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Efficient chemical synthesis of both anomers of ADP L-*glycero*- and D-*glycero*-D-*manno*-heptopyranose

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Abstract

A series of anomeric phosphates and ADP-activated L-*glycero*- and D-*glycero*-D-*manno*-heptopyranoses has been prepared in high overall yields, which provided model compounds and substrates in the elucidation of biosynthetic pathways and glycosyl transfer reactions of nucleotide-activated bacterial heptoses. The α-anomers of the heptosyl phosphates were obtained in high yield and selectivity using the phosphoramidite procedure, whereas the β-phosphates were formed preferentially employing acylation of reducing heptoses with diphenyl phosphorochloridate. An efficient route to the formation of the nucleotide diphosphate sugars was elaborated by coupling of the *O*-acetylated phosphates with AMP-morpholidate followed by alkaline deprotection to furnish ADP-L- and D-*glycero*-α-D-*manno*-heptose in 84 and 89% yield, respectively. Deacetylation of the *O*-acetylated β-configured ADP heptoses was conducted at strictly controlled conditions (−28 °C at pH 10.5) to suppress formation of cyclic heptose-1,2-phosphodiesters with concomitant release of AMP. Isolation of the unstable β-configured ADP-heptoses by anion-exchange chromatography and gel-filtration afforded ADP L- and D-*glycero*-β-D-*manno*-heptose in high yields.

Keywords: Heptose; ADP heptose; Lipopolysaccharide; Sugar nucleotides; Sugar phosphates

1. Introduction

1.1. Occurrence of *glycero*-D-*manno*-heptoses

Lipopolysaccharides (LPS) are structurally complex amphipathic and microheterogeneous glycolipids which are essential components of the outer membrane of Gram-negative bacteria.[1] Furthermore, LPS are mediators of numerous immunological and pathophysiological effects in bacterial infections and are thus responsible for high mortality rates associated with septic shock. Due to an increasing resistance of many bacterial strains against conventional antibiotics, considerable effort is being devoted to the development of novel drugs, which may prevent the assembly of a functionally intact bacterial cell wall by inhibition of its biosynthesis.

Heptoses of the L-*glycero*-D-*manno* configuration are common constituents of the inner core-region of LPS and have been found in *Salmonella*, *Escherichia coli*, *Shigella*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Neisseria*, *Vibrio*, *Bordetella*, *Yersinia*, *Campylobacter* and many other pathogenic bacteria.[2] L-*Glycero*-D-*manno*-heptoses—up to now always present in the α-anomeric configuration—have also been found in the outer core, e.g., of *E. coli* K-12, *Haemophilus ducreyi*, *Haemophilus influenzae* and *Proteus penneri* strains[3–6] and have also been identified in a few cases in repeating units of O-antigens, such as in *Yokenella regensburgei* and *Pseudomonas cepacia* strains.[7,8]

Similar to the L-*glycero*-D-*manno* forms, D-*glycero*-D-*manno*-heptoses are present as α-pyranosides in inner core oligosaccharides, as illustrated in LPS from *Rhodococcus gelatinosus*, *Yersinia enterocolitica*, *Coxiella burnetii*, *Mannheimia haemolytica*, *Aeromonas hydro-*

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phila and *Vibrio salmonicida*.[9–14] Moreover, D-*glycero*-D-*manno*-heptoses are frequently found as side chain units in the outer core of *Proteus* strains,[15] *H. influenzae* LPS,[16] but also as short, α-(1→3 or →2)-linked oligomeric side chains attached to the core units of *Helicobacter pylori* LPS,[17] and *Klebsiella pneumoniae* LPS.[18]

O-Antigens from *Vibrio cholerae* strains harbor D-*glycero*-D-*manno*-heptoses in both anomeric configurations. Thus, the O-antigen polysaccharide from serogroup O:3 contains α-heptosyl residues as constituent of a tetrasaccharide repeating unit,[19] whereas serogroup O:21 contains a β-linked heptosyl moiety.[20] In addition, β-linked D-*glycero*-D-*manno*-heptoses and 6-deoxy-D-*manno*-heptoses are present in the O-antigen from *Plesiomonas shigelloides* O54:H2.[21]

In the S-layer glycoprotein glycan from a Gram-positive microorganism, *Aneurinibacillus thermoaerophilus* DSM 10155, a disaccharide repeating unit →3)-D-*glycero*-β-D-*manno*-Hep*p*-(1→4)-α-L-Rha*p*-(1→ had been identified, substantiating similarities of S-layer glycans to Gram-negative LPS structures.[22] Very recently, a glycoprotein mediating adhesion as an autotransporter protein in enterotoxigenic *E. coli* strains has been found which is substituted on average with 19 heptose residues. The heptose is transferred to the protein by a glycosyl transferase—an autotransporter heptosyl transferase (AAH)—in the presence of ADP-*glycero*-*manno*-heptose.[23] The heptosyl transferase has been cloned and partially characterized. A second glycoprotein, termed TibA has been identified in enterotoxigenic *E. coli*, which acts as an adhesin and whose carbohydrate modification is introduced upon the action of a similar heptosyl transferase.[24]

1.2. Biosynthesis of nucleotide activated *glycero*-D-*manno*-heptoses

The concept of the biosynthetic pathway leading to nucleotide-activated heptose dates back to the fundamental work of Eidels and Osborn who proposed a sequence of enzymatic reactions starting from sedoheptulose 7-phosphate, which is first converted by the action of an isomerase to yield D-*glycero*-D-*manno*-heptose 7-phosphate.[25] A postulated phosphomutase would then furnish the anomeric heptosyl phosphate, which is transformed into a nucleotide activated derivative. Later, Kontrohr and Kocsis provided evidence that substrates of LPS core heptosyl transferases correspond to ADP derivatives of D- and L-*glycero*-D-*manno*-heptose.[26] In the final step, an epimerase effects the inversion of configuration at C-6 to furnish ADP L-*glycero*-D-*manno*-heptose. Based on previous findings by Valvano[27] and the complete elucidation of the biosynthesis of GDP-heptose in the Gram-positive microorganism *A. thermoaerophilus* DSM 10155,[28] two biosynthetic pathways involving a kinase/phosphatase sequence (instead of the mutase step) and utilizing different anomers of D-*glycero*-D-*manno*-heptose 1-phosphates were unambiguously established (Fig. 1).[29] The biosynthesis of GDP heptose has recently been shown to be also valid for the GDP derivative of 6-deoxy-heptose in *Yersinia pseudotuberculosis*.[30]

For the full elucidation of the biosynthesis of these nucleotide-activated heptoses, well defined synthetic compounds were needed to serve as biochemical probes. Herein a high yield synthesis of both anomeric forms of D-*glycero*-D-*manno*-heptose 1-phosphate—occurring as intermediates in both biosynthetic pathways—and ADP L-*glycero*-D-*manno*-heptose **1** and **3** as well as ADP D-*glycero*-D-*manno*-heptose **2** and **4** (Fig. 2) as substrates or substrate analogs for bacterial heptosyl transferases is described. A preliminary account of this work has been published previously.[31]

2. Results and discussion

Since glycosyl phosphates are key intermediates in the biosynthetic pathways, an efficient route for the stereoselective preparation of both anomeric forms of the heptosyl phosphates was required. Among other routes, glycosyl phosphates have been prepared by glycosylation of phosphoric acid diester derivatives with an activated glycosyl donor or by reaction of a free hydroxyl group at the anomeric centre with an activated phosphate precursor.[32] Following the latter approach, the L-*glycero*-D-*manno*-heptopyranose derivative **6**[33] was prepared by anomeric *O*-de-acetylation of the peracetate **5** using ammonium acetate/*N*,*N*-diisopropylethylamine (DIPEA)/DMF in high yield, thereby avoiding the use of the equally effective but toxic hydrazinium acetate (Scheme 1).[34] The 2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-D-*manno*-heptopyranose **16** was similarly obtained in 95% yield from the peracetate derivative **15** (Scheme 2). Treatment of **6** with bis(benzyloxy)(diisopropylamino)phosphine[35] with 1*H*-tetrazole as catalyst, and subsequent oxidation with *t*BuOOH, afforded the α-anomeric phosphotriester **7** as the major isomer (α/β ratio of the product mixture ~ 9:1) in 85% isolated yield after separation from the β-phosphate **9** (9%) (Scheme 1).

Separation of the anomers was also achieved at the stage of the heptosyl phosphites, which may be utilized as potential glycosyl donors. The phosphites were subsequently separately oxidised to give the phosphates in similar overall yield.

By using the phosphoramidite approach, the equilibrium could be shifted from the thermodynamically preferred formation of α-phosphite towards the kinetically controlled generation of β-product in the presence of an excess of 4-*N*,*N*-dimethylaminopyridine (DMAP)

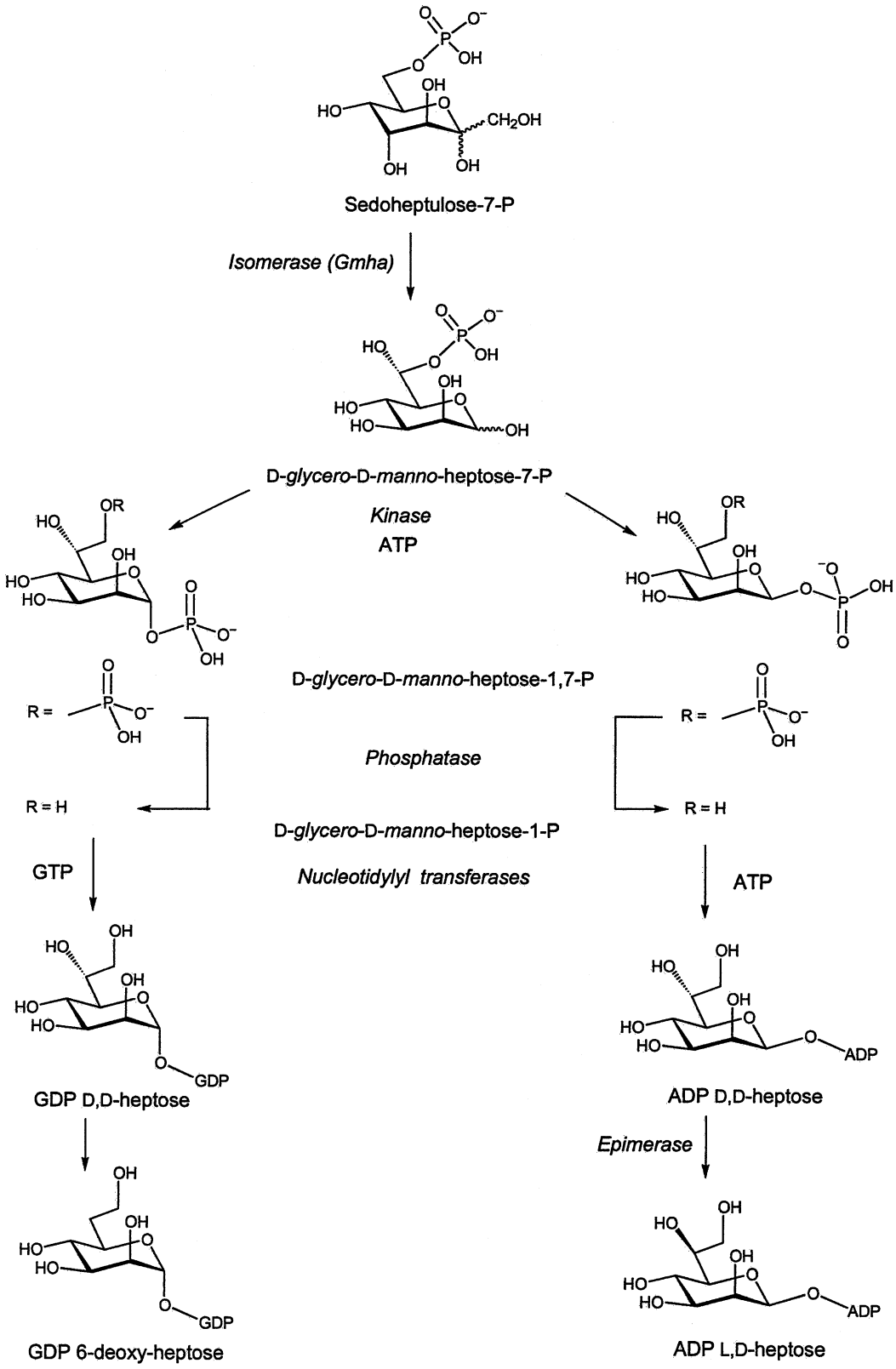


Fig. 1. General biosynthetic pathway for ADP- and GDP-heptose.

by very slow addition of dibenzyl phosphorotetrazolidite prepared in situ. After oxidation with *tert*-BuOOH and chromatographic separation, the α- and β-configured heptosyl phosphotriesters **7** and **9** were obtained in

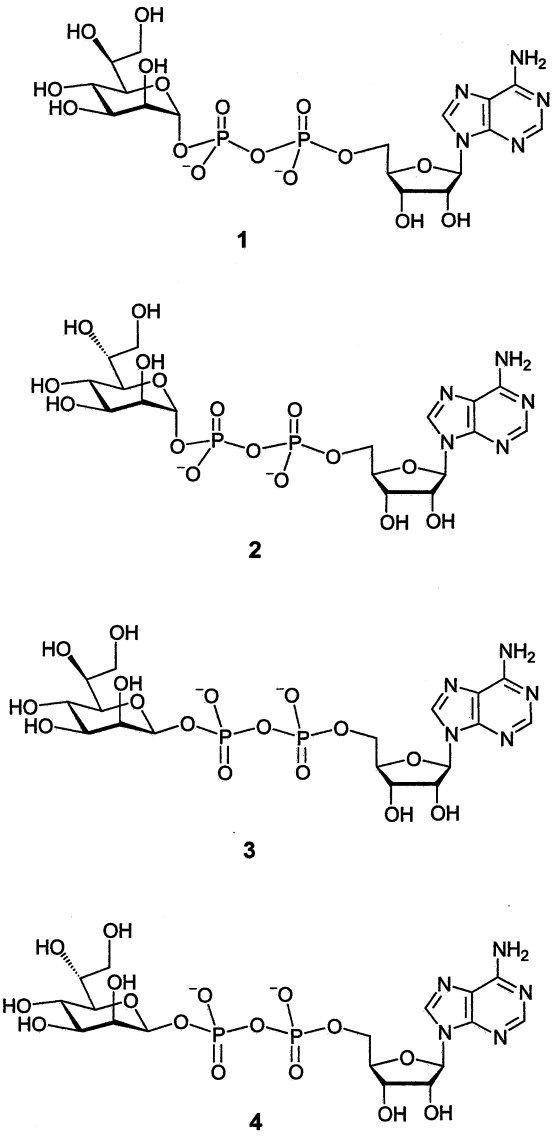
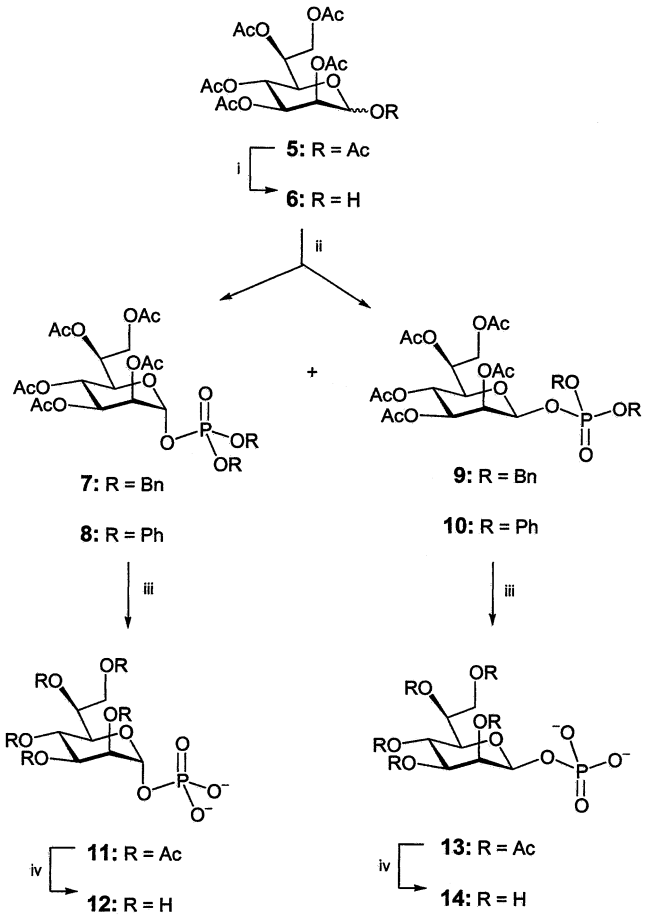


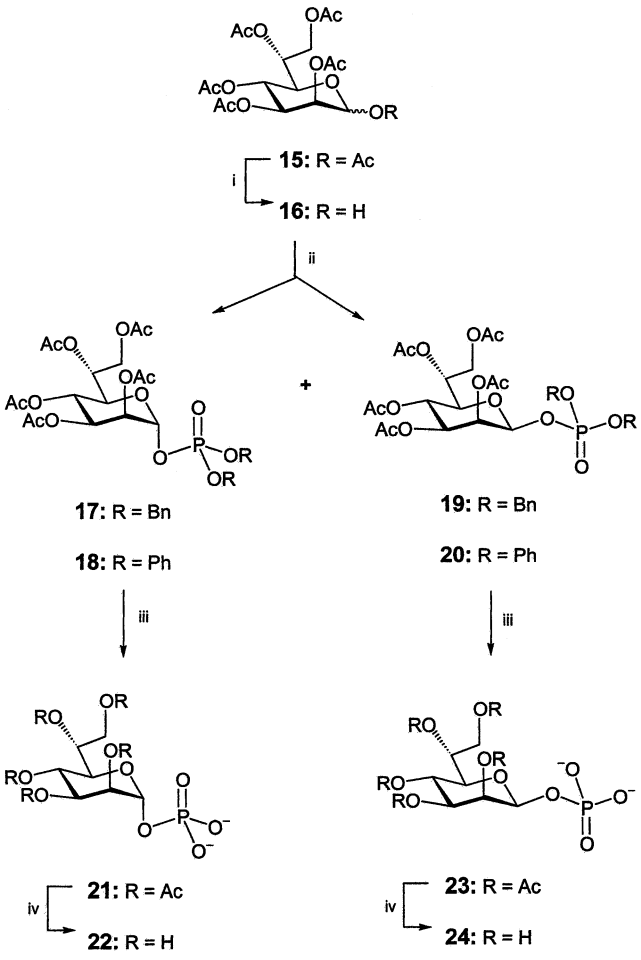
Fig. 2. Structures of ADP L-*glycero*- and D-*glycero*-D-*manno*-heptoses **1**–**4**.



Scheme 1. (i) *N*,*N*-Diisopropylethylamine, NH_4OAc, DMF, 95%; (ii) bis(benzyloxy)(diisopropylamino)phosphine, 1*H*-tetrazole (3.5% in MeCN), CH_2Cl_2, then ^{t}BuOOH (to furnish 85% of **7** and 9% of **9**) or 4-*N*,*N*-dimethylaminopyridine, dibenzyl phosphorotetrazolidite (soln in 1:1 CH_2Cl_2–CH_3CN), CH_2Cl_2, then ^{t}BuOOH (to furnish 56% of **7** and 34% of **9**) or $ClP(O)(OPh)_2$, DMAP, CH_2Cl_2 (to furnish 9% of **8** and 84% of **10**); (iii) Pd/C, H_2, MeOH, Et_3N, 99% (for deprotection of **7** and **9**) or PtO_2, H_2, MeOH, 98% (for deprotection of **8** and **10**); (iv) 7:3:1 MeOH–water–Et_3N, 97–98%.

56 and 34% yield, respectively (Scheme 1). This procedure was also applied to the synthesis of α- and β-phosphates in the D,D-series (Scheme 2).

For the selective synthesis of the β-anomeric heptosyl phosphates, the higher nucleophilicity of the equatorially oriented 1-OH group in **6** was exploited in a direct acylation step using diphenyl phosphorochloridate in the presence of excess of DMAP.[36] Thus, **10** was formed in 84% yield, whereas the α-anomer **8** was obtained in 9% yield. Comparable results were observed for the synthesis of the D-*glycero*-D-*manno* isomers **18** and **20**, which were isolated in 15 and 81% yield, respectively. The ^{1}H NMR data of benzyl-protected heptosyl phosphotriesters **7**, **9**, **17** and **19** are summarised in Table 1. The ^{1}H NMR spectra show a doublet of doublets for H-1, in which the coupling constant $J_{H\text{-}1,P}$ is in the range 6.4–6.6 Hz for α-anomers and 7.7–7.8 Hz for β-configured heptosyl phosphates. The anomeric configuration of the phosphates was unambiguously established on the basis of the values of the heteronuclear coupling constant $J_{C\text{-}1,H\text{-}1}$, which was 185–187 Hz for α-phosphates and 163–165 Hz for β-anomers. The assignment of the anomeric configuration of heptosyl phosphates could also be substantiated from the chemical shift of H-5, which is 0.31–0.37 ppm upfield for the β-anomers in comparison to the corresponding α-counterparts. The facile cleavage of the phosphate protecting groups of benzyl-protected phosphotriesters **7**, **9** in the L,D-series (Scheme 1) and **17**, **19** in the D,D-series (Scheme 2) by catalytic hydrogenation in the presence of Pd/C afforded penta-*O*-acetyl-heptosyl phosphates



Scheme 2. (i) *N*,*N*-Diisopropylethylamine, NH_4OAc, DMF, 95%; (ii) 4-*N*,*N*-dimethylaminopyridine, dibenzyl phosphorotetrazolidite (soln in 1:1 CH_2Cl_2–CH_3CN), CH_2Cl_2, then ^{t}BuOOH (to furnish 58% of **17** and 34% of **19**) or $ClP(O)(OPh)_2$, DMAP, CH_2Cl_2 (to furnish 9% of **18** and 84% of **20**); (iii) Pd/C, H_2, MeOH, Et_3N, 99% (for deprotection of **17** and **19**) or PtO_2, H_2, MeOH, 98% (for deprotection of **18** and **20**); (iv) 7:3:1 MeOH–water–Et_3N, 98%.

11, **13** and **21**, **23** in quantitative yields. Confirmation of their anomeric purity was provided by ^{1}H NMR data (Table 1) and on the basis of the measurement of heteronuclear coupling constants $J_{C\text{-}1,H\text{-}1}$ which were determined from the proton-coupled HMQC spectra and revealed ~180 Hz for α-configured phosphates and ~164 Hz for β-anomers.

Catalytic hydrogenation of the phenyl-protected β-phosphate **10** in the L,D-series (Scheme 1) and **20** in the D,D-series (Scheme 2) in the presence of PtO_2 led to partial (~5–10%) deacetylation of the resulting penta-*O*-acetyl-heptosyl phosphates **13** and **23**. The latter were either purified by chromatography or directly used as partially deacetylated mixtures in the following coupling with AMP-morpholidate, which did not exert any negative influence on the yield of the diphosphate forming reaction.

Removal of the acetyl groups of the *O*-acetylated heptosyl phosphates with triethylamine in MeOH–water furnished the L,D-heptopyranosyl phosphates **12** and **14** as well as the D,D-configured compounds **22** and **24** as triethylammonium salts in near quantitative yields, respectively. The NMR data of all four heptosyl phosphates are in full agreement with the assigned structures (Tables 2 and 3). The downfield shifts (Table 2) of H-5 for α-anomers **12** and **22** (3.92 and 3.83 ppm) in comparison to the related shifts of the β-phosphates **14** and **24** (3.38 and 3.40 ppm) and the measurement of the heteronuclear coupling constant ($J_{C\text{-}1,H\text{-}1}$ ~170 Hz for **12** and **22**, and ~160 Hz for **14** and **24**) confirmed the assignment of the anomeric configuration. The ^{13}C NMR data displayed downfield shifts for C-3 at 73.74 and 73.54 ppm for β-phosphates **14** and **24** (Table 3). The data of the previously reported D-*glycero*-α-D-*manno*-heptosyl phosphate compare favorably with those recorded for compound **22**.[37]

Both anomeric phosphates of D-*glycero*-D-*manno*-heptose have been used in the elucidation of the biosynthesis of nucleotide-activated heptoses, wherein only the β-phosphate **24** serves as a substrate for the adenylyl transfer reaction to yield ADP heptose with retention of configuration. Conversely, in the activating step leading to the formation of GDP heptose, only the α-anomer **22** is accepted by the enzyme.[28,29]

One of the most frequently used methods for the stereospecific chemical synthesis of sugar nucleoside diphosphates employs the coupling of the reducing sugar phosphate with nucleoside monophosphomorpholidate.[38] The yields, however, are mostly moderate even after prolonged reaction times, although improvements have been reported.[39,40] Following the procedure developed for the synthesis of GDP-Fucose,[41] the target compound ADP L-*glycero*-α-D-*manno*-heptose **1** (Fig. 1) was initially prepared in 15% yield by the coupling of 4-morpholine *N*,*N*′-dicyclohexylcarboxamidinium salt of adenosine monophosphomorpholidate (AMP-morpholidate) **25** to α-heptosyl phosphate **12**. The major difficulty was encountered in the poor solubility of heptosyl phosphate **12** (as triethyl- or tri-*n*-butylammonium salt) in anhydrous pyridine, the preferred medium for the phosphomorpholidate coupling, leading to heterogeneous reaction conditions. A homogeneous solution in anhydrous pyridine was obtained using tri-*n*-octylammonium salt of **12**, which, however, also did not significantly increase the yield (25%) of the diphosphate forming reaction, probably due to micelle-like aggregate formation, rendering the phosphate moiety less accessible to the phosphomorpholidate. As an alternative to sluggishly reactive heptosyl phosphate **12**, its penta-*O*-acetyl derivative **11** was used in the coupling with AMP-morpholidate **25** (Scheme 3). The advantageous application of acylated sugar intermediates in the synthesis of sugar-diphosphates has been

Table 1
^{1}H NMR data of acetylated heptosyl phosphates (in $CDCl_3$ for **7**, **9**, **17**, **19** and in D_2O for **11**, **13**, **21** and **23**) [a]

	H-1	H-2	H-3	H-4	H-5	H-6	H-7a	H-7b	CH_2	CH_3CO
7	5.59 (dd) $^3J_{1,2} < 1$ $^3J_{1,P}$ 6.4	5.25 (bs)	5.23 (dd) $^3J_{3,4}$ 9.5	5.25 (t) $^3J_{4,5}$ 9.5	4.11(dd) $^3J_{5,6}$ 2.8	5.26 (m) $^3J_{6,7a}$ 3.9 $^3J_{6,7b}$ 8.4	4.22 (dd) $^2J_{7a,7b}$ 12.0	4.09 (dd)	5.90 (2 d, 4 H) $^2J_{H,P}$ 9.5	2.17, 2.12, 2.03, 2.0, 1.99 (5 s)
9	5.38 (dd) $^3J_{1,2}$ 1.1 $^3J_{1,P}$ 7.7	5.45 (dd) $^3J_{2,3}$ 3.3	5.02 (dd) $^3J_{3,4}$ 10.1	5.30 (t) $^3J_{4,5}$ 10.1	3.76 (dd) $^3J_{5,6}$ 2.4	5.28 (ddd) $^3J_{6,7a}$ 5.1 $^3J_{6,7b}$ 7.8	4.27 (dd) $^2J_{7a,7b}$ 11.6	4.09 (dd)	5.07 (d, 2 H) 5.03, 5.01 (2 d) $^2J_{H,P}$ 8.3	2.16, 2.10, 2.23, 2.20, 1.98 (5 s)
11	5.52 (dd) $^3J_{1,2}$ 1.8 $^3J_{1,P}$ 7.8	5.36 (dd) $^3J_{2,3}$ 3.2	5.43 (dd) $^3J_{3,4}$ 10.1	5.21 (t) $^3J_{4,5}$ 10.1	4.48 (dd) $^3J_{5,6}$ 1.9	5.38 (ddd) $^3J_{6,7a}$ 3.9 $^3J_{6,7b}$ 8.4	4.32 (dd) $^2J_{7a,7b}$ 12.1	4.40 (dd)		2.22, 2.18, 2.10, 2.07, 2.02 (5 s)
13	5.44 (dd) $^3J_{1,2} < 1$ $^3J_{1,P}$ 9.0	5.54 (dd) $^3J_{2,3}$ 3.0	5.34 (dd) $^3J_{3,4}$ 10.0	5.15 (t) $^3J_{4,5}$ 10.0	4.17 (dd) $^3J_{5,6}$ 1.8	5.35 (m) $^3J_{6,7a}$ 4.0 $^3J_{6,7b}$ 8.0	4.35 (dd) $^2J_{7a,7b}$ 12.0	4.44 (dd)		2.26, 2.18, 2.07, 2.09, 2.01 (5 s)
17	5.58 (dd) $^3J_{1,2}$ 2.0 $^3J_{1,P}$ 6.6	5.20 (dd) $^3J_{2,3}$ 2.6	5.28 (dd) $^3J_{3,4}$ 9.7	5.31 (t) $^3J_{4,5}$ 9.7	4.16 (dd) $^3J_{5,6}$ 2.2	5.18 (ddd) $^3J_{6,7a}$ 3.8 $^3J_{6,7b}$ 7.8	4.34 (dd) $^2J_{7a,7b}$ 12.0	4.20 (dd)	5.10 (d, 2 H), 5.09 (d, 2 H) $^2J_{H,P}$ 8.8	2.15, 2.10, 2.04, 2.0, 1.94 (5 s)
19	5.49 (dd) $^3J_{1,2}$ 1.4 $^3J_{1,P}$ 7.8	5.44 (dd) $^3J_{2,3}$ 3.2	5.06 (dd) $^3J_{3,4}$ 9.5	5.27 (t) $^3J_{3,4}$ 9.5	3.85 (dd) $^3J_{5,6}$ 3.7	5.27 (ddd) $^3J_{6,7a}$ 3.6 $^3J_{6,7b}$ 7.0	4.43 (dd) $^2J_{7a,7b}$ 12.1	4.27 (dd)	5.12 (d, 2 H), 5.06 (d, 2 H), $^2J_{H,P}$ 7.8	2.15, 2.11, 2.06, 2.03, 2.01 (5 s)
21	5.44 (dd) $^3J_{1,2}$ 1.8 $^3J_{1,P}$ 7.6	5.26 (dd) $^3J_{2,3}$ 2.7	5.34 (dd) $^3J_{3,4}$ 9.6	5.29 (t) $^3J_{4,5}$ 9.6	4.37 (dd) $^3J_{5,6}$ 2.8	5.21 (ddd) $^3J_{6,7a}$ 4.0 $^3J_{6,7b}$ 7.3	4.26 (dd) $^2J_{7a,7b}$ 12.2	4.41 (dd)		2.14, 2.10, 2.08, 2.04, 1.98 (5 s)
23	5.29 (dd) $^3J_{1,2}$ 1.3 $^3J_{1,P}$ 8.8	5.37 (dd) $^3J_{2,3}$ 2.8	5.17 (dd) $^3J_{3,4}$ 9.5	5.13 (t) $^3J_{4,5}$ 9.5	4.00 (dd) $^3J_{5,6}$ 2.5	5.14 (ddd) $^3J_{6,7a}$ 4.0 $^3J_{6,7b}$ 7.0	4.20 (dd) $^2J_{7a,7b}$ 12.2	4.32 (dd)		2.09, 2.0, 1.99, 1.95, 1.88 (5 s)

[a] Coupling constants (in Hz) are first order values.

Table 2
^{1}H NMR data of heptosyl phosphates **12**, **14**, **22** and **24** in D_2O

	H-1	H-2	H-3	H-4	H-5	H-6	H-7a	H-7b
12	5.33(dd) $^3J_{1,2}$ 1.5 $^3J_{1,P}$ 6.6	3.93(m) n.d. [a]	3.95(m) n.d. [a]	3.90(m) $^3J_{4,5}$ 9.5	3.81(m) $^3J_{5,6}$ 1.6	4.0(ddd) $^3J_{6,7a}$ 7.9 $^3J_{6,7b}$ 6.1	3.73(dd) $^2J_{7a,7b}$ 11.4	3.59(dd)
14	5.08(dd) $^3J_{1,2}$ 0.9 $^3J_{1,P}$ 8.5	4.01(dd) $^3J_{2,3}$ 3.2	3.70(dd) $^3J_{3,4}$ 9.7	3.80(dd) $^3J_{4,5}$ 9.6	3.38(dd) $^3J_{5,6}$ 1.9	3.96(ddd) $^3J_{6,7a}$ 5.2 $^3J_{6,7b}$ 6.6	3.75(dd) $^2J_{7a,7b}$ 11.5	3.70(dd)
22	5.27(dd) $^3J_{1,2}$ 1.7 $^3J_{1,P}$ 7.9	3.87(dd) $^3J_{2,3}$ 2.8	3.82 (dd) $^3J_{3,4}$ 9.3	3.67(dd) $^3J_{4,5}$ 9.5	3.83(dd) $^3J_{5,6}$ 1.8	3.92(ddd) $^3J_{6,7a}$ 7.1 $^3J_{6,7b}$ 5.0	3.70(dd) $^2J_{7a,7b}$ 12.1	3.65(dd)
24	4.99(dd) $^3J_{1,2}$ 1.0 $^3J_{1,P}$ 8.6	3.92(dd) $^3J_{2,3}$ 2.6	3.59(m)	3.59(m)	3.40(m)	3.95(ddd) $^3J_{6,7a}$ 6.6 $^3J_{6,7b}$ 4.2	3.70–3.68 (m)	3.70–3.68 (m)

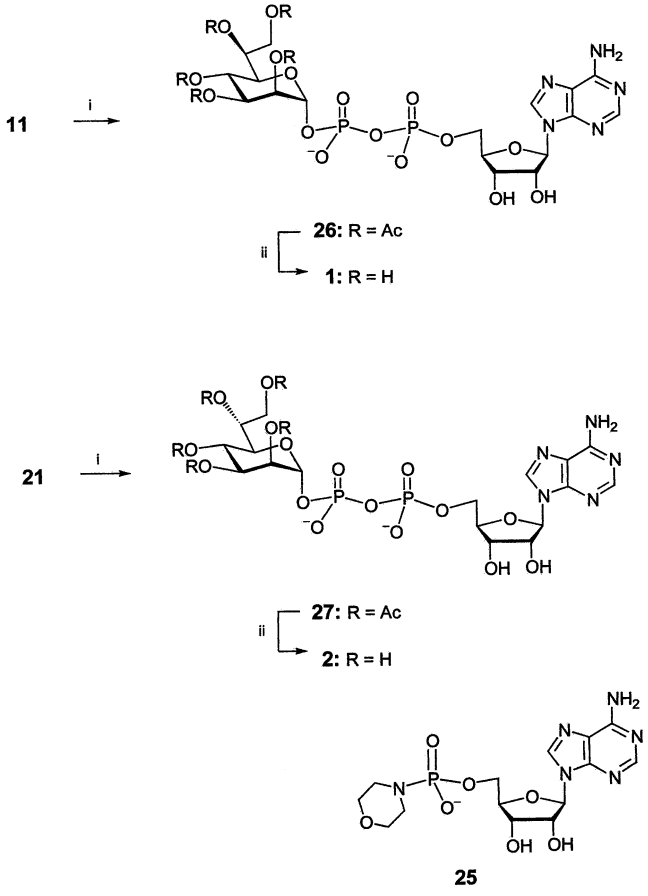
[a] n.d. not determined.

Table 3
^{13}C NMR data of heptosyl phosphates in D_2O

Carbon	**12**	**14**	**22**	**24**
C-1	96.15 $^2J_{1,P}$ 5.5	96.03 $^2J_{1,P}$ 3.8	96.06 $^2J_{1,P}$ 5.3	96.01 $^2J_{1,P}$ 4.0
C-2	71.52 $^3J_{2,P}$ 9.7	72.06 $^3J_{2,P}$ 6.0	71.19 $^3J_{2,P}$ 7.6	71.70 $^3J_{2,P}$ 6.0
C-3	71.20	73.74	70.92	73.54
C-4	66.85	66.66	68.44	67.92
C-5	71.53	75.70	73.48	77.72
C-6	69.02	69.51	72.88	72.01
C-7	62.53	63.11	62.60	62.22

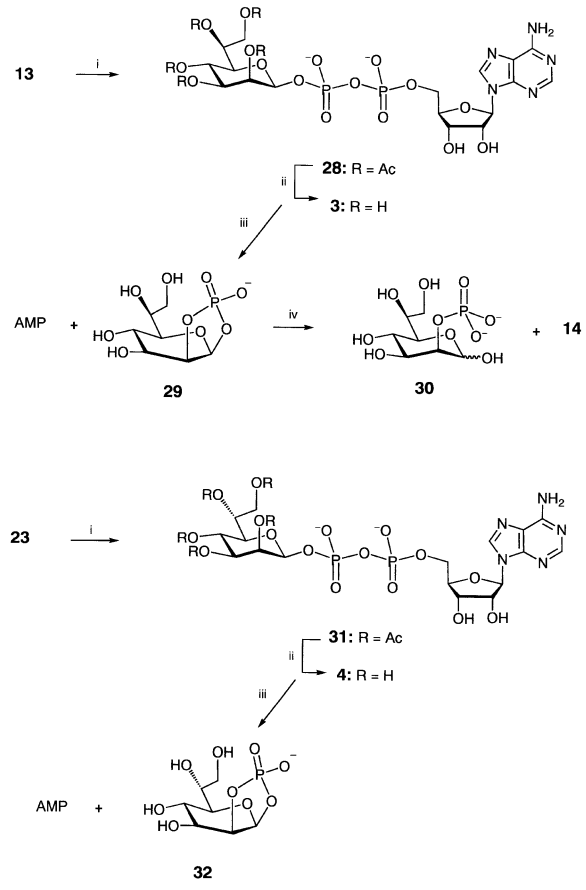
previously reported.[42] The reaction proceeded rapidly to give approx 70% of desired product **26** within 1 day (as judged by TLC), then slowed down and needed two more days to be driven to completion. The acetylated ADP L-*glycero*-α-D-*manno*-heptose **26** was isolated in 91% yield as triethylammonium salt by anion-exchange chromatography.

Coupling of the other three *O*-acetylated heptosyl phosphates **13**, **21**, **23** to AMP-morpholidate **25** gave, after isolation and purification by anion-exchange chromatography, similar and reproducible yields (85–95%) of adenosine heptosyl diphosphates **27**, **28** and **31**, respectively (Schemes 3 and 4). The structures of the acetylated ADP-L- and D-*glycero*-D-*manno*-heptopyranoses **26**, **27**, **28** and **31** were unambiguously confirmed by ^{1}H (Table 4) and ^{31}P NMR experiments. Removal of the acetyl groups from the heptosyl moiety of the α-configured nucleoside diphosphates **26** and **27** with aqueous ammonia or an aqueous methanolic solution of Et_3N at pH 12 furnished the ADP-heptoses **1** and **2**, respectively, in quantitative yields. The overall yield for



Scheme 3. (i) 4′-Morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt of **25**, pyridine, anion exchange on BioRad (Q) 5 mL cartridge (HCO_3^- form), elution 0.01→0.2 M TEAB, 91% for **26** and 86% for **27**; (ii) 7:3:1 MeOH–water–Et_3N, 98%.

the preparation of **1** starting from **5** was 71%. Cleavage of the acetate groups from β-configured sugar nucleotide **28** using these conditions, however, resulted in the



Scheme 4. (i) 4′-Morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt of **25**, pyridine, anion exchange on BioRad (Q) 5 mL cartridge (HCO_3^- form), elution 0.01→0.2 M TEAB, 92% for **28** and 95% for **31**; (ii) 4:3:0.05 0.1 M aq TEAB–MeOH–Et_3N, −28 °C; anion exchange on BioRad (Q) 5 mL cartridge (HCO_2O^- form), elution 0.01→0.2 M TEAB; gel filtration on Superdex Peptide HR 10/30 Pharmacia column, elution 0.1 M TEAB; Dowex 50 (H^+-form), than Et_3N; (iii) 7:3:1 MeOH–water–Et_3N, 85% for **29** and 80% for **32**; (iv) silica gel, 10:3:3 MeOH–water–25% aq NH_4OH, 85%.

formation of the β-heptosyl 1,2-cyclic phosphate **29** along with a minor amount of heptose 2-phosphate **30** and 1-phosphate **14** with release of AMP at the expense of β-heptose nucleoside diphosphate (Scheme 4). The 1,2-cyclic phosphate **29** was isolated by anion-exchange chromatography, and the compound was found to be unstable under extended contact with silica gel to furnish 2-phosphate **30** predominantly. The formation of the five membered ring of the cyclic phosphodiester **29** was evident from the large heteronuclear coupling constant $J_{H\text{-}1,P}$ ~25 Hz, the ^{31}P NMR chemical shift of **29** (δ 17.8), the results of an $^1H,^{31}P$ HMQC experiment and the downfield shift of C-2 in ^{13}C NMR-spectrum (δ 79.5). Additional proof was provided by subsequent alkaline (1 M NaOH) hydrolysis of **29** which gave a 1:1 mixture of 1-phosphate **14** and 2-phosphate **30**. The NMR spectra of heptose 2-phosphate **30** were in agreement with previously reported data.[43]

Cleavage of the diphosphate bond and cyclisation under neighbouring group participation of the hydroxyl group at C-2 of the heptose moiety was found to occur also under milder reaction conditions [MeOH/water/Et_3N, pH 10.5 at 4 °C or 2 M triethylammonium bicarbonate (TEAB) buffer, pH 10, 25 °C] and the cyclic phosphate **29** was the major product after the deacetylation step. Careful adjustment of the conditions for the basic hydrolysis of the acetate groups of β-configured ADP-heptoses **28** and **31**, however, finally resulted in the isolation of **3** and **4** in 85–90% overall yields, based on acetylated heptosyl phosphates **13** and **23**. When deacetylation was conducted at −28 °C in MeOH–0.1-M aq TEAB–Et_3N (pH 10–11) for 20 h followed by immediate neutralisation with Dowex H^+-resin, the formation of only 5–10% of cyclic phosphates **29** and **32** was observed (Scheme 4).

The ADP-heptoses **3** and **4** were eventually purified by anion-exchange chromatography on Bio-Rad High Q resin using a linear TEAB gradient, and by subsequent gel chromatography on Superdex Peptide using the same buffer to remove residual AMP. Purified ADP-L-*glycero*- and D-*glycero*-β-D-*manno*-heptoses may be stored in a frozen solution (pH 5, triethylammonium salt) or after lyophilisation, for several months at −20 °C without noticeable decomposition. In solution, diphosphate **3** (as triethylammonium salt at pD 5) slowly decomposed at room temperature (~30% in 10 days, Fig. 3).

The structure and purity of all four isomers of ADP-heptose was unambiguously confirmed by NMR data. Homonuclear $^1H,^1H$-COSY- and heteronuclear $^1H,^{13}C$-HMQC- and $^1H,^{31}P$-HMQC-experiments allowed the complete assignment of all proton (Table 5) and carbon (Table 6) signals of the carbohydrate residues.

Decoupled ^{31}P NMR spectra showed two doublets (at −10.8 ppm and in the range of −12.7 to −13.2 ppm) with a coupling constant $J_{P,P}$ ~21 Hz for each compound; the signals of the diphosphodiester group were correlated to the anomeric proton of the heptose and to H-5 of the ribose, respectively. The ^{13}C NMR spectra of **1**, **2**, **3** and **4** clearly showed a doublet signal for C-1 at around 96.5–97.0 ppm. The coupling constants $J_{C\text{-}1,P}$ were in the range of 5.8–6.0 Hz for α-anomers and 4.1–4.8 Hz for the β-counterparts. The signals of C-2 of the heptose residues were also coupled to phosphorus with coupling constants of 7–10 Hz. ^{13}C NMR signals of C-5 and C-4 of ribose appeared as doublets with heteronuclear coupling constants $J_{C,P}$ 4.5–5.8 and $J_{C,P}$ ~9 Hz respectively. The anomeric proton coupling constants of **1** and **2** ($J_{H\text{-}1,H\text{-}2}$ 1.9 and 2.0 and $J_{H\text{-}1,P}$ 7.4 and 7.9 Hz) are characteristic of α-anomers, whereas the corresponding values of **3** and **4** ($J_{H\text{-}1,H\text{-}2}$ 1.0, $J_{H\text{-}1,P}$ 8.5 and 8.7 Hz) confirm the β-

Table 4
^{1}HgNMR data of acetylated ADP-heptoses **26**, **27**, **28** and **31** in D_2O

	H-1	H-2	H-3	H-4	H-5	H-6	H-7a	H-7b	CH_3CO	Ade
26 Hep*p*	5.60(dd) $^3J_{1,2}$ 1 $^3J_{1,P}$ 7.7	5.39(dd) $^3J_{2,3}$ 3.3	5.33(dd) $^3J_{3,4}$10.1	5.13(t) $^3J_{4,5}$10.1	4.42(dd)^3 $J_{5,6}$ 1.6	5.21(m)	4.35(m)	4.31(m)	2.18, 2.12, 2.00, 1.96, 1.94 (5 s)	
Rib*f*	6.15(d) $^3J_{1,2}$ 5.5	4.70(dd) $^3J_{2,3}$ 4.8	4.51(dd) $^3J_{3,4}$ 4.1	4.39(m)	4.23(m, 2 H)					8.60, 8.27
27 Hep*p*	5.58(dd) $^3J_{1,2}$ 1.9 $^3J_{1,P}$ 8.2	5.34(m)	5.27(m)	5.11(t) $^3J_{4,5}$ 10.0	4.35(dd) $^3J_{5,6}$ 4.0	5.21(m) $^3J_{6,7a}$ 4.5 $^3J_{6,7b}$ 7.8	4.29(dd) $^2J_{7a,7b}$ 12.1	4.17(dd)	2.13, 2.08, 2.03, 2.02, 1.93 (5 s)	
Rib*f*	6.12(d) $^3J_{1,2}$ 5.4	4.70(dd) $^3J_{2,3}$ 5.0	4.49(dd) $^3J_{3,4}$ 4.2	4.36(m)	4.24(m, 2 H)					8.56, 8.26
28 Hep*p*	5.47(dd) $^3J_{1,2}$ 1 $^3J_{1,P}$ 9.3	5.54(dd) $^3J_{2,3}$ 3.0	5.12(dd) $^3J_{3,4}$ 10.0	5.07(t) $^3J_{4,5}$ 10.0	3.86(dd) $^3J_{5,6}$ 1.8	5.20(ddd) $^3J_{6,7a}$ 4.9 $^3J_{6,7b}$ 8.6	4.38(dd) $^2J_{7a,7b}$ 12.2	4.26(dd)	2.07, 2.01, 1.92, 1.88, 1.83 (5 s)	
Rib*f*	6.15(dd) $^3J_{1,2}$ 5.9	4.70(dd) $^3J_{2,3}$ 4.9	4.50(dd) $^3J_{3,4}$ 3.8	4.38(m)	4.22(m, 2H)					8.50, 8.29
31 Hep*p*	5.51(dd) $^3J_{1,2}$ 1.2 $^3J_{1,P}$ 11.8	5.52(bs) $^3J_{2,3}$ 2.8	5.16(dd) $^3J_{3,4}$ 9.6	5.23(dd) $^3J_{4,5}$ 9.4	3.97(dd) $^3J_{5,6}$ 3.0	5.20(m) $^3J_{6,7a}$ 4.1 $^3J_{6,7b}$ 7.3	4.40(dd) $^2J_{7a,7b}$ 12.0	4.40(dd)	2.20, 2.11, 2,10, 2.06, 1.99 (5 s)	
Rib*f*	6.16(dd) $^3J_{1,2}$ 5.7	4.70(dd) $^3J_{2,3}$ 4.9	4.51(dd) $^3J_{3,4}$ 3.7	4.38(m)	4.23(m, 2 H)					8.59, 8.36

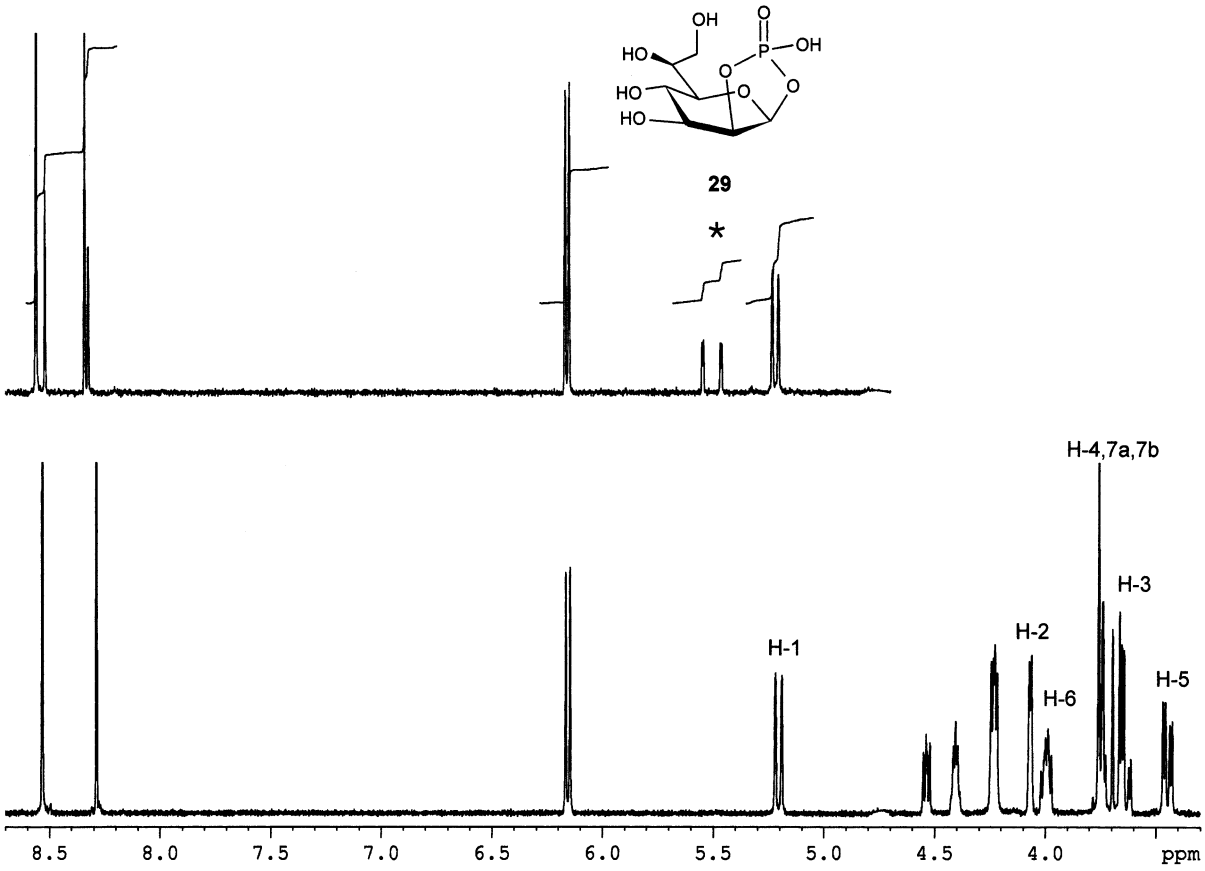


Fig. 3. 300 MHz ^{1}H NMR spectrum and proton assignments of the heptose unit of purified ADP L-*glycero*-β-D-*manno*-heptose **3** (bottom line). Upon standing at room temperature at pH 5 for 10 days, the 1,2-cyclophosphate **29** was formed (insert), which is readily identified by the large heteronuclear coupling constant $J_{H\text{-}1,P}$ displayed by the anomeric proton (asterix).

anomeric configuration (Table 5). The upfield-shifted signals at 3.37 and 3.45 ppm attributed to H-5 of **3** and **4**, respectively, are also consistent with the β-*manno* configuration of the heptose residue.

The α- and β-anomeric configurations were also confirmed using 'gated decoupled' ^{13}C NMR measurements ($J_{C\text{-}1,H\text{-}1}$ ~173 Hz for α-anomers **1** and **2** and ~163 Hz for β-derivatives **3** and **4**). The downfield shifts of heptose C-3 and C-5 for β-anomers **3** and **4** (at 73.34 and 73.31 ppm for C-3 and at 75.66 and 77.64 ppm for C-5, respectively) in comparison with the α-diphosphates **1** and **2** (C-3 at 70.81 and 70.75 ppm and C-5 at 72.70 and 74.35 ppm, respectively) fully confirm the structural assignments. Furthermore, the α-anomers of the heptose 1-phosphates as well as ADP heptoses showed dextrorotation in contrast to their β-configured analogs.

In conclusion, the chemical synthesis of both, α- and β-configured sugar nucleotides ADP-L- and D-*glycero*-D-*manno*-heptopyranose was performed in a high-yield procedure using acetyl-protected heptosyl phosphates in the coupling step to AMP-morpholidate followed by base-catalysed deacetylation. The merit of this procedure pertains to its simplicity and reproducibility giving 85–90% yield of the title compounds for the coupling and deprotection step. Whereas the ADP-α-heptoses **1** and **2** are stable to either mildly acidic (pH 3) or strongly basic conditions (up to pH 13), the substrates of enterobacterial heptosyltransferases—the β-configured ADP L-*glycero*- and D-*glycero*-D-*manno*-heptoses **3** and **4**—are highly susceptible to hydrolysis of the diphosphate linkage.

3. Experimental

3.1. General methods

Column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck). Anion-exchange chromatography was performed on BioRad anion-exchange resin (Pharmacia). Reactions were monitored by TLC on Silica Gel 60 F_{254} HPTLC precoated glass plates with 2.5 cm concentration zone (E. Merck), unless stated otherwise; spots were visualised by spraying with anisaldehyde–H_2SO_4; phosphorus-containing compounds were additionally detected with a molybdate solution (0.02 M solution of ammonium cerium(IV)sul-

Table 5
^{1}H NMR data of ADP-heptoses **1**,**2**,**3** and **4** in D_2O

	H-1	H-2	H-3	H-4	H-5	H-6	H-7a	H-7b	Adenine
1 Hep*p*	5.51(dd) $^3J_{1,2}$ 1.9 $^3J_{1,P}$ 7.4	4.02(dd) $^3J_{2,3}$ 3.2	3.92(m)	3.85(m)	3.83(m)	3.99(ddd) $^3J_{6,7a}$ 6.2 $^3J_{6,7b}$ 7.1	3.73(dd) $^2J_{7a,7b}$ 11.6	3.64(dd)	8.51(H-8) 8.26(H-2)
Rib*f*	6.14(d) $^3J_{1,2}$ 6.0	4.70(m) $^3J_{2,3}$ 5.1	4.52(dd) $^3J_{3,4}$ 3.5	4.39(m)	4.22(m, 2 H)				
2 Hep*p*	5.56(dd) $^3J_{1,2}$ 2.0 $^3J_{1,P}$ 7.9	4.09(dd) $^3J_{2,3}$ 3.2	3.94(dd) $^3J_{3,4}$ 9.3	3.83(t) $^3J_{4,5}$ 9.3	3.97 $^3J_{5,6}$ 3.0	4.05(ddd) $^3J_{6,7a}$ 4.0 $^3J_{6,7b}$ 6.5	3.83(dd) $^2J_{7a,7b}$ 12.1	3.78(dd)	8.56(H-8) 8.29(H-2)
Rib*f*	6.19(dd) $^3J_{1,2}$ 6.0	4.70(m) $^3J_{2,3}$ 5.1	4.59(dd) $^3J_{3,4}$ 3.5	4.46(m)	4.28(m, 2 H)				
3 Hep*p*	5.23(dd) $^3J_{1,2}$ 1.0 $^3J_{1,P}$ 8.5	4.07(dd) $^3J_{2,3}$ 3.2	3.67(dd) $^3J_{3,4}$ 9.7	3.82(t) $^3J_{4,5}$ 9.7	3.37(dd) $^3J_{5,6}$ 1.7	3.95(ddd) $^3J_{6,7a}$ 6.0 $^3J_{6,7b}$ 5.0	3.75(dd) $^2J_{7a,7b}$ 12.3	3.71(dd)	8.60(H-8) 8.37(H-2)
Rib*f*	6.18(d) $^3J_{1,2}$ 5.8	4.70(m) $^3J_{2,3}$ 5.2	4.55(dd) $^3J_{3,4}$ 3.5	4.42(m)	4.25(m, 2 H)				
4 Hep*p*	5.21(dd) $^3J_{1,2}$ 1.0 $^3J_{1,P}$ 8.7	4.07(dd) $^3J_{2,3}$ 3.3	3.63(dd) $^3J_{3,4}$ 9.4	3.69(t) $^3J_{4,5}$ 9.4	3.45(dd) $^3J_{5,6}$ 3.3	3.99(m)	3.75(m)	3.75(m)	8.53(H-8) 8.29(H-2)
Rib*f*	6.16(d) $^3J_{1,2}$ 5.8	4.73(m) $^3J_{2,3}$ 4.9	4.54(dd) $^3J_{3,4}$ 3.8	4.41(m)	4.23(m, 2 H)				

Table 6
^{13}C NMR data of ADP-heptoses in D_2O [a]

	1	**2**	**3**	**4**
C-1 Hep*p*	97.23 $^2J_{1,P}$ 6.0	97.08 $^2J_{1,P}$ 5.8	96.51 $^2J_{1,P}$ 4.8	96.52 $^2J_{1,P}$ 4.1
Rib*f*	87.55	87.48	88.26	87.61
C-2 Hep*p*	70.92 $^3J_{2,P}$ 10	70.74 $^3J_{2,P}$ 8.7	71.37 $^3J_{2,P}$ 7.1	71.22 $^3J_{2,P}$ 9.9
Rib*f*	74.98	74.96	75.23	75.01
Ade	153.05	153.48	152.20	152.68
C-3 Hep*p*	70.81	70.75	73.34	73.31
Rib*f*	71.06	71.09	70.99	71.09
C-4 Hep*p*	66.58	67.89	66.28	67.78
Rib*f*	84.59 $^3J_{4,P}$ 9.0	84.60 $^3J_{4,P}$ 9.2	84.80 $^3J_{4,P}$ 9.0	84.65 $^3J_{4,P}$ 9.1
Ade	149.76	149.78	149.19	149.73
C-5 Hep*p*	72.70	74.35	75.66	77.64
Rib*f*	65.87 $^2J_{5,P}$ 5.0	65.91 $^2J_{5,P}$ 5.0	65.82 $^2J_{5,P}$ 5.8	65.91 $^2J_{5,P}$ 4.5
Ade	119.20	119.27	119.13	119.30
C-6 Hep*p*	69.24	72.89	69.31	72.74
Ade	155.98	156.23	156.55	156.15
C-7 Hep*p*	63.10	62.65	62.98	62.30
C-8 Ade	140.62	140.48	142.34	140.83

[a] Recorded at pD 5.

fate dihydrate and ammonium molybdate(VI)tetrahydrate in aqueous H_2SO_4), adenosine-containing compounds were detected by examination under UV light. Concentration of solutions was performed at diminished pressure at temperatures $<30\,^{\circ}C$. Triethylamine, CH_2Cl_2, dry pyridine were purchased from E. Merck, and were dried by refluxing with CaH_2 (5 g/L) for 16 h, then distilled and stored under argon. Toluene was distilled from phosphorus pentaoxide and redistilled from CaH_2. The liquids were stored over molecular sieves 0.4 nm. DMF was stirred with CaH_2 (5 g/L) for 16 h at 20 °C, distilled under reduced pressure and stored over activated molecular sieves 0.3 nm. Triethylammonium bicarbonate (TEAB) buffer, was prepared by passing a stream of CO_2-gas through a cooled (ice-water bath) solution of Et_3N (2 M) in de-ionized water until a near neutral solution (pH 7.5) was obtained. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. $[\alpha]_D^{20}$ values are given in units of 10^{-1} deg cm^3 g^{-1}. NMR-spectra were recorded at 297 K in D_2O as solvent (unless stated otherwise) with a Bruker DPX 300 spectrometer (^{1}H at 300.13 MHz, ^{13}C at 75.47 MHz and ^{31}P at 121.50 MHz) using standard Bruker NMR software. ^{1}H NMR spectra were referenced either to tetramethylsilane or 2,2-dimethyl-2-silapentane-5-sulfonic acid. ^{13}C NMR spectra were referenced to 1,4-dioxane (δ 67.40), ^{31}P NMR spectra to 85% aq H_3PO_4 in separate experiments prior to measurements. Ele-

mental analyses were provided by Dr J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, Universität Wien. MALDI-TOF-MS-ionisation spectra were recorded on a Dynamo (Thermo BioAnalysis) instrument in the positive or negative ion mode using MeCN or water, both with 2% 2,5-dihydroxybenzoic acid as matrix.

3.2. 2,3,4,6,7-Penta-*O*-acetyl-L-*glycero*-D-*manno*-heptopyranose (6)

A soln of heptopyranosyl peracetate **5**[33a] (300 mg, 0.65 mmol) in a mixture of dry DMF (5 mL) and *N*,*N*-diisopropylethylamine (1 mL) was stirred with ammonium acetate (200 mg); crystals were prewashed with dry ether (50 mL) and dried for 10 min under diminished pressure) for 16 h at room temperature (rt). The reaction mixture was decanted from the undissolved crystals of ammonium acetate, diluted with CH_2Cl_2 (300 mL) and washed with satd aq $NaHCO_3$ (2 × 20 mL), water (10 mL), dried ($MgSO_4$) and concentrated under diminished pressure. The residue was purified by silica gel chromatography (3:1 toluene–EtOAc) to give amorphous **5** (260 mg, 95%). NMR data were in accordance with the previously published data.[33b]

3.3. Dibenzyl (2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl) phosphate (7) and dibenzyl (2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-β-D-*manno*-heptopyranosyl) phosphate (9)

3.3.1. Method A. Heptose pentaacetate **6** (180 mg, 0.43 mmol) and bis(benzyloxy)(diisopropylamino)phosphine (190 mg, 0.55 mmol) were dried by repeated evaporations with dry toluene (3 × 10 mL) and then under diminished pressure for 10 h. The flask was fitted with a septum cap, flushed with N_2 and charged with CH_2Cl_2 (5 mL). A soln of 1*H*-tetrazole in MeCN (1 mmol, 2 mL) was added and the mixture was stirred at rt for 2 h under N_2 atmosphere. Monitoring of the reaction by TLC and ^{31}P NMR spectroscopy showed the formation of intermediate phosphite triesters. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and the soln was washed with 0.5 M aq TEAB (2 × 20 mL), water (20 mL), brine (20 mL), dried (cotton plug) and concentrated. The anomeric phosphites were isolated by chromatography on silica gel (3:1 *n*-hexane–ether → ether) to provide dibenzyl (2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl)phosphite as faster-eluting isomer. Yield: 242 mg (0.36 mmol, 85%); R_f 0.45 (1:2 *n*-hexane–ether); $[\alpha]_D^{20}$ +26° (*c* 1.0, $CHCl_3$); ^{31}P NMR ($CDCl_3$): δ 140.35; ^1H NMR ($CDCl_3$): δ 7.25–7.37 (m, 10 H, 2 C_6H_5), 5.47 (dd, 1 H, $J_{1,2}$ 1.8, $J_{1,P}$ 8.1 Hz, H-1), 5.34 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 5.27 (t, 1 H, $J_{3,4} = J_{4,5}$ 10.1 Hz, H-4), 5.24 (ddd, 1 H, $J_{5,6}$ 2.2 Hz, H-6), 5.22 (dd, 1 H, H-2), 4.90 (d, 2 H, $J_{H,P}$ 8.0 Hz, $J_{H,H} <$ 1 Hz, CH_2Ph), 4.93 (dd, 1 H, $J_{H,P}$ 8.5 Hz, $J_{H,H} < 1$ Hz, CH_2Ph), 4.94 (dd, 1 H, $J_{H,P}$ 8.5 Hz, $J_{H,H} < 1$ Hz, CH_2Ph), 4.19 (dd, 1 H, $J_{6,7a}$ 4.6, $J_{7a,7b}$ 12.2 Hz, H-7_a), 4.11 (dd, 1 H, $J_{6,7b}$ 7.8 Hz, H-7_b), 4.10 (dd, 1 H, H-5), 2.16, 2.11, 2.02, 1.99 and 1.98 (5 s, each 3 H; 5 CH_3);

Further elution afforded dibenzyl (2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-β-D-*manno*-heptopyranosyl)phosphite as a syrup. Yield: 25 mg (0.039 mmol, 9%): R_f 0.38 (1:2 *n*-hexane–ether); ^{31}P NMR ($CDCl_3$): δ 141.20; ^1H NMR ($CDCl_3$): δ 7.27–7.36 (m, 10 H, 2 C_6H_5), 5.43 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 5.30 (t, 1 H, $J_{3,4} = J_{4,5}$ 10.1 Hz, H-4), 5.27 (ddd, 1 H, $J_{5,6}$ 2.5 Hz, H-6), 5.17 (dd, 1 H, $J_{1,2}$ 1.0, $J_{1,P}$ 8.0 Hz, H-1), 5.03 (dd, 1 H, H-3), 4.90 (dd, 2 H, $J_{H,P}$ 8.5 Hz, $J_{H,H} < 1$ Hz, CH_2Ph), 4.87 (dd, 2 H, $J_{H,P}$ 8.5 Hz, $J_{H,H} < 1$ Hz, CH_2Ph), 4.29 (dd, 1 H, $J_{6,7a}$ 4.9, $J_{7a,7b}$ 11.6 Hz, H-7a), 4.12 (dd, 1 H, $J_{6,7b}$ 7.8 Hz, H-7b), 3.69 (dd, 1 H, H-5), 2.16, 2.11, 2.02, 1.99 and 1.98 (5 s, each 3 H; 5 CH_3).

Procedure without isolation of phosphites from heptose pentaacetate **6** (195 mg, 0.46 mmol): the reaction mixture containing phosphite triesters was cooled to 0 °C and a soln of *tert*-BuOOH (150 μL of 80% soln in di-*tert*-butyl peroxide) in CH_2Cl_2 (1 mL) was added gradually over a period of 20 min, the reaction mixture was warmed to rt and stirred for 3 h. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and successively washed with 1 M aq TEAB (pH 8, 2 × 20 mL), water (20 mL) and brine (20 mL). The organic phase was dried (cotton) and concentrated. The α- and β-phosphates were separated by chromatography on silica gel (3:1 *n*-hexane–ether → ether). Appropriate fractions were pooled, concentrated and purified by a second chromatography (1:1 toluene–EtOAc) to give **7** (268 mg, 85%) as a syrup, R_f 0.6 (ether) or 0.6 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ +19° (*c* 1.0, $CHCl_3$); ^{13}C NMR($CDCl_3$): δ 170.44, 170.15, 169.61, 169.45, 169.40 (5 C, CO, Ac), 135.13, 135.05 (2 C, C_6H_5, Bn), 128.83, 128.70, 128.37, 128.27 (10 C, C_6H_5, Bn), 95.22 (C-1, $J_{1,P}$ 5.5 Hz), 70.68 (C-5), 70.17, 70.10 (2 C, $J_{C,P} < 1$ Hz, CH_2, Bn), 68.79 (C-2, $J_{2,P}$ 11.6 Hz), 68.38 (C-3), 66.88 (C-6), 64.12 (C-4), 62.89 (C-7), 20.71, 20.63, 20.52 (5 C, CH_3, Ac); $J_{C\text{-}1,H\text{-}1}$ 185 Hz; ^{31}P NMR ($CDCl_3$): δ −2.1. Anal. Calcd for $C_{31}H_{37}O_{15}P$ (680.6): C, 54.71; H, 5.48; P, 4.55. Found: C, 54.76; H, 5.37; P, 4.66.

Further elution gave **9** (28 mg, 9%) as a syrup, R_f 0.4 (ether) or 0.5 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ −23° (*c* 0.4, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 170.82, 170.64, 170.36, 170.10, 169.82 (5 C, CO, Ac), 135.64, 135.18 (2 C, C_6H_5, Bn), 129.10, 129.01, 128.56, 128.44 (10 C, C_6H_5, Bn), 95.14 (C-1, $J_{1,P}$ 2.8 Hz), 73.79 (C-5), 71.10 (C-3), 70.21, 70.22 (2 C, $J_{C,P} < 1$ Hz, CH_2), 68.90 (C-2, $J_{2,P}$ 8.5 Hz), 66.95 (C-6), 64.41 (C-4), 62.46 (C-7), 21.10, 20.98, 20.88 (5 C, CH_3, Ac); $J_{C\text{-}1,H\text{-}1}$ 163 Hz; ^{31}P NMR ($CDCl_3$): δ −2.1; Anal. Calcd for $C_{31}H_{37}O_{15}P$ (680.6): C, 54.71; H, 5.48. Found: C, 54.80; H, 5.68.

3.3.2. Method B. Heptose pentaacetate **6**, bis(benzyloxy)(diisopropylamino)phosphine and DMAP were separately dried by repeated evaporations with dry toluene (3 × 10 mL each). A solution of dibenzyl phosphorotetrazolidite was prepared in situ by adding a soln of 1*H*-tetrazole in CH_3CN (2 mL, 1 mmol) to a stirred soln of bis(benzyloxy)(diisopropylamino)phosphine (173 mg, 0.5 mmol) in CH_2Cl_2 (2 mL) under N_2. A clear soln of dibenzyl phosphorotetrazolidite in 1:1 CH_2Cl_2–MeCN (4 mL) (which was decanted from the precipitated diisopropylammonium tetrazolide) was added dropwise during 2 h to the stirred solution of **6** (155 mg, 0.37 mmol) and 4-*N*,*N*-dimethylaminopyridine (98 mg, 0.8 mmol) in CH_2Cl_2 (5 mL) through a rubber septum with a syringe under N_2. The reaction mixture was cooled to 0 °C, and a soln of *tert*-BuOOH (120 μL of 80% soln in di-*tert*-butyl peroxide) in CH_2Cl_2 (1 mL) was added gradually over 20 min, the reaction mixture was warmed to rt and stirred for 3 h, diluted with CH_2Cl_2 (200 mL) and successively washed with 1 M aq TEAB (pH 8, 2 × 20 mL), water (20 mL) and brine (20 mL). The organic phase was dried (cotton) and concentrated. The phosphotriesters were isolated in the same fashion as described in method A, which furnished first **7** (140 mg, 56%) and finally **9** (85 mg, 34%).

3.4. Diphenyl (2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl) phosphate (8) and diphenyl (2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-β-D-*manno*-heptopyranosyl) phosphate (10)

Diphenyl phosphorochloridate and **6** were dried by coevaporation with dry toluene (2 × 10 mL each), and dried under diminished pressure. To a stirred soln of **6** (40 mg, 0.095 mmol) and 4-*N*,*N*-dimethylaminopyridine (56 mg, 0.46 mmol) in CH_2Cl_2 (3 mL), a soln of diphenyl phosphorochloridate (25 μL, 0.12 mmol) in CH_2Cl_2 (2 mL) was added dropwise during 2 h at ambient temperature under N_2. The reaction mixture was diluted with CH_2Cl_2 (100 mL), washed successively with 1 M aq TEAB buffer (2 × 20 mL), water (10 mL) and brine (20 mL), the organic phase was dried (cotton) and concentrated. The residue was fractionated by chromatography on silica gel (3:1 *n*-hexane–ether → ether). Appropriate fractions were pooled, concentrated and purified by a second chromatography (49:1 → 7:3 toluene–EtOAc) which gave **8** as faster eluting isomer. Yield: 5 mg (9%), syrup, R_f 0.8 (ether) or 0.7 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ +18° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.43–7.20 (m, 10 H, 2 C_6H_5), 5.90 (dd, 1 H, $J_{1,2}$ 1.2, $J_{1,P}$ 7.0 Hz, H-1), 5.40 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 10.0 Hz, H-3), 5.36 (m, 1 H, H-4), 5.35 (m, 1 H, H-2), 5.3 (ddd, 1 H, $J_{6,7a}$ 6.1, $J_{6,7b}$ 8.3 Hz, H-6), 4.29 (dd, 1 H, $J_{5,6}$ 2.0, $J_{4,5}$ 9.0 Hz, H-5), 4.16 (m, 2 H, H-7_a, H-7_b), 2.20, 2.14, 2.25, 2.22 and 1.95 (5 s, each 3 H, 5 Ac). ^{13}C NMR ($CDCl_3$): δ 170.80, 170.55, 170.11, 169.91, 169.83 (5 C, CO, Ac), 150.55, 150.45 (2 C, Ph), 130.48, 126.32, 120.56, 120.49, 120.42 (10 C, C_6H_5), 96.57 (C-1, $J_{1,P}$ 5.6 Hz), 71.53 (C-5), 69.20 (C-2, $J_{2,P}$ 11.2 Hz), 68.73 (C-3), 67.20 (C-6), 64.46 (C-4), 62.89 (C-7), 21.13, 21.04, 20.96 (5 C, CH_3, Ac); $J_{C\text{-}1,H\text{-}1}$180 Hz. Anal. Calcd for $C_{29}H_{33}O_{15}P$ (652.5): C, 53.38; H, 5.10. Found: C, 53.62; H, 5.36.

Further elution gave **10** (52 mg, 84%), R_f 0.5 (ether) or 0.6 (1:2 toluene–EtOAc); $[\alpha]_D^{20}$ −24° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.40–7.15 (m, 10 H, 2 C_6H_5), 5.58 (dd, 1 H, $J_{1,2}$ 1.2, $J_{1,P}$ 7.0 Hz, H-1), 5.54 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 5.34 (t, 1 H, $J_{3,4} = J_{4,5}$ 10.1 Hz, H-4), 5.29 (ddd, 1 H, $J_{5,6}$ 2.4 Hz, H-6), 5.07 (dd, 1 H, H-3), 4.28 (dd, 1 H, $J_{6,7a}$ 5.3, $J_{7a,7b}$ 11.5 Hz, H-7a), 4.12 (dd, 1 H, $J_{6,7b}$ 7.7 Hz, H-7_b), 3.84 (dd, 1 H, H-5), 2.14, 2.15, 2.04, 2.03, 2.0 (5 s, each 3 H, 5 CH_3); ^{13}C NMR ($CDCl_3$): δ 170.38, 170.16, 169.78, 169.70, 169.41 (5 C, CO, Ac), 150.23, 150.15 (2 C, Ph), 129.90–120.19 (12 C, C_6H_5), 95.34 (C-1, $J_{1,P}$ 5.6 Hz), 73.46 (C-5), 70.56 (C-3), 68.21 (C-2, $J_{2,P}$ 8.7 Hz), 66.55 (C-6), 63.95 (C-4), 61.98 (C-7), 20.64, 20.45 (5 C, CH_3, Ac); $J_{C\text{-}1,H\text{-}1}$ 167 Hz. Anal. Calcd for $C_{29}H_{33}O_{15}P$ (652.5): C, 53.38; H, 5.10. Found: C, 53.30; H, 5.11.

3.5. 2,3,4,6,7-Penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl phosphate (monotriethylammonium salt) (11)

A soln of **7** (68 mg, 0.10 mmol) in dry MeOH (20 mL) was hydrogenated in the presence of 10% Pd/C (20 mg) for 10 h at atmospheric pressure. After completion of the reaction, the catalyst was removed by filtration through a pad of Celite and washed with MeOH (20 mL). The free phosphate was neutralised by addition of Et_3N (0.2 mmol). The filtrate was concentrated and the residue was lyophilised from water (10 mL) to give the monotriethylammonium salt of **11** (59 mg, 99%) as a white fluffy solid which was used in the next step without further purification. R_f 0.5 (60:35:6:1 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ +10° (*c* 1.0, water); ^{31}P NMR: δ −2.0; ^{13}C NMR: δ 174.28, 173.58, 173.52, 173.15, 172.98 (5 C, CO), 93.65 (d, C-1, $J_{1,P}$ 5.1 Hz), 70.75 (d, C-2, $J_{2,P}$ 10.6 Hz), 69.82 (C-3), 69.58 (C-5), 68.29 (C-6), 65.43 (C-4), 63.91 (C-7), 47.09 (CH_2, Et_3N), 20.54, 20.51, 20.45 (5 C, CH_3), 8.63 (CH_3, Et_3N); $J_{C\text{-}1,H\text{-}1}$ 178 Hz. Anal. Calcd for $C_{23}H_{39}NO_{15}P \cdot H_2O$ (618.5): C, 44.66; H, 6.68; N, 2.26. Found: C, 44.85; H, 6.56; N, 2.11.

3.6. L-*Glycero*-α-D-*manno*-heptopyranosyl phosphate (monotriethylammonium salt) (12)

A soln of **11** (25 mg, 0.04 mmol) in 7:3:1 MeOH–water–Et_3N (2 mL, pH 12) was stirred at ambient temperature for 3 h. The reaction mixture was concentrated and lyophilised from water (2 × 20 mL) to give 15 mg (0.038 mmol, 97%) of **12** as a white amorphous solid; R_f 0.2

(5:7:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ +21° (*c* 1.0, water); ^{31}P NMR: δ −0.4; ^{13}C NMR: δ 47.72 (CH_2, Et_3N), 9.26 (CH_3, Et_3N), other signals see Table 3; $J_{C\text{-}1,H\text{-}1}$ 173 Hz. Anal. Calcd for $C_{13}H_{29}NO_{10}P\cdot H_2O$ (408.2): C, 38.24; H, 7.65. Found: C, 38.90; H, 7.41.

3.7. 2,3,4,6,7-Penta-*O*-acetyl-L-*glycero*-β-D-*manno*-heptopyranosyl phosphate (monotriethylammonium salt) (13)

3.7.1. Method A. Compound **13** was prepared from **9** (78 mg, 0.11 mmol) in the same manner as described for the synthesis of **11** from **7**. Yield: 68 mg (99%); R_f 0.4 (60:35:6:1 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ −31° (*c* 1.0, water); ^{31}P NMR: δ −1.5; ^{13}C NMR (D_2O): δ 174.27, 173.72, 173.59, 173.53, 172.93 (5 C, CO), 93.98 (d, C-1, $J_{1,P}$ 3.2 Hz), 72.50 (C-5), 71.52 (C-3), 70.46 (d, C-2, $J_{2,P}$ 6.3 Hz), 67.96 (C-6), 65.38 (C-4), 63.74 (C-7), 47.10 (CH_2, Et_3N), 20.55, 20.51, 20.48, 20.47, 20.36 (5 C, CH_3), 8.64 (CH_3, Et_3N); $J_{C\text{-}1,H\text{-}1}$ 164 Hz. Anal. Calcd for $C_{23}H_{39}NO_{15}P\cdot 2\ H_2O$ (636.6): C, 43.40; H, 6.81; N, 2.20. Found: C, 43.21; H, 5.97; N, 2.32.

3.7.2. Method B. A soln of **10** (40 mg, 0.06 mmol) in dry MeOH (20 mL) was hydrogenated in the presence of PtO_2 (10 mg) for 10 h at atmospheric pressure. After addition of Et_3N (0.12 mmol), the catalyst was removed by filtration through a pad of Celite and washed with MeOH (20 mL). The combined filtrates were concentrated. The partially deacetylated (~5–10% according to ^1H NMR data) phosphate **13** may be used in the next steps without further purification. For characterisation, crude **13** was purified by flash chromatography on silica gel (30:20:1→25:25:1 $CHCl_3$–MeOH–water). Appropriate fractions were concentrated to 10 mL volume, the solution was cooled to 0 °C and the pH was adjusted to 4.5 with Dowex 50 (H^+) resin. The resin was removed by filtration, the total eluate was made neutral by addition of Et_3N, concentrated to 5 mL volume (resulting in a pH of ~5) at 25 °C and lyophilised to give the monotriethylammonium salt of **13** (35 mg, 93%) as a white fluffy solid.

3.8. L-*Glycero*-β-D-*manno*-heptopyranosyl phosphate (triethylammonium salt) (14)

Compound **14** was prepared from **13** (32 mg, 0.05 mmol) as described for the synthesis of **12**. Yield: 21 mg (0.05 mmol, 98%); R_f 0.15 (5:7:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ −11° (*c*, 0.4, water); ^{31}P NMR: δ −2.0, ^{13}C NMR: δ 47.57 (CH_2, Et_3N), 9.07 (CH_3, Et_3N), other signals see Table 3; $J_{C\text{-}1,H\text{-}1}$ 161 Hz. Anal. Calcd for $C_{13}H_{29}NO_{10}P\cdot 0.5\ H_2O$ (399.4): C, 39.10; H, 7.57. Found: C, 38.89; H, 8.15.

3.9. 2,3,4,6,7-Penta-*O*-acetyl-D-*glycero*-D-*manno*-heptopyranose (16)

Compound **16** was prepared from heptosyl peracetate **15**[33] (110 mg, 0.24 mmol) in the same fashion as described for **6**. Yield: 95 mg (95%); $[\alpha]_D^{20}$ +27° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.40 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 9.6 Hz, H-3), 5.29 (dd, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 5.23 (m, 3 H, H-1, H-2, H-6), 4.39 (dd, 1 H, $J_{6,7}$ 3.6, $J_{7a,7b}$ 12.0 Hz, H-7a), 4.28 (dd, 1 H, $J_{6,7b}$ 4.8 Hz, H-7b), 4.21 (dd, 1 H, $J_{5,6}$ 2.8 Hz, H-5), 3.18 (d, 1 H, OH), 2.18, 2.11, 2.09 and 2.02 (4 s, 15 H, 5 Ac). Anal. Calcd for $C_{17}H_{24}O_{12}$ (420.37): C, 48.57; H, 5.75. Found: C, 48.52; H, 6.07.

3.10. Dibenzyl (2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-αD-*manno*-heptopyranosyl) phosphate (17) and dibenzyl (2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-β-D-*manno*-heptopyranosyl) phosphate (19)

Compounds **17** and **19** were prepared from heptosyl pentaacetate **16** (110 mg, 0.26 mmol) in the fashion described for the synthesis of **7** and **9** using method B.

Yield for **17** (103 mg, 0.15 mmol, 58%); syrup, R_f 0.6 (ether); $[\alpha]_D^{20}$ +42.5° (*c* 1.0, $CHCl_3$); ^{31}P NMR ($CDCl_3$): δ −2.7; ^{13}C NMR ($CDCl_3$): δ 170.94, 170.31, 170.11, 170.05, 169.83, (5 C, CO, Ac), 135.63, 135.54 (2 C, C_6H_5), 129.21, 129.14, 129.11, 128.57, 128.37 (10 C, C_6H_5), 95.19 (C-1, $J_{1,P}$ 5.4 Hz), 72.33 (C-5), 70.42 (1 C, $J_{C,P}$ 5.5 Hz, CH_2Ph), 70.24 (1 C, $J_{C,P}$ 5.5 Hz, CH_2Ph), 70.09 (C-6), 68.96 (C-2, $J_{2,P}$ 10.8 Hz), 68.69 (C-3), 66.21 (C-4), 61.90 (C-7), 21.12, 21.09, 20.98 (5 C, CH_3, Ac); $J_{C\text{-}1,H\text{-}1}$ 187 Hz. Anal. Calcd for $C_{31}H_{37}O_{15}P$ (680.6): C, 54.71; H, 5.48. Found: C, 54.80; H, 5.26.

Yield for **19**: 60 mg (34%), syrup, R_f 0.45 (ether); $[\alpha]_D^{20}$ −16.5° (*c* 1.0, $CHCl_3$); ^{31}P NMR ($CDCl_3$): δ −2.14; ^{13}C NMR ($CDCl_3$): δ 171.24, 170.82, 170.15, 170.0, 169.65, (5 C, CO, Ac), 135.94, 135.65 (2 C, C_6H_5Ph), 129.14, 129.04, 129.01, 128.93, 128.40, 128.36 (10 C, C_6H_5Ph), 95.20 (C-1, $J_{1,P}$ 7.3 Hz), 74.38 (C-5), 70.29 (1 C, $J_{C,P}$ 7.7 Hz, CH_2Ph), 69.93 (1 C, $J_{C,P}$ 8.5 Hz, CH_2Ph), 69.85, 69.82 (C-3, C-6), 68.64 (C-2, $J_{2,P}$ 12.4 Hz), 65.94 (C-4), 61.52 (C-7), 21.21, 21.12, 20.91 (5 C, CH_3, Ac); $J_{C\text{-}1,H\text{-}1}$ 165 Hz. MALDI-TOF-MS: *m/z*: 704.97 $[M+Na]^+$.

3.11. Diphenyl (2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-α-D-*manno*-heptopyranosyl) phosphate (18) and diphenyl (2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-β-D-*manno*-heptopyranosyl) phosphate (20)

Compounds **18** and **20** were prepared from **16** (19.3 mg, 0.046 mmol) in the same manner as described for the synthesis of **8** and **10**.

Yield for **18**: 4.5 mg (15%); R_f 0.55 (4:1 ether–hexane); $[\alpha]_D^{20}$ +39° (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.40–7.20 (m, 10 H, 2 C_6H_5), 5.85 (dd, 1 H, $J_{1,2}$ 2.1,

$J_{1,P}$ 6.4 Hz, H-1), 5.38 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.7 Hz, H-3), 5.37 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 5.30 (dd, 1 H, H-2), 5.16 (ddd, 1 H, $J_{6,7a}$ 4.1, $J_{6,7b}$ 7.8 Hz, H-6), 4.38 (dd, 1 H, $J_{7a,7b}$ 12.2 Hz, H-7a), 4.24 (dd, 1 H, $J_{5,6}$ 2.4, $J_{4,5}$ 9.8 Hz, H-5), 4.22 (dd, 1 H, H-7_b), 2.15, 2.11, 2.03, 2.01 (5 s, each 3 H; 5 CH_3); ^{13}C NMR ($CDCl_3$): δ 170.52, 169.97, 169.65, 169.42 (5 C, CO, Ac), 150.30, 149.98 (2 C, Ph), 129.96, 125.87, 125.81, 120.29, 120.22, 120.15 (10 C, Ph), 96.75 (C-1, $J_{1,P}$ 5.9 Hz), 72.35 (C-5), 69.59 (C-6), 68.45 (C-2, $J_{2,P}$ 12.0 Hz), 68.21 (C-3), 65.72 (C-4), 61.36 (C-7), 20.68, 20.57, 20.54 (5 C, CH_3, Ac); $J_{C\text{-}1,H\text{-}1}$ 177 Hz. MALDI-TOF-MS: m/z: 675.88 $[M+Na]^+$.

Yield for **20**: 24.4 mg (81%), R_f 0.45 (4:1 ether–hexane); $[\alpha]_D^{20}$ −5.6° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.38–7.09 (m, 10 H, 2 C_6H_5), 5.59 (dd, 1 H, $J_{1,2}$ 1.7, $J_{1,P}$ 7.2 Hz, H-1), 5.37 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 5.22 (ddd, 1 H, $J_{5,6}$ 4.6 Hz, H-6), 5.19 (t, 1 H, $J_{3,4} = J_{4,5}$ 8.7 Hz, H-4), 5.0 (dd, 1 H, H-3), 4.32 (dd, 1 H, $J_{6,7a}$ 3.5, $J_{7a,7b}$ 12.2 Hz, H-7a), 4.12 (dd, 1 H, $J_{6,7b}$ 6.5 Hz, H-7_b), 3.80 (dd, 1 H, H-5), 2.03, 2.02, 1.99, 1.98, 1.93 (5 s, each 3 H; 5 CH_3); ^{13}C NMR ($CDCl_3$): δ 170.478, 169.82, 169.69, 169.65, 169.52 (5 C, CO, Ac), 150.28, 150.0 (2 C, Ph), 129.88–120.11 (10 C, Ph), 94.70 (C-1, $J_{1,P}$ 4.7 Hz), 74.01 (C-5), 69.63 (C-3), 67.38 (C-2, $J_{2,P}$ 8.1 Hz), 69.47 (C-6), 65.85 (C-4), 61.30 (C-7), 20.82, 20.69, 20.54, 20.47 (5 C, CH_3, Ac); $J_{C\text{-}1,H\text{-}1}$ 169 Hz. Anal. Calcd for $C_{29}H_{33}O_{15}P$ (652.5): C, 53.38; H, 5.10. Found: C 53.36; H 5.43.

3.12. 2,3,4,6,7-Penta-*O*-acetyl-D-*glycero*-α-D-*manno*-heptopyranosyl phosphate (monotriethylammonium salt) (21)

Compound **21** was prepared from **17** (56 mg, 0.08 mmol) in the same fashion as described for the synthesis of **11** from **7**. Yield: 47.5 mg (99%); R_f 0.4 (60:35:6:1 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ +39° (*c* 0.8, water); ^{31}P NMR: δ −1.7; ^{13}C NMR (D_2O): δ 174.17, 173.49, 173.37, 173.17, 173.08 (5 C, CO), 93.30 (d, C-1, $J_{1,P}$ 4.8 Hz), 70.93 (C-6), 70.13 (C-5), 70.12 (d, C-2, $J_{2,P}$ 9.9 Hz), 70.03 (C-3), 67.01 (C-4), 62.41 (C-7), 47.09 (CH_2, Et_3N), 20.72, 20.61, 20.57, 20.53, 20.48 (5 C, CH_3), 8.63 (CH_3, Et_3N); $J_{C\text{-}1,H\text{-}1}$182 Hz. Anal. Calcd for $C_{23}H_{39}NO_{15}P\cdot 1.5\ H_2O$ (627.6): C, 44.02; H, 6.75; N, 2.23. Found: C, 43.96; H, 6.54; N, 2.49.

3.13. D-*Glycero*-α-D-*manno*-heptopyranosyl phosphate (triethylammonium salt) (22)

Compound **22** was prepared from **21** (19 mg, 0.03 mmol) in the way as described for the synthesis of **12**. Yield: 12 mg (98%); R_f 0.15 (5:7:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ +20° (*c* 0.7, water); lit.[37] +33° (*c* 0.5, 1:1 water–THF); ^{31}P NMR: δ 0.5; ^{13}C NMR: δ 47.51 (CH_2, Et_3N), 9.04 (CH_3, Et_3N), other signals see Table 3; $J_{C\text{-}1,H\text{-}1}$ 170 Hz. Anal. Calcd for $C_{13}H_{29}NO_{10}P\cdot H_2O$ (408.4): C, 38.24; H, 7.65; N, 3.43. Found: C, 38.39; H, 7.93; N, 3.62.

3.14. 2,3,4,6,7-Penta-*O*-acetyl-D-*glycero*-β-D-*manno*-heptopyranosyl phosphate (monotriethylammonium salt) (23)

3.14.1. Method A. Compound **23** was prepared from **19** (30 mg, 0.05 mmol) in the same way as described for the synthesis of **11** from **7**. Yield: 29 mg (98%); R_f 0.33 (60:35:6:1 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ −1.4° (*c* 1.0, water); ^{31}P NMR: δ −1.6; ^{13}C NMR: δ 174.18, 173.54, 173.35, 173.25, 172.95 (5 C, CO), 93.64 (d, C-1, $J_{1,P}$ 3.3 Hz), 73.09 (C-5), 71.63 (C-3), 70.90 (C-6), 70.13 (d, C-2, $J_{2,P}$ 6.2 Hz), 66.98 (C-4), 62.35 (C-7), 47.09 (CH_2, Et_3N), 20.70, 20.60, 20.56, 20.47, 20.38 (5 C, CH_3), 8.63 (CH_3, Et_3N); $J_{C\text{-}1,H\text{-}1}$ 164 Hz. MALDI-TOF-MS: m/z: 523.54 $[M+Na]^+$.

3.14.2. Method B. Compound **23** was prepared from **20** (8 mg, 0.012 mmol) in the same fashion as described for the synthesis of **13** from **10**. Yield: 5.8 mg (93%).

3.15. D-*Glycero*-β-D-*manno*-heptopyranosyl phosphate (triethylammonium salt) (24)

Compound **24** was prepared from **23** (25 mg, 0.04 mmol) in the way described for the synthesis of **12**. Yield: 16 mg (98%); R_f 0.1 (5:7:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ −35° (*c* 0.7, water); ^{31}P NMR: δ 1.3; ^{13}C NMR: δ 47.07 (CH_2, Et_3N), 8.62 (CH_3, Et_3N), other signals see Table 3; $J_{C\text{-}1,H\text{-}1}$ 160 Hz. MALDI-TOF-MS: m/z: 335.71 $[M+2Na]^{2+}$.

3.16. Adenosine 5′-(2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-α-D-*manno*-heptopyranosyl)diphosphate (triethylammonium salt) (26)

Pentaacetyl heptosyl phosphate **11** (25 mg, 0.042 mmol) was made anhydrous by repeated dissolution in dry pyridine and evaporation of solvent (4 × 10 mL). After each evaporation step, dry N_2 was flushed into the rotary evaporator. AMP-morpholidate (4′-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt) **25** (44 mg, 0.06 mmol) was dissolved in dry pyridine and evaporated to dryness and the process was repeated three times with exclusion of moisture under N_2. Both components were finally dissolved in pyridine and combined in a one-neck round-bottom flask under N_2 atmosphere. The reaction mixture was repeatedly evaporated from pyridine (3 × 10 mL) and flushed with dry N_2. A final amount of pyridine (10 mL) was added to give a clear solution. About 70% of pyridine was removed by concentration and the reaction vessel was sealed under N_2. The solution was vigorously stirred and the progress of the reaction was monitored by TLC-

analysis (35:20:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water). The reaction was generally complete within 2–3 days as judged by the appearance of a major UV- and P_i-positive spot of ADP-Hep (always as two spots with ΔR_f approx 0.2 corresponding to two different salt forms of **26**: ammonium salt with R_f 0.3 and 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt with R_f 0.5). The reaction was stopped by evaporation of pyridine. The diphosphate **26** was isolated using silica gel or anion-exchange chromatography.

3.16.1. Purification using anion-exchange chromatography (Method A). The crude reaction products were dissolved in 10 mL water and the solution was allowed to slowly adsorb on a resin bed of BioRad anion-exchange column (prepacked cartridge 5 mL, HCO_3^--form) connected to an FPLC-system. The column was operated at 0.7 mL/min, fractions (1 mL) were collected. The column was washed first with water (20 mL) and then developed with a linear gradient of TEAB buffer, pH 8 (0.01 → 0.25 M). The eluate was monitored at 280 nm, **26** was eluted at a concentration of 0.15 M TEAB. The fractions containing ADP-Hep were pooled, concentrated to 10 mL vol, the solution was cooled to 0 °C, and the pH was adjusted to 4.5 with Dowex 50 (H^+) resin. The resin was removed by filtration, the total eluate was made neutral by addition of Et_3N, concentrated to 5 mL vol at 25 °C and lyophilised to give **26** as white solid. Yield: 36 mg (91%); R_f 0.37 (35:20:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ +8° (*c* 1.0, water); ^{31}P NMR: δ −10.5 (d, $J_{P,P}$ 25.2 Hz; P_{Rib}), −13.9 (d, P_{Hep}); ^{13}C NMR: δ 174.13, 173.56, 173.42, 172.84, 172.79 (5 C, CO), 153.59 (C-6_{Ade}), 149.85 (C-2_{Ade}), 149.13 (C-4_{Ade}), 141.45 (C-8_{Ade}), 118.93 (C-5_{Ade}), 94.16 (C-1_{Hep}, $J_{1,P}$ 4.9 Hz), 87.87 (C-1_{Rib}), 84.19 (C-4_{Rib}, $J_{4,P}$ 9.2 Hz), 75.18 (C-2_{Rib}), 70.58 (C-3_{Hep}), 69.91 (C-2_{Hep}, $J_{2,P}$ 11.4 Hz), 69.73 (C-5_{Hep}), 68.27 (C-6_{Hep}), 65.55 (C-5_{Rib}, $J_{5,P}$ 4.5 Hz), 65.32 (C-4_{Hep}), 64.09 (C-7_{Hep}), 47.07 (CH_2, Et_3N), 20.52, 20.47, 20.42, 20.36, 20.33 (5 C, CH_3, Ac), 8.62 (CH_3, Et_3N).

3.16.2. Purification procedure using silica gel chromatography (Method B). The reaction mixture containing **26** was fractionated on a silica gel column using stepwise gradient 60:40:1:1 → 50:50:1:1 $CHCl_3$–MeOH–25% aq NH_4OH–water as eluent. Fractions containing ADP-Hep were combined, concentrated to dryness, redissolved in water, cooled to 0 °C, and the pH was adjusted to 4.5 with Dowex 50 (H^+) resin. The resin was removed by filtration, the filtrate was adjusted to pH 6 with Et_3N, concentrated at 25 °C to about 5 mL and lyophilised to furnish **26** (28 mg, 72%).

3.17. Adenosine 5′-(2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-α-D-*manno*-heptopyranosyl)diphosphate (triethylammonium salt) (27)

Compound **27** was prepared from **21** (20 mg, 0.033 mmol) as described for the synthesis of **26** and purified using method A. Yield 28 mg (0.03 mmol, 86%); R_f 0.4 (35:20:2:2 $CHCl_3$–MeOH–25% NH_4OH–water); $[\alpha]_D^{20}$ +4° (*c* 0.5, water); ^{31}P NMR: δ −10.5 (d, $J_{P,P}$ 24.5 Hz; P_{Rib}), −13.5 (d, P_{Hep}).

3.18. Adenosine 5′-(2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-β-D-*manno*-heptopyranosyl)diphosphate (triethylammonium salt) (28)

Compound **28** was prepared from **13** (30 mg, 0.05 mmol) in the same fashion as described for the synthesis of **26**, and isolated using method A. Yield 43 mg (92%); R_f 0.30 (35:20:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ −19° (*c* 0.5, water); ^{31}P NMR: δ −10.6 (d, $J_{P,P}$ 19.0 Hz; P_{Rib}), −13.4 (d, P_{Hep}).

3.19. L-*Glycero*-β-D-*manno*-heptopyranosyl 1,2-cyclophosphate (triethylammonium salt) (29)

A soln of **28** (9 mg, 0.01 mmol) in 7:3:1 MeOH–water–Et_3N (pH 12, 2 mL) was stirred at ambient temperature for 3 h. The reaction mixture was diluted with water (20 mL), concentrated to a vol of 10 mL, and lyophilised to furnish a mixture of monotriethylammonium salts of **29** and AMP. The residue was dissolved in water (5 mL) and packed on a strong anion-exchange resin (5 mL Bio-Rad Econo-pack Q cartridge, HCO_3^- form). A gradient 0.01 M TEAB (pH 7.5) → 0.2 M TEAB was used, the eluate was monitored at 280 nm and checked by TLC for the presence of **29**. Fractions containing **29** were pooled and lyophilised to give, after desalting on a BioGel P-2 column (2 × 50 cm), **29** as a white solid (3 mg, 85%): R_f 0.6 (5:7:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water); ^{1}H NMR: δ 5.53 (dd, 1 H, $J_{1,2}$ 1.9, $J_{1,P}$ 25.2 Hz, H-1), 4.62 (m, 1 H, H-2), 4.01 (ddd, 1 H, H-6), 3.88 (m, 2 H, H-3, H-4), 3.71 (dd, 1 H, $J_{6,7a}$ 7.8, $J_{7a,7b}$ 11.6 Hz, H-7_a), 3.70 (dd, 1 H, $J_{6,7b}$ 6.3 Hz, H-7_b), 3.40 (m, 1 H, H-5), 3.20 (m, 6 H, CH_2, Et), 1.28 (t, 9 H, CH_3, Et); ^{31}P NMR: δ 17.8; ^{13}C NMR: δ 97.9 (C-1), 79.5 (C-2), 74.2 (C-5), 72.5 (C-3), 69.6 (C-6), 66.5 (C-4), 63.5 (C-7).

3.20. L-*Glycero*-D-*manno*-heptopyranose 2-phosphate (ammonium salt) (30)

A suspension of **29** (6.5 mg, 0.022 mmol) and silica gel (0.5 g) in 10:3:3 MeOH–water–25% aq NH_4OH (5 mL) was stirred for 4 h at rt. Silica gel was separated on a filter and the filtrate was concentrated. Chromatography of the residue on silica gel (10:12:1:1 → 10:12:3:3

$CHCl_3$–MeOH–25% aq NH_4OH–water) afforded phosphate **30** (5.5 mg, 85%) as a white solid. R_f 0.2 (5:7:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water); ^1H NMR: δ 5.28 (d, 0.7 H, $J_{1\alpha,2}$ 1.5 Hz, H-1_α), 4.81 (d, 0.3 H, $J_{1\beta,2} < 1$ Hz, 0.3 H, H-1_β), 4.39 (dd, 0.3 H, $J_{2\beta,3}$ 3.0, $J_{2\beta,P}$ 7.7 Hz, 0.3 H, H-2_β), 4.29 (ddd, $J_{2\alpha,3}$ 2.9, $J_{2\alpha,P}$ 8.5 Hz, H-2_α), 4.05–3.90 (m, 1 H, H-$6_{\alpha,\beta}$), 3.89 (t, 1 H, $J_{4,5} = J_{3,4}$ 9.5 Hz, H-$4_{\alpha,\beta}$), 3.82 (dd, 0.7 H, H-3_α), 3.81 (dd, 0.3 H, H-3_β), 3.73 (dd, 0.7 H, $J_{5\alpha,6}$ 1.5 Hz, H-5_α), 3.68 (dd, 0.7 H, $J_{6,7\alpha\text{-a}}$ 6.7 Hz, 0.7 H, H-$7_{\alpha\text{-a}}$), 3.60–3.70 (m, 0.3 H, H-$7_{\beta\text{-a}}$, H-$7_{\beta\text{-b}}$), 3.67 (dd, 0.7 H, $J_{6,7\alpha\text{-b}}$ 5.5, $J_{7\alpha\text{-a},7\alpha\text{-b}}$ 12.0 Hz, H-$7_{\alpha\text{-b}}$), 3.33 (dd, 0.3 H, $J_{5\beta,6}$ 1.6, $J_{5\beta,4}$ 9.8 Hz, H-5_β); ^{31}P NMR: δ 5.1 (β-anomer), 3.5 (α-anomer); ^{13}C NMR (D_2O): δ 95.7 (C-1β), 94.2 (C-1α), 76.0 (C-2β), 75.0 (C-2α), 74.5 (C-5β), 73.0 (C-5α), 69.6 (C-6), 68.0 (C-4), 63.8 (C-7).

3.21. Adenosine 5′-(2,3,4,6,7-penta-*O*-acetyl-D-*glycero*-β-D-*manno*-heptopyranosyl)diphosphate (triethylammonium salt) (31)

Compound **31** was prepared from **23** (15 mg, 0.025 mmol) in the same way as described for the synthesis of **26**, and isolated using method A. Yield: 22 mg (95%); R_f 0.35 (35:20:2:2 $CHCl_3$–MeOH–25% NH_4OH–water); $[\alpha]_D^{20}$ −4° (*c* 1.0, water); ^{31}P NMR: δ −10.6 (d, $J_{P,P}$ 17.8 Hz; P_{Rib}), −13.6 (d, P_{Hep}).

3.22. D-*Glycero*-β-D-*manno*-heptopyranosyl 1,2-cyclophosphate (monotriethylammonium salt) (32)

Compound **32** was obtained from **31** (7 mg, 0.008 mmol) in the same way as described for **29**. Yield 2.0 mg (0.006 mmol, 80%); R_f 0.6 (5:7:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water; ^1H NMR: δ 5.52 (dd, 1 H, $J_{1,2}$ 2.2, $J_{1,P}$ 24.8 Hz, H-1), 4.61 (m, 1 H, H-2), 4.06 (ddd, 1 H, $J_{5,6}$ 2.0 Hz, H-6), 3.84 (m, 2 H, H-3, H-4), 3.81 (dd, 1 H, $J_{6,7a}$ 4.2, $J_{7a,7b}$ 11.9 Hz, H-7_a), 3.74 (dd, 1 H, $J_{6,7b}$ 7.2 Hz, H-7_b), 3.49 (m, 1 H, H-5), 3.20 (m, 6 H, CH_2, Et), 1.28 (t, 9 H, CH_3, Et); ^{31}P NMR: δ 17.5; ^{13}C NMR (D_2O): δ 97.7 (C-1), 79.1 (C-2), 76.3 (C-5), 72.5 (C-6), 72.2 (C-3), 67.4 (C-4), 62.4 (C-7).

3.23. Adenosine 5′-(L-*glycero*-α-D-*manno*-heptopyranosyl) diphosphate (monotriethylammonium salt) (1)

3.23.1. Method A. A soln of **26** (17 mg, 0.018 mmol) in 7:3:1 MeOH–water–Et_3N (pH 12, 2 mL) was stirred at ambient temperature for 3 h. The reaction mixture was diluted with water (20 mL), concentrated to a volume of 10 mL and lyophilised to give **1** (13 mg, 98%) as a white solid. R_f 0.27 (10:12:3:3 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ +4.5° (*c* 1.0, water); ^{31}P NMR: δ −10.7 (d, $J_{P,P}$ 21.1 Hz; P_{Rib}), −13.1 (d, P_{Hep}); $J_{C\text{-}1,H\text{-}1}$ 174 Hz. MALDI-TOF-MS: *m*/*z* 618.31 $[M-H]^-$. Calcd for $C_{17}H_{26}N_5O_{16}P_2^-$, $[M-H]^-$: 618.10. Anal. Calcd for $C_{23}H_{42}N_6O_{16}P_2 \cdot 2.5\ H_2O$ (765.6): C, 36.08; H, 6.19; N, 10.98. Found: C, 36.39; H, 5.88; N, 10.44.

3.23.2. Method B. A solution of **26** (9 mg, 0.01 mmol) in 1:5 25% aq NH_4OH–water (pH 12, 2 mL) was kept at 4 °C for 48 h, the reaction mixture was diluted with water (30 mL), concentrated to 20 mL vol and lyophilised to give ammonium salt of **1** (7 mg, 0.0097 mmol, 98%) as white solid.

3.24. Adenosine 5′-(D-*glycero*-α-D-*manno*-heptopyranosyl) diphosphate (2) (monotriethylammonium salt)

Compound **2** was prepared from **27** (12 mg, 0.013 mmol) as described for **1** using method A to give **2** (9 mg, 0.012 mmol, 98%) as a white solid. R_f 0.32 (10:12:3:3 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ +4.5° (*c* 1.0, water); ^{31}P NMR: δ −10.8 (d, $J_{P,P}$ 20.8 Hz; P_{Rib}), −13.2 (d, P_{Hep}); $J_{C\text{-}1,H\text{-}1}$ 173 Hz. MALDI-TOF-MS: *m*/*z* 618.42 $[M-H]^-$. Calcd for $C_{17}H_{26}N_5O_{16}P_2^-$, $[M-H]^-$: 618.10. Anal. Calcd for $C_{23}H_{42}N_6O_{16}P_2 \cdot 0.5\ H_2O$ (729.6): C, 37.87; H, 5.94; N, 11.52. Found: C, 38.08; H, 6.09; N, 11.07.

3.25. Adenosine 5′-(L-*glycero*-β-D-*manno*-heptopyranosyl)diphosphate (monotriethylammonium salt) (3)

A solution of adenosine 5′-diphospho-heptose **28** (10 mg, 0.011 mmol) in 4:3:0.05 0.1 M aq TEAB–MeOH–Et_3N (3 mL) was kept at −28 °C for 24 h. The reaction mixture was diluted with water (20 mL), the pH was adjusted to 4.7 with Dowex 50 (H^+-form) at 0 °C. The resin was filtered off, washed excessively with water, the combined filtrates were concentrated to 20 mL vol and lyophilised. The mixture contained approx 95% of **3** (R_f 0.6, 3: 2 CH_3CN–0.1 M NH_4HCO_3) and 5% of **29** (R_f 0.7) and AMP (R_f 0.5) (based on ^1H NMR data). The residue was redissolved in 10 mL water and applied on a strong anion-exchange resin (5 mL Bio-Rad Econo-pack Q cartridge, HCO_3^--form). A gradient 0.01 M TEAB → 0.2 M TEAB was used and the eluate was monitored at 280 nm. Fractions containing **3** (R_f 0.7, PEI-Cellulose, 0.25 M TEAB, visualisation under UV-light) and AMP (R_f 0.58) were pooled, and diluted with water to a total vol of 20 mL. The pH of the solution was adjusted to 5 with Dowex 50 (H^+) at 0 °C, the resin was removed by filtration and the filtrate was lyophilised. The residue was purified by gel filtration on Superdex Peptide HR 10/30 column (Pharmacia), connected to FPLC-system, using 0.05 M TEAB as eluent, at 0.2 mL/min elution rate. The fractions were checked for the absence of AMP by TLC (**3**, R_f 0.3 and AMP, R_f 0.45; HPTLC Cellulose F, 5:9:1:3 $CHCl_3$–MeOH–water–0.3 M

TEAB, visualisation under UV-light). Fractions containing pure **3** were pooled, concentrated to 10 mL vol, the soln was cooled to 0 °C and the pH was adjusted to 4.5 with Dowex 50 (H^+) resin. The resin was removed by filtration, the filtrate was adjusted to pH 6 with Et_3N at 0 °C, concentrated to a vol of 5 mL at 25 °C and lyophilised to furnish the monotriethylammonium salt of **3** (7 mg, 94%) as a white solid. R_f 0.28 (Cellulose F, HPTLC, 5:9:1:3 $CHCl_3$–MeOH–water–0.3 M TEAB); $[\alpha]_D^{20}$ −31° (*c* 0.3, water); $J_{C\text{-}1,H\text{-}1}$ 163 Hz; ^{31}P NMR: δ −10.8 (d, $J_{P,P}$ 20.8 Hz; P_{Rib}), −12.8 (d, P_{Hep}). MALDI-TOF-MS (neg. mode): *m/z* 618.63 $[M-H]^-$. Anal. Calcd for $C_{17}H_{26}N_5O_{16}P_2^-$, $[M-H]^-$: 618.10.

3.26. Adenosine 5′-(D-*glycero*-β-D-*manno*-heptopyranosyl)diphosphate (monotriethylammonium salt) (4)

Compound **4** was prepared from **31** (12 mg, 0.013 mmol) as described for **3**. Yield 9 mg (95%); R_f 0.3 (HPTLC Cellulose F, 5:9:1:3 $CHCl_3$–MeOH–water–0.3 M TEAB); $[\alpha]_D^{20}$ −22° (*c* 0.3, water); $J_{C\text{-}1,H\text{-}1}$ 163 Hz; ^{31}P NMR: δ −10.8 (d, $J_{P,P}$ 20.9 Hz; P_{Rib}) and −12.7 (d, P_{Hep}); MALDI-TOF-MS (neg. mode): *m/z* 618.51 $[M-H]^-$. Anal. Calcd for $C_{17}H_{26}N_5O_{16}P_2^-$, $[M-H]^-$: 618.10.

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