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SYNTHESIS AND N.M.R. CHARACTERIZATION OF INTERMEDIATES IN THE L-TYPE PENTOSE PHOSPHATE CYCLE

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ABSTRACT

The major contributing structures in aqueous solution of D-ribulose 5-phosphate, D-xylulose 5-phosphate, D-allose 6-phosphate, and D-*manno*-heptulose 7-phosphate have been established by ^{13}C-n.m.r. spectroscopy. Syntheses of D-allose 6-phosphate and D-*manno*-heptulose 7-phosphate are described.

INTRODUCTION

In conjunction with studies on the pentose phosphate pathway in rat liver[1], we recently investigated the structures of D-*altro*-heptulose 1,7-diphosphate, and D-*glycero*-D-*ido*- and D-*glycero*-D-*altro*-octulose 1,8-diphosphates by ^{13}C-n.m.r. spectroscopy[2]. Several additional metabolites are formed during the *in vitro* dissimilation of D-ribose 5-phosphate by liver enzymes[3]. Their role in the pentose phosphate pathway is not clear, since the ^{14}C methodology used to study these intermediates does not allow unequivocal determination of label distribution. The use of specifically labelled ^{13}C precursors, combined with ^{13}C-n.m.r. spectroscopy, offers a non-invasive method of following the distribution of ^{13}C label to the various intermediates of the pathway. In order to carry out this experiment, it was necessary to assign the ^{13}C resonances to the carbon atoms of all known metabolites of the *in vitro* dissimilation of D-ribose 5-phosphate to hexose 6-phosphates. The structures and ^{13}C-n.m.r. characteristics of D-ribulose‡ 5-phosphate[3], D-xylulose‡ 5-phosphate[3], D-allose 6-phosphate[4], and D-*manno*-heptulose 7-phosphate[3] are reported here.

RESULTS AND DISCUSSION

D-Ribulose 5-phosphate and D-xylulose 5-phosphate. — ^{13}C-N.m.r. spectroscopy of both phosphates in aqueous solution shows them to exist only as the free

‡Systematic IUPAC names are D-*erythro*-pentulose ("D-ribulose") and D-*threo*-pentulose ("D-xylulose").

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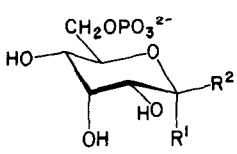
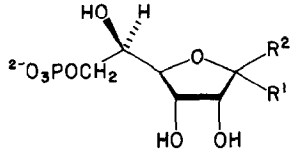
keto-sugars (Table I), as evidenced by the chemical shifts of their C-2 atoms, at 213.2 and 213.7 p.p.m., respectively. No hydrated, dimeric, or oligomeric forms are detectable by ^{13}C-n.m.r. spectroscopy. The assignment of ^{13}C resonances to individual carbon atoms is clear cut, because of the observation of two-bond POC ($^{2}J_{C,P}$ 4.1 and 5.9 Hz respectively) and three-bond POCC ($^{3}J_{C,P}$ 7.3 Hz) coupling-constants.

D-*Allose 6-phosphate.* — This compound was prepared from D-ribose 5-phosphate according to the method of Serianni[5]. Condensation of cyanide with D-ribose 5-phosphate in aqueous solution gave a mixture of the C-2 epimeric allono- and altrono-nitrile phosphates. This was hydrogenolyzed over a palladium catalyst to give a mixture of D-allose 6-phosphate and D-altrose 6-phosphate, in the ratio of 59:41 by ^{13}C-n.m.r. spectroscopy. These were separated by chromatography on DEAE-Sephadex A25. D-Allose 6-phosphate was isolated in 20% yield.

The ^{13}C-n.m.r. spectrum of D-allose 6-phosphate indicates a mixture of two pyranose and two furanose forms in aqueous solution. The β-pyranose **2** predominates (77%), whereas the α-pyranose **1** (15%), the α-furanose **3** (3%), and β-furanose **4** (5%) are minor contributors. The assignment of ^{13}C resonances (Table I) is based on comparison with the known chemical-shift values of all forms of D-allose[6]. The observed ^{13}C chemical shifts of both pyranoses of D-allose 6-phosphate are in good agreement with those expected from the replacement of the equatorial hydroxyl group on C-3 of α- and β-D-glucose 6-phosphate by an axial one[7]. The observation of the three-bond POCC coupling-constant ($^{3}J_{C,P}$ 7.3 Hz) allows identification of C-5 in the β-pyranose **2**, which supports the assignment of C-5 in β-D-allose given by Perlin[7] as against that proposed by Roberts[8].





	R¹	R²
1	OH	H
2	H	OH

	R¹	R²
3	OH	H
4	H	OH

D-manno-*Heptulose 7-phosphate.* — There is some evidence that D-manno-heptulose 7-phosphate acts as a regulator of the L-type pathway and that its accumulation in the *in vitro* dissimilation of pentose phosphate by liver enzymes slows down the formation of hexose phosphate[3]. Enzymic methods failed to provide adequate amounts of this compound for ^{13}C-n.m.r. analysis or for biological experiments. The chemical synthesis of D-*manno*-heptulose 7-phosphate was therefore pursued by two alternative routes as outlined next.

A. Via methyl D-manno-*heptuloside* (**6**). — Fischer glycosidation of D-*manno*-

TABLE I

^{13}C CHEMICAL SHIFTS[a] AND ^{31}P–^{13}C COUPLING CONSTANTS (Hz) OF D-RIBULOSE 5-PHOSPHATE, D-XYLULOSE 5-PHOSPHATE, AND D-ALLOSE 6-PHOSPHATE

Structure	*C-1*	*C-2*	*C-3*	*C-4* ($^3J_{C,P}$)	*C-5* ($^2J_{C,P}$ *or* $^3J_{C,P}$)	*C-6* ($^2J_{C,P}$)
D-Ribulose 5-phosphate	67.2	213.2	75.5	72.3(7.3)	64.9(5.9)	
D-Xylulose 5-phosphate	66.8	213.7	75.9	71.7(7.3)	65.1(4.1)	
D-Allose 6-phosphate **1**	93.9	67.8	72.3	66.5	[b]	64.3(4.4)
2	94.3	72.0[c]	71.8[c]	67.2	73.6(7.3)	64.6(4.4)
3	96.7	[b]	[b]	84.7	70.0[d]	66.1[d]
4	101.5	76.1	[b]	82.9	71.5[d]	66.1[d]

[a]In p.p.m. relative to Me_4Si. [b]Not resolved. [c]C-2 and C-3 assignments could be interchanged. [d]Signal broad, *J* values not determinable.

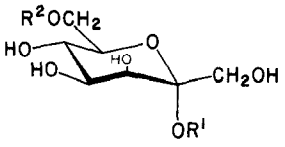
heptulose (**5**) with methanol gave methyl α-D-*manno*-heptulopyranoside (**6**) exclusively. Its ^{13}C-n.m.r. spectrum closely resembles that of D-*manno*-heptulose[9] **5**, except for the C-1 signal, which is shifted upfield by 7.1 p.p.m. and that of C-2, which is shifted downfield by 3.6 p.p.m. (Table II). Some ambiguity in the assignment of C-3–C-4 (70.8 and 71.7 p.p.m.) remains.

Phosphorylation of methyl α-D-*manno*-heptuloside (**6**) occurred preferentially, but not exclusively, at the primary 7-position. When **6** was treated with 1.2 equivalents of diphenyl chlorophosphate, a mixture of one mono- and one di-substituted product was formed, together with unreacted **6**. From this mixture methyl α-D-*manno*-heptuloside 7-diphenyl phosphate (**7**) was isolated by chromatography in 56% yield. The disubstituted product was shown to be methyl α-D-*manno*-heptuloside 1,7-bis(diphenyl phosphate) by the ^1H-n.m.r. spectrum of its triacetate. It became the major product when 2.2 equivalents of phosphorylating reagent were used. The structure of **7** follows from the ^1H-n.m.r. spectrum of its tetraacetate. Signals for H-3, H-4, and H-5 are clearly identifiable on acetylation, thus excluding all phosphate esters other than those of the 1 or 7 positions. The differentiation between the latter two is only possible after hydrogenolysis of **7** to give methyl α-D-*manno*-heptuloside 7-phosphate (**8**). Its ^{13}C-n.m.r. spectrum (Table II) clearly indicates the position of the phosphate group by showing C-6 and C-7 coupled to phosphorus by three-bond POCC-6 ($^3J_{C,P}$ 7.8 Hz) and two-bond POC-7 ($^2J_{C,P}$ 4.0 Hz) coupling-constants. Acid hydrolysis of **8** gave D-*manno*-heptulose 7-phosphate in 27% overall yield. Like its parent compound D-*manno*-heptulose, the 7-phosphate exists exclusively as the α-pyranose **9**, as shown by its ^{13}C-n.m.r. spectrum (Table II).

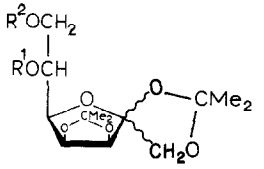
B. Via 1,2:3,4-di-O-isopropylidene-protected D-manno-*heptulose* (**11**). — The lack of selectivity in the phosphorylation of **6** necessitating the use of chromatography, and the low overall yield of method A, led us to explore the use of protecting groups for the preparation of D-*manno*-heptulose 7-phosphate. Selective isopropylidenation of **6** was not successful, but when fully protected 1,2:3,4:6,7-tri-

O-isopropylidene-D-*manno*-heptulofuranose (**10**), prepared from **5** in 56% yield, was treated with dilute acid, crystalline 1,2:3,4-di-*O*-isopropylidene-D-*manno*-heptulofuranose (**11**) was obtained. Phosphorylation of **11** with a slight excess of diphenyl chlorophosphate gave a very polar, water-soluble product, which was not isolated. Hydrogenation of this material followed by acid hydrolysis of the protecting groups gave D-*manno*-heptulose 7-phosphate (**9**) in 85% yield from **10**, identical in all respects with material obtained by method A.

Method B obviates the need for chromatographic separation following phosphorylation. The fully protected heptulose **10** was separated by distillation from products of lower substitution and from starting material. The structures of **10** and **11** were confirmed by their ^{1}H-n.m.r. and mass-spectral data (see Experimental). The coupling constants observed in the ^{1}H-n.m.r. spectra and the fragmentation patterns observed in the mass spectra of **10** and **11** are clearly indicative of a furanose ring. The position of the free hydroxyl groups in **11** is evidenced by the large downfield shifts of H-6, H-7, and H-7′ in the ^{1}H-n.m.r. spectrum of its diacetate **12**. ^{1}H-N.m.r. and mass-spectral data do not provide information on the configuration of the anomeric centre. A second tri-*O*-isopropylidene derivative **10a**, formed together with **10** from D-*manno*-heptulose and acetone in 3% yield, was shown to differ from **10** only in the configuration at the anomeric centre. This



	R¹	R²
5	H	H
6	CH_3	H
7	CH_3	$PO(OPh)_2$
8	CH_3	PO_3^{2-}
9	H	PO_3^{2-}



10, 10a R¹, R² = $\diagdown$CMe$_2$

11 R¹ = R² = H

12 R¹ = R² = Ac

TABLE II

^{13}C CHEMICAL SHIFTS[a] AND ^{31}P–^{13}C COUPLING CONSTANTS (Hz) OF DERIVATIVES OF D-*manno*-HEPTULOSE

Structure	*OCH_3*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6* ($^3J_{C,P}$)	*C-7* ($^2J_{C,P}$)
5		64.9	98.5	71.7[b]	70.8[b]	67.7	73.8	61.9
6	48.5	57.8	102.1	71.8[b]	69.8[b]	67.5	74.5	61.9
8	48.6	57.8	102.5	71.6[b]	69.8[b]	66.9	73.7(7.8)	64.5(4.0)
9	65.0	98.8	71.5[b]	71.0[b]	67.4	73.1(7.9)	64.5[c]	

[a]In p.p.m. relative to Me_4Si. [b]C-3 and C-4 assignments could be interchanged. [c]Signal broad, *J* value not determinable.

isomer codistills with **10** but could be isolated by chromatography on silica gel and shows ^{1}H-n.m.r. and mass-spectroscopic properties consistent with the 1,2:3,4:6,7-substitution pattern of the furanoid structure **10a**.

EXPERIMENTAL

General methods. — ^{13}C-N.m.r. spectra were recorded at 25° with a Jeol FX-200 n.m.r. spectrometer operated at 50.3 MHz. Samples of sugar phosphates were dissolved in D_2O and adjusted to pH 7.0 (uncorrected) by the addition of 0.1M NaOH or 0.1M HCl as required. Methanol was added to all samples as the internal standard. Its chemical-shift value was taken[2] as 49.7 p.p.m. with respect to Me_4Si. ^{1}H-N.m.r. spectra (100 MHz) were recorded with a Varian HA-100 spectrometer. All spectra were run in $CDCl_3$ with Me_4Si as the internal standard. Mass spectra were recorded with a VG Miromass 7070 F instrument. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. Elemental analyses were carried out by the Microanalytical Unit, Australian National University. D-Ribose 5-phosphate, D-ribulose 5-phosphate, D-xylulose 5-phosphate, and D-*manno*-heptulose were of commercial origin (Sigma) and were used without purification. All reagents used were of analytical purity.

D-*Allose 6-phosphate and* D-*altrose 6-phosphate.* — A solution of potassium cyanide (198 mg, 3 mmol) in water (30 mL) was cooled to 5°. The pH was adjusted to 8.0 by addition of 6M acetic acid. A solution of D-ribose 5-phosphate (668 mg, 2.4 mmol) in water (5 mL) was added and the pH was maintained between 7.5 and 8. After 15 min, the pH of the mixture was adjusted to 4 by addition of 6M acetic acid and the solution was concentrated to ~10 mL. A suspension of palladium-on-barium sulfate (5%, 200 mg) in water (5 mL) was reduced with hydrogen for 15 min at atmospheric pressure and 25° with efficient stirring. The solution of D-allono- and D-altrononitrile 6-phosphate (pH 1.7) was added to this suspension. The reduction vessel was evacuated 3 times and filled with hydrogen prior to a final charging with hydrogen. After 16 h, 40 mL of hydrogen had been consumed. The suspension was filtered through Celite and the filtrate treated with Dowex 50W × 8 (H^+) resin. The resin was removed by filtration and the pH of the solution adjusted to 6.5 before freeze-drying. After ^{13}C-n.m.r. spectroscopic analysis of the mixture had been performed, it was applied to a column (2.6 × 100 cm) of DEAE-Sephadex A25 that was eluted with a linear gradient of 0.05–0.4M ammonium tetraborate. Fractions containing hexose phosphates (using the anthrone reaction as described by Graham and Smydzuk[10]) were combined, and evaporated *in vacuo* repeatedly with addition of methanol. Complete desalting was achieved by passing solutions of D-allose 6-phosphate and D-altrose 6-phosphate through Sephadex G10 resin, using deionized water as eluent. D-Allose 6-phosphate and D-altrose 6-phosphate were obtained in 20 and 14% yields, respectively, as determined by the anthrone[10] and phosphate[11] analyses.

D-*Altrose 6-phosphate.* — ^{13}C-N.m.r. data: β-pyranose (39%): δ 64.5 (bd,

C-6), 66.1 (C-4), 71.3 and 71.6 (C-2 and C-3), 74.0 (d, 7.3 Hz, C-5), and 92.8 (C-1); α-pyranose (29%): δ 63.9 (d, 4.4 Hz, C-6), 65.6 (C-4), 71.1 and 71.2 (C-2 and C-3), 71.7 (partly overlapped, C-5), and 94.7 (C-1); α-furanose (20%): δ 65.6 (broad, C-6), 76.5 (C-3), 82.1 (C-2), 84.4 (C-4), and 101.9 (C-1); and β-furanose (12%): δ 65.6 (broad, C-6), 72.6 (d, 5.9 Hz, C-5), 75.8 (C-3), 77.5 (C-2), 82.0 (C-4), and 96.1 (C-1).

Methyl α-D-manno-*heptulopyranoside* (**6**). — D-*manno*-Heptulose (2.0 g) was stirred with dry methanol (20 mL) containing catalytic amounts of hydrogen chloride. After 3 h, two chromatographically distinguishable methyl heptulosides in approximately equal concentration were formed, which gave, after 17 h at 25°, a single compound. Hydrogen chloride was removed by the addition of AG 1 × 8 (OH^-) resin. The resin was filtered and washed with methanol, and the filtrate evaporated, to give **6** (2.02 g, 95%), as a glass. It was characterized as its penta-acetate.

*Methyl 1,3,4,5,7-penta-*O*-acetyl-*α-D-manno-*heptulopyranoside.* — This compound had $[\alpha]_D^{22}$ +48.0° (*c* 1.4, chloroform); ^{1}H-n.m.r.: δ 1.95, 2.01, 2.03, 2.08, 2.12 (5 s, 15 H, 5 OAc), 3.31 (s, 3 H, OCH_3), 3.88 (ddd, 1 H, $J_{5,6}$ 10.0, $J_{6,7}$ 3.4, $J_{6,7'}$ 5.6 Hz, H-6), 4.13 (dd, 1 H, $J_{7,7'}$ 12.6, $J_{6,7}$ 3.4 Hz, H-7), 4.19 (s, 2 H, H-1 and H-1′), 4.29 (dd, 1 H, $J_{7,7'}$ 12.6, $J_{6,7'}$ 5.6 Hz, H-7′), 5.22 (t, 1 H, $J_{4,5} \approx J_{5,6}$ 10 Hz, H-5), 5.38 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-3), and 5.43 (dd, 1 H, $J_{3,4}$ 3.4, $J_{4,5}$ 10 Hz, H-4).

Anal. Calc. for $C_{18}H_{26}O_{12}$: C, 49.8; H, 6.0. Found: C, 50.0; H, 6.4.

Methyl α-D-manno-*heptuloside 7-diphenylphosphate* (**7**). — To a solution of **6** (1.9 g) in dry pyridine (20 mL) at 0° a solution of diphenyl chlorophosphate (2.7 g, 1.2 equiv.) in pyridine (10 mL) was added. The mixture was allowed to warm to 25° and kept for 17 h. The excess of reagent was hydrolyzed with a few drops of water and solvent removed *in vacuo*; the residue was chromatographed on silica gel (4:1 chloroform–methanol) to give **7** (2.2 g, 57%) as a syrup; $[\alpha]_D^{22}$ +30.9° (*c* 0.6, methanol); ^{1}H-n.m.r.: δ 2.91 (s, 3 H, OCH_3), 3.2–4.8 (m, 12 H, H-1, H-1′, H-3,4,5,6,7, and 7′, 4 × OH), and 6.7–7.4 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{20}H_{25}PO_{10}$: C, 52.6; H, 5.5; P, 6.8. Found: C, 53.1; H, 5.6; P, 7.2.

*Methyl 1,3,4,5-tetra-*O*-acetyl* α-D-manno-*heptuloside 7-diphenylphosphate.* — ^{1}H-N.m.r.: δ 1.93, 2.00, 2.06 (3 s, 12 H, 4 OAc), 3.19 (s, 3 H, OCH_3), 3.92 (dt, 1 H, $J_{5,6}$ 9.6, $J_{6,7} \approx J_{6,7'}$ 5.6 Hz, H-6), 4.09, 4.10 (2 s, 2 H, H-1 and 1′), 4.28 (d, 1 H, $J_{6,7}$ 5.6 Hz, H-7), 4.31 (d, 1 H, $J_{6,7'}$ 5.6 Hz, H-7′), 5.17 (t, 1 H, $J_{4,5} \approx J_{5,6}$ 9.6 Hz, H-5), 5.36 (d, 1 H, $J_{3,4}$ 2.8 Hz, H-3), 5.42 (dd, 1 H, $J_{3,4}$ 2.8, $J_{4,5}$ 9.6 Hz, H-4), and 7.1–7.5 (m, 10 H, 2 Ph).

Methyl α-D-manno-*heptuloside 7-phosphate, disodium salt* (**8**). — A solution of **7** (2.09 g) in abs. methanol (50 mL) was hydrogenated in the presence of platinum oxide (150 mg) at atmospheric pressure for 16 h at 25°. Hydrogen (750 mL, 91% of the calculated amount) was consumed. The catalyst was removed by filtration and the solution evaporated *in vacuo*. The residue was dissolved in water (20 mL), made neutral with 0.1M NaOH to pH 7.0, filtered, and freeze dried to give **8** (1.24 g, 78%).

D-manno-*Heptulose 7-phosphate, disodium salt* (**9**). — A solution of **8** (1.24 g) in dilute aqueous sulfuric acid (0.33%, 20 mL) was heated for 30 min on a steam bath. The solution was cooled and made neutral with an excess of solid barium carbonate. The salts were filtered, thoroughly washed with water, and the filtrate evaporated *in vacuo* to give 1.03 g of material. A portion of the residue (500 mg) in water was applied to a column (18 × 300 mm) of AG 1 × 8, 200–400 mesh (formate) resin that was eluted with a linear gradient of 0–4M formic acid–M ammonium formate. Fractions containing **9** (by the cysteine–sulfuric acid reaction described by Paoletti *et al.* [12]) were pooled and passed through a column of Dowex 50W (H^+) resin. Formic acid was removed by continuous extraction with ether and the aqueous layer made neutral with 0.1M NaOH to give **9** (370 mg, 62%). ^{31}P-N.m.r. (D_2O, external H_3PO_4): δ 5.16.

1,2:3,4:6,7-Tri-O-*isopropylidene*-D-manno-*heptulofuranose* (**10**). — A mixture of D-*manno*-heptulose (2.0 g), anhydrous copper sulfate (4.0 g), acetone (50 mL), and sulfuric acid (0.5M, 0.1 mL) was stirred for 24 h at 25°. The mixture was made neutral by the addition of solid sodium hydrogencarbonate, filtered and concentrated *in vacuo*. The residue was distilled (0.1 mm, 175° bath temperature) by Kugelrohr distillation to give a mixture of **10** and **10a** (1.76 g, 56%). Analytically pure **10** was obtained by chromatography on silica gel (chloroform containing 2% methanol); $[\alpha]_D^{22}$ +41.7° (*c* 1.5, chloroform); ^{1}H-n.m.r.: δ 1.33, 1.39, 1.43, 1.45 (4 s, 18 H, 3 CMe_2), 3.89 (dd, 1 H, $J_{4,5}$ 3.4, $J_{5,6}$ 8.2 Hz, H-5), 3.98 (dd, 1 H, $J_{6,7}$ 4.9, $J_{7,7'}$ 8.7 Hz, H-7), 3.99 (d, 1 H, $J_{1,1}$ 9.5 Hz, H-1), 4.11 (dd, 1 H, $J_{6,7'}$ 5.8, $J_{7,7'}$ 8.7 Hz, H-7'), 4.25 (d, 1 H, $J_{1,1'}$ 9.5 Hz, H-1'), 4.37 (ddd, 1 H, $J_{5,6}$ 8.2, $J_{6,7}$ 4.9, $J_{6,7'}$ 5.8 Hz, H-6), 4.61 (d, 1 H, $J_{3,4}$ 5.8 Hz, H-3), and 4.83 (dd, 1 H, $J_{3,4}$ 5.8, $J_{4,5}$ 3.4 Hz, H-4); *m/z*: 331 (2), 316 (4), 315 (28), 257 (4), 229 (2), 215 (2), 199 (4), 197 (3), 157 (2), 156 (3), 143 (3), 142 (6), 141 (10), 139 (13), 126 (4), 117 (3), 113 (15), 101 (29), 85 (13), 72 (12), 59 (23), and 43 (100).

Anal. Calc. for $C_{16}H_{26}O_7$: C, 58.2; H, 7.9. Found: C, 58.4; H, 7.8.

1,2:3,4:6,7-Tri-O-*isopropylidene*-D-manno-*heptulofuranose* (**10a**) *(minor isomer).* — ^{1}H-N.m.r. ($CDCl_3$): δ 1.38, 1.43, 1.49, 1.60 (4 s, 18 H, 3 CMe_2), 3.80 (dd, 1 H, $J_{4,5}$ 4.7, $J_{5,6}$ 7.8 Hz, H-5), 3.90 (d, 1 H, $J_{1,1'}$ 9.0 Hz, H-1), 4.04 (d, 1 H, $J_{1,1'}$ 9.0 Hz, H-1'), 4.08 (d, 1 H, $J_{6,7}$ 5.9 Hz, H-7), 4.09 (d, 1 H, $J_{6,7'}$ 5.0 Hz, H-7'), 4.48 (ddd, 1 H, $J_{5,6}$ 7.8, $J_{6,7}$ 5.9, $J_{6,7'}$ 5.0 Hz, H-6), 4.51 (d, 1 H, $J_{3,4}$ 6.2 Hz, H-3), and 4.79 (dd, 1 H, $J_{3,4}$ 6.2, $J_{4,5}$ 4.7 Hz, H-4); *m/z*: 315 (47), 279 (2), 257 (13), 229 (10), 214 (3), 199 (9), 171 (6), 156 (6), 149 (15), 141 (29), 139 (15), 126 (36), 117 (13), 113 (22), 101 (100), 98 (21), 85 (19), 72 (32), and 59 (33).

1,2:3,4-Di-O-*isopropylidene*-D-manno-*heptulofuranose* (**11**). — To a solution of **10** (contaminated with **10a**, 1.47 g) in methanol (40 mL) a saturated solution of hydrogen chloride in methanol (0.7 mL) was added dropwise and the mixture maintained for 30 min at 25°. Anion-exchange [AG 1× 8 (OH^-)] resin was added to neutrality, the resin was filtered off, and washed with methanol, and the filtrate evaporated *in vacuo*. The residue gave **11** (0.87 g, 67%); m.p. 69–70° (from ethyl acetate–hexane), $[\alpha]_D^{22}$ +58.3° (*c* 0.8, chloroform); ^{1}H-n.m.r.: δ 1.32, 1.38, 1.42 (3

s, 12 H, 2 CMe_2), 2.47, 3.04 (broad, 2 H, 2 × OH), 3.5–4.0 (m, 4 H, H-5,6,7, and 7′), 4.01 (d, 1 H, $J_{1,1'}$ 9.8 Hz, H-1), 4.26 (d, 1 H, $J_{1,1'}$ 9.8 Hz, H-1′), 4.61 (d, 1 H, $J_{3,4}$ 6.6 Hz, H-3), and 4.90 (dd, 1 H, $J_{3,4}$ 6.6, $J_{4,5}$ 3.4 Hz, H-4); *m/z*: 275 (46), 259 (4), 229 (10), 175 (9), 157 (11), 142 (50), 139 (33), 117 (74), 113 (53), 97 (35), 86 (47), 84 (49), 59 (96), and 43 (100).

Anal. Calc. for $C_{13}H_{22}O_7$: C, 53.8; H, 7.6. Found: C, 53.5; H, 7.6.

*6,7-Di-*O*-acetyl-1,2:3,4-di-*O*-isopropylidene-*D-manno*-heptulofuranose* (**12**). — ^{1}H-n.m.r.: δ 1.27, 1.38, 1.41 (3 s, 12 H, 2 CMe_2), 2.02, 2.04 (2 s, 6 H, 2 OAc), 4.01 (d, 1 H, $J_{1,1'}$ 9.5 Hz, H-1), 4.10 (dd, 1 H, $J_{4,5}$ 3.6, $J_{5,6}$ 8.4 Hz, H-5), 4.20 (dd, 1 H, $J_{6,7}$ 5.0, $J_{7,7'}$ 12.8 Hz, H-7), 4.24 (dd, 1 H, $J_{1,1'}$ 9.5 Hz, H-1′), 4.55 (dd, 1 H, $J_{6,7'}$ 2.3, $J_{7,7'}$ 12.8 Hz, H-7′), 4.58 (d, 1 H, $J_{3,4}$ 5.8 Hz, H-3), 4.76 (dd, 1 H, $J_{3,4}$ 5.8, $J_{4,5}$ 3.6 Hz, H-4), and 5.27 (ddd, 1 H, $J_{5,6}$ 8.4, $J_{6,7}$ 5.0, $J_{6,7'}$ 2.3 Hz, H-6).

D-manno-*Heptulose 7-phosphate, disodium salt* (**9**). — To a solution of **11** (0.6 g) in dry pyridine (10 mL) at 0° a solution of diphenyl chlorophosphate (0.67 g, 1.2 equiv.) in pyridine (5 mL) was added. The mixture was kept for 17 h at 25°. A few drops of water were added and the solvent removed *in vacuo*. The residue was dissolved in water (50 mL) and hydrogenated in the presence of platinum oxide (100 mg) at 25° and atmospheric pressure for 16 h. The uptake of hydrogen was 360 mL. The catalyst was removed by filtration and the solution evaporated *in vacuo*. The residue was dissolved in aqueous acetic acid (80%, 20 mL) and the solution kept for 3 h at 90°. Acetic acid was removed by distilling toluene from the residue. The residue was dissolved in water (2 mL) and purified by anion-exchange chromatography, as already described, to give **9** (580 mg, 85%).

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