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Synthesis of a D,D- and L,D-heptose-containing hexasaccharide corresponding to a structure from *Haemophilus ducreyi* lipopolysaccharides

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Abstract

The synthesis of a linear hexasaccharide, 2-(4-trifluoroacetamidophenyl)ethyl (β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(D-*glycero*-α-D-*manno*-heptopyranosyl)-(1→6)-(β-D-glucopyranosyl)-(1→4)-L-*glycero*-α-D-*manno*-heptopyranoside, corresponding to a structure found in *Haemophilus ducreyi* LPS, is described. A Barbier reaction between benzyloxymethyl chloride and a properly protected 6-aldo-1-thio-mannopyranoside yielded both the D,D- and the L,D-heptopyranoside (**2** and **3**, ratio 2:3), which were separated and both used in the synthesis. *p*-Methoxybenzyl and chloroacetyl groups were employed as temporary protecting groups, selectively removed in the presence of the persistent benzyl, acetyl, benzoyl and isopropylidene groups by treatment with DDQ/H_2O and hydrazine dithiocarbonate, respectively. Thioglycosides were utilised as donors throughout using either NIS/TfOH or DMTST as promoters. The introduction of the spacer into thioglycoside **5** was high-yielding (95%) but with low stereoselectivity (α:β 5:3). All other glycosylations are completely stereoselective. The target hexasaccharide is obtained via a 3+3 block approach with the yield in the final NIS/TfOH-promoted coupling between an *N*,*N*-diacetyl-trisaccharide thioglycosyl donor **20** and a 4′′-OH trisaccharide acceptor **13** being 75%.

1. Introduction

Haemophilus ducreyi is a gram-negative bacterium causing genital chancroids. Outer membrane lipopolysaccharide (LPS) structures from *H. ducreyi* have been shown to cause substantial tissue damage if injected into animals,[1] but the exact role of these structures in the infection and the development of the disease is not known. Recently, *H. ducreyi* LPS structures from various strains have been elucidated.[2,3] As for *Haemophilus influenzae* the structures lack the polymeric O-antigen and a substantial structural

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heterogeneity is found. Both *H. influenzae* and *ducreyi* LPSs seem to contain a common core structure, a branched pentasaccharide built up of one β-D-glucose, three L-*glycero*-α-D-*manno*-heptose and one α-Kdo residues (Fig. 1). They differ, however, in the substitution pattern of the common core, both considering phosphate and glycan groups. The *H. ducreyi* structures found are generally substituted at the 6-position of the glucose moiety with various oligosaccharides; a number of these are also found in human glycoconjugate structures. We have earlier synthesised a lactose-containing linear trisaccharide found in *H. ducreyi* LPS and also branched parts of the common core structure.[4,5] We now describe the synthesis of a linear hexasaccharide, (β-D-galactopyranosyl)-(1→4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-(D-*glycero*-α-D-*manno*-heptopyranosyl)-(1→6)-(β-D-glucopyranosyl)-(1→4)-L-*glycero*-α-D-*manno*-heptopyranoside, corresponding to a structure found in *H. ducreyi* LPS, containing both D- and L-*glycero*-α-D-*manno*-heptopyranose as synthetic challenges. The hexasaccharide is synthesised as its 2-(4-trifluoroacetamidophenyl)ethyl glycoside to facilitate the formation of immunogenic glycoconjugates by the coupling of the spacer amino group to a protein, to allow various biological experiments.

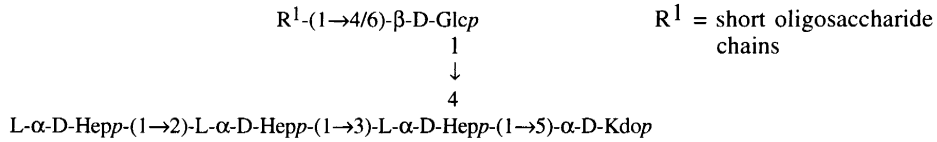
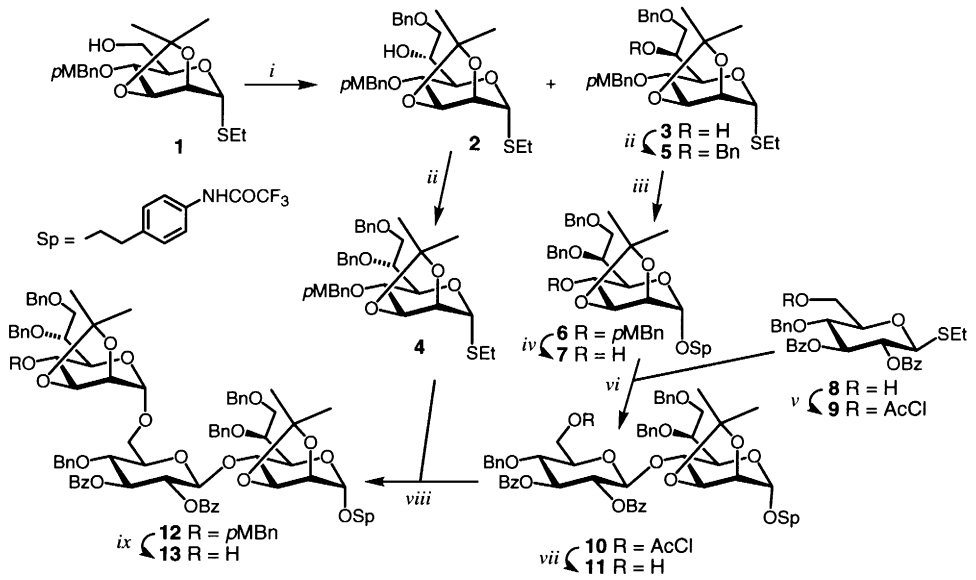


Fig. 1. Generalised structure of the dephosphorylated LPS of *H. ducreyi* without the lipid A moiety

2. Results and discussion

Reactions of organomagnesium reagents of various silyl- and alkoxymethyl chlorides with 1,6-dialdomannopyranosides generally give good yields of heptopyranosides with a strong preponderance for the L-*glycero*-D-*manno*-heptose isomer, due to complexation of the magnesium reagent to the ring oxygen.[6] Initially the D,D-heptose moiety was therefore planned to be obtained through an inversion at C-6 of an L,D-heptose derivative. A precursor **1**, containing a free 6-OH group to allow oxidation to the aldehyde and subsequent carbon elongation, and an orthogonal *p*-methoxybenzyl group to allow later coupling in the 4-position, was therefore synthesised according to methods described by Grzeszczyk and Zamojski in the preparation of the corresponding benzyl glycoside.[7] When the carbon elongation was performed, using bensyloxymethyl chloride and Barbier reaction conditions,[5] a good yield of the heptopyranoside was obtained, but surprisingly almost equal amounts of the 6-D and the 6-L form, **2** and **3** (ratio 2:3, 52%), were obtained (Scheme 1). The stereochemistry was determined by transformation into the perbenzylated thioglycosides and comparison with a derivative with known L,D-configuration. Grignard conditions gave almost identical results. The ratio between the two stereoisomers is known to be dependent on the protecting group pattern of the aldehyde precursor,[6] but the identically protected benzyl glycoside gave a 2:7 ratio in a reaction with the allyloxymethyl Grignard reagent,[7] showing that probably also the nature of the aglycon affects the stereochemical outcome of the reaction. Since both a 4-linked D,D- and L,D-heptose was part of the target structure, this however meant that suitable precursors for both of these residues were obtained directly in the carbon elongation reaction.

The hexasaccharide was designed to be synthesised through a 3+3 block synthesis. To construct the acceptor trisaccharide block containing both heptose residues, both **2** and **3** were benzylated at the 6-position to give **4** (86%) and the previously known[8] compound **5** (82%), respectively (Scheme 1).

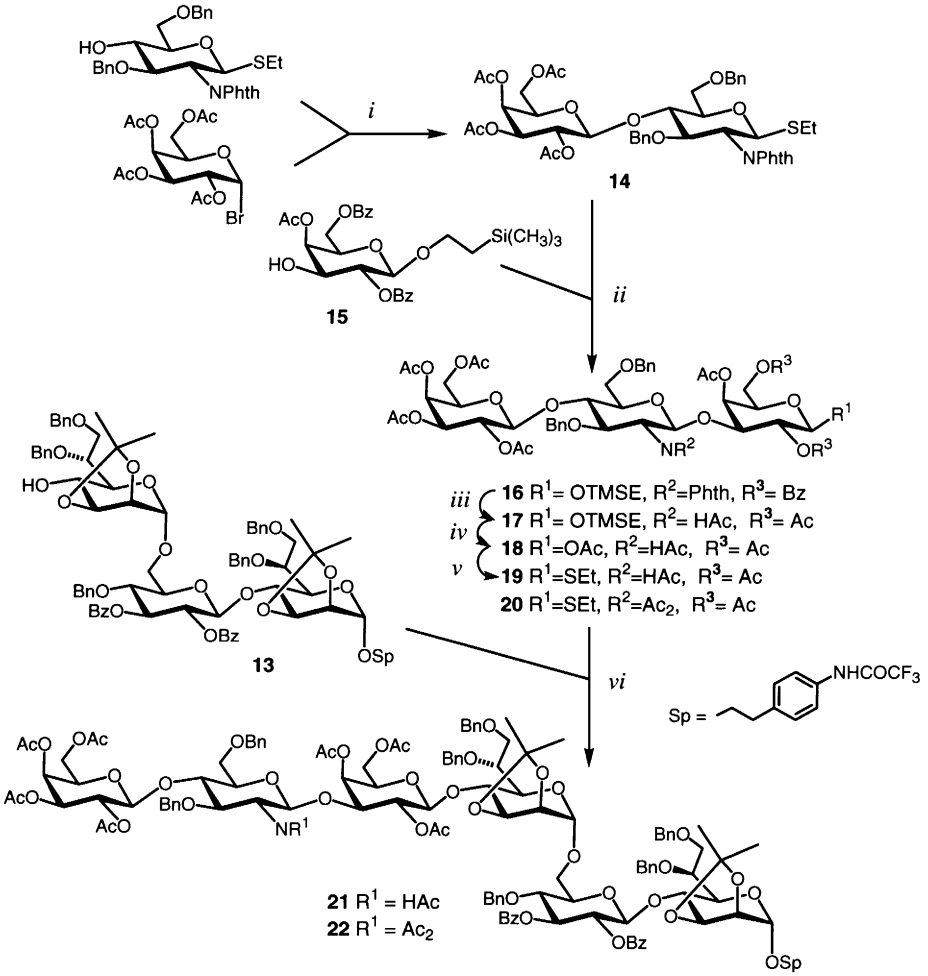


Scheme 1. (i) (a) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, (b) $BnOCH_2Cl$, Mg, THF (52%, **2**:**3** 2:3); (ii) BnBr, NaH, DMF [86% (**4**), 82% (**5**)]; (iii) *p*-$CF_3CONHPhCH_2CH_2OH$, NIS/AgOTf, CH_2Cl_2/MeCN (95%, α:β 5:3); (iv) DDQ, H_2O, CH_2Cl_2 (80%); (v) ClAcCl, CH_2Cl_2/pyridine (79%); (vi) NIS/AgOTf, CH_2Cl_2 (95%); (vii) HDTC, THF/DMF (89%); (viii) DMTST, Et_2O (71%); (ix) DDQ, H_2O, CH_2Cl_2 (69%)

Thioglycoside **5** was transformed into a spacer glycoside through an NIS/AgOTf-promoted[9] coupling with 2-(4-trifluoroacetamidophenyl)ethanol to give an excellent yield of glycoside (95%), but with low stereoselectivity (α:β 1.7:1) as found earlier in similar couplings.[10] This time, in contrast to our earlier experiences, it was not possible to anomerise the β-glycoside to the α-anomer by BF_3–etherate treatment. Removal of the *p*-methoxybenzyl group using DDQ from the α-glycoside **6** yielded the 4-OH acceptor **7** (80%). Reductive benzylidene opening (borane–trimethylamine complex/$AlCl_3$ in CH_2Cl_2/diethyl ether)[11] of the known ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[12] gave **8** (84%), chloroacetylation of which afforded **9** (79%). NIS/AgOTf-promoted coupling between **9** and **7** yielded the (1→4)-β-linked disaccharide **10** (95%), from which the chloroacetate was removed selectively by hydrazine dithiocarbonate[13] (HDTC) to give the new acceptor **11** (89%). Coupling between **11** and the D,D-heptosyl donor **4**, this time with dimethyl(methylthio) sulfonium triflate (DMTST) as promoter,[14] rendered the exclusively α-(1→6)-linked trisaccharide block **12**, which was turned into the 4′′-OH acceptor **13** by orthogonal deprotection of the *p*-methoxybenzyl group with DDQ (69%).

The other trisaccharide block, containing, inter alia, lactosamine, was designed to be a thioglycoside donor, because of our successful experiences with the use and couplings of thioglycoside building blocks, and synthesised by a consecutive synthesis starting from monosaccharides. Initially, ethyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside[15] was chosen as a precursor acceptor, but, in spite of the deactivating benzoyl groups, severe problems with transglycosylations and hydrolysis in both the couplings to give the target trisaccharide made a change to a more resistant anomeric protecting group necessary. Hence, the corresponding (trimethylsilyl)ethyl (TMSE) glycoside **15**[16] was synthesised following the same protecting group protocol. Acceptor **15** was glycosylated with the known[15] disaccharide thioglycoside donor **14** using NIS/AgOTf as promoter to give the trisaccharide **16**, which was subsequently converted into the thioglycoside donor **19** in three steps (Scheme 2). Finally, the trisaccharide donor **19** was coupled to the trisaccharide acceptor **13** promoted by NIS/AgOTf, to give the target hexasaccharide **21** in a rather sluggish reaction where 38% of unreacted acceptor

could be recovered (45% yield of **21**, 72% calculated on consumed acceptor). Observations have been published where couplings involving acetamido-containing donors were found to be more high-yielding if diacetylamino donors were used.[17] When this approach was tried in the coupling to give the hexasaccharide using the diacetylamino donor **20** and the same acceptor **13**, a more effective glycosylation took place and the hexasaccharide could be isolated in a 75% yield.



Scheme 2. (i) AgOTf, toluene/CH_2Cl_2 (93%); (ii) NIS/TfOH Et_2O/CH_2Cl_2 (70%); (iii) (a) NaOMe, MeOH, (b) H_2NNH_2, EtOH/dioxane, (c) Ac_2O, pyridine, DMAP (81%); (iv) BF_3–Et_2O, Ac_2O, toluene (42%); (v) EtSH, BF_3–Et_2O CH_2Cl_2 (73%); (vi) NIS/TfOH, CH_2Cl_2 [45% (**21**), 75% (**22**)]

3. Experimental

3.1. General methods

These were as described earlier.[5]

3.2. *Ethyl 2,3*-O-*isopropylidene-4*-O-p-*methoxybenzyl-1-thio*-α-D-*mannopyranoside* ***1***

Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside[18] (44.3 g, 113 mmol) was dissolved in MeOH (500 mL). The pH was adjusted to 10 by addition of a 1 M solution of sodium methoxide in MeOH. After stirring for 4 h the mixture was neutralised by Dowex H+, filtered and concentrated. The residue was co-evaporated twice from dry pyridine and dissolved in the same solvent (500 mL), whereafter triphenylmethyl chloride (34.6 g, 124 mmol) and 4-dimethylaminopyridine (1 g) were added. The solution was stirred overnight and then concentrated. The residue was dissolved in $CHCl_3$ (400 mL) and washed with H_2O (7×150 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was dissolved in DMF (200 mL), 2,2-dimethoxypropane (100 mL) and *p*-toluenesulfonic acid (5 g) were added, and the solution was stirred at room temperature for 2 h, and then diluted with toluene (500 mL). The organic phase was stirred with $NaHCO_3$ (aq., satd, 300 mL) for 1 h, separated, washed once with H_2O, dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified on a silica gel column, which was packed in CH_2Cl_2, whereafter the crude substance was applied and washed into the gel. The top of the column was equipped with a reflux condenser which was in turn connected to a 500 mL round-bottomed flask containing CH_2Cl_2 (250 mL). The outlet of the column was connected to the flask via a teflon tube, and the flask content was brought to boiling and the outflow from the column was carefully adjusted to maintain the solvent level above the gel. As soon as the desired substance was eluting (as monitored by TLC of the outcoming flow) the flask was exchanged for an identical one containing Et_2O (250 mL). The apparatus was refluxed and eluted until no more substance was detected. The ethereal solution was then concentrated to yield ethyl 2,3-*O*-isopropylidene-1-thio-6-*O*-triphenylmethyl-α-D-mannopyranoside (45.6 g, 90 mmol, 80%). To a flask containing washed (hexane) sodium hydride (10.9 g, 0.27 mol, 60% dispersion in oil) was added dry DMF (200 mL). The solution was cooled (0°C) and a solution of the above residue (45.6 g, 90 mmol) in DMF (150 mL) was slowly added dropwise during 30 min. After an additional 1 h, a solution of *p*-methoxybenzyl bromide (29.8 g, 148 mmol) in DMF (50 mL) was added dropwise, and the mixture was stirred for 2 h at 0°C. The reaction was carefully quenched by the addition of MeOH (20 mL), and the mixture diluted with toluene (500 mL), washed with H_2O (3×200 mL), dried (Na_2SO_4), filtered, concentrated, and purified by the same silica gel column procedure as above, to give ethyl 2,3-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl-1-thio-6-*O*-triphenylmethyl-α-D-mannopyranoside (45.0 g, 80%). ^{13}C NMR ($CDCl_3$): δ 14.2, 23.9 (CH_3CH_2S), 26.5, 28.0 [$(CH_3)_2C$], 55.2 ($ArCH_2O$), 63.1, 69.2, 72.7, 76.1, 76.5, 78.7, 78.9, 86.4 (C-1–6, $ArCH_2O$, Ph_3CO), 109.3 [$(CH_3)_2C$], 113.5–159.0 (aromatic C). To a solution of this derivative (18.0 g, 28.7 mmol) in EtOAc (140 mL) was added formic acid (85% aq., 70 mL). The mixture was stirred for 2.5 h and neutralized by addition of K_2CO_3 (aq., satd, 200 mL). The organic phase was separated, dried ($MgSO_4$) and evaporated. Purification of the crude product on a silica gel column (toluene:EtOAc, 4:1) gave 6.83 g (17.8 mmol, 62%) of **1**. $[\alpha]_D$ +160 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 14.5, 24.3 (CH_3CH_2S), 26.5, 28.0 [$(Me)_2C$], 55.3 ($ArCH_2O$), 62.5, 69.0, 72.7, 76.1, 76.7, 78.5, 79.6 (C-1–6, $ArCH_2O$), 109.4 [$(Me)_2C$], 113.8, 129.7, 130.2, 159.3 (aromatic C). Anal. calcd for $C_{19}H_{28}O_6S$: C, 59.35; H, 7.34. Found: C, 59.34; H, 7.35.

3.3. *Ethyl 7*-O-*benzyl-2,3*-O-*isopropylidene-4*-O-p-*methoxybenzyl*-D-*thio-1*-glycero-α-D-manno-*hepto-pyranoside* ***2*** *and 7*-O-*benzyl-2,3*-O-*isopropylidene-4*-O-p-*methoxybenzyl-1-thio*-L-glycero-α-D-manno-*hepto-pyranoside* ***3***

Swern-oxidation of **1** was performed under standard conditions: A solution of oxalyl chloride (1.42 mL, 16.5 mmol) in dry CH_2Cl_2 (mL) was cooled to −60°C. DMSO (2.4 mL, 31.1 mmol) in CH_2Cl_2

was added dropwise during 10 min. Stirring was continued for 5 min when **1** (5.45 g, 14.2 mmol) in CH_2Cl_2 was added during 10 min. After an additional 30 min at −60°C, Et_3N (10 mL, 72 mmol) was added dropwise and the solution was slowly brought to room temperature (1 h). The solution was diluted (CH_2Cl_2) and washed twice with water. Drying ($MgSO_4$), concentration and co-evaporation three times from sodium-dried toluene yielded crude ethyl 1,6-dialdo-2,3-*O*-isopropylidene-4-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside which was used directly in the next step. Freshly acid-washed and dried magnesium turnings (1.03 g, 42.4 mmol) were added to a 100 mL round-bottomed flask equipped with a dropping funnel, a magnetic stirrer and an internal thermometer. To the dropping funnel was added the above aldehyde (from 14.2 mmol of **1**) and benzyloxymethyl chloride (3.9 mL, 28 mmol) in dry THF (30 mL). $HgBr_2$ (70 mg, 0.19 mmol) was added to the Mg turnings and after 10 min the solid material was covered with dry THF. The formation of the organomagnesium compound was initiated by the addition of a few drops of neat benzyloxymethyl chloride. When the exothermic reaction had started, the flask was partially immersed into an ice-water bath (0°C). The aldehyde/alkyl halide solution was added dropwise at such a rate (approx. 2 mL/min) that the internal reaction temperature was kept between 10 and 12°C. The mixture was then left stirring overnight, slowly attaining room temperature. The mixture was diluted (Et_2O) and transferred to an Ehrlenmeyer flask containing a freshly made (0°C) saturated NH_4Cl solution (100 mL). After 2 h of stirring the organic phase was separated, dried ($MgSO_4$) and concentrated. Silica gel purification of the residue (toluene:EtOAc 6:1) gave first **2** (1.52 g, 3.01 mmol, 21%) followed by **3** (2.20 g, 4.36 mmol, 31%). Compound **2**: $[\alpha]_D$ +113 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 14.2, 24.1 (CH_3CH_2S), 26.4, 28.1 [(*Me*)$_2$C], 55.3 (MeO), 68.4, 70.9, 72.4, 72.5, 73.5, 76.5, 77.8, 78.7, 79.6 (C-1–7, $ArCH_2O$), 109.5 [(Me)$_2$*C*], 113.8, 127.6, 127.7, 128.3, 129.8, 129.9, 138.2, 159.4 (aromatic C); compound **3**: $[\alpha]_D$ +71 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 14.3, 24.2 (CH_3CH_2S), 26.5, 28.0 [(*Me*)$_2$C], 55.3 (MeO), 69.7, 68.6, 71.7, 73.0, 73.4, 75.3, 76.5, 78.8, 79.9 (C-1–7, $ArCH_2O$), 109.4 [(Me)$_2$*C*], 113.8–159.2 (aromatic C).

3.4. Ethyl 6,7-di-O-*benzyl-2,3*-O-*isopropylidene-4*-O-p-*methoxybenzyl-1-thio*-D-glycero-α-D-manno-*heptopyranoside* ***4***

Sodium hydride (310 mg, 60% dispersion in oil) was washed once with dry light petroleum (bp 40–65°C). DMF (4 mL) was added and a solution of **2** (961 mg, 1.90 mmol) in DMF (8 mL) was added dropwise at 0°C. After 2 h, benzyl bromide (905 μL, 7.61 mmol) in DMF (2 mL) was added, and the solution was allowed to attain room temperature. After 2 h, MeOH (0.4 mL) was carefully added and the mixture was diluted with toluene, washed three times with H_2O, dried (Na_2SO_4) and concentrated in vacuo. Purification on a silica gel column (toluene:EtOAc 12:1) gave **4** (970 mg, 1.63 mmol, 86%). $[\alpha]_D$ +109 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 14.4, 23.8 (CH_3CH_2S), 26.5, 28.0 [(*Me*)$_2$C], 55.2 (MeO), 69.3, 70.6, 72.4, 73.2, 76.0, 76.4, 76.6, 79.0, 79.4 (C-1–7, $ArCH_2O$), 109.3 [(Me)$_2$*C*], 113.6–159.1 (aromatic C). Anal. calcd for $C_{34}H_{42}O_7S$: C, 68.66; H, 7.12. Found: C, 68.34; H, 7.13.

3.5. Ethyl 6,7-di-O-*benzyl-2,3*-O-*isopropylidene-4*-O-p-*methoxybenzyl-1-thio*-L-glycero-α-D-manno-*heptopyranoside* ***5***

Following the procedure described above for **4**, **3** (1.27 g, 2.52 mmol) was benzylated to give **5** (1.23 g, 2.07 mmol, 82%). $[\alpha]_D$ +109 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 14.4, 24.2 (CH_3CH_2S), 26.5, 28.0 [(*Me*)$_2$C], 55.2 (MeO), 69.4, 70.8, 71.9, 73.4, 73.6, 75.1, 75.5, 76.5, 79.0, 80.0 (C-1–7, $ArCH_2O$), 109.4 [(Me)$_2$*C*], 113.7–159.1 (aromatic C).

3.6. 2-(4-Trifluoroacetamidophenyl)ethyl 6,7-di-O-benzyl-2,3-O-isopropylidene-4-O-p-methoxybenzyl-L-glycero-α-D-manno-hepto-pyranoside **6** and 2-(4-trifluoroacetamidophenyl)ethyl 6,7-di-O-benzyl-2,3-O-isopropylidene-4-O-p-methoxybenzyl-L-glycero-β-D-manno-heptopyranoside **7**

To a solution of **5** (804 mg, 1.35 mmol) and 2-(4-trifluoroacetamidophenyl)ethanol (426 mg, 1.83 mmol) in CH_2Cl_2:MeCN (5:1, 30 mL) under argon was added powdered molecular sieves (4 Å). The solution was stirred for 1 h at room temperature when *N*-iodosuccinimide (NIS, 410 mg, 1.82 mmol) followed by a catalytic amount of silver trifluoromethanesulfonate (AgOTf) were added. After stirring for 1.5 h the mixture was filtered through Celite and diluted with CH_2Cl_2. The organic phase was washed with a mixture of $NaHCO_3$ (aq., satd, 10 mL) and $Na_2S_2O_3$ (10% aq., 10 mL), dried (Na_2SO_4), concentrated and purified by silica gel chromatography (toluene/EtOAc 14→20%). Concentration of appropriate fractions gave 624 mg of the α-isomer **6** (0.814 mmol, 60%) followed by 363 mg of β-isomer (0.474 mmol, 35%). Compound **6**: $[\alpha]_D$ +32 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 26.4, 27.9 [(*Me*)$_2$C], 35.2 (Ar*C*H$_2$CH$_2$), 55.2 (MeO), 67.9, 68.2, 70.2, 71.8, 73.3, 73.5, 74.6, 75.1, 75.6, 79.2 (C-2–7, ArCH$_2$O, ArCH$_2$*C*H$_2$O), 97.3 (C-1, $J_{C,H}$ 171 Hz), 109.4 [(Me)$_2$*C*], 113.7–159.1 (aromatic C); β-isomer: $[\alpha]_D$ +7 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 26.3, 27.4 [(*Me*)$_2$C], 35.5 (Ar*C*H$_2$CH$_2$), 55.2 (MeO), 69.9, 70.1, 71.6, 73.3, 73.4, 73.8, 74.3, 74.6, 75.5, 80.3 (C-2–7, ArCH$_2$O, ArCH$_2$*C*H$_2$), 98.7 (C-1, $J_{C,H}$ 159 Hz), 110.8 [(Me)$_2$*C*], 113.7–159.2 (aromatic C).

3.7. 2-(4-Trifluoroacetamidophenyl)ethyl 6,7-di-O-benzyl-2,3-O-isopropylidene-L-glycero-α-D-manno-heptopyranoside **7**

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 251 mg, 1.11 mmol) and H_2O (2 mL) were added to a solution of **6** (578 mg, 0.755 mmol) in CH_2Cl_2 (40 mL) and the mixture was left stirring for 2 h. Separation of the organic phase followed by washing ($Na_2S_2O_3$, 5% aq.), drying (Na_2SO_4) and concentration gave a residue, which was purified on a silica gel column (toluene/EtOAc 0→33%) to give **7** (392 mg, 607 μmol, 80%). $[\alpha]_D$ +30 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 26.3, 28.1 [(*Me*)$_2$C], 35.3 (Ar*C*H$_2$CH$_2$), 67.9, 68.7, 69.0, 69.6, 73.1, 73.3, 74.4, 75.6, 78.4 (C-2–7, ArCH$_2$O, ArCH$_2$*C*H$_2$O), 97.3 (C-1), 109.4 [(Me)$_2$*C*], 120.5–138.2 (aromatic C). Anal. calcd for $C_{34}H_{38}F_3NO_8$: C, 63.25; H, 5.93; N, 2.17. Found: C, 63.28; H, 5.87; N, 2.15.

3.8. Ethyl 2,3-di-O-benzoyl-4-O-benzyl-1-thio-β-D-glucopyranoside **8**

Ethyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside[11] (6.0 g, 11.5 mmol) and borane–trimethylamine complex (33.6 g, 461 mmol) were dissolved in dry CH_2Cl_2:Et_2O (5:2, 280 mL). Powdered molecular sieves (4 Å) were added and the mixture was stirred for 1 h and then cooled to 0°C. To another cooled flask (0°C) with 50 mL of dry Et_2O was slowly and carefully added $AlCl_3$ (6.3 g, 47 mmol). This solution was then added dropwise to the above mixture at 0°C. The mixture was stirred for an additional 30 min, when it was filtered (Celite) and stirred with aqueous sulfuric acid (1 M, 100 mL). The organic phase was separated and washed with H_2O followed by $NaHCO_3$ (aq., satd), dried ($MgSO_4$), filtered and concentrated. The residue was dissolved in boiling Et_2O (600 mL) and left overnight. Filtration gave 21 g of unreacted borane–trimethylamine complex (as was determined by NMR). The filtrate was concentrated and purified on a silica gel column (toluene/EtOAc 0→33%) to yield **8** (5.02 g, 9.61 mmol, 84%). $[\alpha]_D$ +53 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 14.9, 24.5 (CH_3CH_2S), 61.7, 70.9, 74.8, 75.4, 76.2, 79.7, 83.8 (C-1–6, PhCH$_2$O), 127.0–137.1 (aromatic C), 165.4, 165.7 (PhCO). Anal. calcd for $C_{29}H_{30}O_7S$: C, 66.65; H, 5.79. Found: C, 66.58; H, 5.76.

3.9. *Ethyl 2,3-di-*O*-benzoyl-4-*O*-benzyl-6-*O*-chloroacetyl-1-thio-β-*D*-glucopyranoside* **9**

Monochloroacetyl chloride (318 μL, 4.00 mmol) was added to a cooled (10°C) solution of **8** (1.023 g, 1.96 mmol) in CH_2Cl_2:pyridine (15:1, 25 mL). After 1 h the solution was washed with H_2O, dried ($MgSO_4$), filtered and concentrated. After silica gel column chromatography (toluene/EtOAc 0→10%), **9** (930 mg, 1.55 mmol, 79%) was obtained. $[\alpha]_D$ +70 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 14.9, 24.4 (CH_3CH_2S), 40.7 (Cl*C*H_2CO), 64.3, 70.6, 74.7, 75.2, 76.4, 76.8, 83.7 (C-1–6, PhCH_2O), 128.2–136.7 (aromatic C), 165.4, 165.6, 166.9 (PhCO, ClCH_2*C*O).

3.10. *2-(4-Trifluoroacetamidophenyl)ethyl (2,3-di-*O*-benzoyl-4-*O*-benzyl-6-*O*-chloroacetyl-β-*D*-gluco-pyranosyl)-(1→4)-6,7-di-*O*-benzyl-2,3-*O*-isopropylidene-*L*-glycero-α-*D*-manno-heptopyranoside* **10**

A solution of **9** (208 mg, 347 μmol) and **7** (159 mg, 246 μmol) in dry CH_2Cl_2 (20 mL) was stirred with powdered molecular sieves (4 Å) under argon for 40 min. The mixture was cooled to 0°C and NIS (86 mg, 382 μmol) and a catalytic amount of AgOTf were added. After 10 min the cooling bath was removed and stirring was continued for 1 h. The reaction was quenched by the addition of Et_3N (50 μl) and the mixture filtered through Celite, washed ($Na_2S_2O_3$, aq., satd) and dried ($MgSO_4$). Filtration and concentration yielded a residue, which was subjected to silica gel column chromatography (toluene/EtOAc 0→20%) to give **10** (277 mg, 234 μmol, 95%). $[\alpha]_D$ +54 (*c* 1.0, $CHCl_3$); mp 144–146°C (corr.); ^{13}C NMR ($CDCl_3$): δ 26.4, 27.9 [(*Me*)$_2$C], 35.1 (Ar*C*H_2CH_2), 40.7 (Cl*C*H_2CO), 63.9, 67.2, 67.8, 69.9, 72.2, 72.6, 72.8, 73.0, 73.3, 75.0, 75.3, 76.8 (C-2–7, C-2′–6′, PhCH_2O, ArCH_2*C*H_2O), 96.5 ($J_{C,H}$ 169 Hz, C-1), 100.5 ($J_{C,H}$ 161 Hz, C-1′), 109.2 [(Me)$_2$*C*], 121.0–138.7 (aromatic C), 165.6, 165.7, 167.0 (PhCO, ClCH_2*C*O).

3.11. *2-(4-Trifluoroacetamidophenyl)ethyl (2,3-di-*O*-benzoyl-4-*O*-benzyl-β-*D*-glucopyranosyl)-(1→4)-6,7-di-*O*-benzyl-2,3-*O*-isopropylidene-*L*-glycero-α-*D*-manno-heptopyranoside* **11**

To a solution of **10** (275 mg, 233 μmol) in THF:DMF (4:1, 15 mL) was added hydrazine dithiocarbonate (2.0 mL of a stock solution, approx 350 mM). After stirring for 1 h at room temperature the solution was diluted with CH_2Cl_2 and washed with H_2O. Drying (Na_2SO_4), filtration, concentration and purification by silica gel column chromatography (toluene:EtOAc 4:1) then gave **11** (229 mg, 207 μmol, 89%). $[\alpha]_D$+38 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 26.0, 27.7 [(*Me*)$_2$C], 35.1 (Ar*C*H_2CH_2), 62.9, 66.9, 67.0, 70.0, 72.7, 72.9, 73.0, 74.3, 74.5, 74.6, 74.9, 75.8, 77.8, 78.5 (C-2–7, C-2′–6′, PhCH_2O, ArCH_2*C*H_2O), 95.8, 101.2 (C-1,1′), 109.2 [(Me)$_2$*C*], 121.3–138.7 (aromatic C), 165.7, 166.0 (PhCO). Anal. calcd for $C_{61}H_{62}F_3NO_{15}$: C, 66.24; H, 5.65; N, 1.27. Found: C, 66.13; H, 5.77; N, 1.25.

3.12. *2-(4-Trifluoroacetamidophenyl)ethyl (6,7-di-*O*-benzyl-2,3-*O*-isopropylidene-4-*O*-p-methoxy-benzyl-*D*-glycero-α-*D*-manno-heptopyranosyl)-(1→6)-(2,3-di-*O*-benzoyl-4-*O*-benzyl-β-*D*-glucopyran-osyl)-(1→4)-6,7-di-*O*-benzyl-2,3-*O*-isopropylidene-*L*-glycero-α-*D*-manno-heptopyranoside* **12**

Compounds **11** (129 mg, 117 μmol) and **4** (90 mg, 151 μmol) were dissolved in dry benzene (2 mL), and the solution concentrated and dried under vacuum. The residue was dissolved in dry Et_2O (10 mL) and powdered molecular sieves (4 Å) were added. The mixture was stirred for 40 min under argon, whereafter dimethyl(methylthio)sulfonium triflate (DMTST, 125 mg, 484 μmol) was added. Continued stirring for 3 h followed by filtration and concentration gave a crude product which was purified by silica gel column chromatography (toluene:EtOAc 6:1) to yield **12** (136 mg, 83.0 μmol, 71%). $[\alpha]_D$ +37 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 26.3, 26.5, 27.9, 28.0 [(*Me*)$_2$C], 35.2 (Ar*C*H_2CH_2), 55.2 (MeO), 64.3,

67.3, 67.9, 69.1, 70.0, 70.7, 72.3, 72.6, 72.8, 72.9, 73.2, 74.4, 74.5, 75.0, 75.2, 75.3, 75.5, 76.9, 78.4, 79.3 (C-2–7, C-2′–6′, C-2′′–7′′, $PhCH_2O$, $ArCH_2CH_2O$), 96.7 ($J_{C,H}$ 169 Hz), 97.5 ($J_{C,H}$ 172 Hz) (C-1,1′′), 100.7 ($J_{C,H}$ 165 Hz, C-1′), 109.1, 109.5 [$(Me)_2C$], 113.6–159.1 (aromatic C), 165.6, 165.8 (PhCO).

3.13. *2-(4-Trifluoroacetamidophenyl)ethyl (6,7-di*-O-*benzyl-2,3*-O-*isopropylidene*-D-glycero-*α*-D-manno-*heptopyranosyl)-(1→6)-(2,3-di*-O-*benzoyl-4*-O-*benzyl-β*-D-*glucopyranosyl)-(1→4)-6,7-di*-O-*benzyl-2,3*-O-*isopropylidene*-L-glycero-*α*-D-manno-*heptopyranoside* **13**

A solution of **12** (165 mg, 101 μmol) and DDQ (34 mg, 150 μmol) in CH_2Cl_2 (10 mL) and H_2O (0.5 mL) was stirred at room temperature overnight. The mixture was washed with H_2O and $Na_2S_2O_3$ (aq., satd), dried ($MgSO_4$) and concentrated. The crude product was purified (silica gel column, toluene:EtOAc 3:1) to give **13** (105 mg, 69.1 μmol, 69%). $[\alpha]_D$ +30 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 26.3, 27.9, 28.1 [$(Me)_2C$], 35.2 ($ArCH_2CH_2$), 64.7, 67.3, 67.8, 68.3, 69.5, 70.0, 71.2, 72.5, 72.6, 72.8, 72.9, 73.5, 74.4, 74.5, 74.9, 74.97, 75.02, 75.2, 75.3, 77.2, 78.0, 80.2 (C-2–7, C-2′–6′, C-2′′–7′′, $PhCH_2O$, $ArCH_2CH_2O$), 96.7, 97.8, 100.8 (C-1,1′,1′′), 109.0, 109.6 [$(CH_3)_2C$], 121.0–138.7 (aromatic C), 165.6, 165.8 (PhCO). Anal. calcd for $C_{85}H_{90}F_3NO_{21}$: C, 67.23; H, 5.97; N, 0.92. Found: C, 67.63; H, 5.68; N, 1.07.

3.14. *Ethyl 2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-1-thio-β*-D-*glucopyranoside* **14**

2,3,4,6-Tetra-*O*-acetyl-β-D-galactosyl bromide[19] (1.645 g, 4.00 mmol) and 3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[15] (1.063 g. 1.99 mmol) were dissolved in a mixture of dry toluene and CH_2Cl_2 (1:1, 18 mL). Powdered molecular sieves (4 Å) were added and the mixture was stirred under argon for 1 h. The flask was wrapped in aluminium foil and cooled to −45°C by means of a CO_2/MeCN bath. AgOTf (1.15 g, 4.48 mmol) dissolved in dry toluene (12 mL) was added during 1 h under the exclusion of light. After additional stirring for 30 min at −45°C solid $Na_2S_2O_3 \cdot 5\ H_2O$ (2 g, 8 mmol) was added at the same temperature. The mixture was stirred for 5 min, whereafter the cooling bath was removed and toluene (30 mL) and H_2O (20 mL) were added. The mixture was stirred for 10 min and then transferred to a separatory funnel via a Celite-packed glass filter funnel. The organic phase was separated, dried (Na_2SO_4), filtered, and concentrated. Purification of the residue by silica gel column chromatography (toluene containing/EtOAc 10→40%) gave 1.595 g (1.85 mmol, 93%) of **14**. NMR was in agreement with published data.[15]

3.15. *2-(Trimethylsilyl)ethyl 4*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*galactopyranoside* **15**

2-(Trimethylsilyl)ethyl β-D-galactopyranoside[16] (1.07 g, 3.82 mmol) was dissolved in acetone (10 mL). The pH was adjusted to 1 by means of the addition of *p*-toluenesulfonic acid. After stirring for 2 h the solution was neutralised by pyridine. The mixture was concentrated and co-evaporated once from dry pyridine. The residue was then dissolved in pyridine (4 mL) and benzoyl chloride (2.0 mL, 17.2 mmol) was added. The solution was immediately cooled in a water bath and CH_2Cl_2 (3 mL) was added. Stirring for another 45 min followed by dilution (toluene), washing (twice with H_2O), filtration through a glass filter funnel containing $MgSO_4$ and concentration gave a crude residue, which was purified on a silica gel column (toluene/EtOAc 0→10%) to give, in the order of elution, the tetrabenzoyl derivative, followed by the benzoylated 3,4-isopropylidene isomer [1.45 g, 2.74 mmol, 72%; ^{13}C NMR ($CDCl_3$): δ −1.6 (MeSi), 17.8 (CH_2Si), 26.3, 27.6 [$(Me)_2C$], 63.7, 67.0, 70.9, 73.6, 73.7, 77.3 (C-2–6, OCH_2CH_2Si), 99.9 (C-

1), 110.8 [$(Me)_2C$], 128.2–133.6 (aromatic C), 165.3, 166.3 (PhCO)], and lastly the 4,6-isopropylidene derivative. A solution of the 3,4-*O*-isopropylidene derivative (890 mg, 1.68 mmol) in HOAc (80% aq., 10 mL) was stirred for 40 min at 90°C, whereafter the solution was cooled and concentrated. Co-evaporation from toluene gave crude crystals of 2-(trimethylsilyl)ethyl 2,6-di-*O*-benzoyl-β-D-galactopyranoside, which were re-crystallised from diethyl ether–light petroleum (40–65°C). Yield 783 mg (1.60 mmol, 95%). To a solution of this derivative (2.00 g, 4.09 mmol) in dry MeCN (14 mL) was added trimethyl orthoacetate (1.13 mL) and then *p*-toluenesulfonic acid until acidic reaction (pH 1). The solution was stirred for 30 min and then concentrated. The residue was co-concentrated once from toluene, dried on a vacuum pump, and dissolved in dry MeCN (14 mL). 500 μL of trifluoroacetic acid (90% aq.) was added and the solution was stirred for 30 min. Neutralisation (1.3 mL of pyridine) followed by concentration yielded a crude residue which was dissolved in toluene, washed once with water, dried (Na_2SO_4), filtered, concentrated, and finally purified on a silica gel column (toluene:EtOAc 4:1) to give **15** (1.72 g, 3.25 mmol, 79%). $[\alpha]_D$ −24 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ −1.6 (CH_3Si), 17.9 (CH_2Si), 20.8 (CH_3CO), 62.2, 67.5, 69.9, 71.0, 71.6, 73.5 (C-2–6, CH_2CH_2Si), 100.5 (C-1), 128.3–133.3 (aromatic C), 166.0, 166.8 (PhCO), 171.0 (CH_3CO).

*3.16. 2-(Trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-phthalimido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-4-*O*-acetyl-2,6-di-*O*-benzoyl-β-D-galactopyranoside **16***

A solution of **14** (1.59 g, 1.84 mmol) and **15** (1.07 g, 2.02 mmol) in dry CH_2Cl_2 (10 mL) and Et_2O (13 mL) was stirred with powdered molecular sieves under argon for 30 min. The mixture was then cooled (0°C) and NIS (502 mg, 2.23 mmol) and TfOH (25 μL, 0.28 mmol) were added, and the reddish brown mixture was stirred at 0°C for 20 min and then filtered through Celite. The solids were washed with additional CH_2Cl_2 and the combined organic phases were washed with $Na_2S_2O_3$ (aq., satd). Drying (Na_2SO_4), filtration, concentration and gradient silica gel column chromatography (toluene/EtOAc 10→40%) gave **16** (1.92 g, 1.44 mmol, 70%). $[\alpha]_D$ +32 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ −1.7 (CH_3Si), 17.7 (CH_2Si), 20.5, 20.6, 20.7, 20.8 (CH_3CO), 55.5, 60.7, 62.9, 66.9, 67.3, 67.7, 69.5, 70.5, 70.97, 71.05, 71.2, 71.4, 73.5, 74.2, 74.9, 76.2, 77.6 (C-2–6, C-2′–6′, C-2′′–6′′, $PhCH_2O$, CH_2CH_2Si), 99.0 ($J_{C,H}$ 169 Hz), 100.3 ($J_{C,H}$ 163 Hz), 100.7 ($J_{C,H}$ 159 Hz) (C-1–1′′), 122.9–138.4 (aromatic C), 164.4, 166.1 (PhCO), 169.1, 170.0, 170.1, 170.3, 170.4 (CH_3CO). HRMS: calcd for $C_{69}H_{77}NO_{24}SiNa$: 1354.4503. Found: 1354.4521.

*3.17. Ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside **19***

Compound **16** (1.55 g, 1.16 mmol) was dissolved in dry MeOH. The pH was adjusted to 11 by the addition of a methanolic 1 M NaOMe solution. After stirring for 2 h when TLC ($CHCl_3$:MeOH 5:1) showed one major product, the mixture was neutralised by adding Dowex H^+ ion exchange resin, then filtrated and concentrated to give a residue, which was dissolved in EtOH:1,4-dioxane (1:1, 40 mL). Hydrazine monohydrate (4 mL) was added and the solution was stirred at reflux temperature for 3 h until TLC ($CHCl_3$:MeOH 5:1, ninhydrin evolution) indicated a free amine. The solution was cooled, carefully concentrated, and co-evaporated twice from EtOH, once from toluene:EtOH 1:1, and finally once from pyridine. The residue was dissolved in pyridine:Ac_2O (1:1, 40 mL) at 0°C. Complete acetylation was eventually effected after addition of an unexpectedly large amount of 4-dimethylaminopyridine (approximately 2 g). After stirring for 30 min the solution was concentrated,

redissolved in toluene (100 mL), washed with H_2O, dried (Na_2SO_4), filtered, and concentrated. Purification by silica gel column chromatography (toluene:EtOAc 1:1) gave 2-(trimethylsilyl)ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (**17**, 1.05 g, 0.937 mmol, 81%); ^{13}C NMR ($CDCl_3$): δ −1.4 (CH_3Si), 17.9 (CH_2Si), 20.6, 20.8, 21.1 (CH_3CO), 23.4 (CH_3CON), 57.2, 60.8, 62.4, 66.9, 67.3, 68.1, 69.5, 70.6, 70.8, 70.9, 71.3, 73.5, 74.3, 74.6, 76.6, 77.0 (C-2–6, C-2′–6′, C-2′′–6′′, $PhCH_2O$, CH_2CH_2Si), 99.6, 100.0, 100.7 (C-1–1′′), 123.2–138.7 (aromatic C), 169.3, 169.7, 170.0, 170.1, 170.2, 170.5, 170.6 (CH_3CO). Compound **17** (403 mg, 360 μmol) was dissolved in dry toluene (4 mL). Ac_2O (0.4 mmol) and BF_3–Et_2O (36 μL, 0.29 mmol) were added at room temperature, and the solution was heated to 40°C. After 1 h more BF_3–Et_2O (36 μL, 0.29 mmol) was added. The heating bath was removed, and the solution was stirred at rt for an additional 2 h, then diluted (CH_2Cl_2), washed with $NaHCO_3$ (aq., satd), dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by gradient silica gel column chromatography (toluene/EtOAc 33→100%) to yield (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-1,2,4,6-tetra-*O*-acetyl-β-D-galactopyranose (**18**, 159 mg, 150 μmol, 42%); ^{13}C NMR ($CDCl_3$): δ 20.6, 20.8 (CH_3CO), 23.3 (CH_3CON), 56.7, 60.8, 62.1, 66.9, 68.1, 69.1, 69.4, 69.7, 70.6, 70.8, 72.4, 73.5, 74.2, 74.7, 76.1, 76.3, 76.9 (C-2–6, C-2′–6′, C-2′′–6′′, $PhCH_2O$), 92.2 ($J_{C,H}$ 161 Hz), 99.7 ($J_{C,H}$ 169 Hz), 100.0 ($J_{C,H}$ 163 Hz) (C-1–1′′), 127.6–138.6 (aromatic C), 169.1, 169.3, 169.5, 169.9, 170.0, 170.1, 170.2, 170.4 (CH_3CO). A solution of **18** (159 mg, 150 μmol) in dry CH_2Cl_2 (3 mL) was stirred with powdered molecular sieves for 30 min. The mixture was cooled (−10°C) and EtSH (30 μL), followed by BF_3–Et_2O (50 μL), were added. After 30 min stirring, more EtSH (40 μL) and BF_3–Et_2O (50 μL) were added and stirring was continued for another 1.5 h (temp. +10°C). Then the mixture was filtered through Celite, the filtrate diluted with CH_2Cl_2, washed ($NaHCO_3$, aq., satd), dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (toluene/EtOAc 10→75%) to yield **19** (116 mg, 109 μmol, 73%); $[\alpha]_D$ +8 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 14.8 (CH_3CH_2S), 20.5, 20.6, 20.9 (CH_3CO), 23.3, 24.0 (CH_3CH_2S, CH_3CON), 56.9, 60.6, 62.4, 66.7, 68.0, 68.9, 69.3, 69.5, 70.5, 70.7, 73.3, 74.1, 74.6, 75.1, 76.4, 76.7 (C-2–6, C-2′–6′, C-2′′–6′′, $PhCH_2O$), 83.8 ($J_{C,H}$ 154 Hz), 99.5 ($J_{C,H}$ 167 Hz), 99.9 ($J_{C,H}$ 163 Hz) (C-1–1′′), 127.4–138.6 (aromatic C), 169.2, 169.7, 169.8, 170.0, 170.3 (CH_3CO). HRMS: calcd for $C_{50}H_{66}NO_{22}S$: 1064.3797. Found: 1064.3822.

*3.18. 2-(4-Trifluoroacetamidophenyl)ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-galacto-pyranosyl)-(1→4)-(6,7-di-*O*-benzyl-2,3-*O*-isopropylidene-D-glycero-α-D-manno-heptopyranosyl)-(1→6)-(2,3-di-*O*-benzoyl-4-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-6,7-di-*O*-benzyl-2,3-*O*-isopropylidene-L-glycero-α-D-manno-heptopyranoside **21***

Compounds **13** (77 mg, 51 μmol) and **19** (85 mg, 80 μmol) were co-evaporated and dried from dry toluene and then dissolved in freshly distilled CH_2Cl_2 (4 mL) under argon. Powdered molecular sieves (4 Å) were added and the mixture was stirred at rt for 1 h. After cooling (−13°C) the mixture, NIS (24 mg, 0.11 mmol) and TfOH (2 μL, 23 μmol) were added. Stirring was continued for 20 min at −10°C and then at rt for additional 20 min. The reaction mixture was then filtered through Celite down into a separatory funnel containing $Na_2S_2O_3$ (10% aq.) and the solids were washed with additional CH_2Cl_2. The organic phase was separated, dried (Na_2SO_4), filtered and concentrated. Purification of the residue by silica gel chromatography (toluene/EtOAc 10→50%) gave 29 mg (19 μmol, 38%) of unreacted **13** followed by 58 mg (23 μmol, 45%) of target hexasaccharide **21** (72% based on consumed aglycon); $[\alpha]_D$ +29 (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 20.6, 20.8, 20.9 (CH_3CO), 23.4 (CH_3CON), 26.3, 26.6, 27.9, 28.2

(Me_2C), 35.1 (OCH_2CH_2Ar), 57.2, 60.8, 62.1, 63.9, 66.9, 67.3, 67.8, 68.2, 68.4, 69.5, 69.6, 69.9, 70.5, 70.6, 70.9, 71.5, 72.5, 72.7, 72.9, 73.1, 73.3, 73.5, 74.3, 74.4, 74.7, 74.9, 75.1, 76.8, 78.3, 78.8 (C-2–7, C-2′–6′, C-2′′–7′′, C-2′′′–6′′′, C-2′′′′–6′′′′, C-2′′′′′–6′′′′′, $PhCH_2O$, $ArCH_2CH_2O$), 96.7, 97.5, 99.7, 100.0, 100.7, 100.9 (C-1–1′′′′′), 109.0 ($(Me)_2C$), 121.0–138.7 (aromatic C), 165.6, 165.8 (PhCO), 169.3, 169.9, 170.0, 170.18, 170.23, 170.5 (CH_3CO).

*3.19. 2-(4-Trifluoroacetamidophenyl)ethyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-(2-diacetylamino-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1→4)-(6,7-di-*O*-benzyl-2,3-*O*-isopropylidene-*D*-glycero-α-*D*-manno-heptopyranosyl)-(1→6)-(2,3-di-*O*-benzoyl-4-*O*-benzyl-β-*D*-glucopyranosyl)-(1→4)-6,7-di-*O*-benzyl-2,3-*O*-isopropylidene-*L*-glycero-α-*D*-manno-heptopyranoside **22***

Ethyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2-diacetylamino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (**20**, 32 mg, 28.9 μmol) and **13** (30 mg, 19.8 μmol) were dissolved in 4 mL of dry CH_2Cl_2 under argon, and stirred with powdered molecular sieves (4 Å) at rt for 1 h. The mixture was then cooled to 0°C and NIS (10 mg, 44 μmol) and TfOH (1.0 μL, 11 μmol) were added consecutively. Stirring of the increasingly violet solution was continued at 0°C for 30 min and at rt for 10 min, whereafter the mixture was filtered through Celite, washed ($Na_2S_2O_3$, 10% aq.), dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel chromatography (toluene/EtOAc 10→50%) to give **22** (38 mg, 15 μmol, 75%). HRMS: calcd for $C_{135}H_{151}N_2O_{44}F_3$: 2560.9592. Found: 2560.9768.

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