Synthesis of a hexasaccharide fragment of the capsular polysaccharide of *Streptococcus pneumoniae* type 3

Dirk J. Lefeber, Eneko Aldaba Arévalo, Johannis P. Kamerling, and Johannes F.G. Vliegenthart

Abstract: In the framework of the development of a new generation of neoglycoconjugate vaccines against *Streptococcus pneumoniae*, the synthesis is described of a spacer-containing hexasaccharide fragment related to the capsular polysaccharide of *S. pneumoniae* type 3. Hexasaccharide β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→O-$(CH_2)_3NH_2$) (**1**), comprised of three repeating units, was synthesized via a blockwise strategy employing suitably protected disaccharide building blocks. Carboxylic groups were introduced by selective oxidation with TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) in the last reaction steps. Deprotection afforded target hexasaccharide **1**.

Key words: oligosaccharide synthesis, *Streptococcus pneumoniae* type 3, TEMPO oxidation.

Résumé : Dans le cadre du développement d'une nouvelle génération de vaccins néoglycoconjugués actifs contre le *Streptococcus pneumoniae* et faisant appel à une stratégie de synthèse par blocs à l'aide de disaccharides protégés de façon appropriée, on a réalisé la synthèse d'un fragment hexasaccharidique apparenté au polysaccharide capsulaire du *S. pneumoniae* de type 3 et contenant un espaceur, soit le β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→O-$(CH_2)_3NH_2$) (**1**) qui trois motifs. Les groupes carboxyliques ont été introduits en procédant à une oxydation sélective à l'aide de TEMPO (2,2,6,6-tétraméthyl-1-pipéridinyloxy) dans les dernières étapes réactionnelles. La déprotection fournit l'hexasaccharide recherché, **1**.

Mots clés : synthèse d'oligosaccharide, *Streptococcus pneumoniae* de type 3, oxydation par TEMPO.

[Traduit par la Rédaction]

Introduction

Diseases caused by encapsulated bacteria can be prevented by vaccination with neoglycoconjugate vaccines as proven for *Haemophilus influenzae* type b (1), *Neisseria meningitidis* type C (2), and *Streptococcus pneumoniae* serotypes (3). These vaccines are prepared by conjugation of an isolated polysaccharide or a mixture of polysaccharide-derived oligosaccharides to a protein carrier. For a detailed investigation of the immune response to these types of vaccines, neoglycoproteins with a well-defined carbohydrate structure need to be available.

Neoglycoconjugates related to *S. pneumoniae* type 3 have been prepared by coupling di-, tri-, or tetrasaccharide fragments in several carbohydrate–protein ratios to a nontoxic mutant of diphtheria toxin (CRM_{197}) (4). While immunization experiments with these conjugates in mice showed that increasing IgG antibody titres were found with increasing oligosaccharide chain length, no difference was found with respect to the oligosaccharide loading (5).

The use of conjugates that contain larger oligosaccharide fragments than in the study described above might lead to an improved immunogenicity. Furthermore, human antibodies can better recognize larger epitopes on the polysaccharide than do animal antibodies, as shown for neoglycoproteins consisting of synthetic oligosaccharides related to the capsular polysaccharide of *S. pneumoniae* type 23F (6). For these reasons, a program was started for the synthesis of a *S. pneumoniae* type 3 related hexasaccharide fragment (**1**), comprised of three cellobiuronic acid repeating units (Fig. 1).

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Dedicated to the memory of Ray Lemieux, one of the most inspiring glycoscientists.

D.J. Lefeber, E. Aldaba Arévalo, J.P. Kamerling, and J.F.G. Vliegenthart.[1] Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, P.O. Box 80.075, NL-3508 TB Utrecht, The Netherlands.

[1]Corresponding author (fax: (+31) 30 2 540 980; e-mail: j.f.g.vliegenthart@chem.uu.nl).

Results and discussion

The synthesis for tetrasaccharide acceptor **11** was previously described (4). Two disaccharide donors (**6** and **10**, Scheme 1) were tested for the ability to couple with **11** to afford protected hexasaccharide **12** (Scheme 2).

For the synthesis of disaccharide thioethyl donor **6**, first monosaccharide acceptor **4** was prepared by debenzylidenation of **2** (7) using trifluoroacetic acid (→**3**, 88%) and subsequent selective benzoylation using benzoyl chloride

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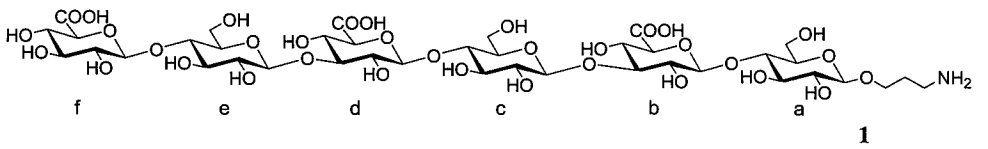
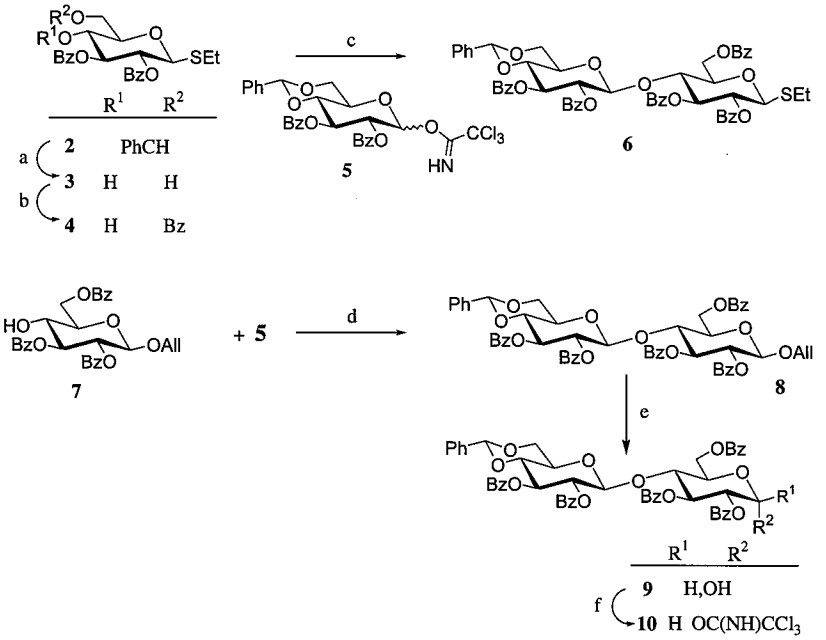
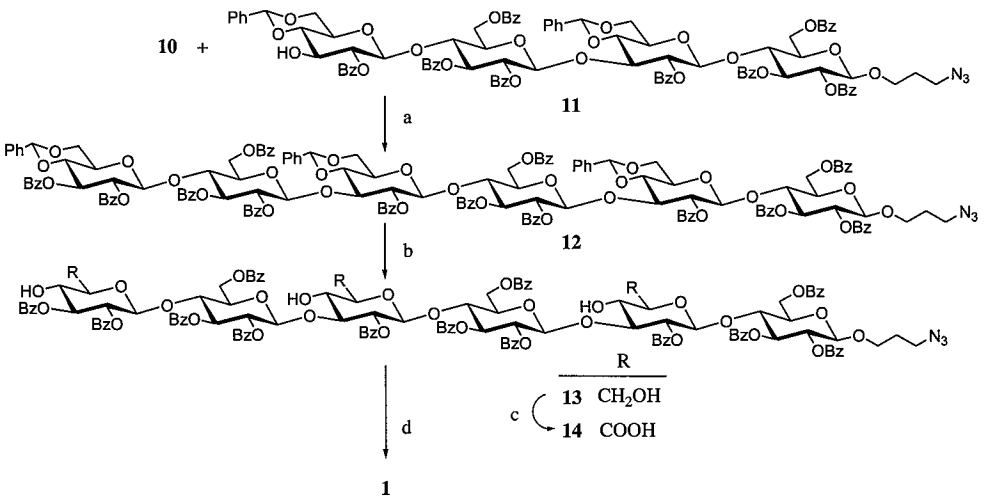
Fig. 1. Target hexasaccharide **1** comprising three repeating units of the capsular polysaccharide of *S. pneumoniae* type 3.



Scheme 1. Synthesis of disaccharide donors **6** and **10**. Reagents and conditions: (*a*) CF_3COOH, H_2O, CH_2Cl_2, 88%; (*b*) PhCOCl, Et_3N, CH_2Cl_2, 50%; (*c*) 10% TMSOTf, CH_2Cl_2, 75%; (*d*) 11% TMSOTf, CH_2Cl_2, 83%; (*e*) (*i*) $(PPh_3)_3Rh(I)Cl$, DABCO, $CH_3C_6H_5$–EtOH–CH_2Cl_2 (10:5:1), (*ii*) NIS, H_2O, THF, 68%; (*f*) Cl_3CCN, DBU, CH_2Cl_2, 86%.



Scheme 2. Synthesis of hexasaccharide **1**. Reagents and conditions: (*a*) 10% TMSOTf, CH_2Cl_2, 63%; (*b*) CF_3COOH, H_2O, CH_2Cl_2, 75%; (*c*) TEMPO, aqueous NaOCl, KBr, Bu_4NBr, aqueous NaCl, aqueous $NaHCO_3$, CH_2Cl_2, 70%; (*d*) (*i*) NaOMe, MeOH (pH 10), (*ii*) $NaBH_4$, 10% Pd/C, 0.05 M NaOH, 61%.



(→**4**, 50%). Coupling of **4** with imidate donor **5** (4) using 10% TMSOTf as a promoter gave **6** in a yield of 75%. For the synthesis of disaccharide imidate donor **10**, first **8** was prepared in 83% yield by coupling of acceptor **7** (4) and donor **5** with 11% TMSOTf as a promoter. Then, deallylation of **8** using tris(triphenylphosphine)rhodium(I) chloride and *N*-iodosuccinimide (→**9**, 68%) and subsequent trichloroacetimidation gave **10** (86%).

For the preparation of protected hexasaccharide **12** (Scheme 2), tetrasaccharide acceptor **11** (4) was coupled

Table 1. 500 MHz ^{1}H NMR data of **1** at 305 K (in ppm).

	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
Glc[a]	4.50 (7.8)	3.33	3.64	3.61	n.d.[a]	3.98	3.80
GlcA[b]	4.53 (8.1)	3.56	3.79	n.d.	n.d.		
Glc[c]	4.82 (8.0)	3.38	3.65	3.62	n.d.	3.98	3.80
GlcA[d]	4.53 (8.0)	3.56	3.79	n.d.	n.d.		
Glc[e]	4.82 (8.0)	3.38	3.65	3.62	n.d.	3.98	3.80
GlcA[f]	4.50 (8.2)	3.36	3.51	3.51	3.75		

Note: The signals for the 3-aminopropyl spacer are: δ = 4.05 and 3.83 ($OCH_2CH_2CH_2NH_2$), 3.16 ($OCH_2CH_2CH_2NH_2$), 2.01 ($OCH_2CH_2CH_2NH_2$). $J_{1,2}$ couplings are presented in parentheses.
[a]n.d. = not determined.

with donor **6** using *N*-iodosuccinimide and triflic acid as a promoter system. Low yields of product (<8%) were obtained when the reaction was performed at 0°C or lower temperatures. Hexasaccharide **12** could, however, be obtained in a yield of 63% by coupling **11** with disaccharide imidate donor **10** using 10% TMSOTf as a promoter at room temperature. After debenzylidenation of **12** using trifluoroacetic acid (→**13**, 75%), the free primary hydroxyl functions were oxidized. Treatment of **13** with TEMPO and sodium hypochlorite (8) was found to be efficient for the oxidation of the three primary hydroxyl groups in the presence of secondary functions, and **14** was obtained in 70% yield. ^{1}H NMR analysis after methylation with diazomethane showed three singlets at δ = 3.39, 3.30, and 3.23 ($COOCH_3$). Debenzoylation of **14** with sodium methoxide and reduction of the azide using sodium borohydride and 10% Pd/C afforded target hexasaccharide **1** in 61% yield. The identity of the compound was established by ^{1}H NMR spectroscopy (Table 1) and mass spectrometry.

In conclusion, a versatile synthetic route was developed using a block wise strategy for the preparation of hexasaccharide fragment **1**, representing three repeating units of the capsular polysaccharide of *S. pneumoniae* type 3. The carboxylic acids were efficiently introduced by selective oxidation using TEMPO.

Experimental section

General

All chemicals were of reagent grade and were used without any further purification. Reactions were monitored by TLC on Silica gel 60 F254 (Merck); compounds were visualized, after examination under UV light, by heating with 10% (v/v) ethanolic H_2SO_4, orcinol (2 mg mL^{-1}) in 20% (v/v) methanolic H_2SO_4, or ninhydrin (1.5 mg mL^{-1}) in BuOH–H_2O–HOAc (38:1.75:0.25). In the work-up procedures of the reaction mixtures, the organic solutions were washed with appropriate amounts of the indicated aqueous solutions, then dried ($MgSO_4$), and concentrated under reduced pressure at 20–40°C on a water bath. Column chromatography was performed on Silica gel 60 (Merck, 0.063–0.200 mm). Optical rotations were measured in $CHCl_3$, unless stated otherwise, with a PerkinElmer 241 polarimeter, using a 10 cm, 1 mL cell. ^{1}H NMR spectra in $CDCl_3$ were recorded at 27°C with a Bruker AC 300 spectrometer; the δ values are given in ppm relative to the signal for internal Me_4Si (δ = 0). ^{13}C (APT, 75 MHz) NMR spectra in $CDCl_3$ were recorded at 27°C with a Bruker AC 300 spectrometer; indicated ppm values for δ are relative to the signal of $CDCl_3$ (δ = 76.9). The ^{1}H NMR spectrum of **1** was recorded in D_2O at 32°C with a Bruker AMX 500 spectrometer, and the δ values are given in ppm relative to the signal for internal acetone (δ = 2.225). Two-dimensional TOCSY and ROESY spectra were recorded using a Bruker AMX 500 apparatus (500 MHz) to assign the spectra of compounds **1**, **12**, **13**, and **14**. Residues *a–f* (Fig. 1) were assigned on the basis of interglycosidic ROESY cross-peaks. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) spectra were obtained on a Voyager-DE™ mass spectrometer using 2,4-dihydroxybenzoic acid (DHB) in H_2O as a matrix. Elemental analyses were carried out by H. Kolbe Mikroanalytisches Laboratorium (Mülheim an der Ruhr, Germany).

Ethyl 2,3-di-O-benzoyl-1-thio-β-D-glucopyranoside (3)

To a solution of ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**2**) (7) (1.37 g, 2.63 mmol) in CH_2Cl_2 (36 mL) were added CF_3COOH (1.5 mL) and H_2O (0.2 mL). The mixture was stirred for 16 h, diluted with CH_2Cl_2, washed with 10% (w/v) aqueous $NaHCO_3$ until neutral pH and 10% (w/v) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (CH_2Cl_2–MeOH, 98:2) to obtain **3** (1.0 g, 88%). $[\alpha]_D^{20}$ +76 (*c* = 1). R_f = 0.05 (CH_2Cl_2–EtOAc, 9:1). ^{1}H NMR ($CDCl_3$) δ: 7.97–7.93 and 7.53–7.34 (2m, 10H, 2*Ph*CO), 4.74 (d, $J_{1,2}$ = 9.8 Hz, 1H, H-1), 2.76–2.73 (m, 2H, SCH_2CH_3), 1.26 (t, 3H, SCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ: 167.4 and 165.2 (2PhCO); 129.1 and 128.7 (2*Ph*CO, quaternary C); 83.6 (C-1); 79.9, 78.2, 70.1, and 69.9 (C-2–C-5); 62.3 (C-6); 24.3 (SCH_2CH_3); 14.8 (SCH_2CH_3). Anal. calcd. for $C_{22}H_{24}O_7S$ (432.4): C 61.11, H 5.59; found C 60.99, H 5.74.

Ethyl 2,3,6-tri-O-benzoyl-1-thio-β-D-glucopyranoside (4)

To a solution of **3** (0.95 g, 2.2 mmol) in CH_2Cl_2 (50 mL) and Et_3N (0.37 mL) at 0°C was added dropwise a solution of benzoyl chloride (0.27 mL, 2.3 mmol) in CH_2Cl_2 (10 mL). After stirring for 24 h, the mixture was diluted with CH_2Cl_2, washed with 10% (w/v) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The crude residue was purified by column chromatography (CH_2Cl_2–EtOAc, 95:5) to give **4** (0.6 g, 50%). $[\alpha]_D^{20}$ +60 (*c* = 1). R_f = 0.51 (CH_2Cl_2–EtOAc, 9:1). ^{1}H NMR ($CDCl_3$) δ: 8.05–7.91 and 7.46–7.28 (2m, 15H, 3*Ph*CO), 5.60 and 5.47 (2t, each 1H, H-2,3), 4.82 (d, $J_{1,2}$ = 9.9 Hz, 1H, H-1), 2.82–2.64 (m, 2H, SCH_2CH_3), 1.24 (t, 3H, SCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ: 166.8, 166.7, and 165.2 (3PhCO); 83.4 (C-1); 78.2, 77.2, 70.2, and 69.3 (C-2–C-5); 63.6 (C-6); 24.1 (SCH_2CH_3); 14.8 (SCH_2CH_3). Anal. calcd. for $C_{29}H_{28}O_8S$ (536.5): C 64.92, H 5.26; found C 65.05, H 5.31.

Ethyl (2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-1-thio-β-D-glucopyranoside (6)

A solution of **4** (50 mg, 0.09 mmol) and 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-D-glucopyranosyl trichloroacetimidate **5** (4) (87 mg, 0.14 mmol) in dry CH_2Cl_2 (2 mL), containing 4 Å molecular sieves, was stirred under Ar for 1 h. Then, TMSOTf (2.5 μL, 14 μmol) was added. After 30 min, the

mixture was neutralized with dry pyridine, filtered over cotton, diluted with CH_2Cl_2, and washed with 10% (w/v) aqueous NaCl. The organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene–EtOAc, 95:5) to obtain **6** (67 mg, 75%). $[\alpha]_D^{20}$ +41 (c = 1). R_f = 0.76 (CH_2Cl_2–EtOAc, 9:1). ^{1}H NMR ($CDCl_3$) δ: 8.05–7.85 and 7.49–7.31 (2m, 30H, *Ph*CH, 5*Ph*CO), 5.74 and 5.61 (2t, $J_{1,2}$ = 9.9 Hz, each 1H, H-2,3), 5.44 (dd, $J_{1',2'}$ = 7.7, $J_{2',3'}$ = 9.5 Hz, 1H, H-2′), 5.42 (t, 1H, H-3′), 5.20 (s, 1H, PhC*H*), 4.83 (d, 1H, H-1′), 4.68 (d, 1H, H-1), 4.49 (dd, $J_{5',6a'}$ = 2.1, $J_{6a',6b'}$ = 12.2 Hz, 1H, H-6a), 4.40 (dd, $J_{5',6b'}$ = 4.8 Hz, 1H, H-6b), 2.70-2.56 (m, 2H, S$CH_2$$CH_3$), 1.16 (t, 3H, SCH_2CH_3). ^{13}C NMR ($CDCl_3$) δ: 136.4 (*Ph*CH, quaternary C); 101.7 and 101.1 (PhCH, C-1′); 83.3 (C-1); 67.5 (C-6′); 62.5 (C-6); 24.2 (S$CH_2$$CH_3$); 14.8 ($SCH_2CH_3$). Anal. calcd. for $C_{56}H_{50}O_{15}S$ (995.0): C 67.59, H 5.06; found C 67.56, H 5.01.

Allyl (2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (8)

A solution of allyl 2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside **7** (4) (0.48 g, 0.93 mmol) and 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-D-glucopyranosyl trichloroacetimidate **5** (4) (1.02 g, 1.64 mmol) in dry CH_2Cl_2 (15 mL), containing 4 Å molecular sieves, was stirred under Ar for 1 h. Then, TMSOTf (35 µL, 0.19 mmol) was added. After 45 min, the mixture was neutralized with dry pyridine, filtered over cotton, diluted with CH_2Cl_2, and washed with 10% (w/v) aqueous NaCl. The organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene–EtOAc, 95:5) to obtain **8** (0.76 g, 83%). $[\alpha]_D^{20}$ +27 (c = 1). R_f = 0.37 (toluene–EtOAc, 9:1). ^{1}H NMR ($CDCl_3$) δ: 8.05–7.85 and 7.50–7.25 (2m, 30H, *Ph*CH, 5*Ph*CO), 5.78–5.65 (m, 1H, OCH_2CH=CH_2), 5.72 and 5.60 (2t, $J_{2,3}$ = 9.6, $J_{2',3'}$ = 9.6 Hz, each 1H, H-3,3′), 5.43 and 5.40 (2dd, $J_{1,2}$ = 7.7, $J_{1',2'}$ = 7.8 Hz, each 1H, H-2,2′), 5.21 (s, 1H, PhC*H*), 5.17–5.10 and 5.08–5.03 (2m, each 1H, OCH_2CH=CH_2), 4.83 and 4.72 (2d, each 1H, H-1,1′), 4.49 (dd, $J_{5,6a}$ = 2.1, $J_{6a,6b}$ = 12.1 Hz, 1H, H-6a), 4.39 (dd, $J_{5,6b}$ = 4.5 Hz, 1H, H-6b), 4.27–4.20 and 4.07–3.99 (2m, each 1H, O$CH_2$$CH$=$CH_2$), 4.11 and 3.63 (2t, each 1H, H-4,4′), 3.76 (ddd, 1H, H-5), 3.61 (dd, $J_{5',6a'}$ = 4.6, $J_{6a',6b'}$ = 10.6 Hz, 1H, H-6a′), 3.31 (ddd, 1H, H-5′), 2.83 (t, $J_{5',6b'}$ = 10.6 Hz, 1H, H-6b′). ^{13}C NMR ($CDCl_3$) δ: 165.4, 165.2, 165.0, 164.9, and 164.7 (5PhCO); 136.4 (*Ph*CH, quaternary C); 117.5 (OCH_2CH=CH_2); 101.7, 101.0, and 99.2 (PhCH, C-1,1′); 69.7 (O$CH_2$$CH$=$CH_2$); 67.4 (C-6′); 62.2 (C-6). Anal. calcd. for $C_{57}H_{50}O_{16}$ (991.0): C 69.08, H 5.08; found C 69.16, H 5.05.

(2,3-Di-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-D-glucopyranose (9)

To a solution of **8** (1.5 g, 1.5 mmol) in absolute EtOH (40 mL), toluene (80 mL), and CH_2Cl_2 (8 mL) were added a catalytic amount of diazabicyclo[2.2.2]octane (DABCO) and tris(triphenylphosphine)rhodium(I) chloride (0.4 g). After stirring at reflux for 4 h, the mixture was concentrated. The residue was dissolved in THF (75 mL), and water (10 mL) and NIS (0.6 g) were added. After 20 min, the mixture was concentrated, diluted with CH_2Cl_2, washed with 10% (w/v) aqueous $NaHSO_3$ (2×) and 10% (w/v) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The crude residue was purified by column chromatography. Impurities were eluted with toluene–EtOAc (95:5), and **9** was obtained as a light brown syrup by elution with toluene–EtOAc (9:1) (0.99 g, 68%). R_f = 0.17$^{\alpha}$/0.08$^{\beta}$ (toluene–EtOAc, 8:2). ^{1}H NMR ($CDCl_3$) δ: 8.08–7.85 and 7.49–7.23 (2m, 30H, *Ph*CH, 5*Ph*CO), 5.63 (t, 1H, H-3′), 5.46 (dd, $J_{1',2'}$ = 7.7, $J_{2',3'}$ = 9.5 Hz, 1H, H-2′), 5.23 (s, 1H, PhC*H*), 4.89 (d, 1H, H-1′), 4.49 (dd, $J_{5,6a}$ = 2.0, $J_{6a,6b}$ = 12.3 Hz, 1H, H-6a), 4.40 (dd, $J_{5,6b}$ = 3.8 Hz, 1H, H-6b), 3.63 (dd, $J_{5',6a'}$ = 4.9, $J_{6a',6b'}$ = 10.6 Hz, 1H, H-6a′), 3.32 (dt, $J_{5',6b'}$ = 10.4 Hz, 1H, H-5′), 2.94 (t, 1H, H-6b′). ^{13}C NMR ($CDCl_3$) δ: 165.8, 165.4, 165.0 (2C), and 164.9 (5PhCO); 136.5 (*Ph*CH, quaternary C); 101.9 and 101.1 (PhCH, C-1′); 95.5 and 90.1 (C-1$^{\alpha}$,1$^{\beta}$); 67.7 (C-6′); 62.0 (C-6). Anal. calcd. for $C_{54}H_{46}O_{16}$ (951.0): C 68.21, H 4.87; found C 68.16, H 5.02.

(2,3-Di-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate (10)

To a solution of **9** (1.0 g, 1.1 mmol) in dry CH_2Cl_2 (14 mL) were added Cl_3CCN (1.2 mL) and DBU (40 µL). After stirring for 16 h, the mixture was concentrated and the residue was purified by column chromatography (toluene–EtOAc, 88:12) to yield **10** (0.99 g, 86%). $[\alpha]_D^{20}$ +83 (c = 1). R_f = 0.32 (toluene–EtOAc, 9:1). ^{1}H NMR ($CDCl_3$) δ: 8.55 (s, 1H, N*H*), 8.08–7.85 and 7.47–7.16 (2m, 30H, *Ph*CH, 5*Ph*CO), 6.68 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 6.12 (dd, $J_{2,3}$ = 10.2, $J_{3,4}$ = 8.6 Hz, 1H, H-3), 5.66 (t, $J_{2',3'}$ = $J_{3',4'}$ = 9.6 Hz, 1H, H-3′), 5.49 (dd, 1H, H-2), 5.48 (dd, $J_{1',2'}$ = 7.7 Hz, 1H, H-2′), 5.25 (s, 1H, PhC*H*), 4.95 (d, 1H, H-1′), 4.54 (dd, $J_{5,6a}$ = 2.0, $J_{6a,6b}$ = 12.2 Hz, 1H, H-6a), 4.44 (dd, $J_{5,6b}$ = 3.8 Hz, 1H, H-6b), 4.30 (ddd, $J_{4,5}$ = 10.0 Hz, 1H, H-5), 4.22 (dd, 1H, H-4), 3.69 (t, $J_{4',5'}$ = 9.5 Hz, 1H, H-4′), 3.64 (dd, $J_{5',6a'}$ = 4.8, $J_{6a',6b'}$ = 10.5 Hz, 1H, H-6a′), 3.34 (dt, $J_{5',6b'}$ = 10.3 Hz, 1H, H-5′), 2.99 (t, 1H, H-6b′). ^{13}C NMR ($CDCl_3$) δ: 165.4, 165.3 (2C), and 164.8 (2C) (5PhCO); 160.3 (OC(NH)CCl_3); 136.4 (*Ph*CH, quaternary C); 101.8 and 101.1 (PhCH, C-1′); 92.8 (C-1), 67.6 (C-6′); 61.6 (C-6).

3-Azidopropyl (2,3-di-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-O-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-O-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (12)

A solution of **10** (70 mg, 63 µmol) and **11** (4) (65 mg, 35 µmol) in dry CH_2Cl_2 (1 mL), containing 4 Å molecular sieves, was stirred under Ar for 1 h. Then, TMSOTf (1.1 µL, 6 µmol) was added. After 30 min, the mixture was neutralized with dry pyridine, filtered over cotton, diluted with CH_2Cl_2, and washed with 10% (w/v) aqueous NaCl. The organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene–EtOAc, 95:5) to obtain **12** (59 mg, 63%). $[\alpha]_D^{20}$ +59 (c = 1). R_f = 0.46 (toluene–EtOAc, 9:1). ^{1}H NMR ($CDCl_3$) δ: 5.56 (t, 1H, H-3^a), 5.45 (t, 1H, H-3^f), 5.38 (t, 1H, H-3^e), 5.29 (t, 1H, H-3^c), 5.26 (2H, H-2^a,2^f), 5.16 (1H, H-2^e), 5.15 (1H, H-2^b), 5.14 (1H, H-2^c), 5.12 (2H) and 5.09 (2s, 3PhC*H*), 5.06

(1H, H-2^d), 4.67 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1^e), 4.64 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1^c), 4.59 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1^f), 4.54 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1^a), 4.50 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1^b), 4.34 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1^d), 3.97 (t, 1H, H-4^e), 3.90 (t, 2H, H-4^a,3^b), 3.85 (t, 1H, H-3^d), 3.84 (t, 1H, H-4^c), 3.77 and 3.45 (OCH_2CH$_2$CH$_2$N$_3$), 3.55 (H-5^a), 3.25 (H-5^e), 3.21 (H-5^c), 3.12 (OCH$_2$CH$_2$CH_2N$_3$), 3.05 (H-5^b), 2.93 (H-5^d), 2.66 (H-6^f), 2.65 (H-6^b), 2.53 (H-6^d), 1.72-1.57 (m, 2H, OCH$_2$CH_2CH$_2$N$_3$). ^{13}C NMR (CDCl$_3$) δ: 136.4 (*Ph*CH, quaternary C); 101.4, 101.3 (2C), 101.1, 100.9 (2C), 100.6, 99.8, and 99.7 (3PhCH, C-1^a,1^b,1^c,1^d,1^e,1^f); 66.2 (OCH$_2$CH$_2$CH$_2$N$_3$); 47.6 (OCH$_2$CH$_2$CH$_2$N$_3$); 28.7 (OCH$_2$CH$_2$CH$_2$N$_3$). MALDI-TOF-MS *m/z*: 2713 [M + Na]$^+$.

3-Azidopropyl (2,3-di-O-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-O-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-O-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (13)

To a solution of **12** (84 mg, 31 μmol) in CH_2Cl_2 (0.85 mL) were added CF_3COOH (75 μL) and H_2O (75 μL). The mixture was stirred for 4 h, diluted with CH_2Cl_2, washed with 10% (w/v) aqueous $NaHCO_3$ until neutral pH and 10% (w/v) aqueous NaCl, and the organic layer was dried, filtered, and concentrated. The residue was purified by column chromatography (toluene–EtOAc, 6:4→4:6) to obtain **13** (55 mg, 75%). $[\alpha]_D^{20}$ +45 (*c* = 1). R_f = 0.21 (toluene–EtOAc, 1:1). ^{1}H NMR (CDCl$_3$) δ: 5.57 (t, 1H, H-3^e), 5.50 (t, 1H, H-3^a), 5.39 (t, 1H, H-3^c), 5.33 (dd, 1H, H-2^f), 5.31 (dd, 1H, H-2^a), 5.30 (dd, 1H, H-2^e), 5.25 (t, 1H, H-3^f), 5.23 (dd, 1H, H-2^c), 5.03 (br t, 2H, H-2^b,2^d), 4.76 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1^f), 4.63 (2H, H-1^e,6^e), 4.53 (d, 1H, H-1^a), 4.52 (d, 1H, H-1^c), 4.43 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1^b), 4.42 (d, $J_{1,2}$ = 8.0 Hz, 1H, H-1^d), 4.31 (3H, H-6^a,6a^c,6a^e), 4.18 (dd, 1H, H-6b^a), 4.14 (dd, 1H, H-6b^c), 3.99 (t, 1H, H-4^e), 3.98 (t, 1H, H-4^a), 3.83 (t, 1H, H-4^c), 3.82 (H-5^e), 3.78 and 3.45 (OCH_2CH$_2$CH$_2$N$_3$), 3.73 (H-4^f), 3.58 (t, 1H, H-3^d), 3.56 (H-5^c), 3.54 (H-5^a), 3.51 (t, 1H, H-3^b), 3.36 (H-4^d), 3.32 (H-4^b), 3.23 (H-5^f), 3.15–3.09 (m, 2H, OCH$_2$CH$_2$CH_2N$_3$), 3.00 (H-5^d), 2.96 (H-5^b), 1.72–1.55 (m, 2H, OCH$_2$CH_2CH$_2$N$_3$). ^{13}C NMR (CDCl$_3$) δ: 101.1 (2C), 100.6 (3C), and 100.5 (C-1^a,1^b,1^c,1^d,1^e,1^f); 66.2 (OCH$_2$CH$_2$CH$_2$N$_3$); 62.2, 62.1, 62.0 (2C), 61.8, and 61.3 (C-6^a,6^b,6^c,6^d,6^e,6^f); 47.6 (OCH$_2$CH$_2$CH$_2$N$_3$); 28.7 (OCH$_2$CH$_2$ CH$_2$N$_3$). MALDI-TOF-MS *m/z*: 2440 [M + Na]$^+$.

3-Azidopropyl (2,3-di-O-benzoyl-β-D-glucopyranosyluronic acid)-(1→4)-(2,3,6-tri-O-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-β-D-glucopyranosyluronic acid)-(1→4)-(2,3,6-tri-O-benzoyl-β-D-glucopyranosyl)-(1→3)-(2-O-benzoyl-β-D-glucopyranosyluronic acid)-(1→4)-2,3,6-tri-O-benzoyl-β-D-glucopyranoside (14)

To a solution of **13** (50 mg, 21 μmol) in CH_2Cl_2 (0.22 mL) were added TEMPO (catalytic amount), and 40 μL of a solution of KBr (2.4 mg) and Bu_4NBr (3.2 mg) in saturated aqueous $NaHCO_3$ (0.4 mL). The mixture was stirred vigorously at 0°C, when 122 μL (total) of a solution of saturated aqueous NaCl (0.44 mL), saturated aqueous $NaHCO_3$ (0.22 mL), and aqueous NaOCl (13% Cl active, 0.56 mL) was added dropwise. After 45 min, the mixture was acidified with 4 M HCl (pH 4), diluted with CH_2Cl_2, and the organic layer was washed with 10% (w/v) aqueous NaCl, dried, filtered, and concentrated. The residue was purified by column chromatography. Impurities were eluted with CH_2Cl_2–acetone (8:2), and **14** (35 mg, 70%) was eluted with CH_2Cl_2–acetone–HOAc, (8:2:0.5). R_f = 0.05 (CH_2Cl_2–acetone–HOAc, 8:2:1). MALDI-TOF-MS *m/z*: 2490 [M + Na]$^+$, 2512 [M – H + 2Na]$^+$, 2534 [M – 2H + 3Na]$^+$, 2556 [M – 3H + 4Na]$^+$.

A small amount was methylated with diazomethane in methanol for analysis. ^{1}H NMR (CDCl$_3$) δ: 5.55 (t, 1H, H-3^e), 5.54 (t, 1H, H-3^a), 5.39 (t, 1H, H-3^c), 5.36 (2H, H-2^f,3^f), 5.26 (dd, $J_{1,2}$ = 7.8, $J_{2,3}$ = 9.6 Hz, 1H, H-2^e), 5.20 (dd, $J_{1,2}$ = 7.8, $J_{2,3}$ = 9.5 Hz, 1H, H-2^a), 5.15 (dd, $J_{2,3}$ = 9.7 Hz, 1H, H-2^c), 5.08 (2t, each 1H, H-2^b,2^d), 4.83 (d, $J_{1,2}$ = 7.6 Hz, 1H, H-1^f), 4.65 (d, 1H, H-1^e), 4.61 (dd, 1H, H-6^e), 4.53 (d, $J_{1,2}$ = 8.0 Hz, 2H, H-1^b,1^c), 4.52 (d, 1H, H-1^a), 4.48 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1^d), 4.38 (dd, 1H, H-6a^a), 4.33 (dd, 1H, H-6a^c), 4.28 (dd, 1H, H-6b^e), 4.17 (dd, 1H, H-6b^a), 4.13 (dd, 1H, H-6b^c), 4.10 (t, 1H, H-4^e), 4.04 (t, 1H, H-4^a), 3.91 (H-4^f), 3.90 (t, 1H, H-4^c), 3.81 (H-5^e), 3.78 and 3.44 (OCH_2 CH$_2$CH$_2$N$_3$), 3.76 (H-4^d), 3.74 (H-4^b), 3.69 (H-5^f), 3.63 (t, 1H, H-3^d), 3.57 (t, 1H, H-3^b), 3.56 (H-5^c), 3.55 (H-5^a), 3.51 (d, $J_{4,5}$ = 9.6 Hz, 1H, H-5^d), 3.49 (d, $J_{4,5}$ = 9.6 Hz, 1H, H-5^b), 3.39, 3.30, and 3.23 (3s, each 3H, COOCH_3), 3.09 (OCH$_2$CH$_2$CH_2N$_3$), 1.70–1.59 (m, 2H, OCH$_2$CH_2CH$_2$N$_3$).

3-Aminopropyl (β-D-glucopyranosyluronic acid)-(1→4)-(β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyluronic acid)-(1→4)-(β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyluronic acid)-(1→4)-β-D-glucopyranoside (1)

To a solution of **14** (20 mg, 8 μmol) in MeOH (3 mL) was added NaOMe until pH 10, and after 5 h, water (1 mL) was added. After stirring for 16 h, TLC (EtOAc–MeOH–H_2O, 12:5:3) showed the formation of a new spot (R_f = 0.1), and the mixture was neutralized with Dowex H$^+$, filtered, and concentrated. A solution of the residue in water was washed with CH_2Cl_2 (3×), and the aqueous layer was concentrated. Then, a solution of the residue in 0.1 M NaOH (0.4 mL) was added dropwise to a suspension of 10% Pd/C (0.8 mg) and $NaBH_4$ (3.5 mg) in doubly distilled water (0.3 mL). After 2 h, when TLC (EtOAc–MeOH–H_2O, 12:5:3) showed the formation of a ninhydrin-positive spot on the baseline, the mixture was acidified with Dowex H$^+$ (pH 4), then loaded on a short column of Dowex 50 W × 2 (H$^+$, 200–400 mesh). After elution of contaminants with water, elution with 1% NH_4OH afforded **1** after concentration and lyophilization from water (2×) (5.4 mg, 61%). $[\alpha]_D^{20}$ = –17 (*c* = 0.4, H_2O). MALDI-TOF-MS *m/z*: 1091 [M + H]$^+$, 1112 [M + Na]$^+$, 1134 [M – H + 2Na]$^+$, 1156 [M – 2H + 3Na]$^+$. For ^{1}H NMR data, see Table 1.

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