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Synthesis of the conjugation ready, downstream disaccharide fragment of the O-PS of *Vibrio cholerae* O:139

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Dedicated to Professor Dr. András Lipták on the occasion of his 75th birthday

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ABSTRACT

The linker-equipped disaccharide, 8-amino-3,6-dioxaoctyl 2,6-dideoxy-2-acetamido-3-*O*-β-D-galactopyranosyluronate-β-D-glucopyranoside (**10**), was synthesized in eight steps from acetobromogalactose and ethyl 4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-1-thio-β-D-glucopyranoside. The hydroxyl group present at C-4^{II} in the last intermediate, 8-azido-3,6-dioxaoctyl 4-*O*-benzyl-6-bromo-2,6-dideoxy-2-trichloroacetamido-3-*O*-(benzyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate)-β-D-glucopyranoside (**9**), is positioned to allow further build-up of the molecule and, eventually, construction of the complete hexasaccharide. Global deprotection (**9**→**10**) was done in one step by catalytic hydrogenolysis over palladium-on-charcoal.

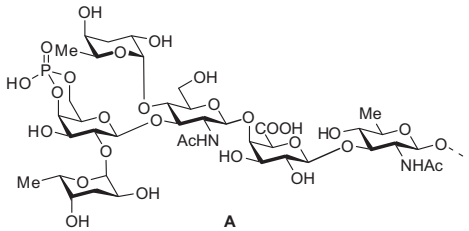
1. Introduction

Cholera is a serious, both endemic and epidemic diarrheal disease, caused by *Vibrio cholerae*. Mainly affected are the third world countries. A potent vaccine for cholera that would provide long term protection from cholera for general population, including the very young and old, is yet to be developed. We have been involved in development of a vaccine for cholera from synthetic carbohydrate antigens for a number of years. Our work,[1] as well as that of others[2,3] has been focused mainly on cholera caused by the O1 strain. Work toward a vaccine caused by the other causative agent of cholera, *V. cholerae* O:139, has gained momentum since the outbreaks of cholera caused by this non-O1 strain. The work by Knirel et al.[4,5] led to elucidation of the structure of the *V. cholerae* O:139 O-antigen, which was established to be the phosphorylated hexasaccharide **A** shown in Figure 1. It prompted efforts aimed at developing immunogens for anti *V. cholerae* O:139 antibodies from synthetic antigens. Synthesis of the hexasaccharide **A** (Fig. 1) is a formidable task and thus far only oligosaccharide fragments mimicking partial structures of its upstream end have been synthesized.[6–9]

Here we describe the first synthesis of the disaccharide **10**, which mimics the downstream[10] end of the hexasaccharide. Structural fragments of **A** are important ligands for inhibition studies and mapping of combining areas of the homologous antibodies. We have used synthetic fragments of the O-specific polysaccharides to determine the mode of antigen–antibody binding in the *V. cholerae* O:1, serotype Inaba and Ogawa systems.[11,12] The disaccharide described herein is equipped with a linker (spacer) allowing conjugation to proteins. The hydroxyl group at C-4^{II} in the intermediate alcohol **9** is positioned to allow further build-up of the molecule and, eventually, construction of the complete hexasaccharide.

2. Results and discussion

The synthesis of **10** was planned with the future construction of the hexasaccharide **A** (Fig. 1) in mind, and conjugation of the latter to protein carriers using, for example, squaric acid chemistry.[13,14]



A

Fig. 1.

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Accordingly, one of its intermediates should allow extension of the oligosaccharide chain at HO-4^{II}, and protecting groups present should be stable during a variety of chemical transformations leading to the hexasaccharide. We anticipate that the last step before conversion of the antigenic carbohydrate to the squaric acid monoester[14] would be reduction of azide in the spacer into amine. Therefore, benzyl-protecting groups seemed to be the natural choice, as those would be removed simultaneously with the reductive azide→amine conversion.

The synthesis of **10** (Scheme 1) started with the known thioglycoside **3**, prepared as described,[15] except that the glycosylation reaction of halide **1** and acceptor **2** was carried out at almost neutral conditions. This minimized formation of byproducts[15] due to hydrolysis of the benzylidene ring in **3**. Treatment of **3** with 8-azido-3,6-dioxaoctan-1-ol[6] gave the spacer-equipped disaccharide **4** whose benzylidene acetal underwent Hanessian–Hullar ring opening[16,17] to regioselectively form **5**, a bromide at the 6 position, in 77% yield. After deacylation of **5**, subsequent *p*-methoxybenzylidenation gave acetal **6** (76% over two steps). Benzylation (BnBr/NaH/DMF) of **6**, when conducted at low temperture[15] was chemoselective, leaving the 6-bromide and the trichloroacetamido group intact (→**7**). Hydrolysis of the *p*-methoxybenzylidene group in the fully protected compound **7** gave diol **8**, whose oxidation with TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxyl) and [bis(acetoxy)iodo]benzene (BAIB) was regioselective at 6^{II}. The formed carboxylic acid was conveniently isolated as the corresponding benzyl ester **9**, the latter being obtained through regio- and chemoselective benzylation.[18] Global deprotection by hydrogenolysis over Pd/C effected one-pot transformation of azide to amine, dehalogenation at C-6^{I} as well as of the *N*-trichloroacetyl group, and debenzylation, to give the title compound **10**.

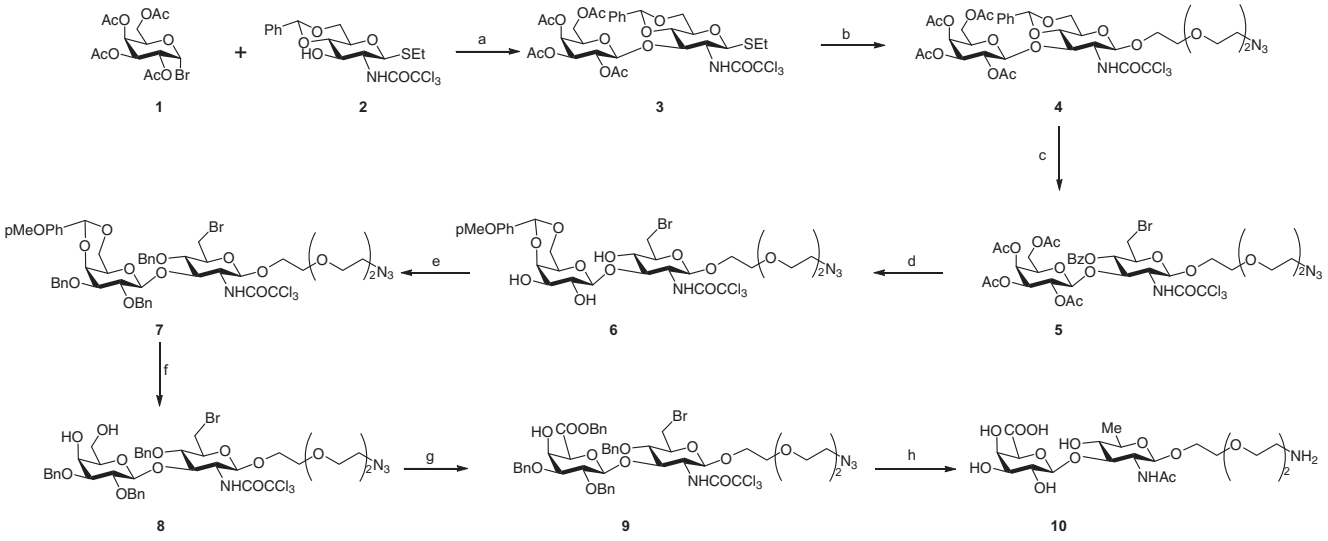
3. Experimental

3.1. General methods

Unless stated otherwise, optical rotations were measured at ambient temperature for solution in $CHCl_3$ with a Perkin–Elmer automatic polarimeter, Model 341. All reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 coated glass slides. Column chromatography was performed by elution from prepacked (Varian, Inc.) columns of silica gel with the CombiFlash Companion Chromatograph (Isco, Inc.) or Isolera Flash Chromatograph (Biotage), the latter being connected to the external Evaporative Light Scattering Detector, Model 380-LC (Varian, Inc.). Nuclear Magnetic Resonance (NMR) spectra were measured at 400 MHz (^{1}H) and 100 MHz (^{13}C) or at 600 MHz (^{1}H) and 150 MHz (^{13}C) with Bruker Avance spectrometers. Assignments of NMR signals were made by homonuclear and heteronuclear two-dimensional correlation spectroscopy, run with the software supplied with the spectrometers. When reporting assignments of NMR signals, nuclei associated with the spacer are denoted with a prime; sugar residues are serially numbered, beginning with the one bearing the aglycon, and are identified by a Roman numeral superscript in listings of signal assignments. Liquid Chromatography–Electron Spray-Ionization Mass Spectrometry (ESI-MS) was performed with a Hewlett–Packard 1100 MSD spectrometer. Attempts have been made to obtain correct combustion analysis data for all new compounds. However, some compounds tenaciously retained traces of solvents, despite exhaustive drying, and analytical figures for carbon could not be obtained within 0.4%. Structures of these compounds follow unequivocally from the mode of synthesis, NMR spectroscopic data and *m*/*z* values found in their mass spectra, and their purity was verified by TLC and NMR spectroscopy. Palladium-on-charcoal catalyst (5%) (Escat™ 103) was purchased from Engelhard Industries. Rubber septa used to close reaction flasks containing organic solvents were protected with a thin Teflon™ sheet, to avoid leaching. Solutions in organic solvents were dried with anhydrous Na_2SO_4, and concentrated at 40 °C/2 kPa.

3.2. Ethyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-thio-2-trichloroacetamido-β-D-glucopyranoside (3)

A mixture of acetobromogalactose (**1**, 11 g, 26.7 mmol), glycosyl acceptor,[15] ethyl 4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-1-thio-β-D-glucopyranoside (**2**, 8.8 g, 19 mmol), *sym*-collidine (3.2 mL, 24 mmol), and 4 Å MS (1.5 g) in CH_2Cl_2 (300 mL) was stirred for 30 min under N_2, cooled to −30 °C, and powdered AgOTf (8.2 g, 32 mmol) was added. With continued stirring, the mixture was allowed to warm up to room temperature during 4 h. Et_3N



Scheme 1. Reagents and conditions: (a) NIS, AgOTf, CH_2Cl_2, 90%; (b) 8-azido-3,6-dioxaoctan-1-ol, NIS, AgOTf, CH_2Cl_2, 83%; (c) NBS, $BaCO_3$, CCl_4—$Cl_2CHCHCl_2$, 77%; (d) (1) MeONa, MeOH; (2) *p*MePhCH(OMe)$_2$, CSA, CH_3CN, 76% (2 steps); (e) BnBr, NaH, DMF, 76%; (f) 60% AcOH, 87%; (g) (1) TEMPO, BAIB, DCM; (2) BnBr, K_2CO_3, DMF, 69% (2 steps); (h) Pd/C, MeOH, pH 7 buffer, 85%.

(2.0 mL) was added, followed by CH_2Cl_2 (300 mL), the mixture was filtered through a pad of Celite, the filtrate was washed successively with 0.5 M aq HCl (2 × 100 mL), saturated $NaHCO_3$ aq (100 mL) and brine (100 mL). After concentration, chromatography (1:2 EtOAc–Hexane) afforded **3** (13.7 g, 90%) as white foam whose NMR characteristics were identical as those reported.[15]

3.3. 8-Azido-3,6-dioxaoctyl 4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (4)

A mixture of **3** (10.6 g, 13.5 mmol), 8-azido-3,6-dioxaoctan-1-ol[6] (3.54 g, 20.2 mmol), and 4 Å MS (5 g) in CH_2Cl_2 (100 mL) was stirred for 30 under N_2. The mixture was cooled to −10 °C and NIS (4.54 g, 20.2 mmol) followed by powder AgOTf (1.73 g, 6.75 mmol) was added with stirring. After 1 h, the mixture was treated with Et_3N (3.0 mL), filtered through Celite, the filtrate was concentrated, and chromatography (2:1, hexane–acetone) gave **4** (10.6 g, 83%) as oil. $[\alpha]_D$ −15 (*c* 1.8, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ: 7.48–7.37 (m, 5H, Ph), 7.18 (d, J = 7.4 Hz, 1H, NH), 5.55 (s, 1H, PhCH), 5.31 (dd, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 0.9 Hz, 1H, H-4^{II}), 5.11 (d, $J_{1,2}$ = 8.2 Hz, H-1^{I}), 4.91 (dd, $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.5 Hz, 1H, H-3^{II}), 4.74 (d, $J_{1,2}$ = 8.0 Hz, H-1^{II}), 4.55 (t, J = 9.6 Hz, 1H, H-3^{I}), 4.34 (dd, $J_{5,6}$ = 5.2 Hz, $J_{6a,b}$ = 10.7 Hz, H-6$^{I}_{a}$)), 4.11 (dd, $J_{5,6a}$ = 7.2 Hz, $J_{6a,b}$ = 11.3 Hz, H-6$^{II}_{a}$), 3.99 (dd, $J_{5,6b}$ = 6.7 Hz, $J_{6a,b}$ = 11.3 Hz, H-6$^{II}_{b}$), 3.9 (m, 1H, H-1′$_a$), 3.93–3.79 (m, 2H, H-1$^{I}_{b}$, H-1′$_b$), 3.74–3.71 (m, 2H, H-4^{I}, H-5^{II}), 3.64–3.61 (m, 8H, H-2′, H-3′, H-4′, H-5′), 3.55–3.52 (m, 2H, H-2^{I}, H-5^{I}), 3.41 (t, J = 5.0 Hz, 2H, H-6′), 2.11 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$), 1.91 (s, 3H, $COCH_3$); NMR (150 MHz, $CDCl_3$) δ: 160.6 (CO), 160.5 (CO), 160.4 (CO), 159.9 (CO), 153.1 (CO), 130.8 (C_q), 123.9, 123.0, 121.3, 99.0 (PhCH), 97.4 (C-1^{I}), 97.3 (C-1^{II}), 79.0 (C-4^{I}), 76.3 (C-3^{I}), 71.8 (C-3^{II}), 71.5 (2C, 2CH_2), 71.4 (C-5^{II}), 71.3 (CH_2), 70.9 (CH_2), 69.9 (C-2^{II}), 69.8 (C-1′$_b$), 68.1 (C-4^{II}), 67.5 (C-5^{I}), 63.0 (C-6^{II}), 60.8 (C-2^{I}), 53.6 (C-6′), 26.7 (4C, 4CO*CH*$_3$); TOF-HRMS, *m*/*z*: calcd for $C_{35}H_{45}Cl_3N_4O_{17}Na$ [M+Na]$^+$: 921.1743, found: 921.1750. Anal. Calcd for $C_{35}H_{45}Cl_3N_4O_{17}$: C, 46.70; H, 5.04; N, 6.22. Found: C, 46.85; H, 5.14; N, 6.22.

3.4. 8-Azido-3,6-dioxaoctyl 4-*O*-benzoyl-6-bromo-2,6-dideoxy-2-trichloroacetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (5)

NBS (3.12 g, 17.5 mmol) was added at 100 °C with stirring to a mixture of **4** (10.6 g, 11.7 mmol), $BaCO_3$ (11.7 g, 59 mmol) in 3:1 CCl_4–tetrachloroethane (400 mL). After 2 h, the mixture was cooled to room temperature and filtered. The filtrate was concentrated and the residue was chromatographed (30:1→17:1 CH_2Cl_2–acetone) to afford **5** (8.9 g, 77%), mp 88–90 °C (hexane–acetone); $[\alpha]_D$ −28 (*c* 2.1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ: 8.08–7.61 (m, 5H, Ph), 7.29 (d, J = 7.6 Hz, 1H, NH), 5.20 (d, $J_{3,4}$ = 3.6 Hz, 1H, H-4^{II}), 5.15 (t, J = 9.5 Hz, 1H, H-4^{I}), 5.08 (d, $J_{1,2}$ = 8.2 Hz, H-1^{I}), 5.05 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.5 Hz, 1H, H-2^{II}), 4.82 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.6 Hz, 1H, H-3^{II}), 4.63 (d, $J_{1,2}$ = 7.8 Hz, H-1^{II}), 4.59 (t, J = 9.0 Hz, 1H, H-3^{I}), 4.01–3.99 (m, 1H, H-1′$_a$), 3.92–3.89 (m, 2H, H-1′$_b$, H-5^{I}), 3.78–3.66 (m, 11H, H-2′, H-3′, H-4′, H-5′, H-2^{I}, H-5^{II}, H-6$^{II}_{a}$), 3.55 (dd, J = 2.6, 11.4 Hz, 1H, H-6$^{I}_{a}$)), 3.51 (dd, J = 5.1, 9.0 Hz, 1H, H-6$^{II}_{b}$), 3.49–3.44 (m, 3H, H-6′, H-6$^{I}_{b}$), 2.05 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.91 (s, 6H, 2 $COCH_3$); NMR (150 MHz, $CDCl_3$) δ: 170.2 (CO), 170.1 (CO), 170.0 (CO), 169.4 (CO), 164.9 (CO), 161.9 (CO), 133.5 (C_q), 129.9, 129.4, 128.4, 99.8 (C-1^{II}), 99.3 (C-1^{I}), 75.4 (C-3^{I}), 73.6 (C-5^{I}), 71.6 (C-4^{I}), 70.9 (C-3^{II}), 70.7 (CH_2), 70.5 (CH_2), 70.4 (C-5^{II}), 70.3 (CH_2), 69.8 (CH_2), 68.6 (C-2^{II}), 68.5 (C-1′), 66.4 (C-4^{II}), 60.5 (C-6^{II}), 58.2 (C-2^{I}), 50.5 (C-6′), 31.1 (C-6^{I}), 20.5 (4C, 4CO*CH*$_3$); TOF-HRMS, *m*/*z*: calcd for $C_{35}H_{45}BrCl_3N_4O_{17}$ [M+H]$^+$: 977.1029, found: 977.0996. Anal. Calcd for $C_{35}H_{44}BrCl_3N_4O_{17}$: C, 52.94; H, 4.53; N, 5.72. Found: C, 43.07; H, 4.68; N, 5.66.

3.5. 8-Azido-3,6-dioxaoctyl 6-bromo-2,6-dideoxy-2-trichloroacetamido-3-*O*-(4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (6)

Acetate **5** (3.4 g, 3.5 mmol) was treated with 1 M NaOMe–MeOH (1.0 mL) in MeOH (40 mL) overnight. The mixture was neutralized with Amberlite IR-120 and filtered. After concentration, a solution of the residue in CH_3CN (40 mL) was treated with *p*-methoxybenzaldehyde dimethyl acetal (1.2 mL, 7 mmol) and CSA (200 mg, 0.86 mmol) for 2 h. The reaction was quenched with Et_3N (2.0 mL), concentrated, and chromatography (2:1, CH_2Cl_2–acetone) afforded **6** (2.3 g, 76% over two steps), mp 132–133 °C (hexane–EtOAc); $[\alpha]_D$ −13 (*c* 1.7, $CHCl_3$); ^1H NMR (600 MHz, CD_3OD) δ: 7.46–6.90 (m, 4H, aromatic protons), 5.57 (s, 1H, 4-MeOPhC*H*), 4.78 (d, $J_{1,2}$ = 8.4 Hz, 1H, H-1^{I}), 4.45 (d, $J_{1,2}$ = 7.7 Hz, 1H, H-1^{II}), 4.21–4.12 (m, 3H, H-4^{II}, H-6^{II}), 3.99 (dd, J = 8.2, 10.5 Hz, 1H, H-3^{I}), 3.96–3.93 (m, 1H, H-1′$_a$), 3.82 (dd, J = 2.1, 11.2 Hz, 1H, H-6$^{I}_{a}$)), 3.80–3.72 (m, 5H, OCH_3, H-1′$_b$, H-2^{I}), 3.68–3.63 (m, 9H, H-2^{II}, H-2′, H-3′, H-4′, H-5′), 3.60–3.57 (m, 3H, H-6$^{I}_{b}$, H-3^{II}, H-5^{II}), 3.53–3.46 (m, 2H, H-4^{I}, H-5^{I}), 3.38 (t, J = 5. 0 Hz, H-6′); NMR (150 MHz, CD_3OD) δ: 164.4 (CO), 161.8, 132.0, 129.0, 114.5, 104.9 (C-1^{II}), 102.4 (4-MeOPhCH), 101.9 (C-1^{I}), 82.4 (C-3^{I}), 77.4 (C-4^{II}), 76.3 (C-5^{I}), 73.6 (C-3^{II}), 72.9 (C-4^{I}), 72.0 (C-2^{II}), 71.9, 71.7, 71.3 (C-2′, C-3′, C-4′, C-5′), 70.4 (C-1′), 70.3 (C-6^{II}), 68.6 (C-5^{II}), 58.6 (C-2^{I}), 55.9 (OCH_3), 52.0 (C-5′), 33.8 (C-6^{II}); TOF-HRMS, *m*/*z*: calcd for $C_{28}H_{39}BrCl_3N_4O_{13}$ [M+H]$^+$: 823.0763, found: 827.0732. Anal. Calcd for $C_{28}H_{38}BrCl_3N_4O_{13}$: C, 40.77; H, 4.64; N, 6.79. Found: C, 40.59; H, 4.75; N, 6.67.

3.6. 8-Azido-3,6-dioxaoctyl 4-*O*-benzyl-6-bromo-2,6-dideoxy-2-trichloroacetamido-3-*O*-(2,3-di-*O*-benzyl-4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside (7)

NaH (372 mg, 9.3 mmol, 60% in oil) was added at −25 °C to a stirred solution of **6** (1.7 g, 2.0 mmol) in DMF (15 mL). After 5 min, BnBr (1.1 mL, 9.3 mmol) was added and, with continued stirring, the mixture was allowed to warm to room temperature. After total reaction time of 40 min, the mixture was cooled to −30 °C and reaction was terminated by addition of MeOH (1.5 mL). After warming to room temperature, the mixture was diluted with CH_2Cl_2 (200 mL), washed with brine, concentrated, and chromatography (3:2→1:1, hexane–EtOAc) gave **7** (1.7 g, 76%) as syrup. $[\alpha]_D$ +17 (*c* 0.9, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ: 7.47–7.24 (m, 17H, aromatic protons), 7.10 (d, J = 7.1 Hz, 1H, NH), 6.84–6.82 (m, 2H, aromatic protons), 5.45 (s, 1H, 4-MeOPhC*H*), 5.18 (d, J = 10.5 Hz, 1H, PhC*H*), 5.16 (d, $J_{1,2}$ = 7.1 Hz, 1H, H-1^{I}), 4.90 (d, J = 10.5 Hz, 1H, PhC*H*), 4.79–4.73 (m, 3H, PhC*H*), 4.68 (d, J = 10.5 Hz, 1H, PhC*H*), 4.50 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1^{II}), 4.46 (dd, $J_{2,3}$ = 6.8 Hz, $J_{3,4}$ = 8.5 Hz, H-3^{I}), 4.20 (dd, J = 1.5, 12.8 Hz, 1H, H-6$^{II}_{a}$)), 4.09 (d, $J_{3,4}$ = 3.7 Hz, 1H, H-4^{II}), 3.97–3.92 (m, 2H, H-1′$_a$, H-6$^{II}_{b}$), 3.85 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.3 Hz, 1H, H-2^{II}), 3.79–3.77 (m, 4H, OCH_3, H-6$^{I}_{a}$)), 3.69–3.65 (m, 4H, H-1′$_B$, H-6$^{I}_{b}$, H-4^{I}, H-5^{I}), 3.64–3.57 (m, 8H, H-2′, H-3′, H-4′, H-5′), 3.47 (dd, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 3.7 Hz, 1H, H-3^{II}), 3.37–3.32 (m, 3H, H-2^{I}, H-6′), 3.23 (br s, 1H, H-5^{II}); NMR (150 MHz, $CDCl_3$) δ: 161.6 (CO), 160.2, 138.8, 138.2, 137.9, 130.7, 129.1, 128.7, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 113.7, 103.6 (C-1^{II}), 101.5 (4-MeOPhCH), 98.2 (C-1^{I}), 79.2 (C-3^{II}), 78.9 (C-2^{II}), 78.6 (C-3^{I}), 78.8 (C-4^{I}), 76.0 ($PhCH_2$), 75.2 ($PhCH_2$), 74.0 (C-5^{I}), 73.7 (C-4^{II}), 71.6, 70.8, 70.2 (C-2′, C-3′, C-4′, C-5′), 69.1 (2C, C-1′, C-6^{II}), 66.7 (C-5^{II}), 58.6 (C-2^{I}), 55.4 (OMe), 50.8 (C-6′), 33.3 (C-6^{I}); TOF-HRMS, *m*/*z*: calcd for $C_{49}H_{57}BrCl_3N_4O_{13}$ [M+H]$^+$: 1093.2171, found: 1093.2135. Anal. Calcd for

$C_{49}H_{56}BrCl_3N_4O_{13}$: C, 53.73; H, 5.15; N, 5.12. Found: C, 53.53; H, 5.18; N, 5.06.

3.7. 8-Azido-3,6-dioxaoctyl 4-*O*-benzyl-6-bromo-2,6-dideoxy-2-trichloroacetamido-3-*O*-(2,3-di-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (8)

The foregoing compound **7** (620 mg, 0.56 mmol) was treated with 60% HOAc aq (25 mL) at 50 °C for 40 min. After concentration, chromatography (3:1 hexane–acetone) afforded **8** (480 mg, 87%). $[\alpha]_D$ +4 (*c* 1.7, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.50–7.20 (m, 15H, aromatic protons), 5.10 (d, J = 10.3 Hz, 1H, PhC*H*), 4.92 (d, J = 11.6 Hz, 1H, PhC*H*), 4.72 (d, J = 11.6 Hz, 1H, PhC*H*), 4.70 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1^{II}), 4.67 (d, J = 11.6 Hz, 1H, PhC*H*), 4.60 (d, $J_{1,2}$ = 8.3 Hz, 1H, H-1^{I}), 4.59 (d, J = 11.6 Hz, 1H, PhC*H*), 4.56 (d, J = 10.3 Hz, 1H, PhC*H*), 4.29 (m, 1H, H-3^{I}), 4.04–4.01 (m, 2H, H-2^{I}, H-4^{II}), 3.94–3.92 (m, 1H, H-1$'_a$), 3.83 (dd, J = 7.4, 11.5 Hz, 1H, H-6$^{II}_a$), 3.75 (dd, J = 1.8, 11.3 Hz, 1H, H-6$^{I}_a$)), 3.74–3.68 (m, 1H, H-1$'_b$), 3.65–3.63 (m, 11H, H-6$^{I}_b$, H-2^{II}, H-6$^{II}_b$, H-2′, H-3′, H-4′, H-5′), 3.53 (m, 2H, H-4^{I}, H-5^{I}), 3.41 (dd, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.4 Hz, 1H, H-3^{II}), 3.38 (m, 3H, H-5^{II} and H-6′); NMR (150 MHz, $CDCl_3$) δ: 163.3 (CO), 140.5, 139.7, 139.1, 130.6, 129.9, 129.3, 129.2, 129.1, 128.9, 128.5, 128.3, 104.2 (C-1^{II}), 102.2 (C-1^{I}), 81.9 (C-3^{II}), 80.1 (C-2^{II}), 78.6 (C-3^{I}), 79.9 (C-4^{I}), 78.2 (C-3^{I}), 77.2 (C-5^{II}), 76.8 (PhCH$_2$), 76.0 (PhCH$_2$), 75.0 (C-5^{I}), 72.7 (PhCH$_2$), 71.7, 71.5, 70.3 (C-1′, C-2′, C-3′, C-4′, C-5′), 67.8 (C-4^{II}), 62.8 (C-6^{II}), 58.9 (C-2^{I}), 51.8 (C-6′), 33.7 (C-6^{I}); TOF-HRMS, m/z: calcd for $C_{41}H_{54}BrCl_3N_5O_{12}$ $[M+NH_4]^+$: 992.2018, found: 992.2009. Anal. Calcd for $C_{41}H_{50}BrCl_3N_4O_{12}$: C, 50.40; H, 5.16; N, 5.73. Found: C, 49.98; H, 5.22; N, 5.64.

3.8. 8-Azido-3,6-dioxaoctyl 4-*O*-benzyl-6-bromo-2,6-dideoxy-2-trichloroacetamido-3-*O*-(benzyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate)-β-D-glucopyranoside (9)

A mixture of TEMPO (200 mg, 1.3 mmol), BIAB (2.09 g, 6.5 mmol) and **8** (2.5 g, 2.6 mmol) in CH_2Cl_2 (20 mL) and water (10 mL) was stirred vigorously at room temperature. EtOAc (200 mL) was added after 24 h, the mixture was washed with brine (3 × 50 mL), and the aqueous solution was extracted with EtOAc (50 mL). The organic phases were combined, dried and concentrated, to give material which was sufficiently pure for the next step.

Anhydrous K_2CO_3 (466 mg, 3.3 mmol), followed by BnBr (401 μl, 3.3 mmol) was added at 0 °C to a solution of the foregoing material in anhydrous DMF (20 mL), stirred at the same temperature for 10 min, and then allowed to warm to room temperature. After having been stirred overnight, the mixture was diluted with EtOAc (300 mL), washed with brine (100 mL), and the aqueous phase was backwashed with EtOAc (80 mL). The organic phases were combined, dried, concentrated, and chromatography (5:1, hexane–acetone) afforded **9** (2.0 g, 69% over two steps). $[\alpha]_D$ +9 (*c* 1.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.37–7.25 (m, 21H, aromatic protons and NH), 5.21 (AB_q, 2H, J = 11.3 Hz, 2H, PhCH_2), 5.03 (d, $J_{1,2}$ = 6.1 Hz, 1H, H-1^{I}), 4.89–4.57 (m, 6H, 3PhCH_2), 4.51 (d, $J_{1,2}$ = 8.2 Hz, 1H, H-1^{II}), 4.36–4.32 (m, 2H, H-3^{I}, H-4^{II}), 4.03 (br s, 1H, H-5^{II}), 3.97–3.94 (m, 1H, H-1$'_a$), 3.80 (m, 2H, H-4^{I}, H-5^{I}), 3.71–3.68 (m, 3H, H-1$'_b$, H-6$^{I}_a$), H-2^{II}), 3.61 (m, 4H, H-5′, H-6$^{I}_b$, H-2^{I}), 3.57–3.53 (m, 6H, H-2′, H-3′, H-4′), 3.51 (dd, $J_{2,3}$ = 9.4 Hz, $J_{3,4}$ = 3.3 Hz, 1H, H-3^{II}), 3.34 (dd, J = 4.7, 5.9 Hz, 2H, H-6′), 2.56 (d, J = 1.4 Hz, 1H, 4^{II}-OH); NMR (150 MHz, $CDCl_3$) δ: 167.2 (CO), 161.4 (CO), 138.0, 137.6, 137.2, 135.1, 128.3, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 102.9 (C-1^{II}), 98.5 (C-1^{I}), 79.6 (C-3^{II}), 78.3 (C-2^{II}), 77.6 (C-3^{I}), 76.8 (C-4^{I}), 75.6 (PhCH$_2$), 74.3 (C-5^{I}), 74.2 (PhCH$_2$), 73.6 (C-5^{II}), 72.3 (PhCH$_2$), 70.6, 70.5, 70.3, 69.9, 68.7 (C-1′, C-2′, C-3′, C-4′, C-5′), 67.6 (C-4^{II}), 67.2 (PhCH$_2$), 50.5 (C-6′), 33.2 (C-6^{I}); TOF-HRMS, m/z: calcd for $C_{48}H_{55}BrCl_3N_4O_{13}$ $[M+H]^+$: 1079.2015, found: 1079.2043. Anal. Calcd for $C_{48}H_{54}BrCl_3N_4O_{13}$: C, 53.32; H, 5.03; N, 5.18. Found: C, 53.42; H, 5.04; N, 5.21.

3.9. 8-Amino-3,6-dioxaoctyl 2,6-dideoxy-2-acetamido-3-*O*-β-D-galactopyranosyluronate-β-D-glucopyranoside (10)

A suspension of **9** (400 mg, 0.37 mmol), Pd/C (200 mg), MeOH (24 mL) and potassium phosphate buffer (24 mL, pH 7, 0.25 M) was stirred at 50 °C under H_2 for 2 days. The mixture was filtered through Celite, and the solids were washed with water–MeOH (1:1, 30 mL). After concentration of the filtrate, chromatography (30:1, MeOH–30% NH_4OH) afforded **10** (160 mg, 85%). Compound **10**, when freeze-dried, formed a hygroscopic solid, which liquified when exposed to air. ^{1}H NMR (600 MHz, D_2O) δ: 4.58 (d, $J_{1,2}$ = 8.7 Hz, 1H, H-1^{I}), 4.44 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1^{II}), 4.22 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.2 Hz, 1H, H-4^{II}), 4.08 (d, $J_{4,5}$ = 1.2 Hz, 1H, H-5^{II}), 4.00 (m, 1H, H-1$'_a$), 3.88 (dd, $J_{1,2}$ = 8.7 Hz, $J_{2,3}$ = 10.3 Hz, 1H, H-2^{I}), 3.79–3.69 (m, 11H, H-1$'_a$, H-2′, H-3′, H-4′, H-5′, H-3^{I}, H-3^{II}), 3.57–3.53 (m, 2H, H-5^{I}, H-2^{II}), 3.35 (t, J = 9.2 Hz, 1H, H-4^{I}), 3.21 (t, J = 5.4 Hz, 2H, H-6′), 2.04 (s, 3H, $COCH_3$), 1.36 (d, $J_{5,6}$ = 6.2 Hz, 3H, H-6^{I}); NMR (150 MHz, D_2O) δ: 174.5 (CO), 174.4 (CO), 103.0 (C-1^{II}), 100.8 (C-1^{I}), 82.5 (C-3^{I}), 75.3 (C-5^{II}), 74.0 (C-4^{I}), 72.6 (C-3^{II}), 71.5 (C-5^{I}), 70.2 (C-2^{II}), 70.0 (C-4^{II}), 69.7–65.3 (5C, C-1′, C-2′, C-3′, C-4′, C-5′), 54.6 (C-2^{I}), 39.1 (C-6′), 22.2 ($COCH_3$), 16.7 (C-6^{I}); TOF-HRMS, m/z: calcd for $C_{20}H_{35}N_2O_{13}$ $[M-H]^+$: 511.2139, found: 511.2132.

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