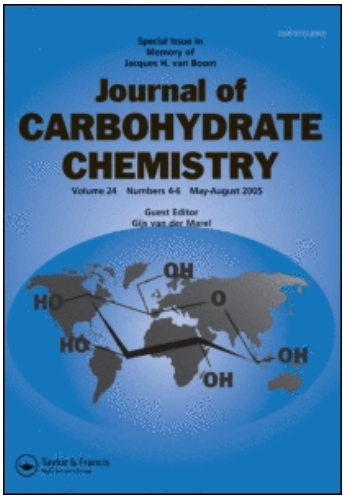

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SYNTHESIS OF NATURALLY OCCURRING LD-HEP*P* CONTAINING DISACCHARIDES

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ABSTRACT

Diastereoselective hydroxymethylation of a suitably protected α-D-*manno*-hexodialdo-1,5-pyranoside with the Grignard reagent derived from (phenyldimethylsilyl)methyl chloride gives, after additional protecting group manipulations, an easy access to one donor and two acceptors of LD-Hep*p*. The latter derivatives could be applied successfully for the preparation of the disaccharides α-D-Glc*p*N-(1-7)-L-α-D-Hep*p*-OMe and L-α-D-Hep*p*-(1-6)-L-α-D-Hep*p*-OMe.

INTRODUCTION

Lipopolysaccharides (LPS) are characteristic cell surface elements[1] of Gram-negative bacteria. The inner core region of LPS consists mainly of L-*glycero*-D-*manno*-heptopyranose (LD-Hep*p*) and 3-deoxy-D-*manno*-octulosonic acid (KDO), one of which may be linked to Lipid A. Many antibodies (poly or monoclonal) raised against LPS of certain Gram-negative bacteria (particularly rough-mutant bacteria) are believed[2] to be directed against the common LD-Hep*p* and KDO containing region of LPS. As part of an ongoing program directed toward the synthesis of immunogenic inner core fragments, we developed suitable methods for the preparation of the non-common sugars LD-Hep*p*[3,4] and KDO[5]. With the objective to widen the scope of our LD-Hep*p* building units, we report here the synthesis of the antigenic disaccharides α-D-Glc*p*N-(1→7)-L-

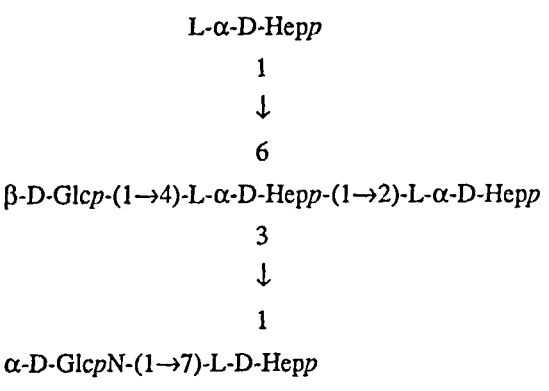
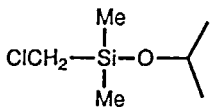


Figure 1.

α-D-Hepp-OMe (*i.e.*, **17**) and L-α-D-Hepp-(1→6)-L-α-D-Hepp-OMe (*i.e.*, **22**) which are fragments (see Figure 1) of the inner core LPS structure from *Aeromonas hydrophila*.[6]

RESULTS AND DISCUSSION

A crucial requirement in a synthetic route to the dimers **17**[7] and **22** is, apart from the stereoselective introduction of the α-interglycosidic linkages, the availability of suitably protected LD-Hepp derivatives. Earlier we reported[3] that LD-Hepp building units were accessible by a two-step Tamao procedure.[8] For example, condensation of the Grignard reagent derived from (isopropyloxydimethylsilyl)methyl chloride (**1**) with the fully benzylated α-D-*manno*-hexodialdo-1,5-pyranoside (**3**) results in the formation of the silane derivative **4** with a high degree of diastereoselectivity. Unfortunately, it was imperative, due to the instability of the carbon-silicon bond, to convert **4** immediately by oxidation into the stable LD-Hepp derivative **5**. Despite this drawback, the usefulness of the hydroxymethylene extension procedure has been nicely illustrated[9,10] in the synthesis of several LD-Hepp containing cell wall oligosaccharides. Nonetheless, it has to be noted that the two-step hydroxymethylene extension procedure affords a vicinal diol function which demands in some cases (*e.g.* introduction of interglycosidic linkages at C-6 or C-7 of LD-Hepp) additional protecting group manipulations.[10] Recently we showed[4,11] that the stability of the newly introduced silicon-carbon bond could be increased by using (phenyldimethylsilyl)methyl chloride (**2**) as the hydroxymethylene reagent. Thus,



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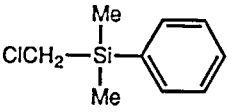

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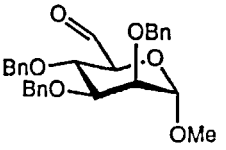

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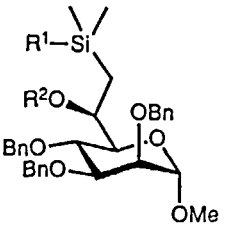
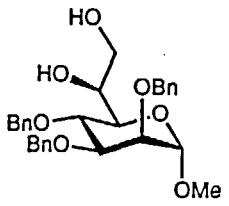


4 R^1= —O— , R^2=H

6 R^1= — , R^2=H

7 R^1= — , R^2=Bn



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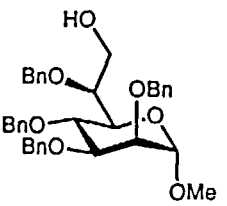

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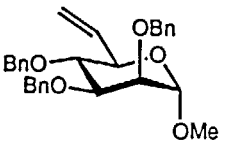

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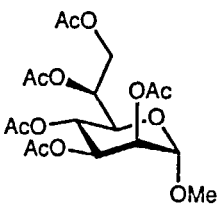

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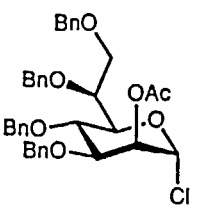

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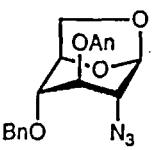

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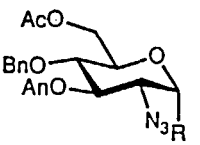
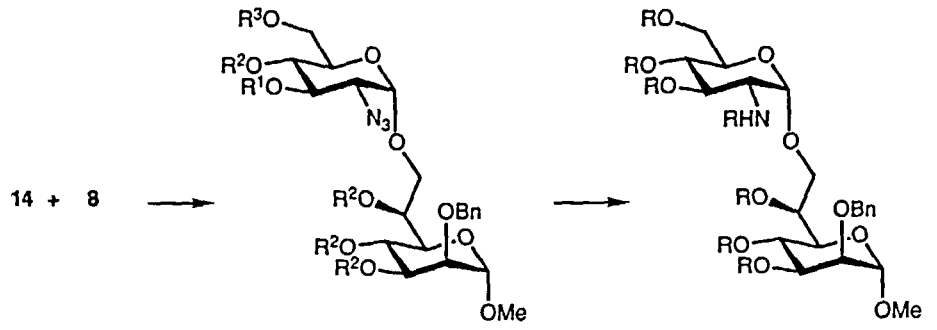


13 R=Ac
14 R=Br

condensation of the Grignard reagent of **2** with the aldehyde **3** gives in a highly diastereoselective manner the stable silane derivative **6**, as evidenced by the following experiments. Cleavage of the carbon-silicon bond[12] in **6** with peracetic acid, in the presence of sodium bromide, gave exclusively the LD-Hepp derivative **5** having the same configuration at C-6 (*i.e.*, L-*glycero*) as in **5** prepared via the Tamao procedure. Further, benzylation[13] of **6** followed by the unmasking of the silyl function in **7**, under the condition mentioned earlier for the conversion of **6**→**5**, resulted in a good recovery of **8**: thus demonstrating the stabilizing effect of the phenyl group on the newly introduced

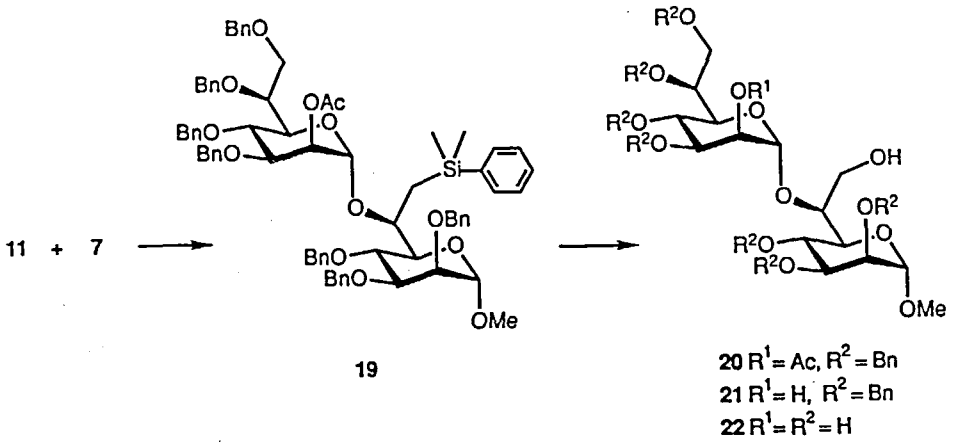


Scheme 1

silicon-carbon bond. In this respect, it is also noteworthy to mention that the unmasking of **7**→**8** was not accompanied by the formation of the Peterson elimination[14] product **9**. Apart from this, three valuable LD-Hep*p* derivatives emerged from the above executed chemical operations. Thus compounds **8** and **6** may serve as acceptors in glycosidations resulting in the disaccharides **17** and **22**, respectively. On the other hand, the LD-Hep*p* derivative **5** can be readily converted into the LD-Hep*p* derivative **11**, which in turn may serve as donor in a condensation reaction leading to disaccharide **22**. Thus hydrogenolysis of **5** followed by acetylation gave **10** which was further elaborated, according to a published[15] procedure, to yield the LD-Hep*p* donor **11**.

At this stage of the synthesis we turned our attention to the preparation of the required disaccharides **17** and **22**. In the first instance, the disaccharide **17** containing the D-glucosamine unit α(1→7)-linked with LD-Hep*p* was obtained as outlined in Scheme 1.

Condensation of the LD-Hep*p* acceptor **8** with glucosyl bromide **14**, prepared *via* acetolysis[16] of the known 1,6-anhydro-3-O-anisoyl-2-deoxy-2-azido-D-glucopyranoside[17] (**12**) and subsequent treatment of **13** with titanium tetrabromide,[16] was executed under Helferich conditions[18] [$Hg(CN)_2/HgBr_2$]. Work up, after three days at ambient temperature, and purification gave the homogeneous α-linked disaccharide **15** in 81% yield. Basic hydrolysis of the acyl protecting groups (**15**→**16**) followed by reduction of the azido function and simultaneous hydrogenolysis of the benzyl groups with palladium on charcoal gave the fully deprotected disaccharide **17**. Acetylation of **17** gave



Scheme 2

exclusively **18**, the ^{1}H- and ^{13}C NMR data of which were in excellent agreement with the same disaccharide recently isolated and fully characterized by Kaca *et al.*.[19]

In the next stage the feasibility (see Scheme 2) to apply the silane derivative **6** as an acceptor in the condensation with donor **11** was explored. Preliminary experiments indicated that the use of the promoter silver triflate[20] was accompanied by Peterson elimination: *i.e.*, conversion of **6** into **9**. However, the latter side-reaction could be avoided by using dry and high-quality silver triflate. Thus coupling of **6** with **11** in the presence of silver triflate afforded, after purification, the fully protected disaccharide **19** in 45% yield. Unmasking of the silyl function by oxidation (**19**→**20**) followed by Zemplén deacetylation (**20**→**21**), and finally hydrogenolysis, resulted in the isolation of homogeneous **22**, the ^{1}H- and ^{13}C NMR data of which were in complete accordance with the proposed structure. In conclusion the data presented in this paper firmly support our earlier reported[4,11] preliminary results. Furthermore, we believe that the commercially available[21] reagent **2** promises[22] to be a very reliable and versatile tool, in terms of diastereoselectivity, protecting group strategy and compatibility with glycosidation conditions, for the preparation of valuable LD-Hepp derivatives.

EXPERIMENTAL

General methods and materials. Dichloromethane, 1,2-dichloroethane and toluene were distilled from P_2O_5 and stored over molecular sieves 4Å. Diethyl ether and

tetrahydrofuran were distilled from CaH_2 and redistilled from $LiAlH_4$ before use. N,N-dimethylformamide was stirred with CaH_2 for 16 h, then distilled under reduced pressure and stored over molecular sieves 4Å. Methanol was dried by refluxing with magnesium methoxide, distilled and stored over molecular sieves 3Å. $TiBr_4$ was sublimed under reduced pressure and stored as a standard solution in dichloromethane. Silver triflate was purchased from Fluka and dried *in vacuo*, with the exclusion of light. Reactions were performed under strict anhydrous conditions unless noted otherwise. Evaporation was carried out below 40 °C under reduced pressure. Column chromatography was performed on columns of silica gel (Merck, 70-230 mesh). Gel filtration was performed on Sephadex LH-20 (Pharmacia). TLC analyses were conducted on Schleicher and Schüll Fertigfolien F 1500 LS 254. Compounds were visualized by UV light (254 nm) or by spraying with conc. H_2SO_4/methanol (1/10, v/v) and charring at 140 °C. Optical rotations were determined with a Perkin-Elmer Model 241 polarimeter at 20 °C. NMR spectra were recorded with a Jeol JNM-FX200 (^1H and ^{13}C 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 2,3,4-tri-O-benzyl-7-(phenyldimethyl)silane-7-deoxy-L-glycero-α-D-manno-pyranoside (6). A small amount of (phenyldimethylsilyl)methyl chloride (11.7 g, 60 mmol) in THF (45 mL) was added under a N_2 atmosphere to dry magnesium turnings (1.46 g, 60 mmol). The reaction mixture was heated until reflux and, after the reaction was initiated by the addition of 1,2-dibromoethane (0.1 mL), the remaining chloride was added at such a rate as to maintain a gentle reflux. The thus obtained Grignard reagent was transferred to a dry round-bottom flask. The aldehyde **3** was coëvaporated with toluene (2 x 20 mL), dissolved in THF (45 mL) and added dropwise to the cooled (0 °C) Grignard reagent . After stirring for 2 h, TLC analysis (ether/pet. ether, 1/2, v/v) indicated complete conversion of **3** in to **6** (Rf 0.5). 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 ($MgSO_4$) and concentrated under reduced pressure. The oil thus obtained was applied onto a column of silica gel (200 g) and eluted with light pet. ether followed by light pet. ether/ethyl acetate (9/1→4/1, v/v). Concentration of the appropiate fractions gave **6** as an oil. Yield 13.1 g (71 %), R_f 0.5 (light pet. ether/ether, 2/1, v/v), [α] +11.5° (c 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.57-7.17 (m, 20H, H-arom), 4.91-4.59 (m, 6H, CH_2 Bn), 4.71 (d, 1H, H-1, $J_{1,2}$=2.0 Hz), 4.05 (m, 2H, H-4, H-6, $J_{4,5}$=$J_{3,4}$=9.5 Hz), 3.84 (dd, 1H, H-3, $J_{2,3}$=3.0 Hz), 3.77 (dd, 1H, H-2), 3.34 (1-H, H-5, $J_{5,6}$=1.5Hz), 1.37 (dd, 1H, H-7a, $J_{7a,6}$=12 Hz, $J_{7a,7b}$=17 Hz), 0.94 (dd, 1H, H-7b, $J_{6,7b}$=5.0 Hz), 0.36, 0.35 (2xs, 6H,

$Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 138.3 (Cq, arom.), 133.5-127.46 (CH arom.), 99.4 (C-1), 80.3, 75.4, 75.1, 74.7, 67.1 (C-2, C-3, C-4, C-5, C-6), 75.3, 72.8, 72.1 (3x CH_2, Bn), 54.7 (OCH_3), 21.7 (C-7), -2.4 ($(CH_3)_2Si$).

Anal. Calcd for $C_{37}H_{44}O_6Si$: C,72.52; H,7.24. Found: C,72.63; H,7.30.

Methyl 2,3,4,-tri-O-benzyl-L-glycero-α-D-manno-heptopyranoside (5). Acetic acid (15 mL), sodium acetate (2.0 g) and potassium bromide (0.25 g, 2.4 mmol) were added to **6** (0.93 g, 2.0 mmol) and the mixture was stirred until the salts were dissolved. The mixture was cooled (10 °C) and peracetic acid (10 mL, 30% in acetic acid) was added dropwise under the exclusion of light. During the addition gas was liberated. After the mixture was stirred for 1.5 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. The mixture was poured in an aqueous solution of sodium thiosulfate (50 mL, 15 %) and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with water (50 mL), aqueous sodium bicarbonate (50 mL, 10 %), water (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was applied onto a column of silica gel (10 g) and eluted with CH_2Cl_2 followed by CH_2Cl_2/methanol (1/99→1/49, v/v). Evaporation of the appropriate fractions afforded **5** as an oil. Yield 0.69 g (72 %), Rf 0.3 (methanol/CH_2Cl_2, 3/97, v/v), [α] +30.5° (c 1, $CHCl_3$).

Methyl 2,3,4,6-tetra-O-benzyl-7-(phenyldimethyl)silane-7-deoxy-L-glycero-α-D-manno-heptopyranoside (7). *N*-tetrabutylammonium iodide (0.74 g, 2.0 mmol), sodium hydride (0.77 g, 32 mmol) and benzyl bromide (3.1 mL, 26 mmol) were added to a cooled (0° C) solution of **6** (13.1 g, 21.5 mmol) in DMF (60 mL). After stirring for 3 h at room temperature, TLC analysis (pet. ether/ether, 2/1, v/v) indicated complete conversion of **6** into **7**. The reaction mixture was quenched with methanol (10 mL) and concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 (150 mL) and water (2x50 mL). The organic layer was dried ($MgSO_4$), and concentrated to an oil, which was applied onto a column of silica gel (200 g) and eluted with light pet. ether followed by light pet. ether /ether (9/1→4/1, v/v). Concentration of the appropiate fractions gave **7** as an oil. Yield 14.3 g (95 %). R_f 0.8 (pet. ether/ether, 2/1, v/v), [α] + 24.6° (c 1, $CHCl_3$). ^1H NMR ($CDCl_3$):δ 7.58-7.10 (m, 25H, H-arom.), 4.85 (d, 1H, H-1, $J_{1,2}$=2.0 Hz), 4.78-4.20 (m, 8H, CH_2, Bn),4.09, t, 1H, H-4, $J_{3,4}$=$J_{4,5}$=9.5 Hz), 4.02 (m,1H, H-6), 3.83 (dd, 1H, H-3, $J_{2,3}$=3.0 Hz), 3.76 (dd, 1H, H-2), 3.46 (dd, 1H, H-5, $J_{5,6}$=1.5 Hz), 3.27 (s, 3H, OCH_3), 1.42 (m, 2H, H-7a, H7b), 0.36, (s, 6H, CH_3Si). ^{13}C NMR ($CDCl_3$): δ 138.8-138.4 (Cq, arom.), 133.6-127.8 (CH, arom.), 99.1 (C-1), 80.4, 75.0, 74.5, 74.4, 72.9 (C-2, C-3, C-4, C-5, C-6), 74.3, 72.3 71.9, 69.8 (4x CH_2, Bn), 55.0 (OCH_3), 17.0 (C-7), -2.1,-2.3 (2x CH_3Si).

Anal. Calcd for $C_{44}H_{50}O_6Si$: C,75.18; H,7.17. Found: C,75.31; H,7.26.

Methyl 2,3,4,6-tetra-O-benzyl-L-glycero-α-D-manno-heptopyranoside (8). Compound **7** (1.4 g, 2.0 mmol) was oxidised with peracetic acid (10 mL) and potassium bromide (0.286 g, 2.4 mmol) in a mixture of acetic acid (15 mL) and sodium acetate (2.0 g), as described for the conversion of **6**→**5**, to give **8** (0.84 g, 72 %), [α] + 37.8° (c 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.48-7.18 (20H, arom. H), 4,84 (d, 1H, H-1, $J_{1,2}$=2.0 Hz), 4.96-4.39 (m, 8H, CH_2, Bn), 4.21 (t, 1H, H-4, $J_{3,4}$=$J_{4,5}$=9.5 Hz), 3.98-3.90 (m, 4H, H-3, H-6, H-7a, H-7b), 3.84 (dd, 1H, H-2, $J_{2,3}$=3.0 Hz), 3.77 (dd, 1H, H-5, $J_{5,6}$=1.0 Hz), 3.36 (OCH_3). ^{13}C NMR ($CDCl_3$): δ138.4-138.0 (Cq, arom.), 128.1-127.3 (CH, arom.), 98.8 (C-1), 80.2, 75.7, 74.1, 74.0, 72.5 (C-2, C-3, C-4, C-5, C-6), 74.4, 72.3, 71.9, 71.6 (4x CH_2, Bn), 62.0 (C-7), 54.6 (OCH_3).

Anal. Calcd for $C_{36}H_{40}O_7$: C,73.95; H,6.90. Found: C,74.08; H,6.82.

1,6-di-O-acetyl-3-O-(4-methoxybenzoyl)-4-O-benzyl-2-azido-2-deoxy-ß-D-glucopyranose (13). Coumpound **12** (9.4 mmol) was dissolved in a mixture of acetic anhydride (135 mL) and trifluoroacetic acid. After stirring for 24 h, TLC analysis (acetone/CH_2Cl_2, 3/97, v/v) indicated complete conversion of the starting compound into a more hydrophilic product (Rf 0.6). The mixture was diluted with toluene (50 mL) and concentrated under reduced pressure to give, after coevaporation (3x50 mL) of the resulting oil, compound **13** as an amorphous material. Yield 4.1 g (100 %), Rf 0.6 (acetone/CH_2Cl_2, 3/97, v/v). ^1H NMR ($CDCl_3$): δ 8.07-6.95 (m, 9H, H-arom.), 6.31 (d, 1H, H-1, $J_{1,2}$=3.0 Hz), 5.81 (t, 1H, H-3, $J_{2,3}$=$J_{3,4}$=10 Hz), 4.61-4.41 (AB, 2H, CH_2, Bn), 4.29 (d, 2H, H6a,b), 4.09 (dt, 1H, H-5, $J_{4,5}$=10 Hz), 3.78 (t, 1H, H-4), 3.87 (s, 3H, OCH_3), 3.61 (dd, 1H, H-2), 2.19, 2.09 (2xs, 2xCH_3, Ac); ^{13}C NMR: δ 170.2, 168.6, 164.9 (3x C=O), 136.6, 121.3 (2xCq, arom.), 131.6, 113.7 (CH, arom), 92.7 (C-1), 74.8 (CH_2, Bn), 75.1, 72.4, 70.9, (C-3, C-4, C-5), 60.8 (C-6), 60.8 (C-2), 55.4 (OCH_3), 20.9, 20.7 (2xCH_3, Ac).

Anal. Calcd for $C_{25}H_{27}O_9N_3$: C,58.48; H,5.30. Found: C,58.57; H,5.37.

3-O-(4-methoxybenzoyl)-4-O-benzyl-2-azido-2-deoxy-α-D-glucopyranosyl bromide (14). A solution of $TiBr_4$ (2.4 mmol, 4 mL) in CH_2Cl_2 (0.23g/mL), was added to a solution of **13** (0.65 g, 1.2 mmol) in CH_2CL_2 (8 mL) and ethyl acetate (0.8 mL). The dark red solution was stirred overnight at 20 °C under an atmosphere of dry nitrogen. TLC analysis (acetone/CH_2Cl_2, 3/97, v/v) showed complete conversion of the starting material into bromide **14** (Rf 0.9). Dry sodium acetate was added until the solution became colourless. The mixture was taken up in dry toluene and filtrated over a pad of Celite and concentrated to dryness, to afford **14** as a colourless oil which was used directly in the next step.

Methyl 2,3,4,6-tetra-O-benzyl-7-O-(3-O-(4-methoxybenzoyl)-4-O-benzyl-6-O-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-L-glycero-α-D-manno-heptopyranoside

(15). To a mixture of **8** (0.47 g, 0.8 mmol), $Hg[CN]_2$ (0.20 g, 0.87 mmol), $HgBr_2$ (0.025 g, 0.07 mmol) and powdered molecular sieves (0.5 g, 4 Å) in CH_2Cl_2 (4 mL) was added dropwise a solution of the bromide **14** (0.58 g, 1.2 mmol) in CH_2Cl_2. After stirring for 3 days at room temperature, TLC analysis (acetone/CH_2Cl_2, 1/99, v/v) showed the absence of acceptor **8**. The mixture was diluted with CH_2Cl_2 (25 mL), filtrated over a pad of Celite and the filtrate was washed with aqueous potassium bromide (2x10 mL, 20%), aqueous sodium bicarbonate (10 mL, 10%) and water (10 mL). The organic layer was dried ($MgSO_4$), concentrated under reduced pressure and the residue was applied onto a column of silica gel (10 g) and eluted with CH_2Cl_2 followed by acetone/CH_2Cl_2 (1/99→1/49, v/v). Concentration of the appropiate fractions afforded **15** as an oil. Yield 0.68 g (81 %), $[\alpha]_D$ = 61.1 (c 1, $CHCl_3$), Rf 0.5 (acetone/CH_2Cl_2, 1/99, v/v). ^1H NMR ($CDCl_3$): δ 8.1 (d, 2H, H-2, H-6 An.), 7.43-7.15 (m, 25H, H-Bn), 6.93 (d, 2H, H-3, H-4, An.), 5.86 (dd, 1H, H-3', $J_{2',3'}$=10.5 Hz, $J_{3',4'}$=9.0 Hz), 5.03 (d, 1H, H-1', $J_{1',2'}$=3.5 Hz), 4.83 (d, 1H, H-1, $J_{1,2}$=2.0 Hz), 4.93-4.36 (m, 6H, 3x CH_2, Bn), 4.27 (d, 2H, H-6'ab, $J_{5',6'}$=3.2 Hz), 4.22 (t, 1H, H-4, $J_{3,4}$=$J_{4,5}$=9.5 Hz), 4.13 (m, 1H, H-5'), 3.97 (m, 1H, H-6), 3.93 (m, 2H, H-4, H-4'), 3.86 (s, 3H, OCH_3), 3.80 (dd, 2H, H-7ab), 3.61 (m, 1H, H-5), 3.43 (dd, 1H, H-2'), 2.05 (s, 3H, CH_3, Ac.). ^{13}C NMR ($CDCl_3$): δ 169.9 (C=O, Ac.), 164.6, (C=O, An.), 163.3 (C-4, An.), 138.3-136.6 (Cq, Bn), 131.4 (C-2, C-6, An.), 121.3 (C-1, An.), 113.3 (C-3, C-5, An.),99.1, 98.2 (C-1, C-1'), 80.3, 75.9, 75.0, 74.4, 74.1,72.5, 71.7, 68.9 (C-2, C-3, C-4, C-5, C-6, C-3', C-4', C-5'), 74.6, 74.2, 72.8, 72.4, 71.9 (5x CH_2, Bn), 69.0 (C-7), 62.5, (C-6'), 61.6 (C-2'), 54.9, 54.5 (2x OCH_3), 20.3 (CH_3, Ac.).

Anal. Calcd for $C_{59}H_{63}O_{14}N_3$: C,68.26; H,6.12. Found: C,68.37; H,6.13.

Methyl 2,3,4,6-tetra-O-benzyl-7-(4-O-benzyl-2-azido-2-deoxy-α-D-glucopyranosyl)-L-glycero-α-D-manno-heptopyranoside (16). Compound **15** (0.68 g, 0.66 mmol) was dissolved in a mixture of dioxane, methanol and aqueous NaOH (16 mL) and left for 3 h at 50 °C. TLC analysis (acetone/CH_2Cl_2, 1/19, v/v) indicated complete conversion of the starting material into a more hydrophilic product (R_f 0.5). The mixture was diluted with water (10 mL) and extracted with CH_2Cl_2 (25 mL). The organic phase was dried ($MgSO_4$), concentrated under reduced pressure and the residual oily product was applied onto a column of silica gel (10 g) and eluted with CH_2Cl_2 followed by acetone/CH_2Cl_2 (1/49→1/19, v/v). Concentration of the appropiate fractions afforded **16** as an oil. Yield 0.52 g (92 %), [α] 1.071 (c 1, $CHCl_3$), Rf 0.5 (acetone/CH_2Cl_2, 1/19, v/v). ^1H NMR ($CDCl_3$): δ 7.41-7.12 (25H, H-arom.), 5.03 (d, 1H, H-1', $J_{1',2'}$=3.5 Hz), 4.22 (d, 1H, H-1, $J_{1,2}$=2.0 Hz), 4.93-4.35 (m, 8H, CH_2, Bn), 4.22-3.42 (m, 13H, H-2 - H7a,b, H-2' - H6'a,b), 3.85 (s, 3H, OCH_3).

Anal. Calcd for $C_{49}H_{55}O_{11}N_3$: C,68.28; H,6.43. Found: C,68.21; H,6.52.

Methyl 2,3,4,6-tetra-O-acetyl-7-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl)-L-glycero-α-D-manno-heptopyranoside (18). A mixture of **16** (90 mg, 0.21 mmol) and 10 % Pd-C (90 mg) in *tert*-butanol (5 mL), acetic acid (1 mL) and water (1 mL) was stirred under 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. The remaining residue was concentrated with pyridine (2x10 mL) and dissolved in a mixture of pyridine (5 mL) and acetic anhydride (5 mL). After stirring for 18 h, the mixture was diluted with toluene (10 mL) and concentrated under reduced pressure. The crude oil was dissolved in CH_2Cl_2 (25 mL) and washed with aqueous sodium bicarbonate (10 mL) and water (10 mL). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure and the resulting material was applied onto a column of silica gel (1 g) and eluted with CH_2Cl_2 followed by CH_2Cl_2/methanol (99/1, v/v). Concentration of the appropriate fractions gave **18** as an oil. Yield 0,12 g (89%), Rf 0.5 (methanol/CH_2Cl_2, 3/97, v/v), [α] 1.071 (c 1, $CHCl_3$) +62.3° (c 1, $CHCl_3$). ^1H NMR: δ 6.08 (d, 1H, NH, $J_{NH,2'}$=9.2 Hz), 5.68 (t, 1H, H-4, $J_{3,4}$=$_{4,5}$=9.9 Hz), 5.58 (dd, 1H, H-3, $J_{2,3}$=3.3 Hz), 5.50 (m, 2H, H-2, H-3'), 5.42 (ddd, 1H, H-6, $J_{6,7a}$=$_{3.6}$ Hz, $J_{6,7b}$=8.1 Hz, $J_{5,6}$=2.5 Hz), 5.31 (t, 1H, H-4', $J_{3',4'}$=$J_{4',5'}$=9.8 Hz), 4.80 (d, 1H, H-1'), 4.56 (ddd, 1H, H-2', $J_{1'2'}$=3.8 Hz, $J_{2',3'}$=10.1 Hz), 4.50 (d, 1H, H-1, $J_{1,2}$=1.8 Hz), 4.39 (dd, 1H, H6'a, $J_{5',6'a}$=5.1 Hz, $J_{6'a,6'b}$=12.3 Hz), 4.16 (dd, 1H, H6'b, $J_{5',6'b}$=2.6 Hz), 4.00 (ddd, 1H, H-5'), 3.94 (dd, 1H, H-5), 3.80 (dd, 1h, H-7a, $J_{7a,b}$=11.6 Hz), 3.68 (dd, 1H, H7b), 3.01 (OCH_3), 1.87, 1.81, 1.76, 1.72, 1.71, 1.67 (24H, CH_3, Ac).

Anal. Calcd for $C_{26}H_{43}O_{18}N$: C,47.49; H,6.59. Found: C,47.57; H,6.63.

Methyl 2,3,4-tri-O-benzyl-6-O-(2-O-acetyl-3,4,6,7-tetra-O-benzyl-L-glycery-D-manno-heptopyranosyl)-7-deoxy-7-(phenyldimethyl)silane-L-glycero-D-manno-heptopyranoside (19). To a solution of compound **6** (0.165 g, 0.27 mmol) in CH_2Cl_2 (5 mL) was added activated powdered molecular sieves (0.5 g) and the mixture was stirred for 2.5 h in an atmosphere of nitrogen. Silver triflate (0.13 g 0.5 mmol) was then added under the exclusion of light. The mixture was cooled to -30 °C and the chloride **11** (0.30 g, 0.45 mmol) in CH_2Cl_2 (2 mL) was added over a period of 30 min. The reaction mixture was stirred at -20 °C for 16 h. The mixture was filtrated and the filtrate was diluted with CH_2Cl_2 (50 mL) and washed with aqueous sodium thiosulfate (15 mL), sodium bicarbonate (15 mL), water (20 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was applied to a column of silica gel (2g) and eluted with light pet. ether/ethylacetate (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). The appropiate fractions were concentrated

to give pure **19** in a yield of 0.145 g (45 %) as an oil. Rf 0.3 (acetone/CH_2Cl_2, 3/97, v/v), $[\alpha]_D$ +18.2° (c 0.95, $CHCl_3$). ^{13}C NMR ($CDCl_3$): δ 170.7 (C=0, Ac.), 139.6-138.4 (Cq, Arom.), 134.1-127.8 (CH, Arom.), 99.4, 95.3 (C-1, C-1'), 80.6, 76.3, 74.4, 74.3, 72.9, 69.5 (C-2, C-3, C-4, C-5, C-6, C-2', C-3', C-4', C-5', C-6'), 73.8, 73.7, 72.9, 70.8, (CH_2, Bn., C-7'), 55.9 (OCH_3), 21.9 (CH_3, Ac.), 16.8 (C-7), -1.4, -1.8 ($(CH_3)_2Si$); $J_{C1\text{-}H1}$ = 167 Hz, $J_{C1'\text{-}H1'}$ = 171 Hz.

Anal. Calcd for $C_{74}H_{82}O_{13}Si$: C,73.61; H,6.84. Found: C,73.69; H,6.90.

Methyl 2,3,4-tri-O-benzyl-6-O-(2-O-acetyl-3,4,6,7-tetra-O-benzyl-L-glycero-α-D-manno-heptapyranosyl)-L-glycero-α-D-manno-heptopyranoside (20). Compound **19** (65 mg, 0.05 mmol) was oxidised, as described earlier for **6→5**, to give, after further work up and purification homogeneous **20** in a yield of 48 mg (88 %), Rf 0.4 (acetone/CH_2Cl_2, 3/97, v/v), [α] + 29.9° (c 0.96, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.32-7.33 (35H, H-arom.), 5,43 - 3.87 (H-1 - H-$7_{a,b}$, H-1' - H-$7'_{a,b}$, 7xCH_2, Bn.), 3.25 (s, 3H, OCH_3), 2.03 (s, 3H, CH_3, Ac.).

Anal. Calcd for $C_{66}H_{72}O_{14}$: C,72.77; H,6.66. Found: C,72.89; H,6.82.

Methyl 2,3,4-tri-O-benzyl-6-O-(3,4,6,7-tetra-O-benzyl-L-glycero-α-D-manno-heptopyranosyl)-L-glycero-α-D-manno-heptopyranoside (21). To a solution of compound **20** (46 mg, 0.04mmol) in methanol (2.5 mL) was added potassium *tert.* butoxide. After stirring for 2 h, the solution was neutralized with Dowex W50 (H^+-form) and the filtrate was concentrated under reduced pressure. The oil thus obtained was applied on a column of silica gel, and the column was eluted with CH_2Cl_2 followed by CH_2Cl_2/acetone (99/1, v/v). Concentration of the appropriate fractions gave **21** (40 mg, 0.038 mmol) as an oil. Rf 0.3 (acetone/CH_2Cl_2, 3/97, v/v), [α] +26.1° (c 1, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.32-7.21 (35H, H-arom.), 4.84-3.87 (H-1 - H-7a,b, H-1' - H-7'a,b, 7xCH_2, Bn.), 3.27 (s, 3H, OCH_3).

Anal. Calcd for C_{64} $H_{70}O_{13}$; C,73.40; H,6.74. Found: C.74.38, H,6.71.

Methyl 6-O-(L-glycero-α-D-manno-heptoyranosyl)-L-glycero-α-D-manno-heptopyranoside (22). Dimer **21** (40 mg, 0.038 mmol) was dissolved in methanol (5 mL) and hydrogenolysis was performed on palladium on active carbon. After stirring for 48 h, the catalyst was removed by filtration and washed with water. The combined filtrates were concentrated under reduced pressure to give **22** (10 mg, 63%) as an amorphous material. Rf 0.1 (methanol/ethyl acetate/water, 3/5/2, v/v/v), [α] +45.7° (c 1, water). ^1H NMR (D_2O): δ 5.16 (d, 1H, H-1, $J_{1,2}$=1.8 Hz), 4.74 (d, 1H, H-1, $J_{1,2}$=1.9 Hz), 4.12 (dt, 1H, H-6, $J_{6,7ab}$=5.8 Hz, $J_{5,6}$=1.0 Hz), 4.02 (ddd, 1H, H-6', $J_{5',6'}$=1.3 Hz, $J_{6',7'a}$=5.8 Hz, $J_{6',7'b}$=7.1 Hz), 3.98 (dd, 1H, H-2, $J_{2,3}$=3.0 Hz), 3.91 (dd, 1H, H-2', $J_{2',3'}$=3.7 Hz), 3.90-3.7- (10H, H-3, H-4, H-5, H7a,b, H3', H4', H-5', H7'a,b); ^{13}C NMR (D_2O): δ 102.0 (C-

1), 99.3 (C-1'), 73.7, 73.4, 72.0, 71.9, 71.4, 71.2, 70.7, (C-2, C-3, C-4, C-5, C-6, C-2', C-3', C-4', C-5', C-6') 69.9, 69.7 (C-7, C-7'), 55.8 (OCH_3).

Anal. Calcd for $C_{15}H_{28}O_{13}$: C,43.27; H,6.78. Found: C,43.18; H6.70.

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21. (Phenyldimethylsilyl)methyl chloride is commercially available from Aldrich chemical company.

22. An alternative approach in which a terminal olefinic precursor of LD-Hepp, prepared by stereospecific condensation of a similarly protected aldehyde **3** with vinyl magnesium bromide, plays a central role was published recently [see: Y. Chaplear *et al.*, *J. Chem. Soc. Perkin Trans I*, 3091 (1990)]. However, it has to be seen whether the presence of a terminal olefinic function in the above LD-Hepp precursor is compatible with an allyl protecting group strategy.