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Total Synthesis of the *Escherichia coli* O111 *O*-Specific Polysaccharide Repeating Unit

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Abstract: The first total synthesis of the *O*-antigen pentasaccharide repeating unit from Gram-negative bacteria *Escherichia coli* O111 was achieved starting from four monosaccharide building blocks. Key to the synthetic approach was a bis-glycosylation reaction to combine trisaccharide **10** and colitose **5**. The colitose building block (**5**) was obtained *de novo* from non-carbohydrate precursors. The pentasaccharide was equipped at the reducing end with an amino spacer to provide a handle for subsequent conjugation to a carrier protein in anticipation of immunological studies.

Keywords: antigens · carbohydrates · *Escherichia coli* · glycosylation · vaccines

Introduction

The bacterial contamination of food can result in great concern in the general population at a time when safe food is often taken for granted in developed countries. Infections caused by *Escherichia coli* bacteria are rather common, but have received increased attention in recent years owing to the emergence of new strains. A new pathogenic strain of the bacteria *Escherichia coli*, serotype O111, has emerged as a significant cause for enteropathogenic, enterotoxigenic, and enterohemorrhagic diseases in humans. For example, enteropathogenic *E. coli* O111 (EPEC) causes diarrhea in children, particularly in the developing world. Enterotoxigenic *E. coli* (ETEC) O111 is responsible for watery diarrhea in infants and is associated with heat-labile (cholera-like) toxin, whilst enterohemorrhagic *E. coli* (EHEC) O111, which generally carries at least one Shinga-toxin gene, is one of the most common non-O157 causes of bloody diarrhea and hemolytic-uremic syndrome in developed countries.[1] The largest outbreak of Shinga-toxin-producing *E. coli* O111 in U.S. history occurred in 2008 and affected 341 persons of all age groups. One patient out of the 70 that were hospitalized died.[2]

The problem with antibiotic resistance of EHEC O111 is evident due to their excessive use in the livestock industry.[3] The prevention of bacterial diseases by vaccination against *E. coli* O111 would circumvent this multidrug-resistance problem. Serum antibodies to the *O*-specific polysaccharide of O111 lipopolysaccharide protect mice and dogs against infection with this pathogen type, which suggests that the induction of antibodies by a carbohydrate-conjugate vaccine would be protective.[4] The structure of the *O*-specific polysaccharide of O111 *E. coli* contains a pentasaccharide repeating unit with two rare colitose (3,6-dideoxy-L-*xylo*-hexopyranose) residues as a distinguishing feature (Figure 1).[5] The *O*-specific polysaccharide from *E. coli* O111 is also found on other enteric pathogens, such as *Salmonella adelaide*[6] and *Salmonella enterica* O35.[7]

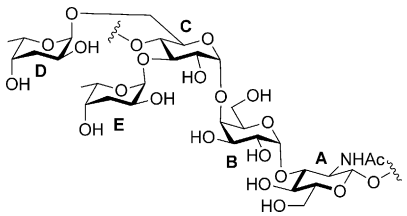

Figure 1. Pentasaccharide repeating unit in the *O*-specific polysaccharide of *E. coli* O111.

Pure pentasaccharide **1** is required to evaluate the immunological properties of the *O*-specific polysaccharide of *E. coli* O111 and to explore the potential of an oligosaccharide-conjugate vaccine (see Scheme 1). This pentasaccharide constitutes a significant synthetic challenge, because it contains two colitose units that are naturally not accessible and are labile in the context of an oligosaccharide. Herein, we report the first total synthesis of a pentasaccharide repeating unit that contains an aminopentanol handle at its reducing end for attachment to any surface or carrier proteins.

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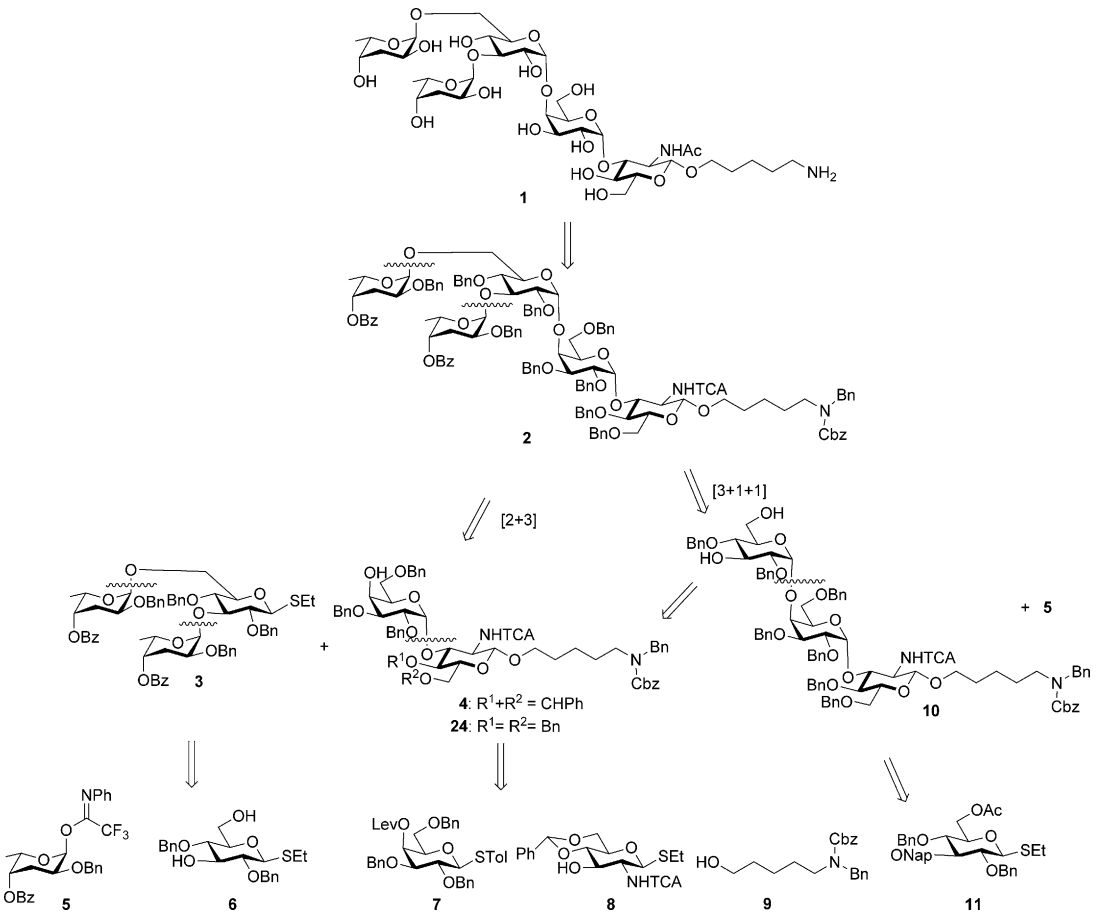
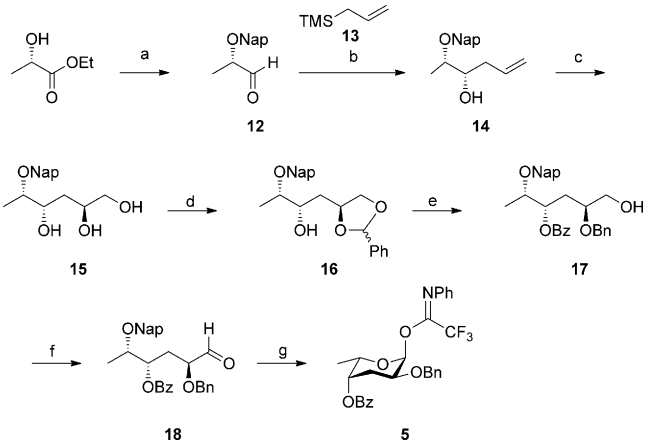
Results and Discussion

Initial attempt to synthesize pentasaccharide 1: Pentasaccharide **1** has several synthetic challenges, the most prominent of which is the α-linkages of the colitoses. Our retrosynthetic analysis of pentasaccharide **1** revealed a convergent [2+3] approach to be attractive (Scheme 1). The reducing end aminopentanol linker will allow for straightforward conjugation. Target structure **1** may be obtained from fully protected pentasaccharide **2** through a three-step deprotection sequence, including conversion of the trichloroacetamide into the corresponding acetamide, cleavage of the ester groups, and hydrogenolysis. In turn, pentasaccharide **2** will be assembled from disaccharide building block **4** and trisaccharide **3**. Disaccharide **4** can be derived from *N*-benzyl-benzyloxycarbonyl-5-aminopentanol **9**, glucosamine thioglycoside **8**, and galactose thioglycoside **7**. The synthesis of a colitose-containing trisaccharide analogue of compound **1** has already been reported by Bundle and co-workers,[8] who employed a halide-assisted glycosylation procedure.[9] The lability of dideoxyglycosyl halides and the need for a reaction time of 10 days prompted us to consider *N*-phenyltrifluoroacetimidate colitose **5** as a precursor of trisaccharide **3**. Thus, we envisaged that we could access trisaccharide **3** from glucose **6** and colitose imidate **5**.

Synthesis of the building blocks: Most of the building blocks that are needed for the synthesis of pentasaccharide repeating unit **1** can be accessed by using modified literature procedures. The synthesis of colitose building block **5** benefits from a *de novo* synthesis that is more efficient than known procedures.[10] Fully functionalized L-colitose glycosylating agent **5** was synthesized in 10 steps, starting from commercially available (*S*)-ethyl lactate, which cements the C5 position of L-colitose (Scheme 2).[11] Protection of the alcohol group on (*S*)-ethyl lactate as a 2-naphthylmethyl (Nap) ether, followed by reduction of the ester moiety with DIBAL, afforded aldehyde **12** in 77% yield over two steps. Cram chelated allylation[12] of aldehyde **12** stereoselectively provided homoallylic alcohol **14** in 91% yield. Upjohn oxidation[13] of alkene **14** resulted in a 1:1 diastereoisomeric mixture affording triol **15** in 49% yield. Having set and defined all of the necessary stereocenters in triol **15**, the synthesis of L-colitose **5** was completed. Strategically, a building block with a non-participating benzyl (Bn) ether protecting group at C2 position was targeted, as L-colitose is found in nature only bearing α-linkages to other sugars. Furthermore, an electron-withdrawing benzoyl (Bz) protecting group was to be placed at the C4 position to stabilize the electron-rich deoxysugar building block. Thus, triol **15** was converted selectively into 5-membered benzylidene acetal **16**, which was



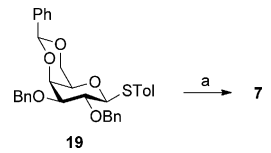
Scheme 1. Retrosynthetic analysis of pentasaccharide **1**.



Scheme 2. New synthesis of colitose building block **5**: a) i) NapBr, NaH, DMF, 0°C to RT; ii) DIBAL, CH_2Cl_2, −78°C, 90 min, 77% yield (2 steps); b) compound **13**, $SnCl_4$, CH_2Cl_2, −78°C, 2 h, 91% yield; c) NMO, OsO_4 (cat.), THF/H_2O (2:1 v/v), RT, 24 h, 49% yield; d) PhCH(OMe)$_2$, CSA, $CuSO_4$, MeCN/THF (1:1 v/v), RT, 10 min; e) i) BzCl, pyridine, RT, 2 h; ii) BH_3·THF, TMSOTf, CH_2Cl_2, 0°C to RT, 2 h, 71% yield (3 steps); f) Dess–Martin periodinane, pyridine, CH_2Cl_2, RT, 1 h, 92% yield; g) i) DDQ, MeOH, CH_2Cl_2, 0°C to RT, 2 h; ii) $CF_3C(=NPh)Cl$, Cs_2CO_3, CH_2Cl_2, RT, 15 h, 68% yield (2 steps). DIBAL = diisobutylaluminum hydride, NMO = *N*-methylmorpholine-*N*-oxide, CSA = (±)-10-camphorsulfonic acid, TMSOTf = trimethylsilyl trifluoromethanesulfonate, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

benzoylated and subjected to regioselective benzylidene-opening to furnish primary alcohol **17** in 71% yield over three steps. Following Dess–Martin oxidation,[14] aldehyde **18** was converted into the corresponding hemiacetal by removal of the Nap ether protecting group with DDQ. This time, we opted to convert the colitose hemiacetal into *N*-trifluorophenylacetimidate[15] **5**, which is an attractive alternative to the previously synthesized colitose trichloroacetimidate, owing to its more moderate reactivity[16] and its lower tendency to undergo O→N isoamide-to-amide rearrangement at the anomeric center.

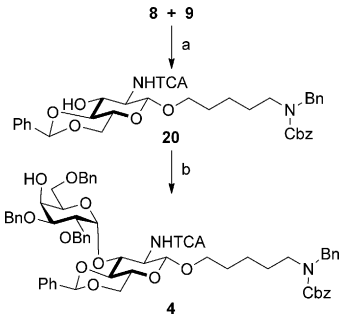
Having established a synthetic route to colitose glycosylating agent **5**, the remaining building blocks were prepared. *N*-Benzyl-benzyloxycarbonyl-5-aminopentanol **9** and glucosamine building block **8** (Scheme 1) were synthesized by following literature procedures.[17,18] Galactose building block **7** (Scheme 1), which was equipped with an orthogonal levulinic ester at the C4 position in anticipation of chain-elongation, was obtained from known benzylidene galactoside **19**,[19] presenting non-participating benzyl ethers at the C2 and C3 positions (Scheme 3). Regioselective reductive opening of the benzylidene group on compound **19** with triethylsilane in the presence of triflic acid and triflic anhydride, followed by the protection of the C-4 hydroxy group as a levulinoyl ester afforded galactose building block **7** in 69% yield over two steps.



Scheme 3. Synthesis of galactose building block **7**: a) i) Et_3SiH, TfOH, Tf_2O, CH_2Cl_2, 0°C to RT, 3 h; ii) LevOH, DCC, DMAP, CH_2Cl_2, 0°C to RT, 15 h, 69% yield (2 steps). Lev = levulinoyl, DCC = *N*,*N*′-dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine.

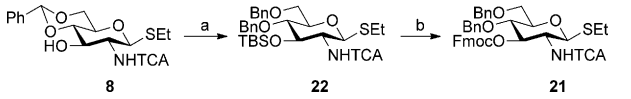
Finally, known glucose building block **6**[20] (Scheme 1), containing free hydroxy groups at the C3 and C6 positions, served as a branching building block.

Synthesis of disaccharide acceptor 4: With all of the required building blocks in hand, the assembly of the pentasaccharide repeating unit through a [2+3] strategy was explored, commencing from the reducing end (Scheme 4). Thioglycoside **8** was treated with an excess of linker **9** in the presence of NIS and TfOH at −20°C to afford target glucosamine **20**. The presence of the trichloroacetamide as a participating group at the C2 position ensured the selective formation of the β-glycosidic linkage.[18] Furthermore, at −20°C, only the primary alcohol acted as a nucleophile in the coupling reaction. To aid in purification, any excess linker was tritylated to afford glucosamine **20** in 70% yield after flash column chromatography on silica gel. Galactopyranose **7** was appended onto β-glucosamine **20** by using a DMF-modulated glycosylation procedure[21] to direct the stereochemical course of the reaction. In our hands, the DMF-modulated glycosylation, followed by removal of the levulinoyl ester under Zemplén conditions,[22] provided the target disaccharide (**4**) in only 14% yield. This low yield was explained by the long glycosylation time, which favored cleavage of the benzylidene acetal on the target molecule and the formation of byproducts.



Scheme 4. Initial attempts towards reducing end disaccharide **4**: a) i) NIS, TfOH, CH_2Cl_2, 4 Å M.S., −20°C, 30 min; ii) TrtCl, pyridine, overnight, 70% yield (2 steps); b) i) compound **7**, DMF, NIS, TMSOTf, CH_2Cl_2, 4 Å M.S., −10°C, 12 h; ii) NaOMe, MeOH, 14% yield (2 steps). NIS = *N*-iodosuccinimide, Trt = triphenylmethyl.

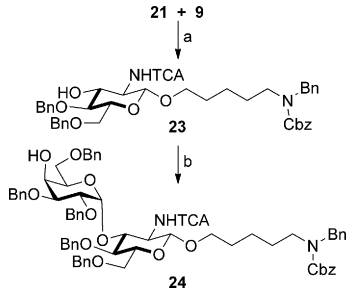
To circumvent the problems that were encountered in the synthesis of disaccharide **4**, glucosamine building block **8** was converted into glycosylating agent **21** presenting an Fmoc protecting group at the C3 position for chain-elongation and stable benzyl ethers at the C4 and C6 positions (Scheme 5). We expected that the use of building block **21** would facilitate the isolation of compound **23**, which would perform better as acceptor in the coupling reaction with galactopyranose **7**. Following the placement of a TBS silyl ether as a protecting group on the hydroxy group at the C3 position of thioglycoside **8**, regioselective reductive opening



Scheme 5. Synthesis of glucosamine building block **21**: a) i) TBSCl, imidazole, CH_2Cl_2; ii) BH_3·THF, TMSOTf, CH_2Cl_2; iii) BnBr, NaH, DMF, 77% yield (3 steps); b) i) BF_3·OEt_2, MeCN; ii) FmocCl, pyridine, CH_2Cl_2, 92% yield (2 steps). TBS = *tert*-butyldimethylsilyl, Fmoc = fluorenylmethyloxycarbonyl.

of the benzylidene acetal, and subsequent benzylation of the hydroxy group at the C6 position provided glucosamine **22** in 77% yield over three steps. Removal of the silyl ether at the C3 position in the presence of BF_3·OEt_2 and protection of the free hydroxy group with Fmoc provided the new glucosamine glycosylating agent **21** in 92% yield over two steps.

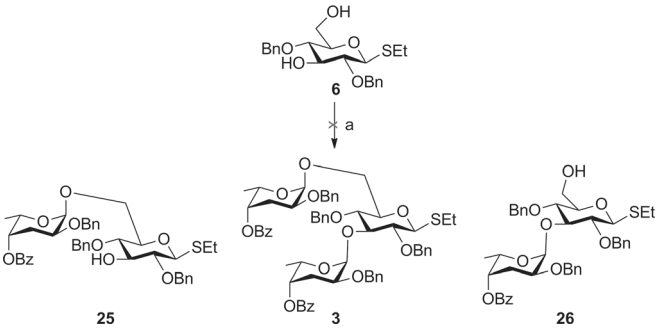
Thioglycoside **21** was successfully coupled to *N*-benzylbenzyloxycarbonyl-5-aminopentanol **9** at −20 °C upon activation by NIS and TfOH in CH_2Cl_2 (Scheme 6). Fmoc cleav-



Scheme 6. Synthesis of "reducing end" disaccharide **24**: a) i) NIS, TfOH, CH_2Cl_2, 3 Å M.S. AW, −20 °C, 1 h; ii) Et_3N, CH_2Cl_2, RT, 3 h, 71% yield (2 steps); b) i) compound **7**, NIS, TfOH, Et_2O/CH_2Cl_2 (3:1 v/v), 3 Å M.S. AW, 0 °C, 30 min; ii) NaOMe, MeOH, RT, overnight, 48% yield (2 steps). AW = acid washed.

age by treatment with triethylamine afforded β-glucosamine nucleophile **23** in 71% yield over two steps. The $^1J_{C1,H1}$ coupling constant of 164.8 Hz was characteristic for the formation of a β-glycosidic linkage. The coupling of nucleophile **23** with thiogalactoside **7** by activation with NIS and TfOH in Et_2O/CH_2Cl_2, followed by cleavage of the levulinoyl ester, afforded target disaccharide **24** in 48% yield over two steps. The configuration of the newly formed glycosidic bond was assigned based on the coupling constant between the C1 and H1 atoms on the galactopyranose residue ($^1J_{C1,H1}$=171.3 Hz).

Attempts to synthesize trisaccharide 3: With thioglucoside **6** and L-colitose *N*-trifluorophenylacetimidate **5** in hand, we attempted the synthesis of trisaccharide **3** (Scheme 7). Owing to the high reactivity of deoxysugars,[16] the reaction was performed at −50 °C with 1.25 equivalents of glycosylating agent **5** per nucleophile. After 1 h, the target trisaccharide was mainly observed, along with deletion sequences. A prolonged reaction time (2 h) produced α-(1,6)-disaccharide

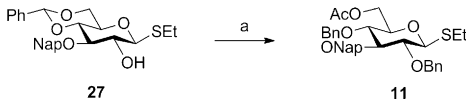


Scheme 7. Attempts towards trisaccharide **4**: a) compound **5** (2.5 equiv), TMSOTf, CH_2Cl_2, 4 Å M.S., −50 °C.

25 at the expense of the desired trisaccharide (**3**). The degradation of electron-rich colitose residues in acidic media has also been reported by Oscarson et al.[10b] The instability of 3,6-dideoxysugars in acidic media rendered the assembly of the target pentasaccharide by using a [2+3] approach unfeasible, because the colitose residues would be exposed to acidic conditions twice.

Total synthesis of the *Escherichia coli* O111 *O*-antigen: To circumvent the degradation pathways, an alternative retrosynthetic analysis was designed (Scheme 1). In this approach, the colitose residues would be introduced at a late stage of the synthesis through a bis-glycosylation reaction with trisaccharide **10**. Trisaccharide **10** was further disconnected to disaccharide **24** and thioglucoside building block **11**, equipped with an acetate ester at the C6 position as a remote protecting group favoring the formation of the α-linkage.[23]

To install residue C on disaccharide **24** (Figure 1), glucose building block **11** was synthesized starting from known thioglucoside **27**[24] (Scheme 8). Building block **11** contains non-

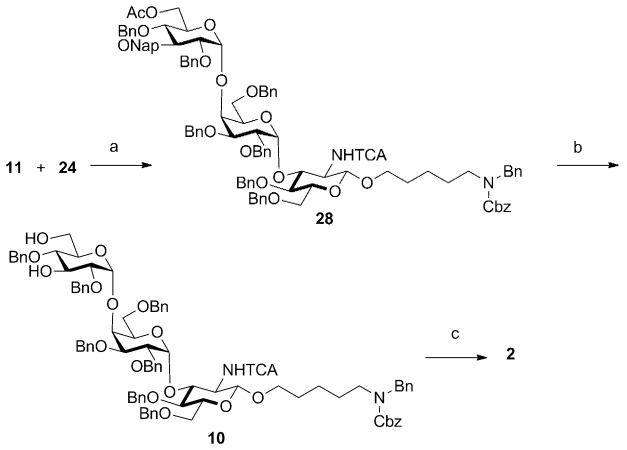


Scheme 8. Synthesis of thioglucoside **11**: a) i) BnBr, NaH, DMF; ii) BH_3·THF, TMSOTf, CH_2Cl_2; (iii) Ac_2O, pyridine, 74% yield (3 steps).

participating ether groups at the C2, C3, and C4 position, as well as a participating acetate ester group at the C6 position, to favor the formation of an α-glycosidic linkage. The orthogonal Nap ether at the C3 position would allow for branching at this position. To access fully functionalized glucose **11**, first, the free hydroxy group of glucose **27** was benzylated. Regioselective opening of the benzylidene acetal with BH_3·THF and TMSOTf afforded the primary alcohol as an intermediate, which was further acetylated to yield glycosylating agent **11** in 74% over three steps.

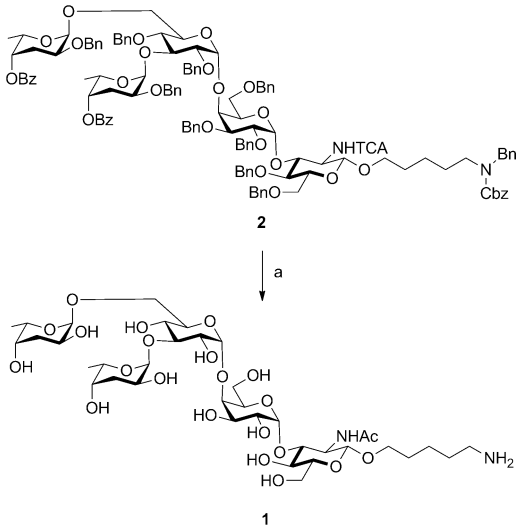
The coupling of thioglucoside **11** with disaccharide **24** was performed at −50 °C by activation with NIS in the presence

of TfOH in Et_2O/CH_2Cl_2 to promote α selectivity (Scheme 9). This procedure afforded α-trisaccharide **28** in 97% yield. Analysis of the coupling constant of the appended glucose moiety ($^1J_{C1,H1}=172.4$ Hz) allowed for the assignment of the α stereochemistry at the newly formed center. Cleavage of the acetate protecting group at the C6 position under Zemplén conditions, followed by removal of the Nap ether at the C3 position by treatment with DDQ, afforded diol **10** in 73% yield over two steps. Finally, the conversion of diol **10** into the corresponding fully protected pentasaccharide (**2**) was achieved through a bis-glycosylation reaction by using four equivalents of colitose *N*-phenyltrifluoroacetimidate **5** in the presence of TMSOTf at −50°C. Quenching of the reaction after 15 min resulted in the formation of the target pentasaccharide (**2**) in 63% yield. The formation of the two α-linkages was confirmed by analysis of the anomeric C–H coupling constants (see the Supporting Information).



Scheme 9. Synthesis of colitose-containing pentasaccharide **2**: a) NIS, TfOH, Et_2O/CH_2Cl_2 (3:1 v/v), 4 Å M.S., −50°C, 1 h, 97% yield; b) i) NaOMe, MeOH, RT, 3 h; ii) DDQ, CH_2Cl_2/MeOH (9:1 v/v), 0°C, 3 h, 73% yield (2 steps); c) compound **5**, TMSOTf, CH_2Cl_2, 4 Å M.S., −50°C, 15 min, 63% yield.

Final deprotection of the target pentasaccharide (**2**) was achieved in three steps (Scheme 10). To decrease the number of transformations, the conversion of the trichloroacetamide group into the corresponding acetamide group by hydrogenolysis (H_2, $Pd(OH)_2$, THF/water) was attempted; however, this reaction resulted in incomplete conversion. To convert the TCA protecting group into the corresponding acetyl group, pentasaccharide **2** was heated at 90°C with Bu_3SnH and AIBN. Removal of the benzoate esters on the colitose residues proceeded slowly under Zemplén conditions, but heating the intermediary pentasaccharide at 40°C overnight drove the reaction to completion. Particular care was taken during the final hydrogenolysis reaction to avoid acidic conditions, which could result in the degradation of the colitose residue. Cleavage of the benzyl ethers and the Cbz carbamate (Cbz = carboxybenzyl) were achieved by hydrogenolysis on $Pd(OH)_2$ to provide target pentasaccharide **1** in 64% yield over three steps.



Scheme 10. Deprotection of pentasaccharide **2** to produce compound **1**: a) i) Bu_3SnH, AIBN, toluene, 90°C, 2 h; ii) NaOMe, MeOH, 40°C, overnight; iii) $Pd(OH)_2$, THF/H_2O (1:1 v/v), 24 h, 64% yield (3 steps). AIBN = azobisisobutyronitrile.

Conclusion

The first total synthesis of the pathogenic *E. coli* 0111 *O*-antigen repeating pentasaccharide unit, starting from inexpensive (*S*)-ethyl lactate, has been achieved in 21 linear steps and 1.5% overall yield. The key step of this approach is a bis-glycosylation reaction to install the acid-labile L-colitose residues (Scheme 9). The pentasaccharide repeating unit contains a terminal amine linker at its reducing end for immobilization on any surface or conjugation to carrier proteins for subsequent immunological evaluation.

Experimental Section

General experimental details: All chemicals were of reagent grade and used as supplied unless otherwise noted. Molecular sieves were activated prior to use by heating and drying under high vacuum. All reactions were performed in oven-dried glassware under an argon atmosphere unless otherwise noted. DMF, CH_2Cl_2, toluene, and THF were purified by using a Cycle-Tainer Solvent Delivery System unless otherwise noted. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). The TLC plates were visualized by UV light or by dipping the plate in a solution of cerium sulfate ammonium molybdate (CAM) or in a 1:1 mixture of H_2SO_4 (2 M) and resorcine monomethylether (0.2%) in EtOH. Flash column chromatography was performed by using forced flow of the indicated solvent through Fluka Kieselgel 60 (230–400 mesh). Preparative HPLC purification was performed on an Agilent 1200 Series. ^1H and ^{13}C NMR spectra were recorded on Varian Mercury 400 (400 MHz) or 600 (600 MHz) spectrometers in $CDCl_3$ or D_2O; chemical shifts are referenced to internal standards ($CDCl_3$: ^1H $\delta=7.26$ ppm, ^{13}C $\delta=77.0$ ppm; D_2O with acetone as internal standard: ^1H $\delta=2.05$ ppm, ^{13}C $\delta=29.84$ or 206.26 ppm) unless otherwise

stated. Splitting patterns are indicated as s singlet, d doublet, t triplet, q quartet, m multiplet, bs broad singlet, as apparent singlet, ad apparent doublet, at apparent triplet, and aq apparent quadruplet for ^{1}H NMR data. NMR chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hz. HRMS was performed by the MS service of the Department of Organic Chemistry, Free University Berlin, on an Agilent 6210 ESI-TOF (Agilent Technologies, Santa Clara, CA, USA). IR spectra were recorded on a Perkin–Elmer 1600 FTIR spectrometer. Optical rotations were measured on a UniPol L 1000 polarimeter (Schmidt & Haensch, Berlin, Germany) with concentrations expressed in g/100 mL.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl-4,6-di-*O*-benzyl-2-trichloroacetamido-β-D-glucopyranoside (23)**: A mixture of glucosamine building block **21** (0.60 g, 0.78 mmol), linker **9** (0.38 g, 1.17 mmol, 1.5 equiv), freshly activated molecular sieves (3 Å AW, 1.8 g), and NIS (0.35 g, 1.56 mmol, 2 equiv) in CH_2Cl_2 (16 mL) was stirred for 15 min before being cooled to −20 °C. After the addition of TfOH (7 μL, 78 μmol, 0.1 equiv), the mixture was stirred at −20 °C for 1 h. After removal of the molecular sieves by filtration through a pad of celite, the reaction mixture was diluted with CH_2Cl_2 and washed with a saturated aqueous solution of $NaHCO_3$. The organic layer was washed with a saturated aqueous solution of $Na_2S_2O_3$ and water, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (hexanes/EtOAc, 80:20 to 50:50) to yield the crude product (0.61 g). The crude material was dissolved in CH_2Cl_2 (12 mL) and treated with Et_3N (1.6 mL, 11.7 mmol). The solution was stirred for 3 h at RT and then concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (hexanes/EtOAc, 90:10 to 50:50) to give target compound **23** as an oil (0.45 g, 0.55 mmol, 71% yield over 2 steps). R_f = 0.25 (hexanes/EtOAc, 70:30); $[\alpha]_D^{20} = -2.4$ (c = 0.35, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.23 (m, 20H), 7.17 (s, 1H), 5.16 (s, 2H), 4.74 (d, J = 11.2 Hz; 1H), 4.69–4.61 (m, 3H), 4.55 (d, J = 12.0 Hz; 1H), 4.48 (bs, 2H), 4.13–4.04 (m, 1H), 3.85–3.71 (m, 3H), 3.63–3.47 (m, 3H), 3.43–3.37 (m, 1H), 3.23–3.17 (m, 2H), 2.99 (s, 1H), 1.56–1.49 (m, 4H), 1.43–1.14 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.3, 138.06, 138.03, 137.8, 128.6 (3C), 128.5 (3C), 128.5, 128.4 (3C), 128.0 (3C), 127.9, 127.8 (4C), 127.7, 127.3, 127.2, 99.66 ($^1J_{C1A,H1A}$ = 164.8 Hz; C_1A-β), 92.6, 92.0, 78.3, 74.9, 74.5, 73.5 (2C), 72.9, 69.5, 69.1, 67.2, 58.4, 50.6, 50.3, 47.1, 46.2, 29.1, 27.9, 27.3, 23.2 ppm; IR (thin film): $\tilde{\nu}$ = 3436, 3327, 2924, 2862, 1697, 1101, 1062 cm^{-1}; HRMS (ESI): m/z calcd for $C_{42}H_{47}Cl_3N_2O_8Na$: 835.2296 $[M+Na]^+$; found: 835.2271.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-trichloroacetamido-β-D-glucopyranoside (24)**: A mixture of β-thiogalactoside **7** (0.40 g, 0.60 mmol, 1.3 equiv), glucosamine acceptor **23** (0.38 g, 0.47 mmol), NIS (0.16 g, 0.7 mmol, 1.5 equiv), and freshly activated molecular sieves (3 Å AW, 1 g) in Et_2O/CH_2Cl_2 (16 mL, 3/1 v/v) was stirred at RT for 30 min. Then, the mixture was cooled to 0 °C and TfOH (4 μL, 47 μmol, 0.1 equiv) was added. The mixture was stirred for 30 min at 0 °C. After removal of the molecular sieves by filtration through a pad of celite, the reaction mixture was diluted with Et_2O and washed with a saturated aqueous solution of $Na_2S_2O_3$. The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel (hexanes/EtOAc, 90:10 to 70:30) to yield the crude disaccharide (0.35 g). The crude material was then dissolved in MeOH (12 mL) and treated with a 0.5 M solution of NaOMe in MeOH (90 μL, 45 μmol, 0.2 equiv). The mixture was stirred at RT overnight and then acidified to pH 6 with Amberlite IR 120-H$^+$ resin. The solvents were concentrated in vacuo and the crude material was purified by flash column chromatography on silica gel (hexanes/EtOAc, 90:10 to 75:25) to give the target disaccharide as an oil (280 mg, 0.22 mmol, 48% yield over 2 steps). R_f = 0.35 (hexanes/EtOAc, 70:30); $[\alpha]_D^{20} = +27.8$ (c = 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ = 8.02 (d, J = 6.4 Hz, 1H; NHCOCl$_3$), 7.31–7.22 (m, 30H), 7.16–7.09 (m, 5H), 5.14 (s, 2H; CO_2CH_2), 5.08–4.93 (m, 3H; CH, H_1A, H_1B), 4.77–4.67 (m, 3H; CH_2), 4.63–4.35 (m, 8H; CH_2), 4.35–4.28 (m, 1H; H_3A), 4.17–4.14 (m, 1H; H_5B), 3.92 (bs, 1H; H_4B), 3.85–3.78 (m, 2H; H_2B, H_3B), 3.77–3.59 (m, 6H; OCH_2, H_{6a}A, H_{6b}A, H_{6a}B, H_4A, H_5A), 3.55 (d, J = 9.2 Hz, 1H; H_{6b}B), 3.39 (bs, 2H; H_2A, OCH_2), 3.17–3.10 (m, 2H; NCH_2), 2.49 (s, 1H; OH), 1.51–1.36 (m, 4H; CH_2), 1.30–1.12 ppm (m, 2H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, rotamers): δ = 161.4 (CO), 138.3 (2C), 138.1, 137.9, 137.8, 137.7, 137.4, 128.4 (3C), 128.4 (3C), 128.4 (3C), 128.4 (3C), 128.3 (3C), 128.2 (3C), 128.0 (3C), 127.9, 127.8 (3C), 127.7 (4C), 127.7 (4C), 127.5, 127.4, 98.6 ($^1J_{C1A,H1A}$ = 166.0 Hz, C_1A-β), 97.7 ($^1J_{C1B,H1B}$ = 171.3 Hz, C_1B-α), 92.7 (NHCOCl$_3$), 80.5 (C_3A), 77.1 (C_4A), 76.7 (C_2B), 75.9 (C_3B), 74.4 (CH_2), 74.0 (C_5A), 73.5 (2C, CH_2), 73.4 (2C; CH_2), 72.5 (CH_2), 70.0 (C_6B), 69.6 (OCH_2), 69.3 (C_5B), 68.9 (C_6A), 68.2 (C_4B), 67.0 (CO_2CH_2Ph), 59.0 (C_2A), 50.4 and 50.1 (NCH_2Ph, rotamers), 47.0 and 46.1 (CH_2, rotamers), 27.9 (CH_2), 27.4 (CH_2), 23.1 ppm (CH_2); IR (thin film): $\tilde{\nu}$ = 3484, 3340, 2923, 2856, 1701, 1523, 1468, 1454, 1091, 1028, 698 cm^{-1}; HRMS (ESI): m/z calcd for $C_{69}H_{75}Cl_3N_2O_{13}Na$: 1267.4232 $[M+Na]^+$; found: 1267.4163.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl-6-*O*-acetyl-2,4-di-*O*-benzyl-3-*O*-2-(naphthylmethyl)-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-trichloroacetamido-β-D-glucopyranoside (28)**: Acceptor **24** (40 mg, 32 μmol) and thioglucoside **11** (28 mg, 48 μmol, 1.5 equiv) were azeotroped with toluene and dried in vacuo. After the addition of NIS (14 mg, 64 μmol, 2.0 equiv) and freshly activated molecular sieves (4 Å, 100 mg), the reaction mixture was dissolved in Et_2O/CH_2Cl_2 (320 μL, 3/1 v/v), cooled to −50 °C, and stirred for 15 min. A solution of TfOH in CH_2Cl_2 (30 μL, 3 μmol, 0.1 equiv) was added dropwise and the mixture was stirred for 1 h at −50 °C. After removal of the molecular sieves by filtration through a pad of celite, the reaction mixture was diluted with Et_2O and washed with a saturated aqueous solution of $Na_2S_2O_3$. Following separation of the layers, the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by NP-HPLC (NP = normal phase) on YMC Pack silica gel (EtOAc/hexanes, 5% (5 min) to 30% (30 min)) to afford the target trisaccharide as a colorless oil (55 mg, 31 μmol, 97% yield). R_f = 0.36 (hexanes/EtOAc, 70:30); $[\alpha]_D^{20} = +18.3$ (c = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, rotamers): δ = 7.90 (d, J = 7.6 Hz, 1H; NHCOCl$_3$), 7.86–7.76 (m, 2H), 7.73–7.70 (m, 2H), 7.51–7.41 (m, 3H), 7.36–7.04 (m, 45H), 5.14 (d, J = 6.0 Hz, 2H; CO_2CH_2Ph), 5.09–5.07 (m, 2H; CH_2Ph, H_1B), 5.00–4.95 (m, 2H; CH_2Ph), 4.88 (d, J = 10.8 Hz, 1H; CH_2Ph), 4.85 (d, J = 3.2 Hz, 1H; H_1C), 4.84–4.77 (m, 2H; H_1A, CH_2Ph), 4.71–4.69 (m, 2H; CH_2Ph), 4.65 (d, J = 12.0 Hz, 1H), 4.63–4.46 (m, 4H), 4.46 (d, J = 10.4 Hz, 1H), 4.42 (bs, 2H; NCH_2Ph), 4.30–4.23 (m, 2H; H_3B, H_3A), 4.20–4.15 (m, 2H; CH_2Ph, H_{6a}C), 4.12–4.09 (m, 1H; H_5C), 4.05 (dd, J = 9.2, 9.6 Hz, 1H; H_3C), 3.98–3.87 (m, 4H; CH_2Ph, H_4B, H_{6b}C, H_2B), 3.83 (dd, J = 10.4, 2.8 Hz, 1H; H_3B), 3.77–3.56 (m, 7H; OCH_2, H_{6a}A, H_{6b}A, H_4A, H_{6a}B, H_5A, H_4C), 3.53 (dd, J = 10.0, 3.2 Hz, 1H; H_2C), 3.51–3.40 (m, 2H; H_{6b}B, H_2A), 3.35–3.32 (m, 1H; OCH_2), 3.16–3.08 (m, 2H; NCH_2), 1.97 (s, 3H; COCH_3), 1.50–1.33 (m, 4H; CH_2), 1.25–1.09 ppm (m, 2H; CH_2); ^{13}C NMR (100 MHz, $CDCl_3$, rotamers): δ = 170.7, 161.5, 138.4, 138.3, 138.2, 138.0, 138.0, 137.7, 136.1, 133.4, 133.0, 128.6, 128.61, 128.5, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.2, 126.6, 126.1, 126.0, 126.0, 99.3 ($^1J_{C1C,H1C}$ = 172.4 Hz, C_1C-α), 99.1 ($^1J_{C1A,H1A}$ = 164.4 Hz, C_1A-β), 97.8 ($^1J_{C1B,H1B}$ = 169.1 Hz, C_1B-α), 92.9 (NHCOCl$_3$), 81.9 (C_3C), 80.77 (C_2C), 79.9 and 79.7 (C_3A, rotamers), 77.6 (C_2B), 77.5 (C_4A), 77.3 (C_4C), 76.4 (C_3B), 75.9 (C_4B), 75.7 (CH_2Ph), 75.1 (CH_2Ph), 74.4 (CH_2Ph), 74.2 (C_5A), 73.9 (CH_2Ph), 73.5 (CH_2Ph), 73.5 (CH_2Ph), 73.1 (CH_2Ph), 72.7 (CH_2Ph), 70.6 (C_5C), 69.7 (OCH_2), 69.5 (C_6B), 69.4 (C_5B), 69.0 (C_6A), 67.2 (CO_2CH_2Ph), 62.8 (C_6C), 58.6 (C_2A), 50.6 and 50.2 (NCH_2Ph, rotamers), 47.2 and 46.2 (NCH_2, rotamers), 29.3 (CH_2), 28.0 and 27.5 (CH_2, rotamers), 23.2 (CH_2), 21.0 ppm (COCH_3); IR (thin film): $\tilde{\nu}$ = 3340, 3062, 3030, 2930, 2867, 1739, 1702, 1454, 1234, 1091, 1060 cm^{-1}; HRMS (ESI): m/z calcd for $C_{102}H_{107}Cl_3N_2O_{19}$: 1791.6431 $[M+Na]^+$; found: 1791.6380.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl-2,4-di-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl- α-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-trichloroacetamido- β-D-glucopyranoside (10)**: A solution of trisaccharide **28** (50 mg, 28 μmol) in MeOH (0.3 mL) was treated with a 0.5 M solution of NaOMe in MeOH (6 μL, 3 μmol, 0.1 equiv). The solution was stirred at RT for 3 h, diluted with MeOH, and then acidified to pH 6 with Amberlite IR 120-H$^+$ resin. The resin was filtered off and

washed several times with MeOH. The filtrate was concentrated in vacuo to give an oil, which was further dissolved in a mixture of CH_2Cl_2/MeOH (3.1 mL, 9/1 v/v). The mixture was cooled at 0 °C and treated with DDQ (21 mg, 84 μmol, 3 equiv). After stirring for 3 h at 0 °C, the reaction was quenched by the addition of a saturated aqueous solution of $Na_2S_2O_3$ and a saturated aqueous solution of $NaHCO_3$. The mixture was extracted with CH_2Cl_2 and the organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (hexanes/EtOAc, 70:30) to give of target diol **10** as a yellow oil (21 μmol, 33 mg, 73 % yield over 2 steps). R_f = 0.46 (hexanes/EtOAc, 50:50); $[\alpha]_D^{18}$ = +70.9 (c = 0.25, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, rotamers): δ = 7.86 (d, J = 6.6 Hz, 1H), 7.42–7.04 (m, 45H), 5.15 (d, J = 6.6 Hz, 2H; CO_2CH_2Ph), 5.02 (s, 1H; H_1B), 4.96 (d, J = 11.0 Hz, 1H; CH_2), 4.84 (d, J = 6.0 Hz, 1H; CH_2), 4.81 (s, 2H; H_1C, H_1A), 4.72–4.65 (m, 4H; CH_2Ph), 4.65–4.58 (m, 2H; CH_2Ph), 4.56 (d, J = 12.2 Hz, 1H; CH_2Ph), 4.50 (d, J = 12.0 Hz, 1H; CH_2Ph), 4.45–4.41 (m, 3H; CH_2Ph, NCH_2Ph), 4.31–4.27 (m, 1H; CH_2Ph), 4.23 (dd, J = 7.8, 7.8 Hz, 1H; H_3A), 4.14–4.08 (m, 2H; H_5B, H_3C), 4.04–4.02 (m, 2H; H_5C, CH_2Ph), 3.86 –3.81 (m, 3H; H_4B, H_3B, H_2B), 3.77–3.44 (m, 10H; OCH_2, $H_{6a}A$, $H_{6b}A$, $H_{6a}B$, H_4A, H_5A, $H_{6a}C$, $H_{6b}C$, $H_{6b}B$, H_2A), 3.41 (dd, J = 9.0, 9.6 Hz, 1H; H_4C), 3.37–3.25 (m, 2H; H_2C, OCH_2), 3.17–3.09 (m, 2H; NCH_2), 2.37 (s, 1H; OH), 1.45–1.40 (m, 4H; CH_2), 1.22–1.15 ppm (m, 2H; CH_2); ^{13}C NMR (150 MHz, $CDCl_3$, rotamers): δ = 161.6, 138.4, 138.3, 138.2, 138.1, 137.9, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.3, 99.2 ($^1J_{C1A,H1A}$ = 165.5 Hz, C_1A-β), 99.1 ($^1J_{C1C,H1C}$ = 173.0 Hz, C_1C-α), 97.9 ($^1J_{C1B,H1B}$ = 173.5 Hz, C_1B-α), 92.9 ($NHCOCCl_3$), 80.5 (C_2C), 79.7 (C_3A), 78.5 (C_2B), 78.0 (C_4C), 77.5 (C_4A), 77.1 (C_3B), 76.2 (C_4B), 74.8 (CH_2Ph), 74.4 (CH_2Ph), 74.3 (C_5A), 73.8 (CH_2Ph), 73.57 (CH_2Ph), 73.56 (CH_2Ph), 73.52 (C_3C), 73.2 (CH_2Ph), 73.0 (CH_2Ph), 71.4 (C_5C), 70.6 (C_5B), 69.8 (C_6B), 69.7 (OCH_2), 69.1 (C_6A), 67.2 (CO_2CH_2Ph), 61.9 (C_6C), 58.7 and 58.5 (C_2A, rotamers), 50.6 and 50.3 (NCH_2Ph, rotamers), 47.2 and 46.3 (NCH_2, rotamers), 29.4 (CH_2), 28.0 and 27.6 (CH_2, rotamers), 23.3 ppm (CH_2); IR (thin film): $\tilde{\nu}$ = 3339, 2835, 1678, 1637, 1091, 1027 cm^{-1}; HRMS (ESI): m/z calcd for $C_{89}H_{97}Cl_3N_2NaO_{18}$: 1609.5700 $[M+Na]^+$; found: 1609.5709.

***N*-(Benzyl)benzyloxycarbonyl-5-amino-pentanyl-3,6-dideoxy-4-*O*-benzoyl-2-*O*-benzyl-α-L-*xylo*-hexopyranosyl-(1→3)-[3,6-dideoxy-4-*O*-benzoyl-2-*O*-benzyl-α-L-*xylo*-hexopyranosyl-(1→6)]-2,4-di-*O*-benzyl-α-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-trichloroacetamido-β-D-glucopyranoside (2)**: Colitose imidate **5** (35 mg, 68 μmol, 4 equiv) and nucleophile trisaccharide **10** (27 mg, 17 μmol) were azeotroped three times with toluene and dried in vacuo. After the addition of freshly activated molecular sieves (4 Å, 80 mg), the mixture was suspended in anhydrous CH_2Cl_2 (340 μL) under an Ar atmosphere. The mixture was cooled to −50 °C and stirred for 15 min. After the dropwise addition of a solution of TMSOTf in CH_2Cl_2 (30 μL, 1.7 μmol, 0.1 equiv), the reaction was stirred for 15 min at −50 °C. After removal of the molecular sieves by filtration through a pad of celite, the reaction mixture was diluted with CH_2Cl_2 and washed with a saturated aqueous solution of $NaHCO_3$. The organic layer was washed with brine and dried over $MgSO_4$. After removal of solvents in vacuo, the crude product was purified by NP-LCMS on YMC Pack silica gel (EtOAc/hexanes, 5 % (5 min) to 30 % (40 min)) to afford the target pentasaccharide as an oil (24 mg, 11 μmol, 63 % yield). R_f = 0.20 (hexanes/EtOAc, 70:30); $[\alpha]_D^{20}$ = +10.3 (c = 0.30, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, rotamers): δ = 8.07 (d, J = 8.0 Hz, 1H; $NHCOCl_3$), 8.04–8.02 (m, 2H), 7.90–7.88 (m, 2H), 7.60–7.51 (m, 3H), 7.49–7.26 (m, 22H), 7.24–7.04 (m, 21H), 6.99–6.94 (m, 4H), 6.80 (d, J = 7.2 Hz, 2H), 5.65 (d, J = 3.2 Hz, 1H; H_1D), 5.26–5.22 (m, 1H; H_4E), 5.13 (s, 2H; CO_2CH_2Ph), 5.05–5.00 (m, 2H; H_1B, CH_2Ph), 4.97–4.91 (m, 3H; 2 CH_2Ph, H_1A), 4.86 (d, J = 3.2 Hz, 1H; H_1C), 4.82–4.77 (m, 2H; H_4D, H_1E), 4.74–4.66 (m, 4H; CH_2Ph), 4.65–4.60 (m, 2H; CH_2Ph), 4.56–4.47 (m, 4H; CH_2Ph), 4.44–4.17 (m, 12H; CH_2Ph, NCH_2Ph, H_3C, H_5B, H_3A, H_5D, H_5E), 4.03–3.92 (m, 4H; $H_{6a}B$, H_5C, H_2B, H_4B), 3.87–3.84 (m, 2H; H_2E, H_4C), 3.80 (dd, J = 10.2, 3.2 Hz, 1H; H_3B), 3.77–3.50 (m, 10H; $H_{6a}C$, H_2D, $H_{6a}A$, $H_{6b}A$, OCH_2, H_2C, H_4A, $H_{6b}C$, H_5A, $H_{6b}B$), 3.37–3.22 (m, 2H; OCH_2, H_2A), 3.19–2.95 (m, 2H, NCH_2), 2.27–2.20 (m, 2H; H_3E), 2.14–2.04 (m, 2H; H_3D), 1.41–1.35 (m, 4H; CH_2), 1.18–1.12 (m, CH_2), 1.06 (d, J = 6.6 Hz, 3H; H_6D), 0.73 ppm (d, J = 6.4 Hz, 3H; H_6E); ^{13}C NMR (150 MHz, $CDCl_3$, rotamers): δ = 166.1, 166.0, 161.7, 138.8, 138.6, 138.6, 138.3, 138.0, 137.8, 137.4, 133.2, 133.1, 130.3, 130.2, 129.8, 129.7, 128.8, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.3, 126.2, 98.8 ($^1J_{C1A,H1A}$ = 166.9 Hz, C_1A-β), 98.6 ($^1J_{C1C,H1C}$ = 170.8 Hz, C_1C-α), 97.9 ($^1J_{C1B,H1B}$ = 171.5 Hz, C_1B-α), 96.7 ($^1J_{C1E,H1E}$ = 171.3 Hz, C_1E-α), 96.5 ($^1J_{C1D,H1D}$ = 173.6 Hz, C_{1D}-α), 93.1 ($NHCOCCl_3$), 82.8 (C_2C), 80.9 (C_3A), 77.6 (C_4C), 77.3 (C_4A), 76.8 (C_2B), 76.4 (C_4B), 75.8 (C_3B), 74.8, 74.7, 74.1, 74.1, 73.5 (C_5A), 73.4 (C_3C), 73.0, 72.9, 72.6 (C_6B), 72.1 (C_4E), 72.0 (C_4D), 71.3 (C_2E), 71.0, 70.9 (C_5B), 70.6 (C_5C), 69.8 (C_2D), 69.2, 69.1 (C_6A), 67.2 ($NHCO_2CH_2Ph$), 65.9 (C_6C), 65.0 (C_5D), 64.8 (C_5E), 59.4 (C_2A), 50.6 and 50.3 (NCH_2Ph, rotamers), 47.2 and 46.3 (NCH_2, rotamers), 29.4 (CH_2), 29.1 (C_3E), 28.3 (C_3D), 28.0 and 27.6 (CH_2, rotamers), 23.2 (CH_2), 16.5 (C_6E), 16.1 ppm (C_6D); IR (thin film): $\tilde{\nu}$ = 3347, 2925, 2855, 1716, 1454, 1270, 1093, 1061, 1027, 984 cm^{-1}; HRMS (ESI): m/z calcd for $C_{129}H_{137}Cl_3N_2NaO_{26}$: 2257.8423 $[M+Na]^+$; found: 2257.8471.

5-Amino-pentanyl 3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→3)-[3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→6)]-α-L-galactopyranosyl)-α-D-glucopyranosyl-(1→4)-α-D-galactopyranosyl-(1→3)-2-*N*-acetyl-β-glucopyranoside (1): A degassed solution of AIBN (15 mg, 89 μmol, 10 equiv) in toluene (2 mL) was added to a solution of pentasaccharide **2** (20 mg, 8.9 μmol) and Bu_3SnH (24 μL, 89 μmol, 10 equiv) in toluene (2 mL). The mixture was stirred at 90 °C for 2 h and then passed through a pad of silica gel (hexanes/EtOAc, 90:10 to 50:50) to give the crude intermediate *N*-acetyl pentasaccharide (17 mg). MS (MALDI): m/z calcd for $C_{129}H_{140}N_2NaO_{26}$: 2155.95 $[M+Na]^+$; found: 2155.89.

The crude *N*-acetyl pentasaccharide was dissolved in MeOH (4 mL) and treated with a 0.5 M solution of NaOMe in MeOH (18 μL, 9 μmol, 1 equiv). The mixture was stirred at 40 °C overnight and then acidified to pH 6 with Amberlite IR 120-H^+ resin. After filtration, the mixture was concentrated in vacuo to give the intermediate diol as an oil. MS (MALDI): m/z calcd for $C_{115}H_{132}N_2NaO_{24}$: 1947.90 $[M+Na]^+$; found: 1947.92.

The oil was taken up in a mixture of THF/water (6 mL, 1/1 v/v). $Pd(OH)_2$ (20 %, 20 mg) was added and the suspension was stirred under a H_2 atmosphere for 24 h. After removal of the solvents, the crude product was dissolved in water and filtered on a cotton pad. Removal of the water by lyophilization, followed by gel-filtration chromatography (Sephadex LH-20, water) afforded the target pentasaccharide (5 mg, 5.6 μmol, 64 % yield over 3 steps). R_f = 0.50 (*i*PrOH/1 M aq. NH_4OAc, 1:2); $[\alpha]_D^{20}$ = +29.3 (c = 0.06, $CHCl_3$); ^{1}H NMR (600 MHz, D_2O): δ = 5.45 (d, J = 2.4 Hz, 1H), 5.15 (d, J = 3.0 Hz, 1H), 4.95 (d, J = 3.6 Hz, 1H), 4.82 (d, J = 3.6 Hz, 1H), 4.54 (d, J = 8.4 Hz, 1H), 4.36–4.28 (m, 2H), 4.09 (s, 1H), 4.05–3.98 (m, 3H), 3.92–3.89 (m, 6H), 3.84–3.58 (m, 13H), 3.47–3.44 (m, 1H), 3.01–2.95 (t, J = 5.2 Hz, 2H), 2.04 (s, 3H), 2.01–1.94 (m, 4H), 1.69–1.64 (m, 2H), 1.62–1.57 (m, 2H), 1.44–1.36 (m, 2H), 1.15 (d, J = 6.6 Hz, 3H), 1.12 ppm (d, J = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, CD_6CO): δ = 174.5, 101.3, 100.5, 99.7, 99.1, 98.9, 80.3, 79.7, 78.4, 75.8, 72.6, 71.6, 71.5, 70.9, 70.4, 69.9, 69.1, 68.9, 68.1, 66.93, 66.90, 66.7, 63.76, 63.73, 60.8, 59.9, 54.5, 39.6, 33.27, 33.21, 28.4, 26.7, 22.6, 22.5, 15.6 ppm (2C); IR (thin film): $\tilde{\nu}$ = 3369, 1649, 1563, 1377, 1347, 1139, 1076 cm^{-1}; HRMS (ESI): m/z calcd for $C_{37}H_{67}N_2O_{22}$: 891.4185 $[M+H]^+$; found: 891.4208.

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