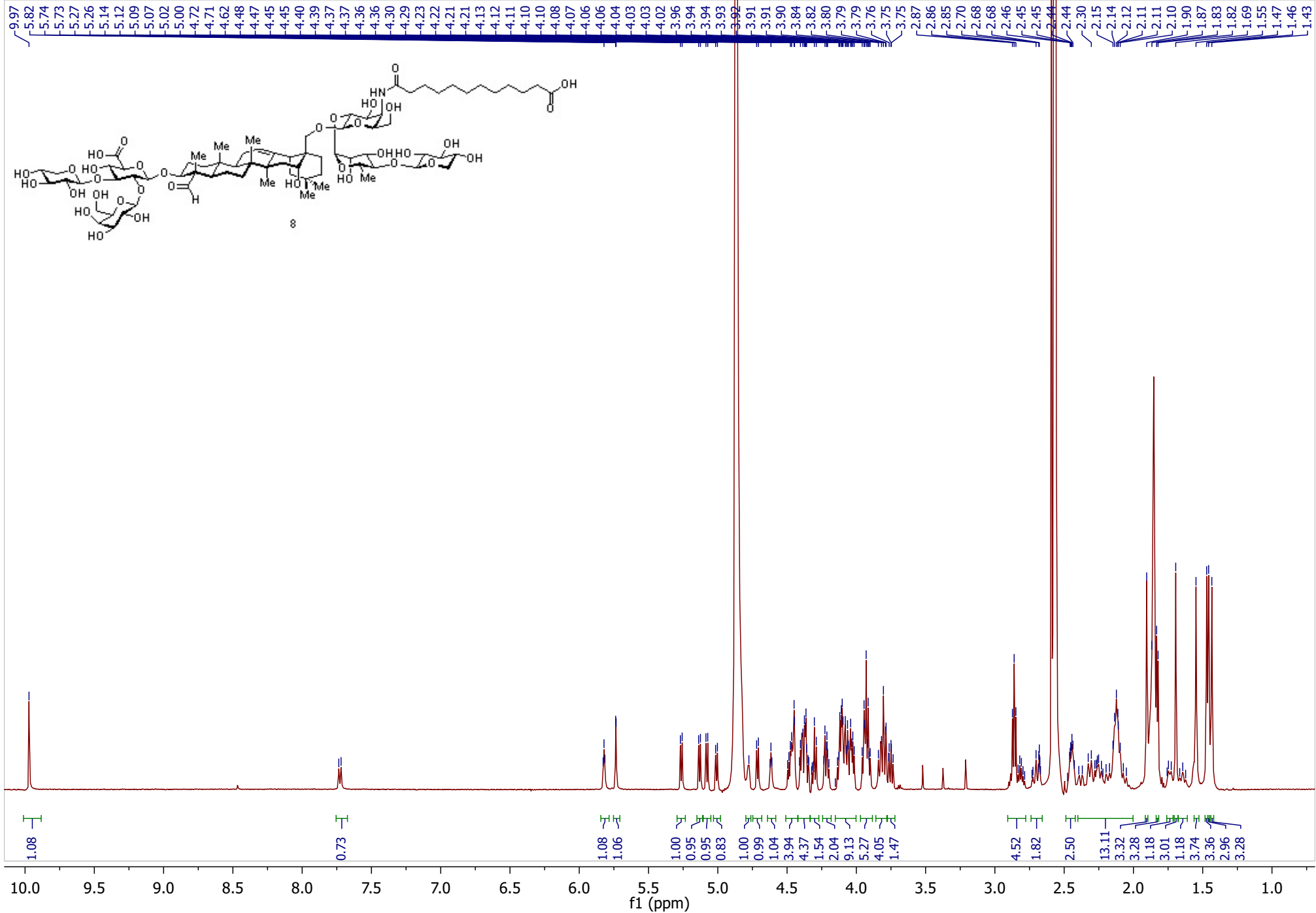


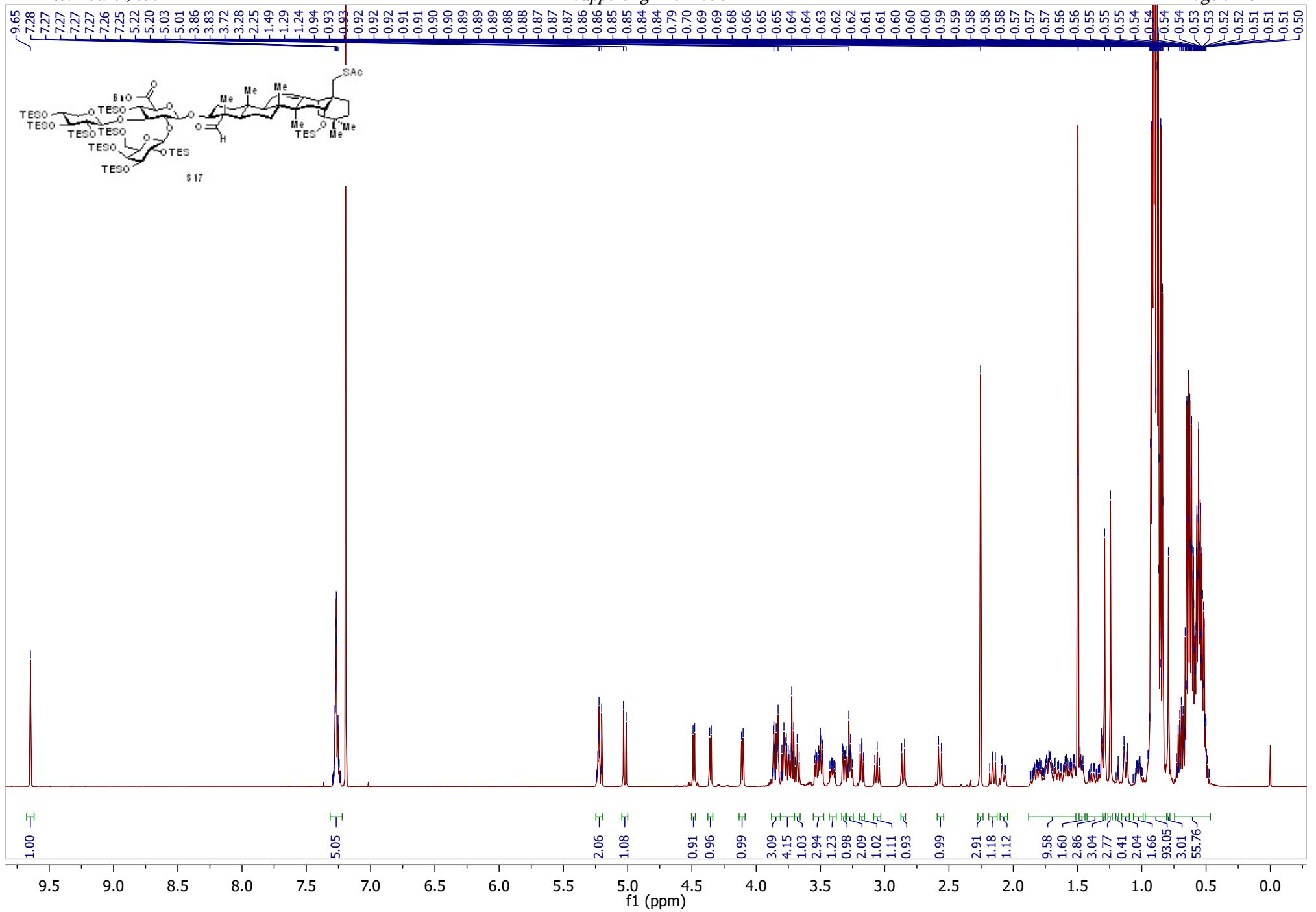


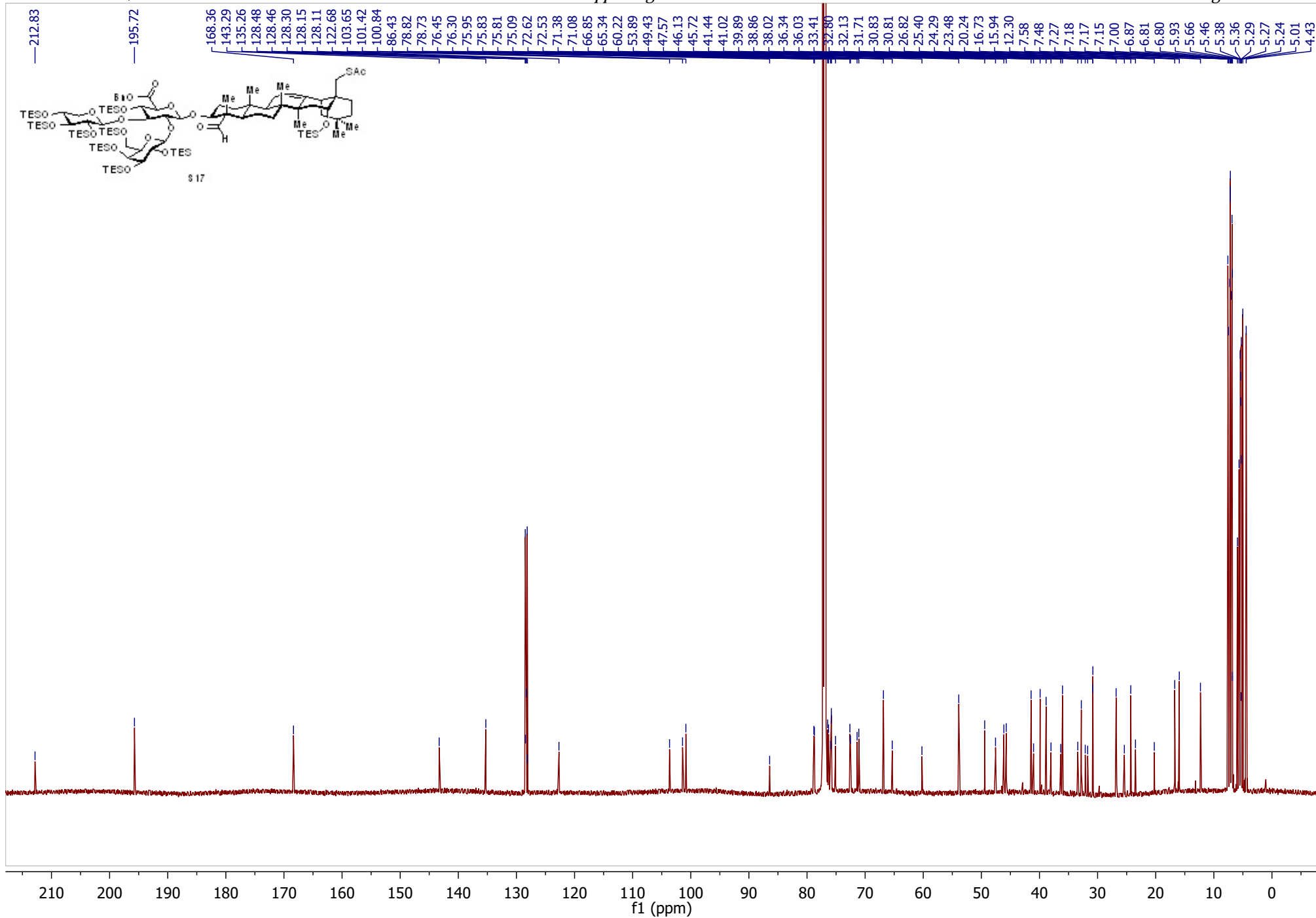


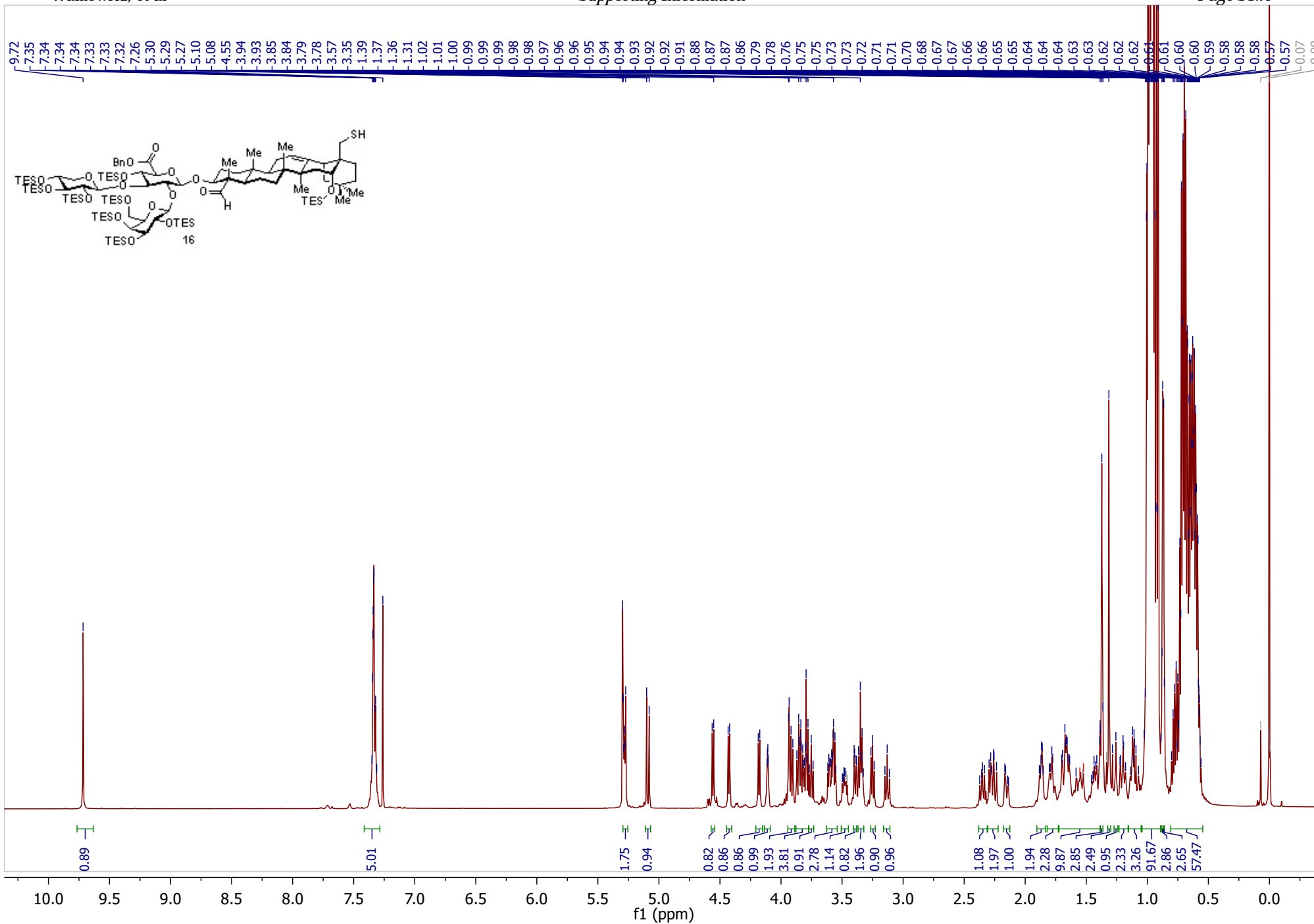


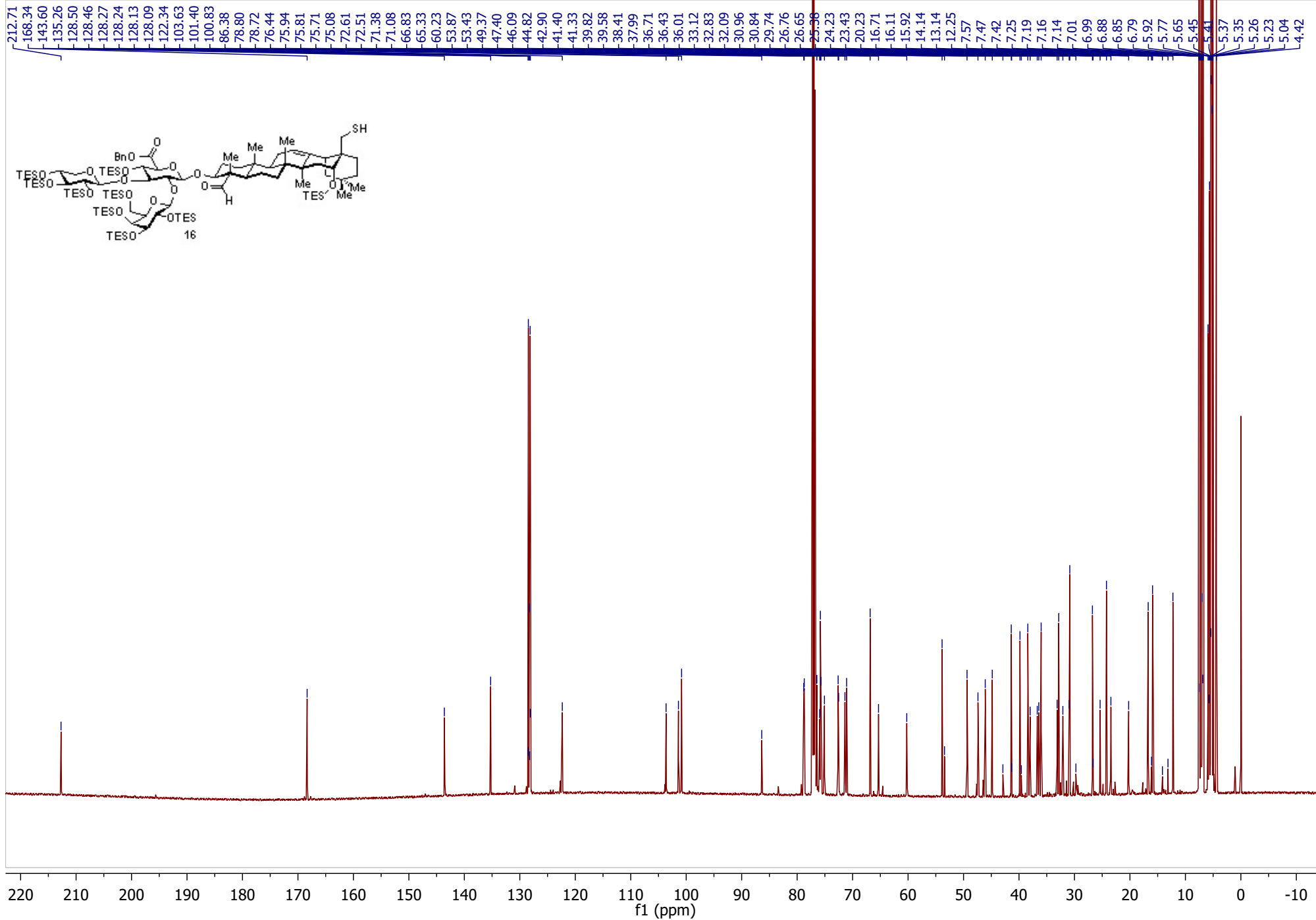


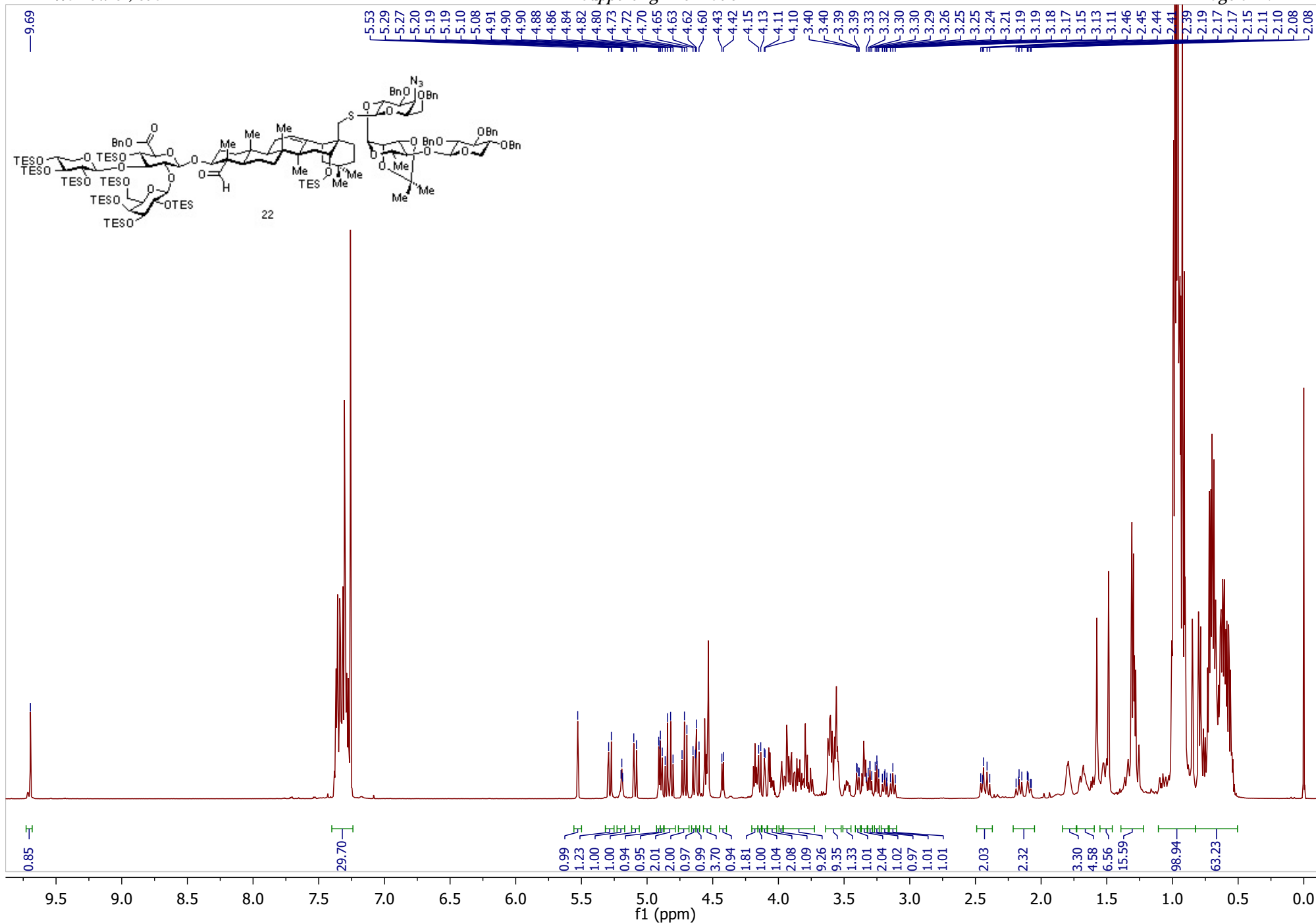


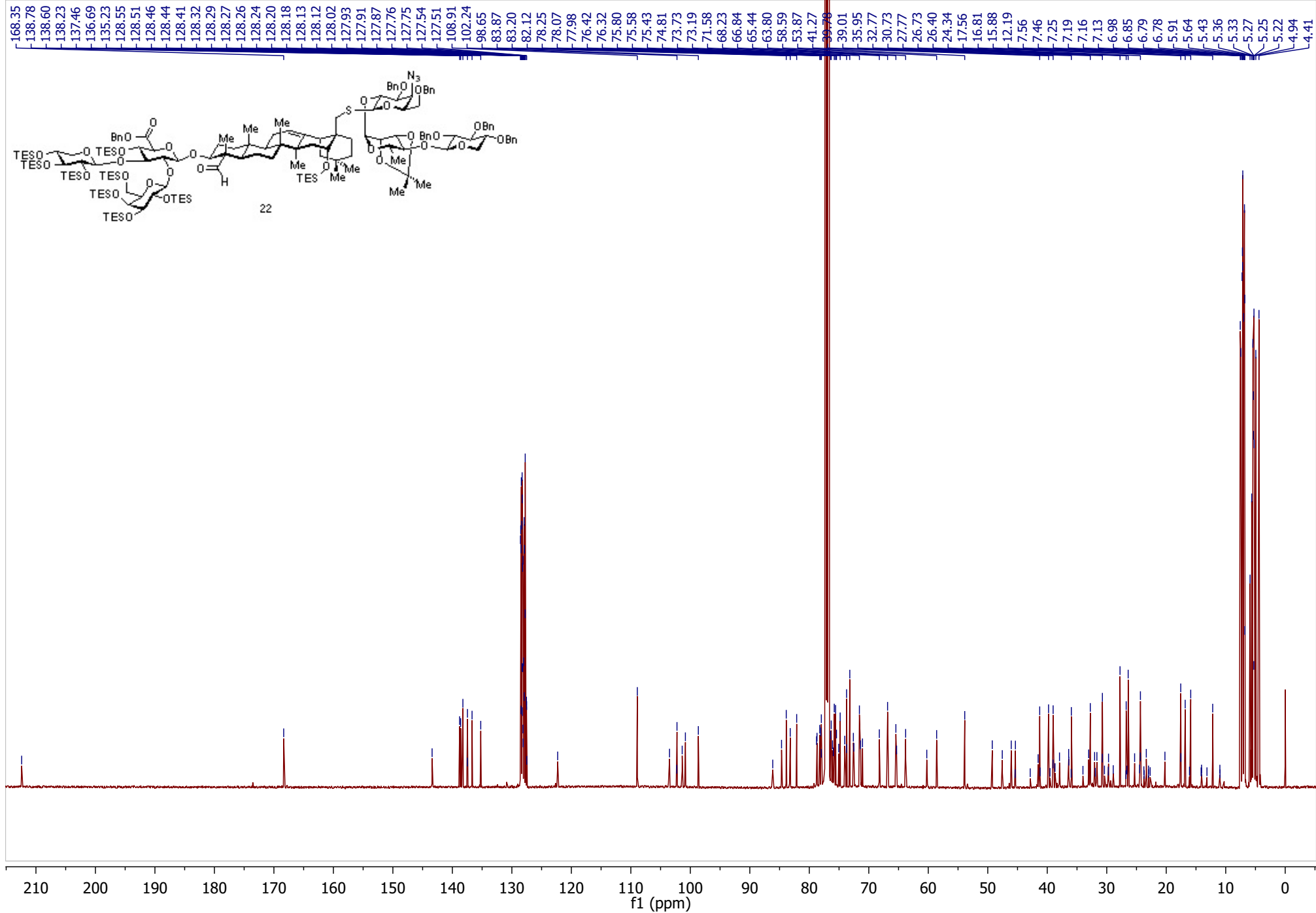


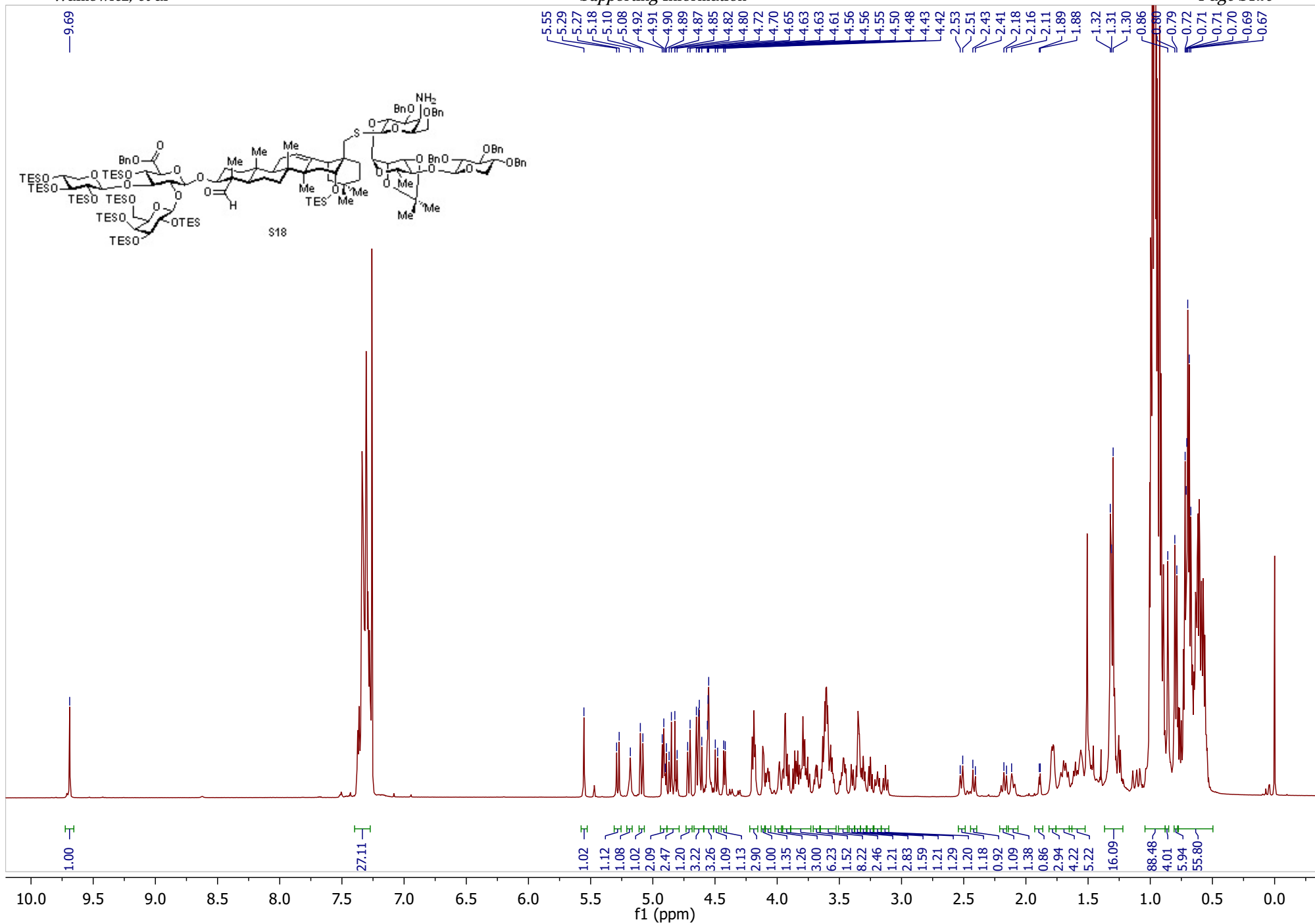


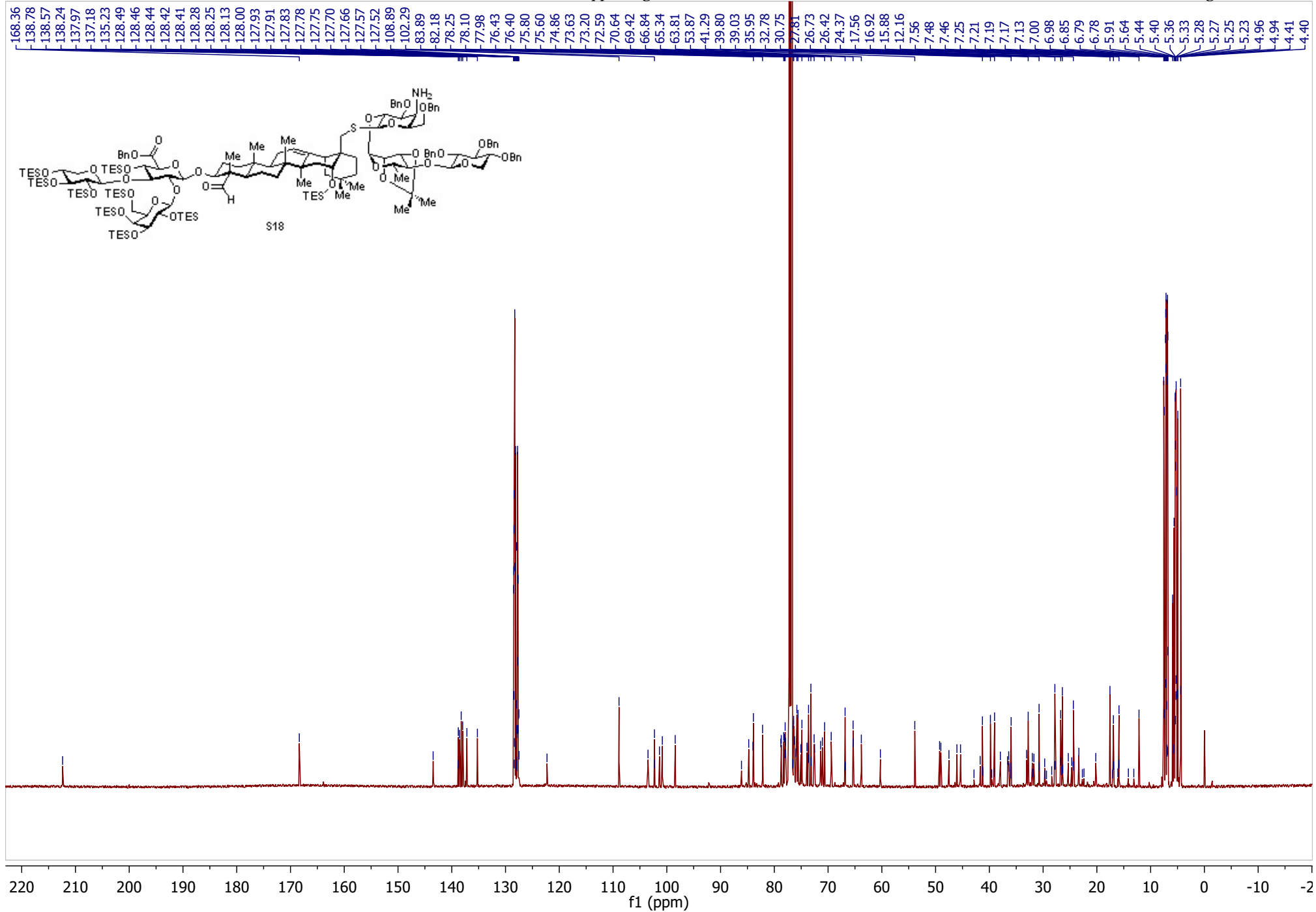


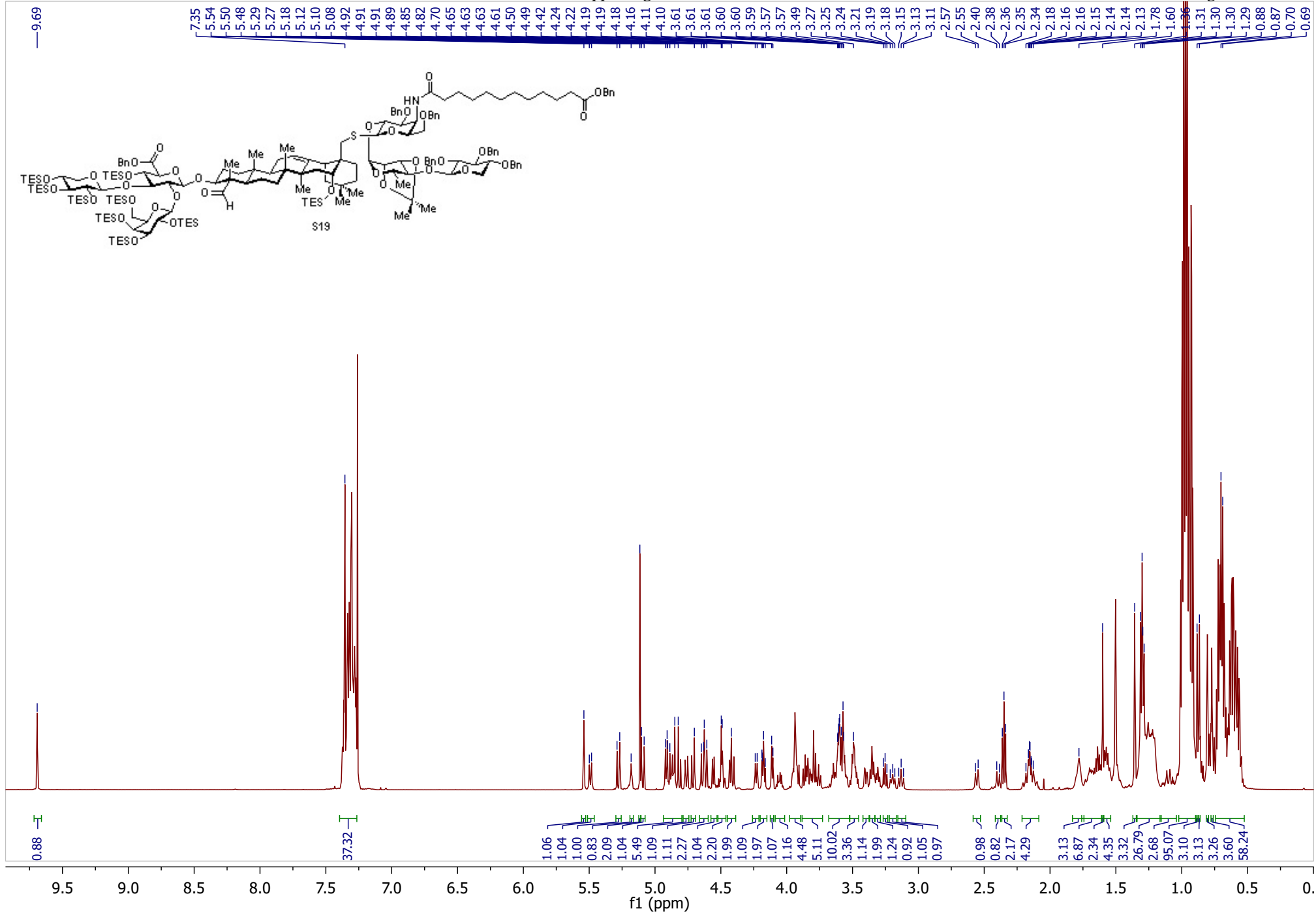


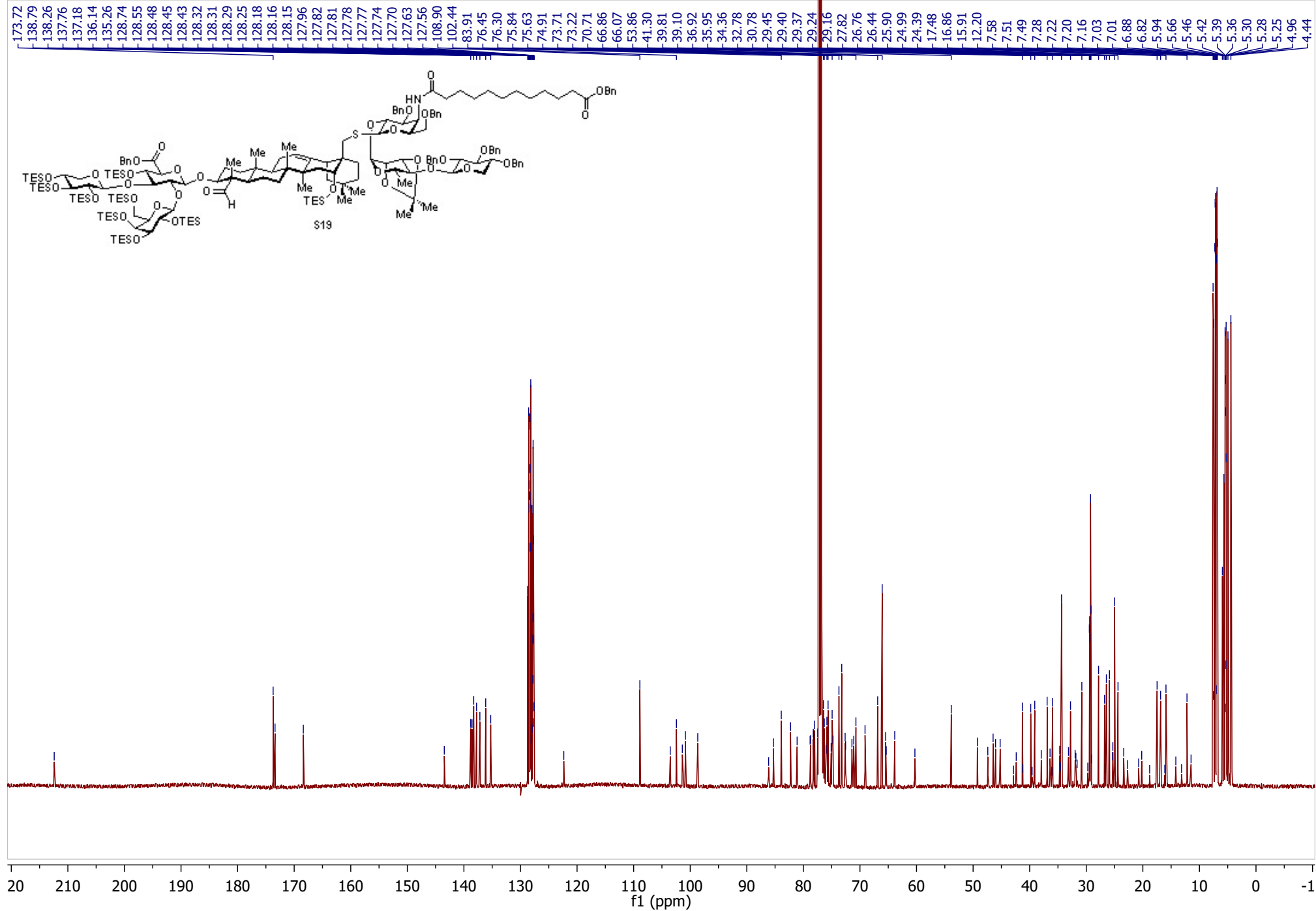


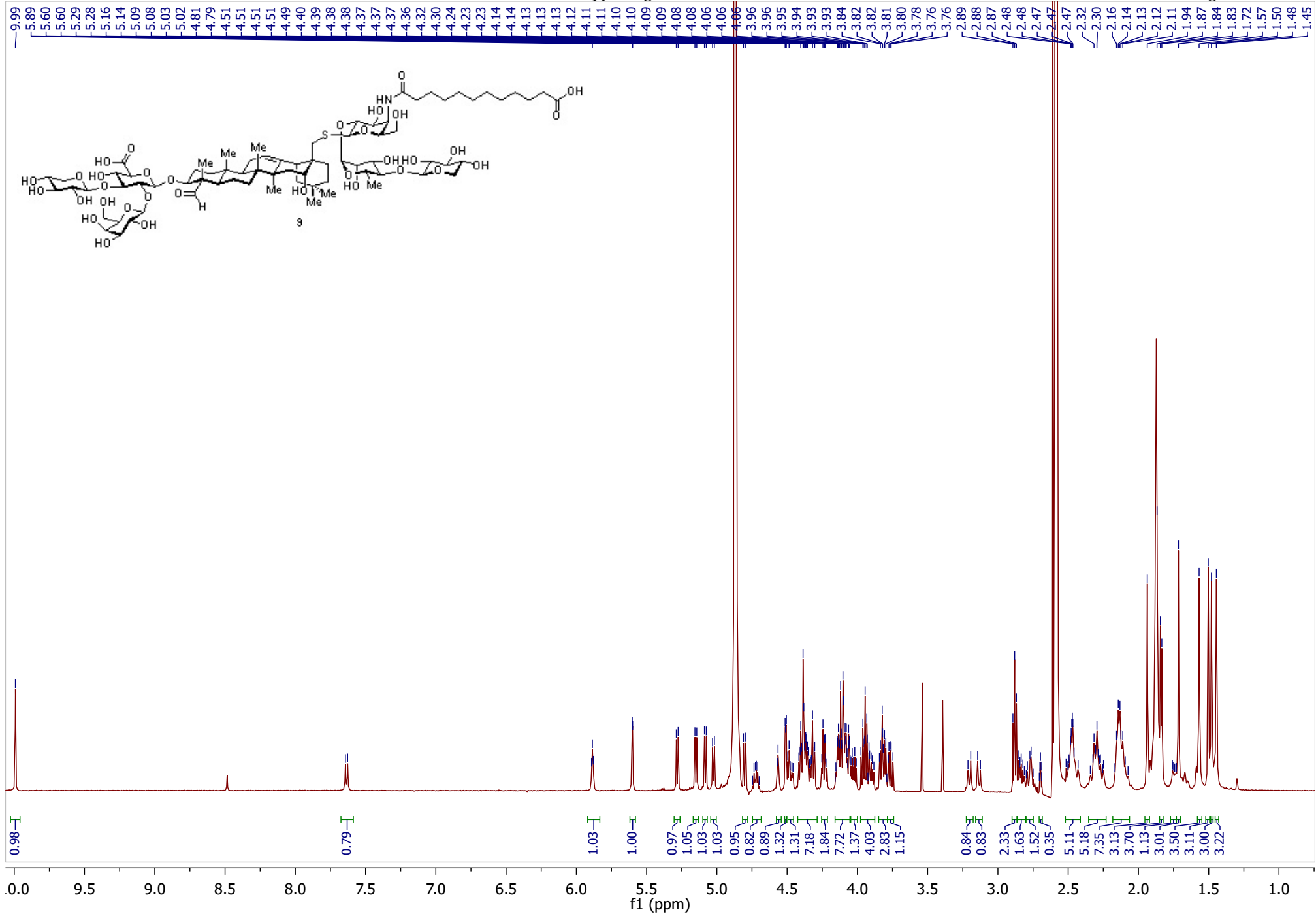












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