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Synthesis of oligosaccharides corresponding to *Streptococcus pneumoniae* type 9 capsular polysaccharide structures

Mia Alpe, Stefan Oscarson*

Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

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Abstract

Two trisaccharides, α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p* and α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*, corresponding to structures from *Streptococcus pneumoniae* capsular polysaccharides type 9A, L, V and type 9N, respectively, have been synthesised as 2-aminoethyl glycosides and as protected TMSE glycosides. Ethyl thioglycosides were used as glycosyl donors and NIS/TfOH (in CH_2Cl_2 for β-linkages) and DMTST (in Et_2O for α-linkages) as promoters in the glycosylations. The β-ManNAc motif was introduced at the disaccharide level by azide displacement of a 2-*O*-triflate with β-D-*gluco* configuration. The protecting group patterns allow continued syntheses of larger structures. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Thioglycosides; Oligosaccharide synthesis; Glycoconjugate vaccines

1. Introduction

The bacteria *Streptococcus pneumoniae* is a major human pathogen.[1] The bacteria is divided into serotypes, each serotype corresponding to a unique structure of the capsular polysaccharide (CPS) surrounding the bacteria.[2] Serotype 9 is one of the most abundant serotypes comprising several variations: 9A, 9L, 9N, and 9V. These vary slightly in their CPS structure (Fig. 1).[3–6] 9V is an acetylated version of 9A, but the acetylation is believed to be not immunologically important.[7]

Streptococcus CPSs have been used as a polyvalent vaccine against bacteria for a long time, and recently a seven-valent glycoconjugate vaccine based on bacterial CPS structures has been licensed in North America.[8] Preliminary results for other serotypes indicate that also much shorter parts of the CPS conjugated to a protein, even down to only one or two repeating units, might be enough to obtain a protective immunity against the bacteria.[9,10] To further investigate this we have synthesised trisaccharide parts (residues III–I in Fig. 1) of the pentasaccharide repeating units of serotypes 9A, L and 9N. The trisaccharides are synthesised as spacer glycosides, to allow conjugation to a carrier protein, as well as TMSE glycosides to make transformation into glycosyl donors possible.[11] Also the protection pattern is designed to permit continued synthesis of the complete pentasaccharide units as well as larger structures.

2. Results and discussion

In spite of the importance of serotype 9 only one synthesis of a type 9 part structure has been published, a trisaccharide corresponding to residues II–V in Fig. 1.[12] Since the biological repeating unit is not known, we decided to choose the β-D-glucopyranosyl residue as reducing end. This would allow starting from a common disaccharide precursor as well as choosing the, at least in theory, most simple glycosidic linkage to construct when later attempting planned block synthesis of oligomers of the pentasaccharide repeating units.

The common disaccharide precursor was prepared as outlined in Schemes 1–3. The known azidoethanol glucoside **1**[13] was benzylidenated and benzylated to give the fully protected derivative **3**, in which the azide was reduced and the resulting amine protected as a benzyl carbamate (→**4**, 45% overall yield from **1**). Regioselective reductive opening of the benzylidene acetal[14] then afforded acceptor **5** almost quantitatively (Scheme 1).

* Corresponding author. Fax: 0046-8-154908
E-mail address: s.oscarson@organ.su.se (S. Oscarson).

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→4)–α-D-Glc*p*-(1→4)-α-D-Glc*p*A-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→ **9A**

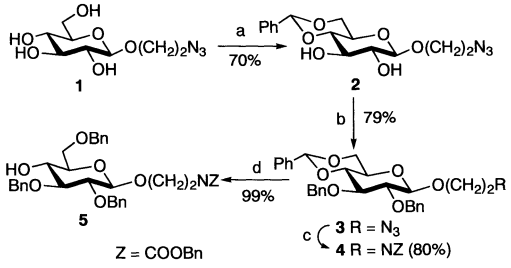
→4)–α-D-Glc*p*NAc-(1→4)-α-D-Glc*p*A-(1→3)-α-D-Gal*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→ **9L**

→4)–α-D-Glc*p*NAc-(1→4)-α-D-Glc*p*A-(1→3)-α-D-Glc*p*-(1→3)-β-D-Man*p*NAc-(1→4)-β-D-Glc*p*-(1→ **9N**

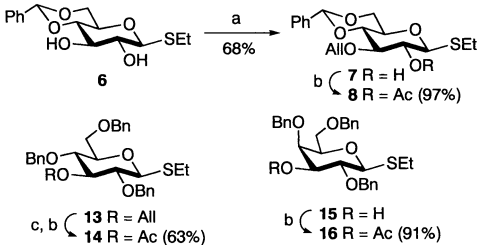
V IV III II I

Fig. 1. Repeating units of *Streptococcus pneumoniae* type 9 CPS.

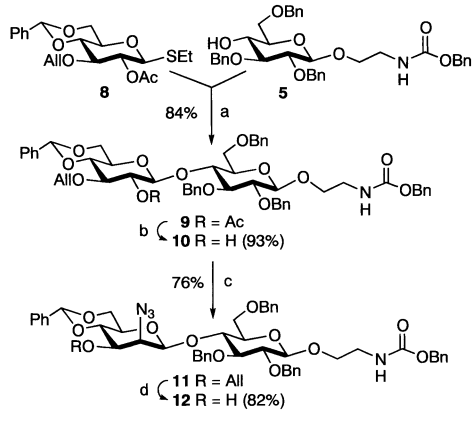
Instead of working with mannosamine-type donors and trying to optimise conditions for β-linkage formation,[12] the other obvious approach, formation of a



Scheme 1. (a) α,α-Dimethoxytoluene, *p*TsOH, DMF; (b) BnBr, NaH, DMF; (c) 1. PPh_3, CH_2Cl_2, 2. H_2O, CH_2Cl_2, reflux, 3. benzylchloroformate, pyridine; (d) $NaCNBH_3$, HCl/Et_2O, THF.



Scheme 2. (a) 1. Bu_2SnO, MeOH, reflux, 2. AllBr, CsF, DMF; (b) Ac_2O, pyridine; (c) 1. $(Ph_3P)_3Rh(I)Cl$, EtOH–toluene–H_2O (6:3:1), 2. $HgBr_2$.



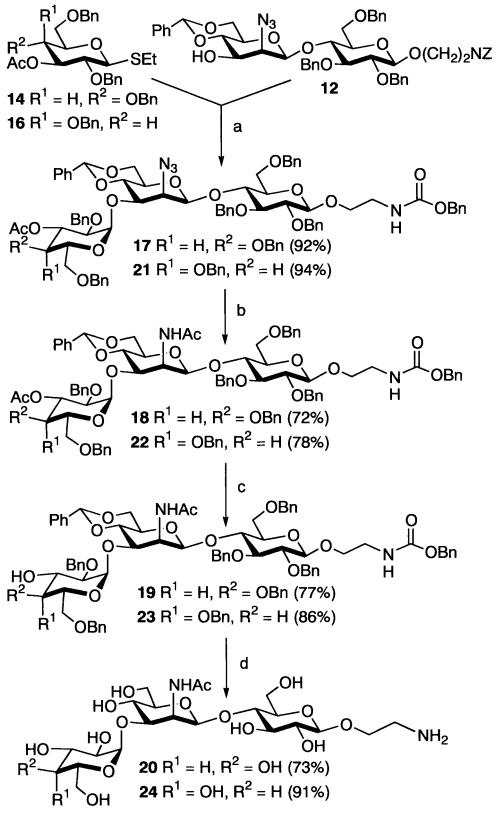
Scheme 3. (a) NIS, TfOH, 4 Å MS, CH_2Cl_2, −30°C; (b) NaOMe, CH_2Cl_2–MeOH; (c) 1. Tf_2O, CH_2Cl_2–pyridine, 0°C, 2. NaN_3, DMF, 70°C; (d) 1. Ir-cat., H_2, THF, 2. NIS, H_2O.

β-glucosidic linkage and consecutive inversion of the configuration at C-2, was employed.[15] Thus, thioglucoside donor **8** was prepared from derivative **6**[16] by regioselective tin-promoted allylation at O-3[17] followed by acetylation (Scheme 2).

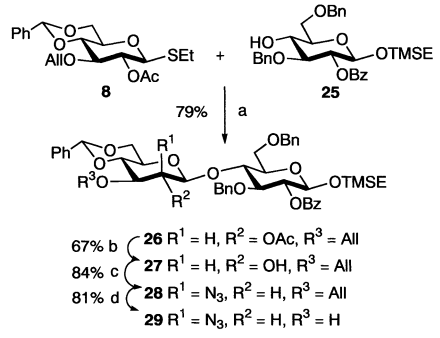
NIS/TfOH-promoted glycosylation in CH_2Cl_2[18,19] of acceptor **5** with donor **8** then gave the β-linked disaccharide **9** (84%), which was transformed into the desired precursor disaccharide acceptor **12**, through deacetylation, triflatation, azide displacement and deallylation (Scheme 3, overall yield 58%).

At this stage the synthesis diverges, to obtain 9A and L structures an α-linked galactose moiety is introduced, whereas coupling with a glucose derivate yields a 9N structure. The 3-*O*-acetyl thioglycoside derivatives **14** and **16** were prepared (Scheme 2) and used in DMTST-promoted couplings in diethyl ether[20,21] with acceptor **12** to produce the α-linked trisaccharides **17** and **21** in excellent yields and stereoselectivity (Scheme 4). Azide reduction followed by acetylation and *O*-deacetylation then gave the two acceptors **19** and **23** ready for further elongation. These derivatives were also fully deprotected by hydrogenolysis to yield the target trisaccharides **20** (73%) and **24** (91%) both with a spacer containing a free amino group for conjugation to a protein to form immunogenic glycoconjugates.

To be able to synthesise larger structures or oligomers of the repeating unit, block donors corresponding to the repeating units or parts of it were necessary. Accordingly, the structures synthesized were assembled not only as spacer glycosides but also as their trimethylsilylethyl (TMSE) glycosides, which can be selectively hydrolysed and transformed into various donors or directly transformed into chloride donors.[11] The only necessary difference in the TMSE-glycosides as compared to the spacer glycosides is the introduction of a participating group at O-2, to ensure β-selectivity in future couplings. The known TMSE-glycoside **25**[22] was coupled with the earlier used donor **8** and under identical conditions to give disaccharide **26**, which was then transformed as discussed for the corresponding spacer derivative **9**, into acceptor **29** (Scheme 5). This time the deacetylation was performed using $Mg(OMe)_2$ instead of NaOMe to avoid concomitant debenzoylation. Since the yield in the chemoselective deacylation



Scheme 4. (a) DMTST, Et_2O; (b) 1. $NaBH_4$, $NiCl_2 \times 6H_2O$, $EtOH–CH_2Cl_2$, 2. Ac_2O; (c) NaOMe, MeOH; (d) H_2, $Pd(OH)_2/C$, AcOH/EtOH.

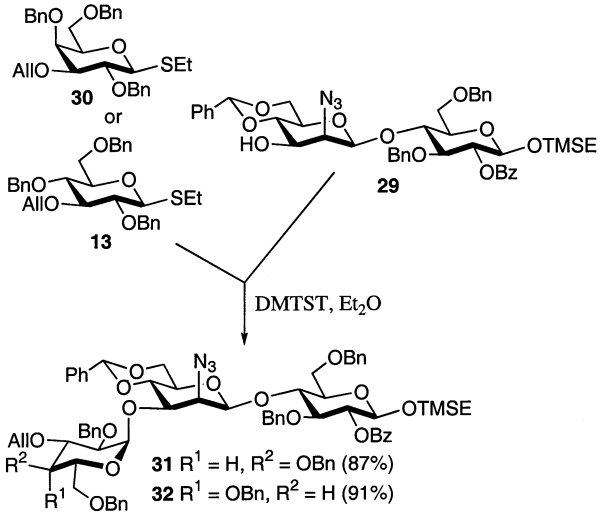


Scheme 5. (a) NIS, TfOH, 4 Å MS, CH_2Cl_2, $-30°C$; (b) $Mg(OMe)_2$, CH_2Cl_2–MeOH; (c) 1. Tf_2O, CH_2Cl_2–pyridine, 0°C, 2. NaN_3, DMF, 70°C; (d) 1. Ir-cat., H_2, THF, 2. NIS, H_2O.

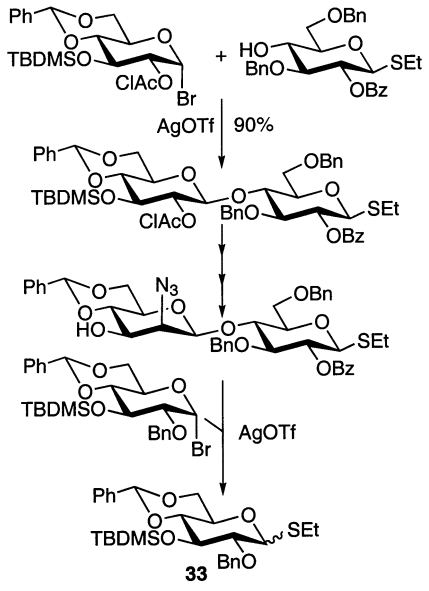
step was not too high (67%) it was decided to use allyl groups instead of acetyl groups as temporary protecting groups in the next donors to ensure orthogonality. Accordingly, the 3-*O*-allyl thioglycosides **13**[23] and **30**[24] were prepared. Once more DMTST-promoted couplings in Et_2O, this time with acceptor **29**, afforded high yields of the α-linked trisaccharides **31** (87%) and **32** (91%) (Scheme 6), both containing the possibility to be elongated at the 3″-position after deallylation as well as at the reducing end after transformation of the TMSE-glycoside.

Originally these trisaccharide donor precursors were designed as thioglycosides, since these can be activated as donors directly.[25] However, in spite of that the first orthogonal coupling to give the corresponding disaccharide proceeded excellent using a bromo sugar donor and silver triflate as promoter (Scheme 7), the next coupling to yield the trisaccharide surprisingly failed, although the same coupling conditions were used. In the latter coupling the thioglycoside acceptor was also activated and the transglycosylated[26] monosaccharide **33** was the main product, showing how even seemingly small differences in donor and acceptor structure can completely change the outcome of a glycosylation.

In summary, trisaccharide structures of *S. pneumoniae* type 9A, L, and N capsular polysaccharides have



Scheme 6.



Scheme 7.

been synthesised, both as deprotected spacer derivatives ready for conjugate formation, as partly protected spacer-equipped acceptors ready for glycosylations, and as differentially protected TMSE-glycosides ready for elongation both at the non-reducing and reducing end.

3. Experimental

General methods.—TLC was carried out on Merck precoated 60 F_{254} plates using UV-light and/or 8% H_2SO_4 for visualization. Column chromatography was performed on silica gel (0.040–0.063 mm, Amicon). NMR spectra were recorded in $CDCl_3$ (internal Me_4Si, $\delta = 0.00$) or D_2O (internal acetone ^{13}C $\delta = 31.0$, ^1H $\delta = 2.21$) at 25 °C on a Varian 300 MHz or 400 MHz instrument. Organic phases were dried over Na_2SO_4 before evaporation, which was performed under reduced pressure.

*2-(*N*-Benzyloxycarbonyl)-aminoethyl 2,3,6-tri*-O*-benzyl-β*-D*-glucopyranoside* (**5**).—A catalytic amount of *p*-TsOH was added to a solution of **1** (4.6 g, 19 mmol)[13] and α,α-dimethoxytoluene (3.37 g, 22 mmol) in DMF (70 mL). The mixture was heated at 50 °C for 24 h, and then co-concentrated with toluene. Silica gel chromatography (1:1 toluene–EtOAc) gave 2-azidoethyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**2**, 4.3 g, 13 mmol, 70%); ^{13}C NMR δ 50.7 ($OCH_2CH_2N_3$), 66.4, 68.6, 68.9, 73.0, 74.5, 80.4 (C-2-6, $OCH_2CH_2N_3$), 101.9 (benzylidene), 103.3 (C-1), and 126.3–136.9 (aromatic C). A solution of **2** (3.48 g, 10.4 mmol) and BnBr (7.10 g, 42 mmol) in dry DMF (40 mL) was added dropwise to a cold (0 °C) suspension of NaH (2.08 g, 52 mmol) in DMF (30 mL). The mixture was stirred at room temperature (rt) overnight before the addition of MeOH (20 mL). Toluene was added and the mixture was washed with a saturated aqueous $NaHCO_3$ and water. The organic layer was dried, concentrated and purified by chromatography (10:1 toluene–EtOAc) to give 2-azidoethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**3**, 4.25 g, 8.2 mmol, 79%) as a white solid; ^{13}C NMR ($CDCl_3$): δ 51.0 ($OCH_2CH_2N_3$), 66.1, 68.5, 68.7, 75.1, 75.4, 80.8, 81.4, 82.1 (C-2-6, $OCH_2CH_2N_3$, $PhCH_2O$), 101.1 (benzylidene), 104.0 (C-1), and 126.0–138.4 (aromatic C). Triphenylphosphine (3.03 g, 12 mmol) was added at rt to a stirred solution of **3** (3.97 g, 7.7 mmol) in CH_2Cl_2 (100 mL) and stirring was continued at rt overnight. Water (50 mL) was added and stirring was continued under reflux for 20 h until all phosphine imine was hydrolysed. The organic layer was concentrated and then co-concentrated twice with toluene. Benzylchloroformate (1.75 mL, 12.3 mmol) was added at 0 °C to a stirred solution of the amine in pyridine (50 mL). More benzylchloroformate (550 μL, 3.85 mmol) was added after 30 min. After 1 h the mixture was diluted with CH_2Cl_2, washed with water, dried and concentrated. The residue was purified on silica gel (9:1 toluene–EtOAc) to give 2-(*N*-benzyloxycarbonyl)-aminoethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (**4**, 3.85 g, 6.2 mmol, 80%); ^{13}C NMR ($CDCl_3$): δ 41.5 ($CH_2NHCOOBn$), 66.5, 67.2, 69.1, 70.0, 75.5, 75.9, 81.4, 81.9, 82.4 (C-2-6, OCH_2CH_2N, $PhCH_2O$), 101.6 (benzylidene), 104.5 (C-1), 126.4–138.8 (aromatic C), and 156.3 ($NHCOOBn$). A solution of **4** (3.80 g, 6. 07 mmol), $NaCNBH_3$ (3.87 g, 61 mmol) and 3 Å molecular sieves in distilled THF (100 mL) was stirred at rt under an argon atmosphere for 30 min. HCl in diethyl ether was added until pH 1. After 4 h the reaction mixture was filtered through a layer of Celite, concentrated and purified on silica gel (3:1 toluene–EtOAc) to give **5** (3.77 g, 6.01 mmol, 99%) as a white solid; $[\alpha]_D$ −19° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 41.4 ($CH_2NHCOOBn$), 66.7, 69.8, 70.0, 71.1, 73.6, 74.0, 74.9, 75.3, 81.7, 83.9 (C-2-6, OCH_2CH_2N, $PhCH_2O$), 104.0 (C-1), 127.7–138.4 (aromatic C), and 156.6 ($NHCOOBn$).

*Ethyl 2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside* (**8**).—A mixture of **6** (9.30 g, 30.0 mmol)[16] and dibutyltin oxide (8.89 g, 35.7 mmol) in MeOH (200 mL) was refluxed. After 1 h the mixture was concentrated. The residue was dissolved in DMF (100 mL) and allyl bromide (3.05 mL, 36.0 mmol) and CsF (5.92 g, 39.0 mmol) were added. The mixture was stirred at rt overnight and then concentrated. The residue was dissolved in CH_2Cl_2 and washed with KF (aq, sat), dried and concentrated. Silica gel chromatography (4:1 toluene–EtOAc) gave ethyl 3-*O*-allyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**7**, 7.16 g, 20.3 mmol, 67%) as a white solid together with some starting material (**6**, 2.23 g, 7.1 mmol, 24%). **7**: ^{13}C NMR ($CDCl_3$): δ 15.3 (SCH_2CH_3), 24.6 (SCH_2CH_3), 68.7, 70.8, 72.9, 73.7, 81.3, 81.3 (C-2-6, allyl CH_2), 86.6 (C-1), 101.3 (benzylidene), 117.4 (allyl), 126.0, 128.3, 129.0, 134.9, and 137.2 (aromatic C, allyl). Compound **7** (1.97 g, 5.59 mmol) was dissolved in pyridine (50 mL) and cooled to 0 °C. Acetic anhydride (50 mL) was added and the mixture was stirred at rt for 1 h, diluted with toluene and concentrated. The residue was purified on a silica gel column (10:1 toluene–EtOAc) to give **8** (2.14 g, 5.41 mmol, 97%); $[\alpha]_D$ −45° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 14.9 (SCH_2CH_3), 21.0 (CH_3CO), 24.0 (SCH_2CH_3), 68.6, 70.7, 71.2, 73.4, 79.8, 81.3 (C-2-6, allyl CH_2), 84.2 (C-1), 101.1 (benzylidene), 116.7 (allyl), 125.9, 128.1, 128.9, 134.6, 137.0 (aromatic C, allyl), and 169.3 (CH_3CO). Anal. Calcd for $C_{20}H_{26}O_6S$: C, 60.89; H, 6.64. Found: C, 61.06; H, 6.74.

*2-(*N*-Benzyloxycarbonyl)-aminoethyl (2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri*-O*-benzyl-β*-D*-glucopyranoside* (**12**).—A mixture of **8** (980 mg, 2.48 mmol) and **5** (1.04 g, 1.66 mmol) in dry CH_2Cl_2 containing powdered molecular

sieves (4 Å) was stirred under argon at rt for 1 h. The solution was cooled to −30 °C, NIS (559 mg, 2.48 mmol) and TfOH (73 μL, 0.83 mmol) were added and the mixture was stirred for 1 h at −30 °C. The mixture was neutralized with NEt_3 and filtered through Celite. The filtrate was washed with $Na_2S_2O_3$ (10% aq), $NaHCO_3$ (aq, sat) and water, dried and evaporated. Purification by silica gel chromatography (3:1 toluene–EtOAc) gave 2-(*N*-benzyloxycarbonyl)-aminoethyl (2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**9**, 1.34 g, 1.40 mmol, 84%); ^{13}C NMR ($CDCl_3$): δ 20.8 (CH_3CO), 41.3 ($CH_2NHCOOBn$), 66.0, 66.6, 67.6, 68.5, 69.7, 73.1, 73.3, 73.6, 74.6, 75.0, 75.3, 76.7, 78.7, 81.3, 81.5, 82.6 (C-2-6, 2′-6′, $PhCH_2O$, OCH_2CH_2N, allyl CH_2), 100.7, 101.1, 103.7 (C-1, -1′, benzylidene), 116.5 (allyl), 126.0–128.9, 134.7, 136.5–138.9 (aromatic C, allyl), 156.4 (NH*C*OOBn), 169.0 (CH_3CO). Compound **9** (1.34 g, 1.40 mmol) was dissolved in 1:1 MeOH–CH_2Cl_2 (50 mL) and the pH was adjusted to 12 by treatment with 1 M methanolic NaOMe. The mixture was stirred at rt for 3 h, neutralized with Dowex 50 (H^+) ion exchange resin, filtered and concentrated. Silica gel chromatography (3:1 toluene–EtOAc) gave 2-(*N*-benzyloxycarbonyl)-aminoethyl (3-*O*-allyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**10**, 1.193 g, 1.30 mmol, 93%) as a white solid; ^{13}C NMR ($CDCl_3$): δ 41.3 ($CH_2NHCOOBn$), 66.3, 66.7, 68.2, 68.5, 69.8, 73.5, 73.6, 74.4, 74.9, 75.1, 75.2, 80.1, 81.2, 81.9, 83.3 (C-2-6, 2′-6′, $PhCH_2O$, OCH_2CH_2N, allyl CH_2), 101.1, 103.5, 103.9 (C-1, -1′, benzylidene), 117.2 (allyl), 125.3–129.0, 134.9, 136.5–138.9 (aromatic C, allyl), 156.4 (NH*C*OOBn). Trifluoromethanesulfonic anhydride (502 μL, 2.98 mmol) was added at 0 °C and under argon to a stirred solution of **10** (1.095 g, 1.19 mmol) in distilled 2:1 CH_2Cl_2–pyridine (18 mL). The reaction mixture was then left stirring overnight, slowly attaining rt. After 20 h the mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ (aq, sat), dried and concentrated. The crude triflate was dried for 2 h in vacuum. The dried triflate was dissolved in dry DMF (40 mL) and NaN_3 (388 mg, 5.96 mmol) was added. The reaction mixture was stirred at 70 °C for 3 h, cooled to 0 °C, diluted with toluene, filtered through Celite and concentrated. Silica gel chromatography (3:1 toluene–EtOAc) gave 2-(*N*-benzyloxycarbonyl)-aminoethyl (3-*O*-allyl-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**11**, 0.85 g, 0.90 mmol, 76%) as a white solid together with some starting material **10** (93 mg, 0.10 mmol, 8%). **11**: ^{13}C NMR ($CDCl_3$): δ 41.3 ($CH_2NHCOOBn$), 63.5 (C-2′), 66.7, 67.2, 68.3, 68.4, 69.8, 71.9, 73.6, 74.1, 75.0, 75.3, 76.6, 78.4, 81.7, 82.9 (C-2-6, 3′-6′, $PhCH_2O$, OCH_2CH_2N, allyl CH_2), 100.2, 101.5, 103.9 (C-1, 1′, benzylidene), 117.1 (allyl), 125.9–129.0, 134.3, 136.5–138.8 (aromatic C, allyl), 156.4 (NH*C*OOBn). Compound **11** (850 mg, 0.90 mmol) was dissolved in distilled THF (10 mL) and palladium on carbon was added. The mixture was stirred for 5 min at rt and then filtered. [Bis(methyldiphenylphosphine)](1,5-cyclooctadiene)iridium(I)PF_6 (76 mg, 90 μmol) was added and the mixture was degassed and then treated with H_2 for 20 min (until the red colour disappeared), then it was degassed again and NIS (1.01 g, 4.51 mmol) and water (4.5 mL, 252 mmol) were added. After 30 min the solution was diluted with CH_2Cl_2 and washed with $Na_2S_2O_3$ (10% aq), $NaHCO_3$ (aq, sat), dried and evaporated. Purification by silica gel chromatography (3:1 toluene–EtOAc) gave **12** (667 mg, 0.74 mmol, 82%); $[\alpha]_D$ −26° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 41.3 ($CH_2NHCOOBn$), 64.7 (C-2′), 66.8, 66.9, 68.3, 69.8, 70.0, 73.7, 74.1, 75.1, 75.3, 77.8, 78.3, 81.7, 82.9 (C-2-6, 3′-6′, $PhCH_2O$, OCH_2CH_2N), 100.6, 102.1, 103.9 (C-1, 1′, benzylidene), 126.2–129.3, 136.5–138.9 (aromatic C), and 156.4 (NH*C*OOBn). Anal. Calcd for $C_{50}H_{54}N_4O_{12}$: C, 66.51; H, 6.03. Found: C, 66.43; H, 6.20.

*Ethyl 3-*O*-acetyl-2,4,6-tri-*O*-benzyl-1-thio-β-*D*-glucopyranoside* (**14**).—To a solution of **13** (550 mg, 1.03 mmol)[23] in 6:3:1 EtOH–toluene–H_2O (30 mL) was added tris(triphenylphosphine)rhodium(I) chloride (381 mg, 0.41 mmol). After 4 h more Wilkinson's catalyst (170 mg) was added. The mixture was refluxed for 24 h and then cooled to rt. $HgBr_2$ (741 mg, 2.06 mmol) was added and the mixture was stirred at rt overnight, filtered through Celite and concentrated. The residue was dissolved in pyridine (10 mL) and cooled to 0 °C. Acetic anhydride (10 mL) was added and the mixture was stirred at rt for 2 h, diluted with toluene and concentrated. Silica gel chromatography (9:1 toluene–EtOAc) gave **14** (348 mg, 0.65 mmol, 63%); $[\alpha]_D$ +6° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 15.1 (SCH_2CH_3), 21.0 (CH_3CO), 25.2 (SCH_2CH_3), 68.7, 73.5, 74.3, 74.8, 76.1, 77.4, 78.9, 79.6 (C-2-6, $PhCH_2O$), 85.1 (C-1), 127.7–138.1 (aromatic C), and 169.9 (CH_3CO).

*Ethyl 3-*O*-acetyl-2,4,6-tri-*O*-benzyl-1-thio-β-*D*-galactopyranoside* (**16**).—To a solution of **15** (270 mg, 0.55 mmol)[27] in pyridine (3 mL) at 0 °C was added acetic anhydride (3 mL). The mixture was stirred at rt for 3 h and then co-concentrated with toluene. Silica gel chromatography (15:1 toluene–EtOAc) gave **16** (267 mg, 0.50 mmol, 91%) as a yellow oil; $[\alpha]_D$ +37° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 15.0 (SCH_2CH_3), 20.9 (CH_3CO), 25.0 (SCH_2CH_3), 68.3, 73.5, 74.6, 74.9, 75.4, 76.6, 76.8 (C-2-6, $PhCH_2O$), 85.3 (C-1), 127.7–138.2 (aromatic C), and 170.3 (CH_3CO). Anal. Calcd for $C_{31}H_{36}O_6S$: C, 69.38; H, 6.76. Found: C, 69.20; H, 6.71.

*2-(*N*-Benzyloxycarbonyl)-aminoethyl (2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside* (**19**).—A solu-

tion of **14** (150 mg, 0.28 mmol) and **12** (126 mg, 0.14 mmol) in 6:1 dry diethyl ether–CH_2Cl_2 (3.5 mL) containing powdered molecular sieves (4 Å) was stirred at rt under an argon atmosphere for 1 h. To the mixture was added DMTST (145 mg, 0.56 mmol) and the stirring was continued for 30 min. After neutralization with NEt_3 (0.5 mL), the mixture was filtered through Celite and concentrated. The residue was purified on a silica gel column (4:1 toluene–EtOAc) to yield 2-(*N*-benzyloxycarbonyl)-aminoethyl (3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**17**, 183 mg, 0.13 mmol, 93%); ^{13}C NMR, δ 21.0 (CH_3CO), 41.3 ($CH_2NHCOOBn$), 64.3 (C-2′), 66.7, 67.1, 68.4, 68.5, 69.7, 70.3, 70.7, 72.7, 73.6, 73.6, 74.2, 74.3, 75.1, 75.2, 75.3, 75.5, 75.8, 76.0, 78.1, 81.7, 82.9 (C-2-6, 3′-6′, 2″-6″, OCH_2CH_2N, $PhCH_2O$), 97.4 (C-1″), 100.0, 102.2, 103.9 (C-1, 1′, benzylidene), 126.2–138.9 (aromatic C), 156.4 ($NHCOOBn$), 169.7 (CH_3CO). To a solution of **17** (187 mg, 0.14 mmol) in EtOH (10 mL) and CH_2Cl_2 (2 mL) were added $NaBH_4$ (10 mg, 0.27 mmol) and a catalytic amount of $NiCl_2{\cdot}6H_2O$. After 3 h more $NaBH_4$ (20 mg) was added. The reaction mixture was stirred at rt for 6 h, when Ac_2O (300 μL) was added. After 30 min the reaction mixture was diluted with toluene and concentrated. Silica gel chromatography (1:1 toluene–EtOAc) gave 2-(*N*-benzyloxycarbonyl)-aminoethyl (3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**18**, 142 mg, 0.10 mmol, 72%); ^{13}C NMR ($CDCl_3$): δ 21.1 (CH_3CO), 22.8 (CH_3CON), 41.3 ($CH_2NHCOOBn$), 51.3 (C-2′), 66.7, 68.2, 68.5, 69.1, 70.0, 70.5, 72.5, 73.2, 73.6, 74.0, 74.6, 75.0, 75.2, 75.6, 76.2, 76.4, 80.0, 82.1, 83.4 (C-2-6, 3′-6′, 2″-6″, OCH_2CH_2N, $PhCH_2O$), 96.8 (C-1″), 100.1, 102.1, 103.9 (C-1, 1′, benzylidene), 126.1–138.8 (aromatic C), 156.4 ($NHCOOBn$), 169.9, 170.6 (CH_3CO and CH_3CON). Compound **18** (80 mg, 57 μmol) was dissolved in 1:1 MeOH–CH_2Cl_2 (4 mL) and the pH was adjusted to 12 by treatment with 1 M methanolic NaOMe. The mixture was stirred at rt for 4 h, neutralized with Dowex 50 (H^+) ion exchange resin, filtered and concentrated. Silica gel chromatography (1:3 toluene–EtOAc) gave **19** (59 mg, 44 μmol, 77%); $[\alpha]_D$ +26° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 23.1 (CH_3CON), 41.3 ($CH_2NHCOOBn$), 52.0 (C-2′), 66.5, 66.7, 68.1, 68.5, 68.9, 69.6, 70.0, 70.6, 72.4, 73.3, 73.5, 73.6, 74.5, 75.0, 75.1, 76.1, 77.5, 77.8, 79.8, 81.9, 83.3 (C-2-6, 3′-6′, 2″-6″, OCH_2CH_2N, $PhCH_2O$), 96.9 (C-1″), 99.9, 102.4, 103.9 (C-1, 1′, benzylidene), 126.3–138.9 (aromatic C), 156.4 ($NHCOOBn$) and 170.2 (CH_3CON). Anal. Calcd for $C_{79}H_{86}N_2O_{18}$: C, 70.21; H, 6.41. Found: C, 69.93; H, 6.52. MALDI-TOF MS: Calcd for $C_{79}H_{86}N_2NaO_{18}$ ($[M+Na]^+$): 1373.58, found 1372.86.

2-Aminoethyl α-D-glucopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-mannopyranosyl-(1→4)-β-D-glucopyranoside (**20**).—To a solution of **19** (41 mg, 30 μmol) dissolved in EtOH (3 mL) and AcOH (2 mL) was added palladium hydroxide on activated carbon powder and the mixture was hydrogenolyzed at 100 psi for 48 h. Additional catalyst was added after 24 h. The mixture was centrifuged and the pellets were washed once with EtOH. The supernatants were combined and concentrated. The residue was dissolved in water and washed with EtOAc, concentrated, and purified on a Bio-Gel P-2 column to give, after freeze-drying, **20** (13 mg, 22 μmol, 73%); $[\alpha]_D$ +123° (*c* 0.83, H_2O); ^{13}C NMR (D_2O): δ 22.7 (CH_3CON), 40.1 (CH_2NH_2), 53.5 (C-2′), 60.6, 61.0, 61.1, 66.5, 67.1, 70.0, 72.2, 72.9, 73.4, 73.5, 74.7, 75.1, 76.9, 79.0, 79.3 (C-2-6, 3′-6′, 2″-6″ OCH_2CH_2), 100.1, 101.2, 102.6 (C-1, 1′, 1″), and 175.5 (CH_3CON); ^{1}H (selected signals), δ 4.50 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.63 (d, 1 H, $J_{2,3}$ 4 Hz, H-2′), 4.88 (s, 1 H, H-1′), 5.22 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1″). MALDI-TOF MS: Calcd for $C_{22}H_{40}N_2NaO_{16}$ ($[M+Na]^+$): 611.23, found 610.87.

2-(N-Benzyloxycarbonyl)-aminoethyl (2,4,6-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-mannopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**23**).—Donor **16** (214 mg, 0.40 mmol) and acceptor **12** (180 mg, 0.20 mmol) was coupled as described above for compounds **14** and **12** to yield 2-(*N*-benzyloxycarbonyl)-aminoethyl (3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**21**, 256 mg, 0.19 mmol, 93%) as a white solid; ^{13}C NMR ($CDCl_3$): δ 21.0 (CH_3CO), 41.3 ($CH_2NHCOOBn$), 64.3 (C-2′), 66.7, 67.1, 68.2, 68.4, 69.9, 70.9, 71.9, 72.4, 73.5, 74.3, 74.4, 74.6, 75.1, 75.4, 75.5, 75.8, 76.6, 77.8, 81.6, 82.8 (C-2-6, 3′-6′, 2″-6″, OCH_2CH_2N, $PhCH_2O$), 98.6 (C-1″), 99.8, 102.1, 103.8 (C-1, 1′, benzylidene), 126.3–138.9 (aromatic C), 156.4 ($NHCOOBn$), and 170.3 (CH_3CO). The azido group in **21** (166 mg, 0.12 mmol) was converted to an acetamido group as described above for compound **17** to give 2-(*N*-benzyloxycarbonyl)-aminoethyl (3-*O*-acetyl-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**22**, 130 mg, 93 μmol, 78%) as a white solid; ^{13}C NMR ($CDCl_3$): δ 21.0 (CH_3CO), 23.1 (CH_3CON), 41.3 ($CH_2NHCOOBn$), 52.1 (C-2′), 66.5, 66.7, 68.2, 68.8, 69.2, 69.6, 70.7, 71.2, 73.0, 73.6, 74.3, 75.0, 75.1, 75.8, 76.2, 79.7, 81.8, 83.1 (C-2-6, 3′-6′, 2″-6″, OCH_2CH_2N, $PhCH_2O$), 97.4 (C-1″), 99.5, 102.2, 103.8 (C-1, 1′, benzylidene), 126.3–138.9 (aromatic C), 156.4 ($NHCOOBn$), 169.9 and 170.8 (CH_3CO and CH_3CON). Compound **22** (89 mg, 64 μmol) was deacetylated as **18** above to yield **23** (75 mg, 55 μmol, 86%); $[\alpha]_D$ +27° (*c* 1.0, $CHCl_3$); ^{13}C

NMR ($CDCl_3$): δ 23.5 (CH_3CON), 41.6 (CH_2NH-COOBn), 52.9 (C-2′), 67.0, 68.4, 68.9, 69.8, 69.9, 70.2, 70.6, 73.3, 73.7, 74.6, 75.2, 75.3, 76.9, 79.9, 82.0, 83.4 (C-2-6, 3′-6′, -2″-6″, OCH_2CH_2N, PhCH_2O), 97.4 (C-1″), 99.6, 102.6, 104.1 (C-1, 1′, benzylidene), 126.5–139.3 (aromatic C), 156.7 (NH$COOBn$), and 170.7 (CH_3CON). MALDI-TOF MS: Calcd for $C_{79}H_{86}N_2NaO_{18}$ ($[M+Na]^+$): 1373.58, found 1372.88. Anal. Calcd for $C_{79}H_{86}N_2O_{18}$: C, 70.21; H, 6.41. Found: C, 70.05; H, 6.55.

2-Aminoethyl α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-mannopyranosyl-(1→4)-β-D-glucopyranoside (**24**).—Compound **23** (33 mg, 24 μmol) was deprotected as descibed for compound **19** above to give, after purification on a Bio-Gel P-2 column, **24** (13 mg, 22 μmol, 91%) after freeze-drying; $[\alpha]_D$ +15° (*c* 1.0, H_2O); ^{13}C NMR (D_2O): δ 22.6 (CH_3CON), 40.1 (CH_2NH_2), 53.5 (C-2′), 60.6, 60.9, 62.2, 66.5, 67.3, 69.0, 69.9, 70.0, 72.0, 73.5, 74.7, 75.2, 77.0, 77.9, 79.3 (C-2-6, 3′-6′, 2″-6″ OCH_2CH_2), 100.1, 101.0, 102.7 (C-1, 1′, 1″) and 175.6 (CH_3CON); ^1H (selected signals), δ 4.52 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.66 (d, 1 H, $J_{2,3}$ 4 Hz, H-2′), 4.91 (s, 1 H, H-1′), and 5.28 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1″). MALDI-TOF MS: Calcd for $C_{22}H_{40}N_2NaO_{16}$ ($[M+Na]^+$): 611.23, found 610.87.

*2-(Trimethylsilyl)ethyl (2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside* (**29**).—Donor **8** (167 mg, 0.42 mmol) and acceptor **25** (159 mg, 0.28 mmol)[22] was coupled as described above for compounds **8** and **5** to give 2-(trimethylsilyl)ethyl (2-*O*-acetyl-3-*O*-allyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (**26**, 200 mg, 0.22 mmol, 79%); ^{13}C NMR ($CDCl_3$): δ −1.5 (CH_3Si), 17.9 (CH_2Si), 20.9 (CH_3CO), 66.1, 67.0, 67.9, 68.5, 73.2, 73.3, 73.4, 73.7, 74.5, 75.3, 78.7, 80.6, 81.4 (C-2-6, 2′-6′, PhCH_2O, allyl CH_2, OCH_2CH_2Si), 100.6, 100.9, 101.1 (C-1, 1′, benzylidene), 116.6 (allyl), 126.0–130.2, 132.8, 134.8, 137.2, 138.1, 138.4 (aromatic C, allyl), 165.0 (PhCO), and 169.2 (CH_3CO). Compound **26** (404 mg, 0.45 mmol) was deacetylated as described for compound **9** but using $Mg(OMe)_2$ instead of NaOMe to obtain 2-(trimethylsilyl)ethyl (3-*O*-allyl-4,6-*O*-benzylidene-β-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (**27**, 257 mg, 0.30 mmol, 67%); ^{13}C NMR ($CDCl_3$): δ −1.5 (CH_3Si), 17.9 (CH_2Si), 66.3, 67.1, 68.2, 68.6, 73.5, 73.6, 74.4, 74.8, 75.1, 77.6, 80.1, 81.3, 81.6 (C-2-6, 2′-6′, PhCH_2O, allyl CH_2, OCH_2CH_2Si), 100.7, 101.1, 103.6 (C-1, 1′, benzylidene), 117.2 (allyl), 126.0–130.0, 132.9, 134.9, 137.3. 137.7, 138.2 (aromatic C, allyl), and 165.0 (PhCO). Compound **27** (239 mg, 0.28 mmol) was converted to 2-(trimethylsilyl)ethyl (3-*O*-allyl-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside (**28**, 208 mg, 0.24 mmol, 84%) as described above for the conversion of **10**→**11**. **28**: ^{13}C NMR ($CDCl_3$): δ −1.5 (CH_3Si), 17.9 (CH_2Si), 63.5 (C-2′), 67.2, 68.3, 68.7, 71.9, 73.5, 73.7, 74.6, 74.7, 77.8, 78.4, 81.2 (C-2-6, 3′-6′, PhCH_2O, allyl CH_2, OCH_2CH_2Si), 100.4, 100.7, 101.5 (C-1, 1′, benzylidene), 117.2 (allyl), 125.3–130.0, 132.9, 134.3, 137.3, 137.9, 138.3 (aromatic C, allyl), and 165.1 (PhCO). Compound **28** (188 mg, 0.21 mmol) was deallylated as described for compound **11** to yield **29** (146 mg, 0.17 mmol, 82%); ^{13}C NMR ($CDCl_3$): δ −1.4 (CH_3Si), 18.0 (CH_2Si), 64.7 (C-2′), 66.9, 67.2, 68.3, 68.5, 70.1, 73.4, 73.7, 74.6, 78.3, 80.9 (C-2-6, 3′-6′, PhCH_2O, OCH_2CH_2Si), 100.6, 100.7, 102.0 (C-1, 1′, benzylidene), 126.1–130.0, 132.8, 136.8, 137.6, 138.2 (aromatic C), and 164.9 (PhCO). MALDI-TOF MS: Calcd for $C_{45}H_{53}N_3NaO_{11}Si$ ($[M+Na]^+$): 862.33, found 861.87.

*2-(Trimethylsilyl)ethyl (3-*O*-allyl-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-(2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside* (**31**).—Donor **13** (38 mg, 71 μmol) and acceptor **29** (30 mg, 36 μmol) was coupled as described above for compounds **14** and **12** to yield **31** (41 mg, 31 μmol, 87%); ^{13}C NMR ($CDCl_3$): δ −1.4 (CH_3Si), 18.0 (CH_2Si), 64.4 (C-2′), 67.1, 68.3, 70.8, 71.2, 73.4, 73.5, 74.3, 74.4, 74.5, 74.6, 74.8, 75.0, 75.9, 77.4, 78.3, 78.3, 80.8, 80.9 (C-2-6, 3′-6′, 2″-6″, PhCH_2O, OCH_2CH_2Si, allyl CH_2), 97.5 (C-1″), 100.1, 100.6, 102.2 (C-1, 1′, benzylidene), 116.5 (allyl), 126.2–138.3 (aromatic C, allyl), and 165.0 (PhCO). MALDI-TOF MS: Calcd for $C_{75}H_{85}N_3NaO_{16}Si$ ($[M+Na]^+$): 1334.56, found 1334.52.

*2-(Trimethylsilyl)ethyl (3-*O*-allyl-2,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside* (**32**).—Donor **30** (42 mg, 79 μmol)[24] and acceptor **29** (33 mg, 39 μmol) was coupled as described above for compounds **14** and **12** to yield **32** (47 mg, 36 μmol, 91%); ^{13}C NMR ($CDCl_3$): δ −1.3 (CH_3Si), 18.0 (CH_2Si), 64.6 (C-2′), 67.0, 67.1, 68.3, 68.4, 70.2, 70.6, 71.2, 72.2, 73.3, 73.5, 74.5, 74.5, 74.8, 75.0, 75.1, 75.3, 77.1, 77.8, 78.0, 80.8 (C-2-6, 3′-6′, 2″-6″, PhCH_2O, OCH_2CH_2Si, allyl CH_2), 98.6 ($J_{C\text{-}1,\ H\text{-}1}$ 174 Hz) (C-1″), 99.7 ($J_{C\text{-}1,\ H\text{-}1}$ 162 Hz), 100.6 ($J_{C\text{-}1,\ H\text{-}1}$ 165 Hz), 101.9 ($J_{C\text{-}1,\ H\text{-}1}$ 157 Hz) (C-1, 1′, benzylidene), 116.2 (allyl), 126.2–138.4 (aromatic C, allyl), 164.9 (PhCO). MALDI-TOF MS: Calcd for $C_{75}H_{85}N_3NaO_{16}Si$ ($[M+Na]^+$): 1334.56, found 1334.89.

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