Synthesis of and molecular dynamics simulations on a tetrasaccharide corresponding to the repeating unit of the capsular polysaccharide from *Salmonella enteritidis*†

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Received 5th January 2009, Accepted 30th January 2009
First published as an Advance Article on the web 4th March 2009
DOI: 10.1039/b823428k

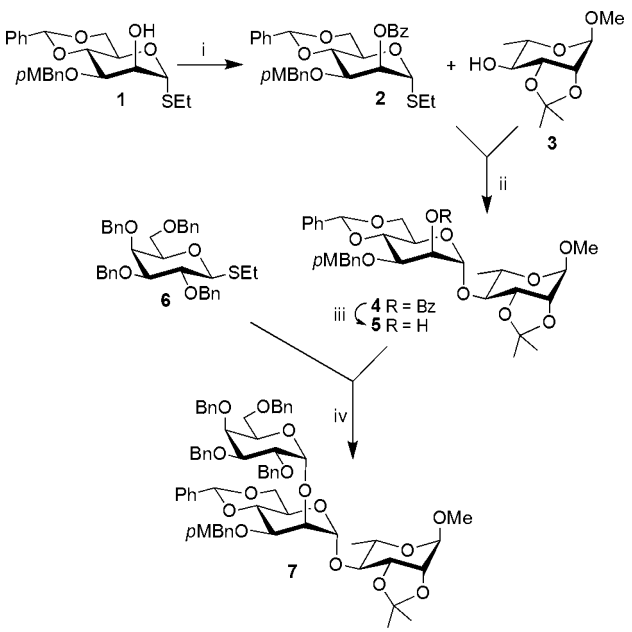
Syntheses of two oligosaccharides as methyl glycosides related to the repeating unit of *S. enteritidis* capsular polysaccharide (CPS) are presented. The trisaccharide corresponds to the backbone of the CPS whereas the tetrasaccharide is a model for the repeating unit which has a branched structure. Molecular dynamics simulations investigating their flexibility and dynamics revealed that the oligosaccharides populate several conformational states and indicate that conformational averaging should be used in describing the accessible conformational space.

Introduction

Salmonella enterica serovar Enteritidis is a food-borne human pathogen causing gasteroentritis. Gram-negative bacteria, to which *S. enteritidis* belong, carry a lipopolysaccharide (LPS) in their outer membrane and, in addition, associated extracellular polysaccharides in some cases. The recent progress in the understanding of the importance of a bacterial capsule, *e.g.*, in immune evasion and survival strategy where it protects the bacterium from desiccation, has spurred novel interest in the structure of capsular polysaccharides (CPSs).[1] Furthermore, recent studies on the interaction of an O-antigen-derived octasaccharide with phage P22 tailspike protein has shed light on the binding process.[2] In *S. enteritidis* the CPS has the same basic repeating unit as that of its LPS, consisting of a tetrasaccharide repeating unit with the following structure: →3)-α-D-Gal*p*-(1→2)[α-Tyv*p*-(1→3)]-α-D-Man*p*-(1→4)-α-L-Rha*p*-(1→, in which Tyv*p* is a 3,6-dideoxy-D-*arabino*-hexopyranosyl group.[3] Structural differences between the LPS and the CPS occur, and comprise the degree and positions of glucosylation, the extent of which has been described as being important for virulence.[4] Synthetic organic chemistry provides an excellent tool to produce well-defined (homogeneous) oligosaccharides[5–7] corresponding to the above repeating unit. We have therefore synthesized a tetrasaccharide as a methyl glycoside, since such a derivative is suitable for *e.g.* solution-state NMR interaction studies.[8] We have also carried out a molecular simulation on this derivative, to gain information on its conformational preferences and flexibility, *e.g.* in comparison to previously reported studies that utilized HSEA calculations on similar oligosaccharides.[9] After we had completed the synthesis and during the analysis of the molecular dynamics (MD) simulations, a different synthesis of the same tetrasaccharide appeared.[10] We therefore judged it timely to report on our syntheses and MD simulations of the tri- and tetrasaccharides.

Results and discussion

Our synthetic strategy, in contrast to the recently published synthesis by Son *et al.*,[10] includes synthesis of the α-D-Gal*p*-(1→2)-α-D-Man*p*-(1→4)-α-L-Rha*p* 'backbone' trisaccharide starting from three known monosaccharide units, and subsequent addition of a 3,6-dideoxy-D-*arabino*-hexopyranoside donor to complete the synthesis of tetrasaccharide **17**. To access mannosyl donor **2**, ethyl 4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (**1**)[11] was benzoylated (Scheme 1).



Scheme 1 *Reagents and conditions*: *i*) BzCl, pyridine, 89%; *ii*) NIS, AgOTf, 4 Å MS, CH_2Cl_2, −30 °C, 92%; *iii*) NaOMe, MeOH/CH_2Cl_2 (2:1), 90%; *iv*) NIS, AgOTf, 4 Å MS, Et_2O, −30 °C, 85%.

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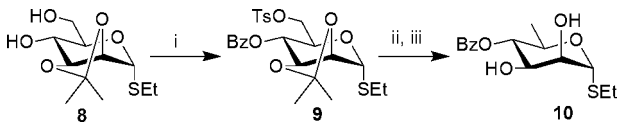
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra and chemical shift assignments of a constituent trisaccharide and the target tetrasaccharide. See DOI: 10.1039/b823428k

NIS-promoted glycosylation at –30 °C with methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**3**)[12] gave the desired disaccharide **4** in 92% yield. Debenzoylation, followed by a second NIS-promoted α-glycosylation with ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**6**)[13] as donor in diethyl ether at –30 °C, gave protected trisaccharide **7** exclusively, in 85% yield.

In the synthesis of the novel tyvelose donor **12**, our first approach was to introduce both deoxy functions in a single reaction. In contrast to Bundle *et al.*,[14] the Barton-McCombie deoxygenation[15] of various 3,6-di-*O*-thiocarbonylimidazole derivatives did not give the desired product in satisfying yield. Neither did hydride addition using $NaBH_4$ to the corresponding 3,6-di-*O*-tosylate or 3,6-di-*O*-trifluoromethanesulfonate compounds. Therefore, this route was abandoned and the deoxy functions were installed separately to afford the desired tyvelose donor. Ethyl 2,3-*O*-isopropylidene-1-thio-α-D-mannopyranoside (**8**) was regioselectively tosylated at O-6, followed by benzoylation (→**9**) (Scheme 2).



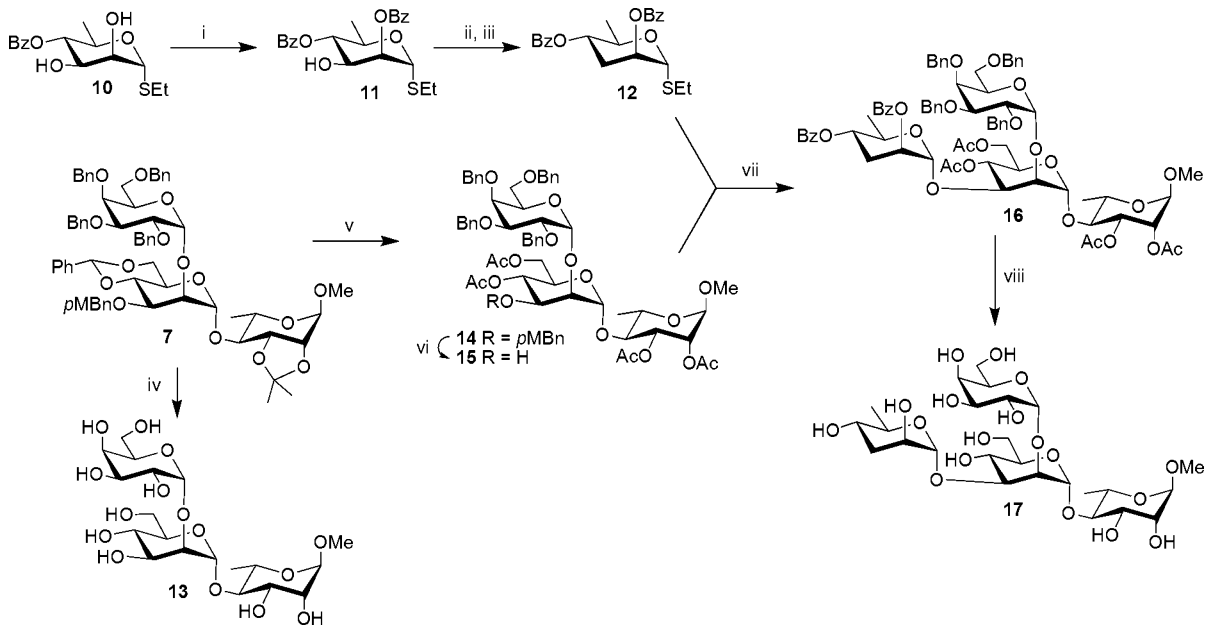
Scheme 2 *Reagents and conditions*: *i*) a) TsCl, Et_3N, CH_2Cl_2 b) BzCl, pyridine, 91%; *ii*) $NaBH_4$, DMF, 50 °C; *iii*) 80% AcOH (aq.), 60 °C, 69% (2 steps).

Hydride addition using $NaBH_4$ in DMF yielded the 6-deoxy sugar, and subsequent acidic hydrolysis of the 2,3-*O*-isopropylidene group gave diol **10**. Insertion of a 2,3-orthoester continued by acidic hydrolysis yielded the 2-*O*-benzoyl derivative **11**. This was followed by a Barton-McCombie deoxygenation of the corresponding 3-*O*-thiocarbonylimidazole derivative, completing the synthesis of the 3,6-dideoxy-D-*arabino*-hexopyranoside (tyveloside) donor **12** (Scheme 3).

The trisaccharide **7** was fully deprotected to yield **13** or alternatively converted into a suitable acceptor for glycosylation with the 3,6-dideoxy donor. To circumvent the potential problems with acidic deprotection of the acid-labile dideoxyhexose-containing tetrasaccharide, both acetal groups were hydrolyzed in acetic acid and the resulting tetraol was acetylated (→**14**) prior to cleavage of the *p*-methoxybenzyl ether using DDQ to yield acceptor **15** in 63% yield. The synthesis of the tetrasaccharide corresponding to the repeating unit of the CPS found in *S. enteritidis* was completed by MeOTf-promoted glycosylation of trisaccharide **15** with tyveloside donor **12** in the presence of DTBMP to give **16** in 62% yield. A subsequent two-step deprotection gave the desired tetrasaccharide **17**, in 63% yield. 1D- and 2D-NMR experiments, the CASPER program[16] and NMR spin simulations (using the PERCH NMR software[17]) were used to fully assign the ^1H and ^{13}C chemical shifts as well as $J_{H,H}$ coupling constants of the target oligosaccharides **13** and **17**.

The two oligosaccharides were studied by MD simulations carried out for 30 ns using a recently developed force field for carbohydrates and explicit water as solvent. The torsion angle analyses are given in Table 1 and the conformations populated are presented as scatter plots (Fig. 1).

At each glycosidic linkage two major conformations are present, which is also indicated by the large RMSDs for, in particular, the ψ torsion angles (Table 1). These correspond for the ϕ torsion angles (H1–C1–O*n*–C*n*, where *n* is the numbering at the glycosidic linkage position) to that of an *exo*-anomeric effect and for the ψ torsion angles (C1–O*n*–C*n*–H*n*) to those alternating between positive and negative values. Population at the non-*exo*-anomeric conformation where ϕ > 0° is limited for the galactosyl group whereas it is higher for the tyvelosyl group (axial hydroxyl group



Scheme 3 *Reagents and conditions*: *i*) a) trimethyl orthobenzoate, CSA, CH_2Cl_2 b) 80% AcOH (aq.), 85%; *ii*) 1,1′-thiocarbonyldiimidazole, toluene, reflux; *iii*) Bu_3SnH, AIBN, benzene, reflux, 75% (two steps); *iv*) a) 70% AcOH (aq.), 80 °C b) Pd/C, H_2, EtOH/H_2O (2:1), 76%; *v*) a) 60% AcOH (aq.), 70 °C b) Pyridine/Ac_2O (1:1), 81%; *vi*) DDQ, CH_2Cl_2/H_2O (14:1), 63%; *vii*) MeOTf, DTBMP, 4 Å MS, Et_2O, 62%; *viii*) a) Pd/C, H_2, EtOAc b) NaOMe, MeOH, 63%.

Table 1 Average glycosidic torsion angles for oligosaccharides **13** and **17** from 30 ns MD simulations. Root-mean-square deviations (RMSDs) are given in parentheses

	Trisaccharide		Tetrasaccharide	
Sugar residue	ϕ/°	ψ/°	ϕ/°	ψ/°
α-D-Gal*p*-(1→2)	−38 (17)	21 (37)	−41 (11)	17 (34)
→2,3)-α-D-Man*p*-(1→	−29 (30)	19 (34)	−31 (26)	26 (34)
→4)-α-L-Rha*p*-OMe	46 (22)	—	46 (21)	—
α-Tyv*p*-(1→3)	—	—	−48 (16)	7 (30)

at C-2) and significant for the mannosyl residue having the same configuration at C2 as the tyvelosyl group. Analysis of the ring conformations of the tyvelosyl group showed that not only was the $^4C_1$ chair conformation present as expected but also the $^1C_4$ chair conformation as well as other transiently observed ring conformations. The ring flexibility of α-Tyv*p* observed in the MD simulations with the PARM/SU01 force field for carbohydrates,[18] which is a CHARMM22 type of force field, agrees well with the accessible conformations deduced from the potential energy minimizations using the MM3(92) force field.[19] The relative energies for the $^4C_1$, $^1C_4$, $^0S_2$ and $^1S_3$ conformers were 0, 1.4, 2.4, and 5.2 kcal/mol, respectively, showing that both chair forms should be anticipated to be populated as well as to some extent skew-boat conformations.

In the tetrasaccharide an anti-ψ conformational state (ψ ≈ 180 °) is populated during the simulation (Fig. 2). This is reached and returned from *via* an intermediate state with ψ ≈ 140°. Only the latter state is transiently populated in the trisaccharide. Whether the transition from the anti-ψ conformation can take more that one path may be addressed by starting a number of MD simulations from this conformation, similar to what has been reported for a disaccharide from a local potential energy well that was higher in energy on the *in vacuo* potential energy surface.[20] The results for the MD simulations indicate that the conformational flexibility as judged by the presence of additional conformations may in fact be higher in what initially may appear to be a sterically more crowded and conformationally restricted oligosaccharide in which the mannosyl residue is vicinally disubstituted, *i.e.*, at O2 and O3, by the galactosyl and tyvelosyl groups, respectively. These results are indeed consistent with the computational and NMR experimental results on α-D-Glc*p*-(1→3)[β-D-Glc*p*-(1→4)]-α-D-Glc*p*-OMe in which it was concluded that an anti-ψ conformational state at the

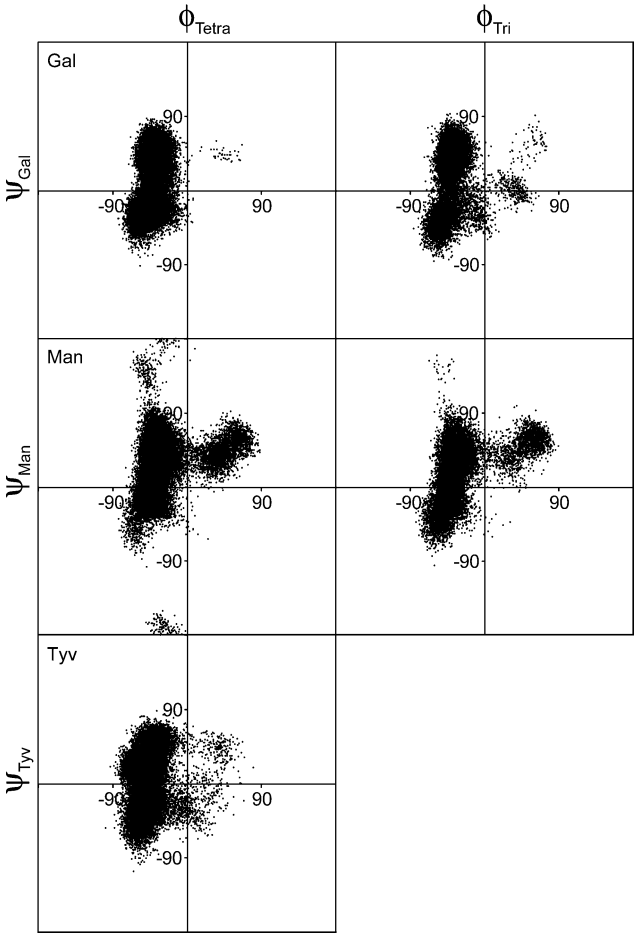


Fig. 1 Scatter plots from the MD simulations of tetrasaccharide **17** (left) and trisaccharide **13** (right).

(1→4)-linkage should be significantly populated.[21] Future analysis based on experimental NMR data may be able to resolve this issue for the tetrasaccharide.

In conclusion, we have presented a concise and alternative synthesis of a tetrasaccharide corresponding the repeating unit of *S. enteritidis* which also should be possible to apply to other serotypes such as A and B having paratose and abequose, respectively, as the 3,6-dideoxy sugar residues. The MD simulations show a complex

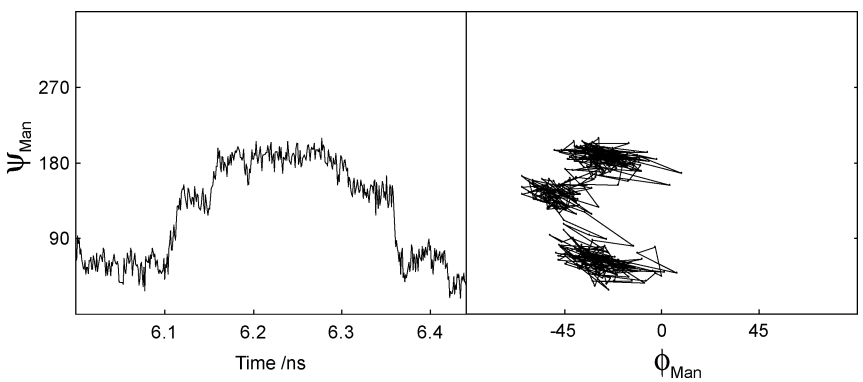


Fig. 2 Trajectory analysis from the MD simulation of tetrasaccharide **17**. The torsion angles in the mannosyl residue for: time *vs.* ψ (left) and ϕ *vs.* ψ (right).

and dynamical behavior at the glycosidic torsion angles of the oligosaccharides, indicating that the O-antigen polysaccharide may behave in a similar way.

Materials and methods

General methods

Normal work-up means drying the organic phase with $MgSO_4$ (s) or Na_2SO_4 (s), filtering and evaporation of the solvent *in vacuo* at ~35 °C. CH_2Cl_2 was distilled over calcium hydride and collected onto 4 Å predried MS. Thin-Layer Chromatography (TLC) was carried out on 0.25 mm precoated silica-gel plates (Merck silica-gel 60 F_{254}); detected with UV-abs (254 nm) and/or by charring with 8% sulfuric acid or AMC (ammonium molybdate (10 g) and cerium sulfate (2 g) dissolved in 10% H_2SO_4 (200 mL)) followed by heating to ~250 °C. FC means Flash Column chromatography using silica gel (Amicon, (0.040–0.063 mm)). ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 400 or Bruker 500 MHz instruments at 25 °C unless otherwise stated. Chemical shifts are given in ppm relative to solvent peaks (δ 77.17 for ^{13}C and δ 7.26 for ^{1}H) in $CDCl_3$. Optical rotation was measured using a Perkin-Elmer 343 polarimeter.

Experimental procedures

Ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (2). Ethyl 4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (**1**) was dissolved in pyridine (30 mL), cooled to 0 °C and BzCl (1.18 mL, 10.14 mmol) was added dropwise. After 14 h, the solution was diluted with CH_2Cl_2 (60 mL) and washed with 1 M HCl (aq.) (2 × 100 mL). Normal work-up, followed by FC (toluene → toluene/EtOAc 19:1) yielded **2** (3.02 g, 5.64 mmol, 89%) as a colourless oil. R_f 0.66 (toluene/EtOAc 9:1). $[\alpha]_D$ +25 (*c* 1.0, $CHCl_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 15.1, 25.8, 55.4, 64.8, 68.8, 71.9, 72.3, 74.0, 79.0, 83.7, 101.8, 113.9, 126.3 (2C), 128.3 (2C), 128.6 (2C), 129.1 (2C), 129.5 (2C), 129.9, 130.0, 130.1 (2C), 133.4, 137.6, 159.4, 165.9. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.31 (t, 3H, J = 7.2 Hz), 2.66 (m, 2H), 3.77 (s, 3H), 3.92 (m, 1H), 4.07 (dd, 1H, J = 3.2, 9.6 Hz), 4.21 (dd, 1H, J = 9.6, 9.6 Hz), 4.26–4.32 (m, 2H), 4.61 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.67 (benzylic d, 1H, J_{gem} = 12.0 Hz), 5.41 (d, 1H, J = 1.2 Hz), 5.65 (dd, 1H, J = 1.2, 3.2 Hz), 5.68 (s, 1H), 6.80 (d, 2H, J = 8.4 Hz, Ar-H), 7.24 (d, 2H, J = 8.4 Hz, Ar-H), 7.37–7.42 (m, 2H, Ar-H), 7.48 (dd, 2H, J = 7.6, 7.6 Hz, Ar-H), 7.53 (dd, 2H, J = 1.6, 7.6 Hz, Ar-H), 7.60 (dd, 2H, J = 7.6, 7.6 Hz, Ar-H), 8.12 (d, 2H, J = 7.6 Hz, Ar-H). HRMS calcd for $C_{30}H_{32}O_7S$: $[M + Na]^+$ 559.1761; Found 559.1759.

Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-α-D-mannopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (4). Methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**3**, 0.384 g, 1.76 mmol) and **2** (0.672 g, 1.25 mmol) were dissolved in dry CH_2Cl_2 (20 mL), 4 Å MS were added and the mixture was stirred under an Ar-atmosphere. After 30 min, the mixture was cooled to −30 °C and NIS (0.420 g, 1.87 mmol) and AgOTf (cat.) were added. After 20 min, the reaction was quenched by adding Et_3N (0.600 mL). The mixture was filtered through a pad of Celite, washed with 10% $Na_2S_2O_3$ (aq., 60 mL) and subjected to normal work-up. FC (toluene/EtOAc 9:1) gave **4** (0.796 g, 1.15 mmol, 92%) as a white foam. R_f 0.72 (toluene/EtOAc 3:1). $[\alpha]_D$ −8 (*c* 1.0, $CHCl_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 17.5, 26.5, 28.2, 54.9, 55.3, 64.0, 64.7, 68.9, 70.6, 71.8, 73.6, 76.1, 76.8, 78.8, 81.2, 98.0 (J_{CH} = 171 Hz), 99.4 (J_{CH} = 173 Hz), 101.7, 109.3, 113.8, 126.3 (2C), 128.2 (2C), 128.5 (2C), 129.0 (2C), 129.3 (2C), 129.8, 130.0 (2C), 130.2, 133.4, 137.7, 159.3, 166.0. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.36 (d, 3H, J = 6.4 Hz), 1.37 (s, 3H), 1.54 (s, 3H), 3.40 (s, 3H), 3.42 (m, 1H), 3.72 (dd, 1H, J = 10.0, 6.0 Hz), 3.78 (s, 3H), 3.90 (m, 1H), 4.11–4.15 (m, 3H), 4.22–4.24 (m, 2H), 4.34 (dd, 1H, J = 3.2, 9.6 Hz), 4.67 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.71 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.88 (s, 1H), 5.03 (d, 1H, J = 1.6 Hz), 5.55 (dd, 1H, J = 1.6, 3.2 Hz), 5.72 (s, 1H), 6.82 (d, 2H, J = 8.4 Hz, Ar-H), 7.28 (d, 2H, J = 8.4 Hz, Ar-H), 7.38–7.44 (m, 3H, Ar-H), 7.48 (dd, 2H, J = 7.6, 7.6 Hz, Ar-H), 7.57–7.62 (m, 3H, Ar-H), 8.14 (d, 2H, J = 7.6 Hz, Ar-H). HRMS calcd for $C_{38}H_{44}O_{12}$: $[M + Na]^+$ 715.2730; Found 715.2748.

Methyl 4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-α-D-mannopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (5). Compound **4** (1.67 g, 2.41 mmol) was dissolved in MeOH/CH_2Cl_2 (12 mL, 2:1). The solution was cooled to 0 °C and NaOMe (cat.) was added. After stirring 6 h at room temperature, the mixture was neutralized with DOWEX-H^+ ion exchange resins, filtered and concentrated. FC (toluene/EtOAc 6:1 → toluene/EtOAc 3:1) afforded **5** (1.27 g, 2.16 mmol, 90%) as a white foam. R_f 0.34 (toluene/EtOAc 3:1). $[\alpha]_D$ +43 (*c* 1.0, $CHCl_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 17.6, 26.6, 28.3, 55.0, 55.4, 63.4, 64.8, 69.0, 70.2, 72.9, 75.3, 76.1, 76.9, 79.0, 80.8, 98.1, 100.8, 101.7, 109.3, 114.0, 126.3 (2C), 128.3 (2C), 129.0 (2C), 129.2, 129.6 (2C), 130.3, 137.8, 159.5. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.26 (d, 3H, J = 6.4 Hz), 1.33 (s, 3H), 1.51 (s, 3H), 2.72 (bs, 1H, -OH), 3.36 (s, 3H), 3.37 (dd, 1H, J = 7.2, 10.0 Hz), 3.64 (dd, 1H, J = 6.4, 10.0 Hz), 3.78–3.83 (m, 4H), 3.88 (dd, 1H, J = 3.6, 9.2 Hz), 4.02 (d, 1H, J = 3.2 Hz), 4.06–4.10 (m, 4H), 4.28 (dd, 1H, J = 3.6, 9.2 Hz), 4.65 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.79 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.84 (s, 1H), 4.93 (d, 1H, J = 1.2 Hz), 5.61 (s, 1H), 6.86 (d, 2H, J = 8.4 Hz, Ar-H), 7.16–7.18 (m, 1H, Ar-H), 7.25–7.29 (m, 3H, Ar-H), 7.35–7.40 (m, 2H, Ar-H), 7.50–7.53 (m, 2H). HRMS calcd for $C_{31}H_{40}O_{11}$: $[M + Na]^+$ 611.2468; Found 611.2495.

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→2)-4,6-*O*-benzylidene-3-*O*-*p*-methoxybenzyl-α-D-mannopyranosyl-(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (7). Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**6**) (0.646 g, 1.10 mmol) and **5** (0.488 g, 0.829 mmol) were dissolved in dry Et_2O (15 mL), 4 Å MS were added and the mixture was stirred under an Ar-atmosphere. After 20 min, the mixture was cooled to −30 °C and NIS (0.270 g, 1.20 mmol) and AgOTf (cat.) were added. After 30 min, the reaction was quenched by adding Et_3N (0.50 mL) and the resulting mixture filtered through a pad of Celite, diluted with CH_2Cl_2 (60 mL), washed with 10% $Na_2S_2O_3$ (aq., 80 mL) and subjected to normal work-up. FC (toluene/EtOAc 19:1 → toluene/EtOAc 9:1) produced **7** (0.783 g, 0.705 mmol, 85%) as a white foam. R_f 0.64 (toluene/EtOAc 3:1). $[\alpha]_D$ +44 (*c* 1.0, CH_2Cl_2). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 17.3, 26.5, 28.2, 54.9, 55.3, 64.5, 64.8, 68.8, 69.2, 70.0, 71.4, 73.3, 73.6, 73.7, 73.9, 74.9, 75.2, 76.1, 76.4, 76.7, 76.9, 78.3, 79.3, 80.4, 97.7 (J_{CH} = 172 Hz), 98.0 (J_{CH} = 168 Hz), 101.0 (J_{CH} = 173 Hz), 101.3, 109.2, 113.8, 126.2, 127.3, 127.4, 127.5 (2C), 127.7, 127.8, 127.9

(2C), 127.9 (2C), 128.1 (2C), 128.3 (2C), 128.3 (2C), 128.4 (2C), 128.5 (2C), 128.5 (2C), 128.9, 129.5 (2C), 130.8, 138.0 (2C), 138.7, 139.1, 139.2, 159.2. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.23 (d, 3H, J = 6.4 Hz), 1.37 (s, 3H), 1.55 (s, 3H), 3.39 (s, 3H), 3.40 (m, 1H), 3.49–3.56 (m, 2H), 3.62 (dd, 1H, J = 6.4, 10.0 Hz), 3.80 (s, 3H), 3.83 (t, 1H, J = 10.0 Hz), 3.97–4.18 (m, 8H), 4.23–4.27 (m, 2H), 4.32 (dd, 1H, J = 10.0, 10.0 Hz), 4.40 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.45 (benzylic d, 1H, J_{gem} = 11.2 Hz), 4.50 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.60 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.68 (benzylic d, 1H, J_{gem} = 11.2 Hz), 4.77 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.81 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.87 (benzylic d, 1H, J_{gem} = 11.2 Hz), 4.88 (s, 1H), 4.93 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.94 (d, 1H, J = 1.2 Hz), 4.98 (benzylic d, 1H, J_{gem} = 11.6 Hz), 5.49 (s, 1H), 5.70 (d, 1H, J = 3.6 Hz), 6.84 (dd, 2H, J = 8.4, 2.0 Hz, Ar-H), 7.18–7.20 (m, 1H, Ar-H), 7.18–7.47 (m, 25H, Ar-H), 7.56 (dd, 2H, J = 8.4, 2.0 Hz, Ar-H). HRMS calcd for $C_{65}H_{74}O_{16}$: [M + Na]$^+$ 1133.4875; Found 1133.4858.

Ethyl 4-*O*-benzoyl-2,3-*O*-isopropylidene-6-*O*-tosyl-1-thio-α-D-mannopyranoside (9). To a cooled (0 °C) solution of ethyl 2,3-*O*-isopropylidene-1-thio-α-D-mannopyranoside[22] (**8**, 0.473 g, 1.79 mmol) in dry CH_2Cl_2 (10 mL) were added Et_3N (0.420 mL, 3.04 mmol) and TsCl (0.443 g, 2.32 mmol). After stirring for 24 h, the mixture was washed with 1 M HCl (aq., 20 mL), sat. $NaHCO_3$ (aq., 20 mL), H_2O (20 mL) and subjected to normal work-up. The crude product was dissolved in pyridine (4 mL) and BzCl (0.416 mL, 3.58 mmol) was added. After 3 h, the solvents were evaporated and co-evaporated with toluene (3 × 10 mL). FC (toluene/EtOAc 19:1 → toluene/EtOAc 9:1) yielded **9** (0.848 g, 1.62 mmol, 91%) as a colourless oil. R_f 0.55 (toluene/EtOAc 9:1). $[\alpha]_D$ +193 (*c* 2.0, $CHCl_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 14.4, 21.7, 24.2, 26.5, 27.8, 67.0, 68.6, 70.6, 75.6, 76.5, 79.3, 110.3, 128.0 (2C), 128.5 (2C), 129.9 (2C), 130.0 (2C), 130.7, 132.6, 133.6, 144.9, 165.6. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.30 (t, 3H, J = 7.2 Hz), 2.35 (s, 3H), 2.54 (dq, J = 7.2, 1.6 Hz), 2.69 (dq, J = 7.2, 1.6 Hz), 4.11–4.08 (m, 2H), 4.21 (dd, 1H, J = 0.8, 5.2 Hz), 4.32 (dd, 1H, J = 5.2, 7.6 Hz), 4.44 (m, 1H), 5.15 (dd, 1H, J = 7.6, 10.4 Hz), 5.58 (s, 1H), 7.19 (dd, 2H, J = 0.8, 8.4 Hz), 7.42–7.69 (m, 6H), 7.99 (dd, 2H, J = 0.8, 8.4 Hz), 8.16 (dd, 2H, J = 1.2, 8.4 Hz). HRMS calcd for $C_{25}H_{31}O_8S_2$: [M + H]$^+$ 523.1455; Found 523.1442.

Ethyl 4-*O*-benzoyl-6-deoxy-1-thio-α-D-mannopyranoside (10). Derivative **9** (0.755 g, 1.45 mmol) and $NaBH_4$ (2.97 g, 11.94 mmol) were dissolved in DMF (10 mL), and the mixture was heated to 50 °C. After 30 h, the mixture was diluted with toluene (30 mL), washed with H_2O (40 mL) and subjected to normal work-up. FC (toluene → toluene/EtOAc 19:1 → toluene/EtOAc 9:1) afforded the 6-deoxy derivative, which was dissolved in acetic acid (80% aq., 25 mL) and the solution stirred at 60 °C. After 3 h, the solution was cooled to r.t, and the solvent evaporated and co-evaporated with toluene (3 × 20 mL). FC (toluene/EtOAc 3:1 → toluene/EtOAc 1:1) gave **10** (0.312 g, 0.998 mmol, 69%). R_f 0.71 (toluene/EtOAc 1:3). $[\alpha]_D$ +225 (*c* 1.0, $CHCl_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 15.0, 17.6, 25.3, 66.4, 71.0, 72.6, 76.6, 83.9, 128.6 (2C), 129.5, 130.0 (2C), 133.6, 167.6. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.28 (d, 3H, J = 6.4 Hz), 1.31 (t, 3H, J = 7.6 Hz), 2.64 (m, 2H), 3.99 (dd, 1H, J = 3.2, 9.6 Hz), 4.10 (dd, 1H, J = 1.2, 3.2 Hz), 3.34 (m, 1H), 5.10 (dd, 1H, J = 9.6, 9.6 Hz), 5. 35 (s, 1H), 7.42–7.60 (m, 3H), 8.03 (dd, 1H, J = 1.6, 8.4 Hz). HRMS calcd for $C_{15}H_{20}O_5S$: [M + H]$^+$ 313.1110; Found 313.1098.

Ethyl 2,4-di-*O*-benzoyl-6-deoxy-1-thio-α-D-mannopyranoside (11). Compound **10** (0.130 g, 0.416 mmol) was dissolved in dry CH_2Cl_2 (5 mL), and trimethyl orthobenzoate (0.143 mL, 0.832 mmol) and CSA (0.010 g, 0.04 mmol) were added. After 60 min, the solvent was evaporated and the residue dissolved in acetic acid (80% aq., 5 mL). After stirring for 45 min, toluene (10 mL) was added and the solvents were evaporated followed by co-evaporation with toluene (2 × 10 mL). FC (toluene/EtOAc 19:1 → toluene/EtOAc 9:1) afforded **11** (0.148 g, 0.355 mmol, 85%). R_f 0.53 (toluene/EtOAc 6:1). $[\alpha]_D$ +29 (*c* 1.0, $CHCl_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 15.1, 17.7, 25.9, 67.0, 69.8, 75.3, 75.9, 82.3, 128.6 (2C), 128,7 (2C), 129.5, 129.6, 130.0 (2C), 130.0 (2C), 133.6 (2C), 166.1, 167.2. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.33 (d, 3H, J = 6.8 Hz), 1.34 (t, 3H, J = 7.2 Hz), 2.58 (bs, 1H), 2.69 (m, 2H), 4.27 (dd, 1H, J = 3.2, 10.0 Hz), 4.42 (m, 1H), 5.30 (dd, 1H, J = 10.0, 10.0 Hz), 5.45 (s, 1H), 5.49 (dd, 1H, J = 1.2, 3.2 Hz), 7.44–7.61 (m, 6H), 8.08 (d, 2H, J = 8.0 Hz), 8.12 (d, 2H, J = 8.0 Hz). HRMS calcd For $C_{22}H_{24}O_6S$: [M + H]$^+$ 417.1372; Found 417.1354.

Ethyl 2,4-di-*O*-benzoyl-3,6-dideoxy-1-thio-α-D-*arabino*-hexopyranoside (12). 1,1′-Thiocarbonyldiimidazole (0.042 g, 0.240 mmol) and **11** (0.050 g, 0.120 mmol) were dissolved in dry toluene (5 mL) and the mixture was refluxed for 2 h. After cooling to room temperature, the solvent was evaporated and FC (toluene/EtOAc 6:1) gave the imidazoylthiocarbonyl intermediate. The intermediate and Bu_3SnH (0.069 mL, 0.255 mmol) were dissolved in dry benzene (6 mL), AIBN (0.003 g, 0.020 mmol) was added and the mixture was heated to reflux. After 20 min, the solution was cooled to room temperature and concentrated. FC (toluene → toluene/EtOAc 19:1) yielded **12** (0.036 g, 0.090 mmol, 75%). R_f 0.69 (toluene/EtOAc 9:1). $[\alpha]_D$ +58 (*c* 1.0, $CHCl_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 15.3, 18.0, 25.5, 30.9, 67.3, 70.9, 72.3, 82.4, 128.6 (4C), 129.8 (2C), 129.9, 130.0 (2C), 130.1, 133.4, 133.5, 165.8, 165.8. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.32 (d, 3H, J = 6.0 Hz), 1.35 (t, 3H, J = 7.2 Hz), 2.17 (ddd, 1H, J = 2.8, 11.2, 14.0 Hz), 2.47 (ddd, 1H, J = 4.8, 4.8, 14.0 Hz), 2.71 (m, 2H), 4.42 (m, 1H), 5.24 (ddd, 1H, J = 4.8, 9.6, 11.2 Hz), 5.34–5.36 (m, 2H), 7.44–7.61 (m, 6H), 8.04–8.13 (m, 4H). HRMS calcd for $C_{22}H_{25}O_5S$: [M + H]$^+$ 401.1417; Found 401.1416.

Methyl α-D-galactopyranosyl-(1→2)-α-D-mannopyranosyl-(1→4)-α-L-rhamnopyranoside (13). A solution of **7** (0.038 g, 0.034 mmol) in acetic acid (70% aq., 5 mL) was stirred at 80 °C for 6 h. The solvent was evaporated followed by co-evaporation with toluene (2 × 10 mL). The residue was dissolved in EtOH/H_2O (3 mL, 2:1) and a catalytic amount of Pd/C (10%) was added. The mixture was stirred under an H_2-atmosphere (100 psi) for 36 h, then filtered through Celite and evaporated. FC (EtOAc/MeOH/H_2O 7:2:1) gave **13** (0.013 g, 0.026 mmol, 76%). R_f 0.22 (EtOAc/MeOH/H_2O 7:2:1). $[\alpha]_D$ +54 (*c* 0.7, H_2O). ^{13}C-NMR and ^{1}H-NMR : See Table S1. HRMS calcd For $C_{19}H_{34}O_{15}$: [M + Na]$^+$ 525.1790; Found 525.1783.

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-*p*-methoxybenzyl-α-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranoside (14). A solution of **7** (0.341 g, 0.307 mmol) in acetic acid (60% aq.) (6 mL) was stirred at 70 °C. After 8 h, the solution was cooled to room

temperature, and the solvent evaporated and co-evaporated with toluene (3 × 10 mL). The crude tetraol was dissolved in pyridine (5 mL) and cooled to 0 °C. Acetic anhydride (5 mL) was added dropwise to the solution, which was stirred for 12 h. The solvent was evaporated and co-evaporated with toluene (3 × 10 mL). FC (toluene/EtOAc 6:1 → toluene/EtOAc 3:1) produced **14** (0.287 g, 0.249 mmol, 81%) as a colourless oil. R_f 0.32 (toluene/EtOAc 3:1). $[\alpha]_D$ +11 (*c* 1.0, $CHCl_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 18.3, 20.8, 21.0, 21.2 (2C), 55.3, 55.5, 62.9, 67.6, 68.1, 69.5, 70.1, 70.3, 70.4, 70.6, 71.8, 72.1, 72.4, 73.6 (2C), 74.9, 75.4, 76.0, 76.3, 77.4, 78.4, 98.1, 98.6, 100.3, 114.1, 127.4, 127.6, 127.6 (2C), 127.8, 127.9 (2C), 128.0, 128.2 (2C), 128.3 (2C), 128.4 (2C), 128.5 (2C), 128.6 (2C), 128.6, 128.6, (2C), 129.6 (2C), 130.1, 138.1, 138.8, 139.0, 139.2, 159.5, 169.5, 170.2 (2C), 171.1. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.22 (d, 3H, *J* = 5.6 Hz), 1.98 (s, 3H), 1.99 (s,3H), 2.03 (s, 3H), 2.04 (s, 3H), 3.37 (s, 3H), 3.43 (dd, 1H, *J* = 6.4, 9.2 Hz), 3.47 (dd, 1H, *J* = 6.4, 9.2 Hz), 3.66–3.70 (m, 2H), 3.78 (s, 3H), 3.80 (m, 1H), 3.90–4.06 (m, 6H), 4.12 (dd, 1H, *J* = 2.4, 12.0 Hz), 4.17 (dd, 1H, *J* = 4.0, 12.0 Hz), 4.40 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.44–4.56 (m, 6H), 4.70 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.80 (benzylic d, 1H, J_{gem} = 12.5 Hz), 4.87 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.92 (benzylic d, 1H, J_{gem} = 11.6 Hz), 5.04 (d, 1H, *J* = 1.6 Hz), 5.16–5.23 (m, 2H), 5.39 (d, 1H, *J* = 3.6 Hz), 5.52 (dd, 1H, *J* = 9.6, 9.6 Hz), 6.81 (d, 2H, *J* = 8.8 Hz), 7.16 (d, 2H, *J* = 8.4 Hz), 7.21–7.36 (m, 21H, Ar-H). HRMS calcd for $C_{63}H_{74}O_{20}$: $[M + Na]^+$ 1173.4671; Found 1173.4708.

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→2)-4,6-di-*O*-acetyl-α-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranoside (15). Compound **14** (0.230 g, 0.200 mmol) was dissolved in CH_2Cl_2/H_2O (14:1, 15 mL) and DDQ (0.050 g, 0.225 mmol) was added. After 8 h, the solution was diluted with CH_2Cl_2 (10 mL), washed with 10% $Na_2S_2O_3$ (aq.) (30 mL) and subjected to normal work-up. FC (toluene/EtOAc 3:1) gave **15** (0.130 g, 0.126 mmol, 63%). R_f 0.24 (toluene/EtOAc 3:1). $[\alpha]_D$ +1 (*c* 2.0, $CHCl_3$). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 18.3, 20.8, 20.9, 21.0, 21.1, 55.3, 63.0, 67.4, 68.8, 68.9, 69.4, 69.6, 70.2, 70.4, 70.5, 73.0, 73.4, 74.4, 74.9, 74.9, 76.2, 77.6, 79.3, 81.3, 98.5, 99.8, 102.2, 127.7 (2C), 127.8, 127.9 (3C), 128.0, 128.1, 128.2 (2C), 128.3 (2C), 128.4 (2C), 128.6 (2C), 128.7 (4C), 137.9 (2C), 138.4, 138.6, 170.0, 170.1, 170.3, 170.8. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.27 (d, 3H, *J* = 6.5 Hz), 1.99 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.09 (s, 3H), 3.36 (s, 3H), 3.43 (d, 2H, *J* = 6.4 Hz), 3.58 (d, 1H, *J* = 12.0 Hz), 3.65–3.81 (m, 4H), 3.89–3.94 (m, 2H), 3.98 (dd, 1H, *J* = 2.8, 10.5 Hz), 4.03–4.09 (m, 3H), 4.18 (dd, 1H, *J* = 4.4, 12.4 Hz), 4.39 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.47 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.53 (benzylic d, 1H, J_{gem} = 11.2 Hz), 4.56 (d, 1H, *J* = 1.6 Hz), 4.68 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.76 (benzylic d, 1H, J_{gem} = 11.6 Hz), 4.82 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.85 (d, 1H, *J* = 3.6 Hz), 4.91 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.93 (benzylic d, 1H, J_{gem} = 11.2 Hz), 5.11 (dd, 1H, *J* = 10.0, 10.0 Hz), 5.17 (dd, 1H, *J* = 3.6, 9.5 Hz), 5.21 (dd, 1H, *J* = 1.6, 3.6 Hz), 7.24–7.41 (m, 20H, Ar-H). HRMS calcd for $C_{55}H_{66}O_{19}$: $[M + Na]^+$ 1053.4096; Found 1053.4049.

Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→2)-[2,4-di-*O*-benzoyl-3,6-dideoxy-α-D-*arabino*-hexopyranosyl-(1→3)]-4,6-di-*O*-acetyl-α-D-mannopyranosyl-(1→4)-2,3-di-*O*-acetyl-α-L-rhamnopyranoside (16). To a solution of **12** (0.029 g, 0.072 mmol), **15** (0.035 g, 0.034 mmol) and DTBMP (0.017 g 0.085 mmol) in dry Et_2O (1.5 mL), 4 Å MS were added, and the mixture was stirred under an Ar-atmosphere. After 30 min, MeOTf (0.023 mL, 0.204 mmol) was added. After 30 h, the reaction was quenched by adding Et_3N (75 μL), an d the mixture diluted with CH_2Cl_2 (5 mL), filtered through a pad of Celite and concentrated. FC (toluene/EtOAc 6:1 → 3:1) afforded **16** (0.029 g, 0.021 mmol, 62%) as an oil. R_f 0.47 (toluene/EtOAc 3:1). $[\alpha]_D$ +18 (*c* 1.0, $CHCl_3$). ^{13}C-NMR (125 MHz, 30 °C, $CDCl_3$): δ = 17.9, 18.1, 20.6, 20.7, 20.9, 20.9, 29.3, 55.0, 62.7, 66.9 (2C), 67.4, 67.8, 69.0, 70.2, 70.3 (3C), 72.2, 73.1, 73.4, 74.8 (2C), 75.2, 75.6, 76.0, 77.0, 78.5, 79.1, 98.3 (J_{CH} = 173 Hz), 98.6 (J_{CH} = 170 Hz), 99.1 (J_{CH} = 171 Hz), 100.4 (J_{CH} = 173 Hz), 127.4, 127.5 (2C), 127.6 (2C), 127.6, 127.7 (2C), 127.9, 128.2–128.5, 129.6 (2C), 129.8 (2C), 133.2, 133.3, 137.7, 138.6 (2C), 138.6, 165.4, 165.6, 169.9, 169.9, 170.0, 170.8. ^{1}H-NMR (500 MHz, 30 °C, $CDCl_3$): δ = 1.25 (d, 3H, *J* = 6.5 Hz), 1.32 (d, 3H, *J* = 6.5 Hz), 1.89 (m, 1H), 1.98 (s, 3H), 1.99 (s, 3H), 2.02 (s, 3H), 2.12 (ddd, 1H, J = 4.0, 4.0, 13.5 Hz), 2.23 (s, 3H), 3.35 (s, 3H), 3.46 (dd, 1H, *J* = 6.0, 9.0 Hz), 3.50 (dd, 1H, *J* = 6.0, 9.5 Hz), 3.66 (dd, 1H, *J* = 9.0, 9.0 Hz), 3.77 (dd, 1H, *J* = 6.0, 10.0 Hz), 3.93 (ddd, 1H, *J* = 3.0, 3.5, 9.5 Hz), 3.96–3.98 (m, 2H), 4.03 (dd, 1H, *J* = 2.5, 9.5 Hz), 4.06 (dd, 1H, *J* = 9.5, 9.5 Hz), 4.13–4.20 (m, 5H), 4.42 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.50 (benzylic d, 1H, J_{gem} = 12.5 Hz), 4.54 (benzylic d, 1H, J_{gem} = 11.5 Hz), 4.56 (d, 1H, *J* = 1.5 Hz), 4.74 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.79 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.82 (benzylic d, 1H, J_{gem} = 12.0 Hz), 4.91 (benzylic d, 1H, J_{gem} = 12.5 Hz), 4.94–5.00 (m, 3H), 5.13 (dd, 1H, *J* = 4.0, 10.5 Hz), 5.16 (d, 1H, *J* = 1.5 Hz), 5.20 (dd, 1H, *J* = 1.5, 4.0 Hz), 5.22 (dd, 1H, *J* = 4.0, 10.0 Hz), 5.39 (d, 1H, *J* = 3.5 Hz), 5.61 (dd, 1H, *J* = 10.0, 10.0 Hz), 7.18–7.59 (m, 26H, Ar-H), 7.92 (dd, 2H, *J* = 1.0, 8.0 Hz), 8.07 (dd, 2H, *J* = 1.0, 8.0 Hz). HRMS calcd for $C_{75}H_{84}O_{24}$: $[M + Na]^+$ 1391.5250; Found 1391.5183.

Methyl-α-D-galactopyranosyl-(1→2)-[3,6-dideoxy-α-D-*arabino*-hexopyranosyl-(1→3)]-α-D-mannopyranosyl-(1→4)-α-L-rhamnopyranoside (17). A solution of **16** (0.041 g, 0.030 mmol) in EtOAc (0.8 mL) was applied to an H-cube. The solution was diluted with additional EtOAc (total volume 15 mL) after injection and the solution was cycled in the H-cube (40 °C, 60 Bar, Flow 0.3 mL/min) over a Pd/C (10%) cartridge. After cycling for 45 min, the system was rinsed an additional 15 min with EtOAc and the solvent was evaporated from the resulting solution. FC (EtOAc → EtOAc/MeOH 19:1 → 9:1) afforded the tetraol intermediate, which was dissolved in MeOH (1.5 mL) and NaOMe (cat.) was added. After stirring 26 h at 25 °C, the mixture was neutralized with DOWEX-H^+ ion exchange resins, filtered and concentrated. FC (EtOAc/MeOH/H_2O 7:2:1) gave **17** (0.012 g, 0.019 mmol, 63%). R_f 0.24 (EtOAc/MeOH/H_2O 7:2:1). $[\alpha]_D$ +66 (*c* 1.0, H_2O). ^{13}C-NMR and ^{1}H-NMR: See Table S2. HRMS calcd for $C_{25}H_{44}O_{18}$: $[M + Na]^+$ 655.2425; Found 655.2449.

Molecular simulations

Molecular dynamics (MD) simulations used NAMD[23,24] (parallel version, 2.6b1 for the tetrasaccharide and 2.6 for the trisaccharide) employing a CHARMM22 type of force field, namely PARM22/SU01 which is a recently modified force field for carbohydrates. Pdb and psf files of the oligosaccharides were created using VEGA ZZ (release 2.0.8) and assigned CHARMM

partial charges. Initial conditions were prepared by placing the oligosaccharides in a previously equilibrated cubic water box following minimization and heating. This procedure resulted in systems with the oligosaccharide and either 1121 or 899 TIP3P water molecules for the tetra- and trisaccharide respectively. The MD simulations were carried out with multiple-time-stepping and 2 fs, 2 fs, and 6 fs were used as the inner, middle and outer time steps. Following a 1 ns equilibration of the system the production run was carried out for a 30 ns duration period performed in the NPT ensemble (P = 1 atm, T = 300 K) with a cutoff distance for non-bond interactions set at 12 Å and periodic boundary conditions giving a cubic box of approximately 32 Å or 30 Å to the side for the tetra- and trisaccharide, respectively. The smooth particle-mesh Ewald (SPME) method was used to calculate the full electrostatic interactions. The temperature and pressure were kept constant using a Langevin thermostat and a Langevin barostat, respectively. All bonds to hydrogen atoms were kept rigid. Data were saved every 500 time steps for analysis. Trajectory analyses were carried out with VEGA ZZ[25] and Excel.

NMR spectroscopy

Prior to NMR analysis tri- and tetrasaccharides **13** and **17** were purified using SEC chromatography. The tri- and tetrasaccharides, 7 and 10 mg, respectively, were lyophilized and subsequently dissolved in D_2O (0.5 mL). The NMR experiments for ^1H and ^{13}C resonance assignments of the tri- and tetrasaccharides were carried out at 298 K on a Bruker Avance III 700 MHz spectrometer equipped with a 5 mm PFG triple-resonance CryoProbe. Standard 1D- and 2D-experiments and the CASPER software were used to assign chemical shifts using internal TSP (0 ppm) as ^1H reference and external dioxane in D_2O (67.4 ppm) as ^{13}C reference. NMR spin simulations were performed using the PERCH NMR software.

Acknowledgements

This work was supported by grants from the Swedish Research Council (VR), The Knut and Alice Wallenberg Foundation, and computing resources at the Center for Parallel Computers (PDC), Stockholm, Sweden.

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