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Chemoenzymatic synthesis of α2–3-sialylated carbohydrate epitopes

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Abstract

Sialic acids are common terminal carbohydrates on cell surface. Together with internal carbohydrate structures, they play important roles in many physiological and pathological processes. In order to obtain α2–3-sialylated oligosaccharides, a highly efficient one-pot three-enzyme synthetic approach was applied. The *P. multocida* α2–3-sialyltransferase (PmST1) involved in the synthesis was a multifunctional enzyme with extremely flexible donor and acceptor substrate specificities. Sialyltransferase acceptors, including type 1 structure (Galβ1–3GlcNAcβProN_3), type 2 structures (Galβ1–4GlcNAcβProN_3 and 6-sulfo-Galβ1–4GlcNAcβProN_3), type 4 structure (Galβ1–3GalNAcβProN_3), type 3 or core 1 structure (Galβ1–3GalNAcαProN_3) and human milk oligosaccharide or lipooligosaccharide lacto-*N*-tetraose (LNT) (Galβ1–3GlcNAcβ1–3Galβ1–4GlcβProN_3), were chemically synthesized. They were then used in one-pot three-enzyme reactions with sialic acid precursor ManNAc or ManNGc, to synthesize a library of natural occurring α2–3-linked sialosides with different internal sugar units. The sialylated oligosaccharides obtained are valuable probes for their biological studies

Keywords

carbohydrate; chemoenzymatic synthesis; sialic acid; sialylation; sialyltransferase

1 Introduction

In nature, terminal sialic acids are commonly α2–3- orα2–6-linked to galactose (Gal) or *N*-acetyl-galactosamine (GalNAc) [1]. Sialic acid residues can also be α2–8- or α2–9-linked to each other [2]. Currently, more than 50 different sialic acid forms have been identified in nature. The presentation of different forms is species and tissue specific. They are developmentally regulated and are believed to be closely related to their biological functions [3–5]. In addition to the naturally existing diversity of sialic acids and sialyl linkages, the internal carbohydrates are quite different which provide additional complexity of sialic acid-containing structures. For example, sialic acids can be α2–3- to the terminal galactose residue in glycans containing disaccharides such as Galβ1–3GlcNAcβ-(type 1 glycan), Galβ1–4GlcNAcβ-(type 2 glycan), Galβ1–3GalNAcα-(core 1 or type 3 glycan, TF or T antigen), Galβ1–3GalNAcβ-(type 4 glycan), and Galβ1–4Glcβ-(type 5 glycan). Sialic acids are also commonly seen α2–6-linked to the terminal galactose residue in glycans containing disaccharides such as Galβ1–3GlcNAcβ-(type 1 glycan), Galβ1–4GlcNAcβ-(type 2 glycan), and Galβ1–4Glcβ-(type 5 glycan). α2–6-Sialylated GalNAcα-(Siaα2–6GalNAcα-, STn antigen) has also been found in the *O*-GalNAc glycans on glycoproteins of certain types of

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cancer cells and is believed to be a potent cancer marker (Figure 1A) [6–8]. Sialylated lipooligosaccharide (LOS) structures have been found in many bacteria, such as *Haemophilus influenzae* [9], possibly *Pasteurella multocida* [10], *Escherichia coli* K1 and K92 [11]. The core structures for sialylation in LOS are lacto-*N*-tetraose (LNT) and lacto-*N*-neotetraose (LNnT). They both can be sialylated and their corresponding sialo-sides are called sialyllacto-*N*-tetraose (LST) a, b, c and d (Figure 1b) [12, 13]. In addition, sialyl Lewis^x and sialyl Lewis^a are two of the most important sialosides that have been broadly studied. They both contain a sialic acid α2–3-linked to a trisaccharide composed of galactose, GalNAc and fucose (Figure 1c), with or without sulfation at galatose or *N*-acetylglucosamine (GlcNAc) [14].

The interaction of sialoside and sialic acid-binding proteins is dependent on the diversity of sialoside structures including sialic acid forms, sialyl linkages, and internal carbohydrate structures [4, 15]. Other than well documented importance of sialic acids and sialyl linkages in host-virus interactions [16–21], the internal carbohydrate structures of sialosides have also been reported to be important for Siglec binding in the immune system [22–25]. For example, among CD-33 related Siglecs, Siglec-8 in human binds strongly only to sialyl Lewis^x structures with *O*-sulfation at *C*-6 of galactose. Sialyl Lewis^x, sialyl LacNAc, and sialyl Lewis^x with *O*-sulfation at *C*-6 of GlcNAc did not bind to Siglec-8 at all [26–28]. However, Siglec-9 favored sialyl Lewis^x structures with *O*-sulfation at the *C*-6 of GlcNAc instead of galactose [29]. Another example of the importance of sialic acid form was shown by preferred binding of Siglec-10 to Neu5Gcα2–6LacNAc rather than to Ne5Acα2–6LacNAc [25].

To better understand the biological significance of sialic acid-containing glycans, homogenous sialosides with different forms of sialic acids and various internal carbohydrates are needed. Chemical sialylation is usually more challenging and more time consuming compared to other chemical glycosylation processes due to the structural complexity of sialic acid [30] including a sterically hindered tertiary anomeric center, lacking of a stereo-directing group adjacent to the anomeric position, and the presence of an electron-withdrawing carboxyl group in sialyl donors. Isolation of sialosides from natural sources is also difficult due to their low abundance, lability, and the complication of diverse sialic acid forms, different linkages, and various internal glycans. To overcome these difficulties, chemoenzymatic methods were developed in our laboratory to obtain homogenous sialosides [31–34].

Herein we report the synthesis of a library of α2–3-sialylated glycans using an efficient one-pot three-enzyme chemoenzymatic approach. Sialyltransferase acceptors, including type 1 (Galβ1–3GlcNAcβProN_3), type 2 (Galβ1–4GlcNAcβProN_3 and 6-sulfo-Galβ1–4GlcNAcβProN_3), type 3 or core 1 (Galβ1–3GalNAcαProN_3), and type 4 (Galβ1–3GalNAcβProN_3) disaccharides as well as type 1 tetrasaccharide (Galβ1–3GlcNAcβ1–3Galβ1–4GlcβProN_3) were chemically synthesized and used in the one-pot three-enzyme system containing a sialic acid aldolase, a CMP-sialic acid synthetase, and an α2–3-sialyltransferase for the production of a library of natural occurring α2–3-linked sialoside epitopes. Two different sialic acid precursors, *N*-acetylmannosamine (ManNAc) and *N*-glycolylmannosamine (ManNGc), were used to produce α2–3-linked sialosides containing *N*-acetylneuraminic acid (Neu5Ac), the most abundant sialic acid form, and *N*-glycolylneuraminic acid (Neu5Gc), a common non-human animal sialic acid form, respectively.

2 Experimental

2.1 General experimental section

^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-300, a Varian Inova-400, or a Varian Inova-600 spectrometer. Chemical shifts were reported in (ppm) unit using ^{13}C and residual ^{1}H signals from deuterated solvents as references. Assignment of ^{1}H NMR spectra was achieved using 2D methods (COSY) when necessary. Low and high resolution electrospray ionization (ESI) mass spectra were obtained at the Mass Spectrometry Facility at the Ohio State University. Silica gel 60 A (40–63 μm, Sorbent technologies) was used for flash column chromatography. Analytical thin layer chromatography was performed on silica gel plates 60 GF_{254} (Sorbent technologies). Anisaldehyde stain was used for detection. Gel filtration chromatography was performed on a column (100 cm ×2.5 cm) packed with BioGel P-2 Fine resins (Bio-Rad, Hercules, CA). All reagents were of analytical grade and were used as supplied without further purification unless specified. Solvents used in chemical reactions were distilled under an inert argon atmosphere.

2.2 Chemical synthesis of sialyltransferase acceptors

2.2.1 3-Azidopropyl β-D-galactopyranosyl-(1–3)-2-acetamido-2-deoxy-β-D-glucopyranoside (Galβ1–3GlcNAcβProN_3, 1)—Compound **7** [35] (0.90 g, 1.62 mmol) was dissolve in dry MeOH (20 mL), NaOMe (100 mg) was then added. The reaction mixture was stirred for overnight, neutralized by Dowex-50 (H^+) resin, and evaporated to dryness. This dried product was dissolved in 10 mL of anhydrous DMF, fol-lowed by the addition of $PhCH(OMe)_2$ (1 mL, 6.57 mmol). The pH of the solution was adjusted to 2–4 by adding D(+)-10-camphorsulfonic acid and the mixture was heated at 60 °C under reducing pressure at 150 mbar. After 1 hour, the reaction mixture was condensed *in vacuo*. The product was purified by flash column chromatography (Hexane: EtOAc = 3:2, by volume) to afford a white foam **8** (0.73 g, 87%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (dd, 1 H, *J* = 5.4 Hz and 3.0 Hz), 7.70 (dd, 1 H, *J* = 6.0 and 3.3 Hz), 7.50–7.49 (m, 2 H), 7.37–7.36 (m, 3 H), 5.56 (s, 1 H), 5.23 (d, 1 H, *J* = 8.4 Hz), 4.64 (t, 1 H, *J* = 9.6 Hz), 4.37 (dd, 1 H, *J* = 10.5 and 4.5 Hz), 4.21 (dd, 1 H, *J* = 10.8 and 2.4 Hz), 3.94–3.91 (m, 1 H), 3.81 (t, 1 H, *J* = 10.5 Hz), 3.64–3.56 (m, 3 H), 3.28–3.19 (m, 2 H), 1.94–1.90 (m, 1 H), 1.82–1.76 (m, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.21, 134.43, 131.85, 129.60, 128.62, 126.57, 102.14, 99.33, 83.38, 68.88, 68.67, 67.48, 66.36, 56.82, 41.48, 32.25.

A solution of compound **8** (0.69 g, 1.32 mmol) and galactosyl trichloroacetimidate **9** [36] (0.79 g, 1.60 mmol) in anhydrous CH_2Cl_2 (10 mL) was stirred with activated 4 A molecular sieves (1.20 g) under argon atmosphere for 30 min. The reaction mixture was cooled to −20 °C and TMSOTf (20μL) was added drop wisely. The reaction mixture was quenched with Et_3N (0.5 mL) after 2 hrs and was filtered through Celite. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (Toluene:EtOAc = 3:1, by volume) to give the glycosylation product **10** (0.60 g, 53%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (dd, 2 H, *J* = 5.4 Hz and 3.0 Hz), 7.75 (dd, 2 H, *J* = 5.4 Hz and 3.0 Hz), 7.48–7.46 (m, 2 H), 7.36–7.35 (m, 3 H), 5.56 (m, 1 H), 5.17 (dd, 1 H, *J* = 3.3 and 0.9 Hz), 5.13 (d, 1 H, *J* = 9.0 Hz), 4.97 (dd, 1 H, *J* = 10.2 and 7.8 Hz), 4.77–4.72 (m, 2 H), 4.53 (d, 1 H, *J* = 8.4 Hz), 4.36 (dd, 1 H, *J* = 10.2 Hz and 4.8 Hz), 4.27 (dd, 1 H, *J* = 10.2 and 8.4 Hz), 4.01 (dd, 1 H, *J* = 10.8 and 7.8 Hz), 3.91–3.88 (m, 1 H), 3.84 (t, 1 H, *J* = 10.2 Hz), 3.81–3.77 (m, 2 H), 3.65–3.62 (m, 1 H), 3.56–3.53 (m, 1 H), 3.49–3.46 (m, 1 H), 3.23–3.20 (m, 1 H), 3.17–3.13 (m, 1 H), 1.94–1.90 (m, 1 H), 1.89 (s, 3 H), 1.82 (s, 3 H), 1.81–1.75 (m, 1 H), 1.54 (s, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.47, 170.26, 170.24, 169.09, 137.23, 134.54, 131.86, 129.50, 128.58, 126.24, 101.68, 100.73, 99.17, 81.16, 75.80, 71.20, 70.51, 69.38, 68.90, 67.35, 66.85, 66.51, 60.99, 55.54, 41.48, 32.25, 20.82, 20.76, 20.65, 20.31.

The protected disaccharide **10** (600 mg, 0.70 mmol) was dissolved in 10 mL of methanol and 100 mg Pd/C was added. The mixture was shaken under H_2 (4 Bar) for overnight, filtered, and dried. The dried product, TBAI (100 mg, 0.27 mmol), and sodium azide (228 mg, 3.5 mmol) were dissolved in 10 mL of anhydrous DMF. After stirred at 60 °C for 3 hours, the reaction mixture was poured into water (50 mL) and extracted by ethyl acetate (30 mL). The organic layer was dried to afford product **11** (485 mg, 95%), which was dissolved in ethanol (5 mL), followed by the addition of hydrazine hydrate (64% hydrazine, 10 mL). After being refluxed at 100°C for 3 hours, the reaction mixture was directly dried *in vacuo*. To the dried white powder was added pyridine (10 mL) and acetic anhydride (2 mL). After overnight stirring, the mixture was concentrated and purified by flash column chromatography (Hexane:Acetone = 1:1, by volume). The purified white foam product was dissolved in dry MeOH (10 mL), and NaOMe (50 mg) was added. The reaction was stirred for overnight, neutralized by Dowex-50 (H^+) resin, and evaporated to dryness to afford Galβ1–3GlcNAcβProN_3 (**1**) (262 mg, 84%). ^{1}H NMR (600 MHz, D_2O) δ 4.51 (d, 1 H, *J* = 8.4 Hz), 4.39 (d, 1 H, *J* = 7.8 Hz), 3.96–3.92 (m, 1 H), 3.90–3.87 (m, 2 H), 3.80–3.59 (m, 8 H), 3.51–3.44 (m, 3 H), 3.35–3.32 (m, 2 H), 2.00 (s, 3 H), 1.80 (pentalet, 2 H, *J* = 6.6 Hz). ^{13}C NMR (75 MHz, D_2O)δ 174.73, 103.64, 101.04, 82.48, 75.44, 75.38, 72.56, 70.77, 68.81, 68.61, 67.26, 61.13, 60.80, 54.66, 47.88, 28.21, 22.32. HRMS (ESI) *m/z* calcd for $C_{17}H_{30}N_4O_{11}$ (M-H) 465.1828, found 465.1833.

2.2.2 2.3-Azidopropyl β-D-galactopyranosyl-(1–4)-2-acetamido-2-deoxy-β-D-glucopyranoside (Galβ1–4GlcNAcβProN_3, 2)—To a solution of compound **7** (2.0 g, 3.91 mmol) in methanol (30 mL) was added NaOMe (100 mg). After stirring at RT for 2 hrs, TLC (Hexane:Acetone = 1:2, by volume) showed the completion of the reaction. The reaction mixture was neutralized by Dowex-50 (H^+) resin, filtered, and evaporated to dryness. The dried product was then dissolved in anhydrous CH_2Cl_2, followed by the addition of imidazole (531 mg, 7.82 mmol) and TBSCl (718 mg, 4.69 mmol). After overnight stirring, the mixture was condensed and purified by flash column chromatography (Hexane:EtOAc = 2:1, by volume) to afford a white foam **12** (1.64 g, 84%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (dd, 2 H, *J* = 5.4 Hz and 3.0 Hz), 7.71 (dd, 2 H, *J* = 5.4 Hz and 3.0 Hz), 5.20 (d, 1 H, *J* = 8.4 Hz), 4.36 (dd, 1 H, *J* = 11.4 Hz and 9.0 Hz), 4.09 (dd, 1 H, *J* = 10.8 Hz and 4.8 Hz), 3.98 (dd, 1 H, *J* = 10.8 Hz and 4.8 Hz), 3.91–3.86 (m, 2 H), 3.63–3.56 (m, 2 H), 3.54–3.51 (m, 1 H), 3.40–3.34 (m, 2 H), 1.96–1.90 (m, 1 H), 1.85–1.80 (m, 1 H), 0.91 (s, 9 H), 0.12 (s, 3 H), 0.11 (s, 3 H).

A solution of acceptor **12** (0.75 g, 1.50 mmol) and trichloroacetimidate donor **9** (0.89 g, 1.80 mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred with activated 4 A molecular sieves (2 g) under argon atmosphere for 30 min. The reaction mixture was cooled to −40 °C and TMSOTf (15 μL) was added drop wisely. The reaction mixture was quenched with Et_3N (0.5 mL) after 10 hours, and filtered through celite. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (Hexane:EtOAc = 2:1, by volume) to afford the coupled product **13** (0.98 g, 79%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75–7.73 (m, 2 H), 7.64–7.62 (m, 2 H), 5.27 (d, 1 H, *J* = 3.0 Hz), 5.14 (dd, 1 H, *J* = 10.2 Hz and 8.4 Hz), 5.11 (d, 1 H, *J* = 8.4 Hz), 4.90 (dd, 1 H, *J* = 10.5 Hz and 3.3 Hz), 4.53 (d, 1 H, *J* = 7.8 Hz), 4.31 (dd, 1 H, *J* = 10.8 Hz and 8.4 Hz), 4.02–3.94 (m, 4 H), 3.92–3.89 (m, 1 H), 3.82–3.77 (m, 2 H), 3.68 (dd, 1 H, *J* = 11.4 Hz and 3.6 Hz), 3.56 (t, 1 H, *J* = 9.0 Hz), 3.51–3.47 (m, 1 H), 3.42 (dd, 1 H, *J* = 7.8 Hz and 2.4 Hz), 3.35–3.28 (m, 2 H), 2.03 (s, 3 H), 2.00 (s, 3 H), 1.88 (s, 3 H), 1.80 (s, 3 H), 1.88–1.70 (m, 2 H), 0.83 (s, 9 H), 0.03 (s, 3 H), 0.00 (s, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.59, 170.18, 169.43, 134.27, 131.89, 101.92, 98.19, 82.12, 74.90, 71.35, 71.08, 69.81, 68.91, 66.99, 65.91, 61.75, 61.70, 56.23, 41.61, 32.52, 26.08, 20.90, 20.76, 20.70, 20.45, 18.48, −4.69, −5.00.

Compound **13** (0.98 g, 1.18 mmol) was treated by TBAF (4 mL, 1 M in THF) in THF (20 mL) for 2 hours. The TLC (Hexane:EtOAc = 1:2, by volume) showed the TBS group was removed. After flash column chromatography purification, the product was dissolved in DMF (10 mL). Sodium azide (325 mg, 5 mmol) and TBAI (50 mg, 0.14 mmol) were added. The mixture was heated at 70 °C for 4 hrs, condensed *in vacuo*, and purified by flash column chromatography (Hexane:EtOAc = 1:2, by volume) to afford compound **14** (647 mg, 76%). Deprotection and *N*-acetylation of compound **14** (647 mg, 0.89 mmol) were achieved by using a similar approach as described for compound **11** to give Galβ1–4GlcNAcβProN$_3$ (**2**) (343 mg, 82%) as a white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.51 (d, 1 H, *J* = 7.8 Hz), 4.45 (d, 1 H, *J* = 7.8 Hz), 3.98–3.95 (m, 2 H), 3.90 (d, 1 H, *J* = 3.6 Hz), 3.81 (dd, 1 H, *J* = 12.3 Hz and 5.1 Hz), 3.76–3.67 (m, 7 H), 3.65 (dd, 1 H, *J* = 9.6 Hz and 3.6 Hz), 3.59–3.56 (m, 1 H), 3.52 (dd, 1 H, *J* = 9.6 Hz and 7.8 Hz), 3.37–3.34 (m, 2 H), 2.03 (s, 3 H), 1.82 (pentalet, 2 H, *J* = 6.6 Hz). ^{13}C NMR (150 MHz, D_2O) δ 174.62, 103.01, 101.24, 78.56, 75.48, 74.88, 72.63, 72.50, 71.09, 68.68, 67.26, 61.16, 60.18, 55.22, 47.90, 28.24, 22.31. HRMS (ESI) *m/z* calcd for $C_{17}H_{30}N_4O_{11}$ (M-H) 465.1828, found 465.1840.

2.2.3 3-Azidopropyl β-D-galactopyranosyl-(1–4)-2-acetamido-2-deoxy-6-O-sulfo-β-D-glucopyranoside (Galβ1–4GlcNAc6SβProN$_3$, 3)—To a solution of compound **13** (500 mg, 0.60 mmol) in pyridine (10 mL) was added acetic anhydride (1 mL). After overnight stirring, the reaction mixture was diluted by EtOAc (100 mL) and washed sequentially with 2N HCl (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL). The organic layer was dried by $MgSO_4$ and evaporated to produce a yellow syrup, which was treated by TBAF and sodium azide (with TBAI) sequentially to give compound **15** (434 mg, 85%) with a free hydroxyl group at the *C*-6 of GlcNAc. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86–7.81 (m, 2 H), 7.74–7.71 (m, 2 H), 5.72 (dd, 1 H, *J* = 10.8 Hz and 9.0 Hz), 5.39 (d, 1 H, *J* = 8.4 Hz), 5.32 (dd, 1 H, *J* = 3.3 and 0.9 Hz), 5.11 (dd, 1 H, *J* = 10.8 and 7.8 Hz), 4.98 (dd, 1 H, *J* = 10.2 and 3.6 Hz), 4.65 (d, 1 H, *J* = 7.8 Hz), 4.15 (dd, 1 H, *J* = 10.8 and 8.4 Hz), 4.09–4.00 (m, 3 H), 3.94–3.92 (m, 1 H), 3.89–3.86 (m, 2 H), 3.80–3.76 (m, 1 H), 3.59–3.57 (m, 1 H), 3.54–3.51 (m, 1 H), 3.23–3.19 (m, 1 H), 3.16–3.12 (m, 1 H), 2.12 (s, 3 H), 2.02 (s, 3 H), 1.95 (s, 3 H), 1.89 (s, 3 H), 1.70–1.67 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.61, 170.45, 170.36, 170.11, 169.46, 134.66, 134.47, 123.78, 101.23, 98.16, 75.79, 75.03, 71.56, 71.18, 70.70, 69.40, 66.91, 66.47, 61.00, 60.67, 55.15, 48.08, 29.03, 20.95, 20.91, 20.87, 20.81.

Compound **15** (434 mg, 0.51 mmol) and pyridine sulfur trioxide (182 mg, 1.02 mmol) were dissolved in pyridine (10 mL) and stirred at room temperature. The reaction was completed in 1.5 h. Purification by flash column chromatography (Hexane:EtOAc = 1:1, by volume) afforded the sulfated product. After this sulfated product was treated similarly as described for compound **11**, the sulfated disaccharide Galβ1–4GlcNAc6SβProN$_3$ (**3**) was obtained as a white foam (184 mg, 66%). ^{1}H NMR (600 MHz, D_2O) δ 4.54 (d, 1 H, *J* = 8.4 Hz), 4.52 (d, 1 H, *J* = 7.8 Hz), 4.38 (dd, 1 H, *J* = 11.1 and 1.5 Hz), 4.31 (dd, 1 H, *J* = 11.1 and 4.5 Hz), 3.97–3.94 (m, 1 H), 3.91 (d, 1 H, *J* = 3.6 Hz), 3.81–3.64 (m, 9 H), 3.51 (dd, 1 H, *J* = 7.8 and 1.8 Hz), 3.37–3.34 (m, 2 H), 2.04 (s, 3 H), 1.84–1.81 (m, 2 H). ^{13}C NMR (75 MHz, D_2O)δ 174.64, 102.65, 101.32, 77.48, 75.49, 72.67, 72.61, 72.40, 71.13, 68.75, 67.41, 66.39, 61.20, 55.22, 47.92, 28.26, 22.32. HRMS (ESI) m/z calcd for $C_{17}H_{29}N_4O_{14}SNa$ (M-Na) 545.1407, found 545.1398

2.2.4 3.3-Azidopropyl β-D-galactopyranosyl-(1–3)-2-acetamido-2-deoxy-α-D-galactopyranoside (Galβ1–3GalNAcαProN$_3$, 4)—To a solution of compound **16** [37] (2.5 g, 5.74 mmol) in 3-chloro-1-propanol (15 mL) was added acetyl chloride (1 mL). The solution was heated to 90 °C. Four hours later, TLC showed the disappearance of the starting material. The mixture was cooled to room temperature and transferred directly to silica gel column for purification (Hexanes:EtOAc = 1:1 followed by EtOAc, and then

Hexanes:Acetone = 1:1, by volume). According to the proton NMR spectroscopy, the purified product was an α/β mixture without any *O*-acetylation. The product was then dissolved in DMF (20 mL), followed by the addition of $PhCH(OMe)_2$ (2 mL). D(+)-10-Camphorsulfonic acid was added to adjust the pH to 2–4. The reaction mixture was heated at 50 °C at 150 mBar for 30 minutes, condensed, and purified by flash column chromatography (Hexane:EtOAc = 3:2, by volume) to afford two products, compound **17** (0.57 g, 21%) and **18** (1.55 g, 57%). For compound **17**, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 (dd, 2 H, *J* = 5.4 Hz and 3.0 Hz), 7.60 (dd, 2 H, *J* = 5.0 Hz and 3.0 Hz), 7.54 (dd, 2 H, *J* = 7.2 Hz and 1.5 Hz), 7.39–7.35 (m, 3 H), 5.61 (s, 1 H), 5.44 (dd, 1 H, *J* = 11.4 Hz and 3.6 Hz), 5.04 (d, 1 H, *J* = 3.0 Hz), 4.65 (dd, 1 H, *J* = 11.4 Hz and 3.6 Hz), 4.37 (d, 1 H, *J* = 4.2 Hz), 4.29 (dd, 1 H, *J* = 12.6 Hz and 1.2 Hz), 4.11 (dd, 1 H, *J* = 12.6 Hz and 1.2 Hz), 3.88–3.84 (m, 2 H), 3.42–3.39 (m, 1 H), 1.94–1.88 (m, 2 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.85, 137.70, 134.28, 131.92, 129.50, 128.53, 126.65, 123.45, 101.59, 98.84, 75.92, 69.72, 64.40, 63.34, 54.27, 41.90, 31.93. For compound **18**, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83–7.80 (m, 2 H), 7.71–7.69 (m, 2 H), 7.55–7.52 (m, 2 H), 7.40–7.36 (m, 3 H), 5.60 (s, 1 H), 5.25 (d, 1 H, *J* = 8.4 Hz), 4.52 (dd, 1 H, *J* = 11.1 Hz and 3.9 Hz), 4.42–4.36 (m, 2 H), 4.28 (d, 1 H, *J* = 3.6 Hz), 4.12 (dd, 1 H, *J* = 12.0 Hz and 1.8 Hz), 3.98–3.95 (m, 1 H), 3.64–3.59 (m, 2 H), 3.42–3.34 (m, 2 H), 1.98–1.93 (m, 1 H), 1.85–1.81 (m, 1 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.85, 137.57, 134.33, 129.57, 128.54, 126.71, 101.73, 98.74, 75.34, 69.45, 68.05, 67.00, 66.14, 54.94, 41.70, 32.37.

Compound **17** (570 mg, 1.20 mmol) was coupled to trichloroacetimidate **9** (710 mg, 1.44 mmol) using a similar approach as described for compound **10**. Purification by flash column chromatography (Hexane:EtOAc = 1:1, by volume) gave product **19** (461 mg, 62%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.85–7.81 (m, 2 H), 7.72–7.68 (m, 2 H), 7.59 (d, 2 H, *J* = 7.2 Hz), 7.39–7.33 (m, 3 H), 5.60 (s, 1 H), 5.36 (dd, 1 H, *J* = 11.7 Hz and 3.3 Hz), 5.32 (d, 1 H, *J* = 3.6 Hz), 5.06 (dd, 1 H, *J* = 10.5 Hz and 2.1 Hz), 5.00 (d, 1 H, *J* = 3.6 Hz), 4.93–4.89 (m, 2 H), 4.81 (d, 1 H, *J* = 8.4 Hz), 4.48 (d, 1 H, *J* = 3.0 Hz), 4.28 (d, 1 H, *J* = 12.6 Hz), 4.19 (dd, 1 H, *J* = 11.7 Hz and 5.7 Hz), 4.10–4.06 (m, 2 H), 3.92 (t, 1 H, *J* = 6.6 Hz), 3.88–3.85 (m, 1 H), 3.82 (s, 1 H), 3.60–3.53 (m, 2 H), 3.43–3.39 (m, 1 H), 2.10 (s, 3 H), 2.04 (s, 3 H), 1.95–1.88 (m, 2 H), 1.86 (s, 3 H), 1.15 (s, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.62, 170.55, 168.88, 168.48, 137.98, 134.50, 134.36, 132.57, 131.20, 129.09, 128.41, 126.54, 123.58, 123.53, 101.82, 100.98, 98.96, 76.50, 72.51, 71.37, 70.87, 69.69, 68.98, 67.26, 64.44, 63.57, 61.63, 51.31, 41.83, 31.88, 21.00, 20.95, 20.73, 19.64.

Compound **19** (461 mg, 0.57 mmol) was dissolved in DMF (10 mL). Sodium azide (195 mg, 3.0 mmol) and TBAI (30 mg, 0.08 mmol) were added. The mixture was heated at 70 °C for 4 hrs, condensed *in vacuo*, diluted by water, and extracted by CH_2Cl_2. The organic layer was evaporated to dryness and 80% HOAc (15 mL) was added to dissolve it. The solution was then heated at 60 °C for 3 hrs to remove the benzylidene protecting group. After removal of the solvent, compound **21** (387 mg, 94%) was obtained without purification. Deprotection and *N*-acetylation of compound **21** (387 mg, 0.54 mmol) were achieved using a similar approach as described for compound **11** to give Galβ1–4GalNAcαProN_3 (**4**) (214 mg, 85%) as the final product. ^{1}H NMR (600 MHz, D_2O) δ 4.90 (d, 1 H, *J* = 3.6 Hz), 4.48 (d, 1 H, *J* = 7.8 Hz), 4.35 (dd, 1 H, *J* = 10.8 Hz and 3.6 Hz), 4.26 (d, 1 H, *J* = 2.4 Hz), 4.04 (dd, 1 H, *J* = 10.8 Hz and 3.0 Hz), 4.00 (t, 1 H, *J* = 6.0 Hz), 3.92 (d, 1 H, *J* = 3.0 Hz), 3.83–3.74 (m, 5 H), 3.68–3.63 (m, 2 H), 3.58–3.45 (m, 4 H), 2.04 (s, 3 H), 1.92 (quintet, 2 H, *J* = 6.6 Hz). ^{13}C NMR (150 MHz, D_2O) δ 174.70, 104.89, 97.41, 77.40, 75.18, 72.71, 70.81, 68.92, 68.79, 65.13, 61.39, 61.19, 48.87, 48.41, 28.17, 22.22. HRMS (ESI) *m/z* calcd for $C_{17}H_{30}N_4O_{11}$ (M-H) 465.1828, found 465.1829.

2.2.5 3-Azidopropylβ-D-galactopyranosyl-(1–3)-2-acetamido-2-deoxy-β-D-galactopyranoside (Galβ1–3GalNAcβProN_3, 5)—Compound **18** (1.0 g, 2.11 mmol)

was coupled to trichloroacetimidate **9** (1.25 g, 2.53 mmol) using a similar approach as described for compound **10**. Purification by flash column chromatography (Hexane:EtOAc = 1:1, by volume) gave product **20** (812 mg, 48%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84–7.82 (m, 2 H), 7.73–7.71 (m, 2 H), 7.57 (d, 2 H, *J* = 7.8 Hz), 7.38–7.31 (m, 3 H), 5.57 (s, 1 H), 5.27 (d, 1 H, *J* = 3.6 Hz), 5.08–5.05 (m, 2 H), 4.80 (dd, 1 H, *J* = 10.5 Hz and 3.3 Hz), 4.75 (dd, 1 H, *J* = 11.4 Hz and 3.0 Hz), 4.68 (dd, 1 H, *J* = 11.4 Hz and 8.4 Hz), 4.54 (d, 1 H, *J* = 8.4 Hz), 4.36 (d, 1 H, *J* = 3.0 Hz), 4.33 (d, 1 H, *J* = 12.6 Hz), 4.10–4.02 (m, 3 H), 3.95–3.92 (m, 1 H), 3.80 (t, 1 H, *J* = 6.6 Hz), 3.57 (s, 1 H), 3.56–3.52 (m, 1 H), 3.35–3.32 (m, 1 H), 3.30–3.26 (m, 1 H), 2.09 (s, 3 H), 2.00 (s, 3 H), 1.97–1.92 (m, 1 H), 1.87 (s, 3 H), 1.81–1.72 (m, 1 H), 1.39 (s, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.48, 170.31, 169.40, 168.96, 167.53, 137.93, 134.60, 134.49, 131.79, 129.10, 128.38, 126.59, 123.82, 123.53, 101.68, 101.08, 98.94, 75.77, 75.04, 71.18, 71.00, 69.44, 68.81, 67.09, 66.96, 65.81, 61.51, 51.93, 41.66, 32.13, 20.95, 20.91, 20.70, 19.99.

Compound **22** (664 mg, 91%) was obtained from compound **20** (812 mg, 1.01 mmol) using a similar approach as described for compound **21**. Deprotection and *N*-acetylation of compound **22** (664 mg, 0.92 mmol) were achieved using a similar approach as described for compound **11** to give Galβ1–4GalNAcβProN$_3$ (**5**) (326 mg, 76%) as the final product. ^{1}H NMR (600 MHz, D_2O) δ 4.51 (d, 1 H, *J* = 8.4 Hz), 4.45 (d, 1 H, *J* = 7.8 Hz), 4.19 (d, 1 H, *J* = 3.0 Hz), 4.02–3.97 (m, 2 H), 3.92 (d, 1 H, *J* = 3.6 Hz), 3.88 (dd, 1 H, *J* = 10.8 Hz and 3.0 Hz), 3.83–3.65 (m, 7 H), 3.62 (dd, 1 H, *J* = 9.6 Hz and 3.0 Hz), 3.53 (dd, 1 H, *J* = 10.2 Hz and 7.8 Hz), 3.41–3.38 (m, 2 H), 2.04 (s, 3 H), 1.86 (pentalet, 2 H, *J* = 6.6 Hz). ^{13}C NMR (150 MHz, D_2O) δ 174.88, 105.00, 101.57, 80.05, 75.15, 74.91, 72.65, 70.77, 68.76, 68.17, 67.19, 61.18, 61.11, 51.47, 47.99, 28.31, 22.45. HRMS (ESI) *m/z* calcd for $C_{17}H_{30}N_4O_{11}$ (M-H) 465.1828, found 465.1835.

2.2.6 3-Azidopropyl β-D-galactopyranosyl-(1–3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1–3)-β-D-galactopyranosyl-(1–4)-β-D-glucopy ranoside (Galβ1–3GlcNAcβ1–3Galβ1–4GlcβProN$_3$, 6)—To a solution of compound **23** [38] (1.00 g, 2.09 mmol) in MeOH (20 mL) was added NaOMe (100 mg). The reaction mixture was stirred for overnight, neutralized by Dowex-50 (H^+) resin, and evaporated to dryness. This dried product was dissolved in 10 mL of anhydrous DMF followed by the addition of $PhCH(OMe)_2$ (1 mL, 6.57 mmol). The pH of the solution was adjusted to 2–4 using D(+)-10-camphorsulfonic acid, and was then heated at 60°C under reducing pressure at 150 mbar. After 1 hour, the reaction mixture was condensed and purified by flash column chromatography (Hexane:EtOAc = 3:2, by volume) to afford a white foam **24** (0.76 g, 83%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87–7.85 (m, 2 H), 7.72–7.71 (m, 2 H), 7.50–7.48 (m, 2 H), 7.38–7.36 (m, 3 H), 5.57 (s, 1 H), 5.40 (d, 1 H, *J* = 10.8 Hz), 4.65 (t, 1 H, *J* = 9.6 Hz), 4.39 (dd, 1 H, *J* = 10.2 Hz and 4.8 Hz), 4.31 (t, 1 H, *J* = 10.5 Hz), 3.80 (t, 1 H, *J* = 10.2 Hz), 3.71–3.67 (m, 1 H), 3.60 (t, 1 H, *J* = 9.3 Hz), 2.72–2.63 (m, 2 H), 1.19 (t, 3 H, *J* = 7.5 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.00, 167.38, 137.17, 134.46, 130.00, 129.61, 129.23, 128.62, 126.55, 102.17, 82.33, 82.10, 70.60, 69.76, 68.86, 55.73, 24.43, 15.11.

Compound **24** (0.76 g, 1.73 mmol) was coupled to trichloroacetimidate **9** (1.02 g, 2.08 mmol) using the same approach as described for compound **10**. Purification by flash column chromatography (Hexane:EtOAc = 1:1, by volume) gave product **25** (1.03 g, 77%) as a white foam. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90–7.77 (m, 4 H), 7.49–7.47 (m, 2 H), 7.40–7.35 (m, 3 H), 5.57 (s, 1 H), 5.28 (d, 1 H, *J* = 10.8 Hz), 5.19 (d, 1 H, *J* = 3.6 Hz), 4.99 (dd, 1 H, *J* = 10.2 Hz and 7.8 Hz), 4.77 (t, 1 H, *J* = 10.2 Hz), 4.74 (dd, 1 H, *J* = 10.2 Hz and 3.6 Hz), 4.55 (d, 1 H, *J* = 7.8 Hz), 4.42–4.37 (m, 2 H), 4.03 (dd, 1 H, *J* = 10.8 Hz and 7.8 Hz), 3.85–3.80 (m, 3 H), 3.72–3.69 (m, 1 H), 3.48 (dd, 1 H, *J* = 7.8 Hz and 6.6 Hz), 2.71–2.62 (m, 2 H), 2.07 (s, 3 H), 1.91 (s, 3 H), 1.84 (s, 3 H), 1.55 (s, 3 H), 1.17 (t, 3 H, *J* = 7.2 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.49, 170.29, 170.27, 169.11, 137.22, 129.53,

128.60, 126.31, 126.25, 101.67, 100.64, 81.93, 91.15, 76.64, 71.20, 70.87, 70.52, 69.40, 68.90, 66.85, 61.02, 54.40, 24.09, 20.85, 20.79, 20.67, 20.33, 15.01.

A solution of glycosyl donor **25** (350 mg, 0.46 mmol) and glycosyl acceptor **26** [39] (400 mg, 0.38 mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred with activated 4 A molecular sieves (0.80 g) under argon for 30 min. The reaction mixture was cooled to −20 °C and NIS (368 mg, 1.64 mmol) was added. After 10 min, TfOH (10 μL) was added. The reaction mixture was quenched with Et_3N (0.5 mL) after 1 hour and filtered through celite. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (Hexanes:EtOAc = 2:1, by volume) to afford tetrasaccharide **27** as an amorphous solid (428 mg, 64%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.10 (dd, 1 H, *J* = 8.4 Hz and 1.2 Hz), 7.99 (dd, 1 H, *J* = 8.4 Hz and 1.2 Hz), 7.90–7.86 (m, 4 H), 7.74 (dd, 1 H, *J* = 8.4 Hz and 1.2 Hz), 7.65–7.60 (m, 3 H), 7.55–7.31 (m, 20 H), 7.25–7.17 (m, 4 H), 6.87 (d, 2 H, *J* = 7.8 Hz), 5.61 (t, 1 H, *J* = 9.6 Hz), 5.51 (d, 1 H, *J* = 3.0 Hz), 5.45 (s, 1 H), 5.35–5.31 (m, 2 H), 5.20 (d, 1 H, *J* = 8.4 Hz), 5.10 (dd, 1 H, *J* = 3.6 Hz and 1.2 Hz), 4.80 (dd, 1 H, *J* = 10.2 Hz and 1.8 Hz), 4.61 (dd, 1 H, *J* = 10.2 Hz and 3.6 Hz), 4.57 (d, 1 H, *J* = 7.8 Hz), 4.55 (d, 1 H, *J* = 7.8 Hz), 4.51 (dd, 1 H, *J* = 10.2 Hz and 9.0 Hz), 4.35–4.33 (m, 2 H), 4.28 (dd, 1 H, *J* = 10.8 Hz and 4.8 Hz), 4.23 (dd, 1 H, *J* = 12.6 Hz and 4.5 Hz), 4.07 (dd, 1 H, *J* = 10.2 Hz and 8.4 Hz), 4.04 (t, 1 H, *J* = 9.6 Hz), 3.97–3.93 (m, 3 H), 3.81–3.78 (m, 1 H), 3.74–3.58 (m, 5 H), 3.51–3.46 (m, 2 H), 3.37 (t, 1 H, *J* = 6.6 Hz), 3.25 (dd, 1 H, *J* = 11.4 Hz and 7.8 Hz), 3.17–3.10 (m, 2 H), 1.99 (s, 3 H), 1.83 (s, 3 H), 1.73 (s, 3 H), 1.721.60 (m, 2 H), 1.37 (s, 3 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.19, 170.29, 170.03, 168.73, 166.02, 165.99, 165.61, 165.57, 165.34, 164.21, 137.33, 133.54–132.88, 130.10–128.45, 128.05, 126.24, 101.55, 101.34, 100.85, 100.27, 99.15, 80.63, 77.41, 75.60, 75.11, 73.25, 72.75, 72.14, 71.88, 71.74, 71.19, 70.46, 69.84, 69.32, 68.64, 66.90, 66.72, 66.52, 62.54, 62.14, 61.00, 55.29, 48.10, 29.81, 20.61, 20.59, 20.46, 20.01.

Compound **27** (428 mg, 0.24 mmol) was dissolved in 80% acetic acid (10 mL) and heated to 60 °C. After 3 hours, the solvent was removed and the residue was dissolved in ethanol (5 mL). Hydrazine hydrate (10 mL, 66.7% in water) was added. The reaction mixture was refluxed at 100 °C for 3 hours, cooled to room temperature and evaporated to dryness. To the dry product in pyridine (10 mL) was added acetic andydride (1 mL). After overnight stirring, the mixture was condensed and applied to flash column chromatography (Hexanes:Acetone = 1:1, by volume) to give the *O*-acetyl/benzoyl protected tetrasaccharide. The *O*-acetyl and *O*-benzoyl were then removed by treating with NaOMe (50 mg) in methanol (10 mL) to afford Galβ1–3GlcNAcβ1–3Galβ1–4GlcβProN_3 (**6**) as a white foam (102 mg, 54%). ^{1}H NMR (600 MHz, D_2O) δ 4.74 (d, 1 H, *J* = 8.4 Hz), 4.50 (d, 1 H, *J* = 7.8 Hz), 4.45 (d, 2 H, *J* = 7.8 Hz), 4.16 (d, 1 H, *J* = 3.6 Hz), 4.03–3.98 (m, 2 H), 3.93–3.89 (m, 3 H), 3.84–3.71 (m, 10 H), 3.67–3.46 (m, 11 H), 3.34–3.30 (m, 1 H), 2.04 (s, 3 H), 1.92 (pentalet, 2 H, *J* = 6.6 Hz). ^{13}C NMR (75 MHz, D_2O) δ 175.19, 103.72, 103.18, 102.75, 102.36, 82.37, 82.22, 78.69, 75.52, 75.44, 75.13, 75.00, 74.62, 73.03, 72.73, 70.93, 70.24, 68.78, 68.72, 68.55, 67.61, 62.81, 61.26, 61.18, 60.78, 60.36, 54.94, 48.14, 28.48, 22.49. HRMS (ESI) *m/z* calcd for $C_{29}H_{50}N_4O_{21}$ (M-H) 789.2884, found 789.2875.

2.3 One-pot three-enzyme synthesis of α2–3-linked sialosides

General Procedure—A prospective acceptor for a multi-functional *Pasteurella multocida* α2–3-sialyltransferase PmST1 (**1–6**, 0.1 mmol), a sialic acid precursor (ManNAc or ManNGc, 1.2 eq), sodium pyruvate (1.8 equiv.), and CTP (1.2 equiv.) were dissolved in water. Stock solutions of Tris-HCl buffer (1 M, pH 8.8, 1 mL) and $MgCl_2 \cdot 6H_2O$ (0.2 M, 1 mL) were added. After the addition of a recombinant *E. coli* sialic acid aldolase [40] (1.96 mg), an *N. meningitidis* CMP-sialic acid synthetase [40] (1.08 mg), and PmST1 [31] (0.62 mg), water was added until the total volume of the reaction mixture reached 10 mL. The

reaction was carried out by incubating the solution in an isotherm incubator for 2 h at 37 °C (or for 15 h at room temperature) with agitation at 140 rpm. The product formation was monitored by TLC developed with EtOAc:MeOH:H_2O:HOAc = 5:2:1:0.2 (by volume, for substrates **1**, **2**, **4**, **5**) or 4:2:1:0.2 (by volume, for substrates **3** and **6**). TLC plates were stained with *p*-anisaldehyde sugar stain. The reaction was quenched by adding the same volume (10 mL) of ice-cold EtOH and incubation at 4 °C for 30 min. The mixture was then centrifuged to remove precipitates. The supernatant was concentrated and passed through a BioGel P-2 gel filtration column with water as the eluant to obtain the crude product. Further purification was performed using flash column chromatography to afford the desired α2–3-linked sialosides.

Neu5Acα2–3Galβ1–3GlcNAcβProN$_3$ (30): From disaccharide **1** (46.6 mg, 0.1 mmol), compound **30** was obtained as a white foam (66 mg, 85%). ^{1}H NMR (600 MHz, D_2O) δ 4.53 (d, 1 H, *J* = 8.4 Hz), 4.47 (d, 1 H, *J* = 7.8 Hz), 4.06 (dd, 1 H, *J* = 9.6 Hz and 3.0 Hz), 3.97–3.45 (m, 20 H), 3.37–3.34 (m, 2 H), 2.73 (dd, 1 H, *J* = 12.3 Hz and 4.5 Hz), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.82 (pentalet, 2 H, *J* = 6.3 Hz), 1.76 (t, 1 H, *J* = 12.3 Hz). ^{13}C NMR (75 MHz, D_2O)δ 175.08, 174.70, 174.04, 103.57, 101.02, 99.75, 82.63, 75.72, 75.49, 75.21, 72.92, 71.96, 69.20, 68.84, 68.51, 68.14, 67.36, 67.27, 62.56, 61.14, 60.83, 54.56, 51.77, 47.90, 39.87, 28.22, 22.43, 22.18. HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{19}Na$ (M-Na) 756.2782, found 756.2783.

Neu5Gcα2–3Galβ1–3GlcNAcβProN$_3$ (31): From disaccharide **1** (46.6 mg, 0.1 mmol), compound **31** was obtained as a white foam (65 mg, 83%). ^{1}H NMR (600 MHz, D_2O) δ 4.53 (d, 1 H, *J* = 8.4 Hz), 4.48 (d, 1 H, *J* = 7.8 Hz), 4.10 (s, 2 H), 4.07 (dd, 1 H, *J* = 9.6 Hz and 3.0 Hz), 3.98–3.44 (m, 20 H), 3.37–3.34 (m, 2 H), 2.75 (dd, 1 H, *J* = 12.3 Hz and 4.5 Hz), 2.02 (s, 3 H), 1.82 (pentalet, 2 H, *J* = 6.3 Hz), 1.78 (t, 1 H, *J* = 12.3 Hz). ^{13}C NMR (75 MHz, D_2O)δ 175.87, 174.71, 174.08, 103.59, 101.04, 99.77, 82.63, 75.73, 75.51, 75.22, 72.64, 72.04, 69.21, 68.84, 68.25, 68.07, 67.36, 67.28, 62.53, 61.15, 60.85, 54.58, 51.48, 47.92, 39.97, 28.24, 22.44. HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{20}Na$ (M-Na) 772.2731, found 772.2728.

Neu5Acα2–3Galβ1–4GlcNAcβProN$_3$ (32): From disaccharide **2** (46.6 mg, 0.1 mmol), compound **32** was obtained as a white foam (65 mg, 84%). ^{1}H NMR (600 MHz, D_2O) δ 4.53 (d, 1 H, *J* = 7.8 Hz), 4.50 (d, 1 H, *J* = 7.8 Hz), 4.09 (dd, 1 H, *J* = 9.9 Hz and 3.3 Hz), 3.99–3.93 (m, 3 H), 3.88–3.81 (m, 4 H), 3.73–3.53 (m, 13 H), 3.37–3.33 (m, 2 H), 2.73 (dd, 1 H, *J* = 12.6 Hz and 4.2 Hz), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.82 (pentalet, 2 H, *J* = 6.3 Hz), 1.78 (t, 1 H, *J* = 12.6 Hz). ^{13}C NMR (75 MHz, D_2O) δ 175.12, 174.61, 174.03, 102.69, 101.28, 99.93, 78.37, 75.58, 75.28, 74.86, 73.00, 72.46, 71.89, 69.50, 68.48, 68.20, 67.59, 67.25, 62.68, 61.15, 60.13, 55.20, 51.80, 47.89, 39.74, 28.23, 22.29, 22.17. HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{19}Na$ (M-Na) 756.2782, found 756.2789.

Neu5Gcα2–3Galβ1–4GlcNAcβProN$_3$ (33): From disaccharide **2** (46.6 mg, 0.1 mmol), compound **33** was obtained as a white foam (64 mg, 80%). ^{1}H NMR (600 MHz, D_2O) δ 4.53 (d, 1 H, *J* = 7.8 Hz), 4.50 (d, 1 H, *J* = 8.4 Hz), 4.11 (dd, 1 H, *J* = 9.3 Hz and 3.3 Hz), 4.10 (s, 2 H), 3.99–3.54 (m, 20 H), 3.38–3.34 (m, 2 H), 2.75 (dd, 1 H, *J* = 12.6 Hz and 4.8 Hz), 2.02 (s, 3 H), 1.82 (pentalet, 2 H, *J* = 6.0 Hz), 1.80 (t, 1 H, *J* = 12.6 Hz). ^{13}C NMR (75 MHz, D_2O)δ 175.90, 174.19, 174.06, 102.68, 101.27, 99.92, 78.36, 75.57, 75.28, 74.86, 72.71, 72.45, 71.94, 69.51, 68.21, 68.12, 67.57, 67.24, 62.64, 61.08, 60.12, 55.20, 51.49, 47.88, 39.79, 28.22, 22.29. HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{20}Na$ (M-Na) 772.2731, found 772.2730.

Neu5Acα2–3Galβ1–4GlcNAc6SβProN$_3$ (34): From disaccharide **3** (56.8 mg, 0.1 mmol), compound **34** was obtained as a white foam (77 mg, 88%). ^{1}H NMR (400 MHz, D_2O) δ

4.61 (d, 1 H, *J* = 7.6 Hz), 4.54 (d, 1 H, *J* = 8.0 Hz), 4.42–4.32 (m, 2 H), 4.12 (dd, 1 H, *J* = 9.8 Hz and 3.0 Hz), 3.99–3.53 (m, 18 H), 3.39–3.54 (m, 2 H), 2.74 (dd, 1 H, *J* = 12.4 Hz and 4.4 Hz), 2.04 (s, 3 H), 2.03 (s, 3 H), 1.84 (pentalet, 2 H, *J* = 6.2 Hz), 1.80 (t, 1 H, *J* = 12.0 Hz). ^{13}C NMR (75 MHz, D_2O) δ 175.09, 174.67, 174.21, 102.27, 101.36, 99.89, 77.29, 75.47, 75.24, 72.99, 72.66, 72.37, 71.64, 69.60, 68.59, 68.21, 67.62, 67.44, 66.40, 62.65, 61.21, 55.21, 51.86, 47.94, 39.74, 28.26, 22.34, 22.24. HRMS (ESI) *m/z* calcd for $C_{28}H_{45}N_5O_{22}SNa_2$ (M-Na) 858.2174, found 858.2151.

Neu5Gcα2–3Galβ1–4GlcNAc6SβProN$_3$ (35): From disaccharide **3** (56.8 mg, 0.1 mmol), compound **35** was obtained as a white foam (74 mg, 82%). ^{1}H NMR (400 MHz, D_2O) δ 4.60 (d, 1 H, *J* = 7.6 Hz), 4.53 (d, 1 H, *J* = 7.6 Hz), 4.40–4.31 (m, 2 H), 4.12 (dd, 1 H, *J* = 9.6 Hz and 2.4 Hz), 4.10 (s, 2 H), 3.98–3.52 (m, 18 H), 3.38–3.32 (m, 2 H), 2.75 (dd, 1 H, *J* = 12.6 Hz and 4.6 Hz), 2.03 (s, 3 H), 1.83 (pentalet, 2 H, *J* = 6.0 Hz), 1.80 (t, 1 H, *J* = 12.6 Hz). ^{13}C NMR (100 MHz, D_2O) δ 175.90, 174.69, 174.23, 102.36, 101.38, 99.95, 77.46, 75.53, 75.26, 72.73, 72.42, 71.70, 69.63, 68.34, 68.21, 67.66, 67.45, 66.44, 62.64, 61.22, 55.24, 51.63, 47.96, 39.85, 27.27, 22.38. HRMS (ESI) *m/z* calcd for $C_{28}H_{45}N_5O_{23}SNa_2$ (M-Na) 874.2124, found 874.2107.

Neu5Acα2–3Galβ1–3GalNAcαProN$_3$ (36): From disaccharide **4** (46.6 mg, 0.1 mmol), compound **36** was obtained as a white foam (68 mg, 87%). ^{1}H NMR (600 MHz, D_2O) δ 4.88 (d, 1 H, *J* = 3.6 Hz), 4.52 (d, 1 H, *J* = 7.8 Hz), 4.29 (dd, 1 H, *J* = 10.8 Hz and 3.6 Hz), 4.22 (d, 1 H, *J* = 3.0 Hz), 4.05 (dd, 1 H, *J* = 10.8 Hz and 3.6 Hz), 4.02 (dd, 1 H, *J* = 10.8 Hz and 3.0 Hz), 3.97 (t, 1 H, *J* = 6.0 Hz), 3.91 (d, 1 H, *J* = 3.6 Hz), 3.87–3.41 (m, 17 H), 2.73 (dd, 1 H, *J* = 12.3 Hz and 4.5 Hz), 2.01 (s, 3 H), 1.88 (pentalet, 2 H, *J* = 6.3 Hz), 1.76 (t, 1 H, *J* = 12.3 Hz). ^{13}C NMR (150 MHz, D_2O) δ 175.12, 174.71, 174.05, 104.60, 99.85, 97.31, 77.52, 75.78, 74.90, 72.93, 71.96, 70.76, 69.22, 68.70, 68.51, 68.17, 67.52, 65.04, 62.63, 61.35, 61.11, 51.78, 48.81, 48.30, 39.84, 28.10, 22.18. HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{19}Na$ (M-Na) 756.2782, found 756.2801.

Neu5Gcα2–3Galβ1–3GalNAcαProN$_3$ (37): From disaccharide **4** (46.6 mg, 0.1 mmol), compound **37** was obtained as a white foam (60 mg, 76%). ^{1}H NMR (600 MHz, D_2O) δ 4.88 (d, 1 H, *J* = 3.6 Hz), 4.52 (d, 1 H, *J* = 8.4 Hz), 4.30 (dd, 1 H, *J* = 11.1 Hz and 3.9 Hz), 4.22 (d, 1 H, *J* = 3.0 Hz), 4.10 (s, 2 H), 4.06 (dd, 1 H, *J* = 9.6 Hz and 3.6 Hz), 3.97 (t, 1 H, *J* = 6.0 Hz), 3.93–3.41 (m, 18 H), 2.75 (dd, 1 H, *J* = 12.6 Hz and 4.8 Hz), 2.01 (s, 3 H), 1.88 (pentalet, 2 H, *J* = 6.3 Hz), 1.78 (t, 1 H, *J* = 12.3 Hz). ^{13}C NMR (75 MHz, D_2O) δ 175.98, 174.79, 174.13, 104.67, 99.98, 97.42, 77.60, 75.91, 75.03, 72.78, 72.12, 70.87, 69.34, 68.80, 68.34, 68.24, 67.64, 65.21, 62.74, 61.45, 61.23, 51.62, 48.93, 48.44, 40.05, 28.19, 22.30. HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{20}Na$ (M-Na) 772.2731, found 772.2758.

Neu5Acα2–3Galβ1–3GalNAcβProN$_3$ (38): From disaccharide **5** (46.6 mg, 0.1 mmol), compound **38** was obtained as a white foam (63 mg, 81%). ^{1}H NMR (600 MHz, D_2O) δ 4.53 (d, 1 H, *J* = 7.8 Hz), 4.52 (d, 1 H, *J* = 8.4 Hz), 4.19 (d, 1 H, *J* = 3.0 Hz), 4.08 (dd, 1 H, *J* = 10.2 Hz and 3.0 Hz), 4.04–3.98 (m, 2 H), 3.90 (d, 1 H, *J* = 2.4 Hz), 3.87–3.54 (m, 16 H), 3.40 (t, 2 H, *J* = 6.6 Hz), 2.77 (dd, 1 H, *J* = 12.3 Hz and 4.5 Hz), 2.05 (s, 3 H), 2.04 (s, 3 H), 1.86 (pentalet, 2 H, *J* = 6.0 Hz), 1.80 (t, 1 H, *J* = 12.3 Hz). ^{13}C NMR (75 MHz, D_2O) δ 175.24, 174.95, 174.14, 104.80, 101.64, 99.95, 80.24, 75.82, 74.98, 73.04, 72.80, 72.04, 69.26, 68.59, 68.32, 68.12, 67.64, 67.27, 62.89, 62.77, 61.19, 51.93, 51.43, 48.07, 39.99, 28.36, 22.56, 22.31. HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{19}Na$ (M-Na) 756.2782, found 756.2783.

Neu5Gcα2–3Galβ1–3GalNAcβProN$_3$ (39): From disaccharide **5** (46.6 mg, 0.1 mmol), compound **39** was obtained as a white foam (63 mg, 79%). ^{1}H NMR (600 MHz, D_2O) δ 4.53 (d, 1 H, *J* = 7.8 Hz), 4.52 (d, 1 H, *J* = 8.4 Hz), 4.19 (d, 1 H, *J* = 3.6 Hz), 4.14 (s, 2 H),

4.10 (dd, 1 H, *J* = 9.6 Hz and 3.0 Hz), 4.04–3.55 (m, 19 H), 3.41 (t, 2 H, *J* = 6.3 Hz), 2.79 (dd, 1 H, *J* = 12.3 Hz and 4.5 Hz), 2.05 (s, 3 H), 1.87 (pentalet, 2 H, *J* = 6.3 Hz), 1.82 (t, 1 H, *J* = 12.3 Hz). ^{13}C NMR (150 MHz, D_2O) δ 175.92, 174.92, 174.16, 104.79, 101.60, 99.88, 80.21, 75.76, 74.97, 74.93, 72.70, 72.07, 69.19, 68.31, 68.17, 68.06, 67.54, 67.20, 62.64, 61.17, 61.14, 51.56, 51.35, 47.99, 39.99, 28.30, 22.50. HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{20}Na$ (M-Na) 772.2731, found 772.2729.

Neu5Acα2–3Galβ1–3GlcNAcβ1–3Galβ1–4GlcβProN$_3$ (40): From tetrasaccharide **6** (30 mg, 0.038 mmol), compound **40** was obtained as a white foam (37 mg, 88%). ^{1}H NMR (600 MHz, D_2O) δ 4.71 (d, 1 H, *J* = 8.4 Hz), 4.49 (d, 1 H, *J* = 7.8 Hz), 4.46 (d, 1 H, *J* = 7.8 Hz), 4.42 (d, 1 H, *J* = 8.4 Hz), 4.13 (d, 1 H, *J* = 3.6 Hz), 4.06 (dd, 1 H, *J* = 9.6 Hz and 3.0 Hz), 4.00–3.43 (m, 32 H), 3.30–3.27 (m, 1 H), 2.73 (dd, 1 H, *J* = 12.6 Hz and 4.8 Hz), 2.01 (s, 6 H), 1.89 (pentalet, 2 H, *J* = 6.3 Hz), 1.76 (t, 1 H, *J* = 12.6 Hz). ^{13}C NMR (75 MHz, D_2O) δ 175.08, 174.05, 103.51, 103.06, 102.65, 102.23, 99.75, 82.24, 82.05, 78.46, 75.71, 75.31, 75.21, 75.02, 74.89, 74.48, 72.90, 71.97, 70.12, 69.20, 68.51, 68.41, 68.14, 67.48, 67.36, 62.71, 62.56, 61.08, 60.61, 60.14, 54.70, 51.77, 47.97, 39.88, 28.35, 22.42, 22.16. HRMS (ESI) *m/z* calcd for $C_{40}H_{66}N_5O_{29}Na$ (M-Na) 1080.3849, found 1080.3827.

Neu5Gcα2–3Galβ1–3GlcNAcβ1–3Galβ1–4GlcβProN$_3$ (41): From tetrasaccharide **6** (30 mg, 0.038 mmol), compound **41** was obtained as a white foam (35 mg, 88%). ^{1}H NMR (600 MHz, D_2O) δ 4.71 (d, 1 H, *J* = 8.4 Hz), 4.49 (d, 1 H, *J* = 8.4 Hz), 4.46 (d, 1 H, *J* = 7.8 Hz), 4.42 (d, 1 H, *J* = 7.8 Hz), 4.13 (d, 1 H, *J* = 3.6 Hz), 4.10 (s, 2 H), 4.07 (dd, 1 H, *J* = 9.6 Hz and 3.0 Hz), 3.99–3.51 (m, 29 H), 3.47–3.43 (m, 3 H), 3.30–3.27 (m, 1 H), 2.75 (dd, 1 H, *J* = 12.6 Hz and 4.8 Hz), 2.01 (s, 3 H), 1.89 (pentalet, 2 H, *J* = 6.6 Hz), 1.78 (t, 1 H, *J* = 12.6 Hz). ^{13}C NMR (75 MHz, D_2O) δ 175.91, 175.09, 174.10, 103.55, 103.11, 102.67, 102.28, 99.83, 99.77, 82.29, 82.10, 78.56, 75.71, 75.33, 75.25, 75.03, 74.94, 74.48, 72.95, 72.69, 72.07, 70.18, 69.26, 68.57, 68.48, 68.25, 68.15, 67.49, 62.59, 61,13, 60.62, 60.18, 54.76, 51.54, 47.98, 39.97, 28.40, 22.48. HRMS (ESI) *m/z* calcd for $C_{40}H_{66}N_5O_{30}Na$ (M-Na) 1096.3787, found 1096.3783.

3 Results and discussion

3.1 Synthesis of sialyltransferase acceptors

Six sialyltransferase acceptors (**1–6**) including type 1 (Galβ1–3GlcNAcβProN$_3$, **1**), type 2 (Galβ1–4GlcNAcβProN$_3$, **2** and 6-sulfo-Galβ1–4GlcNAcβProN$_3$, **3**), type 3 or core 1 (Galβ1–3GalNAcαProN$_3$, **4**), and type 4 (Galβ1–3GalNAcβProN$_3$, **5**) disaccharides as well as type 1 tetrasaccharide (Galβ1–3GlcNAcβ1–3Galβ1–4GlcβProN$_3$, **6**) were chemically synthesized.

Disaccharide Galβ1–3GalNAcβProN$_3$ (**1**) was prepared as shown in Scheme 1. Deacetylation of glucosamine derivative **7** with NaOMe/MeOH [35] followed by 4,6-*O*-benzylidene protection with benzaldehyde dimethyl acetal produced **8** (87%), which was coupled with 2,3,4,6-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate donor **9** [36] in the presence of TMSOTf to afford disaccharide **10** in a moderate 53% yield. Removal of benzylidene protection group from **10** by hydrogenation followed by S_N2 substitution of the chlorine by azido group gave **11** in 95% yield. Removal of the phthalimido group by treating with hydrazine, followed by acetylation and deacetylation, the disaccharide Galβ1–3GlcNAcβProN$_3$ (**1**) was produced in 84% yield over three steps.

Disaccharides **2** and **3** were synthesized as shown in Scheme 2. Monosaccharide acceptor **12** was synthesized from **7** in 84% yield by deacetylation followed by selective silylation at *O*-6 with *tert*-butyldimethylsilyl chloride. Coupling of trichloroacetimidate **9** with acceptor **12** was performed in dichloromethane using TMSOTf as a promoter to give **13** (79%). Removal

of the *tert*-butyldimethylsilyl and transformation of chlorine to azide afforded **14** in 76% yield. Deprotection of **14** as described for **11** afforded disaccharide Galβ1–4GlcNAcβProN_3 **(2)** in 82% yield over three steps. Acetylation of **13** with Ac_2O in pyridine, followed by removal of TBS group and transformation of chlorine to azide groups produced **15** (85%). Sulfation at C-6 of **15** with SO_3. Pyinpyridine, removal of the 2-*N*-phthalyl group, followed by acetylation and deacetylation produced disaccharide Galβ1–4GlcNAc6SβProN_3 **(3)** in 66% overall yield.

To prepare disaccharides **4** and **5**, GalNAc derivative **16** [37] was treated with 3-chloro-1-propanol and acetyl chloride followed by 4,6-*O*-benzylidenation to produce a mixture of **17** (21%) and **18** (57%). Coupling of trichloroacetimidate **9** with **17** and **18**, respectively, using TMSOTf as a promoter produced disaccharides **19** and **20** in 62% and 48% yields, respectively. Substitution of the chlorine by azide using sodium azide and subsequent removal of benzylidene using acetic acid afforded **21** (94%) and **22** (91%). Deprotection of **21** and **22** as described for **11** afforded disaccharides Galβ1–4GalNAcαProN_3 (**4**) and Galβ1–4GalNAcβProN_3 (**5**) in 85% and 76% yields, respectively.

For the synthesis of type 1 LNnT tetrasaccharide **6**, compound **23** [38] was deacetylated and selectively protected with benzylidene to produce **24** (88%). Disaccharide donor **25** was synthesized by coupling the monosaccharide **24** with trichloroacetimidate donor **9** in the presence of TMSOTf with 77% yield. Glycosylation of **25** with a disaccharide acceptor **26** [39] using NIS TfOH as the promoters produced tetrasaccharide **27** (64%). Global deprotection leaded to the production of the desired LNT tetrasaccharide Galβ1–3GlcNAcβ1–3Galβ1–4GlcβProN_3 (**6**) in 54% yield.

3.2 One-pot three-enzyme approach for the synthesis of sialylated glycans

With chemically synthesized glycan acceptors **1–6** in hands, the preparation of α2–3-linked sialyl oligosaccharides was carried out using an efficient one-pot three-enzyme approach [32] (Figure 2) developed in our laboratory. In this system, *N*-acetylmannosamine (ManNAc) or *N*-glycolylmannosamine (ManNGc) was coupled with pyruvate to give sialic acids Neu5Ac or Neu5Gc by a recombinant *Escherichia coli* K12 sialic acid aldolase-catalyzed reaction [40]. The sialic acid formed was then activated by a recombinant *Neisseria meningitidis* CMP-sialic acid synthetase [40], and transferred to a suitable sialyltransferase acceptor by a *Pasteurella multocida* multifunctional α2–3-sialyltransferase [31] for the formation of sialosides.

As shown in Table 1, all six oligosaccharides **1–6** prepared were very good acceptor substrates for *Pasteurella multocida* α2–3-sialyltransferase. Sialic acids (Neu5Ac or Neu5Gc) were successfully transferred to the galactose (Gal) residue through an α2–3-sialyl linkage by the one-pot three-enzyme reaction system. Twelve sialosides representing commond naturally occurring sialic acid-containing carbohydrate epitopes including α2–3-linked sialy l type 1 Neu5Acα2–3Galβ1–3GlcNAcβProN_3 (30, 85%) and Neu5Gcα2–3Galβ1–3GlcNAcβProN_3 (31, 83%); type 2 Neu5Acα2–3Galβ1–4GlcNAcβProN_3 (32, 8–4 %), Neu5Gcα2–3Galβ1–4GlcNAcβProN_3 (33, 8–0 %), Neu5Acα2–3Galβ1–4GlcNAc6SβProN_3 (34, 88%), and Neu5Gcα2–3Galβ1–4GlcNAc6SβProN_3 (35, 82%); type 3 Neu5Acα2–3Galβ1–3GalNAcαProN_3 (36, 87%) and Neu5Gcα2–3Galβ1–3GalNAcαProN_3 (37, 76%); type 4 Neu5Acα2–3Galβ1–3GalNAcβProN_3 (38, 81%), and Neu5Gcα2–3Galβ1–3GalNAcβProN_3 (39, 79%) glycans as well as α2–3-linked sialyllacto-*N*-tetraose Neu5Acα2–3Galβ1–3GlcNAcβ1–3Galβ1–4GlcβProN_3 (40, 86%) and Neu5Gcα2–3Galβ1–3GlcNAcβ1–3Galβ1–4GlcβProN_3 (41, 81%) were obtained in high yields.

4 Conclusions

In summary, chemoenzymatic method which combines chemical and enzymatic synthetic approaches, was demonstrated once again to be a general and efficient strategy to obtain a library of homogenous sialosides containing different sialic acid forms and various internal carbohydrate structures. The α2–3-linked sialosides obtained in this work are valuable probes to study the biological importance of naturally occurring diverse sialic acid-containing carbohydrate epitopes.

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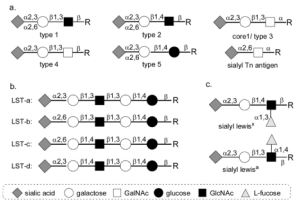


Figure 1.
Common sialic acid-containing structures in nature.

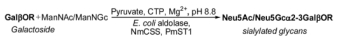
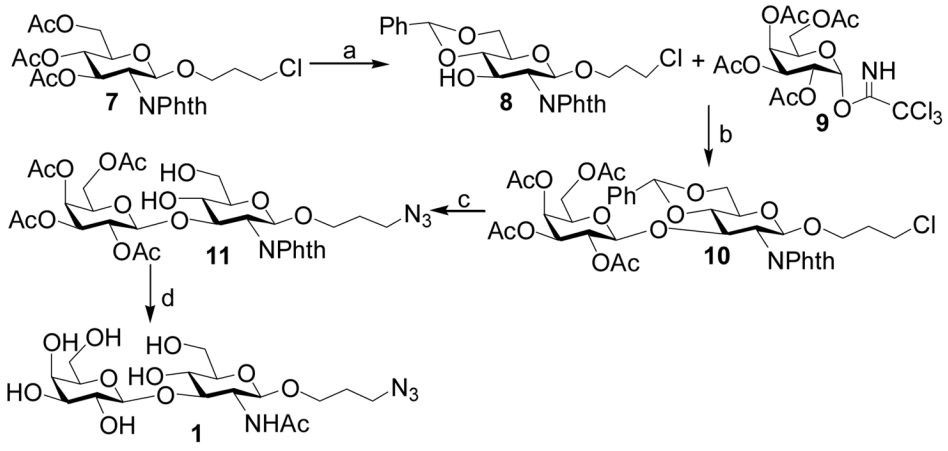


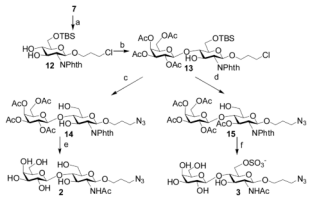
Figure 2.

One-pot three-enzyme synthesis of sialosides containing different sialic acid forms and various internal glycans. Abbreviations: ManNAc, *N*-acetylmannosamine; ManNGc, *N*-glycolylmannosamine; CTP, cytidine 5′-triphosphate. Enzymes: *E. coli* aldolase, *Escherichia coli* K12 sialic acid aldolase; NmCSS, *Neisseria meningitidis* CMP-sialic acid synthetase; PmST1, *Pasteurella multocida* sialyltransferase for the formation of α2–3-linked sialosides.



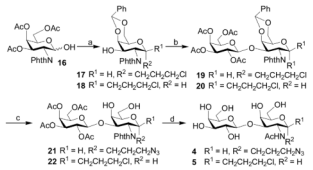
Scheme 1.

Reagents and yields: (a) (i) NaOMe, MeOH; (ii) $PhCH(OMe)_2$, CSA, DMF, 87%; (b) TMSOTf, CH_2Cl_2, 53%; (c) (i) Pd/C, H_2; (ii) NaN_3, TBAI, DMF, 95%; (d) (i) N_2H_4-H_2O, EtOH; (ii) Ac_2O, pyridine; (iii) NaOMe, MeOH, 84%.



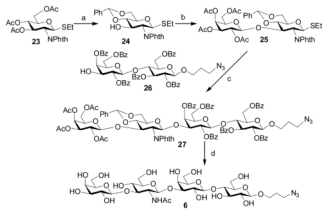
Scheme 2.

Reagents and yields: (a) (i) NaOMe, MeOH (ii) TBSCl, imidazole, CH_2Cl_2, 84%; (b) **9**, TMSOTf, CH_2Cl_2, 79%; (c) (i) TABF, THF; (ii) NaN3, TBAI, DMF, 76%; (d) (i) Ac_2O, pyridine; (ii) TABF, THF; (iii) NaN_3, TBAI, DMF, 85%; (e) (i)) N_2H_4-H_2O, EtOH; (ii) Ac_2O, pyridine; (iii) NaOMe/MeOH, 82%; (f) (i) Py-SO_3, pyridine; (ii) N_2H_4-H_2O, EtOH; (iii) Ac_2O, pyridine; (iv) NaOMe, MeOH, 66%.



Scheme 3.

Reagents and yields: (a) (i) AcCl, 3-chloro-1-propanol; (ii) $PhCH(OMe)_2$, CSA, DMF, **17**:21%; **18**: 57%; (b) **9**, TMSOTf, CH_2Cl_2, **19**:62%; **20**: 48%; (c) (i) NaN_3, TBAI, DMF; (ii) 80% HOAc, **21**:94%; **22**: 91%; (d) (i) N_2H_4-H_2O, EtOH; (ii) Ac_2O, pyridine; (iii) NaOMe, MeOH, **4**:85%; **5**: 76%.

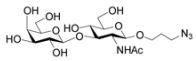
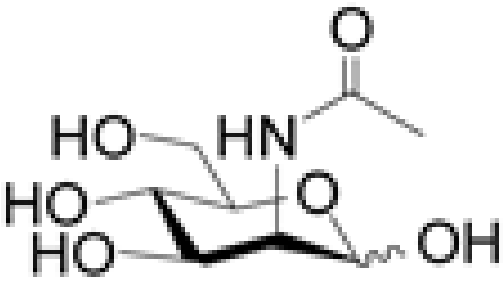
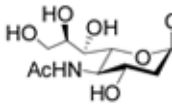
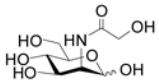
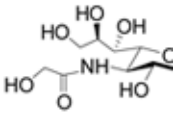
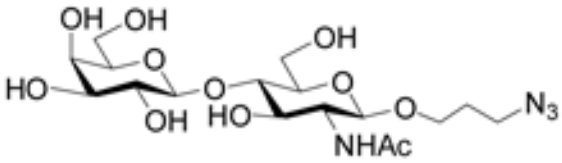
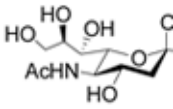
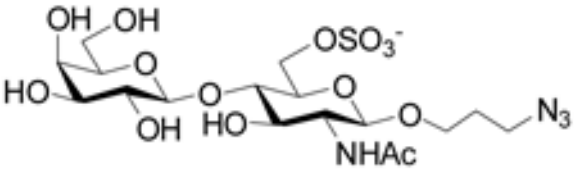
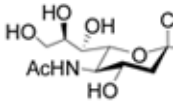
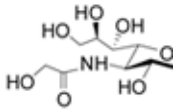
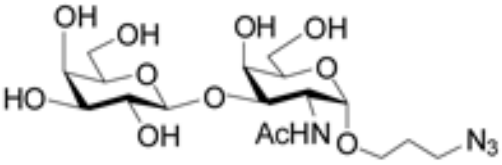
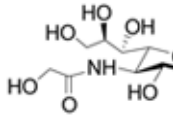
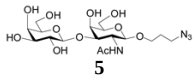


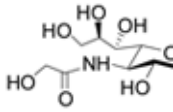
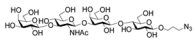
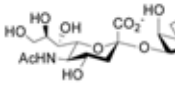
Scheme 4.

Reagents and yields: (a) (i) NaOMe, MeOH; (ii) $PhCH(OMe)_2$, CSA, 88%; (b) **9**, TMSOTf, CH_2Cl_2, 77%; (c) NIS, TfOH, CH_2Cl_2, 64%; (d) (i) 80% HOAc; ii) N_2H_4-H_2O, EtOH; (iii) Ac_2O, pyridine; (iv) NaOMe, MeOH, 54%.

Table 1

α2–3-Linked sialosides synthesized using one-pot three-enzyme system shown in Figure 2

entry	acceptor	sialic acid precurso	α2
a	 **1**	 ManNAc, **28**	
b		 ManNGc, **29**	
c	 **2**	**28**	
d		**29**	
e	 **3**	**28**	
f		**29**	
g	 **4**	**28**	
h		**29**	
i	 **5**	**28**	

entry	acceptor	sialic acid precurso	α2
j		**29**	
k	 **6**	**28**	
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