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Synthesis of a deoxy analogue of ADP L-*glycero*-D-*manno*-heptose

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Abstract—Starting from L-lyxose, indium-mediated chain elongation with allyl bromide followed by acetylation and oxidative cleavage of the double bond and deprotection afforded 2-deoxy-L-*galacto*-heptose as a 2-deoxy analogue of the bacterial carbohydrate L-*glycero*-D-*manno*-heptose in good overall yield. For the synthesis of the ADP-activated derivative, the 2-deoxy-heptose was O-acetylated and transformed into the anomeric bromide derivative, which was then converted into the acetylated heptopyranosyl phosphate by reaction with tetrabutylammonium phosphate. Deprotection and separation of the anomeric phosphates furnished 2-deoxy-β-L-*galacto*-heptopyranosyl phosphate. Coupling of the acetylated heptosyl phosphate with AMP morpholidate afforded the acetylated ADP derivative in good yield. Removal of the acetyl groups gave the target compound ADP 2-deoxy-L-*galacto*-heptopyranose, which may serve as substrate analogue of bacterial ADP heptosyl transferases for biochemical and crystallographic studies.

Keywords: Heptose; ADP Heptose; Lipopolysaccharide; Sugar nucleotide; Deoxy sugar

1. Introduction

Heptoses of the L-*glycero*-D-*manno*- and D-*glycero*-D-*manno*-configuration are common constituents of the core region and various O-antigenic chains of bacterial lipopolysaccharides and have also been detected in capsular polysaccharides.[1] In the assembly of the core region of enterobacterial LPS, ADP L-*glycero*-β-D-*manno*-heptose serves as the substrate of core heptosyl transferases.[2] In contrast, GDP D-*glycero*-α-D-*manno*-heptopyranose has been identified as the substrate for bacterial glycosyltransferases involved in the biosynthesis of the S-layer glycoprotein glycans in *Aneurinobacillus thermoaerophilus* and *Geobacillus tepidamans*.[3] GDP-heptose has also been proposed as the intermediate for GDP 6-deoxy-D-*manno*-heptose biosynthesis in *Yersinia pseudotuberculosis* and *Burkholderia pseudomallei* O-antigen assembly and for GDP D-*glycero*-α-L-*gulo*-heptose in *Campylobacter jejuni* capsular polysaccharide biosynthesis.[4] In both biosynthetic pathways the anomeric D-*glycero*-D-*manno*-heptopyranose 1-phosphates are the precursors for the respective nucleotidylyl transferases.[5,6] Eventually, ADP D-*glycero*-β-D-*manno*-heptopyranose is converted into the 6-epimer by the action of an epimerase belonging to the short chain dehydrogenase/reductase (SDR) family.[7] GDP D-*glycero*-α-D-*manno*-heptopyranose, ADP D-*glycero*-β-D-*manno*-heptopyranose, and ADP L-*glycero*-β-D-*manno*-heptopyranose have recently been synthesized.[8,9] In contrast to the α-configured GDP heptose, the β-anomeric forms of the ADP heptoses are inherently unstable decomposing into AMP and a 1,2-cyclic phosphodiester.[9] The axially disposed hydroxy group at position 2 gives rise to an intramolecular attack at the anomeric phosphate entity. In order to generate stable ADP heptose derivatives, C-glycosidic analogues have previously been prepared.[10] Furthermore, the crystal structure of the *Escherichia coli* heptosyltransferase WaaC complexed to ADP 2-deoxy-2-fluoro-heptose has recently been obtained with a resolution of 2.4 Å.[11] Extending the series of these ADP heptose analogues we have set out to synthesize the corresponding 2-deoxy-heptose analogues. These compounds are of biochemical interest for exploring the substrate specificities of bacterial heptosyl transferases and

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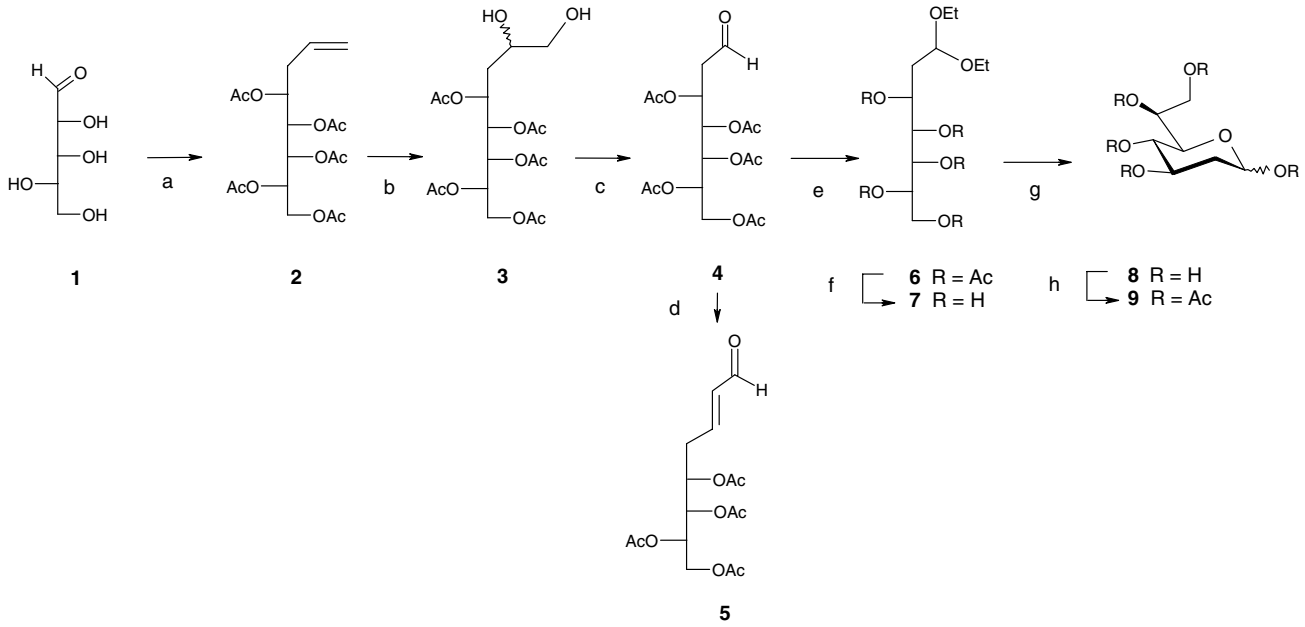
may serve as suitable ligands for cocrystallization experiments for enzymes involved in the biosynthetic pathways of bacterial heptoses.

2. Results and discussion

The indium-mediated allylation reaction of unprotected carbohydrates in aqueous solution has emerged as a versatile approach toward higher-carbon sugars and has been exploited for the synthesis of a variety of heptoses utilizing D-erythrose, D-ribose, L-arabinose, D-xylose, D-lyxose, D-glucose, D-mannose, and D-fructose as educts.[12] Since the addition of allyl species to the carbonyl group should proceed with preferential formation of the *threo*-configured diastereoisomer (with respect to the stereochemistry of the original carbon at position 2), L-lyxose was chosen as starting material to be transformed into the 2-deoxy-derivative of L-*glycero*-D-*manno*-heptopyranose.[13] Thus, **1** was converted into the L-*galacto*-1-octenitol derivative **2** by reaction with indium and allyl bromide in aqueous ethanol for 3 h at room temperature and was then subjected to O-acetylation (Ac_2O–pyridine–DMAP). Compound **2** was isolated as a crystalline solid in 75% yield for the two steps. NMR data measured for the diastereoisomeric mixture indicated a ratio of ~8:1 for the *threo*/*erythro* forms. Catalytic osmylation of the 1-alkene entity in the presence of *N*-methyl-morpholine-*N*-oxide furnished the diastereoisomeric 1,2-diol derivative **3** in 75% yield, which was subsequently reacted with sodium periodate to give the crystalline L-*galacto*-heptose **4** in 83% yield. Treatment of the aldehyde derivative **4** with tetrabutylammonium fluoride in THF afforded the enal derivative **5** in 79% yield.[12b]

Prior to full deprotection, the aldehyde group was masked as diethyl acetal by reaction with triethyl orthoformate catalyzed by sulfuric acid (89% yield). O-Deacetylation of **6** with 1 M methanolic sodium methoxide gave diethyl acetal **7** in 75% yield. Regeneration of the aldehyde group from the diethyl acetal was tested under various conditions. Whereas treatment with acidic cation-exchange resin was not effective, stirring of **7** with 0.05 M hydrochloric acid at elevated temperature for 3 h and neutralization of the soln with anion-exchange resin provided the reducing 2-deoxy-heptose **8** in 87% yield (Scheme 1). ^{1}H NMR spectroscopic data of **8** indicated the presence of α- and β-pyranose and of furanose tautomers in a 1.3:1:0.2 ratio. Removal of the steric interactions of the axial hydroxy group at position 2 thus leads to an increased formation of the β-pyranose and of the furanose forms for the 2-deoxy-heptose. NMR spectra of both L- and D-*glycero*-β-D-*manno*-heptoses display only the presence of ~66% of the α- and ~34% of the β-pyranose similar to the tautomeric equilibrium observed for D-mannose.[14]

For the ensuing introduction of the anomeric phosphate, 2-deoxy-L-*galacto*-heptose **8** was subjected to O-acetylation (Ac_2O/pyridine/DMAP) which afforded the per-O-acetylated 2-deoxy-heptose derivative **9** as an



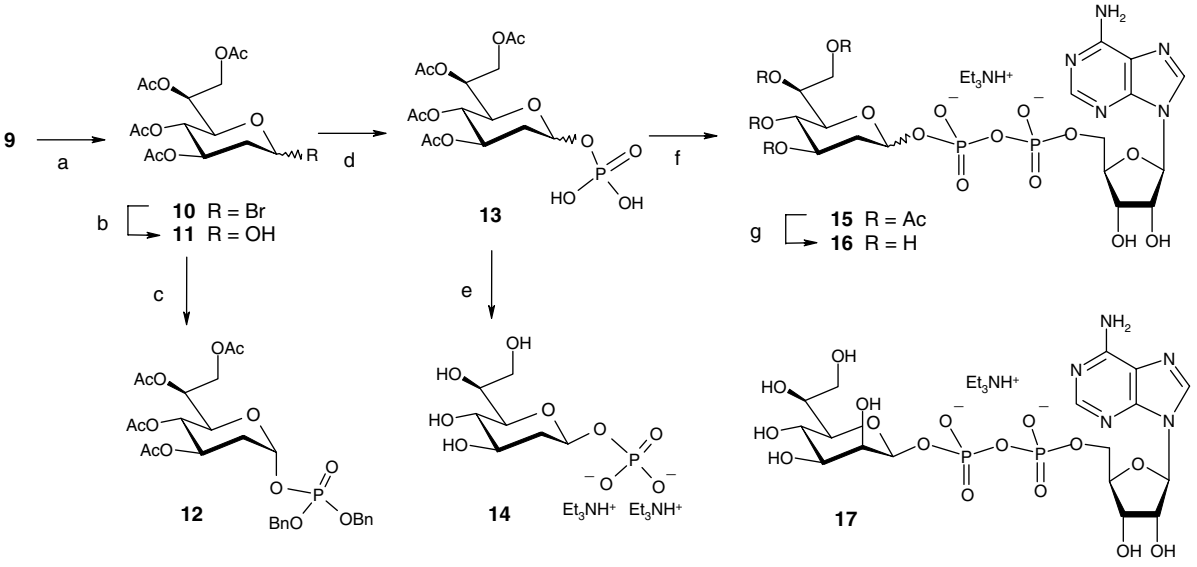
Scheme 1. Reagents and conditions: (a) indium, allyl bromide, aq EtOH, 3 h, rt, then Ac_2O, py., DMAP, 18 h, rt, 75% for **2**; (b) OsO_4, NMO, 2:1 THF–water, 16 h, rt, 75% for **3**; (c) $NaIO_4$, 2:1 THF–water, 3 h, rt, 83% for **4**; (d) Bu_4NF, CH_2Cl_2, 16 h, rt, 79% for **5**; (e) $(EtO)_3CH$, EtOH, cat. H_2SO_4, 2 h, rt, 89% for **6**; (f) 1 M NaOMe, MeOH, 18 h, rt, 75% for **7**; (g) 0.05 M aq HCl, 80 °C, 3 h, 87% for **8**; (h) Ac_2O, py., DMAP, 16 h, rt, 87% for **9**.

anomeric mixture in 87% yield. Attempts to remove the anomeric acetate from **9** using hydrazine acetate, Hünig-Base, basic alumina, or sodium methoxide met with difficulties and led to the formation of several deacetylated products. Best results were obtained in a two-step procedure by converting the anomeric acetyl derivative **9** into 2-deoxy-heptosyl bromide **10** by treatment with titanium tetrabromide followed by hydrolysis in aqueous acetone. Thus, compound **11** was obtained in 76% yield (for two steps).

The introduction of the phosphate at the anomeric position was first attempted using fully protected activated phosphorylating reagents, which were supposed to give rise to an α/β mixture of anomeric phosphotriesters of 2-deoxyheptose. This approach usually allows a facile separation of the anomeric mixture of phosphotriesters to individual anomers by column chromatography. The disadvantage of this strategy was shown to reside in the expected instability and susceptibility to the hydrolysis of β-phosphotriesters of 2-deoxysugars.[15] Indeed, in the case of 2-deoxy-heptose, the β-anomeric phosphotriester derivatives proved to be extremely unstable due to their susceptibility toward hydrolysis. Thus, treatment of the reducing heptose **11** with bis(benzyloxy)-*N*,*N*-diisopropylaminophosphine furnished an anomeric mixture of phosphite-triesters (α/β ratio 1:1 on the basis of integration curves in ^{31}P NMR for signals at 140.68 and 140.52 ppm). Oxidation in situ with *tert*-butylhydroperoxide and subsequent aqueous work-up and isolation of the anomeric phosphotriesters by column chromatography on silica gel furnished only a low amount of the α-anomeric dibenzyl phosphate **12** (15%), the remaining isolated material corresponded to compound **11** formed by hydrolysis of the anomeric phosphotriester derivatives (Scheme 2).

Similarly, attempted direct acylation of compound **11** under various conditions using diphenyl chlorophosphate resulted in the isolation of starting material only. Hence, the direct conversion into the deprotected phosphate derivative **13** was investigated.[16] Reaction of the bromide **10** with tetrabutylammonium phosphate in acetonitrile containing DIPEA afforded a mixture of the anomeric phosphates **13** in 50% yield with the β-anomeric phosphate being the major component (α/β ratio ~1:3.6 on the basis of integration curves of ^{31}P NMR signals δ: 0.78 and 1.42). Deprotection of the acetyl groups of **13** with 7:3:0.5 MeOH–water–triethylamine provided the triethylammonium salts of the anomeric phosphates. In contrast to the acetylated precursors, the anomeric mixture could be separated by silica gel chromatography to afford the β-anomeric 2-deoxy-L-*galacto*-heptose phosphate **14** in 60% isolated yield. Proof of the anomeric configuration was based on the upfield shift of the proton signal attributable to H-5 at 3.24 ppm and on the value of the optical rotation of **14**, which compares favorably to that of the parent L-*glycero*-β-D-*manno*-heptopyranosyl phosphate.[9]

O-Acetylated heptosyl phosphates **13** were then subjected to the coupling with dicyclohexylcarboxamidinium salt of AMP-morpholidate in pyridine under strictly anhydrous conditions. The reaction was finished within 24 h, thin layer chromatography showed com-



Scheme 2. Reagents and conditions: (a) $TiBr_4$, CH_2Cl_2, 4 °C, 18 h, 90% for **10**; (b) 2:1 acetone–water, 3 h, rt, 84% for **11**; (c) bis(benzyloxy)-*N*,*N*-diisopropylaminophosphine, 1*H*-tetrazole (3.5% in CH_3CN), CH_2Cl_2, 0.5 h, rt then ^{t}BuOOH, −20 °C→rt, 1.5 h, 15% for **12**; (d) TBAP, MeCN, DIPEA, −30 °C→rt, 4 h, 50% for **13**; (e) 7:3:0.5 MeOH–water–Et_3N, 4 h, rt, 60% for **14**; (f) 4′-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt of AMP, pyridine, 48 h, rt, BioRad (Q) anion-exchange column (1 × 10 cm, HCO_3^--form), elution 0.025→0.15 M TEAB, 71% for **15**; (g) 7:3:1 MeOH–water–Et_3N, pH 10, 6 h, rt, 91% for **16**.

plete conversion of the starting acetylated anomeric phosphates into heptose diphosphates **15**, which were isolated as anomeric mixture using strong anion-exchange resin (bicarbonate from). Deprotection with aq MeOH/Et_3N (pH 10) for 6 h at room temperature followed by neutralization with Dowex H^+-resin afforded the target ADP compound **16** as an anomeric mixture (α/β ratio ~1:3.4) in 64% yield (for two steps). The MS and NMR data are in full agreement with the structural assignments; the NMR characteristics of the major anomer of **16** compare favorably with the data of the previously reported compound **17** (Table 1).[9] ^{31}P NMR data showed two doublets at −10.30 and −12.52 ppm confirming the presence of the diphosphate unit. In contrast to the ADP L-*glycero*-β-D-*manno*-heptopyranose the corresponding 2-deoxy-heptose analogue was stable upon deacetylation conditions (aqueous methanolic Et_3N solution, pH 10 at room temperature for 6 h); upon storage as a solution in water (pH range of 5.5–7.0) for 2 days at room temperature ADP-2-deoxyheptose was shown to hydrolyze slowly into adenosine-diphosphate and free 2-deoxy-heptose.

In conclusion, the indium-promoted chain elongation of L-lyxose allows for a straightforward preparation of 2-deoxy-heptose, which may be further elaborated into the corresponding 1-phosphate and ADP-derivatives with preferential formation of the β-anomers as required for substrates involved in the bacterial ADP-heptose biosynthetic pathway.

Table 1. ^{13}C NMR data of ADP-heptoses in D_2O[a]

		16	**17**[9]
C-1	Hep*p*	96.27	96.51
		$^2J_{1,P}$ 4.3	$^2J_{1,P}$ 4.8
	Rib*f*	87.54	88.26
C-2	Hep*p*	39.85	71.37
		$^3J_{2,P}$ 7.1	$^3J_{2,P}$ 7.1
	Rib*f*	75.07	75.23
	Ade	153.65	152.20
C-3	Hep*p*	71.27[b]	73.34
	Rib*f*	71.14[b]	70.99
C-4	Hep*p*	70.61	66.28
	Rib*f*	84.74	84.80
		$^3J_{4,P}$ 8.8	$^3J_{4,P}$ 9.0
	Ade	149.89	149.19
C-5	Hep*p*	75.56	75.66
	Rib*f*	66.08	65.82
		$^2J_{5,P}$ 5.1	$^2J_{5,P}$ 5.8
	Ade	119.34	119.13
C-6	Hep*p*	69.19	69.31
	Ade	156.37	156.55
C-7	Hep*p*	63.07	62.98
C-8	Ade	140.65	142.34

[a] Recorded at pD 6.5.
[b] Assignments may be reversed.

3. Experimental

3.1. General

Concentration of solutions was performed under diminished pressure at temperatures <30 °C. Triethylamine, CH_2Cl_2, dry pyridine were purchased from E. Merck, and were dried by refluxing with CaH_2 (5 g per 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 per L) for 16 h at 20 °C, distilled under diminished pressure, and stored over activated molecular sieves 0.3 nm. Triethylammonium bicarbonate (TEAB) buffer was purchased from Aldrich. Column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck). Analytical TLC was performed using silica gel 60 F_{254} HPTLC plates with 2.5 cm concentration zone (E. Merck). Spots were detected by treatment with anisaldehyde–H_2SO_4, adenosine-containing compounds were detected by examination under UV light. Anion-exchange chromatography was performed on BioRad Macro-Prep High Q Support anion-exchange resin. Melting points were determined on a Kofler hot stage microscope and are uncorrected. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. NMR spectra were recorded at 297 K in D_2O and $CDCl_3$ with a Bruker DPX 300 or Avance 400 spectrometer (^1H at 300.13 MHz, ^{13}C at 75.47 MHz, and ^{31}P at 121.50 MHz or ^1H at 400.13 MHz, ^{13}C at 100.61 MHz, and ^{31}P at 161.98 MHz, respectively) using standard Bruker NMR software. ^1H NMR spectra were referenced to tetramethylsilane or 2,2-dimethyl-2-silapentane-5-sulfonic acid. ^{13}C NMR spectra were referenced to chloroform for solutions in $CDCl_3$ (δ 77.00) or dioxane (δ 67.40) for solutions in D_2O. ^{31}P NMR spectra were referenced externally to 85% aq H_3PO_4 (δ 0.0). ESIMS data were obtained on a Waters Micromass Q-TOF Ultima Global instrument. Elemental analyses were provided by Dr. J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, Universität Wien.

3.2. 4,5,6,7,8-Penta-*O*-acetyl-1,2,3-trideoxy-L-*galacto*-oct-1-enitol (2)

To a soln of L-lyxose (1.5 g, 10 mmol) in 4:1 EtOH–water (100 mL), 1.15 g indium powder (10 mmol), and allyl bromide (3.64 mL, 42 mmol) were added. The suspension was sonicated for 3 h at rt, the solvents were removed, the residue was dried under diminished pressure, and redissolved in 1:1 pyridine–Ac_2O (40 mL). After addition of DMAP (10 mg, 0.082 mmol) the mixture was stirred for 18 h at rt. MeOH (1.0 mL) was added at 0 °C and the soln was stirred for 15 min, the

solvents were removed, the residue was partitioned between water (80 mL) and EtOAc (200 mL). The aq phase was extracted with EtOAc (3 × 50 mL), the combined organic phases were dried ($MgSO_4$), and concentrated. The residue was crystallized from *n*-hexane to give *threo* isomer **2** (3.6 g, 75%) as a crystalline white solid; mp 152–154 °C; R_f 0.32 (7:3, *n*-hexane–EtOAc); $[\alpha]_D^{20}$ −93 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.70 (dddd, 1H, $J_{1a,2}$ 6.3, $J_{1b,2}$ 10.2, $J_{2,3a}$ 8.0 Hz, H-2), 5.34–5.26 (m, 3H, H-5, H-6, H-7), 5.12–5.04 (m, 3H, H-1a, H-1b, H-4), 4.29 (dd, 1H, $J_{8a,8b}$ 11.6, $J_{7,8a}$ 4.9 Hz, H-8a), 3.84 (dd, 1H, $J_{7,8b}$ 7.5 Hz, H-8b), 2.33–2.15 (m, 2H, H-3a, H-3b), 2.12, 2.10, 2.08, 2.05, and 2.02 (5s, each 3H, 5 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.83, 170.80, 170.71, 170.32, 170.22 (5C, CH_3CO), 132.92 (C-1), 118.88 (C-2), 69.78 (C-4), 69.54 (C-5), 68.27 and 68.21 (C-6, C-7), 62.72 (C-8), 35.98 (C-3), 21.26, 21.14, 21.08, 21.04, and 21.02 (5C, CH_3CO); Anal. Calcd for $C_{18}H_{26}O_{10}$: C, 53.73; H, 6.51. Found: C, 53.51; H, 6.46.

3.3. 4,5,6,7,8-Penta-*O*-acetyl-3-deoxy-L-*glycero*-D-*manno*/*gluco*-octitol (3)

A soln of **2** (3.25 g, 8.07 mmol) and NMO (2.18 g, 16.14 mmol) in 2:1 THF–water (45 mL) was stirred at rt for 15 min. Then osmium tetraoxide (2 mL, 2% in water) was transferred into the flask and the mixture was stirred 16 h at rt. The mixture was diluted with EtOAc (100 mL), washed subsequently with aq 45% sodium bisulfite (3 × 30 mL), 5 M aq HCl (3 × 50 mL), water (3 × 50 mL), and satd aq $NaHCO_3$ (3 × 50 mL). The organic phase was dried ($MgSO_4$) and concentrated. The residue was crystallized from *n*-hexane–EtOAc to give **3** (2.64 g, 75%) as colorless crystals; mp 132–133 °C; R_f 0.19 (1:4, toluene–EtOAc); $[\alpha]_D^{20}$ −0.4 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.40–5.18 (m, 4H, H-4, H-5, H-6, H-7), 4.28 (dd, 1H, $J_{7,8a}$ 4.9, $J_{8a,8b}$ 11.8 Hz, H-8a), 3.84 (dd, 1H, $J_{7,8b}$ 7.6 Hz, H-8b), 3.66–3.41 (m, 3H, H-1a, H-1b, H-2), 3.33 (br s, 1H, OH), 2.13, 2.12, 2.11, 2.08, and 2.02 (5s, each 3H, 5 × CH_3CO), 1.68–1.40 (m, 2H, H-3a, H-3b); ^{13}C NMR ($CDCl_3$): δ 172.77, 170.43, 170.27, 170.01, 169.83 (5C, CH_3CO), 70.04, 67.85, 67.78, 67.65, 67.61 (5C, C-2, C-4, C-5, C-6, C-7), 66.27 (C-1), 62.13 (C-8), 34.88 (C-3), 21.04, 20.90, 20.67, and 20.56 (5C, CH_3CO); Anal. Calcd for $C_{18}H_{28}O_{12}$: C, 49.54; H, 6.47. Found: C, 49.41; H, 6.38.

3.4. 3,4,5,6,7-Penta-*O*-acetyl-2-deoxy-L-*galacto*-heptose (4)

To a vigorously stirred soln of **3** (1.32 g, 3.03 mmol) in THF–water (2:1, 45 mL), $NaIO_4$ (1.3 g, 6.06 mmol) was added over 1 h at rt. Stirring was continued for 2 h, the mixture was diluted with EtOAc (100 mL) and washed with satd aq $NaHCO_3$ (2 × 50 mL) and water (50 mL). The organic phase was dried ($MgSO_4$) and concentrated, the residue was crystallized from *n*-hexane to give **4** (1.02 g, 83%) as colorless crystals; mp 152–154 °C; R_f 0.44 (3:2, *n*-hexane–EtOAc); $[\alpha]_D^{20}$ −34 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 9.65 (d, 1H, $J_{1,2b}$ 2.1 Hz, H-1), 5.49 (dt, 1H, $J_{2,3}$ 6.5, $J_{3,4}$ 1.8 Hz, H-3), 5.37 (dd, 1H, $J_{5,6}$ 1.8 Hz, H-5), 5.33 (ddd, 1H, H-6), 5.27 (dd, 1H, $J_{4,5}$ 10.0 Hz, H-4), 4.29 (dd, 1H, $J_{6,7a}$ 4.9, $J_{7a,7b}$ 11.8 Hz, H-7a), 3.84 (dd, 1H, $J_{6,7b}$ 7.6 Hz, H-7b), 2.64 (dd, 1H, $J_{2a,2b}$ 17.0 Hz, H-2a), 2.55 (ddd, 1H, H-2b), 2.13 (s, 3H), 2.12 (s, 3H), 2.07 (s, 6H), and 2.02 (s, 3H, 5 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 197.62 (C-1), 170.44, 170.33, 170.23, 170.17, 169.82 (5C, CH_3CO), 69.23 (C-4), 67.72 and 67.65 (C-5, C-6), 65.42 (C-3), 62.23 (C-7), 44.73 (C-2), 20.76, 20.68, and 20.62 (5C, CH_3CO); Anal. Calcd for $C_{17}H_{24}O_{11}$: C, 50.49; H, 5.98. Found: C, 50.35; H, 5.92.

3.5. 2-(*E*)-4,5,6,7-Tetra-*O*-acetyl-2,3-dideoxy-L-*lyxo*-hept-2-enose (5)

A soln of **4** (1.54 g, 3.8 mmol) in CH_2Cl_2 (20 mL) and tetrabutylammonium fluoride trihydrate (TBAF) (300 mg, 0.95 mmol) was stirred for 16 h at rt. The mixture was concentrated, the residue was purified by column chromatography (1:1 toluene–EtOAc) to give **5** as a syrup. Yield: 1.03 g (79%); R_f 0.6 (1:1, toluene–EtOAc); $[\alpha]_D^{20}$ −73.5 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 9.56 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 6.63 (dd, 1H, $J_{2,3}$ 16.0, $J_{3,4}$ 6.2 Hz, H-3), 6.26 (ddd, 1H, $J_{2,4}$ 1.0 Hz, H-2), 5.60 (dt, 1H, $J_{4,5}$ 8.0 Hz, H-4), 5.43 (ddd, 1H, $J_{5,6}$ 3.5, $J_{6,7a}$ 5.5, $J_{6,7b}$ 7.0 Hz, H-6), 5.36 (dd, 1H, H-5), 4.26 (dd, 1H, $J_{7a,7b}$ 11.8 Hz, H-7a), 3.99 (dd, 1H, H-7b), 2.11, 2.10, and 2.05 (3s, 12H, 4 × CH_3CO); ^{13}C NMR ($CDCl_3$): δ 192.27 (C-1), 170.33, 169.88, 169.65, 169.22 (4C, CH_3CO), 148.02 (C-3), 134.42 (C-2), 70.41 (C-5), 69.35 (C-4), 67.89 (C-6), 61.62 (C-7), 20.65, 20.61, and 20.58 (4C, CH_3CO); Anal. Calcd for $C_{15}H_{20}O_9$: C, 52.32; H, 5.85. Found: C, 52.38; H, 5.55.

3.6. 3,4,5,6,7-Penta-*O*-acetyl-2-deoxy-L-*galacto*-heptose diethyl acetal (6)

A soln of **4** (1.0 g, 2.47 mmol) in dry EtOH (37 mL), triethyl orthoformate (3.7 mL, 0.048 mmol), and a catalytic amount (three drops) of sulfuric acid was stirred for 2 h at rt. The mixture was neutralized by the addition of solid $NaHCO_3$, filtered, and the solvent was removed under diminished pressure. The residue was crystallized from *n*-hexane to give **6** (1.05 g, 89%) as colorless crystals; mp 112–113 °C; R_f 0.44 (7:3, *n*-hexane–EtOAc); $[\alpha]_D^{20}$ +8.5 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.32–5.21 (m, 4H, H-3, H-4, H-5, H-6), 4.50 (t, 1H, $J_{1,2}$ 5.8 Hz, H-1), 4.28 (dd, 1H, $J_{6,7a}$ 4.9, $J_{7a,7b}$ 11.8 Hz, H-7a), 3.83 (dd, 1H, $J_{6,7b}$ 7.6 Hz, H-7b), 3.70-3.39 (m, 4H, 2 × CH_2CH_3), 2.11, 2.10, 2.08, 2.06, and

2.02 (5s, each 3H, 5 × CH_3CO), 1.85–1.69 (m, 2H, H-2a, H-2b), 1.19, 1.17 (t, 6H, 2 × CH_2CH_3); ^{13}C NMR ($CDCl_3$): δ 170.49, 170.40, 170.35, 170.00, 169.91 (5C, CH_3CO), 100.08 (C-1), 69.82, 67.80, 67.30 (4C, C-3, C-4, C-5, C-6), 62.50 and 62.37 (2C, C-7, CH_3CH_2), 60.51 (CH_3CH_2), 35.41 (C-2), 20.96, 20.72, 20.64, 20.60 (5C, CH_3CO), 15.24 and 15.21 (2C, $2CH_3CH_2$); Anal. Calcd for $C_{21}H_{34}O_{12}$: C, 52.71; H, 7.16. Found: C, 52.71; H, 6.97.

3.7. 2-Deoxy-L-*galacto*-heptose diethyl acetal (7)

To a soln of **6** (1.42 g, 2.97 mmol) in dry MeOH (20 mL) a 1 M soln of NaOMe in MeOH (0.46 mL) was added. The mixture was stirred for 18 h at rt and then neutralized with AG 50W-X8 resin (H^+-form). The resin was removed by filtration, the solvent was concentrated, the residue was crystallized from *n*-hexane to give **7** (600 mg, 75%); mp 105–107 °C; R_f 0.54 (3:1 EtOAc–MeOH); $[\alpha]_D^{20}$ +10.8 (*c* 0.4, H_2O); ^1H NMR (CD_3OD): δ 4.82 (dd, 1H, $J_{1,2a}$ 4.0, $J_{1,2b}$ 7.9 Hz, H-1), 4.04 (ddd, 1H, $J_{2a,3}$ 9.8, $J_{2b,3}$ 3.8, $J_{3,4}$ 1.4 Hz, H-3), 3.96 (ddd, 1H, $J_{5,6}$ 1.5, $J_{6,7a}$ 5.9, $J_{6,7b}$ 7.3 Hz, H-6), 3.82–3.73 (m, 2H, H-7a, H-7b), 3.69–3.59 (m, 5H, H-5, 2 × CH_2CH_3), 3.51 (dd, 1H, $J_{4,5}$ 9.3 Hz, H-4), 1.96 (ddd, 1H, $J_{2a,2b}$ 14.4 Hz, H-2a), 1.80 (ddd, 1H, H-2b), 1.21, 1.20 (t, 6H, 2 × CH_2CH_3); ^{13}C NMR (CD_3OD): δ 102.17 (C-1), 72.83 (C-4), 71.12 (C-6), 70.33 (C-5), 67.21 (C-3), 64.10 (C-7), 64.06, 63.58 (2C, $2CH_3CH_2$), 38.41 (C-2), 15.16 and 15.15 (2C, $2CH_3CH_2$); Anal. Calcd for $C_{11}H_{24}O_7$: C, 49.24; H, 9.02. Found: C, 48.94; H, 8.72.

3.8. 2-Deoxy-L-*galacto*-heptose (8)

A soln of **7** (300 mg, 1.12 mmol) in 0.05 M HCl (9 mL) was heated at 80 °C for 3 h. After cooling to rt, the reaction mixture was neutralized by the addition of anion-exchange resin (BioRad AG 501-X8), the resin was removed on the filter, the filtrate was concentrated to give **8** as a syrup. Yield: 220 mg (87%); R_f 0.29 (7:3 EtOAc–MeOH); $[\alpha]_D^{20}$ +31.8 (*c* 1.7, H_2O, after 24 h); ^1H NMR (CD_3OD) of the α-pyranose form: δ 5.34 (br d, 1H, $J_{1,2b}$ 3.7 Hz, H-1), 4.04 (dt, 1H, $J_{5,6}$ 1.3, $J_{6,7b}$ 7.1, $J_{6,7a}$ 6.0 Hz, H-6), 3.91 (ddd, 1H, $J_{2a,3}$ 5.1, $J_{2b,3}$ 11.9 Hz, H-3), 3.74 (dd, 1H, $J_{4,5}$ 9.6 Hz, H-5), 3.71–3.65 (m, 2H, H-7a, H-7b), 3.50 (t, 1H, $J_{3,4}$ 9.2 Hz, H-4), 2.10 (ddd, 1H, $J_{1,2a}$ 1.3, $J_{2a,2b}$ 13.3 Hz, H-2a), and 1.67 (ddd, 1H, H-2b); ^{13}C NMR (CD_3OD): δ 91.77 (C-1), 71.11, 71.09 (C-4, C-5), 68.90 (C-6), 68.62 (C-3), 63.44 (C-7), 37.47 (C-2).

^1H NMR (CD_3OD) of the β-isomer: δ 4.88 (dd, 1H, $J_{1,2a}$ 2.0, $J_{1,2b}$ 9.8 Hz, H-1), 3.98 (dt, 1H, $J_{5,6}$ 1.6, $J_{6,7a} \sim J_{6,7b}$ 6.5 Hz, H-6), 3.70–3.65 (m, 2H, H-7a, H-7b), 3.63 (ddd, 1H, $J_{2a,3}$ 5.0, $J_{3,4}$ 7.2 Hz, H-3), 3.46 (dd, 1H, $J_{4,5}$ 8.8 Hz, H-4), 3.32 (dd, 1H, H-5), 2.24 (ddd, 1H, $J_{2a,2b}$ 12.3 Hz, H-2a), and 1.44 (ddd, 1H, $J_{2b,3}$ 9.8 Hz, H-2b); ^{13}C NMR (CD_3OD): δ 94.03 (C-1), 74.87 (C-5), 71.10 (C-3), 70.53 (C-4), 68.93 (C-6), 63.11 (C-7), and 39.81 (C-2); Anal. Calcd for $C_7H_{14}O_6 \cdot 0.5H_2O$: C, 41.38; H, 7.27. Found: C, 41.61; H, 7.33.

3.9. 1,3,4,6,7-Penta-*O*-acetyl-2-deoxy-L-*galacto*-heptopyranose (9)

A soln of **8** (170 mg, 0.875 mmol), DMAP (10 mg, 0.082 mmol) in dry pyridine (4 mL), and Ac_2O (1 mL) was stirred for 16 h at rt. MeOH (2 mL) was added at 0 °C, and the soln was concentrated to dryness. The residue was purified on silica gel (1:1 hexane–EtOAc) to furnish **9** as syrup. Yield: 307 mg (87%); R_f 0.58 (1:1, toluene–EtOAc); $[\alpha]_D^{20}$ +17.5 (*c* 0.5, $CHCl_3$). ^1H NMR of α-pyranose ($CDCl_3$): δ 6.25 (d, 1H, $J_{1,2b}$ 3.7 Hz, H-1), 5.34–5.25 (m, 2H, H-3, H-6), 5.04 (t, 1H, $J_{4,5} \sim J_{3,4}$ 10.0 Hz, H-4), 4.25 (dd, 1H, $J_{6,7a}$ 5.0, $J_{7a,7b}$ 11.8 Hz, H-7a), 4.17–4.08 (m, 2H, H-5, H-7b), 2.27 (ddd, 1H, $J_{1,2a}$ 1.3, $J_{2a,3}$ 5.2, $J_{2a,2b}$ 13.5 Hz, H-2a), 2.13, 2.11, 2.04, and 2.03 (4s, 15H, 5 × CH_3CO), 1.99 (ddd, 1H, $J_{2b,3}$ 3.7 Hz, H-2b); ^{13}C NMR ($CDCl_3$): δ 170.46, 170.27, 170.21, 169.72, 168.64 (5C, CH_3CO), 90.79 (C-1), 70.44 (C-4), 67.91 (C-5), 67.46 (C-3), 66.90 (C-6), 62.39 (C-7), 33.88 (C-2), 20.88, 20.85, 20.70, 20.65, and 20.59 (5C, CH_3CO).

^1H NMR of β-pyranose ($CDCl_3$): δ 5.71 (dd, 1H, $J_{1,2a}$ 2.2, $J_{1,2b}$ 10.0 Hz, H-1), 5.34–5.25 (m, 1H, H-6), 5.02 (t, 1H, $J_{4,5} \sim J_{4,3}$ 9.5 Hz, H-4), 4.31 (dd, 1H, $J_{6,7a}$ 5.0, $J_{7a,7b}$ 11.8 Hz, H-7a), 4.17–4.08 (m, 2H, H-3, H-7b), 3.77 (dd, 1H, $J_{5,6}$ 2.2 Hz, H-5), 2.50 (ddd, 1H, $J_{2a,3}$ 4.8, $J_{2a,2b}$ 12.3 Hz, H-2a), 2.13, 2.11, 2.04, and 2.03 (4s, 15H, 5 × CH_3CO), 1.95–1.80 (m, 1H, H-2b); ^{13}C NMR ($CDCl_3$): δ 170.46, 170.27, 170.21, 169.72, 168.64 (5C, CH_3CO), 91.46 (C-1), 73.12 (C-5), 70.35 (C-3), 68.66 (C-4), 66.78 (C-6), 62.28 (C-7), 34.77 (C-2), 20.88, 20.85, 20.70, 20.65, and 20.59 (5C, CH_3CO); Anal. Calcd for $C_{17}H_{24}O_{11}$: C, 50.49; H, 5.98. Found: C, 50.28; H, 6.01.

3.10. 3,4,6,7-Tetra-*O*-acetyl-2-deoxy-L-*galacto*-heptopyranosyl bromide (10)

A soln of **9** (95 mg, 0.235 mmol) and $TiBr_4$ (200 mg, 0.54 mmol) in dry CH_2Cl_2 (5 mL) was stirred for 18 h at 4 °C. The mixture was diluted with $CHCl_3$ (50 mL), washed with ice-cold satd aq $NaHCO_3$ (2 × 30), and water (20 mL). The organic phase was dried ($MgSO_4$) and concentrated to give crude **10** as a syrup (90 mg, 90%). ^1H NMR ($CDCl_3$): δ 6.57 (dd, 1H, $J_{1,2b}$ 4.0 Hz, H-1), 5.48 (ddd, 1H, $J_{2a,3}$ 5.2, $J_{2b,3}$ 11.5, $J_{3,4}$ 10.0 Hz, H-3), 5.35 (ddd, 1H, $J_{5,6}$ 2.2, $J_{6,7a}$ 5.8, $J_{6,7b}$ 7.8 Hz, H-6), 5.07 (t, 1H, $J_{4,5} \sim J_{3,4}$ 10.0 Hz, H-4), 4.34 (dd, 1H, H-5), 4.22 (dd, 1H, $J_{7a,7b}$ 11.5 Hz, H-7a), 4.08 (dd, 1H,

H-7b), 2.64 (ddd, 1H, $J_{1,2a}$ 1.0, $J_{2a,2b}$ 14.0 Hz, H-2a), 2.31 (ddd, 1H, H-2b), 2.10 (s, 3H), 2.05 (s, 6H), and 2.02 (s, 3H, $4\times CH_3CO$).

3.11. 3,4,6,7-Tetra-*O*-acetyl-2-deoxy-L-*galacto*-heptopyranose (11)

The crude **10** (208 mg, 0.49 mmol) was dissolved in acetone–H_2O (2:1, 30 mL) and the soln was stirred for 3 h at rt. The mixture was diluted with EtOAc (100 mL), the organic phase was washed with satd aq $NaHCO_3$ (2×30 mL) and water (30 mL), dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography (1:1 toluene–EtOAc) to give **11** as a syrup (158 mg, 84%); $[\alpha]_D^{20}$ +24.7 (*c* 4.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) for α-isomer: δ 5.44 (dt, 1H, $J_{1,2b}$ 3.2 Hz, H-1), 5.36 (ddd, 1H, $J_{2a,3}$ 2.0, $J_{2b,3}$ 5.3, $J_{3,4}$ 9.6 Hz, H-3), 5.30 (ddd, 1H, $J_{5,6}$ 2.0, $J_{6,7a}$ 5.1, $J_{6,7b}$ 7.3 Hz, H-6), 4.98 (t, 1H, $J_{4,5}$ 9.6 Hz, H-4), 4.35 (dd, 1H, $J_{7a,7b}$ 11.8 Hz, H-7a), 4.26 (dd, 1H, H-5), 4.12 (dd, 1H, H-7b), 3.44 (dd, 1H, –OH), 2.28 (ddd, $J_{1,2a}$ 1.0, $J_{2a,2b}$ 13.0 Hz, H-2a), 2.13, 2.06, 2.03, 2.02 (4s, each 3H, $4\times CH_3CO$), 1.83 (ddd, 1H, H-2b); ^{13}C NMR ($CDCl_3$): δ 171.14, 170.38, 170.26, 170.00 (4C, CH_3CO), 91.69 (C-1), 68.94 (C-3), 68.54 (C-4), 67.87 (C-5), 67.19 (C-6), 62.58 (C-7), 35.14 (C-2), 20.87, 20.80, 20.74, and 20.65 (4C, CH_3CO). ^{1}H NMR ($CDCl_3$) for β-isomer: δ 5.32–5.25 (m, 1H, H-6), 4.99 (t, 1H, $J_{4,5}$ 9.5 Hz, H-4), 4.91 (m, 1H, H-1), 4.35 (dd, 1H, $J_{6,7a}$ 5.0, $J_{7a,7b}$ 11.8 Hz, H-7a), 4.17 (dd, 1H, $J_{6,7b}$ 7.8 Hz, H-7b), 3.90 (dd, 1H, H-3), 3.68 (dd, 1H, $J_{5,6}$ 2.2 Hz, H-5), 2.41 (ddd, $J_{1,2a}$ 2.2, $J_{2a,3}$ 4.3, $J_{2a,2b}$ 12.5 Hz, H-2a), 2.12, 2.06, 2.03, 2.02 (4s, each 3H, $4\times CH_3CO$), 1.71 (m, 1H, H-2b); ^{13}C NMR ($CDCl_3$): δ 171.14, 170.38, 170.31, 170.26 (4C, CH_3CO), 94.37 (C-1), 72.27 (C-5), 70.50 (C-3), 67.79 (C-4), 66.81 (C-6), 62.58 (C-7), 37.44 (C-2), 20.87, 20.80, 20.74, and 20.65 (4C, CH_3CO); Anal. Calcd for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12. Found: C, 50.04; H, 6.33.

3.12. Dibenzyl (3,4,6,7-tetra-*O*-acetyl-2-deoxy-α-L-*galacto*-heptopyranosyl) phosphate (12)

2-Deoxy-heptose tetraacetate **11** (60 mg, 0.166 mmol) was dried by repeated evaporations with dry toluene (3×5 mL) and then under diminished pressure overnight. Then the flask was charged with dry CH_2Cl_2 (5 mL), bis(benzyloxy)-*N*,*N*-diisopropylaminophosphine (167 μL, 0.497 mmol), and a soln of 1*H*-tetrazole (40.7 mg, 0.581 mmol) in dry CH_3CN (1.3 mL) were added and the mixture was stirred at room temperature for 30 min under Ar. Monitoring of the reaction by TLC showed the formation of the 1:1 α/β mixture of the intermediate phosphite triesters (R_f 0.52 and 0.49 in 97:3 CH_2Cl_2–acetone; ^{31}P MMR: δ 140.68 and 140.52). The mixture was cooled to −20 °C and a soln of *tert*-BuOOH (45 μL of an 80% soln in di-*tert*-butyl peroxide) in CH_2Cl_2 (2 mL) was gradually added over 30 min. The reaction mixture was warmed to room temperature and stirred for 1 h. The solvents were removed, the residue was dissolved in 1:1 Et_2O–EtOAc (100 mL), and washed sequentially with satd aq $NaHCO_3$, water, and brine. The organic phase was dried ($MgSO_4$), concentrated, and the residue was purified by chromatography (3:2 toluene–EtOAc, containing 1% Et_3N) to give **12** (15 mg, 15%) as a syrup. R_f 0.45 (Et_2O); ^{1}H NMR ($CDCl_3$): δ 7.38–7.34 (m, 10H, Ph), 5.79 (dt, 1H, $J_{1,2b}$ 3.8 Hz, H-1), 5.26 (ddd, 1H, $J_{5,6}$ 2.0, $J_{6,7a}$ 3.8, $J_{6,7b}$ 8.5 Hz, H-6), 5.20 (dd, 1H, $J_{2a,3}$ 5.3, $J_{3,4}$ 9.7 Hz, H-3), 5.10–5.03 (d, 4H, $2\times CH_2Ph$), 4.95 (t, 1H, $J_{4,5}$ 9.8 Hz, H-4), 4.17 (dd, 1H, $J_{7a,7b}$ 12.0 Hz, H-7a), 4.08 (dd, 1H, H-5), 4.04 (dd, 1H, H-7b), 2.20 (ddd, 1H, $J_{1,2a}$ 1.0, $J_{2a,2b}$ 13.3 Hz, H-2a), 2.08, 2.01, 2.00, 1.98 (4s, each 3H, $4\times CH_3CO$), 1.83–1.77 (m, 1H, H-2b); ^{13}C NMR ($CDCl_3$): δ 170.45, 170.21, 169.90, 169.73 (4C, CH_3CO), 135.42, 135.33 (2C, $C_{quat.}$ Ph), 128.76, 128.72, 128.67, 128.31, 128.16 (10 C, Ph), 95.74 (C-1), 70.31 (C-5), 69.84, 69.77 (2C, CH_2Ph), 68.04 (C-3), 67.64 (C-4), 67.00 (C-6), 63.12 (C-7), 35.20 (C-2), 20.82, 20.65, and 20.55 (4C, CH_3CO); ^{31}P NMR ($CDCl_3$): δ −1.62.

3.13. 3,4,6,7-Tetra-*O*-acetyl-2-deoxy-L-*galacto*-heptopyranosyl phosphate (triethylammonium salt) (13)

The crude glycosyl bromide **10** (82 mg, 0.193 mmol) was dissolved in dry CH_3CN (3 mL) under Ar, the pH was adjusted to 9 by the dropwise addition of DIPEA (120 μL, 0.71 mmol). After the addition of freshly activated molecular sieves 3 Å (0.3 g), the mixture was cooled to −30 °C, and tetrabutylammonium phosphate (2 mL, 0.577 mmol, ~0.3 M in CH_3CN) was added. The mixture was stirred for 4 h while warming to room temperature, then filtered, diluted with CH_3CN (20 mL), EtOAc (60 mL) and washed with ice-water (3×30 mL). The combined aq phases were lyophilized, and the residue was purified by column chromatography (70:70:4:4 $CHCl_3$–MeOH–water–aq NH_4OH) to afford an anomeric mixture of **13** (α/β = 1:3.6). Yield (62 mg, 50%); R_f 0.28 (70:70:4:4 $CHCl_3$–MeOH–water–aq NH_4OH); $[\alpha]_D^{20}$ −40 (*c* 0.3, H_2O). ^{1}H NMR (D_2O) for α-isomer: δ 5.69 (m, 1H, H-1); ^{13}C NMR (D_2O): δ 91.92 (C-1); ^{31}P NMR (D_2O): δ 0.78. ^{1}H NMR (D_2O) for β-isomer: δ 5.34 (m, 1H, H-6), 5.32 (ddd, 1H, $J_{1,P}$ 8.2 Hz, H-1), 5.21 (m, 1H, H-3), 4.78 (m, 1H, H-4), 4.43–4.29 (m, 2H, H-7a, H-7b), 4.05 (dd, 1H, $J_{4,5}$ 9.9 Hz, H-5), 3.19 (q, 12H, $6\times NCH_2CH_3$), 2.47 (ddd, 1H, $J_{2a,2b}$ 12.0 Hz, H-2a), 2.15, 2.07, 2.06, and 2.05 (4s, each 3H, $4\times CH_3CO$), 1.82 (ddd, 1H, H-2b), and 1.27 (t, 18H, $6\times NCH_2CH_3$); ^{13}C NMR (D_2O): δ 174.60, 174.09, 173.87 (4C, CH_3CO), 95.56 (C-1), 72.69 (C-5), 71.16 (C-3), 69.29 (C-4), 68.42 (C-6), 64.12 (C-7), 47.38 (6C, NCH_2CH_3), 37.11 (C-2), 21.04, 20.86 (4C, CH_3CO),

8.96 (6C, NCH_2CH_3); ^{31}P NMR (D_2O): δ −1.42; QTOF-ES-MS: $m/z = 441.080$ $[M-H]^-$, calcd 441.0798 $[M-H]^-$.

3.14. 2-Deoxy-β-L-*galacto*-heptopyranosyl phosphate bis(triethylammonium) salt (14)

A soln of **13** (27 mg, 0.042 mmol) in 7:3:1 MeOH–water–Et_3N (pH 10, 4.4 mL) was stirred for 4 h at rt. The reaction mixture was diluted with water (10 mL), concentrated to a volume of 5 mL, and lyophilized. The residue was purified by column chromatography (5:10:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water), appropriate fractions were collected, concentrated, and diluted with water (10 mL). The pH of the soln was adjusted to 4.0 with Dowex H^+ resin, the resin was separated on the filter and the pH of the filtrate was adjusted to 7 with Et_3N. The soln was concentrated to 5 mL vol and lyophilized to give **14** as bis-triethylammonium salt (12 mg, 60%). R_f 0.42 (5:10:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ −12.3 (*c* 1.15, water); ^1H NMR (D_2O): δ 4.98 (ddd, 1H, $J_{1,2a}$ 2.0, $J_{1,P}$ 8.0 Hz, H-1), 3.84 (ddd, 1H, $J_{5,6}$ 1.8, $J_{6,7a}$ 6.8 Hz, H-6), 3.64–3.59 (m, 1H, H-3), 3.62 (dd, 1H, $J_{7a,7b}$ 11.5 Hz, H-7a), 3.55 (dd, 1H, $J_{6,7b}$ 6.8 Hz, H-7b), 3.34 (t, 1H, $J_{4,5} \sim J_{3,4}$ 9.8 Hz, H-4), 3.24 (dd, 1H, H-5), 2.94 (q, 12H, $6\times NCH_2CH_3$), 2.17 (ddd, 1H, $J_{2a,3}$ 5.0, $J_{2a,2b}$ 12.3 Hz, H-2a), 1.42 (ddd, 1H, $J_{1,2b}$ 10.0, $J_{2b,3}$ 10.0 Hz, H-2b), and 1.14 (t, 18H, $6\times NCH_2CH_3$); ^{13}C NMR (D_2O): δ 94.63 (C-1), 74.49 (C-5), 70.67 (C-3), 70.12 (C-4), 68.45 (C-6), 62.03 (C-7), 42.19 (CH_2CH_3), 39.68 (C-2), and 9.94 (CH_2CH_3); ^{31}P NMR (D_2O): δ 1.86; QTOF-ES-MS: $m/z = 273.0143$ $[M-H]^-$, calcd 273.0376 $[M-H]^-$.

3.15. Adenosine 5′-(2-deoxy-L-*galacto*-heptopyranosyl)-diphosphate (triethylammonium salt) (16)

Tetraaacetyl heptosyl phosphate **13** (30 mg, 0.047 mmol) was made anhydrous by repeated dissolution in dry pyridine and evaporation of solvent (3×10 mL). After each evaporation step, dry argon was flushed into the rotary evaporator. AMP-morpholidate (4′-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt) (165 mg, 0.233 mmol) was dried byazeotropy with pyridine (3×10 mL) with exclusion of moisture under argon. Both components were combined, repeatedly concentrated from pyridine (2×10 mL), redissolved in 5 mL pyridine and the soln was vigorously stirred at rt. The progress of the reaction was monitored by TLC-analysis (70:70:4:4 $CHCl_3$–MeOH–25% aq NH_4OH–water), the reaction was complete within 48 h as judged by the appearance of a major UV-positive spot of ADP-Hep (as two spots with ΔR_f approx 0.3 corresponding to two different salt forms of **15**: ammonium salt with R_f 0.2 and 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt with R_f 0.5). The reaction was stopped by evaporation of pyridine. The crude reaction products were dissolved in 5 mL water and the soln was allowed to slowly adsorb on a resin bed of BioRad anion-exchange column (1×10 cm, HCO_3^--form). The column was washed first with water (20 mL) and then developed with a stepwise gradient of TEAB buffer, pH 8.4 (0.025→0.15 M), **15** was eluted at a concentration of 0.1 M TEAB. The fractions containing ADP-Hep were pooled, concentrated to 10 mL volume, 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 total eluate was made neutral by addition of Et_3N, concentrated to 5 mL volume and lyophilized to give **15** as white solid. Yield: 32 mg (71%); R_f 0.60 (70:70:4:4, $CHCl_3$–MeOH–water–aq NH_4OH); $[\alpha]_D^{20}$ −9.5 (*c* 1.2, water). ^1H NMR (D_2O), for α-anomer: δ 8.60 (s, 1H, H-8_{Ade}), 8.26 (s, 1H, H-2_{Ade}), 6.13 (d, 1H, $J_{1,2}$ 6.0 Hz, H-1_{Rib}), 5.75 (m, 1H, H-1).

For β-anomer: δ 8.54 (s, 1H, H-8_{Ade}), 8.27 (s, 1H, H-2_{Ade}), 6.15 (d, 1H, $J_{1,2}$ 5.9 Hz, H-1_{Rib}), 5.29 (ddd, 1H, $J_{1,2a}$ 1.9, $J_{1,2b}$ 9.7, $J_{1,P}$ 8.0 Hz, H-1), 5.17 (m, 1H, H-6), 4.94 (m, 1H, H-3), 4.72 (dd, 1H, $J_{2,3}$ 6.1 Hz, H-2_{Rib}), 4.64 (dd, 1H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 4.49 (dd, 1H, $J_{2,3}$ 5.0, $J_{3,4}$ 3.9 Hz, H-3_{Rib}), 4.37 (m, 1H, H-4_{Rib}), 4.34 (d, 2H, H-5_{Rib}), 4.28–4.20 (m, 2H, H-7a, H-7b), 3.71 (dd, 1H, H-5), 3.19 (q, 12H, $6\times CH_2CH_3$), 2.42 (ddd, 1H, $J_{2a,3}$ 5.0, $J_{2a,2b}$ 12.4 Hz, H-2a), 2.09, 2.01, 2.00, and 1.97 (4s, 12H, $4\times CH_3CO$), 1.74 (ddd, 1H, $J_{2b,3}$ 9.7 Hz, H-2b), 1.27 (t, 18H, $6\times CH_2CH_3$); ^{13}C NMR (D_2O): δ 174.44, 174.08, 173.92, 173.56 (4C, CH_3CO), 152.95 (C-2_{Ade}), 140.86 (C-8_{Ade}), 95.80 (C-1, $J_{1,P}$ 4.6 Hz), 87.77 (C-1_{Rib}), 84.48 (C-4_{Rib}, $J_{1,P}$ 9.3 Hz), 75.11 (C-2_{Rib}), 72.68 (C-5), 70.93 (2C, C-3, C-3_{Rib}), 69.07 (C-4), 68.36 (C-6), 66.07 (C-7), 64.37 (C-5_{Rib}), 47.35 (CH_2CH_3), 36.70 (C-2), 20.89, 20.77 (4C, CH_3CO), 8.93 (CH_2CH_3); ^{31}P NMR (D_2O): δ −10.36 (d, $J_{C\text{-}4,P}$ 9.3 Hz, P_{Rib}), −13.06 (d, $J_{C\text{-}1,P}$ 4.6 Hz, P_{Hep}); QTOF-ES-MS: $m/z = 772.1463$ $[M+H]^+$, calcd 772.1480 $[M+H]^+$.

A soln of **15** (32 mg, 0.033 mmol) in 7:3:0.5 MeOH–water–Et_3N (pH 11, 5 mL) was stirred for 6 h at rt. The reaction mixture (pH 10) was diluted with water (10 mL), concentrated to a volume of 5 mL and lyophilized to give **16** as an anomeric mixture (α:β, 1:3.4, 24 mg, 91%) as a solid. R_f 0.61 (5:10:2:2 $CHCl_3$–MeOH–25% aq NH_4OH–water); $[\alpha]_D^{20}$ +43 (*c* 0.33, water). ^1H NMR (D_2O) for α-anomer: δ 8.45 (s, 1H, H-8_{Ade}), 8.20 (s, 1H, H-2_{Ade}), 6.11 (d, 1H, $J_{1,2}$ 4.8 Hz, H-1_{Rib}), 5.69 (ddd, 1H, $J_{1,2a}$ 2.0, $J_{1,2b}$ 5.9 Hz, H-1), 4.01 (m, 1H, H-6), 3.83 (dd, 1H, $J_{4,5}$ 10.0, $J_{5,6}$ 1.4 Hz, H-5), 3.50 (dd, 1H, $J_{4,5}$ 9.7 Hz, H-4), 2.24 (ddd, 1H, $J_{2a,3}$ 5.0, $J_{2a,2b}$ 12.4 Hz, H-2a); ^{31}P NMR (D_2O): δ −10.94 (d, $J_{P,P}$ 20.8 Hz, P_{Rib}) and −12.86 (d, $J_{P,P}$ 20.7 Hz, P_{Hep}).

^{1}H NMR (D_2O) for β-anomer: δ 8.50 (s, 1H, H-8_{Ade}), 8.22 (s, 1H, H-2_{Ade}), 6.12 (d, 1H, $J_{1,2}$ 6.0 Hz, H-1_{Rib}), 5.24 (ddd, 1H, $J_{1,2a}$ 2.3, $J_{1,2b}$ 9.7, $J_{1,P}$ 8.0 Hz, H-1), 4.75 (m, 1H, H-2_{Rib}), 4.52 (dd, 1H, $J_{2,3}$ 5.1, $J_{3,4}$ 3.4 Hz, H-3_{Rib}), 4.39 (m, 1H, H-4_{Rib}), 4.21 (m, 2H, H-5_{Rib}), 3.94 (ddd, 1H, $J_{5,6}$ 1.6, $J_{6,7}$ 6.8 Hz, H-6), 3.68 (m, 3H, H-3, H-7a, H-7b), 3.47 (dd, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 3.33 (dd, 1H, H-5), 3.18 (q, 12H, CH_2CH_3, Et_3N), 2.33 (ddd, 1H, $J_{2a,3}$ 5.0, $J_{2a,2b}$ 12.6 Hz, H-2a), 1.59 (ddd, 1H, $J_{2b,3}$ 9.7 Hz, H-2b), and 1.27 (t, 18H, CH_2CH_3, Et_3N); ^{13}C NMR: see Table 1; ^{31}P NMR (D_2O): δ −10.94 (d, $J_{C\text{-}4,P}$ 9.4, $J_{P,P}$ 20.9 Hz, P_{Rib}), −13.14 (d, $J_{C\text{-}1,P}$ 4.5 Hz, P_{Hep}); QTOF-ESIMS J. Defaye: $m/z = 602.1069$ $[M-H]^-$, calcd 602.0901 $[M-H]^-$.

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