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Five monophosphates of methyl L-*glycero*-α-D-*manno*-heptopyranoside: synthesis, hydrolysis and migrations

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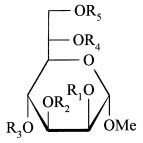
Abstract

From suitably protected methyl α-D-mannopyranosides five methyl L-*glycero*-α-D-*manno*-heptopyranosides were synthesized by the one-carbon-atom chain elongation at C-6 and converted to five monophosphates (**1–5**) having the $PO(OH)_2$ group at O-2, -3, -4, -6 and -7. Compounds **1–5** were exposed to acidic and basic hydrolytic conditions used in lipopolysaccharide analysis and the products and their proportion were determined. Under acidic conditions, besides hydrolysis of the glycoside, migrations and hydrolytic cleavage of the phosphate residue were found. Under basic conditions the phosphates were stable.

Keywords: Bacterial sugars; Methyl L-*glycero*-α-D-*manno*-heptopyranoside monophosphate; Heptose synthesis

1. Introduction

In continuation of the work on L-*glycero*-D-*manno*-heptose (LD-manHep*p*) monophosphates having the $PO(OH)_2$ residue unambiguously located at O-2, -3, -4, -6, and -7 [1] we extended the investigation to a similar series of monophosphates bound to methyl L-*glycero*-α-D-*manno*-heptopyranoside. These compounds enabled the study of phosphoryl group migration under typical conditions used in lipopolysaccharide degradation. The α-glycosidic methyl group eliminated the problems connected with the presence of α,β-anomers and eliminated also side reactions due to the free HO-1 group under, at least, basic or weakly acidic hydrolytic conditions.



1 R_1 = $PO(OH)_2$, R_2 = R_3 = R_4 = R_5 = H
2 R_2 = $PO(OH)_2$, R_1 = R_3 = R_4 = R_5 = H
3 R_3 = $PO(OH)_2$, R_1 = R_2 = R_4 = R_5 = H
4 R_4 = $PO(OH)_2$, R_1 = R_2 = R_3 = R_5 = H
5 R_5 = $PO(OH)_2$, R_1 = R_2 = R_3 = R_4 = H

Synthetic phosphates of L-*glycero*-D-*manno*-heptose are scarcely known; only preparations of LD-manHep*p* 1-phosphate [2], DL-manHep*p*

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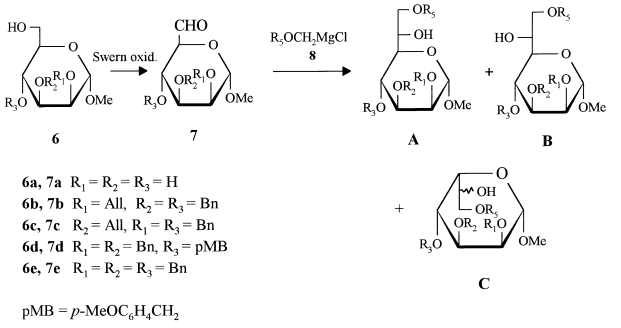
7-phosphate (enantiomer of LD-manHep*p*) [3], 7′-(2-aminoethyl)phosphate [4] and 4- and/or 4′-phosphates of α-(1→3)-linked di-LD-manHep*p* [5] have been described.

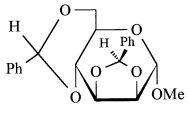
2. Results

Synthesis.—The synthesis of five monophosphates of methyl L-*glycero*-α-D-*manno*-heptopyranoside (**1–5**) was carried out essentially according to the protocol employed previously [1], the main difference being the replacement of benzyl α-D-mannopyranoside as substrate by commercially available methyl α-D-mannopyranoside (**6a**).



Scheme 1.

Below are shortly described the syntheses of specifically blocked methyl L-*glycero*-α-D-*manno*-heptopyranosides permitting a ready access to substrates for phosphorylation having OH groups free at C-2, -3, -4, -6, and -7. In all chain elongation reactions, besides the main product **A** of the LD-*manno* configuration, substantial amounts of the DD-*manno* stereoisomer **B** as well as methyl heptoside **C** derived from C-5 "inverted" aldehyde [1] were obtained. The general course of the syntheses is presented in Scheme 1.



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For the synthesis of the heptoside substrate for phosphorylation at O-2, methyl *exo*-2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside was used (**9**, cf. Scheme 1 in ref. [1]). Selective opening of the 2,3-*O*-benzylidene ring by reduction with $LiAlH_4$–$AlCl_3$, followed by allylation and further selective opening of the 4,6-*O*-benzylidene ring with the same reagent yielded **6b** (Scheme 1), having a free HO-6 group available for the Swern oxidation. After oxidation, the aldehyde **7b** was reacted with benzyloxymethylmagnesium chloride (**8**, R_5 = Bn) to yield methyl 2-*O*-allyl-3,4,7-tri-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (**10**), methyl 2-*O*-allyl-3,4,7-tri-*O*-benzyl-D-*glycero*-α-D-*manno*-heptopyranoside (**14**), and methyl 2-*O*-allyl-3,4,7-tri-*O*-benzyl-D(L)-*glycero*-β-L-*gulo*-heptopyranoside (**15**) (Table 1).

Compound **6c** (Scheme 1) having O-3 blocked with an allyl group and being a precursor of the heptoside substrate for phosphorylation at O-3, was obtained from methyl 6-*O*-trityl-α-D-mannopyranoside by stannylation with Bu_2O followed by allylation with allyl bromide, then benzylation with benzyl bromide at O-2 and O-4, and detritylation. Further steps consisted of a Swern oxidation to aldehyde **7c** and chain elongation with **8** (R_5 = Bn), thus leading to methyl 3-*O*-allyl-2,4,7-tri-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (**16**), methyl 3-*O*-allyl-2,4,7-tri-*O*-benzyl-D-*glycero*-α-D-*manno*-heptopyranoside (**20**), and methyl 3-*O*-allyl-2,4,7-tri-*O*-benzyl-D(L)-*glycero*-β-L-*gulo*-heptopyranoside (**21**) (Table 1).

As a precursor of the heptoside substrate for phosphorylation at O-4 methyl 2,3-di-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (**6d**) was obtained from methyl 4,6-*O*-(*p*-methoxybenzylidene)-α-D-mannopyranoside (**22**) by benzylation and subsequent selective ring opening with Me_3SiCl–$NaBH_3CN$. Chain elongation with **8** (R_5 = Bn) gave methyl 2,3,7-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-L-*glycero*-α-D-*manno*-heptopyranoside (**23**) and methyl 2,3,7-tri-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-D(L)-*glycero*-β-L-*gulo*-heptopyranoside (**27**) (Table 1). In this reaction formation of the DD-*manno* stereoisomer was not detected.

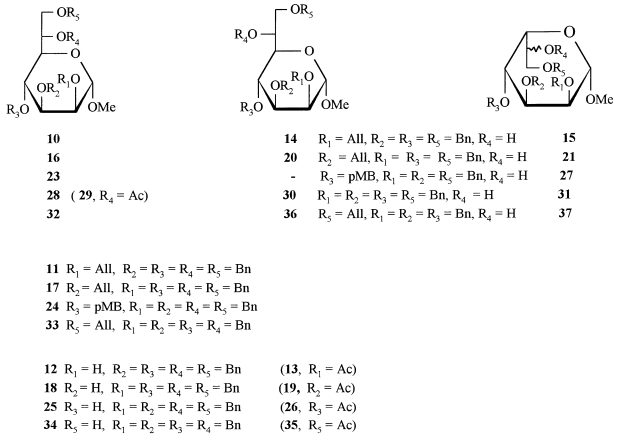
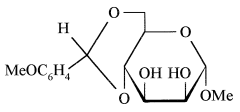


Table 1
Methyl heptosides via chain elongation: yields and HR MS data

Series	Methyl heptoside	No.	Yield[a] %	$[\alpha]_D$[b] (*c*)	HR MS (LSIMS)	
					Calcd	Found
2*P*	LD-*manno*	**10**	32.0	+24 (1.2)	557.25152	557.25093
	DD-*manno*	**14**	15.8	+55 (1.2)		
	D(L)L-*gulo*	**15**	12.4	+11 (1.3)		557.25093
3*P*	LD-*manno*	**16**	36.3	+25 (0.9)		557.25093
	DD-*manno*	**20**[d]	11.5	n.d.[e]		n.d.
	D(L)L-*gulo*	**21**	9.0	+2.2 (0.9)		557.25145
4*P*	LD-*manno*	**23**	28.6	+13 (1.2)	637.27773	637.27921
	D(L)L-*gulo*	**27**	20.2	+6 (1.1)		637.27676
6*P*	LD-*manno*	**28**	40.7	+18 (1.0)	607.26717	607.26715
	DD-*manno*	**30**	16.5	+34 (1.0)	649.27773[f]	649.27739
	D(L)L-*gulo*	**31**	15.2	0 (1.3)	607.26717	607.26943
7*P*	LD-*manno*	**32**	34.5	+21 (1.0)	557.25152	557.25093
	DD-*manno*	**36**	20.8			
	D(L)L-*gulo*	**37**	13.8	+3 (1.3)		

[a] Isolated yield after repeated chromatography.
[b] In $CHCl_3$ at 20 ± 3 °C.
[c] $[M + Na]^+$.
[d] Compound **20** was not analytically pure. Its identity was confirmed by ^1H NMR analysis.
[e] Not determined.
[f] Exact mass determination was made for the acetylated product.



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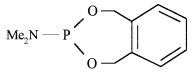


38 (DMABDP)

With respect to the heptoside substrate for phosphorylation at O-6 methyl 2,3,4,7-tetra-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (**28**) was obtained by chain elongation of known methyl 2,3,4-tri-*O*-benzyl-α-D-*manno*-hexodialdo-1,5-pyranoside (**7e**), prepared from compound **6e**, with **8** (R_5 = Bn). Heptoside **28** was accompanied by the DD-stereoisomer **30** and the "inverted" product **31** (Table 1).

For the preparation of the substrate for phosphorylation at O-7, aldehyde **7e** was reacted with allyloxymethylmagnesium chloride (**8**, R_5 = All, cf. Scheme 5 in ref. [1]). Besides the main product, methyl 7-*O*-allyl-2,3,4-tri-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside (**32**) two stereoisomeric products were also formed: methyl 7-*O*-allyl-2,3,4-tri-*O*-benzyl-D-*glycero*-α-D-*manno*-heptopyranoside (**36**) and methyl 7-*O*-allyl-2,3,4-tri-*O*-benzyl-D(L)-*glycero*-β-L-*gulo*-heptopyranoside(**37**)(Table 1).

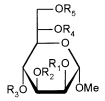
In order to obtain the various final heptoside substrates, first the hydroxyl group at C-6 in **10**, **16**, **23**, and **32** was benzylated to form **11**, **17**, **24**, and **33**, respectively. Then, in a next step, the allyl (or *p*-methoxybenzyl) group was removed to form the substrates **12**, **18**, **25**, and **34**, respectively, for the selective phosphorylation. Obviously, heptoside **28** could be phosphorylated directly. The ^{13}C NMR data of the various products of the L-*glycero*-α-D-*manno* series including the *O*-acetylated derivatives of **12**, **18**, **25**, **28**, and **34** (**13**, **19**, **26**, **29**, and **35**) are collected in Table 2.

For the phosphorylation of **12**, **18**, **25**, **28**, and **34** 2-dimethylamino-5,6-benzo-1,3,2-dioxaphosphepane (DMABDP, **38**) was employed [1], and the products obtained were in situ oxidized with *m*-chloroperoxybenzoic acid to the corresponding 5,6-benzo-2-oxo-1,3,2-phosphepan-2-yl derivatives **39**, **40**, **41**, **42**, and **43** (for ^{13}C NMR data, see Table 3). Exhaustive hydrogenation (H_2, Pd-C) yielded the five methyl L-*glycero*-α-D-*manno*-heptopyranosides **1–5** having phosphoryl groups at O-2, -3, -4, -6, and -7, respectively. Spectral data of these products are collected in Tables 4 and 5.

Hydrolysis and migrations.—Migration of the phosphoryl group in polyol monophosphate systems under acidic conditions is known since a long time [6]. Investigations involved glycerol [7–9], inositol [10], and nucleoside [11,12] phosphates. The mechanism of the $PO(OH)_2$ group migration was carefully studied [13,14].

Although numerous syntheses of sugar phosphates have been described in the literature (e.g. [15–22]), so far the migration of phosphate residues was studied to a very limited extent. Apparently,

Table 2
^{13}C NMR data of protected methyl L-*glycero*-α-D-*manno*-heptopyranosides[a]



	Substituents					^{13}C chemical shifts						
No.	R_1	R_2	R_3	R_4	R_5	C-1	C-2	C-3	C-4	C-5	C-6	C-7
10	All	Bn	Bn	H	Bn	99.45	74.47	80.03	74.18	70.70	67.89	71.44
11	All	Bn	Bn	Bn	Bn	99.04	74.94	80.40	74.13	71.24	74.10	70.15
12	H	Bn	Bn	Bn	Bn	100.39	67.94	80.59	74.80	70.57	73.57	69.98
13	Ac	Bn	Bn	Bn	Bn	98.71	68.37	78.57	74.82	70.93	73.69	69.98
16	Bn	All	Bn	H	Bn	99.39	74.46	79.90	74.14	70.70	67.92	71.06
17	Bn	All	Bn	Bn	Bn	98.97	74.97	80.30	74.16	71.27	74.12	70.15
18	Bn	H	Bn	Bn	Bn	97.87	78.30	72.07	76.05	70.42	75.05	70.12
19	Bn	Ac	Bn	Bn	Bn	98.99	75.73	73.89	73.24	71.08	74.89	70.03
23	Bn	Bn	pMB	H	Bn	99.33	74.59	80.26	74.01	70.80	67.94	71.49
24	Bn	Bn	pMB	Bn	Bn	99.23	75.58	81.19	75.42	71.83	74.41	69.95
25	Bn	Bn	H	Bn	Bn	99.15	75.00	79.94	66.17	71.35	73.75	69.65
26	Bn	Bn	Ac	Bn	Bn	99.27	74.99	77.74	67.81	69.37	73.72	69.33
28	Bn	Bn	Bn	H	Bn	99.34	74.51	80.23	74.23	70.76	67.93	71.44
29	Bn	Bn	Bn	Ac	Bn	98.91	73.90	80.48	73.77	69.43	68.79	67.83
32	Bn	Bn	Bn	H	All	99.33	74.56	80.24	74.21	70.70	67.85	71.32
33	Bn	Bn	Bn	Bn	All	98.95	74.27	80.63	74.19	71.20	74.92	69.88
34	Bn	Bn	Bn	Bn	H	99.12	74.30	80.34	74.22	73.09	75.83	62.52
35	Bn	Bn	Bn	Bn	Ac	99.05	73.98	80.54	73.84	70.99	74.15	62.87

[a] Signals of acetyl, allyl, benzyl and methyl groups occurred at their normal positions and are omitted.

Table 3
^{13}C NMR data of benzyl-protected, phosphorylated methyl L-*glycero*-α-D-*manno*-heptopyranosides[a]

	C-1	C-2	C-3	C-4	C-5	C-6	C-7
39	99.04 (2.8)[b]	73.01 (5.4)	78.34 (4.6)	73.63	71.16	75.23	70.05
40	99.16	76.82	78.86	73.89 (6.4)	71.12	75.01	69.88
41	98.94	74.35	78.36	74.27 (6.3)	70.30 (5.5)	73.84	69.02
42	99.93	73.81	80.68	74.91	69.90 (5.6)	74.60 (5.6)	68.74 (1.7)
43	99.11	73.90[c]	80.48	74.15[c]	70.74	74.85 (7.6)	66.40 (5.7)

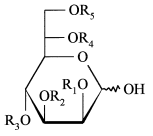
[a] Signals of carbon atoms belonging to benzyl groups and 5,6-benzo-2-oxo-1,3,2-dioxaphosphepane are omitted.
[b] $^3J_{C,P}$ or $^2J_{C,P}$.
[c] Can be interchanged.

the earliest report was by Levene and Raymond [15], who, on phosphorylation of 5-blocked 1,2-*O*-isopropylidene-α-D-xylofuranose with $POCl_3$ and pyridine followed by hydrolysis of the product with acetic and sulfuric acids, obtained only D-xylose 5-phosphate instead of the expected 3-phosphate. Baddiley, Buchanan and Carss [18] found that heating of D-ribitol 5-phosphate in 1 M HCl (15 min, 100 °C) caused migration of the $PO(OH)_2$ group to positions 2 and 3. Pyridine-catalyzed migration of the thiophosphoryl group in sugar β-hydroxyphosphorodithioates was studied by Michalska et al. [23]. More often no migration of the phosphoryl group was noted [2,3,16,19,20,22]. Cawley and Letters [21] synthesized methyl α-D-mannopyranoside 4- and 6-phosphates, and found that under acidic (2 M HCl, 100 °C, 2 h) or alkaline (0.5 M NaOH, 100 °C, 4 h) conditions both compounds were stable and no migration of the $PO(OH)_2$ group was detected.

Table 4
NMR data for methyl L-*glycero*-α-D-*manno*-heptopyranoside monophosphates **1**–**5** and their Na^+ salts[a,b]

Phosphate		Chemical shifts (δ)							
		H-1/C-1	H-2/C-2/2-*P*	H-3/C-3/3-*P*	H-4/C-4/4-*P*	H-5/C-5	H-6/C-6/6-*P*	H-7a/C-7/7-*P*	H-7b
2*P* (**1**)	H^+	4.984	4.370	3.854	3.895	3.598	4.063	3.745	3.745
		100.48	74.40	71.20	67.10	72.00	69.53	63.73	
[c]		100.7	74.10	71.39	67.26	72.06	69.56	63.76	
			0.81						
	Na^+	4.952	4.339	3.811	3.920	3.595	4.065	3.750	3.750
		100.12	75.17	70.82	66.84	71.97	69.47	63.66	
			1.99						
3*P* (**2**)	H^+	4.799	4.143	4.308	3.985	3.641	4.074	3.995	3.995
		101.01	69.75	73.63	66.18	71.31	69.72	63.21	
				0.66					
	Na^+	4.840	4.102	4.219	4.019	3.641	4.094	3.774	3.774
		100.92	69.82	77.36	65.83	71.82	69.61	63.65	
				4.20					
4*P* (**3**)	H^+	4.782	3.968	3.943	4.386	3.704	4.082	3.754	3.754
		101.49	70.52	71.18	71.53	71.00	69.25	63.29	
					1.41				
	Na^+	4.830	3.968	3.972	4.317	3.673	4.169	3.766	3.766
		101.56	70.72	71.05	72.39	70.82	69.23	63.28	
					3.65				
6*P* (**4**)	H^+	4.773	3.935	3.783	3.870	3.731	4.581	3.808	3.896
		102.05	70.79	71.04	66.64	71.94	72.91	62.31	
							1.44		
	Na^+	4.826	3.946	3.852	3.989	3.712	4.524	3.911	3.786
		101.99	70.64	71.30	66.71	71.17	74.49	61.55	
							4.03		
7*P* (**5**)	H^+	4.780	3.928	3.771	3.868	3.628	4.216	4.059	4.059
		101.80	70.79	71.67	66.85	71.51	68.49	66.57	
								1.04	
	Na^+	4.797	3.945	3.791	3.889	3.647	4.220	3.989	3.989
		101.80	70.75	71.61	66.73	71.28	68.00	67.17	
								2.50	

[a] Resonance signals of OCH_3 groups occurred within δ 3.38–3.42 (^1H) and 54.98–55.69 (^{13}C).
[b] The samples possessed the following pH: 2*P* (**1**): 1.3; Na^+ salt: 5.3. 3*P* (**2**): 1.5; Na^+ salt: 6.6. 4*P* (**3**): 1.4; Na^+ salt: 6.0. 6*P* (**4**): 1.2; Na^+ salt: 5.9. 7*P* (**5**): 1.3; Na^+ salt: 6.0.
[c] After KOH treatment of the sample.



44 $R_1 = PO(OH)_2$, $R_2 = R_3 = R_4 = R_5 = H$
45 $R_2 = PO(OH)_2$, $R_1 = R_3 = R_4 = R_5 = H$
46 $R_3 = PO(OH)_2$, $R_1 = R_2 = R_4 = R_5 = H$
47 $R_4 = PO(OH)_2$, $R_1 = R_2 = R_3 = R_5 = H$
48 $R_5 = PO(OH)_2$, $R_1 = R_2 = R_3 = R_4 = H$
49 $R_1 = R_2 = R_3 = R_4 = R_5 = H$

In the present study the methyl L-*glycero*-α-D-*manno*-heptopyranoside monophosphates **1**–**5** have been exposed to different hydrolytic conditions often employed in the degradation of lipopolysaccharides (LPS): (D1) 1 M hydrochloric acid, 100 °C, 2 h; (D2) 4 M trifluoroacetic acid, 100 °C, 4 h; (D3) 4 M potassium hydroxide, 120 °C, 16 h. All reactions were performed in a nitrogen atmosphere. The results of the hydrolyses were studied by high-performance anion-exchange chromatography at high pH (HPAEC); note that the heptose phosphates were stable under alkaline conditions. The elution times of **1**–**5** on CarboPac PA-10 using two concentration gradient systems and of five L-*glycero*-α-D-*manno*-heptopyranose phosphates (**44**–**48**) obtained earlier [1] are collected in Table 6. It must be noted that phosphates **1**, **2**, and **5** displayed very similar elution times in both gradients. Also phosphates **3** and **4** gave a common peak. A better resolution was found for the free heptose phosphates: **44**, **47**, and **48** which gave nicely separated peaks, whereas **45** and **46** formed a joint signal.

Monophosphates **1**–**5** were exposed to the hydrolytic conditions D1–D3 using their free acids and disodium salts. The results of all reactions and proportions of products identified are tabulated in

Table 5
$^{2}J_{P,C}$ and $^{3}J_{P,C}$ coupling constants of methyl L-*glycero*-α-D-*manno*-heptopyranoside phosphates **1**–**5**

	^{13}C-atom					
	C-2	C-3	C-4	C-5	C-6	C-7
2*P* (**1**) H^+	5.1	5.4				
2*P* (**1**) H^+ after KOH	5.1	5.4				
2*P* (**1**) Na^+	3.7	4.4				
3*P* (**2**) H^+		5.5	5.8			
3*P* (**2**) Na^+	2.2	3.3	1.9			
4*P* (**3**) H^+			5.6	6.4		
4*P* (**3**) Na^+		4.3	—	6.1		
6*P* (**4**) H^+				5.7	5.3	
6*P* (**4**) Na^+				4.2	4.0	
7*P* (**5**) H^+					8.5	4.7
7*P* (**5**) Na^+					7.6	4.1

Tables 7–11. Under acidic conditions (D1 and D2) for all phosphates three reactions have been noted: (i) hydrolysis of the glycoside, (ii) migration of the phosphate group, and (iii) hydrolysis of the phosphate. All phosphates were stable under alkaline conditions, which was proven for phosphates **1–4** by ^{13}C NMR analysis before and after the alkaline treatment.

In the case of the 2-phosphate (**1**, Table 7) hydrolysis of the glycoside and of the phosphate were the main reactions under acidic conditions with formation of the free heptose **49** as the dominating product. The presence of LD-Hep*p* 2- and 3(4)-phosphates (**44** and **45**(**46**)) indicated that the hydrolysis of the glycoside was faster than of the phosphate monoester. Migration of the phosphate moiety from O-2 to O-3(O-4), to form **45**(**46**) in ca. 2–3:1 predominance over **44**, demonstrated clearly

Table 6
Elution times of methyl L-*glycero*-α-D-*manno*-heptopyranoside monophosphates **1**–**5** and of L-*glycero*-D-*manno*-heptopyranose monophosphates **44**–**48** and L-*glycero*-D-*manno*-heptopyranose **49** on CarboPac PA-10 (HPAEC)

Heptose phosphate	No.	a[a]	b[b]	No.	b
2*P*	**1**	19.60[c]	12.53	**44**	20.67
3*P*	**2**	19.67[c]	12.73	**45**	21.47[e]
4*P*	**3**	20.40[d]	13.20	**46**	21.47[e]
6*P*	**4**	20.40[d]	13.07	**47**	20.20
7*P*	**5**	19.53[c]	12.53	**48**	18.87
				49	4.67

[a] Gradient a, 1–20% B (cf. Experimental).
[b] Gradient b, 1–50% B.
[c] Mixture of **1**, **2** and **5** could not be separated using gradients a and b.
[d] Mixture of **3** and **4** could not be separated using gradients a and b.
[e] LD-manHep*p* 3*P* (**45**) and 4*P* (**46**) could not be separated.

Table 7
Transformations of methyl L-*glycero*-α-D-*manno*-heptopyranoside 2-phosphate (**1**)

Reaction conditions	Form	Products				
		1	**44**	**45**	**48**	**49**
D1	H^+	10.1	7.2	11.8	—	70.9
	Na^+	16.1	8.9	16.5	—	58.5
D2	H^+	14.6	2.6	7.8	2.0	73.5
	Na^+	15.4	3.2	9.5	2.0	71.9
D3	H^+	100				
	Na^+	100				

that the equatorial phosphate group was more stable than the axial one. Only 10–15% of the unchanged substrate remained in the mixtures. In contrast, 100% of **1** remained unchanged when the compound was exposed to alkaline conditions D3.

Detection of a minute amount of the 7-phosphate **48** in the D2 experiment may indicate that the $PO(OH)_2$ group is capable to migrate through the whole carbon skeleton to form the most stable primary heptose phosphate. Another explanation might also be offered, namely, a direct esterification of HO-7 by the released phosphoric acid. This explanation could be also considered for the acidic hydrolyses of **2** and **3** (vide infra). Therefore, a blank experiment was performed consisting of heating methyl L-*glycero*-α-D-*manno*-heptopyranoside with phosphoric acid in trifluoroacetic acid under the D2 conditions. Besides the free heptose **49** resulting from hydrolysis of the methyl glycoside, not a trace of any heptose phosphate could be detected with HPAEC.

The results of the hydrolysis of the 3-phosphate (**2**, Table 8) were similar to those obtained for the 2-phosphate: 12–16% of the unchanged substrate, two phosphorylated heptoses **44** and **45**(**46**), in a similar proportion as in the case of **1**, large amounts of demethylated and dephosphorylated heptose **49**, and a minute amount of 7-phosphate **48**. The results from Tables 7 and 8 may indicate that **1** and **2** under acidic conditions undergo glycoside

Table 8
Transformations of methyl L-*glycero*-α-D-*manno*-heptopyranoside 3-phosphate (**2**)

Reaction conditions	Form	Products				
		2	**44**	**45**	**48**	**49**
D1	H^+	16.2	9.5	19	—	55.3
	Na^+	13.3	7.4	16.5	—	62.7
D2	H^+	14.9	3.5	8.8	2.6	70.1
	Na^+	12.7	2.7	7.2	2.6	74.7

Table 9
Transformations of methyl L-*glycero*-α-D-*manno*-heptopyranoside 4-phosphate (**3**)

Reaction conditions	Form	Products				
		3	**44**	**45**	**48**	**49**
D1	H^+	—	6.7	19.3	11.2	62.8
	Na^+	—	10.8	33.9	12.1	43.2
D2	H^+	—	3.2	15.5	11.1	70.2
	Na^+	3.3	3.3	16.3	11.6	65.5
D3	H^+	—	—	—	—	—
	Na^+	100	—	—	—	—

hydrolysis in the first step, followed by migration of the phosphate group and, eventually, by hydrolytic cleavage of the $PO(OH)_2$ group. A formal objection remains that—because of peak overlapping—substrate peaks in Tables 7 and 8 may represent, in fact, mixtures of **1**, **2** and **5**. While this can be a real possibility (**1** and **2**, cf. Table 6), the presence of **5** does not appear to be very likely as this phosphate disappears completely under the acidic hydrolysis conditions employed (cf. Table 11).

4-Phosphate **3** remained unchanged under alkaline conditions and disappeared almost completely after acidic treatment (Table 9). The main product was, as above, free heptose **49**. Most interesting was the presence of three (four) heptose phosphates: 2- (**44**), 3- (**45**) (and/or 4- (**46**)), and 7-phosphate (**48**). This means that the phosphoryl residue may migrate in both directions: towards O-2 and O-7, whereby the last direction appears to be preferred.

Hydrogenation of **28** led to 6-phosphate **4**. ^1H and ^{13}C NMR spectra recorded soon after isolation of **4** pointed to a pure substance (cf. Table 4). However, **4** on standing in water solution isomerized partially to **5**; after 2 weeks a mixture was formed containing ca. 62% of **4** and 38% of **5**. The amount of **5** increased gradually on further standing. It appears that the acidity of **4** (higher than phosphoric acid: pH 1.3, cf. [24]) is sufficient

Table 10
Transformations of methyl L-*glycero*-α-D-*manno*-heptopyranoside 6-phosphate (**4**)

Reaction conditions	Form	Products			
		4	**47**	**48**	**49**
D1	H^+	1.0	20.5	63.5	15.0
D2	H^+	—	9.7	80.9	9.4
D3	H^+	100	—	—	—

Table 11
Transformations of methyl L-*glycero*-α-D-*manno*-heptopyranoside 7-phosphate (**5**)

Reaction conditions	Form	Products			
		5	**47**	**48**	**49**
D1	H^+	—	10	84.7	5.3
	Na^+	—	9.6	87.9	2.5
D2	H^+	—	11.4	81.6	7.0
	Na^+	—	9.6	80.5	9.9
D3	H^+	100	—	—	—
	Na^+	100	—	—	—
D4	H^+	100	—	—	—
	Na^+	100	—	—	—

to induce the migration of the $PO(OH)_2$ group towards the primary hydroxyl group. Thus, the location of the phosphoryl group at the primary alcohol center is especially favored.

On acidic (D1 and D2) treatment of **4**, demethylation and migration were the main processes, and the 6- and 7-phosphates **47** and **48** were formed with the latter in a distinct predominance (Table 10). Under alkaline conditions 6-phosphate **4** remained unchanged.

Most interestingly was the behaviour of the 7-phosphate **5** (Table 11). Whereas it was stable under weakly acidic (D4: 1% acetic acid, 100 °C, 2 h) and alkaline (D3) conditions, in trifluoroacetic acid (D2) it underwent a complete demethylation with formation of over 80% of heptose 7-phosphate **48** and ca. 10% of 6-phosphate **47** and free heptose **49**. Formation of 6-phosphate **47** from **5** demonstrated that back migration from O-7 to O-6 was possible, and an equilibrium **47**:**48** = ca. 1:8 was established. Location of the phosphate residue at the primary hydroxyl position is thermodynamically preferred. The reason of this stability might be of steric nature; MM calculations [25] of 6- and 7-phosphates pointed at lower strain energy of the latter.

3. Conclusions

Phosphates **1–5** are stable at room temperature and their phosphoryl groups—with the exception of **4**—do not undergo spontaneous migration. This was proven by recording NMR spectra immediately after the synthesis and after several days.

From the data in Tables 7–11 several conclusions can be drawn. The results of the reactions performed on free acids and on disodium salts

remain—for obvious reasons—the same within the experimental error. Treatment of heptose monophosphates with strong alkali results neither in degradation nor in migration of the phosphate group. Under the harsh acidic conditions employed the methyl glycoside and phosphate ester are cleaved to a substantial extent. For **1**, **2**, and **3** the free heptose is the main product of hydrolysis. The methyl group at the anomeric center is removed faster than the phosphate function. This permits to follow the migrations of the $PO(OH)_2$ group. It is important to note that not only the "1,2-*cis*" migrations (between O-2 and O-3, or O-6 and O-7) but also between O-3 and O-4 ("1,2-*trans*") and between O-4 and O-6 (probably via a dioxaphosphorinane intermediate) are possible. It is well known that for the 1,2-migrations two mechanistic pathways are possible: through a cyclic intermediate or through a direct, H^+-intermediated phosphate transfer [13,14]. We suppose that in case of heptose phosphates both pathways must be involved. In neither case a transient cyclic phosphate was observed.

4. Experimental

The general methods, unless otherwise indicated, were the same as in the previous paper [1]. HPAEC was performed as described [1] with the modification that a column of CarboPac PA-10 (4×240 mm) was used; elutions were carried out with linear concentration gradients of either 1–20% B (eluent A containing 1 M NaOAc, gradient a) or 1–50% B (gradient b) in 0.1 M NaOH (eluent A) at a flow rate of 1 mL/min. Methyl 2,3,4-tri-*O*-benzyl-α-D-mannopyranoside [26], methyl 2,3,4-tri-*O*-benzyl-α-D-*manno*-hexodialdo-1,5-pyranoside [26], methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside [27], methyl 3-*O*-allyl-6-*O*-trityl-α-D-mannopyranoside [28], methyl *exo*-2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranoside (**9**) [29] were obtained according to literature methods.

Methyl 2-O-*allyl-3*-O-*benzyl-4,6*-O-*benzylidene*-α-D-*mannopyranoside*.—To a solution of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside [27] (9.0 g) in DMF (260 mL) cooled to 0 °C was added 50% oil suspension of NaH (3.48 g). After 20 min a solution of allyl bromide (6 mL) in DMF (6 mL) was added dropwise. After 3 h the excess of the hydride was decomposed with MeOH, the mixture was poured into ice-water, extracted with CH_2Cl_2, dried, and concentrated to dryness. The residue was purified by chromatography with 4:1 hexane–ether to yield the allylated product (9.89 g, 99%); $[\alpha]_D$ +51.3° (*c* 2.1, $CHCl_3$); ^{1}H NMR (C_6D_6): δ 5.38 (s, 1 H, PhC*H*), 4.66 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.42 (t, 1 H, H-4), 4.19 (dd, 1 H, $J_{6a,5}$ 4.8, $J_{6a,6b}$ 10.2 Hz, H-6a), 4.07 (dd, 1 H, $J_{3,2}$ 3.2, $J_{3,4}$ 9.9 Hz, H-3), 3.86 (ddd, 1 H, $J_{5,4}$ 9.8, $J_{5,6a}$ 5.0 Hz, H-5), 3.76 (dd, 1 H, H-2), 3.69 (t, 1 H, H-6b).

Methyl 2-O-*allyl-3,4-di*-O-*benzyl*-α-D-*mannopyranoside* (**6b**).—To a solution of methyl 2-*O*-allyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (5.8 g) in CH_2Cl_2 (100 mL) was added $LiAlH_4$ (2.26 g), and a solution of $AlCl_3$ (6.8 g) in ether (20 mL) was added dropwise. The mixture was boiled under reflux for 45 min, whereupon the excess of the hydride was decomposed with EtOAc (10 mL) and water (10 mL). The product was extracted with ether, and the organic extract was dried and concentrated to dryness. The residue was chromatographed with 4:1 hexane–EtOAc to yield **6b** (5.6 g, 96%); $[\alpha]_D$ +44.9° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.71 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.75 (dd, 1 H, $J_{2,3}$ 2.6 Hz, H-2), 3.60 (ddd, 1 H, $J_{5,6a}$ 2.8, $J_{5,6b}$ 4.9, $J_{5,4}$ 9.6 Hz, H-5).

Methyl 4,6-O-(p-*methoxybenzylidene)*-α-D-*mannopyranoside* (**22**).—A solution of methyl α-D-mannopyranoside (19.4 g) in Me_2SO (120 mL) was stirred with 4Å molecular sieves for 20 min, then *p*-methoxybenzaldehyde (16.3 g), $ZnCl_2$ (15.7 g), and *p*-toluenesulfonic acid (2.07 g) were added. After 6 days of stirring at room temperature the mixture was neutralized with aq 25% ammonia (57 mL), filtered through Celite, poured into water, extracted with $CHCl_3$, and the organic layer was dried and concentrated. The residue was chromatographed with 9:1 hexane–EtOAc to yield methyl 2,3:4,6-di-*O*-(*p*-methoxybenzylidene)-α-D-mannopyranoside (13.4 g, 31%) {$[\alpha]_D$ −8.6° (*c* 0.5, $CHCl_3$), *exo:endo* mixture 1:1; Anal. Calcd for $C_{23}H_{26}O_8$: C, 64.17; H, 6.09. Found: C, 64.00; H, 6.19} and **22** (6.8 g, 22%); $[\alpha]_D$ +59.8° (*c* 1.6, $CHCl_3$); ^{1}H NMR (200 MHz) ($CDCl_3$): δ 5.50 (s, 1 H, $MeOC_6H_4CH$), 4.69 (s, 1 H, H-1), 3.8–4.3 (m, 6 H, H-2,3,4,5,6a,6b), 3.79 (s, 3 H, *Me*OC_6H_4CH), 3.37 (s, 3 H, OMe). Anal. Calcd for $C_{15}H_{20}O_7$: C, 57.68; H, 6.46. Found: C, 56.98; H, 6.80.

Methyl 2,3-di-O-*benzyl-4*-O-(p-*methoxybenzyl)*-α-D-*mannopyranoside* (**6d**).—Compound **22** (13.9 g) was conventionally benzylated (NaH, BnBr, DMF, 0 °C) to yield the 2,3-di-*O*-benzyl derivative (20.7 g, 95.1%). A solution of this compound (9.8 g) in

acetonitrile (160 mL) was stirred with 3Å molecular sieves for 10 min. The solution was cooled (ice-water), $NaBH_3CN$ (4.81 g) was added, and after a few minutes a solution of trimethylsilyl chloride (9.7 mL) in acetonitrile (20 mL) was added dropwise. After completion of the reduction (TLC) the mixture was filtered through Celite, poured into dil aq $NaHCO_3$, extracted with CH_2Cl_2, dried, and the organic layer was concentrated to dryness. The residue was chromatographed with 9:1→4:1 hexane–ether. The first compound eluted was methyl 2,3-di-*O*-benzyl-6-*O*-(*p*-methoxybenzyl)-α-D-mannopyranoside (3.13 g, 20%); $[\alpha]_D$ −6.3° (*c* 1.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.77 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.03 (t, 1 H, $J_{4,3} \approx J_{4,5} \approx 9.4$ Hz, H-4). Anal. Calcd for $C_{29}H_{34}O_7 \cdot 1/2H_2O$: C, 69.16; H, 7.01. Found: C, 69.11; H, 7.11. The second one that eluted was **6d** (5.2 g, 34%); $[\alpha]_D$ +32° (*c* 1.2, $CHCl_3$); ^1H NMR (C_6D_6): δ 4.70 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.27 (t, 1 H, $J_{4,5} = J_{4,3} = 9.6$ Hz, H-4), 4.04 (dd, 1 H, $J_{3,2}$ 3.1 Hz, H-3). Anal. Calcd for $C_{29}H_{34}O_7$: C, 70.42; H, 6.93. Found: C, 70.25; H, 7.03.

Synthesis of protected derivatives of methyl L-glycero-α-D-manno-*heptopyranoside. General method.*—A solution of oxalyl chloride (1 mL, 11 mmol) in CH_2Cl_2 (25 mL) was cooled to −50 ÷ −60 °C and a solution of Me_2SO (1.7 mL, 22 mmol) in CH_2Cl_2 (5 mL) was added slowly. After 5 min a solution of suitably protected methyl mannopyranoside with a free HO-6 group (10 mmol) in CH_2Cl_2 (20 mL) was added dropwise. Stirring at −60 °C was continued for 1 h, whereupon Et_3N (7 mL, 50 mmol) was added. After 5 min of stirring, the mixture was allowed to attain room temperature. To the solution was added water (50 mL), and the mixture was extracted with CH_2Cl_2. The organic extract was dried ($MgSO_4$) and concentrated to dryness. The remaining oil was additionally dried by azeotropic distillation (3–4 times) with small portions of anh benzene and was used in the next step without any additional purification.

To dry magnesium turnings (0.384 mg, 16 mmol) was added, under freshly distilled THF (1.5 mL), sublimed $HgCl_2$ (50 mg), and a few drops of neat pure alkoxymethyl chloride, freshly distilled before use, were added while lowering the temperature to −15 °C (for allyloxymethyl chloride) or to 0 ÷ −5 °C (for benzyloxymethyl chloride). When the formation of the Grignard reagent has started, the remaining amount of alkoxymethyl chloride (16 mmol) in THF (3 mL) was added at −18 ÷ −20 °C ($AllOCH_2Cl$) or at 0 ÷ 5 °C ($BnOCH_2Cl$) and stirring was continued for 2 h. The temperature was then lowered to −30 °C ($AllOCH_2MgCl$) or −20 °C ($BnOCH_2MgCl$) and a solution of aldehyde (4 mmol) in abs THF (10 mL) was added dropwise. The mixture was stirred at these temperatures for 2 h and was slowly brought to room temperature while stirring for another 12 h. A cold (0 °C) aq solution of NH_4Cl (150 mL) was added and the products were extracted with ether. The ether extract was dried, concentrated to dryness, and the residue was chromatographed with hexane–EtOAc (9:1 or 85:15) to give derivatives of methyl heptosides in the order: "inverted" first, followed by LD-, and DD-manHep*p* as the last. The assignment of configuration to the stereoisomeric methyl heptosides was based on regularities observed in ^1H NMR coupling constants of H-6, -7a and -7b. For derivatives of LD-manHep*p* the coupling constants were: $J_{6,5}$ 1.0–1.4 Hz, $J_{7a,6}$ 6.3–7.2 Hz, $J_{7b,6}$ 5.8–6.3 Hz, and $J_{7a,7b}$ 9.3–9.6 Hz. For DD-manHep*p*: $J_{6,5}$ 2.0–2.1 Hz, $J_{7a,6}$ 4.5–4.7 Hz, $J_{7b,6}$ 6.7–7.2 Hz, and $J_{7a,7b}$ 10.0–10.7 Hz. It must be added that the configuration of benzyl glycosides of LD-manHep*p* and DD-manHep*p* obtained in the same way was earlier [30,31] confirmed by preparation of diethyl dithioacetals displaying identical physical data as the original compounds. The configuration of methyl 2,3,4-tri-*O*-benzyl-D-*glycero*-α-D-*manno*-heptopyranoside, obtained by deacetylation and deallylation of **36**, was independently confirmed by CD [32]. The L-*gulo* configuration of the "inverted" methyl heptosides follows from ^1H NMR coupling constants within the pyranoside ring, i.e. $J_{1,2}$ 8.2 Hz.

*Methyl 2-*O*-allyl-3,4,7-tri-*O*-benzyl-*D(L)-glycero-β-L-gulo-*heptopyranoside* (**15**).—^1H NMR ($CDCl_3$): δ 4.62 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 3.82 (dd, 1 H, $J_{5,6}$ 9.0, $J_{5,4}$ 1.2 Hz, H-5), 3.79 (t, 1 H, $J_{3,2}$ 3.4, $J_{3,4}$ 3.5 Hz, H-3), 3.75 (dd, 1 H, H-4), 3.70 (dd, 1 H, $J_{7a,6}$ 3.1, $J_{7a,7b}$ 9.6 Hz, H-7a), 3.62 (dd, 1 H, $J_{7b,6}$ 5.4 Hz, H-7b), 3.50 (dd, 1 H, H-2); ^{13}C NMR ($CDCl_3$): δ 101.7 (C-1), 76.14, 74.56, 73.98, 72.45, and 68.01 (C-2,3,4,5,6), 71.12 (C-7). HR MS (LSIMS): Table 1.

*Methyl 2-*O*-allyl-3,4,7-tri-*O*-benzyl-*L-glycero-α-D-manno-*heptopyranoside* (**10**).—^1H NMR ($CDCl_3$): δ 4.68 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.10 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.86 (dd, 1 H, $J_{3,2}$ 3.1, $J_{3,4}$ 9.5 Hz, H-3), 3.72 (dd, 1 H, H-2), 3.66 (dd, 1 H, $J_{7a,6}$ 7.4, $J_{7a,7b}$ 9.4 Hz, H-7a), 3.66 (dd, 1 H, $J_{5,6}$ 1.0 Hz, H-5), 3.57 (dd, 1 H, $J_{7b,6}$ 5.9 Hz, H-7b); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): Table 1.

Methyl 2-O-*allyl-3,4,7-tri*-O-*benzyl*-D-glycero-α-D-manno-*heptopyranoside* (**14**).—^{1}H NMR ($CDCl_3$): δ 4.68 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 3.94 (t, 1 H, $J_{2,3}$ 2.5 Hz, H-2), 3.67 (dd, 1 H, $J_{7a,6}$ 6.7, $J_{7a,7b}$ 10.1 Hz, H-7a), 3.60 (dd, 1 H, $J_{7b,6}$ 3.1, H-7b); ^{13}C NMR ($CDCl_3$): δ 99.09 (C-1), 80.25 (C-3), 76.07, 74.40, 71.99, and 71.01 (C-2,4,5,6), 70.82 (C-7).

Methyl 3-O-*allyl-2,4,7-tri*-O-*benzyl*-D(L)-glycero-β-L-gulo-*heptopyranoside* (**21**).—^{1}H NMR ($CDCl_3$): δ 4.62 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 3.78 (dd, 1 H, $J_{5,6}$ 9.0, $J_{5,4}$ 1.2 Hz, H-5), 3.71 (dd, 1 H, $J_{4,5}$ 1.4, $J_{4,3}$ 3.7 Hz, H-4), 3.71 (dd, 1 H, $J_{7a,6}$ 3.1, $J_{7a,7b}$ 9.8 Hz, H-7a), 3.63 (t, 1 H, $J_{3,2}$ 3.3 Hz, H-3), 3.63 (dd, 1 H, $J_{7b,6}$ 5.5 Hz, H-7b), 3.53 (dd, 1 H, H-2); ^{13}C NMR ($CDCl_3$): δ 101.8 (C-1), 75.85, 74.88, 73.79, 72.48, and 68.00 (C-2,3,4,5,6), 71.17 (C-7). HR MS (LSIMS): Table 1.

Methyl 3-O-*allyl-2,4,7-tri*-O-*benzyl*-L-glycero-α-D-manno-*heptopyranoside* (**16**).—^{1}H NMR ($CDCl_3$): δ 4.69 (bs, 1 H, H-1), 4.12 (t, 1 H, H-4), 3.76 (dd, 1 H, $J_{3,2}$ 3.1, $J_{3,4}$ 9.3 Hz, H-3), 3.74 (dd, $J_{2,1}$ 1.8 Hz, H-2), 3.65 (dd, 1 H, $J_{7a,6}$ 7.2, $J_{7a,7b}$ 9.5 Hz, H-7a), 3.63 (dd, 1 H, $J_{5,6}$ 1.0, $J_{5,4}$ 9.8 Hz, H-5), 3.57 (dd, 1 H, $J_{7b,6}$ 6.0 Hz, H-7b); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): Table 1.

Methyl 3-*O*-allyl-2,4,7-tri-*O*-benzyl-D-*glycero*-α-D-*manno*-heptopyranoside (**20**) was not isolated in analytically pure state.

Methyl 2,3,7-tri-O-*benzyl-4*-O-(p-*methoxybenzyl*)-D(L)-glycero-β-L-gulo-*heptopyranoside* (**27**).—^{1}H NMR ($CDCl_3$): δ 4.67 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.02 (ddd, 1 H, $J_{6,5}$ 8.7, $J_{6,7a}$ 3.1, $J_{6,7b}$ 5.4 Hz, H-6), 3.81 (dd, 1 H, $J_{5,4}$ 1.0 Hz, H-5), 3.97 (t, 1 H, $J_{3,4}$ 3.6 Hz, H-3), 3.62 (dd, 1 H, $J_{7b,7a}$ 9.7 Hz, H-7b), 3.53 (dd, 1 H, $J_{2,3}$ 2.7 Hz, H-2); ^{13}C NMR ($CDCl_3$): δ 101.8 (C-1), 76.10, 74.81, 73.25, 72.41, and 68.06 (C-2,3,4,5,6), 71.16 (C-7). HR MS (LSIMS): Table 1.

Methyl 2,3,7-tri-O-*benzyl-4*-O-(p-*methoxybenzyl*)-L-glycero-α-D-manno-*heptopyranoside* (**23**).—^{1}H NMR ($CDCl_3$): δ 4.62 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.09 (dt, 1 H, $J_{6,5}$ 1.1 Hz, H-6), 4.08 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.79 (dd, 1 H, $J_{3,2}$ 3.1, $J_{3,4}$ 9.5 Hz, H-3), 3.70 (dd, 1 H, H-2), 3.58 (dd, 1 H, $J_{7a,6}$ 7.3, $J_{7a,7b}$ 9.4 Hz, H-7a), 3.55 (dd, 1 H, H-5), 3.49 (dd, 1 H, $J_{7b,6}$ 5.9 Hz, H-7b); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): Table 1.

Methyl 2,3,4,7-tetra-O-*benzyl*-D(L)-glycero-β-L-gulo-*heptopyranoside* (**31**).—^{1}H NMR ($CDCl_3$): δ 4.68 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.04 (ddd, 1 H, $J_{6,7a}$ 3.1, $J_{6,7b}$ 5.3, $J_{6,5}$ 9.0 Hz, H-6), 3.82 (dd, 1 H, H-5), 3.65 (dd, 1 H, $J_{7a,7b}$ 9.5 Hz, H-7b); ^{13}C NMR ($CDCl_3$): δ 101.9 (C-1), 76.07, 74.84, 73.69, 72.43, and 68.03 (C-2,3,4,5,6); HR MS (LSIMS): Table 1.

Methyl 2,3,4,7-tetra-O-*benzyl*-L-glycero-α-D-manno-*heptopyranoside* (**28**).—^{1}H NMR ($CDCl_3$): δ 4.70 (d, 1 H, H-1), 4.17 (dt, 1 H, H-6), 4.17 (t, 1 H, $J_{4,5}$ 9.6 Hz, H-4), 3.88 (dd, 1 H, $J_{3,2}$ 3.1, $J_{3,4}$ 9.4 Hz, H-3), 3.77 (dd, 1 H, $J_{1,2}$ 1.9 Hz, H-2), 3.56 (dd, 1 H, $J_{7a,6}$ 7.2, $J_{7a,7b}$ 9.5 Hz, H-7a), 3.65 (bd, 1 H, H-5), 3.57 (dd, 1 H, $J_{7b,6}$ 6.0 Hz, H-7b); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): Table 1.

The D-*glycero*-D-*manno* stereoisomer (**30**) was characterized as its 6-*O*-acetyl derivative.

Methyl 6-O-*acetyl-2,3,4,7-tetra*-O-*benzyl*-D-glycero-α-D-manno-*heptopyranoside*.—^{1}H NMR ($CDCl_3$): δ 5.56 (ddd, 1 H, $J_{6,5}$ 2.0, $J_{6,7a}$ 4.6 Hz, H-6), 4.71 (bs, 1 H, H-1), 3.98 (t, 1 H, H-4), 3.86 (dd, 1 H, $J_{3,4}$ 9.0, $J_{3,2}$ 3.0 Hz, H-3), 3.80 (dd, 1 H, $J_{5,4}$ 9.9 Hz, H-5), 3.76 (dd, 1 H, $J_{7a,7b}$ 10.7 Hz, H-7a), 3.74 (dd, 1 H, H-2), 3.68 (dd, 1 H, $J_{7b,6}$ 7.2, H-7b); ^{13}C NMR ($CDCl_3$): δ 98.69 (C-1), 80.22 (C-3), 74.74, 74.66, 71.76, and 71.67 (C-2,4,5,6), 68.18 (C-7). HR-MS (LSIMS): Table 1.

Methyl 7-O-*allyl-2,3,4-tri*-O-*benzyl*-D(L)-glycero-β-L-gulo-*heptopyranoside* (**37**).—^{1}H NMR ($CDCl_3$): δ 4.70 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 3.80 (dd, 1 H, $J_{5,6}$ 9.0, $J_{5,4}$ 0.9 Hz, H-5), 3.71 (t, 1 H, $J_{3,4}$ 3.8 Hz, H-3), 3.70 (dd, 1 H, H-4), 3.66 (dd, 1 H, $J_{7a,6}$ 3.1, $J_{7a,7b}$ 9.7 Hz, H-7a), 3.57 (dd, 1 H, $J_{7b,6}$ 5.7 Hz, H-7b), 3.56 (dd, 1 H, $J_{2,3}$ 2.7 Hz, H-2); ^{13}C NMR ($CDCl_3$): δ 101.9 (C-1), 76.10, 74.88, 73.76, 72.56, and 67.94 (C-2,3,4,5,6), 71.10 (C-7). HR MS (LSIMS): Table 1.

Methyl 7-O-*allyl-2,3,4-tri*-O-*benzyl*-L-glycero-α-D-manno-*heptopyranoside* (**32**).—^{1}H NMR ($CDCl_3$): δ 4.71 (bs, 1 H, H-1), 4.17 (t, 1 H, H-4), 4.14 (bt, 1 H, H-6), 3.88 (dd, 1 H, $J_{3,2}$ 3.1, $J_{3,4}$ 9.5 Hz, H-3), 3.78 (dd, 1 H, $J_{2,1}$ 1.9 Hz, H-2), 3.64 (dd, 1 H, $J_{5,6}$ 1.0, $J_{5,4}$ 9.8 Hz, H-5), 3.60 (dd, 1 H, $J_{7a,6}$ 7.3, $J_{7a,7b}$ 9.5 Hz, H-7a), 3.52 (dd, 1 H, $J_{7b,6}$ 6.0 Hz, H-7b); ^{13}C NMR: Table 2. HR MS (LSIMS): Table 1.

The D-*glycero*-α-D-*manno* (**36**) stereoisomer was characterized as its 6-*O*-acetyl derivative

Methyl 6-O-*acetyl-7*-O-*allyl-2,3,4-tri*-O-*benzyl*-D-glycero-α-D-manno-*heptopyranoside*.—^{1}H NMR ($CDCl_3$): δ 5.50 (ddd, 1 H, $J_{6,5}$ 2.1, $J_{6,7a}$ 4.8, $J_{6,7b}$ 6.9 Hz, H-6), 4.72 (bs, 1 H, H-1), 3.99 (t, 1 H, H-4), 3.87 (dd, 1 H, $J_{3,2}$ 3.1, $J_{3,4}$ 9.1 Hz, H-3), 3.79 (dd, 1 H, $J_{5,4}$ 9.9 Hz, H-5), 3.75 (dd, 1 H, $J_{2,1}$ 1.8 Hz, H-2), 3.74 (dd, 1 H, $J_{7a,7b}$ 10.7 Hz, H-7a), 3.64 (dd, 1 H, H-7b); ^{13}C NMR ($CDCl_3$): δ 90.06 (C-1), 80.64 (C-3), 75.14, 75.06, 72.12, and 72.04 (C-2,4,5,6), 68.34 (C-7).

Benzylation at O-6 in methyl L-glycero-α-D-manno-*heptopyranosides* **10**, **16**, **23**, *and* **32**.—To a cooled (0 °C) solution of methyl heptopyranoside (1 mmol) in abs DMF (10 mL) was added NaH (2 mmol, 50% oil suspension). The suspension was stirred for 10 min, then a solution of benzyl chloride (2 mmol) in abs DMF (1 mL) was added. Stirring was continued for 8 h, whereupon the excess of the hydride was decomposed with MeOH and the mixture was poured into ice-water. The solution was extracted with ether, and the organic layer was dried and concentrated to dryness. The residue was purified by chromatography in 95:5 or 9:1 hexane–EtOAc.

*Methyl 2-*O*-allyl-3,4,6,7-tetra-*O*-benzyl-*L*-glycero-α-D-manno-heptopyranoside* (**11**).—Yield: 87.6%; $[\alpha]_D$ +45° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): $C_{39}H_{44}O_7 + Na^+$ $[M+Na]^+$; Calcd: 647.2985. Found: 647.2951.

*Methyl 3-*O*-allyl-2,4,6,7-tetra-*O*-benzyl-*L*-glycero-α-D-manno-heptopyranoside* (**17**).—Yield: 85.5%; $[\alpha]_D$ +34° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): $C_{39}H_{44}O_7 + Na^+$ $[M+Na]^+$; Calcd: 647.2985. Found: 647.2981.

*Methyl 2,3,6,7-tetra-*O*-benzyl-4-*O*-(*p*-methoxybenzyl)-*L*-glycero-α-D-manno-heptopyranoside* (**24**).—Yield: 79.2%; $[\alpha]_D$ +23° (*c* 0.53, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): $C_{44}H_{48}O_8 + Na^+$ $[M+Na]^+$; Calcd: 727.3247. Found: 727.3255.

*Methyl 7-*O*-allyl-2,3,4,6-tetra-*O*-benzyl-*L*-glycero-α-D-manno-heptopyranoside* (**33**).—Yield: 97.2%; $[\alpha]_D$ +38° (*c* 1.4, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): $C_{39}H_{44}O_7 + Na^+$ $[M+Na]^+$; Calcd: 647.2985. Found: 647.2981.

De-allylation of **11**, **17** *and* **33**.—To a solution of heptopyranoside derivative **11**, **17** or **33** (0.65 mmol) in a mixture of EtOH (9 mL), benzene (3 mL), and water (1 mL), was added 1,4-diaminobicyclo[2,2,2]octane (15 mg) and the solution was heated to 80 °C. Wilkinson's catalyst (42 mg) was added, the mixture was boiled under reflux for 3 h, then left at room temperature overnight. The mixture was filtered and the filtrate was concentrated under lowered pressure. The remaining oil was dissolved in 15:1 acetone–water, and to the solution were added HgO (149 mg) and $HgCl_2$ (187 mg). The suspension was stirred (0.5 h) at room temperature, filtered, and concentrated, and the residue was dissolved in ether. The solution was washed with aq 50% KI, aq 10% $NaHSO_3$, and aq 1% $NaHCO_3$, then dried and concentrated. The products were chromatographed with 7:3 light petroleum–ether.

*Methyl 3,4,6,7-tetra-*O*-benzyl-*L*-glycero-α-D-manno-heptopyranoside* (**12**).—Yield: 90.9%; $[\alpha]_D$ +59° (*c* 0.97, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): $C_{36}H_{40}O_7 + Na^+$ $[M+Na]^+$; Calcd: 607.2672. Found: 607.2672. 2-O-*Acetyl derivative* (**13**).—$[\alpha]_D$ +30° (*c* 1.0, $CHCl_3$); HR MS (LSIMS): $C_{38}H_{42}O_8 + Na^+$ $[M+Na]^+$; Calcd: 649.2777. Found: 649.2762. ^{13}C NMR ($CDCl_3$): Table 2.

*Methyl 2,4,6,7-tetra-*O*-benzyl-*L*-glycero-α-D-manno-heptopyranoside* (**18**).—Yield: 99.9%; $[\alpha]_D$ +20° (*c* 1.3, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): $C_{36}H_{40}O_7 + Na^+$ $[M+Na]^+$; Calcd: 607.2672. Found: 607.2672. 3-O-*Acetyl derivative* (**19**). $-[\alpha]_D$ +20° (*c* 1.8, $CHCl_3$). HR MS (LSIMS): $C_{38}H_{42}O_8 + Na^+$ $[M+Na]^+$; Calcd: 649.2777. Found: 649.2774. ^{13}C NMR ($CDCl_3$): Table 2.

*Methyl 2,3,4,6-tetra-*O*-benzyl-*L*-glycero-α-D-manno-heptopyranoside* (**34**).—Yield: 76.0%; $[\alpha]_D$ +38° (*c* 1.3, $CHCl_3$), Lit. [33]: $[\alpha]_D$ +37.8° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 2. *7*-O-*Acetyl derivative* (**35**).—$[\alpha]_D$ +32° (c 1.6, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 2.

*Methyl 2,3,6,7-tetra-*O*-benzyl-*L*-glycero-α-D-manno-heptopyranoside* (**25**).—To a solution of **24** (466 mg) in 9:1 acetonitrile–water (10 mL) was added cerium-ammonium nitrate (743 mg), and the mixture was stirred at room temperature. After 1 h (TLC in 2:1 hexane–ether) the mixture was diluted with CH_2Cl_2 (15 mL) and poured into sat aq $NaHCO_3$ (30 mL). The organic extract was dried and concentrated to dryness. The residue was chromatographed with 3:1 hexane–ether to yield **25** (336 mg, 87%); $[\alpha]_D$ −5.1° (*c* 1.84, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): $C_{36}H_{40}O_7 + Na^+$ $[M+Na]^+$; Calcd: 607.2672. Found: 607.2672. *4*-O-*Acetyl derivative* (**26**).—$[\alpha]_D$ +16° (*c* 1.3, $CHCl_3$). ^{13}C NMR ($CDCl_3$): Table 2. HR MS (LSIMS): $C_{38}H_{32}O_8 + Na^+$ $[M+Na]^+$; Calcd: 649.2777. Found: 649.2780.

*Phosphorylation of methyl tetra-*O*-benzyl-*L*-glycero-α-D-manno-heptopyranosides* **12**, **18**, **25**, **28**, *and* **34** *using 2-dimethylamino-5,6-benzo-1,3,2-dioxaphosphepane* (**38**). *General method.*—To a solution of methyl tetra-*O*-benzyl-*L*-*glycero*-α-D-*manno*-heptopyranoside (585 mg, 1 mmol) in abs CH_2Cl_2 (10 mL) was added tetrazole (210 mg, 3 mmol), and the mixture was stirred. After 10 min a solution of **38** (253 mg, 1.2 mmol) in CH_2Cl_2

(1 mL) was added and the mixture was again stirred. After 2 h at room temperature (TLC in 1:1 hexane–ether) the solution was cooled to −60 °C, and a solution of *m*-$ClC_6H_4CO_3H$ (1.8 mmol) in CH_2Cl_2 (5 mL) was added. The reaction was completed in 15 min (TLC in 1:1 hexane–EtOAc), and the mixture was diluted with EtOAc (20 mL), washed with aq 1% $NaHCO_3$ and aq 1% $NaHSO_3$. The organic layer was dried and concentrated to dryness. The residue was chromatographed with 4:1 hexane–EtOAc to yield pure phosphates.

*Methyl 2-*O*-(5,6-benzo-2-oxo-1,3,2-dioxaphosphepan-2-yl)-3,4,6,7-tetra-*O*-benzyl-*L*-glycero-α-*D*-manno-heptopyranoside* (**39**).—Yield: 92.1%; oil; $[\alpha]_D$ −10.6° (*c* 6.85, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 3. HR MS (LSIMS): $C_{44}H_{47}PO_{10}+Na^+$ $[M+Na]^+$; Calcd: 789.2805. Found: 789.2780.

*Methyl 3-*O*-(5,6-benzo-2-oxo-1,3,2-dioxaphosphepan-2-yl)-2,4,6,7-tetra-*O*-benzyl-*L*-glycero-α-*D*-manno-heptopyranoside* (**40**).—Yield: 73.2%; oil; $[\alpha]_D$ +18° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 3. HR MS (LSIMS): $C_{44}H_{47}PO_{10}+Na^+$ $[M+Na]^+$; Calcd: 789.2805. Found: 789.2780.

*Methyl 4-*O*-(5,6-benzo-2-oxo-1,3,2-dioxaphosphepan-2-yl)-2,3,6,7-tetra-*O*-benzyl-*L*-glycero-α-*D*-manno-heptopyranoside* (**41**).—Yield: 88.1%; oil; $[\alpha]_D$ +11° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 3. HR MS (LSIMS): $C_{44}H_{47}PO_{10}+Na^+$ $[M+Na]^+$; Calcd: 789.2805. Found: 789.2788.

*Methyl 6-*O*-(5,6-benzo-2-oxo-1,3,2-dioxaphosphepan-2-yl)-2,3,4,7-tetra-*O*-benzyl-*L*-glycero-α-*D*-manno-heptopyranoside* (**42**).—Yield: 85.2%; oil; $[\alpha]_D$ −3.5° (*c* 1.1, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 3. HR MS (LSIMS): $C_{44}H_{47}PO_{10}+Na^+$ $[M+Na]^+$; Calcd: 789.2805. Found: 789.2795.

*Methyl 7-*O*-(5,6-benzo-2-oxo-1,3,2-dioxaphosphepan-2-yl)-2,3,4,6-tetra-*O*-benzyl-*L*-glycero-α-*D*-manno-heptopyranoside* (**43**).—Yield: 88.2%; oil; $[\alpha]_D$ +16° (*c* 0.91, $CHCl_3$); ^{13}C NMR ($CDCl_3$): Table 3. HR MS (LSIMS): $C_{44}H_{47}PO_{10}+Na^+$ $[M+Na]^+$; Calcd: 789.2805. Found: 789.2788.

Hydrogenation of methyl O*-(5,6-benzo-2-oxo-1,3,2-dioxaphosphepan-2-yl)-tetra-*O*-benzyl-*L*-glycero-α-*D*-manno-heptopyranosides*.—To a solution of phosphate **39** (**40**, **41**, **42** or **43**) (421 mg, 0.5 mmol) in 9:3 EtOH–EtOAc was added 10% Pd-C (380 mg) and the suspension was hydrogenated overnight. Filtration through Celite and concentration of the filtrate left a foam. The ^1H, ^{13}C and ^{31}P NMR data of the phosphates are collected in Table 4.

Methyl L-glycero-α-D-manno-*heptopyranoside 2-*O*-phosphate* (**1**).—Yield: 99.8%; $[\alpha]_D$ +27.5° (*c* 0.52, H_2O).

Methyl L-glycero-α-D-manno-*heptopyranoside 3-*O*-phosphate* (**2**).—Yield: 94.6%; $[\alpha]_D$ +40.4° (*c* 0.5, H_2O).

Methyl L-glycero-α-D-manno-*heptopyranoside 4-*O*-phosphate* (**3**).—Yield: 98.3%; $[\alpha]_D$ +60.1° (*c* 0.22, H_2O).

Methyl L-glycero-α-D-manno-*heptopyranoside 6-*O*-phosphate* (**4**).—Yield: 97.3%; $[\alpha]_D$ +49.5° (*c* 0.5, H_2O).

Methyl L-glycero-α-D-manno-*heptopyranoside 7-*O*-phosphate* (**5**).—Yield: 98.9%; $[\alpha]_D$ +32° (*c* 0.8, H_2O).

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