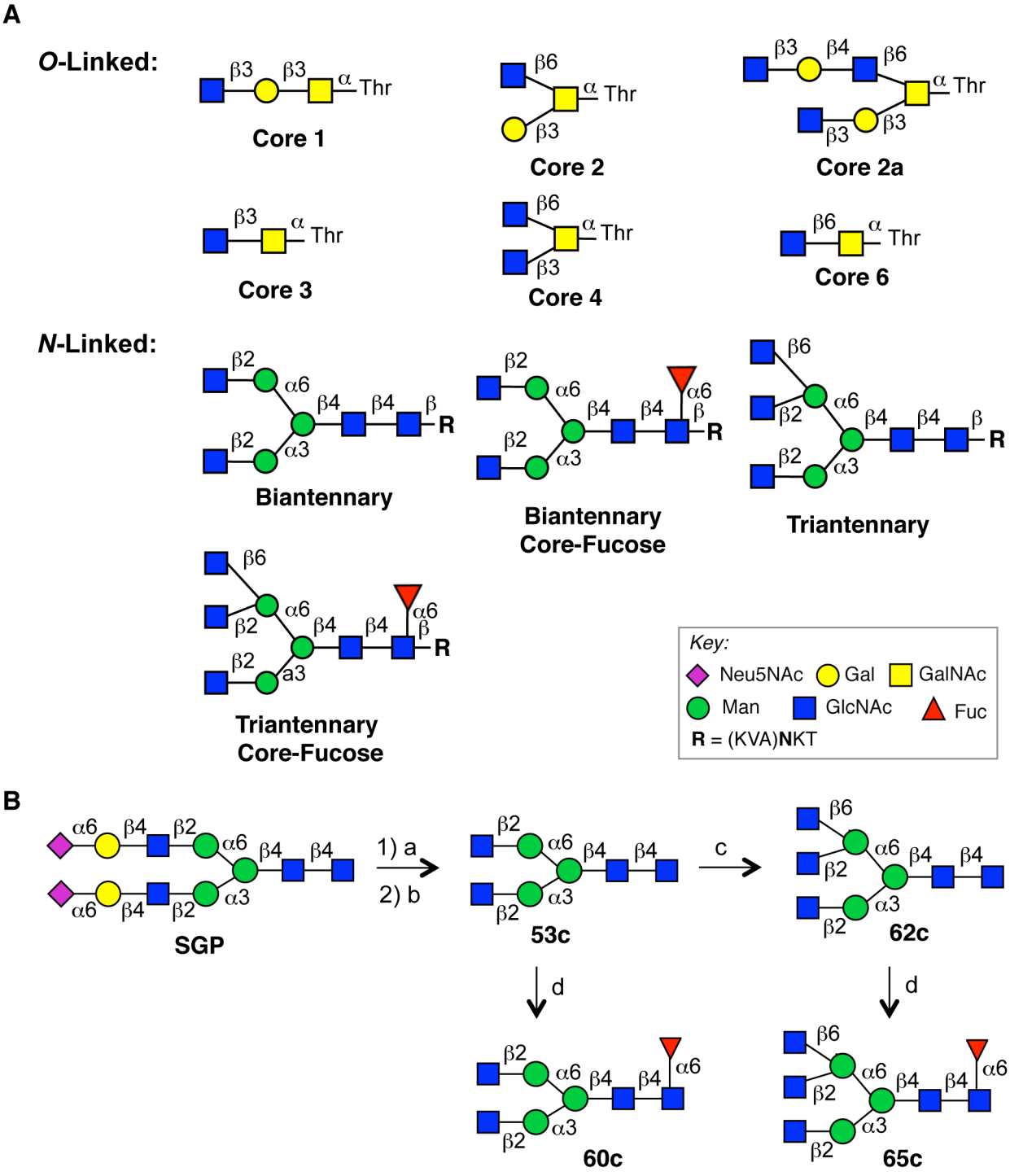
**Supplemental Figures and Legends**

**Figure S1**




*Conditions and reagents:*

a) Neuraminidase from *Arthrobacter ureafaciens*. b) β-Galatosidase from *Aspergillus oryzae*. c) UDP-GlcNAc, GnT-V; d) GDP-Fuc, FUT8.

**Figure S1. O- and N-linked Glycan Core Structures. Related to Figure 1.**
A) O- and N-linked core structures used in this study. GlcNAc residues at the nonreducing end can be extended with poly-LacNAc chains. B) Enzymatic synthesis of N-linked glycan core structures from N-linked glycan **SGP** obtained from egg-yolk.

**Figure S2**

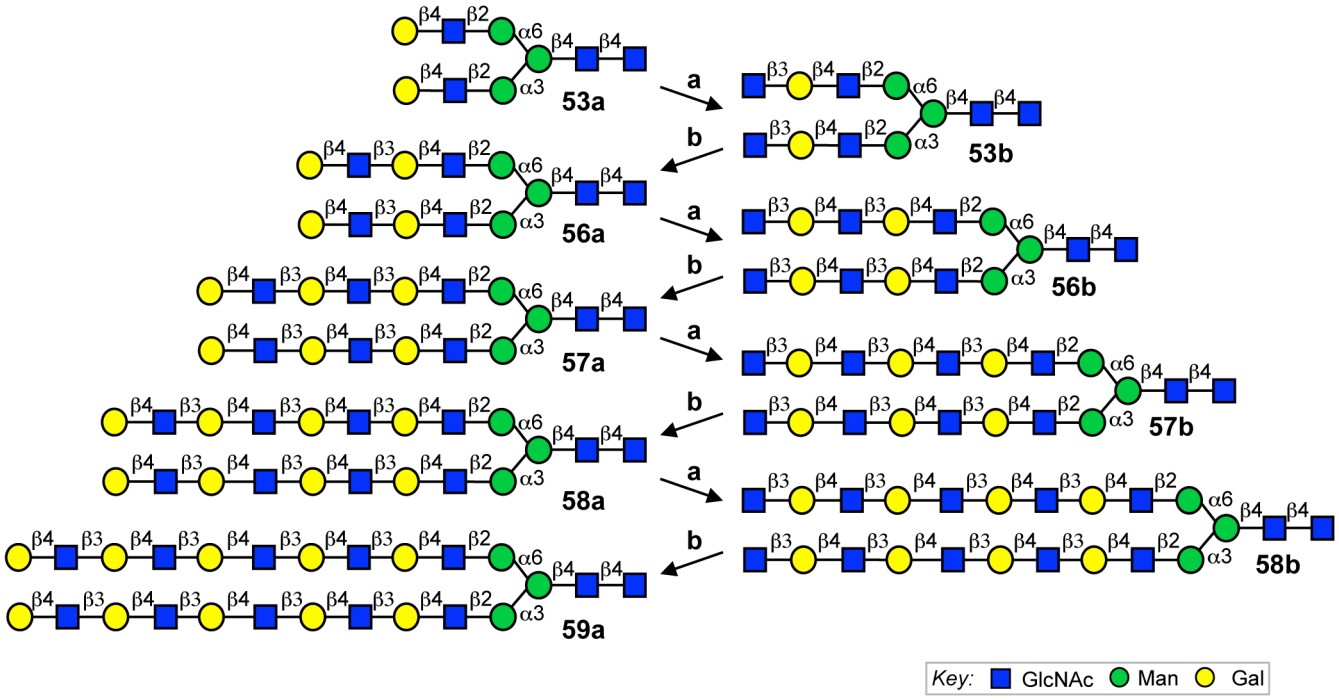



**Figure S2. Example of the Extension of poly-LacNAc Chains on Biantennary N-linked Glycan Core Structures. Related to Figure 1**.

A penta-LacNAc chain was elongated stepwise on a biantennary N-linked glycan using an iterative enzymatic synthesis by HP β1-3GnT and mammalian β1-4GalT-1. The galactoside intermediates could be further sialylated to form either α2-3 or α2-6 sialosides. Conditions and reagents: a, HP β1-3GnT, UDP-GlcNAc; b, GalT-1, UDP-Gal.

**Figure S3**

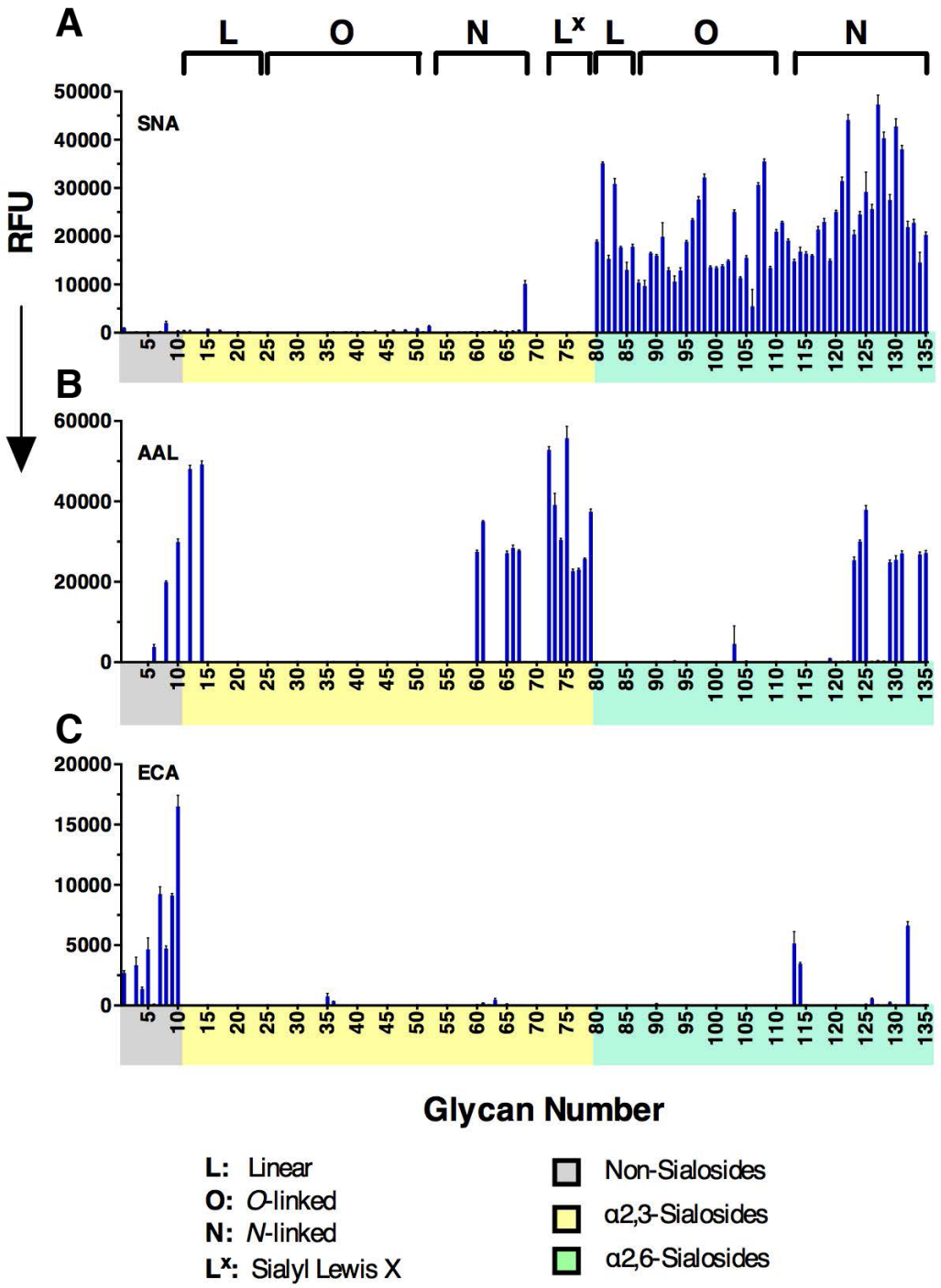



**Figure S3. Binding of Plant Lectins to the Sialoside Microarray. Related to Figure 1B.**
Plant lectins of defined specificity were used to confirm the fidelity of structures on the microarray. Signals are the mean and standard error calculated for six independent replicates on the array after removal of the highest and lowest signals. A) SNA detects all glycans containing the α2-6 sialic acid epitope (**80**-**135**). B) AAL was used to detect fucose residues either in N-linked Core or Lewis X structures. C) ECA recognizes terminal galactose residues (e.g. control glycans **1**-**10**), and did not bind to newly synthesized sialosides except for reference compounds **113**,**114** and **132** with terminal galactose. These controls demonstate that enzymatic sialylation was complete and all terminal galactose residues were efficiently capped.

**Figure S4**

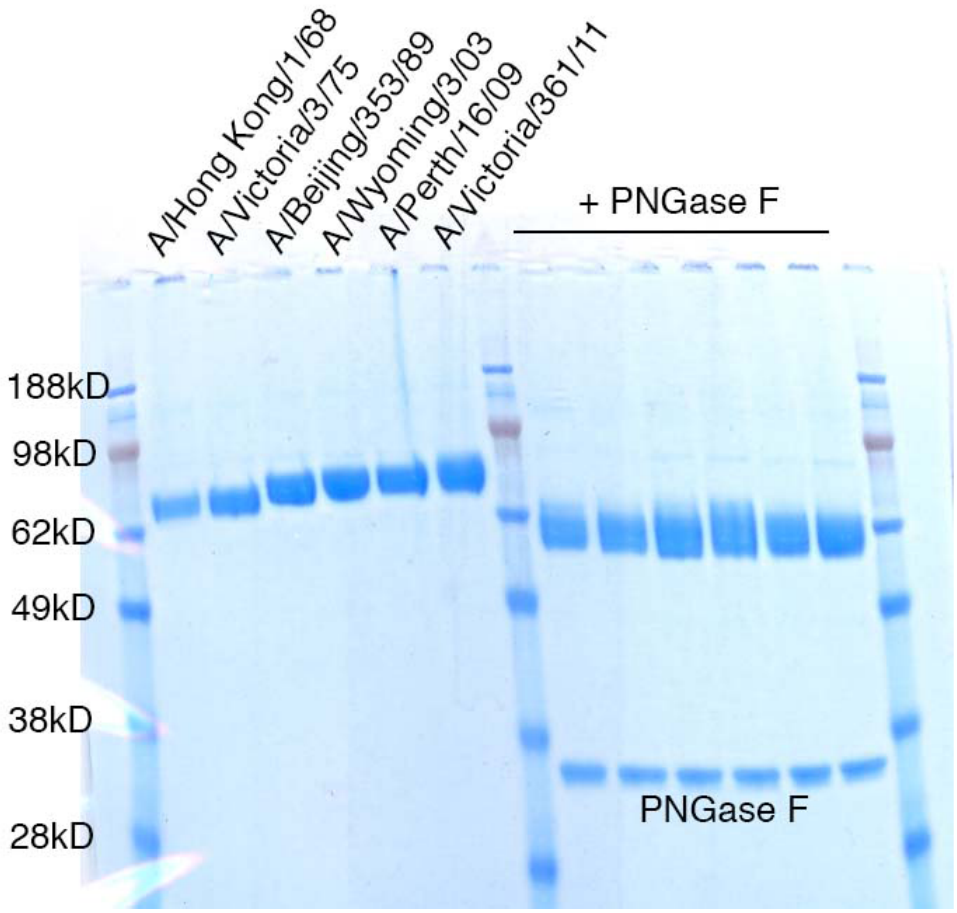



**Figure S4. SDS-PAGE Analysis of N-linked Glycosylation of Recombinant H3 HAs from 1968 to 2011. Related to Figure 2.**

Analysis was performed by incubation of various recombinant HAs with or without PNGase F at 37 °C for 60 mins, followed by non-reducing gel electrophoresis and staining with Coomassie blue. On the right, the lower band represents PNGase F, and the upper band is deglycosylated HA. M.W. standards are shown on the left, center and outer lanes.

**Figure S5**

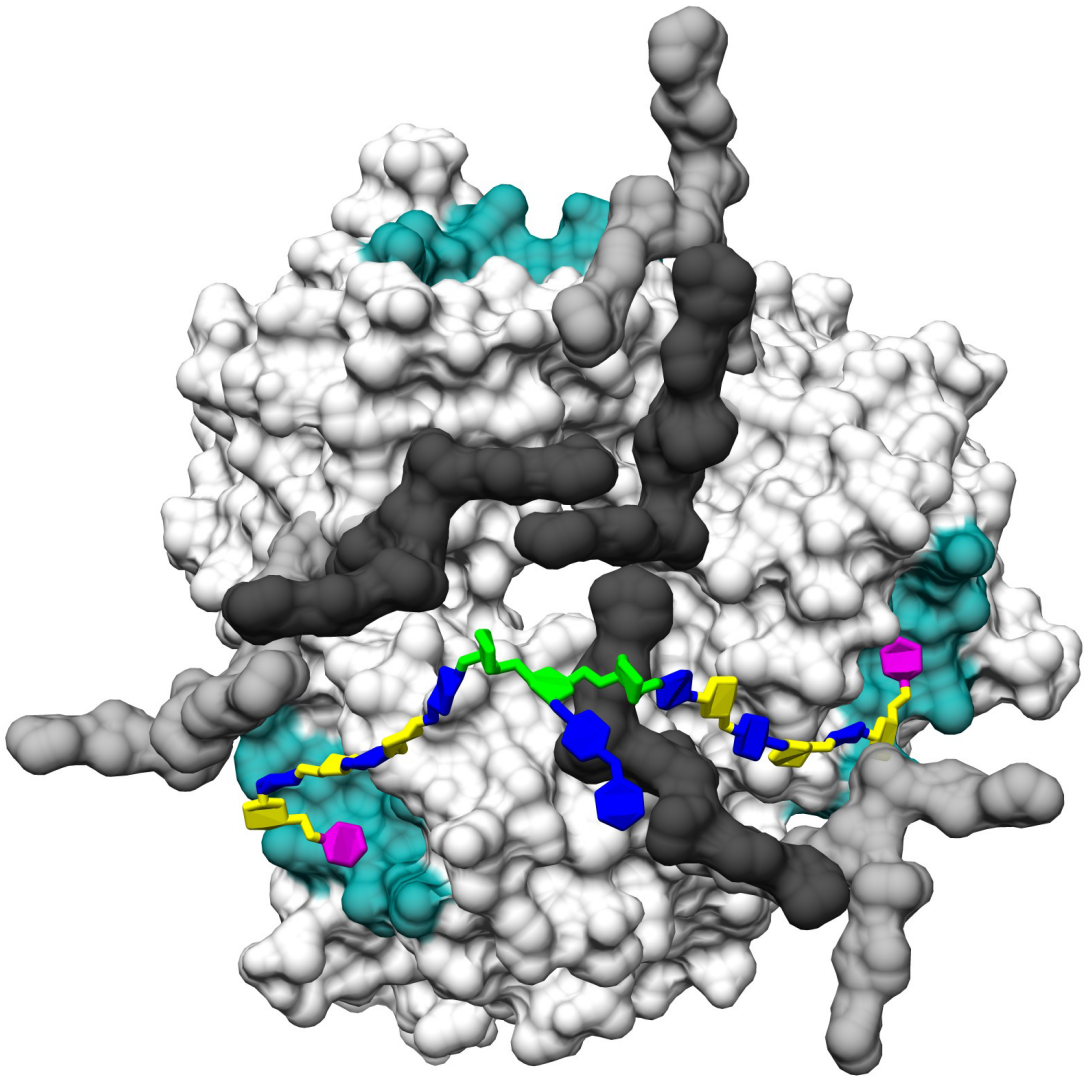
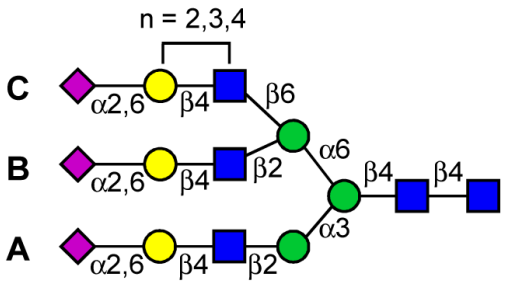


**Figure S5. Bi-dentate Docking of an Extended α2-6 Sialylated N-linked Glycan to an H3 Hemagglutinin. Related to Figure 7.**
A tri-LacNAc biantennary sialoside **120** (in blue/green/yellow/purple) was grafted onto an H3 HA trimer (Victoria/11) with asialo-biantennary N-linked glycans proximal to the RBS modeled at glycosylation sites N165 and N246 (dark and light grey, respectively) on each protomer. The view is looking down on the top of the HA along the threefold axis of the trimer. The biantennary sialoside is able to span two of the three RBS (cyan) of the trimer.

**Figure S6**




| Distance between terminal sialic acids (Å) | | | | | | |
|---|---|---|---|---|---|---|
| **Sialosides** | **Arms** | | | | | |
| | **A-B** | | **B-C** | | **A-C** | |
| | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** |
| **126** (n = 2) | 35 | 46 | 36 | 44 | 19 | 35 |
| **127** (n = 3) | 33 | 66 | 48 | 58 | 20 | 58 |
| **128** (n = 4) | 51 | 82 | 60 | 67 | 31 | 75 |

**Figure S6. Distance between Terminal Sialic Acids of Triantennary N-linked α2-6 Sialosides. Related to Figure 7.**
Distances between the C2 atoms in the sialic acids in each arm of triantennary N-glycans with different numbers of LacNac extensions as calculated from molecular dynamics simulations. The triLacNAc extension is the minimum unit that can span the distance between the two receptor binding sites in the HA trimer (45 Å).

**Figure S7**

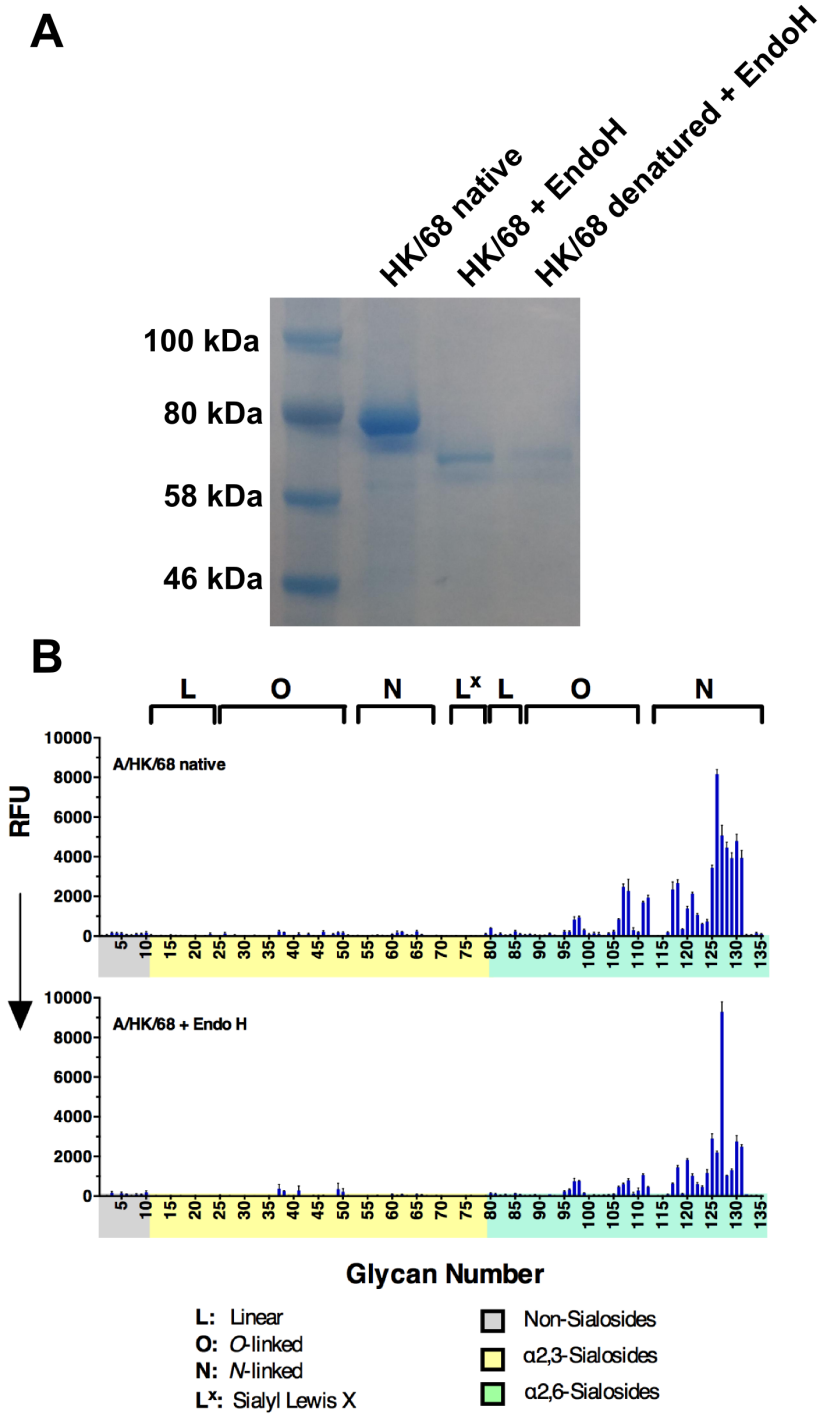



**Figure S7. HA glycosylation has no effect on receptor binding in HK/68. Related to Figure 5 and 6.** (A) HK/68 HA was expressed in 293S ($GnTi^{-/-}$) cells with exclusively high-mannose N-glycans (left) and natively deglycosylated using Endo H (center). As a control, a small HA sample was denatured prior to Endo H treatment (right) to ensure complete deglycosylation of the natively folded protein. (B) Receptor specificities of glycosylated (upper panel) and Endo H-treated (lower panel) HK/68 HA were analyzed by glycan microarray.

**Supplemental Table Legends**

**Table S1. Full Glycan List of Sialoside Microarray Used in this Study. Related to Figure 1, 2, 3, 4, 6, S3 and S7.**

The array contained 135 glycans, including 75 sialosides reported here, and 50 sialosides and non-sialoside glycans reported previously (Tzarum et al., 2015; Wang et al., 2013).

**Table S2. Potential N-linked Glycosylation Sites on H3 HAs during H3N2 Virus Antigenic Drift. Related to Figure 2.** Glycosylation sites on the H3 HA have increased since 1963. In our study, six HAs from 1968 to 2011 were cloned (see red arrow). HA residue numbering as for A/Hong Kong/1/1968.

**Table S2**

| | HA | Subtype | Strain | Year | Glycan (all HAs have conserved glycans at HA1 22, 38, 165, 285; HA2 154) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 81 | 63 | 126 | 246 | 122 | 133 | 144 | 45 |
| | 1 | H3N8 | A/duck/Ukraine/1/1963 | 1963 | + | – | – | – | – | – | – | – |
| → | 2 | H3N2 | A/Hong Kong/1/1968 | 1968 | + | – | – | – | – | – | – | – |
| → | 3 | H3N2 | A/Victoria/3/1975 | 1975 | – | + | + | – | – | – | – | – |
| | 4 | H3N2 | A/Bangkok/1/1979 | 1979 | – | + | + | – | – | – | – | – |
| | 5 | H3N2 | A/Leningrad/360/1986 | 1986 | – | + | + | + | – | – | – | – |
| → | 6 | H3N2 | A/Beijing/353/1989 | 1989 | – | + | + | + | – | – | – | – |
| | 7 | H3N2 | A/Shangdong/9/1993 | 1993 | – | + | + | + | – | – | – | – |
| | 8 | H3N2 | A/Panama/2007/1999 | 1999 | – | + | + | + | + | + | + | – |
| | 9 | H3N2 | A/Moscow/10/1999 | 1999 | – | + | + | + | + | + | – | – |
| → | 10 | H3N2 | A/Wyoming/3/2003 | 2003 | – | + | + | + | + | + | + | – |
| | 11 | H3N2 | A/Brisbane/10/2007 | 2007 | – | + | + | + | + | + | + | – |
| → | 12 | H3N2 | A/Perth/16/2009 | 2009 | – | + | + | + | + | + | – | – |
| → | 13 | H3N2 | A/Victoria/361/2011 | 2011 | – | + | + | + | + | + | + | + |

– no PNG

\+ PNG

→ Candidate HA to test

**Supplemental Movie Legends**

**Movie S1. Molecular Dynamics Simulation of DiLacNAc-Bidendate with Cal04 HA. Related to Figure 7.**
Glycan is an extended α2-6 Sialylated N-linked glycan related to **118** in Table S1.

**Movie S2. Molecular Dynamics Simulation of TriLacNAc-Bidendate with Cal04 HA. Related to Figure 7.**
Glycan is an extended α2-6 Sialylated N-linked glycan related to **120** in Table S1.

**Movie S3. Molecular Dynamics Simulation of TetraLacNAc-Bidendate with Cal04 HA. Related to Figure 7.**
Glycan is an extended α2-6 Sialylated N-linked glycan related to **121** in Table S1.

**Movie S4. Molecular Dynamics Simulation of PentaLacNAc-Bidendate with Cal04 HA. Related to Figure 7.**
Glycan is an extended α2-6 Sialylated N-linked glycan related to **122** in Table S1.

**Supplemental Experimental Procedures**

## 1. Materials and Methods for Glycan Synthesis

The recombinant enzymes, *Helicobacter pylori* β1-3-*N*-acetylglucosaminyltransferase (β3GlcNAcT), rat ST3Gal-III, and human ST6Gal-I were produced and purified as described previously (Nycholat et al., 2012; Nycholat et al., 2013; Peng et al., 2012). *N*-acetylglucosaminyltransferase V (GnT-V) was kindly donated from Dr. Michael Pierce laboratory (CCRC, University of Georgia at Athens). Mammalian α1-6-fucosyltransferase (FUT8) was obtained from the Dr. Naoyuki Taniguchi's laboratory (Riken Advanced Science Institute, Japan) and expressed as previously described (Ihara et al., 2006). Mammalian β1,4-galactosyltransferase from bovine milk (GalT-1) and β-galactosidase (from *Aspergillus oryzae*) were purchased from Sigma-Aldrich. Uridine 5'-diphospho-galactose (UDP-Gal) was purchased from Calbiochem EMD Millipore. Uridine 5'-diphospho-N-acetylglucosamine (UDP-GlcNAc) and guanidine-5-diphospho-fucose (GDP-Fuc) were a gift from Tokyo Research Laboratories, Kyowa Hakko Kogyo Co. Ltd. Neuraminidase (sialidase from *Arthrobacter ureafaciens*; 1 U/100 μL) was purchased from Roche Applied Science (Indianapolis, IN, USA). Mouse anti-Strep2 was purchased from AbCam. Strepavidin-Alexa Fluor488 and anti-mouse-IgG Alexa Fluor488 were purchased from Invitrogen. Biotinylated SNA, AAL and ECL were purchased from VectorLab. Starting materials for *O*-linked glycans were prepared as reported previously (Peng et al., 2012). All chemicals were obtained from commercial suppliers and used as received unless otherwise noted. Whatman TLC plate coated with silica gel 60-$F_{254}$ was purchased from VWR.

All enzymatic reactions were performed in an aqueous, buffered system with the appropriate pH for each enzyme. Water was purified by NanoPure Infinity Ultrapure water system (Barnstead). Reactions were monitored by mass spectrometry analysis and thin layer chromatography (TLC). TLCs were developed with appropriate eluents (*i*PrOH:$NH_4OH$:$H_2O$, 4:3:1 or 5:2:1, v:v:v), and the spots were visualized by UV light or by treatment with 10% sulfuric acid in ethanol followed by heating. Gel filtration chromatography was performed with a column (113 cm × 0.7 cm) or (42 cm × 0.5 cm) packed with Sephadex™ G-25 superfine (GE Healthcare), eluted with 0.1 M $NH_4HCO_3$ (aq). $^1$H NMR spectra were recorded on Bruker DRX-600 (600 M Hz) instruments at 25 °C are reported in parts per million ($\delta$) relative to HOD (4.79 ppm, $D_2O$). Coupling constants ($J$) are reported in Hertz. $^{13}$C NMR spectra were recorded on Bruker DRX-600 (150 MHz) instruments at 25 °C. NMR data were processed with Mnova software. MS data of asialosides were recorded with an Applied Biosystems DE MALDI-TOF using dihydroxybenzoic acid as the matrix. MS data of sialosides were recorded with an Applied Biosystems SCIEX MALDI TOF/TOF 5800 after permethylation. All of the sialosides were permethylated before MS analysis unless otherwise noted.

## 2. Array Quality Control by Plant Lectins

Lectins (10 μg/ml) and Streptavidin-AlexaFluor were pre-mixed for 15 min on ice at 10 μg/ml and 2 μg/ml, respectively in PBS + 0.05% Tween-20. The lectin-Streptavidin complex was loaded on the array surface and incubated in a humidified chamber for 1 hr. Slides were rinsed successively with PBS-T, PBS and deionized $H_2O$. Washed arrays were dried by centrifugation and immediately scanned using an Innoscan 1100AL (Innopsys) confocal microarray scanner. Fluorescent signal intensity was measured using the Mapix software package (Innopsys) and analysed using Prism (Graphpad Software). Signals are expressed as relative fluorescence units (RFU) with the mean and standard error calculated from six independent replicates on the array.

## 3. Molecular Modeling and Simulation Method

*Simulation details* - All simulations were performed with the CUDA implementation of PMEMD (Gotz et al., 2012; Salomon-Ferrer et al., 2013) in the Amber14 software suite (Case et al., 2014a). The carbohydrate was modeled using the GLYCAM06h force field (Kirschner et al., 2008), while the Amber14SB force field (Case et al., 2014b) was employed for the protein. A Berendsen barostat with a time constant of 1 ps was employed for pressure regulation, while a Langevin thermostat with a collision frequency of 2 $ps^{-1}$ was used for temperature regulation. A nonbonded interaction cutoff of 8 Å was employed. Long-range electrostatics were treated with the particle-mesh Ewald (PME) method (Darden et al., 1993). Covalent bonds involving hydrogen were constrained with the SHAKE algorithm allowing a time step of 2 fs (Ryckaert et al., 1977).

*3D structure generation* - Initial conformations for biantennary α2-6 di-, tri-, tetra- and penta-LacNAc glycans were built and energy minimized using GLYCAM-Web (www.glycam.org). Each structure was placed in a periodic box of TIP5P (Mahoney and Jorgensen, 2000) water molecules with an 8 Å buffer between the glycan and the box edge using tleap in AmberTools15 (Case et al., 2014).

The trimeric HA1 domains of H1 Cal/04/09 and H3 Victoria/11 were generated from PDB codes 3UBE and 4O5N, respectively. After alignment, residues before H64 and after Y83 of the stalk region, as well as residues before Q57 and after S270, were removed to reduce computational time. The three NeuAcα2-6Gal disaccharides in the H1 Cal/04/09 structure were added to the H3 Vic/11 HA1 domain after alignment. Biantennary, asialo-monoLacNAc glycans were generated at N165 and N246 of the Vic/11 structure. This structure was then placed in a periodic box of TIP5P (Mahoney and Jorgensen, 2000) water molecules with an 8 Å buffer between the glycan and the box edge using tleap (Case et al., 2014).

*Biantennary co-complexes* - Each N-glycan receptor was grafted into an HA binding site by superimposing the NeuAcα2-6Gal binding motif of one branch onto the crystal structure of the ligand bound to the HA (PDB codes 3UBE and 4O5N). The reported grafting algorithm (Grant et al., 2014; Tessier et al., 2013) was adapted to rotate the glycosidic linkages within normal bounds (Nivedha et al., 2014), while monitoring the distance between the binding motif on the other arm of the glycan and the second HA binding site. The linkages were adjusted in series, beginning from the bound motif. For Cal/04/09, a single optimal structure was selected for each glycan based on the relative orientation and proximity of the second binding motif to the target HA binding site. This structure was subject to refinement via energy minimization and molecular dynamics simulation. The results were independent of whether the 3-arm or the 6-arm of the glycan was grafted onto the bound NeuAcα2-6Gal motif.

For Vic/11, 1,000 snapshots were extracted at regular intervals from the MD simulation. The same procedure as for Cal/04/09 was repeated to assess wether a Tri-LacNAc biantennary receptor glycan could form a bi-dentate co-complex while avoiding atomic overlaps with the N165 and N246 glycans.

*Refinement of the bidentate co-complexes* - A 10 ns production simulation was performed at 300 K (nPT). Cartesian restraints (5 kcal/mol/Å$^2$) were placed on protein Cα atoms. Solvation was treated with the generalized Born approximation (igb=2). During the final 200 ps of equilibration, the NeuAca2-6Gal motif was guided into the binding site by introducing distance restraints (see Supplementary Information S1 and Supplementary Table T1 for details). The stability of the new structure was assessed by continuing MD simulation for 10 ns without restraints.

*Energy minimization and equilibration* - Energy minimization was performed for 20,000 steps (10,000 steepest decent, followed by 10,000 conjugant gradient). The system was then heated from 5 K to 300 K over 100 ps followed by 300 ps of structural equilibration.

*Glycosylated H3 HA1 domain simulations* – A production simulation of 100 ns was performed for the N165 and N246 glycosylated H3 HA1 domain. Cartesian restraints (5 kcal/mol/Å$^2$) were employed on the Cα atoms of the terminal of the HA1 domain.

**4. Virus Infection Protocol**

MDCK cells were grown in MEM (Minimal Essential Media, Gibco) supplemented with 10% FBS (Fetal Bovine Serum), 1x P/S (Pencillin/Streptomycin mix) and 1x L-Gln and used for experiments when monolayers reach 100% confluency in 6-well tissue culture trays (approximately 1 x $10^6$ cells per well). After washing twice with warm PBS (maintained throughout all steps unless otherwise indicated), MDCK monolayers were digested with 50 units (per well) of *C. perfringens* neuraminidase (CPN; New England BioLabs) in 0.5 ml PBS, pH 6.5, 0.1% BSA (Bovine Serum Albumin), 5 mM $CaCl_2$ for 3 hours at 37°C. Cells were then incubated with 3 μM (final) glycolipid ($6SLN_{1\text{-}3}$-L/N-lipid) in 0.5 ml MEM media (0.1% BSA, 1x P/S, 1x L-Gln) for 20 min at 37°C. Following exogenous receptor treatment, cells were incubated with 50 pfu of Vic/11 virus in 0.5ml MEM media for 10 min at 37°C. Finally, MDCK monolayers were washed three times with PBS and incubated with 2 ml MEM media supplemented with 1 μg ml$^{-1}$ TPCK-treated (L-1-Tosylamide-2-Phenylethyl Chloromethyl Ketone) trypsin for 48 hours at 37°C. Control monolayers were treated identically and incubated with media or buffer minus glycolipid, neuraminidase or virus.

After 48 hours, media samples from both control and treated cells were harvested and viral titers were determined via plaque assay. In brief, assay samples were diluted in a $\log_{10}$ series from $10^{-2}$ – $10^{-7}$ in virus dilution media. 0.5ml of each dilution was used to treat confluent monolayers of MDCK cells. Media dilutions were incubated for 1 hour at 37°C with agitation every 15 min to ensure even coverage, before washing in warm PBS. Washed cells were covered in 2 ml 2x EMEM (MEM eagle minus phenol red, Lonza), 2x P/S, 2x L-Gln, supplemented with an equal volume of 3.8% (1.9% final (w/v)) NuSieve GTG agarose (Lonza) and 1 μg $ml^{-1}$ TPCK-trypsin. Agarose overlays were incubated 48 – 72 hours at 37°C to allow plaque development before fixing in 4% (w/v) paraformaldehyde and staining of cell monolayers in 1% (w/v) crystal violet solution. All assays were performed as three independent replicates.

## 5. Experimental Section

The pyranosyl carbohydrate abbreviations were used as: GalNAc, D-N-acetylgalactosamine; GlcNAc, D-N-acetylglucosamine; Neu5Ac, D-N-acetylneuraminic acid; Gal, D-galactose; Fuc, L-fucose.

### 5.1 Synthesis of sialosides with poly-LacNAc extensions.

The poly-LacNAc chain was extended on various N-linked and O-linked core structures (see Figure S1) as previously described (Peng et al., 2012). Briefly, the appropriate galactoside (1.0 - 20.0 mg) and UDP-GlcNAc (2.0 equiv./terminal galactose) were dissolved in HEPES buffer (50 mM, pH 7.2) with 25 mM KCl, 1 mM DTT, and 2 mM $MgCl_2$ in an appropriate microcentrifuge tube. *H. pylori* β-1,3-GlcNAcT and calf intestine alkaline phosphatase (CIAP) were added to the reaction mixture and incubated at 37°C. The product was purified by size-exclusion column with 0.1 M $NH_4HCO_3$ (aq.) eluent.

The GlcNAc-terminated substrate (1.0 - 20.0 mg) and UDP-Gal (2.0 equiv./terminal GlcNAc) were dissolved in Tris buffer (50 mM, pH 7.5) with 10 mM $MnCl_2$ in an appropriate microcentrifuge tube. GalT-1 and CIAP were added to the reaction mixture and incubated at 37°C. The product was purified by size-exclusion column with 0.1 M $NH_4HCO_3$ (aq.) eluent.

The LacNAc-extended glycan and CMP-NeuAc (2.0 equiv./terminal galactose) were dissolved in Tris buffer (50 mM, pH 7.5). Rat ST3Gal-III or human ST6Gal-I and CIAP were added to the reaction mixture and incubated at 37°C. The product was purified by size-exclusion column with 0.1 M $NH_4HCO_3$ (aq.) eluent to give α2-3 or α2-6 sialosides, respectively.**Neu5Acα3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (26).**

Compound **26a** (0.5 mg, 0.589 μmol), CMP-Neu5Ac (0.78 mg, 2.0 equiv.), and rST3Gal-III (3 mU) were dissolved in 118 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.6 mg, 90 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.95 (s, 1H), 4.69 (d, $J$ = 8.0 Hz, 1H), 4.54 (d, $J$ = 7.1 Hz, 1H), 4.48 – 4.42 (m, 2H), 4.28 (d, $J$ = 10.3 Hz, 1H), 4.20 (s, 1H), 4.15 – 4.03 (m, 3H), 4.03 – 3.91 (m, 3H), 3.91 – 3.81 (m, 4H), 3.81 – 3.60 (m, 16H), 3.60 – 3.51 (m, 4H), 2.74 (d, $J$ = 11.8 Hz, 1H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.79 (t, $J$ = 11.3 Hz, 1H), 1.40 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M + $Na^+$] C64H114N4O31Na, calcd 1457, found 1457.6.

**Neu5Acα6Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (87).**

Compound **26a** (0.5 mg, 0.589 μmol), CMP-Neu5Ac (0.78 mg, 2.0 equiv.), and hST6Gal-I (3 mU) were dissolved in 118 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.6 mg, 90 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.96 (s, 1H), 4.73 (d, $J$ = 8.0 Hz, 1H), 4.48 – 4.41 (m, 3H), 4.28 (d, $J$ = 10.3 Hz, 1H), 4.20 (s, 1H), 4.12 (s, 1H), 4.09 – 4.03 (m, 1H), 4.03 – 3.48 (m, 28H), 2.66 (d, $J$ = 11.3 Hz, 1H), 2.03 (s, 6H), 2.01 (s, 3H), 1.71 (t, $J$ = 11.5 Hz, 1H), 1.44 – 1.35 (m, 3H); MALDI-TOF MS: permethylated [M + $Na^+$] C64H114N4O31Na, calcd 1457, found 1457.6.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (27a).**

Compound **26b** (21 mg, 20 μmol), UDP-Gal (24 mg, 2.0 equiv.), GalT-1 (60 mU) and CIAP (0.04 mU) were dissolved in 1 mL Tris-HCl buffer (50 mM, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$). The pH was carefully monitored and adjusted (to pH 7.5) as needed. After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and

lyophilized to give the final product as a white powder (22 mg, 90 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.95 (d, $J$ = 3.9 Hz, 1H), 4.72 – 4.67 (m, 2H), 4.50 – 4.38 (m, 4H), 4.28 (dd, $J$ = 11.1, 3.8 Hz, 1H), 4.19 (d, $J$ = 3.1 Hz, 1H), 4.14 (d, $J$ = 3.3 Hz, 1H), 4.11 (d, $J$ = 3.4 Hz, 1H), 4.06 (dd, $J$ = 7.8, 4.6 Hz, 1H), 4.00 (dd, $J$ = 11.1, 3.0 Hz, 1H), 3.97 – 3.88 (m, 3H), 3.88 – 3.60 (m, 23H), 3.60 – 3.50 (m, 5H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.40 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS: [M + $Na^+$] C46H78N4O33Na, calcd 1237, found 1237.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (27).**

Compound **27a** (0.5 mg, 0.41 μmol), CMP-Neu5Ac (0.54 mg, 2.0 equiv.), and rST3Gal-III (2.5 mU) were dissolved in 103 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.55 mg, 90 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.96 (s, 1H), 4.72 – 4.67 (m, 2H), 4.54 (d, $J$ = 7.8 Hz, 1H), 4.48 – 4.42 (m, 3H), 4.29 (d, $J$ = 11.0 Hz, 1H), 4.20 (s, 1H), 4.15 (s, 1H), 4.13 – 4.04 (m, 3H), 4.00 (d, $J$ = 10.0 Hz, 1H), 3.97 – 3.91 (m, 3H), 3.91 – 3.48 (m, 34H), 2.75 (d, $J$ = 11.6 Hz, 1H), 2.05 – 1.98 (m, 12H), 1.79 (t, $J$ = 11.6 Hz, 1H), 1.40 (d, $J$ = 6.4 Hz, 3H); MALDI-TOF MS: permethylated [M + $Na^+$] C84H149N5O41Na, calcd 1906, found 1906.8.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (88).**

Compound **27a** (0.5 mg, 0.41 μmol), CMP-Neu5Ac (0.54 mg, 2.0 equiv.), and hST6Gal-I (2.5 mU) were dissolved in 103 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.55 mg, 90 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.95 (s, 1H), 4.72 – 4.67 (m, 2H), 4.50 – 4.39 (m, 4H), 4.28 (d, $J$ = 11.8 Hz, 1H), 4.20 (s, 1H), 4.15 (s, 1H), 4.11 (s, 1H), 4.08 – 4.04 (m, 1H), 4.03 – 3.48 (m, 39H), 2.66 (d, $J$ = 12.1 Hz, 1H), 2.10 – 1.97 (m, 12H), 1.71 (t, $J$ = 11.7 Hz, 1H), 1.45 – 1.36 (m, 3H); MALDI-TOF MS: permethylated [M + $Na^+$] C84H149N5O41Na, calcd 1906, found 1906.8.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (27b).**

Compound **27a** (6.0 mg, 5 μmol) was treated with a mixture of 15 mU of HP β3GlcNAcT and UDP-GlcNAc (4.9 mg, 1.5 equiv.) in 0.5 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (6.5 mg, 90 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.95 (d, $J$ = 3.9 Hz, 1H), 4.71 – 4.63 (m, 3H), 4.48 – 4.41 (m, 4H), 4.28 (dd, $J$ = 11.1, 3.8 Hz, 1H), 4.19 (d, $J$ = 3.1 Hz, 1H), 4.17-4.13 (m, 2H), 4.11 (d, $J$ = 3.4 Hz, 1H), 4.06 (dd, $J$ = 7.8, 4.6 Hz, 1H), 4.00 (dd, $J$ = 11.1, 3.0 Hz, 1H), 3.96 – 3.91 (m, 2H), 3.88 (dd, $J$ = 12.4, 2.1 Hz, 1H), 3.85 – 3.60 (m, 25H), 3.59 – 3.52 (m, 6H), 3.49 – 3.41 (m, 2H), 2.03 (s, 3H), 2.02 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.40 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS: [M + $Na^+$] C54H91N5O38Na, calcd 1440, found 1440.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (28a).**

Compound **27b** (11.3 mg, 8 μmol) was treated with a mixture of 20 mU of GalT-1 and UDP-Gal (7.3 mg, 1.5 equiv.) in 0.8 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (12 mg, 94 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.95 (d, $J$ = 3.8 Hz, 1H), 4.72 – 4.66 (m, 3H), 4.48 – 4.39 (m, 5H), 4.28 (dd, $J$ = 11.1, 3.8 Hz, 1H), 4.19 (s, 1H), 4.17-4.13 (m, 2H), 4.11 (s, 1H), 4.06 (dd, $J$ = 7.8, 4.7 Hz, 1H), 3.99 (dd, $J$ = 11.1, 3.0 Hz, 1H), 3.96 – 3.88 (m, 4H), 3.86 – 3.48 (m, 38H), 2.03(s, 3H), 2.02 (s, 6H), 2.01 (s, 3H), 1.39 (d, $J$ = 6.6 Hz, 3H); MALDI-TOF MS: [M + $Na^+$] C60H101N5O43Na, calcd 1602, found 1603.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (28).**

Compound **28a** (0.8 mg, 0.49 μmol), CMP-Neu5Ac (0.65 mg, 2.0 equiv.), and rST3Gal-III (1.6 mU) were dissolved in 245 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.85 mg, 93 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.95 (d, $J$ = 4.1 Hz, 1H), 4.72 – 4.66 (m,

3H), 4.54 (d, *J* = 8.2 Hz, 1H), 4.49 – 4.40 (m, 4H), 4.28 (dd, *J* = 11.3, 3.5 Hz, 1H), 4.19 (d, *J* = 2.3 Hz, 1H), 4.17 – 4.13 (m, 2H), 4.13 – 4.08 (m, 2H), 4.06 (t, *J* = 5.9 Hz, 1H), 4.03 – 3.47 (m, 49H), 2.74 (dd, *J* = 12.0, 4.4 Hz, 1H), 2.08 – 1.95 (m, 15H), 1.78 (t, *J* = 12.1 Hz, 1H), 1.40 (d, *J* = 6.6 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C98H173N5O49Na, calcd 2226, found 2226.9.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (89).**

Compound **28a** (0.4 mg, 0.25 μmol), CMP-Neu5Ac (0.4 mg, 2.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 84 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.4 mg, 85 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.96 (d, *J* = 4.5 Hz, 1H), 4.74 – 4.65 (m, 3H), 4.49 – 4.41 (m, 5H), 4.29 (dd, *J* = 10.7, 3.6 Hz, 1H), 4.20 (s, 1H), 4.17 – 4.13 (m, 2H), 4.11 (d, *J* = 3.6 Hz, 1H), 4.09 – 4.03 (m, 1H), 4.03 – 3.45 (m, 50H), 2.66 (dd, *J* = 12.2, 4.8 Hz, 1H), 2.08 – 1.97 (m, 15H), 1.71 (t, *J* = 12.5 Hz, 1H), 1.40 (d, *J* = 6.8 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C98H173N5O49Na, calcd 2226, found 2226.9.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (28b).**

Compound **28a** (7.7 mg, 4.8 μmol) was treated with a mixture of 28 mU of HP β3GlcNAcT and UDP-GlcNAc (5 mg, 1.5 equiv.) in 0.5 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (8 mg, 90 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.95 (d, *J* = 3.8 Hz, 1H), 4.72-4.64 (m, 4H), 4.48-4.41 (m, 5H), 4.28 (dd, *J* = 11.1, 3.8 Hz, 1H), 4.19 (s, 1H), 4.16 – 4.12 (m, 3H), 4.11 (s, 1H), 4.06 (t, *J* = 6.2 Hz, 1H), 3.99 (dd, *J* = 11.0, 2.8 Hz, 1H), 3.97-3.91 (m, 3H), 3.88 (d, *J* = 12.4 Hz, 1H), 3.86 – 3.40 (m, 43H), 2.03(s, 3H), 2.02 (s, 9H), 2.01 (s, 3H), 1.40 (d, *J* = 6.6 Hz, 1H); $^{13}$C NMR (150 MHz, $D_2O$): *δ* = 175.7(3), 175.6(8), 175.4, 172.7, 105.4, 103.7, 103.6, 103.40, 100.1, 82.9, 82.8, 82.6, 79.0, 78.9, 77.4, 76.4, 75.7, 75.5, 75.3, 74.3, 73.0, 72.0, 70.8, 70.7, 70.5, 70.4, 69.7, 69.2, 69.1, 62.0, 61.7, 61.2, 60.6, 59.9, 56.4, 55.9, 49.3, 23.1, 23.0, 18.9; MALDI-TOF MS: [M + $Na^+$] C68H114N6O48Na, calcd 1805, found 1806.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (29a).**

Compound **28b** (6.7 mg, 3.7 μmol) was treated with a mixture of 20 mU of GalT-1 and UDP-Gal (4.6 mg, 2.0 equiv.) in 0.75 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (7 mg, 96 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.95 (d, *J* = 3.8 Hz, 1H), 4.72 – 4.65 (m, 4H), 4.49-4.41 (m, 6H), 4.28 (dd, *J* = 11.1, 3.8 Hz, 1H), 4.19 (s, 1H), 4.16-4.13 (m, 3H), 4.11 (s, 1H), 4.06 (t, *J* = 6.3 Hz, 1H), 4.00 (dd, *J* = 11.1, 2.9 Hz, 1H), 3.97-3.90 (m, 5H), 3.86 – 3.50 (m, 48H), 2.03(s, 3H), 2.02 (s, 9H), 2.01 (s, 3H), 1.40 (d, *J* = 6.6 Hz, 3H); MALDI-TOF MS: [M + $Na^+$] C74H124N6O53Na, calcd 1967, found 1967.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (29).**

Compound **29a** (2 mg, 1 μmol), CMP-Neu5Ac (1.3 mg, 2.0 equiv.), and rST3Gal-III (2.8 mU) were dissolved in 500 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.1 mg, 91 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.96 (d, *J* = 4.2 Hz, 1H), 4.73 – 4.65 (m, 4H), 4.54 (d, *J* = 7.8 Hz, 1H), 4.48 – 4.40 (m, 5H), 4.28 (dd, *J* = 11.2, 3.7 Hz, 1H), 4.22 – 4.18 (m, 1H), 4.17 – 4.13 (m, 3H), 4.12 – 4.08 (m, 2H), 4.06 (dd, *J* = 8.1, 5.1 Hz, 1H), 4.00 (dd, *J* = 10.9, 2.9 Hz, 1H), 3.97 – 3.90 (m, 5H), 3.90 – 3.49 (m, 54H), 2.74 (dd, *J* = 12.3, 4.7 Hz, 1H), 2.06 – 1.97 (m, 18H), 1.79 (t, *J* = 12.3 Hz, 1H), 1.40 (d, *J* = 6.7 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): *δ* = 175.8, 175.7, 175.4, 174.6, 172.5, 105.4, 103.7, 103.6, 103.5(5), 103.4, 103.3, 100.6, 100.1, 82.9, 82.6, 79.0, 78.9, 78.7, 77.4, 76.3, 76.0, 75.7, 75.3, 73.7, 73.0, 72.5, 72.0, 70.7, 70.5, 70.2, 69.6, 69.2, 69.1, 68.9, 68.3, 63.4, 62.0, 61.8, 61.7, 60.6, 59.8, 55.9, 52.5, 49.3, 40.4, 39.5, 23.1, 23.0, 22.8, 18.9; MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C118H208N6O59Na, calcd 2675, found 2676.1.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (90).**

Compound **29a** (0.5 mg, 0.25 μmol), CMP-Neu5Ac (0.4 mg, 2.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 85 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 89 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.96 (d, $J$ = 3.8 Hz, 1H), 4.74 – 4.65 (m, 4H), 4.49 – 4.41 (m, 6H), 4.29 (dd, $J$ = 11.0, 3.6 Hz, 1H), 4.19 (d, $J$ = 3.3 Hz, 1H), 4.17 – 4.13 (m, 3H), 4.11 (d, $J$ = 3.9 Hz, 1H), 4.06 (dd, $J$ = 7.6, 4.9 Hz, 1H), 4.02 – 3.47 (m, 61H), 2.66 (dd, $J$ = 12.2, 4.7 Hz, 1H), 2.07 – 1.97 (m, 18H), 1.71 (t, $J$ = 12.2 Hz, 1H), 1.40 (d, $J$ = 6.8 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] $C_{118}H_{208}N_6O_{59}Na$, calcd 2675, found 2676.1.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (29b).**

Compound **29a** (6.3 mg, 3.2 μmol) was treated with a mixture of 18 mU of HP β3GlcNAcT and UDP-GlcNAc (4.2 mg, 2.0 equiv.) in 0.64 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (6.5 mg, 93 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.95 (d, $J$ = 3.8 Hz, 1H), 4.72 – 4.65 (m, 5H), 4.49-4.41 (m, 6H), 4.28 (dd, $J$ = 11.0, 3.8 Hz, 1H), 4.19 (s, 1H), 4.17 – 4.13 (m, 4H), 4.11 (s, 1H), 4.06 (t, $J$ = 6.3 Hz, 1H), 4.00 (d, $J$ = 11.2 Hz, 1H), 3.96-3.91 (m, 4H), 3.88 (d, $J$ = 12.1 Hz, 1H), 3.86 – 3.42 (m, 53H), 2.03(s, 3H), 2.02 (s, 12H), 2.01 (s, 3H), 1.40 (d, $J$ = 6.6 Hz, 3H); MALDI-TOF MS: [M + $Na^+$] $C_{82}H_{137}N_7O_{58}Na$, calcd 2170, found 2171.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (30a).**

Compound **29b** (5.5 mg, 2.5 μmol) was treated with a mixture of 15 mU of GalT-1 and UDP-Gal (3.1 mg, 2.0 equiv.) in 0.5 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (5 mg, 86 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.95 (d, $J$ = 4.0 Hz, 1H), 4.72 – 4.65 (m, 5H), 4.49-4.42 (m, 7H), 4.29 (d, $J$ = 11.4 Hz, 1H), 4.19 (s, 1H), 4.17 – 4.13 (s, 4H), 4.11 (s, 1H), 4.06 (d, $J$ = 6.9 Hz, 1H), 4.00 (d, $J$ = 11.2 Hz, 1H), 3.97-3.89 (m, 7H), 3.87 – 3.48 (m, 57H), 2.03(s, 3H), 2.02 (s, 12H), 2.01 (s, 3H), 1.40 (d, $J$ = 6.5 Hz, 2H); MALDI-TOF MS: [M + $Na^+$] $C_{88}H_{147}N_7O_{63}Na$, calcd 2332, found 2332.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (30).**

Compound **30a** (2 mg, 0.85 μmol), CMP-Neu5Ac (1.1 mg, 2.0 equiv.), and rST3Gal-III (2.4 mU) were dissolved in 425 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.0 mg, 91 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.96 (d, $J$ = 3.8 Hz, 1H), 4.72 – 4.65 (m, 5H), 4.54 (d, $J$ = 7.8 Hz, 1H), 4.49 – 4.40 (m, 6H), 4.28 (dd, $J$ = 11.0, 3.8 Hz, 1H), 4.22 – 4.17 (m, 1H), 4.17 – 4.13 (m, 4H), 4.12 – 4.08 (m, 2H), 4.06 (dd, $J$ = 7.9, 5.2 Hz, 1H), 3.99 (dd, $J$ = 10.9, 2.9 Hz, 1H), 3.97 – 3.90 (m, 6H), 3.90 – 3.51 (m, 64H), 2.74 (dd, $J$ = 12.6, 4.9 Hz, 1H), 2.07 – 1.95 (m, 21H), 1.79 (t, $J$ = 12.1 Hz, 1H), 1.40 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] $C_{138}H_{243}N_7O_{69}Na$, calcd 3124, found 3125.1.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3GalNAcα-Thr (91).**

Compound **30a** (0.5 mg, 0.21 μmol), CMP-Neu5Ac (0.3 mg, 2.0 equiv.), and hST6Gal-I (0.6 mU) were dissolved in 42 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 91 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.96 (d, $J$ = 3.8 Hz, 1H), 4.73 – 4.66 (m, 5H), 4.48 – 4.41 (m, 7H), 4.29 (dd, $J$ = 10.9, 3.7 Hz, 1H), 4.19 (d, $J$ = 2.6 Hz, 1H), 4.17 – 4.13 (m, 4H), 4.11 (d, $J$ =

3.9 Hz, 1H), 4.06 (t, *J* = 6.6 Hz, 1H), 4.02 – 3.48 (m, 72H), 2.66 (dd, *J* = 12.4, 4.4 Hz, 1H), 2.07 – 1.97 (m, 21H), 1.71 (t, *J* = 12.2 Hz, 1H), 1.40 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] $C_{138}H_{243}N_7O_{69}Na$, calcd 3124, found 3125.1.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (33b).**

Compound **33a** (2.5 mg, 2 μmol) was treated with a mixture of 14 mU of HP β3GlcNAcT and UDP-GlcNAc (4.2 mg, 2.0 equiv.) in 0.4 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.7 mg, 95 % yield). $^1$H NMR (500 MHz, $D_2O$): *δ* = 4.97 (d, *J* = 3.9 Hz, 1H), 4.74 – 4.67 (m, 2H), 4.59 (d, *J* = 8.3 Hz, 1H), 4.53 – 4.40 (m, 4H), 4.31 (dd, *J* = 11.2, 3.7 Hz, 1H), 4.26 – 4.15 (m, 4H), 4.13 (d, *J* = 11.1 Hz, 1H), 4.08 – 3.89 (m, 6 H), 3.88 – 3.42 (m, 31H), 2.07 (s, 3H), 2.06 (s, 9H), 1.39 (d, *J* = 6.6 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): *δ* = 175.7, 175.6, 175.4, 175.0, 172.5, 105.4, 103.6, 103.5, 101.8, 100.1, 82.8, 79.1, 78.8, 77.2, 76.4, 75.7, 75.6, 75.6, 75.5, 75.3, 74.3, 73.5, 73.2, 72.9, 71.3, 70.8, 70.7, 70.7, 70.4, 69.9, 69.3, 69.1, 61.8, 61.7, 61.2, 60.7, 60.6, 59.9, 56.4, 55.9, 55.7, 49.2, 23.0, 22.9, 19.1; MALDI-TOF MS: [M + $Na^+$] $C_{54}H_{91}N_5O_{38}Na$, calcd 1440, found 1441.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (34a).**

Compound **33b** (2.4 mg, 1.7 μmol) was treated with a mixture of 15 mU of GalT-1 and UDP-Gal (2 mg, 2.0 equiv.) in 0.17 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.5 mg, 92 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (d, *J* = 3.9 Hz, 1H), 4.69 (d, *J* = 8.3 Hz, 2H), 4.56 (d, *J* = 8.4 Hz, 1H), 4.50 – 4.36 (m, 5H), 4.27 (dd, *J* = 11.1, 3.8 Hz, 1H), 4.21 – 4.11 (m, 4H), 4.09 (m, 1H), 4.03 – 3.87 (m, 6H), 3.87 – 3.45 (m, 37H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 6H), 1.35 (d, *J* = 6.8 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): *δ* = 175.7, 175.4, 175.0, 172.5, 105.4, 103.7, 103.6(3), 103.5(5), 101.9, 100.1, 82.9, 82.8, 79.2, 78.9, 77.3, 76.1, 75.8, 75.7, 75.6, 75.3, 73.6, 73.3, 73.0, 71.7, 71.4, 70.9, 70.7, 69.9, 69.4, 69.3, 69.1, 61.82, 61.7(5), 60.8, 60.6, 59.9, 56.0, 55.9, 55.7, 49.3, 23.1, 23.0, 19.1; MALDI-TOF MS: [M + $Na^+$] $C_{60}H_{101}N_5O_{43}Na$, calcd 1602, found 1603.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (34).**

Compound **34a** (0.5 mg, 0.32 μmol), CMP-Neu5Ac (0.5 mg, 2.0 equiv.), and rST3Gal-III (1 mU) were dissolved in 158 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 85 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (d, *J* = 3.5 Hz, 1H), 4.71 – 4.65 (m, 2H), 4.58 – 4.52 (m, 2H), 4.48 – 4.37 (m, 3H), 4.27 (dd, *J* = 11.1, 3.7 Hz, 1H), 4.19 (d, *J* = 3.4 Hz, 1H), 4.18 – 4.13 (m, 3H), 4.13 – 4.07 (m, 2H), 4.04 – 3.52 (m, 60H), 2.74 (dd, *J* = 12.6, 4.5 Hz, 1H), 2.07 – 1.96 (m, 15H), 1.79 (t, *J* = 12.2 Hz, 1H), 1.36 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] $C_{98}H_{173}N_5O_{49}Na$, calcd 2226, found 2226.7.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (94).**

Compound **34a** (0.5 mg, 0.32 μmol), CMP-Neu5Ac (0.5 mg, 2.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 158 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 85 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (d, *J* = 4.2 Hz, 1H), 4.74 – 4.67 (m, 2H), 4.55 (d, *J* = 8.4 Hz, 1H), 4.48 – 4.37 (m, 5H), 4.27 (dd, *J* = 11.2, 3.5 Hz, 1H), 4.19 (d, *J* = 3.4 Hz, 1H), 4.18 – 4.12 (m, 3H), 4.11 – 4.07 (m, 1H), 4.03 – 3.44 (m, 50H), 2.66 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.07 – 1.98 (m, 15H), 1.71 (t, *J* = 12.3 Hz, 1H), 1.35 (d, *J* = 6.8 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] $C_{98}H_{173}N_5O_{49}Na$, calcd 2226, found 2226.7.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (34b).**

Compound **34a** (2.3 mg, 1.46 μmol) was treated with a mixture of 8.5 mU of HP β3GlcNAcT and UDP-GlcNAc (4.2 mg, 2.0 equiv.) in 0.15 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.2 mg, 85 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.93 (d, $J$ = 3.9 Hz, 1H), 4.71-4.66 (m, 3H), 4.56 (d, $J$ = 8.3 Hz, 1H), 4.48 – 4.36 (m, 5H), 4.27 (dd, $J$ = 11.1, 3.8 Hz, 1H), 4.23 – 4.12 (m, 5H), 4.09 (dd, $J$ = 11.0, 3.1 Hz, 1H), 4.04 – 3.86 (m, 7H), 3.84 – 3.39 (m, 41H), 2.04 (s, 3H), 2.02 (s, 6H), 2.01 (s, 6H), 1.35 (d, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.7(4), 175.6(9), 175.4, 175.0, 172.9, 105.4, 103.7, 103.6, 101.9, 100.1, 82.9, 82.8, 79.2, 78.9, 77.3, 76.4, 75.8, 75.7(3), 75.6(7), 75.6, 75.3, 74.3, 73.6, 73.3, 72.9, 71.4, 70.8, 70.8, 70.7(4), 70.6(9), 70.4, 69.9, 69.4, 69.1, 61.8, 61.7, 61.2, 60.8, 60.6, 60.0, 56.4, 55.9, 55.7, 49.3, 23.1, 23.0, 19.1; MALDI-TOF MS: [M + $Na^+$] C68H114N6O48Na, calcd 1805, found 1806.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (35a).**

Compound **34b** (1.9 mg, 1.05 μmol) was treated with a mixture of 15 mU of GalT-1 and UDP-Gal (1.3 mg, 2.0 equiv.) in 0.1 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (1.9 mg, 93 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.93 (d, $J$ = 3.9 Hz, 1H), 4.72-4.66 (m, 3H), 4.55 (d, $J$ = 8.3 Hz, 1H), 4.51 – 4.37 (m, 6H), 4.27 (dd, $J$ = 10.9, 3.8 Hz, 1H), 4.22 – 4.06 (m, 6H), 4.05 – 3.87 (m, 8H), 3.86 – 3.46 (m, 46H), 2.04 (s, 3H), 2.03 – 2.00 (m, 12H), 1.35 (d, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.70, 175.40, 175.03, 172.67, 105.42, 103.67, 103.56, 101.86, 100.14, 82.86, 82.80, 79.16, 78.90, 77.29, 76.14, 75.78, 75.66, 75.60, 75.33, 73.57, 73.28, 72.96, 71.75, 71.36, 70.84, 70.74, 69.91, 69.37, 69.33, 69.11, 61.82, 61.75, 60.80, 60.62, 59.94, 55.97, 55.93, 55.73, 49.27, 23.08, 22.95, 19.11; MALDI-TOF MS: [M + $Na^+$] C74H124N6O53Na, calcd 1967, found 1967.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (35).**

Compound **35a** (2 mg, 1.03 μmol), CMP-Neu5Ac (1.4 mg, 2.0 equiv.), and rST3Gal-III (3 mU) were dissolved in 505 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2 mg, 87 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.93 (d, $J$ = 4.2 Hz, 1H), 4.71 – 4.66 (m, 3H), 4.58 – 4.51 (m, 2H), 4.48 – 4.38 (m, 5H), 4.27 (dd, $J$ = 10.9, 3.7 Hz, 1H), 4.22 – 4.18 (m, 1H), 4.18 – 4.13 (m, 4H), 4.12 – 4.06 (m, 2H), 4.04 – 3.52 (m, 60H), 2.74 (dd, $J$ = 12.1, 4.4 Hz, 1H), 2.06 – 1.96 (m, 18H), 1.79 (t, $J$ = 12.1 Hz, 1H), 1.35 (d, $J$ = 7.1 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C118H208N6O59Na, calcd 2675, found 2676.0.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (95).**

Compound **35a** (0.5 mg, 0.26 μmol), CMP-Neu5Ac (0.4 mg, 2.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 86 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.55 mg, 85 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.93 (d, $J$ = 4.2 Hz, 1H), 4.73 – 4.66 (m, 3H), 4.56 (d, $J$ = 8.3 Hz, 1H), 4.49 – 4.37 (m, 6H), 4.27 (dd, $J$ = 10.9, 3.6 Hz, 1H), 4.19 (d, $J$ = 2.9 Hz, 1H), 4.18 – 4.13 (m, 4H), 4.09 (d, $J$ = 8.3 Hz, 1H), 4.03 – 3.43 (m, 61H), 2.66 (dd, $J$ = 12.2, 4.5 Hz, 1H), 2.07 – 1.97 (m, 18H), 1.71 (t, $J$ = 12.1 Hz, 1H), 1.35 (d, $J$ = 7.0 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C118H208N6O59Na, calcd 2675, found 2676.0.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (36a).**

Compound **35a** (1.6 mg, 0.82 μmol) was treated with a mixture of 8.5 mU of HP β3GlcNAcT and UDP-GlcNAc (4.2 mg, 2.0 equiv.) in 82 μL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was

concentrated, purified by Sephadex™ G-25 and lyophilized to give **35b** as a white powder (1.5 mg, 85 % yield). MALDI-TOF MS: [M + $Na^+$] C82H137N7O58Na, calcd 2170, found 2170.

Compound **35b** (1.22 mg, 0.57 μmol) was treated with a mixture of 15 mU of GalT-1 and UDP-Gal (1.3 mg, 2.0 equiv.) in 57 μL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (1.1 mg, 85 % yield).$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.93 (s, 1H), 4.72-4.66 (m, 4H), 4.56 (d, $J$ = 8.3 Hz, 1H), 4.49-4.41 (m, 6H), 4.39-4.33 (m, 1H), 4.27 (d, $J$ = 7.8 Hz, 1H), 4.23 – 4.06 (m, 8H), 4.03-3.87 (m, 10H), 3.87-3.45 (m, 19H), 2.03 (s, 3H), 2.02 (s, 15H), 1.34 (d, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (151 MHz, $D_2O$): $\delta$ = 175.69, 175.04, 105.42, 103.67, 103.56, 101.86, 100.11, 82.85, 79.16, 78.90, 77.36, 76.14, 75.77, 75.65, 75.33, 73.56, 73.28, 72.96, 71.75, 71.36, 70.74, 69.91, 69.37, 69.33, 69.11, 61.82, 61.75, 60.61, 55.93, 49.31, 23.08, 22.95, 19.10; MALDI-TOF MS: [M + $Na^+$] C88H147N7O63Na, calcd 2332, found 2333.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (36).**

Compound **36a** (2 mg, 0.87 μmol), CMP-Neu5Ac (1.1 mg, 2.0 equiv.), and rST3Gal-III (0.5 mU) were dissolved in 433 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.1 mg, 93 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.93 (d, $J$ = 3.9 Hz, 1H), 4.72 – 4.65 (m, 4H), 4.59 – 4.51 (m, 2H), 4.49 – 4.36 (m, 7H), 4.27 (dd, $J$ = 11.1, 3.8 Hz, 1H), 4.19 (d, $J$ = 4.2 Hz, 1H), 4.18 – 4.12 (m, 6H), 4.12 – 4.07 (m, 2H), 4.03 – 3.36 (m, 69H), 2.74 (dd, $J$ = 12.8, 4.8 Hz, 1H), 2.06 – 1.96 (m, 21H), 1.79 (t, $J$ = 12.1 Hz, 1H), 1.35 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C138H243N7O69Na, calcd 3124, found 3125.1.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ3)GalNAcα-Thr (96).**

Compound **36a** (0.5 mg, 0.22 μmol), CMP-Neu5Ac (0.3 mg, 2.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 72 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 89 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.93 (d, $J$ = 3.6 Hz, 1H), 4.73 – 4.66 (m, 4H), 4.56 (d, $J$ = 8.2 Hz, 1H), 4.49 – 4.36 (m, 7H), 4.27 (dd, $J$ = 11.1, 3.8 Hz, 1H), 4.19 (d, $J$ = 2.7 Hz, 1H), 4.18 – 4.12 (m, 5H), 4.09 (d, $J$ = 8.1 Hz, 1H), 4.04 – 3.44 (m, 72H), 2.66 (dd, $J$ = 12.4, 4.7 Hz, 1H), 2.07 – 1.97 (m, 21H), 1.71 (t, $J$ = 12.3 Hz, 1H), 1.35 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C138H243N7O69Na, calcd 3124, found 3125.2.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(GlcNAcβ3Galβ4GlcNAcβ3Galβ3)GalNAcα-Thr (37d).**

Compound **37c** (1.75 mg, 1.11 μmol) was treated with a mixture of 25 mU of HP β3GlcNAcT and UDP-GlcNAc (2.9 mg, 4.0 equiv.) in 111 μL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give **37d** as a white powder (1.96 mg, 89 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.93 (d, $J$ = 3.8 Hz, 1H), 4.72 – 4.63 (m, 4H), 4.55 (d, $J$ = 8.3 Hz, 1H), 4.49 – 4.36 (m, 5H), 4.27 (dd, $J$ = 11.1, 3.8 Hz, 1H), 4.19 – 4.06 (m, 7H), 4.02 – 3.85 (m, 7H), 3.86 – 3.39 (m, 46H), 2.03 (s, 3H), 2.02 (s, 9H), 2.01 (s, 3H), 2.00 (s, 3H), 1.35 (d, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.7, 175.7, 175.0, 105.4, 103.7, 103.5, 103.4, 101.9, 100.1, 82.8, 82.6, 79.1, 79.0, 78.9, 77.1, 76.4, 75.7, 75.6, 75.3, 74.3, 73.6, 73.0, 70.9, 70.8, 70.7, 70.5, 70.4, 70.0, 69.2, 69.1, 61.8, 61.2, 60.8, 60.6, 59.9, 56.4, 55.9, 55.7, 49.24, 2.12, 23.1, 23.0, 19.1; MALDI-TOF MS: [M + $Na^+$] C76H127N7O53Na, calcd 2008, found 2009.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3)GalNAcα-Thr (37e).**

Compound **37d** (1.76 mg, 0.89 μmol) was treated with a mixture of 15 mU of GalT-1 and UDP-Gal (2.2 mg, 4.0 equiv.) in 89 μL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (1.81 mg, 87 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.92 (d, $J$ = 3.1 Hz, 1H), 4.72-4.67 (m, 4H), 4.55 (d, $J$ = 8.4 Hz, 1H), 4.49 – 4.35 (m, 7H), 4.27 (dd, $J$ = 11.7, 4.0 Hz, 1H), 4.21 – 4.06 (m, 7H), 4.03 – 3.88 (m, 8H), 3.86 – 3.49 (m, 57H), 2.03 (s, 3H), 2.02 (s, 12H), 2.01 (s, 3H), 1.34 (d, $J$ = 6.4 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.7, 175.0, 105.4, 103.7, 103.6, 103.5(6), 103.4, 101.9, 100.1, 82.9, 82.6, 79.2, 78.9, 77.2, 76.1, 75.7, 75.6, 75.3, 73.6, 73.3, 73.0, 71.8, 70.9, 70.7, 70.5, 70.0, 69.3, 69.1, 61.8, 61.7(5), 60.8, 60.6, 60.0, 56.0, 55.7, 49.3, 23.1, 23.1, 23.0, 19.1; MALDI-TOF MS: [M + $Na^+$] $C_{88}H_{147}N_7O_{63}Na$, calcd 2332, found 2333.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3)GalNAcα-Thr (37f).**

Compound **37e** (1.56 mg, 0.68 μmol) was treated with a mixture of 9 mU of HP β3GlcNAcT and UDP-GlcNAc (1.8 mg, 4.0 equiv.) in 68 μL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (1.84 mg, 100 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.92 (d, $J$ = 3.8 Hz, 1H), 4.72 – 4.64 (m, 6H), 4.55 (d, $J$ = 8.3 Hz, 1H), 4.48 – 4.37 (m, 7H), 4.27 (dd, $J$ = 11.3, 3.6 Hz, 1H), 4.20 – 4.07 (m, 9H), 4.03 – 3.36 (m, 75H), 2.03 (s, 3H), 2.02-2.01 (m, 18H), 2.01 (s, 3H), 1.35 (d, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.8, 175.7, 103.7, 103.6, 101.9, 82.8, 78.9, 76.4, 75.7, 75.3, 74.3, 73.0, 70.8, 70.7, 70.5, 69.1, 61.8, 61.2, 60.6, 56.4, 55.9, 23.1, 23.0, 19.1; MALDI-TOF MS: [M + $Na^+$] $C_{104}H_{173}N_9O_{73}Na$, calcd 2738, found 2739.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3)GalNAcα-Thr (37a).**

Compound **37f** (7 mg, 2.58 μmol) was treated with a mixture of 30 mU of GalT-1 and UDP-Gal (6.3 mg, 4.0 equiv.) in 520 μL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (7.5 mg, 95 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.95 – 4.90 (m, 1H), 4.72 – 4.64 (m, 6H), 4.55 (d, $J$ = 8.2 Hz, 1H), 4.48 – 4.37 (m, 9H), 4.27 (d, $J$ = 8.3 Hz, 1H), 4.21 – 4.06 (m, 9H), 4.02 – 3.49 (m, 87H), 2.03 (s, 3H), 2.02-2.01 (m, 18H), 2.01 (s, 3H), 1.35 (d, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.7, 105.4, 103.6, 103.6, 103.6, 101.9, 82.9, 82.6, 79.2, 78.9, 77.2, 76.1, 75.7, 75.3, 73.6, 73.3, 73.0, 71.8, 70.7, 70.5, 70.0, 69.3, 69.1, 61.8, 61.8, 60.6, 56.0, 55.9, 55.7, 49.3, 23.1, 23.0; MALDI-TOF MS: [M + $Na^+$] $C_{116}H_{193}N_9O_{83}Na$, calcd 3062, found 3063.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3)GalNAcα-Thr (37).**

Compound **37a** (0.4 mg, 0.13 μmol), CMP-Neu5Ac (0.4 mg, 4.0 equiv.), and rST3Gal-III (1 mU) were dissolved in 133 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.4 mg, 84 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.93 (s, 1H), 4.72 – 4.66 (m, 6H), 4.58 – 4.52 (m, 3H), 4.48 – 4.38 (m, 7H), 4.28 (d, $J$ = 11.1 Hz, 1H), 4.20 – 4.06 (m, 12H), 4.02 – 3.46 (m, 98H), 2.74 (dd, $J$ = 12.2, 4.2 Hz, 2H), 2.07 – 1.98 (m, 33H), 1.79 (t, $J$ = 12.1 Hz, 2H), 1.35 (d, $J$ = 6.5 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] $C_{194}H_{340}N_{10}O_{97}Na$, calcd 4383, found 4384.6.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3)GalNAcα-Thr (97).**

Compound **37a** (0.5 mg, 0.16 μmol), CMP-Neu5Ac (0.4 mg, 4.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 109 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 85 % yield).$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.92 (s, 1H), 4.73 – 4.66 (m, 6H), 4.55 (d, $J$

= 7.9 Hz, 1H), 4.48 – 4.37 (m, 9H), 4.28 (d, *J* = 11.1 Hz, 1H), 4.21 – 4.08 (m, 10H), 4.03 – 3.49 (m, 100H), 2.66 (dd, *J* = 12.3, 4.4 Hz, 2H), 2.07 – 1.98 (m, 33H), 1.71 (t, *J* = 12.1 Hz, 2H), 1.35 (d, *J* = 6.5 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] $C_{194}H_{340}N_{10}O_{97}Na$, calcd 4383, found 4386.6.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3)GalNAcα-Thr (37b).**

Compound **37a** (7.5 mg, 2.45 μmol) was treated with a mixture of 25 mU of HP β3GlcNAcT and UDP-GlcNAc (6.4 mg, 4.0 equiv.) in 490 μL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (8.2 mg, 96 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (s, 1H), 4.72 – 4.64 (m, 8H), 4.55 (d, *J* = 7.0 Hz, 1H), 4.49 – 4.38 (m, 9H), 4.27 (d, *J* = 11.0 Hz, 1H), 4.22 – 4.06 (m, 11H), 4.04 – 3.38 (m, 97H), 2.03 (s, 3H), 2.02-2.01 (m, 24H), 2.01 (s, 3H), 1.35 (d, *J* = 6.8 Hz, 3H); MALDI-TOF MS: [M + $Na^+$] $C_{132}H_{219}N_{11}O_{93}Na$, calcd 3468, found 3469.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3)GalNAcα-Thr (38a).**

Compound **37b** (3.7 mg, 1.07 μmol) was treated with a mixture of 20 mU of GalT-1 and UDP-Gal (2.6 mg, 4.0 equiv.) in 214 μL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (3.9 mg, 96 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.92 (d, *J* = 3.9 Hz, 1H), 4.72 – 4.64 (m, 8H), 4.55 (d, *J* = 8.2 Hz, 1H), 4.50 – 4.36 (m, 11H), 4.27 (dd, *J* = 10.9, 3.2 Hz, 1H), 4.20 – 4.06 (m, 11H), 4.03 – 3.48 (m, 109H), 2.08 – 1.96 (m, 30H), 1.35 (d, *J* = 6.8 Hz, 3H); MALDI-TOF MS: [M + $Na^+$] $C_{144}H_{239}N_{11}O_{103}Na$, calcd 3792, found 3795.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3)GalNAcα-Thr (38).**

Compound **38a** (2 mg, 0.53 μmol), CMP-Neu5Ac (1.4 mg, 4.0 equiv.), and rST3Gal-III (3 mU) were dissolved in 530 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2 mg, 87 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (d, *J* = 3.5 Hz, 1H), 4.72 – 4.66 (m, 8H), 4.58 – 4.52 (m, 3H), 4.49 – 4.37 (m, 8H), 4.28 (d, *J* = 11.1 Hz, 1H), 4.21 – 4.06 (m, 11H), 4.00 – 3.47 (m, 124H), 2.74 (dd, *J* = 12.5, 4.6 Hz, 2H), 2.06 – 1.96 (m, 36H), 1.79 (t, *J* = 12.2 Hz, 2H), 1.35 (d, *J* = 6.4 Hz, 3H); MALDI-TOF MS: permethylated [M + $H^+$] $C_{241}H_{424}N_{13}O_{119}$, calcd 5402, found 5405.2.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ3)GalNAcα-Thr (98).**

Compound **38a** (0.5 mg, 0.13 μmol), CMP-Neu5Ac (0.4 mg, 4.0 equiv.), and hST6Gal-I (1.5 mU) were dissolved in 88 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 87 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (s, 1H), 4.73 – 4.66 (m, 8H), 4.55 (d, *J* = 8.2 Hz, 1H), 4.48 – 4.38 (m, 12H), 4.28 (d, *J* = 10.5 Hz, 1H), 4.18 – 4.16 (m, 12H), 4.03 – 3.47 (m, 121H), 2.66 (dd, *J* = 12.2, 4.3 Hz, 2H), 2.07 – 1.97 (m, 36H), 1.71 (t, *J* = 12.2 Hz, 2H), 1.35 (d, *J* = 6.5 Hz, 3H); MALDI-TOF MS: permethylated [M + $H^+$] $C_{241}H_{424}N_{13}O_{119}$, calcd 5402, found 5405.6.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (40b).**

Compound **40a** (5.1 mg, 4.85 μmol) was treated with a mixture of 30 mU of HP β3GlcNAcT and UDP-GlcNAc (6.3 mg, 2.0 equiv.) in 490 μL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (5.6 mg, 92 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.91 (d, *J* = 3.8 Hz, 1H), 4.69 – 4.64 (m, 2H), 4.58 (d, *J* = 7.9 Hz, 1H), 4.47 – 4.40 (m, 3H), 4.21 (dd, *J* = 11.2, 3.8 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.14 (dd, *J* = 3.7, 1.6 Hz, 2H), 4.04 (dd,

*J* = 8.3, 4.9 Hz, 1H), 3.97 – 3.90 (m, 3H), 3.90 – 3.85 (m, 1H), 3.85 – 3.65 (m, 21H), 3.60 – 3.52 (m, 5H), 3.50 – 3.40 (m, 2H), 2.05 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 2H), 1.39 (d, *J* = 6.7 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): *δ* = 175.7, 175.6, 175.2, 174.5, 172.7, 103.6(9), 103.6(6), 103.5(5), 103.0, 99.9, 82.9, 82.8, 79.1, 78.9, 77.1, 76.4, 75.7, 75.4, 75.3, 75.3, 74.3, 73.0, 72.8, 71.8, 70.8, 70.7, 70.4, 69.5, 69.1, 69.1, 62.0, 61.7, 61.2, 60.6, 59.9, 56.4, 55.9(2), 55.8(5), 49.1, 23.1, 23.0(3), 22.9(5), 18.9; MALDI-TOF MS: [M+Na$^+$] C48H81N5O33Na, calcd 1278, found 1278.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (41a).**

Compound **40b** (102 mg, 80 μmol) was treated with a mixture of 360 mU of GalT-1 and UDP-Gal (98 mg, 2.0 equiv.) in 8 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (105 mg, 91 % yield).$^1$H NMR (600 MHz, $D_2O$): *δ* = 4.91 (d, *J* = 3.8 Hz, 1H), 4.70 - 4.66 (m, 2H), 4.59 (d, *J* = 8.0 Hz, 1H), 4.49 – 4.39 (m, 4H), 4.21 (dd, *J* = 11.2, 3.7 Hz, 1H), 4.19 – 4.17 (m, 1H), 4.14 (d, *J* = 2.8 Hz, 2H), 4.08 – 4.01 (m, 1H), 3.97 - 3.90 (m, 5H), 3.86 – 3.63 (m, 27H), 3.61 – 3.50 (m, 6H), 2.05 (s, 3H), 2.04 – 2.01 (m, 6H), 2.00 (s, 3H), 1.39 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: [M+Na$^+$] C54H91N5O38Na, calcd 1440, found 1440.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (41).**

Compound **41a** (2 mg, 1.4 μmol), CMP-Neu5Ac (2 mg, 2.0 equiv.), and rST3Gal-III (8 mU) were dissolved in 700 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.1 mg, 88 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.91 (d, *J* = 4.3 Hz, 1H), 4.70 – 4.66 (m, 2H), 4.58 (d, *J* = 7.7 Hz, 1H), 4.54 (d, *J* = 7.7 Hz, 1H), 4.48 – 4.41 (m, 3H), 4.24 – 4.17 (m, 2H), 4.16 – 4.12 (m, 2H), 4.10 (dd, *J* = 9.9, 3.3 Hz, 1H), 4.04 (t, *J* = 6.2 Hz, 1H), 3.98 – 3.90 (m, 5H), 3.90 – 3.49 (m, 39H), 2.74 (dd, *J* = 12.3, 4.6 Hz, 1H), 2.08 – 1.94 (m, 15H), 1.78 (t, *J* = 12.0 Hz, 1H), 1.39 (d, *J* = 6.8 Hz, 3H); MALDI-TOF MS: permethylated [M + Na$^+$] C95H168N6O46Na, calcd 2151, found 2151.9.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (101).**

Compound **41a** (0.5 mg, 0.353 μmol), CMP-Neu5Ac (0.5 mg, 2.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 118 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 83 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.92 (d, *J* = 4.2 Hz, 1H), 4.71 (d, *J* = 7.7 Hz, 1H), 4.68 (d, *J* = 8.7 Hz, 1H), 4.59 (d, *J* = 7.7 Hz, 1H), 4.49 – 4.39 (m, 4H), 4.26 – 4.17 (m, 2H), 4.16 – 4.13 (m, 2H), 4.05 (dd, *J* = 7.8, 4.8 Hz, 1H), 4.02 – 3.46 (m, 45H), 2.66 (dd, *J* = 12.2, 4.8 Hz, 1H), 2.07 – 1.97 (m, 15H), 1.71 (t, *J* = 12.3 Hz, 1H), 1.39 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + Na$^+$] C89H157N5O44Na, calcd 2022, found 2022.8.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (41b).**

Compound **41a** (74 mg, 52.2 μmol) was treated with a mixture of 150 mU of HP β3GlcNAcT and UDP-GlcNAc (68 mg, 2.0 equiv.) in 5.22 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (80 mg, 93 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.92 (d, *J* = 3.9 Hz, 1H), 4.72 – 4.64 (m, 3H), 4.59 (d, *J* = 8.0 Hz, 1H), 4.49 – 4.39 (m, 4H), 4.21 (dd, *J* = 11.1, 3.8 Hz, 1H), 4.18 (d, *J* = 3.5 Hz, 1H), 4.16 – 4.12 (m, 3H), 4.05 (dd, *J* = 8.4, 4.9 Hz, 1H), 3.96 – 3.90 (m, 4H), 3.90 – 3.86 (m, 1H), 3.85 – 3.66 (m, 29H), 3.61 – 3.51 (m, 7H), 3.48 – 3.41 (m, 2H), 2.05 (s, 3H), 2.02 (s, 3H), 2.01 (s, 6H), 2.00 (s, 3H), 1.39 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: [M+Na$^+$] C62H104N6O43Na, calcd 1643, found 1644.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (42a).**

Compound **41b** (42 mg, 25.9 μmol) was treated with a mixture of 150 mU of GalT-1 and UDP-Gal (32 mg, 2.0 equiv.) in 2.6 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble

precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (45 mg, 96 % yield).[1]H NMR (600 MHz, $D_2O$): $\delta$ = 4.92 (d, $J$ = 4.1 Hz, 1H), 4.72 – 4.62 (m, 3H), 4.60 – 4.50 (m, 2H), 4.49 – 4.40 (m, 4H), 4.23 – 4.16 (m, 2H), 4.17 – 4.10 (m, 3H), 4.06 – 3.99 (m, 2H), 3.96 – 3.88 (m, 6H), 3.86 – 3.61 (m, 34H), 3.61 – 3.48 (m, 8H), 2.03 (s, 3H), 2.02 – 2.00 (m, 6H), 1.99 (s, 3H), 1.41 (d, $J$ = 6.8 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] C68H114N6O48Na, calcd 1805, found 1805.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (42).**

Compound **42a** (2 mg, 1.1 μmol), CMP-Neu5Ac (1.5 mg, 2.0 equiv.), and rST3Gal-III (10 mU) were dissolved in 550 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.1 mg, 91 % yield).

[1]H NMR (600 MHz, $D_2O$): $\delta$ = 4.92 (d, $J$ = 4.3 Hz, 1H), 4.73 – 4.66 (m, 3H), 4.58 (d, $J$ = 7.9 Hz, 1H), 4.54 (d, $J$ = 8.0 Hz, 1H), 4.48 – 4.41 (m, 4H), 4.26 – 4.17 (m, 2H), 4.17 – 4.12 (m, 3H), 4.10 (dd, $J$ = 9.7, 3.1 Hz, 1H), 4.04 (dd, $J$ = 8.2, 4.7 Hz, 1H), 3.97 – 3.90 (m, 6H), 3.90 – 3.60 (m, 39H), 3.60 – 3.50 (m, 10H), 2.74 (dd, $J$ = 12.4, 4.6 Hz, 1H), 2.07 – 1.95 (m, 18H), 1.79 (t, $J$ = 12.3 Hz, 1H), 1.39 (d, $J$ = 6.8 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C109H192N6O54Na, calcd 2471, found 2471.9.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (102).**

Compound **42a** (0.5 mg, 0.277 μmol), CMP-Neu5Ac (0.4 mg, 2.0 equiv.), and hST6Gal-I (0.8 mU) were dissolved in 92 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 86 % yield).[1]H NMR (600 MHz, $D_2O$): $\delta$ = 4.92 (d, $J$ = 3.6 Hz, 1H), 4.73 – 4.66 (m, 3H), 4.59 (d, $J$ = 7.6 Hz, 1H), 4.48 – 4.41 (m, 5H), 4.24 – 4.17 (m, 2H), 4.16 – 4.13 (m, 3H), 4.05 (dd, $J$ = 7.8, 4.7 Hz, 1H), 4.01 – 3.48 (m, 56H), 2.66 (dd, $J$ = 12.3, 4.5 Hz, 1H), 2.08 – 1.96 (m, 18H), 1.71 (t, $J$ = 12.1 Hz, 1H), 1.39 (d, $J$ = 6.6 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C109H192N6O54Na, calcd 2471, found 2471.9.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (42b).**

Compound **42a** (10 mg, 5.54 μmol) was treated with a mixture of 15 mU of HP β3GlcNAcT and UDP-GlcNAc (7.2 mg, 2.0 equiv.) in 0.554 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (10 mg, 90 % yield). [1]H NMR (600 MHz, $D_2O$): $\delta$ = 4.91 (d, $J$ = 3.9 Hz, 1H), 4.72 – 4.64 (m, 4H), 4.59 (d, $J$ = 8.0 Hz, 1H), 4.48 – 4.42 (m, 4H), 4.38 (dd, $J$ = 6.7, 2.6 Hz, 1H), 4.25 – 4.15 (m, 2H), 4.14 (d, $J$ = 3.6 Hz, 4H), 4.05 (dd, $J$ = 8.5, 4.8 Hz, 1H), 3.97 – 3.91 (m, 5H), 3.91 – 3.64 (m, 38H), 3.63 – 3.51 (m, 9H), 3.51 – 3.39 (m, 2H), 2.05 (s, 3H), 2.02 (m, 12H), 2.00 (s, 3H), 1.38 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] C76H127N7O53Na, calcd 2008, found 2008.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (43a).**

Compound **42b** (9 mg, 4.48 μmol) was treated with a mixture of 15 mU of GalT-1 and UDP-Gal (5.6 mg, 2.0 equiv.) in 0.458 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (9 mg, 93 % yield).[1]H NMR (600 MHz, $D_2O$): $\delta$ = 4.91 (d, $J$ = 3.9 Hz, 1H), 4.71 – 4.65 (m, 4H), 4.58 (d, $J$ = 8.0 Hz, 1H), 4.51 – 4.39 (m, 6H), 4.21 (dd, $J$ = 11.2, 3.8 Hz, 1H), 4.19 – 4.17 (m, 1H), 4.14 (d, $J$ = 3.5 Hz, 4H), 4.04 (dd, $J$ = 7.9, 5.2 Hz, 1H), 3.98 – 3.89 (m, 7H), 3.87 – 3.61 (m, 44H), 3.61 – 3.49 (m, 9H), 2.05 (s, 3H), 2.04 – 2.01 (m, 12H), 2.00 (s, 3H), 1.39 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS, [M+$Na^+$] C82H137N7O58Na, calcd 2170, found 2170.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (43).**

Compound **43a** (2.2 mg, 1.0 μmol), CMP-Neu5Ac (1.3 mg, 2.0 equiv.), and rST3Gal-III (3 mU) were dissolved in 333 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.3 mg, 93 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.92 (d, $J$ = 3.9 Hz, 1H), 4.71 – 4.65 (d, $J$ = 8.2 Hz, 4H), 4.58 (d, $J$ = 8.2 Hz, 1H), 4.54 (d, $J$ = 7.9 Hz, 1H), 4.48 – 4.41 (m, 5H), 4.25 – 4.17 (m, 2H), 4.17 – 4.13 (m, 4H), 4.10 (dd, $J$ = 9.8, 3.0 Hz, 1H), 4.04 (dd, $J$ = 8.1, 4.9 Hz, 1H), 3.98 – 3.90 (m, 7H), 3.90 – 3.60 (m, 48H), 3.60 – 3.51 (m, 11H), 2.74 (dd, $J$ = 12.2, 4.5 Hz, 1H), 2.08 – 1.95 (m, 21H), 1.79 (t, $J$ = 12.2 Hz, 1H), 1.39 (d, $J$ = 6.7 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.7, 103.7, 103.6, 103.3, 103.0, 100.5, 100.0, 82.8, 79.1, 78.9, 78.7, 77.0, 76.3, 76.0, 75.7, 75.3, 75.3, 73.7, 72.9, 72.5, 71.8, 70.7, 70.2, 69.5, 69.1, 68.9, 63.4, 62.0, 61.7, 60.6, 59.7, 55.9, 52.5, 49.1, 23.1, 23.0(2), 22.9(5), 22.8, 18.9; MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C129H227N7O64Na, calcd 2920, found 2921.0.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3GalNAcα-Thr (103).**

Compound **43a** (0.5 mg, 0.23 μmol), CMP-Neu5Ac (0.3 mg, 2.0 equiv.), and hST6Gal-I (0.8 mU) were dissolved in 77 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 88 % yield).$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.92 (d, $J$ = 3.5 Hz, 1H), 4.73 – 4.66 (m, 4H), 4.59 (d, $J$ = 7.1 Hz, 1H), 4.48 -4.41 (m, 6H), 4.26 – 4.17 (m, 2H), 4.17 – 4.13 (m, 4H), 4.05 (dd, $J$ = 7.9, 5.0 Hz, 1H), 4.02 – 3.60 (m, 55H), 3.62 – 3.48 (m, 12H), 2.66 (dd, $J$ = 12.3, 4.6 Hz, 1H), 2.08 – 1.95 (m, 21H), 1.71 (t, $J$ = 12.2 Hz, 1H), 1.39 (d, $J$ = 6.6 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C129H227N7O64Na, calcd 2920, found 2921.1.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (45b).**

Compound **45a** (33 mg, 18.5 μmol) was treated with a mixture of 15 mU of HP β3GlcNAcT and UDP-GlcNAc (36 mg, 3.0 equiv.) in 3.7 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (35 mg, 95 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.89 (d, $J$ = 4.3 Hz, 1H), 4.72 – 4.65 (m, 4H), 4.61 – 4.53 (m, 2H), 4.48 – 4.42 (m, 4H), 4.37 (dd, $J$ = 6.7, 2.6 Hz, 1H), 4.21 (dd, $J$ = 11.2, 3.8 Hz, 1H), 4.18 (d, $J$ = 3.3 Hz, 1H), 4.15 (dd, $J$ = 3.7, 1.6 Hz, 5H), 4.09 (d, $J$ = 9.4 Hz, 1H), 4.02 – 3.64 (m, 45H), 3.63 – 3.49 (m, 10H), 3.50 – 3.38 (m, 4H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 12H), 1.99 (s, 3H), 1.34 (d, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.7(4), 175.6(9), 175.2, 175.0, 174.5, 103.7, 103.6, 103.0, 101.8, 100.0, 82.9, 82.8, 79.1, 79.1, 78.9, 76.8, 76.4, 75.7, 75.6, 75.3(3), 75.2(6), 74.3, 73.6, 73.0, 72.8, 70.8(4), 70.7(8), 70.7(2), 70.5, 70.4, 69.9, 69.1(1), 69.0(6), 61.8, 61.2, 60.8, 60.6, 59.9, 56.4, 55.9, 55.8, 55.7, 49.0, 23.1(1), 23.0(7), 23.0(2), 22.9(5), 19.1; MALDI-TOF MS: [M+$Na^+$] C84H140N8O58Na, calcd 2211, found 2212.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (46a).**

Compound **45b** (18 mg, 8.22 μmol) was treated with a mixture of 100 mU of GalT-1 and UDP-Gal (20 mg, 4.0 equiv.) in 0.822 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (19 mg, 91 % yield).$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.88 (d, $J$ = 4.3 Hz, 1H), 4.71 – 4.66 (m, 4H), 4.60 – 4.53 (m, 2H), 4.48 – 4.42 (m, 6H), 4.34 (d, $J$ = 5.8 Hz, 1H), 4.22 – 4.06 (m, 8H), 4.03 – 3.90 (m, 9H), 3.86 – 3.63 (m, 50H), 3.62-3.50 (m, 12H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 12H), 2.00 (s, 3H), 1.33 (d, $J$ = 6.6 Hz, 3H); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.7, 103.7, 103.6, 82.9, 78.9, 76.1, 75.7, 75.3, 73.3, 73.0, 71.7, 70.7, 69.3, 69.1, 61.8(2), 61.7(5), 60.6, 56.0, 23.0; MALDI-TOF MS: [M+$Na^+$] C96H160N8O68Na, calcd 2535, found 2537.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (46).**

Compound **46a** (2 mg, 0.8 μmol), CMP-Neu5Ac (2.1 mg, 4.0 equiv.), and rST3Gal-III (8 mU) were dissolved in 800 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.2 mg, 88 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.90 (d, $J$ = 3.7 Hz, 1H), 4.72 – 4.66 (m, 4H), 4.62 – 4.53 (m, 4H), 4.50 – 4.43 (m, 4H), 4.42 – 3.97 (m, 1H), 4.24 – 4.17 (m, 2H), 4.17 – 4.14 (m, 5H), 4.14 – 4.08 (m, 3H), 4.03 – 3.48 (m, 83H), 2.81 – 2.73 (m, 2H), 2.10 – 1.95 (m, 27H), 1.80 (t, $J$ = 12.1 Hz, 2H), 1.36 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] $C_{165}H_{289}N_9O_{82}Na$, calcd 3730, found 3731.3.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (106).**

Compound **46a** (0.5 mg, 0.2 μmol), CMP-Neu5Ac (0.55 mg, 4.0 equiv.), and hST6Gal-I (1.5 mU) were dissolved in 133 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.55 mg, 89 % yield).$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.89 (s, 1H), 4.73 – 4.65 (m, 4H), 4.61 – 4.53 (m, 2H), 4.49 – 4.43 (m, 6H), 4.42 – 4.35 (m, 1H), 4.25 – 4.17 (m, 2H), 4.17 – 4.12 (m, 5H), 4.12 – 4.07 (m, 1H), 4.03 – 3.45 (m, 85H), 2.66 (dd, $J$ = 12.6, 4.8 Hz, 2H), 2.08 – 1.94 (m, 27H), 1.71 (t, $J$ = 12.1 Hz, 2H), 1.34 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] $C_{165}H_{289}N_9O_{82}Na$, calcd 3730, found 3731.4.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (46b).**

Compound **46a** (45 mg, 17.9 μmol) was treated with a mixture of 100 mU of HP β3GlcNAcT and UDP-GlcNAc (47 mg, 4.0 equiv.) in 3.6 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (50 mg, 95 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.88 (d, $J$ = 4.1 Hz, 1H), 4.72 – 4.63 (m, 6H), 4.60 – 4.52 (m, 2H), 4.45 (t, $J$ = 6.2 Hz, 7H), 4.36 (dd, $J$ = 6.6, 2.4 Hz, 1H), 4.22 – 4.16 (m, 2H), 4.14 (d, $J$ = 3.7 Hz, 7H), 4.09 (d, $J$ = 9.4 Hz, 1H), 4.04 – 3.62 (m, 62H), 3.63 – 3.50 (m, 14H), 3.50 – 3.39 (m, 4H), 2.07 – 1.98 (m, 27H), 1.34 (d, $J$ = 6.7 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] $C_{112}H_{186}N_{10}O_{78}Na$, calcd 2941, found 2942.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (47a).**

Compound **46b** (40 mg, 13.7 μmol) was treated with a mixture of 150 mU of GalT-1 and UDP-Gal (34 mg, 4.0 equiv.) in 2.7 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (40 mg, 90 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.88 (d, $J$ = 4.0 Hz, 1H), 4.73 – 4.64 (m, 6H), 4.60 – 4.52 (m, 2H), 4.50 – 4.41 (m, 8H), 4.36 – 4.30 (m, 1H), 4.23 – 4.05 (m, 10H), 4.01 – 3.88 (m, 11H), 3.88 – 3.62 (m, 66H), 3.61 – 3.48 (m, 16H), 2.10 – 1.93 (m, 27H), 1.33 (d, $J$ = 6.6 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] $C_{124}H_{206}N_{10}O_{88}Na$, calcd 3265, found 3266.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (47).**

Compound **47a** (2 mg, 0.62 μmol), CMP-Neu5Ac (1.6 mg, 4.0 equiv.), and rST3Gal-III (1 mU) were dissolved in 620 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.1 mg, 89 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.89 (d, $J$ = 3.6 Hz, 1H), 4.71 – 4.65 (m, 6H), 4.60 – 4.51 (m, 4H), 4.48 – 4.37 (m, 7H), 4.23 – 4.05 (m, 12H), 4.02 – 3.47 (m, 105H), 2.74 (dd, $J$ = 12.4, 4.7 Hz,

2H), 2.07 – 1.94 (m, 33H), 1.79 (t, *J* = 12.3 Hz, 2H), 1.34 (d, *J* = 6.9 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C205H359N11O102Na, calcd 4628, found 4630.0.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (107).**

Compound **47a** (0.5 mg, 0.15 μmol), CMP-Neu5Ac (0.4 mg, 4.0 equiv.), and hST6Gal-I (1.5 mU) were dissolved in 103 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 85 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.89 (d, *J* = 3.4 Hz, 1H), 4.73 – 4.65 (m, 6H), 4.60 – 4.53 (m, 2H), 4.49 -4.38 (m, 9H), 4.23 – 4.06 (m, 10H), 4.03 – 3.47 (m, 107H), 2.66 (dd, *J* = 12.4, 4.7 Hz, 2H), 2.09 – 1.95 (m, 33H), 1.71 (t, *J* = 12.3 Hz, 2H), 1.34 (d, *J* = 6.6 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C205H359N11O102Na, calcd 4628, found 4630.0.

**GlcNAc3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (47b).**

Compound **47a** (9 mg, 2.75 μmol) was treated with a mixture of 8 mU of HP β3GlcNAcT and UDP-GlcNAc (47 mg, 4.0 equiv.) in 0.28 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (9 mg, 90 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.88 (d, *J* = 4.1 Hz, 1H), 4.71 – 4.63 (m, 8H), 4.60 – 4.53 (m, 2H), 4.49 – 4.41 (m, 8H), 4.38 – 4.29 (m, 1H), 4.24 – 4.05 (m, 12H), 4.01 – 3.62 (m, 81H), 3.61 – 3.50 (m, 18H), 3.48 – 3.40 (m, 4H), 2.08 – 1.94 (m, 33H), 1.33 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: C140H232N12O98Na [M+$Na^+$], calcd 3671, found 3672.

**Galβ4GlcNAc3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (48a).**

Compound **47b** (10 mg, 2.72 μmol) was treated with a mixture of 15 mU of GalT-1 and UDP-Gal (6.6 mg, 4.0 equiv.) in 0.55 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (10 mg, 92 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.88 (d, *J* = 4.0 Hz, 1H), 4.72 – 4.64 (m, 8H), 4.59 – 4.52 (m, 2H), 4.51 – 4.36 (m, 11H), 4.24 – 4.06 (m, 12H), 4.01 – 3.88 (m, 13H), 3.88 – 3.62 (m, 82H), 3.62 – 3.48 (m, 20H), 2.10 – 1.94 (m, 33H), 1.34 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] C152H252N12O108Na, calcd 3995, found 3998.

**Neu5Acα3Galβ4GlcNAc3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (48).**

Compound **48a** (2 mg, 0.5 μmol), CMP-Neu5Ac (1.3 mg, 4.0 equiv.), and rST3Gal-III (1 mU) were dissolved in 500 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.1 mg, 92 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.89 (d, *J* = 2.9 Hz, 1H), 4.72 – 4.65 (m, 8H), 4.60 – 4.52 (m, 4H), 4.49 – 4.36 (m, 9H), 4.26 – 4.05 (m, 14H), 4.04 – 3.43 (m, 127H), 2.74 (dd, *J* = 12.5, 4.6 Hz, 2H), 2.10 – 1.94 (m, 39H), 1.79 (t, *J* = 11.9 Hz, 2H), 1.34 (d, *J* = 6.8 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C245H429N13O122Na, calcd 5526, found 5527.9.

**Neu5Acα6Galβ4GlcNAc3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)GalNAcα-Thr (108).**

Compound **48a** (0.5 mg, 0.125 μmol), CMP-Neu5Ac (0.4 mg, 4.0 equiv.), and hST6Gal-I (1.5 mU) were dissolved in 83 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 88 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.89 (s, 1H), 4.73 – 4.65 (m, 8H), 4.60 –

4.53 (m, 2H), 4.49 – 4.34 (m, 11H), 4.26 – 4.05 (m, 12H), 4.03 – 3.42 (m, 129H), 2.66 (dd, *J* = 12.6, 4.7 Hz, 2H), 2.08 – 1.96 (m, 39H), 1.71 (t, *J* = 12.1 Hz, 2H), 1.34 (d, *J* = 6.5 Hz, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] C245H429N13O122Na, calcd 5526, found 5527.9.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6GalNAcα-Thr (49d).**

Compound **49c** (7.8 mg, 7.4 μmol) was treated with a mixture of 30 mU of HP β3GlcNAcT and UDP-GlcNAc (9.6 mg, 2.0 equiv.) in 0.74 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (8.5 mg, 91 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.92 (d, *J* = 3.8 Hz, 1H), 4.70 – 4.65 (m, 2H), 4.56 (d, *J* = 8.4 Hz, 1H), 4.48 – 4.41 (m, 2H), 4.35 (qd, *J* = 6.7, 2.2 Hz, 1H), 4.18 – 4.04 (m, 5H), 4.03 – 3.91 (m, 3H), 3.91 – 3.63 (m, 22H), 3.62 – 3.52 (m, 5H), 3.48 – 3.39 (m, 2H), 2.08 – 1.98 (m, 12H), 1.34 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] C48H81N5O33Na, calcd 1278, found 1279.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6GalNAcα-Thr (49e).**

Compound **49d** (6 mg, 4.77 μmol) was treated with a mixture of 15 mU of GalT-1 and UDP-Gal (5.8 mg, 2.0 equiv.) in 0.48 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (6 mg, 89 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.92 (d, *J* = 3.9 Hz, 1H), 4.71 – 4.66 (m, 2H), 4.58 (d, *J* = 8.3 Hz, 1H), 4.49 – 4.41 (m, 3H), 4.37 (qd, *J* = 6.6, 2.0 Hz, 1H), 4.19 – 4.05 (m, 5H), 4.03 – 3.88 (m, 5H), 3.89 – 3.62 (m, 27H), 3.62 – 3.50 (m, 6H), 2.07 – 1.99 (m, 12H), 1.35 (d, *J* = 6.8 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] C54H91N5O38Na, calcd 1440, found 1441.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6GalNAcα-Thr (49f).**

Compound **49e** (4.1 mg, 2.89 μmol) was treated with a mixture of 8.5 mU of HP β3GlcNAcT and UDP-GlcNAc (3.8 mg, 2.0 equiv.) in 0.29 mL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (4.1 mg, 87 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.92 (d, *J* = 3.8 Hz, 1H), 4.71 – 4.64 (m, 3H), 4.56 (d, *J* = 8.3 Hz, 1H), 4.48 – 4.42 (m, 3H), 4.38 (qd, *J* = 6.6, 2.2 Hz, 1H), 4.18 – 4.04 (m, 6H), 4.02 – 3.64 (m, 34H), 3.62 – 3.51 (m, 7H), 3.50 – 3.38 (m, 2H), 2.08 – 1.97 (m, 15H), 1.35 (d, *J* = 6.8 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] C62H104N6O43Na, calcd 1643, found 1644.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6GalNAcα-Thr (49a).**

Compound **49f** (3.4 mg, 2.1 μmol) was treated with a mixture of 9 mU of GalT-1 and UDP-Gal (2.6 mg, 2.0 equiv.) in 0.42 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (3.3 mg, 89 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.92 (d, *J* = 3.9 Hz, 1H), 4.71 – 4.64 (m, 3H), 4.56 (d, *J* = 8.3 Hz, 1H), 4.50 – 4.41 (m, 4H), 4.36 (dd, *J* = 6.7, 2.6 Hz, 1H), 4.18 – 4.05 (m, 6H), 4.02 – 3.89 (m, 6H), 3.89 – 3.62 (m, 35H), 3.62 – 3.48 (m, 8H), 2.07 – 1.99 (m, 15H), 1.35 (d, *J* = 6.8 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] C68H114N6O48Na, calcd 1805, found 1806.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6GalNAcα-Thr (49).**

Compound **49a** (0.5 mg, 0.28 μmol), CMP-Neu5Ac (0.4 mg, 2.0 equiv.), and rST3Gal-III (2 mU) were dissolved in 93 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 86 % yield).

$^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (d, *J* = 3.7 Hz, 1H), 4.71 – 4.65 (m, 3H), 4.58 – 4.52 (m, 2H), 4.48 – 4.42 (m, 3H), 4.41 – 4.37 (m, 1H), 4.19 – 4.05 (m, 7H), 4.03 – 3.48 (m, 55H), 2.75 (dd, *J* = 12.1, 4.8 Hz, 1H), 2.10

– 1.96 (m, 18H), 1.79 (t, *J* = 12.3 Hz, 1H), 1.35 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M + $Na^+$] $C_{115}H_{203}N_7O_{56}Na$, calcd 2600, found 2601.1.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6GalNAcα-Thr (109).**

Compound **49a** (0.5 mg, 0.28 μmol), CMP-Neu5Ac (0.4 mg, 2.0 equiv.), and hST6Gal-I (0.8 mU) were dissolved in 93 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 86 % yield). MALDI-TOF MS: permethylated [M + $Na^+$] $C_{115}H_{203}N_7O_{56}Na$, calcd 2600, found 2601.1.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6GalNAcα-Thr (49b).**

Compound **49a** (2 mg, 1.12 μmol) was treated with a mixture of 5 mU of HP β3GlcNAcT and UDP-GlcNAc (1.5 mg, 2.0 equiv.) in 112 μL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (2.0 mg, 89 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (d, *J* = 3.8 Hz, 1H), 4.71 – 4.65 (m, 4H), 4.56 (d, *J* = 8.3 Hz, 1H), 4.47 – 4.42 (m, 4H), 4.37 (dd, *J* = 6.7, 2.3 Hz, 1H), 4.18 – 4.07 (m, 7H), 4.02 – 3.65 (m, 42H), 3.3 – 3.52 (m, 10H), 3.49 – 3.39 (m, 2H), 2.10 – 1.97 (m, 18H), 1.35 (dd, *J* = 6.8, 2.3 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] $C_{76}H_{127}N_7O_{53}Na$, calcd 2008, found 2008.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6GalNAcα-Thr (50a).**

Compound **49b** (1.5 mg, 0.75 μmol) was treated with a mixture of 3 mU of GalT-1 and UDP-Gal (1 mg, 2.0 equiv.) in 0.15 mL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 °C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (1.5 mg, 93 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (d, *J* = 3.8 Hz, 1H), 4.68 (d, *J* = 8.7 Hz, 4H), 4.56 (d, *J* = 8.4 Hz, 1H), 4.47 – 4.42 (m, 5H), 4.38 (dd, *J* = 6.6, 2.2 Hz, 1H), 4.19 – 4.05 (m, 7H), 4.01 – 3.89 (m, 7H), 3.88 – 3.63 (m, 43H), 3.62 – 3.50 (m, 10H), 2.07 – 1.97 (m, 18H), 1.35 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: [M+$Na^+$] $C_{82}H_{137}N_7O_{58}Na$, calcd 2170, found 2171.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6GalNAcα-Thr (50).**

Compound **50a** (0.5 mg, 0.23 μmol), CMP-Neu5Ac (0.4 mg, 2.0 equiv.), and rST3Gal-III (2 mU) were dissolved in 46 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 88 % yield).$^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (d, *J* = 3.8 Hz, 1H), 4.71 – 4.65 (m, 4H), 4.58 – 4.52 (m, 2H), 4.47 – 4.42 (m, 5H), 4.40 – 4.35 (m, 1H), 4.19 – 4.05 (m, 8H), 4.03 – 3.51 (m, 65H), 2.74 (dd, *J* = 12.1, 4.3 Hz, 1H), 2.07 – 1.97 (m, 21H), 1.79 (t, *J* = 12.2 Hz, 1H), 1.39 – 1.31 (m, 3H); MALDI-TOF MS: permethylated [M-Thr + $Na^+$] $C_{129}H_{227}N_7O_{64}Na$, calcd 2920, found 2921.1.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6GalNAcα-Thr (110).**

Compound **50a** (0.5 mg, 0.23 μmol), CMP-Neu5Ac (0.4 mg, 2.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 46 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 88 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 4.93 (d, *J* = 3.4 Hz, 1H), 4.74 – 4.66 (m, 4H), 4.56 (d, *J* = 8.4 Hz, 1H), 4.48 – 4.41 (m, 5H), 4.40 – 4.35 (m, 1H), 4.18 – 4.04 (m, 6H), 4.03 – 3.45 (m, 68H), 2.66 (dd, *J* = 12.3, 4.4 Hz, 1H), 2.03 (d, *J* = 14.1 Hz, 21H), 1.71 (t, *J* = 12.1 Hz, 1H), 1.35 (d, *J* = 6.7 Hz, 3H); MALDI-TOF MS: permethylated [M + $Na^+$] $C_{129}H_{227}N_7O_{64}Na$, calcd 2920, found 2921.2.

**Neu5Acα3Galβ3GlcNAcβ3Galβ4GlcNAcβ6(Neu5Acα3Galβ3GlcNAcβ3)Galβ4GlcNAcβ$CH_2CH_2NH_2$ (51)**

Compound **51a** (0.6 mg, 0.39 μmol), CMP-Neu5Ac (1 mg, 4.0 equiv.), and rST3Gal-III (2 mU) were dissolved in 197 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.7 mg, 84 % yield).

**Neu5Acα3Galβ6GlcNAcβ3Galβ6GlcNAcβ3Galβ4GlcNAcβ6(Neu5Acα3Galβ6GlcNAcβ3Galβ6GlcNAcβ3)Galβ4GlcNAcβCH$_2$CH$_2$NH$_2$ (52)**

Compound **52a** (0.5 mg, 0.22 μmol), CMP-Neu5Ac (0.6 mg, 4.0 equiv.), and rST3Gal-III (2 mU) were dissolved in 110 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.55 mg, 87 % yield). MALDI-TOF MS, [M+H$^+$] C108H180N9O77Na, calcd 2834, found 2836.

**Neu5Acα6Galβ6GlcNAcβ3Galβ6GlcNAcβ3Galβ4GlcNAcβ6(Neu5Acα6Galβ6GlcNAcβ3Galβ6GlcNAcβ3)Galβ4GlcNAcβCH$_2$CH$_2$NH$_2$ (111)**

Compound **52a** (0.5 mg, 0.22 μmol), CMP-Neu5Ac (0.6 mg, 4.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 110 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.55 mg, 87 % yield). MALDI-TOF MS: [M+H$^+$] C108H180N9O77Na, calcd 2834, found 2836.

**Neu5Acα6Galβ6GlcNAcβ3Galβ4GlcNAcβ6(Neu5Acα6Galβ6GlcNAcβ3)Galβ4GlcNAcβCH$_2$CH$_2$NH$_2$ (112)**

Compound **112a** (0.4 mg, 0.263 μmol), CMP-Neu5Ac (0.7 mg, 4.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 132 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 91 % yield).

**Galβ4GlcNAcβ2Manα3(Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (53a)**

Egg yolk extraction **SGP** (**116**, 25 mg, 8.7 μmol) was incubated with 0.044 mU neuraminidase (from *Arthrobacter ureafaciens*) in a sodium acetate buffer (220 μL, 50 mM, pH 6.0) at 37 °C overnight. The product was purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (20.0 mg, 100 % yield); MALDI-TOF MS *m/z* calcd for C90H156N13O54 [M+H$^+$] 2283, found 2283.

**GlcNAcβ2Manα3(GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (53c)**

**53a** (12.0 mg, 5.26 μmol) was incubated with pre-dialyzed (to removal of dextrin) β-galactosidase (0.04 U) from *Aspergillus oryzae* and general protease inhibitor cocktail (188 μL) in a total volume of 262 μL of 50 mM GlcNAc, 30 mM NaOAc, pH 5.0 at 37 °C for 4 h. The reaction was monitored by TLC analysis until complete removal of terminal galactose. Longer incubation time may result in the subsequent loss of the six amino acids. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (10.3 mg, 100 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.09 (s, 1H, H-1$_{Man}$), 5.02 (d, $J$ = 9.6 Hz, 1H, H-1$_{Gn}$), 4.90 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.66 (m, 1H, H-1$_{Asn}$), 4.59 (d, $J$ = 7.7 Hz, 1H, H-1$_{Gn}$), 4.53 (d, $J$ = 8.4 Hz, 2H, 2 H-1$_{Gn}$), 4.38 (m, 1H, H-1$_{Lys}$), 4.28 (q, $J$ = 7.4 Hz, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.20 (m, 1H, H-2$_{Thr}$), 4.17 (d, $J$ = 1.9 Hz, 1H, H-2$_{Man}$), 4.12 (m, 1H, H-1$_{Thr}$), 4.08 (m, 2H, H-2$_{Man}$, H-1$_{Val}$), 4.00 – 3.35 (m, 40H), 3.04 – 2.91 (m, 4H, H-5$_{Lys}$), 2.84 (dd, $J$ = 16.2, 5.4 Hz, 1H, H-2$_{Asn}$), 2.72 (dd, $J$ = 16.1, 7.6 Hz, 1H, H-2$_{Asn}$), 2.05 (s, 3H), 2.04 (m, 1H, H-2$_{Val}$), 2.03 (s, 3H), 2.02(9) (s, 3H), 1.98 (s, 3H), 1.88 (m, 1H), 1.75 (m, 1H), 1.71 – 1.52 (m, 6H), 1.50 – 1.33 (m, 4H), 1.35 (d, $J$ = 7.1 Hz, 3H$_{Ala}$), 1.15 (d, $J$ = 6.4 Hz, 3H$_{Thr}$), 0.94 (d, $J$ = 6.8 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 178.4, 177.2, 176.7, 175.6, 175.5, 175.4, 175.2, 174.1, 173.8, 173.1, 172.7, 102.1, 101.2, 100.4, 97.8, 81.2, 80.3, 79.4, 79.0, 77.2, 77.1, 77.0, 76.6, 76.5(7), 75.2, 75.1, 74.3, 74.2, 74.0, 73.6, 72.8, 71.0, 70.7, 70.2, 70.1(7), 68.7, 68.1, 68.0(7), 66.7, 66.5, 62.5, 62.4, 61.4, 60.7, 60.6, 60.0, 56.1, 55.7, 54.9, 54.4, 54.3, 50.8, 50.3, 40.0, 39.9(8), 37.2, 34.5, 31.3, 30.9, 27.5, 27.1, 23.1, 23.0, 22.9, 22.8, 22.7, 22.6(6), 20.1, 19.3, 18.9, 17.5; MALDI-TOF MS *m/z* calcd for C78H136N13O44 [M+H$^+$] 1958, found 1959.

**GlcNAcβ2Manα3(GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (60c)**

**53c** (7.0 mg, 3.58 μmol), FUT8 (0.02 mU), GDP-Fuc (40% purity, 11.3 mg, 2.0 equiv.) and CIAP (0.04 mU) were dissolved in 358 μL Tris-HCl buffer (50 mM, pH 7.5). The pH was carefully monitored and adjusted (to pH 7.5) as needed. After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (7.1 mg, 94 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H, H-1$_{Man}$), 5.04 (d, $J$ = 9.7 Hz, 1H, H-1$_{Gn}$), 4.90 (s, 1H, H-1$_{Man}$), 4.86 (d, $J$ = 3.7 Hz, 1H, H-1$_{Fuc}$), 4.76 (s, 1H, H-1$_{Man}$), 4.67 (m, 2H, H-1$_{Asn}$, H-1$_{Gn}$), 4.54 (d, $J$ = 8.4 Hz, 1H, 2 H-1$_{Gn}$), 4.39 (t, $J$ = 6.9 Hz, 1H, H-1$_{Lys}$), 4.28 (q, $J$ = 7.4 Hz, 1H, H-1$_{Ala}$), 4.24 (s, 1H, H-2$_{Man}$), 4.21 (m, 1H, H-2$_{Thr}$), 4.18 (s, 1H, H-2$_{Man}$), 4.14-4.08 (m, 4H), 4.02 – 3.34 (m, 42H), 2.98 (m, 4H, H-5$_{Lys}$), 2.84 (dd, $J$ = 4.5, 16.4 Hz, 1H, H-2$_{Asn}$), 2.74 (dd, $J$ = 8.2, 16.4 Hz, 1H, H-2$_{Asn}$), 2.08 (s, 3H), 2.06 (m, 1H, H-2$_{Val}$), 2.04 (s, 6H), 1.99 (s, 3H), 1.88 (m, 1H), 1.76 (m, 1H), 1.71 – 1.56 (m, 6H), 1.48-1.32 (m, 3H), 1.36 (d, $J$ = 7.1 Hz, 3H$_{Ala}$), 1.25 (m, 1 H), 1.19 (d, $J$ = 6.6 Hz, 3H$_{Fuc}$), 1.15 (d, $J$ = 6.4 Hz, 3H$_{Thr}$), 0.95 (d, $J$ = 6.8 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 178.3, 177.2, 175.6, 175.55, 175.5, 175.2, 174.1, 173.8, 173.1, 172.8, 101.8, 101.3, 100.4, 100.1, 97.8, 81.2, 80.5, 79.0, 78.9, 77.2, 77.1, 76.6, 76.0, 75.2, 75.1, 74.3, 74.2, 74.1, 73.6, 72.8, 72.6, 71.0, 70.7, 70.3, 70.2, 70.2, 69.0, 68.7, 68.1, 68.1, 67.6, 67.3, 66.5, 62.5, 62.4, 61.4, 60.7, 60.0, 56.1, 55.7, 54.9, 54.4, 50.8, 50.3, 40.0, 39.9(7), 34.5, 31.4, 30.9, 27.5, 27.1, 23.1, 23.0, 22.7, 22.6, 20.1, 19.3, 18.6, 17.5, 16.2; MALDI-TOF MS *m/z* calcd for C84H146N13O48 [M+H$^+$] 2104, found 2105.

**GlcNAcβ2(GlcNAcβ6)Manα6(GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (62c)**

**53c** (4.3 mg, 2.2 μmol), UDP-GlcNAc (7.1 mg, 5.0 equiv.), GnT V (0.015 mU) were dissolved in a total volume of 220 μL of 0.1 mg/mL BSA, 100 mU/mL of calf intestine alkaline phosphatase, HEPES-NaOH buffer (50 mM, pH 7.5). The pH was carefully monitored and adjusted (to pH 7.5) as needed. After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (4.1 mg, 86 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 9.6 Hz, 1H, H-1$_{Gn}$), 4.85 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.69 – 4.64 (m, 1H), 4.59 (d, $J$ = 7.8 Hz, 1H, H-1$_{Gn}$), 4.55 (d, $J$ = 8.3 Hz, 1H, H-1$_{Gn}$), 4.54 (d, $J$ = 8.4 Hz, 1H, H-1$_{Gn}$), 4.51 (d, $J$ = 8.4 Hz, 1H, H-1$_{Gn}$), 4.43 – 4.37 (m, 1H, H-1$_{Lys}$), 4.29 (d, $J$ = 7.2 Hz, 1H, H-1$_{Ala}$), 4.23 (d, $J$ = 2.6 Hz, 1H, H-2$_{Man}$), 4.22 – 4.16 (m, 2H), 4.13 (m, 1H), 4.09 (d, $J$ = 7.8 Hz, 1H, H-1$_{Val}$), 4.07 (d, $J$ = 2.4 Hz, 1H, H-2$_{Man}$), 4.01 – 3.22 (m, 46H), 3.04-2.92 (m, 4H), 2.85 (dd, $J$ = 5.5, 16.3Hz, 1H, H-2$_{Asn}$), 2.73 (dd, $J$ = 7.6, 16.2Hz, 1H, H-2$_{Asn}$), 2.06 (s, 3H), 2.05 (m, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.88 (m, 1H), 1.77 (m, 1H), 1.72 – 1.55 (m, 6H), 1.51-1.35 (m, 4H), 1.36 (d, $J$ = 7.1 Hz, 3H$_{Ala}$), 1.15 (d, $J$ = 6.4 Hz, 3H$_{Thr}$), 0.94 (d, $J$ = 6.7 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 178.4, 177.3, 175.6, 175.5, 175.4, 175.4, 175.3, 175.0, 173.9, 173.8, 173.1, 172.7, 102.5, 102.1, 101.2, 100.5, 100.5, 100.4, 98.0, 81.2, 80.2, 79.4, 79.0, 77.3, 77.3, 77.0, 76.6, 75.3, 75.2, 74.5, 74.4, 74.1, 73.7, 72.7, 72.4, 71.0, 70.7, 70.7, 70.2, 70.2, 68.7, 68.3, 68.1, 66.4, 62.5, 61.6, 61.4, 60.7, 60.0, 56.4, 56.2, 56.1, 55.7, 54.8, 54.4, 54.4, 54.3, 50.8, 50.3, 40.0, 39.9(8), 34.5, 31.3, 30.9, 27.5, 27.1, 23.3, 23.1, 23.0, 22.9, 22.8, 22.7, 22.6, 20.1, 19.3, 18.6, 17.5. MALDI-TOF MS *m/z* calcd for C86H149N14O49 [M+H$^+$] 2161, found 2162.

**GlcNAcβ2(GlcNAcβ6)Manα6(GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (65c)**

**62c** (1.0 mg, 0.46 μmol), 40% GDP-Fuc (1.5 mg, 2.0 equiv.), CIAP (0.008 mU) and FUT8 (0.004 mU) were dissolved in 46 μL Tris-HCl buffer (50 mM, pH 7.5). The pH was carefully monitored and adjusted (to pH 7.5) as needed. After incubation overnight at 37 °C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (1.0 mg, 94 % yield). $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 9.7 Hz, 1H, H-1$_{Gn}$), 4.85 (d, $J$ = 3.6 Hz, 1H, H-1$_{Fuc}$), 4.84 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.70-4.63 (m, 2H, H-1$_{Asn}$, H-1$_{Gn}$), 4.54 (d, $J$ = 8.4 Hz, 1H, H-1$_{Gn}$), 4.53 (d, $J$ = 8.4 Hz, 1H, H-1$_{Gn}$), 4.51(d, $J$ = 8.4 Hz, 1H, H-1$_{Gn}$), 4.38 (m, 1H, H-1$_{Lys}$), 4.28 (m, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.22-4.16 (m, 2H), 4.17 (s, 1H, H-2$_{Man}$), 4.15 – 4.09 (m, 2H), 4.07 (s, 1H, H-2$_{Man}$), 4.00 – 3.30 (m, 58H), 3.03 – 2.91 (m, 4H, H-5$_{Lys}$), 2.83 (dd, $J$ = 4.7, 16.4 Hz, 1H, H-2$_{Asn}$), 2.73 (dd, $J$ = 8.1, 16.1 Hz, 1H, H-2$_{Asn}$), 2.11 (m, 1H, H-2$_{Val}$), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.93 – 1.82 (m, 1H), 1.75 (m, 1H), 1.71-1.52 (m, 6H), 1.50-1.35 (m, 4H), 1.35 (d, $J$ = 7.1 Hz, 3H$_{Ala}$), 1.18 (d, $J$ = 6.5 Hz, 3H$_{Fuc}$), 1.15 (d, $J$ = 6.4 Hz, 3H$_{Thr}$), 0.94 (d, $J$ = 6.8 Hz, 6H$_{Val}$). $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 178.4, 177.3, 177.2, 175.6, 175.4, 175.4, 175.2, 175.0, 173.8, 173.1, 172.8, 102.5, 101.8, 101.2, 100.5, 100.4, 100.4, 100.1, 98.0, 81.2, 80.4, 79.0, 78.9, 77.3, 77.2, 76.6, 76.0, 75.2, 75.2, 74.5, 74.3, 74.1, 73.6, 72.8, 72.6, 72.4, 71.0, 70.7, 70.7, 70.3, 70.2, 70.1(6), 68.9, 68.7, 68.7, 68.3, 68.1, 67.6, 66.4, 63.3, 62.5, 61.6, 61.4, 61.3, 60.7, 59.9, 56.4, 56.2, 56.1, 55.7, 54.9, 54.4, 54.4, 54.3, 50.8, 50.3, 40.0, 39.9(8),

34.5, 31.4, 30.9, 27.5, 27.1, 23.3, 23.1, 23.0, 22.7, 22.6(5), 20.1, 20.0, 19.3, 18.6, 17.5, 16.20. MALDI-TOF MS *m/z* calcd for C92H159N14O53 [M+H$^+$] 2307, found 2308.

**GlcNAcβ3Galβ4GlcNAcβ2Manα3(GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (53b)**

Compound **53a** (6 mg, 2.63 μmol) was treated with a mixture of 0.05 mU of HP β3GlcNAcT and UDP-GlcNAc (3.4 mg, 4.0 equiv.) in 263 μL buffer (50 mM HEPES-NaOH buffer, pH 7.2, with 25 mM KCl, 1 mM DTT, 2 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37 $^oC$. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (6.5 mg, 92 % yield). $^1H$ NMR (600 MHz, $D_2O$): $\delta$ = 4.98 (s, 1H, H-1$_{Man}$), 4.91 (d, $J$ = 9.6 Hz, 1H, H-1$_{Gn}$), 4.80 (s, 1H, H-1$_{Man}$), 4.63 (s, 1H, H-1$_{Man}$), 4.60-4.51 (m, 3H), 4.48 (d, $J$ = 7.6 Hz, 1H, H-1$_{Gn}$), 4.44 (d, $J$ = 7.9 Hz, 2H, 2 H-1$_{Gn}$), 4.32 (d, $J$ = 7.8 Hz, 1H, H-1$_{Gal}$), 4.31 (d, $J$ = 7.8 Hz, 1H, H-1$_{Gal}$), 4.26 (m, 1H, H-1$_{Lys}$), 4.16 (q, $J$ = 7.2 Hz, 1H, H-1$_{Ala}$), 4.12 (s, 1H, H-2$_{Man}$), 4.08 (m, 1H, H-2$_{Thr}$), 4.06 (s, 1H, H-2$_{Man}$), 4.04-4.01 (s, 2H), 4.00 (d, $J$ = 4.2 Hz, 1H, H-1$_{Thr}$), 3.99-3.95 (m, 2H), 3.88 – 3.25 (m, 62H), 2.91 – 2.78 (m, 4H), 2.73 (dd, $J$ = 5.0, 16.0 Hz, 1H, H-2$_{Asn}$), 2.61 (dd, $J$ = 7.6, 16.0 Hz, 1H, H-2$_{Asn}$), 1.99 (m, 1H), 1.94 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.90(6) (s, 3H), 1.90(2) (s, 3H), 1.87 (s, 3H), 1.76 (m, 1H), 1.63 (m, 1H), 1.59 – 1.41 (m, 6H), 1.40-1.19 (m, 4H), 1.24 (d, $J$ = 7.1 Hz, 3H$_{Ala}$), 1.04 (d, $J$ = 6.4 Hz, 3H$_{Thr}$), 0.83 (d, $J$ = 6.8 Hz, 6H$_{Val}$); $^{13}C$ NMR (150 MHz, $D_2O$): $\delta$ = 178.4, 177.2, 175.6, 175.4, 175.3, 175.2, 173.8, 173.0, 172.7, 164.3, 103.6, 102.0, 101.1, 100.4, 100.2, 100.1, 97.7, 82.7, 81.1, 80.1, 79.0, 78.8, 77.0, 76.8, 76.3, 75.5, 75.4, 75.0, 74.2, 73.5, 72.7, 72.6, 70.8, 70.6, 70.3, 70.1, 69.0, 68.5, 68.0, 66.3, 62.3, 61.6, 61.3, 61.1, 60.6, 59.9, 56.3, 55.6, 55.4, 54.7, 54.3, 50.6, 50.2, 40.0, 39.9, 34.5, 31.7, 31.2, 30.7, 27.8, 27.4, 27.3, 27.2, 23.0, 22.9, 22.8, 22.7(9), 22.6, 22.5(9), 20.0, 19.1, 18.5, 18.2, 17.3. MALDI-TOF MS *m/z* calcd for C106H182N15O64 [M+H$^+$] 2688, found 2689.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (56a)**

Compound **53b** (3.4 mg, 1.26 μmol) was treated with a mixture of 0.03 mU of GalT1 and UDP-Gal (3.1 mg, 4.0 equiv.) in 125 μL buffer (50 mM Tris-HCl buffer, pH 7.5, with 0.1 mg/mL BSA, 20 mM $MnCl_2$, 100 mU/mL of calf intestine alkaline phosphatase). The reaction mixture was incubated overnight at 37°C. The insoluble precipitates were removed by centrifugation and the supernatant was concentrated, purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (3.4 mg, 92 % yield). $^1H$ NMR (500 MHz, $D_2O$): $\delta$ = 5.13 (s, 1H, H-1$_{Man}$), 5.06 (d, $J$ = 9.8 Hz, 1H, H-1$_{Gn}$), 4.94 (s, 1H, H-1$_{Man}$), 4.87 (s, 1H, H-1$_{Man}$), 4.76 – 4.68 (m, 3H), 4.63 (d, $J$ = 7.7 Hz, 1H, H-1$_{Gn}$), 4.60 (d, $J$ = 7.8 Hz, 2H, H-1$_{Gn}$), 4.52-4.44 (m, 4H, H-1$_{Gal}$), 4.41 (dd, $J$ = 7.8, 9.6 Hz, 1H, H-1$_{Lys}$), 4.33 (q, $J$ = 7.1 Hz, 1H, H-1$_{Ala}$), 4.27 (s, 1H, H-2$_{Man}$), 4.23 (m, 1H, H-1$_{Thr}$), 4.21 (s, 1H, H-1$_{Man}$), 4.20 – 4.16 (m, 2H), 4.15 (d, $J$ = 4.0 Hz, 1H, H-1$_{Thr}$), 4.14-4.11 (m, 2H), 4.06 – 3.40 (m, 74H), 3.02 – 2.90 (m, 4H, H-5$_{Lys}$), 2.91 – 2.84 (dd, $J$ = 6.6, 16.3 Hz, 1H, H-2$_{Asn}$), 2.76 (dd, $J$ = 7.7, 16.3 Hz, 1H, H-2$_{Asn}$), 2.10 (s, 3H), 2.09 (m, 1H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05(5) (s, 3H), 2.05(3) (s, 3H), 2.02 (s, 3H), 1.91 (m, 1H), 1.78 (m, 1H), 1.72-1.56 (m, 6H), 1.54 – 1.32 (m, 4H), 1.39 (d, $J$ = 7.1 Hz, 3H$_{Ala}$), 1.19 (d, $J$ = 6.3 Hz, 3H$_{Thr}$), 0.98 (d, $J$ = 6.7 Hz, 6H$_{Val}$); $^{13}C$ NMR (125 MHz, $D_2O$): $\delta$ = 176.8, 175.3, 175.1, 174.8, 173.5, 172.3, 103.4, 103.3, 103.2(5), 103.1, 101.7, 100.8, 100.0, 99.9, 97.4, 82.5, 80.8, 79.8, 79.8, 78.9, 78.6, 76.8, 76.7, 76.6, 75.7, 75.3, 75.1, 74.9, 73.9, 73.3, 72.9, 72.6, 72.5, 72.4, 72.3, 71.3, 70.6, 70.3, 69.8, 69.8, 68.9, 68.7, 68.3, 67.7, 66.0, 62.0, 61.4, 61.3, 61.0, 60.4, 60.3, 59.6, 55.6, 55.3, 55.2, 54.5, 54.0, 49.9, 39.8, 34.2, 30.9, 30.5, 22.7, 22.6, 22.6, 22.5, 22.3, 18.9, 18.2, 17.1; MALDI-TOF MS: [M+H$^+$] C118H202N15O74, calcd 3012, found 3013.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (56)**

Compound **56a** (0.5 mg, 0.166 μmol), CMP-Neu5Ac (0.44 mg, 4.0 equiv.), and rST3Gal-III (1.5 mU) were dissolved in 33 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.57 mg, 95 % yield).$^1H$ NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H), 5.03 (d, $J$ = 9.6 Hz, 1H), 4.91 (s, 1H), 4.75 (s, 1H), 4.70 – 4.63 (m, 3H), 4.62 – 4.50 (m, 5H), 4.47 – 4.42 (m, 2H), 4.39 (dd, $J$ = 3.6, 6.6 Hz, 1H), 4.29 (q, $J$ = 7.1 Hz, 1H), 4.26 – 4.04 (m, 10H), 3.99 – 3.44 (m, 86H), 2.99 (t, $J$ = 7.6 Hz, 4H), 2.85 (dd, $J$

= 16.3, 5.0 Hz, 1H), 2.79 – 2.72 (m, 3H), 2.10 – 1.95 (m, 25H), 1.92 – 1.84 (m, 1H), 1.84 – 1.73 (m, 5H), 1.72 – 1.63 (m, 4H), 1.45 – 1.27 (m, 7H), 1.16 (d, *J* = 6.5 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 6H); MALDI-TOF MS: [M+H$^+$] C140H236N17O90, calcd 3594, found 3595.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6) Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (118).**

Compound **56a** (0.5 mg, 0.166 μmol), CMP-Neu5Ac (0.44 mg, 4.0 equiv.), and hST6Gal-I (3.3 mU) were dissolved in 33 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 83 % yield). $^1$H NMR (600 MHz, $D_2O$): *δ* = 5.10 (s, 1H), 5.03 (d, *J* = 9.6 Hz, 1H), 4.91 (s, 1H), 4.75 (s, 1H), 4.71 (d, *J* = 8.0 Hz, 2H), 4.68 – 4.63 (m, 1H), 4.60 (d, *J* = 8.0 Hz, 1H), 4.56 (d, *J* = 7.5 Hz, 2H), 4.48 – 4.42 (m, 4H), 4.39 (t, *J* = 7.2 Hz, 1H), 4.29 (q, *J* = 7.1 Hz, 1H), 4.26 – 4.07 (m, 9H), 4.07 – 3.42 (m, 87H), 3.02 – 2.98 (m, 3H), 2.85 (dd, *J* = 16.1, 4.9 Hz, 1H), 2.75 (dd, *J* = 16.8, 7.8 Hz, 1H), 2.66 (dd, *J* = 12.3, 4.4 Hz, 2H), 2.11 – 1.96 (m, 25H), 1.93 – 1.83 (m, 2H), 1.79 – 1.62 (m, 5H), 1.50 – 1.39 (m, 3H), 1.37 (d, *J* = 7.3 Hz, 3H), 1.30 – 1.20 (m, 1H), 1.16 (d, *J* = 6.5 Hz, 3H), 0.96 (d, *J* = 6.2 Hz, 6H); MALDI-TOF MS: permethylated [M-ThrLysVal + Na$^+$] C181H319N13O86Na, calcd 4072, found 4072.9.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)M anβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (56b)**

This compound was made by the same method used in the preparation of **53b**. Started from 6.0 mg **56a**, 93 % yield. $^1$H NMR (600 MHz, $D_2O$): *δ* = 5.09 (s, 1H, H-1$_{Man}$), 5.02 (d, *J* = 9.7 Hz, 1H, H-1$_{Gn}$), 4.91 (s, 1H, H-1$_{Man}$), 4.74 (s, 1H, H-1$_{Man}$), 4.70-4.63 (m, 5H, H-1$_{Asn}$, 4 H-1$_{Gn}$), 4.59 (d, *J* = 8.0 Hz, 1H, H-1$_{Gn}$), 4.56 (d, *J* = 7.7 Hz, 2H, H-1$_{Gn}$), 4.47-4.40 (m, 4H, H-1$_{Gal}$), 4.38 (dd, *J* = 6.2, 8.1 Hz, 1H, H-1$_{Lys}$), 4.28 (q, *J* = 7.1 Hz, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.20 (dd, *J* = 4.2, 6.4Hz, 1H, H-2$_{Thr}$), 4.17 (d, *J* = 2.8 Hz, 1H, H-2$_{Man}$), 4.15 – 4.12 (m, 4H), 4.11 (d, *J* = 4.2 Hz, 1H, H-1$_{Thr}$), 4.10-4.07 (m, 2H), 3.99 – 3.51 (m, 77H), 3.50-3.40 (m, 7H), 2.97 – 2.86 (m, 4H), 2.85 (dd, *J* = 5.4, 16.2Hz, 1H, H-2$_{Asn}$), 2.73 (dd, *J* = 7.7, 16.2 Hz, 1H, H-2$_{Asn}$), 2.06 (s, 3H), 2.05 (m, 1H), 2.03 (s, 3H), 2.02 (s, 3H), 2.02 (s, 9H), 2.01 (s, 3H), 1.98 (s, 3H), 1.87 (m, 1H), 1.74 (m, 1H), 1.69 – 1.52 (m, 6H), 1.50 – 1.20 (m, 4H), 1.36 (d, *J* = 7.1 Hz, 3H$_{Ala}$), 1.15 (d, *J* = 6.4 Hz, 3H$_{Thr}$), 0.94 (d, *J* = 6.7 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): *δ* = 178.6, 177.2, 175.8, 175.7, 175.5, 175.4, 175.3, 173.9, 173.8(8), 173.1, 172.8, 103.8, 103.7, 103.6(6), 103.5(6), 102.1, 101.2, 100.3, 100.2, 100.2(2), 97.8, 82.8, 82.7(7), 81.2, 80.2, 79.4, 79.3, 79.0, 78.9, 77.2, 77.1, 77.0, 76.4, 75.7, 75.5, 75.4, 75.3, 75.2, 74.3, 73.7, 73.0, 72.9, 72.8, 72.7, 71.0, 70.8, 70.7, 70.5, 70.2, 70.1, 69.1, 68.7, 68.1, 68.0, 66.4, 62.5, 62.4, 61.8, 61.4, 61.2, 60.7, 60.6, 60.0, 56.4, 55.9, 55.7, 55.6, 54.9, 54.5, 50.8, 50.3, 40.2, 40.2, 37.2, 34.7, 31.3, 30.9, 28.5, 27.9, 23.1, 23.0, 22.9(7), 22.9(4), 22.9(1), 22.8, 22.7, 20.0, 19.3, 18.6, 17.5. MALDI-TOF MS: [M+H$^+$] C134H228N17O84, calcd 3418, found 3419.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAc β2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (57a)**

This compound was made by the same method used in the preparation of **56a**. Started from 5.8 mg **56b**, 87% yield. $^1$H NMR (600 MHz, $D_2O$): *δ* = 5.10 (s, 1H, H-1$_{Man}$), 5.03 (d, *J* = 9.4 Hz, 1H, H-1$_{Gn}$), 4.91 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.72-4.63 (m, 5H, H-1$_{Asn}$, 4 H-1$_{Gn}$), 4.60 (d, *J* = 8.4 Hz, 1H, H-1$_{Gn}$), 4.56 (d, *J* = 7.5 Hz, 2H, H-1$_{Gn}$), 4.48-4.41 (m, 6H, H-1$_{Gal}$), 4.38 (dd, *J* = 6.0, 7.8 Hz, 1H, H-1$_{Lys}$), 4.29 (q, *J* = 6.9 Hz, 1H, H-1$_{Ala}$), 4.24 (s, 1H, H-2$_{Man}$), 4.20 (m, 1H, H-2$_{Thr}$), 4.18 (s, 1H, H-2$_{Man}$), 4.16 – 4.13 (m, 6H), 4.12 (d, *J* = 4.2 Hz, 1H, H-1$_{Thr}$), 4.11-4.06 (m, 2H, H-1$_{Val}$, H-2$_{Man}$), 4.00 – 3.40 (m, 94H), 3.03-2.90 (m, 4H, H-5$_{Lys}$), 2.85 (dd, *J* = 6.6, 18.6 Hz, 1H, H-2$_{Asn}$), 2.73 (dd, *J* = 8.4, 18.6 Hz, 1H, H-2$_{Asn}$), 2.10 (m, 1H, H-2$_{Val}$), 2.06 (s, 3H), 2.03 (s, 3H), 2.02(8) (s, 3H), 2.02 (s, 12H), 1.99 (s, 3H), 1.91 – 1.84 (m, 1H), 1.79 – 1.71 (m, 1H), 1.70 – 1.54 (m, 6H), 1.50-1.33 (m, 4H), 1.36 (d, *J* = 7.2 Hz, 3H$_{Ala}$), 1.15 (d, *J* = 6.4 Hz, 3H$_{Thr}$), 0.95 (d, *J* = 6.7 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): *δ* = 178.5, 177.3, 175.7, 175.5, 175.4, 175.3, 173.8(9), 173.8(6), 173.1, 172.8, 103.8, 103.7(4), 103.6(7), 103.6(5), 103.5(7), 102.1, 101.2, 100.4, 100.2, 97.8, 82.9 81.2, 80.3, 79.3, 78.9, 77.2, 77.1, 77.0, 76.1, 75.7, 75.5, 75.3, 75.2, 74.3, 73.7, 73.3, 73.0, 72.9, 72.7, 71.8, 71.0, 70.7, 70.2, 69.3, 69.1, 68.7, 68.1, 66.4, 62.5, 61.8, 61.8, 61.4, 60.6, 60.0, 56.0, 55.9, 55.7, 55.6, 54.9, 54.4, 50.8, 50.3, 40.1, 40.1, 34.6, 31.3, 30.9, 27.4, 23.1, 23.0, 22.9(5), 22.9, 22.7, 22.6(8), 20.1, 19.3, 18.6, 17.5; MALDI-TOF MS: [M+H$^+$] C146H248N17O94, calcd 3742, found 3745.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (57)**

Compound **57a** (0.5 mg, 0.133 μmol), CMP-Neu5Ac (0.4 mg, 4.0 equiv.), and rST3Gal-III (3 mU) were dissolved in 133 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 87 % yield).

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H), 5.03 (d, $J$ = 10.1 Hz, 1H), 4.91 (s, 1H), 4.75 (s, 1H), 4.72 – 4.63 (m, 5H), 4.62 – 4.50 (m, 5H), 4.48 – 4.42 (m, 4H), 4.39 (t, $J$ = 7.1 Hz, 1H), 4.29 (q, $J$ = 7.3 Hz, 1H), 4.26 – 4.05 (m, 14H), 4.01 – 3.40 (m, 106H), 2.99 (t, $J$ = 7.2 Hz, 4H), 2.89 – 2.81 (m, 1H), 2.79 – 2.67 (m, 3H), 2.14 – 1.93 (m, 31H), 1.93 – 1.81 (m, 2H), 1.79 (t, $J$ = 12.2 Hz, 2H), 1.73 – 1.65 (m, 4H), 1.50 – 1.39 (m, 4H), 1.37 (d, $J$ = 7.3 Hz, 3H), 1.16 (d, $J$ = 6.2 Hz, 3H), 0.96 (d, $J$ = 6.4 Hz, 6H); MALDI-TOF MS: [M+H$^+$] C168H282N19O110, calcd 4324, found 4324.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (120)**

Compound **57a** (0.5 mg, 0.133 μmol), CMP-Neu5Ac (0.4 mg, 4.0 equiv.), and hST6Gal-I (2 mU) were dissolved in 133 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 87 % yield).

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H), 5.03 (d, $J$ = 9.6 Hz, 1H), 4.91 (s, 1H), 4.73 – 4.63 (m, 6H), 4.63 – 4.53 (m, 3H), 4.49 – 4.42 (m, 6H), 4.39 (t, $J$ = 7.1 Hz, 1H), 4.29 (q, $J$ = 6.7 Hz, 1H), 4.26 – 4.06 (m, 11H), 4.02 – 3.42 (m, 109H), 3.06 – 2.92 (m, 4H), 2.90 – 2.81 (m, 1H), 2.79 – 2.71 (m, 1H), 2.66 (dd, $J$ = 12.4, 4.7 Hz, 2H), 2.10 – 1.94 (m, 31H), 1.92 – 1.82 (m, 2H), 1.80 – 1.62 (m, 7H), 1.50 – 1.38 (m, 3H), 1.37 (d, $J$ = 7.3 Hz, 3H), 1.16 (d, $J$ = 6.6 Hz, 3H), 0.96 (d, $J$ = 6.7 Hz, 6H); MALDI-TOF MS: [M+H$^+$] C168H282N19O110, calcd 4324, found 4327.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NK (57b)**

This compound was made by the same method used in the preparation of **53b**. Started from 10.5 mg **57a**, 100% yield. MALDI-TOF MS: [M+H$^+$] C152H255N16O101, calcd 3919, found 3921.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NK (58a)**

This compound was made by the same method used in the preparation of **56a**. Started from 10.1 mg **57b**, 91% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.09 (s, 1H, H-1$_{Man}$), 5.02 (d, $J$ = 9.7 Hz, 1H, H-1$_{Gn}$), 4.91 (s, 1H, H-1$_{Man}$), 4.74 (s, 1H, H-1$_{Man}$), 4.68 (d, $J$ = 8.1 Hz, 7H, H-1$_{Asn}$, 6 H-1$_{Gn}$), 4.59 (d, $J$ = 7.5 Hz, 1H, H-1$_{Gn}$), 4.56 (d, $J$ = 7.1 Hz, 2H, H-1$_{Gn}$), 4.45-4.38 (m, 9H), 4.34 (q, $J$ = 7.2 Hz, 1H, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.17 (s, 1H, H-2$_{Man}$), 4.16-4.11 (m, 6H), 4.11-4.07 (m, 2H, H-1$_{Val}$, H-2$_{Man}$), 4.00 – 3.40 (m, 117H), 3.01-2.93 (m, 2H, H-5$_{Lys}$), 2.80 – 2.60 (m, 2H, H-2$_{Asn}$), 2.10 (m, 1 H, H-2$_{Val}$), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 15H), 1.99 (s, 3H), 1.89 (s, 3H), 1.69-1.55 (m, 3H), 1.37 (d, $J$ = 7.5 Hz, 3H$_{Ala}$), 1.38-1.28 (m, 3H), 0.94 (d, $J$ = 6.8 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 182.3, 178.4, 177.4, 175.7, 175.6, 175.5, 175.4, 174.2, 173.8, 103.7, 103.7, 103.6, 103.6, 102.1, 101.2, 100.3, 100.2, 97.8, 82.9, 81.2, 80.2, 79.4, 79.3, 78.9, 77.2, 77.1, 77.0, 76.1, 75.7, 75.5, 75.5, 75.3, 75.2, 74.3, 73.7, 73.6, 73.3, 73.0, 72.9, 72.8, 72.7, 71.8, 71.0, 70.8, 70.2, 69.3, 69.1, 68.1, 66.4, 62.5, 62.4, 61.8, 61.7(5), 60.7, 60.6, 60.1, 56.0, 55.9(3), 55.7, 55.6, 54.8, 54.5, 52.2, 50.3, 40.1, 38.6, 34.6, 30.9, 27.6, 24.1, 23.1, 23.0, 22.9(6), 22.9, 22.7, 19.3, 18.5, 17.6; MALDI-TOF MS: [M+Na$^+$] C164H274N16O111Na, calcd 4265, found 4267.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NK (58)**

Compound **58a** (0.5 mg, 0.118 μmol), CMP-Neu5Ac (0.3 mg, 4.0 equiv.), and rST3Gal-III (2 mU) were dissolved in 24 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 88 % yield).

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H), 5.03 (d, *J* = 9.6 Hz, 1H), 4.91 (s, 1H), 4.75 (s, 1H), 4.68 (d, *J* = 8.2 Hz, 4H), 4.62 – 4.51 (m, 6H), 4.49 – 4.39 (m, 9H), 4.35 (q, *J* = 7.1 Hz, 1H), 4.27 – 4.07 (m, 12H), 4.01 – 3.44 (m, 132H), 3.00 (t, *J* = 7.7 Hz, 2H), 2.80 – 2.66 (m, 4H), 2.10 – 1.95 (m, 37H), 1.94 – 1.85 (m, 2H), 1.79 (t, *J* = 12.1 Hz, 2H), 1.74 – 1.67 (m, 2H), 1.47 – 1.40 (m, 2H), 1.39 (d, *J* = 7.1 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 6H); MALDI-TOF MS: [M+H$^+$] C186H309N18O127, calcd 4825, found 4828.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NK (121).**

Compound **58a** (0.5 mg, 0.118 μmol), CMP-Neu5Ac (0.3 mg, 4.0 equiv.), and hST6Gal-I (2 mU) were dissolved in 24 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 88 % yield).

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H), 5.03 (d, *J* = 9.5 Hz, 1H), 4.91 (s, 1H), 4.75 (s, 1H), 4.73 – 4.65 (m, 6H), 4.63 – 4.52 (m, 4H), 4.50 – 4.38 (m, 9H), 4.35 (q, *J* = 7.0 Hz, 1H), 4.24 (s, 1H), 4.20 – 4.11 (m, 10H), 4.10 (s, 1H), 4.03 – 3.42 (m, 132H), 3.00 (t, *J* = 7.7 Hz, 2H), 2.74 – 2.65 (m, 2H), 2.66 (dd, *J* = 12.3, 4.5 Hz, 2H), 2.10 – 1.95 (m, 37H), 1.92 – 1.82 (m, 2H), 1.71 (t, *J* = 12.0 Hz, 2H), 1.70 – 1.67 (m, 2H), 1.40 (d, *J* = 7.1 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 6H); MALDI-TOF MS: [M+H$^+$] C186H309N18O127, calcd 4825, found 4828.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NK (58b)**

This compound was made by the same method used in the preparation of **53b**. Started from 5.1 mg **58a**, 95% yield. $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H, H-1$_{Man}$), 5.03 (d, *J* = 9.6 Hz, 1H, H-1$_{Gn}$), 4.91 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.72-4.63 (m, 9H, 8 H-1$_{Gn}$, H-1$_{Asn}$), 4.60 (d, *J* = 7.8 Hz, 1H, H-1$_{Gn}$), 4.56 (d, *J* = 6.7 Hz, 2H, H-1$_{Gn}$), 4.50-4.39 (m, 9H, 8 H-1$_{Gal}$, H-1$_{Lys}$), 4.35 (q, *J* = 7.2 Hz, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.17 (s, 1H, H-2$_{Man}$), 4.14 (m, 6H), 4.11 (d, *J* = 7.6 Hz, 1H, H-1$_{Val}$), 4.09 (s, 1H, H-2$_{Man}$), 4.04 – 3.36 (m, 129H), 2.98 (t, *J* = 7.6 Hz, 2H, H-5$_{Lys}$), 2.79 – 2.62 (m, 2H, H-2$_{Asn}$), 2.12 (m, 1 H, H-2$_{Val}$), 2.06 (s, 3H), 2.02 (m, 30H), 1.99 (s, 3H), 1.79 – 1.56 (m, 3H), 1.41-1.32 (m, 2H), 1.38 (d, *J* = 7.1 Hz, 3H$_{Ala}$), 1.32 – 1.09 (m, 1H), 0.95 (d, *J* = 6.7 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 177.3, 175.8, 175.7, 175.6, 175.5, 175.4, 174.3, 174.2, 173.7, 103.7, 103.7, 103.6, 102.1, 101.2, 100.4, 100.2, 97.8, 82.9, 82.8, 81.2, 80.3, 79.5, 79.3, 78.9, 77.2, 77.1, 77.0, 76.4, 75.7, 75.5, 75.3, 75.2, 74.3, 73.7, 73.6, 73.0, 72.8, 72.7, 71.0, 70.8, 70.7(5), 70.5, 70.2, 69.1, 68.1, 66.4, 63.3, 62.4, 61.8, 61.3, 60.7, 60.6, 60.3, 58.2, 56.4, 55.9, 55.7, 55.6, 54.4, 52.2, 50.3, 40.0, 38.6, 33.7, 30.8, 27.3, 23.1, 23.0, 22.9(6), 22.9, 22.4, 19.2, 18.5, 17.6. MALDI-TOF MS: [M+H$^+$] C180H301N18O121, calcd 4649, found 4652.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NK (59a)**

This compound was made by the same method used in the preparation of **56a**. Started from 4.5 mg **58b**, 100% yield. $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H, H-1$_{Man}$), 5.03 (d, *J* = 9.7 Hz, 1H, H-1$_{Gn}$), 4.91 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.72-4.64 (m, 9H, H-1$_{Asn}$, 8H-1$_{Gn}$), 4.60 (d, *J* = 7.5 Hz, 1H, H-1$_{Gn}$), 4.56 (d, *J* = 6.4 Hz, 2H, H-1$_{Gn}$), 4.53 – 4.39 (m, 11H, 10 H-1$_{Gal}$, H-1$_{Lys}$), 4.35 (q, *J* = 7.2 Hz, 1H, H-1$_{Ala}$), 4.24 (s, 1H, H-2$_{Man}$), 4.17 (s, 1H, H-2$_{Man}$), 4.16-4.13 (m, 8H), 4.11 (d, *J* = 7.6 Hz, 1H, H-1$_{Val}$), 4.09 (s, 1H, H-2$_{Man}$), 4.06 – 3.40 (m, 139H), 2.98 (t, *J* = 7.6 Hz, 2H, H-5$_{Lys}$), 2.85 – 2.58 (m, 2H, H-2$_{Asn}$), 2.10 (m, 1 H, H-2$_{Val}$), 2.06 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 2.02 (m, 24H), 1.99 (s, 3H), 1.77 – 1.56 (m, 3H), 1.40-1.30 (m, 2H), 1.38 (d, *J* = 7.1 Hz, 3H$_{Ala}$), 1.20 (m, 1H), 0.95 (d, *J* = 6.7 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 177.3, 175.7, 175.6, 175.5,

175.4, 174.2, 174.2, 173.7, 103.8, 103.7, 103.7, 103.6, 102.1, 101.2, 100.3, 100.2, 97.8, 82.9, 81.2, 80.2, 79.5, 79.3, 78.9, 77.2, 77.1, 77.0, 76.1, 75.7, 75.5, 75.3, 75.2, 74.3, 73.7, 73.3, 73.0, 71.8, 71.0, 70.8, 70.2, 69.3, 69.1, 68.1, 66.4, 63.3, 62.4, 61.8, 61.7(5), 60.7(1), 60.6, 60.2, 56.0, 55.9, 55.7, 55.6, 54.6, 54.5, 52.2, 50.3, 40.0, 38.6, 34.0, 30.9, 27.3, 23.1, 23.0, 22.9, 22.5, 19.2, 18.5, 17.6. MALDI-TOF MS: [M+H$^+$] C192H321N18O131, calcd 4973, found 4976.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NK (59)**

Compound **59a** (0.5 mg, 0.1 μmol), CMP-Neu5Ac (0.3 mg, 4.0 equiv.), and rST3Gal-III (1 mU) were dissolved in 20 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 91 % yield). MALDI-TOF MS: [M+H$^+$] C214H355N20O147, calcd 5555, found 5557.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NK (122)**

Compound **59a** (0.5 mg, 0.1 μmol), CMP-Neu5Ac (0.3 mg, 4.0 equiv.), and hST6Gal-I (2 mU) were dissolved in 20 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 91 % yield). MALDI-TOF MS: [M+H$^+$] C214H355N20O147, calcd 5555, found 5559.

**Galβ4GlcNAcβ2Manα3(Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (8a)**

This compound was made by the same method used in the preparation of **56a**. Started from 5.5 mg **60c**, 92% yield.$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 9.6 Hz, 1H, H-1$_{Gn}$), 4.91 (s, 1H, H-1$_{Man}$), 4.86 (d, $J$ = 3.4 Hz, 1H, H-1$_{Fuc}$), 4.75 (s, 1H, H-1$_{Man}$), 4.67 (overlapped, 1H, H-1$_{Asn}$), 4.66 (d, $J$ = 7.6 Hz, 1H, H-1$_{Gn}$), 4.56 (d, $J$ = 7.7 Hz, 2H, H-1$_{Gn}$), 4.45 (d, $J$ = 7.8 Hz, 1H, H-1$_{Gal}$), 4.44 (d, $J$ = 7.8 Hz, 1H, H-1$_{Gal}$), 4.39 (m, 1H, H-1$_{Lys}$), 4.29 (q, $J$ = 7.2 Hz, 1H, H-1$_{Ala}$), 4.24 (s, 1H, H-2$_{Man}$), 4.21 (m, 1H, H-2$_{Thr}$), 4.18 (s, 1H, H-2$_{Man}$), 4.15 – 4.10 (m, 2H, H-1$_{Thr}$, H-1$_{Val}$), 4.09 (s, 1H, H-2$_{Man}$), 4.02 – 3.42 (m, 56H), 3.08 – 2.91 (m, 4H, H-5$_{Lys}$), 2.84 (dd, $J$ = 16.2, 4.2 Hz, 1H, H-2$_{Asn}$), 2.73 (dd, $J$ = 16.1, 8.1 Hz, 1H, H-2$_{Asn}$), 2.08 (s, 3H), 2.03 (s, 6H), 2.02 (m, 1H), 1.99 (s, 3H), 1.87 (m, 1H), 1.76 (m, 1H), 1.75-1.55 (m, 6H), 1.50-1.35 (m, 4H), 1.36 (d, $J$ = 6.8 Hz, 3H$_{Ala}$), 1.18 (d, $J$ = 6.4 Hz, 3H$_{Fuc}$), 1.15 (d, $J$ = 6.3 Hz, 3H$_{Thr}$), 0.95 (d, $J$ = 6.8 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 177.2, 175.5, 175.4, 175.2, 173.8, 173.1, 172.8, 161.1, 103.7, 103.7, 101.8, 101.3, 100.4, 100.2(4), 100.2(2), 100.1, 97.8, 81.2, 80.4, 79.3, 79.2, 79.0, 78.9, 77.2, 77.1, 76.1, 76.0, 75.5, 75.4(8), 75.2, 75.1, 74.3, 73.6, 73.3, 72.9, 72.7, 72.6, 71.8, 71.0, 70.3, 70.2, 70.1(7), 69.3, 69.0, 68.68, 68.6(5), 68.1, 68.0(6), 67.6, 67.3, 66.5, 62.5, 62.4, 61.8, 61.4, 60.8, 60.0, 55.7, 55.6, 54.8, 54.4, 54.3(7), 50.8, 50.3, 40.0, 34.3, 31.4, 31.3, 30.9, 27.4, 27.0, 27.0, 23.1, 23.0, 22.9, 22.7, 22.6, 20.1, 19.3, 18.6, 17.5, 16.2. MALDI-TOF MS: [M+Na$^+$] C96H165N13O58Na, calcd 2450, found 2451; MALDI-TOF MS: [M-Lys+Na$^+$] C90H153N11O57Na, calcd 2322, found 2324; MALDI-TOF MS: [M-LysValAla+Na$^+$] C82H139N9O55Na, calcd 2152, found 2153.

**GlcNAcβ3Galβ4GlcNAcβ2Manα3(GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (8b)**

This compound was made by the same method used in the preparation of **53b**. Started from 5.5 mg **8a**, 98% yield. $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 8.9 Hz, 1H, H-1$_{Gn}$), 4.90 (s, 1H, H-1$_{Man}$), 4.85 (s, 1H, H-1$_{Fuc}$), 4.75 (s, 1H, H-1$_{Man}$), 4.66 (d, $J$ = 7.5 Hz, 4H, 3 H-1$_{Gn}$, H-1$_{Asn}$), 4.56 (d, $J$ = 6.5 Hz, 2H, H-1$_{Gn}$), 4.45 (d, $J$ = 7.8 Hz, 1H, H-1$_{Gal}$), 4.44 (d, $J$ = 7.8 Hz, 1H, H-1$_{Gal}$), 4.38 (m, 1H, H-1$_{Lys}$), 4.28 (q, $J$ = 7.4 Hz, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.20 (m, 1H, H-2$_{Thr}$), 4.17 (s, 1H, H-2$_{Man}$), 4.15-4.10 (m, , 2H, H-1$_{Thr}$, H-1$_{Val}$), 4.08 (s, 1H, H-2$_{Man}$), 4.02 – 3.36 (m, 68H), 3.05-2.92 (m, 4H, H-5$_{Lys}$), 2.83 (d, $J$ = 15.6 Hz, 1H, H-2$_{Asn}$), 2.73 (dd, $J$ = 15.5, 7.6 Hz, 1H, H-2$_{Asn}$), 2.07 (s, 3H), 2.03 (m, 1 H), 2.02 (s, 12H), 1.99 (s, 3H), 1.89 (m, 1H), 1.83-1.53 (m, 7H), 1.52 – 1.36 (m, 4H), 1.36 (d, $J$ = 6.8 Hz, 3H$_{Ala}$), 1.18 (d, $J$ = 5.8 Hz, 3H$_{Fuc}$), 1.15 (d, $J$ = 5.7 Hz, 3H$_{Thr}$), 0.94 (d, $J$ = 5.4 Hz, 3H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 178.4, 177.2, 175.6, 175.4, 175.3, 175.2, 173.8, 173.0, 172.7, 164.3, 103.6, 102.0, 101.1, 100.2, 100.1, 97.7, 82.7, 81.1, 80.1, 79.0, 78.8, 77.0, 76.8, 76.3, 75.5, 75.4, 75.0, 74.2, 73.5, 73.4(6), 72.7, 72.6, 70.8, 70.6, 70.3, 70.1, 69.0, 68.5, 68.0, 66.3, 62.3, 61.6,

61.3, 61.1, 60.6, 59.9, 56.3, 55.6, 55.4, 54.7, 54.3, 50.6, 50.2, 40.0, 39.9, 37.0, 34.5, 31.7, 31.2, 30.8, 27.8, 27.4, 27.3, 27.2, 23.0, 22.9, 22.8, 22.6, 20.0, 19.1, 18.5, 18.2, 17.3, 17.2; MALDI-TOF MS: [M+$H^+$] $C_{112}H_{192}N_{15}O_{68}$, calcd 2834, found 2835; MALDI-TOF MS: [M-Lys+$H^+$] $C_{106}H_{180}N_{13}O_{67}$, calcd 2706, found 2707; MALDI-TOF MS: [M-LysValAla+$H^+$] $C_{98}H_{166}N_{11}O_{65}$, calcd 2536, found 2537.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (8c)**

This compound was made by the same method used in the preparation of **56a**. Started from 6.0 mg **8b**, 82% yield. $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H, H-$1_{Man}$), 5.03 (d, $J$ = 9.4 Hz, 1H, H-$1_{Gn}$), 4.90 (s, 1H, H-$1_{Man}$), 4.85 (s, 1H, H-$1_{Fuc}$), 4.75 (s, 1H, H-$1_{Man}$), 4.72 – 4.62 (m, 4H, 3 H-$1_{Gn}$, H-$1_{Asn}$), 4.56 (d, $J$ = 7.1 Hz, 2H, H-$1_{Gn}$), 4.51 – 4.41 (m, 4H, H-$1_{Gal}$), 4.38 (m, 1H, H-$1_{Lys}$), 4.28 (q, $J$ = 7.2 Hz, 1H, H-$1_{Ala}$), 4.23 (s, 1H, H-$2_{Man}$), 4.21 (m, 1H, H-$1_{Thr}$), 4.17 (s, 1H, H-$2_{Man}$), 4.16-4.09 (m, 4H), 4.09 (s, 1H, H-$2_{Man}$), 4.03 – 3.36 (m, 78H), 3.05-2.92 (m, 4H, H-$5_{Lys}$), 2.83 (d, $J$ = 15.9 Hz, 1H, H-$2_{Asn}$), 2.73 (dd, $J$ = 16.0, 8.0 Hz, 1H, H-$2_{Asn}$), 2.07 (s, 3H), 2.03 (s, 3H), 2.02 (s, 9H), 2.01 (overlapped, 1H), 1.99 (s, 3H), 1.88 (m, 1H), 1.76 (m, 1H), 1.71 – 1.52 (m, 6H), 1.51-1.38 (m, 4H), 1.36 (d, $J$ = 6.7 Hz, 3$H_{Ala}$), 1.18 (d, $J$ = 6.2 Hz, 3$H_{Fuc}$), 1.15 (d, $J$ = 6.3 Hz, 3$H_{Thr}$), 0.94 (d, $J$ = 6.5 Hz, 3$H_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 177.2, 175.7, 175.5, 175.3, 173.8, 173.1, 172.8, 103.8, 103.7, 103.6, 103.6, 101.8, 101.3, 100.4, 100.2, 100.1, 97.8, 82.9, 81.2, 80.4, 79.4, 79.3, 79.0, 78.9, 77.2, 77.1, 76.1, 76.0, 75.7, 75.5, 75.4(6), 75.3, 75.2, 74.3, 73.6, 73.5, 73.3, 73.0, 72.8, 72.7, 72.6, 71.8, 71.0, 70.7, 70.3, 70.2, 70.2, 69.3, 69.1, 69.0, 68.7, 68.1, 68.1, 67.6, 67.3, 66.6, 66.5, 62.5, 62.4, 61.8, 61.7, 61.4, 60.7, 60.6, 60.0, 56.0, 55.7, 55.6, 54.9, 54.4, 52.1, 50.8, 50.3, 40.0, 37.1, 34.5, 31.3, 30.9, 27.5, 27.1, 23.1, 23.0, 22.9, 22.7, 22.6, 20.1, 19.3, 18.6, 17.5, 16.2; MALDI-TOF MS: [M+$H^+$] $C_{124}H_{212}N_{15}O_{78}$, calcd 3158, found 3159; MALDI-TOF MS: [M-Lys+$H^+$] $C_{118}H_{200}N_{13}O_{77}$, calcd 3030, found 3031; MALDI-TOF MS: [M-LysValAla+$H^+$] $C_{110}H_{186}N_{11}O_{75}$, calcd 2860, found 2861.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (123)**

Compound **8c** (0.5 mg, 0.158 μmol), CMP-Neu5Ac (0.7 mg, 4.0 equiv.), and hST6Gal-I (1.2 mU) were dissolved in 180 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 85 % yield).

MALDI-TOF MS: permethylated [M-ThrLysVal + $Na^+$] $C_{189}H_{333}N_{13}O_{90}Na$, calcd 4246, found 4250.2.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (8d)**

This compound was made by the same method used in the preparation of **53b**. Started from 6.4 mg **8c**, 91% yield. $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H, H-$1_{Man}$), 5.03 (d, $J$ = 9.3 Hz, 1H, H-$1_{Gn}$), 4.90 (s, 1H, H-$1_{Man}$), 4.85 (s, 1H, H-$1_{Fuc}$), 4.75 (s, 1H, H-$1_{Man}$), 4.67 (m, 6H, 5 H-$1_{Gn}$, H-$1_{Asn}$), 4.56 (d, $J$ = 6.9 Hz, 2H, H-$1_{Gn}$), 4.45 (d, $J$ = 7.6 Hz, 4H, H-$1_{Gal}$), 4.39 (m, 1H, H-$1_{Lys}$), 4.28 (m, 1H, H-$1_{Ala}$), 4.23 (s, 1H, H-$2_{Man}$), 4.20 (m, 1H, H-$2_{Thr}$), 4.17 (s, 1H, H-$2_{Man}$), 4.16 – 4.09 (m, 4H), 4.09 (s, 1H, H-$2_{Man}$), 4.03 – 3.36 (m, 90H), 3.04 – 2.93 (m, 4H, H-$5_{Lys}$), 2.83 (d, $J$ = 16.0 Hz, 1H, H-$2_{Asn}$), 2.73 (dd, $J$ = 16.1, 7.9 Hz, 1H, H-$2_{Asn}$), 2.07 (s, 3H), 2.04 (m, 1H, overlapped), 2.03 (s, 3 H), 2.01 (s, 15H), 1.99 (s, 3H), 1.89 (m, 1H), 1.75 (m, 1H), 1.72 – 1.55 (m, 6H), 1.51 – 1.38 (m, 4H), 1.36 (d, $J$ = 6.7 Hz, 3$H_{Ala}$), 1.18 (d, $J$ = 6.2 Hz, 3$H_{Fuc}$), 1.15 (d, $J$ = 6.3 Hz, 3$H_{Thr}$), 0.94 (d, $J$ = 6.6 Hz, 6$H_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 177.2, 175.8, 175.7, 175.5, 175.3, 173.8, 173.1, 172.8, 103.8, 103.7, 103.6(7), 103.6, 101.8, 101.3, 100.4, 100.2, 100.1, 97.8, 82.9, 82.8, 81.2, 80.4, 79.3, 79.2(5), 79.0, 78.9, 77.2, 77.1, 76.4, 76.0, 75.7, 75.5, 75.3, 75.2, 74.3, 73.6, 73.0, 72.8, 72.7, 72.6, 71.0, 70.8, 70.7, 70.5, 70.3, 70.2, 69.1, 69.0, 68.7, 68.1, 68.0(6), 67.6, 67.3, 66.5, 62.4, 61.8, 61.4, 61.2, 60.7, 60.6, 60.0, 56.4, 55.9, 55.7, 55.6, 54.8, 54.4, 50.8, 50.3; MALDI-TOF MS: [M+$H^+$] $C_{140}H_{238}N_{17}O_{88}$, calcd 3564, found 3566; MALDI-TOF MS: [M-Lys+$H^+$] $C_{134}H_{226}N_{15}O_{87}$, calcd 3436, found 3442; MALDI-TOF MS: [M-LysValAla+$H^+$] $C_{126}H_{212}N_{13}O_{85}$, calcd 3266, found 3268.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (60a)**

This compound was made by the same method used in the preparation of **56a**. Started from 6.1 mg **8d**, 98% yield. $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.10 (s, 1H, H-$1_{Man}$), 5.03 (d, $J$ = 9.2 Hz, 1H, H-$1_{Gn}$), 4.90 (s, 1H, H-$1_{Man}$),

4.85 (s, 1H, H-$1_{Fuc}$), 4.75 (s, 1H, H-$1_{Man}$), 4.68 (d, *J* = 8.2 Hz, 6H, 5 H-$1_{Gn}$, H-$1_{Asn}$), 4.56 (d, *J* = 6.7 Hz, 2 H-$1_{Gn}$), 4.51 – 4.41 (m, 6 H-$1_{Gal}$), 4.38 (m, 1H, H-$1_{Lys}$), 4.29 (m, 1H, H-$1_{Ala}$), 4.23 (s, 1H, H-$2_{Man}$), 4.20 (m, 1H, H-$2_{Thr}$), 4.17 (s, 1H, H-$2_{Man}$), 4.16 – 4.10 (m, 6H), 4.09 (s, 1H, H-$2_{Man}$), 4.04 – 3.40 (m, 35H), 3.08-2.94 (m, 4 H-$5_{Lys}$), 2.83 (d, *J* = 15.8 Hz, 1H, H-$2_{Asn}$), 2.73 (dd, *J* = 16.1, 7.9 Hz, 1H, H-$2_{Asn}$), 2.07 (s, 3H), 2.04 (m, 1H, overlapped), 2.03 (s, 3H), 2.02 (s, 15H), 1.99 (s, 3H), 1.89 (m, 1H), 1.82 – 1.55 (m, 7H), 1.51 – 1.35 (m, 4H), 1.36 (d, *J* = 6.7 Hz, $3H_{Ala}$), 1.18 (d, *J* = 6.2 Hz, $3H_{Fuc}$), 1.15 (d, *J* = 6.2 Hz, $3H_{Thr}$), 0.95 (d, *J* = 6.3 Hz, $6H_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): *δ* = 177.2, 175.7, 175.5, 175.2, 174.1, 173.8, 173.1, 172.8, 103.7, 103.6, 103.6, 101.8, 101.3, 100.4, 100.3, 100.2, 97.8, 82.9, 81.2, 80.4, 79.3, 79.3, 79.0, 78.9, 77.2, 77.1, 76.1, 76.0, 75.7, 75.5, 75.3, 75.2, 74.3, 73.6, 73.3, 73.0, 72.8, 72.7, 72.6, 72.0, 71.0, 70.7, 70.3, 70.2, 69.3, 69.1, 69.0, 68.7, 68.1, 67.6, 67.3, 66.6, 66.5, 62.5, 62.4, 61.8, 61.7, 61.4, 61.3, 60.7, 60.6, 60.0, 56.0, 55.9, 55.7, 55.6, 54.8, 54.4, 52.1, 50.8, 50.3, 40.0, 37.1, 34.4, 31.3, 31.3, 30.9, 27.4, 27.0, 23.1, 23.0, 22.6, 20.1, 19.3, 18.6, 17.5, 16.2; MALDI-TOF MS: [M+$H^+$] C152H258N17O98, calcd 3888, found 3892; MALDI-TOF MS: [M-Lys+$H^+$] C146H246N15O97, calcd 3760, found 3764; MALDI-TOF MS: [M-LysValAla+$H^+$] C138H232N13O95, calcd 3590, found 3594.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (60)**

Compound **60a** (0.5 mg, 0.13 μmol), CMP-Neu5Ac (0.35 mg, 4.0 equiv.), and rST3Gal-III (1 mU) were dissolved in 86 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 93 % yield).

MALDI-TOF MS: permethylated [M-ThrLysVal + $Na^+$] C229189H403N15O110Na, calcd 5144, found 5149.2.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (124)**

Compound **60a** (0.5 mg, 0.13 μmol), CMP-Neu5Ac (0.35 mg, 4.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 130 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 93 % yield).

ESI-FT MS: permethylated [M-ThrLysVal + 3x$Na^+$] C229H403N15O110Na3, calcd 1730, found 1731.9 (z = 3).

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (60b)**

This compound was made by the same method used in the preparation of **53b**. Started from 3.7 mg **60a**, 85% yield. $^1$H NMR (600 MHz, $D_2O$): *δ* = 5.10 (s, 1H, H-$1_{Man}$), 5.03 (d, *J* = 9.6 Hz, 1H, H-$1_{Gn}$), 4.91 (s, 1H, H-$1_{Man}$), 4.86 (d, *J* = 3.6 Hz, 1H, H-$1_{Fuc}$), 4.76 (s, 1H, H-$1_{Man}$, overlapped), 4.71 – 4.63 (m, 8H, 7 H-$1_{Gn}$, H-$1_{Asn}$), 4.56 (d, *J* = 6.6 Hz, 2 H-$1_{Gn}$), 4.51 – 4.41 (m, 6 H-$1_{Gal}$), 4.38 (m, 1H, H-$1_{Lys}$), 4.29 (m, 1H, H-$1_{Ala}$), 4.24 (s, 1H, H-$2_{Man}$), 4.20 (m, 1H, H-$2_{Thr}$), 4.17 (s, 1H, H-$2_{Man}$), 4.16-4.10 (m, 8H), 4.09 (s, 1H, H-$2_{Man}$), 4.04 – 3.33 (m, 110H), 3.08 – 2.90 (m, 4 H-$5_{Lys}$), 2.83 (m, 1H), 2.73 (m, 1H), 2.08 (s, 3H), 2.04 (m, 1H, overlapped), 2.03 (s, 3H), 2.02(7) (s, 3H), 2.02 (s, 9H), 2.01 (s, 9H), 1.99 (s, 3H), 1.89(m, 1H), 1.76 (m, 1H), 1.72 – 1.56 (m, 6H), 1.50 – 1.39 (m, 4H), 1.36 (d, *J* = 6.7 Hz, $3H_{Ala}$), 1.18 (d, *J* = 6.3 Hz, $3H_{Fuc}$), 1.15 (d, *J* = 6.6 Hz, $3H_{Thr}$), 0.95 (d, *J* = 6.8 Hz, $6H_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): *δ* = 177.0, 176.9, 176.6, 176.6(3), 174.9, 103.8, 103.7, 10.3.7(1), 103.6, 101.7, 101.2, 100.3, 100.2, 100.1, 97.8, 82.5, 82.4, 80.9, 80.1, 78.9, 78.7, 78.6, 76.8, 76.7, 76.1, 75.5, 75.2, 75.0, 74.9, 74.7, 73.9, 73.1, 72.5, 72.3, 72.2, 72.1, 70.5, 70.3, 70.2, 69.9, 69.8, 69.7, 68.6, 68.5, 68.2, 67.6, 67.1, 65.9, 60.7, 59.3, 55.8, 55.1, 54.9, 54.8, 54.0, 53.6, 49.5, 29.8, 21.8, 21.7, 18.8, 18.0, 17.3, 16.2, 14.8, 14.8(3); MALDI-TOF MS: [M+$H^+$] C168H284N19O108, calcd 4294, found 4300; MALDI-TOF MS: [M-Lys+$H^+$] C162H272N17O107, calcd 4166, found 4172; MALDI-TOF MS: [M-LysValAla+$H^+$] C154H258N15O105, calcd 3996, found 4002.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (61a)**

This compound was made by the same method used in the preparation of **56a**. Started from 3.3 mg **60b**, 86% yield. $^1$H NMR (600 MHz, $D_2O$): *δ* = 5.10 (s, 1H, H-$1_{Man}$), 5.03 (d, *J* = 8.8 Hz, 1H, H-$1_{Gn}$), 4.91 (s, 1H, H-

$1_{Man}$), 4.85 (s, 1H, H-$1_{Fuc}$), 4.75 (s, 1H, H-$1_{Man}$), 4.73 – 4.62 (m, 8H, 7 H-$1_{Gn}$, H-$1_{Asn}$), 4.61 – 4.53 (d, *J* = 7.7 Hz, 2H-$1_{Gn}$), 4.51 – 4.41 (m, 8 H-$1_{Gal}$), 4.38 (m, 1H, H-$1_{Lys}$), 4.30 (m, 1H, H-$1_{Ala}$), 4.24 (s, 1H, H-$2_{Man}$), 4.21 (m, 1H), 4.17 (s, 1H, H-$2_{Man}$), 4.16 – 4.10 (m, 8H), 4.09 (s, 1H, H-$2_{Man}$), 4.02 – 3.35 (m, 122H), 3.07 – 2.89 (m, 4H, H-$5_{Lys}$), 2.3 (m, 1H, H-$2_{Asn}$), 2.74 (m, 1H, H-$2_{Asn}$), 2.08 (s, 3H), 2.04 (m, 1H, overlapped), 2.03(3) (s, 3H), 2.02(7) (s, 3H), 2.01(6) (s, 18H), 2.00 (s, 3H), 1.90 (m, 1H), 1.76 (m, 1H), 1.72 – 1.61 (m, 6H), 1.49 – 1.40 (m, 4H), 1.36 (d, *J* = 6.6 Hz, $3H_{Ala}$), 1.18 (d, *J* = 6.4 Hz, $3H_{Fuc}$), 1.15 (d, *J* = 6.5 Hz, $3H_{Thr}$), 0.95 (d, *J* = 6.7 Hz, $6H_{Val}$); $^{13}$C NMR (151 MHz, $D_2O$): *δ* = 177.2, 175.7, 175.5, 173.8, 103.7, 103.6(5), 103.5(6), 101.7, 101.3, 100.3(5), 100.2, 100.1, 97.8, 82.9, 81.2, 80.4, 79.3, 78.9, 77.2, 77.1, 76.1, 76.0, 75.7, 75.5, 75.4(6), 75.3, 75.2, 74.3, 73.6, 73.3, 73.0, 72.8, 72.7, 72.6, 71.7, 71.0, 70.7, 70.3, 70.2, 69.3, 69.1, 69.0, 68.7, 68.1, 67.6, 67.3, 66.5, 61.4, 59.9, 56.0, 55.9, 55.7, 55.6, 54.8, 54.4, 52.1, 50.7, 50.3, 40.0, 34.5, 32.6, 31.3, 30.9, 27.4, 27.1, 23.1, 23.0, 22.6, 20.1, 19.3, 18.6, 17.5, 16.2; MALDI-TOF MS *m/z* calcd for C180H303N19O118 [M+$Na^+$] 4640, found 4644; MALDI-TOF MS: [M-Lys+$Na^+$] C174H292N17O117Na, calcd 4513, found 4515; MALDI-TOF MS: [M-LysValAla+$Na^+$] C166H277N15O115Na, calcd 4342, found 4346.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (61)**

Compound **61a** (0.5 mg, 0.108 μmol), CMP-Neu5Ac (0.43 mg, 6.0 equiv.), and rST3Gal-III (3 mU) were dissolved in 72 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 89 % yield).

MALDI-TOF MS: permethylated [M+$Na^+$] C269H473N17O130Na, calcd 6042, found 6047.9.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (125)**

Compound **61a** (0.5 mg, 0.108 μmol), CMP-Neu5Ac (0.3 mg, 4.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 72 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 93 % yield); MALDI-TOF MS: [M+$K^+$] C202H337N21O134K, calcd for 5238, found 5242.

**Galβ4GlcNAcβ2(Galβ4GlcNAcβ6)Manα6(Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (9a)**

This compound was made by the same method used in the preparation of **56a**. Started from 7.6 mg **62c**, 87% yield; MALDI-TOF MS: [M+$Na^+$] C104H179N14O64Na, calcd 2670, found 2670.

**GlcNAcβ3Galβ4GlcNAcβ2(GlcNAcβ3Galβ4GlcNAcβ6)Manα6(GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (9b)**

This compound was made by the same method used in the preparation of **53b**. Started from 6 mg **9a**, 80% yield; $^1$H NMR (600 MHz, $D_2O$): *δ* = 5.11 (s, 1H, H-$1_{Man}$), 5.03 (d, *J* = 9.5 Hz, 1H, H-$1_{Gn}$), 4.85 (s, 1H, H-$1_{Man}$), 4.75 (s, 1H, H-$1_{Man}$), 4.68 (m, 1H, H-$1_{Asn}$), 4.70-4.63 (m, 3H-$1_{Gn}$), 4.62-4.55 (m, 3H, H-$1_{Gn}$), 4.53 (d, *J* = 8.2 Hz, 1H), 4.45 (m, 3H, H-$1_{Gal}$), 4.39 (m, 1H, H-$1_{Lys}$), 4.30 (q, *J* = 7.2 Hz, 1H, H-$1_{Ala}$), 4.24 (s, 1H, H-$2_{Man}$), 4.22 – 4.16 (m, 2H), 4.18 (s, 1H, H-$2_{Man}$), 4.16 – 4.09 (m, 4H), 4.07 (s, 1H, H-$2_{Man}$), 4.03 – 3.27 (m, 79H), 3.07 – 2.91 (m, 4H, H-$5_{Lys}$), 2.85 (dd, *J* = 4.8, 16.0 Hz, 1H, H-$2_{Asn}$), 2.73 (dd, *J* = 6.5, 16.0 Hz, 1H, H-$2_{Asn}$), 2.06 (s, 3H), 2.04 (s, 3H), 2.03 (m, 1H), 2.02 (s, 15H), 1.99 (s, 3H), 1.89 (m, 1H), 1.76 (m, 1H), 1.72-1.60 (m, 6H), 1.50-1.35 (m, 4H), 1.37 (d, *J* = 7.1 Hz, $3H_{Ala}$), 1.15 (d, *J* = 6.4 Hz, $3H_{Thr}$), 0.95 (d, *J* = 6.6 Hz, $6H_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): *δ* = 175.8, 175.5, 174.9, 103.76, 103.7, 102.4, 102.1, 101.1, 100.4, 100.3, 98.0, 82.8, 81.0, 80.0, 79.4, 79.3, 79.0, 77.4, 77.2, 77.0, 76.6, 76.4, 75.7, 75.5, 75.2, 74.3, 74.1, 73.6, 73.0, 72.8, 72.7, 72.3, 71.1, 71.0, 70.8, 70.5, 70.2, 69.1, 68.7, 68.3, 68.1, 66.4, 65.9, 62.5, 61.7, 61.2, 60.9, 60.8, 60.6, 59.9, 56.4, 55.8, 55.6, 54.4, 50.8, 50.3, 40.0, 31.3, 30.9, 27.0, 23.3, 23.1, 23.0, 22.6, 20.1; MALDI-TOF MS: [M+$H^+$] C128H219N17O79, calcd 3257, found 3257.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (62a)**

This compound was made by the same method used in the preparation of **56a**. Started from 5.9 mg **9b**, 97% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-$1_{Man}$), 5.03 (d, $J$ = 9.0 Hz, 1H, H-$1_{Gn}$), 4.85 (s, 1H, H-$1_{Man}$), 4.75 (s, 1H, H-$1_{Man}$, overlapped with $H_2O$ peak), 4.68 (d, $J$ = 7.7 Hz, 3H, H-$1_{Gn}$), 4.67 (m, 1H, H-$1_{Asn}$), 4.63 – 4.54 (m, 3H-$1_{Gn}$), 4.52 (d, $J$ = 7.5 Hz, 1H-$1_{Gn}$), 4.50 – 4.41 (m, 6H-$1_{Gal}$), 4.39 (m, 1H, H-$1_{Lys}$), 4.29 (m, 1H, H-$1_{Ala}$), 4.23 (s, 1H, H-$2_{Man}$), 4.22 – 4.19 (m, 1H, H-$2_{Thr}$), 4.18 (s, 1H, H-$2_{Man}$), 4.14 (s, 3H, H-$4_{Gal}$), 4.07 (s, 1H, H-$2_{Man}$), 4.02 – 3.29 (m, 97H), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 15H), 1.99 (s, 3H), 1.36 (d, $J$ = 6.3 Hz, $3H_{Ala}$), 1.15 (d, $J$ = 6.0 Hz, $3H_{Thr}$), 0.95 (d, $J$ = 6.1 Hz, $6H_{Val}$); MALDI-TOF MS: [M+$Na^+$] C146H248N17O94Na, calcd 3765, found 3768.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (62)**

Compound **62a** (0.5 mg, 0.134 μmol), CMP-Neu5Ac (0.53 mg, 6.0 equiv.), and rST3Gal-III (3 mU) were dissolved in 134 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.55 mg, 89 % yield); ESI-FT MS: permethylated [M-ThrLysVal+$K^+$+$Na^+$+$H^+$] C237H417N16O114NaK, calcd 1790.3, found 1791.9 (z = 3).

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (126)**

Compound **62a** (0.5 mg, 0.134 μmol), CMP-Neu5Ac (0.53 mg, 6.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 134 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 89 % yield); ESI-FT MS: permethylated [M-ThrLysVal+$K^+$+2x$Na^+$] C237H416N16O114Na2K, calcd 1797.6, found 1799.3 (z = 3).

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (62b)**

This compound was made by the same method used in the preparation of **53b**. Started from 4.6 mg **62a**, 93% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-$1_{Man}$), 5.03 (d, $J$ = 8.3 Hz, 1H, H-$1_{Gn}$), 4.85 (s, 1H, H-$1_{Man}$), 4.75 (s, 1H, H-$1_{Man}$, overlapped with $H_2O$ peak), 4.71-4.63 (m, 6H-$1_{Gn}$, H-$1_{Asn}$), 4.63-4.54 (m, 3H-$1_{Gn}$), 4.52 (d, $J$ = 7.4 Hz, 1H, H-$1_{Gn}$), 4.48-4.41 (m, 6H-$1_{Gal}$), 4.41-4.36 (m, 1H, H-$1_{Lys}$), 4.32-4.26 (m, 1H, H-$1_{Ala}$), 4.23 (s, 1H, H-$2_{Man}$), 4.22-4.18 (m, 1H, H-$2_{Thr}$), 4.18 (s, 1H, H-$2_{Man}$), 4.17-4.07 (m, 6H-$4_{Gal}$, H-$1_{Thr}$, H-$1_{Val}$), 4.07 (s, 1H, H-$2_{Man}$), 4.04 – 3.29 (m, 112H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 24H), 1.99 (s, 3H), 1.36 (d, $J$ = 6.7 Hz, $3H_{Ala}$), 1.15 (d, $J$ = 6.1 Hz, $3H_{Thr}$), 0.95 (d, $J$ = 6.4 Hz, $6H_{Val}$); MALDI-TOF MS: [M+$H^+$] C170H287N20O109, calcd 4351, found 4355; MALDI-TOF MS: [M-LysValAla+$H^+$] C156H261N16O106, calcd 4053, found 4056.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (63a)**

This compound was made by the same method used in the preparation of **56a**. Started from 4 mg **62b**, 91% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-$1_{Man}$), 5.03 (d, $J$ = 8.0 Hz, 1H, H-$1_{Gn}$), 4.85 (s, 1H, H-$1_{Man}$), 4.75 (s, 1H, H-$1_{Man}$, overlapped with $H_2O$ peak), 4.73-4.63 (m, 6H-$1_{Gn}$, H-$1_{Asn}$), 4.63 – 4.55 (m, 3H-$1_{Gn}$), 4.52 (d, $J$ = 7.4 Hz, 1H, H-$1_{Gn}$), 4.49-4.41 (m, 9H-$1_{Gal}$), 4.41-4.36 (m, 1H, H-$1_{Lys}$), 4.32-4.26 (m, 1H, H-$1_{Ala}$), 4.23 (s, 1H, H-$2_{Man}$), 4.22-4.19 (m, 1H, H-$2_{Thr}$), 4.18 (s, 1H, H-$2_{Man}$), 4.16-4.08 (m, 6H-$4_{Gal}$, H-$1_{Thr}$, H-$1_{Val}$), 4.07 (m, 1H, H-$2_{Man}$), 4.02-3.33 (m, 130H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 24H), 1.99 (s, 3H), 1.36 (d, $J$ = 6.7 Hz, $3H_{Ala}$), 1.15 (d, $J$ = 6.1 Hz, $3H_{Thr}$), 0.95 (d, $J$ = 6.4 Hz, $6H_{Val}$); MALDI-TOF MS: [M+$H^+$] C188H317N20O124, calcd 4837, found 4840; MALDI-TOF MS: [M-LysValAla+$H^+$] C174H290N16O121, calcd 4538, found 4541.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (63)**

Compound **63a** (0.5 mg, 0.103 μmol), CMP-Neu5Ac (0.4 mg, 6.0 equiv.), and rST3Gal-III (1 mU) were dissolved in 100 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 85 % yield); MALDI-TOF MS: permethylated [M-ThrLysVal+Na$^+$] C297H521N19O144Na, calcd 6678, found 6685.9.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (127)**

Compound **63a** (0.5 mg, 0.103 μmol), CMP-Neu5Ac (0.4 mg, 6.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 103 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 85 % yield); ESI-FT MS: permethylated [M-ThrLysVal+4xNa$^+$] C297H521N19O144Na4, calcd 1686.8, found 1686.9 (z = 4).

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (63b)**

This compound was made by the same method used in the preparation of **53b**. Started from 2 mg **63a**, 86% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 8.6 Hz, 1H, H-1$_{Gn}$), 4.85 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$, overlapped with $H_2O$ peak), 4.73-4.63 (m, 9H-1$_{Gn}$, H-1$_{Asn}$), 4.63 – 4.55 (m, 3H-1$_{Gn}$), 4.52 (d, $J$ = 7.4 Hz, 1H, H-1$_{Gn}$), 4.50-4.42 (m, 9H-1$_{Gal}$), 4.24 (s, 1H, H-2$_{Man}$), 4.23-4.20 (m, 1H, H-2$_{Thr}$), 4.18 (s, 1H, H-2$_{Man}$), 4.17-4.11 (m, 9H-4$_{Gal}$), 4.07 (s, 1H, H-2$_{Man}$), 4.01 – 3.34 (m, 145H), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 33H), 1.99 (s, 3H), 1.15 (d, $J$ = 6.1 Hz, 3H$_{Thr}$), 0.95 (d, $J$ = 6.4 Hz, 6H$_{Val}$); MALDI-TOF MS: [M+H$^+$] C212H356N23O139, calcd 5446, found 5448; MALDI-TOF MS: [M-LysValAla+H$^+$] C198H330N19O136, calcd 5148, found 5150.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (64a)**

This compound was made by the same method used in the preparation of **56a**. Started from 1.6 mg **63b**, 85% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 8.6 Hz, 1H, H-1$_{Gn}$), 4.85 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$, overlapped with $H_2O$ peak), 4.73-4.63 (m, 9H-1$_{Gn}$, H-1$_{Asn}$), 4.63 – 4.55 (m, 3H-1$_{Gn}$), 4.52 (d, $J$ = 7.4 Hz, 1H, H-1$_{Gn}$), 4.50-4.42 (m, 12H-1$_{Gal}$), 4.24 (s, 1H, H-2$_{Man}$), 4.23-4.20 (m, 1H, H-2$_{Thr}$), 4.18 (s, 1H, H-2$_{Man}$), 4.17-4.11 (m, 9H-4$_{Gal}$), 4.07 (s, 1H, H-2$_{Man}$), 4.03 – 3.33 (m, 163H), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 33H), 1.99 (s, 3H), 1.15 (d, $J$ = 6.1 Hz, 3H$_{Thr}$), 0.95 (d, $J$ = 6.4 Hz, 6H$_{Val}$); MALDI-TOF MS: [M+H$^+$] C230H386N23O154, calcd 5932, found 5937; MALDI-TOF MS: [M-LysValAla+H$^+$] C216H360N19O151, calcd 5634, found 5639.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (64)**

Compound **64a** (0.5 mg, 0.08 μmol), CMP-Neu5Ac (0.4 mg, 6.0 equiv.), and rST3Gal-III (1 mU) were dissolved in 84 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 88 % yield); MALDI-TOF MS: permethylated [M-LysValAlaAsnLysThr+Na$^+$] C339H593N17O170Na, Calcd 7642, found 7649.4.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4GlcNAcβ-(KVA)NKT (128)**

Compound **64a** (0.5 mg, 0.08 μmol), CMP-Neu5Ac (0.4 mg, 6.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 84 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 88 % yield); MALDI-TOF MS: permethylated [M-LysValAlaAsnLysThr+Na$^+$] C339H593N17O170Na, Calcd 7642, found 7648.5.

**Galβ4GlcNAcβ2(Galβ4GlcNAcβ6)Manα6(Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (10a)**

This compound was made by the same method used in the preparation of **56a**. Started from 7.6 mg **65c**, 91% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 9.3 Hz, 1H, H-1$_{Gn}$), 4.86 (d, $J$ = 3.6 Hz, 1H, overlapped), 4.85 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$, overlapped), 4.68 (m, 1H, H-1$_{Asn}$), 4.66 (d, $J$ = 7.2 Hz, 1H, H-1$_{Gn}$), 4.61 – 4.54 (m, 2H, H-1$_{Gn}$), 4.53 (d, $J$ = 7.5 Hz, 1H, H-1$_{Gn}$), 4.48 – 4.42 (m, 3H, H-1$_{Gal}$), 4.38 (m, 1H, H-1$_{Lys}$), 4.28 (m, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.20 (m, 1H, H-2$_{Thr}$), 4.18 (s, 1H, H-2$_{Man}$), 4.15-4.08 (m, 2H, H-1$_{Thr}$, H-1$_{Val}$), 4.07 (s, 1H, H-2$_{Man}$), 4.03 – 3.33 (m, 68H), 3.04 – 2.89 (m, 4H, H-5$_{Lys}$), 2.83 (m, 1H, H-2$_{Asn}$), 2.73 (m, 1H, H-2$_{Asn}$), 2.07 (s, 3H), 2.06 (m, 1H), 2.03 (s, 3H), 2.02 (s, 6H), 1.99 (s, 3H), 1.36 (d, $J$ = 3.6 Hz, 3H-1$_{Ala}$), 1.18 (d, $J$ = 6.6 Hz, 3H$_{Fuc}$), 1.5 (d, $J$ = 6.6 Hz, 3H$_{Thr}$), 0.94 (d, $J$ = 6.9 Hz, 6H$_{Val}$); $^{13}$C NMR (151 MHz, $D_2O$): $\delta$ = 175.5, 175.4, 175.3, 175.2, 174.9, 173.8, 173.0, 172.7, 103.7, 103.6(6), 102.4, 101.8, 101.2, 100.4, 100.3, 100.2(6), 100.1, 98.0, 81.1, 80.1, 79.4, 79.2(4), 79.2(1), 79.0, 77.3, 77.2, 76.1, 76.0, 75.5, 75.4, 75.2, 74.3, 73.6, 73.5, 73.3, 73.0, 72.7, 72.6, 72.3, 71.7, 71.2, 71.0, 70.4, 70.3, 70.2, 69.3, 68.9, 68.7, 68.3, 68.1, 67.6, 67.3, 66.4, 66.0, 62.5, 61.8, 61.4, 60.9, 60.7, 60.6, 59.9, 55.9, 55.7, 54.8, 54.4, 52.1, 50.8, 50.3, 40.8, 40.0, 37.1, 34.5, 32.6, 31.3, 31.2(8), 30.9, 27.5, 27.1, 23.3, 23.2, 23.0, 22.9, 22.6, 20.1, 19.3, 18.6, 17.5, 16.2; MALDI-TOF MS: [M+H$^+$] C110H189N14O68, calcd 2793, found 2794.

**GlcNAcβ3Galβ4GlcNAcβ2(GlcNAcβ3Galβ4GlcNAcβ6)Manα6(GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (10b)**

This compound was made by the same method used in the preparation of **53b**. Started from 8.4 mg **10a**, 90% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 9.5 Hz, 1H, H-1$_{Gn}$), 4.86 (d, $J$ = 3.0 Hz, 1H, H-1$_{Fuc}$), 4.84 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.66 (d, $J$ = 8.0 Hz, 5H, 4H-1$_{Gn}$, H-1$_{Asn}$), 4.62 – 4.54 (m, 2H, H-1$_{Gn}$), 4.52 (d, $J$ = 7.9 Hz, 1H, H-1$_{Gn}$), 4.47-4.41 (m, 3H, H-1$_{Gal}$), 4.38 (m, 1H, H-1$_{Lys}$), 4.28 (m, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.20 (m, 1H, H-2$_{Thr}$), 4.18 (s, 1H, H-2$_{Man}$), 4.15 – 4.07 (m, 5H, 3 H-4$_{Gal}$, H-1$_{Thr}$, H-1$_{Val}$), 4.06 (s, 1H, H-2$_{Man}$), 4.02 – 3.28 (m, 85H), 3.03 – 2.88 (m, 4H, H-5$_{Lys}$), 2.83 (m, 1H, H-2$_{Asn}$), 2.75 (m, 1H, H-2$_{Asn}$), 2.07 (s, 3H), 2.03 (s, 3H), 2.02 (s, 15H), 1.99 (s, 3H), 1.35 (d, $J$ = 7.0 Hz, 3H$_{Ala}$), 1.18 (d, $J$ = 6.4 Hz, 3H$_{Fuc}$), 1.15 (d, $J$ = 6.3 Hz, 3H$_{Thr}$), 0.94 (d, $J$ = 6.7 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.7, 175.5, 175.3, 174.9, 173.8, 103.7, 103.7, 102.4, 101.8, 101.2, 100.4, 100.3, 100.1, 98.0, 82.8, 81.0, 80.1, 79.4, 79.2, 79.0, 77.4, 77.2, 76.4, 76.0, 75.7, 75.5, 75.2, 74.3, 73.6, 73.0, 72.7, 72.6, 72.3, 71.1, 71.0, 70.8, 70.4, 70.3, 70.2, 69.1, 68.9, 68.7, 68.3, 68.1, 67.6, 67.3, 66.4, 66.0, 62.5, 61.7, 61.4, 61.2, 60.9, 60.7, 59.9, 56.4, 55.8, 55.7, 55.6, 54.9, 54.4, 52.1, 50.8, 50.3, 40.0, 37.1, 34.6, 31.4, 30.9, 27.7, 27.3, 23.3, 23.2, 23.0, 22.7, 20.1, 19.3, 18.6, 17.5, 16.3; MALDI-TOF MS: [M+H$^+$] C134H229N17O83, calcd 3403, found 3403; MALDI-TOF MS: [M-Lys+H$^+$] C128H216N15O82, calcd 3274, found 3275; MALDI-TOF MS: [M-LysValAla+H$^+$] C120H203N13O80, calcd 3105, found 3105.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (65a)**

This compound was made by the same method used in the preparation of **56a**. Started from 9.2 mg **10b**, 90% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 9.3 Hz, 1H, H-1$_{Gn}$), 4.86 (d, $J$ = 3.0 Hz, 1H, H-1$_{Fuc}$), 4.85 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.71 – 4.64 (m, 5H, 4H-1$_{Gn}$, H-1$_{Asn}$), 4.61 – 4.54 (m, 2H, H-1$_{Gn}$), 4.52 (d, $J$ = 7.7 Hz, 1H, H-1$_{Gn}$), 4.49 – 4.41 (m, 6H), 4.38 (m, 1H, H-1$_{Lys}$), 4.28 (m, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.20 (m, 1H), 4.18 (s, 1H, H-2$_{Man}$), 4.16 – 4.07 (m, 5H), 4.06 (s, 1H, H-2$_{Man}$), 4.00 – 3.33 (m, 101H), 2.07 (s, 3H), 2.03 (s, 3H), 2.02 (s, 15H), 1.99 (s, 3H), 1.36 (d, $J$ = 6.8 Hz, 3H$_{Ala}$), 1.18 (d, $J$ = 6.2 Hz, 3H$_{Fuc}$), 1.15 (d, $J$ = 6.3 Hz, 3H$_{Thr}$), 0.94 (d, $J$ = 6.6 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.7, 175.5, 175.3, 174.9, 173.8, 103.8, 103.6, 103.5(6), 102.4, 101.8, 101.2, 100.4, 100.3, 100.1, 98.0, 82.9, 82.8, 81.0, 80.1, 79.4, 79.2, 78.9, 77.4, 77.2, 76.1, 76.0, 75.7, 75.5, 75.3, 75.2, 74.3, 73.6, 73.3, 73.0, 72.7, 72.6, 72.3, 71.7, 71.1, 71.0, 70.7, 70.3, 70.2, 69.3, 69.1, 68.9, 68.7, 68.3, 68.1, 67.6, 67.3, 66.4, 65.9, 62.5, 61.8, 61.7, 61.4, 60.9, 60.7, 60.6, 59.9, 56.0, 55.8, 55.7, 55.6, 54.9, 54.4, 52.1, 50.8, 50.3, 40.0, 37.1, 34.5, 32.7, 31.3, 30.9, 27.5, 27.1, 23.3, 23.2, 23.0, 22.6, 20.1, 19.3, 18.6, 17.5, 16.2; MALDI-TOF MS *m/z* calcd for

C152H259N17O98 [M+H$^+$] 3889, found 3891; MALDI-TOF MS *m/z* calcd for C138H232N13O95Na [M-Lys-Val-Ala+Na$^+$] 3613, found 3615.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (65)**

Compound **65a** (0.5 mg, 0.129 μmol), CMP-Neu5Ac (0.5 mg, 6.0 equiv.), and rST3Gal-III (2 mU) were dissolved in 129 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 81 % yield); MALDI-TOF MS: permethylated [M-LysValAlaAsnLysThr+Na$^+$] C227H397N11O114Na, calcd 5122, found 5124.9.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (129)**

Compound **65a** (0.5 mg, 0.129 μmol), CMP-Neu5Ac (0.5 mg, 6.0 equiv.), and hST6Gal-I (2 mU) were dissolved in 129 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 81 % yield); MALDI-TOF MS: permethylated [M-LysValAlaAsnLysThr+Na$^+$] C227H397N11O114Na, calcd 5122, found 5125.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (65b)**

This compound was made by the same method used in the preparation of **53b**. Started from 7.0 mg **65a**, 93% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 9.3 Hz, 1H, H-1$_{Gn}$), 4.86 (d, $J$ = 3.0 Hz, 1H, H-1$_{Fuc}$), 4.85 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.72-4.63 (m, 8H, 7 H-1$_{Gn}$, H-1$_{Asn}$), 4.62-4.54 (m, 2H, H-1$_{Gn}$), 4.52 (d, $J$ = 7.6 Hz, 1H, H-1$_{Gn}$), 4.50-4.41 (m, 6H, H-1$_{Gal}$), 4.41-4.36 (m, 1H, H-1$_{Lys}$), 4.31-4.26 (m, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.21-4.18 (m, 1H, H-2$_{Thr}$), 4.18 (s, 1H, H-2$_{Man}$), 4.16-4.05 (m, 8H, 6 H-4$_{Gal}$, H-1$_{Thr}$, H-1$_{Val}$), 4.06 (s, 1H, H-2$_{Man}$), 4.00 – 3.37 (m, 116H), 2.07 (s, 3H), 2.04 (s, 3H), 2.02 (s, 24H), 1.99 (s, 3H), 1.36 (d, $J$ = 6.8 Hz, 3H$_{Ala}$), 1.18 (d, $J$ = 6.3 Hz, 3H$_{Fuc}$), 1.15 (d, $J$ = 6.3 Hz, 3H$_{Thr}$), 0.95 (d, $J$ = 6.7 Hz, 6H$_{Val}$); $^{13}$C NMR (150 MHz, $D_2O$): $\delta$ = 175.7, 175.5, 173.8, 103.7, 103.6, 102.4, 101.8, 101.2, 100.4, 100.3, 100.1, 98.0, 82.9, 82.8, 81.0, 80.1, 79.4, 79.2, 78.9, 77.2, 76.4, 76.0, 75.7, 75.5, 75.3, 75.2, 74.3, 73.6, 73.0, 72.7, 72.6, 72.3, 71.0, 70.8, 70.4, 70.3, 70.2, 69.1, 68.9, 68.7, 68.3, 68.1, 67.6, 67.3, 66.4, 62.5, 61.7, 61.4, 61.2, 60.7, 60.6, 60.0, 56.4, 55.9, 55.8, 55.6, 54.8, 54.4, 52.1, 50.8, 50.3, 40.0, 37.1, 34.4, 31.3, 30.9, 27.4, 27.0, 23.3, 23.2, 23.0, 22.9, 22.6, 20.1, 19.3, 18.6, 17.5, 16.3; MALDI-TOF MS *m/z* calcd for C176H298N20O113 [M+H$^+$] 4498, found 4501; MALDI-TOF MS: [M-Lys+H$^+$] C170H286N18O112, calcd 4370, found 4373; MALDI-TOF MS: [M-Lys-Val-Ala+H$^+$] C162H272N16O110, calcd 4200, found 4203.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (66a)**

This compound was made by the same method used in the preparation of **56a**. Started from 7.4 mg **65b**, 96% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 9.0 Hz, 1H, H-1$_{Gn}$), 4.86 (s, 1H, H-1$_{Fuc}$), 4.85 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.73-4.64 (m, 8H, 7 H-1$_{Gn}$, H-1$_{Asn}$), 4.63-4.54 (m, 2H, H-1$_{Gn}$), 4.52 (d, $J$ = 7.1 Hz, 1H, H-1$_{Gn}$), 4.50-4.41 (m, 9H-1$_{Gal}$), 4.41-4.35 (m, 1H, H-1$_{Lys}$), 4.32 – 4.26 (m, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.21-4.18 (m, 1H, H-2$_{Thr}$), 4.17 (s, 1H, H-2$_{Man}$), 4.17-4.07 (m, 8H, 6 H-4$_{Gal}$, H-1$_{Thr}$, H-1$_{Val}$), 4.06 (s, 1H, H-2$_{Man}$), 4.02 – 3.44 (m, 134H), 2.07 (s, 3H), 2.03 (s, 3H), 2.01 (s, 24H), 1.99 (s, 3H), 1.35 (d, $J$ = 6.5 Hz, 3H$_{Ala}$), 1.18 (d, $J$ = 5.9 Hz, 3H$_{Fuc}$), 1.15 (d, $J$ = 6.2 Hz, 3H$_{Thr}$), 0.94 (d, $J$ = 6.5 Hz, 6H$_{Val}$); MALDI-TOF MS: [M+H$^+$] C194H327N20O128, calcd 4983, found 4987; MALDI-TOF MS: [M-Lys+H$^+$] C188H315N18O127, calcd 4855, found 4857; MALDI-TOF MS: [M-Lys-Thr+H$^+$] C184H308N17O125, calcd 4754, found 4757; MALDI-TOF MS: [M-Lys-Val-Ala+H$^+$] C180H301N16O125, calcd 4685, found 4689.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (66)**

Compound **66a** (0.5 mg, 0.1 μmol), CMP-Neu5Ac (0.4 mg, 6.0 equiv.), and rST3Gal-III (1 mU) were dissolved in 100 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 86 % yield); MALDI-TOF MS: permethylated [M-LysValAlaAsnLysThr+Na$^+$] C287H502N14O144Na, calcd 6469, found 6474.9.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (130)**

Compound **66a** (0.5 mg, 0.1 μmol), CMP-Neu5Ac (0.4 mg, 6.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 100 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 86 % yield); MALDI-TOF MS: permethylated [M-LysValAlaAsnLysThr+Na$^+$] C287H502N14O144Na, calcd 6469, found 6475.

**GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (66b)**

This compound was made by the same method used in the preparation of **53b**. Started from 4 mg **66a**, 93% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 8.8 Hz, 1H, H-1$_{Gn}$), 4.86 (s, 1H, H-1$_{Fuc}$), 4.85 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.73-4.64 (m, 11H, 10 H-1$_{Gn}$, H-1$_{Asn}$), 4.63-4.55 (m, 2H, H-1$_{Gn}$), 4.52 (d, $J$ = 6.8 Hz, 1H, H-1$_{Gn}$), 4.50-4.42 (m, 9H-1$_{Gal}$), 4.41-4.36(m, 1H, H-1$_{Lys}$), 4.33-4.25 (m, 1H, H-1$_{Ala}$), 4.23 (s, 1H, H-2$_{Man}$), 4.22-4.20 (m, 1H, H-2$_{Thr}$), 4.18 (s, 1H, H-2$_{Man}$), 4.17-4.08 (m, 11H, 9H-4$_{Gal}$, H-1$_{Thr}$, H-1$_{Val}$), 4.06 (s, 1H, H-2$_{Man}$), 4.00 – 3.36 (m, 149H), 2.07 (s, 3H), 2.03 (s, 3H), 2.02 (s, 33H), 1.99 (s, 3H), 1.36 (d, $J$ = 6.6 Hz, 3H$_{Ala}$), 1.18 (d, $J$ = 6.0 Hz, 3H$_{Fuc}$), 1.15 (d, $J$ = 6.2 Hz, 3H$_{Thr}$), 0.95 (d, $J$ = 6.5 Hz, 6H$_{Val}$); $^{13}$C NMR (151 MHz, $D_2O$): $\delta$ = 177.2, 175.7, 175.6(8), 173.8, 103.7, 103.6(6), 103.5(5), 102.4, 101.8, 101.2, 100.4, 100.3, 100.1, 98.0, 82.8, 82.7(7), 81.0, 80.1, 79.2, 78.9, 77.2, 76.4, 76.0, 75.7, 75.5, 75.3, 74.3, 73.6, 73.0, 72.7, 72.6, 70.8, 70.7, 70.4, 70.3, 70.2, 69.1, 68.9, 68.7, 68.1, 67.6, 67.3, 66.5, 62.5, 61.7, 61.4, 61.2, 60.6, 59.9, 56.4, 55.9, 55.6, 54.9, 54.4, 52.1, 50.8, 50.3, 40.0, 39.9(7), 37.1, 34.5, 32.7, 31.3, 30.9, 27.5, 27.1, 23.3, 23.2, 23.0, 22.7, 22.6, 20.8, 20.2, 20.1, 19.3, 18.6, 17.5, 16.2; MALDI-TOF MS: [M+H$^+$] C218H366N23O143, calcd 5592, found 5597.

**Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (67a)**

This compound was made by the same method used in the preparation of **56a**. Started from 4.2 mg **66b**, 97% yield; $^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.11 (s, 1H, H-1$_{Man}$), 5.03 (d, $J$ = 9.3 Hz, 1H, H-1$_{Gn}$), 4.86 (s, 1H, H-1$_{Fuc}$), 4.85 (s, 1H, H-1$_{Man}$), 4.75 (s, 1H, H-1$_{Man}$), 4.72-4.63 (m, 10H-1$_{Gn}$, H-1$_{Asn}$), 4.60-4.54 (m, 2H-1$_{Gn}$), 4.53 (d, $J$ = 6.6 Hz, 1H-1$_{Gn}$), 4.50-4.41 (m, 12H-1$_{Gal}$), 4.40 – 4.36 (m, 1H, H-1$_{Lys}$), 4.32 – 4.26 (m, 1H, H-1$_{Ala}$), 4.24 (s, 1H, H-2$_{Man}$), 4.22 – 4.19 (m, 1H, H-2$_{Thr}$), 4.18 (s, 1H, H-2$_{Man}$), 4.18 – 4.08 (m, 9H-4$_{Gal}$, H-1$_{Thr}$, H-1$_{Val}$), 4.06 (s, 1H, H-2$_{Man}$), 4.02 – 3.38 (m, 167H), 2.07 (s, 3H), 2.04 (s, 3H), 2.02 (s, 33H), 1.99 (s, 3H), 1.36 (d, $J$ = 7.2 Hz, 3H$_{Ala}$), 1.19 (d, $J$ = 6.4 Hz, 3H$_{Fuc}$), 1.15 (d, $J$ = 6.4 Hz, 3H$_{Thr}$), 0.95 (d, $J$ = 6.8 Hz, 6H$_{Val}$); MALDI-TOF MS: [M+H$^+$] C236H396N23O158, calcd 6078, found 6082.

**Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (67)**

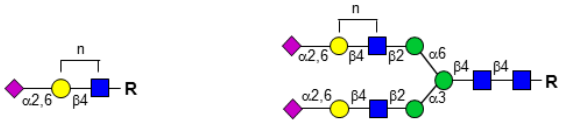
Compound **67a** (0.5 mg, 0.08 μmol), CMP-Neu5Ac (0.4 mg, 6.0 equiv.), and rST3Gal-III (1 mU) were dissolved in 82 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex$^{TM}$ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 88 % yield); MALDI-TOF MS: permethylated [M-LysValAlaAsnLysThr+Na$^+$] C347H607N17O174Na, calcd 7816, found 7822.3.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ6)Manα6(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3)Manβ4GlcNAcβ4(Fucα6)GlcNAcβ-(KVA)NKT (131)**

Compound **67a** (0.5 mg, 0.08 μmol), CMP-Neu5Ac (0.4 mg, 6.0 equiv.), and hST6Gal-I (1 mU) were dissolved in 82 μL buffer (50 mM Tris-HCl, pH 7.5, with 100 mU/mL of calf intestine alkaline phosphatase). After incubation overnight at 37°C, the insoluble precipitates were removed by centrifugation and the supernatant was concentrated and purified by Sephadex™ G-25 and lyophilized to give the final product as a white powder (0.5 mg, 88 % yield); MALDI-TOF MS: permethylated [M-LysValAlaAsnLysThr+$Na^+$] $C_{347}H_{607}N_{17}O_{174}Na$, calcd 7816, found 7821.8.

## 5.2 Synthesis of sialoside derivatives

### 5.2.1 Synthesis of biotinylated α2-6-sialosides used in experiments in Figure 5B.




| Derivatives | | Sialoside Compounds Number | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Linear | | | | Biantennary | | | |
| n = | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| R = | -LCLC-Biotin | **136** | **137** | **138** | **139** | **140** | **141** | **142** | **143** |
| | -PEG2000-DSPE | **144** | **145** | **146** | **NA** | **147** | **148** | **149** | **NA** |

Sialosides (1-10 mg) were mixed with 2 equiv. of EZ-Link™ NHS-LCLC-biotin (ThermoFisher Scientific, Cat #: 21343) and 2 equiv. of DIEA in 200 uL DMSO. The reactions were stirred at room temperature and monitored by TLC. After the starting materials were consumed, the reactions were loaded on Sephadex™ G-25 column. Products were re-purified by HPLC with C18-RP column and eluted with $CH_3CN$-$H_2O$-TFA. The final yield was between 85-98%.

**Neu5Acα6Galβ4GlcNAcβ-ethylamine-LCLC-biotin (136)**

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.61 (dd, $J$ = 7.9, 4.8 Hz, 1H), 4.55 (d, $J$ = 8.4 Hz, 1H), 4.44 (d, $J$ = 7.9 Hz, 1H), 4.42 (dd, $J$ = 7.9, 4.6 Hz, 1H), 4.04 (dd, $J$ = 10.3, 9.0 Hz, 1H), 3.97 (d, $J$ = 10.8 Hz, 1H), 3.94 – 3.79 (m, 8H), 3.77 – 3.59 (m, 9H), 3.57 – 3.50 (m, 2H), 3.37 (t, $J$ = 5.4 Hz, 2H), 3.35 – 3.30 (m, 1H), 3.22 – 3.13 (m, 4 H), 2.99 (dd, $J$ = 13.1, 5.1 Hz, 1H), 2.78 (d, $J$ = 13.1 Hz, 1H), 2.65 (dd, $J$ = 12.7, 4.6 Hz, 1H), 2.27 – 2.21 (m, 6H), 2.05 (s, 3H), 2.03 (s, 3H), 1.81 (t, $J$ = 12.3 Hz, 1H), 1.76-1.68 (m, 1H), 1.68 – 1.63 (m, 1H), 1.62-1.55 (m, 6H), 1.54 – 1.48 (m, 4H), 1.45-1.35 (m, 2H), 1.35 – 1.27 (m, 4H); MALDI-TOF MS: [M + $Na^+$] $C_{49}H_{83}N_7O_{23}SNa$, calcd 1193, found 1193.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ-ethylamine-LCLC-biotin (137)**

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.72 (d, $J$ = 7.9 Hz, 1H), 4.61 (dd, $J$ = 7.9, 5.0 Hz, 1H), 4.53 (d, $J$ = 8.1 Hz, 1H), 4.46 (dd, $J$ = 7.9 Hz, 2H), 4.42 (dd, $J$ = 8.0, 4.5 Hz, 1H), 4.15 (d, $J$ = 3.5 Hz, 1H), 4.02 (t, $J$ = 9.6 Hz, 1H), 4.01 – 3.50 (m, 31H), 3.39 – 3.29 (m, 3H), 3.21 – 3.12 (m, 4H), 2.99 (dd, $J$ = 13.1, 5.1 Hz, 1H), 2.78 (d, $J$ = 13.1 Hz, 1H), 2.65 (dd, $J$ = 12.7, 4.7 Hz, 1H), 2.30 – 2.18 (m, 6H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.82 (t, $J$ = 12.3 Hz, 1H), 1.76 – 1.69 (m, 1H), 1.68 – 1.55 (m, 7H), 1.54 – 1.45 (m, 4H), 1.44 – 1.36 (m, 2H), 1.35-1.27 (m, 4H); ); MALDI-TOF MS: [M + $Na^+$] $C_{63}H_{106}N_8O_{33}SNa$, calcd 1558, found 1558.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ-ethylamine-LCLC-biotin (138)**

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.72 (d, $J$ = 8.5 Hz, 1H), 4.70 (d, $J$ = 8.4 Hz, 1H), 4.61 (dd, $J$ = 7.9, 5.1 Hz, 1H), 4.52 (d, $J$ = 8.0 Hz, 1H), 4.49 – 4.44 (m, 3H), 4.42 (dd, $J$ = 8.0, 4.5 Hz, 1H), 4.15 (m, 2H), 4.04 (t, $J$ = 9.6 Hz, 1H), 4.01 – 3.49 (m, 42H), 3.39 – 3.28 (m, 3H), 3.21 – 3.13 (m, 4H), 2.99 (dd, $J$ = 13.1, 5.1 Hz, 1H), 2.78 (d, $J$ = 13.2 Hz, 1H), 2.65 (dd, $J$ = 12.9, 4.7 Hz, 1H), 2.30 – 2.18 (m, 6H), 2.03 (s, 9H), 2.02 (s, 3H), 1.82 (t, $J$ = 12.2 Hz, 1H), 1.75-1.68 (m, 1H), 1.68 – 1.54 (m, 7H), 1.5-1.47 (m, 4H), 1.44 – 1.36 (m, 2H), 1.35-1.27 (m, 4H); MALDI-TOF MS: [M + $H^+$] $C_{77}H_{130}N_9O_{43}S$, calcd 1901, found 1902.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ-ethylamine-LCLC-biotin (139)**

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 4.72 (d, *J* = 7.8 Hz, 1H), 4.70 (d, *J* = 8.4 Hz, 2H), 4.61 (dd, *J* = 7.9, 5.1Hz, 1H), 4.53 (d, *J* = 7.9 Hz, 1H), 4.49 – 4.44 (m, 4H), 4.42 (dd, *J* = 8.0, 4.5 Hz, 1H), 4.18-4.14 (m, 3H), 4.03 (t, *J* = 9.7 Hz, 1H), 4.01 – 3.50 (m, 55H), 3.41 – 3.30 (m, 3H), 3.22 – 3.12 (m, 4H), 2.99 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.78 (d, *J* = 12.9 Hz, 1H), 2.66 (dd, *J* = 12.5, 4.6 Hz, 1H), 2.28 – 2.20 (m, 6H), 2.05 (s, 3H), 2.03 (s, 9H), 2.02 (s, 3H), 1.79 (t, *J* = 12.2 Hz, 1H), 1.77 – 1.69 (m, 1H), 1.68-1.55 (m, 7H), 1.54-1.48 (m, 4H), 1.45 – 1.36 (m, 2H), 1.35-1.27 (m, 4H); MALDI-TOF MS: [M + $H^+$] C91H153N10O53S, calcd 2266, found 2267.

**Neu5Acα6Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-Asn-LCLC-biotin (140)**

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.13 (s, 1H), 5.05 (d, *J* = 9.7 Hz, 1H), 4.95 (s, 1H), 4.77 (s, 1 H), 4.74 (t, *J* = 6.0 Hz, 1H), 4.64 – 4.57 (m, 4H), 4.45 (dd, *J* = 7.9, 2.4 Hz, 2H), 4.42 (dd, *J* = 7.8, 4.4 Hz, 1H), 4.25 (s, 1H), 4.20 (d, *J* = 3.3 Hz, 1H), 4.13 – 4.10 (m, 1H), 4.06 – 4.00 (m, 1H), 4.00 – 3.47 (m, 64H), 3.35-3.31 (m, 1H), 3.21 – 3.12 (m, 4H), 2.99 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.84 (d, *J* = 6.1 Hz, 2H), 2.78 (d, *J* = 12.9 Hz, 1H), 2.68 – 2.61 (m, 2H), 2.29 (t, *J* = 7.3 Hz, 2H), 2.24 (dt, *J* = 8.9, 7.3 Hz, 4H), 2.08 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.03 (s, 6H), 2.00 (s, 3H), 1.81 (t, *J* = 12.2 Hz, 2H), 1.76 – 1.68 (m, 1H), 1.68 – 1.55 (m, 7H), 1.55 – 1.46 (m, 4H), 1.43 – 1.36 (m, 2H), 1.35-1.27 (m, 4H); MALDI-TOF MS: [M + $H^+$] C110H181N12O68S, calcd 2790, found 2791.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6) Manβ4GlcNAcβ4GlcNAcβ/α-Bifunctional linker-LCLC-biotin (141)**

$^1$H NMR (600 MHz, $D_2O$): δ = 5.20 (s, 0.54H, H-1α), 5.13 (s, 1H), 4.94 (s, 1H), 4.78-4.7 (m, 3.46H), 4.65-4.56 (m, 4H), 4.49-4.41 (m, 5H), 4.26 (s, 1H), 4.22 – 4.14 (m, 5H), 4.12 (s, 1H), 4.06 – 3.45 (m, 90H), 3.40 – 3.30 (m, 1H), 3.21-3.15 (m, 4H), 3.00 (s, 3H), 2.79 (d, *J* = 12.8 Hz, 1H), 2.68 (dd, *J* = 12.3, 4.4 Hz, 2H), 2.33 – 2.19 (m, 6H), 2.09 (s, 3H), 2.08-2.02 (m, 18H), 2.00 (s, 3H), 1.76 (t, *J* = 11.9 Hz, 2H), 1.76-1.69 (m, 1H), 1.70-1.56 (m, 7H), 1.56-1.48 (m, 4H), 1.45-1.37 (m, 2H), 1.37-1.27 (m, 4H); MALDI-TOF MS: [M + $H^+$] C137H229N14O86S, calcd 3478, found 3479.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-Asn-LCLC-biotin (142)**

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.12 (s, 1H), 5.05 (d, *J* = 9.7 Hz, 1H), 4.93 (s, 1H), 4.78-4.65 (m, 7H), 4.66 – 4.53 (m, 4H), 4.52-4.41 (m, 8H), 4.26 (s, 1H), 4.19 (s, 1H), 4.17 (s, 4H), 4.11 (s, 1H), 4.04 – 3.46 (m, 107H), 3.34 (dt, *J* = 9.4, 5.0 Hz, 1H), 3.18 (dt, *J* = 13.4, 6.4 Hz, 4H), 3.00 (dd, *J* = 12.9, 4.8 Hz, 1H), 2.86 – 2.76 (m, 3H), 2.68 (dd, *J* = 12.2, 4.6 Hz, 2H), 2.29 (t, *J* = 7.5 Hz, 2H), 2.27-2.22 (m, 4H), 2.08 (s, 3H), 2.06 (s, 9H), 2.05 (s, 3H), 2.04 (s, 12H), 2.01 (s, 3H), 1.73 (t, *J* = 12.2 Hz, 2H), 1.76-1.69 (m, 1H), 1.70 – 1.56 (m, 7H), 1.55-1.47 (m, 4H), 1.46-1.37 (m, 2H), 1.37-1.27 (m, 4H); MALDI-TOF MS: [M + $Na^+$] C166H272N16O108SNa, calcd 4273, found 4276.

**Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα3(Neu5Acα6Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ3Galβ4GlcNAcβ2Manα6)Manβ4GlcNAcβ4GlcNAcβ-Asn-LCLC-biotin (143)**

$^1$H NMR (600 MHz, $D_2O$): $\delta$ = 5.12 (s, 1H), 5.05 (d, *J* = 9.6 Hz, 1H), 4.93 (s, 1H), 4.76 – 4.65 (m, 8H), 4.65 – 4.53 (m, 4H), 4.51-4.41 (m, 9H), 4.25 (s, 1H), 4.19 (s, 1H), 4.16 (s, 6H), 4.11 (s, 1H), 4.06 – 3.42 (m, 131H), 3.36-3.31 (m, 1H), 3.21-3.14 (m, 4H), 3.00 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.84 (d, *J* = 5.4 Hz, 2H), 2.78 (d, *J* = 13.1 Hz, 1H), 2.67 (dd, *J* = 12.5, 4.7 Hz, 2H), 2.29 (t, *J* = 7.3 Hz, 2H), 2.28-2.21 (m, 4H), 2.08 (s, 3H), 2.07 – 2.01 (m, 30H), 2.00 (s, 3H), 1.75 (t, *J* = 12.2 Hz, 2H), 1.77-1.69 (m, 1H), 1.68-1.57 (m, 7H), 1.56-1.47 (m, 4H), 1.45-1.37 (m, 2H), 1.36-1.28 (m, 4H); MALDI-TOF MS: [M + $Na^+$] C194H318N18O128SNa, calcd 5003, found 5007.

**5.2.2 Synthesis of lipidated α2-6-sialosides in Figure 5C (144-149).**

Sialosides (1-10 mg) were mixed with 1.2 equiv. of NHS-PEG2000-DSPE (NOF America Corp., Cat # DSPE-020GS) and 2 equiv. of DIEA in 200 uL DMSO. The reactions were stirred at room temperature and monitored by TLC. After the starting materials were consumed, the reactions were loaded on Sephadex™ G-100 column and eluted with H2O. The final yield was between 80-90%.

## 6. NMR and MS Spectra

Available upon request.

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