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Rapid Assembly of Oligosaccharides: A Highly Convergent Strategy for the Assembly of a Glycosylated Amino Acid Derived from PSGL-1

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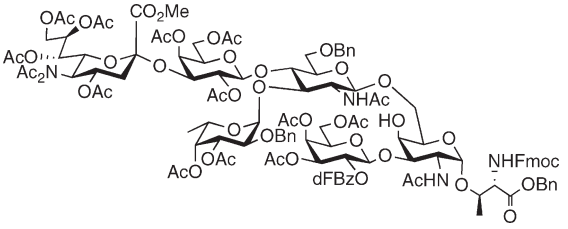

P-Selectin and P-selectin glycoprotein ligand 1 (PSGL-1) are vascular adhesion molecules that play an important role in the recruitment of leukocytes to inflamed tissue by establishing leukocyte−endothelial and leukocyte−platelet interaction. P-Selectin binds to the amino-terminus of PSGL-1 through recognition of a sialyl Lewis^x (SLe^x) moiety linked to a properly positioned core-2 *O*-glycan and three tyrosine sulfate residues. We have developed a highly convergent synthesis of the PSGL-1 oligosaccharide linked to threonine based on the use of trichoroacetimidate donors and thioglycosyl acceptors that give products that can immediately be employed in a subsequent glycosylation step without the need for protecting group manipulations. Furthermore, by employing one-pot multistep glycosylation sequences the number of purification steps could be minimized. The process of oligosaccharide assembly was further streamlined by combining protecting group manipulations and glycosylations as a one-pot multistep synthetic procedure. The resulting PSGL-1 oligosaccharide is properly protected for glycopeptide assembly. It is to be expected that the strategic principles employed for the synthesis of the target compound can be applied for the preparation of other complex oligosaccharides of biological and medical importance.

Introduction

The selectins are a family of three Ca^{2+}-dependent membrane-bound glycoproteins that mediate the adhesion of leukocytes and platelets to vascular surfaces.[1,2] Several studies have demonstrated that they play important roles in inflammation, immune responses, hemeostasis, and wound repair.[3] Selectins also contribute to a broad spectrum of diseases such as arteriosclerosis, thrombosis, organ-transplant rejection, arthritis, sickle cell anemia, and tumor metastasis.[4−6]

Although there are many candidates for selectin ligands, only P-selectin glycoprotein ligand-1 (PSGL-1) has clearly been demonstrated to mediate the adhesion of leukocytes to selectins under flow. P-Selectin binds to the amino-terminus of PSGL-1 through recognition of a sialyl Lewis^x (SLe^x) moiety linked to a properly positioned core-2 *O*-glycan and three tyrosine sulfate residues.[7,8]

Inhibitors of selectins may possess therapeutic properties for the treatment of a number of diseases.[9] In this respect, a recombinant truncated form of a PSGL-1 immunoglobulin fusion protein has already demonstrated effectiveness as

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such an inhibitor.[10−13] This glycoprotein can, however, only be produced in mammalian cells that are cotransfected with fucosyl- and core-2 GlcNAc transferases, making production of even small amounts of glycoprotein difficult. The Davis laboratory is beginning to address these problems by employing a PSGL-1 mimetic by incorporation of azidohomoalanine and cysteine in PSGL-1 using *E. coli B834* as a Met-auxotrophic expression system and selective attachment of sialic acid containing oligosaccharides and a sulfated tyrosine mimic by employing the thiol and azide of the protein as chemical tags.[14] Also, it has been shown that conjugation of sialyl Lewis^x and tyrosine sulfates to a polyacrylamide gave a polymer with high affinity of L-selectin.[15]

The *N*-terminal glycosulfopeptide of PGSL-1 has been obtained by chemoenzymatic approaches.[14,16,17] In these procedures, a glycosulfopeptide that contains an *N*-acetyl galactosamine linked to a threonine moiety was chemically assembled. Subsequently, glycosyl transferases were employed to assemble the complete oligosaccharide. The problems of this approach include difficulties of preparing sufficient quantities of glycosyltransferases, which often require a eukaryotic cell expression system and the need of expensive sugar nucleotides or the use of a complicated in situ recycling system. Furthermore, the high selectivity of glycosyltransferases also complicates the preparation of analogues that may exhibit more desirable pharmacological properties.

Recent progress in chemical oligosaccharide synthesis is beginning to provide opportunities for the efficient and large-scale synthesis of complex oligosaccharides[18−23] and several laboratories are pursuing the preparation of the oligosaccharide of PSGL-1.[24−30] In this respect, Kunz and co-workers have reported the chemical synthesis of a properly protected oligosaccharide of PSGL-1, which was attached to threonine for the preparation of a glycopeptide.[26] Although synthetic problems such as anomeric selectivity and the acid and base sensitivity of the PSGL-1 glycopeptide were addressed by employing properly protected saccharide building blocks, the synthetic approach suffered from poor regioselectivity in key glycosylations and a need for replacement of protecting groups at an advanced stage of synthesis. It is to be expected that a highly convergent approach for the synthesis of PSGL-1 will make it possible to prepare a wide range of glycopeptides structural analogues for structure activity relationships. Furthermore, it may offer an opportunity to make mimetics that have improved pharmacokinetic properties.

As part of a program to prepare the PGSL-1 analogues with improved properties, we report here a highly efficient and convergent synthesis of a properly protected oligosaccharide of PSGL-1 linked to threonine (**1**) that is appropriately protected for solid-phase glycopeptide synthesis. Key features of the approach include an orchestrated use of thioglycosides and trichoroacetimidates[31] for oligosaccharide assembly, which minimized protecting group manipulations and made it possible to employ one-pot multistep glycosylations. The process of oligosaccharide assembly was further streamlined by combining protecting group manipulations and glycosylations as a one-pot multistep synthetic procedure.[22,23,32−34] It is to be expected that the strategic principles employed for the synthesis of the target compound can be applied for the preparation of other complex oligosaccharides of biological and medical importance.

Results and Discussion

The synthesis of target compound **1** is complicated by the fact that *O*-glycosylated peptides are sensitive to acidic and basic conditions. In addition, sufficient quantities of such a complex glycosylated amino acid for glycosulfopeptide assembly can only be obtained by employing a highly convergent synthetic strategy, which uses properly protected monosaccharide building blocks that can be assembled into the target by using a minimal number of synthetic steps. In this respect, strategies such as chemoselective, orthogonal, two-directional, and one-pot multistep glycosylations[22,23,32−34] have engendered an increased efficiency of oligosaccharide synthesis by minimizing the number of protecting group manipulations on advanced intermediates. Furthermore, combining protecting group manipulations with glycosylations as a one-pot procedure can further expand the scope of these procedures.[35−39]

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It was envisaged that **1** could be prepared by a combination of chemo- and regioselective glycosylations and one-pot multistep protocols that combine glycosylations and protecting group manipulations. Thus, the core-2 disaccharide linked to a properly protected threonine (Thr) was prepared by a chemoselective glycosylation of trichloroacetimidate **2** with thioglycoside **3** to give a disaccharide, which immediately can be activated with a thiophilic reagent for coupling with threonine acceptor **4**[40] (Figure 1). Removal of the benzylidene acetal of the resulting compound will give an acceptor for a regioselective coupling with a properly protected SLe^x derivative. It was envisaged the latter compound could be obtained by chemoselective glycosylations and one-pot reactions by using compounds **5**,[41] **6**,[42] and **7**.

Unfortunately, coupling of galactosyl trichloroacetimidate **2a**[43] with thioglycosyl acceptor **3a**[44] in the presence of TMSOTf led to a complex mixture of products that included the required disaccharide, the corresponding ortho-ester,[45] and a thiophenyl galactoside derived from aglycon-transfer[46] (Scheme 1). It is well-known that the use of C-2 benzoyl esters will suppress ortho-ester formation; however, this protecting group is not compatible with glycopeptide synthesis because the rather strong basic conditions required for its removal[47] will result in β-elimination of the *O*-glycopeptide linkage. It has been shown that a 2,5-difluorobenzoyl ester (dFBz) is an efficient neighboring group participant that suppresses ortho-ester formation.[48,49] This protecting group has, however, the advantage that it can be removed under mild basic conditions without affecting threonine and serine glycosides. Thus, dFBz-protected glycosyl donor **2b**,[48,49] having a 2,5-difluorobenzoyl (dFBz) ester at C-2 and acetyl esters at C-3, C-4, and C-6, was coupled with thioglycosyl acceptor **3a**[50] by using TMSOTf as the catalyst.[51] Although ortho-ester formation was suppressed, the aglycon-transfer byproduct was still formed. Recently, it was reported that aglycon transfer of thioglycosyl acceptors can be avoided by employing a 2,6-dimethylthiophenyl glycoside.[52] The rationale of this observation is that the bulky 2,6-dimethylthiophenyl hinders reaction with an activated glycosyl donor, thereby reducing aglycon transfer. Indeed, trimethylsilyl triflate (TMSOTf) promoted glycosylation of **2b** with **3b**[52] gave the corresponding disaccharide **8** in an excellent yield of 90% as only the β-anomer. Next, the core-2 *O*-glycan **9** was obtained in high yield with exclusively α-selectivity by a diphenyl sulfoxide/triflic anhydride-mediated glycosylation[53] of thioglycoside **8** with threonine derivative **4.**

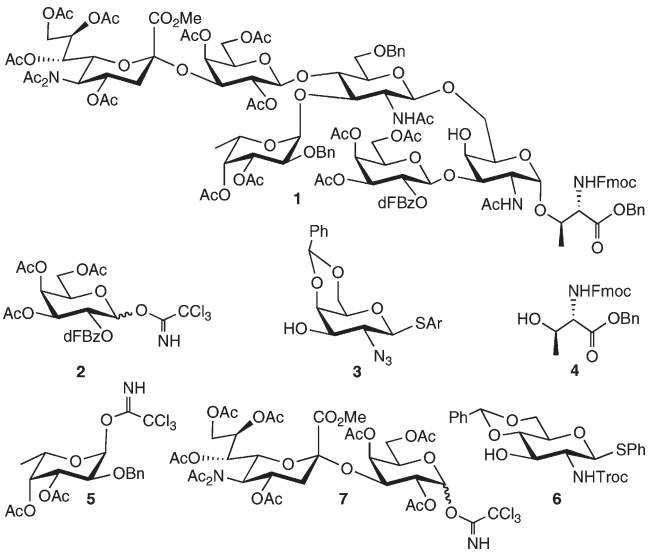

FIGURE 1. Target molecule and building blocks.

Having established efficient reaction conditions for the synthesis of **9**, attention was focused on its preparation by a one-pot procedure. Thus, coupling of galactosyl trichloroacetimidate **2b** with the galactosyl acceptor **3b** in the presence of TMSOTf followed by activation of the resulting thiodisaccharide **8** by addition of diphenyl sulfoxide and triflic anhydride in the presence of DTBMP[53] and coupling with threonine **4** gave oligosaccharide **9** in an overall yield of 61%. Finally, glycosyl acceptor **10** was obtained by the removal of the benzylidene acetal of **9** by using aqueous acetic acid at 70 °C.

The next stage of the synthesis entailed the preparation of properly protected SLe^x glycosyl donor **15** for coupling with glycosyl acceptor **10** to give protected PSGL-1 **16**. Compound **15** was prepared from the readily available saccharide building blocks **5**, **6**, and **7** (Schemes 2 and 3). Thus, fucosyl trichloroacetimidate **5** was coupled with phenyl thioglycosyl acceptor **6** by using TMSOTf as the promoter to give the corresponding disaccharide, exclusively as the α-anomer, which was then treated with triethylsilane and trifluoromethanesulfonic acid (TfOH) for regioselective opening of the benzylidene acetal[39,54] to provide glycosyl acceptor **11** in an overall yield of 84% with excellent stereo- and regioselectivity. The regioselectivity of the latter reaction was confirmed by acetylation of compound **11** and the ^{1}H NMR of the resulting derivative showed a significant downfield shift for H-4 (4.94 ppm).

Glycosyl donor **7** could be obtained in facile manner from the known disaccharide **12**[55] by a four-step reaction sequence. Thus, hydrogenation of **12** over Pd/C to remove the benzylidene acetal was followed by acetylation of the hydroxyls of the resulting compound **13** to give **14** in a quantitative overall yield. Next, the anomeric trimethylsilylethyl

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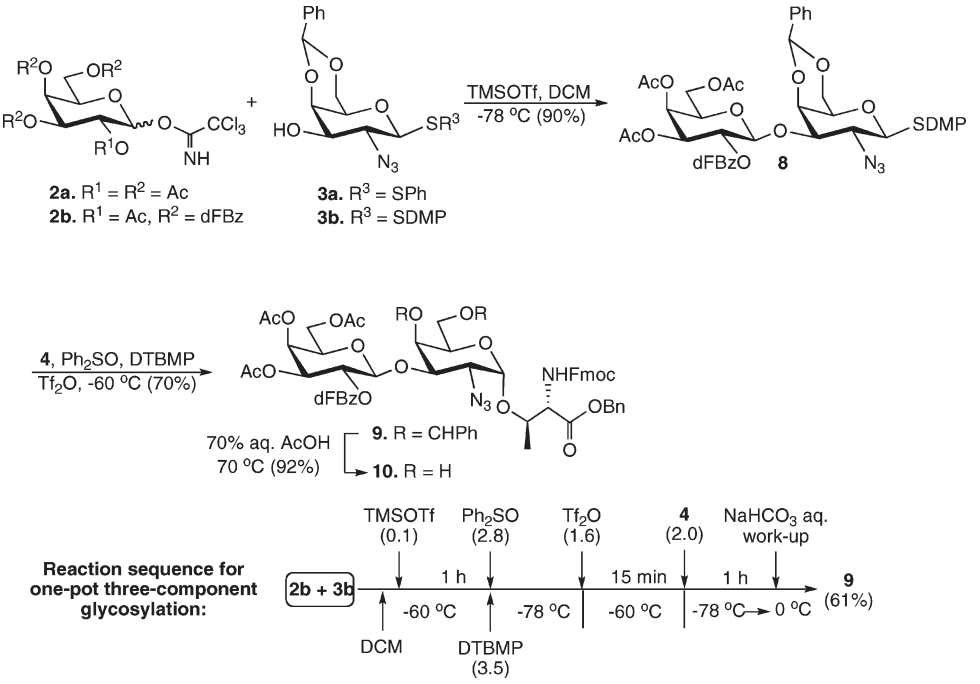
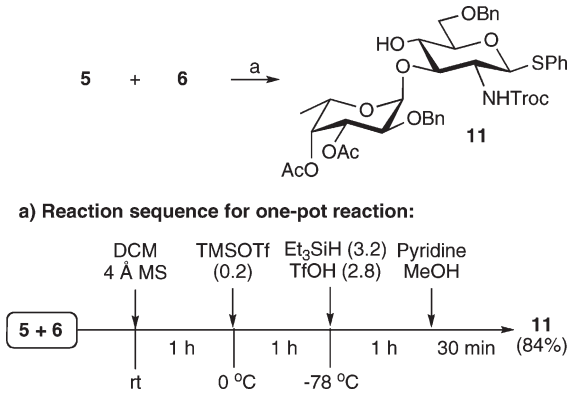
SCHEME 1. One-Pot Three-Component Reaction[a]



[a]SDMP = 2,6-dimethylthiophenyl, dFBz = 2,5-difluorobenzyl.

SCHEME 2. One-Pot Glycosylation Followed by Reductive Opening of the Benzylidene Acetal



moiety of **14** was cleaved by treatment with trifluoroacetic acid in dichloromethane and the resulting lactol was converted into trichloroacetimidate **7** by reaction with trichloroacetonitrile and DBU in dichloromethane.

Next, a TMSOTf-mediated coupling of trichloroactimidate **7** with **11** gave the properly protected SLe^x tetrasaccharide **15** in good yield. Up to this stage of the synthesis, the thiophenyl moiety of **15** has functioned as an effective anomeric protecting group. However, in the next step it was activated with the thiophilic promoter NIS/TfOH for coupling with **10** to give the hexasaccharide **16** in a yield of 55%. As expected, no glycosylation of the less reactive C-4 hydroxyl of **11** was observed, which was confirmed by a range of two-dimensional NMR experiments. Thus, Heteronuclear Multiple Bond Correlation NMR Spectroscopy (HMBC) of **16** showed a cross peak between H-1 of β-GluNTroc (4.62 ppm) and C-6 of α-GalN_3 (69.3 ppm), confirming that the glycosylation had occurred at the C-6 hydroxyl of **10**. The latter was also supported by Nuclear Overhauser Enhancement Spectroscopy (NOESY), which revealed cross peaks between the H-1 of β-GluNTroc and H-6a and H-6b of the α-GalN_3 moiety. Furthermore, due to effective neighboring group participation of the *N*-Troc group of **15** only the β-glycoside was formed, which was confirmed by a large coupling constant between H-1 and H-2 (10.0 Hz).

Finally, the Troc and the azido moiety of **16** were converted into acetamido functions by reduction with Zn/$CuSO_4$ in a mixture of THF, acetic acid, and acetic anhydride[56] to give the target compound **1**. It is envisaged that the benzyl ester of compound **1** can be removed by performing hydrogenolysis over palladium in a mixture of isopropanol and pyridine.[57] The benzyl ethers in the glycan will be removed after glycopeptide assembly by using the previously described "low TfOH" method.[58,59]

In conclusion, a properly protected PSGL-1 oligosaccharide linked to threonine has been described that is appropriately protected for glycosulfopeptide assembly. A highly convergent strategy utilizing six strategically protected building blocks, combined with one-pot chemo- and regioselective glycosylations was employed to minimize the number of protecting group manipulations and purifications during oligosaccharide assembly. The longest linear sequence entailed only seven chemical steps and gave the target compounds in an excellent overall yield of 17%. Previous

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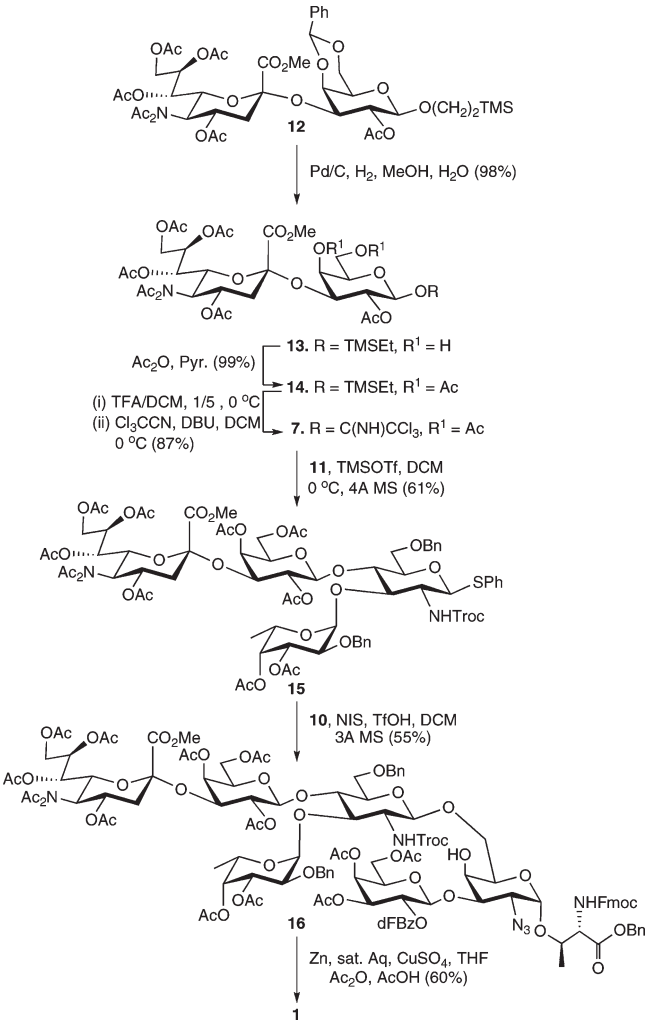
SCHEME 3. Synthesis of Target Molecule



attempts to chemically synthesize the PSGL-1 oligosaccharide suffered from extensive replacement of protecting groups at advanced stages of the synthesis and poor regioselectivities in crucial glycosylation steps compromising the poor overall yield of the target compound.[26] It is to be expected that the strategic principles employed for the synthesis of **1** will be relevant for the synthesis of many other complex oligosaccharides of biological and medical importance.

Experimental Section

2,6-Dimethylphenyl [2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-*β*-D-galactopyranosyl)]-1-thio-α-D-galactopyranoside (8). A mixture of galactosyl acceptor **3b** (93 mg, 0.22 mmol), galactosyl trichloroacetaimidate donor **2b** (200 mg, 0.34 mmol), and 4 Å MS in CH_2Cl_2 (3 mL) was placed under an atmosphere of argon and stirred at room temperature for 1 h. The reaction mixture was then cooled to −78 °C. TMSOTf (0.023 mmol, 0.23 M solution in CH_2Cl_2) was added and the temperature was raised to −15 °C with stirring over a period of 2 h. The progress of the reaction was monitored by TLC and MALDI-ToF MS. The reaction mixture was diluted with CH_2Cl_2 (20 mL), filtered, and washed with saturated aq $NaHCO_3$ solution (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes:EtOAc, 2:1, v:v) to afford compound **8** (170 mg, 90%) as a white foam. Analytical data for **8**: R_f 0.35 (hexanes:EtOAc, 2:1, v:v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52−6.97 (m, 6H, aromatic), 5.49 (dd, 1H, $J_{1',2'} = 8.2$ Hz, $J_{2',3'} = 10.2$ Hz, H-2′), 5.43 (s, 1H, C*H*Ph), 5.38 (br d, 1H, $J = 3.2$ Hz, H-4′), 5.12 (dd, 1H, $J_{3',4'} = 3.1$ Hz, $J_{2',3'} = 10.5$ Hz, H-3′), 4.96 (d, 1H, $J_{1',2'} = 7.9$ Hz, H-1′), 4.18 (m, 2H, H-1, H-4), 4.15−4.05 (m, 3H, H-6a, H-6a′, H-6b′), 3.91−3.85 (m, 2H, H-5′, H-6b), 3.72 (t, 1H, $J_{1,2} = J_{2,3} = 9.9$ Hz, H-2), 3.46 (dd, 1H, $J_{3,4} = 3.1$ Hz, $J_{2,3} = 9.9$ Hz, H-3), 3.17 (br s, 1H, H-5), 2.51 (s, 6H, 2 × CH_3, SDMP), 2.11 (s, 3H, COCH_3), 1.98 (s, 3H, COCH_3), 1.87 (s, 3H, COCH_3) ppm; ^{13}C from HSQC (125.7 MHz, $CDCl_3$) δ 102.3 (C-1′), 101.1 (*C*HPh), 89.4 (C-1), 80.9 (C-3), 75.3 (C-4), 71.4 (C-5′), 71.2 (C-3′), 70.3 (C-2′), 70.0 (C-5), 69.6 (C-6), 67.3 (C-4′), 62.4 (C-2), 61.8 (C-6′), 22.9 (*C*H_3-SDMP), 21.0, 20.9, 20.8 (3 × OAc); HR-MALDI-ToF/MS *m*/*z* calcd for $C_{40}H_{41}F_2N_3O_{13}S$ $[M+Na]^+$ 864.2226, found 864.2231.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-[2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-*β*-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine Benzyl Ester (9). Method A:.** A mixture of disaccharide donor **8** (170 mg, 0.20 mmol), Ph_2SO (114 mg, 0.56 mmol), and 4 Å MS in CH_2Cl_2 (5 mL) was placed under an atmosphere of argon and stirred at room temperature for 1 h. The reaction mixture was then cooled to −60 °C after the addition of 2,6-di-*tert*-butyl-4-methylpyridine (124 mg, 0.60 mmol). Stirring was continued for 10 min at the same temperature followed by the addition of Tf_2O (47 μL, 0.28 mmol). Stirring was continued for another 15 min at the same temperature followed by the addition of a solution of threonine acceptor **4** (173 mg, 0.40 mmol) in CH_2Cl_2 (2 mL). The temperature of the reaction mixture was raised to 0 °C over a period of 1 h. The progress of reaction was monitored by TLC and MALDI-ToF MS. The reaction mixture was diluted with CH_2Cl_2 (30 mL), filtered, and washed with saturated aq $NaHCO_3$ solution (15 mL), water (15 mL), and brine (15 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes:EtOAc, 2:1, v:v) to afford compound **9** (156 mg, 70%) as a white amorphous solid.

Method B:. A mixture of galactosyl acceptor **3b** (65 mg, 0.16 mmol), galactosyl trichloroacetaimidate donor **2b** (120 mg, 0.20 mmol), and 4 Å MS in CH_2Cl_2 (2 mL) was placed under an atmosphere of argon and stirred at room temperature for 1 h. The reaction mixture was then cooled to −60 °C. TMSOTf (0.016 mmol, 0.16 M solution in CH_2Cl_2) was added and stirring was continued for 1 h at the same temperature. The reaction mixture was then cooled to −78 °C followed by addition of Ph_2SO (88 mg, 0.44 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (112 mg, 0.55 mmol). After stirring for 10 min at the same temperature, Tf_2O (37 μL, 0.22 mmol) was added followed by increasing the temperature to −60 °C over a period of 15 min. The reaction mixture was again cooled to −78 °C followed by addition of a solution of threonine acceptor **4** (100 mg, 0.23 mmol) in CH_2Cl_2 (1 mL). The temperature of the reaction mixture was raised to 0 °C over a period of 1 h. The progress of the reaction was monitored by TLC and MALDI-ToF MS. The reaction mixture was diluted with CH_2Cl_2 (20 mL), filtered, and washed with saturated aq $NaHCO_3$ solution (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes:EtOAc, 2:1, v:v) to afford compound **9** (106 mg, 61%) as a white amorphous solid. Analytical data for **9**: R_f 0.25 (hexanes:EtOAc, 2:1, v:v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72−6.87 (m, 21H, aromatic), 5.66 (d, 1H, $J = 9.4$, N*H*Fmoc), 5.51−5.45 (m, 2H, C*H*Ph, H-2′), 5.40 (br d, 1H, H-4′), 5.15 (dd, 1H, $J_{3',4'} = 3.2$ Hz, $J_{2',3'} = 10.2$ Hz, H-3′), 5.10 (br t, 2H, CH_2, Bn), 4.88 (d, 1H, $J_{1',2'} = 7.9$ Hz, H-1′), 4.83 (d, 1H, $J_{1,2} = 3.5$, H-1), 4.46−4.25 (m, 5H, OC*H*CH$_3$ threonine,

CH_2CH-Fmoc, CHCOOBn threonine, H-4), 4.21−4.08 (m, 4H, H-6a′, CH_2CH-Fmoc, H-6b′, H-6a), 3.96−3.92 (m, 3H, H-6b, H-3, H-5′), 3.69 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.8 Hz, H-2), 3.56 (br s, 1H, H-5), 2.11 (s, 3H, OCH_3), 1.97 (s, 3H, COCH_3), 1.87 (s, 3H, COCH_3), 1.23 (d, 3H, OCHCH_3 threonine) ppm; ^{13}C from HSQC (125.7 MHz, $CDCl_3$) δ 102.3 (C-1′), 100.8 (CHPh), 99.4 (C-1), 76.2 (OCHCH$_3$ threonine), 75.9 (C-4), 75.8 (C-3), 71.2 (C-5′), 71.1 (C-3′), 70.3 (C-2′), 69.3 (C-6), 68.0 (CH_2Ph), 67.6 (CH_2CH-Fmoc), 67.3 (C-4′), 63.7 (C-5), 61.5 (C-6′), 59.5 (C-2), 58.9 (CHCOOBn threonine), 47.4 (CH_2CH-Fmoc), 21.0, 20.9, 20.7 (3 × OAc), 19.0 (OCHCH_3 threonine); HR-MALDI-ToF/MS *m*/*z* calcd for $C_{58}H_{56}F_2N_4O_{18}$ [M + Na]$^+$ 1157.3455, found 1157.3460 [M + Na]$^+$.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-[2-azido-2-deoxy-3-*O*-(3,4,-6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine Benzyl Ester (10).** A solution of compound **9** (80 mg, 0.072 mmol) in 5 mL of 70% aq acetic acid was heated at 70 °C for 3 h. The progress of the reaction was monitored by TLC and MALDI-ToF MS. The reaction was cooled to rt and concentrated by coevaporation with toluene in vacuo. The residue was purified by silica gel column chromatography (hexanes:EtOAc, 1:2, v:v) to afford compound **10** (59 mg, 92%) as a white amorphous solid. Analytical data for **10**: R_f 0.25 (hexanes:EtOAc, 1:2, v:v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72−6.94 (m, 16H, aromatic), 5.60 (d, 1H, *J* = 9.5 Hz, NHFmoc), 5.50−5.46 (q, 1H, $J_{1',2'}$ = 8.6 Hz, $J_{3',2'}$ = 10.3 Hz, H-2′), 5.40 (br d, 1H, H-4′), 5.16 (q, 1H, $J_{3',4'}$ = 2.9 Hz, $J_{2'',3'}$ = 10.3 Hz, H-3′), 5.12−5.01 (dd, 2H, CH_2Ph), 4.81 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1′), 4.76 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 4.41−4.35 (m, 3H, OCHCH$_3$ threonine, CHCOOBn threonine, CHH-Fmoc), 4.25−4.21 (m, 1H, CHH-Fmoc), 4.16−4.05 (m, 4H, H-6a′, H-6b′, H-4, CH_2CH-Fmoc), 3.96 (m, 1H, H-5′), 3.91−3.88 (dd, 1H, $J_{3,4}$ = 2.7 Hz, $J_{2,3}$ = 10.7 Hz, H-3), 3.83−3.70 (m, 3H, H-5, H-6a, H-6b), 3.46−3.44 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 2.75 (br s, 1H, C4−OH), 2.32 (br s, 1H, C6−OH), 2.13 (s, 3H, OCH_3), 2.00 (s, 3H, COCH_3), 1.90 (s, 3H, COCH_3), 1.24 (d, 3H, OCHCH_3) ppm; ^{13}C from HSQC (125.7 MHz, $CDCl_3$) δ 101.8 (C-1′), 99.2 (C-1), 78.1 (C-3), 76.2 (OCHCH$_3$ threonine), 71.7 (C-5′), 70.7 (C-3′), 69.8 (C-2′), 69.7 (C-5), 69.3 (C-4), 67.8 (CH_2Ph), 67.4 (CH_2CH-Fmoc), 67.2 (C-4′), 62.7 (C-6), 61.6 (C-6′), 59.0 (C-2), 58.7 (CHCOOBn threonine), 47.4 (CH_2CH-Fmoc), 20.8, 20.7, 20.6 (3 × OAc), 18.6 (OCHCH_3 threonine); HR-MALDI-ToF/MS *m*/*z* calcd for $C_{51}H_{52}F_2N_4O_{18}$ [M + Na]$^+$ 1069.3142, found 1069.3140.

Phenyl 3,4-Di-*O*-acetyl-2-*O*-benzyl-6-deoxy-5-methyl-α-L-fucopyranosyl-(1→3)-2-(2,2,2-trichloroethoxy)carbonylamino-6-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (11). A mixture of glycosyl acceptor **6** (50 mg, 0.09 mmol), trichloroacetimidate donor **5** (63 mg, 0.13 mmol), and 4 Å MS in CH_2Cl_2 (1 mL) was placed under an atmosphere of argon and stirred at room temperature for 1 h. The reaction mixture was then cooled to 0 °C. TMSOTf (0.018 mmol, 0.18 M solution in CH_2Cl_2) was added and stirring was continued for 30 min at the same temperature. The reaction mixture was then cooled to −78 °C followed by addition of TfOH (23 μL, 0.26 mmol) and triethylsilane (48 μL, 0.30 mmol). The reaction mixture was then stirred at −78 °C for 30 min. The progress of the reaction was monitored by TLC and MALDI-ToF MS. The reaction was quenched by the addition of pyridine (25 μL) and MeOH (0.2 mL), diluted with CH_2Cl_2 (20 mL), and washed with saturated aq $NaHCO_3$ solution (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes:EtOAc, 2:1, v:v) to afford compound **11** (67 mg, 84%) as a white amorphous solid. Analytical data for **11**: R_f 0.40 (hexanes:EtOAc, 1:1, v:v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50−7.17 (m, 15H, aromatic), 5.46 (d, 1H, *J* = 6.6 Hz, NHTroc), 5.30−5.27 (m, 2H, H-3′, H-4′), 5.09 (m, 2H, H-1, H-1′), 4.70−4.57 (m, 6H, 2 × CH_2, Bn, OCH_2CCl_3), 4.42 (m, 1H, H-5′), 3.89−3.83 (m, 3H, H-2′, H-3, H-6a), 3.78−3.75 (dd, 1H, H-6b), 3.68 (br s, 1H, C4−OH), 3.58−3.55 (m, 2H, H-4, H-5), 3.31 (m, 1H, H-2), 2.13 (s, 3H, COCH_3), 1.98 (s, 3H, COCH_3), 1.12 (d, 3H, *J* = 6.6 Hz, CH_3 fucose) ppm; ^{13}C from HSQC (125.7 MHz, $CDCl_3$) δ 98.6 (C-1′), 85.9 (C-1), 83.8 (C-3), 78.7 (C-5), 74.1−73.6 (2 × CH_2Ph, CH_2Troc), 73.8 (C-2′), 71.2 (C-3′), 71.0 (C-4), 70.3 (C-4′), 70.2 (C-6), 66.0 (C-5′), 55.7 (C-3), 21.0, 20.8 (2×OAc), 16.4 (C-6′); HR-MALDI-ToF/MS *m*/*z* calcd for $C_{39}H_{44}Cl_3NO_{12}S$ [M+Na]$^+$ 878.1547, found 878.1543.

2-(Trimethylsilyl)ethyl [Methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate]-(2→3)-*O*-β-D-galactopyranoside (13). To a solution of **12** (250 mg, 0.283 mmol) in CH_2Cl_2:MeOH (30:1, v:v; 15 mL) under an argon atmosphere was added Pd, 10 wt % on activated carbon (150 mg), then the mixture was stirred for 20 min at room temperature. The argon was replaced with $H_{2(g)}$, and the reaction was stirred for 8 h. The solution was diluted with CH_2Cl_2 (50 mL) and filtered through Celite. The solvent was removed by evaporation under reduced pressure and the residue was purified by silica gel column chromatography (toluene:acetone, 5:2, v:v) to afford compound **13** (220 mg, 98%) as a white amorphous solid. Analytical data for **13**: R_f 0.48 (toluene:acetone, 1:1, v:v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.52 (ddd, 1H, H-4′), 5.34 (dd, 1H, H-8′), 5.15 (dd, 1H, *J* = 8.2 Hz, H-7′), 4.94 (d, 1H, *J* = 10.3 Hz, H-6′), 4.42 (d, 1H, *J* = 7.6 Hz, H-1), 4.33 (dd, 1H, H-9a′), 4.21 (t, 1H, *J* = 10.2 Hz, H-5′), 4.12 (dd, 1H, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 3.1 Hz, H-3), 4.08 (dd, 1H, H-9b′), 4.02 (m, 1H, OCHH), 3.91 (dd, 1H, H-6), 3.92−3.82 (m, 5H, H-6a, H-6b,COOCH_3), 3.70−3.60 (m, 3H, H-4, H-2, OCHH), 3.55 (t, 1H, H-5), 2.86 (dd, 1H, H-3′eq), 2.35, 2.28 (2*s*, 6H, N(COCH_3)$_2$), 2.10, 2.09, 2.00, 1.97 (4*s*, 12H, 4 × COCH_3), 1.93 (dd, 1H, H-3′ax), 1.13−0.85 (m, 2H, CH_2SiMe$_3$), 0.01 (s, 9H, Si(CH_3)$_3$); ^{13}C from HSQC (125.7 MHz, $CDCl_3$) δ 102.7 (C-1), 77.2 (C-3), 73.8 (C-5), 70.4 (C-6′), 69.5 (C-2), 68.9 (C-4), 68.7 (C-8′), 67.3 ($CH_2CH_2SiMe_3$), 66.9 (C-7′), 66.8 (C-4′), 62.6 (C-6), 62.2 (C-9′), 56.9 (C-5′), 53.7 (COOCH_3), 38.6 (C-3′), 28.3, 26.3 (NAc$_2$), 21.4, 21.1, 21.0 (3 × OAc), 18.3 (CH_2SiMe$_3$); HR-MALDI-ToF/MS *m*/*z* calcd for $C_{33}H_{53}NO_{19}Si$ [M + Na]$^+$ 818.2879, found 818.2880.

2-(Trimethylsilyl)ethyl [Methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-glycero-α-D-galacto-2-nonulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-galactopyranoside) (14). Compound **13** (215 mg, 0.270 mmol) was dissolved in pyridine (10 mL) and acetic anhydride (5 mL) and the reaction was stirred for 14 h at room temperature. The solvent was removed by coevaporation with toluene (3 × 50 mL). Silica gel column chromatography (hexanes:EtOAc, 1:1, v:v) of the residue afforded compound **14** (246 mg, 99%) as a white solid. Analytical data for **14**: R_f 0.55 (hexanes:EtOAc, 1:3, v:v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.53−5.47 (m, 2H, H-4′, H-8′), 5.14 (dd, 1H, H-7′, *J* = 9.3 Hz, 2.4 Hz), 4.98−4.94 (m, 2H, H-3, H-4), 4.58−4.53 (m, 3H, H-2, H-6′, H-1), 4.28−4.25 (m, 2H, H-5′, H-9a′), 4.08−3.98 (m, 3H, H-6a, H-6b, H-9b′), 3.97−3.92 (dt, 1H, OCHHCH$_2$Si(CH$_3$)$_3$), 3.85 (s, 3H, COOCH$_3$), 3.83 (t, 1H, H-5), 3.59−3.54 (dt, 1H, OCHHCH$_2$Si(CH$_3$)$_3$), 2.63 (dd, 1H, *J* = 5.4 Hz, 12.7 Hz, H-3′eq), 2.32, 2.25 (2*s*, 6H, N(COCH$_3$)$_2$), 2.17, 2.15, 2.04, 2.01, 2.00, 1.99, 1.91 (7*s*, 21H, 7 × COCH$_3$), 1.60 (t, 1H, *J* = 12.2 Hz, H-3′ax), 1.01−0.86 (m, 2H, CH_2Si(CH$_3$)$_3$), 0.00 (s, 9H, Si(CH$_3$)$_3$); ^{13}C from HSQC (125.7 MHz, $CDCl_3$) δ 100.8 (C-1), 71.8 (C-2), 70.6 (C-5), 70.4 (C-3), 69.6 (C-6′), 67.9 (C-4), 67.8 (C-4′), 67.6 ($CH_2CH_2SiMe_3$), 67.4 (C-7′), 67.3 (C-8′), 62.7 (C-6), 62.4 (C-9′), 56.5 (C-5′), 53.3 (COOCH_3), 38.7 (C-3′), 28.4, 27.0 (NAc$_2$), 22.0−20.6 (7 × OAc), 18.4 (CH_2SiMe$_3$), 1.3 (SiMe$_3$); HR HR-MALDI-ToF/MS *m*/*z* calcd for $C_{39}H_{59}NO_{22}Si$ [M + Na]$^+$ 944.3196, found 944.3194.

Methyl 5-(*N*-Acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)trichloroacetimidate (7). TFA (2 mL) was added to a solution of compound **14** (240 mg, 0.260 mmol) in CH_2Cl_2 (10 mL) at 0 °C and the reaction was stirred for 4 h at the same temperature. The solvent was removed by coevaporation with toluene (5 × 20 mL). The residue was purified by

silica gel column chromatography (hexanes:EtOAc, 2:5, v:v). Trichloroacetonitrile (130 μL, 1.26 mmol) and 1,8-diazabicyclo-[5.4.0]undec-7-ene (14 μL, 94.8 μmol) were added to a solution of methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2- nonulopyranosylonate-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranoside) (207 mg, 0.252 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred for 1 h and then concentrated in vacuo. Silica gel column chromatography (hexanes:EtOAc, 1:2, v:v) of the syrup afforded compound **7** (220 mg, 87% over two steps, 3:2 α:β) as a white foam. Analytical data for **7**: R_f 0.30 (hexanes:EtOAc, 1:2, v:v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 1H, NH), 8.61 (s, 1H, NH), 6.48 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1α), 5.93 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1β), 5.56−5.50 (m, 2H, H-4′α, H-8′α), 5.37−5.35 (m, 2H, H-4′β, H-8′β), 5.27−5.24 (m, 2H, H-2α, H-2β), 5.15−5.12 (m, 2H, H-7′α, H-7′β), 5.04 (br d, 1H, H-4β), 4.99 (dd, 1H, *J* = 3.4 Hz, 10.5 Hz, H-3α), 4.77 (dd, 1H, *J* = 3.4 Hz, 10.0 Hz, H-3β), 4.62 (m, 2H, H-6′α, H-6′β), 4.32−4.28 (m, 2H, H-5′α, H-6a), 4.23−4.06 (m, 4H, H-5′β, H-6b, H-9′aα, H-9′bα), 4.02−3.95 (m, 2H, H-9′aβ, H-9′bβ), 3.88 (s, 3H, COO-CH_3α), 3.85 (s, 3H, COOCH_3β), 2.70 (dt, 1H, H-3′eq), 2.35, 2.33 (2*s*, 6H, NCOCH_3α), 2.28, 2.27 (2*s*, 6H, NCOCH_3β), 2.16−1.93 (7*s*, 21H, 7 × COCH_3), 1.68 (dd, 1H, H-3′ax); ^{13}C from HSQC (125.7 MHz, $CDCl_3$) δ 96.3 (C-1β), 94.2 C-1α), 72.0 (C-5), 71.2 (C-3β), 69.8 (C-6′), 68.4 (C-2), 68.3 (C-3α), 67.9 (C-4′α), 67.8 (C-8′α), 67.5 (C-4β), 67.3 (C-4′β), 67.2 (C-8′β), 67.1 (C-7′), 62.5 (C-6), 62.3 (C-9′β), 61.8 (C-9′α), 56.6 (C-5′α), 56.1 (C-5′β), 53.3 (COOCH_3), 38.9 (C-3′), 28.4, 28.3 (NAc_2α), 26.9, 26.8 (NAc_2β), 23.0−21.8 (7×OAc α/β);

Phenyl [*O*-Methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-[6-*O*-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside] (15). A mixture of disaccharide acceptor **11** (30 mg, 0.035 mmol) and trichloroacetimidate donor **7** (50 mg, 0.052 mmol) in CH_2Cl_2 (4 mL) was placed under an atmosphere of argon and stirred at room temperature with 4 Å MS for 1 h. The reaction mixture was then cooled to 0 °C. TMSOTf (3.0 μmol, 0.035 M solution in CH_2Cl_2) was added and stirring was continued for 1 h at the same temperature. The progress of reaction was monitored by TLC and MALDI-ToF MS. The reaction was quenched by the addition of pyridine (25 μL), diluted with CH_2Cl_2 (10 mL), filtered, and washed with saturated aq $NaHCO_3$ solution (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$:EtOAc, 1:1, v:v) to afford compound **15** (35 mg, 61%) as a white amorphous solid. Analytical data for **15**: R_f 0.25 (acetone:toluene, 1:3, v:v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43−7.13 (m, 15H, aromatic), 5.56−5.51 (m, 2H, H-8‴, H-4‴), 5.32 (d, 1H, *J* = 7.3 Hz, N*H*Troc), 5.27 (br s, 1H, H-4″), 5.21−5.14 (m, 3H, H-1, H-1″, H-3″), 4.99 (d, 1H, *J* = 1.0 Hz, H-4′), 4.91−4.86 (m, 2H, H-5″, H-2′), 4.83 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1′), 4.79 (d, 1H, C*H*HPh), 4.68−4.90 (m, 7H, CH_2Ph, C*H*HPh, CH_2CCl$_3$, H-3′, H-6″), 4.29 (t, $J_{4',5'} = J_{5',6'}$ = 10.1 Hz, H-5′), 4.23−4.14 (m, 4H, H-6a′, H-6b′, H-9a‴, H-3), 4.03 (m, 1H, H-9b‴), 3.96 (t, 1H, $J_{3,4} = J_{5,4}$ = 9.0 Hz, H-4), 3.87−3.80 (m, 5H, COOCH_3, H-6a, H-6b), 3.78 (m, 1H, H-5′), 3.52(br d, 1H, H-5), 3.10 (br m, 1H, H-2), 2.62 (m, 1H, H-3a‴), 2.33, 2.26 (2s, 6H, 2×NCOCH_3), 2.15−1.92 (9s, 27H, 9×COCH_3), 1.62 (m, 1H, H-3b‴), 1.17 (d, 3H, *J* = 6.6 Hz, CH_3 fucose) ppm; ^{13}C from HSQC (125.7 MHz, $CDCl_3$) δ 99.6 (C-1′), 97.7 (C-1″), 84.5 (C-1), 79.7 (C-5), 75.7 (C-3), 74.6 (CH_2Ph), 74.4 (C-2″), 73.9 (C-4), 73.2 (CH_2Ph), 72.3 (C-4″), 71.8 (C-3′), 71.2 (C-5′), 70.8 (C-5″), 70.6 (C-3″), 69.7 (C-6‴), 68.7 (C-6), 67.8 (C-4′), 67.5 (C-8‴), 67.4 (C-7‴), 67.3 (C-4‴), 64.8 (C-2′), 62.4 (C-9‴), 62.0 (C-6′), 57.9 (C-2), 56.2 (C-5‴), 53.4 (COOCH_3), 38.7 (C-3‴), 28.6, 27.2 (NAc_2), 21.6−20.5 (9×OAc), 16.3 (C-6”); HR-MALDI-ToF/MS *m*/*z* calcd for $C_{73}H_{89}Cl_3N_2O_{33}S$ $[M+Na]^+$ 1681.4032, found 1681.4029.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-[2-azido-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-6-*O*-(*O*-methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-(6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl)-α-D-galactopyranosyl]-L-threonine Benzyl Ester (16).** A mixture of disaccharide acceptor **10** (22 mg, 0.025 mmol) and tetrasaccharide donor **15** (32 mg, 0.019 mmol) in CH_2Cl_2 (2 mL) was placed under an atmosphere of argon and stirred at room temperature with 4 Å MS for 1 h. The reaction mixture was then cooled to 0 °C. *N*-Iodosuccinimide (22 mg, 0.096 mmol) and TfOH (0.019 mmol, 0.20 M solution in CH_2Cl_2) were added sequentially and the mixture was was stirred for 1 h at the same temperature. The progress of the reaction was monitored by TLC and MALDI-ToF MS. The reaction was diluted with CH_2Cl_2 (10 mL), filtered, and washed with saturated aq $NaHCO_3$ solution (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$) and filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes: EtOAc, 1:2, v:v) to afford compound **16** (29 mg, 55%) as a white amorphous solid. Analytical data for **16**: R_f 0.30 (hexanes:EtOAc, 1:2, v:v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77−6.42 (m, 26H, 3 × Bn, Fmoc, dFBz), 5.92−5.88 (m, 2H, H-4‴″, H-8‴″), 5.84 (t, 1H, H-2′), 5.74 (m, 2H, H-3‴, H-4‴), 5.61 (d, 1H, *J* = 3 Hz, H-1‴), 5.47−5.41 (m, 4H, H-2‴″, H-7‴″, H-4′, H-4‴″), 5.34 (m, 1H, H-5‴), 5.27 (m, 1H, H-3′), 5.19 (d, 1H, *J* = 8.1 Hz, H-1‴″), 5.04−4.99 (m, 3H, H-6‴″, H-3‴″, CO-OCH*H*Ph), 4.95 (d, 1H, OC*H*HPh), 4.86−4.77 (m, 3H, CO-OCH*H*Ph, COOC*H*HCCl$_3$, OC*H*HPh), 4.73−4.49 (m, 9H, COOC*H*HCCl$_3$, H-6a‴″, OC*H*HPh, H-1″, H-1, H-5‴″, OC*H*CH$_3$ threonine, OC*H*HPh, H-6b‴″), 4.44−4.18 (m, 9H, CH_2Fmoc, H-9a‴″, H-9b‴″, H-5‴″, C*H*COOBn threonine, H-1′, H-3″, H-4″), 4.15−3.79 (m, 11H, H-6a, H-6b, H-2‴, H-6a′, H-6b′, CH$_2$C*H*Fmoc, H-5, H-4, H-6a′, H-6b′, H-3), 3.77 (s, 3H, COOCH_3), 3.46 (m, 4H, H-2″, H-2, H-5′, H-5″), 2.85 (dd, 1H, H-3‴″), 2.26, 2.22 (2s, 6H, 2 × NCOCH_3), 1.87 (m, 1H, H-3‴″), 1.88−1.65 (11s, 33H, 11 × COCH_3), 1.60 (d, 1.623H, *J* = 6.5 Hz, CH_3 fucose), 1.57 (s, 3H, COCH_3), 1.34 (m, 3H, CH_3 threonine) ppm; ^{13}C from HSQC (125.7 MHz, $CDCl_3$) δ 101.6 (C-1′), 100.7 (C-1″), 100.5 (C-1‴″), 99.9 (C-1), 97.4 (C-1‴), 78.4 (C-3), 76.5 (O*C*HCH$_3$ threonine), 75.6 (C-5”), 74.9 (C-2‴), 74.8 (C-3”), 74.7 (C-4‴), 74.5 (*C*H$_2$Ph), 73.6 (*C*H$_2$Ph), 73.1 (*C*H$_2$Troc), 72.6 (C-4‴), 72.4 (C-3‴″), 71.7 (C-5′), 71.5 (C-5‴″), 71.1 (C-3′), 71.0 (C-2‴″), 70.8 (C-7‴″), 70.6 (C-3‴), 70.5 (C-6‴″), 70.3 (C-2′), 69.9 (C-5), 69.3 (C-6), 69.1 (C-6″), 68.1 (C-4), 67.9 C-4‴″), 67.7 (*C*H$_2$Fmoc), 67.6 (COO*C*H$_2$Ph threonine), 67.5 (C-4′), 67.4 (C-4‴″), 67.3 (C-8‴″), 65.2 (C-5‴), 62.5 (C-9‴″), 62.1 (C-6‴″), 61.6 (C-6’), 59.5 (O*C*HCH$_3$ threonine), 59.3 (C-2), 58.9 (C-2″), 56.3 (C-5‴″), 52.9 (COO*C*H$_3$), 47.6 (CH$_2$*C*HFmoc), 39.0 (C-3‴″), 21.0, 20.9 (NAc_2), 20.8−20.1 (12 × OAc), 18.9 (*C*H$_3$ threonine), 16.5 (C-6‴); HR-MALDI-ToF/MS *m*/*z* calcd for $C_{118}H_{135}Cl_3F_2N_6O_{51}$ $[M + Na]^+$ 2617.7086, found 2617.7091.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-[2-(*N*-acetamido)-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-6-*O*-(*O*-methyl 5-(*N*-acetylacetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-[(3,4-di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-*O*-(6-*O*-benzyl-2-deoxy-2-(*N*-acetamido)-β-D-glucopyranosyl)-α-D-galactopyranosyl]-L-threonine Benzyl Ester (1).** Zn dust (400 mg, 6.12 mmol) and saturated aq $CuSO_4$ (25 μL) were added to a solution of **16** (20 mg, 7.70 μmol) in THF (3 mL), Ac_2O (2 mL), and AcOH (1 mL) and the reaction was stirred at rt for 3 h. The reaction mixture was filtered and coevaporated with toluene (3 × 5 mL). The residue was purified by silica gel column

chromatography ($CHCl_3$:acetone, 2/1, v/v) to afford compound **1** (11.5 mg, 60%) as a white amorphous solid. Analytical data for **1**: R_f 0.30 ($CHCl_3$:acetone, 2/1, v/v); ^{1}H NMR (600 MHz, acetone-d_6) δ 7.75−7.08 (m, 26H, 3 × Bn, Fmoc, dFBz), 6.99 (d, 1H, N*H*, GlcNAc), 6.51 (d, 1H, N*H*, GalNAc), 6.42 (d, 1H, N*H*Fmoc, threonine), 5.5 (m, 1H, H-8′′′′′), 5.45 (m, 1H, H-4′′′′), 5.35 (d, 1H, J = 3.7 Hz, H-1′′′), 5.29 (d, 1H, J = 3.5 Hz, H-4′), 5.25 (dd, 1H, J = 8.1 Hz, J = 10.5 Hz, H-2′), 5.19 (br d, 1H, J = 2.6 Hz, H-4′′′), 5.12−5.08 (m, 3H, H-3′′′, H-7′′′′′, H-3′), 4.99 (br d, 1H, J = 3.7 Hz, H-4′′′′), 4.97−4.93 (dd, 2H, CO-OCH_2Ph, threonine), 4.88 (q, 1H, H-5′′′), 4.84−4.81 (m, 2H, H-2′′′′, H-1′′′′), 4.79 (d, 1H, J = 8.1 Hz, H-1′′), 4.67 (d, 2H, 2 × C*H*HPh), 4.60 (m, 1H, H-3′′′′), 4.57 (d, 1H, J = 2.8 Hz, H-1), 4.55 (dd, 1H, H-6′′′′′), 4.49 (d, 1H, J = 7.5 Hz, H-1′′), 4.45 (d, 1H, CH*H*Ph), 4.38 (d, 1H, CH*H*Ph), 4.35 (m, C*H*HFmoc), 4.27−4.22 (m, 2H, CH*H*Fmoc, H-2), 4.20 (m, 1H, H-5′′′′′), 4.15−4.05 (m, 8H, OC*H*CH_3 threonine, H-6a′′′′, C*H*COOBn threonine, C*H*Fmoc, H-6b′′′′, H-9a′′′′′, H-5′ H-6a′), 4.03−3.91(m, 5H, H-4′′, H-6b′, H-9b′′′′′, H-4, H-3′′), 3.87−3.81 (m, 3H, H-6a′′, H-2′′, H-5), 3.76−3.73 (m, 6H, H-5′′′′, COOCH_3, H-6b′′, H-3), 3.70 (dd, 1H, H-2′′′), 3.54 (m, 2H, H-6a, H-6b), 3.47 (m, 2H, C4−O*H*, H-5′′), 2.48 (dd, 1H, H-3′′′′′), 2.24, 2.20 (2*s*, 6H, NAc_2), 2.14−1.74 (14*s*, 42H, 12 × OAc, 2 × NHAc), 1.43 (t, 1H, H-3′′′′′), 1.16 (d, 3H, CH_3 threonine), 1.03 (d, 3H, J = 6.6 Hz, CH_3 fucose) ppm; ^{13}C from HSQC (150.9 MHz, $CDCl_3$) δ 104.3 (C-1′), 104.1 (C-1′′), 102.2 (C-1′′′′), 101.9 (C-1), 98.6 (C-1′′′), 81.0 C-3), 77.6 (C-5′′), 77.4 (OC*H*CH_3 threonine), 77.3 (C-3′′), 76.3 (C-2′′′), 76.1 (C-4′′′), 75.1 (CH_2Ph), 74.6 (C-4′′′), 74.2 (C-3′′′′), 73.8 (CH_2Ph), 73.6 (C-5′′′′), 73.5 (C-5′), 73.4 (C-3′), 73.1 (C-2′′′′), 72.8 (C-2′), 72.5 (C-3′′′), 72.3 (C-6′′′′′), 72.2 (C-6), 72.1 (C-5), 71.4 (C-4), 71.2 (C-6′′), 70.4 (C-8′′′′′), 70.2 (C-4′′′′), 70.0 (C-4′), 69.8 (C-7′′′′′), 69.5 (COOCH_2Ph), 69.2 (C-4′′′′′), 69.1 (CH_2CHFmoc), 66.4 (C-5′′′), 64.7 (C-9′′′′′), 64.1 (C-6′′′′), 63.9 (C-6′), 61.8 (OC*H*CH_3 threonine), 58.6 (C-2′′), 58.5 (C-5′′′′′), 55.2 (COOCH_3), 50.5 (C-2), 49.8 (CH_2*C*HFmoc), 41.1 (C-3′′′′′), 29.8, 28.4 (NAc_2), 25.5−22.4 (12 × OAc, 2 × NHAc), 21.4 (CH_3 threonine), 18.3 (C-6′′′); HR-MALDI-ToF/MS m/z calcd for $C_{119}H_{140}F_2N_4O_{51}$ $[M + Na]^+$ 2501.8350, found 2501.8353.

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Supporting Information Available: ^{1}H NMR spectra and HSQC of all synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.