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A New Strategy for Stereoselective Synthesis of Sialic Acid-containing Glycopeptide Fragment

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Abstract—Sialic acid donor **5**, which has a thiophenyl group as a stereocontrolling auxiliary and thiomethyl group as a leaving group was prepared and subjected to model glycosylation. Reactions with acceptor substrates **6**, **7**, and **8** gave coupled products **9b**, **10b**, and **11b**, respectively, in a higher efficiency than previously observed for the bromide **1a**. This reaction was further applied to the synthesis of protected glyco-amino acid fragment **12**, that is strategically designed for the synthesis of sialic acid containing glycopeptides. Copyright © 1996 Elsevier Science Ltd

Introduction

Since the use of a thiophenyl (PhS) group as a stereocontrolling auxiliary[1] in sialic acid α-glycoside synthesis was developed in this laboratory,[2] the methodology has been applied to the synthesis of various glycoconjugates.[3] This method, which utilizes the bromide **1a** as a sialic acid donor, proved to be particularly powerful in effecting the glycosylation of unreactive acceptor substrates. In particular, the efficient and fully stereocontrolled construction of the NeuAcα2→8NeuAc unit was realized, which used to be considered as an extremely challenging task,[4] and the first chemical synthesis of disialoganglioside G_{D3} was achieved.[5] Application to the synthesis of mucin-type glycoprotein fragments was subsequently reported by Nakahara et al. by using the corresponding benzyl ester **1b**, thus avoiding basic conditions for final deprotection.[6] In our original procedure, the synthesis of the NeuAc donor **1** from the 2,3-dehydro derivative **3b,c** consisted of a four step process: (1) oxybromination to bromohydrin,[7] (2) replacement of C-3 Br with PhS, (3) base-catalysed epimerization of the C-3 position, and (4) functionalization of the C-2 position into leaving groups (i.e. Br, Cl, F). Among them, the use of the bromide in combination with Hg^{II} salt as a promoter and CCl_4 as a solvent gave the best result.

Results and Discussion

Aiming at further improvement of this transformation, with respect to overall efficiency and operational simplicity, our effort has been directed at the possibility of using other leaving groups than halides. Reported here is (1) the preparation and use of the thiomethyl glycoside **5** and (2) the concise synthesis of a properly protected sialoside fragment **12** that is strategically designed for glycopeptide synthesis. In addition, an easier access to the 3-SPh intermediate **2b** was briefly examined.

The hemiketal **2b** was required as the precursor of the NeuAc donor, which can be synthesized in a multigram quantity by the three-step process described previously.[2] Alternatively, it was expected that the treatment of **3** with a PhS^+-like species might directly give the 2-OH, 3-SPh product. Thus, we turned our attention to $(PhS)_3SbCl_6$[8] as an electrophile, which was used by Franck and co-workers to effect addition to glycals to give access to 2-deoxy glycosides.[9] The stereochemical outcome of this transformation is inconsequential, since the α-SPh product can be readily epimerized to the desired β-isomer under well-established conditions.[2]

The previously described[6] benzyl ester **3b** was prepared from the corresponding free acid **3a**[10] under slightly modified conditions (BnBr, KF/MeCN). The electrophilic addition of $(PhS)_3SbCl_6$ was performed in acetonitrile to afford the C-3α product **2a** in 67% yield together with the β-isomer **2b** (4%). Epimerization at C-3 position of **2a** into the desired C-3β stereochemistry was performed by the action of DBU to give **2b** in 90% yield.

Having the hemiketal functionality at the anomeric position, conversion into a variety of leaving groups should be possible. Among them, the thiomethyl glycoside[3c,4b] was selected for the initial demonstration of the versatility of the synthetic strategy. Thus, acetylation (Ac_2O, DMAP/pyridine) of **2b** into **4**, followed by treatment with TMSSMe-TMSOTf,[11] afforded the thioglycoside **5** as the pure α-isomer presumably due to the participation of the SPh substituent.

Model glycosylation was investigated using glycosyl acceptors **6**, **7**, and **8** (Scheme 1), in order to make a direct comparison with the results obtained previously

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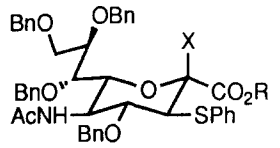
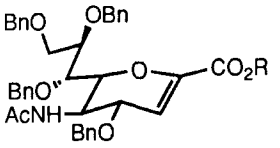
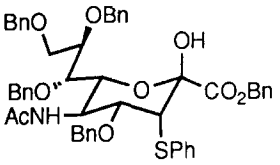


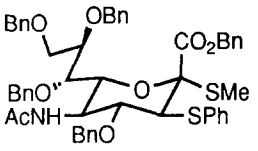
	R	X
1a	Me	Br
b	Bn	Br
2b	Bn	OH
4	Bn	OAc



	R
3a	H
b	Bn
c	Me



2a



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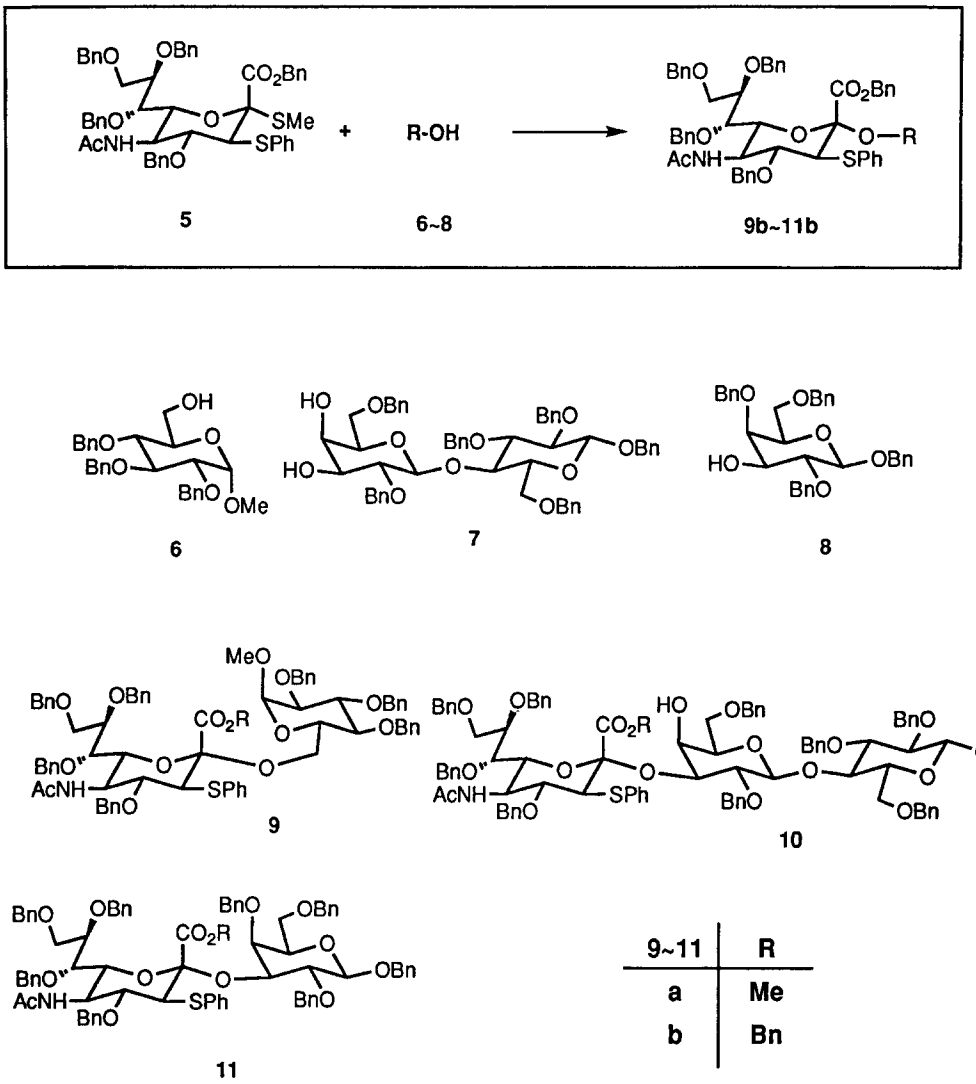
using the bromide **1**. Reactions were promoted by *N*-iodosuccinimide (NIS)-trifluoromethanesulfonic acid (TfOH)[12] and results are summarized in Table 1. As was expected, the coupled products **9b**, **10b**, and **11b** were stereoselectively obtained in good yields. Besides the exclusive formation of the α-anomer, several points deserve comment. First, as in the case of the reactions using corresponding bromide **1a**, the best yields were obtained when carbon tetrachloride was used as the solvent. However, the decrease of the yield observed in other solvents including a polar one (i.e. acetonitrile) was not substantial. For instance, reaction with the lactose derivative **7** as an acceptor afforded the α-2,3-linked trisaccharide **10b** as the only isolable product in 62, 68, 75, and 80% yield in MeCN, CH_2Cl_2, toluene, and CCl_4, respectively (entries 4–7). Aiming at the synthesis of glycoprotein related compounds, this observation is quite important, since the solubility of glycopeptide fragments is not always predictable. Enhancement in efficiency compared with previously obtained results was most clearly seen in the case of a highly hindered substrate **8**. The desired product **11b** was obtained in 40 and 57% yield in MeCN and CCl_4, respectively, which compare quite favorably with that obtained using the bromide (entries 8–11). The stereochemistry of the products were confirmed by comparison of H^1 NMR data with these reported previously for the corresponding methyl esters **9a–11a**.[2] Further support of stereochemical assignments was obtained from $^3J_{C-H}$ values (5.7 Hz) between C-1 and H-3. These values can be compared with those observed for **2b** and **4** (1.5 Hz) which should exist mainly as the β-anomers.

Having established the improved protocol for SPh-assisted stereoselective sialic acid α-glycoside formation, our attention was then turned to the application of the methodology for the preparation of a glyco-amino acid fragment strategically designed for mucin-type glycopeptide synthesis. For this purpose, the protected triglycosyl serine **12** was chosen as the initial target since a closely related compound was already shown to be a valuable synthetic block for mucin-type glycopeptide synthesis.[5] The design of the synthesis is shown in Scheme 2.

The requisite diglycosyl serine fragment **23** was prepared as follows. The galactose portion was designed as the thioglycoside **16** which was synthesized from galactose pentaacetate via the 4,6-*O*-benzylidene derivative **14**. Thus, the triol **15**,[13] obtainable from **14** by reductive opening of the benzylidene group,[14] was subjected to levulinoylation to afford donor The monoglycosyl serine portion was synthesized from previously reported **17**,[15] which was first protected using the monochloroacetyl (MCA) group to give **18**. At this stage, the α- and β-anomers were separated. Coupling of **18**α with the serine derivative **19** gave **20** (93%, α:β = 7.5:1), while the use of **18**β afforded **20** with a nearly identical efficiency (94%, α:β = 5.3:1). Subsequent removal of the MCA group[16] afforded **21**. Reaction with **16** was best achieved by the action of $CuBr_2$–Bu_4NBr–AgOTf[17] and the desired product **22** was obtained in 68% yield. Conversion into the 4,6-diol **23** could be achieved in the conventional manner without any incident.

The introduction of a sialic acid residue on the 4,6-diol **23** was performed in CH_2Cl_2:MeCN (10:1) to afford a 70% yield of **12** which is regioselectively glycosylated at C-6. It is to be noted that the allyl protecting group was compatible with the iodonium ion activation. Subsequent removal of levulinoyl groups[18] was effected by using hydrazine acetate[19] to afford the tetraol **13**.



Scheme 1.

Table 1. Results of glycosylation reactions

Entry	Acceptor	Donor	Solvent	Temperature (°C)	Product	Yield(%)[a]
1	**6**	**1a**	CCl_4	rt	**9a**	72[b]
2	**6**	**5**	MeCN	−42	**9b**	80
3	**7**	**1a**	CCl_4	rt	**10a**	78[b]
4	**7**	**5**	MeCN	−42	**10b**	62
5	**7**	**5**	CH_2Cl_2	−42	**10b**	68
6	**7**	**5**	toluene	−42	**10b**	75
7	**7**	**5**	CCl_4	−15	**10b**	80
8	**8**	**1a**	CCl4	rt	**11a**	24[b]
9	**8**	**5**	MeCN	−42	**11b**	40
10	**8**	**5**	CCl4	−15	**11b**	57

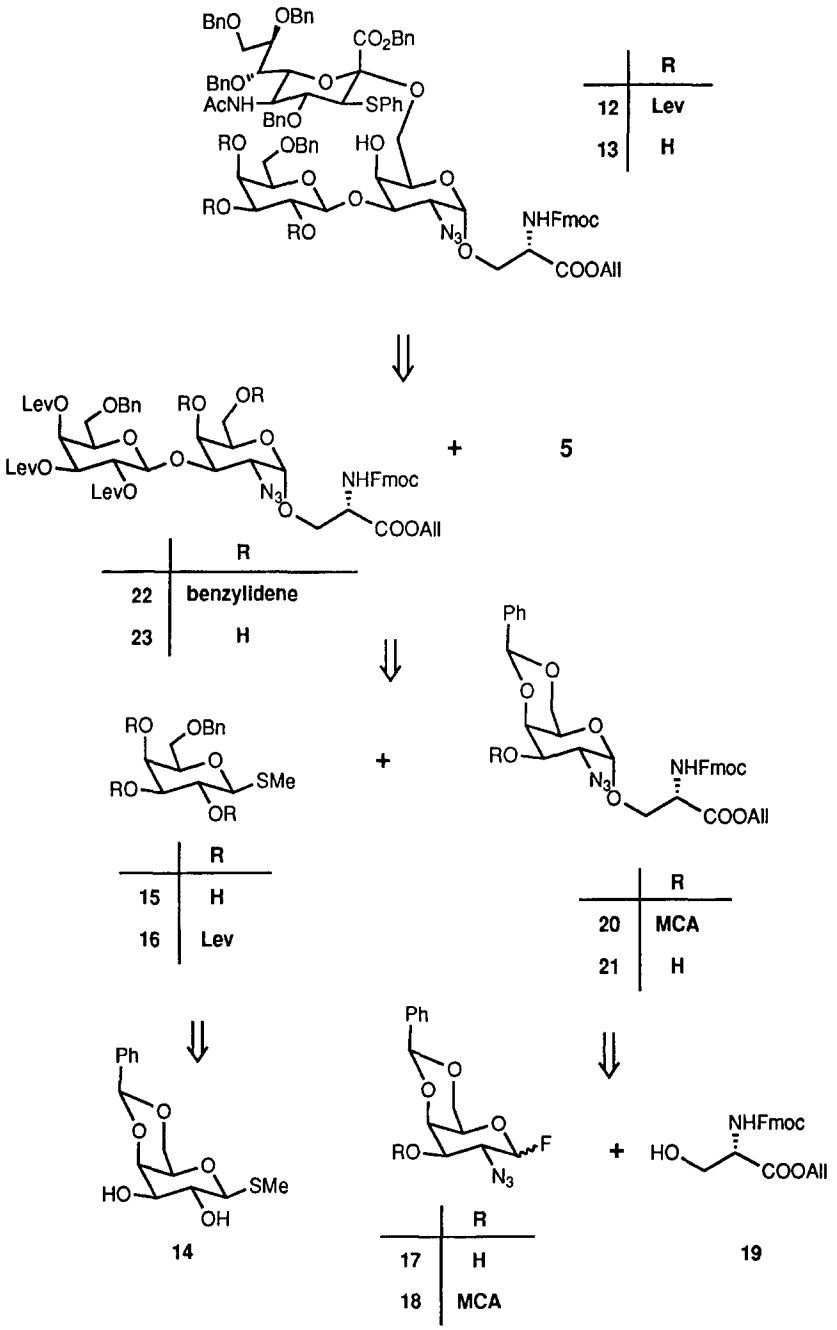
[a]Calculated based on glycosyl donor.
[b]Taken from ref. 2.

The utility of a glycosyl amino acid fragment that is similar to **12** was already demonstrated in the synthesis of mucin-type glycopeptides.[6] Introduction of an additional sialic acid residue at the O-3 position of galactose should be possible in a regioselective manner.[20] Furthermore, the amino acid part is orthogonally protected with respect to the glycan chain, so that elongation of the peptide backbone can be achieved based on conventional Fmoc-based strategy.[21] Since the sialylation strategy described here proved to have higher efficiency than the previously developed one, this method should be a powerful tool for pursuing further studies aimed at glycoprotein related compounds. In addition, the potential usefulness for ganglioside synthesis is apparent.

Experimental

General procedures

Optical rotations were measured at 20 ± 3 °C with JASCO DIP 310 polarimeter. ^{1}H and ^{13}C NMR spectra were measured either with JEOL-EX 270, -GX 400, or -GX 500. Silica-gel column chromatography was performed on columns of silica gel 60 (Merck). Analyt-



Scheme 2.

ical TLC and HPTLC were performed on glass plates coated with silica gel 60 F_{254} (Merck). Gels for size exclusion chromatography (Bio-Beads) were purchased from Bio-Rad. Molecular sieves were purchased from Nakarai Chemical and activated at 180 °C under vacuum immediately prior to use. Glycosylation reactions were performed under an atmosphere of dry Ar, in anhyd solvents. Dichloromethane was distilled from P_2O_5. Acetonitrile, toluene, and carbon tetrachloride were distilled from CaH_2. 1,2-Dichloroethane was dried over molecular sieves 4 Å.

Benzyl 5-acetamido-4,7,8,9-tetra-*O*-benzyl-3,5-dideoxy-3-phenylthio-D-*erythro*-β-L-manno-2-nonulopyranosonate (2a) and benzyl 5-acetamido-4,7,8,9-tetra-*O*-benzyl-3,5-dideoxy-3-phenylthio-D-erythro-β-L-gluco-2-nonulopyranosonate (2b). To a stirred solution of compound **3b** (210 mg, 0.283 mmol) in acetonitrile (5 mL) that was precooled to −42 °C was added a 0.25 M solution of $(PhS)_3SbCl_6$ (1.42 mL, 0.35 mmol) in CH_2Cl_2. The mixture was stirred at the same temperature for 30 min and quenched with aq $NaHCO_3$ soln. Insoluble materials were removed by filtration through Celite and the filtrate was diluted with AcOEt, which was then washed with brine, dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by silica gel column chromatography (toluene:AcOEt 7:3) to afford **2a** (165 mg, 67%) and **2b** (10 mg, 4%).

Compound **2a**. $R_f = 0.25$ (toluene:AcOEt 3:1); $[\alpha]_D$ −2.5° (*c* 1, $CHCl_3$); δ_H (270 MHz, $CDCl_3$) 7.60–6.90 (30 H, m, Ar), 5.21 and 5.12 (each 1H, d, $J = 12.2$ Hz, CO_2CH_2Ph), 3.95 (1H, d, $J = 3.0$ Hz, H-3), 1.62 (3H, s, NHAc); anal.: calcd for $C_{52}H_{53}NO_9S \cdot 0.5H_2O$: C, 71.21; H, 6.20; N, 1.59; found: C, 71.25; H, 6.11; N, 1.59%.

Compound **2b**. $R_f = 0.48$ (toluene:AcOEt 3:1); δ_H (270 MHz, $CDCl_3$) 5.10 and 5.02 (each 1H, d, $J = 12.2$ Hz, CO_2CH_2Ph), 4.26 (1H, q, $J = 9.5$ Hz, H-5), 3.93 (1H, dd, $J = 10.6$ and 9.6 Hz, H-4), 1.72 (3H, s, Ac). This compound was fully characterized as acetate **4** (see below).

Conversion of 2a into 2b. Compound **2a** was converted into **2b** in a manner as described previously for the corresponding methyl ester.[2] Briefly, a solution of compound **2a** (30 mg, 0.034 mmol) in toluene (2 mL) was treated with DBU (1.5 μL, 0.010 mmol) for 1 h to afford 27 mg (90%) of compound **2b**.

Benzyl 5-acetamido-4,7,8,9-tetra-*O*-benzyl-2,3-dehydro-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranosonate (3b). To a solution of compound **3a** (8.00 g, 12.3 mmol) in DMF (60 mL) containing benzyl bromide (8 mL) was added anhyd KF (1.6 g), under ice–water cooling. The mixture was then stirred at ambient temperature overnight, diluted with AcOEt:Et_2O (1:1), washed successively with cold water and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt 3:2) to afford 8.60 g (95%) of compound **3b**; $R_f = 0.26$ (hexane:AcOEt 3:2); δ_H (270 MHz, $CDCl_3$) 6.15 (1H, d, $J = 4.0$ Hz, H-3), 5.25 (1H, d, $J = 7.9$ Hz, NH), 5.21 (2H, s, CO_2CH_2Ph), 4.25 (1H, m, H-5), 4.15 (1H, t, $J = 4$ Hz, H-4), 3.99 (1H, q, $J = 5$ Hz, H-8), 3.88 (1H, dd, $J = 9.9$ and 5.3 Hz, H-9), 3.69 (1H, dd, $J = 9.9$ and 4.3 Hz); δ_C (67.8 MHz, $CDCl_3$)169.56, 161.97, 109.36 (C-3), 78.18, 77.88, 77.23, 74.99, 74.45, 73.40, 72.15, 71.07, 70.94, 68.82, 67.21, 48.10, 23.32.

Benzyl 5-acetamido-2-*O*-acetyl-4,7,8,9-tetra-*O*-benzyl-3,5-dideoxy-3-phenylthio-D-*erythro*-β-L-gluco-2-nonulopyranosonate (4). Compound **2b** (500 mg, 0.576 mmol) was treated with Ac_2O (0.58 mL) and pyridine (0.45 mL) in CH_2Cl_2 (2 mL) containing 10 mg of DMAP at 0 °C. After 1 h, the mixture was diluted with CH_2Cl_2, washed with 0.1 N HCl and aq $NaHCO_3$, successively, dried over Na_2SO_4 and evaporated in vacuo. The residue was subjected to purification by silica gel column chromatography (hexane:AcOEt 2:1) to afford compound **4** (450 mg, 86%); $R_f = 0.59$ (hexane:AcOEt 1:2); $[\alpha]_D$ +15.0° (*c* 2, $CHCl_3$); δ_H (270 MHz, $CDCl_3$) 7.4–7.0 (30 H, m, Ar), 3.61 (1H, q, $J = 9$ Hz, H-5), 3.38 (1H, d, $J = 10.2$ Hz, H-3), 1.92 (3H, s, OAc), 1.59 (3H, s, NAc); δ_C (100 MHz, $CDCl_3$) 170.24, 167.79, 164.78 ($\underline{C}O_2Bn$, $^3J_{C,H} = 1.5$ Hz), 100.25 (C-2), 77.84, 77.64, 75.56, 73.34, 73.10, 72.37, 71.16, 70.90, 67.77, 67.68, 57.14, 53.36, 23.53 (NHCO$\underline{Me}$), 20.75 (O$\underline{C}$OMe); anal.: calcd for $C_{54}H_{55}NO_{10}S$: C, 71.21; H, 6.04; N, 1.54; found: C, 71.04; H, 6.17; N, 1.56%.

Benzyl 5-acetamido-4,7,8,9-tetra-*O*-benzyl-2,3,5-trideoxy-2-methylthio-3-phenylthio-D-*erythro*-α-L-gluco-2-nonulopyranosonate (5). To a mixture of compound **4** (2.04 g, 2.24 mmol), Me_3SiSMe (1.85 mL, 13.4 mmol), and molecular sieves 4 Å (6 g) in 1,2-dichloroethane (40 mL) was added TMSOTf (1.7 mL, 9.0 mmol) and the whole was stirred at 50 °C for 1 h. After cooled down to ambient temperature, the mixture was quenched with aq $NaHCO_3$ and filtered through Celite. The filtrate was diluted with CH_2Cl_2 and washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt 1:1) to afford 1.84 g (91%) of compound **5**; $R_f = 0.48$ (hexane:AcOEt 1:1); $[\alpha]_D$ +87.8° (*c* 2, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 7.8–7.1 (30 H, m, Ar), 5.38 (1H, d, $J = 12.2$ Hz, CO_2CH_2Ph), 5.10 (1H, d, $J = 12.2$ Hz, CO_2CH_2Ph), 4.18 (1H, dd, $J = 10$ and 8 Hz, H-4), 3.32 (1H, d, $J = 10.2$ Hz, H-3), 2.12 (3H, s, SMe), 1.42 (3H, s, NAc); δ_C (125 MHz, $CDCl_3$) 169.84 and 167.55 (NH$\underline{C}$OMe and $\underline{C}O_2Bn$), 87.06 (C-2), 79.34, 79.16, 75.71, 74.56, 74.46, 73.32, 72.84, 72.59, 69.76, 67.60, 61.00 (C-3), 54.18 (C-5), 23.42 (NHCO*Me*), 12.09 (SMe); anal.: calcd for $C_{53}H_{55}NO_8S_2$: C, 70.87; H, 6.17; N, 1.55; found: C, 70.64; H, 6.16; N, 1.49%.

General sialylation procedure. Compound **5**, glycosyl acceptor (**6**–**8**, 1.25–1.5 equiv.) and molecular sieves 4 Å were mixed in a solvent indicated in Table 1. Then, NIS (1–1.5 equiv.) was added and the mixture was cooled down to the temperature specified in Table 1. The reaction was initiated by the addition of TfOH (ca 0.2 equiv.) and monitored by TLC. All reactions were

complete within 5 h, which were quenched with aq $NaHCO_3$ and aq $Na_2S_2O_3$. After processing in the usual manner, the product was isolated by silica gel column chromatography and/or a column of Bio Beads S-X3 or S-X8 in toluene.

Methyl *O*-[benzyl(5-acetamido-4,7,8,9-tetra-*O*-benzyl-3,5,-dideoxy-3-phenylthio-D-*erythro*-α-L-gluco-2-nonulopyranosyl)onate]-(2→6)-2,3,4,-tri-*O*-benzyl-α-D-glucopyranoside (9b). R_f 0.38 (toluene:AcOEt 3:1); $[\alpha]_D$ +10.7° (*c* 2, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 7.6–7.0 (45 H, m, Ar), 5.15 and 5.03 (each 1H, d, J = 12.2 Hz, CO_2CH_2Ph), 4.13 (1H, q, J = 10 Hz, H-5^2), 3.36 (1H, d, J = 9.2 Hz, H-3^2), 3.22 (3H, s, OMe), 1.60 (3H, s, NAc); δ_C (125 MHz, $CDCl_3$) 169.46 (NHCOMe), 167.18 (CO_2Bn, $^3J_{C,H}$ 5.7 Hz), 100.70 (C-1^1), 97.44 (C-2^2), 82.01, 81.23, 79.56, 78.07, 77.58, 75.31, 74.92, 74.54, 74.45, 73.49, 73.23, 72.89, 72.45, 72.15, 69.61, 68.58, 67.23, 62.85, 59.42 (C-3^2), 54.88 (OMe), 51.97 (C-5^2), 23.64 (CO*Me*); anal.: calcd for $C_{80}H_{83}NO_{14}S \cdot 1.5\ H_2O$: C, 71.61; H, 6.46; N, 1.04; found: C, 71.64; H, 6.23; N, 0.98%.

Benzyl *O*-[benzyl(5-acetamido-4,7,8,9-tetra-*O*-benzyl-3,5,-dideoxy-3-phenylthio-D-*erythro*-α-L-gluco-2-nonulopyranosyl)onate]-(2→3)-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4-)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (10b). R_f = 0.60 (toluene:AcOEt 2:1); $[\alpha]_D$ +8.7° (*c* 2, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 7.5–7.0 (60 H, m, Ar), 5.05 and 4,99 (each 1H, d, J = 12 Hz, CO_2CH_2Ph), 4.13 (1H, d, J = 7.9 Hz, H-1^1 or-1^2), 4.03 (1H, m, H-5^3), 1.59 (3H, s, NAc); δ_C (125 MHz, $CDCl_3$) 169.79 (CH_3CO), 167.74 (CO_2Bn, $^3J_{C,H}$ = 5.7 Hz), 102.28 and 102.23 (C-1^1 and C-1^2), 101.00 (C-2^3), 82.81, 81.81, 80.25, 77.93, 76.68, 75.28, 75.14, 74.95, 74.93, 74.62, 74.43, 73.59, 73.20, 73.14, 72.92, 72.83, 72.45, 72.16, 70.80, 69.03, 68.44, 68.02, 67.89, 67.28, 57.90 (C-3^3), 52.03 (C-5^3), 23.54 (COMe); anal.: calcd for $C_{106}H_{109}NO_{19}S$: C, 73.46; H, 6.34; N, 0.80; found: C, 73.21; H, 6.39; N, 0.81%.

The corresponding acetate had $[\alpha]_D$ +25.0° (*c* 0.4, $CHCl_3$); δ_H (400 MHz, $CDCl_3$) 7.6–7.0 (60 H, m, Ar), 5.46 (1H, d, J = 3.4 Hz, H-4^3), 5.23 (1H, d, J = 11.8 Hz, CO_2CH_2Ph), 5.10 (1H, d, J = 11.8 Hz, $CO_{22}CH_2Ph$), 1.77 (3H, s, OAc), 1.57 (3H, s, NAc); anal.: calcd for $C_{108}H_{111}NO_{20}S$: C, 73.06; H, 6.31; N, 0.78; found: C, 72.70; H, 6.49; N, 0.74%.

Benzyl *O*-[benzyl(5-acetamido-4,7,8,9-tetra-*O*-benzyl-3,5,-dideoxy-3-phenylthio-D-*erythro*-α-L-gluco-2-nonulopyranosyl)onate]-(2→3)-2,4,6,-tri-*O*-benzyl-β-D-galactopyranoside (11b). R_f 0.48 (toluene:AcOEt 3:1); $[\alpha]_D$ −4.4° (*c* 0.9, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 7.6–7.0 (50 H, m, Ar), 5.09 (2H, s, CO_2CH_2Ph), 4.16 (1H, d, J = 8.0 Hz, H-1^1), 3.70 (1H, dd, J = 9.8 and 7.6 Hz, H-2^1), 3.62 (1H, d, J = 2 Hz, H-4^1), 3.34 (1H, d, J = 11 Hz, H-3^2), 1.45 (3H, s, Ac); δ_C (125 MHz, $CDCl_3$) 170.11 (NHCOMe), 167.57 (CO_2Bn, $^3J_{C,H}$ = 5.7 Hz), 102.80 (C-1^1), 100.36 (C-2^2), 80.20, 78.92, 77.67, 77.22, 76.77, 75.08, 74.95, 74.88, 74.43, 74.16, 73.26, 72.96, 72.71, 72.31, 70.59, 70.03, 69.20, 67.5, 59.82 (C-3^2), 53.55 (C-5^2), 23.38 (COMe); anal.: calcd for $C_{86}H_{87}NO_{14}S \cdot 1.5\ H_2O$: C, 72.85; H, 6.40; N, 0.98; found: C, 72.59; H, 6.13; N, 0.96%.

Methyl 6-*O*-benzyl-1-deoxy-2,3,4-tri-*O*-levulinoyl-1-thio-β-D-galactopyranoside (16). A mixture of compound **15** (388 mg, 1.29 mmol), levulinic anhydride (838 mg, 5.8 mmol), pyridine (0.6 mL), and DMAP (30 mg) in 1,2-dichloroethane (10 mL) was stirred at room temperature overnight. The solution was diluted with CH_2Cl_2, washed with aq HCl, aq $NaHCO_3$, and brine, successively, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt 3:1 then toluene:AcOEt 1:3) to afford 640 mg (84%) of compound **16**; R_f 0.57 (toluene:AcOEt 1:3); $[\alpha]_D$ −12.3° (*c* 8, $CHCl_3$); FAB-MS 593.53 ($M+H^+$); δ_H (270 MHz, $CDCl_3$) 7.33–7.24 (5H, m, Ar), 5.47 (1H, d, J = 3.3 Hz, H-4), 5.21 (1H, dd, J = 10.8 and 9.6 Hz, H-2), 5.06 (1H, dd, J = 10.8 and 3.3 Hz, H-3), 4.52 and 4.44 (each 1H, d, J = 12 Hz, CH_2Ph), 4.38 (1H, d, J = 9.6 Hz, H-1), 2.8–2.4 (12H, m, $COCH_2CH_2CO$), 2.17 and 2.16 (12H, COMe and SMe); anal.: calcd for $C_{29}H_{38}O_{11}S \cdot 0.5\ H_2O$: C, 57.69; H, 6.54; S, 5.30; found: C, 57.50; H, 6.34; S, 5.43%.

2-Azido-4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-D-galactopyranosyl fluoride (18). Chloroacetic anhydride (1.18 g, 6.92 mmol) was added to a solution of compound **17** (1.12 g, 3.47 mmol) in 1,2-dichloroethane:pyridine (5:1, 12 mL) containing DMAP (0.1 g). The mixture was stirred at room temperature for 0.5 h, diluted with AcOEt, washed successively with 0.5N HCl, aq $NaHCO_3$, and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane:AcOEt 2:1) to afford 0.73 g (52%) of α-isomer (**18α**) and 0.56 g (40%) of β-isomer (**18β**).

18α. R_f 0.58 (hexane:AcOEt 2:1); $[\alpha]_D$ +187.5° (*c* 1, $CHCl_3$); δ_H (270 MHz, $CDCl_3$) 7.6–7.4 (5H, m, Ar), 5.83 (1H, dd, J = 54 and 2.3 Hz, H-1), 5.56 (1H, s, CHPh), 5.32 (1H, dd, J = 10.8 and 3.3 Hz, H-3), 4.58 (1H, d, J = 3 Hz, H-4), 4.17 (2H, s, $ClCH_2CO$), 4.01 (1H, bs, H-5); anal.: calcd for $C_{15}H_{15}N_3O_5ClF$: C, 48.42; H, 4.03; N, 11.29; found: C, 48.55; H, 4.16; N, 10.81%.

18β. R_f 0.32 (hexane:AcOEt 2:1); $[\alpha]_D$ +61.9° (*c* 1, $CHCl_3$); δ_H (270 MHz, $CDCl_3$) 7.6–7.4 (5H, m, Ar), 5.55 (1H, s, CHPh), 5.16 (1H, dd, J = 52 and 7.6 Hz, H-1), 4.81 (1H, ddd, J = 10.9, 3.6 and 1.0 Hz, H-3), 4.38 (1H, d, J = 2.7 Hz, H-4), 4.16 (2H, s, $ClCH_2CO$), 3.62 (1H, bs, H-5); anal.: found: C, 48.65; H, 4.14; N, 10.80%.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2-azido-4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-α-D-galactopyranosyl)-L-serine allyl ester (20).** To a stirred mixture of Cp_2ZrCl_2 (783 mg, 2.68 mmol), $AgClO_4$ (1.109 g, 5.36 mmol) and molecular sieves 4A (4 g) in 1,2-dichloroethane (10 mL) was added dropwise a solution of compounds **18α** (712 mg, 1.79 mmol) and **19** (918 mg, 2.50 mmol) in 1,2-dichloroethane (5 mL)

at −25 °C. The stirring was continued for 5 h, while the mixture was gradually warmed to +5 °C. The reaction was quenched with aq $NaHCO_3$ and insoluble materials were removed by filtration through Celite. The filtrate was diluted with AcOEt, washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography (toluene:AcOEt 3:1) to afford 1.01 g (82%) of compound **20** together with the corresponding β-isomer (0.13 g, 11%).

The same reaction was performed by using **18**β to afford 79% of **20** and 15% of the β-isomer.

Compound 20. $R_f = 0.47$ (toluene:AcOEt 3:1); $[\alpha]_D$ +165.5° (*c* 0.5, $CHCl_3$); δ_H (270 MHz, $CDCl_3$) 7.8–7.0 (13 H, m, Ar), 6.0–5.8 (2H, m, C<u>H</u>=CH_2 and NH), 5.49 (1H, s, C<u>H</u>Ph), 5.40 (2H, m, CH=C<u>H</u>$_2$), 5.04 (1H, d, $J = 3.3$ Hz, H-1), 4.18 (2H, s, $ClCH_2CO$), 3.78 (1H, bs, H-5); δ_C (67.8 MHz, $CDCl_3$) 100.66 (<u>C</u>HPh), 99.66 (C-1), 77.20, 72.85, 71.03, 69.69, 68.84, 67.39, 66.65, 62.89, 57.07, 54.50, 47.01, 40.61, 21.42; anal.: calcd for $C_{36}H_{35}N_4O_{10}Cl$: C, 60.12; H, 4.87; N, 7.79; Cl, 4.93; found: C, 60.22; H, 5.05; N, 7.72; Cl, 5.06%.

Corresponding β-isomer. $R_f = 0.31$ (toluene:AcOEt 3:1); $[\alpha]_D$ +55.8° (*c* 1, $CHCl_3$); δ_H (270 MHz, $CDCl_3$) 7.8–7.0 (13 H, m, Ar), 6.0–5.8 (2H, m, C<u>H</u>=CH_2 and NH), 5.50 (1H, s, C<u>H</u>Ph), 5.33 (1H, dd, $J = 17$ and 1.3 Hz, CH=C<u>H</u>$_2$), 5.19 (1H, dd, $J = 11$ and 1.3 Hz, CH=C<u>H</u>$_2$), 4.40 (1H, d $J = 8.3$ Hz, H-1), 4.17 (2H, s, $ClCH_2CO$), 3.46 (1H, bs, H-5); δ_C (67.8 MHz, $CDCl_3$) 102.26 (C-1), 100.88 (<u>C</u>HPh), 77.20, 73.55, 72.15, 69.62, 68.66, 66.42, 66.24, 60.15, 54.27, 47.05, 40.65; anal.: found: C, 60.31; H, 5.06; N, 7.69; Cl, 5.06%.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2-azido-4,6-*O*-benzylidene-2-deoxygalactopyranosyl)-L-serine allyl ester (21)**. A solution of compound **20** (300 mg, 0.40 mmol) and thiourea (150 mg, 2.0 mmol) in DMF (5 mL) was stirred at 90 °C for 45 min. Resultant mixture was evaporated in vacuo and the residue was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by silica gel column chromatography (toluene-AcOEt 3:1) to afford 187 mg (74%) of compound **21**; R_f 0.48 (toluene:AcOEt 3:1); $[\alpha]_D$ +102.8° (*c* 0.7, $CHCl_3$); δ_H (270 MHz, $CDCl_3$) 7.8–7.0 (13 H, m, Ar), 5.95 (1H, m, C<u>H</u>=CH_2), 5.85 (1H, d, $J = 7.9$ Hz, NH), 5.49 (1H, s, C<u>H</u>Ph), 4.98 (1H, d, $J = 3.3$ Hz, H-1), 3.74 (1H, bs, H-5); FAB-MS 643.1 ($M+H^+$); anal.: calcd for $C_{34}H_{34}N_4O_9$: C, 63.54; H, 5.33; N, 7.72; found: C, 63.43; H, 5.38; N, 7.83%.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-[*O*-(6-*O*-benzyl-2,3,4-tri-*O*-levulinoyl-β-D-galactopyranosyl)-(1→3)-(2-azido-4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-galactopyranosyl)]-L-serine allyl ester (22)**. To the stirred mixture of $CuBr_2$ (311 mg, 1.39 mmol), AgOTf (357 mg, 1.39 mmol), *n*-Bu_4NBr (45 mg, 0.14 mmol) and molecular sieves 4 Å (0.6 g) in 1,2-dichloroethane (10 mL) was added a solution of compounds **16** (158 mg, 0.279 mmol) and **21** (120 mg, 0.186 mmol) under ice–water cooling. The mixture was warmed up to room temperature, stirred for 3 h and then quenched with aq $NaHCO_3$. Insoluble materials were filtered off by the aid of Celite and the filtrate was diluted with AcOEt, washed with aq $NaHCO_3$ and brine, successively, dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by silica gel column chromatography (toluene:AcOEt 1:3) to afford 150 mg (68%) of compound **22**; R_f 0.52 (toluene:AcOEt 1:3); $[\alpha]_D$ +76.5° (C 1, $CHCl_3$); FAB-MS: 1188 (M^+), 1211 ($M+Na^+$); δ_H (270 MHz, $CDCl_3$) 7.8–7.0 (18 H, m, Ar), 6.0–5.8 (2H, m, C<u>H</u>=CH_2 and NH), 5.40 (1H, s, C<u>H</u>Ph), 5.39 (1H, d, $J = 2.9$ Hz, H-4'), 5.28 (1H, dd, $J = 17$ and 1.3 Hz, CH=C<u>H</u>$_2$), 5.22 (1H, dd, $J = 11$ and 1.3 Hz, CH=C<u>H</u>$_2$), 5.03 (1H, d, $J = 3.3$ Hz, H-1'), 2.76–2.44 (12H, m, $COCH_2CH_2CO$), 2.16, 2.14, and 2.12 (each 3H, s, Ac); δ_C (67.8 MHz, $CDCl_3$) 102.22 (C-1'), 100.38 (CHPh), 100.05 (C-1').

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-[*O*-(6-*O*-benzyl-2,3,4-tri-*O*-levulinoyl-β-D-galactopyranosyl)-(1→3)-(2-azido-3-*O*-chloroacetyl-2-deoxygalactopyranosyl)]-L-serine allyl ester (23)**. Under ice–water cooling, 80% aq CF_3COOH (1.4 mL) was added to the solution of compound **22** (137 mg, 0.115 mmol) in CH_2Cl_2 (6 mL). The mixture was stirred at 0 °C for 1.5 h and then evaporated in vacuo at ambient temperature. The residue was purified by silica gel column chromatography (toluene:AcOEt:MeOH 25:100:1) to afford 105 mg (82%) of compound **23**; $[\alpha]_D$ +26.7° (*c* 1, $CHCl_3$); FAB-MS: 1101.5 (M^+), 1124 ($M+Na^+$); δ_H (400 MHz, $CDCl_3$) 7.8–7.3 (13 H, m, Ar), 6.0–5.8 (2H, m, C<u>H</u>=CH_2 and NH), 5.34 (1H, d, $J = 3.4$ Hz, H-4'), 5.14 (1H, dd, $J = 10.3$ and 7.8 Hz, H-2'), 4.98 (1H, dd, $J = 10.3$ and 3.4 Hz H-3'), 4.84 (1H, d, $J = 3.4$ Hz, H-1'), 2.8–2.5 (12H, m, $COCH_2CH_2CO$), 2.16, 2.15, and 2.14 (each 3H, s, Ac); δ_C (100 MHz, $CDCl_3$) 101.41, 99.55; anal.: calcd for $C_{55}H_{64}N_4O_{20}$: C, 59.99; H, 5.86; N, 5.08; found: C, 59.94; H, 5.87; N, 4.73%.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-{*O*-[benzyl(5-acetamido-4,7,8,9-tetra-*O*-benzyl-3,5-dideoxy-3-phenylthio-D-*erythro*-α-L-gluco-2-nonulopyranosyl)onate]-(1→6)-*O*-[(6-*O*-benzyl-2,3,4-tri-*O*-levulinoyl-β-D-galactopyranosyl)-(1→3)]-*O*-(2-azido-3-*O*-chloroacetyl-2-deoxygalactopyranosyl)}-L-serine allyl ester (12)**. To the mixture of compounds **5** (80 mg, 0.089 mmol) and **23** (40 mg, 0.036 mmol), NIS (20 mg, 0.089 mmol), and molecular sieves 4 Å (0.2 g) in CH_2Cl_2:MeCN (10:1, 5 mL) was added 0.15 M solution of CF_3SO_3H in CH_2Cl_2 (0.12 mL, 0.018 mmol), while the mixture was stirred at −45 °C. After being stirred at the same temperature for 1 h, the mixture was quenched by aq $NaHCO_3$ and aq $Na_2S_2O_3$ and then filtered through Celite. The filtrate was diluted with AcOEt, washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by a column of Bio Beads S-X3 (Bio Rad) in toluene to afford 48 mg (70%) of compound **12**; $R_f = 0.57$ (toluene:AcOEt 1:3); $[\alpha]_D$ +29.7° (*c* 1.4, $CHCl_3$); FAB-MS: 1951 (M^+), 1974 ($M+Na^+$); δ_H (270 MHz, $CDCl_3$) 7.8–6.9 (38 H, m, Ar), 6.07 (1H, d, $J = 9$ Hz, NH), 5.85 (1H, m,

CH=CH_2), 5.41 (1H, d, J = 3.0 Hz, H-4^2), 5.27 (1H, d, J = 17.6 Hz, CH=CH_2), 5.21 (1H, d, J = 10.3 Hz, CH=CH_2), 5.11 (1H, d, J = 12.2 Hz, CH_2Ph), 4.96 (dd, J = 10.2 and 3.9 Hz, H-3^2), 3.82 (1H, d, J = 9 Hz, H-3^3), 2.9–2.4 (12H, m, $COCH_2CH_2CO$), 2.16, 2.15 and 2.13 (each 3H, s, COMe), 1.70 (3H, s, NHAc); δ_c (67.8 MHz, $CDCl_3$) 101.03, 100.20, 98.10 (anomeric carbons).

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-{*O*-[benzyl(5-acetamido-4,7,8,9-tetra-*O*-benzyl-3,5-dideoxy-3-phenylthio-D-*erythro*-α-L-gluco-2-nonulopyranosyl)onate]-(1→6)-*O*-[(6-*O*-benzyl-β-D-galactopyranosyl)-(1→3)]-*O*-(2-azido-3-*O*-chloroacetyl-2-deoxygalactopyranosyl)}-L-serine allyl ester (13).** To the ice–water cold solution of compound **12** (28 mg, 0.014 mmol) in toluene (0.8 mL) was added hydrazine acetate (23 mg, 0.25 mmol) in EtOH (4 mL). The mixture was stirred at room temperature for 1 h, diluted with $CHCl_3$, washed successively with aq $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was passed through a short column of silica gel ($CHCl_3$-MeOH 10:1) to afford 17 mg (73%) of compound **13**; $[\alpha]_D$ +27.6° (*c* 0.5, $CHCl_3$); FAB-MS: 1656 (M^+); δ_H (270 MHz, $CDCl_3$) 8.0–7.0 (38 H, m, Ar), 6.2–5.9 (2H, m, NH and CH=CH_2), 5.45 (1H, d, J = 17.1 Hz, CH=CH_2), 5.38 (1H, d, J = 11.5 Hz, CH=CH_2), 5.27 (1H, d, J = 11.9 Hz, CH_2Ph), 5.20 (1h, d, J = 11.9 Hz, CH_2Ph), 4.94 (1H, d, J = 3 Hz, H-1^1), 1.61 (3H, s, NHAc).

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References

1. For the use of a PhS group for 2-deoxyglycoside synthesis, see: Nicolaou, K. C.; Ladduwahetty, T.; Landall, J. L.; Chucholowski, A. *J. Am. Chem. Soc.* **1986**, *108*, 3987.

2. Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 3987; *idem. Tetrahedron* **1990**, *46*, 89.

3. For investigations on PhS-directed sialylation from other groups, see: (a) Kondo, T.; Abe, H.; Goto, T. *Chem. Lett.* **1988**, 1657; (b) Nicolaou, K. C.; Hummel, C. W.; Beckovich, N. J.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1991**, 870; (c) Ercegovic, T.; Magnusson, G. *J. Chem. Soc., Chem. Commun.* **1994**, 831.

4. (a) Okamato, K.; Kondo, T.; Goto, T. *Tetrahedron* **1987**, *44*, 1291; (b) Kanie, O.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1988**, *7*, 501.

5. Ito, Y.; Numata, M.; Sugimoto, M.; Ogawa, T. *J. Am. Chem. Soc.* **1989**, *111*, 8508; Ito, Y.; Nunomura, S.; Shibayama, S.; Ogawa, T. *J. Org. Chem.* **1992**, *57*, 1821.

6. Nakahara, Y.; Iijima, H.; Ogawa, T. *ACS Symp. Ser.* **1994**, *560*, 249.

7. Okamoto, K.; Kondo, T.; Goto, T. *Bull. Chem. Soc. Jpn* **1987**, *43*, 5919.

8. Gybin, A. S.; Smit, W. A.; Bogdanov, V. S. *Tetrahedron Lett.* **1980**, *21*, 383.

9. Ramesh, S.; Kaila, N.; Grewal, G.; Frank, R. W. *J. Org. Chem.* **1990**, *55*, 5.

10. For the preparation of **3a** from methyl 5-acetamido-2,3-dehydro-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranosonate [$PhCH_2Br$ (12 equiv.), KOH (11.6 equiv.), BaO (5.8 equiv.), Bu_4NI (0.06 equiv.)/DMSO, 79% in 1.7 g scale[2] and 77% (74% after crystallization) in 7 g scale], it is critical to use freshly distilled (both from CaH_2) $PhCH_2Br$ and DMSO. In addition, the water content of powdered KOH may differ from bottle to bottle, and the optimum molar ratios of reagents may be variable. Other conditions were also investigated: $PhCH_2Br$ (16 equiv.)-BaO (32 equiv.)-$Ba(OH)_2$ (4 equiv.), 45% (isolated as methyl ester after treatment with CH_2N_2) and 61% yield in DMF and THF-DMSO (5:2), respectively. For benzylation of corresponding 8,9-O-benzylidene derivative[5], the use of CaH_2 in place of BaO gave the best result (70%).

11. Pozsgay, V.; Jennings, H. J. *Tetrahedron Lett.* **1987**, *28*, 1375.

12. Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331; Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313.

13. Prepared in the same manner as described for corresponding ethylthio glycoside: Sato, S.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1986**, *155*, c1.

14. Ek, M.; Garegg, P. J.; Hulyberg, H.; Oscarsson, S. *J. Carbohydr. Chem.* **1983**, *2*, 305.

15. Macindoe, W. M.; Iijima, H.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1995**, *269*, 227.

16. Glaudemans, C. P. J.; Bertolini, M. *Methods Carbohydr. Chem.* **1970**, *8*, 271.

17. Sato, S.; Mori, M.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1986**, *155*, c6.

18. Koeners, H. J.; Verhoeven, J.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1981**, *100*, 65.

19. Nakano, T.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1993**, *243*, 43.

20. For example, see: Murase, T.; Kameyama, A.; Kartha, K. P. P.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1989**, *8*, 265; Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Oriyama, T.; Griffith, D. A. *J. Am. Chem. Soc.* **1992**, *114*, 8329.

21. Matsuo, I.; Nakahara, Yu.; Ito, Y.; Nukada, T.; Nakahara, Y.; Ogawa, T. *Bioorg. Med. Chem.* **1995**, *3*, 1455, and references cited therein.

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