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Synthesis of the Repeating Unit of Capsular Polysaccharide *Staphylococcus aureus* Type 5 To Study Chemical Activation and Conjugation of Native CP5

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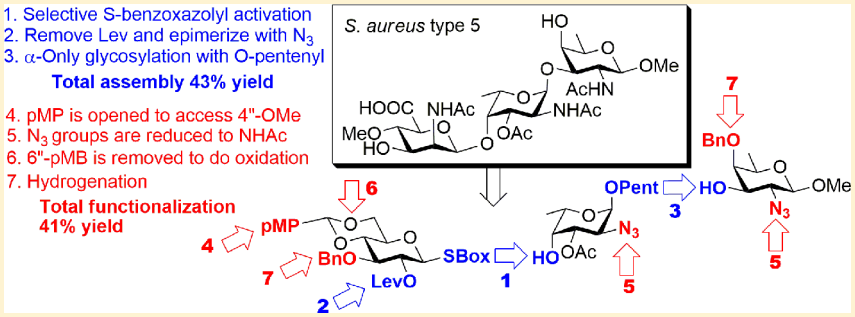
Supporting Information



ABSTRACT: The chemical synthesis of the repeating unit of *S. aureus* capsular polysaccharide type 5 equipped with capping methyl groups at the points of propagation of the polysaccharide sequence is described. This model compound was designed to study activation of the full length polysaccharide for conjugation to a carrier protein.

INTRODUCTION

Staphylococcus aureus[1] is one of the most frequent causes of infections in newborns, surgical patients, and immunocompromised individuals.[2] The majority of *S. aureus* strains express either capsular polysaccharide type 5 (CP5) or type 8 (CP8), which coat the cell for the purpose of immune evasion and define its serotype.[3] These polysaccharides are composed of many repeats of a unique arrangement of monosaccharides (the repeat unit) specific to a given bacterial serotype. The chemical composition of both types has been established,[4] and chemical syntheses of trisaccharides related to CP5[5,6] and CP8 have been reported.[7] The trisaccharide repeating unit of CP5 consists of the terminal D-mannosamine uronic acid (ManNAcA) that is β-(1→4)-linked to 2,3-di-*N*,*O*-acetylated L-fucosamine (3Ac-L-FucNAc, Figure 1). The latter is α-(1→3)-linked to D-fucosamine (D-FucNAc). In the bacterial polysaccharide, D-FucNAc is then linked to another D-ManNAcA via β-(1→4) linkage, etc.[4] Though this is a natural product, chemical synthesis of CP5 has been achieved by[5,6] targeting a trisaccharide repeating unit equipped with the conjugation amenable spacer group at the reducing end (Figure 1). Each building block required total synthesis from common sugars as the monosaccharide components are not available commercially.

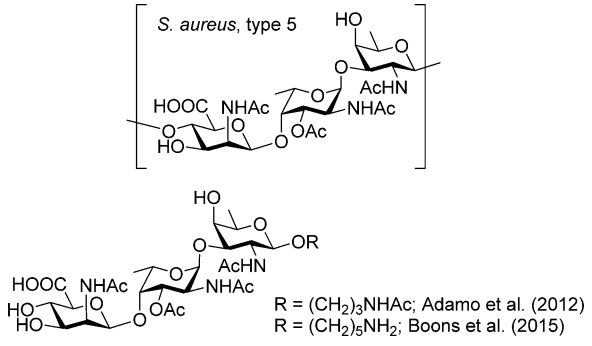


Figure 1. *S. aureus* type 5 and previously synthesized trisaccharides.

Conjugates of the purified native *S. aureus* capsular polysaccharides (CPs) derived from fermentation with two different protein carriers have been tested in human clinical trials.[8,9] Fattom et al. reported on the preparation of conjugates

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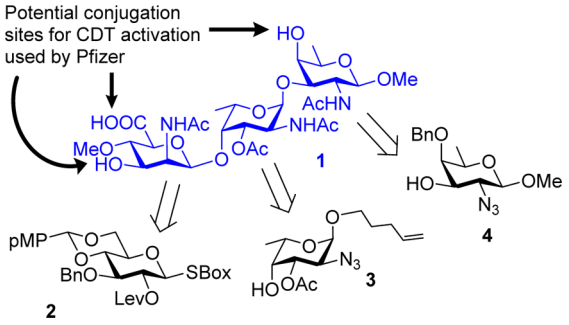
of CP5 and CP8 with *Pseudomonas aeruginosa* endotoxin A (ETA).[10] Freese et al. reported the preparation of conjugates of CP5 and CP8 with cross-reacting material 197 (CRM_{197}).[11] Covalent bond formation between native CPs and protein carriers requires chemically mediated activation of the CPs, and in some instances the protein carrier. The reagents used to activate the polysaccharides in the aforementioned CP5 and CP8 conjugates included 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide (EDC), carbonyldiimidazole (CDI), and carbonylditriazole (CDT). Characterization of activation products derived from purified native CPs by spectroscopic methods is very difficult owing to their large size (MW > 50 kDa). Overactivation and side reactions can lead to a loss of the epitopes required for immunogenicity of CP conjugate vaccines leading to product failure. The study of small model compounds based on the structure of the repeat unit offers a means to fully characterize the structure of the activated CP, identify and quantify sites of activation, and understand side reactions occurring after CP activation. Reported herein is the synthesis of the trisaccharide **1** that on one hand mimics the repeat unit of CP5 and on the other hand preserves the potential conjugation sites as in the native polysaccharide. For this purpose, trisaccharide **1** will be equipped with capping methyl groups (methyl) at the points of propagation of the polysaccharide sequence, C-1 (anomeric position) and C-4″ (Scheme 1).

Scheme 1. Retrosynthetic Analysis of *S. aureus* Type 5 for the CDT Activation Studies



It should be noted that although the direct introduction of the β-manno linkage was proven feasible by Boons et al.,[6] it was rather ineffective in our hands. We relate this disappointing result to a fairly low reactivity profile of 4,6-acetalated mannosyl donors tested and 3-*O*-acetylated L-fucosyl acceptor **3**. Instead, β-glycosylation with *S*-benzoxazolyl (SBox) glycosyl donor **2** followed by the C-2 epimerization, an approach executed in our previous synthesis of CP8, was adopted instead.[7]

The SBox leaving group[12,13] gave us far superior results in activation over the *O*-pentenyl moiety of acceptor **3** compared to SEt. The activation of the SEt group over the pentenyl moiety is also feasible, but it requires the use of MeOTf as promoter,[14] which in this case was ineffective due to the disarming protecting group pattern of the donor. Protecting groups in building blocks **2**−**4** were selected to ensure both selective deprotection for site-specific functionalization and the survival of the base-labile *O*-acetyl group that needs to be retained throughout the entire synthesis. The levulinoyl group was selected at C-2 of donor **2** for two reasons: first, to assist in β-glucosylation, and second, to give access to selective deprotection followed by epimerization at the disaccharide level. *p*-Methoxybenzylidene was found to be suitable for gaining ready access to the 4″-*O*-methylation position via the reductive opening at the trisaccharide level. The resulting 6″-*O*-*p*-methoxybenzyl group would then provide straightforward access to 6″-OH to be oxidized at the later stage of the synthesis. Finally, benzyl and azide protecting groups were found suitable for temporary protection of the remaining *O*- and *N*-positions because their removal would not affect 3′-*O*-acetyl group.

RESULTS AND DISCUSSION

In accordance with our strategic plan, the synthesis of glucosyl donor **2** began from known thioglycoside **5**.[15] Accordingly, the 2-hydroxyl derivative **5** was reacted with levulinic acid (LevOH), dicyclohexycarbodiimide (DCC), and 4-(dimethylamino)pyridine (DMAP) to afford 2-levulinoyl derivative **6** in 94% yield (Scheme 2). Reaction of thioglycoside **6** with bromine followed by the introduction of the SBox leaving group with KSBox[12,16] in the presence of 18-crown-6 in acetone yielded the first key building block, glycosyl donor **2**, in 78% yield over two steps. The synthesis of L-fucosyl acceptor **3** originated from the previously described *O*-pentenyl glycoside **7**.[7] Regioselective acetylation of the equatorial 3-hydroxyl in 3,4-diol **7** with acetyl chloride in the presence of pyridine in toluene at 0 °C gave the desired L-fucosyl acceptor **3** in 91% yield. Selective activation of the SBox leaving group in glycosyl donor **2** over the *O*-pentenyl anomeric moiety of glycosyl acceptor **3** was achieved in the presence of silver trifluoromethanesulfonate (AgOTf). This glycosylation afforded the desired β-linked disaccharide **8** in 78% yield.

Disaccharide **8** was then subjected to a three-step synthetic sequence to epimerize the C-2′ stereocenter of the nonreducing end monosaccharide in order to create the desired D-manno configuration. As previously refined for the synthesis of CP8, the 2′-*O*-levulinoyl group in **8** was removed with hydrazine acetate in methanol and dichloromethane (1/20, v/v) to afford intermediate **9** in 86% yield. Trifluormethanesulfonation of 2′-OH in compound **9** was affected with triflic anhydride in the presence of pyridine in dichloromethane at 0 °C. The resulting 2′-*O*-triflate was directly subjected to the nucleophilic displacement by reaction with sodium azide in DMF at 60 °C. As a result, disaccharide glycosyl donor **10** was isolated in 81% yield over two steps. The synthesis of the D-fucosyl acceptor **4** originated from the previously described methyl fucoside **11**.[7] The 3,4-diol in compound **11** was first protected as the benzylidene acetal upon treatment with dimethoxytoluene (DMT) in the presence of a catalytic amount of camphorsulfonic acid (CSA) to afford compound **12** in 79% yield. Regioselective benzylidene ring opening in **12** was conducted with $NaCNBH_3$ in the presence of 2 M HCl in diethyl ether and THF and led to the desired D-fucosyl acceptor **4** in 87% yield. Glycosylation of acceptor **4** with disaccharide donor **10** involved activation of the *O*-pentenyl leaving group with *N*-iodosuccinimide (NIS) and TfOH in the presence of molecular sieves (3 Å) in 1,2-dichloroethane at 0 °C. This approach allowed us to obtain the desired trisaccharide **13** as a pure α-anomer in 79% yield.

With the key trisaccharide intermediate **13** in hand, we then endeavored to identify a series of protecting and functional group modifications to obtain the target trisaccharide **1**. After thorough experimentation, the following reaction sequence was developed with the main goal of avoiding a spontaneous C-2″,6″ lactamization that was encountered when the carboxyl (or

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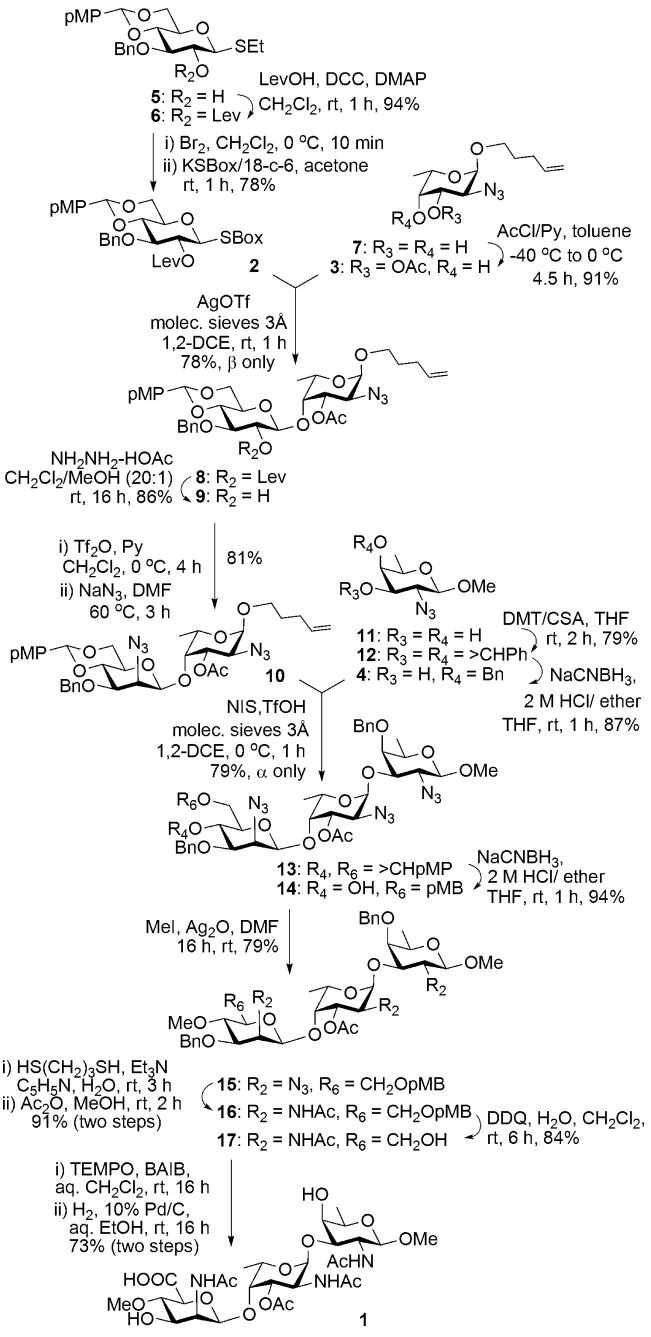
Scheme 2. Synthesis of Trisaccharide 1 of *S. aureus* Type 5 from Building Blocks 2−4



carboxylate) and amine functional groups were present concomitantly. First, the 4″,6″-*p*-methoxybenzylidine acetal in **13** was regioselectively opened with $NaCNBH_3$ in the presence of 2 M HCl in diethyl ether and THF. As a result, 6″-*p*-methoxybenzyl-protected trisaccharide **14** was obtained in 94% yield. The 4″-OH group in **14** was then methylated with MeI in the presence of silver(I) oxide in CH_2Cl_2 to yield compound **15** in 79% yield. The neutral reaction conditions for the methylation step were essential for survivability of 3′-*O*-acetyl group.

After that, all three azide groups in trisaccharide **15** were reduced with propane-1,3-dithiol and triethylamine in wet pyridine.[17] The resulting triamine was acetylated with Ac_2O in methanol to afford trisaccharide **16** in 91% yield over two steps. The 6″-O-PMB protecting group in **16** was then removed with DDQ in wet dichloromethane to give trisaccharide **17** in 84% yield. The primary alcohol in **17** was oxidized with (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) and (diacetoxyiodo)-benzene (BAIB) in wet dichloromethane,[18] and the remaining 3″- and 4-*O*-benzyl groups were removed by hydrogenation in the presence of 10% palladium on charcoal in wet ethanol to obtain the target trisaccharide **1** in 73% yield over two steps.

CONCLUSIONS

As a result of the elaborated synthetic strategy, we accomplished an efficient synthesis of CP5, trisaccharide **1**. The developed strategy allowed assembly of the key trisaccharide intermediate **13** from building blocks **2**−**4** with a 43% yield overall. It should be noted that this yield presents a notable improvement over the assembly stage of the synthesis reported by Adamo and co-workers[5] (28%) employing a similar concept of the indirect ManNAc introduction via β-glycosylation−epimerization. Our assembly was at least as efficient as the assembly stage reported by Boons and co-workers,[6] who employed a direct mannosylation approach (total yield for the assembly stage 42%). The protecting group strategy for selective functionalization employed in our synthesis allowed for an efficient modification of the key intermediate **13** into the target compound **1**. This sequence was accomplished in 41% yield overall, which surpasses the yields of the deprotection-functionalization sequences achieved by Adamo (26%) and Boons (21%). This synthesis provided tool compounds useful for understanding chemical activation of native CP5 and the propensity for side reactions. This research represents a novel approach to the synthesis of conjugation amenable repeating units designed to increasing process and product understanding of conjugate vaccines.

EXPERIMENTAL SECTION

General Methods. The reactions were performed using commercial reagents, and the ACS-grade solvents were purified and dried according to standard procedures. Column chromatography was performed on silica gel 60 (70−230 mesh), and reactions were monitored by TLC on Kieselgel 60 F_{254}. The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in methanol. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2 and $ClCH_2CH_2Cl$ were distilled from CaH_2 directly prior to application. Molecular sieves (3 Å), used for reactions, were crushed and activated in vacuo at 390 °C during 8 h in the first instance and then for 2−3 h at 390 °C directly prior to application. AgOTf was coevaporated with toluene (3 × 10 mL) and dried in vacuo for 2−3 h directly prior to application. Optical rotations were measured at polarimeter. ^{1}H NMR spectra were recorded at 300 MHz, and ^{13}C NMR spectra were recorded at 75 or 150 MHz. The ^{1}H chemical shifts are referenced to the signal of the residual $CHCl_3$ (δ_H = 7.24 ppm) for solutions in $CDCl_3$ or the signal of the residual CH_3OH (δ_H = 4.78 ppm) for solutions in CD_3OD. The ^{13}C chemical shifts are referenced to the central signal of $CDCl_3$ (δ_C = 77.23 ppm) for solutions in $CDCl_3$ or the central signal of CD_3OD (δ_C = 49.24 ppm) for solutions in CD_3OD. HRMS determinations were made with the use of a mass spectrometer with FAB ionization and ion-trap detection.

*Preparation of Monosaccharide Building Blocks **2**−**4**. Ethyl 3-O-Benzyl-2-O-levulinoyl-4,6-O-(p-methoxybenzylidene)-1-thio-β-D-glucopyranoside (**6**).* Levulinic acid (1.10 g, 9.23 mmol), DCC (1.77 g, 8.58 mmol), and DMAP (0.11 g, 0.92 mmol) were added to a stirring solution of ethyl 3-*O*-benzyl-4,6-*O*-(*p*-methoxybenzylidene)-1-thio-β-D-glucopyranoside[15] (**5**, 2.0 g, 4.62 mmol) in CH_2Cl_2 (60 mL), and the resulting mixture was stirred for 1 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~200 mL) and washed

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with satd aq $NaHCO_3$ (50 mL) and water (2 × 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane gradient elution) to afford the title compound as a white amorphous solid in 94% yield (2.31 g, 4.35 mmol). Analytical data for **6**: R_f = 0.43 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{24}$ −46.1 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, 3H, *J* = 7.5 Hz, CH_2CH_3), 2.15 (s, 3H, $COCH_3$), 2.45−2.55 (m, 2H, $COCH_2$), 2.55−2.80 (m, 4H, SCH_2CH_3, $COCH_2$), 3.46 (m, 1H, H-5), 3.65−3.75 (m, 3H, H-3, 4, 6a), 3.79 (s, 3H, OCH_3), 4.32 (dd, 1H, $J_{5,6b}$ = 4.9 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6b), 4.44 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 4.78 (dd, 2H, ^2J = 12.0 Hz, CH_2Ph), 5.04 (dd, 1H, $J_{2,3}$ = 8.9 Hz, H-2), 5.52 (s, 1H, >CHPh), 6.80−7.50 (m, 9H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.9, 24.0, 28.0, 29.9, 37.9, 55.3, 68.5, 70.7, 71.7, 74.3, 79.6, 81.4, 84.2, 101.2, 113.7 (×2), 127.4 (×2), 127.7, 128.0 (×2), 128.3 (×2), 129.7, 138.3, 160.1, 171.5, 206.2; HR-FAB MS $[M + Na]^+$ calcd for $C_{28}H_{34}NaO_8S$ 553.1872, found 553.1875.

*Benzoxazolyl 3-O-Benzyl-2-O-levulinoyl-4,6-O-(p-methoxybenzylidene)-1-thio-β-D-glucopyranoside (**2**).* A mixture of **6** (2.30 g, 4.34 mmol) and activated molecular sieves (3 Å, 2.1 g) in CH_2Cl_2 (70 mL) was stirred under argon for 1 h. A freshly prepared solution of Br_2 in CH_2Cl_2 (45 mL, 1/165, v/v) was then added, and the reaction mixture was kept for 10 min at rt. After that, the solid was filtered off, and the filtrate was concentrated under reduced pressure at rt and then dried in vacuo for 2 h. The crude residue was then dissolved in dry acetone (30 mL), KSBox[19] (1.64 g, 8.67 mmol) and 18-crown-6 (0.23 g, 0.87 mmol) were added, and the resulting mixture was stirred for 1 h under argon at rt. After that, the reaction mixture was concentrated under reduced pressure. The residue was redissolved in CH_2Cl_2 (250 mL) and washed with 1% aq NaOH (50 mL) and water (3 × 50 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane graduate elution) to afford the title compound in 78% yield (2.10 g, 3.39 mmol) as a white foam. Analytical data for **2**: R_f = 0.46 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{24}$ +54.3 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.11 (s, 3H, $COCH_3$), 2.35−2.58 (m, 2H, $COCH_2$), 2.60−2.80 (m, 2H, $COCH_2$), 3.60−3.90 (m, 7H, H-3, 4, 5, 6a, OCH_3), 4.37 (dd, 1H, $J_{5,6a}$ = 3.8 Hz, $J_{6a,6b}$ = 9.4 Hz, H-6b), 4.81 (dd, 2H, ^2J = 11.9 Hz, CH_2Ph), 5.24 (dd, 1H, $J_{2,3}$ = 8.2 Hz, H-2), 5.55 (s, 1H, > CHPh), 5.67 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 7.80−7.70 (m, 13H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.0, 30.0, 37.9, 55.5, 68.5, 71.1, 71.5, 74.6, 79.5, 81.2, 84.3, 101.5, 110.3, 113.8 (×2), 119.1, 124.7 (×2), 127.5 (×2), 127.9, 128.2 (×2), 128.5 (×2), 129.7, 138.1, 141.7, 152.1, 160.3, 161.4, 171.7, 206.0; HR-FAB MS $[M + Na]^+$ calcd for $C_{33}H_{33}NaNO_9S$ 642.1774, found 642.1781.

*4-Pentenyl 3-O-Acetyl-2-azido-2-deoxy-α-L-fucopyranoside (**3**).* 4-Pentenyl 2-azido-2-deoxy-α-L-fucopyranoside[7] (7, 1.50 g, 5.83 mmol) in pyridine (23 mL) was stirred under argon at −40 °C. A solution of acetyl chloride (0.43 mL, 6.07 mmol) in dry toluene (6.3 mL) was then added dropwise, and the resulting mixture was stirred at −40 °C for 30 min. Then the temperature was gradually increased to 0 °C, and the reaction mixture was stirred for additional 4 h. The reaction mixture was diluted with CH_2Cl_2 (250 mL), and the organic layer was washed with water (50 mL), satd aq $NaHCO_3$ (50 mL), and water (2 × 50 mL). The organic phase was separated, dried, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (acetone−dichloromethane gradient elution) to yield the title compound as a white amorphous solid in 91% yield (1.59 g, 5.31 mmol). Analytical data for **3**: R_f = 0.51 (acetone/dichloromethane, 1/9, v/v); $[\alpha]_D^{30}$ −134.3 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (d, 3H, CH_3), 1.63−1.72 (m, 2H, CH_2), 2.07−2.15 (m, 2H, CH_2), 2.12 (s, 3H, $COCH_3$), 2.53 (s, 1H, OH), 3.39−3.46 (m, 1H, CH_2), 3.57 (dd, 1H, $J_{2,3}$ = 11.1 Hz, H-2), 3.62−3.70 (m, 1H, CH_2), 3.88 (br. s, 1H, H-4), 3.97−4.04 (dd, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 4.86 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.90−5.02 (m, 2H, CH═CH_2), 5.22 (dd, 1H, $J_{3,4}$ = 3.0 Hz, H-3), 5.69−5.78 (m, 1H, CH═CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.0, 21.0, 28.6, 30.2, 57.1, 65.7, 67.8, 70.0, 71.2, 98.2, 115.1, 137.9, 170.3; HR-FAB MS $[M + Na]^+$ calcd for $C_{13}H_{21}O_5NaN_3$ 322.1379, found 322.1375.

*Methyl 2-Azido-3,4-O-benzylidene-2-deoxy-β-D-fucopyranoside (**12**).* Dimethoxytoluene (1.38 mL, 9.15 mmol) and camphorsulfonic acid (58 mg, 0.031 mmol) were added to a solution of methyl 2-azido-2-deoxy-β-D-fucopyranoside[7] (**11**, 0.62 g, 3.05 mmol) in THF (20 mL), and the resulting mixture was stirred under argon for 2 h at rt. After that, triethylamine (~0.3 mL) was added, and the volatiles were removed in vacuo. The residue was diluted with CH_2Cl_2 (~200 mL) and washed with water (40 mL), satd aq $NaHCO_3$ (40 mL), and water (2 × 40 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane gradient elution) to yield the title compound as a white amorphous solid in 79% yield (0.70 g, 2.41 mmol). Analytical data for **12**: R_f = 0.46 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{27}$ +44.2 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (d, 3H, $J_{5,6}$ = 6.6 Hz, C-6), 3.37 (dd, 1H, $J_{2,3}$ = 8.2 Hz, H-2), 3.53 (s, 3H, OCH_3), 3.85 (m, 1H, H-5), 3.96 (dd, 1H, $J_{4,5}$ = 5.7 Hz, H-4), 4.02 (dd, 1H, $J_{3,4}$ = 2.1 Hz, H-3), 4.12 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 5.91 (s, 1H, > CHPh), 7.25−7.62 (m, 5H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.5, 56.8, 65.5, 68.8, 76.8, 78.0, 102.5, 104.5, 126.6 (×2), 128.5 (×2), 129.5, 137.2; HR-FAB MS $[M + Na]^+$ calcd for $C_{14}H_{17}O_4NaN_3$ 314.1117, found 314.1123.

*Methyl 2-Azido-4-O-benzyl-2-deoxy-β-D-fucopyranoside (**4**).* A mixture of **12** (0.70 g, 2.41 mmol) and activated molecular sieves (3 Å, 800 mg) in THF (20 mL) was stirred under argon for 1 h at rt. $NaCNBH_3$ (1.20 g, 19.3 mmol) was added followed by a dropwise addition of 2 M HCl in diethyl ether (9.6 mL, 19.3 mmol), and the resulting mixture was stirred under argon for 1 h at rt. After that, the solids were filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~100 mL) was washed with water (20 mL), satd aq $NaHCO_3$ (20 mL), and water (2 × 20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane gradient elution) to yield the title compound as a white amorphous solid in 87% (0.62 g, 2.10 mmol). Analytical data for **4**: R_f = 0.39 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{30}$ +75.0 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (d, 3H, $J_{5,6}$ = 6.5 Hz, C-6), 2.17 (m, 1H, OH), 3.40−3.60 (m, 7H, H-2, 3, 4, 5, OCH_3), 4.12 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.77 (dd, 2H, ^2J = 11.6 Hz, CH_2Ph), 7.20−7.45 (m, 5H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.1, 57.1, 64.8, 71.0, 73.2, 76.2, 78.7, 103.2, 114.2, 128.3 (×2), 128.8 (×2), 138.1; HR-FAB MS $[M + Na]^+$ calcd for $C_{14}H_{19}O_4NaN_3$ 316.1273, found 316.1274.

*Assembly of Trisaccharide **13** from Building Blocks **2**, **3**, and **4**. 4-Pentenyl O-(3-O-Benzyl-2-O-levulinoyl-4,6-O-p-methoxybenzylidene-β-D-glucopyranosyl)-(1→4)-3-O-acetyl-2-azido-2-deoxy-α-L-fucopyranoside (**8**).* A mixture of donor **2** (2.0 g, 3.23 mmol), glycosyl acceptor **3** (0.74 g, 2.48 mmol), and freshly activated molecular sieves (3 Å, 2.8 g) in $ClCH_2CH_2Cl$ (60 mL) was stirred under argon for 2 h. Freshly conditioned AgOTf (1.66 g, 6.46 mmol) was added, and the resulting mixture was stirred for 1 h at rt. The reaction mixture was then diluted with CH_2Cl_2, the solid was filtered off, and the residue was rinsed successively with CH_2Cl_2. The combined filtrate (~200 mL) was washed with satd aq $NaHCO_3$ (40 mL) and water (2 × 40 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane gradient elution) to afford the title compound as a white foam in 78% yield (1.48 g, 1.93 mmol). Analytical data for **8**: R_f = 0.47 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{24}$ −110.2 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (d, 3H, $J_{5,6}$ = 6.6 Hz, C-6), 1.70 (m, 2H, CH_2), 1.95- 2.20 (m, 8H, 2 × $COCH_3$, CH_2), 2.35−2.90 (m, 4H, $COCH_2CH_2CO$), 3.25−3.50 (m, 2H, H-5′, OCH_2^a), 3.55−3.80 (m, 5H, H-2, 3′, 4′, 6a′, OCH_2^b), 3.82 (s, 3H, OCH_3), 3.95 (m, 1H, H-5), 4.06 (d, 1H, $J_{4,5}$ = 3.0 Hz, H-4), 4.29 (dd, 1H, $J_{5',6b'}$ = 4.9 Hz, $J_{6a',6b'}$ = 10.2 Hz, H-6b′), 4.40 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.78 (dd, 2H, ^2J = 12.1 Hz, CH_2Ph), 4.81 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.90−5.05 (m, 4H, H-2′, 3, CH═CH_2), 5.11 (dd, 1H, $J_{3,4}$ = 8.6 Hz, H-3), 5.52 (s, 1H, > CHPh), 5.80 (m, 1H, CH═CH_2), 6.75−7.50 (m, 9H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.2, 21.4, 28.1, 28.7, 30.0, 30.4, 38.2, 55.5, 57.1, 65.5, 66.5, 67.8, 68.6, 70.6, 73.4, 74.2, 76.1, 78.3, 81.7, 98.0, 101.5, 102.2, 113.8 (×2), 115.3, 127.5 (×2), 127.8, 128.1 (×2), 128.4 (×2), 129.6, 138.1, 138.4, 160.3,

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170.6, 171.5, 206.4; HR-FAB MS [M + Na]$^+$ calcd for $C_{39}H_{49}NaO_{13}N_3$ 790.3163, found 790.3167.

*4-Pentenyl O-(3-O-Benzyl-4,6-O-p-methoxybenzylidene-β-D-glucopyranosyl)-(1→4)-3-O-acetyl-2-azido-2-deoxy-α-L-fucopyranoside (**9**).* Hydrazine acetate (0.34 g, 3.78 mmol) was added to a stirred solution of **8** (1.45 g, 1.89 mmol) in CH_2Cl_2/methanol (20/1, v/v, 21 mL), and the resulting mixture was stirred for 16 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (~200 mL) and washed with satd aq $NaHCO_3$ (40 mL) and water (2 × 40 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane gradient elution) to afford the title compound as a white foam in 86% yield (1.09 g, 1.62 mmol). Analytical data for **9**: R_f = 0.54 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{25}$ −124.4 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (d, 3H, $J_{5,6}$ = 6.6 Hz, C-6), 1.55−1.85 (m, 2H, CH_2), 2.07 (s, 3H, $COCH_3$), 2.08−2.20 (m, 2H, CH_2), 3.34 (m, 1H, H-5′), 3.44 (m, 2H, OCH_2[a]), 3.55−3.75 (m, 6H, H-2, 2′, 3′, 4′, 6a′, OCH_2[b]), 3.78 (s, 3H, OCH_3), 4.01 (m, 1H, H-5), 4.10 (d, 1H, $J_{4,5}$ = 2.9 Hz, H-4), 4.25 (dd, 1H, $J_{5',6b'}$ = 4.7 Hz, $J_{6a',6b'}$ = 9.9 Hz, H-6b′), 4.32 (d, 1H, $J_{1',2'}$ = 6.9 Hz, H-1′), 4.85 (dd, 2H, ^2J = 11.6 Hz, CH_2Ph), 4.88 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.93−5.10 (m, 3H, H-3, CH═CH_2), 5.49 (s, 1H, > CHPh), 5.78 (m, 1H, CH═CH_2), 6.80−7.50 (m, 9H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.1, 21.4, 28.7, 30.4, 55.7, 57.3, 65.9, 66.5, 67.9, 68.7, 70.6, 74.6, 74.9, 76.8, 80.7, 81.4, 98.0, 101.4, 104.6, 113.8 (×2), 115.3, 127.5 (×2), 127.9, 128.1 (×2), 128.5 (×2), 129.7, 138.0, 138.6, 160.2, 170.5; HR-FAB MS [M + Na]$^+$ calcd for $C_{34}H_{43}NaO_{11}N_3$ 692.2795, found 692.2802.

*4-Pentenyl O-(2-Azido-3-O-benzyl-2-deoxy-4,6-O-p-methoxybenzylidene-β-D-mannopyranosyl)-(1→4)-3-O-acetyl-2-azido-2-deoxy-α-L-fucopyranoside (**10**).* A solution of **9** (1.07 g, 1.60 mmol) in CH_2Cl_2 (40 mL) and pyridine (4 mL) was cooled to 0 °C. Trifluoromethanesulfonic anhydride (0.67 mL, 3.99 mmol) was slowly added, and the resulting mixture was stirred for 4 h at 0 °C. After that, the reaction was quenched with ice-cold water (~10 mL). The mixture was diluted with CH_2Cl_2 (~150 mL), washed with cold satd aq $NaHCO_3$ (20 mL) and cold water (2 × 20 mL), and the organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The crude residue was dissolved in DMF (5 mL); NaN_3 (0.52 g, 7.99 mmol) was added, and the resulting suspension was stirred for 3 h at 60 °C. After that, ethyl acetate (~100 mL) and water (~20 mL) were added. The organic phase was separated and washed with brine (2 × 20 mL). The combined aqueous phase was extracted with ethyl acetate (3 × 60 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane gradient elution) to afford the title compound as a white foam in 81% (0.91 g, 1.29 mmol). Analytical data for **10**: R_f = 0.59 (ethyl acetate/hexane, 2/3, v/v); $[\alpha]_D^{25}$ −187.8 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (d, 3H, $J_{5,6}$ = 6.6 Hz, C-6), 1.70 (m, 2H, CH_2), 2.01−2.20 (m, 5H, $COCH_3$, CH_2), 3.22 (m, 1H, H-5′), 3.45 (m, 1H, CH_2[a]), 3.58−3.68 (m, 2H, H-3′, CH_2[b]), 3.70−3.85 (m, 5H, H-2, 6a′, OCH_3), 3.95 (dd, 1H, $J_{4',5'}$= 9.4 Hz, H-4′), 3.99 (m, 1H, H-5), 4.06 (d, 1H, $J_{2',3'}$ = 3.5 Hz, H-2′), 4.16 (d, 1H, $J_{4,5}$ = 3.1 Hz, H-4), 4.23 (dd, 1H, $J_{5',6a'}$ = 4.8 Hz, $J_{6a',6b'}$ = 10.2 Hz, H-6b′), 4.50 (s, 1H, H-1′), 4.77 (dd, 2H, ^2J = 12.4 Hz, CH_2Ph), 4.85 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 4.92−5.10 (m, 3H, H-3, CH═CH_2), 5.51 (s, 1H, > CHPh), 5.78 (m, 1H, CH═CH_2), 6.80−7.50 (m, 9H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.5, 21.4, 28.6, 30.3, 55.4, 57.0, 62.8, 65.3, 67.6, 67.9, 68.3, 70.2, 73.0, 76.0, 76.5, 78.4, 98.0, 101.5, 101.7, 113.7 (×2), 115.2, 127.4 (×2), 127.8 (×2), 128.1, 128.6 (×2), 129.7, 137.8, 138.0, 160.2, 170.7; HR-FAB MS [M + Na]$^+$ calcd for $C_{34}H_{42}NaO_{10}N_6$ 717.2860, found 717.2869.

*Methyl O-(2-Azido-3-O-benzyl-2-deoxy-4,6-O-p-methoxybenzylidene-β-D-mannopyranosyl)-(1→4)-O-(3-O-acetyl-2-azido-2-deoxy-α-L-fucopyranosyl)-(1→3)-2-azido-4-O-benzyl-2-deoxy-β-D-fucopyranoside (**13**).* A mixture of donor **10** (0.90 g, 1.30 mmol), acceptor **4** (0.35 g, 1.18 mmol), and freshly activated molecular sieves (3 Å, 800 mg) in $ClCH_2CH_2Cl$ (30 mL) was stirred under argon for 2 h. The reaction mixture was cooled to 0 °C, NIS (0.58 g, 2.59 mmol) and TfOH (23 μL, 0.26 mmol) were added, and the resulting mixture was stirred for 1 h at 0 °C. After that, the solid was filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~50 mL) was washed with 10% aq $Na_2S_2O_3$ (15 mL) and water (2 × 15 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (acetone−dichloromethane gradient elution) to afford the title compound as a white foam in 79% yield (0.85 g, 0.94 mmol). Analytical data for **13**: R_f = 0.49 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]_D^{28}$ −130.7 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (d, 3H, $J_{5',6'}$ = 6.5 Hz, C-6′), 1.32 (d, 3H, $J_{5,6}$ = 6.4 Hz, C-6), 2.14 (s, 3H, $COCH_3$), 3.23 (m, 1H, H-5″), 3.37−3.58 (m, 6H, H-3, 4, 5, OCH_3), 3.65 (dd, 1H, $J_{3'',4''}$ = 9.6 Hz, H-3″), 3.74−3.91 (m, 7H, H-1, 2′, 5′, 6a″, OCH_3), 3.97 (dd, 1H, $J_{4'',5''}$ = 9.2 Hz, H-4″), 4.06 (d, 1H, $J_{2'',3''}$ = 3.7 Hz, H-2″), 4.01−4.18 (m, 2H, H-1, 4′), 4.25 (dd, 1H, $J_{5',6b'}$ = 4.8 Hz, $J_{6a',6b'}$ = 10.2 Hz, H-6b′), 4.50 (s, 1H, H-1″), 4.65−4.78 (m, 2H, CH_2Ph), 4.82−4.93 (m, 2H, CH_2Ph), 5.03 (dd, 1H, $J_{2',3'}$ = 11.3 Hz, $J_{3',4'}$ = 3.1 Hz, H-3′), 5.31 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1′), 5.54 (s, 1H, > CHPh), 6.80−7.50 (m, 14H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.6, 17.2, 21.4, 55.4, 56.8, 57.2, 62.8, 63.8, 66.1, 67.6, 68.3, 69.8, 70.9, 73.0, 75.1, 75.9, 76.4, 78.1, 78.4, 79.1, 99.4, 101.5, 101.8, 103.6, 113.7 (×2), 127.5 (×2), 127.9 (x 3), 128.0 (×2), 128.1, 128.5 (×2), 128.7 (×2), 129.6, 137.8, 138.1, 160.2, 170.6; HR-FAB MS [M + Na]$^+$ calcd for $C_{43}H_{51}O_{13}N_9Na$ 924.3504, found 924.3512.

*Final Functionalization of **13** into Target Trisaccharide **1**. Methyl O-(2-Azido-3-O-benzyl-2-deoxy-6-O-p-methoxybenzyl-β-D-mannopyranosyl)-(1→4)-O-(3-O-acetyl-2-azido-2-deoxy-α-L-fucopyranosyl)-(1→3)-2-azido-4-O-benzyl-2-deoxy-β-D-fucopyranoside (**14**).* A mixture of **13** (0.81 g, 0.90 mmol) and activated molecular sieves (3 Å, 1.50 g) in THF (32 mL) was stirred under argon for 1 h at rt. $NaCNBH_3$ (0.45 g, 7.19 mmol) was added followed by a dropwise addition of 2 M HCl in diethyl ether (2.7 mL, 5.4 mmol), and the resulting mixture was stirred under argon for 1 h at rt. After that, the solids were filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~200 mL) was washed with water (20 mL), satd aq $NaHCO_3$ (20 mL), and water (2 × 20 mL). The organic phase was separated, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane gradient elution) to yield the title compound as a white amorphous solid in 94% yield (0.77 g, 0.85 mmol). Analytical data for **14**: R_f = 0.56 (acetone/dichloromethane, 1/19, v/v); $[\alpha]_D^{31}$ −149.6 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.04 (d, 3H, $J_{5',6'}$ = 6.5 Hz, C-6′), 1.24 (d, 3H, $J_{5,6}$ = 6.3 Hz, C-6), 2.03 (s, 3H, $COCH_3$), 2.68 (d, 1H, *J* = 2.1 Hz, 4″−OH), 3.19 (m, 1H, H-5″), 3.28 (dd, 1H, $J_{3'',4''}$ = 9.1 Hz, H-3″), 3.33−3.49 (m, 6H, H-3, 4, 5, OCH_3), 3.58 (m, 2H, H-6a″, 6b″), 3.70 (s, 3H, OCH_3), 3.71−3.82 (m, 4H, H-2, 2′, 4″, 5′), 3.91 (d, 1H, $J_{2'',3''}$ = 3.5 Hz, H-2″), 4.02−4.10 (m, 2H, H-1, 4′), 4.34−4.41 (m, 3H, H-1″, CH_2Ph), 4.55−4.70 (m, 3H, 11/2 CH_2Ph), 4.82 (d, 1H, ^2J = 11.6 Hz, 1/2 CH_2Ph), 4.99 (dd, 1H, $J_{2',3'}$ = 11.3 Hz, $J_{3',4'}$ = 3.1 Hz, H-3′), 5.22 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1′), 6.65−7.50 (m, 14H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.6, 17.1, 21.2, 55.4, 56.9, 57.1, 60.7, 63.9, 66.3, 68.2, 69.5, 69.8, 70.9, 72.1, 73.4, 74.9, 75.1, 75.3, 78.1, 79.1, 79.8, 99.4, 100.8, 103.6, 114.0 (×2), 127.9, 128.0 (×4), 128.3, 128.5 (×2), 128.8 (×2), 129.6 (×2), 129.7, 137.6, 138.1, 159.5, 170.8; HR-FAB MS [M + Na]$^+$ calcd for $C_{43}H_{53}O_{13}N_9Na$ 926.3661, found 926.3668.

*Methyl O-(2-Azido-3-O-benzyl-2-deoxy-6-O-p-methoxybenzyl-4-O-methyl-β-D-mannopyranosyl)-(1→4)-O-(3-O-acetyl-2-azido-2-deoxy-α-L-fucopyranosyl)-(1→3)-2-azido-4-O-benzyl-2-deoxy-β-D-fucopyranoside (**15**).* Silver oxide (1.95 g, 8.41 mmol) was added to a stirring solution of **14** (0.76 g, 0.84 mmol) and MeI (0.79 mL, 12.6 mmol) in DMF (5 mL), and the resulting mixture was stirred for 16 h at rt. After that, the solid was filtered off and rinsed successively with CH_2Cl_2. The combined filtrate (~150 mL) was washed with satd aq $NaHCO_3$ (20 mL) and water (2 × 20 mL). The organic phase was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (ethyl acetate−hexane gradient elution) to afford the title compound as a white amorphous solid in 79% yield (0.61 g, 0.66 mmol). Analytical data for **15**: R_f = 0.62 (acetone/hexane, 1/1, v/v); $[\alpha]_D^{28}$ −79.2 (*c* = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.11 (d, 3H, $J_{5',6'}$ = 6.5 Hz, C-6′), 1.31 (d, 3H, $J_{5,6}$ = 6.4 Hz, C-6), 2.13 (s, 3H, $COCH_3$), 3.17 (m,

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1H, H-5″), 3.37−3.58 (m, 16H, H-3, 3″, 4, 4″, 5, 2 × OCH_3), 3.60−3.65 (m, 2H, H-6a″, 6b″), 3.78 (s, 3H, OCH_3), 3.80−3.92 (m, 3H, H-2, 2′, 5′), 3.98 (d, 1H, $J_{2'',3''}$ = 3.5 Hz, H-2″), 4.09−4.18 (m, 2H, H-1, 4′), 4.40 (s, 1H, H-1″), 4.47 (dd, 2H, ^2J = 11.5 Hz, CH_2Ph), 4.65−4.74 (m, 3H, 11/2 CH_2Ph), 4.90 (d, 1H, ^2J = 11.6 Hz, 1/2 CH_2Ph), 5.07 (dd, 1H, $J_{2',3'}$ = 11.3 Hz, $J_{3',4'}$ = 3.1 Hz, H-3′), 5.30 (d, 1H, $J_{1',2'}$ = 3.6 Hz, H-1′), 6.75−7.50 (m, 14H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.7, 17.2, 21.1, 31.0, 55.4, 56.9, 57.1, 61.1, 61.4, 63.9, 66.3, 68.7, 69.4, 70.9, 72.1, 73.1, 75.1 (×2), 75.8, 78.1, 79.1, 80.5, 99.5, 100.8, 103.6, 113.9 (×2), 127.9 (×3), 128.1 (×3), 128.6 (×2), 128.7 (×2), 129.6 (×2), 130.0, 137.8, 138.1, 159.4, 171.1; HR-FAB MS [M + Na]$^+$ calcd for $C_{44}H_{55}O_{13}N_9Na$ 940.3817, found 940.3822.

*Methyl O-(2-Acetamido-3-O-benzyl-2-deoxy-6-O-p-methoxybenzyl-4-O-methyl-β-D-mannopyranosyl)-(1→4)-O-(2-acetamido-3-O-acetyl-2-deoxy-α-L-fucopyranosyl)-(1→3)-2-acetamido-4-O-benzyl-2-deoxy-β-D-fucopyranoside (**16**).* Water (5.0 mL), triethylamine (4.0 mL), and 1,3-propanedithiol (3.95 mL, 39.2 mmol) were added to a solution of trisaccharide **15** (0.60 g, 0.65 mmol) in pyridine (15.0 mL), and the resulting mixture was stirred for 3 h at rt. After that, the reaction mixture was concentrated and dried in vacuo. The crude residue was dissolved in MeOH (5.0 mL), Ac_2O (0.74 mL, 0.78 mmol) was added, and the resulting suspension was stirred under argon for 2 h at rt. The reaction mixture was then concentrated in vacuo. The residue was purified by column chromatography on silica gel (methanol−dichloromethane gradient elution) to afford the title compound as a white foam in 91% yield (0.57 g, 0.59 mmol). Analytical data for **16**: R_f = 0.52 (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{28}$ −92.3 (c = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10 (d, 3H, $J_{5',6'}$ = 6.4 Hz, C-6′), 1.31 (d, 3H, $J_{5,6}$ = 6.3 Hz, C-6), 1.92, 2.00, 2.04, 2.16 (4s, 12H, 4 × $COCH_3$), 3.17 (m, 1H, H-5″), 3.30−3.83 (m, 16H, H-3, 3″, 4, 4″, 5, 6a″, 6b″, 3 × OCH_3), 3.85−4.05 (m, 2H, H-4′, 5′), 4.28−4.60 (m, 7H, H-1, 1′, 2, 2′, 1 1/2 CH_2Ph), 4.70−5.00 (m, 6H, H-1″, 2″, 3′, 1 1/2 CH_2Ph), 6.10 (d, 1H, $J_{2',NH}$ = 9.3 Hz, 2′-NH), 6.84−6.88 (m, 2H, aromatic), 6.92 (d, 1H, $J_{2'',NH}$ = 9.3 Hz, 2″-NH), 7.05 (d, 1H, $J_{2,NH}$ = 9.3 Hz, 2-NH), 7.18−7.50 (m, 12H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.5, 17.2, 20.9, 21.1, 23.3, 23.4, 23.6, 46.2, 49.7, 51.5, 55.4 (×2), 56.0, 61.0, 67.0, 67.9, 70.7, 70.9, 71.2, 73.2, 74.7, 75.1, 75.6, 78.3, 80.9, 99.7, 100.0, 101.7, 113.9 (×2), 127.4 (×2), 127.6, 127.7, 128.2 (×2), 128.4 (×2), 128.5 (×2), 129.4, 129.9 (×2), 138.3, 138.5, 159.5, 171.2 (×2), 172.0, 172.5; HR-FAB MS [M + Na]$^+$ calcd for $C_{50}H_{67}O_{16}N_3Na$ 988.4419, found 988.4432.

*Methyl O-(2-Acetamido-3-O-benzyl-2-deoxy-4-O-methyl-β-D-mannopyranosyl)-(1→4)-O-(2-acetamido-3-O-acetyl-2-deoxy-α-L-fucopyranosyl)-(1→3)-2-acetamido-4-O-benzyl-2-deoxy-β-D-fucopyranoside (**17**).* 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 0.27 g, 1.18 mmol) was added to a mixture of **16** (0.57 g, 0.59 mmol) in CH_2Cl_2 (10.0 mL) and water (0.5 mL), and the resulting mixture was stirred for 6 h at rt. After that, the reaction mixture was diluted with CH_2Cl_2 (∼150 mL) and washed with water (2 × 20 mL), the organic phase was separated and dried over $MgSO_4$, and the reaction mixture was concentrated in vacuo. Then the residue was purified by column chromatography on silica gel (methanol−dichloromethane gradient elution) to afford the title compound as a white amorphous solid in 84% yield (0.42 g, 0.50 mmol). Analytical data for **17**: R_f = 0.36 (methanol/dichloromethane, 1/9, v/v); $[\alpha]_D^{26}$ −90.9 (c = 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10 (d, 3H, $J_{5',6'}$ = 6.4 Hz, C-6′), 1.40 (d, 3H, $J_{5,6}$ = 6.5 Hz, C-6), 1.94, 2.03, 2.10, 2.11 (4s, 12H, 4 × $COCH_3$), 3.10−3.25 (m, 2H, H-4″, 5″), 3.38−3.60 (m, 8H, H-3″, 4, 2 × OCH_3), 3.61−4.00 (m, 6H, H-3, 4′, 5, 5′, 6a″, 6b″), 4.25−4.40 (m, 2H, H-1, 2), 4.43−4.58 (m, 2H, H-1″, 1/2 CH_2Ph), 4.67 (dd, 1H, $J_{1',2'}$ = 3.5 Hz, $J_{2',3'}$ = 11.4 Hz, H-2′), 4.70−5.00 (m, 6H, H-1′, 2″, 3′, 1 1/2 CH_2Ph), 5.67 (d, 1H, $J_{2,NH}$ = 9.3 Hz, 2-NH), 6.29 (d, 1H, $J_{2',NH}$ = 9.3 Hz, 2′-NH), 6.39 (d, 1H, $J_{2'',NH}$ = 9.3 Hz, 2″-NH), 7.20−7.50 (m, 10H, aromatic); ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.5, 17.3, 21.2, 23.3, 23.6 (×2), 46.7, 49.9, 51.6, 55.9 (×2), 61.1 (×2), 61.8, 67.4, 70.8, 71.2, 71.6, 74.6, 75.8, 77.4, 78.2, 80.7, 99.7, 100.1, 101.4, 127.5 (×2), 127.7, 128.1 (×2), 128.5 (×2), 128.6 (×4), 138.2, 138.4, 171.4, 171.7, 171.8; HR-FAB MS [M + Na]$^+$ calcd for $C_{42}H_{59}O_{15}N_3Na$ 868.3844, found 868.3831.

*Methyl O-(2-Acetamido-3-O-benzyl-2-deoxy-4-O-methyl-β-D-mannopyranosyluronic acid)-(1→4)-O-(2-acetamido-3-O-acetyl-2-deoxy-α-L-fucopyranosyl)-(1→3)-2-acetamido-4-O-benzyl-2-deoxy-β-D-fucopyranoside (**1**).* Water (4.0 mL), 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO, 11.4 mg, 0.07 mmol), and diacetoxyiodobenzene (BAIB, 295.1 mg, 0.92 mmol) were added to a solution of trisaccharide **17** (0.31 g, 0.37 mmol) in CH_2Cl_2 (4.0 mL), and the resulting mixture was stirred for 16 h at rt. After that, the reaction mixture was passed through a pad of silica gel, concentrated in vacuo, and dried. The crude residue was dissolved in 90% aq ethanol (5.0 mL), 10% Pd on activated charcoal (200 mg) was added, and the resulting suspension was vigorously stirred under H_2 atmosphere for 16 h at rt. After that, the solids were filtered off and rinsed successively with methanol. The combined filtrate (∼35 mL) was concentrated in vacuo. The residue was purified by column chromatography on silica gel (dichloromethane−methanol gradient elution) to afford the title compound as a white amorphous solid in 73% yield (143.6 mg, 0.21 mmol). Analytical data for **1**: R_f = 0.43 (methanol/dichloromethane, 1/1, v/v); $[\alpha]_D^{25}$ −75.6 (c = 0.6, CH_3OH); ^{1}H NMR (300 MHz, CH_3OD) δ 1.19 (d, 3H, $J_{5',6'}$ = 6.5 Hz, C-6′), 1.29 (d, 3H, $J_{5,6}$ = 6.3 Hz, C-6), 1.88, 1.93, 2.08, 2.11 (4s, 12H, 4 × $COCH_3$), 3.27 (dd, 1H, $J_{4'',5''}$ = 9.7 Hz, H-4″), 3.42, 3.48 (2s, 6H, 2 × OCH_3), 3.45−3.52 (m, 3H, H-5″, 6a″, 6b″), 3.57−3.68 (m, 4H, H-3, 3′, 4, 5), 4.00−4.08 (m, 2H, H-2, 4′), 4.12 (m, 1H, H-5′), 4.20 (dd, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.40 (dd, 1H, $J_{1',2'}$ = 8.4 Hz, H-1′), 4.52 (s, 1H, H-1″), 4.56 (d, 1H, $J_{2'',3''}$ = 4.0 Hz, H-2″), 4.82- 4.90 (m, 1H, H-1′), 4.98 (dd, 1H, $J_{3',4'}$ = 2.7 Hz, H-3′); ^{13}C NMR (150 MHz, CH_3OD) δ 16.9 (×2), 21.5, 23.1 (×2), 23.3, 52.6, 54.7, 57.1 (×2), 60.8 (×2), 67.6, 71.1, 71.9, 72.2, 73.8, 78.9, 79.1, 79.6, 81.7, 100.8, 102.2, 103.9, 173.2, 173.4, 173.7, 174.8, 177.1; HR-FAB MS [M + Na]$^+$ calcd for $C_{28}H_{45}O_{16}N_3Na$ 702.2698, found 702.2695.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00910.

Spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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