Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information

Improvement of the Immune Efficacy of Carbohydrate Vaccines by Chemical Modification on GM3 Antigen

Xiu-Jing Zheng, Fan Yang, Mingwei Zheng, Chang-Xin Huo, Ye Zhang, and Xin-Shan Ye*

State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Xue Yuan Rd #38, Beijing 100191, China

E-mail: xinshan@bjmu.edu.cn

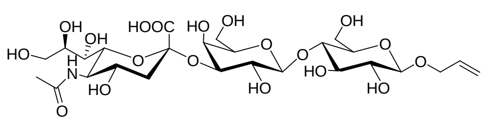
Table of Contents

1. Supplementary Methods and Results on Chemical Synthesis

1.1. General information

Unless otherwise noted, all reactions were carried out in oven-dried glassware under an atmosphere of argon or nitrogen. Acetonitrile and dichloromethane were distilled from calcium hydride. Methanol was dried by refluxing with magnesium and then distilled. *N*, *N*-Dimethylformamide was dried over P_2O_5 and distilled under vacuum. Reactions were monitored by analytical thin-layer chromatography (TLC) on Merck silica gel $60F_{254}$ plates (0.25 mm), visualized by ultraviolet light and/ or by staining with ceric ammonium molybdate or ninhydrin. ^{1}H NMR spectra were obtained on Varian INOVA-500 or JEOL JNM-AL300 spectrometer at ambient temperature. Data were reported as follows: chemical shift on the δ scale (using either TMS or residual proton solvent as internal standard), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, and coupling constant(s) in hertz. ^{13}C NMR spectra were obtained with proton decoupling on a Varian INOVA-500 (125 MHz) and JEOL JNM-AL-300 (75 MHz) spectrometer and were reported in ppm with residual solvent for internal standard (77.0 for $CDCl_3$). High resolution spectra were obtained on a PE SCLEX QSTAR spectrometer.

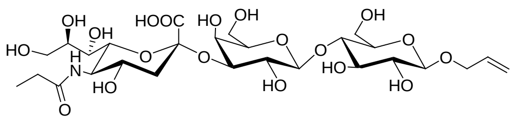
1.2. Compound characterization

Compound **2**[1] 

To a stirred solution of **22** (178.0 mg, 0.167 mmol) in methanol (10 mL) was added a sodium methoxide solution in methanol (30%, 0.02 g, 0.11 mmol). The mixture was stirred at r.t. for 4 h. The solvent was concentrated in vacuum, and then an aqueous NaOH solution (1 N, 3 mL) was added. After the reaction finished within 12-24 h, as indicated by TLC, the mixture was neutralized with 1N HCl in methanol to PH = 6-8. The reaction mixture was concentrated in vacuum, and then purified on a Biogel P-2 column with H_2O as the eluent to afford **2** (105.0 mg, yield = 93%). $[\alpha]_D$ = -7.97 (*c* = 1.1, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.03-5.93 (m, 1H), 5.38 (dq, 1H, *J* = 1.5 Hz, 17.5 Hz), 5.28 (dd, 1H, J_1 = 1.0 Hz, J_2 = 10.5 Hz), 4.52 (d, 1H, *J* = 8.5 Hz, anomeric H), 4.51 (d, 1H *J* = 7.0 Hz, anomeric H), 4.39 (ddt, 1H, J_1 = J_2 = 1.0 Hz, J_3 = 5.5 Hz, J_4 = 13.0 Hz), 4.22 (ddt, 1H, J_1 = J_2 = 1.5 Hz, J_3 = 6.5

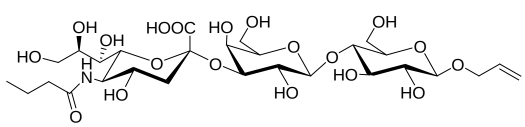
Hz, J_4 = 13.0 Hz), 4.11 (dd, 1H, J_1 = 5.0 Hz, J_2 = 3.5 Hz), 3.97 (m, 2H), 3.92-3.54 (m, 15H), 3.33 (dd, 1H, J_1 = 8.0 Hz, J_2 = 9.0 Hz), 2.75 (dd, 1H, J_1 = 4.5 Hz, J_2 = 12.5 Hz, sialH-3eq), 2.02 (s, 3H), 1.80 (t, 1H, J_1 = J_2 = 12.0 Hz, sialH-3ax); ^{13}C-NMR (125 MHz, D_2O) δ 177.64, 176.53, 135.90, 121.42, 105.27, 103.67, 102.43, 80.85, 78.11, 77.79, 77.40, 77.03, 75.50, 75.44, 74.41, 73.30, 72.00, 70.99, 70.72, 70.10, 65.20, 63.66, 62.69, 54.30, 42.26, 24.68; HRMS (*m/z*): $[M+H]^+$ calcd. for $[C_{26}H_{44}NO_{19}]^+$, 674.2502; found, 674.2505.

Compound **3**

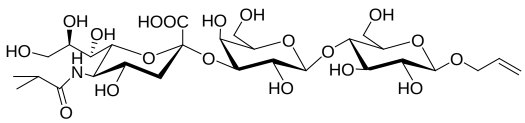


Yield = 90%; $[\alpha]_D$ = -8.04 (*c* = 0.9, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.02-5.93 (m, 1H), 5.38 (m, 1H), 5.28 (m, 1H), 4.52 (d, 1H, *J* = 8.0 Hz, anomeric H), 4.51 (d, 1H, *J* = 8.0 Hz, anomeric H), 4.39 (m, 1H), 4.22 (dd, 1H, J_1 = 6.5 Hz, J_2 = 12.5 Hz), 4.11 (dd, 1H, J_1 = 11.0 Hz, J_2 = 2.0 Hz), 3.98 (dd, 1H, J_1 = 12.0 Hz, J_2 = 2.0 Hz), 3.95 (d, 1H, *J* = 3.5 Hz), 3.89-3.54 (m, 15H), 3.32 (t, 1H, J_1 = J_2 = 9.0 Hz), 2.75 (dd, 1H, J_1 = 4.5 Hz, J_2 = 12.5 Hz, sialH-3eq), 2.29 (q, 2H, *J* = 7.5 Hz), 1.85 (t, 1H, J_1 = J_2 = 12.5 Hz, sialH-3ax), 1.11 (t, 3H, *J* = 7.5 Hz); ^{13}C-NMR (125 MHz, D_2O) δ 179.73, 173.52, 134.01, 119.50, 103.53, 101.79, 99.96, 78.99, 76.20, 75.81, 75.49, 75.14, 73.78, 73.55, 72.10, 71.40, 70.10, 68.91, 68.56, 68.30, 63.49, 61.68, 60.80, 52.26, 40.07, 29.98, 10.22; HRMS (*m/z*): $[M+NH_4]^+$ calcd. for $[C_{27}H_{49}N_2O_{19}]^+$, 705.2924; found, 705.2935.

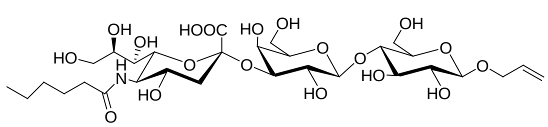
Compound **4**



Yield = 84%; $[\alpha]_D$ = -6.26 (*c* = 0.8, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.02-5.93 (m, 1H), 5.38 (dq, 1H, *J* = 1.5 Hz, 17.5 Hz), 5.28 (m, 1H), 4.52 (d, 1H, *J* = 7.5 Hz, anomeric H), 4.51 (d, 1H, *J* = 7.5 Hz, anomeric H), 4.39 (ddt, 1H, J_1 = J_2 = 1.5 Hz, J_3 = 5.5 Hz, J_4 = J_3 = 12.5 Hz), 4.22 (ddt, 1H, J_1 = J_2 = 1.0 Hz, J_3 = 7.5 Hz, J_4 = 12.5 Hz), 4.11 (dd, 1H, J_1 = 10.0 Hz, J_2 = 3.0 Hz), 3.98 (dd, 1H, J_1 = 12.0 Hz, J_2 = 2.0 Hz), 3.95 (d, 1H, *J* = 3.0 Hz), 3.92-3.78 (m, 4H), 3.78-3.54 (m, 11H), 3.32 (dd, 1H, J_1 = 8.0 Hz, J_2 = 9.0 Hz), 2.75 (dd, 1H, J_1 = 4.0 Hz, J_2 = 12.0 Hz, sialH-3eq), 2.26 (t, 2H, J_1 = J_2 = 7.5 Hz), 1.80 (t, 1H, $J_1 = J_2$ = 12.0 Hz, sialH-3ax), 1.60 (hexad, 2H, J = 7.5 Hz), 0.90 (t, 3H, $J_1 = J_2$ = 7.5 Hz); ^{13}C-NMR (125 MHz, D_2O) δ 178.88, 174.6, 134.02, 119.49, 103.38, 101.79, 100.53, 78.99, 76.22, 75.91, 75.50, 75.14, 73.65, 73.55, 72.50, 71.40, 70.11, 68.96, 68.93, 68.19, 63.39, 61.76, 60.81, 52.33, 40.53, 38.549, 19.74, 13.56; HRMS (*m/z*): $[M+Na]^+$ calcd. for $[C_{28}H_{47}NNaO_{19}]^+$, 724.2634; found, 724.2637.


Compound **5**

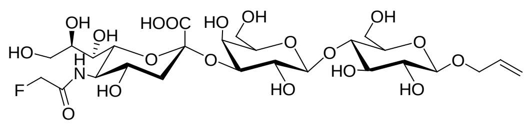
Yield = 90%; $[\alpha]_D$ = -8.11 (c = 1.0, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.02-5.93 (m, 1H), 5.38 (dd, 1H, J_1 = 1.5 Hz, J_2 = 17.5 Hz), 5.28 (dd, 1H, J_1 = 1.0 Hz, J_2 = 10.5 Hz), 4.53 (d, 1H, J = 8.0 Hz, anomeric H), 4.52 (d, 1H, J = 8.0 Hz, anomeric H), 4.39 (dd, 1H, J_1 = 5.5 Hz, J_2 = 12.5 Hz), 4.22 (dd, 1H, J_1 = 6 Hz, J_2 = 12.5 Hz), 4.11 (dd, 1H, J_1 = 10.0 Hz, J_2 = 3.0 Hz), 3.98 (dd, 1H, J_1 = 12.5 Hz, J_2 = 2.0 Hz), 3.95 (d, 1H, J = 3.5 Hz), 3.94-3.50 (m, 15H), 3.32 (t, 1H, $J_1 = J_2$ = 9.0 Hz), 2.75 (t, 1H, J_1 = 5.0 Hz, J_2 = 12.0 Hz, sialH-3eq), 2.54 (heptad, 1H, J = 7.0 Hz), 1.80 (t, 1H, $J_1 = J_2$ = 12.0 Hz, sialH-3ax), 1.12 (d, 3H, J = 7.0 Hz), 1.10 (d, 3H, J = 7.5 Hz); ^{13}C-NMR (125 MHz, D_2O) δ 182.92, 174.64, 134.01, 119.50, 103.39, 101.79, 100.52, 78.99, 76.22, 75.91, 75.50, 75.14, 73.66, 73.54, 72.50, 71.40, 70.10, 68.95, 68.81, 68.19, 63.26, 61.76, 60.80, 52.19, 40.55, 35.95, 19.69, 19.16; HRMS (*m/z*): $[M+K]^+$ calcd. for $[C_{28}H_{47}NKO_{19}]^+$, 740.2374; found, 740.2340.


Compound **6**

Yield = 86%; $[\alpha]_D$ = -7.91 (c = 1.0, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.02-5.93 (m, 1H), 5.38 (dd, 1H, J_1 = 1.5 Hz, J_2 = 17.0 Hz), 5.28 (d, 1H, J = 10.5 Hz), 4.53 (d, 1H, J = 8.0 Hz, anomeric H), 4.53 (d, 1H, J = 8.0 Hz, anomeric H), 4.86 (dd, 1H, J_1 = 6.0 Hz, J_2 = 13.0 Hz), 4.22 (dd, 1H, J_1 = 6.5 Hz, J_2 = 13.0 Hz,), 4.11 (dd, 1H, J_1 = 3.5 Hz, J_2 = 10.0 Hz), 3.98 (d, 1H, J = 10.0 Hz), 3.95 (d, 1H, J = 3.5 Hz), 3.90-3.55 (m, 15H), 3.33 (dd, 1H, J_1 = 8.0 Hz, J_2 = 9.0 Hz), 2.76 (dd, 1H, J_1 = 4.5 Hz, J_2 = 12.5

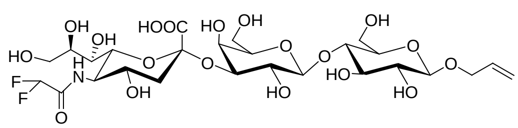
Hz, sialH-3eq), 2.27 (t, 2H, $J_1 = J_2 = 7.0$ Hz), 1.81 (t, 1H, $J_1 = J_2 = 12.0$ Hz, sialH-3ax), 1.59 (pentad, 2H, $J = 7.0$ Hz), 1.32-1.23 (m, 4H), 0.86 (t, 3H, $J_1 = J_2 = 7.0$ Hz)； ^{13}C-NMR (125 MHz, D_2O) δ 178.96, 174.32, 133.81, 119.34, 103.20, 101.60, 100.27, 78.77, 76.04, 75.72, 75.33, 74.96, 73.51, 73.36, 72.30, 71.24, 69.94, 68.83, 68.68, 68.02, 63.18, 61.59, 60.61, 52.13, 40.32, 36.46, 31.08, 25.65, 22.23, 13.77; HRMS (*m/z*): $[M+Na]^+$ calcd. for $[C_{30}H_{51}NNaO_{19}]^+$, 752.2947; found, 752.2940.

Compound **7**



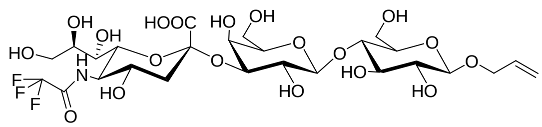
Yield = 75%; $[\alpha]_D$ = -8.64 (c = 0.9, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 5.89-5.82 (m, 1H), 5.26 (d, 1H, $J = 17.5$ Hz), 5.18 (d, 1H, $J = 10.5$ Hz), 4.80 (d, 2H, $J_{F\text{-}H} = 46.0$ Hz), 4.42 (d, 2H, $J = 8.0$ Hz, overlapped anomeric H)，4.28 (dd, 1H, $J_1 = 5.5$ Hz, $J_2 = 12.5$ Hz), 4.11 (dd, 1H, $J_1 = 6.5$ Hz, $J_2 = 12.5$ Hz), 4.01 (dd, 1H, $J_1 = 10.0$ Hz, $J_2 = 3.0$ Hz), 3.90-3.40 (m, 17H), 3.21 (t, 1H, $J_1 = J_2 = 8.5$ Hz), 2.66 (dd, 1H, $J_1 = 5.0$ Hz, $J_2 = 12.5$ Hz, sialH-3eq), 1.71 (dd, 1H, $J_1 = J_2 = 12.0$ Hz, sialH-3ax)； ^{13}C-NMR (125 MHz, D_2O) δ 174.47, 171.40 (d, 1C, $J_{F\text{-}C} = 18.5$ Hz), 133.33, 118.77, 109.68, 102.68, 101.09, 99.86, 80.0(d, 1C, $J_{F\text{-}C} = 180.6$ Hz), 78.32, 75.52, 75.19, 74.79, 74.43, 72.84, 72.50, 71.89, 70.69, 69.39, 68.20, 68.04, 67.48, 62.61, 61.04, 60.12, 51.33, 39.70; HRMS (*m/z*): $[M+Na]^+$ calcd. for $[C_{26}H_{42}FNNaO_{19}]^+$, 714.2227; found, 714.2251.

Compound **8**

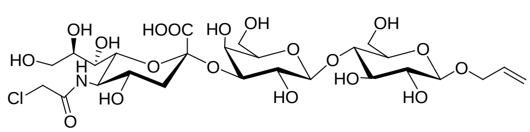


Yield = 86%; $[\alpha]_D$ = -8.85 (c = 1.2, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.16 (t, 1H, $J_{F\text{-}H} =$ 54.0 Hz), 6.01-5.93 (m, 1H), 5.38 (dt, 1H, J = 1.5 Hz, 17.5 Hz), 5.28 (dq, 1H, J = 1.0 Hz, 10.5 Hz), 4.52 (d, 1H, $J = 8.5$ Hz, anomeric H), 4.51 (d, 1H, $J = 7.0$ Hz, anomeric H), 4.39 (ddt, 1H, $J_1 = J_2 = 1.0$ Hz, $J_3 = 5.5$ Hz, $J_4 = 13.0$ Hz), 4.22 (ddt, 1H, $J_1 = J_2 = 1.5$ Hz, $J_3 = 6.5$ Hz, $J_4 = 13.0$ Hz), 4.11 (dd, 1H, $J_1 = 10.0$ Hz, $J_2 = 3.0$ Hz), 4.02-3.94 (m, 3H), 3.92-3.54 (m, 14H), 3.32 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 9.0$ Hz), 2.77 (dd, 1H, $J_1 = 4.0$ Hz, $J_2 = 12.0$ Hz, sialH-3eq), 1.83 (t, 1H, $J_1 = J_2 = 12.0$ Hz, sialH-3ax);

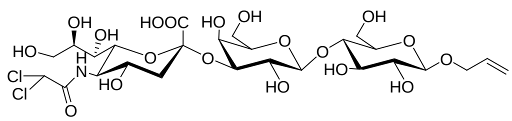
^{13}C-NMR (125 MHz, D_2O) δ 174.38, 166.28 (t, 1C, $J_{F\text{-}C}$ = 25.75 Hz), 134.03, 119.50, 109.01 (t, 1C, $J_{F\text{-}C}$ = 247 Hz), 103.38, 101.80, 100.48, 79.01, 76.23, 75.88, 75.50, 75.14, 73.55, 73.02, 72.56, 71.40, 70.11, 68.80, 68.72, 68.18, 63.31, 61.75, 60.82, 52.46, 40.33; HRMS (*m/z*): $[M+Na]^+$ calcd. for $[C_{26}H_{41}F_2NNaO_{19}]^+$, 732.2133; found, 732.2147.

Compound **9** 

Yield = 70%; $[\alpha]_D$ = -8.61 (*c* = 1.1, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.02-5.93 (m, 1H), 5.38 (dd, 1H, J_1 = 3.0 Hz, J_2 = 17.5 Hz), 5.28 (d, 1H, *J* = 10.5 Hz), 4.53 (d, 2H, *J* = 8.5 Hz, overlapped anomeric H), 4.39 (dd, 1H, J_1 = 5.5 Hz, J_2 = 12.5 Hz), 4.22 (dd, 1H, J_1 = 6.5 Hz, J_2 = 12.5 Hz), 4.12 (dd, 1H, J_1 = 10.0 Hz, J_2 = 3.0 Hz), 4.03-3.96 (m, 3H), 3.92-3.56 (m, 14H), 3.33 (t, 1H, J_1 = J_2 = 8.5 Hz), 2.78 (dd, 1H, J_1 = 5.0 Hz, J_2 = 12.5 Hz, sialH-3eq), 1.83 (t, 1H, J_1 = J_2 = 12.0 Hz, sialH-3eq); ^{13}C-NMR (125 MHz, D_2O) δ 174.51, 160.13 (q, 1C, $J_{F\text{-}C}$ = 38.0 Hz), 134.02, 119.50, 116.51 (q, 1C, $J_{F\text{-}C}$ = 285.0 Hz), 103.38, 101.80, 100.58, 79.02, 76.24, 75.90, 75.50, 75.15, 73.55, 72.81, 72.66, 71.41, 70.11, 68.78, 68.15, 63.26, 61.76, 60.82, 52.98, 40.42; HRMS (*m/z*): $[M+Na]^+$ calcd. for $[C_{26}H_{40}F_3NNaO_{19}]^+$, 750.2039; found, 750.2039.

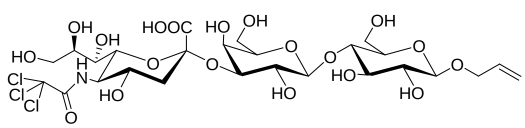
Compound **10** 

Yield = 71%; $[\alpha]_D$ = -10.64 (*c* = 0.9, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.03-5.93 (m, 1H), 5.38 (dq, 1H, *J* = 1.5 Hz, 17.5 Hz), 5.28 (dq, 1H, *J* = 1.5 Hz, 10.5 Hz), 4.52 (d, 1H, *J* = 8.0 Hz, anomeric H), 4.51 (d, 1H, *J* = 8.0 Hz, anomeric H), 4.39 (ddt, 1H, J_1 = J_2 = 1.5 Hz, J_3 = 5.5 Hz, J_4 = 12.5 Hz), 4.22 (ddt, 1H, J_1 = J_2 = 1.5 Hz, J_3 = 5.5 Hz, J_4 = 12.5 Hz), 4.16 (d, 2H, *J* = 14.0 Hz, $-COCH_2Cl$), 4.11 (dd, 1H, J_1 = 10 Hz, J_2 = 3.5 Hz), 3.98 (dd, 1H, J_1 = 12.5 Hz, J_2 = 2.5 Hz), 3.95 (d, 1H, *J* = 3.5 Hz), 3.95-3.55 (m, 15H), 3.33 (dd, 1H, J_1 = 8.0 Hz, J_2 = 9.0 Hz), 2.75 (dd, 1H, J_1 = 4.5 Hz, J_2 = 12.0 Hz, sialH-3eq), 1.80 (dd, 1H, J_1 = J_2 = 12.0 Hz, sialH-3ax); ^{13}C-NMR (125 MHz, D_2O) δ 174.61, 171.18, 134.02, 119.50, 103.38, 101.79, 100.55, 79.00, 76.23, 75.90, 75.50, 75.14, 73.54, 73.27, 72.59, 71.40, 70.11, 68.91, 68.80, 68.18, 63.30, 61.76, 60.81, 52.84, 43.04, 40.42; HRMS (*m/z*): $[M+Na]^+$ calcd. for $[C_{26}H_{42}ClNNaO_{19}]^+$, 730.1932; found, 730.1906.



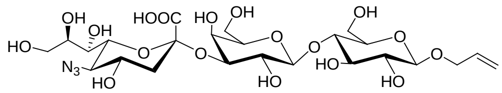
Compound **11**

Yield = 89%; $[\alpha]_D$ = -11.64 (*c* = 1.2, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.30 (s, 1H), 5.96 (dq, 1H, *J* = 1.5 Hz, 17.5 Hz), 5.37 (dd, 1H, J_1 = 1.5 Hz, J_2 = 10.5 Hz), 4.52 (d, 1H, *J* = 8.0 Hz, anomeric H), 4.51 (d, 1H, *J* = 8.0 Hz, anomeric H), 4.38 (dd, 1H, J_1 = 5.5 Hz, J_2 = 12.5 Hz), 4.38 (dd, 1H, J_1 = 6.5 Hz, J_2 = 13.0 Hz), 4.14 (dd, 1H, J_1 = 3.0 Hz, J_2 = 10.0 Hz), 4.00-3.55 (m, 18H), 3.32 (t, 1H, *J* = 8.5 Hz), 2.75 (dd, 1H, J_1 = 5.0 Hz, J_2 = 13.0 Hz, sialH-3eq), 1.88 (t, 1H, J_1 = J_2 = 12.0 Hz, sialH-3ax); ^{13}C-NMR (75MHz, D_2O) δ 172.80, 168.02, 133.75, 119.32, 103.10, 101.56, 99.48, 78.70, 75.96, 75.56, 75.28, 74.92, 73.33, 73.14, 71.82, 71.21, 69.90, 68.75, 67.99, 66.71, 6332, 61.45, 60.57, 52.89, 39.78; HRMS (*m/z*): $[M+Na]^+$ calcd. for $[C_{26}H_{41}Cl_2NNaO_{19}]^+$, 764.1542; found, 764.1550.

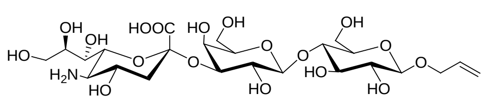


Compound **12**

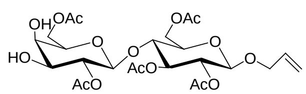
Yield = 81%; $[\alpha]_D$ = -14.20 (*c* = 1.3, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.03-5.93 (m, 1H), 5.35 (dd, 1H, J_1 = 1.5 Hz, J_2 = 17.5 Hz), 5.28 (dd, 1H, J_1 = 1.0 Hz, J_2 = 10.5 Hz), 4.53 (d, 2H, *J* = 8.0 Hz, overlapped anomeric H), 4.39 (dd, 1H, J_1 = 5.5 Hz, J_2 = 12.5 Hz), 4.22 (dd, 1H, J_1 = 6.5 Hz, J_2 = 12.5 Hz), 4.13 (dd, 1H, J_1 = 10.0 Hz, J_2 = 3.0 Hz), 4.03-3.46 (m, 17H), 3.36-3.31 (m, 1H), 2.80 (dd, 1H, J_1 = 5.0 Hz, J_2 = 12.5 Hz, sialH-3eq), 1.83 (t, 1H, J_1 = J_2 = 12.0 Hz, sialH-3ax); ^{13}C-NMR (125 MHz, D_2O) δ 174.58, 165.50, 134.01, 119.50, 103.38, 101.79, 100.55, 79.00, 76.21, 75.89, 75.49, 75.13, 73.54, 72.91, 72.75, 71.40, 70.10, 69.04, 68.55, 68.13, 63.26, 61.75, 60.82, 54.44, 40.74; HRMS (*m/z*): $[M+Na]^+$ calcd. for $[C_{26}H_{40}Cl_3NNaO_{19}]^+$, 798.1152; found, 798.1161.

Compound **13** 

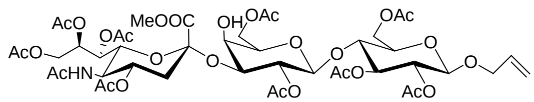
A suspension of sodium azide (44.0 mg, 0.67 mmol) in 8 mL of pyridine[2] was cooled in ice bath. Then triflic anhydride (150.0 mg, 0.56 mmol) was added to the mixture by a syringe in about 5 minutes while stirring. The reaction was maintained for 2 h in ice bath to give a TfN_3-containing solution (~0.07 mmol TfN_3, based on 100% conversion of triflic anhydride), 1 mL (~0.07 mmol) of which was then added directly to the solution of **14** (20.0 mg, 0.032 mmol) in 2 mL MeOH, and $CuSO_4$ (0.5 mg, 0.003 mmol) was added. The diazotransfer reaction was finished in 1 h, as indicated by TLC. The mixture was condensed under reduced pressure. The residue was purified by C-18 reversed-phase column eluted by H_2O, then H_2O/MeOH, and then with Biogel P-2 column with H_2O as the eluent, to afford **13** (13.0 mg, yield = 62%); $[\alpha]_D$ = -18.70 (c = 1.0, MeOH); ^{1}H-NMR (500 MHz, D_2O) δ 6.03-5.93 (m, 1H), 5.35 (dq, 1H, J = 1.5 Hz, 17.5 Hz), 5.31-5.27 (m, 1H), 4.53 (d, 1H, J = 8.0 Hz, anomeric H), 4.52 (d, 1H, J = 8.0 Hz, anomeric H), 4.39 (dd, 1H, J_1 = 5.5 Hz, J_2 = 12.5 Hz), 4.22 (dd, 1H, J_1 = 6.5 Hz, J_2 = 12.5 Hz), 4.10 (dd, 1H, J_1 = 10.0 Hz, J_2 = 3.0 Hz), 4.03-3.46 (m, 17H), 3.33 (t, 1H, J_1 = J_2 = 8.5 Hz), 2.74 (dd, 1H, J_1 = 5.0 Hz, J_2 = 12.5 Hz, sialH-3eq), 1.80 (t, 1H, J_1 = J_2 = 12.0 Hz, sialH-3ax); ^{13}C-NMR (125 MHz, D_2O) δ 174.47, 134.02, 119.50, 103.38, 101.80, 100.54, 79.01, 76.20, 75.88, 75.50, 75.14, 73.58, 73.55, 72.68, 71.40, 70.23, 70.08, 69.13, 68.10, 63.33, 63.28, 61.75, 60.82, 40.23; HRMS (m/z): $[M+Na]^+$ calcd. for $[C_{24}H_{39}N_3O_{18}Na]^+$, 680.2121; found, 680.2133.

Compound **14** 

A stirred solution of **2** (165.0 mg, 0.245 mmol) in aqueous NaOH solution (2 N, 5 mL) was heated at 90 °C for 4-6 h, until the conversion was completed (detected by TLC). The mixture was neutralized with 1N HCl in methanol to PH = 6-8. The reaction mixture was concentrated in vacuo, extracted by MeOH. The organic layer was concentrated in vacuum to give a residue, which was employed for the next reactions. The crude product (10%) was purified by Biogel P-2 column with H_2O as the eluent, and then by C-18 reversed-phase column with H_2O as the eluent, to afford **14** (15.0 mg, 0.0233 mmol, yield = 95%). $[\alpha]_D$ = -18.80 (c = 1.0, MeOH); ^{1}H-NMR (400 MHz, D_2O) δ 5.97-5.86 (m, 1H), 5.33 (d, 1H, J = 17.2 Hz, allyl H), 5.22 (d, 1H, J = 10.4 Hz, allyl H), 4.47 (d, 1H, J = 8.0 Hz, anomeric H), 4.45 (d, 1H, J = 8.0 Hz, anomeric H), 4.32 (dd, 1H, J_1 = 5.6 Hz, J_2 = 12.8 Hz, allyl H), 4.22 (dd, 1H, J_1 = 6.2 Hz, J_2 = 12.4 Hz, allyl H), 4.03 (dd, 1H, J_1 = 10.0 Hz, J_2 = 3.2 Hz), 3.94-3.46 (m, 16H), 3.26 (m, 1H), 3.19 (t, 1H, J_1 = J_2 = 10.0 Hz), 2.74 (dd, 1H, J_1 = 4.8 Hz, J_2 = 12.4 Hz, sialH-3eq), 1.80 (t, 1H, J_1 = J_2 = 12.4 Hz, sialH-3ax); ^{13}C-NMR (125 MHz, D_2O) δ 133.33, 118.77, 102.66, 101.10, 99.76, 72.83, 71.54, 71.46, 70.69, 69.36, 67.81, 67.24, 66.74, 62.23, 61.01, 60.16, 52.19, 39.89; HRMS (m/z): $[M+H]^+$ calcd. for $[C_{24}H_{42}NO_{18}]^+$, 632.2396; found, 632.2396.

Compound **20**[1] 

Compound **21** (100.0 mg, 0.237 mmol) was dissolved in pyridine (2 mL) and acetic anhydride (1.8 mL), DMAP (15 mg) was added. The mixture was stirred overnight. The reaction mixture was concentrated on reduced pressure. The residue was dissolved in 90% TFA aqueous solution (5 mL) at -20 °C. The mixture was stirred at -20 °C for 10~20 min until TLC analysis indicated that the reaction had completed. The reaction mixture was immediately poured into saturated $KHCO_3$ aqueous solution at -20 °C. The mixture was extracted with ethyl acetate (50 mL×3). The organic phase was combined, washed with H_2O (15 mL), dried over Na_2SO_4, and condensed under reduced pressure. The residue was purified by column chromatography on silica gel (acetone: petroleum ether = 1:3), to afford **20** as an oil (120.0 mg, yield = 94%). ^{1}H-NMR (500 MHz, D_2O) δ 5.89-5.79 (m, 1H), 5.25 (dq, 1H, J_1 = 1.5 Hz, 17.5 Hz), 5.20 (dd, 1H, J_1 = 1.5 Hz, J_2 = 10.5 Hz), 5.17 (t, 1H, J_1 = J_2 = 9.5 Hz), 4.95 (dd, 1H, J_1 = 8.5 Hz, J_2 = 10.0 Hz), 4.91 (dd, 1H, J_1 = 8.5 Hz, J_2 = 8.0 Hz), 4.52 (d, 1H, J = 8.0 Hz, anomeric H), 4.48 (dd, 1H, J_1 = 2.0 Hz, J_2 = 12.0 Hz), 4.32 (d, 1H, J = 8.0 Hz, anomeric H), 4.35-4.29 (m, 2H), 4.23 (dd, 1H, J_1 = 7.5 Hz, J_2 = 11.5 Hz), 4.16 (dd, 1H, J_1 = 5.0 Hz, J_2 = 12.0 Hz), 4.08 (ddt, 1H, J_1 = J_2 = 1.5 Hz, J_3 = 6.5 Hz, J_4 = 13.0 Hz), 3.87 (s, br, 1H), 3.75 (t, 1H, J_1 = J_2 = 9.0 Hz), 3.65-3.58 (m, 3H), 3.55-3.42 (m, 2H), 2.119 (s, 3H), 2.116 (s, 3H), 2.108 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); ^{13}C-NMR (125 MHz, D_2O) δ 171.29, 170.98, 170.66, 170.55, 169.59, 133.37, 117.61, 100.91, 99.42, 76.35, 73.34, 72.77, 72.65, 72.30, 71.44, 70.02, 68.52, 62.46, 62.20, 20.90, 20.83, 20.70.

Compound **22**[1] 

A mixture of **20** (1.0 eq.), **15** (1.5 eq.), and activated 4 Å molecular sieves (50 mg/1mL THF) in dry THF was stirred for 0.5 h at r.t. under N_2 protection before it was cooled to -72 °C, then TMSOTf (0.15 eq.) was added by drop in three batches. The reaction mixture was stirred for 2-4 h until TLC analysis indicated that the reaction had completed. Triethylamine (2 eq.) was added, the solids were filtered off, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (gradient acetonitrile in chloroform) to afford **22** as a white foam. Compound **22** was the only isolated isomer. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.89-5.79 (m, 1H), 5.54-5.49(m, 1H), 5.40 (dd, 1H, J_1 = 2.5 Hz, J_2 = 9.0 Hz), 5.25 (dq, 1H, J = 1.5 Hz, 17.5 Hz), 5.19 (dq, 1H, J = 1.5 Hz, 10.5 Hz), 5.17 (t, 1H, J = 9.5 Hz), 5.06 (d, 1H, J = 10.0 Hz), 4.97 (dd, 1H, J_1 = 8.0 Hz, J_2 = 10.0 Hz), 4.94 (dd, 1H, J_1 = 8.0 Hz, J_2 = 10.0 Hz), 4.81 (ddd, 1H, J_1 = 4.5 Hz, J_2 = 10.5 Hz, J_3 = 12.5 Hz), 4.54 (d, 1H, J = 8.0 Hz, anomeric H), 4.52 (d, 1H J = 8.0 Hz, anomeric H), 4.44 (dd, 1H, J_1 = 2.0 Hz, J_2 = 12.0 Hz), 4.39 (dd, 1H, J_1 = 2.5 Hz, J_2 = 12.5 Hz), 4.32-4.22 (m, 4H), 4.18 (dd, 1H, J_1 = 5.5 Hz, J_2 = 12.0 Hz), 4.11-4.00 (m, 3H), 3.93 (dd, 1H, J_1 = 2.5 Hz, J_2 = 10.5 Hz), 3.85 (t, 1H, J = 9.0 Hz), 3.78 (s, 3H, $COOCH_3$), 3.63 (dd, 1H, J_1 = 6.0 Hz, J_2 = 6.5 Hz), 3.59 (ddd, 1H, J_1 = 2.0 Hz, J_2 = 5.0 Hz, J_3 = 9.5 Hz), 3.42-3.36 (m, 2H), 2.75 (dd, 1H, J_1 = 4.5 Hz, J_2 = 12.5 Hz, sialH-3eq), 2.21 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.09 (s, 9H, OAc), 2.06 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.87 (s, 3H, NAc), 1.83 (t, 1H, J = 12.0 Hz, sialH-3ax); HRMS (*m/z*): $[M+Na]^+$ calcd. for $[C_{45}H_{63}NNaO_{28}]^+$, 1088.3429; found, 1088.3391.

2. Supplementary Methods and Results on Biological Assay

2.1. Procedure for coupling carbohydrates with protein:

Modified GM3 and native GM3 were coupled with KLH to immune mice. A solution of carbohydrate (**2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, 5.0 mg each), was oxidized with ozone to give the corresponding aldehyde, which was coupled to KLH (5.0 mg) in the presence of $NaBH_3CN$ (5.0 mg) in phosphate buffered saline (PBS 0.4 mL, 0.1 M, pH = 7.6).

For the preparation of carbohydrate-BSA conjugates, the recovered antigen aldehydes were employed to make full use of them. Thus, the glycoconjugation reaction mixture of modified GM3-KLH or native GM3-KLH was firstly dialyzed against small amount of PBS (0.85 mL, 0.1 M, pH = 7.6, 4 ^oC, molecular weight cut-off value 14,000 Da) for 4 h. The obtained dialysate, which included the unreacted antigen aldehyde, was then added with 1 mg of BSA and 3 mg of $NaBH_3CN$.

The reaction mixture of modified GM3-BSA or native GM3-BSA was allowed to be gently shaken in the dark for 12-16 h at r.t., before being dialyzed against PBS at 4 ^oC (molecular weight cut-off value 14,000 Da, 1 L × 6 times PBS). The reaction mixture of modified GM3-KLH or native GM3-KLH was allowed to be gently shaken in the dark for 12-16 h at r.t., then first dialyzed against small amount of PBS to recover antigen aldehydes as mentioned above, and then dialyzed against 1 L × 6 times PBS.

2.2. Analysis of the carbohydrate loading levels of the glycoconjugates:

The epitope ratios of the glycoconjugates (including carbohydrate-KLH and carbohydtate-BSA) were determined by estimating protein content by BCA assay[3] and sialic acid content using the resorcinol method described by Svennerholm[4] (**13-**KLH and **13-**BSA cannot develop legible color by this method, their carbohydrate contents were estimated by the phenol-sulfuric acid method[5]). The glycoconjugate (100 μL) was mixed well with the resorcinol reagent (100 μL) and heated in a boiling water bath for 30 min, then cooled on ice for 10 min. An extraction solution (1-butanol acetate and 1-butanol, 85:15 v/v, 250 μL) was added to the mixture. The mixture was kept standing still for 15 min after it was shaken vigorously to allow the organic layer to separate well from the inorganic layer. The absorbance at 580 nm of organic layer was determined by an UV-vis spectrometer, using a blank organic solution as the control. The trisaccharide content of the glycoconjugate was determined against a calibration curve created with the corresponding allyl-trissachrides solutions analyzed under the same conditions[4]. The carbohydrate loading of each glycoconjugate was calculated according to the equation shown below:

$$\text{Loading of GM3 or modified GM3 (\%)} = \frac{\text{content of trisaccharide (mg) in the sample}}{\text{content of trisaccharide (mg) in the sample + content of protein (mg) in the sample}} \cdot 100\%$$

Supplementary Table 1. Carbohydrate Loading of Glycoconjugates

sample	KLH conjugates loading (%)	BSA conjugates loading (%)
2	9.05	3.59
3	6.68	2.81
4	9.35	4.78
5	8.89	4.72
6	6.85	3.57
7	6.57	4.53
8	6.82	2.66
9	7.02	4.48
10	8.32	4.09
11	7.42	3.23
12	8.11	4.71
13	11.00	5.47
14	7.03	2.80

2.3. Immunization of mice and serologic assays

Pathogen-free BALB/c female mice aged 6–8 weeks (Number: SCXKjing2007-0001, SPF/VAF) were obtained from Department of Laboratory Animal Science, Peking University of Health Science Center. Groups of six mice were immunized four times at 2-week intervals with unmodified-GM3-KLH or modified-GM3-KLH glycoconjugates (each containing 2 μg of μ carbohydrate in PBS). The vaccines were administered intraperitoneally over the lower abdomen. Mice were bled prior to the initial vaccination, 13 days after the second and the third vaccinations, and 14 days after the fourth vaccination. Blood was clotted to obtain sera, which were stored at -80 ^{o}C.

The total antigen-specific antibody titers of the pooled sera were assessed by means of ELISA. ELISA plate was coated with 100 μL of 2-BSA（including 0.02 μg of GM3）overnight at 4 ^{o}C (0.1 M bicarbonate buffer, pH = 9.6). After three washed with PBST (0.05% Tween20 in PBS), microwells were blocked with 3% BSA. After the plate was washed, serially diluted sera were added to microwells (100 μL /well) and incubated for 1 h at 37 ^{o}C. The plate was washed and incubated with 1:5000 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (γ-chain specific) or IgM (μ-chain specific) (Southern Biotechnology Associates, Inc., Buckingham, AL) for 1 h at 37 ^{o}C. The plate was washed, developed with *o*-phenylenediamine (OPD) substrate in the dark for 15 min, terminated with 2 M H_2SO_4, and then was read at 490 nm. The antibody titer was defined as the highest dilution showing an absorbance of 0.1, after subtracting background.

Meanwhile, the anti-modified-GM3 antibody titers（sera from 13 days after the 3rd vaccination）were determined by ELISA, with plate coated by the corresponding modified-GM3-BSA conjugates instead. The results were summarized in Supplementary Table 5.

Supplementary Table 2. ELISA titers (obtained from pooled sera 13 days after 2nd vaccination) against 2-BSA

Group	IgG	IgM
immunized with **2-**KLH	12,252	762
immunized with **3-**KLH	106,740	24,930
immunized with **4-**KLH	30,326	<500
immunized with **5-**KLH	44,563	5109
immunized with **6-**KLH	1986	1315
immunized with **7-**KLH	17,073	<500
immunized with **8-**KLH	25,402	<500
immunized with **9-**KLH	24,990	<500
immunized with **10-**KLH	4121	<500
immunized with **11-**KLH	10,573	<500
immunized with **12-**KLH	<2500	<500
immunized with **13-**KLH	<2500	<500
immunized with **14-**KLH	2500	<500

Supplementary Table 3. ELISA titers (obtained from pooled sera 13 days after 3rd vaccination) against 2-BSA

Group	IgG	IgM
immunized with **2-**KLH	63,902	3727
immunized with **3-**KLH	277,143	37,437
immunized with **4-**KLH	53,333	7800
immunized with **5-**KLH	58,182	9719
immunized with **6-**KLH	5109	10,235
immunized with **7-**KLH	63,964	<500
immunized with **8-**KLH	97,419	3651
immunized with **9-**KLH	50,612	<500
immunized with **10-**KLH	22,009	2225
immunized with **11-**KLH	32,264	3220
immunized with **12-**KLH	7338	<500
immunized with **13-**KLH	<2500	<500
immunized with **14-**KLH	5173	3542

Supplementary Table 4. ELISA titers (obtained from pooled sera 14 days after 4th vaccination) against 2-BSA

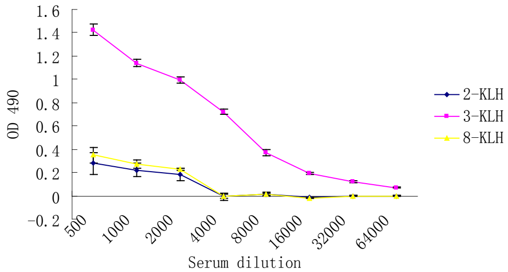
Group	IgG	IgM
immunized with **2-**KLH	335,732	9697
immunized with **3-**KLH	1,233,305	64,265
immunized with **4-**KLH	387,615	14,012
immunized with **5-**KLH	280,535	17,056
immunized with **6-**KLH	18,493	6121
immunized with **7-**KLH	315,634	4688
immunized with **8-**KLH	529,891	5118
immunized with **9-**KLH	186,877	5684
immunized with **10-**KLH	113,837	6002
immunized with **11-**KLH	87,777	9963
immunized with **12-**KLH	50,158	<1000
immunized with **13-**KLH	<5000	<1000
immunized with **14-**KLH	22,662	1220

Supplementary Table 5. The anti-modified-GM3 antibody titers（serum from 13 days after 3rd vaccination）

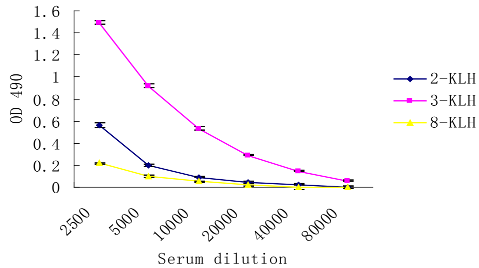
Group	IgG
immunized with **2-**KLH	63,902
immunized with **3-**KLH	642,890
immunized with **4-**KLH	105,387
immunized with **5-**KLH	306,499
immunized with **6-**KLH	128,827
immunized with **7-**KLH	80,041
immunized with **8-**KLH	231,026
immunized with **9-**KLH	139,406
immunized with **10-**KLH	21,654
immunized with **11-**KLH	89,989
immunized with **12-**KLH	72,869
immunized with **13-**KLH	127,621
immunized with **14-**KLH	112,163

Supplementary Figure 1

a



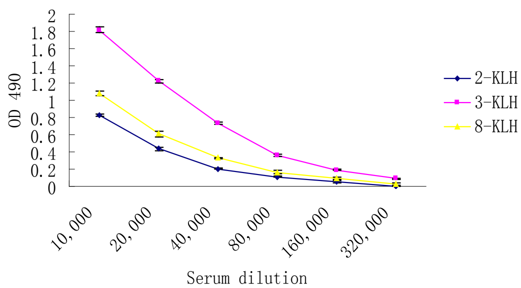
b



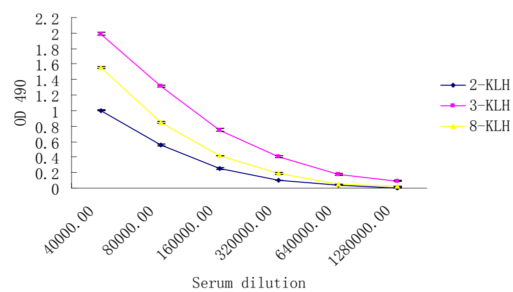
IgM antibody titers of pooled sera immunized with 3-KLH, 8-KLH and 2-KLH by ELISA. (a) Pooled sera obtained from 13 days after the 3rd vaccination. (b) Pooled sera obtained from 14 days after the 4th vaccination. All of the data points were the mean of three parallel measurement data. Some error bars are smaller than the symbol width.

Supplementary Figure 2

a



b



IgG antibody titers of pooled sera immunized with 3-KLH, 8-KLH and 2-KLH by ELISA. (a) Pooled sera obtained from 13 days after the 3rd vaccination. (b) Pooled sera obtained from 14 days after the 4th vaccination. All of the data points were the mean of three parallel measurement data. Some error bars are smaller than the symbol width.

Supplementary Table 6. Antibody titers against 2-BSA, 24-BSA of the pooled sera of mice immunized with 3-KLH, 8-KLH and 2-KLH after the 4th vaccination

Vaccine	2-BSA		24-BSA	
	IgG	IgM	IgG	IgM
2-KLH	335,732	9697	<250	<250
3-KLH	1,233,305	64,265	<250	<250
8-KLH	529,891	5118	<250	<250

The antibody titers against **2-**BSA, **24-**BSA of the pooled sera of mice immunized with **2-**KLH, **3-**KLH and **8-**KLH after the 4th vaccination were assessed by means of ELISA. For the detailed method, see Serological Assays of main text. ELISA plate was coated with 100 μL of **2-**BSA or **24-**BSA (including 0.02 μg of **2** or **24** respectively). When the plate was coated with **24-**BSA, the antibody titers could not be detected (<250).

Supplementary Table 7. IgM antibody titer against 2-BSA of individual mouse immunized with 2-KLH and 3-KLH after the 3rd vaccination

Mice	vaccine	
	2-KLH	**3**-KLH*
1	697	139,456
2	2881	38,158
3	475	8737
4	2775	3932
5	1075	3227
6	414	7352

In the analysis, there were six mice per group (n = 6). The IgM antibody titer of individual mouse serum was separately detected twice, and three parallel pores were arranged in plates for each serum-diluted concentration. The titer represented the average of two detections. The data of titers were dealt with logarithmic function to base 10, and then statistical analysis was performed by independent two sample *t* test with equal variances. Value of $p < 0.05$ was considered to be statistically significant and was identified by *. The results indicated that the IgM level for the conjugate of compound **3** exhibited a remarkable increase relative to **2** after the 3rd immunizations. The statistical analysis was performed with SAS software (version 9.1).

Supplementary Table 8. IgM antibody titer against 2-BSA of individual mouse immunized with 2-KLH and 3-KLH after the 4th vaccination

Mice	vaccine	
	2-KLH	**3**-KLH*
1	6520	341,868
2	41,771	99,239
3	8332	27,122
4	18,329	23,127
5	11,386	32,431
6	1780	72,281

In the analysis, there were six mice per group (n = 6). The IgM antibody titer of individual mouse serum was separately detected twice, and three parallel pores were arranged in plates for each serum-diluted concentration. The titer represented the average of two detections. The data of titers were dealt with logarithmic function to base 10, and then statistical analysis was performed by independent two sample *t* test with equal variances. Value of $p < 0.05$ was considered to be statistically significant and was identified by *. The results indicated that the IgM level for the conjugate of compound **3** exhibited a remarkable increase relative to **2** after the 4th immunizations. The statistical analysis was performed with SAS software (version 9.1).

Supplementary Table 9. IgG antibody titer against 2-BSA of individual mouse immunized with 2-KLH and 3-KLH after the 3rd vaccination

Mice	vaccine	
	2-KLH	**3**-KLH*
1	128,655	48,187
2	13,396	280,615
3	75,999	245,915
4	35,796	1,269,419
5	122,157	209,357
6	101,716	154,989

In the analysis, there were six mice per group (n = 6). The IgG antibody titer of individual mouse serum was separately detected twice, and three parallel pores were arranged in plates for each serum-diluted concentration. The titer represented the average of two detections. The data of titers were dealt with logarithmic function to base 10, and then statistical analysis was performed by independent two sample *t* test with equal variances. Value of $p < 0.05$ was considered to be statistically significant and was identified by *. The results indicated that the IgG level for the conjugate of compound **3** exhibited a remarkable increase relative to **2** after the 3rd immunizations. The statistical analysis was performed with SAS software (version 9.1).

Supplementary Table 10. IgG antibody titer against 2-BSA of individual mouse immunized with 2-KLH and 3-KLH after the 4th vaccination

Mice	vaccine	
	2-KLH	**3**-KLH
1	557,686	508,305
2	137,575	2,530,941
3	198,967	823,202
4	193,535	3,599,717
5	2,554,387	709,632
6	875,698	610,904

In the analysis, there were six mice per group (n = 6). The IgG antibody titer of individual mouse serum was separately detected twice, and three parallel pores were arranged in plates for each serum-diluted concentration. The titer represented the average of two detections. Even if statistical significance could not be obtained here, the IgG level for the conjugate of compound **3** still exhibited an obviously increased tendency relative to **2** after the 4th immunization.

3. References

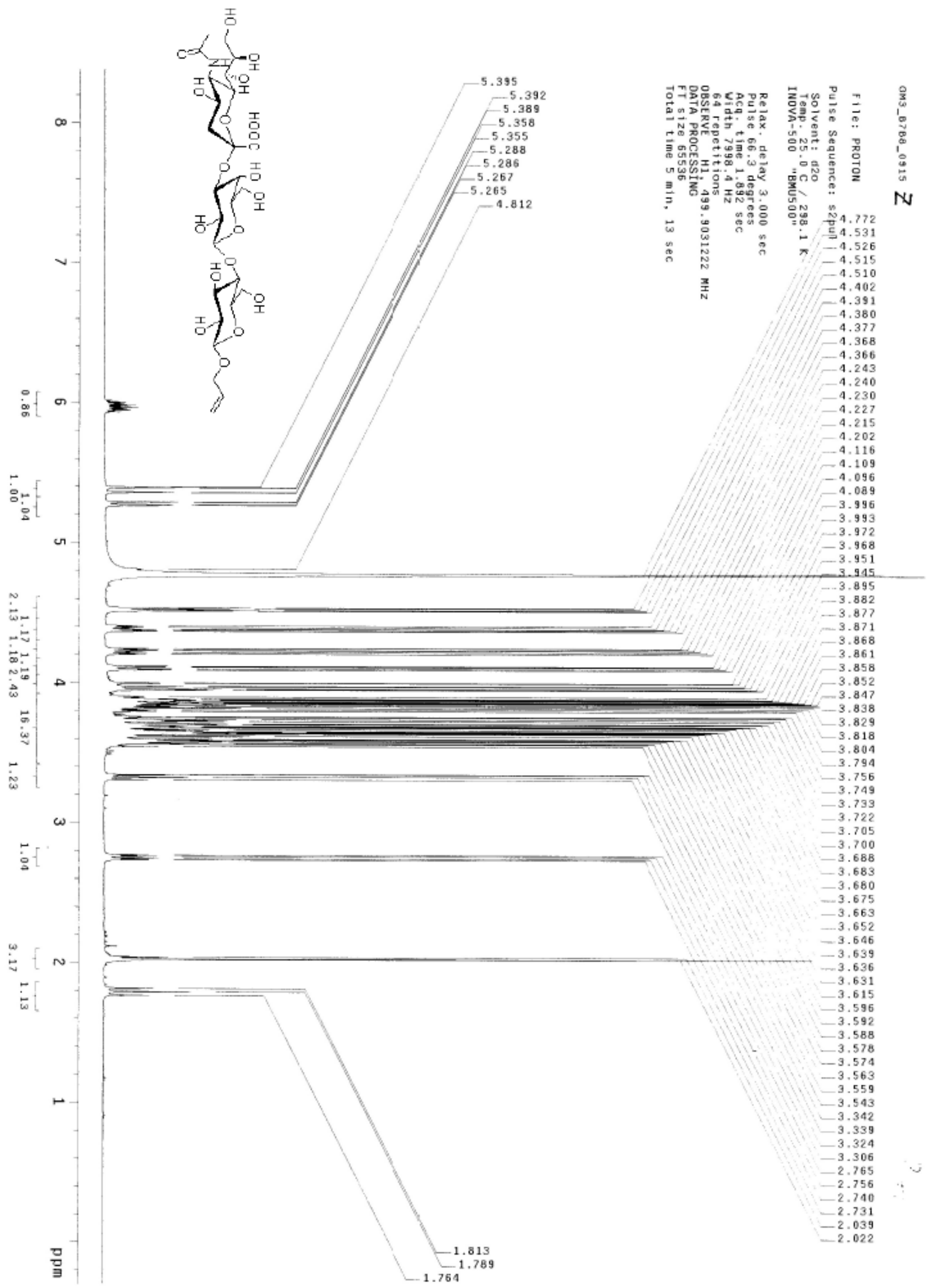
[1] T. Ogawa, M. Sugimoto, S. Yoshiyasu, M. Ito, *U.S. Patent* **1986**, No. 4968786.

[2] R.-B. Yan, F. Yang, Y. Wu, L.-H. Zhang, X.-S. Ye, *Tetrahedron Lett.* **2005**, *46*, 8993-8995.

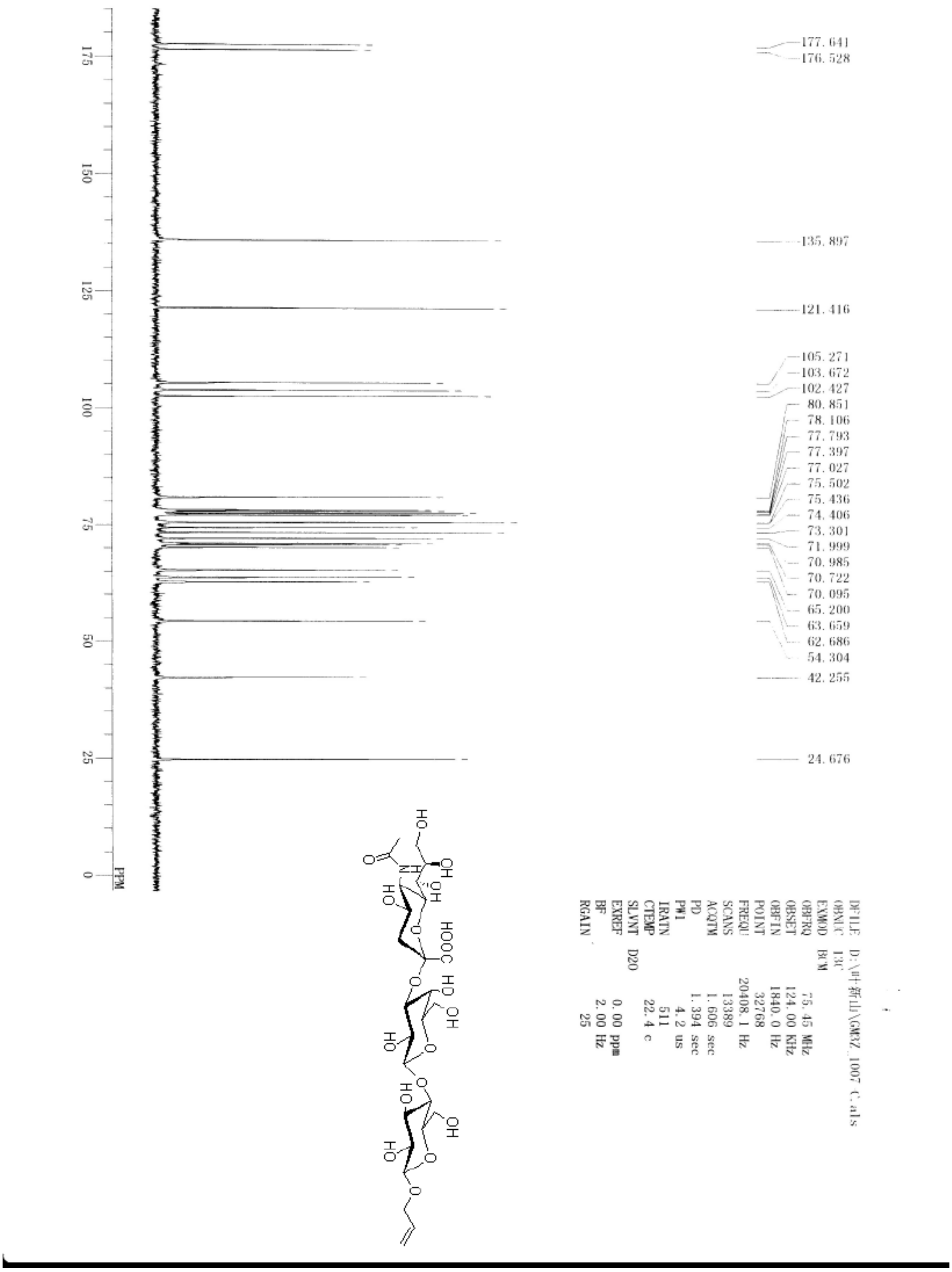
[3] (a) V. S. Hornsey, C. V. Prowse, D. Pepper, *Journal of Immunological Methods* **1986**, *93*, 83-88; (b) P. K. Smith, R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, D. C. Klenk, *Analytical Biochemistry* **1985**, *150*, 76-85.

[4] L. Svennerholm, *Biochim. Biophy. Acta* **1957**, *24*, 604-611.

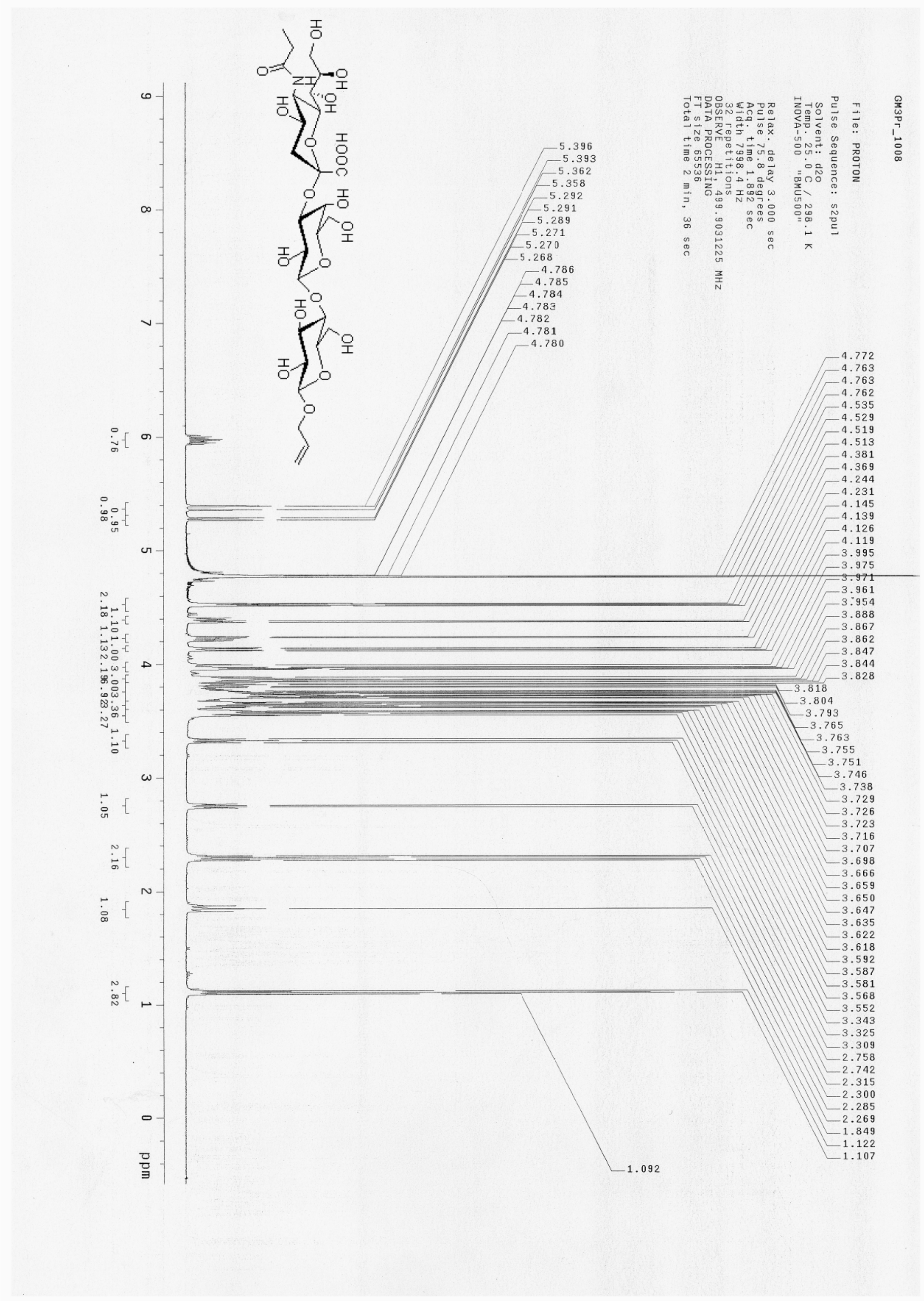
[5] M. Dubois, J. K. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith, *Anal. Chem.* **1956**, *28*, 350-356.



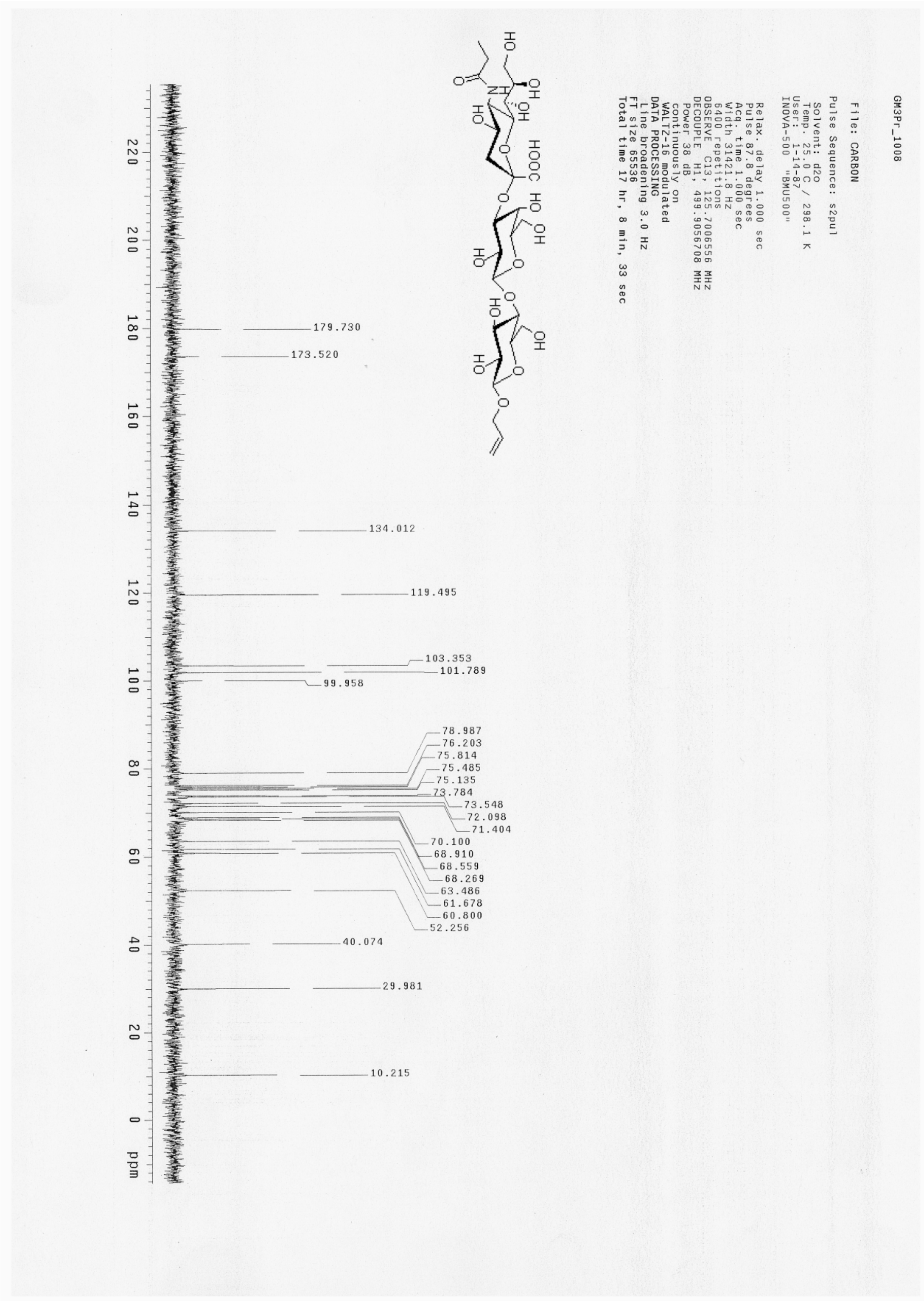
[1]H NMR of Compound **2**



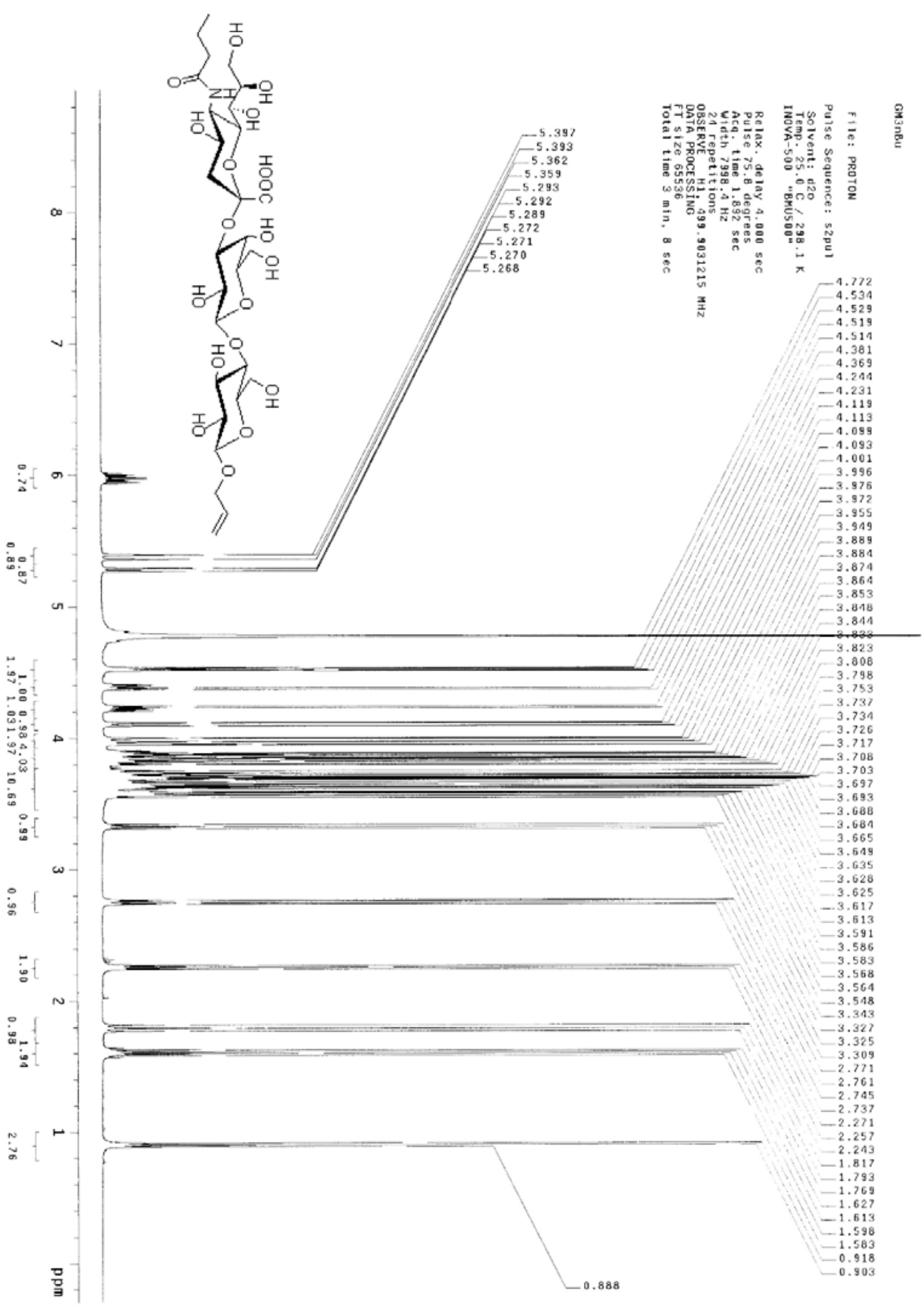
^{13}C NMR of Compound **2**



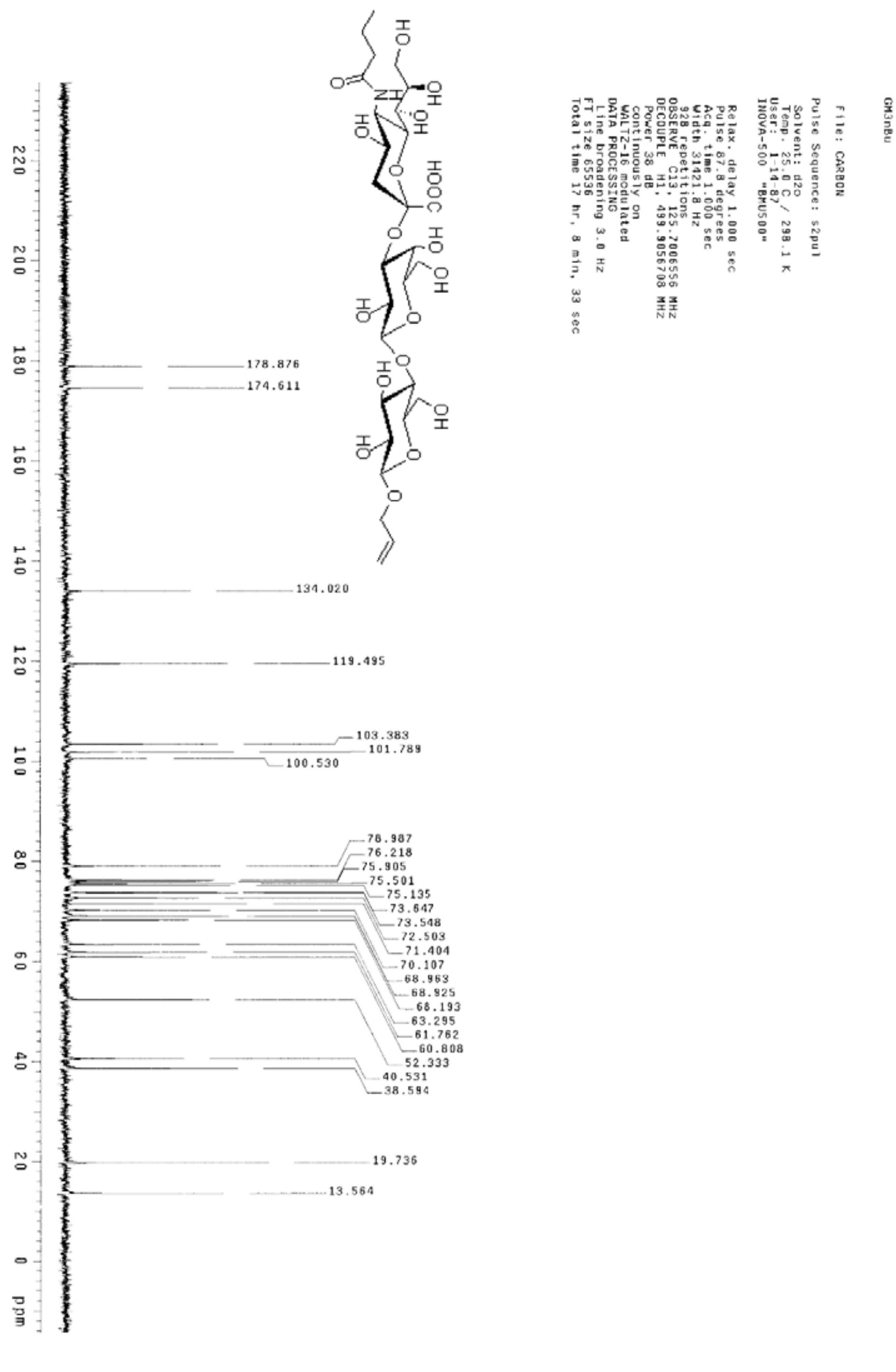
[1]H NMR of Compound **3**



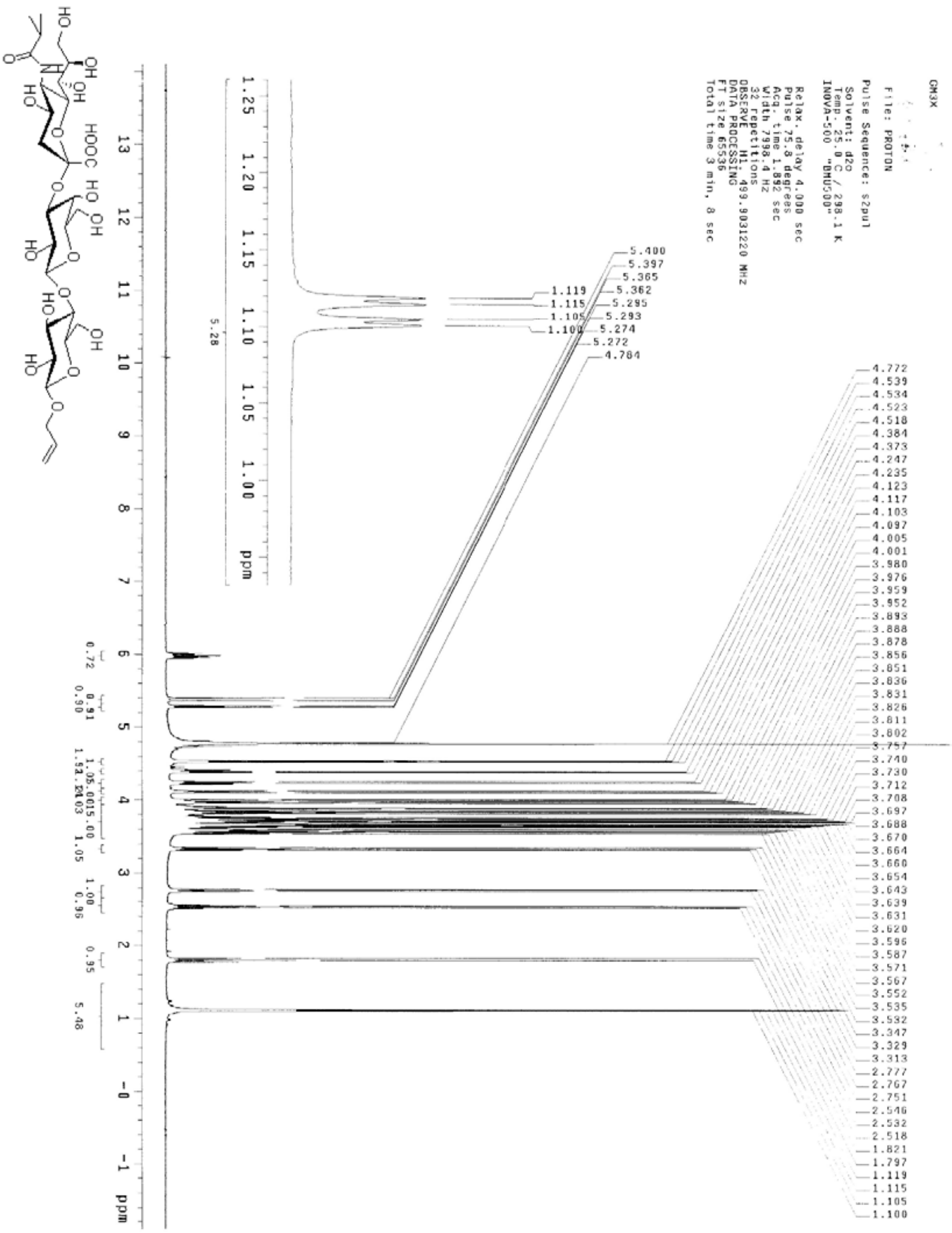
^{13}C NMR of Compound **3**



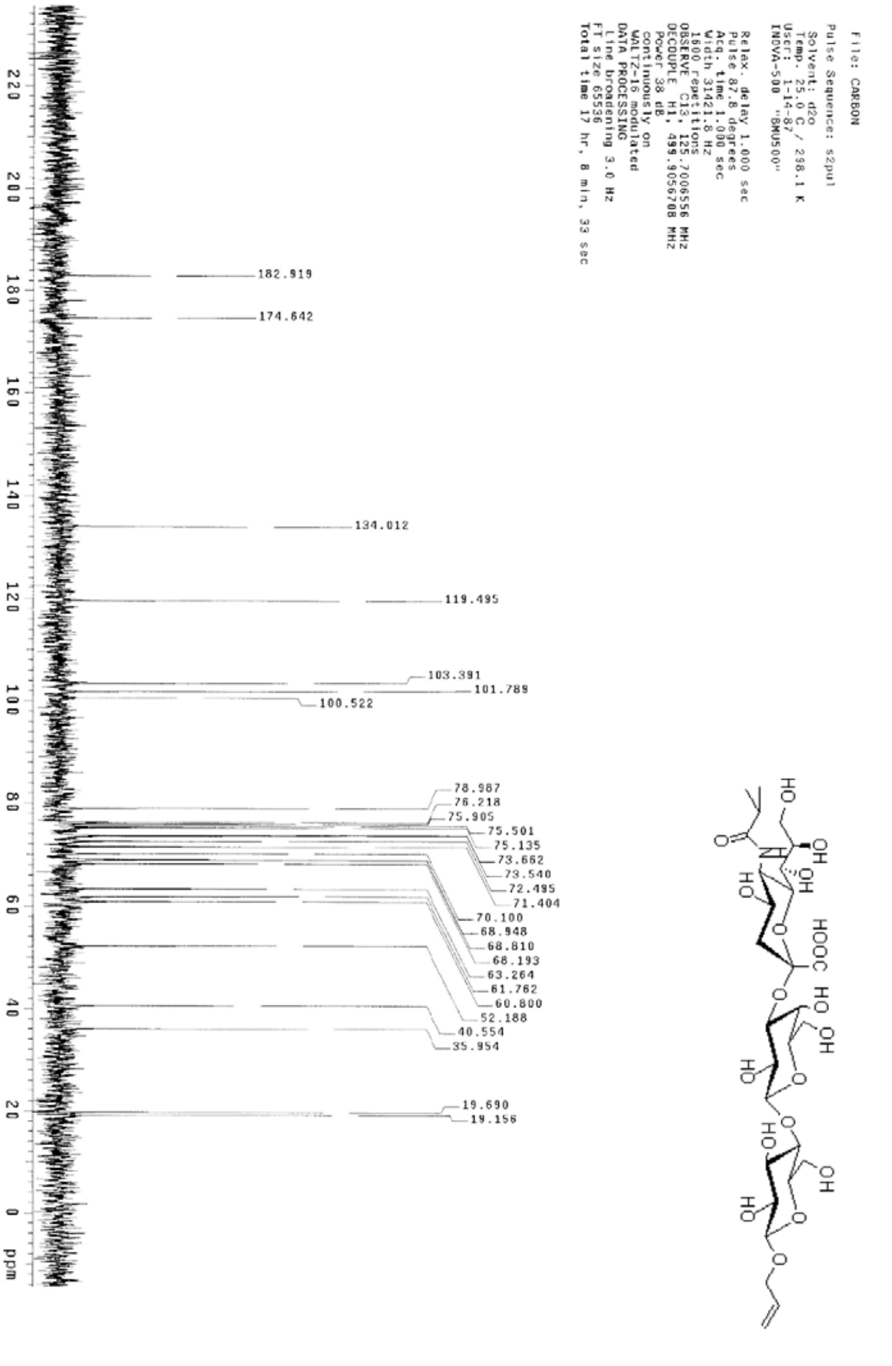
^{1}H NMR of Compound **4**



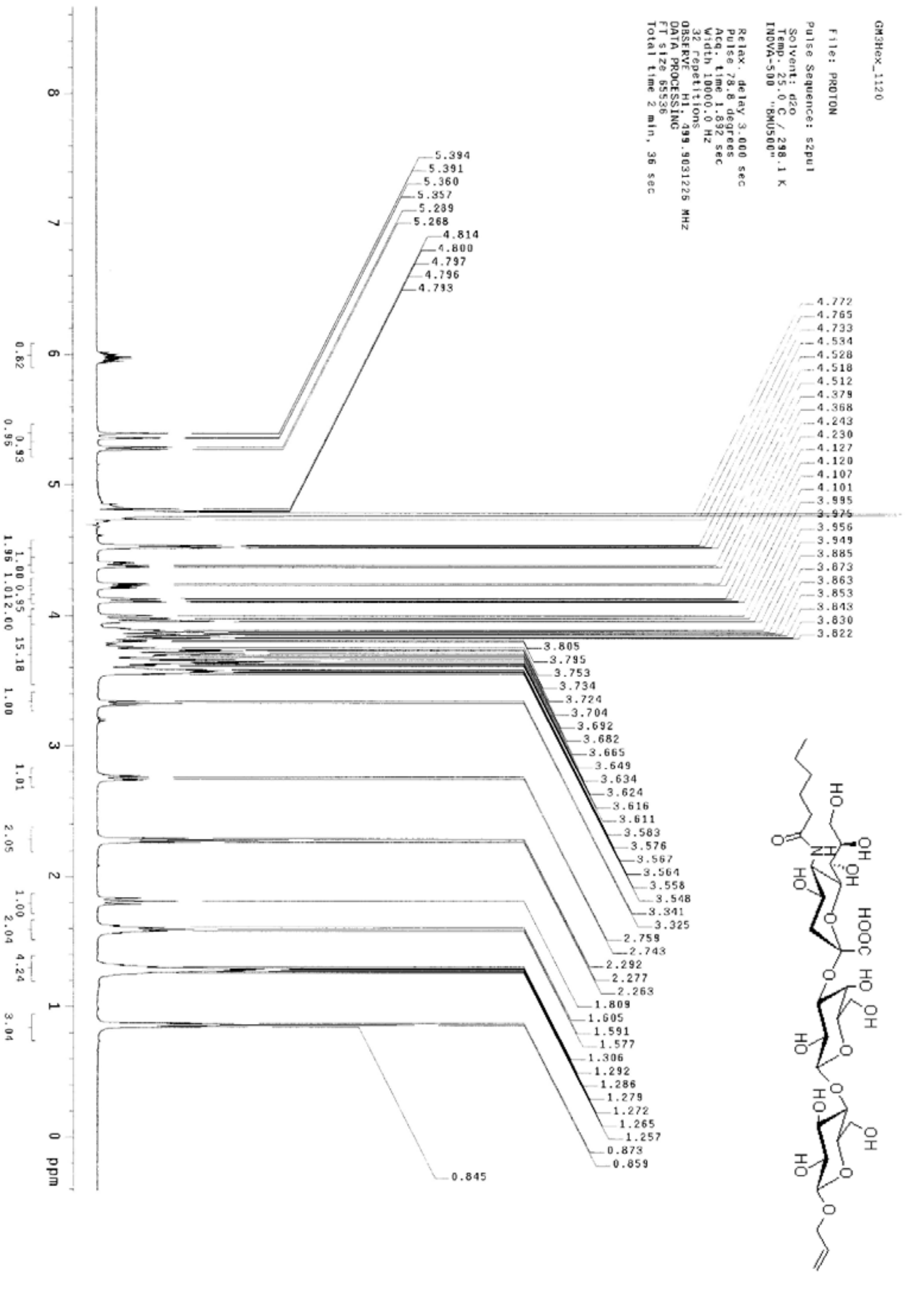
^{13}C NMR of Compound **4**



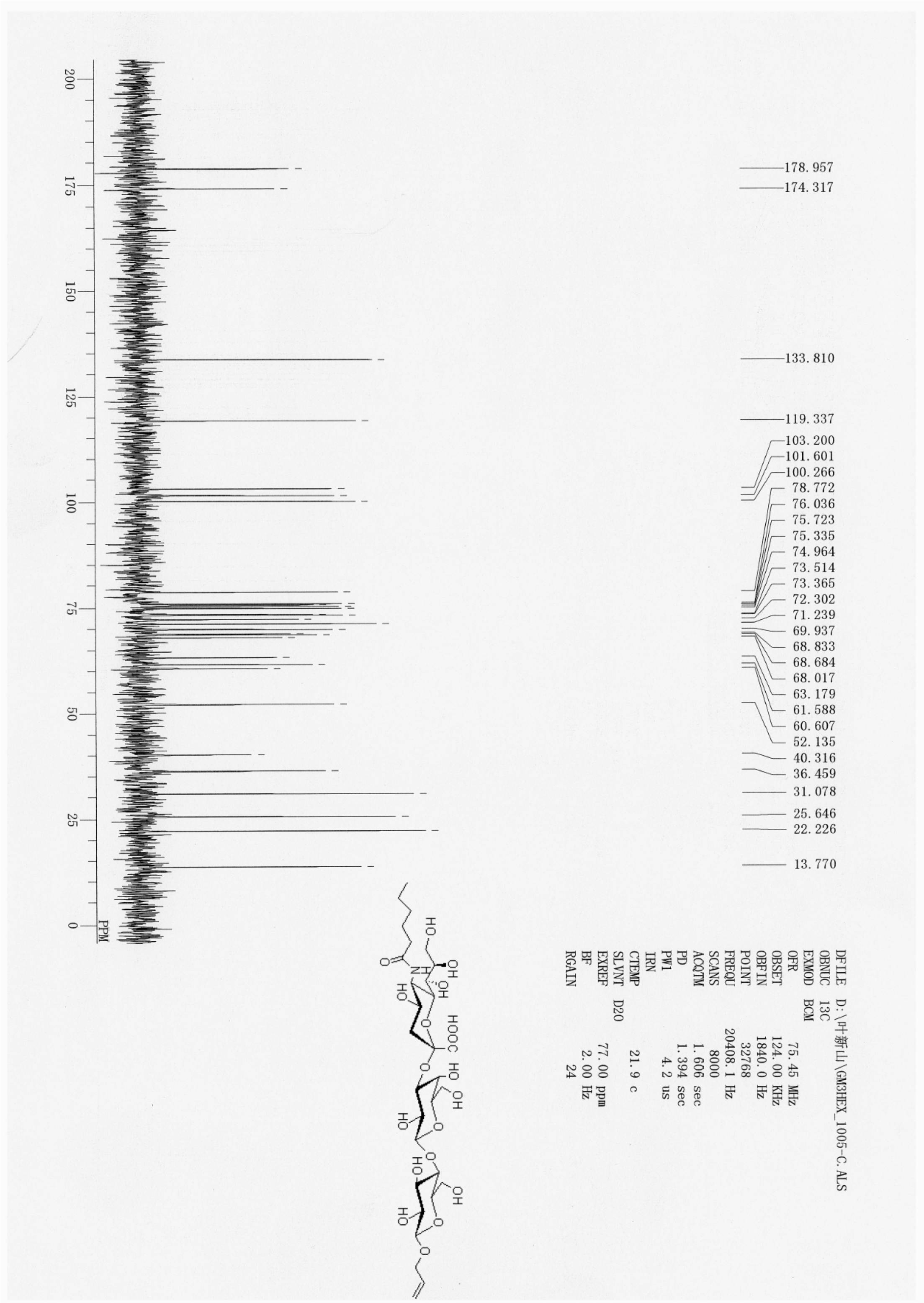
^{1}H NMR of Compound **5**



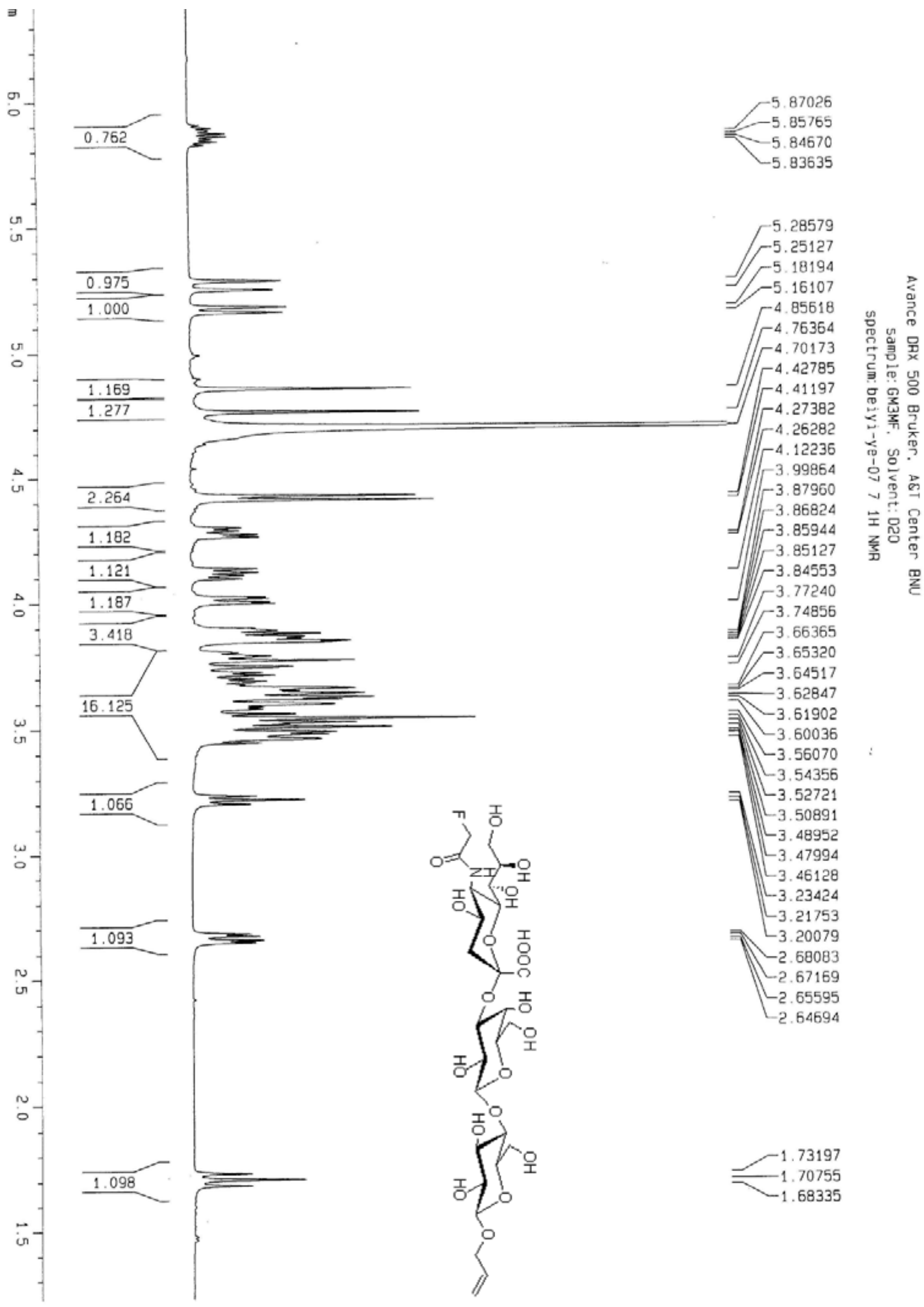
^{13}C NMR of Compound **5**



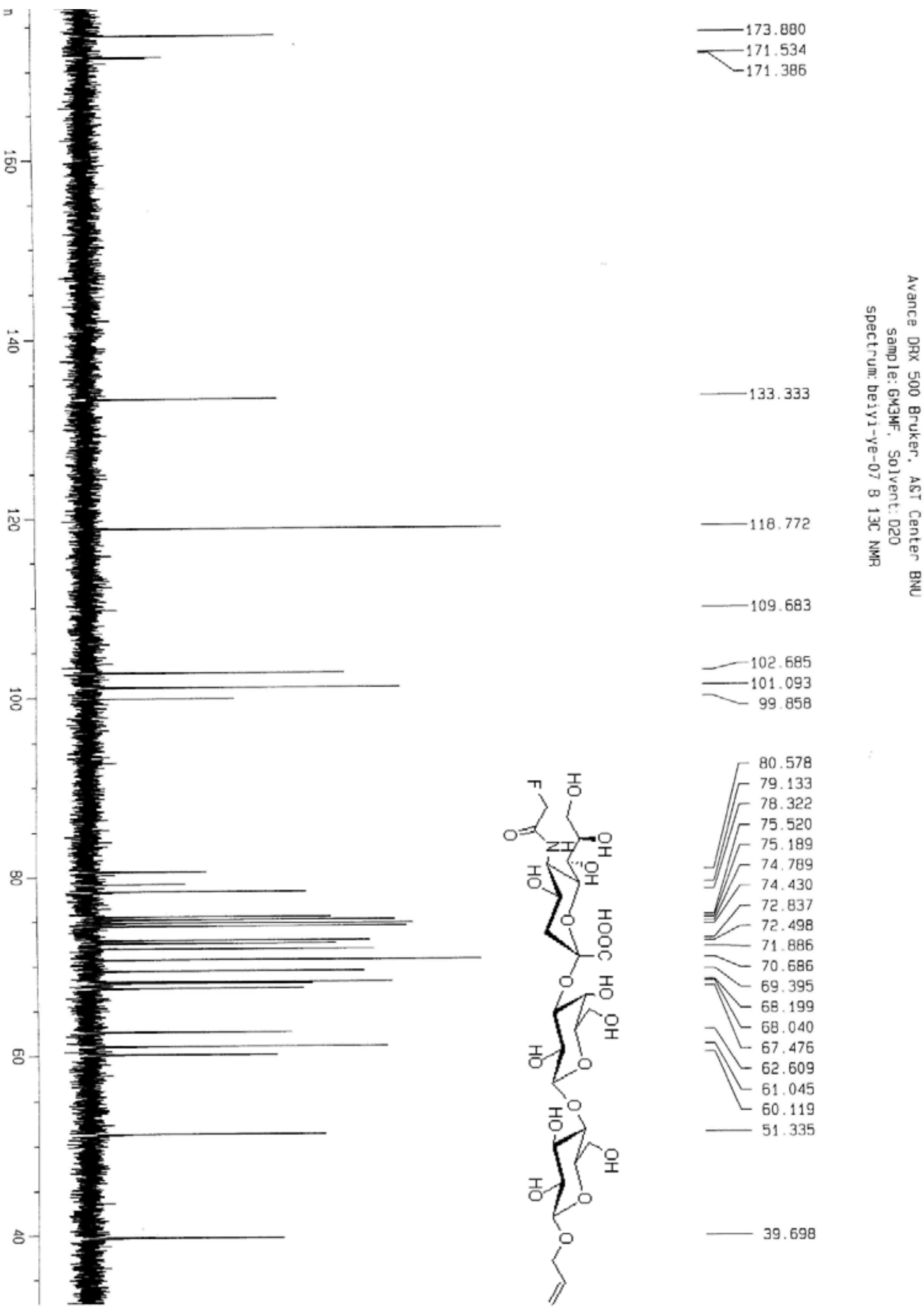
^{1}H NMR of Compound **6**



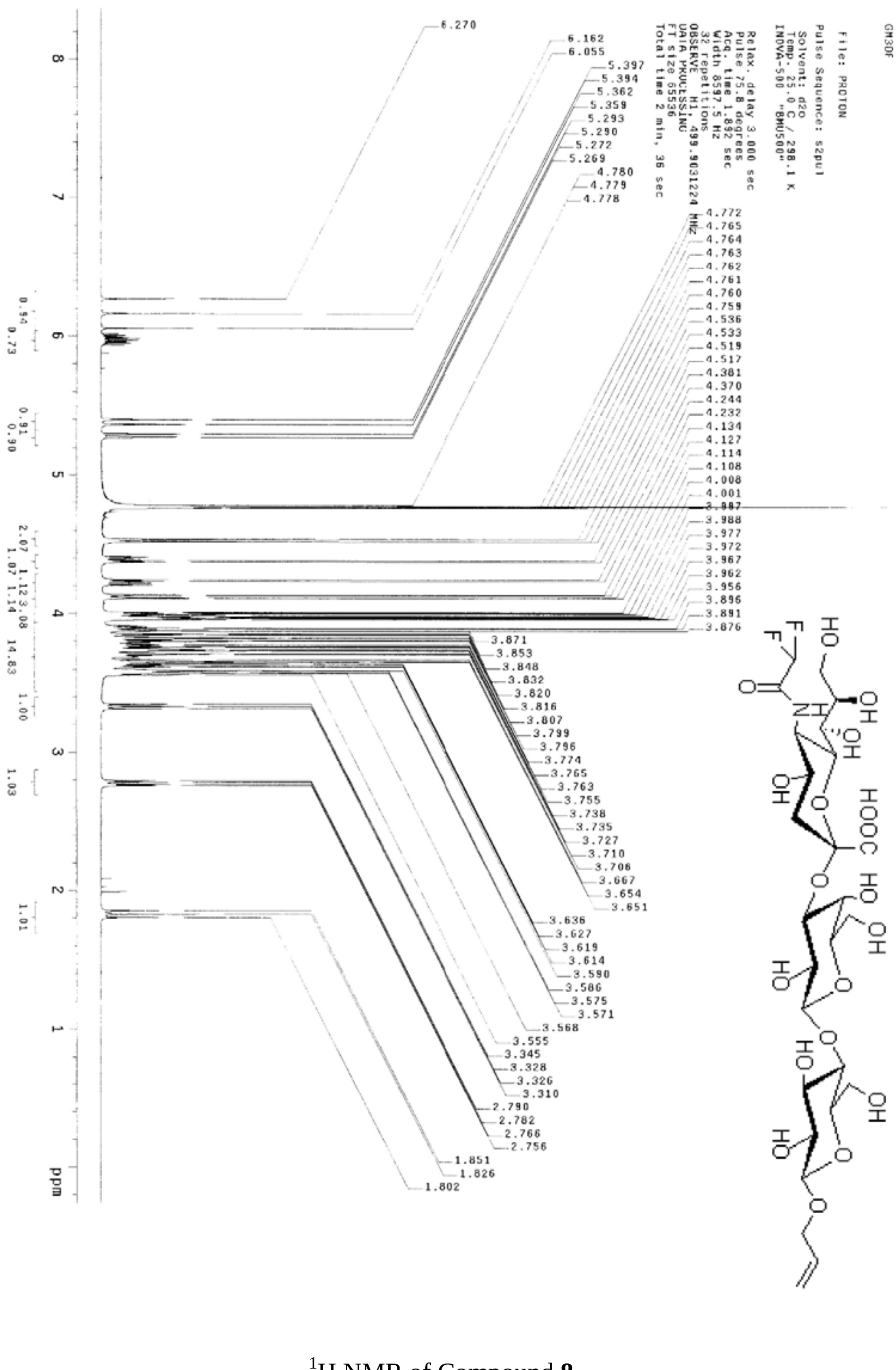
^{13}C NMR of Compound **6**



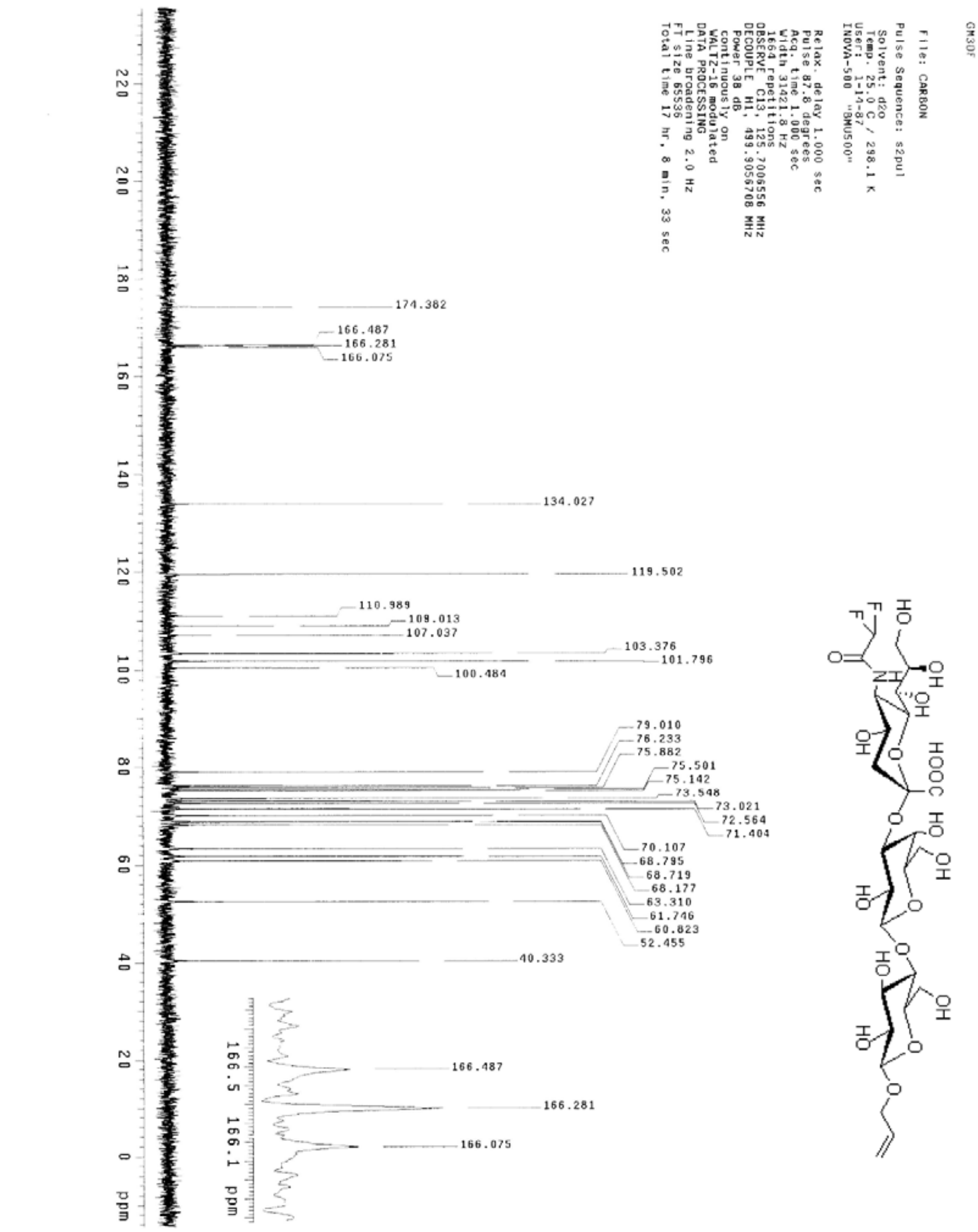
^{1}H NMR of Compound **7**



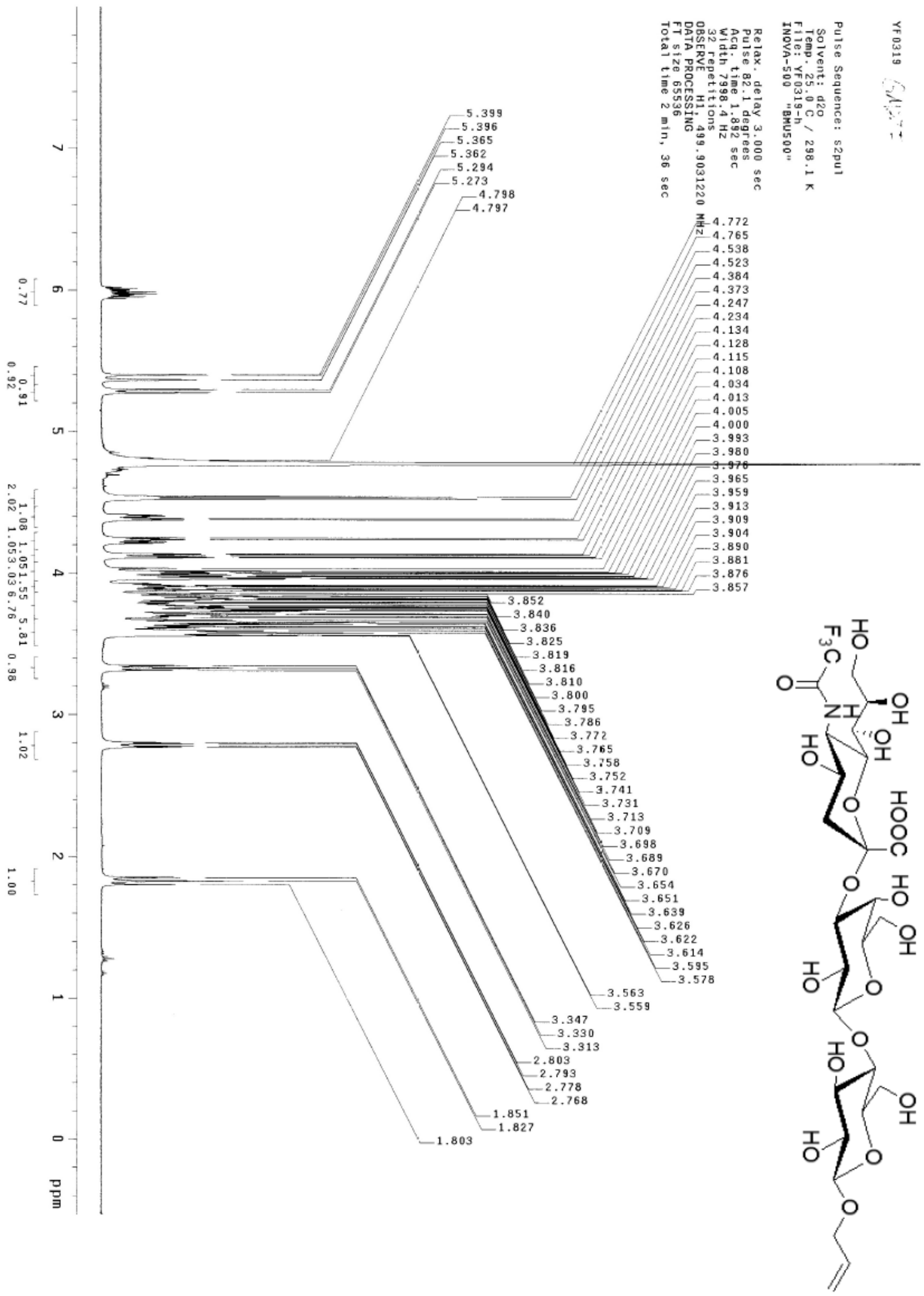
^{13}C NMR of Compound **7**



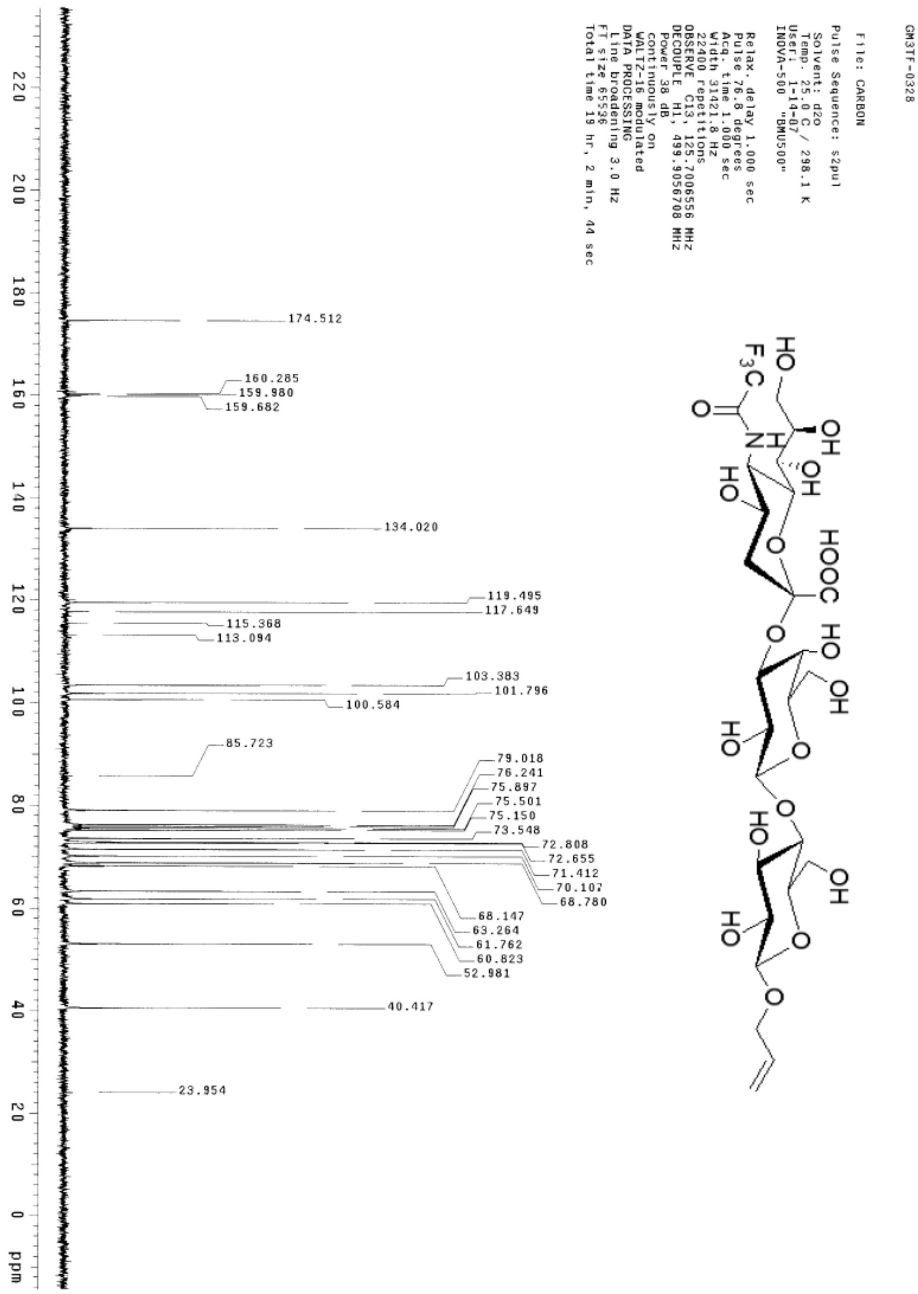
[1]H NMR of Compound **8**



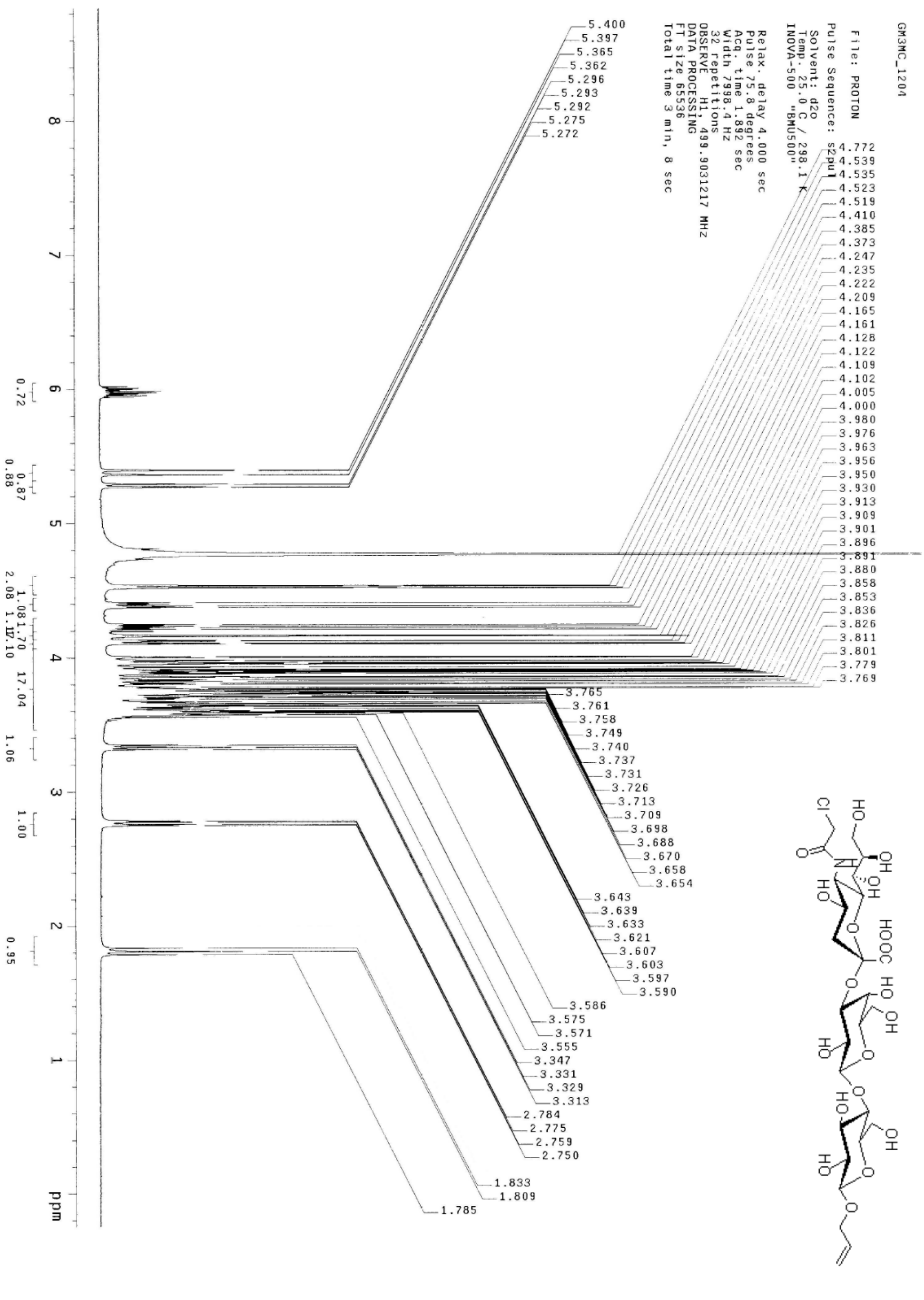
^{13}C NMR of Compound **8**



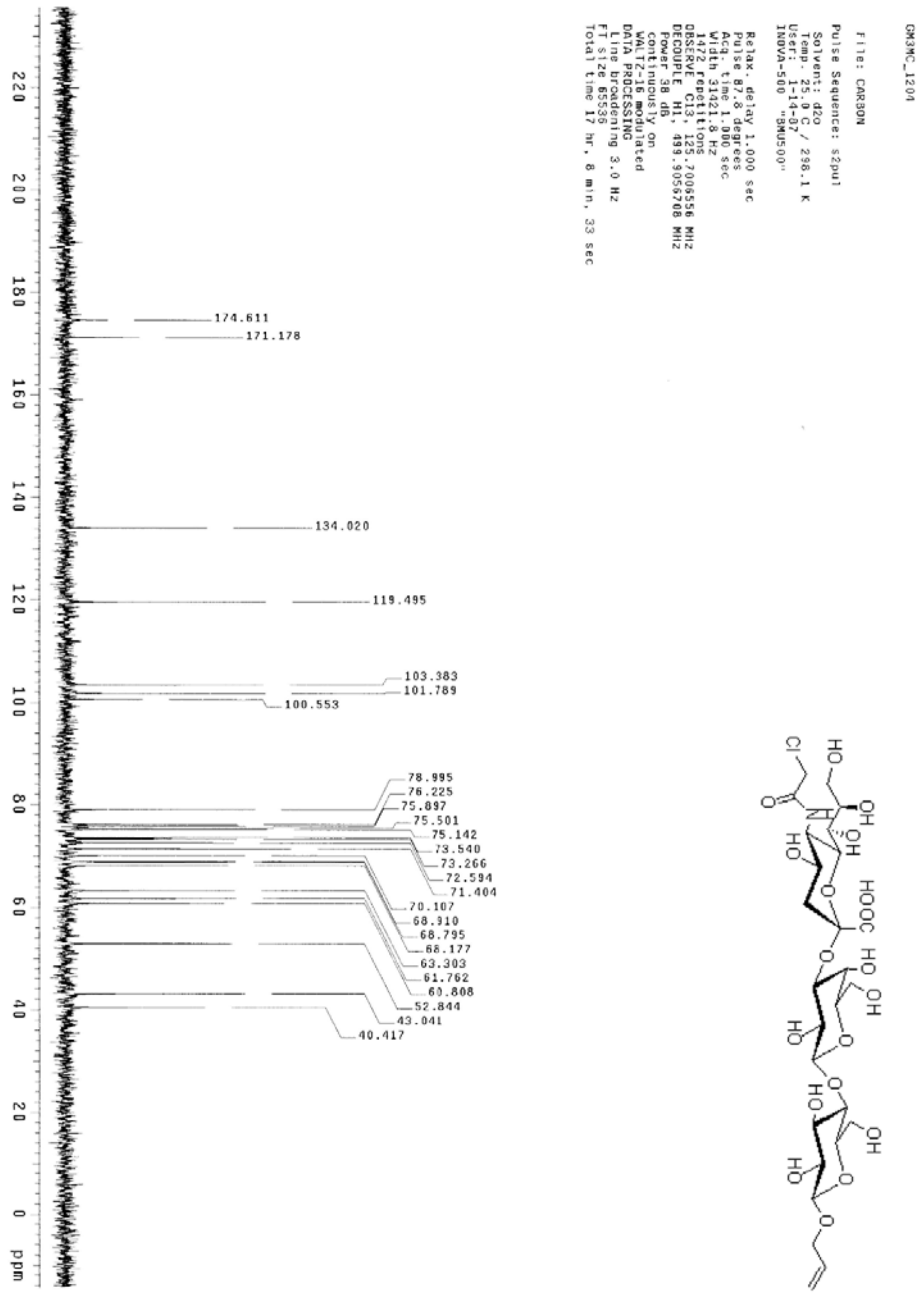
^{1}H NMR of Compound **9**



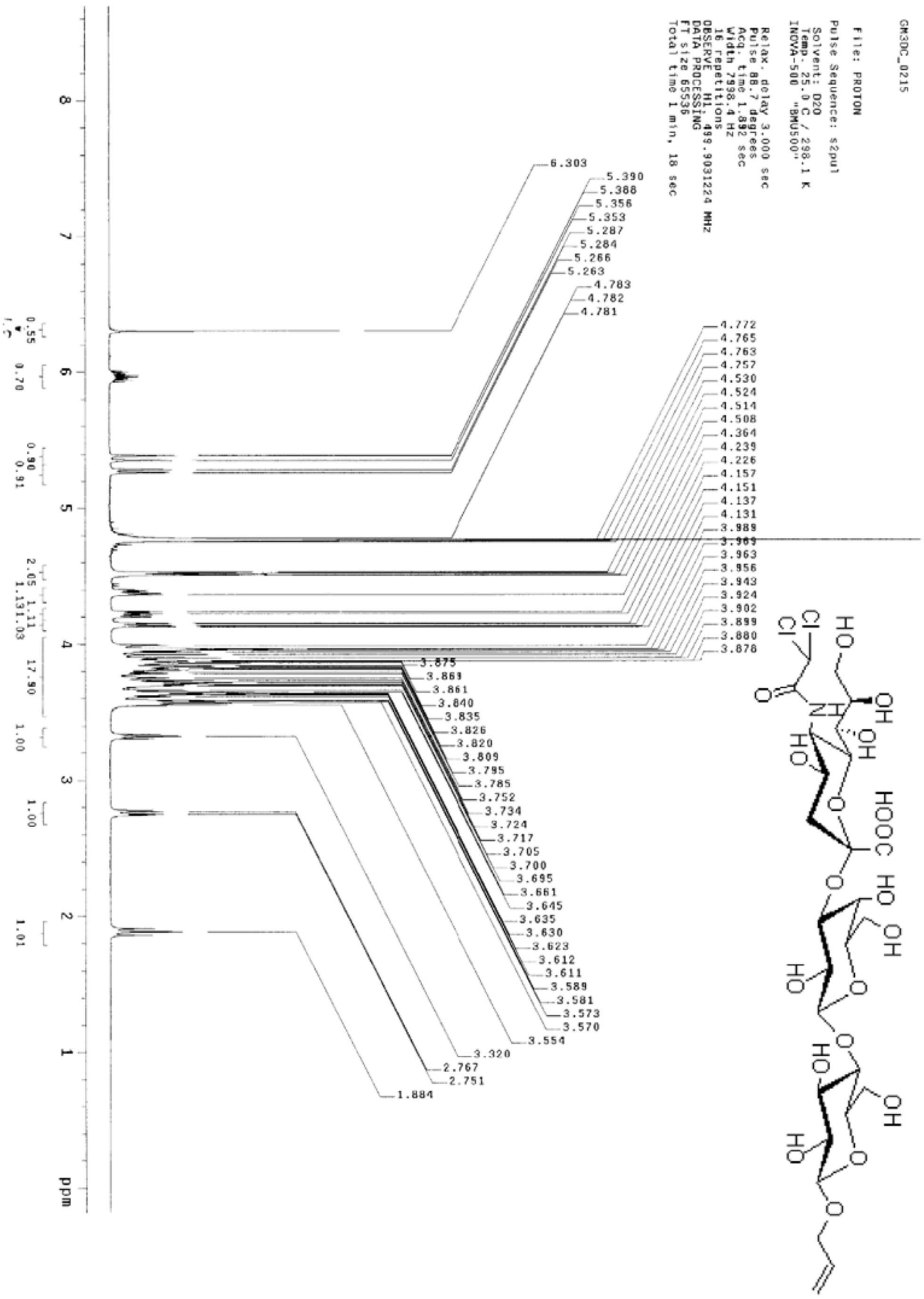
^{13}C NMR of Compound **9**



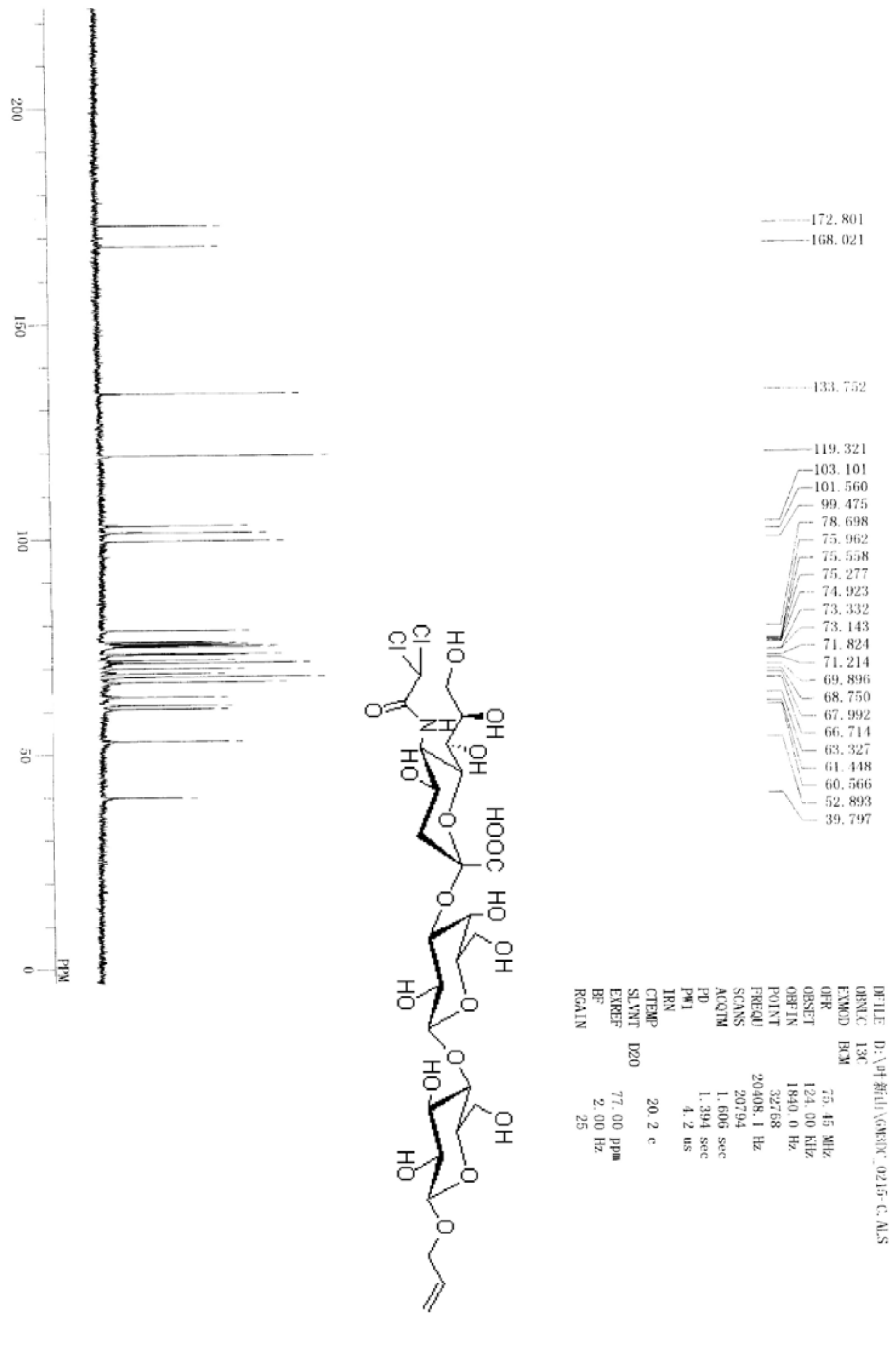
[1]H NMR of Compound **10**



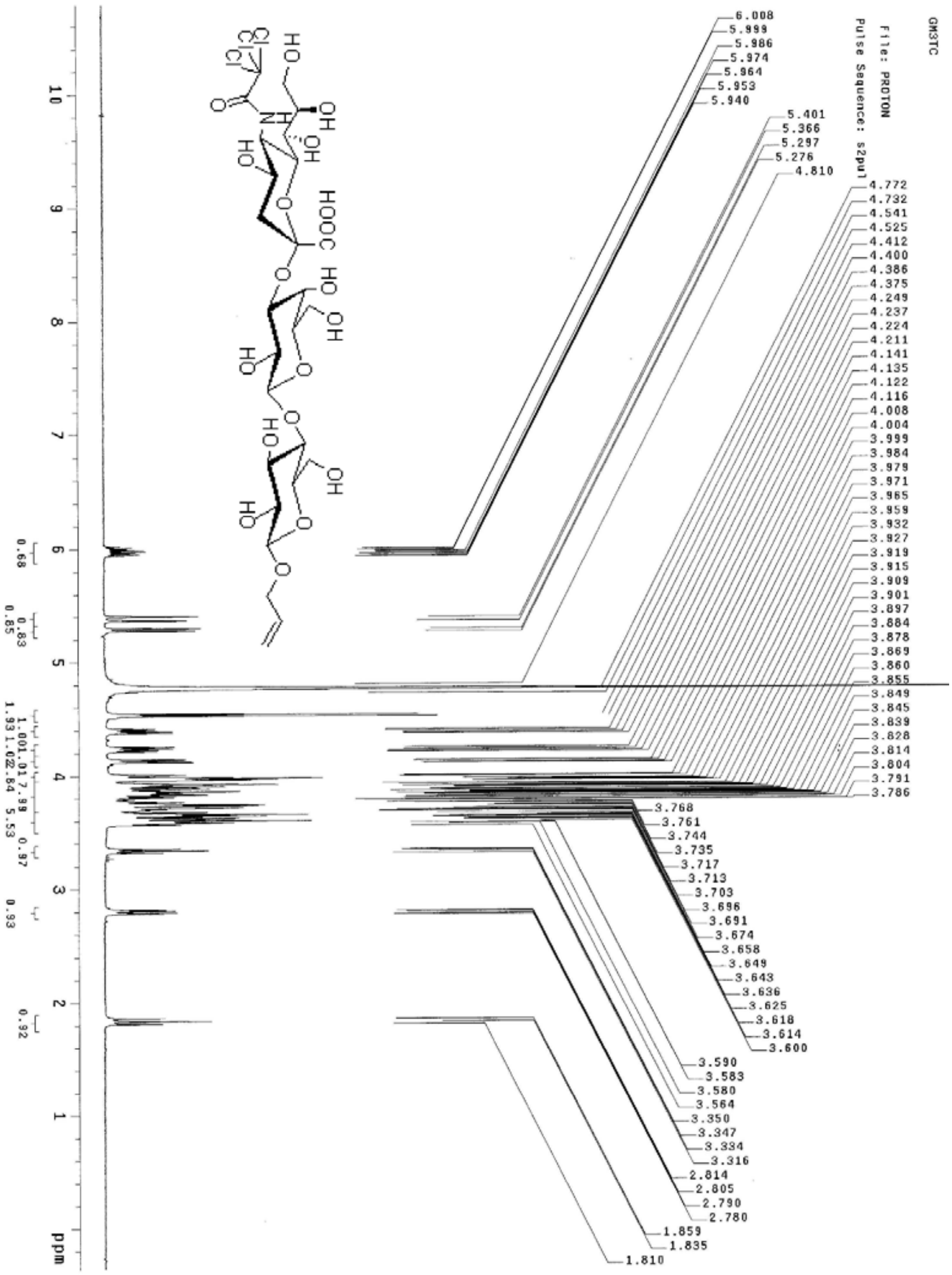
^{13}C NMR of Compound **10**



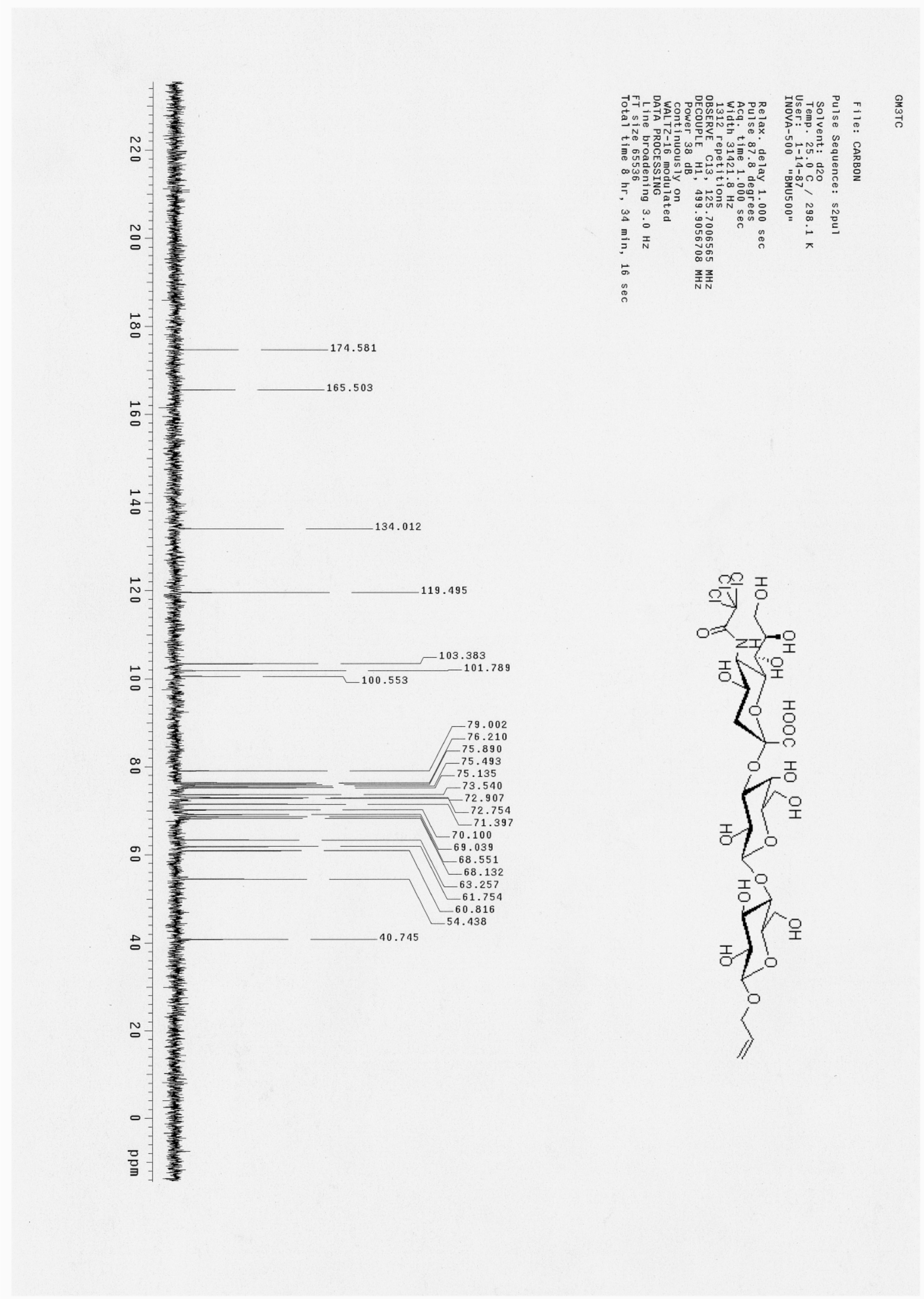
[1]H NMR of Compound **11**



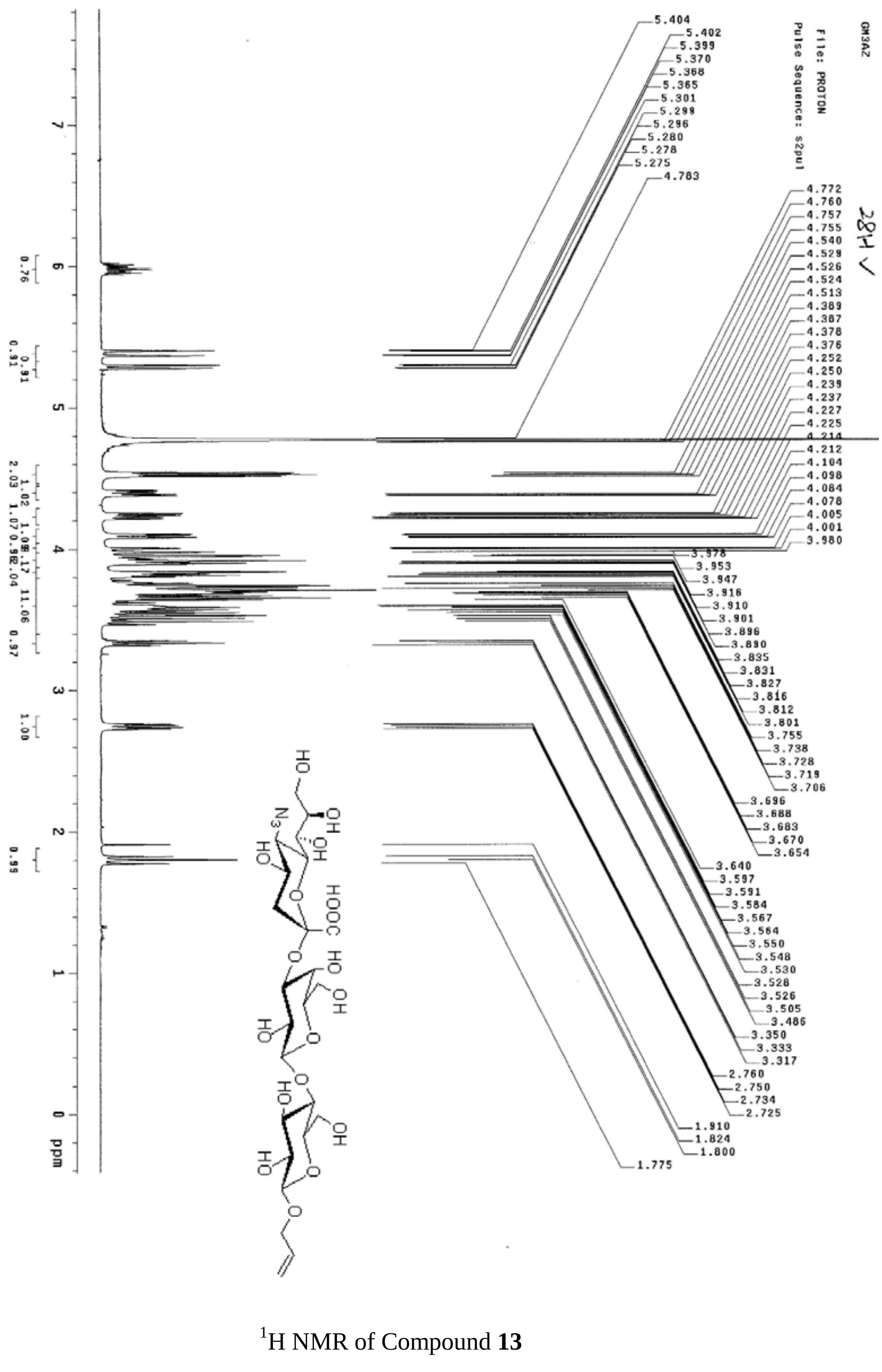
^{13}C NMR of Compound **11**



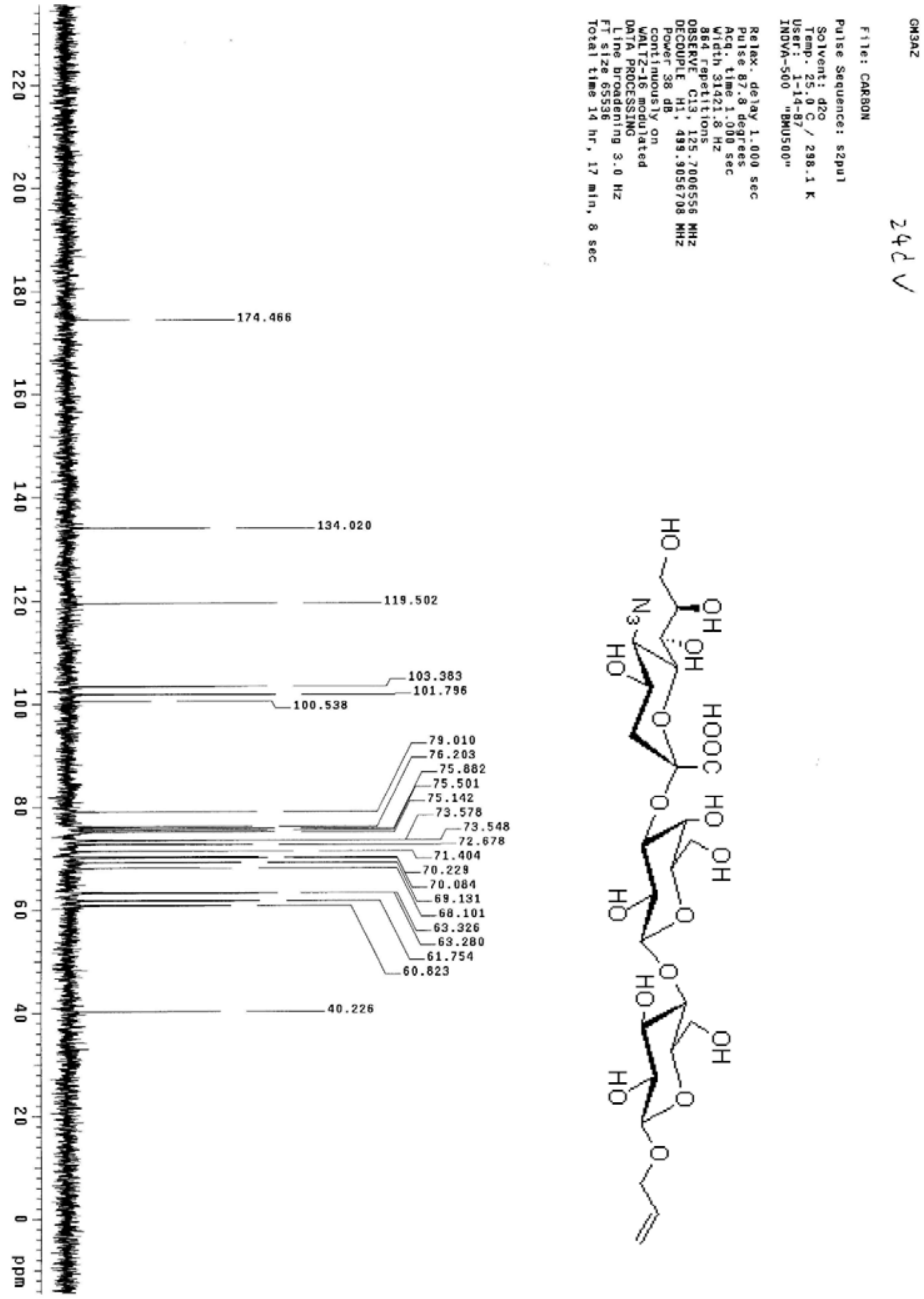
^{1}H NMR of Compound **12**



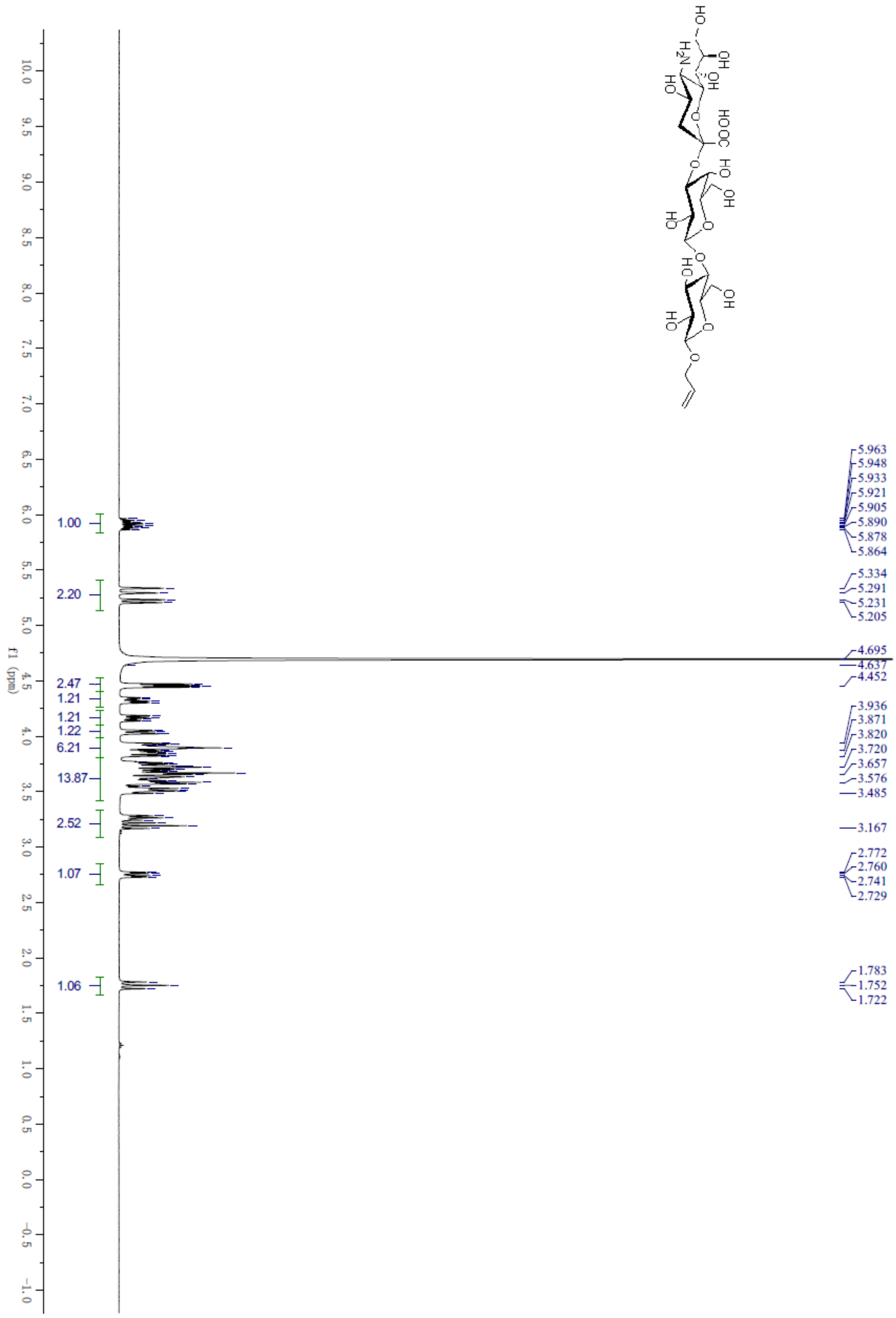
[13]C NMR of Compound **12**



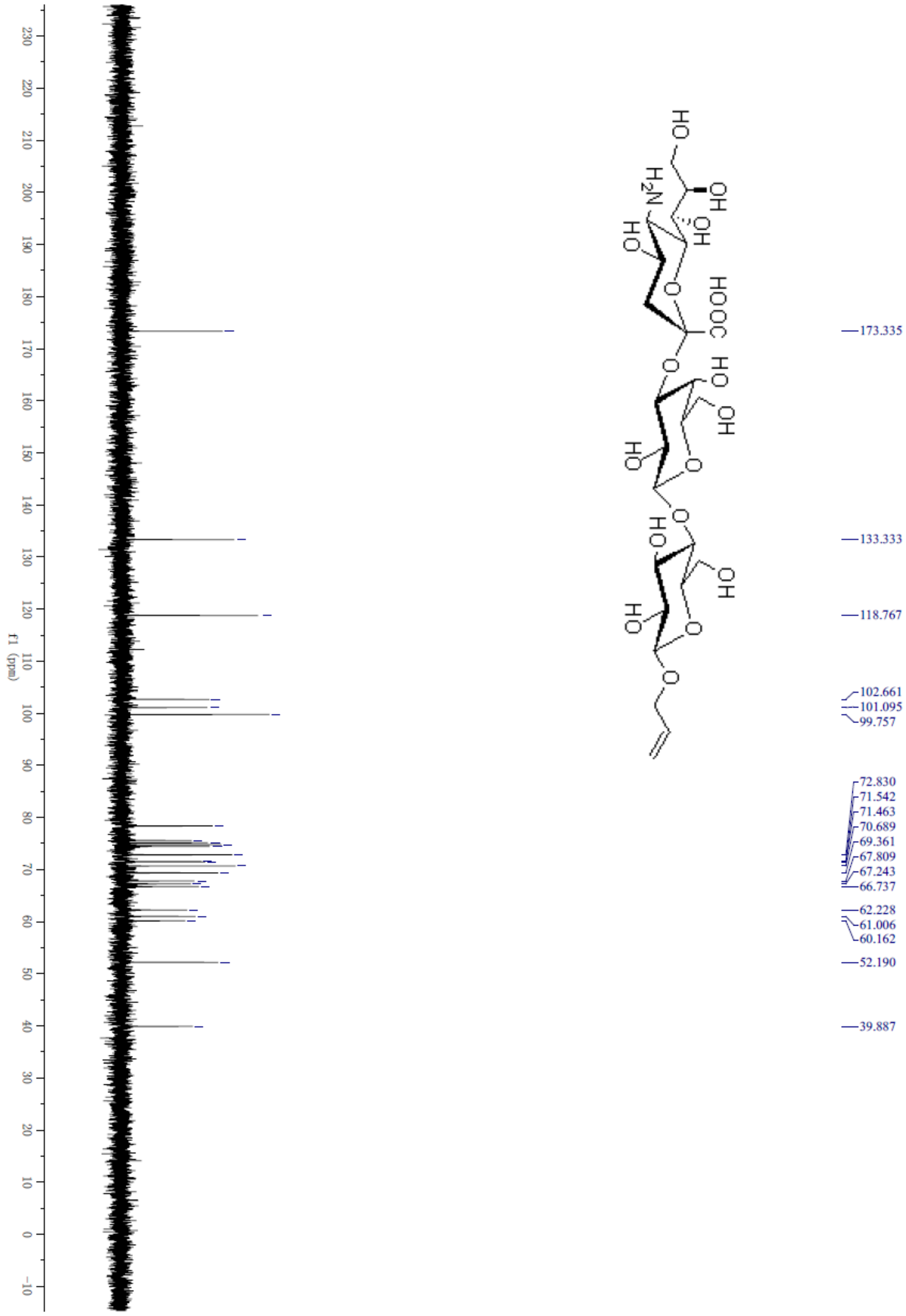
[1]H NMR of Compound **13**



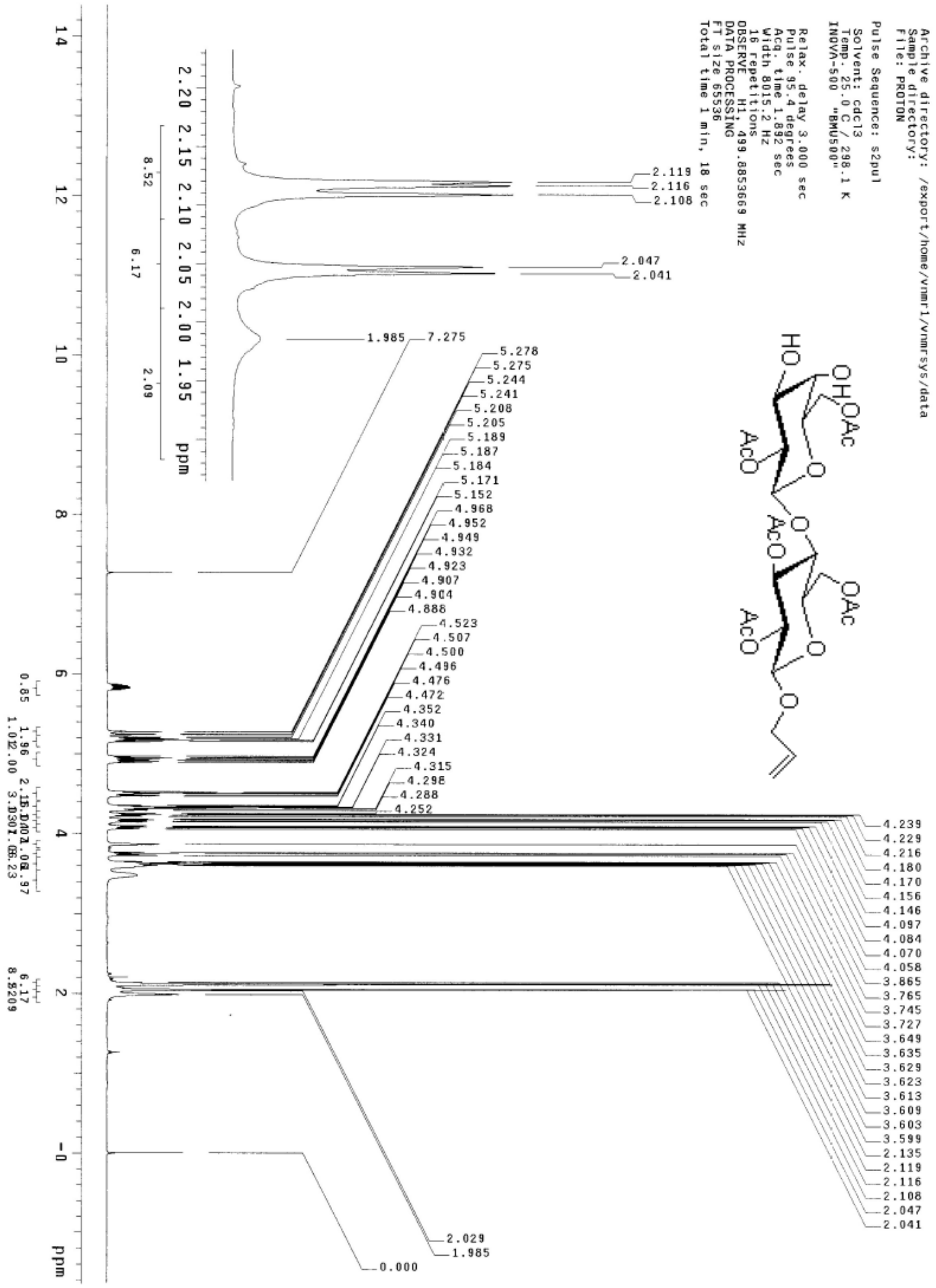
^{13}C NMR of Compound **13**



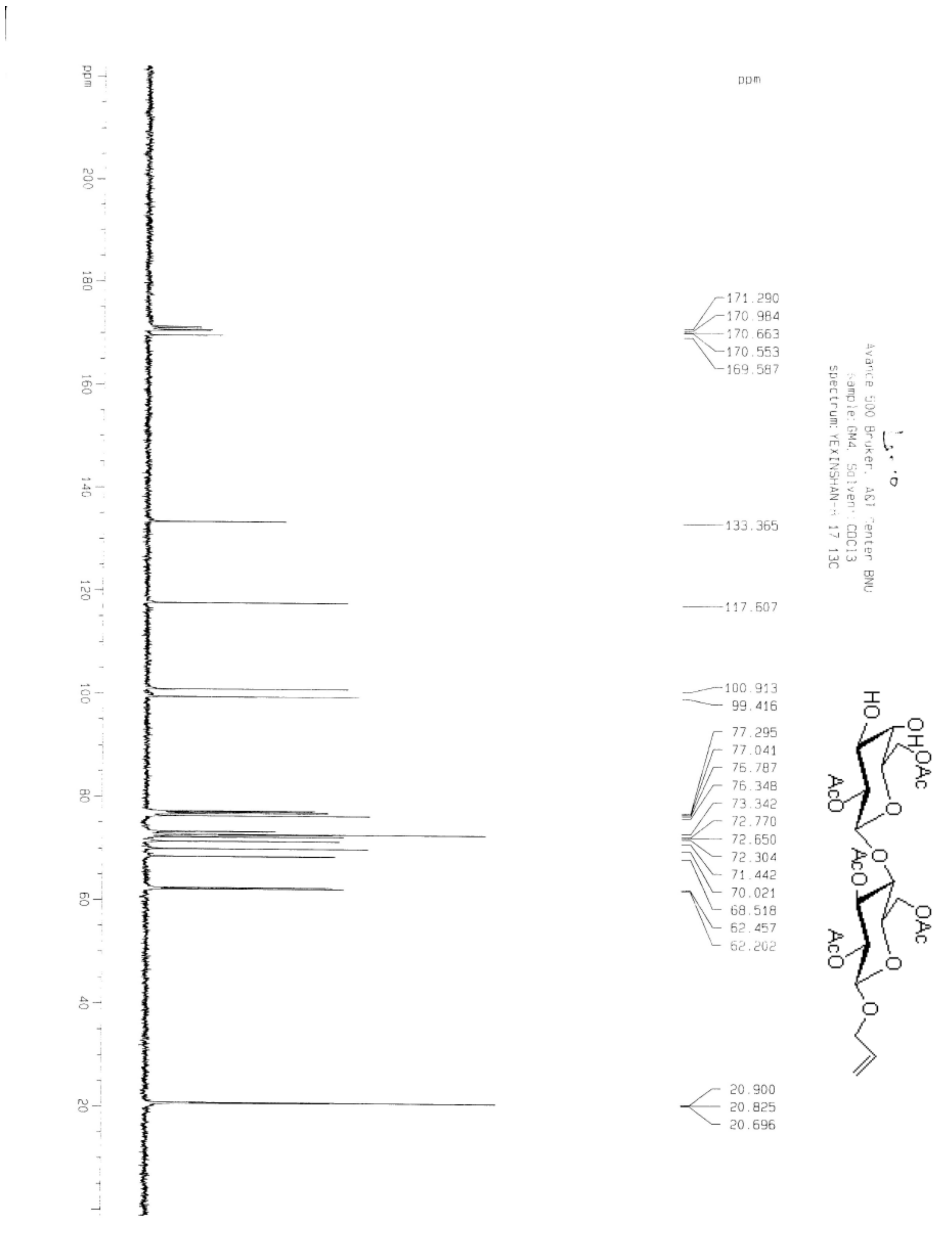
^{1}H NMR of Compound **14**



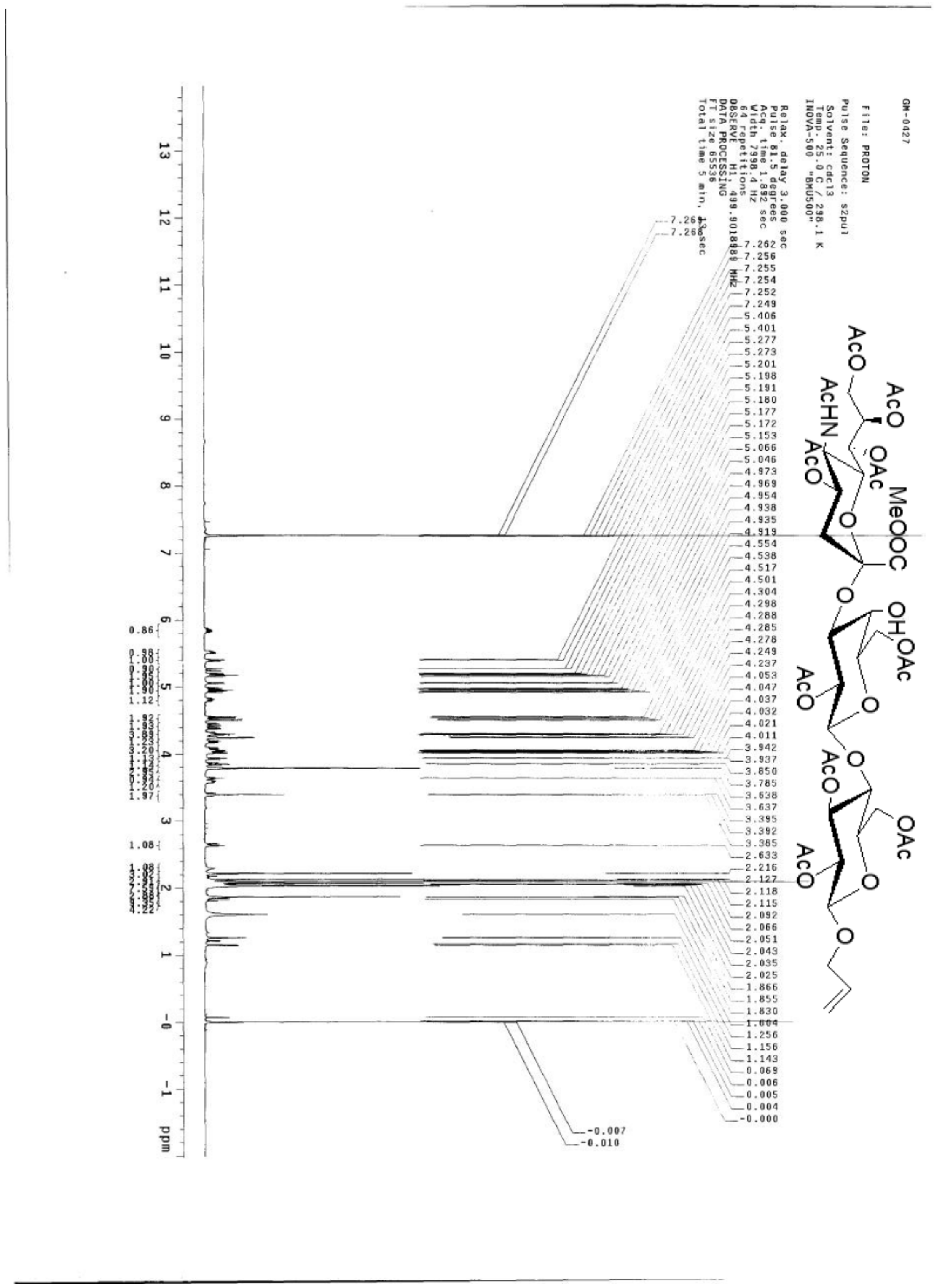
^{13}C NMR of Compound **14**



[1]H NMR of Compound **20**



^{13}C NMR of Compound **20**



^{1}H NMR of Compound **22**