Supporting Information for

Unprecedented Role of Hybrid *N*-Glycans as Ligands for HIV-1 Broadly Neutralizing Antibodies

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Table of Contents

I. Supporting Figures

1. Schematic illustration of HIV-1 gp160 glycosylation.....S3
2. Hybrid glycan simulations.....S4

II. Synthesis of hybrid type oligosaccharides

1. Material and methods.....S5
2. General procedures.....S5
3. Synthesis of hybrid form G1-G12 …….....S7

III. Glycan array analysis

1. Microarray fabrication.....S30
2. Antibody binding assay.....S30
3. Analyzing glycan binding specificity of HIV-1 bNAbs on NHS-coated glass slide…...…S31
4. Determination of surface dissociation constants on ACG slide.....S32

IV. References…….…….....S45

V. NMR characterization data……..S46

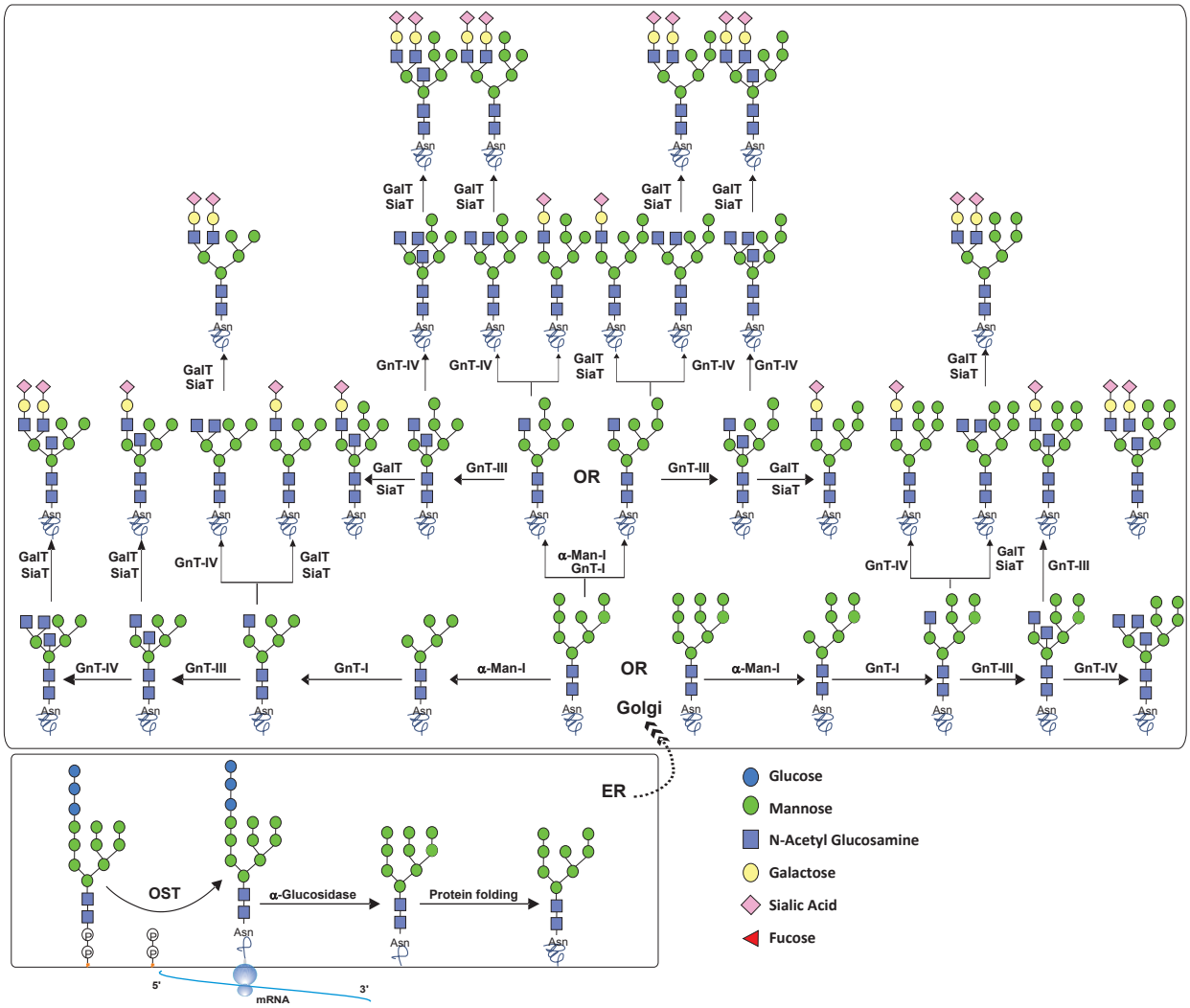


Figure S1. HIV-1 gp160 *N*-linked glycosylation. The schematic illustration of possible hybrid *N*-glycans those could be formed on HIV surface depending on the location of glycosyltransferases and glycosidases, and the timing of glycoform exposure to these various enzymes.

(a) *Ref: Nat. Struct. Mol. Biol. 2013, 20, 804.*

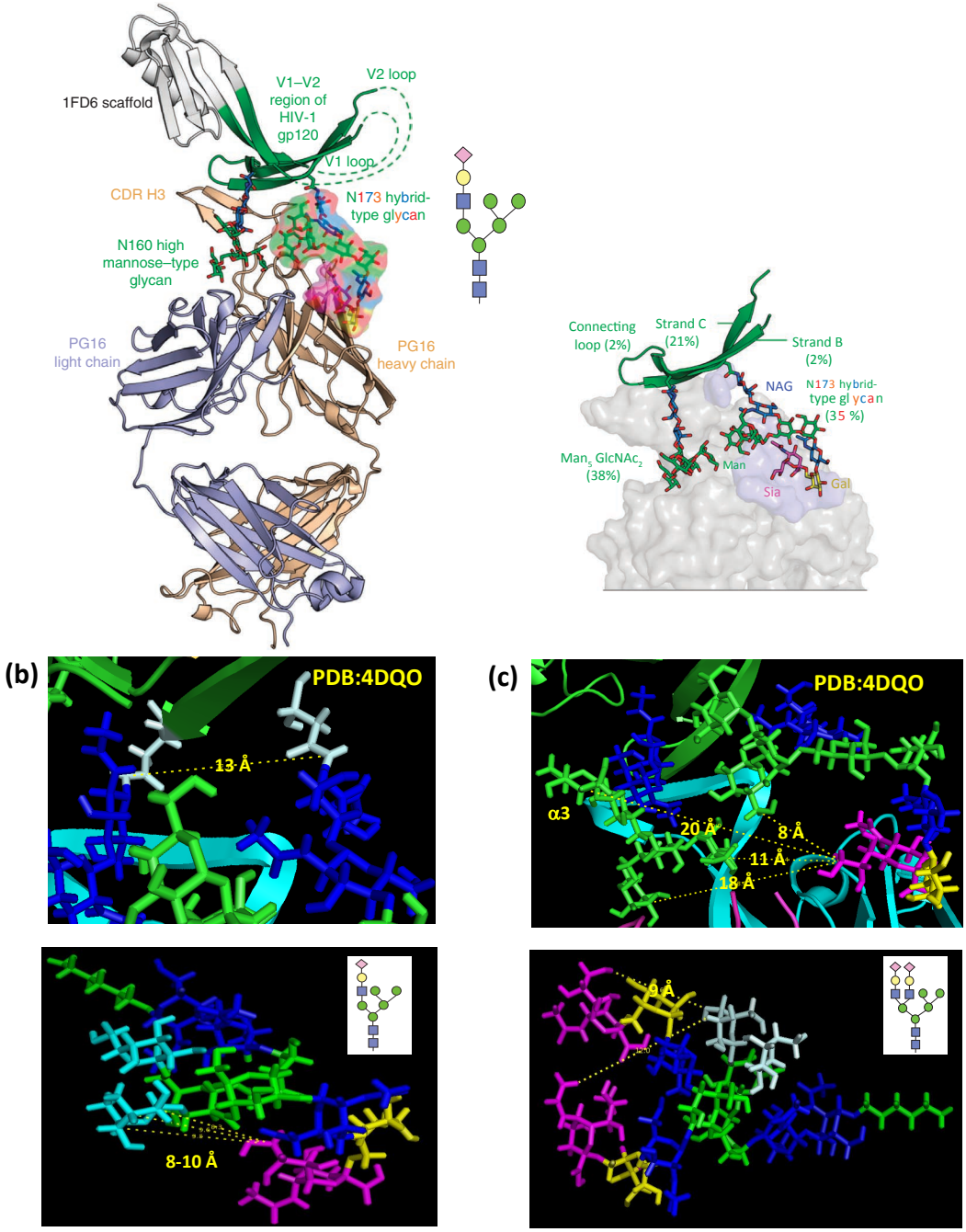


Figure S2. Hybrid type glycan simulations. a) From ref [1]: PG16 interactions with hybrid type glycan at residue 156 or 173 on gp120. (b, c) PDB ID 4DQO processed using pymol to determine distance between reducing end GlcNAc (13 Å) and terminal sialic acid of hybrid glycan at N173 and closest mannose either on the same glycan or mannose residues of adjacent Man_5 at N160 (8-10 Å). (d, e) DFT and AM1 based computational methods to determine distance between complex and mannose arms of synthetic hybrid glycans[2,3].

2. Synthesis of hybrid type oligosaccharides

i. Materials and Methods

All reagents were purchased from Sigma Aldrich, Across and used without further purification. Dry solvents were purchased from a commercial source without further distillation. Pulverized Molecular Sieves MS-4 Å (Aldrich) for glycosylation was activated by heating at 350 ºC for 3 h. Reactions were monitored by analytical thin-layer chromatography (TLC) in EM silica gel 60 F254 plates and visualized under UV (254 nm) and/or by staining with acidic ceric ammonium molybdate or *p*-anisadehyde. Flash chromatography was performed on silica gel (Merck) of 40-63µm particle size. ^{1}HNMR spectra were recorded on a Bruker AVANCE 400 and 600 (400 and 600 MHz) spectrometer at 25 ºC. All ^{1}H Chemical shifts (in ppm) were assigned according to $CDCl_3$ (δ = 7.24 ppm) and D_2O (δ = 4.80 ppm). ^{13}C NMR spectra were obtained with Bruker AVANCE 600 spectrometer and were calibrated with $CDCl_3$ (δ = 77.00 ppm). Coupling constants (*J*) are reported in hertz (Hz). Splitting patterns are described by using the following abbreviations: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet. ^{1}H NMR spectra are reported in the following order: chemical shift, multiplicity, coupling constant(s) and number(s) of protons. High resolution ESI mass spectra were recorded on a Bruker Daltonics spectrometer. NHS coated glass slides were purchased from SCHOTT (Nexterion H). Broadly neutralizing HIV antibodies were kindly gifted by Prof. Peter Kwong, NIH and by Prof. Dennis Burton, TSRI. Secondary antibody DyLight649-conjugated donkey anti-Human IgG was purchased from Jackson Immuno Research. Cytidine 5'-triphosphate (CTP), *N*-Acetylneuraminic acid (Neu5Ac), were purchased from Sigma-Aldrich.

ii. General Procedures.

1. Enzyme expression, purification and reactions:

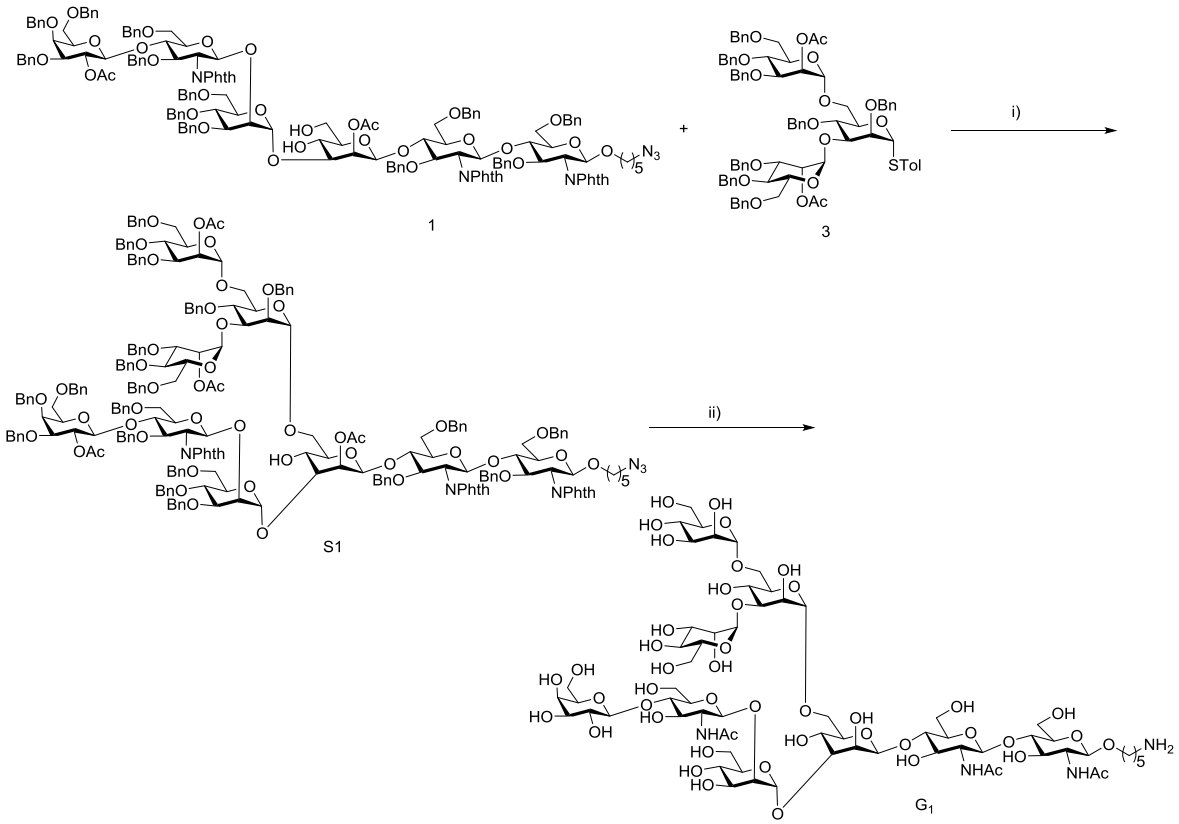
The functional domains of α-2,3 sialyltransferase (JTFAJ-16)[4] and α-2,6 sialyltransferase (JT-ISH-224)[5] were obtained according to our previous report[6]. The enzymes used in this work including pyruvate kinase (PK), pyrophosphatase (PPA), cytidine monophosphate kinase (CMK), and CMP-sialic acid synthetases (CSS) are examples of suitable recombinant enzymes that are expressed and purified in our laboratory. Enzymatic reactions with cofactor regeneration were carried out according to the procedure reported previously [7].

2. Global deprotection: A mixture of protected glycans (50 mmol) and 10 mL of ethylene diamine: *n*-BuOH (1: 4) was stirred at 90 ºC for overnight. Volatiles were then evaporated and the crude product was reacted with 10 mL Ac_2O/pyridine (1:2) for overnight. The solvents were removed using high vacuum and product was purified by flash column chromatography (acetone: toluene, 2/8, v/v). Product was de-acetylated using sodium methoxide in MeOH (10 mL) for overnight. The reaction mixture was neutralized by using IR-120, filtered and concentrated in *vacuo.* The residue was purified by flash column chromatography (acetone: toluene, 3/7, v/v). Product was dissolved in 10 mL MeOH: H_2O: HCOOH (6:3:1), $Pd(OH)_2$ (50% by weight) was added and the reaction mixture was hydrogenated for overnight. The reaction mixture was filtered through Celite and concentrated in *vacuo.* The residue was purified by Bio-Gel P-2 (BIO-RAD) column chromatography using water as eluent, and the product was the lyophilized to get desired oligosaccharides as a white color powder.

3. Enzymatic sialylation with cofactor regeneration: Glycans (5 μmol), Neu5Ac (10 μmol), ATP (0.05 μmol), CTP (1 μmol), phosphoenolpyruvate (10 μmol, monopotassium salt), cytidine monophosphate kinase (CMK, 80 units), CMP-sialic acid synthetases (CSS, 120 units), pyruvate kinase (PK, 40 units), pyrophosphatase (PPA, 40 units) and α 2,6/2,3 sialyltransferase (150 units)

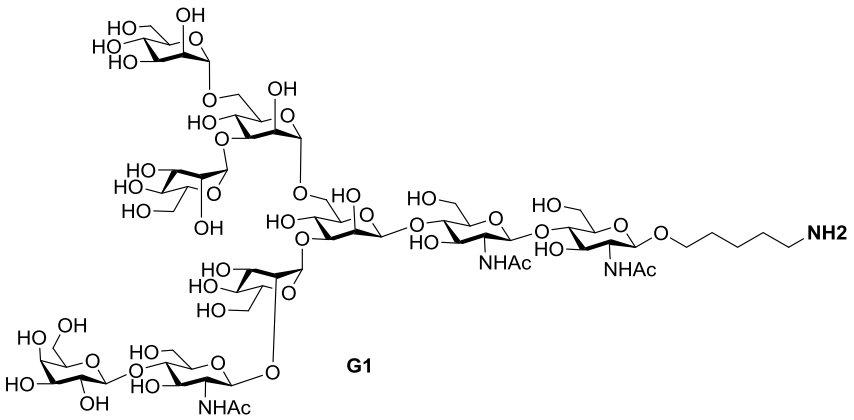
were dissolved in 50 μM Tris buffer (25 mmol, pH 7.5). The reaction was incubated at 37 °C with gentle agitation. Complete consumption of starting material was confirmed by mass spectrometric analysis. The reaction mixture was centrifuged and the supernatant subjected to gel filtration over P2-Biogel (eluent water). Fractions containing the product were combined and lyophilized to give the respective products as amorphous white solids.

4. Preparation of hybrid glycan G1



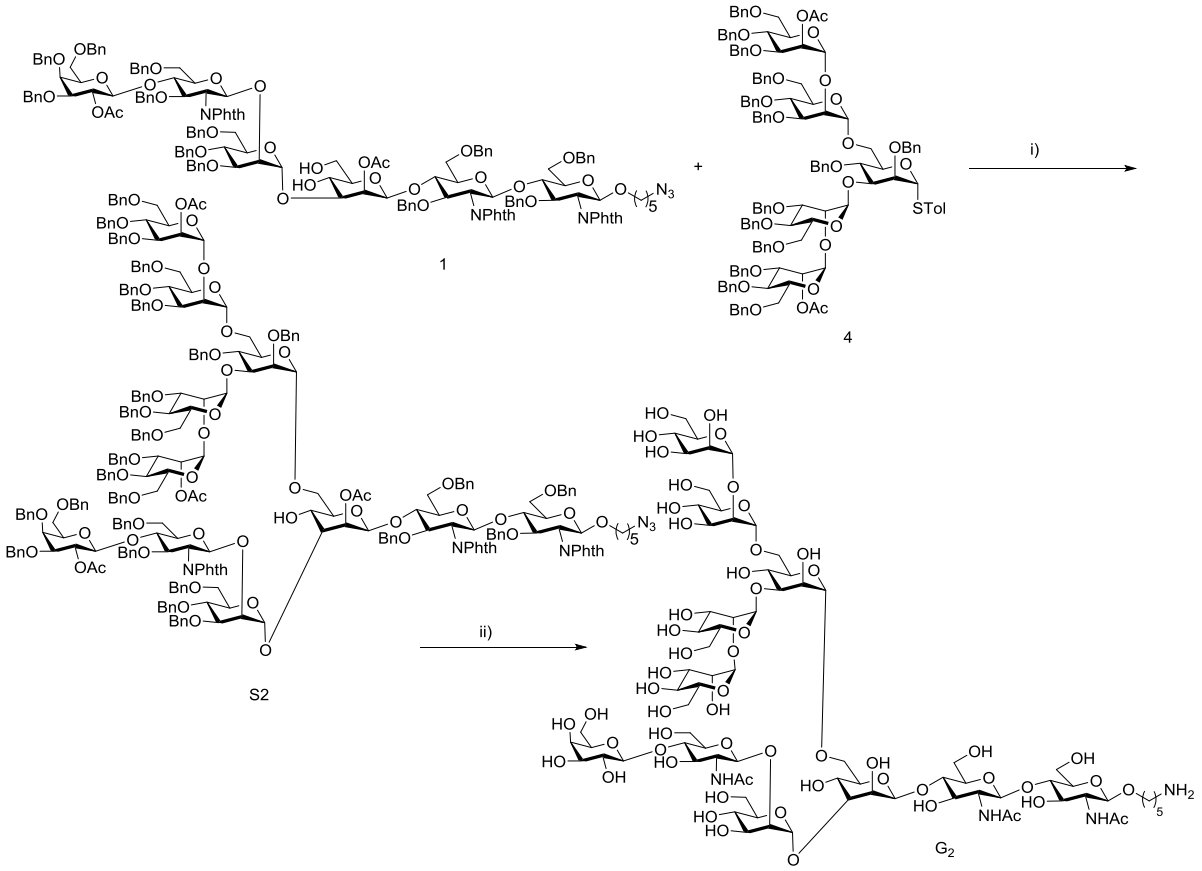
Scheme S1. Reagents and reaction conditions. i) $(BrC_6H_4)_3NSbCl_6$, CH_3CN, 4 Å MS, -10 ºC, overnight, 39%; ii) (1) $NH_2CH_2CH_2NH_2$, *n*-BuOH, 90 ºC, overnight; (2) Ac_2O, pyridine, overnight; (3) NaOMe, MeOH, overnight; (4) $Pd(OH)_2$, MeOH: H_2O : HCOOH (5:3:2), H_2; 55 %; $(BrC_6H_4)_3NSbCl_6$: Tris (4-bromophenyl) ammoniumyl hexachloroantimonate.

Compound S1: A mixture of hexasaccharide acceptor **1** (0.190 g, 0.071 mmol), thiomannoside donor **3** (0.121 g, 0.085 mmol) and activated 4 Å molecular sieves (0.5 g) in CH_3CN (10 mL) was stirred at room temperature for 1 h. The resulting mixture was cooled to -10 °C, tris (4-bromophenyl) aminium hexachloroantimonate (0.120 g, 0.142 mmol) was added and stirred for overnight. TLC (acetone : toluene, 1/9) indicated formation of product with consumption of starting material, reaction was quenched by Et_3N. The reaction mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with aqueous $NaHCO_3$ (2 x 50 mL), and a brine (50 mL) solution. The organic layer was dried over Na_2SO_4 and concentrated in *vacuo*. The residue was purified by flash column chromatography (0% to 10% acetone in toluene) to afford **S1** (0.110 g, 39%) as colorless foam. TLC: (acetone: toluene = 1/9, v/v): R_f = 0.51; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.72-7.45 (m, 12H, Ar-H), 7.37-7.28 (m, 5H, Ar-H), 7.28-7.12 (m, 67H, Ar-H), 7.09-7.02 (m, 11H, Ar-H), 6.94-6.90 (m, 7H, Ar-H), 6.80-6.78 (m, 4H, Ar-H), 6.69-6.68 (m, 3H, Ar-H), 6.62-6.61 (m, 3H, Ar-H), 5.49 (s, 1H), 5.48 (s, 1H), 5.35 (t, *J* = 8.4 Hz, 1H), 5.28 (d, *J* = 8.4 Hz, 1H, H-1), 5.17 (d, *J* = 8.4 Hz, 1H, H-1), 5.09 (d, *J* = 6.2 Hz, 1H), 5.00 (s, 1H, H-1), 4.92 (d, *J* = 3.1 Hz, 1H), 4.90-4.72 (m, 12H), 4.69-4.72 (m, 23H), 4.26-4.17 (m, 5H), 4.13-3.97 (m, 12H), 3.89-3.82 (m, 12H), 3.72 (t, 1H), 3.68-3.62 (m, 1H), 3.65-3.55 (m, 7H), 3.54-3.45 (m, 4H), 3.43-3.40 (m, 4H), 3.40-3.29 (m, 9H), 3.23-3.19 (m, 3H), 3.17-3.09 (m, 2H), 2.91-2.79 (m, 3H), 2.68 (s, 1H), 2.28 (s, 3H, -C(O)CH_3), 2.05 (s, 3H, -C(O)CH_3), 2.01 (s, 3H, -C(O)CH_3), 1.99 (s, 3H, -C(O)CH_3), 1.34-1.22 (m, 4H, linker), 1.06-1.00 (m, 2H, linker); ^{13}C NMR (150 MHz, $CDCl_3$): δ 171.37, 170.38, 170.29, 169.54, 168.49, 168.23, 167.97, 167.53, 139.28, 139.11, 139.03, 138.97, 138.94, 138.67, 138.63, 138.58, 138.53, 138.41, 138.38, 138.25, 138.10, 128.74, 128.70, 128.67, 128.63, 128.58, 128.55, 128.53, 128.51, 128.48, 128.44, 128.40, 128.37, 128.34, 128.30, 128.15, 128.03, 128.00, 127.95, 127.92, 127.84, 127.77, 127.75, 127.70, 127.64, 127.61, 101.17, 100.92, 98.84, 98.28, 97.71, 97.45, 75.49, 75.36, 75.26, 75.17, 75.06, 74.96, 74.82, 74.75, 74.65, 74.60, 74.38, 74.30, 73.99, 73.77, 73.73, 73.59, 73.25, 73.25, 73.06, 72.99, 72.91, 72.26, 72.18, 71.98, 71.82, 71.57, 71.45, 70.52, 70.03, 69.92, 69.56, 69.10, 68.90, 68.85, 68.57, 68.48, 68.37, 66.64, 66.06, 56.75, 56.09, 55.99, 51.37, 34.25, 29.99, 28.94, 28.54, 25.90, 23.27, 21.86, 21.42, 21.33; HRMS (MALDI-TOF): *m/z* calcd for $C_{231}H_{238}N_6O_{54}$; 3945.6071; found 3968.6006 $(M + Na)^+$.



Glycan G1: Compound **S1** (0.110 g, 0.027 mmol) was deprotected by following general procedure 2 to get the desired heptasaccharide **G1** (0.025 g, 55%) as a white solid. ^{1}HNMR (400 MHz, D_2O): δ 5.14 (s, 1H), 5.11 (s, 1H), 4.93 (d, *J* = 1.2 Hz, 1H), 4.91 (d, *J* = 1.2 Hz, 1H), 4.60 (d, *J* = 6.4 Hz, 2H), 4.50 (t, *J* = 8 Hz, 2H), 4.27 (s, 1H), 4.23 (s, 1H), 2.22 (s, 1H), 4.09–4.08 (m, 1H), 4.00–3.52 (m, 53H), 3.00 (t, *J* = 8 Hz, 2H, linker), 2.09 (s, 3H, $C(O)CH_3$), 2.07 (s, 3H, $C(O)CH_3$), 2.05 (s, 3H, $C(O)CH_3$), 1.72–1.52 (m, 4H, linker), 1.45–1.39 (m, 2H, linker). ^{13}C NMR (150 MHz, D_2O): 174.67, 174.40, 171.00, 102.88, 102.27, 101.46, 101.02, 100.41, 99.87, 99.51, 99.40, 99.21, 80.39, 79.51, 79.22, 78.58, 78.42, 76.31, 75.31, 74.69, 74.49, 74.39, 74.32, 73.52, 73.28, 72.66, 72.45, 72.32, 71.90, 70.92, 70.79, 70.54, 70.30, 70.19, 70.09, 69.91, 69.41, 69.34, 68.49, 67.23, 65.98, 65.57, 65.11, 61.66, 60.90, 60.04, 59.93, 54.94, 54.88, 54.82, 39.28, 28.01, 26.36, 22.29, 22.16, 22.09, 22.06; ESI-MS: *m/z* calculated for $C_{65}H_{112}N_4O_{46}$; 1684.65 found 854.3255 $(M+Na+H)^{2+}$.

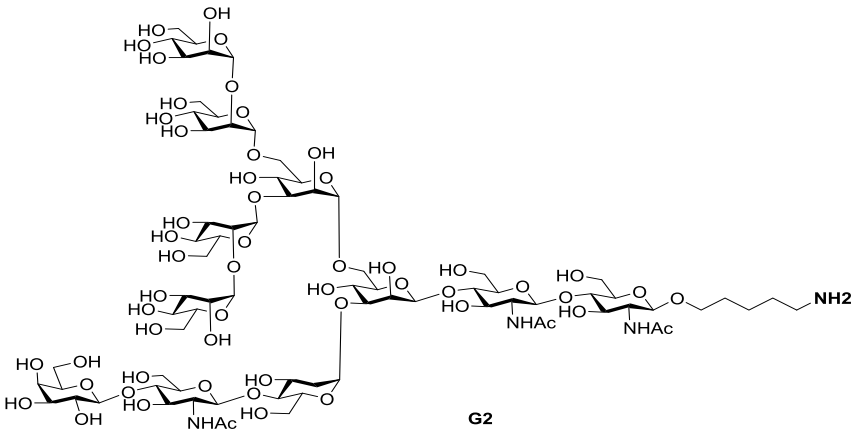
4. Preparation of hybrid glycan G2



Scheme S2. Reagents and reaction conditions. i) $(BrC_6H_4)_3NSbCl_6$, CH_3CN, 4 Å MS, -10 ºC, overnight, 29%; ii) (1) $NH_2CH_2CH_2NH_2$, *n*-BuOH, 90 ºC, overnight; (2) Ac_2O, pyridine, overnight; (3) NaOMe, MeOH, overnight; (4) $Pd(OH)_2$, MeOH: H_2O : HCOOH (5:3:2), H_2; 40%.

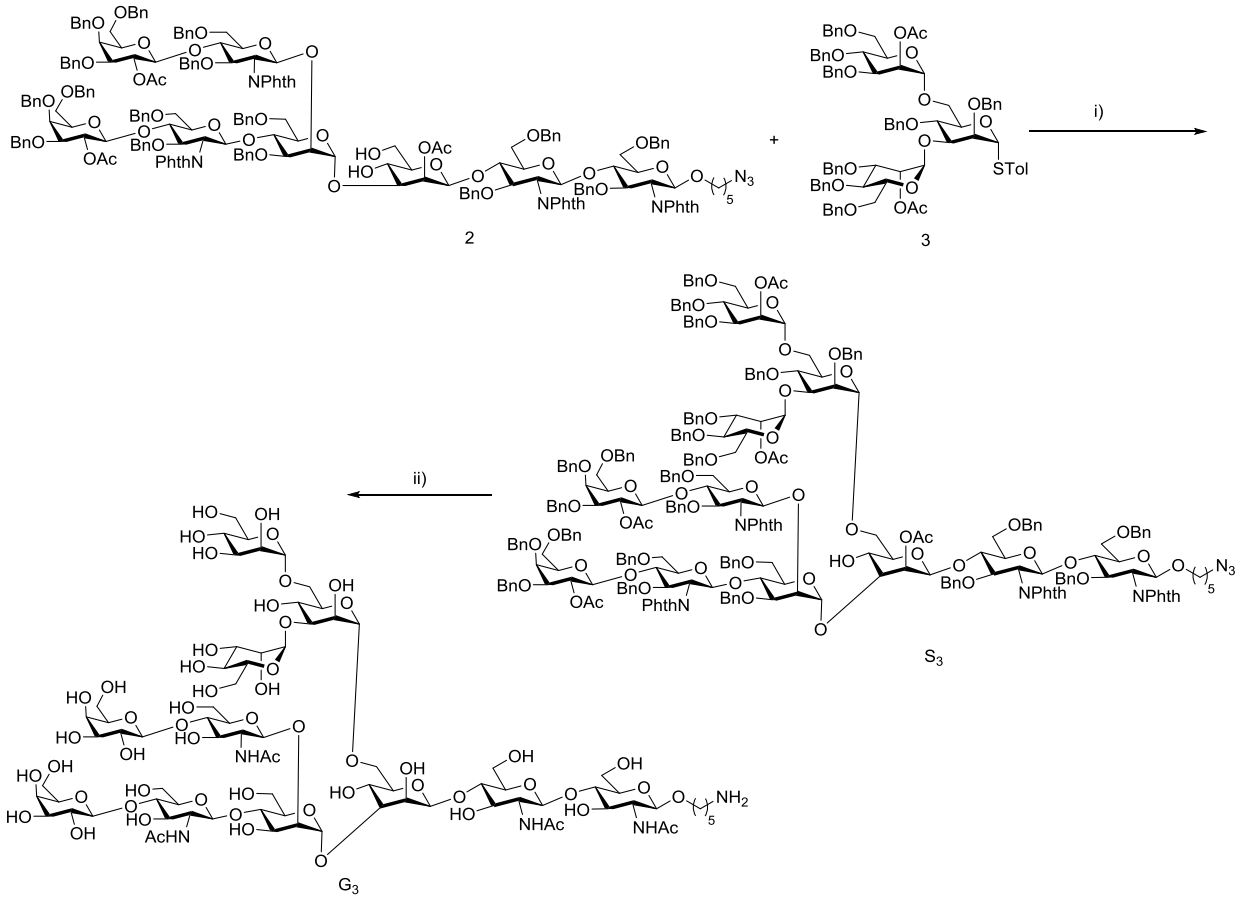
Compound S2: A mixture of hexasaccharide acceptor **1** (0.250 g, 0.094 mmol), thiomannoside donor **4** (0.257 g, 0.113 mmol) and activated 4 Å molecular sieves (0.5 g) in CH_3CN (10 mL) was stirred at room temperature for 1 h. The resulting mixture was cooled to -10 °C, tris (4-bromophenyl) aminium hexachloroantimonate (0.159 g, 0.188 mmol) was added and stirred for overnight. TLC (acetone : toluene, 1.5/8.5) indicated formation of α/β (7/3) mixture of isomers with consumption of starting material, reaction was quenched by Et_3N. The reaction mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with aqueous $NaHCO_3$ (2 x 50 mL), and a brine (50 mL) solution. The organic layer was dried over Na_2SO_4 and concentrated in *vacuo*. The desired α isomer was separated by flash column chromatography (0%

to 15% acetone in toluene) to afford **S2** (0.130 g, 29%) as colorless foam. TLC: (acetone: toluene = 1.5/8.5, v/v): R_f = 0.63; ^{1}H NMR (400 MHz, $CDCl_3$): δ7.67 – 7.49 (m, 12H), 7.35 – 6.96 (m, 110H), 6.88 – 6.83 (m, 10H), 6.79 – 6.69 (m, 10H,), 5.46 (d, *J* = 12 Hz, 2H), 5.32 (t, *J* = 7.8 Hz, 1H), 5.20 – 5.09 (m, 6H), 5.01 (d, *J* = 12.2 Hz, 3H), 4.89 – 3.10 (m, 115H), 2.85 – 2.95 (m, 4H), 2.08 (s, 3H), 2.02 (s, 3H), 1.89 (s, 3H), 1.88 (s, 3H), 1.05 – 0.99 (m, 4H), 0.87 – 0.78 (m, 2H). HRMS (MALDI-TOF): *m/z* calcd for $C_{285}H_{294}N_6O_{63}$; 4811.3863 found 2433.3255 $(M+Na+4H)^{2+}$.



Glycan G2: Compound **S2** (0.130 g, 0.027 mmol) was deprotected by following general procedure 2 to get the desired heptasaccharide **G2** (0.022 g, 40%) as a white solid. ^{1}HNMR (400 MHz, D_2O): δ 5.32 (s, 1H), 5.07 (s, 1H), 5.03 (s, 1H), 4.98 (s, 1H), 4.96 (s, 1H), 4.50 (d, *J* = 5.6 Hz, 2H), 4.40 (t, *J* = 8 Hz, 2H), 4.23 (s, 1H), 4.16 (s, 1H), 4.08 (s, 1H), 3.99 (s, 2H), 3.90 – 3.49 (m, 67H), 2.90 (t, *J* = 8 Hz, 2H, linker), 1.99 (s, 3H, -C(O)CH_3), 1.97 (s, 3H, -C(O)CH_3), 1.95 (s, 3H, -C(O)CH_3), 1.60 – 1.49 (m, 4H, linker), 1.33 – 1.29 (m, 4H, linker). ^{13}CNMR (150 MHz, D_2O): 174.68, 174.48, 174.40, 102.89, 102.24, 101.41, 101.02, 100.60, 100.26, 99.53, 99.44, 97.97, 80.61, 79.26, 78.90, 78.60, 78.40, 76.37, 75.30, 74.66, 74.47, 74.04, 73.50, 73.29, 73.17, 72.69, 72.45, 72.34,71.90, 71.14, 70.92, 70.26, 70.07, 70.00, 69.91, 69.39, 68.50, 67.26, 66.85, 66.80, 65.50, 65.06, 61.67, 61.13, 60.98, 60.04, 59.91, 54.95, 54.82, 43.40, 39.27, 28.01, 26.33, 22.30, 22.14, 22.05. ESI-MS: *m/z* calculated for $C_{77}H_{132}N_4O_{56;}$ 2008.76 found 1016.3766 $(M+Na)^{2+}$.

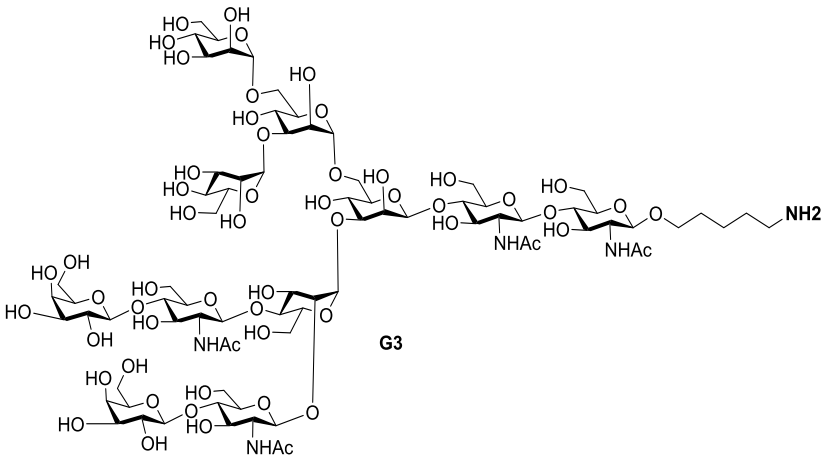
5. Preparation of hybrid glycan G3



Scheme S3: Reagents and reaction conditions. i) $(BrC_6H_4)_3NSbCl_6$, CH_3CN, 4 Å MS, -10 °C, overnight, 40%; ii) (1) $NH_2CH_2CH_2NH_2$, *n*-BuOH, 90 °C, overnight; (2) Ac_2O, pyridine, overnight; (3) NaOMe, MeOH, overnight; (4) $Pd(OH)_2$, MeOH: H_2O : HCOOH (5:3:2), H_2; 45%.

Compound S3: A mixture of thioglycoside donor **3** (0.119 g, 0.085 mmol), acceptor **2** (0.250 g, 0.071 mmol) and activated 4Å molecular sieves (0.5 g) in CH_3CN (10 mL) was stirred at room temperature for 1 h. The resulting mixture was cooled to -10 °C, tris (4-bromophenyl) aminium hexachloroantimonate (0.120 g, 0.142 mmol) was added and stirred for overnight. TLC (acetone : toluene, 1/9) indicated formation of product with consumption of starting material, reaction was quenched by Et_3N. The reaction mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with aqueous $NaHCO_3$ (2 x 50 mL), and a brine (50 mL) solution. The organic layer was dried over Na_2SO_4 and concentrated in *vacuo*. The desired product was

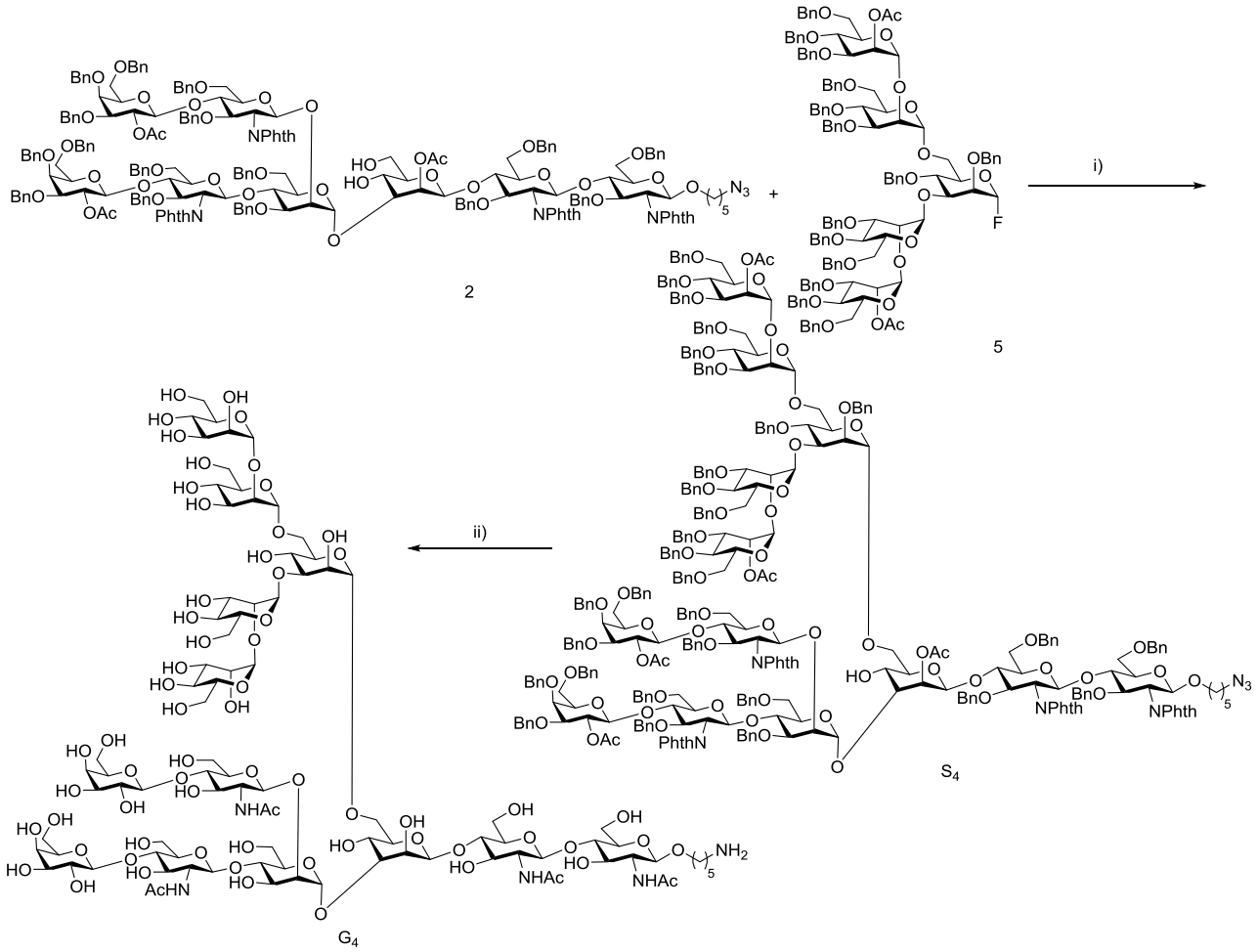
separated by flash column chromatography (0% to 15% acetone in toluene) to afford **S3** (0.140 g, 40%) as colorless foam and acceptor was recovered (0.085 g). TLC: (acetone: toluene = 1/9, v/v): R_f = 0.33; ^{1}H NMR (600 MHz, $CDCl_3$): δ7.68 – 7.41 (m, 16H), 7.34 – 7.05 (m, 95H), 6.99 – 6.70 (m, 25H), 5.47 (s, 2H,), 5.30 – 5.25 (m, 2H), 5.19 – 5.03 (m, 3H), 4.87 –2.82 (m, 122H), 2.13 (m, 6H), 1.94 (s, 3H), 1.88 (s, 3H), 1.78 (s, 3H),1.05 – 1.01 (m, 4H), 0.88 – 0.78 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$):170.45, 170.36, 169.58, 169.46, 169.34, 168.56, 168.33 167.78, 167.58, 140.14, 139.45, 139.27, 139.09, 139.00, 138.91, 138.69, 138.56, 138.43, 138.33, 138.26, 138.21, 138.17, 138.03, 137.76, 134.04, 133.82, 131.69, 128.83, 128.62, 128.55, 128.49, 128.42, 128.39, 128.30, 128.25, 128.22, 128.19, 128.13, 128.05, 128.00, 127.95, 127.89, 127.85, 127.76, 127.61, 127.51, 127.48, 127.34, 127.25, 127.16, 127.11, 126.97, 126.89, 123.39, 103.23, 101.06, 100.74, 98.32, 98.15, 97.22, 80.60, 75.32, 75.17, 75.08, 75.01, 74.77, 74.65, 74.61, 74.36, 74.24, 73.90, 73.86, 73.65, 73.61, 73.49, 73.42, 73.32, 73.24, 73.05, 72.90, 72.17, 72.10, 71.88, 71.66, 71.46, 71.39, 68.80, 68.54, 68.29, 68.20, 55.93, 51.30, 29.92, 29.58, 28.88, 28.47, 23.20, 22.91, 21.54, 21.36, 21.31, 21.20, 20.87, 14.34. ESI-MS: *m/z* calculated for $C_{281}H_{287}N_7O_{65}$; 4802.2934 found 888.6632.



Glycan G3: Compound **S3** (0.140 g, 0.029 mmol) was deprotected by following general procedure 2 to get the desired heptasaccharide **G3** (0.027 g, 45%) as a white solid. ^{1}HNMR (400 MHz, D_2O): δ 5.13 (s, 1H), 5.10 (s, 1H), 4.92 (s, 1H), 4.89 (s, 1H), 4.59 – 4.55 (m, 4H), 4.49 – 4.47 (m, 3H), 4.23 (d, *J* = 6.4 Hz, 2H), 4.08 – 3.50 (m, 65H), 2.99 (t, *J* = 8 Hz, 2H, linker), 2.09 (s, 3H, -C(O)CH_3), 2.80 (s, 3H, -C(O)CH_3), 2.06 (s, 3H, -C(O)CH_3), 2.04 (s, 3H, -C(O)CH_3), 1.71 – 1.57 (m, 4H, linker), 1.42 – 1.38 (m, 2H, linker). ^{13}C NMR (150 MHz, D_2O): 174.67,

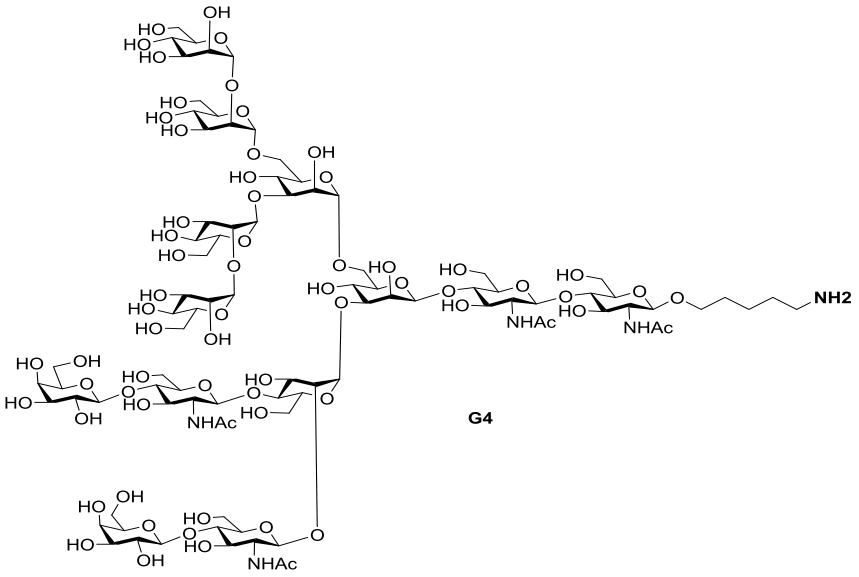
174.54, 174.40, 102.89, 102.85, 102.26, 101.54, 101.45, 101.01, 100.37, 99.89, 99.48, 99.21, 99.09, 80.31, 79.47, 79.21, 8.55, 78.45, 78.19, 78.04, 75.92, 75.31, 74.77, 74.70, 74.49, 74.39, 74.31, 73.29, 72.66, 72.43, 72.32, 72.11, 71.91, 70.90, 70.80, 70.54, 70.31, 70.15, 70.09, 69.91, 69.41, 68.50, 68.05, 66.70, 66.67, 65.98, 65.59, 65.11, 61.62, 61.23, 60.99, 60.90, 60.76, 60.05, 59.88, 55.12, 54.95, 54.88, 54.78, 39.28, 28.01, 26.35, 22.36, 22.16, 22.12, 22.09, 22.06; ESI-MS: *m/z* calculated for $C_{79}H_{135}N_5O_{56}$; 2049.79 found 1048.3772 $(M+2Na)^{2+}$.

6. Preparation of hybrid glycan G4



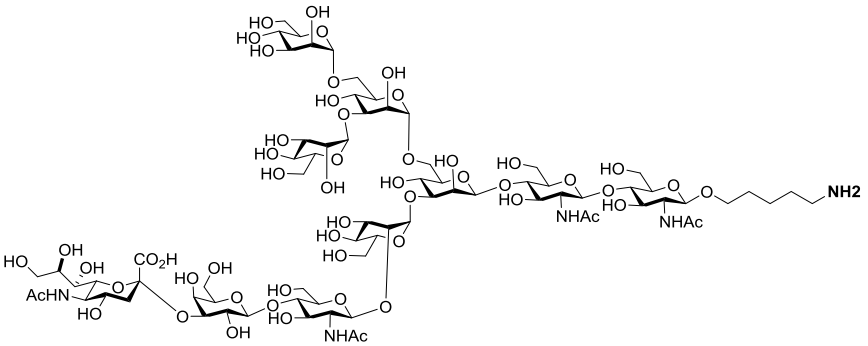
Scheme S4: Reagents and reaction conditions. i) Cp_2HfCl_2, AgOTf, 4 Å MS, -50 °C, 3 h, 72%; ii) (1) $NH_2CH_2CH_2NH_2$, *n*-BuOH, 90 °C, overnight; (2) Ac_2O, pyridine, overnight; (3) NaOMe, MeOH, overnight; (4) $Pd(OH)_2$, MeOH: H_2O : HCOOH (5:3:2), H_2; 41%.

Compound S4: A mixture of Silver triflate (0.108 g, 0.425 mmol), Bis(cyclopentadienyl) hafnium dichloride (0.112 g, 0.297 mmol) and 4 Å activated molecular sieves in dry toluene (10 mL) was stirred at room temperature for 1 h. The reaction mixture was then cooled to -50 °C, a solution of donor **5** (0.223 g, 0.102 mmol) and acceptor **2** (0.300 g, 0.085 mmol) in 5 mL toluene was added. The mixture was stirred for 3 h, quenched with Et_3N, diluted with EA and filtered through celite. The filtrate was washed with aqueous $NaHCO_3$ (2 x 50 mL), and a brine (50 mL) solution. The organic layer was dried over Na_2SO_4 and concentrated in *vacuo*. The residue was purified by flash column chromatography (0% to 10 % acetone in toluene) to afford **S4** (0.350 g, 72%) as colorless foam. TLC: (acetone: toluene = 1/9, v/v): R_f = 0.46; ^{1}H NMR (600 MHz, $CDCl_3$): δ7.62 – 7.41 (m, 16H), 7.32 – 6.95 (m, 120H), 6.87 – 6.68 (m, 10H), 5.49 (dd, J = 12.2 Hz, 2H,), 5.28 (m, 2H), 5.12 (m, 4H), 4.93 (m, 3H), 4.90 – 3.26 (m, 115H), 2.90 – 2.85 (m, 4H), 2.11 (m, 3H), 2.09 (s, 3H), 2.01 (s, 3H),1.92 (s, 3H), 1.73 (s, 3H), 1.09-1.02 (m, 4H), 0.87-0.81 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) 224.54, 223.66, 200.88, 196.34, 194.48, 191.42, 179.24, 178.00, 169.25, 167.56, 163.42, 152.02, 148.61, 139.51, 138.83, 138.71, 138.35, 138.10, 135.35, 133.64, 133.47, 128.60, 128.41, 128.35, 128.25, 128.22, 128.14, 128.09, 127.95, 127.85, 127.76, 127.67, 127.50, 127.39, 127.29, 127.16, 126.66, 78.28, 77.57, 74.90, 74.76, 74.40, 74.23, 73.40, 73.27, 73.09, 72.76, 72.11, 71.80, 71.66, 71.28, 71.07, 69.16, 68.82, 68.72, 67.97. ESI-MS: *m/z* calculated for $C_{335}H_{343}N_7O_{75;}$ 5667.3098 found 1887.2030 $(M)^{3+}$.



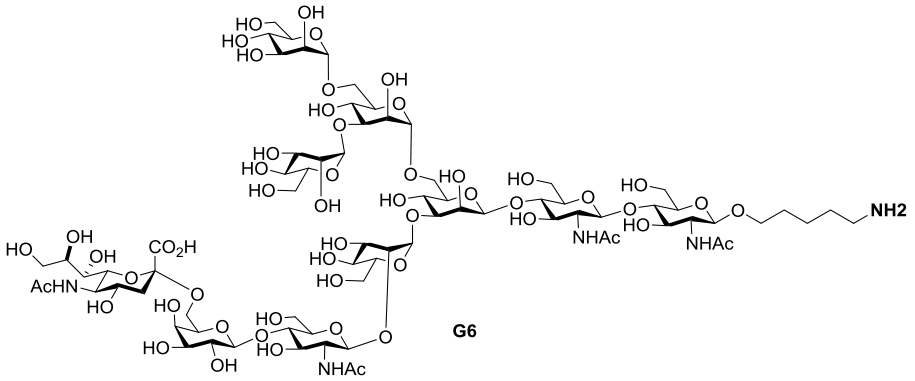
Glycan G4: Compound **S4** (0.350 g, 0.061 mmol) was deprotected by following general procedure 2 to get the desired heptasaccharide **G4** (0.060 g, 41%) as a white solid. ^{1}HNMR (400 MHz, D_2O): δ 5.40 (s, 1H), 5.14 (s, 1H), 5.09 (s, 1H), 5.04 (s, 1H), 5.03 (s, 1H), 4.59 – 4.52 (m, 4H), 4.49 – 4.45 (m, 3H), 4.24 (s, 1H), 4.20 (s, 1H), 4.15 (s, 1H), 4.07 – 3.40 (m, 77H), 2.97 (t, *J* = 8 Hz, 2H, linker), 2.07 (s, 3H, -C(O)CH_3), 2.06 (s, 3H, -C(O)CH_3), 2.04 (s, 3H, -C(O)CH_3), 2.02 (s, 3H, linker), 1.68 – 1.55 (m, 4H, linker), 1.42 – 1.35 (m, 2H, linker). ^{13}C NMR (150 MHz, D_2O): 177.24, 177.22, 177.14, 173.74, 105.65, 105.58, 105.04, 104.95, 104.33, 104.16, 103.76, 103.69, 103.41, 102.95, 102.27, 102.24, 101.89, 100.70, 83.39, 82.00, 81.70, 81.59, 81.52, 81.33, 81.18, 81.13, 80.76, 78.65, 78.05, 77.51, 77.41, 77.21, 76.77, 76.09, 75.99, 75.91, 75.43, 75.25, 75.18, 75.17, 75.08, 74.89, 74.66, 73.91, 73.65, 73.01, 72.94, 72.81, 72.72, 72.66, 72.13, 71.24, 70.85, 69.66, 69.60, 68.23, 67.74, 67.67, 63.96, 63.86, 63.76, 63.73, 63.64, 62.78, 62.63, 57.87, 57.69, 57.52, 42.02, 30.75, 29.07, 25.11, 24.88, 24.83, 24.80.; ESI-MS: *m/z* calculated for $C_{91}H_{155}N_5O_{66}$; 2373.89 found 1199.4434 $(M+Na+2H)^{2+}$.

Enzymatic sialylation to prepare hybrid glycan G5-G12



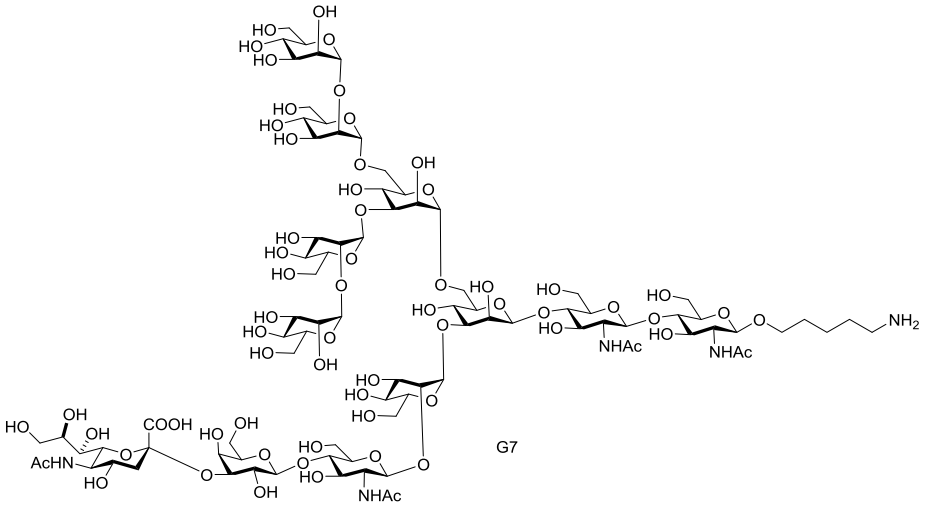
Glycan G5: Enzymatic α-2, 3 sialylation of glycan G**1** (6 mg, 0.0035 mmol) was performed according to general method 2 to get glycan **G5** (4.5 mg, 64%) as white solid after lypholization. ^{1}H NMR (400 MHz, D_2O): δ 5.01 (s, 1H), 4.97 (dd, *J* = 3.2, & 7.2 Hz, 1H), 4.84 (s, 1H), 4.81 (s, 1H), 4.75 (s, 1H), 4.62 (s, 1H), 4.52 (d, *J* = 7.8 Hz, 1H), 4.97 (d, *J* = 9.2 Hz, 1H), 4.42 (d, *J* = 8.3 Hz, 1H), 4.39 (d, *J* = 8.3 Hz, 1H), 4.22 (t, *J* = 7.8 Hz, 2H), 4.09 (s, 1H), 4.06 (d, *J* = 7.4 Hz, 1H), 4.03 (d, *J* = 7.3 Hz, 1H), 4.00 (d, *J* = 3.2 Hz, 1H), 3.96-3.85 (m, 10H), 3.85-3.71 (m, 19H), 3.72-3.52 (m, 26H), 3.51-3.48 (m, 2H,), 3.38-3.370 (m, 2H), 2.90 (t, *J* = 7.9 Hz, 2H, linker), 2.68 (dd, *J* = 4.8, & 12.0 Hz, 1H, H-3 equi. Sialic acid), 2.01 (s, 3H, -C(O)CH3), 1.99 (s, 3H, -C(O)CH3),

1.96 (s, 3H, -C(O)CH3), 1.95 (s, 3H, -C(O)CH3), 1.73 (t, J = 8.6 Hz, 1H, H-3 axial sialic acid), 1.61-1.56 (m, 2H, linker), 1.54-1.46 (m, 2H, linker), 1.34-1.33 (m, 2H, linker); ^{13}CNMR (150 MHz, D_2O): δ 171.94, 171.81, 171.37, 170.80, 113.44, 99.86, 99.53, 99.27, 98.42, 97.99, 97.38, 97.08, 96.74, 96.21, 75.74, 75.66, 75.52, 73.56, 72.85, 72.41, 72.10, 71.62, 71.45, 71.36, 70.27, 69.81, 69.62, 69.28, 69.14, 69.02, 68.90, 68.69, 67.91, 67.82, 67.50, 67.20, 67.08, 66.87, 66.36, 65.46, 65.29, 65.01, 64.39, 64.32, 63.68, 62.47, 62.09, 61.02, 59.50, 59.39, 58.64, 57.96, 57.87, 57.10, 56.91, 52.12, 51.91, 48.60, 42.85, 40.32, 36.54, 36.25, 35.04, 25.27, 24.98, 23.34, 20.60, 19.53, 19.13, 19.03, 18.95, 13.70; ESI-MS (negative mode): *m/z* calcd for $C_{76}H_{129}N_5O_{54}$; 1975.7418 found 1974.7671 $(M - H)^-$.

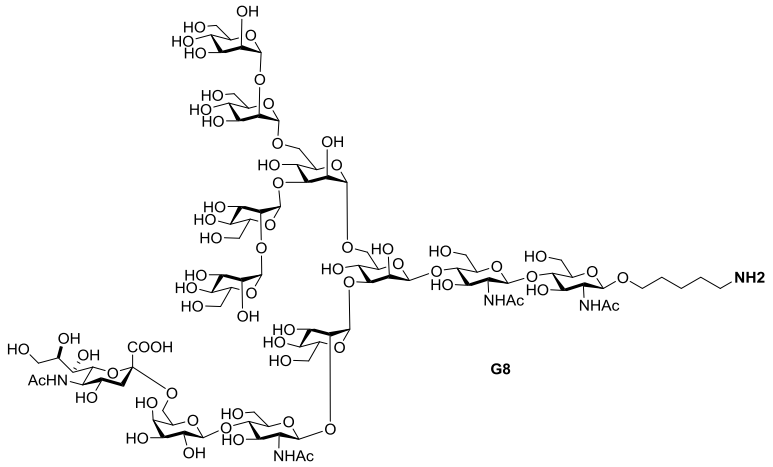


Glycan G6: Enzymatic α-2, 6 sialylation of glycan G**1** (8 mg, 0.0047 mmol) was performed according to general method 2 to get glycan **G6** (7.5 mg, 80%) as white solid after lypholization. ^{1}HNMR (400 MHz, D_2O): δ 5.14 (s, 1H), 5.10 (d, J = 1.2 Hz, 1H), 4.92 (s, 1H), 4.88 (s, 1H), 4.61 (t, J = 7.6 Hz, 2H), 4.50 (d, J = 8 Hz, 1H), 4.46 (d, J = 8 Hz, 1H), 4.26 (s, 1H), 4.21 (s, 1H), 4.16 (s, 1H), 4.8 (d, J = 7.6 Hz, 1H), 3.99–3.50 (m, 60H), 2.99 (t, J = 7.6 Hz, 2H, linker), 2.78 (dd, J = 4.8 and J = 12 Hz, 1H, H-3 equi. sialic acid), 2.09 (s, 3H, -C(O)CH3), 2.08 (s, 3H, -C(O)CH3), 2.05 (s, 3H, -C(O)CH3), 2.04 (s, 3H, -C(O)CH3), 1.76–1.52 (m, 5H, linker + H-3 axial sialic acid), 1.44–1.38 (m, 2H, linker). ^{13}C NMR (150 MHz, D_2O): 174.87, 174.70, 174.40, 173.52, 172.00, 103.51, 102.28, 101.46, 101.02, 100.39, 100.09, 99.88, 99.53, 99.22, 80.74, 80.48, 79.50, 79.27, 78.60, 76.31, 74.48, 74.39, 73.66, 73.50, 73.28, 72.49, 72.32, 72.01, 71.94, 71.67, 70.79, 70.69, 70.54, 70.30, 70.19, 70.09, 70.05, 69.91, 69.38, 68.35, 68.16, 67.22, 66.67, 65.99, 65.58, 65.50, 65.11, 63.29, 62.60, 61.68, 60.97, 60.22, 60.05, 59.88, 54.93, 54.56 51.83,

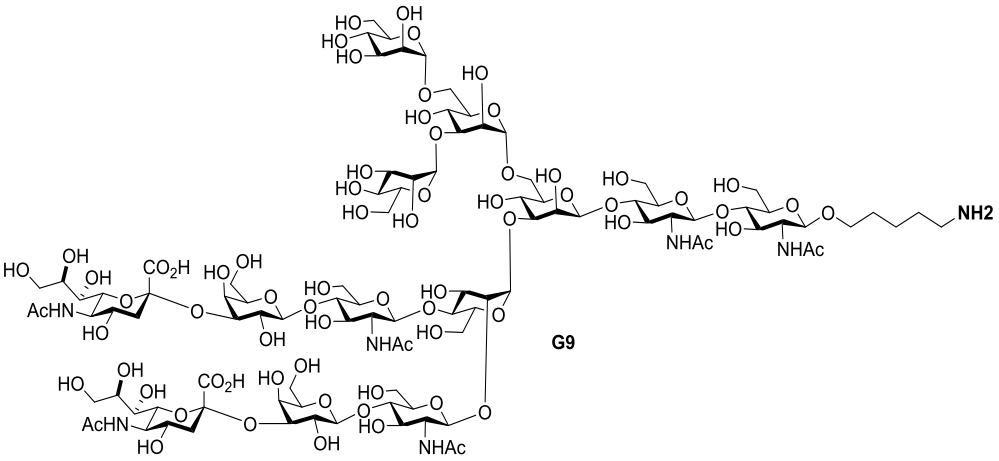
40.01, 39.27, 28.00, 26.33, 22.16, 22.06, 22.00.; ESI-MS (negative mode): *m/z* calculated for $C_{76}H_{129}N_5O_{54}$; 1975.75 found 986.683 $(M\text{-H})^{2-}$.



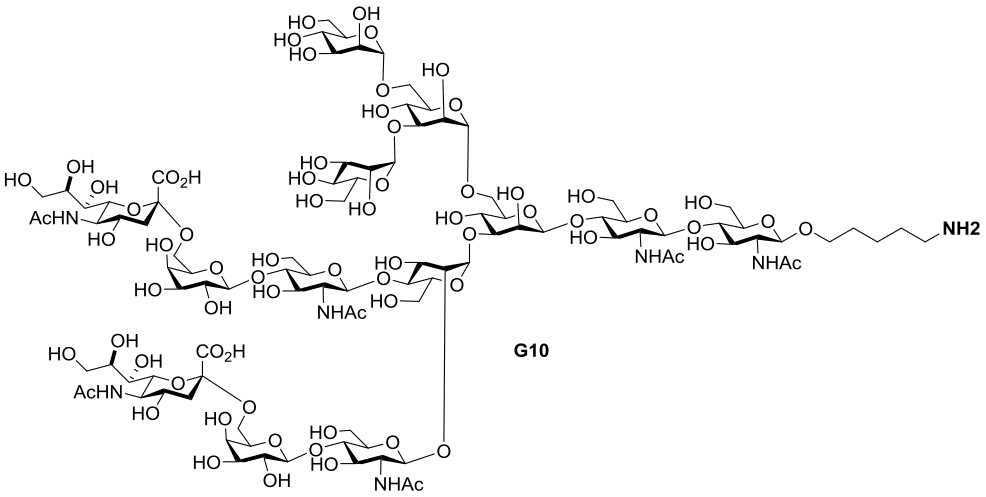
Glycan G7: Enzymatic α-2, 3 sialylation of glycan G**2** (3.5 mg, 0.0017 mmol) was performed according to general method 2 to get glycan **G7** (2.7 mg, 60%) as white solid after lypholization. ^{1}HNMR (400 MHz, D_2O): δ 5.43 (s, 1H), 5.17 (s, 1H), 5.13 (s, 1H), 5.08 (s, 1H), 5.06 (s, 1H), 4.89 (s, 1H), 4.62 – 4.56 (m, 3H), 4.51 (d, *J* = 8 Hz, 1H), 4.23 (dd, *J* = 3.6 and J = 12 Hz, 2H), 4.18 – 3.51 (m, 74H), 3.00 (t, *J* = 8 Hz, 2H), 2.78 (dd, *J* = 4 and *J* = 12 Hz, 1H, H-3 axial sialic acid), 2.10 (s, 3H, -C(O)CH3), 2.07 (s, 6H, -C(O)CH3), 2.06 (s, 3H, -C(O)CH3), 1.83 (t, *J* = 12 Hz, 1H, H3 equi. sialic acid), 1.73 – 1.58 (m, 4H, linker), 1.45 – 1.43 (m, 2H, linker). ^{13}C NMR (150 MHz, D_2O): 174.96, 174.39, 173.85, 102.56, 102.23, 101.02, 100.60, 100.25, 99.76, 99.50, 97.96, 79.29, 78.62, 78.58, 75.41, 75.11, 74.67, 74.47, 73.28, 73.16, 72.83, 72.68, 72.33, 71.88, 71.72, 70.27, 70.06, 70.00, 69.91, 69.35, 68.32, 68.04, 67.42, 66.92, 66.85, 66.79, 65.43, 65.05, 62.52, 61.68, 61.11, 60.99, 60.89, 60.03, 59.88, 54.93, 54.80, 51.62, 39.27, 28.00, 26.35, 22.29, 22.14, 22.08, 22.05, 21.98. ESI-MS (negative mode): *m/z* calculated for $C_{88}H_{149}N_5O_{64}$; 2299.86 found 1149.4205 $(M\text{-H})^{2-}$.



Glycan G8: Enzymatic α-2, 6 sialylation of glycan G**2** (4.0 mg, 0.0019 mmol) was performed according to general method 2 to get glycan **G8** (2.9 mg, 64%) as white solid after lypholization. ^{1}HNMR (400 MHz, D_2O): δ 5.41 (s, 1H), 5.15 (s, 1H), 5.12 (s, 1H), 5.06 (s, 1H), 5.04 (s, 1H), 4.88 (s, 1H), 4.61 (t, *J* = 7.6 Hz, 3H), 4.49 (t, *J* = 4 Hz, 1H), 4.55 (d, *J* = 8.2 Hz, 1H), 4.25 (s, 1H), 4.23 (s, 1H), 4.16 (s, 1H), 4.00 – 3.50 (m, 72H), 2.99 (t, *J* = 7.6 Hz, 2H), 2.67 (dd, *J* = 4 and *J* = 12.4 Hz, 1H, H3 equi. sialic acid), 2.08 (s, 6H, -C(O)CH3), 2.03 (s, 6H, -C(O)CH3), 1.76–1.56 (m, 5H, linker + H3 axial sialic acid), 1.43–1.41 (m, 2H, linker). ^{13}C NMR (150 MHz, D_2O): 174.87, 174.70, 174.47, 174.40, 173.52, 103.55, 102.23, 101.43, 101.02, 100.59, 100.26, 100.10, 99.61, 99.29, 97.97, 96.79, 82.05, 80.72, 79.33, 78.89, 78.59, 76.33, 74.47, 74.37, 73.66, 73.50, 73.29, 73.17, 72.69, 72.49, 72.34, 72.03, 71.90, 71.67, 70.6, 70.06, 69.91, 69.37, 68.36, 68.17, 67.25, 66.93, 66.85, 66.79, 65.53, 62.62, 61.67, 61.13, 60.91, 60.07, 54.94, 54.58, 51.83, 40.02, 39.28, 28.01, 22.39, 22.00. ESI-MS (negative mode): *m/z* calculated for $C_{88}H_{149}N_5O_{64}$; 2299.86 found 1149.4184 $(M\text{- H})^{2-}$.

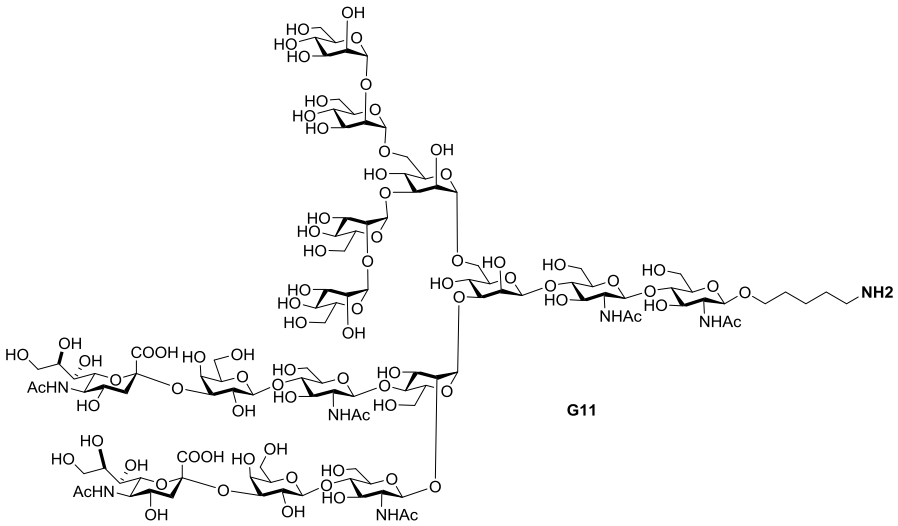


Glycan G9: Enzymatic α-2, 3 sialylation of glycan G**3** (4 mg, 0.0019 mmol) was performed according to general method 2 to get glycan **G9** (3.3 mg, 73%) as white solid after lypholization. ^{1}HNMR (400 MHz, D_2O): δ 5.41 (s, 1H), 5.14 (s, 1H), 5.09 (s, 1H), 5.05 (s, 1H), 5.04 (s, 1H), 4.87 (s, 1H), 4.59 – 4.48 (m, 6H), 4.24 – 3.54 (m, 81H), 2.98 (t, *J* = 8 Hz, 2H), 2.77 (dd, *J* = 4. and *J* = 10 Hz, 2H, H3 equi. sialic acids), 2.08 (s, 3H, -C(O)CH3), 2.07 (s, 3H, -C(O)CH3), 2.05 (s, 6H, -C(O)CH3), 2.03 (s, 6H, -C(O)CH3), 1.80 (t, *J* = 12, 2H, H3-axial sialic acids), 1.70 – 1.59 (m, 4H, linker), 1.43 – 1.39 (m, 2H, linker). ^{13}C NMR (150 MHz, D_2O): 175.19, 174.96, 174.63, 174.42, 173.88, 102.53, 102.27, 101.50, 101.02, 99.89, 99.75, 99.22, 99.08, 79.48, 79.24, 78.23, 75.95, 75.38, 75.12, 74.75, 74.50, 74.39, 74.32, 72.84, 72.66, 72.31, 72.11, 71.91, 71.73, 70.80, 70.54, 70.31, 70.05, 69.91, 69.33, 68.35, 68.04, 67.42, 66.68, 65.58, 65.12, 62.52, 60.99, 60.06, 59.88, 54.95, 54.78, 51.63, 39.57, 39.28, 28.01, 26.33, 22.36, 22.06, 21.99.; ESI-MS (negative mode): *m/z* calculated for $C_{101}H_{169}N_7O_{72}$; 2631.98 found 1315.4736 $(M\text{- H})^{2-}$.



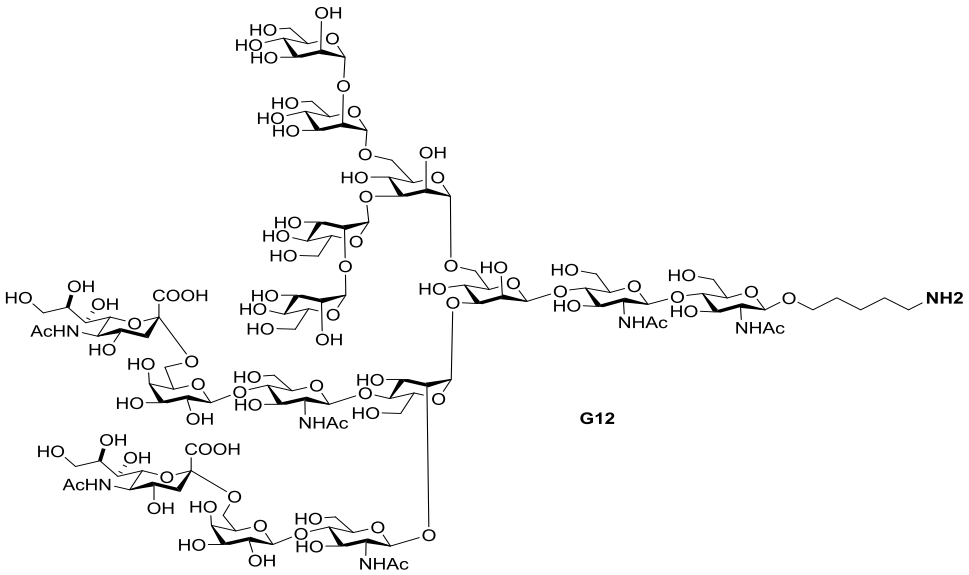
Glycan G10: Enzymatic α-2, 6 sialylation of glycan G**3** (5 mg, 0.0024 mmol) was performed according to general method 2 to get glycan **G10** (5.2 mg, 81%) as white solid after lypholization. ^{1}HNMR (400 MHz, D_2O): δ 5.41 (s, 1H), 5.14 (s, 1H), 5.10 (s, 1H), 5.05 (s, 1H), 5.03 (s, 1H), 4.87 (s, 1H), 4.59–4.53 (m, 3H), 4.50–4.43 (m, 3H), 4.25 (s, 1H), 4.21 (s, 1H), 4.15 (s, 1H), 4.07 –3.51 (m, 78H), 2.98 (t, *J* = 8 Hz, 2H, linker), 2.66 (dd, *J* = 4.8 and *J* = 11 Hz, 2H, H3-equi. Sialic acids), 2.10 (s, 3H, -C(O)CH3), 2.07 (s, 3H, -C(O)CH3), 2.06 (s, 3H, -C(O)CH3), 2.05 (s, 3H, -C(O)CH3), 2.03 (s, 6H, -C(O)CH3), 1.75–1.55 (m, 6H, linker + H3-axial sialic acids), 1.43 – 1.39 (m, 2H, linker). ^{13}C NMR (150 MHz, D_2O): 174.90, 174.71, 174.61, 174.43, 173.34, 103.57,

102.42, 102.28, 101.47, 101.11, 101.04, 100.96, 100.37, 100.04, 99.92, 99.38, 99.26, 99.14, 80.83, 80.36, 79.48, 79.31, 78.59, 75.98, 74.53, 74.49, 74.44, 74.36, 74.28, 73.67, 73.32, 72.69, 72.55, 72.41, 72.38, 72.33, 72.27, 72.07, 71.99, 71.63, 70.85, 70.72, 70.68, 70.58, 70.36, 70.13, 70.07, 69.95, 69.45, 68.41, 68.12, 66.72, 66.03, 65.65, 65.57, 65.18, 63.32, 62.68, 61.27, 61.02, 60.94, 60.36, 60.12, 59.92, 59.31, 54.99, 54.93, 54.57, 51.86, 39.99, 39.31, 28.04, 26.35, 22.09, 22.04.; ESI-MS (negative mode): *m/z* calculated for $C_{101}H_{169}N_7O_{72}$; 2631.98 found 1315.4757(*M*- H)$^{2-}$.



Glycan G11: Enzymatic α-2, 3 sialylation of glycan G**4** (3.5 mg, 0.0014 mmol) was performed according to general method 2 to get glycan **G11** (3.0 mg, 69%) as white solid after lypholization. ^{1}HNMR (400 MHz, D_2O): δ 5.41 (s, 1H), 5.14 (s, 1H), 5.09 (s, 1H), 5.05 (s, 1H), 5.03 (s, 1H), 4.86 (s, 1H), 4.59 – 4.88 (m, 6H), 4.24 – 3.54 (m, 98H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.79-2.71 (m, 2H, linker), 2.07 (s, 6H, -C(O)CH_3), 2.06 (s, 3H, -C(O)CH_3), 2.04 (s, 3H, -C(O)CH_3), 2.03 (s, 3H, -C(O)CH_3), 2.02 (s, 3H, -C(O)CH_3), 1.80 (t, *J* = 12 Hz, 2H, H3-axial sialic acids). 1.70–1.55 (m, 4H, linker), 1.43–1.39 (m, 2H, linker), ^{13}C NMR (150 MHz, D_2O): 177.69, 177.37, 177.21, 177.13, 176.61, 105.26, 105.03, 104.95, 104.30, 104.15, 103.75, 103.40, 102.94, 102.49, 102.28, 101.88, 100.70, 83.35, 82.01, 81.69, 81.58, 81.49, 81.32, 81.14, 80.99, 80.61, 78.70, 78.13, 78.04, 77.85, 77.51, 77.22, 76.79, 76.08, 75.99, 75.90, 75.57, 75.42, 75.16, 75.06, 74.88, 74.64, 74.47,

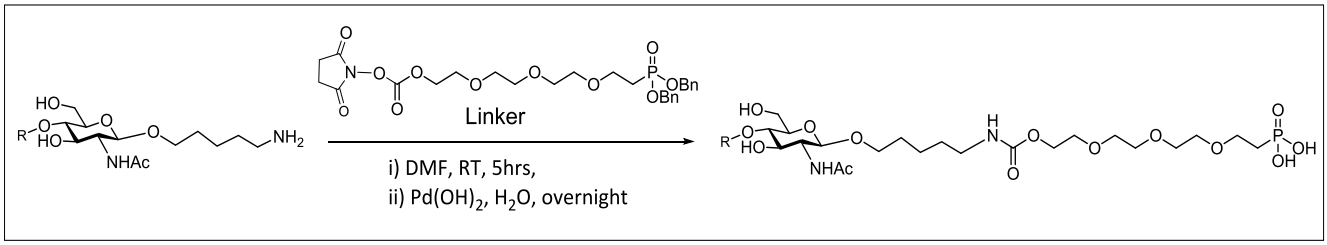
73.91, 73.01, 72.94, 72.79, 72.72, 72.66, 72.10, 71.24, 71.09, 70.78, 70.17, 69.59, 68.24, 67.74, 65.26, 63.95, 63.85, 63.72, 63.63, 62.79, 62.62, 57.87, 57.69, 57.52, 56.24, 55.85, 54.37, 46.39, 46.22, 42.31, 42.01, 37.18, 30.75, 29.06, 25.11, 24.87, 24.83, 24.80, 24.73.; ESI-MS (negative mode): *m/z* calculated for $C_{113}H_{189}N_7O_{82}$; 2956.08 found 1477.5268 $(M\text{- } 2H)^{2-}$.



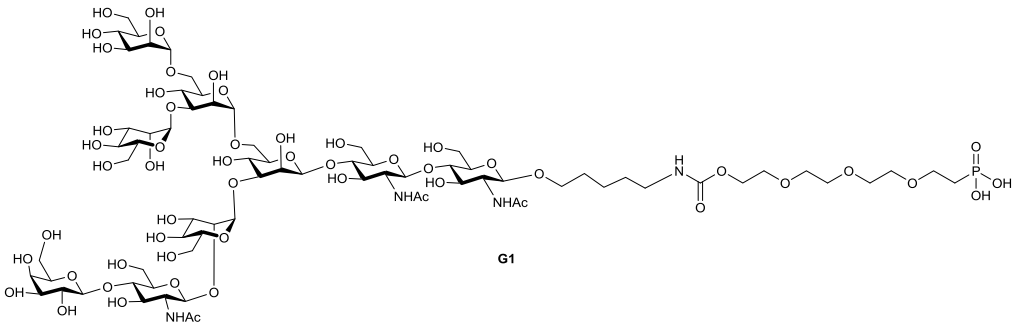
Glycan G12: Enzymatic α-2, 6 sialylation of glycan G**4** (5 mg, 0.0021 mmol) was performed according to general method 2 to get glycan **G12** (5.5 mg, 88%) as white solid after lypholization. ^{1}HNMR (400 MHz, D_2O): δ 5.41 (s, 1H), 5.14 (s, 1H), 5.10 (s, 1H), 5.05 (s, 1H), 5.03 (s, 1H), 4.86 (s, 1H), 4.59–4.43 (m, 6H), 4.25 (s, 1H), 4.21 (s, 1H), 4.15 (s, 1H), 4.07–3.51 (m, 96H), 2.98 (t, *J* = 7.6 Hz, 2H), 2.66 (dd, *J* = 4.8 and J = 12 Hz, 2H, H3-equi. sialic acids), 2.10 (s, 3H, -C(O)CH_3), 2.07 (s, 3H, -C(O)CH_3), 2.06 (s, 3H, -C(O)CH_3), 2.05 (s, 3H, -C(O)CH_3), 2.02 (s, 6H, -C(O)CH_3), 1.75 – 1.59 (m, 6H, linker + H3-axial sialic acids), 1.55–1.39 (m, 2H, linker), ^{13}C NMR (150 MHz, D_2O): 174.68 174.47, 174.38, 173.50, 103.57, 102.20, 101.01, 100.12, 100.08, 97.95, 74.48, 73.64, 73.16, 72.48, 72.31, 71.90, 71.66, 70.26, 70.19, 70.04, 69.91, 68.35, 68.16, 66.85, 62.60, 61.11, 60.89, 54.91, 51.82, 40.04, 39.27, 28.00, 26.32, 22.46, 22.24, 22.13, 21.80, 19.80.; ESI-MS (negative mode): *m/z* calculated for $C_{113}H_{189}N_7O_{82}$; 2956.08 found 1477.5364 $(M\text{- } 2H)^{2-}$.

Conjugation of phosphonic acid tail to glycans G1-12.

General procedure: To a solution of sugars with amine tail (3-5 μmol) in DMF (400 μL) was added linker [2(2(2(bis(benzyloxy)phosphoryl)ethoxyethoxy)ethyl(2,5-dioxopyrrolidin-1-yl)carbonate] (15-25 μmol), and the resulting reaction mixture was stirred at rt for 5 h. DMF was removed by using high vacuum and the product was purified by using Bio-Gel P-2 chromatography (eluent H_2O). The solid was dissolved in 2 mL of H_2O, added $Pd(OH)_2$ (50% by weight) and hydrogenated for overnight. The reaction mixture was filtered through Celite and concentrated in *vacuo.* The residue was purified by Bio-Gel P-2 (BIO-RAD) column chromatography using water as eluent. The product was the lypholysed to obtain the desired sugar with phosphonic acid tail as white color powder.

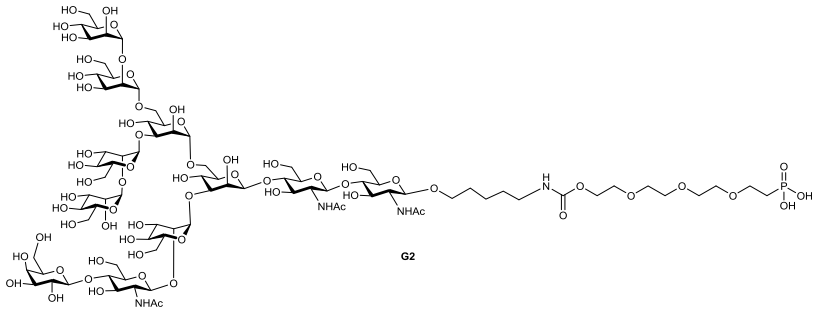


Scheme S5 | Preparation of sugars with phosphonic acid linker. i, (1) Linker, DMF, RT, 5 h; (2) $Pd(OH)_2$, H_2O, H_2, RT, overnight.

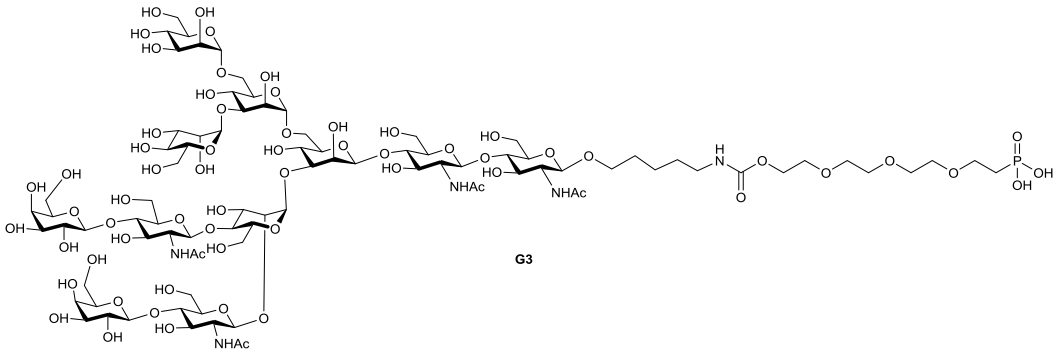


G1 with phosphonic acid linker. G1 with amine linker was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O): δ 5.11 (s, 2H), 5.02 (d, J = 7.8Hz, 2H), 4.68 (d, J = 3.2 Hz, 2H), 4.48 (d, J = 7.2 Hz, 1H), 4.45 (d, J = 7.2 Hz, 1H), 4.27 (s, 2H), 4.22 (s, 2H), 4.15 (s, 1H), 4.08 (s, 2H), 4.00-3.30 (m, 64H), 3.10 (t, J = 7.8 Hz, 2H), 2.08 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H),

1.93-1.87 (m, 2H), 1.56-1.54 (m, 2H), 1.51-1.47 (m, 2H), 1.33-1.30 (m, 2H); ESI-MS: *m/z* calculated for $C_{74}H_{129}N_4O_{54}P$; 1968.71 found 1968.71.

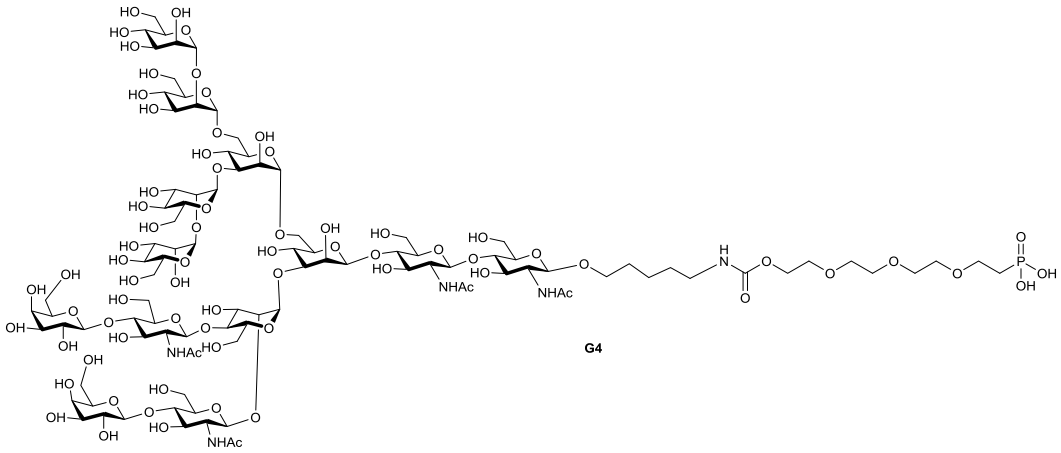


G2 with phosphonic acid linker. G2 with amine linker was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O). δ 5.33 (s, 1H), 5.08 (s, 1H), 5.04 (s, 1H), 4.98 (d, *J* = 9.8 Hz, 1H), 4.80 (s, 1H), 4.52 (t, *J* = 8.2 Hz, 2H), 4.42 (d, *J* = 7.2 Hz, 1H), 4.39 (d, *J* = 8.2 Hz, 1H), 4.17 – 3.48 (m, 85H), 3.04 (t, *J* = 9.2 Hz, 2H), 1.99 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.91 – 1.86 (m, 2H), 1.50 – 1.48 (m, 2H), 1.44 – 1.48 (m, 2H), 1.25 – 1.27 (m, 2H). ESI-MS: *m/z* calculated for $C_{86}H_{149}N_4O_{64}P$ 2292.83 found 2292.82

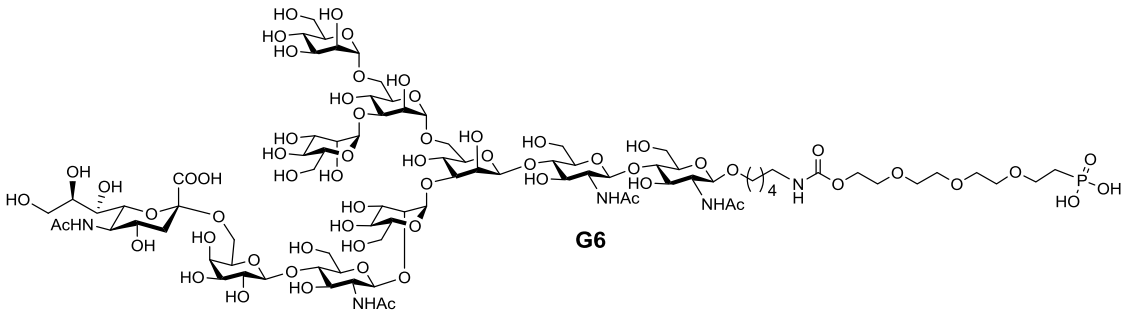


G3 with phosphonic acid linker. G3 with amine linker was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O):. δ 5.04 (s, 1H), 5.02 (s, 1H), 4.84 (s, 2H), 4.80 (s, 1H), 4.52 – 4.47 (m, 4H), 4.41 – 4.39 (m, 2H), 4.16 – 3.48 (m, 85H), 3.04 (t, *J* = 9.2 Hz, 2H), 2.01 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.91 – 1.89 (m, 2H), 1.50 – 1.48 (m, 2H), 1.44 –

1.41 (m, 2H), 1.27 – 1.23 (m, 2H). ESI-MS: *m/z* calculated for $C_{88}H_{152}N_5O_{64}P$, 2335.13, found 1168.44 $(M + H)^{2+}$.

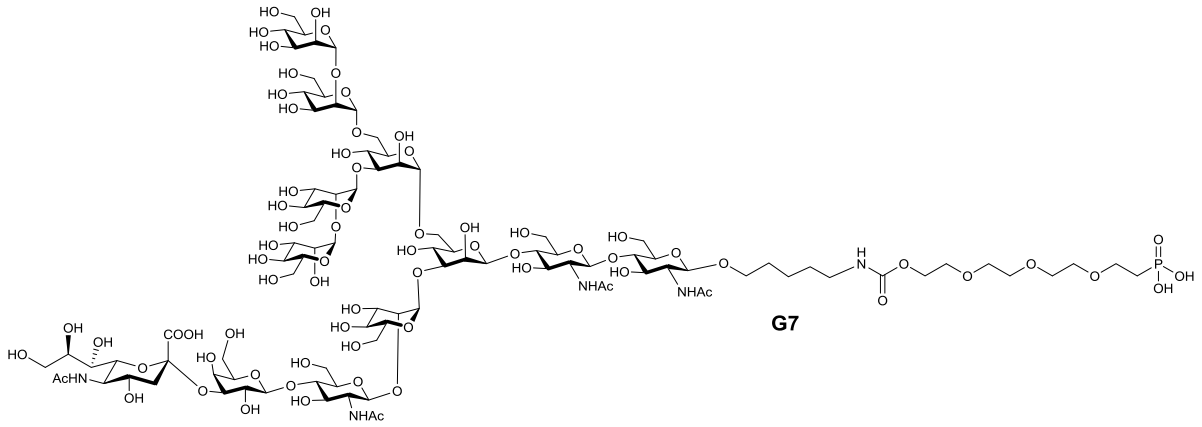


G4 with phosphonic acid linker. G4 with amine linker was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O): δ 5.34 (s, 1H), 5.07 (s, 1H), 5.02 (s, 1H), 4.97 (d, J = 9 Hz, 2H), 4.80 (s, 1H), 4.53 – 4.46 (m, 4H), 4.42 – 4.39 (m, 2H), 4.17 (s, 1H), 4.13 (s, 2H), 4.08 (s, 1H), 4.00 – 3.48 (m, 93H), 2.01 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.97 – 1.89 (m, 2H), 1.50 – 1.48 (m, 2H), 1.44 – 1.41 (m, 2H), 1.27 – 1.23 (m, 2H). ESI-MS: *m/z* calculated for $C_{100}H_{172}N_5O_{74}P$, 2657.96 found 1330.4918 $(M+ 3H)^{2+}$

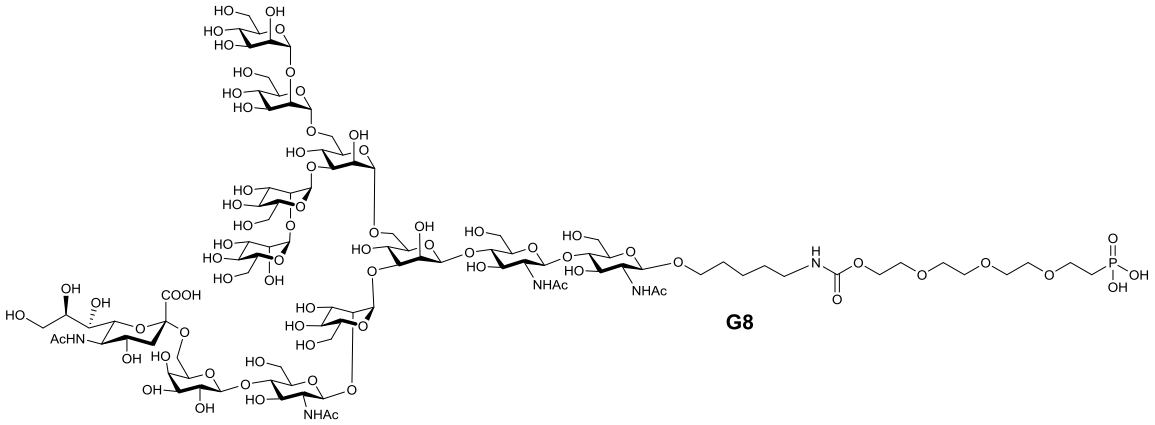


G6 with phosphonic acid linker. G6 with amine linker was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O): δ 5.08 (d, J = 10.2 Hz, 1H), 5.03 (s, 1H), 4.94 (s, 2H),

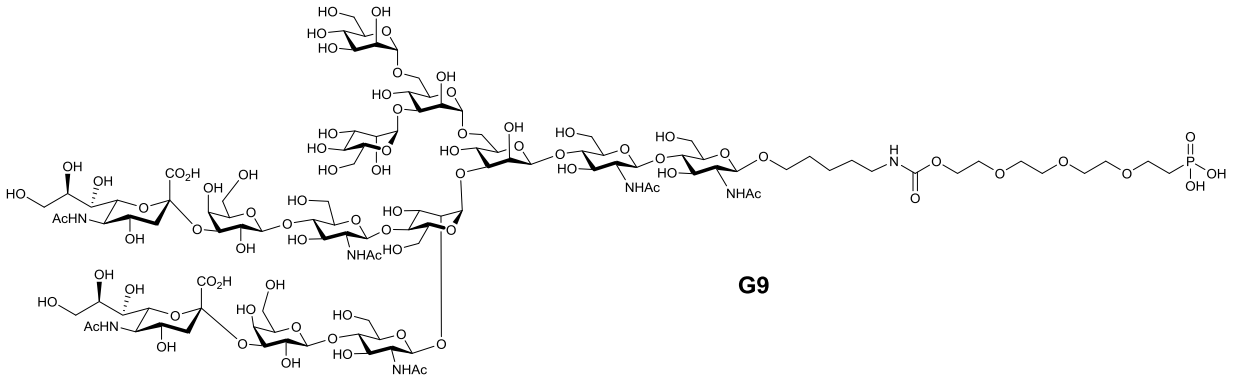
4.91 (s, 1H), 4.62 (s, 1H), 4.51 (d, J = 6.6 Hz, 1H), 4.46 (d, J = 7.8 Hz, 1H), 4.30 (s, 2H), 4.25 (s, 2H), 4.17 (s, 1H), 4.06 (s, 1H), 4.00-3.20 (m, 73H), 2.70 (dd, J = 3.2, 7.8 Hz, 1H), 2.11 (s, 6H), 2.05 (s, 6H), 2.03-1.97 (m, 2H), 1.14 (t, J = 12.6 Hz, 1H), 1.58-1.51 (m, 4H), 1.36-1.31 (m, 2H). ESI-MS: *m/z* calculated for $C_{85}H_{146}N_5O_{62}P$; 2259.8163 found 1129.40 $(M + H)^{2+}$.



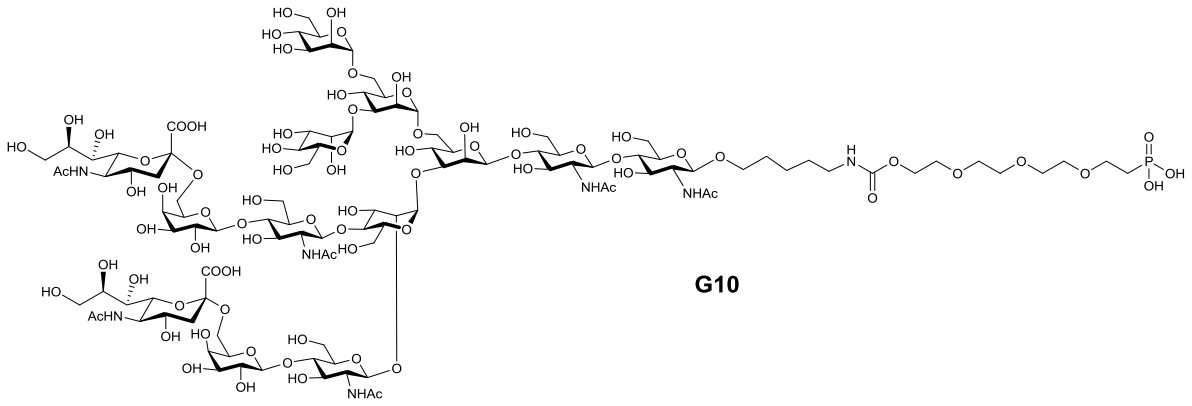
G7 with phosphonic acid linker. G7 with amine linker was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O): δ 5.33 (s, 1H), 5.07 (s, 1H), 5.03 (s, 1H), 4.98 (d, J = 8.2 Hz, 2H), 4.81 (s, 1H), 4.53 (t, J = 7.8 Hz, 1H), 4.50 (d, J = 7.2 Hz, 1H), 4.42 (d, J = 3.2 Hz, 1H), 4.17 – 3.48 (m, 93H), 3.04 (t, J = 8.2 Hz, 2H), 2.69 (dd, J = 2.3 and J = 7.5 Hz, 1H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96 (s, 6H), 1.77 – 1.93 (m, 2H), 1.74 (t, J = 7.2 Hz, 1H), 1.50 – 1.48 (m, 2H), 1.44 – 1.41 (m, 2H), 1. 27 – 1.23 (m, 2H). ESI-MS: *m/z* calculated for $C_{97}H_{166}N_5O_{72}P$; 2583.92 found 2583.92.



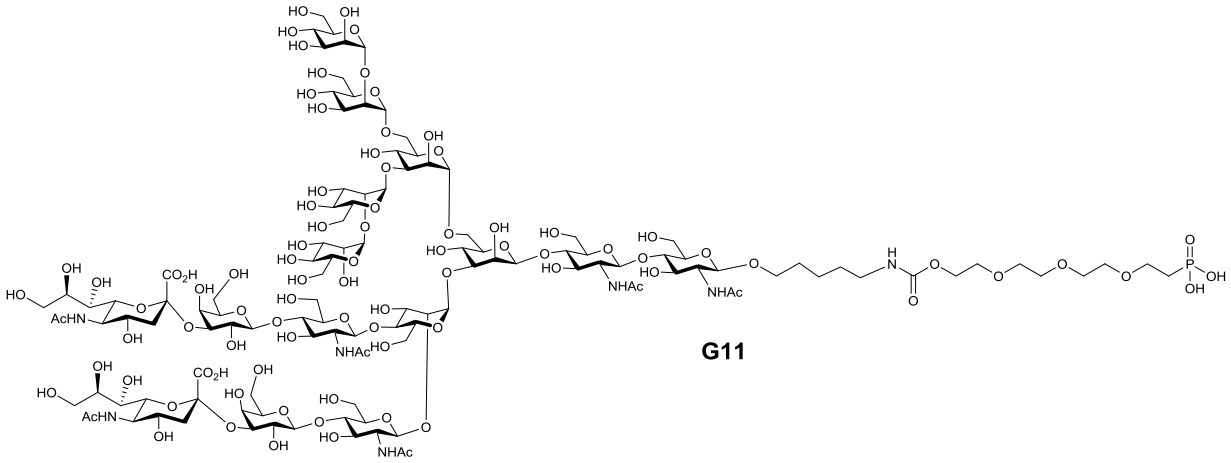
G8 with phosphonic acid linker. G8 with amine linker was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O): δ 5.33 (s, 1H), 5.08 (s, 1H), 5.05 (s, 1H), 4.97 (d, *J* = 8.2 Hz, 2H), 4.80 (s, 1H), 4.53 (t, *J* = 9.2 Hz, 1H), 4.42 (d, *J* = 2.1 Hz, 1H), 4.37 (d, *J* = 7.2 Hz, 1H), 4.15 – 3.48 (m, 93H), 3.04 (t, *J* = 10.2 Hz, 2H), 2.61 (dd, *J* = 4.8 and J = 12 Hz, 1H), 2.00 (s, 6H), 1.96 (s, 6H), 1.91 – 1.87 (m, 2H), 1.65 (t, *J* = 12 Hz, 1H), 1.50 – 1.48 (m, 2H), 1.44 – 1.41 (m, 2H), 1.27 – 1.23 (m, 2H). ESI-MS: *m/z* calculated for $C_{97}H_{166}N_5O_{72}P$; 2583.92 found 2583.92.



G9 with phosphonic acid linker. G9 with amine linker was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O): δ 5.03 (d, *J* = 8.5 Hz, 1H), 4.84 (s, 1H), 4.80 (s, 1H), 4.52 – 4.47 (m, 4H), 4.41 (d, *J* = 6.2 Hz, 1H), 4.15 – 3.48 (m, 100H), 3.04 (t, *J* = 10.9 Hz, 2H), 2.69 (dd, *J* = 4.2 &9.2Hz, 2H), 2.06 (s, 3H), 1.99 (s, 3H), 1.97 (s, 6H), 1.96 (s, 6H), 1.95 – 1.90 (m, 2H), 1.79 – 1.74 (m, 2H), 1.50 – 1.48 (m, 2H), 1.44 – 1.41 (m, 2H), 1.27 – 1.23 (m, 2H). ESI-MS: *m/z* calculated for $C_{110}H_{186}N_7O_{80}P$; 2917.64 found 1460.03 $(M + 2H)^{2+}$.

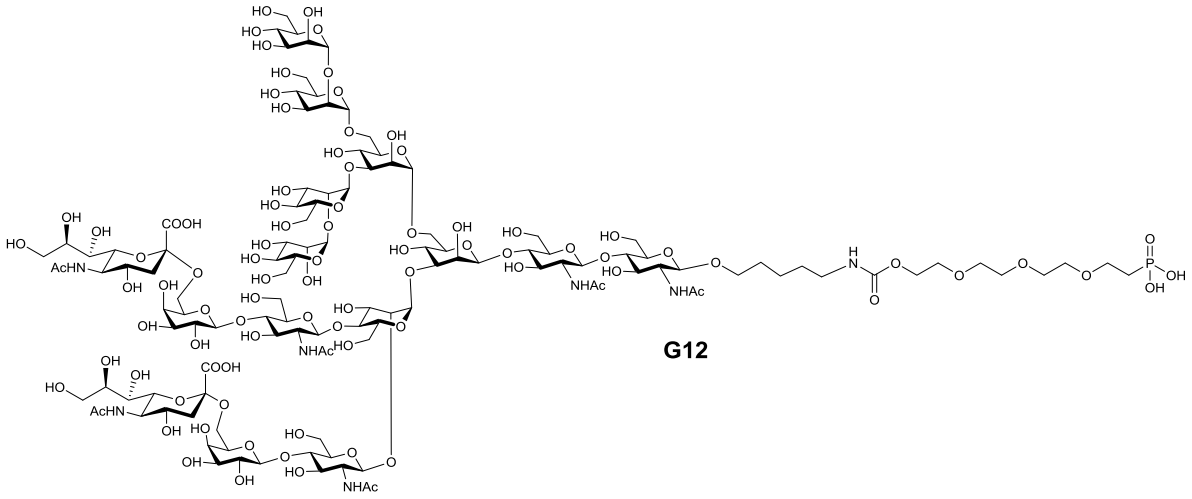


G10 with phosphonic acid linker. G10 with amine linker was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O):. δ 5.05 (s, 1H), 5.02 (s, 1H), 4.84 (s, 1H), 4.80 (s, 1H), 4.52 – 4.49 (m, 2H), 4.42 (s, 1H), 4.37 (s, 1H), 4.16 – 3.48 (m, 94H), 3.04 (t, *J* = 10.2 Hz, 2H), 2.59 (dd, *J* = 4.2 and *J* = 10.2 Hz, 2H), 2.03 (s, 3H), 2.00 (s, 6H), 1.99 (s, 9H), 1.72 – 1.67 (m, 2H), 1.50 – 1.48 (m, 2H), 1.44 – 1.42 (m, 2H), 1.27 – 1.23 (m, 2H). ESI-MS: *m/z* calculated for $C_{110}H_{186}N_7O_{80}P$; 2917.64 found 1459.53 $(M + H)^{2+}$.



G11 with phosphonic acid linker. G11 with amine linker was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O): δ 5.34 (s, 1H), 5.07 (s, 1H), 5.02 (s, 1H), 4.97 (d, *J*=9.6, 2H), 4.52 – 4.47 (m, 5H), 4.17 – 3.47 (m, 112H), 3.04 (t, *J* = 9.2 Hz, 12H), 2.68 (dd, *J* = 2 & 8 Hz, 2H), 2.05 (s, 6H), 1.98 (s, 6H), 1.96 (s, 6H), 2.01–1.95 (m, 2H), 1.75 (t, *J* = 9.2 Hz, 2H),

1.50 – 1.48 (m, 2H), 1.44–1.41 (m, 2H), 1.25–1.27 (m, 2H). ESI-MS: *m/z* calculated for $C_{122}H_{206}N_7O_{90}P$; 3240.15 found 1621.59 $(M + 3H)^{2+}$.



G12 with phosphonic acid linker. G12 was modified by above general procedure. ^{1}H NMR (600 MHz, D_2O):. δ 5.34 (s, 1H), 5.07 (s, 1H), 5.03 (s, 1H), 4.97 (d, *J* = 7.2 Hz, 2H), 4.80 – 4.72 (m, 2H), 4.56 – 4.52 (m, 2H), 4.42 (d, *J* = 8.2 Hz, 2H), 4.18 – 3.48 (m, 105H), 3.04 (t, *J* = 10.2 Hz, 2H), 2.64 (dd, *J* = 4.2 and J = 10.2 Hz, 2H), 2.03 (s, 3H), 2.02 (s, 6H), 2.00 (s, 9H), 1.96 – 1.90 (m, 2H), 1.69–1.68 (m, 2H), 1.48–1.46 (m, 2H), 1.44–1.41 (m, 2H), 1.27–1.23 (m, 2H). ESI-MS: *m/z* calculated for $C_{122}H_{206}N_7O_{90}P$; 3240.15 found 1621.58 $(M + 3H)^{2+}$.

2. Glycan Array Analysis

i. Microarray Fabrication

a) Fabrication of *N*-hydroxy succinimide-coated glass slide: All monovalent glycans (**G1-62**) were prepared in stock buffer (pH 7.4, sodium phosphate 5 mM) 10 mM concentration individually and served as mother solutions which are to be diluted with printing buffer to prepare a working solution. Microarrays were printed (BioDot Cartesian Technologies) by robotic pin (SMP3: TeleChem International) deposition of ~ 0.6 nL of 100 μM concentration of amine-containing glycans in printing buffer. The printed slides were allowed to react in an atmosphere of 80% humidity for an hour followed by desiccation overnight. These slides were designed for 16 grids in one slide, and stored at room temperature in a desiccator prior to use. Before the binding assay, these slides were blocked with blocking solution for 1 h. The slides were then washed with PBST-BSA buffer prior to use. Unless otherwise stated, reagents were obtained from commercial suppliers and used without purification. All aqueous solutions were prepared from distilled de-ionized water filtered with a Milli-Q purification system and sterile filtered through a 0.2 μm filter. Buffers used in the experiment include the printing buffer (pH 8.5, 300 mM sodium phosphate buffer containing 0.01% triton), the blocking buffer (superblock blocking buffer in PBS, Pierce), the washing buffer (PBST buffer; PBS and 0.05% Tween-20) and the binding buffer (PBS buffer and 3% BSA). Printing buffer, blocking buffer, and binding buffer were prepared freshly before use.

b) Fabrication of aluminium oxide coated glass slide.

A detailed procedure for fabrication of aluminium oxide coated glass slide has been reported previously[8]. A fluorescence intensity of sugar-protein binding on the substrates was found to be more sensitive on aluminum oxide glass substrate provides than that of transparent glass slide. As reported, an aluminum coated (300 nm) on glass slide (1 x 75.5 x 24.5 mm) was fabricated using E-Beam VDP coater by the Thin Film Technology Division, Instrument Technology Research Center at National Applied Research Laboratories, (Hsinchu Science Park, Hsinchu, Taiwan, R.O.C.). The slide after Al-coating was immediately packed and vacuum sealed in air-tight laminated foil pouches until the moment for surface anodization. Surface anodization of the aluminum coated glass slide has been conducted in-house via electrochemical reaction in a 0.3 M oxalic acid aqueous solution in a 4 °C chamber. The anodization reaction was controlled by

voltage and reaction time. Under proper reaction conditions, a layer of approximately 50 to 65 nm of smooth anodized aluminum oxide (AAO) can be grown consistently on top of aluminum coated glass slide.

ii. Antibody binding assay:

Glycan microarrays were blocked with blocking buffer (Superblock, Thermo Fisher Scientific, USA) for 1 hour and washed with PBST buffer (PBS buffer with 0.05% Tween 20). The HIV-1 broadly neutralizing antibodies (25 μg/mL in BSA contained PBS buffer, 3% w/v) were pre-complexed with Donkey Anti-Human IgG (Alexa Fluor647 conjugated, Jackson Immuno Research, USA) in 1:1 ratio and incubated at 4°C for 30 minutes. The pre-complexed mixture was added to the glycan array and incubated at 4°C overnight. The slides were washed sequentially in PBST buffer (PBS and 0.05% Tween-20), and de-ionized water. Slides were spin dried prior to scanning with a GenePix 4300A reader (Molecular Devices, USA) and analyzed with GenePix Pro 7.0 software (Molecular Devices, USA).

iii. Image processing and data analysis:

The slide was scanned at 635 nm with a microarray fluorescence chip reader (GenePix® 4300A, Molecular Devices Corporation). Scanned images were analyzed with GenePix Pro 6.0 analysis software (Molecular Devices Corporation,). The image resolution was set to 5 μm per pixel. Spots were defined as circular features with maximum diameter of 100 μm. The values of total intensity were chosen for data processing, performed with Graphpad Prism® 6.0. The intensities was calculated and averaged. Error bars represent the standard deviation for all data points reported.

iv. Mapping glycan epitopes of HIV-1 broadly neutralizing antibodies

a. Using ACG slide: The slide for the study of bNAbs was prepared by printing glycan **G1-29** (Figure S3a) on the aluminum oxide coated surface through acid base condensation of phosphonic acid with aluminum hydroxyl groups. Slide images obtained by fluorescent scan after secondary antibody incubation.

b. Using NHS glass slide: The slide for the study of bNAbs was prepared by printing glycans **G1-62** (Figure S3b) on the *N*-hydroxysuccinimide coated surface through covalent bond formation.

vi. Determination of surface dissociation constants (KD.surf) on NHS slide:

The quantitative measurement of hybrid glycan and HIV-1 bNAbs interactions ($K_{D,surf}$) was perfomed by following our previously published protocol.[9] In perticular, the *N*-hydroxysuccinimide coated coated glass slides for the determination of dissociation constants were spotted with 400, 300, 200, 150, 100, 75, and 50 μM concentrations of hybrid type glycans G6, G8, G10 and G12. Various glycan printing concentrations can change their density on array surface which further affect on atibody recognition. Antibodies PG9, PG16, 35O22, VRC26.25, VRC26.09, PGT121, PGT128-3C, PGDM1400, and PGT121 was serially diluted to 2.13, 1.07, 0.53, 0.27, 0.13, 0.07, 0.03, and 0.02 μM with 3% BSA-PBS buffer. DyLight 649-conjugated Donkey Anti-Human IgG antibody was pre-complexed with primary antibody (1:1 by weight). The pre-complexed solution (100 μL) was applied to each well and incubated at 4 °C for overnight in the dark. Finally, slides were washed with PBST, spin dried and scanned at 635 nm with a microarray fluorescence chip reader. Scanned images were analyzed with GenePix Pro 6.0 analysis software. The curves were analyzed as Langmuir isotherms, assuming that the system reached equilibrium during incubation.

$$F = \frac{F_{\max}\,[\mathrm{P}]}{[\mathrm{P}] + K_{\mathrm{D,surf}}}$$

Where, F_{max} is the maximum fluorescence intensity, a measure of the amount of active carbohydrate on the surface, [P] is the total antibody concentration, and $K_{D,surf}$ is the equilibrium dissociation constant for surface carbohydrate and antibody. The binding curves for the HIV bNAbs are shown along with total microarray data. The $K_{D,surf}$ values are summarized in Table 1.

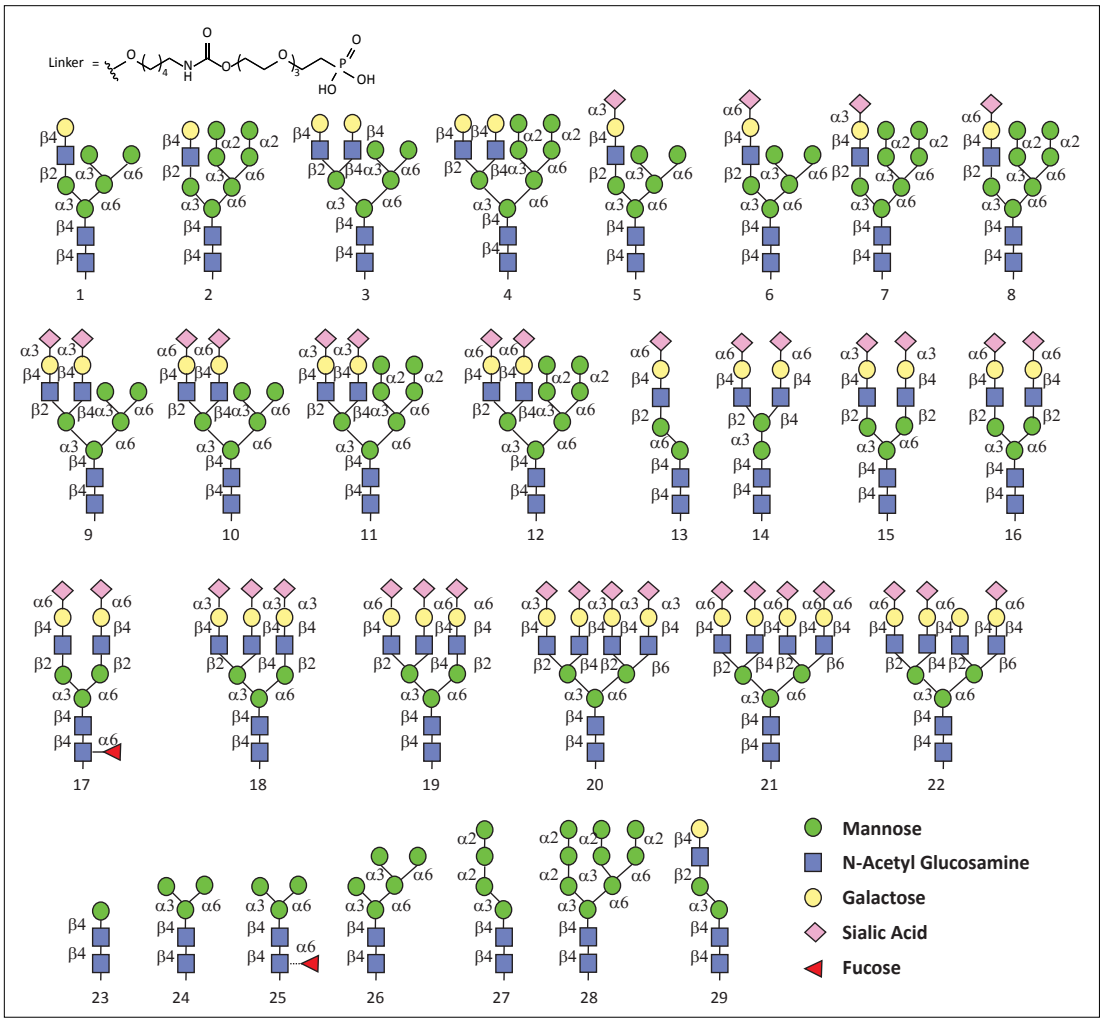


Figure S3a | Schematic representation of *N*-glycans printed on ACG glass slide. Glycans from this work were combined with in-house array.

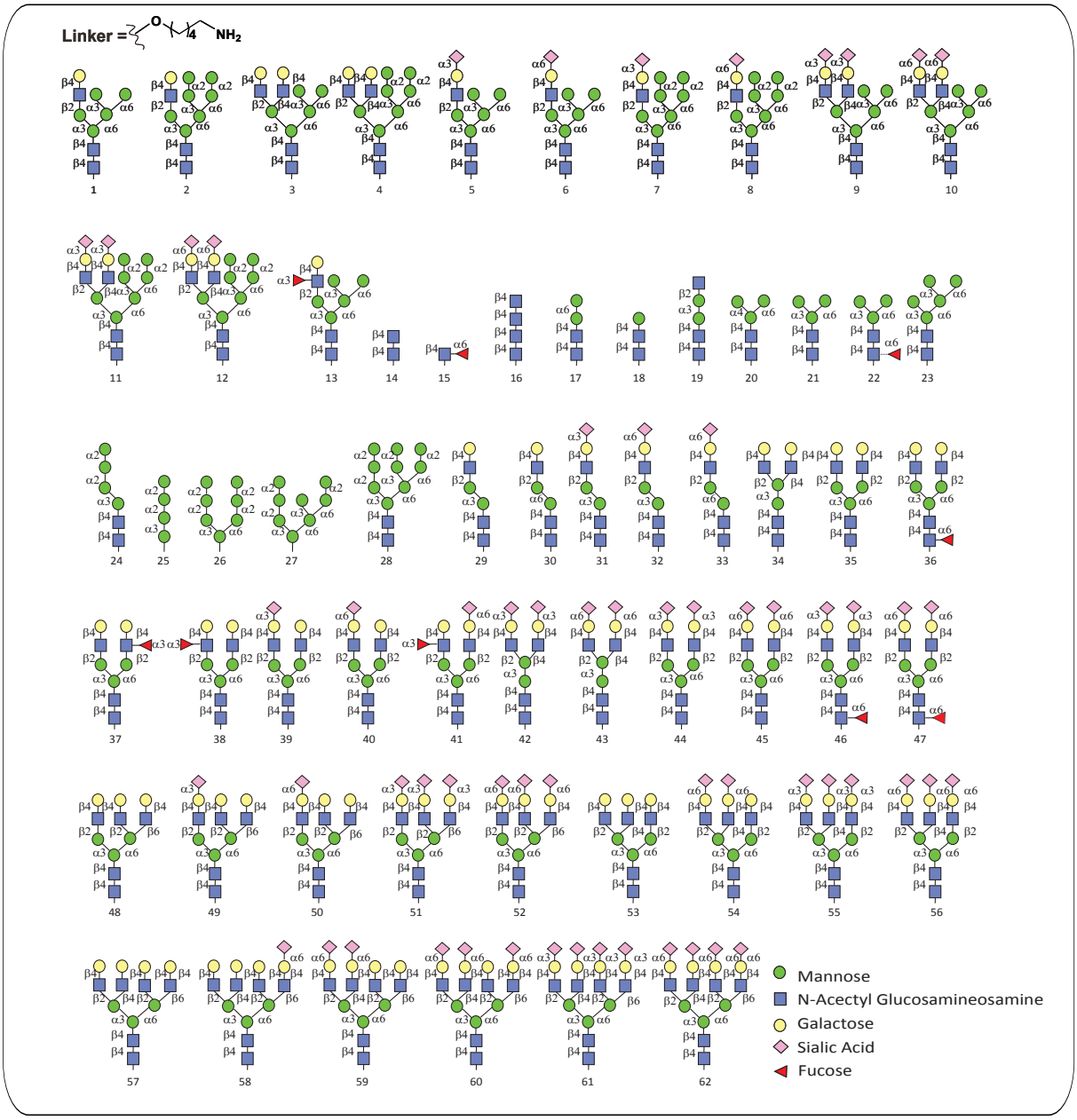


Figure S3bl Schematic representation of *N*-glycans printed on NHS coated glass slide. Glycans from this work were combined with in-house array.

Binding specificity profiling of PG9 on single and mixed ACG arrays

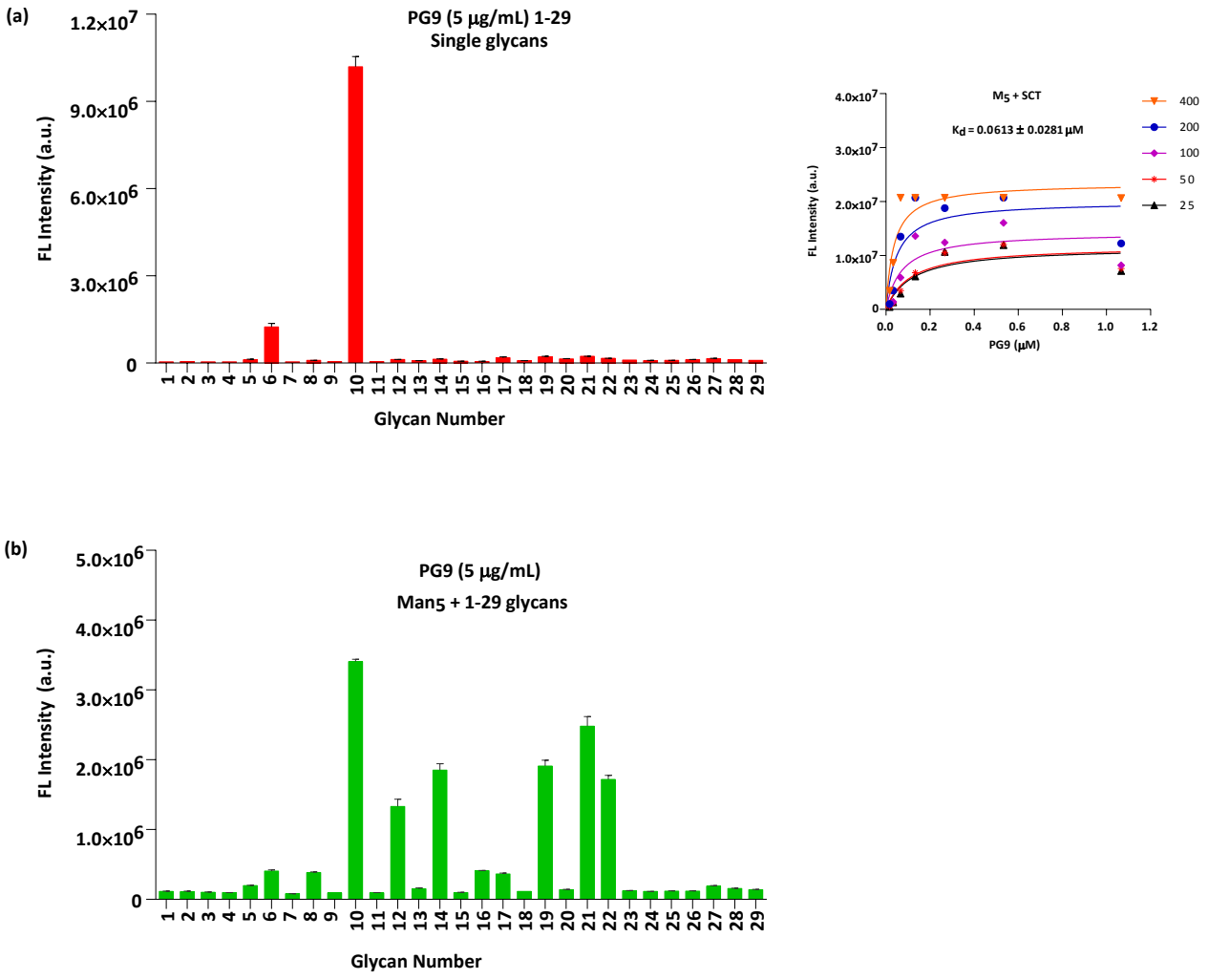


Figure S4. Binding behavior of PG9 using ACG glass slide. (a) Glycans **G1-29** with phosphonic acid tails were covalently immobilized on the ACG slide. (b) For mixed glycan arrays, solutions of $Man_5GlcNAc_2$ mixed with every glycan from G1-29 in a 1 : 1 mole ratio, were printed on ACS array. (c) The slides for the determination of dissociation constants were spotted with 400, 300, 200, 150, 100, 75, and 50 µM concentrations of a mixture of Man5 with SCT glycan. Antibody PG9 was serially diluted to 2.13, 1.07, 0.53, 0.27, 0.13, 0.07, 0.03, and 0.02 µM with 3% BSA-PBS buffer. DyLight 649-conjugated Donkey Anti-Human IgG antibody was pre-complexed with primary antibody (1 : 1 by weight). Antibody concentrations used here are depicted in legends.

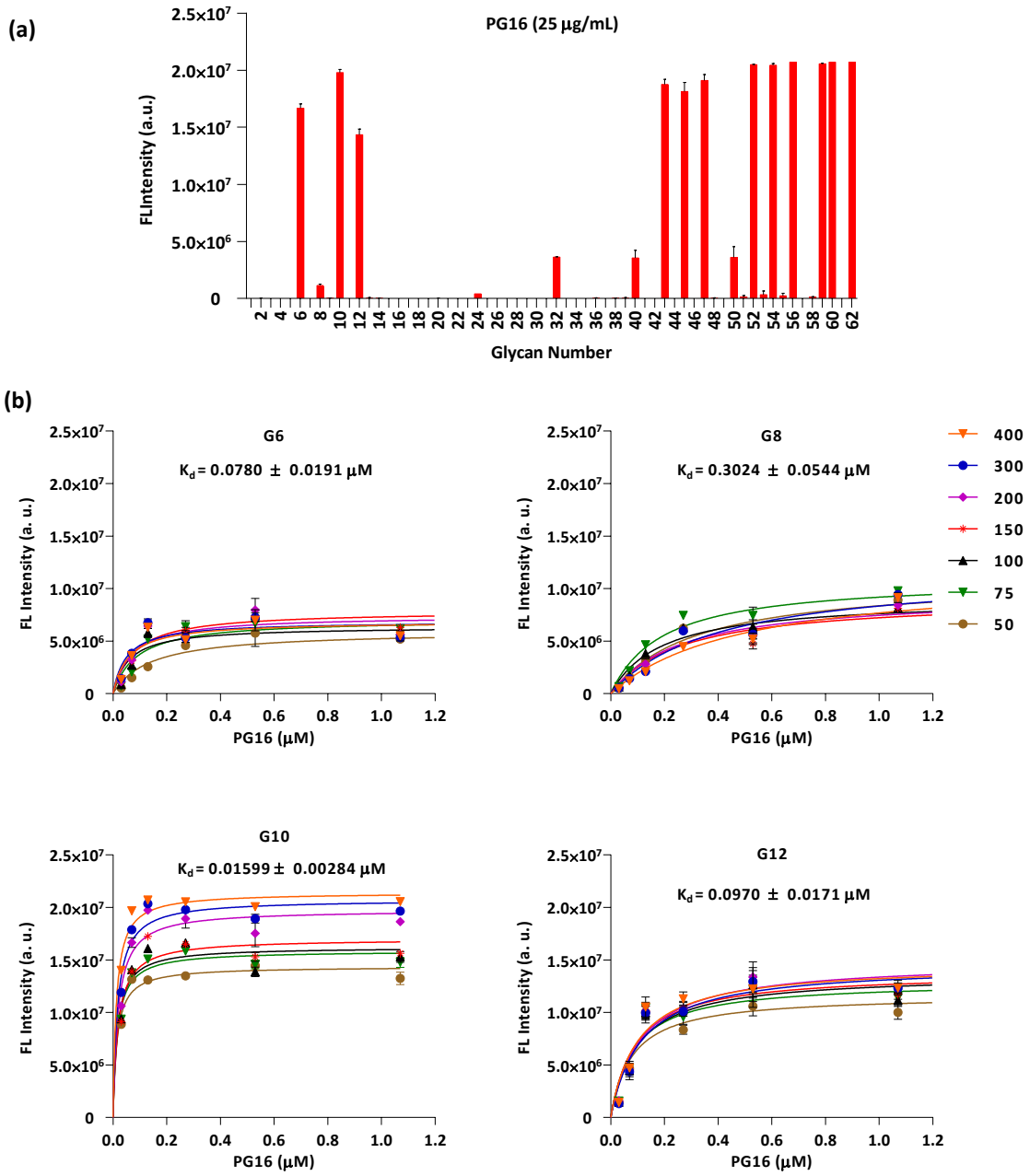


Figure S5. (a) Binding of PG16 towards panel of *N*-glycans on NHS glass slide. Antibody concentrations used here are 25 µg/mL. (b) Binding curves observed for hybrid glycans **G6, G8**, **G10** and **G12** at various concentrations. The curves were obtained by using DyLight649-conjugated donkey anti-Human IgG secondary antibody.

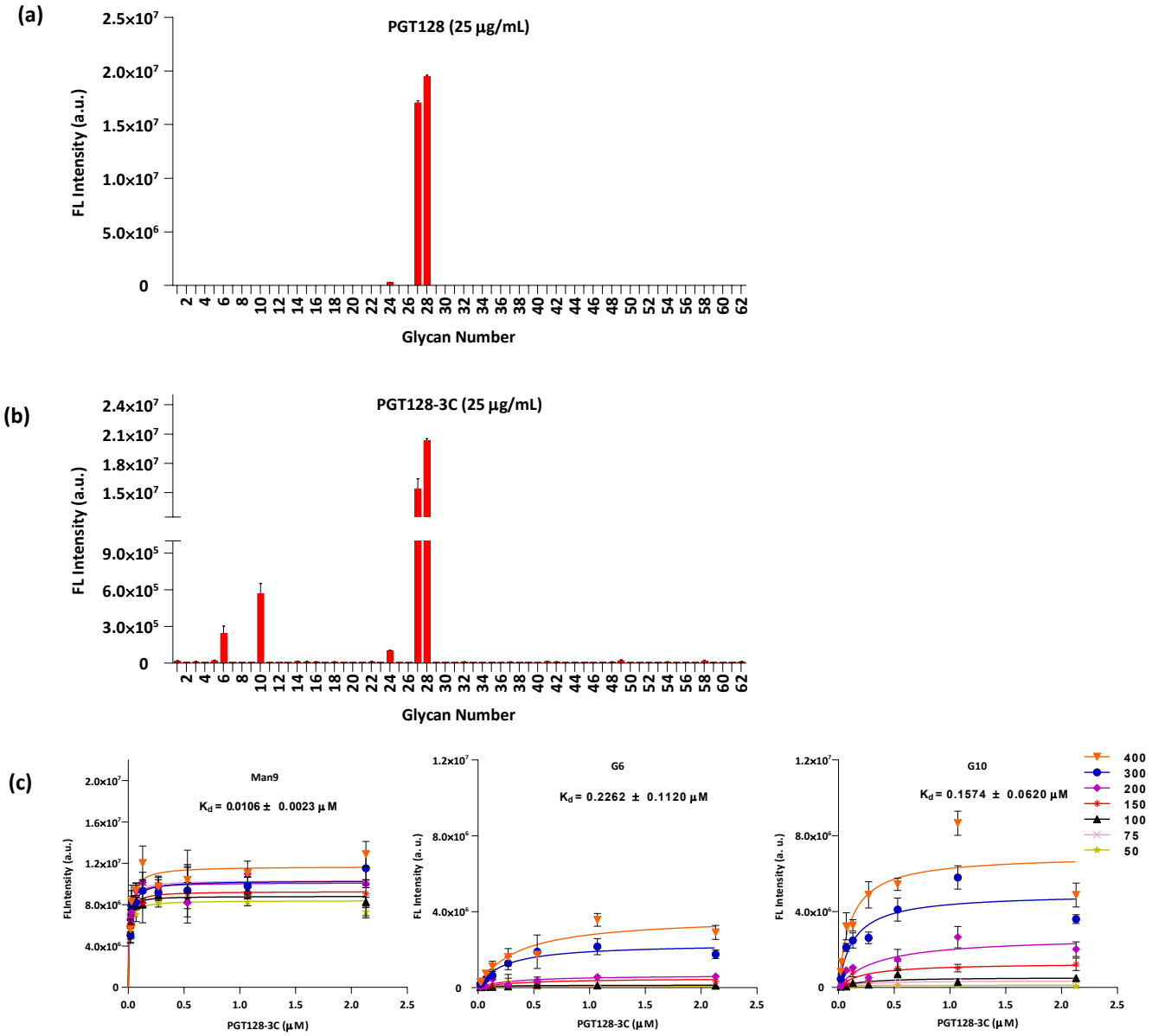


Figure S6. Binding behavior of (a) PGT128 and (b) PGT128-3C using glycans **G1-62** printed on NHS-coated glass slide. Antibody concentrations used here are 25 µg/mL. (c) Determination of dissociation constants. The binding curves for glycans Man_9, **G6** and **G10** printed on array are shown and K_D values are summarized in Table 1.

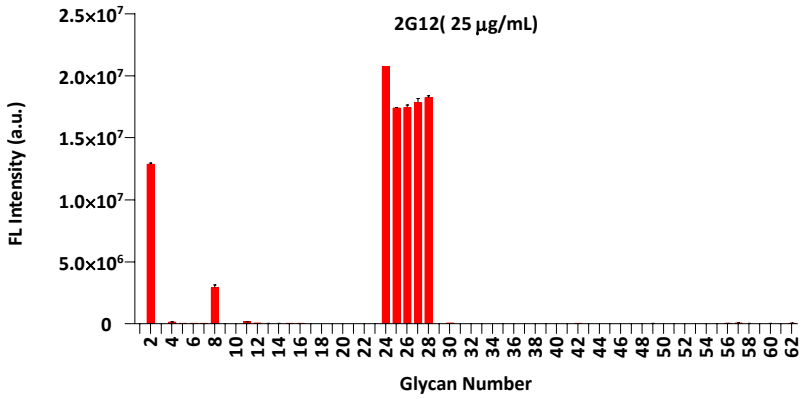


Figure S7. Binding behavior of 2G12 towards glycans **G1-62** represented in bar chart.

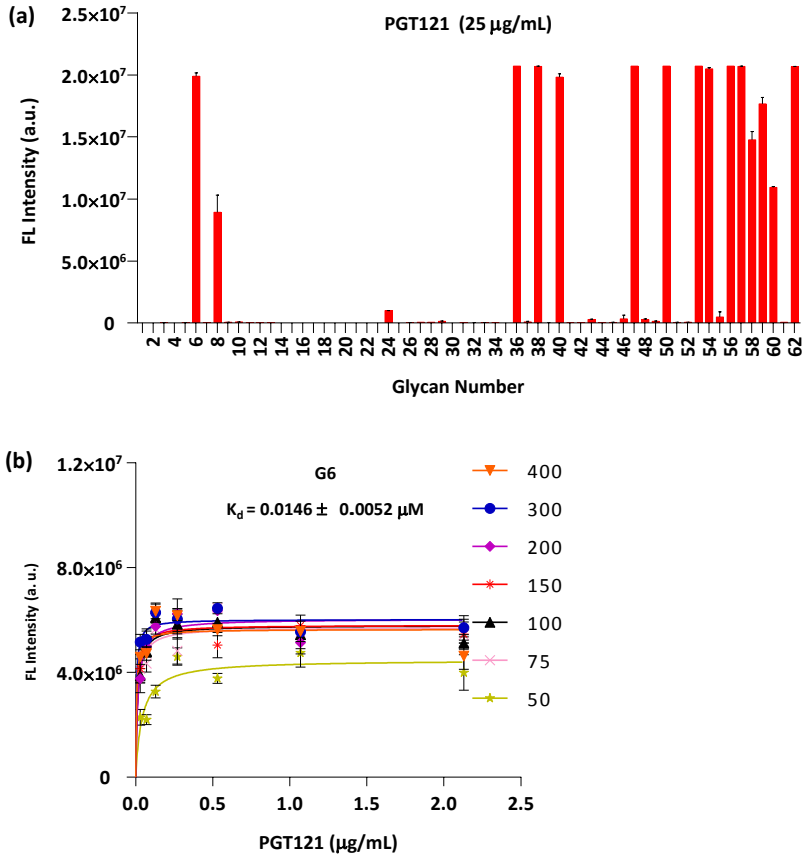


Figure S8. (a) Binding of PGT121 using NHS glass slide. Antibody concentrations used here are 25 µg/mL. (b) Determination of dissociation constant. Binding curves observed for glycan **G6** at various concentrations is shown and *a* K_D value is summarized in Table 1.

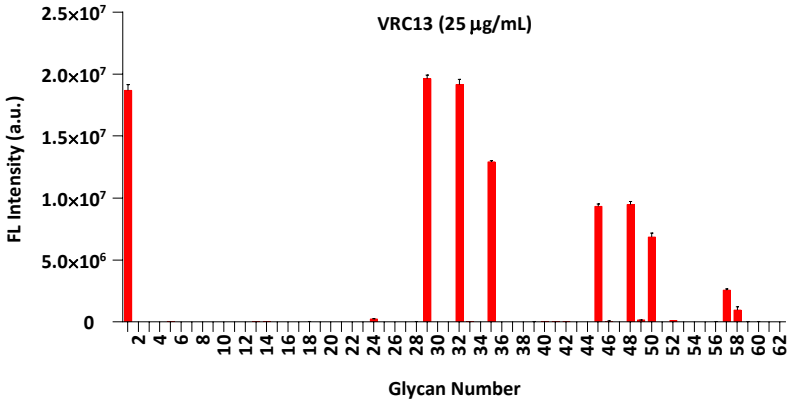


Figure S9a. Binding of VRC13 using NHS glass slide. The binding was detected for hybrid glycan **G1** and series of truncated bi-antennary structures.

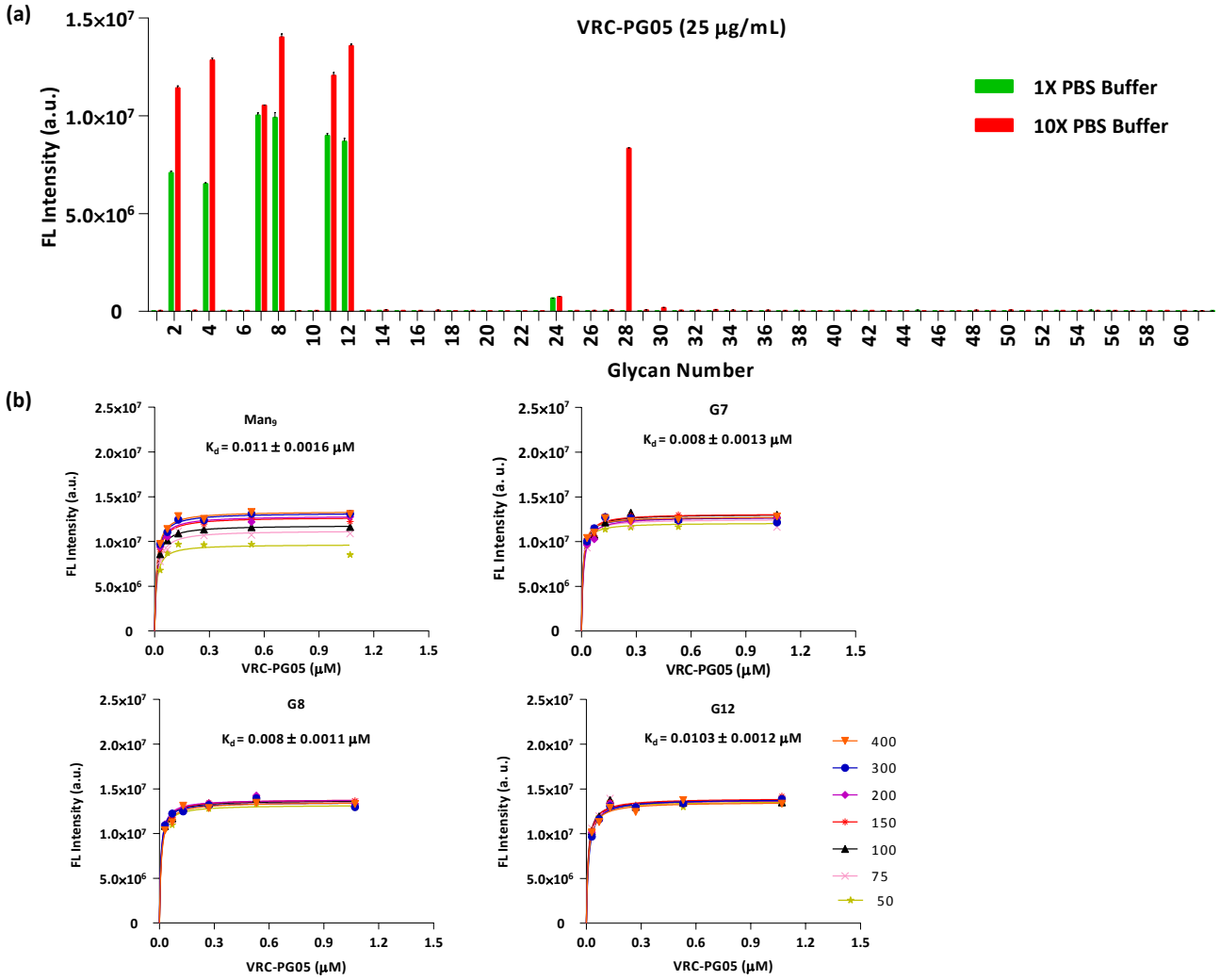


Figure S9b. (a) Binding of VRC-PG05 using NHS glass slide. (b) Determination of dissociation constant. Binding curves observed for glycan Man_9, **G7, G8,** and **G12** at various concentrations are shown and K_D value are summarized in Table 1

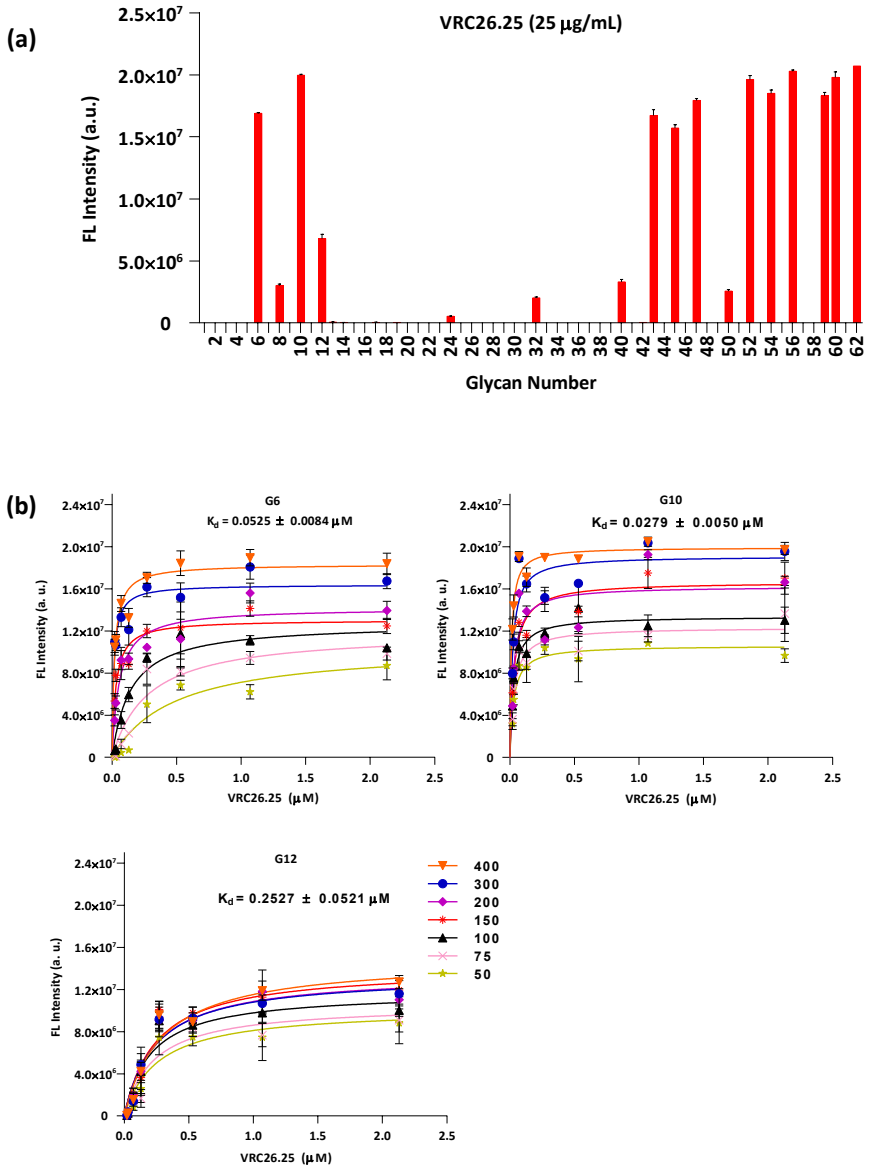


Figure S10. (a) Binding of VRC26.25 using NHS glass slide. Antibody concentrations used here are 25 µg/mL. (b) Determination of dissociation constant. Binding curves observed for glycan **G6, G10,** and **G12** at various concentrations are shown and K_D value are summarized in Table 1.

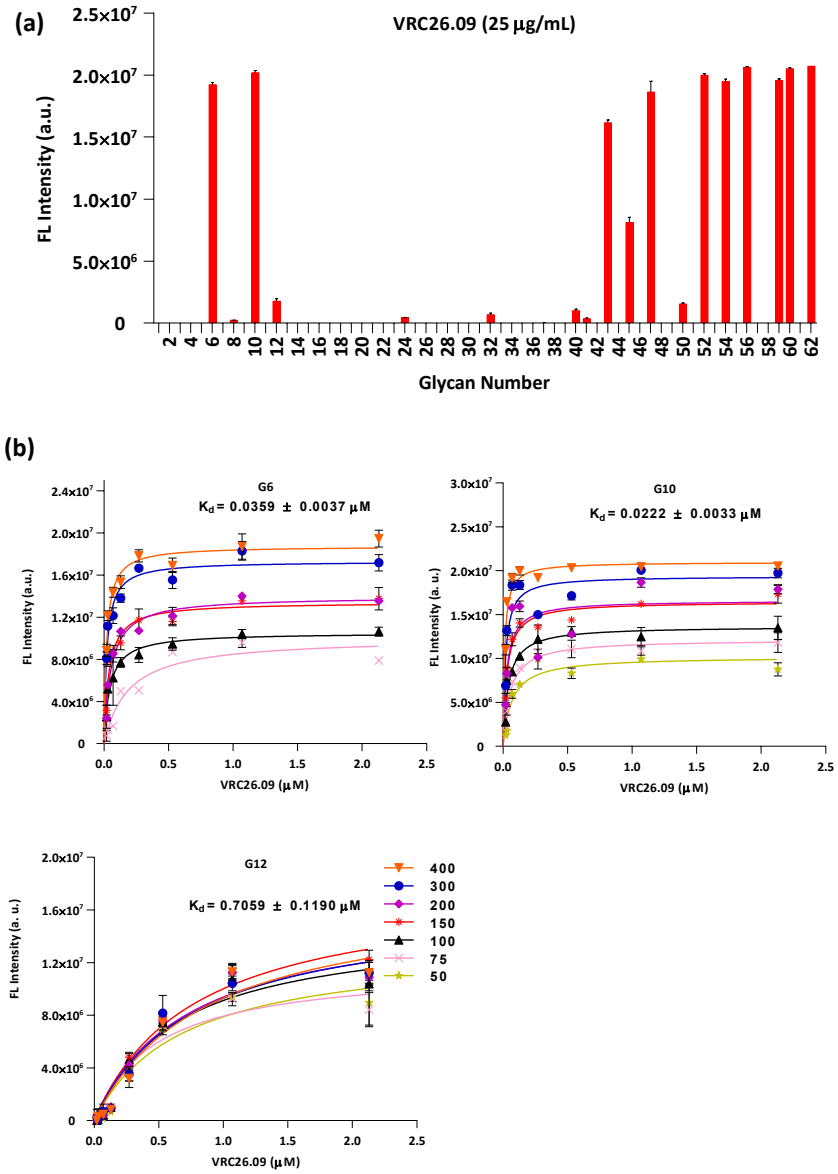


Figure S11. (a) Binding of VRC26.09 using NHS glass slide. Antibody concentrations used here are 25 µg/mL. (b) Determination of dissociation constant. Binding curves observed for glycan **G6, G10,** and **G12** at various concentrations are shown and K_D value are summarized in Table 1.

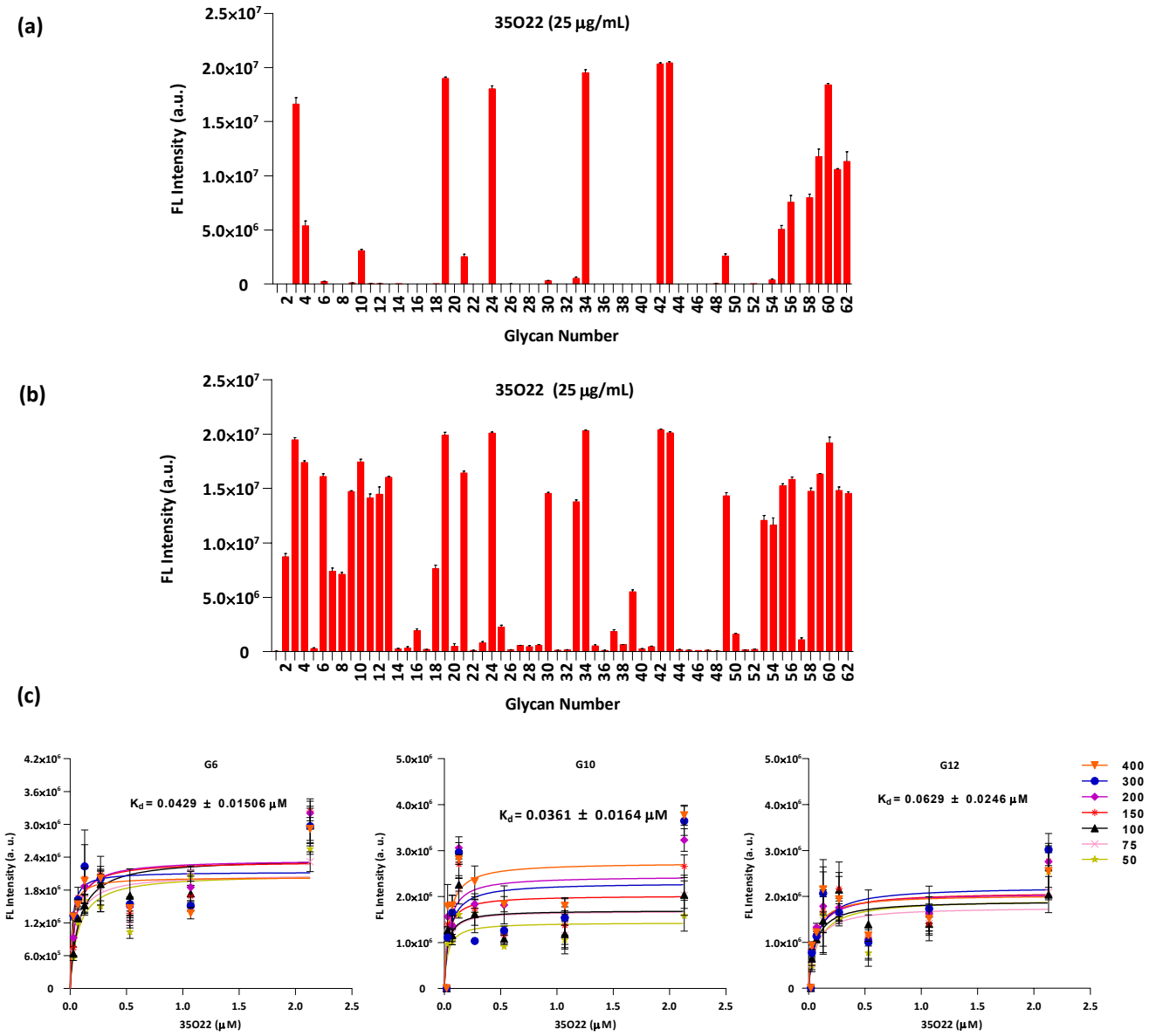


Figure S12. (a,b) Binding of 35O22 on NHS glass slide in various buffer concentrations (a, 1XPBS; b,10XPBS). (c) Binding curves observed for glycans **G6, G10** and **G12** at various concentrations. The curves were obtained by using DyLight649-conjugated donkey anti-Human IgG secondary antibody.

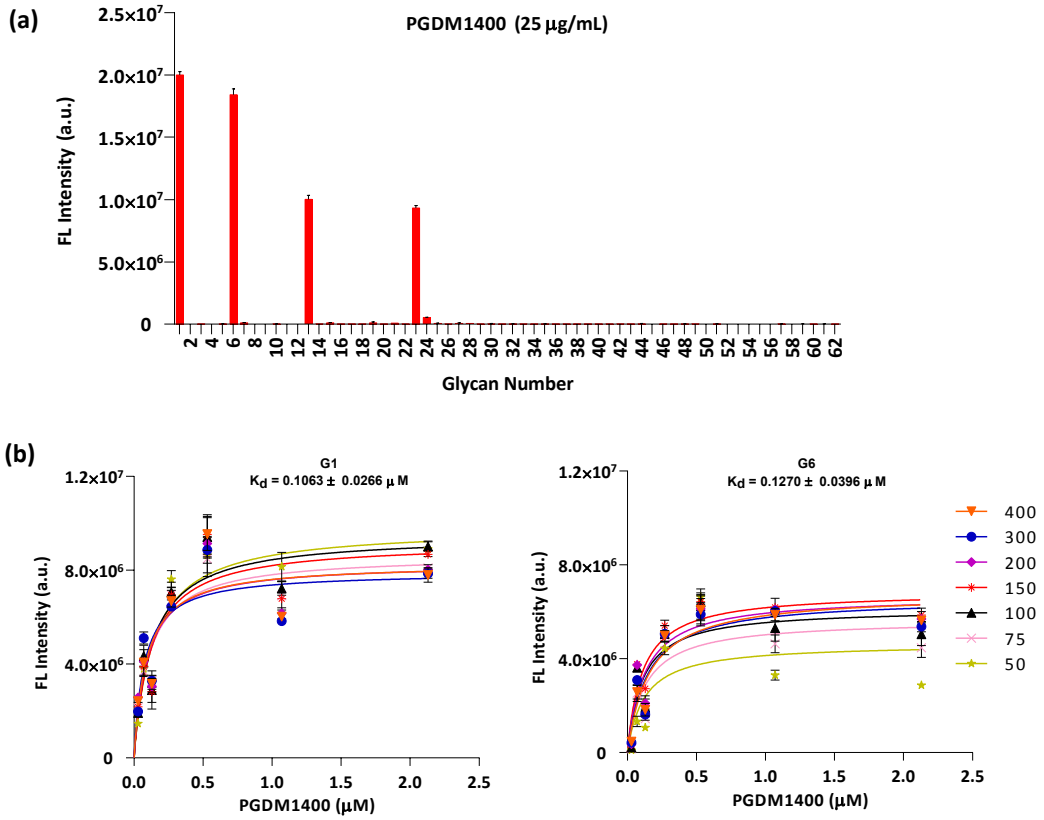


Figure S13. (a) Binding of PGDM1400 bNAb on NHS glass slide. (b) Binding curves observed for glycans **G1,** and **G6** at various concentrations. The curves were obtained by using DyLight649-conjugated donkey anti-Human IgG secondary antibody.

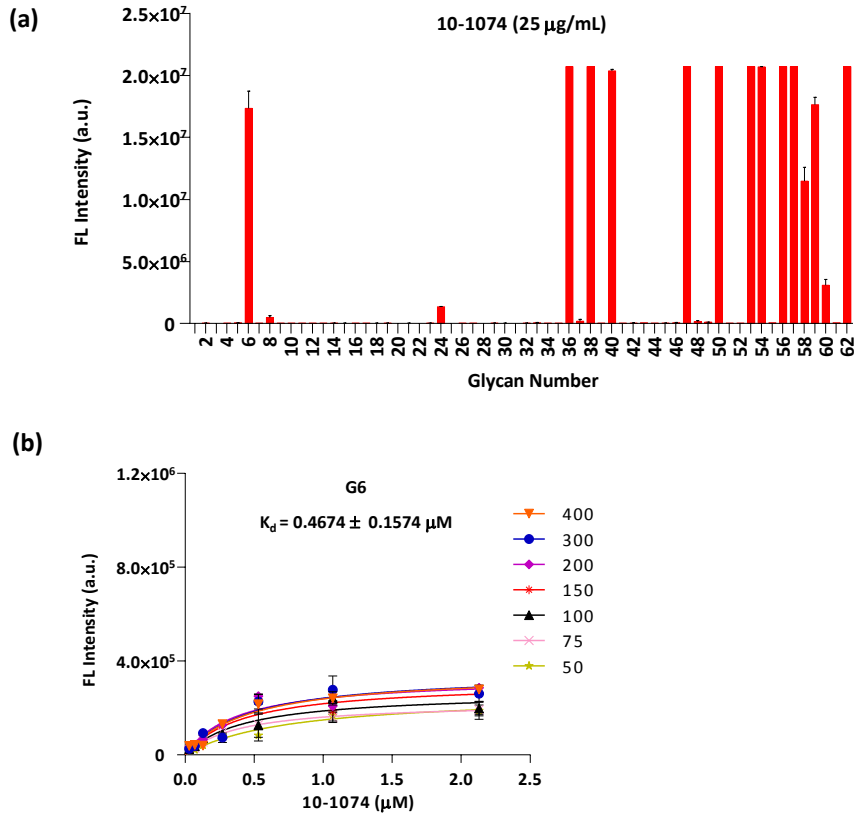


Figure S14. (a) Binding of 10-1074 bNAb on NHS glass slide. (b) Binding curve observed for glycan **G6** at various concentrations. The curve was obtained by using DyLight649-conjugated donkey anti-Human IgG secondary antibody.

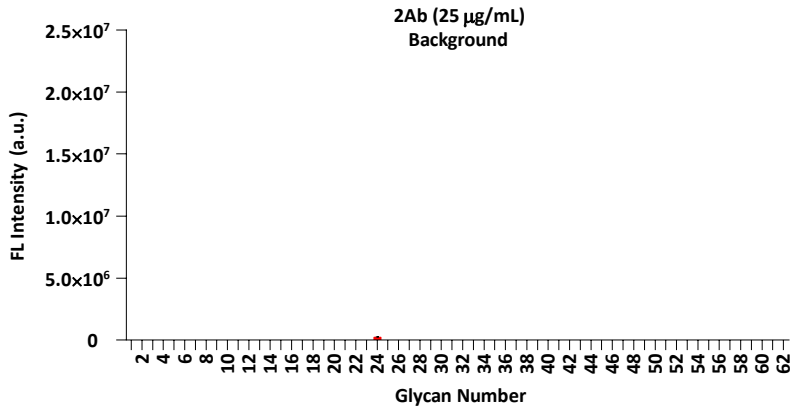


Figure S15. Experimental proof of secondary antibody (DyLight649-conjugated donkey anti-Human IgG) secondary antibody. binding to glycan 24 (Man_4)

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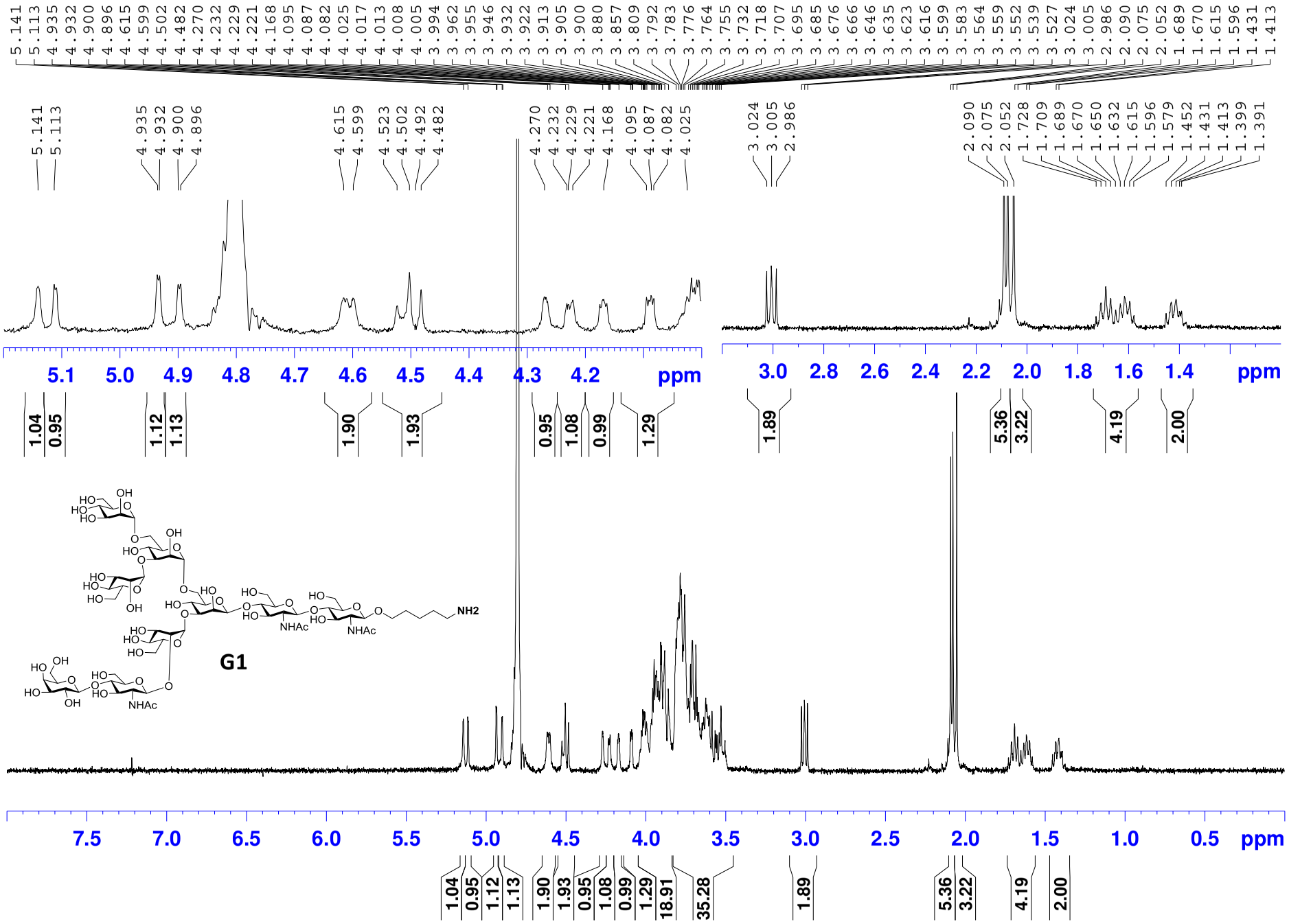
V. NMR characterization data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NH2
NHAc
ppm

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174.396
171.000

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102.273
101.456
101.018
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99.403
99.211
80.391
79.509
79.217
78.584
78.422
76.307
75.308
74.685
74.486
74.389
74.321
73.522
73.280
72.660
72.446
72.319
71.904
70.917
70.788
70.535
70.304
70.186
70.088
69.906
69.412
69.341
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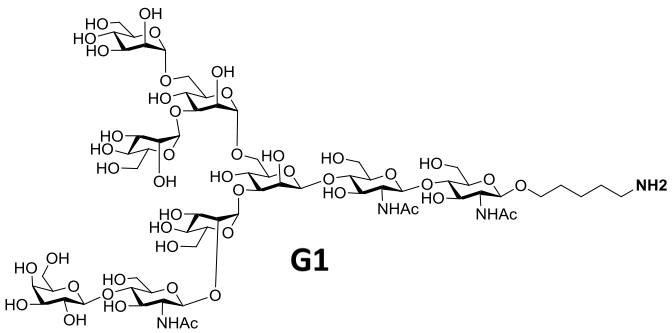
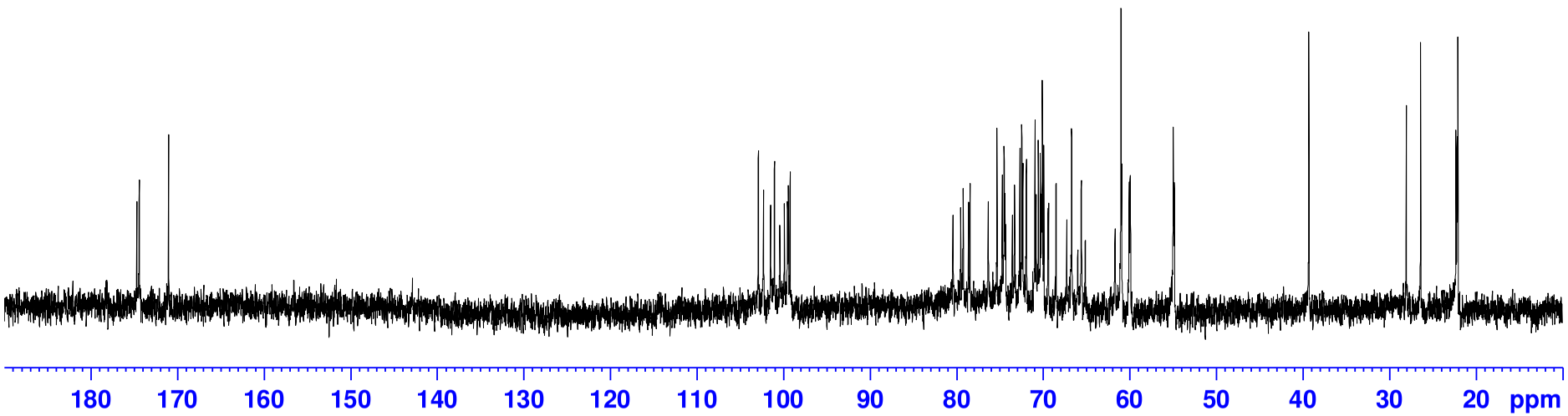
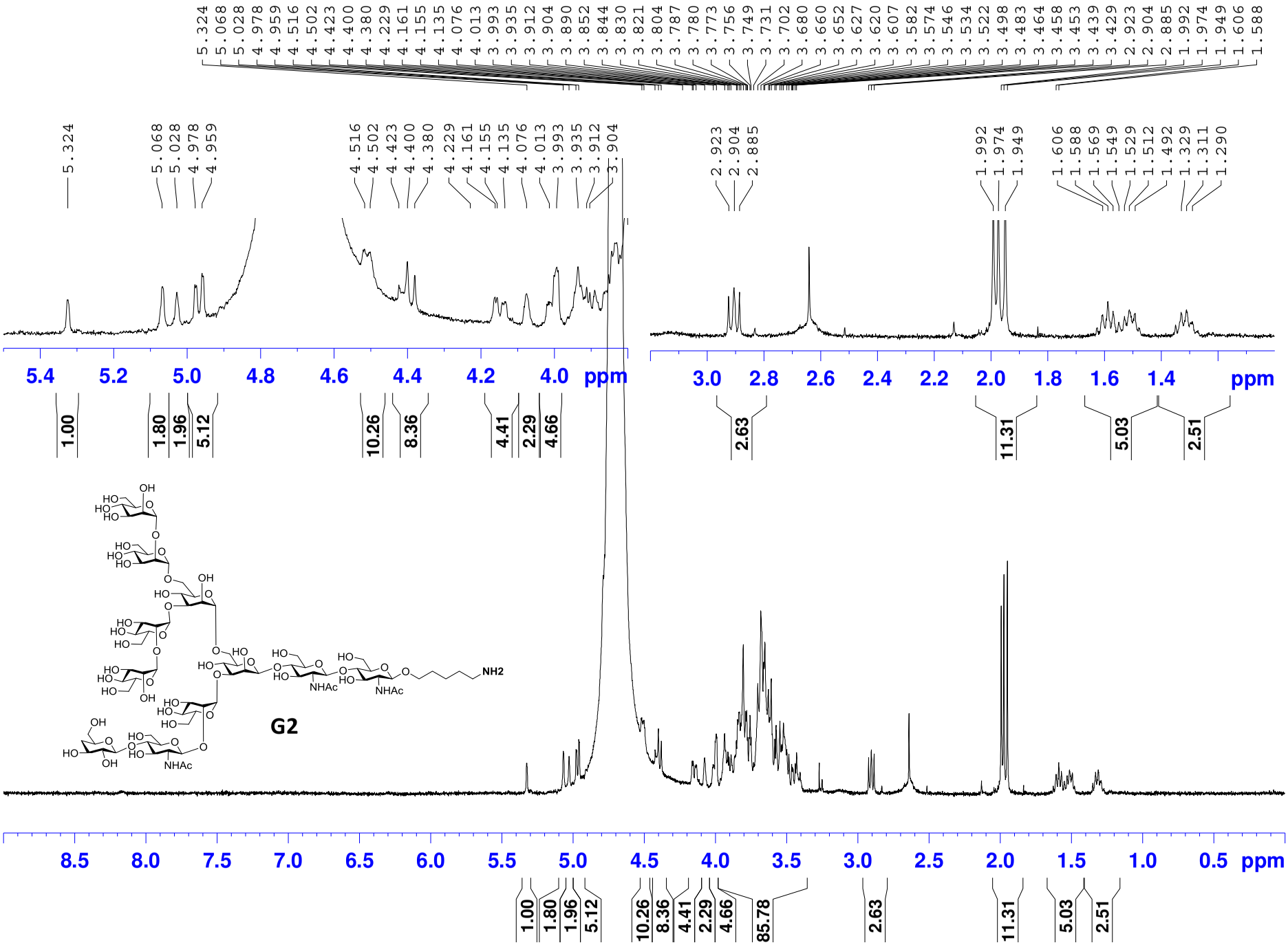
66.695
65.979
65.573
65.111
61.665
60.898
60.036
59.932



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5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 ppm
1.00 1.80 1.96 5.12 10.26 8.36 4.41 2.29 4.66
3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 ppm
2.63 11.31 5.03 2.51
G2
NH2
NHAc
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00 1.80 1.96 5.12 10.26 8.36 4.41 2.29 4.66 85.78 2.63 11.31 5.03 2.51

174.681
174.476
174.403

102.886
102.235
101.414
101.017
100.598
100.262
99.527
99.443
97.970
80.606
79.263
78.896
78.603
78.400
76.374
75.304
74.660
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74.040
73.504
73.286
73.168
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71.144
70.921
70.262
70.069
70.005
69.908
69.386
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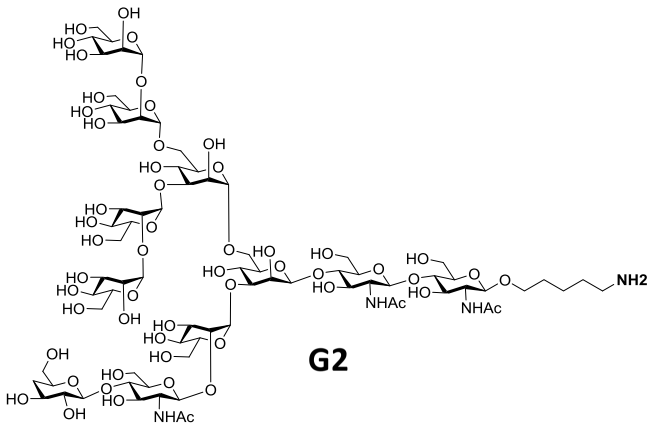
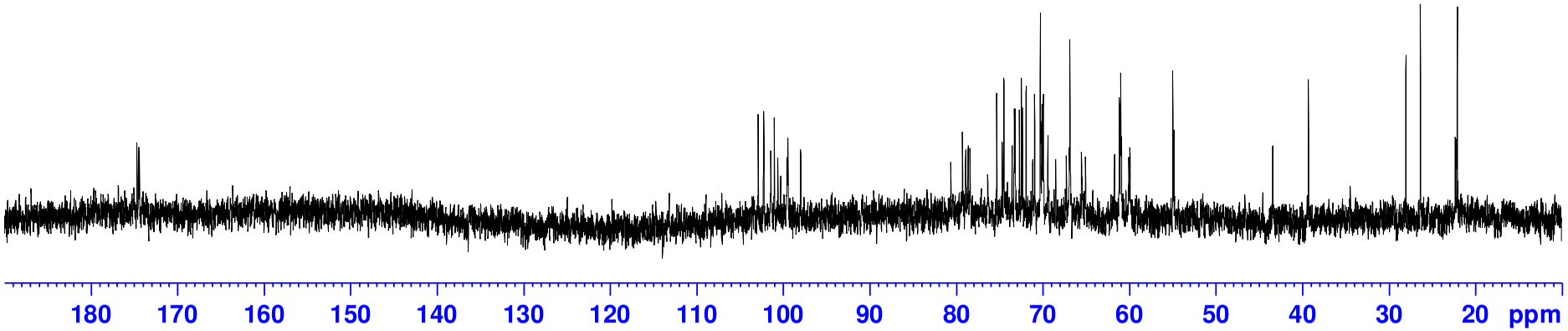
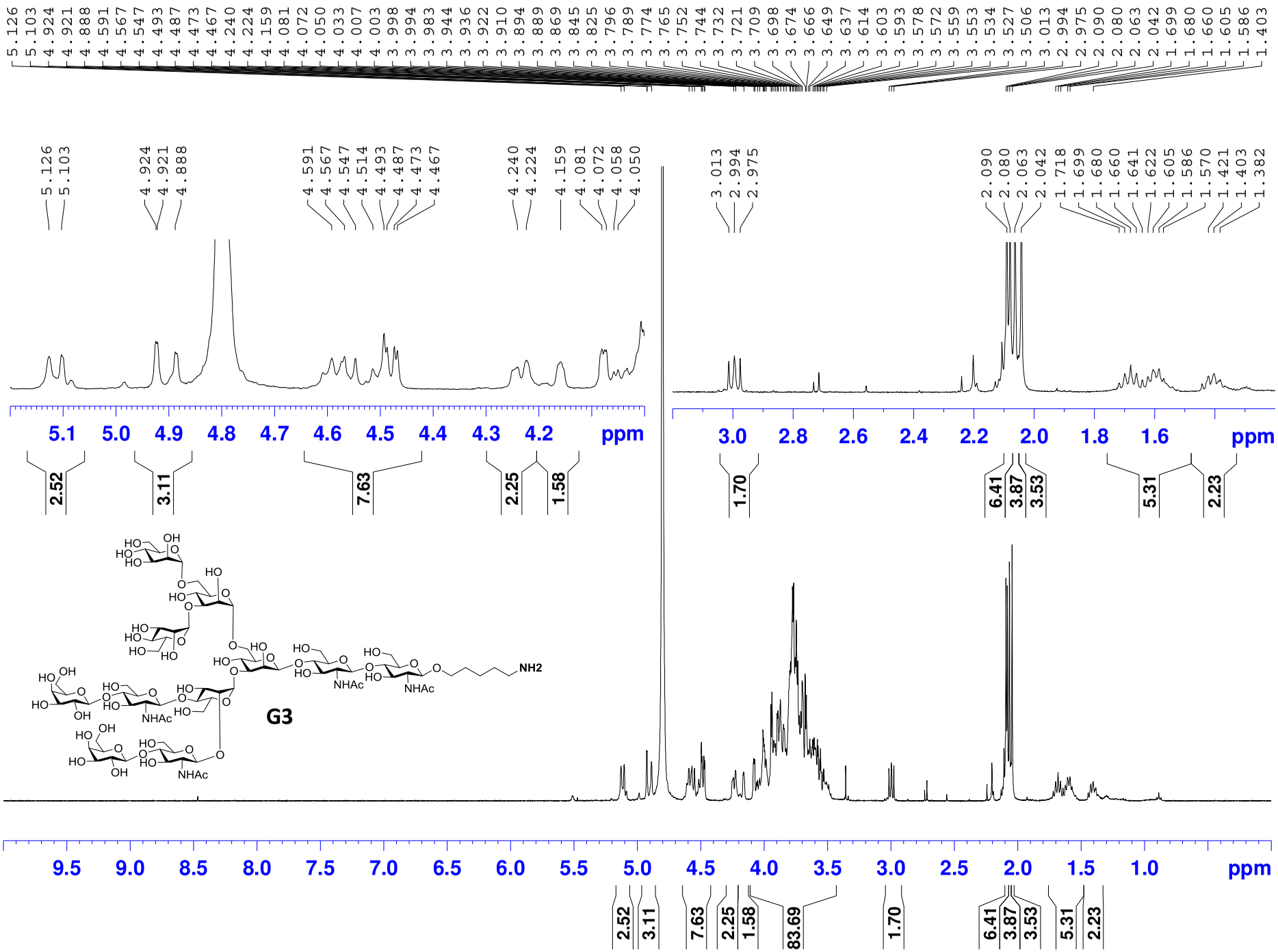


G2




G3
NH2
NHAc
ppm

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174.398
102.894
102.850
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101.537
101.454
101.015
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99.889
99.478
99.213
99.086
79.208
78.548
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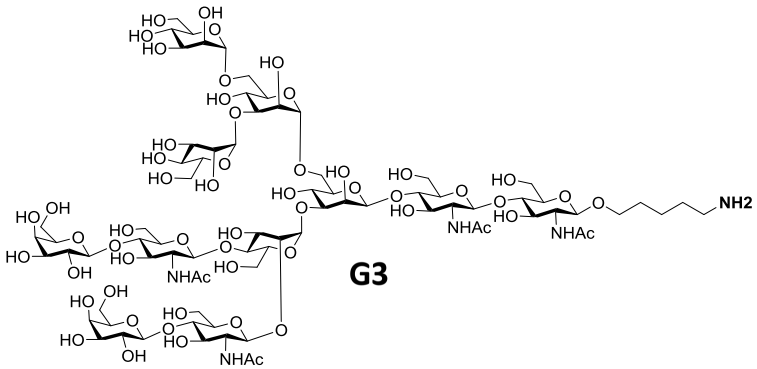

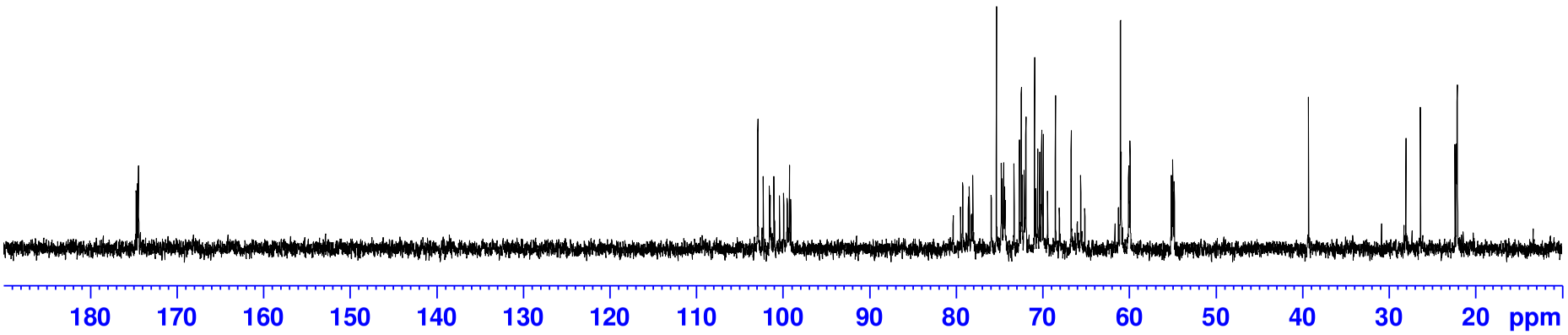

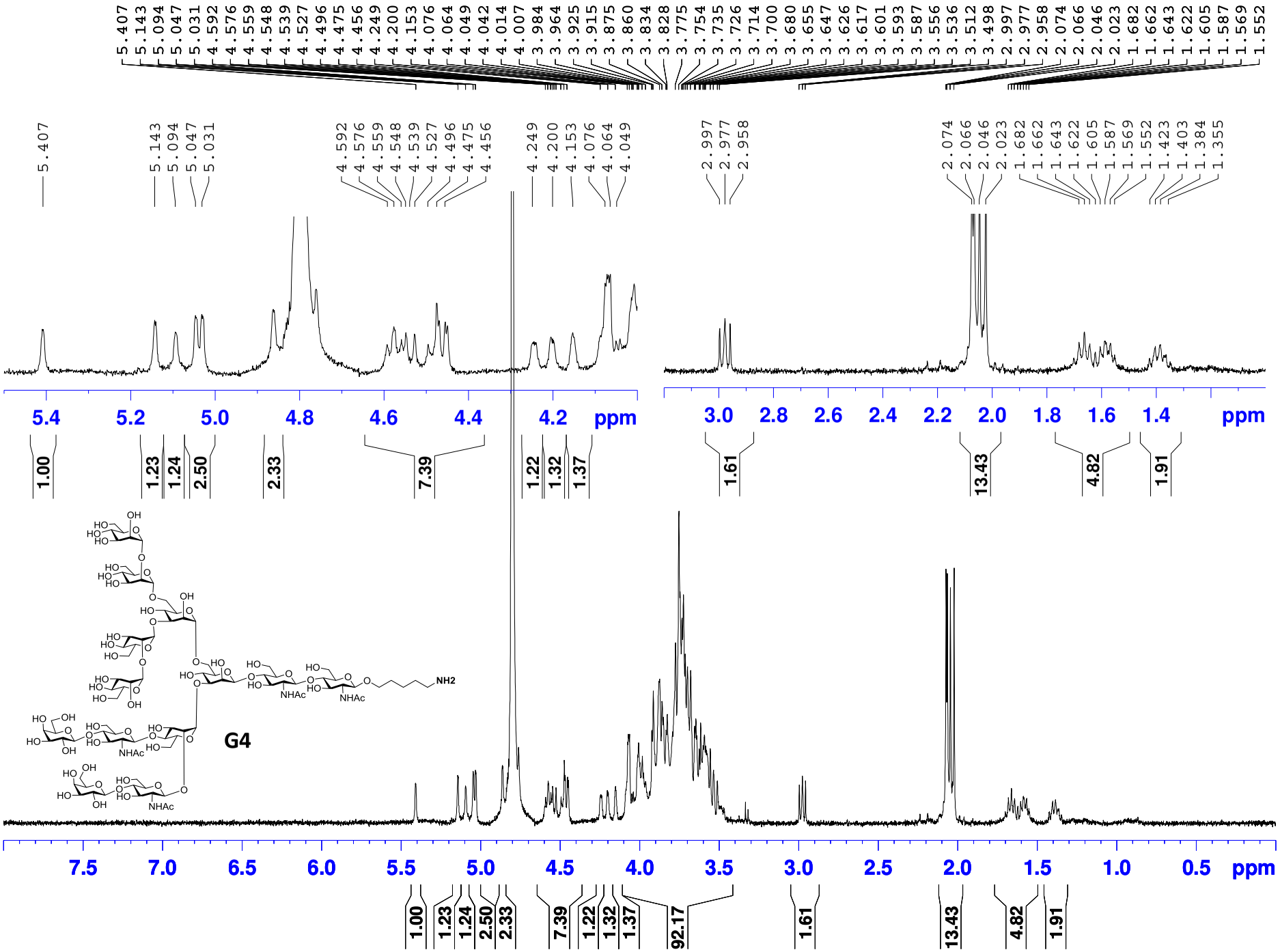
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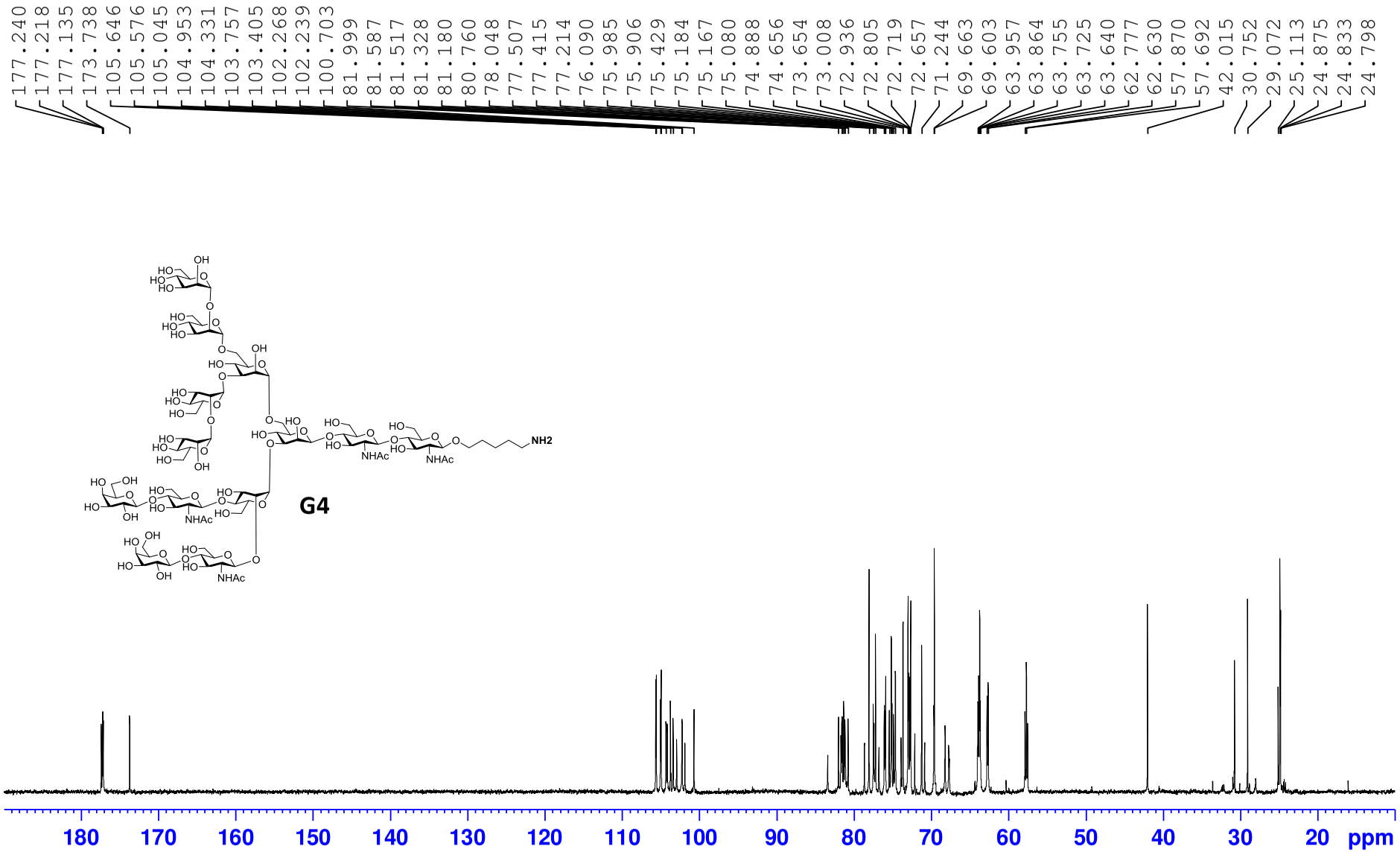
NH2
NHAc
ppm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102.239
100.703
81.999
81.587
81.517
81.328
81.180
80.760
78.048
77.507
77.415
77.214
76.090
75.985
75.906
75.429
75.184
75.167
75.080
74.888
74.656
73.654
73.008
72.936
72.805
72.719
72.657
71.244
69.663
69.603
63.957
63.864
63.755
63.725
63.640
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62.630
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29.072
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24.833
24.798
G4
NH2
NHAc
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160
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130
120
110
100
90
80
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40
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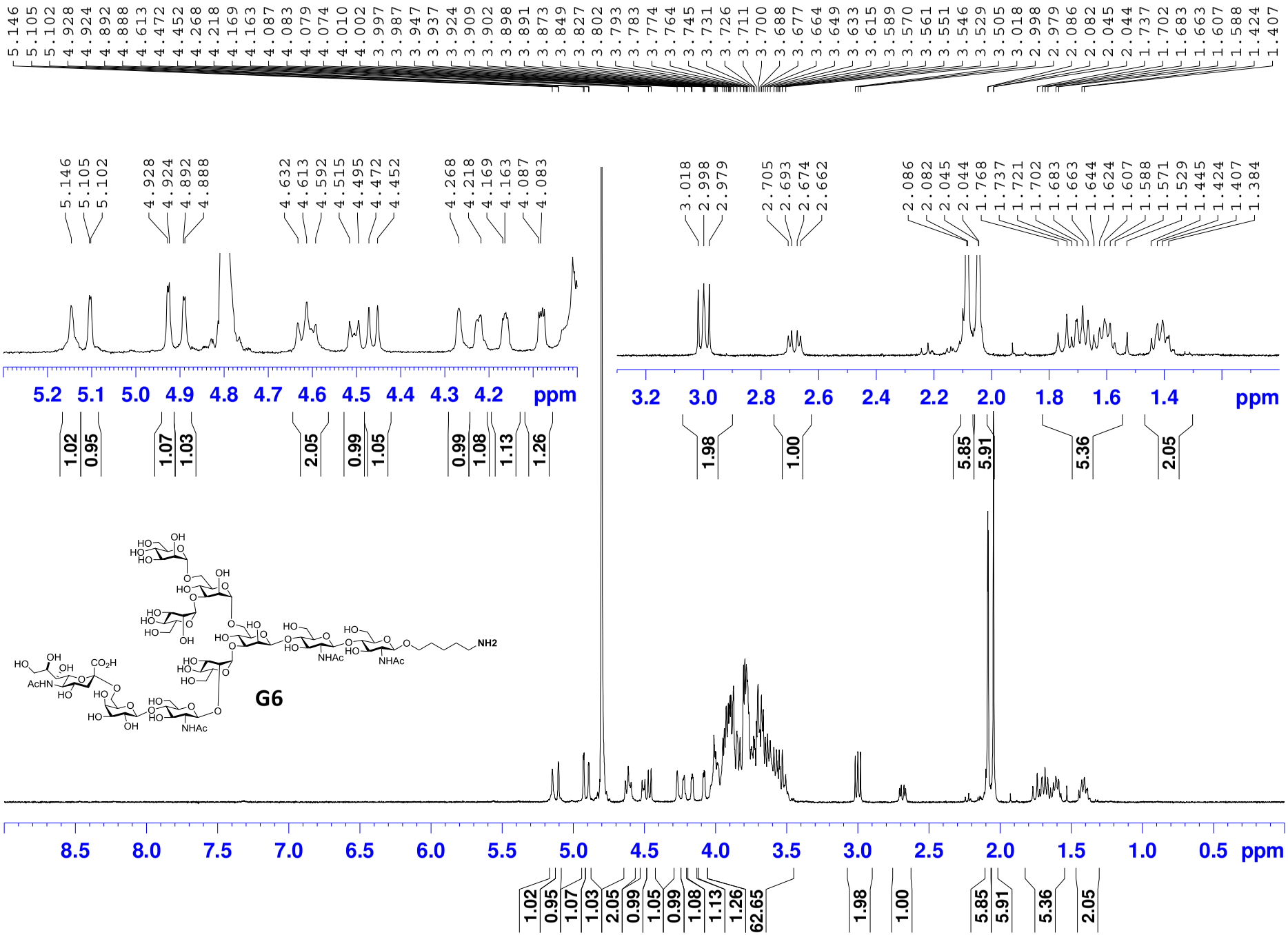
20
ppm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NHAc
AcHN
CO2H
ppm

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100.086
99.534
99.218
80.480
79.500
79.269
78.598
74.482
74.391
73.664
73.277
72.656
72.488
72.319
72.013
71.937
71.668
70.688
70.538
70.304
70.189
70.093
70.052
69.906
69.383
68.355
68.162
67.221
66.673
65.583
65.504
65.114
63.292
62.600
61.675
60.972
60.901
60.046
59.879
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54.560
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39.273
28.005
26.329
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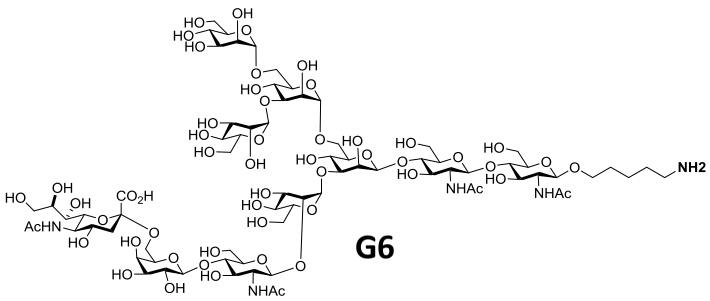
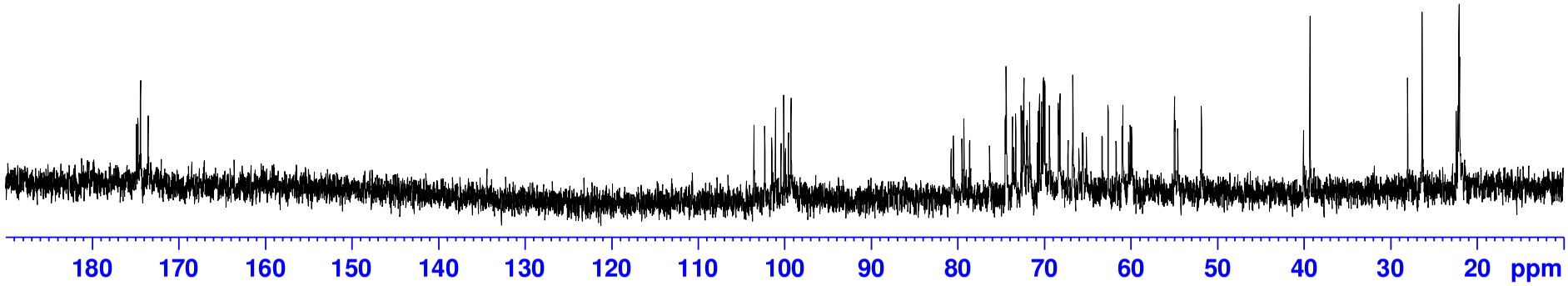
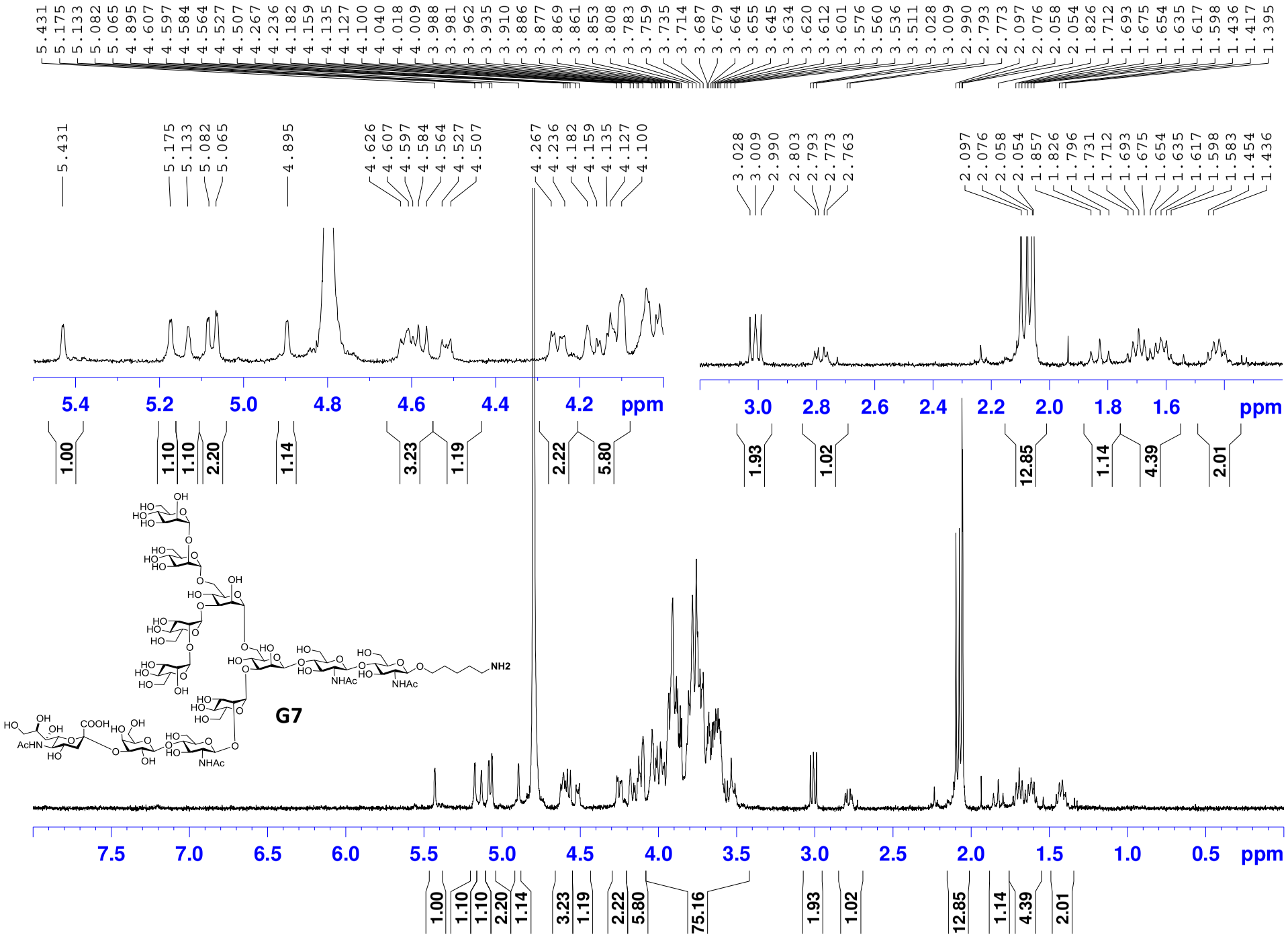
22.160
22.057
21.997






G7
NH2
NHAc
AcHN
COOH
ppm

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174.393
173.848

102.556
102.234
101.015
100.595
100.251
99.758
99.502
97.956
79.287
78.624
78.582
75.408
75.113
74.668
74.467
73.278
73.162
72.833
72.679
72.334
71.884
71.725
70.265
70.056
69.997
69.909
69.345
68.322
68.037
67.418
66.918
66.845
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65.047
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61.114
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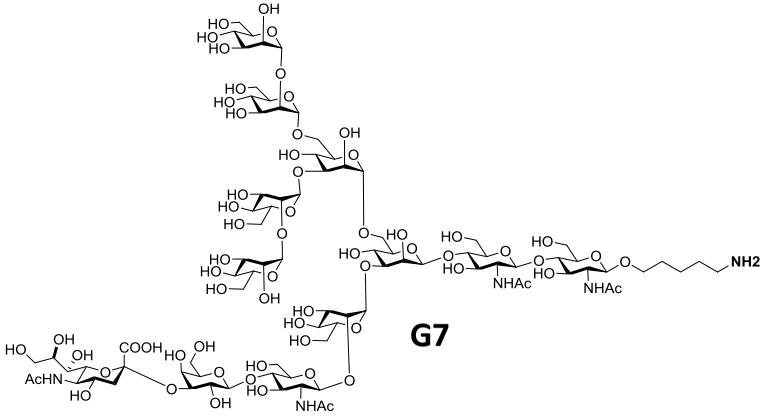
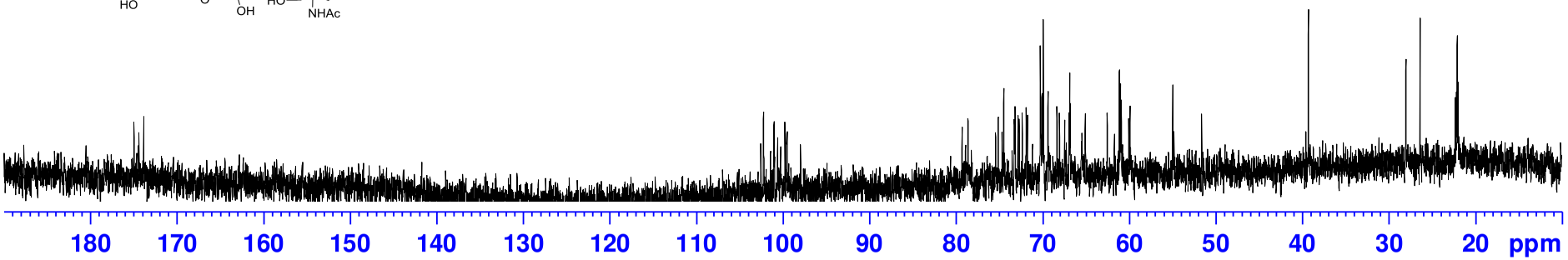
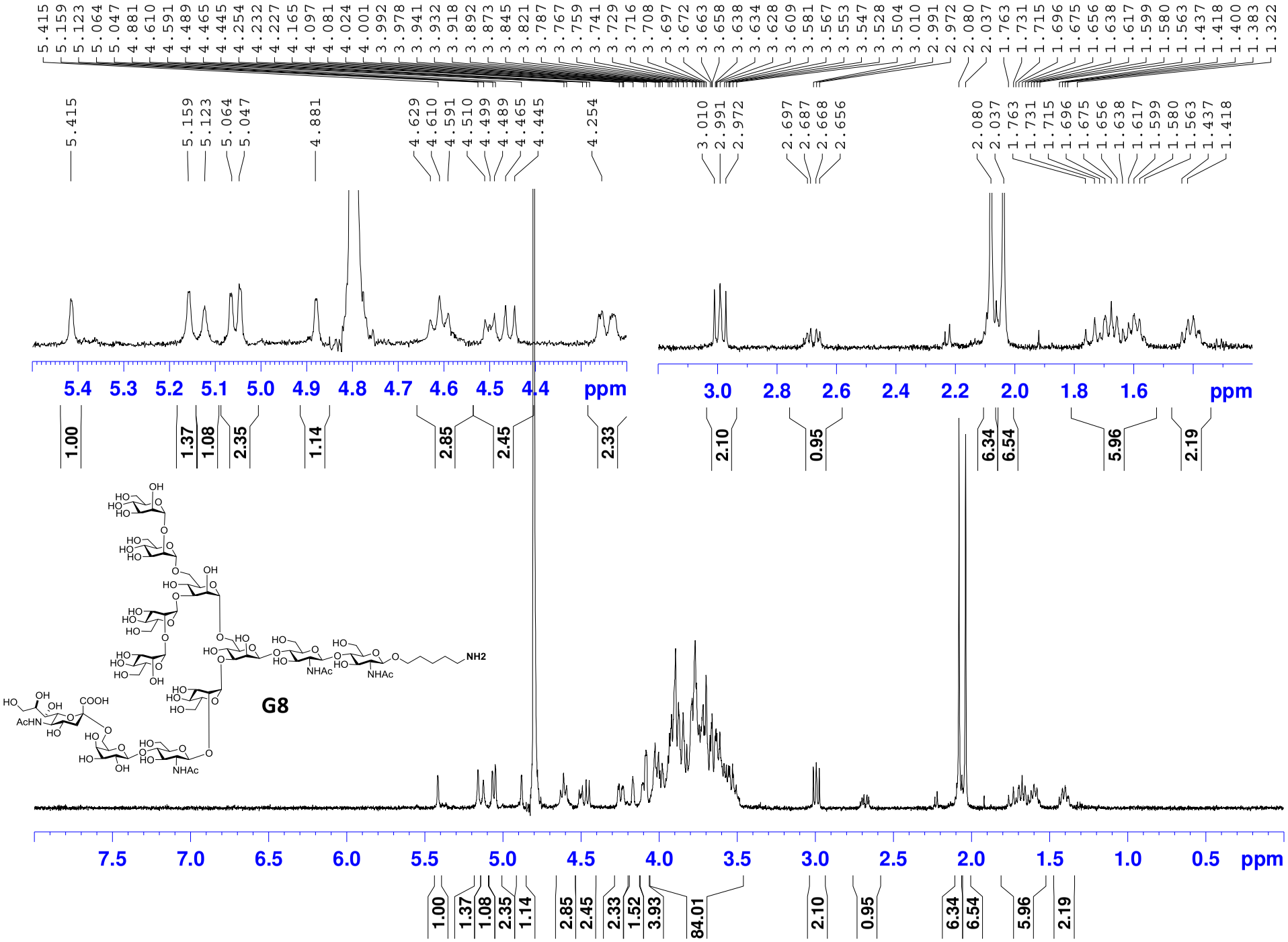
60.893
60.034
59.877
54.933






G8
ppm

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174.699
174.467
174.401
173.518

103.548
102.234
101.426
101.024
100.595
100.255
100.098
99.607
99.289
97.972
96.792
82.046
80.715
79.330
78.895
78.592
76.334
74.469
74.366
73.661
73.499
73.286
73.169
72.690
72.491
72.340
72.027
71.904
71.674
70.694
70.063
69.907
69.375
68.361
68.170
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66.854
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65.531
62.624
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61.132
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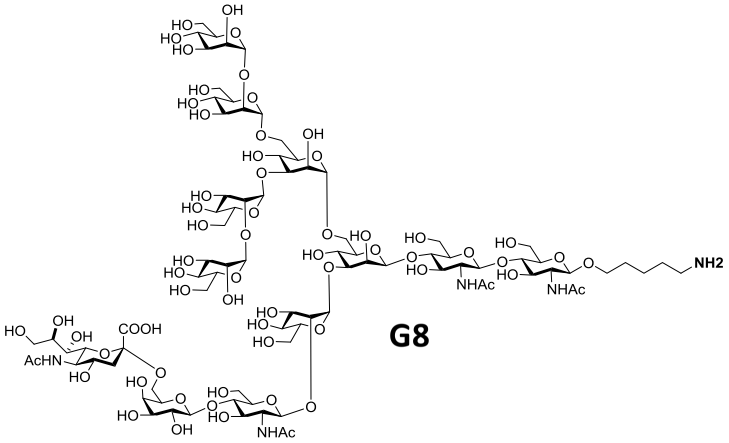
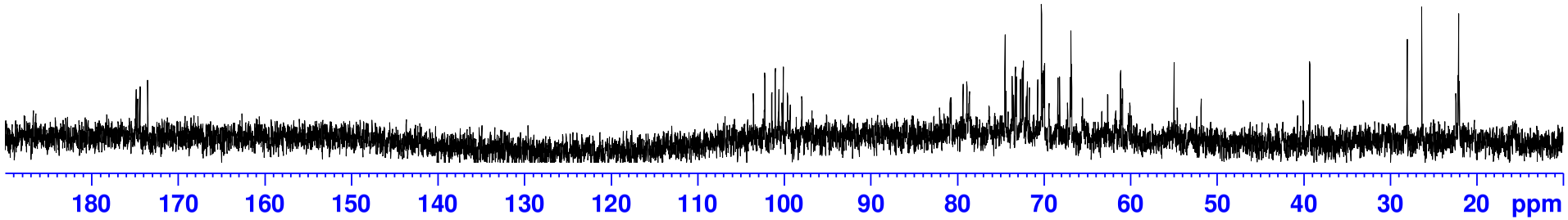
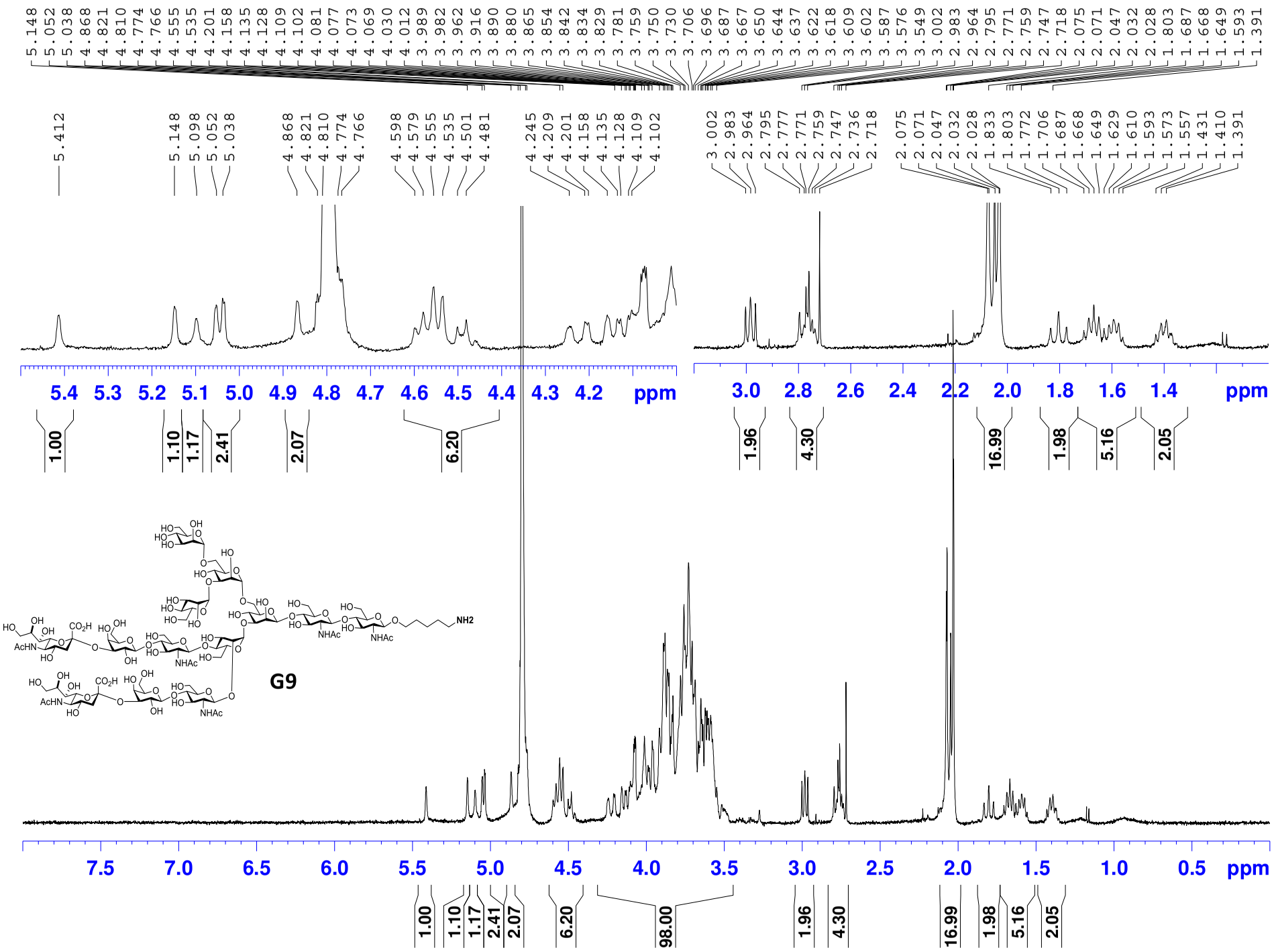
60.069
54.943






G9
ppm

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174.418
173.881

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101.498
101.016
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99.755
99.219
99.078
79.476
79.237
78.226
75.948
75.382
75.116
74.750
74.501
74.395
74.318
72.839
72.657
72.305
72.110
71.912
71.727
70.803
70.541
70.312
70.047
69.907
69.327
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62.522
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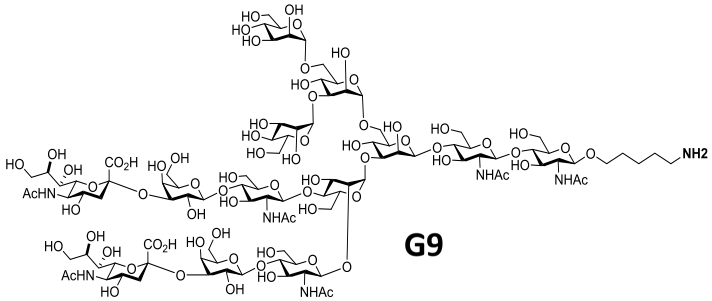
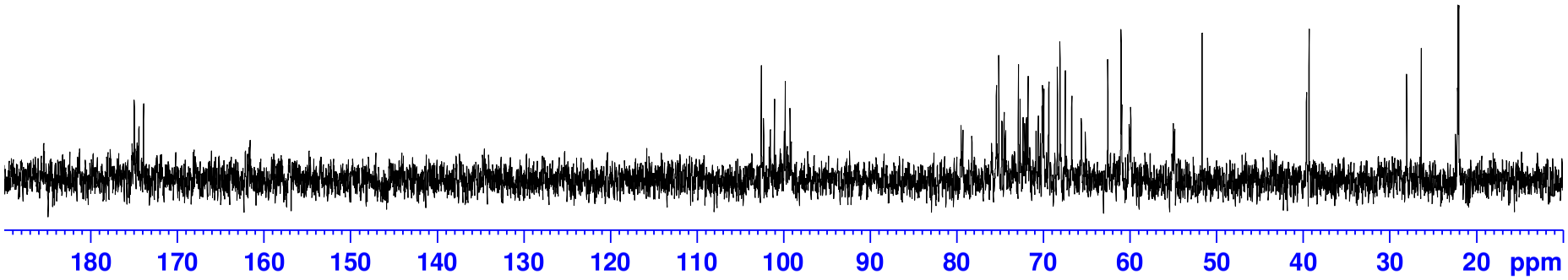
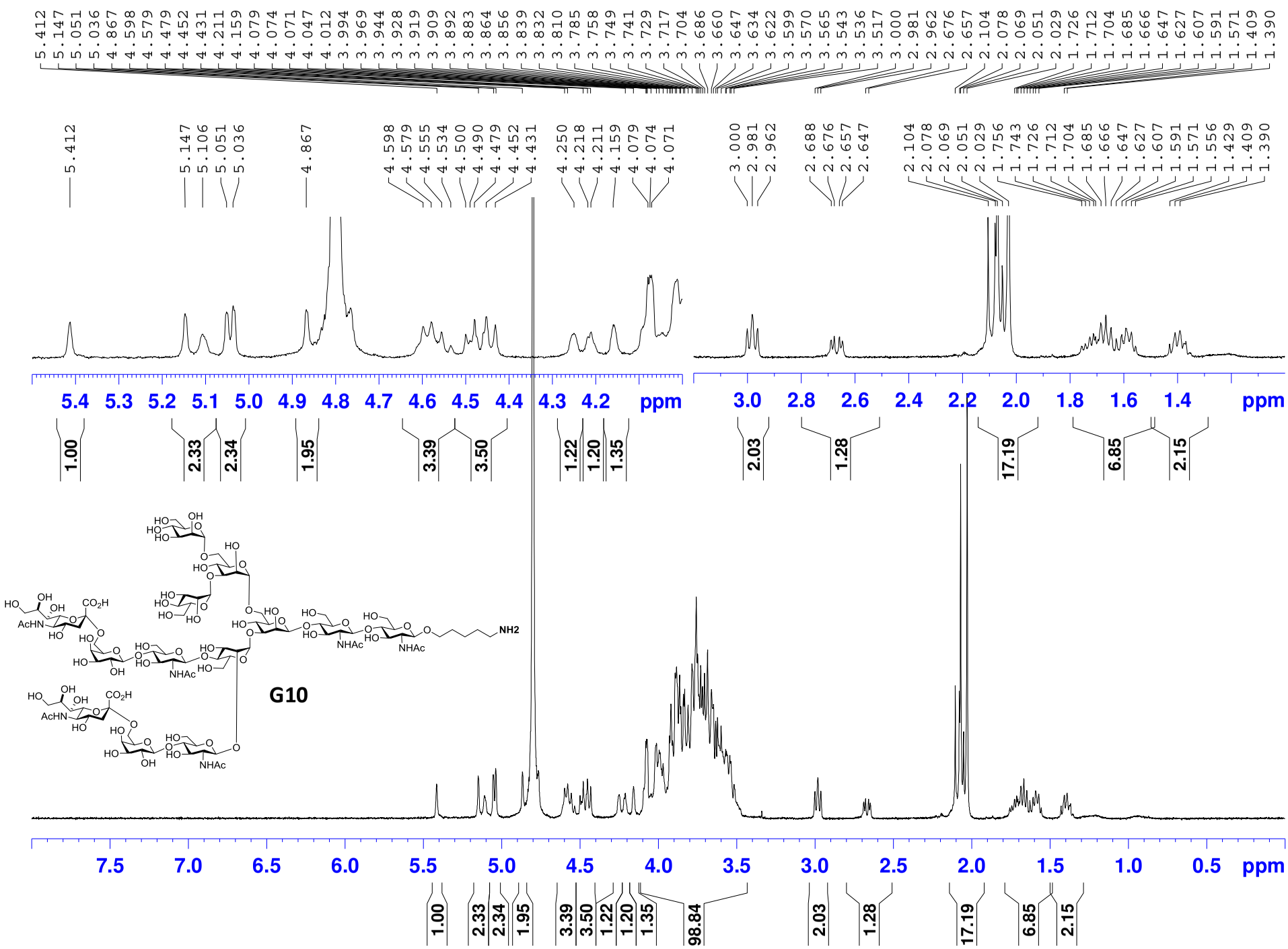
60.062
59.879
54.949
54.782



G9




G10
ppm

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174.434
103.573
102.278
101.474
101.044
100.039
99.924
99.381
99.264
80.359
79.313
74.530
74.487
74.440
73.666
73.318
72.688
72.553
72.413
72.378
72.329
72.274
72.072
71.986
71.632
70.849
70.725
70.682
70.580
70.357
70.129
70.068
69.947
69.446
68.408
68.368
68.122
66.721
65.650
63.324
62.684
61.022
60.945
60.122
59.922
59.313
54.995
54.928
54.570
51.862
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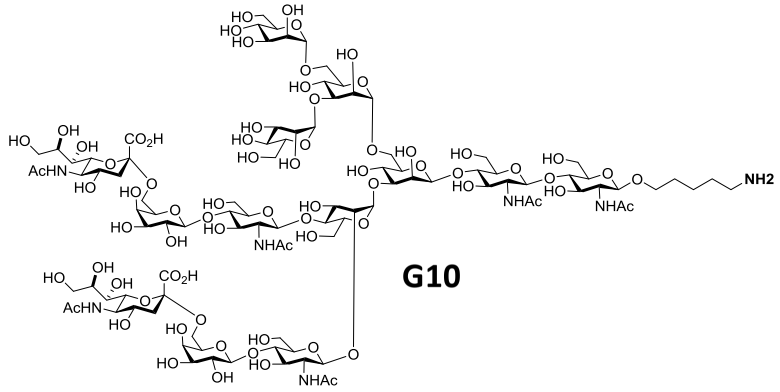
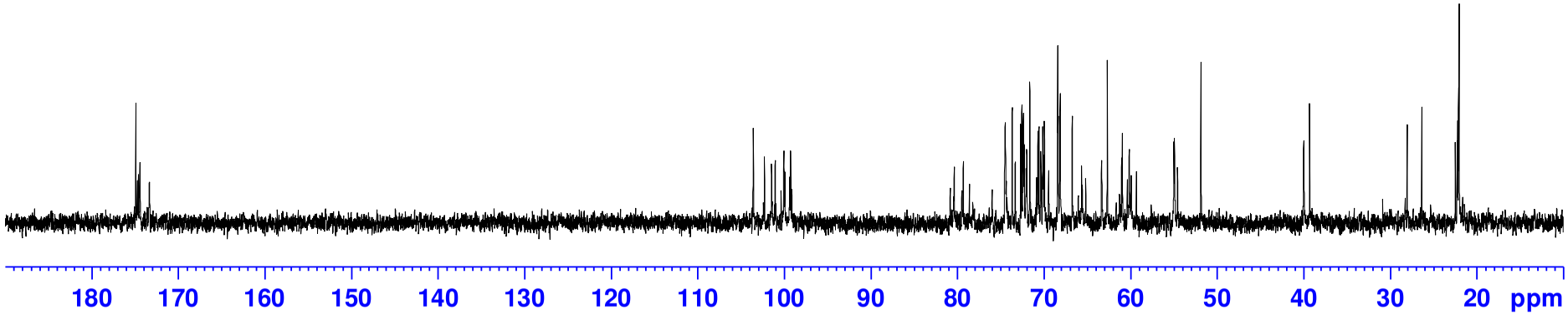
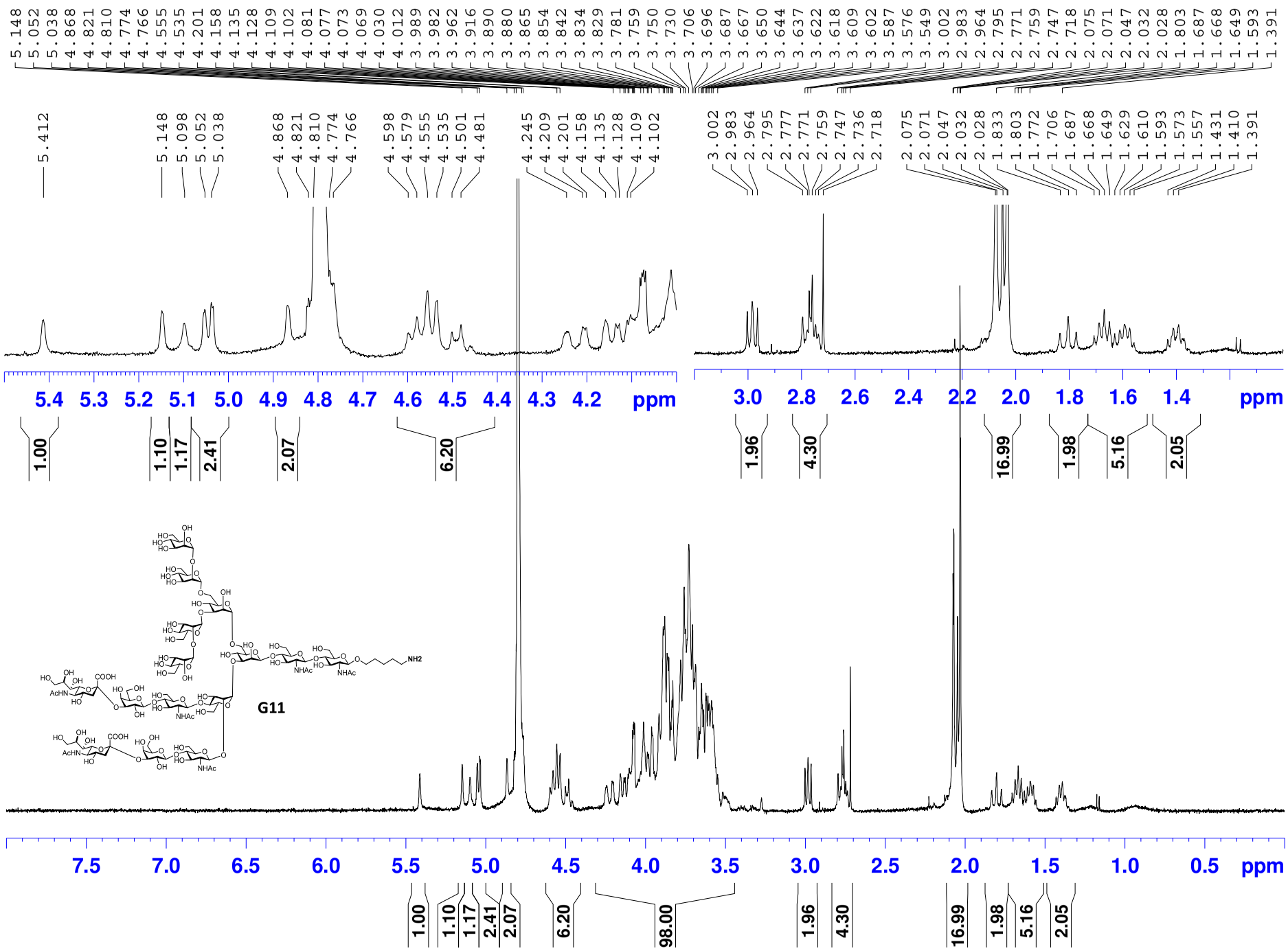
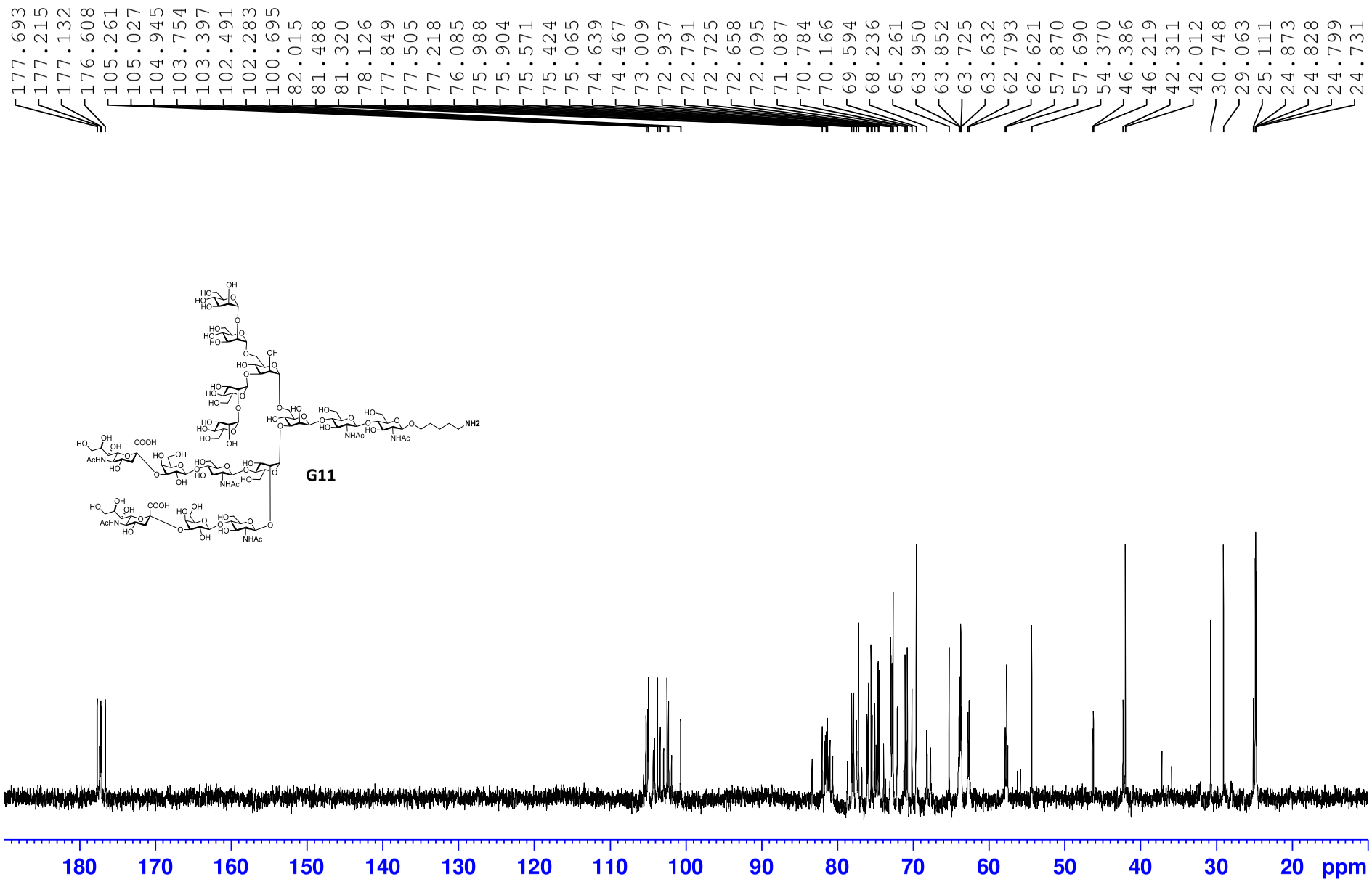
39.314
28.037
26.348
22.090
22.038






G11
NH2
NHAc
COOH
AcHN
ppm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177.132
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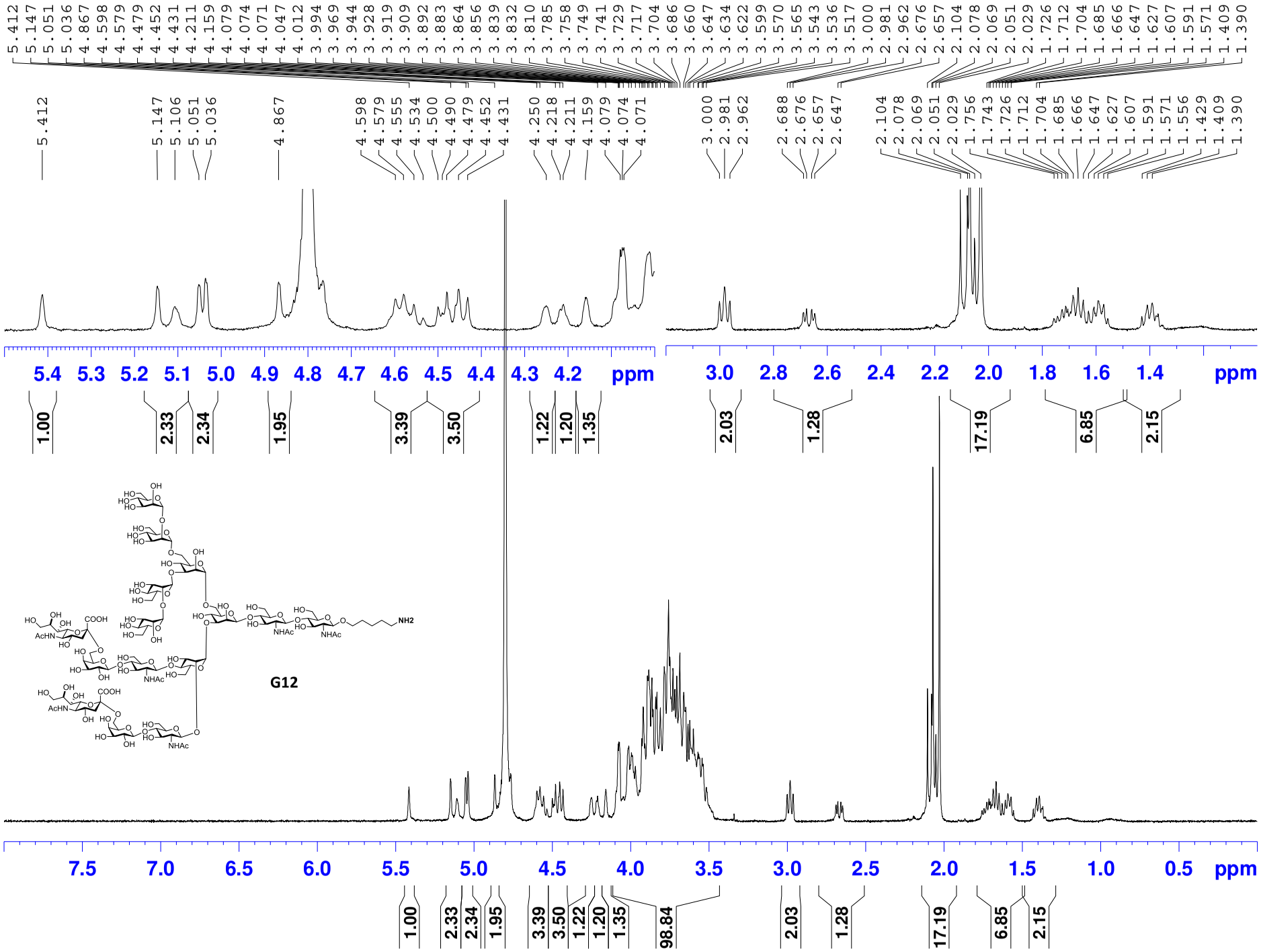
105.027
104.945
103.754
103.397
102.491
102.283
100.695
82.015
81.488
81.320
78.126
77.849
77.505
77.218
76.085
75.988
75.904
75.571
75.424
75.065
74.639
74.467
73.009
72.937
72.791
72.725
72.658
72.095
71.087
70.784
70.166
69.594
68.236
65.261
63.950
63.852
63.725
63.632
62.793
62.621
57.870
57.690
54.370
46.386
46.219
42.311
42.012
30.748
29.063
25.111
24.873
24.828
24.799
24.731
G11
NH2
NHAc
COOH
AcHN
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm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ppm

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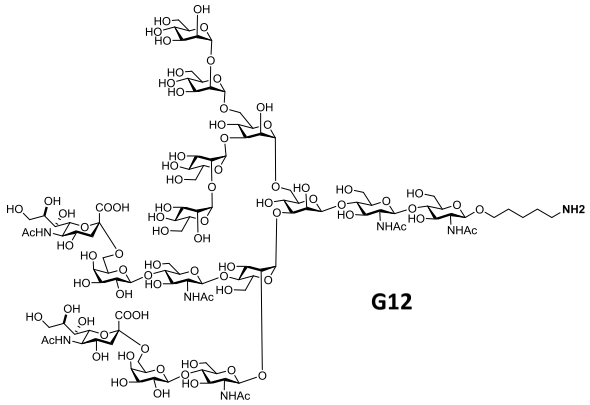
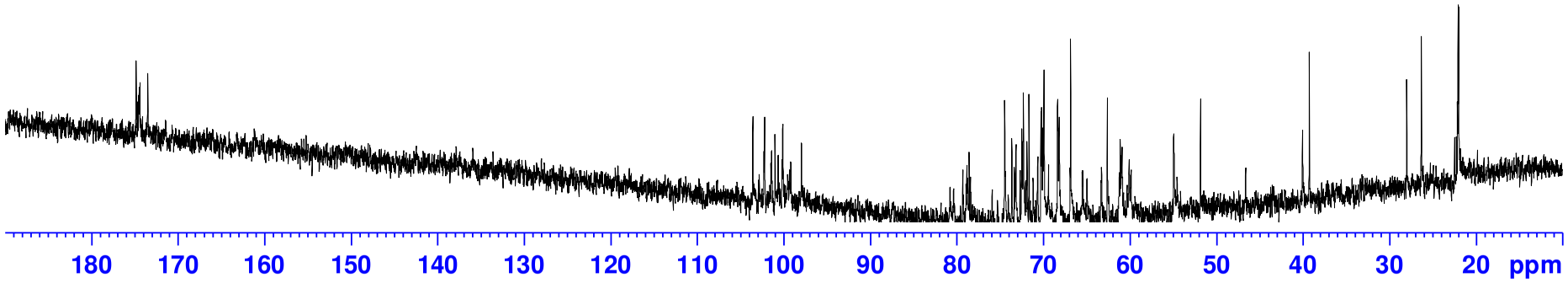
174.383
173.502

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100.121
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73.639
73.159
72.483
72.312
71.902
71.660
70.263
70.190
70.038
69.914
68.352
68.155
66.849
62.601
61.107
60.888
54.908
51.822
40.041
39.267
28.002
26.315
22.457
22.244
22.127
21.802
19.800