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Phosphite-based sialic acid donors in the synthesis of α(2→9) oligosialic acids

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ABSTRACT

The combination of a phosphite donor and an anomeric thiocresol-protected acceptor, both with a TFA protecting group at C-5 of the sialic acid, provides good α-selectivity and yield in sialylation. Although the convergent synthetic strategy of using a phosphite disialo-donor and a disialo-acceptor assembles tetra-sialic acid efficiently, overcoming the low α-selectivity of α-anomer and purifying it remain to be achieved. Furthermore, mono- and di-sialic acids were, respectively, conjugated on carrier protein, keyhole limpet hemocyanin. The enzymatic hydrolysis method is recommended for estimating the amount of sialic acid on a protein conjugate.

1. Introduction

As a diverse, chiral pool of nine-carbon carboxylated saccharides,[1] sialic acids are commonly attached to the non-reducing terminus of oligosaccharide chains in glycolipids or glycoproteins on the surface of a cell.[2] Since they are at the terminal positions of glycan chains, sialic acids have very important roles in biological events on the surface of the cell, serving as a mask for cell-surface glycan receptors, ligands for proteins, and markers of some cancers.[1–3] Sialic acid-containing glycoconjugates exist in nature as α-configuration sialosides. The linear homopolymer of sialic acids is linked internally via contiguous α-2–8, α-2–9, or alternating α-2–8 and α-2–9 linkages, and functions as a virulence factor. These chains are found as capsular polysaccharides on the surface of bacterial cells such as *Escherichia coli* K1 and *Neisseria meningitidis* groups B and C, making them potential antigens for the development of antibacterial vaccine.[4] Polysaccharides that are adopted to prepare vaccines are typically isolated from their natural sources, and so can be heterogeneous and/or contaminated with other antigenic components. Therefore, the development of synthetic polysaccharide vaccines with high immunogenicity has attracted much attention.[5]

A challenging issue in synthesizing sialoconjugates is anomeric α-selectivity during the chemical sialylation of sialic acids. An electron-withdrawing carboxyl group at the anomeric center and the lack of participating functionality at the C-3 of sialyl donor cause the sialylation reaction commonly to proceed with low to moderate yield of product, low α-selectivity, and the formation of an undesirable 2,3-elimination product. Additionally, the low reactivity of hydroxyl groups at the C-8/C-9 of sialic acid in glycosylation[6] slows the synthesis of homo-oligosialic acids.

Recently, many attempts[7] have been made to achieve high α-selectivity in sialylation. Most of these studies have focused on changing the anomeric leaving group, installing an auxiliary group at the C-1 or C-3 position, or modifying the amine protecting group at the C-5 position of the sialyl donor.[8] Although moderate to high α-selectivities with variable yields have been obtained using halide, sulfide, selenide, or oxygen substituent at the C-3 position[9] or C-1[10] auxiliaries of the donors, additional steps are required to remove these substituents at the end of the synthesis. However, the replacement of the acetyl group at the C-5 *N*-acetyl group of the sialyl donor to a 2,2,2-trichloroethoxycarbonyl (NTroc),[8c,d] trifluoroacetyl (*N*HTFA),[8b] azido (N_3),[8e,f] Fmoc,[8g] phthalimide[8h] or diacetyl[8a] group has been studied and demonstrated by the improved yield of product or α-selectivity during sialylation. Recently, 1,5-lactamized-sialyl acceptors have been developed and shown to increase reactivity of the C-8 hydroxyl group in glycosylation.[11] Despite the potential of these sialyl donors, their use has been limited to the synthesis of α(2,8)-disialosides, and glycosylation with other saccharide acceptors. Recently, Takahashi et al. adopted 5-*N*,4-*O*-carbonyl-protected sialyl donor to synthesize first α(2,8) tetra-sialic acid,[12] and later α(2,9) tri-sialic acid via one-pot glycosylation and polymer-assisted deprotection[13] with exclusive α-selectivity in sialylations. However, the α-selectivity toward some primary hydroxyl acceptors remained low and is still a challenge.

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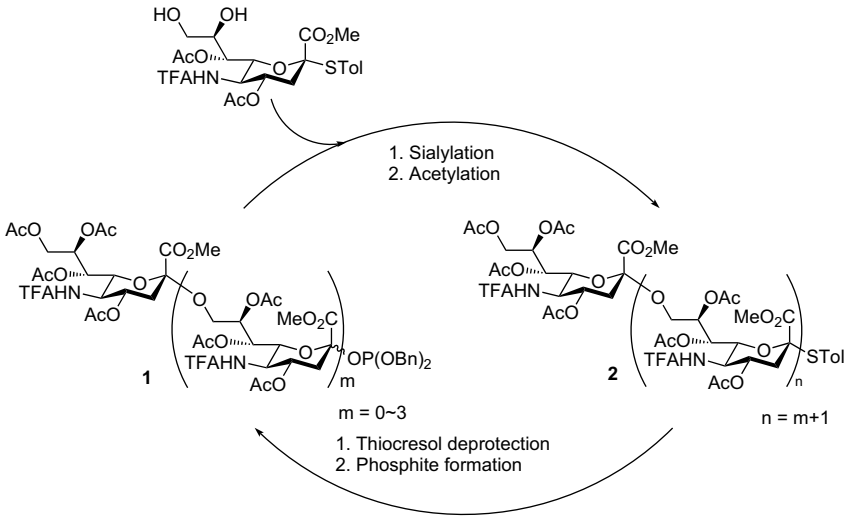


Scheme 1. Oligosialic acid synthesis through iterative sialylation.

Our previous study employed *N*-trifluoroacetyl-protected sialyl phosphite as a general glycosyl donor in the synthesis of α(2,9)-oligosialic acids and has studied their α-selectivity in sialylations (Scheme 1).[14] α(2,9) Penta-sialic acid was synthesized using phosphite donor and iterative sialylations to elongate the sugar chain from the non-reducing end to the reducing end. However, the synthesis of homo-oligosaccharide using a convergent approach is more practical and efficient. Thus, tetra-sialic acid can conceivably be prepared by realization of the glycosylation of disaccharide donor and acceptor, a [2+2] coupling strategy. Herein, we describe the synthesis of tetra-sialic acid by coupling disaccharide phosphite donor with disaccharide acceptor. Also, mono- and di-sialic acids are conjugated with keyhole limpet hemocyanin (KLH) to synthesize α(2,9) sialic acid-based vaccine. The conjugation efficiency is examined by using the enzymatic or acidic hydrolysis of sialic acid from carrier protein, followed by Warren's method.

2. Results and discussion

2.1. Design of sialyl donors and acceptors

The outcomes of sialylations are well known to depend primarily on the nature of the protecting groups on glycosyl donors and acceptors.[7–14] In the design of sialic acid donors and acceptors, many efforts have been made to discover the best combination of the protecting and leaving groups at the C-5 amino functional group and the anomeric center, respectively. Based on our recent findings,[14] the strong electron-withdrawing nature of the C-5 *N*HTFA group was expected to enhance the α-selectivity of sialic acid donors and the primary C-9 hydroxyl group of sialic acid acceptors, as shown in Figure 1. Additionally, the TFA group can be introduced efficiently and removed easily under mild reaction conditions.

Although various leaving groups are known to increase the α-selectivity of sialyl donors,[15] per-*O*-acetyl-*N*-acetylneuraminic acid methyl ester derivatives with either phosphites[15d,e] or aryl sulfides[15b,c] at the anomeric center are mainly used in chemical sialylation. Notably, the order of activation of donors can be tuned, not only by the protecting groups, but also by a combination of activators and leaving groups on them.[8g,14] The C-7, C-8, and C-9 hydroxyl groups on the exocyclic chain of sialyl acceptors and donors can be selectively protected as the bulky *tert*-butyldimethylsilyl (TBDMS)[8g,14] group or as the electron-withdrawing chloroacetyl ($ClCH_2CO$)[16] group. While the α-phenylthiosialoside-based donors are relatively stable, the phosphite-based donors exhibit high activity when a catalytic amount of promoter (typically trimethylsilyl trifluoromethanesulfonate, TMSOTf) is used. Based on these

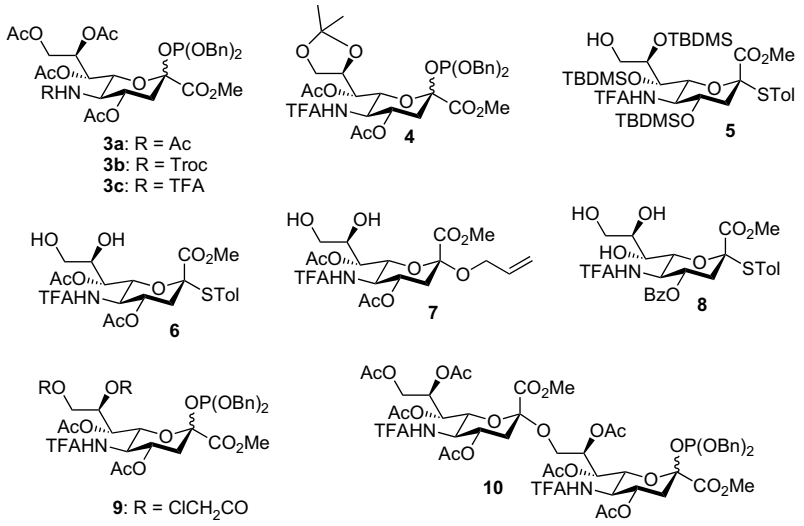
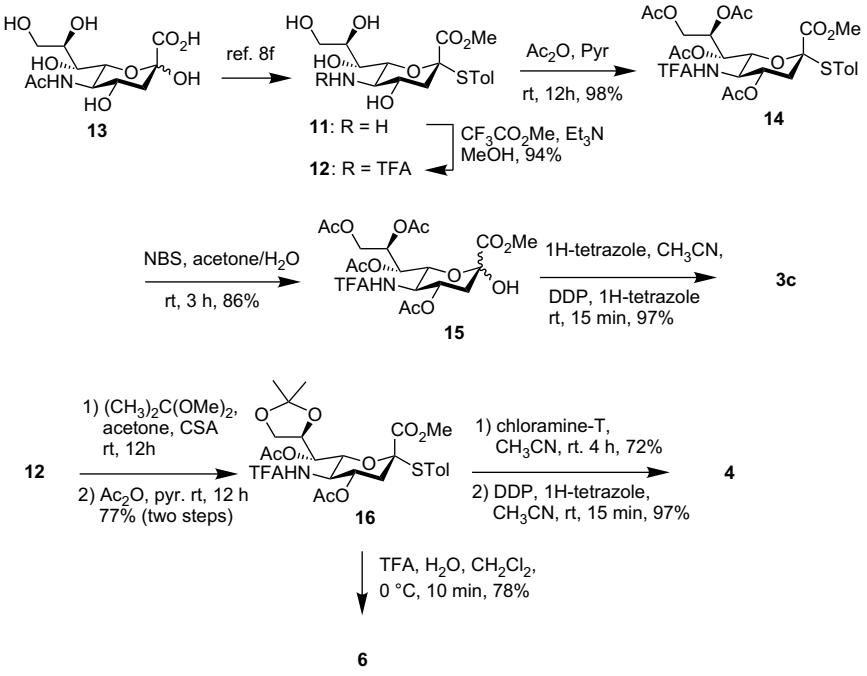


Figure 1. Structure of sialyl donors (**3**, **4**, **9**, and **10**) and acceptors (**5**–**8**).



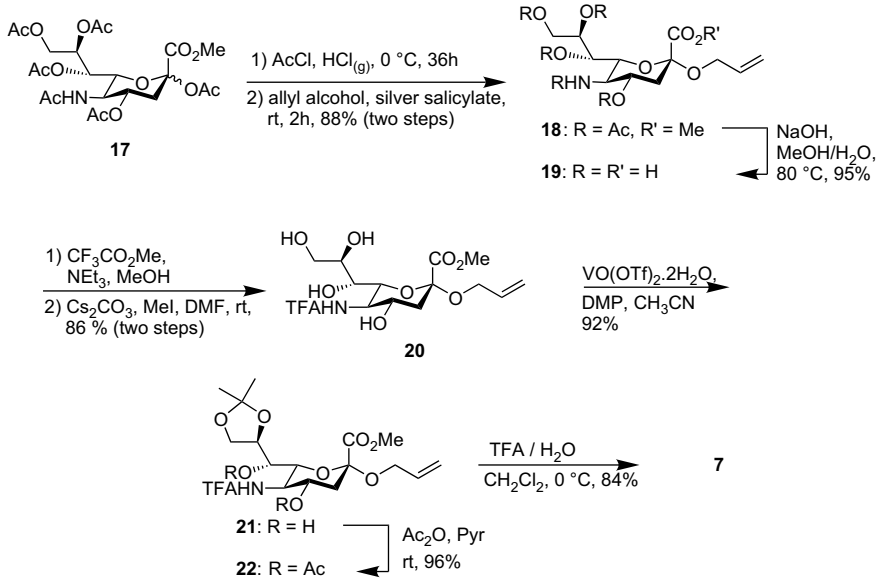
Scheme 2. Synthesis of donors **3c** and **4**, and acceptor **6**.

considerations, C-5 *N*HTFA-protected sialyl phosphites (**3**, **4**, **9**, and **10**) were synthesized as building blocks for constructing the α(2-9)-linked oligosialic acids, while thiocresol was chosen as an anomeric protecting group (Fig. 1).

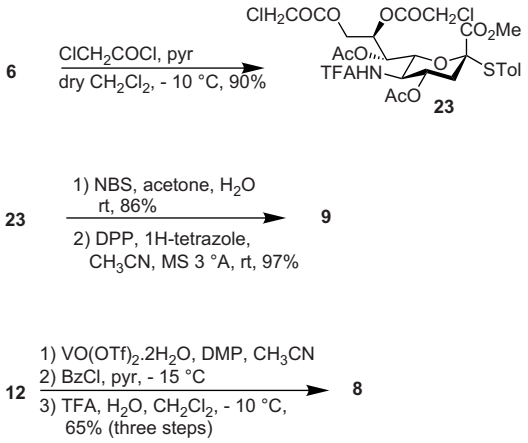
2.2. Synthesis of donors and acceptors

As presented in Scheme 2, the synthesis of donors **3c**[14] and **4**, and acceptor **6**[14] commenced from the key intermediate **12**, which was prepared by the acetylation of **11** obtained from Neu5Ac **13** by following reported procedures.[8f] The per-*O*-acetylation of **12** afforded **14** in almost quantitative yield. Donor **3c** was then efficiently obtained by the *N*-bromosuccinimide (NBS)-mediated removal of thiocresol-protecting group[17] to give anomeric alcohol **15** (86%) and then phosphite was formed using dibenzyl diisopropylphosphoramidite (DDP) in CH_3CN at room temperature in 97% yield. Selective 8,9-*O*-isopropylidenation of **12** was followed by acetylation of secondary hydroxyl groups to give thioglycoside **16** in 77% overall yield in two steps. The aryl sulfide group without the deprotection of the acetonide in **16** was selectively removed using chloramine-T.[18] Notably, the use of NBS led to the deprotection of thiocresol and isopropylidene. The resulting free hydroxyl group at the anomeric center was converted to phosphite **4** (97%) using DDP at room temperature in a very short reaction period (~15 min). Meanwhile, the acid-catalyzed de-*O*-isopropylidenation of **16** provided the desired acceptor **6** in 78% yield.

The synthesis of acceptor **7** began with the known compound **18**[19] (Scheme 3). Saponification of **18** followed by *N*-trifluoroacetylation[8c] with $CF_3CO_2Me/Et_3N/MeOH$ and then esterification using MeI in the presence of Cs_2CO_3 gave TFA derivative **20**.[20] The desired acceptor **7**



Scheme 3. Synthesis of acceptor **7**.



Scheme 4. Synthesis of donor **9** and acceptor **8**.

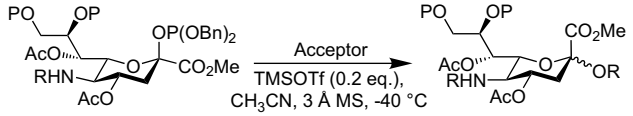
was easily obtained by selective protection of terminal diol using our developed vanadyl triflate-catalyzed[21] isopropylidenation followed by *O*-acetylation with Ac_2O/pyridine and then acid (aq CF_3CO_2H in CH_2Cl_2)-catalyzed de-O-isopropylidenation.

The 8,9-*O*-dichloroacetyl-protected phosphite donor **9** was synthesized from readily available acceptor **6** in three steps. Acylation of **6** with chloroacetyl chloride in CH_2Cl_2 followed by thiocresol deprotection (NBS, acetone/H_2O) and subsequent phosphite formation of the resulting anomeric hydroxyl group using DDP in CH_3CN gave the desired donor **9** in overall 80% yield (Scheme 4). 8,9-*O*-isopropylidenation of **12** followed by regioselective benzoylation with benzoyl chloride in pyridine at −15 °C and then de-O-isopropylidenation (aq CF_3CO_2H in CH_2Cl_2) provided the acceptor **8**[22] in 65% overall yield in three steps (Scheme 4).

2.3. Evaluation of sialylation

With the sialyl phosphites **3**–**4** and **9**, and acceptors **5**–**8** in hand, we examined the sialylation reactions of these sialyl donors and acceptors. The combination of TMSOTf as an activator and CH_3CN as solvent in the presence of molecular sieves (3 Å) at −40 °C was used in the sialylation because it is known to improve α-selectivity.[23] The choice of an appropriate donor–acceptor combination was responsible for the obtaining of the predominantly α-(2,9)-linked disialosides **25**–**32** in 33–77% yields (Fig. 2 and Table 1).

Table 1
Sialylations of C-5 *N*-protected sialyl phosphites



Entry	Donor	Acceptor	Product	Yield (%)	α/β
1	**3a**	**5**	**24**	83	—
2	**3b**	**5**	**25**	33	1.5/1
3	**3a**	**6**	**26**	55	4.7/1
4	**3c**	**6**	**27**	77	α
5	**3c**	**7**	**28**	70	6/1
6	**4**	**6**	**29**	68	3/1
7	**4**	**7**	**30**	64	1.3/1
8	**9**	**6**	**31**	75	2.5/1
9	**3c**	**8**	**32**	72	α

According to Table 1, the bulky acceptor **5**[24] coupled with donor **3b** to give the desired dimer **25** in a modest yield with poor selectivity (entry 2); only 2,3-elimination product **24** (entry 1) was obtained when **3a** was used as a donor. However, when less

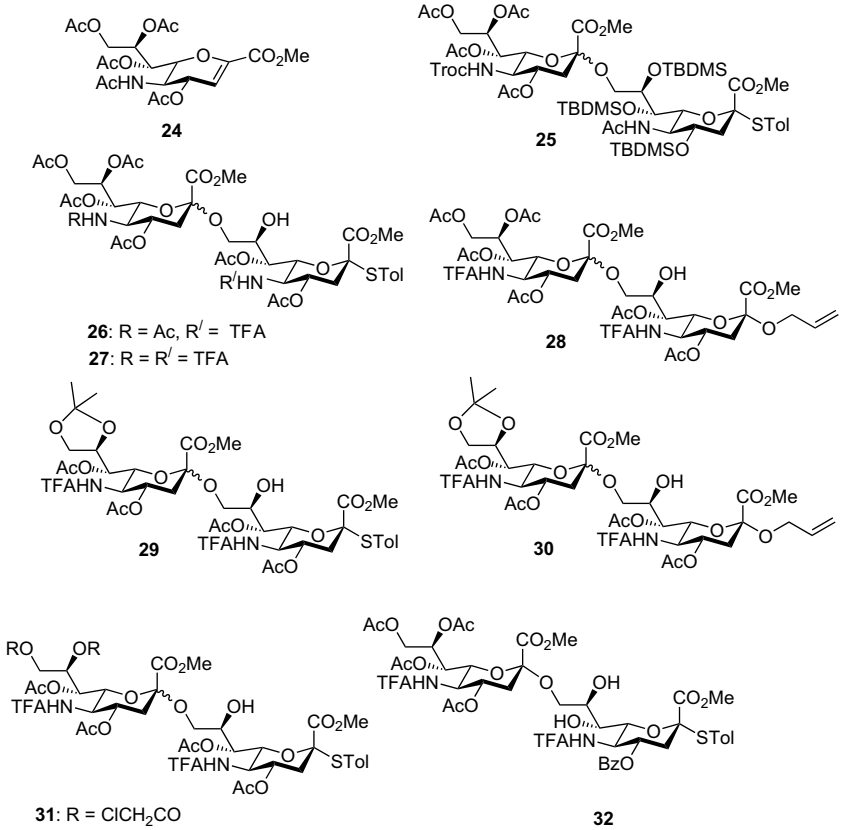


Figure 2. Products of sialylations.

sterically hindered acceptor **6** was reacted with **3a**, both the α-selectivity and the product yield were markedly increased (entry 3). These results suggest that the combination of the donor with the electron-withdrawing group at the C-5 position and a less sterically hindered acceptor can improve the α-selectivity and product yield. Therefore, a TFA-protected, donor **3c** was used to react with **6**; TFA is a more strongly electron-withdrawing protecting group. As expected, disialoside **27** was obtained with exclusive α-anomer in 77% yield (entry 4). Surprisingly, when the anomeric protecting group STol of **6** was changed to an allyl group (**7**), the α-selectivity of sialylation with donor **3c** was reduced (entry 5). Notably, when the acetonide-protected donor **4** reacted with **6**, the α-selectivity also declined (entry 6). Additionally, low α-selectivity was observed when **4** reacted with **7** (entry 7). Moreover, although the *O*-chloroacetyl-protecting sialyl donor exhibited good α-selectivity,[16] the 8,9-*O*-dichloroacetyl-protected phosphite donor **9** reacted with acceptor **6** to give disialoside **31** in 75% yield with relatively low anomeric selectivity (entry 8). However, the sialylation of phosphite donor **3c** with thiosialoside **8** under the aforementioned conditions resulted in 72% yield of disialoside **32** (entry 9) with exclusive α-anomer. These results show that the α-selectivity was seriously affected by the protecting group on the donor and the protecting group at the anomeric center of the acceptor. The combination of TFA as the C-5 protecting group, phosphite as the leaving group of the donor, and STol as the anomeric protecting group of the acceptor ensured good α-selectivity and yield in sialylation.

The configurations of the newly formed glycosidic bond of disialosides were determined by ^{1}H NMR spectroscopy from the chemical shifts of H-3e of the non-reducing end sialic acid and relevant empirical rules.[15a,25] The chemical shifts of H-3e of α-glycosides were more downfield than those of β-glycosides. In the case of compound **34** (Fig. 3(a)), only one isomer was obtained, and the long-range $J_{C\text{-}1,H\text{-}3ax}$ coupling constant was measured to verify the stereochemistry at the anomeric center. In a selective proton decoupling ^{13}C NMR experiment,[26] the coupling pattern of non-reducing C-1 gave a doublet signal with a 5.6 Hz coupling constant. A similar method was applied to compound **28** to determine the stereochemistry. As presented in Figure 3(b), two doublet signals of C-1s with coupling constants 6.0 and 6.3 Hz, respectively, were observed, verifying the assignment of two α-glycosidic bonds.

2.4. Synthesis of oligosialic acid

Although penta-sialic acid was successfully synthesized by elongating the sugar chain from the non-reducing end to the reducing end using monosaccharide acceptor,[14] this approach gave a low overall yield because of the consecutive linear synthetic strategy. To assemble sugar chain efficiently, the [2+2] convergent strategy was adopted to synthesize tetra-sialoside **36**. The synthesis of tetra-sialoside depends on access to a suitable disialoside to serve as a glycosyl donor and an acceptor. Therefore, disialyl phosphite donor **10** and acceptor **33**, as shown in Scheme 5, were selected. Disialoside **10** was prepared by the same methods as described for the synthesis of phosphite donor **3c**, while disialyl acceptor **33** was obtained by the method used to prepare acceptor **6**. The optimized reaction conditions supported the synthesis of disialosides **10** and **33** in very high yields.

Scheme 5 outlines a new route to tetra-sialoside **36** by a convergent [2+2] strategy from disialyl phosphite donor **10** and acceptor **33**. As anticipated, upon *O*-sialylations under standard conditions (with CH_3CN as solvent and a catalytic amount of TMSOTf as promoter at −40 °C), the glycosylation proceeded with reasonable selectivity (α/β=1.6/1), giving tetra-sialoside **36** in moderate yield (58%). The newly generated glycosidic bond of the tetra-sialoside **36** was identified by the analysis of ^{1}H and ^{13}C NMR data of **36**. Notably, however, α and β anomers of **36** remained unseparable under many chromatographic conditions.

2.5. Conjugation of sialic acid with carrier protein

To synthesize an α(2–9) sialic acid-based vaccine, sialic acid was conjugated with KLH and the conjugation efficiency was analyzed. KLH is an extra-cellular respiratory protein that is widely recognized as a hapten carrier and an immune stimulant. It has also been found to be an effective immunogenic carrier protein for carbohydrate antigens.[27] Sialic acid antigen was conjugated with KLH using a bifunctional linker *m*-maleimidobenzoyl-*N*-hydroxysuccinimide (MBS). As described by Scheme 6, the terminal olefin contained in sialosides **37** was firstly transformed to thioacetate by the radical addition of thiolacetic acid in the presence of AIBN[27,28] to yield quantitatively the desired thioacetates **38**. The basic removal of the acetates using sodium methoxide in methanol followed by

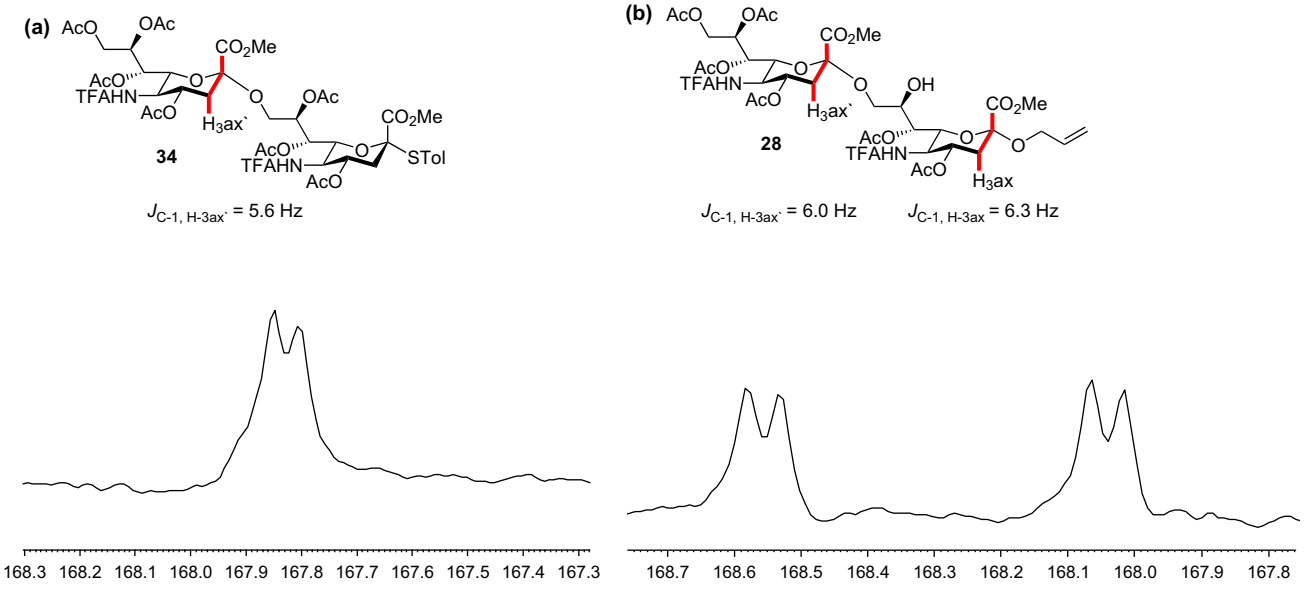
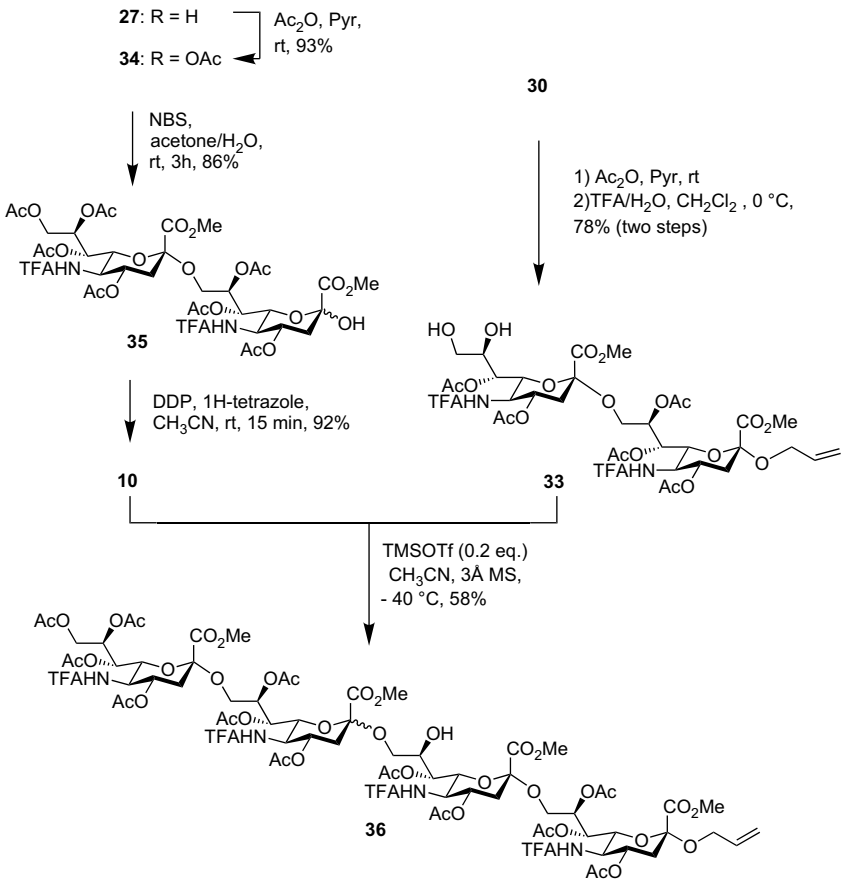


Figure 3. C-1 portion of selective proton-decoupled ^{13}C NMR spectra of **28** and **34**.



Scheme 5. Synthesis of α-(2→9) linked tetra-sialoside **36**.

saponification of the methyl ester and TFA amide yielded the fully deprotected sialosides. Finally, per-O-acetylation and *N*-acetylation with acetic anhydride and pyridine followed by global deacetylation under basic conditions (NaOMe in MeOH) gave the target thiosialoglycosides **39a** as a dimer and **39b** as a monomer.

To covalently attach sialo-antigens to carrier protein, the lysine residues of KLH were converted to maleimide by reacting with MBS, as shown in Scheme 6. Conjugation to the carrier protein was achieved through the Michael addition of the thiol nucleophile of antigens **39**, which was pretreated with $NaBH_4$ to give the vaccine constructs **40**. Warren's method[29] was adopted to assay the amounts of sialic acid on KLH. We used two methods to release free sialic acid from carrier protein (see Experimental section for details). Firstly, the sialic acid was hydrolyzed by sulfuric acid and then reacted with thiobarbituric acid to give a chromophoric product, which was measured by UV. Alternatively, sialic acid was hydrolyzed by the sialidase and the resulting mixture was then assayed by Warren's method. Surprisingly, the amount of sialic acid obtained by sialidase hydrolysis was 10 times higher than that obtained by acid hydrolysis. The amount of sialic acid estimated by sialidase hydrolysis is 22.7 μg per 100 μg of vaccines **40a**, while acidic hydrolysis only gives 1.9 μg. This difference may arise because periodate oxidation of sialic acid in strong acidic solution may lead to the elimination product Neu5Ac2,3ene, and thereby reducing in the production of the final chromophoric compound.

3. Conclusion

The combination of phosphite donor and anomeric thiocresol-protected acceptor, both with a TFA protecting group at the C-5 of sialic acid, provided better α-selectivity and yield in sialylation. The protecting group on both donor and acceptor strongly affected α-selectivity. Although the [2+2] convergent strategy was an efficient means of assembling tetra-sialic acid, the low α-selectivity and purification of α-anomer remain to be solved. The assay of the sialic acid amount on synthetic vaccine by acid hydrolysis may be interrupted by the formation of Neu5Ac2,3ene. Enzymatic hydrolysis is recommended to assay sialic acid on the carrier protein. The syntheses of oligosialic acid-conjugated vaccines and the evaluation of their immunogenicities are underway and will be described in the near future.

4. Experimental section

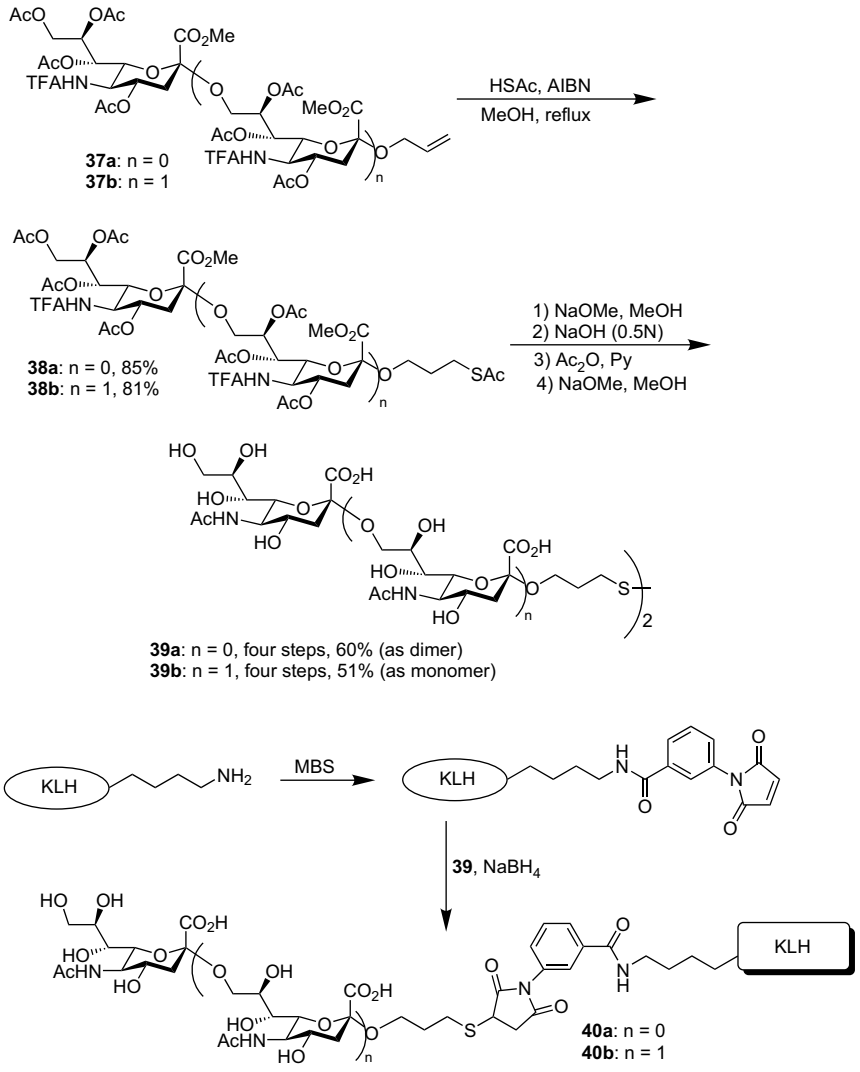
4.1. General methods

^1H and ^{13}C NMR spectra were recorded on Bruker AV-400 or 500 MHz. Assignment of ^1H NMR spectra was achieved using 2D methods (COSY). Chemical shifts were expressed in parts per million using residual $CHCl_3$ as reference. High-resolution mass spectra were obtained by means of a Micromass (Autospec) mass spectrometer. Analytical thin-layer chromatography (TLC) was performed on precoated plates (Merck silica gel 60 F-254). Silica gel 60 (E. Merck Co.) was employed for all flash chromatographies. All solvents are commercially available and dried by general methods.

Compounds **3a**,[14] **3b**,[14] **3c**,[14] **6**,[14] **8**,[22] **12**,[14] **16**,[14] **18**,[19] **19**,[20] **21**,[21b] **25**,[14] **26**,[14] and **27**[14] have been previously prepared and reported in the cited papers and their NMR spectra are in good agreement with the literature data.

*4.1.1. Methyl (4-methylphenyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-2-thio-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranoside)onate (**14**)*

To a solution of **11** (308 mg, 0.64 mmol) in pyridine (4 mL) was added acetic anhydride (2 mL) at 0 °C. The reaction was stirred for



Scheme 6. Functionalization of allyl glycosialosides en route to KLH conjugation.

12 h at room temperature and then concentrated in vacuo. The residue was diluted with EtOAc and the resulting solution was washed with 10% HCl, saturated $NaHCO_3$, and saturated NaCl in sequence. The organic phase was dried ($MgSO_4$) and filtered, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane=1/4) to afford **14** (301 mg, 98%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (dd, J=11.5, 12.9 Hz, 1H), 2.00 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.14 (s, 3H), 2.36 (s, 3H), 2.84 (dd, J=4.7, 12.9 Hz, 1H), 3.6 (s, 3H), 3.84 (ddd, J=9.6, 10.2, 10.2 Hz, 1H), 4.06 (dd, J=1.7, 10.2 Hz, 1H), 4.23 (m, 1H), 4.42 (dd, J=1.7, 11.5 Hz, 1H), 4.99 (ddd, J=4.7, 10.2, 11.5 Hz, 1H), 5.24 (br, 2H), 6.42 (d, J=9.6 Hz, 1H), 7.14 (d, J=8.0 Hz, 2H), 7.33 (d, J=8.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.50, 20.66, 20.87, 21.26, 37.81, 49.78, 52.72, 61.91, 67.59, 69.15, 73.91, 87.5, 115.37, 124.75, 129.66, 136.50, 140.39, 157.48, 167.76, 169.79, 170.58, 170.64, 171.01; HRMS (FAB) calcd for $C_{27}H_{33}F_3NO_{12}S$ $(M+H)^+$: 652.1676, found: 652.1664.

4.1.2. *Methyl (4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosid)onate (**15**)*

To a solution of **14** (230 mg, 0.35 mmol) in acetone (4.5 mL) and water (0.4 mL) was added NBS (62 mg, 0.53 mmol). The reaction was stirred for 30 min at room temperature. Then, the solution was concentrated under reduced pressure. The residue was diluted with EtOAc and the resulting solution was washed with H_2O, saturated $NaHCO_3$, and saturated NaCl in sequence. The organic phase was dried ($MgSO_4$) and filtered, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane=1/2) to afford **15** (165 mg, 86%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H), 2.02 (s, 3H), 2.09 (s, 3H), 2.14 (s, 3H), 2.26 (m, 2H), 3.84 (s, 3H), 4.01 (dd, J=8.7, 12.0 Hz, 1H), 4.15 (q, J=10.2 Hz, 1H), 4.48 (d, J=10.2 Hz, 1H), 4.71 (d, J=12.0 Hz, 1H), 5.22 (td, J=5.2, 10.2 Hz, 1H), 5.31 (d, J=8.7 Hz, 1H), 5.36 (s, 1H), 5.57 (s, 1H), 7.84 (d, J=10.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.04, 20.09, 20.32, 20.34, 35.82, 49.32, 52.85, 67.84, 68.47, 70.67, 72.19, 94.58, 115.04, 157.35, 168.25, 168.25, 169.40, 170.08, 171.16, 172.38; HRMS (FAB) calcd for $C_{20}H_{27}F_3NO_{13}$ $(M+H)^+$: 546.1435, found: 546.1436.

4.1.3. *Dibenzyl [Methyl (4,7-di-O-acetyl-3,5-dideoxy-8,9-O-isopropylidene-5-trifluoroacetamido-D-glycero-α/β-D-galacto-non-2-ulopyranosid)onate] phosphite (**4**)*

To a solution of **16** (121 mg, 0.2 mmol) in MeCN (2 mL) was added chloramine-T (85 mg, 0.3 mmol). The reaction was stirred for 4 h at room temperature. Then the solution was concentrated in vacuo. The residue was diluted with EtOAc and the resulting solution was washed with H_2O, saturated $NaHCO_3$, and saturated NaCl in sequence. The organic phase was dried ($MgSO_4$) and filtered, and the filtrate was evaporated. The residue was purified by silica gel column

chromatography to afford hydroxyl compound (72%). To a solution of the above hydroxyl compound (65 mg, 0.14 mmol) in MeCN (3.7 mL) were added DDP (0.45 mL, 1.4 mmol) and 1*H*-tetrazole (0.1 g, 1.4 mmol) at −40 °C under argon atmosphere. After being stirred for 5 min at −40 °C, the solution was further stirred for 15 min at room temperature. The solution was diluted with EtOAc, and the resulting solution was washed with saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography to give **4** (97%): some selected peaks of **4β**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.29 (s, 3H), 1.32 (s, 3H), 1.93 (dd, *J*=11.8, 13.0 Hz, 1H), 1.96 (s, 3H), 2.06 (s, 3H), 2.42 (dd, *J*=4.9, 13.0 Hz, 1H), 3.50 (dd, *J*=2.2, 10.5 Hz, 1H), 3.72 (s, 3H), 3.86 (dd, *J*=7.8, 8.7 Hz, 1H), 3.94 (ddd, *J*=10.2, 10.5, 10.5 Hz, 1H), 3.99 (dd, *J*=6.5, 8.7 Hz, 1H), 4.10 (ddd, *J*=3.3, 6.5, 7.8 Hz, 1H), 4.91 (ddd, *J*=4.9, 10.5, 11.8 Hz, 1H), 4.95–5.07 (m, 5 H), 5.58 (d, *J*=10.2 Hz, 1H), 7.24–7.44 (m, 10H).

4.1.4. Methyl (2-allyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosid)onate (**20**)

Compound **19** (2.56 g, 8.34 mmol) was dissolved in MeOH (80 mL). Triethylamine (9.36 mL, 66.72 mmol) and methyl trifluoroacetate (3.36 mL, 33.36 mmol) were added to the solution dropwise at room temperature, respectively. The solution was stirred for 1.5 h at room temperature. After the reaction was completed, the solution was evaporated and to the residue were added DMF (80 mL), Cs_2CO_3 (2.7 g, 8.34 mmol), and MeI (2.6 mL, 41.7 mmol) sequentially. The reaction mixture was allowed to proceed for 12 h at room temperature. After completion of the reaction, the mixture was evaporated and the resulting residue was purified by column chromatography (EtOAc/hexane=1/1, with 15% MeOH) to give product **20** (3.0 g, 86% for two steps): ^{1}H NMR (400 MHz, CD_3OD) δ 1.78 (dd, *J*=12.4, 12.4 Hz, 1H), 2.69 (dd, *J*=4.6, 12.4 Hz, 1H), 3.46 (dd, *J*=1.4, 8.6 Hz, 1H), 3.63 (dd, *J*=6.4, 11.9 Hz, 1H), 3.71 (ddd, *J*=4.6, 10.0, 12.4 Hz, 1H), 3.81 (s, 3H), 3.82–3.84 (m, 2H), 3.87 (dd, *J*=1.4, 10.5 Hz, 1H), 3.98 (dd, *J*=10.0, 10.5 Hz, 1H), 4.00 (dddd, *J*=1.6, 1.6, 5.4, 12.8 Hz, 1H), 4.30 (dddd, *J*=1.6, 1.6, 5.4, 12.8 Hz, 1H), 5.12 (dddd, *J*=1.6, 1.6, 1.6, 10.5 Hz, 1H), 5.24 (dddd, *J*=1.6, 1.6, 1.6, 17.2 Hz, 1H), 5.87 (dddd, *J*=5.4, 5.4, 10.5, 17.2 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 41.83, 53.48, 54.02, 64.85, 66.43, 68.55, 70.27, 72.85, 73.95, 100.23, 117.12, 117.54, 135.56, 159.79, 170.92; HRMS (FAB) calcd for $C_{15}H_{23}F_3NO_9$ $(M+H)^+$: 418.1325, found: 418.1317.

4.1.5. Methyl (2-allyl-4,7-di-O-acetyl-3,5-dideoxy-8,9-O-isopropylidene-5-trifluoroacetamido-α-D-glycero-D-galacto-2-nonulopyranosid)onate (**22**)

Compound **22** (96%) was obtained from **21** by the method described in the synthesis of **14**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.99 (dd, *J*=12.0, 13.0 Hz, 1H), 2.03 (s, 3H), 2.16 (s, 3H), 2.72 (dd, *J*=5.0, 13.0 Hz, 1H), 3.83 (s, 3H), 3.97–4.00 (m, 2H), 4.01 (dd, *J*=6.5, 8.5 Hz, 1H), 4.07 (dd, *J*=2.0, 9.5 Hz, 1H), 4.08 (dd, *J*=6.5, 8.5 Hz, 1H), 4.26 (dddd, *J*=1.5, 1.5, 5.5, 13.0 Hz, 1H), 4.38 (ddd, *J*=4.0, 6.5, 6.5 Hz, 1H), 5.11 (ddd, *J*=5.0, 10.0, 12.0 Hz, 1H), 5.20 (dddd, *J*=1.5, 1.5, 1.5, 10.5 Hz, 1H), 5.29 (dddd, *J*=1.5, 1.5, 1.5, 17.0 Hz, 1H), 5.37 (dd, *J*=2.0, 4.0 Hz, 1H), 5.87 (dddd, *J*=5.5, 5.5, 10.5, 17.0 Hz, 1H), 6.64 (d, *J*=9.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 20.83, 21.00, 25.49, 26.54, 31.14, 37.67, 53.13, 53.79, 62.40, 65.70, 65.95, 68.92, 69.54, 72.08, 72.96, 99.07, 115.70, 117.67, 113.50, 158.00, 168.45, 170.46, 171.15; HRMS (FAB) calcd for $C_{22}H_{31}F_3NO_{11}$ $(M+H)^+$: 542.1849, found: 542.1834.

4.1.6. Methyl (2-allyl-4,7-di-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosid)onate (**7**)

A mixture of trifluoroacetic acid (1.5 mL) and water (0.25 mL) was added dropwise to a solution of **22** (0.87 mmol) in dichloromethane (15 mL) at 0 °C. After being stirred for 10 min, the reaction mixture was diluted with dichloromethane (10 mL), and then washed with saturated $NaHCO_3$ (30 mL). The organic phase was dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane=1/1) to give **7** (85%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.04 (dd, *J*=13.0, 11.9 Hz, 1H), 2.79 (dd, *J*=5.0, 13.0 Hz, 1H), 3.0 (br, 2H), 3.49 (dd, *J*=3.7, 12.5 Hz, 1H), 3.70 (dd, *J*=2.5, 12.5 Hz, 1H), 3.87 (s, 3H), 3.90–3.96 (m, 2H), 4.11 (dd, *J*=2.0, 10.5 Hz, 1H), 4.22 (ddd, *J*=9.9, 10.2, 10.5 Hz, 1H), 4.28 (dddd, *J*=1.6, 1.6, 5.6, 12.6 Hz, 1H), 4.97 (ddd, *J*=5.0, 10.5, 11.9 Hz, 1H), 5.07 (dd, *J*=2.0, 7.5 Hz, 1H), 5.19 (dddd, *J*=1.6, 1.6, 1.6, 10.7 Hz, 1H), 5.28 (dddd, *J*=1.6, 1.6, 1.6, 17.2 Hz, 1H), 5.86 (dddd, *J*=5.6, 5.6, 10.7, 17.2 Hz, 1H), 7.76 (d, *J*=9.9 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 20.83, 21.05, 37.63, 49.65, 53.79, 62.31, 65.87, 68.86, 69.34, 69.57, 72.06, 98.71, 115.80, 117.71, 133.40, 158.13, 169.89, 171.12, 171.72; HRMS (FAB) calcd for $C_{19}H_{27}F_3NO_{11}$ $(M+H)^+$: 502.1536, found: 502.1534.

4.1.7. Methyl (4-methylphenyl 4,7-di-O-acetyl-8,9-di-O-chloroacetyl-3,5-dideoxy-2-thio-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranoside)onate (**23**)

To the solution of **6** in dry CH_2Cl_2 and pyridine was added chloroacetyl chloride at −10 °C. The reaction was stirred for 3 h at −10 °C. Then the reaction was quenched with MeOH, and the reaction mixture was diluted with dichloromethane (10 mL), and then washed with saturated 10% HCl, $NaHCO_3$, and saturated NaCl in sequence. The organic phase was dried ($MgSO_4$) and filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give **23** (90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (s, 3H), 2.00 (dd, *J*=11.6, 13.0 Hz, 1H), 2.14 (s, 3H), 2.35 (s, 3H), 2.82 (dd, *J*=4.8, 13.0 Hz, 1H), 3.60 (s, 3H), 3.88–4.02 (m, 2H), 4.02 (d, *J*=15.2 Hz, 1H), 4.03 (s, 2H), 4.10 (d, *J*=15.2 Hz, 1H), 4.26 (dd, *J*=4.8, 12.4 Hz, 1H), 4.52 (dd, *J*=2.0, 12.4 Hz, 1H), 4.91 (ddd, *J*=4.8, 10.4, 11.6 Hz, 1H), 5.25 (dd, *J*=7.6, 2.0 Hz, 1H), 5.29 (tdd, *J*=7.6, 4.8, 2.0 Hz, 1H), 6.37 (br d, *J*=9.2 Hz, 1H), 7.14 (d, *J*=8.0 Hz, 2H), 7.31 (d, *J*=8.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.53, 20.61, 21.33, 37.95, 40.61, 40.82, 50.07, 52.96, 62.89, 66.76, 68.86, 70.77, 73.41, 87.45, 115.35, 124.41, 129.90, 136.48, 140.75, 157.91, 166.44, 167.02, 167.70, 169.94, 170.83; HRMS (FAB) calcd for $C_{27}H_{31}Cl_2F_3NO_{12}S$ $(M+H)^+$: 720.0896, found: 720.0904.

4.1.8. Dibenzyl [methyl (4,7-di-O-acetyl-8,9-O-chloroacetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α/β-D-galacto-non-2-ulopyranosid)onate] phosphite (**9**)

To a solution of **23** (0.38 mmol) in acetone (4.5 mL) and water (0.4 mL) was added NBS (62.30 mg, 0.53 mmol). The reaction was stirred for 30 min at room temperature. Then the solution was concentrated in vacuo. The residue was diluted with EtOAc and was washed with H_2O, saturated $NaHCO_3$, and saturated NaCl in sequence. The organic phase was dried ($MgSO_4$) and filtered, and the filtrate was evaporated. The residue was purified by silica gel column chromatography to afford hydroxyl compound (86%). To a solution of the above hydroxyl compound (0.2 mmol) in MeCN (3.7 mL) were added DDP (0.45 mL, 0.14 mmol) and 1*H*-tetrazole (0.1 g, 1.4 mmol) at −40 °C under argon atmosphere. After being stirred for 5 min at −40 °C, the solution was further stirred for 30 min at room temperature. The solution was diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography to give **9** (97%, α/β=1/2): some selected peaks of **9α**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.99 (s, 3H), 2.09 (s, 3H), 2.71 (dd, *J*=4.8, 13.1 Hz, 1H), 3.69 (s, 3H), 4.20 (d, *J*=15.2 Hz, 1H), 4.32 (dd, *J*=2.0, 10.6 Hz, 1H), 5.31 (dd, *J*=2.0, 7.5 Hz, 1H), 6.95 (d, *J*=9.9 Hz, 1H, NH). Some selected peaks of **9β**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.09 (s, 3H), 2.46 (dd, *J*=5.0, 13.2 Hz, 1H), 3.71 (s, 3H), 3.99 (s, 2H), 4.05 (d, *J*=15.2 Hz, 1H), 4.12 (d, *J*=15.2 Hz, 1H), 4.26 (dd, *J*=7.4, 12.5 Hz, 1H), 4.70 (dd, *J*=2.5, 12.5 Hz, 1H), 5.10 (dd, *J*=2.5, 4.1 Hz, 1H), 5.15 (dd, *J*=10.0, 12.5 Hz, 1H), 5.25 (ddd, *J*=2.5, 4.1, 7.0 Hz, 1H), 5.66 (d, *J*=10.0 Hz, 1H, NH).

*4.1.9. Methyl [2-allyl 4,7-di-O-acetyl-3,5-dideoxy-9-O-(methyl-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-5-trifluoroacetamido-D-glycero-α/β-D-galacto-2-nonulopyranosid]onate (**28**)*

A mixture of donor **3c** (0.074 mmol), acceptor **7** (0.049 mmol), and activated molecular sieves (3 Å, 120 mg) in MeCN (3.0 mL) was stirred for 15 min under argon at room temperature. The mixture was cooled to −40 °C for 15 min and TMSOTf (1.47 μmol) was added. The reaction mixture was stirred until TLC analysis indicated that reaction was completed. The residue was concentrated in vacuo and purified by silica gel column chromatography to afford **28** (70%, α/β=6/1): **28α** ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.99–2.04 (m, 2H), 2.01 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.14 (s, 9H), 2.63 (dd, *J*=4.5, 13.0 Hz, 1H), 2.74 (dd, *J*=4.5, 13.0 Hz, 1H), 3.55 (dd, *J*=3.5, 10.5 Hz, 1H), 3.81 (s, 3H), 3.82 (dd, *J*=6.5, 10.5 Hz, 1H), 3.85 (s, 3H), 3.98 (dd, *J*=5.5, 13.0 Hz, 1H), 3.99 (ddd, *J*=10.0, 10.0, 10.0 Hz, 1H), 4.07–4.11 (m, 1H), 4.08 (ddd, *J*=10.0, 10.0, 10.0 Hz, 1H), 4.14 (dd, *J*=6.5, 12.5 Hz, 1H), 4.22 (dd, *J*=1.5, 10.0 Hz, 1H), 4.24 (br d, *J*=10.0 Hz, 1H), 4.27 (dd, *J*=5.5, 13.0 Hz, 1H), 4.36 (dd, *J*=2.5, 12.5 Hz, 1H), 4.99–5.03 (m, 3H), 5.19 (dd, *J*=1.5, 10.5 Hz, 1H), 5.27 (dd, *J*=1.5, 9.0 Hz, 1H), 5.28 (dd, *J*=1.5, 17.0 Hz, 1H), 5.38 (ddd, *J*=3.5, 6.5, 9.0 Hz, 1H), 5.86 (ddd, *J*=5.5, 10.5, 17.0 Hz, 1H), 6.51 (d, *J*=10.0 Hz, 1H), 7.01 (d, *J*=10.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.79, 20.98, 21.28, 37.13, 37.50, 50.16, 50.38, 53.25, 53.44, 62.57, 65.76, 65.95, 67.42, 68.13, 68.51, 68.79, 68.90, 68.95, 69.28, 69.33, 72.09, 72.45, 98.81, 98.99, 115.65, 115.76, 117.81, 133.39, 157.83, 168.04, 169.08, 170.12, 170.13, 170.43, 170.86, 171.11, 171.40; HRMS (FAB) calcd for $C_{39}H_{50}F_6N_2NaO_{23}$ $(M+Na)^+$: 1051.2606, found: 1051.2628.

*4.1.10. Methyl [4-methylphenyl-4,7-di-O-acetyl-3,5-dideoxy-2-thio-9-O-(methyl-4,7-di-O-acetyl-3,5-dideoxy-8,9-O-isopropylidene-5-trifluoroacetamido-D-glycero-α/β-D-galacto-2-nonulopyranosylonate)-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosid]onate (**29**)*

Compound **29** (68%, α/β=3/1) was obtained from donor **4** and acceptor **6** by the method described in the synthesis of **28**: **29α** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 3H), 1.36 (s, 3H), 1.97 (dd, *J*=12.0, 13.0 Hz, 1H), 2.02 (s, 6H), 2.06 (dd, *J*=11.5, 13.0 Hz, 1H), 2.14 (s, 3H), 2.16 (s, 3H), 2.39 (s, 3H), 2.70 (dd, *J*=5.0, 13.0 Hz, 1H), 2.87 (dd, *J*=5.0, 13.0 Hz, 1H), 3.53 (dd, *J*=3.4, 10.5 Hz, 1H), 3.70 (dd, *J*=6.2, 10.5 Hz, 1H), 3.71 (s, 3H), 3.85 (s, 3H), 3.90 (dd, *J*=2.0, 10.5 Hz, 1H), 3.93–4.00 (m, 2H), 4.01–4.10 (m, 4H), 4.35 (ddd, *J*=4.3, 4.3, 6.4 Hz, 1H), 4.95 (ddd, *J*=5.0, 10.5, 11.5 Hz, 1H), 5.01 (dd, *J*=2.0, 8.0 Hz, 1H), 5.14 (ddd, *J*=5.0, 10.2, 12.0 Hz, 1H), 5.30 (dd, *J*=2.0, 4.3 Hz, 1H), 6.72 (d, *J*=9.2 Hz, 1H), 6.84 (d, *J*=10.0 Hz, 1H), 7.17 (d, *J*=7.6 Hz, 2H), 7.39 (d, *J*=8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.77, 20.81, 20.91, 20.95, 21.57, 31.12, 37.47, 49.58, 49.68, 49.75, 53.67, 53.74, 53.83, 69.12, 69.22, 69.68, 72.18, 72.77, 74.18, 74.33, 115.40, 124.50, 124.57, 130.04, 130.07, 136.82, 141.26, 141.31, 157.91, 158.20, 169.36, 169.61, 170.14, 170.40, 171.74, 172.19; HRMS (FAB) calcd for $C_{42}H_{52}F_6N_2NaO_{20}S$ $(M+Na)^+$: 1073.2636, found: 1073.2634. **29β** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 1.98 (s, 3H), 1.99–2.05 (m, 4H), 2.08 (dd, *J*=11.2, 12.8 Hz, 1H), 2.10 (s, 3H), 2.16 (s, 3H), 2.19 (s, 3H), 2.35 (s, 3H), 2.37 (dd, *J*=5.2, 14.0 Hz, 1H), 2.89 (dd, *J*=4.4, 12.8 Hz, 1H), 3.34 (dd, *J*=9.2, 12.0 Hz, 1H), 3.43–3.45 (m, 1H), 3.46 (s, 3H), 3.62–3.66 (m, 2H), 3.76 (dd, *J*=2.0, 10.0 Hz, 1H), 3.81 (s, 3H), 4.05–4.16 (m, 4H), 4.63 (ddd, *J*=4.4, 11.2, 11.2 Hz, 1H), 4.80 (d, *J*=10.0 Hz, 1H), 4.89 (ddd, *J*=5.2, 11.2, 10.4 Hz, 1H), 5.42 (dd, *J*=2.0, 8.8 Hz, 1H), 7.09 (d, *J*=10 Hz, 1H), 7.14 (d, *J*=8.0 Hz, 2H), 7.36 (d, *J*=8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.60, 20.68, 20.78, 21.49, 21.51, 31.51, 31.12, 37.68, 48.14, 48.81, 52.96, 54.32, 60.61, 64.08, 68.57, 68.83, 69.45, 69.73, 87.06, 98.56, 115.72, 115.88, 167.52, 170.11, 170.43, 170.73, 172.81; HRMS (FAB) calcd for $C_{42}H_{52}F_6N_2NaO_{20}S$ $(M+Na)^+$: 1073.2636, found: 1073.2627.

*4.1.11. Methyl [2-allyl-4,7-di-O-acetyl-3,5-dideoxy-9-O-(methyl-5-trifluoroacetamido-4,7-di-O-acetyl-3,5-dideoxy-8,9-O-isopropylidene-D-glycero-α-D-galacto-2-nonulopyranosylonate)-5-trifluoroacetamido-D-glycero-α/β-D-galacto-2-nonulopyranosid]onate (**30**)*

Compound **30** (64%, α/β=1.3/1) was obtained from donor **4** and acceptor **7** by the method described for the synthesis of **28**: **30α** ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 (s, 3H), 1.35 (s, 3H), 1.98 (dd, *J*=12.5, 13.0 Hz, 1H), 2.06 (dd, *J*=12.5, 13.0 Hz, 1H), 2.70 (dd, *J*=5.0, 13.0, Hz, 1H), 2.78 (dd, *J*=5.0, 13.0 Hz, 1H), 3.58 (dd, *J*=3.4, 10.5 Hz, 1H), 3.77 (dd, *J*=6.1, 10.5 Hz, 1H), 3.85 (s, 3H), 3.87 (s, 3H), 3.91 (ddd, *J*=9.7, 10.4, 10.4, Hz, 1H), 3.94–4.00 (m, 1H), 4.02 (ddd, *J*=8.0, 10.4, 10.4 Hz, 1H), 4.05–4.12 (m, 4H), 4.25–4.30 (m, 2H), 4.35 (ddd, *J*=4.3, 4.3, 6.5 Hz, 1H), 4.96 (ddd, *J*=5.0, 10.4, 12.5 Hz, 1H), 5.05 (br d, *J*=8.8 Hz, 1H), 5.15 (ddd, *J*=5.0, 10.4, 12.5 Hz, 1H), 5.21 (br d, *J*=10.8 Hz, 1H), 5.26 (dd, *J*=1.6, 4.3, 1H), 5.35 (dddd, *J*=1.7, 1.7, 1.7, 17.5 Hz, 1H), 5.87 (dddd, *J*=5.4, 5.4, 10.8, 17.5 Hz, 1H), 6.34 (d, *J*=9.7 Hz, 1H), 6.54 (d, *J*=8.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.78, 20.80, 20.96, 20.97, 25.46, 26.62, 31.14, 37.59, 50.25, 50.88, 53.19, 53.74, 65.88, 65.97, 68.76, 68.79, 69.12, 69.20, 70.47, 72.11, 72.85, 75.50, 98.86, 99.21, 109.00, 115.47, 115.63, 117.82, 133.32, 157.68, 157.99, 166.97, 168.28, 170.16, 170.54, 170.55, 170.96; HRMS (FAB) calcd for $C_{38}H_{50}F_6N_2NaO_{21}$ $(M+Na)^+$: 1007.2708, found: 1007.2690.

*4.1.12. Methyl [4-methylphenyl-4,7-di-O-acetyl-3,5-dideoxy-2-thio-9-O-(methyl-4,7-di-O-acetyl-8,9-O-chloroacetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α/β-D-galacto-2-nonulopyranosylonate)-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosid]onate (**31**)*

Compound **31** (75%, α/β=2.5/1) was obtained from donor **9** and acceptor **6** by the method described for the synthesis of **28**: **31α** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98–2.00 (m, 1H), 1.99 (s, 3H), 1.99–2.01 (m, 1H), 2.00 (s, 3H), 2.12 (s, 3H), 2.14 (s, 3H), 2.36 (s, 3H), 2.61 (dd, *J*=4.7, 13.0 Hz, 1H), 2.86 (dd, *J*=4.9, 13.0 Hz, 1H), 3.39 (dd, *J*=3.0, 10.5 Hz, 1H), 3.69 (s, 3H), 3.69–3.72 (m, 1H), 3.80 (s, 3H), 3.91 (dd, *J*=1.9, 10.5 Hz, 1H), 3.94–4.25 (m, 7H), 4.15 (d, *J*=15.2 Hz, 1H), 4.26 (d, *J*=15.2 Hz, 1H), 4.43 (ddd, *J*=2.4, 4.8, 12.5 Hz, 1H), 4.90–4.98 (m, 2H), 5.03 (dd, *J*=1.7, 8.3 Hz, 1H), 5.26 (dd, *J*=1.7, 8.3 Hz, 1H), 5.44 (ddd, *J*=2.5, 8.3, 13.5 Hz, 1H), 6.46 (d, *J*=9.8 Hz, 1H, NH), 6.67 (t, *J*=9.6 Hz, 1H, NH), 7.16 (d, *J*=7.9 Hz, 2H), 7.38 (d, *J*=7.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.54, 20.55, 20.56, 20.81, 21.40, 37.20, 37.28, 40.59, 40.60, 41.04, 49.72, 50.11, 53.18, 53.33, 63.56, 65.27, 66.70, 68.13, 68.70, 69.26, 69.81, 71.66, 74.09, 86.71, 98.63, 115.35, 115.43, 124.36, 129.86, 136.69, 141.01, 157.54, 157.60, 166.86, 167.22, 168.07, 169.05, 169.78, 170.05, 170.60, 170.92; **31β** ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.84 (dd, *J*=11.8, 13.2 Hz, 1H), 1.99 (s, 3H), 2.00 (s, 3H), 2.06 (dd, *J*=11.0. 13.1 Hz, 1H), 2.13 (s, 3H), 2.17 (s, 3H), 2.36 (s, 3H), 2.47 (dd, *J*=4.9, 13.2 Hz, 1H), 2.90 (dd, *J*=4.8, 13.1 Hz, 1H), 3.51(d, *J*=9.9 Hz, 1H), 3.62 (dd, *J*=2.3, 9.9 Hz, 1H), 3.71 (s, 3H), 3.74–3.76 (m, 1H), 3.75 (s, 3H), 3.97–4.21 (m, 8H), 4.48 (dd, *J*=2.3, 10.6 Hz, 1H), 4.67 (ddd, *J*=2.3, 12.8, 14.8 Hz, 1H), 4.87 (tdd, *J*=2.5, 4.8, 11.0 Hz, 1H), 5.01 (ddd, *J*=4.9, 11.8, 16.0 Hz, 1H), 5.26 (dd, *J*=1.1, 4.5 Hz, 1H), 5.31–5.40 (m, 2H), 7.16 (d, *J*=8.0 Hz, 2H), 7.36 (d, *J*=8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.53, 20.57, 21.05, 21.35, 21.37, 37.30, 40.61, 40.93, 48.31, 49.31, 52.89, 53.52, 53.57, 60.39, 63.82, 64.29, 67.23, 68.41, 68.93, 69.30, 70.04, 71.80, 74.88, 86.75, 97.88, 115.42, 115.43, 124.17, 129.91, 136.62, 141.21, 157.68, 158.06, 166.62, 166.85, 167.40, 169.56, 169.82, 170.39, 170.94, 171.85; HRMS (FAB) calcd for $C_{43}H_{51}Cl_2F_6N_2O_{22}S$ $(M+H)^+$: 1163.1935, found: 1163.1954.

*4.1.13. Methyl [4-methylphenyl 4-O-benzoyl-3,5-dideoxy-2-thio-9-O-(methyl-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosid]onate (**32**)*

Compound **32** (72%, only α) was obtained from donor **3c** and acceptor **8** by the method described for the synthesis of **28**: ^{1}H NMR

(500 MHz, $CDCl_3$) δ 1.96 (dd, J=11.9, 12.6 Hz, 1H), 2.02 (s, 3H), 2.04 (s, 3H), 2.13 (s, 3H), 2.14 (s, 3H), 2.23 (dd, J=11.3, 12.3 Hz, 1H), 2.37 (s, 3H), 2.68 (dd, J=4.8, 12.6 Hz, 1H), 3.04 (dd, J=4.9, 12.3 Hz, 1H), 3.58 (d, J=7.7 Hz, 1H), 3.64 (s, 3H), 3.71 (d, J=7.7 Hz, 1H), 3.76 (d, J=10.3 Hz, 1H), 3.81 (s, 3H), 3.90–3.96 (m, 2H), 3.99 (q, J=10.2 Hz, 1H), 4.13 (dd, J=6.1, 12.5 Hz, 1H), 4.27 (dd, J=2.0, 10.2 Hz, 1H), 4.38 (q, J=10.3 Hz, 1H), 4.42 (dd, J=3.0, 12.5 Hz, 1H), 5.07 (ddd, J=4.8, 10.2, 11.9 Hz, H), 5.22 (ddd, J=4.9, 10.3, 11.3 Hz, 1H), 5.31 (dd, J=2.0, 7.0 Hz, 1H), 5.37 (ddd, J=3.0, 6.1, 7.1 Hz, 1H), 6.86 (d, J=10.2 Hz, 1H, NH), 7.17 (d, J=7.0 Hz, 2H), 7.38 (d, J=10.3 Hz, 1H, NH), 7.39 (d, J=7.0 Hz, 2H), 7.46 (t, J=7.5 Hz, 2H), 7.61 (t, J=7.5 Hz, 1H), 7.99 (d, J=7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.50, 20.54, 20.62, 21.02, 21.35, 37.18, 37.25, 50.13, 50.80, 52.93, 53.90, 62.26, 66.14, 67.32, 68.29, 68.36, 69.41, 69.97, 70.44, 71.78, 75.09, 86.54, 98.63, 115.38, 115.41, 124.51, 128.57, 128.63, 129.80, 129.82, 133.90, 136.59, 140.86, 157.55, 158.49, 166.96, 168.02, 168.89, 170.16, 170.71, 170.95, 170.97; HRMS (FAB) calcd for $C_{43}H_{51}Cl_2F_6N_2O_{22}SNa$ $(M+Na)^+$: 1137.2585, found: 1137.2582.

4.1.14. *Methyl [2-allyl-4,7,8-tri-O-acetyl-3,5-dideoxy-9-O-(methyl-4,7-di-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosylonate)-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosid]onate (**33**)*

Compound **33** (84%) was obtained from **30α** by the methods described in the synthesis of **14** and **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (t, J=12.8 Hz, 2H), 2.03 (s, 3H), 2.04 (s, 3H), 2.13 (s, 3H), 2.17 (s, 6H), 2.69 (dd, J=4.4, 12.8 Hz, 1H), 2.72 (dd, J=5.2, 12.8 Hz, 1H), 3.50 (br, 1H), 3.62 (dd, J=2.8, 10.8 Hz, 1H), 3.70 (br, 1H), 3.81 (s, 3H), 3.84–3.91 (m, 3H), 3.88 (s, 3H), 3.93 (q, J=10.0 Hz, 1H), 4.02 (br d, J=10.0 Hz, 1H), 4.15 (ddd, J=10.0, 10.0, 10.0 Hz, 1H), 4.25–4.30 (m, 1H), 4.26 (dd, J=2.0, 10.0 Hz, 1H), 4.95 (ddd, J=5.2, 10.0, 12.8 Hz, 1H), 5.00 (br d, J=8.0 Hz, 1H), 5.04 (ddd, J=4.4, 10.0, 12.8 Hz, 1H), 5.19 (dddd, J=1.6, 1.6, 1.6, 10.8 Hz, 1H), 5.28 (dd, J=2.0, 9.2 Hz, 1H), 5.29 (dddd, J=1.6, 1.6, 1.6, 17.2 Hz, 1H), 5.38 (ddd, J=3.2, 4.8, 8.0 Hz, 1H), 5.86 (dddd, J=5.6, 5.6, 10.8, 17.2 Hz, 1H), 6.57 (d, J=10.0 Hz, 1H), 7.04 (d, J=10.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.78, 20.79, 20.94, 20.99, 21.32, 37.20, 38.16, 49.95, 50.64, 53.05, 53.91, 62.59, 63.36, 66.16, 67.54, 68.44, 68.64, 68.99, 69.01, 69.55, 71.46, 72.27, 98.46, 98.60, 115.63, 117.62, 133.62, 157.88, 168.34, 169.20, 170.13, 170.36, 170.96, 171.16, 171.52; HRMS (FAB) calcd for $C_{37}H_{48}F_6N_2NaO_{22}$ $(M+Na)^+$: 1009.2501, found: 1009.2520.

4.1.15. *Methyl [4-methylphenyl 4,7,8-tri-O-acetyl-3,5-dideoxy-2-thio-9-O-(methyl-5-trifluoroacetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-5-trifluoroacetamido-D-glycero-α-D-galacto-non-2-ulopyranosid]onate (**34**)*

Compound **34** (93%) was obtained from **27b** by the method described for the synthesis of **14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93 (t, J=12.8 Hz, 1H), 1.95 (t, J=12.8 Hz, 1H), 2.00 (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 2.11 (s, 3H), 2.12 (s, 3H), 2.15 (s, 3H), 2.17 (s, 3H), 2.39 (s, 3H), 2.62 (dd, J=4.6, 12.8 Hz, 1H), 2.80 (dd, J=4.6, 12.8 Hz, 1H), 3.62 (s, 3H), 3.77 (dd, J=2.6, 11.4 Hz, 1H), 3.80 (s, 3H), 3.88 (dt, J=9.7, 10.7 Hz, 1H), 3.95 (dd, J=6.3, 11.4 Hz, 1H), 4.02 (dt, J=9.7, 10.7 Hz, 1H), 4.09–4.15 (m, 2H), 4.22 (dd, J=2.2, 10.7 Hz, 1H), 4.33 (dd, J=2.6, 12.4 Hz, 1H), 4.94–5.01 (m, 2H), 5.16 (dd, J=2.2, 6.3 Hz, 1H), 5.25 (ddd, J=2.6, 6.3, 6.3 Hz, 1H), 5.32 (dd, J=2.2, 8.1 Hz, 1H), 5.38 (ddd, J=2.6, 8.1, 10.5 Hz, 1H), 6.65 (d, J=9.7 Hz, 1H), 6.96 (d, J=9.7 Hz, 1H), 7.17 (d, J=8.0 Hz, 2H), 7.45 (d, J=8.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.78, 21.20, 21.29, 21.55, 37.58, 37.99, 50.14, 50.20, 52.99, 53.27, 62.52, 63.19, 67.39, 67.98, 68.70, 68.86, 69.32, 70.73, 71.87, 73.52, 116.18, 117.15, 125.23, 129.87, 136.59, 140.52, 157.55, 157.66, 168.09, 170.02, 170.14, 170.69, 170.81, 171.20, 171.25; HRMS (FAB) calcd for $C_{45}H_{55}F_6N_2O_{23}S$ $(M+H)^+$: 1137.2821, found: 1137.2826.

4.1.16. *Methyl [4,7,8-tri-O-acetyl-3,5-dideoxy-9-O-(methyl-5-trifluoroacetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-5-trifluoroacetamido-D-glycero-β-D-galacto-non-2-ulopyranosid]onate (**35**)*

Compound **35** (93%) was obtained from **34** by the method described for the synthesis of **15**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.86 (dd, J=12.4, 13.2 Hz, 1H), 1.92 (br, 1H), 1.99 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.11 (s, 3H), 2.14 (s, 3H), 2.15 (s, 3H), 2.18 (s, 3H), 2.24 (dd, J=12.4, 13.2 Hz, 1H), 2.34 (dd, J=4.8, 13.2 Hz, 1H), 2.43 (dd, J=5.2, 13.2 Hz, 1H), 3.57 (dd, J=6.8, 10.8 Hz, 1H), 3.81 (s, 3H), 3.82 (dd, J=2.0, 6.8 Hz, 1H), 3.86 (m, 1H), 3.88 (s, 3H), 4.06–4.20 (m, 3H), 4.57–4.61 (m, 2H), 5.22–5.30 (m, 4H), 5.33 (br, 1H), 5.39 (br, 1H), 7.35 (d, J=9.6 Hz, 1H), 7.79 (d, J=10.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.40, 20.78, 20.83, 20.85, 21.07, 21.09, 21.26, 36.37, 36.47, 49.39, 50.21, 53.08, 53.57, 60.62, 62.34, 63.33, 67.94, 68.78, 68.82, 68.89, 70.58, 71.61, 95.46, 98.56, 115.81, 115.87, 157.76, 158.11, 166.43, 169.89, 170.08, 170.59, 170.71, 170.92, 171.05; HRMS (FAB) calcd for $C_{38}H_{48}F_6N_2NaO_{24}$ $(M+Na)^+$: 1053.2399, found: 1053.2406.

4.1.17. *Dibenzyl {methyl [4,7,8-tri-O-acetyl-3,5-dideoxy-9-O-(methyl-5-trifluoroacetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate)-5-trifluoroacetamido-D-glycero-α/β-D-galacto-non-2-ulopyranosid]onate} phosphite (**10**)*

To a solution of **35** (0.14 mmol) in MeCN (3.7 mL) were added DDP (0.45 mL, 1.4 mmol) and 1*H*-tetrazole (0.1 g, 1.4 mmol) at −40 °C under argon atmosphere. After being stirred for 5 min at −40 °C, the solution was further stirred for 15 min at room temperature. The solution was diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography to give **10** (92%): some selected peaks of **10β**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.85 (dd, J=12.7, 13.1 Hz, 1H), 1.86 (dd, J=12.7, 13.1 Hz, 1H), 1.97 (s, 3H), 1.98 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 2.12 (s, 3H), 2.46 (dd, J=4.9, 13.1 Hz, 1H), 2.55 (dd, J=4.3, 13.1 Hz, 1H), 3.70 (s, 3H), 3.76 (s, 3H), 3.94 (ddd, J=10.2, 10.5, 10.5 Hz, 1H), 3.96 (ddd, J=9.7, 10.5, 10.5 Hz, 1H), 4.14 (dd, J=2.2, 10.6 Hz, 1H), 4.20 (dd, J=2.2, 10.6 Hz, 1H), 4.25 (dd, J=2.6, 12.5 Hz, 1H), 4.87 (ddd, J=4.3, 10.5, 12.7 Hz, 1H), 4.91 (d, J=7.8 Hz, 1H), 4.93 (d, J=8.0 Hz, 1H), 4.95 (d, J=7.8 Hz, 1H), 5.08 (ddd, J=4.9, 10.5, 13.1 Hz, 1H), 5.25 (dd, J=2.2, 8.4 Hz, 1H), 5.32 (ddd, J=2.6, 5.5, 8.4 Hz, 1H), 6.08 (d, J=9.7 Hz, 1H), 6.48 (d, J=10.2 Hz, 1H), 7.14–7.37 (m, 10H).

4.1.18. *Tetramer (**36**)*

Compound **36** (58%) was obtained from donor **10** and acceptor **33** by the method described for the synthesis of **28**: some selected peaks of **36**: ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.51, 20.67, 20.77, 20.96, 21.02, 29.57, 35.59, 36.25, 36.39, 36.58, 36.89, 37.31, 37.49, 37.89, 48.78, 49.10, 49.32, 49.54, 49.61, 49.79, 49.92, 50.03, 50.24, 50.30, 52.75, 52.89, 53.02, 53.17, 53.44, 53.84, 62.08, 62.46, 63.03, 63.15, 63.40, 63.52, 63.65, 63.82, 65.24, 65.88, 66.93, 67.23, 67.41, 67.66, 67.87, 68.26, 68.39, 68.53, 68.76, 69.17, 69.57, 69.92, 71.17, 71.26, 71.39, 71.65, 71.83, 72.02, 72.13, 72.47, 98.33, 98.38, 98.48, 98.57, 112.01, 114.30, 116.60, 117.32, 118.89, 133.34, 133.39, 157.13, 157.43, 157.73, 158.2, 166.60, 167.76, 167.86, 168.08, 168.13, 168.41, 168.97, 169.29, 169.67, 169.81, 169.89, 170.06, 170.17, 170.51, 170.68, 170.74, 170.83, 171.09, 171.30, 171.46; HRMS (FAB) calcd for $C_{75}H_{94}N_4F_{12}NaO_{45}$ $(M+Na)^+$: 2021.4896, found: 2021.4882.

4.1.19. *Methyl(2-allyl-3,5-dideoxy-4,7,8,9-tetra-O-acetyl-5-trifluoroacetamido-α-D-glycero-D-galacto-2-nonulopyranosid)onate (**37a**)*

Compound **37a** (95%) was obtained from **20** by the method described for the synthesis of **14**: ^{1}H NMR (400 MHz, $CDCl_3$)

δ 1.98 (dd, J=12.3, 12.9 Hz, 1H), 2.03 (s, 3H), 2.05 (s, 3H), 2.16 (s, 3H), 2.18 (s, 3H), 2.69 (dd, J=4.7, 12.9 Hz, 1H), 3.81 (s, 3H), 3.89 (dddd, J=1.4, 1.4, 5.2, 12.8 Hz, 1H), 3.99 (ddd, J=10.4, 10.4, 10.4 Hz, 1H), 4.14 (dd, J=5.4, 12.6 Hz, 1H), 4.27–4.29 (m, 2H), 4.32 (dd, J=2.6, 12.6 Hz, 1H), 5.01 (ddd, J=4.7, 10.4, 12.3 Hz, 1H), 5.18 (dddd, J=1.4, 1.4, 1.4, 10.5 Hz, 1H), 5.29 (dddd, J=1.4, 1.4, 1.4, 17.2 Hz, 1H), 5.30 (dd, J=2.2, 8.1 Hz, 1H), 5.42 (ddd, J=2.6, 5.4, 8.1 Hz, 1H), 5.86 (dddd, J=5.2, 5.2, 10.5, 17.2, Hz, 1H), 6.36 (d, J=10.4 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 20.77, 20.89, 21.32, 38.22, 50.57, 53.03, 62.34, 66.29, 67.34, 68.50, 68.68, 71.83, 75.66, 98.68, 117.67, 117.54, 159.70, 168.32, 170.19, 170.39, 170.87, 171.06; HRMS (FAB) calcd for $C_{23}H_{30}F_3NNaO_{13}$ $(M+Na)^+$: 608.1567, found: 608.1575.

*4.1.20. Methyl[2-(3-acetylsulfanyl-propoxy)-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonulopyranosid]onate (**38a**)*

HSAc (0.39 mL, 5.1 mmol) and AIBN (14 mg, 0.085 mmol) were added to a solution of compound **37a** (200 mg, 0.34 mmol) in dry MeOH (15 mL). The mixture was then degassed for three times and then stirred at 75 °C for 24 h. The mixture was concentrated in vacuo and then purified by flash silica gel chromatography (EtOAc/hexane=1/3) to yield product **38a** (191.2 mg, 85%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.78–1.83 (m, 2H), 1.89 (dd, J=12.4, 12.9 Hz, 1H), 1.97 (s, 3H), 1.99 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.27 (s, 3H), 2.57 (dd, J=4.7, 12.9 Hz, 1H), 2.82–2.94 (m, 2H), 3.21–3.28 (m, 1H), 3.76 (s, 3H), 3.77 (m, 1H), 3.97 (q, J=10.0 Hz, 1H), 4.09 (dd, J=6.0, 12.4 Hz, 1H), 4.25 (dd, J=2.0, 10.0 Hz, 1H), 4.31 (dd, J=2.4, 12.4 Hz, 1H), 4.93 (ddd, J=4.7, 10.0, 12.4 Hz, 1H), 5.26 (dd, J=2.0, 7.5 Hz, 1H), 5.32 (ddd, J=2.4, 6.0, 7.5 Hz, 1H), 7.00 (d, J=10.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.47, 20.54, 20.61, 20.98, 25.61, 29.37, 30.50, 37.78, 49.94, 52.73, 62.16, 63.33, 67.16, 68.47, 69.14, 71.71, 98.62, 168.10, 169.86, 170.37, 170.63, 170.98, 195.77; HRMS (FAB) calcd for $C_{25}H_{35}F_3NO_{14}S$ $(M+H)^+$: 662.1730, found: 662.1721.

*4.1.21. 3-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosidonic acid)-oxypropyldisulfide (**39a**)*

To a stirred solution of compound **38a** (0.11 mmol) in dry MeOH (1.5 mL) was added NaOMe (catalytic amount). After being stirred at room temperature for 2 h, the solution was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to dryness. To the residue was then added 0.5 N NaOH (1 mL) and allowed to stir at room temperature for 30 min. The solution was neutralized with Dowex 50W×8 (H^+) resin, filtered, and concentrated. To a solution of the residue in pyridine (2 mL) was added acetic anhydride (1 mL) at 0 °C. The reaction mixture was stirred for 12 h at room temperature and then concentrated in vacuo. The residue left was diluted with EtOAc and was washed with 10% HCl, saturated $NaHCO_3$, and saturated NaCl sequentially. The organic phase was dried ($MgSO_4$) and filtered, and the filtrate was evaporated. The residue was purified by silica gel column chromatography to afford peracetyl compound. To a stirred solution of peracetyl compound in dry MeOH (1.5 mL) was added NaOMe (catalytic amount). After being stirred at room temperature for 2 h, the solution was neutralized with Amberlite IR-120 (H^+) resin, filtered, and concentrated to dryness. The dried residue was purified by P2 biogel to give dimer product **39a** (60%): ^{1}H NMR (500 MHz, CD_3OD) δ 1.60 (t, J=12.0 Hz, 1H), 1.89–1.94 (m, 2H), 2.03 (s, 3H), 2.73–2.76 (m, 2H), 2.77 (dd, J=4.5, 12.0 Hz, 1H), 3.54 (dd, J=6.5, 16.0 Hz, 2H), 3.59 (br d, J=9.5 Hz, 1H), 3.64–3.71 (m, 2H), 3.82–3.89 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD) δ 22.98, 30.40, 35.92, 42.16, 53.76, 63.93, 63.99, 69.44, 69.88, 73.01, 74.49, 101.85, 171.22, 176.07; HRMS (FAB) calcd for $C_{28}H_{48}N_2NaO_{18}S_2$ $(M+Na)^+$: 787.2241, found: 787.2236.

*4.1.22. Methyl[2-(3-acetylsulfanyl-propoxy)-4,7,8-tri-O-acetyl-3,5-dideoxy-9-O-(methyl-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-trifluoroacetamido-D-glycero-α-D-galacto-2-nonlopyranosylonate)-5-trifluoroacetamido-D-glycero-α/β-D-galacto-2-nonulopyranosid]onate (**38b**)*

Compound **38b** (81%) was obtained from **28** by the methods described in the synthesis of **14** and **38a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.79–1.85 (m, 2H), 1.91 (t, J=12.1 Hz, 1H), 1.93 (t, J=12.1 Hz, 1H), 2.02 (s, 6H), 2.05 (s, 3H), 2.13 (s, 3H), 2.14 (s, 3H), 2.15 (s, 3H), 2.17 (s, 3H), 2.33 (s, 3H), 2.62 (dd, J=4.7, 12.1 Hz, 1H), 2.63 (dd, J=4.7, 12.1 Hz, 1H), 2.94 (ddd, J=7.4, 7.4, 13.7 Hz, 1H), 2.97 (ddd, 6.4, 7.8, 13.7 Hz, 1H), 3.32 (ddd, J=5.0, 7.4, 9.7 Hz, 1H), 3.58 (dd, J=2.8, 11.2 Hz, 1H), 3.81(s, 3H), 3.83 (s, 3H), 3.88 (ddd, J=7.4, 7.8, 9.7 Hz, 1H), 3.90 (dd, J=6.4, 11.2 Hz, 1H), 3.97 (q, J=10.5 Hz, 1H), 4.00 (q, J=10.5 Hz, 1H), 4.14 (dd, J=5.2, 12.1 Hz, 1H), 4.19 (dd, J=2.0, 10.5 Hz, 1H), 4.32 (dd, J=2.0, 10.5 Hz, 1H), 4.35 (dd, J=2.2, 12.1 Hz, 1H), 4.97 (ddd, J=4.7, 10.5, 12.1 Hz, 1H), 5.00 (ddd, J=4.7, 10.5, 12.1 Hz, 1H), 5.20 (dd, J=2.0, 8.0 Hz, 1H), 5.32 (dd, 2.0, 7.7 Hz, 1H), 5.33–5.39 (m, 2H), 6.59 (d, J=10.5 Hz, 1H), 6.81 (d, J=10.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.81, 20.87, 21.27, 21.33, 25.99, 29.72, 30.83, 37.65, 38.16, 50.30, 50.50, 62.54, 63.55, 63.75, 67.45, 67.57, 68.54, 68.66, 68.89, 69.15, 71.47, 72.07, 98.51, 98.83, 115.62, 115.72, 157.79, 157.84, 167.97, 168.58, 169.96, 170.13, 170.25, 170.83, 170.96, 171.08, 171.24, 195.98; HRMS (FAB) calcd for $C_{43}H_{56}F_6N_2NaO_{25}S$ $(M+Na)^+$: 1169.2695, found: 1169.2688.

*4.1.23. 2-(3-Mercapto-propoxy)-5-acetamido-3,5-dideoxy-9-O-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosidonic acid)-D-glycero-α-D-galacto-2-nonulopyranosidonic acid (**39b**)*

Compound **39b** (51%) was obtained as a monomer from **38b** by the method described in the synthesis of **39a**: ^{1}H NMR (500 MHz, D_2O) δ 1.70 (t, J=12.5 Hz, 1H), 1.88 (t, J=12.5 Hz, 1H), 1.99–2.03 (m, 2H), 2.08 (s, 3H), 2.09 (s, 3H), 2.79 (dd, J=5.0, 12.5 Hz, 1H), 2.84–2.91 (m, 3H), 3.57–3.63 (m, 1H), 3.67–3.77 (m, 5H), 3.78–4.08 (m, 10H); ^{13}C NMR (125 MHz, CD_3OD) δ 23.12, 23.17, 29.77, 35.41, 40.52, 41.59, 53.04, 53.05, 63.41, 63.79, 64.13, 67.90, 68.37, 69.13, 69.55, 71.34, 71.74, 72.63, 73.55, 101.52, 101.74, 171.14, 172.87, 174.97, 176.14; HRMS (FAB) calcd for $C_{25}H_{42}N_2NaO_{17}S$ $(M+Na)^+$: 697.2102, found: 697.2091.

4.2. Conjugation of sialic acid with carrier protein (KLH)

The solution of KLH (5 mg) in 0.01 M PBS buffer (pH=7) was put inside a dialysis membrane, and then the membrane was put inside a 4 L 0.01 M PBS buffer and stirred for more than 4 h. The process was repeated three times. After that, KLH was taken out and dissolved in 70 μL *m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester (MBS)/DMF. The solution was stirred for 30 min at room temperature. PD-10 column was applied to purify the maleimide-linked KLH, and 0.05 M PBS buffer (pH=6) was used as the eluent. All tubes containing protein, which was indicated by Coomassie Brilliant Blue, were combined together. To the solution of disulfide-linked sialic acid **39** (5 mg) in PBS (0.05 M, 1 mL) was added $NaBH_4$ (10 equiv). The solution was stirred for 20 min and then added to the combined protein solution. The mixture was adjusted to pH 7.3 and stirred for 3 h. Then, the mixture was dialyzed and lyophilized to obtain sialic acid-linked KLH.

4.3. Determination of sialic acid amount on sialic acid-linked KLH by Warren's method

Four kinds of solution were prepared. Solution A: a solution of periodic acid (0.025 M) in diluted sulfuric acid (0.063 M, pH 1.2). Solution B: 2% solution of sodium arsenite in diluted hydrochloric acid (0.5 M). Solution C: thiobarbituric acid (0.1 M) adjusted to pH

9.0 with NaOH. Solution D: 1-butanol containing 5% hydrochloric acid (12 M). To the solution of sialic acid-linked KLH (10 μg, 200 μg, 300 μg, and 400 μg) in 500 μL water was added 250 μL of solution A. The resulting mixture was heated in a water bath at 37 °C for 30 min. Then, 200 μL of solution B was added to it at room temperature for 2 min. To this, solution C (2 mL) was added, and the mixture was heated in boiling water bath for 15 min. After that, the solution was cooled to 0 °C and extracted by solution D (3 mL). The solution was centrifuged at 1000 rpm for 5 min. The upper clear layer was separated and measured by UV.

Alternatively, the sialic acid was removed from the sialic acid-linked KLH by sialidase. In this method, the above A solution was changed to sialidase (*Arthrobacter ureafaciens*, 8 mU) solution and the reaction time was extended to 12 h at 37 °C.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.04.022.

References and notes

1. (a) Schauer, R.; Kamerling, J. P. In *Glycoproteins II*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier: Amsterdam, 1997; pp 243–402; (b) Angata, T.; Varki, A. *Chem. Rev.* **2002**, *102*, 439–469.
2. (a) Bomsel, M.; Alfsen, A. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 57–68; (b) Allende, M. L.; Proia, R. *Curr. Opin. Struct. Biol.* **2002**, *12*, 587–592; (c) Crocker, P. R. *Curr. Opin. Struct. Biol.* **2002**, *12*, 609–615; (d) *Biology of the Sialic Acids*; Rosenberg, A., Ed.; Plenum: New York, NY, 1995.
3. (a) Kiefel, M. J.; von Itzstein, M. *Chem. Rev.* **2002**, *102*, 471–490; (b) Inoue, Y.; Inoue, S. *Pure Appl. Chem.* **1999**, *71*, 789–800.
4. Jennings, H. J. In *Carbohydrate-based Drug Discovery*; Wong, C. H., Ed.; Wiley-VCH: Weinheim, 2003; pp 357–380.
5. (a) Verez-Bencome, V.; Fernández-Santana, V.; Hardy, E.; Toledo, M. E.; Rodríguez, M. C.; Heynngnezz, L.; Rodriguez, A.; Baly, A.; Herrera, L.; Izquierdo, M.; Villar, A.; Valdés, Y.; Cosme, K.; Deler, M. L.; Montane, M.; Garcia, E.; Ramos, A.; Aguilar, A.; Medina, E.; Yoraño, G.; Sosa, I.; Hernandez, I.; Martínez, R.; Muzachio, A.; Carmenates, A.; Costa, L.; Cardoso, F.; Campa, C.; Diaz, M.; Roy, R. *Science* **2004**, *305*, 522–525; (b) Phalipon, A.; Costachel, C.; Grandjean, C.; Thuizat, A.; Guerreiro, C.; Tanguy, M.; Nato, F.; Vulliez-Le Normand, B.; Belot, F.; Wright, K. *J. Immunol.* **2006**, *176*, 1686–1694; (c) Pozsgay, V.; Kubler-Kielb, J.; Schneerson, R.; Robbins, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 14478–14482; (d) Xin, H.; Dziadek, S.; Bundle, D. R.; Cutler, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 13526–13531.
6. Castro-Palomino, J. C.; Tsvetkov, Y. E.; Schneider, R.; Schmidt, R. R. *Tetrahedron Lett.* **1997**, *38*, 6837–6840.
7. For recent reviews of glycosidations of sialic acid, see: (a) Boons, G. J.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539–4565; (b) Lin, C. H.; Lin, C. C. *The Molecular Immunology of Complex Carbohydrates 2*; Kluwer–Plenum: New York, NY, 2001; (c) Halcomb, R. L.; Cappell, M. D. *J. Carbohydr. Chem.* **2002**, *21*, 723–768; (d) Ress, D. K.; Linhardt, R. J. *Curr. Org. Synth.* **2004**, *1*, 31–46; (e) De Meo, C.; Priyadarshani, U. *Carbohydr. Res.* **2008**, *343*, 1540–1552.
8. (a) Demchenko, A. V.; Boons, G. J. *Chem.—Eur. J.* **1999**, *5*, 1278–1283; (b) De Meo, C.; Demchenko, A. V.; Boons, G. J. *J. Org. Chem.* **2001**, *66*, 5490–5498; (c) Ren, C. T.; Chen, C.-S.; Wu, S.-H. *J. Org. Chem.* **2002**, *67*, 1376–1379; (d) Ando, H.; Koike, Y.; Ishida, H.; Kiso, M. *Tetrahedron Lett.* **2003**, *44*, 6883–6886; (e) Lu, K.-C.; Tseng, S.-Y.; Lin, C.-C. *Carbohydr. Res.* **2002**, *337*, 755–760; (f) Yu, C.-S.; Niikura, K.; Lin, C.-C.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2001**, *40*, 2900–2903; (g) Tanaka, H.; Adachi, M.; Takahashi, T. *Chem.—Eur. J.* **2005**, *11*, 849–862; (h) Tanaka, K.; Goi, T.; Fukase, K. *Synlett* **2005**, 2958–2962.
9. (a) Okamoto, K.; Kondo, T.; Goto, T. *Tetrahedron* **1987**, *43*, 5909–5918; (b) Ercégovec, T.; Magnusson, G. *J. Org. Chem.* **1995**, *60*, 3378–3384; (c) Ito, Y.; Ogawa, T. *Tetrahedron* **1990**, *46*, 89–102; (d) Martichonok, V.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 8187–8191; (e) Ercégovec, T.; Magnusson, G. *J. Org. Chem.* **1996**, *61*, 179–184; (f) Castro-Palomino, J. C.; Tsvetkov, Y. E.; Schmidt, R. R. *J. Am. Chem. Soc.* **1998**, *120*, 5434–5440; (g) Hossain, N.; Magnusson, G. *Tetrahedron Lett.* **1999**, *40*, 2217–2220; (h) Okamoto, K.; Kondo, T.; Goto, T. *Tetrahedron* **1987**, *43*, 5919–5928.
10. (a) Takahashi, T.; Tsukamoto, H.; Yamada, H. *Tetrahedron Lett.* **1997**, *38*, 8223–8226; (b) Danishefsky, S. J.; DeNinno, M. P.; Chen, S. *J. Am. Chem. Soc.* **1988**, *110*, 3929–3940; (c) Haberman, J. M.; Gin, D. Y. *Org. Lett.* **2001**, *3*, 1665–1668; (d) Ye, X.-S.; Huang, X.; Wong, C.-H. *Chem. Commun.* **2001**, 974–975; (e) Haberman, J. M.; Gin, D. Y. *Org. Lett.* **2003**, *5*, 2539–2541; (f) Ishiwata, A.; Ito, Y. *Synlett* **2003**, 1339–1343.
11. Ando, H.; Koike, Y.; Koizumi, S.; Ishida, H.; Kiso, M. *Angew. Chem., Int. Ed.* **2005**, *44*, 6759–6763.
12. Tanaka, H.; Nishiura, Y.; Takahashi, T. *J. Am. Chem. Soc.* **2006**, *128*, 7124–7125.
13. Tanaka, H.; Tateno, Y.; Nishiura, Y.; Takahashi, T. *Org. Lett.* **2008**, *10*, 5597–5600.
14. Lin, C.-C.; Huang, K.-T.; Lin, C.-C. *Org. Lett.* **2005**, *7*, 4169–4172.
15. (a) Kanie, O.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1988**, *7*, 501–506; (b) Marra, A.; Sinay, P. *Carbohydr. Res.* **1989**, *187*, 35–42; (c) Roy, R.; Andersson, F. O.; Letellier, M. *Tetrahedron Lett.* **1992**, *33*, 6053–6056; (d) Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123–6126; (e) Kondo, H.; Icjikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *411*, 8748–8750; (f) Cai, S.; Yu, B. *Org. Lett.* **2003**, *5*, 3827–3830; (g) Liu, Y.; Ruan, X.; Li, X.; Li, Y. *J. Org. Chem.* **2008**, *73*, 4287–4290.
16. Tsvetkov, Y. E.; Nifantiev, N. E. *Synlett* **2005**, 1375–1380.
17. Lin, C.-C.; Hsu, T.-S.; Lu, K.-C.; Huang, I.-T. *J. Chin. Chem. Soc.* **2000**, *47*, 921–928.
18. Misra, A. K.; Agnihotri, G. *Carbohydr. Res.* **2004**, *339*, 885–890.
19. Eby, R.; Schuerch, C. *Carbohydr. Res.* **1982**, *102*, 131–138.
20. Fan, G.-T.; Lee, C.-C.; Lin, C.-C.; Fang, J.-M. *J. Org. Chem.* **2002**, *67*, 7565–7568.
21. (a) Chen, C.-T.; Weng, S.-S.; Kao, J.-Q.; Lin, C.-C.; Jan, M.-D. *Org. Lett.* **2005**, *7*, 3343–3346; (b) Lin, C.-C.; Jan, M.-D.; Weng, S.-S.; Lin, C.-C.; Chen, C.-T. *Carbohydr. Res.* **2006**, *341*, 1948–1953.
22. Yan, M.-C.; Chen, Y.-N.; Wu, H.-T.; Lin, C.-C.; Chen, C.-T.; Lin, C.-C. *J. Org. Chem.* **2007**, *72*, 299–302.
23. Hasegawa, A.; Nagahama, T.; Ohki, H.; Hotta, K.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1991**, *10*, 493–498.
24. For a representative synthesis, see: Chen, M.-Y.; Patkar, L.-N.; Lu, K.-C.; Lee, S.-Y.; Lin, C.-C. *Tetrahedron Lett.* **2004**, *45*, 635–639.
25. (a) Hasegawa, A.; Ohki, H.; Nagahama, T.; Ishida, H.; Kiso, M. *Carbohydr. Res.* **1991**, *212*, 277–281; (b) Okamoto, K.; Kondo, T.; Goto, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 637–643; (c) Van der Vleugel, D. J. M.; Van Heeswijk, W. A. R.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1982**, *102*, 121–130; (d) Dabrowski, U.; Friebolin, H.; Brossmer, R.; Supp, M. *Tetrahedron Lett.* **1979**, *48*, 4637–4640.
26. Hori, H.; Nakajima, T.; Nishida, Y.; Ohrui, H.; Meguro, H. *Tetrahedron Lett.* **1988**, *29*, 6317–6320.
27. Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 837–863.
28. Roy, R.; Baek, M.-G.; Rittenhouse-Olson, K. *J. Am. Chem. Soc.* **2001**, *123*, 1809–1816.
29. (a) Warren, L. *J. Biol. Chem.* **1959**, *234*, 1971–1975; (b) Romero, E. L.; Pardo, M. F.; Porro, S.; Alonso, S. *J. Biochem. Biophys. Methods* **1997**, *35*, 129–134.