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Note

5-Azido neuraminic acid thioglycoside as sialylation donor

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Abstract

5-Azido neuraminic acid thioglycoside with *O*-benzyl protecting groups was synthesized. The sialylations of this new donor type showed good α-selectivities for certain primary hydroxyls. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: 5-Azidoneuraminic acid; Sialic acid; Thioglycoside; Sialylation

N-Acetylneuraminic acid (sialic acid, Neu5Ac) is found as the terminal sugar of many oligosaccharide chains on the cell surface and is among the most important residue for interactions with their receptors.[1] Recent findings show that synthetic *N*-acyl-modified D-mannosamines could be taken up by cells, efficiently metabolized in the promiscuous sialic acid biosynthetic pathway, and incorporated into cell surface sialoglycoconjugates.[2] Applications of this approach in different biological systems have revealed important and unexpected functions of the *N*-acyl side chain of sialic acids, including its crucial roles for the interactions of different viruses with their sialylated host cell receptors.[3] Also, the immunogenicity of the group B menigococcal polysaccharide has been successfully improved by substituting *N*-acetyl with *N*-propionyl groups in the polysialic acid.[4] Therefore, engineering of the side chain of sialic acid offers a new tool to study its biological relevance and to exploit it as a tag for therapeutic and diagnostic applications.

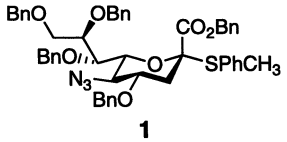

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Fig. 1. Structure of **1**.

In order to tackle the importance of different functionalities of sialic acid, its derivatives are needed to study its interactions with various proteins. Although many *N*-substituted sialic acid analogs have been synthesized by either chemoenzymatic or chemical methods,[5,6] they did not serve as good substrates for CMP sialic acid synthetase. To develop a universal sialic acid donor which can be easily functionalized at C-5 for chemical sialylation is of current interest. Modifications of NHAc of sialic acid donors have been reported to have influence on the reactivities and selectivities of sialylations.[7] We proposed that the use of 5-azido sialic acid as donor for the chemical preparation of *N*-substituted sialic acid conjugates: In our previous studies on peracetylated 5-azido sialic acid donor, demonstrated it to be a good donor.[8] In this note, we describe our studies of benzyl protected 5-azido sialic acid, **1** (Fig. 1), as sialylation donor.

Preparation of azido thioglycoside donor **1**.—Synthesis of 5-azido benzyl-protected thioglycoside **1** is shown in Scheme 1. The readily available compound **2** synthesized as described in Ref. 9 was treated with acetyl chloride[10] to give glycosyl chloride **3**. Substitution of the chlorine atom in compound **3** with *p*-thiocresol in the presence of Hunig's base[11] afforded the α-thioglycoside **4** (84%) which was then *N*,*O*-deacetylated with methanesulfonic acid in MeOH for 24 h at 60 °C[12] to give compound **5** in 81% yield. Treatment of **5** with trifluoromethanesulfonyl azide (TfN_3)[13] as the diazo transfer reagent furnished azido derivative **6** (84%),

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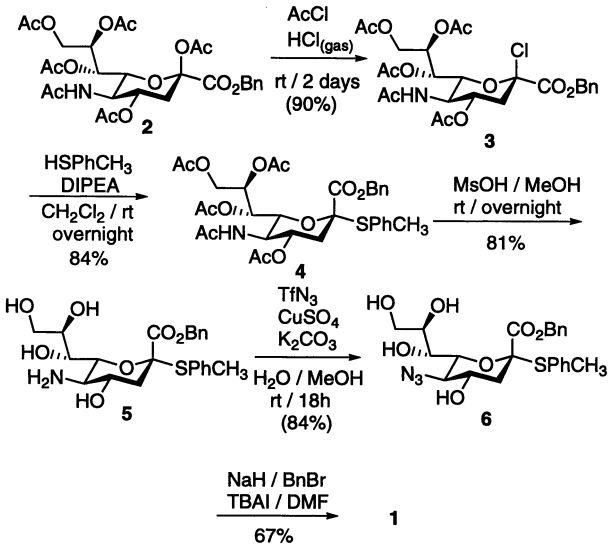


Scheme 1. Synthesis of donor **1**.

which was followed by per-benzylation to obtain azido thioglycoside donor **1** in 67% yield. The configurational assignment at C-5 was confirmed by the ^{1}H NMR spectroscopic data ($J_{4,5}$ 9.2, $J_{5,6}$ 10.4 Hz).

Acceptors used in the present study.—As shown in Fig. 2, acceptors **7**–**10** were chosen for the studies on the steric effects of acceptors in sialylations. The sialylation products of donor **1** with acceptor **7**/**8** and **9**/**10** gave STn and pseudo GM_3 precursors, respectively. Acceptor **11** was used for the synthesis of (α2-9) sialic acid dimer. Secondary hydroxyl acceptors **12** and **13** were used for testing the sialylation reactions with more hindered acceptors (primary vs. secondary acceptor). Their products can serve as precursors for the synthesis of GM_3 and (α2-8) sialic acid dimer. Also, due to the change of 5-NHAc to N_3, the 8 and/or 9-hydroxy groups could not form intramolecular hydrogen bond with the azido group. Thus, acceptors **11** and **13** could be potentially better acceptors than their 5-NHAc parents. Acceptors **7**[14]–**9**[15] and **12**[16] were obtained by following reported procedures. Compounds **11** and **13** were synthesized by modification of Demchenko's[7b] and Yu's[8] procedures.

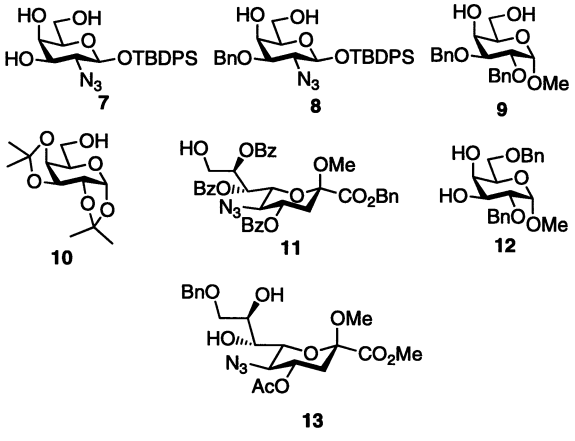
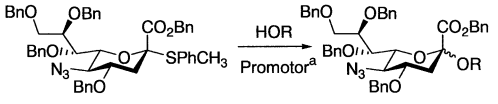


Fig. 2. Acceptors for sialylation.

Evaluation of 5-azido thioglycoside donor **1**.—Sialylation reactions were proceeded under NIS/TfOH reaction conditions.[17] Acceptors **7**–**13** (1 equiv) were reacted with donor **1** (2 equiv) in the presence of NIS (3 equiv to donor), TfOH (3–10 mol% to NIS), and molecular sieve (3 Å) in MeCN at −40 °C to give disaccharides **19**–**24**, as shown in Table 1. The results showed that the primary hydroxyl acceptors gave better α-selectivities and yields than those of the secondary acceptors. For the primary acceptor, the less hindered one afforded better α-selectivity. The selectivities and yields decreased dramatically when secondary hydroxyl acceptors were used, e.g. for acceptor **13**, no desired product was observed except elimination product from donor. These results may be attributed to the low reactivity of donor **1**. The anomeric configurations of these disaccharides were determined by NMR spectroscopy based on the chemical shifts of H-3e of sialic acid and empirical rules.[18] The chemical shifts of H-3e of α-glycosides were more downfield than those of β-glycosides. The α anomers of **19**, **20**, and **22** were further confirmed by measurements of the long-range $J_{C\text{-}1,\ H\text{-}3ax}$ coupling constants.[19] By selective proton decoupled ^{13}C NMR experiments, the coupling pattern of C-1 of α anomer gave a doublet C-1 signal and the coupling constants of **19**α, **20**α, and **22**α were 6.4, 6.6, and 7.3 Hz, respectively.

In summary, the 5-azido thioglycoside donor **1** showed good α-selectivities for certain primary hydroxyl acceptors. The selectivity tendency of donor **1** was the same as its parent per-*O*-acetylated 5-azido sialyl donor.[8] However, further studies are needed to understand how the protecting groups affect the reac-

Table 1
Sialylation reactions



Entry	Acceptor	Promotor	Product (α/β)	Yield (%)
1	**7**	NIS/TfOH	**14** (only α)	43
2	**8**	NIS/TfOH	**15** (9:1)	61
3	**9**	NIS/TfOH	**16** (4:1)	57
4	**10**	NIS/TfOH	**17** (3.3:1)	64
5	**11**	NIS/TfOH	**18** (7:3)	42
6	**12**	NIS/TfOH	**19** (1.4:1)	17
7	**13**	NIS/TfOH		

[a] Key: NIS, TfOH, 3 Å MS, CH_3CN, −40 °C.

tivity and selectivity. Use of donor **1** to synthesize sialo-antigens with modification of sialic acid at C-5 is in progress.

1. Experimental

General methods.—^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-400 or 500 MHz. Assignment of ^{1}H NMR spectra was achieved using 2D methods (COSY). Chemical shifts are expressed in ppm using residual $CHCl_3$ as reference. High-resolution mass spectra were obtained by means of a Micromass (Autospec) mass spectrometer. Optical rotation was measured at 22 °C. Analytical thin-layer chromatography (TLC) was performed on precoated plates (Silica Gel 60 F-254). Silica gel 60 (E. Merck) was employed for all flash chromatography. All reactions were carried out in oven-dried glassware (120 °C) under an atmosphere of nitrogen unless indicated otherwise. All solvents were dried and distilled by standard techniques.

Benzyl (5-acetamido-4,7,8,9-tetra-O-*acetyl-2-chloro-3,5-dideoxy*-D-glycero-α-D-galacto-*non-2-ulpyranosid)onate* (**3**).—AcCl (80 mL) was added to the flask which contained compound **2**[9] (8.08 g, 13.3 mmol) and then purged with HCl gas for 10 min. The mixture was stirred for 2 days at rt and the concentrated, diluted with EtOAc, washed with water and satd $NaHCO_3$, and concentrated in vacuo to give compound **3** (6.89 g, 90%) which was used without further purification; R_f 0.3 (1:1 EtOAc–hexane containing 10% MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.91 (s, 3 H, NHAc), 2.02 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.12 (s, 3 H, OAc), 2.29 (dd, 1 H, $J_{3ax,4}$ 11.2, J_{gem} 14.0 Hz, H-3ax), 2.78 (dd, 1 H, $J_{3aq,4}$ 4.8, J_{gem} 14.0 Hz, H-3eq), 4.08 (dd, 1 H, $J_{9a,8}$ 5.6, J_{gem}12.4 Hz, H-9a), 4.20 (ddd, 1 H, $J_{5,4}$ 10.8, $J_{5,6}$ 10.8, $J_{5,NH}$ 10.8 Hz, H-5), 4.36 (dd, 1 H, $J_{6,7}$ 2.4, $J_{6,5}$ 10.8 Hz, H-6), 4.40 (dd, 1 H, $J_{9b,8}$ 2.4, J_{gem}12.4 Hz, H-9b), 5.18 (ddd, 1 H, $J_{8,9b}$ 2.4, $J_{8,9a}$ 5.6, $J_{8,7}$ 6.4 Hz, H-8), 5.23 (d, 1 H, J_{gem} 12.0 Hz, Bn), 5.36 (d, 1 H, J_{gem} 12.0 Hz, Bn), 5.37–5.44 (m, 2 H, H-4, NH), 5.47 (dd, 1 H, $J_{7,6}$ 2.4, $J_{7,8}$ 6.4 Hz, H-7), 7.35–7.41 (m, 5 H).

*Benzyl (*p-*tolyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy-2-thio*-D-glycero-α-D-galacto-*non-2-ulpyranosid)onate* (**4**).—To a solution of sialic acid chloride **3** (6.89 g, 11.8 mmol) and thiocresol (2.20 g, 17.6 mmol) in CH_2Cl_2 (75 mL) was added *N*-ethyldiisopropylamine (Hunig's base, 3.1 mL, 17.6 mmol) at rt, and then stirred for overnight. When the reaction showed to be complete, the mixture was concentrated and then diluted with EtOAc, washed with water, satd $NaHCO_3$, brine, and then concentrated. The residue was purified by silica gel column chromatography (1:1 EtOAc–hexane containing 5% MeOH) to give compound **4** (6.65 g, 84%). R_f 0.2 (1:1 EtOAc–hexane containing 5% MeOH); $[\alpha]_D^{23}$ +2.66° (*c* 0.019, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.85 (s, 3 H, NHAc), 2.00–2.07 (m, 1 H, H-3ax), 2.01 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.07 (s, 3 H, OAc), 2.13 (s, 3 H, OAc), 2.35 (s, 3 H, OAc), 2.82 (dd, 1 H, $J_{3eq,4}$ 4.4, J_{gem} 12.8 Hz, H-3eq), 3.87 (dd, 1 H, $J_{6,7}$ 2.0, $J_{6,5}$ 10.4 Hz, H-6), 3.97 (ddd, 1 H, $J_{5,4}$ 10.4, $J_{5,6}$ 10.4, $J_{5,NH}$ 10.4 Hz, H-5), 4.21 (dd, 1 H, $J_{9a,8}$ 6.4, J_{gem}12.4 Hz, H-9a), 4.38 (dd, 1 H, $J_{9b,8}$ 2.8, J_{gem}12.4 Hz, H-9b), 4.81 (ddd, 1 H, $J_{4,3eq}$ 4.4, $J_{4,5}$ 10.4, $J_{4,3ax}$ 11.6 Hz, H-4), 5.03 (s, 2 H, Bn), 5.13 (d, 1 H, $J_{NH,5}$ 10.4 Hz, NH), 5.16 (ddd, 1 H, $J_{8,9b}$ 2.8, $J_{8,9a}$ 6.4, $J_{8,7}$ 6.4 Hz, H-8), 5.27 (dd, 1 H, $J_{7,6}$ 2.0, $J_{7,8}$ 6.4 Hz, H-7), 7.08–7.10 (m, 2 H), 7.28–7.39 (m, 7 H); ^{13}C (100 MHz, $CDCl_3$): δ 20.73, 20.81, 20.81, 20.93, 21.32, 23.14, 38.12, 49.25, 62.04, 67.80, 67.85, 69.69, 70.54, 75.01, 87.26, 124.96, 128.48, 128.48, 128.48, 128.48, 128.60, 128.60, 129.67, 129.67, 136.94, 136.94, 140.16, 167.43, 170.05, 170.15, 170.16, 170.59, 170.76. HRMS (FAB) Calcd for $C_{33}H_{40}NO_{12}S$ $[M+H]^+$: 674.2272 Found: 674.2265.

*Benzyl (*p-*tolyl 5-amino-3,5-dideoxy-2-thio*-D-glycero-α-D-galacto-*non-2-ulpyrnosid)onate* (**5**).—To a stirred solution of compound **4** (1.0 g, 1.5 mmol) in dry MeOH (50 mL) was added methanesulfonic acid (MsOH, 0.2 mL, 22.2 mmol). The mixture was stirred for 24 h at 60 °C, then neutralized with Dowex 1 × 8 (OH^-) resin, after which the suspension was filtered. The filtrate was concentrated and purified by silica gel column chromatography (3:7 MeOH–$CHCl_3$) to give compound **5** (557.3 mg, 81%). R_f 0.3 (3:7 MeOH–$CHCl_3$); $[\alpha]_D^{23}$ −30.77° (*c* 0.004, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.85 (dd, 1 H, $J_{3ax,4}$ 11.2, J_{gem} 12.8 Hz, H-3ax), 2.33 (s, 3 H, $SPhCH_3$), 2.86 (dd, 1 H, $J_{3eq,4}$ 4.4, J_{gem} 12.8 Hz, H-3eq), 3.04 (t, 1 H, $J_{5,4} = J_{5,6}$ 10.0 Hz, H-5), 3.46–3.52 (m, 1 H, H-4), 3.53 (dd, 1 H, $J_{9a,8}$ 2.0, J_{gem}10.0 Hz, H-9a), 3.63–3.66 (m, 1 H, H-6), 3.66 (dd, 1 H, $J_{9b,8}$ 6.4, J_{gem}10.0 Hz, H-9b), 3.79–3.83 (m, 2 H, H-7, H-8), 5.12 (s, 2 H, Bn), 7.09 (d, *J* 7.6 Hz, 2 H), 7.26 (d, *J* 8.0 Hz, 2 H), 7.34–7.42 (m, 5 H); ^{13}C (100 MHz, $CDCl_3$): δ 21.48, 41.72, 54.26, 64.58, 69.24, 69.74, 70.20, 73.51, 77.46, 87.96, 126.45, 129.85, 129.85, 129.90, 129.90, 130.09, 130.09, 130.80, 130.80, 137.94, 137.94, 141.87, 170.43. HRMS (FAB) Calcd for $C_{23}H_{30}NO_7S$ $[M+H]^+$: 464.1744 Found: 464.1737.

*Benzyl (*p-*tolyl 5-azido-3,5-dideoxy-2-thio*-D-glycero-α-D-galacto-*non-2-ulpyranosid)onate* (**6**).—A solution of NaN_3 (2.3 g, 35.3 mmol) in water (5.7 mL) was cooled in an ice bath and treated with CH_2Cl_2 (3 mL). The resulting biphasic mixture was stirred vigorously and then treated with Tf_2O (2.5 g, 8.8 mmol) over a period of 5 min. The reaction was stirred in an ice bath for 2 h, the organic layer was separated and aqueous phase was washed with CH_2Cl_2 as less volume as possible. The organics were washed once with satd $NaHCO_3$ solution and used without further purification.

Compound **5** (1.2 g, 4.06 mmol) was dissolved in water (13.6 mL) and treated with potassium carbonate (K_2CO_3, 731.0 mg, 5.3 mmol) and $CuSO_4 \cdot 5\ H_2O$ (8.8 mg, 0.04 mmol). To the sugar solution was added MeOH (20 mL) and TfN_3 solution. Then, more MeOH (8 mL) was added to homogenity. The reaction was allowed to stirred for 18 h and the solvent was removed. The residue was purified by silica gel column chromatography (1:4 MeOH–$CHCl_3$) to give azido product **6** (1.45 g, 84%). R_f 0.4 (1:1 EtOAc–hexane containing 10% MeOH); $[\alpha]_D^{23}$ −26.47° (*c* 0.002, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.86 (dd, 1 H, $J_{3ax,4}$ 11.2, J_{gem} 12.8 Hz, H-3ax), 2.33 (s, 3 H, $SPhCH_3$), 2.80 (dd, 1 H, $J_{3eq,4}$ 3.6, J_{gem} 12.8 Hz, H-3eq), 3.27–3.31 (m, 1 H, H-6), 3.49 (t, 1 H, $J_{5,4} = J_{5,6}$ 9.6 Hz, H-5), 3.51–3.57 (m, 1 H, H-8), 3.60 (dd, 1 H, $J_{9a,8}$ 5.6, J_{gem}11.2 Hz, H-9a), 3.66 (dd, 1 H, $J_{7,6}$ 1.2, $J_{7,8}$ 8.8 Hz, H-7), 3.73–3.77 (m, 1 H, H-4), 3.80 (dd, 1 H, $J_{9b,8}$ 2.4, J_{gem}11.2 Hz, H-9b), 5.08 (s, 2 H, Bn), 7.09 (d, *J* 8.4 Hz, 2 H), 7.26 (d, *J* 8.0 Hz, 2 H), 7.33–7.42 (m, 5 H); ^{13}C (100 MHz, $CDCl_3$): δ 21.48, 41.45, 64.27, 64.72, 69.17, 70.61, 71.41, 73.62, 77.04, 87.80, 126.54, 129.82, 129.82, 129.88, 129.88, 130.15, 130.15, 130.76, 130.76, 137.92, 137.92, 141.79, 170.38. HRMS (FAB) Calcd for $C_{23}H_{28}N_3O_7S$ $[M+H]^+$: 490.1649 Found: 490.1638.

*Benzyl (*p*-tolyl 5-azido-4,7,8,9-tetra*-O*-benzyl-3,5-dideoxy-2-thio*-D-glycero-α-D-galacto-*non-2-ulpyranosid)onate* (**1**).—DMF (4.0 mL) was added to a flask containing compound **6** (200 mg, 0.4 mmol) and tetrabutyl ammonium iodine (TBAI 10 mg, 0.03 mmol), then cooled to 0 °C. NaH (80.0 mg, 3.3 mmol) was added to the solution and stirred for 30 min. BnBr (0.3 mL, 2.4 mmol) was added dropwise and then the mixture was stirred for overnight. When the reaction was completed, it was cooled to 0 °C, and quenched by water. The solvent was removed and then diluted with EtOAc followed by washing with water and brine, and then dried over $MgSO_4$. The mixture was purified by silica gel column chromatography (1:1 EtOAc–hexane) to afford donor **1** (234 mg, 67%); R_f 0.25 (1:1 EtOAc–hexane); $[\alpha]_D^{23}$ −8.0° (*c* 0.006, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.73 (dd, 1 H, $J_{3ax,4}$ 11.6, J_{gem} 12.8 Hz, H-3ax), 2.25 (s, 3 H, $SPhCH_3$), 2.80 (dd, 1 H, $J_{3eq,4}$ 4.4, J_{gem} 12.8 Hz, H-3eq), 3.41 (ddd, 1 H, $J_{4,3eq}$ 4.4, $J_{4,5}$ 9.2, $J_{4,3ax}$ 11.6 Hz, H-4), 3.50–3.58 (m, 1 H, H-5), 3.54 (dd, 1 H, $J_{6,7}$ 9.2, $J_{6,5}$ 10.4 Hz, H-6), 3.73 (dd, 1 H, $J_{9a,8}$ 4.4, J_{gem} 10.4 Hz, H-9a), 3.84–3.91 (m, 2 H, H-8, H-9b), 3.85 (dd, 1 H, $J_{7,8}$ 7.2, $J_{7,6}$ 9.2 Hz, H-7), 4.37 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.41 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.43 (d, 1 H, J_{gem} 11.2 Hz, Bn), 4.51–4.56 (m, 4 H, Bn), 4.78 (d, 1 H, J_{gem} 11.2 Hz, Bn), 4.91 (d, 1 H, *J* 12.4 Hz, Bn), 4.98 (d, 1 H, *J* 12.4 Hz, Bn), 6.92–6.94 (m, 2 H), 7.22–7.37 (m, 27); ^{13}C (100 MHz, $CDCl_3$): δ 21.20, 37.31, 61.06, 67.36, 69.68, 71.42, 72.31, 73.29, 74.34, 76.00, 76.68, 77.31, 78.46, 86.72, 125.49, 127.15, 127.40, 127.59, 127.59, 127.59, 127.63, 127.63, 127.63, 127.83, 127.83, 127.94, 127.98, 127.98, 128.08, 128.08, 128.26, 128.26, 128.30, 128.30, 128.43, 128.43, 128.58, 128.58, 128.58, 128.58, 129.44, 129.44, 135.09, 136.64, 136.64, 137.26, 138.24, 138.51, 139.32, 139.76, 167.81. HRMS (FAB) Calcd for $C_{51}H_{51}N_3O_7SNa$ $[M+H]^+$: 872.3345 Found: 872.3321.

tert-*Butyldiphenylsilyl [benzyl (5-azido-4,7,8,9-tetra*-O*-benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*non-2-ulpyranosid)onate]-α-(2,6)-2-azido-3*-O*-benzyl-2-deoxy-β-*D*-galactopyranoside* (**15**).—General procedures for the sialylation reaction: NIS (53.1 mg, 0.2 mmol) was added into the flask which contained donor **1** (50 mg, 0.06 mmol), acceptor **8**[14] (62.8 mg, 0.12 mmol) and 3 Å MS (113 mg) in CH_3CN (1.5 mL) at −40 °C under Ar and then stirred for 30 min. TfOH (0.3 L, 0.003 mmol) was added to the mixture solution at −40 °C. After stirred for 30 min Et_3N (one drop) was added and the solvent was removed. The mixture was diluted with EtOAc and washed with $Na_2S_2O_3$, satd. $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (1:2 EtOAc–hexane) to give disaccharide **15** (α/ = 9:1, 45.0 mg, 61%); R_f 0.33 (1:2 EtOAc–hexane); $[\alpha]_D^{23}$ −19.35° (*c* 0.012, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.07 (s, 9 H, 3 CH_3), 1.60 (dd, 1 H, $J_{3ax,4}$ 11.6, J_{gem} 12.8 Hz, H-3ax), 2.44 (br, 1 H, OH), 2.65 (dd, 1 H, $J_{3eq,4}$ 3.6, J_{gem} 12.8 Hz, H-3eq), 3.07 (dd, 1 H, $J_{3',4'}$ 3.2, $J_{3',2'}$ 10.0 Hz, H-3′), 3.10–3.12 (m, 1 H, H-5′), 3.43–3.48 (m, 2 H, H-4, H-6′a), 3.61–3.79 (m, 8 H, H-5, H-6, H-8, H-9a, H-9b, H-2′, H-4′, H-6′b), 3.95 (d, 1 H, $J_{7,8}$ 7.6 Hz, H-7), 4.26 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.42 (d, 1 H, J_{gem} 11.2 Hz, Bn), 4.46–4.67 (m, 7 H, Bn), 4.76 (d, 1 H, J_{gem} 11.2 Hz, Bn), 4.97 (d, 1 H, J_{gem} 12.0 Hz, Bn), 5.08 (d, 1 H, J_{gem} 12.0 Hz, Bn), 7.22–7.36 (m, 34 H), 7.68–7.71 (m, 6 H); ^{13}C (100 MHz, $CDCl_3$): δ 19.13, 26.78, 26.78, 26.78, 36.57, 60.96, 61.55, 64.26, 65.31, 67.38, 68.59, 71.48, 71.65, 72.25, 72.66, 73.25, 73.76, 74.63, 76.06, 76.73, 77.99, 79.36, 96.71, 98.83, 127.31, 127.31, 127.36, 127.36, 127.51, 127.51, 127.51, 127.69, 127.69, 127.69, 127.69, 127.86, 127.86, 127.92, 127.92, 127.92, 127.96, 127.96, 127.96, 128.01, 128.01, 128.01, 128.20, 128.20, 128.31, 128.31, 128.46, 128.46, 128.46, 128.46, 128.68, 128.68, 129.66, 129.66, 129.87, 129.78, 132.85, 133.04, 134.99, 135.82, 135.82, 135.96, 135.96, 137.30, 137.37, 137.93, 138.34, 138.88, 177.16. HRMS (FAB) Calcd for $C_{73}H_{78}N_6O_{12}SiNa$ $[M+H]^+$: 1281.5344 Found: 1281.5313.

tert-*Butyldiphenylsilyl [benzyl (5-azido-4,7,8,9-tetra*-O*-benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*non-2-ulpyranosid)onate]-α-(2,6)-2-azido-2-deoxy-β-*D*-galactopyranoside* (**14**).—For α form; R_f 0.20 (1:3 EtOAc–hexane); $[\alpha]_D^{23}$ − 19.35° (*c* 0.003, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (br, 1 H, OH), 1.68 (dd, 1 H, $J_{3ax,4}$ 11.6, J_{gem} 12.8 Hz, H-3ax), 2.43 (d, 1 H, *J* 9.6 Hz, OH), 2.56 (dd, 1 H, $J_{3eq,4}$ 4.8, J_{gem} 12.8 Hz, H-3eq), 3.08 (m, 1 H, H-3′), 3.19 (ddd, 1 H, $J_{4,3eq}$ 4.8, $J_{4,5}$ 9.6,

$J_{4,3ax}$ 11.6 Hz, H-4), 3.30 (t, 1 H, $J_{5,4} = J_{5,6}$ 9.6 Hz, H-5), 3.40–3.49 (m, 4 H, H-2′, H-5′, H-6′a, H-6′b), 3.63–3.72 (m, 3 H, H-6, H-8, H-4′), 3.77–3.80 (m, 2 H, H-9a, H-9b), 3.98 (d, 1 H, $J_{7,8}$ 7.6 Hz, H-7), 4.27 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1′), 4.40 (d, 1 H, J_{gem} 11.2 Hz, Bn), 4.46 (d, 1 H, J_{gem} 11.2 Hz, Bn), 4.50–4.58 (m, 2 H, Bn), 4.51 (d, 1 H, J_{gem} 12.0 Hz, Bn), 4.55 (d, 1 H, J_{gem} 12.0 Hz, Bn), 4.57 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.76 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.94 (d, 1 H, J_{gem} 12.0 Hz, Bn), 5.04 (d, 1 H, J_{gem} 12.0 Hz, Bn), 7.20–7.41 (m, 30 H), 7.67–7.71 (m, 5 H); ^{13}C (100 MHz, $CDCl_3$): δ 18.70, 26.37, 26.37, 26.37, 34.92, 60.39, 60.58, 66.01, 66.87, 67.13, 67.71, 71.18, 71.52, 71.74, 72.30, 73.01, 74.34, 75.29, 76.35, 76.77, 77.42, 96.41, 98.60, 126.87, 126.87, 127.03, 127.10, 127.10, 127.14, 127.14, 127.24, 127.43, 127.43, 127.48, 127.48, 127.58, 127.83, 127.83, 127.93, 127.93, 127.93, 127.96, 127.96, 127.96, 127.96, 128.05, 128.05, 128.13, 128.13, 128.31, 128.31, 128.35, 129.34, 129.40, 132.34, 132.79, 134.38, 135.41, 135.41, 135.51, 135.51, 136.78, 137.00, 137.67, 138.19, 167.10. HRMS (FAB) Calcd for $C_{66}H_{72}N_6O_{12}SiNa$ $[M+H]^+$: 1191.4874 Found: 1191.4869.

Methyl [benzyl (5-azido-4,7,8,9-tetra-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*non-2-ulpyranosid)-onate]-α-(2,6)-2,3-di*-O-*benzyl*-α-D-*galactopyranoside* (**16**).—For α form; R_f 0.34 (1:3 EtOAc–hexane); $[\alpha]_D^{23}$ +48.08° (*c* 0.010, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.71 (dd, 1 H, $J_{3ax,4}$ 11.6, J_{gem} 12.8 Hz, H-3ax), 2.44 (br, 1 H, OH), 2.73 (dd, 1 H, $J_{3eq,4}$ 4.0, J_{gem} 12.8 Hz, H-3eq), 3.29 (s, 3 H, OMe), 3.43–3.47 (m, 1 H, H-4), 3.51 (t, 1 H, $J_{5,4} = J_{5,6}$ 10.0 Hz, H-5), 3.66–3.88 (m, 9 H, H-6, H-8, H-9a, H-9b, H-2′, H-3′, H-5′, H-6′a, H-6′b), 3.95–3.97 (m, 2 H, H-7, H-4′), 4.42 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.50 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.50 (s, 2 H, H-1′, Bn), 4.57 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.61 (d, 1 H, J_{gem} 12.0 Hz, Bn), 4.60–4.63 (m, 2 H, Bn), 4.63 (d, 1 H, J_{gem} 12.0 Hz, Bn), 4.65 (d, 1 H, J_{gem} 11.2 Hz, Bn), 4.70 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.75 (d, 1 H, J_{gem} 12.0 Hz, Bn), 4.78 (d, 1 H, J_{gem} 12.0 Hz, Bn), 5.01 (d, 1 H, J_{gem} 12.4 Hz, Bn), 5.11 (d, 1 H, J_{gem} 12.4 Hz, Bn), 7.24–7.37 (m, 35 H); ^{13}C (100 MHz, $CDCl_3$): δ 36.83, 55.32, 60.96, 62.79, 66.89, 67.39, 68.03, 69.32, 71.40, 72.41, 72.51, 73.28, 73.48, 74.07, 74.41, 75.57, 76.22, 77.21, 77.52, 78.26, 98.55, 99.06, 126.89, 127.33, 127.46, 127.65, 127.65, 127.73, 127.73, 127.73, 127.73, 127.79, 127.79, 127.95, 127.95, 127.95, 128.01, 128.01, 128.01, 128.01, 128.19, 128.19, 128.28, 128.28, 128.37, 128.37, 128.37, 128.37, 128.41, 128.41, 128.41, 128.45, 128.45, 128.45, 128.50, 128.50, 128.68, 128.68, 135.04, 137.32, 137.97, 138.28, 138.41, 138.98, 167.61. HRMS (FAB) Calcd for $C_{65}H_{69}N_3O_{13}Na$ $[M+H]^+$: 1122.4727 Found: 1122.4733.

1,2,3,4-Di-O-*isopropylidene-6*-O-*[benzyl (5-azido-4,7,8,9-tera*-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*non-2-ulpyranosid)onate]-α*-D-*galactopyranoside* (**17**).—For α form; R_f 0.20 (1:4 EtOAc–hexane); $[\alpha]_D^{23}$ −46.15° (*c* 0.001, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25 (s, 3 H, CH_3), 1.30 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 1.73 (dd, 1 H, $J_{3ax,4}$ 12.4, J_{gem} 12.4 Hz, H-3ax), 2.83 (dd, 1 H, $J_{3eq,4}$ 4.4, J_{gem} 12.4 Hz, H-3eq), 3.43 (ddd, 1 H, $J_{4,3eq}$ 4.4, $J_{4,5}$ 9.6, $J_{4,3ax}$ 12.4 Hz, H-4), 3.56 (t, 1 H, $J_{5,4} = J_{5,6}$ 10.0 Hz, H-5), 3.69 (dd, 1 H, $J_{6,7}$ 1.6, $J_{6,5}$ 10.0 Hz, H-6), 3.69–3.74 (m, 2 H, H-6′a, H-6′b), 3.89–3.93 (m, 4 H, H-8, H-9a, H-9b, H-5′), 3.95 (dd, 1 H, $J_{7,6}$ 1.6, $J_{7,8}$ 6.8 Hz, H-7), 4.15 (dd, 1 H, $J_{4',3'}$ 1.2, $J_{4',5'}$ 7.6 Hz, H-4′), 4.27 (dd, 1 H, $J_{2',3'}$ 2.4, $J_{2',1'}$ 5.6 Hz, H-2′), 4.40 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.49–4.59 (m, 5 H, H-3′, Bn), 4.62 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.66 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.75 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.97 (d, 1 H, J_{gem} 12.0 Hz, Bn), 5.18 (d, 1 H, J_{gem} 12.0 Hz, Bn), 5.48 (d, 1 H, $J_{1',2'}$ 5.2 Hz, H-1′), 7.24–7.38 (m, 25 H); ^{13}C (100 MHz, $CDCl_3$): δ 24.55, 24.92, 26.01, 26.11, 37.02, 61.02, 62.8, 66.56, 67.21, 69.86, 70.56, 70.67, 71.26, 72.54, 73.31, 74.29, 76.43, 76.79, 76.79, 77.29, 78.68, 94.25, 99.01, 108.59, 109.11, 127.27, 127.40, 127.66, 127.78, 127.78, 127.80, 127.80, 127.89, 127.96, 127.96, 128.00, 128.00, 128.17, 128.17, 128.25, 128.25, 128.30, 128.30, 128.34, 128.34, 128.43, 128.43, 128.55, 128.68, 128.68, 135.16, 137.38, 138.10, 138.59, 139.17, 167.52. HRMS (FAB) Calcd for $C_{56}H_{63}N_3O_{13}Na$ $[M+H]^+$: 1008.4258 Found: 1008.4284.

Methyl {methyl 5-azido-4,7,8-tri-O-*benzoyl-9*-O-*[benzyl (5-azido-4,7,8,9-tetra*-O-*benzyl-3,5-dideoxy*-D-glycero-D-galacto-*non-2-ulpyra-nosid)onate]-3,5-dideoxy*-D-glycero-α-D-galacto-*non-2-ulpyranosid}onate* (**18**).—For α form; R_f 0.23 (1:3 EtOAc–hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.51–1.57 (m, 1 H, H-3ax), 1.77 (dd, 1 H, $J_{3'ax,4'}$ 11.6, J_{gem} 12.8 Hz, H-3′ax), 2.59 (dd, 1 H, $J_{3eq,4}$ 4.0, J_{gem} 12.8 Hz, H-3eq), 2.66 (dd, 1 H, $J_{3'eq,4'}$ 5.2, J_{gem} 12.8 Hz, H-3′eq), 3.05 (s, 3 H, OMe), 3.31–3.39 (m, 2 H, H-4, H-9a), 3.41 (t, 1 H, $J_{5,4} = J_{5,6}$ 10.0 Hz, H-5), 3.52 (t, 1 H, $J_{5',4'} = J_{5',6'}$ 10.0 Hz, H-5′), 3.54–3.58 (m, 2 H, H-6, H-9b), 3.62–3.65 (m, 1 H, H-8), 3.76 (s, 3 H, CO_2Me), 3.83 (dd, 1 H, $J_{7,6}$ 1.6, $J_{7,8}$ 7.2 Hz, H-7), 3.87 (dd, 1 H, $J_{6',7'}$ 0.8, $J_{6',5'}$ 10.0 Hz, H-6′), 4.02 (dd, 1 H, $J_{9'a',8'}$ 3.6, J_{gem} 11.2 Hz, H-9′a), 4.08 (dd, 1 H, $J_{9'b,8'}$ 3.2, J_{gem} 11.2 Hz, H-9′b), 4.24 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.33 (m, 4 H, Bn), 4.37 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.48 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.60 (d, 1 H, J_{gem} 11.6 Hz, Bn), 4.66 (d, 1 H, J_{gem} 12.0 Hz, Bn), 5.02 (d, 1 H, J_{gem} 12.0 Hz, Bn), 5.46 (ddd, 1 H, $J_{4',3'eq}$ 5.2, $J_{4',5'}$ 10.0, $J_{4',3'ax}$ 11.6 Hz, H-4′), 5.68 (ddd, 1 H, $J_{8',9'b}$ 3.2, $J_{8',9'a}$ 3.6, $J_{8',7'}$ 8.8 Hz, H-8′), 6.09 (dd, 1 H, $J_{7',6'}$ 0.8, $J_{7',8'}$ 8.8 Hz, H-7′), 7.13–7.43 (m, 31 H), 7.47–7.57 (m, 3 H), 7.97–8.00 (m, 4 H), 8.08–8.10 (m, 2 H); ^{13}C (100 MHz, $CDCl_3$): δ 36.19, 36.93, 51.18, 52.59, 60.67, 60.81, 62.10, 67.10, 68.75, 68.96, 70.18, 70.22, 70.98, 71.53, 72.23, 73.19, 73.99, 74.05, 76.19, 77.21, 78.06, 98.70, 98.81, 127.23, 127.47, 127.53, 127.72, 127.72, 127.72, 127.72, 127.72, 127.72, 127.72, 127.72, 127.80, 127.80, 127.86, 127.86, 128.10, 128.10,

128.23, 128.23, 128.29, 128.29, 128.29, 128.29, 128.29, 128.36, 128.36, 128.45, 128.45, 128.50, 128.50, 128.50, 128.58, 128.58, 128.58, 129.31, 129.40, 129.58, 129.76, 129.76, 130.19, 130.19 133.37, 133.37, 135.04, 137.33, 138.25, 138.46, 139.04, 165.16, 165.29, 165.46, 167.27, 167.36. HRMS (FAB) Calcd for $C_{76}H_{75}N_6O_{18}Na$ $[M+H]^+$: 1381.4955 Found: 1381.4922.

Methyl [benzyl (5-azido-4,7,8,9-tetra-O-*benzyl-3,5-dideoxy*-D-glycero-α-D-galacto-*non*-2-*ulpyranosid)-onate]*-α-*(2,3)*-2,6-*di*-O-*benzyl*-α-D-*galactopyranoside* (**19**).—For α form; R_f 0.2 (1:3 EtOAc–hexane); $[\alpha]_D^{23}$ +3.03° (*c* 3.3×10^{-3}, EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.81 (dd, 1 H, $J_{3ax,4}$ 11.6, J_{gem} 13.6 Hz, H-3ax), 2.71 (dd, 1 H, $J_{3eq,4}$ 4.6, J_{gem} 13.6 Hz, H-3eq), 3.08 (d, 1 H, *J* 2.4 Hz, OH), 3.27 (s, 3 H, OMe), 3.35 (dd, 1 H, $J_{6'a,5'}$ 4.4, $J_{6'a,6'b}$ 10.4 Hz, H-6′a), 3.41 (dd, 1 H, $J_{6'b,5'}$ 5.6, $J_{6'b,6'a}$ 10.4 Hz, H-6′b), 3.49 (m, 1 H, H-5′), 3.62 (t, $J_{5,4}=J_{5,6}$ 9.6 Hz, H-5), 3.67 (dd, 1 H, $J_{9a,8}$ 4.8, J_{gem} 10.4 Hz, H-9a), 3.73 (dd, 1 H, *J* 3.6, *J* 10.0 Hz, H-3′), 3.88–3.93 (m, 3 H, H-6, H-9a, H-4′), 4.04–4.13 (m, 4 H, H-4, H-7, H-8, H-2′), 4.37 (d, 1 H, J_{gem} 12.0 Hz, Bn), 4.36–4.66 (m, 7 H, H-1′, Bn), 4.43 (d, J_{gem} 12.0 Hz, Bn), 4.49 (d, J_{gem} 11.2 Hz, Bn), 4.53 (d, J_{gem} 11.2 Hz, Bn), 4.65 (d, J_{gem} 12.0 Hz, Bn), 4.74 (d, J_{gem} 11.6 Hz, Bn), 5.07 (d, J_{gem} 12.0 Hz, Bn), 5.17 (d, J_{gem} 12.0 Hz, Bn), 7.22–7.38 (m, 35 H); ^{13}C (100 MHz, $CDCl_3$): δ 36.03, 55.19, 61.37, 67.77, 67.93, 69.38, 70.38, 70.55, 71.68, 72.41, 72.98, 73.25, 73.54, 73.99, 74.21, 74.84, 75.90, 76.29, 77.20, 79.80, 98.65, 99.74, 127.33, 127.33, 127.41, 127.41, 127.41, 127.47, 127.47, 127.47, 127.63, 127.63, 127.77, 127.77, 127.77, 127.88, 127.88, 128.13, 128.13, 128.13, 128.26, 128.26, 128.26, 128.26, 128.26, 128.26, 128.35, 128.35, 128.35, 128.35, 128.42, 128.42, 128.42, 128.55, 128.55, 128.77, 128.77, 134.97, 137.78, 137.79, 137.92, 138.44, 138.55, 138.95, 167.48. HRMS (FAB) Calcd for $C_{65}H_{69}N_3O_{13}Na$ $[M+H]^+$: 1122.4727 Found: 1122.4704.

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