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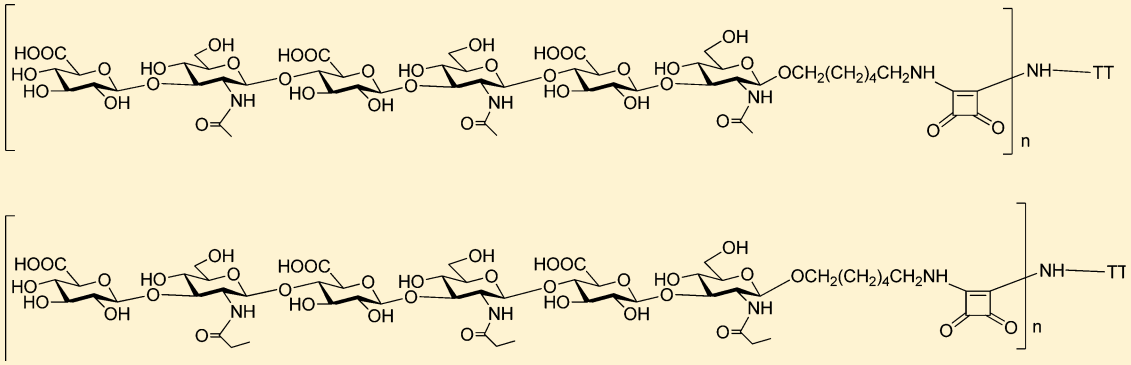
Synthesis and Immunological Characterization of Modified Hyaluronic Acid Hexasaccharide Conjugates

Guofeng Gu,[†] Pal John Pal Adabala,[‡] Monica G. Szczepina,[‡] Silvia Borrelli,[‡] and B. Mario Pinto*[,‡]

[†]National Glycoengineering Research Center, Shandong University, Jinan 250100, PR China

[‡]Department of Chemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

Supporting Information



ABSTRACT: The synthesis of a tetanus toxoid (TT)-conjugate of a hyaluronic acid (HA) hexasaccharide is described. The compound was intended for use in monitoring HA levels as a disease marker and as a potential vaccine against Group A *Streptococcus* (GAS) infections. We also report the synthesis of a chemically modified HA-hexasaccharide-TT conjugate in which the *N*-acetyl moiety of the *N*-acetyl-D-glucosamine residue is replaced with an *N*-propionyl unit in order to enhance immunogenicity. The oligosaccharides are synthesized in a convergent manner. The TT-conjugate syntheses rely on the reaction of the amines on the 6-aminohexyl aglycon of the hexasaccharides with diethyl squarate to give the monoethyl squarate adducts. Subsequent reactions with lysine ε-amino groups on TT then give the glycoconjugates containing an average of 8 hexasaccharide haptens per TT molecule. Immunological studies in mice show very similar antibody responses with both conjugates, suggesting that the *N*-acetyl groups of the glucosaminyl residues of the HA-hexasaccharide are not a critical part of the epitope recognized by the anti-HA polyclonal immune response. Furthermore, it would appear that the *N*-acyl moieties are not in close contact with the amino acid residues of the antibody combining sites.

INTRODUCTION

Hyaluronan (or hyaluronic acid, HA) is a linear polysaccharide composed of repeating disaccharide units of D-glucuronic acid and *N*-acetyl-D-glucosamine, which can reach more than 10 000 repeating units of $[-\beta(1,4)$-D-glucuronic acid$-\beta(1,3)$-*N*-acetyl-D-glucosamine$]_n$. The molecular weight of HA is about 4000 kDa, and it has an average extended length of about 10 μm (e.g., n = 10 000).[1]

HA has been described in all living organisms, from prokaryotes to eukaryotes, and is located in the extracellular and pericellular matrix, but it is also found intracellularly.[1] In mammals, HA can be released by many cell types, although connective tissue cells are believed to be the predominant source of HA, which is synthesized by membrane-bound hyaluronan synthases using the activated nucleotide sugars (UDP-D-glucuronic acid and UDP-*N*-acetyl-D-glucosamine) as substrates.[1,2] These enzymes are located on the inner surface of the plasma membrane, and the chains synthesized are secreted through pore-like structures into the extracellular space.[2] The biological roles of HA in humans are based mainly on its hydrophilic and hydrodynamic properties by which it retains water and plays structural and/or lubricant roles, for example, in joint synovial and eye vitreous fluid, skin, umbilical cord, and water transport, heart valves, skeletal tissues, supramolecular assembly of proteoglycans in the extracellular matrix, and control of tissue hydration.[1−3] HA also plays important receptor-mediated roles (interacting with a variety of binding proteins), in cell detachment, mitosis, migration, tumor development, metastasis, and inflammation.[1,2] It is now clear that specific biological roles of HA are related to the length of the carbohydrate chain and its molecular weight[1,2] In addition, HA is produced in mass during tissue injury, tissue repair, wound healing, and inflammation,[2] and it has also been found in blood serum in high concentrations.[4] Consequently, serum hyaluronan was described as a disease marker of many pathological conditions such as liver cirrhosis, rheumatoid arthritis and other joint diseases, septicemia, uraemia, and renal failure.[4] Urine

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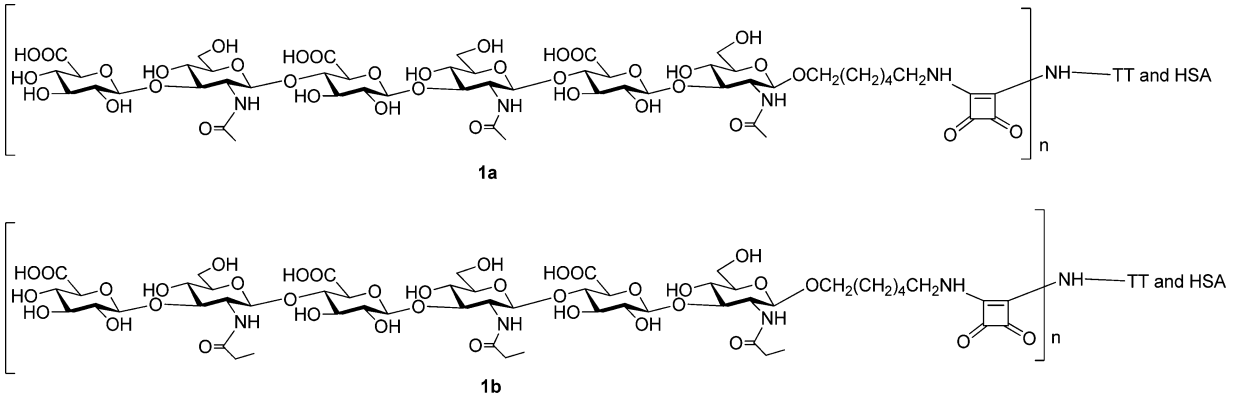
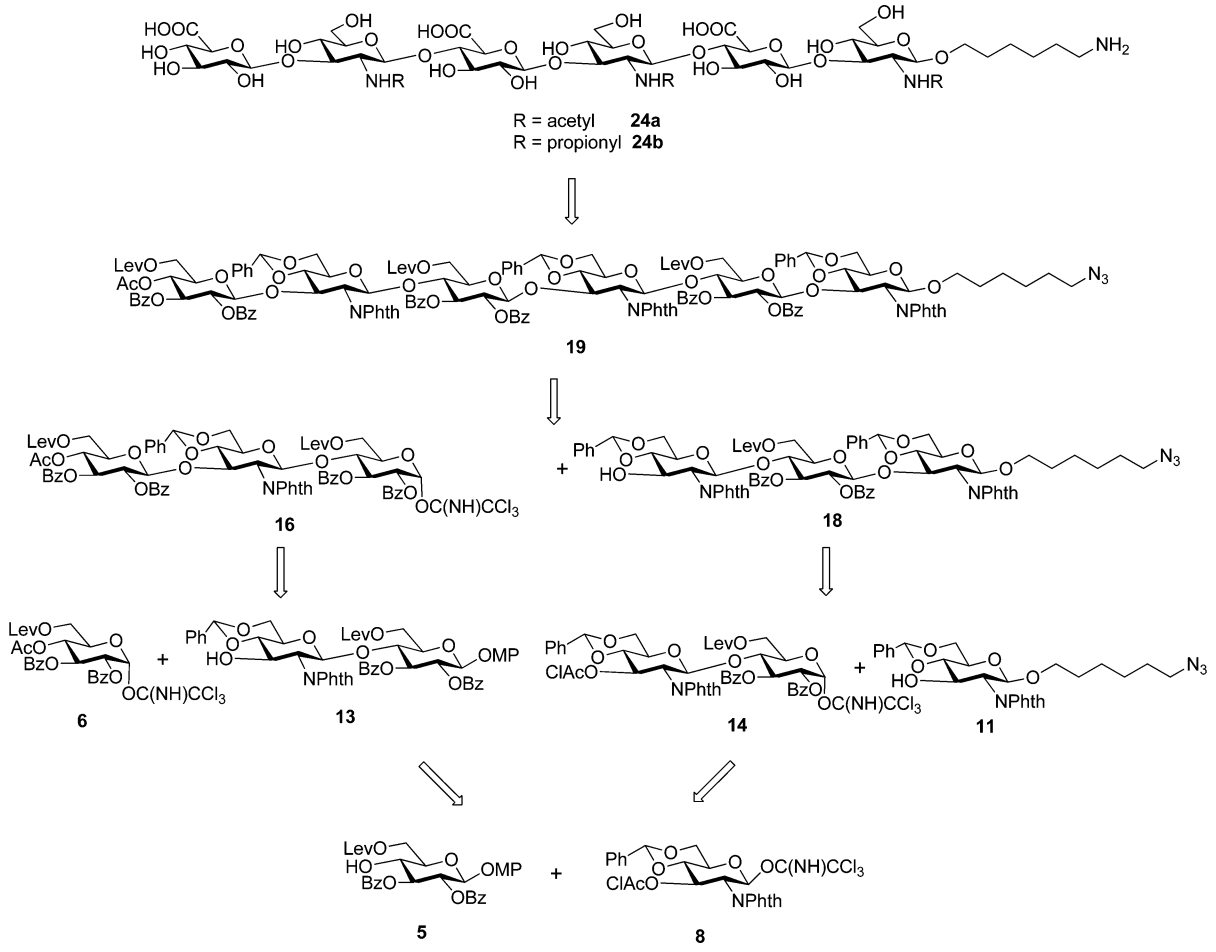


Figure 1. Target protein conjugates **1a** and **1b**.

Scheme 1. Retrosynthetic Analysis of the Hexasaccharides 24a and 24b



HA has been found in patients with bladder cancer angiogenesis and metastasis; thus, HA appears to be a useful marker in the diagnosis of these pathologies.[5] HA is nonantigenic and nonimmunogenic because of its highly conserved structure among species and weak interaction with blood components.[1]

Streptococcus pyogenes, or Group A *Streptococcus* (GAS) is the etiological agent of a number of human diseases ranging from trivial pharyngitis, to lethal necrotizing fasciitis, and streptococcal toxic shock syndrome, leading in some cases to delayed sequelae such as rheumatic fever and rheumatic heart disease.[6] GAS causes ~700 million human infections each year, resulting in over 500 000 deaths.[7] Rapid administration of penicillin or amoxicillin therapy shortens the clinical course, decreases the incidence of sequelae and the risk of transmission, and prevents acute rheumatic fever.[8] The risk of antibiotic-resistant bacteria[9] makes a vaccine protocol an attractive alternative to the present antibiotic therapy.[10] A safe and effective commercial GAS vaccine has yet to be developed.[11] Synthetic oligosaccharide vaccine candidates against GAS infections have been reported recently,[12] but good protection against heavily encapsulated strains may be difficult to establish. The surface-anchored GAS M proteins and its peptides are capable of eliciting protective immunity,[11,13] but cross-reactive antibodies against human tissue antigens have been elicited, raising vaccine safety concerns.[11] GAS is an

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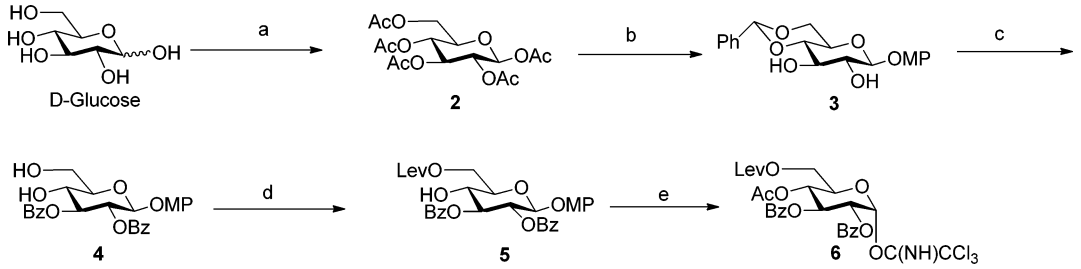
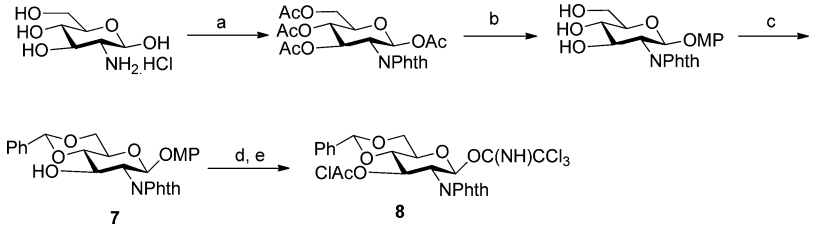
Scheme 2. Synthesis of the Glucose-Derived Monosaccharide Building Blocks 5 and 6[a]



[a](a) NaOAc, Ac_2O, 140 °C. (b) (i) 4-Methoxyphenol, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (ii) 1 N NaOMe, MeOH; (iii) $PhCH(OMe)_2$, *p*-TsOH, DMF, 70 °C. (c) (i) BzCl, pyridine, CH_2Cl_2, 0 °C to rt, 83 %; (ii) 80% HOAc, $CHCl_3$, reflux, 78%. (d) Levulinic acid, 2-chloro-1-methylpyridinium iodide, 1,4-diazabicyclo[2,2,2]octane, 1,2-dichloroethane, rt, 90%. (e) (i) Ac_2O, pyridine; (ii) $(NH_4)_2Ce(NO_3)_6$, CH_3CN/H_2O (4:1, v/v); (iii) Cl_3CCN, DBU, CH_2Cl_2, 0 °C, 70% (3 steps).

Scheme 3. Synthesis of the Glucosamine-Derived Monosaccharide Building Block 8[a]



[a](a) (i) Phthalic anhydride, aq NaOH; (ii) Ac_2O, pyridine. (b) (i) 4-Methoxyphenol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, reflux; (ii) 1 N NaOMe, MeOH. (c) $PhCH(OMe)_2$, *p*-TsOH, DMF, 70 °C. (d) Chloroacetic anhydride, pyridine/CH_2Cl_2, 0 °C, 85%. (e) (i) $(NH_4)_2Ce(NO_3)_6$, CH_3CN/H_2O (4:1, v/v), 0 °C; (ii) Cl_3CCN, DBU, CH_2Cl_2, 61% (2 steps).

encapsulated bacteria, and its capsule is composed of HA (also present in Group C *Streptococcus*), which confers resistance to phagocytosis. GAS HA is chemically similar to that found in human connective tissue and is therefore a poor immunogen; antibodies to GAS HA have been quite difficult to detect in humans, although they have been elicited in rabbits and mice immunized with encapsulated GAS.[6] Previous work on the immunogenicities of low molecular weight HA in the prevention and treatment of Group A and C *Streptococcus* infections has shown that these antibodies are directed against the nonreducing end of the glucuronic acid moiety of low-molecular weight HA.[14] Of greater utility would be an antibody that recognizes the internal epitopes of HA. Ideally, a portion of HA could be used as part of an antigen to elicit the appropriate antibodies that could then be used to monitor HA levels as a disease marker. This oligosaccharide, when conjugated to carrier protein, could also be used in a vaccine preparation. Alternatively, the antibodies could be used as therapeutic agents in passive immunization protocols to treat GAS infections. Various strategies have been reported for the synthesis of diverse molecular weight HA oligosaccharides, ranging from enzymatic[15,16] to chemical methods,[17−25] with the aim of studying HA-protein interactions and structure−activity relationships. We report here the synthesis of a hexasaccharide unit of HA coupled to tetanus toxoid (TT) for use as an antigen to raise antibodies for diagnostic purposes. We also report the synthesis of a chemically modified HA unit and its conjugation to TT to be used as a vaccine in active immunization protocols. In the latter we replace the *N*-acetyl moiety of the *N*-acetyl-D-glucosamine unit with an *N*-propionyl unit, a nonself structural element. This modification has been shown to work in the case of the polysialic acid antigen of *Neisseria meningitidis* B, which shows enhanced immunogenicity.[26−29] We also report the immunological studies in mice with both vaccine candidates, which show very similar antibody responses for both conjugates, suggesting that the *N*-acetyl groups of the glucosaminyl residues of HA-hexasaccharide are not in close contact with the amino acid residues of the antibody combining site. Toward this end, we designed the synthesis of two tetanus toxoid (TT) and two human serum albumin (HSA) conjugates of the HA-hexasaccharides **1a** and **1b** (Figure 1).

RESULTS AND DISCUSSION

Retrosynthetic analysis indicated that the desired hexasaccharides **24a** and **24b** could be synthesized through a convergent (3 + 3′) strategy (Scheme 1). Thus, the hexasaccharide derivative **19** could be synthesized by coupling of the trisaccharide donor **16** and trisaccharide acceptor **18**, which could be assembled, in turn, from the following four monosaccharide building blocks: glucose-derived monosaccharide donor **6**, disaccharide acceptor **13**, disaccharide donor **14** and glucosamine-derived acceptor **11**. Multiple protecting group manipulations of **19**, i.e., removal of benzylidene, levulinoyl and acyl groups, conversion of the *N*-phthalimido group to *N*-acyl groups, PDC oxidation of 6-OH of the glucose residues to carboxyl acids and reduction of the azide to an amine, would then generate the target hexasaccharides **24a**/**b** (Scheme 1). The conjugates of hexasaccharides **24a** and **24b** with tetanus toxoid (TT) or human serum albumin (HSA) could then be prepared, using diethyl squarate[30−32] as a linker, to afford the corresponding hexasaccharide neoglycoproteins (**TT-1a**/**b**, **HSA-1a**/**b**).

The synthesis of the four key monosaccharide precursors **5**, **6**, **8**, and **11** was examined first. The monosaccharide acceptor **5** was synthesized using a similar procedure described in the literature (Scheme 2).[33] Benzoylation of 4-methoxyphenyl 4,6-di-*O*-benzylidene-*β*-D-glucopyranoside (**3**) with benzoyl chloride in pyridine, followed by removal of the benzylidene group

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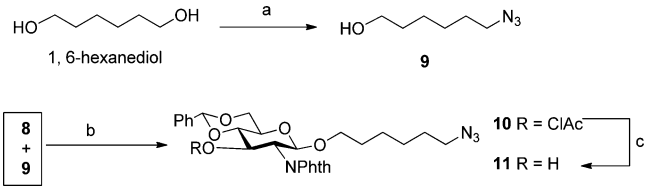
with 80% HOAc in $CHCl_3$, furnished the diol **4** in 61% yield for two steps. Selective protection of the 6-OH with levulinic acid in 1,2-dichloroethane afforded the glucopyranosyl acceptor **5** in 90% yield. Acetylation of **5**, followed by removal of the 4-methoxyphenyl (MP) group at C-1 with cerium ammonium nitrate,[34] and activation of the anomeric hydroxyl as its trichloroacetimidate,[35] followed by chromatographic purification, gave the α-anomer of donor **6** as the major product in a total yield of 70% (Scheme 2).

4-Methoxyphenyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside[36,37] (**7**) was prepared from D-glucosamine hydrochloride as indicated in Scheme 3. Then, protection of the 3-OH with chloroacetic anhydride in pyridine/dichloromethane at 0 °C, followed by removal of the methoxyphenyl (MP) group at C-1, and anomeric activation of the hemiacetal as the trichloroacetimidate furnished the donor **8**, after chromatography, with the β-configuration as the major product in 61% yield (Scheme 3). The β-configuration was confirmed by the coupling constant of H-1 (δ 6.72 ppm, $J_{1,2}$ 8.7 Hz) in the ^{1}H NMR spectrum.

6-Azido-1-hexanol (**9**)[38−40] was prepared from commercially available 1,6-hexanediol by (i) selective tosylation with tosyl chloride in pyridine/DMF (1:2, v/v) at 0 °C and (ii) displacement of the tosylate with sodium azide in DMF at 70 °C. Glycosylation of **9** with the donor **8** in dichloromethane, with promotion by trimethylsilyl trifluoromethanesulfonate (TMSOTf), generated compound **10** in 92% yield. Dechloroacetylation of **10** was carried out with thiourea[41] to afford the acceptor **11** in 83% yield (Scheme 4).

Scheme 4. Synthesis of the Acceptor 11[a]



[a](a) (i) TsCl, pyridine/DMF, 0 °C; (ii) NaN_3, DMF, 70 °C. (b) TMSOTf, CH_2Cl_2, 4 Å MS, −30 to 0 °C, 92%. (c) Thiourea, 2,6-lutidine, MeOH/CH_2Cl_2 (4:1 v/v), 83%.

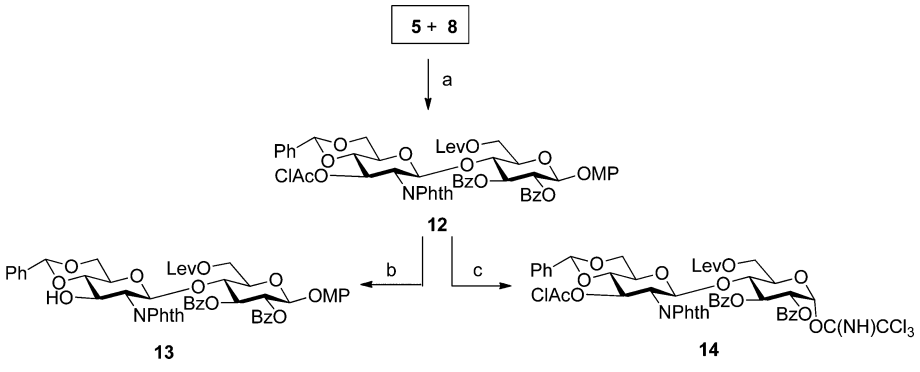
With synthons **5**, **6**, **8** and **11** in hand, the trisaccharides were next assembled. First, coupling of the acceptor **5** and trichoroacetimidate **8** in dichloromethane with TMSOTf as promoter afforded the β-(1 → 4)-linked disaccharide **12** in 81% yield (Scheme 5). The chemical shifts of H-1′ (δ 5.49 ppm, $J_{1',2'}$ 8.2 Hz) and C-1′ (δ 98.9 ppm) in the NMR spectra confirmed the β-linkage. Removal of the chloroacetyl group from **12** with thiourea gave the disaccharide acceptor **13** (73%). On the other hand, cerium ammonium nitrate-promoted cleavage of the MP group on the anomeric carbon of **12**, followed by trichloroacetimidate formation with Cl_3CCN/DBU in dichloromethane, generated the disaccharide donor **14** with the α-configuration as the major product in 67% yield for the two steps (Scheme 5).

The coupling reaction of trichloroacetimidate **6** and the disaccharide acceptor **13** in dichloromethane was carried out with promotion of TMSOTf to yield the trisaccharide **15** in 82% yield (Scheme 6). Then, cleavage of the MP group with cerium ammonium nitrate followed by activation of the hemiacetal with trichloroacetonitrile/DBU in CH_2Cl_2 furnished the α-anomer of trichloroacetimidate **16** as the major product, after chromatography, in 70% yield. Condensation of the acceptor **11** and the disaccharide donor **14**, promoted by TMSOTf at −40 °C, then furnished the trisaccharide **17** in 83% yield. Thiourea-promoted dechloroacetylation of **17** generated the trisaccharide acceptor **18** in 78% yield (Scheme 6).

We next turned our attention to the synthesis of the hexasaccharides. The hexasaccharide **19** was obtained in 70% yield by condensation of the trisaccharide donor **16** with the trisaccharide acceptor **18** under standard coupling conditions (Scheme 7).

Debenzylidenation of **19** with 80% HOAc, followed by acetylation with acetic anhydride in pyridine, furnished the hexasaccharide **20** in 78% yield for two steps (Scheme 8). Delevulinoylation of **20** was carried out smoothly with hydrazine acetate[42] in ethanol/toluene (2:1, v/v) to afford the hexasaccharide-triol **21** in 89% yield. Oxidation of the triol **21** with pyridinium dichromate[43,44] (PDC) in dichloromethane in the presence of acetic anhydride gave the desired product **22** in 67% yield. The correct structure of **22** was confirmed by spectral analysis of its methyl-esterified derivative, **22a**, whose ^{1}H NMR spectrum showed three singlets at δ 3.42, 3.44, and 3.71 ppm, together with the MALDI-TOFMS spectrum, which showed a molecular-ion peak at 2528 $[M + Na]^+$, indicating the presence of three methyl-esters. Dephthaloylation of **22** with ethylenediamine[45] in *n*-butanol, followed by acylation with acetic anhydride or propionic anhydride in pyridine, and then de-*O*-acetylation of

Scheme 5. Synthesis of the Disaccharide Acceptor 13 and Donor 14[a]



[a](a) TMSOTf, CH_2Cl_2, −40 to 0 °C, 81%. (b) Thiourea, 2,6-lutidine, MeOH/CH_2Cl_2, reflux, 73%. (c) (i) $(NH_4)_2Ce(NO_3)_6$, CH_3CN/H_2O (4:1), 0 °C; (ii) Cl_3CCN, DBU, CH_2Cl_2, 67% (2 steps).

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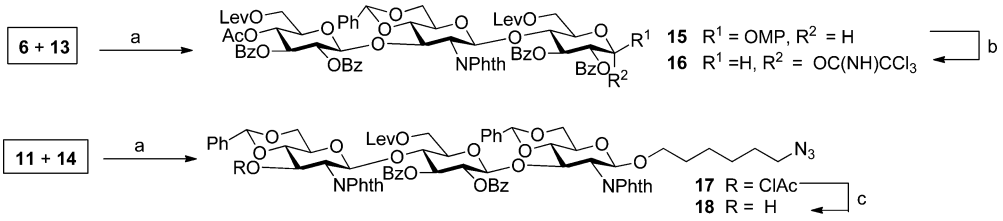
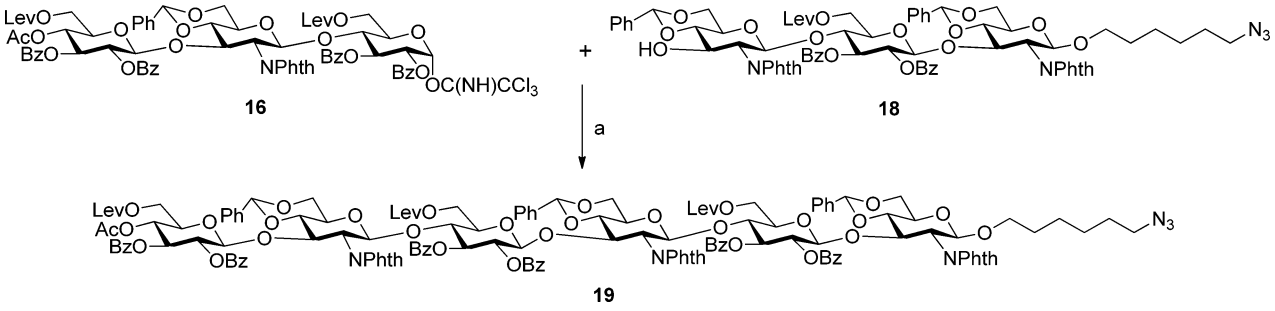
Scheme 6. Synthesis of the Trisaccharide Donor 16 and Acceptor 18[a]



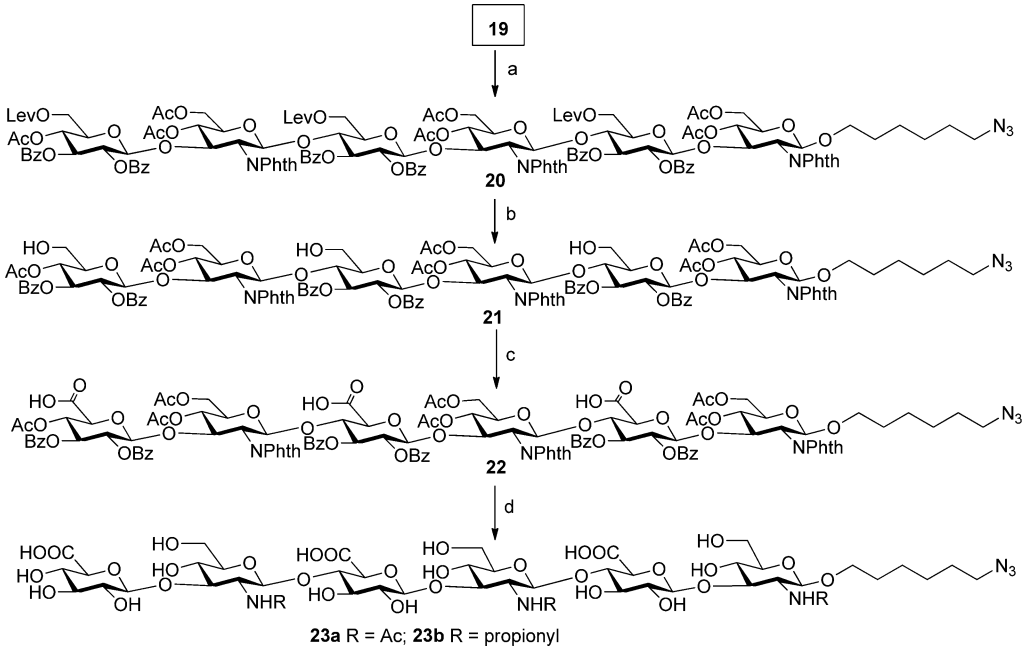
[a](a) TMSOTf, CH_2Cl_2, −40 to 0 °C, 82% for **15**, 83% for **17**. (b) (i) $(NH_4)_2Ce(NO_3)_6$, CH_3CN/H_2O, 0 °C; (ii) Cl_3CCN, DBU, CH_2Cl_2. (c) Thiourea, $MeOH/CH_2Cl_2$, 2,4-lutidine, 78%.

Scheme 7. Synthesis of the Hexasaccharide 19[a]



[a](a) TMSOTf, CH_2Cl_2, −40 to 0 °C, 70%.

Scheme 8. Synthesis of the Hexasaccharides 23a and 23b[a]



[a](a) 80% HOAc, 70 ° C, then Ac_2O, pyridine, 78%. (b) Hydrazine acetate, 2:1 EtOH−toluene, 89%. (c) PDC/Ac_2O, CH_2Cl_2, 67%. (d) (i) $H_2NCH_2CH_2NH_2$, 1-butanol; (ii) Ac_2O or $(CH_3CH_2CO)_2O$, pyridine; (iii) 1 N LiOH, THF, 0 °C, 72% for **23a**, 78% for **23b**.

the resulting products using 1 N LiOH[46] in THF at 0 °C, afforded the free hexasaccharides **23** (72% for **23a**; 78% for **23b**) (Scheme 8).

Reduction of the azide function in compounds **23a** or **23b** with 10% Pd−C and $NaBH_4$[30] in 0.05 M aqueous NaOH gave the amino compounds **24** (85% for **24a**; 81% for **24b**; Scheme 9). Reaction of **24a** or **24b** with diethyl squarate[31,32,47,48] in 1:1 EtOH−phosphate buffer (50 mM, pH 7.3) then generated the monoethyl squarate derivatives **25a** or **25b** after purification using size-exclusion chromatography. Compounds **25a** or **25b** were then directly conjugated to tetanus toxoid (TT) or human serum albumin (HSA) in 0.1 M carbonate buffer at pH 10.0.[47] After dialysis and lyophilization of the crude samples, the hexasaccharide neoglycoproteins (**TT-1a/b**, **HSA-1a/b**) were obtained as white powders. The average number of hexasaccharide units incorporated in TT or HSA was assessed by MALDI-TOF MS analysis (Table 1).

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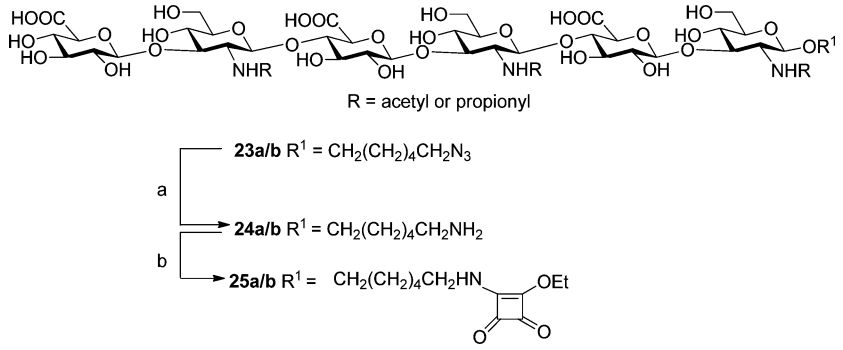
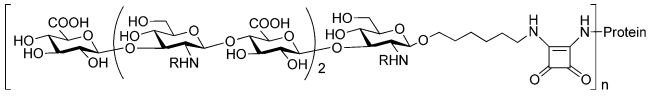
Scheme 9. Synthesis of the Monoethyl Squarate Adducts 25a and 25b[a]



[a](a) 10% Pd-C, $NaBH_4$, 0.05 M NaOH. (b) Diethyl squarate, pH 7.3

Table 1. TT and HSA-Hexasaccharide Conjugates



neoglycoprotein	R group	molar ratio of protein/ squarate derivative	hapten residues/ protein molecule (*n*)	incorporation efficiency (%)
TT-1a	Ac	1:60	10.3	17.2
TT-1b	propionyl	1:60	7.0	11.7
HSA-1a	Ac	1:50	7.8	15.6
HSA-1b	propionyl	1:50	6.9	13.8

Immunological Studies. The immunogenicity of the *N*-propionylated and *N*-acetylated (native form) HA-hexasaccharides-TT conjugates (compounds **1b** and **1a**) in mice was investigated by ELISA using the homologous hexasaccharide-human serum albumin (HSA) conjugates as solid-phase antigens. The absorbances corresponding to antisera on day 38 are shown (Figures 2 and 3).

The HA hexasaccharideNCOPr-TT **1b**-specific IgG response is shown (Figure 2A) with the HA-hexasaccharideNCOPr-HSA as a solid-phase antigen on the ELISA plate. The conjugate induced a strong immune IgG response in virtually all mice except for one animal. When the same sera were measured for activity against the native form of the HA-hexasaccharide, the IgG response was very similar (Figure 2B), indicating that *N*-propionylation of the glucosaminyl groups of the oligosaccharides residues did not affect the recognition of the HA-hexasaccharideNCOPr-specific IgG for the native HA-hexasaccharide.

Conversely, the native HA-hexasaccharideNAc-TT (compound **1a**)-specific IgG response is shown (Figure 3A) with the HA-hexasaccharideNCOPr-HSA conjugate as an antigen on the ELISA plate. All sera (except for one) recognized the HA-hexasaccharideNCOPr immobilized on the plate. When the same sera were measured against the native form of the HA-hexasaccharide, the IgG response was very similar (Figure 3B). These data indicate that *N*-propionylation of the glucosaminyl groups of the oligosaccharides residues of HA does not affect the binding of IgG elicited against either form, NCOPr- or NAc-, of the hexasaccharides. This surprising result may indicate that the NAc groups of the glucosaminyl residues of the HA-hexasaccharide do not form a critical part of the natural epitope recognized by a polyclonal response, and that the *N*-acetyl moiety does not make close contacts with the complementarity

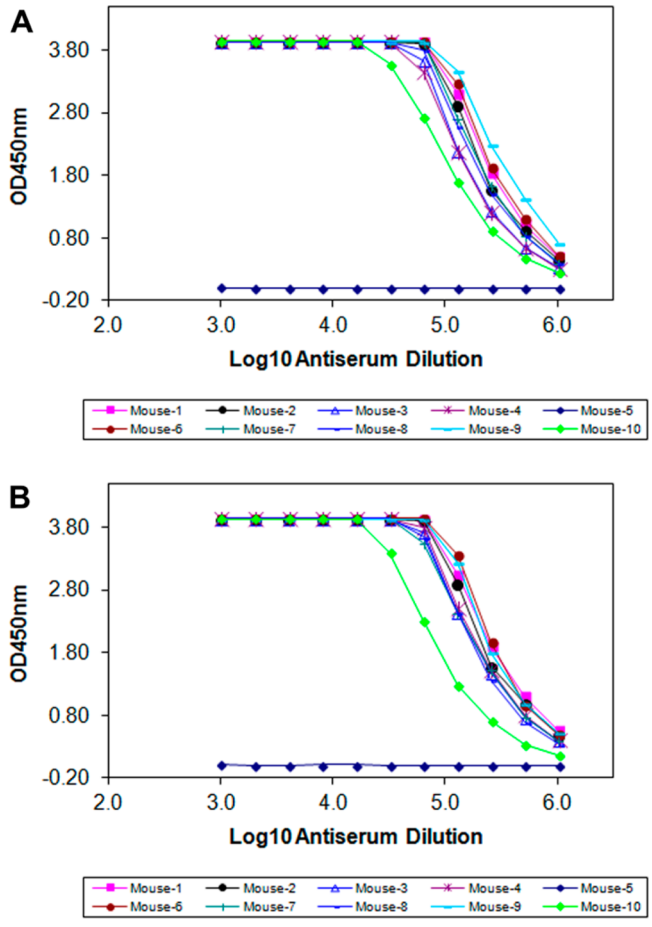


Figure 2. Immunogenicity of the HA-hexasaccharideNCOPr-TT conjugate (compound TT-**1b**): antibody titers (IgG) (A) to HA-hexasaccharideNCOPr-HSA coated plates and (B) to HA-hexasaccharideNAc-HSA coated plates.

determining regions (CDR) residues within the antibody combining site.

CONCLUSIONS

In summary, hyaluronic acid-related hexasaccharide derivative **24a** and its *N*-propionyl analogue **24b** were efficiently synthesized by a highly convergent strategy using glycosyl trichloroacetimidates as glycosyl donors. These two oligosaccharide derivatives were linked to tetanus toxoid (TT) or human serum albumin (HSA) through a squarate linker to provide their

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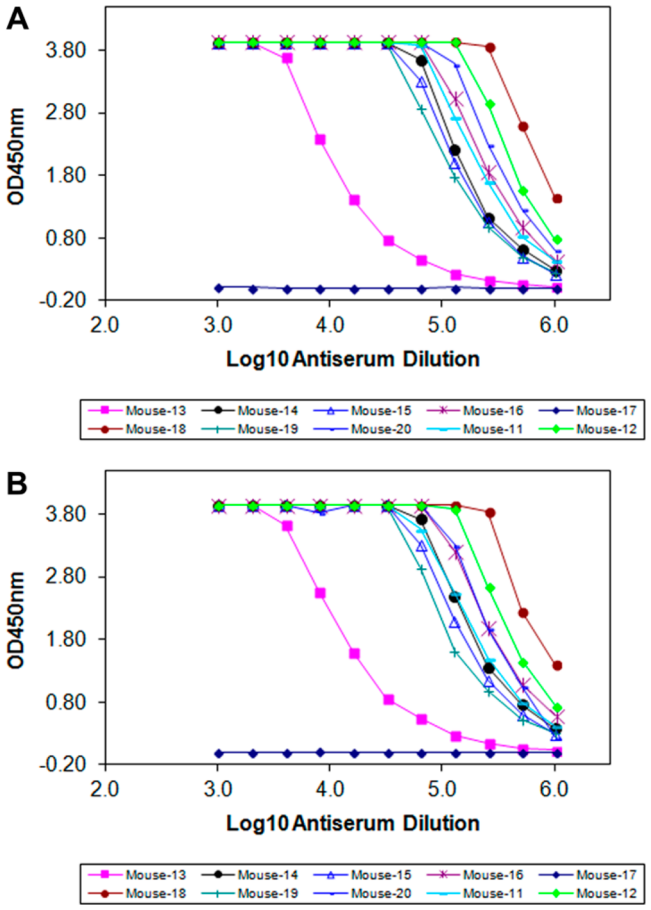

Figure 3. Immunogenicity of the HA-hexasaccharideNAc-TT conjugate (compound TT-**1a**): antibody titers (IgG) (A) to HA-hexasaccharideNCOPr-HSA coated plates and (B) to HA-hexasaccharideNAc-HSA coated plates.

neoglycoproteins. The TT conjugates were intended for use as vaccines candidates against Group A *Streptococcus* infections or to elicit antibodies directed against HA in order to monitor HA levels as disease markers in blood serum and urine samples. The chemically modified *N*-propionyl hexasaccharide conjugate was also intended to enhance immunogenicity, but studies in mice showed similar immunological results for both conjugates, negating the hypothesis that this modification would differentiate between self HA antigens and nonself, modified-HA antigens. The results suggest that the NAc groups of the glucosaminyl residues of the HA-hexasaccharide are not a critical part of the epitope recognized by the set of anti-HA polyclonal antibodies. Furthermore, it would appear that the *N*-acyl moieties are not in close contact with the amino acid residues of the antibody combining sites.

EXPERIMENTAL SECTION

General Methods. Optical rotations were measured at 23 °C. ^{1}H and ^{13}C NMR spectra were recorded at 600 and 150 MHz for proton and carbon respectively. Peak and coupling constant assignments are based on ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H gCOSY, ^{1}H−^{13}C gHSQC, and ^{1}H−^{13}C gHMBC experiments. ^{1}H NMR experiments for the three hexasaccharides **23a**, **23b** and **24b** were recorded at 800 MHz (D_2O; 25 °C). These included gHMBC, gHSQC, TOCSY, ROESY, COSY. The ^{13}C NMR spectra were acquired at 600 MHz, as was the ^{1}H NMR spectrm of **24a**. Severe overlap of resonances prevented accurate measurement of coupling constants. MALDI-TOF mass spectra were obtained for samples dispersed in a 2,5-dihydroxybenzoic acid matrix. High resolution mass spectra were obtained by the electrospray ionization method, using TOF LC−MS high resolution mass spectrometer. Column chromatography was performed with Silica gel 60 (230−400 mesh), or size-exclusion gel (Sephadex G-10/Bio Gel P-2). Solvents were evaporated under reduced pressure below 50 °C.

***p*-Methoxyphenyl 2,3-di-*O*-benzoyl-*β*-D-glucopyranoside (4).** To a mixture of 1,2,3,4,6-penta-*O*-acetyl-*β*-D-glucopyranose (**2**) (20 g, 51.3 mmol) and *p*-methoxyphenol (7.0 g, 56.4 mmol) in dichloromethane (150 mL) was added borontrifluoride etherate (20 mL) at 0 °C. The reaction mixture was stirred for 0.5 h and then warmed to rt, and stirring was continued for another 2 h. The mixture was poured into cold aqueous saturated $NaHCO_3$ (300 mL) and extracted with dichloromethane (500 mL). The organic layer was washed with water and brine, and then dried with anhydrous $MgSO_4$, and filtered. The filtrate was concentrated to give a syrupy product, which was dissolved in methanol (200 mL), and 1 M NaOMe in methanol (20 mL) was added. The reaction mixture was stirred at rt overnight, neutralized with HOAc, and concentrated to give the crude product as a white solid. This solid was suspended in EtOAc/hexane (300 mL, v/v 1: 1) and filtered to give the crude tetrahydroxy compound as a solid (14g, 95%).[49] To a solution of this crude product (14 g) in DMF (100 mL) was added $PhH(OMe)_2$ (12 mL) and a catalytic amount of *p*-TsOH (500 mg). The mixture was heated to 70 °C and stirred under reduced pressure for 1 h and then neutralized with triethylamine, and the solvent was removed under reduced pressure. This benzylidene product[33] was then dissolved in pyridine (50 mL) and dichloromethane (50 mL), and benzoyl chloride (13 mL) was added dropwise over 20 min at 0 °C. The reaction mixture was warmed to rt and stirred overnight. Methanol (2 mL) was then added to decompose the excess BzCl, and the resulting mixture was filtered to give part of the desired compound as a white solid (15 g). The filtrate was concentrated to yield a yellow solid and then suspended in EtOAc/hexane (50 mL, v/v 1:2). After filtration, *p*-methoxyphenyl 2,3-di-*O*-benzoyl-4,6-di-*O*-benzylidene-*β*-D-glucopyranoside was obtained (21 g). To a solution of this compound in chloroform (100 mL) was added 80% HOAc (100 mL), and the mixture was heated to 80 °C and refluxed overnight. The solvent was removed under reduced pressure, and purification of the resulting residue by flash column (EtOAc/hexane 2:1) gave compound **4** as white crystals (18 g, 78%): $[\alpha]_D^{23}$ = +96.0 (*c* 1.0, $CHCl_3$); mp 88−90 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98 (ddd, *J* = 15.1, 8.2, 1.1 Hz, 4H, Ar-*H*), 7.55−7.50 (m, 2H, Ar-*H*), 7.41−7.36 (m, 4H, Ar-*H*), 6.93−6.89 (m, 2H, Ar-*H*), 6.80−6.76 (m, 2H, Ar-*H*), 5.66 (dd, $J_{2,3}$ = 9.7, $J_{1,2}$ = 8.0 Hz, 1H, H-2), 5.47 (t, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, 1H, H-3), 5.21 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.07−4.03 (m, 2H, H-6a, H-4), 3.94 (dd, $J_{6a,6b}$ = 12.0, $J_{5,6b}$ = 4.8 Hz, 1H, H-6b), 3.75 (s, 3H, −OC*H*$_3$), 3.71 (ddd, $J_{4,5}$ = 9.4, $J_{5,6b}$ = 4.7, $J_{5,6a}$ = 3.4 Hz, 1H, H-5), 3.30 (br s, 1H, −O*H*); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.7 (−*C*OPh), 165.4 (−*C*OPh), 155.8 (Ar-*C*), 151.1 (Ar-*C*), 133.8 (Ar-*C*), 133.4 (Ar-*C*), 130.1 (Ar-*C*), 129.8 (Ar-*C*), 129.3 (Ar-*C*), 128.8 (Ar-*C*), 128.6 (Ar-*C*), 128.5 (Ar-*C*), 118.7 (Ar-*C*), 114.7 (Ar-*C*), 100.7 (C-1), 77.3 (C-3), 76.2 (C-5), 71.4 (C-2), 70.0 (C-4), 62.3 (C-6), 55.7 (−OCH_3); HRMS *m*/*z* calcd for $C_{27}H_{30}NO_9$ $[M + NH_4]^+$ 512.1915, found 512.1924.

***p*-Methoxyphenyl 2,3-di-*O*-benzoyl-6-*O*-levulinoyl-*β*-D-glucopyranoside (5).** To a solution of compound **4** (15 g, 31 mmol) in chloroform (20 mL) and 1,2-dichloroethane (100 mL) was added levulinic acid (6.4 mL, 62 mmol) and 2-chloro-1-methylpyridinium iodide (15.8 g, 62 mmol). The reaction mixture was stirred at rt for 20 min, and 1,4-diazabicyclo[2,2,2] octane (10.4 g, 93 mmol) was added. The resulting mixture was stirred for another 30 min, at which time TLC (EtOAc/hexane 2:1) showed the complete consumption of diol **4**. The reaction mixture was filtered through Celite, diluted with dichloromethane (500 mL), and then washed with 10% NaCl (200 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. Column chromatography (EtOAc/hexane 1:2) of the residue afforded **5** as a white solid (16.2 g, 88%): $[\alpha]_D^{23}$ = +66.0 (*c* 1.5, $CHCl_3$); mp 147−149 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01−7.96 (m, 4H, Ar-*H*), 7.55−7.49 (m, 2H, Ar-*H*), 7.41−7.35 (m, 4H, Ar-*H*), 6.96−6.91 (m, 2H, Ar-*H*), 6.80−6.75 (m, 2H, Ar-*H*), 5.66 (dd, $J_{2,3}$ = 9.7, $J_{1,2}$ = 7.9 Hz, 1H, H-2), 5.50 (t, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, 1H, H-3), 5.15 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.58 (dd, $J_{6a,6b}$ = 12.1, $J_{5,6a}$ = 4.8 Hz, 1H, H-6a), 4.44

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(dd, $J_{6a,6b}$ = 12.1, $J_{5,6b}$ = 2.1 Hz, 1H, H-6b), 3.95 (td, $J_{3,4} = J_{4,5}$ = 9.5, $J_{4,OH}$ = 2.5 Hz, 1H, H-4), 3.80 (ddd, $J_{4,5}$ = 9.7, $J_{5,6a}$ = 4.7, $J_{5,6b}$ = 2.2 Hz, 1H, H-5), 3.74(s, 3H, $-OCH_3$), 3.47 (d, $J_{4,OH}$ = 3.7 Hz, 1H, $-OH$), 2.80−2.77 (m, 2H, $-COCH_2CH_2$), 2.65 (t, J = 6.4 Hz, 2H, $-COCH_2CH_2$), 2.19 (s, 3H, $-COCH_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.9 ($-CH_2COCH_3$), 173.3 ($-OCOCH_2CH_2$), 167.3 (−COPh), 165.3 (−COPh), 155.8 (Ar-*C*), 151.2 (Ar-*C*), 133.6 (Ar-*C*), 133.4 (Ar-*C*), 130.1 (Ar-*C*), 129.8 (Ar-*C*), 129.3 (Ar-*C*), 129.0 (Ar-*C*), 128.5 (Ar-*C*), 128.5 (Ar-*C*), 118.9 (Ar-*C*), 114.6 (Ar-*C*), 100.9 (C-1), 76.4 (C-3), 74.5 (C-5), 71.4 (C-2), 69.3 (C-4), 63.1 (C-6), 55.7 ($-OCH_3$), 38.1 ($-COCH_2CH_2$) 29.9 ($-COCH_3$), 28.0 ($-COCH_2CH_2$); HRMS *m*/*z* calcd for $C_{32}H_{36}NO_{11}$ $[M + NH_4]^+$ 610.2283, found 610.2296.

4-*O*-Acetyl-2,3-di-*O*-benzoyl-6-*O*-levulinoyl-α-D-glucopyranosyl trichloroacetimidate (6). To a solution of compound **5** (1.04 g, 1.76 mmol) in pyridine (5 mL) at rt was added acetic anhydride (3 mL). The reaction mixture was stirred overnight, and the solvent was coevaporated with toluene under reduced pressure. The resulting residue was purified by flash column with 1:2 EtOAc/hexane as the eluent to give the acetylated product as a white solid (1.06 g). To a solution of this product (1.06 g, 1.67 mmol) in CH_3CN/H_2O (20 mL, v/v 4:1) at 0 °C was added cerium ammonium nitrate (2.75 g, 5.01 mmol). The reaction mixture was stirred for 30 min at 0 °C and then diluted with EtOAc (100 mL). The resulting mixture was washed with aqueous saturated $NaHCO_3$ and water, and the organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column with 1:1 EtOAc/hexane as the eluent to yield the hemiacetal as a yellow foam. The product was dissolved in dichloromethane (10 mL), trichloroacetonitrile (0.85 mL) and DBU (100 μL) were added, and the reaction mixture was stirred at 0 °C for 2 h. After concentration, purification of the residue by flash column (EtOAc/hexane 1:2) afforded the trichloroacetimidate **6** as a white foam (828 mg, 74%): $[\alpha]_D^{23}$ = +105.3 (*c* 1.5, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.62 (s, 1H, −N*H*), 7.96−7.92 (m, 4H, Ar-*H*), 7.53−7.49 (m, 2H, Ar-*H*), 7.40−7.34 (m, 4H, Ar-*H*), 6.76 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 6.05 (t, $J_{2,3} = J_{3,4}$ =10.0 Hz, 1H, H-3), 5.50 (dd, $J_{2,3}$ = 10.2, $J_{1,2}$ = 3.5 Hz, 1H, H-2), 5.45 (t, $J_{3,4} = J_{4,5}$ =9.9 Hz, 1H, H-4), 4.38−4.34 (m, 1H, H-5,), 4.34 (dd, $J_{6a,6b}$ = 12.0, $J_{5,6a}$ = 4.5, 1H, H-6a), 4.24 (dd, $J_{6a,6b}$ = 12.0, $J_{5,6a}$ = 3.0, 1H, H-6b), 2.84−2.71 (m, 2H, $-COCH_2CH_2$), 2.68−2.64 (m, 2H, $-COCH_2CH_2$), 2.21 (s, 3H, $-COCH_3$), 1.97 (s, 3H, $-COCH_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.4 ($-CH_2COCH_3$), 172.4 ($-OCOCH_2CH_2$), 169.5 ($-OCOCH_3$), 165.7 (−COPh), 165.5 (−COPh), 160.6 (−CNH), 133.6 (Ar-*C*), 133.5 (Ar-*C*), 130.0 (Ar-*C*), 129.9 (Ar-*C*), 128.6 (Ar-*C*), 128.5 (Ar-*C*), 93.2 (C-1), 90.7 ($-CCl_3$), 70.6 (C-2), 70.5 (C-5), 70.3 (C-3), 67.6 (C-4), 61.8 (C-6), 37.9 ($-COCH_2CH_2$), 29.9 ($-COCH_3$), 27.9 ($-COCH_2CH_2$), 20.6 ($-COCH_3$); HRMS *m*/*z* calcd for $C_{29}H_{32}Cl_3N_2O_{11}$ $[M + NH_4]^+$ 689.1066, found 689.1052; *m*/*z* calcd for $C_{58}H_{60}Cl_6N_3O_{22}$ $[2M + NH_4]^+$ 1360.1794, found 1360.1769.

***p*-Methoxyphenyl 4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (7).** A solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (15 g, 31.4 mmol), *p*-methoxyphenol (4.3 g, 34.54 mmol) and borontrifluoride etherate (15 mL) was refluxed for 1h. The reaction mixture was cooled to rt, poured into cold aqueous saturated $NaHCO_3$ (50 mL), and then extracted with dichloromethane (300 mL). The organic layer was washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated to give light yellow foam, which was used directly in next step. To a solution of this product in methanol (150 mL) was added 1 M NaOMe/methanol (10 mL) at rt. The reaction mixture was stirred for 3 h and neutralized with Amberlyst 15 ion-exchange resin (H^+). The resulting mixture was filtered and concentrated. The generated residue was then dissolved in DMF (80 mL), and $PhH(OMe)_2$ (10 mL) was added. The reaction mixture was stirred with a catalytic amount of *p*-TsOH (300 mg) at 70 °C under diminished pressure for 1.5 h. The reaction was neutralized with triethylamine and concentrated under reduced pressure. Column chromatography (EtOAc/hexane 1:1) gave compound **7** as white foam (10.3 g, 63%): $[\alpha]_D^{23}$ = +12.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90−7.84 (m, 2H, Ar-*H*), 7.76−7.72 (m, 2H, Ar-*H*), 7.52−7.49 (m, 2H, Ar-*H*), 7.41−7.35 (m, 3H, Ar-*H*), 6.87−6.83 (m, 2H, Ar-*H*), 6.76−6.72 (m, 2H, Ar-*H*), 5.81 (d, $J_{1,2}$ = 8.5 Hz, 1H, H-1), 5.60 (s, 1H, PhC*H*), 4.71 (dd, $J_{2,3}$ = 10.5, $J_{3,4}$ = 8.6 Hz, 1H, H-3), 4.51 (dd, $J_{2,3}$ 10.6, $J_{1,2}$ = 8.5 Hz, 1H, H-2), 4.41 (dd, $J_{6a,6b}$ = 10.5, $J_{5,6a}$ = 4.6 Hz, 1H, H-6a), 3.93−3.83 (m, 1H, H-6b), 3.76−3.68 (m, 5H, H-5, H-4, $-OCH_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.2 (−COPhth), 155.7 (Ar-*C*), 150.6 (Ar-*C*), 136.9 (Ar-*C*), 134.3 (Ar-*C*), 131.7 (Ar-*C*), 129.5 (Ar-*C*), 128.5 (Ar-*C*), 126.4 (Ar-*C*), 123.7 (Ar-*C*), 118.7 (Ar-*C*), 114.6 (Ar-*C*), 102.1 (PhCH), 98.2 (C-1), 82.1 (C-4), 68.8 (C-3), 68.7 (C-6), 66.4 (C-5), 56.5 (C-2), 55.7 ($-OCH_3$); HRMS *m*/*z* calcd for $C_{28}H_{26}NO_8$ $[M + H]^+$ 504.1653, found 504.1665; *m*/*z* calcd for $C_{28}H_{29}N_2O_8$ $[M + NH_4]^+$ 521.1918, found 521.1930.

4,6-Di-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (8). To a mixture of compound **7** (9 g, 17.3 mmol) in dichloromethane (50 mL) and pyridine (10 mL) at 0 °C was added chloroacetic anhydride (3.26 g, 19 mmol). The reaction mixture was stirred for 40 min at 0 °C, at which time TLC (EtOAc/hexane 1:1) indicated the consumption of **7** and the formation of a new compound. The reaction mixture was then diluted with dichloromethane (100 mL) and washed with 1 N HCl, water and brine. The organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated and purified by flash column with 1:1 EtOAc/hexane as eluent to afford the 3-chloroacetylated compound as a light-yellow foam (8.8 g, 85%). To a solution of this product in CH_3CN/H_2O (100 mL, v/v 4:1) at 0 °C was added cerium ammonium nitrate (14.4 g, 26.3 mmol). The reaction mixture was stirred for 40 min at 0 °C, at which time TLC indicated the disappearance of the starting material. The resulting mixture was diluted with EtOAc (300 mL), washed with aqueous saturated $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was recrystallized from EtOAc/hexane (1:2) to yield the crude hemiacetal as a light-yellow solid (5 g). This compound was dissolved in dichloromethane (40 mL), and trichloroacetonitrile (7 mL) was added. The reaction mixture was stirred with a catalytic amount of DBU (0.7 mL) at 0 °C for 2 h. After concentration, the residue was purified on a flash column with EtOAc/hexane (1:2) as the eluent to afford compound **8** as white crystals (6 g, 56%): $[\alpha]_D^{23}$ = +31.3 (*c* 1.5, $CHCl_3$); mp 179−181 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.67 (s, 1H, −N*H*), 7.85 (dd, *J* = 5.2, 2.9 Hz, 2H, Ar-*H*), 7.76−7.71 (m, 2H, Ar-*H*), 7.47−7.46 (m, 2H, Ar-*H*), 7.40−7.36 (m, 3H, Ar-*H*), 6.72 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1), 6.08 (t, $J_{2,3} = J_{3,4}$ = 9.7 Hz,1H, H-3), 5.57 (s, 1H, −C*H*Ph), 4.66 (dd, $J_{2,3}$ = 10.3, $J_{1,2}$ = 8.8 Hz, 1H, H-2), 4.51 (dd, $J_{6a,6b}$ = 10.5, $J_{5,6a}$ = 4.7 Hz, 1H, H-6a), 4.00−3.87 (m, 5H, H-5, H-4, H-6b, $-COCH_2Cl$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.5 (−COPhth), 166.7 ($-COCH_2Cl$), 160.7 (−CNH), 136.6 (Ar-*C*), 134.6 (Ar-*C*), 131.2 (Ar-*C*), 129.4 (Ar-*C*), 128.4 (Ar-*C*), 126.3 (Ar-*C*), 123.9 (Ar-*C*), 101.9 (−CHPh), 94.0 (C-1), 90.1 ($-CCl_3$), 78.8(C-4), 71.2 (C-3), 68.5 (C-6), 66.9 (C-5), 54.1 (C-2), 40.4 ($-COCH_2Cl$); HRMS *m*/*z* calcd for $C_{25}H_{21}Cl_4N_2O_8$ $[M + H]^+$ 617.0047, found 617.0043.

6-Azidohexyl 4,6-di-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (10). To a mixture of compound **8** (360 mg, 0.582 mmol), 6-azido-1-hexanol (**9**) (84 mg, 0.582 mmol) and molecular sieves 4 Å (500 mg) in dichloromethane (5 mL) at −30 °C was added under N_2 trimethylsilyl trifluoromethanesulfonate (11 μL, 60 μmol). The reaction mixture was stirred with the temperature slowly warming to 0 °C for 1 h, at which time TLC (EtOAc/hexane 1:2) indicated the completion of the reaction. The reaction mixture was then neutralized with triethylamine and flitered through Celite. The flitrate was evaporated under reduced pressure, and the residue was purified by column chromatography (EtOAc/hexane 1:2) to give compound **10** as a foam (320 mg, 92%): $[\alpha]_D^{23}$ = −11.4 (*c* 0.35, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88 (br s, 2H, Ar-*H*), 7.76 (dd, *J* = 5.3, 2.6 Hz, 2H, Ar-*H*), 7.47−7.42 (m, 2H, Ar-*H*), 7.38−7.34 (m, 3H, Ar-*H*), 5.96 (t, $J_{2,3} = J_{3,4}$ = 9.8 Hz, 1H, H-3), 5.54 (s, 1H, −C*H*Ph), 5.42 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1), 4.43 (dd, $J_{6a,6b}$ = 10.5, $J_{5,6a}$ = 4.8 Hz, 1H, H-6a), 4.34 (dd, $J_{2,3}$ = 10.3, $J_{1,2}$ = 8.5 Hz, 1H, H-2), 3.91 (d, *J* = 1.9 Hz, 2H, $-COCH_2Cl$), 3.88−3.80 (m, 3H, H-4, H-6b, $OCHH(CH_2)_5N_3$), 3.77−3.73 (m, 1H, H-5), 3.46−3.42 (m, 1H, $OCHH(CH_2)_5N_3$), 3.05 (t, *J* = 7.0 Hz, 2H, $-OCH_2$ $(CH_2)_4CH_2N_3$), 1.50−1.37 (m, 2H, $-OCH_2CH_2(CH_2)_4N_3$), 1.32−1.20 (m, 2H, $-O(CH_2)_4CH_2CH_2N_3$), 1.20−1.02 (m, 4H, $-O-(CH_2)_2(CH_2)_2(CH_2)_2N_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.8 ($-COCH_2Cl$), 136.8 (Ar-*C*), 134.6 (Ar-*C*), 134.5 (Ar-*C*), 131.4 (Ar-*C*),

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129.3 (Ar-*C*), 128.4 (Ar-*C*), 126.3 (Ar-*C*), 123.7 (Ar-*C*), 101.8 (−*C*HPh), 98.8 (C-1), 79.3 (C-4), 71.6 (C-3), 70.2 (−O*C*H$_2$(CH$_2$)$_5$N$_3$), 68.8 (C-6), 66.2 (C-5), 55.2 (C-2), 51.2 (−O(CH$_2$)$_5$*C*H$_2$N$_3$), 40.5 (−*C*OCH$_2$Cl), 29.2 (−CH$_2$), 28.7 (−CH$_2$), 26.3 (−CH$_2$), 25.4 (−CH$_2$); HRMS *m*/*z* calcd for $C_{29}H_{35}ClN_5O_8$ [M + NH$_4$]$^+$ 616.2169, found 616.2171.

6-Azidohexyl 4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (11). To a solution of compound **10** (381 mg, 0.636 mmol) in dichloromethane/methanol (15 mL, v/v 1:4) at rt was added thiourea (245 mg, 3.18 mmol) and 2,4-lutidine (50 μL). The reaction mixture was refluxed for 4 h. The solvent was removed under reduced pressure, and the resulting residue was dissolved in dichloromethane (80 mL) and washed with 1 N HCl, water and brine. The organic layer was dried over MgSO$_4$, filtered, and concentrated. Purification of the residue on flash column with EtOAc/hexane (2:3) as the eluent gave the acceptor **11** as a white foam (277 mg, 83%): $[\alpha]_D^{23}$ = −36.0 (*c* 1.0, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) *δ* 7.88−7.86 (m, 2H, Ar-*H*), 7.78−7.72 (m, 2H, Ar-*H*), 7.53−7.47 (m, 2H, Ar-*H*), 7.41−7.35 (m, 3H, Ar-*H*), 5.57 (s, 1H, −C*H*Ph), 5.27 (d, $J_{1,2}$ = 8.5 Hz, 1H, H-1), 4.68−4.59 (m, 1H, H-3), 4.40 (dd, $J_{6a,6b}$ = 10.6, $J_{5,6a}$ = 4.5 Hz, 1H, H-6a), 4.25 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 8.6 Hz, 1H, H-2), 3.89−3.79 (m, 2H, −OC*H*H(CH$_2$)$_5$N$_3$, H-6b), 3.68−3.58 (m, 2H, H-4, H-5), 3.43 (dt, *J* = 9.7, 6.5 Hz, 1H, −OCH*H*(CH$_2$)$_5$N$_3$), 3.05 (t, *J* = 7.0 Hz, 2H, −OCH$_2$(CH$_2$)$_4$C*H*$_2$N$_3$), 2.50 (d, *J* = 3.5 Hz, 1H, −O*H*), 1.50−1.36 (m, 2H, −OCH$_2$C*H*$_2$(CH$_2$)$_4$N$_3$), 1.32−1.21 (m, 2H, −O(CH$_2$)$_4$C*H*$_2$CH$_2$N$_3$), 1.17−1.05 (m, 4H, −O(CH$_2$)$_2$(*CH*$_2$)$_2$(CH$_2$)$_2$N$_3$); ^{13}C NMR (150 MHz, CDCl$_3$) *δ* 168.2 (−*C*OPhth), 137.0 (Ar-*C*), 134.3 (Ar-*C*), 131.7 (Ar-*C*), 129.5 (Ar-*C*), 128.5 (Ar-*C*), 126.4 (Ar-*C*), 123.6 (Ar-*C*), 102.1 (−*C*HPh), 99.0 (C-1), 82.4 (C-4), 69.9 (−O*C*H$_2$(CH$_2$)$_5$N$_3$), 68.8 (C-6), 68.7 (C-3), 66.3 (C-5), 56.7 (C-2), 51.2 (−O(CH$_2$)$_5$*C*H$_2$N$_3$), 29.2 (−CH$_2$), 28.8 (−CH$_2$), 26.3 (−CH$_2$), 25.4 (−CH$_2$); HRMS *m*/*z* calcd for $C_{27}H_{31}N_4O_7$ [M + H]$^+$ 523.2187, found 523.2202; *m*/*z* calcd for $C_{27}H_{34}N_5O_7$ [M + NH$_4$]$^+$ 540.2453, found 540.2465.

***p*-Methoxyphenyl 4,6-di-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1 → 4)-2,4-di-*O*-benzoyl-6-*O*-levulinoyl-*β*-D-glucopyranoside (12).** To a mixture of compound **5** (1.48 g, 2.49 mmol), **8** (1.85 g, 3.0 mmol) and molecular sieves 4 Å (3 g) in dichloromethane (20 mL) at −30 °C was added dropwise trimethylsilyl trifluoromethanesulfonate (50 μL) under N$_2$. The reaction mixture was stirred for 2 h, with the temperature slowly warming to 0 °C, and neutralized with Et$_3$N. The resulting mixture was filtered through Celite, and the filtrate was concentrated and purified by flash column with EtOAc/hexane (2:3) as eluent to yield the disaccharide **12** as a white foam (2.12 g, 85%): $[\alpha]_D^{23}$ = +18.0 (*c* 1.0, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) *δ* 8.09−8.04 (m, 2H, Ar-*H*), 7.95−7.90 (m, 2H, Ar-*H*), 7.88−7.84 (m, 2H, Ar-*H*), 7.76−7.74 (m, 2H, Ar-*H*), 7.58 (t, *J* = 7.4 Hz, 1H, Ar-*H*), 7.50 (t, 7.5 Hz, 1H, Ar-*H*), 7.47 (t, 7.7 Hz, 2H, Ar-*H*), 7.36 (t, *J* = 7.8 Hz, 2H), 7.33−7.28 (m, 5H, Ar-*H*), 6.83 (d, *J* = 9.1 Hz, 2H, Ar-*H*), 6.71 (d, *J* = 9.1 Hz, 2H, Ar-*H*), 5.79 (t, $J_{2',3'}$ = $J_{3',4'}$ = 9.8 Hz, 1H, H-3′), 5.67 (t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1H, H-3), 5.53 (dd, $J_{2,3}$ = 9.1, $J_{1,2}$ = 7.7 Hz, 1H, H-2), 5.49 (d, $J_{1',2'}$ = 8.2 Hz, 1H, H-1′), 5.20 (s, 1H, −C*H*Ph), 5.04 (d, $J_{1,2}$ = 7.6 Hz, 1H, H-1), 4.31−4.25 (m, 2H, H-2′, H-6a), 4.16 (t, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, 1H, H-4), 3.83 (s, 2H, −COC*H*$_2$Cl), 3.73−3.69 (m, 4H, H-5, −OC*H*$_3$), 3.62 (m, 2H, H-6′a, H-6b), 3.52 (t, $J_{3',4'}$ = $J_{4',5'}$ = 9.4 Hz, 1H, H-4′), 3.44 (m, 1H, H-5′), 2.75 (t, $J_{6'a,6'b}$ = 10.3 Hz, 1H, H-6′b), 2.69 (t, *J* = 6.8 Hz, 2H, −COC*H*$_2$CH$_2$), 2.53−2.38 (m, 2H, −COC*H*$_2$CH$_2$), 2.21 (s, 3H, −OC*H*$_3$); ^{13}C NMR (150 MHz, CDCl$_3$) *δ* 206.2 (−CH$_2$COCH$_3$), 171.9 (−OCOCH$_2$CH$_2$), 166.7 (−COCH$_2$Cl), 165.2 (−*C*OPh), 165.1 (−*C*OPh), 155.8 (Ar-*C*), 150.9 (Ar-*C*), 136.6 (Ar-*C*), 134.6 (Ar-*C*), 133.6 (Ar-*C*), 133.3 (Ar-*C*), 131.2 (Ar-*C*), 129.9 (Ar-*C*), 129.3 (Ar-*C*), 129.2 (Ar-*C*), 128.7 (Ar-*C*), 128.5 (Ar-*C*), 128.3 (Ar-*C*), 126.3 (Ar-*C*), 124.0 (Ar-*C*), 118.9 (Ar-*C*), 114.5 (Ar-*C*), 101.5 (−*C*HPh), 100.3 (C-1), 98.9 (C-1′), 78.4 (C-4′), 76.5 (C-4), 73.5 (C-3), 72.6 (C-5), 71.8 (C-2), 71.5 (C-3′), 67.8 (C-6′), 65.7 (C-5′), 61.9 (C-6), 55.6 (−OCH$_3$), 55.2 (C-2′), 40.3 (−COCH$_2$Cl), 38.0 (−COCH$_2$CH$_2$), 29.9 (−COCH$_3$), 27.7 (−COCH$_2$CH$_2$); HRMS *m*/*z* calcd for $C_{55}H_{54}ClN_2O_{18}$ [M + NH$_4$]$^+$ 1065.3055, found 1065.3090.

***p*-Methoxyphenyl 4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1 → 4)-2,3-di-*O*-benzoyl-6-*O*-levulinoyl-*β*-D-glucopyranoside (13).** To a solution of the disaccharide **12** (966 g, 0.92 mmol) in dichloromethane/methanol (20 mL, v/v 1:4) at rt was added thiourea (350 mg, 4.6 mmol) and 2,4-lutidine (100 μL). The reaction mixture was refluxed for 6 h, the solvent was removed under reduced pressure, and the resulting residue was dissolved in dichloromethane (100 mL) and washed with 1 N HCl, water, and brine. The organic layer was dried over MgSO$_4$, filtered, and concentrated. Purification of the residue on flash column with 1:1 EtOAc/hexane as the eluent gave the disaccharide acceptor **13** as a white foam (654 g, 73%): $[\alpha]_D^{23}$ = +20.0 (*c* 1.0, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) *δ* 8.06−8.05 (m, 2H, Ar-*H*), 7.93−7.91 (m, 2H, Ar-*H*), 7.86−7.84 (m, 2H, Ar-*H*), 7.76−7.73 (m, 2H, Ar-*H*), 7.59−7.54 (m, 1H, Ar-*H*), 7.53−7.48 (m, 1H, Ar-*H*), 7.45 (t, *J* = 7.8 Hz, 2H, Ar-*H*), 7.38−7.34 (m, 4H, Ar-*H*), 7.33−7.31 (m, 3H, Ar-*H*), 6.84−6.81 (m, 2H, Ar-*H*), 6.72−6.69 (m, 2H, Ar-*H*), 5.68 (t, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, 1H, H-3), 5.52 (dd, $J_{2,3}$ = 9.2, $J_{1,2}$ = 7.6 Hz, 1H, H-2), 5.35 (d, $J_{1',2'}$ = 8.3 Hz, 1H, H-1′), 5.24 (s, 1H, −C*H*Ph), 5.04 (d, $J_{1,2}$ = 7.6 Hz, 1H, H-1), 4.48 (dd, $J_{2',3'}$ = 10.4, $J_{3',4'}$ = 8.6 Hz, 1H, H-3′), 4.30 (dd, $J_{6a,6b}$ = 11.9, $J_{5,6a}$ = 1.6 Hz, 1H, H-6a), 4.18 (dd, $J_{2',3'}$ = 10.5, $J_{1',2'}$ = 8.3 Hz, 1H, H-2′), 4.16−4.13 (m, 1H, H-4), 3.74−3.70 (m, 1H, H-5), 3.71 (s, 3H, −OC*H*$_3$), 3.68 (dd, $J_{6a,6b}$ = 11.9, $J_{5,6b}$ = 4.2 Hz, 1H, H-6b), 3.58−3.55 (m, 1H, H-6′a), 3.33−3.29 (m, 2H, H-4′, H-5′), 2.82−2.77 (m, 1H, H-6′b), 2.67 (t, *J* = 6.9 Hz, 2H, −COC*H*$_2$CH$_2$), 2.48−2.39 (m, 2H, −COCH$_2$*CH*$_2$), 2.33 (s, 1H, −O*H*), 2.20 (s, 3H, −COC*H*$_3$); ^{13}C NMR (150 MHz, CDCl$_3$) *δ* 206.2 (−CH$_2$COCH$_3$), 171.9 (−OCOCH$_2$CH$_2$), 168.1 (−COPhth), 165.2 (−COPh), 165.1 (−COPh), 155.7 (Ar-*C*), 150.9 (Ar-*C*), 136.8 (Ar-*C*), 134.4 (Ar-*C*), 133.5 (Ar-*C*), 133.3 (Ar-*C*), 131.5 (Ar-*C*), 129.9 (Ar-*C*), 129.4 (Ar-*C*), 129.2 (Ar-*C*), 128.6 (Ar-*C*), 128.5 (Ar-*C*), 128.4 (Ar-*C*), 126.3 (Ar-*C*), 123.9 (Ar-*C*), 118.9 (Ar-*C*), 114.5 (Ar-*C*), 101.7 (−CHPh), 100.3 (C-1′), 99.2 (C-1), 81.5 (C-4′), 76.4 (C-4), 73.5 (C-3), 72.7 (C-5), 71.8 (C-2), 68.5 (C-3′), 67.8 (C-6′), 65.9 (C-5′), 61.9 (C-6), 56.7 (C-2′), 55.6 (−OCH$_3$), 38.0 (−COCH$_2$CH$_2$), 29.9 (−COCH$_3$), 27.7 (−COCH$_2$CH$_2$); HRMS *m*/*z* calcd for $C_{53}H_{53}N_2O_{17}$ [M + NH$_4$]$^+$ 989.3339, found 989.3373.

4,6-Di-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1 → 4)-2,4-di-*O*-benzoyl-6-*O*-levulinoyl-*α*-D-glucopyranosyl trichloroacetimidate (14). To a solution of compound **12** (917 mg, 0.875 mmol) in CH$_3$CN/H$_2$O (20 mL, v/v 4:1) at 0 °C was added cerium ammonium nitrate (1.52 g, 2.62 mmol). The reaction mixture was stirred for 40 min at 0 °C, diluted with EtOAc (100 mL), washed with aqueous saturated NaHCO$_3$ and water. The organic layer was dried over MgSO$_4$ and filtered. The filtrate was concentrated, and the residue was purified by flash column with EtOAc/hexane (2:1) as eluent to afford the disaccharide hemiacetal as a yellow foam. The compound was dissolved in dichloromethane (5 mL), trichloroacetinitrile (0.4 mL) and DBU (50 μL) were added, and the reaction mixture was stirred at 0 °C for 3 h. The solvent was removed under reduced pressure, and the residue was purified by flash column with EtOAc/hexane (1:1) to yield the disaccharide trichloroacetimidate **14** as a white foam (637 mg, 67%): $[\alpha]_D^{23}$ = +65.0 (*c* 1.0, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) *δ* 8.52 (s, 1H, −N*H*), 8.06 (d, *J* = 7.3 Hz, 2H, Ar-*H*), 7.90 (d, *J* = 7.3 Hz, 2H, Ar-*H*), 7.87−7.82 (m, 2H, Ar-*H*), 7.77−7.75 (m, 2H, Ar-*H*), 7.57 (t, *J* = 7.4 Hz, 1H, Ar-*H*), 7.49−7.45 (m, 3H, Ar-*H*), 7.34−7.30 (m, 7H, Ar-*H*), 6.61 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 6.01 (t, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, 1H, H-3), 5.77 (t, $J_{2',3'}$ = $J_{3',4'}$ = 9.8 Hz, 1H, H-3′), 5.58 (d, $J_{1',2'}$ = 8.2 Hz, 1H, H-1′), 5.40 (dd, $J_{2,3}$ = 10.2, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 5.23 (s, 1H, −C*H*Ph), 4.32−4.29 (m, 2H, H-2′, H-6a), 4.19 (t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 1H, H-4), 4.13−4.09 (m, 1H, H-5), 3.83 (s, 2H, −COC*H*$_2$Cl), 3.61 (dd, $J_{6a,6b}$ = 12.2, $J_{5,6b}$ = 3.2 Hz, 1H, H-6b), 3.58 (dd, $J_{6'a,6'b}$ = 10.1, $J_{5',6'a}$ = 4.3 Hz, 1H, H-6′a), 3.55 (t, $J_{3',4'}$ = $J_{4',5'}$ = 9.4 Hz, 1H, H-4′), 3.40 (td, $J_{4',5'}$ = 9.8, $J_{5',6'}$ = 5.2 Hz, 1H, H-5′), 2.91 (t, $J_{6'a,6'b}$ = 10.4 Hz, 1H, H-6′b), 2.75−2.68 (m, 2H, −COC*H*$_2$CH$_2$), 2.50 (dt, *J* = 17.0, 6.8 Hz, 1H, −COCH$_2$C*H*H), 2.39 (dt, *J* = 17.0, 6.8 Hz, 1H, −COCH$_2$CH*H*), 2.23 (s, 3H, −COC*H*$_3$); ^{13}C NMR (150 MHz, CDCl$_3$) *δ* 206.2 (−CH$_2$COCH$_3$), 171.9 (−OCOCH$_2$CH$_2$), 166.8 (−COCH$_2$Cl), 165.5 (−COPh), 165.0 (−COPh), 160.7 (−CNH), 136.6 (Ar-*C*), 134.6 (Ar-*C*), 133.6 (2C, Ar-*C*) 131.2 (br, Ar-*C*), 130.0 (Ar-*C*), 129.8 (2C, Ar-*C*), 129.3 (Ar-*C*), 128.8 (Ar-*C*), 128.5 (Ar-*C*),

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128.3 (Ar-*C*), 126.3 (Ar-*C*), 124.0 (Ar-*C*), 101.5 (−*C*HPh), 99.2 (C-1′), 93.1 (C-1), 90.7 (−*C*Cl$_3$), 78.3 (C-4′), 76.3 (C-4), 71.6 (C-3′), 70.8 (C-2), 70.7 (C-3, C-5), 67.9 (C-6′), 65.8 (C-5′), 61.5 (C-6), 55.4 (C-2′), 40.3 (−COCH$_2$Cl), 38.1 (−COCH$_2$), 29.9 (−COCH$_3$), 27.7 (−OCOCH$_2$); HRMS *m*/*z* calcd for $C_{50}H_{48}Cl_4N_3O_{17}$ [M + NH$_4$]$^+$ 1102.1732, found 1102.1776.

***p*-Methoxyphenyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-2,3-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-glucopyranoside (15).** To a mixture of compound **13** (642 mg, 0.661 mmol), **6** (534 mg, 0.793 mmol) and molecular sieves 4 Å (1.2 g) in dichloromethane (10 mL) at −40 °C was added dropwise trimethylsilyl trifluoromethanesulfonate (15 μL, 80 μmol) under N$_2$. The reaction mixture was stirred for 3 h, with the temperature slowly warming to 0 °C, and neutralized with Et$_3$N. The resulting mixture was filtered through Celite, and the filtrate was concentrated. Column chromatography of the residue with EtOAc/hexane (2:1) as the eluent yielded the trisaccharide **15** as a white foam (804 mg, 82%): $[\alpha]_D^{23}$ = +78.0 (*c* 1.0, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.04−8.03 (m, 2H, Ar-*H*), 7.91−7.90 (m, 2H, Ar-*H*), 7.71−7.69 (m, 2H, Ar-*H*), 7.59−7.56 (m, 1H, Ar-*H*), 7.50−7.44 (m, 5H, Ar-*H*), 7.42−7.33 (m, 11H, Ar-*H*), 7.30−7.29 (m, 2H, Ar-*H*), 7.25−7.23 (m, 2H, Ar-*H*), 7.16−7.14 (m, 2H, Ar-*H*), 6.80−6.77 (m, 2H, Ar-*H*), 6.69−6.66 (m, 2H, Ar-*H*), 5.61 (t, $J_{2,3} = J_{3,4}$ = 9.0 Hz, 1H, H-3), 5.48 (dd, $J_{2,3}$ = 9.2, $J_{1,2}$ = 7.6 Hz, 1H, H-2), 5.31 (s, 1H, −C*H*Ph), 5.28 (t, $J_{2'',3''} = J_{3'',4''}$ = 9.6 Hz, 1H, H-3″), 5.19 (d, $J_{1',2'}$ = 8.4 Hz, 1H, H-1′), 5.14 (t, $J_{3'',4''} = J_{4'',5''}$ = 9.7 Hz, 1H, H-4″), 5.12 (dd, $J_{1'',2''}$ = 8.0, $J_{2'',3''}$ = 9.7 Hz, 1H, H-2″), 4.98 (d, $J_{1,2}$ = = 7.6 Hz, 1H, H-1), 4.74 (d, $J_{1'',2''}$ = 8.0 Hz, 1H, H-1″), 4.62 (dd, $J_{2',3'}$ = 10.3, $J_{3',4'}$ = 8.8 Hz, 1H, H-3′), 4.25 (dd, $J_{2',3'}$ = 10.3, $J_{1',2'}$ = 8.4 Hz, 1H, H-2′), 4.13 (dd, $J_{6a,6b}$ = 11.9, $J_{5,6a}$ = 1.6 Hz, 1H, H-6a), 4.05 (t, $J_{3,4} = J_{4,5}$ = 9.0 Hz 1H, H-4), 3.92 (dd, $J_{6''a,6''b}$ = 12.1, $J_{5'',6''a}$ = 3.2 Hz, 1H, H-6″a), 3.86 (dd, $J_{6''a,6''b}$ = 12.1, $J_{5'',6''a}$ = 2.2 Hz, 1H, H-6″b), 3.69 (s, 3H, −OC*H*$_3$), 3.63 (t, $J_{3',4'} = J_{4',5'}$ = 9.1 Hz, 1H, H-4′), 3.61−3.59 (m, 1H, H-5), 3.57 (dd, $J_{6'a,6'b}$ = 10.6, $J_{5',6'a}$ = 4.8 Hz, 1H, H-6′a) 3.48 (dd, $J_{6a,6b}$ = 12.0, $J_{5,6b}$ = 4.1 Hz, 1H, H-6b), 3.34 (td, $J_{4',5'}$ = 9.7, $J_{5',6'}$ = 4.8 Hz 1H, H-5′), 3.23 (dt, $J_{4'',5''}$ = 10.0, $J_{5'',6''}$ = 2.8 Hz, 1H, H-5″), 2.82 (t, $J_{6'a,6'b}$ = 10.4 Hz, 1H, H-6′b), 2.74 (td, *J* = 6.6, 3.2 Hz, 2H, −COC*H*$_2$CH$_2$), 2.64−2.54 (m, 4H, −COC*H*$_2$CH$_2$, −COCH$_2$C*H*$_2$), 2.42−2.30 (m, 2H, −COCH$_2$C*H*$_2$), 2.21 (s, 3H, −COC*H*$_3$), 2.18 (s, 3H, −COC*H*$_3$), 1.79 (s, 3H, −COC*H*$_3$); ^{13}C NMR (150 MHz, CDCl$_3$) δ 206.6 (−CH$_2$COCH$_3$), 206.2 (−CH$_2$COCH$_3$), 172.5 (−OCOCH$_2$CH$_2$), 171.7 (−OCOCH$_2$CH$_2$), 169.2 (−COCH$_3$), 165.7 (−COPh), 165.2(−COPh), 165.0 (−COPh), 164.4 (−COPh), 155.7 (Ar-*C*), 150.9 (Ar-*C*), 136.9 (Ar-*C*), 133.9 (Ar-*C*), 133.5 (Ar-*C*), 133.3 (Ar-*C*), 133.3 (Ar-*C*), 132.8 (Ar-*C*), 130.8 (Ar-*C*), 129.9−129.5 (m, Ar-*C*), 129.2 (Ar-*C*), 128.7−128.3 (m, Ar-*C*), 128.1 (Ar-*C*), 126.2 (Ar-*C*), 123.5 (Ar-*C*), 118.9 (Ar-*C*), 114.5(Ar-*C*), 101.6 (−*C*HPh), 100.2 (C-1), 100.0 (C-1″), 98.8 (C-1′), 80.5 (C-4′), 76.3 (C-4), 75.9 (C-3′), 73.4 (C-3), 73.3 (C-3″), 72.5 (C-5), 72.1 (C-2″), 71.7 (C-2), 71.4 (C-5″), 67.9 (C-6′), 67.7 (C-4″), 65.9 (C-5′), 61.8 (C-6), 61.4 (C-6″), 55.6 (−OCH$_3$), 55.2 (C-2′), 37.9 (−COCH$_2$CH$_2$ X 2), 29.9 (−COCH$_3$ X 2), 27.9 (−COCH$_2$CH$_2$), 27.6 (−COCH$_2$CH$_2$), 20.5 (−COCH$_3$); HRMS *m*/*z* calcd for $C_{80}H_{79}N_2O_{27}$ [M + NH$_4$]$^+$ 1499.4865, found 1499.4895; *m*/*z* calcd for $C_{80}H_{83}N_3O_{27}$ [M + 2NH$_4$]$^{2+}$ 758.7601, found 758.7623.

4-*O*-Acetyl-2,3-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-2,3-di-*O*-benzoyl-6-*O*-levulinoyl-α-D-glucopyranosyl trichloroacetimidate (16). To a solution of compound **15** (720 mg, 0.486 mmol) in CH$_3$CN/H$_2$O (10 mL, v/v 4:1) at 0 °C was added cerium ammonium nitrate (845 mg, 1.46 mmol). The reaction mixture was stirred for 1 h at 0 °C, at which time TLC (EtOAc/hexane 3:1) indicated completion of the reaction. The reaction mixture was diluted with EtOAc (100 mL), and washed with aqueous saturated NaHCO$_3$ and water. The organic layer was dried over MgSO$_4$, filtered, and concentrated. The residue was purified by flash column with EtOAc/hexane (5:1) as the eluent to give the hemiacetal as a yellow foam. This compound was then dissolved in dichloromethane (5 mL), trichloroacetonitrile (0.3 mL) and DBU (50 μL) were added at 0 °C, and the reaction mixture was stirred for 2 h, and concentrated. Column chromatography of the residue (EtOAc/hexane 1:1) gave the trisaccharide trichloroacetimidate **16** as a white foam (502 mg, 70%): $[\alpha]_D^{23}$ = +110.0 (*c* 1.0, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.48 (s, 1H, −N*H*), 8.06−8.02 (m, 2H, Ar-*H*), 7.89−7.87 (m, 2H, Ar-*H*), 7.70 (dd, *J* = 8.3, 1.2 Hz, 2H, Ar-*H*), 7.57−7.54 (m, 1H, Ar-*H*), 7.48−7.37 (m, 15H, Ar-*H*), 7.34−7.23 (m, 5H, Ar-*H*), 7.16 (t, *J* = 7.8 Hz, 2H, Ar-*H*), 6.55 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 5.94 (t, $J_{2,3} = J_{3,4}$ = 9.6 Hz, 1H, H-3), 5.34 (s, 1H, −C*H*Ph), 5.33 (dd, $J_{1,2}$ = 3.7, $J_{2,3}$ = 9.6 Hz, 1H, H-2), 5.29−5.26 (m, 2H, H-1′, H-3″), 5.14 (t, $J_{3'',4''} = J_{4'',5''}$ = 9.7, 1H, H-4″), 5.11 (dd, $J_{1'',2''}$ = 8.0, $J_{2'',3''}$ = 9.6, 1H, H-2″), 4.74 (d, $J_{1'',2''}$ = 8.0 Hz, 1H, H-1″), 4.59 (dd, *J* = $J_{2',3'}$ = 10.2, $J_{3',4'}$ = 8.8 Hz, 1H, H-3′), 4.27 (dd, $J_{2',3'}$ = 10.3, $J_{1',2'}$ = 8.4 Hz, 1H, H-2′), 4.17 (dd, $J_{6a,6b}$ = 12.1, $J_{5,6a}$ = 1.5 Hz, 1H, H-6a), 4.08 (t, $J_{3,4} = J_{4,5}$ = 9.7, 1H, H-4), 4.01−3.98 (m, 1H, H-5), 3.92 (dd, $J_{6''a,6''b}$ = 12.1, $J_{5'',6''a}$ = 3.1 Hz, 1H, H-6″a), 3.87 (dd, $J_{6''a,6''b}$ = 12.1, $J_{5'',6''b}$ = 2.2 Hz, 1H, H-6″b), 3.68 (t, $J_{3',4'} = J_{4',5'}$ = 9.1 Hz, 1H, H-4′), 3.59 (dd, $J_{6'a,6'b}$ = 10.6, $J_{5',6'a}$ = 4.9 Hz, 1H, H-6′a), 3.48 (dd, $J_{6a,6b}$ = 12.2, $J_{5,6b}$ = 3.3 Hz, 1H, H-6b), 3.32 (td, $J_{4',5'}$ = 9.7, $J_{5',6'}$ = 4.8 Hz, 1H, H-5′), 3.22 (dt, $J_{4'',5''}$ = 9.8, $J_{5'',6''}$ =2.9 Hz, 1H, H-5″), 3.00−2.95 (m, 1H, H-6′b), 2.74 (td, *J* = 6.5, 3.5 Hz, 2H, −COC*H*$_2$CH$_2$), 2.65 (q, *J* = 6.8 Hz, 2H, −COC*H*$_2$CH$_2$), 2.59−2.55 (m, 2H, −COCH$_2$C*H*$_2$), 2.42−2.39 (m, 1H, −COCH$_2$C*H*H), 2.33−2.31 (m, 1H, −COCH$_2$CH*H*), 2.21 (s, 3H, −COC*H*$_3$), 2.20 (s, 3H, −COC*H*$_3$), 1.79 (s, 3H, −COC*H*$_3$); ^{13}C NMR (150 MHz, CDCl$_3$) δ 206.6 (−CH$_2$COCH$_3$), 206.2 (−CH$_2$COCH$_3$), 172.5 (−OCOCH$_2$CH$_2$), 171.7 (−OCOCH$_2$CH$_2$), 169.2 (−COCH$_3$), 165.7 (−COPh), 165.5 (−COPh), 164.9 (−COPh), 164.4 (−COPh), 160.7 (−CNH), 136.9 (Ar-*C*), 134.0 (Ar-*C*), 133.5 (Ar-*C*), 133.3 (Ar-*C*), 132.9 (Ar-*C*), 130.8 (Ar-*C*), 129.9−129.5 (m, Ar-*C*), 128.7−128.3 (m, Ar-*C*), 128.1 (Ar-*C*), 126.2 (Ar-*C*), 123.5 (Ar-*C*), 101.6 (−*C*HPh), 99.9 (C-1′), 98.9 (C-1″), 92.9 (C-1), 90.4 (−*C*Cl3), 80.4 (C-4′), 76.0 (C-4), 75.9 (C-3′), 73.4 (C-3″), 72.1 (C-2″), 71.4 (C-5″), 70.8 (C-5), 70.7 (C-2), 70.5 (C-3), 68.0 (C-6′), 67.7 (C-4″), 65.9 (C-5′), 61.4 (C-6, C-6″), 55.3 (C-2′), 38.0 (−COC*H*$_2$CH$_2$), 37.9 (−COC*H*$_2$CH$_2$), 29.9 (−COCH$_3$), 29.9 (−COCH$_3$), 28.0 (−COCH$_2$CH$_2$), 27.6 (−COCH$_2$CH$_2$), 20.5 (−COCH$_3$); HRMS *m*/*z* calcd for $C_{75}H_{73}Cl_3N_3O_{26}$ [M + NH$_4$]$^+$1536.3542, found1536.3604.

6-Azidohexyl 4,6-di-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 4)-2,4-di-*O*-benzoyl-6-*O*-levulinoyl-β-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (17). To a mixture of compound **14** (543 mg, 0.50 mmol) and **11** (236 mg, 0.452 mmol), containing molecular sieves 4 Å (1 g), in dichloromethane (8 mL) at −40 °C was added dropwise trimethylsilyl trifluoromethanesulfonate (9 μL, 50 μmol) under N$_2$. The reaction mixture was stirred for 2 h, with the temperature slowly warming to 0 °C, and neutralized with Et$_3$N. The resulting mixture was filtered through Celite, and the filtrate was concentrated. Column chromatography of the residue with EtOAc/hexane (3:2) as eluent yielded the trisaccharide **17** as a white foam (535 mg, 82%): $[\alpha]_D^{23}$ = +42.0 (*c* 1.0, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ 7.88−7.81 (m, 6H, Ar-*H*), 7.51−7.45 (m, 2H, Ar-*H*), 7.40−7.29 (m, 7H, Ar-*H*), 7.29−7.26 (m, 8H, Ar-*H*), 7.14−7.11 (m, 5H, Ar-*H*), 5.68 (dd, $J_{2'',3''}$ = 10.2, $J_{3'',4''}$ = 9.4 Hz, 1H, H-3″), 5.51 (s, 1H, −C*H*Ph), 5.33 (t, $J_{2',3'} = J_{3',4'}$ = 9.3 Hz, 1H, H-3′), 5.32 (d, $J_{1'',2''}$ = 8.2 Hz, 1H, H-1″), 5.12 (s, 1H, −C*H*Ph), 5.08 (d, $J_{1,2}$ = 8.5 Hz, 1H, H-1), 5.05 (dd, $J_{2',3'}$ = 9.7, $J_{1',2'}$ = 8.2 Hz, 1H, H-2′), 4.68 (d, $J_{1',2'}$ = 8.1 Hz, 1H, H-1′), 4.63 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 8.7 Hz, 1H, H-3), 4.30 (dd, $J_{6a,6b}$ = 10.6, $J_{5,6b}$ = 4.8 Hz, 1H, H-6a), 4.21 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 8.6 Hz, 1H, H-2), 4.11 (dd, $J_{2'',3''}$ = 10.2, $J_{1'',2''}$ = 8.1 Hz, 1H, H-2″), 3.96 (dd, $J_{6'a,6'b}$ = 12.1, $J_{5',6'a}$ = 1.5 Hz, 1H, H-6′a), 3.88 (t, $J_{3',4'} = J_{4',5'}$ = 9.3 Hz, 1H, H-4′), 3.85 (t, $J_{6a,6b}$ = 10.3 Hz, 1H, H-6b), 3.79 (s, 2H, −C*H*$_2$Cl), 3.77 (t, $J_{3,4} = J_{4,5}$ = 9.1 Hz, 1H, H-4), 3.77−3.71 (m, 1H, −OC*H*H(CH$_2$)$_5$N$_3$), 3.59 (td, $J_{4,5}$ = 9.8, $J_{5,6}$ = 4.9 Hz, 1H, H-5), 3.48 (dd, $J_{6''a,6''b}$ = 10.8, $J_{5'',6''a}$ = 4.9 Hz, 1H, H-6″a), 3.41 (t, $J_{3'',4''} = J_{4'',5''}$ = 9.4 Hz, 1H, H-4″), 3.32 (td, $J_{4'',5''}$ = 9.7, $J_{5'',6''}$ = 4.8, 1H, H-5″), 3.31−3.27 (m, 1H, −OCH*H*(CH$_2$)$_5$N$_3$), 3.00 (dd, $J_{6'a,6'b}$ = 12.1, $J_{5',6'b}$ = 2.5 Hz, 1H, H-6′b), 2.96 (t, *J* = 7.0 Hz, 2H, −OCH$_2$(CH$_2$)$_4$CH$_2$N$_3$), 2.89 (dt, $J_{4',5'}$ = 9.7, $J_{5',6'}$ = 2.4, 1H, H-5′), 2.83−2.71 (m, 2H, −COC*H*$_2$CH$_2$), 2.59 (t, $J_{6''a,6''b}$ = 10.4 Hz, 1H, H-6″b), 2.52−2.39 (m, 2H, −COCH$_2$C*H*$_2$), 2.27 (s, 3H, −COC*H*$_3$), 1.41−1.30 (m, 1H, −OCH$_2$C*H*H(CH$_2$)$_4$N$_3$), 1.30−1.22 (m, 1H, −OCH$_2$CH*H*(CH$_2$)$_4$N$_3$), 1.22−1.08 (m, 2H, −O(CH$_2$)$_4$C*H*$_2$CH$_2$N$_3$), 1.07−0.86 (m, 4H, −O(CH$_2$)$_2$(C*H*$_2$)$_2$(CH$_2$)$_2$N$_3$); ^{13}C NMR (150 MHz, CDCl$_3$) δ 206.4 (−CH$_2$COCH$_3$), 172.0 (−OCOCH$_2$CH$_2$), 166.7

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(−$COCH_2Cl$), 164.9 (−COPh), 164.5 (−COPh), 136.9 (Ar-*C*), 136.6 (Ar-*C*), 134.6 (br, Ar-*C*), 133.8 (Ar-*C*), 133.3 (Ar-*C*), 132.8 (Ar-*C*), 131.1 (br, Ar-*C*), 129.8 (Ar-*C*), 129.6 (Ar-*C*), 129.5 (Ar-*C*), 129.3 (Ar-*C*), 129.2 (Ar-*C*), 128.6 (Ar-*C*), 128.5 (Ar-*C*), 128.3 (Ar-*C*), 128.2 (Ar-*C*), 128.1 (Ar-*C*), 126.2 (Ar-*C*), 126.1 (Ar-*C*), 101.9 (−*C*HPh), 101.4 (−*C*HPh), 100.2 (C-1′), 98.6 (C-1″, C-1), 81.4 (C-4), 78.3 (C-4″), 76.3 (C-3), 75.8 (C-4′), 73.7 (C-3′), 72.3 (C-2′), 71.9 (C-5′), 71.4 (C-3″), 69.7 (−$OCH_2(CH_2)_5N_3$), 68.9 (C-6), 67.6 (C-6″), 66.3 (C-5), 65.5 (C-5″), 60.9 (C-6′), 55.1 (C-2), 55.0 (C-2″), 51.1 (−$O(CH_2)_5CH_2N_3$), 40.2 (−$COCH_2Cl$), 38.2 (−$COCH_2$), 30.1 (−$COCH_3$), 29.0 (−$OCH_2CH_2(CH_2)_4N_3$), 28.6 (−$O(CH_2)_4CH_2CH_2N_3$), 27.8 (−$OCOCH_2$), 26.2 (−$O(CH_2)_2CH_2(CH_2)_3N_3$), 25.3 (−$O(CH_2)_3CH_2(CH_2)_2N_3$); HRMS *m*/*z* calcd for $C_{75}H_{76}ClN_6O_{23}$ [M + NH_4]$^+$ 1463.4645, found 1463.4689.

6-Azidohexyl 4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1 → 4)-2,4-di-*O*-benzoyl-6-*O*-levulinoyl-*β*-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (18). To a solution of compound **17** (491 mg, 0.34 mmol) in dichloromethane/methanol (20 mL, 1:4 v/v) at rt was added thiourea (130 mg, 1.7 mmol) and 2,4-lutidine (50 μL). The reaction mixture was refluxed for 6 h, the solvent was removed under reduced pressure, and the resulting residue was dissolved in dichloromethane (100 mL) and washed with 1 N HCl, water, and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. Purification of the residue on flash column with EtOAc/hexane (3:2) as eluent gave the trisaccharide acceptor **18** as a white foam (363 mg, 78%): $[\alpha]_D^{23}$ = +54.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87−7.83 (m, 4H, Ar-*H*), 7.81 (dd, *J* = 5.5, 3.0 Hz, 2H, Ar-*H*), 7.48−7.45 (m, 2H, Ar-*H*), 7.40−7.27 (m, 15H, Ar-*H*), 7.17−7.11 (m, 5H, Ar-*H*), 5.53 (s, 1H, −*CH*Ph), 5.34 (t, $J_{2',3'} = J_{3',4'}$ = 9.3 Hz, 1H, H-3′), 5.20 (d, $J_{1'',2''}$ = 8.3 Hz, 1H, H-1″), 5.16 (s, 1H, −*CH*Ph), 5.08 (d, $J_{1,2}$ = 8.5 Hz, 1H, H-1), 5.05 (dd, $J_{2',3'}$ = 9.6, $J_{1',2'}$ = 8.2 Hz, 1H, H-2′), 4.68 (d, $J_{1',2'}$ = 8.1 Hz, 1H, H-1′), 4.63 (dd, $J_{2,3}$ = 10.4, $J_{3,4}$ = 8.7 Hz, 1H, H-3), 4.36 (dd, $J_{3'',4''}$ = 8.8, $J_{2'',3''}$ = 10.5 Hz, 1H, H-3″), 4.30 (dd, $J_{6a,6b}$ = 10.6, $J_{5,6b}$ = 4.8 Hz, 1H, H-6a), 4.21 (dd, $J_{2,3}$ = 10.4, $J_{1,2}$ = 8.6 Hz, 1H, H-2), 4.02 (dd, $J_{1'',2''}$ = 8.3, $J_{2'',3''}$ = 10.4 Hz, 1H, H-2″), 4.00−3.98 (m, 1H, H-6′a), 3.87 (t, $J_{3',4'} = J_{4',5'}$ = 9.4, Hz, 1H, H-4′), 3.86−3.83 (m, 1H, H-6b), 3.78−3.71 (m, 2H, H-4, −OC*H*H($CH_2)_5N_3$), 3.59 (td, $J_{4,5}$ = 9.7, $J_{5,6}$ = 4.9 Hz, 1H, H-5), 3.44 (dd, $J_{6''a,6''b}$ = 10.8, $J_{5'',6''a}$ = 4.7 Hz, 1H, H-6″a), 3.29 (ddd, *J* = 9.9, 7.4, 5.6 Hz, 1H, −OCH*H*($CH_2)_5N_3$), 3.22 (t, $J_{3'',4''} = J_{4'',5''}$ = 9.0 Hz, 1H, H-4″), 3.18 (td, $J_{4'',5''}$ = 9.4, $J_{5'',6''}$ = 4.7 Hz, 1H, H-5″), 3.08 (dd, $J_{6'a,6'b}$ = 12.1, $J_{5',6'a}$ = 2.6 Hz, 1H, H-6′b), 2.96 (t, *J* = 7.0 Hz, 2H, −$OCH_2(CH_2)_4CH_2N_3$), 2.93 (dt, $J_{4',5'}$ = 9.9, $J_{5',6'}$ = 2.2 Hz, 1H, H-5′), 2.80−2.69 (m, 2H, −$COCH_2CH_2$), 2.65−2.62 (m, 1H, H-6″b), 2.48−2.37 (m, 2H, −$COCH_2CH_2$), 2.27 (s, 3H, −$COCH_3$), 1.34−1.31 (m, 1H, −OCH_2CH*H*$(CH_2)_4N_3$), 1.29−1.23 (m, 1H, −OCH_2CH*H*$(CH_2)_4N_3$), 1.20−1.09 (m, 2H, −$O(CH_2)_4CH_2CH_2N_3$), 1.07−0.88 (m, 4H, −$O(CH_2)_2(CH_2)_2(CH_2)_2N_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.4 (−CH_2COCH_3), 172.0 (−$OCOCH_2CH_2$), 164.9 (−COPh), 164.5 (−COPh), 136.9 (Ar-*C*), 136.8 (Ar-*C*), 134.4 (Ar-*C*), 133.8 (Ar-*C*), 133.2 (Ar-*C*), 132.7 (Ar-*C*), 131.5 (Ar-*C*), 129.9−129.4 (m, Ar-*C*), 128.6 (Ar-*C*), 128.4−128.3 (m, Ar-*C*), 128.1 (Ar-*C*), 126.3 (Ar-*C*), 126.1 (Ar-*C*), 123.8 (Ar-*C*), 101.9 (−*C*HPh), 101.7 (−*C*HPh), 100.1 (C-1′), 98.8 (C-1″), 98.6 (C-1), 81.4 (C-4″), 81.4 (C-4), 76.2 (C-3), 75.7 (C-4′), 73.7 (C-3′), 72.4 (C-2′), 72.1 (C-5′), 69.7 (−$OCH_2(CH_2)_5N_3$), 68.9 (C-6), 68.4 (C-3″), 67.7 (C-6″), 66.3 (C-5), 65.7 (C-5″), 60.9 (C-6′), 56.6 (C-2″), 55.1 (C-2), 51.1 (−$O(CH_2)_5CH_2N_3$), 38.2 (−$COCH_2$), 30.1 (−$COCH_3$), 29.1 (−$OCH_2CH_2(CH_2)_4N_3$), 28.6 (−$O(CH_2)_4CH_2CH_2N_3$), 27.8 (−$OCOCH_2$), 26.2 (−$O(CH_2)_2CH_2(CH_2)_3N_3$), 25.3 (−$O(CH_2)_3CH_2(CH_2)_2N_3$); HRMS *m*/*z* calcd for $C_{73}H_{75}N_6O_{22}$ [M + NH_4]$^+$ 1387.4929, found 1387.4973.

6-Azidohexyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-levulinoyl-*β*-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1 → 4)-2,3-di-*O*-benzoyl-6-*O*-levulinoyl-*β*-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1 → 4)-2,4-di-*O*-benzoyl-6-*O*-levulioyl-*β*-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (19). A mixture of the trisaccharide trichloroacetimidate **16** (415 mg, 0.273 mmol), the trisaccharide acceptor **18** (312 mg, 0.228 mmol) in dichloromethane (8 mL) at rt was stirred with molecular sieves 4 Å (1 g) for 1 h under N_2. Trimethylsilyl trifluoromethanesulfonate (5 μL, 27 μmol) was then added at −40 °C. The reaction mixture was stirred for 2 h, with temperature slowly warming to 0 °C, at which time TLC (EtOAc/hexane 2:1) indicated the complete consumption of the trisaccharide trichloroacetimidate **16**. The reaction mixture was neutralized with Et_3N, filtered through Celite, the solvent was evaporated under reduced pressure, and the resulting residue was purified by flash column with EtOAc/hexane (2:1) as the eluent to yield the hexasaccharide **19** as a white foam (435 mg, 70%): $[\alpha]_D^{23}$ = +91.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79−7.76 (m, 4H, Ar-*H*), 7.68−7.66 (m, 3H, Ar-*H*), 7.48−7.28 (m, 24H, Ar-*H*), 7.26−7.22 (m, 7H, Ar-*H*), 7.19−7.03 (m, 16H, Ar-*H*), 7.00−6.99 (m, 3H, Ar-*H*), 5.45 (s, 1H, −*CH*Ph), 5.23 (t, $J_{2,3} = J_{3,4}$ = 9.3 Hz, 1H, H-3^B), 5.22 (t, $J_{2,3} = J_{3,4}$ = 9.6 Hz, 1H, H-3^F), 5.21 (s, 1H, −*CH*Ph), 5.15 (t, $J_{2,3} = J_{3,4}$ = 9.3 Hz, 1H, H-3^D), 5.10 (t, $J_{3,4} = J_{4,5}$ = 9.7 Hz, 1H, H-4^F), 5.05 (dd, 1H, $J_{1,2}$ = 8.0, $J_{2,3}$ = 9.5 Hz, H-2^F), 5.05 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1^A), 5.04 (s, 1H, −*CH*Ph), 5.00 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1^E), 4.99 (d, $J_{1,2}$ = 8.6 Hz, 1H. H-1^C), 4.97 (dd, $J_{1,2}$ = 8.3, $J_{2,3}$ = 9.5 Hz, 1H, H-2^B), 4.88 (dd, $J_{1,2}$ = 8.3, $J_{2,3}$ = 9.5 Hz, 1H, H-2^D), 4.68 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1^F), 4.60 (d, $J_{1,2}$ = 8.3 Hz, 1H, H-1^B), 4.57 (dd, $J_{2,3}$ = 10.2, $J_{3,4}$ = 8.8 Hz, 1H, H-3^A), 4.47 (t, $J_{2,3} = J_{3,4}$ = 10.0 Hz, 1H, H-3^E), 4.46 (d, $J_{1,2}$ = 8.3 Hz, 1H, H-1^D), 4.35 (dd, $J_{2,3}$ = 10.0, $J_{3,4}$ = 9.0 Hz, 1H, H-3^C), 4.27 (dd, $J_{6a,6b}$ = 10.6, $J_{5,6a}$ = 4.8 Hz, 1H, H-6a^A), 4.16 (dd, $J_{2,3}$ = 10.3, $J_{1,2}$ = 8.6 Hz, 1H, H-2^A), 4.04 (dd, $J_{2,3}$ = 10.2, $J_{1,2}$ = 8.5 Hz, 1H, H-2^E), 3.98 (dd, $J_{2,3}$ = 10.2, $J_{1,2}$ = 8.5 Hz, 1H, H-2^C), 3.88−3.78 (m, 5H, H-6a^F, 6b^A, 6a^B, 6a^D, 6b^F), 3.74−3.69 (m, 1H, 4H, H-4^{A,B,D}, −OC*H*H$(CH_2)_4CH_2N_3$), 3.54 (td, $J_{4,5}$ = 9.7, $J_{5,6}$ = 4.9 Hz, 1H, H-5^A), 3.50 (t, $J_{3,4} = J_{4,5}$ = 9.1 Hz, 1H, H-4^E), 3.43 (dd, $J_{6a,6b}$ = 10.7, $J_{5,6a}$ = 4.7 Hz, 1H, H-6a^E), 3.36−3.33 (m, 2H, H-4^C, H-6a^C), 3.29−3.25 (m, 1H, OCH*H*$(CH_2)_4CH_2N_3$), 3.21 (td, *J* = 9.7, 4.9 Hz, 1H, H-5^E), 3.17−3.11 (m, 2H, H-5^{C,F}), 2.95 (t, *J* = 7.0 Hz, 2H, −$OCH_2(CH_2)_4CH_2N_3$), 2.86−2.80 (m, 3H, H-5^B, H-6b^{B,D}), 2.74−2.64 (m, 7H, H-5^D, 3 × −$COCH_2CH_2$), 2.61 (t, $J_{6a,6b}$ = 10.6 Hz, 1H, H-6b^E), 2.57−2.51 (m, 3H, H-6b^C, −$COCH_2CH_2$), 2.42−2.28 (m, 4H, 2 × −$COCH_2CH_2$), 2.24 (s, 3H, −$COCH_3$), 2.23 (s, 3H, −$COCH_3$), 2.18 (s, 3H, −$COCH_3$), 1.77 (s, 3H, −$COCH_3$), 1.35−1.29 (m, 1H, −OCH_2CHH-$(CH_2)_4N_3$) 1.26−1.22 (m, 1H, −OCH_2CHH$(CH_2)_4N_3$), 1.19−1.08 (m, 2H, −$O(CH_2)_4CH_2CH_2N_3$), 1.05−0.87 (m, 4H, −$O(CH_2)_2(CH_2)_2(CH_2)_2N_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.6 (−CH_2COCH_3), 206.4 (−CH_2COCH_3), 206.3 (−CH_2COCH_3), 172.4 (−$OCOCH_2CH_2$), 171.9 (−$OCOCH_2CH_2$), 171.8 (−$OCOCH_2CH_2$), 169.2 (−$COCH_3$), 167.9 (br, −COPhth), 166.5 (br, −COPhth), 165.7 (−COPh), 164.8 (−COPh), 164.7 (−COPh), 164.5 (−COPh), 164.4 (−COPh), 164.3 (−COPh), 136.9 (2C, Ar-*C*), 136.6 (Ar-*C*), 133.9−133.8 (m, Ar-*C*), 133.3 (Ar-*C*), 133.2 (Ar-*C*), 132.8 (2C, Ar-*C*), 132.7 (Ar-*C*), 130.9 (br, Ar-*C*), 130.4 (br, Ar-*C*), 129.8−129.3 (m, Ar-*C*), 128.7−128.1 (m, Ar-*C*), 126.1 (Ar-*C*), 126.0 (Ar-*C*), 125.9 (Ar-*C*), 123.4 (br, Ar-*C*), 101.9 (−*C*HPh), 101.6 (−*C*HPh), 101.5 (−*C*HPh), 100.1 (C-1^B), 99.9 (C-1^F), 99.6 (C-1^D), 98.6 (C-1^A), 98.5 (2C, C-1^{C,E}), 81.3 (C-4^A), 80.4 (2 C, C-4^{C,E}), 76.2 (C-3^A), 75.9 (C-3^E), 75.7 (C-3^C), 75.6 (C-4^B), 75.5 (C-4^D), 73.5 (2 C, C-3^{B,D}), 73.3 (C-3^F), 72.3 (C-2^B), 72.2 (C-2^D), 72.1 (C-2^F), 71.9 (C-5^B), 71.7 (C-5^D), 71.3 (C-5^F), 69.7 (−$OCH_2(CH_2)_5N_3$), 68.8 (C-6^A), 67.8 (C-6^E), 67.7 (2 C, C-4^F and C-6^C), 66.3 (C-5^A), 65.7 (C-5^E), 65.6 (C-5^C), 61.3 (C-6^F), 60.8 (C-6^B), 60.8 (C-6^D), 55.1 (C-2^A), 54.9 (C-2^E), 54.9 (C-2^C), 51.1 (−$O(CH_2)_5CH_2N_3$), 38.1, 38.1, 37.9, 30.1 (−$COCH_3$), 30.0 (−$COCH_3$), 29.9 (−$COCH_3$), 29.1 (−$OCH_2CH_2(CH_2)_4N_3$), 28.5 (−$O(CH_2)_4CH_2CH_2N_3$), 27.9, 27.8, 27.7, 26.1 (−$O(CH_2)_2CH_2(CH_2)_3N_3$), 25.3 (−$O(CH_2)_3CH_2(CH_2)_2N_3$), 20.5 (−$COCH_3$); HRMS *m*/*z* calcd for $C_{146}H_{142}N_7O_{47}$ [M + NH_4]$^+$ 2744.8931, found 2744.8934; *m*/*z* calcd for $C_{146}H_{146}N_8O_{47}$ [M + 2NH_4]$^{2+}$ 1381.9651, found 1381.9659.

6-Azidohexyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-6-*O*-levulinoyl-*β*-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1 → 4)-2,3-di-*O*-benzoyl-6-*O*-levulinoyl-*β*-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1 → 4)-2,4-di-*O*-benzoyl-6-*O*-levulinoyl-*β*-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (20). A solution of the hexasaccharide **19** (400 mg, 0.147 mmol) in 80% HOAc (20 mL) was stirred at 80 °C for 5 h, at which time TLC indicated the

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Table 2. ^{1}H and ^{13}C NMR Data for Compounds 23a, 23b, 24a, and 24b[a]

resonance	**23a** δ (ppm)	**23b** δ (ppm)	**24a** δ (ppm)	**24b** δ (ppm)
CH_3			22.0, 22.3	
CH_3 (3C)	22.3, 22.5	8.9, 9.3		9.0, 9.4
$CH_2\varepsilon/CH_2\zeta$	24.8, 25.7	24.6, 25.4		
$CH_2\delta/CH_2\gamma$	28.1, 28.5	27.8, 28.3	24.4, 25.1	24.6, 25.1
$CH_2\beta/CH_2\varepsilon$			26.5, 28.1	26.5, 28.3
CH_2-carbonyl		29.1, 29.3		
CH_2-carbonyl (3C)				29.2, 29.3
$CH_2\beta$	51.2	51.0		
$CH_2\zeta$			39.2	39.3
C-2 CE			54.1, 54.1	54.0, 54.1
C-2 A			54.4	54.4
C-2 ACE	54.3, 54.4, 54.6	54.0, 54.1, 54.3		
C-6 ACE	60.6, 60.8	60.3, 60.5		
C-6 CE			60.2, 60.3	
C-6 A			60.5	
C-6 ACE (3C)				60.4, 60.6
C-4 A			68.1	
C-4 CE			68.3, 68.3	
C-4 ACE	68.3, 68.4, 68.6	68.1, 68.2, 68.4		68.3, 68.4, 68.5
$CH_2\alpha$	70.5	70.3	70.2	70.3
C-4 F	71.4	71.3	71.5	71.6
C-2BD			72.2, 72.3	72.3, 72.3
C-2F			72.5	72.6
C-2 BDF	72.3, 72.3, 72.6	72.1, 72.4		
C-3 BD	73.7	73.3	73.3, 73.4	73.4
C-5 BDF	74.5	74.9		75.6, 76.0, 76.2
C-5 ACE/C-3F	75.2, 75.5, 75.5	75.2	75.1, 75.1, 75.2, 75.2	75.1, 75.2, 75.3
C-5F			75.6	
C-5 BD			76.1, 76.2	
C-4 BD	80.1	79.3	79.7, 79.8	79.4, 79.5
C-3 CE			81.8, 82.2	
C-3ACE	82.4, 82.7,82.8			
C-5 ACE		81.8, 82.1, 82.2		81.6, 82.0, 82.6
C-3 A			82.8	
C-1 ACE	101.0, 101.1			
C-1 CE		100.5	100.4, 100.5	100.3, 100.3
C-1 A		100.8	100.8	100.9
C-1 BDF	102.8, 103.0, 103.1	102.5,102.7,102.9	102.8, 102.9, 103.0	102.7, 102.8, 102.9
C═O (6C)	174.6,174.9, 174.9	178.2, 178.4	174.0,174.0,174.3, 174.8, 175.3	173.8, 173.9,175.1, 178.2, 178.6
CH_3		0.99		0.99
H-ζ/H-ε/H-δ/H-γ (8H)	1.37, 1.57, 1.61	1.47, 1.42, 1.22	1.27, 1.47, 1.56	1.22, 1.43, 1.47
CH_2 (6H)		2.16		2.16
CH_3 (9H)	2.03		1.93	
H-ζ (2H)			2.89	3.19
H-β (2H)	3.33	3.19		
H-2 F	3.36	3.22	3.23	3.27
H-2 BD	3.38	3.25	3.26	3.30
H-5 A	3.47	3.33	3.36	3.40
H-5 CE	3.50	3.36	3.39	3.42
H-4 A	3.52	3.38	3.41	3.42
H-4 F/H-3 F			3.41	3.43
H-4 C/H-4 E			3.45	
H-3 F	3.53	3.38		
H-4 CE	3.56	3.45		3.47
H-4 F	3.57	3.44		
H-α	3.62	3.53	3.50	
H-3 BD	3.64	3.48	3.49	3.50
H-3 ACE	3.74	3.64		3.70
H-6 ACE	3.78	3.70		
H-4 BD	3.80	3.69		
H-5 BD			3.61	

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Table 2. continued

resonance	**23a** δ (ppm)	**23b** δ (ppm)	**24a** δ (ppm)	**24b** δ (ppm)
H-3 CE			3.62	
H-3 A			3.64	
H-5 BDF				
H-α				3.53
H-5 F			3.64	3.66
H-5 BD				3.70
H-6 ACE				3.70
H-4 BD			3.66	3.70
H-6 A			3.66	
H-6 CE			3.68	
H-2 A	3.82	3.69	3.72	3.75
H-2 CE	3.86	3.74	3.75	3.81
H-5 BDF	3.90	3.81		
H-α	3.91	3.84	3.81	3.84
H-6 ACE	3.92	3.85		3.85
H-6 A			3.82	
H-6 CE			3.82	
H-1 BD				4.40
H-1 F			4.37	4.42
H-1 A	4.53	4.47	4.42	4.46
H-1 BD			4.39	
H-1 BDF	4.54	4.45		
H-1 CE	4.59	4.53	4.47	4.52

[a] ^{1}H NMR spectra for **23a**, **23b**, and **24b** were recorded at 800 MHz. All ^{13}C NMR spectra and the ^{1}H NMR spectrum for the hexasaccharide **24a** were recorded at 150 and 600 MHz, respectively.

disappearance of the starting material. The solvent was coevaporated with toluene/methanol (3 × 20 mL, v/v 1:1). The resulting residue was then dissolved in pyridine (5 mL), and acetic anhydride (3 mL) was added. The reaction mixture was stirred at rt overnight and then concentrated with toluene. Purification of the resulting residue by flash column using EtOAc/hexane (5:1) as eluent gave compound **20** as a colorless foam (293 mg, 78%): $[\alpha]_D^{23}$ = +17.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75−7.60 (m, 13H, Ar-*H*), 7.50−7.35 (m, 13H, Ar-*H*), 7.26−7.14 (m, 16H, Ar-*H*), 5.33 (t, *J* = 9.6 Hz, 1H), 5.23 (t, *J* = 9.0 Hz, 1H), 5.18 (t, *J* = 9.0 Hz, 1H), 5.13 (t, *J* = 9.8 Hz, 1H), 5.11 (dd, *J* = 7.9, 9.7 Hz, 1H), 5.03−4.99 (m, 2H), 4.96 (dd, *J* = 9.2, 7.8 Hz, 1H), 4.87 (d, *J* = 8.3 Hz, 1H), 4.83 (t, *J* = 8.8 Hz, 2H), 4.77 (t, *J* = 9.4 Hz, 1H), 4.69 (t, *J* = 9.1 Hz, 1H), 4.64 (dd, *J* = 9.1, 10.6 Hz, 1H), 4.54 (dd, *J* = 9.1, 10.7 Hz, 1H), 4.44 (dd, *J* = 9.2, 10.5 Hz, 1H), 4.42−4.39 (m, 2H), 4.27 (d, *J* = 7.6 Hz, 1H), 4.19 (dd, *J* = 12.0, 4.5 Hz, 1H), 4.14−4.00 (m, 5H), 3.98 (dd, *J* = 10.8, 8.4 Hz, 1H), 3.90−3.80 (m, 4H), 3.70−3.58 (m, 4H), 3.56−3.46 (m, 7H), 3.25−3.15 (m, 3H,), 2.95 (t, *J* = 7.1 Hz, 2H, $-OCH_2(CH_2)_4CH_2N_3$), 2.80−2.65 (m, 6H), 2.61−2.42 (m, 6H), 2.21 (s, 3H, $-COCH_3$), 2.20 (s, 3H, $-COCH_3$), 2.17 (s, 3H, $-COCH_3$), 2.06 (s, 3H, $-COCH_3$), 1.98 (s, 3H, $-COCH_3$), 1.88 (s, 3H, $-COCH_3$), 1.85 (s, 3H, $-COCH_3$), 1.83 (s, 6H, $-COCH_3$ x 2), 1.78 (s, 3H, $-COCH_3$), 1.30−1.24 (m, 1H, $-OCH_2CHH(CH_2)_4N_3$), 1.21−1.16 (m, 1H, $-OCH_2CHH(CH_2)_4N_3$), 1.15−1.06 (m, 2H, m, 2H, $-O(CH_2)_4CH_2CH_2N_3$), 0.99−0.80 (m, 4H, $-O-(CH_2)_2(CH_2)_2(CH_2)_2N_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ 206.3 ($-CH_2COCH_3$), 206.1 ($-CH_2COCH_3$), 206.0 ($-CH_2COCH_3$), 172.4 ($-OCOCH_2CH_2$), 171.8 ($-OCOCH_2CH_2$), 171.8 ($-OCOCH_2CH_2$), 170.9 ($-COCH_3$), 170.6 (2C, $-COCH_3$), 169.3 ($-COCH_3$), 169.3 ($-COCH_3$), 169.2 ($-COCH_3$), 169.0 ($-COCH_3$), 165.8 (−COPh), 165.0 (2C, −COPh), 164.9 (−COPh), 164.9 (2C, −COPh), 134.4 (Ar-*C*), 134.2 (Ar-*C*), 133.5 (Ar-*C*), 133.1 (2C, Ar-*C*), 132.8 (Ar-*C*), 132.6 (2C, Ar-*C*), 130.9 (Ar-*C*), 130.7 (Ar-*C*), 130.6 (Ar-*C*), 129.9−129.3 (m, Ar-*C*), 128.5 (Ar-*C*), 128.4 (Ar-*C*), 128.2−128.1 (m, Ar-*C*), 124.0 (Ar-*C*), 123.6 (br, Ar-*C*), 101.0, 100.9, 100.7, 98.2, 97.5 (2C), 76.5, 75.9, 75.6, 74.7, 74.5, 73.0, 72.6 (2C), 72.5, 72.4, 72.2, 72.1 (2C), 72.0 (2C), 71.9 (2C), 69.4 ($-OCH_2(CH_2)_5N_3$), 69.3, 68.9, 68.8, 67.8, 62.4 (2C), 62.3, 62.0, 61.8, 61.7, 55.4 (2C), 55.3, 51.1 ($-O(CH_2)_5CH_2N_3$), 37.8 (3C), 30.0 (2C, $-COCH_3$), 29.9 ($-COCH_3$), 29.0 ($-OCH_2CH_2(CH_2)_4N_3$), 28.5 ($-O(CH_2)_4CH_2CH_2N_3$), 27.8, 27.7, 27.6, 26.1 ($-O(CH_2)_2CH_2(CH_2)_3N_3$), 25.3 ($-O-(CH_2)_3CH_2(CH_2)_2N_3$), 20.9 (2C, $-COCH_3$), 20.8 ($-COCH_3$), 20.7 ($-COCH_3$), 20.6 ($-COCH_3$), 20.5 (2C, $-COCH_3$); HRMS *m*/*z* calcd for $C_{137}H_{142}N_7O_{53}$ $[M + NH_4]^+$ 2732.8626, found 2732.8661; *m*/*z* calcd for $C_{137}H_{146}N_8O_{53}$ $[M + 2NH_4]^{2+}$ 1375.9499, found 1375.9515.

6-Azidohexyl 4-*O*-acetyl-2,3-di-*O*-benzoyl-*β*-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1 → 4)-2,3-di-*O*-benzoyl-*β*-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranosyl-(1 → 4)-2,4-di-*O*-benzoyl-*β*-D-glucopyranosyl-(1 → 3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranoside (21). To a solution of hexasaccharide **20** (248 mg, 91.3 μmol) in ethanol/toluene (21 mL, v/v 2:1) at rt was added hydrazine acetate (120 mg, 1.38 mmol). The reaction mixture was stirred at rt for 3 h, and the solvent was removed under reduced pressure. The residue was then dissolved in dichloromethane (50 mL) and washed with 1 N HCl, water, and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. Column chromatography of the residue using EtOAc/hexane (10:1) as the eluent afforded the hexasaccharide triol **21** as a white foam (198 mg, 89%): $[\alpha]_D^{23}$ = +36.0 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.78−7.71 (m, 6H, Ar-*H*), 7.66−7.60 (m, 7H, Ar-*H*), 7.54 (dd, *J* = 8.3, 1.2 Hz, 2H, Ar-*H*), 7.51−7.40 (m, 12H, Ar-*H*), 7.30−7.17 (m, 15H, Ar-*H*), 5.43 (t, *J* = 9.6 Hz, 1H), 5.31 (t, *J* = 8.9 Hz, 1H), 5.28 (t, *J* = 8.8 Hz, 1H), 5.10 (dd, *J* = 9.7, 7.7 Hz, 1H), 5.06−5.02 (m, 3H), 5.00 (d, *J* = 8.4 Hz, 1H), 4.94 (dd, *J* = 8.9, 7.4 Hz, 1H), 4.89−4.84 (m, 3H), 4.77 (t, *J* = 9.4 Hz, 1H), 4.69 (dd, *J* = 9.2, 10.6 Hz, 1H), 4.65 (dd, *J* = 9.0, 10.8 Hz, 1H), 4.57 (d, *J* = 7.7 Hz, 1H), 4.51 (dd, *J* = 9.1, 10.8 Hz, 1H), 4.49 (d, *J* = 7.3 Hz, 1H), 4.39 (d, *J* = 7.2 Hz, 1H), 4.22−4.06 (m, 5H), 3.89 (td, *J* = 9.2, 3.5 Hz, 2H), 3.74−3.69 (m, 2H), 3.65−3.62 (m, 1H), 3.58−3.50 (m, 5H), 3.47−3.43 (m, 1H), 3.40 (d, *J* = 11.4 Hz, 1H), 3.37−3.35 (m, 1H), 3.27−3.21 (m, 2H), 3.20−3.17 (m, 1H), 3.15−3.10 (m, 3H), 2.96 (t, *J* = 7.0 Hz, 2H, $-OCH_2(CH_2)_4CH_2N_3$), 2.06 (s, 3H, $-COCH_3$), 1.94 (s, 3H, $-COCH_3$), 1.90 (s, 3H, $-COCH_3$), 1.89 (s, 6H, $-COCH_3$ x 2), 1.87 (s, 3H, $-COCH_3$), 1.78 (s, 3H, $-COCH_3$), 1.34−1.28 (m, 1H, $-OCH_2CHH(CH_2)_4N_3$), 1.25−1.19 (m, 1H, $-OCH_2CHH(CH_2)_4N_3$), 1.17−1.08 (m, 2H, $-O-(CH_2)_4CH_2CH_2N_3$), 1.04−0.84 (m, 4H, $-O-(CH_2)_2(CH_2)_2(CH_2)_2N_3$); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.9

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(−$COCH_3$), 170.6 (−$COCH_3$), 170.6 (−$COCH_3$), 170.2 (−$COCH_3$), 169.8 (−$COCH_3$), 169.7 (−$COCH_3$), 169.5 (−$COCH_3$), 165.7 (−COPh), 164.9 (4C, −COPh) 164.8 (−COPh), 134.5 (Ar-*C*), 134.3 (Ar-*C*), 134.2 (Ar-*C*), 133.5 (Ar-*C*), 133.2 (2C, Ar-*C*), 132.9 (Ar-*C*), 132.8 (Ar-*C*), 131.1 (Ar-*C*), 130.9 (Ar-*C*), 129.9−129.6 (m, Ar-*C*), 129.3−129.2 (m, Ar-*C*), 128.6 (Ar-*C*), 128.4−128.2 (m, Ar-*C*), 123.5 (br, Ar-*C*), 99.8 (2C), 99.6, 98.4, 97.9 (2C), 76.2, 75.9, 75.2, 74.8, 74.7, 74.6, 74.4, 74.3, 73.4, 72.9, 72.6, 72.3, 72.0, 71.9, 69.6 (−$OCH_2(CH_2)_5N_3$), 69.4, 68.9, 68.7 (2C), 62.3, 61.6 (2C), 61.2, 60.5 (2C), 55.7, 55.5 (2C), 51.2 (−$O(CH_2)_5CH_2N_3$), 29.0 (−$OCH_2CH_2(CH_2)_4N_3$), 28.6 (−$O(CH_2)_4CH_2CH_2N_3$), 26.1 (−$O(CH_2)_2CH_2(CH_2)_3N_3$), 25.3 (−$O(CH_2)_3CH_2(CH_2)_2N_3$), 20.9 (2C, −$COCH_3$), 20.8 (2C, −$COCH_3$), 20.7 (2C, −$COCH_3$), 20.6 (−$COCH_3$); HRMS *m*/*z* calcd for $C_{122}H_{124}N_7O_{47}$ $[M + NH_4]^+$ 2438.7523, found 2438.7610; *m*/*z* calcd for $C_{122}H_{128}N_8O_{47}$ $[M + 2NH_4]^{2+}$ 1228.8947, found 1228.8937.

6-Azidohexyl (4-*O*-acetyl-2,3-di-*O*-benzoyl-β-D-glucopyranosyluronic acid)-(1 → 3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 4)-(2,3-di-*O*-benzoyl-β-D-glucopyranosyluronic acid)-(1 → 3)-(4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 4)-(2,4-di-*O*-benzoyl-β-D-glucopyranosyluronic acid)-(1 → 3)-4,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (22). To a solution of the triol **21** (170 mg, 70.2 μmol) in dichloromethane (10 mL) was added pyridinium dichromate (200 mg, 532 μmol) and Ac_2O (150 μL). The mixture was stirred at rt for 8 h, at which time TLC (EtOAc/HOAc 10:1) indicated the completion of reaction. The reaction mixture was diluted with EtOAc (15 mL), and the suspended mixture was transferred to column chromatography (EtOAc/HOAc 10:1 → 5:1) to yield the triacid **22** as a brown solid (116 mg, 67%): $[\alpha]_D^{23}$ = +120.0 (*c* 0.5, $CHCl_3$); HRMS *m*/*z* calcd for $C_{122}H_{118}N_7O_{50}$ $[M + NH_4]^+$ 2480.6901, found 2480.6939; *m*/*z* calcd for $C_{122}H_{122}N_8O_{50}$ $[M + 2NH_4]^{2+}$ 1249.8636, found 1249.8661.

Esterification of a small amount of **22** with diazomethane in ether gave the methyl ester **22a**, which was used for analysis: Selected ^{1}H NMR data (500 MHz, $CDCl_3$) δ 4.85, 4.83, 4.79 (3 d, 3 × 1H, $J_{1,2}$ = 8.5 Hz, H-$1^{A,C,E}$), 4.45, 4.32, 4.20 (3 d, 3 × 1H, $J_{1,2}$ = 7.8 Hz, H-$1^{B,D,F}$), 3.71, 3.44, 3.42 (3 s, 3 × 3H, 3 CH_3O−), 2.95 (t, 2H, *J* = 7.0 Hz, −$CH_2CH_2N_3$); MALDI-TOF MS *m*/*z* calcd for $C_{125}H_{120}N_6NaO_{50}$ $[M + Na]^+$ 2527.69, found 2528.08.

6-Azidohexyl (β-D-glucopyranosyluronic acid)-(1 → 3)-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 4)-(β-D-glucopyranosyluronic acid)-(1 → 3)-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 4)-(β-D-glucopyranosyluronic acid)-(1 → 3)-2-deoxy-2-acetamido-β-D-glucopyranoside (23a). To a solution of compound **22** (50 mg, 34.9 μmol) in *n*-butanol (10 mL) was added ethylenediamine (8 mL). The reaction mixture was stirred at 90 °C for 20 h under N_2. The solvent was coevaporated with toluene (3 × 10 mL) under reduced pressure, and the residue was then dissolved in pyridine (10 mL). Acetic anhydride (10 mL) was added to the above solution. The reaction mixture was then stirred at rt overnight, concentrated, and coconcentrated with toluene and methanol (3 × 10 mL). The yellow residue was then dissolved in THF (15 mL), and aqueous 1 N LiOH (5 mL) was added at 0 °C. The reaction mixture was stirred for 20 h with temperature slowly warming to rt, and then neutralized with aqueous 1 N HCl (5 mL), and the resulting mixture was concentrated. Purification of the residue by gel filtration on Sephadex G-10 (water) gave **23a**, isolated after lyophilization as a white foam (18.7 mg, 72%): $[\alpha]_D^{23}$ = −42 (*c* 0.1, H_2O); The ^{1}H and ^{13}C NMR resonances are listed in Table 2; HRMS *m*/*z* calcd for $C_{48}H_{80}N_7O_{34}$ $[M + NH_4]^+$ 1298.4741, found 1298.4724; *m*/*z* calcd for $C_{48}H_{78}N_6O_{34}$ $[M + 2H]^{2+}$ 641.2274, found 641.2253.

6-Azidohexyl (β-D-glucopyranosyluronic acid)-(1 → 3)-(2-deoxy-2-propionamido-β-D-glucopyranosyl)-(1 → 4)-(β-D-glucopyranosyluronic acid)-(1 → 3)-(2-deoxy-2-propionamido-β-D-glucopyranosyl)-(1 → 4)-(β-D-glucopyranosyluronic acid)-(1 → 3)-2-deoxy-2-propionamido-β-D-glucopyranoside (23b). Compound **23b** (20.9 mg, 78%) was prepared by the same process as for **23a** by using propionic anhydride; it was obtained, after lyopholization, as a white foam: $[\alpha]_D^{23}$ = −60 (*c* 0.1, H_2O); The ^{1}H and ^{13}C NMR resonances are listed in Table 2; HRMS *m*/*z* calcd for $C_{51}H_{83}N_6O_{34}$ $[M + H]^+$ 1323.4945, found 1323.4999; *m*/*z* calcd for $C_{51}H_{84}N_6O_{34}$ $[M + 2H]^{2+}$ 662.2509, found 662.2540.

6-Aminohexyl (β-D-glucopyranosyluronic acid)-(1 → 3)-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 4)-(β-D-glucopyranosyluronic acid)-(1 → 3)-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1 → 4)-(β-D-glucopyranosyluronic acid)-(1 → 3)-2-deoxy-2-acetamido-β-D-glucopyranoside (24a). To a solution of 10% Pd−C (2 mg) and $NaBH_4$ (6 mg) in water (1 mL) was added a solution of compound **23a** (12 mg, 9.4 μmol) in 50 mM aqueous NaOH (1.5 mL). The reaction mixture was stirred at rt for 5 h and then filtered through Celite. The filtrate was loaded on a size-exclusion column (Bio-Gel P-2, 50 mM NH_4HCO_3) and chromatographed to afford the free-amine **24a**, after lyophilization, as a white foam (10 mg, 85%): $[\alpha]_D^{23}$ = −45 (*c* 0.1, H_2O); The ^{1}H and ^{13}C NMR resonances are listed in Table 2; HRMS *m*/*z* calcd for $C_{48}H_{79}N_4O_{34}$ $[M + H]^+$ 1255.4570, found 1255.4525; *m*/*z* calcd for $C_{48}H_{80}N_4O_{34}$ $[M + 2H]^{2+}$ 628.2321, found 628.2293.

6-Aminohexyl (β-D-glucopyranosyluronic acid)-(1 → 3)-(2-deoxy-2-propionamido-β-D-glucopyranosyl)-(1 → 4)-(β-D-glucopyranosyluronic acid)-(1 → 3)-(2-deoxy-2-propionamido-β-D-glucopyranosyl)-(1 → 4)-(β-D-glucopyranosyluronic acid)-(1 → 3)-2-deoxy-2-propionamido-β-D-glucopyranoside (24b). Compound **24b** (6.5 mg, 81%) was prepared as a white foam using a similar protocol as **24a** from compound **23b** (8.2 mg, 6.2 μmol): $[\alpha]_D^{23}$ = −40.0 (*c* 0.1, H_2O); The ^{1}H and ^{13}C NMR resonances are listed in Table 2; HRMS *m*/*z* calcd for $C_{51}H_{85}N_4O_{34}$ $[M + H]^+$ 1297.5040, found 1297.5067; *m*/*z* calcd for $C_{51}H_{86}N_4O_{34}$ $[M + 2H]^{2+}$ 649.2556, found 649.2569.

Preparation of the Hexasaccharide−Tetanus Toxoid (TT) Conjugates (1a, 1b) and Hexasaccharide−Human Serum Albumin (HSA) Conjugates. To a solution of **24a**/**b** (5 mg) in 50 mM phosphate buffer (500 μL, pH 7.3) was added a solution of diethyl squarate (2 equiv) in ethanol (500 μL). The mixture was stirred at rt for 20 h, at which time TLC (1-butanol:EtOH:H_2O:HOAc 4:2:2:0.5) indicated the disappearance of starting material and formation of one major product. The solvent was removed under reduced pressure, and size-exclusion chromatography (Bio-Gel P-2, 50 mM NH_4HCO_3) of the residue yielded the monoethyl squarate compounds **25a**/**b** (∼5.2 mg), which were used directly for preparation of the protein conjugates. A portion of the hexasaccharide (2.5 mg, 40−60 equiv of protein) was added to a solution of protein (4.5 mg for **TT**; 3.0 mg for **HSA**) in 0.1 M carbonate buffer (pH 10, 100 μL). After incubation for 7 days at rt, analysis by MALDI-TOF mass spectrometry (sinapinic acid matrix) indicated no further increase in mass had occurred. The reaction mixture was dialyzed against distilled water (3 × 5 mL) using an Amicon ulfrafiltration cell equipped with a Diaflo membrane. The residue was taken up in water and lyophilized to give the corresponding protein-hexasaccharide conjugates (4.4 mg for **TT-1a**; 4.2 mg for **TT-1b**; 2.9 mg for **HSA-1a** and 2.8 mg for **HSA-1b**) as white powders.

MALDI-TOF MS data (sinapinic acid matrix, 0.1% TFA in 1:1 CH_3CN/H_2O): TT-hexasaccharide **1a** conjugate (163 907 Da); TT-hexasaccharide **1b** conjugate (159 816 Da); HSA-hexasaccharide **1a** conjugate (76 482 Da); HSA-hexasaccharide **1b** conjugate (75 548 Da). The number of haptens per protein for each conjugate is listed in Table 1.

ELISA Solid-Phase Antigens. HSA-hexasaccharideNAc **1a**, HSA-hexasaccharideNCOPr **1b** conjugates were used as solid-phase antigens for the enzyme-linked immunosorbent assays (ELISA).

Experimental Groups of Mice and Immunization Protocol. Groups of 10 female CD1 outbred mice (4−6 weeks old) were immunized subcutaneously with 50 μg (based on the protein amount in the conjugate) of the synthetic hexasaccharideNAc-TT conjugates **1a** and **1b**. They received two doses of the conjugate vaccine at day 0 and day 28. The first dose was formulated with Freund's complete adjuvant (FCA, Sigma Chemical Co., St. Louis, MO) and the second dose with Incomplete Freund's adjuvant (IFA, Sigma). Sera from mice were collected on days 0, 28, and 38 and stored frozen until they were analyzed for HA-specific IgG titer.

ELISA. Antibody titers to hexasaccharideNAc-HSA and hexasaccharideNCOPr-HSA in sera from mice were determined before vaccination (day 0) and on days 28 and 38 by enzyme-linked immunosorbent assay

(ELISA). Serum samples were titered against the HSA conjugates in 96-well plates (NUNC-Polysorp, Rochester, NY). Wells were coated with 100 μL of HSA conjugates (1 μg/mL) in PBS (pH 7.4, Quality Biologicals, Gaithersburg, MD) per well for 1 h at 37 °C; the plates were previously covered with adhesive film. Plates were aspirated and washed three times with a washing buffer of 0.05% Tween 20 (Sigma Chemical Co., St. Louis, MO) in PBS (PBS-T). The wells were blocked by the addition of 150 μL of 0.5% BSA (Sigma) in PBS for 1 h at rt. This was followed by aspiration of blocking buffer solution and washing the plates (×3) as above. Plates were stored at 4 °C until further use. Antisera serially diluted in PBS-T to a final 1:500 dilution (starting at 1:20) were added (100 μL well) and incubated for 1 h at rt. This was followed by washing three times the plates as above, followed by the addition of 100 μL of peroxidase-labeled goat antimouse IgG (H+L) conjugate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) (1:2500) in PBS-T; plates were incubated for 1 h at rt. Plates were then washed five times as above, and this was followed by addition of 100 μL per well of substrate solution (SureBlue Reserve TMB, Kirkegaard & Perry Laboratories), and plates were incubated for 5−10 min at rt. This was followed by the addition of 100 μL of stop solution (1 N HCl). Plates were scanned at 450 nm in a microplate reader. Absorbances corresponding to antisera from day 38 were plotted versus log of serum dilutions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +1 778 782 4152. Fax: +1 778 782 4860. E-mail: bpinto@sfu.ca.

Notes

The authors declare no competing financial interest.

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