Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 343 (2008) 607–614
Carbohydrate RESEARCH

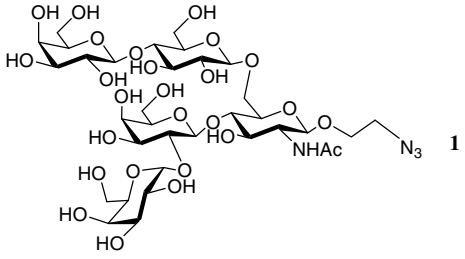
Facile synthesis of a pentasaccharide mimic of a fragment of the capsular polysaccharide of *Streptococcus pneumoniae* type 15C

Chen Yong-Xiang, Zhao Wei, Zhao Lei, Zhao Yu-Fen and Li Yan-Mei,*

Key Laboratory of Bioorganic Phosphorous Chemistry and Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084, PR China

Received 12 October 2007; received in revised form 5 December 2007; accepted 6 December 2007
Available online 14 January 2008

Abstract—A pentasaccharide mimic of a fragment of the capsular polysaccharide of *Streptococcus pneumoniae* type 15C β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-[α-D-Gal*p*-(1→2)-β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc-(1→$OCH_2CH_2N_3$) (**1**) was synthesized in a regio- and stereoselective manner. The 2-azidoethyl-spacered pentasaccharide mimic **1** can be used to construct a neoglycoconjugate antigen.



1

Keywords: *Streptococcus pneumoniae*; Oligosaccharide synthesis

1. Introduction

The pathogenic bacterium *Streptococcus pneumoniae*, which causes infections of the lung (pneumonia), middle ear (otitis media) and meninges (meningitis) remains a major cause of death all over the world.[1] With the increasing incidence of antibiotic resistance in this organism, vaccination has become an effective means of protection. A vaccine that consists of a mixture of the capsular polysaccharides (CPSs) from 23 of the 90 serotypes of *S. pneumoniae* has been available for more than 20 years.[2] However, the T-cell-independent CPSs vaccines are ineffective in the most important high-risk groups, such as infants, small children, immuno-compromised patients, and the elderly.[3] Conjugation of *S. pneumoniae* carbohydrate antigens to a protein carrier results in a T-cell dependent neoglycoconjugate antigen that gives an efficient immune response in the high-risk groups.[4]

These neoglycoproteins were prepared by conjugation of isolated capsular polysaccharides or a mixture of polysaccharide-derived oligosaccharides to a protein carrier.[5,6] The studies with *S. pneumoniae* type 6B neoglycoproteins showed that a synthetic tetrasaccharide fragment, that is, one repeating unit of the 6B CPS, coupled to keyhole limpet hemocyanin (KLH) was sufficient to generate a protective antibody response in mice.[7] Similar results were shown for *S. pneumoniae* type 3 neoglycoproteins, consisting of di-, tri- and tetrasaccharides

* Corresponding author. Fax: +86 10 62781695; e-mail: liym@mail.tsinghua.edu.cn

0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.12.002

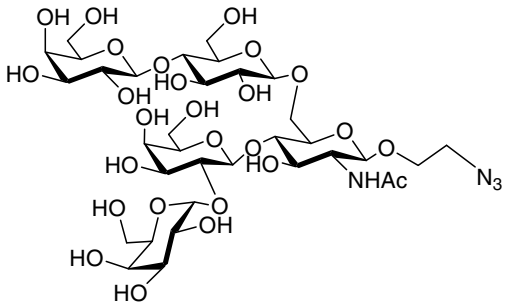


Figure 1. Overview of pentasaccharide mimic **1** with a 2-azidoethyl spacer group, representing fragments of the repeating unit of the *S. pneumoniae* type 15C capsular polysaccharide.

coupled to the cross-reacting material (CRM_{197}) of modified diphtheria toxin in different molar carbohydrate–protein ratios.[8] Kamerling's group[9–13] has reported on the chemoenzymatic synthesis of spacered oligosaccharide fragments of the CPS of *S. pneumoniae* type 14. And the tetrasaccharide containing neoglycoconjugate (CRM_{197} as carrier protein) showed, particularly, promising immunological data when tested in mice models.[14]

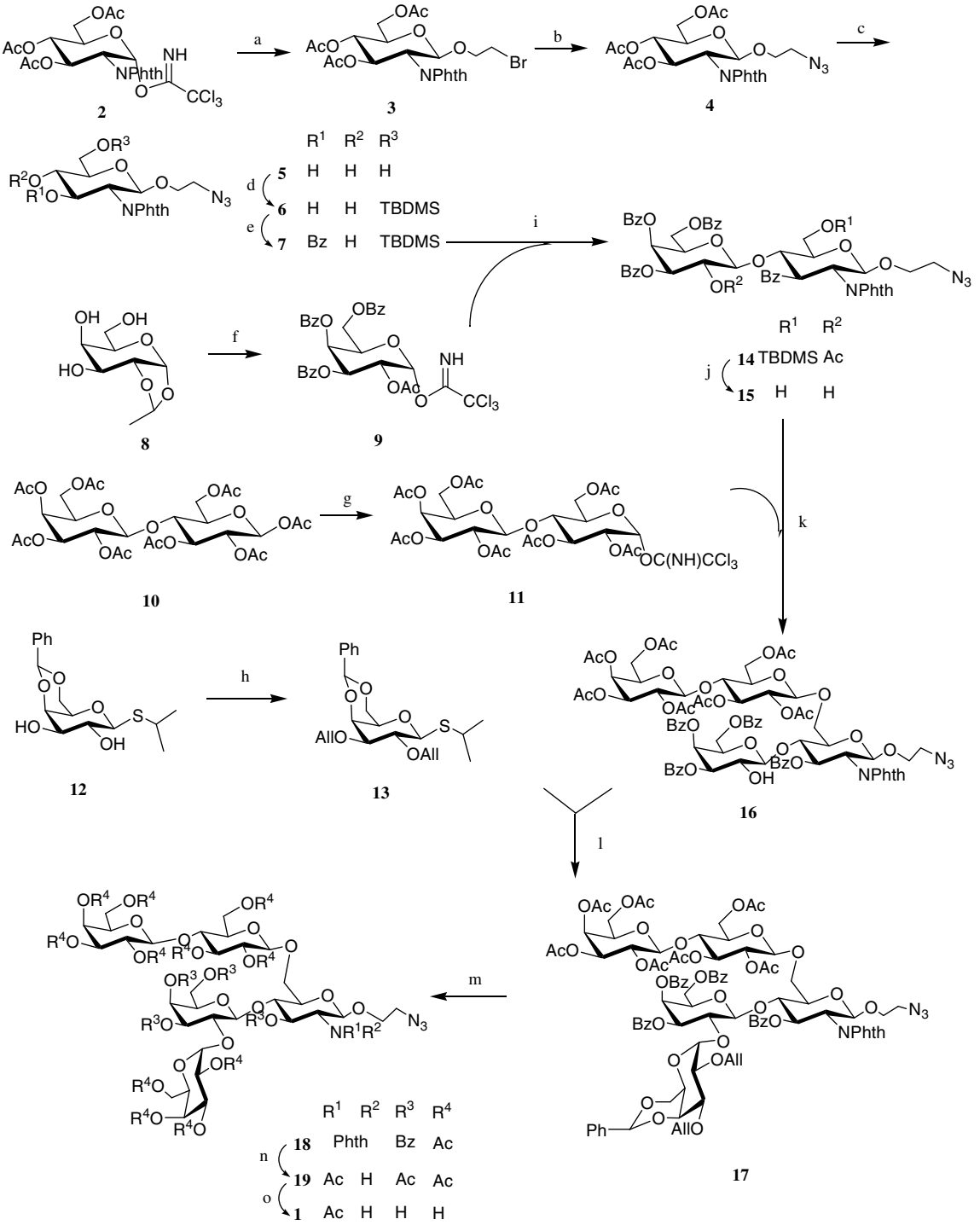
In this paper, we report an original and efficient approach proposed for the chemical synthesis of 2-azidoethyl-spacered pentasaccharide mimic **1** (Fig. 1) (without a glycerol-2-phosphate substituent) of a fragment of the CPS of *S. pneumoniae* type 15C,[15] which is suitable for the construction of a novel neoglycoconjugate antigen. The introduction of an azide group can be useful to construct neoglycoconjugates through a highly efficient coupling method: the copper (I)-catalyzed 1,2,3-triazole formation from azides and terminal acetylenes (click chemistry), developed by Sharpless and co-workers.[16]

2. Results and discussion

The CPS of *S. pneumoniae type* 15C is built up from the pentasaccharide repeating unit→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-[α-D-Gal*p*-(1→2)-β-D-Gal*p*(3-OR)-(1→4)]-β-D-Glc*p*NAc-(1→(R is glycerol-2-phosphate substituent). Pentasaccharide **1** (Fig. 1) represents one repeating unit. For the synthesis of **1**, four building blocks were designed: the glucosamine acceptor **7**, the galactose trichloroacetimidate donor **9**, the lactose trichloroacetimidate donor **11** and the thiogalactoside donor **13** (Scheme 1).

The synthesis of monoglucosamine acceptor **7** involved, as the first step, the coupling of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl trichloroacetimidate **2**[17] to the spacer 2-bromoethan-1-ol in dichloromethane (DCM) using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst (→**3**, 73%). Then, the bromo group in the spacer was further substituted by an azido group from sodium azide in *N*,*N*-dimethylformamide (DMF) (→**4**, 94%). After deacetylation (→**5**, 85%), product **5** was regioselectively *tert*-butyldimethylsilylated at O-6 with *tert*-butyldimethylsilyl chloride in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP), pyridine and triethylamine to give **6** in 78% yield. Subsequently, product **6** was regioselectively benzoylated at O-3 with a stoichiometric amount of benzoyl chloride in the presence of silver(I) oxide and a catalytic amount of potassium iodide to give the acceptor **7** with a free hydroxyl group at O-4 in high regioselectivity and in good yield (81%). Ye's group[18] has also reported the silver(I) oxide-mediated selective monoprotection of 2,3-diols in pyranosides. Here, silver(I) oxide was used to mediate selective monoprotection of 3,4-diols in the pyranoside. The acceptor **7** was verified by ESIMS and NMR spectroscopy (1D ^{1}H and 2D ^{1}H COSY). The galactose trichloroacetimidate donor **9** with regioselective protective groups was prepared in the following way: Benzoylation of 1,2-*O*-ethylidene-α-D-galactopyranoside (**8**),[19] followed by sequent de-ethylidenation, acetylation, 1-*O*-selective deacetylation and trichloroacetimidation afforded monosaccharide donor **9** (35% over five steps). The lactose donor **11**[20] was easily prepared from fully protected lactose **10**, in which the acetyl group at O-1 was selectively removed by benzylamine in tetrahydrofuran (THF), followed by activation to give the α-trichloroacetimidate **11** (50% over two steps). Allylation of isopropyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**12**)[21] with allyl bromide in the presence of sodium hydride afforded the thiogalactoside donor **13** in 80% yield.

Based on these building blocks, the target pentasaccharide **1** was synthesized. Kamerling's group[13,11,12] has developed enzymic methods to connect galactose residues to the internal *N*-acetyl-β-D-glucosamine residues with a β-(1→4) glycosidic linkage by using bovine milk β-1,4-galactosyltransferase. Here, we took the chemical coupling method. Condensation of the galactose trichloroacetimidate donor **9** with the monoglucosamine acceptor **7** in DCM using TMSOTf as a catalyst afforded the β-(1→4) linked disaccharide **14** in acceptable yield (67%). Subsequent selective removal of 2-*O*-acetyl and 6-*O*-*tert*-butyldimethylsilyl groups by methanolysis with MeCOCl–MeOH–CH_2Cl_2 afforded the disaccharide acceptor **15** (79%) with two free hydroxyl groups at O-2 of the galactosyl residue and O-6 of GlcNAc. Then, a stoichiometric amount of lactose trichloroacetimidate donor **11** was regioselectively coupled with the acceptor **15** at the primary hydroxyl group in the presence of TMSOTf to give the β-(1→6) linked tetrasaccharide **16** (50%) with a free hydroxyl group at O-2, which was characterized by ESIMS and NMR spectroscopy (1D ^{1}H and 2D ^{1}H COSY). Finally, condensation of the tetrasaccharide **16** with isopropyl 2,3-di-*O*-allyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside



Scheme 1. Synthesis of pentasaccharide **1**. Reagents and conditions: (a) CH_2BrCH_2OH, TMSOTf, CH_2Cl_2, −10 °C, 73%; (b) NaN_3, DMF, 55 °C, 94%; (c) MeONa, MeOH, 85%; (d) TBDMSCl, DMAP, Pyr, Et_3N, CH_2Cl_2, 78%; (e) BzCl, Ag_2O, KI, CH_2Cl_2, 81%; (f) (1) BzCl/Pyr, (2) 80% HOAc, (3) Ac_2O/Pyr, (4) benzyl amine, THF, (5) CCl_3CN, K_2CO_3, CH_2Cl_2; 35% over five steps; (g) (1) $PhCH_2NH_2$, THF, (2) CCl_3CN, K_2CO_3, CH_2Cl_2; 50% over two steps; (h) AllBr/NaH, DMF, 80%; (i) TMSOTf, CH_2Cl_2, −10 °C, 67%; (j) AcCl, MeOH/CH_2Cl_2, 79%; (k) TMSOTf, CH_2Cl_2, −10 °C, 50%; (l) TMSOTf, NIS, CH_2Cl_2, −10 °C, 68%; (m) (1) F_3CCO_2H/H_2O, CH_2Cl_2, (2) Ac_2O/Pyr, (3) $PdCl_2$, CH_2Cl_2/MeOH, (4) Ac_2O/Pyr; 86% over four steps; (n) (1) MeONa, MeOH, (2) $NH_2CH_2CH_2NH_2$, butanol, 80 °C, (3) Ac_2O/Pyr; 77% over three steps; (o) MeONa, MeOH, 84%.

donor **13** using TMSOTf and *N*-iodosuccinimide (NIS) as catalysts afforded the α-(1→2)-linked pentasaccharide **17** (68%).

In the deprotection sequence, the fully protected pentasaccharide **17** was firstly O-debenzylidenated and O-reacetylated, followed by O-deallylation and

O-reacetylation, to give the fully acylated pentasaccharide **18** (86% over four steps). Subsequently, **18** was O-deacylated and N-dephthaloylated, followed by N,O-reacetylation to yield the *N*-acetyl protected derivative **19** (77% over three steps). Complete conventional O-deacetylation of **19** to give the final product **1** in 84% yield required long reaction times (48 h), as indicated by NMR spectroscopy.

3. Conclusion

In summary, based on readily accessible building blocks **7**, **9**, **11** and **13**, a convergent and very efficient synthesis of 2-azidoethyl-spacered pentasaccharide β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-[α-D-Gal*p*-(1→2)-β-D-Gal*p*-(1→4)]-β-D-Glc*p*NAc-(1→$OCH_2CH_2N_3$) (**1**) was achieved in a regio- and stereoselective manner. Conjugation of the oligosaccharide **1** to peptides or proteins to form neoglycoconjugates and immunological studies are in progress.

4. Experimental

4.1. General methods

1D ^{1}H NMR and 2D ^{1}H COSY NMR spectra were recorded with Jeol ECA-600 and Jeol ECP-300 spectrometers for solutions in $CDCl_3$ or D_2O as indicated. Chemical shifts are given in ppm downfield from internal Me_4Si. The ^{1}H NMR resonance assignments have been performed mainly according to the peak shapes, the coupling constants and chemical shifts in combination of 2D ^{1}H COSY. Mass spectra were recorded with Bruker Esquire-LC mass spectrometer in the ESI mode. High-resolution mass spectra were recorded on a Thermo Electron LTQ Orbitrap (ESI; source voltage 3.8 kV) spectrometer. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV detector. Column chromatography was conducted by elution of a column (16 × 240 mm, 18 × 300 mm, 35 × 400 mm) of silica gel (200–300 mesh) with EtOAc–petroleum ether (bp 60–90 °C) as the eluent. Solutions were concentrated at <50 °C under reduced pressure.

4.2. General procedure for the glycosylations

The mixture of donor and acceptor was dried under high vacuum for 2 h, then dissolved in dry CH_2Cl_2. TMSOTf (0.05 equiv) was added dropwise at −10 °C under an argon atmosphere. The reaction mixture was stirred for 3 h, during which time the temperature was gradually raised to ambient temperature. Then the mixture was neutralized with Et_3N. Concentration of the reaction mixture, followed by purification on a silica gel column, gave the desired product.

4.3. 2-Bromoethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (3)

As described in the general procedure, **2** (1.31 g, 2.26 mmol) and 2-bromoethan-1-ol (185 μL, 2.60 mmol) were coupled, and the product was purified by column chromatography with 1:1 petroleum ether–EtOAc as the eluent to give **3** (890 mg, 1.64 mmol, 73%) as a foamy solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88 (s, 3H, O*Ac*); 2.04 (s, 3H, O*Ac*); 2.12 (s, 3H, O*Ac*); 3.30–3.38 (m, 2H, BrCH_2); 3.76 (m, 1H, OCH_2); 3.88 (m, 1H, H-5); 4.12 (m, 1H, OCH_2); 4.18 (dd, 1H, $J_{6b,5} = 2.4$ Hz, $J_{6b,6a} = 12.4$ Hz, H-6b); 4.28–4.38 (m, 2H, H-2, H-6a); 5.18 (dd, H-1, $J_{4,3} = 9.0$ Hz, $J_{4,5} = 10.0$ Hz, H-4); 5.42 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1); 5.81 (dd, 1H, $J_{3,2} = 10.6$ Hz, $J_{3,4} = 9.0$ Hz, H-3); 7.7–7.9 (m, 4H, *Ph*).

ESIMS: calcd for $C_{22}H_{24}BrNNaO_{10}$ $[M+Na]^+$ 564.05, found 563.9.

4.4. 2-Azidoethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4)

To a solution of **3** (890 mg, 1.64 mmol) in 20 mL DMF was added NaN_3 (1.07 g, 16.4 mmol), and the mixture was stirred at 55 °C overnight. After the solvent was co-evaporated with toluene under reduced pressure, the residue was dissolved in a mixture of water and EtOAc. The organic phase was separated from the water phase. The water phase was extracted two times with EtOAc. The combined organic layers were washed with water two times and then dried with Na_2SO_4. The solvent was evaporated, and the crude product was purified by silica gel flash chromatography (1:1 petroleum ether–EtOAc) to give **4** (780 mg, 1.55 mmol, 94%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.87 (s, 3H, O*Ac*); 2.04 (s, 3H, O*Ac*); 2.12 (s, 3H, O*Ac*); 3.18 (m, 1H, N_3CH_2); 3.40 (m, 1H, N_3CH_2); 3.66 (m, 1H, OCH_2); 3.90 (m, 1H, H-5); 4.02 (m, 1H, OCH_2); 4.21 (dd, 1H, $J_{6b,5} = 2.1$ Hz, $J_{6b,6a} = 12.3$ Hz, H-6b); 4.28–4.38 (m, 2H, H-2, H-6a); 5.19 (dd, H-1, $J_{4,3} = 9.6$ Hz, $J_{4,5} = 9.6$ Hz, H-4); 5.47 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1); 5.79 (dd, 1H, $J_{3,2} = 10.6$ Hz, $J_{3,4} = 9.6$ Hz, H-3); 7.70–7.80 (m, 4H, *Ph*).

ESIMS: calcd for $C_{22}H_{24}N_4NaO_{10}$ $[M+Na]^+$ 527.14, found 527.2.

4.5. 2-Azidoethyl 2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (6)

To a solution of **4** (780 mg, 1.55 mmol) in 15 mL of methanol was added MeONa (pH 10). The mixture was stirred for 0.5 h at room temperature, then neutralized with Dowex 50WX8 (H^+), filtered and concentrated. The residue was eluted on a Sephadex LH-20 column with MeOH to give **5** (500 mg, 1.32 mmol,

85%) as a white solid. To a solution of **5** (385 mg, 1.02 mmol) in 15 mL of dry CH_2Cl_2 was added *tert*-butyldimethylsilyl chloride (TBDMSCl) (216 mg, 1.43 mmol), 658 μL pyridine, a catalytic amount of DMAP and 187 μL Et_3N. The mixture was stirred overnight, and 15 mL of cold water was then added. The organic phase was separated from the water phase. The water phase was extracted two times with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4. The solvent was evaporated, and the residue was purified by silica gel flash chromatography (1:1 petroleum ether–EtOAc) to give a product **6** (390 mg, 0.792 mmol, 78%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.12 (s, 3H, SiCH_3); 0.13 (s, 3H, SiCH_3); 0.92 (s, 3 × 3H, SiC(CH_3)$_3$); 3.16 (m, 1H, N_3CH_2); 3.36 (m, 1H, N_3CH_2); 3.51–3.68 (m, 3H, H-5, OCH_2, H-4); 3.85–4.03 (m, 3H, OCH_2, H-6b, H-6a); 4.15 (dd, 1H, $J_{2,1} = 8.5$ Hz, $J_{2,3} = 11.0$ Hz, H-2); 4.35 (dd, 1H, $J_{3,2} = 11.0$ Hz, $J_{3,4} = 8.2$ Hz, H-3); 5.31 (d, 1H, $J_{1,2} = 8.5$ Hz, H-1); 7.70–7.85 (m, 4H, *Ph*). ESIMS: calcd for $C_{22}H_{32}N_4NaO_7Si$ $[M+Na]^+$ 515.19, found 515.1.

4.6. 2-Azidoethyl 3-*O*-benzoyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (7)

To a solution of **6** (354 mg, 0.719 mmol) in 15 mL of dry CH_2Cl_2 was added benzoyl chloride (92 μL, 0.791 mmol) in 5 mL of dry CH_2Cl_2, Ag_2O (250 mg, 1.08 mmol), KI (25.8 mg, 0.155 mmol) and 390 μL of pyridine. The mixture was stirred overnight, and then the solvent was removed. The residue was purified by silica gel flash chromatography (1:1 petroleum ether–EtOAc) to give product **7** (350 mg, 0.587 mmol, 81%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.12 (s, 3H, SiCH_3); 0.13 (s, 3H, SiCH_3); 0.92 (s, 3 × 3H, SiC(CH_3)$_3$); 3.19 (m, 1H, N_3CH_2); 3.37 (m, 1H, N_3CH_2); 3.63–3.75 (m, 2H, H-5, OCH_2); 3.91–4.05 (m, 4H, H-4, OCH_2, H-6b, H-6a); 4.43 (dd, 1H, $J_{2,1} = 8.2$ Hz, $J_{2,3} = 10.6$ Hz, H-2); 5.51 (d, 1H, $J_{1,2} = 8.2$ Hz, H-1); 5.90 (dd, 1H, $J_{3,2} = 10.6$ Hz, $J_{3,4} = 8.6$ Hz, H-3); 7.30–7.90 (m, 4H, *Ph*). ESIMS: calcd for $C_{29}H_{36}N_4NaO_8Si$ $[M+Na^+]$ 619.22, found 619.2.

4.7. 2-*O*-Acetyl-3,4,6-tri-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (9)

To a solution of 1,2-*O*-ethylidene-α-D-galactopyranoside (**8**, 250 mg, 1.21 mmol) in 15 mL of pyridine was added 3 mL of benzoyl chloride at 0 °C, and then the mixture was stirred at room temperature overnight. After the solvent was evaporated, the residue was dissolved in 80% CH_3CO_2H, and stirred for one day, followed by neutralization with Et_3N. After the solvent was removed, the residue was redissolved in a certain amount of EtOAc and water. The organic phase was separated from the water phase. The water phase was extracted two times with EtOAc. The combined organic layers were dried with Na_2SO_4. The solvent was evaporated, and the residue was purified by silica gel flash chromatography (1:2 petroleum ether–EtOAc) to give a pure product, which was dissolved in 15 mL of pyridine. After adding 10 mL of Ac_2O at 0 °C, the mixture was stirred at room temperature overnight. The solvent was evaporated, and the residue was purified by silica gel flash chromatography (3:2–1:1 petroleum ether–EtOAc) to give 1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzoyl-β-D-galactopyranoside, which was dissolved in 20 mL dry THF, and benzylamine (3 mL, 27.4 mmol) was added. The mixture was stirred at rt for 8 h and then neutralized with 2 N HCl. After the solvent was removed, the residue was dissolved in a mixture of EtOAc and water. The organic phase was separated from the water phase. The water phase was extracted two times with EtOAc. The combined organic layers were dried with Na_2SO_4. The solvent was evaporated, and the residue was purified by silica gel flash chromatography (1:1–1:2 petroleum ether–EtOAc) to give 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-β-D-galactopyranoside, which was dissolved in 15 mL of CH_2Cl_2. Then, 3 mL of trichloroacetonitrile and 3 g K_2CO_3 were added to the solvent, and the mixture was stirred overnight. After filtration, the mixture was concentrated. The residue was purified by silica gel flash chromatography (1:1–2:3 petroleum ether–EtOAc) to give product **9** (296 mg, 0.42 mmol). The yield over five steps was 35%.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.98 (s, 1H, O*Ac*); 4.42 (dd, 1H, $J_{6b,6a} = 11.3$ Hz, $J_{6b,5} = 6.9$ Hz, H-6b); 4.57 (dd, 1H, $J_{6a,6b} = 11.3$ Hz, $J_{6a,5} = 6.9$ Hz, H-6a); 4.80 (t, $J_{5,6a} = J_{5,6b} = 6.9$ Hz, H-5); 5.72 (dd, 1H, $J_{2,3} = 10.6$ Hz, $J_{2,1} = 3.4$ Hz, H-2); 5.85 (dd, 1H, $J_{3,2} = 10.6$ Hz, $J_{3,4} = 3.4$ Hz, H-3); 6.10 (d, 1H, $J_{4,3} = 2.7$ Hz, H-4); 6.78 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1); 7.28–8.09 (m, 15H, *Ph*CO); 8.71 (s, 1H, C=N*H*).

ESIMS: calcd for $C_{31}H_{26}Cl_3NNaO_{10}$ $[M+Na]^+$ 700.05, found 700.2.

4.8. Isopropyl 2,3-di-*O*-allyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (13)

To a solution of isopropyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**12**, 500 mg, 1.53 mmol) in 10 mL DMF was added allyl bromide (135 μL, 1.56 mmol) twice and NaH (74 mg, 3.07 mmol) at 0 °C. The mixture was stirred at 0 °C for 0.5 h, and then at rt for 4 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in a mixture of EtOAc and water. The organic phase was separated from the water phase. The water phase was extracted two times with EtOAc. The combined organic layers were dried with Na_2SO_4. The solvent was evaporated and the residue was purified

by silica gel flash chromatography (2:1–3:2 petroleum ether–EtOAc) to give **13** (527 mg, 1.23 mmol, 80%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (d, 3H, $J = 6.9$ Hz, CH_3); 1.37 (d, 3H, $J = 6.9$ Hz, CH_3); 3.28 (m, 1H, SC*H*); 3.38 (d, 1H, $J_{4,3} = 3.5$ Hz, H-4); 3.47 (dd, 1H, $J_{3,2} = 9.3$ Hz, $J_{3,4} = 3.5$ Hz, H-3); 3.68 (dd, 1H, $J_{2,1} = 9.3$ Hz, $J_{2,3} = 9.3$ Hz, H-2); 4.00 (dd, 1H, $J_{6b,5} = 2.1$ Hz, $J_{6b,6a} = 12.4$ Hz, H-6b); 4.19–4.39 (m, 6H, H-6b, H-5, 2 × C=CCH_2); 4.45 (d, 1H, $J_{1,2} = 9.3$ Hz, H-1); 5.17 (m, 2H, 2 × C*H*b=CC); 5.30 (m, 2H, 2 × C*H*a=CC); 5.51 (s, 1H, PhC*H*); 5.96 (m, 2H, 2 × C=C*H*C); 7.31–7.56 (m, 5H, *Ph*C).

ESIMS: calcd for $C_{22}H_{30}O_5NaS$ $[M+Na]^+$ 429.17, found 429.3.

4.9. 2-Azidoethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-2-phthalimido-6-*O*-tert-butyldimethylsilyl-β-D-glucopyranoside (14)

As described in the general procedure, **7** (74 mg, 0.124 mmol) and **9** (92.6 mg, 0.136 mmol) were coupled, and the product was purified by column chromatography with 2:1 petroleum ether–EtOAc as the eluent to give **14** (102 mg, 0.092 mmol, 67%) as a foamy solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.14 (s, 6H, 2 × SiCH_3); 0.91 (s, 3 × 3H, SiC(CH_3)$_3$); 1.98 (s, 3H, O*Ac*); 3.21 (m, 1H, N_3CH_2); 3.38 (m, 1H, N_3CH_2); 3.60–3.74 (m, 3H, H^I-5, OCH_2, H^{II}-6b); 3.84–4.04 (m, 5H, H^{II}-6a, H^{II}-5, H^I-6b, H^I-6a, OCH_2); 4.24 (dd, 1H, $J_{4,3} = 9.0$ Hz, $J_{4,5} = 9.6$ Hz, H^I-4); 4.43 (dd, 1H, $J_{2,1} = 8.6$ Hz, $J_{2,3} = 10.6$ Hz, H^I-2); 4.95 (d, 1H, $J_{1,2} = 7.9$ Hz, H^{II}-1); 5.22 (dd, 1H, $J_{3,2} = 10.3$ Hz, $J_{3,4} = 3.0$ Hz, H^{II}-3); 5.38 (d, 1H, $J_{2,1} = 7.9$ Hz, $J_{2,3} = 10.3$ Hz, H^{II}-2); 5.51 (d, 1H, $J_{1,2} = 8.6$ Hz, H^I-1); 5.71 (d, 1H, $J_{4,3} = 3.0$ Hz, H^{II}-4); 6.13 (dd, 1H, $J_{3,2} = 10.6$ Hz, $J_{3,4} = 9.0$ Hz, H^I-3); 7.10–8.10 (m, 24H, *Ph*). ESIMS: calcd for $C_{58}H_{60}N_4NaO_{17}Si$ $[M+Na]^+$ 1135.36, found 1135.1.

4.10. 2-Azidoethyl 3,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (15)

To a solution of **14** (350 mg, 0.314 mmol) in 10 mL of 1:1 MeOH–CH_2Cl_2 was added 500 μL of AcCl at 0 °C. The mixture was stirred at rt for 24 h and then neutralized with Et_3N. The solvent was evaporated, and the residue was dissolved in the mixture of EtOAc and water. The organic phase was separated from the water phase. The water phase was extracted two times with EtOAc. The combined organic layers were dried with Na_2SO_4. The solvent was evaporated, and the residue was purified by silica gel flash chromatography (1:1–1:2 petroleum ether–EtOAc) to give **15** (237 mg, 0.248 mmol, 79%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.21 (m, 1H, N_3CH_2); 3.38 (m, 1H, N_3CH_2); 3.60–3.74 (m, 3H, H^I-6b, OCH_2, H^I-6a); 3.81–3.93 (m, 2H, H^I-5, H^{II}-5); 4.01–4.19 (m, 4H, H^{II}-2, OCH_2, H^{II}-6b, H^{II}-6a); 4.33 (dd, 1H, $J_{4,3} = 9.0$ Hz, $J_{4,5} = 9.6$ Hz, H^I-4); 4.50 (dd, 1H, $J_{2,1} = 8.6$ Hz, $J_{2,3} = 10.6$ Hz, H^I-2); 4.82 (d, 1H, $J_{1,2} = 7.9$ Hz, H^{II}-1); 5.34 (dd, 1H, $J_{3,2} = 10.3$ Hz, $J_{3,4} = 3.0$ Hz, H^{II}-3); 5.61 (d, 1H, $J_{1,2} = 8.6$ Hz, H^I-1); 5.67 (d, 1H, $J_{4,3} = 3.0$ Hz, H^{II}-4); 6.24 (dd, 1H, $J_{3,2} = 10.6$ Hz, $J_{3,4} = 9.0$ Hz, H^I-3); 7.10–8.10 (m, 24H, *Ph*). ESIMS: calcd for $C_{50}H_{44}N_4NaO_{16}$ $[M+Na]^+$ 979.27, found 979.4.

4.11. 2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→6)-[3,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)]-3-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (16)

As described in the general procedure, **15** (237 mg, 0.248 mmol) and **11** (203 mg, 0.260 mmol) were coupled, and the product was purified by column chromatography with 1:1–1:2 petroleum ether–EtOAc as the eluent to give **16** (197 mg, 0.125 mmol, 50%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 1.96 (s, 3H, O*Ac*); 2.01 (s, 3H, O*Ac*); 2.03 (s, 3H, O*Ac*); 2.04 (s, 3H, O*Ac*); 2.08 (s, 3H, O*Ac*); 2.10 (s, 3H, O*Ac*); 2.12 (s, 3H, O*Ac*); 2.14 (s, 3H, O*Ac*); 3.22 (m, 1H, N_3CH_2); 3.40 (m, 1H, N_3CH_2); 3.53 (dd, 1H, $J_{6b,5} = 7.4$ Hz, $J_{6b,6a} = 11.8$ Hz; H^{II}-6b); 3.62–3.74 (m, 3H, H^{III}-5, H^{II}-5, OCH_2); 3.76-4.17 (m, 2H, H^{II}-6a, H^{III}-4, H^{IV}-5, H^I-5, H^I-6b, OCH_2, H^{II}-2, H^I-4, H^{III}-6b, H^{IV}-6b, H^{IV}-6a); 4.29 (dd, 1H, $J_{6a,5} = 1.2$ Hz, $J_{6a,6b} = 9.6$ Hz, H^I-6a); 4.48 (dd, 1H, $J_{2,1} = 8.9$ Hz, $J_{2,3} = 11.0$ Hz; H^I-2); 4.53 (d, 1H, $J_{1,2} = 8.3$ Hz, H^{IV}-1); 4.59 (d, 1H, $J_{1,2} = 7.6$ Hz, H^{II}-1); 4.64 (dd, 1H, $J_{6a,5} = 1.2$ Hz, $J_{6a,6b} = 10.3$ Hz, H^{III}-6a); 4.95–5.00 (m, 2H, H^{IV}-3, H^{III}-2); 5.11 (dd, 1H, $J_{2,1} = 8.3$ Hz, $J_{2,3} = 10.3$ Hz, H^{IV}-2); 5.21 (dd, 1H, $J_{3,2} = 8.9$ Hz, $J_{3,4} = 8.9$ Hz, H^{III}-3); 5.27 (dd, 1H, $J_{3,2} = 10.4$ Hz, $J_{3,4} = 3.5$ Hz, H^{II}-3); 5.35 (d, 1H, $J_{4,3} = 3.4$ Hz, H^{IV}-4); 5.52 (d, 1H, $J_{1,2} = 8.2$ Hz, H^I-1); 5.65 (d, 1H, $J_{4,3} = 2.8$ Hz, H^{II}-4); 6.15 (dd, 1H, $J_{3,2} = 11.0$ Hz, $J_{3,4} = 8.9$ Hz, H^I-3); 7.10-8.10 (m, 24H, *Ph*).

ESIMS: calcd for $C_{76}H_{79}N_4O_{33}$ $[M+H]^+$ 1597.44, found 1597.8.

4.12. 2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→6)-[2,3-di-*O*-allyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)]-3-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (17)

To a solution of the donor **13** (36 mg, 0.086 mmol) and the tetrasaccharide acceptor **16** (90 mg, 0.057 mmol) in

10 mL dry CH_2Cl_2 was added 50 mg of dry 4 Å molecular sieves under argon. The mixture was stirred at rt for 0.5 h, followed by the addition of 10 μL of TMSOTf and NIS (24 mg, 0.107 mmol) at −10 °C. The mixture was stirred at rt for 2 h, neutralized with Et_3N and filtered. The filtrate was concentrated. The residue was purified by silica gel flash chromatography (1:1–1:2 petroleum ether–EtOAc) to give **17** (75 mg, 0.039 mmol, 68%).

^{1}H NMR (600 MHz, $CDCl_3$): δ 1.97 (s, 3H, O*Ac*); 2.05 (s, 6H, 2 × O*Ac*); 2.06 (s, 3H, O*Ac*); 2.15 (s, 6H, 2 × O*Ac*); 2.17 (s, 3H, O*Ac*); 3.16 (d, 1H, $J_{6b,6a}$ = 12.6 Hz, H^V-6b); 3.27 (m, 1H, N_3CH_2); 3.38–3.44 (m, 2H, H^V-5, N_3CH_2); 3.56–3.64 (m, 2H, H^{III}-5, OCH_2) 3.75 (d, 1H, $J_{4,3}$ = 3.5 Hz, H^V-4); 3.86–3.94 (m, 4H, H^{III}-4, H^I-6b, H^{IV}-5 H^V-2); 4.00–4.35 (m, 14H, H^{II}-6a, H^I-5, H^{II}-5, H^{III}-6b, 2 × C=CCH_2, OCH_2, H^{II}-2, H^I-4, H^I-6a, H^{IV}-6b, H^{IV}-6a); 4.47 (dd, 1H, J_1 = 8.9 Hz, $J_{2,3}$ = 11.0 Hz, H^I-2); 4.52 (d, 1H, $J_{1,2}$ = 8.1 Hz, H^{IV}-1); 4.58 (d, 1H, $J_{6a,6b}$ = 11.6 Hz, H^{III}-6a); 4.69 (d, 1H, $J_{1,2}$ = 8.06 Hz, H^{II}-1); 4.79 (d, 1H, $J_{1,2}$ = 8.2 Hz, H^{III}-1); 4.96–5.00 (m, 2H, H^{IV}-3, H^{III}-2); 5.10–5.14 (m, 2H, H^{IV}-2, C*H*=CC); 5.19 (s, 1H, PhC*H*); 5.20–5.26 (m, 2H, H^{III}-3, C*H*=CC); 5.32–5.42 (m, 4H, C*H*=CC, H^{IV}-4, CH=C*H*, H^V-1); 5.47–5.53 (m, 2H, H^I-1, H^{II}-3); 5.67 (d, 1H, $J_{4,3}$ = 3.5 Hz, H^{II}-4); 5.84–6.02 (m, 2H, 2 × C=C*H*C); 6.05 (dd, 1H, $J_{3,2}$ = 11.0 Hz, $J_{3,4}$ = 9.1 Hz, H^I-3); 7.16–8.03 (m, 29H, *Ph*). ESIMS: calcd for $C_{95}H_{100}N_4NaO_{38}$ $[M+Na]^+$ 1927.59, found 1927.6.

4.13. 2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→4)]-3-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (18)

To a solution of pentasaccharide **17** (70 mg, 0.037 mmol) in 5 mL of CH_2Cl_2 were added 400 μL of CF_3CO_2H and 250 μL of water. The mixture was stirred at rt for 24 h, followed by neutralization with Et_3N. After the solvent was evaporated, the residue was dissolved in the mixture of EtOAc and water. The organic phase was separated from the water phase. The water phase was extracted two times with EtOAc. The combined organic layers were dried with Na_2SO_4. The solvent was evaporated, and the crude product was dissolved in 5 mL of pyridine, followed by the addition of 5 mL of Ac_2O at 0 °C. The mixture was stirred at rt overnight and then concentrated. The residue was purified by silica gel flash chromatography (1:1–1:2 petroleum ether–EtOAc) to give an intermediate product, which was dissolved in 10 mL of 1:1 MeOH–CH_2Cl_2. After the addition a catalytic amount of $PdCl_2$, the mixture was stirred at rt for 8 h. After the filtration of the catalyst, the filtrate was concentrated. The residue was dissolved in 5 mL of pyridine, followed by the addition of 5 mL of Ac_2O at 0 °C. The mixture was stirred at rt overnight and then concentrated. The residue was purified by silica gel flash chromatography (1:1–1:2 petroleum ether–ethyl acetate) to give **18** (61 mg, 0.032 mmol). The total yield was 86%.

^{1}H NMR (600 MHz, $CDCl_3$): δ 1.80 (s, 3H, O*Ac*); 1.969 (s, 3H, O*Ac*); 1.973 (s, 3H, O*Ac*); 2.04 (s, 3H, O*Ac*); 2.061 (s, 3H, O*Ac*); 2.065 (s, 3H, O*Ac*); 2.07 (s, 3H, O*Ac*); 2.16 (s, 6H, 2 × O*Ac*); 2.18 (s, 3H, O*Ac*); 2.28 (s, 3H, O*Ac*); 3.25 (m, 1H, N_3CH_2); 3.41 (m, 1H, $J_{6b,5}$ = 8.2 Hz, $J_{6b,6a}$ = 11 Hz, N_3CH_2); 3.66–3.70 (m, 3H, H^{III}-5, OCH_2, H^I-6b); 3.76–3.79 (m, 2H, H^I-5, H^V-5); 3.87–3.96 (m, 3H, H^{II}-3, H^{IV}-5, H^{III}-4); 4.02–4.18 (m, 9H, H^{II}-6a, H^I-6a, OCH_2, H^{IV}-6b, H^{IV}-6a, H^V-6b, H^V-6a, H^{III}-6b, H^{II}-5); 4.28 (t, 1H, $J_{4,3}$ = $J_{4,5}$ = 9 Hz, H^I-4); 4.48 (dd, 1H, $J_{2,1}$ = 8.2 Hz, $J_{2,3}$ = 11.0 Hz, H^I-2); 4.56 (d, 1H, $J_{1,2}$ = 7.6 Hz, H^{IV}-1); 4.66 (dd, 1H, $J_{6a,5b}$ = 2.0 Hz, $J_{6a,6b}$ = 12.4 Hz, H^{III}-6a); 4.78 (d, 2H, $J_{1,2}$ = 7.6 Hz, H^{II}-1, H^{III}-1); 4.97–5.02 (m, 2H, H^{III}-2, H^{IV}-3); 5.06 (dd, 1H, $J_{3,2}$ = 11.0 Hz, $J_{3,4}$ = 3.4 Hz, H^V-3); 5.12–5.16 (m, 2H, H^{IV}-2, H^V-1); 5.18 (dd, $J_{2,1}$ = 2.8 Hz, $J_{2,3}$ = 11.0 Hz, 1H, H^V-2); 5.24 (t, 1H, $J_{3,2}$ = $J_{3,4}$ = 9.0 Hz, H^{III}-3); 5.35 (d, 1H, $J_{4,3}$ = 3.4 Hz, H^V-4); 5.36 (d, 1H, $J_{4,3}$ = 2.8 Hz, H^{IV}-4); 5.47 (d, 1H, $J_{1,2}$ = 8.2 Hz, H^I-1); 5.49 (dd, 1H, $J_{3,2}$ = 10.3 Hz, $J_{3,4}$ = 3.5 Hz, H^{II}-3); 5.68 (d, 1H, $J_{4,3}$ = 3.5 Hz, H^{II}-4); 6.08 (dd, 1H, $J_{3,2}$ = 11.0 Hz, $J_{3,\ 4}$ = 9.0 Hz, H^I-3); 7.16–8.03 (m, 29H, *Ph*). ESIMS: calcd for $C_{90}H_{96}N_4NaO_{42}$ $[M+Na]^+$ 1927.54, found 1927.7.

4.14. 2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)]-2-acetamido-3-*O*-acetyl-2-deoxy-β-D-glucopyranoside (19)

To a solution of **18** (50 mg, 0.026 mmol) in 5 mL of MeOH was added NaOMe (pH 10), and the mixture was stirred for 24 h. After neutralization with Dowex 50WX8 (H^+) and filtration, the mixture was concentrated. To a solution of the residue in 10 mL of 1-BuOH was added 2 mL of 1,2-diaminoethane, and the mixture was stirred overnight at 80 °C, then, co-concentrated with toluene. A solution of the residue in 10 mL of pyridine and 10 mL of Ac_2O was stirred overnight, then co-concentrated with toluene. The residue was purified by silica gel flash chromatography (1:1–1:2 petroleum ether–EtOAc) to give **19** (32 mg, 0.020 mmol). The total yield was 77%.

^{1}H NMR (600 MHz, $CDCl_3$): δ 1.95 (s, 3H, NH*Ac*); 1.97 (s, 3H, O*Ac*); 1.987 (s, 3H, O*Ac*); 1.991 (s, 3H, O*Ac*); 2.047 (s, 6H, 2 × O*Ac*); 2.055 (s, 3H, O*Ac*); 2.06 (s, 9H, 3 × O*Ac*); 2.09 (s, 3H, O*Ac*); 2.13 (s, 3H,

O*Ac*); 2.14 (s, 6H, 2 × O*Ac*); 2.15 (s, 3H, O*Ac*); 2.16 (s, 3H, O*Ac*); 3.32 (m, 1H, N_3CH_2); 3.46–3.53 (m, 2H, N_3CH_2, H^{III}-5); 3.63–3.69 (m, 3H, OCH_2, H^{II}-2, H^{III}-4, OCH_2); 3.78 (m, 1H, H^{I}-2); 3.88–4.18 (m, 14H, H^{II}-6b, H^{II}-5, H^{I}-6b, H^{I}-5, OCH_2, H^{IV}-6b, H^{IV}-6a, H^{IV}-5, H^{V}-6b, H^{V}-6a, H^{V}-5, H^{III}-6b, H^{III}-6a, H^{I}-4); 4.28 (m, 1H, H^{II}-6a); 4.52–4.57 (m, 3H, H^{IV}-1, H^{II}-1, H^{I}-6a); 4.68 (d, 1H, $J_{1,2} = 6.8$ Hz, H^{III}-1); 4.71 (d, 1H, $J_{1,2} = 7.6$ Hz, H^{I}-1); 4.70 (dd, 1H, $J_{2,1} = 6.8$ Hz, $J_{2,3} = 8.1$ Hz, H^{III}-2); 4.97 (dd, 1H, $J_{3,2} = 10.3$ Hz, $J_{3,4} = 3.4$ Hz, H^{IV}-3); 5.05 (dd, 1H, $J_{3,2} = 9.7$ Hz, $J_{3,4} = 4.1$ Hz, H^{II}-3); 5.07 (dd, 1H, $J_{2,1} = 3.4$ Hz, $J_{2,3} = 10.3$ Hz, H^{V}-2); 5.12 (dd, 1H, $J_{2,3} = 10.3$ Hz, $J_{2,1} = 8.2$ Hz, H^{IV}-2); 5.16–5.24 (m, 3H, H^{III}-3, H^{V}-3, H^{I}-3); 5.35–5.32 (m, 3H, H^{IV}-4, H^{II}-4, H^{V}-1); 5.61 (d, 1H, $J_{NH,HI-2} = 8.3$ Hz). ESIMS: calcd for $C_{64}H_{88}N_4NaO_{41}$ $[M+Na]^+$ 1591.48, found 1591.9.

4.15. 2-Azidoethyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-[α-D-galactopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

To a solution of **19** (30 mg, 0.019 mmol) in 5 mL of MeOH was added NaOMe (pH 10), and the mixture was stirred for 48 h. After neutralization with Dowex 50WX8 (H^+) and filtration, the mixture was concentrated. The residue was eluted on a Sephadex LH-20 column with MeOH to give **1** (15 mg, 0.016 mmol, 84%) as a white solid.

^{1}H NMR (300 MHz, D_2O): δ 1.95 (s, 3H, NH*Ac*); 3.25–4.04 (m, 20H); 4.17–4.24 (m, 2H); 4.38 (d, 1H, $J = 7.6$ Hz); 4.46 (d, 1H, $J = 8.2$ Hz); 4.50 (d, 1H, $J = 7.9$ Hz); 4.60–4.80 (m, H_2O); 5.32 (d, 1H, $J = 3.7$ Hz). ESIMS: calcd for $C_{34}H_{58}N_4NaO_{26}$ $[M+Na]^+$ 961.32, found 961.5.

HRESIMS: calcd for $C_{34}H_{59}N_4O_{26}$ $[M+H]^+$ 939.34120, found 939.34215.

Acknowledgements

This work was supported by The National Natural Science Foundation of China (Projects 20472041 and 20532020).

Supplementary data

NMR spectra of **7**, **14**, **16**, **17**, **18**, **19** and **1**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.12.002.

References

1. Fine, M. J.; Smith, M. A.; Carson, C. A.; Mutha, S. S.; Sankey, S. S.; Weissfeld, L. A.; Kapoor, W. N. *J. Am. Med. Assoc.* **1996**, *275*, 134–141.
2. Robbins, J. B.; Austrian, R.; Lee, C.-J.; Rastogi, S. C.; Schiffman, G.; Henrichsen, J.; Makela, P. H.; Broome, C. V.; Facklam, R. R.; Tiesjema, R. H.; Parke, J. C. *J. Infect. Dis.* **1983**, *148*, 1136–1159.
3. Lee, C. J.; Wang, T. R. *Crit. Rev. Microbiol.* **1994**, *20*, 1–12.
4. Alonsodevelasco, E.; Verheul, A. F. M.; Verhoef, J.; Snippe, H. *Microbiol. Rev.* **1995**, *59*, 591–603.
5. Kamerling, J. P. In *Streptococcus pneumoniae*; Mary Ann Liebert: New York, 2000.
6. Rennels, M. B.; Edwards, K. M.; Keyserling, H. L.; Reisinger, K. S.; Hogerman, D. A.; Madore, D. V.; Chang, I.; Paradiso, P. R.; Malinoski, F. J.; Kimura, A. *Pediatrics* **1998**, *101*, 604–611.
7. Jansen, W. T. M.; Gootjes, J.; Zelle, M.; Madore, D. V.; Verhoef, J.; Snippe, H.; Verheul, A. F. M. *Clin. Diagn. Lab. Immunol.* **1998**, *5*, 703–710.
8. Benaissa-Trouw, B.; Lefeber, D. J.; Kamerling, J. P.; Vliegenthart, J. F. G.; Kraaijeveld, K.; Snippe, H. *Infect. Immun.* **2001**, *69*, 4698–4701.
9. Niggemann, J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Bioorg. Med. Chem.* **1998**, *6*, 1605–1612.
10. Niggemann, J.; Kamerling, J. P.; Vliegenthart, J. F. G. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3011–3020.
11. Joosten, J. A. F.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **2003**, *338*, 2611–2627.
12. Joosten, J. A. F.; Lazet, B. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **2003**, *338*, 2629–2651.
13. Michalik, D.; Vliegenthart, J. F. G.; Kamerling, J. P. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1973–1981.
14. Mawas, F.; Niggemann, J.; Jones, C.; Corbel, M. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Infect. Immun.* **2002**, *70*, 5107–5114.
15. Jones, C.; Lemercinier, X. *Carbohydr. Res.* **2005**, *340*, 403–409.
16. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
17. Grundler, G.; Schmidt, R. R. *Carbohydr. Res.* **1985**, *135*, 203–218.
18. Wang, H. S.; She, J.; Zhang, L. H.; Ye, X. S. *J. Org. Chem.* **2004**, *69*, 5774–5777.
19. Betaneli, V. I.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1980**, *84*, 211–224.
20. Koeman, F. A. W.; Meissner, J. W. G.; Vanritter, H. R. P.; Kamerling, J. P.; Vliegenthart, J. F. G. *J. Carbohydr. Chem.* **1994**, *13*, 1–25.
21. Du, Y. G.; Gu, G. F.; Wei, G. H.; Hua, Y. X.; Linhardt, R. J. *Org. Lett.* **2003**, *5*, 3627–3630.