Recl. Trav. Chim. Pays-Bas 111, 144–148 (1992) 0165–0513/92/03144–05$1.75

Synthesis of two branched heptopyranoside- (L,D-Hep*p*)-containing trisaccharides of the inner-core region of *Citrobacter PCM* 1487

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(Received October 10th, 1991)

Abstract. The diastereogenic addition of (phenyldimethylsilyl)magnesium chloride to methyl 3-*O*-allyl-2,4-di-*O*-benzyl-α-D-*manno*-hexodialdo-1,5-pyranoside gave a L-*glycero*-D-*manno*-heptopyranoside (L,D-Hep*p*) derivative having at C-7 the phenyldimethylsilyl function as a hydroxy-masking group. The L,D-Hep*p* derivative was converted into two acceptors which could be applied successfully for the preparation of the trisaccharides L-α-D-Hep*p*-(1→3)-*O*-[L-α-D-Hepp-(1→7)]-L-α-D--Hep*p*-O-Me **20** and α-D-Glc*p*-(1→3)-*O*-[L-α-D-Hep*p*-(1→7)]-L-D-Hep*p*-O-Me **26**.

Introduction

Lipopolysaccharides (LPS) are constituents[2] of the outer membrane of Gram-negative bacteria. They are composed of a lipid part, the Lipid A moiety, and a polysaccharide, comprising an oligomeric repeating unit (the O-specific chain) and a core oligosaccharide. The core connects the O-specific chain to lipid A which tethers the LPS into the cell envelope. Core oligosaccharides have a rather conserved composition among different Gram-negative bacteria and are characterised by the presence of the uncommon seven- and eight-carbon sugars L-*glycero*-D-*manno*-heptopyranoside (L,D-Hep*p* see for derivatives *e.g.* **4**, **9**) and 3-deoxy-D-*manno*-2-octulosonic acid (KDO), respectively. The immunoreactivity of core oligosaccharides[3] has received special attention in relation to endotoxin neutralization by antibodies directed against the inner-core LPS region.

As part of an ongoing program directed toward the synthesis of immunogenic inner-core fragments, we have developed convenient methods for the preparation of these rare sugars L,D-Hep*p*[3,4] and KDO[5].

With the objective of widening the scope of our synthetic route to L,D-Hep*p* building units, we now report in detail[6] the assembly of the trisaccharides **20** and **26** which have been proposed[7] (Figure 1) to be part of the inner-core region of *Citrobacter* PCM 1487.

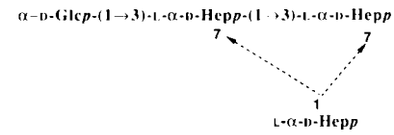


Figure 1. Alternative locations of the branched terminal heptose are marked by dashed lines.

Results and discussion

The availability of suitably protected L,D-Hep*p* donors and acceptors is a key element in the assembly of trisaccharides **20** and **26**. Recently, we showed[5] that L,D-Hep*p* derivatives are easily accessible by a two-step stereoselective hydroxymethylation method. In this approach, *anti*-Cram addition[8] of (phenyldimethylsilyl)methylmagnesium chloride (**1**) to the fully benzylated methyl α-D-*manno*-hexodialdo-1,5-pyranoside **2** constitutes the key carbon-bond-forming step: the resulting α-hydroxy silane adduct **3** can be converted to homologous L,D-Hep*p* **4** by oxidative generation[9] of the hydroxy group at C-7 from the silyl moiety. On the other hand, unmasking of the phenyldimethylsilyl (PDMSi) moiety can also be executed at an advanced stage of the synthesis. The latter option opens the way to introduce glycosidic bonds at C-6 or C-7 of the L,D-Hep*p* acceptors.

The usefulness of the above cited stereoselective L,D-Hep*p* synthesis can be extended further by using a D-*manno*--hexodialdo-1,5-pyranoside carrying different protecting groups in the carbon-bond-forming step: additional protective-group manipulations of the resulting Grignard addition

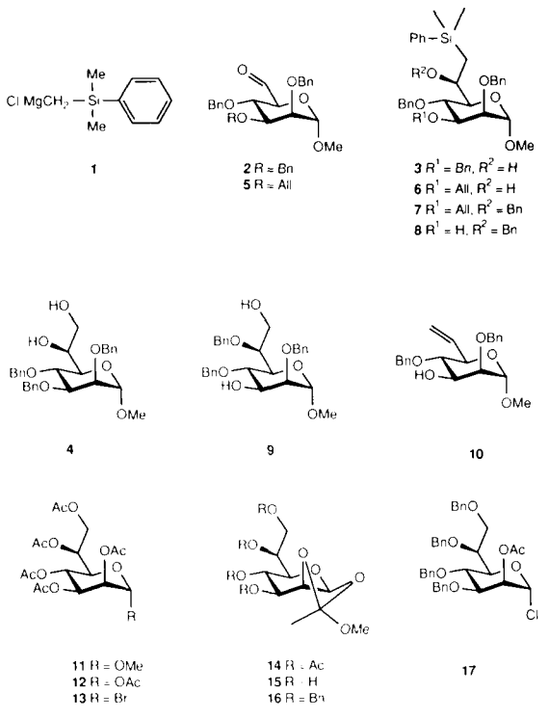
Abbreviations:

All = allyl
Bn = benzyl
DMF = *N*,*N*-dimethylformamide
Glc*p* = glucopyranoside
KDO = 3-deoxy-D-*manno*-2-octulosonic acid
L,D-Hep*p* = L-*glycero*-D-*manno*-heptopyranoside
LPS = lipopolysaccharides
PDMSi = phenyldimethylsilyl (IUPAC order: dimethylphenylsilyl)
THF = tetrahydrofuran
TLC = thin-layer chromatography
triflate = trifluoromethanesulfonate

product may give access to L,D-Hep*p* acceptors which allow glycosylation of the ring and external hydroxy groups.

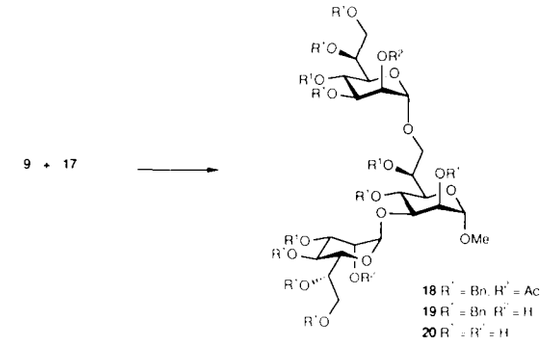
For example, the L,D-Hep*p* derivative **6**, which can be obtained by condensing **1** with known[10] methyl 3-*O*-allyl--2,4-di-*O*-benzyl-α-D-*manno*-hexodialdo-1,5-pyranoside (**5**), gives access to the acceptors **8** and **9** which, in turn, may serve as L,D-Hep*p* building units in the preparation of trisaccharides **20** and **26**, respectively. In addition, the L,D-Hep*p* derivative **4** can be readily converted into L,D-Hep*p* derivative **17**, which proved to be a suitable donor in the synthetic routes to the target trisaccharides.

Thus, addition of **1** in 1,3-dioxolane to **5** at 0° occurred with high diastereofacial selectivity to give **6** (d.p. 95%) in 72% yield. Benzylation of **6** according to *Czernecki* et al.[11] gave **7**. Deallylation[12] of **7** with palladium on carbon (10%) in the presence of *p*-toluenesulfonic acid in a mixture of MeOH + H_2O at 70° for 8 h gave the glycosyl acceptor **8** in 70% yield. In this respect, it is interesting to note that removal of the allyl ether was accompanied by the formation of a small amount (6%) of the *Peterson* elimination[13] product **10**. Moreover, the residual sulfur-containing impurities, resulting from the Swern oxidation of methyl 3-*O*--allyl-2,4-di-*O*-benzyl-α-D-mannopyranoside to give **5**, impeded the deallylation of **7**. Moreover, an increase in the formation of the unwanted product **10** was observed.



The second glycosyl acceptor **9** was isolated, following the procedure of *Flemming*[9], in a good yield by generating the hydroxy group from the PDMSi moiety in **8** with peroxyacetic acid and potassium bromide in AcOH + NaOAc with the exclusion of light. On the other hand, glycosyl donor **17** was synthesised in an overall yield of 65% starting from **11**, which was obtained[4] in three steps from **4**. Acetolysis (HOAc + H_2SO_4) of **11** gave **12**, which was quantitatively converted (HBr + AcOH) into the known[4] α-glycosyl bromide **13**. Transformation of **13** into the α-glycosyl chloride **17** was executed via intermediates **14**, **15** and **16**, according to *Paulsen* et al.[14].

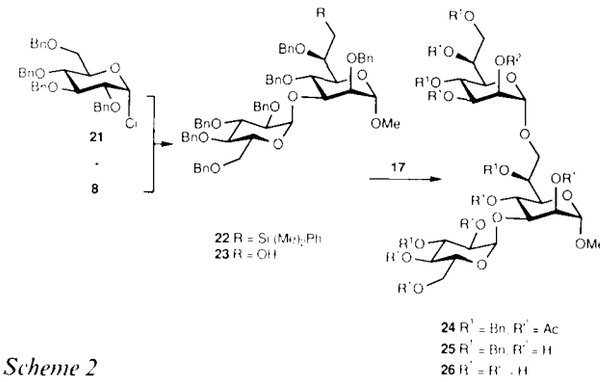
Having the key L,D-Hep*p* donors and acceptors in hand, we now turned our attention to the assembly of the target trisaccharides **20** and **26**. In the first instance, the trisaccharide **20** was obtained by glycosylation of C-3,7 hydroxyls in the L,D-Hep*p* acceptor **9** with L,D-Hep*p* donor **17**, followed by removal of all protective groups (Scheme 1). Thus, condensation of acceptor **9** with excess donor **17** under the conditions described by *Hanessian* and *Banoub*[15] in the absence of 1,1,3,3-tetramethylurea gave a 71% yield of trisaccharide **18**.



Scheme 1

Zemplén deacylation of **18** led to the isolation of **19** in 95% yield. Hydrogenolysis of **19** in the presence of 10% Pd-C in EtOH gave the target methyl *O*-L-*glycero*-α-D-*manno*-heptopyranosyl-(1→3)-*O*-[L-*glycero*-α-D-*manno*-heptopyranoside-(1–7)-L-*glycero*-D-*manno*-heptopyranoside **20** in 85% yield. The structural integrity of **20** was fully established by homo- and heteronuclear-correlation NMR spectroscopy.

In the next stage, the more complex trisaccharide **26** was assembled as outlined in Scheme 2. Thus, α-stereospecific coupling of glucosyl chloride **21**[16] with L,D-Hep*p* acceptor **8** could be accomplished using the promoter silver triflate[15] to give, after purification, anomerically pure disaccharide **22** in 90% yield indicating that the PDMSi masking group in acceptor **8** was compatible with the glycosylation conditions. In order to introduce the required glycosidic bond at C-7, the PDMSi function in **22** was unmasked by oxidation[9]



Scheme 2

to give **23**. Silver triflate promoted coupling of acceptor **23** with glycosyl chloride donor **17** gave the fully protected trisaccharide **24** in a yield of 89%. Deblockling of **24** to give the fully deprotected trisaccharide **26** was executed by Zemplén deacylation of **24** and hydrogenolysis of **25** to yield homogeneous **26**, the ^1H- and ^{13}C-NMR data of which were in complete accordance with the proposed structure.

In conclusion, the steroselective addition of (dimethylphenylsilyl)methylmagnesium chloride to properly protected α-D-*manno*-hexodialdo-1,5-pyranosides gives easy access to valuable L,D-Hep*p* units suitable for oligosaccharide synthesis.

Experimental

General methods and materials

Dichloromethane, 1,2-dichloroethane and toluene were distilled from P_2O_5 and stored over molecular sieves 4 Å. Diethyl ether and THF were distilled from CaH_2 and redistilled from $LiAlH_4$ before use. DMF was stirred with CaH_2 for 16 h, then distilled under reduced pressure and stored over molecular sieves 4 Å. Methanol was dried by refluxing with magnesium methoxide, distilled and stored over molecular sieves 3 Å. $TiBr_4$ was sublimed under reduced pressure and stored as a standard solution in dichloromethane. Silver triflate was purchased from Fluka and dried, with the exclusion of light, under reduced pressure for 18 h before use. Reactions were performed under strict anhydrous conditions unless noted otherwise. Evaporation was carried out below 40°C under reduced pressure.

Column chromatography was performed using silica gel (Merck, 70–230 mesh). Gel filtration was performed on Sephadex LH-20 (Pharmacia). TLC analyses were conducted on Schleicher and Schüll DC fertigfolien F 1500 LS 254. Compounds were visualized by UV light (254 nm) or by spraying with concd. H_2SO_4/methanol (1/10, v/v) and charring at 140°C.

Optical rotation was determined with a Perkin–Elmer Model 241 polarimeter at 20°C. NMR spectra were recorded with a Yeol JNM-FX200 (^{13}C and ^1H at 200 and 80.7 MHz, respectively), or a Bruker WM-300 spectrometer equipped with an Aspect-2000 computer (^1H, 300 MHz). Chemical shifts are given in ppm (δ) relative to TMS as internal standard.

Methyl 3-O-allyl-2,4-di-O-benzyl-7-(phenyldimethylsilyl)-7-deoxy-L-glycero-α-D-manno-heptopyranoside (**6**)

Aldehyde **5** (4.54 g, 11 mmol) was dissolved in THF (45 ml) and the mixture was added dropwise to a cooled (0°C) solution of Grignard reagent **1** (25 mmol) in THF (25 ml). After stirring the reaction mixture for 2 h at 0°C, TLC analysis (acetone/CH_2Cl_2, 1/19, v/v) indicated complete conversion of **5** into **6** (R_f 0.8). The mixture was slowly poured into aqueous ammonium chloride (50 ml, 20%) and extracted with CH_2Cl_2 (200 ml). The organic layer was washed with water (50 ml), dried over $MgSO_4$ and concentrated under reduced pressure. The residual oil was applied onto a column of silica gel (45 g) and eluted with CH_2Cl_2 followed by CH_2Cl_2/acetone (99/1→49/1, v/v). Concentration of the appropriate fractions gave **6** as an oil. Yield 3.6 g (58%), R_f (light petroleum/ether, 2/1, v/v), $[\alpha]_D$ +25.0° (*c* 0.8, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.60–7.25 (m, 15H, H-arom), 5.95 (m, 1H, CH=, All), 5.30 (m, 2H, $=CH_2$, All), 4.95–4.60 (m, 4H, CH_2, Bn), 4.74 (s, 1H, H-1), 4.12 (2H, OCH_2, All), 4.08–3.29 (5H, H-2, H-3, H-4, H-5, H-6), 3.28 (s, 3H, OCH_3), 1.40 (dd, 1H, H-7a), 0.9 (dd, 1H, H-7b), 0.41 (2× s, 6H, CH_3Si). ^{13}C NMR ($CDCl_3$): δ 138.4, 138.3 (Cq, arom), 134.9 (CH=, All), 133.7–133.6 (CH, arom), 116.5 ($=CH_2$, All), 99.5 (C-1), 79.9, 75.3, 72.8, 71.0 (C-2, C-3, C-4, C-5), 67.2 (C-6), 69.7 (CH_2O, All), 54.6 (OCH_3), 21.8 (C-7), −2.0, −2.2 ($SiCH_3$). Anal. calcd. for $C_{33}H_{42}O_6Si$: C 70.43, H 7.52; found: C 70.38, H 7.50%.

Methyl 3-O-allyl-2,4,6-tri-O-benzyl-7-(phenyldimethylsilyl)-7-deoxy-L-glycero-α-D-manno-heptopyranoside (**7**)

Tetra-*n*-butylammonium iodide (0.36 g, 0.97 mmol), sodium hydride (0.35 g, 14.5 mmol) and benzyl bromide (1.7 ml, 14.5 mmol) were added to a cooled (0°C) solution of **6** (5.45 g, 9.7 mmol) in MF (30 ml). After stirring the reaction mixture for 3 h at 20°C, TLC analysis (light-petroleum/ether, 2/1, v/v) indicated complete conversion of **6** into **7** (R_f 0.7). The reaction mixture was quenched by the addition of methanol (5 ml) and the solvents were evaporated. The residue was partitioned between CH_2Cl_2 (150 ml) and water (2× 50 ml). The organic layer was dried over $MgSO_4$ and concentrated to an oil, which was applied onto a column of silica gel (100 g). Elution of the column was effected with light-petroleum/ether (9/1→4/1, v/v). The appropriate fractions were concentrated *in vacuo* to give **7** as an oil. Yield 6.12 g (97%); R_f 0.7 (light-petroleum/ether, 2/1, v/v), $[\alpha]_D$ +24.6° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.51–7.12 (m, 20H, H-arom), 5.90 (m, 1H, CH=, All), 5.16 (m, 2H, $=CH_2$, All), 4.83–4.54 (m, 6H, CH_2, Bn), 4.73 (s, 1H, H-1), 4.12 (2H, OCH_2, All), 4.24–3.30 (5H, H-2, H-3, H-4, H-5, H-6), 3.26 (s, 3H, OCH_3), 1.42 (m, 2H, H-7a,b), 0.32 (s, 6H, CH_3Si). ^{13}C NMR ($CDCl_3$): δ 138.9, 138.3 (Cq, arom), 134.9 (CH=, All), 129.3–127.6 (CH, arom), 116.4 ($=CH_2$, All), 99.1 (C-1), 80.1, 74.9, 74.5, 74.3, 72.9 (C-2, C-3, C-4, C-5, C-6), 74.3, 72.3, 70.7 (3× CH_2, Bn), 69.8 (CH_2O, All), 54.9 (OCH_3), 17.1 (C-7), −2.0, −2.2 ($SiCH_3$). Anal. calcd. for $C_{40}H_{48}O_6Si$: C 73.59, H 7.41; found: C 73.48, H 7.52%.

Methyl 2,4,6-tri-O-benzyl-7-(phenyldimethylsilyl)-7-deoxy-L-glycero-α-D-manno-heptopyranoside (**8**)

p-Toluenesulfonic acid (1.8 g) and palladium on active carbon (1.8 g, 10%) was added to **7** (6.12 g, 9.4 mmol) in a mixture of methanol/water (120 ml, 5/1, v/v). After heating the reaction for 8 h under reflux, TLC analysis (acetone/CH_2Cl_2, 1/99, v/v) showed the absence of **7**. The reaction mixture was cooled to room temperature, neutralized with triethylamine, filtered over a pad of celite and the filtrate was concentrated under reduced pressure. The oily product was dissolved in CH_2Cl_2 (75 ml) and washed with water (25 ml). The organic phase was dried over $MgSO_4$ and concentrated to an oil which was applied onto a column of silica gel (60 g). Elution of the column was effected with a mixture of light-petroleum/ethyl acetate (5/95, 15/85→v/v). The appropriate fractions were concentrated to give **8** as an oil. Yield 4.2 g (73%); R_f 0.6 (light-petroleum/ether, 2/1, v/v), $[\alpha]_D$ +21.6° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.52–7.20 (20H, H-arom), 4.90 (d, 1H, H-1, $J_{1,2}$ 1.8 Hz), 4.80–4.20 (m, 6H, 3× CH_2, Bn), 4.02 (m, 1H, H-6, $J_{5,6}$ 1.3 Hz, $J_{6,7a}$ 1.7 Hz, $J_{6,7b}$ 4.0 Hz), 3.90 (bs, 1H, H-3), 3.77 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.2 Hz), 3.68 (dd, 1H, H-2, $J_{2,3}$ 3.7 Hz), 3.44 (dd, 1H, H-5), 3.29 (s, 3H, OCH_3), 2.25 (bs, 1H, OH), 1.43, 1.41 (2× d, 2H, H-7a, H-7b). Anal. calcd. for $C_{37}H_{44}O_6Si$: C 72.52, H 7.24; found: C 72.61, H 7.48%.

Methyl 2,4,6-tri-O-benzyl-L-glycero-α-D-manno-heptopyranoside (**9**)

Acetic acid (5 ml), sodium acetate (0.65 g) and potassium bromide (95 mg, 0.8 mmol) were added to **8** (0.39 g, 0.64 mmol) and the mixture was stirred until the salts were dissolved. The solution was cooled (10°C) and peracetic acid (3.3 ml, 30% in acetic acid) was added dropwise under exclusion of ight. During the addition, gas was liberated. After the mixture was stirred for 1 h at 20°C, TLC analysis (acetone/CH_2Cl_2, 3/97, v/v) indicated complete conversion of the starting material into a more hydrophilic product (R_f 0.2). The mixture was diluted with CH_2Cl_2 (25 ml) and poured in an aqueous solution of sodium thiosulfate (5 ml, 15%). The organic layer was washed with aqueous sodium bicarbonate (10 ml, 10%) and water (10 ml), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in toluene (2× 10 ml) and concentrated. The oily product was applied onto a column of silica gel (10 g) and elution was effected with CH_2Cl_2 followed by CH_2Cl_2/methanol (99/1→49/1, v/v). The appropriate fractions were concentrated to give **9** as an oil. Yield 0.26 g (82%), R_f 0.2 (methanol/CH_2Cl_2, 3/97, v/v), $[\alpha]_D$ +3.7° (*c* 1, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 138.2–137.5 (Cq, arom), 128.4–127.3 (CH, arom), 97.7 (C-1), 80.0, 75.8, 71.7, 71.4 (C-2, C-3, C-4, C-5, C-6), 74.0, 72.4, 71.9 (3× CH_2, Bn), 61.9 (C-7), 54.6 (OCH_3). Anal. calcd. for $C_{29}H_{34}O_7$: C 70.43, H 6.93; found: C 70.56, H 6.93%.

Methyl O-(2-O-acetyl-3,4,6,7-tetra-O-benzyl-L-glycero-D-manno-heptopyranosyl)-(1→3)-O-[2-O-acetyl-3,4,6,7-O-L-glycero-D-manno-heptopyranosyl(1→7)]-2,3,6-tri-O-benzyl-L-glycero-α-D-manno-heptopyranoside (**18**)

Activated powdered molecular sieves (0.5 g) was added to a solution of compound **9** (0.1 g, 0.17 mmol) in CH_2CCl_2 (3 ml) and the mixture was stirred for 2½ h in an atmosphere of nitrogen. Silver triflate (0.15 g, 0.6 mmol) was added under the exclusion of light. The mixture was cooled to −30°C and chloride **17** (0.4 g, 0.64 mmol) in CH_2Cl_2 (2 ml) was added dropwise over a period of 30 min. The reaction mixture was stirred at −20°C for 2 h. The mixture was filtrated and the filtrate was diluted with CH_2Cl_2 (15 ml) and washed with aqueous sodium thiosulfate (10 ml), aqueous sodium bicarbonate (10 ml) and water (10 ml), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was applied onto a column of silica gel (2 g) and eluted with light-petroleum/ethyl-acetate (85/15, v/v). Fractions containing the required dimer were concentrated under reduced pressure and the residual oil was applied on a LH-20 Sephadex column (eluent, CH_2Cl_2/methanol, 1/1, v/v). Concentration of the appropriate fractions gave pure **18**

in a yield of 0.205 g (71%). R_f 0.6 (acetone/CH_2Cl_2, 3/97, v/v), $[\alpha]_D$ +31.6° (*c* 0.95, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.35–7.05 (m, 55H, H-arom), 5.43 (t, 1H, H-2′, $J_{2,3}$, $J_{1,2}$ 2.1 Hz), 5.26 (t, 1H, H-2″, $J_{2,3}$, $J_{1,2}$ 2.5 Hz), 5.17 (d, 1H, H-1′), 4.82 (d, 1H, H-1″), 4.48 (d, H, H-1, $J_{1,2}$ 1.9), 3.91 (2× dd,, 2H, H-3′, H-3″), 3.60 (dd, H-2, $J_{2,3}$ 3.2 Hz), 4.79–3.43 (m, 38H, 11× CH_2, Bn. H-3–H-7a,b, H-4′–H-7′a,b, H-4″–H-7″a,b), 3.09 (OCH_3), 2.10, 1.98 (2× s, 6H, CH_3, Ac). ^{13}C NMR ($CDCl_3$): δ 170.1, 169.7 (C=O, Ac), 138.8–137.4 (Cq, arom), 128.3–137.4 (CH, arom), 100.1, 98.2 (C-1, C-1′, C-1″), 78.4, 77.9, 76.9, 75.1, 74.8, 73.6, 71.8, 71.5, 86.3, (C-2 to C-6, C-2′ to C-6′, C-2″ to C-6″), 74.5, 74.2, 73.2, 72.8, 72.6, 72.5, 72.2, 71.2, 70.3, 66.8 (CH_2, Bn, C-7, C-7′, C-7″), 54.6 (OCH_3), 20.9, 20.8 (CH_3, Ac). Anal. calcd. for $C_{103}H_{110}O_{21}$: C 73.46, H 6.58; found: C 73.55, H 6.53%.

Methyl O-*(3,4,6,7-tetra*-O-*benzyl*-L-glycero-α-D-manno-*heptopyranosyl)-(*1→3*)*-O-*[3,4,6,7-tetra*-O-*benzyl*-L-glycero-α-D-manno-*heptopyranosyl-(*1→7*)]-2,4,6-tri*-O-*benzyl*-L-glycero-α-D-manno-*heptopyranoside* (**19**)

Methanolic sodium methoxide (1M, 0.05 ml) was added to a solution of **18** (0.205 g, 0.12 mmol) in methanol (5 ml) and the reaction mixture was stirred for 2 h at 20°C. The mixture was neutralized with Dowex 50 XW4 (H^+ form) resin (100–200 mesh), filtered and concentrated under reduced pressure. The oily residue was applied on to a column of silica gel (2 g) and eluted with CH_2Cl_2 followed by a mixture of acetone/CH_2Cl_2 (1/99 → 3/97, v/v). Concentration of the appropriate fractions gave **19** as an oil. Yield 0.18 g (94%); R_f 0.5 (acetone/CH_2Cl_2, 3/97, v/v). ^{13}C NMR ($CDCl_3$): δ 138.7–137.5 (Cq, arom), 128.3–127.0 (CH, arom), 99.8 (C-1, C-1′, C-1″), 80.0, 75.1, 74.3, 73.4, 71.3, 71.0, 67.2, 67.8 (C-2–C-6, C-2′–C-6′, C-2″–C-6″), 74.4, 74.2, 73.2, 72.8, 72.4, 71.7, 71.4, 70.2, 66.88 (CH_2, Bn, C-7, C-7′, C-7″), 54.6 (OCH_3). Anal. calcd. for $C_{99}H_{106}O_{19}$: C 74.32, H 6.68; found: C 74.35, H 6.63%.

*Methyl (*L-glycero-α-D-manno-*heptopyranosyl)-(*1→3*)*-O-*[*L-glycero-α-D-manno-*heptopyranosyl-(*1→7*)]*-L-glycero-α-D-manno-*heptopyranosyl* (**20**)

Compound **19** (0.18 g) was dissolved in methanol and after addition of 200 mg Pd/C (10%), the suspension was kept under an atmosphere of hydrogen during 42 h at 1 atmosphere. The catalyst was removed by filtration and washed with water. The combined filtrates were concentrated under reduced pressure to give **20** (50 mg) as an amorphous material. R_f 0.2 (methanol/ethyl-acetate/water, 3/5/2, v/v), $[\alpha]_D$ +114.2° (*c* 1, water). ^1H NMR (D_2O): δ 5.11 (d, 1H, H-1, $J_{1,2}$ 1.8 Hz), 4.87 (d, 1H, H-1″, $J_{1,2}$ 1.7 Hz), 4.71 (d, 1H, H-1′, $J_{1,2}$ 1.7), 4.17 (m, 1H, H-6′), 4.03 (dd, 1H, H-2, $J_{2,3}$ 3.0 Hz), 3.99 (dd, 1H, H-2′, $J_{2,3}$ 3.0 Hz), 3.96 (dd, 1H, H-2″, $J_{2,3}$ 3.0 Hz), 3.93 (t, 1H, H-4′, $J_{3,4}$, $J_{4,5}$ 9.7 Hz), 3.85 (dd, 1H, H-3, $J_{3,4}$ 7.8 Hz), 3.80 (dd, 1H, H-3′), 3.68 (t, 1H, H-4, $J_{4,5}$ 7.3), 3.54 (dd, 1H, H-5′, $J_{5,6}$ 1.7 Hz), 3.36 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$): δ 103.4 (C-1), 101.9 (C-1′), 101.6 (C-1″), 72.4 (C-5′), 71.0 (C-2), 68.2 (C-2), 79.1, 72.7, 72.3, 71.7, 71.4, 70.0, 69.7, 67.0, 66.5, 63.9, 63.8 (C-3–C-5, C-7, C-2′–C-4′, C-6′, C-7′, C-2′–C-7″), 55.7 (OCH_3). $J_{C-1,H-1}$ 172.9, $J_{C-1',H-1'}$ 174.4 Hz, $J_{C-1'',H-1''}$ 171.5 Hz.

Methyl 2,4,6-tri-O-*benzyl-3*-O-*(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)-7-deoxy-7-(phenyldimethylsilyl)*-L-glycero-D-manno-*heptopyranoside* (**22**)

Activated powdered molecular sieves (0.5 g) was added to a solution of compound **8** (0.17 g, 0.28 mmol) in diethylether (2 ml) and the mixture was stirred for 2 h in an atmosphere of nitrogen. Silver triflate (0.13 g, 0.52 mmol) was added with the exclusion of light. The mixture was cooled to −30°C and the chloride **21** (0.44 mmol) in diethyl ether (2 ml) was added over a period of 30 min. The reaction mixture was stirred at −20°C for 16 h. The mixture was filtered and the filtrate was diluted with CH_2Cl_2 (25 ml) and washed with aqueous sodium thiosulfate (10 ml), aqueous sodium bicarbonate (10 ml), water (10 ml), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was applied to a column of silica gel (3 g) and eluted with light-petroleum/ethyl-acetate (95/5 → 85/15, v/v). The dimer containing fractions were concentrated under reduced pressure and the residual oil was applied on a LH-20 Sephadex column (eluent, CH_2Cl_2/methanol, 1/1, v/v). Concentration of the appropriate fractions gave pure **22** in a yield of 0.29 g (90%). R_f 0.5 (acetone/CH_2Cl_2, 1/97, v/v): $[\alpha]_D$ +32.1° (*c* 0.95, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.49–7.07 (40H, H arom), 5.12 (d, 1H, H-1′, $J_{1,2}$ 3.4 Hz), 4.88 (d, 1H, H-1, $J_{1,2}$ 2.7), 4.15 (t, 1H, H-4, $J_{3,5}$, $J_{4,5}$ 11.8 Hz), 4.07 (dd, 1H, H-3), 4.01 (m, 1H, H-6), 3.89 (t, 1H, H-2, $J_{1,2}$ 2.3 Hz), 3.55 (dd, 1H, H-3), 3.49 (q, 1H, H-2′), 5.05–3.45 (19H, 8× CH_2, Bn, H-3′ to H-6′a,b), 3.27 (s, 3H, OCH_3), 1.42 (d, 2H, H-7a,b, $J_{6,7}$ 7.0 Hz), 0.33, 0.32 (2× s, 6H, CH_3Si). ^{13}C NMR ($CDCl_3$): δ 139.1–137.8 (Cq, arom), 133.4–126.6 (CH, arom), 98.8, 97.0 (C-1, C-1′), 81.6, 79.6, 77.7, 77.4, 75.2, 74.6, 72.9, 70.8 (C-2–C-6, C-2′–C-5′), 75.2, 74.5, 73.7, 73.2, 72.6, 71.4, 69.6, 68.6 (CH_2, Bn, C-6′), 54.9 (OCH_3), 17.8 (C-7), −2.3, −2.4 (CH_3Si). Anal. calcd. for $C_{71}H_{78}O_{11}$: C 75.10, H 6.92; found: C 75.31, H 6.93%.

Methyl 2,4,6-tri-O-*benzyl-3*-O-*(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)*-L-glycero-α-D-manno-*heptopyranoside* (**23**)

Compound **22** (0.29 g, 0.25 mmol) was oxidized, as described earlier for **8→9**, to give, after further work up and purification, homogeneous **23** in a yield of 140 mg (53%), R_f 0.4 (acetone/CH_2Cl_2, 1/97, v/v); $[\alpha]_D$ +41.7° (*c* 0.1, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 139.5–138.2 (Cq, arom), 129.8–126.9 (CH, arom), 98.8, 97.2 (C-1, C-1′), 81.6, 79.5, 77.7, 77.2, 75.7, 74.6, 73.9, 73.1, 70.9 (C-2–C-6, C-2′–C-5′), 75.3, 74.5, 73.3, 72.7, 71.7, 71.4 (CH_2, Bn), 68.6 (C-6′), 62.3 (C-7), 54.8 (OCH_3). Anal. calcd. for $C_{63}H_{68}O_{12}$: C 74.39, H 6.74; found: C 75.37, H 6.81%.

Methyl O-*(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(*1→3*)*-O-*[2-O-acetyl-3,4,6,7-tetra*-O-*benzyl*-α-L-glycero-D-manno-*heptopyranosyl-(*1→7*)]-2,4,6-tri*-O-*benzyl*-L-glycero-α-D-manno-*heptopyranoside* (**24**)

A mixture of compound **23** (0.140 mg, 0.09 mmol) and activated powdered molecular sieves (0.5 g) in CH_2Cl_2 (3 ml) was stirred for $2\frac{1}{2}$ h in an atmosphere of nitrogen. Under the exclusion of light, silver triflate (65 mg, 0.25 mmol) was added. The mixture was then cooled to −30°C and chloride **17** (0.135 g, 0.25 mmol) in CH_2Cl_2 (1 ml) was added over a period to 30 min. The reaction mixture was stirred at −20°C for 16 h. The mixture was filtered and the filtrate was diluted with CH_2Cl_2 (15 ml), washed with aqueous sodium thiosulfate (10 ml), sodium bicarbonate (10 ml) and water (10 ml). The organic phase dried over $MgSO_4$, was concentrated under reduced pressure and the residue was applied onto a column of silica gel (2 g). Elution of the column was effected with CH_2Cl_2 followed by a CH_2Cl_2/acetone mixture (99/1 → 97/2, v/v). Fractions containing the required dimer were concentrated under reduced pressure and the residual oil was applied on a LH-20 Sephadex column (eluent, CH_2Cl_2/methanol, 1/1, v/v). The appropriate fractions were concentrated to give pure **24** as an oil. Yield 0.20 g (89%); R_f 0.6 (acetone/CH_2Cl_2, 3/97, v/v), $[\alpha]_D$ +34.7° (*c* 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.40–7.10 (55H, H-arom), 5.35 (t, 1H, H-2″, $J_{1,2}$, $J_{2,3}$ 2.3 Hz), 5.15 (d, 1H, H-1′, $J_{1,2}$ 3.5 Hz), 4.90 (d, 1H, H-1′, $J_{1,2}$ 1.9 Hz), 4.79 (s, 1H, H-1), 4.24 (t, 1H, H-4, $J_{3,4}$, $J_{4,5}$ 1.9 Hz), 4.23 (t, 1H, H-4, $J_{3,4}$, $J_{4,5}$ 9.4 Hz), 4.13 (dd, 1H, H-3, $J_{2,3}$ 2.9 Hz), 3.98 (dd, 1H, H3″, $J_{3,4}$ 7.7 Hz), 3.90 (d, 1H, H-2, $J_{2,3}$ 2.3 Hz), 3.62 (d, 1H, H-5), 3.53 (dd, 1H, H-2′), $J_{2,3}$ 9.8 Hz), 4.91–3.49 (35H, 11× CH_2, Bn, H-6, H-7a,b, H-3′–H-6′a,b, H-4″–H-7″a,b), 3.21 (s, 3H, OCH_3), 2.15 (s, 3H, CH_3, Ac). ^{13}C NMR ($CDCl_3$): δ 170.2 (C=O, Ac), 139.2–137.7 (Cq, arom), 128.5–127.1 (CH, arom), 99.0, 98.3 (C-1, C-1′, C-1″), 81.9, 79.8, 78.3, 77.6, 75.0, 73.9, 71.5, 71.3, 68.5 (C-2–C-6, C-2′–C-5′, C-2″–C-6″), 75.6, 74.8, 73.8, 73.5, 72.8, 72.6, 72.0, 71.7, 70.6, 70.2, 67.8 (11× CH_2, Bn, C-7, C-7″, C-6′), 55.1 (OCH_3), 21.1 (CH_3, Ac). Anal calcd. for $C_{100}H_{106}O_{19}$: C 74.51, H 6.63; found: C 74.39, H 6.61%.

Methyl O-*(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(*1→3*)*-O-*[3,4,6,7-tetra*-O-*benzyl*-L-glycero-α-D-manno-*heptopyranosyl-(*1→7*)]*-L-glycero-α-D-manno-*heptopyranoside* (**25**)

Potassium *tert*-butoxide (5 mg) was added to a solution of compound **24** (0.150 g, 0.09 mmol) in methanol (3 ml). After stirred for 2 h, the reaction mixture was neutralized with Dowex 50 XW4 (H^+ form) resin (100–200 mesh), filtered and concentrated under reduced pressure. The residue was purified by silica gel (3 g) column chromatography (eluent: CH_2Cl_2 followed by acetone/CH_2Cl_2, 1/99 → 3/99, v/v). Concentration of the appropriate fractions gave **25** as an oil. Yield 0.115 g (81%); R_f 0.5

(acetone/CH_2Cl_2, 3/97, v/v); $[\alpha]_D$ +43.8° (*c* 1.05, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 139.0–137.6 (cq, arom), 128.4–126.9 (CH, arom), 99.7, 90.0, 97.8 (C-1, C-1′, C-1″), 80.1, 79.5, 77.7, 77.3, 74.8, 73.8, 73.5, 71.2, 70.9, 67.9 (C-2–C-6, C-2′–C-5′, C-2″–C-6″), 75.4, 74.5, 74.1, 73.4, 73.3, 72.6, 71.6, 71.4, 70.5, 68.5, 67.7 (CH_2, Bn. C-7, C-7″, C-6′), 54.9 (OCH_3). Anal. calcd. for $C_{98}H_{104}O_{18}$: C 74.98, H 6.68; found: C 74.87, H 6.74%.

Methyl O-*(α-D-glucopyranosyl)-(***1→3***)*-O-*[α-L*-glycero-*D*-manno-*-heptopyranosyl-(***1→7***)]-L*-glycero-*α-D*-manno-*heptopyranoside* (**26**)

A mixture of **25** (0.115 g, 0.07 mmol) and 10% Pd-C (0.7 g) in methanol (3 ml) was stirred under an atmosphere of H_2 for 48 h at 20°C. The catalyst was removed by filtration and washed with water. The combined filtrates were concentrated under reduced pressure to give **26** (31 mg) as an amorphous material. R_f 0.3 (methanol/ethyl-acetate/water, 3/5/2, v/v/v), $[\alpha]_D$ +112.3° (*c* 1, water). ^1H NMR (D_2O): δ 5.19 (d, 1H, H-1′, $J_{1,2}$ 3.9 Hz), 4.88 (d, 1H, H-1″, $J_{1,2}$ 1.8 Hz), 4.71 (d, 1H, H-1, $J_{1,2}$ 1.9 Hz), 4.17 (ddd, 1H, H-6, $J_{5,6}$ 1.5 Hz, $J_{6,7a}$ 7.0 Hz, $J_{6,7b}$ 8.0 Hz), 4.06 (dd, 1H, H-2, $J_{2,3}$ 3.3 Hz), 4.02 (ddd, 1H, H-6″, $J_{5,6}$ 5.0 Hz, $J_{6,7a}$ 1.5 Hz, $J_{6,7b}$ 7.5 Hz), 4.01 (t, 1H, H-4, $J_{3,4}$, $J_{4,5}$ 9.8 Hz), 3.96 (dd, 1H, H-2″, $J_{2,3}$ 3.0 Hz), 3.84 (dd, 1H, H-3), 3.82 (dd, 1H, H-3″), 3.78 (dd, 1H, H-5′, $J_{5,6}$ 1.7 Hz, $J_{4,5}$ 9.7 Hz), 3.76 (t, 1H, H-3′, $J_{3,4}$, $J_{2,3}$ 9.7 Hz), 3.65 (dd, 1H, H-5″, $J_{4,5}$ 11.3 Hz), 3.40 (t, 1H, H-4′), 3.87–3.59 (8H, H-7a,b, H-6′a,b, H-4″–H-7″), 3.36 (s, 3H, OCH_3). ^{13}C NMR (D_2O): δ 101.6 (C-1), 101.4 (C-1″), 101.3 (C-1′), 79.7 (C-3), 72.5 (C-2″), 70.5 (C-2), 70.3 (C-4′), 68.2 (C-6), 73.6, 73.0, 72.2, 72.1, 69.5, 66.8, 66.0 (C-4, C-3′, C-5′, C-3″–C-6″), 69.8, 63.7, 61.2 (C-7, C-6′, C-7″), 55.5 (OCH_3).

Acknowledgement

We thank the World Health Organization (WHO) for financial support.

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