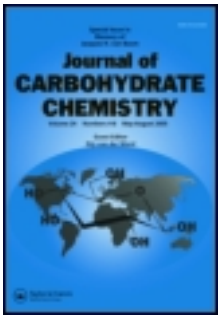

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Shin-ichi Tanaka [a] , Takashi Goi [a] , Katsunori Tanaka [a] & Koichi Fukase [a]
[a] Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka, Japan
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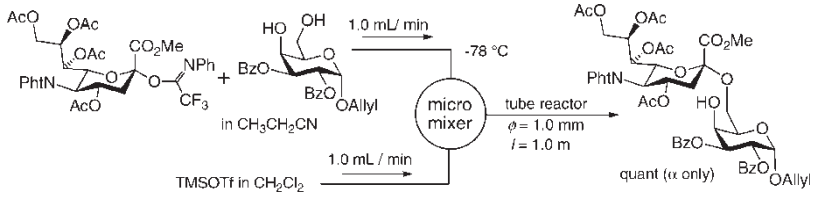
Highly Efficient α-Sialylation by Virtue of Fixed Dipole Effects of *N*-Phthalyl Group: Application to Continuous Flow Synthesis of α(2-3)- and α(2-6)-Neu5Ac-Gal Motifs by Microreactor

Shin-ichi Tanaka, Takashi Goi, Katsunori Tanaka, and Koichi Fukase

Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka, Japan

Highly α-selective sialylation of sialic acid *N*-phenyltrifluoroacetimidate with various galactose and lactose acceptors has been achieved by introducing the C-5 *N*-phthalyl group on the donor. The "fixed dipole effect" of the *N*-phthalyl group was proposed to explain the high reactivity and α-selectivity. The microfluidic system was applied to the present α-sialylation, which is amenable to large-scale synthesis. The *N*-phthalyl group was removed by treatment with methylhydrazine acetate, for which protocol can be readily applied to the synthesis of a variety of sialic acid-containing oligosaccharides.



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Address correspondence to Koichi Fukase, Department of Chemistry, Graduate School of Science, Osaka University, Machikaneyama 1-1, Toyonaka, Osaka 560-0043, Japan. E-mail: koichi@chem.sci.osaka-u.ac.jp

Keywords α-Sialylation, *N*-Phthalyl group, Fixed dipole effects, Microreactor, Oligosaccharides

N-Acetylneuramic acid (Neu5Ac), the most abundant sialic acid congener in nature, is found at the termini of glycoproteins and glycolipids on mammalian cell surfaces, usually linked with galactose or *N*-acetylgalactosamine through α(2-3) or α(2-6) sialoglycoside bonds. Since Neu5Ac on the cell surfaces plays diverse and important roles in cell/cell interaction processes,[1] such as pathogen/host recognition, tumor metastasis, and cell differentiation/proliferation, much effort has been devoted to the development of efficient and stereoselective synthesis of α(2-3) and α(2-6)-Neu5Ac-Gal units in order to further investigate their biological functions.[2,3] However, an efficient and general sialylation with high α-selectivity still has not been fully realized, because (1) the presence of the electron-withdrawing carboxylic acid groups at C-2 of the sialyl acid donors deactivates the oxocarbenium ions, thus electronically and sterically interfering with efficient sialylation. This leads to the formation of a significant amount of the dehydrated byproduct, 2,3-glycal. In addition, (2) the absence of the neighboring participation group at C-3 cannot ensure the stereochemical outcome for the α-selective sialylation. This inherent reactivity of the sialyl donor renders the α-selective sialylation as one of the most difficult[4] and challenging topics in the field of oligosaccharide synthesis.[5]

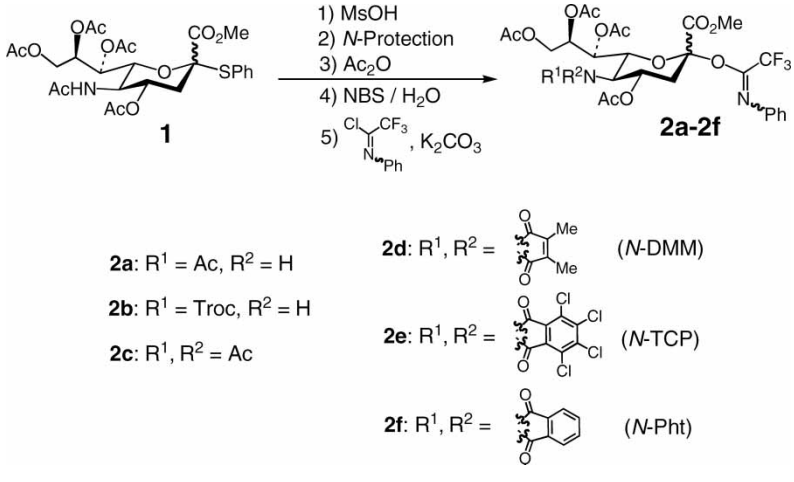
Recently, Cai and Yu have successfully enhanced the reactivity of sialic acid donors by utilizing phenyltrifluoroacetimidate as a leaving group, and achieved efficient sialylation with a variety of acceptors in 59% to 90% yields: while (2-3)-Neu5Ac-Gal synthesis has been realized in 81% yield ($\alpha:\beta = 3:1$), the corresponding α-selective (2-6)-sialylation was achieved in 61% yield.[6] Kiso and coworkers[7] and Takahashi and coworkers[8] have used the C-5-*N*-Troc-protected thiophenyl derivatives as the sialic acid donors and achieved good α-selectivity, namely, $\alpha:\beta = 54:10$ for the (2-3)-sialylation case.[7] Apparently, one of the most exciting achievements in this field is the α-(2-8)-linkage formation between the sialosides, quite recently realized by Kiso's[9a] and Takahashi's[9b] groups. Especially, Takahashi's group has utilized the 4-*O*,5-*N*-oxazolidinone-protecting group both on the sialoside donor and the acceptor and realized both α-(2-9)- and α-(2-8)-oligosialoside synthesis with excellent selectivity.[9b]

In our program directed toward the establishment of a general and practical synthesis of *N*-linked oligosaccharides and other neuramic acid-containing natural products, highly yielding and α-selective sialylation is essential. The recent successful precedents mentioned above led us to utilize the phenyltrifluoroacetimidate donor and to pursue the possibility of increasing the α-selectivity by modifying the C-5-*N*-protecting groups of the sialic acid donors (i.e., the amide groups of which dipole moments direct the stereochemical course of glycosylation and/or the carbonyls take part in the neighboring group

participation). In this paper, we disclose in detail[10] highly efficient sialylation with excellent α-selectivity by using the *N*-phthalyl group, which led to a general synthesis of the α(2-3)- and α(2-6)-Neu5Ac-Gal moieties. The method is applied to microfluidic α-sialylation in pursuit of a scaled-up synthesis of neuramic acid-containing compounds. The possible mechanisms for the enhanced α-selectivity based on the conformational analysis and electronic property calculations are also described.

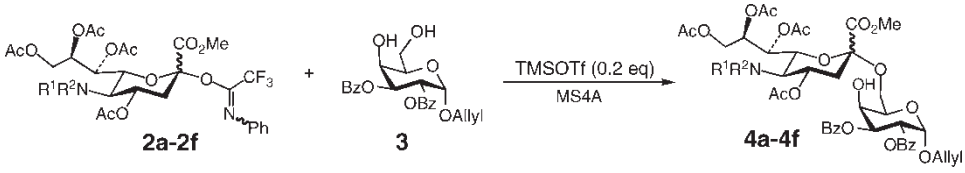
For the sialic acid donors, we planned six differently protected derivatives on the C-5 nitrogen, namely, acetyl (**2a**), Troc (**2b**), bis-acetyl (**2c**), dimethylmaleoyl (DMM, **2d**), tetrachlorophthalyl (TCP, **2e**), and phthalyl (Pht, **2f**) (Sch. 1). These donors were easily prepared from thioglycoside **1**,[11] according to Higuchi's procedure:[3e] (1) deacetylation, (2) protection of the C-5 nitrogen, (3) peracetylation of the remaining hydroxyls, (4) hydrolysis of the thioglycoside, and (5) phenyltrifluoroacetimidate formation.

α-Selective (2-6)-sialylation trials using donors **2a**–**2f** thus prepared and 1-*O*-allyl-2,3-*O*-benzoylgalactose **3**, appropriately protected for further functional group manipulation, are shown in Table 1. First, the reactivity of the sialy donors **2a**–**2f** was screened using 50 mg of each sample (Table 1). All reactions were performed at -78°C in the presence of TMSOTf as the Lewis acid activator, using 1.5 equivalents of acceptor **3** with respect to the donors. Propionitrile was used as the optimal solvent, by taking advantage of "nitrile solvent effect."[13] The reaction of *N*-mono-acetyl donor **2a** with acceptor **3** provided the corresponding disacchalide **4a** in good yield (entry 1, 93%) and with moderate selectivity (α:β = 77:23). It is noteworthy that, when *N*-Troc derivative **2b** was used (entry 2), the reaction was significantly accelerated (6 h for *N*-Ac and



Scheme 1: Preparation of sialic acid donors **2a**–**f** bearing a variety of *N*-protected groups.

Table 1: α-Selective (2-6)-sialylation Using **2a–f**.[a]



Entry	Donor	Solvent	Temp	Time	Product	Yield (%)[b]	α:β[c]
1	**2a**	EtCN	−78°C	6 h	**4a**	93	77:23
2	**2b**	EtCN	−78°C	30 min	**4b**	84	92:8
3	**2c**	EtCN	−78°C	<5 min	**4c**	75	72:28
4	**2d**	EtCN	−78°C	<5 min	**4d**	94	96:4
5	**2e**	EtCN	−78°C	<5 min	**4e**	87	96:4
6	**2f**	EtCN	−78°C	30 min	**4f**	92[d]	α only
7	**2f**	CH_2Cl_2	rt	<5 min	**4f**	87	9:91
8	**2f**	EtCN	−78°C	30 min	**4f**	60[e]	α only

[a]All sialylations were performed using 1.5 equiv of acceptor **3** relative to donors **2a**–**2f**. The mixture of anomeric stereoisomers for imidates **2a**–**2f** was used.
[b]Isolated yields.
[c]The α/β- ratio was determined by NMR analysis: α- and β-isomers were identified based on the empirical rule for their characteristic proton chemical shifts.[12]
[d]Yield at 50 mg- scale of **3**.
[e]Yield at 100 mg- scale of **3**.

30 min for *N*-Troc), and α-selectivity also increased up to α:β = 92:8. This observation is in accordance with that of Kiso and coworkers.[7]

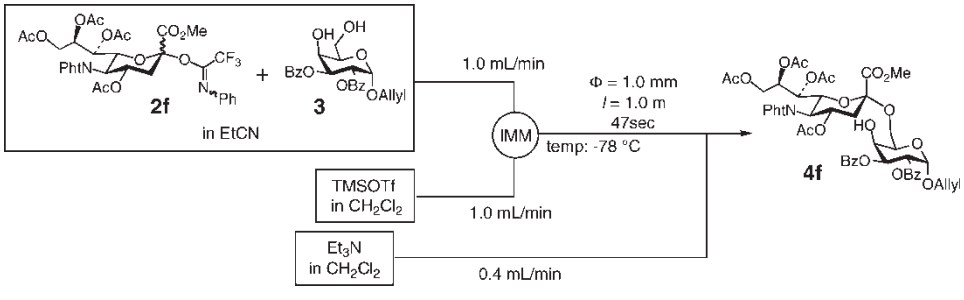
Encouraged by these promising results, we then examined the *N*-bis-acylated donors **2c**–**2f**. Bis-*N*-acetyl derivative **2c** gave the corresponding disacchalide **4c** in moderate yield and with moderate α-selectivity (75% and α:β = 72:28, entry 3), similar to that observed for monoacylate **2a** (entry 1). However, we were glad to find that, when two acyl groups on the C-5 nitrogen were fixed within the five-membered rings, the α-selectivity increased dramatically. Thus, *N*-DMM- and *N*-TCP-protected donors **2d** and **2e** successfully provided disacchalides **4d** and **4e** in 94% and 87% yields, respectively, and both with excellent α-selectivity (α:β = 96:4, entries 4 and 5). Furthermore, the utilization of the *N*-Pht-protected donor **2f** resulted in perfect α-selectivity and excellent yield (92% on 50-mg scale, entry 6). It is worthwhile mentioning that, when the solvent was exchanged from propionitrile to the noncoordinating dichloromethane, the stereoselectivity was reversed (entry 7, α:β = 9:91), indicating the importance of the "nitrile solvent effect" in order to obtain good α-selectivity. However, when the scale of the reaction was increased by only two, the product yield markedly decreased, although the α-selectivity remained high. Thus, the 100-mg

scale reaction of **3** gave only 60% of α-sialoside accompanied by a significant amount of a glycal byproduct (entry 8).

The decrease in sialylation efficiency observed in entry 8 might be due to the high reactivity of **2f**. For such a case, precise reaction control is very difficult under the conventional batch process conditions, especially when the reaction is scaled up. Thus, the disorder of the reaction factors in the scaled-up batch reaction, that is, (1) precise temperature control; (2) mixing efficiency between acceptor, donor, and Lewis acid; and (3) reaction time might lead to the glycal production. In order to circumvent these problems, we used a continuous flow microreactor, which is reported to realize efficient mixing and fast heat transfer and, therefore, is recognized as innovative technology in recent organic synthesis.[14] Once the reaction conditions are optimized for the small-scale reaction, the same conditions are directly applied to a large-scale synthesis, since the reaction is conducted under the flow process conditions.[15] An application of the microfluidic system to the glycosylation reaction was first reported by Seeberger and coworkers on α-mannosylation.[16a] We also have established an efficient microfluidic glycosylation in combination with the affinity separation method.[16b]

For the present microfluidic sialylation, a propionitrile solution of sialyl donor **2f** and acceptor **3** with various concentrations was mixed with TMSOTf solution in dichloromethane at −78°C using an IMM micromixer[17] at the flow rate of 1.0 mL/min (Table 2). After the reaction mixture was allowed to flow at −78°C for additional 47 sec through a reactor tube ($\Phi = 1.0$ mm,

Table 2: Optimization of α-(2-6)-sialylation between donor **2f** and acceptor **3** using microreactor.



Entry	Donor 2f (M)	Acceptor 3 (M)	TMSOTf (M)	Yield of 4f (%)	$\alpha:\beta^a$
1	0.15	0.1	0.08	14	α only
2	0.15	0.1	0.15	88	α only
3	0.2	0.1	0.15	>99	α only

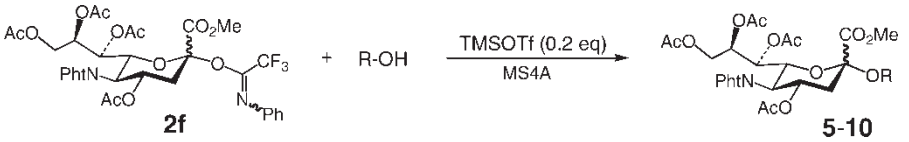
[a]Based on ^{1}H NMR analysis.

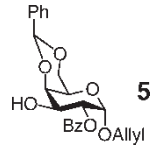
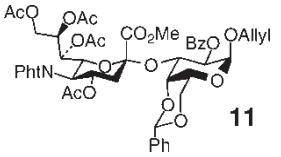
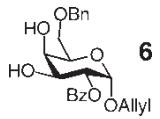
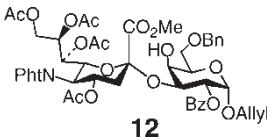
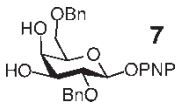
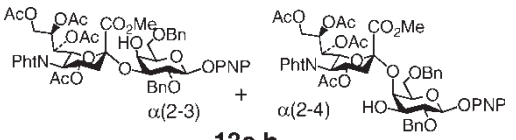
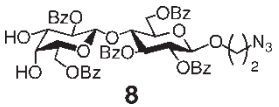
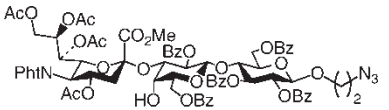
$l = 1.0$ m), the mixture was quenched by another flow of excess triethylamine dissolved in dichloromethane by using a T-shaped mixer at -78°C. CH_2Cl_2 was used as a cosolvent for the microfluidic sialylation in order to avoid blockages during the micromixing. By using the mixed solvent system of $EtCN/CH_2Cl_2$ (1:1), the same yield and α-selectivity in batch sialylation was observed as in Table 1, entry 6. When the concentrations of the donor **2f**, acceptor **3**, and TMSOTf were adjusted to 0.15 M, 0.1 M, and 0.08 M, respectively, disaccharide **4f** was obtained in only 14% yield and an excess amount of acceptor **3** was recovered (entry 1). However, we were pleased to find that the yield of **4f** dramatically increased (88%) when the concentration of the Lewis acid was increased up to 0.15 M (entry 2). Finally, the desired α-sialoside **4f** was obtained in quantitative yield by increasing the concentration of the donor **2f** to 0.2 M (entry 3). The use of excess amounts of donor **2f** or TMSOTf under the batch reaction conditions did not improve the sialylation yield, but rather resulted in a large amount of glycal production. It is noted that, by this continuous microflow reaction, single α-sialoside **4f** was reproducibly obtained without any decrease of the yield. The microfluidic reaction successfully controlled the high reactivity of the sialyl donor **2f** for α-sialylation, and efficient and large-scale procedures have now been realized.

The excellent α-selectivity achieved for α(2-6)-sialoglycosidation using the *N*-Pht donor **2f** was also applied to the (2-3)-sialylation cases (Table 3). Although poor selectivity (α:$\beta = 65$:35) was obtained by the reaction of **2f** with the sterically hindered 4,6-benzylidene-protected monosaccharaide acceptor **5** (entry 1), excellent α-selectivity (97:3) was achieved by the reaction with the 1-*O*-allyl-2-*O*-benzoyl-6-*O*-benzyl-protected acceptor **6** (entry 2). A slightly lower yield of 77%, compared with the (2-6)-sialylation case, is due to the decreased reactivity of the C-3 hydroxyl of acceptor **6**, thus giving rise to the 2,3-glycal production (16%). In order to prevent the glycal formation for such a (2-3)-sialylation case, we further examined the more reactive 2-benzyl-protected acceptor **7** (entry 3). Although the reaction rapidly proceeded and the glycal byproduct could not be detected from the crude mixtures, both (2-3)-and (2-4)-sialoglycosides **13a** and **13b** (**13a**:**13b** = 2:1) were produced in 85% total yield as their single α-isomers. The C2-α-configuration in (2-3)-and (2-4)-sialoglycosides **13a** and **13b** was assigned from the NOEs between methyl protons of the ester and H-4 and/or H-6 in the neuramic acid moiety.

The method was also applied to the lactose-derived disaccharide acceptors, providing the corresponding trisaccharides with excellent α(2-3)-selectivity and moderate yields (entries 4–6). The reaction of **2f** with the perbenzoyl-protected lactoses **8** and **9** gave α(2-3)-trisaccahride derivatives **14** and **15** as the single stereoisomers in 38% and 43% yields, respectively (entries 4 and 5). The yield was increased up to 50% by the reaction with more reactive perbenzylated acceptor **10**, although the α-selectivity slightly decreased (entry 6,

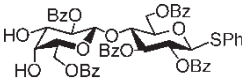
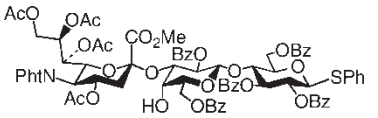
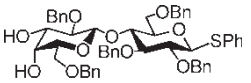
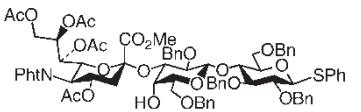
Table 3: α-Selective (2-3)-sialylation between **2f** and a variety of acceptors.[a]



Entry	Acceptor	Condition	Product	Yield (%)[b]	α:β[c]
1	 **5**	EtCN, −78°C, 5 min	 **11**	84%	65:35
2	 **6**	EtCN, −78°C, 5 min	 **12**	77%	97:3
3[d,e]	 **7**	EtCN, −78°C, 5 min	 α(2-3) + α(2-4) **13a,b**	85%	α only both for α(2-3) and α(2-4)
4	 **8**	EtCN, −78°C, 15 min	 **14**	38%	α only

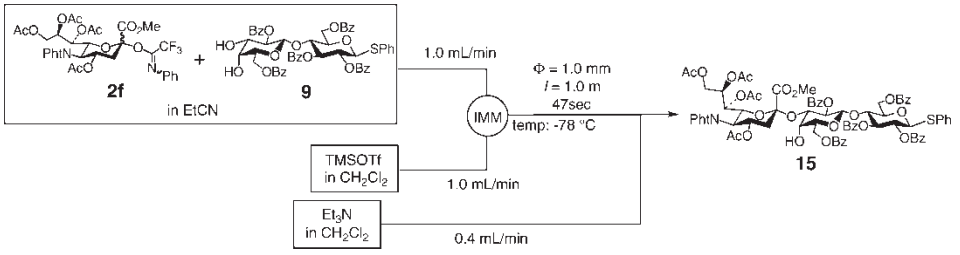
(*continued*)

Table 3: Continued.

Entry	Acceptor	Condition	Product	Yield (%)[b]	α:β[c]
5	 **9**	EtCN, −78°C, 15 min	 **15**	43%	α only
6	 **10**	EtCN, −78°C, 1 h	 **16**	50%	93:7

[a]All sialylations were performed using 1.5 equiv of acceptor **5**–**10** relative to donor **2f**.
[b]Isolated yields.
[c]The α/β-ratio was determined by NMR analysis.[12]
[d]Products ratio of α(2-3) and β(2-4) sialosides was 2:1. Their structures were analyzed after benzoylation.
[e]PNP = *p*-Nitrophenyl.

Table 4: Optimization of α-(2-3)-sialylation between donor **2f** and acceptor **9** using microreactor.



Entry	Donor 2f (M)	Acceptor 9 (M)	TMSOTf (M)	Yield of 15 (%)	$\alpha:\beta^a$
1	0.1	0.15	0.05	21	α only
2	0.3	0.1	0.15	50	α only
3	0.3	0.1	0.3	62	α only

[a]Based on ^{1}H NMR analysis.

$\alpha:\beta = 93:7$). The main byproduct of the sialylation using the disaccharide acceptors **8** and **9** was the glycal derivative of **2f**. The sterically bulkier disaccharides would not react smoothly with the donor cation derived from **2f**, but instead the 3H proton of the donor cation was subtracted to give rise to the glycal.

In anticipation of the efficient mixing effects between donor, acceptor, and acid, the microfluidic system was again applied to this case (Table 4). The perbenzoylated lactose **9** was used for the microfluidic sialylation trials, since the functional group manipulation after the glycosylation was easier than the corresponding perbenzylated acceptor **10**. After the optimization of the micromixing conditions, trisaccharide **15** was obtained in 62% yield as a single α-stereoisomer (entry 3), when 0.3 M of the donor **2f**, 0.1 M of the acceptor **9**, and 0.3 M of TMSOTf were combined by the IMM micromixer. This is about a 20% increase in the yield compared with that of the batch reaction (43% yield in Table 3, entry 5). By taking the results obtained in Table 2 and Table 4 together, the microfluidic reaction enabled α-selective sialylation for both α-(2-6) and α-(2-3) cases in higher yields than the conventional batch process.

In order to understand the good reactivity and α-selectivity observed for the C-5-*N*-phthalyl- and the other protected donors, conformational analysis of the intermediary oxocarbenium ions **17a**–**17f**, derived from the imidates **2a** (*N*-Ac), **2b** (*N*-Troc), **2c** (*N*-Ac_2), and **2f** (*N*-Pht), was performed based on the molecular mechanics calculations with the MMFF94s/MonteCarlo method using Spartan 02 software (Fig. 1a–d).[18,19] The boat-like folded conformations were found as the lowest-energy conformations for both

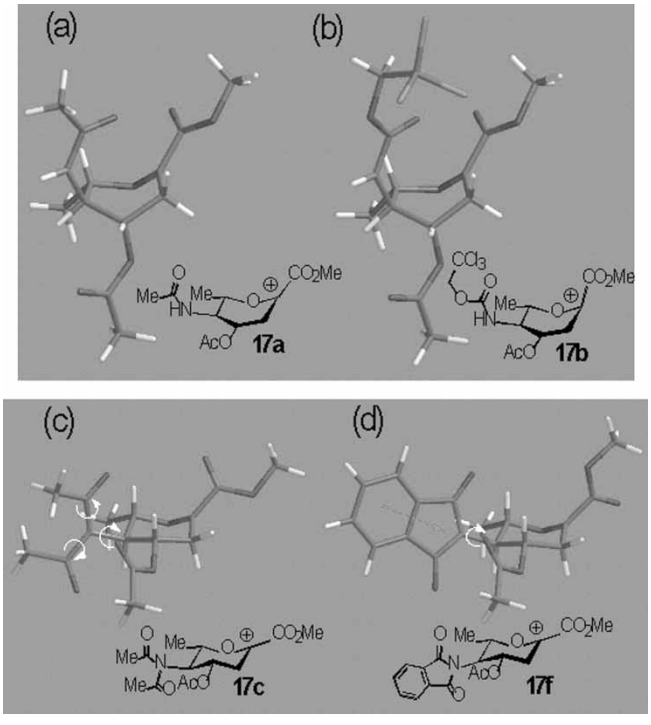


Figure 1: Optimized structures of oxocarbenium ions **17a**–**17f** by molecular mechanics calculation with MMFF94s/MonteCarlo Method Using Spartan 02 Software. The most stable conformations are shown in (a–d): Based on the experimental results in Table 1, the calculation was mainly performed to provide a rationale for the electronic effects of 5*N*-substitutents on oxocarbenium ion. Accordingly, the C-6 alkyl side chain was replaced by a simple methyl group in order to simplify the conformational analysis. Among the optimized conformations of **17c**, the *trans*-diacetyl conformation was obtained as the second lowest structure at 0.1 kcal/mol higher energy level.

monoacylates **17a** (*N*-Ac) and **17b** (*N*-Troc) (Fig. 1a, b). For these energy-minimized conformations, both *N*-acetyl and *N*-Troc carbonyls are thought to be participating in the electrostatic interaction with the oxocarbenium ions, thus stabilizing the intermediates. Although the observed α-selectivity is mainly caused by the nitrile solvent effects as exemplified by both Kiso's and Takahashi's experiments with 5-*N*-Troc derivatives,[7,8] the additional conformational preferences of Figures 1a and 1b may also lead to the attack of the acceptor from the backside of the molecule and thus high α-selective sialylation would result. The carbonate (O-C=O) group would be involved in a stronger interaction with the carbenium ion than the acyl (C=O) group; thereby, the higher α-selectivity of Troc-protected **2b** would result.

On the other hand, chair-like extended conformations were obtained for both *N*-bis-acyl derivatives **17c** (*N*-Ac_2) and **17f** (*N*-Pht) as their energy-minimized conformations (Fig. 1c, d). Since the *N*-Ac_2 and *N*-Pht moieties determine the totally different reactivity and selectivity toward the glycosylation

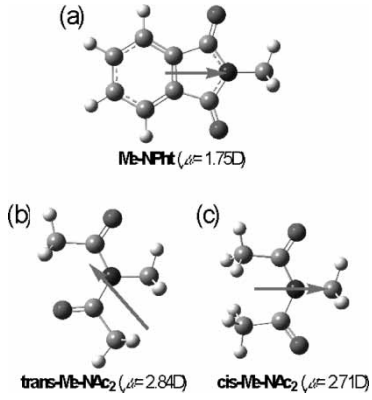


Figure 2: Optimized structures and dipole moments of simplified *N*-Pht and *N*-Ac_2 moieties by DFT/BLYP/6-31G* level calculation using Gaussian 03W software.

observed in Table 1, their local conformations and electronic properties were further investigated (Fig. 2). Thus, the geometries of the simplified Me-*N*Pht and Me-*N*-Ac_2 models were optimized at the DFT/BLYP/6-31G* level, and on these obtained minima, the dipole moments (μ) were then calculated by using Gaussian 03W Software.[20]

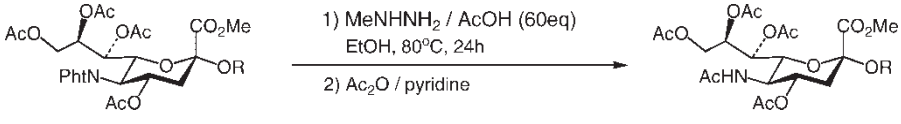
While one minimum was found, as expected, for the conformationally rigid Me-*N*Pht (Figure 2a, the dipole moment shown with a red arrow), two absolute minima were found for Me-*N*-Ac_2, where the *trans* isomer is more stable than the *cis* isomer by 3.57 kcal/mol (Fig. 2b, c). In the *trans* isomer, the dipole moment is directed from the nitrogen atom toward one of the carbonyls while it is aligned toward the *N*-Me direction in the *cis* isomer.

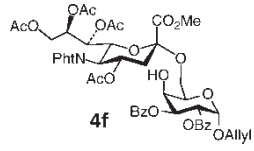
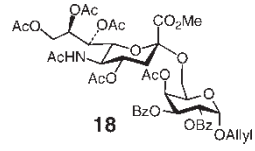
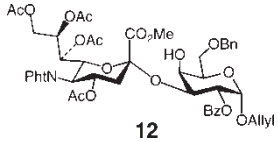
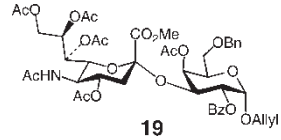
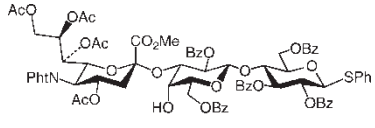
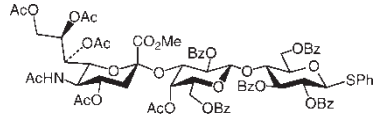
Therefore, in the oxocarbenium ion intermediate **17f** (Fig. 1d), the fixed dipole of the *N*-Pht may possibly facilitate the nitrile solvent participation from the β-face of the molecule. Namely, the fixed dipole moment in *N*-Pht aligned on the same plane as the sugar six-membered ring would interact with the dipoles of the attacking nitrile solvent due to the favorable dipole/dipole arrangement in space. It is noted that the rotation around the C-N bond in **17f** does not change the direction of the dipole of *N*-Pht (see yellow arrow in Fig. 1d); thus, the strong dipole/dipole effects may be anticipated. Furthermore, the fixed dipole may also stabilize the cation intermediate **17f** by interacting with the dipole of oxocarbonium ion. This stabilized cation can be trapped by the nitrile solvent from the β-side of the molecule, namely, due to the stereoelectronic effect, which in turn is attacked by the acceptor from the α-face of the molecule via S_N2-type substitution reaction. The significant solvent effects observed in Table 1 (entries 6 and 7) support this rationale. A similar dipole stabilized carbocation intermediate has also been proposed by Wong and coworkers in order to explain the highly α-selective sialylation using the C 5-*N*-azide sialoside donor.[2p]

On the other hand, no such strong dipole/dipole interactions can be expected for the conformationally flexible bis-*N*-acylate oxocarbenium ion **17c**. As predicted from the dipole orientations of *N*-Ac_2 in Figures 2b and 2c, when two acetyl groups on the nitrogen and the N-C bond rotate freely (see yellow arrows in Fig. 1c), the dipoles are randomized; that is, the dipole is not fixed as parallel to the pyrane plane. This offset of the dipole orientations of *N*-Ac_2 might therefore decrease the reactivity and α-selectivity of **17c**, compared with that of **17f**. Although many other effects, including the solvation and temperature effects, operate the total reactivity and stereochemical results on the sialylation, the conformational and "fixed dipole" analysis performed on **17c** and **17f** supported the experimentally observed high α-selectivity of **2f**, the 5-*N* phthalyl-protected sialoside donor.

Finally, removal of the *N*-phthalyl group of di- and trisaccharides **4f**, **12**, **15**, and **16** was attempted (Table 5). Hydrazine acetate, a common reagent for the deprotection of the *N*-phthalyl group, unexpectedly hydrogenated the 1-*O*-allyl moiety of α(2-6)-disaccharide **4f** into the *O*-propyl group. The problem was circumvented by using the corresponding methylhydrazine acetate. The reaction of **4f** with excess methylhydrazine acetate in ethanol at 80°C provided a

Table 5: Removal of *N*-phthalyl group.



Entry	Substrate	Product	Yield (%)
1	 **4f**	 **18**	84
2	 **12**	 **19**	73[a]
3	 **15**	 **20**	45

[a]Corresponding hydrazone was obtained in 11% yield as a byproduct.

complex mixture of the products consisting of partially deacetylated compounds, which without the purification was acetylated with Ac_2O in pyridine to give *N*-Ac derivative **18** in 84% yield (entry 1). Similarly, the treatment of α(2-3)-disaccharide and trisaccharides **12** and **15** yielded the corresponding *N*-Ac derivatives **19** and **20** in 73% and 45% yields, although the corresponding hydrazone derivatives were obtained as the main byproducts for these α(2-3)-sialoside cases.

In summary, we have achieved highly efficient α-selective sialylation toward the synthesis of the α(2-6)- and α(2-3)-Neu5Ac-Gal units by tuning the electronic properties of the C-5 nitrogen-protecting groups. We proposed the *fixed dipole moment* concept in order to explain the high reactivity and excellent α-selectivity observed when the *N*-phthalyl group was utilized. Furthermore, the microfluidic system was applied to the present α-sialylation, and an efficient route to large-scale synthesis was established. The C5-*N*-phthalyl group utilized for α-selective sialylation was readily removed (i.e., by treatment with methylhydrazine acetate), thereby enabling efficient and general procedures for easy access to the sialic acid-containing library or complex *N*-linked glycans. Research directed along this line is now in progress in our laboratory.

EXPERIMENTAL

All commercially available reagents were used without further purification. Dichloromethane and propionitrile were refluxed over and distilled from CaH_2. Preparative separation was usually performed by column chromatography on silica gel (FUJI silysia LTD, BW-200 and BW-300) and by thin layer chromatography on silica gel (Merck, 20 × 20 cm, Silica gel 60 F_{254}, 1 mm). ^{1}H NMR spectra were recorded on a JEOL α-500 spectrometer and chemical shifts were represented as δ values relative to the internal standard TMS. ESI-MS was measured on an Applied Biosystems Mariner. High-resolution mass spectra (HRMS) were measured on a JEOL JMS-T100LC mass spectrometer.

Representative Procedure for *N*-protected Sialyl Donors

*Methyl 4,7,8,9-Tetra-O-acetyl-3,5-dideoxy-5-phthalimido-D-glycero-D-galacto-2-nonulopyranosylonate-2-N-phenyltrifluoroacetimidate (**2f**):*

To a solution of methyl (phenyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (1.5 g, 2.6 mmol) in MeOH (25 mL) was added methanesulfonic acid (190 μL, 2.6 mmol) and the resulting mixture was stirred at 60°C for 24 h. After the solution was cooled to rt, the mixture was concentrated in vacuo. Without purification of the intermediary amine derivative, NaOMe (530 μL, 2.6 mmol) was added to

a crude material dissolved in anhydrous THF (25 mL) to neutralize at rt under Ar atmosphere. After being stirred at rt for 10 min, phthalic anhydride (760 mg, 5.1 mmol) and Et_3N (720 μL, 5.1 mmol) were added and the reaction mixture was stirred at 70°C overnight. After the solution was cooled to rt, the mixture was concentrated in vacuo. The residue was dissolved in EtOAc; washed with saturated aqueous $NaHCO_3$, 1 M HCl, and brine; dried over Na_2SO_4; filtered; and concentrated in vacuo to give the crude product, which was roughly purified by column chromatography on silica gel (6% MeOH in chloroform) to give the intermediate *N*-Phth derivative as colorless needles (798 mg, 62%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.93–7.91 (m, 1H, SPh), 7.66–7.64 (m, 2H, Phth), 7.62–7.60 (m, 1H, SPh), 7.57–7.52 (m, 2H, Phth), 7.45–7.40 (m, 3H, SPh), 4.77 (d, $J = 10.5$ Hz, 1H, H-6), 4.33 (ddd, $J = 4.70$, 9.80, 11.2 Hz, 1H, H-4), 4.18 (brt, $J = 10.3$ Hz, 1H, H-5), 4.13 (brd, $J = 8.25$ Hz, 1H, H-7), 3.91–3.85 (m, 3H, H-8, H-9a, H-9b), 3.60 (s, 3H, CO_2CH_3), 2.76 (dd, $J = 4.55$, 13.5 Hz, 1H, H-3eq), 2.05 (dd, $J = 11.7$, 13.4 Hz, 1H, H-3ax); ESI-MS m/z calcd for $C_{24}H_{25}NO_9SNa$ $(M + Na)^+$ 526.11, found 526.11.

To a solution of *N*-Phth derivative obtained above (280 mg, 560 μmol) in pyridine (1 mL) was added Ac_2O (1 mL) at rt under Ar atmosphere. After the solution was stirred at rt overnight, the mixture was concentrated in vacuo and the residue was coevaporated with toluene three times. The residue was dissolved in EtOAc, and the organic layer was washed with saturated aqueous $NaHCO_3$, 1 M HCl solution, and brine; dried over Na_2SO_4; filtered; and concentrated in vacuo to give the crude product. The residue was purified by silica-gel column chromatography (17% ethyl acetate in toluene) to give *N*-Phth tetraacetate as colorless needles (61% for three steps): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (m, 2H, Phth), 7.68–7.66 (m, 2H, Phth), 7.53–7.51 (m, 2H, SPh), 7.32–7.28 (m, 3H, SPh), 5.89 (td, $J = 5.15$, 10.8, 10.8 Hz, 1H, H-4), 5.71 (dd, $J = 2.45$, 10.5 Hz, 1H, H-6), 5.20 (dd, $J = 2.45$, 3.65 Hz, 1H, H-7), 4.93 (ddd, $J = 2.85$, 7.00, 8.23 Hz, 1H, H-8), 4.36 (dd, $J = 2.60$, 12.3 Hz, 1H, H-9a), 4.14 (t, $J = 10.4$ Hz, 1H, H-5), 3.96 (dd, $J = 7.75$, 12.4 Hz, 1H, H-9b), 3.62 (s, 3H, CO_2CH_3), 2.72 (dd, $J = 5.50$, 13.8 Hz, 1H, H-3eq), 2.04 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.87 (s, 3H, Ac), 1.75 (s, 3H, Ac), 2.03 (dd, $J = 11.3$, 14.4 Hz, 1H, H-3ax); ESI-MS m/z calcd for $C_{32}H_{33}NO_{13}SNa$ $(M + Na)^+$ 694.16, found 694.16.

To a solution of *N*-Phth tetraacetate obtained above (50 mg, 74 μmol) in acetone were added NBS (27 mg, 150 μmol) and water (4.0 mg, 220 μmol) at rt. After the solution was stirred at rt for 1 h, the mixture was quenched by 10% aqueous $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give the crude product. The residue was purified by column chromatography on silica gel (chloroform) to give the hydrolyzed derivative as colorless needles (33 mg, 75%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88–7.80 (m, 2H, Phth), 7.74–7.72 (m, 2H, Phth), 5.82 (ddd,

$J = 5.63$, 10.5, 10.5 Hz, 1H, H-4), 5.40 (ddd, $J = 2.58$, 6.17, 8.12 Hz, 1H, H-8), 5.26 (dd, $J = 2.01$, 10.5 Hz, 1H, H-6), 5.13 (dd, $J = 2.04$, 7.92 Hz, 1H, H-7), 4.24 (dd, $J = 2.64$, 12.5 Hz, 1H, H-9a), 4.22 (t, $J = 10.5$ Hz, 1H, H-5), 4.03 (dd, $J = 6.22$, 12.4 Hz, 1H, H-9b), 3.89 (s, 3H, $CO_2C\underline{H_3}$), 2.36 (dd, $J = 5.57$, 13.0 Hz, 1H, H-3eq), 2.32–2.29 (m, 1H, H-3ax), 2.17 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.12 (s, 3H, Ac), 1.95 (s, 3H, Ac); ESI-MS m/z calcd for $C_{26}H_{29}NO_{14}Na$ $(M + Na)^+$ 602.15, found 602.16.

To solution of the compound obtained above (55 mg, 95 μmol) in acetone (1.0 mL) were added *N*-phenyltrifluoroacetoimidoylchloride (200 mg, 950 μmol) and K_2CO_3 (39 mg, 290 μmol) at rt under Ar atmosphere. After being stirred at rt for 3 h, the mixture was filtered and the filtrate was concentrated in vacuo to give the crude product. The residue was purified by column chromatography on silica gel (17% ethyl acetate in toluene) to give **2f** as a colorless needles (70 mg, 99%). Since the imidate product was labile under the acidic condition, after the identification by ESI-MS, it was immediately used for the sialylation: ESI-MS m/z calcd for $C_{32}H_{33}NO_{13}SNa$ $(M + Na)^+$ 773.18, found 773.14.

*Methyl 4,7,8,9-Tetra-O-acetyl-3,5-dideoxy-5-dimethylmaleimido-D-glycero-D-galacto-2-nonulopyranosylonate-2-N-phenyltrifluoroacetimidate (**2d**):*

ESI-MS m/z calcd for $C_{32}H_{35}F_3N_2O_{14}Na$ $(M + Na)^+$ 751.19, found 751.18. Data for precursor of imidate: ^1H NMR (500 MHz, $CDCl_3$) δ 5.66 (ddd, $J = 5.53$, 10.5, 10.5 Hz, 1H, H-4), 5.35 (ddd, $J = 2.44$, 6.41, 8.03 Hz, 1H, H-8), 5.09–5.05 (m, 2H, H-6, H-7), 4.26 (dd, $J = 2.49$, 12.4 Hz, 1H, H-9a), 4.03 (dd, $J = 6.43$, 12.3 Hz, 1H, H-9b), 3.99 (t, $J = 10.6$ Hz, 1H, H-5), 3.87 (s, 3H, $CO_2C\underline{H_3}$), 2.30 (dd, $J = 5.51$, 13.0 Hz, 1H, H-3eq), 2.25–2.21 (m, 1H, H-3ax), 2.14 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.95 (s, 6H, $C\underline{H_3}CH{=}CHC\underline{H_3}$); ESI-MS m/z calcd for $C_{24}H_{31}NO_{14}Na$ $(M + Na)^+$ 580.16, found 580.17.

*Methyl 4,7,8,9-Tetra-O-acetyl-3,5-dideoxy-5-tetrachlorophthalimido-D-glycero-D-galacto-2-nonulopyranosylonate-2-N-phenyltrifluoroacetimidate (**2e**):*

ESI-MS m/z calcd for $C_{24}H_{29}Cl_4F_3N_2O_{14}Na$ $(M + Na)^+$ 909.02, found 909.04. Data for precursor of imidate: ^1H NMR (500 MHz, $CDCl_3$) δ 5.69 (ddd, $J = 6.13$, 10.4, 10.4 Hz, 1H, H-4), 5.31 (ddd, $J = 2.41$, 5.93, 8.18 Hz, 1H, H-8), 5.18 (dd, $J = 2.06$, 10.5 Hz, 1H, H-6), 5.05 (dd, $J = 2.23$, 8.04 Hz, 1H, H-7), 4.20 (dd, $J = 2.52$, 12.4 Hz, 1H, H-9a), 4.12 (t, $J = 10.4$ Hz, 1H, H-5), 3.95 (dd, $J = 6.03$, 12.4 Hz, 1H, H-9b), 3.83 (s, 3H, $CO_2C\underline{H_3}$), 2.27 (dd, $J = 6.11$, 12.9 Hz, 1H, H-3eq), 2.26–2.23 (m, 1H, H-3ax), 2.05 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.91 (s, 3H, Ac), 1.80 (s, 3H, Ac); ESI-MS m/z calcd for $C_{26}H_{25}Cl_4NO_{14}Na$ $(M + Na)^+$ 737.99, found 738.00.

Representative Procedure of Sialylation

To a solution of the donor **2f** (50 mg, 67 μmol), acceptor **3** (45 mg, 100 μmol), and MS4A (70 mg) in propionitrile (1.0 mL) was added TMSOTf (2.5 μL, 13 μmol) at −78°C under Ar atmosphere. After the mixture was stirred for 30 min at this temperature, the reaction was quenched by a saturated $NaHCO_3$ solution. After MS4A was removed by filtration, filtrate was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give the crude product. The residue was purified by column chromatography on silica gel (chloroform) to afford the α-sialoside **4f** (61 mg, 92%).

*Allyl 2,3-Di-O-benzoyl-6-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-phthalimido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranoside (**4f**):*

(α-isomer) ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97–7.92 (m, 4H, aromatic), 7.76–7.75 (m, 2H, Phth), 7.66–7.65 (m, 2H, Phth), 7.45–7.42 (m, 4H, aromatic), 7.32–7.28 (m, 2H, aromatic), 5.87 (ddd, J = 5.51, 10.7, 22.3 Hz, 1H, $OCH_2C\underline{H}{=}CH_2$), 5.71 (m, 2H, H-2,3), 5.53 (ddd, J = 5.03, 10.6, 11.1 Hz, 1H, H-4′), 5.45 (dd, J = 2.71, 5.55, 8.42 Hz, 1H, H-8′), 5.34 (m, 1H, H-4), 5.31 (dd, J = 1.73, 17.2 Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.18 (dd, J = 2.42, 8.31 Hz, 1H, H-7′), 5.15 (dd, J = 1.44, 10.4 Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.10 (dd, J = 2.34, 10.6 Hz, 1H, H-6′), 4.43 (s, 1H, H-1), 4.29 (dd, J = 2.73, 9.76 Hz, 1H, H-9′a), 4.27 (dd, J = 5.09, 13.2 Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 4.23 (t, J = 10.6 Hz, 1H, H-5′), 4.19 (t, 1H, H-5), 4.00 (dd, J = 6.00, 13.3 Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 4.05–4.02 (m, 2H, H-9′b, H-6a), 3.91 (s, 3H, $CO_2C\underline{H}_3$) 3.87 (dd, J = 6.50, 10.0 Hz, 1H, H-6b), 3.03 (s, 1H, O<u>H</u>), 2.80 (dd, J = 5.14, 12.9 Hz, 1H, H-3′eq), 2.15 (s, 3H, Ac), 2.13 (s, 3H, Ac), 1.88 (s, 3H, Ac), 1.84 (s, 3H, Ac), 2.01 (m, 1H, H-3′ax); HRMS m/z calcd for $C_{49}H_{51}NO_{21}Na$ $(M + Na)^+$ 1012.2851, found 1012.2873. The α-configuration of **4f** was confirmed by converting to the corresponding *N*-Ac derivatives. (β-isomer) ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17–8.15 (m, 2H), 8.00–7.95 (m, 4H), 7.72–7.69 (m, 2H), 7.49–7.43 (m, 2H), 7.37–7.29 (m, 2H), 5.86–5.76 (m, 1H), 5.69–5.65 (m, 1H), 5.46–5.44 (m, 1H), 4.83 (s, 1H), 4.65 (dd, J = 2.50, 12.5 Hz, 1H), 4.52–4.43 (m, 2H), 4.23–3.65 (m, 7H), 3.78 (s, 3H), 3.75–3.763 (1H, m), 2.60 (dd, J = 5.50, 12.5 Hz, 1H), 2.05 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.92 (s, 3H, Ac).

*Allyl 2,3-Di-O-benzoyl-6-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-dimethylmaleimido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranoside (**4d**)*:

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03–7.98 (m, 4H, aromatic), 7.52–7.48 (m, 2H, aromatic), 7.39–7.35 (m, 4H, aromatic), 5.86 (m, 1H, $OCH_2C\underline{H}{=}CH_2$), 5.70 (m, 2H, H-2,3), 5.42 (ddd, J = 2.83, 6.14, 8.45 Hz, 1H,

H-8′), 5.36 (ddd, $J = 5.12, 10.2, 10.2$ Hz, 1H, H-4′), 5.34–5.33 (m, 1H, H-4), 5.31 (dd, $J = 1.65, 15.6$ Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.12 (dd, $J = 2.41, 8.24$ Hz, 1H, H-7′), 5.15–5.13 (m, 1H, $OCH_2CH{=}C\underline{H}_2$), 4.92 (dd, $J = 2.16, 10.8$ Hz, 1H, H-6′), 4.41 (s, 1H, H-1), 4.31 (dd, $J = 2.87, 12.5$ Hz, 1H, H-9′a), 4.26 (dd, $J = 5.13, 13.3$ Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 4.17 (t, 1H, H-5), 4.07–3.97 (m, 4H, H-5′, H-6a, H-9′b, $OC\underline{H}_2CH{=}CH_2$), 3.87 (s, 3H, $CO_2C\underline{H}_3$), 3.85 (dd, $J = 6.74, 10.0$ Hz, 1H, H-6b), 2.75 (dd, $J = 5.13, 12.9$ Hz, 1H, H-3′eq), 2.12 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.93 (s, 3H, Ac), 1.91, (s, 3H, Ac), 1.94 (m, 1H, H-3′ax), 1.94 (s, 6H, $C\underline{H}_3CH{=}CHC\underline{H}_3$); ESI-MS m/z calcd for $C_{47}H_{53}NO_{21}Na$ $(M + Na)^+$ 990.30, found 990.37.

*Allyl 2,3-Di-O-benzoyl-6-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-tetrachlorophthalimido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranoside (**4e**):*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03–7.98 (m, 4H, aromatic), 7.51–7.50 (m, 2H, aromatic), 7.39–7.35 (m, 4H, aromatic), 5.87 (m, 1H, $OCH_2C\underline{H}{=}CH_2$), 5.71 (m, 2H, H-2,3), 5.49–5.43 (m, 2H, H-4′, H-8′), 5.34 (m, 1H, H-4), 5.31 (dd, $J = 1.77, 15.5$ Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.18 (dd, $J = 2.49, 8.18$ Hz, 1H, H-7′), 5.15 (dd, $J = 1.66, 10.6$ Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.05 (dd, $J = 2.65, 10.8$ Hz, 1H, H-6′), 4.42 (d, $J = 0.90$ Hz, 1H, H-1), 4.32 (dd, $J = 2.91, 12.5$ Hz, 1H, H-9a), 4.27 (dd, $J = 5.15, 13.3$ Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 4.23 (t, $J = 10.5$ Hz, 1H, H-5′), 4.19 (t, $J = 6.33$ Hz, 1H, H-5), 4.07 (dd, J = 6.07, 13.3 Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 4.03 (dd, $J = 5.51, 12.6$ Hz, 1H, H-9′b), 4.07–4.02 (m, 1H, H-6a), 3.90 (s, 3H, $CO_2C\underline{H}_3$) 3.86 (dd, $J = 6.76, 10.1$ Hz, 1H, H-6b), 2.80 (dd, $J = 5.22, 13.0$ Hz, 1H, H-3′eq), 2.14 (s, 3H, Ac), 2.13 (s, 3H, Ac), 1.92 (s, 3H, Ac), 1.88 (s, 3H, Ac), 2.03 (m, 1H, H-3′ax); ESI-MS m/z calcd for $C_{49}H_{47}Cl_4NO_{21}Na$ $(M + Na)^+$ 1148.13, found 1148.13.

*Allyl 4,6-O-Benzylidene-3-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-phthalimido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranoside (**11**):*

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83–7.81 (m, 2H, Phth), 7.72–7.71 (m, 2H, Phth), 7.50–7.48 (m, 2H, aromatic), 7.34–7.31 (m, 3H, aromatic), 5.99–5.92 (m, 1H, $OCH_2C\underline{H}{=}CH_2$), 5.50 (ddd, $J = 4.94, 10.5, 11.8$ Hz, 1H, H-4′), 5.44 (s, 1H, $PhC\underline{H}$), 5.41 (ddd, $J = 2.92, 4.84, 9.13$ Hz, 1H, H-8′), 5.34 (dd, $J = 1.61, 17.2$ Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.18 (dd, $J = 1.8{,}3$ 10.6 Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.16 (dd, $J = 1.93, 9.23$ Hz, 1H, H-7′), 5.11 (d, $J = 3.55$ Hz, 1H, H-1), 5.04 (dd, $J = 1.91, 10.9$ Hz, 1H, H-6′), 4.65 (dd, $J = 3.35, 10.4$ Hz, 1H, H-2), 4.27 (dd, $J = 5.05, 13.2$ Hz, $OC\underline{H}_2CH{=}CH_2$), 4.24–4.18 (m, 2H, H-6a, H-9′a), 4.20 (t, $J = 10.4$ Hz, 1H, H-5′), 4.16–4.12 (m, 3H, H-3, H-4, H-6b), 4.09 (dd, $J = 5.53, 13.3$ Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 4.05 (dd, $J = 4.92, 12.5$ Hz, 1H, H-9′b), 3.75 (s, 1H, H-5), 3.71 (s, 3H, $CO_2C\underline{H}_3$), 2.61 (s, 1H, $O\underline{H}$),

2.87 (dd, $J = 5.08$, 13.0 Hz, 1H, H-3′eq), 2.17 (s, 3H, Ac), 2.15 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.80 (s, 3H, Ac), 2.02 (dd, $J = 11.8$, 13.1 Hz, 1H, H-3′ax); ESI-MS m/z calcd for $C_{42}H_{47}NO_{19}Na$ $(M + Na)^+$ 892.81, found 892.26.

*Allyl 2-O-Benzoyl-6-O-benzyl-3-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-phthalimido-D-glycero-β-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranoside (**12**)*:

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13–8.12 (m, 4H, PhCO-), 7.83–7.82 (m, 2H, Phth), 7.74–7.73 (m, 2H, Phth), 7.59–7.56 (m, 4H, PhCO-), 7.47 (m, 2H, PhCO-), 7.37–7.32 (m, 5H, $PhCH_2$-), 5.90 (ddd, $J = 4.56$, 9.73, 22.1 Hz, 1H, -$OCH_2CH{=}CH_2$), 5.52 (td, $J = 5.00$, 15.9 Hz, 1H, H-4′), 5.43–5.39 (m, 2H, H-8′, H-2), 5.31 (m, 1H, -$OCH_2CH{=}CH_2$), 5.24 (d, $J = 2.64$ Hz, 1H, H-1), 5.16–5.12 (m, 2H, H-7′, -$OCH_2CH{=}CH_2$), 5.02 (dd, $J = 1.72$, 10.9 Hz, 1H, H-6′), 4.76 (dd, $J = 3.58$, 10.3 Hz, 1H, H-9′a), 4.64 (d, $J = 8.53$ Hz, 1H, $PhCH_2$-), 4.62 (d, $J = 8.51$ Hz, 1H, $PhCH_2$-), 4.21 (m, 1H, $OCH_2CH{=}CH_2$), 4.21 (m, 1H, H-3), 4.17 (m, 1H, H-5′), 4.19 (m, 1H, H-5), 4.06 (dd, $J = 6.01$, 13.3 Hz, 1H, -$OCH_2CH{=}CH_2$), 4.00 (dd, $J = 5.43$, 12.4 Hz, 1H, H-9′b), 3.89–3.78 (m, 2H, H-6), 3.82 (s, 3H, -$COOCH_3$), 2.95 (brs, 1H, -OH), 2.62 (dd, $J = 4.93$, 13.3 Hz, 1H, H-3′eq), 2.12 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.91 (s, 3H, Ac), 1.80 (s, 3H, Ac), 2.07 (m, 1H, H-3′ax); ESI-MS m/z calcd for $C_{49}H_{53}NO_{20}Na$ 998.31 $(M + Na)^+$, found 998.34.

*p-Nitrophenyl 4-O-Benzoyl-2,6-di-O-benzyl-3-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-phthalimido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranoside (Benzoyl Derivative of **13a**)*:

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20–8.18 (m, 2H, aromatic), 8.07–8.05 (m, 2H, aromatic), 7.83–7.82 (m, 2H, Phth), 7.73–7.72 (m, 2H, Phth), 7.62–7.59 (m, 1H, aromatic), 7.50–7.47 (m, 2H, aromatic), 7.40–7.39 (m, 2H, aromatic), 7.31–7.22 (m, 8H, aromatic), 7.11–7.09 (m, 2H, aromatic), 5.59–5.57 (m, 2H, H-4, H-8′), 5.49 (ddd, $J = 5.00$, 10.5, 11.6 Hz, 1H, H-4′), 5.33 (dd, $J = 3.32$, 10.1 Hz, 1H, H-7′), 5.26 (d, $J = 3.51$ Hz, 1H, H-1), 5.20 (dd, $J = 2.22$, 8.53 Hz, 1H, H-3), 4.90 (d, $J = 12.5$ Hz, 1H, CH_2Ph), 4.78–4.75 (m, 2H, H-2, H-6′), 4.65 (d, $J = 12.7$ Hz, 1H, CH_2Ph), 4.54 (d, $J = 11.9$ Hz, 1H, CH_2Ph), 4.50 (t, $J = 6.02$ Hz, 1H, H-5), 4.46 (d, $J = 11.9$ Hz, 1H, CH_2Ph), 4.29 (dd, $J = 2.81$, 12.6 Hz, 1H, H-9′a), 4.14 (dd, $J = 4.31$, 12.7 Hz, 1H, H-9′b), 4.00–3.96 (t, 1H, H-5′), 3.96 (s, 3H, CO_2CH_3), 3.57–3.55 (m, 2H, H-6a,b), 2.52 (dd, $J = 5.02$, 12.6 Hz, 1H, H-3′eq), 2.18 (s, 3H, Ac), 2.15 (s, 3H, Ac), 1.90 (s, 3H, Ac), 1.73 (s, 3H, Ac); ESI-MS m/z calcd for $C_{59}H_{58}N_2O_{22}Na$ $(M + Na)^+$ 1169.34, found 1169.32.

*p-Nitrophenyl 3-O-Benzoyl-2,6-di-O-benzyl-4-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-phthalimido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranoside (Benzoyl Derivative of **13b**):*

^{1}H NMR (500 MHz $CDCl_3$) δ 8.19–8.17 (m, 2H, aromatic), 8.07–8.05 (m, 2H, aromatic), 7.82–7.80 (m, 2H, Phth), 7.73–7.72 (m, 2H, Phth), 7.60–7.57 (m, 1H, aromatic), 7.47–7.44 (m, 2H, aromatic), 7.31–7.05 (m, 12H, aromatic), 5.70 (ddd, J = 2.83, 5.04, 9.41 Hz, 1H, H-8′), 5.51 (ddd, J = 5.02, 10.5, 11.7 Hz, 1H, H-4′), 5.45 (dd, J = 3.33, 13.0 Hz, 1H, H-2), 5.02–4.97 (m, 1H, H-3), 4.99 (d, J = 12.6 Hz, 1H, C$\underline{H}_2$Ph), 4.73 (dd, J = 12.6, 10.6 Hz, 1H, H-6′), 4.73 (d, J = 12.6 Hz, 1H, C$\underline{H}_2$Ph), 4.55 (d, J = 11.9 Hz, 1H, C$\underline{H}_2$Ph), 4.47 (d, J = 11.9 Hz, 1H, C$\underline{H}_2$Ph), 4.30 (dd, J = 2.71, 12.5 Hz, 1H, H-9′a), 4.12 (t, J = 6.21 Hz, 1H, H-5), 4.03–3.97 (m, 2H, H-5′H-9′b), 4.03 (s, 3H, $CO_2C\underline{H}_3$), 3.66 (dd, J = 6.31, 9.93 Hz, 1H, H-6a), 3.59 (dd, J = 6.22, 9.93 Hz, 1H, H-6b), 2.57 (dd, J = 5.01, 12.6 Hz, 1H, H-3′eq), 2.21 (s, 3H, Ac), 2.15 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.74 (s, 3H, Ac); ESI-MS m/z calcd for $C_{59}H_{58}N_2O_{22}Na$ $(M + Na)^+$ 1169.34, found 1169.32.

*2-Azidoethyl 2,3,6-O-Benzoyl-4-O-[2,6-benzoyl-4-hydroxyl-3-O-(methyl 4,7,8,9,-tetra-O-acetyl-3,5-dideoxy-5-phthalimido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl]-β-D-glucopyranoside (**14**):*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10–8.08 (m, 4H), 7.99–7.97 (m, 2H), 7.93–7.91 (m, 4H), 7.84–7.82 (m, 2H), 7.75–7.73 (m, 2H), 7.65–7.63 (m, 1H), 7.58–7.33 (m, 14H), 5.73 (t, J = 9.46 Hz, 1H), 5.46–5.36 (m, 3H), 5.27–5.23 (m, 1H), 5.09 (dd, J = 1.53 Hz, 9.46 Hz, 1H), 4.79 (dd, J = 1.53 Hz, 10.9 Hz, 1H), 4.73 (d, J = 7.78 Hz, 1H), 4.64 (dd, J = 1.98 Hz, 13.0 Hz 1H), 4.61 (d, J = 7.93 Hz, 1H), 4.49 (dd, J = 4.43 Hz, 12.1 Hz, 1H), 4.39 (d, J = 2.75 Hz, 1H), 4.21 (t, J = 9.46 Hz, 1H), 4.19 (t, J = 10.7 Hz, 1H), 4.11 (dd, J = 3.05 Hz, 12.7 Hz, 1H), 3.98 (dd, J = 4.58 Hz, 12.7 Hz, 1H), 3.95–3.91 (m, 2H), 3.85–3.80 (m, 1H), 3.81 (s, 3H), 3.67–3.61 (m, 3H), 2.83 (dd, 1H, J = 5.04 Hz, 13.0 Hz), 2.09 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.02–1.98 (m, 1H), 1.92 (s, 3H, Ac), 1.82 (s, 3H, Ac).

*Phenyl 2,3,6-O-Benzoyl-4-O-[2,6-benzoyl-4-hydroxyl-3-O-(methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-phthalimido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl]-1-thio-β-D-glucopyranoside (**15**):*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (m, 2H), 8.03 (m, 2H), 7.98–7.94 (m, 5H), 7.77–7.30 (m, 17H), 7.18 (t, J = 7.35, 1H), 7.09–7.05 (m, 2H), 5.68 (t, J = 9.30 Hz, 1H), 5.57 (ddd, J = 2.20 Hz, 4.73 Hz, 9.73 Hz, 1H), 5.40 (t, J = 9.80 Hz, 1H), 5.35–5.25 (m, 2H), 5.03 (dd, J = 2.55 Hz, 9.63 Hz, 1H), 4.87–4.84 (m, 3H), 4.67 (d, J = 11.3 Hz, 1H), 4.50–4.47 (m, 2H), 4.22 (dd, J = 2.30 Hz, 13.0 Hz, 1H), 4.14 (t, J = 9.65 Hz, 1H), 4.03–4.00 (m, 1H), 3.95 (dd, J = 4.85 Hz, 12.6 Hz, 1H), 3.89 (t, J = 10.4 Hz, 1H), 3.82-3.79 (m, 1H),

3.70 (s, 3H), 3.65–3.56 (m, 2H), 3.44–3.43 (m, 1H), 2.69 (dd, $J = 5.05$ Hz, 12.6 Hz, 1H), 1.93 (3H, s, Ac), 1.93 (3H, s, Ac), 1.77 (3H, s, Ac), 1.48 (3H, s, Ac); HRMS m/z calcd for $C_{79}H_{73}NO_{28}SNa$ $(M + Na)^+$ 1538.3937, found 1538.3953.

*Phenyl 2,3,6-O-Benzyl-4-O-[2,6-benzyl-4-hydroxyl-3-O-(methyl 4,7,8,9,-tetra-O-acetyl-3,5-dideoxy-5-phthalimido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-1-thio-β-D-galactopyranosyl]-β-D-glucopyranoside (**16**):*

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (bs, 2H), 7.73–7.72 (m, 2H), 7.56–7.54 (m, 2H), 7.41–7.16 (m, 14H), 5.53–5.47 (m, 2H), 5.17 (dd, $J = 2.30$ Hz, 8.78 Hz), 5.06–5.03 (m, 2H), 4.82–4.69 (m, 5H), 4.62 (d, $J = 9.75$ Hz, 1H), 4.61 (d, $J = 7.60$ Hz, 1H), 4.51 (bs, 2H), 4.45 (d, $J = 12.1$ Hz, 1H), 4.36 (t, $J = 11.9$ Hz, 1H), 4.23–4.16 (m, 3H), 4.02 (t, $J = 9.50$ Hz, 1H), 3.95 (dd, $J = 5.20$ Hz, 12.6 Hz, 1H), 3.86–3.84 (bs, 1H), 3.85 (s, 3H), 3.70–3.67 (m, 1H), 3.61 (t, $J = 8.85$ Hz, 1H), 3.57–3.49 (m, 3H), 3.44 (t, $J = 9.60$ Hz, 1H), 3.40–3.37 (m, 1H), 2.73 (dd, $J = 5.00$ Hz, 13.0 Hz, 1H), 2.11 (s, 3H, Ac), 1.93 (s, 3H, Ac), 1.88 (s, 3H, Ac), 1.82 (s, 3H, Ac), 2.12–1.98 (m, 1H); ESI-MS m/z calcd for $C_{79}H_{83}NO_{23}SNa$ $(M + Na)^+$ 1468.50, found 1468.51.

Deprotection Procedure of *N*-phthalyl Group

To a solution of **4f** (1.0 mg, 1.0 μmol) in anhydrous EtOH (200 μL) were added methylhydrazine (3.2 μL, 61 μmol) and AcOH (3.5 μL, 61 μmol) at rt under Ar atmosphere. After the solution was stirred 24 h at 80°C and cooled to rt, the mixture was concentrated in vacuo. A crude product obtained was dissolved in Ac_2O and pyridine at rt. After being stirred overnight, the mixture was concentrated in vacuo and coevaporated with toluene three times. The residue was dissolved in EtOAc; washed with saturated aqueous $NaHCO_3$ solution, 1 M HCl solution, and brine; dried over Na_2SO_4; filtered; and concentrated in vacuo to give the crude product. The residue was purified by silica-gel column chromatography (from 0 to 2% MeOH in chloroform) to give **18** as colorless needles (800 μg, 84%).

*Allyl 4-O-Acetyl-2,3-di-O-benzoyl-6-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyloate)-α-D-galactopyranoside (**18**):*

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96–7.91 (m, 4H), 7.45–7.41 (m, 4H), 7.32–7.26 (m, 4H), 5.89–5.81 (m, 1H), 5.39–5.28 (3H, m), 5.17–512 (m, 2H), 4.93–4.88 (m, 1H), 4.40–4.37 (2H, m), 4.27–4.23 (2H, m), 4.18–4.02 (5H, m), 3.93 (dd, $J = 5.50$, 9.50 Hz, 1H), 3.83–3.80 (m, 1H), 3.82 (s, 3H), 2.17 (s, 3H, Ac), 2.18 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.88 (s, 3H, Ac), 2.06–2.03 (1H, m); ESI-MS m/z calcd for $C_{45}H_{53}NO_{21}Na$ $(M + Na)^+$ 966.30, found 966.43.

*Allyl 4-O-Acetyl-2-O-benzoyl-6-O-benzyl-3-O-(methyl 5-acetamido- 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyloate)-α-D-galactopyranoside (**19**):*

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12-8.10 (m, 2H), 7.62–7.19 (m, 8H), 5.86–5.79 (m, 1H), 5.41–5.38 (m, 1H), 5.29 (dd, $J = 2.50$, 7.50 Hz, 1H), 5.25–5.12 (m, 4H), 5.02 (t, $J = 10.1$ Hz, 1H), 5.02 (dd, $J = 5.50$, 10.0 Hz, 1H), 4.88–4.82 (m, 1H), 4.50 (d, $J = 11.5$ Hz, 1H), 4.42 (d, $J = 11.5$ Hz, 1H), 4.33 (dd, $J = 2.50$, 12.5 Hz, 1H), 4.25 (brt, $J = 6.00$ Hz, 1H), 4.20–4.17 (m, 1H), 4.09 (dd, $J = 2.50$, 12.5 Hz, 1H), 4.02 (q, $J = 10.0$ Hz, 1H), 3.98–3.94 (m, 1H), 3.68 (dd, $J = 2.00$, 10.8 Hz, 1H), 3.44 (dd, $J = 2.50$, 6.00 Hz, 2H), 2.42 (dd, $J = 5.00$, 12.8 Hz, 1H), 2.03 (3H, s, Ac), 1.99 (3H, s, Ac), 1.96 (3H, s, Ac), 1.89 (3H, s, Ac), 1.82 (3H, s, Ac), 1.79 (3H, s, Ac), 1.73 (t, $J = 12.5$ Hz, 1H) ESI-MS m/z calcd for $C_{45}H_{55}NO_{20}Na$ 952.32 $(M + Na)^+$, found 951.54.

*Phenyl 2,3,6-O-Benzoyl-4-O-[2,6-benzoyl-4-O-acetyl-3-O-(methyl 6-acetoamide-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-acetoamido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl]-1-thio-β-D-glycopyranoside (**20**)***:

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95–7.92 (m, 2H, Bz), (m, 6H, Bz), 7.74–7.73 (m, 2H, Bz), 7.54–7.16 (m, 15H, Bz), 7.11–7.06 (m, 4H, SPh), 6.97 (t, $J = 8.00$ Hz, 2H, SPh), 5.70 (t, $J = 9.00$ Hz, 1H, H-3), 5.48–5.43 (m, 2H, H-2′, H-4′), 5.40–5.37 (m, 1H, H-8″), 5.32–5.26 (m, 2H, H-2, H-3′), 5.23 (dd, $J = 1.00$, 8.75 Hz, 1H, H-9″a), 4.89 (d, $J = 8.00$ Hz, 1H, H-1′), 4.86 (d, $J = 10.0$ Hz, 1H, H-1), 4.80–4.75 (m, 1H, H-8″), 4.58 (dd, $J = 2.00$, 11.8 Hz, 1H, H-6a), 4.50 (dd, $J = 6.50$ Hz, 12.0 Hz, 1H, H-6b), 4.33 (dd, $J = 3.00$ Hz, 12.0 Hz, 1H, H-7″), 4.18 (t, $J = 10.0$ Hz, 1H, H-4), 4.06–4.03 (m, 2H, H-9″b, H-6″), 3.96–3.90 (m, 1H, H-5″), 3.80–3.77 (m, 1H, H-6′a), 3.71 (s, 3H, CO_2Me), 3.44 (dd, $J = 5.50$ Hz, 10.8 Hz, 1H, H-6′b), 2.76–2.72 (m, 1H, H-5′), 2.37 (dd, $J = 4.50$ Hz, 12.8 Hz, 1H, H-3″eq), 2.14 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.94 (s, 3H, Ac), 1.86 (s, 3H, Ac), 1.83 (s, 3H, Ac), 1.77–1.72 (m, 1H, H-3″ax); ESI-MS m/z calcd for $C_{73}H_{73}NO_{27}SNa$ 1492.41 $(M + Na)^+$, found 1492.56.

Representative Procedure of Microfluidic Reaction

A solution of TMSOTf (54 μL, 0.30 mmol, 0.15 M) in CH_2Cl_2 (2.0 mL) was injected in advance to the micromixer by using a syringe pump at the flow rate of 1.0 mL/min. Subsequently, a solution of donor **2f** (150 mg, 0.20 mmol, 0.20 M) and the acceptor **3** (43 mg, 0.10 mmol, 0.10 M) dissolved in EtCN (1.0 mL) was also injected to the IMM micromixer by another syringe pump at the flow rate of 1.0 mL/min and mixed at −78°C. After the reaction mixture was allowed to flow at −78°C for an additional 47 sec through a stainless reactor tube ($\Phi = 1.0$ mm, $l = 1.0$ m), the mixture was quenched by

another flow of an excess triethylamine dissolved in CH_2Cl_2 at −78°C. It takes about 2 to 3 min to consume 43 mg of the acceptor **3** under the above conditions. The mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give the crude product. The excess glycal obtained as a byproduct was removed by preparative TLC on silica gel (4% MeOH in chloroform) and the yield of **4f** was analyzed by ^1H NMR. The conditions established herein can be readily applicable to the scale-up synthesis simply by preparing the stock solutions of substrate and reagents and pumping them continuously into the micromixer (i.e., α-sialylation using 2.3 g of the acceptor **3** under the present microfluidic system has been realized for 10 min).

Computational Methods

The conformational distribution search for **17a**–**17c** and **17f** in Figure 1 has been performed by the Spartan02 package[18,19] using the MMFF94s molecular mechanics force field. The most stable conformations are shown in Figure 1. Similarly, the preliminary conformational distribution search for simplified *N*-Pht and *N*-Ac_2 moieties in Figure 2 was first performed by the Spartan02 package using the MMFF94s molecular mechanics force field. The real minimum energy conformers found by molecular mechanics have been further fully optimized at the DFT/B3LYP/6-31G* level as implemented in the Gaussian03 package.[20] These calculations gave one minima for *N*-Pht and two absolute minima for *N*-Ac_2 (*trans* isomer is more stable than *cis* isomer by 3.57 kcal/mol). The dipole moment (μ) was calculated on these obtained three absolute minima by means of DFT methods using the hybrid B3LYP functional and the 6-31G* basis set as available within Gaussian03.

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