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Chemical Synthesis of a Complex-Type *N*-Glycan Containing a Core Fucose

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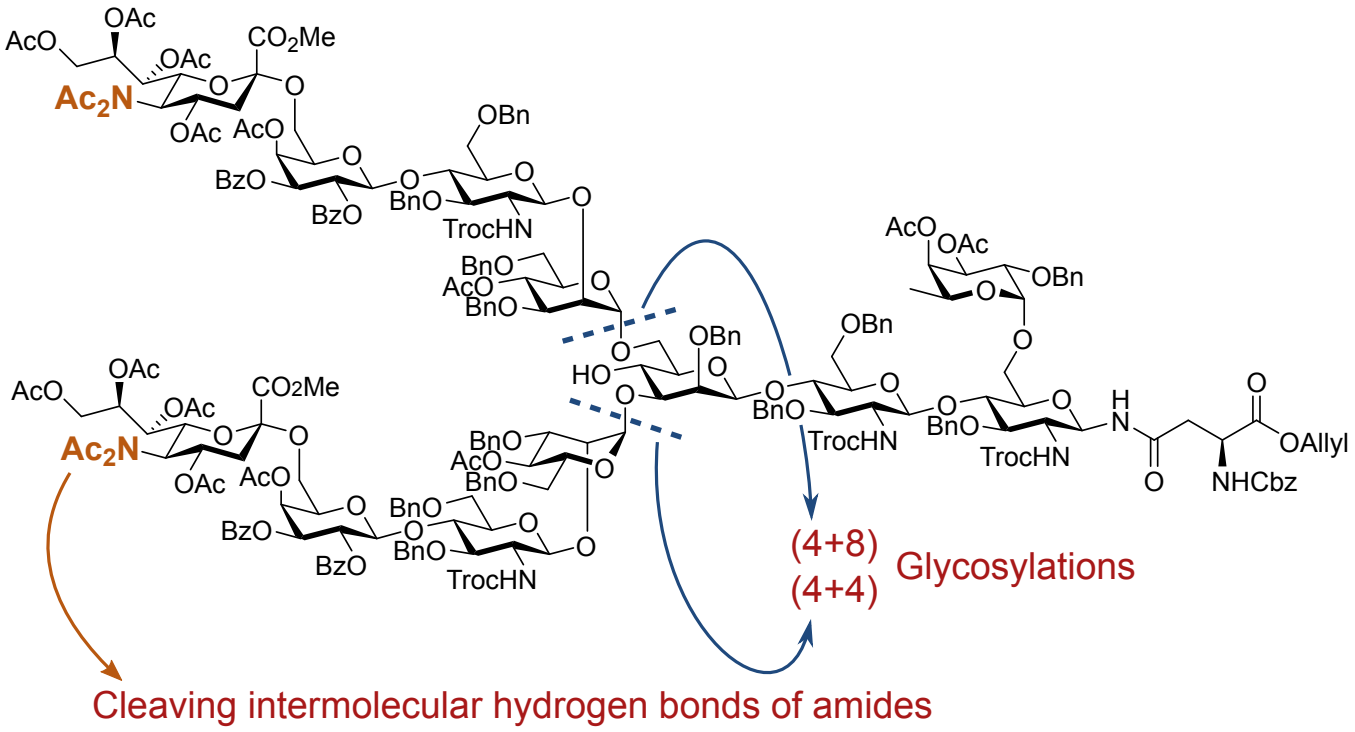
Abstract Graphic



Abstract

A chemical synthesis of a core fucose containing *N*-glycan was achieved. The asparagine was introduced at the early-stage of the synthesis and the sugar chain was convergently elongated. As for the fragment synthesis, we reinvestigated α-sialylation, β-mannosylation, and *N*-glycosylation to reveal that precise temperature control was essential for these glycosylations. Intermolecular hydrogen bonds involving acetamide groups were found to reduce the reactivity in glycosylations: the protection of NHAc as NAc_2 dramatically improved the reactivity. The dodecasaccharide-asparagine framework was constructed via the (4+4) glycosylation and the (4+8) glycosylation using the tetrasaccharide donor and the tetrasaccharide-asparagine acceptor. An ether-type solvent enhanced the yields of these key glycosylations between large substrates. After the whole deprotection of the dodecasaccharide, the target *N*-glycan was obtained.

Introduction

Asparagine-linked glycans (*N*-glycans) in glycoproteins are oligosaccharides that are found in eukaryotes and some prokaryotes and display a broad structural diversity. They are divided into three types: high mannose-type, complex-type, and hybrid-type. *N*-Glycans are generally heterogeneous, even at one specific glycosylation site. This structural diversity, which is common in natural glycans, is called glycoform.

Complex-type *N*-glycans play important roles in various biological events and diseases, including the regulation of glycoprotein dynamics,[1,2] cell development,[3] immunity,[4,5] and cancer invasion.[6,7] The structures of the *N*-glycans influence the function and dynamics of glycoproteins *in vivo*. For example, Tanaka et al. conducted *in vivo* PET and fluorescence imaging of glycoproteins and glycoclusters to reveal the remarkable dependence of the *in vivo* dynamics and bio-distributions of these compounds on the glycan structure. They found that trimming the non-reducing end structure or linking sialic acid to the galactose 3-OH or 6-OH positions affected the distribution of the glycoproteins.[8,9]

Fucose residues linked to the reducing end GlcNAc through an α linkage form a core fucose structure that comprises one of the major modifications of the complex-type *N*-glycans. Mammalian core fucose is transferred to complex-type *N*-glycans by fucosyl transferase 8 (FUT8) to form an α(1-6) fucosyl linkage.[10] The core fucose has been shown to play an important role in various physiological and pathological events. *Fut8* knockout mice exhibited severe growth retardation, with a mortality rate 70% during the 3 postnatal days.[11] Core fucosylated immunoglobulin G (IgG) showed the antibody-dependent cell-mediated cytotoxicity (ADCC) 100-fold weaker than that without a core fucose structure.[12] The functional significance of the core fucosylation has been noted in a variety of the pathophysiological steps involved in carcinogenesis and tumor progression.[13] Human colon cancers with reduced levels of core fucosylation were found to be resistant to TRAIL-induced apoptosis and escaped immune surveillance. Core fucosylated α-fetoprotein levels were found to be significantly elevated in

hepatocellular carcinoma and are useful as a liver cancer marker.[14] The up-regulation of core fucosylation on growth factor receptors, such as epidermal growth factor receptor (EGFR) and fibroblast growth factor receptor (FGFR), has been found to activate these receptors.[11,15,16]

The functions of core fucose and its mechanisms of action have not yet been elucidated well. Core fucose binding lectin has not been identified in mammals, although several core fucose-binding lectins are found in plants,[17-19] fungi,[20,21] and bacteria.[22] Homogeneous preparations of core fucosylated *N*-glycans are needed to elucidate the biological functions of these structures, including the core fucose-dependent biodynamics and the identification of lectins responsible for core fucose recognition. The isolation of core fucosylated *N*-glycans from natural sources has proven to be difficult, although certain series of *N*-glycans are available from natural sources.[23-25] Motivated by this unmet need, we investigated the chemical synthesis of *N*-glycans containing core fucose.

Several syntheses of *N*-glycans have been studied in an effort to investigate their biological functions. Danishefsky et al. succeeded in synthesizing various types of *N*-glycans, including a core-fucosylated glycan and a triantennary glycan.[26-28] Ito et al. synthesized high-mannose/complex-type glycans.[29-31] Unverzagt et al. reported the syntheses of *N*-glycans with various structures, including core fucose.[32-35] Chemoenzymatic approaches using a variety of glycosidases or glycosyltransferases have been employed for the synthesis of *N*-glycan libraries by Ito et al.,[36] Boons et al.,[37] Wang et al.,[38] and Wong et al.[39,40] Schmidt et al. carried out the synthesis of complex-type *N*-glycans not only in liquid phase but also on solid phase.[41,42] Our research group has reported the solid-phase synthesis of a sialic acid containing glycan.[43] Convergent synthetic strategies have been employed for the liquid phase syntheses of glycans, and stepwise strategies have been employed for the solid phase syntheses. As for the convergent *N*-glycan syntheses, two strategies have been adopted. In path A, two donors possessing a glucosamine residue at the reducing end were introduced to acceptors containing trimannosyl core

(Figure 1). Stereoselective glycosylation was secured via neighboring group participation of a 2-*N*–protecting group on the glucosamine residue. In path B, the glycan structure was constructed through glycosylation at two branch positions (Figure 1). The reaction steps in path B were smaller than those applied in path A, although neighboring group participation could not be used in glycosylations between the stem and branch fragments in path B.

In this study, we selected path B to synthesize a core fucose containing complex-type *N*-glycan **1**, which has an asparagine-linked dodecasaccharide structure (Figure 1). We employed a new synthetic strategy based on the early-stage introduction of asparagine into the glycan part. Previous reports of the syntheses of *N*-glycans have generally introduced asparagine into the glycan after preparing the deprotected glycan.[44-46] The advantage of our method is the facile preparation of various *N*-glycans possessing an asparagine residue because any conversion is not necessary after the deprotection and the protected glycosyl asparagines are easy to handle. *N*-Glycan **1** was synthesized by the successive coupling of two branched tetrasaccharide donors **3** with the tetrasaccharide-Asn fragment **2**, followed by the global deprotection. Hydroxy groups at the 3- and 6-positions of the branched mannose of **2** were orthogonally protected: 3-OH was protected by 4-azido-3-chlorobenzyl (ClAzb) developed by our group,[47,48] whereas 6-OH was protected by a 4,6-benzylidene acetal. The key intermediates **2** and **3** were synthesized by coupling **4** with **5** and **6** with **7**, respectively.

The other important key to our synthesis involved the application of an amide protection strategy in which 5-NHAc of the sialic acid residue was protected with another acetyl group. We recently reported that protection of the amide group of 5-NHAc sialic acid significantly improved the reactivity of glycosylation at a position away from the sialic acid residues.[49] This strategy enabled efficient glycosylation using sialic acid containing fragments, which sometimes have a low reactivity.

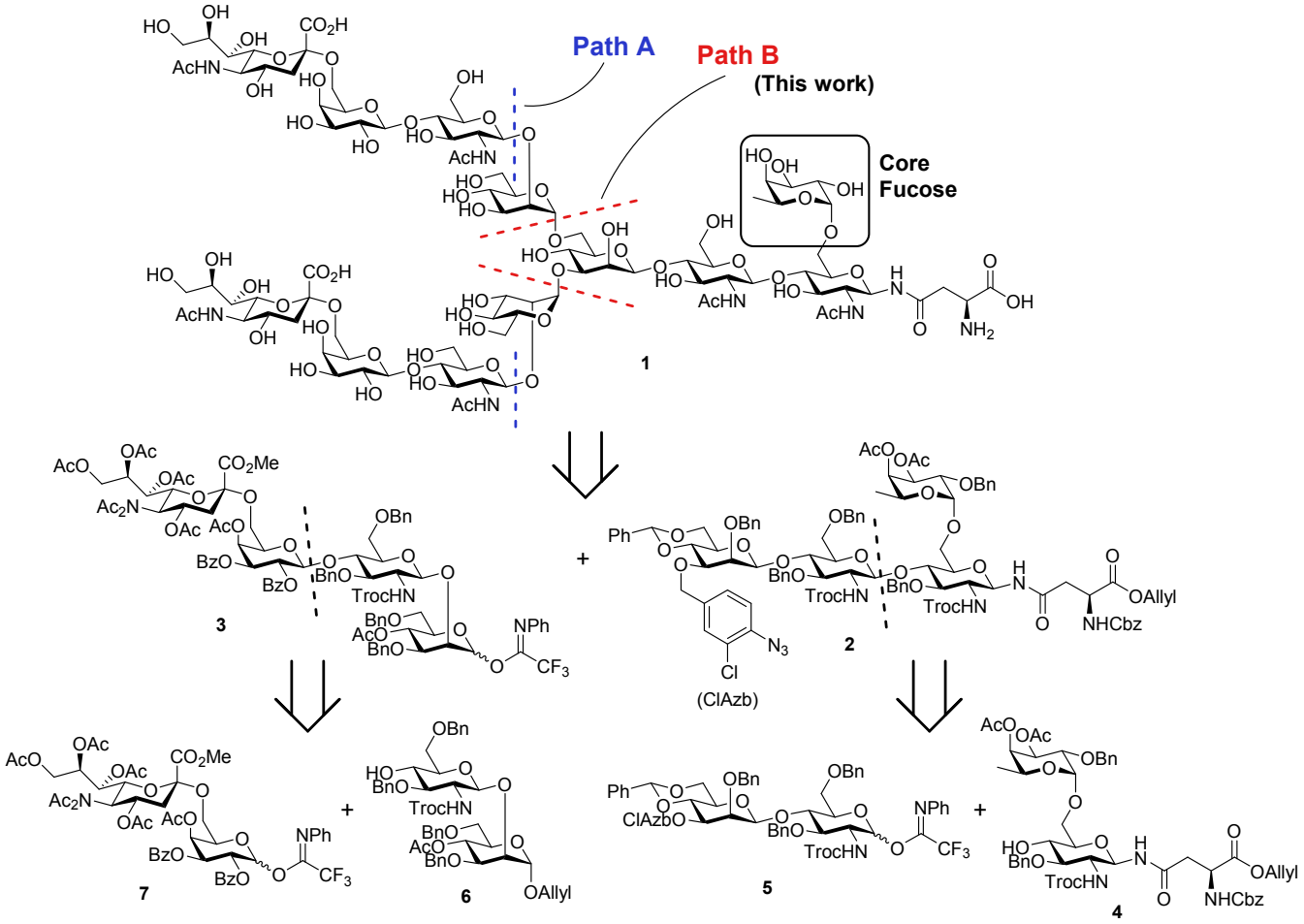


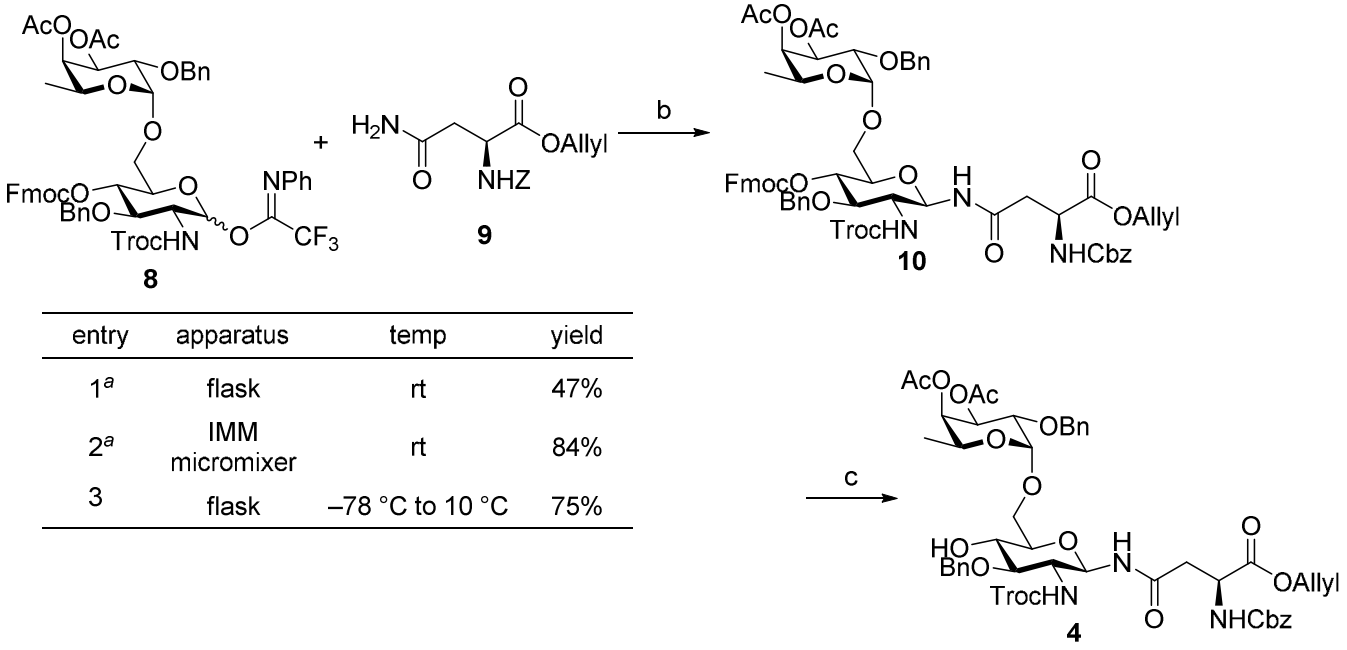
FIGURE 1. Structure and synthetic strategy for achieving the *N*-glycan 1. Troc = 2,2,2-trichloroethoxycarbonyl.

Results and Discussion

The tetrasaccharide-Asn fragment **2** was prepared as shown in Scheme 1. We first prepared the asparagine-linked disaccharide **10** through chemical *N*-glycosylation. *N*-Glycosylation of asparagine was first developed by Kahne[50] and then improved by Tanaka and Takahashi.[51] Tanaka and Takahashi found that nitromethane was a suitable solvent for *N*-glycosylation, whereas the reaction in CH_2Cl_2 afforded lower yields. Nitromethane, however, is highly flammable. We therefore investigated the

N-glycosylation in CH_2Cl_2 and established efficient *N*-glycosylation conditions under integrated microfluidic/batch conditions to obtain the asparagine-linked mono- and disaccharide fragments in high yields.[52] *N*-Phenyltrifluoroacetimidate glycosyl donors were mixed with an asparagine acceptor using a micromixer at room temperature. The mixture was then transferred to a flask. The reaction mixture was stirred until the reaction had reached completion to give the desired **10** in 84% yield (Scheme 1, entry 2). The yield was low under batch conditions, as reported by Tanaka and Takahashi (Scheme 1, entry 1)[52]; however, the key to obtaining *N*-glycosylation was the removal of the reaction heat, predominantly the neutralization heat, during the addition of the Lewis acid. The disaccharide-Asn was obtained in 75% yield under batch conditions through careful addition of diluted TMSOTf in CH_2Cl_2 to the reaction mixture containing the disaccharide donor **8** with the protected asparagine **9** at low temperatures (Scheme 1, entry 3). The yield was much better than that obtained using the batch procedure reported in our previous study, but was still lower than the yield obtained from the integrated microfluidic/batch procedure. The Fmoc group of disaccharide-Asn **11** was then removed using 15% Et_3N to give the disaccharide acceptor **4**.

SCHEME 1. Synthesis of the disaccharide acceptor 4 via *N*-glycosylation.



entry	apparatus	temp	yield
1[a]	flask	rt	47%
2[a]	IMM micromixer	rt	84%
3	flask	–78 °C to 10 °C	75%

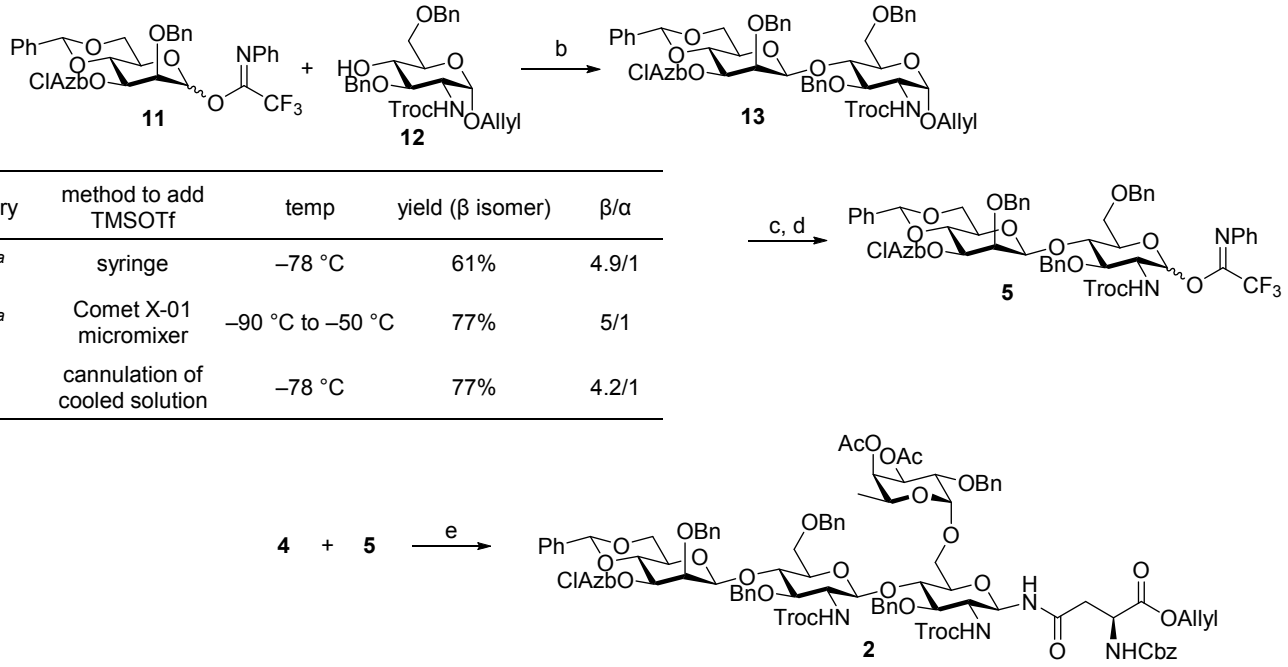
[a]Ref. 52. Reagents and conditions: (b) TMSOTf, MS4A, CH_2Cl_2; (c) 15% Et_3N/CH_2Cl_2, rt, 2 h, 90%.

Stereoselective β-mannosylation using the donor **11** and the acceptor **12** posed another challenge because neighboring group participation is unavailable, and the α-isomer is thermodynamically favored. β-Mannosylation has been achieved using a variety of methods, including intramolecular aglycon delivery,[53] S_N2-like substitution of an α-glycosyl triflate intermediate,[54,55] and the use of a bulky Lewis acid,[56] among others.[57-60] We employed the glycosyl *N*-phenyltrifluoroacetimidate **11** as a donor and TMSOTf as an activator. The glycosylation was expected to proceed via the α-glycosyl triflate. The reaction must be carried out under low temperatures in order to obtain a high β-selectivity; however, this step was not easy in the context of a large-scale synthesis. We developed a method for obtaining integrated microfluidic/batch conditions that provided the β-product **13** in 77% yield on the gram scale (Scheme 2, entry 2).[61] We also found that the key to the β-mannosylation was the removal of the reaction heat during the addition of TMSOTf. The yield under batch conditions improved, as observed with the *N*-glycosylation through the careful addition of diluted TMSOTf to the reaction mixture. The

desired stereoisomer was thus obtained in 77% yield (Scheme 2, entry 3). The allyl group of **12** was then isomerized using an Ir complex,[62] and the resulting compound was oxidatively cleaved using I_2 and H_2O[63] to give the 1-OH compound, which was converted to the *N*-phenyltrifluoroacetimidate **5**.[64]

The glycosyl donor **5** and acceptor **4** were then coupled using TMSOTf as a catalyst to give the tetrasaccharide-Asn fragment **2** in good yield.

SCHEME 2. Synthesis of the tetrasaccharide-Asn fragment 2.



entry	method to add TMSOTf	temp	yield (β isomer)	β/α
1[a]	syringe	–78 °C	61%	4.9/1
2[a]	Comet X-01 micromixer	–90 °C to –50 °C	77%	5/1
3	cannulation of cooled solution	–78 °C	77%	4.2/1

[a]Ref. 61. Reagents and conditions: (b) TMSOTf, MS4A, CH_2Cl_2; (c) $[Ir(cod)(PPh_2Me)_2]PF_6$, H_2, THF, rt, 30 min; then I_2, H_2O, rt, 15 min, 97%; (d) *N*-phenyltrifluoroacetimidoyl chloride, K_2CO_3, acetone, rt, 5 h, 88%; (e) TMSOTf, MS4A, CH_2Cl_2, −20 °C, 20 min, 98%. cod = 1,5-cyclooctadiene.

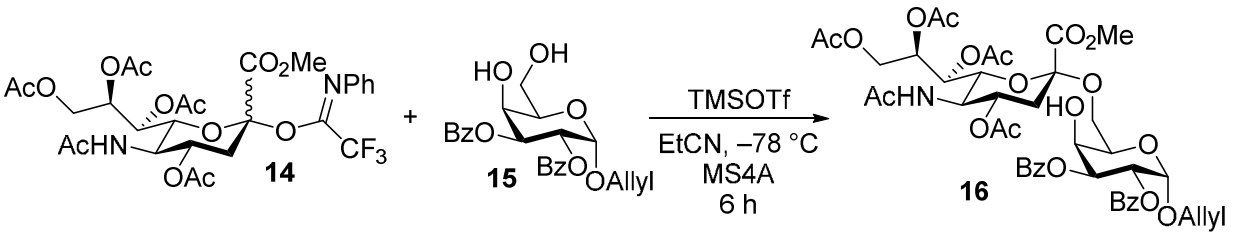
α-Selective sialylation is a critical issue for the synthesis of sialylated glycans[65] because no neighboring participation is available, the formation of unnatural β-sialoside is thermodynamically more favorable, and glycals are readily formed as by-products. Recent advances in α-sialylation have featured

substituent effects at the 5-position, since Boons et al. found that the 5-NAc_2 donor showed a high reactivity.[66,67] Glycosylation using NH-TFA,[68,69] NHTroc,[70] and N_3[71] donors provided higher reactivities with improved α-selectivity compared to the NHAc donor. The 4,5-oxazolidinone sialyl donor, in particular, provided almost perfect α-selectivity for various glycosyl acceptors.[72-75] Our research group developed the 5-NPhth donor, which provided excellent yields and selectivities.[76] We deployed the 5-NPhth and 5-N_3 donors in the microflow reactor to obtain the disaccharides in almost quantitative yield and perfect α-selectivity.[77,78] The α-orienting solvent effect of nitrile has been used for sialylation, except in the context of an oxazolidinone sialyl donor. The lower reaction temperature needed for sialylation affords better α-selectivity in general, because α-sialylation is a kinetically controlled reaction. The key to successful microflow sialylation was, therefore, the rapid removal of the reaction heat to maintain the reaction temperature at −78 °C.

These substituents were converted into natural forms, such as NHAc, after sialylation. By contrast, the 5-NHAc donors were readily derived from commercially available sialic acids and did not require *N*-derivatization after sialylation. The critical point is that the reaction should be carried out around −80 °C to obtain a high α-selectivity. Although NHAc sialyl donors have a lower reactivity than the corresponding 5-*N*-modified sialyl donors, Yu's *N*-phenyltrifluoroacetimidate donor **14** had a reactivity high enough to allow the reaction to proceed around −80 °C.[79] We thus investigated the practical α-sialylation using the 5-NHAc donor **14** with the galactose acceptor **15** to obtain the disaccharide **16** (Table 1). Previously, we reported that a reaction of **14** and **15** gave **16** with moderate selectivity (α/β = 77/23) (entry 1);[76] however, we then realized that the reaction temperature might not have been well-controlled, because TMSOTf was added in a single step using a micropipette. The careful addition of TMSOTf using a microsyringe required substoichiometric amounts of TMSOTf, and the selectivity was quite high (entry 2).[80] The large-scale α-sialylation was achieved using the integrated

microfluidic/batch procedure to afford the disaccharide **16** in 89% yield with a high selectivity (α/β = 94/6) in the gram scale (entry 3).[80] Because the key to the integrated microfluidic/batch procedure was the removal of the reaction heat during the addition of TMSOTf, similar results were obtained under batch conditions by delivering a pre-cooled TMSOTf solution via cannula to the substrate solution at −78 °C (entry 4). These results indicated that efficient removal of the reaction heat was important for the α-selective sialylation. Thus, the α-sialyl disaccharide **16** was obtained efficiently on the multi-gram scale, even in a flask, although the microflow system offered better reproducibility and scalability.

TABLE 1. Investigation of the α-sialylation.



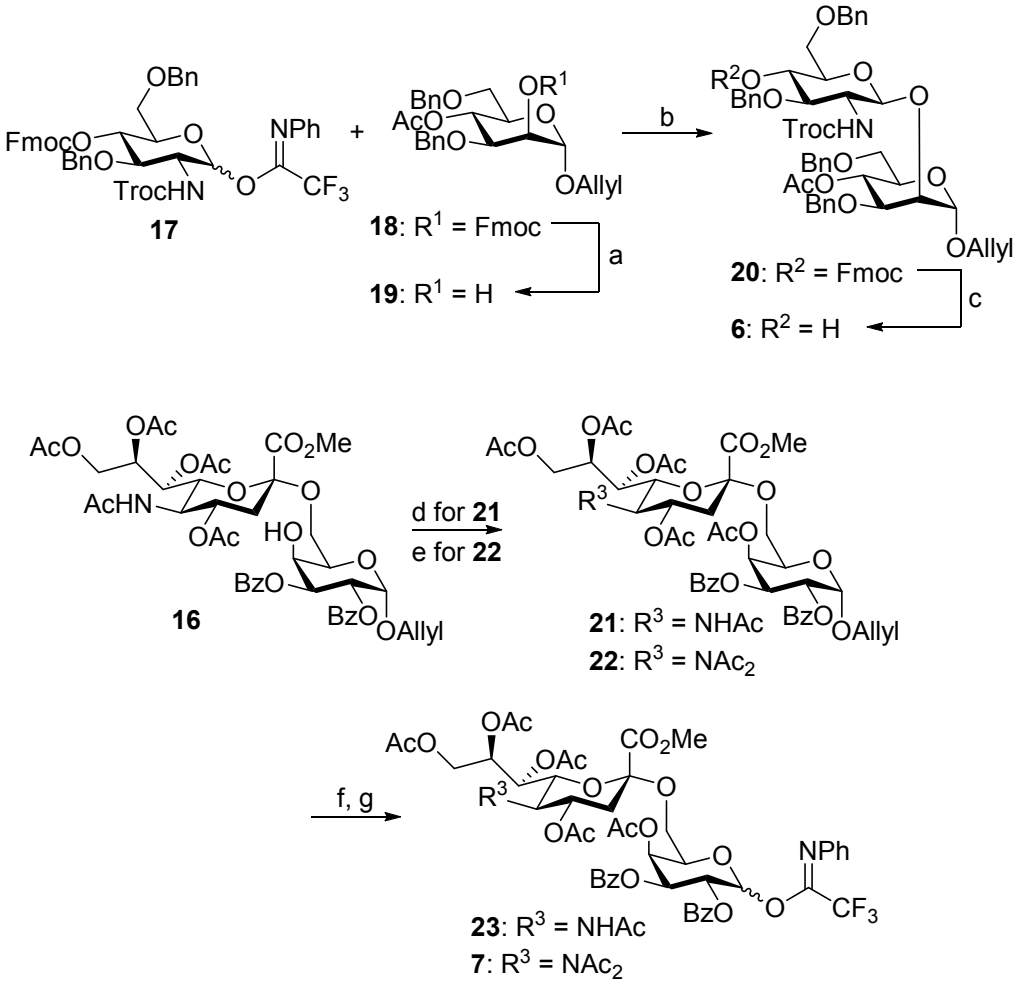
entry	scale	TMSOTf (eq)	apparatus	method to add TMSOTf	yield	α/β
1[a]	50 mg	0.2	flask	micropipette	93%	77/23
2[b]	30 mg	1.0	flask	microsyringe	86%	93/7
3[b]	1 g	1.0	Comet X-01	microfluidic	89%	94/6
4	4.5 g	0.9	flask	cannulation of cooled TMSOTf solution	85%	95/5

[a]Ref. 76. [b]Ref. 80.

Tetrasaccharides containing a sialic acid residue were then synthesized by coupling the disaccharide acceptor **6** and the disaccharide donors **23** and **7** via a (2+2) pathway. The acceptor **6** and donors **23** and **7** were prepared as shown in Scheme 3. The Fmoc group of the protected mannose **18**[43] was removed,

and the resulting alcohol **19** was glycosylated with the GlcNAc donor **17**[43] to obtain the disaccharide **20**. The Fmoc group in **20** was cleaved to give the disaccharide acceptor **6**. Two types of donor, **23** and **7**, were prepared from the α-sialyl disaccharide **16** by acetylation under two sets of conditions. Acetic anhydride in pyridine gave the 4-*O*-acetate **21** whereas isopropenyl acetate with *p*-TsOH[66] gave the 4-*O*-acetyl diacetylimide **22**. Both **21** and **22** were converted into the glycosyl imidates **23** and **7** via two-step reactions.

SCHEME 3. Preparation of the disaccharide acceptor 6 and the disaccharide donors 23/7.

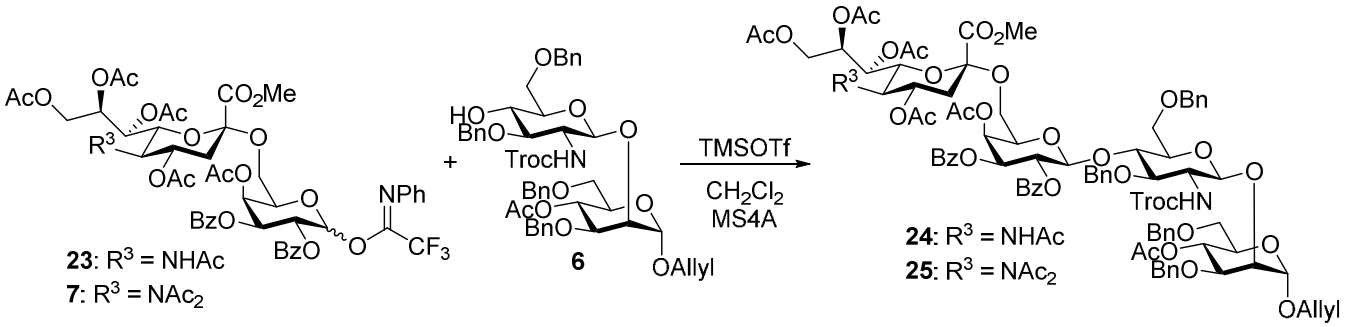


Reagents and conditions: (a) 15% Et_3N/CH_2Cl_2, rt, 3 h, quant; (b) TMSOTf, MS4A, CH_2Cl_2, −78 °C to rt, overnight, 96%; (c) 15% Et_3N/CH_2Cl_2, rt, 5.5 h, quant; (d) Ac_2O, pyridine, 0 °C to rt, overnight; (e) isopropenyl acetate, *p*-TsOH,

95 °C, quant; (f) $[Ir(cod)(PPh_2Me)_2]PF_6$, H_2, THF, rt, then I_2, H_2O, rt; (g) *N*-phenyltrifluoroacetimidoyl chloride, K_2CO_3, acetone, rt, 95% for **23** from **16**, 75% for **7** from **22**.

The syntheses of the tetrasaccharides via (2+2) glycosylation were investigated using the donors **23** and **7** (Table 2). The donor **7**, with a NAc_2 group, showed good reactivity, and the desired tetrasaccharide **25** was obtained in 96% yield. On the other hand, the donor **23**, with a NHAc group, showed a much lower reactivity. The reaction required a greater quantity of TMSOTf and a higher temperature to give tetrasaccharide **24** in 52% yield.

TABLE 2. Comparison of the two donors 23 and 7 in the context of the (2+2) glycosylation.

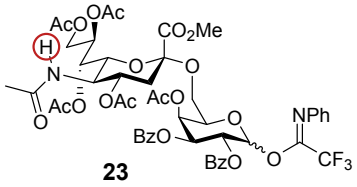


entry	donor	R^3	TMSOTf (eq)	temp	time	yield
1	**23**	NHAc	0.2 + 0.2	0 °C to rt	1.5 h + 1 h	52%
2	**7**	NAc_2	0.2	0 °C	20 min	96%

The difference between the reactivities of the donors **23** and **7** was attributed to an intermolecular hydrogen bond formed by the NHAc group. As mentioned above, the 5-NHAc sialyl donor displayed a lower reactivity than the 5-N-modified sialyl donors. The formation of a donor cluster via intermolecular hydrogen bonds involving 5-NHAc appeared to contribute to the low reactivity of the 5-NHAc donor. In

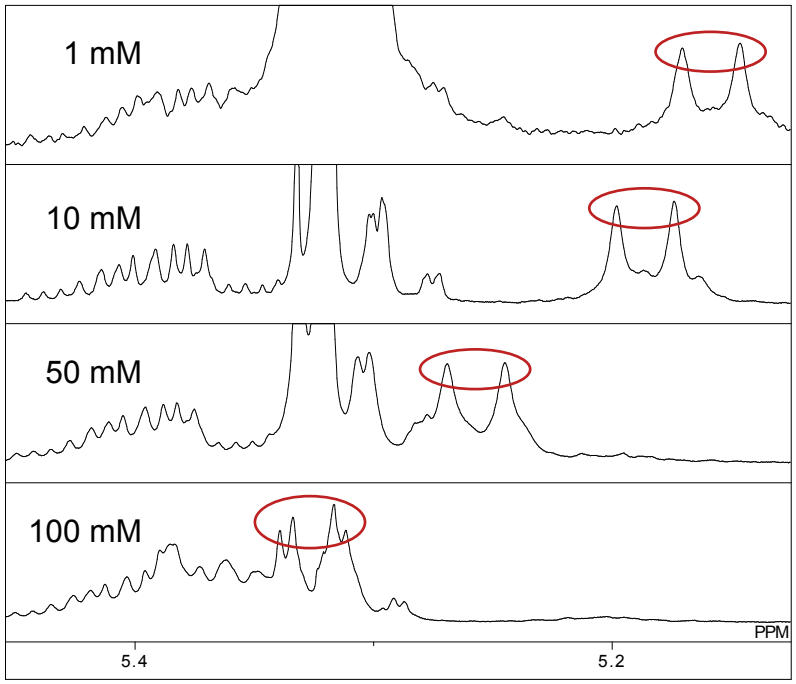
fact, Kononov and co-workers[81,82] reported that a 5-NHAc sialic acid monosaccharide forms a dynamic cluster-like structure in solution that affects the reactivity and stereoselectivity of sialylation. We hypothesized that this effect may arise from intermolecular hydrogen bonds formed by the NHAc group in the disaccharide. This hypothesis was tested by collecting the ^{1}H NMR spectra of the 5-NHAc donor **23** at various concentrations (Figure 2a). The chemical shifts of the 5-NHAc proton moved downfield at higher substrate concentrations, indicating the formation of intermolecular hydrogen bonds. The intermolecular hydrogen-bonding network may form a cluster-like structure among the molecules containing 5-NHAc sialic acid (Figure 2b) and reduce the reactivity of the donor **23** by inhibiting molecular motion and intermolecular reactions.

(a)



23

^{1}H NMR in CD_2Cl_2



(b)

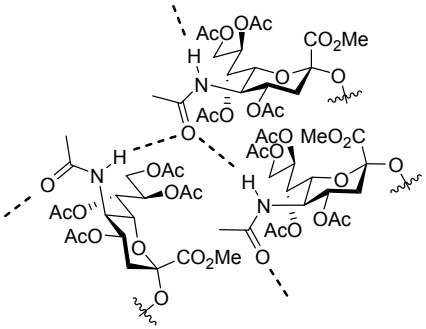
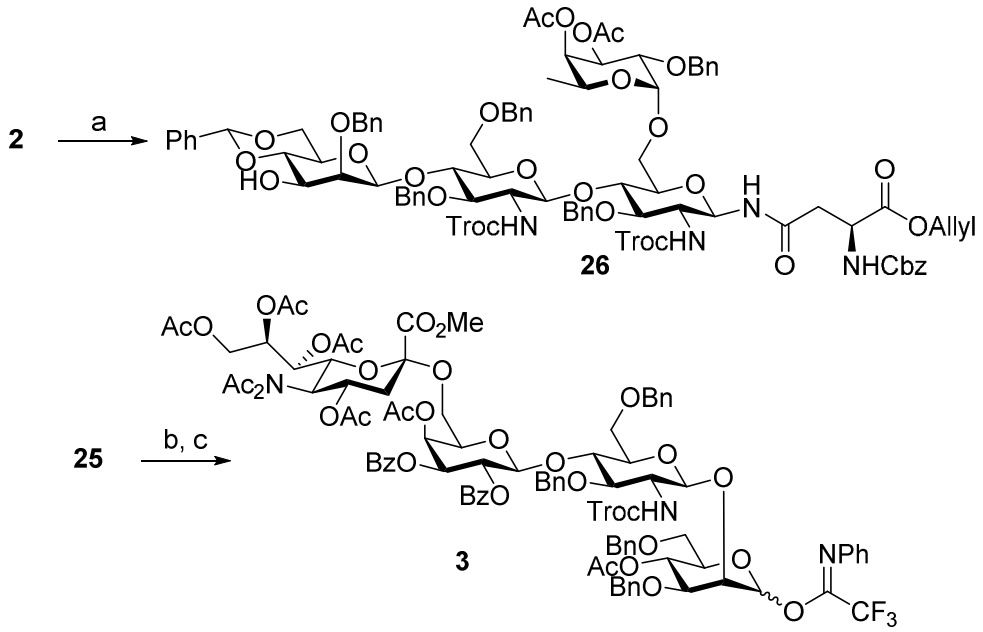


FIGURE 2. (a) ^{1}H NMR of the disaccharide donor 23 at various concentrations. (b) Plausible intermolecular hydrogen bonds formed by the NHAc groups in sialic acids.

Our research group recently observed similar hydrogen-bonding effects in the synthesis of a disialylated tetrasaccharide motif.[49] These results indicated that intermolecular hydrogen bonding in sialic acid residues greatly affects the reactivity of glycosylation. Glycosylation between larger oligosaccharide fragments should be more sensitive to intermolecular hydrogen bonds, considering the lower mobility of the larger molecules. Therefore, the use of 5-NAc_2 sialic acid should improve the efficiency of the large oligosaccharide synthesis.

With both the reducing and non-reducing end tetrasaccharide fragments in hand, we next prepared the tetrasaccharide-Asn acceptor **26** and the tetrasaccharide donor **3** for the synthesis of dodecasaccharide-Asn (Scheme 4). The ClAzb group of the tetrasaccharide-Asn fragment **2** was cleaved via formation of iminophosphorane with phosphine, followed by DDQ oxidation, to give the tetrasaccharide-Asn acceptor **26**. On the other hand, the 1-allyl group of the tetrasaccharide fragment **25** was converted into glycosyl imidate to obtain the donor **3**.

SCHEME 4. Synthesis of the tetrasaccharide-Asn acceptor 26 and the tetrasaccharide donor 3.

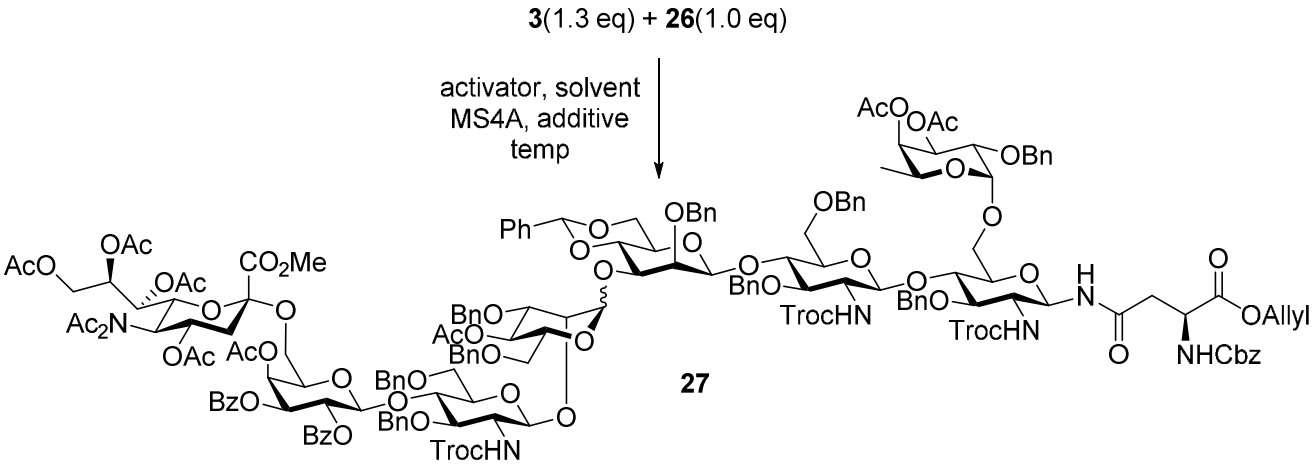


Reagents and conditions: (a) PPh_3, CH_2Cl_2, rt, 1 h, then DDQ, AcOH, H_2O, rt, 20 min, 89%; (b) $[Ir(cod)(PPh_2Me)_2]PF_6$, H_2, THF, rt, 1.5 h, then I_2, H_2O, rt, 10 min, 98%; (c) *N*-phenyltrifluoroacetimidoyl chloride, K_2CO_3, acetone, rt, 1 h, 99%.

The synthesis of the octasaccharide-Asn **27** was carried out via (4+4) coupling using the tetrasaccharide donor **3** and the tetrasaccharide-Asn acceptor **26** (Table 3). In previous studies of *N*-glycan synthesis, CH_2Cl_2 or toluene was used as a solvent for the coupling of large fragments, and a variety of glycosyl donors and activation methods have been investigated. We first carried out the reaction at 0 °C in CH_2Cl_2 to give the desired product in 56% yield with α/β = 3/1 (entry 1). Glycosylation in MeCN at 0 °C resulted in a moderate yield and low selectivity (entry 2). In general, coupling between large fragments is difficult in comparison to coupling of small fragments, because both the mobility of the molecules and the accessibility of the glycosyl acceptor to oxocarbenium ion intermediate are expected to be low. We postulated that the coordination of ether to the intermediate oxocarbenium ion should stabilize the cationic intermediate and prolong its lifetime to enable the attack of the large acceptor to the activated large donor before degradation of the activated donor. In fact,

glycosylation in cyclopentylmethylether (CPME) provided an exceedingly high (91%) yield (entry 3). The more strongly coordinating THF reduced the stereoselectivity (entry 4). The temperature had little effect on the selectivity (entry 5). The selectivity was decreased when $TMSClO_4$ was used as a promoter (entry 6). TMSI was not strong enough to activate the donor **3** (entry 7). DMF was added to the reaction mixture to generate a DMF adduct *in situ*;[83,84] however, the donor degraded (entries 8, 9).

TABLE 3. Investigation of the (4+4) glycosylation.



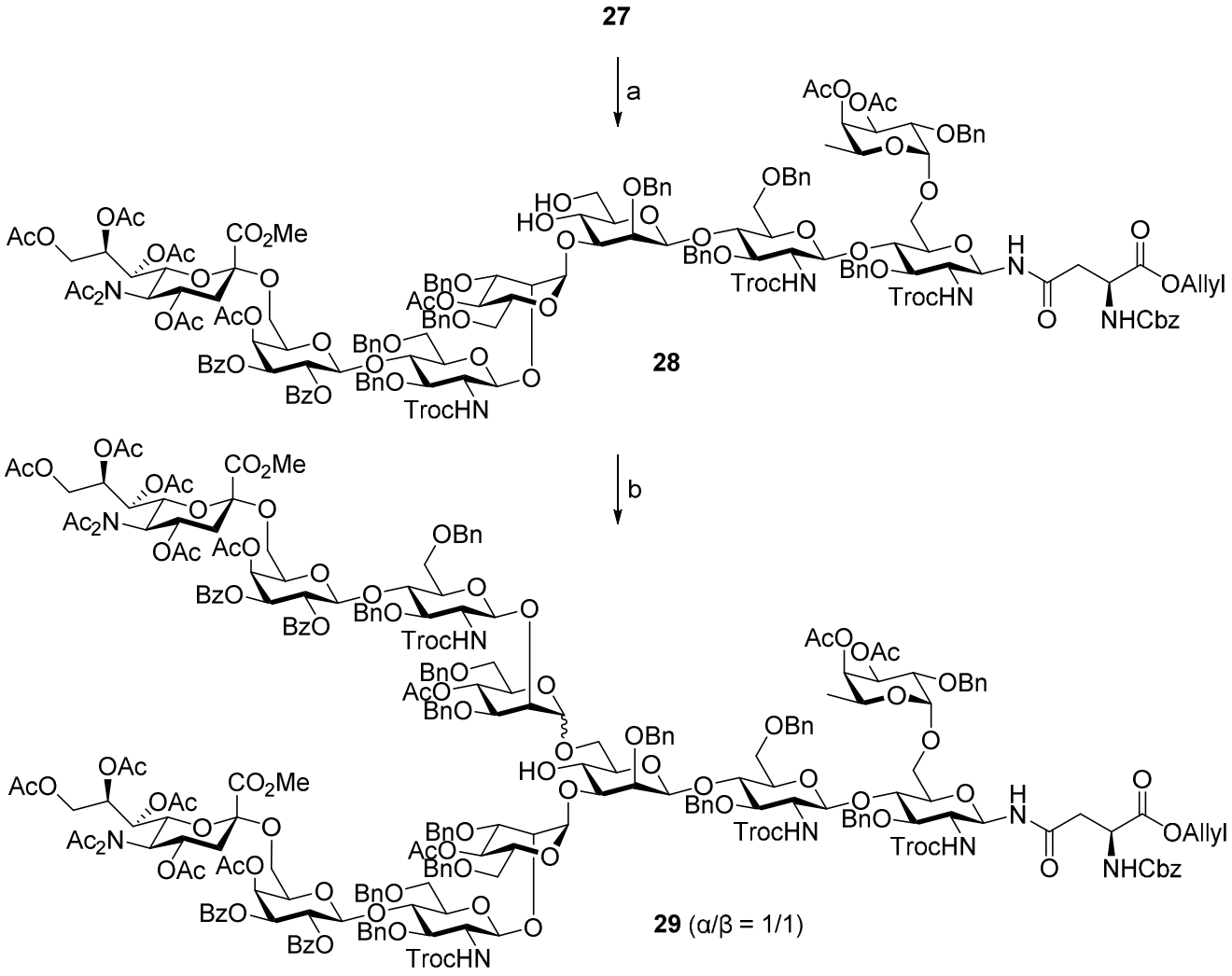
entry	activator	solvent	temp	additive (eq)	yield	α/β
1	TMSOTf (0.5)	CH_2Cl_2	0 °C	—	56%	3/1
2	TMSOTf (1.0)	MeCN	0 °C	—	59%	5/3
3	TMSOTf (0.5)	CPME	0 °C	—	91%	3/1
4	TMSOTf (0.5)	CPME/THF = 1/1	0 °C	—	68%	5/3
5	TMSOTf (0.5)	CPME	rt	—	63%	3/1
6	$TMSClO_4$[a] (0.5)	CPME	0 °C	—	81%	5/3
7	TMSI (0.5 + 2.0)	CPME	0 °C to rt	—	0%[b]	—

8	TMSOTf (0.5)	CPME	0 °C to rt	DMF (5.0)	0%[c]	—
9	TMSOTf (0.5)	CH_2Cl_2	0 °C to rt	DMF (5.0)	0%[c]	—

[a]generated *in situ* by TMSCl/$AgClO_4$. [b]no reaction. [c]hydrolysis and β-elimination of **3** occurred.

The octasaccharide-Asn **27** was treated with TFA to cleave benzylidene acetal. After purification by column chromatography, the α-isomer **28** was isolated in 58% yield. Glycosylation of the donor **3** with **28** was carried out in CPME to obtain the dodecasaccharide-Asn **29** in a quite good 87% yield with α/β = 1/1 (Scheme 5). The stereoisomers could be separated at a later stage in the synthesis. The use of the 5-NAc_2–sialylated donor suppressed cluster formation by the donor via intermolecular hydrogen bonds. Coupling of the large oligosaccharide fragments was thereby achieved with a high efficiency. The low selectivity was attributable to the incompatibility between the donor and the acceptor. This issue is expected to be addressed in the next generation synthesis.

SCHEME 5. (4+8) glycosylation to provide the dodecasaccharide-Asn 29.

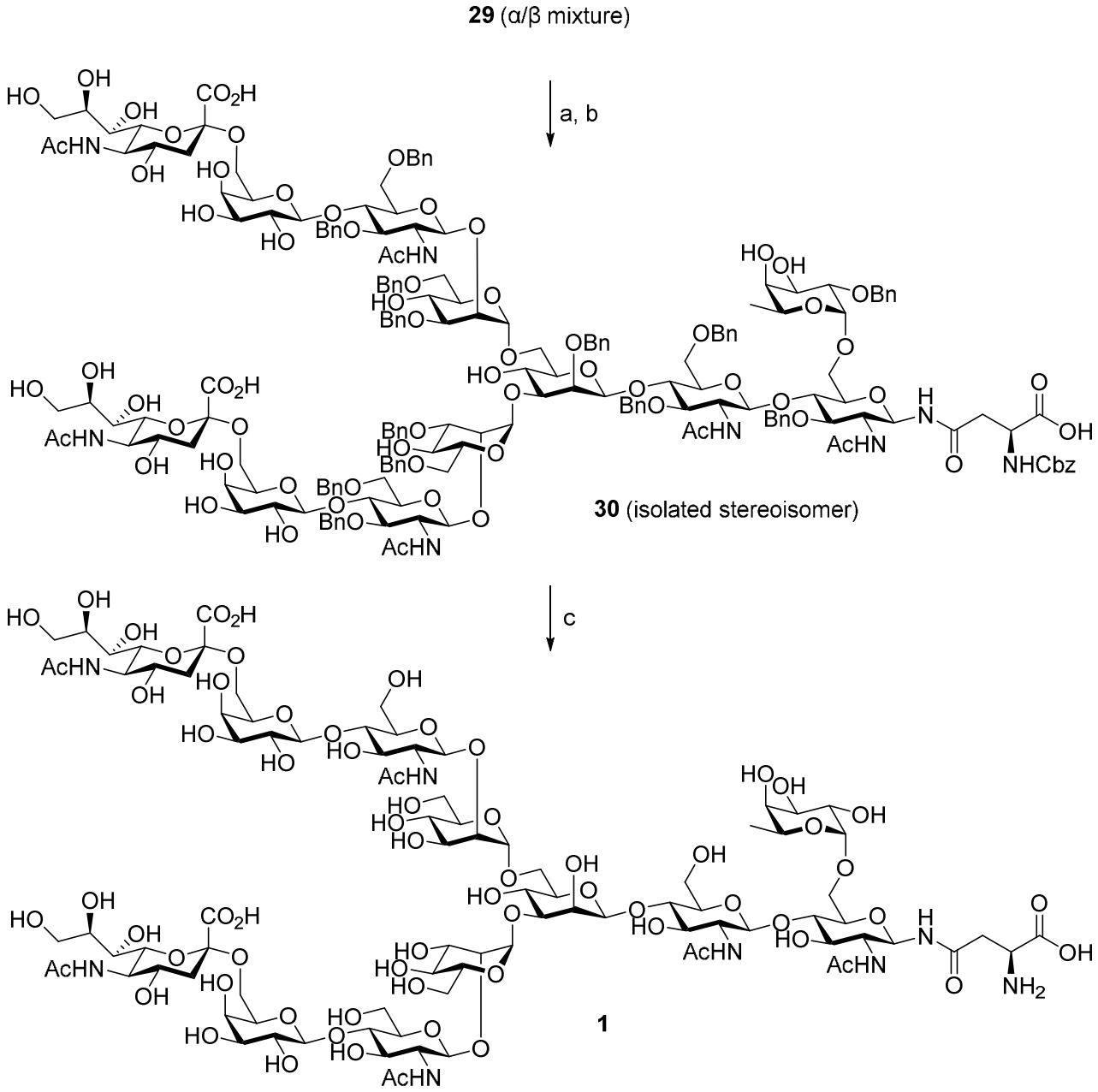


Reagents and conditions: (a) TFA, H_2O, CH_2Cl_2, 0 °C to rt, 1.5 h, 58% for α isomer (14% for β isomer); (b) **3**, TMSOTf, CPME, MS4A, 0 °C, 10 min, 87% as α/β mixture.

With the protected *N*-glycan **29** in hand, global deprotection of **29** was carried out (Scheme 6). Cleavage of the *N*-Troc group with aqueous LiOH was identified by our research group.[85] This method appeared to be applicable to the dodecasaccharide-Asn **29**; however, our investigation suggested that aspartimide formed under basic conditions. This side reaction was expected to occur via nucleophilic attack by the nitrogen in the side chain on the allyl ester. This ester was selectively cleaved using a Pd catalyst.[86] The resulting carboxylic acid was treated with aqueous LiOH, and subsequent *N*-acetylation

gave compound **30** without aspartimide formation. Compound **30** was purified by reverse-phase HPLC, and the two stereoisomers generated in the (4+8) glycosylation were separated in this step. Finally, the desired isomer **30** was hydrogenated to obtain the target *N*-glycan **1**.

SCHEME 6. Deprotection of dodecasaccharide-Asn.



Reagents and conditions: (a) $Pd(OAc)_2$, PPh_3, sodium 2-ethylhexanoate, acetone, rt, 2 h; (b) 1) 3 M LiOH aq, THF, dioxane, rt, overnight; 2) Ac_2O, $NaHCO_3$, H_2O, rt, 1 h × 2, then $LiOH•H_2O$, rt, 2 h, 27% for **30** (27% for β isomer) from **29**; (c) 20% $Pd(OH)_2/C$, H_2, *t*-BuOH, H_2O, AcOH, rt, overnight, 60%.

In summary, the chemical synthesis of a core fucose containing *N*-glycan **1** was achieved. We developed a universal route to various asparagine-linked *N*-glycans based on several new synthetic strategies. An asparagine residue was introduced by *N*-glycosylation during the early step of the synthesis, whereas asparagine was introduced during the final step of the synthesis by coupling glycosyl amine with the aspartic acid residue in previous studies. Protection of 5-acetamide in sialic acid using an additional acetyl group dramatically increased the reactivity of glycosylation during fragment coupling by avoiding intermolecular hydrogen bonding involving the 5-NHAc group. The fragment coupling strategy successfully reduced the total reaction steps. The stepwise coupling of the branch tetrasaccharides at the 3- and 6-positions of mannose in the stem tetrasaccharide asparagine was the key to this synthetic strategy. High yields of the fragment coupling reaction were obtained by stabilizing the intermediate oxocarbenium ion through coordination of the ether solvent. The low selectivity of the glycosylation during fragment coupling represents an issue that will be addressed in future work. The present study enables the efficient synthesis of various *N*-glycans and precise biological studies using synthetic *N*-glycans.

Experimental Section

General procedures. ^1H and ^{13}C NMR spectra were recorded in an indicated solvent with a 400 MHz spectrometer, a 500 MHz spectrometer, or a 600 MHz spectrometer equipped with a cryoprobe. For ^1H NMR analysis, the chemical shifts in $CDCl_3$ are given δ values from tetramethylsilane (TMS) as an internal standard. Acetone (δ = 2.22 ppm) is used as an internal standard for the measurement in D_2O.

CHD_2OD (δ = 3.30 ppm), CHD_2COCD_3 (δ = 2.05 ppm), and $CHDCl_2$ (δ = 5.32 ppm) are used as references for the measurements in CD_3OD, acetone-D_6, and CD_2Cl_2, respectively. High-resolution mass spectra were obtained on an ESI-Orbitrap (FTMS) or an ESI-TOF mass spectrometer. Unless otherwise noted, reactions in anhydrous solvent were carried out under argon atmosphere. Distilled CH_2Cl_2 was distilled from calcium hydride. MS4A were activated with a microwave oven and dried *in vacuo* three times before use. All other commercially available reagents and solvents were used as purchased.

*N^{α}-Benzyloxycarbonyl-N^{γ}-(6-O-(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-3-O-benzyl-2-deoxy-4-O-(9-fluorenylmethoxycarbonyl)-2-(2,2,2-trichloroethyloxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (**10**).* Disaccharide donor 6-*O*-(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-3-*O*-benzyl-2-deoxy-4-*O*-(9-fluorenylmethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl *N*-phenyltrifluoroacetimidate (**8**)[52] (1.00 g, 0.863 mmol) and asparagine acceptor N^{α}-benzyloxycarbonyl-L-asparagine allyl ester (**9**)[52] (529 mg, 1.73 mmol) were lyophilized from benzene and activated MS4A powder was added. To the mixture was added dist. CH_2Cl_2 (17.3 mL). To the solution was added TMSOTf (31.3 μL, 0.173 mmol) at − 78 °C and the solution was stirred for 20 min at the same temperature. The solution was warmed up to 10 °C and stirred for 21 h. The reaction was quenched by sat. aqueous $NaHCO_3$ and insoluble materials were filtered. The filtrate was poured into sat. aqueous $NaHCO_3$ and the aqueous layer was extracted with $CHCl_3$. The organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 4/1 to 2/1) was carried out to obtain product **10** (831 mg, 75%) as a white solid. For analytical data, see ref. 52.

*N^{α}-(Benzyloxycarbonyl)-N^{γ}-(6-O-(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-3-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (**4**).* To a solution of Disaccharide-Asn **10** (1.00 g, 0.784 mmol) in CH_2Cl_2 (26.7 mL) was added Et_3N (4.7 mL).

After stirring for 2 h at rt, the reaction mixture was diluted with toluene and concentrated *in vacuo*. The residue was co-evaporated four times with toluene to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 2/1 to 1/1) was carried out to obtain **4** (745 mg, 90%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.40-7.28 (m, 15H), 6.92 (d, 1H, *J* = 7.9 Hz), 5.91 (d, 1H, *J* = 8.7 Hz), 5.87-5.79 (m, 1H), 5.30 (dd, 1H, *J* = 10.4, 3.4 Hz), 5.28-5.24 (m, 2H), 5.18 (dd, 1H, *J* = 10.4, 1.1 Hz), 5.13-5.07 (m, 2H), 4.84 (d, 1H, *J* = 3.6 Hz), 4.81-4.77 (m, 2H), 4.75 (dd, 1H, *J* = 11.1, 11.1 Hz), 4.71 (d, 1H, *J* = 4.2 Hz), 4.67 (s, 1H), 4.63 (dd, 1H, *J* = 11.1, 11.1 Hz), 4.59-4.55 (m, 5H), 4.15 (q, 1H, *J* = 6.5 Hz), 3.90-3.85 (m, 2H), 3.83 (d, 2H, *J* = 4.7 Hz), 3.53-3.44 (m, 3H), 3.25 (dd, 1H, *J* = 10.2, 8.9 Hz), 2.85 (dd, 1H, *J* = 16.6, 3.3 Hz), 2.68 (dd, 1H, *J* = 16.6, 3.9 Hz), 2.13 (s, 3H), 2.00 (s, 3H), 1.08 (d, 3H, *J* = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$); δ = 170.6, 170.4, 170.0, 156.1, 137.9, 137.6, 136.3, 131.6, 128.8, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 118.5, 98.2, 95.3, 80.1, 79.0, 74.8, 74.8, 73.9, 73.8, 73.8, 72.9, 71.5, 70.5, 68.5, 67.0, 66.2, 64.9, 55.1, 50.5, 37.7, 20.8, 20.7, 15.8. HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{48}H_{56}Cl_3N_3O_{17}$ $[M+Na]^+$ 1074.2573, found 1074.2589.

*Allyl 4-O-(3-O-(4-azido-3-chlorobenzyl)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranoside (**13**).* Mannose donor 3-*O*-(4-azido-3-chlorobenzyl)-2-*O*-benzyl-4,6-*O*-benzylidene-D-mannopyranosyl *N*-phenyltrifluoroacetimidate (**11**)[61,78] (2.00 g, 2.88 mmol) and GlcN acceptor allyl 3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranoside (**12**)[56] (1.66 g, 2.88 mmol) were co-evaporated with toluene three times and activated MS4A powder was added. To the mixture was added dist. CH_2Cl_2 (40 mL), and the mixture was cooled to −80 °C. A solution of TMSOTf (156 μL, 0.864 mmol) in dist. CH_2Cl_2 (18 mL) was dried over activated MS4A pellets and cooled to −

78 °C. To the solution of **11** and **12** was added the solution of TMSOTf via cannula and the mixture was stirred for 2.5 d at −80 °C. The reaction was quenched by Et_3N (1.0 mL) and insoluble materials were filtered. The filtrate was concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 15/1 to 10/1) was carried out to obtain product **13** (2.41 g, 77%) as a yellow solid. For analytical data, see ref. 61, 78.

4-O-(3-O-(4-azido-3-chlorobenzyl)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose. A suspension of $[Ir(cod)(PPh_2Me)_2]PF_6$ (157 mg, 0.185 mmol) in anhydrous THF (24 mL) was stirred under H_2 atmosphere for 5 min to give a yellow solution. The solution was added to a solution of disaccharide allyl glycoside **13** (4.00 g, 3.70 mmol) in anhydrous THF (50 mL) and the mixture was stirred for 30 min at rt. To the reaction solution was added H_2O (20 mL) and I_2 (1.88 g, 7.40 mmol) and the mixture was stirred for additional 15 min. The reaction was quenched by 20% aqueous $Na_2S_2O_3$ and the aqueous layer was extracted by EtOAc. The organic layer was washed with sat. aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene 100% to toluene/EtOAc = 3/1) was carried out to give the 1-OH product (3.72 g, 97%) as a brown solid of α/β mixture. ^1H NMR (500 MHz, $CDCl_3$) of major isomer: δ = 7.45-7.41 (m, 4H), 7.39-7.34 (m, 4H), 7.34-7.27 (m, 12H), 7.25-7.22 (m, 3H), 7.18 (dd, 1H, *J* = 8.2, 1.9 Hz), 7.05 (d, 1H, *J* = 8.2 Hz), 5.48 (s, 1H), 5.32 (t, 1H, *J* = 3.7 Hz), 5.05 (d, 1H, *J* = 7.2 Hz), 5.03 (d, 1H, *J* = 11.5 Hz), 4.84 (s, 2H), 4.72 (d, 1H, *J* = 11.7 Hz), 4.67-4.60 (m, 3H), 4.48-4.46 (m, 2H), 4.40 (d, 1H, *J* = 12.0 Hz), 4.08 (dd, 1H, *J* = 10.5, 4.8 Hz), 4.05 (dd, 1H, *J* = 9.5, 9.5 Hz), 3.97 (d, 1H, *J* = 3.0 Hz), 3.96 (s, 1H), 3.90 (td, 1H, *J* = 9.7, 3.0 Hz), 3.70 (td, 1H, *J* = 9.2, 3.2 Hz), 3.62-3.56 (m, 2H), 3.50 (dd, 1H, *J* = 10.3, 10.3 Hz), 3.33 (dd, 1H, *J* = 9.8, 2.9 Hz), 3.10 (td, 1H, *J* = 9.6, 4.9 Hz), 2.90 (d, 1H, *J* = 2.7 Hz).HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{50}H_{50}Cl_4N_4O_{12}$ $[M+Na]^+$ 1061.2077, found 1061.2091.

*4-O-(3-O-(4-azido-3-chlorobenzyl)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl N-phenyltrifluoroacetimidate (**5**).* To a solution of 4-*O*-(3-*O*-(4-azido-3-chlorobenzyl)-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (100 mg, 0.0961 mmol) in acetone (1.9 mL) were added *N*-phenyltrifluoroacetimidoyl chloride (39.9 mg, 0.192 mmol) and K_2CO_3 (39.8 mg, 0.288 mmol). After stirring for 5 h at rt, insoluble materials were filtered and the filtrate was concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 30/1 to 10/1) was carried out to obtain **5** (103 mg, 88%) as a yellowish solid of α/β mixture. ^{1}H NMR (500 MHz, $CDCl_3$) of major isomer: δ = 7.45 (dd, 2H, *J* = 7.6, 1.9 Hz), 7.42 (dd, 2H, *J* = 7.6, 1.0 Hz), 7.39-7.35 (m, 4H), 7.32 (q, 8H, *J* = 6.9 Hz), 7.27 (d, 7H, *J* = 8.4 Hz), 7.20 (dd, 1H, *J* = 8.2, 1.8 Hz), 7.10 (tt, 1H, *J* = 7.4, 1.1 Hz), 7.06 (d, 1H, *J* = 8.2 Hz), 6.78 (d, 2H, *J* = 7.7 Hz), 6.32 (br s, 1H), 5.50 (s, 1H), 5.03 (d, 1H, *J* = 11.6 Hz), 4.88 (d, 1H, *J* = 11.9 Hz), 4.82 (d, 1H, *J* = 11.9 Hz), 4.75 (d, 1H, *J* = 12.1 Hz), 4.71 (d, 1H, *J* = 8.1 Hz), 4.67 (d, 1H, *J* = 3.3 Hz), 4.64 (d, 1H, *J* = 2.6 Hz), 4.63-4.60 (m, 1H), 4.51 (d, 1H, *J* = 12.7 Hz), 4.49 (s, 1H), 4.49 (s, 1H), 4.38 (d, 1H, *J* = 12.1 Hz), 4.12 (dd, 1H, *J* = 10.5, 4.8 Hz), 4.07 (dd, 1H, *J* = 9.5, 9.5 Hz), 4.07 (d, 1H, *J* = 8.9 Hz), 4.03 (s, 1H), 3.72 (d, 2H, *J* = 2.8 Hz), 3.65 (dd, 1H, *J* = 9.8, 9.8 Hz), 3.59-3.51 (m, 3H), 3.35 (dd, 1H, *J* = 9.9, 2.9 Hz), 3.13 (td, 1H, *J* = 9.7, 4.8 Hz). HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{58}H_{54}Cl_4F_3N_5O_{12}$ $[M+Na]^+$ 1232.2373, found 1232.2384.

*N^{α}-benzyloxycarbonyl-N^{γ}-(6-O-(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-4-O-(4-O-(3-O-(4-azido-3-chlorobenzyl)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (**2**).* Disaccharide donor **5** (1.26 g, 1.04 mmol) and disaccharide-Asn acceptor **4** (1.00 g, 0.949 mmol) were lyophilized from benzene and

activated MS4A powder was added. To the mixture was added dist. CH_2Cl_2 (19 mL). To the mixture was added TMSOTf (34.4 μL, 0.190 mmol) at −20 °C and the mixture was stirred for 20 min at the same temperature. The reaction was quenched by sat. aqueous $NaHCO_3$ at 0 °C and insoluble materials were filtered. The filtrate was poured into sat. aqueous $NaHCO_3$ and the aqueous layer was extracted by $CHCl_3$. The organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography ($CHCl_3$/acetone = 20/1 to 8/1) was carried out to obtain product **2** (1.94 g, 98%) as a yellowish solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.43-7.38 (m, 6H), 7.37-7.27 (m, 16H), 7.24-7.12 (m, 16H), 7.03 (d, 1H, *J* = 8.2 Hz), 6.91 (d, 1H, *J* = 7.4 Hz), 6.15 (d, 1H, *J* = 8.1 Hz), 5.90 (d, 1H, *J* = 8.8 Hz), 5.85-5.77 (m, 1H), 5.45 (s, 1H), 5.37 (dd, 1H, *J* = 10.5, 3.3 Hz), 5.24 (dddd, 1H, *J* = 17.2, 1.3, 1.3, 1.3 Hz), 5.19 (dd, 1H, *J* = 3.1, 0.8 Hz), 5.16 (dddd, 1H, *J* = 10.5, 1.3, 1.3, 1.3 Hz), 5.12 (d, 1H, *J* = 12.2 Hz), 5.04 (d, 1H, *J* = 6.0 Hz), 5.02 (d, 1H, *J* = 5.2 Hz), 4.98 (d, 1H, *J* = 2.3 Hz), 4.89-4.76 (m, 5H), 4.73-4.64 (m, 4H), 4.61-4.48 (m, 6H), 4.42 (d, 1H, *J* = 12.3 Hz), 4.39 (d, 1H, *J* = 7.0 Hz), 4.38 (d, 1H, *J* = 12.3 Hz), 4.34 (d, 1H, *J* = 11.5 Hz), 4.25 (d, 1H, *J* = 12.1 Hz), 4.23 (dd, 1H, *J* = 13.6, 5.5 Hz), 4.08 (dd, 1H, *J* = 12.1, 3.4 Hz), 4.06 (dd, 1H, *J* = 9.2, 9.2 Hz), 4.00 (dd, 1H, *J* = 9.8, 9.8 Hz), 3.98 (dd, 1H, *J* = 10.2, 5.2 Hz), 3.86-3.80 (m, 3H), 3.69 (d, 1H, *J* = 3.2 Hz), 3.68 (dd, 1H, *J* = 8.6, 2.5 Hz), 3.63 (d, 1H, *J* = 9.5 Hz), 3.54 (dd, 2H, *J* = 10.9, 2.9 Hz), 3.50 (dd, 2H, *J* = 9.8, 2.5 Hz), 3.43-3.39 (m, 3H), 3.32 (dd, 1H, *J* = 9.5, 9.5 Hz), 3.25 (dd, 1H, *J* = 9.8, 3.1 Hz), 3.04 (td, 1H, *J* = 9.6, 4.9 Hz), 2.83 (dd, 1H, *J* = 16.7, 3.4 Hz), 2.62 (dd, 1H, *J* = 16.7, 4.0 Hz), 2.05 (s, 3H), 1.78 (s, 3H), 1.00 (d, 3H, *J* = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5, 170.5, 170.3, 169.9, 156.2, 156.1, 154.3, 139.1, 138.3, 138.1, 137.5, 137.1, 136.7, 136.2, 136.2, 131.6, 129.4, 129.0, 128.9, 128.8, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.2, 127.1, 126.7, 126.0, 124.9, 119.4, 118.5, 103.6, 102.0, 101.4, 97.2, 95.6, 95.2, 81.4, 81.0, 80.4, 78.5, 78.4, 78.0, 77.7, 77.3, 76.2, 75.9, 74.9, 74.8, 74.7, 74.7, 74.1, 73.7, 73.6, 73.4, 73.4, 71.7, 71.0, 69.6, 68.9, 68.4, 67.3, 67.0,

66.2, 64.9, 57.5, 55.9, 50.4, 37.6, 20.8, 20.6, 15.5. HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{98}H_{104}Cl_7N_7O_{28}$ $[M+Na]^+$ 2094.4647, found 2094.4646.

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 ***(16)****.* Sialic acid donor methyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranosylonate *N*-phenyltrifluoroacetimidate (**14**)[76] (4.50 g, 6.80 mmol) and galactose acceptor allyl 2,3-di-*O*-benzoyl-α-D-galactopyranoside (**15**)[76] (4.39 g, 10.2 mmol) were lyophilized from benzene and activated MS4A powder was added. To the mixture was added dist. EtCN (120 mL), and the mixture was cooled to −78 °C. A solution of TMSOTf (1.11 mL, 6.12 mmol) in dist. EtCN (16 mL) was dried over activated MS4A pellets and cooled to −78 °C. The solution of TMSOTf was added to the solution of **14** and **15** via cannula and the mixture was stirred for 6 h at −78 °C. The reaction was quenched by Et_3N (5.0 mL) and insoluble materials were filtered. The filtrate was concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 1/1 to 1/5) was carried out to obtain **16** (85%, α/β = 95/5) as a white solid. For analytical data, see ref. 80.

Allyl 4-O-acetyl-3,6-di-O-benzyl-α-D-mannopyranoside ***(19)****.* To a solution of protected mannose allyl 4-*O*-acetyl-3,6-di-*O*-benzyl-2-*O*-(9-fluorenylmethoxycarbonyl)-α-D-mannopyranoside (**18**)[43] (1.50 g, 2.33 mmol) in CH_2Cl_2 (79 mL) was added Et_3N (14 mL). The mixture was stirred for 3 h at rt and diluted with toluene. The resulting solution was concentrated *in vacuo* and co-evaporated four times with toluene to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 5/1 to 3/1) was carried out to obtain product **19** (1.03 g, quant) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.36-7.24 (m, 10H, aromatic), 5.95-5.87 (m, 1H), 5.29 (dddd, 1H, *J* = 17.2, 1.5, 1.5, 1.5 Hz), 5.24 (dd, 1H, *J* = 9.8, 9.8 Hz), 5.20 (dddd, 1H, *J* = 10.5, 1.5, 1.5, 1.5 Hz), 4.95 (d, 1H, *J* = 1.8 Hz), 4.66 (d, 1H, *J*

= 12.0 Hz), 4.53 (d, 1H, *J* = 12.0 Hz), 4.53 (s, 2H), 4.21 (dddd, 1H, *J* = 13.0, 5.2, 1.5, 1.5 Hz), 4.05 (m, 1H), 4.01 (dddd, 1H, *J* = 13.0, 6.3, 1.5, 1.5 Hz), 3.86 (m, 1H), 3.81 (dd, 1H, *J* = 9.8, 3.5 Hz), 3.57 (dd, 1H, *J* = 10.8, 5.5 Hz), 3.53 (dd, 1H, *J* = 10.8, 3.5 Hz), 2.60 (d, 1H, *J* = 2.0 Hz, 1H), 1.89 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.9, 138.0, 137.7, 133.6, 128.5, 128.3, 128.3, 127.9, 127.7, 127.6, 127.5, 117.6, 98.2, 77.1, 73.5, 71.8, 69.7, 69.6, 68.3, 68.2, 68.1, 20.8. HR ESI-TOF MS: *m*/*z* calcd for $C_{25}H_{30}O_7$ $[M+Na]^+$ 465.1884, found 465.1893.

*Allyl 4-O-acetyl-3,6-di-O-benzyl-2-O-(3,6-di-O-benzyl-4-O-(9-fluorenylmethyloxycarbonyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-α-D-mannopyranoside (**20**).* GlcNAc donor 3,6-di-*O*-benzyl-2-deoxy-4-*O*-(9-fluorenylmethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl *N*-phenyltrifluoroacetimidate (**17**)[43] (659 mg, 0.710 mmol) and mannose acceptor **19** (377 mg, 0.852 mmol) were lyophilized from benzene and activated MS4A powder was added. To the mixture was added dist. CH_2Cl_2 (7.1 mL). To the mixture was added TMSOTf (25.0 μL, 0.142 mmol) at −78 °C and the mixture was stirred for 15 min at rt. The reaction was quenched by sat. aqueous $NaHCO_3$ and insoluble materials were filtered. CH_2Cl_2 was evaporated and the residual mixture was poured into sat. aqueous $NaHCO_3$. The aqueous layer was extracted by EtOAc and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Silica-gel column chromatography (toluene/EtOAc = 20/1 to 5/1) was carried out to give **20** (805 mg, 96%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.74 (dd, 2H, *J* = 7.6, 3.2 Hz, Fmoc aromatic), 7.56 (ddd, 2H, *J* = 22.1, 7.6, 0.8 Hz, Fmoc aromatic), 7.39-7.14 (m, 24H, aromatic), 5.92-5.84 (m, 1H, -CH_2-C<u>H</u>=CH_2), 5.42 (br s, 1H, N<u>H</u>Troc), 5.26 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 5.23 (dddd, 1H, *J* = 17.3, 1.5, 1.5, 1.5 Hz, -CH_2-CH=C<u>H$_2$</u>), 5.18 (dddd, 1H, *J* = 10.5, 1.5, 1.5, 1.5 Hz, -CH_2-CH=C<u>H$_2$</u>), 5.07 (br d, 1H, *J* = 6.5 Hz, H-1'), 4.83 (s, 1H, H-1), 4.81 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4'), 4.71 (d, 1H, *J* = 12.0 Hz, -C<u>H$_2$</u>Ph), 4.66 (d,

1H, *J* = 12.0 Hz, -C$\underline{H_2}$Ph), 4.59 (d, 1H, *J* = 12.0 Hz, -C$\underline{H_2}$Ph), 4.58 (s, 2H, -C$\underline{H_2}$Ph), 4.52 (s, 2H, -C$\underline{H_2}$Ph), 4.47 (d, 1H, *J* = 12.0 Hz, -C$\underline{H_2}$Ph), 4.45 (d, 1H, *J* = 12.0 Hz, -NHCO$_2$C$\underline{H_2}$CCl$_3$), 4.42 (d, 1H, *J* = 12.0 Hz, -NHCO$_2$C$\underline{H_2}$CCl$_3$), 4.33 (br s, 1H, H-3'), 4.29 (d, 2H, *J* = 7.4 Hz, -COC$\underline{H_2}$-fluorenyl), 4.18 (dd, 1H, *J* = 3.2, 3.2 Hz, H-2), 4.16 (dddd, 1H, *J* = 13.0, 6.3, 1.5, 1.5 Hz, -C$\underline{H_2}$-CH=CH$_2$), 4.10 (t, 1H, *J* = 7.4 Hz, Fmoc fluorenyl), 3.94 (dddd, 1H, *J* = 13.0, 6.0, 1.5, 1.5 Hz, -C$\underline{H_2}$-CH=CH$_2$), 3.84 (dd, 1H, *J* = 9.5, 3.2 Hz, H-3), 3.79 (m, 1H, H-5), 3.71 (m, 1H, H-5'), 3.60-3.51 (m, 4H, H-6, H-6'), 3.14 (br s, 1H, H-2'), 1.92 (s, 3H, Ac). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.7, 154.3, 153.9, 143.3, 143.1, 141.3, 141.2, 138.2, 138.0, 137.8, 133.6, 129.0, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.6, 127.6, 127.1, 125.3, 125.1, 125.0, 120.0, 117.5, 95.5, 75.1, 74.2, 73.6, 73.4, 73.0, 72.8, 71.2, 70.5, 70.0, 69.8, 68.7, 68.3, 57.8, 46.7, 20.9. HR ESI-TOF MS: *m*/*z* calcd for $C_{63}H_{64}Cl_3NO_{15}$ $[M+Na]^+$ 1202.3234, found 1202.3234.

*Allyl 4-O-acetyl-3,6-di-O-benzyl-2-O-(3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-α-D-mannopyranoside (**6**).* To a solution of protected disaccharide **20** (200 mg, 0.169 mmol) in CH_2Cl_2 (5.8 mL) was added Et_3N (1.0 mL) and the solution was stirred for 5.5 h at rt. The reaction solution was concentrated *in vacuo* and co-evaporated three times with toluene to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 4/1 to 3/1) was carried out to give product **6** (162 mg, quant) as a colorless solid. ^{1}H NMR (400 MHz, acetone-D_6): δ = 7.37-7.19 (m, 20H), 6.96 (d, 1H, *J* = 8.6 Hz), 5.99-5.89 (m, 1H), 5.25 (dddd, 1H, *J* = 17.3, 1.6, 1.6, 1.6 Hz), 5.15 (dd, 1H, *J* = 9.8, 9.8 Hz), 5.13 (dddd, 1H, *J* = 10.9, 1.6, 1.6, 1.6 Hz), 4.99 (d, 1H, *J* = 1.4 Hz), 4.89-4.76 (m, 5H), 4.60 (d, 1H, *J* = 13.7 Hz), 4.57 (d, 1H, *J* = 10.2 Hz), 4.53 (s, 4H), 4.46 (d, 1H, *J* = 12.0 Hz), 4.30 (t, 1H, *J* = 2.3 Hz), 4.19 (dddd, 1H, *J* = 13.2, 5.2, 1.6, 1.6 Hz), 3.98 (dddd, 1H, *J* = 13.2, 5.8, 1.6, 1.6 Hz), 3.89 (dd, 1H, *J* = 10.7, 2.0 Hz), 3.83 (dd, 1H, *J* = 9.4, 3.3 Hz), 3.82 (ddd, 1H, *J* = 12.9, 6.2, 3.3 Hz), 3.77-3.69 (m, 2H),

3.65-3.50 (m, 5H), 1.92 (s, 3H). ^{13}C NMR (100 MHz, acetone-D_6): δ = 170.0, 155.2, 140.2, 139.8, 139.6, 135.2, 129.0, 129.0, 128.9, 128.8, 128.5, 128.4, 128.2, 128.1, 128.1, 128.0, 127.9, 117.0, 101.0, 97.8, 97.1, 82.9, 82.9, 76.6, 76.5, 76.0, 74.8, 74.7, 74.0, 73.9, 73.6, 72.1, 72.0, 71.3, 71.2, 71.1, 70.5, 69.3, 68.7, 58.0, 20.9. HR ESI-Orbitrap MS: *m/z* calcd for $C_{48}H_{54}Cl_3NO_{13}$ $[M+Na]^+$ 980.2558, found 980.2567.

*Allyl 4-O-acetyl-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 (**21**).* To a solution of α-Sialyl disaccharide **16** (706 mg, 0.783 mmol) in pyridine (15 mL) was added Ac_2O (15 mL). The solution was stirred overnight at rt and the reaction was quenched by MeOH. The mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc and poured into H_2O. The aqueous layer was extracted with EtOAc and the organic layer was washed with brine three times, dried over Na_2SO_4, filtered, concentrated *in vacuo*, and co-evaporated with toluene four times to give a crude product. The crude product was roughly purified by silica-gel column chromatography ($CHCl_3$/MeOH = 50/1 to 20/1). The resulting product was used for the next reaction without further purification.

*4-O-acetyl-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-galactopyranosyl N-phenyltrifluoroacetimidate (**23**)*[43]. A suspension of $[Ir(cod)(PPh_2Me)_2]PF_6$ (133 mg, 0.157 mmol) in anhydrous THF (7.8 mL) was stirred for 5 min under H_2 atmosphere to give a yellow solution. The solution was added to a solution of the crude disaccharide **21** (0.783 mmol) in anhydrous THF (7.8 mL) under Ar atmosphere and the mixture was stirred overnight at rt. To the solution were added H_2O (6 mL) and I_2 (398 mg, 1.57 mmol), and the mixture was stirred for 1.5 h at rt. The reaction was quenched by 20% aqueous $Na_2S_2O_3$ and THF was evaporated under reduced pressure. The residue

was poured into sat. aqueous $NaHCO_3$ and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a crude product. The crude product was roughly purified by silica-gel column chromatography ($CHCl_3$ only to $CHCl_3$/MeOH = 20/1). To a solution of the crude product in acetone (7.8 mL) were added *N*-phenyltrifluoroacetimidoyl chloride (325 mg, 1.57 mmol) and K_2CO_3 (325 mg, 2.35 mmol). The mixture was stirred overnight at rt, filtered, and concentrated in vacuo. Silica-gel column chromatography (toluene/EtOAc = 1/3 to 1/10) was carried out to obtain **23** (804 mg, 95%) as a brown solid of α/β mixture. ^{1}H NMR (400 MHz, CD_2Cl_2) of major isomer: δ = 7.98 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.90 (dd, 2H, *J* = 8.3, 1.2 Hz), 7.61-7.52 (m, 2H), 7.45-7.38 (m, 5H), 7.30 (t, 1H, *J* = 7.8 Hz), 7.17 (t, 2H, *J* = 7.7 Hz), 7.04 (t, 1H, *J* = 7.4 Hz), 6.50 (d, 1H, *J* = 5.5 Hz), 5.83 (d, 2H, *J* = 2.1 Hz), 5.55-5.51 (m, 1H), 5.45-5.37 (m, 1H), 5.30 (dd, 1H, *J* = 2.1, 0.6 Hz), 5.19 (d, 1H, *J* = 9.6 Hz), 4.93-4.84 (m, 1H), 4.51 (dd, 1H, *J* = 5.6, 5.6 Hz), 4.23 (dd, 1H, *J* = 12.4, 3.0 Hz), 4.12-3.96 (m, 4H), 3.80 (s, 3H), 3.38 (dd, 1H, *J* = 10.0, 6.2 Hz), 2.61 (dd, 1H, *J* = 12.8, 4.7 Hz), 2.17 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (dd, 1H, *J* = 4.2, 3.6 Hz), 1.84 (s, 3H). HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{50}H_{53}F_3N_2O_{21}$ $[M+Na]^+$ 1097.2985, found 1097.2982.

*Allyl 4-O-acetyl-2,3-di-O-benzoyl-6-O-(Methyl 4,7,8,9-tetra-O-acetyl-5-(N-acetylacetamide)-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-α-D-galactopyranoside (**22**).* To α-sialyl disaccharide **16** (4.00 g, 4.44 mmol) were added isopropenyl acetate (220 mL) and *p*-TsOH (761 mg, 4.00 mmol). The mixture was stirred for 2 h at 95 °C under reflux. Then, the reaction mixture was cooled to 0 °C and Et_3N (3 mL) was added. The mixture was concentrated *in vacuo* and co-evaporated twice with toluene to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 3/1 to 1/1) was carried out to obtain **22** (4.38g, quant) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.98 (dd, 2H, *J* = 8.4, 1.2 Hz), 7.88 (dd, 2H, *J*

= 8.4, 1.4 Hz), 7.53-7.47 (m, 2H), 7.37 (dt, 4H, *J* = 14.8, 6.7 Hz), 5.90-5.82 (m, 1H), 5.82 (dd, 1H, *J* = 10.8, 3.3 Hz), 5.73 (dd, 1H, *J* = 3.3, 1.1 Hz), 5.58 (dd, 1H, *J* = 10.8, 3.7 Hz), 5.51 (ddd, 1H, *J* = 10.5, 10.5, 5.4 Hz), 5.35-5.30 (m, 3H), 5.17-5.15 (m, 2H), 4.94 (dd, 1H, *J* = 10.1, 1.8 Hz), 4.38-4.35 (m, 1H), 4.31 (dddd, 1H, *J* = 13.4, 4.5, 1.5, 1.5 Hz), 4.29 (dd, 1H, *J* = 12.5, 2.9 Hz), 4.17 (dd, 1H, *J* = 10.0, 10.0 Hz), 4.15 (dd, 1H, *J* = 12.5, 5.2 Hz), 4.08 (dddd, 1H, *J* = 13.4, 5.9, 1.5, 1.5 Hz), 3.94 (dd, 1H, *J* = 10.2, 6.3 Hz), 3.82 (s, 3H), 3.50 (dd, 1H, *J* = 10.2, 7.3 Hz), 2.73 (dd, 1H, *J* = 13.1, 5.4 Hz), 2.38 (s, 3H), 2.31 (s, 3H), 2.19 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.85 (dd, 1H, *J* = 13.1, 10.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.5, 173.6, 170.5, 170.1, 169.9, 169.8, 169.6, 167.3, 166.0, 165.4, 133.5, 133.3, 133.1, 129.8, 129.5, 129.5, 129.3, 128.4, 128.3, 117.5, 98.7, 95.6, 77.2, 69.8, 69.0, 68.7, 68.6, 68.5, 68.3, 67.6, 67.1, 66.8, 62.5, 61.9, 57.1, 52.8, 38.7, 27.9, 25.9, 21.0, 21.0, 20.7, 20.7, 20.6. HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{47}H_{55}NO_{22}$ $[M+Na]^+$ 1008.3113, found 1008.3119.

4-O-acetyl-2,3-di-O-benzoyl-6-O-(Methyl 4,7,8,9-tetra-O-acetyl-5-(N-acetylacetamide)-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-D-galactopyranose. A suspension of $[Ir(cod)(PPh_2Me)_2]PF_6$ (25.5 mg, 0.0301 mmol) in anhydrous THF (3.0 mL) was stirred for 5 min under H_2 atmosphere to give a yellow solution. The solution was added to a solution of allyl glycoside **22** (297 mg, 0.301 mmol) in anhydrous THF (3.0 mL) under Ar atmosphere and stirred for 1 h at rt. To the reaction solution were added H_2O (2 mL) and I_2 (153 mg) and the mixture was stirred for additional 1 h. The reaction was quenched by 20% aqueous $Na_2S_2O_3$ and THF was evaporated. The aqueous layer was extracted by EtOAc. The organic layer was washed with sat. aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography ($CHCl_3$/acetone = 15/1 to 10/1) was carried out to give the 1-OH product (225 mg, 79%) as a yellow solid of α/β mixture. ^{1}H NMR (500 MHz, $CDCl_3$) of major isomer: δ = 8.00 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.90 (dd, 2H, *J* = 8.4, 1.2 Hz), 7.52-7.48 (m, 2H), 7.39-7.35

(m, 4H), 5.91 (dd, 1H, *J* = 10.7, 3.4 Hz), 5.82 (dd, 1H, *J* = 3.4, 1.2 Hz), 5.68 (dd, 1H, *J* = 3.2, 3.2 Hz), 5.57 (ddd, 1H, *J* = 10.7, 3.6, 1.1 Hz), 5.54 (dd, 1H, *J* = 4.7, 1.7 Hz), 5.54-5.48 (m, 1H), 5.36 (ddd, 1H, *J* = 7.5, 7.5, 2.5 Hz), 5.17 (dd, 1H, *J* = 6.9, 1.5 Hz), 5.02 (dd, 1H, *J* = 10.1, 1.5 Hz), 4.70 (ddd, 1H, *J* = 9.1, 5.3, 1.0 Hz), 4.62 (dd, 1H, *J* = 3.0, 1.3 Hz), 4.41 (dd, 1H, *J* = 12.1, 2.6 Hz), 4.11 (ddd, 1H, *J* = 10.2, 10.2, 10.2 Hz), 3.85 (s, 3H), 3.80 (dd, 1H, *J* = 11.2, 5.4 Hz), 3.57 (dd, 1H, *J* = 11.1, 9.4 Hz), 2.75 (dd, 1H, *J* = 13.2, 5.3 Hz), 2.38 (s, 3H), 2.33 (s, 3H), 2.30 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.89 (dd, 1H, *J* = 13.2, 10.9 Hz). HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{44}H_{51}NO_{22}$ $[M+Na]^+$ 968.2800, found 968.2810.

4-O-acetyl-2,3-di-O-benzoyl-6-O-(Methyl 4,7,8,9-tetra-O-acetyl-5-(N-acetylacetamide)-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-D-galactopyranosyl N-phenyltrifluoroacetimidate (7). To a solution of 1-OH disaccharide 4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-D-galactopyranose (3.03 g, 3.20 mmol) in acetone (32 mL) were added *N*-phenylacetimidoyl chloride (1.33 g, 6.40 mmol) and K_2CO_3 (1.33 g, 9.60 mmol). The mixture was stirred for 30 min under rt and insoluble materials were filtered. The filtrate was concentrated *in vacuo* to give a crude product. Silica-gel column chromatography ($CHCl_3$/acetone = 30/1 to 15/1) was carried out to give the product **7** (3.41 g, 95%) as a yellowish solid of α/β mixture. ^{1}H NMR (500 MHz, $CDCl_3$) of major isomer: δ = 7.98 (dd, 2H, *J* = 8.4, 1.2 Hz, Bz), 7.90 (dd, 2H, *J* = 8.3, 1.3 Hz, Bz), 7.57 (tt, 1H, *J* = 7.4, 1.4 Hz, Bz), 7.51 (tt, 1H, *J* = 7.4, 1.4 Hz, Bz), 7.43-7.36 (m, 4H, Bz), 7.14-7.08 (m, 2H, NPh), 7.01 (t, 1H, *J* = 7.4 Hz, NPh), 6.74 (d, 1H, *J* = 5.7 Hz, H-1'), 6.44 (d, 2H, *J* = 4.4 Hz, NPh), 5.87-5.76 (m, 3H, H-2', 3', 4'), 5.52 (ddd, 1H, *J* = 11.0, 11.0, 5.2 Hz, H-4), 5.35 (ddd, 1H, *J* = 8.2, 5.0, 2.7 Hz, H-8), 5.17 (dd, 1H, *J* = 8.2, 1.8 Hz, H-7), 4.94 (dd, 1H, *J* = 10.0, 1.8 Hz, H-6), 4.52 (dd, 1H, *J* = 6.2, 6.2 Hz, H-5'), 4.29 (dd, 1H, *J* =

12.5, 2.7 Hz, H-9a), 4.17 (dd, 1H, *J* = 10.0, 10.0 Hz, H-5), 4.14 (dd, 1H, *J* = 12.5, 5.0 Hz, H-9b), 4.03 (dd, 1H, *J* = 10.1, 6.2 Hz, H-6a'), 3.83 (s, 3H, CO_2Me), 3.53 (dd, 1H, *J* = 10.1, 6.2 Hz, H-6b'), 2.75 (dd, 1H, *J* = 13.0, 5.2 Hz, H-3_{eq}), 2.38 (s, 3H, *N*Ac), 2.31 (s, 3H, *N*Ac), 2.17 (s, 3H, *O*Ac), 2.17 (s, 3H, *O*Ac), 2.13 (s, 3H, *O*Ac), 2.02 (s, 3H, *O*Ac), 1.98 (s, 3H, *O*Ac), 1.86 (dd, 1H, *J* = 13.0, 11.0 Hz, H-3_{ax}). HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{52}H_{55}F_3N_2O_{22}$ $[M+Na]^+$ 1139.3096, found 1139.3107.

*Allyl 4-O-acetyl-2-O-(4-O-(4-O-acetyl-2,3-di-O-benzoyl-6-O-(Methyl 5-acetamide-4,7,8,9-tetra-O-acetyl-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-O-benzyl-α-D-mannopyranoside (**24**).* 5-NHAc disaccharide donor **23** (20.0 mg, 0.0186 mmol) and disaccharide acceptor **6** (21.4 mg, 0.0223 mmol) were lyophilized from benzene and activated MS4A powder was added. To the mixture was added dist. CH_2Cl_2 (0.36 mL). A solution of TMSOTf (67 μL, 0.372 mmol) in dist. CH_2Cl_2 (1.0 mL) was dried over activated MS4A pellets. To the solution of **23** and **6** was added the solution of TMSOTf (10 μL, 3.72 μmol of TMSOTf) at 0 °C and the mixture was stirred for 20 min at the same temperature. The mixture was allowed to warm up to rt and stirred for another 1 h. Then, another portion of the solution of TMSOTf (10 μL, 3.72 μmol of TMSOTf) was added and the mixture was stirred for 30 min. The reaction was quenched by sat. aqueous $NaHCO_3$ and insoluble materials were filtered. The filtrate was poured into sat. aqueous $NaHCO_3$ and the aqueous layer was extracted with $CHCl_3$. The organic layer was washed by brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 1/1 to 2/3) was carried out to obtain **24** (17.7 mg, 52%) as a colorless solid. ^{1}H NMR (500 MHz, acetone-D_6): δ = 7.95 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.87 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.60-7.55 (m, 2H), 7.50 (dd, 2H, *J* = 8.0, 0.9 Hz), 7.43 (q, 4H, *J* = 8.0 Hz), 7.40-7.29 (m, 14H), 7.27-7.17 (m, 4H), 6.99 (d, 1H, *J* = 8.5 Hz), 6.87 (d, 1H, *J* = 9.7 Hz), 5.95-5.87 (m, 1H), 5.63 (dd, 1H, *J* = 3.5, 0.8 Hz), 5.60 (dd, 1H, *J* =

10.4, 8.0 Hz), 5.50 (dd, 1H, *J* = 10.4, 3.5 Hz), 5.44 (ddd, 1H, *J* = 8.5, 6.0, 3.0 Hz), 5.35 (dd, 1H, *J* = 8.2, 2.1 Hz), 5.22 (dddd, 1H, *J* = 17.4, 1.6, 1.6, 1.6 Hz), 5.20 (d, 1H, *J* = 7.7 Hz), 5.13 (dd, 1H, *J* = 9.8, 9.8 Hz), 5.10 (dddd, 1H, *J* = 10.4, 1.6, 1.6, 1.6 Hz), 5.09 (d, 1H, *J* = 10.8 Hz), 4.94 (br s, 1H), 4.86-4.76 (m, 5H), 4.63 (d, 1H, *J* = 11.7 Hz), 4.52 (d, 1H, *J* = 12.0 Hz), 4.52 (s, 2H), 4.40 (d, 1H, *J* = 12.0 Hz), 4.32 (dd, 1H, *J* = 12.7, 2.6 Hz), 4.30 (d, 1H, *J* = 12.2 Hz), 4.26 (dd, 1H, *J* = 3.0, 2.0 Hz), 4.21 (dd, 1H, *J* = 10.7, 2.2 Hz), 4.19-4.14 (m, 2H), 4.12-4.05 (m, 3H), 3.94 (dddd, 1H, *J* = 13.1, 5.7, 1.6, 1.6 Hz), 3.92-3.86 (m, 2H), 3.80-3.77 (m, 6H), 3.73 (dd, 1H, *J* = 11.5, 2.7 Hz), 3.71 (d, 1H, *J* = 4.5 Hz), 3.59-3.49 (m, 2H), 3.44-3.41 (m, 2H), 2.54 (dd, 1H, *J* = 12.8, 4.8 Hz), 2.10 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.91 (s, 3H), 1.78 (s, 3H), 1.76 (dd, 1H, *J* = 12.8, 12.3 Hz). HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{90}H_{101}Cl_3N_2O_{33}$ $[M+2Na]^{2+}$ 944.2568, found 944.2559.

*Allyl 4-O-acetyl-2-O-(4-O-(4-O-acetyl-2,3-di-O-benzoyl-6-O-(Methyl 4,7,8,9-tetra-O-acetyl-5-(N-acetylacetamide)-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyr anosyl)-3,6-di-O-benzyl-α-D-mannopyranoside (**25**).* 5-NAc_2 disaccharide donor **7** (1.39 g, 1.24 mmol) and disaccharide acceptor **6** (1.30 g, 1.36 mmol) were lyophilized from benzene and activated MS4A powder and dist. CH_2Cl_2 (25 mL) were added. To the mixture was added TMSOTf (45 μL, 0.248 mmol) at 0 °C and the mixture was stirred at the same temperature for 20 min. Sat. aqueous $NaHCO_3$ was added to the reaction mixture and insoluble materials were filtered. The filtrate was poured into sat. aqueous $NaHCO_3$ and the aqueous layer was extracted with $CHCl_3$. The organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene/EtOAc = 5/1 to 3/2) was carried out to give the product **25** (2.25 g, 96%) as a white solid. ^{1}H NMR (400 MHz, acetone-D_6): δ = 7.94 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.88 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.61-7.54 (m, 2H), 7.51-7.36 (m, 10H), 7.34-7.30 (m, 9H), 7.29-7.12 (m, 5H), 6.98 (d, 1H, *J* =

8.7 Hz), 5.96-5.86 (m, 1H), 5.65 (dd, 1H, *J* = 3.5, 0.9 Hz), 5.62 (d, 1H, *J* = 14.9 Hz), 5.61 (dd, 1H, *J* = 7.4, 3.0 Hz), 5.51 (ddd, 1H, *J* = 10.8, 10.8, 5.0 Hz), 5.50 (dd, 1H, *J* = 10.4, 3.4 Hz), 5.36 (ddd, 1H, *J* = 8.3, 5.0, 2.3 Hz), 5.22 (dddd, 1H, *J* = 17.2, 1.7, 1.7, 1.7 Hz), 5.19 (d, 1H, *J* = 8.0 Hz), 5.17 (dd, 1H, *J* = 5.2, 2.2 Hz), 5.13 (d, 1H, *J* = 9.6 Hz), 5.11 (dddd, 1H, *J* = 10.4, 1.7, 1.7, 1.7 Hz), 5.09 (d, 1H, *J* = 10.9 Hz), 5.00 (dd, 1H, *J* = 10.1, 1.7 Hz), 4.95 (d, 1H, *J* = 1.1 Hz), 4.89-4.76 (m, 4H), 4.63 (d, 1H, *J* = 12.3 Hz), 4.56 (d, 1H, *J* = 12.1 Hz), 4.53 (s, 2H), 4.41 (d, 1H, *J* = 12.0 Hz), 4.38 (dd, 1H, *J* = 12.3, 3.5 Hz), 4.33 (d, 1H, *J* = 12.0 Hz), 4.32 (dd, 1H, *J* = 10.2, 10.2 Hz), 4.20-4.09 (m, 3H), 4.06 (dd, 1H, *J* = 7.6, 6.0 Hz), 3.95 (dddd, 1H, *J* = 13.1, 5.8, 1.7, 1.7 Hz), 3.95 (dd, 1H, *J* = 10.2, 5.7 Hz), 3.86 (dd, 1H, *J* = 10.1, 10.1 Hz), 3.82-3.78 (m, 5H), 3.76-3.69 (m, 2H), 3.61-3.49 (m, 4H), 3.40 (ddd, 1H, *J* = 9.7, 4.1, 2.3 Hz), 2.69 (dd, 1H, *J* = 12.9, 5.0 Hz), 2.37 (s, 3H), 2.34 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.92 (s, 3H), 1.81 (dd, 1H, *J* = 12.9, 10.8 Hz). ^{13}C NMR (100 MHz, acetone-D_6): δ = 175.2, 174.6, 170.8, 170.7, 170.4, 170.1, 170.1, 170.0, 168.4, 165.7, 165.7, 155.1, 140.2, 139.7, 139.6, 139.5, 135.1, 134.3, 134.1, 130.3, 130.3, 130.3, 130.2, 129.7, 129.5, 129.3, 129.2, 129.0, 128.8, 128.8, 128.6, 128.4, 128.2, 128.1, 128.0, 128.0, 117.0, 100.9, 100.8, 99.8, 97.8, 97.0, 80.5, 77.4, 76.0, 75.5, 74.8, 74.5, 74.1, 73.9, 73.6, 72.9, 72.6, 71.4, 71.2, 71.1, 71.0, 70.5, 69.7, 69.6, 69.3, 68.7, 68.1, 67.1, 62.7, 58.0, 57.5, 53.3, 39.3, 28.0, 26.0, 21.2, 21.1, 20.9, 20.7, 20.7, 20.6. HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{92}H_{103}Cl_3N_2O_{34}$ $[M+Na]^+$ 1907.5356, found 1907.5353.

*N^{α}-benzyloxycarbonyl-N^{γ}-(6-O-(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-3-O-benzyl-4-O-(3,6-di-O-benzyl-4-O-(2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (**26**).* To a solution of protected tetrasaccharide-Asn **2** (509 mg, 0.245 mmol) in anhydrous CH_2Cl_2 (49 mL) was added PPh_3 (193 mg, 0.735 mmol) and the solution was stirred for 1 h at rt. To the reaction solution were added AcOH (422 μL, 7.35 mmol), H_2O (132 μL, 7.35

mmol), and DDQ (195 mg, 0.858 mmol) and the mixture was stirred for another 20 min. The resulting mixture was diluted by $CHCl_3$ and the remaining DDQ was reduced by 5% aqueous ascorbic acid. The aqueous layer was extracted with $CHCl_3$ twice and the gathered organic layer was washed by sat. aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography ($CHCl_3$/acetone = 15/1 to 10/1) was carried out to give **26** (412 mg, 89%) as a brown solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.44-7.39 (m, 4H), 7.36-7.27 (m, 20H), 7.24-7.16 (m, 12H), 6.94 (d, 1H, *J* = 7.7 Hz), 6.15 (d, 1H, *J* = 8.7 Hz), 5.91 (d, 1H, *J* = 8.5 Hz), 5.85-5.78 (m, 1H), 5.39 (s, 1H), 5.35 (dd, 1H, *J* = 10.6, 3.4 Hz), 5.25 (dddd, 1H, *J* = 17.2, 1.4, 1.4, 1.4 Hz), 5.18 (br s, 1H), 5.16 (dddd, 1H, *J* = 10.4, 1.4, 1.4, 1.4 Hz), 5.12 (d, 1H, *J* = 12.3 Hz), 5.06-4.97 (m, 4H), 4.88 (d, 1H, *J* = 13.2 Hz), 4.86 (d, 1H, *J* = 14.4 Hz), 4.79-4.65 (m, 6H), 4.59-4.55 (m, 6H), 4.50 (d, 1H, *J* = 11.8 Hz), 4.42 (d, 1H, *J* = 7.2 Hz), 4.33 (d, 1H, *J* = 13.7 Hz), 4.31 (d, 1H, *J* = 11.8 Hz), 4.22 (ddd, 1H, *J* = 6.5, 6.5, 6.5 Hz), 4.12-4.06 (m, 2H), 4.00 (dd, 1H, *J* = 10.6, 4.8 Hz), 3.86-3.81 (m, 4H), 3.72 (d, 1H, *J* = 8.7 Hz), 3.65-3.60 (m, 4H), 3.51 (ddd, 1H, *J* = 10.2, 10.2, 10.2 Hz), 3.52-3.30 (m, 5H), 3.03 (ddd, 1H, *J* = 9.5, 9.5, 4.8 Hz), 2.83 (dd, 1H, *J* = 16.5, 3.4 Hz), 2.62 (dd, 1H, *J* = 16.5, 4.2 Hz), 2.29 (d, 1H, *J* = 5.4 Hz), 2.04 (s, 3H), 1.72 (s, 3H), 1.00 (d, 3H, *J* = 6.5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5, 170.5, 170.3, 169.8, 156.2, 156.1, 154.3, 139.1, 138.1, 137.3, 137.3, 137.1, 136.2, 131.6, 129.0, 129.0, 128.8, 128.6, 128.5, 128.5, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.8, 127.2, 127.2, 126.3, 118.5, 103.6, 101.9, 101.8, 97.1, 95.6, 95.2, 81.3, 81.0, 80.3, 79.1, 78.5, 77.8, 77.5, 77.3, 75.9, 75.4, 74.8, 74.8, 74.6, 73.8, 73.7, 73.5, 73.4, 71.7, 70.7, 69.6, 68.6, 68.4, 67.0, 66.9, 66.7, 66.2, 64.9, 57.4, 55.9, 50.4, 37.6, 20.7, 20.5, 15.5. HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{91}H_{100}Cl_6N_4O_{28}$ $[M+Na]^+$ 1929.4553, found 1929.4554.

4-O-acetyl-2-O-(4-O-(4-O-acetyl-2,3-di-O-benzoyl-6-O-(Methyl 4,7,8,9-tetra-O-acetyl-5-(N-acetylacetamide)-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate

)-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-O-benzyl-D-mannopyranose. A suspension of $[Ir(cod)(PPh_2Me)_2]PF_6$ (44.8 mg, 0.0530 mmol) in anhydrous THF (5 mL) was stirred for 10 min under H_2 atmosphere to give a yellow solution. To a solution of allyl glycoside **25** (2.00 g, 1.06 mmol) in anhydrous THF (16 mL) was added the solution of activated Ir complex under Ar atmosphere and stirred for 1.5 h at rt. To the reaction solution were added H_2O (5 mL) and I_2 (538 mg, 2.12 mmol) and the solution was stirred for another 10 min. The reaction was quenched by 20% aqueous Na_2SO_4 and THF was evaporated. The residual mixture was poured into 20% aqueous Na_2SO_4 and the aqueous layer was extracted with EtOAc. The organic layer was washed by sat. aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene/acetone = 6/1 to 4/1) was carried out to obtain the 1-OH product (1.93 g, 98%) as a yellow solid of α/β mixture. ^{1}H NMR (400 MHz, acetone-D_6) of major isomer: δ = 7.94 (dd, 2H, *J* = 8.2, 1.1 Hz), 7.87 (dd, 2H, *J* = 8.2, 1.1 Hz), 7.57 (qt, 2H, *J* = 9.3, 1.5 Hz), 7.50 (d, 2H, *J* = 7.1 Hz), 7.46-7.38 (m, 7H), 7.36-7.30 (m, 10H), 7.28-7.12 (m, 5H), 6.96 (d, 1H, *J* = 8.7 Hz), 5.68 (d, 1H, *J* = 4.1 Hz), 5.64 (d, 1H, *J* = 3.5 Hz), 5.61 (s, 2H), 5.60 (d, 1H, *J* = 5.8 Hz), 5.54-5.47 (m, 2H), 5.36 (ddd, 1H, *J* = 7.8, 6.0, 3.2 Hz), 5.25 (br s, 1H), 5.21-5.08 (m, 4H), 5.00 (dd, 1H, *J* = 10.1, 1.6 Hz), 4.86-4.76 (m, 3H), 4.61 (d, 1H, *J* = 12.7 Hz), 4.57 (d, 1H, *J* = 12.3 Hz), 4.48 (s, 2H), 4.43-4.29 (m, 4H), 4.24 (dd, 1H, *J* = 2.2, 2.2 Hz), 4.16-4.04 (m, 3H), 4.01 (ddd, 1H, *J* = 10.0, 6.0, 3.8 Hz), 3.95 (dd, 1H, *J* = 9.7, 5.5 Hz), 3.89-3.85 (m, 2H), 3.82 (s, 3H), 3.76-3.69 (m, 2H), 3.60-3.46 (m, 3H), 3.41 (ddd, 1H, *J* = 9.8, 4.2, 2.2 Hz), 2.69 (dd, 1H, *J* = 12.8, 5.2 Hz), 2.37 (s, 3H), 2.34 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 1.81 (dd, 1H, *J* = 12.8, 10.9 Hz). HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{89}H_{99}Cl_3N_2O_{34}$ $[M+Na]^+$ 1867.5043, found 1867.5054.

*4-O-acetyl-2-O-(4-O-(4-O-acetyl-2,3-di-O-benzoyl-6-O-(Methyl 4,7,8,9-tetra-O-acetyl-5-(N-acetylacetamide)-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-O-benzyl-D-mannopyranosyl N-phenyltrifluoroacetimidate (**3**)*. To a solution of 1-OH tetrasaccharide 4-*O*-acetyl-2-*O*-(4-*O*-(4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-(Methyl 4,7,8,9-tetra-*O*-acetyl-5-(*N*-acetylacetamide)-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-D-mannopyranose (3.45 g, 1.87 mmol) in acetone (37 mL) were added *N*-phenyltrifluoroacetimidoyl chloride (776 mg, 3.74 mmol) and K_2CO_3 (1.29 g, 9.35 mmol). The mixture was stirred for 1 h at rt and insoluble materials were filtered. The filtrate was concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene/acetone = 7/1 to 5/1) to obtain **3** (3.72 g, 99%) as a white solid of α/β mixture. ^{1}H NMR (500 MHz, acetone-D_6) of major isomer: δ = 7.96 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.88 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.61 (tt, 1H, *J* = 7.5, 1.4 Hz), 7.56 (tt, 1H, *J* = 7.4, 1.4 Hz), 7.51-7.36 (m, 11H), 7.35-7.19 (m, 16H), 7.05 (tt, 2H, *J* = 7.5, 1.1 Hz), 6.77 (d, 2H, *J* = 7.7 Hz), 6.28 (br s, 1H), 5.65 (dd, 1H, *J* = 3.5, 1.0 Hz), 5.61 (dd, 1H, *J* = 10.4, 7.9 Hz), 5.53-5.48 (m, 1H), 5.36 (ddd, 1H, *J* = 7.0, 6.0, 3.5 Hz), 5.23 (dd, 1H, *J* = 9.6, 9.6 Hz), 5.19 (d, 1H, *J* = 7.9 Hz), 5.17 (dd, 1H, *J* = 7.3, 1.7 Hz), 5.09 (d, 1H, *J* = 10.9 Hz), 5.00 (dd, 1H, *J* = 10.1, 1.7 Hz), 4.89-4.81 (m, 2H), 4.77 (d, 2H, *J* = 12.2 Hz), 4.76 (d, 2H, *J* = 10.7 Hz), 4.57 (d, 2H, *J* = 12.0 Hz), 4.54 (s, 2H), 4.48 (d, 1H, *J* = 11.7 Hz), 4.37 (dd, 1H, *J* = 12.0, 3.5 Hz), 4.35 (d, 1H, *J* = 12.2 Hz), 4.31 (d, 1H, *J* = 10.1 Hz), 4.14 (dd, 1H, *J* = 12.2, 6.0 Hz), 4.11 (dd, 1H, *J* = 9.6, 8.8 Hz), 4.07 (ddd, 1H, *J* = 8.0, 5.5, 0.9 Hz), 3.96-3.92 (m, 2H), 3.90-3.85 (m, 2H), 3.82 (s, 3H), 3.74-3.73 (m, 2H), 3.58 (d, 2H, *J* = 4.5 Hz), 3.52 (dd, 1H, *J* = 8.9, 8.9 Hz), 3.38 (ddd, 1H, *J* = 9.5, 3.3, 3.3 Hz), 2.69 (dd, 1H, *J* = 12.7, 5.2 Hz), 2.37 (s, 3H), 2.34 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.81

(dd, 1H, J = 12.7, 11.3 Hz). HR ESI-Orbitrap MS: m/z calcd for $C_{97}H_{103}Cl_3F_3N_3O_{34}$ $[M+Na]^+$ 2038.5338, found 2038.5328.

*N^{α}-benzyloxycarbonyl-N^{γ}-(6-O-(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-3-O-benzyl-4-O-(3,6-di-O-benzyl-4-O-(3-O-(4-O-acetyl-2-O-(4-O-(4-O-acetyl-2,3-di-O-benzoyl-6-O-(Methyl 4,7,8,9-tetra-O-acetyl-5-(N-acetylacetamide)-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-O-benzyl-D-mannopyranosyl)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (**27**).* Tetrasaccharide donor **3** (1.27 g, 0.628 mmol) and tetrasaccharide-Asn acceptor **26** (1.00 g, 0.523 mmol) were lyophilized from benzene and activated MS4A powder was added. To the mixture was added anhydrous CPME (9.0 mL). A solution of TMSOTf (47.4 μL, 0.262 mmol) in anhydrous CPME (1.0 mL) was dried over activated MS4A pellets. Both solutions were cooled to 0 °C and the solution of TMSOTf was added to the solution of **3** and **26** via cannula. The reaction mixture was stirred for 20 min at 0 °C and sat. aqueous $NaHCO_3$ was added to the mixture. Insoluble materials were filtered and the filtrate was poured into sat. aqueous $NaHCO_3$. The aqueous layer was extracted with EtOAc and the organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography (toluene/acetone = 6/1 to 4/1) was carried out to obtain **27** as a yellowish solid of α/β mixture (1.79 g, 91%, α/β = 3/1, estimated by ^{1}H NMR). HR ESI-Orbitrap MS: m/z calcd for $C_{180}H_{197}Cl_9N_6O_{61}$ $[M+2Na]^{2+}$ 1889.4739, found 1889.4762.

N^{α}-benzyloxycarbonyl-N^{γ}-(6-O-(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-3-O-benzyl-4-O-(3,6-di-O-benzyl-4-O-(3-O-(4-O-acetyl-2-O-(4-O-(4-O-acetyl-2,3-di-O-benzoyl-6-O-(Methyl

*4,7,8,9-tetra-O-acetyl-5-(N-acetylacetamide)-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyr anosyl)-3,6-di-O-benzyl-α-D-mannopyranosyl)-2-O-benzyl-β-D-mannopyranosyl)-2-deoxy-2-(2,2,2-trich loroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-gl ucopyranosyl)-L-asparagine allyl ester (**28**)*. To a solution of octasaccharide-Asn **27** (1.73 g, 0.463 mmol) in CH_2Cl_2 (18 mL) were added H_2O (1.8 mL) and TFA (3.5 mL) at 0 °C. The solution was stirred for 1.5 h at rt and neutralized by sat. aqueous $NaHCO_3$. The mixture was poured into sat. aqueous $NaHCO_3$ and the aqueous layer was extracted twice with $CHCl_3$. The organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography ($CHCl_3$/MeCN = 5/1 to 3/1) was carried out to obtain **28** (974 mg, 58%, α isomer) as a yellow solid and the β isomer at 1^E- position (244 mg, 14%) as a white solid. ^{1}H NMR (500 MHz, acetone-D_6): δ = 7.95 (dd, 2H, *J* = 8.3, 1.1 Hz), 7.87 (dd, 2H, *J* = 8.4, 1.3 Hz), 7.83 (d, 1H, *J* = 9.5 Hz), 7.61-7.55 (m, 2H), 7.49 (d, 2H, *J* = 7.1 Hz), 7.47-7.41 (m, 4H), 7.39-7.15 (m, 50H), 6.99 (d, 1H, *J* = 8.4 Hz), 6.98 (d, 1H, *J* = 5.9 Hz), 6.88 (d, 1H, *J* = 9.2 Hz), 6.47 (d, 1H, *J* = 8.7 Hz), 5.94-5.86 (m, 1H), 5.63 (d, 1H, *J* = 3.6 Hz), 5.60 (dd, 1H, *J* = 10.3, 7.9 Hz), 5.49 (d, 1H, *J* = 10.5 Hz), 5.48 (dd, 1H, *J* = 10.5, 2.0 Hz), 5.36-5.29 (m, 4H), 5.22-5.00 (m, 13H), 4.99 (dd, 1H, *J* = 10.1, 1.7 Hz), 4.87-4.66 (m, 12H), 4.63-4.56 (m, 5H), 4.52-4.48 (m, 4H), 4.43 (d, 1H, *J* = 11.2 Hz), 4.39-4.24 (m, 7H), 4.13 (dd, 1H, *J* = 12.2, 6.1 Hz), 4.12-4.01 (m, 4H), 3.94-3.45 (m, 28H), 3.39 (dd, 1H, *J* = 11.5, 5.9 Hz), 3.24 (ddd, 1H, *J* = 9.7, 4.3, 2.3 Hz), 3.02 (ddd, 1H, *J* = 9.2, 5.6, 3.3 Hz), 2.81 (d, 2H, *J* = 5.7 Hz), 2.72 (t, 1H, *J* = 6.3 Hz), 2.68 (dd, 1H, *J* = 12.7, 5.2 Hz), 2.36 (s, 3H), 2.33 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.88 (s, 3H), 1.80 (dd, 1H, *J* = 12.7, 10.5 Hz), 1.77 (s, 3H), 1.02 (d, 3H, *J* = 6.4 Hz). ^{13}C NMR (125 MHz, acetone-D_6): δ = 175.2, 174.6, 171.6, 170.9, 170.9, 170.8, 170.8, 170.4, 170.3, 170.1, 170.1, 170.0, 168.4, 165.7, 165.7, 156.8, 155.8, 155.3, 155.3, 155.2, 140.6, 140.6, 140.3,

140.2, 139.7, 139.4, 139.4, 139.3, 138.0, 134.3, 134.2, 133.3, 130.4, 130.3, 130.3, 130.2, 129.6, 129.4, 129.3, 129.2, 129.2, 129.2, 128.9, 128.8, 128.8, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 128.0, 127.9, 127.6, 127.6, 117.9, 102.3, 102.3, 101.4, 101.1, 101.1, 100.9, 97.5, 97.1, 97.0, 96.9, 82.0, 81.8, 81.8, 81.8, 80.6, 79.8, 79.7, 78.2, 77.7, 77.5, 77.2, 76.9, 76.3, 75.5, 75.4, 75.3, 75.0, 74.9, 74.8, 74.5, 74.4, 74.2, 73.9, 73.8, 73.5, 73.1, 72.9, 72.6, 72.4, 71.6, 71.4, 71.2, 71.0, 70.8, 69.7, 69.7, 69.4, 68.6, 68.2, 67.1, 66.9, 66.3, 66.1, 65.1, 63.0, 62.7, 58.8, 57.9, 57.6, 57.4, 57.4, 53.3, 51.5, 39.3, 38.2, 38.1, 30.2, 30.1, 28.0, 26.0, 21.2, 21.1, 20.9, 20.9, 20.7, 20.7, 20.6, 20.5, 16.1. HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{173}H_{193}Cl_9N_6O_{61}$ $[M+2Na]^{2+}$ 1845.4583, found 1845.4594.

*N^{α}-benzyloxycarbonyl-N^{γ}-(6-O-(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-3-O-benzyl-4-O-(3,6-di-O-benzyl-4-O-(3,6-bis-O-(4-O-acetyl-2-O-(4-O-(4-O-acetyl-2,3-di-O-benzoyl-6-O-(Methyl 4,7,8,9-tetra-O-acetyl-5-(N-acetylacetamide)-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-3,6-di-O-benzyl-D-mannopyranosyl)-2-O-benzyl-β-D-mannopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-L-asparagine allyl ester (**29**)*. Tetrasaccharide donor **3** (8.29 mg, 4.11 μmol) and octasaccharide-Asn acceptor **28** (10.0 mg, 2.74 μmol) were lyophilized from benzene and activated MS4A powder was added. To the mixture was added anhydrous CPME (132 μL). A solution of TMSOTf (30 μL, 0.166 mmol) in anhydrous CPME (1.0 mL) was dried over activated MS4A pellets. The solution of TMSOTf (5.0 μL, 0.822 μmol of TMSOTf) was added to the solution of **3** and **28** at 0 °C and the mixture was stirred for 10 min at the same temperature. The reaction was quenched by sat. aqueous $NaHCO_3$ and insoluble materials were filtered. The filtrate was poured into sat. $NaHCO_3$ and the aqueous layer was extracted with EtOAc. The organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product. Silica-gel column chromatography was

carried out to give **29** as a white solid of α/β mixture (13.1 mg, 87%, α/β = 1/1, estimated by ^{1}H NMR). The mixture was used for the next reaction without further purification. HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{262}H_{290}Cl_{12}N_8O_{94}$ $[M+3Na]^{3+}$ 1846.8032, found 1846.8000.

*N^{α}-benzyloxycarbonyl-N^{γ}-(2-acetamide-4-O-(2-acetamide-4-O-(3,6-bis-O-(2-O-(2-acetamide-4-O-(6-O-(5-acetamide-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-β-D-galactopyranosyl)-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-benzyl-α-D-manno pyranosyl)-2-O-benzyl-β-D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-3-O-benzyl -6-O-(2-O-benzyl-α-L-fucopyranosyl)-2-deoxy-β-D-glucopyranosyl)-L-asparagine (**30**)*. Solutions of $Pd(OAc)_2$ (1.23 mg, 5.46 μmol) in acetone (200 μL), PPh_3 (7.16 mg, 0.0273 mmol) in acetone (200 μL), and sodium 2-ethylhexanoate (30.2 mg, 0.182 mmol) in acetone (400 μL) were prepared. To a solution of dodecasaccharide-Asn **29** (10.0 mg, 1.82 μmol) in acetone (122 μL) were added the solution of $Pd(OAc)_2$ (20 μL, 0.546 μmol of $Pd(OAc)_2$), the solution of PPh_3 (20 μL, 2.73 μmol of PPh_3), and the solution of sodium 2-ethylhexanoate (20 μL, 9.10 μmol of sodium 2-ethylhexanoate). The reaction solution was stirred for 2 h at rt, diluted with EtOAc, and poured into brine. The aqueous layer was extracted with EtOAc and the organic layer was washed by brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a crude product of the carboxylic acid. The crude carboxylic acid was dissolved in THF (455 μL)/dioxane (303 μL) and 3M aqueous LiOH (152 μL) was added to the solution. The mixture was stirred overnight at rt to produce the tetramine. To the reaction mixture were added H_2O (76 μL), $NaHCO_3$ (61.2 mg, 0.728 mmol), and Ac_2O (34.4 μL, 0.364 mmol) and the mixture was stirred for another 1 h. Then, another portion of $NaHCO_3$ (61.2 mg, 0.728 mmol) and Ac_2O (34.4 μL, 0.364 mmol) were added to the reaction mixture and the mixture was stirred for 1 h. To the resulting mixture was added $LiOH•H_2O$ (30.5 mg, 0.728 mmol) and the mixture was stirred for 2 h. The reaction mixture was neutralized by dry ice and concentrated *in vacuo*. The residue was roughly purified on

diaion™ HP20 resin (H_2O/MeOH = 3/2 to 1/2) to give a crude product. HPLC purification (COSMOSIL 5C_{18}-AR-300 10 × 250 mm) was carried out to obtain pure **30** (1.88 mg, 27%) and the β isomer at 1^E-position (1.88 mg, 27%) as white solids. Eluting condition: H_2O + 0.1% TFA/MeCN + 0.1% TFA as mobile phase, 4 mL^{-1} isocratic flow of 53% MeCN, 7.1 min for **30**, 10.9 min for the β isomer. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.42-7.17 (m, 54H), 7.15-7.05 (m, 16H), 5.20 (s, 1H), 5.10-5.01 (m, 4H), 4.97-4.91 (m, 4H), 4.87-4.67 (m, 11H), 4.61-4.48 (m, 9H), 4.46-4.36 (m, 7H), 4.34-4.28 (m, 3H), 4.08-3.87 (m, 17H), 3.85-3.69 (m, 22H), 3.67-3.34 (m, 32H), 3.17 (d, 1H, *J* = 7.0 Hz), 2.70-2.61 (m, 4H), 2.00 (s, 6H), 1.82 (s, 3H), 1.81 (s, 3H), 1.81 (s, 3H), 1.77-1.69 (m, 5H), 1.12 (d, 3H, *J* = 6.4 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ = 175.3, 174.5, 174.0, 173.6, 173.5, 173.3, 172.6, 171.8, 171.8, 140.9, 140.6, 140.2, 140.2, 140.0, 139.8, 139.7, 139.7, 139.6, 139.5, 138.0, 129.6, 129.6, 129.5, 129.5, 129.5, 129.4, 129.3, 129.2, 129.2, 129.2, 129.1, 129.0, 129.0, 128.9, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 104.7, 104.4, 101.7, 101.5, 101.1, 100.9, 100.9, 100.8, 100.3, 100.2, 98.6, 98.3, 83.3, 81.8, 81.6, 81.3, 80.5, 79.8, 79.6, 78.6, 78.3, 78.0, 77.9, 77.6, 77.1, 76.9, 76.6, 76.4, 76.3, 76.1, 75.8, 75.6, 75.3, 75.1, 75.1, 74.9, 74.9, 74.5, 74.4, 74.3, 74.2, 74.1, 74.0, 73.9, 73.5, 73.2, 73.1, 73.0, 72.6, 72.2, 71.7, 71.6, 71.4, 71.2, 70.3, 70.1, 69.9, 69.8, 69.5, 68.8, 68.4, 67.8, 67.7, 67.5, 67.2, 66.8, 64.5, 64.5, 63.3, 57.4, 56.5, 56.3, 54.9, 51.9, 40.9, 40.5, 38.5, 33.0, 30.7, 23.7, 23.4, 23.4, 22.9, 22.7, 16.6. HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{193}H_{238}N_8O_{70}$ $[M+3Na]^{3+}$ 1285.4995, found 1285.4995.

*N^{γ}-(2-acetamide-4-O-(2-acetamide-4-O-(3,6-bis-O-(2-O-(2-acetamide-4-O-(6-O-(5-acetamide-3,5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl)-β-D-mannopyranosyl)-2-deoxy-β-D-glucopyranosyl)-2-deoxy-6-O-(α-L-fucopyranosyl)-β-D-glucopyranosyl)-L-asparagine (**1**).* To a solution of dodecasaccharide-Asn **30** (5.00 mg, 1.32 μmol) in *t*-BuOH (314 μL)/dist. H_2O (314 μL)/AcOH (63 μL) was added a suspension of 20% $Pd(OH)_2$/C (35.0 mg) in *t*-BuOH (314 μL)/dist. H_2O

(314 μL). The mixture was stirred under H_2 (2.0 MPa) atmosphere overnight at rt. After the reaction, insoluble materials were filtered through Hyflo Super Cel® and washed by dist. H_2O + 0.1% TFA and MeOH. The filtrate was concentrated *in vacuo* and lyophilized from H_2O to give a crude product. The crude product was purified on Sephadex LH-20 (H_2O/MeOH = 1/2 as an eluent) to obtain *N*-glycan **1** (1.97 mg, 60%) as a white solid. ^{1}H NMR (600 MHz, D_2O): δ = 5.03 (s, 1H), 4.97 (d, 1H, *J* = 9.4 Hz), 4.84 (s, 1H), 4.77 (d, 1H, *J* = 3.7 Hz), 4.68 (s, 1H), 4.58 (d, 1H, *J* = 7.8 Hz), 4.50 (d, 2H, *J* = 7.4 Hz), 4.34 (d, 2H, *J* = 7.9 Hz), 4.16 (s, 1H), 4.10 (d, 1H, *J* = 2.4 Hz), 4.04-3.99 (m, 3H), 3.91-3.38 (m, 68H), 2.81-2.74 (br m, 1H), 2.69-2.63 (br m, 1H), 2.59-2.54 (m, 2H), 1.99 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.93 (s, 6H), 1.91 (s, 3H), 1.62 (dd, 2H, *J* = 12.3, 12.1 Hz), 1.10 (d, 3H, *J* = 6.5 Hz). ^{13}C NMR from HSQC/HMBC spectra (150 MHz, D_2O): δ = 180.1, 174.81, 173.1, 174.6, 174.48, 103.35 ($^1J_{CH}$ = 160.8 Hz, C-$1^{I/J}$), 100.83 ($^1J_{CH}$ = 160.7 Hz, C-1^{C}), 100.5 ($^1J_{CH}$ = 159.7 Hz, C-1^{D}), 99.8, 99.4 ($^1J_{CH}$ = 169.3 Hz, C-1^{F}), 99.25 ($^1J_{CH}$ = 168.3 Hz, C-1^{B}), 99.2 ($^1J_{CH}$ = 160.4 Hz, C-$1^{G/H}$), 96.77 ($^1J_{CH}$ = 170.5 Hz, C-1^{E}), 80.55, 80.4, 79.7, 77.92 ($^1J_{CH}$ = 155.3 Hz, C-1^{A}), 76.07, 76.02, 74.39, 74.3, 74.22, 73.63, 73.42, 73.3, 73.27, 72.7, 72.4, 72.39, 72.1, 72.04, 71.99, 71.9, 71.7, 70.67, 70.1, 69.4, 69.3, 69.3, 68.3, 68.26, 68.2, 68.0, 67.27, 67.2, 66.8, 66.3, 65.9, 63.26, 62.6, 61.54, 61.5, 60.13, 59.9, 54.68, 54.56, 53.45, 51.6, 51.19, 48.2, 40.0, 36.0, 15.0. HR ESI-Orbitrap MS: *m*/*z* calcd for $C_{94}H_{154}N_8O_{68}$ $[M+2H]^{2+}$ 1242.4492, found 1242.4505.

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Associated Content

Supporting information. ^{1}H and ^{13}C NMR spectra for new compounds. This material is available free of charge from ACS website.

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