DOCUMENT S1

Systematic Synthesis of Bisecting N-glycans and Unique Recognitions by Glycan-Binding Proteins

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1. MATERIALS

1.1 Key Resource Table (Table S1)

REAGENT OR RESOURCE	SOURCE	CAT#
Antibodies		
Mouse monoclonal anti-human galectin-3 (clone#194805)	R&D Systems	MAB11541
Mouse monoclonal anti-human CD15s (clone#CSLEX1)	BD Biosciences	551344
Mouse monoclonal anti-His AF647-conjugated (Clone #AD1.1.10R)	R&D Systems	IC0501R
Goat polyclonal anti-mouse IgG(H+L) AF647-conjugated	Invitrogen	A21235
Goat polyclonal anti-human IgG(H+L) AF647-conjugated	Invitrogen	A21445
Glycan-binding proteins		
Recombinant Calsepa, N-terminal His tag	This study	N/A
Phaseolus vulgaris erythroagglutinin (PHA-E), Biotinylated	Vector Laboratories	B-1125-2
Phaseolus vulgaris leucoagglutinin (PHA-L), Biotinylated	Vector Laboratories	B-1115-2
Datura stramonium lectin (DSA, DSL), Biotinylated	Vector Laboratories	B-1185-2
Maackia amurensis lectin I (MAL-I), Biotinylated	Vector Laboratories	B-1315-2
Sambucus nigra lectin (SNA), Biotinylated	Vector Laboratories	B-1305-2
Aleuria aurantia lectin (AAL), Biotinylated	Vector Laboratories	B-1395-1
Lotus Tetragonolobus Lectin (LTL), Biotinylated	Vector Laboratories	B1325-2
Ulex europaeus agglutinin I (UEA-I), Biotinylated	Vector Laboratories	L-1065-2
Ricinus communis agglutinin I (RCA-I), Biotinylated	Vector Laboratories	B-1085-5
Erythrina cristagalli lectin (ECL), Biotinylated	Vector Laboratories	B-1145-5
Wisteria floribunda lectin (WFA, WFL), Biotinylated	Vector Laboratories	B-1355-2
Wheat germ agglutinin (WGA), Biotinylated	Vector Laboratories	B-1025-5
Concanavalin A (Con A), Biotinylated	Vector Laboratories	B-1005-5
Galanthus nivalis lectin (GNL), Biotinylated	Vector Laboratories	B-1245-2
Griffonia simplicifolia lectin II (GS-II), Biotinylated	Vector Laboratories	B-1215-2
Recombinant Human Galectin-3	R&D Systems	1154-GA
Recombinant human Siglec-3/CD33 Fc Chimera	R&D Systems	1137-SL
Recombinant human Siglec-10 Fc Chimera	R&D Systems	2130-SL
Recombinant Human E-Selectin/CD62E Fc Chimera Protein	R&D Systems	724-ES
Recombinant Human P-Selectin/CD62P Fc Chimera Protein	R&D Systems	137-PS
Recombinant Human L-Selectin/CD62L Fc Chimera Protein	R&D Systems	728-PS
Recombinant Rat CLEC4A2 protein (rDCIR2)	SinoBiological	80267-R07H
Recombinant A/Puerto Rico/8/1934 (H1N1) HA protein C-His (PR8)	bei Resources	NR-19240
Recombinant A/New York/18/2009 (H1N1) HA protein C-His (NY18)	bei Resources	NR-19441
Recombinant A/St. Petersburg/27/2011 (H1N1) pdm09 HA protein C-His (SP27)	bei Resources	NR-41637
Recombinant A/Czech Republic/32/2011 (H1N1) pdm09 HA protein C-His (CR32)	bei Resources	NR-42486
Recombinant A/Anhui/1/2013 (H7N9) HA protein C-His (A1)	bei Resources	NR-44365
Other Reagents		
Streptavidin - Cyanine 5	Invitrogen	434316
Streptavidin - Cyanine 3	Invitrogen	SA1010
Calsepa expression plasmid pET15b-His-Calsepa	GeneUniversal	N/A
Nexterion H NHS functionalized slides	Schott AG	1070936

1.2 Other Materials

Unless otherwise stated, chemicals were purchased and used without further purification. Sugar nucleotides, such as uridine 5'-diphospho-galactose (UDP-Gal),[1] guanosine 5'-diphospho-L-fucose (GDP-Fuc),[2] and uridine 5'-diphosphate-*N*-acetylglucosamine (UDP-GlcNAc)[3] were prepared as reported previously. Enzymes including *E. coli* β-galactosidase (LacZ),[4] *Neisseria meningitidis* CMP-sialic acid synthetase (NmCSS),[5] *Pasteurella multocida* α2-3 sialyltransferase 1 mutant E271F/R313Y (PmST1-DM),[6] *Pasteurella multocida* α2-3 sialyltransferase mutant M144D (PmST1-M144D),[7] *Photobacterium damsela* α2-6 sialyltransferase (Pd26ST)[8], *Helicobacter pylori* β1-3 *N*-acetylglucosaminyltransferase (HpLgtA),[9] *Helicobacter pylori* α1-3fucosyltransferase C-terminal 66 amnio acid truncation (Hp3FT),[10] were expressed and purified as previously described. Bovine β1-4 galactosyltransferase (b4GalT) was purchased from Sigma.

1.3 Cloning and expression of His-tagged Calsepa

Full-length *Calystegia sepium* lectin mRNA (GenBank: U56820.1) was codon optimized (target host: *E. coli*) and cloned into expression vector pET15b using restriction sites NdeI and BamHI (GeneUniversal, Newark, DE). The plasmid pET15b-His-Calsepa was then transformed into *E. coli* BL21(DE3) (New England BioLabs, Ipswich, MA) for overexpression. The strain was grown at 37°C in 1 L of LB medium (Lennox) with 100 μg/mL Ampicillin until OD600 reached 0.6–0.8. After cooling the culture on ice for 20 min, isopropyl 1-thio-β-D-galactopyranoside (IPTG) was added to a final concentration of 0.2 mM. Expression was allowed to proceed at 16°C for 20 h. Cells were harvested by brief centrifugation and stored at −20°C until use. To purify soluble recombinant proteins, cells were resuspended in buffer A (20 mM Tris–HCl, pH 8.0, 0.3 M NaCl, 15 mM imidazole), and disrupted by a cell disruption X2 pro (Constant Systems Ltd. UK). The lysate was cleared by centrifugation (12,000 × g, 30 min, 4°C) and loaded onto a 3 mL Ni-NTA gravity column preequilibrated with buffer A. The column was then washed with 200 mL of buffer A and 200 mL of buffer B (20 mM Tris–HCl, pH 8.0, 0.3M NaCl, 40 mM imidazole). The protein was finally eluted with buffer C (20 mM Tris–HCl, pH 8.0, 0.3 M NaCl, 250 mM imidazole) and desalted against buffer D (50 mM Tris–HCl, pH 8.0, 0.1 M NaCl) for use.

>Codon optimized Calsepa DNA:

GCTGTGCCTATGGATACCATTAGCGGTCCGTGGGGTAATAATGGCGGTAATTTTTGGAGCTTTCGTC
CGGTTAATAAGATTAATCAGATTGTGATTAGCTACGGCGGCGGCGGTAATAATCCGATTGCCCTGAC
CTTTAGTAGCACCAAAGCAGATGGCAGTAAAGATACCATTACCGTGGGTGGTGGTGGTCCGGATAG
TATTACCGGTACCGAAATGGTGAATATTGGCACCGATGAATATCTGACCGGCATTAGTGGCACCTTT
GGCATCTATCTGGATAATAATGTTCTGCGTAGTATTACCTTTACCACCAATCTGAAAGCACATGGCC
CGTATGGCCAGAAAGTTGGTACCCCGTTTAGTAGCGCAAATGTGGTTGGTAATGAAATTGTTGGCTT
TCTGGGTCGCAGTGGTTATTATGTGGATGCAATTGGTACCTATAATCGTCATAAATAA

>Peptide sequence of His6-tagged Calsepa

MGSSHHHHHHSSGLVPRGSHMAVPMDTISGPW
GNNGGNFWSFRPVNKINQIVISYGGGGNNPIALT
FSSTKADGSKDTITVGGGGPDSITGTEMVNIGTD
EYLTGISGTFGIYLDNNVLRSITFTTNLKAHGPYG
QKVGTPFSSANVVGNEIVGFLGRSGYYVDAIGTY
NRHK

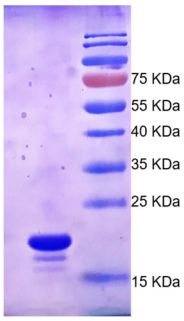


Figure S1 SDS-PAGE of purified His6-Calsepa.

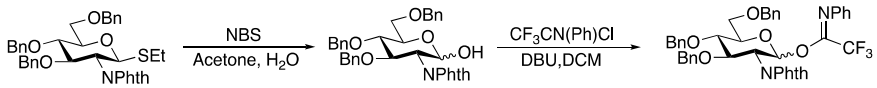
2. CHEMICAL SYNTHESIS OF CORE STRUCTURES 7 AND 7i

ESI-mass spectrometry were performed on an LTQ-Orbitrap Elite mass spectrometer (Thermo Fisher) equipped with EASY-spray source and nano-LC Ultimate 3000 high-performance liquid chromatography system (Thermo Fisher). Samples were transmitted into MS with a silica column. LTQ-Orbitrap Elite mass spectrometer was operated in the data-dependent mode. A full-scan survey MS experiment (*m/z* range was set according to the molecular weight of N-glycan; automatic gain control target, 1,000,000 ions; resolution at 400 *m/z*, 240,000; maximum ion accumulation time, 200 ms) was acquired by the Orbitrap mass spectrometer.

MALDI-TOF MS analyses were performed on UltrafleXtreme MALDI TOF/TOF Mass Spectrometer (Bruker). Scan range of MS was set according to the molecular weight of N-glycans, and reflector mode was used for glycan analysis. Mass spectra were obtained in both positive and negative extraction mode with the following voltage settings: ion source 1 (19.0 kV), ion source 2 (15.9 kV), and lens (9.3 kV). The reflector voltage was set to 20 kV. The laser was pulsed at 7 Hz and the pulsed ion extraction time was set at 400 ns. The laser power was kept in the range of 40–90%.

Anhydrous dichloromethane (DCM), TMSOTf, TfOH, PTSA×H_2O, solid sodium methoxide and FmocOSu was purchased from Sigma Aldrich. TFA was purchased from Alfa Aesar. ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 (400 MHz) or Bruker AVANCE 600 (600 MHz) spectrometer at 25°C. All ^1H Chemical shifts (in ppm) were assigned according to $CDCl_3$ (δ= 7.24 ppm) and D_2O (δ = 4.79 ppm) and all ^{13}C NMR was calibrated with $CDCl_3$ (δ = 77.00 ppm).

Compound 27:



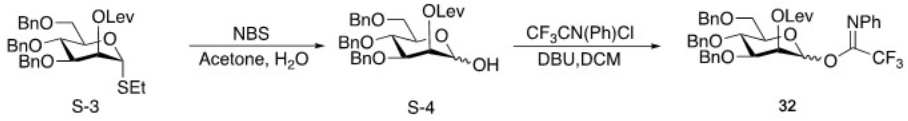
S-1 S-2 27

Comp. **S-1**[11] (21.0 g, 33.7 mmol) was dissolved in mixed solvents of acetone (500 ml) and H_2O (50 ml). The solution was then cooled to -20°C before NBS (30.00 g, 168.5 mmol) was added。After about 5 mins, **S-1** was completely converted to **S-2**. Saturated $Na_2S_2O_3$ solution was added to the mixture to quench the reaction. The solution was concentrated by rotavapor to remove acetone. Then dichloromethane (DCM) was added to the solution and the aq. layer was extracted with DCM twice. The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. Crude **S-2** was directly used for next step without purification.

S-2 was dissolved in anhydrous DCM (200 ml) and the solution was cooled to 0°C. CF_3(NPh)COCl (27.4 mL, 168.5mmol) was added to the above solution before DBU (5.10 mL, 33.7 mmol) was slowly added under 0°C. The reaction was slowly warmed to 25°C and stirred for 2 h. The solution was directly concentrated under reduced pressure and the mixture was purified by silica gel flash column chromatography using hexanes and ethyl acetate to obtain compound **27** (17.1 g) with a yield of 67% over the 2 steps.

^1H NMR (400 MHz, Chloroform-*d*) δ 7.76 – 7.62 (m, 4H), 7.41 – 7.25 (m, 8H), 7.25 – 7.13 (m, 4H), 7.06 – 6.94 (m, 3H), 6.92 – 6.80 (m, 3H), 6.63 (s, 2H), 4.87 – 4.74 (m, 2H), 4.71 – 4.60 (m, 2H), 4.57 (d, *J* = 12.1 Hz, 1H), 4.43 (d, *J* = 12.3 Hz, 3H), 3.87 (t, *J* = 9.0 Hz, 1H), 3.81 – 3.63 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.48, 142.89, 137.78, 137.68, 133.84, 131.33, 128.49, 128.45, 128.41, 128.35, 128.01, 127.91, 127.86, 127.82, 127.81, 127.67, 127.49, 127.36, 126.88, 124.27, 123.35, 119.20, 93.32, 78.81, 78.62, 77.32, 77.00, 76.68, 75.66, 74.98, 74.82, 73.37, 67.75, 54.90, -0.07. HRMS (ESI) m/z calcd. for $C_{43}H_{37}F_3N_2O_7Na$ $(M + Na)^+$ 773.2451; found 773.2420.

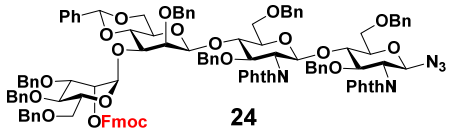
Compound 32:



Compound **32** was prepared with a similar manner of **27** from the corresponding Ethyl thio donor **S-3**[12] (5 g, 5.8 mmol). Yield (4.9 g, 82%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.40 – 7.28 (m, 15H), 7.25 – 7.20 (m, 2H), 7.15 – 7.09 (m, 1H), 6.82 (d, *J* = 7.8 Hz, 2H), 5.54 – 5.47 (m, 1H), 4.90 (d, *J* = 10.6 Hz, 1H), 4.76 (d, *J* = 11.1 Hz, 1H), 4.70 (d, *J* = 12.0 Hz, 1H), 4.62 (d, *J* = 11.1 Hz, 1H), 4.57 (dd, *J* = 11.3, 4.2 Hz, 2H), 4.05 (dd, *J* = 8.9, 3.3 Hz, 1H), 4.02 – 3.92 (m, 2H), 3.86 (dd, *J* = 11.1, 3.9 Hz, 1H), 3.81 – 3.74 (m, 1H), 2.78 – 2.71 (m, 4H), 2.16 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.08, 171.72, 143.19, 138.06, 137.99, 137.52, 128.70, 128.40, 128.35, 128.23, 128.04, 127.90, 127.82, 127.78, 127.65, 124.37, 119.35, 77.41, 77.21, 77.00, 76.79, 75.41, 73.95, 73.58, 73.42, 72.08, 68.44, 67.50, 37.90, 29.74, 28.02. HRMS (ESI) m/z calcd. for $C_{40}H_{40}F_3NO_8Na$ $(M + Na)^+$ 742.2604; found 742.2630.

Compound 24:

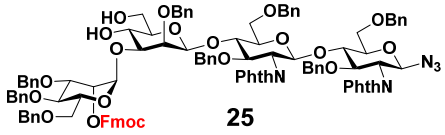


24

Compound **22** (4.23 g, 3.20 mmol)[13], **23** (3.43 g, 4.80 mmol)[14] and powdered 4Å molecular sieves (10 g) was dissolved in anhydrous DCM (100 ml). The solution was stirred at 25°C for 30 mins and then cooled to -20°C. NIS (1.0 g, 4.8 mmol) and AgOTf (82.2 mg, 0.32 mmol) was added to the solution at -20°C. The reaction was allowed to reach RT and stirred until completion. The solution was filtrated to remove molecular sieves and quenched by 100 mL of saturated 1:1 saturated aq. $Na_2S_2O_3$/$NaHCO_3$. The aq. layer was extracted with DCM (2×150 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was purified by silica gel flash column chromatography using hexanes and ethyl acetate to obtain compound **24** (5.81 g, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 1H), 7.81-7.61(m, 11H), 7.48 – 7.15 (m, 39H), 6.96 (dd, *J* = 22.5, 9.1 Hz, 7H), 6.81 (s, 3H), 5.54 – 5.40 (m, 3H), 5.33 (d, *J* = 7.9 Hz, 1H), 5.21 (d, *J* = 9.2 Hz, 1H), 5.00 – 4.82 (m, 5H), 4.78 – 4.47 (m, 10H), 4.40 (dd, *J* = 15.9, 12.7 Hz, 3H), 4.34 – 3.96 (m, 12H), 3.91-3.73 (m, 5H), 3.63 (t, *J* = 12.7 Hz, 2H), 3.54-3.41 (dt, *J* = 18.6, 10.1 Hz, 4H), 3.26 (d, *J* = 9.6 Hz, 1H), 3.11 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.61, 167.69, 154.74, 143.66, 143.49, 141.40, 141.33, 138.96, 138.57, 138.49, 138.35, 138.30, 137.99, 137.93, 137.38, 133.90, 131.67, 130.37, 128.84, 128.73, 128.57, 128.45, 128.38, 128.14, 128.06, 127.99, 127.96, 127.92, 127.84, 127.80, 127.72, 127.67, 127.61, 127.51, 127.30, 127.11, 127.04, 126.08, 125.55, 125.37, 125.10, 123.81, 123.47, 120.10, 120.08, 101.56, 101.36, 100.10, 98.54, 97.20, 85.71, 78.90, 78.80, 78.58, 78.11, 77.36, 76.93, 76.63, 75.80, 75.62, 75.38, 74.90, 74.73, 74.40, 73.63, 73.37, 72.94, 72.58, 72.38, 71.78, 70.35, 69.25, 68.53, 67.87, 67.04, 56.68, 55.35, 46.70; HRMS (ESI) m/z calcd. for $C_{118}H_{110}N_5O_{24}$ $(M + H)^+$ 1980.7541; found 1980.7536.

Compound 25:

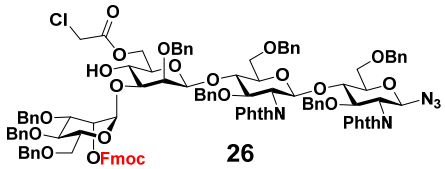


25

$TsOH.H_2O$ (100 mg, 0.53 mmol) and EtSH (1.18 mL, 15.9 mmol) was added to a solution of compound **24** (5.40 g, 2.70 mmol) in DCM (50 ml). The solution was stirred at 25°C until completion and quenched by adding saturated $NaHCO_3$(aq). The aq. layer was extracted with DCM (2×100 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was purified by silica gel flash column chromatography using hexanes and ethyl acetate to obtain compound **25** (4.30 g, 84%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.87 (d, *J* = 7.3 Hz, 1H), 7.76 (dq, *J* = 7.6, 0.9 Hz, 3H), 7.69 – 7.63 (m, 4H), 7.59 (ddq, *J* = 16.4, 7.5, 0.9 Hz, 3H), 7.40 (tdd, *J* = 7.5, 2.6, 1.1 Hz, 2H), 7.36 – 7.24 (m, 26H), 7.22 – 7.15 (m, 8H), 7.07 – 7.02 (m, 1H), 6.97 (dd, *J* = 7.3, 2.2 Hz, 2H), 6.88 – 6.84 (m, 3H), 6.82 – 6.79 (m, 2H), 6.78 – 6.74 (m, 3H), 5.62 (d, *J* = 1.9 Hz, 1H), 5.28 (dd, *J* = 5.7, 2.7 Hz, 1H), 5.23 (dd, *J* = 3.2, 1.9 Hz, 1H), 5.17 (d, *J* = 9.4 Hz, 1H), 4.95 (d, *J* = 11.6 Hz, 1H), 4.88 (dd, *J* = 11.8, 6.6 Hz, 3H), 4.74 (dd, *J* = 11.5, 4.3 Hz, 2H), 4.59 – 4.49 (m, 10H), 4.44 (d, *J* = 12.1 Hz, 1H), 4.38 – 4.32 (m, 1H), 4.29 (d, *J* = 12.2 Hz, 1H), 4.26 – 4.14 (m, 7H), 4.08 – 3.93 (m, 5H), 3.82 (d, *J* = 3.4 Hz, 1H), 3.76 – 3.71 (m, 2H), 3.64 (dt, *J* = 11.2, 2.1 Hz, 2H), 3.61 – 3.55 (m, 2H), 3.48 (ddd, *J* = 9.8, 7.4, 3.1 Hz, 2H), 3.45 – 3.38 (m, 3H), 3.23 (dt, *J* = 10.0, 2.6 Hz, 1H), 3.19 (d, *J* = 4.6 Hz, 1H), 3.05 (ddd, *J* = 9.2, 5.7, 3.3 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.45, 167.51, 154.64, 143.44, 143.21, 141.23, 141.17, 138.48, 138.38, 138.20, 138.16, 137.79, 137.72, 137.55, 134.06, 133.81, 131.68, 131.50, 131.34, 128.57, 128.41, 128.30, 128.27, 128.24, 128.18, 128.05, 127.89, 127.82, 127.77, 127.66, 127.42, 127.36, 127.33, 127.24, 127.13, 127.05, 126.99, 126.93, 125.40, 125.16, 123.64, 123.31, 123.10, 119.97, 119.93, 101.05, 97.07, 85.53, 80.76, 79.11, 78.59, 77.86, 76.79, 76.47, 75.81, 75.30, 75.01, 74.67, 74.49, 73.56, 73.28, 72.93, 72.75, 71.86, 71.67, 70.22, 69.47, 67.69, 67.59, 66.32, 62.55, 56.42, 55.18, 46.49. HRMS (ESI) m/z calcd. for $C_{111}H_{106}N_5O_{24}$ $(M + H)^+$ 1892.7228; found 1892.7265.

Compound 26:



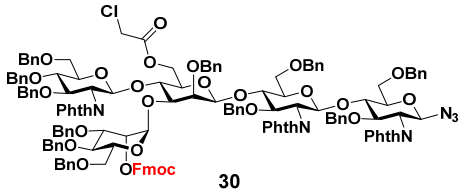
26

Chloroacetic anhydride (410 mg, 2.40 mmol) and *s*-collidine (1.33mL, 10.0 mmol) was added to a solution of compound **25** (3.80 g, 2.00 mmol) in DCM (100 ml) at 0°C. The solution was stirred for 1h at 0°C and stirred at RT until completion before quenched by adding 1 mL of MeOH. Brine was added the solution and the aq. layer was extracted with DCM (2×100 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was purified by silica gel flash column chromatography using hexanes and ethyl acetate to obtain compound **26** (3.59 g, 91%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.84 (d, *J* = 7.4 Hz, 1H), 7.76 (dd, *J* = 7.6, 1.1 Hz, 3H), 7.70 (t, *J* = 7.4 Hz, 1H), 7.67 – 7.56 (m, 7H), 7.39 (tdd, *J* = 7.5, 2.6, 1.0 Hz, 2H), 7.35 – 7.14 (m, 36H), 7.08 – 7.02 (m, 1H), 6.97 – 6.93 (m, 2H), 6.81 – 6.73 (m, 9H), 5.62 (d, *J* = 1.9 Hz, 1H), 5.28 – 5.23 (m, 2H), 5.16 (d, *J* = 9.4 Hz, 1H), 4.98 (d, *J* = 11.6 Hz, 1H), 4.91 – 4.83 (m, 3H), 4.73 (dd, *J* = 19.9, 11.5 Hz, 2H), 4.60 – 4.54 (m, 5H), 4.51 (td, *J* = 12.3, 11.8, 4.6 Hz, 5H), 4.45 (d, *J* = 12.1 Hz, 1H), 4.40 – 4.33 (m, 2H), 4.31 (d, *J* = 12.7 Hz, 1H), 4.26 – 4.12 (m, 8H), 4.07 – 4.02 (m, 3H), 4.02 – 3.98 (m, 1H), 3.96 – 3.90 (m, 1H), 3.88 (d, *J* = 2.7 Hz, 2H), 3.84 (q, *J* = 3.6, 2.7 Hz, 1H), 3.74 (ddd, *J* = 9.6, 5.5, 3.5 Hz, 2H), 3.64 (dd, *J* = 11.3, 1.8 Hz, 1H), 3.62 – 3.54 (m, 2H), 3.51 – 3.44 (m, 2H), 3.44 – 3.37 (m, 2H), 3.35 (d, *J* = 4.7 Hz, 1H), 3.20 (ddt, *J* = 9.9, 7.6, 2.2 Hz, 2H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.26, 167.55, 154.61, 143.40, 143.20, 141.21, 141.15, 138.80, 138.37, 138.35, 138.18, 138.12, 137.76, 137.47, 133.89, 133.70, 131.71, 131.45, 131.33, 128.57, 128.53, 128.45, 128.41, 128.34, 128.28, 128.24, 128.20, 128.14, 128.09, 127.99, 127.97, 127.90, 127.86, 127.82, 127.79, 127.76, 127.71, 127.65, 127.63, 127.51, 127.45, 127.37, 127.33, 127.29, 127.11, 127.09, 126.92, 126.75, 125.35, 125.13, 123.48, 123.28, 123.05, 119.95,

119.92, 101.49, 97.13, 96.93, 85.49, 80.36, 79.07, 78.85, 77.85, 76.89, 76.71, 76.43, 75.16, 75.00, 74.60, 74.55, 74.42, 74.39, 73.78, 73.56, 73.22, 72.81, 72.70, 71.83, 71.64, 70.19, 69.45, 67.65, 65.74, 64.85, 56.38, 55.14, 46.47, 40.65, -0.05.HRMS (ESI) m/z calcd. for $C_{113}H_{107Cl}N_5O_{25}$ $(M + H)^+$ 1968.6944; found 1968.7000.

Compound 30:

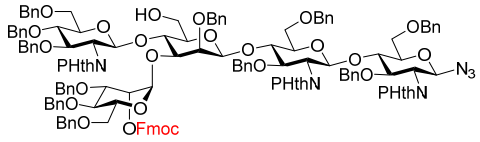


30

Compound **26** (3.23 g, 1.64 mmol), **27** (2.46g, 3.28 mmol) and powdered 4Å molecular sieves (10 g) was dissolved in anhydrous DCM (100 mL). The solution was stirred at 25°C for 30 mins and then cooled to -30°C. TfOH (58 µL, 0.66 mmol) was added to the solution at -30° and the solution was stirred at -30°C for 1h and slowly warmed to 25°C within 2h. The solution was quenched by 50 uL of Et_3N and filtrated to remove molecular sieves. Saturated $NaHCO_3$(aq) was added to the solution and the aq. layer was extracted with DCM (2×100 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was purified by silica gel flash column chromatography using toluene and ethyl acetate to obtain compound **30** (3.68 g, 89%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (d, *J* = 7.8 Hz, 1H), 7.81 (d, *J* = 7.2 Hz, 1H), 7.75 (dd, *J* = 7.5, 3.0 Hz, 3H), 7.70 (d, *J* = 7.5 Hz, 2H), 7.65 (s, 5H), 7.59 (d, *J* = 6.7 Hz, 2H), 7.41 – 7.35 (m, 2H), 7.33 – 7.28 (m, 18H), 7.24 – 7.17 (m, 11H), 7.17 – 7.05 (m, 11H), 7.00 (q, *J* = 7.8 Hz, 3H), 6.92 – 6.91 (m, 2H), 6.84 – 6.80 (m, 3H), 6.76 – 6.66 (m, 11H), 5.77 (s, 1H), 5.34 (s, 1H), 5.23 (d, *J* = 8.2 Hz, 1H), 5.14 (d, *J* = 9.4 Hz, 1H), 5.04 (d, *J* = 8.2 Hz, 1H), 4.92 (d, *J* = 10.9 Hz, 1H), 4.81 (dd, *J* = 12.6, 6.5 Hz, 2H), 4.77 – 4.71 (m, 4H), 4.67 – 4.62 (m, 2H), 4.59 (dd, *J* = 11.7, 3.1 Hz, 2H), 4.55-4.52 (m, 3H), 4.50 – 4.46 (m, 4H), 4.45 – 4.34 (m, 7H), 4.26 – 4.22 (m, 3H), 4.16 – 4.12 (m, 5H), 4.08 (d, *J* = 10.9 Hz, 1H), 4.01 (dd, *J* = 15.6, 6.0 Hz, 2H), 3.92– 3.78 (m, 7H), 3.75 – 3.66 (m, 6H), 3.51 (t, *J* = 10.0 Hz, 2H), 3.38 (dd, *J* = 13.1, 6.0 Hz, 3H), 3.21 (dd, *J* = 10.9, 2.3 Hz, 1H), 3.14 (d, *J* = 9.9 Hz, 1H), 2.65 (dd, *J* = 9.6, 4.1 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.58, 166.67, 155.29, 144.49, 143.62, 141.37, 141.12, 138.90, 138.60, 138.52, 138.33, 138.25, 138.21, 138.06, 137.93, 137.85, 133.78, 128.48, 128.40, 128.36, 128.30, 128.26, 128.24, 128.03, 128.00, 127.93, 127.88, 127.84, 127.77, 127.73, 127.63, 127.57, 127.52, 127.45, 127.42, 127.36, 127.22, 127.14, 126.93, 126.84, 126.04, 125.40, 119.95, 100.63, 99.77, 97.12, 96.55, 85.53, 80.16, 79.22, 78.22, 78.19, 77.68, 76.72, 76.67, 75.87, 75.39, 75.14, 74.99, 74.65, 74.57, 74.43, 74.35, 74.00, 73.42, 73.39, 72.93, 72.77, 72.67, 72.25, 72.05, 71.44, 71.28, 70.75, 69.68, 69.61, 67.66, 67.56, 63.96, 56.47, 55.95, 55.22, 46.89, 40.53. HRMS (ESI) m/z calcd. for $C_{148}H_{138}ClN_6O_{31}$ $(M + H)^+$ 2529.9095; found 2529.9142.

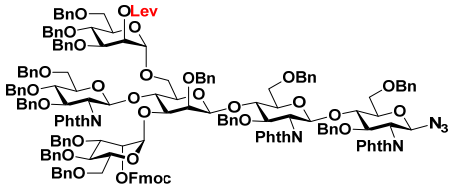
Compound 31:



Thiourea (1.81 g, 23.8 mmol) and 2,6-lutidine (2.78 mL, 23.8 mmol) was added to a solution of compound **30** (3.00 g, 1.19 mmol) in MeOH (50 ml) at 25°C. The solution was warmed to 65°C and stirred for 12 h until no compound **30** was detected by TLC. The solution was concentrated under reduced pressure to remove MeOH and DCM, brine was added to the mixture. The aq. layer was extracted with DCM (2×100 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was purified by silica gel flash column chromatography using hexanes and ethyl acetate to obtain compound **31** (2.54 g, 87%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85 (t, *J* = 8.2 Hz, 2H), 7.76 – 7.63 (m, 15H), 7.39 – 7.27 (m, 20H), 7.23 – 7.11 (m, 16H), 7.08 - 6.99 (m, 6H), 6.93 - 6.90 (m, 3H), 6.86 – 6.82 (m, 5H), 6.74 – 6.67 (m, 8H), 5.79 (s, 1H), 5.34 (s, 1H), 5.25 (d, *J* = 8.4 Hz, 1H), 5.15 (d, *J* = 9.4 Hz, 1H), 5.02 (d, *J* = 8.2 Hz, 1H), 4.92 (d, *J* = 11.0 Hz, 1H), 4.83 (dd, *J* = 12.8, 5.6 Hz, 2H), 4.79 (d, *J* = 12.9 Hz, 1H), 4.75 (d, *J* = 6.8 Hz, 1H), 4.72 (d, *J* = 10.1 Hz, 1H), 4.69 (d, *J* = 7.4 Hz, 1H), 4.65 (dd, *J* = 9.3, 5.3 Hz, 2H), 4.59 (dd, *J* = 11.6, 8.9 Hz, 2H), 4.56 – 4.48 (m, 7H), 4.46 - 4.37 (m, 4H), 4.34 (dd, *J* = 12.1, 7.4 Hz, 2H), 4.30 – 4.24 (m, 3H), 4.21 (s, 1H), 4.18 (d, *J* = 9.0 Hz, 2H), 4.15 (d, *J* = 5.1 Hz, 2H), 4.10 (dd, *J* = 11.1, 9.0 Hz, 1H), 4.04 – 4.00 (m, 2H), 3.93 (d, *J* = 9.7 Hz, 1H), 3.89 – 3.83 (m, 4H), 3.81 – 3.78 (m, 1H), 3.76 – 3.71 (m, 2H), 3.68 (d, *J* = 10.2 Hz, 1H), 3.65 (d, *J* = 2.6 Hz, 1H), 3.52 (dd, *J* = 14.0, 10.8 Hz, 2H), 3.41 – 3.33 (m, 4H), 3.23 (dd, *J* = 10.8, 2.4 Hz, 1H), 3.17 (d, *J* = 10.0 Hz, 1H), 3.10 (s, 1H), 2.51 – 2.49 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.41, 167.55, 155.24, 144.47, 143.63, 141.35, 141.10, 138.63, 138.49, 138.37, 138.29, 138.19, 138.09, 138.05, 137.69, 133.73, 128.44, 128.42, 128.34, 128.33, 128.30, 128.28, 128.23, 128.07, 128.04, 128.00, 127.94, 127.90, 127.87, 127.86, 127.81, 127.75, 127.67, 127.65, 127.59, 127.53, 127.46, 127.37, 127.20, 127.11, 126.91, 126.02, 125.41, 123.32, 119.91, 119.78, 100.31, 99.82, 97.18, 96.65, 85.53, 80.20, 79.08, 78.26, 77.91, 76.67, 75.51, 75.21, 75.11, 74.82, 74.70, 74.63, 74.54, 74.45, 74.39, 74.20, 73.36, 73.19, 72.99, 72.79, 72.18, 72.09, 71.43, 70.69, 69.64, 67.68, 67.44, 61.54, 56.46, 55.96, 55.22, 46.88. HRMS (ESI) m/z calcd. for $C_{146}H_{137}N_6O_{30}$ $(M + H)^+$ 2453.9379; found 2453.9298

Compound 33:

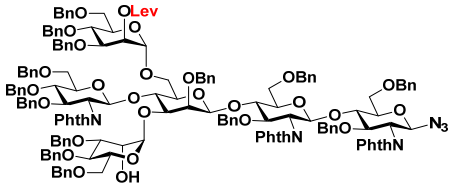


Compound **31** (2.20 g, 0.90 mmol), **32** (1.29 g, 1.80 mmol) and 4Å molecular sieve (5 g) was dissolved in anhydrous DCM (50 mL). The solution was stirred at 25°C for 30 mins and then cooled to -78°C. TfOH (32 µL, 0.66 mmol) was added to the solution at -78° and the solution was stirred at -78°C for 1h and slowly warmed to 25°C within 2h. The solution was quenched by Et_3N and filtrated to remove molecular sieve. Saturated $NaHCO_3$(aq) was added to the solution and the aq. layer was extracted with DCM (2×50 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was purified by silica gel flash column chromatography to obtain compound **33** (2.23 g, 83%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.86 (dd, *J* = 7.4, 1.0 Hz, 1H), 7.77 – 7.59 (m, 12H), 7.48 (d, *J* = 7.3 Hz, 1H), 7.40 – 7.22 (m, 32H), 7.22 – 7.10 (m, 28H), 7.09 – 7.02 (m, 7H), 6.97 (td, *J* = 7.6, 2.2 Hz, 3H), 6.89 – 6.86 (m, 2H), 6.83 – 6.80 (m, 2H), 6.80 – 6.76 (m, 1H), 6.72 – 6.66 (m, 6H), 6.66 – 6.60 (m, 3H), 6.58 – 6.54 (m, 2H), 5.79 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.35 – 5.31 (m, 2H), 5.18 (dd, *J* = 19.0, 8.1 Hz, 2H), 5.11 (d, *J* = 9.4 Hz, 1H), 4.95 (d, *J* = 1.8 Hz, 1H), 4.88 (dd, *J* = 25.2, 11.7 Hz, 2H), 4.81 – 4.75 (m, 3H), 4.73 – 4.62 (m, 7H), 4.60 – 4.44 (m, 14H), 4.40 – 4.35 (m, 4H), 4.33 (d, *J* = 11.2 Hz, 1H), 4.30 (d, *J* = 6.5 Hz, 2H), 4.28 – 4.21 (m, 3H), 4.20 – 4.13 (m, 3H), 4.11 – 4.06 (m, 3H), 4.05 – 3.96 (m, 3H), 3.94 – 3.84 (m, 7H), 3.84 – 3.77 (m, 4H), 3.76 – 3.70 (m, 4H), 3.69 – 3.64 (m, 2H), 3.61 (d, *J* = 12.1 Hz, 1H), 3.46 (ddd, *J* = 16.6, 9.9, 2.3 Hz, 3H), 3.36 (dd, *J* = 9.8, 2.9 Hz, 1H), 3.33 – 3.28 (m, 2H), 3.19 (dd, *J* = 11.1, 2.9 Hz, 1H), 3.09 (dt, *J* = 9.8, 2.3 Hz, 1H), 2.55 (ddd, *J* = 9.8, 3.7, 1.5 Hz, 1H), 2.29 – 2.20 (m, 2H), 2.18 – 2.08 (m, 2H), 1.87 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 206.33, 170.92, 167.96, 167.38, 155.28, 144.61, 143.69, 141.36, 141.08, 139.02, 138.82, 138.73, 138.69, 138.53, 138.49, 138.43, 138.25, 138.17, 138.09, 138.04, 137.84, 133.74, 133.51, 131.79, 131.51, 129.07, 128.67, 128.46, 128.36, 128.30, 128.27, 128.24, 128.19, 128.11, 128.03, 127.97, 127.91, 127.83, 127.76, 127.70, 127.67, 127.63, 127.57, 127.52, 127.47, 127.41, 127.35, 127.31, 127.27, 127.20, 127.16, 127.10, 127.07, 126.88, 126.10, 125.43, 123.39, 123.10, 119.90, 119.76, 101.18, 99.98, 98.26, 97.12, 96.47, 85.47, 80.13, 79.12, 79.01, 78.78, 78.40, 78.13, 77.26, 77.05, 76.84, 76.30, 75.91, 75.47,

75.10, 75.04, 74.83, 74.65, 74.60, 74.48, 74.39, 74.23, 73.70, 73.58, 73.38, 73.35, 72.89, 72.71, 72.13, 72.08, 71.64, 71.38, 70.75, 70.44, 69.54, 69.08, 68.44, 67.66, 67.60, 65.33, 56.51, 55.89, 55.20, 46.91, 37.87, 29.78, 29.61, 27.93, 0.03. HRMS (ESI) m/z calcd. for $C_{178}H_{171}N_6O_7$ $(M + H)^+$ 2984.1684; found 2984.1675.

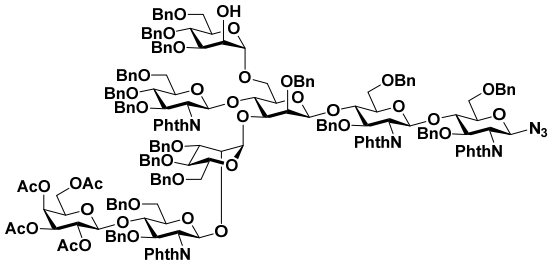
Compound 34:



To a solution of compound **33** (2.00 g, 0.67 mmol) in DCM (30 ml) was added Et_3N (10 ml) at 25°C. The solution was stirred for 24h until no compound **33** was detected by TLC. The solution was concentrated under reduced pressure and the mixture was directly purified by silica gel flash column chromatography using hexanes and ethyl acetate to obtain compound **34** (1.54 g, 84%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.3 Hz, 2H), 7.70 - 7.58 (m, 8H), 7.50 (d, *J* = 7.3 Hz, 1H), 7.41 (d, *J* = 7.2 Hz, 2H), 7.31 – 7.27 (m, 16H), 7.25 – 7.08 (m, 38H), 7.05 – 7.03 (m, 1H), 6.95 (dd, *J* = 7.7, 1.5 Hz, 2H), 6.90 - 6.85 (m, 5H), 6.80 (d, *J* = 7.0 Hz, 2H), 6.75 – 6.70 (m, 3H), 6.65 (t, *J* = 7.3 Hz, 1H), 6.56 (t, *J* = 7.5 Hz, 1H), 5.31 (d, *J* = 2.2 Hz, 1H), 5.29 (dd, *J* = 3.1, 1.9 Hz, 1H), 5.22 (d, *J* = 8.2 Hz, 1H), 5.17 (d, *J* = 8.1 Hz, 1H), 5.13 (d, *J* = 9.4 Hz, 1H), 4.93 (d, *J* = 1.5 Hz, 1H), 4.89 (q, *J* = 12.4 Hz, 2H), 4.82 - 4.73 (m, 5H), 4.69 (d, *J* = 13.1 Hz, 1H), 4.66 (d, *J* = 11.8 Hz, 1H), 4.62 – 4.58 (m, 2H), 4.57 – 4.55 (m, 3H), 4.53 – 4.46 (m, 6H), 4.44 (d, *J* = 2.0 Hz, 1H), 4.42 - 4.37 (m, 8H), 4.33 – 4.32 (m, 1H), 4.30 – 4.27 (m, 2H), 4.18 (dd, *J* = 10.8, 8.3 Hz, 2H), 4.11 – 4.09 (m, 2H), 4.04 – 3.99 (m, 1H), 3.91 (dd, *J* = 9.8, 8.2 Hz, 1H), 3.86 – 3.73 (m, 11H), 3.69 – 3.62 (m, 5H), 3.58 (d, *J* = 11.5 Hz, 1H), 3.51 (dd, *J* = 13.8, 7.0 Hz, 2H), 3.46 (d, *J* = 9.7 Hz, 1H), 3.40 (dd, *J* = 12.2, 3.9 Hz, 1H), 3.34 - 3.30 (m, 2H), 3.27 (dd, *J* = 11.1, 2.8 Hz, 1H), 3.09 (d, *J* = 9.6 Hz, 1H), 2.76 (dd, *J* = 9.5, 2.5 Hz, 1H), 2.35 – 2.16 (m, 4H), 1.91 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.36, 171.17, 168.01, 167.43, 139.03, 138.79, 138.63, 138.57, 138.55, 138.45, 138.30, 138.21, 138.10, 137.98, 137.86, 137.73, 134.05, 133.80, 133.58, 131.86, 131.57, 128.66, 128.55, 128.50, 128.48, 128.42, 128.41, 128.32, 128.31, 128.30, 128.20, 128.13, 128.11, 128.06, 127.88, 127.86, 127.85, 127.83, 127.76, 127.73, 127.70, 127.66, 127.62, 127.58, 127.49, 127.42, 127.40, 127.33, 127.31, 126.97, 126.94, 123.55, 123.46, 123.16, 101.74, 101.07, 98.04, 97.75, 97.14, 85.53, 79.61, 79.43, 79.18, 79.12, 78.71, 77.98, 77.61, 76.84, 76.76, 76.02, 75.51, 75.25, 75.21, 75.01, 74.93, 74.83, 74.72, 74.37, 74.34, 74.26, 74.22, 73.45, 73.40, 73.29, 73.06, 72.79, 72.34, 72.19, 71.74, 71.47, 69.74, 69.12, 68.74, 68.54, 68.48, 67.68, 65.43, 56.58, 56.53, 55.27, 37.91, 29.70, 28.03. HRMS (ESI) m/z calcd. for $C_{163}H_{161}N_6O_{35}$ $(M + H)^+$ 2762.1003; found 2762.0928

Compound 36:



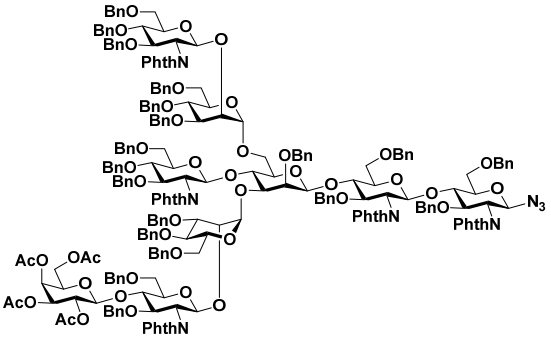
Compound **34** (700 mg, 0.25 mmol), **35** (471 mg, 0.50 mmol) [15] and 4Å molecular sieves (1.5 g) was dissolved in anhydrous DCM (15 mL). The solution was stirred at 25°C for 30 mins and then cooled to -30°C. TfOH (5 µL, 0.10 mmol) was added to the solution at -30° and the solution was stirred at -30°C for 1h and slowly warmed to 25°C

within 2h. The solution was quenched by 5 uL of Et_3N and filtrated to remove molecular sieves. Saturated $NaHCO_3$(aq) was added to the solution and the aq. layer was extracted with DCM (2×20 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude was used in the next step without purification.

To a solution of above crude compound in DCM (10 ml) and MeOH (1ml) was added N_2H_4.HOAc (22 mg, 0.24 mmol) at 25 °C. The solution was stirred for 4h and monitored by TLC. The solution was quenched by saturated $NaHCO_3$(aq) and the aq. layer was extracted with DCM (2×30 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was purified by silica gel flash column chromatography using hexanes and ethyl acetate to obtain compound **36** (392 mg, 49% over two steps).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.2 Hz, 2H), 7.69 – 7.62 (m, 8H), 7.51 (d, *J* = 7.1 Hz, 4H), 7.30 – 7.26 (m, 11H), 7.25– 7.01 (m, 48H), 6.98 – 6.89 (m, 12H), 6.86 – 6.84 (m, 3H), 6.77 (d, *J* = 7.0 Hz, 2H), 6.72 – 6.70 (m, 3H), 6.64 (t, *J* = 7.3 Hz, 1H), 6.58 (t, *J* = 7.4 Hz, 2H), 5.57 (d, *J* = 8.4 Hz, 1H), 5.25 (d, *J* = 3.4 Hz, 1H), 5.18 – 5.12 (m, 5H), 4.99 (d, *J* = 1.2 Hz, 1H), 4.90 (d, *J* = 12.2 Hz, 1H), 4.86 - 4.81 (m, 5H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.75 - 4.70 (m, 6H), 4.63 (d, *J* = 11.0 Hz, 1H), 4.55 – 4.48 (m, 5H), 4.45 – 4.36 (m, 13H), 4.33 (dd, *J* = 11.5, 4.9 Hz, 2H), 4.27 – 4.18 (m, 7H), 4.10 – 3.91 (m, 14H), 3.84 (s, 2H), 3.78 – 3.67 (m, 8H), 3.63 (d, *J* = 9.4 Hz, 1H), 3.60 – 3.45 (m, 7H), 3.37 (d, *J* = 10.2 Hz, 1H), 3.33 – 3.30 (m, 2H), 3.20 (dd, *J* = 11.0, 3.3 Hz, 2H), 3.01 (d, *J* = 9.8 Hz, 1H), 2.76 (dd, *J* = 10.3, 7.0 Hz, 1H), 2.64 (s, 1H), 2.06 (s, 3H), 2.04 (s, 3H), 1.95 (s, 3H), 1.77 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.38, 170.26, 170.05, 169.20, 168.05, 167.49, 138.89, 138.39, 138.32, 138.26, 138.14, 138.05, 137.83, 133.91, 133.77, 133.47, 131.97, 131.56, 128.54, 128.49, 128.36, 128.33, 128.26, 128.20, 128.14, 128.06, 128.02, 127.90, 127.82, 127.79, 127.75, 127.69, 127.62, 127.55, 127.45, 127.40, 127.37, 123.35, 101.03, 100.37, 99.58, 99.30, 97.11, 97.09, 96.26, 85.46, 81.09, 80.34, 78.92, 78.33, 75.50, 75.44, 75.26, 74.71, 74.60, 74.50, 74.44, 74.24, 74.18, 73.76, 73.26, 72.89, 72.75, 72.55, 72.11, 71.32, 71.19, 70.77, 70.50, 69.87, 69.72, 69.19, 68.75, 67.70, 67.57, 67.07, 66.88, 60.90, 60.60, 56.52, 56.07, 56.01, 55.57, 55.22, 29.73, 20.74, 20.63, 20.60, 20.57. HRMS (ESI) m/z calcd. for $C_{184}H_{198}N_7O_{48}$ $(M + H)^+$ 3273.3268; found 3273.3351

Compound 37:

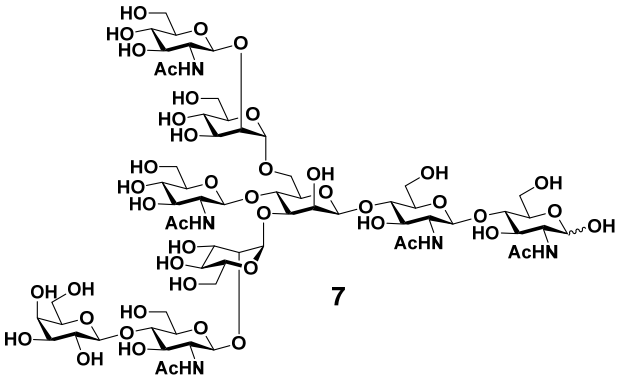


Compound **36** (350 mg, 0.11 mmol), **27** (165 mg, 0.22 mmol) and 4Å molecular sieves (1.0 g) was dissolved in anhydrous DCM (10 mL). The solution was stirred at 25°C for 30 mins and then cooled to -20°C. TfOH (2.5 μL, 0.044 mmol) was added to the solution at -20° and the solution was stirred at -20°C for 1h and slowly warmed to 25°C within 2h. The solution was quenched by 3 uL of Et_3N and filtrated to remove molecular sieves. Saturated $NaHCO_3$(aq) was added to the solution and the aq. layer was extracted with DCM (2×20 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was purified by silica gel flash column chromatography using hexanes and ethyl acetate to obtain Compound **37** (290 mg, 70%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.82 (d, *J* = 7.4 Hz, 1H), 7.76 (s, 1H), 7.71 – 7.59 (m, 7H), 7.53 (s, 2H), 7.46 – 7.37 (m, 6H), 7.31 – 7.13 (m, 42H), 7.11 – 6.96 (m, 25H), 6.95 – 6.79 (m, 26H), 6.77 – 6.72 (m, 3H), 6.48 (d, *J*

= 6.3 Hz, 3H), 5.60 (d, *J* = 8.3 Hz, 1H), 5.26 – 5.21 (m, 1H), 5.16 (dd, *J* = 10.4, 8.0 Hz, 1H), 5.12 (dd, *J* = 8.9, 2.2 Hz, 2H), 5.09 (d, *J* = 8.1 Hz, 1H), 4.88 (d, *J* = 12.2 Hz, 1H), 4.86 – 4.75 (m, 7H), 4.73 – 4.65 (m, 7H), 4.63 (d, *J* = 11.9 Hz, 2H), 4.55 (d, *J* = 13.4 Hz, 2H), 4.51 (d, *J* = 8.6 Hz, 2H), 4.49 – 4.43 (m, 5H), 4.42 (d, *J* = 13.3 Hz, 2H), 4.39 – 4.32 (m, 7H), 4.32 – 4.20 (m, 11H), 4.07 (dtd, *J* = 10.8, 5.0, 2.9 Hz, 5H), 4.04 – 3.97 (m, 6H), 3.94 (d, *J* = 6.6 Hz, 5H), 3.92 – 3.87 (m, 4H), 3.82 – 3.76 (m, 2H), 3.66 (dd, *J* = 8.7, 3.2 Hz, 1H), 3.63 – 3.48 (m, 7H), 3.45 – 3.41 (m, 2H), 3.40 – 3.36 (m, 2H), 3.36 – 3.27 (m, 6H), 3.27 – 3.21 (m, 2H), 2.98 (d, *J* = 10.0 Hz, 1H), 2.62 (s, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 1.94 (s, 3H), 1.74 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.33, 170.22, 169.98, 169.15, 168.17, 167.66, 167.38, 139.26, 139.17, 139.03, 138.94, 138.85, 138.73, 138.65, 138.50, 138.25, 138.04, 137.86, 133.69, 133.33, 131.92, 131.82, 131.70, 131.48, 129.21, 128.44, 128.26, 128.19, 128.09, 128.03, 128.00, 127.97, 127.93, 127.88, 127.78, 127.65, 127.49, 127.43, 127.37, 127.32, 127.22, 126.96, 126.87, 126.67, 123.30, 123.12, 101.74, 100.21, 99.73, 98.24, 96.92, 96.79, 96.02, 85.37, 80.87, 80.17, 79.04, 78.89, 78.69, 78.31, 77.95, 77.66, 76.59, 75.41, 74.99, 74.72, 74.62, 74.56, 74.46, 74.39, 74.28, 74.14, 73.66, 73.34, 72.79, 72.68, 72.59, 72.38, 71.90, 71.13, 70.51, 70.38, 69.65, 68.81, 68.55, 68.35, 67.48, 67.13, 67.02, 60.82, 56.81, 56.50, 55.98, 55.41, 55.14, 20.69, 20.57. HRMS (ESI) m/z calcd. for $C_{235}H_{228}N_8O_{54}$ $(M + Na)^+$ 4048.5239; found 4048.5235.

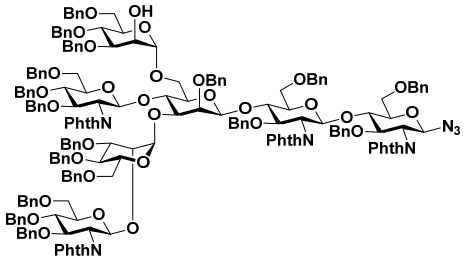
Compound 7:



To a solution of compound **37** (250 mg, 66.0 μmol) in n-butanol (10 ml) was added ethylenediamine (3 ml) at 25°C. The solution was stirred for 8 h at 90°C. Then the solution was directly concentrated under reduced pressure and co-evaporated twice with toluene (5 ml). To the above syrup was added pyridine (10 ml) and acetic anhydride (10 ml). The solution was stirred for 10 h at 25°C. The solution was directly concentrated under reduced pressure and co-evaporated twice with toluene (5 ml). The crude liquid was purified by silica gel flash column chromatography. To the obtained intermedia was added MeOH (10 ml) and NaOMe (20 mg). The solution was stirred with a stable pH 9-10 for 10 h at 25°C. The solution was quenched by Dowex $50H^+$ and filtrated to remove the resin. The obtained solution was concentrated under reduced pressure. To the above syrup in a mixed solvent of MeOH (10 ml) and H_2O (1 ml) was added 10% Pd $(OH)_2$/C (200 mg). The solution was stirred for 24 h under H_2 atmosphere at 25°C. The solid was filtered off and the solution was concentrated to give compound **7** (55 mg, 50 % for 4 steps).

^{1}H NMR (600 MHz, D_2O) δ 5.11 (d, *J* = 2.4 Hz, 1H), 4.98 (s, 1H), 4.93 (s, 1H), 4.54-4.52 (m, 2H), 4.47 (d, *J* = 8.4 Hz, 1H), 4.40 – 4.36 (m, 2H), 4.18 (s, 1H), 4.10 (d, *J* = 2.7 Hz, 1H), 4.07 (s, 1H), 4.00 (t, *J* = 9.8 Hz, 1H), 3.92 – 3.30 (m, 50H), 3.18 (t, *J* = 9.3 Hz, 1H), 1.99 (dt, *J* = 10.9, 8.8 Hz, 15H). ^{13}C NMR (150 MHz, D_2O) δ 174.72, 174.61, 174.53, 174.45, 102.89, 101.41, 100.56, 100.10, 99.69, 99.49, 97.67, 94.79, 90.43, 79.65, 79.19, 78.88, 78.49, 78.09, 76.41, 76.17, 75.71, 75.33, 74.69, 74.56, 74.25, 73.48, 73.43, 73.31, 72.44, 71.95, 71.66, 71.11, 70.94, 70.33, 69.98, 69.86, 69.49, 69.31, 69.23, 68.55, 67.56, 67.36, 65.38, 62.48, 61.90, 61.83, 61.63, 61.03, 60.54, 59.99, 59.83, 56.10, 55.24, 54.84, 53.63, 22.43, 22.36, 22.21, 22.15, 22.12, 21.86. HRMS (ESI) m/z calcd. for $C_{64}H_{108}N_5O_{46}$ $(M + H)^+$ 1682.6265; found 1682.6350

Compound 38:

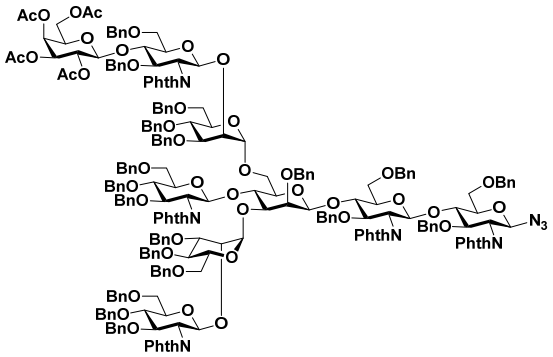


Compound **34** (700 mg, 0.25 mmol), **27** (375 mg, 0.50 mmol) and 4Å molecular sieves (1.5 g) was dissolved in a mixture of anhydrous DCM (15 mL) and anhydrous acetonitrile (2 mL). The solution was stirred at 25 °C for 30 mins and then cooled to -20°C. TfOH (5 µL, 0.10 mmol) was added to the solution at -20° and the solution was slowly warmed to 25°C within 1h. The solution was quenched by 5 uL of Et_3N and filtrated to remove molecular sieves. Saturated $NaHCO_3$(aq) was added to the solution and the aq. layer was extracted with DCM (2×20 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was used in the next step without purification.

To the crude compound in mixture of solvents DCM (10 ml) and MeOH (1ml) was added N_2H_4.HOAc (22 mg, 0.24 mmol) at 25 °C. The solution was stirred for 4h. The solution was quenched by saturated $NaHCO_3$(aq) and the aq. layer was extracted with DCM (2×30 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was purified by silica gel flash column chromatography using hexanes and ethyl acetate to obtain compound **38** (350 mg, 43%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.74 (d, *J* = 7.4 Hz, 2H), 7.70 – 7.56 (m, 7H), 7.53 – 7.43 (m, 5H), 7.27 – 7.13 (m, 48H), 7.13 – 6.86 (m, 38H), 6.79 (dd, *J* = 9.4, 7.1 Hz, 5H), 6.73 – 6.68 (m, 3H), 6.64 (d, *J* = 7.3 Hz, 1H), 6.58 (t, *J* = 7.4 Hz, 2H), 5.70 (d, *J* = 8.3 Hz, 1H), 5.18 (d, *J* = 8.2 Hz, 1H), 5.14 – 5.09 (m, 2H), 5.01 – 4.92 (m, 2H), 4.87 (t, *J* = 11.0 Hz, 2H), 4.84 – 4.80 (m, 2H), 4.80 – 4.74 (m, 4H), 4.72 (d, *J* = 11.2 Hz, 1H), 4.69 – 4.65 (m, 3H), 4.64 (s, 1H), 4.57 – 4.53 (m, 3H), 4.50 (dt, *J* = 14.1, 5.2 Hz, 6H), 4.46 (dd, *J* = 10.3, 3.4 Hz, 3H), 4.44 – 4.40 (m, 3H), 4.40 – 4.34 (m, 7H), 4.28 – 4.22 (m, 4H), 4.22 – 4.15 (m, 3H), 4.09 (d, *J* = 12.0 Hz, 2H), 4.08 – 4.04 (m, 2H), 4.04 – 4.00 (m, 3H), 4.00 – 3.97 (m, 1H), 3.93 – 3.85 (m, 5H), 3.78 (q, *J* = 10.0 Hz, 5H), 3.71 (d, *J* = 8.9 Hz, 2H), 3.70 – 3.58 (m, 9H), 3.52 – 3.46 (m, 2H), 3.43 (d, *J* = 10.3 Hz, 1H), 3.36 (dd, *J* = 10.5, 6.9 Hz, 2H), 3.33 – 3.26 (m, 4H), 3.08 – 3.03 (m, 1H), 2.96 (d, *J* = 10.0 Hz, 1H), 2.77 (dd, *J* = 10.4, 6.8 Hz, 1H), 2.58 (d, *J* = 9.7 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.98, 167.43, 138.97, 138.88, 138.76, 138.68, 138.54, 138.37, 138.25, 138.16, 138.10, 138.02, 137.92, 133.80, 133.71, 133.50, 131.95, 131.45, 128.47, 128.33, 128.28, 128.21, 128.16, 128.08, 128.03, 127.96, 127.91, 127.87, 127.80, 127.77, 127.73, 127.63, 127.55, 127.49, 127.46, 127.43, 127.37, 127.33, 127.26, 127.21, 127.14, 126.82, 126.74, 123.28, 100.72, 99.43, 98.60, 97.60, 97.03, 96.51, 85.38, 81.04, 80.16, 79.57, 79.36, 79.16, 78.77, 77.94, 77.21, 77.00, 76.79, 76.24, 75.46, 75.39, 75.15, 75.08, 74.89, 74.78, 74.71, 74.60, 74.43, 74.15, 74.03, 73.55, 73.19, 72.80, 72.64, 72.50, 72.16, 71.20, 70.51, 69.83, 69.69, 69.14, 67.64, 67.50, 64.66, 56.43, 56.33, 55.98, 55.13. HRMS (ESI) m/z calcd. for $C_{193}H_{186}N_7O_{39}$ $(M + H)^+$ 3225.2786; found 3225.2801.

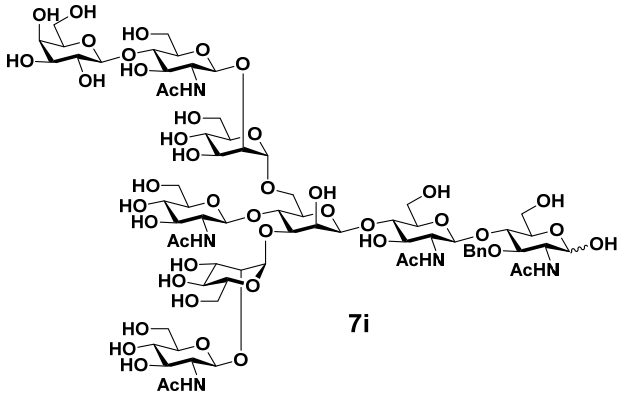
Compound 39:



Compound **38** (320 mg, 0.10 mmol), **35** (198 mg, 0.21 mmol) and 4Å molecular sieves (1.0 g) was dissolved in anhydrous DCM (10 mL). The solution was stirred at 25°C for 30 mins and then cooled to 0°C. TfOH (2.5 μL, 0.044 mmol) was added to the solution at 0° and the solution was stirred at 0°C for 1h and slowly warmed to 25°C within 2h. The solution was quenched by 2.5 uL of Et_3N and filtrated to remove molecular sieves. Saturated $NaHCO_3$(aq) was added to the solution and the aq. layer was extracted with DCM (2×20 mL). The organic layer was combined, dried over Na_2SO_4 and concentrated under reduced pressure. The crude liquid was purified by silica gel flash column chromatography using hexanes and ethyl acetate to obtain compound **39** (246 mg, 65%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.62 (d, *J* = 31.4 Hz, 8H), 7.50 – 7.34 (m, 8H), 7.30 – 7.12 (m, 42H), 7.12 – 6.98 (m, 26H), 6.95 – 6.80 (m, 27H), 6.76 – 6.70 (m, 3H), 6.49 (dd, *J* = 5.0, 1.8 Hz, 3H), 5.59 (d, *J* = 8.3 Hz, 1H), 5.24 – 5.21 (m, 1H), 5.15 (dd, *J* = 10.3, 8.0 Hz, 1H), 5.11 (dt, *J* = 8.2, 6.7 Hz, 3H), 4.90 – 4.85 (m, 2H), 4.85 – 4.80 (m, 4H), 4.80 – 4.75 (m, 2H), 4.74 – 4.66 (m, 7H), 4.65 – 4.60 (m, 3H), 4.57 (s, 1H), 4.53 – 4.46 (m, 6H), 4.44 (d, *J* = 11.3 Hz, 2H), 4.41 (s, 2H), 4.39 – 4.32 (m, 8H), 4.32 – 4.27 (m, 6H), 4.27 – 4.19 (m, 4H), 4.14 – 4.07 (m, 3H), 4.07 – 3.97 (m, 10H), 3.97 – 3.93 (m, 4H), 3.92 – 3.88 (m, 4H), 3.87 – 3.76 (m, 4H), 3.71 (s, 1H), 3.68 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.62 – 3.53 (m, 6H), 3.51 – 3.39 (m, 6H), 3.39 – 3.28 (m, 7H), 3.25 (dd, *J* = 11.2, 3.3 Hz, 1H), 3.07 (dd, *J* = 11.2, 4.8 Hz, 1H), 3.01 (d, *J* = 10.0 Hz, 1H), 2.65 (dd, *J* = 10.5, 7.0 Hz, 1H), 2.04 (s, 3H), 2.02 (s, 3H), 1.93 (s, 3H), 1.73 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.25, 170.14, 169.90, 169.10, 167.69, 167.37, 139.28, 139.16, 139.07, 138.95, 138.81, 138.62, 138.36, 138.15, 138.01, 133.68, 133.32, 133.16, 132.05, 131.84, 131.61, 129.19, 128.45, 128.27, 128.19, 128.10, 128.03, 127.95, 127.89, 127.80, 127.66, 127.51, 127.43, 127.36, 127.22, 127.02, 126.93, 126.86, 126.78, 126.68, 123.28, 123.11, 114.10, 101.78, 100.25, 99.81, 98.31, 97.05, 96.83, 96.12, 85.44, 80.98, 80.21, 79.18, 78.92, 78.38, 77.96, 77.82, 76.90, 75.55, 75.13, 74.70, 74.61, 74.49, 74.40, 74.17, 73.74, 73.44, 72.98, 72.88, 72.80, 72.72, 72.65, 72.49, 71.95, 71.21, 70.68, 70.51, 70.37, 69.79, 68.99, 68.81, 68.70, 67.66, 67.17, 60.89, 56.92, 56.60, 56.09, 55.57, 55.28, 20.62, 20.51, 20.47. HRMS (ESI) m/z calcd. for $C_{235}H_{229}N_8O_{54}$ $(M + H)^+$ 4026.5419; found 4026.5430.

Compound 7i:



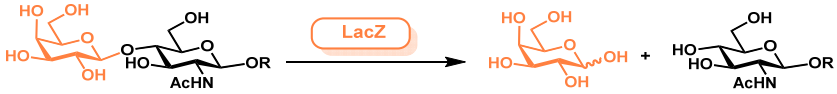
7i

To a solution of compound **39** (220 mg, 58.0 μmol) in n-butanol (10 ml) was added ethylenediamine (3 ml) at 25 °C. The solution was stirred for 8 h at 90 °C. Then the solution was directly concentrated under reduced pressure and co-evaporated twice with toluene (5 ml). To the above syrup was added pyridine (10 ml) and acetic anhydride (10 ml). The solution was stirred for 10 h at 25 °C. The solution was directly concentrated under reduced pressure and co-evaporated twice with toluene (5 ml). The crude liquid was purified by silica gel flash column chromatography. To the obtained intermediate was added MeOH (10 ml) and NaOMe (20 mg). The solution was stirred with a stable pH 9-10 for 10 h at 25 °C. The solution was quenched by Dowex $50H^+$ and filtrated to remove the resin. The obtained solution was concentrated under reduced pressure. To the above syrup in a mixed solvent of MeOH (10 ml) and H_2O (1 ml) was added 10% Pd $(OH)_2/C$ (200 mg). The solution was stirred for 24 h under H_2 atmosphere at 25 °C. The solid was filtered off and the solution was concentrated to give compound **7i** (58 mg, 60 % for 4 steps).

^{1}H NMR (600 MHz, D_2O) δ 5.52 (s, 1H), 5.08 (d, *J* = 2.2 Hz, 1H), 4.99 (d, *J* = 3.4 Hz, 0.4H), 4.95 (s, 1H), 4.91 (s, 1.4H), 4.59 (d, *J* = 11.2 Hz, 2.4H), 4.51 – 4.44 (m, 4H), 4.37 (t, *J* = 8.5 Hz, 2.8H), 4.14 (s, 1H), 4.09 – 3.94 (m, 4.8H), 3.89 – 3.35 (m, 66H), 3.30 (t, *J* = 9.4 Hz, 1.4H), 3.21-3.14 (m, 1.4H), 1.98 – 1.93 (m, 21H). ^{13}C NMR (150 MHz, D_2O) δ 174.75, 174.67, 174.61, 174.57, 174.46, 174.40, 174.23, 102.94, 101.39, 101.22, 100.53, 100.02, 99.90, 99.58, 99.52, 99.33, 97.70, 97.61, 94.77, 90.40, 79.63, 79.15, 78.60, 78.50, 78.24, 76.72, 76.44, 76.21, 75.78, 75.31, 74.54, 74.33, 73.49, 73.31, 72.95, 72.49, 72.20, 72.00, 71.38, 71.08, 70.95, 70.65, 70.47, 70.28, 69.93, 69.73, 69.44, 69.29, 69.21, 68.48, 67.54, 67.32, 65.18, 61.90, 61.83, 61.63, 60.98, 60.58, 60.37, 59.95, 59.87, 56.09, 55.27, 55.20, 54.73, 53.60, 22.40, 22.36, 22.26, 22.16, 22.12, 22.10, 21.87, 21.85. HRMS (ESI) m/z calcd. for $C_{64}H_{108}N_5O_{46}$ $(M + H)^+$ 1682.6265; found 1682.6330

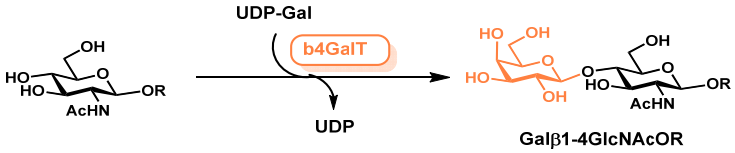
3. ENZYMATIC MODULAR SYNTHESIS AND HPLC PURIFICATION

dG. β-galactosidase digestion with LacZ



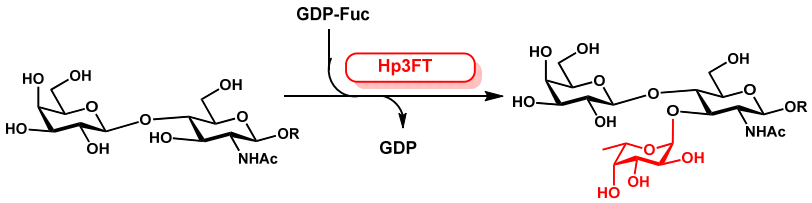
Reaction mixtures contain Tris-HCl (100 mM, pH 7.5), a galactosylated glycan (10 mM), and an appropriate amount of LacZ. Reactions were incubated at 37°C for 3 days and monitored by MALDI-TOF MS. After over 90% glycan conversion, the reaction was quenched, concentrated and subject for HPLC purification. Product-containing fractions were pooled and lyophilized for characterization.

G. β1-4 galactosylation with b4GalT



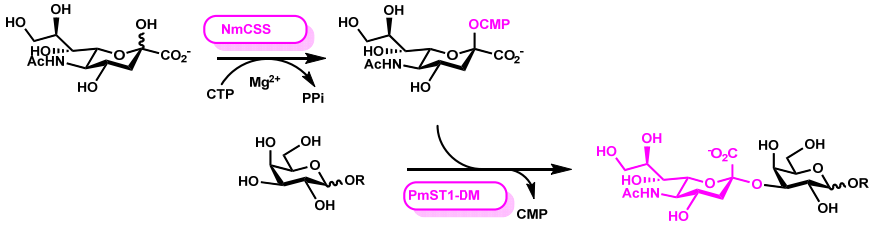
Reaction mixtures contain Tris-HCl (100 mM, pH 7.5), an acceptor glycan (10 mM), UDP-Gal (15 mM), $MgCl_2$ (10 mM), and an appropriate amount of b4GalT. Reactions were incubated at 37 °C 6 h to overnight and monitored by HPLC and/or MALDI-TOF MS. After complete conversion of the acceptor, the reaction was quenched, concentrated and subject for HPLC purification. Product-containing fractions were pooled and lyophilized for characterization and next step modular assembly.

F2. α1-3 fucosylation with Hp3FT



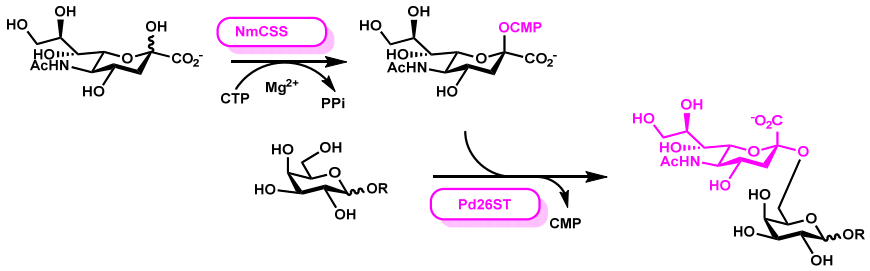
Reaction mixtures contain Tris-HCl (100 mM, pH 7.5), an acceptor glycan (10 mM), GDP-Fuc (15 mM), $MgCl_2$ (10 mM), and appropriate amount of Hp3FT. Reactions were incubated at 37 °C 6 h to overnight and monitored by HPLC and/or MALDI-TOF MS. After complete conversion of the acceptor, the reaction was quenched, concentrated and subject for HPLC purification. Product-containing fractions were pooled and lyophilized for characterization and next step modular assembly

S1. α2-3 sialylation with PmST1-DM



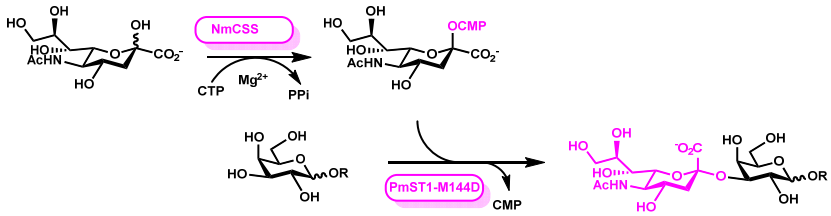
Reaction mixtures contain Tris-HCl (100 mM, pH 8.0), an acceptor glycan (10 mM), CTP (15 mM), Neu5Ac (15 mM), $MgCl_2$ (10 mM), and appropriate amount of NmCSS and PmST1-DM. PmST1-DM-catalyzed reactions were incubated at 37 °C for 15-30 min and monitored by HPLC and/or MALDI-TOF MS. After complete conversion of the acceptor, the reaction was quenched, concentrated and subject for HPLC purification. Product-containing fractions were pooled and lyophilized for characterization and next step modular assembly

S2. α2-6 sialylation with Pd26ST



Reaction mixtures contain Tris-HCl (100 mM, pH 8.0), an acceptor glycan (10 mM), CTP (15 mM), Neu5Ac (15 mM), $MgCl_2$ (10 mM), and appropriate amount of NmCSS and Pd26ST. Reactions were incubated at 37 °C 3 h to 6 h and monitored by HPLC and/or MALDI-TOF MS. After complete conversion of the acceptor, the reaction was quenched, concentrated and subject for HPLC purification. Product-containing fractions were pooled and lyophilized for characterization and next step modular assembly

S3. α2-3 sialylation with PmST1-M144D



Reaction mixtures contain Tris-HCl (100 mM, pH 8.0), an acceptor glycan (10 mM), CTP (15 mM), Neu5Ac (15 mM), $MgCl_2$ (10 mM), and appropriate amount of NmCSS and PmST1-144D. Reactions were incubated at 37 °C 3 h to 6 h and monitored by HPLC and/or MALDI-TOF MS. After complete conversion of the acceptor, the reaction was quenched, concentrated and subject for HPLC purification. Product-containing fractions were pooled and lyophilized for characterization and next step modular assembly

HPLC Analysis and Purification

HPLC analysis of synthesize glycans was performed on a Shimadzu LC-20AD system using a Waters XBridge BEH amide column (130 Å, 5 μm, 4.6 mm × 250 mm) monitored by a UV detector (210 nm). The running solvents are ddH_2O (for non-sialylated N-glycans) or 100 mM ammonium formate pH 3.4 (for sialylated N-glycans) (solvent A) and acetonitrile (solvent B). Gradient elution from 65% solvent B to 50% solvent B within 25 mins, with a flow rate of 1 mL/min.

HPLC purification of synthesized glycans was performed on a Shimadzu LC-20AR semi-preparative system using a analytical XBridge BEH amide column (130 Å, 5 μm, 4.6 mm × 250 mm) (for reactions with 3 mg or less products) or a semi-preparative XBridge BEH amide column (130 Å, 5 μm, 10 mm × 250 mm). Same rounding solvents and elution conditions were used as for analysis with the only difference of flow rate for the semi-preparative column (4.5 mL/mL).

4. MICROARRAY FABRICATION AND ANALYSIS

4.1 AEAB label

N-glycans (50 μg) were dissolved in 50 μL of DMSO and AcOH solution (7:3, v/v) and incubated with AEAB at 60 ºC for 2 h. Then, ice cold acetonitrile was used to precipitate the glycans and the precipitation was dissolved in water and purified by HPLC. Hypercarb™ porous graphitic carbon column (150 mm × 4.6 mm, Thermo Fisher) was used for the purification with ddH_2O containing 0.1% TFA as solvent A and acetonitrile containing 0.1% TFA as solvent B. A gradient elution with solvent B from 15 % to 45 % in 30 mins was used for the purification of AEAB labeled glycans.

4.2 Method for microarray fabrication

The bisecting vs. non-bisecting microarray was printed according to the guidelines of MIRAGE as summarized in **Table S2**. All glycans were prepared at a concentration of 100 μM in the printing buffer (150 mM phosphate, pH 8.5), and printed on Nexterion slide H-3D hydrogel coated glass microarray slides (Schott AG), each for 1 nL in replicates of four as described previously.[16] Non-contact printing was performed at room temperature with a humidity of 60% by a sciFLEXARRAYER S3 spotter (Scienion) with two PDC 80 Piezo Dispense Capillary, and 8 subarrays were printed on each slide. After overnight dehumidification under room temperature, the slides were washed with MilliQ water and subsequently blocked with 50 mM ethanolamine in 100 mM Tris buffer (pH 9.0) for 2 hours. The blocked slides were then washed with MilliQ water twice, dried, and stored desiccated at -20°C until use. Print buffer was printed as a negative control. In addition, biotinylated PEG amine (0.01mg/mL), Mouse IgG (0.1 mg/mL) and Human IgG (0.1 mg/mL) were printed in four replicates in print buffer to serve as a positive control. A marker containing anti-human IgG conjugate with Cy3 (0.01 mg/mL) and anti-human IgG conjugate with Alexa 647 (0.01 mg/mL) was also printed in the replicates of four.

4.3 Method for microarray assay

All assay were performed as previously reported.[16] Biotin-labelled lectins were detected by Cy3-streptavidin (1 μg/mL) (PHA-L, DSA, LTL, UEA-I, WFL, and GS-II) or Cy5-streptavidin (1 μg/mL). Human galectins, Siglecs and selectins were detected by corresponding second antibody with fluorescent label (5 μg/mL). Influenza A Hemagglutinin were detected with mouse anti-His-tag antibody (Alexa Fluor 647 conjugated) and goat anti-mouse IgG antibody (Alexa Fluor 647 conjugated) in a molar ratio of 4:2:1. After binding and washing, the slides were scanned with a GenePix 4000B scanner, and the collected data was analyzed with GenePix Pro.

Table S2. Glycan microarray information based on MIRAGE.

Classification	Guidelines
1. Sample: Glycan Binding Sample	
Description of Sample	Glycan binding proteins (**Table S1**).
Sample modifications	Not applicable.
Assay protocol	Microarray analyses were performed essentially as described Section 4.3 Method for microarray assay.
2. Glycan Library	
Glycan description for defined glycans	All glycans were synthesized as described in body text.
Glycan description for undefined glycans	Not applicable.
Glycan modifications	Glycans were linked with Ser.
3. Printing Surface; e.g., Microarray Slide	
Description of surface	Nexterion slide H-3D hydrogel coated glass microarray slides.
Manufacturer	Schott
Custom preparation of surface	Not relevant.
Covalent Immobilization	Glycans were linked with Ser for robotically arraying and the amine group could be covalently immobilized on NHS ester coated glass slide.

4. Arrayer (Printer)	
Description of Arrayer	sciFLEXARRAYER S3 spotter (Scienion) with two PDC 80 Piezo Dispense Capillary, and 8 subarrays were printed on each slide
Dispensing mechanism	Non-contact liquid delivery.
Glycan deposition	Each glycan probe was printed at 1 deposit in 6 replicates.
Printing conditions	Samples were prepared at a concentration of 100 μM in the printing buffer (150 mM phosphate, pH 8.5), printing was performed at room temperature and relative humidity of 60%.
5. Glycan Microarray with "Map"	
Array layout	Each array slide contained 8 identical subarrays (pads). Each subarray contained up to 74 unique samples.
Glycan identification and QC	Quality control included analyses with plant lectins.
6. Detector and Data Processing	
Scanning hardware	GenePix 4000B Microarray Scanner (Molecular Devices, LLC)
Scanner settings	Laser channel: wavelength 635 nm or 535 nm PMT gain: 600 Scan power:100%
Image analysis software	GnePix Pro (Molecular Devices, LLC)
Data processing	The gpr files were processed with in-house excel macro to obtain basic descriptive statistics. No particular normalization method or statistical analysis was used.
7. Glycan Microarray Data Presentation	
Data presentation	The microarray binding results are in Figure 5,6, S5-S12, and Table 2. Binding results are presented as relative fluorescence intensity units (RFU) of binding in mean and S.D.
8. Interpretation and Conclusion from Microarray Data	
Data interpretation	No software or algorithms were used to interpret processed data.
Conclusions	Described in Results parts.

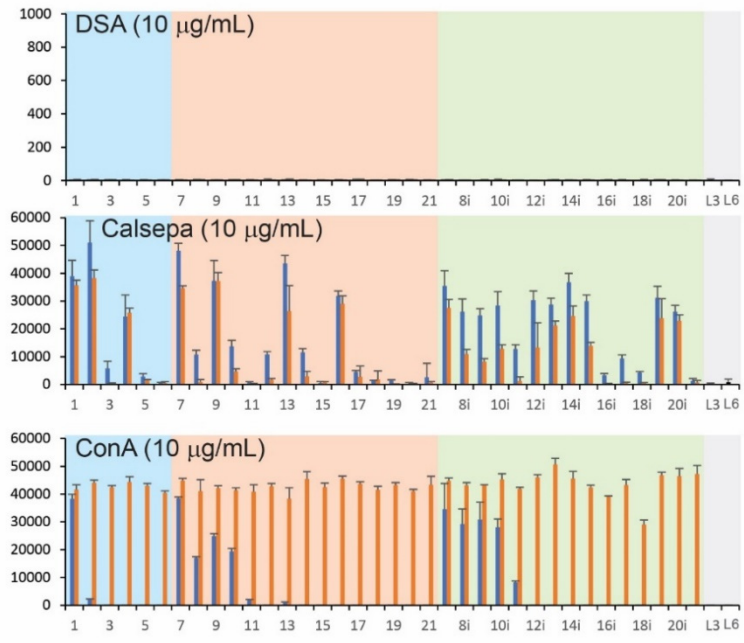
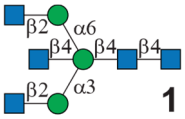


Figure S2. microarray results for Lectins DSA, Calsepa, and ConA at 10 mg/mL

Results are shown as relative fluorescence units (RFUs) by averaging the background-subtracted fluorescence signals of 4 replicate spots, error bars represent the standard deviation (SD). Source data are provided as a Source Data file. L3, 3'SLNnT; L6, 6'SLNnT.

5. HPLC, MASS SPECTROMETRY, AND NMR ANALYSIS

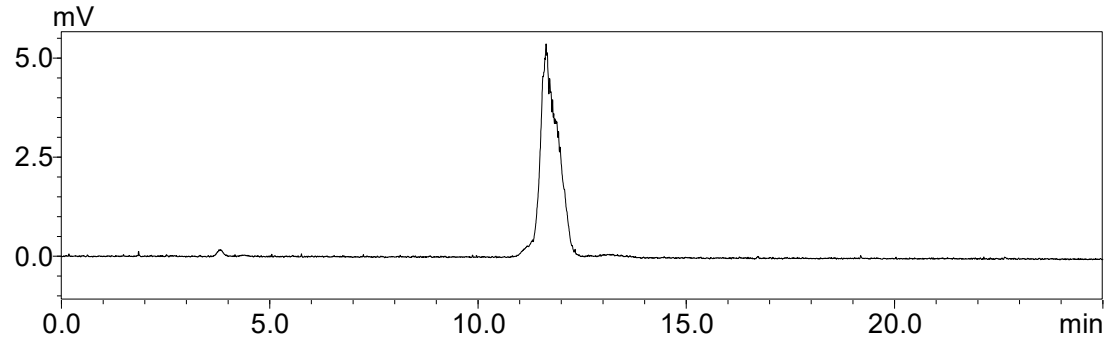


1

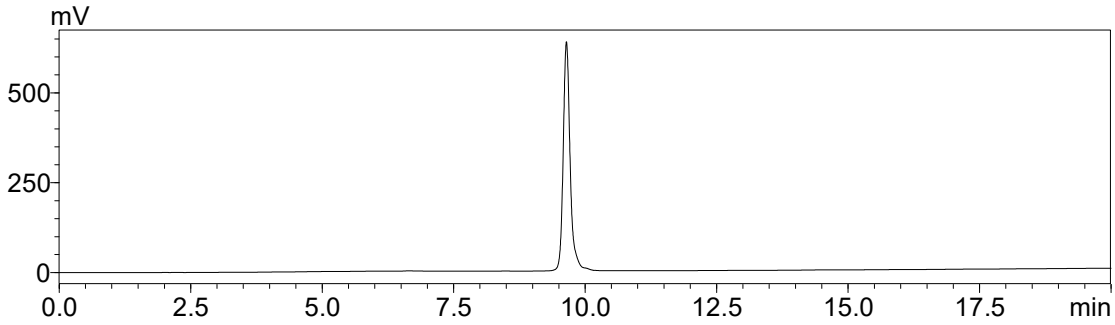
1H NMR (600 MHz, Deuterium Oxide) δ 5.16 (d, J = 2.6 Hz, 1H), 5.03 (s, 1H), 4.97 (s, 1H), 4.66 (s, 2H), 4.61 – 4.55 (m, 2H), 4.55 – 4.49 (m, 2H), 4.44 (d, J = 8.4 Hz, 2H), 4.22 (s, 2H), 4.15 (d, J = 3.2 Hz, 1H), 4.12 (s, 2H), 4.05 (t, J = 9.6 Hz, 2H), 4.00 – 3.40 (m, 50H), 3.37 (d, J = 8.5 Hz, 3H), 3.24 (t, J = 9.3 Hz, 2H), 2.08 – 1.95 (m, 15H).

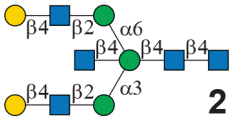
HRMS (ESI) m/z calcd. for C58H97N5O41 1519.5659; found [M+Na]+ 1542.5551.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



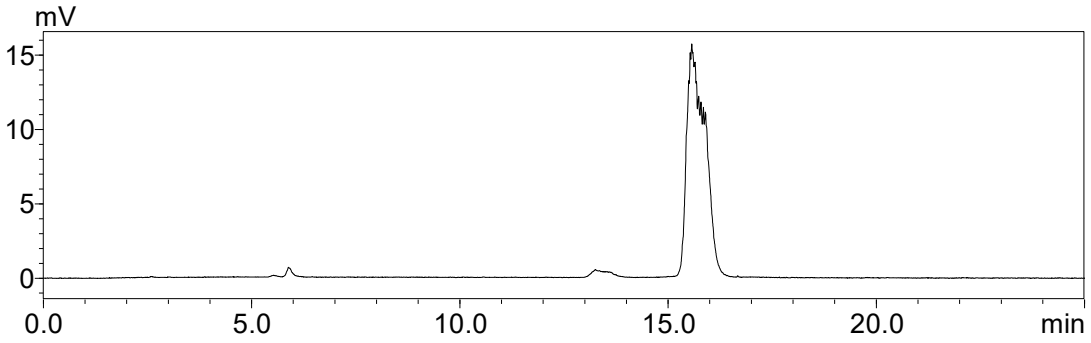


2

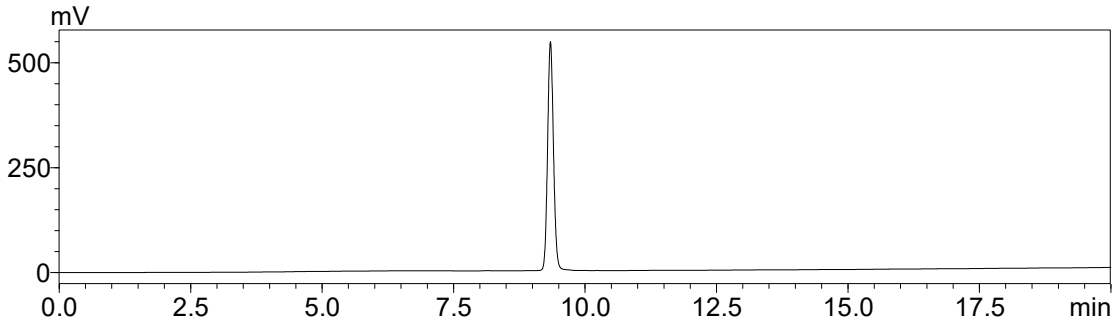
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.6 Hz, 1H), 5.07 (d, J = 1.7 Hz, 1H), 5.02 (t, J = 2.0 Hz, 1H), 4.73 – 4.68 (m, 2H), 4.64 – 4.56 (m, 3H), 4.52 – 4.45 (m, 3H), 4.27 (dd, J = 3.4, 1.7 Hz, 1H), 4.19 (d, J = 3.2 Hz, 1H), 4.15 (dd, J = 3.6, 1.7 Hz, 1H), 4.10 (t, J = 9.8 Hz, 2H), 4.03 – 3.45 (m, 61H), 3.40 (d, J = 9.8 Hz, 1H), 3.27 (dd, J = 9.9, 8.8 Hz, 1H), 2.15 – 2.00 (m, 15H).

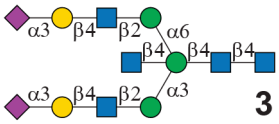
HRMS (ESI) m/z calcd. for C70H117N5O51 1843.6715; found [M+2H]2+ 922.8437.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



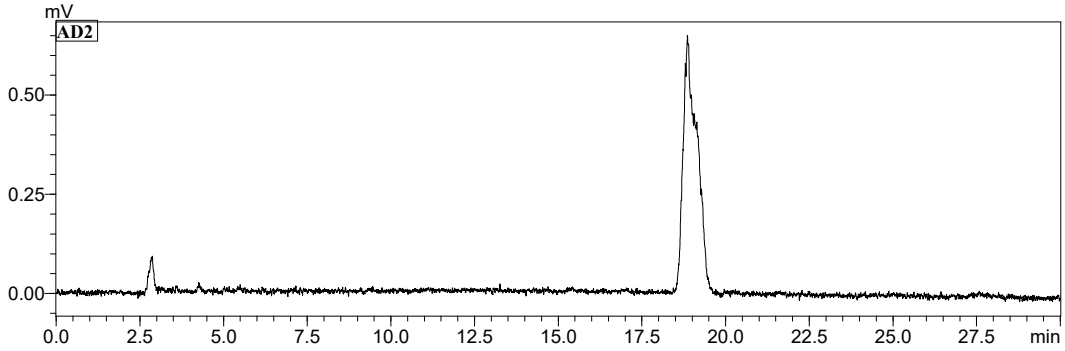


3

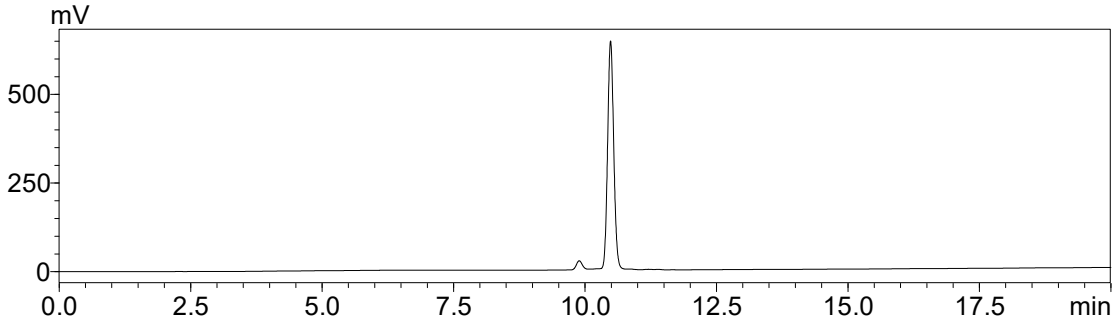
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.5 Hz, 1H), 5.07 (s, 1H), 5.01 (s, 1H), 4.70 (d, J = 3.1 Hz, 1H), 4.61 (dd, J = 8.6, 5.7 Hz, 2H), 4.59 – 4.53 (m, 3H), 4.47 (d, J = 8.3 Hz, 1H), 4.26 (d, J = 3.3 Hz, 1H), 4.20 – 4.15 (m, 2H), 4.13 (dt, J = 9.7, 3.2 Hz, 2H), 4.09 (d, J = 9.7 Hz, 1H), 4.03 – 3.94 (m, 5H), 3.94 – 3.81 (m, 14H), 3.80 – 3.45 (m, 35H), 3.41 (t, J = 8.8 Hz, 2H), 3.27 (t, J = 9.3 Hz, 1H), 2.76 (dt, J = 12.4, 4.7 Hz, 2H), 2.12 – 1.98 (m, 15H), 1.81 (td, J = 12.1, 6.5 Hz, 2H).

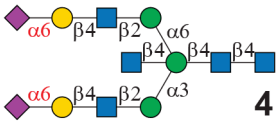
HRMS (ESI) m/z calcd. for C92H151N7O67 2425.8624; found [M-2H]2- 1211.9151.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



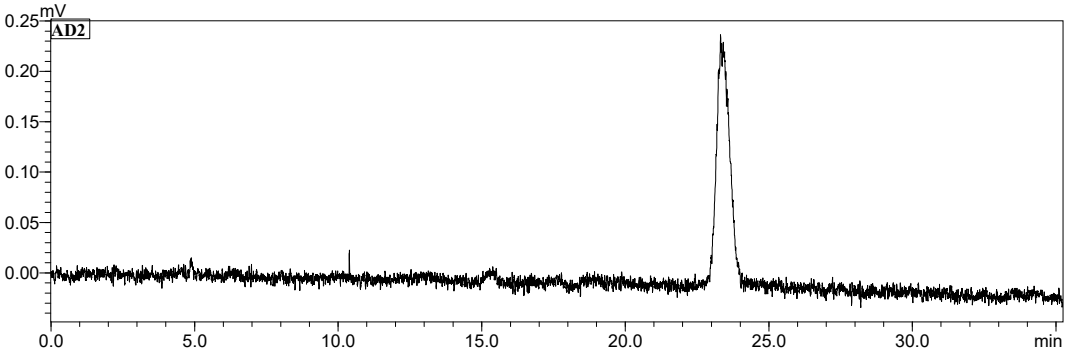


4

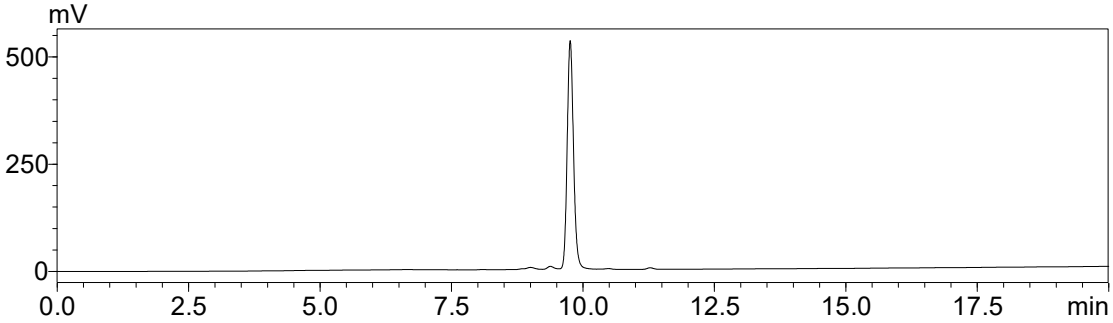
1H NMR (600 MHz, Deuterium Oxide) δ 4.66 (dd, J = 13.1, 8.1 Hz, 1H), 4.45 (s, 1H), 4.38 (s, 1H), 4.31 (d, J = 10.6 Hz, 1H), 4.20 – 3.67 (m, 47H), 3.61 (t, J = 8.5 Hz, 1H), 3.48 (t, J = 9.3 Hz, 1H), 2.88 (tt, J = 8.3, 4.4 Hz, 1H), 2.30 – 2.18 (m, 15H), 1.98 (t, J = 12.1 Hz, 1H), 1.90 (t, J = 12.2 Hz, 1H).

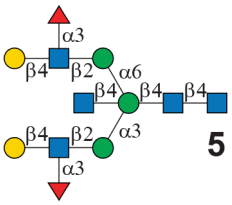
HRMS (ESI) m/z calcd. for C92H151N7O67 2425.8624; found [M-2H]2- 1211.9159.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)

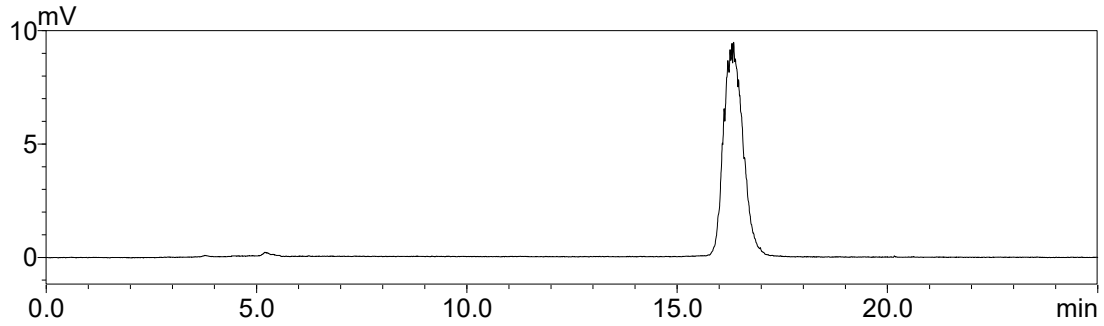




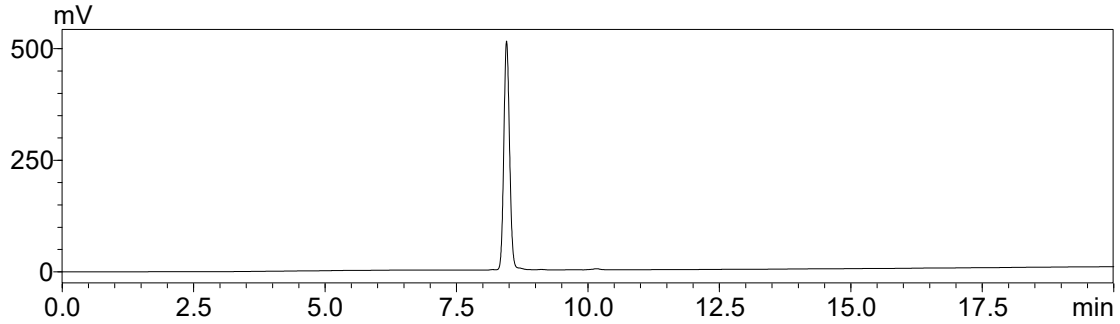
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 2.6 Hz, 1H), 5.15 (dd, J = 8.4, 4.0 Hz, 2H), 5.04 (s, 2H), 4.70 (d, J = 6.2 Hz, 2H), 4.60 (dd, J = 8.3, 5.6 Hz, 4H), 4.51 – 4.43 (m, 4H), 4.27 (d, J = 3.4 Hz, 1H), 4.19 (d, J = 3.1 Hz, 1H), 4.14 (d, J = 3.4 Hz, 1H), 4.07 (t, J = 9.8 Hz, 2H), 4.03 – 3.39 (m, 71H), 3.25 (t, J = 9.3 Hz, 1H), 2.15 – 1.97 (m, 15H), 1.19 (dd, J = 14.8, 6.6 Hz, 6H).

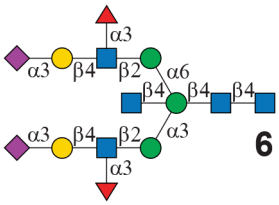
HRMS (ESI) m/z calcd. for C82H137N5O59 2135.7874; found [M+2H]2+ 1068.9021.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)

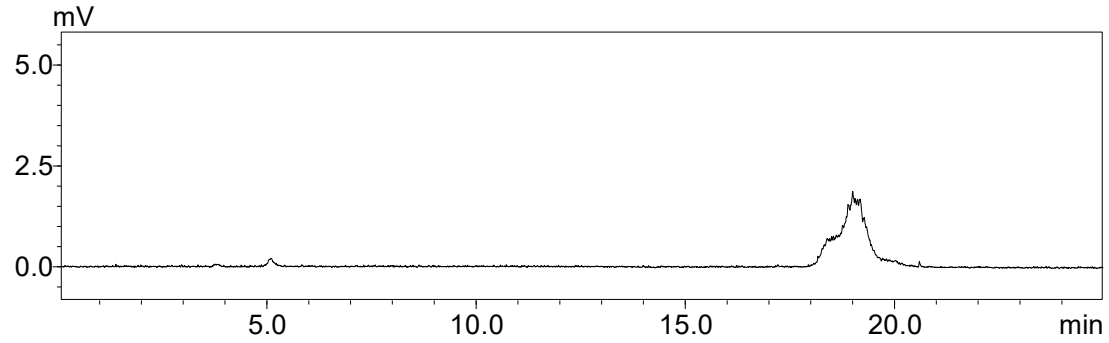




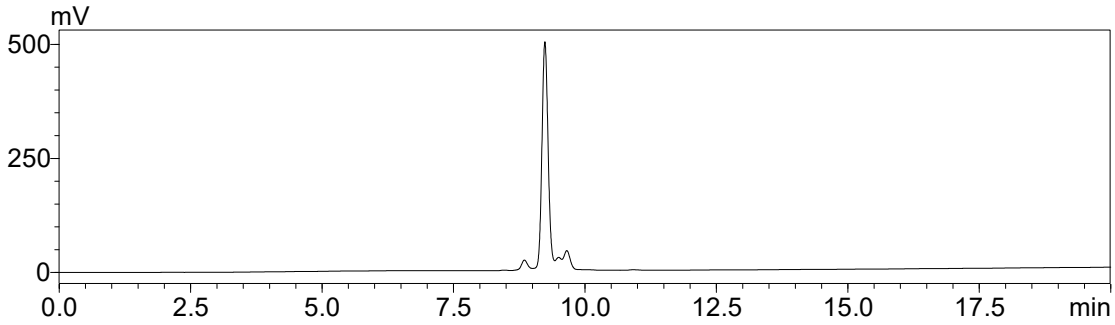
1H NMR (600 MHz, Deuterium Oxide) δ 5.39 (s, 1H), 5.33 (t, J = 4.2 Hz, 3H), 5.23 (d, J = 12.7 Hz, 3H), 5.04 – 4.97 (m, 4H), 4.89 (s, 2H), 4.72 (d, J = 7.9 Hz, 2H), 4.67 (t, J = 8.4 Hz, 3H), 4.44 (s, 2H), 4.38 (s, 1H), 4.33 (s, 2H), 4.28 (d, J = 9.1 Hz, 3H), 4.22 – 3.65 (m, 56H), 3.62 (s, 4H), 3.46 (t, J = 9.2 Hz, 3H), 3.01 – 2.87 (m, 6H), 2.32 – 2.18 (m, 15H), 1.99 (dd, J = 12.1, 5.1 Hz, 5H), 1.42 – 1.31 (m, 7H).

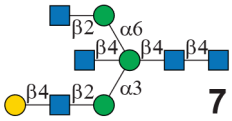
HRMS (ESI) m/z calcd. for C104H171N7O75 2717.9782; found [M-2H]2- 1357.9730.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)





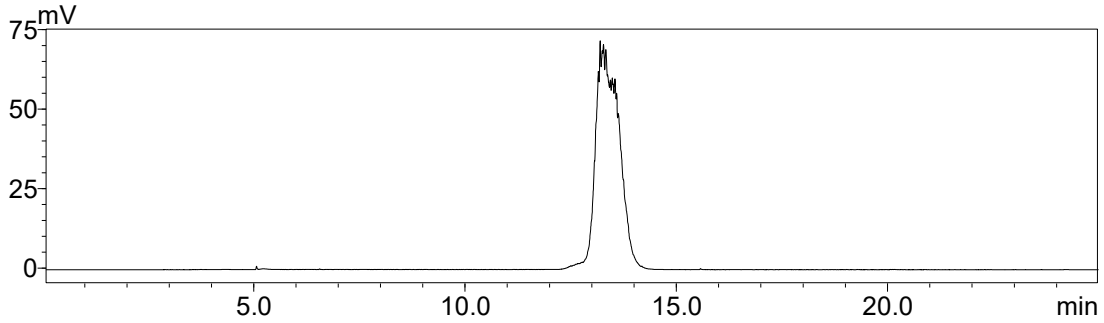
7

1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.7 Hz, 1H), 5.07 (d, J = 1.8 Hz, 1H), 5.01 (t, J = 1.8 Hz, 1H), 4.71 (d, J = 5.4 Hz, 1H), 4.65 – 4.58 (m, 2H), 4.56 (d, J = 8.4 Hz, 1H), 4.48 (dd, J = 8.1, 2.9 Hz, 2H), 4.27 (dd, J = 3.5, 1.7 Hz, 1H), 4.19 (d, J = 3.1 Hz, 1H), 4.16 (dt, J = 3.1, 1.3 Hz, 1H), 4.08 (td, J = 9.8, 1.6 Hz, 1H), 4.03 – 3.45 (m, 50H), 3.42 (tq, J = 8.9, 2.3 Hz, 2H), 3.27 (dd, J = 9.9, 8.7 Hz, 1H), 2.12 – 2.01 (m, 15H).

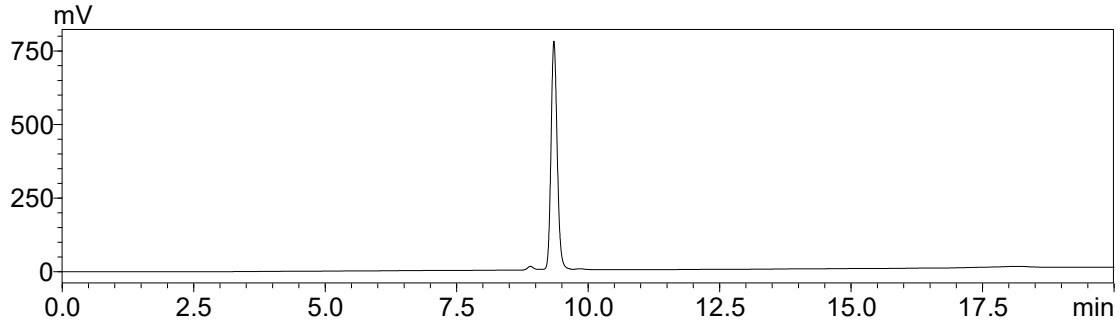
13C NMR (150 MHz, D2O) δ 174.72, 174.61, 174.53, 174.45, 102.89, 101.41, 100.56, 100.10, 99.69, 99.49, 97.67, 94.79, 90.43, 79.65, 79.19, 78.88, 78.49, 78.09, 76.41, 76.17, 75.71, 75.33, 74.69, 74.56, 74.25, 73.48, 73.43, 73.31, 72.44, 71.95, 71.66, 71.11, 70.94, 70.33, 69.98, 69.86, 69.49, 69.31, 69.23, 68.55, 67.56, 67.36, 65.38, 62.48, 61.90, 61.83, 61.63, 61.03, 60.54, 59.99, 59.83, 56.10, 55.24, 54.84, 53.63, 22.43, 22.36, 22.21, 22.15, 22.12, 21.86.

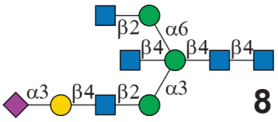
HRMS (ESI) m/z calcd. for C64H107N5O46 1681.6187; found [M+2H]2+ 841.8198.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



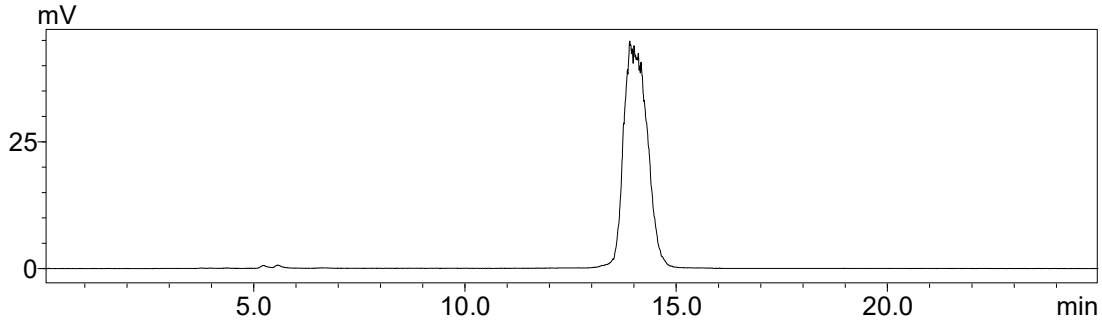


8

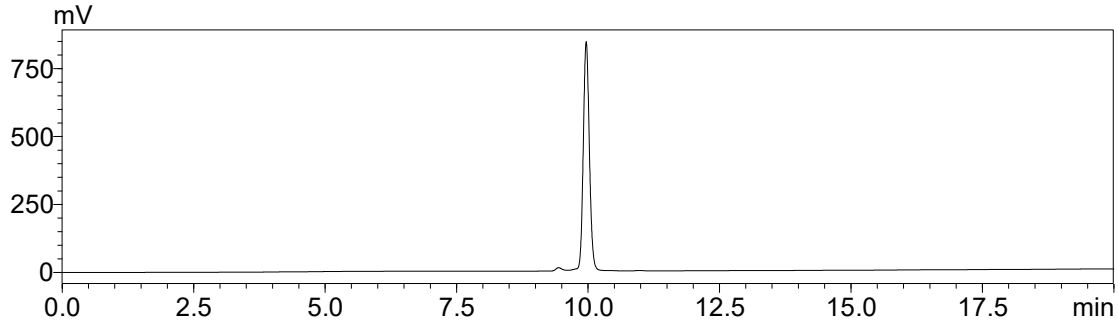
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.6 Hz, 1H), 5.07 (s, 1H), 5.01 (s, 1H), 4.70 (d, J = 3.3 Hz, 1H), 4.60 (td, J = 8.4, 4.2 Hz, 2H), 4.55 (d, J = 8.2 Hz, 2H), 4.47 (d, J = 8.3 Hz, 1H), 4.26 (d, J = 3.2 Hz, 1H), 4.18 (d, J = 3.2 Hz, 1H), 4.17 – 4.14 (m, 1H), 4.12 (dd, J = 9.9, 3.1 Hz, 1H), 4.07 (t, J = 9.8 Hz, 1H), 3.98 (ddd, J = 13.7, 10.9, 7.9 Hz, 4H), 3.94 – 3.45 (m, 45H), 3.45 – 3.37 (m, 2H), 3.26 (t, J = 9.3 Hz, 1H), 2.76 (dd, J = 12.5, 4.7 Hz, 1H), 2.17 – 1.95 (m, 15H), 1.80 (t, J = 12.1 Hz, 1H).

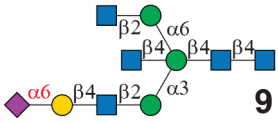
HRMS (ESI) m/z calcd. for C75H124N6O54 1972.7141; found [M-H]- 1971.6954.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



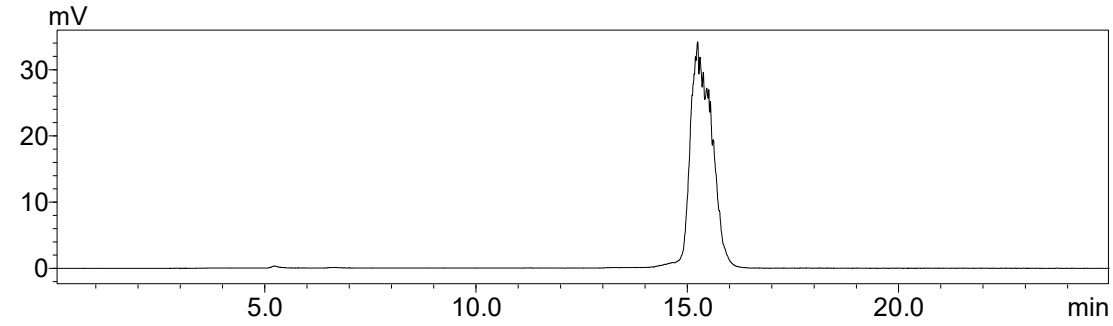


9

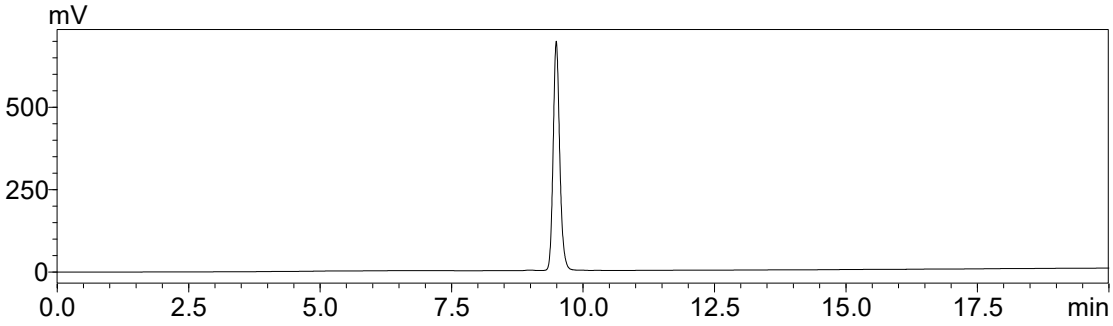
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.8 Hz, 1H), 5.09 (s, 1H), 5.01 (d, J = 2.0 Hz, 1H), 4.71 (d, J = 6.8 Hz, 1H), 4.64 – 4.59 (m, 2H), 4.56 (d, J = 8.4 Hz, 1H), 4.47 (dd, J = 13.8, 8.1 Hz, 2H), 4.29 – 4.25 (m, 1H), 4.20 (d, J = 3.2 Hz, 1H), 4.16 (d, J = 2.5 Hz, 1H), 4.07 (t, J = 9.8 Hz, 1H), 4.03 – 3.45 (m, 51H), 3.41 (d, J = 8.9 Hz, 2H), 3.28 (t, J = 9.3 Hz, 1H), 2.68 (dd, J = 12.4, 4.7 Hz, 1H), 2.12 – 1.99 (m, 16H), 1.73 (t, J = 12.2 Hz, 1H).

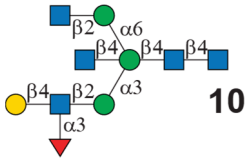
HRMS (ESI) m/z calcd. for C75H124N6O54 1972.7141; found [M-H]- 1971.6943.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



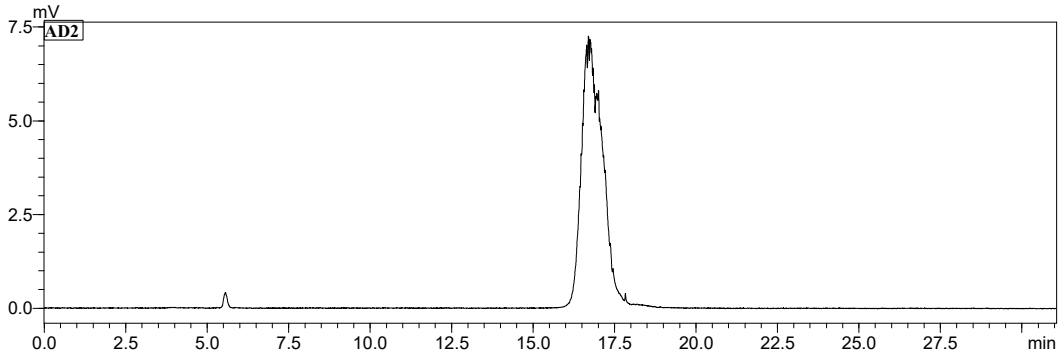


10

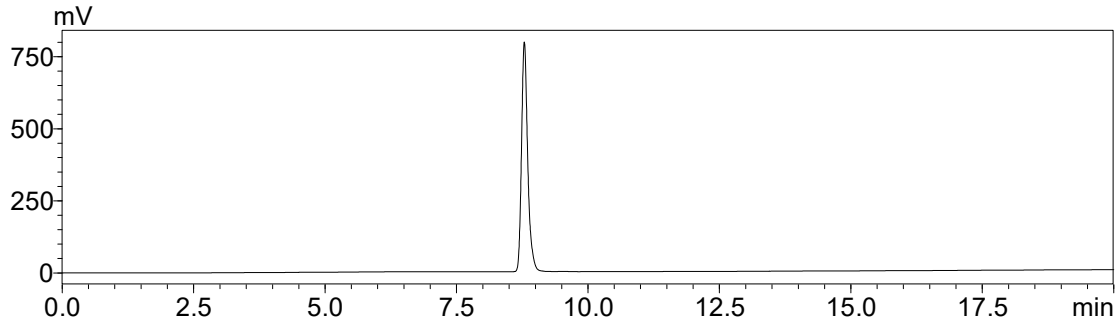
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.6 Hz, 1H), 5.16 (d, J = 4.0 Hz, 1H), 5.04 (s, 1H), 5.01 (d, J = 2.1 Hz, 1H), 4.85 (t, J = 6.8 Hz, 1H), 4.71 (d, J = 5.9 Hz, 2H), 4.64 – 4.58 (m, 2H), 4.56 (d, J = 8.4 Hz, 1H), 4.47 (t, J = 7.6 Hz, 2H), 4.27 (dd, J = 3.5, 1.7 Hz, 1H), 4.19 (d, J = 3.2 Hz, 1H), 4.18 – 4.14 (m, 1H), 4.08 (t, J = 9.8 Hz, 1H), 4.03 – 3.37 (m, 65H), 3.26 (dd, J = 9.9, 8.7 Hz, 1H), 2.13 – 2.00 (m, 15H), 1.18 (d, J = 6.6 Hz, 3H).

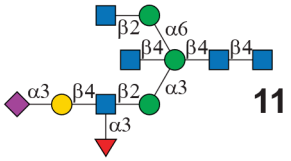
HRMS (ESI) m/z calcd. for C70H117N5O50 1827.6776; found [M+2H]2+ 914.8486.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



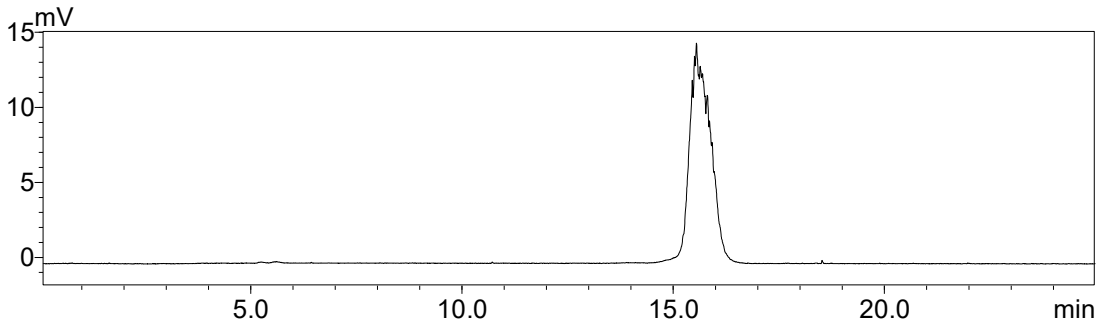


11

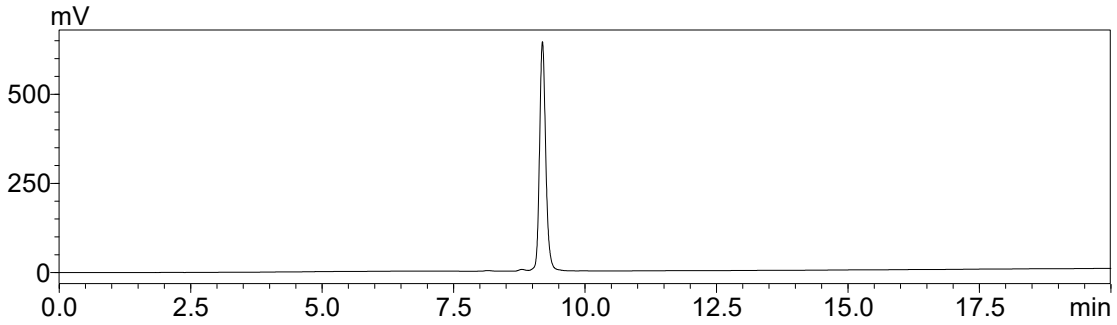
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.7 Hz, 1H), 5.15 (d, J = 4.0 Hz, 1H), 5.05 (s, 1H), 5.01 (d, J = 1.9 Hz, 1H), 4.71 (d, J = 4.7 Hz, 1H), 4.65 – 4.58 (m, 2H), 4.54 (dd, J = 14.0, 8.1 Hz, 2H), 4.47 (d, J = 8.3 Hz, 1H), 4.26 (d, J = 3.1 Hz, 1H), 4.19 (d, J = 3.2 Hz, 1H), 4.16 (dd, J = 3.5, 1.7 Hz, 1H), 4.10 (dd, J = 10.0, 3.3 Hz, 1H), 4.06 (d, J = 9.8 Hz, 1H), 4.03 – 3.37 (m, 53H), 3.26 (t, J = 9.3 Hz, 1H), 2.77 (dd, J = 12.4, 4.6 Hz, 1H), 2.13 – 1.97 (m, 15H), 1.80 (t, J = 12.1 Hz, 1H), 1.17 (d, J = 6.6 Hz, 3H).

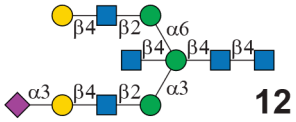
HRMS (ESI) m/z calcd. for C81H134N6O58 2118.7720; found [M-H]- 2117.7599.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



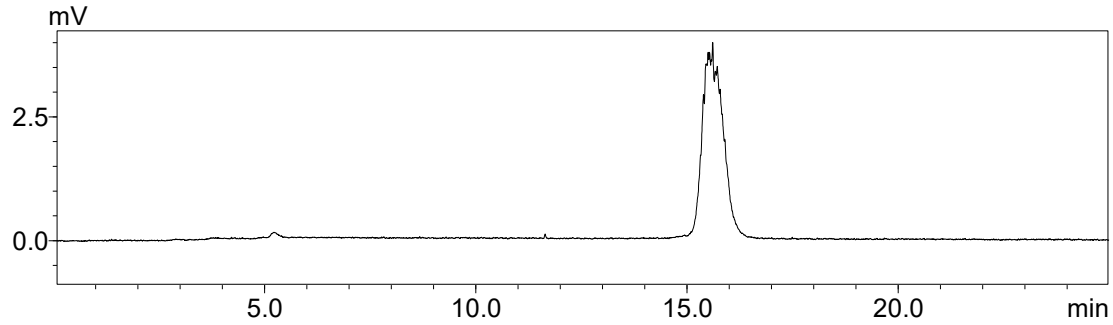


12

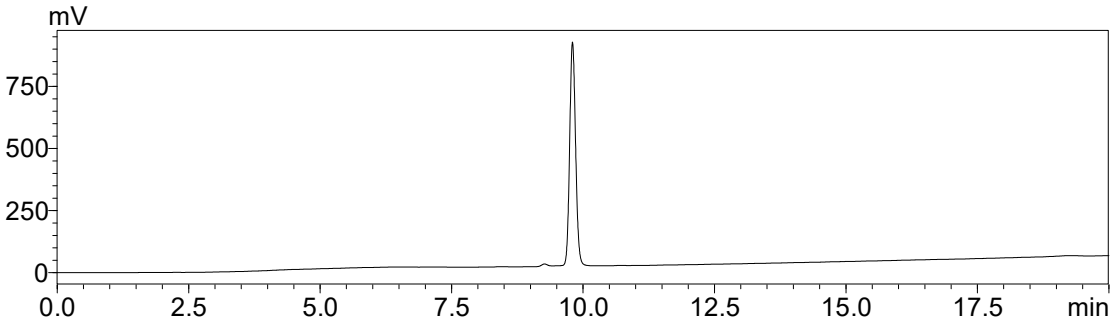
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.7 Hz, 1H), 5.07 (s, 1H), 5.02 (d, J = 1.9 Hz, 1H), 4.70 (d, J = 9.2 Hz, 2H), 4.65 – 4.54 (m, 4H), 4.48 (dd, J = 11.2, 8.0 Hz, 2H), 4.29 – 4.24 (m, 1H), 4.18 (d, J = 3.1 Hz, 1H), 4.15 (dd, J = 3.5, 1.7 Hz, 1H), 4.12 (dd, J = 9.9, 3.1 Hz, 1H), 4.08 (d, J = 9.8 Hz, 1H), 4.03 – 3.52 (m, 52H), 3.49 (td, J = 9.6, 2.0 Hz, 2H), 3.44 – 3.39 (m, 1H), 3.27 (t, J = 9.3 Hz, 1H), 2.76 (dd, J = 12.4, 4.6 Hz, 1H), 2.12 – 2.00 (m, 15H), 1.80 (t, J = 12.1 Hz, 1H), 1.34 (d, J = 6.6 Hz, 1H).

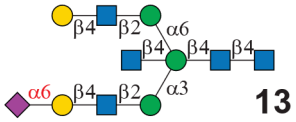
HRMS (ESI) m/z calcd. for C81H134N6O59 2134.7670; found [M-H]- 2133.7560.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



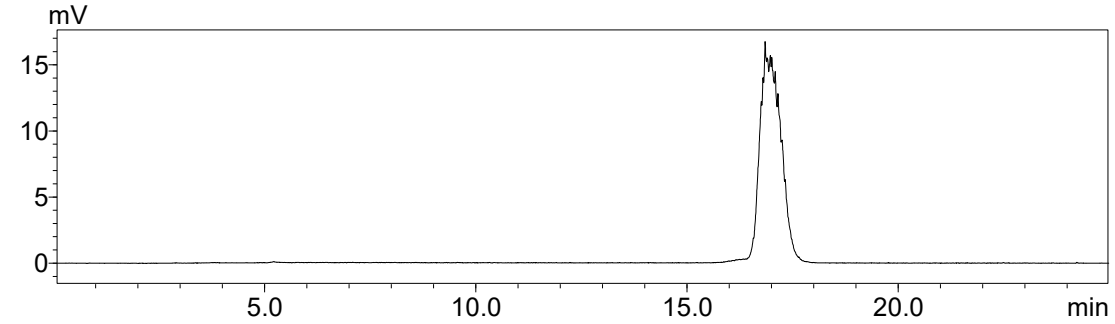


13

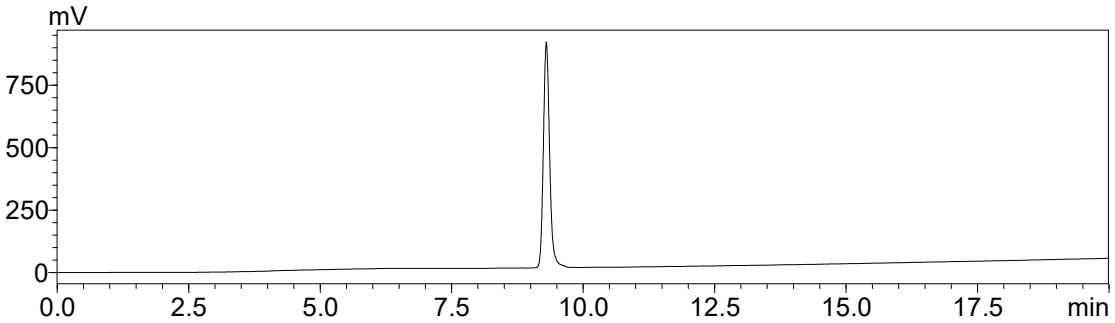
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.7 Hz, 1H), 5.09 (s, 1H), 5.02 (t, J = 1.9 Hz, 1H), 4.71 (d, J = 6.9 Hz, 1H), 4.66 – 4.55 (m, 3H), 4.52 – 4.43 (m, 3H), 4.27 (dd, J = 3.3, 1.6 Hz, 1H), 4.19 (d, J = 3.1 Hz, 1H), 4.16 (dd, J = 3.5, 1.7 Hz, 1H), 4.08 (t, J = 9.8 Hz, 1H), 4.03 – 3.45 (m, 55H), 3.41 (t, J = 8.8 Hz, 1H), 3.28 (t, J = 9.3 Hz, 1H), 2.68 (dd, J = 12.4, 4.6 Hz, 1H), 2.12 – 2.00 (m, 15H), 1.73 (t, J = 12.1 Hz, 1H).

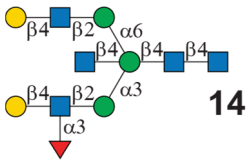
HRMS (ESI) m/z calcd. for C81H134N6O59 2134.7670; found [M-H]- 2133.7514.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



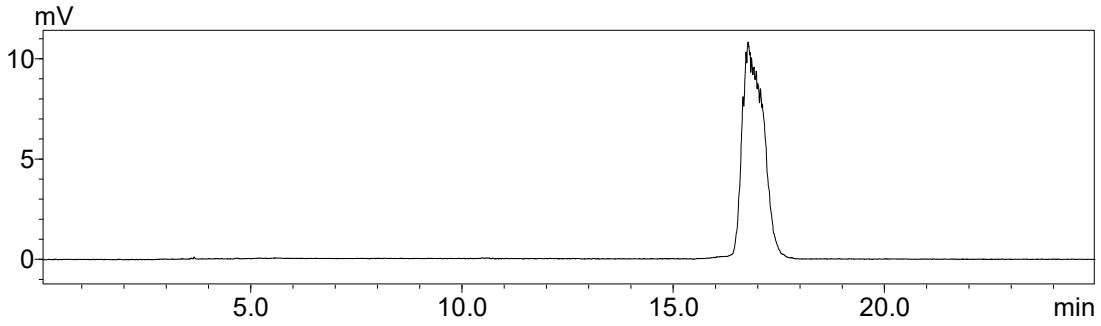


14

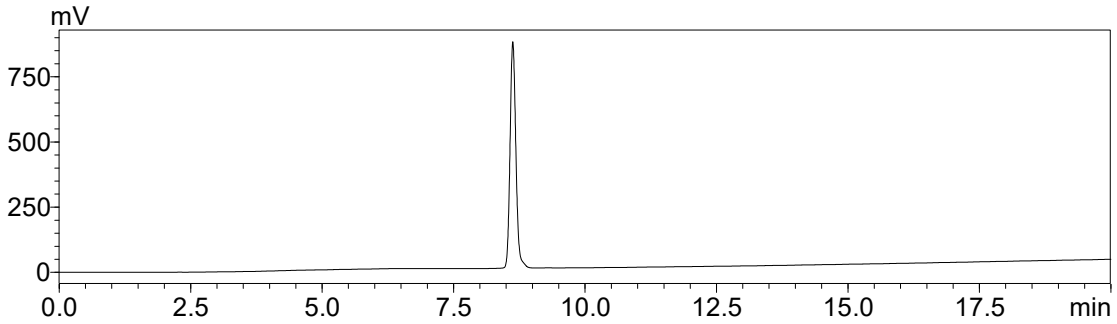
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (s, 1H), 5.15 (d, J = 3.9 Hz, 1H), 5.03 (d, J = 12.7 Hz, 3H), 4.68 (s, 3H), 4.64 – 4.56 (m, 4H), 4.47 (q, J = 7.5 Hz, 4H), 4.26 (d, J = 3.4 Hz, 2H), 4.18 (d, J = 3.1 Hz, 1H), 4.15 (d, J = 3.4 Hz, 1H), 4.08 (t, J = 9.8 Hz, 2H), 4.02 – 3.43 (m, 59H), 3.41 (t, J = 8.9 Hz, 1H), 3.25 (t, J = 9.3 Hz, 2H), 2.18 – 1.96 (m, 15H), 1.17 (d, J = 6.5 Hz, 4H).

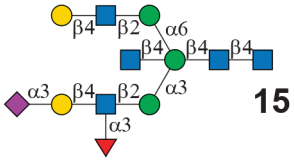
HRMS (ESI) m/z calcd. for C76H127N5O55 1989.7295; found [M+2H]2+ 995.8733

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



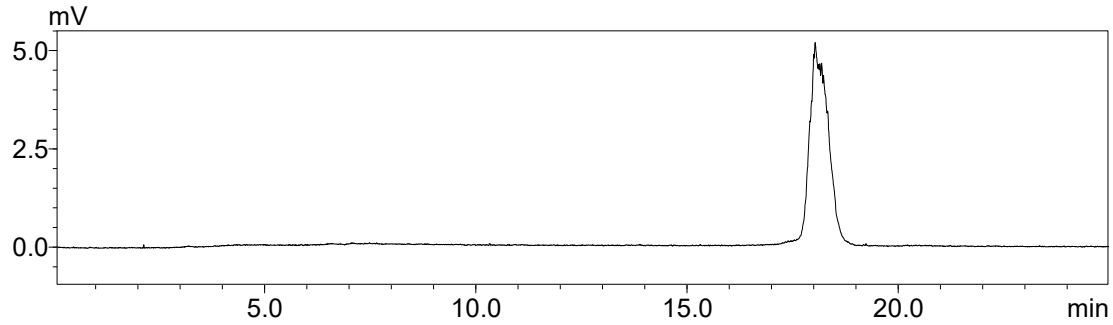


15

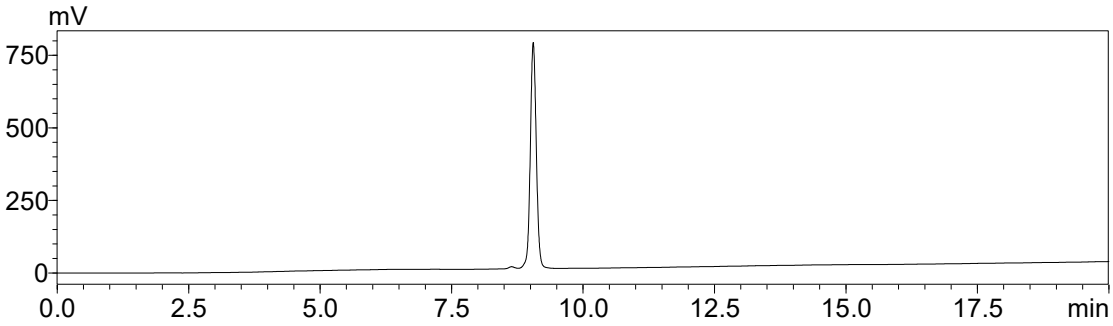
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 2.5 Hz, 1H), 5.15 (d, J = 3.9 Hz, 1H), 5.05 (s, 1H), 5.02 (s, 2H), 4.69 (s, 2H), 4.64 – 4.55 (m, 4H), 4.53 (d, J = 7.8 Hz, 1H), 4.48 (dd, J = 11.3, 8.0 Hz, 2H), 4.26 (d, J = 3.4 Hz, 1H), 4.18 (d, J = 3.2 Hz, 1H), 4.15 (d, J = 3.5 Hz, 1H), 4.12 – 4.04 (m, 2H), 4.02 – 3.50 (m, 53H), 3.47 (q, J = 9.7, 9.2 Hz, 2H), 3.42 (t, J = 8.9 Hz, 1H), 3.25 (t, J = 9.3 Hz, 2H), 2.81 – 2.72 (m, 2H), 2.14 – 1.99 (m, 15H), 1.79 (t, J = 12.0 Hz, 2H), 1.17 (d, J = 6.5 Hz, 4H).

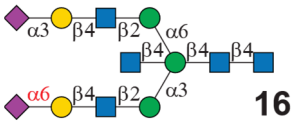
HRMS (ESI) m/z calcd. for C87H144N6O63 2280.8249; found [M-H]- 2279.8147.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



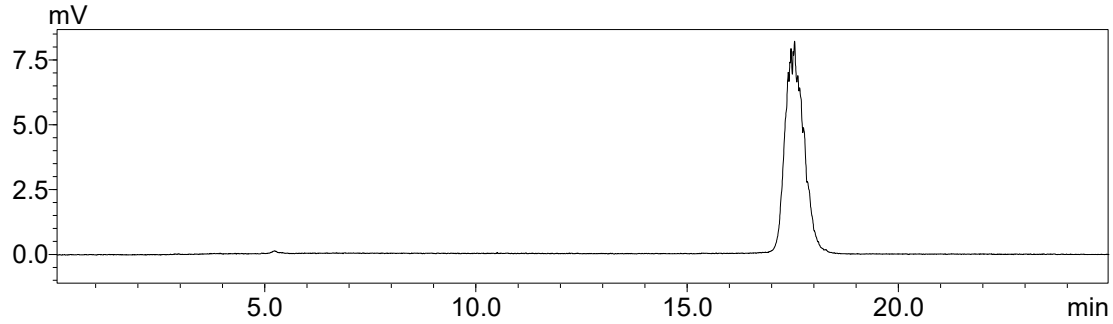


16

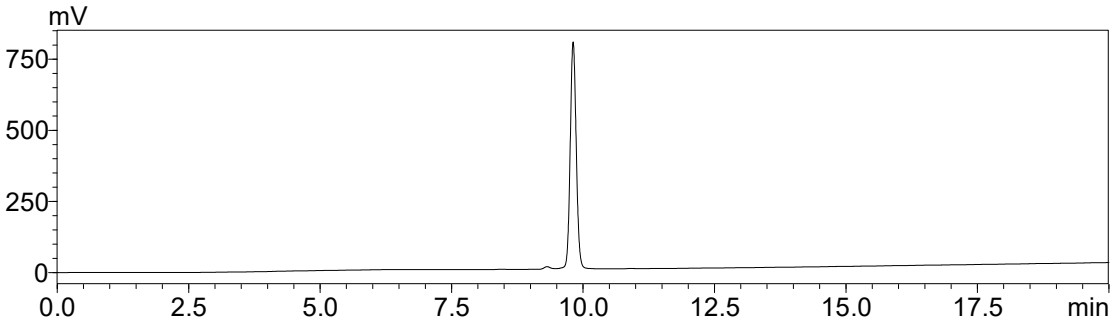
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.6 Hz, 1H), 5.09 (s, 1H), 5.01 (s, 1H), 4.71 (d, J = 7.6 Hz, 1H), 4.66 – 4.59 (m, 2H), 4.57 (dd, J = 8.1, 5.1 Hz, 2H), 4.46 (dd, J = 12.4, 8.1 Hz, 2H), 4.27 (s, 1H), 4.19 (d, J = 3.1 Hz, 1H), 4.17 (s, 1H), 4.14 (d, J = 3.1 Hz, 1H), 4.12 (d, J = 3.2 Hz, 1H), 4.09 (d, J = 9.8 Hz, 1H), 4.03 – 3.44 (m, 50H), 3.41 (t, J = 9.0 Hz, 1H), 3.28 (t, J = 9.3 Hz, 1H), 2.77 (dd, J = 12.4, 4.6 Hz, 1H), 2.68 (dd, J = 12.4, 4.7 Hz, 1H), 2.17 – 1.97 (m, 15H), 1.81 (t, J = 12.1 Hz, 1H), 1.74 (t, J = 12.2 Hz, 1H).

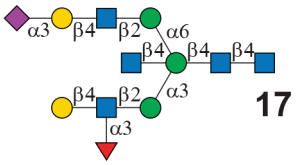
HRMS (ESI) m/z calcd. for C92H151N7O672425.8624; found [M-2H]2- 1211.9152.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



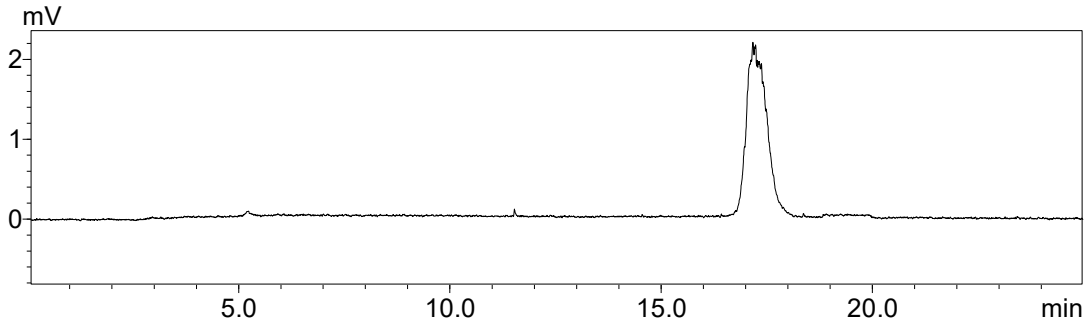


17

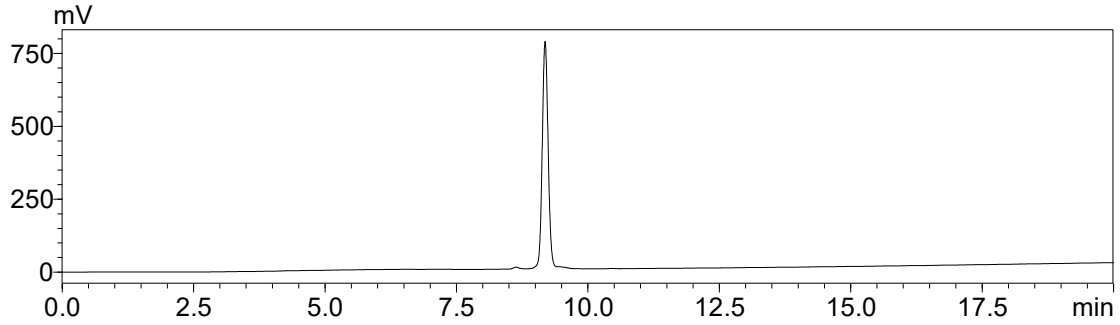
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.5 Hz, 1H), 5.16 (d, J = 4.0 Hz, 1H), 5.05 (s, 1H), 5.02 (s, 1H), 4.70 (d, J = 12.2 Hz, 1H), 4.61 (dd, J = 8.1, 5.3 Hz, 2H), 4.57 (dd, J = 8.1, 5.5 Hz, 2H), 4.47 (dd, J = 8.0, 5.1 Hz, 2H), 4.27 (s, 1H), 4.19 (d, J = 3.1 Hz, 1H), 4.17 (s, 1H), 4.13 (dd, J = 9.9, 3.2 Hz, 1H), 4.09 (d, J = 9.7 Hz, 1H), 4.03 – 3.38 (m, 57H), 3.26 (t, J = 9.3 Hz, 2H), 2.77 (dd, J = 12.4, 4.6 Hz, 1H), 2.18 – 1.96 (m, 16H), 1.81 (t, J = 12.1 Hz, 2H), 1.18 (d, J = 6.6 Hz, 3H).

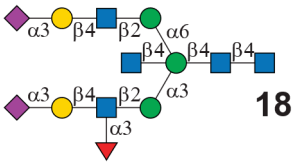
HRMS (ESI) m/z calcd. for C87H144N6O63 2280.8249; found [M-H]- 2279.8148.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



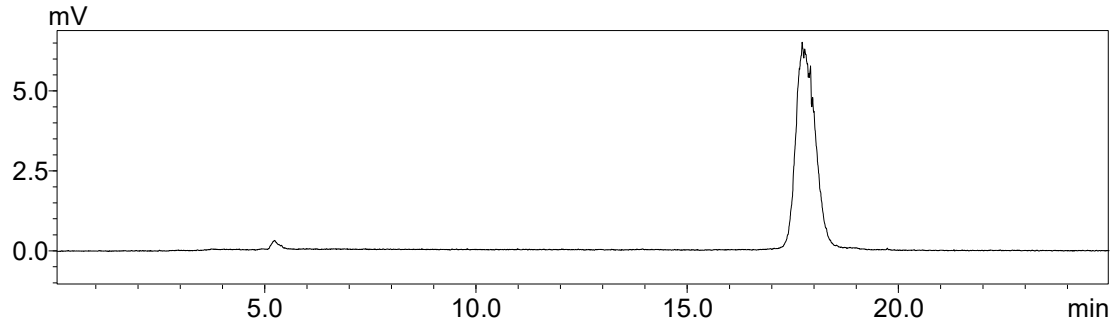


18

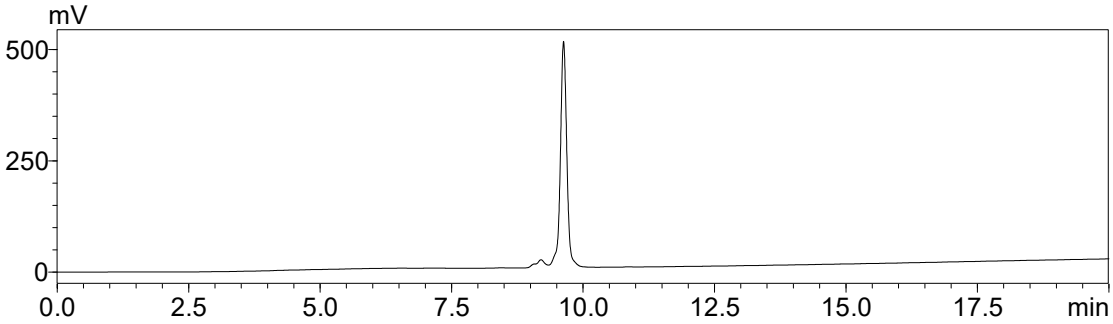
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.6 Hz, 1H), 5.15 (d, J = 4.0 Hz, 1H), 5.05 (s, 1H), 5.01 (s, 1H), 4.70 (d, J = 11.6 Hz, 1H), 4.64 – 4.55 (m, 3H), 4.53 (d, J = 7.8 Hz, 1H), 4.47 (d, J = 8.3 Hz, 1H), 4.26 (s, 1H), 4.20 – 4.15 (m, 2H), 4.15 – 4.05 (m, 3H), 4.04 – 3.44 (m, 47H), 3.42 (t, J = 9.0 Hz, 1H), 3.26 (t, J = 9.3 Hz, 1H), 2.82 – 2.72 (m, 2H), 2.18 – 1.91 (m, 15H), 1.87 – 1.75 (m, 3H), 1.33 (d, J = 6.9 Hz, 2H), 1.17 (d, J = 6.6 Hz, 3H).

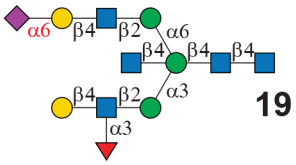
HRMS (ESI) m/z calcd. for C98H161N7O71 2571.9203; found [M-2H]2- 1284.9484.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



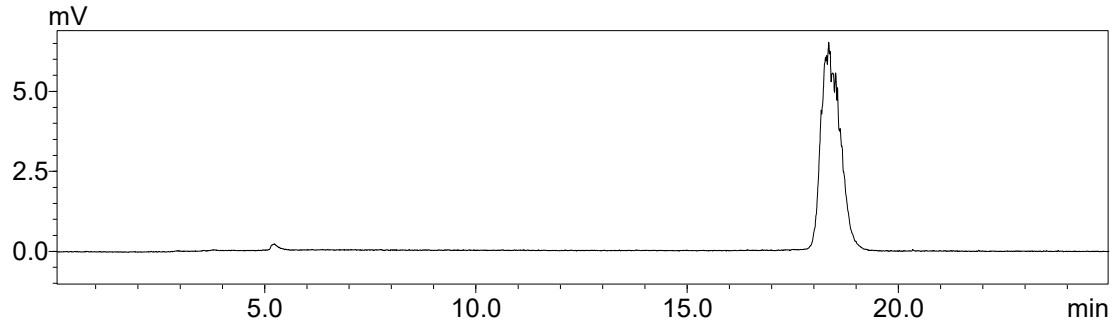


19

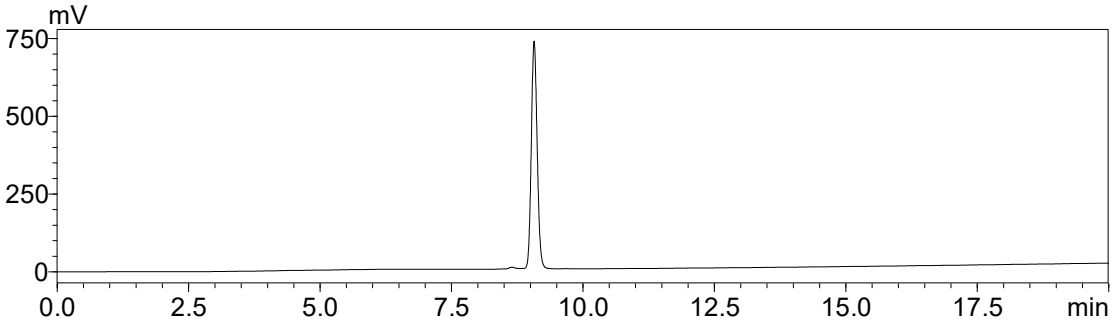
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.7 Hz, 1H), 5.16 (d, J = 4.0 Hz, 1H), 5.04 (s, 2H), 4.85 (d, J = 6.9 Hz, 1H), 4.70 (d, J = 5.8 Hz, 1H), 4.61 (t, J = 8.1 Hz, 3H), 4.51 – 4.42 (m, 3H), 4.27 (s, 1H), 4.19 (d, J = 3.0 Hz, 1H), 4.16 (s, 1H), 4.07 (t, J = 9.7 Hz, 1H), 4.03 – 3.40 (m, 58H), 3.26 (t, J = 9.3 Hz, 1H), 2.69 (dd, J = 12.4, 4.7 Hz, 1H), 2.19 – 1.98 (m, 16H), 1.72 (t, J = 12.2 Hz, 2H), 1.18 (d, J = 6.6 Hz, 3H).

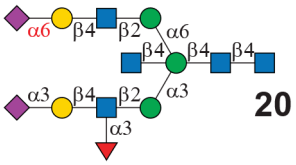
HRMS (ESI) m/z calcd. for C93H154N6O67 2280.8249; found [M-H]- 2279.8142.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



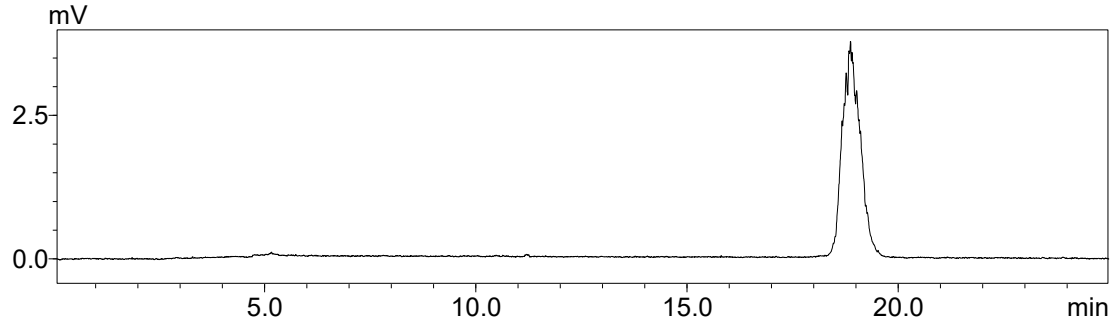


20

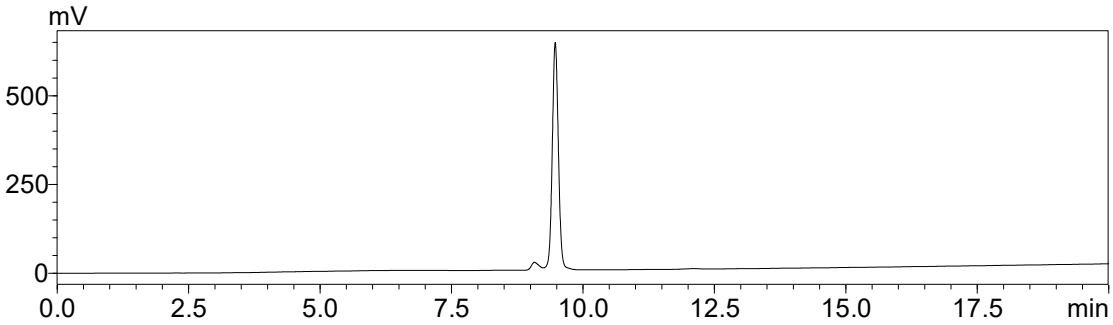
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.7 Hz, 1H), 5.15 (d, J = 4.0 Hz, 1H), 5.05 (d, J = 6.1 Hz, 2H), 4.71 (s, 1H), 4.64 – 4.57 (m, 3H), 4.53 (d, J = 7.7 Hz, 1H), 4.47 (t, J = 7.7 Hz, 2H), 4.26 (s, 1H), 4.19 (d, J = 3.0 Hz, 1H), 4.16 (s, 1H), 4.11 (d, J = 3.0 Hz, 1H), 4.10 – 4.08 (m, 1H), 4.05 (d, J = 9.7 Hz, 1H), 4.03 – 3.40 (m, 52H), 3.26 (t, J = 9.3 Hz, 2H), 2.77 (dd, J = 12.3, 4.6 Hz, 2H), 2.69 (dd, J = 12.4, 4.7 Hz, 2H), 2.16 – 1.96 (m, 15H), 1.80 (t, J = 12.2 Hz, 2H), 1.72 (t, J = 12.2 Hz, 2H), 1.17 (d, J = 6.6 Hz, 3H).

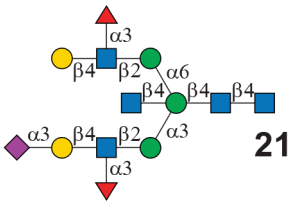
HRMS (ESI) m/z calcd. for C98H161N7O71 2571.9203; found [M-2H]2- 1284.9439.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



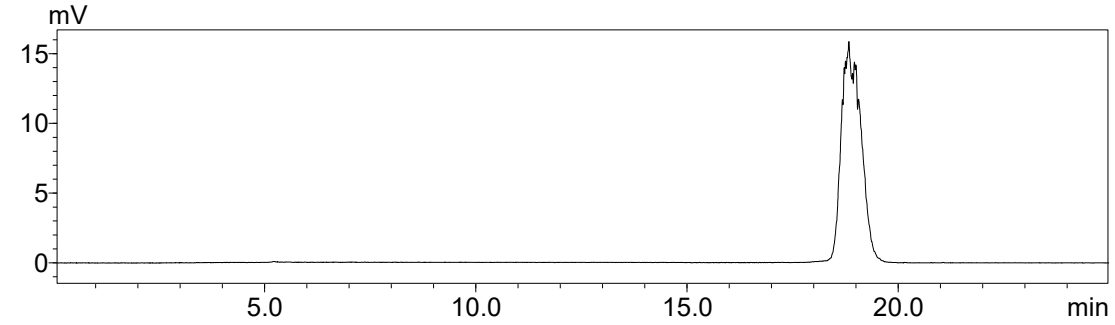


21

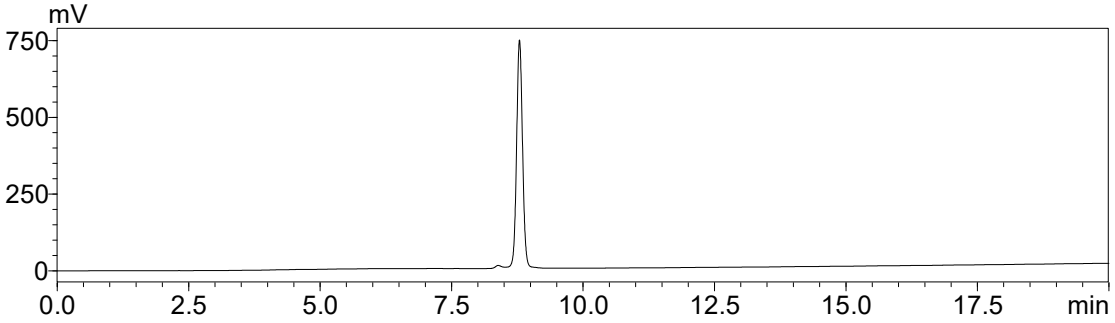
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.8 Hz, 1H), 5.15 (d, J = 4.1 Hz, 2H), 5.05 (d, J = 5.6 Hz, 2H), 4.71 (d, J = 5.0 Hz, 1H), 4.64 – 4.57 (m, 3H), 4.53 (d, J = 7.8 Hz, 1H), 4.48 (t, J = 7.6 Hz, 2H), 4.26 (d, J = 3.1 Hz, 1H), 4.19 (d, J = 3.1 Hz, 1H), 4.14 (d, J = 3.1 Hz, 1H), 4.10 (dd, J = 9.8, 3.1 Hz, 1H), 4.06 (t, J = 9.8 Hz, 1H), 4.02 – 3.39 (m, 57H), 3.26 (t, J = 9.3 Hz, 1H), 2.77 (dd, J = 12.5, 4.6 Hz, 1H), 2.17 – 1.98 (m, 15H), 1.80 (t, J = 12.1 Hz, 2H), 1.19 (dd, J = 19.4, 6.6 Hz, 5H).

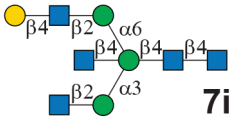
HRMS (ESI) m/z calcd. for C93H154N6O67 2426.8828; found [M-H]- 2425.8742.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)





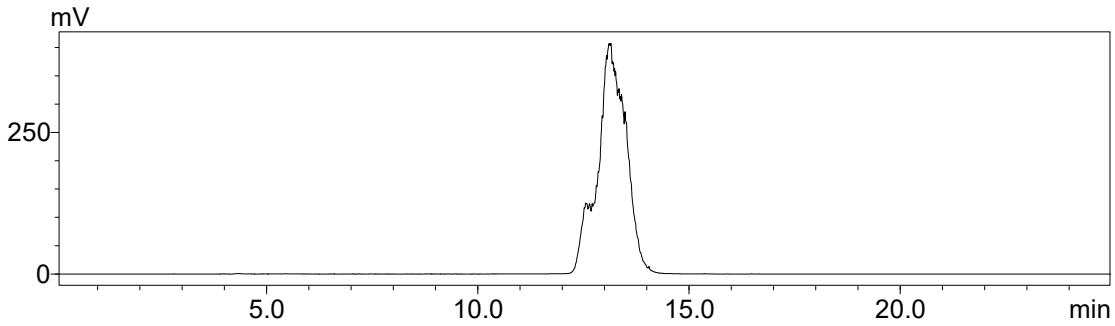
7i

1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 2.5 Hz, 1H), 5.06 (d, J = 1.7 Hz, 1H), 5.02 (d, J = 1.9 Hz, 1H), 4.69 (d, J = 10.2 Hz, 1H), 4.64 – 4.54 (m, 3H), 4.47 (t, J = 8.3 Hz, 2H), 4.25 (dd, J = 3.4, 1.7 Hz, 1H), 4.17 (d, J = 3.2 Hz, 1H), 4.14 (dt, J = 3.3, 1.5 Hz, 1H), 4.11 – 4.05 (m, 1H), 4.01 – 3.44 (m, 49H), 3.40 (ddd, J = 9.9, 7.6, 2.1 Hz, 1H), 3.27 (dd, J = 9.8, 8.8 Hz, 1H), 2.16 – 1.96 (m, 15H).

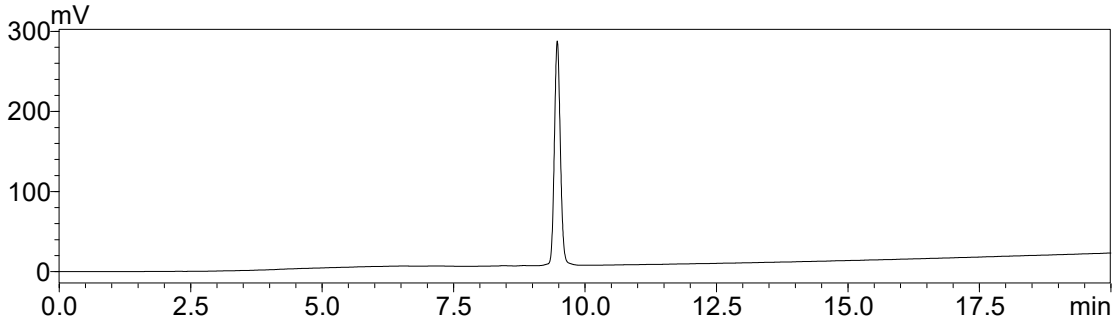
13C NMR (150 MHz, D2O) δ 174.75, 174.67, 174.61, 174.57, 174.46, 174.40, 174.23, 102.94, 101.39, 101.22, 100.53, 100.02, 99.90, 99.58, 99.52, 99.33, 97.70, 97.61, 94.77, 90.40, 79.63, 79.15, 78.60, 78.50, 78.24, 76.72, 76.44, 76.21, 75.78, 75.31, 74.54, 74.33, 73.49, 73.31, 72.95, 72.49, 72.20, 72.00, 71.38, 71.08, 70.95, 70.65, 70.47, 70.28, 69.93, 69.73, 69.44, 69.29, 69.21, 68.48, 67.54, 67.32, 65.18, 61.90, 61.83, 61.63, 60.98, 60.58, 60.37, 59.95, 59.87, 56.09, 55.27, 55.20, 54.73, 53.60, 22.40, 22.36, 22.26, 22.16, 22.12, 22.10, 21.87, 21.85.

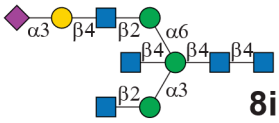
HRMS (ESI) m/z calcd. for C64H107N5O46 1681.6187; found [M+2H]2+ 841.8186.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



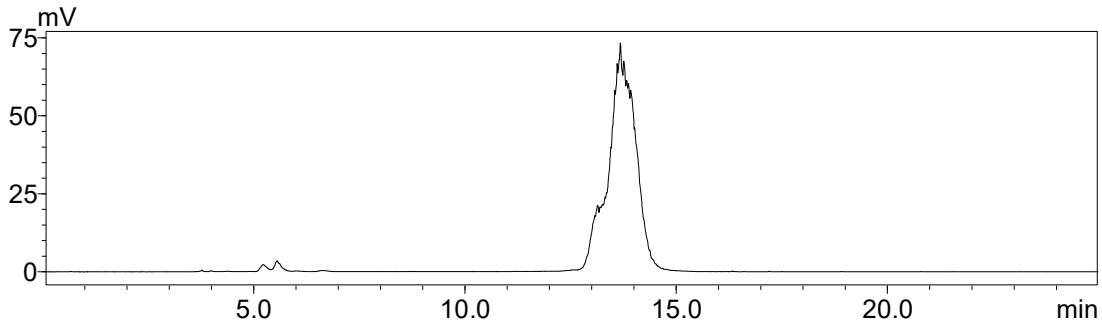


8i

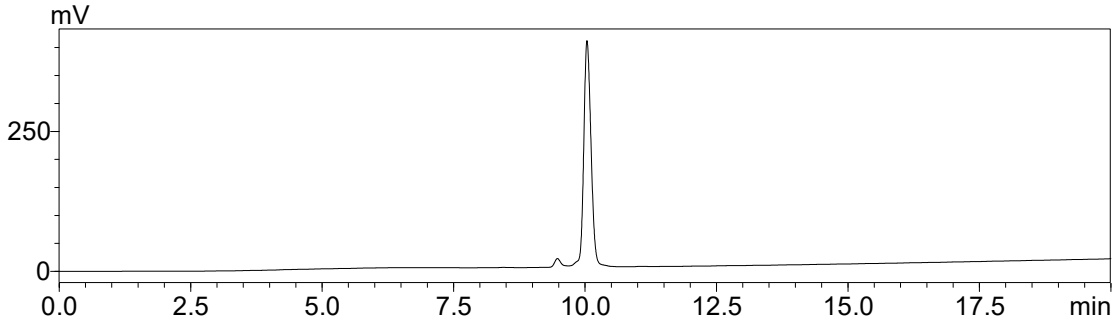
1H NMR (600 MHz, Deuterium Oxide) δ 5.39 (d, J = 2.3 Hz, 1H), 5.25 (s, 1H), 5.19 (s, 1H), 4.89 (s, 2H), 4.66 (d, J = 8.2 Hz, 1H), 4.44 (d, J = 2.9 Hz, 1H), 4.37 (d, J = 3.2 Hz, 1H), 4.34 (s, 1H), 4.33 – 4.26 (m, 2H), 4.22 – 3.63 (m, 45H), 3.60 (t, J = 8.5 Hz, 1H), 3.47 (t, J = 9.3 Hz, 1H), 2.96 (dd, J = 12.4, 4.6 Hz, 1H), 2.35 – 2.16 (m, 15H), 1.99 (t, J = 12.1 Hz, 1H).

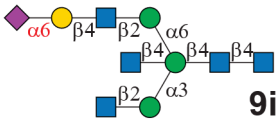
HRMS (ESI) m/z calcd. for C75H124N6O54 1972.7141; found [M-H]- 1971.6948.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



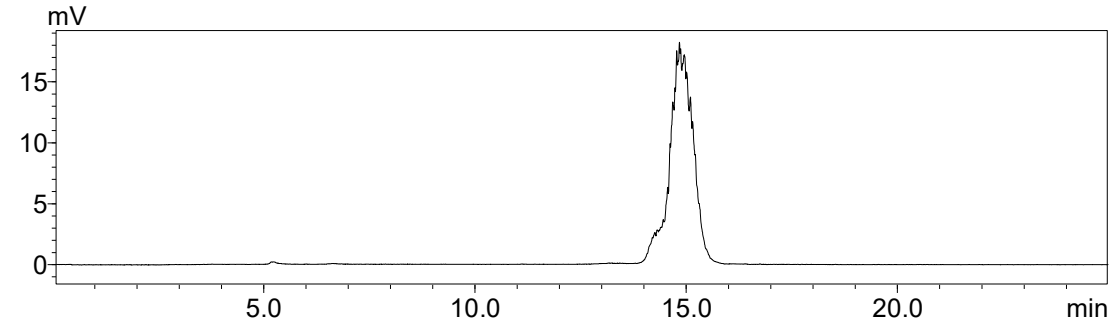


9i

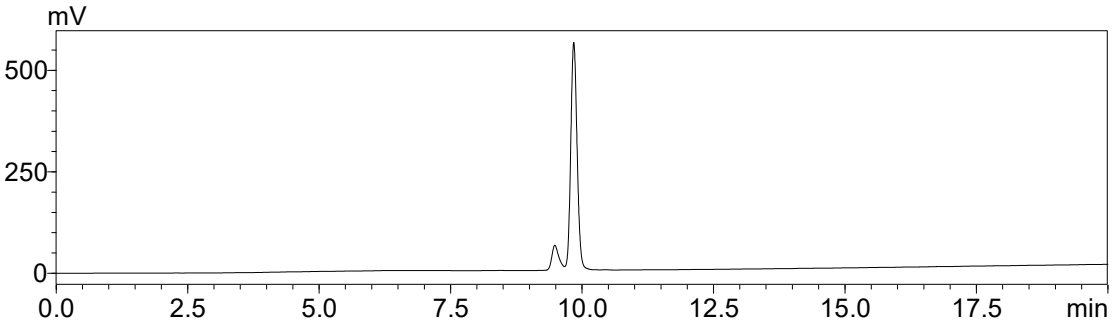
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.6 Hz, 1H), 5.07 (s, 1H), 5.04 (s, 1H), 4.70 (d, J = 7.4 Hz, 1H), 4.61 (t, J = 8.1 Hz, 2H), 4.56 (d, J = 8.4 Hz, 1H), 4.47 (t, J = 8.5 Hz, 2H), 4.26 (d, J = 3.3 Hz, 1H), 4.19 (d, J = 3.1 Hz, 1H), 4.16 (s, 1H), 4.08 (t, J = 10.0 Hz, 1H), 4.03 – 3.36 (m, 49H), 3.27 (t, J = 9.3 Hz, 1H), 2.69 (dd, J = 12.4, 4.7 Hz, 1H), 2.15 – 1.99 (m, 15H), 1.72 (t, J = 12.2 Hz, 1H).

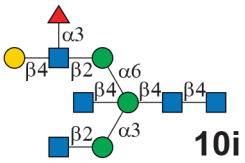
HRMS (ESI) m/z calcd. for C75H124N6O54 1972.7141; found [M-H]- 1971.6982.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



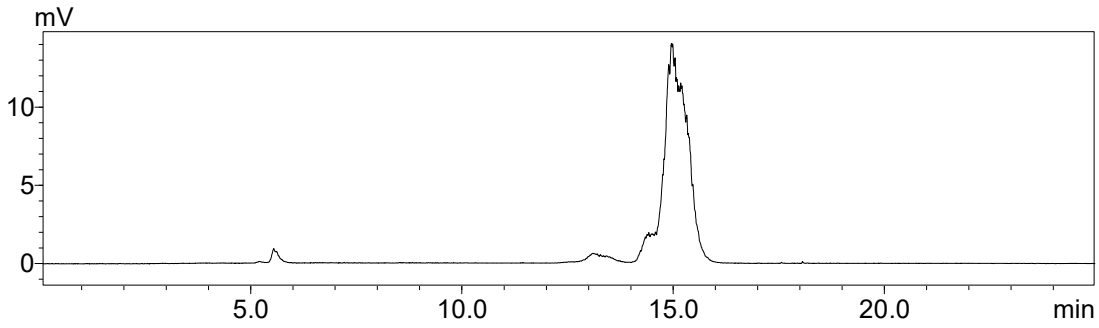


10i

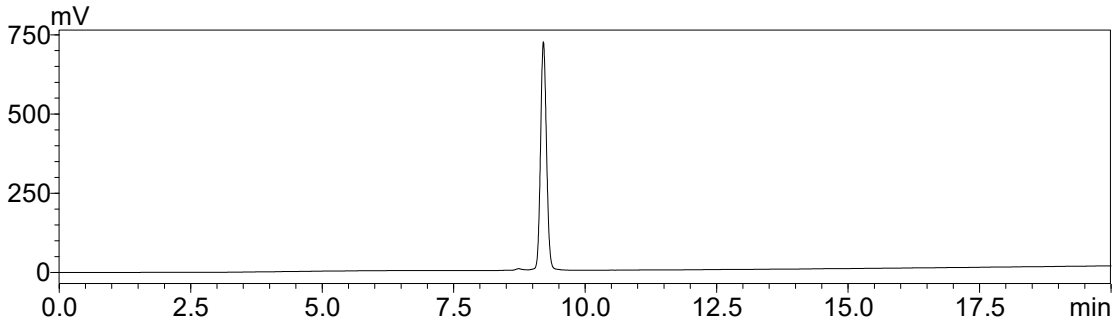
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 2.7 Hz, 1H), 5.14 (d, J = 4.0 Hz, 1H), 5.06 (s, 1H), 5.03 (s, 1H), 4.69 (d, J = 3.7 Hz, 2H), 4.60 (dd, J = 8.3, 5.1 Hz, 2H), 4.55 (d, J = 8.4 Hz, 1H), 4.47 (dd, J = 9.9, 8.0 Hz, 2H), 4.25 (d, J = 3.3 Hz, 1H), 4.18 (d, J = 3.2 Hz, 1H), 4.13 (s, 1H), 4.07 (t, J = 9.9 Hz, 1H), 4.02 – 3.36 (m, 51H), 3.27 (t, J = 9.3 Hz, 2H), 2.17 – 1.95 (m, 15H), 1.19 (d, J = 6.6 Hz, 3H).

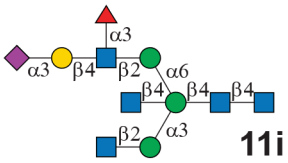
HRMS (ESI) m/z calcd. for C70H117N5O50 1827.6766; found [M+2H]2+ 914.8469.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



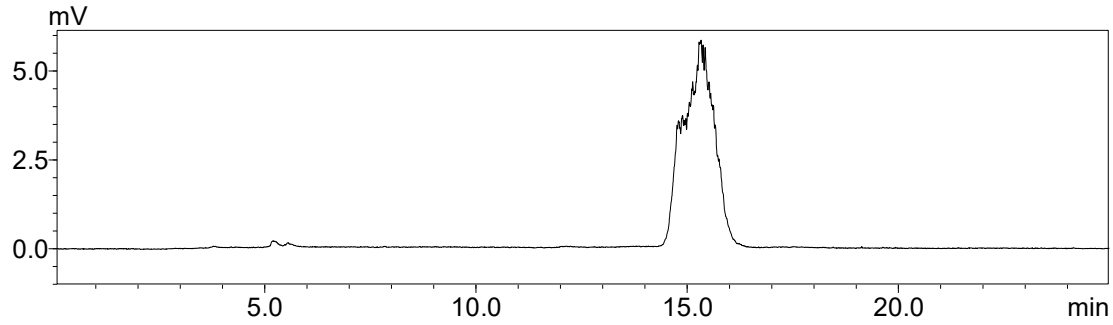


11i

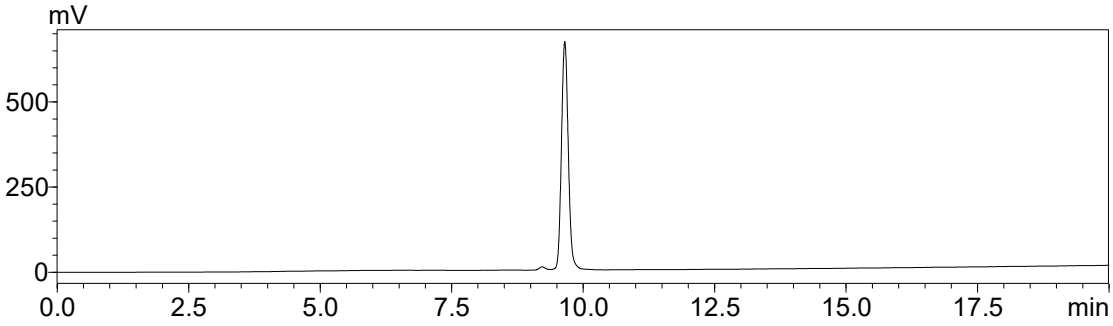
1H NMR (600 MHz, Deuterium Oxide) δ 5.16 (d, J = 2.7 Hz, 1H), 5.11 (d, J = 4.0 Hz, 1H), 5.01 (s, 1H), 4.97 (s, 1H), 4.66 (s, 1H), 4.57 (t, J = 9.2 Hz, 2H), 4.51 (dd, J = 13.3, 8.1 Hz, 2H), 4.43 (d, J = 8.3 Hz, 1H), 4.22 (s, 1H), 4.15 (d, J = 3.1 Hz, 1H), 4.12 (s, 1H), 4.09 – 4.00 (m, 2H), 4.00 – 3.33 (m, 53H), 3.21 (t, J = 9.3 Hz, 1H), 2.73 (dd, J = 12.4, 4.6 Hz, 1H), 2.12 – 1.93 (m, 15H), 1.76 (t, J = 12.1 Hz, 1H), 1.13 (d, J = 6.6 Hz, 3H).

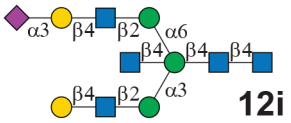
HRMS (ESI) m/z calcd. for C81H134N6O58 2118.7720; found [M-H]- 2117.7585.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



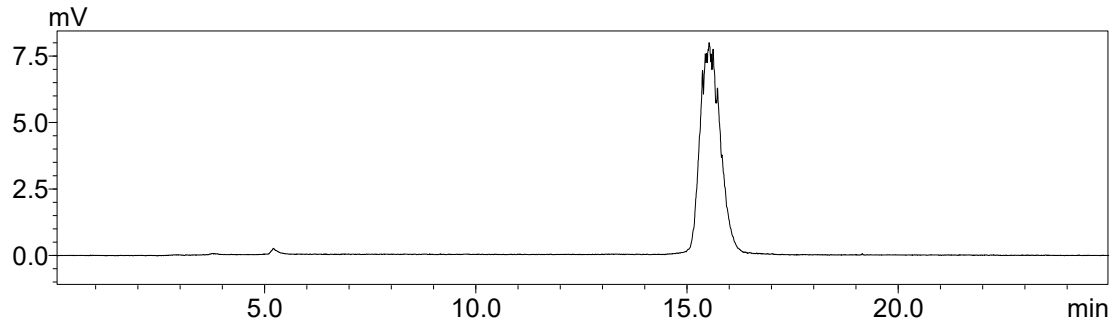


12i

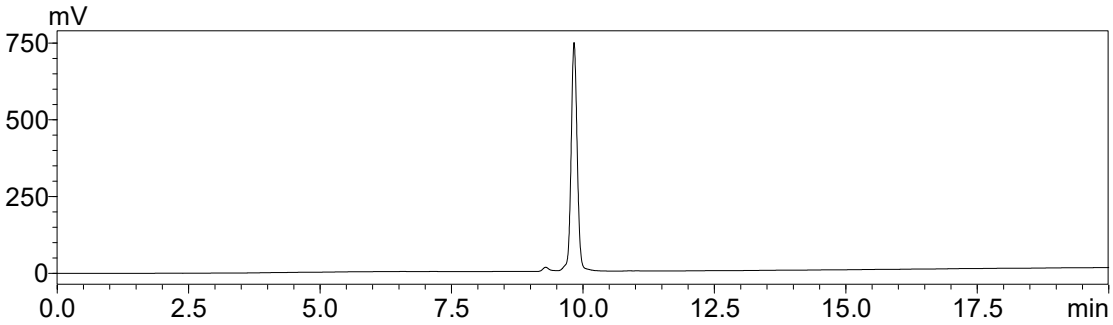
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 2.4 Hz, 1H), 5.06 (s, 1H), 5.03 – 4.97 (m, 1H), 4.69 (d, J = 11.3 Hz, 2H), 4.60 (dd, J = 7.9, 5.1 Hz, 2H), 4.56 (dd, J = 8.1, 5.2 Hz, 2H), 4.46 (dd, J = 8.0, 4.3 Hz, 2H), 4.26 (t, J = 2.5 Hz, 1H), 4.20 – 4.14 (m, 2H), 4.14 – 4.06 (m, 2H), 4.02 – 3.44 (m, 51H), 3.43 – 3.37 (m, 1H), 3.26 (t, J = 9.3 Hz, 1H), 2.76 (dd, J = 12.5, 4.6 Hz, 1H), 2.16 – 1.96 (m, 15H), 1.80 (t, J = 12.1 Hz, 1H).

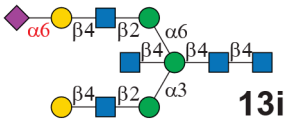
HRMS (ESI) m/z calcd. for C81H134N6O59 2134.7670; found [M-H]- 2133.7560.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



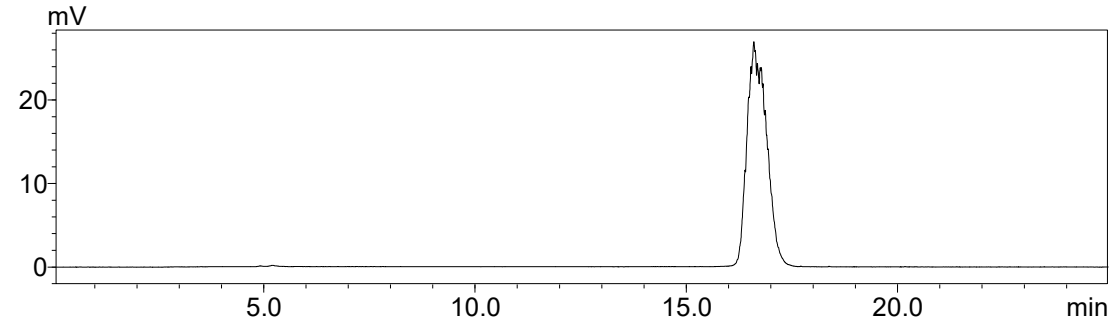


13i

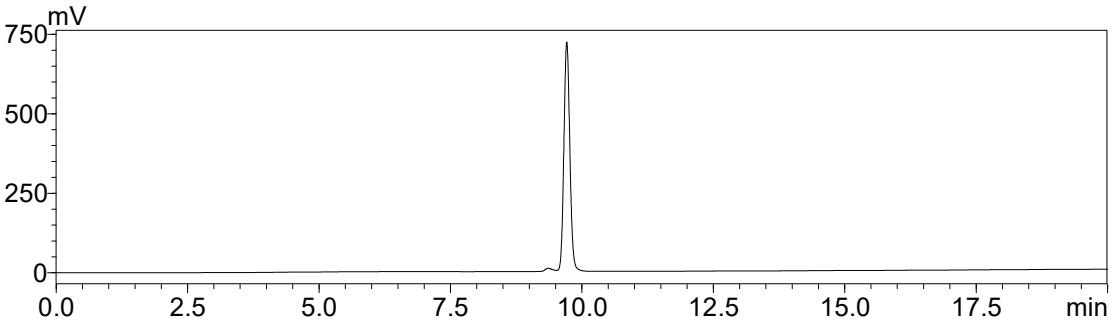
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 2.5 Hz, 1H), 5.06 (d, J = 1.7 Hz, 1H), 5.03 (t, J = 2.1 Hz, 1H), 4.69 (d, J = 6.3 Hz, 1H), 4.64 – 4.56 (m, 3H), 4.46 (dd, J = 10.0, 7.8 Hz, 3H), 4.26 (dd, J = 3.4, 1.7 Hz, 1H), 4.18 (d, J = 3.1 Hz, 1H), 4.15 (dd, J = 3.4, 1.6 Hz, 1H), 4.09 – 4.03 (m, 1H), 4.03 – 3.45 (m, 52H), 3.42 (ddd, J = 9.9, 7.7, 2.1 Hz, 1H), 3.26 (t, J = 9.3 Hz, 1H), 2.68 (dd, J = 12.4, 4.6 Hz, 1H), 2.14 – 1.96 (m, 15H), 1.71 (t, J = 12.2 Hz, 2H).

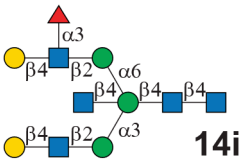
HRMS (ESI) m/z calcd. for C81H134N6O59 2134.7670; found [M-H]- 2133.7567.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



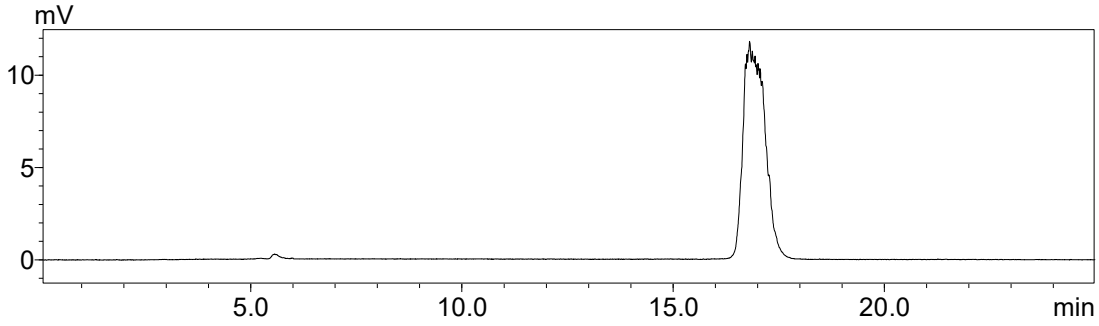


14i

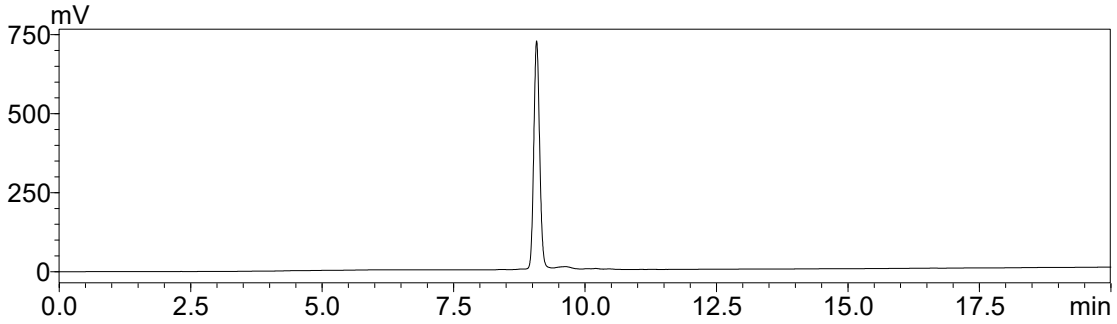
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 2.7 Hz, 1H), 5.14 (d, J = 4.1 Hz, 1H), 5.06 (s, 1H), 5.03 (s, 1H), 4.70 (d, J = 4.9 Hz, 2H), 4.60 (dd, J = 8.2, 5.1 Hz, 3H), 4.50 – 4.43 (m, 3H), 4.26 (s, 1H), 4.18 (d, J = 3.1 Hz, 1H), 4.13 (s, 1H), 4.07 (t, J = 9.8 Hz, 1H), 4.02 – 3.44 (m, 63H), 3.44 – 3.37 (m, 1H), 3.26 (t, J = 9.3 Hz, 1H), 2.17 – 1.94 (m, 15H), 1.19 (d, J = 6.6 Hz, 3H).

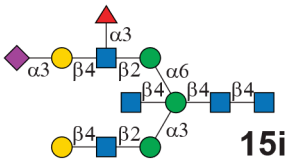
HRMS (ESI) m/z calcd. for C76H127N5O55 1989.7295; found [M+2H]2+ 995.8722.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



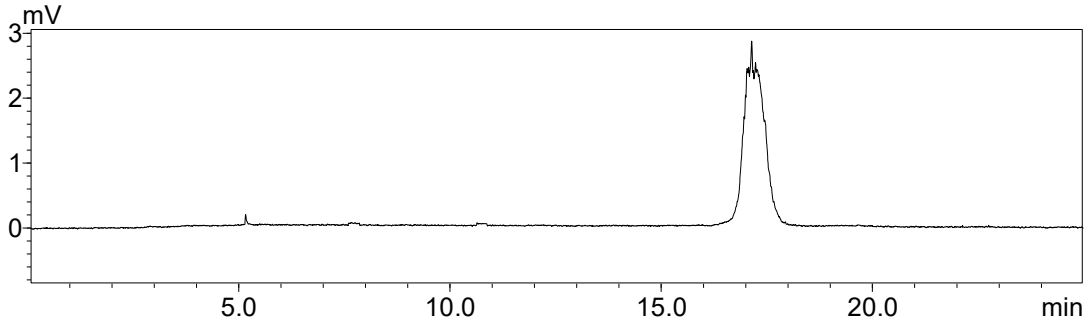


15i

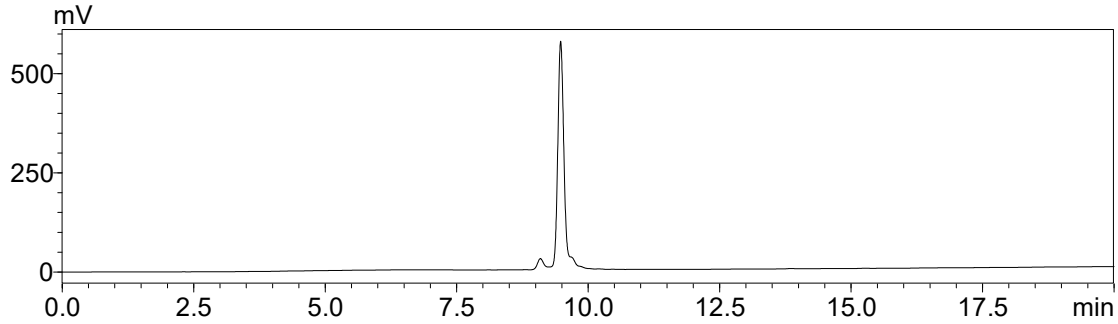
1H NMR (600 MHz, Deuterium Oxide) δ 5.18 (d, J = 2.4 Hz, 1H), 5.13 (d, J = 3.9 Hz, 1H), 5.05 (s, 1H), 5.02 (s, 1H), 4.69 (s, 2H), 4.58 (dt, J = 12.5, 6.7 Hz, 3H), 4.52 (d, J = 7.7 Hz, 1H), 4.46 (d, J = 8.0 Hz, 2H), 4.26 (d, J = 3.5 Hz, 1H), 4.18 (d, J = 2.9 Hz, 1H), 4.13 (d, J = 3.6 Hz, 1H), 4.07 (q, J = 10.9, 8.1 Hz, 2H), 4.02 – 3.36 (m, 55H), 3.25 (t, J = 9.3 Hz, 1H), 2.76 (d, J = 12.0 Hz, 1H), 2.15 – 1.94 (m, 15H), 1.79 (t, J = 12.0 Hz, 2H), 1.18 (d, J = 6.5 Hz, 3H).

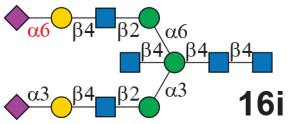
HRMS (ESI) m/z calcd. for C87H144N6O63 2280.8249; found [M-H]- 2279.8140.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



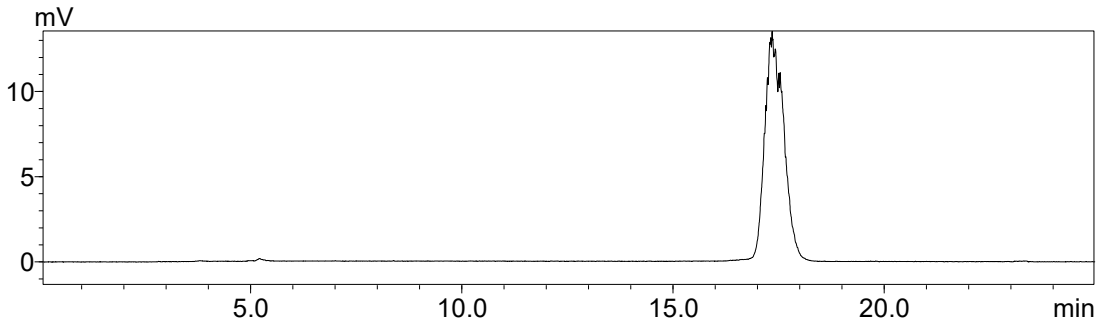


16i

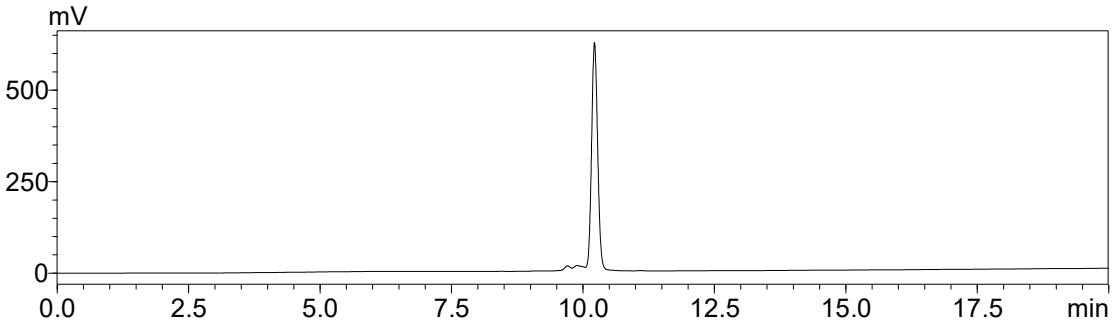
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 2.6 Hz, 1H), 5.06 (s, 1H), 5.03 (s, 1H), 4.69 (d, J = 6.8 Hz, 1H), 4.63 – 4.57 (m, 2H), 4.55 (d, J = 7.8 Hz, 1H), 4.46 (t, J = 7.5 Hz, 2H), 4.25 (s, 1H), 4.18 (d, J = 3.1 Hz, 1H), 4.15 (s, 1H), 4.11 (dd, J = 9.9, 3.1 Hz, 1H), 4.06 (t, J = 9.8 Hz, 1H), 4.03 – 3.45 (m, 47H), 3.42 (t, J = 8.9 Hz, 1H), 3.26 (t, J = 9.3 Hz, 1H), 2.80 – 2.72 (m, 1H), 2.68 (dd, J = 12.4, 4.6 Hz, 1H), 2.15 – 1.95 (m, 14H), 1.79 (t, J = 12.1 Hz, 1H), 1.71 (t, J = 12.2 Hz, 1H).

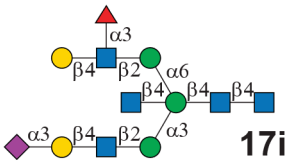
HRMS (ESI) m/z calcd. for C92H151N7O67 2425.8624; found [M-2H]2- 1211.9152.

HLC



HPLC (AEAB-labeled N-glycan with PGC column)



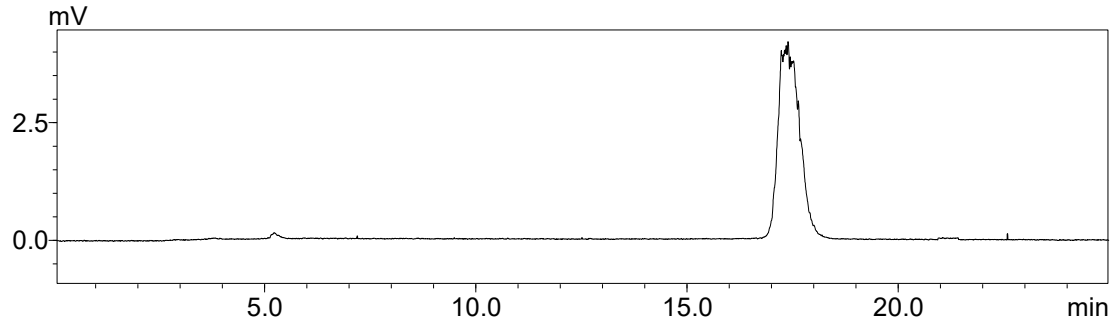


17i

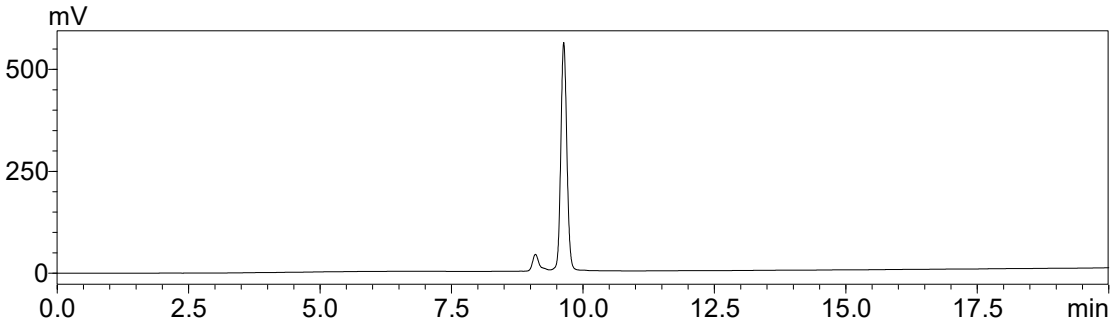
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 2.8 Hz, 1H), 5.14 (d, J = 4.1 Hz, 1H), 5.06 (s, 1H), 5.03 (s, 1H), 4.70 (s, 1H), 4.63 – 4.57 (m, 2H), 4.55 (d, J = 7.8 Hz, 1H), 4.47 (t, J = 7.3 Hz, 2H), 4.25 (s, 1H), 4.18 (d, J = 3.1 Hz, 1H), 4.12 (d, J = 3.9 Hz, 1H), 4.11 (d, J = 3.1 Hz, 1H), 4.06 (t, J = 9.7 Hz, 1H), 4.02 – 3.45 (m, 50H), 3.42 (t, J = 9.0 Hz, 2H), 3.26 (t, J = 9.3 Hz, 2H), 2.75 (dd, J = 12.4, 4.7 Hz, 1H), 2.17 – 1.96 (m, 14H), 1.79 (t, J = 12.1 Hz, 2H), 1.19 (d, J = 6.6 Hz, 3H).

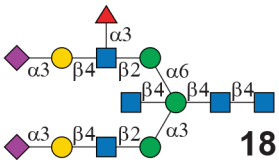
HRMS (ESI) m/z calcd. for C87H144N6O63 2280.8249; found [M-H]- 2279.8150.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



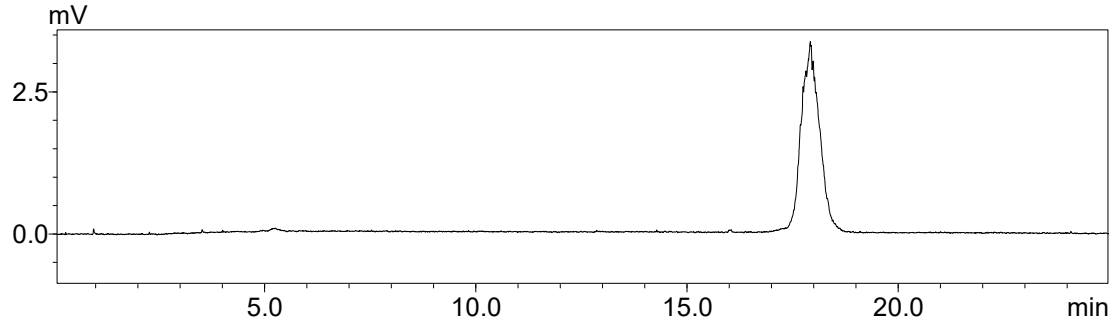


18i

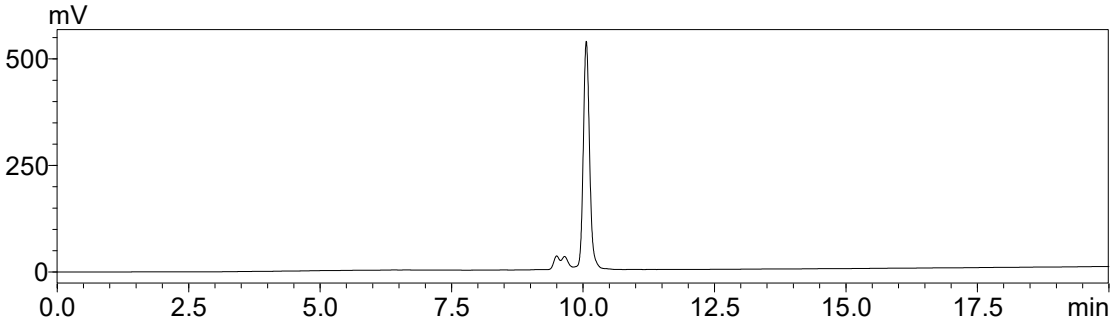
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 2.7 Hz, 1H), 5.13 (d, J = 4.0 Hz, 1H), 5.06 (s, 1H), 5.03 (s, 1H), 4.70 (d, J = 6.2 Hz, 1H), 4.61 – 4.55 (m, 3H), 4.55 – 4.51 (m, 1H), 4.47 (d, J = 8.3 Hz, 1H), 4.25 (s, 1H), 4.18 (s, 1H), 4.14 (s, 1H), 4.12 (d, J = 2.9 Hz, 1H), 4.10 (dd, J = 6.3, 3.2 Hz, 1H), 4.09 – 4.03 (m, 2H), 4.03 – 3.37 (m, 53H), 3.26 (t, J = 9.3 Hz, 2H), 2.80 – 2.72 (m, 2H), 2.15 – 1.98 (m, 15H), 1.80 (td, J = 12.1, 3.9 Hz, 2H), 1.32 (d, J = 6.8 Hz, 1H), 1.19 (d, J = 6.5 Hz, 3H).

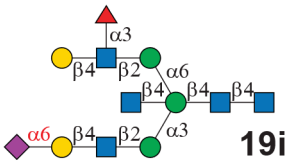
HRMS (ESI) m/z calcd. for C98H161N7O712571.9203; found [M-2H]2- 1284.9440.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



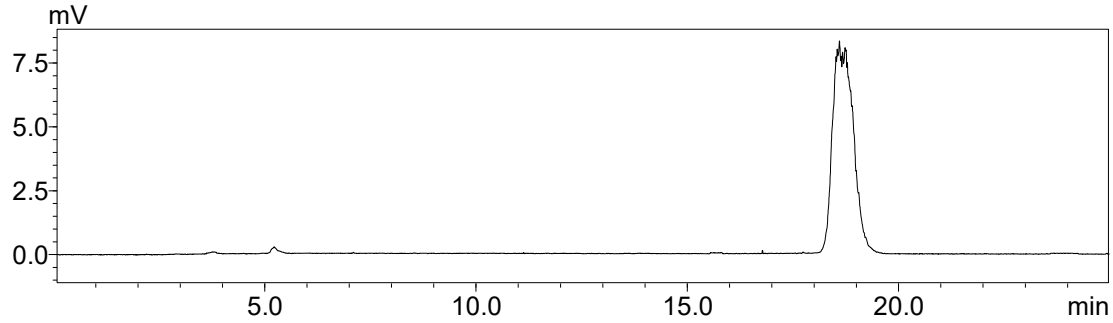


19i

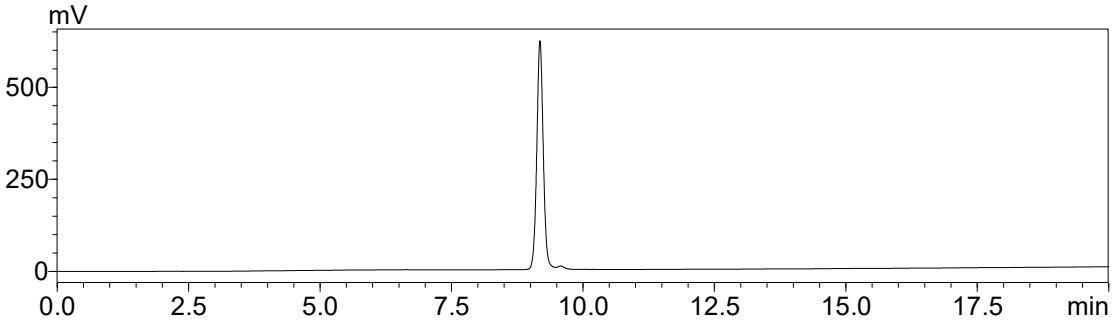
1H NMR (600 MHz, Deuterium Oxide) δ 5.19 (d, J = 3.0 Hz, 1H), 5.14 (d, J = 4.0 Hz, 1H), 5.08 (s, 1H), 5.03 (s, 1H), 4.70 (d, J = 7.3 Hz, 1H), 4.64 – 4.55 (m, 3H), 4.46 (dd, J = 18.7, 9.2 Hz, 3H), 4.26 (s, 1H), 4.19 (d, J = 3.0 Hz, 1H), 4.13 (s, 1H), 4.05 (t, J = 9.7 Hz, 1H), 4.02 – 3.44 (m, 52H), 3.41 (t, J = 8.9 Hz, 1H), 3.27 (t, J = 9.3 Hz, 1H), 2.67 (dd, J = 12.4, 4.7 Hz, 1H), 2.17 – 1.95 (m, 15H), 1.73 (t, J = 12.2 Hz, 2H), 1.19 (d, J = 6.6 Hz, 3H).

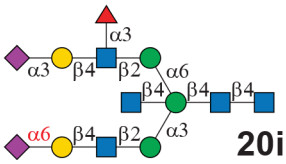
HRMS (ESI) m/z calcd. for C87H144N6O63 2280.8249; found [M-H]- 2279.8154.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)



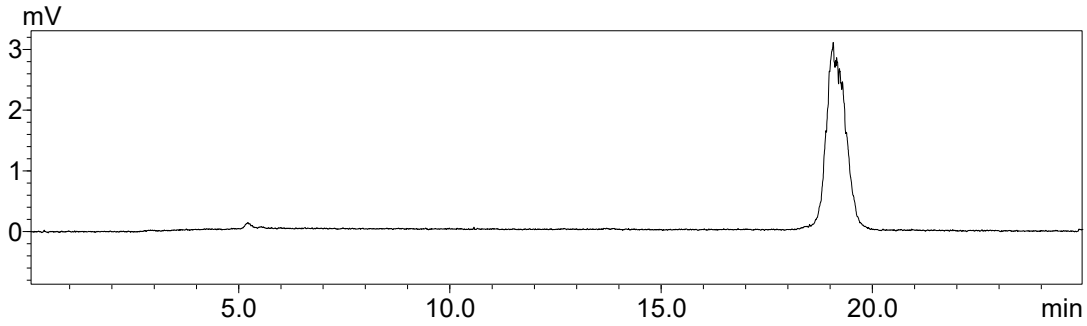


20i

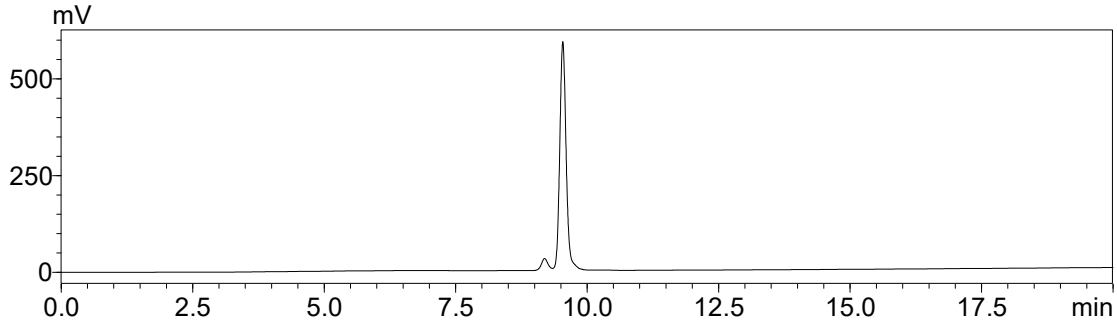
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (d, J = 2.9 Hz, 1H), 5.14 (d, J = 4.0 Hz, 1H), 5.09 (s, 1H), 5.04 (s, 1H), 4.70 (d, J = 5.3 Hz, 1H), 4.61 (dt, J = 14.3, 8.7 Hz, 3H), 4.54 (d, J = 7.8 Hz, 1H), 4.49 (d, J = 8.3 Hz, 1H), 4.45 (d, J = 7.9 Hz, 1H), 4.27 (s, 1H), 4.20 (d, J = 3.0 Hz, 1H), 4.15 (s, 1H), 4.12 – 4.05 (m, 2H), 4.04 – 3.39 (m, 52H), 3.28 (t, J = 9.3 Hz, 2H), 2.77 (dd, J = 12.4, 4.6 Hz, 1H), 2.68 (dd, J = 12.4, 4.7 Hz, 1H), 2.16 – 1.95 (m, 15H), 1.81 (t, J = 12.2 Hz, 2H), 1.74 (t, J = 12.2 Hz, 2H), 1.33 (d, J = 6.9 Hz, 1H), 1.20 (d, J = 6.6 Hz, 3H).

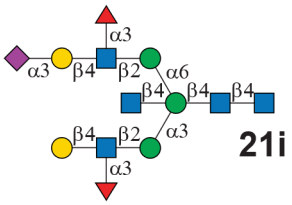
HRMS (ESI) m/z calcd. for C98H161N7O71 2571.9203; found [M-2H]2- 1284.9448.

HPLC (free N-glycan with HILIC column)



HPLC (AEAB-labeled N-glycan with PGC column)

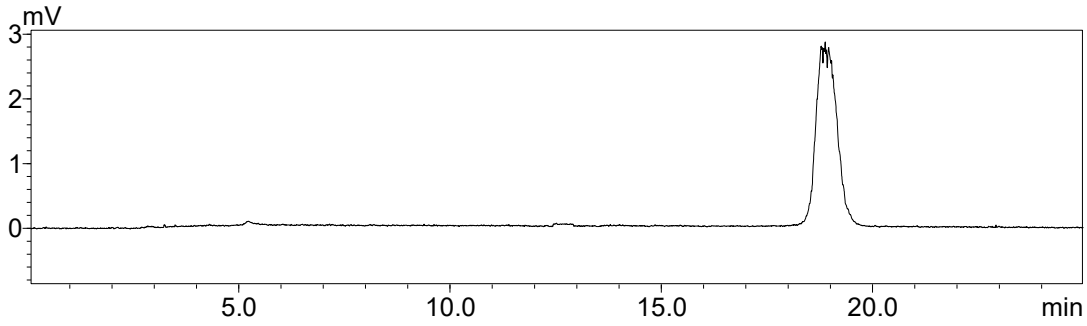




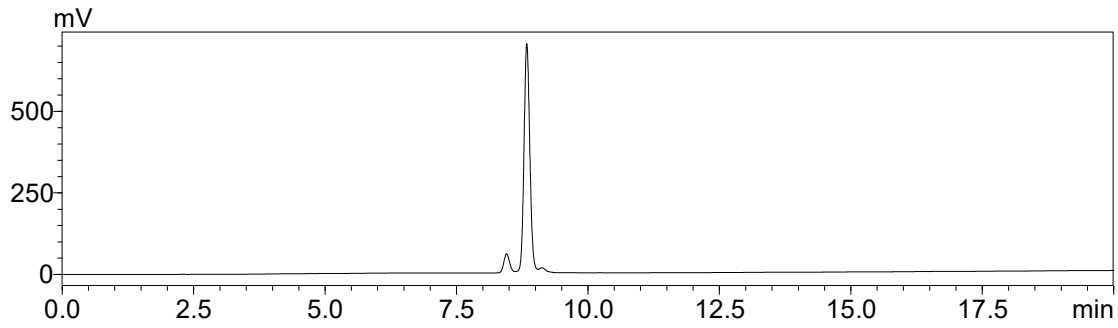
1H NMR (600 MHz, Deuterium Oxide) δ 5.20 (s, 1H), 5.16 (d, J = 4.1 Hz, 2H), 5.14 (d, J = 4.0 Hz, 2H), 5.04 (s, 2H), 4.60 (d, J = 8.4 Hz, 4H), 4.54 (d, J = 7.8 Hz, 2H), 4.51 – 4.44 (m, 3H), 4.27 (s, 2H), 4.19 (s, 2H), 4.15 (s, 2H), 4.13 – 4.06 (m, 3H), 4.05 – 3.39 (m, 67H), 3.26 (t, J = 9.3 Hz, 3H), 2.77 (dd, J = 12.6, 4.5 Hz, 2H), 2.16 – 1.95 (m, 16H), 1.81 (t, J = 12.1 Hz, 3H), 1.19 (dd, J = 11.1, 6.9 Hz, 6H).

HRMS (ESI) m/z calcd. for C93H154N6O67 2426.8828; found [M-H]- 2425.8742.

HPLC (free N-glycan with HILIC column)

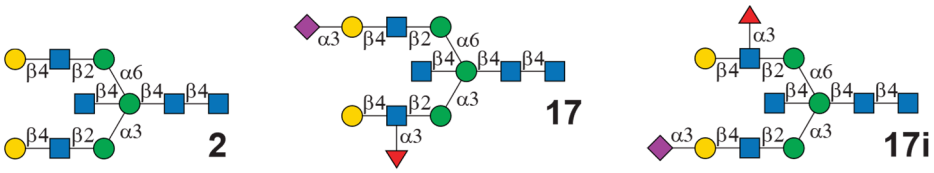


HPLC (AEAB-labeled N-glycan with PGC column)



6. LC-HCD-MS/MS and MSn ANALYSIS OF COMPOUNDS 2, 17, AND 17i

Experiment details:



UPLC-MS Analysis of AEAB labelled glycans: AEAB labeled **2**, **17** or **17i** were resuspended in water to a concentration of 0.5 µg/µL. Experiments were carried out with a linear separation of low-to-high organic (100% Acetonitrile containing 0.1% formic acid) on a Zorbax Eclipse XDB-C18 column (Agilent; 2.1x150 mm, 1,8 µm) connected to a Thermo QExactive HF mass spectrometer. All chemicals used for chromatography were mass-spec grade purity. A constant flow of 0.3mL/min was used into the mass spectrometer. A full FTMS spectrum was collected with the Orbitrap detector at a resolution of 120 000 from m/z 400-1800, with a data dependent scan used to collect HCD fragments of highly abundant ions throughout the separation. An injection of 2µL (1µg) was used for analysis.

Permethylation and MSn analysis of native compounds **2, 17**, and **17i**: Approximately 20 µg of the sample was transferred to a glass vial and resuspended in 200 µL of dimethyl sulfoxide (DMSO). A sodium hydroxide (NaOH)-DMSO base was made by combining 100 µL of 50% v/v NaOH and 200 µL of methanol (MeOH) and shaking. To this 4 mL of DMSO was added and the sample was mixed vigorously and then centrifuged for 5 mins at 3000 g. A white precipitate of carbonates is formed at the top of the DMSO and a clear NaOH-DMSO base at the bottom of the tube. All precipitate and DMSO was removed without disturbing the base. 4 mL of DMSO was added and the base was once again once again mixed vigorously and centrifuged. This process was repeated until precipitate no longer formed. The clear base was then resuspended in 1 mL of DMSO, and 300 µL of this was added to the sample. 150 µL of iodomethane was added to the sample. The sample was then vortexed and allowed to shake on a sample shaker for 25 minutes. The reaction was quenched by adding 2 mL of HPLC grade H_2O. 2 mL of dichloromethane was then added to extract the glycans. The sample was mixed vigorously for 30 seconds and then centrifuged at 3000 g for 1 minute. The upper water layer was then removed, and 2 additional mL of water was added. This process was repeated a total of 4 times. After the final water layer was removed, the glycan containing dichloromethane layer was then transferred to a clean glass tube and dried under a stream of N_2.[17, 18] Permethylated glycans were then resuspended in 20 µL of MeOH, and transferred to an LC target vial and diluted to 0.2 µg/ µL in 50:50 MeOH:H_2O. MS1 and MS2 data were collected on an Orbitrap Fusion Tribrid mass spectrometer equipped with a nanospray ion source and connected to a Dionex binary solvent system (Waltham, MA).[19] MS^4 experiments for compound **2** were done by direct injecting the sample into the Orbitrap Fusion and following the fragmentation pathway outlined in **Figure S3**.

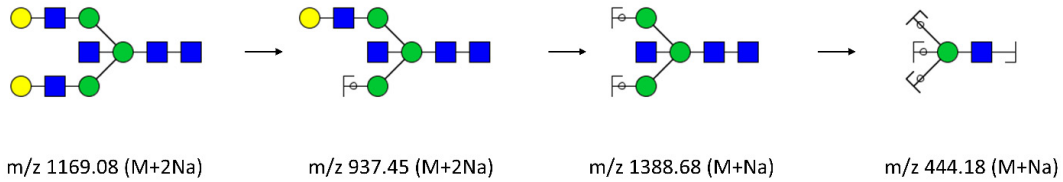


Figure S3. Fragmentation pathway of MS4 experiment done to confirm bisecting GlcNAc**.**

Results:

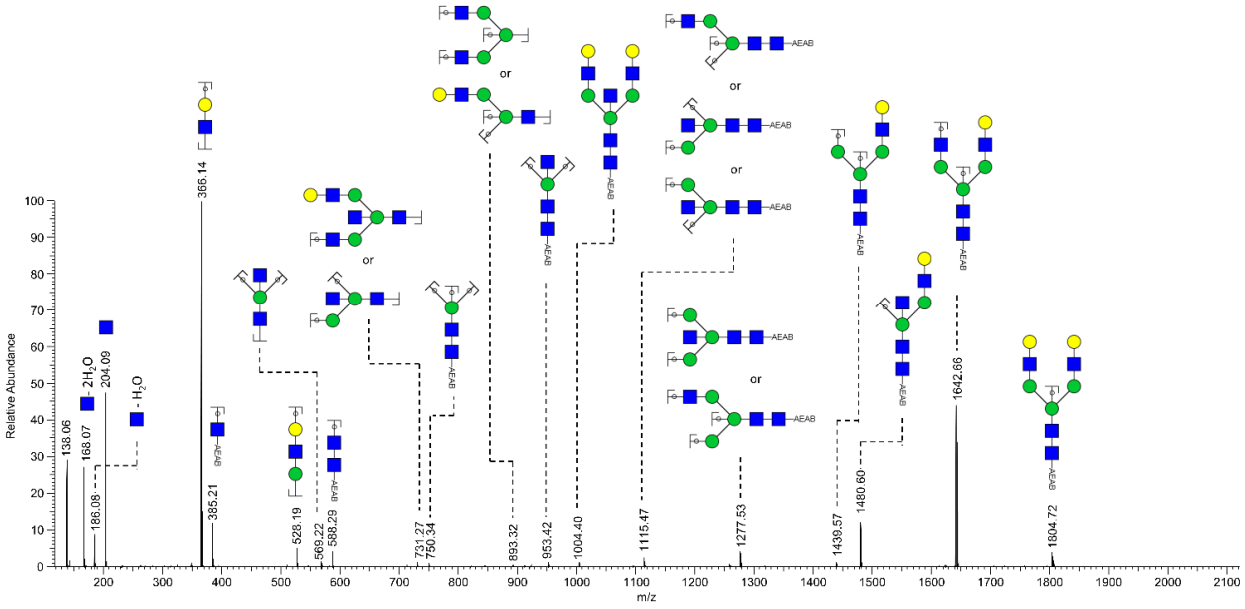


Figure S4. LC-HCD-MS/MS of AEAB labeled **2** (MS/MS of m/z 1004 [M+2H]).

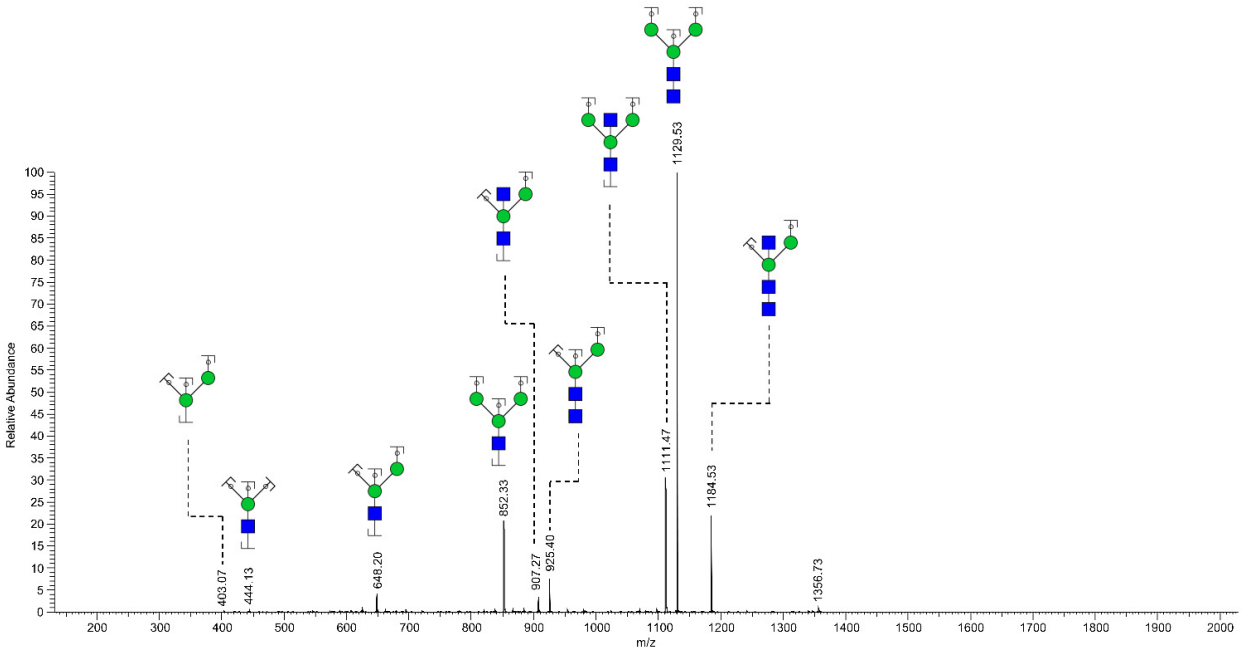


Figure S5: MS^4 of permethylated compound **2**. The fragmentation pathway for this experiment can be seen in **Figure S5**. This data was collected in the ion trap. Fragment at m/z 444.1 confirms the bisecting GlcNAc.

To determine presence of bisecting GlcNAc, an MS^n strategy to trim down the permethylated N-glycan to the trimannose core was utilized.[18, 20] Fragmentation to the core yields a 3-substituted structure at m/z 1388.68 [M+Na], that could correspond to either a tri-antennary structure or a bi-antennary structure with a bisecting GlcNAc. Fragmentation of this ion yields an m/z of 444 [M+Na] for bisecting structures or m/z 458 [M+Na] for tri-antennary glycans.

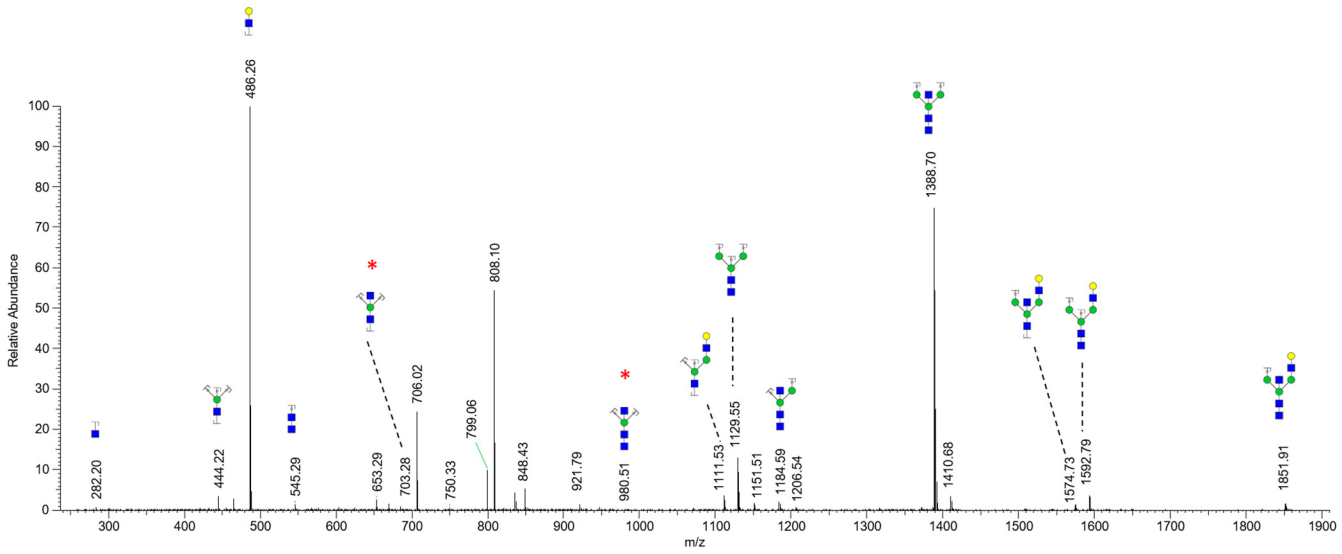


Figure S6: MS^3 of permethylated compound **2** (m/z 1169 [M+2Na] → 937 [+2Na]).

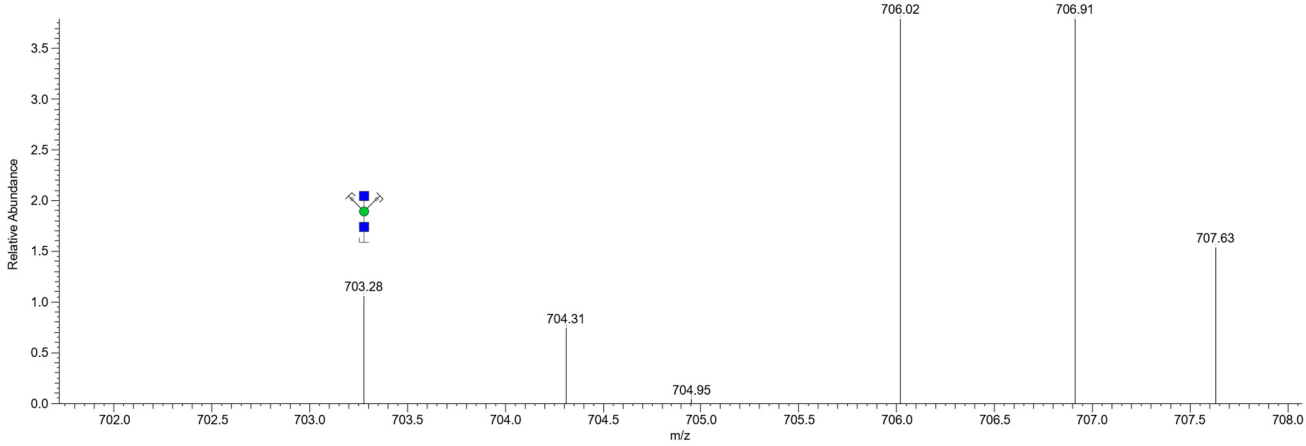


Figure S7: Zoom in of MS^3 of permethylated compound **2** (Ppm error: -56.87).

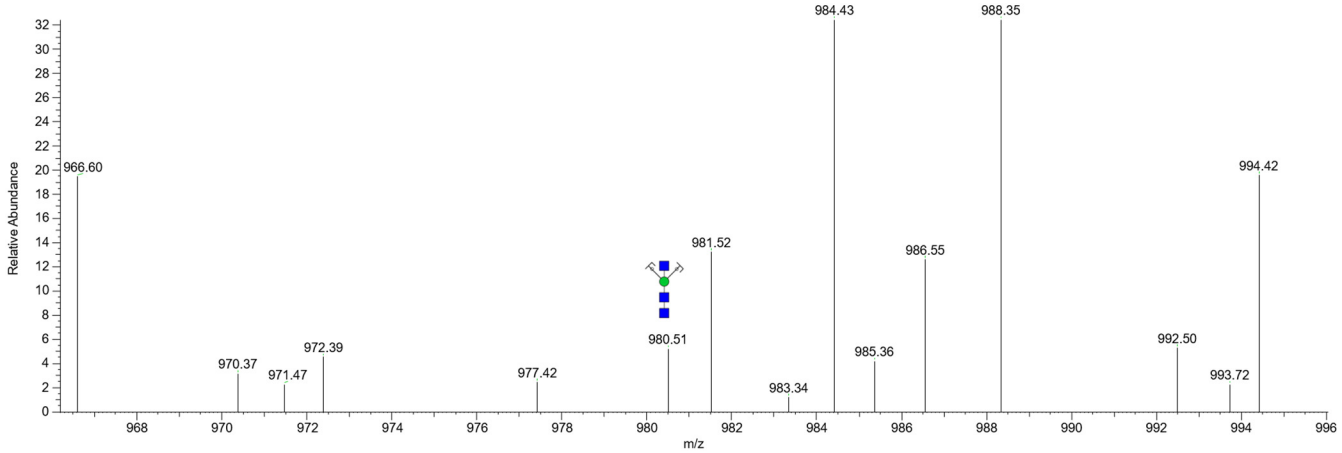


Figure S8: Zoom in of MS^3 of permethylated compound **2** (Ppm error: 30.60).

MS^3 spectrum was obtained using IT-MS mode by fragmenting a 3-substituted structure at m/z 937.45 [M+2Na], that could correspond to either a tri-antennary structure or a bi-antennary structure with a bisecting GlcNAc. The data (Figure S6, S7, S8) support that this structure is the bi-antennary structure with a bisecting GlcNAc. If the bisecting GlcNAc was galactosylated there would be a diagnostic fragment at m/z 966.46, whereas if the GlcNAc is not galactosylated we would not expect to see this fragment and instead would see a fragment at m/z 980.48. A fragment at m/z 980.51 was observed, which is within expected ppm error for ion trap MS. Additionally, a fragment at m/z 703.28 was observed which corresponds to a loss of the reducing end GlcNAc. The bond between the core mannose's is not labile, and it is difficult to achieve these fragments. Because of this the intensities of these fragments are low. The fragment expected for galactosylated bisecting GlcNAc was not observed.

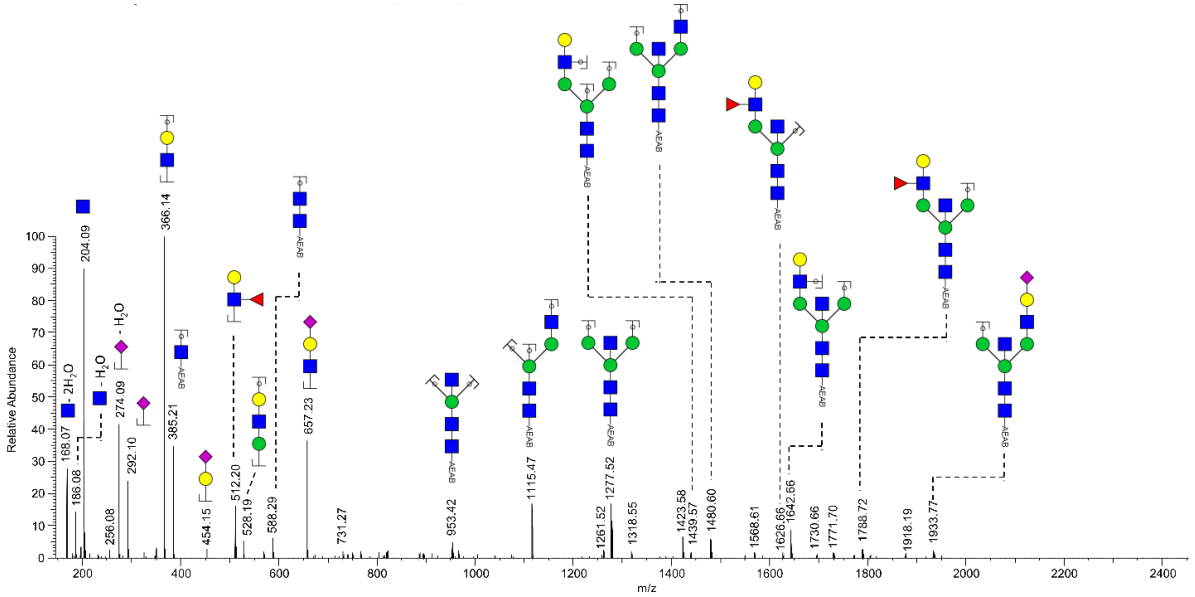


Figure S9. LC-HCD-MS/MS of AEAB labeled **17** (MS/MS of m/z 815 [M+3H]). Fragments at m/z 512 and m/z 657 confirm the sialic acid and fucose are on different branches.

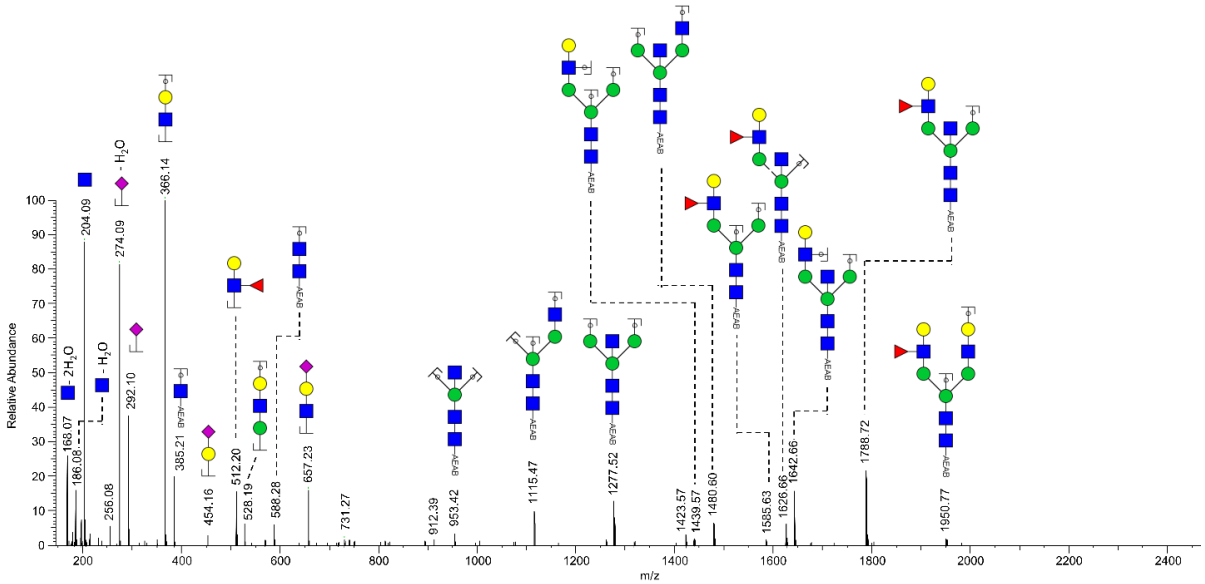


Figure S10. LC-HCD-MS/MS of AEAB labeled **17i** (MS/MS of m/z 815 [M+3H]). Fragments at m/z 512 and m/z 657 confirm the sialic acid and fucose are on different branches.

For compounds **17** and **17i**, the retention times shift confirming these structures are structural isomers (Part 5 HPLC analysis of AEAB labeled **17** and **17i**). For MS/MS, both compounds produced fragment ions at m/z 512 which confirm the fucosylated branch, and m/z 657 which confirm the sialylated branch confirming that **17** and **17i** are positional isomers with respect to the sialic acid placement. An additional water loss would be seen if the fucose and sialic acid were on different branches. This LC-HCD-MS/MS analysis of AEAB labelled compound 17 and 17i confirmed that Module S1 catalyzed reaction only attach α2-3Neu5Ac into the branch without fucosylation. This is further supported by LC-CID-MS/MS data collected on permethylated samples, which produced these same diagnostic fragments (**Figure S11**, **S12**).

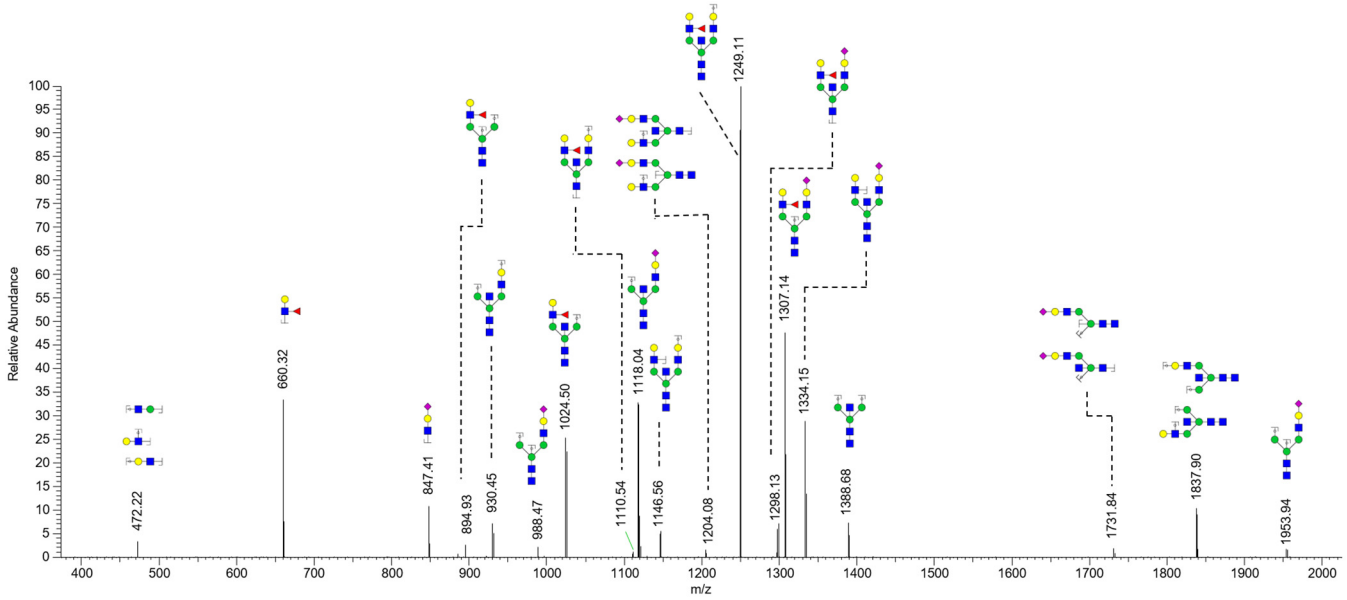


Figure S11. Compound **17** Permethylated MS/MS of m/z 1436.7 [M+2Na]

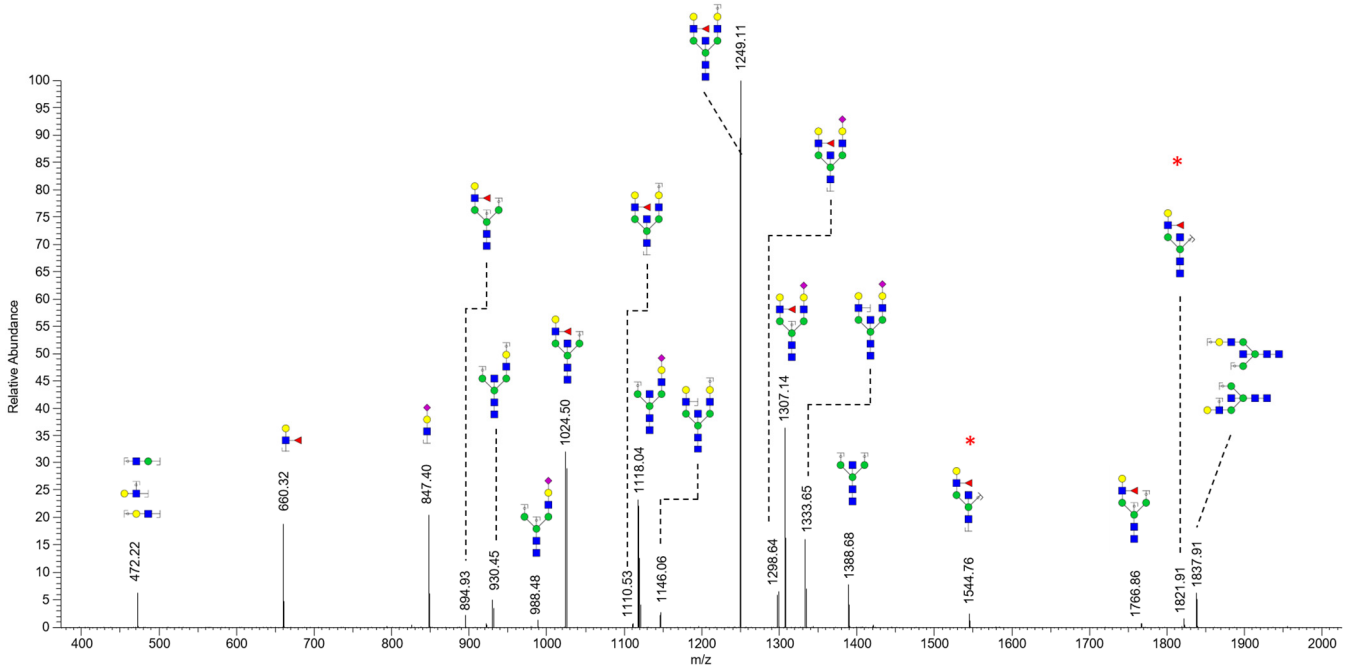
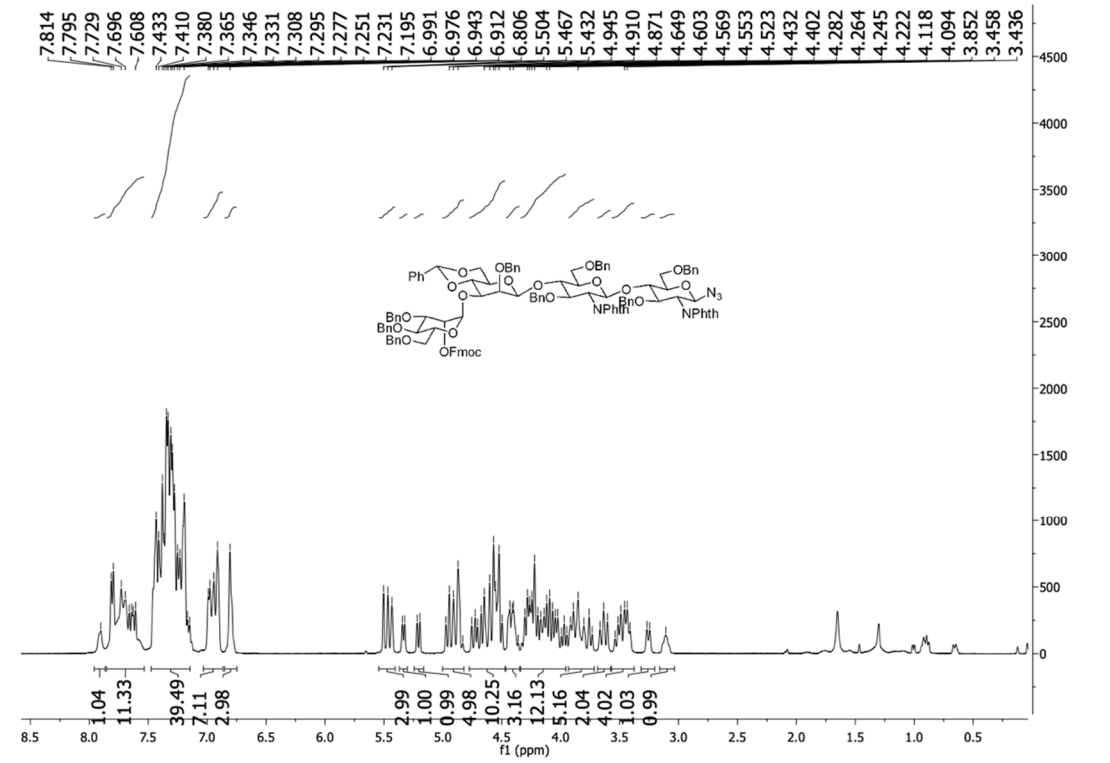


Figure S12. Compound **17i** Permethylated MS/MS of m/z 1436.7 [M+2Na]

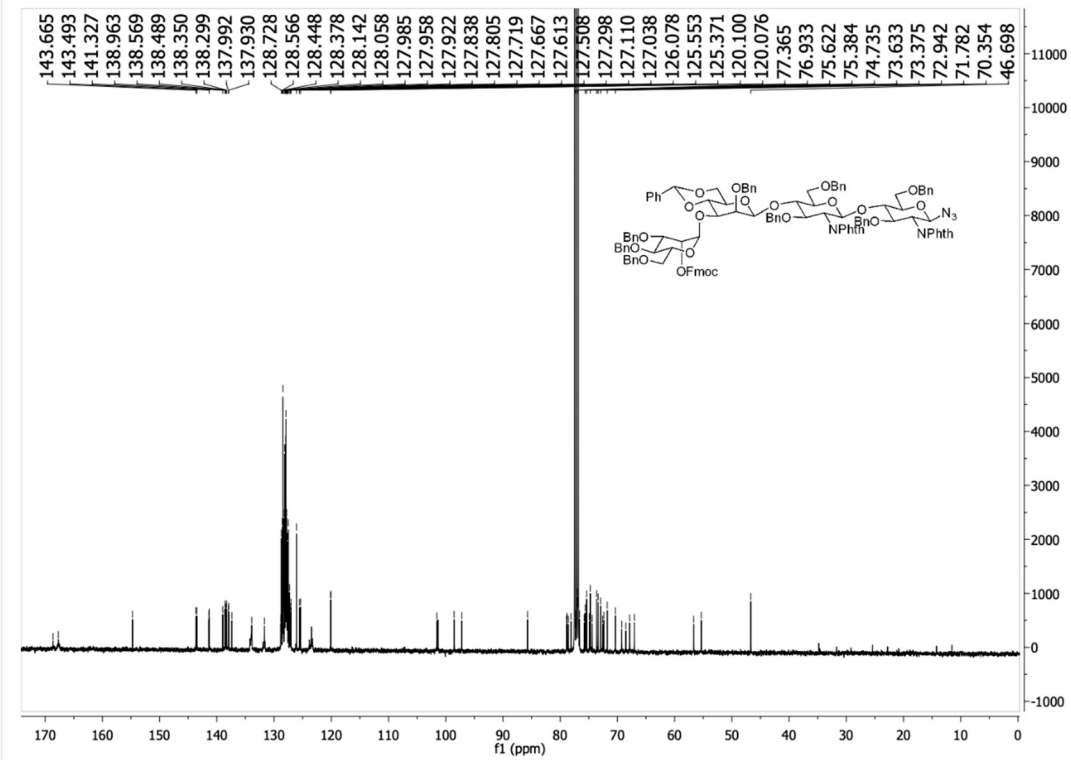
7. NMR SPECTRA

Compound 24

^{1}H NMR (400 MHz, $CDCl_3$)

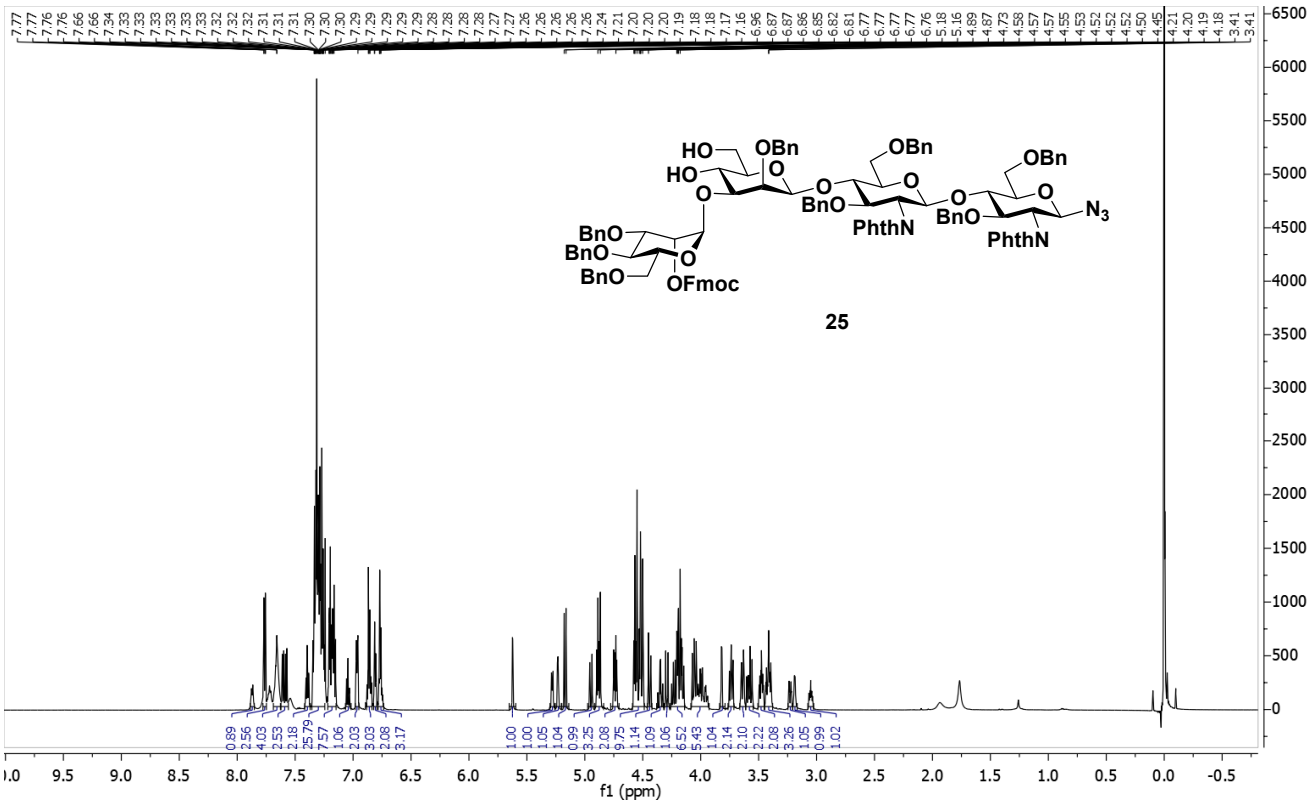


^{13}C NMR (100 MHz, $CDCl_3$)

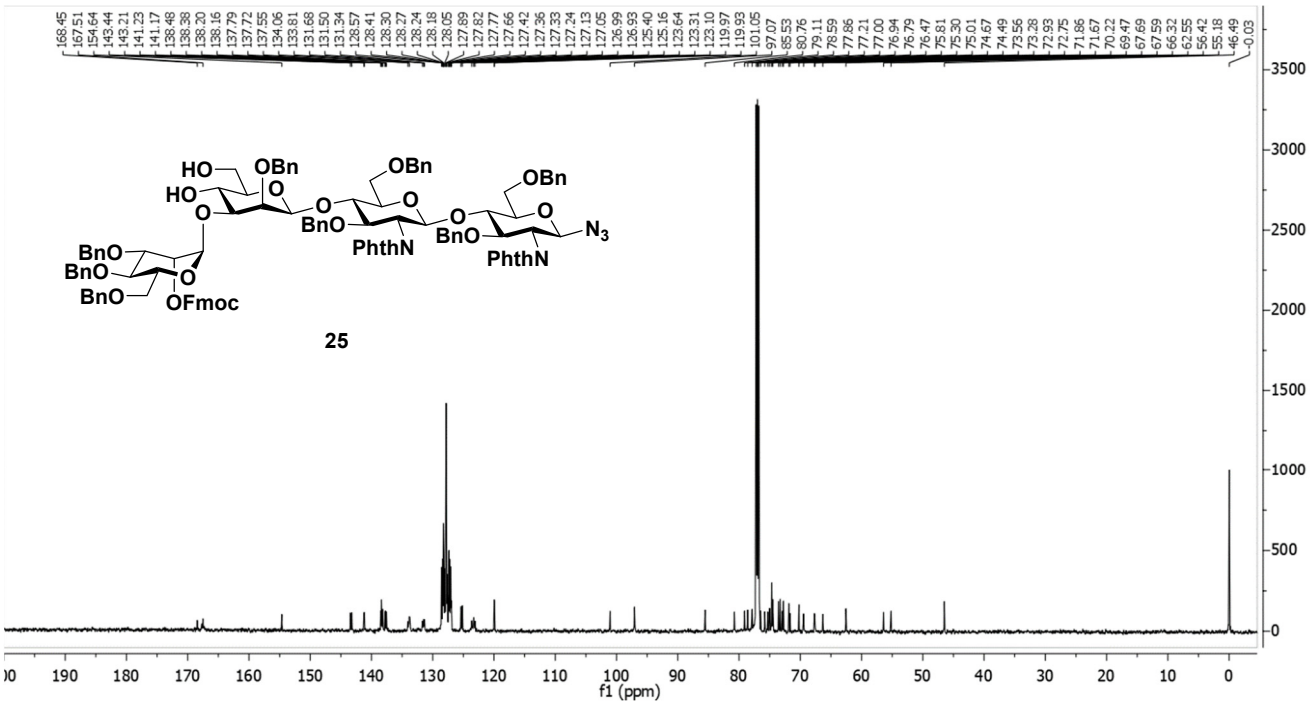


Compound 25

^{1}H NMR (600 MHz, $CDCl_3$)

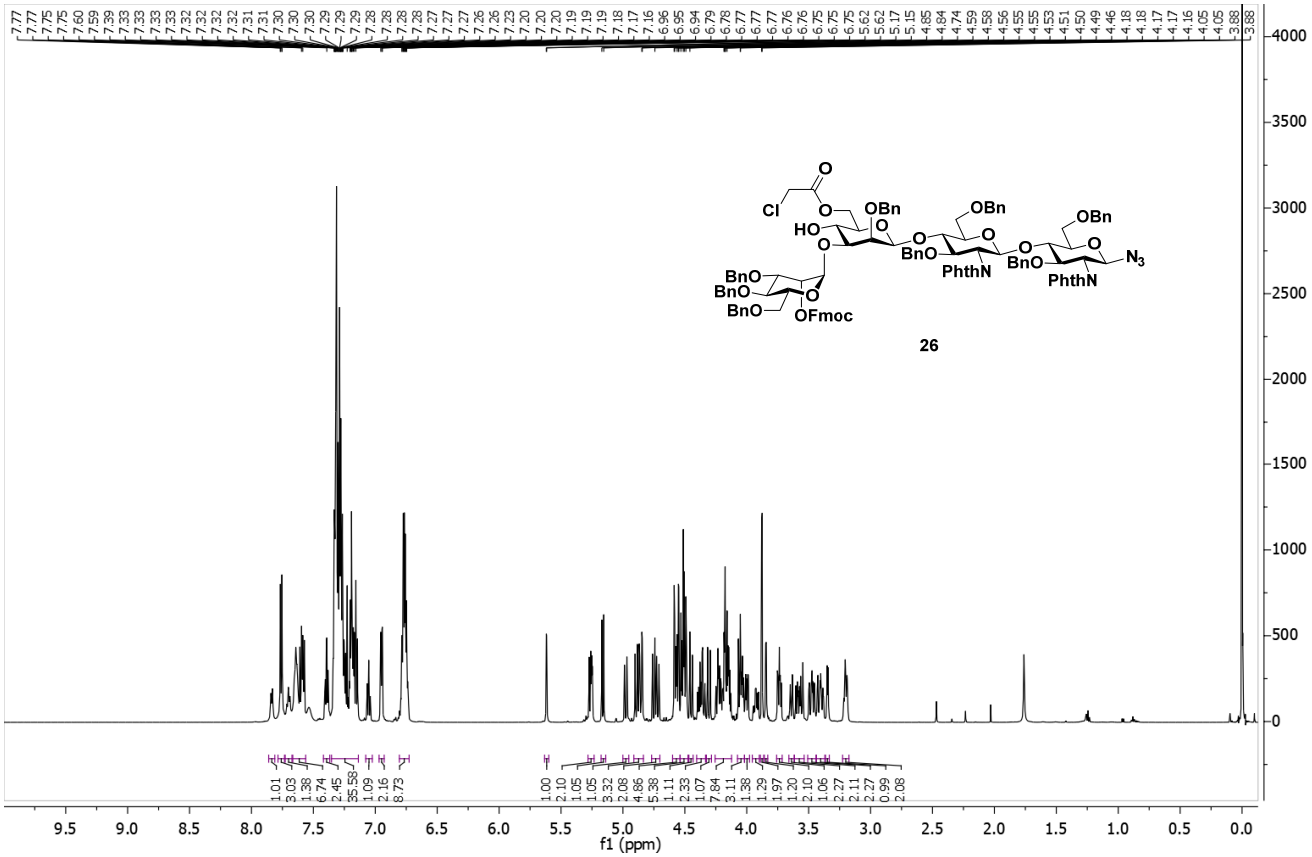


^{13}C NMR (150 MHz, $CDCl_3$)

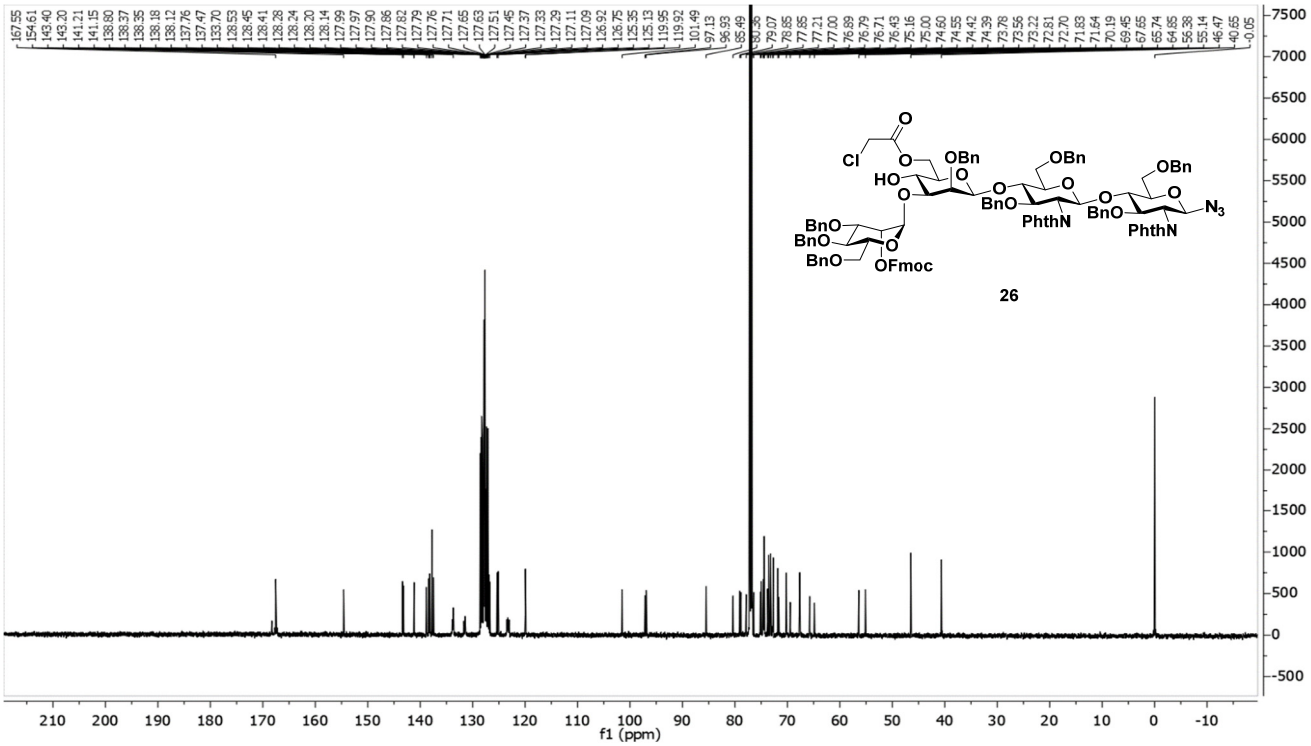


Compound 26

^{1}H NMR (600 MHz, $CDCl_3$)

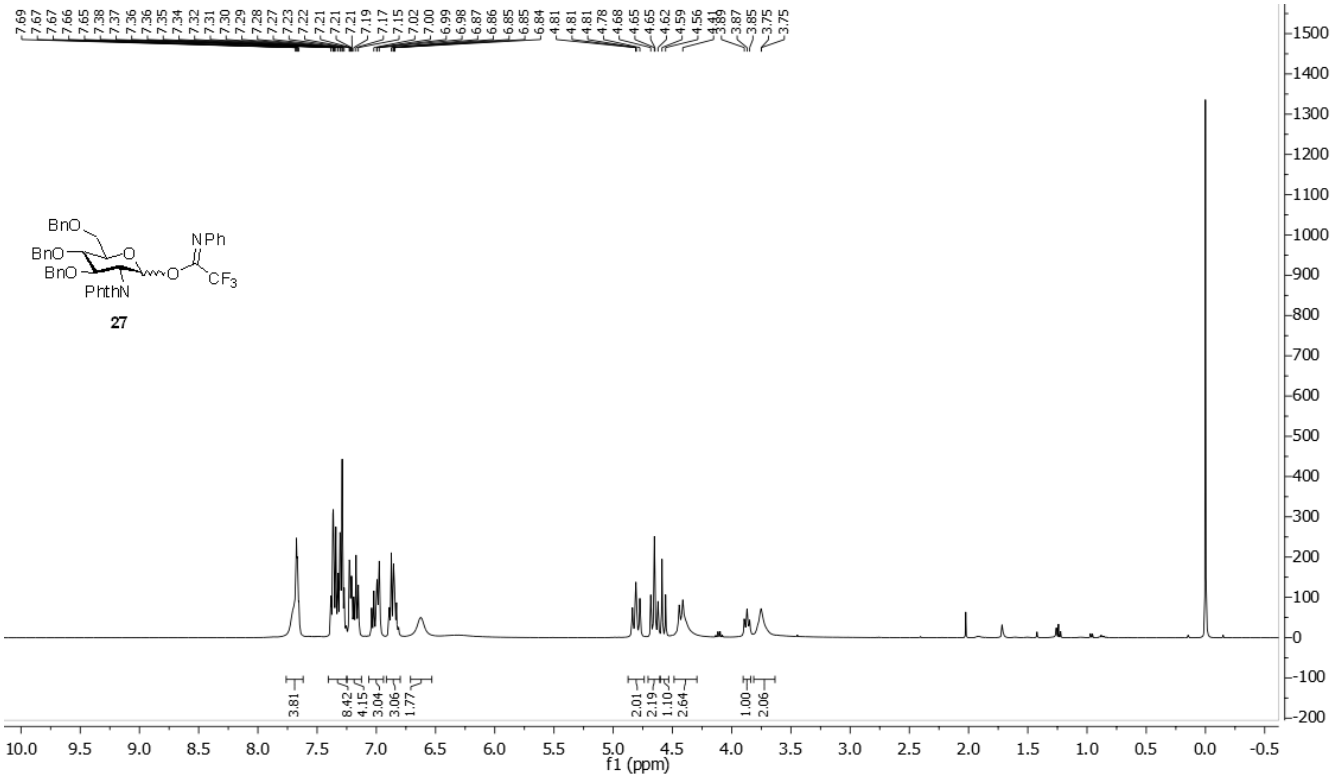


^{13}C NMR (150 MHz, $CDCl_3$)

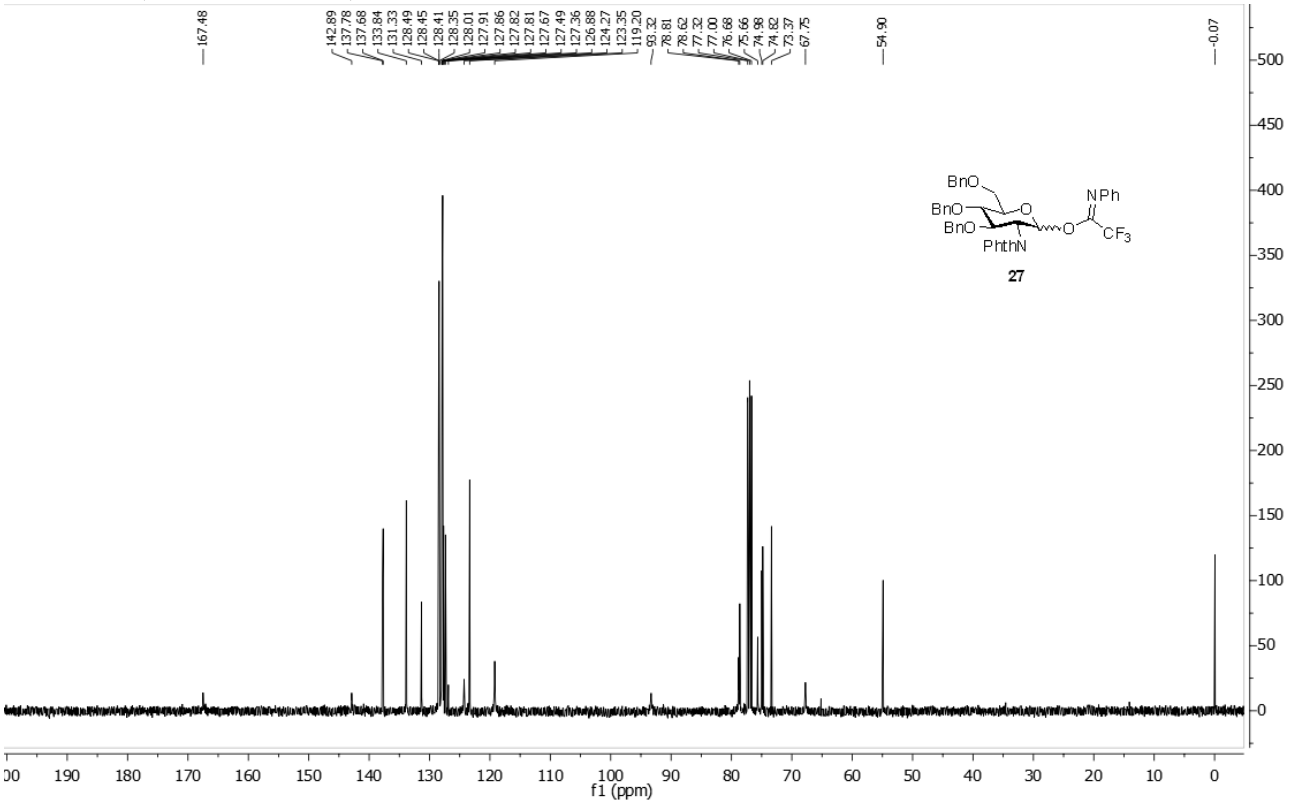


Compound 27

^{1}H NMR (400 MHz, $CDCl_3$)

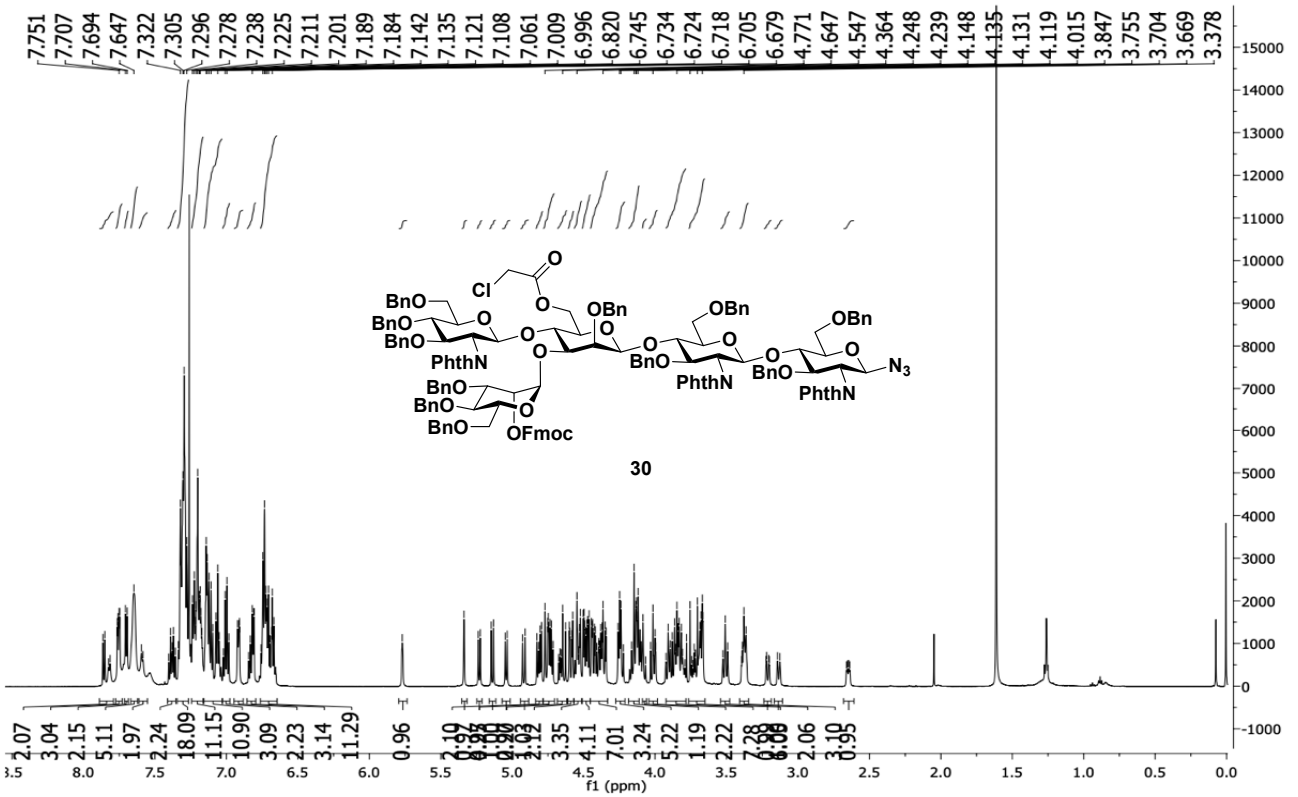


^{13}C NMR (100 MHz, $CDCl_3$)

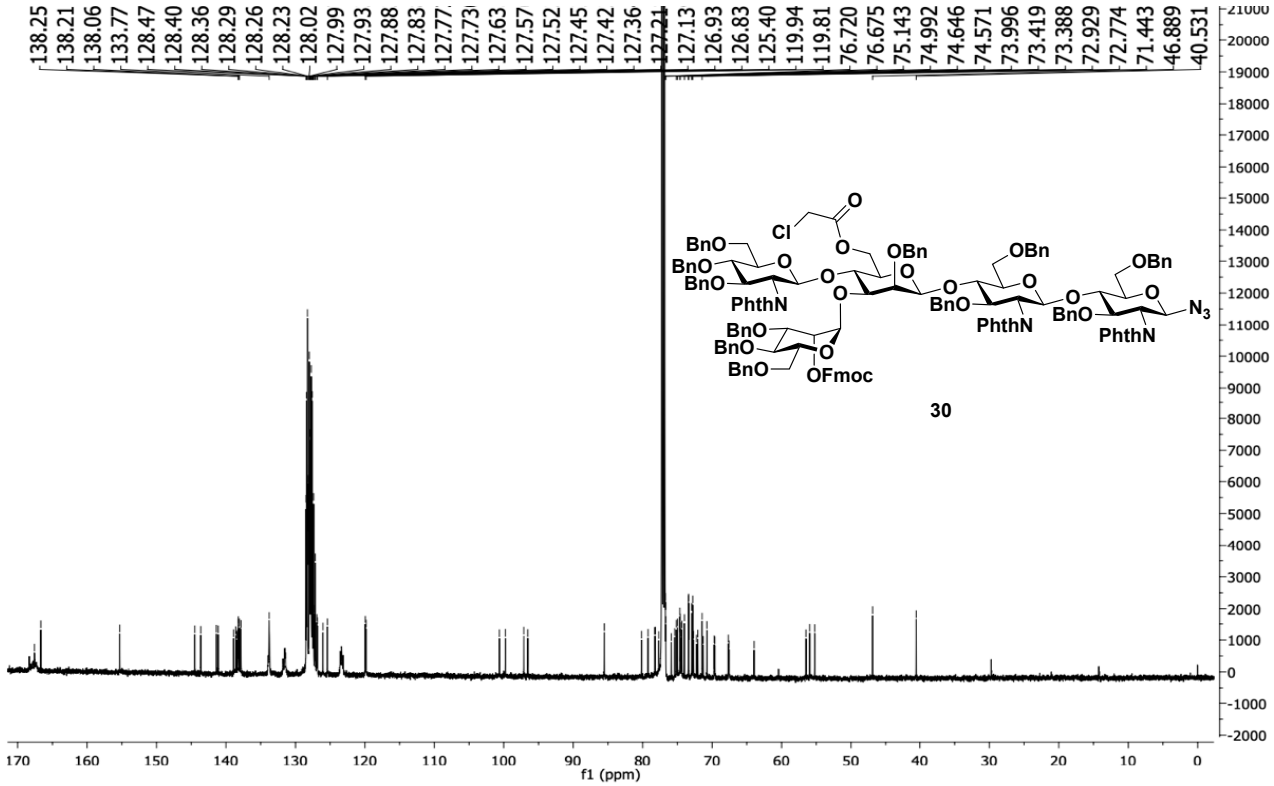


Compound 30

^{1}H NMR (600 MHz, $CDCl_3$)

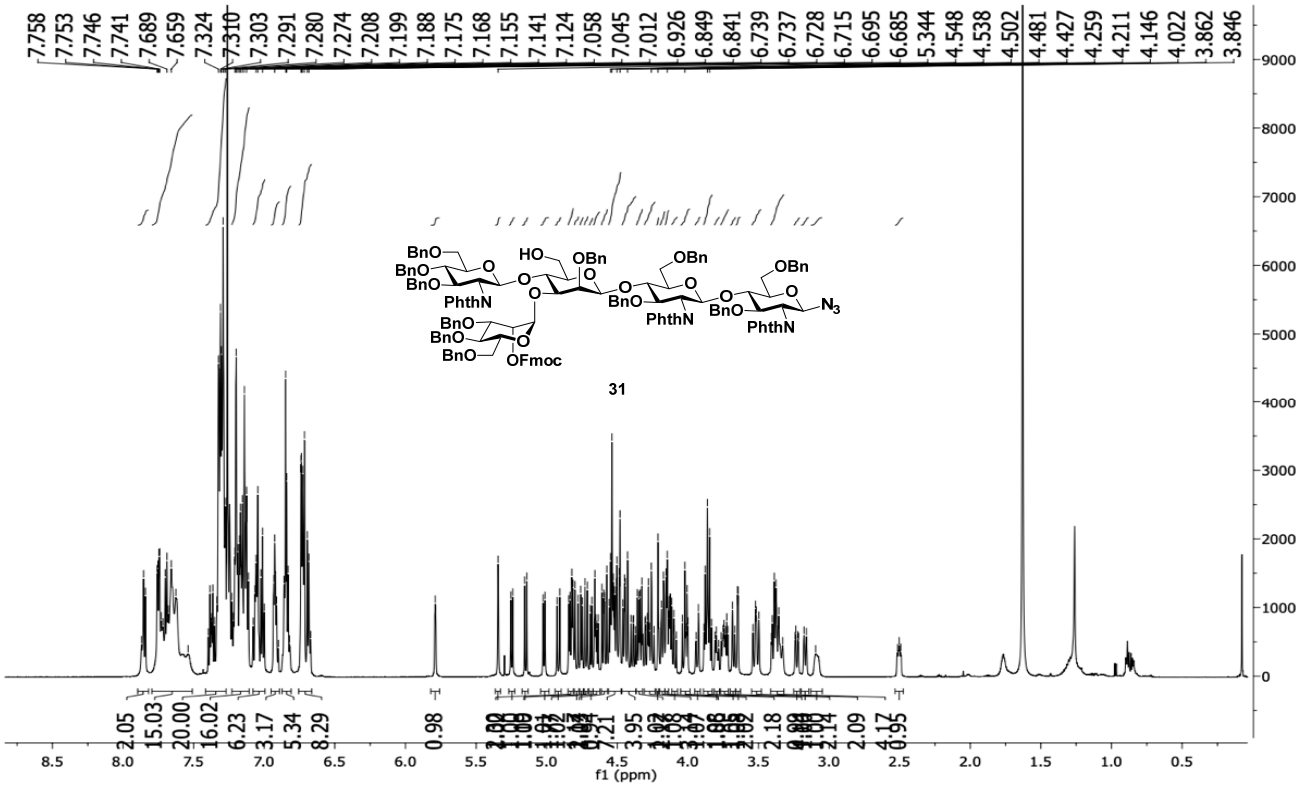


^{13}C NMR (150 MHz, $CDCl_3$)

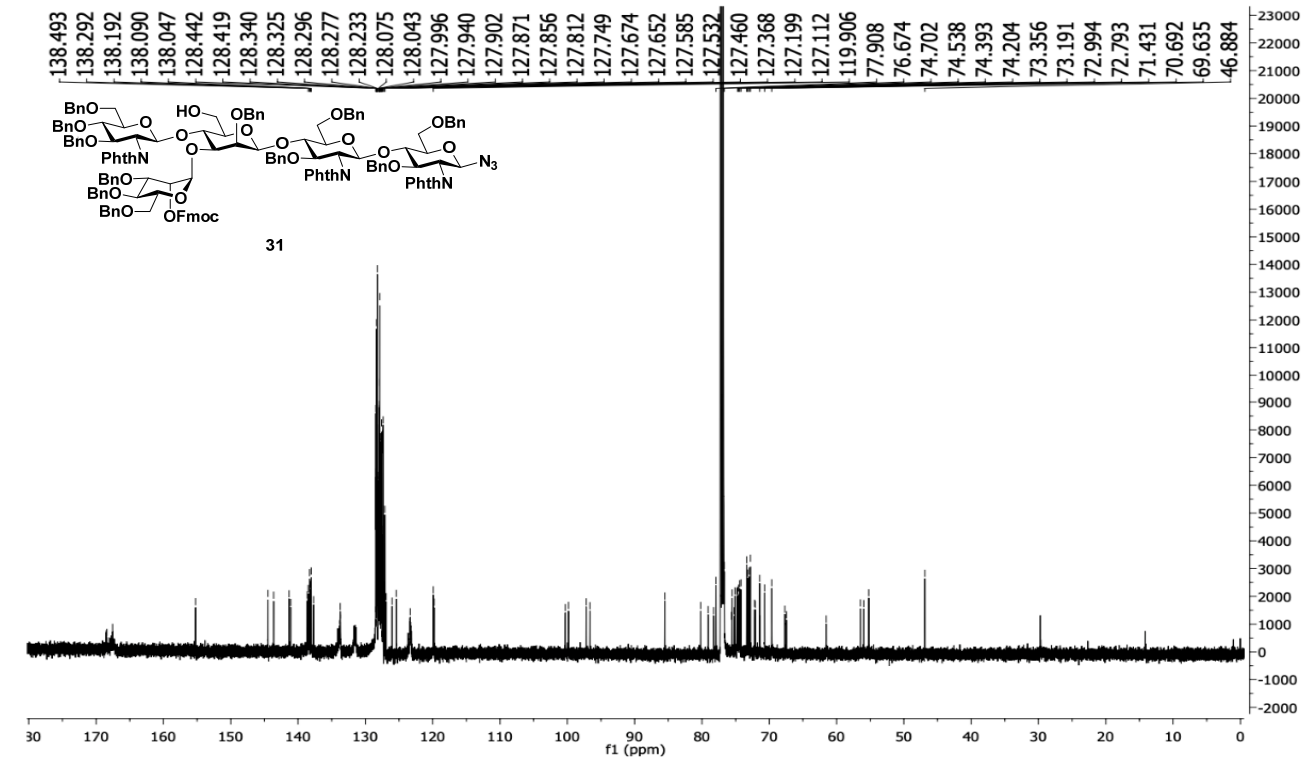


Compound 31

^{1}H NMR (600 MHz, $CDCl_3$)

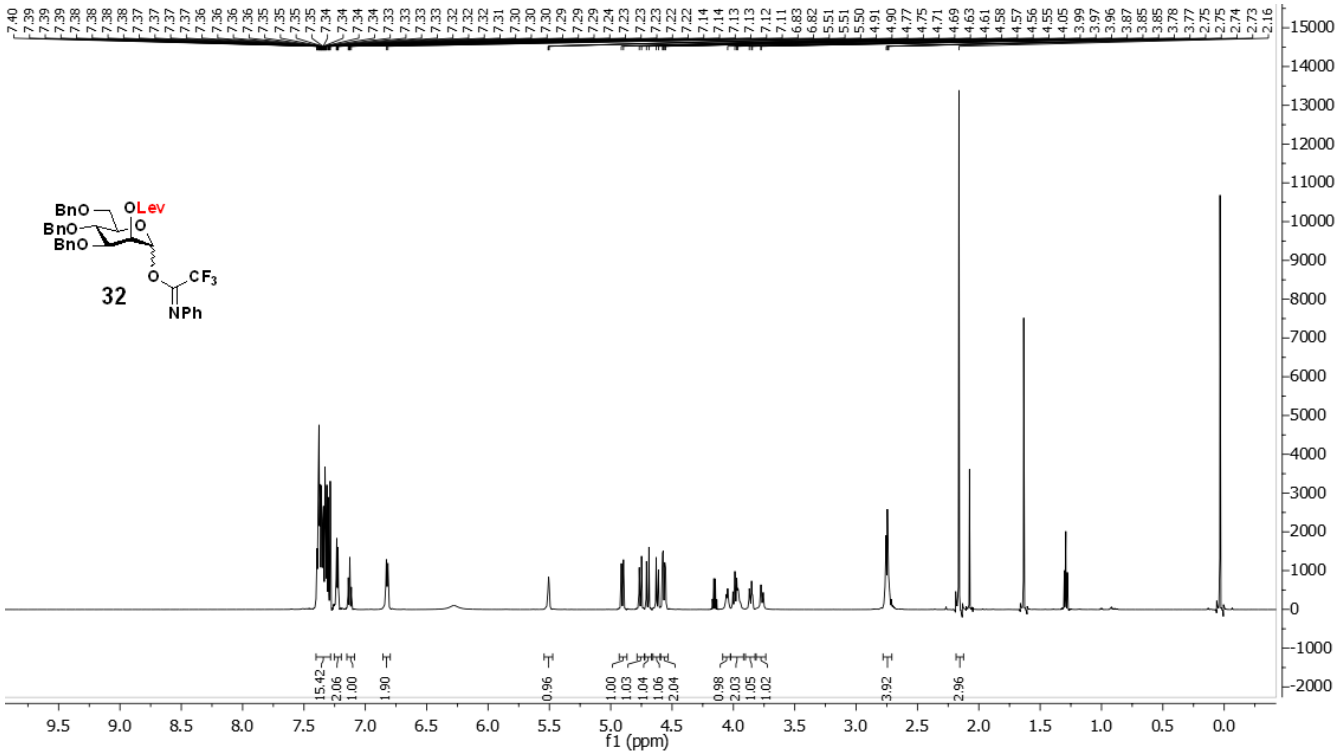


^{13}C NMR (150 MHz, $CDCl_3$)

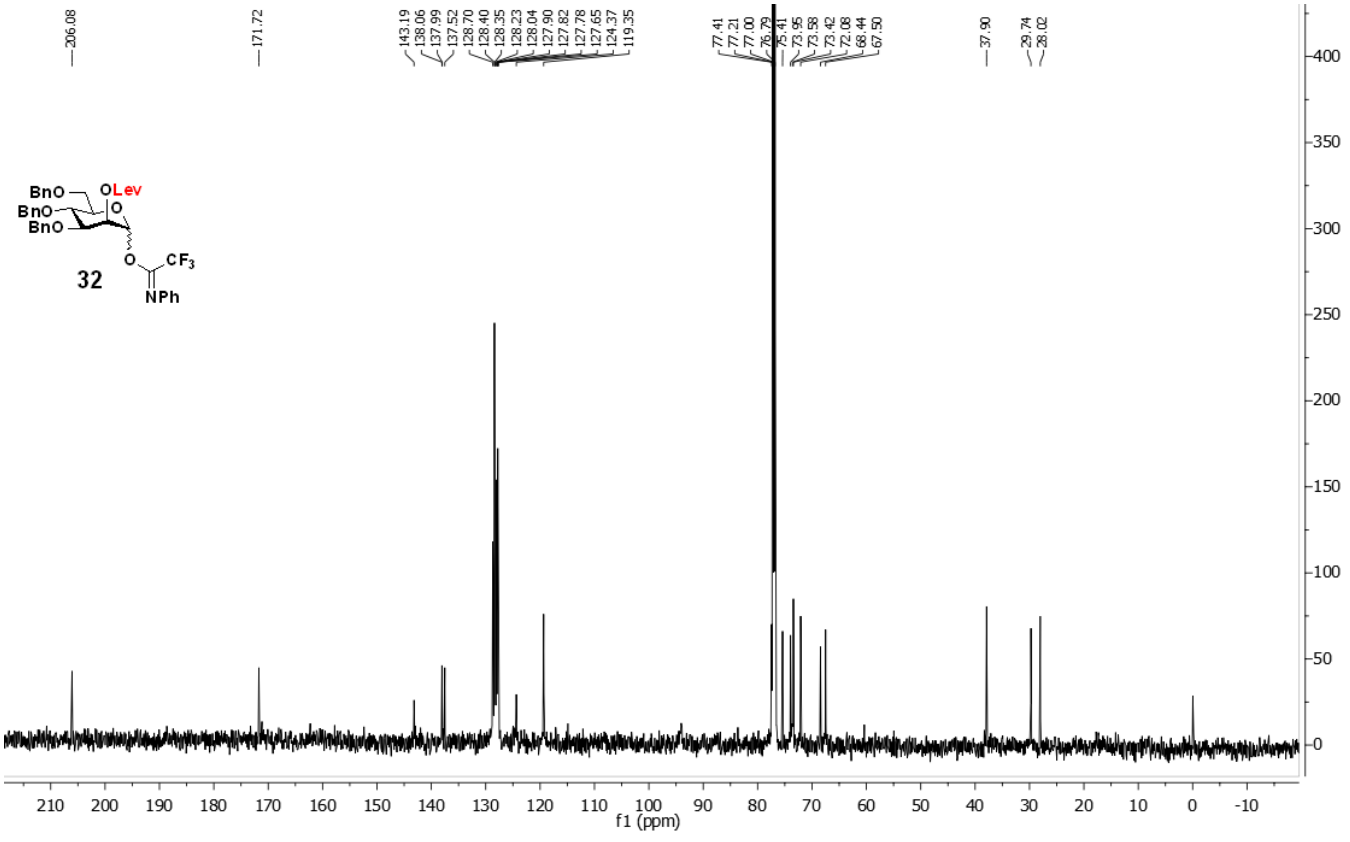


Compound 32

^{1}H NMR (600 MHz, $CDCl_3$)

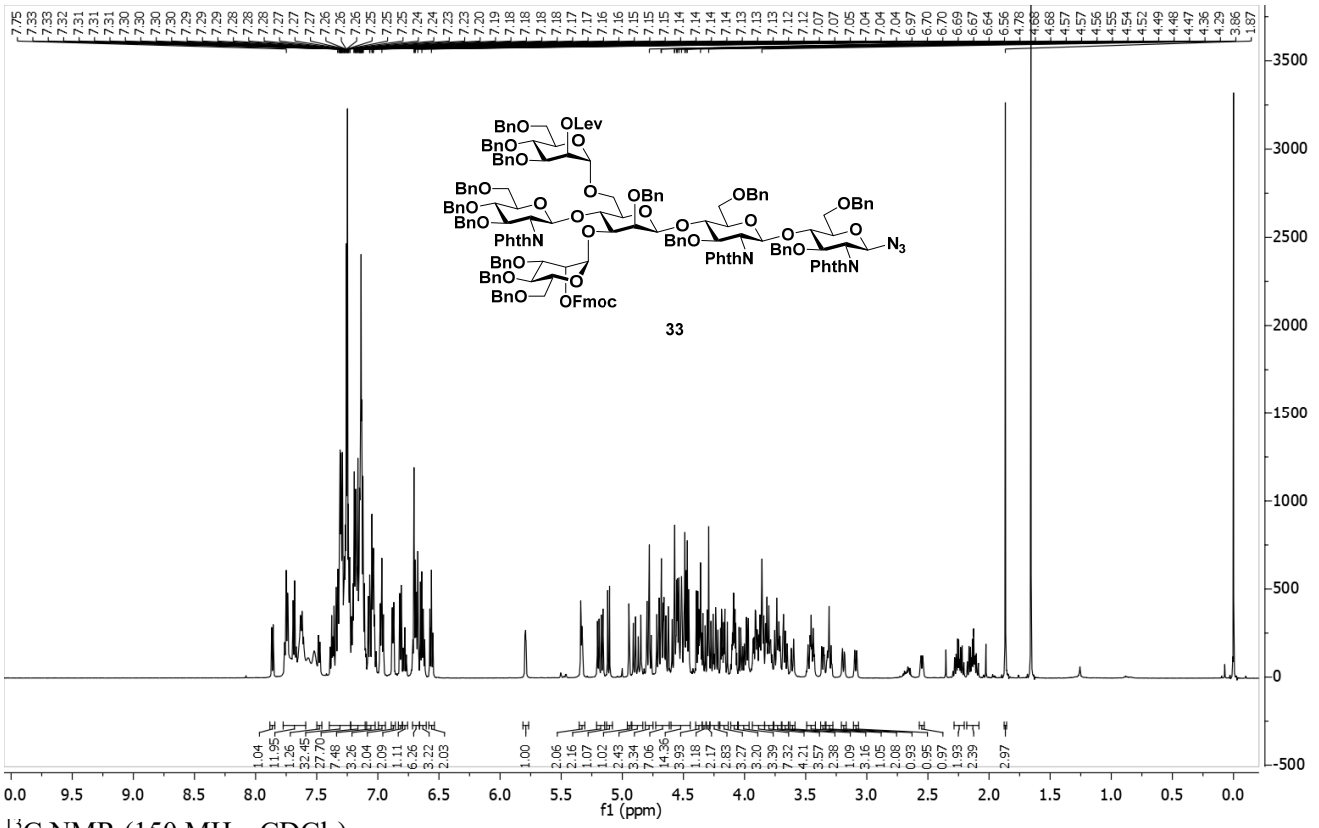


^{13}C NMR (150 MHz, $CDCl_3$)

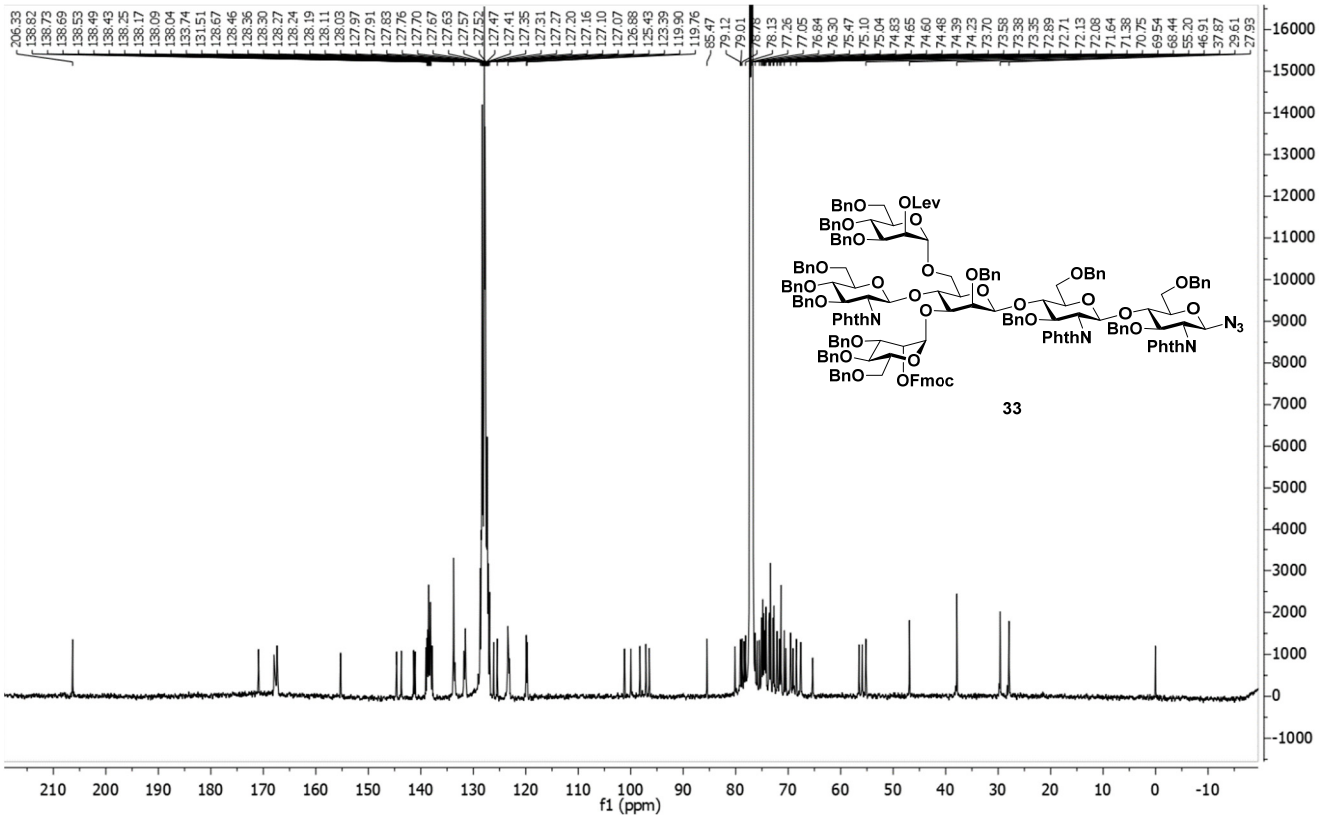


Compound 33

^{1}H NMR (600 MHz, $CDCl_3$)

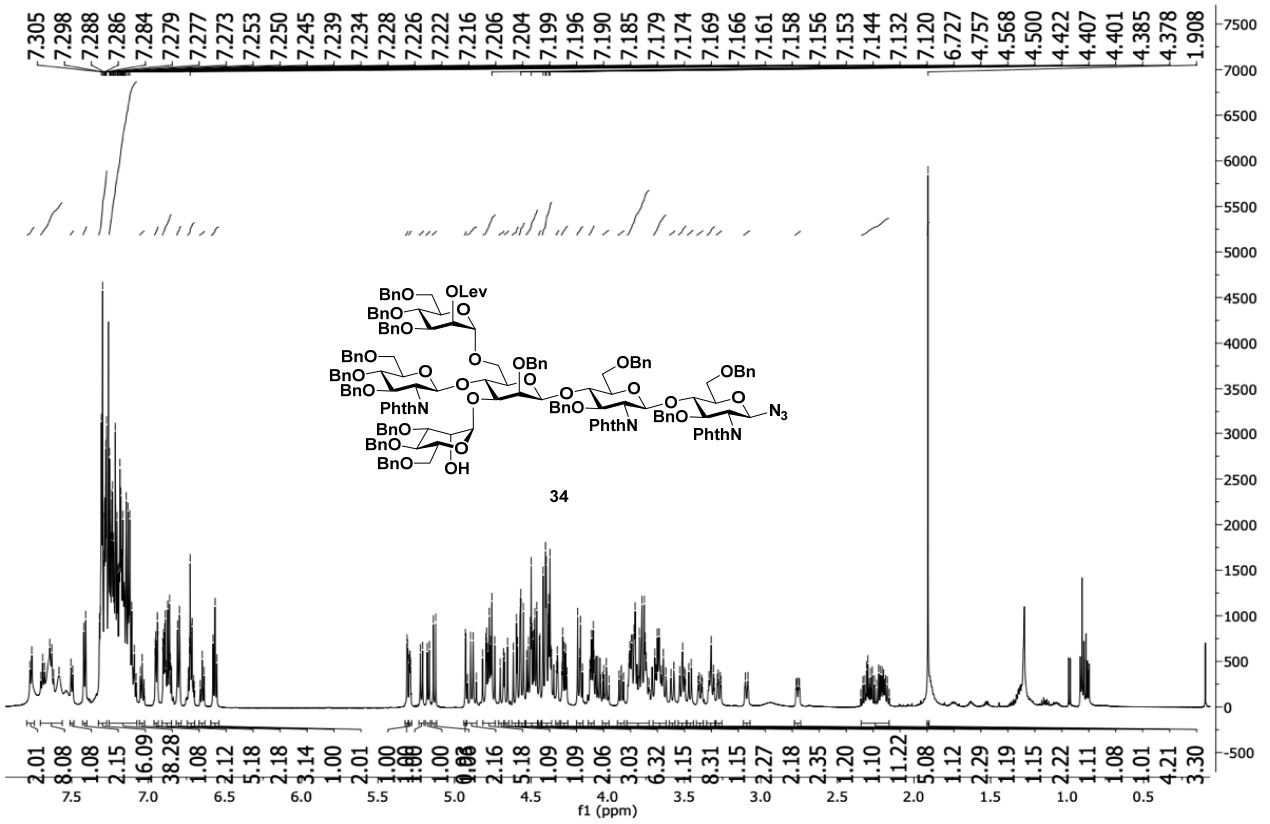


^{13}C NMR (150 MHz, $CDCl_3$)

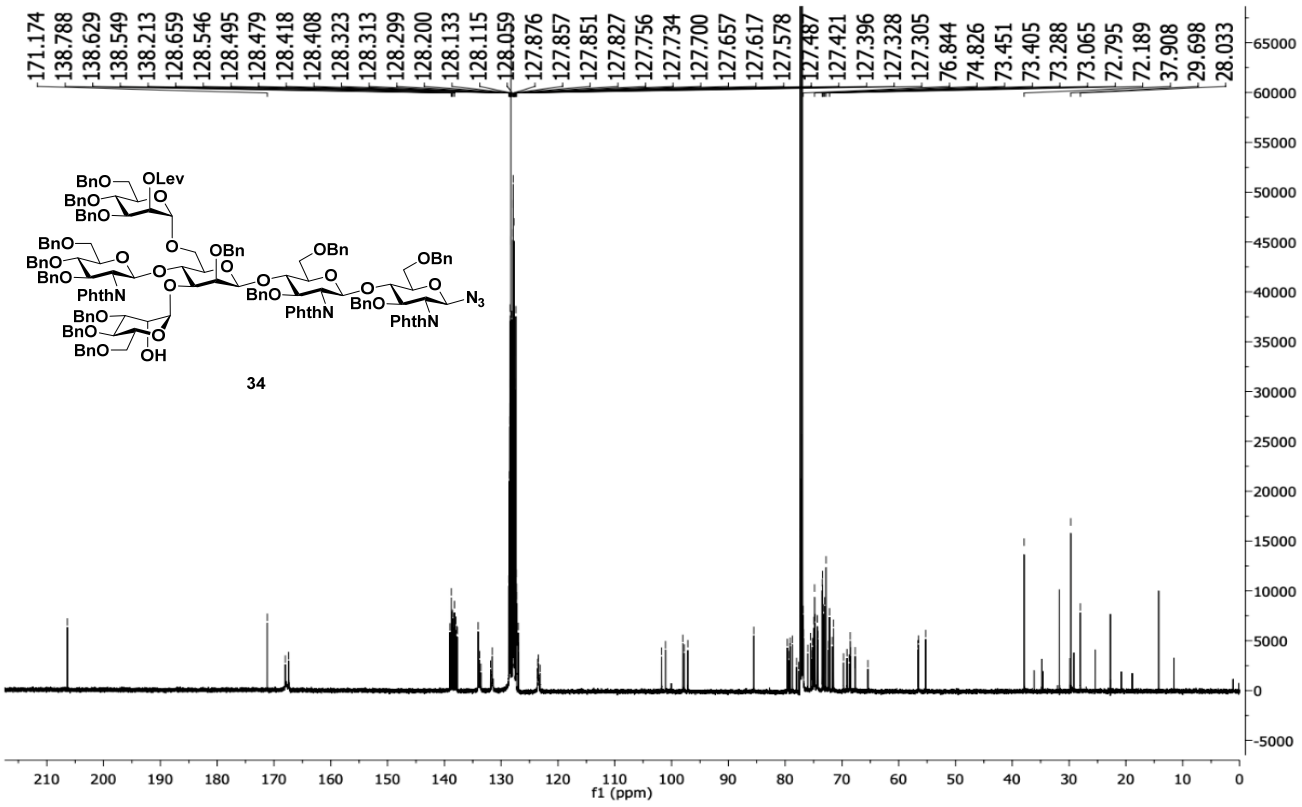


Compound 34

^{1}H NMR (600 MHz, $CDCl_3$)

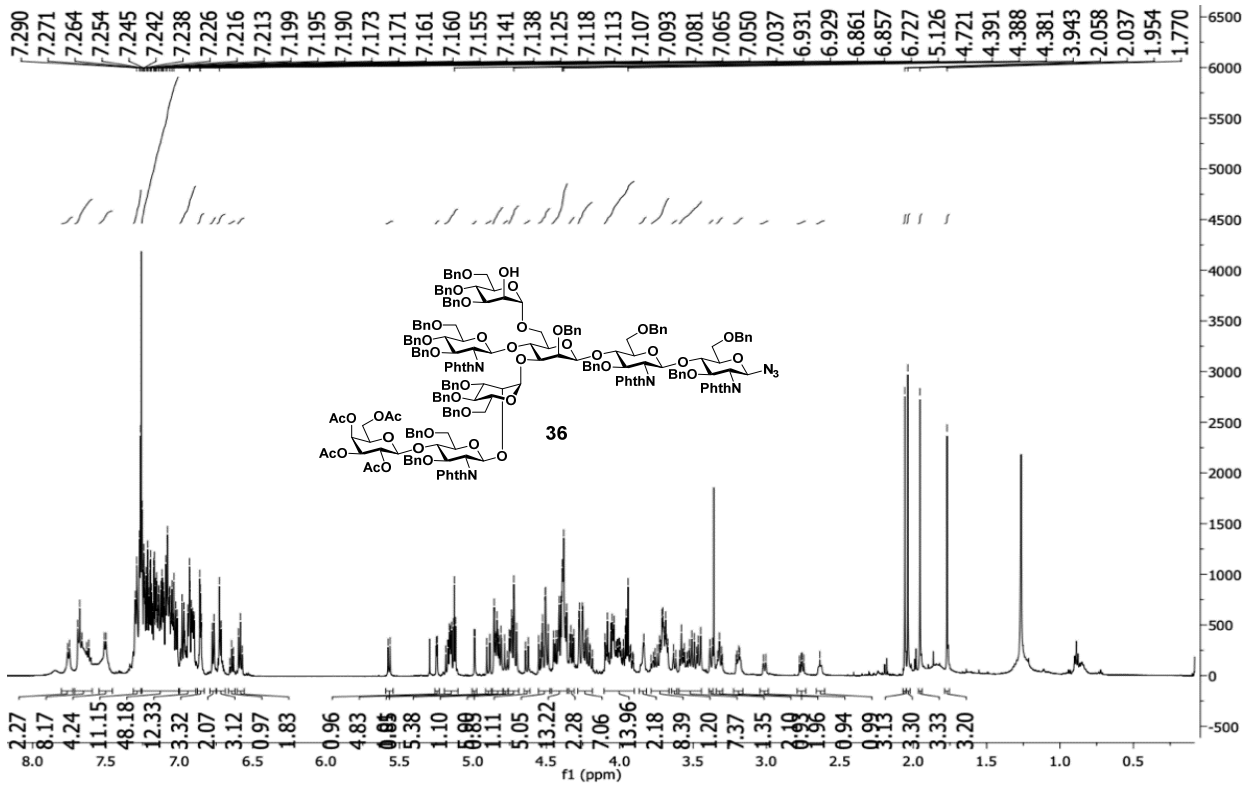


^{13}C NMR (150 MHz, $CDCl_3$)

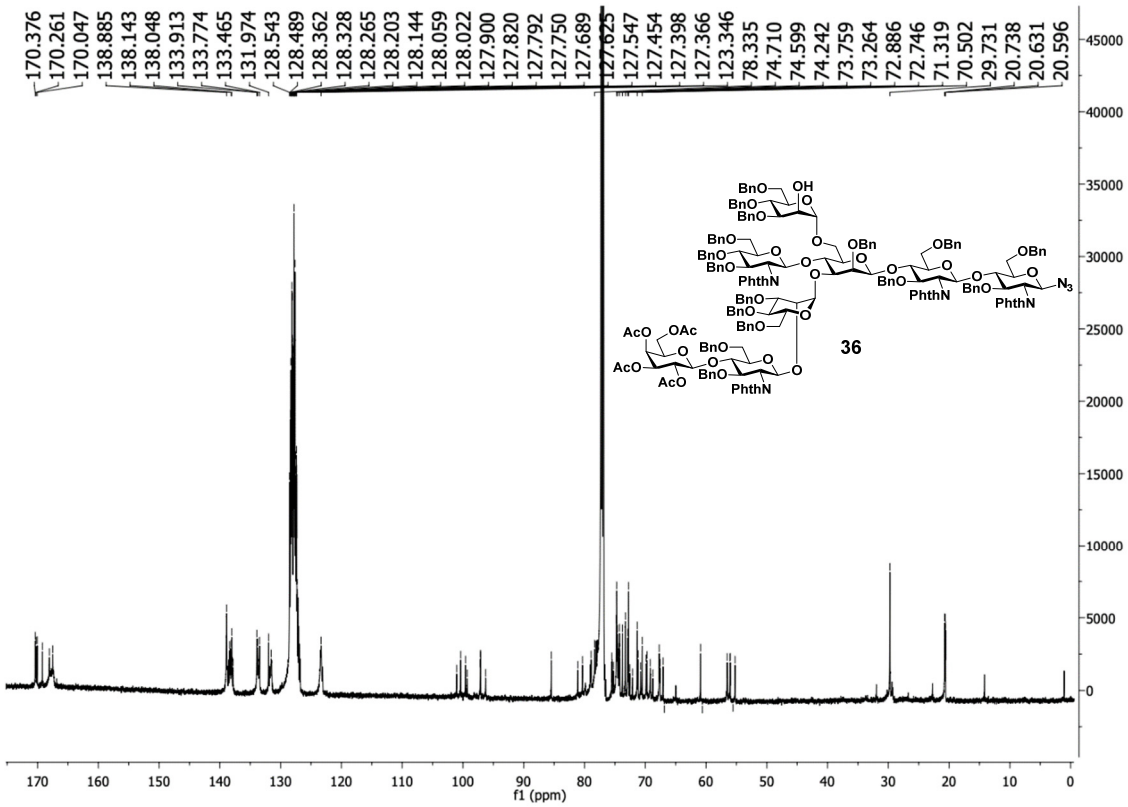


Compound 36

^{1}H NMR (600 MHz, $CDCl_3$)

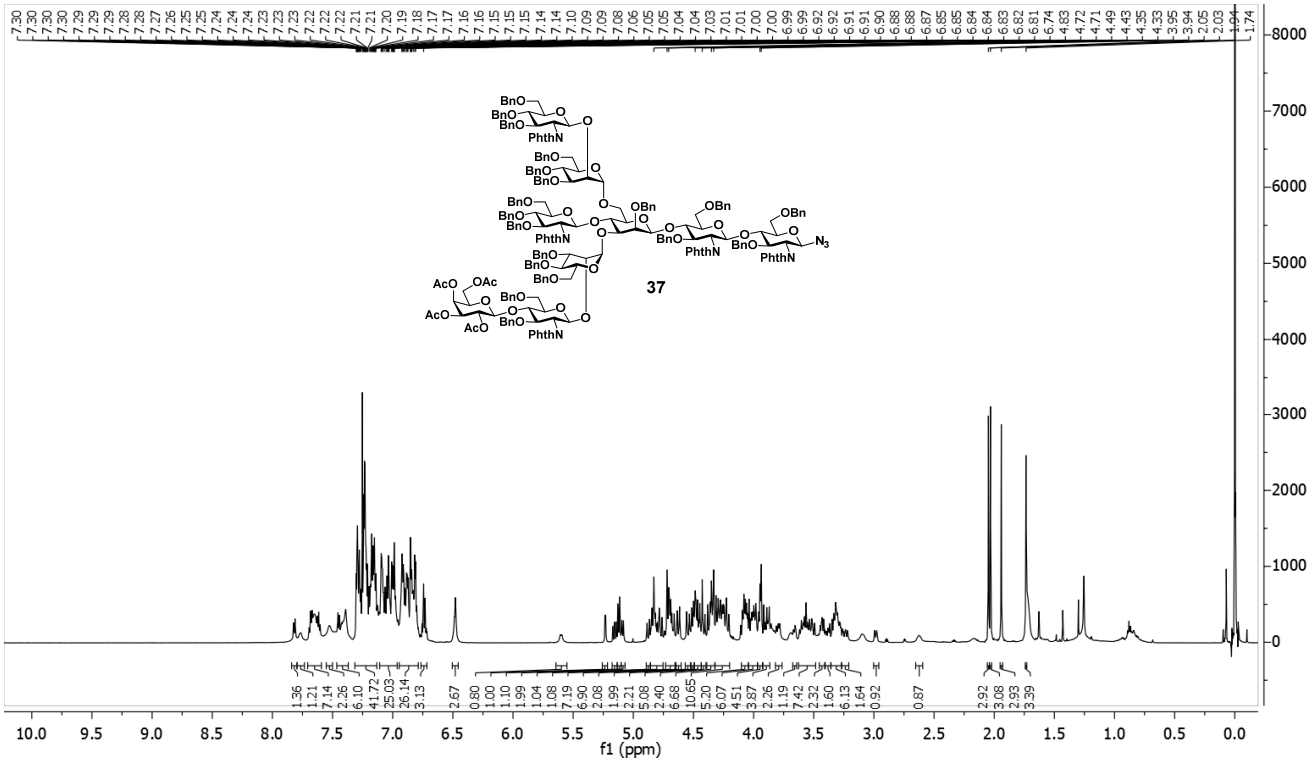


^{13}C NMR (150 MHz, $CDCl_3$)

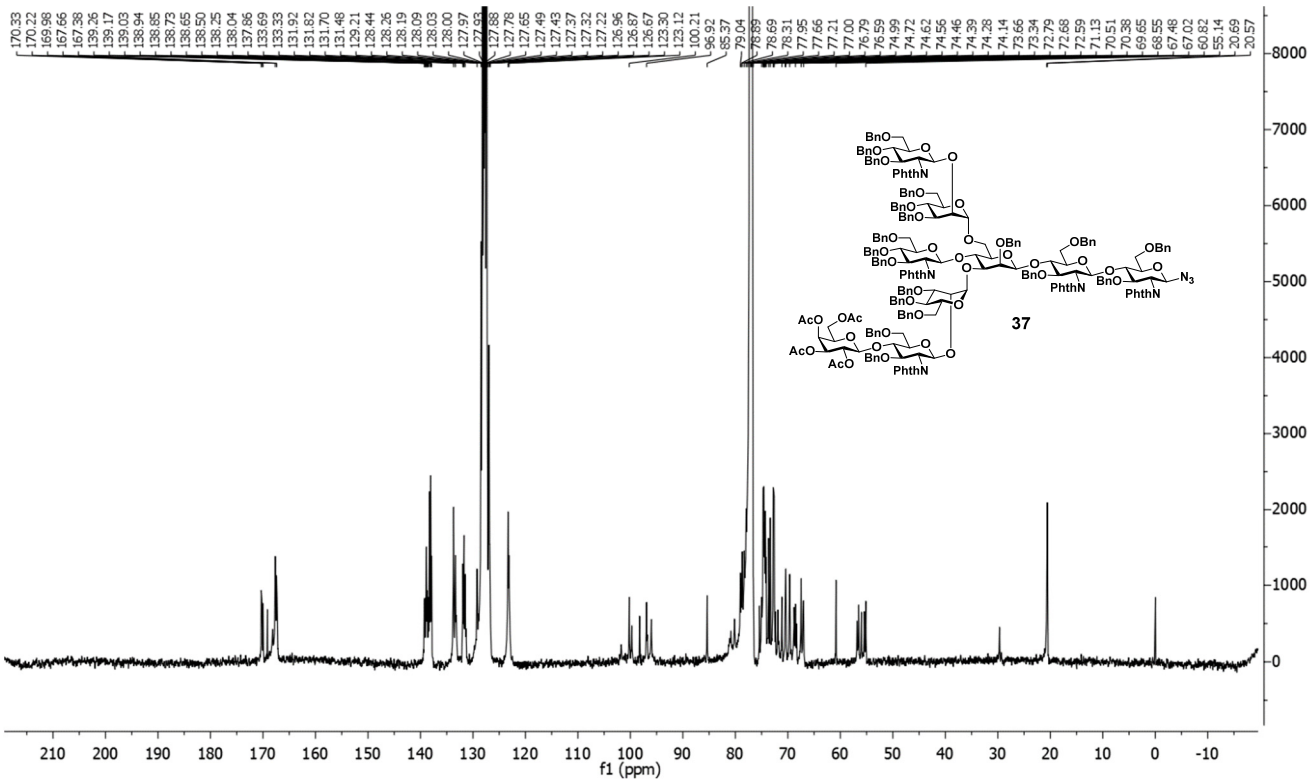


Compound 37

^{1}H NMR (600 MHz, $CDCl_3$)

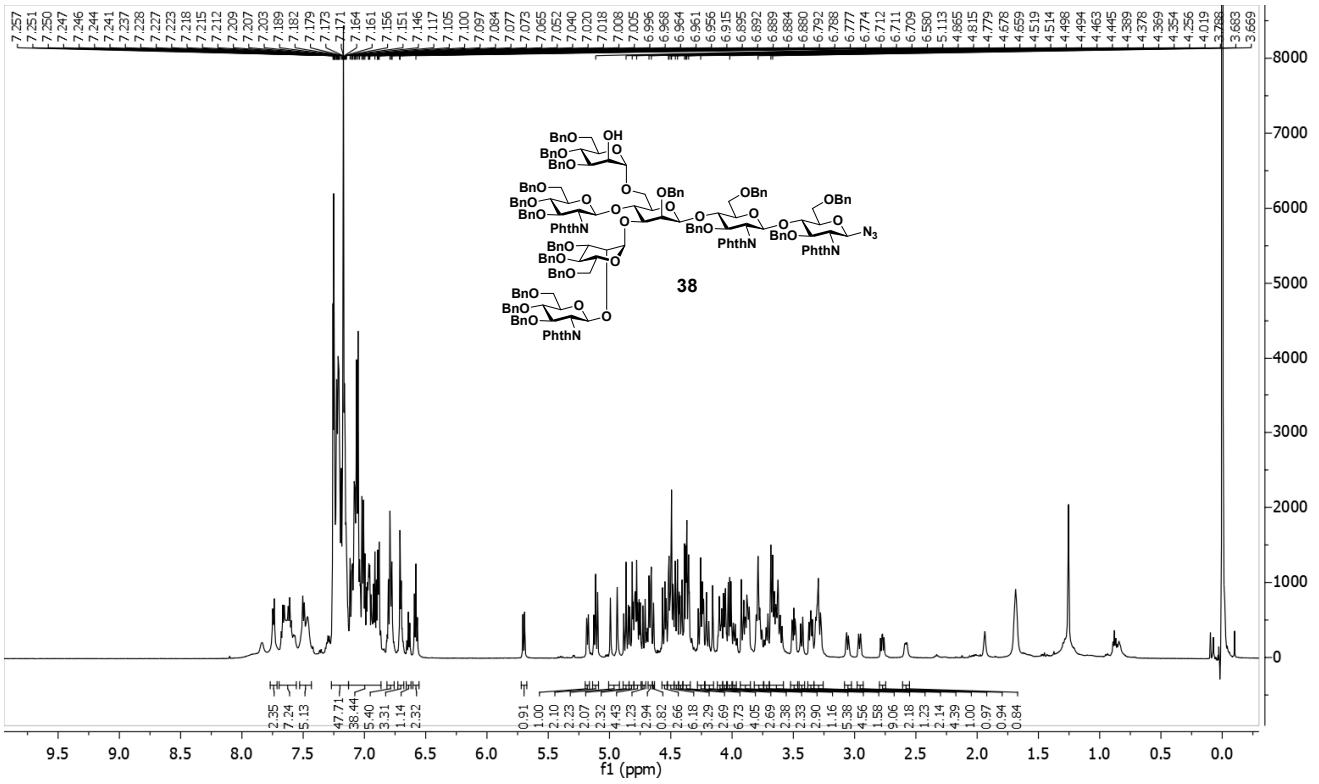


^{13}C NMR (150 MHz, $CDCl_3$)

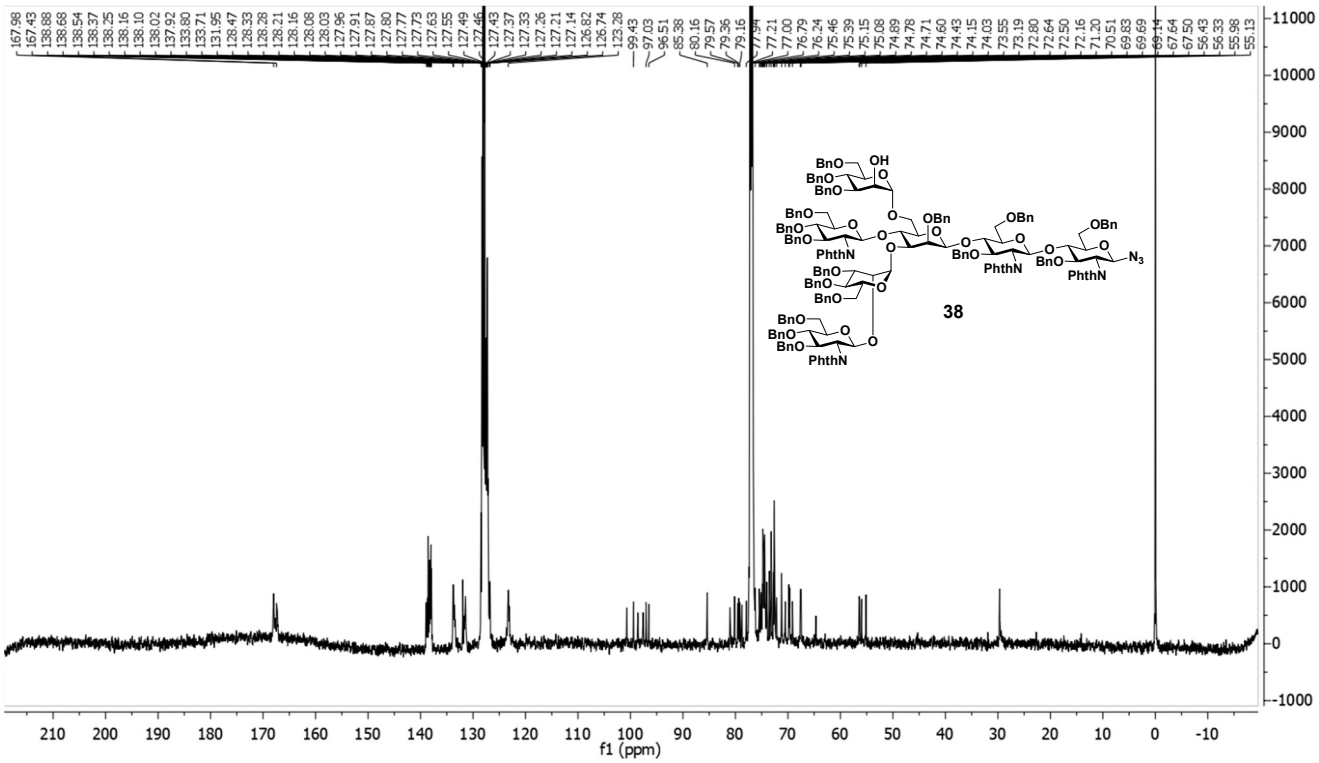


Compound 38

^{1}H NMR (600 MHz, $CDCl_3$)

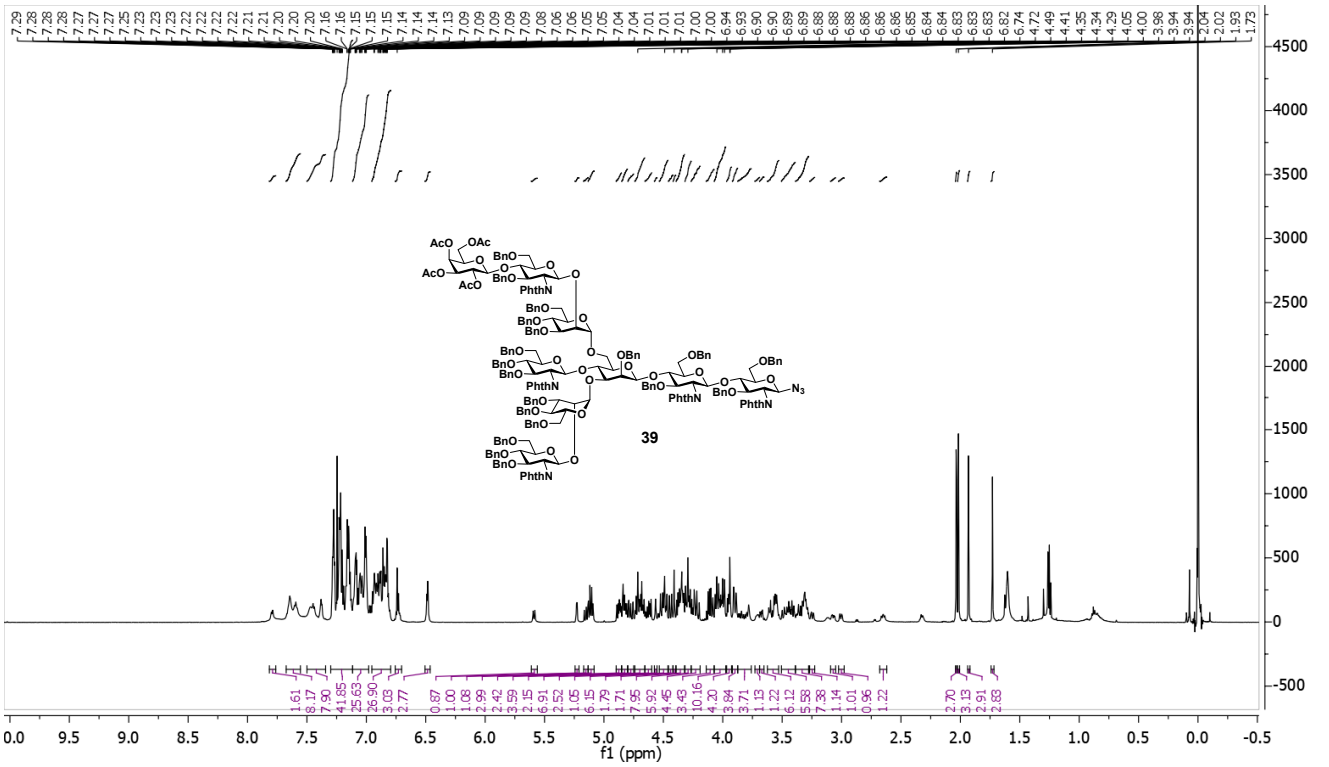


^{13}C NMR (150 MHz, $CDCl_3$)

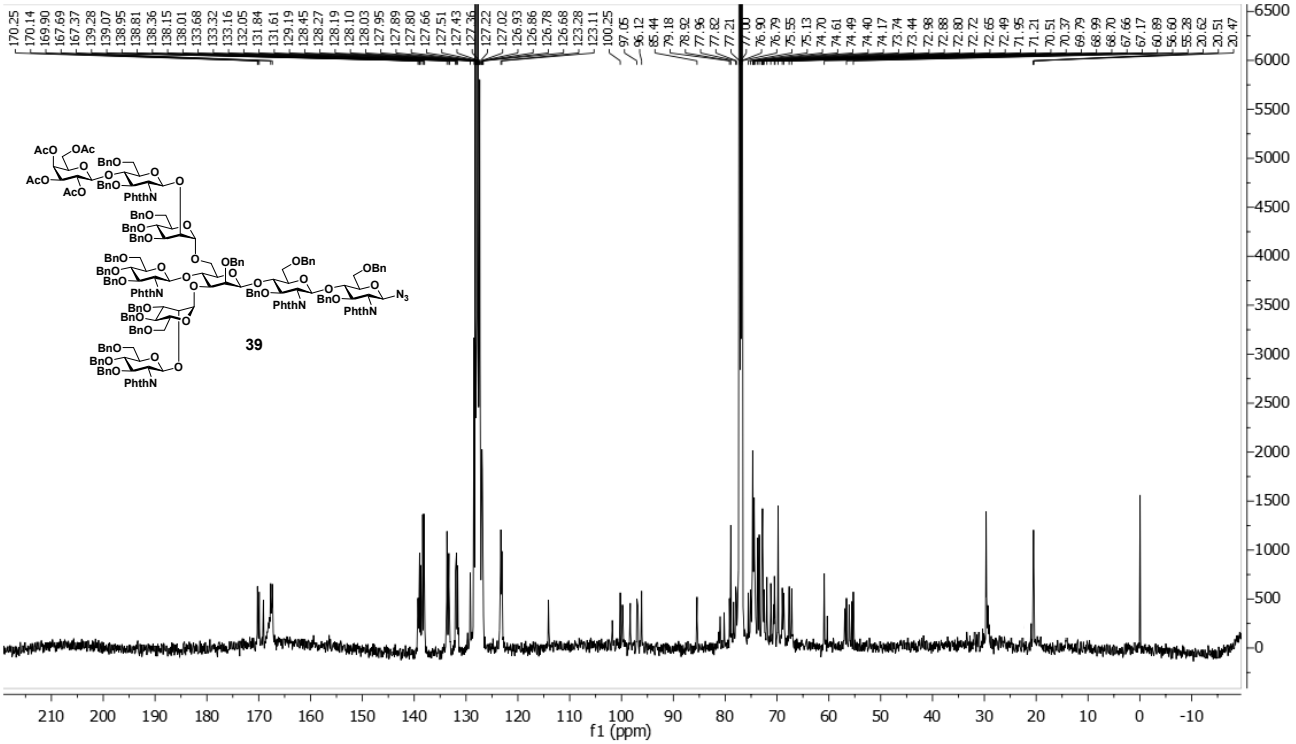


Compound 39

^{1}H NMR (600 MHz, $CDCl_3$)

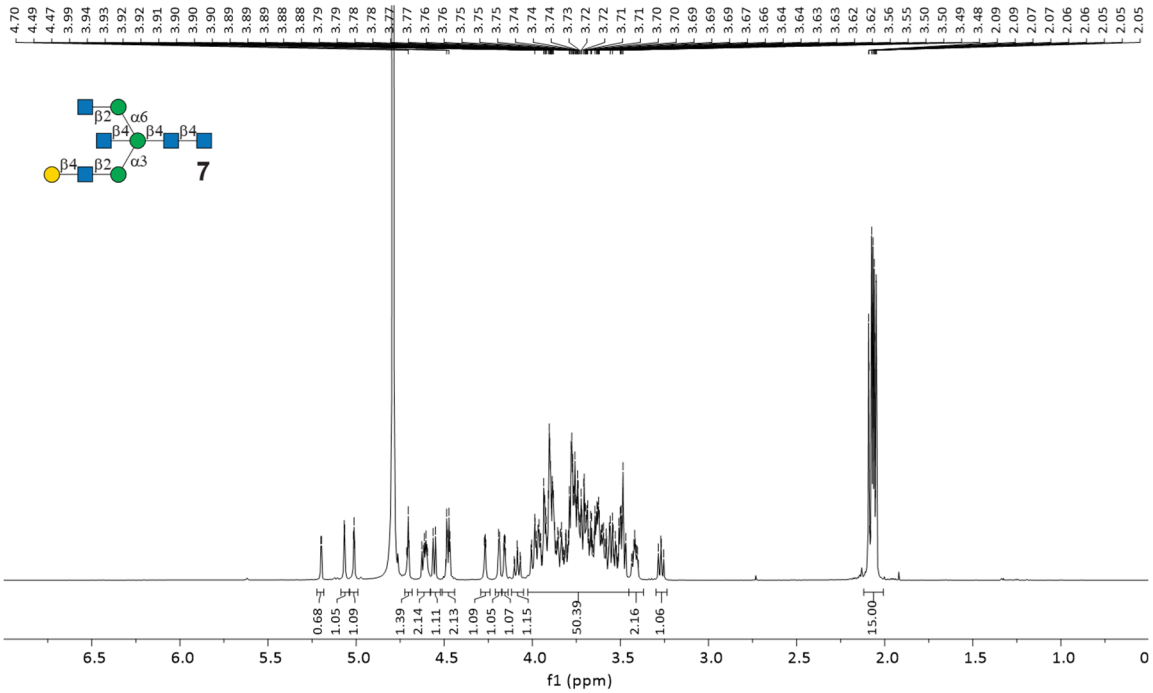


^{13}C NMR (150 MHz, $CDCl_3$)

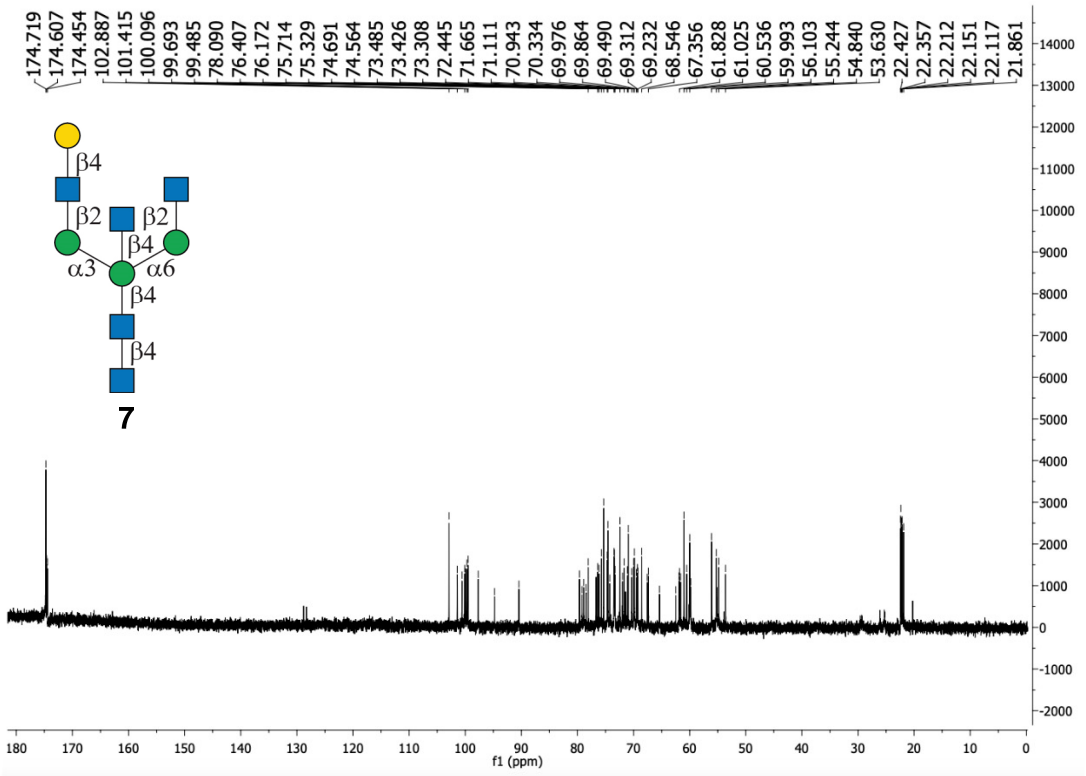


Compound 7

^{1}H NMR (600 MHz, D_2O)

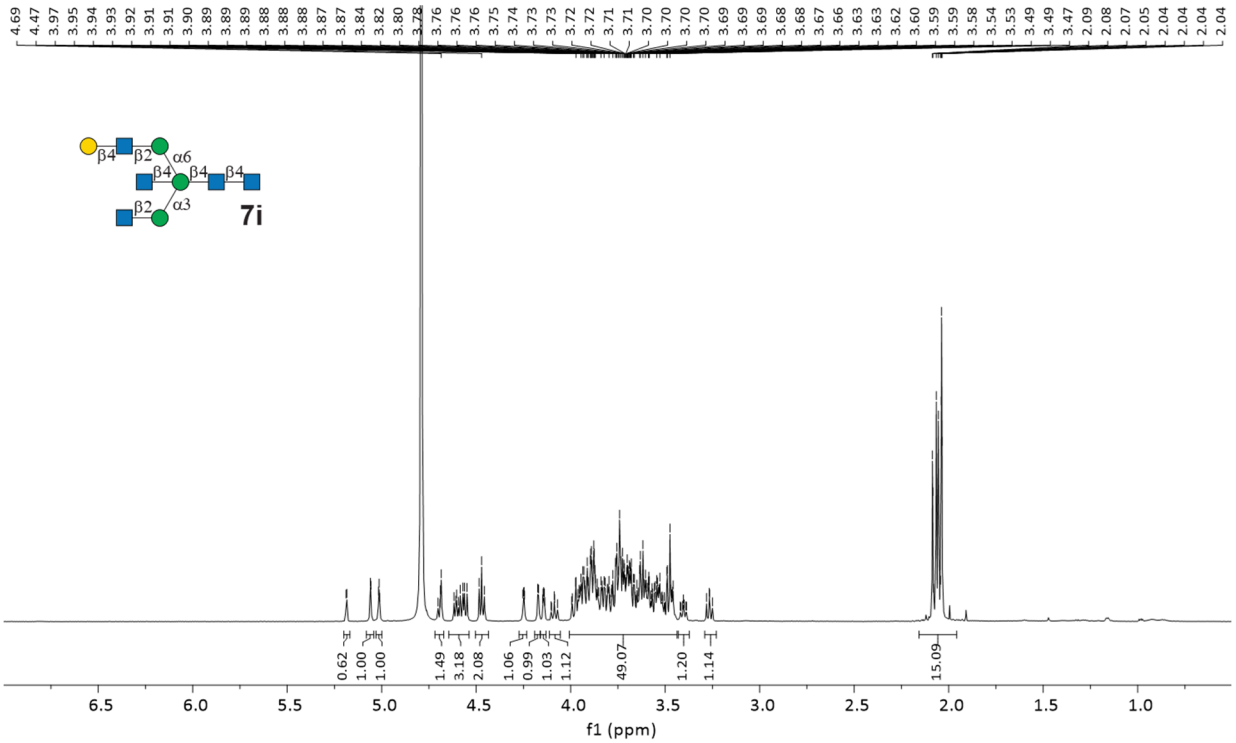


^{13}C NMR (150 MHz, D_2O)

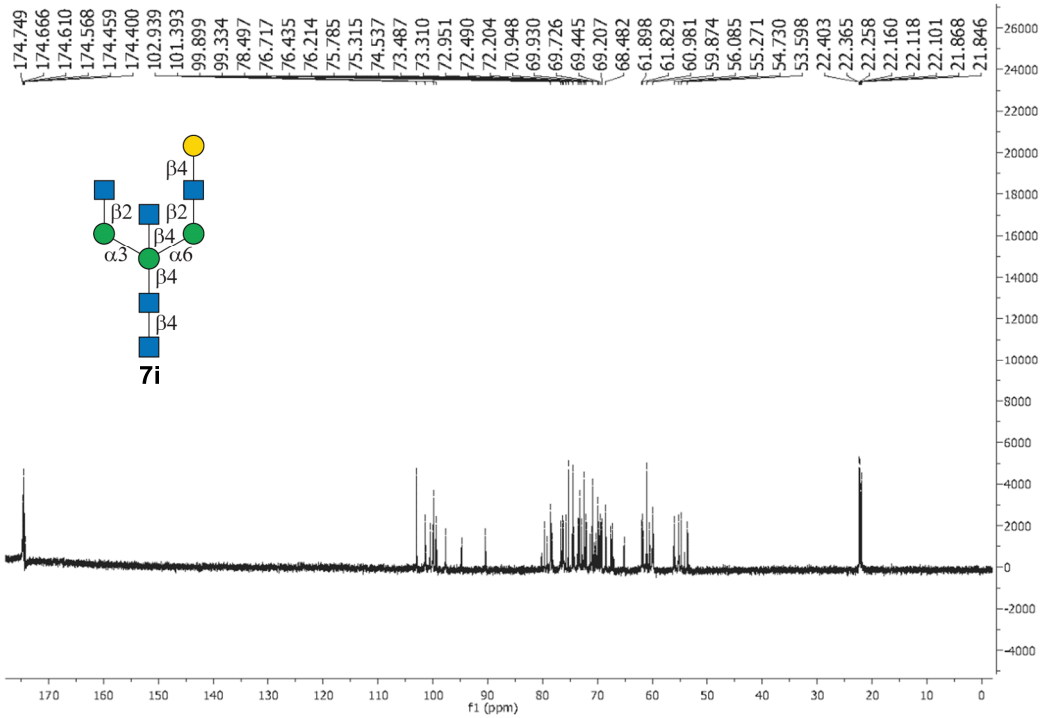


Compound 7i

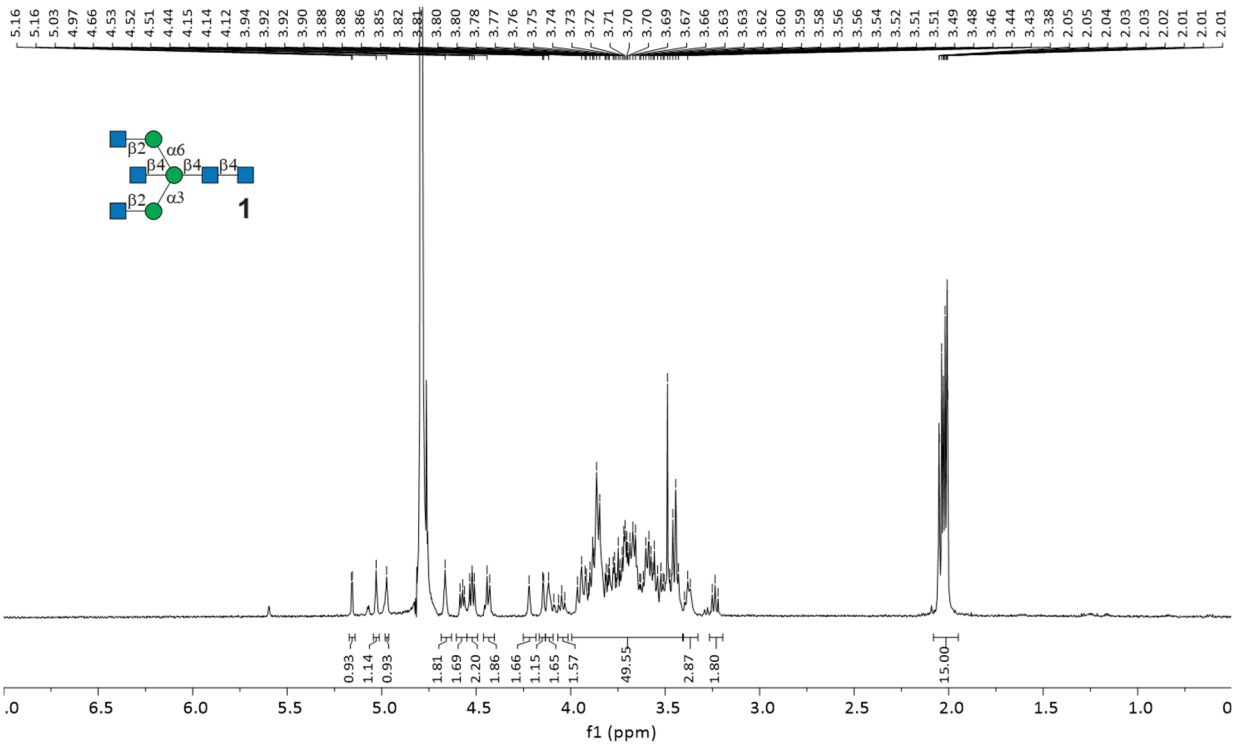
^{1}H NMR (600 MHz, D_2O)



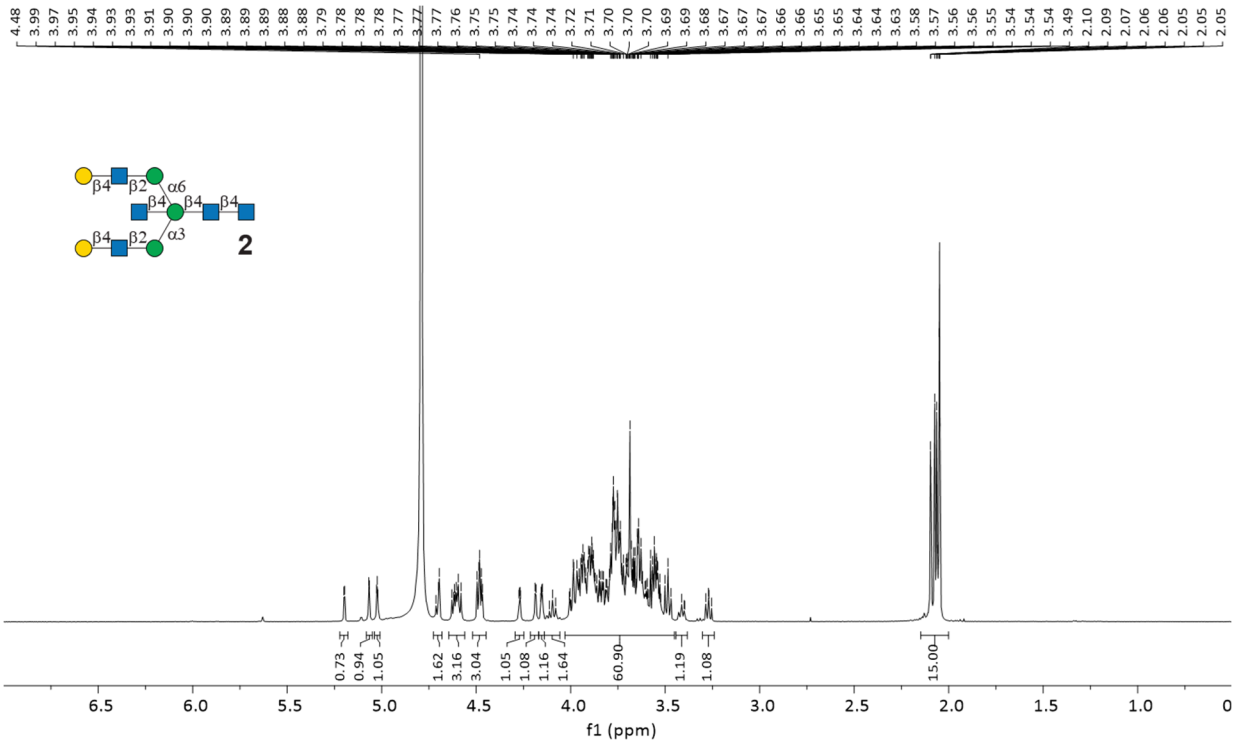
^{13}C NMR (150 MHz, D_2O)



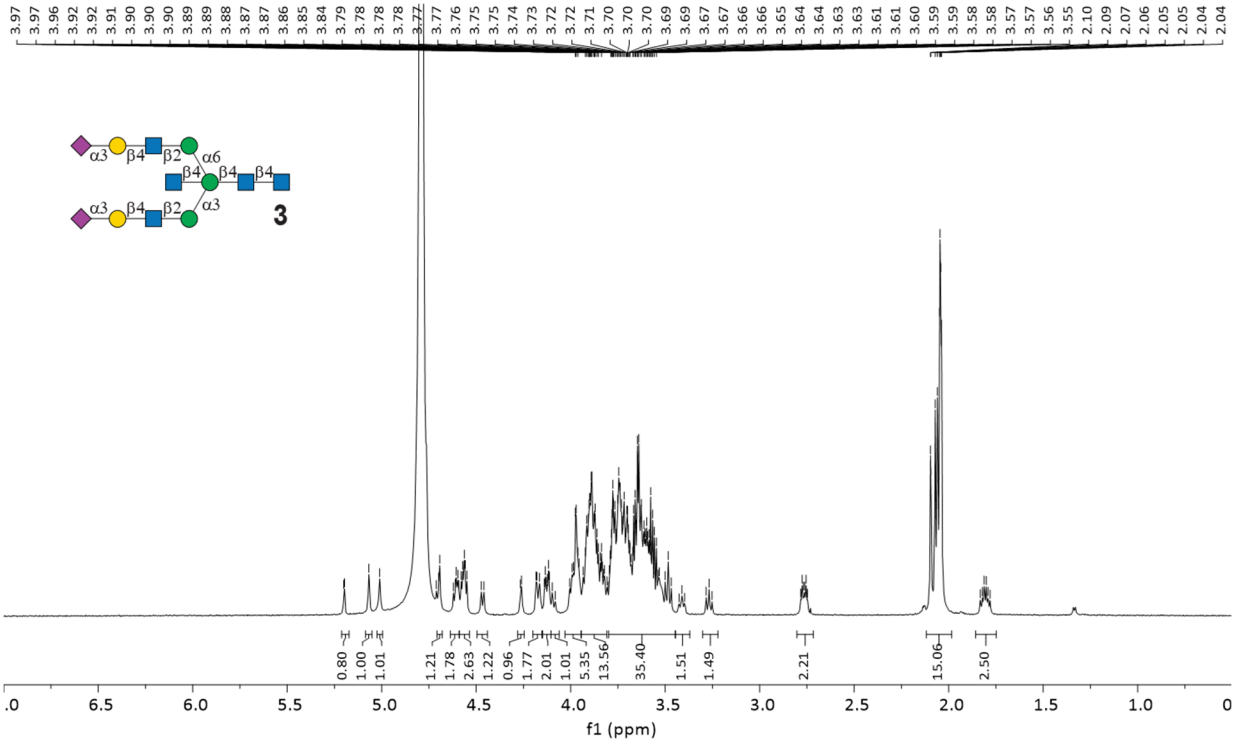
^{1}H NMR (600 MHz, D_2O) of compound **1**



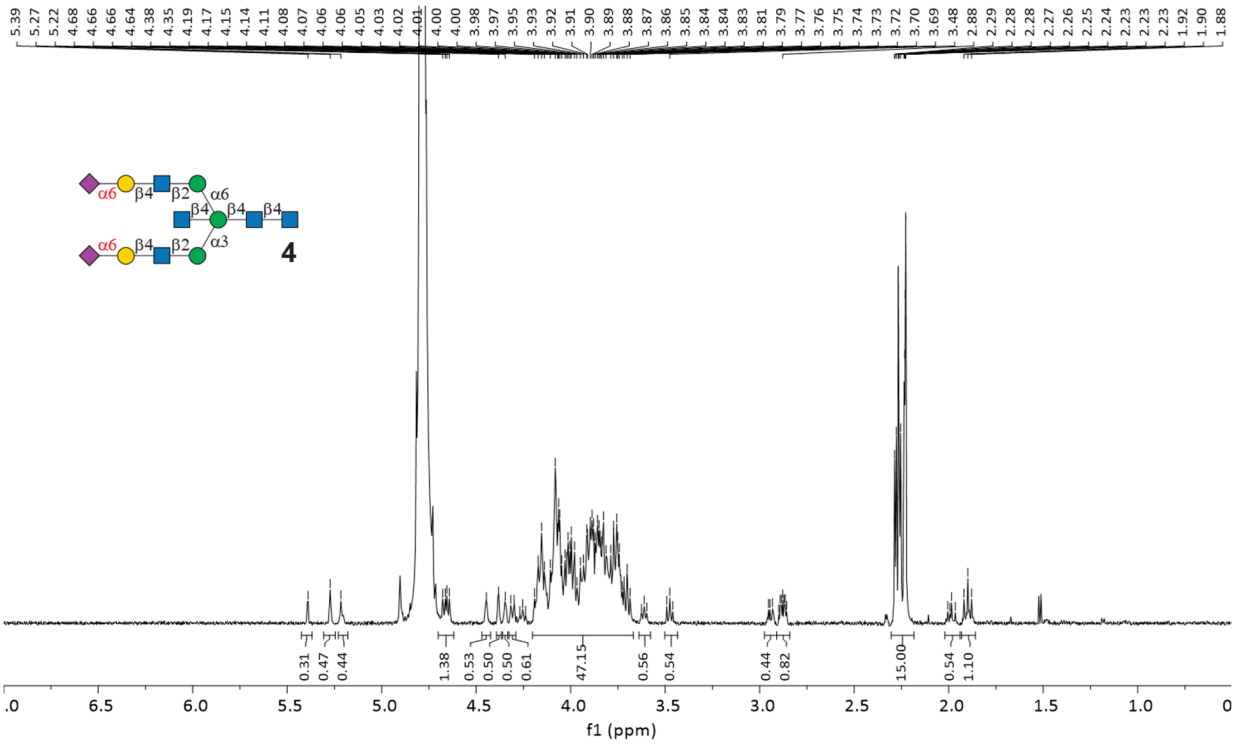
^{1}H NMR (600 MHz, D_2O) of compound **2**



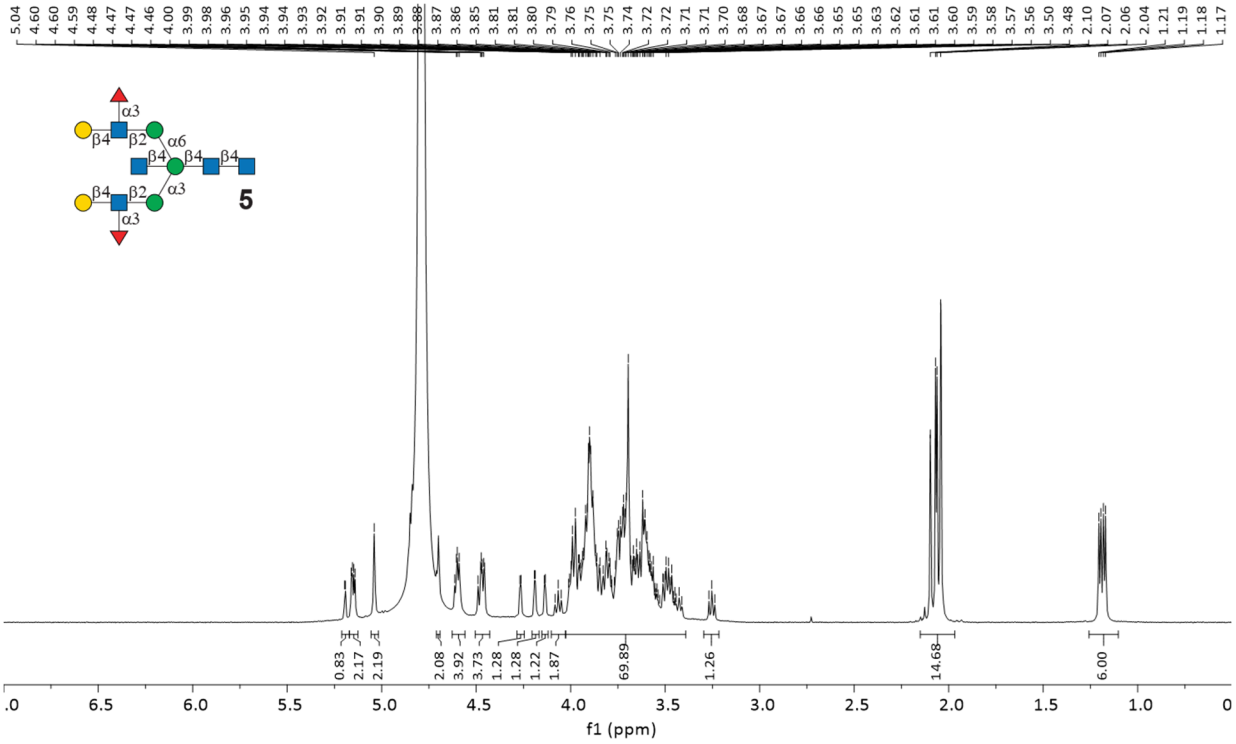
^{1}H NMR (600 MHz, D_2O) of compound **3**



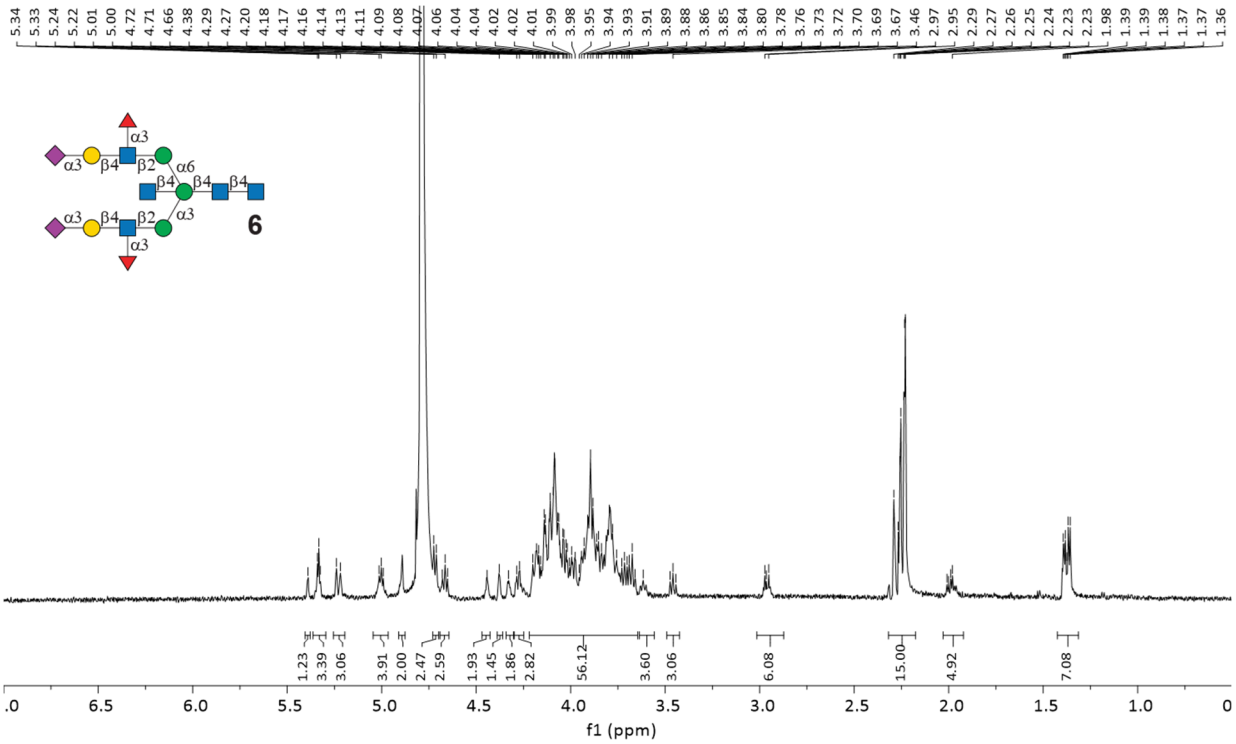
^{1}H NMR (600 MHz, D_2O) of compound **4**



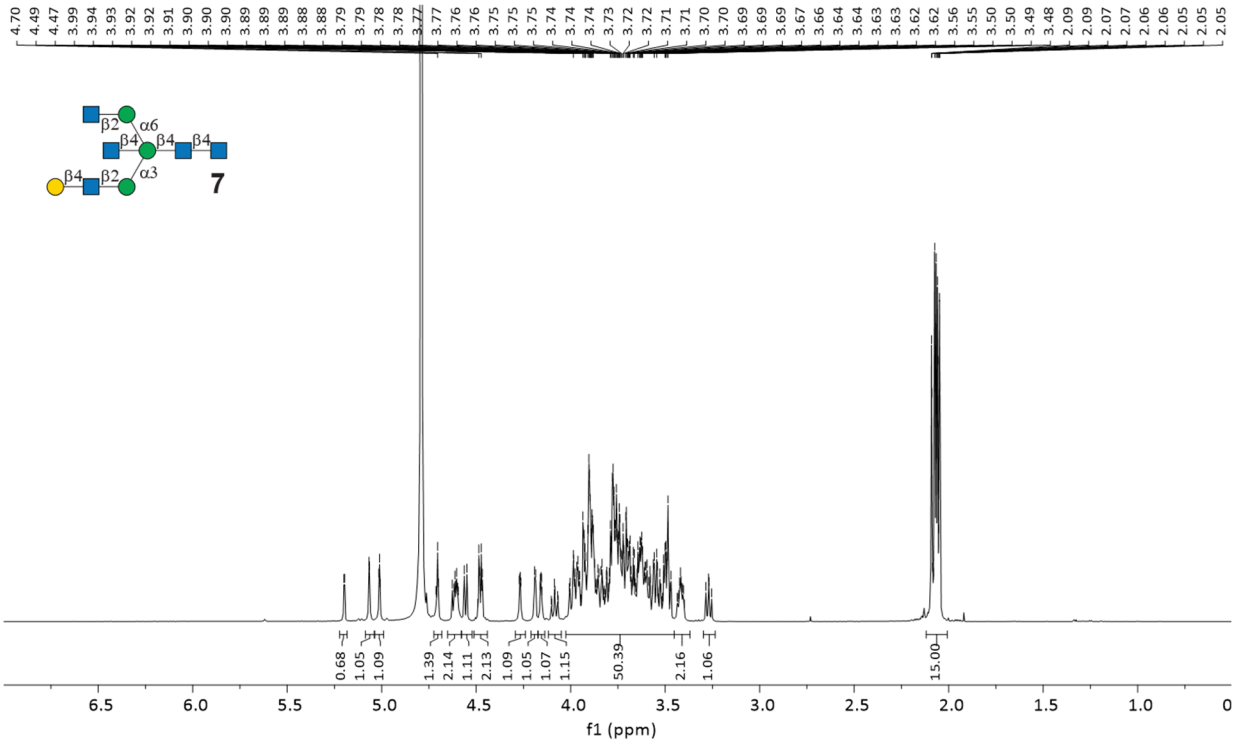
^{1}H NMR (600 MHz, D_2O) of compound **5**



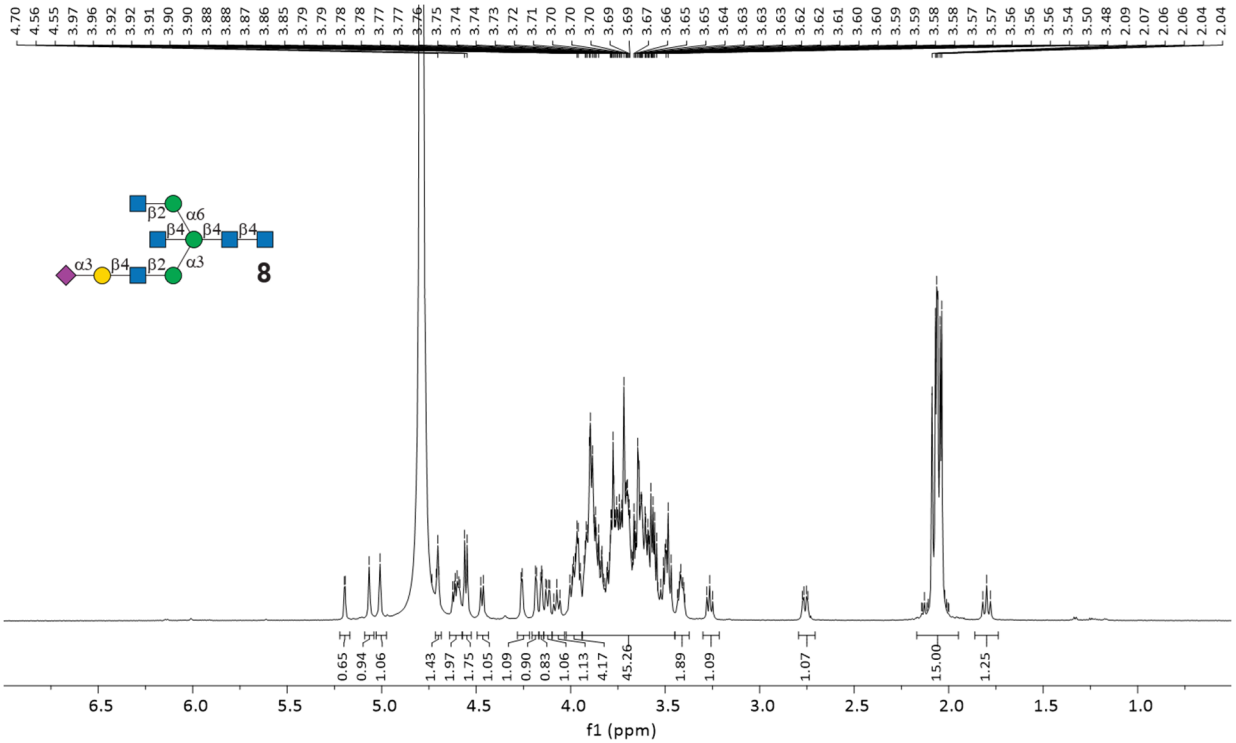
^{1}H NMR (600 MHz, D_2O) of compound **6**



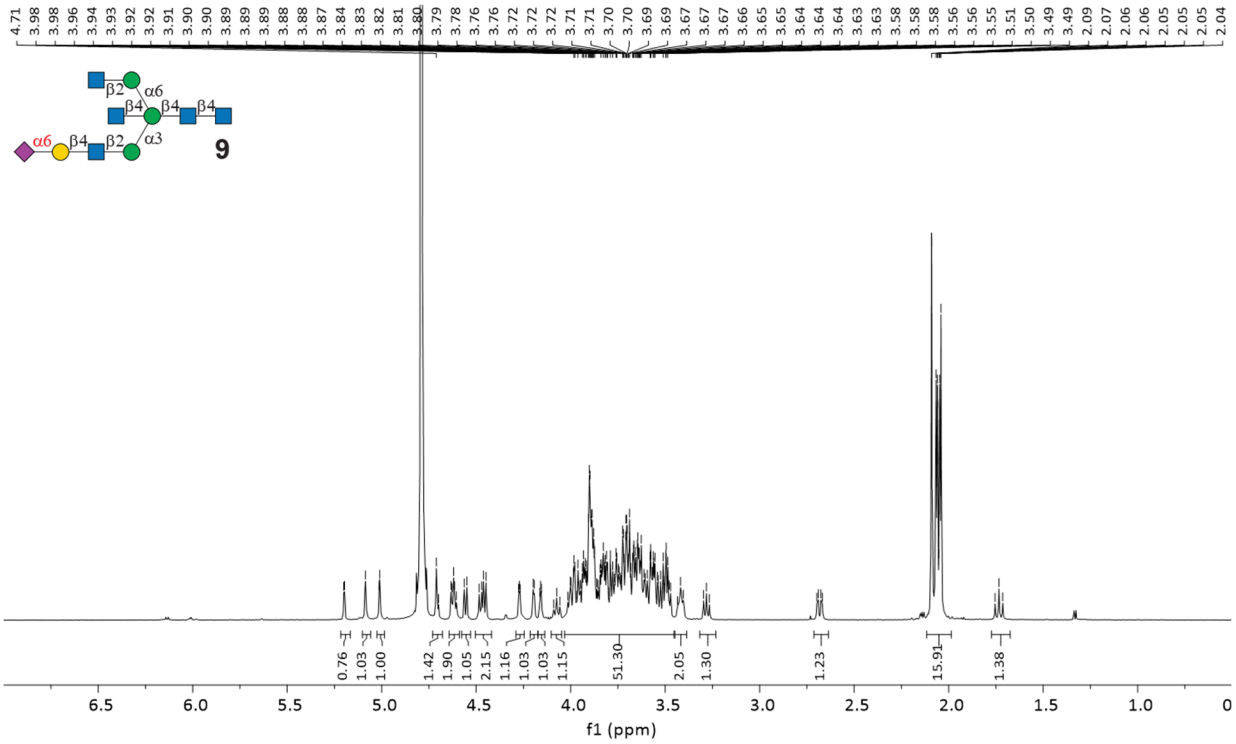
^{1}H NMR (600 MHz, D_2O) of compound **7**



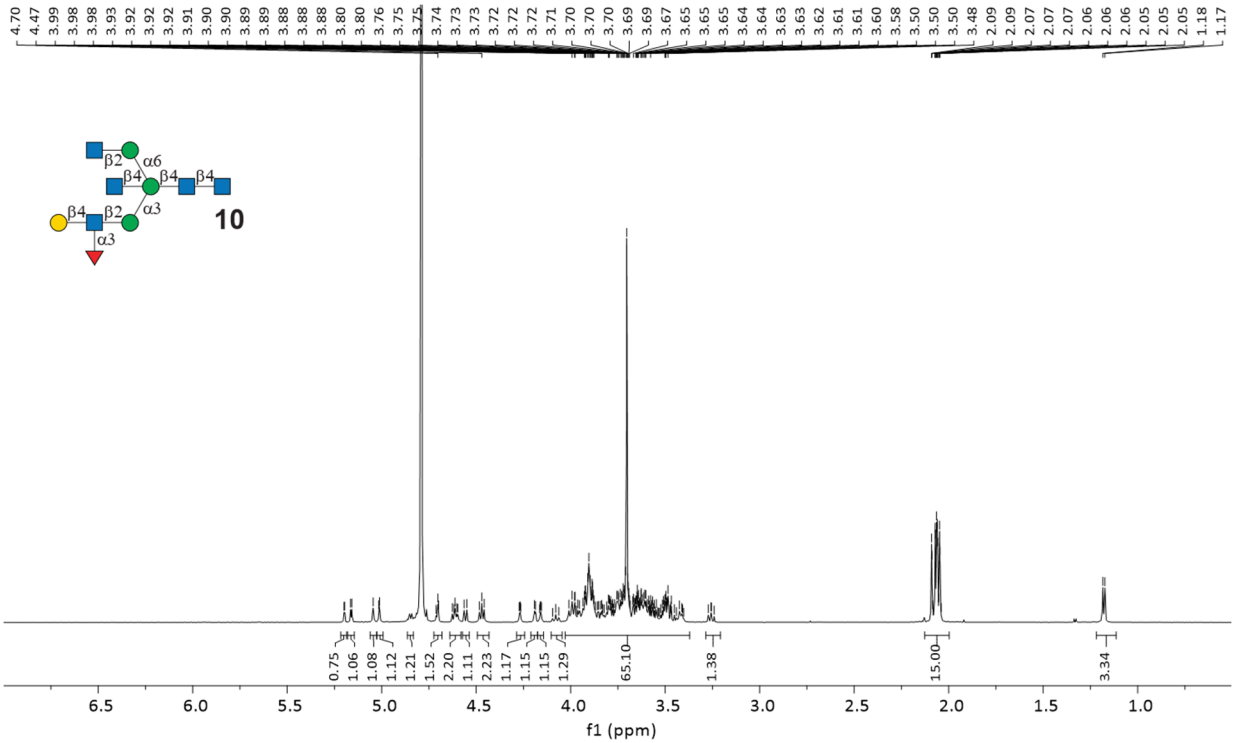
^{1}H NMR (600 MHz, D_2O) of compound **8**



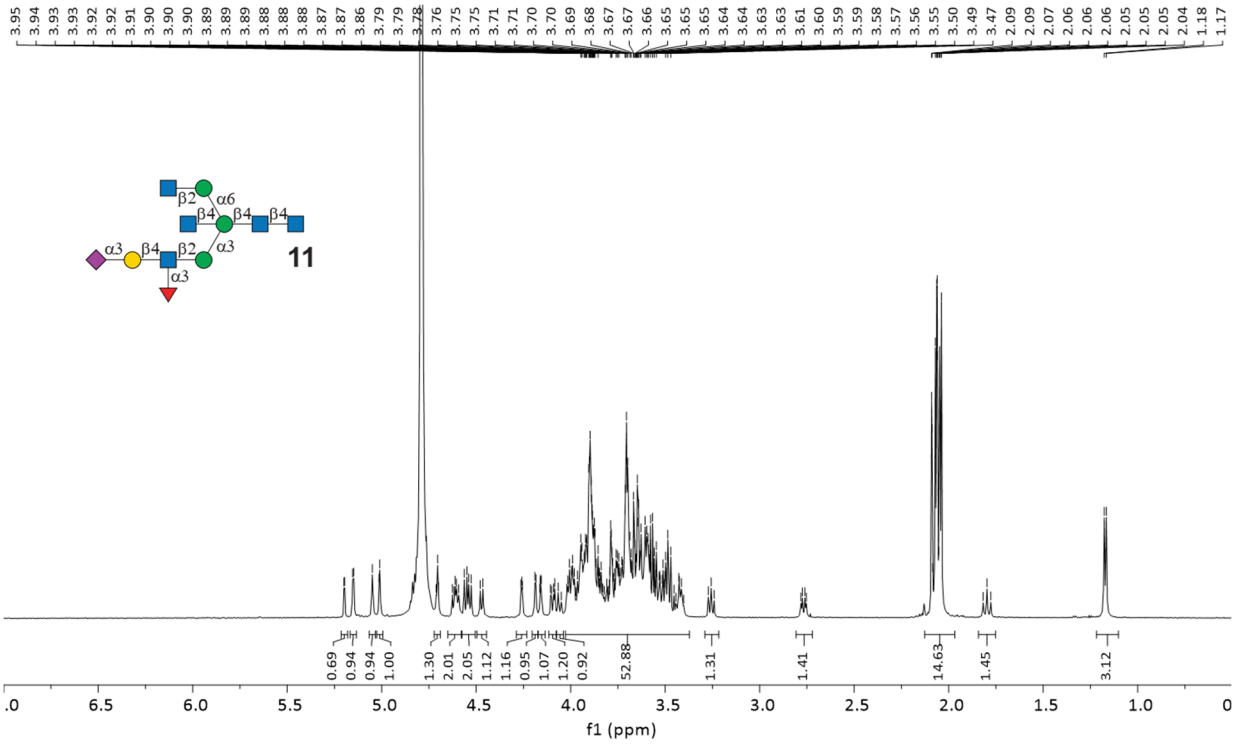
^{1}H NMR (600 MHz, D_2O) of compound **9**



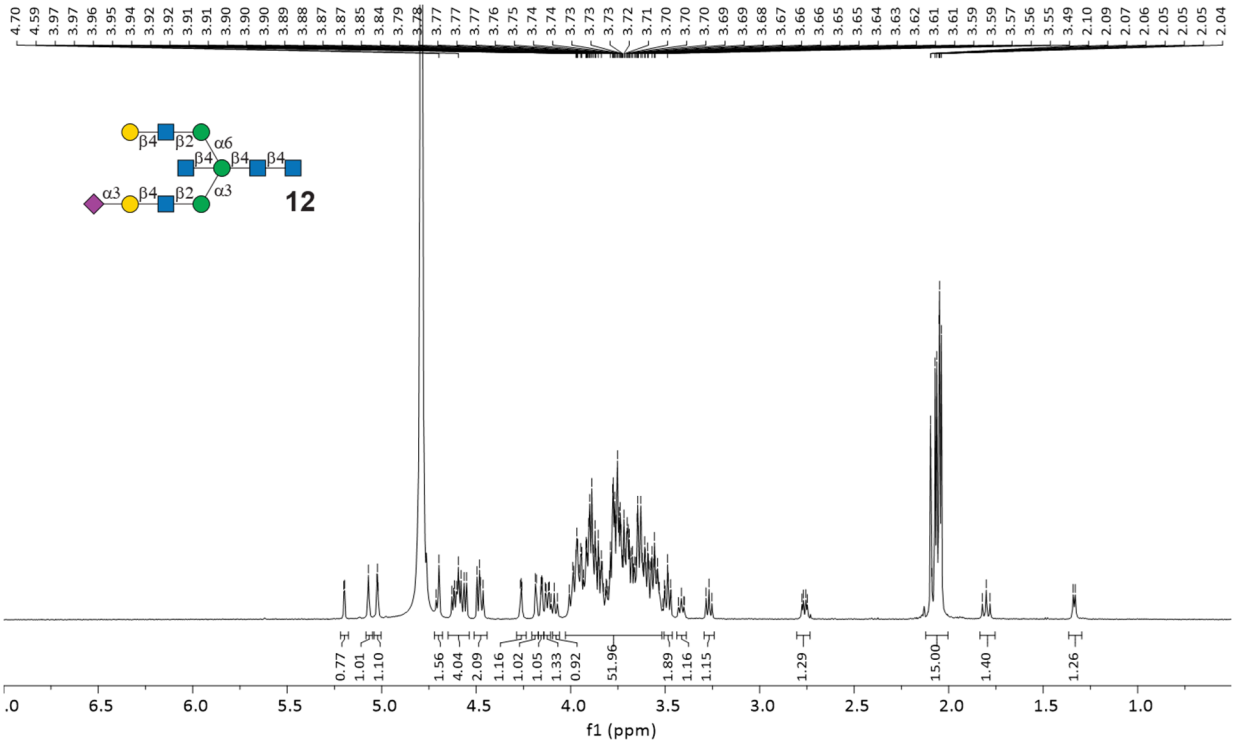
^{1}H NMR (600 MHz, D_2O) of compound **10**



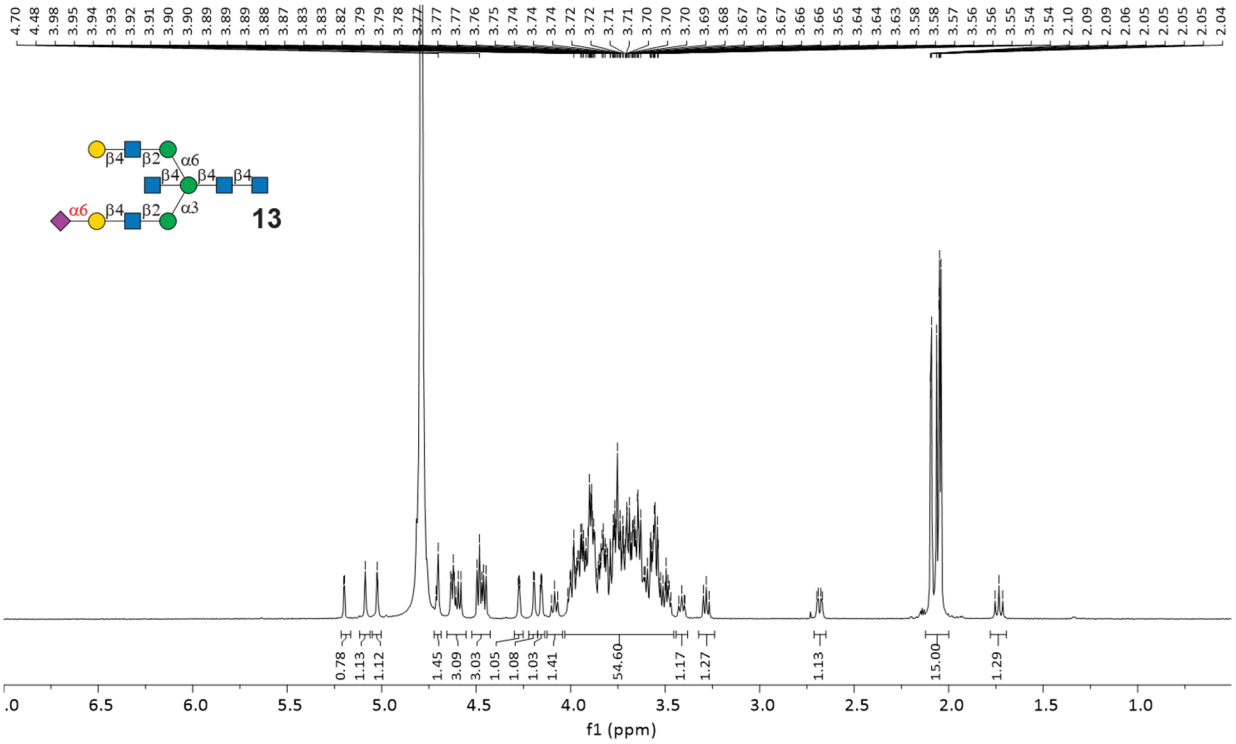
^{1}H NMR (600 MHz, D_2O) of compound **11**



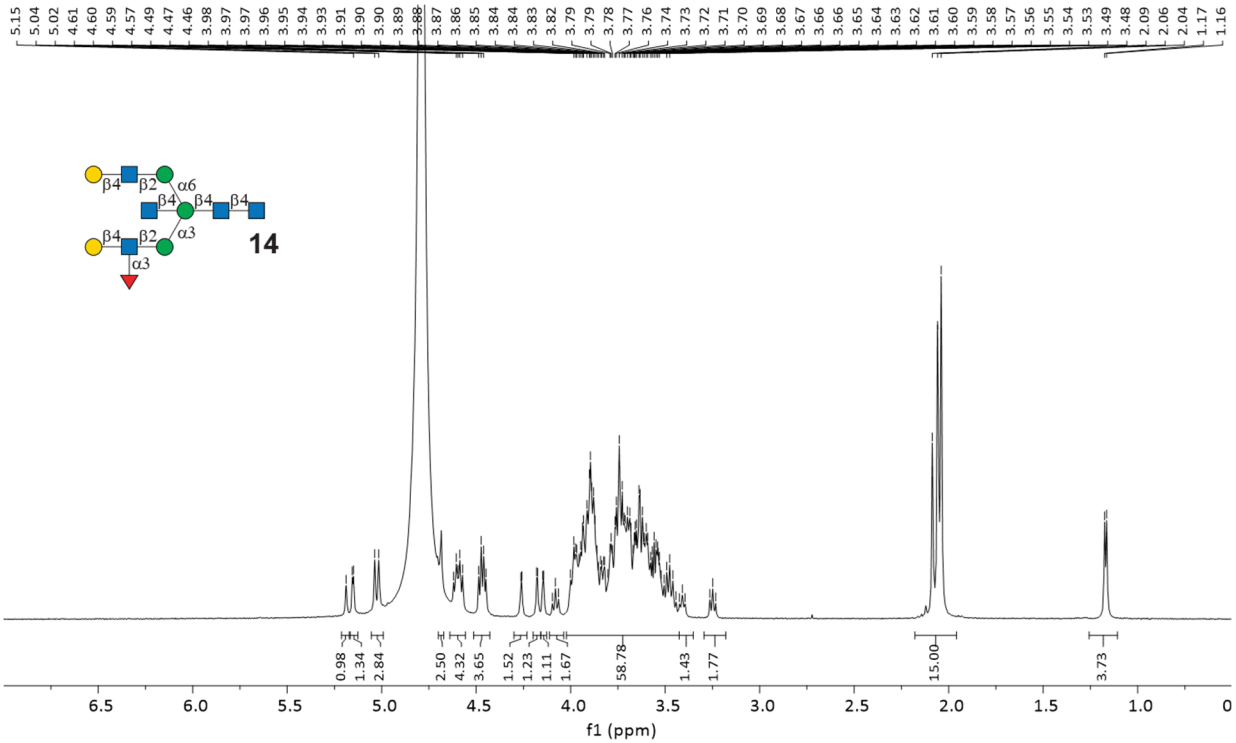
^{1}H NMR (600 MHz, D_2O) of compound **12**



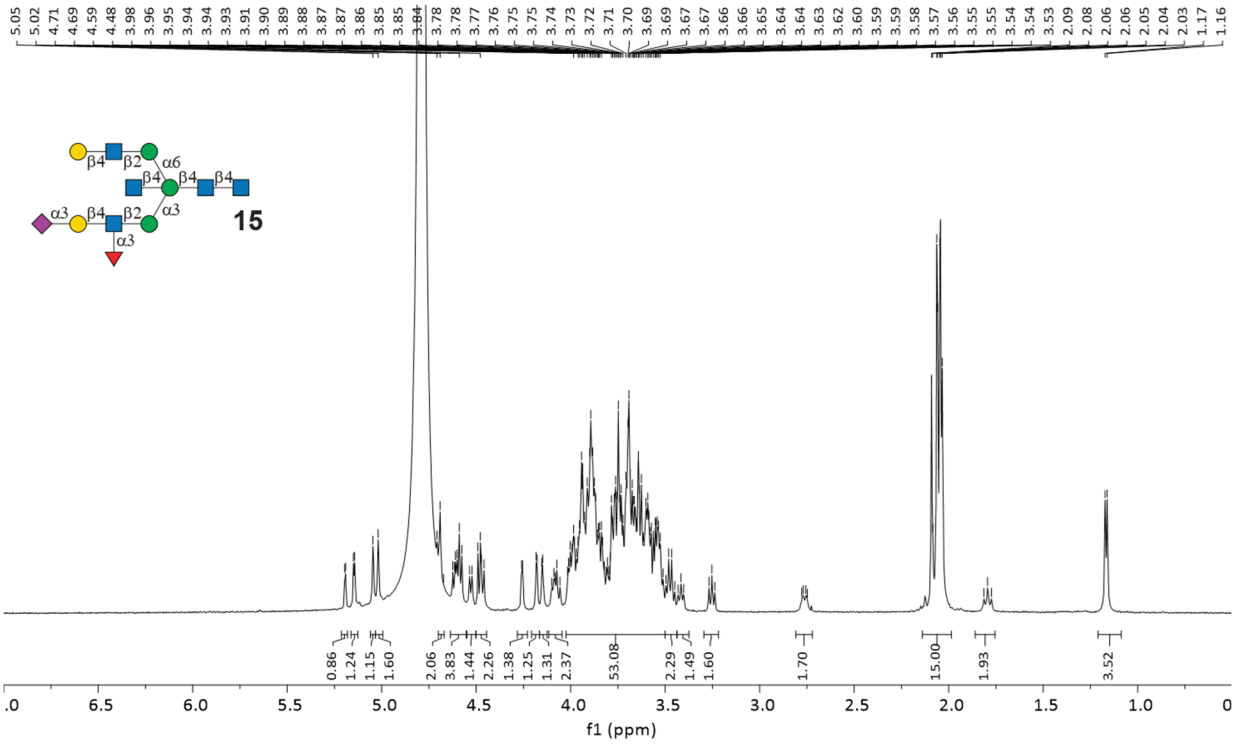
^{1}H NMR (600 MHz, D_2O) of compound **13**



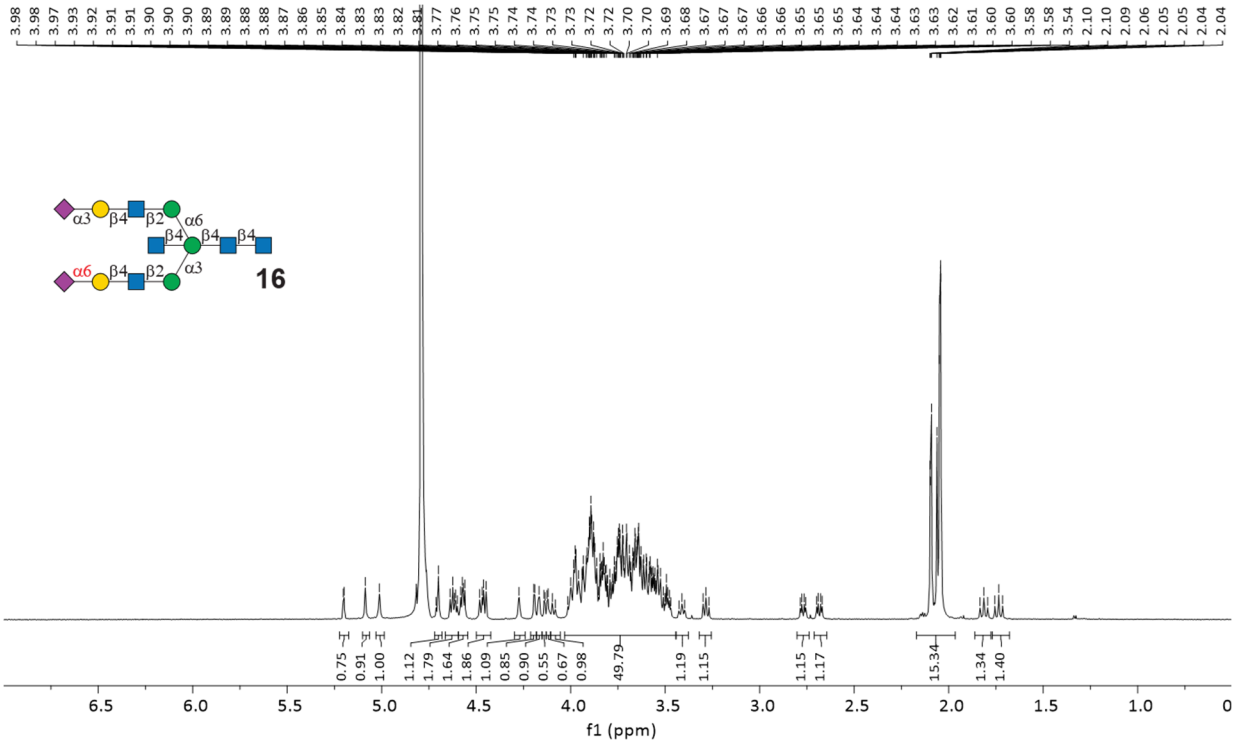
^{1}H NMR (600 MHz, D_2O) of compound **14**



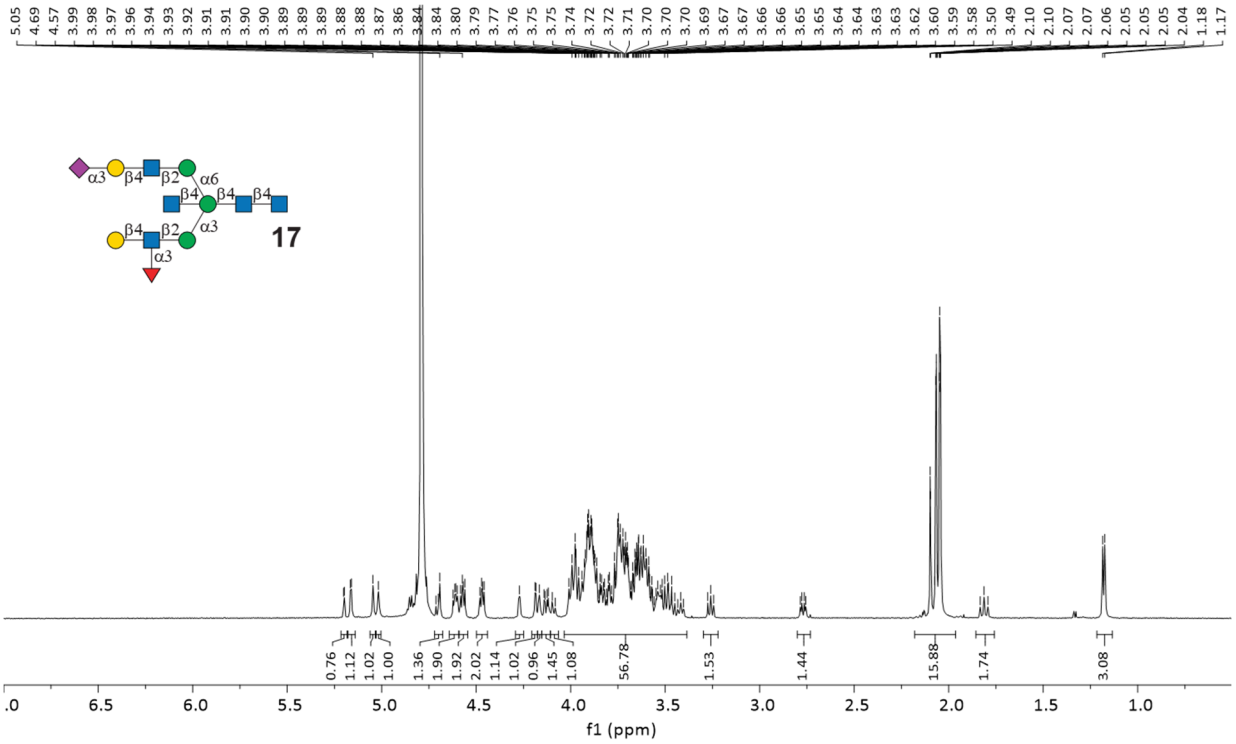
^{1}H NMR (600 MHz, D_2O) of compound **15**



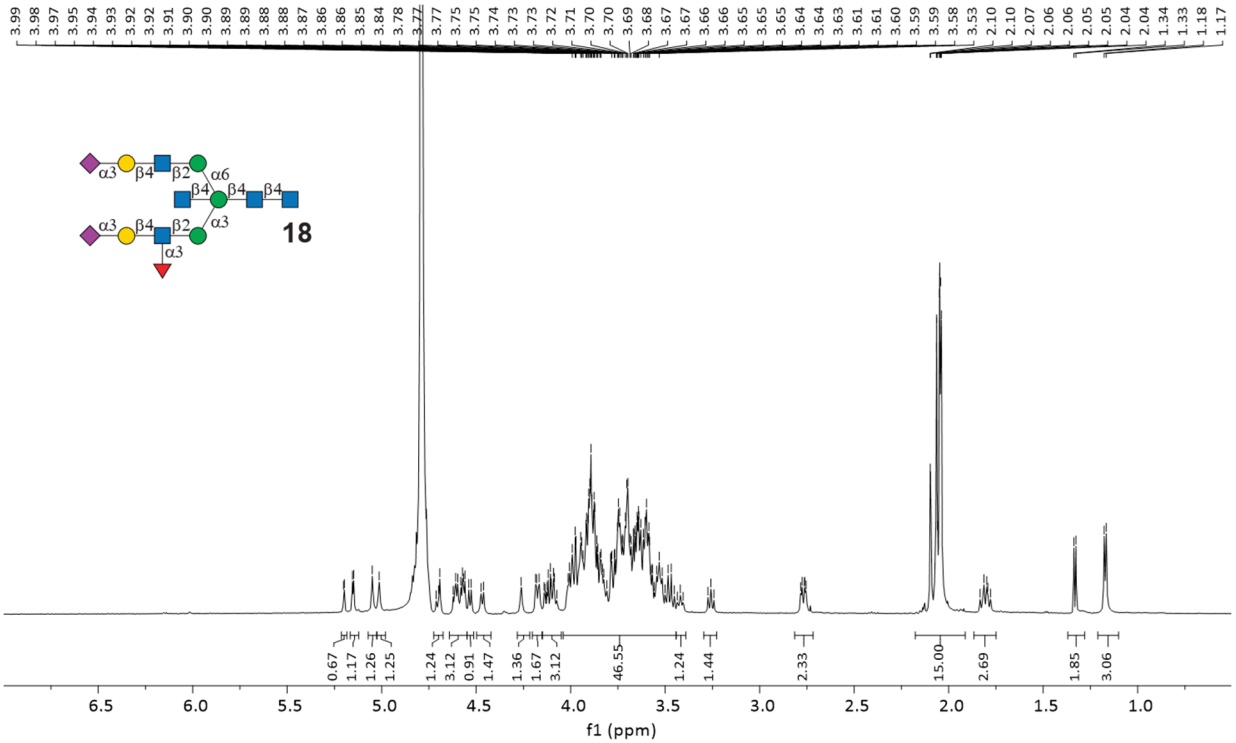
^{1}H NMR (600 MHz, D_2O) of compound **16**



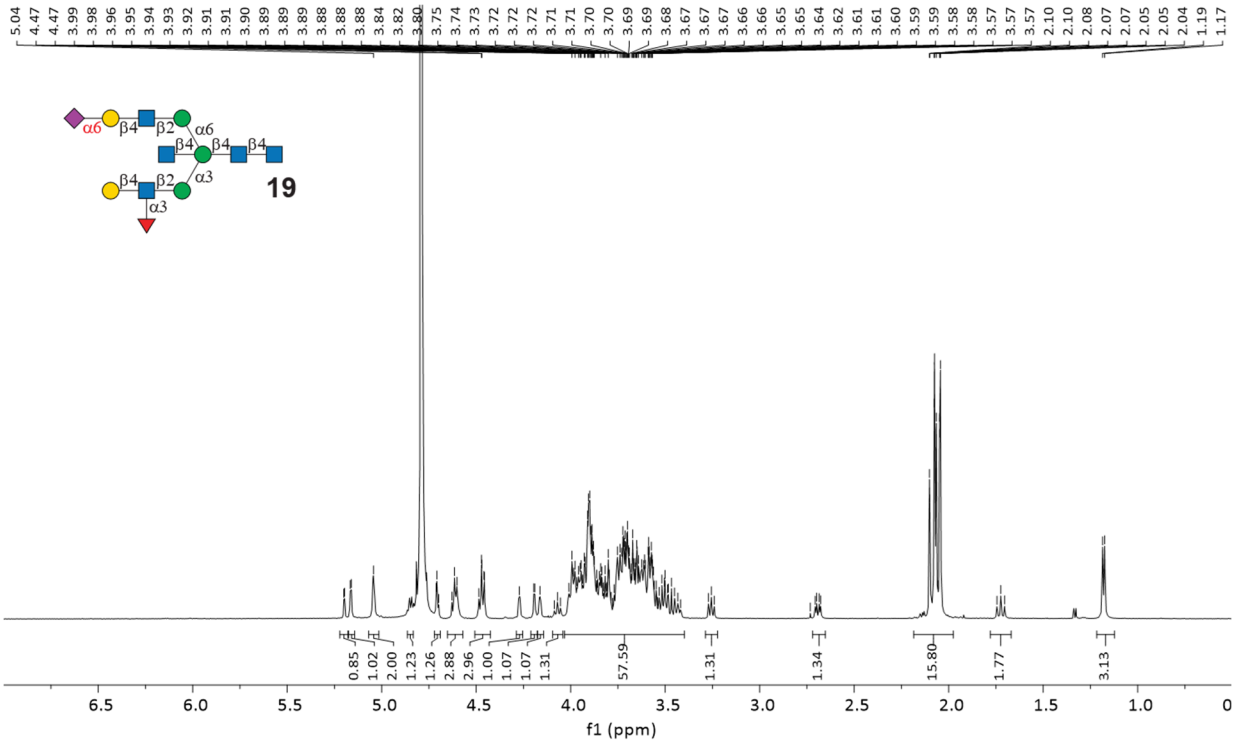
^{1}H NMR (600 MHz, D_2O) of compound **17**



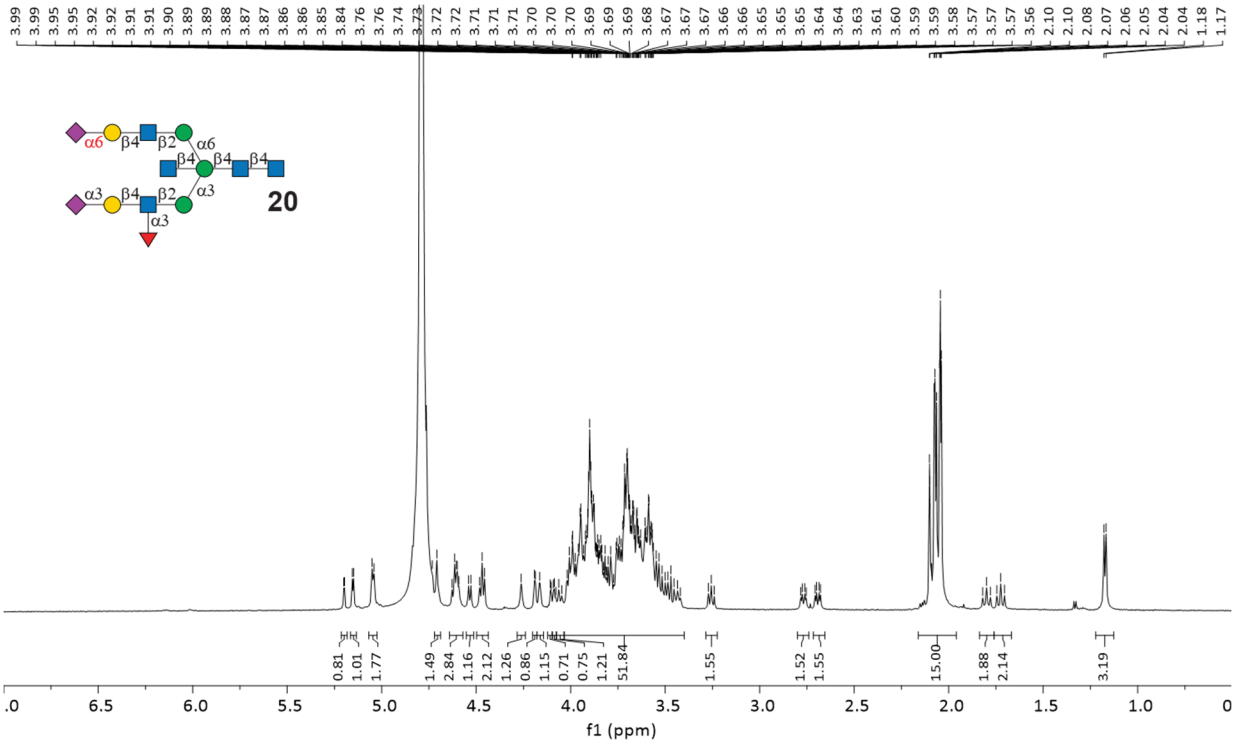
^{1}H NMR (600 MHz, D_2O) of compound **18**



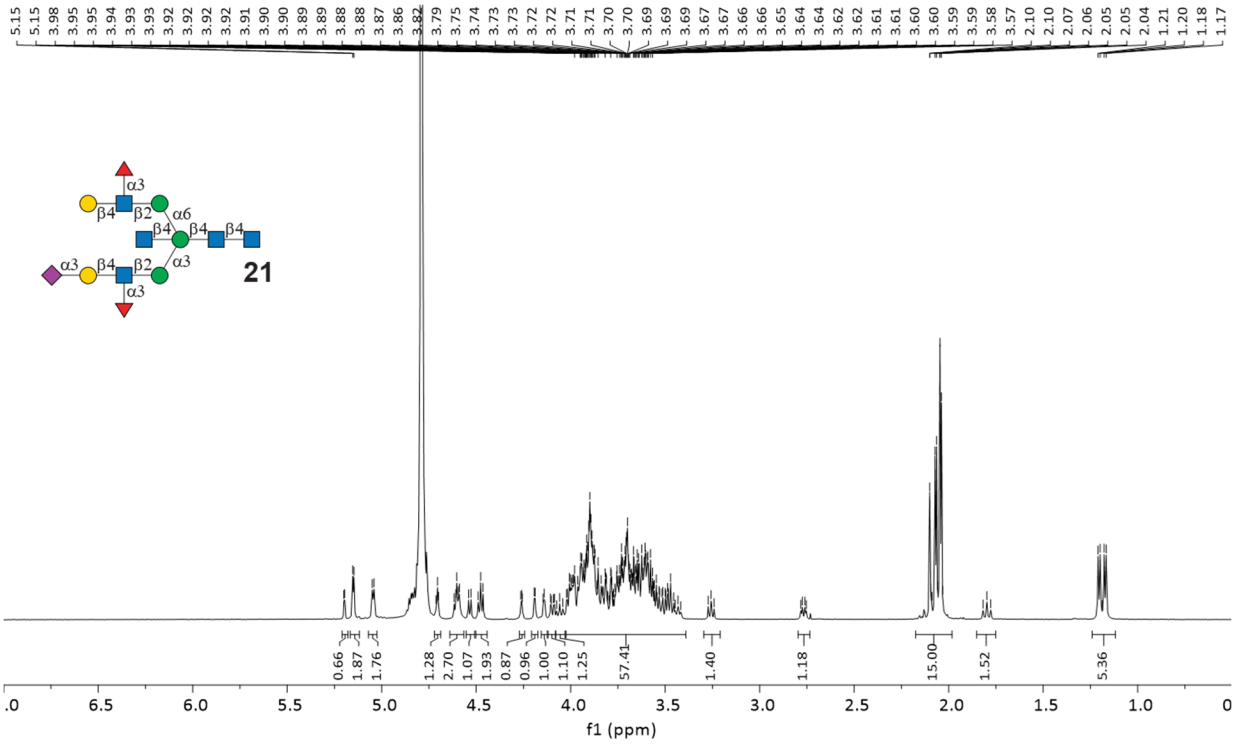
^{1}H NMR (600 MHz, D_2O) of compound **19**



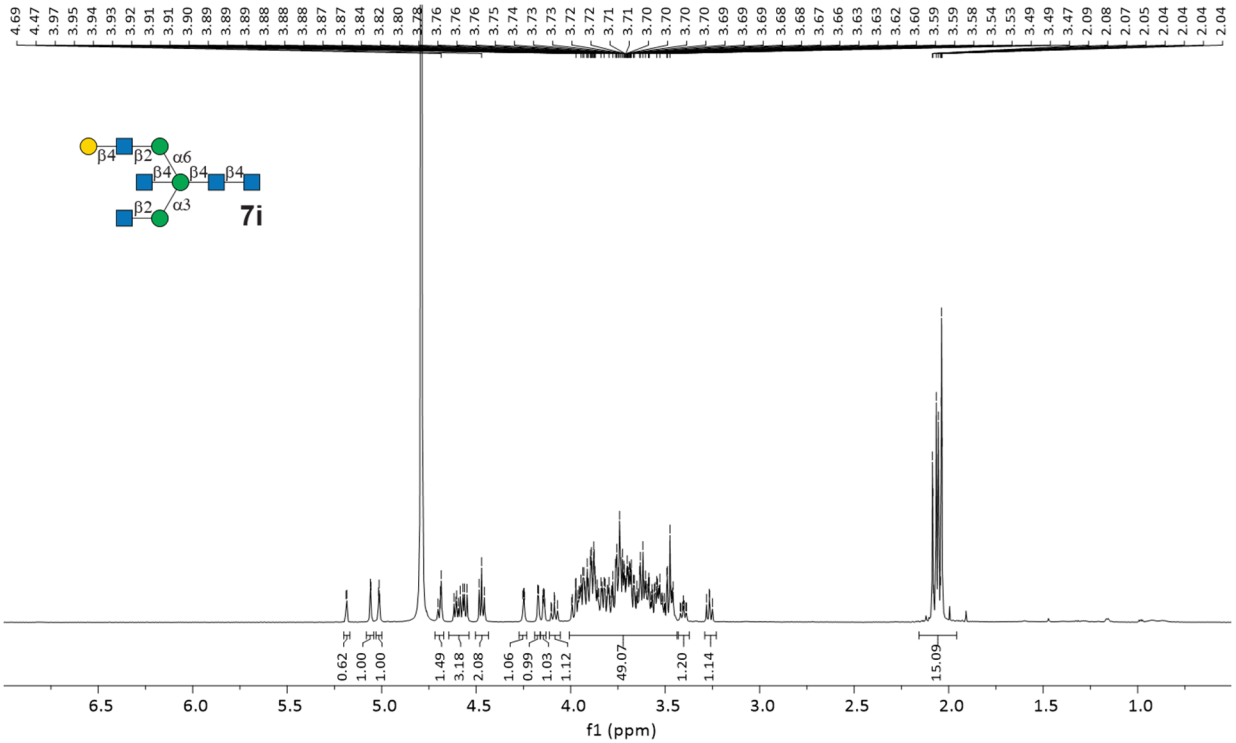
^{1}H NMR (600 MHz, D_2O) of compound **20**



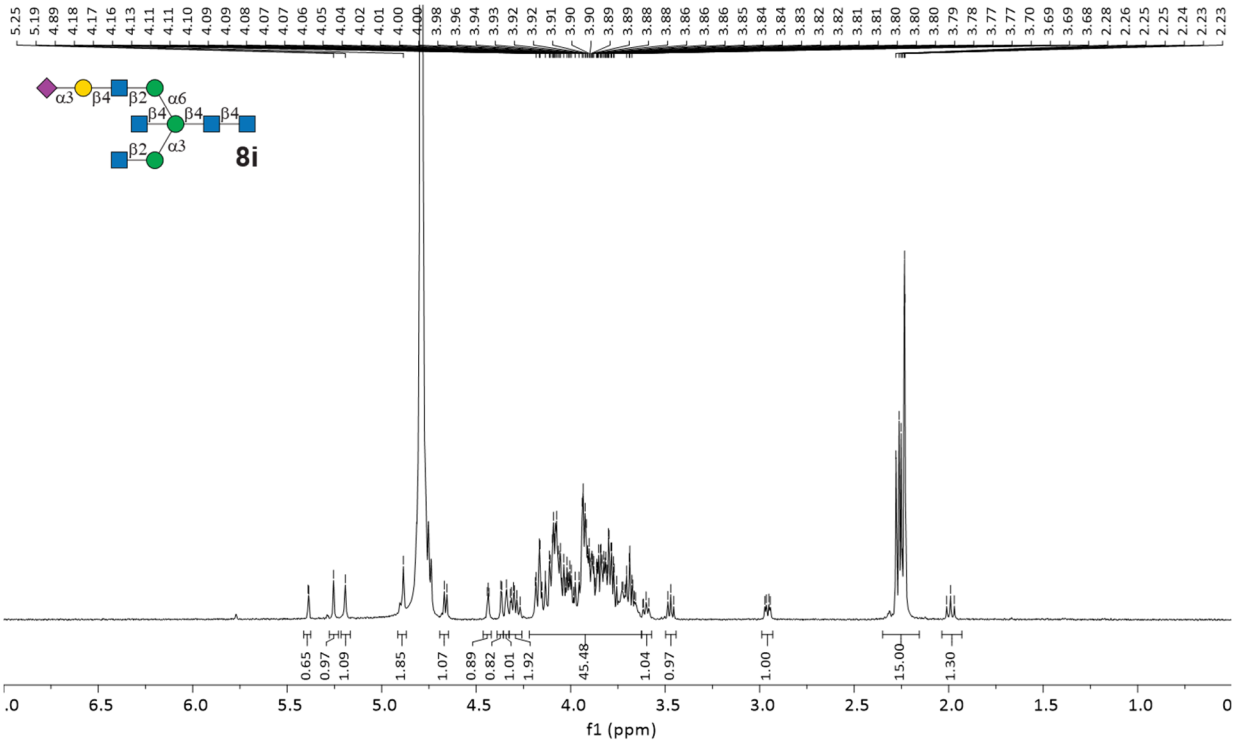
^{1}H NMR (600 MHz, D_2O) of compound **21**



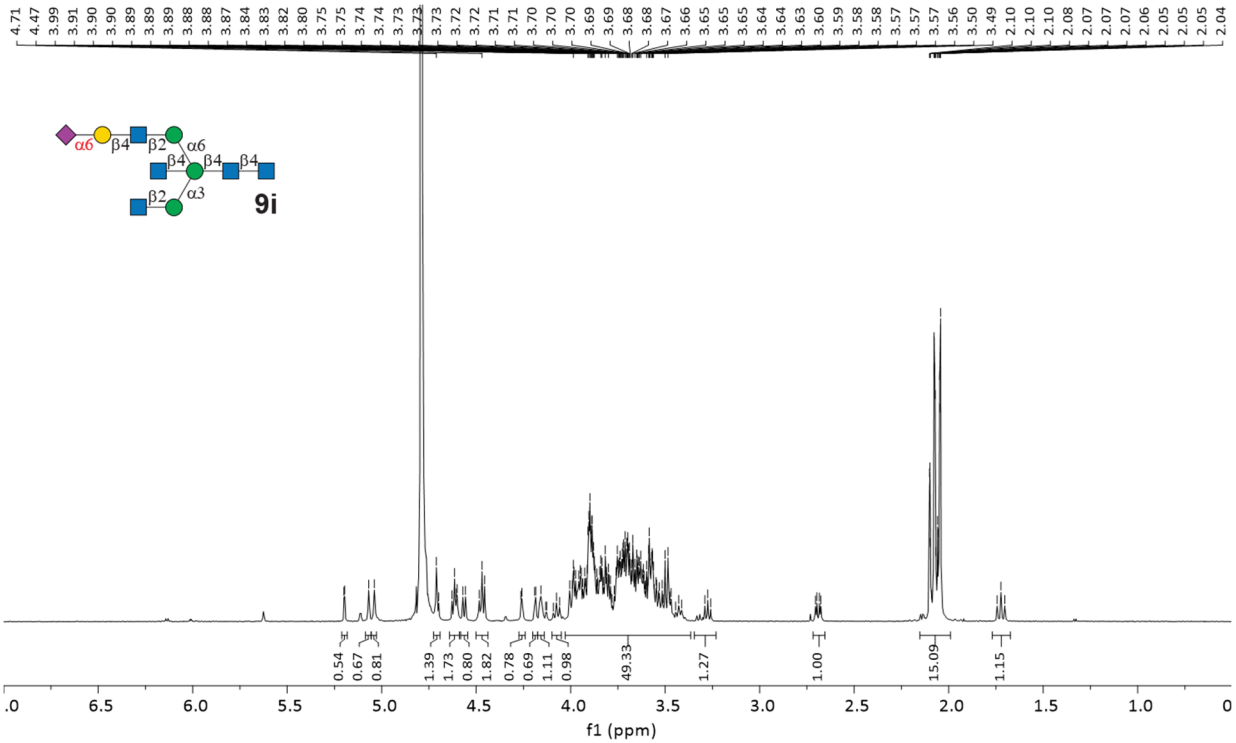
^{1}H NMR (600 MHz, D_2O) of compound **7i**



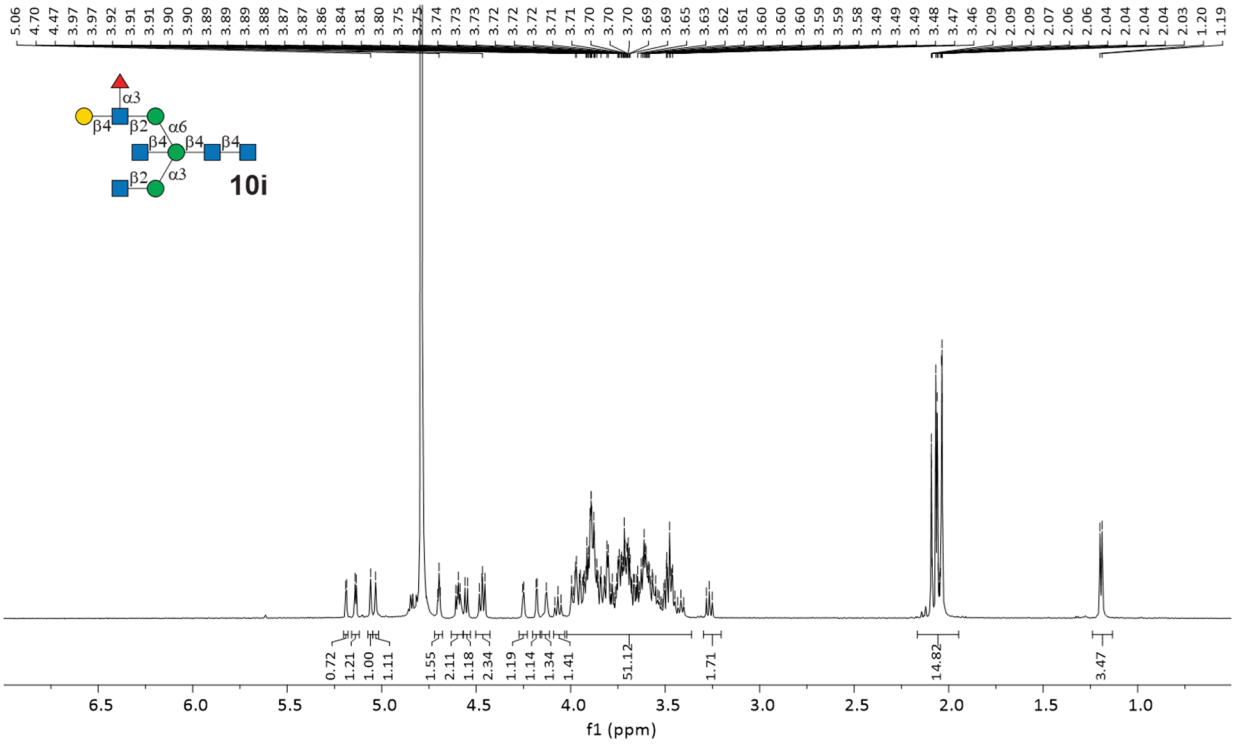
^{1}H NMR (600 MHz, D_2O) of compound **8i**



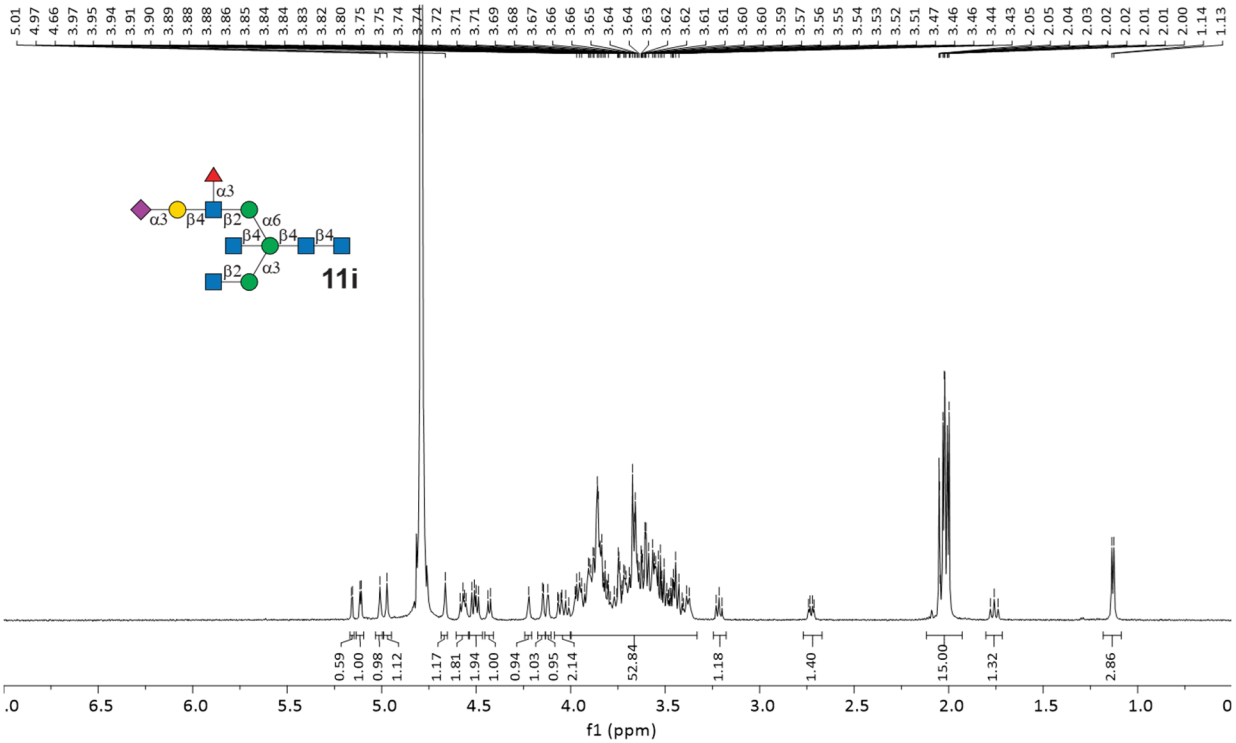
^{1}H NMR (600 MHz, D_2O) of compound **9i**



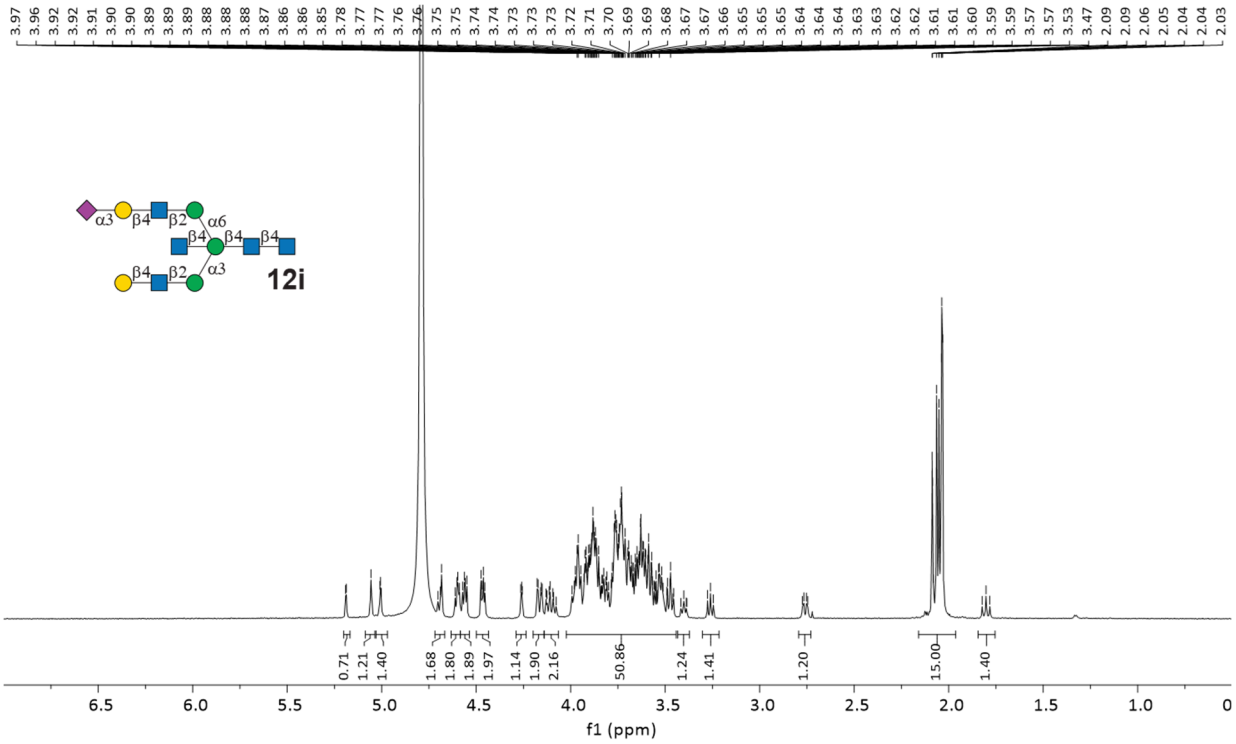
^{1}H NMR (600 MHz, D_2O) of compound **10i**



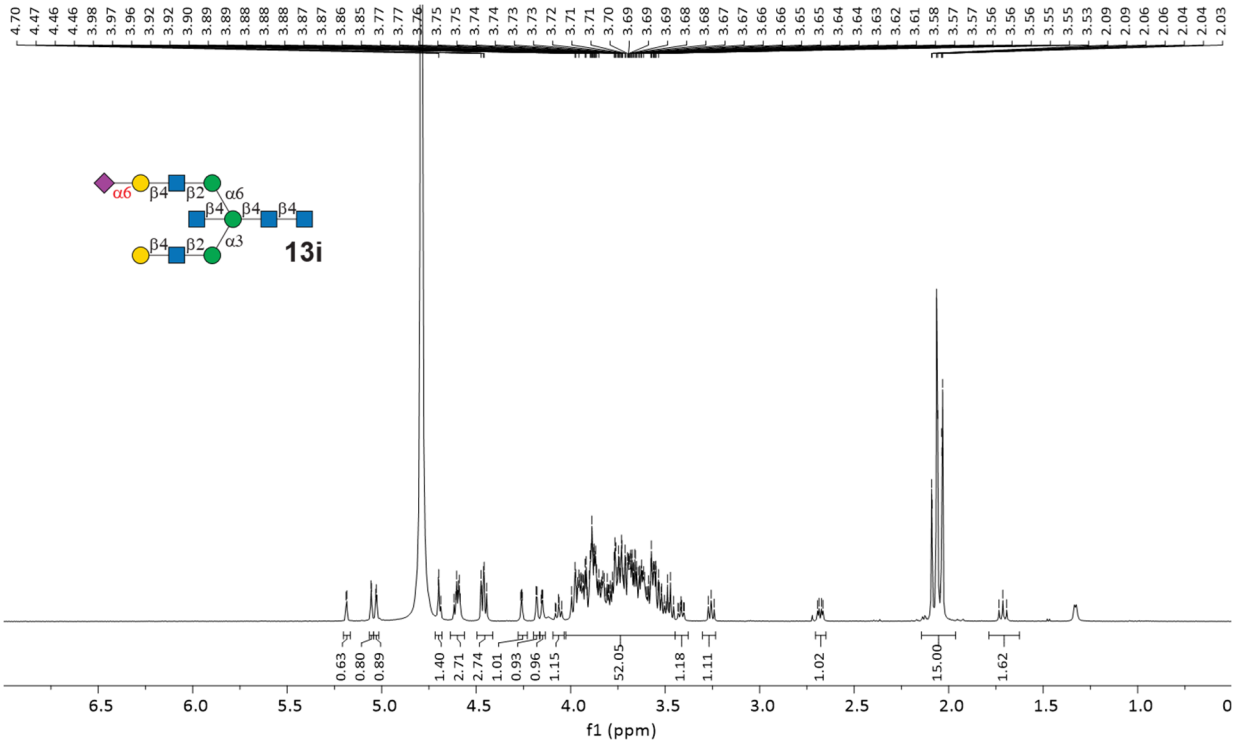
^{1}H NMR (600 MHz, D_2O) of compound **11i**



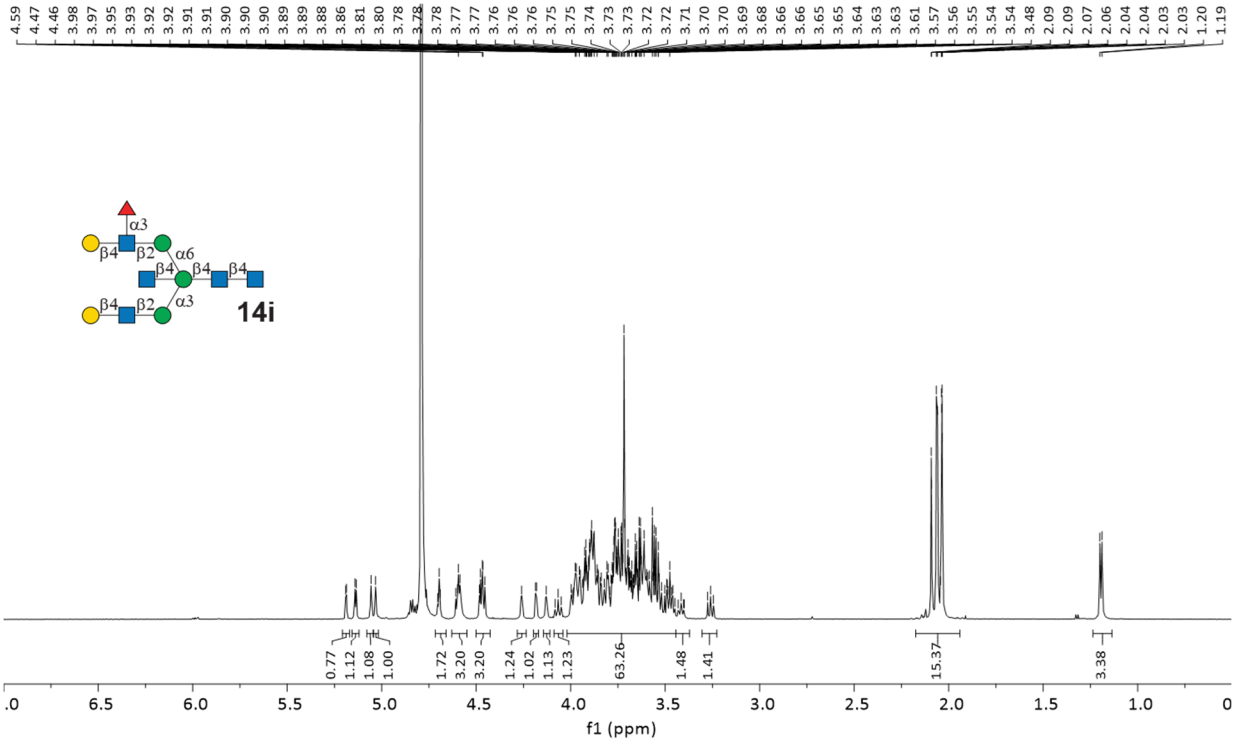
^{1}H NMR (600 MHz, D_2O) of compound **12i**



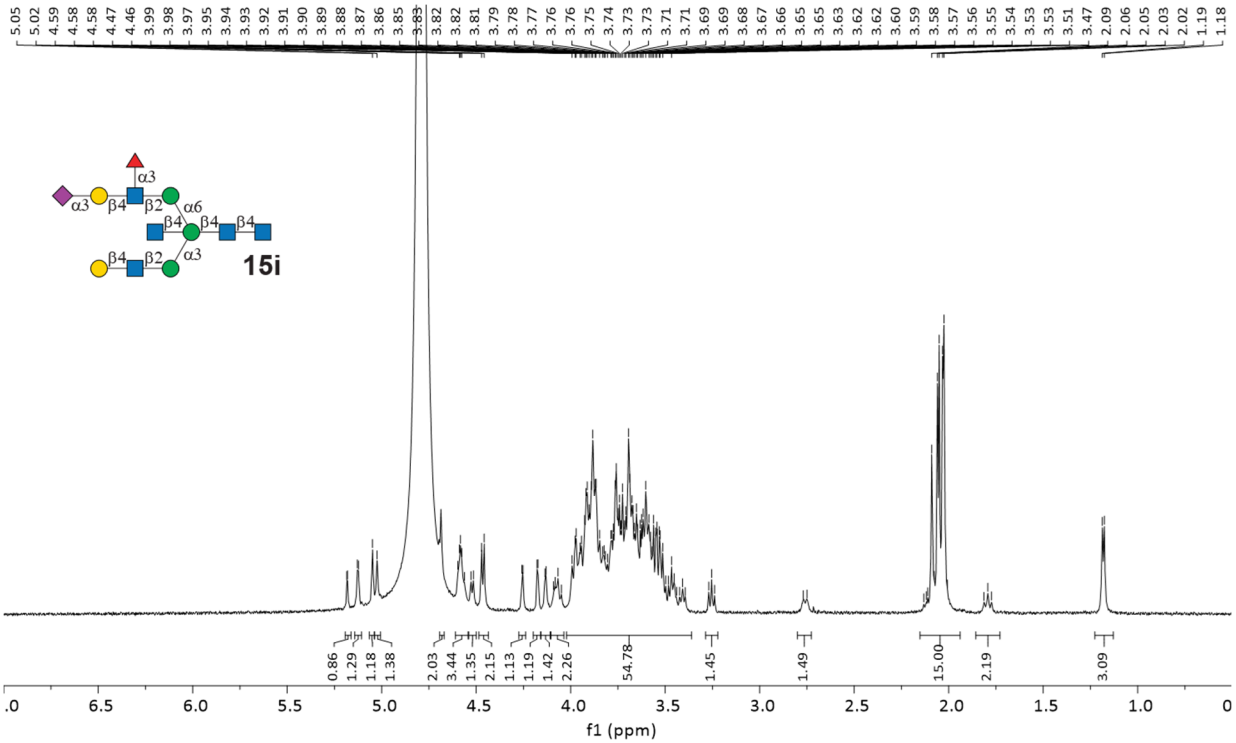
^{1}H NMR (600 MHz, D_2O) of compound **13i**



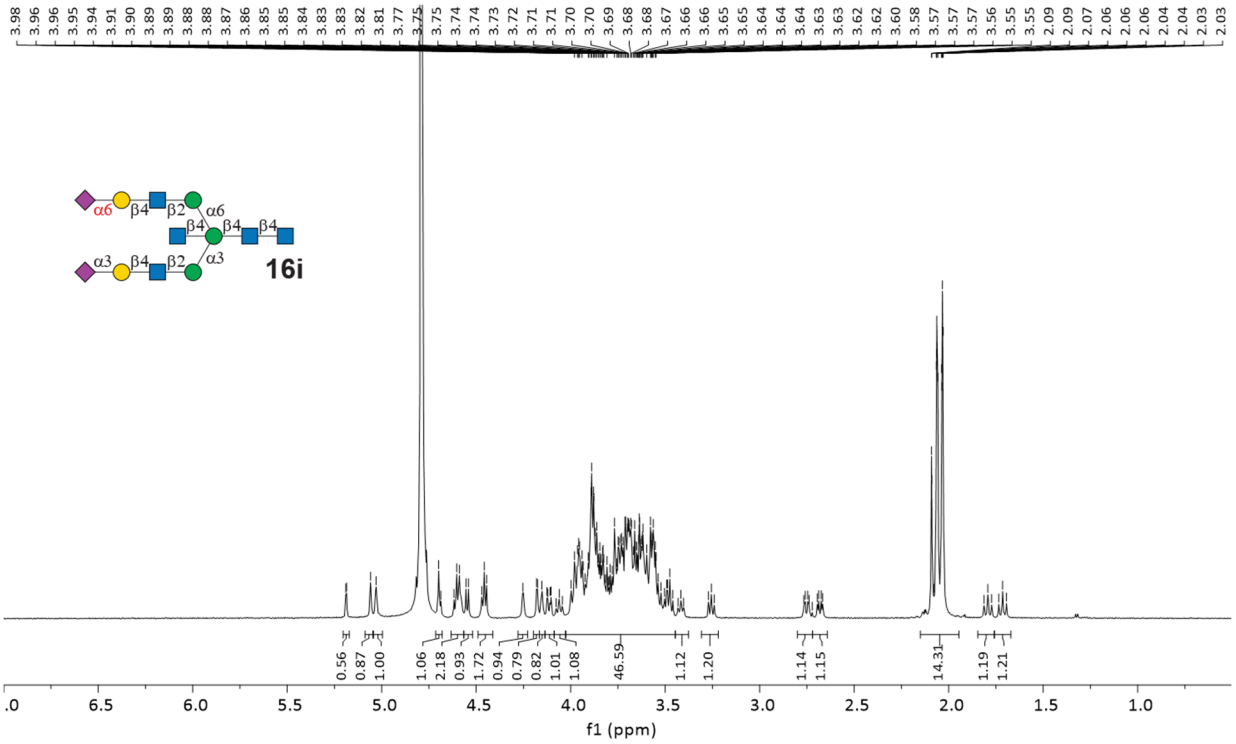
^{1}H NMR (600 MHz, D_2O) of compound **14i**



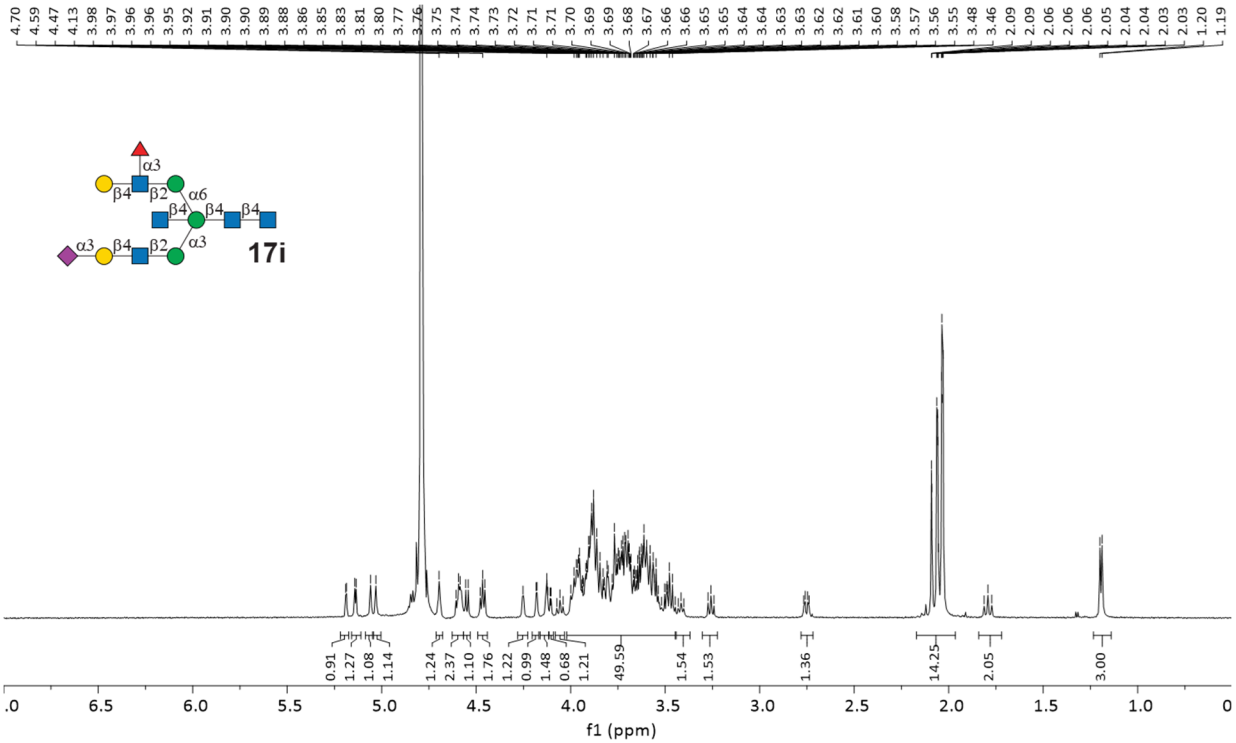
^{1}H NMR (600 MHz, D_2O) of compound **15i**



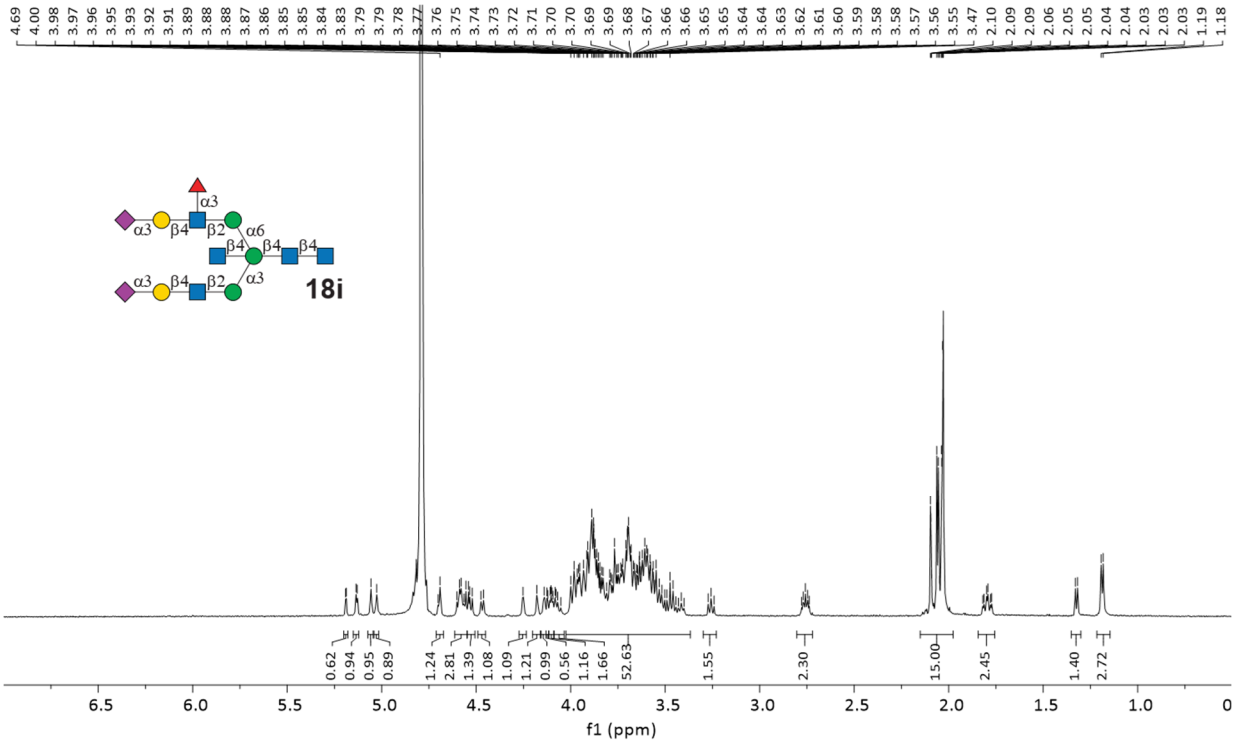
^{1}H NMR (600 MHz, D_2O) of compound **16i**



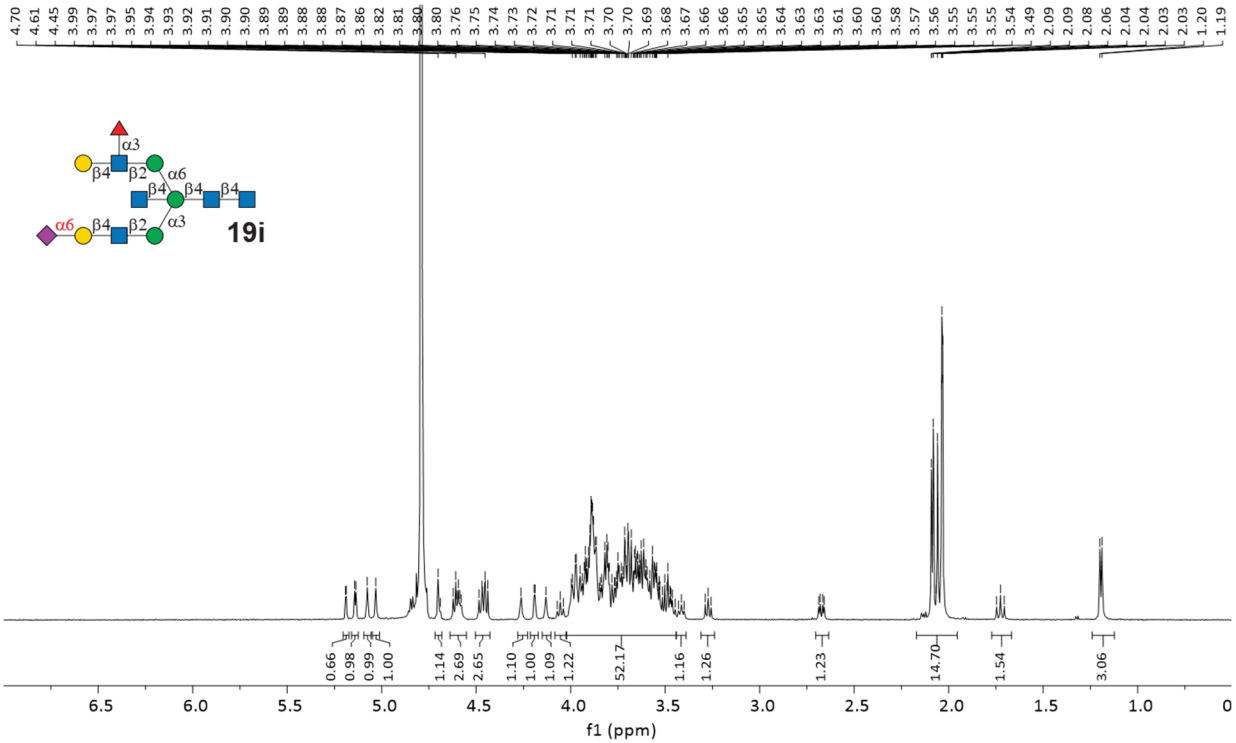
^{1}H NMR (600 MHz, D_2O) of compound **17i**



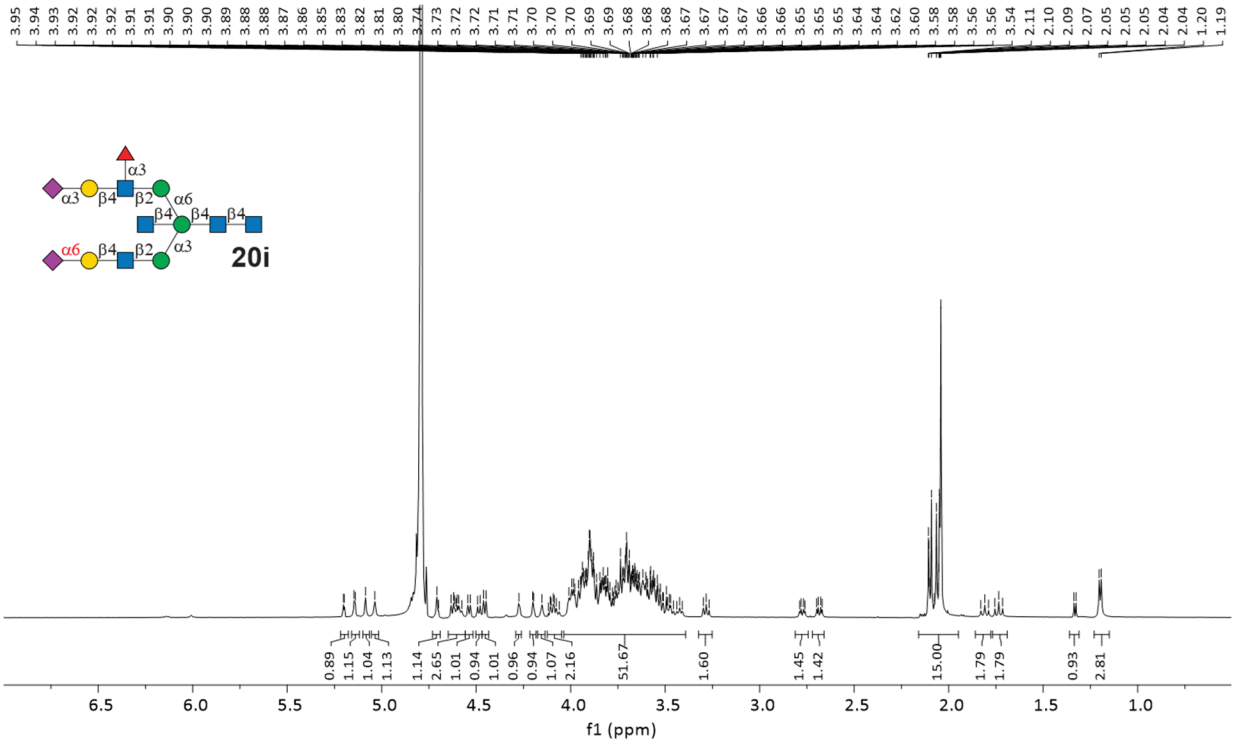
^{1}H NMR (600 MHz, D_2O) of compound **18i**



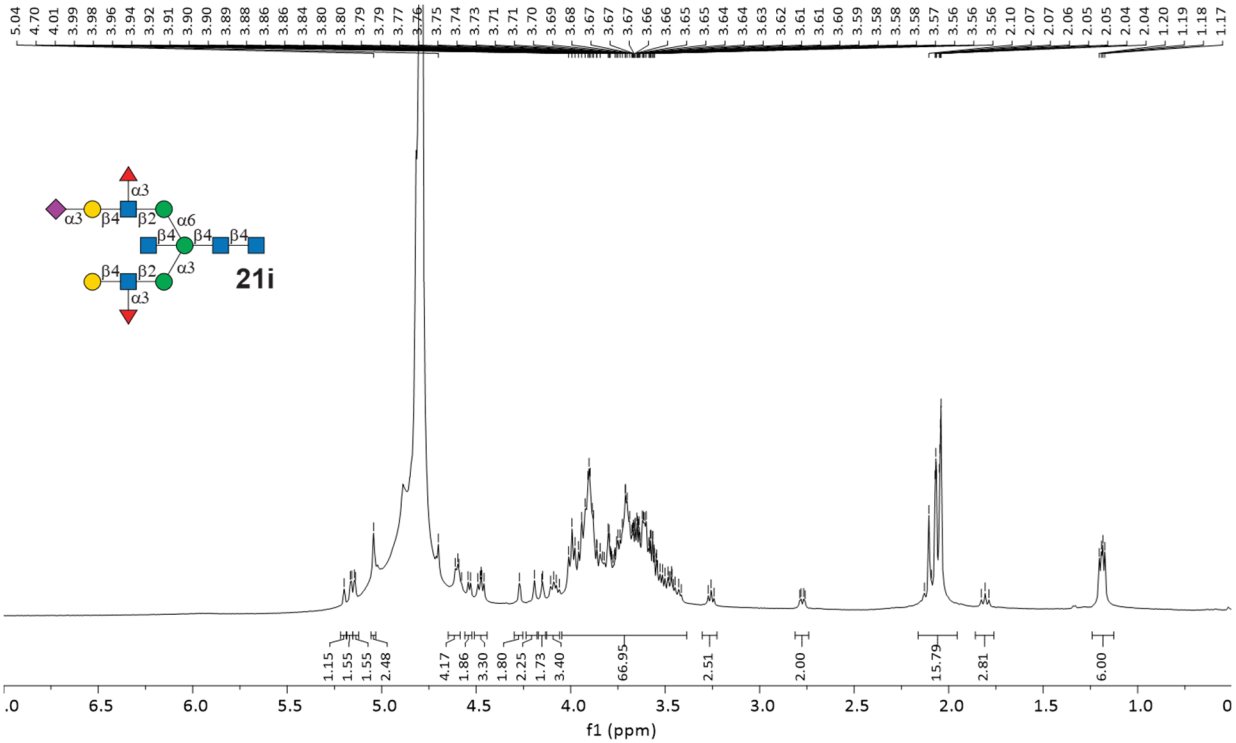
^{1}H NMR (600 MHz, D_2O) of compound **19i**



^{1}H NMR (600 MHz, D_2O) of compound **20i**



^{1}H NMR (600 MHz, D_2O) of compound **21i**



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