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Authors

Kooner, Anoopjit Singh
Diaz, Sandra
Yu, Hai
[et al.](#)

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Chemoenzymatic Synthesis of Sialosides Containing 7-*N*- or 7,9-Di-*N*-Acetyl Sialic Acid as Stable *O*-Acetyl Analogues for Probing Sialic Acid-Binding Proteins

Anoopjit Singh Kooner^a, Sandra Diaz^{b,c}, Hai Yu^a, Abhishek Santra^{a,#}, Ajit Varki^{b,c}, Xi Chen^{a,*}

^aDepartment of Chemistry, University of California, One Shields Avenue, Davis, California 95616, United States

^bDepartment of Medicine, University of California, San Diego, California 92093, United States

^cDepartment of Cellular & Molecular Medicine, Glycobiology Research and Training Center, University of California, San Diego, California 92093, United States

Abstract

A novel chemoenzymatic synthon strategy has been developed to construct a comprehensive library of α 2–3- and α 2–6-linked sialosides containing 7-*N*- or 7,9-di-*N*-acetyl sialic acid, the stable analogue of naturally occurring 7-*O*-acetyl- or 7,9-di-*O*-acetyl-sialic acid. Diazido and triazido-mannose derivatives that were readily synthesized chemically from inexpensive galactose were shown to be effective chemoenzymatic synthons. Together with bacterial sialoside biosynthetic enzymes with remarkable substrate promiscuity, they were successfully used in one-pot multienzyme (OPME) sialylation systems for highly efficient synthesis of sialosides containing multiple azido groups. Conversion of the azido groups to *N*-acetyl groups generated the desired sialosides. The hydrophobic and UV-detectable benzyloxycarbonyl (Cbz) group introduced in the synthetic acceptors of sialyltransferases was used as a removable protecting group for the propylamine aglycon of the target sialosides. The resulting *N*-acetyl sialosides are novel stable probes for sialic acid-binding proteins such as plant lectin MAL II which bond strongly to sialyl T antigens with or without an *N*-acetyl at C7 or at both C7 and C9 in the sialic acid.

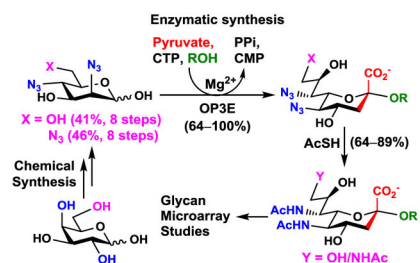
Graphical Abstract

*Corresponding Author: xiichen@ucdavis.edu.

#Current Address: Department of Organic Synthesis and Process Chemistry, CSIR-Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad 500007, India

Supporting Information.

Microarray data, detailed synthetic procedures, nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry (HRMS) data, and NMR spectra of the products. The Supporting Information is available free of charge on the ACS Publications website.



Keywords

biocatalysis; carbohydrate; chemoenzymatic synthesis; *N*-acetyl analogue; *O*-acetyl sialic acid

INTRODUCTION

Sialic acids (Sias) are nine-carbon acidic monosaccharides that are called nonulosonic acids. They are commonly found as the terminal residues of glycan moieties on cell surface glycoproteins and glycolipids of all vertebrates^{1,2} and as components of capsular polysaccharides and lipopolysaccharides of a number of pathogenic bacteria.^{3,4} Sia-terminated glycans and glycoconjugates are involved in various biological and pathogenic processes which are influenced by the natural diversities of Sia structures, sialyl linkages, and internal glycans.⁵ More than 50 structurally distinct forms of Sias have been found in nature.^{1,2,5} *N*-Acetylneuraminic acid (Neu5Ac, **1**, Figure 1) is the most common form of natural Sias. Modifications at one or more hydroxyl groups of Sias at C4, C5, C7, C8, and/or C9 are most commonly by *O*-acetyl group and less commonly by *O*-methyl, *O*-sulfate, or *O*-lactyl group.^{6,7} *O*-Acetylated sialoglycans have been identified as potential cancer markers for acute lymphoblastic leukemia and have been shown to be involved in the regulation of ganglioside-mediated apoptosis.^{8,9} Sialosides containing 7-*O*-acetyl Neu5Ac (Neu5,7Ac₂, **2**) have been found on human immune cells such as human lymphocytes¹⁰ and those containing 7,9-di-*O*-acetyl Neu5Ac (Neu5,7,9Ac₃, **3**) are preferentially recognized by bovine coronavirus.^{11–15}

Even though *O*-acetylated sialoglycans were discovered in as early as 1936,^{16,17} the progress of elucidating their functions has been delayed due to the instability of the *O*-acetyl moiety.^{18,19} *O*-Acetyl group at C7-OH of Sia is notoriously challenging to study. It was shown to spontaneously migrate to C9-OH group even under physiological pH.²⁰ A sialate *O*-acetyltransferase (EC 2.3.1.45) was believed to catalyze the transfer of *O*-acetyl group to C7-OH of the Neu5Ac in sialosides which is then migrated to C9-OH to form the sialosides containing 9-*O*-acetyl Neu5Ac (Neu5,9Ac₂) as the most abundant *O*-acetylated Sia.^{20,21} The *O*-acetyl group at C7 of Neu5,7,9Ac₃ (**3**) was also shown to migrate to C8-OH.²⁰

To overcome the *O*-acetyl instability problem, we showed previously that sialosides containing 9-*N*-acetyl Neu5Ac (Neu5Ac9NAc) are suitable mimics of Neu5,9Ac₂-sialosides.^{22–24} To explore the functions and applications of more challenging sialoside targets containing Neu5,7Ac₂ (**2**) or Neu5,7,9Ac₃ (**3**), we report here the design and synthesis of their structurally stable mimics where the labile *O*-acetyl groups are replaced by

N-acetyl groups. A novel chemoenzymatic synthon strategy was developed based on highly efficient one-pot three-enzyme (OP3E) sialylation systems containing sialoside biosynthetic enzymes with remarkable substrate promiscuities. The produced sialosides containing an *N*-acetyl sialic acid analogue were shown to be stable probes for glycan microarray-based studies for Sia-binding proteins including human Siglec 7 (hSiglec 7)²⁵ and human Siglec 9 (hSiglec 9)^{26,27} as well as plant lectins *Sambucus nigra* lectin (SNA) and *Maackia amurensis* lectin II (MAL II).

RESULTS AND DISCUSSION

Synthesis Design.

In nature, *N*-acetylmannosamine (ManNAc) is the six-carbon sugar precursor for Neu5Ac biosynthesis. For vertebrate cells, Neu5Ac is activated inside the nucleus to form CMP-Neu5Ac which is transported to the Golgi apparatus and used by sialyltransferases to form α -linked sialoglycoconjugates.²⁸ Our design for synthesizing α 2–3- and α 2–6-linked sialosides containing modified Sia is by chemical introduction of the modifications to its six-carbon precursor and using substrate promiscuous sialoside biosynthetic enzymes to convert modified precursors to Sia derivatives which are then activated and transferred to suitable acceptors to form the target sialosides.²³ Sialoside biosynthetic enzymes including a sialic acid aldolase, CMP-sialic acid synthetase, and a sialyltransferase can be used in one-pot for efficient sialylation.^{29,30} As shown in Figure 2, 7-*N*-acetyl Neu5Ac (Neu5Ac7NAc, **4**) was designed as the stable mimic of Neu5,7Ac₂ (**2**). It can be derived from the corresponding azido analogue Neu5,7diN₃ (**5**). Similarly, 7,9-di-*N*-acetyl Neu5Ac (Neu5Ac7,9diNAc, **6**) was proposed as the more stable mimic of Neu5,7,9Ac₃ (**3**) and the corresponding azido analogue is Neu5,7,9triN₃ (**7**). The corresponding six-carbon precursors for these sialic acid derivatives (**4–7**) of a sialic acid aldolase-catalyzed reaction are 2,4-diacetamido-2,4-dideoxy-D-mannose (Man2,4diNAc, **8**), 2,4-diazido-2,4-dideoxy-D-mannose (Man2,4diN₃, **9**), 2,4,6-triacetamido-2,4,6-trideoxy-D-mannose (Man2,4,6triNAc, **10**), and 2,4,6-triazido-2,4,6-trideoxy-D-mannose (Man2,4,6triN₃, **11**). These compounds (**8–11**) were chemically synthesized.

Chemical Synthesis of Mannose Derivatives.

Numerous chemical synthetic methods have been developed to access amino sugars, rare sugars, and sugar derivatives by selective protection and epimerization.^{31–34} However, some of them suffer from long multi-step synthetic routes along with low to moderate yields.³⁵ We envisioned that Man2,4diNAc (**8**) and Man2,4diN₃ (**9**) could be synthesized from commercially available inexpensive D-galactose (**12**) by introducing two azido groups simultaneously with inversion of the stereochemistry at both C2 and C4. As shown in Scheme 1, galactoside **14** was formed in an overall 77% yield over 3 steps by per-*O*-acetylation of D-galactose (**12**) followed by nucleophilic replacement of the anomeric acetate with *para*-methoxyphenyl (*p*MP) under acidic conditions using BF₃·Et₂O, and de-*O*-acetylation using sodium methoxide. Regio-selective benzylation of both C3 and C6-hydroxyl groups of **14** using tributyltin oxide (Bu₃Sn)₂O catalyst^{36,37} formed a partially protected D-galactosyl-2,4-diol intermediate (**15**) in 79% yield. Treatment of **15** with trifluoromethanesulfonic anhydride (Tf₂O) and pyridine followed by simultaneous

displacement (S_N2 reaction) of both triflate groups at C2 and C4 with azido groups using tetrabutylammonium azide (TBAN₃)³⁸ in dry toluene^{30,39} at 90 °C afforded diazido-D-mannose derivative **16** in 92% yield. The azido moieties of **16** were converted to acetamido moieties by reacting with thioacetic acid in pyridine under argon⁴⁰ to produce benzoyl protected glycoside **17** in 89% yield. Debenzoylation under Zemplén reaction conditions in the presence of sodium methoxide and methanol followed by ceric ammonium nitrate (CAN)-catalyzed removal of the *p*MP group⁴¹ produced desired Man2,4diNAc (**8**). On the other hand, removal of the benzoyl groups and the anomeric *p*MP group from **16** formed the desired Man2,4diN₃ (**9**). Altogether, Man2,4diNAc (**8**) and Man2,4diN₃ (**9**) were synthesized from D-galactose (**12**) in nine and eight steps with overall yields of 32% and 41%, respectively.

Man2,4,6triNAc (**10**) and Man2,4,6triN₃ (**11**) were synthesized from galactoside **14** (Scheme 2) using a similar strategy except that only 3-OH group of **14** was regio-selectively protected with a benzoyl group using dimethyltin chloride (Me₂SnCl₂) as a catalyst^{38,42} to produce partially protected triol **20** in 93% yield. Simultaneous triflation of all three hydroxyl groups in **20** with Tf₂O in pyridine followed by concurrent displacement of all three triflates with azido groups via an S_N2 -mechanism worked very well when TBAN₃ in dry toluene^{30,39} was used at 90 °C for 2 h in the second step. The formation of D-mannose derivative **21** was achieved in an overall 88% yield. Conversion of all three azido groups in **21** to acetamido moieties using thioacetic acid and pyridine⁴⁰ produced **22** in 80% yield. Debenzoylation of **22** under Zemplén reaction conditions followed by CAN-catalyzed removal of the *p*MP group⁴¹ produced Man2,4,6triNAc (**10**) in 70% yield. On the other hand, step-wise removal of the benzoyl protecting group (92% yield) and the *p*MP group (80% yield) from **21** produced Man2,4,6triN₃ (**11**). Altogether, Man2,4,6triNAc (**10**) and Man2,4,6triN₃ (**11**) were formed from **14** in six and five steps with overall yields of 46% and 60%, respectively.

Enzymatic Synthesis of Sialic Acid Derivatives.

Mannose derivatives Man2,4diNAc (**8**), Man2,4diN₃ (**9**), Man2,4,6triNAc (**10**), and Man2,4,6triN₃ (**11**) were tested as potential substrates for *Pasteurella multocida* sialic acid aldolase (PmAldolase).⁴³ Among these four compounds, Man2,4diNAc (**8**) and its di-azido derivative Man2,4diN₃ (**9**), as well as the tri-azido derivative Man2,4,6triN₃ (**11**) were suitable substrates for PmAldolase. The corresponding Sia derivatives Neu5Ac7NAc (**4**), Neu5,7diN₃ (**5**), and Neu5,7,9triN₃ (**7**) were synthesized in the presence of five equivalents of sodium pyruvate with 70–72% yields (Scheme 3). In contrast, Man2,4,6triNAc (**10**) was not a suitable substrate for PmAldolase, and the corresponding Neu5Ac7,9diNAc (**6**) could not be obtained directly from **10** by PmAldolase-catalyzed reaction.

One-Pot Multienzyme (OPME) Synthesis of Sialosides Containing An Azido-Modified Sialic Acid.

The Neu5Ac7NAc (**4**), Neu5,7diN₃ (**5**), and Neu5,7,9triN₃ (**7**) obtained from PmAldolase-catalyzed reaction were tested as potential substrates for *Neisseria meningitidis* CMP-sialic acid synthetase (NmCSS).⁴⁴ While both Neu5,7diN₃ (**5**) and Neu5,7,9triN₃ (**7**) were suitable substrates for NmCSS, Neu5Ac7NAc (**4**) was not.

Man_{2,4}diN₃ (**9**) and Man_{2,4,6}triN₃ (**11**) were also tested as chemoenzymatic synthons³⁰ in one-pot three-enzyme (OP3E) sialylation systems²⁹ (Table 1) for enzymatic synthesis of α 2–3- and α 2–6-linked sialosides containing Neu_{5,7}diN₃ or Neu_{5,7,9}triN₃. It was remarkable that all four enzymes including PmAldolase,⁴³ NmCSS,⁴⁴ *Pasteurella multocida* α 2–3-sialyltransferase 1 (PmST1),⁴⁵ and *Photobacterium damsela* α 2–6-sialyltransferase (Pd_{2,6}ST)⁴⁶ were able to tolerate the diazido- and triazido-derivatives of the corresponding substrates.

Lac β ProNHCBz (Type VI glycan, **24**),⁴⁷ LacNAc β ProNHCBz (Type II glycan, **25**), Gal β 3GalNAc β ProNHCBz (Type IV glycan, **26**), Gal β 3GalNAc α ProNHCBz (Type III or Core 1 glycan, **27**), Gal β 3GlcNAc β ProNHCBz (Type I glycan, **28**), Gal β 3GlcNAc α ProNHCBz (**29**), and GalNAc α ProNHCBz (Tn antigen, **30**) were synthesized and used as sialyltransferase acceptors for the construction of a comprehensive library of sialosides (Table 1). The hydrophobic and UV-detectable benzyloxycarbonyl (Cbz) group in **24–30** was used as a removable protecting group for the propylamine aglycon of the target sialosides.

In the OP3E sialylation systems (Table 1), Man_{2,4}diN₃ (**9**) or Man_{2,4,6}triN₃ (**11**) was coupled with pyruvate by a PmAldolase-catalyzed reaction to form Neu_{5,7}diN₃ (**5**) or Neu_{5,7,9}triN₃ (**7**). The resulting azido-Sia derivative was converted by an NmCSS-catalyzed reaction in the presence of cytidine 5'-triphosphate (CTP) and magnesium cation (Mg²⁺) to form the corresponding CMP-activated sugar nucleotide CMP-Neu_{5,7}diN₃ or CMP-Neu_{5,7,9}triN₃, which was used by sialyltransferases such as *Pasteurella multocida* α 2–3-sialyltransferase 1 (PmST1)⁴⁵ and *Photobacterium damsela* α 2–6-sialyltransferase (Pd_{2,6}ST),⁴⁶ respectively, to produce α 2–3- and α 2–6-linked sialosides (**31–56**) from sialyltransferase acceptors **24–30**.

Using PmST1⁴⁵ as the sialyltransferase, α 2–3-linked Neu_{5,7}diN₃-containing (**31**, **35**, **39**, **43**, **47**, and **51**) and Neu_{5,7,9}triN₃-containing glycosides (**32**, **36**, **40**, **44**, **48**, and **52**) were obtained in good to excellent (64–99%) yields with galactosides **24–30** as the sialyltransferase acceptors. Similarly, using Pd_{2,6}ST⁴⁶ as the sialyltransferase, α 2–6-linked Neu_{5,7}diN₃-containing (**33**, **37**, **41**, **45**, **49**, **53**, and **55**) and Neu_{5,7,9}triN₃-containing glycosides (**34**, **38**, **42**, **46**, **50**, **54**, and **56**) were obtained in good to excellent (65–100%) yields.

Chemical Conversion of The Azido Groups in Sialosides to *N*-Acetyl Groups and Removal of The Cbz-Protecting Group.

The azido-containing sialosides (**31–56**) obtained were readily converted to their *N*-acetyl analogues (**57–82**) in 64–89% yields using thioacetic acid and saturated sodium bicarbonate aqueous solution (Table 1), a condition optimized previously.³⁰ The Cbz group in the aglycone of the sialosides **57–82** was removed by hydrogenation using Pd/C catalyst in water-methanol solution for a few hours at room temperature to form sialosides containing a propylamine aglycone in quantitative yields for downstream conjugation reactions. Compared to the previous chemoenzymatic synthon strategy³⁰ of using sialyltransferase acceptors with a propyl chloride aglycone, the Cbz-protected propylamine aglycone has

several advantages. It facilitates reaction monitoring and product purification processes due to its hydrophobic UV-detectability. It also allows selective conversion of the azido group in the enzymatic products (**31–56**) to the desired *N*-acetyl group and permits highly efficient conversion (in quantitative yields) of the aglycon in the sialosides (**57–82**) to a primary propylamine for glycan microarray printing.

Sialosides Containing Neu5Ac7NAc or Neu5Ac7,9diNAc Can Bind to Sia-Binding Proteins as Shown by Sialoglycan Microarray Studies.

The propylamine aglycon in the sialosides obtained made it easy to print these glycans on an array format for protein-binding studies in a high-throughput format. Glycan microarray experiments were carried out using α 2–3- and α 2–6-linked sialosides containing Neu5Ac7NAc or Neu5Ac7,9diNAc synthesized here as well as related sialosides containing Neu5Ac, Neu5,9Ac₂, or Neu5Ac9NAc synthesized previously.^{22,23,45,46,48} The corresponding internal glycans (without the Sia component) were used as negative controls. As shown in Figure 3 and Table S1, the binding of hSiglec 7, hSiglec 9, and SNA to the sialosides containing Neu5Ac (grey bars) or Neu5Ac9NAc (orange bars) was blocked by substituting the C7-OH of sialic acid by a C7-NAc group (Neu5Ac7NAc red bars and Neu5Ac7,9diNAc blue bars, respectively) except for the weak binding retained for Neu5Ac7NAc α 3Gal β 3GalNAc β -glycan (red bars for Js in Figure 3). Interestingly, MAL II bond to sialyl T antigens Sia α 3Gal β 3GalNAc α -glycans (L in Figure 3) strongly⁴⁹ regardless of the Sia forms in the sialosides tested including those containing Neu5Ac (grey bar), Neu5,9Ac₂ (green bar), Neu5Ac9NAc (orange bar), Neu5Ac7NAc (red bar), and Neu5Ac7,9diNAc (blue bar). On the other hand, the binding of MAL II to Neu5Ac α 3Gal β 3GalNAc β -glycan (grey bar in J in Figure 3), a sialoglycan differing from the sialyl T antigen only on the glycosyl linkage remotely from the Sia, was not influenced significantly by Neu5Ac 9-*O*-acetylation (green bar) or 9-NAc substitution (orange bar) but was blocked completely by Neu5Ac 7-NAc (red bar) or 7,9-di-NAc substitution (blue bar).

CONCLUSIONS

In conclusion, a highly efficient chemoenzymatic synthon strategy has been successfully developed to construct a comprehensive library of sialosides containing *N*-acetyl analogues of 7-*O*- or 7,9,-di-*O*-acetylated Neu5Ac. Man₂,4diN₃ (**9**) and Man₂,4,6triN₃ (**11**) are effective chemoenzymatic synthons that are readily accessible via chemical synthesis from inexpensive galactose. We have shown that bacterial sialoside biosynthetic enzymes including PmAldolase, NmCSS, PmST1, and Pd2,6ST have remarkable substrate promiscuity. The OPME systems are highly efficient in using Man₂,4diN₃ and Man₂,4,6triN₃ as effective chemoenzymatic synthons to produce sialosides containing Neu5Ac7N₃ or Neu5Ac7,9diN₃ which are readily converted to the target NAc-containing sialosides by a facile chemical conversion process. The hydrophobic and UV-detectable Cbz group introduced in the synthetic acceptors of sialyltransferases is a convenient removable protecting group for the propylamine aglycon of the target sialosides. The application of the obtained sialosides in glycan microarray studies has demonstrated that these compounds could be valuable stable probes for Sia-binding proteins. The chemoenzymatic synthon

strategy has the potential to be used for the synthesis of other glycans containing *N*-acetyl groups.

EXPERIMENTAL SECTION

Materials and General Methods.

Chemicals were purchased and used without further purification. Nuclear magnetic resonance (NMR) spectra were recorded in the NMR facility of the University of California, Davis on a 600 MHz and 800 MHz Bruker Avance III-NMR spectrometers and a 400 MHz Bruker Avance III HD Nanobay spectrometer. Chemical shifts are reported in parts per million (ppm) on the δ scale. High resolution electrospray ionization (ESI) mass spectra were obtained using a Thermo Electron LTQ-Orbitrap Hybrid mass spectrometer or a Thermo Scientific Q Exactive HF Orbitrap Mass Spectrometer at the mass spectrometry facility in the University of California, Davis or using a LTQ-Orbitrap Elite mass spectrometer at the Georgia State University. Column chromatography was performed using columns manually packed with silica gel 60 Å (230–400 mesh, Sorbent Technologies) or a CombiFlash® Rf 200i system with either RediSep Rf silica columns or an ODS-SM (C18) column (51 g, 50 μ m, 120 Å, Yamazen). Thin layer chromatography (TLC) was performed on silica gel plates (Sorbent Technologies) using anisaldehyde sugar stain or 5% sulfuric acid in ethanol stain for detection. Gel filtration chromatography was performed with a column (100 cm \times 2.5 cm) packed with Bio-Gel P-2 Fine resins (Bio-Rad). *Pasteurella multocida* sialic acid aldolase (PmAldolase),⁴³ *Neisseria meningitidis* CMP-sialic acid synthetase (NmCSS),⁴⁴ *Pasteurella multocida* α 2–3-sialyltransferase 1 (PmST1),⁴⁵ and *Photobacterium damsela* α 2–6-sialyltransferase (Pd2,6ST)⁴⁶ were expressed and purified as described previously.

Chemical Synthesis of 2,4-Diacetamido-2,4-dideoxy-D-mannose (Man2,4diNAc, 8) and 2,4-Diazido-2,4-dideoxy-D-mannose (Man2,4diN₃, 9).

***p*-Methoxyphenyl- β -D-galactopyranoside (14).**—D-Galactopyranose (**12**, 7.00 g, 38.8 mmol) and sodium acetate (3.7 g, 0.045 mole) were dissolved in acetic anhydride (50 mL). The reaction mixture was heated at 120 °C for 1 h and neutralized using sodium bicarbonate. The compound was extracted by washing with dichloromethane. The dichloromethane solution was washed with brine. The organic layer was dried on sodium sulfate and the solvent was removed under a reduced pressure to produce 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose (**13**) which was used in the next step without further purification. Compound **13** (21.67 g, 0.055 mmol) and *para*-methoxyphenol (10.33 g, 0.083 mmol) were added to a round bottom flask (500 mL) and dissolved in dichloromethane (50 mL). The reaction mixture was cooled in an ice-water bath. Boron trifluoride diethyl etherate (11.86 g, 0.0832 mmol) was added slowly into the reaction mixture over a period of 5 minutes.³⁰ The reaction was stirred for 6 h and quenched by adding MeOH (50 mL). The solvent was removed under a reduced pressure and the residue was subjected to silica gel column chromatography (hexane:ethyl acetate = 3:1, by volume) to obtain *p*-methoxyphenyl-2,3,4,6-tetra-*O*-acetyl- β -D-galactopyranoside in 81% yield. The column-purified intermediate was dissolved in dry MeOH (100 mL), and 0.2 mL of sodium methoxide solution (5.4 M) was added. After the reaction was completed, the reaction mixture was neutralized by adding

H⁺-resin. The product was purified by silica gel column chromatography (ethyl acetate: methanol = 20:1, by volume) to produce *p*-methoxyphenyl-β-D-galactopyranoside (**14**) in 95% yield. Compound **14** (8.56 g) was obtained as a white amorphous solid in an overall 77% yield from D-galactose in 3 steps. ¹H NMR (400 MHz, CD₃OD) δ 7.10–7.05 (m, 2H), 6.87–6.82 (m, 2H), 4.74 (d, *J* = 7.7 Hz, 1H), 3.91 (dd, *J* = 3.4, 1.1 Hz, 1H), 3.78 (s, 3H), 3.65 (ddd, *J* = 6.7, 5.3, 1.1 Hz, 1H), 3.57 (dd, *J* = 9.7, 3.4 Hz, 1H), 3.37 (s, 1H), 3.33 (p, *J* = 1.7 Hz, 2H). ¹³C NMR (100 MHz, CD₃OD) δ 156.3, 152.3, 119.3, 115.4, 108.7, 84.6, 83.5, 78.2, 72.2, 64.4, 56.0. HRMS (ESI-Orbitrap) *m/z*: [M + Cl]⁻ Calcd for C₁₃H₁₈ClO₇ 321.0747; found 321.0760.

p-Methoxyphenyl-3,6-dibenzoyl-β-D-galactopyranoside (15).—Compound **14** (0.60 g, 2.10 mmol) and bis(*tri-n*-butyltin) oxide (1.78 g, 3.15 mmol) were dissolved in toluene (70 mL) in a round bottom flask (500 mL). The reaction mixture was heated to 140 °C in an oil bath for 15 minutes. Reaction mixture was cooled down to 0 °C in an ice-water bath, and benzoyl chloride (0.88 g, 6.30 mmol) was added. The reaction was stirred for 6 h at 0 °C and quenched by adding methanol (50 mL). The solvent was removed under a reduced pressure and the residue was purified by silica gel column chromatography (toluene:ethyl acetate = 7:1, by volume) to produce **15** as a white amorphous solid (0.82 g, 79% yield).^{36–38} ¹H NMR (400 MHz, CD₃OD) δ 8.22–8.09 (m, 2H), 8.08–7.99 (m, 2H), 7.64 (dt, *J* = 9.7, 7.5 Hz, 2H), 7.50 (td, *J* = 7.6, 5.3 Hz, 4H), 7.09–6.97 (m, 2H), 6.73–6.62 (m, 2H), 5.15 (dd, *J* = 10.1, 3.4 Hz, 1H), 4.94 (d, *J* = 7.8 Hz, 1H), 4.68 (dd, *J* = 11.4, 8.5 Hz, 1H), 4.59 (bs, 2H), 4.50 (dd, *J* = 11.5, 4.0 Hz, 1H), 4.30 (d, *J* = 3.4 Hz, 1H), 4.20 (td, *J* = 7.3, 2.6 Hz, 2H), 3.69 (s, 3H). ¹³C{¹H} NMR (100 MHz, CD₃OD) δ 167.74, 167.70, 156.6, 153.0, 134.43, 134.36, 131.4, 131.3, 130.9, 130.7, 130.7, 129.6, 129.5, 119.3, 115.3, 103.7, 77.6, 74.1, 69.9, 68.0, 65.1, 56.0.

p-Methoxyphenyl-2,4-diazido-3,6-dibenzoyl-2,4-dideoxy-β-D-mannopyranoside (16).—*p*-Methoxyphenyl-3,6-dibenzoyl-β-D-galactopyranoside (**15**, 0.40 g, 0.81 mmol) was dissolved in dichloromethane (25 mL) in a round bottom flask (100 mL) at 0 °C. Pyridine (0.65 mL, 8.09 mmol) was added followed by slow addition of trifluoromethanesulfonic anhydride (0.68 mL, 4.04 mmol) at 0 °C and the reaction was stirred for 30 min before it was quenched by adding sodium bicarbonate (10 mL). The organic layer was washed with hydrochloric acid (1 N) and brine. Organic layer was combined and concentrated under reduced pressure to produce a crude product, which was used for the next step without any purification. To a solution of 2,4-bistriflate in toluene (20 mL), tetrabutylammonium azide (0.76 g, 2.67 mmol) was added at room temperature. The reaction was stirred at 70 °C for 1 h and then at 90 °C for another 1 h.³⁹ The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (toluene) to produce *p*-methoxyphenyl-2,4-di-azido-3,6-dibenzoyl-2,4-dideoxy-β-D-mannopyranoside (**16**) as a white amorphous solid (0.40 g, 92% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.15 (ddd, *J* = 21.4, 8.4, 1.4 Hz, 4H), 7.71–7.60 (m, 2H), 7.52 (dt, *J* = 11.6, 7.8 Hz, 4H), 7.03 (d, *J* = 9.0 Hz, 2H), 6.75 (d, *J* = 9.1 Hz, 2H), 5.36–5.27 (m, 1H), 5.24 (d, *J* = 1.2 Hz, 1H), 4.81 (dd, *J* = 11.9, 2.4 Hz, 1H), 4.61 (dd, *J* = 11.9, 6.6 Hz, 1H), 4.57 (dd, *J* = 3.7, 1.1 Hz, 1H), 4.13 (t, *J* = 10.1 Hz, 1H), 3.76 (s, 4H). ¹³C{¹H} NMR (100 MHz, CDCl₃) δ 166.1, 165.6, 155.8, 150.4, 134.1, 133.4, 130.1, 129.9, 129.7, 129.1, 128.8, 128.5, 118.5, 114.6, 98.9, 74.1, 73.0, 63.7,

61.6, 57.4, 55.6. HRMS (ESI-Orbitrap) m/z : $[M + Na]^+$ Calcd for $C_{27}H_{24}N_6NaO_7$ 567.1599; found 567.1581.

***p*-Methoxyphenyl-2,4-diacetamido-3,6-dibenzoyl-2,4-dideoxy- β -D-mannopyranoside (17).**—*p*-Methoxyphenyl-2,4-diazido-3,6-dibenzoyl-2,4-dideoxy- β -D-mannopyranoside (**16**, 0.22 g, 0.41 mmol)

was dissolved in pyridine (4.0 mL) in a round bottom flask (50 mL), and thioacetic acid (1.0 mL) was added. The reaction mixture was stirred for 24 h under a reduced pressure at room temperature before the reaction was quenched by adding methanol (10 mL). The solvent was removed under a reduced pressure⁴⁰ and the residue was purified by silica gel column chromatography (toluene: ethyl acetate = 2:1, by volume) to produce *p*-methoxyphenyl-2,4-di-acetamido-3,6-dibenzoyl-2,4-dideoxy- β -D-mannopyranoside (**17**) as a white amorphous solid (0.21 g, 89% yield). ¹H NMR (800 MHz, CDCl₃) δ 8.00 (dd, J = 8.2, 1.5 Hz, 2H), 7.95 (dd, J = 8.1, 1.5 Hz, 2H), 7.62–7.56 (m, 1H), 7.55–7.49 (m, 1H), 7.43 (t, J = 7.8 Hz, 2H), 7.36 (t, J = 7.8 Hz, 2H), 6.92–6.87 (m, 2H), 6.89–6.85 (m, 1H), 6.62–6.52 (m, 3H), 5.43 (dd, J = 10.7, 4.0 Hz, 1H), 5.19 (d, J = 1.8 Hz, 1H), 5.10 (ddd, J = 9.3, 4.0, 1.6 Hz, 1H), 4.66 (dd, J = 11.9, 2.6 Hz, 1H), 4.55 (dd, J = 11.9, 8.6 Hz, 1H), 4.51–4.44 (m, 1H), 4.01–3.95 (m, 1H), 3.65 (s, 3H), 2.06 (s, 3H), 1.89 (s, 3H). ¹³C{¹H} NMR (200 MHz, CDCl₃) δ 171.3, 171.1, 166.8, 166.3, 155.3, 150.6, 133.5, 133.3, 129.9, 129.8, 129.8, 129.3, 128.6, 128.5, 118.2, 114.4, 98.0, 74.4, 72.0, 64.4, 55.5, 50.6, 47.6, 23.5, 23.2. HRMS (ESI-Orbitrap) m/z : $[M + H]^+$ Calcd for $C_{31}H_{33}N_2O_9$ 577.2181; found 577.2167.

***p*-Methoxyphenyl-2,4-diacetamido-2,4-dideoxy- β -D-mannopyranoside (18).**—*p*-

Methoxyphenyl-2,4-diacetamido-3,6-dibenzoyl-2,4-dideoxy- β -D-mannopyranoside (**17**, 0.51 g, 0.88 mmol) was dissolved in dry MeOH (10 mL) in a round bottom flask (50 mL), and 0.2 mL of sodium methoxide solution (5.4 M) was added. After the reaction was completed, the reaction mixture was neutralized using H⁺-resin. The solvent was removed under a reduced pressure and the residue was purified by silica gel column chromatography (ethyl acetate: methanol = 9:1, by volume) to produce *p*-methoxyphenyl-2,4-diacetamido-2,4-dideoxy- β -D-mannopyranoside (**18**) as a white amorphous solid (0.26 g, 80% yield). ¹H NMR (800 MHz, CD₃OD) δ 7.01–6.94 (m, 2H), 6.86–6.76 (m, 2H), 5.12 (d, J = 1.6 Hz, 1H), 4.70 (dd, J = 3.8, 1.6 Hz, 1H), 3.93–3.86 (m, 2H), 3.74 (s, 3H), 3.72–3.66 (m, 2H), 3.40 (ddd, J = 9.7, 4.0, 2.4 Hz, 1H), 2.12 (s, 3H), 2.02 (s, 3H). ¹³C{¹H} NMR (200 MHz, MeOD) δ 175.0, 174.6, 156.7, 152.4, 119.1, 115.5, 99.6, 77.5, 71.7, 62.4, 56.0, 54.5, 49.7, 22.77, 22.75. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{17}H_{23}N_2O_7$ 367.1511; found 367.1507.

2,4-Diacetamido-2,4-dideoxy-D-mannose (Man2,4diNAc, 8).—*p*-

Methoxyphenyl-2,4-di-acetamido-2,4-dideoxy- β -D-mannopyranoside (**18**, 0.125 g, 0.34 mmol) was added to a round bottom flask (50 mL) and dissolved in acetonitrile (6 mL). Ammonium cerium nitrate (0.6 g) was dissolved in water (1.5 mL) and was added slowly while stirring. The reaction was carried out at room temperature for 4.5 h before the reaction mixture was concentrated. The residue was purified by silica gel column chromatography (ethyl acetate: methanol: water = 9:1:0.5, by volume) to produce Man2,4diNAc (**8**) as a white amorphous solid (0.071 g, 80% yield). ¹H NMR (800 MHz, D₂O) δ 5.14 (bs, 0.6H),

4.97 (bs, 0.4H), 4.45 (d, $J = 4.8$ Hz, 0.4H), 4.30 (d, $J = 4.8$ Hz, 0.6H), 4.10 (dd, $J = 10.8, 4.0$ Hz, 0.6H), 3.76–3.99 (m, 2H), 3.52–3.74 (m, 2H), 3.39–3.47 (m, 0.4H), 1.97–2.31 (m, 6H). $^{13}\text{C}\{^1\text{H}\}$ NMR (200 MHz, D_2O) $\delta = 175.7, 174.8, 174.7, 174.6, 92.9, 92.8, 75.3, 70.8, 69.9, 66.6, 60.6, 60.5, 53.4, 52.4, 48.1, 47.8, 21.9, 21.8$. HRMS (ESI-Orbitrap) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_6\text{Na}$ 285.1057; found 285.1058.

***p*-Methoxyphenyl-2,4-di-azido-2,4-dideoxy- β -D-mannopyranoside (19).**—*p*-Methoxyphenyl-2,4-diazido-3,6-dibenzoyl-2,4-dideoxy- β -D-mannopyranoside (**16**) (0.50 g, 0.92 mmol) was dissolved in dry MeOH (5 mL) in a round bottom flask (50 mL), and 0.2 mL of sodium methoxide solution (5.4 M) was added. After the reaction was completed, the reaction mixture was neutralized using H^+ -resin.⁴¹ The product was purified by silica gel column chromatography (toluene: ethyl acetate = 7:1, by volume) to produce *p*-methoxyphenyl-2,4-diazido-2,4-dideoxy- β -D-mannopyranoside (**19**) as a white amorphous solid (0.28 g, 92% yield). ^1H NMR (800 MHz, CDCl_3) δ 6.95 (d, $J = 9.0$ Hz, 2H), 6.83 (d, $J = 9.0$ Hz, 2H), 5.13–5.09 (m, 1H), 4.21–4.15 (m, 1H), 3.93 (ddd, $J = 12.4, 5.9, 2.4$ Hz, 1H), 3.79 (d, $J = 3.6$ Hz, 1H), 3.77 (s, 3H), 3.64 (t, $J = 9.9$ Hz, 1H), 3.24 (ddd, $J = 10.1, 4.7, 2.4$ Hz, 1H), 2.61 (d, $J = 9.2$ Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (200 MHz, CDCl_3) δ 155.6, 150.1, 117.6, 114.7, 98.8, 75.1, 72.4, 63.9, 62.0, 59.4, 55.7. HRMS (ESI-Orbitrap) m/z : $[\text{M} + \text{Cl}]^-$ Calcd for $\text{C}_{13}\text{H}_{16}\text{ClN}_6\text{O}_5$ 371.0876; found 371.0864.

2,4-Diazido-2,4-dideoxy-D-mannose (Man2,4diN₃, 9).—*p*-Methoxyphenyl-2,4-diazido-2,4-dideoxy- β -D-mannopyranoside (**19**, 0.28 g 0.84 mmol) was added to a round bottom flask (50 mL) and dissolved in acetonitrile (6 mL). Ammonium cerium nitrate (1.4 g) dissolved in water (1.5 mL) was added slowly while stirring. The reaction was carried out at room temperature⁴¹ for 2.5 h before the reaction mixture was concentrated. The residue was purified by silica gel column chromatography (toluene:ethyl acetate = 2:1, by volume) to produce Man2,4diN₃ (**9**) as a white amorphous solid (0.15 g, 80% yield). ^1H NMR (800 MHz, D_2O) δ 5.29 (s, 1H), 4.99 (s, 1H), 4.21 (dd, $J = 9.9, 3.8$ Hz, 1H), 4.07 (d, $J = 3.8$ Hz, 1H), 4.03–3.97 (m, 2H), 3.87 (dd, $J = 12.5, 2.2$ Hz, 1H), 3.83 (dd, $J = 12.4, 2.3$ Hz, 1H), 3.78 (dd, $J = 12.4, 4.8$ Hz, 1H), 3.74 (dd, $J = 12.7, 5.2$ Hz, 2H), 3.66 (t, $J = 10.1$ Hz, 1H), 3.54 (t, $J = 10.1$ Hz, 1H), 3.32 (ddd, $J = 10.3, 5.5, 2.2$ Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (200 MHz, D_2O) δ 93.0, 92.2, 74.7, 72.1, 70.7, 69.5, 65.6, 63.9, 61.0, 60.9, 59.1, 58.7. HRMS (ESI-Orbitrap) m/z : $[\text{M} + \text{Cl}]^-$ Calcd for $\text{C}_6\text{H}_{10}\text{ClN}_6\text{O}_4$ 265.0458; found 265.0439.

Chemical Synthesis of 2,4,6-Triacetamido-2,4,6-trideoxy-D-mannose (Man2,4,6triNAc, 10) and 2,4,6-Triazido-2,4,6-trideoxy-D-mannose (Man2,4,6triN₃, 11)

***p*-Methoxyphenyl-3-benzoyl- β -D-galactopyranoside (20).**—Compound **14** (6.14 g, 21.4 mmol) was dissolved in THF (100 mL) in a round bottom flask (500 mL). Diisopropylethylamine (DIPEA) (5.5 g, 42.9 mmol) and a catalytic amount of dimethyltin dichloride (200 mg) were added to the reaction mixture.³⁸ Reaction mixture was cooled down to 0 °C in an ice-water bath and benzoyl chloride (3.32 g, 23.6 mmol) was added drop-wisely. The reaction mixture was stirred for 2 h at 0 °C and quenched by adding methanol (100 mL). The solvent was removed under a reduced pressure and the residue was purified by silica gel column chromatography (toluene: ethyl acetate = 3:1, by volume) to produce **20** as a white amorphous solid (7.77 g, 93% yield). ^1H NMR (400 MHz, CD_3OD)

δ 8.20–8.13 (m, 2H), 7.69–7.60 (m, 1H), 7.51 (dd, J = 8.4, 7.1 Hz, 2H), 7.15–7.06 (m, 2H), 6.91–6.80 (m, 2H), 5.10 (dd, J = 10.1, 3.3 Hz, 1H), 4.93 (d, J = 7.7 Hz, 1H), 4.25 (d, J = 3.3 Hz, 1H), 4.19 (dd, J = 10.2, 7.7 Hz, 1H), 3.85–3.79 (m, 3H), 3.77 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (200 MHz, CD_3OD) δ 167.7, 156.6, 153.1, 134.3, 130.8, 129.4, 119.3, 115.4, 104.0, 77.8, 76.6, 69.9, 67.9, 62.1, 56.0. HRMS (ESI-Orbitrap) m/z : $[\text{M} + \text{Cl}]^-$ Calcd for $\text{C}_{20}\text{H}_{22}\text{ClO}_8$ 425.1009; found 425.1027.

***p*-Methoxyphenyl-2,4,6-tri-azido-3-benzoyl-2,4,6-trideoxy- β -D-**

mannopyranoside (21).—*p*-Methoxyphenyl-3-benzoyl- β -D-galactopyranoside

(**20**, 0.15 g, 0.38 mmol) was dissolved in dichloromethane (10 mL) at 0 °C in a round bottom flask (50 mL). Pyridine (0.31 mL, 3.84 mmol) was added followed by slow addition of trifluoromethanesulfonic anhydride (0.32 mL 1.92 mmol) at 0 °C. After stirring at the same temperature for 30 min, the reaction was quenched by adding sodium bicarbonate (10 mL). The organic layer was washed with hydrochloric acid (1 N) and brine. Organic layer was combined and concentrated under reduced pressure to produce a crude product, which was used for the next step without any purification. To a solution of 2,4,6-trisulfate in toluene (20 mL), tetrabutylammonium azide (0.6 g, 2.1 mmol) was added at room temperature to a round bottom flask (50 mL). The reaction was stirred at 70 °C for 1 h and then at 90 °C for another 1 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (toluene) to produce *p*-methoxyphenyl-2,4,6-tri-azido-3-benzoyl-2,4,6-trideoxy- β -D-mannopyranoside (**21**) as a colorless oil (0.15 g, 88% yield). ^1H NMR (400 MHz, CD_3Cl) δ 8.15 (d, J = 8 Hz, 2H), 7.67 (t, J = 7.6 Hz, 1H), 7.54 (t, J = 8 Hz, 2H), 7.05 (d, J = 8.8 Hz, 2H), 6.91–6.82 (m, 2H), 5.22 (s, 1H), 4.52 (d, J = 3.6 Hz, 1H), 4.08 (t, J = 10 Hz, 1H), 3.80 (s, 3H), 3.69–3.56 (m, 2H), 3.51–3.43 (m, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CDCl_3) δ = 165.5, 150.2, 155.9, 134.0, 130.0, 128.7, 128.4, 118.7, 114.6, 98.8, 74.1, 73.8, 61.5, 57.4, 55.6, 51.5. HRMS (ESI-Orbitrap) m/z : $[\text{M} + \text{Cl}]^-$ Calcd for $\text{C}_{20}\text{H}_{19}\text{ClN}_9\text{O}_5$ 500.1203; found 500.1190.

***p*-Methoxyphenyl-2,4,6-triacetamido-3-benzoyl-2,4,6-trideoxy- β -D-**

mannopyranoside (22).—*p*-Methoxyphenyl-2,4,6-tri-azido-3-benzoyl-2,4,6-trideoxy-

β -D-mannopyranoside (**21**, 0.18 g, 0.38 mmol) was dissolved in pyridine (4.0 mL) in a round bottom flask (50 mL) and thioacetic acid (1.0 mL) was added drop-wisely. The reaction mixture was stirred for 24 h under reduced pressure at room temperature. The reaction was quenched by adding methanol (10 mL) and the solvent was removed under a reduced pressure. The compound was purified by silica gel column chromatography (ethyl acetate: methanol = 20:1, by volume) to produce *p*-methoxyphenyl-2,4,6-tri-acetamido-3-benzoyl-2,4,6-trideoxy- β -D-mannopyranoside (**22**) as a white amorphous solid (0.16 g, 80% yield). ^1H NMR (400 MHz, CD_3OD) δ 7.98–7.92 (m, 2H), 7.59 (t, J = 7.5 Hz, 1H), 7.45 (t, J = 7.7 Hz, 2H), 7.01–6.93 (m, 2H), 6.89–6.80 (m, 2H), 5.28 (d, J = 1.6 Hz, 1H), 5.25 (dd, J = 11.0, 4.2 Hz, 1H), 5.00 (dd, J = 4.2, 1.5 Hz, 1H), 4.34 (t, J = 10.7 Hz, 1H), 3.75 (s, 3H), 3.74–3.68 (m, 1H), 3.61–3.50 (m, 1H), 3.44 (dd, J = 14.2, 2.7 Hz, 1H), 2.06 (s, 3H), 1.96 (s, 3H), 1.88 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (200 MHz, CD_3OD) δ 174.1, 173.7, 173.6, 167.1, 156.9, 152.1, 134.4, 131.1, 130.7, 129.5, 119.3, 115.5, 99.1, 75.0, 73.8, 56.1, 51.9, 48.4, 41.5, 22.7, 22.6, 22.5. HRMS (ESI-Orbitrap) m/z : $[\text{M} + \text{Na}]^+$ Calcd for $\text{C}_{26}\text{H}_{31}\text{N}_3\text{O}_8\text{Na}$ 536.2003; found 536.1995.

2,4,6-Triacetamido-2,4,6-trideoxy-D-mannose (Man2,4,6triNAc, 10).—*p*-Methoxyphenyl-2,4,6-tri-acetamido-3-benzoyl-2,4,6-trideoxy- β -D-mannopyranoside (**22**, 0.1 g, 0.19 mmol) was dissolved in dry MeOH (10 mL) in a round bottom flask (50 mL), and 0.3 mL of sodium methoxide solution (5.4 M) was added. After the reaction was completed, the reaction mixture was neutralized using H⁺-resin. The crude product without further purification was added to a round bottom flask (50 mL) and dissolved in acetonitrile (4 mL). Ammonium cerium nitrate (0.3 g) dissolved in water (1 mL) was added slowly while stirring. The reaction was run for 2.5 h at room temperature. The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (ethyl acetate: methanol: water = 5:1:0.1, by volume) to produce 2,4,6-triacetamido-2,4,6-trideoxy-D-mannopyranose (**10**) as a white amorphous solid (0.04 g, 70% yield). ¹H NMR (800 MHz, D₂O) δ 5.16 (bs, 1H), 4.97 (bs, 1H), 4.48 (dt, J = 2.8, 1.4 Hz, 1H), 4.31 (dd, J = 4.6, 1.5 Hz, 1H), 4.09 (ddd, J = 10.6, 4.6, 1.3 Hz, 1H), 4.01–3.95 (m, 1H), 3.92 (t, J = 10.5 Hz, 1H), 3.88 (ddt, J = 10.7, 4.2, 0.9 Hz, 1H), 3.83 (t, J = 10.5 Hz, 1H), 3.56–3.52 (m, 1H), 3.45–3.38 (m, 3H), 3.36–3.33 (m, 1H), 2.13 (s, 3H), 2.08 (s, 3H), 2.02 (s, 6H), 2.00 (s, 6H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 175.8, 174.9, 174.5, 174.2, 93.0, 73.4, 70.0, 69.0, 66.7, 53.5, 52.6, 49.3, 48.9, 40.0, 39.9, 22.0, 21.9, 21.7. HRMS (ESI-Orbitrap) m/z : [M + Na]⁺ Calcd for C₁₂H₂₁N₃NaO₆ 326.1323; found 326.1308.

***p*-Methoxyphenyl-2,4,6-tri-azido-2,4,6-trideoxy- β -D-mannopyranoside (23).**—*p*-Methoxyphenyl-2,4,6-tri-azido-3-benzoyl-2,4,6-trideoxy- β -D-mannopyranoside (**21**, 0.50 g, 1.1 mmol) was dissolved in dry MeOH (10 mL) in a round bottom flask (50 mL), and 0.2 mL of sodium methoxide solution (5.4 M) was added. The reaction was carried out at room temperature for 6 h. After the reaction was completed, the reaction mixture was neutralized using H⁺-resin. The product was purified by silica gel column chromatography (toluene: ethyl acetate = 35:1, by volume) to produce compound **23** as a white amorphous solid (0.36 g, 92% yield). ¹H NMR (400 MHz, CD₃Cl) δ 6.99 (d, J = 8.8 Hz, 2H), 6.83 (d, J = 8.8 Hz, 2H), 5.06 (s, 1H), 4.19 (bd, J = 3.6 Hz, 1H), 3.75 (s, 1H), 3.75 (s, 3H), 3.60–3.45 (m, 3H), 3.31–3.22 (m, 1H), 2.59 (d, J = 9.2 Hz, 1H). ¹³C{¹H} NMR (100 MHz, CD₃Cl) δ 155.9, 150.1, 118.5, 114.7, 99.4, 74.2, 72.5, 63.9, 60.5, 55.7, 51.7. HRMS (ESI-Orbitrap) m/z : [M + Cl]⁻ Calcd for C₁₃H₁₅ClN₉O₄ 396.0941; found 396.0930.

2,4,6-Triazido-2,4,6-trideoxy-D-mannose (Man2,4,6triN₃, 11).—*p*-Methoxyphenyl-2,4,6-tri-azido-2,4,6-trideoxy- β -D-mannopyranoside (**23**, 0.72 g, 1.99 mmol) was added to a round bottom flask (50 mL) and dissolved in acetonitrile (12 mL). Ammonium cerium nitrate (1.1 g) dissolved in water (3 mL) was added slowly while stirring. The reaction was carried out at room temperature for 2.5 h. The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (toluene: ethyl acetate = 15:1, by volume) to produce compound **11** as a white amorphous solid (0.41 g, 80% yield). ¹H NMR (400 MHz, CD₃OD) δ 5.14–4.86 (m, 2H), 4.20–4.11 (m, 1H), 3.95–3.73 (m, 4H), 3.64–3.36 (m, 6H), 3.35–3.25 (m, 1H). ¹³C{¹H} NMR (100 MHz, CD₃OD) δ 93.7, 92.4, 73.6, 72.4, 69.8, 69.7, 66.2, 64.7, 60.4, 60.0, 51.7, 51.6. HRMS (ESI-Orbitrap) m/z : [M + Na]⁺ Calcd for C₆H₉N₉NaO₃ 278.0721; found 278.0707.

Enzymatic Synthesis of Sia Derivatives 7-Acetamido-7-deoxy-*N*-acetylneuraminic Acid (Neu5Ac7NAc, 4), 5,7-Diazido-5,7-dideoxy-neuraminic Acid (Neu5,7diN₃, 5), and 5,7,9-Triazido-5,7,9-trideoxy-neuraminic Acid (Neu5,7,9triN₃, 7)

7-Acetamido-7-deoxy-*N*-acetylneuraminic acid (Neu5Ac7NAc, 4).—The 2,4-diacetamido-2,4-dideoxy-*D*-mannose (Man2,4diNAc, **8**, 0.27 g, 1.03 mmol) and sodium pyruvate (0.57 g, 5.15 mmol) were dissolved in water in 50 mL centrifuge tube. After the addition of appropriate amount of PmAldolase, water was added to bring the final concentration of mannose derivative to 10 mM. The reaction was carried out by incubating the solution at 30 °C with agitation at 120 rpm in an incubator for 72 h. The product formation was observed by TLC developed with ethyl acetate: methanol: water = 7:1:0.5 (by volume) and stained with *p*-anisaldehyde sugar stain. The reaction was quenched by addition of methanol (15 mL) and mixture was then centrifuged. The supernatant was concentrated and passed through a BioGel P-2 gel filtration (water was used as an eluent). Then the product was further purified by silica gel chromatography (ethyl acetate: methanol: water = 7:1:0.5) to produce 5,7-di-*N*-acetyl-neuraminic acid (**4**) as a white amorphous solid (0.26 g, 72% yield). ¹H NMR (400 MHz, D₂O) δ 4.23 (dd, *J* = 10.5, 2.1 Hz, 1H), 4.00–3.90 (m, 2H), 3.81–3.68 (m, 2H), 3.63 (dd, *J* = 12.1, 2.7 Hz, 1H), 3.46 (dd, *J* = 12.1, 7.0 Hz, 1H), 2.22 (dd, *J* = 13.0, 4.9 Hz, 1H), 2.00 (s, 3H), 1.99 (s, 3H), 1.83 (d, *J* = 13.0 Hz, 1H). ¹³C{¹H} NMR (100 MHz, D₂O) δ 176.6, 174.1, 173.9, 96.5, 70.2, 69.4, 67.5, 62.5, 59.3, 54.7, 49.3, 39.6, 22.1, 21.6. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₁₃H₂₁N₂O₉ 349.1253; found 349.1275.

5,7-Diazido-5,7-dideoxy-neuraminic acid (Neu5,7diN₃, 5).—The 2,4-diazido-2,4-dideoxymannose (Man2,4diN₃, **9**, 0.05 g, 0.22 mmol) and sodium pyruvate (0.12 g, 1.1 mmol) were dissolved in water in 50 mL centrifuge tube. After the addition of appropriate amount of PmAldolase, water was added to bring the final concentration of mannose derivative to 10 mM. The reaction was carried out by incubating the solution at 30 °C with agitation at 120 rpm in an incubator for 48 h. The product formation was observed by TLC developed with ethyl acetate:methanol:water = 7:1:0.5 (by volume) and stained with *p*-anisaldehyde sugar stain. The reaction was quenched by addition of methanol (15 mL) and mixture was then centrifuged. The supernatant was concentrated and passed through a BioGel P-2 gel filtration (water was used as an eluent). Then the product was further purified by silica gel chromatography (ethyl acetate: methanol: water = 7:1:0.5) to produce 5,7-di-azido-5,7-dideoxy-neuraminic acid (**5**) as a white amorphous solid (0.05 g, 72% yield). ¹H NMR (600 MHz, D₂O) δ 4.11 (ddd, *J* = 11.6, 9.5, 5.0 Hz, 1H), 4.05 (dd, *J* = 10.4, 1.8 Hz, 1H), 3.98–3.91 (m, 1H), 3.82–3.74 (m, 2H), 3.70–3.65 (m, 1H), 3.60–3.56 (m, 1H), 2.21 (dd, *J* = 13.1, 5.0 Hz, 1H), 1.97 (d, *J* = 13.1 Hz, 1H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 175.7, 96.4, 72.1, 69.5, 68.5, 63.1, 62.5, 61.4, 39.1. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₉H₁₃N₆O₇ 317.0851; found 317.0830.

5,7,9-Triazido-5,7,9-trideoxy-neuraminic acid (Neu5,7,9triN₃, 7).—Man2,4,6triN₃ (**11**, 0.04 g, 0.16 mmol) and sodium pyruvate (0.086 g, 0.78 mmol) were dissolved in water in a 50 mL centrifuge tube. After the addition of appropriate amount of PmAldolase, water was added to bring the final concentration of mannose derivative to 10 mM. The reaction was carried out by incubating the solution at 30 °C with agitation at 120 rpm

in an incubator for 48 h. The product formation was observed by TLC developed with ethyl acetate:methanol:water = 7:1:0.5 (by volume) and stained with *p*-anisaldehyde sugar stain. The reaction was quenched by addition of methanol (15 mL) and mixture was then centrifuged. The supernatant was concentrated and passed through a Bio-Gel P-2 gel filtration (water was used as an eluent). Then the product was further purified by silica gel chromatography (ethyl acetate: methanol: water = 7:1:0.5) to produce **7** as a white amorphous solid (0.037 g, 70% yield). ¹H NMR (600 MHz, D₂O) δ 4.14–4.06 (m, 2H), 4.04 (dd, *J* = 10.4, 1.8 Hz, 1H), 3.79 (dd, *J* = 8.9, 1.8 Hz, 1H), 3.72 (dd, *J* = 13.4, 2.8 Hz, 1H), 3.60–3.53 (m, 2H), 2.20 (dd, *J* = 13.1, 5.0 Hz, 1H), 1.96 (dd, *J* = 13.2, 11.7 Hz, 1H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 175.6, 96.4, 69.5, 68.5, 68.4, 64.2, 62.1, 53.9, 39.1. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₉H₁₂N₉O₆ 342.0916; found 342.0943.

Chemical Synthesis of NHCbz-tagged Sialyltransferase Acceptors (24–30)

LacβProNHCbz (24)—LacβProNHCbz (**24**) was synthesized as described previously.⁴⁷ Compounds **25–30** were synthesized similarly. Briefly, in a round bottom flask, a propylazide-tagged acceptor²³ (0.085 mmol) was dissolved in water, a catalytic amount of palladium (10%) Pd/C was added. The mixture was stirred under a hydrogen atmosphere for overnight. After the completion of reaction, palladium was removed by filtration. The solvent was removed *in vacuo*. The obtained propylamine-tagged acceptor was used directly for next reaction without any further filtration. The compound (0.085 mmol) was dissolved in 5–10 mL water in a round bottom flask, sodium carbonate (45 mg, 0.43 mmol) was added. Benzyl chloroformate (CbzCl, 30 mg, 0.17 mmol) in acetonitrile was added to the mixture in the flask immersed in ice-water bath. After the completion of reaction, the solvent was removed. The mixture was purified using a C18 column (gradient solvent of CH₃CN in H₂O was used for elute) to produce the Cbz-tagged acceptors (**25–30**).

LacNAcβProNHCbz (25).—39 mg, 80% yield, white amorphous solid. ¹H NMR (400 MHz, D₂O) δ 7.43 (q, *J* = 6.2, 5.7 Hz, 5H), 5.11 (s, 2H), 4.47 (dd, *J* = 7.7, 1.9 Hz, 2H), 4.05–3.46 (m, 14H), 3.17 (d, *J* = 7.1 Hz, 2H), 2.00 (d, *J* = 11.0 Hz, 3H), 1.74 (t, *J* = 6.4 Hz, 2H). ¹³C{¹H} NMR (100 MHz, D₂O) δ 174.5, 158.3, 136.6, 128.8, 128.3, 127.6, 102.9, 101.0, 78.5, 75.3, 74.7, 72.5, 72.4, 71.0, 68.5, 67.6, 66.8, 61.0, 60.1, 55.1, 37.2, 28.8, 22.1. HRMS (ESI-Orbitrap) *m/z*: [M + FA – H][–] Calcd for C₂₆H₃₉N₂O₁₅ 619.2356; found 619.2350.

Galβ3GalNAcβProNHCbz (26).—38 mg, 78% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.48–7.38 (m, 5H), 5.12–5.08 (m, 2H), 4.45–4.40 (m, 2H), 4.16 (d, *J* = 3.2, 1H), 3.98 (dd, *J* = 10.9, 8.5 Hz, 1H), 3.90 (d, *J* = 3.7 Hz, 2H), 3.82 (dd, *J* = 10.9, 3.3 Hz, 1H), 3.81–3.70 (m, 4H), 3.68–3.63 (m, 2H), 3.62–3.58 (m, 2H), 3.52 (dd, *J* = 10.0, 7.8 Hz, 1H), 3.21–3.10 (m, 2H), 1.99 (s, 3H), 1.79–1.69 (m, 2H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 174.7, 158.3, 136.6, 128.7, 128.3, 127.6, 104.8, 101.3, 79.9, 75.0, 74.7, 72.4, 70.5, 68.5, 68.0, 67.5, 66.73, 61.0, 60.9, 51.2, 37.2, 28.7, 22.2. HRMS (ESI-Orbitrap) *m/z*: [M + FA – H][–] Calcd for C₂₆H₃₉N₂O₁₅ 619.2356; found 619.2356.

Galβ3GalNAcαProNHCbz (27).—39 mg, 81% yield, white amorphous solid. ¹H NMR (400 MHz, D₂O) δ 7.52–7.34 (m, 5H), 5.12 (d, *J* = 2.8 Hz, 2H), 4.82 (d, *J* = 3.8 Hz, 1H),

4.43 (d, $J = 7.6$ Hz, 1H), 4.32 (dd, $J = 11.0, 3.7$ Hz, 1H), 4.22 (d, $J = 3.1$ Hz, 1H), 4.06–3.92 (m, 2H), 3.90 (d, $J = 3.3$ Hz, 1H), 3.81–3.66 (m, 5H), 3.65–3.57 (m, 2H), 3.55–3.40 (m, 2H), 3.25 (t, $J = 6.6$ Hz, 2H), 2.01 (s, 3H), 1.80 (dd, $J = 9.8, 4.2$ Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, D_2O) δ 174.5, 158.4, 136.6, 128.8, 128.3, 127.5, 104.7, 97.1, 77.4, 74.9, 72.5, 70.6, 70.6, 68.8, 68.5, 66.7, 65.0, 61.2, 60.9, 48.6, 37.5, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z : $[\text{M} + \text{FA} - \text{H}]^-$ Calcd for $\text{C}_{26}\text{H}_{39}\text{N}_2\text{O}_{15}$ 619.2356; found 619.2351.

Gal β 3GlcNAc β ProNHCBz (28).—39 mg, 80% yield, white amorphous solid. ^1H NMR (400 MHz, D_2O) δ 7.49–7.24 (m, 5H), 5.03 (s, 2H), 4.41 (d, $J = 8.2$ Hz, 1H), 4.34 (d, $J = 7.7$ Hz, 1H), 3.83 (tt, $J = 5.8, 2.9$ Hz, 3H), 3.77–3.60 (m, 6H), 3.59–3.48 (m, 2H), 3.48–3.41 (m, 2H), 3.37 (ddd, $J = 9.9, 5.6, 2.1$ Hz, 1H), 3.09 (hept, $J = 6.9$ Hz, 2H), 1.92 (s, 3H), 1.66 (p, $J = 6.6$ Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, D_2O) δ 174.6, 158.3, 136.6, 128.8, 128.3, 127.6, 103.5, 100.9, 82.4, 75.3, 75.3, 72.5, 70.7, 68.7, 68.5, 67.6, 66.8, 61.0, 60.7, 54.5, 37.2, 28.8, 22.2. HRMS (ESI-Orbitrap) m/z : $[\text{M} + \text{FA} - \text{H}]^-$ Calcd for $\text{C}_{26}\text{H}_{39}\text{N}_2\text{O}_{15}$ 619.2356; found 619.2353.

Gal β 3GlcNAc α ProNHCBz (29).—38 mg, 78% yield, white amorphous solid. ^1H NMR (400 MHz, D_2O) δ 7.43 (q, $J = 6.9$ Hz, 5H), 5.12 (s, 2H), 4.41 (d, $J = 7.7$ Hz, 1H), 4.08 (dd, $J = 10.6, 3.6$ Hz, 1H), 3.98–3.86 (m, 2H), 3.84–3.39 (m, 12H), 3.34–3.14 (m, $J = 7.0$ Hz, 2H), 2.01 (s, 3H), 1.88–1.68 (m, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, D_2O) δ 174.4, 158.4, 136.6, 128.8, 128.3, 127.5, 103.5, 97.0, 80.6, 75.2, 72.5, 71.5, 70.6, 68.6, 68.5, 66.7, 64.9, 60.9, 60.5, 52.4, 37.4, 28.5, 21.9. HRMS (ESI-Orbitrap) m/z : $[\text{M} + \text{FA} - \text{H}]^-$ Calcd for $\text{C}_{26}\text{H}_{39}\text{N}_2\text{O}_{15}$ 619.2356; found 619.2349.

GalNAc α ProNHCBz (30).—42 mg, 78% yield, white amorphous solid. ^1H NMR (400 MHz, CD_3OD) δ 7.45–7.23 (m, 5H), 5.17–5.02 (m, 2H), 4.77 (d, $J = 3.7$ Hz, 1H), 4.27 (dd, $J = 10.9, 3.6$ Hz, 1H), 3.89 (d, $J = 3.2$ Hz, 1H), 3.84–3.66 (m, 5H), 3.42 (dt, $J = 10.7, 6.0$ Hz, 1H), 3.32–3.17 (m, 2H), 2.01 (s, 3H), 1.79 (p, $J = 6.5$ Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, CD_3OD) δ 174.2, 159.0, 138.5, 129.5, 129.0, 128.8, 98.8, 72.5, 70.4, 70.0, 67.4, 65.9, 62.9, 51.6, 38.8, 30.7, 22.7. HRMS (ESI-Orbitrap) m/z : $[\text{M} + \text{FA} - \text{H}]^-$ Calcd for $\text{C}_{20}\text{H}_{29}\text{N}_2\text{O}_{10}$ 457.1828; found 457.1822.

General Procedures for One-Pot Three-Enzyme (OP3E) Preparative-Scale Synthesis of Neu5,7diN $_3\alpha$ 2–3/6-Linked Sialosides (31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53)

Man $_2,4\text{diN}_3$ (**9**, 50–70 mg, 0.22–0.30 mmol), sodium pyruvate (0.75–1.5 mmol), CTP (0.15–0.30 mmol), and an acceptor selected from Lac β ProNHCBz (**24**),⁴⁷ LacNAc β ProNHCBz (**25**), Gal β 1–3GalNAc β ProNHCBz (**26**), Gal β 1–3GalNAc α ProNHCBz (**27**), Gal β 1–3GlcNAc β ProNHCBz (**28**), Gal β 1–3GlcNAc α ProNHCBz (**29**), and GalNAc α ProNHCBz (**30**) (0.10–0.20 mmol), were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.5) and MgCl_2 (20 mM). After adding sialic acid aldolase (0.5–3 mg), NmCSS (0.5 mg), and a sialyltransferase PmST1 (1–3 mg) or Pd $_2,6\text{ST}$ (1–4 mg), water was added to bring the final concentration of Man $_2,4\text{diN}_3$ (**9**) to 10 mM. The reaction mixture was incubated at 30 °C for 24–36 h. The reaction progress was monitored using TLC (ethyl acetate:methanol:water = 6:1:1, by volume) and mass spectrometry. The reaction mixture was diluted with the same volume of ethanol and

incubated at 4 °C for 30 min. The mixture was then centrifuged and concentrated, which was purified using a C18 column (CH₃CN in H₂O gradient was used as running solvents) to produce the sialoside product.

Neu5,7diN₃α2-3LacβProNHCBz (31).—107 mg, 90% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.49–7.36 (m, 5H), 5.09 (s, 2H), 4.50 (d, *J* = 7.9 Hz, 1H), 4.41 (d, *J* = 8.0 Hz, 1H), 4.06 (ddd, *J* = 8.8, 5.6, 2.6 Hz, 1H), 4.03 (dd, *J* = 9.9, 3.2 Hz, 1H), 3.96–3.89 (m, 4H), 3.81–3.73 (m, 4H), 3.71–3.52 (m, 10H), 3.28 (t, *J* = 8.4 Hz, 1H), 3.26–3.17 (m, 2H), 2.72 (dd, *J* = 12.7, 4.7 Hz, 1H), 1.90 (t, *J* = 12.3 Hz, 1H), 1.79 (p, *J* = 6.6 Hz, 2H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 173.3, 158.4, 136.6, 128.8, 128.3, 127.6, 102.6, 102.1, 100.8, 78.4, 75.7, 75.1, 74.7, 74.3, 72.8, 72.4, 70.4, 69.5, 69.3, 67.9, 67.7, 66.8, 62.5, 60.9, 60.3, 60.1, 38.8, 37.3, 28.8. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₂H₄₆N₇O₁₉ 832.2854; found 832.2883.

Neu5,7diN₃α2-6LacβProNHCBz (33).—140 mg, 100% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.46–7.37 (m, 5H), 5.09 (s, 2H), 4.40 (dd, *J* = 17.6, 7.9 Hz, 2H), 4.05 (ddd, *J* = 8.7, 5.5, 2.5 Hz, 1H), 3.96–3.90 (m, 5H), 3.80–3.72 (m, 5H), 3.70–3.62 (m, 4H), 3.61–3.50 (m, 5H), 3.30 (t, *J* = 8.7 Hz, 1H), 3.26–3.18 (m, 2H), 2.71 (dd, *J* = 12.6, 4.7 Hz, 1H), 1.85–1.77 (m, 3H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 173.1, 158.4, 136.6, 128.8, 128.3, 127.7, 103.3, 102.0, 100.6, 80.0, 74.6, 74.5, 73.5, 72.6, 72.4, 72.1, 70.8, 70.3, 69.4, 68.3, 67.7, 66.8, 63.4, 62.5, 60.6, 60.3, 59.3, 39.8, 37.3, 28.8. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₂H₄₆N₇O₁₉ 832.2854; found 832.2879.

Neu5,7diN₃α2-3LacNAcβProNHCBz (35).—26.5 mg, 70% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.46–7.40 (m, 5H), 5.11 (s, 2H), 4.54 (d, *J* = 7.9 Hz, 1H), 4.46 (d, *J* = 8.3 Hz, 1H), 4.11–4.02 (m, 2H), 4.00–3.88 (m, 4H), 3.84 (dd, *J* = 12.4, 5.1 Hz, 1H), 3.80–3.75 (m, 3H), 3.73–3.64 (m, 7H), 3.62–3.51 (m, 4H), 3.24–3.13 (m, 2H), 2.74 (dd, *J* = 12.6, 4.7 Hz, 1H), 2.02 (s, 3H), 1.91 (t, *J* = 12.3 Hz, 1H), 1.74 (p, *J* = 6.5 Hz, 2H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.5, 173.4, 158.3, 136.6, 128.8, 128.3, 127.6, 102.6, 101.1, 100.7, 78.4, 75.7, 75.1, 74.7, 72.4, 70.4, 69.5, 69.3, 67.9, 67.6, 66.7, 62.5, 61.0, 60.4, 60.1, 59.4, 55.0, 38.8, 37.2, 28.8, 22.1. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₄H₄₉N₈O₁₉ 873.3119; found 873.3144.

Neu5,7diN₃α2-6LacNAcβProNHCBz (37).—27 mg, 72% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.51–7.39 (m, 5H), 5.20–5.09 (m, 2H), 4.53 (d, *J* = 8.1 Hz, 1H), 4.43 (d, *J* = 8.0 Hz, 1H), 4.10 (ddd, *J* = 9.5, 5.5, 2.6 Hz, 1H), 4.01–3.89 (m, 5H), 3.84–3.51 (m, 15H), 3.44 (t, *J* = 9.9 Hz, 1H), 3.25–3.12 (m, 1H), 2.69 (dd, *J* = 12.6, 4.8 Hz, 1H), 2.04 (s, 3H), 1.86–1.72 (m, 3H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.2, 173.1, 158.3, 136.6, 128.8, 128.4, 127.6, 103.5, 100.8, 100.4, 80.8, 74.5, 73.6, 72.5, 72.2, 70.8, 70.2, 69.2, 68.4, 67.6, 66.8, 63.7, 63.4, 62.5, 60.4, 60.3, 59.3, 55.0, 39.9, 37.3, 28.7, 22.2. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₄H₄₉N₈O₁₉ 873.3119; found 873.3146.

Neu5,7diN₃α2-3Galβ1-3GalNAcβProNHCBz (39).—24 mg, 99% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.48–7.38 (m, 5H), 5.14–5.07 (m, 2H), 4.49 (d, *J* = 7.8 Hz, 1H), 4.44 (d, *J* = 8.5 Hz, 1H), 4.16 (d, *J* = 3.2 Hz, 1H), 4.09–4.04 (m, 1H), 4.02–3.96 (m, 2H), 3.94–3.88 (m, 3H), 3.83–3.70 (m, 6H), 3.70–3.60 (m, 6H), 3.58 (t,

$J = 9.7$ Hz, 1H), 3.52 (dd, $J = 9.8, 7.8$ Hz, 1H), 3.23–3.12 (m, 2H), 2.73 (d, $J = 12.7, 4.7$ Hz, 1H), 1.99 (s, 3H), 1.88 (t, $J = 12.3$ Hz, 1H), 1.80–1.69 (m, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (200 MHz, D_2O) δ 174.7, 173.4, 158.3, 136.6, 128.8, 128.3, 127.6, 104.6, 101.3, 100.4, 80.3, 75.8, 74.72, 74.7, 72.2, 70.4, 69.5, 68.9, 67.8, 67.6, 67.5, 66.8, 63.2, 62.4, 60.9, 60.3, 51.1, 39.1, 37.2, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{34}\text{H}_{49}\text{N}_8\text{O}_{19}$ 873.3119; found 873.3142.

Neu5,7diN₃α₂-6Galβ1-3GalNAcβProNHCBz (41).—25 mg, 100% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.51–7.39 (m, 5H), 5.19–5.09 (m, 2H), 4.42 (dd, $J = 8.2, 6.1$ Hz, 2H), 4.19 (d, $J = 3.3$ Hz, 1H), 4.07–4.02 (m, 1H), 4.00–3.87 (m, 5H), 3.82–3.69 (m, 8H), 3.67–3.60 (m, 4H), 3.56–3.49 (m, 2H), 3.27–3.10 (m, 2H), 2.73 (dd, $J = 12.6, 4.8$ Hz, 1H), 2.00 (s, 3H), 1.80–1.71 (m, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.7, 173.0, 158.3, 136.6, 128.8, 128.3, 127.6, 104.9, 101.3, 100.8, 80.0, 74.9, 73.1, 72.5, 72.1, 70.54, 70.48, 69.3, 68.6, 67.9, 67.6, 66.8, 63.6, 63.4, 62.5, 60.9, 60.7, 51.1, 40.0, 37.2, 28.8, 22.2. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{34}\text{H}_{49}\text{N}_8\text{O}_{19}$ 873.3119; found 873.3128.

Neu5,7diN₃α₂-3Galβ1-3GalNAcαProNHCBz (43).—47.6 mg, 95% yield, white amorphous solid. ^1H NMR (400 MHz, D_2O) δ 7.50–7.39 (m, 5H), 5.14 (s, 2H), 4.85 (d, $J = 3.8$ Hz, 1H), 4.49 (d, $J = 7.9$ Hz, 1H), 4.30 (dd, $J = 11.1, 3.7$ Hz, 1H), 4.22 (s, 1H), 4.10–4.03 (m, 1H), 4.02–3.89 (m, 5H), 3.84–3.62 (m, 9H), 3.62–3.43 (m, 4H), 3.32–3.21 (m, 2H), 2.73 (ddd, $J = 13.3, 8.6, 4.7$ Hz, 1H), 2.01 (s, 3H), 1.93–1.78 (m, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, D_2O) δ 174.5, 173.4, 158.4, 135.7, 128.8, 128.3, 127.5, 104.5, 100.4, 97.1, 77.9, 75.8, 74.7, 72.3, 70.5, 70.4, 69.5, 69.0, 68.5, 67.7, 66.7, 65.1, 62.4, 61.2, 60.9, 60.3, 59.3, 48.5, 39.1, 37.5, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{34}\text{H}_{49}\text{N}_8\text{O}_{19}$ 873.3119; found 873.3149.

Neu5,7diN₃α₂-6Galβ1-3GalNAcαProNHCBz (45).—34.1 mg, 68% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.55–7.40 (m, 5H), 5.14 (s, 2H), 4.83 (d, $J = 3.7$ Hz, 1H), 4.41 (d, $J = 7.8$ Hz, 1H), 4.31 (dd, $J = 10.9, 3.7$ Hz, 1H), 4.18 (s, 1H), 4.09–3.97 (m, 2H), 3.95–3.85 (m, 4H), 3.81–3.72 (m, 6H), 3.70–3.57 (m, 4H), 3.55–3.45 (m, 3H), 3.34–3.22 (m, 2H), 2.74 (dd, $J = 12.6, 4.5$ Hz, 1H), 2.01 (d, $J = 4.2$ Hz, 3H), 1.89–1.76 (m, 2H), 1.73 (t, $J = 12.3$ Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.5, 173.0, 158.4, 136.6, 128.8, 128.3, 127.6, 104.5, 100.7, 97.1, 77.2, 73.2, 72.3, 72.1, 70.7, 70.5, 70.4, 69.3, 68.8, 68.4, 66.8, 65.0, 63.5, 62.5, 61.5, 60.6, 59.4, 48.6, 40.0, 37.6, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{34}\text{H}_{49}\text{N}_8\text{O}_{19}$ 873.3119; found 873.3150.

Neu5,7diN₃α₂-3Galβ1-3GlcNAcβProNHCBz (47).—66.1 mg, 87% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.49–7.39 (m, 5H), 5.12 (s, 2H), 4.50 (dd, $J = 14.0, 8.2$ Hz, 2H), 4.08–4.00 (m, 2H), 3.96–3.89 (m, 4H), 3.79–3.69 (m, 7H), 3.70–3.65 (m, 3H), 3.64–3.56 (m, 2H), 3.53 (dd, $J = 9.5, 7.7$ Hz, 2H), 3.49–3.43 (m, 1H), 3.24–3.13 (m, 2H), 2.76 (dd, $J = 12.7, 4.8$ Hz, 1H), 2.02 (s, 3H), 1.89 (t, $J = 12.3$ Hz, 1H), 1.76 (p, $J = 6.6$ Hz, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.5, 173.4, 158.3, 136.6, 128.8, 128.3, 127.6, 103.5, 100.8, 100.3, 82.9, 75.8, 75.3, 75.1, 72.2, 70.4, 69.5, 69.0, 68.8, 67.7, 67.5,

66.8, 63.2, 62.4, 60.9, 60.7, 60.4, 59.3, 54.3, 39.2, 37.3, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3150.

Neu5,7diN₃α2-6Galβ1-3GlcNAcβProNHCBz (49).—60.7 mg, 100% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.51–7.39 (m, 5H), 5.12 (s, 2H), 4.51 (d, J = 8.5 Hz, 1H), 4.37 (d, J = 7.8 Hz, 1H), 4.07 (ddd, J = 9.4, 5.5, 2.6 Hz, 1H), 4.00–3.89 (m, 5H), 3.82–3.76 (m, 5H), 3.74–3.66 (m, 3H), 3.65–3.48 (m, 7H), 3.24–3.12 (m, 2H), 2.72 (dd, J = 12.6, 4.8 Hz, 1H), 2.01 (s, 3H), 1.83–1.71 (m, 3H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.6, 173.1, 158.3, 136.6, 128.8, 128.4, 127.6, 104.0, 100.9, 100.5, 84.4, 75.3, 73.5, 72.5, 72.1, 70.6, 70.4, 69.3, 69.1, 68.4, 67.6, 66.8, 63.6, 62.5, 61.4, 61.0, 60.6, 59.3, 54.2, 39.9, 37.2, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3150.

Neu5,7diN₃α2-3Galβ1-3GlcNAcαProNHCBz (51).—46.6 mg, 93% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.48–7.38 (m, 5H), 5.13 (s, 2H), 4.53–4.45 (m, 1H), 4.06 (ddt, J = 9.2, 5.6, 3.1 Hz, 2H), 4.01 (dd, J = 9.8, 3.1 Hz, 1H), 3.95–3.87 (m, 3H), 3.81–3.65 (m, 11H), 3.64–3.47 (m, 5H), 3.33–3.20 (m, 2H), 2.79–2.69 (m, 1H), 2.01 (s, 3H), 1.89 (t, J = 12.3 Hz, 1H), 1.85–1.74 (m, 2H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.4, 173.2, 158.4, 136.7, 128.8, 128.3, 127.5, 118.9, 103.4, 100.5, 96.9, 81.1, 75.9, 75.0, 72.3, 71.5, 70.4, 69.45, 69.37, 69.1, 68.7, 67.5, 66.7, 63.2, 62.4, 60.9, 60.5, 60.4, 59.3, 52.3, 39.1, 37.4, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3142.

Neu5,7diN₃α2-6Galβ1-3GlcNAcαProNHCBz (53).—50 mg, 100% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.52–7.34 (m, 5H), 5.14 (s, 2H), 4.34 (d, J = 7.8 Hz, 1H), 4.12–4.02 (m, 2H), 3.97–3.88 (m, 4H), 3.87–3.70 (m, 9H), 3.68 (dd, J = 9.4, 2.3 Hz, 1H), 3.62–3.54 (m, 3H), 3.53–3.45 (m, 3H), 3.35–3.17 (m, 2H), 2.72 (dd, J = 12.6, 4.8 Hz, 1H), 2.01 (s, 3H), 1.86–1.74 (m, 3H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.4, 173.1, 158.4, 136.6, 128.8, 128.3, 127.5, 103.8, 100.6, 96.8, 82.3, 73.5, 72.5, 72.1, 71.4, 70.6, 70.3, 69.3, 68.9, 68.4, 66.7, 65.0, 63.6, 62.5, 61.4, 60.6, 59.3, 52.2, 39.9, 37.5, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3133.

Neu5,7diN₃α2-6GalNAcαProNHCBz (55).—112 mg, 65% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.46–7.38 (m, 5H), 5.17–5.06 (m, 2H), 4.12 (dd, J = 11.1, 3.6 Hz, 1H), 4.02 (ddd, J = 8.6, 5.4, 2.6 Hz, 1H), 3.99–3.87 (m, 3H), 3.87–3.80 (m, 2H), 3.79–3.58 (m, 7H), 3.50 (t, J = 9.8 Hz, 1H), 3.47–3.37 (m, 1H), 3.30–3.17 (m, 2H), 2.74 (dd, J = 12.7, 4.8 Hz, 1H), 2.02 (s, 3H), 1.82–1.71 (m, 3H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.5, 172.8, 158.3, 136.6, 128.8, 128.3, 127.6, 101.0, 97.1, 72.1, 70.4, 69.4, 69.3, 68.2, 67.6, 66.7, 65.4, 63.5, 62.5, 60.6, 59.3, 49.9, 40.0, 37.5, 28.5, 21.9. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{28}H_{39}N_8O_{14}$ 711.2591; found 711.2605.

General Procedures for One-Pot Three-Enzyme (OP3E) Preparative-Scale Synthesis of Neu5,7,9triN₃α2–3/6-linked Sialosides (32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56)

An acceptor selected from LacβProNHCBz **24**, LacNAcβProNHCBz **25**, Galβ1–3GalNAcβProNHCBz **26**, Galβ1–3GalNAcαProNHCBz **27**, Galβ1–3GlcNAcβProNHCBz **28**, Galβ1–3GlcNAcαProNHCBz **29**, and GalNAcαProNHCBz **30** (0.10–0.20 mmol), Man₂,4,6triN₃ (**11**, 50–70 mg, 0.2–0.30 mmol), sodium pyruvate (1.0–1.5 mmol), CTP (0.3–0.45 mmol) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.5) and MgCl₂ (20 mM). After adding sialic acid aldolase (0.5–3 mg), NmCSS (0.5 mg), and a sialyltransferase PmST1 (1–3 mg) or Pd2,6ST (1–4 mg) water was added to bring the final concentration of Man₂,4,6triN₃ (**11**) to 10 mM. The reaction mixture was incubated at 30 °C for 24–36 h. The reaction progress was monitored by TLC (ethyl acetate:methanol:water = 6:1:1, by volume) and mass spectrometry. The reaction mixture was diluted with the same volume of ethanol and incubated at 4 °C for 30 min. The mixture was then centrifuged and concentrated, which was purified by automated flash chromatography using a C18 column (CH₃CN in H₂O gradient was used as running solvents) to produce sialosides.

Neu5,7,9triN₃α2–3LacβProNHCBz (32).—127 mg, 92% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.46–7.38 (m, 5H), 5.10 (s, 2H), 4.49 (d, *J* = 7.8 Hz, 1H), 4.42 (d, *J* = 8.0 Hz, 1H), 4.19 (ddd, *J* = 9.0, 6.2, 2.7 Hz, 1H), 4.02 (dd, *J* = 9.8, 3.2 Hz, 1H), 3.98–3.88 (m, 3H), 3.83–3.46 (m, 15H), 3.31–3.15 (m, 3H), 2.73 (dd, *J* = 12.7, 4.7 Hz, 1H), 1.89 (t, *J* = 12.3 Hz, 1H), 1.80 (p, *J* = 6.6 Hz, 2H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 173.2, 158.8, 136.6, 128.8, 128.3, 127.6, 102.6, 102.6, 102.1, 78.4, 75.8, 75.1, 74.7, 74.3, 72.8, 72.2, 69.5, 69.3, 69.3, 67.8, 67.7, 66.8, 63.1, 61.3, 60.9, 60.1, 53.1, 39.0, 37.3, 28.8. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₂H₄₅N₁₀O₁₈ 857.2919; found 857.2948.

Neu5,7,9triN₃α2–6LacβProNHCBz (34).—132 mg, 100% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.41 (dd, *J* = 25.3, 7.7 Hz, 5H), 5.08 (s, 2H), 4.40 (t, *J* = 8.9 Hz, 2H), 4.17 (ddd, *J* = 8.7, 5.8, 2.6 Hz, 1H), 3.96–3.90 (m, 4H), 3.80–3.71 (m, 6H), 3.71–3.63 (m, 4H), 3.61–3.51 (m, 5H), 3.31 (t, *J* = 8.7 Hz, 1H), 3.23 (dt, *J* = 12.5, 6.7 Hz, 2H), 2.71 (dd, *J* = 12.6, 4.8 Hz, 1H), 1.85–1.74 (m, 3H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 173.1, 158.3, 136.5, 128.8, 128.3, 127.7, 103.3, 102.0, 100.6, 80.0, 74.6, 74.5, 73.5, 72.6, 72.5, 72.0, 70.8, 69.5, 69.1, 68.3, 67.7, 66.8, 63.4, 63.3, 61.6, 60.4, 53.2, 39.9, 37.4, 28.9. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₂H₄₅N₁₀O₁₈ 857.2919; found 857.2939.

Neu5,7,9triN₃α2–3LacNAcβProNHCBz (36).—12.9 mg, 66% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.52–7.37 (m, 5H), 5.11 (s, 2H), 4.53 (d, *J* = 7.8 Hz, 1H), 4.47 (d, *J* = 8.3 Hz, 1H), 4.21 (ddd, *J* = 9.1, 6.3, 2.7 Hz, 1H), 4.04 (dd, *J* = 9.9, 3.2 Hz, 1H), 3.99 (dd, *J* = 12.4, 2.4 Hz, 1H), 3.95–3.87 (m, 2H), 3.84 (dd, *J* = 12.4, 5.1 Hz, 1H), 3.81–3.75 (m, 3H), 3.73–3.65 (m, 7H), 3.65–3.52 (m, 5H), 3.23–3.08 (m, 2H), 2.74 (dd, *J* = 12.6, 4.7 Hz, 1H), 2.02 (s, 3H), 1.90 (t, *J* = 12.3 Hz, 1H), 1.75 (t, *J* = 6.5 Hz, 2H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.5, 173.3, 158.3, 136.6, 128.8, 128.4, 127.6, 102.6, 101.1, 100.7, 78.5, 75.8, 75.1, 74.7, 72.4, 72.2, 71.0, 69.5, 69.4, 69.3, 67.8, 66.8, 61.3, 60.9, 60.1, 59.3, 55.0, 53.2, 39.0, 37.3, 28.8, 22.1. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₄H₄₈N₁₁O₁₈ 898.3184; found 898.3200.

Neu5,7,9triN₃α2-6LacNAcβProNHCBz (38).—25.4 mg, 65% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.50–7.39 (m, 5H), 5.17–5.08 (m, 2H), 4.52 (d, *J* = 8.2 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.23 (ddd, *J* = 8.8, 5.9, 2.7 Hz, 1H), 4.01–3.88 (m, 3H), 3.86–3.76 (m, 4H), 3.73–3.51 (m, 12H), 3.44 (t, *J* = 12 Hz, 1H), 3.25–3.12 (m, 2H), 2.69 (dd, *J* = 12.6, 4.7 Hz, 1H), 2.06 (s, 3H), 1.84–1.71 (m, 3H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.2, 173.0, 158.3, 136.6, 128.8, 128.4, 127.6, 103.5, 102.9, 100.8, 80.8, 74.5, 73.6, 72.51, 72.47, 72.1, 70.8, 69.2, 68.9, 68.3, 67.6, 66.8, 63.6, 61.2, 60.4, 59.3, 55.0, 53.2, 39.9, 37.3, 28.7, 22.2. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₄H₄₈N₁₁O₁₈ 898.3184; found 898.3202.

Neu5,7,9triN₃α2-3Galβ1-3GalNAcβProNHCBz (40).—7.3 mg, 67% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.47–7.38 (m, 5H), 5.15–5.09 (m, 2H), 4.48 (d, *J* = 7.8 Hz, 1H), 4.44 (d, *J* = 8.6 Hz, 1H), 4.19–4.15 (m, 2H), 4.01–3.96 (m, 2H), 3.94–3.90 (m, 1H), 3.89 (d, *J* = 3.2 Hz, 1H), 3.82–3.73 (m, 5H), 3.72–3.55 (m, 9H), 3.52 (dd, *J* = 9.8, 7.8 Hz, 1H), 3.23–3.11 (m, 2H), 2.73 (dd, *J* = 12.7, 4.7 Hz, 1H), 2.00 (s, 3H), 1.87 (t, *J* = 12.3 Hz, 1H), 1.77–1.72 (m, 2H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 174.7, 173.3, 158.3, 136.6, 128.8, 128.3, 127.6, 104.5, 101.3, 100.3, 80.3, 75.8, 74.7, 72.1, 69.5, 69.3, 68.9, 67.8, 67.5, 66.7, 61.2, 60.92, 60.9, 59.3, 53.1, 51.1, 39.2, 37.2, 29.6, 28.7, 22.2. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₄H₄₈N₁₁O₁₈ 898.3184; found 898.3206.

Neu5,7,9triN₃α2-6Galβ1-3GalNAcβProNHCBz (42).—17.2 mg, 69% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.55–7.36 (m, 5H), 5.25–5.05 (m, 2H), 4.43 (ddd, *J* = 15.5, 11.6, 8.0 Hz, 2H), 4.24–4.12 (m, 2H), 3.93–3.69 (m, 10H), 3.66–3.50 (m, 9H), 3.43–3.29 (m, 1H), 2.90 (s, 1H), 2.77–2.70 (m, 1H), 2.01 (s, 3H), 1.84–1.71 (m, 3H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 173.02, 172.97, 171.0, 158.2, 136.5, 128.8, 128.4, 127.6, 104.9, 104.4, 100.8, 75.0, 72.5, 72.31, 72.28, 72.1, 70.5, 70.4, 69.3, 69.2, 69.1, 68.6, 63.4, 63.2, 61.3, 60.9, 53.2, 51.2, 45.7, 40.0, 28.8, 22.2. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₄H₄₈N₁₁O₁₈ 898.3184; found 898.3212.

Neu5,7,9triN₃α2-3Galβ1-3GalNAcαProNHCBz (44).—29.2 mg, 65% yield, white amorphous solid. ¹H NMR (400 MHz, D₂O) δ 7.44 (q, *J* = 7.3 Hz, 5H), 5.14 (s, 2H), 4.85 (d, *J* = 3.7 Hz, 1H), 4.48 (d, *J* = 7.8 Hz, 1H), 4.29 (dd, *J* = 11.1, 3.7 Hz, 1H), 4.24–4.13 (m, 2H), 4.04–3.86 (m, 4H), 3.81–3.46 (m, 14H), 3.35–3.19 (m, 2H), 2.74 (dd, *J* = 12.7, 4.7 Hz, 1H), 2.01 (s, 3H), 1.92–1.76 (m, 3H). ¹³C{¹H} NMR (100 MHz, D₂O) δ 174.5, 173.4, 158.4, 136.5, 128.8, 128.3, 127.4, 104.5, 100.3, 97.1, 77.9, 77.3, 75.9, 74.7, 72.1, 70.6, 69.5, 69.2, 69.0, 68.5, 67.5, 66.7, 65.1, 63.1, 61.2, 60.9, 53.1, 48.6, 39.3, 37.4, 28.4, 22.0. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₄H₄₈N₁₁O₁₈ 898.3184; found 898.3201.

Neu5,7,9triN₃α2-6Galβ1-3GalNAcαProNHCBz (46).—40.7 mg, 79% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.52–7.31 (m, 5H), 5.13 (s, 2H), 4.82–4.81 (m, 1H), 4.39 (d, *J* = 7.8 Hz, 1H), 4.29 (dd, *J* = 11.1, 3.7 Hz, 1H), 4.20–4.07 (m, 2H), 3.98 (dd, *J* = 11.0, 3.1 Hz, 1H), 3.94–3.82 (m, 3H), 3.79 (dd, *J* = 10.3, 2.3 Hz, 1H), 3.78–3.65 (m, 6H), 3.63 (t, *J* = 6.2 Hz, 1H), 3.60–3.52 (m, 3H), 3.52–3.43 (m, 3H), 3.26 (dq, *J* = 13.5, 6.9 Hz, 2H), 2.71 (dd, *J* = 12.6, 4.8 Hz, 1H), 2.00 (s, 3H), 1.80 (qt, *J* = 13.0, 7.6 Hz, 2H), 1.71 (t, *J* = 12.3 Hz, 1H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 174.5, 173.0, 158.4, 136.6, 128.8,

128.3, 127.6, 104.5, 100.7, 97.1, 77.2, 73.1, 72.3, 72.0, 70.7, 70.5, 69.3, 69.2, 68.8, 68.3, 66.7, 65.0, 63.4, 61.5, 61.5, 59.3, 53.1, 48.6, 40.0, 37.6, 28.4, 22.0. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3216.

Neu5,7,9triN₃α₂-3Galβ1-3GlcNAcβProNHCBz (48).—50 mg, 64% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.71–7.20 (m, 5H), 5.29–5.04 (m, 2H), 4.49 (d, J = 8.5 Hz, 1H), 4.46 (d, J = 7.8 Hz, 1H), 4.15 (ddd, J = 9.0, 6.4, 2.8 Hz, 1H), 4.00 (dd, J = 9.8, 3.2 Hz, 1H), 3.96–3.87 (m, 3H), 3.84–3.77 (m, 1H), 3.75–3.55 (m, 12H), 3.54–3.48 (m, 2H), 3.48–3.40 (m, 1H), 3.24–3.08 (m, 2H), 2.74 (dd, J = 12.6, 4.7 Hz, 1H), 2.01 (s, 3H), 1.86 (t, J = 12.3 Hz, 1H), 1.74 (p, J = 6.5 Hz, 2H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 174.5, 173.3, 158.3, 136.6, 128.8, 128.3, 127.6, 103.4, 100.8, 100.3, 82.9, 75.9, 75.3, 75.0, 72.1, 69.5, 69.3, 69.0, 68.8, 67.6, 67.4, 66.7, 63.1, 61.3, 60.9, 60.7, 54.3, 53.1, 39.3, 37.3, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3201.

Neu5,7,9triN₃α₂-6Galβ1-3GlcNAcβProNHCBz (50).—57.1 mg, 91% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.50–7.41 (m, 5H), 5.16–5.08 (m, 2H), 4.50 (d, J = 8.5 Hz, 1H), 4.37 (d, J = 7.8 Hz, 1H), 4.20 (ddd, J = 9.0, 6.0, 2.7 Hz, 1H), 4.01–3.88 (m, 4H), 3.84–3.74 (m, 6H), 3.71–3.66 (m, 2H), 3.65–3.56 (m, 4H), 3.55–3.45 (m, 4H), 3.26–3.05 (m, 2H), 2.71 (dd, J = 12.6, 4.8 Hz, 1H), 2.01 (s, 3H), 1.82–1.70 (m, 3H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.6, 173.0, 158.3, 136.6, 128.8, 128.4, 127.6, 103.9, 100.9, 100.6, 84.4, 75.3, 73.5, 72.5, 72.0, 70.6, 69.3, 69.1, 69.1, 68.4, 67.6, 66.8, 63.5, 63.5, 61.5, 61.0, 59.3, 54.2, 53.2, 39.9, 37.2, 28.8, 22.2. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3213.

Neu5,7,9triN₃α₂-3Galβ1-3GlcNAcαProNHCBz (52).—44.8 mg, 87% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.53–7.37 (m, 5H), 5.19–5.08 (m, 2H), 4.52–4.39 (m, 1H), 4.21–4.13 (m, 1H), 4.12–4.02 (m, 1H), 3.99 (dd, J = 9.8, 3.2 Hz, 1H), 3.96–3.85 (m, 2H), 3.87–3.81 (m, 1H), 3.81–3.71 (m, 5H), 3.73–3.63 (m, 4H), 3.64–3.43 (m, 7H), 3.35–3.16 (m, 2H), 2.78–2.69 (m, 1H), 2.02 (s, 3H), 1.88 (t, J = 12.3 Hz, 1H), 1.86–1.73 (m, 2H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.4, 173.3, 158.3, 136.7, 128.8, 128.3, 127.5, 103.5, 100.3, 96.9, 81.3, 75.9, 75.0, 72.5, 72.1, 71.5, 70.7, 69.5, 69.3, 69.1, 68.8, 67.4, 66.7, 63.1, 61.3, 60.9, 60.5, 53.1, 52.3, 39.3, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3209.

Neu5,7,9triN₃α₂-6Galβ1-3GlcNAcαProNHCBz (54).—49.5 mg, 96% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.51–7.38 (m, 5H), 5.14 (s, 2H), 4.34 (d, J = 7.9 Hz, 1H), 4.23–4.16 (m, 1H), 4.06 (dd, J = 10.6, 3.6 Hz, 1H), 3.95–3.87 (m, 3H), 3.87–3.78 (m, 3H), 3.78–3.66 (m, 7H), 3.64–3.53 (m, 4H), 3.53–3.43 (m, 3H), 3.39–3.16 (m, 2H), 2.71 (dd, J = 12.6, 4.8 Hz, 1H), 2.01 (s, 3H), 1.90–1.70 (m, 3H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.4, 173.0, 158.4, 136.6, 128.8, 128.3, 127.5, 103.8, 100.6, 96.8, 82.2, 73.4, 72.5, 72.0, 71.4, 70.6, 69.3, 69.1, 68.9, 68.3, 66.7, 65.0, 63.5, 63.3, 61.5, 60.7, 59.3, 53.2, 52.2, 40.0, 37.5, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3209.

Neu5,7,9triN₃a2-6GalNAc_αProNHCBz (56).—108 mg, 65% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.72–7.09 (m, 5H), 5.32–5.02 (m, 2H), 4.22–4.05 (m, 2H), 3.95 (s, 2H), 3.89–3.46 (m, 11H), 3.43 (dt, *J* = 11.3, 6.1 Hz, 1H), 3.29–3.06 (m, 2H), 2.79–2.63 (m, 1H), 2.00 (s, 3H), 1.82–1.65 (m, 3H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 174.5, 158.2, 136.6, 128.7, 128.3, 127.6, 101.3, 97.1, 72.0, 69.4, 69.2, 68.1, 67.6, 66.7, 65.4, 63.4, 63.3, 63.2, 61.5, 53.2, 53.1, 49.9, 40.0, 37.5, 28.5, 21.9. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₂₈H₃₈N₁₁O₁₃ 736.2656; found 736.2672.

General Procedures for Converting Azido-containing Glycosides (31–56) to *N*-Acetyl-containing Glycosides (57–82).

To a sodium bicarbonate saturated solution in water in a round bottom flask (100 mL), an azido-containing glycoside (30–50 mg) was added followed by drop-wise addition of 12–24 equivalents of thioacetic acid under argon at room temperature and stir at 70 °C for 20 h. After the completion of the reaction, the solvent was removed under vacuum. The mixture was passed through a Bio-Gel P-2 gel filtration (water was used as an eluent). The product-containing fractions were concentrated and further purified by silica gel chromatography using a mixed solvent (ethyl acetate: methanol: water = 10:1:0.1, by volume) as an eluent, followed by C18 purification (CH₃CN in H₂O gradient was used as running solvents) to obtain the pure product.

Neu5Ac7NAc_α2-3Lac β ProNHCBz (57).—38 mg, 68% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.55–7.37 (m, 5H), 5.10 (s, 2H), 4.51 (d, *J* = 7.9 Hz, 1H), 4.43 (d, *J* = 8.0 Hz, 1H), 4.13 (ddd, *J* = 10.0, 3.1, 1.0 Hz, 1H), 4.00–3.87 (m, 5H), 3.82–3.67 (m, 7H), 3.66–3.61 (m, 3H), 3.61–3.54 (m, 3H), 3.53–3.49 (m, 1H), 3.29 (t, *J* = 8.4 Hz, 1H), 3.27–3.18 (m, 2H), 2.79 (dd, *J* = 12.5, 4.5 Hz, 1H), 1.98 (s, 3H), 1.93 (s, 3H), 1.80 (p, *J* = 6.6 Hz, 2H), 1.76 (t, *J* = 12.2 Hz, 1H). ¹³C{¹H} NMR (200 MHz, D₂O) δ 174.0, 173.8, 173.8, 158.4, 136.6, 128.8, 128.3, 127.6, 102.6, 102.1, 99.6, 78.3, 75.4, 75.1, 74.7, 74.3, 72.8, 71.8, 71.6, 69.4, 68.6, 67.7, 66.9, 66.8, 62.4, 61.0, 60.1, 51.8, 49.2, 40.1, 37.3, 28.8, 22.1, 21.8. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₆H₅₄N₃O₂₁ 864.3255; found 864.3282.

Neu5Ac7,9diNAc_α2-3Lac β ProNHCBz (58).—42 mg, 80% yield, white amorphous solid. ¹H NMR (600 MHz, D₂O) δ 7.47–7.41 (m, 5H), 5.12 (s, 2H), 4.51 (d, *J* = 7.8 Hz, 1H), 4.45 (d, *J* = 8.1 Hz, 1H), 4.12 (dd, *J* = 9.9, 3.2 Hz, 1H), 3.99–3.95 (m, 4H), 3.87–3.53 (m, 13H), 3.48 (dd, *J* = 14.1, 2.4 Hz, 1H), 3.34–3.23 (m, 3H), 3.09 (dd, *J* = 14.1, 7.5 Hz, 1H), 2.80 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.01 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H), 1.85–1.74 (m, 3H). ¹³C{¹H} NMR (150 MHz, D₂O) δ 174.2, 174.0, 173.83, 173.79, 158.4, 136.6, 128.8, 128.3, 127.6, 102.6, 102.1, 99.6, 78.3, 75.5, 75.1, 74.7, 74.3, 72.8, 71.8, 69.7, 69.4, 68.7, 67.7, 67.0, 66.8, 61.0, 60.1, 51.9, 50.6, 41.8, 40.1, 37.3, 28.8, 22.1, 21.90, 21.88. HRMS (ESI-Orbitrap) *m/z*: [M – H][–] Calcd for C₃₈H₅₇N₄O₂₁ 905.3521; found 905.3554.

Neu5Ac7NAc_α2-6Lac β ProNHCBz (59).—42 mg, 78% yield, white amorphous solid. ¹H NMR (800 MHz, D₂O) δ 7.51–7.34 (m, 5H), 5.10 (s, 2H), 4.43 (dd, *J* = 19.3, 8.0 Hz, 2H), 4.04–3.87 (m, 7H), 3.84–3.72 (m, 3H), 3.71–3.52 (m, 9H), 3.49 (dd, *J* = 12.2, 6.7 Hz, 1H), 3.34 (t, *J* = 8.6 Hz, 1H), 3.27–3.20 (m, 2H), 2.73 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.00

(s, 3H), 1.93 (s, 3H), 1.81 (p, $J = 6.7$ Hz, 2H), 1.75 (t, $J = 12.2$ Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (200 MHz, D_2O) δ 173.93, 173.86, 173.3, 158.4, 136.6, 128.8, 128.3, 127.6, 103.2, 101.9, 100.4, 79.5, 74.6, 73.6, 72.8, 72.4, 71.6, 71.5, 70.7, 68.7, 68.4, 67.7, 66.8, 63.4, 62.4, 60.2, 51.9, 49.2, 40.1, 37.3, 28.8, 22.1, 22.0. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{36}\text{H}_{54}\text{N}_3\text{O}_{21}$ 864.3255; found 864.3280.

Neu5Ac7,9diNAc α 2-6Lac β ProNHCBz (60).—36 mg, 80% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.49–7.37 (m, 5H), 5.12 (s, 2H), 4.45 (dd, $J = 15.6$, 8.0 Hz, 2H), 4.04–3.90 (m, 8H), 3.86–3.54 (m, 12H), 3.45 (dd, $J = 14.1$, 2.5 Hz, 1H), 3.36 (t, $J = 8.6$ Hz, 1H), 3.09 (dd, $J = 14.1$, 7.6 Hz, 1H), 2.75 (dd, $J = 12.4$, 4.6 Hz, 1H), 2.02 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H), 1.83 (p, $J = 6.5$ Hz, 2H), 1.76 (t, $J = 12.2$ Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.2, 174.0, 173.8, 173.3, 158.4, 136.6, 128.8, 128.3, 127.6, 103.2, 102.0, 100.4, 79.5, 74.64, 74.62, 73.6, 72.9, 72.4, 71.6, 70.7, 69.5, 68.7, 68.5, 67.7, 66.8, 63.4, 60.2, 51.9, 50.6, 41.8, 40.1, 37.3, 28.9, 22.14, 22.09, 21.9. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{38}\text{H}_{57}\text{N}_4\text{O}_{21}$ 905.3521; found 905.3555.

Neu5Ac7NAc α 2-3LacNAc β ProNHCBz (61).—10 mg, 67% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.44 (dq, $J = 14.1$, 7.3 Hz, 5H), 5.12 (s, 2H), 4.59–4.53 (m, 1H), 4.48 (d, $J = 8.0$ Hz, 1H), 4.19–4.11 (m, 1H), 4.03–3.96 (m, 2H), 3.91 (ddd, $J = 9.5$, 6.7, 2.3 Hz, 2H), 3.87–3.80 (m, 2H), 3.78–3.64 (m, 9H), 3.63–3.50 (m, 5H), 3.26–3.11 (m, 2H), 2.80 (dd, $J = 12.4$, 4.6 Hz, 1H), 2.03–1.93 (m, 9H), 1.80–1.73 (m, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.5, 174.0, 173.9, 173.8, 158.4, 136.6, 128.8, 128.3, 127.6, 102.5, 101.1, 99.5, 78.4, 75.4, 75.1, 74.7, 72.4, 71.9, 71.6, 69.4, 68.6, 67.6, 67.0, 66.8, 62.4, 61.0, 60.1, 55.0, 51.9, 49.2, 40.1, 37.2, 28.7, 22.1, 21.9. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{38}\text{H}_{57}\text{N}_4\text{O}_{21}$ 905.3521; found 905.3546.

Neu5Ac7,9diNAc α 2-3LacNAc β ProNHCBz (62).—8.5 mg, 67% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.56–7.34 (m, 5H), 5.14 (d, $J = 20.6$ Hz, 2H), 4.54 (dd, $J = 7.9$, 2.3 Hz, 1H), 4.50–4.45 (m, 1H), 4.12 (dd, $J = 9.9$, 3.1 Hz, 1H), 4.03–3.88 (m, 5H), 3.87–3.80 (m, 2H), 3.80–3.65 (m, 7H), 3.65–3.54 (m, 4H), 3.49 (dd, $J = 14.0$, 2.3 Hz, 1H), 3.24–3.13 (m, 2H), 3.09 (dd, $J = 14.1$, 7.5 Hz, 1H), 2.80 (dd, $J = 12.4$, 4.6 Hz, 1H), 2.05–2.02 (m, 6H), 2.01 (s, 3H), 1.96 (s, 3H), 1.87–1.72 (m, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.2, 174.0, 173.8, 158.4, 136.8, 128.8, 128.3, 127.6, 102.5, 101.1, 100.0, 78.3, 75.5, 75.1, 74.7, 72.4, 71.8, 69.7, 69.4, 68.6, 67.6, 67.0, 66.8, 61.0, 60.1, 55.0, 51.9, 50.6, 41.8, 40.1, 37.2, 28.6, 22.12, 22.10, 21.90, 21.88. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{40}\text{H}_{60}\text{N}_5\text{O}_{21}$ 946.3786; found 946.3804.

Neu5Ac7NAc α 2-6LacNAc β ProNHCBz (63).—11.3 mg, 77% yield, white amorphous solid. ^1H NMR (400 MHz, D_2O) δ 7.68–7.29 (m, 5H), 5.14 (d, $J = 12.8$ Hz, 2H), 4.60–4.32 (m, 2H), 4.18–3.39 (m, 21H), 3.36–2.84 (m, 2H), 2.72 (dd, $J = 12.5$, 4.6 Hz, 1H), 2.05 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.87–1.56 (m, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, D_2O) δ 174.5, 173.8, 173.7, 173.4, 158.6, 138.3, 128.8, 128.3, 127.6, 103.3, 100.9, 100.3, 80.0, 74.6, 73.6, 72.4, 71.7, 71.5, 70.7, 68.7, 68.3, 67.6, 66.8, 63.2, 62.5, 60.3, 55.1, 51.9, 49.2, 40.1, 36.9, 28.6, 22.2, 22.1, 22.0. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{38}\text{H}_{57}\text{N}_4\text{O}_{21}$ 905.3521; found 905.3529.

Neu5Ac7,9diNAc α 2-6LacNAc β ProNHCBz (64).—19.3mg, 85% yield, white amorphous solid. ^1H NMR (400 MHz, D_2O) δ 7.44 (td, $J = 7.4, 4.1$ Hz, 5H), 5.14 (d, $J = 12.9$ Hz, 2H), 4.56–4.43 (m, 2H), 4.24–3.31 (m, 20H), 3.27–2.87 (m, 3H), 2.71 (dd, $J = 12.6, 4.7$ Hz, 1H), 2.19–1.91 (m, 12H), 1.77 (h, $J = 11.7$ Hz, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, D_2O) δ 175.2, 174.2, 173.8, 173.7, 173.4, 166.2, 136.1, 128.8, 128.3, 127.6, 103.3, 101.0, 100.4, 79.9, 74.6, 73.5, 72.4, 71.9, 71.6, 70.7, 69.5, 68.7, 68.4, 67.6, 66.8, 63.3, 55.1, 51.9, 50.6, 41.8, 40.1, 36.0, 27.6, 22.1, 22.0, 21.9. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{40}\text{H}_{60}\text{N}_5\text{O}_{21}$ 946.3786; found 946.3805.

Neu5Ac7NAc α 2-3Gal β 1-3GalNAc β ProNHCBz (65).—5.7 mg, 64% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.59–7.34 (m, 5H), 5.15 (d, $J = 22.3$ Hz, 2H), 4.50 (d, $J = 7.8$ Hz, 1H), 4.43 (dd, $J = 15.4, 8.6$ Hz, 1H), 4.16 (d, $J = 3.5$ Hz, 1H), 4.10 (dd, $J = 9.9, 3.2$ Hz, 1H), 4.05–3.96 (m, 2H), 3.95–3.86 (m, 3H), 3.85–3.71 (m, 6H), 3.69–3.55 (m, 6H), 3.51 (dd, $J = 12.0, 6.8$ Hz, 1H), 3.37–3.17 (m, 1H), 2.93 (d, $J = 32.4$ Hz, 2H), 2.81 (dd, $J = 12.3, 4.6$ Hz, 1H), 2.01–1.94 (m, 9H), 1.87–1.69 (m, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.1, 173.6, 173.52, 173.45, 158.0, 136.3, 128.4, 127.9, 127.1, 104.1, 99.0, 96.7, 76.9, 75.2, 74.4, 71.4, 71.3, 70.2, 68.6, 68.3, 68.2, 66.5, 66.3, 64.6, 62.0, 60.9, 60.6, 51.4, 48.9, 48.3, 39.9, 37.1, 28.1, 21.72, 21.66, 21.5. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{38}\text{H}_{57}\text{N}_4\text{O}_{21}$ 905.3521; found 905.3546.

Neu5Ac7,9diNAc α 2-3Gal β 1-3GalNAc β ProNHCBz (66).—4.4 mg, 65% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.44 (dt, $J = 18.5, 6.5, 3.0$ Hz, 5H), 5.15 (d, $J = 22.8$ Hz, 2H), 4.49 (d, $J = 8.0$ Hz, 1H), 4.43 (dd, $J = 14.7, 8.6$ Hz, 1H), 4.17 (d, $J = 3.1$ Hz, 1H), 4.09–3.91 (m, 6H), 3.84–3.72 (m, 7H), 3.67–3.57 (m, 5H), 3.51–3.45 (m, 1H), 3.36–3.13 (m, 2H), 3.09–2.98 (m, 1H), 2.80 (dd, $J = 12.4, 4.5$ Hz, 1H), 2.02–1.99 (m, 6H), 1.95 (s, 6H), 1.84–1.80 (m, 1H), 1.78–1.72 (m, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.1, 173.80, 173.76, 173.61, 173.58, 158.1, 136.4, 128.5, 128.1, 127.3, 103.2, 100.6, 99.2, 82.0, 75.4, 75.2, 74.8, 71.5, 69.5, 68.8, 68.42, 68.36, 67.5, 66.6, 66.5, 60.8, 60.5, 54.3, 51.6, 50.2, 41.4, 40.0, 37.1, 28.5, 21.91, 21.88, 21.7, 21.6. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{40}\text{H}_{60}\text{N}_5\text{O}_{21}$ 946.3786; found 946.3803.

Neu5Ac7NAc α 2-6Gal β 1-3GalNAc β ProNHCBz (67).—10.4 mg, 67% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.61–7.33 (m, 5H), 5.15 (d, $J = 20.8$ Hz, 2H), 4.50–4.36 (m, 2H), 4.20 (dd, $J = 19.0, 3.2$ Hz, 1H), 4.02–3.83 (m, 8H), 3.78–3.59 (m, 9H), 3.56–3.48 (m, 2H), 3.43–3.10 (m, 1H), 3.00–2.87 (m, 2H), 2.84–2.70 (m, 1H), 2.01 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.89–1.63 (m, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.0, 173.8, 173.7, 173.3, 157.9, 136.7, 128.8, 128.4, 127.6, 104.9, 104.4, 100.4, 75.0, 74.7, 73.2, 72.5, 72.4, 71.70, 71.67, 71.5, 71.4, 70.6, 70.4, 68.6, 68.5, 67.4, 66.8, 62.5, 61.2, 61.0, 52.0, 51.2, 49.23, 49.16, 45.7, 40.3, 22.2, 22.1, 21.9. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{38}\text{H}_{57}\text{N}_4\text{O}_{21}$ 905.3521; found 905.3530.

Neu5Ac7,9diNAc α 2-6Gal β 1-3GalNAc β ProNHCBz (68).—10.4 mg, 70% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.55–7.34 (m, 5H), 5.23–5.09 (m, 2H), 4.43 (ddd, $J = 14.7, 9.2, 5.6$ Hz, 2H), 4.23–4.11 (m, 1H), 4.01–3.89 (m, 6H), 3.84–3.53 (m, 11H), 3.47–3.33 (m, 2H), 3.29–3.02 (m, 2H), 2.98–2.88 (m, 1H), 2.80–2.70 (m, 1H), 2.04–1.98

(m, 9H), 1.96 (s, 3H), 1.84–1.62 (m, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.2, 173.99, 173.97, 173.7, 173.3, 158.3, 136.6, 128.8, 128.3, 127.6, 104.9, 100.5, 100.4, 75.0, 73.21, 73.16, 72.5, 71.61, 71.58, 70.6, 70.4, 69.6, 69.4, 68.6, 67.7, 67.4, 66.8, 61.0, 52.0, 51.2, 50.6, 45.7, 41.9, 40.3, 34.3, 28.9, 22.2, 22.1, 21.90, 21.87. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{40}\text{H}_{60}\text{N}_5\text{O}_{21}$ 946.3786; found 946.3805.

Neu5Ac7NAc α 2–3Gal β 1–3GalNAc α ProNHCBz (69).—20 mg, 77% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.50–7.35 (m, 5H), 5.17–5.09 (m, 2H), 4.84 (d, $J = 3.8$ Hz, 1H), 4.56–4.48 (m, 1H), 4.37–4.30 (m, 1H), 4.25–4.19 (m, 1H), 4.11–4.06 (m, 1H), 4.00–3.92 (m, 3H), 3.88 (ddd, $J = 9.5, 6.8, 2.4$ Hz, 1H), 3.82 (dd, $J = 10.5, 3.1$ Hz, 1H), 3.80–3.67 (m, 7H), 3.63–3.54 (m, 4H), 3.53–3.46 (m, 2H), 3.32–3.20 (m, 2H), 2.81 (dd, $J = 12.4, 4.6$ Hz, 1H), 1.99 (s, 6H), 1.95 (s, 3H), 1.85–1.79 (m, 2H), 1.76 (t, $J = 12.1$ Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.5, 174.0, 173.9, 173.8, 158.4, 136.7, 128.8, 128.3, 127.5, 104.5, 99.4, 97.1, 77.2, 75.6, 74.8, 71.73, 71.70, 70.6, 69.0, 68.7, 68.6, 66.9, 66.7, 65.0, 62.3, 61.2, 60.9, 51.8, 49.2, 48.7, 40.2, 37.5, 28.4, 22.1, 22.0, 21.8. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{38}\text{H}_{57}\text{N}_4\text{O}_{21}$ 905.3521; found 905.3536.

Neu5Ac7,9diNAc α 2–3Gal β 1–3GalNAc α ProNHCBz (70).—10.2 mg, 65% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.53–7.31 (m, 5H), 5.17–5.09 (m, 2H), 4.85 (d, $J = 3.8$ Hz, 1H), 4.52–4.42 (m, 1H), 4.32 (dd, $J = 11.1, 3.8$ Hz, 1H), 4.26–4.20 (m, 1H), 4.10–4.00 (m, 2H), 3.99–3.89 (m, 4H), 3.83 (dd, $J = 10.4, 3.1$ Hz, 1H), 3.78–3.68 (m, 6H), 3.63–3.53 (m, 3H), 3.52–3.44 (m, 2H), 3.34–3.21 (m, 2H), 3.04 (dd, $J = 14.0, 7.9$ Hz, 1H), 2.80 (dd, $J = 12.4, 4.6$ Hz, 1H), 2.02–1.94 (m, 12H), 1.87–1.71 (m, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.4, 174.04, 173.99, 173.8, 158.4, 136.7, 128.8, 128.3, 127.4, 104.4, 99.5, 97.0, 77.4, 75.6, 74.7, 71.7, 70.6, 69.7, 69.1, 68.6, 66.8, 66.7, 65.0, 61.2, 60.9, 51.8, 50.5, 48.6, 41.7, 40.2, 37.5, 28.4, 22.1, 22.0, 21.9. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{40}\text{H}_{60}\text{N}_5\text{O}_{21}$ 946.3786; found 946.3804.

Neu5Ac7NAc α 2–6Gal β 1–3GalNAc α ProNHCBz (71).—13.5 mg, 77% yield, white amorphous solid. ^1H NMR (400 MHz, D_2O) δ 7.61–7.29 (m, 5H), 5.28–5.04 (m, 2H), 4.84 (d, $J = 3.7$ Hz, 1H), 4.43 (d, $J = 7.8$ Hz, 1H), 4.32 (dd, $J = 11.0, 3.8$ Hz, 1H), 4.24 (s, 1H), 4.11–3.40 (m, 19H), 3.38–3.19 (m, 2H), 2.78 (dd, $J = 12.5, 4.6$ Hz, 1H), 2.07–1.91 (m, 9H), 1.89–1.75 (m, 2H), 1.65 (t, $J = 12.1$ Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, D_2O) δ 174.3, 173.9, 173.8, 173.3, 158.4, 136.6, 128.8, 128.3, 127.5, 103.9, 100.4, 97.0, 77.7, 73.2, 72.4, 71.7, 71.4, 70.4, 68.6, 68.5, 68.3, 66.7, 65.1, 63.7, 62.4, 61.6, 52.0, 49.1, 48.7, 40.4, 37.6, 28.5, 22.1, 22.0. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{38}\text{H}_{57}\text{N}_4\text{O}_{21}$ 905.3521; found 905.3533.

Neu5Ac7,9diNAc α 2–6Gal β 1–3GalNAc α ProNHCBz (72).—14.6 mg, 72% yield, white amorphous solid. ^1H NMR (400 MHz, D_2O) δ 7.35 (q, $J = 7.5$ Hz, 5H), 5.05 (s, 2H), 4.76 (d, $J = 3.7$ Hz, 1H), 4.34 (d, $J = 7.7$ Hz, 1H), 4.23 (dd, $J = 11.1, 3.8$ Hz, 1H), 4.15 (s, 1H), 3.98–3.29 (m, 18H), 3.26–3.11 (m, 2H), 3.00 (dd, $J = 14.1, 7.3$ Hz, 1H), 2.69 (dd, $J = 12.4, 4.6$ Hz, 1H), 1.98–1.81 (m, 12H), 1.79–1.65 (m, 2H), 1.56 (t, $J = 12.1$ Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz, D_2O) δ 174.5, 174.2, 173.9, 173.7, 173.3, 158.4, 136.6, 128.8, 128.3, 127.5, 103.9, 100.4, 97.2, 77.7, 73.2, 72.4, 71.6, 70.4, 69.4, 68.6, 68.5, 68.3, 66.7,

64.7, 63.7, 61.6, 52.0, 50.5, 48.7, 41.8, 40.4, 22.1, 22.00, 21.96, 21.9. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3793.

Neu5Ac7NAca2-3Gal β 1-3GlcNAc β ProNHCBz (73).—24.2 mg, 73% yield, white amorphous solid. 1H NMR (600 MHz, D_2O) δ 7.43 (h, $J = 7.3$ Hz, 5H), 5.12 (s, 2H), 4.53–4.45 (m, 2H), 4.15–4.07 (m, 1H), 3.98–3.44 (m, 20H), 3.25–3.11 (m, 2H), 2.81 (dd, $J = 12.3, 4.5$ Hz, 1H), 2.04–1.92 (m, 9H), 1.80–1.72 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.5, 174.0, 173.9, 173.8, 158.3, 136.6, 128.8, 128.3, 127.5, 103.6, 100.8, 99.3, 82.4, 75.6, 75.4, 75.1, 71.7, 71.7, 68.9, 68.62, 68.57, 67.7, 66.8, 66.7, 62.3, 61.1, 60.7, 54.5, 51.7, 49.2, 40.2, 37.3, 28.7, 22.2, 22.1, 21.8. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3534.

Neu5Ac7,9diNAca2-3Gal β 1-3GlcNAc β ProNHCBz (74).—11.6 mg, 66% yield, white amorphous solid. 1H NMR (400 MHz, D_2O) δ 7.55–7.35 (m, 5H), 5.12 (s, 2H), 4.48 (dd, $J = 11.2, 8.1$ Hz, 2H), 4.26–3.34 (m, 20H), 3.28–3.10 (m, 2H), 3.02 (dd, $J = 14.2, 7.7$ Hz, 1H), 2.80 (dd, $J = 12.5, 4.6$ Hz, 1H), 2.07–1.88 (m, 12H), 1.76 (d, $J = 10.2$ Hz, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.3, 174.03, 173.99, 173.84, 173.81, 158.3, 136.6, 128.8, 128.3, 127.5, 103.4, 100.8, 99.5, 82.2, 75.6, 75.4, 75.1, 71.7, 69.7, 69.0, 68.7, 68.6, 67.7, 66.8, 66.7, 61.0, 60.7, 54.5, 51.8, 50.5, 41.7, 40.2, 37.3, 28.7, 22.14, 22.11, 21.88, 21.86. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3807.

Neu5Ac7NAca2-6Gal β 1-3GlcNAc β ProNHCBz (75).—25.4 mg, 75% yield, white amorphous solid. 1H NMR (400 MHz, D_2O) δ 7.84–7.09 (m, 5H), 5.13 (d, $J = 10.4$ Hz, 2H), 4.52 (d, $J = 8.5$ Hz, 1H), 4.39 (d, $J = 7.8$ Hz, 1H), 4.22–3.35 (m, 21H), 3.28–3.09 (m, 2H), 2.74 (dd, $J = 12.4, 4.7$ Hz, 1H), 2.10–1.88 (m, 9H), 1.76 (tq, $J = 6.7, 3.1$ Hz, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, D_2O) δ 174.7, 173.9, 173.7, 173.5, 158.3, 136.6, 128.8, 128.4, 127.6, 104.0, 100.9, 100.1, 84.4, 75.6, 73.5, 72.5, 71.6, 71.5, 70.5, 69.0, 68.6, 68.3, 67.6, 66.8, 63.2, 62.4, 60.9, 54.2, 51.9, 49.2, 40.1, 37.2, 28.7, 22.15, 22.13, 22.10. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3531.

Neu5Ac7,9diNAca2-6Gal β 1-3GlcNAc β ProNHCBz (76).—23.7 mg, 70% yield, white amorphous solid. 1H NMR (400 MHz, D_2O) δ 7.50–7.39 (m, 5H), 5.13 (d, $J = 10.8$ Hz, 2H), 4.51 (d, $J = 8.5$ Hz, 1H), 4.39 (d, $J = 7.7$ Hz, 1H), 4.23–3.34 (m, 20H), 3.29–2.99 (m, 3H), 2.73 (dd, $J = 12.4, 4.6$ Hz, 1H), 2.17–1.91 (m, 12H), 1.86–1.64 (m, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, D_2O) δ 174.6, 174.2, 173.9, 173.6, 173.5, 158.3, 136.6, 128.8, 128.4, 127.6, 104.0, 100.9, 100.1, 84.3, 75.6, 73.5, 72.5, 71.6, 70.5, 69.5, 69.0, 68.7, 68.3, 67.6, 66.8, 63.1, 60.9, 54.2, 51.9, 50.6, 41.8, 40.1, 37.2, 28.7, 22.1, 21.9. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3799.

Neu5Ac7NAca2-3Gal β 1-3GlcNAcaProNHCBz (77).—18.4 mg, 89% yield, white amorphous solid. 1H NMR (600 MHz, D_2O) δ 7.48–7.38 (m, 5H), 5.19–5.09 (m, 2H), 4.48 (d, $J = 7.9$ Hz, 1H), 4.13–4.06 (m, 2H), 3.98 (dd, $J = 9.8, 3.0$ Hz, 1H), 3.95–3.68 (m, 12H), 3.66–3.43 (m, 7H), 3.34–3.17 (m, 2H), 2.81 (dd, $J = 12.4, 4.6$ Hz, 1H), 2.02–1.91 (m, 9H), 1.86–1.70 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.3, 174.0, 173.9, 173.8, 158.4, 136.7, 128.8, 128.3, 127.5, 103.4, 99.3, 97.0, 80.2, 75.6, 75.0, 71.75, 71.68, 71.6, 69.0, 68.57, 68.55, 66.8, 66.7, 64.9, 62.3, 61.0, 60.5, 52.5, 51.7, 49.2, 40.2, 37.4, 28.4, 22.1,

22.0, 21.8. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{38}H_{57}N_4O_{21}^-$ 905.3521; found 905.3540.

Neu5Ac7,9diNAc α 2-3Gal β 1-3GlcNAc α ProNHCBz (78).—14.5 mg, 69% yield, white amorphous solid. 1H NMR (600 MHz, D_2O) δ 7.47–7.37 (m, 5H), 5.17–5.09 (m, 2H), 4.52–4.40 (m, 1H), 4.08 (td, $J = 9.8, 3.4$ Hz, 2H), 4.00–3.87 (m, 4H), 3.87–3.68 (m, 8H), 3.62–3.45 (m, 6H), 3.40 (s, 1H), 3.27 (ddt, $J = 26.8, 13.3, 6.6$ Hz, 2H), 3.03 (dd, $J = 14.2, 7.9$ Hz, 1H), 2.80 (dd, $J = 12.4, 4.6$ Hz, 1H), 2.07–1.91 (m, 12H), 1.86–1.78 (m, 2H), 1.75 (t, $J = 12.1$ Hz, 1H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.2, 174.0, 173.84, 173.80, 158.4, 136.7, 128.8, 128.3, 127.4, 103.3, 99.5, 96.9, 80.4, 78.5, 75.6, 74.9, 71.7, 71.6, 69.7, 69.1, 68.62, 68.59, 66.8, 66.7, 64.9, 61.0, 60.5, 55.3, 52.4, 51.8, 50.5, 41.7, 40.3, 37.4, 28.4, 22.1, 21.94, 21.88, 21.86. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3808.

Neu5Ac7NAc α 2-6Gal β 1-3GlcNAc α ProNHCBz (79).—19.3 mg, 72% yield, white amorphous solid. 1H NMR (400 MHz, D_2O) δ 7.50–7.36 (m, 5H), 5.13 (s, 2H), 4.36 (d, $J = 7.8$ Hz, 1H), 4.20–3.40 (m, 22H), 3.34–3.17 (m, 2H), 2.74 (dd, $J = 12.4, 4.7$ Hz, 1H), 2.00 (d, $J = 6.3$ Hz, 6H), 1.98–1.91 (m, 3H), 1.86–1.71 (m, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, D_2O) δ 174.5, 173.9, 173.7, 173.5, 158.4, 136.6, 128.8, 128.3, 127.5, 103.9, 100.1, 96.8, 82.5, 73.4, 72.5, 71.7, 71.6, 71.5, 70.5, 68.8, 68.7, 68.3, 66.7, 65.0, 63.1, 62.4, 60.6, 52.1, 51.9, 49.2, 40.1, 37.5, 28.5, 22.13, 22.09, 21.9. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3531.

Neu5Ac7,9diNAc α 2-6Gal β 1-3GlcNAc α ProNHCBz (80).—13.8 mg, 68% yield, white amorphous solid. 1H NMR (600 MHz, D_2O) δ 7.51–7.37 (m, 5H), 5.14 (t, $J = 9.1$ Hz, 2H), 4.38 (dd, $J = 16.6, 7.9$ Hz, 1H), 4.10 (dd, $J = 10.5, 3.6$ Hz, 1H), 4.05–3.82 (m, 8H), 3.81–3.70 (m, 4H), 3.68–3.38 (m, 8H), 3.37–3.19 (m, 2H), 3.08 (dd, $J = 14.0, 7.3$ Hz, 1H), 2.73 (dd, $J = 12.4, 4.7$ Hz, 1H), 2.07–1.99 (m, 9H), 1.96 (s, 3H), 1.90–1.70 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.5, 174.2, 173.9, 173.6, 173.5, 158.4, 136.6, 128.8, 128.3, 127.5, 103.9, 100.1, 96.9, 82.4, 73.4, 72.5, 71.7, 71.5, 70.5, 69.5, 68.8, 68.7, 68.3, 66.7, 65.0, 63.0, 60.6, 52.1, 51.9, 50.6, 41.8, 40.1, 37.5, 28.5, 22.2, 22.1, 22.0, 21.9. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3794.

Neu5Ac7NAc α 2-6GalNAc α ProNHCBz (81).—32 mg, 84% yield, white amorphous solid. 1H NMR (800 MHz, D_2O) δ 7.47–7.36 (m, 5H), 5.19–5.07 (m, 2H), 4.82 (d, $J = 3.7$ Hz, 1H), 4.12 (dd, $J = 11.1, 3.7$ Hz, 1H), 4.03–3.93 (m, 3H), 3.91–3.84 (m, 4H), 3.75 (dt, $J = 11.5, 5.8$ Hz, 1H), 3.71–3.66 (m, 1H), 3.64 (dd, $J = 12.1, 2.3$ Hz, 1H), 3.60–3.51 (m, 2H), 3.50–3.44 (m, 2H), 3.31–3.19 (m, 2H), 2.75 (dd, $J = 12.5, 4.6$ Hz, 1H), 2.00 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.81 (p, $J = 6.3$ Hz, 2H), 1.65 (t, $J = 12.2$ Hz, 1H). $^{13}C\{^1H\}$ NMR (200 MHz, D_2O) δ 174.5, 174.0, 173.7, 173.3, 158.4, 136.6, 128.8, 128.3, 127.6, 100.2, 97.0, 71.6, 71.5, 69.5, 68.6, 68.5, 67.6, 66.7, 65.4, 64.3, 62.4, 52.0, 49.9, 49.2, 40.4, 37.6, 28.4, 22.1, 21.9, 21.8. HRMS (ESI-Orbitrap) m/z : $[M - H]^-$ Calcd for $C_{32}H_{47}N_4O_{16}$ 743.2993; found 743.3022.

Neu5Ac7,9diNAc α 2-6GalNAc α ProNHCBz (82).—33 mg, 85% yield, white amorphous solid. 1H NMR (600 MHz, D_2O) δ 7.48–7.41 (m, 5H), 5.14 (q, $J = 12.7$ Hz,

2H), 4.83 (d, $J = 3.9$ Hz, 1H), 4.17–4.10 (m, 1H), 3.98–3.86 (m, 6H), 3.80–3.66 (m, 3H), 3.62–3.53 (m, 2H), 3.52–3.43 (m, 2H), 3.33–3.21 (m, 2H), 3.08 (dd, $J = 14.1, 7.3$ Hz, 1H), 2.76 (dd, $J = 12.5, 4.6$ Hz, 1H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.83 (p, $J = 6.3$ Hz, 2H), 1.67 (t, $J = 12.2$ Hz, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (150 MHz, D_2O) δ 174.5, 174.2, 174.0, 173.7, 173.3, 158.4, 136.6, 128.8, 128.3, 127.6, 100.3, 97.0, 71.4, 69.7, 69.5, 68.63, 68.56, 67.6, 66.7, 65.5, 64.3, 52.0, 50.7, 49.9, 41.9, 40.4, 37.6, 28.4, 22.1, 21.91, 21.87, 21.8. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{34}\text{H}_{50}\text{N}_5\text{O}_{16}$ 784.3258; found 784.3285.

General Procedures for Synthesis of Neu5Ac7NAc- and Neu5Ac7,9diNAc-terminated Propylamino-glycosides by Reduction.

To a stirred solution of a glycoside (selected from **57–82**, 1.0–6.0 mg) in water-methanol solution (2 mL, 1:1 by volume), a catalytic amount of 10% palladium on charcoal was added to a round bottom flask (50 mL). The mixture was stirred under a hydrogen environment for 2–5 h. The solution was passed through filter to remove the catalyst. The solvent was removed under vacuum and lyophilized and used directly for microarray studies.

Neu5Ac7NAca2–3Lac β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{28}\text{H}_{48}\text{N}_3\text{O}_{19}$ 730.2887; found 730.2896.

Neu5Ac7,9diNAca2–3Lac β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{30}\text{H}_{51}\text{N}_4\text{O}_{19}$ (M-H) 771.3153; found 771.3170.

Neu5Ac7NAca2–6Lac β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{28}\text{H}_{48}\text{N}_3\text{O}_{19}$ 730.2887; found 730.2898.

Neu5Ac7,9diNAca2–6Lac β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{30}\text{H}_{51}\text{N}_4\text{O}_{19}$ 771.3153; found 771.3172.

Neu5Ac7NAca2–3LacNAc β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{30}\text{H}_{51}\text{N}_4\text{O}_{19}$ 771.3153; found 771.3157.

Neu5Ac7,9diNAca2–3LacNAc β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{32}\text{H}_{54}\text{N}_5\text{O}_{19}$ 812.3418; found 812.3427.

Neu5Ac7NAca2–6LacNAc β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{30}\text{H}_{51}\text{N}_4\text{O}_{19}$ 771.3153; found 771.3151

Neu5Ac7,9diNAca2–6LacNAc β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{32}\text{H}_{54}\text{N}_5\text{O}_{19}$ 812.3418; found 812.3416.

Neu5Ac7NAca2–3Gal β 1–3GalNAc β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{30}\text{H}_{51}\text{N}_4\text{O}_{19}$ 771.3153; found 771.3172.

Neu5Ac7,9diNAca2–3Gal β 1–3GalNAc β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{32}\text{H}_{54}\text{N}_5\text{O}_{19}$ 812.3418; found 812.3427.

Neu5Ac7NAca2–6Gal β 1–3GalNAc β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{30}\text{H}_{51}\text{N}_4\text{O}_{19}$ 771.3153; found 771.3154.

Neu5Ac7,9diNAca2–6Gal β 1–3GalNAc β ProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z : $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{32}\text{H}_{54}\text{N}_5\text{O}_{19}$ 812.3418; found 812.3421.

Neu5Ac7NAca2-3Galβ1-3GalNAcaProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₀H₅₁N₄O₁₉ 771.3153; found 771.3150.

Neu5Ac7,9diNAca2-3Galβ1-3GalNAcaProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₂H₅₄N₅O₁₉ 812.3418; found 812.3424.

Neu5Ac7NAca2-6Galβ1-3GalNAcaProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₀H₅₁N₄O₁₉ 771.3153; found 771.3147.

Neu5Ac7,9diNAca2-6Galβ1-3GalNAcaProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₂H₅₄N₅O₁₉ 812.3418; found 812.3422.

Neu5Ac7NAca2-3Galβ1-3GlcNAcβProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₀H₅₁N₄O₁₉ 771.3153; found 771.3158.

Neu5Ac7,9diNAca2-3Galβ1-3GlcNAcβProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₂H₅₄N₅O₁₉ 812.3418; found 812.3421.

Neu5Ac7NAca2-6Galβ1-3GlcNAcβProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₀H₅₁N₄O₁₉ 771.3153; found 771.3159.

Neu5Ac7,9diNAca2-6Galβ1-3GlcNAcβProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₂H₅₄N₅O₁₉ 812.3418; found 812.3421.

Neu5Ac7NAca2-3Galβ1-3GlcNAcaProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₀H₅₁N₄O₁₉ 771.3153; found 771.3161.

Neu5Ac7,9diNAca2-3Galβ1-3GlcNAcaProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₂H₅₄N₅O₁₉ 812.3418; found 812.3421.

Neu5Ac7NAca2-6Galβ1-3GlcNAcaProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₀H₅₁N₄O₁₉ 771.3153; found 771.3155.

Neu5Ac7,9diNAca2-6Galβ1-3GlcNAcaProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₃₂H₅₄N₅O₁₉ 812.3418; found 812.3421.

Neu5Ac7NAca2-6GaNAcaProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₂₄H₄₁N₄O₁₄ 609.2625; found 609.2631.

Neu5Ac7,9diNAca2-6GalNAcaProNH₂ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M – H][–] Calcd for C₂₆H₄₄N₅O₁₄ 650.2890; found 650.2901.

Sialoglycan Microarray Studies.

Glycans were printed on PolyAn Glass Slide, 3D-NHS (Automate Scientific) at 100 μM in 4 replicates each in phosphate buffer (300 mM, pH 8.4). Printed glycan microarray slides were blocked with ethanolamine (0.05 M) in Tris-HCl (0.1 M, pH 9.0), washed and dried. Slides were fitted in a multiwell microarray hybridization cassette (AHC4X8S, ArrayIt, Sunnyvale, CA, USA) to divide into 8 subarrays. The subarrays were blocked with 1% ovalbumin in PBS, pH 7.4 at room temperature for 1 h with gentle shaking (all incubations were carried out in a humid chamber). The blocking solution was removed followed by incubation at room temperature for 2 h with gentle shaking with 30 μg/mL of Fc-fused human Siglec 7 (hSiglec 7) or Fc-fused human Siglec 9 (hSiglec 9), or 20

$\mu\text{g/mL}$ of *Sambucus nigra* lectin (SNA) or *Maackia amurensis* lectin II (MAL II) (Vector Laboratories, Burlingame, CA) in blocking buffer. The slides were washed and incubated at room temperature for 1 h with goat anti-human IgG-Cy3 (1.5 $\mu\text{g/mL}$ in PBS) (Jackson ImmunoResearch Laboratories, 109-165-088) for Siglecs or streptavidin-Cy3 (1.5 $\mu\text{g/mL}$ in PBS) for lectins (Jackson ImmunoResearch, 109-160-084). The slides were subsequently washed and dried. The microarray slides were then scanned by Genepix 4000B microarray scanner (Molecular Devices Corp., Union City, CA, USA) and data analysis was performed using Genepix Pro 7.0 analysis software (Molecular Devices Corp., Union City, CA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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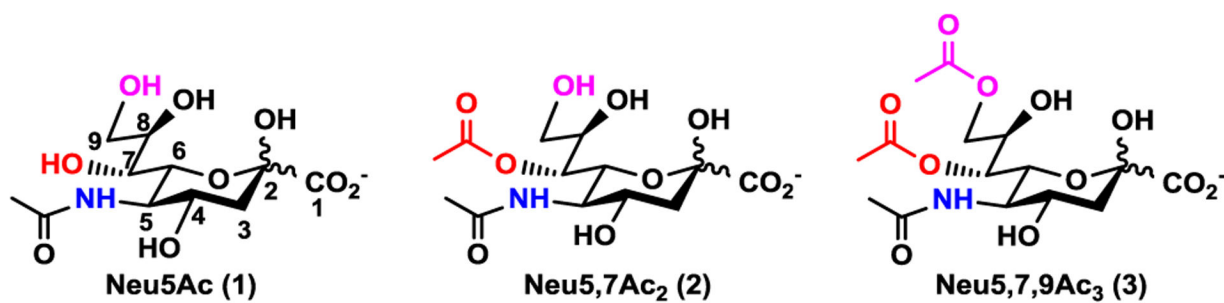


Figure 1.

Structures of *N*-acetylneuraminic acid (Neu5Ac, **1**), the most abundant natural sialic acid form, and its naturally occurring *O*-acetylated forms 7-*O*-acetyl-*N*-acetylneuraminic acid (Neu5,7Ac₂, **2**) and 7,9-di-*O*-acetyl-*N*-acetylneuraminic acid (Neu5,7,9Ac₃, **3**).

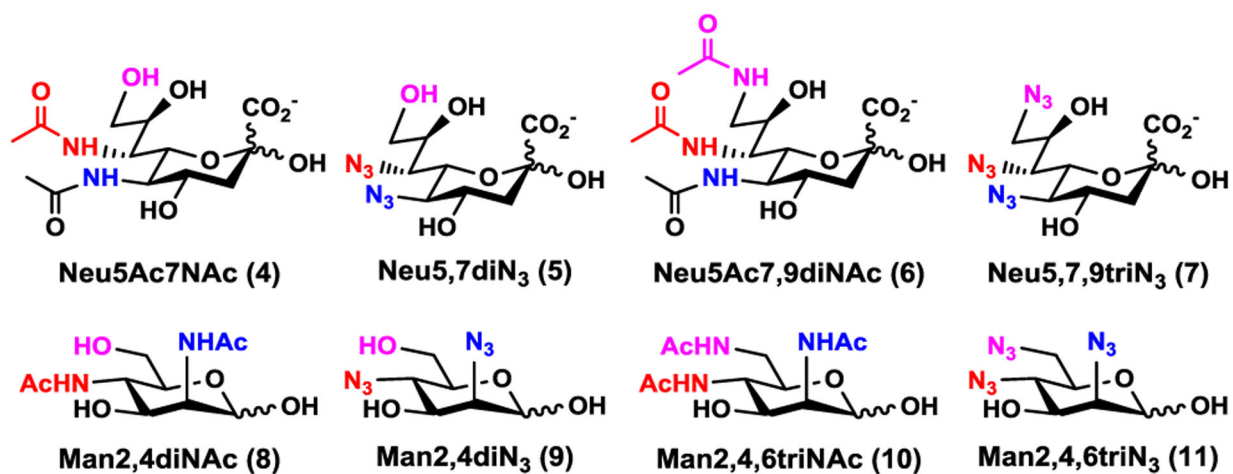


Figure 2.

Structures of *N*-acetyl analogue Neu5Ac7NAc (**4**) and azido derivative Neu5,7diN₃ (**5**) of Neu5,7Ac₂ (**2**); *N*-acetyl analogue Neu5Ac7,9diNAc (**6**) and azido derivative Neu5,7,9triN₃ (**7**) of Neu5,7,9Ac₃ (**3**); as well as their corresponding hexose precursors Man2,4diNAc (**8**), Man2,4diN₃ (**9**), Man2,4,6triNAc (**10**), and Man2,4,6triN₃ (**11**), respectively.

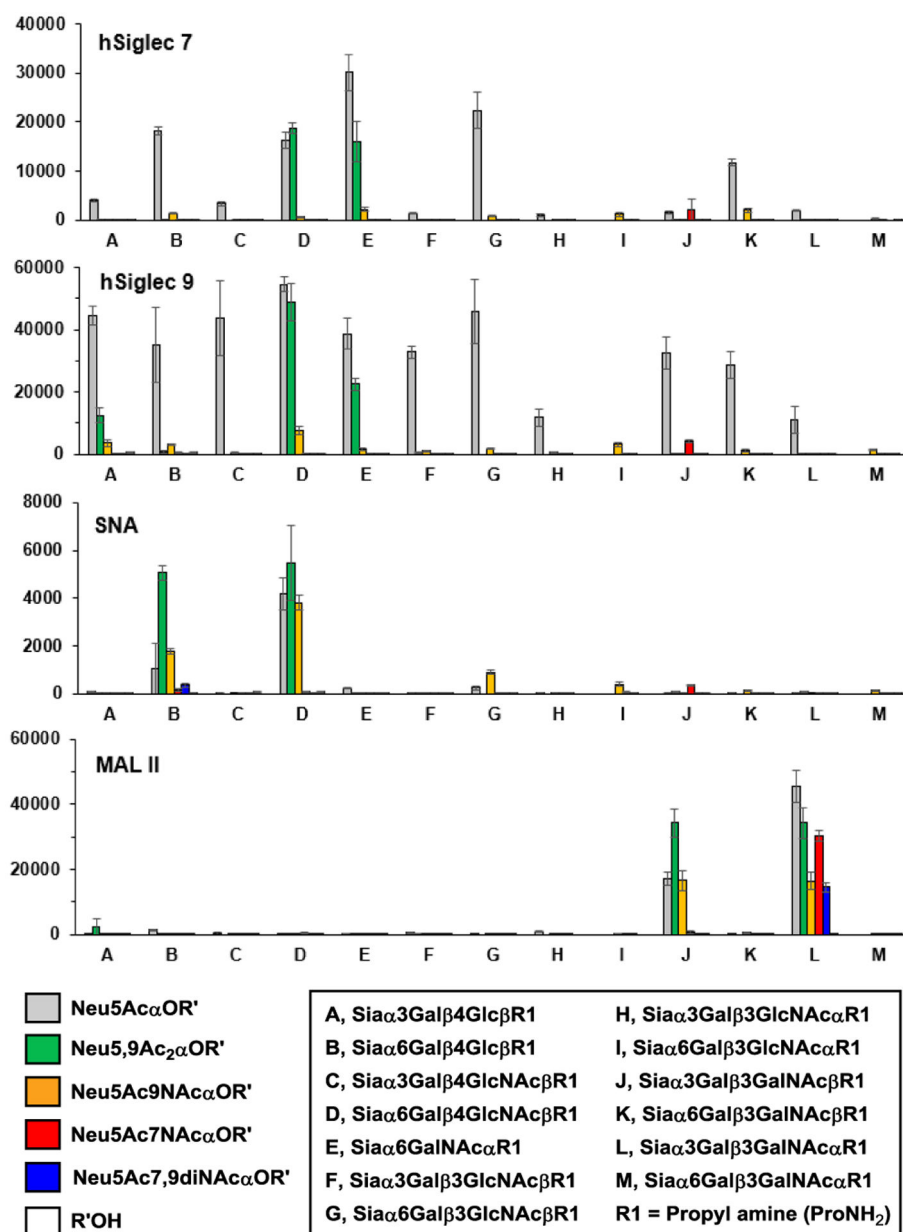
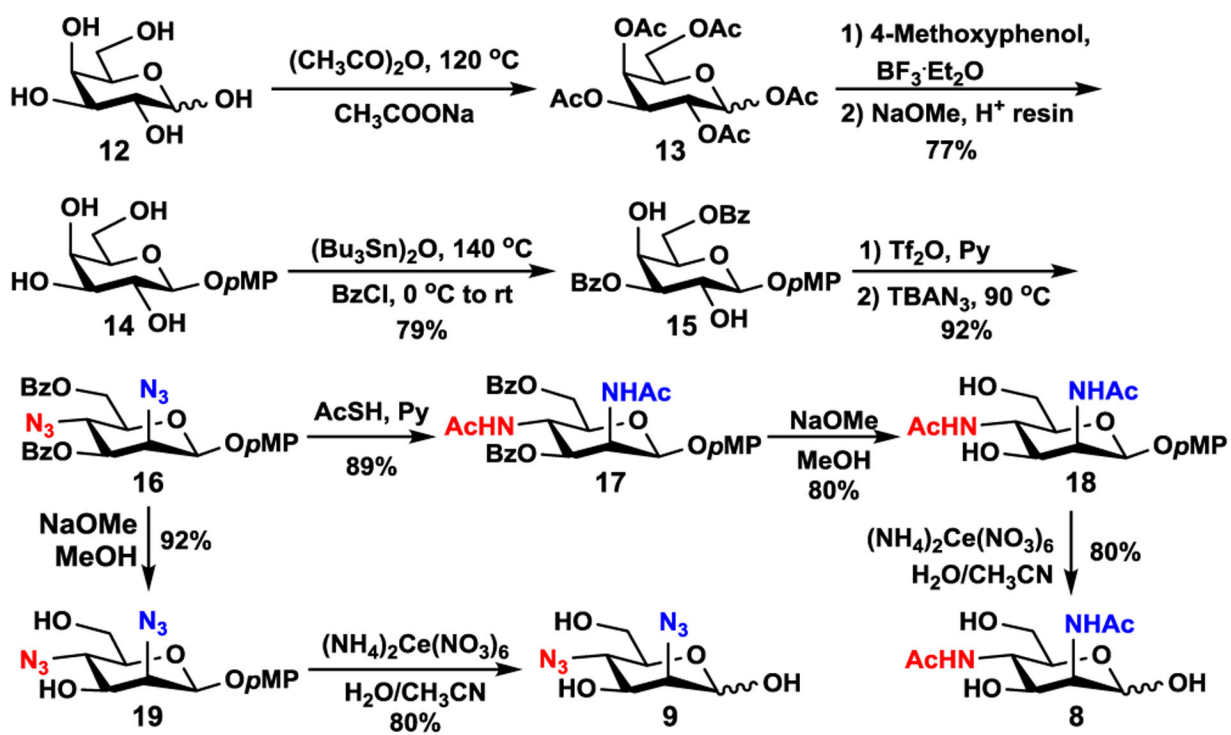
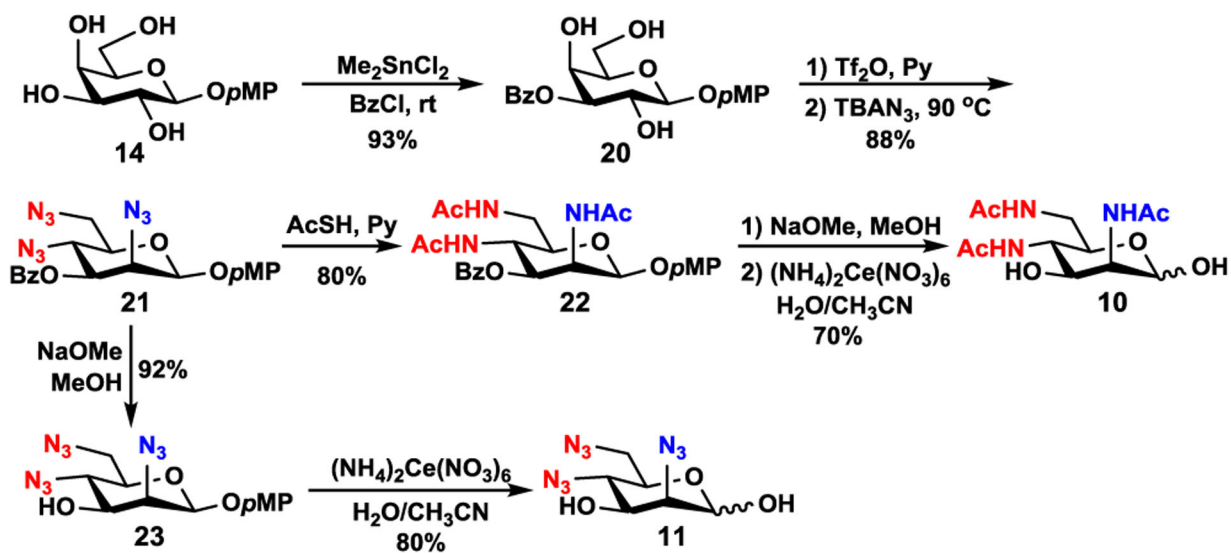


Figure 3. Glycan microarray study results for sialoside binding by hSiglec 7, hSiglec 9, SNA, and MAL II (Numeric data are shown in Table S1). Asialoglycans (R'OH) corresponding to the internal glycans in the sialosides are negative controls shown in white bars. R1 = ProNH $_2$.



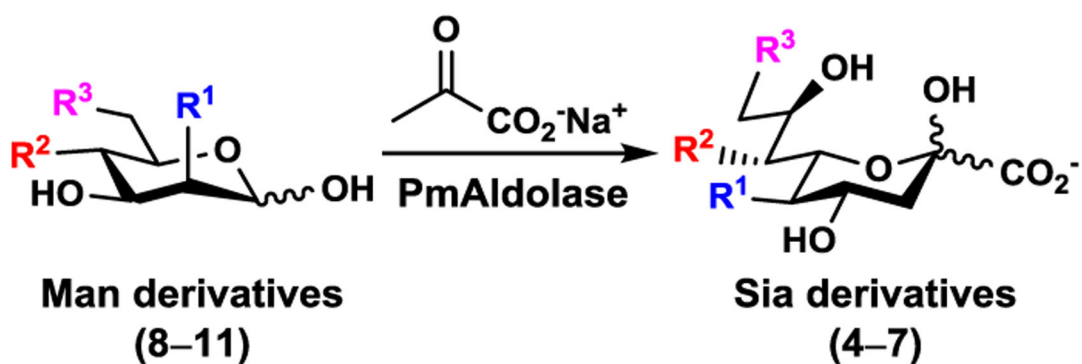
Scheme 1.

Chemical synthesis of Man2,4diNac (**8**) and Man2,4diN₃ (**9**) from galactose (**12**). Ac = acetyl, *p*MP = *para*-methoxyphenyl, Bz = benzoyl.



Scheme 2.

Synthesis of Man2,4,6triNAc (**10**) and Man2,4,6triN₃ (**11**) from galactoside **14**.



4 (72%) or 8, $R^1 = R^2 = \text{NHAc}$, $R^3 = \text{OH}$

5 (72%) or 9, $R^1 = R^2 = \text{N}_3$, $R^3 = \text{OH}$

6 (0%) or 10, $R^1 = R^2 = R^3 = \text{NHAc}$

7 (70%) or 11, $R^1 = R^2 = R^3 = \text{N}_3$

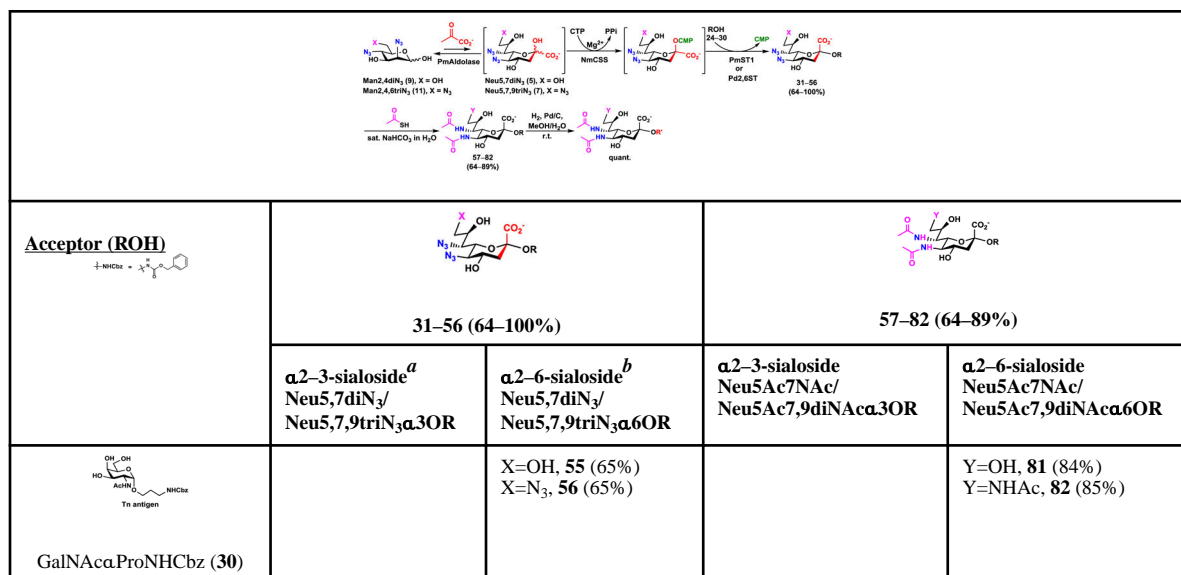
Scheme 3.

PmAldolase-catalyzed preparative-scale synthesis of sialic acid derivatives Neu5Ac7NAc (**4**), Neu5,7diN₃ (**5**), and Neu5,7,9triN₃ (**7**) from the corresponding mannose derivatives Man2,4diNAc (**8**), Man2,4diN₃ (**9**), and Man2,4,6triN₃ (**11**), respectively. Man2,4,6triNAc (**10**) was not a suitable substrate for PmAldolase-catalyzed synthesis of Neu5Ac7,9diNAc (**6**).

Table 1.

One-pot three-enzyme (OP3E) synthesis of sialosides containing Neu5,7diN₃ or Neu5,7,9triN₃ (**31–56**) from Man2,4diN₃ (**9**) or Man2,4,6triN₃ (**11**) and chemical conversion of the azido groups to *N*-acetyl moieties to form sialosides containing desired Neu5Ac7NAc or Neu5Ac7,9diNAc (**57–82**). The Cbz group in the resulting sialosides was removed from the NAc-sialosides in quantitative yields to produce sialosides containing a propylamine aglycone for glycan microarray studies. R' differs from R by replacing the propyl NHCbz aglycon in R with the propylamine (ProNH₂) in R'.

Acceptor (ROH) 	 31–56 (64–100%)		 57–82 (64–89%)	
	α2–3-sialoside^a Neu5,7diN₃/ Neu5,7,9triN₃α3OR	α2–6-sialoside^b Neu5,7diN₃/ Neu5,7,9triN₃α6OR	α2–3-sialoside Neu5Ac7NAc/ Neu5Ac7,9diNAcα3OR	α2–6-sialoside Neu5Ac7NAc/ Neu5Ac7,9diNAcα6OR
 LacβProNHCbz (24)	X=OH, 31 (90%) X=N ₃ , 32 (92%)	X=OH, 33 (quant.) X=N ₃ , 34 (quant.)	Y=OH, 57 (68%) Y=NHAc, 58 (80%)	Y=OH, 59 (78%) Y=NHAc, 60 (80%)
 LacNAcβProNHCbz (25)	X=OH, 35 (70%) X=N ₃ , 36 (66%)	X=OH, 37 (72%) X=N ₃ , 38 (65%)	Y=OH, 61 (67%) Y=NHAc, 62 (67%)	Y=OH, 63 (77%) Y=NHAc, 64 (85%)
 Galβ3GalNAcβProNHCbz (26)	X=OH, 39 (99%) X=N ₃ , 40 (67%)	X=OH, 41 (Quant.) X=N ₃ , 42 (69%)	Y=OH, 65 (64%) Y=NHAc, 66 (65%)	Y=OH, 67 (67%) Y=NHAc, 68 (70%)
 Galβ3GalNAc α ProNHCbz (27)	X=OH, 43 (95%) X=N ₃ , 44 (65%)	X=OH, 45 (68%) X=N ₃ , 46 (79%)	Y=OH, 69 (77%) Y=NHAc, 70 (65%)	Y=OH, 71 (77%) Y=NHAc, 72 (72%)
 Galβ3GlcNAcβProNHCbz (28)	X=OH, 47 (87%) X=N ₃ , 48 (64%)	X=OH, 49 (quant.) X=N ₃ , 50 (91%)	Y=OH, 73 (73%) Y=NHAc, 74 (66%)	Y=OH, 75 (75%) Y=NHAc, 76 (70%)
 Galβ3GlcNAc α ProNHCbz (29)	X=OH, 51 (93%) X=N ₃ , 52 (87%)	X=OH, 53 (quant.) X=N ₃ , 54 (96%)	Y=OH, 77 (89%) Y=NHAc, 78 (69%)	Y=OH, 79 (72%) Y=NHAc, 80 (68%)



^aPmST1 was used as the α 2–3sialyltransferase;

^bPd2,6ST was used as the α 2–6sialyltransferase.