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PAPER

Reinvestigation of the C5-acetamide sialic acid donor for α-selective sialylation: practical procedure under microfluidic conditions†

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Despite the previous literature describing the "low-to-modest" efficiency, the readily available C5-acetamide donor was reinvestigated for its use in α-sialylation under microfluidic conditions. The *N*-phenyltrifluoroacetimidate donor was efficiently mixed with an appropriate amount of TMSOTf to produce the α(2–6) and α(2–3)-sialylation products of galactose and glucosamine acceptors in excellent yields and with nearly perfect α-selectivity.

Introduction

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 and is usually linked to galactose or *N*-acetylgalactosamine through α(2–3) or α(2–6) sialoglycosidic bonds.[1] Because the presence of Neu5Ac on cell surfaces plays diverse and important roles, such as pathogen/host recognition, tumor metastasis, regulation of immunosuppressive signals, and cell differentiation/proliferation, much effort has been devoted to the development of efficient and stereoselective synthetic methods for producing α(2–3) and Neu5Acα(2–6)Gal units to investigate their biological functions.[2] α-Sialylation entails glycosylation at a sterically and electronically unfavorable oxocarbenium ion in such a way that the neighboring groups do not influence the stereochemical outcome. Although this reaction is one of the most difficult in the field of oligosaccharide synthesis, recent advances in glycosylation chemistry, *e.g.*, the development of new leaving and protecting groups, have enabled the efficient construction of α-sialoside linkages.[3]

The novel α-sialoside linkages feature a variety of C5-substituents, such as carbamates, on the sialic acid donors that may be used to tune the reactivity of the oxocarbenium ions. These substituents include *N*-Ac_2,[4] *N*-TFA,[5,6c] *N*-Troc,[6] and azide[7] groups and show improved yields and α-selectivities. Recently, the 5,4-*N*,*O*-cyclic carbamates have been utilized especially for the α(2–8)- and α(2–9)-sialylation case.[8] We have developed *N*-phenyltrifluoroacetimidate donors with C5-phthalimide[9a,b] or azide[9c] groups to apply the fixed dipole moment effects, and we have achieved quantitative sialylation with complete α-selectivity under microfluidic conditions.

In contrast, the *N*-Ac derivatives have not been extensively studied for α-sialylation.[3,10] These are considered to be the simplest sialic acid donors because they are readily available from the commercially available compounds and homologous to the natural 5*N*-Ac sialosides in that they do not require *N*-derivatization after sialylation. *N*-Ac imidate shows high α-selectivity in a few cases, such as α(2–6)-sialylation with the galactose acceptor,[11] although most reports,[3,10] including one of our own,[9a] have described the sialylation using *N*-Ac donors resulted in the modest yields and α-selectivities (~50% and α:β = ~3 : 1, respectively). Recently, Kononov and co-workers has rationalized the "low-to-modest" efficiency using the *N*-Ac donors by proving the hydrogen-bonding network between the N- and O-acetyl moieties; the supramolecular aggregation of the glycosyl donors, which is sensitively affected by the reaction concentrations, attenuates the nucleophilic attack and/or α-face approach of the acceptor.[12]

On the other hands, during a recent investigation of α-sialylation under microfluidic conditions,[9,13] we realized that in the conventional flask reaction, the efficiency and reproducibility of the reaction is quite sensitive to the heat generated during syringe-addition of the Lewis acid to the donor and acceptor. We hypothesized that this point has not been investigated to a great depth for the *N*-Ac case. That is, the efficient sialylation could be achieved by using the *N*-Ac donor, but the heat generated during the inefficient mixing in the flask may lead to poor yield, selectivity, and sometimes the reproducibility. Therefore, we reinvestigated the use the most straightforward C5-acetamide donor in pursuit of a practical route to α-sialylation.

Results and discussion

We initially optimized the α-sialylation of the *N*-Ac imidate **1**[11,14] using the C6-hydroxyl group of the galactose acceptor **2**[9a] in the presence of TMSOTf as an activator in propionitrile at −78 °C (Table 1). Assuming that temperature control was critical for sialylation in the presence of the donor **1**, the reaction was initially performed on a small scale (50–100 mg scale) by

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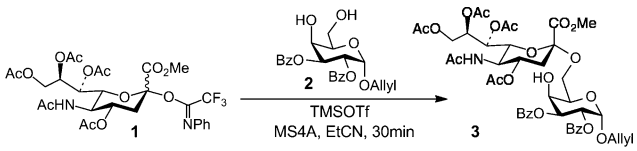
Table 1 Optimization of the α-sialylation of *N*-Ac sialyl imidate using the C6-hydroxy of galactose



Entry	1 (equiv)[a]	2 (equiv)[b]	LA (equiv)	*T*/°C	Apparatus	Yield (%)	α:β[c]
1	2.0	1.0	0.1	−78	Flask	Trace	NA
2	2.0	1.0	0.5	−78	Flask	<10	99 : 1
3	2.0	1.0	1.0	−78	Flask	77	96 : 4
4	2.0	1.0	1.0	−78	Micro	89	94 : 6
5	1.0	1.5	0.2	−78	Flask	Trace	NA
6	1.0	1.5	0.4	−78	Flask	40	99 : 1
7	1.0	1.5	1.0	−78	Flask	86	93 : 7
8	1.0	1.5	1.0	−78	Micro	89	94 : 6
9	1.0	1.5	1.0	−60	Micro	82	86 : 14
10	1.0	1.5	0.2	−20	Flask	89	74 : 26

[a] Entries 1–4: 100 mM, entries 5–10: 50 mM. [b] Entries 1–4: 50 mM, entries 5–10: 75 mM. [c] Determined by ^{1}HNMR.

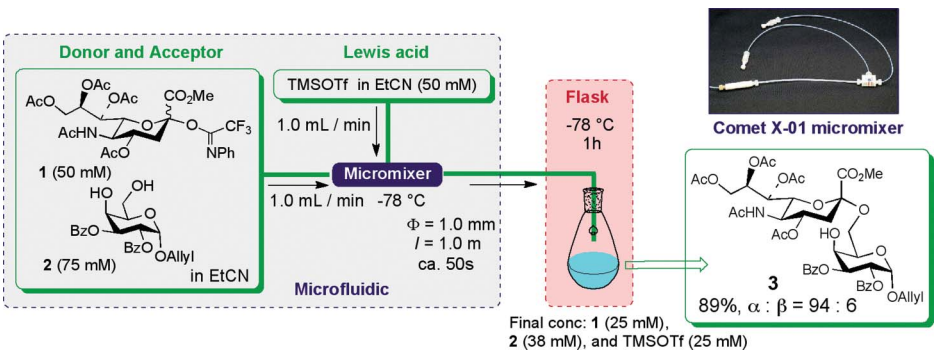


Fig. 1 Practical α-sialylation under microfluidic conditions.

conventional syringe addition of the Lewis acid to a mixture containing the donor and acceptor in a flask apparatus. One goal was to verify that the syringe-addition procedure in the flask did not affect the glycosylation outcome by introducing heterogeneous mixing and local heating that would re-route the reaction pathways away from the desired outcome. To achieve this goal, the bulk reaction was compared with the reaction performed under microfluidic conditions[15,16] using a Comet X-01 apparatus,[17] which produces efficient mixing and fast heat transfer.[13,18–22] Another advantage to performing the reaction in the micromixer is that the optimized conditions are directly applicable to large-scale synthesis under flow. The optimal conditions may be appropriately scaled up to yield a practical α-sialylation of oligosaccharides (*vide infra*).

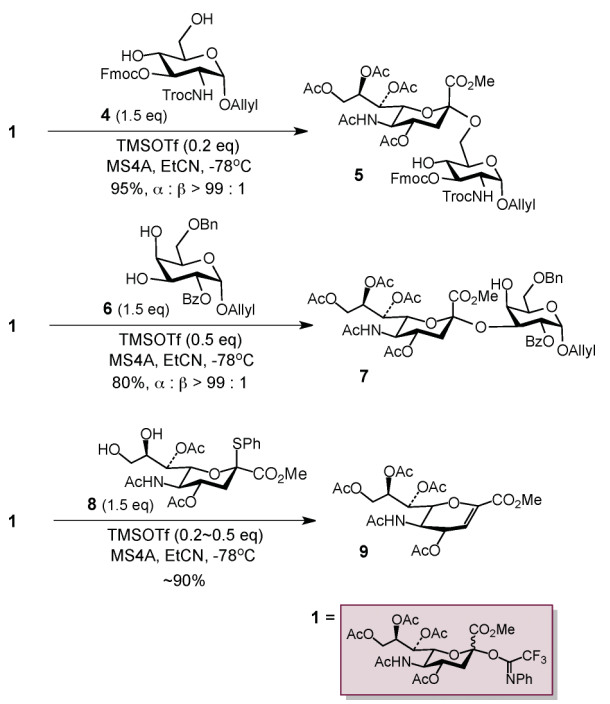
The reaction did not proceed significantly upon addition of catalytic amounts of the acid, *i.e.*, 0.1 equiv TMSOTf, to the solution containing the acceptor **2** (1 equiv, 50 mM) and the donor **1** (2 equiv, 100 mM) (Table 1, entry 1). Increasing the acid to 0.5 equiv gave only a small amount of the disaccharide **3** (entry 2). The reaction stopped after only 5 min, and the donor **1** was recovered intact. Thus, the reaction yield was proportional to the amount of Lewis acid present. We were gratified to find that 1.0 equiv of the Lewis acid resulted in formation of the disaccharide **3** in 77% yield with excellent α-selectivity (α : β = 96 : 4, entry 3). The conditions in entry 3 were reproducible under the microfluidic conditions (entry 4, 89% yield, α : β = 94 : 6, see the reaction system shown in Fig. 1).

Although donor **1** exhibited excellent α-sialylation reactivity in the presence of substoichiometric amounts of the acid under the optimized conditions, the presence of excess donor (2 equiv relative to the acceptor) inhibited purification of the sialoside **3** from the glycal byproduct due to similar polarities on the silica gel. Such a difficulty is incompatible with large-scale synthetic approaches. Therefore, we optimized the conditions by reversing the ratio between the donor and acceptor. One and one-half equivalents of the acceptor **2** (75 mM) were used with donor **1** (50 mM, entries 5–10). The observed trend mirrored the optimization trend described above. Catalytic amounts of TMSOTf (0.2 equiv) yielded essentially no product formation (entry 5). The use of 0.4 equiv acid gave 40% **3** (entry 6), and 1.0 equiv TMSOTf successfully provided the α-sialoside **3** in 86% yield with good selectivity (α : β = 93 : 7, entry 7). The yield and selectivity were validated by performing the sialylation reaction described in entry 7 under microfluidic conditions (89%, α : β = 94 : 6, entry 8). Concentrations of the substrates and Lewis acid did not affect the outcome of the sialylation efficiency under the conditions examined here;[12] although the reactions at higher concentrations than those performed in entry 8 could not be examined due to the

low solubility of the substrates in EtCN at −78 °C, an excellent reactivity of **1** was observed even in the diluted solutions (see Fig. 1 below). It should be noted that the mixing temperature affected the α-selectivity in a non-trivial way. Mixing at −60 °C such that the temperature was precisely adjusted by a micromixer yielded the sialoside **3** in 82% yield but with substantially decreased α-selectivity, α : β = 86 : 14 (entry 9).

As noted previously, most reported flask sialylation trials using *N*-Ac sialyl donors resulted in unsatisfactory results (~50% yields, α : β = ~3 : 1). We previously reported that microfluidic sialylation under the conditions described in entry 5 in Table 1 proceeded with good yields but with moderate selectivity (93%, α : β = 77 : 23).[9a] To resolve these contradictory results (current results: <5% yield (entry 5)), we intentionally added 0.2 equiv acid to the −20 °C solution containing the donor and acceptor in a flask (entry 10). The sialoside **3** was rapidly obtained in 89% yield with α : β = 74 : 26. The contradictory results may have resulted from temperature heterogeneities and/or heat generation during inefficient mixing in the flask, which promote the catalytic reaction at the expense of α-selectivity. To counteract such effects, one might add excess quantities of the Lewis acid in a small-scale reaction. Considering that a slight increase in the mixing temperature non-trivially affected the α-selectivity (entry 9), we concluded that the low efficiency and reproducibility of the *N*-Ac sialyl donors, reported by us and others, was not due to the inherently reactivity of the *N*-Ac donors, but was derived from the mixing inefficiencies associated with a flask apparatus.

After the NeuNAcα(2–6)Gal **3** was found to be obtained efficiently by using *N*-Ac donor **1** under the optimized conditions listed in Table 1, the other important α-sialylation reactions of the natural glycans, including α-sialylation of the C3- and C6-hydroxyls of galactose and glucosamine acceptors, were examined (Scheme 1). To our surprise, in contrast with the results reported in



Scheme 1 α-Selective sialylation with the C3- and C6-hydroxyls of galactose and glucosamine acceptors.

Table 1, even catalytic amounts of the Lewis acid promoted these glycosylation reactions. Hence, the amount of acid was optimized in each case to maximize the glycosylation efficiency. The reaction with the C6-hydroxy of *N*-Troc glucosamine **4** in the presence of 0.2 equiv TMSOTf provided the corresponding sialoside **5** in 95% yield with perfect α-selectivity. Sialylation proceeded smoothly for the more sterically demanding C3-hydroxy of galactose **6** in the presence of 0.5 equiv of the acid, with an α-selectivity that yielded **7** in 80% yield. Reaction with the C9–OH of the sialic acid **8**, a hydroxyl group known for its poor reactivity, resulted in formation of the glycal (**9**). This reaction illustrates the limitations associated with using the *N*-Ac donor **1**.

The pursuit of a sialylation protocol with practical applications to large-scale synthesis led us to integrate microfluidic procedures into the bulk procedures. The concentrations of the substrates and Lewis acid in the final reaction mixture were scaled down to half of those listed in Table 1, entry 8 to prevent solution blockage problems.[18] A propionitrile solution of the sialyl donor **1** (50 mM) and the acceptor **2** (75 mM) was mixed with TMSOTf in propionitrile (50 mM) at −78 °C using a Comet X-01 micromixer with a channel width of 500 μm at a flow rate of 1.0 mL min^{-1} (Fig. 1). The reaction mixture was allowed to flow with cooling at −78 °C for an additional 50 s through the reactor tube (f^3 = 1.0 mm, l = 1.0 m). The mixture was then introduced into a flask apparatus at −78 °C. Continuous stirring for another 1 hr at this temperature successfully provided a gram-scale preparation of the disaccharide **3** without decreasing the efficiency (89%, α : β = 94 : 6).

Conclusion

In conclusion, we explored the utility of the most straightforward C5-acetamide donor **1** for practical α-sialylation. The previously reported poor and non-reproducible sialylation results may have been due not to the inherent reactivity of this donor, but to mixing artifacts in the flask apparatus, in addition to the aggregate formation of the *N*-Ac donors under the certain concentrations.[12] To achieve a large-scale synthesis, we used a microfluidic apparatus to achieve rapid initial mixing, thereby establishing a practical bulk α-sialylation procedure. Other reactive intermediates may have been ignored simply due to technical issues associated with the conventional flask apparatus. These results suggest that several traditional reactions merit reinvestigation in microfluidic devices, which have not been extensively utilized in organic synthesis.

Experimental section

General

All commercially available reagents were used without further purification. Propionitrile was refluxed over and distilled from CaH_2. Preparative separation was performed by column chromatography on silica gel (FUJI silysia LTD, BW-200 and BW-300). ^1H and ^{13}C NMR spectra were recorded on either JEOL JNM-LA 500 spectrometer and chemical shifts were represented as δ-values relative to the internal standard TMS. The melting point was determined on a microscopic melting apparatus and uncorrected. Optical rotation was measured on a PERKIN ELMER models 241 polarimeter. IR spectra were recorded on a JASCO

FT/IR-6100 Fourier Transform Infrared Spectrometer. MALDI-TOF-mass spectra were measured on a PerSeptive Biosystems, Voyager RP-DE/H mass spectrometer equipped with a nitrogen laser ($\lambda = 337$ nm).

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

To a solution of the *N*Ac donor **1** (30.0 mg, 45.3 μmol), the acceptor **2** (29.1 mg, 68.0 μmol), and dry MS4A in EtCN (1.0 mL) was added TMSOTf (8.2 μL, 45.3 μmol) at −78 °C, and the resulting mixture was stirred at this temperature for 6 h. After the reaction was quenched by adding the excess triethylamine at −78 °C, the mixture was filtered and concentrated *in vacuo*. The residue was directly purified by column chromatography on silica gel from 67% to 100% of ethyl acetate in toluene) to give the disaccharide **3** (35.1 mg and 86% (sum of α + β), α : β = 93 : 7). These α- and β-isomers were further separated by column chromatography on silica gel (gradually from 67% to 100% of ethyl acetate in toluene). Data for α-isomer (white solid): $[\alpha]_D^{20}$ +69.0 (*c* 0.1, $CHCl_3$); mp 94–96 °C; IR (neat, cm^{-1}) 3377, 2957, 1742, 1538, 1452; MALDI-TOF-MS *m/z* calcd for $C_{43}H_{51}NO_{20}$ $(M+Na)^+$ 924.3, found 924.3; ^1H NMR (500 MHz, $CDCl_3$) δ 8.01 (ddd, *J* = 16.3, 8.4, 1.3 Hz, 4H, Bz), 7.52–7.48 (m, 2H, Bz), 7.37 (td, *J* = 7.4, 3.6 Hz, 4H, Bz), 5.85 (ddd, *J* = 22.2, 10.5, 5.9 Hz, 1H, $OCH_2C\underline{H}{=}CH_2$), 5.69 (dd, *J* = 2.1, 0.9 Hz, 1H, H-2′), 5.69 (dd, *J* = 2.1, 0.9 Hz, 1H, H-3′), 5.37 (ddd, *J* = 13.7, 7.4, 2.7 Hz, 1H, H-8), 5.34 (dd, *J* = 7.4, 2.0 Hz, 1H, H-7), 5.32 (d, *J* = 2.0 Hz, 1H, H-1′), 5.30 (ddd, *J* = 15.5, 3.2, 1.6 Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.21 (d, *J* = 9.7 Hz, 1H, Ac$\underline{H}$N), 5.14 (ddd, *J* = 10.6, 2.7, 1.1 Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 4.90 (ddd, *J* = 12.0, 10.0, 4.7 Hz, 1H, H-4), 4.39 (bd, *J* = 2.9 Hz, 1H, H-4′), 4.39 (dd, *J* = 12.3, 2.7 Hz, 1H, H-9a), 4.26 (ddt, *J* = 13.2, 4.6, 1.6 Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 4.17 (t, *J* = 6.2 Hz, 1H, H-5′), 4.12 (dd, *J* = 10.7, 2.1 Hz, 1H, H-6), 4.06 (dd, *J* = 12.3, 6.2 Hz, 1H, H-9b), 4.06 (q, *J* = 9.7 Hz, 1H, H-5), 4.05 (ddt, *J* = 13.2, 7.3, 1.6 Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 3.93 (dd, *J* = 9.7, 5.6 Hz, 1H, H-6a′), 3.82 (s, 3H, CO_2Me), 3.82 (dd, *J* = 9.7, 7.3 Hz, 1H, H-6b′), 3.05 (d, *J* = 4.6 Hz, 1H, HO-4′), 2.60 (dd, *J* = 12.7, 4.7 Hz, 1H, H-3eq), 2.14 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.01 (dd, *J* = 12.6, 7.2 Hz, 1H, H-3ax), 1.94 (s, 3H, Ac), 1.88 (s, 3H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.91, 170.87, 170.21, 170.17, 168.08, 165.99, 165.91, 133.62, 133.13, 133.1, 129.81, 129.78, 129.72, 129.58, 128.33, 117.37, 98.73, 95.79, 72.90, 71.15, 69.25, 69, 68.92, 68.55, 68.26, 67.73, 67.48, 62.98, 62.60, 53.01, 49.43, 37.05, 23.15, 21.03, 20.8, 20.76, 20.58; α-Confuguration of the sialoside linkage[23] was determined based on the $^3J_{C1\text{-}3Hax}$ of 6.9 Hz.

Allyl 2-Amino-2-deoxy-3-*O*-9-fluorenylmethoxycarbonyl-2-*N*-2,2,2-trichloroethoxycarbonyl-6-*O*-(methyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-α-D-glucopyranoside 5

To a solution of the donor **1** (88.0 mg, 133 μmol), the acceptor **4** (128 mg, 207 μmol), and dry MS4A in EtCN (1.0 mL) was added TMSOTf (5.4 μL, 29.8 μmol) at −78 °C, and the resulting mixture was stirred at this temperature for 6 h. After the reaction was quenched by adding the excess triethylamine at −78 °C, the mixture was filtered and concentrated *in vacuo*. The residue was directly purified by column chromatography on silica gel (gradually from 50 to 83% of ethyl acetate in toluene) to give the sialoside **5** as an α-isomer based on ^1H NMR analysis (138 mg, 95%) as a white solid: $[\alpha]_D^{20}$ +29.0 (*c* 0.1, $CHCl_3$); mp 100–101 °C; IR (neat, cm^{-1}) 3388, 2955, 1747, 1518, 1452; MALDI-TOF-MS *m/z* calcd for $C_{47}H_{55}Cl_3N_2O_{21}$ $(M+Na)^+$ 1111.2, found 1111.2; ^1H NMR (500 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.4 Hz, 2H, Fmoc), 7.60 (dd, *J* = 7.9, 9.4 Hz, 2H, Fmoc), 7.40 (t, *J* = 7.5 Hz, 2H, Fmoc), 7.32 (tdd, *J* = 7.3, 2.9, 1.2 Hz, 2H, Fmoc), 5.88 (ddd, *J* = 23.2, 10.3, 5.3 Hz, 1H, $OCH_2C\underline{H}{=}CH_2$), 5.37 (ddd, *J* = 11.2, 5.6, 2.6 Hz, 1H, H-8), 5.37 (d, *J* = 9.7 Hz, 1H, TrocN$\underline{H}$), 5.30 (dd, *J* = 10.0, 1.8 Hz, 1H, H-7), 5.30 (dd, *J* = 10.0, 1.8 Hz, 1H, Ac$\underline{H}$N), 5.29 (dq, *J* = 15.6, 1.5 Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.22 (dq, *J* = 10.3, 1.2 Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.07 (dd, *J* = 10.5, 9.7 Hz, 1H, H-3′), 5.03 (td, *J* = 10.5, 4.7 Hz, 1H, H-4), 4.94 (d, *J* = 3.8 Hz, 1H, H-1′), 4.61 (d, *J* = 12.0 Hz, 1H, $Cl_3CC\underline{H}_2$), 4.54 (d, *J* = 12.0 Hz, 1H, $Cl_3CC\underline{H}_2$), 4.42 (dd, *J* = 10.3, 7.6 Hz, 1H, Fmoc), 4.33 (dd, *J* = 10.6, 7.6 Hz, 1H, Fmoc), 4.32 (dd, *J* = 12.0, 3.2 Hz, 1H, H-9a), 4.23 (dd, *J* = 15.0, 5.9 Hz, 1H, Fmoc), 4.23 (dd, *J* = 7.9, 2.1 Hz, 1H, H-6a′), 4.18 (ddt, *J* = 12.6, 5.3, 1.5 Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 4.15 (dd, *J* = 10.9, 1.8 Hz, 1H, H-6), 4.09 (dd, *J* = 12.0, 5.6 Hz, 1H, H-9b), 4.07 (dd, *J* = 10.3, 3.8 Hz, 1H, H-2′), 4.05 (q, *J* = 10.6 Hz, 1H, H-5), 4.00 (ddt, *J* = 12.9, 6.5, 1.2 Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 3.97 (dd, *J* = 10.0, 5.0 Hz, 1H, H-4′), 3.81 (s, 3H, CO_2Me), 3.77 (dd, *J* = 10.9, 2.9 Hz, 1H, H-6b′), 3.77 (ddd, *J* = 10.9, 4.4, 1.8 Hz, 1H, H-5′), 3.48 (d, *J* = 5.0 Hz, 1H, HO-4′), 2.66 (dd, *J* = 13.5, 5.0 Hz, 1H, H-3eq), 2.13 (s, 3H, Ac), 2.11 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.06 (dd, *J* = 12.6, 10.0 Hz, 1H, H-3ax), 2.03 (s, 3H, Ac), 1.90 (s, 3H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.96, 170.63, 170.27, 170.11, 169.75, 168.21, 155.65, 154.23, 143.26, 143.18, 141.25, 133.16, 127.91, 127.21, 125.20, 120.04, 118.32, 97.92, 96.60, 95.31, 77.36, 74.57, 72.28, 71.09, 70.39, 68.78, 68.65, 68.57, 67.79, 67.30, 62.81, 62.3, 54.23, 52.88, 49.84, 46.62, 37.79, 30.86, 23.15, 21.05, 20.85, 20.69, 20.55; α-Confuguration of the sialoside linkage[23] was determined based on the $^3J_{C1\text{-}3Hax}$ of 13.9 Hz.

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

To a solution of the donor **1** (30.0 mg, 45.3 μmol) and the acceptor **6** (28.2 mg, 68.0 μmol), and dry MS4A in EtCN (1.0 mL) was added TMSOTf (4.1 μl, 22.7 μmol) at −78 °C, and the resulting mixture was stirred at this temperature for 6 h. After the reaction was quenched by adding the excess triethylamine at −78 °C, the mixture was filtered and concentrated *in vacuo*. The residue was directly purified by column chromatography on silica gel (gradually from 67% to 100% of ethyl acetate in toluene) to give the disaccharide **7** essentially as an α-isomer based on ^1H NMR analysis (32.3 mg, 80%) as a white solid: $[\alpha]_D^{20}$ +31.0 (*c* 0.1, $CHCl_3$); mp 77–78 °C; IR (neat, cm^{-1}) 3375, 2928, 1748, 1537, 1453; MALDI-TOF-MS *m/z* calcd for $C_{43}H_{53}NO_{19}$ $(M+Na)^+$ 910.3, found 910.2; ^1H NMR (500 MHz, $CDCl_3$) δ 8.09 (dd, *J* = 8.4, 1.4 Hz, 2H, Bz), 7.57 (tt, *J* = 7.4, 1.5 Hz, 1H, Bz), 7.45 (t, *J* = 7.8 Hz, 2H, Bz), 7.36–7.32 (m, 4H, Bn), 7.27 (tt, *J* = 6.6, 2.3 Hz, 1H, Bn), 5.88 (ddd, *J* = 22.2, 10.5, 6.0 Hz, 1H, $OCH_2C\underline{H}{=}CH_2$), 5.40 (dd, *J* = 10.3, 3.7 Hz, 1H, H-2′), 5.32 (dd, *J* = 5.6, 2.4 Hz, 1H, H-8), 5.31 (dd, *J* = 5.0, 2.4 Hz, 1H, H-7), 5.29 (dq, *J* = 17.2,

1.6 Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 5.20 (d, J = 9.7 Hz, 1H, Ac$\underline{H}$N), 5.20 (d, J = 3.9 Hz, 1H, H-1′), 5.12 (dq, J = 10.4, 1.4 Hz, 1H, $OCH_2CH{=}C\underline{H}_2$), 4.89 (ddd, J = 11.9, 10.3, 4.7 Hz, 1H, H-4), 4.66 (dd, J = 10.3, 3.3 Hz, 1H, H-3′), 4.64 (d, J = 11.9 Hz, 1H, $PhC\underline{H}_2$), 4.60 (d, J = 11.6 Hz, 1H, $PhC\underline{H}_2$), 4.32 (dd, J = 12.4, 2.4 Hz, 1H, H-9a), 4.24 (ddt, J = 13.3, 5.2, 1.6 Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 4.14 (t, J = 6.0 Hz, 1H, H-5′), 4.10 (bd, J = 2.0 Hz, 1H, H-4′), 4.08 (q, J = 10.0 Hz, 1H, H-5), 4.05 (ddt, J = 13.5, 6.0, 1.4 Hz, 1H, $OC\underline{H}_2CH{=}CH_2$), 4.02 (dd, J = 12.7, 5.3 Hz, 1H, H-9b), 4.00 (dd, J = 10.9, 2.0 Hz, 1H, H-6), 3.84 (dd, J = 10.2, 5.3 Hz, 1H, H-6a′), 3.77 (dd, J = 10.0, 6.3 Hz, 1H, H-6b′), 3.71 (s, 3H, CO_2Me), 2.89 (d, J = 1.0 Hz, 1H, HO-4′), 2.38 (dd, J = 13.0, 4.7 Hz, 1H, H-3eq), 2.14 (t, J = 12.5 Hz, 1H, H-3ax), 2.09 (s, 3H, Ac), 2.01 (s, 3H, Ac), 2.01 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.88 (s, 3H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.71, 170.50, 170.32, 169.87, 169.84, 168.12, 165.73, 138.27, 133.85, 133.06, 130.03, 129.80, 128.35, 128.32, 127.58, 127.52, 117.30, 98.63, 95.75, 73.51, 72.89, 70.80, 69.60, 69.52, 69.28, 69.08, 68.87, 68.76, 68.47, 67.26, 62.27, 53.02, 49.34, 36.28, 23.12, 21.02, 20.76, 20.68, 20.63; α-Confuguration of the sialoside linkage[23] was determined based on the $^3J_{C1\text{-}3Hax}$ of 16.6 Hz.

Procedure of microfluidic α-sialylation

A solution of TMSOTf (270 μL, 1.50 mmol, 50 mM) in EtCN (30.0 mL) was injected to the Comet X-01 micromixer by using a syringe-pump at the flow rate of 1.0 mL/min. At the same time, a solution of the *N*Ac donor **1** (994 mg, 1.50 mmol, 50 mM) and the galactosyl acceptor **2** (964 mg, 2.25 mmol, 75 mM) dissolved in EtCN (30.0 mL) was also injected to the Comet X-01 micromixer by another syringe-pump flow at the rate of 1.0 mL min^{-1}, and two solutions were mixed at −78 °C in the cooling bath. The resulting mixture was allowed to flow at −78 °C for additional 50 s through a Teflon tube reactor (Φ = 1.0 mm, l = 1.0 m), and then the solution was introduced into a flask, which contained the dry MS4A and cooled down in advance at −78 °C. After the reaction was stirred for another 1 h at this temperature, it was quenched by adding the excess triethylamine −78 °C. The mixture was filtered and concentrated *in vacuo* to gave the crude product, which was purified by the same procedure described above, producing the sialoside **3** (1.17 g, 89%, α : β = 94 : 6).

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