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Brief Communication

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Synthesis of a Conjugation-Ready, Phosphorylated, Tetrasaccharide Fragment of the O-PS of *Vibrio cholerae* O139

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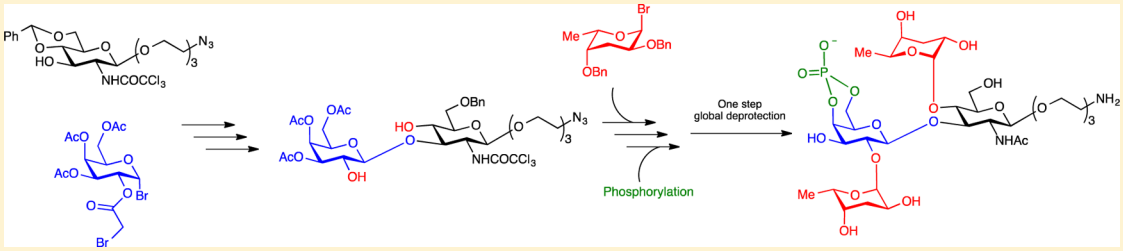
Supporting Information



ABSTRACT: A new pathway to the tetrasaccharide α-Col*p*-(1→2)-4,6-*P*-β-D-Gal*p*-(1→3)-[α-Col*p*-(1→4)]-β-D-Glc*p*NAc-1-$(OCH_2CH_2)_3NH_2$ has been developed. Glycosylation of 8-azido-3,6-dioxaoctyl 4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside with 3,4,6-tri-*O*-acetyl-2-*O*-bromoacetyl-α-D-galactopyranosyl bromide afforded the β-linked disaccharide. Debromoacetylation followed by reductive opening of the benzylidene acetal afforded the disaccharide diol acceptor. Halide-assisted glycosylation with 2,4-di-*O*-benzyl-α-colitosyl bromide gave the 1,2-*cis*-coupling product. Deacetylation followed by regioselective phosphorylation gave isomeric (*R*,*S*)-(P)-4^{II},6^{II}-cyclic phosphates, which were globally deprotected by one-step catalytic (Pd/C) hydrogenation/hydrogenolysis. The target tetrasaccharide, obtained in high overall yield, is amenable for conjugation to proteins.

Cholera is a deadly, infectious enteric disease, endemic to many countries in the Third World. The disease caused by *Vibrio cholerae* O1 has been around for centuries, but in 1992, a new type of cholera emerged, which manifested itself by the same symptoms, i.e., watery diarrhea, but was caused by a newly discovered pathogen *Vibrio cholerae* O139. This prompted the elucidation of the structure[1,2] of the O-specific polysaccharide (O-PS) of this Gram-negative bacterium, which was the first step toward the rational development of a conjugate vaccine for the disease. This laboratory has been involved in development of conjugate vaccines for infectious diseases from synthetic and bacterial carbohydrates for a number of years. To that end, among other things, we have synthesized fragments of the protective carbohydrate antigens of the disease-causing bacterial pathogens and studied their binding with the homologous protective antibodies involved.[3−5] Determination of the minimum structural requirements in the antigen which, when transformed into immunogenic conjugates and used as vaccines elicit protective antibodies, requires assembly of the O-PS fragments in the spacer-equipped form to make them amenable for conjugation. In 2006, Oscarson's[6] and our laboratory[7] reported independent syntheses of the linker-equipped upstream tetrasaccharide [sequence FD(E)C, Figure 1]. Here, we describe a new, high-yielding approach to the synthesis of the same carbohydrate sequence. It provides the desired structure in fewer steps and in an experimentally more convenient manner than by the approaches described previously.

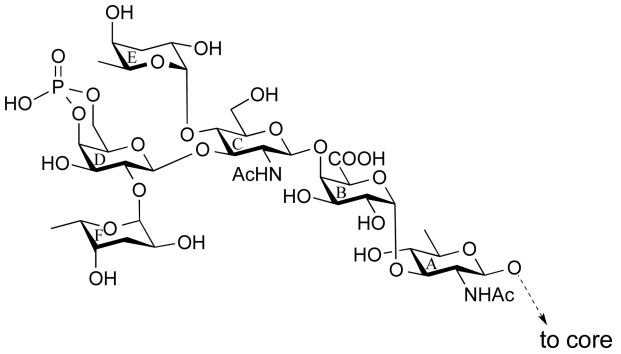


Figure 1. Structure of the O-PS of *Vibrio cholerae* O139.

Each of the previous pathways to the linker-equipped sequence FD(E)C, shown in Figure 1, has its own merits. The disadvantages in the Oscarson approach[6] are that the attachment of a linker requires chemical manipulations with the fully assembled tetrasaccharide, and the synthetic strategy requires two steps for the final deprotection. On the other hand, in the Ruttens approach,[7] the isomers-forming phosphorylation is done in the middle of the whole reaction

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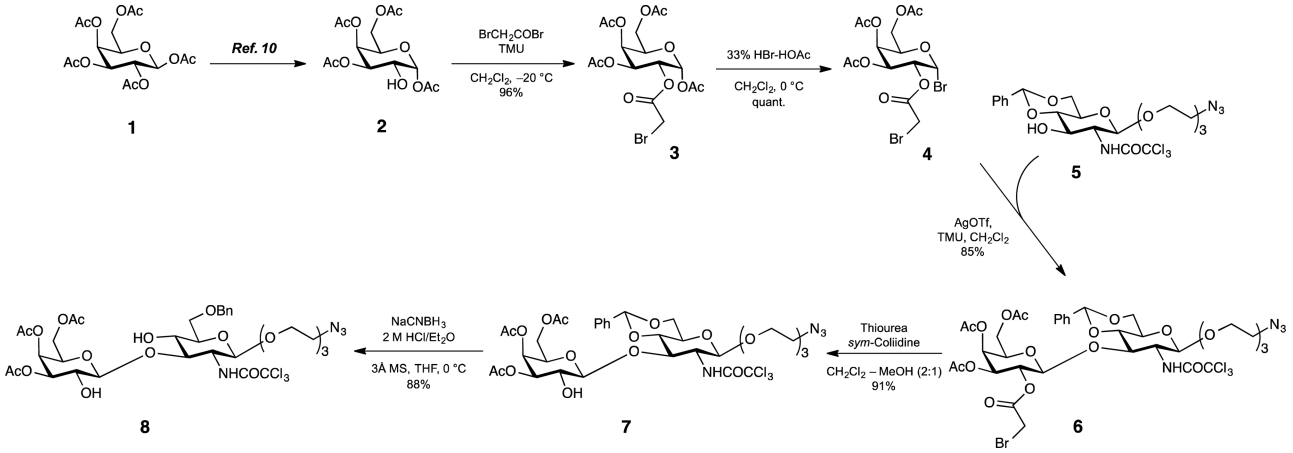
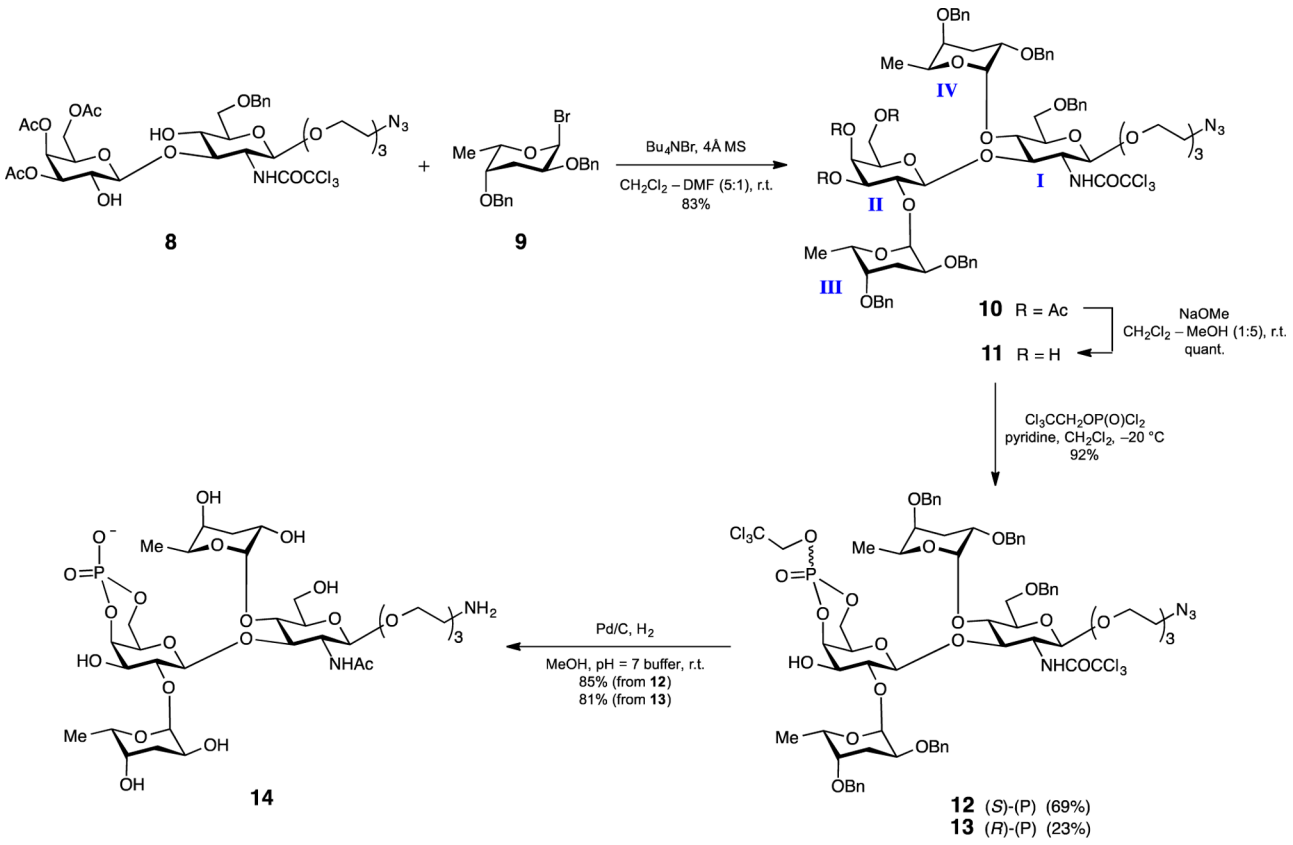
Scheme 1. Synthesis of the Disaccharide Diol Acceptor 8



Scheme 2. Synthesis of the Spacer-Equipped, Phosphorylated Tetrasaccharide 14



sequence. Consequently, the two isomeric phosphates must be resolved to eliminate complications that would arise from having to continue the sequence with a mixture. Thus, one has to either complete the sequence with only one isomer or perform all chemical transformations separately with both phosphates to increase the overall yield of the final product. The present tetrasaccharide buildup starts from the downstream[8] end with the linker-equipped monosaccharide, and the synthesis is designed in the way allowing the phosphorylation to be done as a penultimate step. Thus, separation of isomeric phosphates is not required because the following global deprotection removes the chirality at the P atom yielding, thus, the same target product from both phosphates.

The feasible, just outlined strategy toward the tetrasaccharide fragment **14** (Scheme 2) is an extension of our preparation of the phosphorylated upstream trisaccharide sequence[9] D(E)C, Figure 1. There, and here as well, to minimize the overall number of synthetic steps, the choice of protecting groups allows final, global deprotection involving transformation of several different functional groups to be achieved in one step (catalytic hydrogenation/hydrogenolysis with Pd/C).

Accordingly (Scheme 1), 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (**2**)[10] was bromoacetylated to give **3** (96%), which was converted into the α-glycosyl bromide **4** with 33% HBr–HOAc. Silver trifluoromethanesulfonate (triflate) mediated glycosylation of the linker-equipped glycosyl acceptor 8-azido-3,6-dioxaoctyl 4,6-*O*-benzylidene-2-deoxy-2-trichloroacetami-

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do-β-D-glucopyranoside[11] (**5**) with the α-glycosyl donor **4** was performed using our improved protocol[11,12] (cf. ref 13) to afford the desired β-linked disaccharide **6** in 85% yield. In addition to being a key intermediate here, the foregoing substance is also a generally useful building block in oligosaccharide synthesis, as it allows orthogonal deprotection (debromoacetylation or regioselective opening of the benzylidene acetal). The new methodology using 1,1,3,3-tetramethylurea as an acid scavenger[11,12] obviates the use of molecular sieves, whose use makes it difficult to control the mild acidity of the reaction medium required to prevent side reactions to occur. The β-configuration of the interglycosidic linkage in **6** follows from the ^{1}H NMR spectrum (δ 4.74, d, $J_{1,2}$ = 8.0 Hz, H-1^{II}). In addition, signals for the two anomeric carbons appeared in the ^{13}C NMR spectrum at almost identical chemical shifts (δ 99.4 and 99.8 for C-1^{II} and C-1^{I}, respectively). That the acid-labile benzylidene group was preserved under these conditions was manifested by the presence of the ^{13}C signal at δ 101.2 (PhCH).

Selective *O*-debromoacetylation using thiourea in the presence of a weak, non-nucleophilic organic base to prevent acyl group migration[14] gave the disaccharide acceptor **7** having *O*-2^{II} free, in 91% yield. The upfield shift of the signal for H-2^{II} (δ 3.79 ppm), and the COSY crosspeak between the hydroxyl group (δ 2.59 ppm) and H-2^{II} confirmed that removal of the bromoacetyl group occurred without acetyl group migration.

Subsequent regioselective reductive opening of the 4^{I},6^{I}-*O*-benzylidene ring in disaccharide **7** using sodium cyanoborohydride and HCl–Et_2O in tetrahydrofuran[15] afforded the 6^{I}-*O*-benzyl derivative **8** in 88% yield. The significant upfield shift of the signal for C-4^{I} (by ∼10 ppm, as compared to that in the spectrum of **7**), as well as the COSY crosspeak between H-4^{I} (δ 3.53 ppm) and the newly generated hydroxyl group (δ 4.34 ppm), confirmed that the reductive opening of the benzylidene acetal led to the 6^{I}-benzyl ether and a free 4^{I}-OH group.

The disaccharide diol glycosyl acceptor **8** (Scheme 2) was subjected to halide-assisted glycosylation[16] with the α-colitosyl bromide **9** (made by treatment of the corresponding ethyl thioglycoside[17] with bromine) to give the tetrasaccharide **10** (83%). The identification of the coupling product **10** was based on its ^{1}H and ^{13}C NMR spectra, which included signals characteristic for both the donor and the acceptor moieties. As expected, a downfield shift was observed in the spectrum of **10** for the H-4^{I} and H-2^{II} signals, compared to those in the spectrum of **8**, as a result of glycosylation[18] at those positions. The signals for the two anomeric protons of colitose residues present appeared as doublets at δ 5.22 ppm (J = 3.0 Hz) and 5.07 ppm (J = 3.3 Hz) and confirmed, thus, the exclusive formation of the α-glycosidic linkages.

Subsequent de-*O*-acetylation of **10** with methanolic sodium methoxide afforded 8-azido-3,6-dioxaoctyl 2,4-di-*O*-benzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→4)-[2,4-di-*O*-benzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)]-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (**11**) in virtually theoretical yield. Treatment[19,20] of the latter with the phosphorylating reagent 2,2,2-trichloroethyl phosphorodichloridate gave a mixture of the two isomeric 4^{II},6^{II}-cyclic 2,2,2-trichloroethyl phosphates **12** and **13** (∼3:1, ^{31}P NMR) in combined 92% yield. For identification and characterization, the mixture was resolved, but the global deprotection could be done with a mixture of the isomeric phosphates.

The desired transformation of the benzyl ethers, the 2,2,2-trichloroethyl, *N*-trichloroacetyl, and the azide groups in **12** was accomplished in one step by hydrogenation/hydrogenolysis in the presence of Pd/C catalyst to give the title phosphorylated tetrasaccharide fragment **14** in 85% yield. Considering the lability of the α-colitosyl group to acid hydrolysis, the reaction was conducted in a pH = 7 buffer. In addition to HRMS, presence of the cyclic phosphate in **14** followed from the ^{31}P NMR spectrum, showing $^3J_{P,H}$ = 21.6 Hz.[21] As expected, because the deprotection of the phosphate group canceled the chirality at that site, similar treatment of the phosphate isomer **13** gave the same product **14** (^{1}H NMR, ^{13}C NMR, ^{31}P NMR, TLC, HRMS) in comparable yield.

■ CONCLUSIONS

We have developed an improved, convenient, and high-yielding strategy for the stereoselective synthesis of the phosphorylated tetrasaccharide **14**, which is one of the terminal determinants of the O-specific polysaccharide of *Vibrio cholerae* O139. The final, amino-functionalized, linker-equipped fragment **14** was obtained in a form ready for conjugation to proteins. Many features in the present, successful synthetic approach will be incorporated in the synthesis of the full O-PS, the phosphorylated hexasaccharide [sequence FD(E)CBA, Figure 1], which is in progress.

■ EXPERIMENTAL SECTION

General Methods. Optical rotations were measured at ambient temperature for solution in $CHCl_3$, unless stated otherwise. All reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 coated glass slides. Column chromatography was performed by elution from prepacked columns of silica gel and monitored with the evaporative light scattering detector. Nuclear magnetic resonance (NMR) spectra were measured at 600 MHz for ^{1}H, 150 MHz for ^{13}C, and 162 MHz for ^{31}P. Solvent peaks were used as internal reference relative to TMS for ^{1}H and ^{13}C chemical shifts (ppm); ^{31}P chemical shifts (ppm) are reported relative to 85% H_3PO_4 in D_2O external reference. Assignments of NMR signals were made by homonuclear and heteronuclear two-dimensional correlation spectroscopy, run with the software supplied with the spectrometers. When reporting assignments of NMR signals, nuclei associated with the spacer are denoted with a prime; sugar residues are serially numbered, beginning with the one bearing the aglycon, and are identified by a Roman numeral superscript in listings of signal assignments, with nuclei of the colitose residues as III and IV (see Scheme 2, **10**). The density of 2,2,2-trichloroethyl phosphorodichloridate (Aldrich/Sigma, d ≈ 1.7 g/mL at 20 °C) was determined by weighing of 1 mL of the reagent. Palladium-on-charcoal catalyst (5%, Escat 103) was purchased from Engelhard Industries. 1,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranose (**2**) was prepared from the commercially available 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**1**) as described,[10] except that the compound was crystallized from *i*-PrOH. Solutions in organic solvents were dried with anhydrous Na_2SO_4, and concentrated at 40 °C/2 kPa.

1,3,4,6-Tetra-O-acetyl-2-O-bromoacetyl-α-D-galactopyranose (**3**). To a solution of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (**2**)[10] (5.0 g, 14.35 mmol) and 1,1,3,3-tetramethylurea (3.77 mL, 31.57 mmol) in dry CH_2Cl_2 (45 mL) was added bromoacetyl bromide (2.5 mL, 28.70 mmol) dropwise with stirring under argon at −20 °C. The cooling was removed, and with continued stirring, the mixture was allowed to warm to room temperature. After 8 h, analysis by TLC (12:1 toluene–acetone) indicated complete disappearance of the starting tetraacetate. The mixture was diluted with CH_2Cl_2 (100 mL) and washed successively with ice–water, satd aq $NaHCO_3$, and brine. The organic layer was dried, concentrated, and coevaporated with toluene (twice). The residue was crystallized from isopropyl ether to give **3** (6.45 g, 96%). Mp: 86–87 °C (*i*-Pr_2O). $[\alpha]_D^{20}$ = +92.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.40 (d, 1 H, $J_{1,2}$ = 3.2 Hz,

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H-1), 5.52 (dd, 1 H, $J_{3,4}$ = 2.9, $J_{4,5}$ = 1.2 Hz, H-4), 5.40 (dd, 1 H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 5.37 (dd, 1 H, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 10.8 Hz, H-2), 4.35 (ddd, 1 H, J = 0.8, J = 6.7, J = 7.6 Hz, H-5), 4.14−4.08 (m, 2 H, H-$6_{a,b}$), 3.80−3.75 (m, 2 H, CH_2Br), 2.18, 2.17, 2.05, 2.02 (4 s, 12 H, 4COCH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.3, 170.1, 170.0, 169.9 (4 O*C*OCH$_3$), 166.4 (*C*OCH$_2$Br), 89.2 (C-1), 68.8 (C-5), 68.0 (C-2), 67.4 (C-4), 67.1 (C-3), 61.1 (C-6), 24.7 (*C*H$_2$Br), 20.9, 20.7, 20.6, 20.5 (4OCO*C*H$_3$). HRMS (ESI-TOF): m/z [M + NH_4]$^+$ calcd for $C_{16}H_{25}BrNO_{11}$ 486.0611, found 486.0608. Anal. Calcd for $C_{16}H_{21}BrO_{11}$: C, 40.94; H, 4.51. Found: C, 41.05; H, 4.70.

*8-Azido-3,6-dioxaoctyl 4,6-O-Benzylidene-2-deoxy-3-O-(3,4,6-tri-O-acetyl-2-O-bromoacetyl-β-D-galactopyranosyl)-2-trichloroacetamido-β-D-glucopyranoside (**6**).* To a solution of **3** (6.0 g, 12.80 mmol) in CH_2Cl_2 (100 mL) at 0 °C (ice−water bath) was added 33% HBr−HOAc (60 mL) slowly and with stirring (during ∼30 min). After 4 h at 0 °C, the solution was diluted with CH_2Cl_2 and washed subsequently with ice−water (twice) and satd aq $NaHCO_3$. The organic layer was dried, concentrated, and coevaporated with toluene (twice) to give 3,4,6-tri-*O*-acetyl-2-*O*-bromoacetyl-α-D-galactopyranosyl bromide (**4**) as a syrup in almost theoretical yield, which was used in the next step without purification. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.36 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1), 5.53 (br d, 1 H, J = 3.1 Hz, H-4), 5.46 (dd, 1 H, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 5.31 (dd, 1 H, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 10.6 Hz, H-2), 4.35 (br t, 1 H, J = 6.7 Hz, H-5), 4.17 (dd, 1 H, J = 6.4, J = 11.5 Hz, H-6_a), 4.12 (dd, 1 H, J = 6.7, J = 11.4 Hz, H-6_b), 3.88 (d, 1 H, J = 12.1 Hz, C*H*HBr), 3.84 (d, 1 H, J = 12.2 Hz, CH*H*Br), 2.17, 2.07, 2.02 (3 s, 9 H, 3 COCH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.3, 169.9, 169.7 (3 O*C*OCH$_3$), 166.6 (*C*OCH$_2$Br), 90.5 (C-1), 69.5 (C-2), 69.3 (C-5), 67.2 (C-4), 66.9 (C-3), 60.9 (C-6), 24.7 (*C*H$_2$Br), 20.7, 20.6, 20.5 (3 OCO*C*H$_3$).

A solution of **4** (3.1 g, 6.32 mmol) in anhydrous CH_2Cl_2 (15 mL) was added, at −30 °C in one portion, to a mixture of glycosyl acceptor[11] (**5**, 2.0 g, 3.51 mmol), 1,1,3,3-tetramethylurea (1.0 mL, 8.42 mmol), and powdered AgOTf (1.8 g, 7.02 mmol) in anhydrous CH_2Cl_2 (40 mL). The cooling was removed, and with continued stirring, the mixture was allowed to warm to room temperature. The stirring was continued until TLC (∼8 h, 3:2 hexane−acetone) indicated that all of the acceptor was consumed. Et_3N (0.5 mL) was added, and the mixture was diluted with CH_2Cl_2 (50 mL) and filtered through a Celite pad. The filtrate was washed successively with 0.5 M aq HCl, satd aq $NaHCO_3$, and brine, and dried. After concentration, chromatography (2:1 hexane−acetone) gave the spacer-equipped disaccharide **6** (2.9 g, 85%).

$[\alpha]_D^{20}$ = −11.0 (*c* 0.7, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.50−7.37 (m, 5 H, Ph), 7.13 (d, 1 H, $J_{2,NH}$ = 7.8 Hz, NH), 5.56 (s, 1 H, PhC*H*), 5.34 (d, 1 H, $J_{3,4}$ = 2.9 Hz, H-4^{II}), 5.22 (dd, 1 H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.4 Hz, H-2^{II}), 5.06 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1^I), 4.96 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.4 Hz, H-3^{II}), 4.74 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^{II}), 4.47 (t, 1 H, J = 9.5 Hz, H-3^I), 4.35 (dd, 1 H, J = 4.8, J = 10.6 Hz, H-$6^I{}_b$), 4.12 (dd, 1 H, J = 7.3, J = 11.0 Hz, H-$6^{II}{}_b$), 4.03 (dd, 1 H, J = 6.4, J = 11.2 Hz, H-$6^{II}{}_a$), 3.93−3.89 (m, 1 H, H-$1'_b$), 3.83−3.80 (m, 2 H, H-$6^I{}_a$, H-$1'_a$), 3.77 (t, 1 H, J = 6.9 Hz, H-5^{II}), 3.73−3.68 (m, 3 H, CH_2Br, H-4^I), 3.67−3.58 (m, 9 H, H-2′, H-3′, H-4′, H-5′, H-2^I), 3.53−3.49 (m, 1 H, H-5^I), 3.41 (t, 2 H, J = 5.0 Hz, H-6′), 2.12, 2.01, 1.97 (3 s, 9 H, 3 COCH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.3, 170.1, 170.0 (3 O*C*OCH$_3$), 166.2 (*C*OCH$_2$Br), 161.9 (*C*OCCl$_3$), 137.0 (*ipso* Ph), 129.2−126.0 (Ar), 101.2 (PhCH), 99.8 (C-1^I), 99.4 (C-1^{II}), 92.5 (*C*Cl$_3$), 78.8 (C-4^I), 76.3 (C-3^I), 70.8, 70.62, 70.5 (C-2′, C-3′, C-4′), 70.7 (C-3^{II}), 70.65 (C-5^{II}), 70.2 (C-2^{II}), 70.0 (C-5′), 68.7 (C-1′), 68.5 (C-6^I), 66.8 (C-4^{II}), 66.2 (C-5^I), 61.0 (C-6^{II}), 58.5 (C-2^I), 50.6 (C-6′), 25.3 (CH_2Br), 20.59, 20.57, 20.56 (3 OCO*C*H$_3$). HRMS (ESI-TOF): m/z [M + NH_4]$^+$ calcd for $C_{35}H_{48}BrCl_3N_5O_{17}$ 994.1294, found 994.1298. Anal. Calcd for $C_{35}H_{44}BrCl_3N_4O_{17}$: C, 42.94; H, 4.53; N, 5.72. Found: C, 43.01; H, 4.56; N, 5.63.

*8-Azido-3,6-dioxaoctyl 4,6-O-Benzylidene-2-deoxy-3-O-(3,4,6-tri-O-acetyl-β-D-galactopyranosyl)-2-trichloroacetamido-β-D-glucopyranoside (**7**).* A solution of thiourea (1.1 g, 14.7 mmol) in methanol (50 mL) was added at 0 °C to a stirred solution of **6** (4.8 g, 4.90 mmol) and *sym*-collidine (0.9 mL, 7.35 mmol) in CH_2Cl_2 (100 mL). The mixture was stirred overnight at room temperature, when TLC (6:1 dichloromethane−acetone) showed that all of the starting material was consumed and a single product was formed. The mixture was partitioned between water and CH_2Cl_2, the organic phase was dried and concentrated, and the residue was chromatographed (9:1 dichloromethane−acetone) to give **7** (3.8 g, 91%) as a white foam. $[\alpha]_D^{20}$ = −9.3 (*c* 1.2, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.47−7.26 (m, 6 H, Ph, NH), 5.56 (s, 1 H, PhC*H*), 5.32 (dd, 1 H, $J_{3,4}$ = 3.4, $J_{4,5}$ = 0.9 Hz, H-4^{II}), 5.08 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1^I), 4.83 (dd, 1 H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 3.5 Hz, H-3^{II}), 4.56 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1^{II}), 4.48 (t, 1 H, J = 9.9 Hz, H-3^I), 4.37 (dd, 1 H, J = 5.0, J = 10.6 Hz, H-$6^I{}_b$), 4.11 (dd, 1 H, J = 7.5, J = 11.1 Hz, H-$6^{II}{}_b$), 3.96−3.92 (m, 2 H, H-$6^{II}{}_a$, H-$1'_b$), 3.84−3.80 (m, 2 H, H-$6^I{}_a$, H-$1'_a$), 3.79 (m, 1 H, H-2^{II}), 3.78−3.73 (m, 2 H, H-4^I, H-5^{II}), 3.72−3.61 (m, 9 H, H-2^I, H-2′, H-3′, H-4′, H-5′), 3.56−3.52 (m, 1 H, H-5^I), 3.41 (t, 2 H, J = 5.0 Hz, H-6′), 2.59 (br s, 1 H, 2^{II}−OH), 2.10, 2.01, 1.98 (3 s, 9 H, 3 COCH_3). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.4, 170.3, 170.1 (3 O*C*OCH$_3$), 162.3 (N*C*OCCl$_3$), 136.7 (*ipso* Ph), 129.3−126.0 (Ar), 102.4 (C-1^{II}), 101.5 (PhCH), 100.3 (C-1^I), 92.5 (*C*Cl$_3$), 79.5 (C-4^I), 76.9 (C-3^I), 72.6 (C-3^{II}), 71.0 (C-5^{II}), 70.8, 70.7, 70.5 (C-2′, C-3′, C-4′), 70.0 (C-5′), 68.9 (C-1′), 68.8 (C-2^{II}), 68.6 (C-6^I), 66.9 (C-4^{II}), 66.3 (C-5^I), 61.2 (C-6^{II}), 58.2 (C-2^I), 50.6 (C-6′), 20.7−20.6 (3 OCO*C*H$_3$). HRMS (ESI-TOF): m/z [M + NH_4]$^+$ calcd for $C_{33}H_{47}Cl_3N_5O_{16}$ 874.2083, found 874.2064. Anal. Calcd for $C_{33}H_{43}Cl_3N_4O_{16}$: C, 46.19; H, 5.05; N, 6.53. Found: C, 46.09; H, 5.14; N, 6.45.

*8-Azido-3,6-dioxaoctyl 6-O-Benzyl-2-deoxy-3-O-(3,4,6-tri-O-acetyl-β-D-galactopyranosyl)-2-trichloroacetamido-β-D-glucopyranoside (**8**).* A mixture of the benzylidene acetal **7** (1.12 g, 1.31 mmol) and freshly activated, powdered molecular sieves (3 Å, 1.7 g) in dry THF (35 mL) was stirred under nitrogen for 60 min at room temperature. The solution was cooled to 0 °C, and sodium cyanoborohydride (1.0 g, 15.72 mmol) was added portionwise. After being stirred for 20 min at 0 °C, 2 M HCl−Et_2O was added dropwise at 0 °C until the effervescence ceased and the pH remained acidic. The mixture was stirred for an additional 30 min at room temperature, diluted with CH_2Cl_2 (50 mL), and filtered through Celite. The filtrate was washed with cold satd aq $NaHCO_3$ (25 mL) and brine (25 mL), and the organic extract was dried and concentrated. Chromatography (4:1 toluene−acetone) afforded **8** (990 mg, 88%). $[\alpha]_D^{20}$ = +6.7 (*c* 1.0, acetone). ^{1}H NMR (600 MHz, acetone-d_6): δ = 8.26 (d, 1 H, $J_{2,NH}$ = 8.6 Hz, NH), 7.42−7.28 (m, 5 H, Ph), 5.34 (dd, 1 H, $J_{3,4}$ = 3.5, $J_{4,5}$ = 1.0 Hz, H-4^{II}), 4.93 (dd, 1 H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 3.6 Hz, H-3^{II}), 4.88 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1^I), 4.67 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1^{II}), 4.63 (bs, 1 H, PhCH_2), 4.34 (br s, 1 H, 4^I−OH), 4.33−4.29 (m, 1 H, H-5^{II}), 4.22 (d, 1 H, J = 3.7 Hz, 2^{II}−OH), 4.18−4.12 (m, 2 H, H-$6^{II}{}_{a,b}$), 4.07−4.04 (m, 1 H, H-3^I), 3.95−3.90 (m, 2 H, H-$6^I{}_b$, H-$1'_b$), 3.81 (m, 1 H, H-2^I), 3.78 (m, 1 H, H-2^{II}), 3.76−3.71 (m, 2 H, H-$6^I{}_a$, H-$1'_a$), 3.70−3.60 (m, 8 H, H-2′, H-3′, H-4′, H-5′), 3.56−3.52 (m, 2 H, H-4^I, H-5^I), 3.40 (t, 2 H, J = 5.0 Hz, H-6′), 2.13, 2.00, 1.96 (3 s, 9 H, 3 COCH_3). ^{13}C NMR (150 MHz, acetone-d_6): δ = 170.8, 170.6, 170.4 (3 O*C*OCH$_3$), 162.8 (N*C*OCCl$_3$), 139.9 (*ipso* Ph), 129.1−128.1 (Ar), 104.7 (C-1^{II}), 101.4 (C-1^I), 93.9 (*C*Cl$_3$), 85.6 (C-3^I), 76.5 (C-5^I), 73.8 (PhCH_2), 73.6 (C-3^{II}), 71.8 (C-5^{II}), 71.3, 71.1, 71.0 (C-2′, C-3′, C-4′), 70.7 (C-5′), 70.5 (C-6^I), 70.3 (C-4^I), 69.5 (C-2^{II}), 69.4 (C-1′), 68.2 (C-4^{II}), 62.5 (C-6^{II}), 57.6 (C-2^I), 51.4 (C-6′), 20.7, 20.6, 20,5 (3 OCO*C*H$_3$). HRMS (ESI-TOF): m/z [M + NH_4]$^+$ calcd for $C_{33}H_{49}Cl_3N_5O_{16}$ 876.2240, found 876.2241. Anal. Calcd for $C_{33}H_{45}Cl_3N_4O_{16}$: C, 46.08; H, 5.27; N, 6.51. Found: C, 46.15; H, 5.30; N, 6.51.

*8-Azido-3,6-dioxaoctyl 2,4-Di-O-benzyl-3,6-dideoxy-α-L-xylo-hexopyranosyl-(1→4)-[2,4-di-O-benzyl-3,6-dideoxy-α-L-xylo-hexopyranosyl-(1→2)-3,4,6-tri-O-acetyl-β-D-galactopyranosyl-(1→3)]-6-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (**10**).* Br_2 (360 μL, 6.96 mmol) was added to a solution of ethyl 2,4-di-*O*-benzyl-3,6-dideoxy-1-thio-β-L-*xylo*-hexopyranoside[15] (1.13 g, 3.48 mmol) in CCl_4 (20 mL). The mixture was shaken gently, and after 5 min, hex-1-ene (1.75 mL, 13.92 mmol) was added. After concentration and coevaporation with CCl_4 (3 × 10 mL), a solution in dry CH_2Cl_2 (10 mL) of the crude α-colitosyl bromide **9** thus obtained was added to a stirred mixture of **8** (0.50 g, 0.58 mmol), Bu_4NBr (1.13 g, 3.48 mmol), and powdered molecular sieves (4 Å, 4.5

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g) in 2:1 CH_2Cl_2–DMF (30 mL). After stirring under argon atmosphere for 48 h at room temperature, when TLC (6:1 toluene–acetone) indicated the glycosyl acceptor **8** was still present, more Bu_4NBr (0.75 g, 2.32 mmol) and freshly prepared bromide donor **9** (2.32 mmol/10 mL CH_2Cl_2) were added into the reaction mixture, and stirring was continued at room temperature. When TLC (6:1 toluene–acetone) showed that all glycosyl acceptor **8** had been consumed (48 h), the mixture was diluted with CH_2Cl_2 (25 mL) and filtered through a Celite pad, and the solids were washed with CH_2Cl_2 (2 × 10 mL). The combined filtrate and washings were successively washed with satd aq $NaHCO_3$ (25 mL) and water (25 mL), dried, and concentrated. Chromatography (6:1 chloroform–acetone) gave the title tetrasaccharide **10** (0.71 g, 83%). $[\alpha]_D^{20} = -23.6$ (*c* 0.6, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.34–7.22 (m, 25 H, Ph), 7.09 (d, 1 H, $J_{2,NH}$ = 8.0 Hz, NH), 5.27 (d, 1 H, $J_{3,4}$ = 2.9 Hz, H-4^{II}), 5.22 (d, 1 H, $J_{1,2}$ = 3.0 Hz, H-1^{IV}), 5.07 (d, 1 H, $J_{1,2}$ = 3.3 Hz, H-1^{III}), 4.93 (d, partial overlap, 1 H, $J_{1,2}$ = 7.0 Hz, H-1^{I}), 4.90 (dd, partial overlap, 1 H, $J_{2,3}$ = 10.1, $J_{3,4}$ = 3.5 Hz, H-3^{II}), 4.76 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1^{II}), 4.66–4.62 (m, 1 H, H-5^{III}), 4.57 (d, 1 H, ^2J = 12.1 Hz, PhC*H*H), 4.56 (d, 1 H, ^2J = 12.3 Hz, PhC*H*H), 4.55 (d, 1 H, ^2J = 12.2 Hz, PhC*H*H), 4.52 (d, 1 H, ^2J = 12.2 Hz, PhC*H*H), 4.46 (d, 1 H, ^2J = 12.1 Hz, PhC*H*H), 4.43 (d, 1 H, ^2J = 12.2 Hz, PhC*H*H), 4.41–4.38 (m, 4 H, 3 x PhC*H*H, H-3^{I}), 4.28 (d, 1 H, ^2J = 12.4 Hz, PhC*H*H), 4.29–4.25 (m, 1 H, H-5^{IV}), 4.02–3.95 (m, 4 H, H-4^{I}, H-1′$_a$, H-6$^{II}_{a,b}$), 3.90 (dd, 1 H, $J_{1,2}$ = 8.1, $J_{2,3}$ = 10.1 Hz, H-2^{II}), 3.88–3.84 (m, 3 H, H-2^{III}, H-2^{IV}, H-6$^{I}_a$), 3.76 (t, 1 H, *J* = 6.9 Hz, H-5^{II}), 3.74–3.68 (m, 2 H, H-1′$_b$, H-6$^{I}_b$), 3.65–3.55 (m, 10 H, H-2^{I}, H-2′, H-3′, H-4′, H-5′, H-5^{I}), 3.51 (br s, 1 H, H-4^{IV}), 3.36 (t, 2 H, *J* = 5.1 Hz, H-6′), 3.33 (br s, 1 H, H-4^{III}), 2.08–2.16 (m, 2 H, H-3$^{III}_{eq}$, H-3$^{IV}_{eq}$), 2.01, 1.81, 1.68 (3 s, 9 H, 3 x $COCH_3$), 1.73–1.81 (m, 2 H, H-3$^{III}_{ax}$, H-3$^{IV}_{ax}$), 1.24 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6^{IV}), 1.20 (d, 3 H, $J_{5,6}$ = 6.2 Hz, H-6^{III}). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.4, 169.8, 169.7 (3 $OCOCH_3$), 161.5 ($NCOCCl_3$), 138.5, 138.4, 138.3, 138.1, 138.0 (5 *ipso* Ph), 128.4–127.3 (Ar), 101.7 ($J_{C,H}$ = 161.7 Hz, C-1^{II}), 99.0 ($J_{C,H}$ = 165.0 Hz, C-1^{I}), 97.3 ($J_{C,H}$ = 171.3 Hz, C-1^{IV}), 96.6 ($J_{C,H}$ = 172.1 Hz, C-1^{III}), 92.4 (CCl_3), 75.7 (C-3^{I}, C-4^{IV}), 75.4 (C-4^{III}), 75.2 (C-5^{I}), 73.2 (PhCH_2), 73.1 (C-3^{II}), 72.8 (C-4^{I}), 72.5 (C-2^{II}), 71.4 (C-2^{III}), 71.3 (PhCH_2), 71.2 (PhCH_2), 70.9 (C-2^{IV}), 70.7, 70.6, 70.5 (C-2′, C-3′, C-4′), 70.4 (C-5^{II}), 70.3 (PhCH_2), 70.0 (C-5′), 68.4 (C-6^{I}), 67.6 (C-1′), 67.4 (C-4^{II}), 67.3 (C-5^{IV}), 66.0 (C-5^{III}), 60.6 (C-6^{II}), 59.3 (C-2^{I}), 50.5 (C-6′), 26.7 (C-3^{IV}), 26.4 (C-3^{III}), 20.7, 20.6, 20.4 (3 x $OCOCH_3$), 16.6 (C-6^{III}), 16.3 (C-6^{IV}). HRMS (ESI-TOF): *m*/*z* $[M + NH_4]^+$ calcd for $C_{73}H_{93}Cl_3N_5O_{22}$ 1496.5372, found 1496.5388. Anal. Calcd for $C_{73}H_{89}Cl_3N_4O_{22}$: C, 59.21; H, 6.06; N, 3.78. Found: C, 59.44; H, 6.17; N, 3.69.

*8-Azido-3,6-dioxaoctyl 2,4-Di-O-benzyl-3,6-dideoxy-α-L-xylo-hexopyranosyl-(1→4)-[2,4-di-O-benzyl-3,6-dideoxy-α-L-xylo-hexopyranosyl-(1→2)-β-D-galactopyranosyl-(S)-(P)-4,6-cyclic 2,2,2-trichloroethyl phosphate-(1→3)]-6-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (**12**) and 8-Azido-3,6-dioxaoctyl 2,4-Di-O-benzyl-3,6-dideoxy-α-L-xylo-hexopyranosyl-(1→4)-[2,4-di-O-benzyl-3,6-dideoxy-α-L-xylo-hexopyranosyl-(1→2)-β-D-galactopyranosyl-(R)-(P)-4,6-cyclic 2,2,2-trichloroethyl phosphate-(1→3)]-6-O-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranoside (**13**).* A solution of sodium methoxide in methanol (1 M, ~ 1.2 mL) was added under argon to a solution of **10** (400 mg, 0.27 mmol) in 1:5 CH_2Cl_2–MeOH (30 mL), and the mixture was stirred at room temperature for 2 h. The mixture was neutralized with Amberlite IR-120 (H^+) resin and filtered, and the solids were washed with MeOH (2 × 10 mL). The filtrate was concentrated and coevaporated with toluene (twice) to give compound **11** as an amorphous solid in almost theoretical yield. HRMS (ESI-TOF): *m*/*z* $[M + NH_4]^+$ calcd for $C_{67}H_{87}Cl_3N_5O_{19}$ 1370.5055, found 1370.5061.

To a solution of triol **11** (330 mg, 0.24 mmol) and pyridine (200 μL, 2.44 mmol) in CH_2Cl_2 (5 mL) was added 2,2,2-trichloroethyl phosphorodichloridate (57 μL, 0.36 mmol) dropwise at −20 °C. When TLC (~20 min, 4:1 toluene–acetone) indicated complete conversion of **11**, excess of reagent was destroyed by addition of MeOH (400 μL). The mixture was concentrated, and EtOAc (3 mL) was added to the residue. The precipitate was filtered off and washed with EtOAc (2 × 2 mL). The combined filtrates were concentrated, and chromatography (3:1 toluene–acetone) gave **12** (260 mg, 69%) and **13** (87 mg, 23%), in that order.

*Data for **12**.* $[\alpha]_D^{20} = -16.5$ (*c* 0.5, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.38–7.23 (m, 25 H, Ph), 7.06 (d, 1 H, $J_{2,NH}$ = 7.1 Hz, NH), 5.26 (d, 1 H, $J_{1,2}$ = 3.0 Hz, H-1^{III}), 5.07 (d, 1 H, $J_{1,2}$ = 8.1 Hz, H-1^{I}), 5.04 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1^{IV}), 4.76 (d, 1 H, $J_{3,4}$ = 3.1 Hz, H-4^{II}), 4.72 (q, 1 H, $J_{5,6}$ = 7.0 Hz, H-5^{IV}), 4.68–4.61 (m, 2 H, CH_2CCl_3), 4.64 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1^{II}), 4.60 (dd, partial overlap, 1 H, $J_{2,3}$ = 8.2, $J_{3,4}$ = 2.7 Hz, H-3^{I}), 4.58–4.52 (m, 7 H, 5 x PhC*H*H, H-6$^{II}_{a,b}$), 4.51 (d, 1 H, ^2J = 11.6 Hz, PhC*H*H), 4.48 (d, 1 H, ^2J = 11.7 Hz, PhC*H*H), 4.45 (d, 1 H, ^2J = 12.2 Hz, PhC*H*H), 4.41 (d, 1 H, ^2J = 12.1 Hz, PhC*H*H), 4.36 (d, 1 H, ^2J = 12.2 Hz, PhC*H*H), 4.28 (d, 1 H, *J* = 3.3 Hz, 3^{II}–OH), 4.13 (q, 1 H, $J_{5,6}$ = 7.3 Hz, H-5^{III}), 3.98–3.95 (m, 2 H, H-4^{I}, H-2^{III}), 3.93–3.87 (m, 4 H, H-6$^{I}_b$, H-4^{IV}, H-2^{IV}, H-1′$_b$), 3.83 (dd, 1 H, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.9 Hz, H-2^{II}), 3.73–3.71 (m, 2 H, H-6$^{I}_a$, H-1′$_a$), 3.70–3.66 (m, 1 H, H-3^{II}), 3.63–3.57 (m, 8 H, H-2′, H-3′, H-4′, H-5′), 3.56–3.50 (m, 2 H, H-5^{I}, H-2^{I}), 3.48 (br s, 2 H, H-4^{III}, H-5^{II}), 3.34 (t, 2 H, *J* = 5.1 Hz, H-6′), 2.17 (dt, 1 H, $J_{2,3}$ = $J_{3,4}$ = 3.7, ^2J = 13.1 Hz, 1 H, H-3$^{III}_{eq}$), 2.06 (dt, 1 H, $J_{2,3}$ = $J_{3,4}$ = 3.7, ^2J = 12.6 Hz, 1 H, H-3$^{IV}_{eq}$), 1.85 (dt, 1 H, $J_{3,4}$ = 2.2, $J_{2,3}$ = ^2J = 12.7 Hz, 1 H, H-3$^{IV}_{ax}$), 1.82 (dt, 1 H, $J_{3,4}$ = 2.3, $J_{2,3}$ = ^2J = 13.0 Hz, 1 H, H-3$^{III}_{ax}$), 1.28 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6^{IV}), 1.22 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6^{III}). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.3 ($NCOCCl_3$), 139.5, 138.3, 138.2, 138.1, 136.8 (5 *ipso* Ph), 128.7–127.1 (Ar), 101.9 (C-1^{II}), 98.9 (C-1^{III}), 98.1 (C-1^{I}), 96.7 (C-1^{IV}), 94.9 (d, $J_{C,P}$ = 9.9 Hz, CH_2CCl_3), 92.6 ($COCCl_3$), 78.3 (d, $J_{C,P}$ = 6.9 Hz, C-4^{II}), 77.7 (C-2^{II}), 76.9 (C-4^{IV}), 76.8 (CH_2CCl_3), 75.8 (C-3^{I}), 75.8 (C-5^{I}), 75.2 (C-4^{III}), 73.2 (PhCH_2), 72.4 (PhCH_2), 72.3 (C-4^{I}), 72.1 (C-2^{III}), 71.6 (d, $J_{C,P}$ = 7.1 Hz, C-3^{II}), 71.4 (2 PhCH_2), 71.1 (C-2^{IV}), 70.9 (PhCH_2), 70.8 (d, $J_{C,P}$ = 7.7 Hz, C-6^{II}), 70.7, 70.6, 70.5, 70.0 (C-2′, C-3′, C-4′, C-5′), 68.6 (C-1′), 68.4 (C-5^{III}), 67.6 (C-6^{I}), 66.5 (C-5^{IV}), 66.2 (d, $J_{C,P}$ = 6.3 Hz, C-5^{II}), 60.6 (C-2^{I}), 50.6 (C-6′), 27.5 (C-3^{IV}), 26.9 (C-3^{III}), 16.6 (C-6^{III}), 16.4 (C-6^{IV}). ^{31}P NMR (162 MHz, $CDCl_3$): δ = −10.62. HRMS (ESI-TOF): *m*/*z* $[M + Na]^+$ calcd for $C_{69}H_{83}Cl_6N_4O_{21}PNa$ 1567.3316, found 1567.3302.

*Data for **13**.* $[\alpha]_D^{20} = -8.7$ (*c* 0.5, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.35–7.24 (m, 25 H, Ph), 7.16 (d, 1 H, $J_{2,NH}$ = 7.1 Hz, NH), 5.24 (d, 1 H, $J_{1,2}$ = 2.8 Hz, H-1^{III}), 5.08 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1^{I}), 5.06 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1^{IV}), 4.90 (d, 1 H, $J_{3,4}$ = 3.2 Hz, H-4^{II}), 4.71–4.66 (m, 2 H, H-5^{IV}, H-6$^{II}_b$), 4.65–4.56 (m, 2 H, CH_2CCl_3), 4.63 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1^{II}), 4.58 (d, 1 H, ^2J = 12.2 Hz, PhC*H*H), 4.54–4.39 (m, 9 H, 7 x PhC*H*H, H-3^{I}, H-6$^{II}_a$), 4.38 (d, 1 H, ^2J = 11.8 Hz, PhC*H*H), 4.36 (d, 1 H, ^2J = 11.8 Hz, PhC*H*H), 4.27 (d, 1 H, *J* = 3.9 Hz, 3^{II}–OH), 4.13 (q, 1 H, $J_{5,6}$ = 7.4 Hz, H-5^{III}), 4.00 (t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 8.8 Hz, H-4^{I}), 3.95–3.92 (m, 1 H, H-2^{III}), 3.91–3.86 (m, 2 H, H-6$^{I}_b$, H-1′$_b$), 3.85–3.83 (m, 1 H, H-2^{IV}), 3.79 (dd, 1 H, $J_{1,2}$ = 7.8, $J_{2,3}$ = 9.9 Hz, H-2^{II}), 3.75–3.72 (m, 1 H, H-3^{II}), 3.71–3.66 (m, 2 H, H-6$^{I}_a$, H-1′$_a$), 3.62–3.57 (m, 11 H, H-4^{IV}, H-2′, H-3′, H-4′, H-5′, H-5^{I}, H-2^{I}), 3.52 (br s, 1 H, H-5^{II}), 3.46 (br s, 1 H, H-4^{III}), 3.34 (t, 2 H, *J* = 5.0 Hz, H-6′), 2.16 (dt, 1 H, $J_{2,3}$ = $J_{3,4}$ = 3.1, ^2J = 12.2 Hz, 1 H, H-3$^{IV}_{eq}$), 2.12 (dt, 1 H, $J_{2,3}$ = $J_{3,4}$ = 3.6, ^2J = 12.7 Hz, 1 H, H-3$^{III}_{eq}$), 1.85 (dt, 1 H, $J_{3,4}$ = 2.2, $J_{2,3}$ = ^2J = 12.8 Hz, 1 H, H-3$^{III}_{ax}$), 1.82 (dt, 1 H, $J_{3,4}$ = 2.2, $J_{2,3}$ = ^2J = 12.7 Hz, 1 H, H-3$^{IV}_{ax}$), 1.26 (d, 3 H, $J_{5,6}$ = 6.3 Hz, H-6^{IV}), 1.20 (d, 3 H, $J_{5,6}$ = 6.5 Hz, H-6^{III}). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.4 ($NCOCCl_3$), 138.9, 138.2, 138.1, 138.0, 137.1 (5 *ipso* Ph), 130.9–127.4 (Ar), 102.1 (C-1^{II}), 98.7 (C-1^{III}), 98.2 (C-1^{I}), 96.2 (C-1^{IV}), 94.5 (d, $J_{C,P}$ = 8.2 Hz, CH_2CCl_3), 92.6 ($COCCl_3$), 77.6 (d, $J_{C,P}$ = 2.2 Hz, CH_2CCl_3), 78.3 (C-4^{II}, C-2^{II}, C-3^{I}), 76.5 (C-4^{IV}), 75.3 (C-5^{I}), 75.2 (C-4^{III}), 73.3 (PhCH_2), 72.3 (C-4^{I}), 71.8 (d, partial overlap $J_{C,P}$ = 7.5 Hz, C-3^{II}), 71.7 (C-2^{III}), 71.3 (PhCH_2), 71.2 (PhCH_2), 71.1 (PhCH_2), 71.0 (PhCH_2), 70.8 (C-2^{IV}), 70.7, 70.6, 70.5, 70.0 (C-2′, C-3′, C-4′, C-5′), 70.2 (d, $J_{C,P}$ = 4.4 Hz, C-6^{II}), 68.6 (C-1′), 68.4 (C-5^{III}), 67.9 (C-6^{I}), 67.1 (d, $J_{C,P}$ = 5.6 Hz, C-5^{II}), 66.4 (C-5^{IV}), 60.1 (C-2^{I}), 50.6 (C-6′), 27.0 (C-3^{III}, C-3^{IV}), 16.5 (C-6^{III}), 16.4 (C-6^{IV}). ^{31}P NMR (162 MHz, $CDCl_3$): δ = −1.88. HRMS (ESI-TOF): *m*/*z* $[M + NH_4]^+$ calcd for $C_{69}H_{87}Cl_6N_5O_{21}P$ 1562.3756, found 1562.3749.

*8-Amino-3,6-dioxaoctyl 3,6-Dideoxy-α-L-xylo-hexopyranosyl-(1→4)-[3,6-dideoxy-α-L-xylo-hexopyranosyl-(1→2)-β-D-galactopyranosyl-4,6-cyclic phosphate-(1→3)]-2-deoxy-2-acetamido-β-D-glucopyranoside (**14**).* (a) A mixture of **12** (150 mg, 0.11 mmol) and Pd/

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C (150 mg) in a mixture of MeOH (6 mL) and 0.1 M potassium phosphate buffer (6 mL; pH = 7) was stirred under H_2 (1 atm) at room temperature. After 5 days, when TLC (2:1 *i*-PrOH−30% NH_4OH) showed complete conversion of the starting material into a more polar product, the mixture was filtered through a Celite pad, the catalyst was washed with H_2O (2 × 5 mL), and the filtrate was concentrated. Chromatography (2:1:0.1 *i*-PrOH−H_2O−30% NH_4OH) followed by lyophilization afforded the desired tetrasaccharide **14** (69 mg, 85%). ^{1}H NMR (600 MHz, D_2O): δ = 4.94 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1^{III}), 4.83 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1^{IV}), 4.73 (q, 1 H, $J_{5,6}$ = 6.9 Hz, H-5^{III}), 4.64 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1^{II}), 4.50 (d, 1 H, $J_{3,4}$ = 3.7 Hz, H-4^{II}), 4.35 (d, partial overlap, 1 H, $J_{1,2}$ = 8.8 Hz, H-1^{I}), 4.33−4.27 (m, 2 H, H-6$^{II}_{a,b}$), 4.25−4.22 (m, 1 H, H-5^{III}), 4.13 (br t, 1 H, *J* = 3.1 Hz, H-4^{IV}), 3.84 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3^{I}), 3.95−3.89 (m, 4 H, H-2^{IV}, H-2^{III}, H-6$^{I}_{b}$, H-1′$_b$), 3.84−3.79 (m, 2 H, H-3^{II}, H-6$^{I}_{a}$), 3.77 (dd, 1 H, $J_{1,2}$ = 8.8, $J_{2,3}$ = 10.4 Hz, H-2^{I}), 3.72 (br t, 1 H, *J* = 3.3 Hz, H-4^{III}), 3.66−3.64 (m, 4 H, H-4^{I}, H-1′$_b$, H-5′), 3.62−3.58 (m, 6 H, H-2′, H-3′, H-4′), 3.58−3.56 (m, 1 H, H-5^{I}), 3.55 (dd, partial overlap, 1 H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.5 Hz, H-2^{II}), 3.44 (dt, 1 H, *J* = 3.1, *J* = 9.9 Hz, H-5^{I}), 3.08 (t, 2 H, *J* = 5.1 Hz, H-6′), 2.01−1.98 (dt, partial overlap, 1 H, $J_{3,4}$ = 2.9, $J_{2,3}$ = ^2J = 12.9 Hz, H-3$^{IV}_{ax}$), 1.97 (s, 3 H, COCH_3), 1.84−1.80 (m, 2 H, H-3^{III}), 1.81−1.78 (dt, partial overlap, 1 H, $J_{3,4}$ = 3.4, $J_{2,3}$ = ^2J = 12.9 Hz, H-3$^{IV}_{eq}$), 1.14 (d, 6 H, $J_{5,6}$ = 6.6 Hz, H-6^{III}, H-6^{IV}). ^{13}C NMR (150 MHz, D_2O): δ = 173.6 ($COCH_3$), 101.7 (C-1^{I}), 100.9 (C-1^{II}), 99.4 (C-1^{III}), 97.7 (C-1^{IV}), 76.3 (d, $J_{C,P}$ = 4.6 Hz, C-4^{II}), 76.1 (C-2^{II}), 75.5 (C-5^{I}), 75.3 (C-3^{I}), 72.5 (C-4^{I}), 72.3 (d, $J_{C,P}$ = 7.4 Hz, C-3^{II}), 69.8, 69.7, 69.5 (C-2′, C-3′, C-4′), 69.1 (C-1′), 68.7 (C-4^{III}), 68.6 (d, $J_{C,P}$ = 5.0 Hz, C-6^{II}), 68.3 (C-4^{IV}), 67.3 (d, $J_{C,P}$ = 4.6 Hz, C-5^{II}), 67.1 (C-5′), 66.6 (C-5^{IV}), 66.0 (C-5^{III}), 63.5 (C-2^{III}), 63.3 (C-2^{IV}), 59.5 (C-6^{I}), 55.6 (C-2^{I}), 39.2 (C-6′), 32.7 (C-3^{III}), 32.5 (C-3^{IV}), 22.1 ($COCH_3$), 15.5, 15.3 (C-6^{III}, C-6^{IV}). ^{31}P NMR (162 MHz, D_2O): δ = −3.74 ($^3J_{P,H}$ 21.6 Hz). HRMS (ESI-TOF): *m*/*z* [M − H]$^-$ calcd for $C_{32}H_{56}N_2O_{21}P$ 835.3113, found 835.3109.

(b) Compound **13** (120 mg, 0.08 mmol) when treated with Pd/C (120 mg) and worked up, as described for **12**, afforded **14** (53 mg, 81%), which was in all aspects identical with the compound described above.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b02105.

^{1}H and ^{13}C NMR spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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