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Note

A short synthesis of D-*glycero*-D-*manno*-heptose 7-phosphate

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Dedicated to András Lipták on the occasion of his 70th birthday

Abstract—D-*glycero*-D-*manno*-Heptopyranose 7-phosphate—an intermediate in the biosynthesis of nucleotide-activated heptoses—has been prepared in good overall yield from benzyl 5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-(*Z*)-hept-5-enofuranoside by a short-step synthesis. Phosphitylation using the phosphoramidite procedure followed by in situ oxidation afforded the corresponding 7-*O*-phosphotriester derivative in high yield. Subsequent osmylation proceeded in good diastereoselectivity (4:1) to furnish the D-*glycero*-D-*manno*-configured derivative, which was separated from the L-*glycero*-L-*gulo*-isomer by chromatography. Hydrogenolysis led to simultaneous removal of the benzyl and isopropylidene groups and afforded the target compound in high yield, which serves as a substrate of bacterial heptose 7-phosphate kinases.

Keywords: Heptose; Heptose phosphate; Lipopolysaccharide; Capsular polysaccharide

Heptoses of the D-*glycero*-D-*manno*- and L-*glycero*-D-*manno*-configuration are common constituents of the core region of numerous bacterial lipopolysaccharides and have also been detected in various O-antigenic chains.[1] In the assembly of the core region of enterobacterial LPS, ADP L-*glycero*-β-D-*manno*-heptose serves as a substrate of heptosyl transferases.[2] In addition, GDP D-*glycero*-α-D-*manno*-heptopyranose has been identified as the substrate for the bacterial glycosyltransferase involved in the biosynthesis of the S-layer glycoprotein glycan in *Aneurinobacillus thermoaerophilus* and has been proposed as the intermediate leading to GDP 6-deoxy-D-*manno*-heptose in *Yersinia pseudotuberculosis* and to related heptoses and 6-deoxy-heptoses in *Burkholderia pseudomallei* and *Campylobacter* capsular polysaccharide biosynthesis.[3,4] In both biosynthetic pathways, D-*glycero*-D-*manno*-heptopyranose 7-phosphate is an essential precursor, which by the action of the respective kinases encoded by the genes *hldE* and *hddA*, respectively, either furnishes the β- or the α-linked D-*glycero*-D-*manno*-heptopyranose 1,7-bisphosphate.[5] For a detailed kinetic analysis of the bifunctional heptokinase/ADP heptose transferase reaction of *hldE*, the authentic substrate was needed.[6] Previously, the 7-phosphate derivative of the L-*glycero*-D-*manno*-heptopyranose had been prepared[7] and recently reaction of an α-diazoketone mannopyranoside derivative with dibenzyl phosphoric acid afforded a diastereoisomeric mixture of D-*glycero*- and L-*glycero*-D-*manno*-heptopyranose 7-phosphates.[8] Since in the preparation of D-*glycero*-D-*manno*-heptopyranose following the procedure elaborated by Brimacombe, a terminal hydroxy group is present for further modification, we set out for a short-step synthesis from precursor derivative **1**.[9] Compound **1** is prepared in five high-yielding steps from commercially available 2,3:5,6-di-*O*-isopropylidene mannofuranose.

Hence, the previously described benzyl D-*lyxo*-heptenofuranoside **1** was subjected to phosphitylation using the phosphoramidite method.[10] Treatment of **1** with bisbenzyloxy-*N*,*N*-diisopropylaminophosphine/1*H*-tetrazole and subsequent oxidation with *tert*-BuOOH gave 7-*O*-phosphotriester derivative **2** in 65% yield. Catalytic osmylation of the double bond in the presence of *N*-methyl-morpholine-*N*-oxide afforded a ~4:1 mixture

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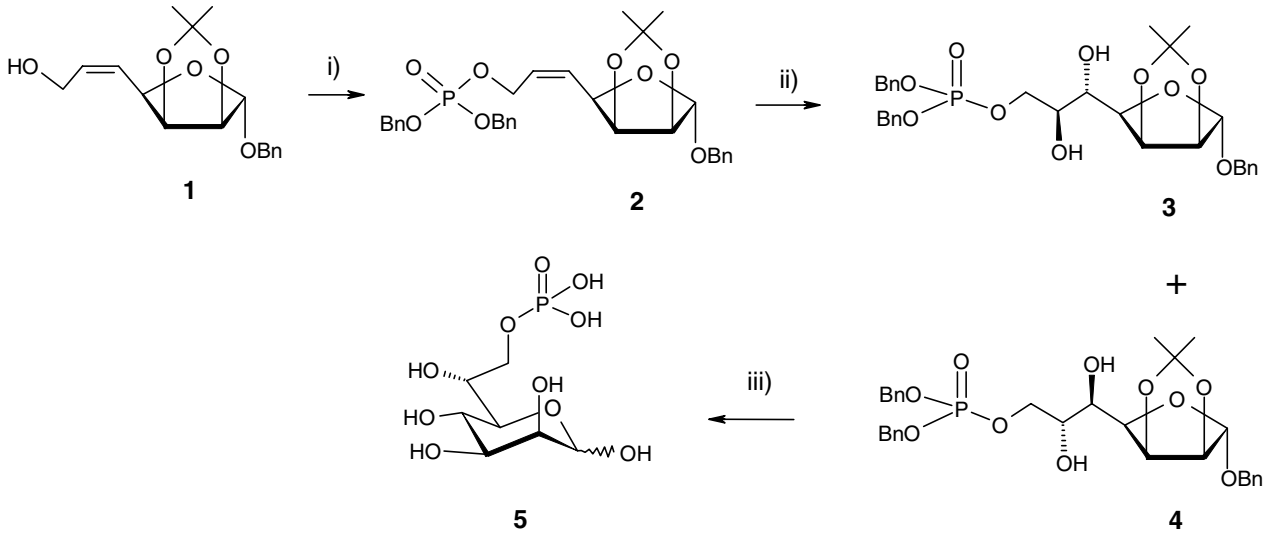

Scheme 1. Reagents and conditions: (i) Bisbenzyloxy-*N*,*N*-diisopropylaminophosphine, 1*H*-tetrazole (3.5% in CH_3CN), 2 h, CH_2Cl_2, then ^{t}BuOOH, 15 h, rt, 65% for **2**; (ii) OsO_4, NMMO, 2:1 dioxane–water, 5 h, rt, 52% for **4**; (iii) 10% Pd/C, H_2, MeOH, 12 h, rt, 81% for **5**.

of the diastereoisomers **3** and **4** in 71% yield, which were separated by silica gel chromatography (Scheme 1). Assignments of the new stereogenic centres at C-5 and C-6 were based on the comparison of the NMR data of **3** and **4** with those of the non-phosphorylated derivatives.[11] In addition, the values of the coupling constants $J_{4,5}$ indicated a cis orientation for H-4 and H-5 for compound **3** ($J_{4,5}$ 2.5 Hz) and a trans configuration for the D-*glycero*-D-*manno*-isomer **4** ($J_{4,5}$ 7.0 Hz).

Catalytic hydrogenation of **4** in the presence of 10% Pd/C resulted in concomitant cleavage of the isopropylidene group and after treatment with triethylamine produced the triethylammonium salt of heptosyl phosphate **5** in 81% yield following desalting on a PD-10 Sephadex G-25 column. The ^{1}H and ^{13}C NMR data of **5** (Table 1) were in full agreement with the structural assignments and revealed a ratio of 65:35 of the α/β pyranose forms. The presence of the phosphomonoester moiety in compound **5** was evident from the chemical shift of the ^{31}P NMR signal at 2.18 ppm and its location was ascertained by the downfield-shifted ^{13}C NMR signal of C-7 (66.26 and 66.14 ppm, respectively) as well as the heteronuclear ^{13}C/^{31}P spin–spin coupling observed for C-7 and C-6. Moreover, the spectra were identical to those of the material obtained by HPLC-purification of the reaction mixture of the enzymatic conversion of sedoheptulose 7-phosphate in the thermophilic bacterium *A. thermoaerophilus*, thereby confirming the

Table 1. NMR data[a] of D-*glycero*-D-*manno*-heptose 7-phosphate **5**

Atom H/C/P (ppm)	1	2	3	4	5	6	7a/7b
α-Pyranose							
^{1}H	5.16	3.90	3.81	3.80	3.86	4.15	4.07/3.96
J (Hz)	1.7	2.9	n.d.[b]	n.d.	n.d.	n.d.	n.d.
^{13}C	94.85	71.35[c]	71.29[c]	68.34	72.90	71.78	66.46
^{31}P							2.18
$J_{C,P}$ (Hz)						8.9	5.05
β-Pyranose							
^{1}H	4.86	3.92	3.62	3.73	3.45	4.15	4.07/3.96
J (Hz)	1.1	3.2	9.5	9.8	3.3	n.d.	n.d.
^{13}C	94.77	71.61	74.06	68.05	76.79	71.55	66.26
^{31}P							2.18
$J_{C,P}$ (Hz)						9.0	5.16
Other signals							
CH_3CH_2N							
^{1}H	3.19	1.27					
^{13}C	47.49	9.04					

[a] ^{13}C NMR data are based on HMQC and HMBC-assignments.
[b] n.d.: not determined.
[c] Assignments may be reversed.

structure of the biosynthetic intermediate.[3a] Except for the signal of C-6, the ^{13}C NMR signals of the heptopyranosyl unit are nearly identical to those of the related L-*glycero*-D-*manno*-heptose 7-phosphate, thus fully confirming the structural assignments.[7]

In conclusion, the method reported herein constitutes a short and efficient strategy for the synthesis of heptose 7-phosphates.

1. Experimental

1.1. General

All solvents were purified and dried by standard procedures. Column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck). Desalting was performed on PD-10 desalting column containing Sephadex™ G-25. Analytical TLC was performed using Silica Gel 60 F_{254} HPTLC plates with 2.5 cm concentration zone (E. Merck). Spots were detected by treatment with anisaldehyde–H_2SO_4. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. NMR spectra were recorded at 297 K in D_2O and $CDCl_3$ with a Bruker DPX 300 spectrometer (^1H at 300.13 MHz, ^{13}C at 75.47 MHz and ^{31}P at 121.50 MHz) using standard Bruker NMR software. ^1H NMR spectra were referenced to tetramethylsilane or 2,2-dimethyl-2-silapentane-5-sulfonic acid. ^{13}C NMR spectra were referenced to chloroform for solns in $CDCl_3$ (δ 77.00) or dioxane (δ 67.40) for solns in D_2O. ^{31}P NMR spectra were referenced externally to 85% aq H_3PO_4 (δ 0.0). ESIMS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument.

1.2. Benzyl 7-*O*-[bis(benzyloxy)phosphoryl]-5,6-dideoxy-2,3-*O*-isopropylidene-α-D-*lyxo*-(*Z*)-hept-5-enofuranoside (2)

Compound **1** (680 mg, 2.22 mmol) and bisbenzyloxy-*N*,*N*-diisopropylaminophosphine (1.87 mL, 5.55 mmol) were dried by repeated evaporation with dry toluene (4 × 10 mL) and then under diminished pressure for 5 h. Then CH_2Cl_2 (6 mL) was added to the sample. The flask was charged with a soln of 1*H*-tetrazole (467 mg, 6.66 mmol) in dry MeCN (3 mL) and stirred at room temperature for 2 h under Ar. Monitoring of the reaction by TLC showed the formation of phosphite triesters (7:3 toluene–EtOAc). The reaction mixture was cooled to 0 °C and a soln of *t*-BuOOH (617 μL, 3.33 mmol, 80% soln in di-*tert*-butyl peroxide) was gradually added. The soln was stirred for 15 h at room temperature and the solvent was evaporated using a stream of argon. The residue was dissolved in 2:1 diethyl ether–EtOAc (50 mL) and washed sequentially with satd $NaHCO_3$–water (until pH 9 was reached) and brine. The organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (1:1, toluene–diethyl ether) to give **2** as a colourless syrup (815 mg, 65%); $[\alpha]_D^{20}$ +26 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36–7.25 (m, 15H, Ph), 5.85 (dd, 1H, $J_{6,5}$ 10.8, $J_{5,4}$ 1.3 Hz, H-5), 5.79 (ddd, 1H, $J_{6,7a} = J_{6,7b}$ 2.3 Hz, H-6), 5.05 (s, 1H, H-1), 5.09–4.94 (m, 4H, CH_2Ph), 4.71–4.59 (m, 5H, H-4, H-7a, H-7b, H-2, H-3), 4.65 and 4.47 (AB system, 2H, $J_{A,B}$ 11.9 Hz, CH_2Ph), 1.43 and 1.27 [s, each 3H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): δ 137.22 (Ph), 135.83 and 135.74 (C-5, C-6), 128.71–127.42 (Ph), 112.56 [$C(CH_3)_2$], 105.32 (C-1), 85.29 (C-3), 81.35 (C-2), 75.61 (C-4), 69.31, 69.24 and 69.05 (CH_2Ph), 63.55 ($J_{C,P}$ 5.3 Hz, C-7), 26.04 and 24.77 [$C(CH_3)_2$]; ^{31}P NMR ($CDCl_3$): δ 0.05. Anal. Calcd for $C_{31}H_{35}O_8P$: C, 65.72; H, 6.23. Found: C, 65.47; H, 6.60.

1.3. Benzyl 7-*O*-[bis(benzyloxy)phosphoryl]-2,3-*O*-isopropylidene-L-*glycero*-β-L-*gulo*-heptofuranoside (3) and benzyl 7-*O*-[bis(benzyloxy)phosphoryl]-2,3-*O*-isopropylidene-D-*glycero*-α-D-*manno*-heptofuranoside (4)

A soln of **2** (815 mg, 1.44 mmol) and NMMO (388.7 mg, 2.88 mmol) in 2:1 dioxane–water (10 mL) and acetone (3 mL) was stirred at rt. Then osmium tetraoxide (1.5 mL, 0.12 mmol; 2% in water) was transferred into the flask and the mixture was stirred for 5 h. The soln was then diluted with $CHCl_3$ (150 mL). After treatment with ice-cold 5 M HCl (6 mL) the mixture was vigorously shaken with 45% aq $Na_2S_2O_5$ (9 mL) and water. The organic phase was dried ($MgSO_4$) and concentrated to a syrupy residue (580 mg, 71%). D-*glycero*-α-D-*manno*-Heptofuranoside (**4**) and the L-*glycero*-β-L-*gulo* diastereoisomer **3** were separated by chromatography on silica gel (2:3 *n*-hexane–EtOAc) to give 445 mg of **4** as colourless crystals, mp 82–83 °C (hexane–EtOAc), R_f 0.62; $[\alpha]_D^{20}$ +40 (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.37–7.26 (m, 15H, Ph), 5.12 (s, 1H, H-1), 5.10–5.04 (m, 4H, $POCH_2Ph$), 4.91 (dd, 1H, $^3J_{3,2}$ 6.0, $^3J_{3,4}$ 3.8 Hz, H-3), 4.70 (s, 2H, CH_2Ph), 4.63 (d, 1H, H-2), 4.61 (dd, 1H, $J_{7a,7b}$ 11.8, $^3J_{7a,6}$ 5.0 Hz, H-7a), 4.45 (m, 1H, H-7b), 4.16 (dd, 1H, $^3J_{4,5}$ 7.0 Hz, H-4), 4.01 (t, 1H, $^3J_{5,6}$ 7.0 Hz, H-5), 3.92 (m, 1H, $^3J_{6,7b}$ 3.0 Hz, H-6), 3.78 (br s, OH), 1.44 and 1.30 [2s, 6H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): δ 137.09 (Cquart. Ph), 128.65–126.99 (Ph), 112.70 [$C(CH_3)_2$], 105.46 (C-1), 84.73 (C-2), 80.44 (C-3), 79.03 (C-4), 72.87 (d, $J_{C6,P}$ 4.9 Hz, C-6), 69.75 (d, $POCH_2Ph$), 69.35, 69.25 and 69.17 (C-5, C-7, $POCH_2Ph$), 65.36 (CH_2Ph), 25.92 and 24.45 [$C(CH_3)_2$]; ^{31}P NMR ($CDCl_3$): δ 1.14. Anal. Calcd for $C_{31}H_{37}O_{10}P$: C, 61.99; H, 6.21. Found: C, 61.46; H, 6.26.

Further elution furnished **3** as a syrup (115 mg), R_f 0.57; $[\alpha]_D^{20}$ +43 (*c* 1.3, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.36–7.26 (m, 15H, Ph), 5.16 (s, 1H, H-1), 5.11–5.04

(dd, 4H, $POCH_2Ph$), 4.82 (dd, 1H, $^3J_{3,2}$ 5.9, $^3J_{3,4}$ 3.6 Hz, H-3), 4.66 (d, 1H, H-2), 4.67 and 4.49 (AB-system, 2H, $J_{A,B}$ 11.7 Hz, CH_2Ph), 4.33 (dd, 1H, $J_{7a,7b}$ 11.0, $^3J_{7a,6}$ 2.6 Hz, H-7a), 4.28 (dd, 1H, $^3J_{4,5}$ 2.5 Hz, H-4), 4.23 (dd, 1H, $^3J_{7b,6}$ 5.0 Hz, H-7b), 3.99 (dd, 1H, H-5), 3.89 (m, 1H, H-6), 2.60 (br s, OH), 1.47 and 1.30 [2s, 6H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): δ 137.42, 135.84, 135.72 (Cquart. Ph), 128.76–128.02 (Ph), 112.91 [$C(CH_3)_2$], 104.88 (C-1), 85.54 (C-2), 81.87 (C-3), 76.65 (C-4), 70.96 (d, $J_{C6,P}$ 4.9 Hz, C-6), 69.85, 69.82, 69.78 and 69.73 (C-5, C-7, $POCH_2Ph$), 69.23 (CH_2Ph), 25.77 and 24.09 [$C(CH_3)_2$]; ^{31}P NMR ($CDCl_3$): δ 1.36. Anal. Calcd for $C_{31}H_{37}O_{10}P$: C, 61.99; H, 6.21. Found: C, 61.48; H, 6.37.

1.4. D-*glycero*-D-*manno*-Heptopyranose 7-phosphate (triethylammonium salt) (5)

A soln of **4** (125 mg, 0.21 mmol) in dry MeOH (5.5 mL) was stirred at room temperature in the presence of 10% Pd/C (45 mg) for 12 h under hydrogen at atmospheric pressure. After completion of the reaction, the catalyst was removed by filtration through Celite and washed with MeOH. The filtrate was concentrated, redissolved in water (4 mL, the compound was acidic: pH 1) and stirred for 5 h at room temperature. Then the pH was adjusted to 5.5 by adding triethylamine (200 μL) and the soln was lyophilized to give **5** as amorphous material. Final purification was achieved by elution on a PD-10 column (water) to furnish 66 mg (81%) of **5**. $[\alpha]_D^{20}$ +6.5 → +9.7 (*c* 0.85, water); R_f 0.36 (10:10:3 MeOH–$CHCl_3$–water); NMR data see Table 1. ESI-MS: 291.0508 $[MH]^+$; Calcd for $C_7H_{15}O_{10}P$: 291.0476.

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