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Stereoselective Synthesis of the Trisaccharide Moiety of Ganglioside HLG-2

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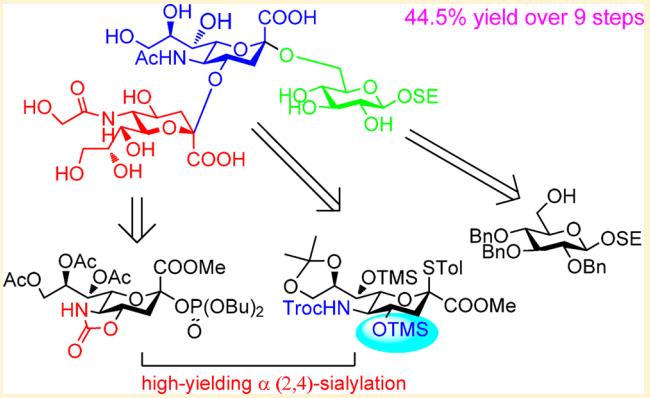
Supporting Information

ABSTRACT: The glycan portion of ganglioside HLG-2, which was identified in the extracts of the sea cucumber *Holothuria leucospilota*, was synthesized in a highly efficient and stereoselective manner. The unusual sequence of the trisaccharide moiety, α-*N*-glycolylsialyl-(2,4)-α-*N*-acetylsialyl-(2,6)-glucoside, was assembled by stereoselective coupling of a 5-*N*,4-*O*-carbonyl-protected sialyl phosphate donor, a *N*-2,2,2-trichloroethoxycarbonyl (Troc)-protected sialyl acceptor, and a (trimethylsilyl)ethyl-β-glucosyl acceptor in high yield. The synthesis featured the high-yielding construction of two α-sialyl linkages.



Echinodermatous gangliosides, one kind of sialic acid-containing glycosphingolipids, have recently attracted much attention because of their specific structures and their neuritogenic activity,[1] which is similar to that of mammalian gangliosides. However, their structure−activity relationships have not been investigated yet, mainly because of the lack of homogeneous gangliosides.

Ganglioside HLG-2, which was first isolated from the sea cucumber *Holothuria leucospilota* by Higuchi and co-workers,[2] showed neuritogenic activity toward the rat pheochromocytoma cell line PC-12 in the presence of nerve growth factor.[1] What attracts us more is the fact that the glycan portion of HLG-2 contains two unique structural features shown in Figure 1. An α(2,4) linkage between sialic acids is only found in rare

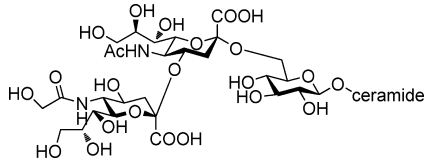


Figure 1. Structure of the novel disialyl ganglioside HLG-**2**.

natural products such as the HLG series gangliosides[2] and the ganglioside HPG series.[3−5] It is known that a sialylation reaction in high yield and with complete α stereoselectivity is a great challenge[6] because of the presence of a destabilizing electron-withdrawing carboxylic group together with a tertiary anomeric center and the lack of a participating group. In addition, the low nucleophilic activity of 4-OH of the sialic acid as an acceptor makes it harder to build the α(2,4) linkage between sialic acids.[7] As a result, there are few methods to date to synthesize such a linkage efficiently. The other remarkable characteristic of the glycan moiety of HLG-2 is the tandem of *N*-glycolylsialic acid (NeuGc) and *N*-acetylsialic acid (NeuAc). All of these features lead to difficulties in synthesizing the glycan portion of HLG-2.

Previously, Kiso and co-workers reported the synthesis of the glycan moiety of HLG-2 in 4.7% overall yield in 14 steps using a novel 1,5-lactamized sialyl acceptor[7] with the fixed boat form, which could result in the increased activity of the C4-hydroxyl group. In that process, the α/β ratio was 66/18 for the formation of the (2,4) linkage between sialic acids. By the same strategy, they completed the total synthesis of the ganglioside HLG-2.[8]

Our group has been interested in the development of new methodologies for chemical synthesis of sialosides[9,10] and their synthetic applications. In fact, in the past decades, various sialylation methods have been developed.[6,9−12] Among all of these sialyl donors, an *N*-2,2,2-trichloroethoxycarbonyl (Troc)-protected thiophenyl sialyl donor[12,13] showed high α stereoselectivity and good yield toward glycosylation promoted by NIS/TfOH, especially when the primary alcohol of glucose was used as the acceptor.[14] Recently, Wong and co-workers identified 5-*N*,4-*O*-carbonyl-protected sialyl phosphates[15,16] to be more efficient donors than the corresponding 5-*N*,4-*O*-carbonyl-protected thiophenyl donors[17−22] developed earlier. In this work, we tried to develop a more efficient strategy to assemble the ganglioside HLG-2 glycan moiety (**1**).

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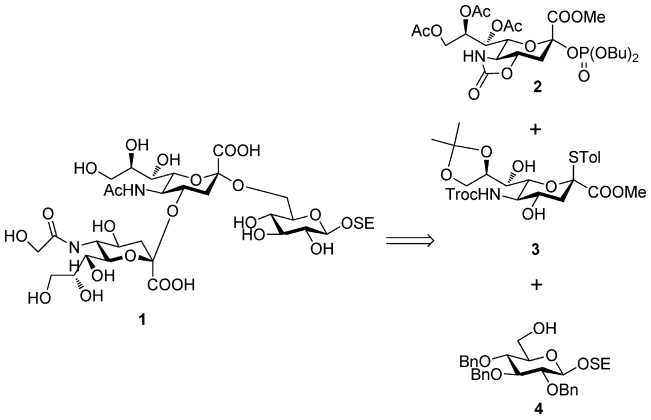
For this purpose, 5-*N*,4-*O*-carbonyl-protected sialyl phosphate donor **2**, Troc-protected sialyl building block **3**, and (trimethylsilyl)ethyl (SE)-protected *β*-glucosyl acceptor **4**[23] were chosen as building blocks (Scheme 1). The reason why we

Scheme 1. Retrosynthetic Analysis of the Trisaccharide Portion of HLG-2

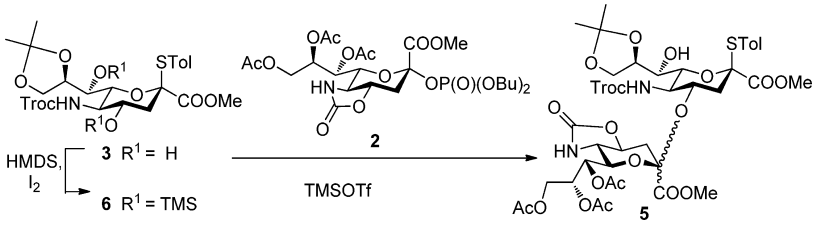


chose the 5-*N*,4-*O*-carbonyl-protected sialyl phosphate donor and the *N*-Troc-protected thiophenylsialyl block is that they both have proved to be good *α*-sialylation donors, allowing us to construct the glycan structure more efficiently. In addition, the Troc and oxazolidinone protecting groups could be orthogonally removed under certain conditions to afford the amino functionality, which could be further transformed into the corresponding *N*-glycolylsialic acid and *N*-acetylsialic acid, respectively.

We tried to couple sialyl phosphate donor **2**[15,16] with *N*-Troc-protected acceptor **3** to produce disaccharide **5**. However, the glycosylation reaction showed a poor stereoselective ratio (*α*:*β* = 1:1.26) even though it gave a high yield (96%) (Table 1, entry 1). In this process, we did not obtain the disaccharide with the (2,7) linkage because of the low reactivity of the 7-OH group. The configuration of the disaccharide was examined by NMR spectroscopy, and the newly formed *α*-glycosidic bond was confirmed by a $^{3}J_{C1-H3ax}$ coupling constant of 6.0 Hz.[6,24] Other solvents and additives were tried, but unfortunately, the results were not improved (Table 1). We hypothesized that the poor stereoselectivity may come from the low nucleophilic reactivity of the C4-hydroxyl group in the sialyl acceptor. On the other hand, the trimethylsilyloxy moiety is very attractive in the chemistry of protecting groups because of its selective introduction and selective removal under mild conditions. Actually, trimethylsilyl (TMS) ethers were previously used as acceptors in some glycosylation reactions.[25−27] In our case, we presumed that trimethylsilylation of the C4-hydroxyl group could improve its nucleophilic reactivity as an acceptor, which might lead to good stereoselectivity. Therefore, the sialyl block **6** was prepared quantitatively from acceptor **3** by treatment with 0.55 equiv of hexamethyldisilazane (HMDS) and a catalytic amount (up to 2 mol %) of iodine under solvent-free conditions at room temperature.[28] Although compound **6** seems not to be a typical acceptor, it may be used as a glycosyl acceptor because the TMS protecting group is acid-sensitive and may be easily removed in situ under glycosylation conditions such as promoted by trimethylsilyl trifluoromethanesulfonate (TMSOTf). As expected, the glycosylation reaction of sialyl phosphate donor **2** and sialyl acceptor **6** yielded the corresponding sialyl-*α*(2,4)sialyl sequence **5** in a highly stereoselective manner. However, when the reaction was quenched with triethylamine, the C7-hydroxyl group in disaccharide **5** was trimethylsilylated (up to 50% yield) because the promoter TMSOTf can be used as the reagent for silylation in an organic alkali environment.[29] To avoid this problem, saturated $NaHCO_3$ aqueous solution was used to quench the reaction; finally, disaccharide **5** was successfully isolated in 95% yield as the pure *α* isomer (Table 1, entry 8).

With the success of the efficient construction of disaccharide **5**, we tried to directly conjugate **5** with the glucosyl acceptor **4**. However, the result did not meet our expectation, as the trisaccharide **7** was collected in only 36% isolated yield (Scheme 2), and all of our attempts to improve the yield failed. We supposed that the low yield may be related to the 8,9-*O*-isopropylidene group. Therefore, the 8,9-*O*-isopropyli-

Table 1. Coupling Reaction of Sialyl Phosphate Donor 2 and *N*-Troc-Protected Sialyl Acceptor 3 or 6



entry[a]	acceptor	additive	solvent[b]	*α*/*β* ratio[c]	yield (%)[d]
1	**3**	–	CH_2Cl_2	1:1.26	96
2	**3**	–	CH_2Cl_2/MeCN	1.75:1	82
3	**3**	–	CH_2Cl_2/Et_2O	3.96:1	34[e]
4	**3**	–	CH_2Cl_2/THF	2.25:1	<10
5	**3**	TTBP[f]	CH_2Cl_2	<1:10	<30
6	**3**	Ph_2SO	CH_2Cl_2	*β*	19
7	**3**	Me_2SO	CH_2Cl_2	–	–[g]
8	**6**	–	CH_2Cl_2	*α*	95

[a]All of the glycosylation reactions were conducted at −72 °C. [b]The ratio of all solvent mixtures was 3:2. [c]Anomeric ratios were determined by ^{1}H NMR analysis of the crude reaction mixtures. [d]Isolated yields. [e]Most of the donor was transformed into glycal. [f]TTBP = 2,4,6-tri-*tert*-butylpyridine. [g]No reaction.

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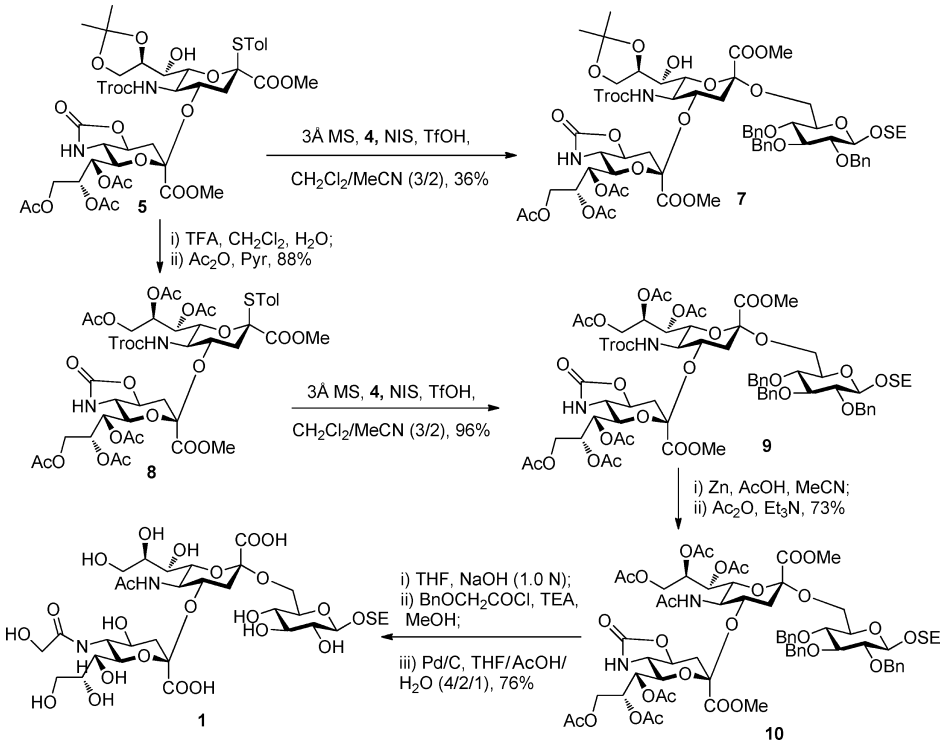
Scheme 2. Synthesis of the Trisaccharide Portion of HLG-2



dene functionality in **5** was manipulated to give 7,8,9-tri-*O*-acetyl-protected disaccharide **8** in the hope that the glycosylation efficiency would be improved. Fortunately, the glycosyl coupling of disaccharide **8** with monosaccharide **4** afforded trisaccharide **9** in 96% isolated yield with only the α isomer. Finally, as shown in Scheme 2, after replacement of the Troc group in trisaccharide **9** by an acetyl group,[12] the oxazolidinone ring was removed to expose the amino group, which was then functionalized with a glycolyl group. Full deprotection of the benzyl groups then yielded the target molecule ganglioside HLG-2 glycan structure **1** smoothly. The structure of compound **1** was confirmed by NMR spectral analyses.

In conclusion, we have completed the efficient synthesis of the glycan moiety of ganglioside HLG-2 in 44.5% overall yield in nine steps by using a 5-*N*,4-*O*-carbonyl-protected sialyl phosphate donor, an *N*-Troc-protected sialyl block, and a (trimethylsilyl)ethyl-β-glucosyl acceptor. The two key glycosylation reactions were performed in high yields with excellent α-stereoselectivity. It is noteworthy that in this process, trimethylsilylation of the hydroxyl group was applied to improve the reactivity of the sialyl acceptor, enhancing the yield and stereoselectivity of the glycoslylation reaction. By means of the same strategy, some other important natural products with similar structures such as ganglioside HPG-7 and the lipopolysaccharide of *Legionella pneumophila* serogroup 1[30] could be synthesized to study their structure−activity relationships.

EXPERIMENTAL SECTION

General. Chemicals were purchased as reagent grade and used without further purification, except as otherwise noted. Dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN), and pyridine were distilled over calcium hydride (CaH_2). Methanol was distilled from magnesium. Pulverized 4 Å and 3 Å molecular sieves (MS) for glycosylation were activated by heating at 400 °C for 6 h. Reactions were monitored by thin-layer chromatography (TLC) analysis, which was visualized by UV light (254 nm) and acidic ceric ammonium molybdate. Solvents were evaporated under reduced pressure and below 40 °C (water bath). Column chromatography was performed on silica gel or RP-18. ^{1}H NMR and ^{13}C NMR spectra were recorded on a 400 or 600 MHz spectrometer at room temperature. Chemical shifts (in ppm) were calibrated with the solvent residual peak. $J_{C1-H3ax}$ values were measured from the selective-proton-decoupling ^{13}C NMR spectrum recorded using a 600 MHz spectrometer. HRMS (ESI) data were obtained using a Fourier transform ion cyclotron resonance mass spectrometer.

Dibutyl (Methyl 5-Amino-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galactonon-2-ulopyranosyl)onate) Phosphate (2). A mixture of methyl (*p*-tolyl 5-amino-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-2-thio-D-glycero-α-D-galactonon-2-ulopyranoside)onate[19] (539.6 mg, 1.0 mmol), dibutyl phosphate (0.6 mL, 3.0 mmol), and activated pulverized 4 Å MS in CH_2Cl_2 (18 mL) was stirred at room temperature for 3 h. After the solution was cooled to 0 °C, NIS (445.0 mg, 2.0 mmol) and TfOH (27 μL, 0.3 mmol) were added. The reaction mixture was stirred for 6 h, and then the reaction was quenched with 10% $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ solution. The reaction mixture was filtered through a pad of Celite, and the filtrate was diluted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel, eluting with acetone/petroleum ether (1:4) to give product **2** (598.9 mg, 96%) as a yellowish syrup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.42 (s, 1H), 5.35 (ddd, J = 9.6, 3.2, 2.4 Hz, 1H), 5.13 (dd, J = 9.6, 1.2 Hz, 1H), 4.45 (dd, J = 10.0, 1.6 Hz, 1H), 4.36 (dd, J = 12.4, 2.0 Hz, 1H), 4.29 (dd, J = 12.4, 3.6 Hz, 1H), 4.15−4.00 (m, 5H), 3.82 (s, 3H), 3.24 (td, J = 10.4, 1.2 Hz, 1H), 2.92 (dd, J = 12.0, 4.0 Hz, 1H), 2.65 (t, J = 12.4 Hz, 1H), 2.19 (s, 3H), 2.15 (s, 3H), 2.06 (s, 3H), 1.72−1.63 (m, 4H), 1.47−1.37 (m, 4H), 0.95 (td, J = 7.2, 2.0 Hz, 6H). The spectroscopic data coincide with those in the previous report.[15]

Methyl (*p*-Tolyl 8,9-*O*-Isopropylidene-3,5-dideoxy-2-thio-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-β-D-galactonon-2-ulopyranoside)onate (3). A stirred solution of methyl (*p*-

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tolyl 5-acetamino-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galactonon-2-ulopyranoside)onate[31] (1.51 g, 2.53 mmol) in dry methanol (20 mL) was treated with methanesulfonic acid (0.51 mL) at room temperature, and the mixture was refluxed under an argon atmosphere for 10 h. After the reaction mixture was cooled to room temperature, the reaction was quenched with triethylamine (TEA), and the mixture was concentrated in vacuo. The residue was dissolved in MeOH (12 mL), and TrocCl (1.7 mL, 12.65 mmol) and TEA (0.71 mL, 5.06 mmol) were added. The reaction mixture was stirred for 3 h and concentrated under reduced pressure. The residue was dissolved in EtOAc, and the solution was washed with water. The aqueous layer was extracted twice with EtOAc, and the combined organic layers were washed successively with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was redissolved in CH_3CN (10 mL), and 2,2-dimethoxypropane (DMP, 0.3 mL, 5.06 mmol) and camphorsulfonic acid (CSA, 26.7 mg, 0.25 mmol) were added. The reaction mixture was stirred for 2 h, and then the reaction was quenched with TEA. The mixture was concentrated in vacuo, and the residue was purified by column chromatography on silica gel eluting with ethyl acetate/petroleum ether (2:3) to yield **3** as a white foam (459.4 mg, 76%). $[\alpha]_D^{31}$ −160.8° (*c* 3.14, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.0 Hz, 2H), 7.08 (d, *J* = 8.0 Hz, 2H), 5.52 (d, *J* = 8.8 Hz, 1H), 4.92 (d, *J* = 12.0 Hz, 1H), 4.63 (d, *J* = 12.0 Hz, 1H), 4.39 (d, *J* = 10.4 Hz, 1H), 4.19−4.10 (m, 2H), 3.98 (d, *J* = 5.2 Hz, 2H), 3.75 (q, *J* = 10.0 Hz, 1H), 3.67 (d, *J* = 7.6 Hz, 1H), 3.51 (s, 3H), 2.78 (dd, *J* = 13.6, 4.8 Hz, 1H), 2.33 (s, 3H), 2.09 (dd, *J* = 13.2, 12.0 Hz, 1H), 1.41 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 155.9, 140.1, 136.3, 129.7, 126.0, 109.2, 95.5, 90.1, 74.9, 74.7, 72.5, 70.6, 67.6, 67.3, 55.0, 52.6, 41.1, 27.0, 25.6, 21.5; HMRS (ESI) calcd for $C_{23}H_{34}Cl_3N_2O_9S$ $[M + NH_4]^+$ 619.1045, found 619.1039.

2-Trimethylsilylethyl 2,3,4-Tri-*O*-benzyl-β-D-glucopyranoside (4). This compound was prepared according to the known procedure.[23] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.26 (m, 15H), 4.95 (d, *J* = 11.2 Hz, 1H), 4.93 (d, *J* = 10.8 Hz, 1H), 4.86 (d, *J* = 10.8 Hz, 1H), 4.81 (d, *J* = 10.8 Hz, 1H), 4.73 (d, *J* = 10.8 Hz, 1H), 4.64 (d, *J* = 10.8 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.02−3.95 (m, 1H), 3.87 (ddd, *J* = 11.8, 5.6, 2.7 Hz, 1H), 3.73−3.54 (m, 4H), 3.43−3.35 (m, 2H), 1.92 (dd, *J* = 7.6, 6.0 Hz, 1H), 1.08−0.99 (m, 2H), 0.03 (s, 9H). The spectroscopic data coincide with those in the previous report.[23]

Methyl (*p*-Tolyl 8,9-*O*-Isopropylidene-4,7-di-*O*-trimethylsilyl-3,5-dideoxy-2-thio-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-β-D-galactonon-2-ulopyranoside)onate (6). To compound **3** (602.9 mg, 1.0 mmol) was added iodine (5.1 mg, 0.02 mmol) followed by hexamethyldisilazane (HMDS) (0.089 g, 0.55 mmol). The mixture was stirred at room temperature until the starting material was completely consumed. The crude reaction mixture was dissolved in *tert*-butyl methyl ether (TBME) (4 mL) and stirred with the addition of finely powered $Na_2S_2O_3$ until the disappearance of iodine was achieved. The solids were filtered off, and the solvent was evaporated under the reduced pressure, yielding **6** as a light-yellow foam quantitatively. $[\alpha]_D^{31}$ −123.4° (*c* 3.40, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 2H), 4.96 (d, *J* = 8.0 Hz, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.66 (t, *J* = 12.4 Hz, 2H), 4.44 (td, *J* = 10.4, 4.8 Hz, 1H), 4.23 (s, 1H), 3.87−3.85 (m, 1H), 3.77 (t, *J* = 7.8 Hz, 1H), 3.61 (s, 3H), 3.52 (t, *J* = 7.6 Hz, 1H), 3.28 (q, *J* = 10.0 Hz, 1H), 2.54 (dd, *J* = 14.0, 4.4 Hz, 1H), 2.32 (s, 3H), 1.95 (dd, *J* = 14.0, 11.2 Hz, 1H), 1.41 (s, 3H), 1.25 (s, 3H), 0.14 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 153.8, 140.1, 136.3, 129.7, 126.2, 107.5, 95.4, 89.3, 74.8, 73.0, 70.9, 67.4, 64.9, 55.6, 52.4, 41.5, 26.4, 24.7, 21.4, 1.1, 0.3; HMRS (ESI) calcd for $C_{29}H_{50}Cl_3N_2O_9SSi_2$ $[M + NH_4]^+$ 763.1836, found 763.1843.

Methyl (*p*-Tolyl 8,9-*O*-Isopropylidene-3,5-dideoxy-4-(methyl 7,8,9-Tri-*O*-acetyl-5-amino-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galactonon-2-ulopyranosylonate)-2-thio-5-(2,2,2-trichloroethoxy Carbonylamino)-D-glycero-β-D-galactonon-2-ulopyranoside)onate (5). A mixture of 5-*N*,4-*O*-carbonyl-protected sialyl phosphate donor **2** (96.6 mg, 0.154 mmol), Troc-protected sialyl block **6** (73.8 mg, 0.098 mmol), and activated pulverized 4 Å MS (1.01 g) in CH_2Cl_2 (10 mL) was stirred at room temperature for 3 h. Then trimethylsilyl trifluoromethanesulfonate (TMSOTf, 28.3 μL, 0.154 mmol) was added at −72 °C, and the solution was continuously stirred at the same temperature for 3 h. The reaction was quenched with saturated $NaHCO_3$ solution, and the mixture was filtered through a pad of Celite. After the aqueous layer was extracted with two portions of ethyl acetate, the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography on silica gel (ethyl acetate/petroleum ether = 1:3 → 1:2) to give **5** (95.6 mg, 95%) as a white foam. $[\alpha]_D^{32}$ −87.5° (*c* 2.24, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 2H), 6.33 (d, *J* = 8.4 Hz, 1H), 5.54 (d, *J* = 6.4 Hz, 1H), 5.49 (s, 1H), 5.25 (dd, *J* = 8.8, 2.8 Hz, 1H), 5.08 (d, *J* = 12.0 Hz, 1H), 4.58 (d, *J* = 12.4 Hz, 1H), 4.37−4.24 (m, 4H), 4.10 (dd, *J* = 9.6, 2.8 Hz, 1H), 4.07−4.02 (m, 1H), 4.00−3.96 (m, 1H), 3.94 (s, 3H), 3.90−3.87 (m, 1H), 3.83−3.80 (m, 2H), 3.73 (q, *J* = 9.6 Hz, 1H), 3.46 (s, 3H), 3.40 (d, *J* = 6.4 Hz, 1H), 3.19 (t, *J* = 10.4 Hz, 1H), 3.00 (dd, *J* = 12.0, 3.2 Hz, 1H), 2.46 (dd, *J* = 14.0, 4.0 Hz, 1H), 2.35 (s, 3H), 2.25 (s, 3H), 2.24 (s, 3H), 2.10−2.01 (m, 5H), 1.42 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 170.9, 170.8, 168.4, 167.6 (d, $J_{C1-H3ax}$ = 6.0 Hz), 159.1, 156.2, 140.2, 136.6, 129.6, 125.8, 108.8, 100.5, 95.7, 89.7, 75.2, 74.63, 74.57, 73.7, 71.1, 69.9, 69.7, 67.3, 66.6, 62.2, 57.8, 53.6, 52.9, 52.5, 39.7, 37.5, 26.9, 25.7, 21.5, 21.4, 21.2, 20.8; HMRS (ESI) calcd for $C_{40}H_{51}Cl_3N_2NaO_{20}S$ $[M + Na]^+$ 1039.1714, found 1039.1729.

Methyl (8,9-*O*-Isopropylidene-3,5-dideoxy-4-(methyl 7,8,9-Tri-*O*-acetyl-5-amino-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galactonon-2-ulopyranosylonate)-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-α-D-galactonon-2-ulopyranoside)-onate-(2→6)-(trimethylsilyl)ethyl 2,3,4-Tri-*O*-benzyl-β-D-glucopyranoside (7). A mixture of disaccharide **5** (234.2 mg, 0.23 mmol), compound **4** (191.0 mg, 0.35 mmol), and activated pulverized 3 Å MS (2.60 g) in CH_2Cl_2/CH_3CN (3:2, 25 mL) was stirred at room temperature for 30 min. NIS (164.0 mg, 0.69 mmol) and TfOH (6.2 μL, 0.069 mmol) were added at −72 °C, and the solution was continuously stirred at the same temperature for 3 h. The reaction was quenched with saturated $NaHCO_3$ solution and 10% $Na_2S_2O_3$ solution, and the mixture was filtered through a pad of Celite. After the aqueous layer was extracted with two portions of ethyl acetate, the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate/petroleum ether = 1:3) to give compound **7** (119.7 mg, 36%) as a colorless syrup. $[\alpha]_D^{24}$ −16.2° (*c* 0.29, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35−7.26 (m, 15H), 6.06 (d, *J* = 7.2 Hz, 1H), 5.42 (d, *J* = 8.4 Hz, 1H), 5.39 (s, 1H), 5.18 (d, *J* = 9.0 Hz, 1H), 5.04 (d, *J* = 12.6 Hz, 1H), 4.94 (d, *J* = 10.8 Hz, 1H), 4.91 (d, *J* = 10.8 Hz, 1H), 4.81 (d, *J* = 10.8 Hz, 1H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.72−4.68 (m, 2H), 4.59 (d, *J* = 12.0, 1H), 4.34 (d, *J* = 7.8 Hz, 1H), 4.28 (dd, *J* = 12.6, 3.6 Hz, 1H), 4.21−4.19 (m, 1H), 4.16 (d, *J* = 12.6 Hz, 1H), 4.12 (d, *J* = 10.2 Hz, 1H), 4.04−4.02 (m, 1H), 4.00−3.94 (m, 3H), 3.88 (s, 3H), 3.86−3.84 (m, 1H), 3.81−3.79 (m, 2H), 3.78 (s, 3H), 3.76−3.74 (m, 1H), 3.67 (t, *J* = 9.6 Hz, 1H), 3.63−3.56 (m, 3H), 3.48−3.46 (m, 2H), 3.39−3.34 (m, 2H), 3.13 (t, *J* = 10.8 Hz, 1H), 2.97 (dd, *J* = 12.0, 3.6 Hz, 1H), 2.46 (dd, *J* = 12.6, 4.2 Hz, 1H), 2.21 (s, 3H), 2.20 (s, 3H), 2.06 (s, 3H), 2.03 (t, *J* = 12.6 Hz, 1H), 1.74 (t, *J* = 12.6 Hz, 1H), 1.36 (s, 3H), 1.34 (s, 3H), 1.02 (t, *J* = 9.0 Hz, 2H), 0.03 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.4, 170.7, 170.6, 168.2, 167.5, 159.1, 156.5, 138.7, 138.3, 128.6, 128.52, 128.48, 128.3, 128.2, 128.0, 127.9, 127.8, 108.7, 103.3, 100.2, 99.0, 95.8, 84.9, 82,4, 75.9, 75.1, 74.9, 74.7, 74.44, 74.40, 74.0, 71.4, 69.4, 69.1, 67.7, 66.8, 66.5, 63.3, 62.1, 57.8, 53.6, 52.8, 52.3, 38.8, 37.7, 27.0, 26.0, 21.4, 21.2, 20.8, 18.7, 0.1, −1.3; HMRS (ESI) calcd for $C_{65}H_{85}Cl_3N_2NaO_{26}Si$ $[M + Na]^+$ 1465.4118, found 1465.4129.

Methyl (*p*-Tolyl 7,8,9-Tri-*O*-acetyl-3,5-dideoxy-4-(methyl 7,8,9-tri-*O*-acetyl-5-amino-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galactonon-2-ulopyranosylonate)-2-thio-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-β-D-galactonon-2-ulopyranoside)onate (8). A stirred solution of **5** (101.8 mg, 0.10 mmol) in CH_2Cl_2 (2 mL) was treated with trifluoroacetic acid (20 μL) and two drops of water at room temperature. After the mixture was stirred for about 3 h, the reaction was quenched with excess triethylamine, and the mixture was concentrated under reduced pressure. The concentrate was dissolved in pyridine (2 mL), and Ac_2O

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(2 mL) was added. The mixture was stirred under room temperature for 8 h and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/petroleum ether = 1:3) to give **8** (97.2 mg, 88% over two steps) as a white foam. $[\alpha]$ −79.9° (*c* 4.13, $CHCl_3$); ^1H NMR (400 MHz, DMSO-d_6) δ 7.71 (d, *J* = 10.0 Hz, 1H), 7.59 (s, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 7.19 (d, *J* = 8.0 Hz, 2H), 5.35 (s, 1H), 5.26 (ddd, *J* = 7.6, 5.2, 2.8 Hz, 1H), 5.14 (d, *J* = 7.6 Hz, 1H), 4.83 (d, *J* = 12.4 Hz, 1H), 4.70−4.65 (m, 2H), 4.58 (dd, *J* = 10.4, 1.6 Hz, 1H), 4.44 (td, *J* = 10.4, 4.8 Hz, 1H), 4.38 (dd, *J* = 12.4, 2.4 Hz, 1H), 4.29−4.22 (m, 2H), 4.06−3.99 (m, 2H), 3.89 (dd, *J* = 12.0, 9.2 Hz, 1H), 3.67 (s, 3H), 3.56 (s, 3H), 3.44 (q, *J* = 10.1 Hz, 1H), 3.16 (t, *J* = 10.4 Hz, 1H), 2.66−2.63 (m, 2H), 2.37 (t, *J* = 12.4 Hz, 1H), 2.30 (s, 3H), 2.22 (s, 3H), 2.08 (s, 3H), 2.01 (s, 6H), 1.97 (s, 4H), 1.93 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.22, 170.15, 170.0, 169.7, 169.4, 169.3, 167.9, 167.3, 158.7, 154.0, 139.7, 135.5, 129.8, 125.1, 100.7, 95.9, 88.4, 76.1, 73.5, 73.2, 72.5, 72.3, 69.8, 68.8, 68.7, 68.4, 62.0, 61.2, 56.6, 53.1, 52.4, 51.2, 33.5, 20.8, 20.73, 20.70, 20.50, 20.48, 20.4; HMRS (ESI) calcd for $C_{43}H_{57}Cl_3N_3O_{23}S$ $[M + NH_4]^+$ 1120.2164, found 1120.2166.

Methyl (7,8,9-Tri-*O*-acetyl-3,5-dideoxy-4-(methyl 7,8,9-Tri-*O*-acetyl-5-amino-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galactonon-2-ulopyranosylonate)-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-α-D-galactonon-2-ulopyranoside)onate-(2→6)-(trimethylsilyl)ethyl 2,3,4-Tri-*O*-benzyl-β-D-glucopyranoside (9). A mixture of disaccharide **8** (255.0 mg, 0.23 mmol), compound **4** (191.0 mg, 0.35 mmol), and activated pulverized 3 Å MS (2.60 g) in CH_2Cl_2/CH_3CN (3:2, 25 mL) was stirred at room temperature for 30 min. NIS (164.0 mg, 0.69 mmol) and TfOH (6.2 μL, 0.069 mmol) were added at −72 °C, and the solution was continuously stirred at the same temperature for 3 h. The reaction was quenched with saturated $NaHCO_3$ solution and 10% $Na_2S_2O_3$ solution, and the mixture was filtered through a pad of Celite. After the aqueous layer was extracted with two portions of ethyl acetate, the combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel (ethyl acetate/petroleum ether = 1:4 → 1:3) to give **9** (339.0 mg, 96%) as a colorless syrup. $[\alpha]_D^{32}$ −10.3° (*c* 0.91, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.35−7.26 (m, 15H), 5.74 (d, *J* = 10.0 Hz, 1H), 5.54 (s, 1H), 5.47 (s, 1H), 5.38 (s, 2H), 5.22 (dd, *J* = 8.4, 3.6 Hz, 1H), 4.93 (d, *J* = 11.2 Hz, 1H), 4.88−4.84 (m, 2H), 4.82−4.71 (m, 4H), 4.61 (d, *J* = 12.0 Hz, 1H), 4.34 (d, *J* = 7.6 Hz, 1H), 4.31−4.25 (m, 2H), 4.19−4.15 (m, 2H), 4.03−3.91 (m, 4H), 3.87 (s, 3H), 3.82−3.73 (m, 2H), 3.75 (s, 3H), 3.67−3.51 (m, 5H), 3.39−3.35 (m, 2H), 3.17 (t, *J* = 10.4 Hz, 1H), 2.97 (dd, *J* = 12.0 Hz, 3.2 Hz, 1H), 2.35 (dd, *J* = 12.8, 4.0 Hz, 1H), 2.22 (s, 3H), 2.17 (s, 3H), 2.14 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.99 (t, *J* = 12.8 Hz, 1H), 1.85 (s, 3H), 1.79 (d, *J* = 12.4 Hz, 1H), 1.05−1.00 (m, 2H), 0.03 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 170.9, 170.8, 170.6, 170.0, 169.9, 167.9 (d, $J_{C1-H3ax}$ = 7.5 Hz), 167.6 (d, $J_{C1-H3ax}$ = 6.0 Hz), 159.0, 154.7, 138.8, 138.7, 138.6, 128.53, 128.48, 128.2, 128.01, 127.95, 127.8, 127.7, 110.1, 103.3, 99.9, 98.7, 95.7, 84.7, 82.4, 77.4, 75.9, 75.0, 74.9, 74.6, 74.5, 73.9, 72.6, 71.3, 69.9, 68.2, 67.6, 67.5, 67.3, 63.7, 62.3, 62.0, 57.7, 53.5, 52.4, 52.0, 39.6, 37.5, 29.7, 21.4, 21.3, 20.9, 20.8, 20.7, 18.7, 0.1, −1.3; HRMS (ESI) calcd for $C_{68}H_{91}Cl_3N_3O_{29}Si$ $[M + NH_4]^+$ 1546.4568, found 1546.4524.

Methyl (5-Acetamino-7,8,9-tri-*O*-acetyl-3,5-dideoxy-4-(methyl 7,8,9-Tri-*O*-acetyl-5-amino-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galactonon-2-ulopyranosylonate)-D-glycero-α-D-galactonon-2-ulopyranoside)onate-(2→6)-(trimethylsilyl)ethyl 2,3,4-Tri-*O*-benzyl-β-D-glucopyranoside (10). Zinc powder (5.0 g, excess) was added to a solution of **9** (241.0 mg, 0.16 mmol) in MeCN (2.0 mL) and acetic acid (0.5 mL) under argon, and the reaction mixture was stirred for about 3 h at ambient temperature. The reaction mixture was then filtered through a pad of Celite, and the pad was washed with MeCN. The filtrate and washings were combined and extracted with EtOAc. Then the organic layer was successively washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, concentrated, and dried in vacuo for 30 min. Then the residue was dissolved in CH_2Cl_2 (20 mL), and acetic anhydride (149 μL, 1.6 mmol) and triethylamine (111 μL, 7.9 mmol) were added. The reaction mixture was stirred for 3 h under argon, and the reaction was quenched with MeOH. The mixture was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel (ethyl acetate/petroleum ether = 1:3 → 1:2) to give **10** (159.0 mg, 73%) as a colorless syrup. $[\alpha]_D^{32}$ −6.0° (*c* 0.22, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.35−7.26 (m, 15H), 5.90 (d, *J* = 9.6 Hz, 1H), 5.64−5.59 (m, 1H), 5.44−5.38 (m, 2H), 5.27 (s, 1H), 5.12 (dd, *J* = 9.2, 2.4 Hz, 1H), 4.93 (d, *J* = 11.2 Hz, 1H), 4.86 (d, *J* = 11.2 Hz, 1H), 4.80 (d, *J* = 10.4 Hz, 1H), 4.77−4.70 (m, 3H), 4.43 (dd, *J* = 10.4, 2.0 Hz, 1H), 4.33 (d, *J* = 8.0 Hz, 1H), 4.24 (dd, *J* = 12.0, 2.0 Hz, 1H), 4.17 (dd, *J* = 10.8, 4.0 Hz, 1H), 4.07−3.91 (m, 6H), 3.85 (s, 3H), 3.79−3.73 (m, 4H), 3.67−3.55 (m, 4H), 3.51 (d, *J* = 10.0 Hz, 1H), 3.39−3.35 (m, 2H), 3.03−2.95 (m, 2H), 2.29 (dd, *J* = 13.2, 4.4 Hz, 1H), 2.19 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.11 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H), 1.97 (t, *J* = 13.2 Hz, 1H), 1.85 (s, 3H), 1.84 (t, *J* = 12.8 Hz, 1H), 1.04−0.99 (m, 2H), 0.03 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.6, 171.2, 170.8, 170.5, 170.3, 170.2, 170.0, 167.6, 167.4, 159.1, 138.8, 138.7, 138.6, 128.52, 128.49, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 103.3, 100.0, 98.7, 84.7, 82.4, 77.5, 75.9, 75.1, 75.0, 73.9, 73.6, 72.5, 72.1, 69.8, 68.4, 67.6, 67.4, 66.3, 63.5, 63.2, 62.5, 57.6, 53.5, 52.4, 49.1, 39.4, 37.6, 23.6, 21.40, 21.35, 21.0, 20.94, 20.91, 20.7, 18.7, 0.1, −1.3; HRMS (ESI) calcd for $C_{67}H_{92}N_3O_{28}Si$ $[M + NH_4]^+$ 1414.5631, found 1414.5582.

5-Glycolylamino-3,5-dideoxy-D-glycero-α-D-galactonon-2-ulopyranosylonate-(2→4)-5-acetamino-3,5-dideoxy-D-glycero-α-D-galactonon-2-ulopyranoside)onate-(2→6)-(trimethylsilyl)-ethyl-β-D-glucopyranoside (1). A solution of **10** (174.0 mg, 0.12 mmol) in THF (20 mL) was stirred at 40 °C. NaOH (1 N, aq., 2.0 mL) was added, and then the reaction mixture was stirred for 4 h. After completion, the reaction was quenched with aqueous HCl (1 N), and the mixture was concentrated under reduced pressure. The residue was dissolved in MeOH (20 mL), and benzyloxyacetyl chloride (102 μL, 0.6 mmol) and triethylamine (177 μL, 1.2 mmol) were added at 0 °C. The mixture was stirred for about 3 h at room temperature and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 10:1 → 8:1 → 6:1) to give the crude product, which was dissolved in THF/AcOH/H_2O (4:2:1, 2.8 mL). Then 10% Pd/C (excess) was added, and the reaction mixture was stirred for 8 h under a hydrogen atmosphere (4 atm). The reaction mixture was filtered and then concentrated under reduced pressure. The residue was purified by RP-18 silica gel (H_2O → MeOH/H_2O = 1:10), and then the H^+ exchange resin column was eluted with H_2O to give compound **1** (83.0 mg, 76% over three steps) as a colorless syrup. $[\alpha]_D^{32}$ −40.8° (*c* 0.50, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.43 (d, *J* = 8.0 Hz, 1H), 4.24 (td, *J* = 10.8, 4.4 Hz, 1H), 4.10 (s, 2H), 4.01−3.80 (m, 10H), 3.78−3.71 (m, 3H), 3.68−3.61 (m, 2H), 3.59−3.56 (m, 2H), 3.50−3.46 (m, 1H), 3.44−3.41 (m, 2H), 3.22 (t, *J* = 8.4 Hz, 1H), 2.71−2.65 (m, 2H), 2.06 (s, 3H), 1.79 (t, *J* = 12.0 Hz, 1H), 1.61 (t, *J* = 12.0 Hz, 1H), 1.05 (td, *J* = 12.4, 5.6 Hz, 1H), 0.96 (td, *J* = 12.8, 5.6 Hz, 1H), 0.01 (s, 9H); ^{13}C NMR (150 MHz, D_2O) δ 176.6, 175.6, 173.1 (d, $J_{C1-H3ax}$ = 6.8 Hz), 172.5 (d, $J_{C1-H3ax}$ = 6.2 Hz), 102.3, 99.80, 98.79, 76.6, 74.9, 73.8, 73.4, 73.3, 71.8, 71.7, 70.7, 70.2, 69.2, 69.1, 68.9, 68.2, 63.6, 61.7, 52.2, 50.8, 40.5, 39.0, 23.0, 18.4, −1.8; HRMS (ESI) calcd for $C_{33}H_{57}N_2O_{23}Si$ $[M - H]^-$ 877.3121, found 877.3126.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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