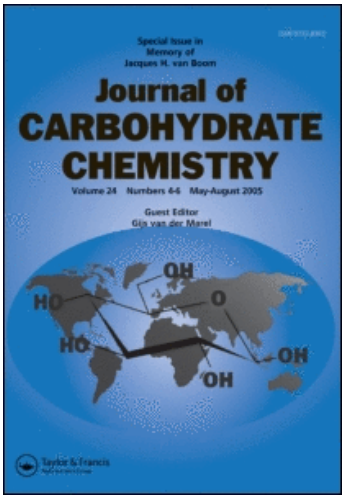

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Chemical-Enzymatic Synthesis of A Branched Hexasaccharide Fragment of Type Ia Group B *Streptococcus* Capsular Polysaccharide

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CHEMICAL-ENZYMATIC SYNTHESIS OF A BRANCHED HEXASACCHARIDE FRAGMENT OF TYPE Ia GROUP B *STREPTOCOCCUS* CAPSULAR POLYSACCHARIDE

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ABSTRACT

A branched hexasaccharide fragment of type Ia group B streptococcal polysaccharide, α-NeuAc(2→3)-β-D-Gal(1→4)-β-D-GlcNAc(1→3)-[β-D-Glc(1→4)]-β-D-Gal(1→4)-β-D-Glc-OMe (**13**), has been synthesized by chemical-enzymatic procedures. Chemical synthesis of a pentasaccharide, β-D-Gal(1→4)-β-D-GlcNAc(1→3)-[β-D-Glc(1→4)]-β-D-Gal(1→4)-β-D-Glc-OMe (**12**), was achieved from glycosyl donor, 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**9**), and acceptor, methyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,6-di-*O*-benzyl-β-D-galacto-pyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6**), by block condensation in 41% yield. Following enzymatic sialylation of **12** at the 3-*O*-position of its terminal galactopyranosyl residue using recombinant α-(2→3)-sialyltransferase and CMP-NeuAc afforded **13** in 59% yield.

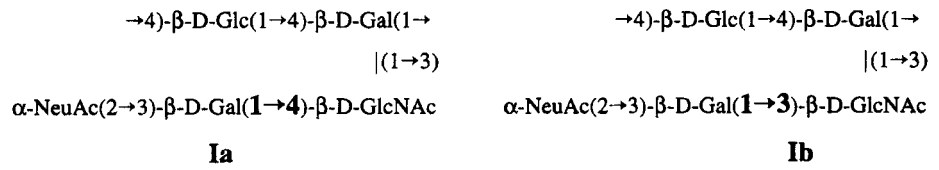


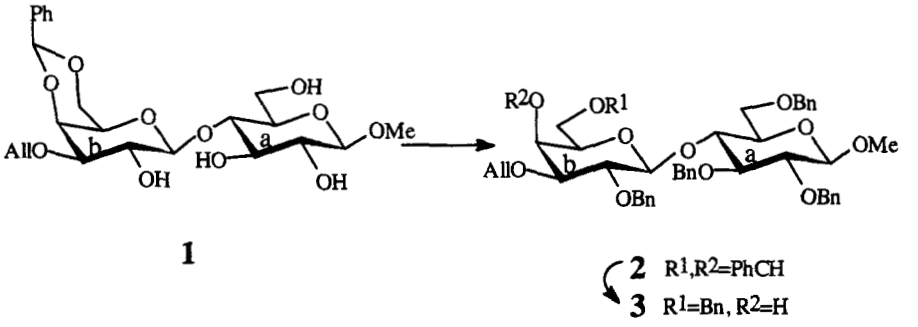
Figure 1. Repeating-unit structures of GBS type Ia and Ib polysaccharides

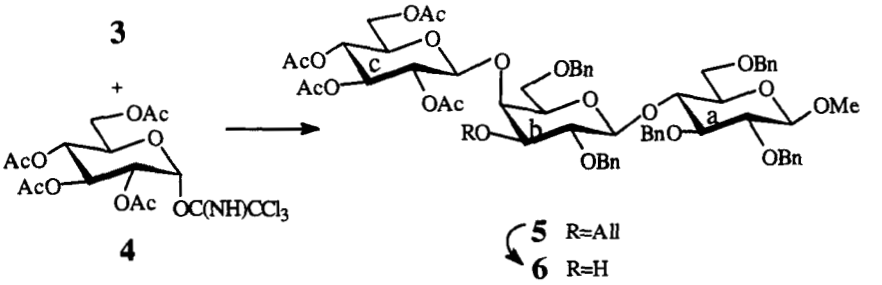
INTRODUCTION

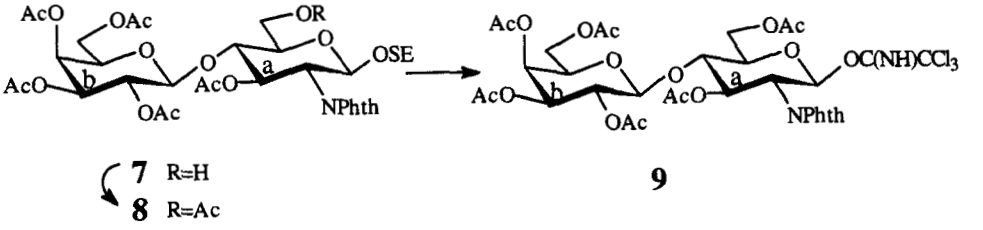
Group B *Streptococcus* (GBS) is the leading cause of bacterial infection among neonates.[1] Organisms of capsular types Ia and Ib together account for 40-50% of the cases of early-onset GBS disease.[1] The type Ia and Ib capsular polysaccharides have chemically similar structures.[2,3] The sole difference between these polysaccharides is the bond between the galactose and *N*-acetylglucosamine residues in the side chain. These sugars are β(1→4)-linked in type Ia and β(1→3)-linked in type Ib (see Figure 1). Although the two polysaccharides are structural isomers, they are antigenically distinct; whereas antisera raised to type Ia organisms reacts strongly with type Ia polysaccharide, it reacts to a much lesser extent with type Ib polysaccharide.[3] As a consequence of this, both polysaccharides will be required as components of any future comprehensive vaccine against meningitis caused by group B streptococci.[4] Interestingly, in contrast to type Ib polysaccharide, type Ia produces a distinct population of antibodies entirely dependent on the presence of terminal sialic acid.[5] This has been attributed to a sialic acid-controlled conformational epitope exhibited by type Ia polysaccharide but not by type Ib polysaccharide.[5] Therefore, as part of our program to understand the role of sialic acid in the formation of conformational epitopes of capsular polysaccharides, a branched hexasaccharide fragment of type Ia polysaccharide was required to serve as a probe to define the epitope. Here, we describe the synthesis of this unit by a combined chemical-enzymatic protocol; the chemical synthesis of branched pentasaccharide **12** is followed by enzymatic sialylation with recombinant α-(2→3)-sialyltranferase and CMP-NeuAc to afford hexasaccharide **13**.

RESULTS AND DISCUSSION

Methyl 4-*O*-(3-*O*-allyl-2-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**2**) was obtained in 78% yield from lactose derivative **1**[6] by conventional benzylation with BnBr/NaH in DMF. Reductive ring-opening of benzylidene acetal by treatment **2** with sodium cyanoborohydride[7] and HCl in THF afforded compound **3** in 69% yield. Condensation of **3** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**4**) in dichloromethane at -45 °C in the presence of trimethylsilyl triflate gave methyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-*O*-(3-*O*-allyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5**) in 75% yield. Removal of the 3^b-*O*-allyl group in **5** was then achieved by treatment with $PdCl_2$ in methanol,[8] to afford **6** in 71% yield.







Although compound **9** has been previously synthesized from lactosamine,[6,9-11] we prepared this compound from 2-(trimethylsilyl)ethyl 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**7**)[12] in three steps. Conversion of **7** into compound **8** by acetylation at the 6^b-*O*-position with acetic anhydride in pyridine (81% yield) followed by removal of the 2-(trimethylsilyl)ethyl group with boron trifluoride etherate,[13] and finally treatment with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[14] to furnish compound **9** in 40% yield (two steps). The yield of **9** from **8** can be improved to 77% through the use of trifluoroacetic acid to remove 2-(trimethylsilyl)ethyl group[13] and anhydrous K_2CO_3 as base in the formation of trichloroacetimidate.[15] The ^{1}H NMR and physical data of this compound were in accordance with those reported in the literature.[9-11]

Condensation of **6** with **9** in dichloromethane at -45 °C using trimethylsilyl triflate as promoter afforded methyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-*O*]-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**10**) in 41% yield. The ^{13}C NMR spectrum of **10** showed five anomeric carbon resonances at 99.99, 101.17, 101.31, 101.61, and 104.64 ppm, and its FABMS spectrum was also consistent with the proposed structure.

Compound **10** was treated with hydrazine hydrate in 95% ethanol at reflux temperature for 16 h to remove the phthalimido group and the *O*-acetyl groups. The obtained product was then fully acetylated with acetic anhydride in pyridine overnight to give **11** in 63% yield. Removal of protecting groups (*O*-acetyl groups and *O*-benzyl groups) was then performed by treatment of **11** with 0.1% NaOMe/MeOH for 2 h, following catalytic hydrogenation (Pd/C) to give the pentasaccharide **12** in 79% yield. Enzymatic sialylation at the 3^e-*O*-position of the terminal galactopyranosyl residue of **12** was achieved using recombinant α-(2→3)-sialyltransferase[16,17] and CMP-NeuAc, which afforded branched hexasaccharide **13** in 59% yield.

The ^{1}H NMR spectra of both compounds **12** and **13** are shown in Figure 2. Five anomeric proton (H-1) signals were observed in the ^{1}H NMR spectrum of **12** at 4.395 (H-1^a, Glc), 4.453 (H-1^b, Gal), 4.477 (H-1^e, Gal), 4.722 (H-1^d, GlcNAc), and 4.877 (H-1^c, Glc) ppm (the extra singlet under H-1^a is the resonance of H-4^b), whereas the equivalent

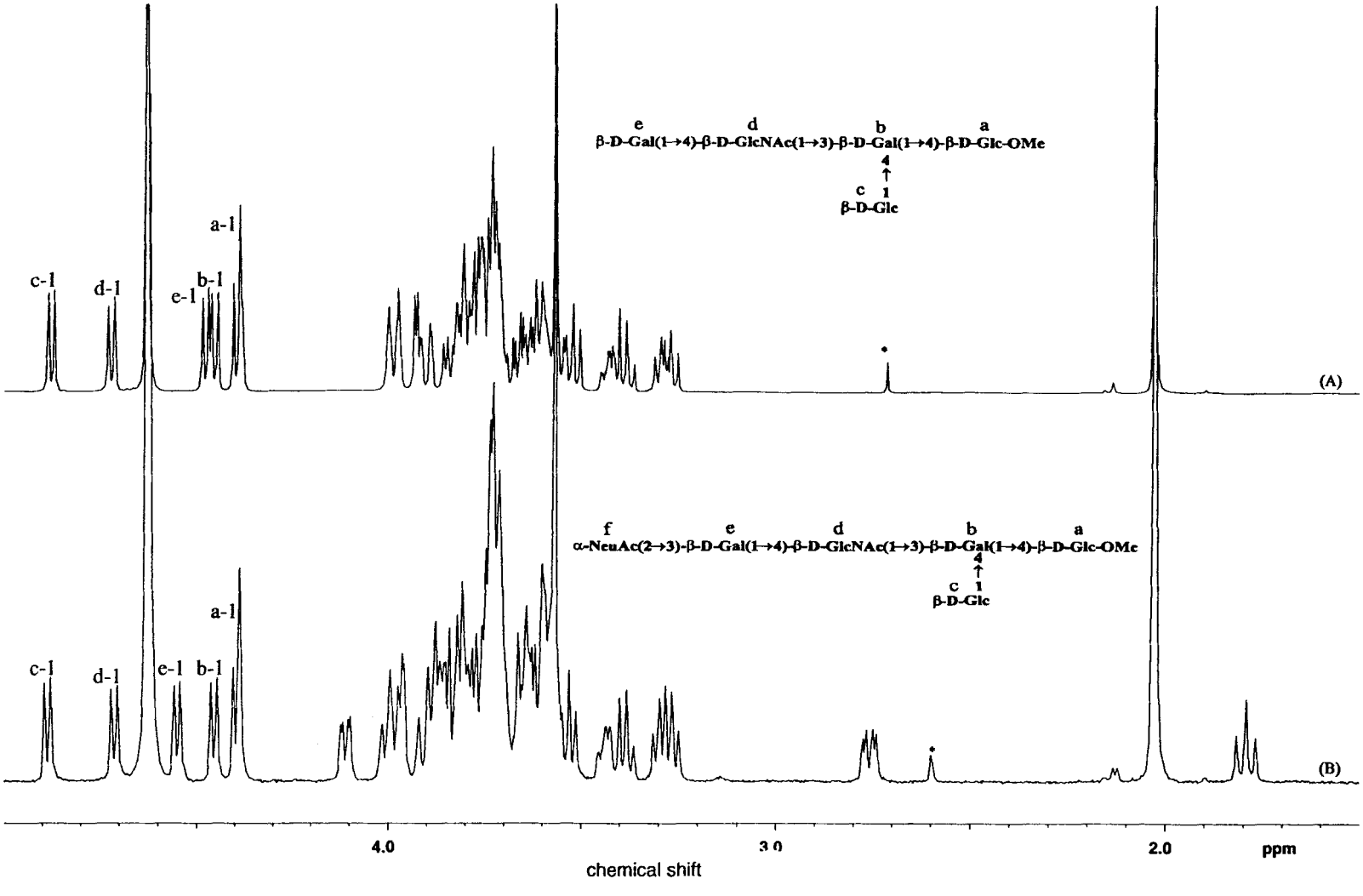
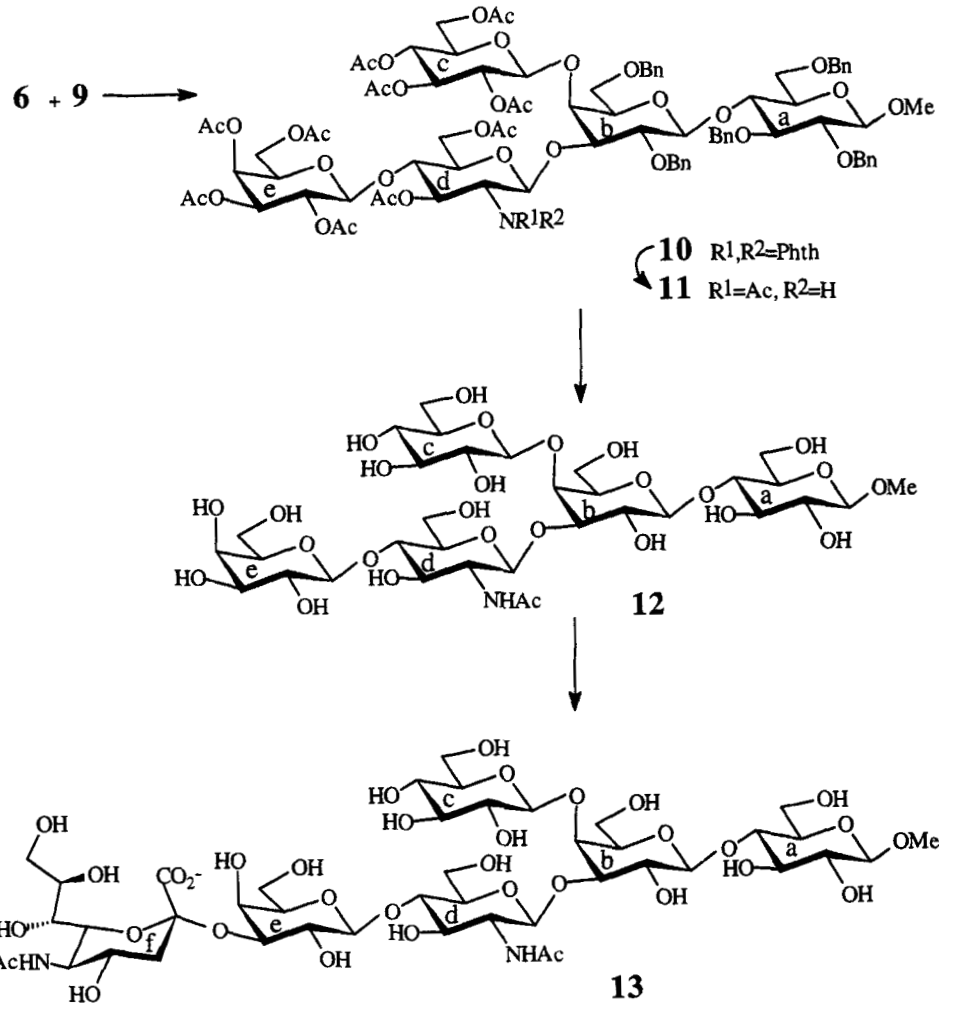


Figure 2. 500 MHz ^{1}HNMR spectra of compound **12** (A) and compound **13** (B) recorded in D_2O at 310 K.

anomeric signals of **13** were at 4.396 (H-1^a, Glc), 4.455 (H-1^b, Gal), 4.551 (H-1^c, Gal), 4.713 (H-1^d, GlcNAc), and 4.886 (H-1^c, Glc) ppm. Thus, sialylation at the 3-*O*-position resulted in a large increase in chemical shift (0.074 ppm) of the signal of H-1^e. More detailed conformational studies of **12** and **13** will be reported elsewhere.



EXPERIMENTAL

General methods. Optical rotations were measured at room temperature with a Perkin-Elmer 243 polarimeter, using a 10-cm 1-mL cell. ^1H and ^{13}C NMR spectra were

recorded at 500 MHz and 125 MHz, respectively, with a Bruker AMX 500 instrument at 300 K unless otherwise noted. Chemical shifts are given in ppm relative to the signal of internal Me_4Si or indirectly to solvent signals 7.25 ($CDCl_3$), 2.225 (acetone in D_2O) for ^1H NMR spectra, and to the solvent signals 76.9 ($CDCl_3$), 31.55 (internal acetone) for ^{13}C NMR spectra. The ^1H NMR resonances of oligosaccharides were assigned on the basis of 2D ^1H-homonulear chemical-shift correlated (^1H-COSY) experiments. FAB and ES (electron spray) mass spectroscopic analyses were performed with a JEOL JMS-AX505H and a VG QUATTRO mass spectrometer, respectively.

Column chromatography was performed on Silica gel 60 (Merck, 230-400 mesh) and fractions were monitored by TLC on Silica gel 60 F_{254} (Merck) unless otherwise noted. Detection was effected by examination under UV light and by charring with 5% sulphuric acid solution in ethanol. Solutions were concentrated at or below 40 °C and dried with anhydrous Na_2SO_4.

Methyl 4-*O*-(3-*O*-Allyl-2-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (2). To a solution of compound **1** (0.8 g, 1.65 mmol) in DMF (15 mL) was added NaH (50%, 0.4 g, 8.33 mmol). The mixture was stirred at rt for 0.5 h. Benzyl bromide (1 mL, 8.42 mmol) was added to the mixture and the stirring was continued for another 3 h. Methanol (0.5 mL) was added to the mixture to destroy excess NaH, and then cold water (50 mL) was added. The solution was extracted with EtOAc (2 x 40 mL). The organic solution was subsequently washed with water, aq $NaHCO_3$, and water, then dried and concentrated to a residue. Purification by chromatography (EtOAc/hexane 1:2) gave **2** (1.1g, 78%) as a solid: $[\alpha]_D$ +1.6° (*c* 1.49, MeOH); ^1H NMR ($CDCl_3$) δ 3.006 (s, 1H, H-5^b), 3.317 (dd, 1H, H-3^b, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.4 Hz), 3.368 (dd, 1H, H-5^a), 3.418 (dd, 1H, H-2^a, $J_{2,3}$ = 8.5 Hz), 3.552 (s, 3H, OMe), 3.622 (dd, 1H, H-3^a, $J_{3,4}$ = 9.0 Hz), 3.710 (dd, 1H, H-2^b, $J_{2,3}$ = 9.5 Hz), 3.726 (m, 1H, H-6^b), 3.860-3.883 (m, 2H, H-6^a, 6$^{b'}$), 3.976 (dd, 1H, H-4^a, $J_{4,5}$ = 9.3 Hz), 4.104 (d, 1H, H-4^b, $J_{3,4}$ = 3.4 Hz), 4.170-4.226 (m, 3H, OC*H*$_2$CH=CH$_2$, H-6$^{a'}$), 4.303 (d, 1H, H-1^a, $J_{1,2}$ = 8.3 Hz), 4.348 and 4.539 (2d, 2H, C*H*$_2$Ph, J = 12.5 Hz), 4.476 (s, 1H, H-1^b, $J_{1,2}$ = 8.4 Hz), 4.670-4.882 (m, 5H, 2.5 x C*H*$_2$Ph), 5.163 (d, 2H, one of C*H*$_2$Ph and one of CH$_2$CH=C*H*$_2$, J = 10.8 Hz), 5.283 (d, 1H, one of OCH$_2$CH=C*H*$_2$, J = 16.6 Hz), 5.492 (s, 1H, PhC*H*), 5.927 (m, 1H, OCH$_2$C*H*=CH$_2$), 7.159-7.498 (m, 25H, 5 x Ph).

Methyl 4-*O*-(3-*O*-Allyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3). To a mixture of sodium cyanoborohydride (1.0 g, 16 mmol), powdered molecular sieves 3Å (3.0 g), and compound **2** (1.0 g, 1.18 mmol) in THF (15 mL) a saturated solution of HCl in ethyl ether was added dropwise at 0 °C until the mixture became acidic (pH 3 with pH paper). Then the mixture was stirred further at 0 °C until the reaction was complete (2 h). The mixture was diluted with EtOAc (50 mL) and filtered through Celite. The filtrate was subsequently washed with water, aq $NaHCO_3$, and water, then dried and concentrated to a residue. Purification by chromatography (EtOAc/hexane 1:3) gave syrupy **3** (0.7 g, 69%): $[\alpha]_D$ +27.1° (*c* 0.8, MeOH); ^{1}H NMR ($CDCl_3$) δ 2.340 (s, 1H, OH-4^b), 3.271 (dd, 1H, H-3^b, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 3.4 Hz), 3.537 (s, 3H, OMe), 4.266 (d, 1H, H-1^a, $J_{1,2}$ = 7.8 Hz), 4.364-4.548 (m, 5H, 2 x C*H*$_2$Ph and H-1^b), 4.678-4.967 (m, 6H, 3 x C*H*$_2$Ph), 5.161 (d, 1H, one of CH_2CH=C*H*$_2$, J = 10.0 Hz), 5.263 (d, 1H, one of OCH_2CH=C*H*$_2$, J = 17.2 Hz), 5.910 (m, 1H, OCH_2CH=CH_2), 7.198-7.364 (m, 25H, 5 x Ph); HRFABMS Calcd for $C_{51}H_{58}O_{11}Li$ (M+Li): 853.4139. Found: 853.4131.

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-*O*-(3-*O*-allyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (5). A mixture of **3** (0.6 g, 0.7 mmol), **4** (0.7 g, 1.4 mmol), and powdered molecular sieves 4Å (1.0 g) in dichloromethane (15 mL) was stirred at rt for 1 h. The mixture was cooled to -45 °C and trimethylsilyl triflate (150 μL) was added. The mixture was stirred at -45 °C for 1 h, and was then neutralized with a solution of 2,6-lutidine (0.5 mL) in dichloromethane (20 mL). The filtrate was subsequently washed with water, 1M HCl, and water, then dried and concentrated to a residue. Purification by chromatography (EtOAc/hexane 2:3) gave **5** (0.73 g, 75%) as a solid: $[\alpha]_D$ +25.9° (*c* 1.1, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.973, 1.990, 2.008, 2.049 (4s, 3H each, 4 x OAc), 3.230 (dd, 1H, H-3^b, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.4 Hz), 3.528 (s, 3H, OMe), 4.851 (d, 1H, H-1^c, $J_{1,2}$ = 8.1 Hz), 4.981 (dd, 1H, H-2^c, $J_{2,3}$ = 9.5 Hz), 5.100 (t, 1H, H-4^c, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz), 5.161-5.243 (m, 2H, H-3^c and one of OCH_2CH=C*H*$_2$), 5.316 (d, 1H, one of OCH_2CH=C*H*$_2$, J = 17 Hz), 5.910 (m, 1H, OCH_2CH=CH_2), 7.195-7.466 (m, 25H, 5 x Ph); HRFABMS Calcd for $C_{65}H_{76}O_{20}Li$ (M+Li): 1183.5090. Found: 1183.5098.

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6). A mixture of **5** (0.45 g, 0.38 mmol) and $PdCl_2$ (0.6 g) in methanol (10 mL) was stirred at rt until the starting material was consumed (2 h). The mixture was filtered through Celite and the solid residue was extracted with EtOAc (50 mL). The combined organic solution was subsequently washed with water, aq $NaHCO_3$, and water, then dried and concentrated to a residue. Purification by chromatography (EtOAc/hexane 1:1) gave **6** (0.31 g, 71%) as a solid: $[\alpha]_D$ +14.5° (*c* 1.6, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.944, 1.965, 2.000, 2.017 (4s, 3H each, 4 x OAc), 2.298 (d, 1H, OH-3^b, J = 4.4 Hz), 3.529 (s, 3H, OMe), 4.261 (d, 1H, H-1^a, $J_{1,2}$ = 7.7 Hz), 4.394 (d, 1H, H-1^b, $J_{1,2}$ = 8.3 Hz), 4.861 (d, 1H, H-1^c, $J_{1,2}$ = 8.4 Hz), 4.986 (dd, 1H, H-2^c, $J_{2,3}$ = 9.5 Hz), 5.092 (t, 1H, H-4^c, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz), 5.195 (t, 1H, H-3^c, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz), 7.198-7.444 (m, 25H, 5 x Ph); HRFABMS Calcd for $C_{62}H_{72}O_{20}Li$ (M+Li): 1143.4777. Found: 1143.4783.

2-(Trimethylsilyl)ethyl 4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (8). A mixture of compound **7** (0.2 g, 0.26 mmol) in acetic anhydride/pyridine (1:1, 2 mL) was stirred overnight. The solvent was removed by co-distillation with toluene. The residue was purified by chromatography (EtOAc/hexane 1:1) to give crystalline **8** (0.17 g, 81%): mp 133-134 °C (EtOAc/Hexane); $[\alpha]_D$ +12.9° (*c* 0.49, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.159 (s, 9H, $SiMe_3$), 0.714, 0.788 (2m, 1H each, $CH_2CH_2SiMe_3$), 1.877, 1.941, 2.017, 2.044, 2.112 (2) (5s, 6 x OAc), 4.522 (d, 1H, H-1^b, $J_{1,2}$ = 7.4 Hz), 5.308 (bs, 1H, H-4^b) 5.355 (d, 1H, H-1^a, $J_{1,2}$ = 8.4 Hz), 5.708 (t, 1H, H-3^a, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz), 7.702, 7.821 (2bs, 2H each, Phth); HRFABMS Calcd for $C_{37}H_{49}NO_{18}SiLi$ (M+Li): 830.2879. Found: 830.2875.

Anal. Calcd for $C_{37}H_{49}O_{18}Si$: C 53.9; H 6.0; N 1.7. Found: C 53.7; H 6.1; N 1.8.

4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (9). Method 1: To a stirred solution of **8** (0.16 g, 0.19 mmol) in dichloromethane (5 mL) was added boron trifluoride etherate (0.4 mL). The stirring was continued at 0 °C for 2 h, and the solution was then diluted with dichloromethane (20 mL), washed subsequently with water, aq $NaHCO_3$, and water, then dried and concentrated to a residue. To a solution of the above residue and

trichloroacetonitrile (0.4 mL) in dichloromethane (5 mL) at 0 °C was added DBU (50 μL). The mixture was stirred for 2 h at 0 °C, and then concentrated. Purification by chromatography (EtOAc/hexane 1:1) gave **9** (66 mg, 40%) as a solid: $[\alpha]_D$ +39.6° (*c* 2.5, CH_2Cl_2); lit. $[\alpha]_D$ +40.9°,[9] $[\alpha]_D$ +43°;[11] ^{1}H NMR ($CDCl_3$) δ 1.904, 1.937, 2.032, 2.039, 2.112, 2.123 (6s, 3H each, 6 x OAc), 4.532 (d, 1H, H-1^b, $J_{1,2}$ = 7.9 Hz), 5.314 (d, 1H, H-4^b, $J_{3,4}$ = 2.9 Hz), 5.858 (dd, 1H, H-3^a, $J_{2,3}$ = 8.0 Hz, $J_{3,4}$ = 10.0 Hz), 6.594 (d, 1H, H-1^a, $J_{1,2}$ = 8.8 Hz), 7.691, 7.810 (2m, 2H each, Phth), 8.618 (s, 1H, NH).

Method 2: To a solution of **8** (0.10 g, 0.12 mmol) in dichloromethane (4 mL) was added trifluoroacetic acid (2 mL). The mixture was stirred for 2 h at rt. EtOAc (20 mL) was added and the solution was subsequently washed with water, aq $NaHCO_3$, and water, then dried and concentrated to a residue. To a solution of the above residue and trichloroacetonitrile (0.5 mL) in dichloromethane (8 mL) was added anhydrous K_2CO_3 (0.1 g). The mixture was stirred for 4 h and the filtrate was concentrated. Purification by chromatography (EtOAc/hexane 1:1) gave **9** (80 mg, 77%).

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-*O*]-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (10). A mixture of **6** (60 mg, 0.053 mmol), **9** (60 mg, 0.069 mmol), and powdered molecular sieves 4Å (0.1 g) in dichloromethane (3 mL) was stirred at rt for 1 h. The mixture was cooled to -45 °C and trimethylsilyl triflate (20 μL) was added. The mixture was stirred at that temperature for 1.5 h, and was then neutralized with a solution of 2,6-lutidine (0.5 mL) in dichloromethane (20 mL). The filtrate was subsequently washed with water, 1M HCl, and water, then dried and concentrated to a residue. Purification by chromatography (EtOAc/hexane 1:1) gave **10** (40 mg, 41 %) as a solid: $[\alpha]_D$ +18° (*c* 2.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 1.853, 1.857, 1.954, 1.986, 1.987, 2.005, 2.038, 2.066, 2.078, 2.117 (10s, 3H each, 10 x OAc), 3.428 (s, 3H, OMe), 4.558 (d, 1H, H-1^e, $J_{1,2}$ = 8.0 Hz), 4.611 (d, 1H, H-1^c, $J_{1,2}$ = 9.9 Hz), 5.125 (bt, 2H, H-2^e, 4^c), 5.340 (m, 2H, H-3^c, 4^e), 5.589 (d, 1H, H-1^d, $J_{1,2}$ = 8.4 Hz), 5.735 (dd, 1H, H-3^d, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 8.5 Hz), 6.821-7.506 (m, 29H, Phth and 5 x Ph); ^{13}C NMR ($CDCl_3$) δ 99.99, 101.17, 101.31, 101.61, 104.64 (5 x C-1); FABMS Calcd for $C_{94}H_{107}NO_{37}Li$ (M+Li):1848.67. Found: 1848.80.

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-*O*]-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11). A mixture of **10** (38 mg, 0.021 mmol) and hydrazine hydrate (0.5 mL) in 95% EtOH (3 mL) was refluxed for 16 h. Upon cooling, the solvent was evaporated and the residue was treated with acetic anhydride/pyridine (1:1, 2 mL) overnight. The solvent was removed by co-distillation with toluene, and the residue was purified by chromatography (EtOAc/hexane 3:1) to give **11** (23 mg, 62%) as a solid: $[\alpha]_D$ +13.7° (*c* 2.2, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.702 (NAc), 1.953, 1.956, 1.967, 2.000, 2.011, 2.028, 2.037 (2), 2.045, 2.127 (9s, 10 x OAc), 3.486 (s, 3H, OMe), 4.406 (d, 1H, H-1^b, $J_{1,2}$ = 7.4 Hz), 4.476 (d, 1H, H-1^e, $J_{1,2}$ = 8.3 Hz), 5.080 (d, 1H, H-1^d, $J_{1,2}$ = 9.0 Hz), 5.122 (t, 1H, H-4^c, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz), 5.336 (m, 2H, H-3^c, 4^e), 7.186-7.457 (m, 25H, 5 x Ph); ^{13}C NMR ($CDCl_3$) δ 20.74-24.98 (11 x *C*H_3CO), 100.19, 101.32, 102.31, 102.52, 104.67 (5 x C-1), 169.28-170.75 (11 x CH_3*C*O); FABMS Calcd for $C_{88}H_{107}NO_{36}Li$ (M+Li): 1760.67. Found: 1760.76.

Methyl *O*-(β-D-Galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-[β-D-glucopyranosyl-(1→4)-*O*]-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (12). A solution of **11** (20 mg, 11.4 μmol) in 0.1% NaOMe/MeOH (3 mL) was stirred at rt for 2 h. The solution was neutralized with ion-exchange resin Dowex 50 (H^+), and the filtrate was concentrated to a residue. A mixture of above residue and 10% Pd/C (50% water, 30 mg) in H_2O-MeOH-AcOH (1:1:1, 3 mL) was subjected to a hydrogenation (40 psi) for 3 h. The filtrate was concentrated and purified by passage through a Sephadex G-10 column. The fractions containing the major component were lyophilized to give **12** (8.0 mg, 79%) as an amorphous solid: $[\alpha]_D$ -7.5° (*c* 0.8, H_2O); ^{1}H NMR (D_2O, 310 K) δ 2.029 (s, 3H, NAc), 3.277 (dd, 1H, H-2^c, $J_{2,3}$ = 8.7 Hz), 3.303 (dd, 1H, H-2^a, $J_{2,3}$ = 8.3 Hz), 3.568 (s, 3H, OMe), 3.939 (d, 1H, H-4^e, $J_{3,4}$ = 3.1 Hz), 4.387 (bs, 1H, H-4^b), 4.395 (d, 1H, H-1^a, $J_{1,2}$ = 8.2 Hz), 4.453 (d, 1H, H-1^b, $J_{1,2}$ = 8.1 Hz), 4.477 (d, 1H, H-1^e, $J_{1,2}$ = 7.9 Hz), 4.722 (d, 1H, H-1^d, $J_{1,2}$ = 8.2 Hz), 4.877 (d, 1H, H-1^c, $J_{1,2}$ = 8.0 Hz); ^{13}C NMR (D_2O) δ 23.51 (*C*H_3CON), 56.56 (C-2^d), 58.47 (OMe), 61.28, 61.45, 62.08 (2), 69.82, 71.02, 71.59, 72.24, 73.48, 73.79, 74.02, 74.94, 75.64 (2), 75.93, 76.05, 76.32, 76.63, 76.93, 77.18, 79.56, 79.70, 81.17, 103.36, 104.17,

104.28, 104.33, 104.40 (5 x C-1), 176.09 (CH_3CON); FABMS Calcd for $C_{33}H_{57}NO_{26}Na$ (M+Na): 906.80. Found: 906.80.

Methyl *O*-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-(β-D-galactopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-[β-D-glucopyranosyl-(1→4)-*O*]-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (13). A solution of **12** (4.0 mg, 4.5 μL), CMP-NeuAc (3 mg) in water (0.5 mL) was adjusted to pH 7.5 by 1M sodium cacodylate. To the above solution were added 1% HSA (10 μL), alkaline phosphatase (5 U) and α-(2→3)-sialyltransferase (30 mU) and the pH was adjusted again to 7.5 using 1 M sodium cacodylate solution, and the mixture was kept at rt for 24 h. Additional CMP-NeuAc (3 mg) was added and the mixture was incubated for another 24 h, when TLC (*n*-BuOH/AcOH/H_2O 2:1:1) indicated the reaction was almost complete. The mixture was purified on a column of Bio-gel P-2 (0.02 M acetic acid/pyridine buffer, pH 5.5). The fractions containing major product were lyophilized to give **13** (3.2 mg, 59%) as an amorphous solid: $[\alpha]_D$ -4.0° (*c* 0.3, H_2O); ^1H NMR (D_2O, 310 K) δ 1.794 (t, 1H, H-3a^f, $J_{3a,4} = J_{3a,3e} = 12.1$ Hz), 2.027 and 2.032 (2s, 3H each, 2 x NAc), 2.767 (dd, 1H, H-3e^f, $J_{3e,4} = 4.5$ Hz, $J_{3a,3e} = 12.1$ Hz), 3.267 (dd, 1H, H-2^c, $J_{2,3} = 9.3$ Hz), 3.283 (dd, 1H, H-2^a, $J_{2,3} = 8.3$ Hz), 3.569 (s, 3H, OMe), 4.103 (dd, 1H, H-3^e, $J_{3,4} = 2.8$ Hz, $J_{2,3} = 9.8$ Hz), 4.388 (bs, 1H, H-4^b), 4.396 (d, 1H, H-1^a, $J_{1,2} = 7.9$ Hz), 4.455 (d, 1H, H-1^b, $J_{1,2} = 7.8$ Hz), 4.551 (d, 1H, H-1^e, $J_{1,2} = 7.8$ Hz), 4.713 (d, 1H, H-1^d, $J_{1,2} = 8.2$ Hz), 4.886 (d, 1H, H-1^c, $J_{1,2} = 8.0$ Hz); ^{13}C NMR (D_2O) δ 22.87, 23.08 (2 x CH_3CON), 40.50 (C-3^f), 52.54 (C-5^f), 56.13 (C-2^d), 58.04 (OMe), 60.87, 61.04, 61.64, 61.86, 63.48, 68.34, 68.97, 69.17, 70.22, 70.62, 71.16, 72.62, 73.01, 73.59, 73.75, 74.53, 75.23, 75.52, 75.63, 75.88, 76.04, 76.37, 76.50, 76.77, 79.17, 82.78, 100.67 (C-2^f), 102.91, 103.46, 103.85, 103.91, 104.03 (5 x C-1), 175.66, 175.89 (2 x CH_3CON); ESMS Calcd for $C_{44}H_{73}N_2O_{34}Na$: 1197.1 [M]. Found: 1197.3 $[M]^+$ (positive mode); 1174.0 $[M-Na]^-$ (negative mode).

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REFERENCES

1. C. J. Baker and M. S. Edwards, in J. S. Remington and J. O. Klein (ed.), *Infectious Diseases of the Fetus and Newborn Infant.* The W. B. Saunders Co., Philadelphia, 1990, p742.
2. H. J. Jennings, E. Katzenellenbogen, C. Lugowski, and D. L. Kasper, *Biochemistry*, **22**, 1258 (1983).
3. R. C. Lancefield, *J. Exp. Med.*, **67**, 25 (1938).
4. M. R. Wessels, L. C. Paoletti, A. K. Rodewald, F. Michon, J. DiFabio, H. J. Jennings, and D. L. Kasper, *Infection and Immunity*, **61**, 4760 (1993).
5. H. J. Jennings, E. Katzenellenbogen, C. Lugowski, F. Michon, R. Roy, and D. L. Kasper, *Pure Appl. Chem.*, **56**, 893 (1984), and references cited therein.
6. A. Maranduba and A. Veyrieres, *Carbohydr. Res.*, **151**, 105 (1986).
7. P. J. Garegg, H. Hultberg, and S. Wallin, *Carbohydr. Res.*, **108**, 97 (1982).
8. G. Ekborg, T. Curenton, N. Rama Krishna, and L. Roden, *J. Carbohydr. Chem.*, **9**, 15 (1990).
9. K. K. Sadozai, T. Nukada, Y. Ito, Y. Nakahara, T. Ogawa, and A. Kobata, *Carbohydr. Res.*, **157**, 101 (1986).
10. G. Grundler and R. R. Schmidt, *Carbohydr. Res.*, **135**, 203 (1985).
11. H. Ammann and G. Dupius, *Can J. Chem.*, **66**, 1651 (1988).
12. W. Zou, Jean-Robert Brisson, Qing-Ling Yang, Mark van der Zwan, and H. J. Jennings, *To be published.*
13. T. Murase, H. Ishida, M.Kiso, and A. Hasegawa, *Carbohydr. Res.*, **184**, C1 (1988); **188**, 71(1989); K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori, and K. Stenvall, *J. Org. Chem.*, **53**, 5629 (1988).
14. S. Sato, Y. Ito, T. Nukada, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, **167**, 197 (1987).
15. B. Wegman and R. R. Schmidt, *J. Carbohydr. Chem.*, **6**, 357 (1987); R. R Schmidt, J. Michel, and M. Roos, *Liebigs Ann. Chem.*, 1343 (1984); R. R. Schmidt and M. Stumpp, *Liebigs Ann. Chem.*, 1249 (1983).
16. C. Unverzagt, H. Kunz, J. C. Paulson, *J. Am. Chem. Soc.*, **112**, 9308 (1990).
17. R. L. Halcomb, H. Huang, and C.-H. Wong, *J. Am. Chem. Soc.*, **116**, 11315 (1994).