One-Pot Synthesis of Sialo-Containing Glycosyl Amino Acids by Use of an *N*-Trichloroethoxycarbonyl-β-thiophenyl Sialoside

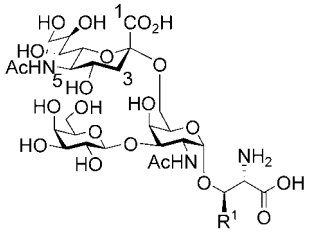
Hiroshi Tanaka, Masaatsu Adachi, and Takashi Takahashi*[a]

Abstract: We describe an efficient synthesis of 2,6- and 2,3-sialyl T antigens linked to serine in a one-pot glycosylation. We first investigated the glycosidation of thiosialosides by varying the *N*-protecting group. Modification of the C-5 amino group of β-thiosialosides into the *N*-9-fluorenylmethoxycarbonyl, *N*-2,2,2-trichloroethoxycarbonyl (*N*-Troc), and *N*-trichloroacetyl derivatives enhanced the reactivity of these compounds towards glycosidation. Addition of a minimum amount of 3 Å molecular sieves was also effective in improving the yield of α-linked sialosides. Next, we conducted one-pot syntheses of the glycosyl amino acids by using the *N*-Troc sialyl donor. The *N*-Troc derivative can be converted into the *N*-acetyl derivative without racemization of the amino acids. Branched-type one-pot glycosylation, initiated by regioselective glycosylation of the 3,6-dihydroxy galactoside with the *N*-Troc-β-thiophenyl sialoside, provided the protected 2,6-sialyl T antigen in good yield. Linear-type one-pot glycosylation, initiated by chemoselective glycosylation of galactosyl fluoride with the *N*-Troc-β-thiophenyl sialoside, afforded the protected 2,3-sialyl T antigen in excellent yield. Both protected glycosyl amino acids were converted into the fully deprotected 2,6- and 2,3-sialyl T antigens linked to serine in good yields.

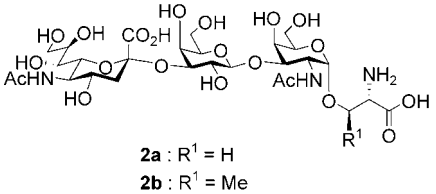
Keywords: glycosyl amino acids · glycosylation · oligosaccharides · sialic acids · thioglycosides

Introduction

Sialic acids such as *N*-acetylneuraminic acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Glc), and 3-deoxy-D-glycero-D-galacto-non-2-ulopyranosonic acid (KDN) are often attached at the nonreducing end of glycoconjugates on the cell surface through α-glycosidic bonds and they play a central role in cell-surface recognition phenomena.[1] For example, Galβ(1→3)–[Neu5Acα(2→6)]–GalNAcα(1→3)–Ser (**1a**) or -Thr (**1b**) and Neu5Acα(2→3)–Galβ(1→3)–GalNAcα(1→3)–Ser (**2a**) or -Thr (**2b**) are important tumor-associated antigens carried on the MUC1 and MUC4 proteins (Scheme 1).[2–4] Additionally, various related glycoforms containing α-sialosides are found in related antigens.[5] Therefore, sialo-containing glycosyl amino acids and peptides have been synthesized to generate tumor-selective immunostimulating antigens.[6]



1a : R^1 = H
1b : R^1 = Me



2a : R^1 = H
2b : R^1 = Me

Scheme 1. Structures of 2,6- and 2,3-sialyl T antigens **1** and **2**.

One-pot glycosylation[7–11] to form two or more glycosidic bonds by chemoselective activation of glycosyl donors is an

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Supporting information for this article is available on the WWW under http://www.chemeurj.org/ or from the author.

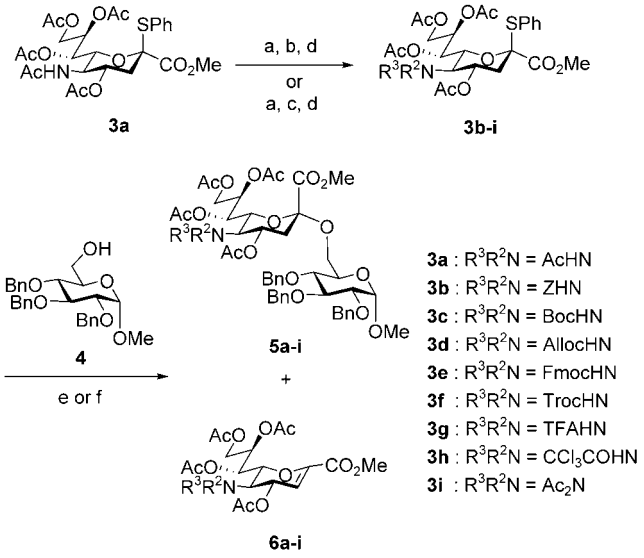
 DOI: 10.1002/chem.200400840

effective solution-phase method, not only for the high-speed synthesis of a target oligosaccharide but also for the parallel synthesis of oligosaccharide libraries[11] because it circumvents the need for purification of the synthetic intermediates. Wong and co-workers have reported the Optimer method, involving sequential activation of thioglycosides whose reactivity was tuned by the protecting groups on the basis of the armed and disarmed concept.[8j,l] We have investigated one-pot glycosylation based on chemoselective activation of glycosyl donors with different leaving groups[8a,c,k,9–11] by appropriate activators. The order of activation of glycosyl donors can be tuned, not only by their protecting groups but also by the combination of their leaving groups and activators. If sialylation could be adapted to one-pot glycosylation, this would be an effective and attractive way for the synthesis of sialo-containing oligosaccharides. However, α-selective sialylation is a problematic step in the chemical synthesis of oligosaccharides.[12] Therefore, the successful synthesis of glycosyl amino acids by one-pot glycosylations including sialylation requires the development of a new effective sialylation method. Herein we report an effective sialylation method that uses *N*-Troc-, *N*-Fmoc-, and *N*-trichloroacetyl-β-thiophenyl sialosides and the application of the *N*-Troc sialyl donor to the synthesis of two sialo-containing glycosyl amino acids, **1** and **2**, by using branched and linear one-pot glycosylations.[13]

Results and Discussion

In 1998, Demchenko and Boons reported that di-*N*-acetylthiomethyl sialoside was effective for glycosidation in terms of reactivity in comparison with *N*-acetyl-thiomethyl sialoside.[14] Recently, modification of the C-5 amino group of the sialyl donor into an azido group[15] or an *N*-TFA group[16] has been reported to improve reactivity toward glycosidation. Based on this information, we first investigated glycosidation of β-thiophenyl sialosides **3a–i** with various *N*-protecting groups (Scheme 2). Although β-thiophenyl sialosides are less reactive to glycosidation than α-thiophenyl sialosides, they can be simply prepared from *N*-acetylneuraminic acid (Neu5Ac).[17] Thiosialosides **3b–h** were synthesized by removal of all acetyl groups from the *N*-acetyl thiosialoside **3a** under acidic conditions,[18] followed by selective acylation of the amino group with various acid derivatives[19,20] and acetylation of the remaining hydroxy groups. All compounds except for *N*-Fmoc derivative **3e** were obtained in good yields (63–89% in three steps).

Glycosylation of the primary alcohol of glucose **4** with the sialyl donors **3a–i** was examined. Acetonitrile was used as the solvent because it is known to improve α selectivity in sialylation.[21] We first examined activation of thiosialosides **3a–i** by DMTST,[22] which is a mild activator for thioglycosides. Treatment of donors **3b–i** with 1.5 equivalents of acceptor **4** and DMTST in the presence of 3 Å molecular sieves (MS; 10 g mmol^{-1}) in CH_3CN at −10 °C resulted in no significant improvement in the yields of **5b–i** in comparison



Scheme 2. Synthesis and glycosidation of β-thiophenyl sialosides **3a–i**. Reagents and conditions: a) MsOH, MeOH, 60 °C, 12 h; b) Z-OSu, Alloc-OSu, Fmoc-OSu, Troc-OSu, or $(Boc)_2O$, NEt_3, CH_3CN/H_2O, RT, 5.5 h; c) CF_3CO_2Me or CCl_3CO_2Me, NEt_3, MeOH, RT, 1 h; d) Ac_2O, Py, DMAP, 63% for **3b**, 76% for **3c**, 68% for **3d**, 27% for **3e**, 89% for **3f**, 77% for **3g**, and 70% for **3h** in three steps from **3a**; e) **3a–i** (1.00 equiv), **4** (1.50 equiv), NIS (1.20 equiv), TfOH (0.20 equiv), CH_3CN, 3 Å MS (10 g mmol^{-1}), −35 °C, 1 h; f) **3a–i** (1.00 equiv), **4** (1.50 equiv), DMTST (1.50 equiv), CH_3CN, 3 Å MS (10 g mmol^{-1}), −10 °C, 8 h. Alloc = allyloxycarbonyl, Bn = benzyl, Boc = *tert*-butoxycarbonyl, DMAP = 4-dimethylaminopyridine, DMTST = (dimethylthio)methylsulfonium trifluoromethanesulfonate, Fmoc = 9-fluorenylmethoxycarbonyl, MsOH = methanesulfonic acid, NIS = *N*-iodosuccinimide, Py = pyridine, Su = succinimidyl, Tf = trifluoromethanesulfonyl, TFA = trifluoroacetyl, Troc = 2,2,2-trichloroethoxycarbonyl, Z = benzyloxycarbonyl.

with the result of glycosidation of *N*-acetyl derivative **3a** (Table 1). We next applied NIS and TfOH to the glycosidation; these are the most common and strongest reagents for activation of thioglycosides, and the *N*-Boc and *N*-Alloc protecting groups, unfortunately, might not survive these conditions. The sialyl donors **3a–i** were treated with acceptor **4** (1.5 equiv) and NIS (1.20 equiv)/TfOH (0.20 equiv)[23] in the presence of 3 Å MS (10 g mmol^{-1}) in CH_3CN at −35 °C (Table 2). Glycosidation of *N*-TFA derivative **3g** provided

Table 1. Glycosylation of **4** (1.50 equiv) with β-thiophenyl sialosides **3a–i** (1.00 equiv) by DMTST.

Entry	Donor	Disaccharide	Yield of **5** [%]	α:β[a]	Glycal	Yield of **6** [%]
1	**3a**	**5a**	40	85:15	**6a**	41
2	**3b**	**5b**	24	84:16	**6b**	–
3	**3c**	**5c**	–	–	**6c**	–
4	**3d**	**5d**	56	84:16	**6d**	39
5	**3e**	**5e**	55	86:14	**6e**	40
6	**3f**	**5f**	40	85:15	**6f**	35
7	**3g**	**5g**	35	87:13	**6g**	42
8	**3h**	**5h**	41	76:24	**6h**	38
9	**3i**	**5i**	36	75:25	**6i**	47

[a] The α:β ratio was determined by HPLC analysis based on refractive-index detection.

Table 2. Glycosylation of **4** (1.50 equiv) with β-thiophenyl sialosides **3a–i** (1.00 equiv) by NIS/TfOH.

Entry	Donor	Disaccharide	Yield of **5** [%]	α:β[a]	Glycal	Yield of **6** [%]
1	**3a**	**5a**	47	85:15	**6a**	28
2	**3b**	**5b**	68	84:16	**6b**	8
3	**3c**	**5c**	–	–	**6c**	–
4	**3d**	**5d**	44	88:12	**6d**	20
5	**3e**	**5e**	91	86:14	**6e**	7
6	**3f**	**5f**	91	89:11	**6f**	6
7	**3g**	**5g**	92	92:8	**6g**	5
8	**3h**	**5h**	83	91:9	**6h**	4
9	**3i**	**5i**	65	62:38	**6i**	23

[a] The α:β ratio was determined by HPLC analysis based on refractive-index detection.

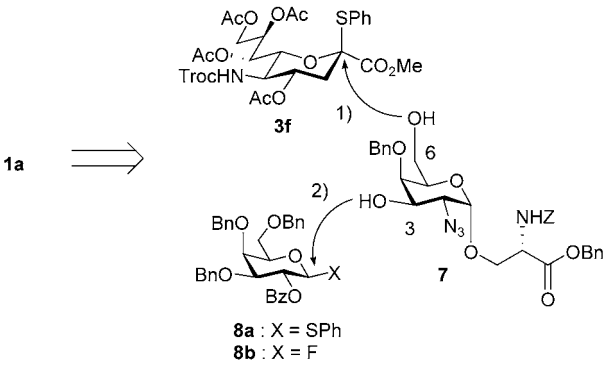
disaccharide **5g** in excellent yield (92%, α:β = 92:8) along with a small amount of glycal **6g** in 5% yield. The *N*-Fmoc and *N*-Troc derivatives **3e** and **3f**[24] served as effective glycosyl donors to afford the corresponding disaccharides **5e** and **5f** in excellent yields and with acceptable α selectivity (91%, α:β = 86:14 for **5e**; 91%, α:β = 89:11 for **5f**). The *N*-trichloroacetyl derivative **3h** was converted into disaccharide **5h** in good yield. The anomeric configurations of the resulting sialosides were determined by analysis of ^{1}H NMR spectra based on the chemical shift values of the H-3eq and H-4 signals, the coupling constant $J_{7,8}$, and the value of $\Delta\delta$(H-9′−H-9), which were in accordance with the empirical rules for defining the anomeric configuration of *N*-acetyl sialic acid glycosides (Table 3).[25] Additionally, the structural confirmation for the α- and β-*N*-Troc sialosides **5f** was accomplished by transformation of these compounds into the corresponding α- and β-*N*-acetyl sialosides **5a**.

Table 3. Partial ^{1}H NMR signal assignment for disaccharides **5e–g**.

R^3R^2N		$\delta_{H\text{-}3eq}$ [ppm]	$\delta_{H\text{-}4}$ [ppm]	$J_{7,8}$ [Hz]	$\Delta\delta$(H-9′−H-9) [ppm]
AcHN	**5a-α**	2.65	4.84	9.3	0.29
	5a-β	2.48	5.17	1.9	0.94
FmocHN	**5e-α**	2.70	4.90	9.2	0.31
	5e-β	2.54	5.21	8.7	0.93
TrocHN	**5f-α**	2.72	4.98	–	0.27
	5f-β	2.54	5.25	4.8	0.91
TFAHN	**5g-α**	2.70	4.99	8.8	0.30
	5g-β	2.45	5.21	–	0.91

To adapt the sialylation to the synthesis of glycosyl amino acids and peptides, removal of the C-5 protecting group without racemization of the amino acid is required. Unfortunately, deprotection of the *N*-TFA group requires relatively strongly basic conditions, in which racemization of the amino acid would occur. On the other hand, the *N*-Fmoc and *N*-Troc groups can be converted into various *N*-acyl groups under mildly acidic or basic conditions. Therefore, the *N*-Fmoc and *N*-Troc-β-thiophenyl sialosides **3e** and **3f** would be suitable for the synthesis of glycosyl amino acids and glycopeptides. Recently, Kiso, Ando, and co-workers have reported the effectiveness of an *N*-Troc-α-thiophenyl sialoside for the synthesis of sialo-containing glycosides.[26]

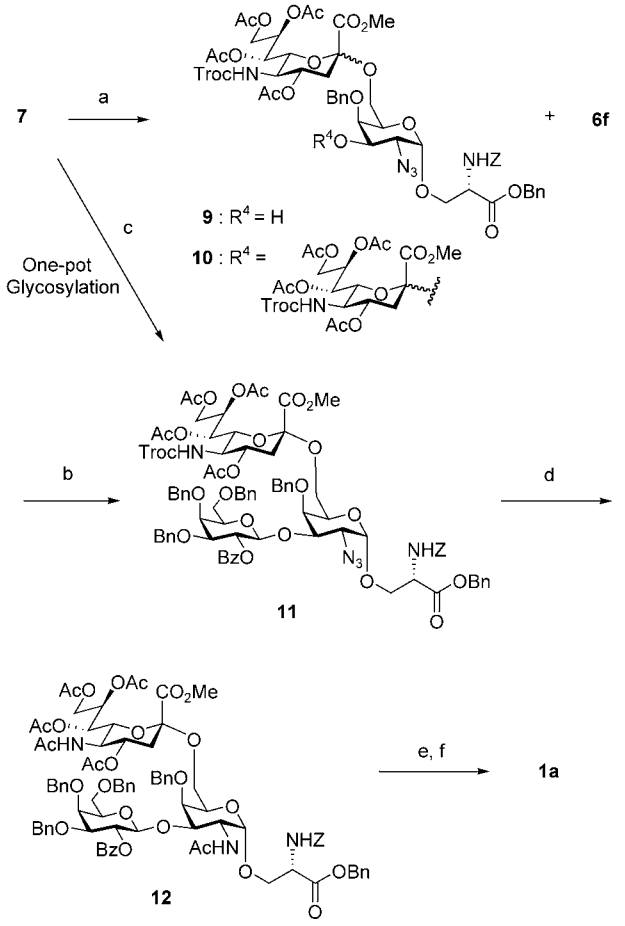
To demonstrate the feasibility of the sialylation method, we planned the one-pot syntheses of **1** and **2**. We first examined a one-pot two-step synthesis of 2,6-sialyl T antigen **1a**.[27] Our strategy for the synthesis of **1a** involved a branched-type one-pot glycosylation initiated by regioselective glycosylation (Scheme 3). 3,6-Dihydroxy galactoside **7** linked to serine was chosen as the glycosyl acceptor.[27d,e,28,29]



Scheme 3. Strategy for the one-pot two-step synthesis of **1a**. Bz = benzoyl.

The *N*-Troc-β-thiophenyl sialoside **3f** was selected as the glycosyl donor because the availability of **3f** from *N*-acetylneuraminic acid was better than that of the *N*-Fmoc derivative **3e**. Regioselective glycosylation of the primary alcohol on position 6 of **7** with the *N*-Troc derivative **3f**, followed by glycosylation of the alcohol on position 3 with galactoside **8** would provide the protected 2,6-sialyl T antigen. Transformation of the azido and *N*-Troc groups into *N*-acetyl groups followed by deprotection of all protecting groups should be achieved without racemization of the amino acid to provide the fully deprotected 2,6-sialyl T antigen **1a**.

Stepwise synthesis of the protected trisaccharide **11** was undertaken (Scheme 4). We first examined the glycosylation of the primary alcohol of **7** with an equivalent of *N*-Troc-β-thiophenyl sialoside **3f** (Table 4). Treatment of diol **7** with the sialyl donor **3f** (1 equiv) in the presence of NIS (1.20 equiv), TfOH (0.20 equiv), and 3 Å MS (10 g mmol^{-1}) in CH_3CN (10 mL mmol^{-1}) at −35 °C for 1 h provided disaccharide **9** in moderate yield (57%, α:β = 84:16), along with disialyl trisaccharide **10** in 4% yield and glycal **6f** in 20% yield (Table 4, entry 1).[30,31] Further optimization of the reaction revealed that the quantity of 3 Å MS and the concentration of the substrates influenced the efficiency of the glycosidation. Glycosylation of **7** with **3f** (1 equiv) in the presence of TfOH (0.20 equiv) and 3 Å MS (0.50 g mmol^{-1}) in CH_3CN (5 mL mmol^{-1}) provided disaccharide **9** in 75% yield (α:β = 80:20), along with disialyl trisaccharide **10** in 4% yield and glycal **6f** in 8% yield (entry 7). Use of propio-



Scheme 4. Synthesis of 2,6-sialyl T antigen **1a** by a a stepwise process and by a branched-type one-pot glycosylation. Reagents and conditions: a) **3f** (1.20 equiv), NIS (1.44 equiv), TfOH (0.10 equiv), CH_3CN (5 mL mmol^{-1}), 3 Å MS (0.50 g mmol^{-1}), −35 °C, 93 %, α:β = 78:22; b) **8a** (1.50 equiv), NIS (2.00 equiv), TfOH (0.20 equiv), CH_2Cl_2/CH_3CN (9:1, 5 mL mmol^{-1}), 3 Å MS (0.50 g mmol^{-1}), −30 °C, 82 %, α:β = 6:94; c) **3f** (1.20 equiv), **7** (1.00 equiv), NIS (1.44 equiv), TfOH (0.10 equiv), CH_3CN (5 mL mmol^{-1}), 3 Å MS (0.50 g mmol^{-1}), −35 °C; then **8a** (1.80 equiv), NIS (2.70 equiv), TfOH (0.20 equiv), CH_2Cl_2 (45 mL mmol^{-1}), −30 °C, 77 %, α:β = 78:22; d) Zn dust, THF, AcOH, Ac_2O, 0 °C, 1 h, 92 %; e) $Pd(OH)_2$, THF, MeOH, AcOH, H_2O, 2 h; f) 0.1 M aq. NaOH, MeOH; then H_2O, 85 % over two steps. THF = tetrahydrofuran.

nitrile as the solvent requires a higher reaction temperature (−25 °C) and longer reaction time (3 h) and resulted in a reduced yield of **9** (58 %, α:β = 82:18; entry 8). Finally, minimization of the amount of remaining acceptor **7** and the side products **10** and **6f** was accomplished by glycosidation of sialyl donor **3f** (1.2 equiv) with NIS (1.44 equiv), TfOH (0.10 equiv), and 3 Å MS (0.50 g mmol^{-1}) in CH_3CN (5 mL mmol^{-1}) at −35 °C to provide disaccharide **9** in 93 % yield (α:β = 78:22) along with trisaccharide **10** (6 %) and glycal **6f** (entry 9). The yield of glycal **6f** was 12 %, based on donor **3f**.

Next, glycosylation of the remaining secondary alcohol of **9** with galactoside **8** was examined. In the one-pot glycosylation, galactosylation should be achieved after the sialylation. Therefore, we first examined glycosylation of **9α** with **8** in CH_3CN. Galactosyl fluoride **8b** was not completely activated by $ZrCp_2Cl_2/AgOTf$[32] in CH_3CN. On the other hand, treatment of disaccharide **9α** with thiogalactoside **8a** in the presence of NIS/TfOH in CH_3CN at room temperature provided trisaccharide **11** in 60 % yield and with β selectivity (α:β = 19:81).[33,34] The anchimeric effect of the C-2 acyl protecting group to provide 1,2-*trans* glycosidic linkage did not work well under these reaction conditions. After optimization of the reaction conditions, we found that treatment of disaccharide **9α** with **8a** (1.50 equiv) in CH_2Cl_2/CH_3CN (9:1) at −30 °C afforded trisaccharide **11** in 82 % yield and with acceptable selectivity (α:β = 6:94).

One-pot glycosylation with **3f**, **7**, and **8a** was examined. Treatment of diol **7** with the *N*-Troc-β-thiophenyl sialoside **3f** under the above-described conditions provided disaccharide **9**. Dilution of the reaction mixture with CH_2Cl_2 followed by addition of thioglactoside **8a** and additional NIS/TfOH afforded the protected trisaccharide **11**. Purification of the crude mixture was achieved by silica-gel column chromatography and gel-permeation chromatography (GPC) to provide trisaccharide **11** in 77 % overall yield, based on **7**, and with good selectivity (α:β = 78:22). The analytical data of the trisaccharides **11α** and **11β** were identical to those of authentic sample synthesized by the stepwise synthesis.

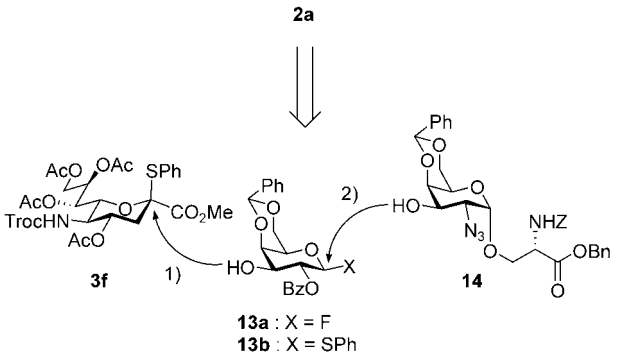
Transformation of **11α** into **1a** proceeded as follows. The simultaneous reduction of the azido and *N*-Troc groups of **11α**,[35] followed by acetylation, provided the corresponding *N*-acetyl derivative **12** in 92 % yield. Complete deprotection of **12** was accomplished in two steps, involving hydrogenolysis of the benzyl ethers and subsequent hydrolysis of the benzoates, acetyls, and the methyl ester, to provide the fully deprotected 2,6-sialyl T antigen **1a** in 85 % overall yield. The analytical data (^{1}H NMR spectroscopy, optical rotation) were identical to those previously reported.[27a]

Table 4. Optimization of regioselective sialylation of diol **7** with the *N*-Troc-β-thiophenyl sialoside **3f**.[a]

Entry	Donor [equiv]	TfOH [equiv]	Concentration [mL mmol^{-1}]	Solvent	3 Å MS [g mmol^{-1}]	Yield of **9** [%]	α:β[b]	Yield of **10** [%]	Yield of **6f** [%][c]
1	1.0	0.2	10	CH_3CN	10	57	84:16	4	20
2	1.0	0.2	10	CH_3CN	5.0	65	78:22	5	12
3	1.0	0.2	10	CH_3CN	2.5	55	79:21	4	17
4	1.0	0.2	10	CH_3CN	0.50	71	80:20	4	12
5	1.0	0.2	10	CH_3CN	0.25	69	81:19	5	10
6	1.0	0.2	5	CH_3CN	0.50	70	79:21	5	17
7	1.0	0.1	5	CH_3CN	0.50	75	80:20	4	8
8[d]	1.0	0.1	5	EtCN	0.50	58	82:18	6	25
9[e]	1.2	0.1	5	CH_3CN	0.50	93	78:22	6	12

[a] Conditions: NIS (1.2 equiv based on **3f**) and TfOH at −35 °C for 1 h. [b] The α:β ratio was determined by HPLC analysis based on refractive-index detection. [c] Yields are based on donor **3f**. [d] The reaction was carried out at −25 °C for 3 h. [e] 1.44 equivalents of NIS were used.

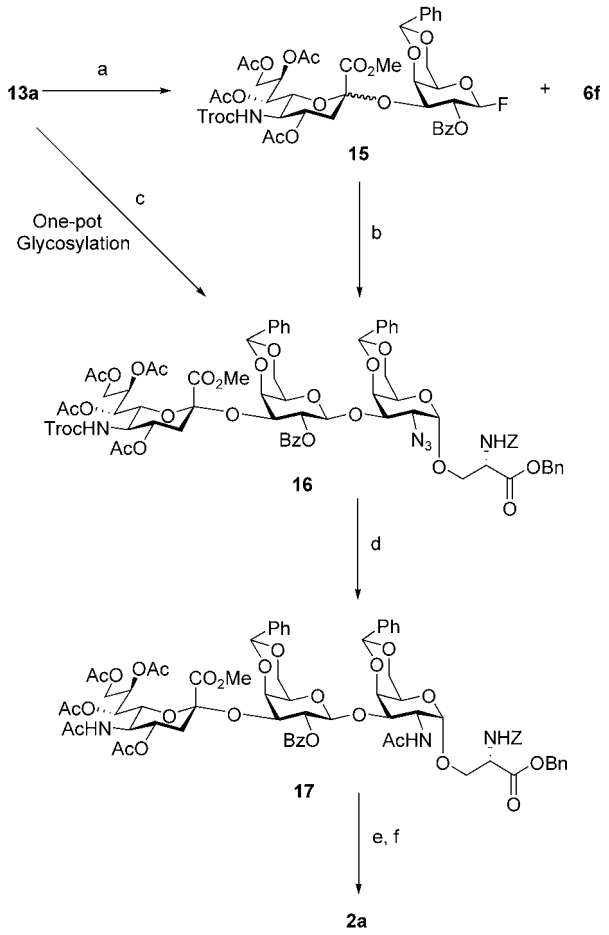
Next, we planned to conduct the one-pot synthesis of **2a** by a method involving chemoselective glycosylation of galactoside **13** with thiosialoside **3f** (Scheme 5).[36] Subsequent



Scheme 5. Strategy for the one-pot two-step synthesis of **2a**.

coupling of the resulting disaccharide and the galactosyl serine **14**[29,37] would provide the protected 2,3-sialyl-T antigen. The glycosyl acceptor **13** must survive under the activation conditions for **3f**. Additionally, the resulting sialo-containing disaccharide should smoothly undergo glycosidation by the secondary hydroxy group. The glycosyl fluoride **13a** and thioglycoside **13b** were candidate acceptors in the glycosylation. We selected the glycosyl fluoride **13a** as the glycosyl acceptor in the chemoselective glycosidation with thiosialoside **3f**[38,39] and avoided the use of thioglycoside **13b** as it is tuned to have lower reactivity than the sialoside. Deactivation of the acceptor by the protecting groups in comparison with the sialoside **3f** would result in a reduced yield of the following glycosylation because sialosides are known to be one of the most unreactive glycosyl donors.

Stepwise synthesis of the protected trisaccharide **16** was examined (Scheme 6). We first conducted the chemoselective glycosylation of galactosyl fluoride **13a** with the *N*-Troc-β-thiophenyl sialoside **3f** (Table 5). Treatment of the galactosyl fluoride **13a** with the sialyl donor **3f** (1 equiv) in the presence of NIS (1.35 equiv), TfOH (0.30 equiv), and 3 Å MS (0.50 g mmol^{-1}) in CH_3CN (5 mL mmol^{-1}) at −30 °C for 15 min provided disaccharide **15** in 56% yield with relatively low anomeric selectivity (α:β = 70:30), along with glycal **6f** in 35% yield (Table 5, entry 1).[40,41] Further optimization of the reaction revealed that control of the reaction temperature was critical for improving the α selectivity of the glycosylation. Finally, we found that glycosylation of **13a** with an equivalent of **3f** in CH_3CN/CH_2Cl_2 (2:3) at −78 °C provided disaccharide **15** in moderate yield (65%) with excellent selectivity (α:β = 93:7), along with glycal **6f** in 31% (entry 11). Use of CH_2Cl_2 as the solvent resulted in a reduced yield (17%) of disaccharide **15** but provided products with moderately α-selective glycosylation (entry 12). These results indicated that acetonitrile would be effective for improving the coupling yield as well as the α selectivity. Minimization of the remaining acceptor **13a** was accomplished by glycosidation of sialyl donor **3f** (1.5 equiv)



Scheme 6. Synthesis of 2,3-sialyl T antigen **2a** by a stepwise process and by a linear-type one-pot glycosylation. Reagents and conditions: a) **3f** (1.50 equiv), NIS (2.00 equiv), TfOH (0.30 equiv), CH_3CN/CH_2Cl_2 (2:3, 5 mL mmol^{-1}), 3 Å MS (0.50 g mmol^{-1}), −78 °C, 86%, α:β = 93:7; b) **14** (1.30 equiv), $ZrCp_2Cl_2$ (1.50 equiv), AgOTf (3.00 equiv), CH_3CN/CH_2Cl_2 (2:3, 5 mL mmol^{-1}), 3 Å MS (0.50 g mmol^{-1}), −5 °C, 96%; c) **3f** (1.50 equiv), **13a** (1.00 equiv), NIS (2.00 equiv), TfOH (0.30 equiv), CH_3CN/CH_2Cl_2 (2:3, 5 mL mmol^{-1}), 3 Å MS (0.50 g mmol^{-1}), −78 °C; then **14** (1.50 equiv), $ZrCp_2Cl_2$ (2.00 equiv), AgOTf (4.00 equiv), −5 °C, 88%, α:β = 93:7; d) Zn dust, THF, AcOH, Ac_2O, 0 °C, 1 h, 93%; e) $Pd(OH)_2$, THF, MeOH, AcOH, H_2O, 2 h; f) 0.1 N NaOH, MeOH; then H_2O, 90% over two steps.

with NIS (2.00 equiv), TfOH (0.30 equiv), and 3 Å MS (0.50 g mmol^{-1}) in CH_3CN/CH_2Cl_2 (2:3; 5 mL mmol^{-1}) at −78 °C to provide disaccharide **15** in 86% yield (α:β = 93:7), along with glycal **6f** (entry 13). The yield of glycal **6f** was 44%, based on donor **3f**. This chemoselective glycosylation method should be effective for the preparation of various glycosyl fluorides attached to α-sialosides.

Next, glycosylation of the glycosyl amino acid **14** with disaccharide **15** was examined. In the one-pot glycosylation, the galactosylation should be achieved after the sialylation. Therefore, we examined glycosylation of **14** with disaccharide **15α** in CH_3CN/CH_2Cl_2 (2:3). Treatment of the glycosyl fluoride **15α** with the glycosyl amino acid **14** in the presence of $[ZrCp_2Cl_2]$/AgOTf in CH_3CN/CH_2Cl_2 (2:3) at −5 °C pro-

Table 5. Optimization of regio-selective sialylation of galactosyl fluoride **13a** with the *N*-Troc β-thiophenyl sialoside **3f**.[a]

Entry	Donor [equiv]	Acid [equiv]	Solvent	*T* [°C]	Yield of **15** [%]	α:β[b]	Yield of **6f** [%][c]
1	1.0	TfOH (0.3)	CH_3CN	−30	56	70:30	35
2	1.0	TfOH (0.3)	CH_3CN	−35	61	75:25	31
3	1.0	TMSOTf[d] (0.3)	CH_3CN	−35	55	75:25	35
4[e]	1.0	TfOH (0.5)	EtCN	−30	45	72:28	47
5	1.0	TfOH (0.3)	CH_3CN/CH_2Cl_2 5:1	−35	45	75:25	45
6	1.0	TfOH (0.3)	CH_3CN/CH_2Cl_2 1:1	−50	45	84:16	46
7	1.0	TfOH (0.3)	CH_3CN/CH_2Cl_2 1:1	−55	63	85:15	31
8	1.0	TfOH (0.3)	CH_3CN/CH_2Cl_2 1:1	−60	64	87:13	28
9	1.0	TfOH (0.3)	CH_3CN/CH_2Cl_2 1:1	−65	65	91:9	25
10	1.0	TfOH (0.3)	CH_3CN/CH_2Cl_2 2:3	−70	66	91:9	30
11	1.0	TfOH (0.3)	CH_3CN/CH_2Cl_2 2:3	−78	65	93:7	31
12[f]	1.0	TfOH (0.3)	CH_2Cl_2	−78	17	62:38	82
13[g]	1.5	TfOH (0.3)	CH_3CN/CH_2Cl_2 2:3	−78	86	93:7	44

[a] Conditions: NIS (1.35 equiv based on **3f**) for 15 min. [b] The α:β ratio was determined by HPLC analysis based on refractive-index detection. [c] Yields were based on donor **3f**. [d] TMS = trimethylsilyl. [e] The reaction time was 3 h. [f] The reaction time was 5 h. [g] 2.00 equivalents of NIS were used.

vided trisaccharide **16** in 96% yield and with excellent β selectivity (α:β = < 1:99).

One-pot glycosylation with **3f**, **13a**, and **14** was examined next. Chemoselective glycosylation of the glycosyl fluoride **13a** with the *N*-Troc-β-thiophenyl sialoside **3f** under the above-described conditions provided disaccharide **15**. Subsequent addition of acceptor **14**, $[ZrCp_2Cl_2]$, and AgOTf afforded the protected trisaccharide **16** as two diastereomers in 88% overall yield, based on **13a**, and with excellent selectivity (α:β = 93:7). The analytical data of the trisaccharides **16α** and **16β** were identical to those of authentic samples synthesized by the stepwise synthesis.

Transformation of **16α** into **2a** proceeded as follows. Simultaneous reduction of the azido and *N*-Troc groups of **16α** followed by acetylation provided the corresponding *N*-acetyl derivative **17** in 93% yield. Complete deprotection of **17** was accomplished in two steps, involving hydrogenolysis of the benzyl ethers followed by hydrolysis of the benzoates, acetates, and the methyl ester, to provide the fully deprotected 2,3-sialyl T antigen **2a** in 90% overall yield. The analytical data (^{1}H NMR spectroscopy) were identical to those previously reported.[42]

Conclusion

We have described an effective sialylation method that utilizes the *N*-Fmoc, *N*-Troc, and *N*-trichloroacetyl-β-thiophenyl sialosides **3e**, **3f**, and **3h**. Additionally, use of a minimum amount of 3 Å molecular sieves improved the yield of the coupled products. An application of this sialylation with *N*-Troc-β-thiosialoside **3f** to the synthesis of glycosyl amino acids by one-pot glycosylation was demonstrated. A branched-type one-pot two-step glycosylation initiated by regioselective glycosidation of **3f** on the primary alcohol of acceptor **7** provided the protected 2,6-sialyl T antigen **11** in good yield. A linear-type one-pot glycosylation initiated by chemoselective glycosylation of glycosyl fluoride **13a** with the sialyl donor **3f** afforded the protected 2,3-sialyl T antigen **16** in excellent yield. Modification of the *N*-Troc and azido groups into *N*-acetyl groups followed by removal of all protecting groups without racemization of the α position of the amino acid was accomplished to provide sialo-containing glycosyl amino acids **1a** and **2a**. Application of the glycosylation method to the synthesis of an oligosaccharide library is in progress.

Experimental Section

General: NMR spectra were obtained on a JEOL model ECP-400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) instrument in the indicated solvent. Chemical shifts are reported in parts per million (ppm) relative to the signal (0 ppm) for internal tetramethylsilane for solutions in $CDCl_3$. ^{1}H NMR spectral data are reported as follows: $CDCl_3$ (7.26 ppm) or D_2O (4.7015 ppm at 303 K; as an internal standard with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as anexternal standard). ^{13}C NMR spectral data are reported as follows: $CDCl_3$ (77.0 ppm) or $[D_6]$acetone (30.3 ppm; as an internal standard for D_2O). 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. FT-IR spectra were recorded on a Perkin–Elmer Spectrum One spectrometer. Data are given in cm^{-1} with only significant diagnostic bands reported. Optical rotations were measured with a JASCO P-1020 polarimeter. Column chromatography was performed with silica gel (Merck). Gel-permeation chromatography (GPC) for qualitative analyses was performed on a Japan Analytical Industry model LC908 (recycling preparative HPLC) instrument, with a Japan Analytical Industry model RI-5 refractive-index detector and a Japan Analytical Industry model 310 UV detector, on a polystylene gel column (JAIGEL-1H, 20×600 mm), with chloroform as the solvent (Flow rate: 3.5 mL min^{-1}). HPLC was performed on a Waters apparatus with a Senshu Pak Silica-3301-N column and a Waters 2414 refractive-index detector. Dry tetrahydrofuran (THF), hexane, benzene, toluene, diethyl ether, and 1,2-dimethoxyethane (DME) were distilled from sodium wire contained with a catalytic amount of benzophenone. Dry dichloromethane was distilled from P_2O_5. Dry *N,N*-dimethylformamide (DMF), triethylamine, and pyridine were distilled from CaH_2. Dry methanol and ethanol were distilled from magnesium contained with a catalytic amount of iodine.

Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-5-(benzyloxycarbonylamino)-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (3b): Methanesulfonic acid (0.66 mL, 10.2 mmol) was added to a stirred solution of methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (**3a**; 595 mg, 1.02 mmol) in methanol (20 mL) at room temperature. After being stirred at 60 °C for 24 h, the reaction mixture was neutralized with triethylamine and concentrated in vacuo. The residue was used for the next reaction without further purification. Benzyl succinimidyl carbonate (254 mg, 1.02 mmol) and triethylamine (0.21 mL, 1.53 mmol) were added to a stirred solution of the residue in CH_3CN/H_2O (17:1; 9.00 mL) at 0 °C. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated in

vacuo. The residue was used for the next reaction without further purification. Acetic anhydride (0.86 mL, 6.12 mmol) and a catalytic amount of DMAP were added to a stirred solution of the residue in pyridine (0.99 mL, 12.2 mmol) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was poured into ice-cooled water. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with 1 M HCl, saturated aq. $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (98:2) to give **3b** (426 mg, 0.630 mmol, 63 % over three steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.27–7.45 (m, 10H, aromatic), 5.55 (brs, 1H, H-7), 5.36 (ddd, 1H, H-4, $J_{3ax,4}$ = 11.7, $J_{3eq,4}$ = 4.4, $J_{4,5}$ = 10.3 Hz), 5.17 (d, 1H, J_{gem} = 12.2 Hz), 5.01 (brd, 1H, H-8, J = 8.3 Hz), 4.93 (d, 1H, J_{gem} = 12.2 Hz), 4.86 (d, 1H, NH, J = 10.6 Hz), 4.61 (dd, 1H, H-6, $J_{5,6}$ = 11.0, $J_{6,7}$ = 2.0 Hz), 4.48 (brd, 1H, H-9′, J_{gem} = 12.2 Hz), 4.03 (dd, 1H, H-9, $J_{8,9}$ = 8.8, J_{gem} = 12.2 Hz), 3.81 (ddd, 1H, H-5, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$ = 10.3 Hz), 3.59 (s, 3H, OMe), 2,69 (dd, 1H, H-3eq, $J_{3eq,4}$ = 4.4, J_{gem} = 13.7 Hz), 2.08 (dd, 1H, H-3ax, $J_{3ax,4}$ = 11.7 Hz), 1.84, 1.98, 2.07, 2.13 (4s, 12H, Ac) ppm; IR (KBr): $\tilde{\nu}$ = 3480, 2801, 1703, 1489, 1460, 1045, 861 cm^{-1}.

Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-5-(*tert*-butoxycarbonylamino)-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (3c): Methanesulfonic acid (1.32 mL, 20.3 mmol) was added to a stirred solution of methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (**3a**; 1.12 g, 2.03 mmol) in methanol (40 mL) at room temperature. After being stirred at 60 °C for 24 h, the reaction mixture was neutralized with triethylamine and concentrated in vacuo. The residue was used for the next reaction without further purification. Di-*tert*-butyldicarbonate (2.20 g, 10.2 mmol) and triethylamine (0.85 mL, 6.09 mmol) were added to a stirred solution of the residue in dioxane/MeOH/H_2O (1:1:1; 9.00 mL) at 0 °C. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated in vacuo. The residue was used for the next reaction without further purification. Acetic anhydride (1.71 mL, 12.1 mmol) and a catalytic amount of DMAP were added to a stirred solution of the residue in pyridine (1.97 mL, 24.4 mmol) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was poured into ice-cooled water. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with 1 M HCl, saturated aq. $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (98:2) to give **3c** (889 mg, 1.38 mmol, 68 % over three steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30–7.45 (m, 5H, aromatic), 5.57 (brs, 1H, H-7), 5.33 (ddd, 1H, H-4, $J_{3ax,4}$ = 10.8, $J_{3eq,4}$ = 4.9, $J_{4,5}$ = 10.8 Hz), 5.00 (brd, 1H, H-8, J = 8.8 Hz), 4.67 (m, 1H, NH), 4.59 (dd, 1H, H-6, $J_{5,6}$ = 10.8, $J_{6,7}$ = 2.4 Hz), 4.53 (dd, 1H, H-9′, $J_{8,9'}$ = 1.5, J_{gem} = 12.2 Hz), 4.00 (dd, 1H, H-9, $J_{8,9}$ = 8.8, J_{gem} = 12.2 Hz), 3.81 (ddd, 1H, H-5, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$ = 10.7 Hz), 3.59 (s, 3H, OMe), 2.70 (dd, 1H, H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 13.7 Hz), 2.11 (dd, 1H, H-3ax, $J_{3ax,4}$ = 10.8 Hz), 1.84, 1.97, 2.05, 2.17 (4s, 12H, Ac), 1.40 (s, 9H, Me) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 170.4, 170.2, 169.7, 168.2, 155.2, 136.1, 129.6, 129.0, 128.8, 88.8, 80.0, 73.2, 72.8, 69.3, 68.9, 62.7, 54.5, 50.7, 37.6, 28.6, 21.0, 20.7, 20.7, 20.6 ppm; IR (KBr): $\tilde{\nu}$ = 3373, 2979, 1741, 1524, 1370, 1235, 1037, 752 cm^{-1}.

Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-5-(allyloxycarbonylamino)-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (3d): Methanesulfonic acid (0.434 mL, 6.70 mmol) was added to a stirred solution of methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (**3a**; 391 mg, 0.670 mmol) in methanol (13 mL) at room temperature. After being stirred at 60 °C for 24 h, the reaction mixture was neutralized with triethylamine and concentrated in vacuo. The residue was used for the next reaction without further purification. A 0.5 M dioxane solution of allyl succinimidyl carbonate (1.35 mL, 0.670 mmol) and triethylamine (0.14 mL, 1.01 mmol) were added to a stirred solution of the residue in CH_3CN/H_2O (9:1; 6.00 mL) at 0 °C. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated in vacuo. The residue was used for the next reaction without further purification. Acetic anhydride (0.65 mL, 4.20 mmol) and a catalytic amount of DMAP were added to a stirred solution of the residue in pyridine (0.65 mL, 8.04 mmol) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was poured into ice-cooled water. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with 1 M HCl, saturated aq. $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (98:2) to give **3d** (319 mg, 0.510 mmol, 67 % over three steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.45 (m, 5H, aromatic), 5.88 (dddd, 1H, J = 5.8, J = 5.8, J = 10.3, J = 10.3 Hz), 5.54 (brs, 1H, H-7), 5.39 (ddd, 1H, H-4, $J_{3ax,4}$ = $J_{3eq,4}$ = $J_{4,5}$ = 10.2 Hz), 5.26 (dd, 1H, J = 10.3, J_{gem} = 1.0 Hz), 5.20 (dd, 1H, J = 10.3, J_{gem} = 1.0 Hz), 5.00 (brd, 1H, H-8, J = 8.3 Hz), 4.87 (d, 1H, NH, J = 10.2 Hz), 4.62 (dd, 1H, H-6, $J_{5,6}$ = 10.7, $J_{6,7}$ = 2.4 Hz), 4.57 (dd, 1H, J = 5.8, J_{gem} = 13.6 Hz), 4.48 (dd, 1H, H-9′, $J_{8,9'}$ = 2.0, J_{gem} = 12.2 Hz), 4.44 (dd, 1H, J = 5.8, J_{gem} = 13.6 Hz), 4.02 (ddd, 1H, H-5, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$ = 10.2 Hz), 3.60 (s, 3H, OMe), 2.72 (dd, 1H, H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 14.2 Hz), 2.11 (dd, 1H, H-3ax, $J_{3ax,4}$ = 11.2 Hz), 1.97, 2.03, 2.06, 2.11 (4s, 12H, Ac) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 170.5, 170.3, 170.0, 168.2, 155.8, 136.1, 132.6, 129.7, 129.1, 128.8, 117.6, 88.8, 73.1, 72.8, 69.0, 65.9, 62.6, 52.5, 51.5, 37.5, 21.0, 20.8, 20.7, 20.7 ppm; IR (KBr): $\tilde{\nu}$ = 3454, 2998, 2911, 1730, 1487, 1123, 790 cm^{-1}.

Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-(9-fluorenylmethoxycarbonylamino)-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (3e): Methanesulfonic acid (0.86 mL, 13.2 mmol) was added to a stirred solution of methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (**3a**; 770 mg, 1.32 mmol) in methanol (26 mL) at room temperature. After being stirred at 60 °C for 24 h, the reaction mixture was neutralized with triethylamine and concentrated in vacuo. The residue was used for the next reaction without further purification. 9-Fluorenylmethyl succinimidyl carbonate (445 mg, 1.32 mmol) was added to a stirred solution of the residue in CH_3CN/H_20 (11:1; 12.0 mL) at 0 °C. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated in vacuo. The residue was used for the next reaction without further purification. Acetic anhydride (1.10 mL, 7.92 mmol) and a catalytic amount of DMAP were added to a stirred solution of the residue in pyridine (1.30 mL, 15.8 mmol) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was poured into ice-cooled water. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with 1 M HCl, saturated aq. $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (98:2) to give **3e** (296 mg, 0.387 mmol, 27 % over three steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30–7.77 (m, 13H, aromatic), 5.61 (brs, 1H, H-7), 5.45 (ddd, 1H, H-4, $J_{3ax,4}$ = 10.7, $J_{3eq,4}$ = 4.8, $J_{4,5}$ = 10.7 Hz), 5.00 (brd, 1H, H-8, J = 8.3 Hz), 4.89 (d, 1H, NH, J = 10.2 Hz), 4.67 (dd, 1H, H-6, $J_{5,6}$ = 10.2, $J_{6,7}$ = 1.9 Hz), 4.49 (dd, 1H, H-9′, $J_{8,9'}$ = 2.0, J_{gem} = 12.2 Hz), 4.35 (m, 1H), 4.17–4.25 (m, 2H), 4.03 (dd, 1H, H-9, $J_{8,9}$ = 8.8, J_{gem} = 12.2 Hz), 3.84 (ddd, 1H, H-5, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$ = 10.2 Hz), 3.60 (s, 3H, OMe), 2.74 (dd, 1H, H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 13.7 Hz), 2.13 (dd, 1H, H-3ax, $J_{3ax,4}$ = 10.7 Hz), 1.96 × 2, 2.05, 2.13 (3s, 12H, Ac) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 170.6, 170.2, 170.0, 168.2, 155.9, 144.3, 143.5, 141.3, 141.1, 136.1, 129.7, 129.1, 128.8, 127.6, 127.6, 127.0, 126.4, 125.0, 119.9, 119.8, 88.8, 77.2, 73.2, 72.9, 68.9, 67.5, 62.6, 52.5, 51.5, 46.9, 37.5, 21.0, 20.8, 20.3 × 2 ppm; IR (KBr): $\tilde{\nu}$ = 3354, 3022, 2952, 1740, 1534, 1234, 741 cm^{-1}.

Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-β-D-galacto-2-nonulopyranosid)onate (3f): Methanesulfonic acid (0.46 mL, 7.07 mmol) was added to a stirred solution of methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (**3a**; 421.8 mg, 0.707 mmol) in methanol (14 mL) at room temperature. After being stirred at 60 °C for 24 h, the reaction mixture was neutralized with triethylamine and concentrated in vacuo. The residue was used for the next reaction without further purification. Succinimidyl 2,2,2-trichloroethyl carbonate (205 mg, 0.707 mmol) and triethylamine (0.15 mL, 1.06 mmol) were added to a stirred solution of the residue in CH_3CN/H_20 (15:1; 6.40 mL) at 0 °C. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated in vacuo. The residue was used for the next reaction without further purification. Acetic anhydride

(0.40 mL, 4.24 mmol) and a catalytic amount of DMAP were added to a stirred solution of the residue in pyridine (0.69 mL, 4.84 mmol) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was poured into ice-cooled water. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with 1 M HCl, saturated aq. $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (98:2) and recrystallized from ethyl acetate/hexane to give **3f** (450 mg, 0.64 mmol, 89% over three steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31–7.46 (m, 5H, aromatic), 5.51 (dd, 1H, H-7, $J_{6,7}$ = 1.9, $J_{7,8}$ = 2.4 Hz), 5.45 (ddd, 1H, H-4, $J_{3ax,4}$ = 11.2, $J_{3eq,4}$ = 4.9, $J_{4,5}$ = 10.3 Hz), 5.09 (d, 1H, NH, J = 10.2 Hz), 5.01 (d, 1H, J_{gem} = 12.2 Hz), 4.67 (dd, 1H, H-6, $J_{5,6}$ = 10.8, $J_{6,7}$ = 1.9 Hz), 4.49 (d, 1H, J_{gem} = 12.2 Hz), 4.46 (dd, 1H, H-9′, $J_{8,9'}$ = 2.0, J_{gem} = 12.7 Hz), 4.03 (dd, 1H, H-9, $J_{8,9}$ = 8.3, J_{gem} = 12.7 Hz), 3.76 (ddd, 1H, H-5, $J_{4,5}$ = 10.3, $J_{5,6}$ = 10.8, $J_{5,NH}$ = 10.3 Hz), 3.60 (s, 3H, OMe), 2.73 (dd, 1H, H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 13.6 Hz), 2.07 (dd, 1H, H-3ax, $J_{3ax,4}$ = 11.2 Hz), 1.83, 2.01, 2.05, 2.12 (4s, 12H, Ac) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.0, 170.4, 170.3, 170.0, 168.1, 154.3, 136.1, 129.7, 129.1, 128.7, 95.4, 88.8, 77.2, 74.5, 72.8, 69.0, 68.6, 62.5, 51.7, 37.5, 21.0, 20.8, 20.7, 20.7 ppm; IR (KBr): $\tilde{\nu}$ = 3329, 2954, 1743, 1540, 1371, 1253, 1039, 755, 694 cm^{-1}; elemental analysis calcd (%) for $C_{27}H_{32}Cl_3NO_{13}S$: C 45.23, H 4.50, N 1.95; found: C 45.04, H 4.48, N 1.95.

Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-5-trifluoroacetamido-D-glycero-β-D-galacto-2-nonulopyranosid)onate (3g): Methanesulfonic acid (0.86 mL, 13.3 mmol) was added to a stirred solution of methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (**3a**; 775 mg, 1.33 mmol) in methanol (26 mL) at room temperature. After being stirred at 60 °C for 24 h, the reaction mixture was neutralized with triethylamine and concentrated in vacuo. The residue was used for the next reaction without further purification. Trifluoroacetic acid methyl ester (1.34 mL, 13.3 mmol) and triethylamine (0.37 mL, 2.66 mmol) were added to a stirred solution of the residue in methanol (13 mL) at 0 °C. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated in vacuo. The residue was used for the next reaction without further purification. Acetic anhydride (0.75 mL, 7.98 mmol) and a catalytic amount of DMAP were added to a stirred solution of the residue in pyridine (1.30 mL, 16.0 mmol) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was poured into ice-cooled water. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with 1 M HCl, saturated aq. $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (98:2) and recrystallized from ethyl acetate/hexane to give **3g** (656 mg, 1.03 mmol, 77% over three steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.43 (m, 6H, NH, aromatic), 5.55 (ddd, 1H, H-4, $J_{3ax,4}$ = 13.7, $J_{3eq,4}$ = 4.9, $J_{4,5}$ = 10.0 Hz), 5.51 (brs, 1H, H-7), 5.02 (ddd, 1H, H-8, $J_{7,8}$ = 2.0, $J_{8,9'}$ = 2.4, $J_{8,9}$ = 8.0 Hz), 4.86 (dd, 1H, H-6, $J_{5,6}$ = 10.8, $J_{6,7}$ = 2.4 Hz), 4.53 (dd, 1H, H-9′, $J_{8,9'}$ = 2.0, J_{gem} = 10.2 Hz), 4.13 (ddd, 1H, H-5, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$ = 10.2 Hz), 4.05 (dd, 1H, H-9, $J_{8,9}$ = 8.8, J_{gem} = 10.2 Hz), 3.57 (s, 3H, OMe), 2.73 (dd, 1H, H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 12.9 Hz), 2.16 (dd, 1H, H-3ax, $J_{3ax,4}$ = 13.7 Hz), 1.95, 2.07, 2.11, 2.12 (4s, 12H, Ac) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.7, 171.1, 170.3×2, 169.9, 167.9, 136.5, 136.0, 130.0, 129.8, 129.1, 128.9, 128.6, 89.1, 73.2, 72.1, 68.5, 68.4, 62.5, 52.6, 50.0, 37.4, 21.0, 20.7, 20.6, 20.5 ppm; IR (KBr): $\tilde{\nu}$ = 3056, 2971, 1708, 1410, 1205, 760 cm^{-1}.

Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-5-trichloroacetamido-D-glycero-β-D-galacto-2-nonulopyranosid)onate (3h): Methanesulfonic acid (1.95 mL, 30.0 mmol) was added to a stirred solution of methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-β-D-galacto-2-nonulopyranosid)onate (**3a**; 1.75 g, 3.00 mmol) in methanol (30 mL) at room temperature. After being stirred at 60 °C for 24 h, the reaction mixture was neutralized with triethylamine and concentrated in vacuo. The residue was used for the next reaction without further purification. Trichloroacetic acid methyl ester (3.57 mL, 30.0 mmol) and triethylamine (0.84 mL, 6.00 mmol) were added to a stirred solution of the residue in methanol (30 mL) at 0 °C. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated in vacuo. The residue was used for the next reaction without further purification. Acetic anhydride (1.70 mL, 18.0 mmol) and a catalytic amount of DMAP were added to a stirred solution of the residue in pyridine (2.90 mL, 36.0 mmol) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was poured into ice-cooled water. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with 1 M HCl, saturated aq. $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (98:2) and recrystallized from ethyl acetate/hexane to give **3h** (1.44 g, 2.10 mmol, 70% over three steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31–7.47 (m, 5H, aromatic), 7.19 (d, 1H, NH, J = 10.3 Hz), 5.57 (ddd, 1H, H-4, $J_{3ax,4}$ = 10.8, $J_{3eq,4}$ = 4.9 Hz), 5.53 (dd, 1H, H-7, $J_{6,7}$ = 2.4, $J_{7,8}$ = 2.4 Hz), 5.07 (ddd, 1H, H-8, $J_{7,8}$ = 2.4, $J_{8,9'}$ = 2.0, $J_{8,9}$ = 8.8 Hz), 4.89 (dd, 1H, H-6, $J_{5,6}$ = 10.7, $J_{6,7}$ = 2.4 Hz), 4.55 (dd, 1H, H-9′, $J_{8,9'}$ = 2.0, J_{gem} = 12.7 Hz), 4.08 (dd, 1H, H-9, $J_{8,9}$ = 8.3, J_{gem} = 12.7 Hz), 4.06 (ddd, 1H, H-5), 3.58 (s, 3H, OMe), 2.72 (dd, 1H, H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 12.7 Hz), 2.12 (dd, 1H, H-3ax, $J_{3ax,4}$ = 10.8 Hz), 1.92, 2.04, 2.11, 2.14 (4s, 12H, Ac) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.0, 170.5, 170.2, 170.0, 167.8, 162.4, 136.2, 129.8, 129.1, 128.9, 128.8, 128.1, 92.0, 89.2, 73.6, 72.3, 68.5, 68.1, 62.5, 52.6, 51.4, 37.5, 21.1, 20.8, 20.6, 20.5 ppm; IR (KBr): $\tilde{\nu}$ = 3319, 2953, 1744, 1526, 1232, 1038, 941, 751 cm^{-1}.

General procedure for sialylation of methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (4) with thiosialosides 3a–i:

Method A (NIS/TfOH, CH_3CN): A mixture of the appropriate thiosialoside (**3a–i**; 1.00 equiv), methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**; 1.50 equiv; azeotroped three times with toluene), and pulverized activated 3 Å MS (10 g mmol^{-1}) in dry CH_3CN was stirred at room temperature for 10 min under argon to remove any trace amounts of water. The reaction mixture was then cooled to −35 °C. *N*-Iodosuccinimide (1.20 equiv) and a catalytic amount of trifluoromethanesulfonic acid (0.20 equiv) was added to the reaction mixture at −35 °C. After being stirred at the same temperature for 1.0 h, the reaction mixture was neutralized with triethylamine and filtered through a pad of celite. The filtrate was poured into a mixture of saturated aq. $NaHCO_3$ and saturated aq. $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with saturated aq. $NaHCO_3$/$Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (97:3) to give the respective disaccharide (**5a–i**). The α:β ratio was determined by HPLC analysis (Senshu Pak Silica-3301-N column; eluent: hexane/2-propanol 94:6; flow rate: 3.0 mL min^{-1}).

Method B (DMTST, CH_3CN): A mixture of the appropraite thiosialoside (**3a–i**; 1.00 equiv), methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**; 1.50 equiv; azeotroped three times with toluene), and pulverized activated 3 Å MS (10 g mmol^{-1}) in dry CH_3CN was stirred at room temperature for 10 min under argon to remove any trace amounts of water. The reaction mixture was then cooled to −10 °C. A 1.5 M CH_3CN solution of dimethyl(methylthio)sulfonium trifluoromethanesulfonate (1.50 equiv) was added to the reaction mixture at −10 °C. After being stirred at the same temperature for 8 h, the reaction mixture was neutralized with triethylamine and filtered through a pad of celite. The filtrate was poured into saturated aq. $NaHCO_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with saturated aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (97:3) to give the respective disaccharide (**5a–i**). The α:β ratio was determined by HPLC analysis (conditions as for method A).

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-D-galacto-2-nonulopyranosylonate)-α-D-glucopyranoside (5f): In accordance with method A, thiosialoside **3f** (17.2 mg, 0.0239 mmol) was treated with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**; 16.7 mg, 0.0360 mmol) and *N*-iodosuccinimide (10.8 mg, 0.0478 mmol) in CH_3CN (0.25 mL) to provide disaccharide **5f** (23.2 mg, 0.0217 mmol, 91%, α:β = 89:11). In accordance with method B, thiosialoside **3f** (17.7 mg, 0.0247 mmol) was treated with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**; 17.2 mg, 0.037 mmol)

and DMTST (25 μL, 0.037 mmol, 1.50 equiv) in CH_3CN (0.25 mL) to provide disaccharide **5f** (55.0 mg, 0.0136 mmol, 55%, α:β = 86:14).

5f α isomer: R_t = 19.2 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30–3.74 (m, 15H, aromatic), 5.31 (brs, 2H, Neu-H-7, H-8), 4.98 (ddd, 1H, Neu-H-4, $J_{3ax,4}$ = 12.7, $J_{3eq,4}$ = 4.9 Hz), 4.91 (d, 1H, J_{gem} = 10.8 Hz), 4.89 (d, 1H, J_{gem} = 12.2 Hz), 4.85 (d, 1H, J_{gem} = 10.8 Hz), 4.84 (d, 1H, Neu-NH, J = 10.7 Hz), 4.79 (d, 1H, J_{gem} = 12.7 Hz), 4.78 (d, 1H, J_{gem} = 10.7 Hz), 4.74 (d, 1H, J_{gem} = 11.2 Hz), 4.66 (d, 1H, J_{gem} = 12.2 Hz), 4.61 (d, 1H, Glc-H-1, $J_{1,2}$ = 3.9 Hz), 4.44 (d, 1H, J_{gem} = 12.2 Hz), 4.21 (dd, 1H, Glc-H-6′, $J_{5,6'}$ = 3.9, J_{gem} = 11.2 Hz), 4.18 (brd, 1H, Neu-H-6, $J_{5,6}$ = 10.2 Hz), 4.00 (brd, 1H, Neu-H-9′, J_{gem} = 10.2 Hz), 3.95 (dd, 1H, Glc-H-3, $J_{2,3}$ = 9.3, $J_{3,4}$ = 9.3 Hz), 3.73–3.80 (m, 5H, Neu-OMe, H-5, H-9), 3.60 (dd, 1H, Glc-H-4, $J_{3,4}$ = 9.3, $J_{4,5}$ = 9.3 Hz), 3.56 (ddd, 1H, Glc-H-5, $J_{4,5}$ = 9.3, $J_{5,6'}$ = 3.9, $J_{5,6}$ = 2.0 Hz), 3.52 (dd, 1H, Glc-H-2, $J_{1,2}$ = 3.9, $J_{2,3}$ = 9.3 Hz), 3.42 (dd, 1H, Glc-H-6, $J_{5,6}$ = 2.0, J_{gem} = 11.2 Hz), 3.36 (s, 3H, Glc-OMe), 2.72 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 12.7 Hz), 1.89 (dd, 1H, Neu-H-3ax, $J_{3ax,4}$ = 12.7 Hz), 1.82, 2.01 × 2, 2.12 (3s, 12H, Ac) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6, 170.4, 169.9, 169.7, 167.8, 154.0, 138.8, 138.6, 138.2, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 98.4, 98.2, 95.3, 82.0, 82.0, 79.4, 77.7, 75.8, 74.7, 74.5, 73.4, 71.7, 69.5, 68.4, 67.4, 66.9, 63.5, 61.7, 55.2, 52.8, 51.6, 38.3, 21.1, 20.8, 20.4 ppm; IR (KBr): $\tilde{\nu}$ = 3333, 2927, 1744, 1535, 1454, 1369, 1218, 1042, 735, 699 cm^{-1}.

5f β isomer: R_t = 24.9 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29–7.42 (m, 15H, aromatic), 5.44 (brs, 1H, Neu-H-7), 5.31 (brd, 1H, Neu-H-8, J = 9.3 Hz), 5.25 (ddd, 1H, Neu-H-4, $J_{3ax,4}$ = 11.7, $J_{3eq,4}$ = 5.4 Hz), 5.06 (brd, 2H, Neu-NH, H-9′), 4.97 (d, 1H, J_{gem} = 10.8 Hz), 4.95 (d, 1H, J_{gem} = 12.2 Hz), 4.89 (d, 1H, Glc-H-1, $J_{1,2}$ = 3.4 Hz), 4.87 (d, 1H, J_{gem} = 10.8 Hz), 4.83 (d, 1H, J_{gem} = 11.2 Hz), 4.80 (d, 1H, J_{gem} = 11.7 Hz), 4.76 (d, 1H, J_{gem} = 11.2 Hz), 4.70 (d, 1H, J_{gem} = 10.8 Hz), 4.43 (d, 1H, J_{gem} = 12.2 Hz), 4.39 (dd, 1H, Neu-H-6, $J_{5,6}$ = 10.8, $J_{6,7}$ = 2.0 Hz), 4.13 (dd, 1H, Neu-H-9, $J_{8,9}$ = 9.3, J_{gem} = 12.2 Hz), 3.97 (dd, 1H, Glc-H-3, $J_{2,3}$ = 8.8, $J_{3,4}$ = 8.8 Hz), 3.71–3.84 (m, 8H, Neu-OMe, H-5, Glc-H-4, H-5, H-6′, H-6), 3.63 (dd, 1H, Glc-H-2, $J_{1,2}$ = 3.4, $J_{2,3}$ = 9.8 Hz), 3.35 (s, 3H, Glc-OMe), 2.54 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 5.4, J_{gem} = 13.2 Hz), 1.98, 2.00, 2.04, 2.15 (4s, 12H, Ac), 1.83 (dd, 1H, Neu-H-3ax, $J_{3ax,4}$ = 11.7 Hz) ppm; IR (KBr): $\tilde{\nu}$ = 3428, 2927, 1745, 1568, 1454, 1229, 1215, 1005, 733 cm^{-1}.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-(9-fluorenylmethoxycarbonylamino)-D-glycero-D-galacto-2-nonulopyranosylonate)-α-D-glucopyranoside (5e): In accordance with method A, thiosialoside **3e** (23.0 mg, 0.030 mmol) was treated with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**; 21.0 mg, 0.0452 mmol) and *N*-iodosuccinimide (8.12 mg, 0.0361 mmol) in CH_3CN (0.30 mL) to provide disaccharide **5e** (30.7 mg, 0.0275 mmol, 91%, α:β = 86:14). In accordance with method B, thiosialoside **3e** (20.0 mg, 0.0262 mmol) was treated with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**; 18.2 mg, 0.0393 mmol) and DMTST (26 μL, 0.039 mmol) in CH_3CN (0.25 mL) to provide disaccharide **5e** (16.4 mg, 0.0147 mmol, 56%, α:β = 86:14).

5e α isomer: R_t = 19.3 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, 2H, aromatic), 7.53–7.60 (m, 2H, aromatic), 7.30–7.40 (m, 19H, aromatic), 5.31–5.41 (m, 2H, Neu-H-7, H-8), 4.91 (d, 1H, J_{gem} = 11.2 Hz), 4.90 (m, 1H, Neu-H-4), 4.87 (d, 1H, J_{gem} = 10.8 Hz), 4.80 (d, 1H, J_{gem} = 12.2 Hz), 4.79 (d, 1H, J_{gem} = 11.2 Hz), 4.75 (d, 1H, J_{gem} = 10.7 Hz), 4.66 (d, 1H, J_{gem} = 12.2 Hz), 4.60 (d, 1H, Glc-H-1, $J_{1,2}$ = 3.4 Hz), 4.59 (d, 1H, Neu-NH, J = 11.2 Hz), 4.10–4.34 (m, 5H, Neu-H-6, Glc-H-6′), 4.03 (dd, 1H, Neu-H-9′, $J_{8,9'}$ = 2.0, J_{gem} = 12.7 Hz), 3.95 (dd, 1H, Glc-H-3, $J_{2,3}$ = 9.8, $J_{3,4}$ = 9.8 Hz), 3.73–3.77 (m, 5H, Neu-H-9, OMe, Glc-H-5), 3.71 (m, 1H, Neu-H-5), 3.60 (dd, 1H, Glc-H-4, $J_{3,4}$ = 9.8, $J_{4,5}$ = 3.4 Hz), 3.51 (dd, 1H, Glc-H-2, $J_{1,2}$ = 3.4, $J_{2,3}$ = 9.8 Hz), 3.43 (brd, 1H, Glc-H-6, J = 9.8 Hz), 3.36 (s, 3H, Glc-OMe), 2.70 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 4.4, J_{gem} = 12.7 Hz), 1.83, 1.93, 1.97, 2.13 (4s, 12H, Ac), 1.92 (dd, 1H, Neu-H-3ax) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6 × 2, 169.8, 167.9, 155.7, 155.7, 144.2, 143.4, 141.3, 141.1, 138.8, 138.6, 138.2, 128.6, 128.4, 128.3, 128.1, 127.9, 127.9, 127.7, 127.5, 127.0, 125.2, 124.9, 119.9, 119.9, 98.5, 98.2, 82.0, 75.8, 74.8, 73.3, 72.3, 69.5, 68.8, 67.6, 67.4, 66.8, 63.5, 61.8, 55.2, 52.8, 51.4, 46.9, 38.3, 21.0, 20.8, 20.7, 20.5 ppm; IR (KBr): $\tilde{\nu}$ = 3385, 2928, 1745, 1532, 1452, 1217, 1040, 740 cm^{-1}.

5e β isomer: R_t = 23.9 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75 (d, 2H, aromatic), 7.56–7.63 (m, 2H, aromatic), 7.26–7.47 (m, 19H, aromatic), 5.54 (brs, 1H, Neu-H-7), 5,32 (brd, 1H, Neu-H-8, J = 8.8 Hz), 5.21 (ddd, 1H, Neu-H-4, $J_{3ax,4}$ = 11.7, $J_{3eq,4}$ = 4.9 Hz), 5.09 (dd, 1H, Neu-H-9′, $J_{8,9'}$ = 1.5, J_{gem} = 12.2 Hz), 4.98 (d, 1H, J_{gem} = 11.2 Hz), 4.92 (d, 1H, Glc-H-1, $J_{1,2}$ = 3.4 Hz), 4.90 (d, 1H, Neu-NH, J = 10.2 Hz), 4.89 (d, 1H, J_{gem} = 11.2 Hz), 4.83 (d, 1H, J_{gem} = 10.7 Hz), 4.79 (2d, 2H), 4.71 (d, 1H, J_{gem} = 11.2 Hz), 4.20–4.38 (m, 4H, Neu-H-6), 4.15 (dd, 1H, Neu-H-9, $J_{8,9}$ = 9.3, J_{gem} = 12.2 Hz), 3.98 (dd, 1H, Glc-H-3, $J_{2,3}$ = 9.3, $J_{3,4}$ = 9.3 Hz), 3.71–3,86 (m, 8H, Neu-H-5, OMe, Glc-H-4, H-5, H-6′, H-6), 3.65 (dd, 1H, Glc-H-2, $J_{1,2}$ = 3.4, $J_{2,3}$ = 9.3 Hz), 3.36 (s, 3H, Glc-OMe), 2.54 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 12.7 Hz), 1.93, 1.97, 2.02, 2.17 (4s, 12H, Ac), 1.86 (dd, 1H, Neu-H-3ax, $J_{3ax,4}$ = 11.7 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7, 170.6, 170.4, 170.3, 167.2, 155.9, 144.3, 143.5, 141.3, 138.7, 138.5, 138.2, 128.6, 128.4, 128.3, 128.0, 128.0, 127.8, 127.6, 127.5, 127.0, 125.4, 125.0, 119.9, 97.9, 97.8, 82.0, 80.1, 77.3, 75.8, 75.1, 73.6, 73.2, 71.7, 69.2, 69.1, 68.8, 67.4, 62.9, 62.1, 54.9, 52.7, 51.4, 47.0, 37.6, 29.7, 21.0, 20.9, 20.8, 20.7 ppm; IR (KBr): $\tilde{\nu}$ = 3387, 2928, 11742, 1518, 1452, 1370, 1224, 1074, 741, 698 cm^{-1}.

Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-D-galacto-2-nonulopyranosylonate)-α-D-glucopyranoside (5g): In accordance with method A, thiosialoside **3g** (22.5 mg, 0.0353 mmol) was treated with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**; 24.6 mg, 0.0529 mmol) and *N*-iodosuccinimide (9.50 mg, 0.0424 mmo) in CH_3CN (0.35 mL) to provide disaccharide **5g** (32.1 mg, 0.0323 mmol, 92%, α:β = 92:8). In accordance with method B, thiosialoside **3g** (17.7 mg, 0.0277 mmol) was treated with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**4**; 19.3 mg, 0.0416 mmol) and DMTST (28 μL, 0.042 mmol) in CH_3CN (0.28 mL) to provide disaccharide **5g** (10.9 mg, 0.011 mmol, 40%, α:β = 85:15).

5g α isomer: R_t = 22.6 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.18–7.39 (m, 15H, aromatic), 6.50 (d, 1H, Neu-NH, J = 10.3 Hz), 5.31 (ddd, 1H, Neu-H-8, $J_{7,8}$ = 8.8, $J_{8,9'}$ = 2.0, $J_{8,9}$ = 4.9 Hz), 5.22 (dd, 1H, Neu-H-7, $J_{6,7}$ = 2.0, $J_{7,8}$ = 8.8 Hz), 4.99 (m, 1H, Neu-H-4), 4.92 (d, 1H, J_{gem} = 11.2 Hz), 4.80–4.84 (2d, 2H), 4.79 (d, 1H, J_{gem} = 11.7 Hz), 4.75 (d, 1H, J_{gem} = 11.2 Hz), 4.66 (d, 1H, J_{gem} = 12.2 Hz), 4.60 (d, 1H, Glc-H-1, $J_{1,2}$ = 3.4 Hz), 4.25 (dd, 1H, Neu-H-6, $J_{5,6}$ = 10.7, $J_{6,7}$ = 2.0 Hz), 4.20 (dd, 1H, Glc-H-6′, $J_{5,6'}$ = 3.9, J_{gem} = 10.8 Hz), 4.06 (dd, 1H, Neu-H-9′, $J_{8,9'}$ = 2.0, J_{gem} = 10.2 Hz), 3.95 (dd, 1H, Glc-H-3, $J_{2,3}$ = 9.8, $J_{3,4}$ = 9.8 Hz), 3.90 (ddd, 1H, Neu-H-5, $J_{5,6}$ = 10.7, $J_{5,NH}$ = 10.3 Hz), 3.76 (dd, 1H, Neu-H-9, $J_{8,9}$ = 4.9, J_{gem} = 10.2 Hz), 3.74 (s, 3H, Neu-OMe), 3.58 (dd, 1H, Glc-H-4, $J_{3,4}$ = 9.8, $J_{4,5}$ = 9.3 Hz), 3.51 (dd, 1H, Glc-H-2, $J_{1,2}$ = 3.4, $J_{2,3}$ = 9.8 Hz), 3.45 (dd, 1H, Glc-H-6, $J_{5,6}$ = 1.4, J_{gem} = 10.8 Hz), 3.36 (s, 3H, Glc-OMe), 2.70 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 13.2 Hz), 1.84, 1.99, 2.00, 2.13 (4s, 12H, Ac), 1.96 (dd, 1H, Neu-H-3ax, $J_{3ax,4}$ = 12.7 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.8, 170.6, 169.9, 167.7, 157.7, 157.3, 138.7, 138.5, 138.1, 128.9, 128.6, 128.4, 128.3, 128.2, 128.1, 127.9, 127.9, 127.7, 127.7, 127.6, 98.6, 98.2, 81.9, 79.4, 79.1, 75.8, 74.7, 73.3, 71.4, 69.4, 68.2, 67.8, 66.6, 63.6, 61.7, 55.2, 52.9, 50.2, 38.0, 21.0, 20.6, 20.5, 20.3 ppm; IR (KBr): $\tilde{\nu}$ = 3318, 2929, 1751, 1562, 1456, 1371, 1216, 1056, 752 cm^{-1}.

5g β isomer: R_t = 27.0 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.10–7.33 (m, 15H, aromatic), 7.02 (d, 1H, Neu-NH, J = 10.3 Hz), 5.27 (brs, 1H, Neu-H-7), 5.15–5.24 (m, 2H, Neu-H-4, H-8), 4.97 (dd, 1H, Neu-H-9′, $J_{8,9'}$ = 2.4, J_{gem} = 12.7 Hz), 4.90 (d, 1H, J_{gem} = 11.2 Hz), 4.79 (d, 1H, J_{gem} = 12.7 Hz), 4.78 (d, 1H, Glc-H-1, $J_{1,2}$ = 3.9 Hz), 4.77 (d, 1H, J_{gem} = 10.7 Hz), 4.74 (2d, 2H), 4.61 (d, 1H, J_{gem} = 12.7 Hz), 4.39 (dd, 1H, Neu-H-6, $J_{5,6}$ = 10.7, $J_{6,7}$ = 2.0 Hz), 4.04 (dd, 1H, Neu-H-9, $J_{8,9}$ = 9.3, J_{gem} = 12.7 Hz), 3.88–4.01 (m, 2H, Neu-H-5, Glc-H-3), 3.64–3.75 (m, 7H, Neu-OMe, Glc-H-4, H-5, H-6′, H-6), 3.27 (s, 3H, Glc-OMe), 2.45 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 5.4, J_{gem} = 12.7 Hz), 1.90, 1.92, 1.98, 2.07 (4s, 12H, Ac), 1.80 (dd, 1H, Neu-H-3ax, $J_{3ax,4}$ = 12.2 Hz) ppm; IR (KBr): $\tilde{\nu}$ = 3374, 2926, 2855, 2749, 1456, 1372, 1229, 1029, 803, 745, 699 cm^{-1}.

***N*-(Benzyloxycarbonyl) 3-*O*-(2-azido-4-*O*-benzyl-2-deoxy-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranosyl)-L-serine benzyl ester (9)**: A mixture of thiosialoside **3f** (20.0 mg, 0.0279 mmol), *N*-(benzyloxycarbonyl)-3-*O*-(2-azido-4-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-L-serine benzyl ester (**7**; 14.1 mg, 0.0232 mmol; azeotroped three times with toluene), and pulverized activated 3 Å MS (11.6 mg) in dry CH_3CN (0.116 mL) was stirred at room temperature for

10 min under argon to remove any trace amounts of water. The reaction mixture was then cooled to −35 °C. *N*-iodosuccinimide (7.52 mg, 0.0334 mmol) and a catalytic amount of trifluoromethanesulfonic acid (0.20 μL, 4.64 μmol, 0.10 equiv) were added to the reaction mixture at −35 °C. After being stirred at the same temperature for 1 h, the reaction mixture was neutralized with triethylamine and filtered through a pad of celite. The filtrate was poured into a mixture of saturated aq. $NaHCO_3$ and saturated aq. $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with saturated aq. $NaHCO_3$/$Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (97:3) and further purified by gel-permeation chromatography to give **9** (26.2 mg, 2.16 μmol, 93 %, α:β = 78:22). The α:β ratio was determined by HPLC analysis (Senshu Pak Silica-3301-N column; eluent: hexane/2-propanol 90:10; flow rate: 3.0 mL min^{-1}; retention times: α isomer = 10.7 min, β isomer = 11.7 min). The α,β isomers were separated by chromatography on silica gel with toluene/acetone (86:14) to give the α isomer and with toluene/acetone (88:12) to give the β isomer.

9α isomer: $[\alpha]_D^{21}$ +43.4 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30–7.60 (m, 15H, aromatic), 5.89 (d, 1H, Ser-NH, J = 8.3 Hz), 5.40 (ddd, 1H, Neu-H-8), 5.36 (dd, 1H, Neu-H-7, $J_{6,7}$ = 1.4, $J_{7,8}$ = 8.8 Hz), 5.35 (d, 1H, J_{gem} = 12.2 Hz), 5.14 (d, 1H, J_{gem} = 12.6 Hz), 5.11 (2d, 2H), 5.00 (br dd, 1H, Neu-H-4, $J_{3ax,4}$ = 4.9, $J_{3eq,4}$ = 10.8 Hz), 4.94 (d, 1H, Neu-Troc-NH, J = 10.3 Hz), 4.90 (d, 1H, J_{gem} = 12.2 Hz), 4.79 (d, 1H, J_{gem} = 11.7 Hz), 4.78 (d, 1H, GalNAc-H-1, $J_{1,2}$ = 3.4 Hz), 4.65 (d, 1H, J_{gem} = 11.2 Hz), 4.60 (m, 1H, Ser-α), 4.36 (d, 1H, J_{gem} = 12.2 Hz), 4.24 (d, 1H, Neu-H-9′, $J_{8,9'}$ = 1.9, J_{gem} = 12.7 Hz), 4.16 (br d, 1H, Neu-H-6, J = 10.8 Hz), 4.06 (dd, 1H, Neu-H-9, $J_{8,9}$ = 4.9, J_{gem} = 12.7 Hz), 3.91–4.01 (m, 3H, GalNAc-H-6′, Ser-β), 3.79–3.85 (m, 3H, GalNAc-H-3, H-4, H-6), 3.65–3.69 (m, 4H, Neu-H-5, OMe), 3.52 (br dd, 1H, GalNAc-H-5), 3.36 (dd, 1H, GalNAc-H-2, $J_{1,2}$ = 3.0, $J_{2,3}$ = 10.3 Hz), 2.66 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 13.2 Hz), 2.25 (d, 1H, GalNAc-3OH, J = 8.8 Hz), 1.97, 2.00, 2.09, 2.11 (4s, 12H, Ac), 1.92 (dd, 1H, Neu-H-3ax, $J_{3ax,4}$ = 10.8 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.0, 170.7, 170.5, 169.8, 166.6, 156.0, 154.5, 138.0, 136.0, 134.9, 128.6, 128.6, 128.5, 128.4, 128.1, 127.9, 127.8, 98.7, 98.2, 95.5, 77.9, 77.2, 75.4, 74.4, 72.3, 71.8, 70.7, 69.2, 68.4, 68.2, 68.1, 67.9, 67.2, 63.3, 62.7, 60.4, 53.8, 52.7, 50.9, 37.0, 20.9, 20.8, 20.7, 20.6 ppm; IR (KBr): $\tilde{\nu}$ = 3347, 2534, 2109, 1745, 1523, 1369, 1218, 1038, 738, 738, 698 cm^{-1}; elemental analysis calcd (%) for $C_{52}H_{60}Cl_3N_5O_{22}$: C 41.47, H 4.98, N 5.77; found: C 51.03, H 5.02, N 5.47.

9β isomer: $[\alpha]_D^{22}$ +37.5 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30–7.39 (m, 15H, aromatic), 6.36 (d, 1H, Ser-NH, J = 8.8 Hz), 6.22 (d, 1H, Neu-Troc-NH, J = 10.3 Hz), 5.54 (ddd, 1H, Neu-H-4, $J_{3ax,4}$ = 12.2, $J_{3eq,4}$ = 4.4 Hz), 5.47 (br s, 1H, Neu-H-7), 5.37 (d, 1H, J_{gem} = 13.9 Hz), 5.34 (m, 1H, Neu-H-8), 5.12 (d, 1H, J_{gem} = 13.9 Hz), 5.11 (2d, 2H), 4.96 (br d, 1H, Neu-H-9′, J = 11.2 Hz), 4.89 (d, 1H, GalNAc-H-1, $J_{1,2}$ = 2.9 Hz), 4.76 (m, 1H, Ser-α), 4.75 (d, 1H, J_{gem} = 12.2 Hz), 4.69 (2d, 2H), 4.53 (d, 1H, J_{gem} = 11.7 Hz), 4.10 (m, 1H, Ser-β′), 4.03 (dd, 1H, Neu-H-9, $J_{8,9}$ = 8.8, J_{gem} = 12.7 Hz), 3.94 (m, 2H, Neu-H-6, H-5), 3.62 (m, 9H, Neu-H-5, OMe, GalNAc-H-3, H-4, H-6′, H-6), 3.36 (dd, 1H, GalNAc-H-2, $J_{1,2}$ = 2.9, $J_{2,3}$ = 10.8 Hz), 2.51 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 4.4, J_{gem} = 13.9 Hz), 1.96 (d, 1H, GalNAc-3OH), 1.91, 1.97×2, 2.01 (3s, 12H, Ac), 1.83 (dd, 1H, Neu-H-3ax, $J_{3ax,4}$ = 12.2 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7, 170.3, 170.0, 169.8, 169.7, 167.7, 156.0, 154.0, 138.0, 156.0, 138.0, 136.1, 135.1, 128.5, 128.5, 128.1, 128.0, 128.0, 127.8, 99.3, 98.4, 95.3, 76.6, 75.1, 74.4, 71.9, 69.6, 69.1, 68.3, 68.0, 67.9, 67.5, 67.3, 67.0, 63.0, 62.2, 60.8, 54.4, 52.8, 51.4, 37.7, 21.0, 20.8, 20.7, 20.6 ppm; IR (KBr): $\tilde{\nu}$ = 3368, 2955, 2110, 1744, 1530, 1370, 1223, 1036, 944, 736, 696 cm^{-1}.

***N*-(Benzyloxycarbonyl) 3-*O*-(2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-D-galactopyranosyl)-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranosyl)-L-serine benzyl ester (11)**: A mixture of **9α** (21.4 mg, 0.0176 mmol), phenylthio 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**8a**; 17.1 mg, 0.0265 mmol; azeotroped three times with toluene), and pulverized activated 3 Å MS (8.80 mg) in dry CH_3CN/CH_2Cl_2 (1:9; 2.00 mL) was stirred at room temperature for 10 min under argon to remove any trace amounts of water. The reaction mixture was then cooled to −30 °C. *N*-iodosuccinimide (7.90 mg, 0.0352 mmol) and a catalytic amount of trifluoromethanesulfonic acid (0.50 μL, 8.80 μmol) were added to the reaction mixture at −30 °C. After being stirred at the same temperature for 1 h, the reaction mixture was neutralized with triethylamine and filtered through a pad of celite. The filtrate was poured into a mixture of saturated aq. $NaHCO_3$ and saturated aq. $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with saturated aq. $NaHCO_3$/$Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (97:3) and further purified by gel-permeation chromatography to give **11** (25.3 mg, 0.0145 mmol, 82 %, α:β = 6:94). The α–α:α–β ratio was determined by HPLC analysis (Senshu Pak Silica-3301-N column; eluent: hexane/2-propanol 95:5; flow rate: 3.0 mL min^{-1}; retention times: α–α isomer = 12.9 min, α–β isomer = 16.1 min). The α–α,α–β isomers were separated by chromatography on silica gel with toluene/acetone (91:9) to give the α–α isomer and with toluene/acetone (90:10) to give the α–β isomer.

11α–β isomer: $[\alpha]_D^{21}$ +23.8 (*c* 1.36, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05 (d, 2H, aromatic), 7.13–7.57 (m, 33H, aromatic), 5.74 (dd, 1H, Gal-H-2, $J_{1,2}$ = 7.3, $J_{2,3}$ = 8.2 Hz), 5.74 (d, 1H, Ser-NH, J = 6.8 Hz), 5.39 (m, 1H, Neu-H-8), 5.35 (dd, 1H, Neu-H-7, $J_{6,7}$ < 1, $J_{7,8}$ = 8.3 Hz), 5.09–5.17 (m, 4H), 5.05 (d, 1H, J = 11.2 Hz), 4.97 (ddd, 1H, Neu-H-4, $J_{3ax,4}$ = 12.2, $J_{3aq,4}$ = 4.9, $J_{4,5}$ = 9.6 Hz), 4.94 (d, 1H, J = 11.7 Hz), 4.90 (d, 1H, J = 12.7 Hz), 4.87 (d, 1H, J = 10.8 Hz), 4.77 (d, 1H, Gal-H-1, $J_{1,2}$ = 7.8 Hz), 4.69 (d, 1H, GalNAc-H-1, $J_{1,2}$ = 3.4 Hz), 4.67 (d, 1H, J = 12.2 Hz), 4.61 (d, 1H, J = 11.2 Hz), 4.58 (d, 1H, J = 11.7 Hz), 4.56 (m, 1H, Ser-α), 4.53 (d, 1H, J = 12.7 Hz), 4.46 (d, 1H, J = 12.2 Hz), 4.45 (d, 1H, J = 12.2 Hz), 4.29 (dd, 1H, Neu-H-9′, J_{gem} = 11.2 Hz), 4.14 (dd, 1H, Neu-H-9, $J_{8,9}$ = 4.9, J_{gem} = 11.2 Hz), 4.13 (dd, 1H, Neu-H-6, $J_{5,6}$ = 9.8, $J_{6,7}$ < 1 Hz), 4.05 (br dd, 1H, Gal-H-4), 3.89–3.99 (m, 4H, Ser-β, GalNAc-H-3, H-6′), 3.67–3.69 (m, 6H, GalNAc-H-4, H-6, Gal-H-3, H-5, H-6, H-6′), 3.59–3.64 (m, 4H, Neu-H-5, OMe), 3.50 (dd, 1H, GalNAc-H-2, $J_{1,2}$ = 3.0, $J_{2,3}$ = 11.2 Hz), 3.20 (ddd, 1H, GalNAc-H-5, J = 4.9, J = 9.3 Hz), 2.56 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 9.3, J_{gem} = 12.7 Hz), 1.97, 1.99, 2.07, 2.11 (4s, 12H, Ac), 1.84 (dd, 1H, Neu-H-3ax, $J_{3ax,4}$ = 12.2 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6, 170.3, 170.1, 169.8, 169.6, 169.6, 167.7, 165.3, 156.0, 154.0, 138.6, 138.5, 137.7, 137.6, 136.2, 135.1, 132.8, 132.8, 130.2, 129.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 127.3, 102.6, 99.3, 98.4, 95.4, 79.7, 77.2, 76.8, 75.3, 74.5, 74.4, 74.3, 73.6, 73.4, 72.6, 72.1, 72.0, 71.8, 69.8, 68.7, 68.6, 68.4, 68.3, 67.5, 67.1, 63.8, 62.1, 59.4, 54.5, 52.7, 51.5, 37.7, 21.0, 20.7, 20.7 ppm; IR (KBr): $\tilde{\nu}$ = 3347, 2952, 2109, 1745, 1520, 1454, 1218, 1040, 737, 698 cm^{-1}.

11α–α isomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.88–7.91 (m, 35H, aromatic), 5.89 (d, 1H, Ser-NH, J = 7.9 Hz), 5.61 (dd, 1H, Gal-H-2, $J_{1,2}$ = 3.4, $J_{2,3}$ = 10.3 Hz), 5.55 (d, 1H, Gal-H-1, $J_{1,2}$ = 3.4 Hz), 5.36 (br s, 2H, Neu-H-7, H-8), 5.20 (d, 1H, J_{gem} = 12.2 Hz), 5.10 (d, 1H, J_{gem} = 12.3 Hz), 5.09 (2d, 2H), 4.91 (m, 3H, Neu-H-4), 4.81 (d, 1H, Neu-Troc-NH, J = 10.3 Hz), 4.79 (d, 1H, GalNAc-H-1, $J_{1,2}$ = 3.4 Hz), 4.63 (d, 1H, J_{gem} = 12.3 Hz), 4.62 (2d, 2H), 4.57 (d, 1H, J_{gem} = 12.0 Hz), 4.52 (d, 1H, J_{gem} = 11.7 Hz), 4.50 (m, 1H, Ser-α), 4.46 (d, 1H, J_{gem} = 12.2 Hz), 4.42 (d, 1H, J_{gem} = 12.2 Hz), 3.98–4.26 (m, 8H, Neu-H-6, H-9′, H-9, GalNAc-H-3, H-4, Gal-H-3, H-4, Ser-β′), 3.88 (dd, 1H, Ser-β, $J_{a,b}$ = 2.9, J_{gem} = 12.2 Hz), 3.54–3.81 (m, 7H, Neu-H-5, GalNAc-H-2, H-6′, H-6, Gal-H-5, H-6′, H-6), 3.25 (dd, 1H, GalNAc-H-5, J = 8.3 Hz), 2.51 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 12.2 Hz), 1.97, 2.03, 2.06×2 (3s, 12H, Ac), 1.76 (dd, 1H, Neu-H-3ax, $J_{3ax,4}$ = 11.2 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6, 170.3, 170.1, 169.6, 169.6, 167.5, 166.4, 156.1, 154.0, 138.5, 138.1, 138.0, 136.3, 135.3, 133.1, 130.9, 129.9, 129.5, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.1, 127.9, 127.9, 127.6, 127.1, 126.7, 99.3, 98.3, 95.4, 94.7, 77.2, 74.9, 74.5, 74.5, 74.1, 73.9, 73.5, 73.0, 72.8, 71.9, 71.7, 69.7, 69.1, 68.4, 68.3, 68.1, 68.1, 67.3, 67.3, 67.0, 62.5, 62.0, 59.4, 54.5, 52.7, 51.5, 38.0, 21.0, 20.8, 20.8, 20.7 ppm; IR (KBr): $\tilde{\nu}$ = 3473, 2850, 2115, 1707, 1554, 1493, 1212, 1059, 594, 690 cm^{-1}.

One-pot glycosylation procedure for the synthesis of *N*-(benzyloxycarbonyl) 3-*O*-(2-azido-4-*O*-benzyl-2-deoxy-3-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-6-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-D-galacto-2-non-

ulopyranosylonate)-α-D-galactopyranosyl)-L-serine benzyl ester (11): A mixture of thiosialoside **3f** (24.0 mg, 0.0335 mmol), *N*-(benzyloxycarbonyl)-3-*O*-(2-azido-4-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-D-serine benzyl ester (**7**; 16.9 mg, 0.0279 mmol; azeotroped three times with toluene), and pulverized activated 3 Å MS (14.0 mg) in dry CH_3CN (0.140 mL) was stirred at room temperature for 30 min under argon to remove any trace amounts of water. The reaction mixture was then cooled to −35 °C. *N*-iodosuccinimide (9.40 mg, 0.0419 mmol) and a catalytic amount of trifluoromethanesulfonic acid (0.50 μL) were added to the reaction mixture at −35 °C. After being stirred at the same temperature for 1 h, the reaction mixture was warmed to −30 °C. A solution of phenylthio 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**8a**; 32.5 mg, 0.0502 mmol; azeotroped three times with toluene) in dry CH_2Cl_2 (1.25 mL) was added to the reaction mixture. After 10 min, *N*-iodosuccinimide (17.0 mg, 0.0753 mmol) and a catalytic amount of trifluoromethanesulfonic acid (0.50 μL, 5.58 μmol) were added to the reaction mixture at −30 °C. After being stirred at the same temperature, the reaction mixture was neutralized with triethylamine and filtered through a pad of celite. The filtrate was poured into a mixture of saturated aq. $NaHCO_3$ and saturated aq. $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with saturated aq. $NaHCO_3$/$Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (97:3) and further purified by gel-permeation chromatography to give **11** (37.3 mg, 0.0214 mmol, 77 %, α:β = 78:22). The α:β ratio was determined by HPLC analysis (Eluent: hexane/2-propanol 95:5; flow rate: 3.0 mL min^{-1}; retention times: α isomer = 15.2 min, β isomer = 12.4 min). The α isomer was separated by chromatography on silica gel with toluene/acetone (92:8).

***N*-(Benzyloxycarbonyl) 3-*O*-(2-acetamido-4-*O*-benzyl-2-deoxy-3-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-6-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranosyl)-L-serine benzyl ester (12)**: Activated zinc dust (30 mg) was added to a solution of trisaccharide **11α** (15.0 mg, 8.57 μmol) in dry THF/AcOH/Ac_2O (3:2:1; 0.60 mL) at 0 °C. After being stirred at the same temperature for 1 h, the reaction mixture was filtered through a pad of celite. The filtrate was concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (98:2) to give **12** (12.8 mg, 7.85 μmol, 92 %); $[\alpha]_D^{27}$ +24.8 (*c* 0.985, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00 (d, 2H, aromatic), 7.14–7.57 (m, 33H, aromatic), 5.74 (d, 1H, Ser-NH, *J* = 11.2 Hz), 5.73 (d, 1H, GalNAc-NH, *J* = 7.3 Hz), 5.68 (dd, 1H, Gal-H-2, $J_{1,2}$ = 7.8, $J_{2,3}$ = 9.8 Hz), 5.38 (m, 1H, Neu5Ac-H-8), 5.30 (dd, 1H, Neu5Ac-H-7, $J_{6,7}$ = 2.0, $J_{7,8}$ = 7.8 Hz), 5.16 (m, 2H, Neu5Ac-NH), 5.09 (m, 2H), 5.03 (d, 1H, J_{gem} = 12.2 Hz), 4.99 (d, 1H, J_{gem} = 12.2 Hz), 4.84 (m, 2H, Neu5Ac-H-4, GalNAc-H-1), 4.76 (d, 1H, Gal-H-1, $J_{1,2}$ = 7.8 Hz), 4.38–4.68 (m, 6H, GalNAc-H-2, Gal-H-4, H-5, H-6′, H-6, Ser-α), 4.31 (dd, 1H, Neu5Ac-H-9′, $J_{8,9'}$ = 2.4, J_{gem} = 12.7 Hz), 4.11 (m, 1H, Neu5Ac-H-9, $J_{8,9}$ = 5.8, J_{gem} = 12.2 Hz), 3.51–4.05 (m, 9H, Neu5Ac-H-5, H-6, GalNAc-H-3, H-4, H-6′, H-6, Gal-H-3, Ser-β′, Ser-β), 3.48 (s, 3H, Neu5Ac-OMe), 3.27 (dd, 1H, GalNAc-H-5), 2.52 (dd, 1H, Neu5Ac-H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 12.2 Hz), 2.00 (m, 1H, Neu5Ac-H-3ax), 1.87, 1.98×2, 2.02, 2.07, 2.10 (6s, 18H, Ac) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 170.6, 170.2×2, 170.0, 169.9, 167.8, 165.1, 155.9, 138.9, 138.2, 137.5, 137.4, 136.1, 134.9, 133.1, 130.1, 130.0, 129.9, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 126.9, 100.9, 98.8, 98.6, 79.7, 72.2, 75.1, 74.8, 74.2, 74.0, 73.5, 72.6, 72.1, 71.7, 69.5, 69.1, 69.0, 68.8, 68.3, 67.5, 67.4, 67.2, 63.2, 62.3, 54.6, 52.6, 49.4, 37.7, 23.2, 22.6, 21.0, 20.8, 20.8, 20.7 ppm; IR (KBr): $\tilde{\nu}$ = 3358, 2923, 2856, 1732, 1660, 1530, 1455, 1368, 1213, 1036, 736, 697 cm^{-1}.

3-*O*-(2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-6-*O*-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-α-D-galactopyranosyl)-L-serine (1a): $Pd(OH)_2$ (30 mg) was added to a solution of **12** (13.3 mg, 8.15 μmol) in THF/MeOH/AcOH/H_2O (100:80:10:7; 1.97 mL). The reaction mixture was hydrogenolyzed for 2 h under an H_2 atmosphere. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue was used for the next reaction without further purification. 0.1 M aq. NaOH (350 μL) was added to a stirred solution of the residue in methanol (1.20 mL) at 0 °C. After being stirred at the same temperature for 4 h, H_2O (0.30 mL) was added and the reaction mixture was neutralized with Dowex 50W-4X. After filtration and evaporation of the solvent, the residue was purified by reversed-phase column chromatography (Bond Elut-C18 column) to give **1a** (5.28 mg, 6.94 μmol, 85 % over two steps); $[\alpha]_D^{26}$ +67.0 (*c* 0.16, H_2O); ^{1}H NMR (400 MHz, D_2O, 303 K): δ = 4.88 (d, 1H, GalNAc-H-1, $J_{1,2}$ = 3.9 Hz), 4.43 (d, 1H, Gal-H-1, $J_{1,2}$ = 7.8 Hz), 4.31 (dd, 1H, GalNAc-H-2, $J_{1,2}$ = 3.4, $J_{2,3}$ = 11.2 Hz), 4.22 (brd, GalNAc-H-4, *J* = 2.9 Hz), 4.10 (dd, 1H, Ser-β′, $J_{a,b'}$ = 2.4, J_{gem} = 10.7 Hz), 4.04 (dd, 1H, GalNAc-H-3, $J_{2,3}$ = 11.2, $J_{3,4}$ = 2.9 Hz), 4.01 (m, 1H, GalNAc-H-5), 3.97 (dd, 1H, Ser-α, $J_{\alpha,\beta'}$ = 2.4, $J_{\alpha,\beta}$ = 4.9 Hz), 3.90 (dd, 1H, Ser-β, $J_{a,b}$ = 4.9, J_{gem} = 10.7 Hz), 3.61–3.88 (12H, Neu5Ac-H-4, H-5, H-6, H-7, H-8, H-9′, H-9, GalNAc-H-6′, H-6, Gal-H-5, H-6′), 3.58 (dd, 1H, Gal-H-3, $J_{2,3}$ = 10.3, $J_{3,4}$ = 3.9 Hz), 3.55 (dd, 1H, Gal-H-6, $J_{5,6}$ = 1.0, J_{gem} = 10.3 Hz), 3.48 (dd, 1H, Gal-H-2, $J_{1,2}$ = 7.8, $J_{2,3}$ = 10.3 Hz), 2.70 (dd, 1H, Neu5Ac-H-3eq, $J_{3eq,4}$ = 4.9, J_{gem} = 12.7 Hz), 2.00, 2.01 (2s, 6H, Ac), 1.65 (dd, 1H, Neu5Ac-H-3ax, $J_{3ax,4}$ = 12.2 Hz) ppm; ^{13}C NMR (100 MHz, D_2O, acetone-d_6): δ = 175.8, 175.4, 173.9, 172.0, 105.5, 100.9, 99.1, 77.4, 75.8, 73.5, 73.4, 72.4, 71.4, 70.3, 69.4, 69.3, 69.7, 68.9, 67.3, 64.6, 63.5, 61.8, 54.8, 52.6, 49.2, 40.9, 22.9×2 ppm; IR (KBr): $\tilde{\nu}$ = 3330, 1641, 1572, 1393, 1121, 1055, 930, 669 cm^{-1}; HRMS (ESI-TOF): calcd for $C_{28}H_{48}N_3O_{21}$ $[M+H]^+$: 762.2775; found: 762.2772.

2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl fluoride (15): A mixture of thiosialoside **3f** (45.0 mg, 0.0627 mmol), 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl fluoride (**13a**; 15.6 mg, 0.0418 mmol; azeotroped three times with toluene), and pulverized activated 3 Å MS (21.0 mg) in dry CH_3CN/CH_2Cl_2 (9:13; 0.22 mL) was stirred at room temperature for 10 min under argon to remove any trace amounts of water. The reaction mixture was then cooled to −78 °C. *N*-iodosuccinimide (18.8 mg, 0.0836 mmol) and a catalytic amount of trifluoromethanesulfonic acid (1.10 μL, 0.0125 mmol) were added to the reaction mixture at −78 °C. After being stirred at the same temperature for 1.5 min, the reaction mixture was neutralized with triethylamine and filtered through a pad of celite. The filtrate was poured into a mixture of saturated aq. $NaHCO_3$ and saturated aq. $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with saturated aq. $NaHCO_3$/$Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (97:3) and further purified by gel-permeation chromatography to give **15** (35.2 mg, 0.0360 mmol, 86 %, α:β = 93:7). The α:β ratio was determined by HPLC analysis (Senshu Pak Silica-3301-N column; eluent: hexane/2-propanol 89:11; flow rate: 3.0 mL min^{-1}; retention times: α isomer = 20.6 min, β isomer = 22.7 min). The α,β isomers were separated by chromatography on silica gel with toluene/acetone (86:14) to give the α isomer and with toluene/acetone (88:12) to give the β isomer.

15α isomer: $[\alpha]_D^{23}$ +59.6 (*c* 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13 (d, 2H, aromatic), 7.34–7.62 (m, 8H, aromatic), 5.51–5.63 (m, 3.5H, Neu-H-8, Gal-H-1′, Gal-H-2), 5.45 (d, 0.5H, Gal-H-1, $J_{1,2}$ = 7.3 Hz), 5.40 (s, 1H, benzylic-H), 5.31 (dd, 1H, Neu-H-7, $J_{6,7}$ = 1.4, $J_{7,8}$ = 9.8 Hz), 4.85 (d, 1H, J_{gem} = 12.2 Hz), 4.83 (m, 1H, Neu-H-4), 4.75 (d, 1H, Neu-Troc-NH, *J* = 9.8 Hz), 4.61 (dd, 1H, Gal-H-3, $J_{2,3}$ = 10.3, $J_{3,4}$ = 3.4 Hz), 4.44 (d, 1H, J_{gem} = 12.2 Hz), 4.37 (brd, 1H, Gal-H-6′, *J* = 12.7 Hz), 4.32 (dd, 1H, Neu-H-9′, $J_{8,9}$ = 2.4, J_{gem} = 12.7 Hz), 4.17 (brd, 1H, Gal-H-6, *J* = 12.7 Hz), 4.05 (brs, 1H, Gal-H-4), 4.03 (dd, 1H, Neu-H-9, $J_{8,9}$ = 6.3, J_{gem} = 12.2 Hz), 3.97 (dd, 1H, Neu-H-6, $J_{5,6}$ = 10.8, $J_{6,7}$ = 2.0 Hz), 3.71 (brs, 1H, Gal-H-5), 3.59 (s, 3H, Neu-OMe), 3.52 (ddd, 1H, Neu-H-5, $J_{5,6}$ = 10.8, $J_{5,NH}$ = 9.8 Hz), 2.68 (dd, 1H, Neu-H-3eq, $J_{3eq,4}$ = 4.4, J_{gem} = 12.2 Hz), 1.83, 1.94, 2.07, 2.22 (4s, 12H, Ac), 1.66 (dd, 1H, Neu-H-3ax, $J_{3ax,4}$ = 12.7 Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.8, 170.4, 170.2, 169.9, 168.5, 165.0, 154.0, 137.4, 133.2, 129.9, 129.8, 129.0, 128.3, 128.1, 126.3, (108.6, 106.4 ($J_{C\text{-}F}$ = 217 Hz)), 100.9, 96.5, 95.2, 74.4, 72.7, 71.9, 71.3, 71.2, 70.3, 70.0, 68.6, 68.2, 67.3, 67.0, 66.5, 66.4, 62.5, 52.8, 51.2, 38.3, 21.4, 20.7, 20.7, 20.7 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): δ = −144.2 (dd, $J_{1,F}$ = 55.0, $J_{2,F}$ = 14.7 Hz) ppm; IR (KBr): $\tilde{\nu}$ = 3319, 2959, 1743, 1715, 1549, 1337, 1265, 1097, 821, 737, 716 cm^{-1}; elemental analysis calcd (%) for $C_{41}H_{45}Cl_3FNO_{19}$: C 50.19, H 4.62, N 1.43; found: C 49.99, H 4.71, N 1.39;

HRMS (ESI-TOF): calcd for $C_{41}H_{45}Cl_3NO_{19}Na$ $[M+Na]^+$: 1004.1533; found: 1004.1528.

15β isomer: $[\alpha]_D^{24}$ +81.2 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=8.03$ (d, 2H, aromatic), 7.39–7.65 (m, 8H, aromatic), 5.68 (s, 1H, benzylic-H), 5.65 (m, 1H, Gal-H-2), 5.45 (dd, 1H, Gal-H-1, $J_{1,2}=7.8$, $J_{H\text{-}F}=53.5$ Hz), 5.34 (ddd, 1H, Neu-H-8, $J_{7,8}=2.9$, $J_{8,9'}=2.9$, $J_{8,9}=8.8$ Hz), 5.27 (brs, 1H, Neu-H-7), 5.10 (ddd, 1H, Neu-H-4, $J_{3ax,4}=12.7$, $J_{3eq,4}=4.4$, $J_{4,5}=10.8$ Hz), 5.06 (dd, 1H, Neu-H-9′, $J_{8,9'}=2.9$, $J_{gem}=12.6$ Hz), 4.83 (d, 1H, $J_{gem}=12.2$ Hz), 4.49 (brs, 1H, Gal-H-4), 4.41 (brd, 1H, Gal-H-6′, $J=11.7$ Hz), 4.38 (d, 1H, $J_{gem}=12.2$ Hz), 4.29 (dd, 1H, Gal-H-3, $J_{2,3}=10.3$, $J_{3,4}=3.9$ Hz), 4.17 (brd, 1H, Gal-H-6, $J=11.7$ Hz), 4.03 (dd, 1H, Neu-H-6, $J_{5,6}=12.7$, $J_{6,7}=2.4$ Hz), 3.90 (dd, 1H, Neu-H-9, $J_{8,9}=8.8$, $J_{gem}=12.6$ Hz), 3.78 (brs, 1H, Gal-H-5), 3.71 (ddd, 1H, Neu-H-5), 3.36 (s, 3H, Neu-OMe), 2.58 (dd, 1H, Neu-H-3eq, $J_{3eq,4}=4.4$, $J_{gem}=13.7$ Hz), 1.97, 2.05, 2.09×2 (3s, 12H, Ac), 1.77 (dd, 1H, Neu-H-3ax, $J_{3ax,4}=12.7$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=171.1$, 170.8, 170.3, 169.7, 166.9, 164.7, 154.3, 137.3, 133.8, 129.8, 129.7, 129.5, 128.6, 126.1, (108.1, 105.1 ($J_{C\text{-}F}=217$ Hz)), 99.2, 95.3, 74.5, 73.8, 73.7, 73.5, 73.0, 71.5, 70.6, 70.3, 68.9, 68.7, 68.1, 66.7, 66.7, 62.6, 52.7, 51.2, 36.5, 20.9, 20.8, 20.8, 20.7 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): $\delta=-144.4$ (dd, $J_{1,F}=57.4$, $J_{2,F}=13.4$ Hz) ppm; IR (KBr): $\tilde{\nu}=3397$, 2952, 2896, 1742, 1516, 1369, 1238, 1218, 1095, 1033, 709 cm^{-1}; elemental analysis calcd (%) for $C_{41}H_{45}Cl_3FNO_{19}$: C 50.19, H 4.62, N 1.43; found: C 49.83, H 4.62, N 1.46; HRMS (ESI-TOF): calcd for $C_{41}H_{45}Cl_3NO_{19}Na$ $[M+Na]^+$: 1004.1533; found: 1002.1528.

***N*-(Benzyloxycarbonyl)-3-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-α-D-galactopyranosyl)-L-serine benzyl ester (16)**: A mixture of disaccharide **15α** (20.0 mg, 0.0204 mmol), *N*-(benzyloxycarbonyl)-3-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-L-serine benzyl ester (**14**; 16.0 mg, 0.0265 mmol; azeotroped three times with toluene), and pulverized activated 3 Å MS (60.0 mg) in dry CH_3CN/CH_2Cl_2 (2:3; 0.25 mL) was stirred at room temperature for 10 min under argon to remove any trace amounts of water. The reaction mixture was then cooled to −5 °C. $ZrCp_2Cl_2$ (9.00 mg, 0.0309 mmol) and silver trifluoromethanesulfonate (15.7 mg, 0.0612 mmol) were added to the reaction mixture. After being stirred at the same temperature for 2 h, the reaction mixture was neutralized with triethylamine and filtered through a pad of celite. The filtrate was poured into saturated aq. $NaHCO_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with saturated aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with toluene/acetone (90:10) to give **16** (30.6 mg, 0.0195 mmol, 96%); $[\alpha]_D^{24}$ +63.4 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=8.11$ (d, 2H, aromatic), 7.18–7.54 (m, 23H, aromatic), 5.86 (d, 1H, Ser-NH, $J=8.3$ Hz), 5.52 (m, 2H, Neu-H-8, Gal-H-2), 5,46 (s, 1H, benzylic-H), 5.38 (s, 1H, benzylic-H), 5.32 (dd, 1H, Neu-H-7, $J_{6,7}=1.4$, $J_{7,8}=9.3$ Hz), 5.16 (2d, 2H), 5.11 (2d, 2H), 5.07 (d, 1H, Gal-H-1, $J_{1,2}=7.8$ Hz), 4.85 (d, 1H, $J_{gem}=9.3$ Hz), 4.83 (d, 1H, GalNAc-H-1, $J_{1,2}=2.9$ Hz), 4.80 (m, 1H, Neu-H-4), 4.72 (d, 1H, Neu-Troc-NH, $J=10.2$ Hz), 4.57 (dd, 1H, Gal-H-3, $J_{2,3}=10.2$, $J_{3,4}=3.4$ Hz), 4.52 (m, 1H, Ser-α), 4.43 (d, 1H, $J_{gem}=12.7$ Hz), 4.40 (brs, 1H, GalNAc-H-4), 4.31 (2brdd, 2H, Gal-H-6′, Neu-H-9′), 4.16 (brd, 1H, Gal-H-6), 4.00–4.11 (m, 5H, Neu-H-9, Gal-H-4, GalNAc-H-3, H-6′, Ser-β′), 3.99 (brd, 1H, Neu-H-6, $J=10.7$ Hz), 3.93 (dd, 1H, Ser-β, $J_{\alpha,\beta}=2.4$, $J_{gem}=10.8$ Hz), 3.84 (brd, 1H, GalNAc-H-6, $J=12.2$ Hz), 3.66 (brs, 1H, Gal-H-5), 3.62 (dd, 1H, GalNAc-H-2, $J_{1,2}=3.4$, $J_{2,3}=10.8$ Hz), 3.55 (s, 3H, Neu-OMe), 3.44–3.51 (m, 2H, Neu-H-5, GalNAc-H-5), 2.65 (dd, 1H, Neu-H-3eq, $J_{3eq,4}=4.4$, $J_{gem}=12.7$ Hz), 1.83, 1.92, 2.06, 2.22 (4s, 12H, Ac), 1.62 (dd, 1H, Neu-H-3ax, $J_{3ax,4}=12.7$ Hz) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.8$, 170.3, 170.2, 170.0, 169.6, 168.7, 165.1, 155.9, 154.0, 137.8, 137.5, 136.1, 135.0, 132.8, 130.4, 129.8, 129.0, 128.6, 128.6, 128.4, 128.3, 128.2, 127.9, 126.4, 126.0, 101.4, 100.9, 100.4, 100.2, 96.5, 95.3, 77.2, 75.6, 74.5, 73.1, 72.9, 72.2, 71.9, 70.2, 69.8, 69.1, 68.8, 68.2, 67.7, 67.5, 67.1, 67.0, 66.2, 63.6, 62.3, 58.6, 54.6, 52.7, 51.3, 38.4, 21.4, 20.8, 20.7, 20.5 ppm; IR (KBr): $\tilde{\nu}=3353$, 2951, 2110, 1736, 1521, 1367, 1211, 1088, 1037, 741, 698 cm^{-1}; elemental analysis calcd (%) for $C_{72}H_{76}Cl_3N_5O_{28}$: C 55.23, H 4.89, N 4.47; found: C 55.01, H 5.00, N 4.35; MS (ESI-TOF): calcd for $C_{72}H_{76}Cl_3N_5O_{28}$ $[M+NH_4]^+$: 1583.4; found: 1583.4.

One-pot glycosylation procedure for the synthesis of *N*-(benzyloxycarbonyl)-3-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(methyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-(2,2,2-trichloroethoxycarbonylamino)-D-glycero-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-α-D-galactopyranosyl)-L-serine benzyl ester (16): A mixture of thiosialoside **3f** (43.1 mg, 0.0602 mmol), 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl fluoride (**13a**; 15.0 mg, 0.0401 mmol; azeotroped three times with toluene), and pulverized activated 3 Å MS (20.0 mg) in dry CH_3CN/CH_2Cl_2 (2:3; 0.50 mL) was stirred at room temperature for 30 min under argon to remove any trace amounts of water. The reaction mixture was then cooled to −78 °C. *N*-iodosuccinimide (18.3 mg, 0.0812 mmol) and a catalytic amount of trifluoromethanesulfonic acid (1.10 μL, 0.0120 mmol, 0.30 equiv) were added to the reaction mixture at −78 °C. After being stirred at the same temperature for 1.5 min, the reaction mixture was warmed to −5 °C. *N*-(Benzyloxycarbonyl)-3-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-L-serine benzyl ester (**14**; 36.4 mg, 0.0602 mmol) was added to the reaction mixture. After 10 min, $ZrCp_2Cl_2$ (23.4 mg, 0.0802 mmol) and silver trifluoromethanesulfonate (41.2 mg, 0.1604 mmol) were added to the reaction mixture at −5 °C. After being stirred at the same temperature for 2 h, the reaction mixture was neutralized with triethylamine and filtered through a pad of celite. The filtrate was poured into a mixture of saturated aq. $NaHCO_3$ and saturated aq. $Na_2S_2O_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with saturated aq. $NaHCO_3/Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (97:3) and further purified by gel-permeation chromatography to give **16** (55.5 mg, 0.0355 mmol, 88%, α:β = 93:7). The α,β isomers were separated by chromatography on silica gel with toluene/acetone (90:10) to give the α isomer and with toluene/acetone (92:8) to give the β isomer.

***N*-(Benzyloxycarbonyl)-3-*O*-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-α-D-galactopyranosyl)-L-serine benzyl ester (17)**: Activated zinc dust (100 mg) was added to a solution of trisaccharide **16α** (113 mg, 0.0722 mmol) in dry THF/AcOH/Ac_2O (3:2:1) (1.20 mL) at 0 °C. After being stirred at the same temperature for 1 h, the reaction mixture was filtered through a pad of celite. The filtrate was concentrated in vacuo. The residue was purified by chromatography on silica gel with chloroform/methanol (97:3) to give **17** (97.4 mg, 0.0672 mmol, 93%); $[\alpha]_D^{27}$ +68.3 (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta=8.12$ (d, 2H, aromatic), 7.11–7.60 (m, 23H, aromatic), 5.96 (d, 1H, GalNAc-NH, $J=6.4$ Hz), 5.91 (d, 1H, Ser-NH, $J=8.8$ Hz), 5.52 (m, 2H, Neu5Ac-H-8, Gal-H-2), 5.39 (s, 1H, benzylic-H), 5.26 (s, 1H, benzylic-H), 5.21 (dd, 1H, Neu5Ac-H-7), 5.17 (d, 1H, Gal-H-1, $J_{1,2}=8.2$ Hz), 5.13 (d, 1H, $J_{gem}=11.2$ Hz), 5.12 (brs, 1H, GalNAc-H-1), 5.07 (d, 1H, Neu5Ac-NH, $J=10.2$ Hz), 5.06 (d, 1H, $J_{gem}=11.2$ Hz), 5.05 (d, 1H, $J_{gem}=12.2$ Hz), 5.00 (d, 1H, $J_{gem}=11.2$ Hz), 4.74 (m, 1H, Neu5Ac-H-4), 4.58 (dd, 1H, Gal-H-3, $J_{2,3}=9.8$, $J_{3,4}=2.9$ Hz), 4.23–4.38 (m, 6H, Neu5Ac-H-9′, Gal-H-6′, GalNAc-H-2, H-3, H-4, Ser-α), 4.14 (2brd, 2H), 3.81–4.05 (m, 6H, Neu5Ac-H-5, H-6, H-9, Gal-H-4, GalNAc-H-6, Ser-β′), 3.72 (brd, 1H, Ser-β, $J=11.7$ Hz), 3.70 (brs, 1H, Gal-H-5), 3.62 (s, 3H, Neu-OMe), 3.42 (brs, 1H, GalNAc-H-5), 2.60 (dd, 1H, Neu5Ac-H-3eq, $J_{3eq,4}=4.4$, $J_{gem}=12.7$ Hz), 1.71, 1.81×2, 1.94, 2.04, 2.22 (6s, 18H, Ac), 1.72 (dd, 1H, Neu5Ac-H-3ax) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.8$, 170.8, 170.4, 170.2, 170.1×2, 169.8, 168.7, 165.3, 156.1, 137.5, 137.5, 136.3, 135.0, 133.3, 130.0, 129.8, 129.2, 128.6, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 126.4, 126.1, 101.3, 100.7, 99.7, 99.1, 96.8, 77.2, 75.0, 73.5, 72.2, 72.0, 69.9, 69.5, 69.4, 69.2, 68.7, 67.5, 66.9, 66.9, 66.4, 63.4, 62.7, 54.8, 52.8, 49.0, 48.7, 38.1, 23.1, 22.9, 21.4, 20.8, 20.7, 20.4 ppm; IR (KBr): $\tilde{\nu}=3356$, 2955, 1723, 1661, 1518, 1453, 1369, 1269, 1031, 696 cm^{-1}; HRMS (ESI-TOF): calcd for $C_{73}H_{81}N_3O_{28}Na$ $[M+Na]^+$: 1470.4899; found: 1470.4899.

3-*O*-(2-Acetamido-2-deoxy-3-*O*-(3-*O*-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-β-D-galactopyranosyl)-α-D-

galactopyranosyl)-L-serine (2a): $Pd(OH)_2$ (30 mg) was added to a solution of trisaccharide **17** (26.0 mg, 0.0180 mmol) in THF/MeOH/AcOH/H_2O (100:80:7:10; 1.97 mL). The reaction mixture was hydrogenolyzed for 2 h under an H_2 atmosphere. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue was used for the next reaction without further purification. 0.1 M aq. NaOH (120 μL) was added to a stirred solution of the residue in methanol (1.00 mL) at 0 °C. After being stirred at the same temperature for 4 h, H_2O (0.35 mL) was added and the reaction mixture was neutralized with Dowex 50W-4X. After filtration and evaporation of the solvent, the residue was purified by reversed-phase column chromatography (Bond Elut-C18 column) to give **2a** (12.3 mg, 0.0162 mmol, 90% over two steps); ^{1}H NMR (400 MHz, D_2O, 303 K): $\delta = 4.90$ (d, 1H, GalNAc-H-1, $J_{1,2} = 2.4$ Hz), 4.50 (d, 1H, Gal-H-1, $J_{1,2} = 7.8$ Hz), 4.32 (dd, 1H, GalNAc-H-2, $J_{1,2} = 2.4$, $J_{2,3} = 10.3$ Hz), 4.20 (br s, 1H, GalNAc-H-4), 4.11 (br d, 1H, Ser-β′, $J = 11.2$ Hz), 4.02–4.05 (m, 2H, Gal-H-3, GalNAc-H-3), 3.57–4.01 (m, 16H), 3.50 (dd, 1H, Gal-H-2, $J_{1,2} = 7.8$, $J_{2,3} = 8.8$ Hz), 2.73 (dd, 1H, Neu5Ac-H-3eq, $J_{3eq,4} = 4.9$, $J_{gem} = 12.7$ Hz), 2.00 (s, 6H, Ac), 1.75 (dd, 1H, Neu5Ac-H-3ax, $J_{3ax,4} = 12.7$ Hz) ppm; ^{13}C NMR (100 MHz, D_2O, acetone-d_6): $\delta = 175.8$, 175.5, 174.7, 172.4, 105.3, 100.5, 99.0, 77.0, 76.5, 75.6, 73.6, 72.7, 72.0, 71.7, 69.8, 69.4, 69.1, 68.9, 68.2, 67.3, 63.4, 62.0, 61.8, 55.4, 52.5, 49.1, 40.6, 23.0, 22.9 ppm; IR (KBr): $\tilde{\nu} = 3332$, 1614, 1401, 1031, 932, 823, 668 cm^{-1}; HRMS (ESI-TOF): calcd for $C_{28}H_{48}N_3O_{21}$ $[M+H]^+$: 762.2775; found: 762.2767.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research on Priority Area (S) from the Ministry of Education, Culture, Sports, Science, and Technology (Grant-in-Aid No.: 14103013).

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[31] Partial ^{1}H NMR signal assignment for disaccharide **9**: For the **9α** isomer: $\delta_{H\text{-}3eq} = 2.67$ ppm, $\delta_{H\text{-}4} = 5.00$ ppm, $J_{7,8} = 8.8$ Hz, $\Delta\delta$(H-9′–H-9) = 0.17 ppm. For the **9β** isomer: $\delta_{H\text{-}3eq} = 2.52$ ppm, $\delta_{H\text{-}4} = 5.55$ ppm, $\Delta\delta$(H-9′–H-9) = 0.94 ppm.

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[34] Selected analytical date for sialoside **11**: For the **11α** isomer: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.61$ (dd, 1H, Gal-H-2, $J_{1,2} = 3.4$, $J_{2,3} = 10.3$ Hz), 5.55 (d, 1H, Gal-H-1, $J_{1,2} = 3.4$ Hz) ppm. For the **11β** isomer: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 5.74$ (dd, 1H, Gal-H-2, $J_{1,2} = 7.3$, $J_{2,3} = 8.2$ Hz), 4.77 (d, 1H, Gal-H-1, $J_{1,2} = 7.8$ Hz) ppm.

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[41] Partial ^{1}H NMR signal assignment for disaccharide **15**: For the **15α** isomer: $\delta_{H\text{-}3eq} = 2.69$ ppm, $\delta_{H\text{-}4} = 4.83$ ppm, $J_{7,8} = 9.8$ Hz, $\Delta\delta$(H-9′–H-9) = 0.30 ppm. For the **15β** isomer: $\delta_{H\text{-}3eq} = 2.58$ ppm, $\delta_{H\text{-}4} = 5.10$ ppm, $J_{7,8} = 2.9$ Hz, $\Delta\delta$(H-9′–H-9) = 1.15 ppm.

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Received: August 13, 2004
Published online: December 6, 2004