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Synthesis of *C*-glycosides related to *glycero*-β-D-*manno*-heptoses

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Abstract—Reducing heptopyranoses of the D-*glycero*-D-*manno*- and L-*glycero*-D-*manno*-configuration were converted in good overall yields into β-anomeric *C*-glycosides via condensation with pentane-2,4-dione, followed by sodium methoxide-induced enrichment of the β-anomeric products and subsequent *O*-acetylation. The resulting 2-oxo-propyl glycosides were further transformed into separable ~1:1 diastereomeric mixtures of (*S*)-and (*R*)-2-hydroxy derivatives. The configuration of the additional stereogenic centre was assigned on the basis of NMR data obtained from the corresponding Dale–Mosher esters. Finally, phosphorylation of the D-*glycero*-D-*manno*-heptose analogues using the phosphoramidite procedure and deprotection furnished the (*S*)- and (*R*)-2-phosphate derivatives in good yields. The compounds serve as substrate analogues for enzymes involved in bacterial ADP heptose biosynthesis and glycosyl transfer reactions.
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1. Introduction

Lipopolysaccharides (LPS) are structurally complex amphipathic and microheterogeneous glycolipids, which are essential components of the outer membrane of Gram-negative bacteria.[1] In many cases, lipopolysaccharides display a tripartite structure, comprising lipid A with a bisphosphorylated and acylated glucosamine disaccharide backbone, the core region containing 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo), heptoses of the L-*glycero*-D-*manno*- and D-*glycero*-D-*manno*- configuration and a regular polysaccharide chain harbouring the O-antigen.[2] Since the higher-carbon sugars do not occur in mammalian systems, the inhibition of the biosynthesis of the nucleotide-activated sugars and the respective glycosyl transferase reactions is regarded as an attractive target for the design of novel antimicrobial agents.

The biosynthesis of the nucleotide-activated heptoses of the D-*glycero*- and L-*glycero*-D-*manno*- configuration has recently been fully elucidated.[3,4] Heptoses transferred to the inner core of *Enterobacteriaceae*—but also onto hitherto structurally not characterized bacterial adhesins[5]—are activated as ADP-linked sugars.[6] An epimerase reaction inverts the stereochemistry at carbon 6 of the precursor ADP-D-*glycero*-β-D-*manno*-heptose to provide ADP-L-*glycero*-β-D-*manno*-heptose—the common substrate for inner core enterobacterial heptosyl transferases (Fig. 1).[7,8] Furthermore, crystal structures of the ADP-L-*glycero*-β-D-*manno*-heptose 6-epimerase and of ADP-L-*glycero*-β-D-*manno*-heptose transferase II from *Escherichia coli* have recently been reported.[9,10] So far, structural data of these enzymes complexed to appropriate substrates have not been obtained. ADP heptopyranoses of the β-anomeric configuration are unstable due to neighbouring group participation of the axial 2-OH group resulting in the formation of heptose 1,2-cyclo-phosphate and release of AMP.[11] To overcome the inherent hydrolytic lability of the ADP heptoses we set out to synthesize stable *C*-glycosides in the β-anomeric configuration to be used as versatile building blocks for the synthesis of substrate analogues.

2. Results and discussion

The chemical synthesis of *C*-glycosides has been developed considerably over the past few years.[12] The construction of *C*-glycosides in the β-*manno*-configured cases, however, has met with difficulties, similar to their *O*-glycosidic counterparts. By contrast, the direct assembly of *C*-glycosides by aqueous Knoevenagel-type condensation of unprotected sugars with β-dicarbonyl compounds has been reported to proceed with

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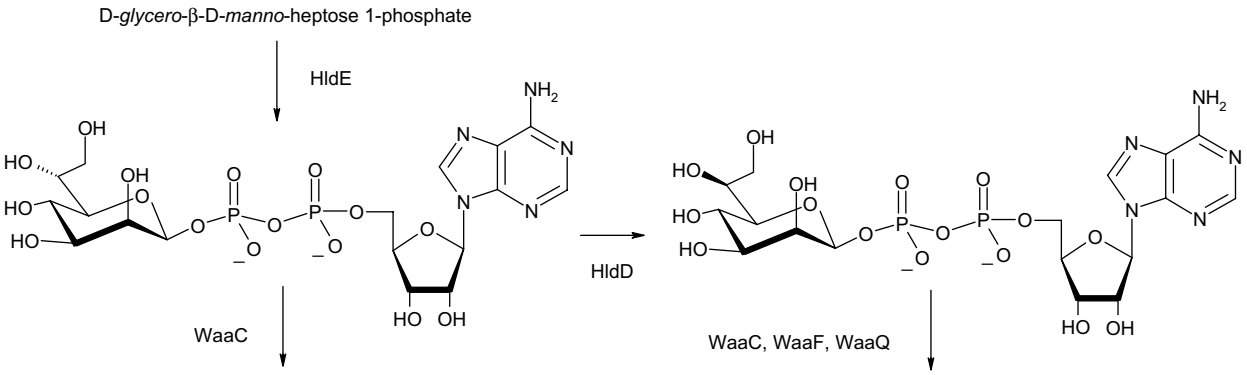
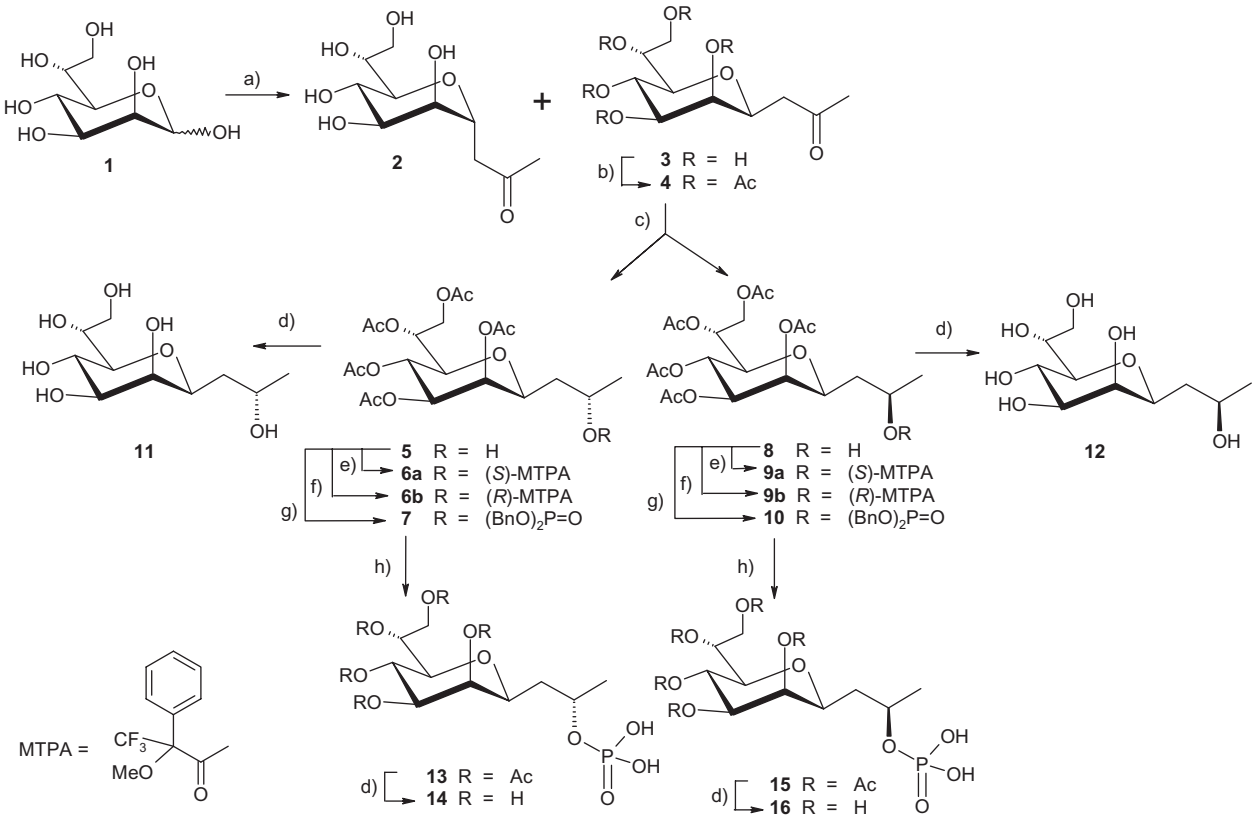


Figure 1. Structure of ADP heptoses and enzymes involved in heptose activation and heptosyl transfer reactions. HldE: Heptose 1-phosphate adenylyltransferase; HldD: ADP-D-*glycero*-β-D-*manno*-heptose epimerase; WaaC, WaaF, WaaQ: inner core ADP-heptosyl transferases.

pronounced β-anomeric stereoselectivity.[13–15] Furthermore, subsequent alkali-induced anomeric epimerization gave β-2′-carbonylalkyl glycosides in high yield. These compounds may now be exploited for further transformation and for coupling reactions to mono- or diphosphonucleoside derivatives. Reaction of D-*glycero*-D-*manno*-heptose[16] with an aqueous solution of pentane-2,4-dione gave an anomeric mixture of the α- and β-configured *C*-glycosidic ketones **2** and **3** in a 2:1 ratio and two other minor components. The assignments were based on the ^{1}H NMR chemical shift of H-8, which was shifted upfield for the β-anomeric heptopyranose derivative (3.36 ppm) in comparison to the α-anomer (3.54 ppm). In order to enrich the β-isomer **3**, the mixture was treated with methanolic sodium methoxide, which also led to a conversion of the higher-running by-products into the target compound **3**. Following acetylation with acetic anhydride in pyridine to facilitate chromatographic separation, the penta-*O*-acetyl derivative **4** was obtained in 65% overall yield. Deprotection of **4** by treatment with triethylamine in aqueous MeOH furnished the dec-2-ulopyranose derivative **3** in 92% yield.

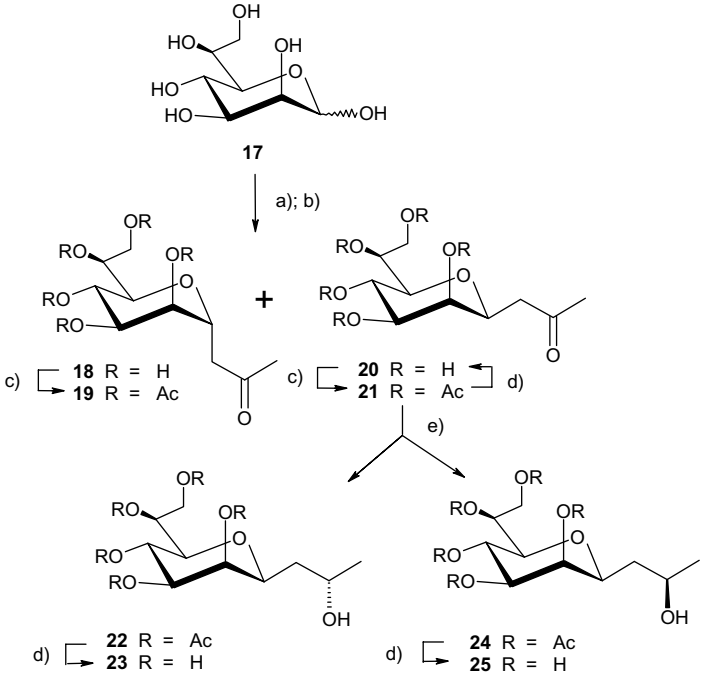
Reduction of the β-*C*-glycoside derivative **4** with borane–ammonia furnished a ~1:1.2 mixture of the diastereomeric alcohols **5** and **8** (90% yield), which could be partially separated by column chromatography. In order to assign the stereochemistry at the new stereogenic centre, the mixture of compounds being enriched in one diastereoisomer was converted into the (*S*)- and (*R*)-2-methoxy-2-phenyl-3-trifluoro-1-propanoic esters[17] as the Mosher ester pairs **6a**, **9a** and **6b**, **9b**, respectively. According to Mosher's rules, the higher running derivative **5** was assigned as the (*S*)-configured derivative based on the ^{1}H NMR chemical shifts observed in the (*S*)-MTPA esters for the methyl group of the decitol moiety of **6a** (δ 1.36) in comparison with the value observed for **9a** (δ 1.28), whereas the (*R*)-MTPA esters **6b**, **9b** displayed an inverse relation of chemical shifts (δ 1.29 for **6b**, δ 1.36 for **9b**). In addition, the dextrorotatory value measured for the specific rotation of diastereoisomer **5** (+2) versus that for compound **8** (−19) is consistent with the assignments (Scheme 1). Better separation of the mixture was achieved following phosphitylation using the phosphoramidite procedure with dibenzyl-*N*,*N*-diisopropyl-phosphoramidite/1*H*-tetrazole and subsequent oxidation with *tert*-BuOOH in 70% yield.[18] Again, the $[\alpha]_D^{20}$ values (+2.5 for **7**, −8 for **10**) were consistent with the (*S*)- and (*R*)-assignment, respectively. Deprotection of the penta-*O*-acetyl derivatives **5** and **8** under alkaline conditions furnished the β-*C*-glycosides **11** and **12** in high yield, respectively. Deprotection of the phosphotriester derivatives **7** and **10** was performed in two steps and in 90% yield by hydrogenolysis of the benzyl groups with 10% Pd–carbon followed by removal of the acetyl groups with triethylamine in aqueous MeOH. NMR evidence for the presence of the 2-phosphate was provided by the downfield shift of the ^{1}H NMR signal H-2 and the ^{31}P chemical shifts (δ 2.53 for **14**, δ 2.54 for **16**). The 2-*O*-phosphoryl group present in compounds **14** and **16** may be regarded as mimic of the diphosphate bridge in the nucleotide sugar and—although lacking the charged moiety of the glycosyl phosphate—could provide a proper formal distance to fit into the binding site of the glycosyl transferase, an important aspect to maintain binding potency of synthetic inhibitors.[19]

In addition to the analogues of D-*glycero*-D-*manno*-heptose, *C*-glycosides of the L-*glycero*-D-*manno*- configuration were prepared in a similar fashion. Thus, condensation of **17**[20] with pentane-2,4-dione proceeded in good yield to afford a complex mixture containing the anomeric 2-oxo-propyl heptopyranosides **18** and **20**. Acetylation of the mixture afforded **19** and **21**, which were partially separated and characterized. Measurements of the heteronuclear coupling constant $J_{C4,H4}$ were consistent with the assignment of the anomeric configuration and showed 152 Hz for the α-anomer **19** and 142 Hz for the β-anomer **21**. Similar to the D-*glycero*-D-*manno* derivatives, the proportion of **21** could be substantially increased via alkaline epimerization of the crude reaction mixture containing **18** and **20** with sodium methoxide and *O*-acetylation to give the β-anomer **21** in 56% yield.[21] Deprotection furnished the β-*C*-glycoside **20** in 98% yield (Scheme 2). Reduction of the keto-group with $NaBH_4$ gave the (*S*)- and (*R*)-alcohols **22** and **24** in a ~10:9 ratio, which were more readily separable in comparison to **5** and **8**. The stereochemical assignments were based on the similarity of ^{13}C NMR chemical shifts for C-1 and C-3 with those for **5** and **8**.



Scheme 1. Synthesis of D-*glycero*-D-*manno*-heptopyranosyl analogues. Reagents and conditions: (a) aq $NaHCO_3$, pentane-2,4-dione; (b) NaOMe, MeOH; then Ac_2O, pyridine, 65% for **4**; (c) NH_3–BH_3, MeOH, 90%; (d) Et_3N, aq MeOH, 92%; (e) (*S*)-MTPA-Cl, pyridine; (f) (*R*)-MTPA-Cl, pyridine; (g) *N*,*N*-(*i*Pr)$_2$NP(OBn)$_2$, 1*H*-tetrazole, MeCN–CH_2Cl_2, then *t*-BuOOH, 38% for **10**, 31% for **17**; (h) 10% Pd–C, MeOH, 95%.

Zemplén de-*O*-acetylation of **22** and **24** afforded the fully deprotected 4,8-anhydro-decitol derivatives **23** and **25** in high yield, respectively. The NMR-spectral characteristics as well as the values for the specific rotation [−9 for the 2-(*S*)-configured alcohol versus −46 for the (*R*)-isomer; the value measured for the known



Scheme 2. Synthesis of L-*glycero*-D-*manno*-heptopyranosyl analogues. Reagents and conditions: (a) aq $NaHCO_3$, pentane-2,4-dione; (b) MeONa, MeOH; (c) Ac_2O, pyridine, DMAP, 56% for **21** (three steps); (d) NaOMe, MeOH, 98% for **20**, 85% for **23**, 97% for **25**; (e) $NaBH_4$, MeOH, 94%.

methyl L-*glycero*-β-D-*manno*-heptopyranoside[21] is −40 and for (*S*)-1,2-propanediol +16] were in agreement with the previous assignments.

Conversion of the analogues into nucleoside derivatives and biological results will be reported in due course.

3. Conclusion

The condensation of pentane-2,4-dione with reducing *glycero*-D-*manno*-heptoses followed by alkaline epimerization provides an easy access to β-configured *C*-alkylcarbonyl glycosides as educts for further transformations, such as reduction to give diastereomeric alcohols and further elaboration into isosteric analogues of nucleotide-activated sugars.

4. Experimental

4.1. General methods

Column chromatography was performed on silica gel 60 (230–400 mesh, Merck), HPLC was performed on silica gel 60 (10 μm). Anion-exchange chromatography was performed on a strong anion-exchange resin (Bio-Rad, 5 mL cartridge, HCO_3^- form). Reactions were monitored by TLC on silica gel 60 F_{254} precoated glass plates (Merck) or on silica gel 60 F_{254} HPTLC precoated glass plates with 2.5 cm concentration zone (Merck); spots were visualised by spraying with anisaldehyde–H_2SO_4; phosphorus-containing compounds were additionally detected with a molybdate solution [0.02 M solution of ammonium cerium(IV)sulfate dihydrate and ammonium molybdate(VI)tetrahydrate in aqueous H_2SO_4]. Concentration of solutions was performed at reduced pressure at temperatures <25 °C. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. $[\alpha]_D^{20}$ values are given in units of 10^{-1} deg cm^3 g^{-1}. NMR spectra were recorded at 297 K in $CDCl_3$ (unless stated otherwise) with a Bruker DPX 300 spectrometer (^{1}H at 300.13 MHz, ^{13}C at 75.47 MHz and ^{31}P at 121.50 MHz) using standard Bruker NMR software. ^{1}H NMR spectra were referenced to tetramethylsilane or 2,2-dimethyl-2-silapentane-5-sulfonic acid. ^{13}C NMR spectra were referenced to chloroform (δ 77.00) or external 1,4-dioxane (δ 67.40). ^{31}P NMR spectra were referenced externally to 85% aq H_3PO_4 (δ 0.0). Elemental analyses were provided by Dr. J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, Universität Wien. TOF-ES-MS spectra were recorded on a Waters Micromass Q-TOF Ultima Global instrument. MALDI-TOF-MS spectra were recorded on a Thermo-BioAnalysis Dynamo MALDI-TOF instrument in the positive ion mode with 2% 2,5-dihydroxybenzoic acid as matrix. Melting points were determined with a Kofler hot stage microscope and are uncorrected.

4.1.1. 5,6,7,9,10-Penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-D-*erythro*-D-*galacto*-dec-2-ulose 4. A solution of **1** (113 mg, 0.54 mmol), $NaHCO_3$ (69 mg, 0.82 mmol) and pentane-2,4-dione (68 μL, 0.65 mmol) in water (10 mL) was stirred at 90 °C for 14 h. The solution was diluted with water and neutralized with Dowex 50 resin (H^+ form). The resin was filtered off, and the filtrate was lyophilized. R_f 0.16 (4:2:0.25 EtOAc/2-propanol/water); ^{1}H NMR (D_2O) for **2**: δ 4.36 (ddd, 1H, H-4), 4.05 (ddd, 1H, $^3J_{9,8}$ = 5.0 Hz, H-9), 3.87 (t, 1H, $^3J_{7,6} = {}^3J_{7,8}$ = 7.7 Hz, H-7), 3.82 (m, 2H, H-5, H-6), 3.76 (dd, 1H, $^3J_{9,10a}$ = 3.3 Hz, H-10a) 3.62 (dd, 1H, $^3J_{9,10b}$ = 7.2 Hz, $^2J_{10a,10b}$ = 12.0 Hz, H-10b), 3.54 (dd, 1H, H-8), 3.02 (dd, 1H, $^2J_{3a,3b}$ = 17.0 Hz, $^3J_{3a,4}$ = 9.0 Hz, H-3a), 2.85 (dd, 1H, $^3J_{3b,4}$ = 4.9 Hz, H-3b) and 2.25 (s, 3H, H-1); ^{13}C NMR (D_2O): δ 213.04 (C-2), 75.73 (C-8), 73.50 (C-4), 72.10 (C-9), 71.45 (C-5), 70.86 (C-6), 68.68 (C-7), 62.92 (C-10), 45.24 (C-3) and 30.63 (C-1). The mixture was dissolved in dry MeOH (50 mL) and stirred with 1.3 M methanolic NaOMe (2 mL) for 12 h at room temperature. The solution was concentrated and the residue (110 mg) was dissolved in dry pyridine (10 mL). Acetic anhydride was added (2.0 mL, 21.2 mmol) at 0 °C and the solution was stirred for 12 h at room temperature. MeOH (1 mL) was added, and the mixture was diluted with ethyl acetate (200 mL). The organic phase was washed sequentially with ice-cold 6 M HCl, water, satd aq $NaHCO_3$, water and brine and dried (Na_2SO_4). The solution was concentrated and the residue was purified on silica gel (1:9 *n*-hexane/diethyl ether), which afforded **4** as a pallid yellow syrup. Yield: 161 mg (65% for three steps); $[\alpha]_D^{20} = -10$ (*c* 0.9, $CHCl_3$); ^{1}H NMR: δ 5.30 (dd, 1H, $^3J_{5,4}$ = 1.0 Hz, H-5), 5.21 (t, 1H, $^3J_{7,8} = {}^3J_{7,6}$ = 9.7 Hz, H-7), 5.12 (ddd, 1H, $^3J_{9,8}$ = 3.0 Hz, H-9), 5.07 (dd, 1H, $^3J_{6,5}$ = 3.5 Hz, H-6), 4.34 (dd, 1H, $^3J_{10a,9}$ = 3.7 Hz, H-10a), 4.21 (dd, 1H, $^3J_{10b,9}$ = 7.3 Hz, $^2J_{10b,10a}$ = 12.0 Hz, H-10b), 4.13 (ddd, 1H, H-4), 3.68 (dd, 1H, H-8), 2.71 (dd, 1H, $^2J_{3a,4}$ = 8.0 Hz, H-3a), 2.41 (dd, 1H, $^2J_{3b,4}$ = 5.0 Hz, $^2J_{3a,3b}$ = 17.0 Hz, H-3b), 2.16, 2.06, 2.05, 2.04, 1.96 (5s, 15H, CH_3, Ac), 2.14 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ = 204.61 (C-2), 171.04, 170.74, 170.35, 170.25, 170.22 (5C, CO, Ac), 77.57 (C-8), 73.24 (C-4, $J_{C\text{-}4,H\text{-}4}$ = 143 Hz), 72.51 (C-6), 70.69 (C-9), 70.17 (C-5), 66.89 (C-7), 62.01 (C-10), 44.40 (C-3), 31.01 (C-1), 21.25, 21.09, 21.06, 20.91 (5C, CH_3, Ac); Anal. Calcd for $C_{20}H_{28}O_{12}$ (460.43): C, 52.17; H, 6.13. Found: C, 52.10; H, 6.24.

4.1.2. 4,8-Anhydro-1,3-dideoxy-D-*erythro*-D-*galacto*-dec-2-ulose 3. A solution of **4** (30 mg, 0.065 mmol) in 3:2:1 MeOH–water–Et_3N (2 mL) was stirred at room temperature for 3 h. The reaction mixture was evaporated and purified by a flash chromatography (4:2:0.25 ethylacetate/2-propanol/water) to give **3** (15 mg, 92%, 0.060 mmol) as a colourless syrup. $[\alpha]_D^{20} = -13$ (*c* 0.7, H_2O); ^{1}H NMR (D_2O) for **3**: δ 3.99 (ddd, 1H, $^3J_{5,4}$ = 1.0 Hz, H-4), 3.95 (ddd, 1H, $^3J_{9,8}$ = 3.6 Hz, $^3J_{9,10b}$ = 7.2 Hz, H-9), 3.83 (dd, 1H, $^3J_{5,6}$ = 3.0 Hz, H-5), 3.73 (dd, 1H, $^3J_{9,10a}$ = 3.5 Hz, H-10a), 3.68 (t, 1H, $^3J_{7,6} = {}^3J_{7,8}$ = 9.5 Hz, H-7), 3.66 (dd, 1H, $^2J_{10a,10b}$ = 12.0 Hz, H-10b), 3.65 (dd, 1H, H-6), 3.36 (dd, 1H, H-8), 2.91 (dd, 1H, $^2J_{3a,3b}$ = 17.0 Hz, $^3J_{3a,4}$ = 8.5 Hz, H-3a), 2.75 (dd, 1H, $^3J_{3b,4}$ = 4.5 Hz, H-3b) and 2.24 (s, 3H, H-1); ^{13}C NMR (D_2O): δ 213.38

(C-2), 80.40 (C-8), 74.67, 74.59 (C-4, C-6), 72.66 (C-9), 70.98 (C-5), 68.27 (C-7), 62.29 (C-10), 44.83 (C-3) and 30.19 (C-1). Anal. Calcd for $C_{10}H_{18}O_7 \cdot H_2O$: C, 44.77; H, 7.51. Found: C, 45.36; H, 7.61.

4.1.3. 5,6,7,9,10-Penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-D-*ribo*-L-*manno*-decitol 5 and 5,6,7,9,10-penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-D-*ribo*-L-*gluco*-decitol 8. A solution of **4** (200 mg, 0.43 mmol) in dry MeOH (15 mL) was treated with borane–ammonia (23 mg, 0.74 mmol) at 0 °C. Then the mixture was stirred under Ar for 15 h at rt. The solution was concentrated to give a mixture of diastereomers (180 mg, 90%), which was directly used for the next step. Alternatively, the diastereoisomers can be separated by silica gel chromatography using 7:3 diethyl ether/CH_2Cl_2 as eluant, followed by several HPLC steps. Data for **5**:R_f 0.20 (1:1, diethyl ether/CH_2Cl_2); $[\alpha]_D^{20} = +2$ (*c* 1.0, MeOH); ^{1}H NMR (MeOD): δ 5.37 (dd, 1H, $^3J_{5,6}$ = 3.3 Hz, $^3J_{5,4}$ = 1.0 Hz, H-5), 5.20 (dd, 1H, $^3J_{7,6}$ = 9.8 Hz, H-7), 5.13 (m, 1H, H-9), 5.10 (dd, 1H, H-6), 4.39 (dd, 1H, $^3J_{10a,9}$ = 3.5 Hz, $^2J_{10a,10b}$ = 12.0 Hz, H-10a), 4.20 (dd, 1H, $^3J_{10b,9}$ = 7.5 Hz, H-10b), 3.91 (ddd, 1H, $^3J_{4,3b}$ = 6.0 Hz, $^3J_{4,3a}$ = 7.5 Hz, H-4), 3.80 (m, 1H, H-2), 3.79 (dd, 1H, $^3J_{8,9}$ = 3.0 Hz, $^3J_{8,7}$ = 9.8 Hz, H-8), 2.15, 2.06, 2.05, 2.02, 1.94 (5s, 15H, CH_3, Ac) 1.77 (ddd, 1H, $^3J_{3a,2}$ = 6.5 Hz, $^2J_{3a,3b}$ = 14.0 Hz, H-3a), 1.49 (ddd, 1H, $^3J_{3b,2}$ = 5.5 Hz, H-3b), 1.16 (d, 3H, $^3J_{1,2}$ = 6.2 Hz, CH_3); ^{13}C NMR (MeOD): δ 172.40, 172.23, 171.72, 171.68, 171.53 (5C, CO, Ac), 78.26 (C-8), 75.69 (C-4), 73.99 (C-6), 71.84 (C-9), 71.05 (C-5), 68.18 (C-7), 65.38 (C-2), 62.84 (C-10), 40.31 (C-3), 23.26 (C-1), 20.79, 20.71, 20.63, 20.53, 20.49 (5C, CH_3, Ac). Data for **8**: R_f 0.14 (1:1, diethyl ether/CH_2Cl_2); $[\alpha]_D^{20} = -19$ (*c* 0.5, MeOH); ^{1}H NMR (MeOD): δ 5.27 (dd, 1H, $^3J_{5,6}$ = 3.3 Hz, $^3J_{5,4}$ = 0.9 Hz, H-5), 5.19 (dd, 1H, $^3J_{7,8}$ = 9.8 Hz, H-7), 5.13 (m, 1H, H-9), 5.10 (dd, 1H, $^3J_{6,7}$ = 9.8 Hz, H-6), 4.39 (dd, 1H, $^3J_{10a,9}$ = 3.5 Hz, H-10a), 4.20 (dd, 1H, $^3J_{10b,9}$ = 7.5 Hz, $^2J_{10b,10a}$ = 12.0 Hz, H-10b), 3.93 (ddd, 1H, $^3J_{4,3b}$ = 9.5 Hz, H-4), 3.88 (m, 1H, H-2), 3.78 (dd, 1H, $^3J_{8,9}$ = 3.0 Hz, H-8), 2.06, 2.05, 2.02, 2.01, 1.94 (5s, 15H, CH_3, Ac), 1.65 (ddd, 1H, $^3J_{3a,2}$ = 3.4 Hz, $^3J_{3a,4}$ = 10.0 Hz, $^2J_{3a,3b}$ = 14.0 Hz, H-3a), 1.36 (ddd, 1H, $^3J_{3b,2}$ = 3.0 Hz, H-3b), 1.17 (d, 3H, $^3J_{1,2}$ = 6.3 Hz, CH_3); ^{13}C NMR (MeOD): δ 172.43, 172.18, 171.76, 171.68, 171.55 (5C, CO, Ac), 78.13 (C-8), 74.95 (C-4), 74.09 (C-6), 72.12, 71.99 (C-9, C-5), 68.24 (C-7), 64.93 (C-2), 62.99 (C-10), 41.31 (C-3), 24.20 (C-1), 20.80, 20.72, 20.62, 20.54, 20.51 (5C, CH_3, Ac).

Conversion into Dale–Mosher esters **6a** and **9a**: A solution of (*S*)-MTPA chloride (15 μL, 80 μmol) in dry pyridine (1 mL) was charged with CCl_4. The mixture containing **5** and **8** (17 mg, 0.037 mmol, enriched with **5**) dissolved in 2 mL of dry pyridine was added and stirred for 12 h at room temperature. Diethylamine (12 μL, 0.11 mmol) was added and the suspension was diluted with ether. The organic phase was extracted with ice-cold HCl, water, Na_2CO_3, water, brine, dried (Na_2SO_4) and concentrated. ^{1}H NMR (MeOD) for **6a**: δ 1.85 (m, H-3a), 1.65 (m, H-3b), 1.36 (d, 3H, $^3J_{1,2}$ = 6.3 Hz, H-1); data for **9a**: δ 1.85 (m, H-3a), 1.65 (m, H-3b), 1.28 (d, 3H, $^3J_{1,2}$ = 6.2 Hz, H-1).

Conversion into Dale–Mosher esters **6b** and **9b**: A solution of (*R*)-MTPA chloride (15 μL, 80 μmol) in dry pyridine (1 mL) was charged with CCl_4. The mixture containing **5** and **8** (17 mg, 0.037 mmol, enriched with **5**) dissolved in 2 mL dry pyridine was added and treated as described for **6a** and **9a**. ^{1}H NMR (MeOD) for **6b**: δ 1.98 (m, H-3a), 1.68 (m, H-3b), 1.29 (d, 3H, $^3J_{1,2}$ = 6.3 Hz, H-1); data for **9b**: δ 1.80 (m, H-3a), 1.52 (m, H-3b), 1.36 (d, 3H, $^3J_{1,2}$ = 6.2 Hz, H-1).

4.1.4. 5,6,7,9,10-Penta-*O*-acetyl-4,8-anhydro-2-*O*-[bis(benzyloxy)phosphoryl]-1,3-dideoxy-D-*ribo*-L-*manno*-decitol 7 and 5,6,7,9,10-penta-*O*-acetyl-4,8-anhydro-2-*O*-[bis(benzyloxy)phosphoryl]-1,3-dideoxy-D-*ribo*-L-*gluco*-decitol 10. The diastereomeric mixture of **5** and **8** (134 mg, 0.29 mmol) and dibenzyl-*N*,*N*-diisopropylphosphoramidite (0.234 mL, 0.72 mmol) were dried by repeated evaporation with dry toluene (3 × 10 mL) and then under diminished pressure for 10 h. Then the flask was charged with CH_2Cl_2 (5 mL), a solution of 1*H*-tetrazole (61 mg, 0.87 mmol) in dry CH_3CN (3 mL) was added and the mixture was stirred at room temperature for 3 h under N_2. Monitoring of the reaction by TLC showed the formation of intermediate phosphite triesters (R_f 0.54; 1:1, *n*-hexane/EtOAc). The reaction mixture containing phosphite triesters was cooled to 0 °C and a solution of *t*-BuOOH (72 μL of an 80% solution in di-*tert*-butyl peroxide) in CH_2Cl_2 (3 mL) was gradually added (~20 min). The reaction mixture was warmed to room temperature and stirred for 12 h. The solvent was evaporated using a stream of N_2. The residue was redissolved in diethyl ether and washed sequentially with saturated $NaHCO_3$–water and brine. The organic phase was dried (Na_2SO_4) and concentrated. The phosphates were separated by chromatography on silica gel (several columns with 1:9 *n*-hexane/diethyl ether and 1:1 EtOAc/*n*-hexane) and final HPLC purification to give **10** (79 mg, 38%, 0.10 mmol) as a colourless syrup, R_f 0.15; $[\alpha]_D^{20} = -8$ (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40–7.30 (m, 10H, Ph), 5.13 (d, 1H, $^3J_{5,6}$ = 3.5 Hz, H-5), 5.12 (dd, 1H, $^3J_{7,8}$ = 9.8 Hz, H-7), 5.10 (m, 1H, H-9), 5.05 (d, 2H, $^3J_{H,P}$ = 8.0 Hz, CH_2Ph), 5.02 (d, 2H, $^3J_{H,P}$ = 8.0 Hz, CH_2Ph), 4.86 (dd, 1H, $^3J_{6,7}$ = 9.8 Hz, H-6), 4.65 (m, 1H, H-2), 4.34 (dd, 1H, $^3J_{10a,9}$ = 3.5 Hz, $^2J_{10a,10b}$ = 12.0 Hz, H-10a), 4.22 (dd, 1H, $^3J_{10b,9}$ = 7.5 Hz, H-10b), 3.58 (br d, 1H, J = ~9.0 Hz, H-4), 3.43 (dd, 1H, $^3J_{8,9}$ = 3.0 Hz, H-8), 2.16, 2.13, 2.08, 2.04, 1.96 (5s, 15H, CH_3, Ac), 1.62 (m, 1H, H-1a), 1.48 (m, H-3b), 1.30 (d, 3H, $^3J_{1,2}$ = 6.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.86, 170.56, 170.41, 170.10, 170.02 (5C, CO, Ac), 136.02, 135.66 (2C, Ph), 128.84, 128.77, 128.72, 128.69, 128.64 (10C, Ph), 77.33 (C-8), 72.94 (C-4), 72.90 (1C, $^3J_{2,P}$ = 5.8 Hz, C-2), 72.52 (C-6), 70.40, 70.33 (C-5, C-9), 69.39, (1C, $^2J_{C,P}$ = 6.0 Hz, *C*H_2Ph), 69.32 (1C, $^2J_{C,P}$ = 6.0 Hz, *C*H_2Ph), 66.62 (C-7), 62.09 (C-10), 39.29 (1C, $^3J_{C,P}$ = 6.2 Hz, C-3), 22.43 (1C, $^3J_{C,P}$ = 2.3 Hz, C-1), 21.05, 20.91, 20.85, 20.74 (5C, CH_3, Ac); ^{31}P NMR ($CDCl_3$): δ −0.8; Anal. Calcd for $C_{34}H_{43}O_{15}P$ (722.67): C, 56.51; H, 6.00. Found: C, 56.49; H, 6.28.

Further elution gave **7** (65 mg, 31%, 0.09 mmol) as a colourless syrup, R_f 0.10; $[\alpha]_D^{20} = +2.5$ (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.37–7.30 (m, 10H, Ph), 5.27 (dd,

1H, $^3J_{5,4}$ = 0.8 Hz, H-5), 5.16 (dd, 1H, $^3J_{7,6}$ = 10.0, $^3J_{7,8}$ = 10.0 Hz, H-7), 5.09 (m, 1H, H-9), 5.05 (d, 2H, $^3J_{H,P}$ = 8.8 Hz, CH_2Ph), 5.04 (d, 2H, $^3J_{H,P}$ = 8.8 Hz, CH_2Ph), 4.86 (dd, 1H, $^3J_{6,5}$ = 4.0 Hz, H-6), 4.56 (m, 1H, H-2), 4.36 (dd, 1H, $^3J_{10a,9}$ = 3.5 Hz, H-10a), 4.18 (dd, 1H, $^3J_{10b,9}$ = 7.5 Hz, $^2J_{10b,10a}$ = 12.0 Hz, H-10b), 3.66 (dd, 1H, $^3J_{4,3b}$ = 6.0 Hz, $^3J_{4,3a}$ = 7.5 Hz, H-4), 3.52 (dd, 1H, $^3J_{8,9}$ = 2.7 Hz, H-8), 2.12, 2.08, 2.05, 2.04, 1.97 (5s, 15H, CH_3, Ac), 1.87 (ddd, 1H, $^3J_{3a,2}$ = 6.5 Hz, $^2J_{3a,3b}$ = 14.0 Hz, H-3a), 1.55 (ddd, 1H, $^3J_{3b,2}$ = 5.5 Hz, H-3b), 1.30 (d, 3H, $^3J_{1,2}$ = 6.3 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.76, 170.51, 170.16, 170.04 (5C, CO, Ac), 136.10, 136.02 (2C, Ph), 128.76, 128.73, 128.20, 128.13 (10C, Ph), 77.35 (C-8), 73.51 (C-4), 72.94 (1C, $^3J_{2,P}$ = 5.8 Hz, C-2), 72.45 (C-6), 70.39 (C-9), 69.81 (C-5), 69.52 (1C, $^2J_{C,P}$ = 6.0 Hz, *C*H_2Ph), 69.44 (1C, $^2J_{C,P}$ = 6.0 Hz, *C*H_2Ph), 66.62 (C-7), 61.76 (C-10), 37.68 (1C, $^3J_{C,P}$ = 6.8 Hz, C-3), 21.32 (1C, $^3J_{C,P}$ = 2.5 Hz, C-1), 21.05, 20.90, 20.80, 20.75 (5C, CH_3, Ac); ^{31}P NMR ($CDCl_3$): δ −0.9; Anal. Calcd for $C_{34}H_{43}O_{15}P$ (722.67): C, 56.51; H, 6.00. Found: C, 55.82; H, 6.26.

4.1.5. 4,8-Anhydro-1,3-dideoxy-D-*ribo*-L-*manno*-decitol 11.

A solution of **5** (25 mg, 0.065 mmol) in 3:2:1 MeOH–water–Et_3N (2 mL) was stirred at room temperature for 3 h. The reaction mixture was evaporated and purified by flash chromatography (4:2:0.25 EtOAc/2-propanol/water) to give **11** (15 mg, 92%, 0.060 mmol) as a colourless syrup. $[\alpha]_D^{20} = -11$ (*c* 0.8, H_2O); ^{1}H NMR (D_2O): δ 3.96 (m, 1H, H-2), 3.96 (m, 1H, H-9), 3.82 (br d, 1H, $^3J_{5,6}$ = 3.3 Hz, H-5), 3.74 (dd, 1H, $^3J_{9,10a}$ = 3.6 Hz, $^2J_{10a,10b}$ = 12.0 Hz, H-10a), 3.65 (dd, 1H, $^3J_{9,10b}$ = 7.0 Hz, H-10b), 3.64 (t, 1H, $^3J_{7,8}$ = $^3J_{7,6}$ = 9.5 Hz, H-7), 3.62 (m, 1H, H-6), 3.59 (ddd, 1H, H-4), 3.33 (dd, 1H, $^3J_{8,9}$ = 3.6 Hz, H-8), 1.83 (ddd, 1H, $^3J_{3a,4}$ = 5.3 Hz, $^3J_{3a,2}$ = 5.3 Hz, $^2J_{3a,3b}$ = 14.2 Hz, H-3a), 1.62 (ddd, 1H, $^3J_{3b,4}$ = 7.7 Hz, $^3J_{3b,2}$ = 7.7 Hz, H-3b), 1.17 (d, 3H, $^3J_{1,2}$ = 6.2 Hz, H-1); ^{13}C NMR (D_2O): δ 80.95 (C-8), 77.18 (C-6), 75.27 (C-4), 72.96 (C-9), 71.44 (C-5), 68.89 (C-7), 66.31 (C-2), 62.69 (C-10), 39.58 (C-3) and 22.75 (C-1). TOF-ES-MS: *m*/*z* = 253.103 $[M+H]^+$. Calcd 253.128 $[M+H]^+$.

4.1.6. 4,8-Anhydro-1,3-dideoxy-D-*ribo*-L-*gluco*-decitol 12.

A solution of **8** (25 mg, 0.065 mmol) in 3:2:1 MeOH–water–Et_3N (2 mL) was stirred at room temperature for 3 h. The reaction mixture was evaporated and purified by a flash chromatography (4:2:0.25 EtOAc/2-propanol/water) to give **12** (16 mg, 97%, 0.063 mmol) as a colourless syrup. $[\alpha]_D^{20} = -35$ (*c* 0.6, H_2O); ^{1}H NMR (D_2O): δ 3.96 (m, 1H, H-2), 3.96 (m, 1H, H-9), 3.75 (br d, 1H, $^3J_{5,6}$ = 4.9 Hz, H-5), 3.74 (dd, 1H, $^3J_{9,10a}$ = 3.9 Hz, $^2J_{10a,10b}$ = 12.0 Hz, H-10a), 3.65 (dd, 1H, $^3J_{9,10b}$ = 7.0 Hz, H-10b), 3.64 (t, 1H, $^3J_{7,6}$ = 10.0 Hz, H-7), 3.63 (m, 1H, H-6), 3.59 (ddd, 1H, H-4), 3.32 (dd, 1H, $^3J_{8,9}$ = 3.9 Hz, $^3J_{8,7}$ = 9.0 Hz, H-8), 1.79 (ddd, 1H, $^3J_{3a,4}$ = 3.5 Hz, $^3J_{3a,2}$ = 10.0, $^2J_{3a,3b}$ = 14.0 Hz, H-3a), 1.49 (ddd, 1H, $^3J_{3b,4}$ = 3.2 Hz, $^3J_{3b,2}$ = 9.2 Hz, H-3b), 1.17 (d, 3H, $^3J_{1,2}$ = 6.3 Hz, H-1); ^{13}C NMR (D_2O): δ 80.51 (C-8), 75.84 (C-6), 75.31 (C-4), 73.21 (C-9), 72.26 (C-5), 69.18 (C-7), 65.19 (C-2), 62.73 (C-10), 40.33 (C-3) and 23.41 (C-1). TOF-ES-MS: *m*/*z* = 253.103 $[M+H]^+$.

4.1.7. Triethylammonium 5,6,7,9,10-penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-D-*ribo*-L-*manno*-decitol 2-phosphate 13.

A solution of **7** (40 mg, 0.055 mmol) in dry MeOH (11 mL) was hydrogenated in the presence of 10% Pd/C (11 mg) for 10 h at atmospheric pressure. After the completion of the reaction, the catalyst was removed by filtration through a pad of Celite and washed with MeOH. The combined filtrates were neutralized by addition of Et_3N (9 μL) and concentrated. The residue was lyophilized from water to give the monotriethylammonium salt of **13** (34 mg, 95%) as an amorphous solid. R_f 0.75 (5:10:2:2, $CHCl_3$/MeOH/25% aq NH_4OH/H_2O); $[\alpha]_D^{20} = +1$ (*c* 0.2, MeOH); ^{1}H NMR (MeOD): δ 5.45 (br s, 1H, H-5), 5.17 (d, 1H, $^3J_{6,7}$ = 7.0 Hz, H-6) 5.16 (dd, 1H, $^3J_{7,8}$ = 9.0 Hz, H-7), 5.12 (m, 1H, H-9), 4.42 (dd, 1H, $^3J_{10a,9}$ = 3.4 Hz, $^2J_{10a,10b}$ = 12.0 Hz, H-10a), 4.34 (m, 1H, H-2), 4.20 (dd, 1H, $^3J_{10b,9}$ = 8.2 Hz, H-10b), 4.04 (br t, 1H, H-4), 3.78 (dd, 1H, $^3J_{8,9}$ = 3.0 Hz, H-8), 3.16 (q, ∼6H, CH_2, Et_3N), 2.15, 2.06, 2.05, 2.03, 1.95 (5s, 15H, CH_3, Ac) 1.84 (m, 1H, H-3a), 1.62 (m, H-3b), 1.33 (d, 3H, $^3J_{1,2}$ = 7.2 Hz, CH_3), 1.27 (t, ∼9H, CH_3, Et_3N); ^{13}C NMR (MeOD): δ = 171.49, 171.30, 170.77, 170.65, (5C, CO, Ac), 77.26 (C-8), 73.96 (C-4), 73.01 (C-6), 70.87, 70.39 (C-5, C-9), 68.52 (1C, $^3J_{2,P}$ = 5.3 Hz, C-2), 67.44 (C-7), 61.89 (C-10), 46.67 (CH_2, Et_3N), 38.32 (1C, $^3J_{C,P}$ = 5.4 Hz, C-3), 21.16 (1C, $^3J_{C,P}$ = 2.7 Hz, C-1), 19.85, 19.75, 19.68, 19.57, 19.56 (5C, CH_3, Ac), 8.26 (CH_3, Et_3N); ^{31}P NMR (MeOD): δ 0.9; TOF-ES-MS: *m*/*z* = 543.118 $[M+H]^+$. Calcd 543.147 $[M+H]^+$.

4.1.8. Triethylammonium 5,6,7,9,10-penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-D-*ribo*-L-*gluco*-decitol 2-phosphate 15.

A solution of **10** (30 mg, 0.041 mmol) in dry MeOH (9 mL) was hydrogenated in the presence of 10% Pd/C (9 mg) for 10 h at atmospheric pressure. After the completion of the reaction, the catalyst was removed by filtration through a pad of Celite and washed with MeOH. The combined filtrates were neutralized by addition of Et_3N (7 μL) and concentrated. The residue was lyophilized from water to give the monotriethylammonium salt of **15** (25 mg, 95%, 0.039 mmol) as an amorphous solid. R_f 0.75 (5:10:2:2, $CHCl_3$/MeOH/25% aq NH_4OH/H_2O); $[\alpha]_D^{20} = -11.0$ (*c* 0.2, MeOH); ^{1}H NMR (MeOD): δ 5.26 (d, 1H, $^3J_{5,6}$ = 3.0 Hz, H-5), 5.19 (dd, 1H, $^3J_{7,8}$ = 9.8 Hz, H-7), 5.15 (m, 1H, H-9), 5.12 (dd, 1H, $^3J_{6,7}$ = 9.8 Hz, H-6), 4.42 (dd, 1H, $^3J_{10a,9}$ = 3.4 Hz, $^2J_{10a,10b}$ = 12.0 Hz, H-10a), 4.36 (m, 1H, H-2), 4.21 (dd, 1H, $^3J_{10b,9}$ = 8.2 Hz, H-10b), 4.05 (br d, 1H, H-4), 3.85 (dd, 1H, $^3J_{8,9}$ = 3.0 Hz, H-8), 3.16 (q, ∼6H, CH_2, Et_3N), 2.15, 2.07, 2.06, 2.02, 1.94 (5s, 15H, CH_3, Ac) 1.68 (m, 1H, H-3a), 1.52 (m, H-3b), 1.33 (d, 3H, $^3J_{1,2}$ = 7.2 Hz, CH_3), 1.27 (t, ∼9H, CH_3, Et_3N); ^{13}C NMR (MeOD): δ 171.55, 171.29, 171.10, 170.72, 170.58 (5C, CO, Ac), 76.82 (C-8), 73.75 (C-4), 73.13 (C-6), 71.39, 71.08 (C-5, C-9), 68.96 (1C, $^3J_{2,P}$ = 5.5 Hz, C-2), 67.33 (C-7), 62.30 (C-10), 46.68 (CH_2, Et_3N), 40.08 (1C, $^3J_{C,P}$ = 6.5 Hz, C-3), 22.06 (1C, $^3J_{C,P}$ = 2.0 Hz, C-1), 19.97, 19.77, 19.69, 19.57, 19.56 (5C, CH_3, Ac), 8.29 (CH_3, Et_3N); ^{31}P NMR (MeOD): δ 0.3; TOF-ES-MS: *m*/*z* = 543.127 $[M+H]^+$.

4.1.9. Triethylammonium 4,8-anhydro-1,3-dideoxy-D-*ribo*-L-*manno*-decitol 2-phosphate 14. A solution of **13** (25 mg, 0.039 mmol) in 7:3:1 MeOH–water–Et_3N (2 mL) was stirred at room temperature for 3 h at pH 12. The reaction mixture was diluted with water and purified on an anion-exchange column. The eluate was concentrated and lyophilized to give **14** (16 mg, 95%, 0.037 mmol) as a white fluffy solid. R_f 0.15 (5:10:2:2, $CHCl_3$/MeOH/25% aq NH_4OH/H_2O); $[\alpha]_D^{20} = -5.0$ (*c* 1.4, H_2O); ^{1}H NMR (D_2O): δ 4.27 (m, 1H, H-2), 3.95 (m, 1H, H-9), 3.92 (br s, 1H, H-5), 3.75 (dd, 1H, $^3J_{10a,9} = 5.0$ Hz, $^2J_{10a,10b} = 12.0$ Hz, H-10a), 3.68 (m, 1H, H-6), 3.66 (dd, 1H, $^3J_{10b,9} = 3.6$ Hz, H-10b), 3.63 (m, 1H, H-7), 3.63 (m, 1H, H-4), 3.36 (dd, 1H, $^3J_{8,9} = 4.0$ Hz, $^3J_{8,7} = 9.5$ Hz, H-8), 3.17 (q, ~12H, CH_2, Et_3N), 1.86 (m, 1H, H-3a), 1.72 (m, 1H, H-3b), 1.25 (t, ~18H, CH_3, Et_3N), 1.24 (s, 3H, CH_3); ^{13}C NMR (D_2O): δ 80.80 (C-8), 76.34 (C-6), 75.22 (C-4), 73.11 (C-9), 71.31 (C-5), 69.26 (1C, $^3J_{2,P} = 4.9$ Hz, C-2), 68.96 (C-7), 62.70 (C-10), 47.49 (CH_2, Et_3N), 38.83 (1C, $^3J_{C,P} = 4.8$ Hz, C-3), 22.01 (C-1, $J_{C\text{-}1,P} = 2.5$ Hz), 9.08 (CH_3, Et_3N); ^{31}P NMR (D_2O): δ 2.53. TOF-ES-MS: *m*/*z* = 333.100 $[M+H]^+$. Calcd. 333.095 $[M+H]^+$.

4.1.10. Triethylammonium 4,8-anhydro-1,3-dideoxy-D-*ribo*-L-*gluco*-decitol 2-phosphate 16. A solution of **15** (34 mg, 0.053 mmol) in 7:3:1 MeOH–water–Et_3N (2 mL) was stirred at room temperature for 3 h at pH 12. The reaction mixture was diluted with water and purified on an anion-exchange column. The eluate was concentrated and lyophilized to give **16** (22 mg, 95%, 0.050 mmol) as a white fluffy solid. R_f 0.15 (5:10:2:2, $CHCl_3$/MeOH/25% aq NH_4OH/H_2O); $[\alpha]_D^{20} = -15$ (*c* 1.7, H_2O); ^{1}H NMR (D_2O): δ 4.32 (m, 1H, H-2), 3.94 (m, 1H, H-9), 3.83 (d, 1H, $^3J_{4,5} = < 1.0$ Hz, H-5), 3.78 (dd, 1H, $^3J_{10a,9} = 6.0$, $^2J_{10a,10b} = 12.0$ Hz, H-10a), 3.75 (dd, 1H, $^3J_{10b,9} = 3.4$ Hz, H-10b) 3.73 (m, 1H, H-6), 3.68 (m, 1H, H-7), 3.65 (m, 1H, H-4), 3.41 (dd, 1H, $^3J_{8,9} = 4.0$, $^3J_{8,7} = 9.0$ Hz, H-8), 3.19 (q, ~12H, CH_2, Et_3N), 1.72 (m, 2H, H-3), 1.26 (s, 3H, CH_3), 1.27 (t, ~18H, CH_3, Et_3N); ^{13}C NMR (D_2O): δ 79.61 (C-8), 75.92 (C-6), 75.06 (C-4), 73.20 (C-9), 71.81 (C-5), 69.47 (C-7), 69.24 (1C, $^3J_{2,P} = 4.8$ Hz, C-2), 62.24 (C-10), 47.09 (CH_2, Et_3N), 39.70 (1C, $^3J_{C,P} = 5.8$ Hz, C-3), 22.63 (C-1, $J_{C\text{-}1,P} = 2.4$ Hz), 8.26 (CH_3, Et_3N); ^{31}P NMR (D_2O): $\delta = 2.5$; TOF-ES-MS: *m*/*z* = 333.100 $[M+H]^+$. Calcd 333.095 $[M+H]^+$.

4.1.11. 5,6,7,9,10-Penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-L-*threo*-D-*galacto*-dec-2-ulose 21. A solution of **17** (300 mg, 1.43 mmol), $NaHCO_3$ (180 mg, 2.5 mmol) and pentane-2,4-dione (180 μL, 1.72 mmol) in water (10 mL) was stirred at 90 °C for 16 h. The solution was diluted with water and neutralized with Dowex 50 resin (H^+ form). The resin was filtered off, and the filtrate was lyophilized. Yield: 261 mg (73%). An aliquot of the mixture (100 mg) was dissolved in dry MeOH (3 mL) and stirred with 1.0 M methanolic NaOMe (2 mL) for 15 h at room temperature. The solution was made neutral by addition of Dowex 50 resin (H^+ form), filtered and concentrated. The residue was dissolved in dry pyridine (4 mL), acetic anhydride (2 mL) and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine was added. The solution was stirred for 15 h at room temperature, MeOH (2 mL) was added, and the mixture was coevaporated three times with toluene. The residue was purified on a column of silica gel (diethylether), which afforded **21** as the major compound. Yield: 140 mg (77%), colourless syrup; $[\alpha]_D^{20} = -52$ (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.32 (dd, 1H, $^3J_{5,4} = 0.9$ Hz, H-5), 5.30–5.21 (m, 1H, $^3J_{7,8} = 9.9$ Hz, $^3J_{7,6} = 10.1$ Hz, H-7), 5.30–5.21 (m, 1H, H-9), 5.08 (dd, 1H, $^3J_{6,5} = 3.4$ Hz, H-6), 4.27 (dd, 1H, $^3J_{10a,9} = 5.3$ Hz, H-10a), 4.17 (ddd, 1H, H-4), 4.08 (dd, 1H, $^3J_{10b,9} = 7.8$ Hz, $^2J_{10b,10a} = 11.5$ Hz, H-10b), 3.72 (dd, 1H, $^3J_{8,9} = 2.5$ Hz, H-8), 2.82 (dd, 1H, $^3J_{3a,4} = 8.2$ Hz, H-3a), 2.44 (dd, 1H, $^3J_{3b,4} = 4.3$ Hz, $^2J_{3a,3b} = 17.4$ Hz, H-3b), 2.21 (s, 3H, CH_3), 2.15, 2.11, 2.05, 1.99, 1.96 (5s, 15H, CH_3, Ac); ^{13}C NMR ($CDCl_3$): δ 204.03 (C-2), 170.68, 170.63, 170.49, 170.11, 169.78 (5C, CO, Ac), 76.90 (C-8), 73.72 (C-4, $J_{C\text{-}4,H\text{-}4} = 142$ Hz), 72.51 (C-6), 70.18 (C-5), 67.21 (C-9), 64.87 (C-7), 62.27 (C-10), 44.04 (C-3), 30.74 (C-1), 20.93, 20.91, 20.83, 20.78, 20.71 (5C, CH_3, Ac); Anal. Calcd for $C_{20}H_{28}O_{12}$: C, 52.17; H, 6.13. Found: C, 52.19; H, 6.05.

Acetylation of the mixture: An aliquot of the lyophilized mixture of **18** and **20** (15 mg) was dissolved in pyridine (3 mL). Acetic anhydride (1 mL) and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine were added and the solution was stirred for 15 h at room temperature. After addition of MeOH (0.1 mL), the solution was coevaporated with toluene and the residue was purified by silica gel chromatography to give **21** as the higher running component (12 mg) followed by **19** (13 mg); colourless syrup; $[\alpha]_D^{20} = -26$ (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$): δ 5.34 (ddd, 1H, $^3J_{8,9} = 2.3$ Hz, H-9), 5.29–5.14 (m, 3H, H-5, H-6, H-7), 4.55 (ddd, 1H, $^3J_{5,4} = 2.9$ Hz, H-4), 4.25 (dd, 1H, $^3J_{10a,9} = 4.9$ Hz, H-10a), 4.07 (dd, 1H, $^3J_{10b,9} = 7.0$ Hz, $^2J_{10b,10a} = 11.7$ Hz, H-10b), 3.89 (dd, 1H, $^3J_{8,7} = 7.7$ Hz, H-8), 2.87 (dd, 1H, $^3J_{3a,4} = 9.5$ Hz, H-3a), 2.68 (dd, 1H, $^3J_{3b,4} = 5.2$ Hz, $^2J_{3a,3b} = 15.0$ Hz, H-3b), 2.20 (s, 3H, CH_3), 2.15, 2.13, 2.05, 2.03, 2.02 (5s, 15H, CH_3, Ac); ^{13}C NMR ($CDCl_3$): δ 203.41 (C-2), 170.56, 170.34, 170.07, 169.96, 169.42 (5C, CO, Ac), 71.48 (C-8), 71.25 (C-4, $J_{C\text{-}4,H\text{-}4} = 151$ Hz), 69.85 (C-6), 68.83 (C-5), 66.92 (C-9), 65.50 (C-7), 62.20 (C-10), 43.14 (C-3), 29.80 (C-1), 20.91, 20.75 (d.i.), 20.69, 20.63 (5C, CH_3, Ac).

4.1.12. 4,8-Anhydro-1,3-dideoxy-L-*threo*-D-*galacto*-dec-2-ulose 20. A solution of **21** (450 mg, 0.98 mmol) in dry MeOH (10 mL) was stirred with 0.1 M NaOMe (0.5 mL) for 15 h at room temperature. The solution was made neutral by adding Dowex 50 resin (H^+ form), filtered and concentrated, which furnished **20** as amorphous solid. Yield: 240 mg (98%); $[\alpha]_D^{20} = -15$ (*c* 0.5, MeOH); ^{1}H NMR (D_2O): δ 4.00 (ddd, 1H, $^3J_{5,4} = 1.0$ Hz, H-4), 3.94 (ddd, 1H, $^3J_{9,8} = 1.5$ Hz, $^3J_{9,10a} = 6.2$, $^3J_{9,10b} = 7.7$ Hz, H-9), 3.83 (dd, 1H, $^3J_{5,6} = 3.7$ Hz, H-5), 3.77 (t, 1H, $^3J_{7,6} = {}^3J_{7,8} = 9.6$ Hz, H-7), 3.66 (dd, 1H, H-6), 3.64–3.59 (m, 2H, H-10a, H-10b), 3.29 (dd, 1H, H-8), 2.94 (dd, 1H, $^2J_{3a,3b} = 16.6$ Hz, $^3J_{3a,4} = 9.4$ Hz, H-3a), 2.71 (dd, 1H, $^3J_{3b,4} = 3.8$ Hz, H-3b) and 2.24 (s, 3H, H-1); ^{13}C NMR (D_2O): δ 214.08

(C-2), 79.31 (C-8), 75.12 (C-4), 74.96 (C-6), 71.65 (C-5), 69.56 (C-9), 66.91 (C-7), 63.79 (C-10), 45.18 (C-3) and 30.56 (C-1); Anal. Calcd for $C_{10}H_{18}O_7$. 0.5 H_2O: C, 46.33; H, 7.39. Found: C, 45.88; H, 7.85.

4.1.13. 5,6,7,9,10-Penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-L-*lyxo*-L-*manno*-decitol 22 and 5,6,7,9,10-penta-*O*-acetyl-4,8-anhydro-1,3-dideoxy-L-*lyxo*-L-*gluco*-decitol 24. A solution of **21** (80 mg, 0.17 mmol) in dry MeOH (10 mL) was cooled to −10 °C. $NaBH_4$ (90 mg) and AcOH (130 μL) were added. The solution was stirred for 30 min, then concentrated. The residue was dissolved in EtOAc (100 mL). The organic phase was washed with water, dried (Na_2SO_4) and concentrated. The remainder was purified on a column of silica gel (1:1 CH_2Cl_2/Et_2O), which afforded **22** as the first fraction. Yield: 40 mg (50%); $[\alpha]_D^{20} = -50$ (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.36 (dd, 1H, $^3J_{5,4}$ = 1.0 Hz, H-5), 5.28 (t, 1H, $^3J_{7,6} = {}^3J_{7,8}$ = 10.0 Hz, H-7), 5.24 (ddd, 1H, $^3J_{9,8}$ = 2.5 Hz, H-9), 5.06 (dd, 1H, $^3J_{5,6}$ = 3.5 Hz, H-6), 4.38 (dd, 1H, $^3J_{9,10a}$ = 5.3 Hz, $^2J_{10a,10b}$ = 11.5 Hz, H-10a), 4.11 (dd, 1H, $^3J_{9,10b}$ = 7.3 Hz, H-10b), 3.93 (m, 1H, H-2), 3.85 (ddd, 1H, $^3J_{3a,4}$ = 8.3 Hz, H-4), 3.68 (dd, 1H, H-8), 2.20, 2.12, 2.06, 2.01, 1.97 (5s, 15H, Ac), 1.85 (dt, 1H, $^2J_{3a,3b}$ = 14.4 Hz, H-3a), 1.55 (ddd, 1H, $^3J_{3b,2}$ = 4.0 Hz, $^3J_{3b,4}$ = 5.1 Hz, H-3b), 1.19 (d, 3H, $^3J_{1,2}$ = 6.2 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 170.58 (d.i.), 170.37, 170.14, 169.55 (5C, CO, Ac), 77.07 (C-8), 76.87 (C-4), 72.51 (C-6), 69.75 (C-5), 67.05 (C-9), 65.57 (C-2), 64.80 (C-7), 62.17 (C-10), 39.11 (C-3), 23.59 (C-1), 20.76 (d.i.), 20.71, 20.64, 20.59 (5C, CH_3, Ac). Anal. Calcd for $C_{20}H_{30}O_{12}$: C, 51.94; H, 6.54. Found: C, 51.97; H, 6.53.

Further elution of the column gave **24** as a colourless syrup; yield: 35 mg (44%); $[\alpha]_D^{20} = -60$ (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.31 (dd, 1H, $^3J_{5,4}$ = 1.0 Hz, H-5), 5.28 (t, 1H, $^3J_{7,6} = {}^3J_{7,8}$ = 10.0 Hz, H-7), 5.21 (ddd, 1H, $^3J_{9,8}$ = 2.3 Hz, H-9), 5.07 (dd, 1H, $^3J_{5,6}$ = 3.5 Hz, H-6), 4.57 (dd, 1H, $^3J_{9,10a}$ = 6.5 Hz, $^2J_{10a,10b}$ = 11.3 Hz, H-10a), 4.03 (dd, 1H, $^3J_{9,10b}$ = 8.7 Hz, H-10b), 4.03 (m, 1H, H-2), 3.92 (ddd, 1H, $^3J_{3a,4}$ = 10.1 Hz, H-4), 3.61 (dd, 1H, H-8), 3.48 (m, 1H, OH), 2.19, 2.13, 2.07, 2.01, 1.96 (5s, 15H, Ac), 1.81 (ddd, 1H, $^2J_{3a,3b}$ = 14.4 Hz, $^3J_{3a,2}$ = 2.6 Hz, H-3a), 1.35 (ddd, 1H, $^3J_{3b,4}$ = 2.7 Hz, $^3J_{3b,2}$ = 10.3 Hz, H-3b), 1.20 (d, 3H, $^3J_{1,2}$ = 6.2 Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 170.81, 170.55, 170.42, 170.10, 169.62 (5C, CO, Ac), 76.39 (C-8), 74.70 (C-4), 72.71 (C-6), 70.73 (C-5), 67.19 (C-9), 64.99 (C-7), 63.77 (C-2), 61.50 (C-10), 39.63 (C-3), 24.18 (C-1), 20.81 (t.i.), 20.65, 20.60 (5C, CH_3, Ac). Anal. Calcd for $C_{20}H_{30}O_{12}$: C, 51.94; H, 6.54. Found: C, 51.78; H, 6.24.

4.1.14. 4,8-Anhydro-1,3-dideoxy-L-*lyxo*-L-*manno*-decitol 23. A solution of **22** (15 mg, 0.032 mmol) in dry MeOH (2 mL) was stirred with 0.1 M methanolic NaOMe (0.1 mL) for 3 h at room temperature. The solution was diluted with MeOH (10 mL), neutralized by addition of Dowex 50 resin (H^+ form), filtered and concentrated. The residue was purified on LH-20 to furnish **23** as colourless solid. Yield: 7.0 mg (85%); $[\alpha]_D^{20} = -9$ (*c* 0.6, MeOH); ^{1}H NMR (D_2O): δ 3.98 (m, 1H, H-2), 3.95 (ddd, 1H, $^3J_{9,8}$ = 1.8 Hz, H-9), 3.82 (dd, 1H, $^3J_{4,5}$ = 1.0 Hz, $^3J_{5,6}$ = 3.5 Hz, H-5), 3.78 (t, 1H, $^3J_{7,8} = {}^3J_{7,6}$ = 9.7 Hz, H-7), 3.71–3.63 (m, 3H, H-4, H-10a, H-10b), 3.63 (dd, 1H, H-6), 3.27 (dd, 1H, H-8), 1.89 (ddd, 1H, $^2J_{3a,3b}$ = 14.3 Hz, $^3J_{3a,4}$ = 8.9 Hz, $^3J_{3a,2}$ = 6.9 Hz, H-3a), 1.61 (ddd, $^3J_{3b,4}$ = 4.5 Hz, $^3J_{3b,2}$ = 5.9 Hz, H-3b), 1.19 (d, 3H, $^3J_{1,2}$ = 6.3 Hz, H-1); ^{13}C NMR (D_2O): δ 79.36 (C-8), 76.83 (C-4), 75.30 (C-6), 71.98 (C-59), 69.66 (C-9), 67.17 (C-7), 66.42 (C-2), 63.93 (C-10), 39.61 (C-3) and 22.46 (C-1). Anal. Calcd for $C_{10}H_{20}O_7 \cdot H_2O$: C, 44.44; H, 8.20. Found: C, 44.69; H, 7.12.

4.1.15. 4,8-Anhydro-1,3-dideoxy-L-*lyxo*-L-*gluco*-decitol 25. A solution of **24** (15 mg) was treated as described above. Yield of **25**: 8.0 mg (97%) as colourless solid. $[\alpha]_D^{20} = -46$ (*c* 0.8, MeOH); ^{1}H NMR (D_2O): δ 3.99–3.87 (m, 2H, H-2, H-9); 3.73 (t, 1H, $^3J_{7,8} = {}^3J_{7,6}$ = 9.7 Hz, H-7), 3.77–3.63 (m, 5H, H-4, H-5, H-6, H-10a, H-10b), 3.23 (dd, 1H, $^3J_{8,9}$ = 1.6 Hz, H-8), 1.80 (ddd, 1H, $^2J_{3a,3b}$ = 14.6 Hz, $^3J_{3a,4}$ = 10.3 Hz, $^3J_{3a,2}$ = 3.5 Hz, H-3a), 1.47 (ddd, $^3J_{3b,4}$ = 2.7 Hz, $^3J_{3b,2}$ = 9.3 Hz, H-3b), 1.16 (d, 3H, $^3J_{1,2}$ = 6.4 Hz, H-1); ^{13}C NMR (D_2O): δ 78.96 (C-8), 75.46 (C-4), 75.40 (C-6), 72.60 (C-5), 69.72 (C-9), 67.17 (C-7), 65.35 (C-2), 63.65 (C-10), 40.36 (C-3) and 23.45 (C-1). Anal. Calcd for $C_{10}H_{20}O_7 \cdot 0.4H_2O$: C, 46.61; H, 8.04. Found: C, 46.16; H, 7.67.

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