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Chemical synthesis of GDP-L-galactose and analogues

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Abstract

Succinct syntheses for L-galactose, 3-deoxy-L-*xylo*-hexose (3-deoxy-L-galactose), 6-deoxy-L-galactopyranose (L-fucose) and 3,6-dideoxy-L-*xylo*-hexose (3,6-dideoxy-L-galactose) have been developed starting from commercially available L-galactono-1,4-lactone. L-Galactose and variants were then converted to the guanosine diphosphate derivatives, via the formation of the anomeric phosphates and coupling to guanosine monophosphate morpholidate. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: L-Galactono-1,4-lactone; GDP-β-L-galactose and analogues; 3-Deoxy-L-galactose; 3,6-Dideoxy-L-galactose

1. Introduction

Oligosaccharides conjugated to lipids or proteins have an important role in biological systems and are found in the cytoplasm, on cell membranes and in extra-cellular fluids and matrices. Some of the many biological functions in which glycoconjugates are involved include blood clotting, lubrication, structural support, immunological protection, cell adhesion, hormone activity and many other recognition events [1]. Oligosaccharides modify the structure dynamics and physical properties of proteins to which they are covalently bound via nitrogen to asparagine (N-linked) or oxygen to serine or threonine (O-linked).

L-Fucose terminates the oligosaccharide chain of various glycoconjugates and may therefore be of particular importance. In order to elucidate the role of L-fucose, a series of L-fucose derivatives have already been prepared for biological tests [2]. The synthesis of guanosine diphosphate (GDP) β-L-fucose, the substrate for fucosyl transferases has also been well documented by enzymatic [3] and chemical [4] procedures. In this paper, we now describe the synthesis of GDP-β-L-galactose, GDP-3-deoxy-β-L-galactose and GDP-3,6-dideoxy-β-L-galactose, which were prepared as mimics of GDP-β-L-fucose for transferase enzymatic studies. L-Galactose can be favourably compared to L-fucose with a functional group difference at C-6.

Previously, approaches towards such deoxygenated derivatives were usually multi-step starting from the parent hexopyranoses [5]. Each route was expressly designed to give the desired site of deoxygenation highlighted by a specific protecting group, demanding extensive usage of protection

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and deprotection chemistry. To obtain the deoxy sugar, this subsequent step would be a radical or alternative deoxygenation. The overall yields for the syntheses of deoxygenated derivatives are in the range of 10% from expensive starting materials and therefore an alternative, more direct procedure is required. A suitable starting point is commercially available L-galactono-1,4-lactone, which can be manipulated in two steps to the deoxygenated species, followed by a reduction of the lactone functionality to afford the desired compounds in 80% yield. L-Galactono-1,4-lactone can also be easily reduced to L-galactose in one step. The activation of the anomeric centre via the phosphates finally gave the GDP derivatives.

2. Results and discussion

Preparation of L-*galactose*.—A two step synthesis of L-galactose has already been documented, whereby L-galactono-1,4-lactone (**1**) is reduced to L-galactose by the use of sodium amalgam [6]. Alternatively, careful reduction of L-galactono-1,4-lactone (Amberlite IR-120 H^+, pH 3–5, sodium borohydride) [7] afforded L-galactose as a mixture with the over-reduced product L-galactitol. Treatment of this mixture with typical acetylation conditions gave the desired acetylated L-galactose (**2**), in 71% yield after purification.

Preparation of 3-deoxy-L-*galactose*.—Under basic conditions, acetylated aldonolactones suffer elimination reaction to give α,β-unsaturated lactones, which can be further transposed to 3-deoxy sugars, but with the complication of a further spontaneous elimination [8]. However, a more efficient procedure to 3-deoxyaldonolactones has been described, which involves treatment of aceylated aldonolactones (generated by acidic acetylation conditions) with hydrogen using palladium catalyst (5% Pd/C) in the presence of triethylamine [9]. This route combines firstly a base catalysed elimination with a subsequent reduction of the ensuing α,β-unsaturated lactone.

Applying this strategy, L-galactono-1,4-lactone (**1**) was converted to tetra-*O*-acetyl-L-galactono-1,4-lactone (**3**) using acetic anhydride and a catalytic amount of acid, which was then subsequently treated with hydrogen in the presence of 5% Pd/C and triethylamine for 18 h to furnish 2,4,5-tri-*O*-acetyl-3-deoxy-L-galactono-1,4-lactone (**4**) in 88% yield. It should be noted that high quality Pd/C and freshly distilled triethylamine should be used to avoid production of side products and long reaction times. Lactone (**4**) was then treated with the highly selective reducing agent disiamylborane (DIIAMB) [10], (prepared in situ) to give furanose (**5**) in 81% yield. Conversion to the pyranose was achieved by Zemplén O-deacetylation conditions and subsequent treatment with benzoyl chloride in the presence of pyridine, affording 1,2,4,6-tetra-*O*-benzoyl-3-deoxy-β-L-galactopyranose (**6**), a precursor in the preparation of GDP-3-deoxy-β-L-galactose (**28**).

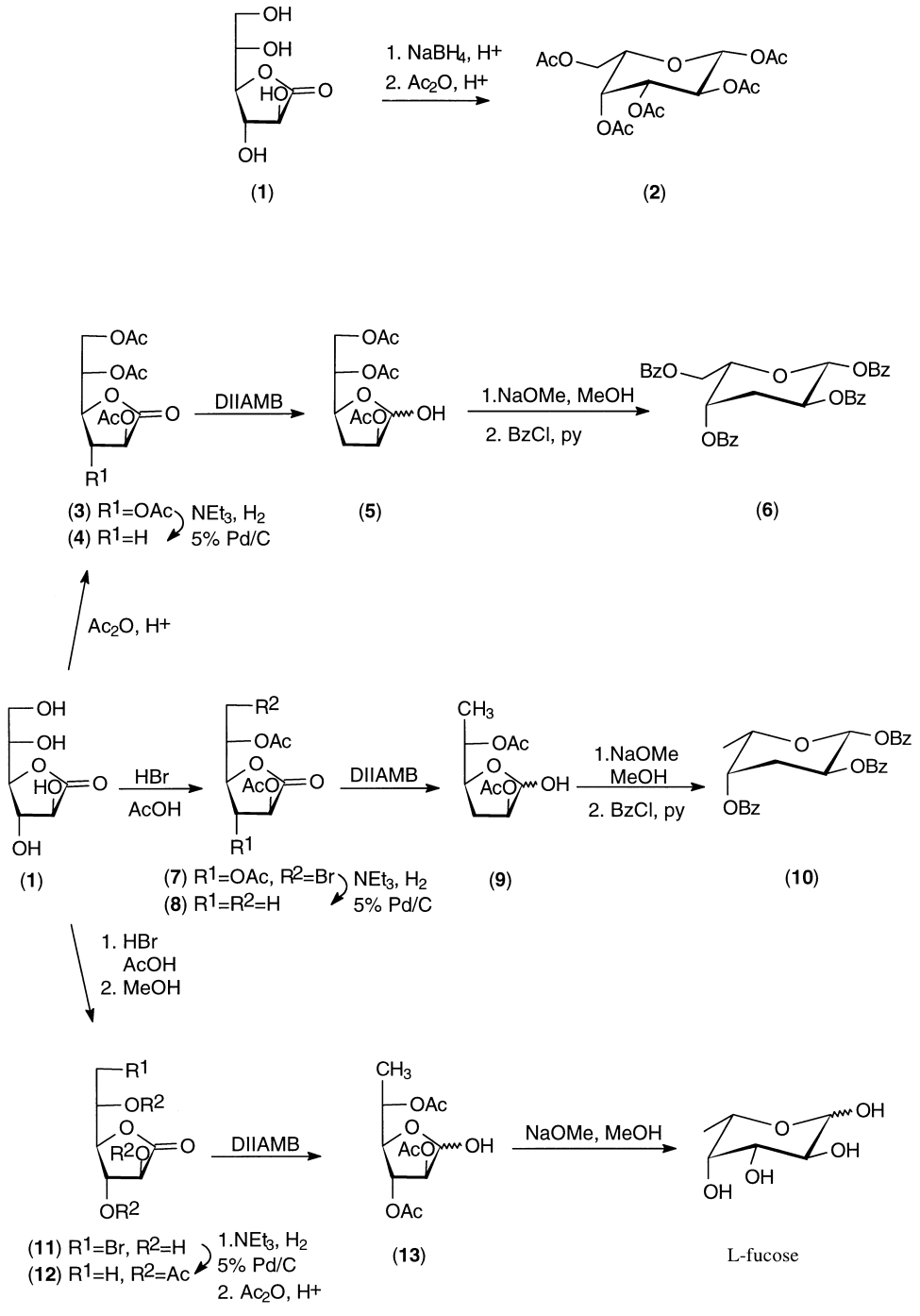
Preparation of L-*fucose and 3,6-dideoxy*-L-*galactose*.—Known methodology published by Pedersen et al. allows the transformation of 6-OH groups in aldonolactone systems to bromides, which can be further modified by a hydrogenation reaction to the 6-deoxy derivative [11]. Treatment of L-galactono-1,4-lactone with HBr in acetic acid, as described for the D-galactonolactone [12], gave bromide (**7**) in good yield. The next step towards the synthesis of 3,6-dideoxy-L-galactose was a hydrogenation reaction in the presence of triethylamine, which in one step provided the C-3 and C-6 dideoxy functionality leading to 3,6-dideoxylactone (**8**) in 79% yield. To complete the synthesis, lactone (**8**) underwent reduction with DIIAMB to give furanose (**9**), deprotection of the ester groups, followed by benzoylation gave 1,2,4-tri-*O*-benzoyl-3,6-dideoxy-β-L-galactose (**10**) to be used for the formation of GDP-3,6-dideoxy-β-L-galactose (**29**).

In the fucose case, once the bromide had been synthesised, it was necessary at this stage to remove the ester protecting groups (accomplished by addition of methanol on work up of the bromination reaction) to prevent any elimination occurring during the hydrogenation reaction furnishing lactone (**11**). Hydrogenation of 6-bromolactone (**11**) gave lactone (**12**), which was acetylated and then reduced with DIIAMB to furnish 2,3,5-tri-*O*-acetyl-6-deoxy-β-L-galactofuranose (**13**) in 76% yield. Finally the remaining protecting groups were removed by the Zemplén O-deacetylation conditions to give L-fucose (Scheme 1).

Preparation of GDP-β-L-*galactose, GDP-3-deoxy*-β-L-*galactose and GDP-3,6-dideoxy*-β-L-*galactose*.—There are many reported routes for the synthesis of GDP-fucose and GDP-arabinose [3,4] and we intended to base our strategy for the targets, GDP-β-L-galactose, GDP-3-deoxy-β-L-galactose and GDP-3,6-dideoxy-β-L-galactose on such published work. For such an approach, we

would couple the sugar containing one of the phosphorus moieties in an activated form with the correct anomeric configuration, to guanosine monophosphate morpholidate *N,N′*-dicyclohexylcarboxaminide (GMP). Although this type of reaction has been well described for GDP-fucose, the coupling reaction had until recently suffered from poor yields in the range of 25 to 48%. Wong *et al.* have recently described an improvement in the synthesis of these GDP-sugars, whereby 1*H*-tetrazole was employed as a catalyst in the coupling reaction, boosting the yields to 76–91% and minimising reaction times [13]. However, at the time this improvement was not known and our chemistry was carried out in accordance with typical coupling conditions for the formation of GDP-fucose.

The formation of the phosphate must be stereospecific, allowing access only to the desired



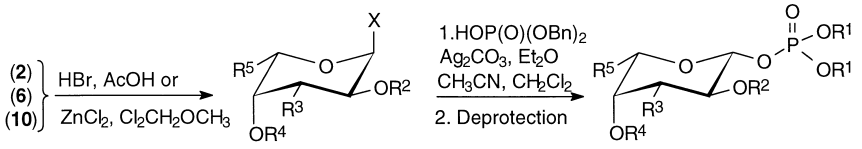
Scheme 1.

β-phosphate. This can be achieved by treating the sugar, activated at the anomeric position, with the nucleophilic reagent dibenzyl phosphate in the presence of silver carbonate [2]. Activation of the sugar at the anomeric position is usually attained by the formation of a glycosyl halide. Synthesis of α-bromides (**14**) and (**15**) was achieved in 85 and 63% yield, respectively, from the preceding acetate (**2**) and benzoate (**6**) using hydrogen bromide in acetic acid. In the case of the 3-deoxygalactose compound, the synthesis of 3,6-dideoxy-L-galactose phosphate via bromide has been documented, but the phosphate was generated as a 5.4:1 β/α anomeric mixture in low yield [4]. Due to both the instability of the glycosyl bromide and the formation of both anomers of the subsequent phosphonate, the more stable 3,6-dideoxy-L-galactosyl chloride (**16**) was prepared following a procedure using zinc chloride in dichloromethyl-methylether [5] and used immediately without any purification. With halides (**14**), (**15**) and (**16**) in hand, conversion to the desired β-phosphates (**17**), (**18**), and (**19**) was achieved by reaction with dibenzyl phosphate in the presence of silver carbonate, followed by purification by silica gel column chromatography in 89, 90 and 55% yields (over two steps), respectively. Full deprotection was performed by hydrogenolysis of the benzyl ester groups using Pd/C as catalyst, followed by basic hydrolysis of the benzoate or acetate groups also allowing for the formation of the disodium salt of L-galactosyl phosphate (**20**), the dilithium salt for both 3-deoxy-L-galactosyl phosphate (**21**) and 3,6-dideoxy-L-galactosyl phosphate (**22**) in 69, 79 and 65% yields, respectively (Scheme 2).

Before the final step, phosphates (**20**), (**21**) and (**22**) were converted to the tributylammonium salts to increase solubility in dimethylformamide. GMP (**26**) was then allowed to react with phosphates (**23**), (**24**) and (**25**) under strictly anhydrous conditions for 7–10 days. Purification of the reaction mixture in each case was performed by ion-exchange and then desalting of the residue using Sephadex G-10 chromatography to give GDP-L-galactose (**27**) (29%), GDP-3-deoxy-L-galactose (**28**) (27%) and GDP-3,6-dideoxy-L-galactose (**29**) (19%) (Scheme 3).

3. Experimental

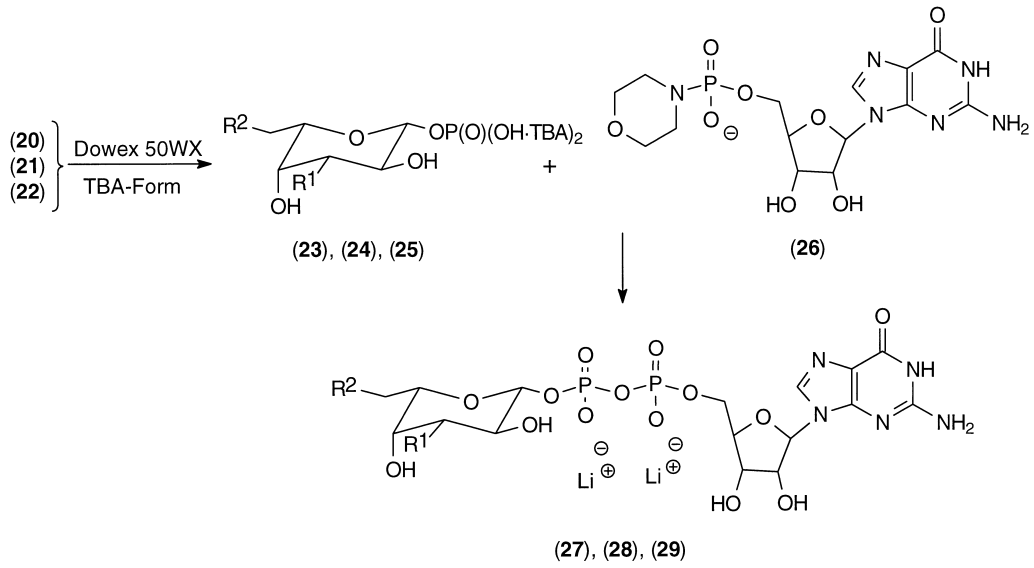
General methods.—Optical rotations were measured using a Perkin–Elmer 241 polarimeter at 22 ± 2 °C. Analytical TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by UV absorption and/or by charring with H_2SO_4. All commercial reagents were used as supplied and chromatography solvents were distilled prior to use. Column chromatography was performed on Silica Gel 60 (40–60 μm, E. Merck, Darmstadt). ^{1}H NMR spectra were recorded at 500 MHz (Bruker AMX-500), 360 MHz (Bruker WM-360) or 300 MHz (Bruker AM-300) with either $(Me)_4Si$ (δ 0, for solutions in $CDCl_3$ and CD_3OD) or DOH (δ



	X	R^2	R^3	R^4	R^5
(**2**)→(**14**)	Br	Ac	OAc	Ac	CH_2OAc
(**6**)→(**15**)	Br	Bz	H	Bz	CH_2OBz
(**10**)→(**16**)	Cl	Bz	H	Bz	Me

	R^1	R^2	R^3	R^4	R^5
(**17**) ↓ (**20**)	Bn	Ac	OAc	Ac	CH_2OAc
	Na	H	OH	H	CH_2OH
(**18**) ↓ (**21**)	Bn	Bz	H	Bz	CH_2OBz
	Li	H	H	H	CH_2OH
(**19**) ↓ (**22**)	Bn	Bz	H	Bz	Me
	Li	H	H	H	Me

Scheme 2.



	R1	R2
(**20**), (**23**), (**27**)	OH	OH
(**21**), (**24**), (**28**)	H	OH
(**22**), (**25**), (**29**)	H	H

Scheme 3.

4.80, for solutions in D_2O) as internal references. ^{13}C NMR spectra were recorded at 75.5 MHz (Bruker AM-300) and 125.7 MHz (Bruker AMX-500) with internal $(Me)_4Si$ (δ 0, for solutions in $CDCl_3$ and CD_3OD). ^1H NMR data are reported as though they were first order. Assignments of ^{13}C chemical shifts are tentative and are based on comparison with published spectral data. Unless otherwise stated, all reactions are carried out at room temperature.

*1,2,3,4,6-Penta-*O*-acetyl-*β*-*L*-galactopyranose* (**2**).—To L-galactono-1,4-lactone (1.0 g, 5.55 mmol) in MeOH (6 mL) and water (25 mL) at 0 °C was added Amberlite IR 120 (H^+) resin (5 mL). Sodium borohydride (0.22 g, 5.61 mmol) was added portionwise, keeping the pH between 3 and 5. Once the addition of $NaBH_4$ was complete, the reaction mixture was stirred for 1 h, warming to room temperature, then the resin was removed by filtration. Methanol was removed under reduced pressure and the resulting solid was dissolved in MeOH (50 mL) and then concentrated under reduced pressure. This procedure was repeated twice. The residue was then dissolved in Ac_2O (30 mL), 60% $HClO_4$ (0.5 mL) was added and the soln stirred for 1 h. Ice water was added and, after 0.5 h, the mixture was extracted with CH_2Cl_2. The organic extract was washed with water, sat $NaHCO_3$, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography using 1:3 EtOAc–toluene as eluent to give the title compound (**2**) (1.54 g, 71%) as an oil. $[\alpha]^{20}_D$ −173.1 (*c* 2.1 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 5.74 (d, 1 H, $J_{1,2}$ 7.1 Hz, H-1), 5.58 (dd∼t, 1 H, $J_{2,1}$ 7.1, $J_{2,3}$ 6.6 Hz, H-2), 5.48 (d, 1 H, $J_{4,3}$ 3.0 Hz, H-4), 4.73 (dd, 1 H, $J_{3,2}$ 6.6, $J_{3,4}$ 3.0 Hz, H-3), 4.49 (dd, 1 H, $J_{6a,6b}$ 11.7, $J_{6a,5}$ 5.0 Hz, H-6a), 4.39 (dd, 1 H, $J_{6a,6b}$ 11.7, $J_{6b,5}$ 6.6 Hz, H-6b), 4.25 (m, 1 H, $J_{5,6b}$ 6.6, $J_{5,6a}$ 5.0 Hz, H-5), 2.30–2.10 (5×s, 15 H, 5×OCO*CH*$_3$) ppm.; Anal. Calcd. for $C_{16}H_{22}O_{11}$ (390.4): C, 49.23; H, 5.68. Found: C, 50.14; H, 5.64.

*2,5,6-Tri-*O*-acetyl-3-deoxy-*L*-xylo-hexono-1,4-lactone* (**4**).—To 2,3,5,6-tetra-*O*-acetyl-L-galactono-1,4-lactone (**3**) (1.0 g, 2.91 mmol) in EtOAc (10 mL) containing triethylamine (1 mL) was added 5% Pd/C (0.1 g) and the soln was stirred for 18 h under an atmosphere of hydrogen (3 atm). Once the reaction was complete, the reaction mixture was filtered and washed with 2 N HCl (2×10 mL), then the organic phase was dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound (**4**) (731 mg, 88%) as an oil. $[\alpha]^{20}_D$ −93 (*c* 1.7 in $CHCl_3$); ^1H NMR (500 MHz,

$CDCl_3$): δ 5.48 (dd, 1 H, $J_{2,3b}$ 10.2, $J_{2,3a}$ 9.0 Hz, H-2), 5.16 (dt, 1 H, $J_{5,6b}$ 6.4, $J_{5,6a}$ 4.5, $J_{5,4}$ 4.5 Hz, H-5), 4.64 (ddd, 1 H, $J_{4,3b}$ 11.4, $J_{4,3a}$ 6.0, $J_{4,5}$ 4.5 Hz, H-4), 4.34 (dd, 1 H, $J_{6a,6b}$ 12.2, $J_{6a,5}$ 4.5 Hz, H-6a), 4.15 (dd, 1 H, $J_{6b,6a}$ 12.2, $J_{6b,5}$ 6.4 Hz, H-6b), 2.72 (ddd, 1 H, $J_{3a,3b}$ 13.0, $J_{3a,2}$ 9.0, $J_{3a,4}$ 6.0 Hz, H-3a), 2.09, 2.00 (2 × s, 6 H, 2 × $OCOCH_3$), 1.95 (m, 4 H, H-3b, 3 × $OCOCH_3$); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 171.4 (C-1), 74.34 (C-4), 70.53 (C-2), 67.66 (C-5), 61.90 (C-6), 30.43 (C-3) ppm. Anal. Calcd. for $C_{12}H_{16}O_8$ (288.3):C, 50.00; H, 5.59. Found: C, 49.81; H, 5.69.

The ^{1}H and ^{13}C data for (**4**) are consistent with the reported data for 2,3,6-tri-*O*-acetyl-3-deoxy-D-galactono-1,4-lactone [9].

2,5,6-Tri-O-*acetyl-3-deoxy*-L-xylo-*hexofuranose* (**5**).—To a solution of borane–dimethyl sulfide complex (3.2 mL, 0.03 mol) in CH_2Cl_2 (10 mL) was added dropwise under an atmosphere of N_2, a soln of 2-methyl-2-butene (3.8 mL, 0.07 mol) in CH_2Cl_2 (15 mL) and then stirred for 3 h. After this time, the reaction mixture was cooled to 0 °C, a soln of 2,5,6-tri-*O*-acetyl-3-deoxy-L-*xylo*-hexono-1,4-lactone (**4**) (731 mg, 2.53 mmol) in CH_2Cl_2 (10 mL) was added and the reaction mixture stirred for 18 h. Water (10 mL) was added, the reaction mixture was stirred for a further 1 h and then concentrated under reduced pressure. The residue was co-evaporated with water (3×15 mL) and then MeOH (3×15 mL) and then purified by column chromatography using 1:2 EtOAc–toluene as eluent to give the title compound (**5**) (596 mg, 81%) as an oil (α/β 6:1). ^{1}H NMR (500 MHz, $CDCl_3$) α-anomer: δ 5.33 (dd∼t, 1 H, $J_{1,OH}$ 3.6, $J_{1,2}$ 3.1 Hz, H-1), 5.11 (m, 1 H, H-5), 4.96 (ddd, 1 H, $J_{2,3a}$ 7.0, $J_{2,1}$ 3.1, $J_{2,3b}$ 2.5 Hz, H-2), 4.40 (ddd∼dt, 1 H, $J_{4,3a}$ 8.2, $J_{4,5}$ 5.7, $J_{4,3b}$ 5.4 Hz, H-4), 4.29 (dd, 1 H, $J_{6a,6b}$ 12.1, $J_{6a,5}$ 3.4 Hz, H-6a), 4.11 (dd, 1 H, $J_{6b,6a}$ 12.1, $J_{6b,5}$ 8.5 Hz, H-6b), 3.96 (d, ^{1} H, $J_{OH,1}$ 3.6 Hz, OH), 2.49 (ddd, 1 H, $J_{3a,3b}$ 14.8, $J_{3a,4}$ 8.2, $J_{3a,4}$ 7.0 Hz, H-3a), 2.10, 2.01, 1.96 (3 × s, 9 H, 3 × $OCOCH_3$), 1.69 (ddd, 1 H, $J_{3b,3a}$ 14.8, $J_{3b,4}$ 5.4, $J_{3b,2}$ 2.5 Hz, H-3b); ^{13}C NMR (125.76 MHz, $CDCl_3$) β-anomer: δ 100.59 (C-1), 77.91 (C-4), 76.03 (C-2), 71.58 (C-5), 62.96 (C-6), 31.38 (C-3) ppm. Anal. Calcd. for $C_{12}H_{18}O_8$ (290.3):C, 49.65; H, 6.25. Found: C, 49.12; H, 6.43.

1,2,4,6-Tetra-O-*benzoyl-3-deoxy*-β-L-xylo-*hexopyranose* (**6**).—2,5,6-Tri-*O*-acetyl-3-deoxy-L-*xylo*-hexofuranose (**5**) (1.2 g, 4.10 mmol) was treated with 0.1 M NaOMe in dry MeOH (10 mL) for 24 h. After neutralisation with Amberlite IR 120 (Cl^- form) the mixture was filtered, concentrated under reduced pressure to yield 3-deoxy-L-*xylo*-hexopyranose. This residue (0.5 g, 3.05 mmol) was dissolved in dry pyridine (10 mL) and dry CH_2Cl_2 (2 mL), cooled to −40 °C and benzoyl chloride (390 μL, 3.35 mmol) was added dropwise over 5 h under an atmosphere of argon. The reaction was quenched with water (10 mL), the mixture extracted with CH_2Cl_2 and the organic layer concentrated under reduced pressure. The residue was then purified by column chromatography using as eluent, 1:10 EtOAc–toluene, to give the title compound (**6**) (1.39 g, 58%) as colourless syrup. $[\alpha]^{20}$D −168.6 (*c* 1.9 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20−7.14 (m, 20 H, Aryl-H), 6.24 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.70−5.52 (m, 2 H, H-2, H-4), 4.79 (m, 1 H, H-5), 4.73 (dd, 1 H, $J_{6a,6b}$ 13.8, $J_{6a,5}$ 3.8 Hz, H-6a), 4.60 (dd, 1 H, $J_{6b,6a}$ 13.8, $J_{6b,5}$ 7.0 Hz, H-6b), 2.81 (m, 1 H, H-3e), 2.21 (m, 1 H, H-3a) ppm.; Anal. Calcd. for $C_{34}H_{28}O_9$ (580.6): C, 70.34; H, 4.86. Found: C, 70.31; H, 4.97.

2,3,5-Tri-O-*acetyl-6-bromo-6-deoxy*-L-*galactono-1,4-lactone* (**7**).—L-Galactono-1,4-lactone (**1**) (1.0 g, 5.61 mmol) was dissolved in HBr (30 mL, 30% in glacial acetic acid) and stirred for 4 h. Acetic anhydride (10 mL) was added, and the mixture was stirred for a further 1 h. The reaction mixture was poured on ice water and after 30 min, the mixture was extracted with CH_2Cl_2. The organic extract was washed with water, sat $NaHCO_3$ soln, dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound (**7**) (1.87 g, 91%) as colourless crystals. mp 97 °C; $[\alpha]^{20}$D−67 (*c* 1.4 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.59 (d, 1 H, $J_{2,3}$ 7.0 Hz, H-2), 5.36 (dd∼t, 1 H, $J_{3,2}$ 7.0, $J_{3,4}$ 6.7 Hz, H-3), 5.18 (dt, 1 H, $J_{5,6}$ 6.5, $J_{5,4}$ 2.3 Hz, H-5), 4.80 (dd, 1 H, $J_{4,3}$ 6.7, $J_{4,5}$ 2.3 Hz, H-4), 3.50 (d, 2 H, $J_{5,6}$ 6.5 Hz, H-6), 2.09, 2.02, 1.96 (3×s, 9 H, 3×$OCOCH_3$); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 171.4 (C-1), 77.27 (C-4), 72.33 (C-3), 72.00 (C-2), 70.14 (C-5), 27.25 (C-6) ppm.; Anal. Calcd. for $C_{12}H_{15}$ BrO_8 (367.2): C, 39.26; H, 4.12; Br, 21.76. Found: C, 38.99; H, 4.07; Br, 21.59.

The ^{1}H and ^{13}C data found for (**7**) are consistent with the reported data for 2,3,5-tri-O-acetyl-6-bromo-6-deoxy-D-galactono-1,4-lactone [9].

2,5-Di-O-*acetyl-3,6-dideoxy*-L-xylo-*hexono-1,4-lactone* (**8**).—2,3,5-Tri-*O*-acetyl-6-bromo-6-deoxy-L-galactono-1,4-lactone (**7**) (0.5 g, 1.36 mmol) in EtOAc (15 mL) and triethylamine (0.75 mL) were stirred for 20 h under an atmosphere of hydrogen (5 atm) in the presence of 5% Pd/C (0.1 g). Once

the reaction was complete, the reaction mixture was filtered and washed with 2 N HCl (2×10 mL), the organic phase was dried ($MgSO_4$) and concentrated under reduced pressure to give the title compound (**8**) (246 mg, 79%) as colourless crystals. mp 82–84 °C; $[\alpha]^{20}_D$ −33.4 (*c* 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.48 (dd, 1 H, $J_{2,3b}$ 10.3, $J_{2,3a}$ 8.9 Hz, H-2), 5.00 (dq∼m, 1 H, $J_{5,6}$ 6.5, $J_{5,4}$ 5.4 Hz, H-5), 4.42 (ddd, 1 H, $J_{4,3b}$ 10.3, $J_{4,3a}$ 6.1. $J_{4,5}$ 5.4 Hz, H-4), 2.69 (ddd, 1 H, $J_{3a,3b}$ 12.6, $J_{3a,2}$ 8.9, $J_{3a,4}$ 6.1 Hz, H-3a), 2.12, 2.08, 2.01 (3×s, 9 H, 3×OCOCH_3), 1.94 (td, 1 H, $J_{3b,3a}$ 12.6, $J_{3b,2}$ 10.3, $J_{3b,4}$ 10.3 Hz, H-3b), 1.31 (dd, 3 H, $J_{6,5}$ 6.5 Hz, H-6); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 171.52 (C-1), 77.41 (C-4), 77.00 (C-2), 68.02 (C-5), 30.76 (C-3), 15.64 (C-6) ppm.; Anal. Calcd. for $C_{10}H_{14}O_6$ (230.2): C, 52.17; H, 6.13. Found: C, 52.11; H, 6.10.

The ^{1}H and ^{13}C data found for (**8**) are consistent with the reported data for 2,5-di-*O*-acetyl-3,6-dideoxy-D-galactono-1,4-lactone [9].

2,5-Di-O-*acetyl-3,6-dideoxy*-L-xylo-*furanose* (**9**).—Lactone (**8**) (246 mg, 1.07 mmol) was reduced in a similar manner to that described for the preparation of (**5**). The residue was purified by column chromatography using 1:3 EtOAc–toluene as eluent to give the title compound (**9**) (191 mg, 77%) as a colourless syrup (α:β 5:1). ^{1}H NMR (500 MHz, $CDCl_3$) α-anomer: 5.36 (t, 1 H, $J_{1,2}$ 3.0, $J_{1,OH}$ 3.0 Hz, H-1), 5.00 (ddd, 1 H , $J_{2,3a}$ 6.7, $J_{2,1}$ 3.0, $J_{2,3b}$ 2.1 Hz, H-2), 4.90 (dd∼t, 1 H, $J_{5,6}$ 6.3, $J_{5,4}$ 5.6 Hz, H-5), 4.26 (ddd∼dt, 1 H, $J_{4,3a}$ 7.9, $J_{4,3b}$ 6.0, $J_{4,5}$ 5.6 Hz, H-4), 3.69 (d, 1 H, $J_{OH,1}$ 3.0 Hz, OH), 2.49 (ddd, 1 H, $J_{3a,3b}$ 14.2, $J_{3a,4}$ 7.9, $J_{3a,2}$ 6.7 Hz, H-3a), 2.01, 2.00 (2×s, 6 H, 2×OCOCH_3), 1.62 (ddd, 1 H, $J_{3b,3a}$ 14.2, $J_{3b,4}$ 6.0, $J_{3b,2}$ 2.1 Hz, H-3b), 1.19 (d, 3 H, $J_{6,5}$ 6.3 Hz, H-6); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 100.84 (C-1), 79.64 (C-4), 78.17 (C-2), 71.64 (C-5), 32.00 (C-3), 16.34 (C-6) ppm.; Anal. Calcd. for $C_{10}H_{16}O_6$ (232.2): C, 51.72; H, 6.94. Found: C, 52.15; H, 6.81.

1,2,4-Tri-O-*benzoyl-3,6-dideoxy*-β-L-xylo-*hexopyranose* (**10**).—2,5-Di-*O*-acetyl-3,6-dideoxy-L-*xylo*-furanose (**9**) (1.9 g, 8.32 mmol) was treated with 0.1 M NaOMe in dry MeOH (10 mL) for 24 h. After neutralisation with Amberlite 120 (Cl^- form) the mixture was filtered, concentrated under reduced pressure to yield 3,6-dideoxy-L-*xylo*-hexopyranose. This residue (1.0 g, 6.72 mmol) was dissolved in dry pyridine (10 mL) and dry CH_2Cl_2 (2 mL), cooled to −40 °C and benzoyl chloride (860 μL, 7.30 mmol) was added dropwise over 5 h under an atmosphere of argon. The reaction was quenched with water (10 mL), the mixture extracted with CH_2Cl_2 and the organic layer concentrated under reduced pressure. The residue was then purified by column chromatography using as eluent, 1:10 EtOAc–toluene to give the title compound **10** (2.44 g, 64%) as a colourless syrup;$[\alpha]^{20}_D$ −184.3 ($_c$ 1.9 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.25–7.22 (m, 15 H, Aryl-H), 6.17 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.61 (m, 1 H, H-2), 5.40 (m, 1 H, H-4), 4.62 (dq, 1 H, $J_{5,6}$ 6.4, $J_{5,4}$ 4.7 Hz, H-5), 2.79 (ddd, 1 H, $J_{3a,3e}$ 15.1, $J_{3a,2}$ 7.5, $J_{3a,4}$ 5.8 Hz, H-3a), 2.13 (ddd, 1 H, $J_{3a,3e}$ 15.1, $J_{3e,2}$ 5.8, $J_{3e,4}$ 3.5 Hz, H-3e), 1.43 (d, 3 H, H-6) ppm.; Anal. Calcd. for $C_{27}H_{24}O_7$ (460.5): C, 70.43; H, 5.25. Found: C, 70.08; H, 5.33.

6-Bromo-6-deoxy-L-*galactono-1,4-lactone* (**11**).—To L-galactono-1,4-lactone (**1**) (1.0 g, 5.61 mmol) was added HBr (15 mL, 30% in glacial acetic acid) and the reaction mixture was stirred for 4 h. Methanol (40 mL) was then added and the reaction mixture stirred for additional 12 h. After concentration and co-evaporation twice with MeOH (30 mL) and water (30 mL), the title compound (**11**) (1.13 g, 84%) was obtained as colourless crystals. mp 105–106 °C; $[\alpha]^{20}_D$ −105 (*c* 2.3 in H_2O); ^{13}C NMR (125.76 MHz, D_2O): δ 176.22 (C-1), 81.33 (C-4), 74.54 (C-2), 74.04 (C-3), 70.40 (C-5), 33.74 (C-6) ppm.; Anal. Calcd. for $C_6H_9O_5Br$ (241.1): C, 29.90; H, 3.76; Br, 33.15. Found: C, 30.17; H, 3.83; Br, 33.03.

The ^{13}C data found for (**11**) was consistent with the reported data for 6-bromo-D-galactono-1,4-lactone [12].

2,3,5-Tri-O-*acetyl-6-deoxy*-L-*galactono-1,4-lactone* (**12**).—6-Bromo-6-deoxy-L-galactono-1,4-lactone (**11**) (0.5 g, 2.07 mmol) in EtOAc (10 mL) and triethylamine (1 mL) was stirred in the presence of 5% Pd/C (0.1 g) under an atmosphere of H_2 (3 atm) for 16 h. Once the reaction was complete, the reaction mixture was filtered and washed with 2 N HCl (2×10 mL), the organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. Acetic anhydride (20 mL) and 60% $HClO_4$ (0.5 mL) were added and the soln stirred for 1 h. Ice water was added and the reaction mixture was extracted with CH_2Cl_2. The organic extract was washed with water, saturated $NaHCO_3$ soln, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography using 1:2 EtOAc–toluene as eluent to give the title compound (**12**) (482 mg, 81%) as an oil; $[\alpha]^{20}_D$ −29.8 (*c* 1.3 in $CHCl_3$); ^{1}H NMR

(500 MHz, $CDCl_3$): δ 5.71 (d, 1 H, $J_{2,3}$ 6.8 Hz, H-2), 5.38 (t, 1 H, $J_{3,2}$ 6.8, $J_{3,4}$ 6.7 Hz, H-3), 5.11 (dq, 1 H, $J_{5,6}$ 6.6, $J_{5,4}$ 3.1 Hz, H-5), 4.31 (dd, 1 H, $J_{4,3}$ 6.7, $J_{4,5}$ 3.1 Hz, H-4), 2.11, 2.08, 2.06 (3×s, 9 H, 3×OCOCH_3), 1.39 (d, 3 H, $J_{6,5}$ 6.6 Hz, H-6); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 168.9 (C-1), 80.4 (C-4), 72.1 (C-2), 71.9 (C-3), 67.8 (C-5), 15.4 (C-6) ppm.; Anal. Calcd. for $C_{12}H_{16}O_8$ (288.3): C, 50.00; H, 5.59. Found: C, 49.77; H, 5.11.

*2,3,5-Tri-*O*-acetyl-6-deoxy-*L*-galactofuranose* (**13**).—A solution of diisoamylborane was prepared by addition of 2-methyl-2-butene (3.8 mL, 68 mmol) in CH_2Cl_2 (15 mL) to the borane–dimethylsulfide complex (3.2 mL, 34 mmol) at 0 °C under nitrogen atmosphere. After 3 h, 2,3,5-tri-*O*-acetyl-6-deoxy-L-galactono-1,4-lactone (**12**) (482 mg, 1.68 mmol) was added and the mixture was stirred for 18 h at room temperature. Water (15 mL) was added and the mixture was stirred for 1 h, the soln concentrated and the residue was co-evaporated three times with water at 40 °C and three times with MeOH leaving the reduced hydroxyaldehyde. This residue was purified by column chromatography using as eluent 1:2 EtOAc–toluene to give the title compound (**13**) (368 mg, 76%) as a colourless oil ($\alpha:\beta$ 5:1). ^{1}H NMR (500 MHz, $CDCl_3$) α-anomer: 5.38 (t, 1 H, $J_{1,OH}$ 3.7, $J_{1,2}$ 3.2 Hz, H-1), 5.12 (m, 1 H, H-5), 5.02 (m, 2 H, H-2, H-3), 4.22 (t, 1 H, $J_{4,5}$ 5.1, $J_{4,3}$ 5.0 Hz, H-4), 3.00 (d, 1 H, $J_{OH,1}$ 3.7 Hz, OH), 2.09, 2.07, 2.06 (3×s, 9 H, 3×OCOCH_3), 1.29 (d, 3 H, $J_{6,5}$ 6.5 Hz, H-6); ^{13}C NMR (125.76 MHz, $CDCl_3$): α 100.57 (C-1), 83.65 (C-4), 82.14 (C-2), 77.78 (C-3), 69.07 (C-5), 16.14 (C-6) ppm.; Anal. Calcd. for $C_{12}H_{18}O_8$ (290.3): C, 49.65; H, 6.25. Found: C, 49.57; H, 6.21.

*2,3,4,6-Tetra-*O*-acetyl-α-L-galactopyranosyl bromide* (**14**).—1,2,3,4,6-Penta-*O*-acetyl-β-L-galactopyranose (**2**) (750 mg, 1.92 mmol) was dissolved in HBr (10 mL, 30% in glacial acetic acid) and acetic anhydride (10 mL) and the soln stirred for 2 h. The reaction mixture was then poured onto ice water (20 mL) and extracted with CH_2Cl_2 (2×100 mL). The combined organic extract was washed with sat aq $NaHCO_3$ (3×50 mL), brine soln (50 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The resulting yellow oil was purified by column chromatography using as 1:2 EtOAc–toluene + 2% triethylamine to give the title compound (**14**) (670 mg, 85%) as an oil. $[\alpha]^{20}_D$ −164 (*c* 2.4 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): d 6.51 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 5.31 (dd, 1 H, $J_{4,3}$ 3.2, $J_{4,5}$ 1.0 Hz, H-4), 5.24 (dd, 1 H, $J_{3,2}$ 10.6, $J_{3,4}$ 3.2 Hz, H-3), 4.86 (dd, 1 H, $J_{2,3}$ 10.6, $J_{2,1}$ 3.9 Hz, H-2), 4.29 (ddd∼t, 1 H, $J_{5,6b}$ 6.8, $J_{5,6a}$ 6.2 Hz, H-5), 4.02 (dd, 1 H, $J_{6a,6b}$ 11.5, $J_{6a,5}$ 6.2 Hz, H-6a), 3.97 (dd, 1 H, $J_{6b,6a}$ 11.5, $J_{6b,5}$ 6.8 Hz H-6b) ppm.; Anal. Calcd. for $C_{14}H_{19}BrO_9$ (411.2): C, 40.89; H, 4.66; Br 19.43. Found: C, 40.47; H, 4.53; Br, 19.24.

*Dibenzyl-(2,3,4,6-tetra-*O*-acetyl-β-L-galactopyranosyl)-phosphate* (**17**).—2,3,4,6-Tetra-*O*-acetyl-α-L-galactopyranosyl bromide (**14**) (670 mg, 1.63 mmol) was dissolved in a mixture of dry CH_2Cl_2 (10 mL), dry CH_3CN (10 mL) and dry Et_2O (10 mL) containing crushed 3 Å molecular sieves (1.0 g) and the soln was stirred over 20 min. To the slurry was added dibenzyl phosphate (873 mg, 3.14 mmol) and silver carbonate (865 mg, 3.14 mmol) and this mixture was stirred for 16 h with exclusion of light. The reaction mixture was then filtered, concentrated under reduced pressure and the residue purified by column chromatography using as eluent 1:2 EtOAc–toluene + 2% triethylamine to give the title compound (**17**) (839 mg, 89%) as a colourless syrup. $[\alpha]^{20}_D$ −67 (*c* 2.4 in $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6): δ 7.45–7.20 (m, 10 H, Ar-H), 5.97 (dd, 1 H, $J_{2,3}$ 10.0, $J_{2,1}$ 7.6 Hz, H-2), 5.70 (t, 1 H, $J_{1,2}$ 7.6, $^3J_{1,P}$ 7.6 Hz, H-1), 5.60 (dd, 1 H, $J_{4,3}$ 3.0, $J_{4,5}$ 1.0 Hz, H-4), 5.34 (dd, 1 H, $J_{3,2}$ 10.0, $J_{3,4}$ 3.0 Hz, H-3), 5.30–5.15 (m, 4 H, 2×CH_2Ph), 4.24 (d, 2 H, $J_{6,6}$ 6.6 Hz, 2×H-6), 3.52 (dt, 1 H, $J_{5,6}$ 6.6, $J_{5,4}$ 1.0 Hz, H-5), 1.92, 1.90, 1.78, 1.76 (4×s, 12 H, 4×OCOCH_3); ^{13}C NMR (125.76 MHz, $CDCl_3$): δ 96.2 (C-1), 71.1 (C-5), 69.8 (C-3), 69.1, 69.0 (2×PhCH_2), 68.1 (C-2), 66.1 (C-4), 60.5 (C-6) ppm.

β-L-Galactopyranosylphosphate disodium salt (**20**).—A soln of dibenzyl-(2,3,4,6-tetra-*O*-acetyl-β-L-galactopyranosyl)phosphate (**17**) (950 mg, 1.64 mmol) in EtOH (10 mL) and 1 M $NaHCO_3$ (2 mL) was treated with 10% Pd/C (0.2 g) and stirred under an atmosphere of hydrogen for 24 h. The reaction mixture was then filtered, concentrated under reduced pressure, diluted in water (40 mL) and washed with CH_2Cl_2 (2×40 mL). The aq layer was separated, cooled to 0 °C and NaOH (2 M) was added until pH 12. The soln was stirred at this pH for 4 h and was then carefully neutralised with Dowex 50WX H^+ resin, filtered and lyophilised. The residue was purified by Sephadex G10 chromatography using water as eluent ar a rate of 10 mL/h. The appropriate fractions were pooled and lyophilised to yield the title

compound (**20**) (309 mg, 69%) as an amorphous lather. ^{1}H NMR (400 MHz, D_2O): δ 4.84 (t, 1 H, $J_{1,2}$ 7.6, $^3J_{1,P}$ 7.6 Hz, H-1), 3.90 (d, 1 H, $J_{4,3}$ 3.0 Hz, H-4), 3.73–3.65 (m, 4 H, H-3, H-5, 2×H-6), 3.52 (dd, 1 H, $J_{2,3}$ 9.9, $J_{2,1}$ 7.6 Hz, H-2) ppm.

2,4,6-Tri-O-*benzoyl-3-deoxy*-α-L-xylo-*hexopyranosyl bromide* (**15**).—1,2,4,6-Tetra-*O*-benzoyl-3-deoxy-β-L-*xylo*-hexopyranose (**6**) (1.0 g, 1.72 mmol) was dissolved in HBr (5 mL, 30% in glacial acetic acid) and Ac_2O (5 mL) and the soln stirred for 2 h. The reaction mixture was then poured onto ice water (20 mL) and extracted with CH_2Cl_2 (2×100 mL). The combined organic extract was washed with sat $NaHCO_3$ (3×50 mL), brine soln (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure. The resulting oil was purified by column chromatography using as 1:2 EtOAc–toluene + 2% triethylamine to give the title compound (**15**) (583 mg, 63%) as an oil. $[\alpha]^{20}_D$ −147.3 (*c* 1.8 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15–7.18 (m, 15 H, Ar-H), 6.93 (d, 1 H, $J_{1,2}$ 2.7 Hz, H-1), 5.64 (d, 1 H, $J_{4,3a}$ 2.8 Hz, H-4), 5.35 (ddd, 1 H, $J_{2,3a}$ 11.8, $J_{2,3e}$ 4.2, $J_{2,1}$ 2.7 Hz, H-2), 4.66 (dd, 1 H, $J_{5,6a}$ 7.1, $J_{5,6b}$ 5.7 Hz, H-5), 4.57 (dd, 1 H, $J_{6a,6b}$ 11.6, $J_{6a,5}$ 7.1 Hz, H-6a), 4.48 (dd, 1 H, $J_{6b,6a}$ 11.6, $J_{6b,5}$ 5.7 Hz, H-6b), 2.56 (ddd, 1 H, $J_{3a,3e}$ 14.0, $J_{3a,2}$ 11.8, $J_{3a,4}$ 2.8 Hz, H-3a), 2.42 (m, 1 H, H-3e) ppm.; Anal. Calcd. for $C_{27}H_{23}BrO_7$ (539.4): C, 60.12; H, 4.30; Br, 14.81. Found: C, 57.41; H, 4.11; Br, 14.53 (labile compound).

Dibenzyl-(2,4,6-tri-O-*benzoyl-3-deoxy*-β-L-xylo-*hexopyranosyl)-phosphate* (**18**).—2,4,6-Tri-*O*-benzoyl-3-deoxy-α-L-*xylo*-hexopyranosyl bromide (**15**) (583 mg, 1.08 mmol) was dissolved in a mixture of dry CH_2Cl_2 (10 mL), dry CH_3CN (10 mL) and dry Et_2O (10 mL) containing crushed 3 Å molecular sieves (1.0 g) and the soln was stirred over 20 min. To this slurry was added dibenzyl phosphate (585 mg, 2.10 mmol) and silver carbonate (580 mg, 2.10 mmol) and this mixture was stirred for 18 h with exclusion of light. The reaction mixture was then filtered, concentrated under reduced pressure and the residue purified by column chromatography using as eluent 1:2 EtOAc–toluene + 2% triethylamine to give the title compound (**18**) (685 mg, 90%) as a colourless syrup. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14–7.30 (m, 25 H, Ar-H), 5.67 (t, 1 H, $J_{1,2}$ 7.5, $^3J_{1,P}$ 7.5 Hz, H-1), 5.54 (d, 1 H, $J_{4,3a}$ 2.8 Hz, H-4), 5.45 (ddd, 1 H, $J_{2,3a}$ 12.0, $J_{2,1}$ 7.5, $J_{2,3e}$ 5.3 Hz, H-2), 5.07–4.81 (m, 4 H, 2×CH_2Ph), 4.54 (dd, 1 H, $J_{6a,6b}$ 11.4, $J_{6a,5}$ 7.1 Hz, H-6a), 4.46 (dd, 1 H, $J_{6b,6a}$ 11.4, $J_{6b,5}$ 5.5 Hz, H-6b), 4.36 (dd, 1 H, $J_{5,6a}$ 7.1, $J_{5,6b}$ 5.5 Hz, H-5), 2.76 (m, 1 H, H-3e), 2.07 (ddd, 1 H, $J_{3a,3e}$ 14.1, $J_{3a,2}$ 12.0, $J_{3a,4}$ 2.8 Hz, H-3a) ppm.

3-Deoxy-β-L-xylo-hexopyranosylphosphate dilithium salt (**21**).—A soln of dibenzyl-(2,3,4,6-tri-*O*-benzoyl-3-deoxy-β-L-*xylo*-hexopyranosyl)-phosphate (**18**) (500 mg, 0.71 mmol) in EtOH (10 mL) and 1 M $NaHCO_3$ (2 mL) was treated with 5% Pd/C (0.2 g) and stirred under an atmosphere of H_2 for 1 h. The reaction mixture was then filtered, concentrated under reduced pressure, diluted with CH_2Cl_2 (40 mL) and washed with water (2×40 mL). The organic layer was separated, concentrated under reduced pressure, dissolved in 2:1 water–MeOH and aq NaOH was added until pH 12. The reaction mixture was stirred for 3 h, then neutralised with AcOH (1M) and this soln was further diluted with water (200 mL) and absorbed onto a resin ion exchange column Dowex 2×8 (1.5×20 cm, Cl^-). The column was washed with water (200 mL) then with a gradient eluent of LiCl solution (0–0.8 M) at a flow rate of 1.5 mL/min. Fractions containing the desired product were pooled and lyophilised and then the residue was desalted using Sephadex G10 chromatography (100×1.6 cm) with a flow rate of 1 mL/min using water as eluent. The appropriate fractions were pooled and lyophilised to yield the title compound (**21**) (143 mg, 79%) as a colourless syrup. ^{1}H NMR (500 MHz, D_2O): δ 4.89 (t, 1 H, $J_{1,2}$ 7.6, $^3J_{1,P}$ 7.6 Hz, H-1), 4.03 (m, 1 H, H-4), 3.85–3.75 (m, 3 H, H-5, H-6a, H-6b), 3.73 (m, 1 H, H-2), 2.21 (ddd, 1 H, $J_{3e,3a}$ 13.8, $J_{3e,4}$ 4.5, $J_{3e,2}$ 3.8, Hz, H-3e), 1.79 (ddd, 1 H, $J_{3a,3e}$ 13.8, $J_{3a,2}$ 12.2, $J_{3a,4}$ 2.9 Hz H-3a) ppm.

Dibenzyl-(2,4-di-O-*benzoyl-3,6-dideoxy*-β-L-xylo-*hexopyranosyl)-phosphate* (**19**).—To a soln of 1,2,4-tri-*O*-benzoyl-3,6-dideoxy-β-L-*xylo*-hexopyranose (**10**) (481 mg, 1.05 mmol) in dichloromethyl methyl ether (2.5 mL) was added a catalytic amount of zinc chloride (50 mg, 0.36 mmol), and the reaction mixture was stirred for 2 h under an atmosphere of argon. The reaction mixture was quenched by adding toluene (5 mL) and then concentrated under reduced pressure. The residue was extracted with CH_2Cl_2, washed with 2 N H_2SO_4, sat $NaHCO_3$, water, dried ($MgSO_4$) and concentrated under reduced pressure. Chloride (**16**) was used directly in the preparation of the glycosyl phosphate (**19**) without any further purification and was dissolved in a mixture of dry CH_2Cl_2 (10 mL), dry CH_3CN (10 mL) and dry

Et_2O (10 mL) containing crushed 3 Å molecular sieves (1.0 g) and stirred for 20 min. To this slurry was added dibenzyl phosphate (584 mg, 2.10 mmol) and silver carbonate (580 mg, 2.10 mmol) and this mixture was stirred for 18 h with exclusion of light. The reaction mixture was then filtered, concentrated under reduced pressure and the residue purified by column chromatography using as eluent 1:2 EtOAc–toluene + 1% triethylamine to give the title compound (**19**) (356 mg, 55%) as a colourless syrup. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.25–7.11 (m, 20 H, Aryl-H), 5.62 (dd, 1 H, $J_{1,2}$ 7.9, $^3J_{1,P}$ 7.0 Hz, H-1), 5.40 (ddd, 1 H, $J_{2,3a}$ 12.2, $J_{2,1}$ 7.9, $J_{2,3e}$ 5.5 Hz, H-2), 5.31 (dd, 1 H, $J_{4,3e}$ 3.5, $J_{4,3a}$ 3.1, $J_{4,5}$ 1.5 Hz, H-4), 5.13–4.81 (m, 4 H, 2×CH_2Ph), 4.11 (dq, 1 H, $J_{5,6}$ 6.5 Hz, H-5), 2.69 (ddd, 1 H, $J_{3e,3a}$ 14.0, $J_{3e,2}$ 5.5, $J_{3e,4}$ 3.5 Hz, H-3e), 2.08 (ddd∼dt, 1 H, $J_{3a,3e}$ 14.0, $J_{3a,2}$ 12.2, $J_{3a,4}$ 3.1 H-3a), 1.26 (d, 3 H, $J_{6,5}$ 6.5 Hz, H-6) ppm.

The ^{1}H data found for (**19**) was consistent with that reported for dibenzyl-(2,4-di-*O*-benzoyl-3,6-dideoxy-β-L-*xylo*-hexopyranosyl)- phosphate [2,5].

3,6-Dideoxy-β-L-xylo-hexopyranosylphosphate dilithium salt (**22**).—Dibenzyl-(2,4-di-*O*-benzoyl-3,6-dideoxy-β-L-*xylo*-hexopyranosyl)-phosphate (**19**) (356 mg, 0.57 mmol) in EtOH (10 mL) and 1 M $NaHCO_3$ (2 mL) was treated with 5% Pd/C (0.2 g) and stirred under an atmosphere of H_2 for 1 h. The reaction mixture was then filtered, concentrated under reduced pressure, diluted in CH_2Cl_2 (40 mL) and washed with water (2×40 mL). The organic layer was separated, concentrated under reduced pressure, dissolved in 2:1 water–MeOH and aq NaOH was added until pH 12. The reaction mixture was stirred for 3 h, then neutralised with acetic acid (1M) and this soln was further diluted with water (200 mL) and absorbed onto a resin ion exchange column Dowex 2×8 (1.5×20 cm, Cl^-). The column was washed with water (200 mL) then with a gradient eluent of LiCl solution (0–0.8 M) at a flow rate of 1.5 mL/min. Fractions containing the desired product were pooled and lyophilised and then the residue was desalted using Sephadex G10 chromatography (100×1.6 cm) with a flow rate of 1 mL/min using water as eluent. The appropriate fractions were pooled and lyophilised to yield the title compound (**22**) (91 mg, 65%) as an amorphous lather. ^{1}H NMR (500 MHz, D_2O): β 4.80 (t, 1 H, $J_{1,2}$ 7.5, $^3J_{1,P}$ 7.5 Hz, H-1), 3.81 (dq, 1H, $J_{5,6}$ 6.5, $J_{5,4}$ 1 Hz, H-5), 3.63 (ddd, 1 H, $J_{2,3a}$ 12.0, $J_{2,1}$ 7.5, $J_{2,3e}$ 4.9 Hz, H-2), 3.41 (m, 1 H, H-4), 2.14 (ddd, 1 H, $J_{3e,3a}$ 14.0, $J_{3e,2}$ 4.9 Hz, $J_{3e,4}$ 4.0 Hz, H-3e), 1.75 (ddd, 1 H, $J_{3a,3e}$ 14.0, $J_{3a,2}$ 12.0, $J_{3a,4}$ 3.0 Hz, H-3a), 1.23 (d, 3 H, $J_{6,5}$ 6.5 Hz, H-6) ppm.

^{1}H NMR data are consistent with that reported in the literature [4].

Guanosine-5′-(β-L-galactopyranosyl)-diphosphate dilithium salt (**27**).—A soln of β-L-galactopyranosyl-phosphate disodium salt (**20**) (264 mg, 0.87 mmol) in water (50 mL) was slowly adsorbed onto a resin bed of Dowex 50W × 8 (tributylammonium-form, 2.4×28 cm) and slowly washed with water. Fractions containing the desired compound were pooled and lyophilised. Galactopyranosyl-phosphate bis-(tributylammonium) salt (**23**) and guanosine-5′-monophosphate morpholidate (**26**) were combined in dry 1:1 pyridine–dry DMF (10 mL) and evaporated under reduced pressure in order to dry the compounds. This procedure was repeated twice. The heterogeneous reaction was then stirred for 8 days under an atmosphere of argon. Evaporation of the solvent gave a syrup, which was diluted to 200 mL with water and passed through a column of Dowex 1 × 8 (200–400 mesh) resin (Cl^-, 2.4×28 cm). The column was washed with water (200 mL) and then eluted with a linear gradient of LiCl (0–0.8 M) using a flow rate of 4 mL/min. GDP-L-galactose eluted at a concentration of 0.17–0.19 M LiCl. The relevant fractions were pooled, concentrated to 6 mL and passed through a Sephadex G10 column (100×1.6 cm) using water as eluent at a rate of 10 mL/h. Fractions containing the product were pooled and lyophilised, affording the title compound (**27**) (155 mg, 29%) as an amorphous lather. ^{1}H NMR (400 MHz, D_2O): δ 8.10 (s, 1 H, H-8), 5.97 (d, 1 H, $J_{1',2'}$ 6.2 Hz, H-1′), 4.95 (t, 1 H, $J_{1''2''}$ 7.8, $^3J_{1'',P}$ 7.8 Hz, H-1″), 4.80 (m, 1 H, H-2′), 4.55 (m, 1 H, H-3′), 4.35 (m, 1 H, H-4′), 4.22–4.18 (m, 2 H, 2×H-5′), 4.01 (d, 1 H, $J_{4'',3''}$ 3.0 Hz, H-4″), 3.89 (dd, 1 H, $J_{3'',2''}$ 10.6, $J_{3'',4''}$ 3.0 Hz, H-3″), 3.84–3.73 (m, 3 H, H-5″, 2×H-6″), 3.70 (dd, 1H, $J_{2'',3''}$ 10.6, $J_{2'',1''}$ 7.8 Hz, H-2″); ^{13}C NMR (125.76 MHz, D_2O): δ 159.93 (C-6), 154.85 (C-2), 152.72 (C-4), 138.73 (C-8), 117.13 (C-5), 99.36 (d, $J_{1'',P}$ 6.3 Hz, C-1″), 87.60 (C-1′), 84.68 (d, $J_{4',P}$ 8.9 Hz, C-4′), 76.70 (C-4″), 74.49 (C-2′), 73.15 (C-3″), 72.12 (d, $J_{2'',P}$ 7.6 Hz, C-2″), 71.33 (C-3′), 69.48 (C-5″), 66.17 (d, $J_{5',P}$ 5.6 Hz, C-5′), 62.05 (C-6″) ppm.

Guanosine-5′-(3″-deoxy-β-L-xylo-hexopyranosyl)-diphosphate dilithium salt (**28**).—3-Deoxy-β-L-*xylo*-hexopyranosylphosphate dilithium salt (**21**)

(143 mg, 0.49 mmol) was treated as described in the synthesis of (**27**). Ion-exchange chromatography and subsequent desalting on Sephadex G 10 column afforded the title compound (**28**) (80 mg, 27%) as an amorphous lather. ^{1}H NMR (500 MHz, D_2O): δ 8.13 (s, 1 H, H-8), 5.97 (d, 1 H, $J_{1',2'}$ 6.1 Hz, H-1′), 5.01 (t, 1 H, $J_{1'',2''}$ 7.9, $^3J_{1'',P''}$ 7.9 Hz, H-1″), 4.83 (m, 1 H, H-2′), 4.57 (m, 1 H, H-3′), 4.40 (m, 1 H, H-4′), 4.25 (m, 2 H, 2×H-5′), 4.20 (m, 1 H, H-4″), 3.85–3.76 (m, 3 H, H-5″, H-6a″, H-6b″), 3.73 (ddd, 1 H, $J_{2'',3a''}$ 11.5, $J_{2'',1''}$ 7.9, $J_{2'',3e''}$ 3.8 Hz, H-2″), 2.22 (ddd, 1 H, $J_{3e'',3a''}$ 13.5, $J_{3e'',4''}$ 4.7, $J_{3e'',2''}$ 3.8 Hz, H-3e″), 1.77 (ddd, 1 H, $J_{3a'',3e''}$ 13.5, $J_{3a'',2''}$ 11.5, $J_{3a'',4''}$ 3.4 Hz H-3a″); ^{13}C NMR (125,76 MHz, D_2O): δ 159.84 (C-6), 154.84 (C-2), 152.58 (C-4), 138.37 (C-8), 101.09 (d, C-1″, $J_{1'',P}$ 6.3 Hz), 87.53 (C-1′), 84.55 (d, C-4′, $J_{4',P}$ 8.8 Hz), 79.78 (C-4″), 74.37 (C-2′), 71.19 (C-3′), 66.68 (d, C-2″, $J_{2'',P}$ 7.6 Hz), 66.36 (C-5″), 66.07 (d, C-5′, $J_{5',P}$ 6.3 Hz), 62.15 (C-6″), 37.13 (C-3″) ppm.

Guanosine-5′-(3″,6″-dideoxy-β-L-xylo-*hexopyranosyl)-diphosphat dilithium salt* (**29**).—3,6-Dideoxy-*β*-L-*xylo*-hexopyranosylphosphate dilithium salt (**22**) (91 mg, 0.43 mmol) was treated as described in the synthesis of (**27**). Ion-exchange chromatography and desalting on Sephadex G 10 afforded the title compound (**29**) (47 mg, 19%) as an amorphous lather. ^{1}H NMR (500 MHz, D_2O): β 8.11 (s, 1 H, H-8), 5.96 (d, 1 H, $J_{1',2'}$ 6.0 Hz, H-1′), 5.03 (t, 1 H, $J_{1'',2''}$ 8.0, $^3J_{1'',P''}$ 8.0 Hz, H-1″), 4.79 (m, 1 H, H-2′), 4.53 (m, 1 H, H-3′), 4.37 (m, 1 H, H-4′), 4.21 (m, 2 H, 2×H-5′), 3.82–3.65 (m, 3 H, H-2″, H-4″, H-5″), 2.19 (ddd, 1 H, $J_{3e'',3a''}$ 13.5, $J_{3e'',2''}$ 5.0, $J_{3e'',4''}$ 4.1 Hz, H-3e″), 1.69 (ddd, 1 H, $J_{3a'',3e''}$ 13.5, $J_{3a'',2''}$ 12.0, $J_{3a'',4''}$ 3.1 Hz H-3a″), 1.19 (d, 3H, $J_{6'',5''}$ 6.4 Hz, H-6″) ppm.

The analytical data found for (**29**) are consistent with those reported [4].

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