Synthesis of oligosaccharides corresponding to *Vibrio cholerae* O139 polysaccharide structures containing dideoxy sugars and a cyclic phosphate

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A spacer-equipped tetrasaccharide, *p*-aminocyclohexylethyl α-L-Col*p*-(1→2)-β-D-Gal*p*-(1→3)-[α-L-Col*p*-(1→4)]-β-D-Glc*p*NAc, containing a 4,6-cyclic phosphate in the galactose residue, has been synthesised. The structure corresponds to a part of the repeating unit of the capsular (and lipo-) polysaccharide of the endemic bacteria *Vibrio cholerae* type O139 synonym Bengal. The synthetic strategy allows continuous syntheses of the complete O139 hexasaccharide repeating unit as well as of the structurally related repeating unit of serotype O22. Starting from ethyl 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside, a thioglycoside tetrasaccharide donor block was constructed through two orthogonal glycosylations with glycosyl bromide donors. First, a properly protected galactose moiety was introduced using silver triflate as promoter and subsequently the two colitose residues, carrying electron-withdrawing protecting groups for stability reasons, under halide-assisted conditions. The tetrasaccharide block was then linked to the spacer in a NIS–TMSOTf-promoted coupling. Transformation of the azido group into an acetamido group using H_2S followed by removal of temporary protecting acetyl groups gave a 4′,6′-diol, which was next phosphorylated with methyl dichlorophosphate and deprotected to yield the 4,6-cyclic phosphate tetrasaccharide target structure.

Introduction

A new form of the bacterium *Vibrio cholerae*, serotype O139 containing the cholera toxin, has rather recently been found to be the causative agent of endemics of cholera in Asia.[1–4] The surface carbohydrate structures of this serotype are completely different from those of the only other endemic serotype O1. The structure of both the lipo- and the capsular polysaccharide of serotype O139 has been elucidated,[5–8] and was found to contain identical structures, a hexasaccharide repeating unit containing, *i.a.*, dideoxy sugars (Col = colitose = 3,6-dideoxy-L-*xylo*-hexose) and a 4,6-cyclic phosphate (Fig. 1).

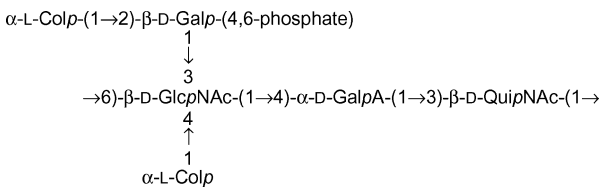

Fig. 1 Repeating unit of *V. cholerae* type O139.

This structure shows a strong resemblance to the capsular polysaccharide structure of the non-endemic serotype O22 (Fig. 2)[7] differing only in the anomeric configuration of the GlcNAc residue, and in the nature and substituents of position 4 and 6 in the galactose moiety.

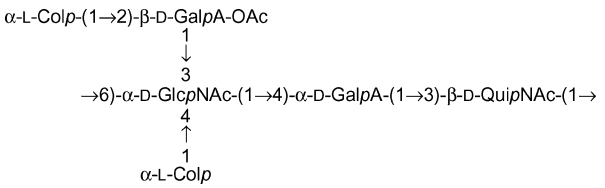

Fig. 2 Repeating unit of *V. cholerae* type O22.

To evaluate the immunological properties of these surface carbohydrate structures, and to investigate if glycoconjugates of part structures of the pathogenic form O139 could function as a vaccine against the bacteria, synthetic derivatives were in demand. In an earlier publication we have described the synthesis of two colitose-containing trisaccharides and one tetrasaccharide.[9] This paper describes the synthesis of an O139 tetrasaccharide structure, containing both colitose residues and the cyclic phosphate. The synthetic strategy also allows syntheses of larger parts of the O139 structures as well as of O22 structures.

Results and discussion

In our former synthesis[9] of colitose-containing structures from *Vibrio cholerae* O139, the perbenzylated ethyl thioglycoside was used as colitosyl donor in glycosylations with dimethyl-(methylthio)sulfonium trifluoromethanesulfonate (DMTST)[10] as promoter. Due to the activating benzyl protecting groups in combination with the deoxy functions this residue is quite acid labile, and care has to be taken both in the glycosylation reactions as well as in subsequent steps so as not to lose colitosyl moieties.

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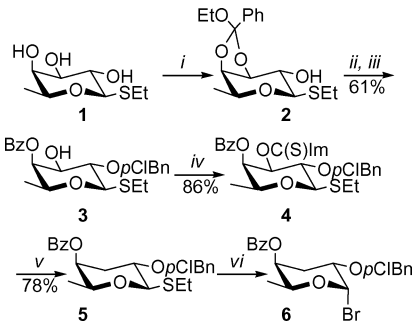
Consequently, a differently protected, more stable colitose derivative was desired. Since the colitose residues are α-linked in the target substances, a non-participating group has to be chosen in the 2-position. After several attempts a derivative, **5** (**6**), was developed,[11] which functioned well as a donor and was stable when introduced into an oligosaccharide structure. This compound was more efficiently synthesised from ethyl 1-thio-β-L-fucopyranoside[12] (**1**) than the corresponding perbenzylated derivative. Recently, Buttens and Kováč published a similar approach towards colitose thioglycosides.[13]

Formation of the 3,4-*O*-orthobenzoate of **1** (→**2**), followed by 2-*O*-*p*-chlorobenzylation and subsequent opening of the orthoester gave compound **3** with a free 3-OH group in an overall yield of 61% (Scheme 1). Deoxygenation[14] of this through formation of the thiocarbonylimidazole derivate **4** and ensuing tin hydride reduction then gave **5** (67%).

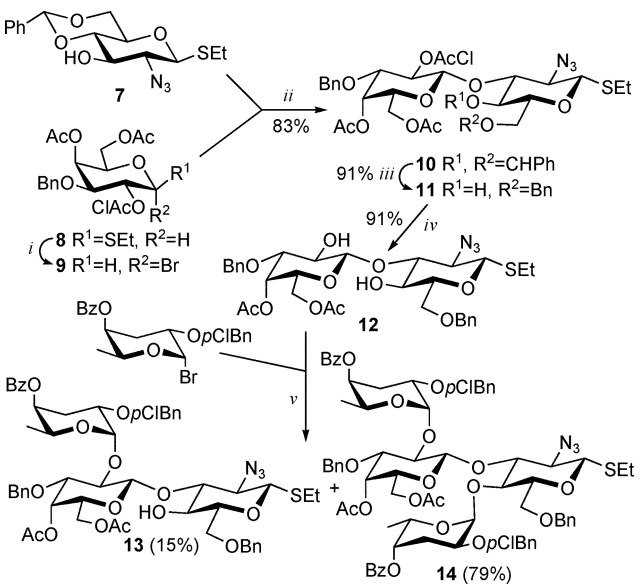


Scheme 1 (i) $PhC(EtO)_3$, TsOH, CH_3CN; (ii) *p*ClBnCl, NaH, DMF; (iii) TFA (90% aq.), CH_3CN; (iv) $C(S)Im_2$, imidazole, $(CH_2Cl)_2$; (v) Bu_3SnH, AIBN, toluene, reflux; (vi) Br_2, CH_2Cl_2.

As compared to the earlier synthesis, the protecting group pattern in the galactosyl part also had to be altered to allow introduction of the cyclic phosphate in the 4,6-positions. In our strategy, this modification was planned to be performed as one of the last steps in the synthesis, just prior to deprotection. Furthermore, a synthetic pathway suitable also for the synthesis of the complete hexasaccharide repeating unit was desired, and, hence, a thioglycoside tetrasaccharide building block was designed, which can be glycosylated either with a spacer to give the spacer-equipped tetrasaccharide structure or with a GalA–QuiNAc-disaccharide acceptor to give the complete hexasaccharide repeating unit. Experiences from a structurally similar donor block, used in the synthesis of Lewis b structures,[15] made us hesitant to use a phthalimide as amino protecting group, since problems were encountered both with the stability of the thioglycoside block (elimination of ethyl mercaptan) and in deprotection steps, both deacetylation and dephthalimidisation. This prompted us to select an azido group as an amino equivalent instead, and choose the known derivative **7**[16] as the GlcNAc residue precursor. The probable lower β-stereoselectivity in glycosylations with the non-participating azido group as compared to a phthalimido derivative was considered as a not too severe problem, since this would implement the simultaneous formation of structures corresponding to the O22 serotype.

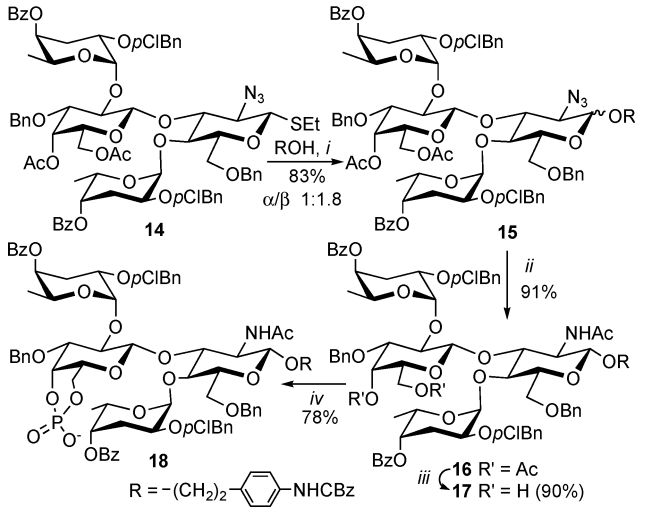
Originally, a 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPS) group, in combination with a 2-*O*-acetate, was chosen as a temporary protecting group for the galactosyl 4- and 6-hydroxyl groups to be phosphorylated. However, later in the synthesis, during the introduction of the colitose residues, it was found that this bulky protecting group prohibited the colitosylation at the 4-position of the GlcNAc-residue resulting only in 2′-glycosylation and a trisaccharide product. In the native structure, the 4,6-cyclic phosphate is found in very close vicinity of the 4-O-linked colitose residue,[17] which indicates similar conformations for the native and the protected oligosaccharide. Consequently, the TIPS-group was exchanged for acetyl protecting groups, which made the use of a chloroacetyl group in the 2-position necessary. Monochloroacetylation of ethyl 3-*O*-benzyl-4,6-benzylidene-1-thio-β-D-galactopyranoside[18] followed by acetal hydrolysis, acetylation (→**8**, 60%), and finally treatment with bromine afforded donor **9**.

Coupling between donor **9** and acceptor **7** (Scheme 2) promoted by silver triflate gave the β-linked disaccharide **10** in 83% yield. Reductive opening ($NaBH_3CN$–HCl[19,20]) of the benzylidene acetal in **10** produced the 4-OH regioisomer **11** (91%), from which the monochloroacetyl group was removed chemoselectively by hydrazine acetate treatment[21] to yield the 2′,4-diol **12** (91%). Glycosylation of this acceptor with the new colitose donor, was best performed using the bromosugar **6** and halide-assisted conditions.[22] Also this time glycosylation in the 4-position was sluggish,[9] and 15% of the trisaccharide **13** was isolated together with the tetrasaccharide **14** (79%).



Scheme 2 (i) Br_2, CH_2Cl_2; (ii) AgOTf, CH_2Cl_2, −50 °C; (iii) $NaBH_3CN$, HCl–Et_2O, THF; (iv) H_2NNHAc, CH_2Cl_2–EtOAc; (v) Et_4NBr, CH_2Cl_2–DMF.

Introduction of a spacer into block **14** was then investigated (Scheme 3), the α-anomer of interest for the O22 and the β-anomer for the O139 serotype structures. Two analogues of the 2-(4-aminophenyl)ethanol, the nitro and the Cbz protected derivatives, were tried as spacers with similar results. NIS–TMSOTf[23,24] as promoter in CH_2Cl_2 gave predominantly the β-anomer (α/β 1 : 2, 83%), whereas DMTST in diethyl ether gave slightly more of the α-anomer (α/β 1 : 1.2–1.8, 73–92%) in accordance with earlier findings.[25] The corresponding bromosugar of **14** promoted by the heterogeneous catalyst silver zeolite[26] gave, as expected, the by far best β-selectivity (α/β 1 : 7, 74%). Perhaps most important was that the separation of the anomers by silica gel chromatography caused



Scheme 3 (i) NIS–TMSOTf, CH_2Cl_2, 0 °C; (ii) (a) H_2S, Et_3N, pyridine; (b) Ac_2O, pyridine; (iii) NaOMe, MeOH; (iv) $MeOP(O)Cl_2$, pyridine.

no problems, since the α- and β-anomers showed a ΔR_f-value of about 0.2 (pentane–EtOAc 2 : 1).

Further transformations were performed on the β-linked spacer-equipped derivative **15**β. Reduction of the azido group and subsequent acetylation of the resulting amino group was carried out through reduction with H_2S followed by acetylation to give compound **16** (91%). Removal of the acetyl groups was first accomplished by treatment with HCl in MeOH,[27] to prevent cleavage of the benzoyl groups. However, it was later found that these benzoates are unusually base stable and the selective removal could easily be performed with NaOMe in MeOH in a 90% yield (→**17**). Finally, treatment of **17** with methyl dichlorophosphate in pyridine[28] followed by work-up with triethylamine introduced the 4′,6′-cyclic phosphate as its triethylammonium salt (→**18**, 78%). The cyclic structure was proven by MALDI-TOF in combination with the $^3J_{PH}$ coupling constant, which was 22 Hz[8] as compared to 5–10 Hz in phosphate monoesters.[29]

Deprotection starting with catalytic hydrogenolysis of benzyl protecting groups afforded no distinct product. However, by changing the order of the deprotection steps and start with the deacylation, effective deprotection was accomplished (Scheme 4). As mentioned the benzoyl groups were quite base-stable and required prolonged (4 d) methoxide treatment to be removed completely (→**19**, 82%). Catalytic hydrogenolysis over palladium on charcoal afforded the target structure **20** in 76% yield. The cyclic phosphate had survived the prolonged base treatment as shown by ^{31}P-NMR ($^3J_{PH}$ = 22 Hz[8]).

Conclusions

In conclusion, a working methodology, including the introduction of stable colitose residues and a cyclic phosphate, has been developed for the synthesis of a tetrasaccharide part of the repeating unit of the *Vibrio cholerae* type O139 capsular polysaccharide. The strategy also includes the possibility of continued synthesis of the complete hexasaccharide structure as well as O22 serotype structures.

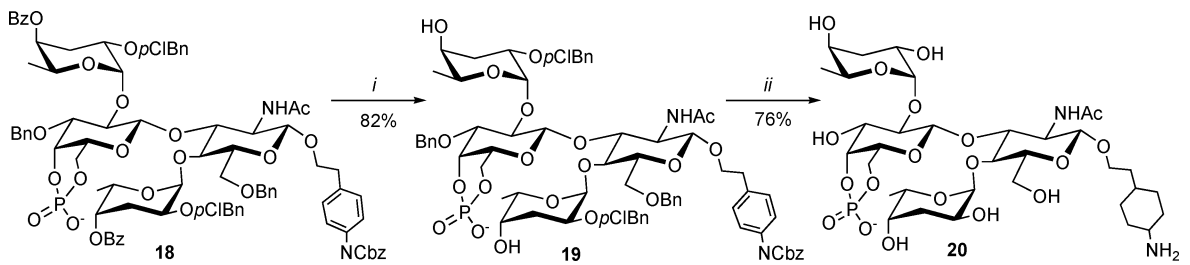
Experimental

General methods

Organic solutions were concentrated under reduced pressure at <40 °C (bath temp). NMR spectra were recorded at 25 °C at 400 MHz (^{1}H), 100 MHz (^{13}C) in $CDCl_3$ with Me_4Si as internal standard (δ = 0 ppm) or 162 MHz (^{31}P) in H_2O with H_3PO_4 as external standard (δ = 0 ppm), unless otherwise stated. TLC was performed on Silica Gel F_{254} (E. Merck) with detection by UV light and/or by charring with 8% sulfuric acid. Silica gel (0.040–0.063 mm, Amicon) was used for column chromatography.

Ethyl 4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-1-thio-β-L-fucopyranoside (3). Triethyl orthobenzoate (4.05 mL, 17.9 mmol) and TsOH (136 mg, 715 μmol) were added to a solution of ethyl 1-thio-β-L-fucopyranoside[11] (**1**, 2.483 g, 11.9 mmol) in MeCN (30 mL). After 2 h the reaction was made basic by addition of Et_3N and concentrated. The crude ethyl 3,4-*O*-(1-ethoxybenzylidene)-1-thio-β-L-fucopyranoside and *p*-chlorobenzyl chloride (**2**, 2.888 g, 17.9 mmol) in DMF (30 mL) were added dropwise to NaH (95%, 500 mg, 20.8 mmol), covered with DMF, at 0 °C. After 30 min the reaction mixture was brought to room temperature, and after one additional hour MeOH was carefully added. The mixture was diluted with H_2O and then extracted with toluene three times. The combined organic phases were washed with H_2O, dried ($MgSO_4$), filtered and concentrated to yield crude ethyl 2-*O*-*p*-chlorobenzyl-3,4-*O*-(1-ethoxybenzylidene)-1-thio-β-L-fucopyranoside, which was dissolved in MeCN (33 mL) and 90% aqueous TFA (5 mL). After 15 min the reaction mixture was concentrated and purified by flash chromatography (toluene→10 : 1 toluene–EtOAc) to afford **3** (3.171 g, 61%). $[a]_D$ −3.5 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 15.0 (SCH_2CH_3), 16.6 (C-6), 24.7 (SCH_2CH_3), 73.0, 73.7, 73.8, 74.3, 78.5 (C-2, 3, 4, 5 and CH_2Ph), 84.4 (C-1), 128.1–136.4 (aromatic *C*) and 166.5 (*C*O).

Ethyl 4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-1-thio-β-L-*xylo*-hexopyranoside (5). Compound **3** (1.433 g, 3.28 mmol),



Scheme 4 (i) NaOMe, MeOH; (ii) H_2, Pd/C, H_2O–MeOH.

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imidazole (91 mg, 1.34 mmol) and thiocarbonyldiimidazole (1.174 g, 6.59 mmol) in 1,2-dichloroethane (30 mL) were heated for 18 h at 75 °C. After concentration, the residue was purified by flash chromatography (15 : 1, toluene–EtOAc) to give ethyl 4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3-*O*-thiocarbonylimidazole-1-thio-β-L-fucopyranoside (**4**, 1.539 g, 86%). $[\alpha]_D$ −138 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 15.0 (SCH_2CH_3), 16.6 (C-6), 25.2 (SCH_2CH_3), 70.3, 73.0, 74.5, 76.1, 82.2 (C-2-5 and CH_2Ph), 84.9 (C-1), 117.5–136.2 (imidazole and aromatic *C*), 165.4 (*C*O) and 182.5 (*C*S). Compound **4** and a catalytic amount of AIBN, dissolved in toluene (8.5 mL), were added dropwise to a refluxing solution of Bu_3SnH (1.2 mL, 4.53 mmol) in toluene (7.5 mL). After 35 min the reaction mixture was concentrated and partitioned between MeCN–hexane. The layers were separated and the hexane layer extracted with MeCN twice. The MeCN phases were combined and concentrated, and the residue was purified by flash silica gel chromatography (20 : 1, toluene–EtOAc) to yield **5** (919 mg, 78%). $[\alpha]_D$ +23.5 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 14.9 (SCH_2CH_3), 16.9 (C-6), 24.5 (SCH_2CH_3), 35.5 (C-3), 71.2, 71.4, 72.6, 75.0 (C-2, 4, 5 and CH_2Ph), 86.5 (C-1), 128.3–136.1 (aromatic C) and 165.7 (CO). Anal. calcd for $C_{22}H_{25}ClO_4S$: C, 62.77; H, 5.99. Found C, 62.62; H, 6.00.

Ethyl 4,6-di-*O*-acetyl-3-*O*-benzyl-2-*O*-chloroacetyl-1-thio-β-D-galactopyranoside (8). A solution of ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[16] (3.884 g, 9.65 mmol) and pyridine (3.9 mL, 48.5 mmol) in CH_2Cl_2 (97 mL) was cooled to 0 °C and chloroacetyl chloride (1.15 mL, 14.5 mmol) in CH_2Cl_2 (20 mL) was added dropwise. After 25 min ice-water was added to the reaction mixture, the phases were separated and the organic layer was washed with 1 M HCl and H_2O, dried ($MgSO_4$), filtered and concentrated to yield ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-chloroacetyl-1-thio-β-D-galactopyranoside $[\alpha]_D$ +11.1 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 14.8 (SCH_2CH_3), 22.6 (SCH_2CH_3), 40.7 ($COCH_2Cl$), 69.1, 69.8, 69.9, 71.2, 73.2, 78.0, 82.2 (C-1-6 and CH_2Ph), 101.1 (*C*HPh), 126.1–137.5 (aromatic C) and 165.6 ($COCH_2Cl$). This derivative was dissolved in CH_2Cl_2 (30 mL) and TFA (60 mL) was added at 0 °C. After 20 min ice was added. The organic phase was washed with H_2O, 3 × $NaHCO_3$ (aq. satd) and H_2O, dried ($MgSO_4$), filtered and concentrated. The residue, ethyl 3-*O*-benzyl-2-*O*-chloroacetyl-1-thio-β-D-galactopyranoside, was dissolved in pyridine (40 mL) and Ac_2O (20 mL) together with a catalytic amount of DMAP. After 17 h at room temperature the reaction mixture was concentrated, co-evaporated twice with toluene and partitioned between toluene–H_2O. The organic phase was washed with 1 M HCl and H_2O, dried ($MgSO_4$), filtered, concentrated and purified by flash chromatography (8 : 1 toluene–EtOAc) and gravity chromatography (4 : 1 toluene–EtOAc) to yield **8** (2.762 g, 60%). $[\alpha]_D$ +37.7 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 14.9 (SCH_2CH_3), 20.7, 20.8 ($COCH_3$), 24.1 (SCH_2CH_3), 40.6 ($COCH_2Cl$), 62.0, 66.0, 70.5, 71.3, 74.6, 77.2, 83.4 (C-1-6 and CH_2Ph), 127.8–137.0 (aromatic C), 165.7 ($COCH_2Cl$), 170.0 and 170.2 ($COCH_3$). Anal. calcd for $C_{21}H_{27}ClO_8S$: C, 53.11; H, 5.73. Found C, 52.92; H, 5.81.

Ethyl (4,6-di-*O*-acetyl-3-*O*-benzyl-2-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (10). Bromine (150 μL, 2.93 mmol) was added to a cooled (0 °C) solution of ethyl 4,6-di-*O*-acetyl-3-*O*-benzyl-2-*O*-chloroacetyl-1-thio-β-D-galactopyranoside (**8**, 930 mg, 1.96 mmol) in CH_2Cl_2 (15 mL). After 1.5 h the reaction mixture was concentrated and co-evaporated with toluene. The residue, crude **9**, and ethyl 2-azido-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside[14] (**7**, 410 mg, 1.22 mmol) and crushed 4 Å molecular sieves in CH_2Cl_2 (15 mL), were stirred under nitrogen for 30 min and then cooled to −50 °C prior to addition of AgOTf (805 mg, 2.47 mmol). After 30 min Et_3N (1 mL) was added, the mixture was filtered through a pad of Celite and concentrated. The crude product was purified by flash chromatography (10 : 1→5 : 1, toluene–EtOAc) to yield the pure β-anomer (**10**, 754 mg, 83%). $[\alpha]_D$ −20.1 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 15.1 (SCH_2CH_3), 20.6, 20.7 ($COCH_3$), 24.9 (SCH_2CH_3), 40.8 ($COCH_2Cl$), 61.5, 65.5, 65.6, 68.2, 70.3, 71.0, 71.3, 72.7, 76.4, 79.0, 80.7, 85.5 (C-1-6, 2′-6′ and CH_2Ph), 100.6, 101.0 (C-1′ and *C*HPh), 125.0–137.5 (aromatic C), 165.6 ($COCH_2Cl$), 169.9 and 169.9 ($COCH_3$). Anal. calcd for $C_{34}H_{40}ClN_3O_{12}S$: C, 54.43; H, 5.37; N, 5.60. Found C, 54.25; H, 5.49; N, 5.72.

Ethyl (4,6-di-*O*-acetyl-3-*O*-benzyl-2-*O*-chloroacetyl-β-D-galactopyranosyl)-(1→3)-2-azido-6-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (11). $NaBH_3CN$ (1.039 g, 16.5 mmol) and crushed 3 Å molecular sieves (6.3 g) were added to a stirred solution of **10** (878 mg, 1.17 mmol) in THF (83 mL). After 5 min, HCl–Et_2O was added drop-wise until evolution of gas ceased. After 25 min the mixture was filtered through Celite, concentrated and purified by flash chromatography (4 : 1→3 : 1, toluene–EtOAc) to yield **11** (797 mg, 91%). $[\alpha]_D$ +19.8 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 15.1 (SCH_2CH_3), 20.5, 20.7 ($COCH_3$), 24.7 (SCH_2CH_3), 40.6 ($COCH_2Cl$), 61.9, 64.7, 65.5, 68.6, 69.4, 71.4, 71.6, 71.9, 73.4, 76.3, 79.4, 84.4, 87.7 (C-1-6, 2′-6′ and 2 CH_2Ph), 101.5 (C-1′), 127.3-138.0 (aromatic C), 165.8 ($COCH_2Cl$), 169.9 and 170.1 ($COCH_3$).

Ethyl (4,6-di-*O*-acetyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-azido-6-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (12). Hydrazine acetate (78 mg, 1.17 mmol) in MeOH (4.2 mL) was added to **11** (176 mg, 234 μmol) in CH_2Cl_2–EtOAc (1 : 1, 8.4 mL). After 22 h at room temperature the mixture was concentrated and purified by flash chromatography (2 : 1, toluene–EtOAc) to yield **12** (144 mg, 91%). $[\alpha]_D$ −7.2 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 15.1 (SCH_2CH_3) 20.5, 20.7 ($COCH_3$), 24.8 (SCH_2CH_3), 62.2, 64.8, 65.7, 68.7, 69.4, 70.3, 71.5, 72.0, 73.3, 78.6, 79.3, 84.1, 88.5 (C-1-6, 2′-6′ and 2 CH_2Ph), 104.0 (C-1′), 127.3–138.0 (aromatic C), 169.9 and 170.2 ($COCH_3$). Anal. calcd for $C_{32}H_{41}N_3O_{11}S$: C, 56.88; H, 6.12; N, 6.22. Found C, 56.62; H, 6.15; N, 6.20. Anal. calcd for $C_{72}H_{79}Cl_2N_3O_{19}S$: C, 62.06; H, 5.71; N, 3.02. Found C, 61.95; H, 5.78; N, 2.83.

Ethyl (4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1→2)-(4,6-di-*O*-acetyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→4)]-2-azido-6-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (14). Bromine (150 μL, 2.93 μmol) was added to a cooled (0 °C) solution of ethyl 4-*O*-benzoate-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-β-L-*xylo*-1-thio-hexopyranoside (**5**, 1.05 g, 2.49 mmol) in CH_2Cl_2 (10 mL). After 20 min the reaction mixture was concentrated, co-evaporated with toluene, dissolved in CH_2Cl_2 (3.0 mL) and added to a solution of **12** (420 mg, 637 μmol), Et_4NBr (546 mg, 2.60 mmol) and crushed 4 Å molecular sieves in CH_2Cl_2 (1.0 mL) and DMF (400 μL). After

7 d the reaction was quenched by addition of Et_3N (0.5 mL) and MeOH (0.5 mL), diluted with CH_2Cl_2, filtered through Celite, concentrated and purified by flash chromatography (4 : 1→1 : 1, toluene–EtOAc) to yield the tetrasaccharide **14** (699 mg, 79%) and the trisaccharide **13** (98 mg, 15%). **14**: $[\alpha]_D$ −60.9 (*c* 1.0, $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 15.2 (SCH_2CH_3), 16.1, 16.3 (C-6″, 6‴), 20.8, 20.9 ($COCH_3$), 24.9 (SCH_2CH_3), 27.8, 28.3 (C-3″, 3‴), 61.5, 65.0, 65.6, 65.8, 67.2, 67.7, 69.3, 69.4, 70.1, 70.5, 70.9, 71.3, 71.9, 72.0, 72.5, 73.4, 74.1, 77.9, 80.1, 80.7, 85.4 (C-1-6, 2′–6′, 2″, 4″–5″, 2‴, 4‴–5‴and 4 CH_2Ph), 96.9, 97.7 (C-1″, 1‴), 100.9 (C-1′), 126.6–138.1 (aromatic C), 165.8, 165.9 (*C*OPh), 170.2 and 170.5 ($COCH_3$). Anal. calcd for $C_{72}H_{79}Cl_2N_3O_{19}S$: C, 62.06; H, 5.71; N, 3.02. Found C, 61.95; H, 5.78; N, 2.83.

2-(4-Benzyloxyamidophenyl)ethanol. 2-(4-Aminophenyl)ethanol (3.00 g, 21.9 mmol) and NaOH (892 mg, 22.3 mmol) were dissolved in H_2O (170 mL). Benzyl chloroformate (3.1 mL, 21.7 mmol) was added, with another addition after 30 min (1 mL, 7.0 mmol). The reaction mixture was cooled to 0 °C and the precipitate was collected by filtration. NaOH (487 mg, 12.3 mmol) and benzyl chloroformate (2 mL, 14.0 mmol) was added to the mother liquor and after another 40 min the precipitate was collected by filtration. The combined precipitates were recrystallized from toluene–EtOAc to yield white needles (3.807 g, 64%); mp 124.0 °C; NMR (CD_3OD) ^{13}C, δ 39.5 ($HOCH_2CH_2Ar$), 64.1 ($HOCH_2CH_2Ar$), 67.3 (CH_2Ph), 119.5–138.0 (aromatic C) and 155.6 (*C*O).

2-(4-Benzyloxyamidophenyl)ethyl (4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1→2)-(4,6-di-*O*-acetyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→4)]-2-azido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (15). 2-(4-Benzyloxyamidophenyl)ethanol (154 mg, 568 μmol), NIS (128 mg, 569 μmol) and 4 Å molecular sieves (1.13 g) were added to a solution of tetrasaccharide **14** (395 mg, 284 μmol) in CH_2Cl_2 (19 mL). After 50 min stirring under argon the solution was cooled to 0 °C and 12 drops of TMSOTf in CH_2Cl_2 (2 drops TMSOTf in 1 mL CH_2Cl_2) were added. After 1 h the reaction mixture was filtered through Celite, washed with $Na_2S_2O_3$ (aq. satd), H_2O, dried (Na_2SO_4), filtered and concentrated. The crude product was purified by flash chromatography (6 : 1 toluene–acetone) and the α and β anomers were separated by gravity chromatography (2 : 1 → 1.5 : 1, pentane–EtOAc) to yield **15**α (134 mg, 30%) and **15**β (242 mg, 53%). **15**β: $[\alpha]_D$ −46.3 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 16.0, 16.3 (C-6″, 6‴), 20.8, 20.9 ($COCH_3$), 27.9, 28.3 (C-3″, 3‴), 35.5 ($HOCH_2CH_2Ar$), 61.5, 65.0, 65.6, 65.8, 66.9, 67.0, 67.5, 69.3, 69.5, 70.1, 70.5, 70.7, 70.9, 71.4, 72.0, 72.1, 72.6, 73.5, 73.9, 75.7, 76.3, 80.9 (C-2-6, 2′–6′, 2″, 4″–5″, 2‴, 4‴–5‴, $HOCH_2CH_2Ar$ and 4 CH_2Ph), 96.8, 97,5 (C-1″, 1‴), 100.9, 102.1 (C-1, 1′), 126.6–138.0 (aromatic C), 153.4 (NH*C*OOPh), 166.8, 166.0 (*C*OPh), 170.2 and 170.5 ($COCH_3$).

2-(4-Benzyloxyamidophenyl)ethyl (4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1→2)-(4,6-di-*O*-acetyl-3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (16). H_2S was bubbled through a solution of **15**β (218 mg, 136 μmol) in pyridine (3.0 mL) and triethylamine (1.5 mL) for 3 h and the solution was concentrated. Pyridine (0.30 mL) and Ac_2O (0.10 mL) were added to a solution of the residue in dry CH_2Cl_2 (5 mL), stirred for 30 min, concentrated and coevaporated with toluene. Purification by flash chromatography (1 : 1→1 : 2, pentane–EtOAc) yielded **16** (201 mg, 91%). $[\alpha]_D$ −42.4 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 16.2, 16.4 (C-6″, 6‴), 20.8, 20.8 ($COCH_3$), 23.5 ($NHCOCH_3$), 28.1, 28.5 (C-3″, 3‴), 35.3 ($HOCH_2CH_2Ar$), 58.0, 61.6, 64.9, 65.5, 65.9, 66.9, 67.9, 69.3, 69.7, 70.0, 70.4, 70.7, 71.0, 71.4, 71.5, 72.0, 72.1, 72.9, 73.0, 73.4, 75.1, 76.6, 80.9 (C-2-6, 2′–6′, 2″, 4″–5″, 2‴, 4‴–5‴, $HOCH_2CH_2Ar$ and 4 CH_2Ph), 96.3, 96.6 (C-1″, 1‴) 99.1, 101.3 (C-1, 1′), 118.9–138.1 (aromatic C), 153.5 (NH*C*OOPh), 165.8, 165.9 (*C*OPh), 170.2, 170.3 and 170.5 ($COCH_3$, $NHCOCH_3$). Anal. calcd for $C_{88}H_{94}Cl_2N_2O_{23}$: C, 65.30; H, 5.85; N, 1.73. Found C, 65.13; H, 5.83; N, 1.78.

2-(4-Benzyloxyamidophenyl)ethyl (4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1→2)-(3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (17). **16** (141 mg, 87 μmol), was deacetylated with 1 M NaOMe (4 drops) in MeOH (4 mL) and CH_2Cl_2 (1.5 mL). Dowex H^+ ion-exchange resin was added after 1 h, the reaction mixture was filtered, concentrated and purified by flash chromatography (50 : 1, $CHCl_3$–MeOH) to yield **17** (120 mg, 90%). $[\alpha]_D$ −49.4 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 16.2, 16.3 (C-6″, 6‴), 23.5 ($NHCOCH_3$), 28.1, 28.3 (C-3″, 3‴), 35.3 ($HOCH_2CH_2Ar$), 56.2, 62.3, 65.4, 65.5, 66.2, 66.9, 68.1, 69.4, 69.8, 70.0, 70.5, 71.0, 71.5, 72.2, 72.3, 72.6, 73.3, 73.5, 74.4, 74.7, 74.8 (C-2-6, 2′–6′, 2″, 4″–5″, 2‴, 4‴–5‴, $HOCH_2CH_2Ar$ and 4 CH_2Ph), 95.6, 96.9 (C-1″, 1‴), 100.1, 101.3 (C-1, 1′), 118.9–138.1 (aromatic C), 153.5 (NH*C*OOPh), 165.9, 166.0 (*C*OPh) and 170.2 ($NHCOCH_3$).

2-(4-Benzyloxyamidophenyl)ethyl (4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1→2)-(4,6-*O*-cyclic phosphate-3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[4-*O*-benzoyl-2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside, triethylammonium salt (18). Diol **17** (98 mg, 63.9 μmol) was coevaporated three times with pyridine, dissolved in pyridine (1.2 mL) and added to a 0 °C solution of methyl dichlorophosphate (40 μL, 400 μmol) in pyridine (800 μL). After 10 min the reaction was allowed to attain room temperature and 70 min later 10% Et_3N in H_2O (1 mL) was added. The mixture was diluted with $CHCl_3$ and washed with 3 × NaCl (aq. satd). The combined water phases were extracted with $CHCl_3$, and the combined organic phases were dried (Na_2SO_4), filtered, concentrated and purified twice by flash chromatography (6 : 1→3 : 1, EtOAc–MeOH) and (100 : 1→15 : 1, $CHCl_3$–MeOH) to yield the cyclic phosphate **18** (85 mg, 78%). $[\alpha]_D$ −41.9 (*c* 1.0 $CHCl_3$); NMR ($CDCl_3$) ^{13}C, δ 8.6 (Et_3N), 16.4, 16.4 (C-6″, 6‴), 23.1 ($NHCOCH_3$), 28.2, 28.7 (C-3″, 3‴), 35.4 ($HOCH_2CH_2Ar$), 45.7 (Et_3N), 57.8, 65.1, 65.7, 66.4, 68.0, 68.2, 69.1, 69.2, 69.4, 69.8, 70.0, 70.4, 70.6, 70.9, 71.1, 71.2, 71.6, 71.8, 72.3, 72.6, 72.7, 73.1, 73.5, 80.7, 80.8 (C-2-6, 2′–6′, 2″, 4″–5″, 2‴, 4‴–5‴, $HOCH_2CH_2Ar$, 4 CH_2Ph), 94.8, 96.0 (C-1″, 1‴), 99.8, 102.5 (C-1, 1′), 118.6–138.3 (aromatic C), 153.6 (NH*C*OOPh), 165.5, 165.6 (*C*OPh) and 169.8 ($NHCOCH_3$); ^{31}P, δ −4 ($^3J_{PH}$ 22 Hz). MALDI-TOF MS: Calcd for $C_{84}H_{88}Cl_2N_2Na_2O_{23}P$ ($[M + 2Na]^+$): 1639.47. Found 1639.46.

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2-(4-Benzyloxyamidophenyl)ethyl (2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1→2)-(4,6-*O*-cyclic phosphate-3-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[2-*O*-*p*-chlorobenzyl-3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (19). The triethylammonium salt of **18** (72 mg, 42 μmol) was dissolved in MeOH (3 mL) and 1 M NaOMe (300 μL) was added. After 4 d of stirring, the solution was neutralized with Dowex H^+ ion-exchange resin, filtered and concentrated. The residue was purified by flash chromatography (100 : 1→10 : 1, CH_2Cl_2–MeOH) to produce **19** (48 mg, 82%). $[\alpha]_D$ −40.6 (*c* 0.6 $CHCl_3$); NMR (CD_3OD) ^{13}C, δ 15.4 (C-6″, 6‴), 22.1 (NHCO*C*H_3), 30.4, 31.5 (C-3″, 3‴), 35.0 (OCH$_2$*C*H$_2$Ar), 55.8, 66.0, 66.1, 66.4, 67.6, 67.9, 68.3, 68.5, 68.5, 68.9, 68.9, 69.5, 69.8, 70.1, 70.9, 71.9, 72.0, 72.2, 73.0, 73.2, 73.3, 75.6, 76.0, 80.3, 80.4 (C-2-6, 2′–6′, 2″, 4″–5″, 2‴, 4‴–5‴, HO*C*H$_2$CH$_2$Ar, 4 *C*H$_2$Ph, and impurities), 96.8, 97.3 (C-1″, 1‴), 101.1, 101.7 (C-1, 1′), 118.6–138.4 (aromatic C), 154.6 (NH*C*OOPh) and 171.7 (NH*C*OCH$_3$); MALDI-TOF MS: Calcd for $C_{70}H_{81}Cl_2N_2O_{21}P$ ([M − H^+]$^-$): 1385.44, found 1385.38.

2-(Aminocyclohexyl)ethyl (3,6-dideoxy-α-L-*xylo*-hexopyranosyl)-(1→2)-(4,6-*O*-cyclic phosphate-β-D-galactopyranosyl)-(1→3)-[3,6-dideoxy-α-L-*xylo*-hexopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (20). A catalytic amount of 10% Pd/C was added to a solution of **19** (42 mg, 30 μmol) in MeOH (1.5 mL) and H_2O (0.8 mL) and the reaction was stirred under H_2 (1 atm) for 20 h. The mixture was filtered and the obtained solution concentrated. The residue was filtered through a short reversed phase column and the combined fractions concentrated to afford **20** (19 mg, 76%). $[\alpha]_D$ −36.0 (*c* 0.5 H_2O); NMR (D_2O) ^1H (selected data), δ 1.18, 1.20 (2s, 6H, H-6″, H-6‴), 2.02 (s, 3H, NHCOC*H*$_3$), 4.56 (d, 1H, J = 2.9 Hz, H-4′), 4.70 (d, 1H, J = 8.0 Hz), 4.75 (d, 1H, J = 7.0 Hz), 4.89 (d, 1H, J = 3.0 Hz), 4.99 (d, 1H, J = 3.3 Hz); NMR (D_2O) ^{13}C, δ 15.5, 15.7 (C-6″, 6‴), 22.1 (NHCO*C*H$_3$), 26.1–32.9 (cyclohexyl), 29.9, 30.0 (C-3″, 3‴), 34.0 (OCH$_2$*C*H$_2$R), 49.1, 50.6, 55.9, 59.7, 63.4, 63.7, 66.2, 66.8, 67.2, 68.5, 68.6, 68.8, 72.5, 72.8, 75.7, 76.3, 76.5 (C-2-6, 2′–6′, 2″, 4″–5″, 2‴, 4‴–5‴, O*C*H$_2$CH$_2$R), 97.9, 99.6, 101.1, 102.0 (C-1, 1′, 1″, 1‴) and 173.4 (NH*C*OCH$_3$);^{31}P, δ −3.20 ($^3J_{PH}$ 22 Hz); MALDI-TOF MS: calcd for $C_{34}H_{59}N_2O_{19}P$ ([M − H^+]$^-$): 829.34 found 829.35.

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