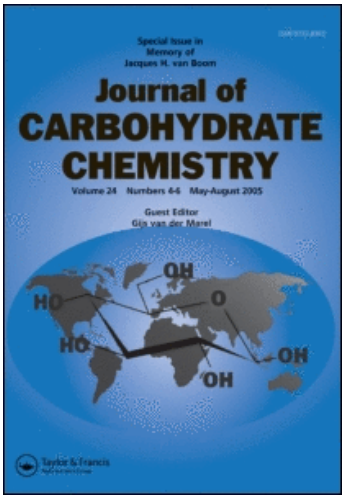

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Block Synthesis of *Streptococcus pneumoniae* Type 14 Capsular Polysaccharide Structures

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Block Synthesis of *Streptococcus pneumoniae* Type 14 Capsular Polysaccharide Structures

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Synthesis of a thioglycoside tetrasaccharide block, β-D-Gal*p*-(1 → 4)-β-DGlc*p*-(1 → 6)-[β-D-Gal*p*-(1 → 4)]-β-D-GlcNPhth*p*-(1 → SEt, corresponding to the repeating unit of *Streptococcus pneumoniae* serotype 14 CPS is described. Coupling of this block with a spacer followed by removal of an isopropylidene acetal yielded an acceptor, which was elongated with the donor block to give a protected dimer of the repeating unit. Iteration of this methodology yielded the trimer. Deprotection then produced an octa and a dodecasaccharide derivative ready for conjugation to proteins to afford immunoactive glycoconjugates.

Keywords Oligosaccharide synthesis, Block synthesis, Glycoconjugate vaccines

INTRODUCTION

The bacterium *Streptococcus pneumoniae* is a major human pathogen.[1] The bacterium is divided into serotypes, each serotype corresponding to a unique structure of the capsular polysaccharide (CPS) surrounding the bacteria.[2]

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In memory of Professor Jacques H. van Boom.
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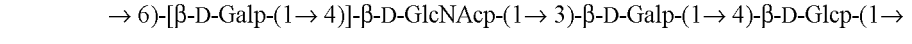


Figure 1: Repeating units of *S. pneumoniae* type 14 CPS.

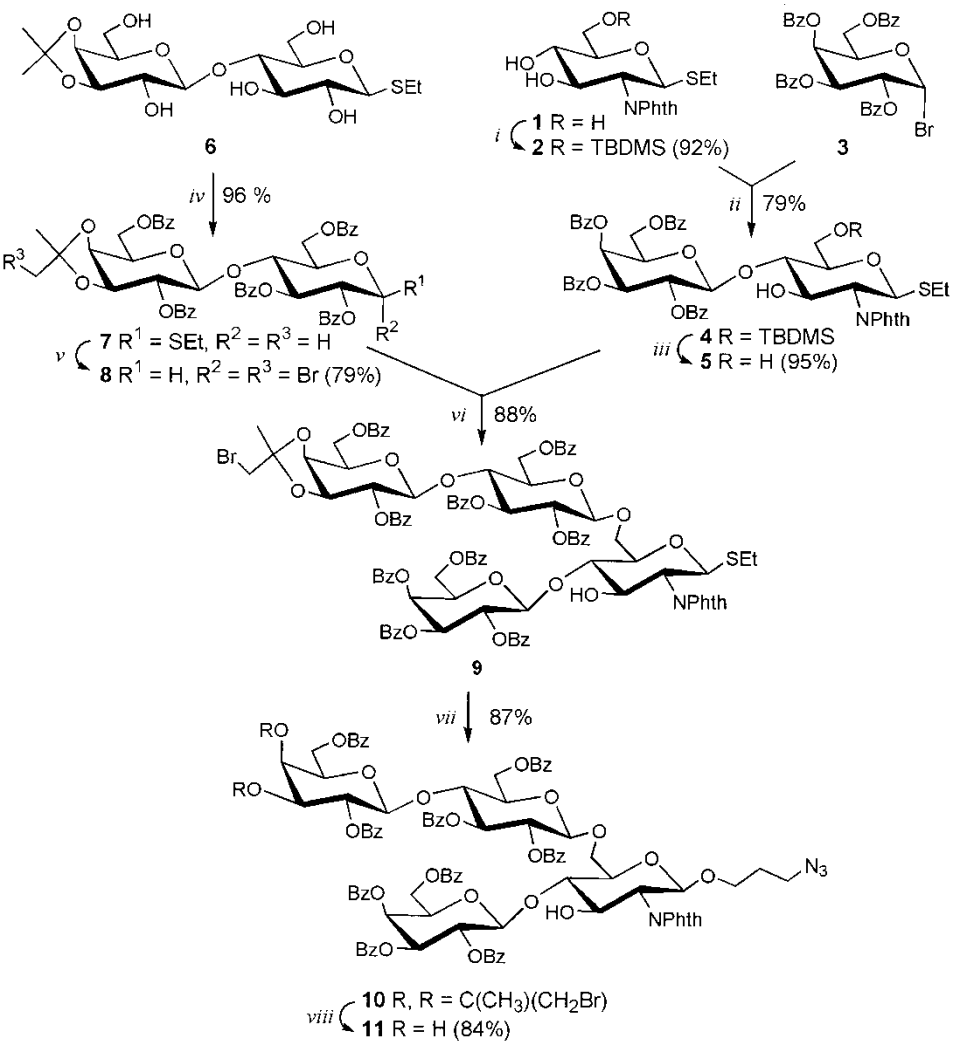
Serotype 14 is one of the most abundant serotypes with a tetrasaccharide repeating unit corresponding to a branched Lacto-*N*-tetraose structure (Fig. 1).[3]

Both polysaccharide and glycoconjugate vaccines against *S. pneumoniae* are now commercial. The polysaccharide vaccine comprises 23 serotypes, whereas the conjugate vaccine contains seven serotypes, both including serotype 14. Regarding polysaccharide vaccines, there is a consensus that large structures are needed, but with glycoconjugate vaccines there is a debate on the saccharide size needed to give protection.[4–6] To further investigate this, Vliegenthart and coworkers have synthesised type 14 structures up to octasaccharides and investigated the immunology of some of their protein conjugates.[7–9] Also, in Jennings' syntheses of *Streptococcus* Type III Group B structures (which is a 3-*O*-sialylated variant, →6)-[α-NeuAc*p*-(2 → 3)-β-D-Gal*p*-(1 → 4)]-β-D-GlcNAc*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1→, of type 14,[10]), up to dimeric structures (octasaccharide) of the type 14 CPS have been prepared.[11] In addition, this group has made a thorough interpretation of the NMR spectra of both type 14 and Type III Group B oligo- and polysaccharides.[12] In the '80s Kochetkov and coworkers performed polycondensation of a tritylated tetrasaccharide cyanoorthoester block (β-D-Gal*p*-(1 → 4)-β-D-GlcNAc*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-) to obtain mixtures of polysaccharides with an average degree of polymerization of about 10.[13,14]

We now report the synthesis of a tetrasaccharide block donor comprising the type 14 repeating unit and its efficient use in the synthesis of spacer-containing dimer (octasaccharide) and trimer (dodecasaccharide) derivatives, to be used in conjugate formation and immunological studies. The synthetic approach allows further elongation either with a new tetrasaccharide block or smaller fragments to obtain derivatives with different nonreducing end groups.

RESULTS AND DISCUSSION

Our strategy involves the use of the branched tetrasaccharide β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 6)-[β-D-Gal*p*-(1 → 4)]-β-D-GlcNPhth*p*-(1 → SEt (**9**) as the target building block. Regioselective silylation of compound **1**[15] (→**2**) followed by regioselective galactosylation afforded disaccharide acceptor **4**, which after desilylation (→**5**) was regioselectively glycosylated at the primary position with donor bromide **8**, using silver triflate promotion, to yield the desired tetrasaccharide thioglycoside building block **9** in high yield



Scheme 1: (i) TBDMSCl, pyridine, 0°C; (ii) AgOTf, CH_2Cl_2, −55°C; (iii) $Et_3N(HF)_3$, CH_2Cl_2; (iv) BzCl, pyridine, 0°C; (v) Br_2, CH_2Cl_2, 0°C; (vi) AgOTf, CH_2Cl_2, −40°C; (vii) $HO(CH_2)_3N_3$, NIS, AgOTf, CH_2Cl_2, −25°C; (viii) TFA (90% aqueous).

(61% from **1** over four steps, Sch. 1). Bromide **8** was obtained from the corresponding thioglycoside **7**. One advantage of thioglycosides is their easy transformation into other type of donors under mild conditions. Thus, even instable bromo sugars, for example, containing acid-labile groups, can be synthesized. When derivative **7** was treated with bromine in CH_2Cl_2, the bromo sugar was efficiently formed and the acetal was not cleaved off. However, one of the methyl groups of the isopropylidene acetal was concomitantly monobrominated to give a mixture of diastereomers (ratio ~3 : 1). There is precedence in

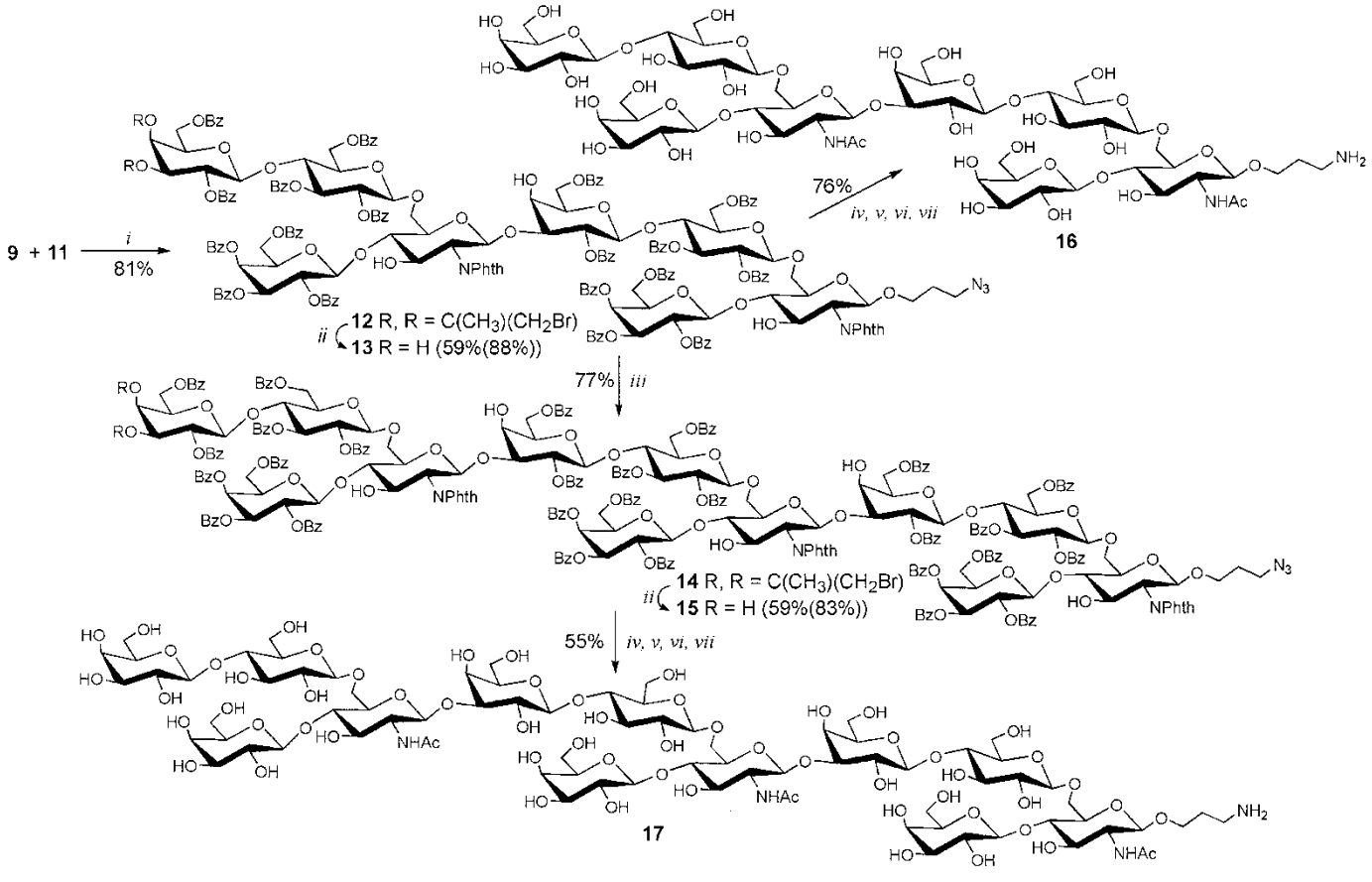
the literature of similar reactions,[16] and since it did not interfere with the synthetic plans the strategy was not altered.

With the desired building block in hand, the assembly of oligomers could start. First, a spacer-containing acceptor was synthesized by coupling 3-azidopropanol[17] to compound **9** using NIS/AgOTf as promotor to yield spacer glycoside **10** (87%), from which the bromoisopropylidene group was removed by acid hydrolysis to afford the $3,3^{IV},4^{IV}$-triol acceptor **11** (84%, Sch. 1). NIS-promoted couplings (together with a catalytic amount of either TfOH or AgOTf) between **11** and thioglycoside donor block **9** were attempted, but the regioselectivity was low and a mixture of octasaccharides was obtained. This problem was solved by transforming the thioglycoside **9** into the corresponding bromide and use of this donor in a silver triflate-promoted glycosylation.[18] Excellent regioselectivity was observed and the desired octasaccharide **12** was obtained in 81% yield (Sch. 2). The formed $(1 \rightarrow 3)$-linkage was proven by NMR. Proton H-4^{IV} could be easily identified by its shift and coupling constant, thus revealing the H-3^{IV} (3.52 ppm) from a COSY experiment. H-1^{V} was found to have a significantly stronger NOE to the H-3^{IV} as compared to H-4^{IV}. Further proof for the identity of all synthesized derivatives was obtained from the excellent agreement of the ^{1}H NMR data of the deprotected target structures with published data of the native polysaccharide and other synthesized type 14 oligosaccharides.[7,8,12]

The synthetic procedure was iterated to yield the trimer. A minor problem was encountered in the removal of the isopropylidene group, which did not go to completion. Hence, the reaction was worked up after 4 hr to afford the dimer acceptor **13** (59%, 88% calculated on consumed starting material) together with starting material **12** (33%), which were easily separated on a silica gel column. Formation of a trimer using the same coupling conditions, that is, the bromo sugar of donor **9** and silver triflate promotion, afforded the dodecasaccharide **14** in 77% yield (Sch. 2). Once more, acidic removal of the isopropylidene acetal was sluggish, yielding 59% of **15** (83% calculated on consumed starting material), which again included the possibility of elongation with different donors and the formation of larger structures.

Deprotection of the dimer **13** and the trimer **15** was performed in three steps (Sch. 2). Debenzoylation under Zemplén conditions was followed by removal of the phthalimido groups using ethylenediamine in EtOH. Finally, N-acetylation with acetic anhydride in MeOH afforded the azido-spacer derivatives in 81% and 73% yield, respectively. The obtained azido derivatives can be used directly in conjugations with propargyl-activated proteins.[19] To be able to use other conjugation methodologies, reduction of the azido groups was accomplished through catalytic hydrogenolysis to yield the amino derivatives **16** and **17**, respectively.

In conclusion, an effective block synthesis of *S. pneumoniae* type 14 CPS structures has been worked out. Gram quantities of the building blocks and



Scheme 2: (i) a. Br_2, CH_2Cl_2, 0°C; b. AgOTf, CH_2Cl_2, −40°C; (ii) TFA (90% aqueous); (iii) a. **9** Br_2, CH_2Cl_2, 0°C; b. AgOTf, CH_2Cl_2, −40°C; (iv) NaOMe, MeOH; (v) Et_2N, EtOH, reflux; (vi) Ac_2O, MeOH; (vii) H_2, Pd/C, HCl, MeOH.

the protected dimers and trimers have been synthesized; further, their high-yielding transformation into target structures ready for conjugation to proteins is reported.

EXPERIMENTAL

General Methods

Organic solutions were dried over $MgSO_4$ or Na_2SO_4 before concentration, which was performed under reduced pressure at $<45^{\circ}C$ (bath temperature). NMR spectra were recorded at 25°C and 400 MHz (^1H) in $CDCl_3$ with Me_4Si as internal standard ($\delta = 0.00$) or 75 or 100 (^{13}C) MHz in $CDCl_3$ with $CDCl_3$ as internal standard ($\delta = 77.16$). TLC was performed on silica gel 60 F254 with detection by charring with 8% sulfuric acid or ninhydrin. Silica gel (0.040–0.063 mm) was used for column chromatography.

Ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (5). Pyridine (9 mL) and TBDMS-Cl (9.35 g, 57.0 mmol) were added to a solution of ethyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[15] (**1**, 18.27 g, 51.8 mmol) in CH_2Cl_2 (140 mL) at 0°C. The reaction was stirred at rt over night. MeOH (30 mL) was added and the mixture was concentrated and purified by silica gel column chromatography (toluene-EtOAc 2 : 1) to give ethyl 6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**2**, 28.16 g, 47.7 mmol, 92%). $[\alpha]_D + 7.7^{\circ}$ (*c* 0.9, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ −5.2, −5.2 ($SiCH_3$), 15.1 (SCH_2CH_3), 18.4 ($C(CH_3)_3$), 24.1 (SCH_2CH_3), 26.1 ($C(CH_3)_3$), 55.5, 64.9, 72.7, 74.6, 78.4 (C-2-6), 81.1 (C-1), 123.5–134.3 (aromatic C). Compound **2** (4.22 g, 7.14 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (**3**, 4.75 g, 7.24 mmol) were dried together under vacuum and then dissolved in CH_2Cl_2 (90 mL) under an argon atmosphere. 4 Å molecular sieves were added and the reaction mixture was cooled to −55°C. After stirring for 25 min, AgOTf (2.80 g, 10.9 mmol) was added and the reaction mixture was stirred for another 45 min before triethylamine (5.5 mL) was added. After filtration through Celite and concentration of the filtrate, the residue was purified by silica gel column chromatography (toluene - EtOAc 5 : 1) to give ethyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4**, 6.60 g, 5.64 mmol, 79%). $[\alpha]_D + 79^{\circ}$ (*c* 0.5, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ −5.3. −5.1 ($SiCH_3$), 14.9 (SCH_2CH_3), 18.3 ($C(CH_3)_3$), 23.4 (SCH_2CH3), 25.9 ($C(CH_3)_3$), 55.4, 61.6, 62.5, 68.1, 69.7, 70.6, 71.8, 72.4, 78.7, 80.5 (C-2^I-6^I, 2^{II}-6^{II}), 81.6 (C-1^I), 101.9 (C-1^{II}), 123.3–137.9 (aromatic C), 165.1–166.2 (C=O, Bz), 167.8, 168.3 (C=O, Phth).

Compound **4** (6.60 g, 5.64 mmol) was dissolved in CH_2Cl_2 (60 mL) and $Et_3N \cdot (HF)_3$ (1.0 mL) was added. After stirring for 6 d, the reaction mixture

was diluted with toluene, washed with water, dried, concentrated, and purified by silica gel chromatography (toluene-EtOAc 2 : 1) to give **5** (4.98 g, 5.35 mmol, 95%). ^{13}C NMR ($CDCl_3$): δ 15.0, 24.4 (SCH_2CH_3), 55.2, 60.9, 62.8, 68.2, 69.7, 70.7, 71.7, 72.5, 78.2, 81.5 (C-2^{I}-6^{I}, 2^{II}-6^{II}), 82.2 (C-1^{I}), 102.3 (C-1^{II}), 123.4–134.2 (aromatic C), 165.3–166.2 (C═O, Bz), 167.9, 168.4 (C═O, Phth).

[2,6-Di-*O*-benzoyl-3,4-*O*-(1-bromomethylethylidene)-β-D-galactopyranosyl]-(1 → 4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl bromide (8). Benzoyl chloride (45 mL) was added to a solution of ethyl (3,4-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-1-thio-β-D-glucopyranoside[20] (**6**, 22.30 g, 52.7 mmol) in pyridine (110 mL) at 0°C. After stirring for 40 min, ice (50 mL) and toluene (200 mL) were added and the mixture was stirred for another 60 min. The organic phase was washed with water and $NaHCO_3$ solution (sat.), dried, concentrated, and purified by silica gel chromatography (toluene-EtOAc 6 : 1) to produce ethyl [2,6-di-*O*-benzoyl-3,4-*O*-(1-methylethylidene)-β-D-galactopyranosyl]-(1 → 4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (**7**, 47.86 g, 50.6 mmol, 96 %). $[\alpha]_D + 39°$ (*c* 1.1, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 15.3, 24.8 (SCH_2CH_3), 26.6, 27.8 (C(CH_3)$_2$), 63.2, 63.3, 71.1, 71.7, 73.6, 74.1, 74.2, 75.7, 77.5, 77.8 (C-2^{I}-6^{I}, 2^{II}-6^{II}), 84.1 (C-1^{I}), 100.5 (C-1^{II}), 111.3 (C(CH_3)$_2$), 128.5–133.8 (aromatic C), 165.3–166.4 (C═O, Bz). Compound **7** (8.56 g, 9.04 mmol) was dissolved in CH_2Cl_2 (55 mL) and bromine (1.7 mL, 33 mmol) was added at 0°C. The reaction was stirred for 80 min and then quenched by the addition of cyclohexene (1.5 mL) and triethylamine (2 mL). The solution was concentrated, coevaporated with toluene, and purified by silica gel column chromatography (pentane-toluene 3:1 to 1 : 10) to give **8** (7.42 g, 7.12 mmol, 79%). ^{13}C NMR ($CDCl_3$): δ 24.5 (C(CH_3)(CH_2Br)), 37.2 (C(CH_3)(CH_2Br)), 61.8, 62.7, 70.3, 71.2, 71.6, 73.2, 73.5, 74.5, 74.6, 78.0 (C-2^{I}-6^{I}, 2^{II}-6^{II}), 86.9 (C-1^{I}), 100.5 (C -1^{II}), 109.4 (C(CH_3)(CH_2Br)), 128.4–133.8 (aromatic C), 165.0–166.0 (C═O, Bz).

Ethyl [2,6-di-*O*-benzoyl-3,4-*O*-(1-bromomethylethylidene)-β-D-galactopyranosyl]-(1 → 4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)]-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (9). A solution of **5** (3.12 g, 3.35 mmol) and **8** (3.36 g, 3.22 mmol) in CH_2Cl_2 (25 mL) containing 4 Å molecular sieves was stirred for 30 min. AgOTf (1.05 g, 4.11 mmol) was added and the mixture stirred for 1 hr at −40°C. Triethylamine was added and the mixture filtered through Celite, concentrated, and dried under reduced pressure. The residue was purified by silica gel column chromatography (toluene-EtOAc 5 : 1) to give **9** (5.36 g, 2.83 mmol, 88%). ^{13}C NMR ($CDCl_3$): δ 14.6, 23.6 (SCH_2CH_3), 24.4 (C(CH_3)(CH_2Br)), 36.9 (C(CH_3)(CH_2Br)), 54.7, 62.3, 62.5, 62.6, 67.3, 67.8, 69.9, 70.8, 71.1, 71.1, 71.6, 71.9, 72.4, 72.5, 73.3, 74.6, 75.2, 76.5, 77.9, 81.0 (C-$2^{I\text{-}IV}$-$6^{I\text{-}IV}$), 82.6, 100.4, 100.8, 101.6 (C-$1^{I\text{-}IV}$), 109.4 (C(CH_3)(CH_2Br)), 123.0–137.7 (aromatic C), 164.6–165.9 (C═O, Bz and Phth).

3-Azidopropyl (2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)]-2-deoxy-2-phthalimido-β-D-glucopyranoside (11). A solution of **9** (742 mg, 392 μmol) and 3-azidopropanol[17] (104 mg, 1.03 mmol) in CH_2Cl_2 (10 mL) containing 4 Å molecular sieves was stirred for 30 min. The mixture was then cooled to −35°C, NIS (140 mg, 622 μmol) and a catalytic amount of AgOTf were added, and stirring was continued for another 25 min at −25°C. Triethylamine was added and the mixture filtered through Celite and concentrated. The residue was purified by silica gel column chromatography (toluene-EtOAc 3 : 1) to yield 3-azidopropyl [2,6-di-*O*-benzoyl-3,4-*O*-(1-bromomethylethylidene)-β-D-galactopyranosyl]-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)]-2-deoxy-2-phthalimido-β-D-glucopyranoside (**10**, 659 mg, 341μmol, 87%). ^{13}C NMR ($CDCl_3$): δ 24.6 (C(*C*H_3)(CH_2Br)), 28.7 ($OCH_2CH_2CH_2N_3$), 37.0 (C(CH_3)(*C*H_2Br)), 48.0 ($OCH_2CH_2CH_2N_3$), 55.6, 62.3, 62.6, 66.1, 67.2, 67.8, 69.8, 70.0, 71.1, 71.3, 71.6, 71.9, 72.4, 72.6, 72.6, 73.4, 74.7, 75.3, 78.0, 82.7 (C-$2^{I\text{-}IV}$-$6^{I\text{-}IV}$, O*C*$H_2CH_2CH_2N_3$), 98.5, 100.5, 100.9, 101.7 (C -$1^{I\text{-}IV}$), 109.5 (*C*(CH_3)(CH_2Br)), 125.3–137.9 (aromatic C), 164.6–166.0 (C═O, Bz and Phth). Compound **10** (1.603 g, 829 μmol) was dissolved in 90% TFA (20 mL), stirred for 40 min, concentrated, and purified by silica gel column chromatography (toluene-EtOAc 3 : 1) to give **11** (1.263 g, 696 μmol, 84%). $[\alpha]_D$ +57° (*c* 0.7, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 28.8 ($OCH_2CH_2CH_2N_3$), 48.1 ($OCH_2CH_2CH_2N_3$), 55.7, 62.0, 62.5, 62.6, 66.2, 67.2, 67.9, 68.6, 69.9, 70.1, 71.2, 71.7, 72.0, 72.4, 72.7, 72.7, 72.8, 72.8, 73.8, 75.8, 82.7 (C-$2^{I\text{-}IV}$-$6^{I\text{-}IV}$, O*C*$H_2CH_2CH_2N_3$), 98.6, 100.9, 101.1, 101.8 (C-$1^{I\text{-}IV}$), 123.4–138.0 (aromatic C), 164.8–166.4 (C═O Bz, Phth).

3-Azidopropyl (2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*benzoyl-β-D-glucopyranosyl)-(1 → 6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)]-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)]-2-deoxy-2-phthalimido-β-D-glucopyranoside (13). Bromine (0.20 mL, 4.0 mmol) was added to a solution of **9** (1.061 g, 561 μmol) in CH_2Cl_2 (10 mL) at 0°C. After stirring for 90 min at 0°C, the reaction mixture was concentrated, coevaporated with toluene, and dried under vacuum. The residue was redissolved in CH_2Cl_2 (20 mL) containing 4 Å molecular sieves together with **11** (910 mg, 501 μmol), and the mixture was stirred for 30 min at rt. The mixture was cooled to −40°C and AgOTf (285 mg, 1.117 mmol) was added. Triethylamine was added after 25 min and the mixture was filtered through Celite and concentrated. The residue was purified by silica gel column chromatography (toluene -EtOAc 6 : 1) to produce 3-azidopropyl [2,6-di-*O*-benzoyl-3,4-*O*-(1-bromomethylethylidene)-β-D-galactopyranosyl]-(1→4)-

(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)]-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)]-2-deoxy-2-phthalimido-β-D-glucopyranoside (**12**, 1.480 g, 406 μmol, 81%). ^{13}C NMR ($CDCl_3$): δ 24.7 (C(*C*H_3)(CH_2Br)), 28.8 ($OCH_2CH_2CH_2N_3$), 37.1 (C(CH_3)(*C*H_2Br)), 48.1 ($OCH_2CH_2CH_2N_3$), 53.6, 55.2, 55.6, 61.7, 62.3, 62.4, 62.6, 64.1, 66.1, 67.1, 67.9, 67.9, 68.8, 69.4, 69.7, 69.8, 70.0, 70.8, 70.9, 71.1, 71.2, 71.6, 71.9, 72.1, 72.2, 72.6, 72.8, 72.9, 73.2, 73.4, 74.6, 75.1, 75.7, 78.0, 80.9, 82.6, 83.3 (C-2$^{I\text{-}VIII}$-6$^{I\text{-}VIII}$, O*C*$H_2CH_2CH_2N_3$), 98.5, 98.6, 100.8, 100.8, 101.6, 101.7, 101.8 (C-1$^{I\text{-}VIII}$), 109.5 (*C*(CH_3)(CH_2Br)),128.4–134.9 (aromatic C), 164.0–166.2 (C═O, Bz and Phth). A solution of compound **12** (1.202 g, 330 μmol) in TFA (25 mL, 85% aqueous) was stirred for 3.5 hr, then concentrated, coevaporated with toluene, and purified by silica gel column chromatography (toluene-EtOAc 4 : 1) to give **13** (687 mg, 195 μmol, 59%) and unreacted **12** (397 mg, 109 μmol, 33%). $[\alpha]_D + 62^\circ$ (*c* 1.1, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 28.8 ($OCH_2CH_2CH_2N_3$), 48.1 ($OCH_2CH_2CH_2N_3$), 55.1, 55.6, 61.7, 62.5, 62.7, 64.3, 66.1, 67.1, 67.9, 68.0, 68.5, 69.2, 69.5, 69.7, 69.9, 70.0, 70.8, 71.1, 71.2, 71.6, 72.0, 72.2, 72.2, 72.4, 72.6, 72.8, 73.0, 73.1, 73.2, 73.7, 75.1, 76.0, 80.9, 82.6, 83.6 (C-2$^{I\text{-}VIII}$-6$^{I\text{-}VIII}$, O*C*$H_2CH_2CH_2N_3$), 98.5, 100.8, 101.4, 101.7, 101.8, 101.8 (C -1$^{I\text{-}VIII}$), 128.3–137.9 (aromatic C), 164.0–166.3 (C═O, Bz and Phth). Anal. calcd. for $C_{193}H_{165}N_5O_{61}$: C, 65.66; H, 4.71; N, 1.98. Found: C, 65.53; H, 4.74; N, 1.86.

3-Azidopropyl [2,6-di-*O*-benzoyl-β-D-galactopyranosyl]-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)]-(2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)]-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)]-2-deoxy-2-phthalimido-β-D-glucopyranoside (15). Bromine (0.10 mL, 2.0 mmol) was added to a solution of compound **9** (303 mg, 160 μmol) in CH_2Cl_2 (5 mL) and the reaction mixture was stirred at 0°C for 90 min, then concentrated, coevaporated with toluene, and dried under vacuum. The residue was dissolved in CH_2Cl_2 (8 mL) followed by the addition of **13** (450 mg, 128 μmol) and 4 Å molecular sieves and stirred for 50 min at rt. AgOTf (85 mg, 333 μmol) was added and the mixture was stirred for 30 min at −40°C. Triethylamine was added and the mixture was filtered through Celite and concentrated. The residue was purified by silica gel chromatography (toluene-EtOAc 6 : 1) to produce 3-azidopropyl [2,6-di-*O*-benzoyl-3,4-*O*-(1-bromomethylethylidene)-β-D-galactopyranosyl]-(1 → 4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 6)-[(2,

3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)]-(2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(2,3, 6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1 → 6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)]-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-*O*-benzoyl-β-D-galactopyranosyl)-(1 → 4)-(2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-(1→6)-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)]-2-deoxy-2-phthalimido-*b*-D-glucopyranoside (**14**, 531 mg, 99 μmol, 77%). ^{13}C NMR ($CDCl_3$): δ 24.7 (C(*C*H_3)(CH_2Br)), 28.8 ($OCH_2CH_2CH_2N_3$), 37.1 (C(CH_3)(*C*H_2Br)), 48.1 ($OCH_2CH_2CH_2N_3$), 55.2, 55.6, 62.4, 62.7, 64.0, 66.1, 67.1, 67.7, 67.8, 67.9, 68.0, 69.4, 69.5, 69.5, 69.6, 69.6, 69.7, 69.9, 70.0, 70.3, 70.9, 71.1, 71.2, 71.3, 71.4, 71.6, 71.7, 71.8, 71.8, 71.9, 72.1, 72.2, 72.3, 72.4, 72.4, 72.5, 72.6, 72.7, 72.8, 72.9, 73.0, 73.1, 73.2, 73.4, 73.4, 78.1, 80.6, 81.0, 81.1, 82.5, 82.5, 82.6, 82.7, 82.9, 83.1 (C-2$^{I\text{-}XII}$-6$^{I\text{-}XII}$, O*C*$H_2CH_2CH_2N_3$), 100.8, 101.0, 101.1, 101.1, 101.2, 101.3, 101.4, 101.5, 101.7, 101.7, 102.0 (C-1$^{I\text{-}XII}$), 128.0–135.0 (aromatic C), 164.0–168.2 (C=O, Bz and Phth). Compound **14** (490 mg, 91 μmol) was dissolved in TFA (20 mL, 85% aqueous). The mixture was stirred for 4 hr, then evaporated, coevaporated with toluene, and purified by silica gel column chromatography (toluene-EtOAc 4 : 1) to give **15** (281 mg, 54 μmol, 59%) and unreacted **14** (142 mg, 26 μmol, 29%). $[\alpha]_D + 72^\circ$ (*c* 3, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 28.8 ($OCH_2CH_2CH_2N_3$), 48.1 ($OCH_2CH_2CH_2N_3$), 55.2, 55.6, 61.5, 61.7, 62.0, 62.3, 62.6, 63.9, 63.9, 66.1, 67.0, 67.6, 67.8, 67.8, 67.9, 68.0, 68.3, 68.9, 69.4, 69.6, 69.7, 69.7, 69.8, 70.0, 70.8, 71.1, 71.2, 71.6, 71.7, 71.9, 72.1, 72.2, 72.4, 72.4, 72.6, 72.7, 72.8, 72.9, 72.9, 73.0, 73.2, 75.0, 75.2, 75.8, 77.7, 80.6, 81.0, 82.6, 83.4, (C-2$^{I\text{-}XII}$-6$^{I\text{-}XII}$, O*C*$H_2CH_2CH_2N_3$), 98.5, 98.9, 100.8, 101.0, 101.2, 101.3, 101.6, 101.7, 101.7, 101.8 (C-1$^{I\text{-}XII}$), 128.3–135.0 (aromatic C), 164.0–168.2 (C=O, Bz and Phth). Anal. calcd. for $C_{288}H_{244}N_6O_{91}$: C, 65.95; H, 4.69; N, 1.60. Found: C, 65.78; H, 4.60; N, 1.48.

3-Aminopropyl (β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-[(β-D-galactopyranosyl)-(1→4)]-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-[(β-D-galactopyranosyl)-(1→4)]-2-deoxy-2-acetamido-β-D-glucopyranoside (16). Derivative **13** (36 mg, 10 μmol) was dissolved in MeOH (6 mL) and 1 M NaOMe (100 μL in MeOH) was added. The solution was stirred overnight, neutralized with dry ice, and concentrated. The residue was passed through a reversed phase column (H_2O), concentrated, dissolved in EtOH (10 mL) and ethylenediamine (1 mL), and refluxed for 4 d. The reaction mixture was concentrated, and the formed crude product filtered through a reversed phase column (H_2O) and then purified on a Bio -Gel P-2 column (1% 1-butanol in H_2O). The combined carbohydrate -containing fractions were concentrated and the residue was dissolved in MeOH (5 mL) and acetic anhydride (50 μL) was added. After stirring for 10 min the solution was concentrated, coevaporated

with MeOH and toluene, purified on a Bio-Gel P-2 column (1% 1-butanol in H_2O), and concentrated. Pd/C (catalytic amount) and 1 M HCl (40 μL) were added to the product from the last step dissolved in MeOH (1.5 mL), stirred for 15 min under H_2 (1 atm.), filtered through a reversed phase column, concentrated, coevaporated with water, and dried under vacuum to yield **16** (11 mg, 7.6 μmol, 76%). $[\alpha]_D -1.7^\circ$ (*c* 0.8, H_2O). ^1H NMR (D_2O) (selected data): δ 1.93 (t, 2H, CH_2 3-aminopropyl), 2.02, 2.03 (2s, 6H, NHAc), 3.07 (t, 2H), 3.35 (t, 2H), 4.15 (d, 1H, H-4^{IV}), 4.27 (t, 2H, H-6^{I}, 6^{V}), 4.43 (t, 2H, H-1^{IV}, 1^{VIII}), 4.51–4.55 (m, 5H, H-1^{I}, 1^{II}, 1^{III}, 1^{VI}, 1^{VII}), 4.69 (d, 1H, H-1^{V}). MALDI-TOF MS: Calcd. for $C_{55}H_{95}N_3O_{41}$ ($[M + Na]^+$): 1476.5; found 1477.8.

3-aminopropyl (β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-[(β-D-galactopyranosyl)-(1→4)]-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-[(β-D-galactopyranosyl)-(1→4)]-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→6)-[(β-D-galactopyranosyl)-(1→4)]-2-deoxy-2-acetamido-β-D-glucopyranoside (17). Compound **15** (31 mg, 6 μmol) was deprotected as described for compound **13** above to give **17** (7 mg, 3.3 μmol, 55 %). $[\alpha]_D -2.1^\circ$ (*c* 0.3, H_2O). 1H NMR (D_2O) (selected data): δ 1.97 (t, 2H, CH_2 3-aminopropyl), 2.02, 2.02, 2.03 (3s, 9H, NHAc), 4.15 (2d, 2H, H-4^{IV}, 4^{VIII}), 4.26 (2t, 3H, H-6^{I}, 6^{V}, 6^{IX}), 4.43 (2t, 3H, H-1^{IV}, 1^{VIII}, 1^{XII}), 4.51–4.55 (m, 7H, H-1^{I}, 1^{II}, 1^{III}, 1^{VI}, 1^{VII}, 1^{X}, 1^{XI}), 4.69 (2d, 2H, H-1^{V}, 1^{IX}). MALDI-TOF MS: Calcd. for $C_{81}H_{138}N_4O_{61}$ ($[M + Na]^+$): 2165.8; found 2168.3.

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