Synthesis of α-(2 → 5)Neu5Gc Oligomers

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Abstract: A facile synthesis of the sialic acid oligomers α-(2 → 5)Neu5Gc (**1**) is presented. Monosaccharides **2**–**4** with suitable functionality were used as the building blocks. After selective removal of the paired carboxyl and amine protecting groups, the fully protected oligomers were assembled through consecutive coupling of the building blocks by well established peptide coupling techniques. By this approach, fully protected oligomers as large as an octasaccharide were synthesized. Deprotection of these fully protected oligomers was conducted in two steps (LiCl in refluxing pyridine and 0.1N NaOH) to afford the desired products in high yield. Enzymatic degradation of the octamer with neuraminidase, monitored by capillary electrophoresis (CE), was also accomplished. The stepwise *exo*-cleavage adducts were all well separated and identified in the CE spectrum. The strategy described here for solution-phase synthesis also provides the basis for future solid-phase synthesis of poly-α-(2 → 5)-Neu5Gc.

Keywords: carbohydrates · electrophoresis · neuraminidase · sialic acids

Introduction

Sialic acids comprise a family of more than 40 acidic sugars. *N*-Acetylneuraminic acid (Neu5Ac) is the most common sialic acid and seems to be the precursor for all other neuraminic acid derivatives.[1] *N*-Glycolylneuraminic acid (Neu5Gc), formed by the substitution of one of the hydrogen atoms in the acetyl moiety of Neu5Ac by a hydroxy group, is one of these Neu5Ac derivatives. Neu5Gc has been found as a component of all types of glycoconjugates in animals of the deuterostomate lineage.[2] The formation of CMP-Neu5Gc in vivo is through the introduction—by the action of a CMP-Neu5Ac hydroxylase (monooxygnease), a cytosolic enzyme—of a single oxygen atom to the *N*-acetyl group of CMP-Neu5Ac.[3] According to previous reports,[3, 4] this is the only biosynthetic pathway for the production of Neu5Gc in biological systems. A mutation resulting in the inactivation of this enzyme in the human genome can explain the almost complete lack of Neu5Gc in human tissues.[5]

In the marine deuterostomes, including sea urchins, starfish, and sea cucumbers, Neu5Gc is often the main sialic acid and is widely distributed in glycoproteins and gangliosides, suggesting that it is involved in a number of important biological functions in these marine invertebrates.[6] The *N*-glycolyl group of Neu5Gc can serve as an acceptor site for *O*-acetylation, *O*-methylation, and glycosylation to form some unusual compounds.[7, 8] Besides the α-(2 → 8)- or α-(2 → 9)-linked Neu5Ac polymers found in microbial and vertebrate neural cells, a polysialic acid (PSA) chain with Neu5Gc residues ketosidically linked to the glycolyl group of NeuGc [(→5-O_{glycolyl}Neu5Gcα2 →)$_n$, called poly-α-(2 → 5)-*N*-glycolylneuraminic acid (NeuGc)] (**1**), has been isolated from the jelly coat of sea urchin egg and found to play an important role in the fertilization of eggs.[8] In order to investigate the biosynthesis of this kind of PSA and the mechanism of fertilization involving the PSA, α-(2 → 5)Neu5Gc oligomers were synthesized as described below.

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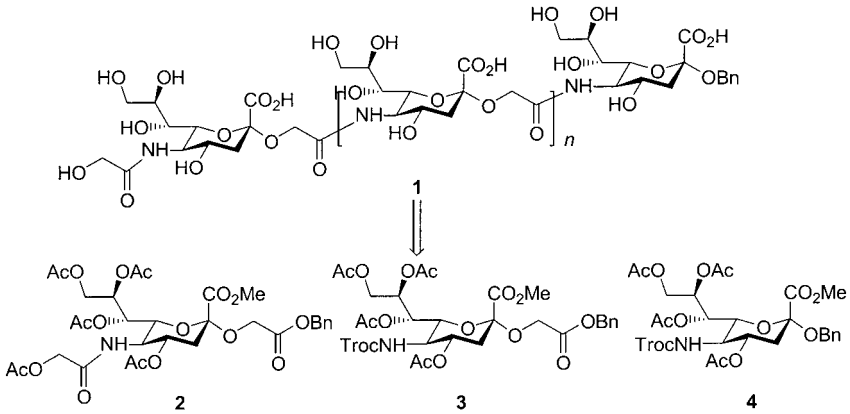
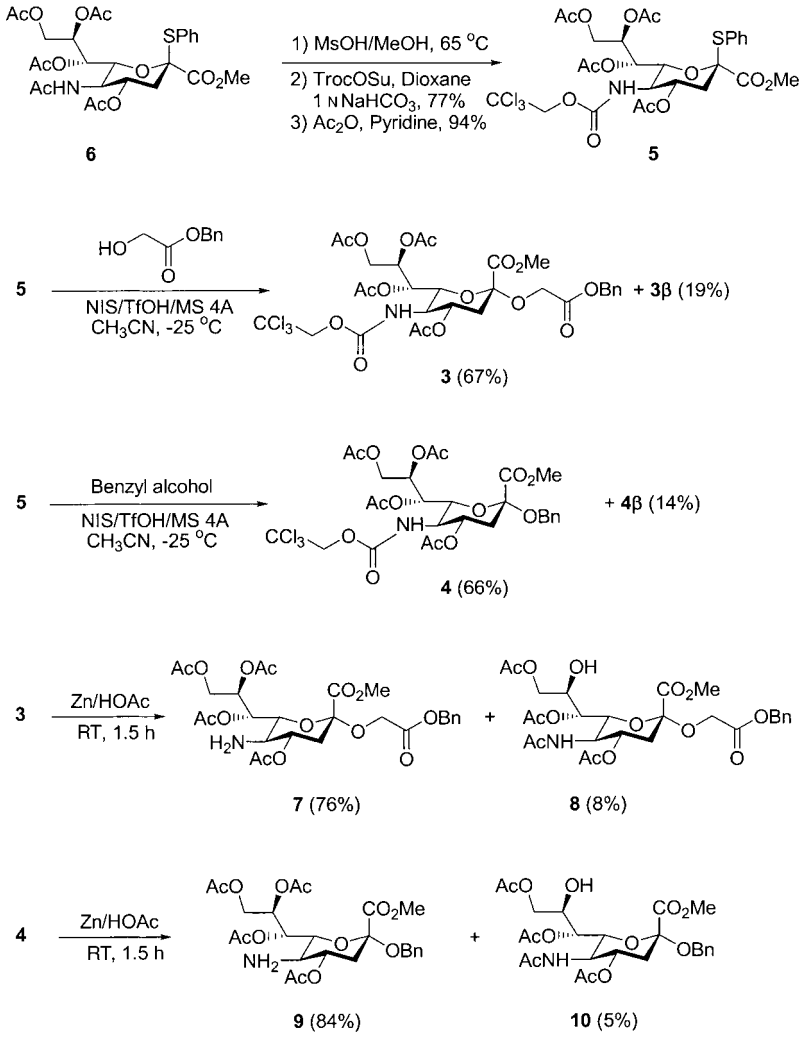
Results and Discussion

Because of its linear structure, oligo-α-(2→5)Neu5Gc (compound **1**) should be accessible from three suitable monosaccharides: namely, the non-reducing end building block **2**, the internal building block **3**, and the reducing end unit **4**[9a] (Scheme 1). Compounds **2**–**4** can be regarded as amino acid equivalents. By this synthetic strategy the stereoselective sialylation[9b] should be performed at an early stage to avoid the use of two sialic acid intermediates (donor and acceptor) and the potential difficulty in separation of anomeric oligomers. More importantly, our approach essentially translates a normally problematic oligosaccharide synthesis into a peptide synthesis by assembling these building blocks through amide bond linkages by use of well established peptide coupling techniques.[10]



Scheme 1. Retrosynthesis of α-(2→5)Neu5Gc oligomers **1**.

As it was to be employed for sequential chain elongation, the internal building block **3** was designed with a pair of carboxyl and amine protecting groups that could be selectively removed under mild conditions. The *N*-(2,2,2-trichloroethoxy)-carbonyl (*N*-Troc) group was chosen to protect amino moieties because *N*-Troc is compatible with both sialylation and amide condensation and can be easily removed by zinc powder under acidic conditions. On the other hand, the benzyl ester in **2** and **3** can be converted into the corresponding carboxylic acid by catalytic hydrogenation.



Scheme 2. Synthesis of the amino building blocks.

Preparation of monosaccharide building blocks: The protected building blocks (**2**–**4**) were all prepared from thiosialoside **5**[9a] (Scheme 2), which had previously been prepared from **6**.[11] Treatment of **5** with benzyl glycolate in the presence of *N*-iodosuccinimide (NIS)/TfOH[12] at −25 °C afforded, after chromatographic separation, the desired α-anomer **3** as a major product (67%), together with the *β*-anomer **3***β* (19%). Similarly, **4** was obtained by treatment of **5** with benzyl alcohol in 66% yield, together with 14% of the *β*-anomer **4***β*. One of the major difficulties encountered in the previous synthesis had been in obtaining the desired sialosides with high anomeric purity. The *N*-Troc[14] group introduced here appears to facilitate the separation of two sialoside anomers by silica gel chromatography, which allowed us to obtain pure **3** and **4** on multigram scales. In order to prepare building block **2**, the *N*-Troc group

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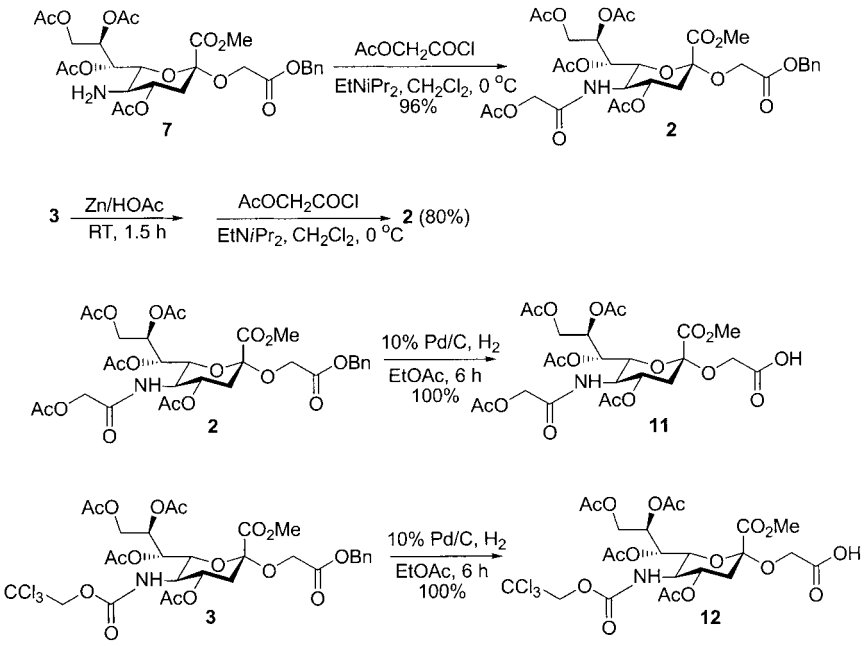
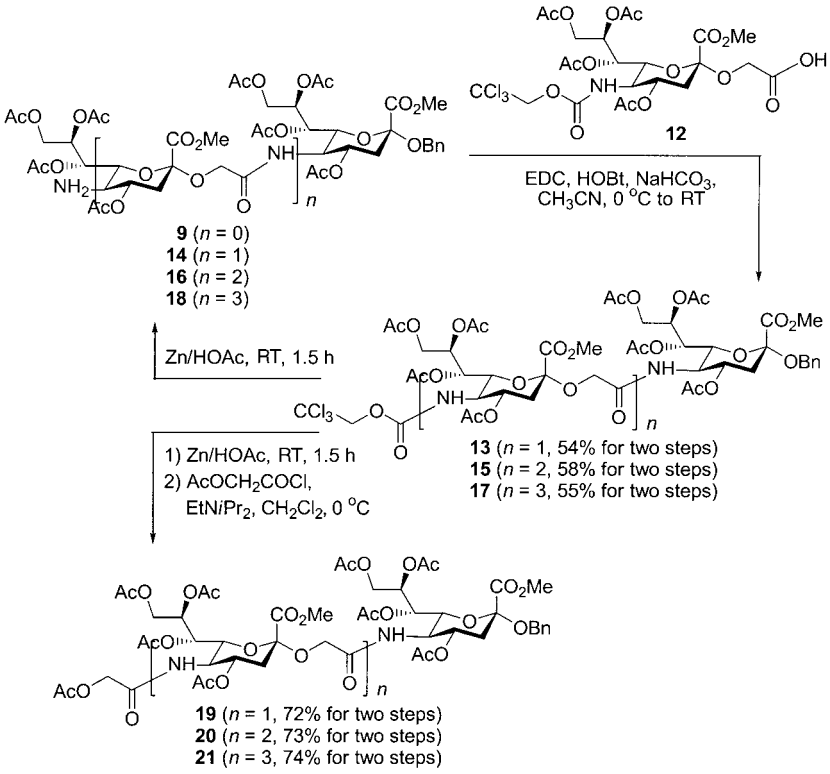
in **3** was first removed with Zn/HOAc to give the amine **7** (76%), which was in turn treated with acetoxyacetyl chloride and Hunig's base in CH_2Cl_2 at 0°C to afford **2** in 96% yield. However, we prepared **2** more practically from **3** in 80% yield by direct acetoxyacetylation of the crude **7** (Scheme 3), to minimize the *O*–*N* acetyl migration observed in the process after the removal of *N*-Troc. Although the expected **7** was indeed the major product (76%) from **3**, we also isolated *N*-acetylated **8** in 8% yield, an intramolecular *O*–*N* acetyl migration apparently having taken place. The same migration was also observed in the removal of *N*-Troc from **4**, which led to the major product **9** (84%) and a by-product **10** (5%) as shown in Scheme 2. It was speculated that the regioselective migration is caused by the proximity of the 8-OAc group and the 5-NH_2 group in compounds **7** and **9**. Furthermore, it is noteworthy that spontaneous *O*–*N* acetyl migrations were also observed in the pure compounds **7** and **9**[15, 16] even during storage at −20°C, resulting in about 50% formation of **8** and **10**, respectively, within two months. Protected building blocks **2** and **3**, after catalytic hydrogenation (Pd/C, H_2) in ethyl acetate, were converted in quantitative yield into **11** and **12**, respectively, each with a free carboxylic group for amide coupling.



Scheme 3. Synthesis of the carboxy building blocks.

With building blocks **7**, **9**, **11**, and **12** to hand we have two coupling routes towards α-(2→5)Neu5Gc oligomers, either by construction from the non-reducing end, by use of **7** and **11**, or by starting from the reducing end, with **9** and **12**. We decided to try both routes for the syntheses of two tetramers (**17** and **27**) and to perform a final [4+4] coupling to provide an octasaccharide **28** (see Scheme 4 and Scheme 5). In the process, we would be able to evaluate the overall efficiency of both procedures.

Assembly of the oligosaccharides from the reducing end: Condensation of amine **9** and **12** was performed in CH_3CN in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC; 1.5 equiv) and activating reagent HOBt[17] (0.2 equiv), which led to the formation of an amide bond between the 5-NH_2 group of **9** and the aglycon carboxylic group of **12** and afforded the fully protected disaccharide **13** in 54% yield based on **4** (Scheme 4). For further elongation, the *N*-Troc moiety in **13** was removed (Zn/HOAc) to generate amine **14**, which was in turn again coupled with **12** under same conditions to give the corresponding trisaccharide **15** in 58%



Scheme 4. Assembly of the oligomers from the reducing end.

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yield. After repetition of the above procedure (i.e., *N*-Troc removal and amide coupling), tetrasaccharide **17** was obtained from **15** (56%) in two steps. To terminate the elongation cycle, the unstable amines (**14**, **16**, and **18**) derived from removal of the *N*-Troc group were converted into acetoxyacetamides **19**–**21** in 72–74% yields by use of acetoxyacetyl chloride and Hunig's base. As previously discussed with compounds **7** and **9**, there was also significant *O*–*N* acetyl migration associated with these oligomer intermediates, which not only made their purification difficult but also effectively prevented further elongation. To minimize these adverse effects these amine compounds were used immediately in the crude state in the coupling reactions, without chromatographic purification.

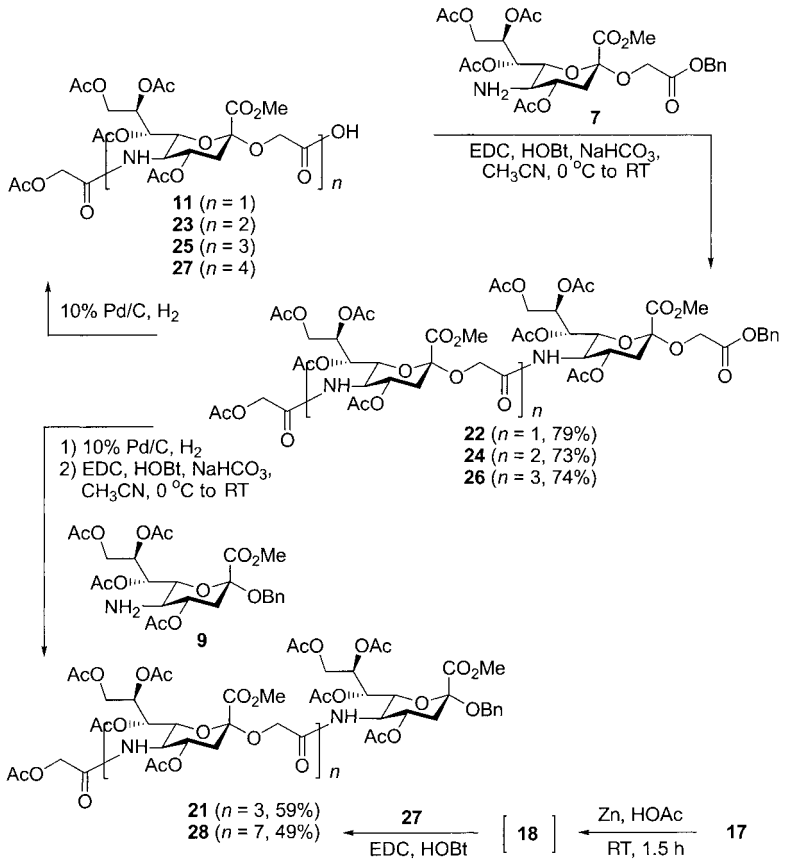
Assembly of the oligosaccharides from the non-reducing end: The alternative route for elongation, with carboxylic acid **11** and amine **7**, was also examined (Scheme 5). The coupling of **7** and **11** (EDC/HOBt) gave the desired disaccharide **22** (79%). The benzyl ester protecting group was removed by catalytic hydrogenolysis (Pd-C/H_2) to afford the carboxylic acid **23** for further coupling with **7**. By repetition of this deprotection and coupling cycle, trimer **24** and tetramer **26** were obtained in yields of about 75% from **23** and **25**, respectively. For the introduction of a reducing end monosaccharide unit, the carboxylic acid **25**, derived from **24**, was coupled with **9** to give the fully protected tetramer **21** (59%).

With two tetrasaccharide fragments to hand, one carboxylic acid **27** and the other *N*-Troc-protected amine **17**, we were able to perform a convergent coupling. Thus, the *N*-Troc group of **17** was removed to produce **18**, which was condensed with **27** to provide octamer **28** in 49% yield (two steps). The major difficulty encountered was the purification of these oligomers, more specifically the amines, due to *O*-*N* acetyl migration.[18] Fortunately, the fully protected oligomers could be isolated in high purity by crystallization. On comparison of the two procedures it is clear that the construction of α-(2→5)-linked Neu5Gc oligomers from the non-reducing end gives a higher overall coupling yield. Such difference is very probably the result of the lower yield in the *N*-Troc removal step because of the acetyl migration. In addition, the use of amine **7** as a building block instead of the generation of amine groups on oligomers for each elongation step also simplified the purification.

Deprotection of the fully protected oligo-α-(2→5)Neu5Gc compounds: To complete the synthesis, an efficient deprotection method for these protected oligomers was required. A model study on a protected disaccharide **29** showed that the Zemplen removal of acetyl groups resulted in two positional isomers: the desired product **31** and the amide migration product **32** in a ratio of 1:4 (Scheme 6). Both structures were characterized by various NMR techniques. By HMBC, the heteronuclear cross-signal pairs ^2J(C,H) A-NH/B-C1 and ^3J(C,H) B-CH_3/B-C22 were observed for **32** (Figure 1). This compound was probably formed through a cyclic imide intermediate **30** (Scheme 6), followed by a nucleophilic attack on the carbonyl group of the glyocolyl amide. Because the glycolyl amide is sterically more accessible than the C1 amide carbonyl group, competitive ring-opening by NaOMe gave the undesired **32** as the major product.

As an alternative (Scheme 7), we first removed the methyl ester groups in **19**, **21**, and **28** with LiCl in refluxing pyridine,[19] to obtain the corresponding lithium salts **33**–**35** (86–89%), which effectively prevented cyclization and subsequent amide migration. Basic hydrolysis of **33**–**35** with 0.1N NaOH followed, to remove the *O*-acetyl groups and to afford sodium salts **36**–**38** in 87–89% yield after purification on Sephadex LH-20.

Characterization of synthetic α-(2→5)Neu5Gc oligomers with neuraminidase: As one of the *exo*-glycosidases, neuraminidase recognizes the carboxyl group of sialic acid and specifically releases the α-linked sialic acid from the non-reducing terminal.[20] We should therefore be able to characterize the synthetic oligomers



Scheme 5. Assembly of the fully protected oligomers from the non-reducing end.

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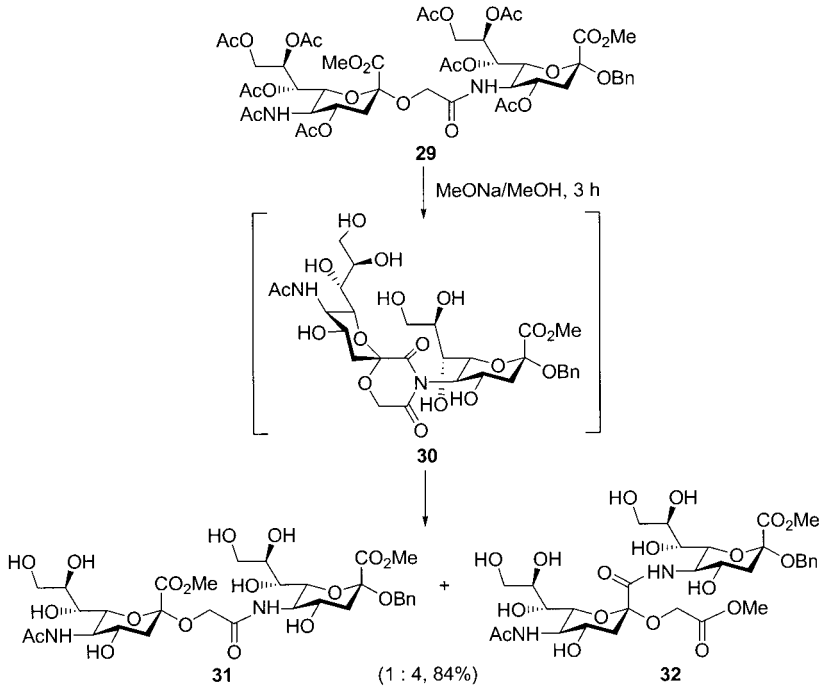


Scheme 6. Acyl migration during basic deprotection.

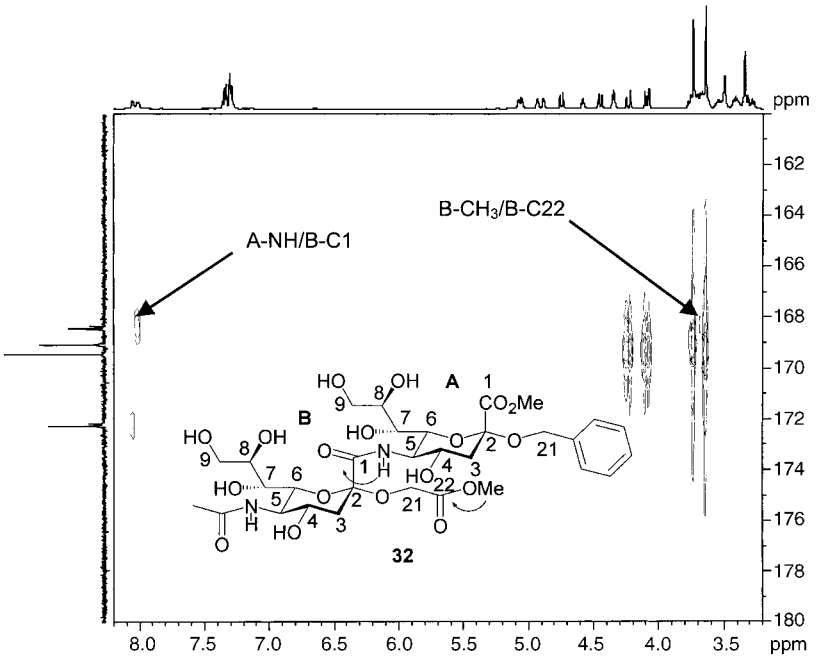


Figure 1. Partial HMBC spectra (500 MHz) of **32** in [D_6]DMSO.

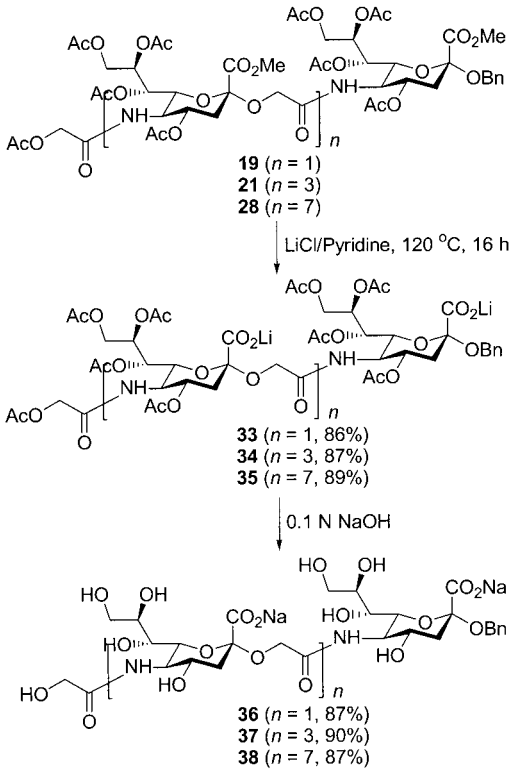
such as **38** by use of neuraminidase and thus verify the α configuration. High-performance capillary electrophoresis (HPCE)[21] should allow the determination of the number of repeating units. As shown in Figure 2, neuraminidase degraded α-(2→5)Neu5Gc octamer **38** (peak a) into heptamer (peak b), hexamer (peak c), pentamer (peak d), tetramer **37** (peak e), trimer (peak f), dimer **36** (peak g), and monomer-OBn (peak h). Each peak was compared with authentic α-(2-8)Neu5Ac oligomers by coinjection, and synthetic α-(2→5)Neu5Gc oligomers **36** and **37** were used to identify the dimer and tetramer derived from neuraminidase degradation. Within 24 h, octamer **38** was completely converted to Neu5Gc (peak i) by neuraminidase. This stepwise digestive process clearly confirms both the purity and the α configuration of octamer **38**.

In summary, we have efficiently synthesized α-(2→5)-Neu5Gc oligomers of up to eight repeating units, by a process in which the amide linkage, rather than the glycosidic bond, was assembled by peptide coupling techniques. The method should be applicable to the construction of higher oligomers. Access to these oligosaccharides should allow us to study further the biological roles played by poly-α-(2→5)-Neu5Gc and to identify the sialyltransferase involved in the biosynthesis of Neu5Gc PSA.

Experimental Section

General: NMR spectra were recorded with Bruker AM 400 (400 MHz) and Bruker DMX 500 (500 MHz) spectrometers. Assignment of ^{1}H NMR spectra was achieved by 2D methods (COSY, NOESY, HMQC, HMBC). Chemical shifts are expressed in ppm with residual $CHCl_3$ or CHD_2OD as reference. High-resolution FAB-MS were recorded with a JEOL SX-120 mass spectrometer. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) spectra were obtained on a Voyager-DE™ mass spectrometer. Reactions were monitored by TLC on aluminaor glass plates coated with silica gel (60 F_{254}, Merck) and visualized either by use of UV light or by charring with a molybdate solution (a 0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Column chromatography was performed on silica gel 60 (Merck 70–230 mesh). Methanol was dried by heating under reflux with magnesium methoxide and distilled immediately before use. Dioxane, acetonitrile, and pyridine were freshly distilled under N_2 over CaH_2. Molecular sieves (4 Å) were activated in vacuo at 300 °C (16 h) and stored under N_2. Neuraminidase from *Anthrobacter ureafaciens* was purchased from Nacalai Tesque (Kyoto, Japan).

General procedure for the glycosidation reactions of thioglycoside 5 with benzyl glycolate and benzyl alcohol—preparation of the building blocks 3 and 4:[9a] A mixture of thioglycoside **5** (6.484 g, 9.04 mmol), benzyl glycolate (2.252 g, 13.56 mmol), and molecular sieves (4 Å, 9.0 g) in acetonitrile (40 mL) was stirred under N_2 for 1 h. After the solution had



Scheme 7. Deprotection of the fully protected oligomers.

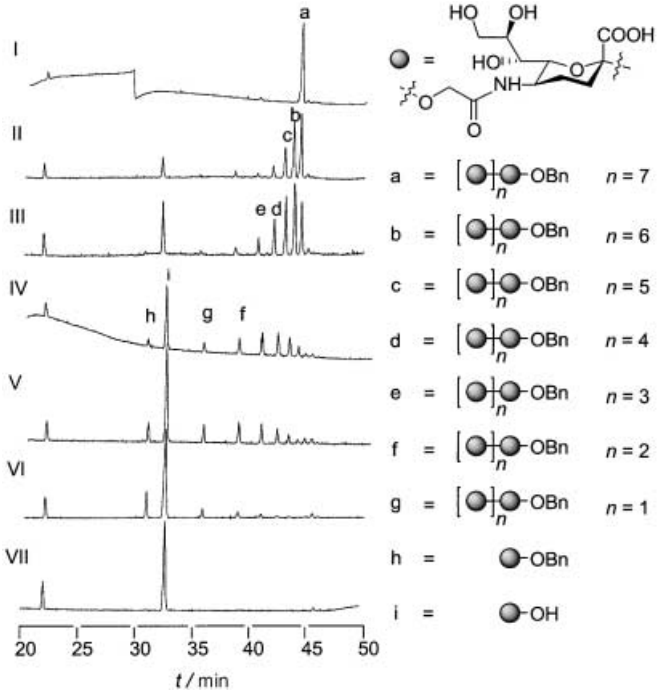


Figure 2. The products obtained from the hydrolysis of α-(2→5)Neu5Gc octamer **38** by neuraminidase were analyzed by CE at different times intervals (I, 0 min; II, 1 h; III, 90 mins; IV, 3 h; V, 4 h; VI, 6 h; VII, 1 day). Peaks a–h were assigned to octamer, heptamer, hexamer, pentamer, tetramer, trimer, dimer (2→5)Neu5Gc-2-OBn, and monomer Neu5Gc-2-OBn respectively, peak i was monomer Neu5Gc-2-OH.

been cooled to −40 °C, NIS (3.051 g, 13.56 mmol) and TfOH (0.15 mL, 1.71 mmol) were added successively. The reaction mixture was stirred at −25 °C for 1 h, diluted with CH_2Cl_2 (200 mL), and filtered through a pad of celite. The celite was washed with CH_2Cl_2 (3 × 50 mL), and the combined filtrate was washed with saturated aqueous $Na_2S_2O_3$ (15 mL) and saturated aqueous $NaHCO_3$ (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (hexane/CH_2Cl_2/acetone 10:10:1) to afford the α anomer **3** (4.682 g, 67%) and the β anomer **3**β (1.327 g, 19%) as white foams.

Compound 3: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.28 (m, 5H), 5.34 (s, 2H), 5.15 (dd, J = 12.3, 23.6 Hz, 2H), 5.04 (ddd, J = 12.4, 10.4, 4.6 Hz, 1H), 4.90 (d, J = 10.6 Hz, 1H), 4.87 (d, J = 12.0 Hz, 1H), 4.45 (d, J = 12.0 Hz, 1H), 4.34 (d, J = 16.3 Hz, 1H), 4.23 (d, J = 16.3 Hz, 1H), 4.22 (d, J = 12.4 Hz, 1H), 4.08 (dt, J = 12.4, 2.2 Hz, 1H), 4.02 (d, J = 10.4 Hz, 1H), 3.72 (s, 3H), 3.60 (q, 10.4 Hz, 1H), 2.75 (dd, J = 12.4, 4.6 Hz, 1H), 2.11 (s, 3H), 2.09(s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.93 (t, J = 12.4 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.63 (C), 170.37 (C), 170.10 (C), 169.88 (C), 169.08 (C), 167.39 (C), 154.04 (C), 135.41 (C), 128.53 (CH), 128.35 (CH), 98.05 (C), 95.36 (C), 74.50 (CH_2), 71.93 (CH), 68.30 (CH), 67.98 (CH), 67.20 (CH), 66.64 (CH_2), 62.08 (CH_2), 61.90 (CH_2), 52.98 (CH_3), 51.56 (CH), 37.69 (CH_2), 21.03 (CH_3), 20.82 (CH_3), 20.78 (CH_3), 20.71 (CH_3) ppm; LRMS (FAB): *m*/*z* (%): 796 (2) $[M+Na+2]^+$, 794 (2) $[M+Na]^+$, 774 (1) $[M+H+2]^+$, 772 (1) $[M+H]^+$, 714 (6), 712 (6), 607 (1), 548 (28), 546 (28), 488 (5), 486 (5), 368 (7), 366 (7), 330 (8), 328 (8), 154 (12), 137 (16), 91 (100); HRMS (FAB, $[M+H]^+$): found 772.1174; $C_{30}H_{37}Cl_3O_{16}N$ calcd 772.1178.

Compound 3β: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.30 (m, 5H), 5.29 (dd, J = 4.4, 1.8 Hz, 1H), 5.24 (ddd, J = 11.8, 10.6, 4.9 Hz, 1H), 5.20 (ddd, J = 7.5, 4.4, 2.4 Hz, 1H), 5.16 (dd, J = 25.8, 12.0 Hz, 2H), 4.86 (d, J = 12.0 Hz, 1H), 4.72 (d, J = 9.8 Hz, 1H), 4.66 (dd, J = 12.4, 2.4 Hz, 2H), 4.45 (d, J = 12.0 Hz, 1H), 4.26 (s, 2H), 4.14 (dd, J = 10.6, 1.8 Hz, 1H), 3.98 (dd, J = 12.4, 7.5 Hz, 1H), 3.72 (q, J = 10.6 Hz, 1H), 3.72 (s, 3H), 2.52 (dd, J = 13.0, 4.9 Hz, 1H), 2.10 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.90 (dd, J = 13.0, 11.8 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.60 (C), 170.37 (C), 170.25 (C), 169.89 (C), 169.04 (C), 166.64 (C), 154.29 (C), 135.21 (C), 128.78 (CH), 128.72 (CH), 98.42 (C), 95.39 (C), 74.58 (CH_2), 71.86 (CH), 71.25 (CH), 68.42 (CH), 68.30 (CH), 67.10 (CH_2), 62.39 (CH_2), 61.61 (CH_2), 52.89 (CH_3), 51.34 (CH), 36.96 (CH_2), 20.88 (CH_3), 20.81 (CH_3), 20.70 (CH_3) ppm; LRMS (FAB): *m*/*z* (%): 796 (1) $[M+Na+2]^+$, 794 (1) $[M+Na]^+$, 774 (1) $[M+H+2]^+$, 772 (1) $[M+H]^+$, 714 (2), 712 (2), 607 (1), 548 (30), 546 (30), 488 (5), 486 (5), 368 (8), 366 (8), 330 (9), 328 (9), 137 (35), 91 (100); HRMS (FAB, $[M+H]^+$): found 772.1166; $C_{30}H_{37}Cl_3O_{16}N$ calcd 772.1178.

Under the same glycosidation conditions,[9a] compound **5** (5.201 g, 7.25 mmol) and benzyl alcohol (1.10 mL, 10.87 mmol) afforded the α-anomer **4** (3.407 g, 66%) and the β-anomer **4**β (0.702 g, 14%) as white foams after purification by flash silica gel chromatography (10–30% gradient EtOAc in hexane).

General procedure for *N*-Troc removal—preparation of free amino compounds 7 and 9: Freshly activated zinc dust (1.51 g) was added to a solution of compound **3** (0.254 g, 0.33 mmol) in glacial acetic acid (3.0 mL). The mixture was stirred at room temperature for 1.5 h, diluted with ethyl acetate (100 mL), and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash silica gel chromatography (50–100% gradient EtOAc in hexane) to afford **7** (150 mg, 76%) and **8** (16 mg, 8%) as white foams.

Compound 7: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.25 (m, 6H), 5.50 (dd, J = 3.5, 2.0 Hz, 1H), 5.44 (dd, J = 9.4, 1.0 Hz, 1H), 5.38 (ddd, J = 9.4, 3.0, 2.2 Hz, 1H), 5.15 (dd, J = 22.2, 12.3 Hz, 1H), 4.66 (ddd, J = 12.2, 9.9, 4.6 Hz, 1H), 4.32 (d, J = 16.3 Hz, 1H), 4.25 (dd, J = 12.7, 2.2 Hz, 1H), 4.20 (dd, J = 12.7, 3.0 Hz, 1H), 4.20 (d, J = 16.3 Hz, 1H), 3.70 (s, 3H), 3.62 (dd, J = 9.9, 1.0 Hz, 1H), 2.77 (dd, J = 12.3, 4.6 Hz, 1H), 2.57 (t, J = 9.9 Hz, 1H), 2.13 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 1.76 (t, J = 12.3 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.71 (C), 170.62 (C), 170.32 (C), 169.91 (C), 169.16 (C), 167.46 (C), 128.50 (CH), 128.30 (CH), 128.28 (C), 98.02 (C), 74.90 (CH), 71.80(CH), 67.66 (CH), 66.54 (CH_2), 61.89 (CH_2), 61.67 (CH_2), 52.77 (CH_3), 50.98 (CH), 37.12 (CH_2), 21.04 (CH_3), 21.00 (CH_3), 20.72 (CH_3), 20.67 (CH_3) ppm; MS (MALDI-TOF): *m*/*z*: found 598.2129; $[C_{27}H_{36}O_{14}N]^+$ calcd 598.2136.

Compound 8: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.25 (m, 6H), 5.33 (d, J = 9.7 Hz, 1H), 5.14 (s, 2H), 5.07 (dd, J = 8.6, 2.1 Hz, 1H), 4.89 (ddd, J = 11.8, 10.3, 4.8 Hz, 1H), 4.30 (s, 2H), 4.15–4.00 (m, 4H), 3.70 (dd, J = 10.6,

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2.1 Hz, 1H), 3.70 (s, 3H), 2.80 (dd, J = 13.0, 4.9 Hz, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 2.02–1.98 (m, 4H), 1.84 (s, 3H) PPM; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.88 (C), 170.28 (C), 168.86 (C), 135.07 (C), 128.64 (CH), 128.57 (CH), 128.42 (CH), 98.26 (C), 72.86 (CH), 69.23(CH), 68.82 (CH), 68.00 (CH), 66.95 (CH_2), 64.71 (CH_2), 61.54 (CH_2), 53.76 (CH_3), 49.02 (CH), 36.94 (CH_2), 23.06 (CH_3), 20.83 (CH_3); MS (MALDI-TOF): m/z: Found 598.2135; $[C_{27}H_{36}O_{14}N]^+$ calcd 598.2136.

Under the same deprotection conditions, compound **4** (235 mg, 0.33 mmol) in glacial acetic acid (5.0 mL) afforded **9** (151 mg, 84%) and **10** (9 mg, 5%) as white foams after purification by flash silica gel chromatography (50–100% gradient EtOAc in hexane).

Compound 9: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.25 (m, 6H), 5.50 (dd, J = 3.5, 2.0 Hz, 1H), 5.46 (dd, J = 10.0, 2.0 Hz, 1H), 4.80–4.77 (m, 1H), 4.79 (d, J = 8.0 Hz, 1H), 4.44 (d, J = 8.8 Hz, 1H), 4.34 (dd, J = 12.6, 2.0 Hz, 1H), 4.29 (dd, J = 12.6, 3.5 Hz, 1H), 3.97 (d, J = 10.0 Hz, 1H), 3.70 (s, 3H), 2.77 (dd, J = 12.5, 4.6 Hz, 1H), 2.64 (t, J = 10.0 Hz, 1H), 2.19 (s, 3H), 2.18 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 1.78 (t, J = 12.5 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.09 (C), 170.68 (C), 170.42 (C), 170.08 (C), 168.05 (C), 137.34 (C), 128.17 (CH), 127.75 (CH), 127.59 (CH), 98.35 (C), 74.12 (CH), 71.21(CH), 67.99 (CH), 66.83 (CH), 66.60 (CH_2), 61.98 (CH_2), 52.54 (CH_3), 51.17 (CH), 37.74 (CH_2), 21.11 (CH_3), 20.97 (CH_3), 20.93 (CH_3), 20.77 (CH_3), 20.71 (CH_3) ppm; LRMS (FAB): m/z (%): 580 (33) $[M+41]^+$, 540 (35) $[M+H]^+$, 480 (8), 432 (5), 372 (5), 192 (8), 126 (8), 91 (100); HRMS (FAB, MH^+): found 540.2090; $C_{25}H_{34}O_{12}N$ calcd 540.2081.

Compound 10: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.25 (m, 6H), 5.24 (d, J = 10.0 Hz, 1H), 5.12 (dd, J = 8.5, 1.9 Hz, 1H), 4.86 (ddd, J = 11.8, 10.5, 4.8 Hz, 1H), 4.80 (d, J = 11.8 Hz, 1H), 4.48 (d, J = 11.8 Hz, 1H), 4.20–4.03 (m, 4H), 3.92 (dd, J = 10.5, 1.9 Hz, 1H), 3.76 (s, 3H), 2.75 (dd, J = 13.1, 4.8 Hz, 1H), 2.13–2.06 (m, 7H), 2.02 (s, 3H), 1.86 (s, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.94 (C), 170.36 (C), 170.29 (C), 169.61 (C), 136.64 (C), 128.37 (CH), 127.94 (CH), 127.62 (CH), 98.73 (C), 72.85 (CH), 69.42 (CH), 68.93 (CH), 68.21 (CH), 66.58 (CH_2), 64.69 (CH_2), 53.52 (CH_3), 49.12 (CH), 37.41 (CH_2), 23.12 (CH_3), 20.82 (CH_3) ppm; MS (MALDI-TOF): m/z: found 562.1883; $[C_{25}H_{33}O_{12}NNa]^+$ calcd 562.1901.

Methyl [2-(2′-benzyloxy-2′-oxoethyl)-5-acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosid]onate (2): Freshly activated zinc dust (2.16 g) was added to a solution of compound **3** (0.585 g, 0.76 mmol) in glacial acetic acid (5.0 mL). The mixture was stirred at room temperature for 1.5 h, diluted with ethyl acetate (100 mL), and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in CH_2Cl_2 (4.0 mL) and cooled to 0 °C, and acetoxyacetyl chloride (0.10 mL, 0.93 mmol) was added, followed by EtN^iPr_2 (0.25 mL, 1.44 mmol). After the mixture had been stirred for 2 h it was quenched with saturated aqueous $NaHCO_3$ (2.0 mL), diluted with CH_2Cl_2 (50 mL), washed with saturated aqueous $NaHCO_3$ (8 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash silica gel chromatography (50–100% gradient EtOAc in hexane) to afford **2** (422 mg, 80%). Alternatively, **7** (151 mg, 0.25 mmol) was acetoxyacetylated under the same reaction conditions to yield **2** (168 mg, 96%).

Compound 2: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35–7.29 (m, 5H), 5.85 (d, J = 9.3 Hz, 1H), 5.35 (ddd, J = 8.2, 5.4, 2.9 Hz, 2H), 5.22 (dd, J = 8.2, 1.3 Hz, 1H), 5.16 (dd, J = 12.3, 23.9 Hz, 2H), 5.00 (m, 1H), 4.56 (d, J = 15.3 Hz, 1H), 4.34 (d, J = 16.4 Hz, 1H), 4.27 (d, J = 15.3 Hz, 1H), 4.25 (d, J = 16.4 Hz, 1H), 4.24 (dd, J = 12.4, 2.9 Hz, 1H), 4.05 (dd, J = 12.4, 5.4 Hz, 1H), 4.03–3.99 (m, 2H), 3.73 (s, 3H), 2.71 (dd, J = 12.9, 4.7 Hz, 1H), 2.16 (s, 3H), 2.10(s, 3H), 2.09 (s, 3H), 2.02–1.96 (m, 7H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.02 (C), 170.60 (C), 170.26 (C), 170.00 (C), 169.62 (C), 169.09 (C), 167.63 (C), 167.57 (C), 135.41 (C), 128.53 (CH), 128.35 (CH), 98.23 (C), 72.45 (CH), 68.39 (CH), 68.05 (CH), 67.23 (CH), 66.65 (CH_2), 62.76 (CH_2), 62.22 (CH_2), 61.90 (CH_2), 53.02 (CH_3), 49.43 (CH), 37.69 (CH_2), 21.01 (CH_3), 20.83 (CH_3), 20.74 (CH_3), 20.68 (CH_3) ppm; LRMS (FAB): m/z (%): 720 (2) $[M+Na]^+$, 699 (1) $[M+H+1]^+$, 698 (2) $[M+H]^+$, 638 (18), 532 (7), 472 (56), 430 (4), 351 (3), 254 (9), 193 (5), 137 (6), 91 (100); HRMS (FAB): found 698.2295 $[M+H]^+$; $C_{31}H_{40}O_{17}N$ calcd 698.2296.

General procedure for the catalytic hydrogenation of benzyl esters—preparation of the carboxyl compounds 11 and 12: Pd on carbon (10%, 18 mg) was added to a solution of compound **2** (0.456 g, 0.65 mmol) in EtOAc (20 mL). The mixture was stirred under hydrogen at one atmosphere for 6–20 h until TLC analysis indicated that the reaction had gone to completion. The catalyst was removed by filtration through a pad of celite, and the cake was washed with EtOAc (3 × 20 mL). The filtrate was concentrated under reduced pressure to give **11** as a crystalline solid (0.394 g, 100%).

By the same procedure, **3** (0.461 g, 0.58 mmol) afforded **12** as a crystalline solid (0.412 g, 100%).

Compound 11: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.93 (d, J = 9.7 Hz, 1H), 5.36 (ddd, J = 8.4, 5.8, 2.7 Hz, 2H), 5.21 (dd, J = 8.4, 2.0 Hz, 1H), 5.04 (ddd, J = 11.6, 10.2, 4.6 Hz, 1H), 4.58 (d, J = 15.3 Hz, 1H), 4.35 (d, J = 16.6 Hz, 1H), 4.29 (d, J = 15.3 Hz, 1H), 4.27 (dd, J = 12.4, 2.7 Hz, 1H), 4.24 (d, J = 16.6 Hz, 1H), 4.11 (dd, J = 10.7, 2.0 Hz, 1H), 4.04 (dd, J = 12.4, 5.8 Hz, 1H), 3.98 (q, J = 10.2 Hz, 1H), 3.81 (s, 3H), 2.70 (dd, J = 13.0, 4.6 Hz, 1H), 2.17 (s, 3H), 2.13 (s, 3H), 2.03 (s, 3H), 2.01 (dd, J = 13.0, 11.6 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.48 (C), 171.01 (C), 170.81 (C), 170.36 (C), 170.30 (C), 169.67 (C), 167.81 (C), 167.61 (C), 98.33 (C), 72.46 (CH), 68.37 (CH), 67.98 (CH), 67.21 (CH), 62.75 (CH_2), 62.33 (CH_2), 61.74 (CH_2), 53.21 (CH_3), 49.53 (CH), 37.44 (CH_2), 21.11 (CH_3), 20.80 (CH_3), 20.75 (CH_3), 20.71 (CH_3), 20.66 (CH_3) ppm; LRMS (FAB): m/z (%): 630 (100) $[M+Na]^+$, 608 (7) $[M+H]^+$, 548 (22), 472 (73), 430 (11), 310 (6), 254 (16), 193 (12), 136 (46), 101 (31); HRMS (FAB, MH^+): found 608.1818; $C_{24}H_{34}O_{17}N$ calcd 608.1827.

Compound 12: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.32–5.40 (m, 2H), 5.08 (ddd, J = 12.4, 11.6, 4.6 Hz, 1H), 4.97 (d, J = 9.6 Hz, 1H), 4.88 (d, J = 12.1 Hz, 1H), 4.46 (d, J = 12.1 Hz, 1H), 4.35 (d, J = 16.7 Hz, 1H), 4.25 (dd, J = 12.1, 1.8 Hz, 1H), 4.23 (d, J = 16.7 Hz, 1H), 4.12 (d, J = 11.6, Hz, 1H), 4.09 (dd, J = 12.1, 4.5 Hz, 1H), 3.80 (s, 3H), 3.60 (q, J = 11.6 Hz, 1H), 2.74 (dd, J = 13.0, 4.6 Hz, 1H), 2.14 (s, 3H), 2.12(s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.95 (dd, J = 13.0, 12.4 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.58 (C), 170.81 (C), 170.36 (C), 170.19 (C), 167.46(C), 154.08 (C), 98.18 (C), 95.34 (C), 74.52 (CH_2), 72.06 (CH), 68.24 (CH), 68.06 (CH), 67.20 (C), 62.20 (CH_2), 61.79 (CH_2), 61.34 (CH_2), 53.20 (CH_3), 51.57 (CH), 37.46 (CH_2), 21.04 (CH_3), 20.81 (CH_3), 20.72 (CH_3) ppm; MS (MALDI-TOF): m/z: found 704.0513; $[C_{23}H_{30}Cl_3NO_{16}Na]^+$ calcd 704.0527.

Elongation of the α-(2→5)-linked oligosaccharides from reducing end (4)—preparation of 13, 15, and 17: Freshly activated zinc dust (2.04 g) was added to a solution of compound **4** (0.60 g, 0.84 mmol) in glacial acetic acid (7.5 mL). The mixture was stirred at room temperature for 1.5 h, diluted with ethyl acetate (200 mL), and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated. Without purification, compound **12** (0.482 g, 0.71 mmol) in acetonitrile (8.0 mL), $NaHCO_3$ (140 mg, 1.67 mmol), HOBt (20.2 mg, 0.15 mmol), and EDC (241 mg, 1.26 mmol) were added sequentially to the residue **9**. The resulting mixture was stirred at room temperature for 4–18 h. The solvent was removed under reduced pressure, and the residue was purified by flash silica gel chromatography (20–100% gradient EtOAc in hexane) to give compound **13** (0.548 g, 54%) as a colorless powder.

By the same procedure, **13** (400 mg, 0.33 mmol) afforded **15** (327 mg, 58%), and **15** (262 mg, 0.16 mmol) afforded **17** (188 mg, 56%) as colorless powders.

Compound 13: ^{1}H NMR (400 MHz, [D$_6$]benzene): δ = 7.42 (d, J = 7.4 Hz, 2H), 7.15–7.12 (m, 2H), 7.04 (t, J = 7.4 Hz, 1H), 6.42 (d, J = 10.0 Hz, 1H), 5.93 (dt, J = 7.2, 2.7 Hz, 1H), 5.70 (dd, J = 7.2, 2.2 Hz, 1H), 5.64 (ddd, J = 7.8, 5.4, 2.5 Hz, 1H), 5.51 (dd, J = 7.8, 1.9 Hz, 1H), 5.28 (ddd, J = 12.0, 10.3, 4.7 Hz, 1H), 5.12 (d, J = 12.1 Hz, 1H), 4.78–4.69 (m, 4H), 4.65 (d, J = 10.2 Hz, 1H), 4.63 (d, J = 10.6, Hz, 1H), 4.49 (dd, J = 10.6, 2.2 Hz, 1H), 4.37–4.26 (m, 4H), 4.16 (d, J = 14.5 Hz, 1H), 4.00–3.96 (m, 2H), 3.88 (q, J = 10.2 Hz, 1H), 3.48 (s, 3H), 3.18 (s, 3H), 2.93 (dd, J = 12.4, 4.6 Hz, 1H), 2.58 (dd, J = 12.4, 4.6 Hz, 1H), 2.19 (t, J = 12.4 Hz, 1H), 2.07 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.83 (s, 3H), 1.76 (t, J = 12.4 Hz, 1H), 1.74 (s, 3H), 1.72 (s, 3H), 1.61 (s, 3H) ppm; ^{13}C NMR (100 MHz, [D$_6$]benzene): δ = 170.49 (C), 170.36 (C), 170.13 (C), 170.02 (C), 169.95 (C), 169.86 (C), 169.56 (C), 168.79 (C), 168.68 (C), 168.02 (C), 154.48 (C), 137.93 (C), 128.43(CH), 128.17 (C), 99.28 (C), 98.79 (C), 96.08 (C), 74.77 (CH_2), 73.51 (CH), 73.04 (CH), 69.81 (CH), 69.45 (CH), 69.01 (CH), 68.56 (CH), 68.26 (CH), 67.48 (CH), 67.23 (CH_2), 64.48 (CH_2), 63.17 (CH_2), 62.66 (CH_2), 52.84 (CH_3), 52.09 (CH_3), 51.22 (CH), 49.62 (CH), 38.88 (CH_2), 37.33 (CH_2), 21.12 (CH_3), 20.97 (CH_3), 20.74 (CH_3), 20.55 (CH_3), 20.45 (CH_3), 20.38 (CH_3), 20.27 (CH_3) ppm; LRMS (FAB): m/z (%): 1227 (2) $[M+Na+2]^+$, 1225 (2)

$[M+Na]^+$, 1207 (8) $[M+H+2]^+$, 1205 (7) $[M+H]^+$, 1145 (8), 1143 (7), 1037 (10), 1035 (9), 598 (6), 430 (28), 368 (6), 366 (6), 154 (55), 136 (43), 91 (100); HRMS (FAB): found 1203.2588 $[M+H]^+$; $C_{48}H_{62}Cl_3O_{27}N_2$ calcd 1203.2606.

Compound 15: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.40-7.24$ (m, 5H), 6.11 (d, $J = 9.5$ Hz, 3H), 5.46 (ddd, $J = 8.7$, 6.4, 2.8 Hz, 1H), 5.38–5.29 (m, 3H), 5.26–5.21 (m, 2H), 5.02 (ddd, $J = 10.3$, 7.2, 3.1 Hz, 1H), 4.97–4.86 (m, 4H), 4.79 (d, $J = 12.0$ Hz, 1H), 4.48 (d, $J = 12.1$ Hz, 1H), 4.43 (d, $J = 12.0$ Hz, 1H), 4.32 (dd, $J = 12.3$, 2.9 Hz, 1H), 4.29–4.20 (m, 3H), 4.20–4.03 (m, 9H), 3.87 (s, 3H), 3.85 (s, 3H), 3.81 (d, $J = 14.8$ Hz, 2H), 3.68 (s, 3H), 3.64 (q, $J = 10.3$ Hz, 1H), 2.75–2.66 (m, 3H), 2.14–2.10 (m, 18H), 2.05–1.93 (m, 21H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.63$ (C), 170.53 (C), 170.44 (C), 170.29 (C), 170.20 (C), 170.03 (C), 169.96 (C), 169.78 (C), 168.60 (C), 168.56 (C), 167.78 (C), 167.53 (C), 154.08 (C), 137.23 (C), 128.24 (CH), 127.82 (CH), 127.68 (CH), 98.63 (C), 98.55 (C), 98.39 (C), 95.36 (C), 74.58 (CH_2), 73.16 (CH), 72.70 (CH), 72.42 (CH), 68.73 (CH), 68.62 (CH), 68.42 (CH), 68.23 (CH), 67.57 (CH), 67.44 (CH), 67.33 (CH), 66.87 (CH_2), 63.92 (CH_2), 62.66 (CH_2), 62.50 (CH_2), 62.08 (CH_2), 53.27 (CH_3), 52.62 (CH_3), 51.51 (CH), 48.92 (CH), 48.66 (CH), 38.26 (CH_2), 37.65 (CH_2), 21.14 (CH_3), 21.00 (CH_3), 20.96 (CH_3), 20.87 (CH_3), 20.75 (CH_3) ppm; MS (MALDI-TOF): m/z: found 1714.3930; $[C_{68}H_{88}Cl_3N_3O_{40}Na]^+$ calcd 1714.3905.

Compound 17: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.40-7.24$ (m, 5H), 6.12 (t, $J = 7.9$ Hz, 3H), 5.46 (ddd, $J = 8.5$, 6.3, 2.8 Hz, 1H), 5.38–5.29 (m, 4H), 5.26–5.21 (m, 3H), 5.02 (ddd, $J = 10.3$, 7.2, 3.1 Hz, 1H), 4.97–4.86 (m, 6H), 4.81 (d, $J = 12.0$ Hz, 1H), 4.47 (d, $J = 12.1$ Hz, 1H), 4.42 (d, $J = 12.0$ Hz, 1H), 4.34–4.02 (m, 21H), 3.87 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.81(d, $J =$ 14.8 Hz, 3H), 3.68 (s, 3H), 3.64 (q, $J = 10.3$ Hz, 1H), 2.75–2.66 (m, 4H), 2.15–2.10 (m, 25H), 2.05–1.93 (m, 27H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.62$ (C), 170.52 (C), 170.42 (C), 170.27 (C), 170.16 (C), 170.00 (C), 169.94 (C), 169.73 (C), 168.59 (C), 168.48 (C), 167.73 (C), 167.49 (C), 154.05 (C), 137.20 (C), 128.21 (CH), 127.80 (CH), 127.66 (CH), 98.60 (C), 98.53 (C), 98.36 (C), 95.33 (C),74.55 (CH_2), 73.14 (CH), 72.67 (CH), 72.39 (CH), 68.66 (CH), 68.59 (CH), 68.35 (CH), 68.20 (CH), 67.53 (CH), 67.40 (CH), 67.30 (CH), 66.85 (CH_2), 63.96 (CH_2), 62.63 (CH_2), 62.48 (CH_2), 62.05 (CH_2), 53.26 (CH_3), 52.60 (CH_3), 51.48 (CH), 48.89 (CH), 48.66 (CH), 38.24 (CH_2), 37.57 (CH_2), 21.12 (CH_3), 21.00 (CH_3), 20.95 (CH_3), 20.85 (CH_3), 20.72 (CH_3) ppm; MS (MALDI-TOF): m/z: found 2203.5376; $[C_{88}H_{115}Cl_3N_4O_{53}Na]^+$ calcd 2203.5388.

General procedure for the conversion of *N*-Troc-containing oligomers 13, 15, and 17 into fully protected α-(2→5)-linked Neu5Gc oligomers—preparation of 19–21: Freshly activated zinc dust (1.03 g) was added to a solution of compound **13** (0.201 g, 0.17 mmol) in glacial acetic acid (3.0 mL). The mixture was stirred at room temperature for 1.5 h, diluted with ethyl acetate (100 mL), and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in CH_2Cl_2 (4.0 mL) and cooled to 0 °C, and acetoxyacetyl chloride (0.05 mL, 0.46 mmol) was added, followed by EtN^iPr_2 (0.15 mL, 0.86 mmol). After the mixture had been stirred for 2 h it was quenched with saturated aqueous $NaHCO_3$ (2.0 mL), diluted with CH_2Cl_2 (50 mL), washed with saturated aqueous $NaHCO_3$ (8 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash silica gel chromatography (50–100% gradient EtOAc in hexane) to afford **19** (0.136 g, 72%).

By the same procedure, **15** (200 mg, 0.12 mmol) afforded **20** (139 mg, 73%), and **17** (98 mg, 0.045 mmol) afforded **21** (70 mg, 74%) as colorless powders.

Compound 19: ^{1}H NMR (400 MHz, [D$_6$]benzene): $\delta = 7.42$ (d, $J = 7.4$ Hz, 2H), 7.15–7.12 (m, 2H), 7.04 (t, $J = 7.4$ Hz, 1H), 6.53 (d, $J = 10.0$ Hz, 1H), 5.92 (dt, $J = 7.2$, 2.7 Hz, 1H), 5.70 (dd, $J = 7.2$, 2.1 Hz, 1H), 5.67 (m, 1H), 5.57 (dd, $J = 6.8$, 2.2 Hz, 1H), 5.56 (d, $J = 9.1$ Hz, 1H), 5.29 (ddd, $J = 12.1$, 4.6 Hz, 1H), 5.12–5.03 (m, 2H), 4.77–4.62 (m, 4H), 4.51–4.47 (m, 2H), 4.42–4.32 (m, 4H), 4.28 (dd, $J = 10.6$, 2.0 Hz, 1H), 4.22–4.16 (m, 2H), 3.46 (s, 3H), 3.19 (s, 3H), 2.92 (dd, $J = 12.4$, 4.6 Hz, 1H), 2.58 (dd, $J = 12.4$, 4.6 Hz, 1H), 2.18 (t, $J = 12.4$ Hz, 1H), 2.08 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H), 1.90 (t, $J = 12.4$ Hz, 1H), 1.83 (s, 3H), 1.82 (s, 3H), 1.74 (s, 6H), 1.73 (s, 3H) ppm; ^{13}C NMR (100 MHz, [D$_6$]benzene): $\delta = 170.94$ (C), 170.48 (C), 170.35 (C), 170.16 (C), 169.64 (C), 168.82 (C), 168.70 (C), 168.16 (C), 167.62(C), 137.92 (C), 128.45(CH), 128.17 (C), 99.31 (C), 99.00 (C), 73.59 (CH), 73.54 (CH), 70.98 (CH_2), 70.05 (CH), 69.87 (CH), 69.01 (CH), 68.37 (CH), 67.74 (CH), 67.20 (CH_2), 64.46 (CH_2), 63.23 (CH_2), 62.95 (CH_2), 62.74 (CH_2), 52.91 (CH_3), 52.13 (CH_3), 49.63 (CH), 49.41 (CH), 38.86 (CH_2), 37.49 (CH_2), 21.13 (CH_3), 20.97 (CH_3), 20.73 (CH_3), 20.47 (CH_3), 20.40 (CH_3), 20.14 (CH_3) ppm; LRMS (FAB): m/z (%): 1151 (6) $[M+Na]^+$, 1129 (12) $[M+H]^+$, 1069 (14), 961 (16), 598 (4), 532 (9), 472 (71), 430 (31), 307 (10), 254 (15), 154 (60), 91 (100); HRMS (FAB): found 1129.3732 $[M+H]^+$; $C_{49}H_{65}O_{28}N_2$ calcd 1129.3724.

Compound 20: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.36-7.24$ (m, 5H), 6.13 (t, $J = 8.3$ Hz, 2H), 5.85 (d, $J = 10.0$ Hz, 1H), 5.46 (ddd, $J = 8.5$, 6.1, 2.8 Hz, 1H), 5.36–5.29 (m, 2H), 5.26–5.20 (m, 3H), 5.02–4.86 (m, 3H), 4.81(d, $J = 12.0$ Hz, 1H), 4.58 (d, $J = 15.3$ Hz, 1H), 4.42 (d, $J = 12.0$ Hz, 1H), 4.34–4.01 (m, 15H), 3.87 (s, 3H), 3.86 (s, 3H), 3.81 (d, $J = 14.9$ Hz, 1H), 3.80(d, $J = 14.9$ Hz, 1H), 3.68 (s, 3H), 2.73–2.64 (m, 3H), 2.17 (s, 3H), 2.15(s, 3H), 2.12 (s, 6H), 2.11 (s, 3H), 2.10 (s, 6H), 2.04–1.97 (m, 21H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.95$ (C), 170.55 (C), 170.22 (C), 170.10 (C), 169.97 (C), 169.83 (C), 169.62 (C), 168.61 (C), 167.71 (C), 137.22 (C), 128.24 (CH), 127.82 (CH), 127.69 (C), 98.56 (C), 98.56 (C),73.14 (CH), 72.92 (CH), 72.68 (CH), 68.62 (CH), 68.38 (CH), 67.94 (CH), 67.57 (CH), 67.45 (CH), 67.32 (CH), 66.87 (CH_2), 63.91 (CH_2), 62.80 (CH_2), 62.66 (CH_2), 62.52 (CH_2), 62.19 (CH_2), 53.34 (CH_3), 53.29 (CH_3), 52.63 (CH_3), 49.37 (CH), 48.91 (CH), 48.68 (CH), 38.25 (CH_2), 37.60 (CH_2), 21.14 (CH_3), 21.03 (CH_3), 20.72 (CH_3) ppm; MS (MALDI-TOF): m/z: found 1640.5023; $[C_{69}H_{91}O_{41}N_3Na]^+$ calcd 1640.5026.

Compound 21: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.40-7.24$ (m, 5H), 6.14 (d, $J = 8.4$ Hz, 2H), 6.12 (d, $J = 9.0$ Hz, 1H), 5.85 (d, $J = 9.8$ Hz, 1H), 5.46 (ddd, $J = 8.2$, 5.8, 3.4 Hz, 1H), 5.35–5.29 (m, 3H), 5.27–5.21 (m, 4H), 5.03–4.86 (m, 4H), 4.81 (d, $J = 12.0$ Hz, 1H), 4.58 (d, $J = 15.3$ Hz, 1H), 4.42 (d, $J =$ 12.0 Hz, 1H), 4.34–4.24 (m, 4H), 4.23–4.02 (m, 16H), 3.87 (s, 3H), 3.86 (s, 6H), 3.82 (d, $J = 14.8$ Hz, 2H), 3.81 (d, $J = 14.8$ Hz, 1H), 3.68 (s, 3H), 2.73–2.64 (m, 4H), 2.17 (s, 3H), 2.14 (s, 3H), 2.12–2.09 (m, 21H), 2.05–1.97 (m, 28H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.93$ (C), 170.54 (C), 170.44 (C), 170.18 (C), 170.06 (C), 169.96 (C), 169.83 (C), 169.78 (C), 169.60 (C), 168.60 (C), 168.49 (C), 167.69 (C), 137.21 (C), 128.22 (CH), 127.81 (CH), 127.67 (CH), 98.62 (C), 98.55 (C),73.14 (CH), 72.91 (CH), 72.67 (CH), 68.62 (CH), 68.35 (CH), 67.93 (CH), 67.55 (CH), 67.42 (CH), 67.30 (CH), 66.86 (CH_2), 63.88 (CH_2), 62.79 (CH_2), 62.64 (CH_2), 62.50 (CH_2), 62.17 (CH_2), 53.33 (CH_3), 53.28 (CH_3), 52.61 (CH_3), 49.36 (CH), 48.90 (CH), 48.67 (CH), 38.24 (CH_2), 37.58 (CH_2), 21.13 (CH_3), 21.02 (CH_3), 20.85 (CH_3), 20.79 (CH_3), 20.71 (CH_3) ppm; MS (MALDI-TOF): m/z: found 2129.6506; $[C_{89}H_{118}O_{54}N_4Na]^+$ calcd 2129.6508.

Elongation of the α-(2→5)-linked oligosaccharides from non-reducing end (11)—preparation of 22, 24, and 26: $NaHCO_3$ (84 mg, 1.00 mmol), HOBt (12 mg, 0.09 mmol), and EDC (142 mg, 0.74 mmol) were added sequentially to a solution of compound **11** (0.30 g, 0.49 mmol) and compound **7** (0.31 g, 0.51 mmol) in acetonitrile (8.0 mL). The resulting mixture was stirred at room temperature for 4–18 h. The solvent was removed under reduced pressure, and the residue was purified by flash silica gel chromatography (20–100% gradient EtOAc in hexane) to give compound **22** (0.465 g, 79%) as a colorless powder.

By the same procedure, **23** (318 mg, 0.290 mmol) afforded **24** (357 mg, 73.5%), and **25** (360 mg, 0.227 mmol) afforded **26** (366 mg, 74.5%) as colorless powders.

Compound 22: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.38-7.26$ (m, 5H), 6.12 (d, $J = 10.0$ Hz, 1H), 5.86 (d, $J = 9.8$ Hz, 1H), 5.37 (ddd, $J = 8.2$, 6.2, 2.8 Hz, 1H), 5.31 (ddd, $J = 8.0$, 5.4, 2.4 Hz, 1H), 5.24 (dd, $J = 8.0$, 2.0 Hz, 1H), 5.20 (dd, $J = 8.2$, 2.1 Hz, 1H), 5.16 (dd, $J = 23.9$, 12.3 Hz, 2H), 5.01–4.92 (m, 2H), 4.57 (d, $J = 15.3$ Hz, 1H), 4.37–4.18 (m, 6H), 4.14–3.97 (m, 6H), 3.86 (s, 3H), 3.80 (d, $J = 14.9$ Hz, 1H), 3.74 (s, 3H), 2.74 (dd, $J = 12.8$, 4.6 Hz, 1H), 2.66 (dd, $J = 12.8$, 4.6 Hz, 1H), 2.17 (s, 3H), 2.11 (s, 6H), 2.10 (s, 3H), 2.09 (s, 3H), 2.04–1.97 (m, 14H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 170.96 (C), 170.52 (C), 170.19 (C), 169.94 (C), 169.14 (C), 168.57 (C), 167.70(C), 135.40(C), 128.53 (CH), 128.35 (CH), 98.51 (C), 98.26 (C), 72.87 (CH), 72.68 (CH), 68.63 (CH), 68.42 (CH), 67.94 (CH), 67.38 (CH), 67.29 (CH), 66.64 (CH), 63.85 (CH_2), 62.78 (CH_2), 62.57 (CH_2), 62.17 (CH_2), 61.92 (CH_2), 53.34 (CH_3), 52.97 (CH_3), 49.34 (CH), 48.80 (CH), 37.70 (CH_2), 37.56 (CH_2), 21.09 (CH_3), 21.04 (CH_3), 20.72(CH_3) ppm; LRMS (FAB): m/z (%): 1209 (8) $[M+Na]^+$, 1187 (15) $[M+H]^+$, 1127 (22), 961 (50), 663 (5), 532 (15), 472 (93), 430 (58), 254 (26), 154 (75), 136 (61), 91 (100); HRMS (FAB): found 1187.3789 $[M+H]^+$; $C_{51}H_{67}O_{30}N_2$ calcd 1187.3779.

Compound 23: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.20$ (d, $J = 9.4$ Hz, 1H), 5.86 (d, $J = 9.8$ Hz, 1H), 5.37 (ddd, $J = 8.2$, 6.1, 2.2 Hz, 1H), 5.31 (ddd, $J =$ 8.1, 5.4, 2.2 Hz, 1H), 5.25 (dd, $J = 8.2$, 1.3 Hz, 1H), 5.20 (d, $J = 8.1$ Hz, 1H),

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5.05–4.95 (m, 2H), 4.58 (d, J = 15.3 Hz, 1H), 4.38–4.16 (m, 7H), 4.14–4.00 (m, 6H), 3.86 (s, 3H), 3.84–3.77 (m, 5H), 2.73 (dd, J = 13.0, 4.6 Hz, 1H), 2.66 (dd, J = 13.0, 4.6 Hz, 1H), 2.17 (s, 3H), 2.12 (s, 12H), 2.05–1.99 (m, 14H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.98 (C), 170.71 (C), 170.44 (C), 170.20 (C), 170.00 (C), 169.65 (C), 168.75 (C), 167.81 (C), 167.69(C), (C), 98.55 (C), 98.41 (C), 72.88 (CH), 72.75 (CH), 68.69 (CH), 68.53 (CH), 68.41 (CH), 67.99 (CH), 67.41 (CH), 67.32 (CH), 63.82 (CH_2), 62.79 (CH_2), 62.69 (CH_2), 62.19 (CH_2), 61.84 (CH_2), 53.33 (CH_3), 53.15 (CH_3), 49.35 (CH), 48.94 (CH), 37.50 (CH_2), 37.44 (CH_2), 21.07 (CH_3), 21.02 (CH_3), 20.77 (CH_3), 20.72(CH_3) ppm; LRMS (FAB): m/z (%): 1119 (32) $[M+Na]^+$, 1097 (8) $[M+H]^+$, 1037 (7), 961 (7), 532 (7), 472 (48), 430 (17), 307 (15), 154 (100), 136 (70), 69 (50); HRMS (FAB): found 1097.3315 $[M+H]^+$; $C_{44}H_{61}O_{30}N_2$ calcd 1097.3309.

Compound 24: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.31 (m, 5H), 6.10 (d, J = 8.6 Hz, 1H), 6.08 (d, J = 9.6 Hz, 1H), 5.82 (d, J = 9.8 Hz, 1H), 5.37 (ddd, J = 8.2, 5.4, 2.9 Hz, 2H), 5.34–5.29 (m, 2H), 5.26–5.18 (m, 3H), 516 (dd, J = 23.7, 12.3 Hz, 2H), 5.01–4.90 (m, 3H), 4.58(d, J = 15.3 Hz, 1H), 4.35 (d, J = 16.4 Hz, 1H), 4.30–4.00 (m, 18H), 3.98 (dd, J = 10.8, 2.2 Hz, 1H), 3.86 (s, 6H), 3.82 (d, J = 14.8 Hz, 1H), 3.81 (d, J = 14.8 Hz, 1H), 3.74 (s, 3H), 2.74 (dd, J = 12.8, 4.6 Hz, 1H), 2.70 (dd, J = 12.8, 4.6 Hz, 1H), 2.66 (dd, J = 12.8, 4.6 Hz, 1H), 2.17 (s, 3H), 2.11(s, 6H), 2.10 (s, 9H), 2.09 (s, 3H), 2.05–1.98 (m, 20H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.93 (C), 170.61 (C), 170.45 (C), 170.15 (C), 170.06 (C), 169.89 (C), 169.79 (C), 169.59 (C), 169.12 (C), 168.58 (C), 167.68 (C), 167.61 (C), 135.49 (C), 128.52 (CH), 128.33 (CH), 98.54 (C), 98.27 (C),73.12 (CH), 72.92 (CH), 72.71 (CH), 68.69 (CH), 68.63 (CH), 68.40 (CH), 67.92 (CH), 67.39 (CH), 67.30 (CH), 66.61 (CH_2), 63.87 (CH_2), 62.79 (CH_2), 62.56 (CH_2), 62.49 (CH_2), 62.17 (CH_2), 61.92 (CH_2), 53.33 (CH_3), 53.26 (CH_3), 52.95 (CH_3), 49.36 (CH), 48.82 (CH), 48.67 (CH), 37.72 (CH_2), 37.58 (CH_2), 21.07 (CH_3), 21.01 (CH_3), 20.79 (CH_3), 20.70 (CH_3) ppm; MS (MALDI-TOF): m/z: found 1698.5078; $[C_{71}H_{93}O_{43}N_3Na]^+$ calcd 1698.5081.

Compound 25: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.29 (br, 2H), 5.86 (d, J = 9.6 Hz, 1H), 5.41 (m, 1H), 5.37–5.21 (m, 5H), 5.16 (d, J = 7.5 Hz, 1H), 5.06 (m, 1H), 4.97–4.85 (m, 2H), 4.57(d, J = 15.3 Hz, 1H), 4.35–4.02 (m, 21H), 3.87 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.82–3.77 (m, 2H), 2.76 (dd, J = 13.0, 4.6 Hz, 1H), 2.67 (dd, J = 12.8, 4.6 Hz, 1H), 2.62 (dd, J = 12.8, 3.9 Hz, 1H), 2.17 (s, 3H), 2.11–2.12 (m, 12H), 2.10 (s, 9H), 2.09 (s, 3H), 2.07–1.94 (m, 21H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.91 (C), 170.82 (C), 170.66 (C), 170.57 (C), 170.42 (C), 170.32 (C), 170.22 (C), 170.10 (C), 170.02 (C), 169.87 (C), 169.62 (C), 168.68 (C), 168.14 (C), 167.82 (C), 167.73 (C), 167.68 (C), 98.71 (C), 98.27 (C),72.97 (CH), 72.89 (CH), 72.69 (CH), 68.68 (CH), 68.34 (CH), 68.24 (CH), 67.38 (CH), 67.33 (CH), 67.19 (CH), 63.98 (CH_2), 63.61 (CH_2), 62.80 (CH_2), 62.55 (CH_2), 62.18 (CH_2), 61.50 (CH_2), 53.34 (CH_3), 53.21 (CH_3), 53.17 (CH_3), 49.14 (CH), 48.90 (CH), 48.29 (CH), 37.60 (CH_2), 37.44 (CH_2), 37.36 (CH_2), 21.01 (CH_3), 20.75 (CH_3), 20.66 (CH_3) ppm; MS (MALDI-TOF) m/z: found 1608.4609; $[C_{64}H_{87}O_{43}N_3Na]^+$ calcd 1608.4611.

Compound 26: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.31 (m, 5H), 6.10 (d, J = 9.5 Hz, 2H), 6.08 (d, J = 10.2 Hz, 1H), 5.82 (d, J = 9.8 Hz, 1H), 5.37 (ddd, J = 8.0, 4.8, 2.4 Hz, 1H), 5.34–5.29 (m, 3H), 5.26–5.18 (m, 4H), 516 (dd, J = 23.9, 12.3 Hz, 2H), 4.98–4.90 (m, 4H), 4.58 (d, J = 15.3 Hz, 1H), 4.35 (d, J = 16.4 Hz, 1H), 4.30–4.21 (m, 6H), 4.19–3.96 (m, 14H), 3.86 (s, 9H), 3.81 (d, J = 14.8 Hz, 2H), 3.80 (d, J = 14.8 Hz, 1H), 3.73 (s, 3H), 2.74 (dd, J = 12.8, 4.6 Hz, 1H), 2.72–2.64 (m, 3H), 2.17 (s, 3H), 2.11 (s, 6H), 2.10 (s, 15H), 2.09 (s, 3H), 2.05–1.98 (m, 28H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.92 (C), 170.61 (C), 170.53 (C), 170.43 (C), 170.13 (C), 170.04 (C), 169.88 (C), 169.80 (C), 169.59 (C), 169.12 (C), 168.58 (C), 167.68 (C), 167.61 (C), 135.49 (C), 128.52 (CH), 128.33 (CH), 98.54 (C), 98.26 (C),73.14 (CH), 72.92 (CH), 72.71 (CH), 68.64 (CH), 68.44 (CH), 68.34 (CH), 67.92 (CH), 67.39 (CH), 67.29 (CH), 66.61 (CH_2), 63.86 (CH_2), 62.79 (CH_2), 62.56 (CH_2), 62.48 (CH_2), 62.16 (CH_2), 61.92 (CH_2), 53.33 (CH_3), 53.27 (CH_3), 52.95 (CH_3), 49.36 (CH), 48.82 (CH), 48.66 (CH), 37.72 (CH_2), 37.58 (CH_2), 21.08 (CH_3), 21.02 (CH_3), 20.80 (CH_3), 20.70 (CH_3) ppm; MS (MALDI-TOF): m/z: found 2187.6560; $[C_{91}H_{120}O_{56}N_4Na]^+$ calcd 2187.6563.

Compound 27: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.56 (br, 1H), 6.42 (br, 1H), 6.21 (d, J = 10.0 Hz, 1H), 5.84 (d, J = 9.8 Hz, 1H), 5.46 (m, 1H), 5.35–5.23 (m, 6H), 5.14–5.05 (m, 2H), 4.98 (dt, J = 11.6, 4.6 Hz, 1H), 4.89–4.72 (m, 2H), 4.58 (d, J = 15.3 Hz, 1H), 4.30–4.00 (m, 21H), 3.88 (s, 3H), 3.86 (s, 3H), 3.83–3.80 (m, 7H), 3.72 (d, J = 15.3 Hz, 2H), 2.80 (dd, J = 13.0, 4.6 Hz, 1H), 2.70–2.57 (m, 3H), 2.17 (s, 3H), 2.12–2.07 (m, 27H), 2.04–1.94 (m, 25H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.92 (C), 170.63 (C), 170.53 (C), 170.39 (C), 170.31 (C), 170.16 (C), 170.04 (C), 169.93 (C), 169.83 (C), 169.71 (C), 169.61 (C), 168.58 (C), 167.80 (C), 167.74 (C), 167.68 (C), 167.58 (C), 98.91 (C), 98.66 (C), 98.05 (C), 97.93 (C), 73.09 (CH), 72.91 (CH), 72.62 (CH), 69.01 (CH), 68.89 (CH), 68.75 (CH), 68.68 (CH), 68.48 (CH), 68.24 (CH), 67.87 (CH), 67.36 (CH), 67.21 (CH), 64.18 (CH_2), 63.99 (CH_2), 63.31 (CH_2), 62.78 (CH_2), 62.62 (CH_2), 62.47 (CH_2), 62.17 (CH_2), 61.03 (CH_2), 53.37 (CH_3), 53.25 (CH_3), 53.19 (CH_3), 53.10 (CH_3), 49.30 (CH), 48.95 (CH), 48.32 (CH), 47.76 (CH), 37.79 (CH_2), 37.67 (CH_2), 37.47 (CH_2), 37.07 (CH_2), 21.00 (CH_3), 20.69 (CH_3), 20.62 (CH_3) ppm; MS (MALDI-TOF): m/z: found 2097.6091; $[C_{84}H_{114}O_{56}N_4Na]^+$ calcd 2097.6094.

Synthesis of the fully protected octamer 28: Freshly activated zinc dust (1.0 g) was added to a solution of compound **17** (0.251 g, 0.12 mmol) in glacial acetic acid (3.0 mL). The mixture was stirred at room temperature for 1.5 h, diluted with ethyl acetate (100 mL), and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and concentrated. The residue and compound **27** (0.204 g, 0.10 mmol) were dissolved in acetonitrile (4.0 mL), and $NaHCO_3$ (19 mg, 0.23 mmol), HOBt (4 mg, 0.03 mmol), and EDC (52 mg, 0.27 mmol) were added sequentially. The resulting mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure, and the residue was purified by flash silica gel chromatography (0–10% gradient MeOH in EtOAc) to give relatively pure compound **28** (0.274 g). This material was recrystallized from 20% EtOAc in hexane to afford **28** (0.231 g, 49%) as a colorless powder.

Compound 28: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.24 (m, 5H), 6.13 (d, J = 9.3 Hz, 6H), 6.11 (d, J = 9.0 Hz, 1H), 5.83 (d, J = 9.8 Hz, 1H), 5.46 (ddd, J = 8.2, 5.8, 3.4 Hz, 1H), 5.36–5.28 (m, 7H), 5.28–5.19 (m, 8H), 5.03–4.86 (m, 8H), 4.81 (d, J = 12.0 Hz, 1H), 4.58 (d, J = 15.3 Hz, 1H), 4.43 (d, J = 12.0 Hz, 1H), 4.34–4.24 (m, 8H), 4.23–4.02 (m, 28H), 3.87 (s, 21H), 3.82(d, J = 10.9 Hz, 7H), 3.68 (s, 3H), 2.73–2.64 (m, 8H), 2.17 (s, 3H), 2.15 (s, 3H), 2.14–2.06 (m, 42H), 2.06–1.94 (m, 59H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.90 (C), 170.52 (C), 170.40 (C), 170.13 (C), 170.01 (C), 169.82 (C), 169.58 (C), 168.67 (C), 168.47 (C), 167.69 (C), 137.21 (C), 128.20 (CH), 127.78 (CH), 127.64 (CH), 98.53 (C), 73.11 (CH), 72.89 (CH), 72.65 (CH), 68.61 (CH), 68.33 (CH), 67.91 (CH), 67.54 (CH), 67.39 (CH), 66.83 (CH_2), 63.85 (CH_2), 62.77 (CH_2), 62.62 (CH_2), 62.47 (CH_2), 62.16 (CH_2), 53.25 (CH_3), 52.58 (CH_3), 49.34 (CH), 48.88 (CH), 48.65 (CH), 38.22 (CH_2), 37.56 (CH_2), 21.10 (CH_3), 21.00 (CH_3), 20.77 (CH_3), 20.69 (CH_3) ppm; MS (MALDI-TOF): m/z: found 4085.2420; $[C_{169}H_{225}O_{106}N_8Na]^+$ calcd 4085.2356.

Deprotection of 29 with MeONa in methanol—preparation of 31 and 32: A methanolic sodium methoxide solution (0.05 mL, 1N) was added under N_2 at room temperature to a solution of **29** (79 mg, 0.07 mmol) in dry MeOH (3.0 mL). After 2 h, Amberlite IR-120 (H^+) ion exchange resin was added. The mixture was filtered, and the resin was washed with methanol (2 × 5 mL). The combined filtrate was concentrated, and the residue was purified by flash silica gel chromatography (0–20% MeOH in EtOAc) to give **31** (10mg, 17%) and **32** (36 mg, 67%) as colorless powders.

Compound 31: MS (MALDI-TOF): m/z: found 757.2665; $[C_{31}H_{46}N_2O_{18}Na]^+$ calcd 757.2643.

Compound 32: MS (MALDI-TOF): m/z: found 757.2635; $[C_{31}H_{46}N_2O_{18}Na]^+$ calcd 757.2643.

For NMR data for compounds **31** and **32** see Table 1.

General procedure for deprotection of methyl esters with LiCl—preparation of the lithium salts 33–35: LiCl (21 mg, 0.50 mmol) was added to a solution of **19** (174 mg, 0.15 mmol) in pyridine (4.0 mL). The solution was heated at 120°C for 16 h, and the solvent was removed under reduced pressure. The dark brown residue was purified by chromatography over Sephadex LH-20 with MeOH to afford the dilithium salt **33** as a light brown powder (147 mg, 85%). By the same procedure, **21** (150 mg, 0.07 mmol) afforded **34** (131 mg, 89%), and **28** (99 mg, 0.02 mmol) afforded **35** (87 mg, 89%) as light brown powders.

Compound 33: ^{1}H NMR (400 MHz, D_2O): δ = 7.56–7.44 (m, 5H), 5.57 (ddd, J = 8.0, 5.3, 2.6 Hz, 1H), 5.50 (dd, J = 8.1, 1.8 Hz, 1H), 5.49–5.45 (m, 1H), 5.43 (dd, J = 8.4, 1.8 Hz, 1H), 5.10 (dt, J = 11.4, 4.7 Hz, 2H), 4.88 (d, J = 10.9 Hz, 1H), 4.77 (d, J = 10.9 Hz, 1H), 4.66–4.59 (m, 4H), 4.53 (dd, J = 10.2, 2.1 Hz, 1H), 4.44 (dd, J = 12.6, 2.4 Hz, 1H), 4.38–4.32 (m, 3H), 4.12 (t, J = 10.5 Hz, 1H), 4.07 (t, J = 10.5 Hz, 1H), 3.98 (d, J = 15.0 Hz, 1H), 2.87 (dd, J = 12.4, 4.7 Hz, 1H), 2.79 (dd, J = 12.4, 4.7 Hz, 1H), 2.30 (s, 3H), 2.29 (s, 6H), 2.28 (s, 3H), 2.27 (s, 3H), 2.20 (s, 3H), 2.19 (s, 3H), 2.16 (s,

Table 1. 500 MHz ^{1}H NMR data (300 K) and 100 MHz ^{13}C NMR data (300 K) for the disaccharides **31** and **32**. Two-dimensional COSY, NOESY, HMQC, and HMBC experiments were used for the assignments.

	^{1}H NMR chem. shift [ppm]				^{13}C NMR chem. shift [ppm]				
	31		**32**			**31**		**32**	
unit	A	B	A	B	unit	A	B	A	B
H3eq	2.56	2.54	2.55	2.71	C1	169.15	168.55	169.08	168.46
H3ax	1.71	1.63	1.65	1.47	C2	98.17	98.28	98.33	98.93
H4	3.60	3.71	3.71	3.50	C3	40.47	40.15	40.70	39.63
H5	3.61	3.68	3.73	3.50	C4	66.13	65.65	65.29	65.75
H6	3.44	3.67	3.76	3.50	C5	51.78	51.91	52.68	52.30
H7	3.59	3.67	3.28	3.34	C6	73.42	72.93	72.69	73.99
H8	3.27	3.30	3.68	3.55	C7	70.27	71.01	69.13	67.85
H9	3.39	3.63	3.65	3.41	C8	68.73	68.73	71.10	71.00
NH	7.68	7.97	8.01	8.05	C9	63.43	63.32	63.13	63.57
Ac-CH_3	–	1.87	–	1.89	C21	65.04	62.67	65.70	60.45
CH_2	4.75, 4.45	4.19, 3.98	4.75, 4.45	4.24, 4.10	C22	–	169.88	–	169.45
CO_2Me	3.75	3.73	3.64	3.74	C51	–	171.38	–	172.27
					C52	–	22.61	–	22.53
					CH_3	52.63	52.63	52.67	51.54

6H), 1.99 (t, $J = 12.4$ Hz, 1H), 1.89 (dt, $J = 12.4$, 1.8 Hz, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): $\delta = 174.49$ (C), 174.40 (C), 173.74 (C), 173.66 (C), 173.46 (C), 173.17 (C), 173.00 (C), 172.74 (C), 171.15 (C), 138.04 (C), 129.30 (CH), 129.22 (CH), 128.84 (CH), 101.60 (C), 101.01 (C), 71.81 (CH), 71.73 (CH), 71.06 (CH), 70.67 (CH), 69.75 (CH), 69.00 (CH), 68.89 (CH), 68.43 (CH), 67.75 (CH_2), 63.95 (CH_2), 63.35 (CH_2), 63.01 (CH_2), 49.58 (CH), 49.49 (CH), 38.75 (CH_2), 37.86 (CH_2), 21.35 (CH_3), 21.08 (CH_3), 21.06 (CH_3), 20.94 (CH_3), 20.86 (CH_3), 20.63 (CH_3) ppm; MS (MALDI-TOF): *m*/*z*: found 1105.50; $[C_{47}H_{58}O_{28}N_2Li]^-$ calcd 1105.33.

Compound 34: ^{1}H NMR (400 MHz, D_2O): $\delta = 7.56-7.44$ (m, 5H), 5.54 (ddd, $J = 8.0$, 5.3, 2.6 Hz, 1H), 5.49–5.38 (m, 7H), 5.11–5.01 (m, 4H), 4.84 (d, $J = 10.5$ Hz, 1H), 4.74 (dd, $J = 10.5$, 2.1 Hz, 1H), 4.70–4.53 (m, 8H), 4.49 (dd, $J = 12.5$, 2.6 Hz, 1H), 4.42–4.26 (m, 10H), 4.11–3.87 (m, 8H), 2.86–2.71 (m, 4H), 2.26 (s, 12H), 2.25 (s, 3H), 2.24 (s, 9H), 2.23 (s, 3H), 2.16 (s, 9H), 2.15 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 1.95 (dt, $J = 12.1$, 3.2 Hz, 1H), 1.83 (t, $J = 12.4$ Hz, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): $\delta = 174.40$ (C), 173.73 (C), 173.40 (C), 173.01 (C), 172.68 (C), 171.13 (C), 137.96 (C), 129.25 (CH), 128.81 (CH), 101.59(C), 100.97 (C), 71.68 (CH), 70.93 (CH), 70.60 (CH), 69.63 (CH), 68.82 (CH), 68.47 (CH), 68.37 (CH), 67.76 (CH_2), 63.91 (CH_2), 63.30 (CH_2), 63.13 (CH_2), 62.97 (CH_2), 49.55 (CH), 49.47 (CH), 49.27 (CH), 38.76 (CH_2), 37.83 (CH_2), 21.33 (CH_3), 21.04 (CH_3), 20.91 (CH_3), 20.81 (CH_3), 20.56 (CH_3) ppm; MS (ESI): *m*/*z*: found 2051.93 $[M+H]^+$; $[C_{85}H_{111}N_4O_{54}]^+$ calcd 2051.61; MS (MALDI-TOF): *m*/*z*: found 2031.75; $[C_{83}H_{103}N_4O_{53}Li_4]^-$ calcd 2031.61.

Compound 35: ^{1}H NMR (400 MHz, D_2O): $\delta = 7.58-7.46$ (m, 5H), 5.58–5.40 (m, 16H), 5.16–5.02 (m, 8H), 4.86 (d, $J = 11.5$ Hz, 1H), 4.76–4.53 (m, 13H), 4.52–4.27 (m, 29H), 4.15–3.89 (m, 16H), 2.88–2.73 (m, 8H), 2.30–2.22 (m, 51H), 2.20–2.13 (m, 48H), 2.01–1.92 (m, 7H), 1.86 (t, $J = 12.0$ Hz, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): $\delta = 174.45$ (C), 173.76 (C), 173.53 (C), 173.46 (C), 173.19 (C), 173.06 (C), 172.71 (C), 171.16 (C), 138.06 (C), 129.33 (CH), 129.24 (CH), 129.07 (CH), 128.86 (CH), 101.61 (C), 100.97 (C), 71.75 (CH), 71.01 (CH), 70.68 (CH), 69.77 (CH), 68.91 (CH), 68.56 (CH), 67.75 (CH_2), 63.96 (CH_2), 63.37 (CH_2), 61.59 (CH_2), 49.60 (CH), 49.50 (CH), 49.30 (CH), 38.46 (CH_2), 37.92 (CH_2), 21.39 (CH_3), 21.11 (CH_3), 20.98 (CH_3), 20.89 (CH_3), 20.67 (CH_3) ppm; MS (ESI): *m*/*z*: found 3954.25 $[M+H]^+$; $[C_{161}H_{211}N_8O_{106}]^-$ calcd 3954.39.

General procedure for the alkaline hydrolysis of lithium salts 33–35—preparation of the oligomer sodium salts 36–28: Compound **33** (147 mg, 0.13 mmol) was treated with aqueous NaOH (0.1M, 15 mL) at room temperature for 3 h. The mixture was lyophilized to give the crude product as a light brown solid. The crude product was purified by chromatography over Sephadex LH-20 with water, and the fractions containing the product were lyophilized to afford the disodium salt **36** as a light brown powder (88 mg, 87%). By the same procedure, **34** (130 mg, 0.06 mmol) afforded **37** (79 mg, 88%), and **35** (87 mg, 0.02 mmol) afforded **38** (52 mg, 86%) as light brown powders.

Compound 36: ^{1}H NMR (400 MHz, D_2O): $\delta = 7.56-7.42$ (m, 5H), 4.88 (d, $J = 10.9$ Hz, 1H), 4.65 (d, $J = 10.9$ Hz, 1H), 4.43 (d, $J = 15.3$ Hz, 1H), 4.24 (d, $J = 15.3$ Hz, 1H), 4.24 (s, 2H), 4.08–3.90 (m, 12H), 3.79–3.67 (m, 4H), 2.90 (ddd, $J = 12.1$, 7.7, 4.6 Hz, 2H), 1.93 (t, $J = 12.1$ Hz, 1H), 1.81 (t, $J = 12.0$ Hz, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): $\delta = 176.40$ (C), 174.17 (C), 173.75 (C), 173.54 (C), 137.78 (C), 129.33 (CH), 129.30 (CH), 128.88 (CH), 101.62 (C), 101.05 (C), 73.11 (CH), 72.98 (CH), 72.52 (CH), 72.20 (CH), 68.80 (CH), 68.73 (CH), 68.61 (CH), 68.56 (CH), 67.66 (CH_2), 63.73 (CH_2), 63.25 (CH_2), 61.64 (CH_2), 52.46 (CH), 52.09 (CH), 41.13 (CH_2), 40.17 (CH_2) ppm; MS (MALDI-TOF): *m*/*z*: found 743.42; $[C_{29}H_{40}O_{19}N_2Na]^-$ calcd 743.21.

Compound 37: ^{1}H NMR (400 MHz, D_2O): $\delta = 7.54-7.42$ (m, 5H), 4.87 (d, $J = 10.9$ Hz, 1H), 4.64 (d, $J = 10.9$ Hz, 1H), 4.41 (d, $J = 15.2$ Hz, 3H), 4.22 (d, $J = 15.2$ Hz, 3H), 4.22 (s, 2H), 4.06–3.88 (m, 24H), 3.77–3.65 (m, 8H), 2.92–2.84 (m, 4H), 1.90 (dt, $J = 12.2$, 4.9 Hz, 3H), 1.79 (t, $J = 12.0$ Hz, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): $\delta = 176.37$ (C), 174.15 (C), 173.75 (C), 173.48 (C), 137.73 (C), 129.32 (CH), 129.29 (CH), 128.88 (CH), 101.58 (C), 101.01 (C), 73.07 (CH), 72.97 (CH), 72.48 (CH), 72.32 (CH), 72.17 (CH), 68.67 (CH), 68.57 (CH), 68.51 (CH), 68.44 (CH), 67.63 (CH_2), 63.68 (CH_2), 63.28 (CH_2), 63.21 (CH_2), 61.61 (CH_2), 52.45 (CH), 52.26 (CH), 52.06 (CH), 41.09 (CH_2), 40.09 (CH_2) ppm; MS (MALDI-TOF): *m*/*z*: found 1401.15; $[C_{51}H_{72}O_{37}N_4Na_3]^-$ calcd 1401.36.

Compound 38: ^{1}H NMR (400 MHz, D_2O): $\delta = 7.52-7.42$ (m, 5H), 4.84 (d, $J = 10.9$ Hz, 1H), 4.61 (d, $J = 10.9$ Hz, 1H), 4.39 (d, $J = 15.2$ Hz, 7H), 4.18 (d, $J = 15.2$ Hz, 9H), 4.03–3.88 (m, 48H), 3.72–3.63 (m, 16H), 2.89–2.80 (m, 8H), 1.89 (t, $J = 12.2$ Hz, 1H), 1.87 (t, $J = 12.2$ Hz, 6H), 1.76 (t, $J = 12.0$ Hz, 1H) ppm; ^{13}C NMR (100 MHz, D_2O): $\delta = 176.37$ (C), 174.15 (C), 173.74 (C), 173.44 (C), 137.72 (C), 129.32 (CH), 129.28 (CH), 128.87 (CH), 101.58(C), 101.01 (C), 73.06 (CH), 72.96 (CH), 72.50 (CH), 72.33 (CH), 72.17 (CH), 68.66 (CH), 68.57 (CH), 68.42 (CH), 67.64 (CH_2), 63.68 (CH_2), 63.30 (CH_2), 61.60 (CH_2), 52.44 (CH), 52.25 (CH), 52.04 (CH), 40.10 (CH_2), 38.52 (CH_2) ppm; MS (ESI, MH^+): *m*/*z*: found 2566.43; $[C_{95}H_{145}N_8O_{73}]^-$ calcd 2565.79.

Neuraminidase hydrolysis: α-(2→5)Neu5Gc octamer **38** (20 μg) dissolved in 30 μL of 100 mM ammonium acetate buffer (pH 5) was digested with neuraminidase (0.1 mU) from *Anthrobacter ureafaciens* at 37°C for different time intervals. The progress of hydrolysis was monitored by HPCE at each time interval.

High-performance capillary electrophoresis chromatographic conditions (HPCE): Capillary electrophoresis (CE) was performed on a Beckman capillary electrophoresis system (P/ACE 2100) on a fused silica capillary (107 cm × 75 μm (inner diameter)) and carried out by applying 20 kV at 25°C. Phosphate buffer (50 mM, pH 7.0) was used as the running buffer. The spectra were monitored by their UV absorption at 200 nm. Samples were injected into the capillary under high nitrogen pressure (1.3 bar) for 3 s. The capillary was regenerated by washing with 0.1N NaOH for 7 min and then with double distilled water for 5 min.

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