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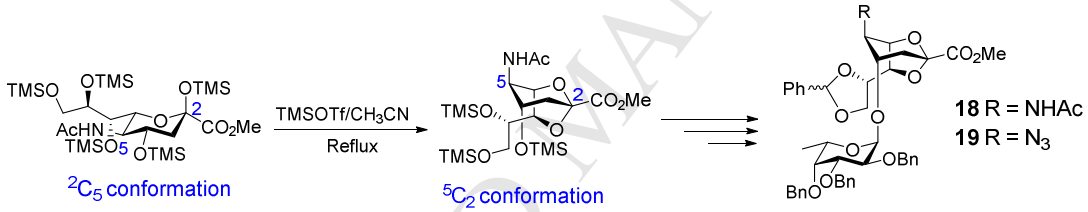
Graphical abstract



Concise Synthesis of 2,7-Anhydrosialic Acid Derivatives and Its Application

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Abstract

In *N*-acetylneuraminic acid, apart from O9 and O8, a possible glycosylation site is the O4 position. For example, gangliosides HLG-2 and HPG-7 are considered to be potential lead compounds for carbohydrate-based drug development to treat neural disorders. However, the construction of their $\alpha(1\rightarrow4)$ fucosyl sialic acid and $\alpha(2\rightarrow4)$ linkages between sialic acids is difficult because of the regioselectivity problem. Herein, *N*-acetyl-2,7-anhydroneuraminic acid was synthesized in three steps from Neu5Ac methyl ester through per-*O*-trimethylsilylation, heating-assisted intramolecular anomeric protection (iMAP) and desilylation. The iMAP simultaneously circumvents both the 2- and 7-OH protection. Upon protecting the 8- and 9-OH groups as a benzylidene acetal, only 4-OH is free for glycosylation. These 2,7-anhydro-8,9-*O*-benzylidenesialic acid derivatives were examined as acceptor for an α-selective fucosylation to construct the glycosidic linkage of fucosyl $\alpha(1\rightarrow4)$ 2,7-anhydroneuraminic acid.

Keywords:

N-Acetyl-2,7-anhydroneuraminic acid, fucosyl $\alpha(1\rightarrow4)$ sialic acid linkage, ganglioside HPG-7

1. Introduction

Sialic acids are the most prevalent nine-carbon monosaccharides that are incorporated in a wide variety of oligosaccharides and glycoconjugates on cell surfaces of vertebrates and invertebrates [1, 2]. More than 50 structural derivatives of sialic acid exist in nature, among which *N*-acetylneuraminic acid (Neu5Ac) is the most studied. Neu5Ac exists in a variety of glycosidic linkages in biologically active glycoconjugates, most typically $\alpha(2\rightarrow3)$ and $\alpha(2\rightarrow6)$ to galactose (or lactose), $\alpha(2\rightarrow8)$, $\alpha(8\rightarrow9)$ and/or $\alpha(2\rightarrow9)$ to polysialic acids [3,4], $\alpha(2\rightarrow4)$ linkage to sialic acids [5-9], fucosyl $\alpha(1\rightarrow4)$ and galactosyl $\alpha(1\rightarrow4)$ to Neu5Ac [6,10] as well as 2-O linked to C-7 in 2,7-anhydroNeu5Ac [11].

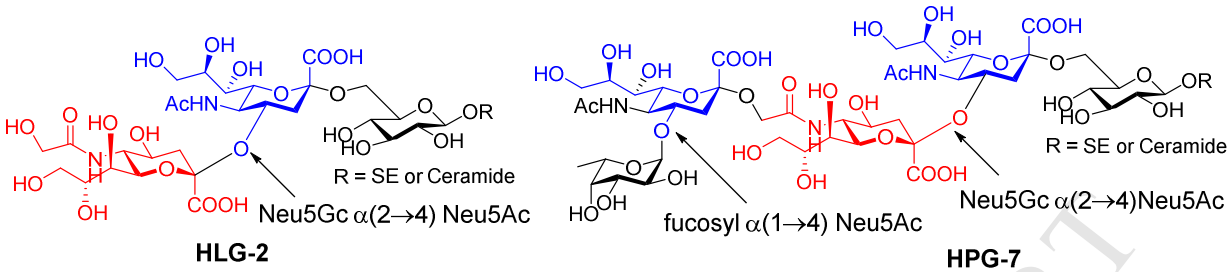


Figure 1. Gangliosides HLG-2 and HPG-7

Gangliosides are neuraminic acid-containing glycosphingolipids and widely found in the brain of vertebrates. Neu5Ac and *N*-glycolylneuraminic acid (Neu5Gc) are the most common sialic acids in gangliosides HLG-2 (isolated from sea cucumber *Holothuria leucospilota*) and HPG-7 (isolated from the sea cucumber *Holothuria pervicax*) (Figure 1) [12]. Gangliosides HLG-2 and HPG-7 have significant biological roles in mammals [13] and exhibit neuritogenic activity toward the rat adrenal pheochromocytoma cell (PC-12) in the presence of nerve growth factor [14-16]. However, their structure-activity relationships have not been described because of the inaccessibility of homogeneous gangliosides. As shown in Figure 1, the glycan moiety of ganglioside HPG-7 contains a fucosyl *α*(1→4) linkage to Neu5Ac and an *α*(2→4) linkage between sialic acids (also present in HLG-2). However, the synthesis of *α*-linked sialic acid derivatives is highly challenging because of the lack of neighboring C-3 functionality, high susceptibility to 2,3-elimination, and the presence of a sterically hindered anomeric center bearing a destabilizing electron-withdrawing carboxylic acid group [17]. Another common concern in the synthesis of Neu5Ac containing oligosaccharides is the poor reactivity of its hydroxyl groups, particularly the 4-,7- and 8-OH groups, which is probably due to hydrogen bonding with the C-5 acetamide group [18]. Owing to the low reactivity of the 4-OH of the Neu5Ac towards glycosylation, it is difficult to construct the *α*(2→4) between sialic acids [7,9] and fucosyl *α*(1→4) linkages to Neu5Ac [6]. To solve this problem, Kiso *et al* reported the first total synthesis of ganglioside HLG-2 with high stereoselectivity and yield (69%), using a 1,5-lactamized sialyl acceptor and *N*-2,2,2-trichloroethoxycarbonyl *(N*-Troc)-protected thiosialoside donor [8]. Furthermore, Ye and co-workers described a stereoselective synthesis of HLG-2 with a 45% yield over nine steps by employing a *N*-Troc-protected sialyl acceptor and a 5-*N*-4-*O*-carbonyl-protected sialyl phosphate donor [7]. Herein, we developed a new method through which the poor reactivity of 4-OH of the Neu5Ac can be improved by using 2,7-anhydroNeu5Ac, which can be an acceptor for the construction of the glycosidic linkages of

fucosyl α(1→4)Neu5Ac in HPG-7, Neu5Gc α(2→4)Neu5Ac in HLG-2 or HPG-7 (Figure 1) and galactosyl α(1→4)Neu5Ac in AG2 [10].

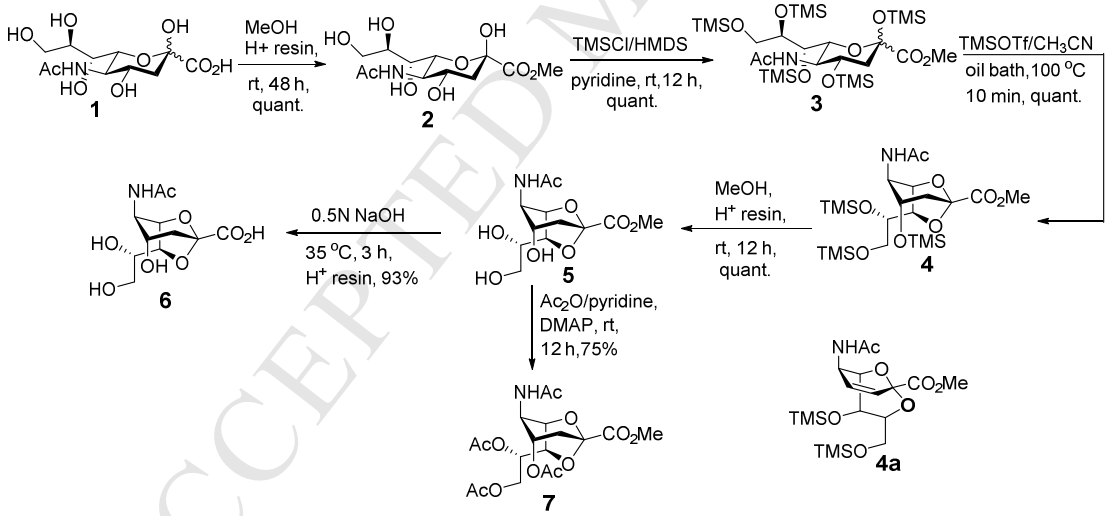
The C-4 and C-7 positions of sialic acid bear secondary alcohols that have a similar steric environment; hence, it is difficult to differentiate them. These alcohols can be easily distinguished by selective protection of the 7-OH group as 2,7-anhydroNeu5Ac by intramolecular glycosylation when the adjacent 8- and 9-OH groups are protected as benzylidene acetals or acetonide. Nevertheless, only a few syntheses of Neu5Ac containing intramolecular glycosides were reported in the literature. Ogura *et al* synthesized 2,7-anhydroNeu5Ac from thiosialoside donors by using silver triflate and palladium (II) salt [19], or dimethyl(methylthio)sulfonium triflate [20] as promoters. However, these protocols were mainly limited to thioglycoside donors and involved numerous protection group manipulations which required tedious purifications and isolation steps for the corresponding intermediates. Hence, the overall efficiency and yield of the 2,7-anhydroNeu5Ac obtained by these methods were low. Recently, Juge described a membrane-enclosed multienzyme approach to obtain 2,7-anhydrosialic acid derivatives from glycoproteins and evaluated their biological significance and potential applications in carbohydrate based-drug discovery [21]. The aim of this project was to develop a concise chemical synthesis of 2,7-anhydroNeu5Ac **6** from per-*O*-trimethylsilylated sialic acid methyl ester **3** under trimethylsilyl trifluoromethanesulfonate (TMSOTf) catalysis without installing a good leaving group at C-2 center in order to differentiate the 4- and 7-OH groups of sialic acid for the construction of the fucosyl α(1→4)Neu5Ac linkage present in gangloside HPG-7.

2. Results and discussion

Recently, our group has developed new methodologies for chemical synthesis of 1,6-anhydro sugar from silylated sugars using TMSOTf and TfOH as catalysts and have described their synthetic applications in natural products [22]. Synthesis of 1,6-anhydro and 2,7-ahydro sugars for the preparation of building blocks have numerous advantages: since there is no anomeric isomer, tedious α/β purification and isolation can be avoided. Similarly, protections at the anomeric position and O6 in 1,6-anhydro [23] and O7 of the 2,7-ahydro sugars can be circumvented.

2.1. Synthesis of 2,7-anhydrosialic acid methyl ester and its derivatives

Treatment of Neu5Ac **1** [24] with acidified MeOH afforded sialic acid methyl ester **2** in quantitative yield. Ether silylation of compound **2** was conducted according to the protocol reported by Gervay-Hague *et al*, by using hexamethyldisilazane (HMDS) and chlorotrimethylsilane (TMSCl) in pyridine, which yielded compound **3** in quantitative yield (Scheme 1) [25]. Heating-assisted intramolecular anomeric protection (iMAP) of **3** was performed in anhydrous acetonitrile (CH_3CN) by adding a catalytic amount of TMSOTf at room temperature, followed by refluxing for ten min at 100 °C in a preheated oil bath. During the formation of 2,7-anhydroNeu5Ac, refluxing at 100 °C unfortunately led to the cleavage of some of the TMS groups. Subsequent addition of three equivalents of HMDS to the same reaction mixture reinstalled the cleaved TMS groups, affording compound **4** in quantitative yield in a milligram scale reaction (Scheme 1). However, a ten gram scale iMAP of **3** required a higher reaction temperature (up to 120 ^{o}C) and longer reaction time (40 to 60 minutes) to give **4** (89%) along with 2,8-anhydrosaialic acid **4a** (5%) as side product. The structures of products **4** and **4a** were confirmed using 1D and 2D NMR spectroscopy.



Scheme 1. Synthesis of 2,7-Anhydrosialic Acid **6** and **7**

The formation of the 2,7-anhydro backbone could be readily characterized through 2D NMR spectroscopic data including ^{1}H-^{1}H COSY, HSQC, and HMBC spectra. The vicinal coupling constants of **4** were small, indicating the conversion of $^{2}C_5$ conformation in **3** to $^{5}C_2$ in **4.** Treatment of **4** with Amberlite IR-120(H^+) in anhydrous MeOH yielded **5** in a quantitative amount. To confirm the formation of 2,7-anhydro skeleton, triol **5** was acetylated with acetic

anhydride (Ac_2O) in pyridine to afford **7** in 75% yield. As depicted in the HMBC spectrum of **7**, the H-7, H-6 and H-4 showed three-bond correlation with C-2. Similarly, H-4, H-5, H-8 and H-9 revealed three-bond correlation with the acetyl carbonyl carbons (Figure 2). The synthesis of 2,7-anhydroNeu5Ac **6** was completed by saponification of **5** with 0.5 N aquesous NaOH, followed by treatment with Amberlite IR-120 (H^+) and **6** was afforded in 93% yield as a white powder (Scheme 1).

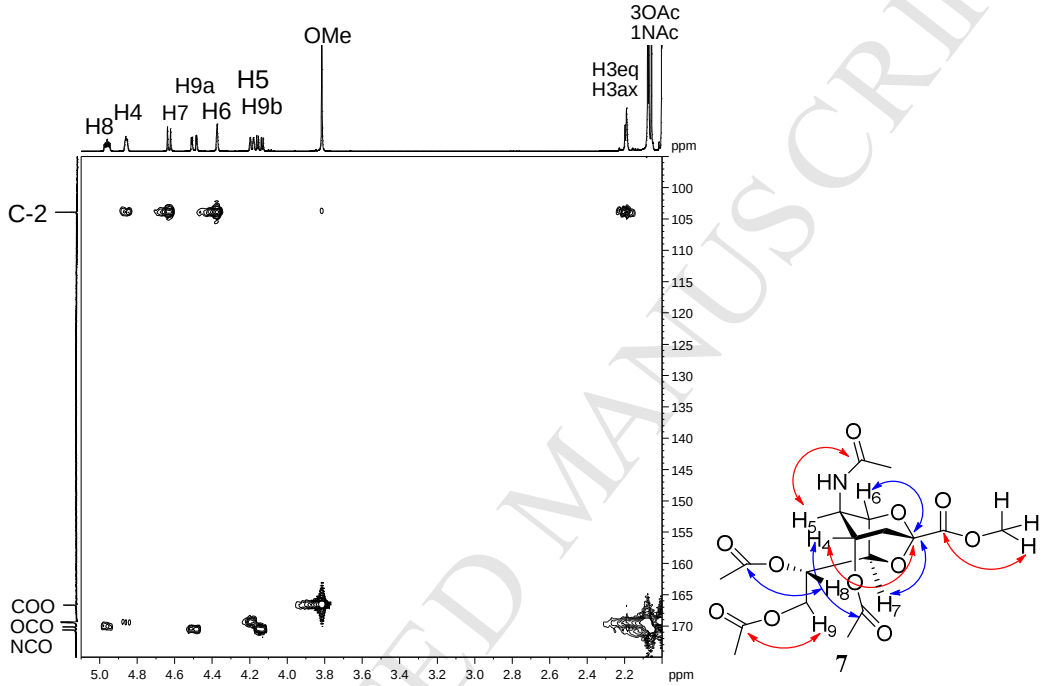
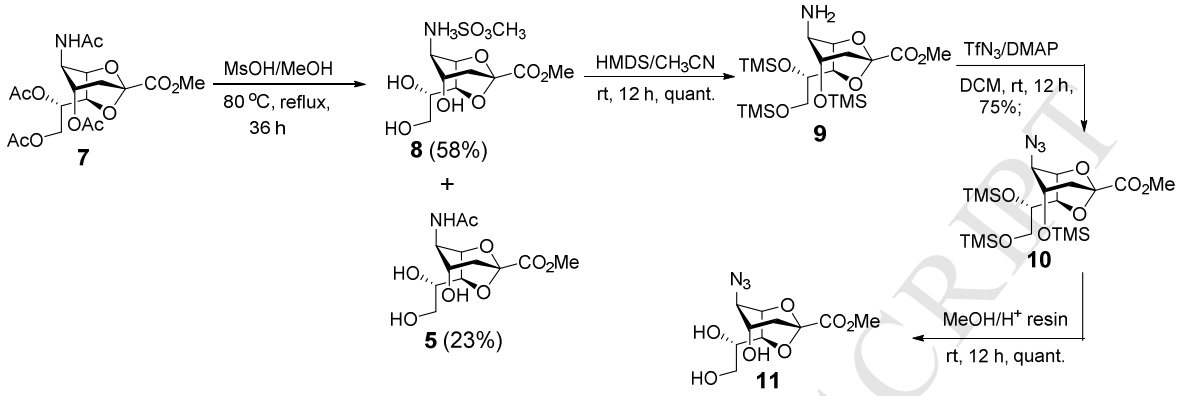


Figure 2. HMBC spectrum of **7**

2.2. Synthesis of 2,7-anhydrosialic acid acceptors and fucosyl donors

To circumvent any undesirable hydrogen bonding interaction with NHAc of **7**, the acetamido moiety was replaced with the azido group (Scheme 2) and its glycosylation yield was compared with that of the mono-*N*-acetylated counterparts. Hence, compound **7** was deacetylated with methanesulfonic acid (MsOH) in MeOH for 36 h at 80 °C to furnish compounds **8** in 58% and **5** in 23% yields. CH_3CN-promoted chemoselective silylation and azidation of **8** was conducted following the protocol developed in our lab [26]. Treatment of **9** with trifluoromethanesulfonyl azide (TfN_3) as the diazo transfer reagent afforded azido derivative **10** (75%). The introduction of azide group at C-5 position was confirmed through NMR and IR spectroscopic data; for example, the signals of H-5 and H-6 of **10** became more downfield shifted compared with that of **9** and the IR spectrum showed an azide peak at 2117.5 cm^{-1}. Next,

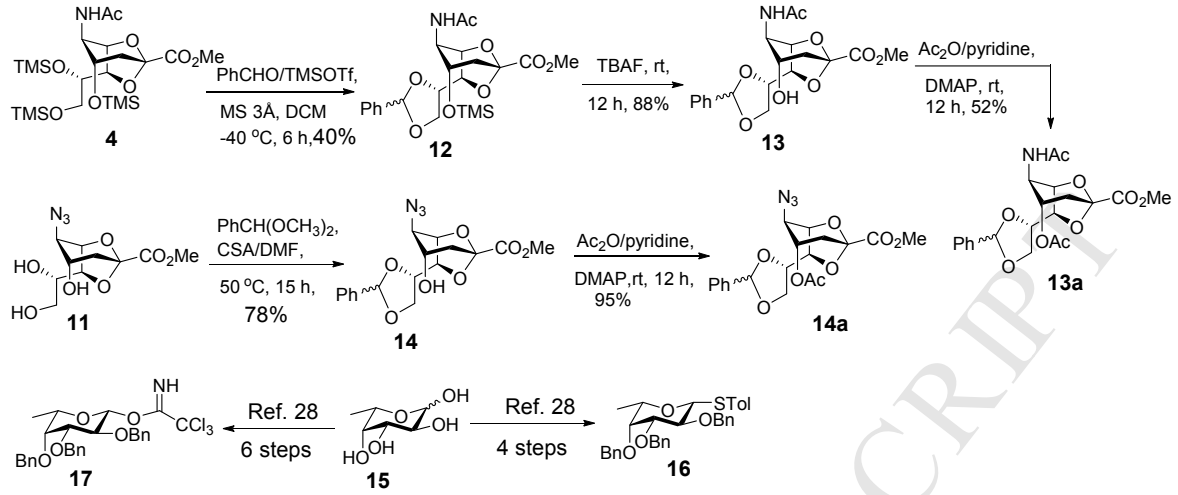
the TMS groups on compound **10** were cleaved by treating it with Amberlite IR-120 (H^+) in MeOH and **11** was afforded in 76% yield.



Scheme 2. Synthesis of 5-azido-protected-2,7-anhydrosialic acid **11**

As shown in Scheme 3, the *O*-8 and *O*-9 of 2,7-anhydrosialic acid derivative was selectively protected as a benzylidene by adapting the protocol reported in the literature [27] so that only the 4-OH/OTMS group was available for fucosylation. Accordingly, compound **4** was first treated with benzaldehyde in the presence of TMSOTf as a catalyst for 6 h at -40 ^oC to provide **12** in 40% and **13** in 28% yields. After the ring formation of arylidene acetal at oxygens 8 and 9 was completed, the 4-OTMS was totally cleaved using TBAF at rt to afford acceptor **13** in 88 % yield. On the other hand, the 8,9-*O*-bezylidenation of triol **11** was achieved with benzaldehyde dimethyl acetal ($PhCH(OCH_3)_2$) and camphor sulfonic acid (CSA) in *N*,*N*-dimethylformamide (DMF) and **14** was obtained in 78% yield (Scheme 3). The positions of acetal functionality in **13** and **14** were confirmed to be at 8- and 9-*O* by NMR spectroscopy after acetylation. The ^1H NMR spectrum of **13a** and **14a** showed that the signals for the H-4 were shifted to downfield; for example, comparing **13a** with **13**, the signal of H-4 moved to 4.88 from 4.01 ppm. Through this method, the 4- and 7-OH groups of Neu5Ac were easily differentiated; only the 4-OH is left free for glycosylation. Also, the change in orientation of 4-OH from equatorial to axial in 2,7 anhydro ring results in ablation of hydrogen bonding thereby enhancing its reactivity.

Donors **16** and **17** were obtained in 4 and 6 steps, respectively from commericially available fucose **15** according to the reported procedure [28].



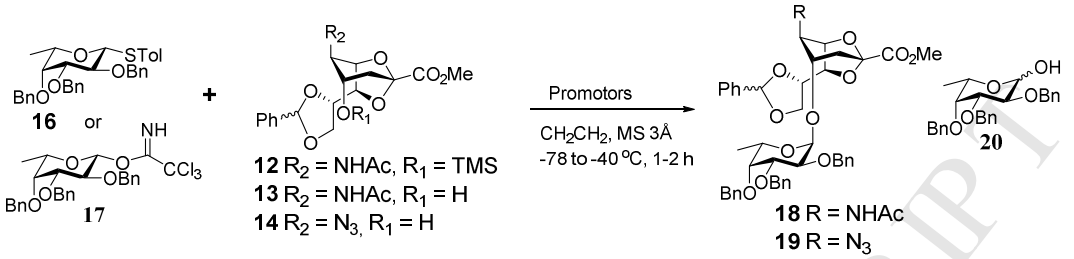
Scheme 3. Synthesis of acceptors (**12-14**) and donors (**16-17**).

2.3. Fucosylation of 2,7-anhydrosialic acid derivatives

Table 1 shows the construction of fucosyl $\alpha(1\rightarrow4)$sialyl glycosidic linkage present in ganglioside HPG-7. Thioglycoside **16** can be activated using NIS/TfOH promoter system [29]. The glycosylation reaction between thioglycoside donor **16** and 4-OH acceptor **13** was initially attempted using 1.0 equiv of NIS and 0.4 equiv of TfOH in CH_2CH_2 in the presence of 3Å molecular sieve at -78 ^oC and disaccharide **18** was afforded in 37% yield accompanied with hydrolyzed product **20** in 15% yield (entry 1). Varying the amount of NIS and/or TfOH employed did not led to significant improvement in disaccharide yield (entries 2-3). The yield of glycosylation product **18** was slightly improved to 51% when small excess of acceptor **13** was used (entry 4). Gratifyingly, 71% of disaccharide **18** was formed using 1.0 equiv of NIS, 0.2 equiv of TfOH and 1.2 equiv of acceptor **13** (entry 5).

Next, we focused in synthesizing the disaccharide **18** using the imidate donor **17** and 4-OTMS acceptor **12**. It has been reported that trichloroacetimidate donors **17** can be activated by catalytic amount of $BF_3.OEt_2$ or TMSOTf [30, 31]. However, TMSOTf, $BF_3.OEt_2$, TfOH (0.2 equiv) and NIS/TfOH failed to promote glycosylation of acetimidate donor **17** and acceptor **12** (entries 6-8 and 10). In these reactions, the trichloroacetimidate donor **17** was hydrolyzed to **20** and a significant amount of the unreacted acceptor **12** was recovered. However, increasing the amount of TfOH to 0.3 equivalent furnished 31% of disaccharide **18** under the same conditions (entry 9).

Table 1. Fucosylation study at the 4-position of 2,7-anhydrosialic acid methyl ester



Entry	Promotor (equiv)	Donor (equiv)	Acceptor (equiv)	Product	Yield[a] (%)	(α/β)[b]
1	NIS(1.0)/TfOH(0.4)	**16** (1.0)	**13** (1.0)	**18**	37	α
2	NIS(1.5)/TfOH(0.4)	**16** (1.0)	**13** (1.0)	**18**	36	α
3	NIS(1.5)/TfOH(0.2)	**16** (1.0)	**13** (1.0)	**18**	44	α
4	NIS(1.3)/TfOH(0.2)	**16** (1.0)	**13** (1.2)	**18**	51	α
5	NIS(1.0)/TfOH(0.2)	**16** (1.0)	**13** (1.2)	**18**	71	α
6	TMSOTf(0.6)	**17** (1.0)	**12** (1.0)	**18**	trace	--
7	$BF_3.OEt_2$ (0.6)	**17** (1.0)	**12** (1.0)	**18**	--	--
8	TfOH(0.2)	**17** (1.0)	**12** (1.0)	**18**	trace	--
9	TfOH(0.3)	**17** (1.0)	**12** (1.0)	**18**	31	α
10	NIS(1.0)/TfOH(0.2)	**17** (1.2)	**12** (1.0)	**18**	trace	--
11	NIS(1.0)/TfOH(0.2)	**16** (1.5)	**14** (1.0)	**19**	83	α

[a] Isolated yield, MS = molecular sieves.
[b]The ratio of α/β was determined by ^{1}H NMR spectroscopy.

Furthermore, the fucosyl *α*(1→4)2,7-anhydroNeu5N_3 linkage of compound **19** was built with the same amount of NIS/TfOH used in the synthesis of disaccharide **18**. The NIS/TfOH-promoted glycosylation reaction of 5-azido acceptor **14** and fucosyl thioglycoside donor **16** afforded **19** in 83% yield with excellent α-stereoselectivity. A higher yield of disaccharide **19** was obtained with 5-azido acceptor **14,** compared with mono-*N*-acetylated acceptor **13**. The structures of the disaccharides **18** and **19** were confirmed through COSY and HSQC NMR experiments.

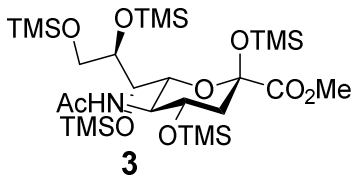
3. Conclusion

We described a concise synthesis of the 2,7-anhydrosialic acid in three steps from per-*O*-trimethylsilylated sialic acid methyl ester without introducing leaving group at the anomeric center. The 4- and 7-OH groups of neuraminic acid were differentiated by tying 2-C and 7-OH as an 2,7-anhydro ring, leaving only 4-OH free for glycosylation. Different reaction temperatures and glycosylation protocols were examined for the synthesis of fucosyl α(1→4)2,7-anhydroneuraminic acid and the corresponding disaccaharides were obtained in high yields with excellent α-stereoselectivity from the NIS/TfOH-promoted glycosylation reactions. This protocol can be applied to synthesize important gangliosides upon successful cleavage of the 2,7-anhydro backbone of the disaccharides. Currently, the synthesis of the glycan moiety of gangliosides HLG-2 and HPG-7 using 2,7-anhydroneuraminic acid acceptors is in progress, and the results will be reported in due course.

4. Experimental Section

4.1. General

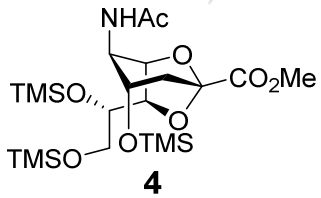
All dry solvents and chemicals were purchased from commercial sources, and were used without further purification unless otherwise mentioned. All moisture-sensitive reactions were conducted in flame-dried glasswares under dry nitrogen atmosphere. Flash column chromatography was carried out as recommended with Silica Gel 60 (230-400 mesh, E. Merck). TLC was performed on pre-coated glass plates of Silica Gel 60 F254 (0.25 mm, E. Merck); detection was executed by UV (254 nm) or spraying with a solution of $Ce(NH_4)_2(NO_3)_6$, $(NH_4)_6M_7O_{24}$, as well as H_2SO_4 in water and subsequent heating on a hot plate. Specific rotations were measured on Jasco P-2000 digital polarimeter using a 100 mm cell at 589 nm and at ambient temperature conditions and reported in $10^{-1}\cdot deg\cdot cm^2\cdot g^{-1}$; the sample concentrations are in $g\cdot dL^{-1}$. ^1H and ^{13}C NMR spectra were recorded with Bruker AVIII-400 or AV500 or N600 MHz instruments. Chemical shifts are in ppm from Me_4Si, generated from the $CDCl_3$ and CD_3OD lock signals at δ 7.24 and 3.31 for ^1H spectra and 77.16, and 49.00 for ^{13}C spectra, respectively. Multiplicities are reported by using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; *J* = coupling constant values in Hertz. Proton peaks were assigned based on 2D NMR spectra (1H−1H COSY, HSQC, HMBC and NOESY). Mass spectra were obtained with a JEOL JMS-700 mass spectrometer in FAB mode or Waters Premier XE mass spectrometer in ESI or APCI mode. IR spectra were taken with a Perkin-Elmer Paragon 1000 FT-IR spectrometer.



3

4.2. Methyl 5-acetamido-2, 4,7, 8, 9-penta-*O*-trimethylsilyl-3, 5-dideoxy-*β*-D-*glycero*-D-*galacto*-2-nonulopyranosonate (3).

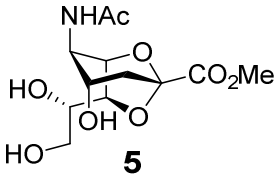
Sialic acid methyl ester **2** (15 g, 46.40 mmol, 1 equiv) was placed in a 1 L round bottom flask and azeotroped with toluene three times and placed under high vacuum overnight. Then, it was dissolved in pyridine (350 mL) under N_2, followed by the addition of hexamethyldisilazane (58.35 mL, 278.40 mmol, 6 equiv) and chlorotrimethylsilane (29.44 mL, 232 mmol, 5 equiv) dropwise. The reaction mixture was stirred overnight at room temperature. After the reaction completed, the solution was concentered through the addition of toluene. The crude product was diluted with hexanes (530 mL), then washed and partitioned with ice water (500 mL). The organic layer was washed with brine (300 mL) and collected. The aqueous solution was extracted twice with hexanes (600 mL) and the organic layers were combined, dried with anhydrous $MgSO_4$, filtered and concentered to afford a hygroscopic white foam product **3** (27 g, quant). $[\alpha]^{25}_D$ -32 (c = 0.75, $CHCl_3$); IR ($CHCl_3$) *v* 2955, 1752, 1655, 1560, 1251, 1166, 843, 753 cm-^{1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.54 (d, J = 8.08 Hz, 1H, NH), 3.75 (d, J = 4.96 Hz, 1H, H-7), 4.22 (d, J = 2.84 1H, H-6), 3.52 (q, J = 18.4, 9.68 Hz, 1H, H-5), 4.19 (d, J = 2.84 Hz, 1H, H-4), 3.90 (dd, J = 3.36, 10.04 Hz, 1H, H-9a), 3.79 (br m,1H, H-8), 3.72 (s, OCH_3), 3.43 (dd, J = 6.72, 10.08 Hz, 1H, H-9b), 2.09 (dd, 4.6, 8.04 Hz, 1H, H-3eq), 1.93 (s, 3H, NAc), 1.89 (t, J = 11.24 Hz, 1H, H-3ax), 0.14 (s, 9H, TMS), 0.13 (s, 9H, TMS), 0.11 (s, 9H, TMS), 0.10 (s, 9H, TMS), 0.08 (s, 9H, TMS), 0.07(s, 9H, TMS); ^{13}C NMR (100 MHz, CDCl3): δ = 172.96 (CO), 169.78 (CO), 96.83 (C-2), 75.47 (CH), 75.23 (CH), 71.64 (CH), 68.26 (CH), 63.94 (CH_2), 55.27 (CH), 52.46 (OCH_3), 43.22 (CH_2), 24.02 (CH_3), 1.39 (TMS), 0.75 (TMS), 0.62 (TMS), 0.40 (TMS), -0.44 (TMS); HRMS (ESI): *m/z* calcd for $C_{27}H_{61}NO_9Si_5$ ($[M+Na]^+$): 706.3090, found: 706.3088.



4

4.3. Methyl 5-acetamido-2,7-anhydro-4, 8, 9-tri-*O*-trimethylsilyl-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosonate (4)

The per-*O*-trimethylsilylated sialic acid derivative **3** (6.13 g, 8.96 mmol, 1 equiv) was dissolved in dry CH_3CN (120 mL), followed by the addition of TMSOTf (486 µL, 2.69 mmol, 0.3 equiv). The mixture was subjected to a preheated oil bath and refluxed at 110 °C for 40 min. The consumption of the starting material was confirmed by TLC. The reaction was treated with HMDS (5.6 mL, 26.88 mmol, 3.0 equiv) and further stirred for 30 min at room temperature. The mixture was evaporated under reduced pressure to afford a white foam hygroscopic 2,7-anhydro sialic acid methyl ester **4** (4.18 g, 89%) and **4a** (0.25 g, 5%). $[\alpha]^{25}_D$ +18 (c = 1.0, $CHCl_3$); IR ($CHCl_3$) *v* 2957, 1754, 1656, 1253, 1092, 843, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 6.08 (d, J = 8.40 Hz, 1H, NH), 4.65 (d, J = 6.88 Hz, 1H, H-7), 4.42 (s, 1H, H-6), 3.94 (br d, J = 7.92 Hz, 1H, H-5), 3.89 (br dd, J = 1.16, 3.68 Hz, 1H, H-4), 3.80 (s, OCH_3), 3.66 (dd, J = 3.12, 10.40 Hz, 1H, H-9a), 3.59 (br m,1H, H-8), 3.53 (dd, J = 5.72, 10.36 Hz, 1H, H-9b), 2.08 (dd, J = 5.2,14.52 Hz, 1H, H-3eq), 1.98 (s, 3H, NAc), 1.94 (br d, J = 1.96 Hz, 1H, H-3ax), 0.13 (s, 9H, TMS), 09 (s, 9H, TMS), 0.08 (s, 9H, TMS); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.28 (CO), 167.77 (CO), 104.08 (C-2), 77.98 (CH), 77.36 (CH), 74.01 (CH), 67.46 (CH), 64.38 (CH_2), 52.95 (OCH_3), 52.32 (CH), 36.86 (CH_2), 23.38 (CH_3), 0.64 (TMS), 0.05 (TMS), 0.42 (TMS); HRMS (ESI): *m/z* calcd for $C_{21}H_{43}NO_8Si_3$ ($[M+Na]^+$): 544.2194, found: 544.2189.

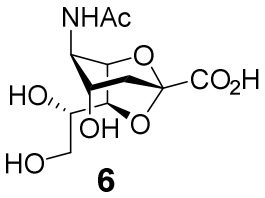


5

4.4. Methyl 5-acetamido-2,7-anhydro-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-*2*-nonulopyranosonate (5)

Compound **4** (500 mg, 0.958 mmol, 1 equiv) and Amberlite IR-120 (H^+) resin (0.4 g) was dissolved in anhydrous methanol (5 mL) and stirred for 30 min at room temperature. Reaction completion was determined by TLC analysis ($CHCl_3$:CH_3OH, 4:1). The solvent was concentrated through the addition of toluene to give a solid white foam product (268 mg, 92%). $[\alpha]^{25}_D$ +53 (c = 1.0, CH_3OH); IR (CH_3OH) *v* 3357, 1750, 1644, 1544, 1206, 1086, 1047, 888, 741 cm^{-1}; ^1H NMR (500 MHz, D_2O): δ = 4.58 (s, 1H, H-6), 4.48 (d, J = 8.85 Hz, 1H, H-7), 3.95 (br s, 1H, H-4), 3.86 (br s, 1H, H-5), 3.79 (s, OCH_3), 3.74 (dd, J = 3.05, 11.55 Hz, 1H, H-9a),

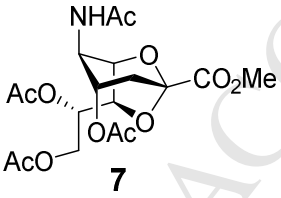
3.58 (dd, J = 5.65,11.50 Hz, 1H, H-9b), 3.41 (m, 1H, H-8), 2.27 (dd, J = 5.5, 14.90 Hz, 1H, H-3eq), 2.04 (d, J = 15.5 Hz, 1H, H-3ax), 1.99 (s, 3H, NAc); ^{13}C NMR (125 MHz, D_2O): δ = 172.82 (CO), 169.33 (CO), 105.12 (C-2) , 79.68 (CH), 78.75 (CH), 73.43 (CH), 68.20 (CH), 64.16 (CH_2), 53.67 (OCH_3), 53.29 (CH), 36.47 (CH_2), 22.40 (CH_3). HRMS (ESI): *m/z* calcd for $C_{12}H_{19}NO_8$ ($[M+H]^+$): 306.1189, found: 306.1196.



6

4.5. 5-Acetamido-2,7-anhydro-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-*2*-nonulopyranosonic acid (6)

A solution of **5** (19.7 mg, 0.06 mmol) in 0.5 N NaOH (0.5 mL) was stirred at 35 ^{o}C for 3 h. After the reaction completed, the solution was diluted with water (10 mL) and neutralized with Amberlite IR-120 (H^+) resin. Then, the filtered solution was lyophilized to give a white powder compound **6** (17.4 mg, 93%). $[\alpha]^{25}_{D}$ +65 (c = 0.5, CH_3OH); IR (CH_3OH) *v* 3250, 3025, 1751, 1438, 1000 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD): δ = 4.57 (br s, 1H, H-6), 4.49 (d, J = 8.5 Hz, 1H, H-7), 3.87 (dd, J = 1.3, 5.5 Hz, 1H, H-4), 3.95 (br s, 1H, H-5), 3.74 (dd, J = 3.5, 11.55 Hz, 1H, H-9a), 3.59 (dd, J = 5.45, 11.55 Hz, 1H, H-9b), 3.46 (br m, 1H, H-8), 2.25 (dd, J = 5.55, 14.95 Hz, 1H, H-3eq), 2.03 (d, J = 15.15 Hz, 1H, H-3ax), 1.99 (s, 3H, NAc); ^{13}C NMR (125 MHz, CD_3OD): δ = 172.79 (CO), 170.65 (CO), 105.05 (C-2), 79.63 (CH), 78.92 (CH), 73.47 (CH), 68.28 (CH), 64.22 (CH_2), 52.23 (CH), 36.31 (CH_2), 22.45 (CH_3); HRMS (ESIneg): *m/z* calcd for $C_{11}H_{17}NO_8$ ($[M-H]^-$): 290.0876, found: 290.0872.

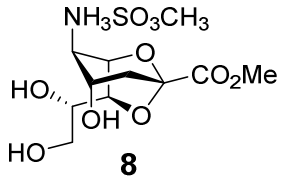


7

4.6. Methyl 5-acetamido-2,7-anhydro-4,8,9-tri-*O*-acetyl-3, 5-dideoxy-*α*-D-*glycero*-D-*galacto*-*2*-nonulopyranosonate (7)

Compound **5** (5.2 g, 12.1 mmol, 1 equiv) and 4-(dimethylamino)pyridne (0.25 g, 2.41 mmol, 0.2 equiv) was dissolved in dry pyridine (110 mL). To this solution, acetic anhydride (30 mL) was added dropwise and kept for 12 h at room temperature. After the reaction completed, the solvent

was evaporated through the addition of toluene. Then, the crude product was dissolved in saturated copper sulfate pentahydrated aqueous solution and extracted three times with EtOAc. The extract was dried over anhydrous $MgSO_4$, filtered and evaporated to dryness in vacuo. The crude product was purified in a column of silica gel with EtOAc: hexane (4: 1) to give **7** (5.5 g, 75%) as a hygroscopic white foam. $[\alpha]^{25}_D$ +70 (c = 0.5, $CHCl_3$); IR ($CHCl_3$) *v* 1744, 1660, 1537, 1372, 1226, 1053, 820 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 6.13 (d, J = 8.85 Hz, 1H, NH), 4.98 (m, 1H, H-8), 4.86 (br d, J = 2.05 Hz, 1H, H-4), 4.64 (d, J = 7.95 Hz, 1H, H-7), 4.51 (dd, J = 2.55, 12.35 Hz, 1H, H-9a), 4.37 (s, 1H, H-6), 4.2 (d, J = 8.9 Hz, 1H, H-5), 4.16 (dd, J = 4.75, 12.35 Hz, 1H, H-9b), 3.81 (s, OCH_3), 2.19 (d, J = 4.35 Hz, 2H, H-3eq, H-3ax), 2.08, 2.07, 2.06, 2.00 (each s, 12H, 4Ac); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.64 (CO), 170.14 (CO), 169.47 (CO), 169.36 (CO), 166.67 (CO), 103.90 (C-2), 78.69 (CH), 75.37 (CH), 71.01 (CH), 68.57 (CH), 62.20 (CH_2), 53.39 (OCH_3), 48.90 (CH), 33.33 (CH_2), 23.31, 21.31, 21.09, 20.84 (each CH_3). HRMS (ESI): *m/z* calcd for $C_{18}H_{25}NO_{11}$ ($[M+Na]^+$): 454.1325, found: 454.1317.

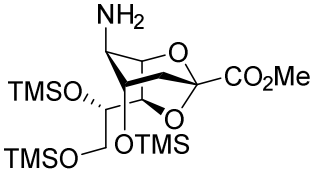


8

4.7. Methyl 5-ammoniummethanesulfonate-2,7-anhydro-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-*2*-nonulopyranosonate (8)

Compound **7** (1.98 g, 4.59 mmol, 1 equiv) was dissolved in dry MeOH (50 mL) followed by addition of methanesulfonic acid (MsOH, 1.8 mL, 27.54 mmol, 6 equiv) at room temperature. The mixture was stirred at 80 °C for 36 h. After the reaction get completed, it was neutralized with Amberlite IRN-78 (OH^-) resin, after which the suspension was filtered. The filtrate was azeotroped with toluene three times and purified by silica gel column chromatography using MeOH-$CHCl_3$ (1:3) to give a hygroscopic white foam **8** (700 mg, 58%) and **5** (180 mg, 23%) compounds. $[\alpha]^{25}_D$ +3 (c = 0.55, CH_3OH); IR (CH_3OH) *v* 1740, 1729, 1061, 1038, 804 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 4.77 (s, 1H, H-6), 4.54 (d, J = 8.82 Hz, 1H, H-7), 4.04 (br d, J = 5.41 Hz, 1H, H-4), 3.81(s, 3H, OCH_3), 3.74 (dd, J = 2.92, 11.50 Hz, 1H, H-9a), 3.58 (dd, J = 5.22, 11.54 Hz, 1H, H-9b), 3.45 (m, 1H, H-8), 3.35 (s, 1H, H-5), 2.70 (s, 3H, SO_3CH_3), 2.36 (dd, J = 5.65, 15.18 Hz, 1H, H-3eq), 2.15 (d, J = 15.28 Hz, 1H, H-3ax); ^{13}C NMR (125 MHz, CD_3OD): δ = 168.69 (CO), 105.31 (C-2), 78.56 (CH), 77.98 (CH), 73.13 (CH), 66.07 (CH),

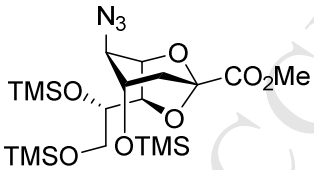
63.88 (CH_2), 53.44 (OCH_3), 39.47 (OCH_3), 36.14 (CH_2); HRMS (ESI): *m/z* calcd for $C_{11}H_{21}NO_{10}S$ ($[M-H]^-$): 358.0808, found: 358.0817.



9

4.8. Methyl 5-amino-2,7-anhydro-4,8,9-tri-*O*-trimethylsilyl-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosonate (9)

To a suspension of **8** (1.07 g, 2.97 mmol, 1 equiv) in CH_3CN (25 mL) was added HMDS (2.5 mL, 4 equiv) at room temperature under N_2 atmosphere and stirred overnight. The reaction was monitored by TLC analysis employing hexane-EtOAc (1:1). When the reaction completed, the salt was removed by filtration. The filtrate solution was evaporated in vacuo under reduced pressure to furnish the desired product **9** as a pale yellow syrup compound (1.458 g, quant). $[\alpha]^{25}_D$ +6(c = 0.33, $CHCl_3$); IR ($CHCl_3$) *v* 2956, 1748, 1688, 1605, 1306, 1095, 842, 749 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.45 (d, J = 7.89 Hz, 1H, H-7), 4.40 (s, 1H, H-6), 3.79 (br d, J = 5.24 Hz, 1H, H-4), 3.78 (s, 3H, OCH_3), 3.71 (dd, J = 2.27, 10.35 Hz, 1H, H-9a), 3.55-3.49 (m, 2H, H-8, H-9b), 2.76 (s, 1H, H-5), 2.16 (dd, J = 5.18, 14.77 Hz, 1H, H-3eq), 1.92 (d, J = 14.64 Hz, 1H, H-3ax), 0.11 (s, 9H, OTMS), 0.08 (s, 18 H, OTMS); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 168.03 (CO), 104.07 (C-2), 80.59 (CH), 74.29 (CH), 71.29 (CH), 64.40 (CH_2), 55.07 (CH), 52.86 (OCH_3), 36.12 (CH_2), 0.76, -0.03, -0.04 (each TMS); HRMS (ESI): *m/z* calcd for $C_{19}H_{41}NO_7Si_3$ ($[M+Na]^+$): 502.2089, found: 502.2095.

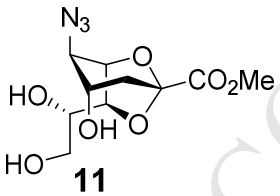


10

4.9. Methyl 5-azido-2,7-anhydro-4,8,9-tri-*O*-trimethylsilyl-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosonate (10)

Sodium azide (2.0 g, 29.74 mmol, 9.84 equiv) was dissolved in water (6 mL) and cooled in an ice bath and treated with CH_2Cl_2 (10 mL). Trifluoromethanesulfonic anhydride (1000 µL, 6.35 mmol, 2.1 equiv) was added slowly at 0 ^oC to the resulting biphasic mixture. The reaction was stirred at 0 ^oC for 2 h, the organic layer was separated and aqueous phase was washed with

CH_2Cl_2 as less volume as possible. The combined organic layers were washed with saturated sodium bicarbonate solution, dried over anhydrous $MgSO_4$, filtered and used without further purification. To a stirred solution of compound **9** (1.45 g, 3.02 mmol, 1 equiv) in CH_2Cl_2 (3 mL) was added 4-(dimethylamino)pyridine (1.16 g, 9.1 mmol, 3 equiv). The generated trifluoromethanesulfonic azide (TfN_3) solution was added dropwise at 0 ^oC. The ice-bath was removed and stirred overnight at room temperature. The mixture was quenched with glycine (0.6 g, 1.5 equiv) over 30 min, then diluted with water (7 mL). The desired product was extracted with CH_2Cl_2 from the aqueous phase. The combined organic layers were dried over anhydrous $MgSO_4$ and filtered. The volume of CH_2Cl_2 solution was reduced by evaporation in vacuo and resilylated by adding HMDS (2.5 mL, 9.06 mmol, 3 equiv) in the presence of catalytic amount of TMSOTf (55 µL, 0.302 mmol, 0.1 equiv). After the reaction completed, the reaction mixture was concentrated in vacuo and purified by a short pad of silica gel column chromatography (4:1 hexane-EtOAc) to furnish azido product **10** (1.1 g, 75%) as a colorless syrup. $[\alpha]^{25}_D$ +74 (c = 0.31, $CHCl_3$); IR ($CHCl_3$) *v* 2102, 1755, 1307, 1253, 1214, 1140, 843, 748, 667 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 4.60 (s, 1H, H-6), 4.38 (d, J = 7.93 Hz, 1H, H-7), 3.98 (br d, J = 4.98 Hz, 1H, H-4), 3.78 (s, 3H, OCH_3), 3.70 (d, J = 8.68 Hz, 1H, H-9a), 3.58-3.51 (m, 2H, H-8, H-9b), 3.30 (s, 1H, H-5), 2.21 (dd, J = 5.59, 14.65 Hz, 1H, H-3eq), 1.98 (d, J = 14.72 Hz,1H, H-3ax), 0.12 (s, 9H, OTMS), 0.11 (s, 9H, OTMS), 0.09 (s, 9H, OTMS); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 167.50 (CO), 103.89 (C-2), 77.59 (CH), 77.34 (CH), 73.85 (CH), 67.22 (CH), 64.23 (CH_2), 63.10 (CH), 52.93 (OCH_3), 37.14 (CH_2), 0.69, -0.07. -0.39 (each TMS); HRMS (ESI): *m/z* calcd for $C_{19}H_{39}N_3O_7Si_3$ ($[M+Na]^+$): 528.1994, found: 528.1999.

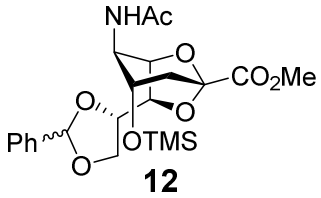


11

4.10. Methyl 5-azido-2,7-anhydro-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosi-donate (11)

Compound **10** (1.09g, 2.16 mmol, 1 equiv) and Amberlite IR-120 (H^+) resin (900 mg) was dissolved in anhydrous CH_3OH (25 mL) and stirred for 30 min at room temperature. Reaction completion was determined by TLC analysis ($CHCl_3$:CH_3OH, 6:1). The solvent was concentrated through the addition of toluene to give a hygroscopic sticky brown product **11**

(659.4 mg, quant). $[\alpha]^{25}_{D}$ +68 (c = 0.55, CH_3OH); IR (CH_3OH) *v* 3389, 2952, 2105, 1749, 1637, 1441, 1307, 1252, 1206, 1157, 1089, 1045, 999, 842, 741 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ = 4.74 (s, 1H, H-6), 4.40 (d, J = 8.94 Hz, 1H, H-7), 4.02 (br dd, J = 1.53, 5.58 Hz, 1H, H-4), 3.80 (s, 3H, OCH_3), 3.72 (dd, J = 2.99, 11.63 Hz, 1H, H-9a), 3.56 (dd, J = 5.51, 11.67 Hz, 1H, H-9b), 3.45 (s, 1H, H-5), 3.44-3.42 (m, 1H, H-8), 2.21 (dd, J = 5.68, 15.00 Hz, 1H, H-3eq), 2.06 (d, J = 15.00 Hz, 1H, H-3ax); ^{13}C NMR (125 MHz, CD_3OD): δ = 169.03 (CO), 105.09 (C-2), 79.28 (CH), 78.54 (CH), 73.21 (CH), 67.49 (CH), 64.03 (CH_2), 63.25 (CH), 53.37 (OCH_3), 36.95 (CH_2); HRMS (ESI): *m/z* calcd for $C_{10}H_{15}N_3O_7$ ($[M+Na]^+$): 312.0808, found: 312.0803.

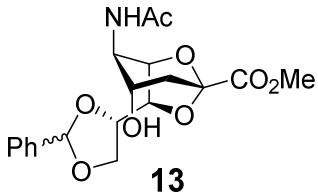


12

4.13. Methyl 5-acetamido-2,7-anhydro-4-*O*-trimethylsilyl-8,9-*O*-benzylidene-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosidonate (12)

To a solution of compound **4** (413.1 mg, 0.792 mmol, 1 equiv) in dry CH_2Cl_2 (6 mL) was added 3 Å molecular sieves (400 mg), and benzaldehyde (0.2 mL, 1.98 mmol, 2.5 equiv) under N_2 atmosphere. The reaction mixture was stirred for 30 min at room temperature. The mixture was cooled to -78 °C. TMSOTf (28.66 μL, 0.158 mmol, 0.8 equiv) was added at -78 °C and the mixture was stirred for 8 h at this temperature. When reaction was completed according to TLC, the ice bath was removed and the reaction solution was neutralized by $NaHCO_3$ aqueous solution. The reaction mixture was filtered by passing through a short pad Celite bed. The desired product was extracted from the aqueous phases with CH_2Cl_2 (three times). Then the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography with EtOAc: hexane (1:1) to give 4-OTMS **12** (150 mg, 40%) as white foam compound and 4-OH **13** (106 mg, 28%). $[\alpha]^{25}_{D}$ +21 (c = 0.44, $CHCl_3$); IR ($CHCl_3$) *v* 2950, 1753, 1656, 1253, 1204, 1090, 1059, 1028, 907, 866, 843, 760 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.42-7.41 (m, 3H, Ar), 7.38-7.34 (m, 5H, Ar), 6.06 (t, J = 8.42 Hz, NH), 5.94, 5.77 (each s, 1H, CH), 4.69 (d, J = 9.01 Hz, 1H, H-7), 4.62 (d, J = 9.30 Hz, H-7), 4.58 (br s, 1H, H-6), 4.55 (br s, 1H, H-6), 4.25-4.19 (overlap, 2H, H-9a), 4.11-4.02 (overlap, 4H, H-9b, H-8, H-5), 3.97 (dd, J = 6.0, 8.75 Hz, 1H, H-9b), 3.92-3.89 (overlap, 3H, H-4, H-5), 3.83, 3.82 (each s, 2H, OCH_3), 2.10-1.99 (m, 4H, H-3eq, H-3ax), 1.98, 1.96 (each s, 6H, 2NAc), 0.17,

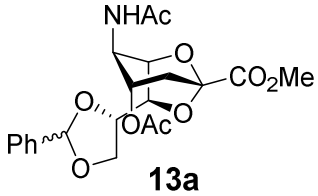
0.14 (each s, 18H, 2TMS); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.31 (CO), 167.46 (CO), 167.41 (CO), 137.96 (C), 137.18 (C), 129.74 (CH), 129.38 (CH), 128.66 (CH), 128.53 (CH), 126.62 (CH), 126.44 (CH), 104.75 (C-2), 104.29 (CH), 104.14 (CH), 79.42 (CH), 79.06 (CH), 77.76 (CH), 77.57 (CH), 75.94 (CH), 75.90 (CH), 68.55 (CH_2), 68.46 (CH_2), 67.49 (CH), 67.34 (CH), 53.22 (OCH_3), 52.17 (CH), 52,06 (CH), 36.63 (CH_2), 23.33 (CH_3), 0.01 (TMS); HRMS (ESI): *m/z* calcd for $C_{22}H_{31}NO_8Si$ ($[M+Na]^+$): 488.1717, found: 488.1713.



13

4.14. Methyl 5-acetamido-2,7-anhydro-8,9-*O*-benzylidene-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosidonate (13)

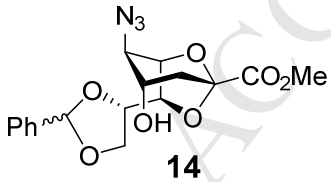
To the solution of **12** in CH_2Cl_2, TBAF (1 mL, 1.6 mmol, 2 equiv) was added and stirred at room temperature overnight. When reaction was completed, the mixture neutralized with water and the product extracted with EtOAc (three times) from the aqueous layer. Then the combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography (60% EtOAc in hexane) to afford the desired product as a colorless sticky compound **13** (0.273 g, 88 % yield %). $[\alpha]^{25}_D$ +6 (c = 0.6, $CHCl_3$); IR ($CHCl_3$) *v* 2963, 2878, 1752, 1657, 1541, 1459, 1441, 1378, 1260, 1223, 1205, 1157, 1030, 974,759, 742, 700, 665, 638 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.42-7.41 (m, 3H, Ar), 7.38-7.34 (m, 4H, Ar), 6.05 (t, J = 8.62 Hz, 2H, NH), 5.94, 5.75 (each s, 1H, CH), 4.68 (d, J = 9.33 Hz, 1H, H-7), 4.65, 4.61 (each s, 1H, H-6), 4.59 (d, J = 8.77 Hz, 1H, H-7), 4.24-4.20 (m, 2H, H-9a), 4.16-3.96 (overlap, 7H, H-9b, H-8, H-4), 3.84, 3.83 (each s, 6H, OCH_3), 2.94, 2.83 (each d, J = 5.10 Hz, 1H, H-5), 2.16-2.14 (m, 3H, H-3eq, H-3ax), 1.99, 1.97 (each s, 6H, 2NAc); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.66 (CO), 167.21 (CO), 136.87 (C), 129.80 (CH), 129.43 (CH), 128.68 (CH), 128.55 (CH), 126.71 (CH), 126.44 (CH), 104.87 (CH), 104.31 (CH), 79.03 (CH), 78.20 (CH), 77.85 (CH), 75.55 (CH), 68.48 (CH_2), 67.39 (CH), 53.29 (OCH_3), 52.04 (CH), 35.81 (CH_2), 29.85 (CH_2), 23.34 (CH_3); HRMS (ESI): *m/z* calcd for $C_{19}H_{23}NO_8$ ($[M+Na]^+$): 416.1321, found: 416.1316.



13a

4.15. Methyl 5-acetamido-2,7-anhydro-4-*O*-acetyl-8,9-*O*-benzylidene-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosidonate (13a)

Compound **13** (33.4 mg, 0.085 mmol, 1 equiv) and 4-(dimethylamino)pyridine (1.0 mg, 0.0085 mmol, 0.1 equiv) was dissolved in dry pyridine (1 mL). To this solution, acetic anhydride (0.2 mL) was added dropwise and stirred overnight at room temperature. After the reaction completed, the solution was azeotroped with toluene three times. The crude product was purified by column chromatography with EtOAc: hexane (3:2) to give **13a** (19.1 mg, 52%) as a colorless syrup product. $[\alpha]^{25}_{D}$ +31 (c = 0.5, $CHCl_3$); IR ($CHCl_3$) *v* 2955, 1746, 1657, 1539, 1450, 1372, 1234, 1207, 1163, 1093, 1070, 1026, 761, 744, 715, 700 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.42-7.34 (m, 8H, Ar), 6.13 (t, J = 9.93 Hz, 1H, NH), 5.98, 5.77 (each s, 1H, CH), 4.88-4.85 (m, 2H, H-4), 4.65, 4.63 (each br s, 1H, H-5), 4.50 (d, J = 8.98 Hz, 1H, H-7), 4.39 (d, J = 8.63 Hz, 1H, H-7), 4.26-4.18 (m, 2H, H-6, H-9a), 4.14-4.08 (overlap, 4H, H-8, H-9b), 3.82, 3.81 (each s, 3H, OCH_3), 2.19-2.15 (m, 3H, H-3eq, H-3ax), 2.05, 1.98 (each s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.74 (CO), 169.32 (CO), 167.01 (CO), 166.97 (CO), 138.03 (C), 137.00 (C), 129.88 (CH), 129.49 (CH), 128.79 (CH), 128.64 (CH), 126.64 (CH), 126.45 (CH), 104.84 (C-2), 104.35 (C-2), 103.97 (CH), 79.57 (CH), 79.25 (CH), 78.28 (CH), 77.96 (CH), 75.60 (CH), 68.74 (CH), 68.57 (CH), 68.50 (CH_2), 68.18 (CH_2), 53.42 (OCH_3), 33.81 (CH_2), 33.76 (CH_2), 23.35, 21.52 (each CH_3); HRMS (ESI): *m/z* calcd for $C_{21}H_{25}NO_9$ ($[M+Na]^+$): 458.1427, found: 458.1423.

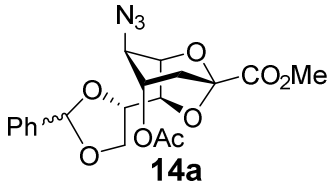


14

4.11. Methyl 5-azido-2,7-anhydro-8,9-*O*-benzylidene-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosidonate (14)

To a solution of compound **11** (74.3 mg, 256 mmol, 1 equiv) in dry DMF (2 mL) was added $PhCH(OMe)_2$ (96.4 µL, 0.64 mmol, 2.5 equiv) and CSA (11.8 mg, 0.054 mmol, 0.2 equiv) under

N_2 atmosphere at 50 ^oC and stirred for 15 h at the same temperature. When reaction was completed according to TLC, the oil bath was removed, and the mixture was neutralized with Et_3N and concentrated. The crude product was purified by column chromatography (60% EtOAc in hexane) to afford colorless syrup compound **14** (75.1 mg, 78%). $[\alpha]^{25}_D$ +21 (c = 0.56, $CHCl_3$); IR ($CHCl_3$) *v* 2103, 1752, 1626, 1220, 1206, 1157, 1091, 1072, 1027, 979, 924, 893, 761, 741, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.43-7.41 (m, 4H, Ar), 7.38-7.35 (m, 5H, Ar), 5.96, 5.77 (each s, 2H, CH), 4.85, 4.80 (each br s, 2H, H-6), 4.49 (d, J = 8.97 Hz, 1H, H-7), 4.38 (d, J = 8.84 Hz, 1H, H-7), 4.23-4.15 (m, 4H, H-9a, H-9b), 4.13-4.10 (m, 2H, H-8), 4.08-4.02 (m, 2H, H-4), 3.82, 3.81 (each s, 3H, OCH_3), 3.58 (d, J = 1.46 Hz, 1H, H-5), 3.46 (d, J = 1.34 Hz, 1H, H-5), 2.35-2.29 (m, 2H, H-3eq), 2.16 (d, J = 15.13 Hz, 2H, H-3ax); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 166.82 (CO), 166.78 (CO), 137.70 (C), 136.89 (C), 129.79 (CH), 129.51 (CH), 128.65 (CH), 128.59 (CH), 126.63 (CH), 126.42 (CH), 104.83 (CH), 104.26 (CH), 78.83 (CH), 78.58 (CH), 78.22 (CH), 77.89 (CH), 75.20 (CH), 75.14 (CH), 68.42 (CH_2), 68.15 (CH_2), 67.46 (CH), 61.15 (CH), 53.32 (OCH_3), 36.31 (CH_2); HRMS (ESI): *m/z* calcd for $C_{17}H_{19}N_3O_7$ ($[M+Na]^+$): 400.1121, found: 400.1129.

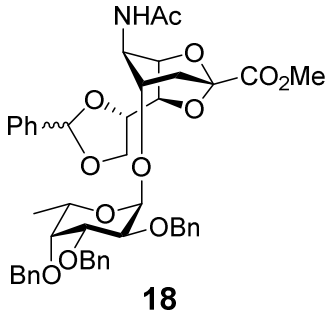


14a

4.12. Methyl 5-azido-2,7-anhydro-4-*O*-acetyl-8,9-*O*-benzylidene-3,5-dideoxy-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosidonate (14a)

Compound **14** (19.3 mg, 0.051 mmol, 1 equiv) and 4-(dimethylamino)pyridine (1.5 mg, 0.01 mmol, 0.2 equiv) was dissolved in dry pyridine (0.4 mL). To this solution, acetic anhydride (0.12 mL) was added dropwise and stirred overnight at room temperature. After the reaction completed, the solution was azeotroped with toluene three times. The crude product was purified by column chromatography with EtOAc: hexane (3:2) to give **14a** (20.3 mg, 95%) as a colorless syrup product. $[\alpha]^{25}_D$ +31 (c = 1.0, $CHCl_3$); IR ($CHCl_3$) *v* 2104, 1746, 1458, 1439, 1230, 1207, 1093, 1069, 1026, 761, 742, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.44-7.41 (m, 4H, Ar), 7.39-7.35 (m, 5H, Ar), 5.99, 5.80 (each s, 2H, CH), 5.06 (dd, J = 1.25, 5.85 Hz, 1H, H-4), 5.02 (dd, J = 1.25, 5.75 Hz, 1H, H-4), 4.83, 4.78 (each br s, 1H, H-6), 4.30 (d, J = 9.06 Hz, 1H, H-7), 4.24-4.20 (m, 2H, H-9a), 4.17-4.12 (m, 4H, H-9b, H-7, H-8), 4.00 (dd, J = 3.25, 8.7 Hz, 1H, H-

9b), 3.82, 3.81 (each s, 3H, OCH_3), 3.54 (d, J = 1.1 Hz, 1H, H-5), 3.35 (d, J = 1.05 Hz, 1H, H-5), 2.36-2.29 (overlap, 2H, H-3eq), 2.19-2.15 (overlap, 2H, H-3ax), 2.12, 2.05 (each, s, CH_3);^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.85 (CO), 169.84 (CO), 166.68 (CO), 166.64 (CO), 137.85 (C), 137.23 (C), 129.73 (CH), 129.48 (CH), 128.65 (CH), 128.61 (CH), 126.43 (CH), 126.35 (CH), 104.54 (C-2), 104.31(CH), 103.60 (CH), 78.53 (CH), 78.28 (CH), 78.10 (CH), 77.93 (CH), 75.38 (CH), 68.49 (CH_2), 68.05 (CH_2), 67.99 (CH), 67.90 (CH), 59.22 (CH), 59.16 (CH), 53.34 (OCH_3), 33.71 (CH_2), 21.35, 21.29 (each CH_3); HRMS (APCI): *m/z* calcd for $C_{19}H_{21}N_3O_8$ ($[M+H]^+$): 420.1407, found: 420.1404.

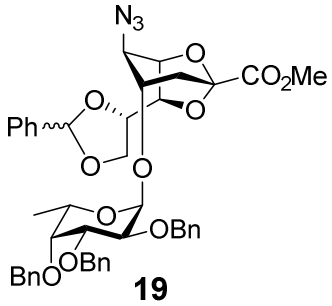


18

4.16. Methyl (5-acetamido-2,7-anhydro-8,9-*O*-benzylidene-3,5-dideoxy-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (18)

Fucosyl donor **16** (107 mg, 0.198 mmol, 1 equiv) and acceptor **13** (94 mg, 0.238 mmol, 1.2 equiv) were azeotroped with toluene at 35 °C and dissolved in dry CH_2Cl_2 (5 mL). Freshly activated 3Å molecular sieves (1.8 g) were added, and the mixture was stirred at room temperature for 1 h under N_2. The reaction mixture was cooled to -78 °C and added *N*-iodosuccinimide (NIS) (45 mg, 0.198 mmol, 1 equiv) and trifluoromethanesulfonic acid (TfOH) (3.484 µL, 0.04 mmol, 0.2 equiv) and stirred for 1 h. The completion of reaction was confirmed by TLC analysis and allowed to warm to 0 °C over 0.5 h. The reaction solution was neutralized with saturated aqueous solution of $NaHCO_3$ (pH = 8) at 0 °C, diluted with CH_2Cl_2 and filtered through a Celite and the filter bed was washed with EtOAc. The organic layer was washed with 10% $Na_2S_2O_3$ aqueous solution, treated with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification of the crude product by flash chromatography on silica gel (3:1 hexanes-EtOAc) afforded disaccharide **18** (113 mg, 71%) as colorless syrup. $[\alpha]^{25}_D$ -5 (c = 0.46, $CHCl_3$); IR ($CHCl_3$) *v* 3031, 2931, 1752, 1713, 1656, 1526, 1497, 1205, 1162, 1136, 1088, 1042, 1027, 739, 697, 667, 641 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39-7.05 (m, 35H, Ar), 6.09-6.07 (overlap, 2H, NH), 5.68, 5.51 (each s, 1H, CH), 5.21 (d, J = 3.45 Hz, 1H, H-1), 5.09

(d, J = 3.29 Hz, 1H, H-1), 4.96-4.49 (overlap, 14H, OCH_2, H'-6, H'-7), 4.16 (d, J = 8.20 Hz, 2H, H'-5), 4.11-4.06 (m, 2H, H'-9a), 3.99-3.74 (m, 16H, H-2, H-3, H-5, H'-9b, H'-8, H'-4, OCH_3), 3.63, 3.57 (each s, 2H, H-4), 2.17-2.05 (m, 3H, H'-3eq, H'-3ax), 1.93 (s, 5H, CH_3), 1.06, 1.02 (each d, J = 6.55 Hz, 5H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.28 (CO), 167.36 (CO), 139.19 (C), 139.14 (C), 138.78 (C), 138.65 (C), 138.55 (C), 138.10 (C), 136.82 (C), 129.60 (CH), 129.18 (CH), 128.46 (CH), 128.36 (CH), 128.30 (CH), 128.25 (CH), 128.17 (CH), 128.05 (CH), 127.66 (CH), 127.60 (CH), 127.55 (CH), 127.52 (CH), 127.44 (CH), 127.37 (CH), 126.80 (CH), 126.38 (CH), 104.92 (CH), 104.04 (C-2), 103.93 (C-2), 103.87 (CH), 99.27 (CH), 98.48 (CH), 79.58 (CH), 79.49 (CH), 79.19 (CH), 78.85 (CH), 78.34 (CH), 78.24 (CH), 77.97 (CH), 77.34 (CH), 75.91 (CH), 75.82 (CH), 75.02 (OCH_2), 74.89 (OCH_2), 74.43 (CH), 73.74 (OCH_2), 73.64 (OCH_2), 73.57 (CH), 73.43 (OCH_2), 72.57 (OCH_2), 68.16 (CH_2), 67.32 (CH), 67.26 (CH), 53.15 (OCH_3), 50.06 (CH), 49.51 (CH), 35.13 (CH_2), 35.02 (CH_2), 23.22, 16.74, 16.64 (each CH_3); HRMS (ESI): *m/z* calcd for $C_{46}H_{51}NO_{12}$ ($[M+Na]^+$): 832.3309, found: 832.3308.



19

4.17. Methyl (5-azido-2,7-anhydro-8,9-*O*-benzylidene-3,5-dideoxy-4-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-*α*-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (19)

Fucosyl donor **16** (56.6 mg, 0.158 mmol, 1.5 equiv) and acceptor **14** (39.5 mg, 0.105 mmol, 1 equiv) were azeotroped with toluene at 35 °C and dissolved in dry CH_2Cl_2 (2.5 mL). Freshly activated 3Å molecular sieve (120 mg) was added, and the mixture was stirred at room temperature for 1 h under N_2. The reaction mixture was cooled to -78 °C and added NIS (23.6 mg, 0.105 mmol, 1 equiv) and TfOH (1.8 μL, 0.021 mmol, 0.2 equiv) and stirred for another 1 h. The completion of reaction was confirmed by TLC analysis and allowed to warm to 0 °C over 0.5 h. The reaction solution was neutralized with saturated aqueous solution of $NaHCO_3$ (pH = 8) at 0 °C, diluted with CH_2Cl_2 and filtered through a Celite and the filter bed was washed with EtOAc. The organic layer was washed with 10% $Na_2S_2O_3$ aqueous solution, treated with brine, dried over anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification of the crude

product by flash chromatography on silica gel (EtOAc-hexanes, 1:4) afforded disaccharide **19** (40.9 mg, 83%) as colorless syrup. $[\alpha]^{25}_{D}$ -15 (c = 0.52, $CHCl_3$); IR ($CHCl_3$) *v* 2101, 1685, 1655, 1053, 1028, 1012, 761, 743, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 4.74-7.26 (m, 20H, Ar), 5.72 (s, 1H, CH), 4.99 (d, J = 11.55 Hz, 1H, OCH_2), 4.89-4.60 (m, 9H, OCH_2, H-1, H'-6), 4.20 (dd, J = 6.39, 8.61 Hz, 1H, H'-9a), 4.09-4.04 (overlap, 2H, H-2, H'-8), 3.96 (t, J = 6.24 Hz, 1H, H-5), 3.91 (dd, J = 2.4, 10.18 Hz, 2H, H'-7, H-3), 3.85-3.82 (overlap, 2H, H'-4, H'-9b), 3.80 (s, 3H, OCH_3), 3.68 (s, 1H, H-4), 3.33 (1H, H'-5), 2.24-2.14 (overlap, 2H, H'-3eq, H'-3ax), 1.10 (d, J = 6.44 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.13 (CO), 138.89 (C), 138.71 (C), 138.66 (C), 137.98 (C), 129.40 (CH), 128.79 (CH), 128.58 (CH), 128.54 (CH), 128.41 (CH), 128.18 (CH), 128.09 (CH), 127.83 (CH), 127.74 (CH), 127.48 (CH), 126.46 (CH), 104.09 (CH), 103.81 (C-2), 99.05(CH), 79.65 (CH), 78.67 (CH), 77.92 (CH), 77.36 (CH), 75.69 (CH), 75.13 (OCH_2), 74.70 (OCH_2), 73.34 (OCH_2), 73.08 (CH), 68.46 (CH_2), 67.64 (CH), 58.66 (CH), 53.19 (OCH_3), 35.08 (CH_2), 16.72 (CH_3); HRMS (ESI): *m/z* calcd for $C_{44}H_{47}N_3O_{11}$ ($[M+Na]^+$): 816.3108, found: 816.3109.

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Appendix A. Supplementary data

Supplementary data including ^1H NMR and ^{13}C NMR spectra of all final products and intermediates are available at xxxx.

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Concise Synthesis of 2,7-Anhydrosialic Acid Derivatives and its application

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Highlights

- *N*-acetyl-2,7-anhydroneuraminic acid was synthesized in three steps through heating-assisted intramolecular anomeric protection of per-*O*-trimethylsilylated sialic acid.
- The 4- and 7-OH groups of neuraminic acid were differentiated in the form of 2,7-anhydrosugar.
- Fucosylation at 4-OH position of the 2,7-anhydrosialic acid afforded the corresponding $\alpha(1\rightarrow4)$ fucosyl sialic acid linkage in higher yield and with excellent stereoselectivity.