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Synthesis of colitose-containing oligosaccharide structures found in polysaccharides from *Vibrio cholerae* O139 synonym Bengal using thioglycoside donors

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Abstract

The syntheses of the two colitose-containing trisaccharides 8-methoxycarbonyloctyl (3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1 → 2)-β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-glucopyranoside and 8-methoxycarbonyloctyl β-D-galactopyranosyl-(1 → 3)-[(3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1 → 4)]-2-acetamido-2-deoxy-β-D-glucopyranoside, and tetrasaccharide 8-methoxycarbonyloctyl (3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1 → 2)-β-D-galactopyranosyl-(1 → 3)-[(3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1 → 4)]-2-acetamido-2-deoxy-β-D-glucopyranoside are described. The oligosaccharides correspond to structures found in the capsular polysaccharide and the lipopolysaccharide of *Vibrio cholerae* O139 and also to lipopolysaccharide structures of *E. coli* O55 and *Salmonella greenside*. The colitose residues were introduced via dimethyl(methylthio)sulfonium trifluoromethanesulfonate promoted glycosylations using colitose thioglycosides as glycosyl donors. © 1997 Elsevier Science Ltd.

Keywords: Vibrio cholerae; Capsular polysaccharide; Lipopolysaccharide; 3,6-Dideoxy-L-*xylo*-hexose; Colitose; Thioglycosides; Oligosaccharide synthesis

1. Introduction

The venerable diarrhoeal disease cholera is caused by the Gram-negative bacteria *Vibrio cholerae*. Earlier pandemics were found to be due to only one serotype, *V. cholerae* serogroup O1. Recently, new epidemics of cholerae have started in Asia, in which the causative agent was found to be none of the known serotypes of *V. cholerae* [1,2]. This new serotype, *V. cholerae* O139 synonym Bengal [3], was similar in many aspects to certain types of *V. cholerae* O1, but the carbohydrate part of the cell envelope showed large differences. The new serotype has the ability to synthesize a capsular polysaccharide. The repeating unit structure of the capsule is also found as part of the lipopolysaccharide. This structure has been elucidated [4–6] and found to contain, i.a., 3,6-dideoxy-L-*xylo*-hexose (colitose, Col), D-galacturonic acid, 2-acetamido-2,6-dideoxy-D-glucose (*N*-

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α-Col*p*-(1→2)-β-D-Gal*p* (4,6-Phosphate)
1
↓
3
→6)-β-D-Glc*p*NAc-(1→4)-α-D-Gal*p*A-(1→3)-β-D-Qui*p*NAc-(1→
4
↑
1
α-Col*p*

Fig. 1.

α-Col*p*-(1→2)-β-D-Gal*p*
1
↓
3
→6)-β-D-Glc*p*NAc-(1→3)-α-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→

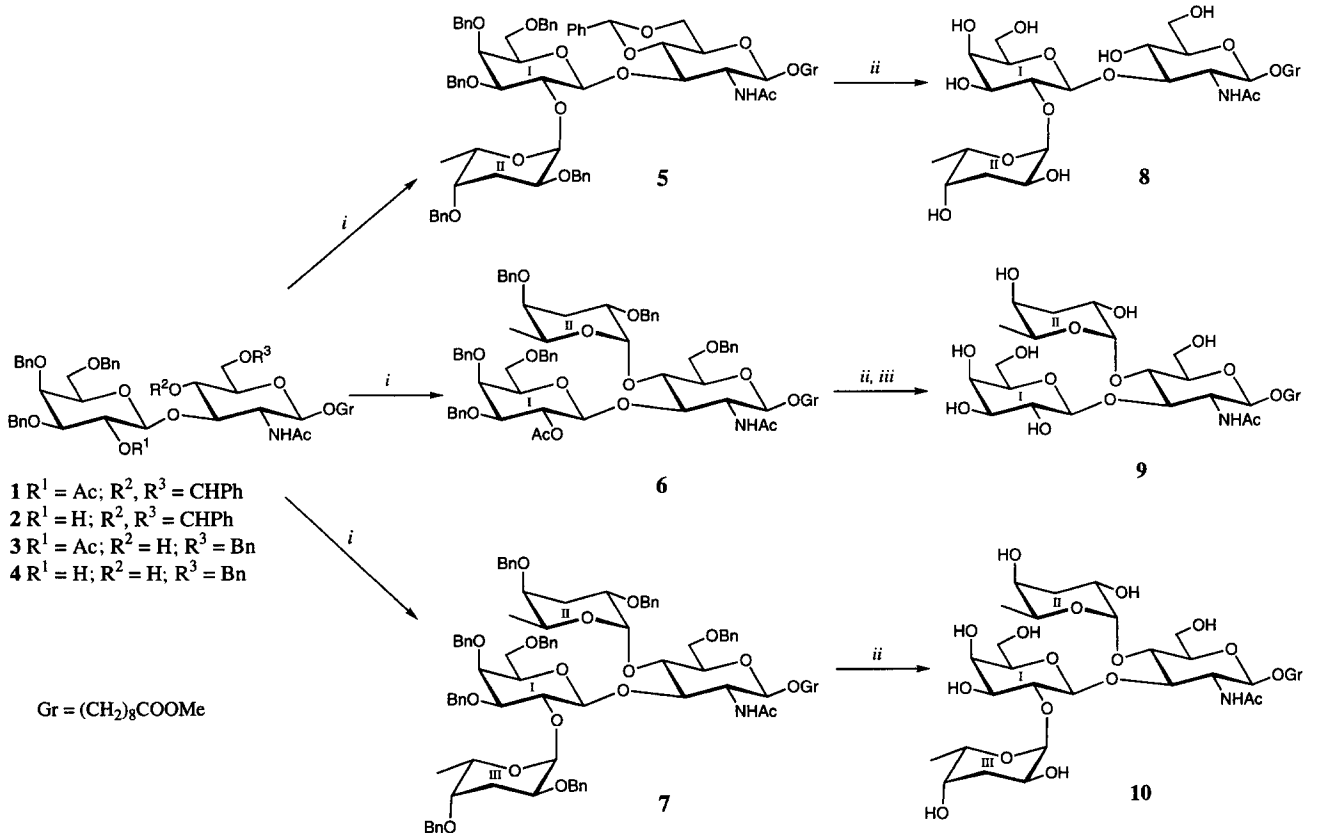
Fig. 2.

acetyl-D-quinovosamine, D-QuiNAc) and a cyclic phosphate. The complete repeating unit is shown in Fig. 1.

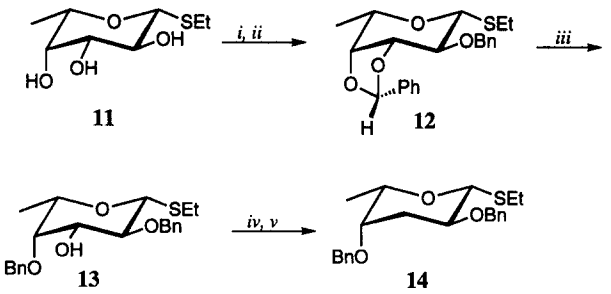
Dideoxysugars have earlier been shown, in connection with *Salmonella typhimurium* O-antigenic structures, to be immuno-dominant [7]. Therefore, structures containing the colitose residues were chosen as primary synthetic targets to be used in the evaluation of the immune response towards *V. cholerae* O139. The synthesis of the two trisaccharides **8** and **9**, containing either one of the colitose residues, and the tetrasaccharide **10**, containing both colitose residues, is described (Scheme 1). Structure **8** is also part of the O-antigen of *Salmonella greenside* [8] and *E. coli* O55 [9], whose structure is shown in Fig. 2, and which have been shown to crossreact with antibodies raised against *V. cholerae* O139 [5].

2. Results and discussion

Because of the similarity between the *V. cholerae* structures to be synthesised and the Lewis b and H type 1 determinant of human glycoconjugates, intermediates from syntheses of the latter structures by Lemieux et al. [10–12] could be used as convenient precursors in the present synthesis. Thus, disaccharide **1** was synthesised according to the literature [10] and deacylated using Zemplén conditions to give the first acceptor **2**. The benzylidene acetal in derivatives **1** and **2** was regioselectively opened with sodium cyanoborohydride and etheral hydrogen chloride [13]



Scheme 1. (*i*) **14**, DMTST; (*ii*) H_2, Pd–C; (*iii*) MeO^-.



Scheme 2. (*i*) α,α-dimethoxytoluene, *p*-TsOH, 8 min; (*ii*) BnBr, NaH; (*iii*) $NaCNBH_3$–HCl; (*iv*) Im_2CS; (*v*) Bu_3SnH, AIBN.

to yield the two other acceptors, the 4-OH derivative **3** (77%) and the 2^1,4-diol **4** (63%, overall yield from **1**) (Scheme 1).

Syntheses of colitose and of colitose-containing oligosaccharides have previously been performed by Bundle et al. [14,15]. They synthesised colitose as its methyl glycoside, which was then converted to the glycosyl chloride with dichloromethyl methyl ether and zinc chloride and used in halide-assisted glycosylations. We decided to synthesise colitose as its ethyl thioglycoside, this should be an excellent precursor to the labile dideoxyglycosyl halides, due to the mild conditions used in the transformation of thioglycosides into glycosyl halides [16]. Furthermore, the thioglycosides can themselves be used directly as glycosyl donors [17] which gives flexibility in the coupling reactions.

Ethyl 1-thio-β-L-fucopyranoside [18] (**11**) was stereoselectively benzylidenated in neat α,α-dimethoxytoluene in the presence of *p*-toluenesulfonic acid to give the kinetic *endo*-product [19], which was directly benzylated to give compound **12** (Scheme 2). The short time necessary to obtain predominantly the *endo*-benzylidene derivative resulted in incomplete benzylidenation, but the starting material is cheap (and can be recovered from the water-phase), and derivative **12** could conveniently be obtained pure by direct crystallisation after work-up of the reaction mixture, giving an easy access to this key intermediate. Regioselective reductive opening [13] of the *endo*-benzylidene acetal in **12** gave the expected 4-*O*-benzyl ether **13** (85%). Deoxygenation of the 3-OH group was accomplished by formation of the thiocarbonyl imidazole carbamate using thiocarbonyldiimidazole and subsequent reduction with tributyltin hydride and α,α'-azaisobutyronitrile (AIBN) [20] to give the colitose thioglycoside derivative **14** (66%, 88% calculated on consumed **13**) (Scheme 2).

In the coupling reactions, the use of the thioglycoside **14** as donor using thiophilic promoters was explored. Initial attempts were made with acceptor **4** and *N*-iodosuccinimide (NIS) as promoter with or without silver triflate as a catalyst [21,22]. Coupling products were obtained, but the main product isolated in about 50% yield was identified as the *N*-succinimide glycoside derivative of the donor by ^{13}C NMR [δ 16.8 (C-6), 28.3 (CH_2CO), 29.7 (C-3), 69.0, 71.3, 71.4, 71.6, 75.1, and 76.7 (C-1,2,4,5 and CH_2Ph), 127.5–129.0, 138.0 and 138.2 (aromatic C), 177.9 (CO)]. This type of product has previously been found in our laboratory when NIS is used as a promoter in couplings with reactive thioglycoside donors and unreactive (or no) acceptors [23,24]. With dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST) [25] as promoter care had to be taken with the reaction time. If the reaction was allowed to continue for a longer time, the already formed glycosides started to decompose and the yields of the desired tri- and tetra-saccharides were low. If, however, the reaction was quenched after a short time (~ 15 min), high yields of coupling products were obtained. Thus, coupling of acceptor **2** with donor **14**, using DMTST as promoter gave trisaccharide **5** in 85% yield, whereas acceptor **3** gave trisaccharide **6** in 82% yield. The diol acceptor **4** afforded the tetrasaccharide **7** in 59% yield, i.e. about 77% yield in each glycosidation (Scheme 1). Recently, similar yields have been reported with other dideoxy thioglycoside donors in the synthesis of various *Salmonella* O-antigen structures [26]. Interestingly, the authors used, i.a., NIS as promoter, apparently without the formation of the *N*-succinimide glycoside byproducts.

Deprotection of the derivatives **5**, **6** and **7** was accomplished with catalytic hydrogenolysis in the presence of a basic ion-exchange resin (Amberlite OH^-) to avoid loss of the colitose residues, and, when necessary, Zemplén deacetylation to give the target products **8**, **9** and **10**.

3. Experimental

General methods.—These were as described [27].

*Ethyl 2-O-benzyl-*endo*-3,4-O-benzylidene-1-thio-β-L-fucopyranoside* (**12**).—A mixture of ethyl 1-thio-β-L-fucopyranoside [18] (**11**; 3.5 g, 16.8 mmol), α,α-dimethoxytoluene (15 mL) and *p*-toluenesulfonic acid (120 mg) in DMF (1.5 mL) was stirred for 9 min, then diluted with CH_2Cl_2 (150 mL) and washed with aq sat $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The residue was dissolved in DMF (4

mL), then benzyl bromide (4 mL, 33 mmol) was added, and the mixture was added dropwise at 0 °C to a stirred suspension of sodium hydride (80%, 2 g, 66 mmol) in DMF (10 mL). When TLC (6:1 toluene–EtOAc) showed a complete reaction, the mixture was again cooled to 0 °C and MeOH (10 mL) was added dropwise. The mixture was diluted with toluene (100 mL), filtered through Celite, washed with water and brine, dried (Na_2SO_4), and concentrated. Crystallisation from diethyl ether–light petroleum bp 60–70 °C gave **12** (1.718 g, 4.45 mmol, 26%). The mother liquor was concentrated and purified on a silica gel column (10:1 toluene–EtOAc) to give additional **12** after recrystallisation (441 mg, 1.14 mmol, 8%); mp 87 °C; $[\alpha]_D$ −0.1° (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 14.8 (CH_3CH_2S), 16.8 (C-6), 24.3 (CH_3CH_2S), 72.4, 73.9, 76.5, 77.0, and 77.5 (C-2,3,4,5 and CH_2Ph), 83.5 (C-1), 104.3 (PhCH), 126.9–137.8 (aromatic C); ^1H, δ 1.28 (t, 3 H, CH_3CH_2S), 1.46 (d, 3 H, H-6), 2.69 (q, 2 H, CH_3CH_2S), 3.48 (dd, 1 H, $J_{2,3}$ 6.6 Hz, H-2), 3.91 (dd, 1 H, $J_{5,6}$ 6.6 Hz, H-5), 4.15 (dd, 1 H, $J_{4,5}$ 2.2 Hz, H-4), 4.35 (t, 1 H, $J_{3,4}$ 6.2 Hz, H-3), 4.44 (d, 1 H, $J_{1,2}$ 9.6 Hz, H-1), 4.7 (q, 2 H, CH_2Ph), 5.93 (s, 1 H, CHPh), 7.3 (m, 10 H, aromatic H). Anal. Calcd for $C_{22}H_{26}O_4S$: C, 68.37; H, 6.78. Found: C, 68.35; H, 6.83.

*Ethyl 2,4-di-O-benzyl-3,6-dideoxy-1-thio-β-*L*-xylo-hexopyranoside* (**14**).—Compound **12** (1.50 g, 3.89 mmol) and sodium cyanoborohydride (1.5 g, 23.8 mmol) were dissolved in THF (20 mL) containing crushed 3Å molecular sieves and stirred for 30 min, whereafter etheral hydrogen chloride was added dropwise until gas evolution ceased. After an additional 30 min, the mixture was filtered through Celite and the filter was washed with CH_2Cl_2. The filtrate was concentrated and purified by silica gel chromatography (two columns) (6:1 toluene–EtOAc) to give ethyl 2,4-di-*O*-benzyl-1-thio-β-L-fucopyranoside (**13**; 1.286 g, 3.31 mmol, 85%); $[\alpha]_D$ −14.7° (*c* 0.95, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 14.9 (CH_3CH_2S), 17.2 (C-6), 24.9 (CH_3CH_2S), 74.7, 75.3, 75.5, 75.9, and 79.3 (2 C) (C-2,3,4,5 and 2 CH_2Ph), 84.7 (C-1), 127.8–138.4 (aromatic C); ^1H, δ 1.28 (d, 3 H, H-6), 1.32 (t, 3 H, CH_3CH_2S), 2.76 (2 H, CH_3CH_2S), 3.49–3.61 (3 H, H-2,3,5), 3.66 (dd, 1 H, H-4), 4.37 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 4.68 (d, 1 H), 4.75 (s, 2 H), and 4.95 (d, 1 H) (2 CH_2Ph), 7.35 (m, 10 H, aromatic H).

A mixture of compound **13** (1.29 g, 3.31 mmol), *N,N'*-thiocarbonyldiimidazole (1.18 g, 6.6 mmol) and imidazole (100 mg) was boiled under reflux in 1,2-dichloroethane. After 1 h an additional amount of *N,N'*-thiocarbonyldiimidazole (0.59 g, 3.3 mmol) was added and the mixture was boiled under reflux overnight, then cooled, washed with 1 M HCl, aq sat $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. The residue and a catalytical amount of AIBN were dissolved in toluene (10 mL), and the solution was added dropwise, via a syringe, to a refluxing solution of tributyltin hydride (1.4 mL, 5.2 mmol) in toluene (20 mL). After 3 h, the mixture was concentrated and the residue dissolved in *n*-hexane and extracted with MeCN several times. The combined MeCN extracts were washed with *n*-hexane and concentrated. The residue was purified on a silica gel column (10:1 toluene–EtOAc) to give first **14** (808 mg, 2.17 mmol, 66%) and then recovered **13** (303 mg, 25%). Compound **14**: $[\alpha]_D$ +49.1° (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$): ^{13}C, δ 14.9 (CH_3CH_2S), 17.0 (C-6), 24.3 (CH_3CH_2S), 34.2 (C-3), 71.1, 72.5, 72.7, 75.1, and 76.3 (C-2,4,5 and 2 CH_2Ph), 86.6 (C-1), 127.6–138.4 (aromatic C); ^1H, δ 1.24 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.30 (t, 3 H, CH_3CH_2S), 1.45 (ddd, 1 H, $J_{3,3}$ 14, $J_{3,2}$ 11, $J_{3,4}$ 3 Hz, H-3ax), 2.41 (ddd, 1 H, $J_{3,2} \approx J_{3,4} = 3$–4 Hz, H-3eq), 2.73 (2 H, CH_3CH_2S), 3.39 (br s, 1 H, H-4), 3.60 (m, 2 H, H-2,5), 4.46 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 4.37 (d, 1 H), 4.54 (d, 1 H), and 4.71 (d, 2 H) (2 CH_2Ph), 7.30 (m, 10 H, aromatic H). Anal. Calcd for $C_{22}H_{28}O_3S$: C, 70.93; H, 7.58. Found: C, 70.68; H, 7.52.

*8-Methoxycarbonyloctyl (3,6-dideoxy-α-*L*-xylo-hexopyranosyl)-(1 → 2)-β-*D*-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**8**).—8-Methoxycarbonyloctyl (3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside [10,11] (**2**; 112 mg, 0.12 mmol), **14** (65 mg, 0.17 mmol) and 2,6-di-*tert*butyl-4-methylpyridine (50 mg, 0.24 mmol) were dissolved in CH_2Cl_2 (20 mL) containing crushed 4Å molecular sieves and the mixture was stirred for 30 min, whereafter DMTST (54 mg, 0.21 mmol) was added. After 16 min NEt_3 (1 mL) was added and the mixture was concentrated and purified on a silica gel column (20:20:1 toluene–EtOAc–MeOH) to give 8-methoxycarbonyloctyl (2,4-di-*O*-benzyl-3,6-dideoxy-α-L-*xylo*-hexopyrano- syl)-(1 → 2)-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**5**; 128 mg, 0.10 mmol, 85%); $[\alpha]_D$ −34.5° (*c* 1.22, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 16.4 (C-6^{II}), 23.5 (Me NHAc), 24.9, 25.7, 29.1 (2 C), 29.5, and 34.0 (CH_2 spacer), 27.6 (C-3^{II}), 51.4 (OMe), 58.0 (C-2), 66.4, 66.7, 68.7 (2 C), 70.2, 70.8, 70.9, 71.0, 72.0,

72.7, 73.1, 73.5, 74.5, 74.7, 75.7, 76.4, and 78.8 (C-3,4,5,6, C-2^{I},3^{I},4^{I},5^{I},6^{I}, C-2^{II},4^{II},5^{II}, CH_2Ph and CH_2O spacer), 96.8, 100.9 (2 C), and 101.2 (C-$1,1^{I},1^{II}$ and CHPh), 126.4–138.5 (aromatic C), 171.8 and 174.2 (CO).

Compound **5** (120 mg, 0.098 mmol) was dissolved in 2:1 EtOAc–EtOH (15 mL) and hydrogenolysed over Pd–C, in the presence of Amberlite OH^- ion-exchange resin, at 90 psi overnight. The mixture was filtered through Celite and the filtrate concentrated. The residue was purified on a Bio-Gel P-2 column eluted with 99:1 water–*n*-butanol to give, after freeze-drying, **8** (34 mg, 0.05 mmol, 51%); $[\alpha]_D$ −34.8° (*c* 1.0, H_2O); ^{13}C (acetone, δ 31.0), δ 16.1 (C-6^{II}), 23.0 (Me NHAc), 25.1, 25.8, 29.0, 29.1, 29.3, and 34.6 (CH_2 spacer), 33.5 (C-3^{II}), 52.8 (OMe), 55.6 (C-2), 61.5, 62.0, 64.0, 67.0, 69.2, 69.6, 69.9, 71.3, 74.3, 75.8, 76.2, 77.4, and 78.0 (C-3,4,5,6, C-2^{I},3^{I},4^{I},5^{I},6^{I}, C-2^{II},4^{II},5^{II}, and CH_2O spacer), 99.8, 101.0, and 102.6 (C-$1,1^{I},1^{II}$), 174.2 and 178.6 (CO); ^{1}H (50 °C, HDO, δ 4.53), δ 1.16 (d, 3 H, H-6^{II}), 1.29 (m, 10 H, CH_2), 1.56 (dt, 4 H, CH_2), 1.78–1.99 (m, 2 H, H-3^{II}), 2.04 (s, 3 H, MeCON), 2.39 (t, 2 H, CH_2), 3.45–4.05 (C*H*OH), 3.69 (s, 3 H, OMe), 4.25 (q, 1 H, H-5^{II}), 4.40 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.66 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1^{I}), 5.11 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1^{II}). HRMS Calcd for $C_{30}H_{53}O_{16}N$ $[M-H]^-$: 682.3286; Found: 682.3268.

8-Methoxycarbonyloctyl β-D-galactopyranosyl-(1 → 3)-[(3,6-dideoxy-α-L-xylo-hexopyranosyl)-(1 → 4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (**9**).—8-Methoxycarbonyloctyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside [10] (**3**; 120 mg, 0.13 mmol) and **14** (70 mg, 0.19 mmol) were coupled in the presence of 2,6-di-*tert*butyl-4-methylpyridine (51 mg, 0.25 mmol) and DMTST (65 mg, 0.25 mmol) as described above for compounds **2** and **14**, to give 8-methoxycarbonyloctyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-[(2,4-di-*O*-benzyl-3,6-dideoxy-α-L-*xylo*-hexopyra-nosyl)-(1 → 4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**6**; 131 mg, 0.10 mmol, 82%); $[\alpha]_D$ −27.4° (*c* 1.25, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 16.1 (C-6^{II}), 21.1 (Me OAc), 23.2 (Me NHAc), 24.9, 25.9, 29.0, 29.1, 29.4, and 34.1 (CH_2 spacer), 27.5 (C-3^{II}), 51.4 (OMe), 53.4 (C-2), 66.4, 68.0, 69.3, 70.6, 70.9, 71.0, 71.1, 72.0, 72.8, 73.2, 73.5 (2 C), 74.8, 75.6, 76.2, and 80.3 (C-3,4,5,6, C-2^{I},3^{I},4^{I},5^{I},6^{I}, C-2^{II},4^{II},5^{II}, CH_2Ph and CH_2O spacer), 93.7, 99.6, and 100.3 (C-$1,1^{I},1^{II}$), 127.3–138.7 (aromatic C), 169.4, 169.7, and 174.3 (CO).

Compound **6** (105 mg, 0.083 mmol) was deacetylated overnight using Zemplén conditions, neutralised with Dowex H^+ ion-exchange resin, and then debenzylated in the same way as described for compound **5** to give, after freeze-drying, **9** (29.6 mg, 0.043 mmol, 52%); $[\alpha]_D$ −33.7° (*c* 0.6, water); NMR (D_2O): ^{13}C (acetone, δ 31.0), δ 16.2 (C-6^{II}), 23.1 (Me NHAc), 25.0, 25.8, 28.9, 29.1, 29.3, and 34.5 (CH_2 spacer), 33.3 (C-3^{II}), 52.8 (OMe), 56.6 (C-2), 60.5, 62.5, 63.8, 67.2, 69.2, 69.3, 71.2, 71.4, 73.1, 73.4, 75.7, 76.2, and 76.8 (C-3,4,5,6, C-2^{I},3^{I},4^{I},5^{I},6^{I}, C-2^{II},4^{II},5^{II} and CH_2O spacer), 98.3, 101.7, and 103.6 (C-$1,1^{I},1^{II}$), 175.0 and 178.7 (CO); ^{1}H (70 °C, HDO, δ 4.34), δ 1.12 (d, 3 H, H-6^{II}), 1.30 (m, 10 H, CH_2), 1.56 (dt, 4 H, CH_2), 1.87–2.10 (m, 2 H, H-3^{II}), 2.03 (s, 3 H, MeCON), 2.38 (t, 2 H, CH_2), 3.46–4.18 (C*H*OH), 3.70 (s, 3 H, OCH_3), 4.51 (d, 1 H, $J_{1,2}$ 7.7 Hz) and 4.57 (d, 1 H, $J_{1,2}$ 8.4 Hz) (H-$1,1^{I}$), 4.67 (q, 1 H, H-5^{II}), 4.95 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^{II}). HRMS Calcd for $C_{30}H_{53}O_{16}N$ $[M-H]^-$: 682.3286; Found: 682.3289.

8-Methoxycarbonyloctyl (2,4-di-O-benzyl-3,6-dideoxy-α-L-xylo-hexopyranosyl)-(1 → 2)-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1 → 3)-[(2,4-di-O-benzyl-3,6-dideoxy-α-L-xylo-hexopyranosyl)-(1 → 4)]-2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranoside (**7**).—8-Methoxycarbonyloctyl (3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside [10] (**4**; 445 mg, 0.49 mmol) and **14** (500 mg, 1.34 mmol) were coupled in the presence of 2,6-di-*tert*butyl-4-methylpyridine (330 mg, 1.6 mmol) and DMTST (414 mg, 1.6 mmol) as described above for compounds **2** and **14**, to give **7** (438 mg, 0.29 mmol, 59%); $[\alpha]_D$ −40.0° (*c* 0.49, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 15.9 and 16.3 (C-6^{II},6^{III}), 23.5 (Me NHAc), 24.9, 25.8, 29.8, 29.2, 29.4, and 34.0 (CH_2 spacer), 27.0 and 27.8 (C-3^{II},3^{III}), 51.4 (MeO), 58.4 (C-2), 66.2, 66.6, 68.0, 69.5, 70.5, 70.7, 71.0, 71.2, 71.3, 72.7, 72.8, 73.1, 73.6, 74.9, 75.2, 75.9, 76.0, 76.8, and 84.5 (C-3,4,5,6, C-2^{I},3^{I},4^{I},5^{I},6^{I}, C-2^{II},4^{II},5^{II}, C-2^{III},4^{III},5^{III}, CH_2Ph and CH_2O spacer), 96.1, 96.4, 99.8, and 102.1 (C-$1,1^{I},1^{II},1^{III}$), 125.3–139.0 (aromatic C), 170.3 and 174.2 (CO). Anal. Calcd for $C_{92}H_{111}NO_{19}$: C, 71.99; H, 7.29. Found: C, 71.24; H, 7.14.

8-Methoxycarbonyloctyl (3,6-dideoxy-α-L-xylo-hexopyranosyl)-(1 → 2)-β-D-galactopyranosyl-(1 → 3)-[(3,6-dideoxy-α-L-xylo-hexopyranosyl)-(1 → 4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (**10**).—Compound **7** (438 mg, 0.29 mmol) was deprotected as described above for compound **5** to give **10** (170 mg, 0.21 mmol, 73%); $[\alpha]_D$ −57.0° (*c* 1.00, H_2O);

NMR (D_2O): ^{13}C (acetone, δ 31.0), δ 16.1 and 16.2 (C-6^{II},6^{III}), 23.0 (Me NHAc), 25.1, 28.9 (2 C), 29.1, 29.4 and 34.5 (CH_2 spacer), 33.2 and 33.5 (C-3^{II},3^{III}), 52.8 (MeO), 56.4 (C-2), 60.4, 62.5, 63.9, 64.2, 66.8, 67.3, 69.3, 69.5, 69.6, 71.3, 73.1, 74.5, 75.5 (2 C), 76.2, and 77.2 (C-3,4,5,6, C-2^{I},3^{I},4^{I},5^{I},6^{I}, C-2^{II},4^{II},5^{II}, C-2^{III},4^{III},5^{III} and CH_2O spacer), 98.0, 99.8, 101.4, and 102.7 (C-1,1^{I},1^{II},1^{III}), 174.1 and 178.7 (CO); ^{1}H (50 °C, HDO, δ 4.53), δ 1.21 and 1.22 (2 d, 6 H, H-6^{II},6^{III}), 1.30 (m, 10 H, CH_2), 1.58 (dt, 4 H, CH_2), 1.86–2.10 (4 H, H-3^{II},3^{III}), 2.06 (s, 3 H, MeCON), 2.40 (t, 2 H, CH_2), 3.50–4.22 (C*H*OH), 3.70 (s, 3 H, OCH_3), 4.34 (q, 1 H, $J_{5,6}$ 6.2 Hz, H-5^{III}), 4.41 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.71 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1^{I}), 4.75 (q, 1 H, $J_{5,6}$ 7.3 Hz, H-5^{II}), 4.96 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^{II}), 5.08 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^{III}). HRMS Calcd for $C_{36}H_{63}O_{19}N$ $[M-H]^-$: 812.3916; Found: 812.3861.

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References

[1] M.J. Albert, A.K. Siddique, M.S. Islam, A.S.G. Faruque, M. Ansaruzzaman, S.M. Faruque, and R.B. Sack, *Lancet*, 341 (1993) 704.
[2] T. Ramamurthy, S. Garg, R. Sharma, S.K. Bhattacharya, G.B. Nair, T. Shimada, T. Takeda, T. Karasawa, H. Kurazano, A. Pal, and Y. Takeda, *Lancet*, 341 (1993) 703–704.
[3] M.J. Albert, M. Ansaruzzaman, P.K. Bardhan, A.S.G. Faruque, S.M. Faruque, M.S. Islam, D. Mahalanabis, R.B. Sack, M.A. Salam, A.K. Siddique, M.D. Yunus, and K. Zaman, *Lancet*, 342 (1993) 387–390.
[4] L.M. Preston, Q. Xu, J.A. Johnsson, A. Joseph, D.R. Maneval, Jr., K. Husain, G.P. Reddy, C.A. Bush, and J.G. Morris, Jr., *J. Bacteriol.*, (1995) 835–838.
[5] Y.A. Knirel, L. Paredes, P.-E. Jansson, A. Weintraub, G. Widmalm, and M.J. Albert, *Eur. J. Biochem.*, 232 (1995) 391–396.
[6] A.D. Cox and M.B. Perry, *Carbohydr. Res.*, 290 (1996) 59–65.
[7] O. Lüderlitz, A.M. Staub, and O. Westphal, *Bacteriol. Rev.*, 30 (1966) 192–255.
[8] L. Kenne, B. Lindberg, E. Söderholm, D. Bundle, and D.W. Griffith, *Carbohydr. Res.*, 111 (1982) 289–296.
[9] B. Lindberg, F. Lindh, J. Lönngren, A.A. Lindberg, and S.B. Svenson, *Carbohydr. Res.*, 97 (1981) 105–112.
[10] R.U. Lemieux, D.R. Bundle, and D.A. Baker, U.S. Pat. 4,137,401 (1979).
[11] D.P. Khare, O. Hindsgaul, and R.U. Lemieux, *Carbohydr. Res.*, 136 (1985) 285–305.
[12] U. Spohr and R.U. Lemieux, *Carbohydr. Res.*, 174 (1988) 211–237.
[13] P.J. Garegg, H. Hultberg, and S. Wallin, *Carbohydr. Res.*, 108 (1982) 97–101.
[14] D.R. Bundle and S. Josephsson, *Can. J. Chem.*, 56 (1978) 2686–2690.
[15] T. Iversen and D.R. Bundle, *Can. J. Chem.*, 60 (1982) 299–303.
[16] F. Weygandt, H. Ziemann, and H.J. Bestmann, *Chem. Ber.*, 91 (1958) 2534.
[17] P. Fügedi, P.J. Garegg, H. Lönn, and T. Norberg, *Glycoconjugate J.*, 4 (1987) 97–108.
[18] H. Lönn, *Carbohydr. Res.*, 139 (1985) 105–113.
[19] J. Kerékgyártó and A. Lipták, *Carbohydr. Res.*, 248 (1993) 361–364.
[20] J.R. Rasmussen, *J. Org. Chem.*, 45 (1980) 2725–2727.
[21] G.H. Veeneman, S.H. van Leeuwen, and J.H. van Boom, *Tetrahedron Lett.*, 31 (1990) 1331–1334.
[22] P. Konradsson, U.E. Udodong, B. Fraser-Reid, *Tetrahedron Lett.*, 31 (1990) 4313–4316.
[23] C. Krog-Jensen and S. Oscarson, *J. Org. Chem.*, 61 (1996) 1234–1238.
[24] L. Öhberg, Licentiate Thesis, Stockholm University (1996).
[25] P. Fügedi and P.J. Garegg, *Carbohydr. Res.*, 149 (1986) C9–C12.
[26] K. Zegelaar-Jaarsveld, S.C. van der Plas, G.A. van der Marel, and J.H. van Boom, *J. Carbohydr. Chem.*, 15 (1996) 665–689.
[27] K. Ekelöf and S. Oscarson, *Carbohydr. Res.*, 278 (1995) 289–300.