The first chemical synthesis of UDP-α-D-galactofuranose

Yury E. Tsvetkov and Andrei V. Nikolaev *

Department of Chemistry, University of Dundee, Dundee, UK DD1 4HN

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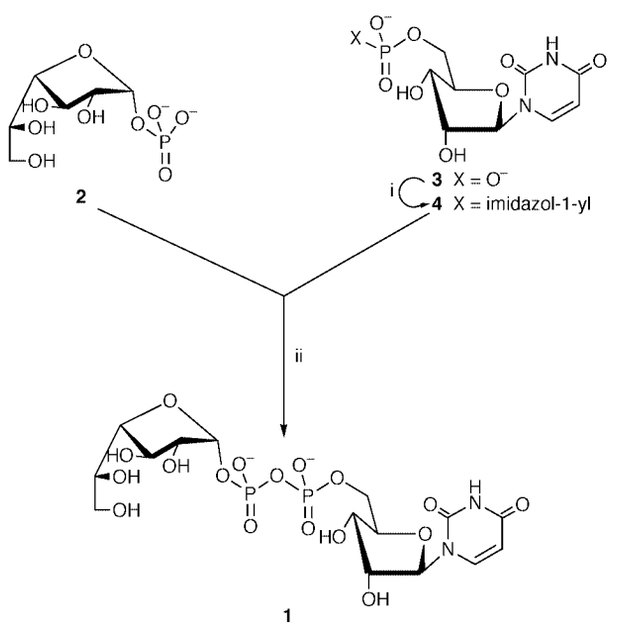
Uridine 5′-diphosphate α-D-galactofuranose, which is likely to be a biochemical donor of D-galactofuranosyl residues in Nature, is synthesized from α-D-galactofuranosyl phosphate and uridine 5′-monophosphate.

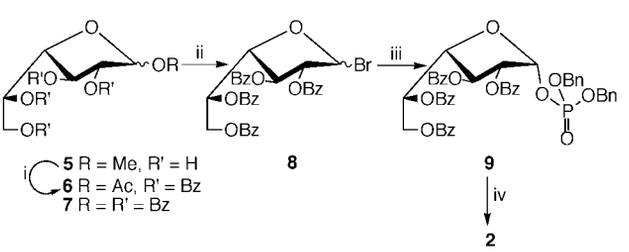
Introduction

D-Galactofuranose (D-Gal*f*) is distributed in Nature as a component of immunologically important glycoconjugates from bacteria, protozoa and fungi, including some clinically significant pathogens. D-Gal*f* residues are structural blocks of the cell wall of *Mycobacterium tuberculosis* and other mycobacteria[1] and principal constituents of the glycosylphosphatidylinositol (GPI) anchor of *Leishmania* lipophosphoglycans.[2] They are also present in lipopeptidophosphoglycans of *Trypanosoma cruzi*,[3] in glycosylinositolphospholipids of *Leishmania*[4] and *Endotrypanum schaundinni*[5] and in a complex and unique peptidophosphogalactomannan of *Penicillium charlesii*.[6] In addition, they are found in *N*-linked glycoproteins of *Crithidia fasciculata*, *Crithidia harmonosa*, *Leishmania samueli*, *Herpetomonas samuelpessoai* and *Trypanosoma cruzi*.[3] D-Gal*f*-containing conjugates are vital for the survival of parasites and bacteria. Since this monosaccharide is not present in humans, the enzymes involved in the biosynthesis and transfer of D-galactofuranose become important drug targets.

It was suggested[7,8] (but not strictly proved) that uridine 5′-diphosphate α-D-galactofuranose (UDP-Gal*f*) **1** is expected to be a substrate for D-galactofuranosyl transferases responsible for the transfer of D-Gal*f* units in the biosynthesis of various glycoconjugates. Compound **1** was first prepared recently[9] by enzymic methods from UDP-α-D-galactopyranose in 3% yield using the enzyme UDP-galactopyranose mutase. The authors claimed the procedure is able 'to produce about 0.5 mg of the UDP-Gal*f* in a week'.[9] Obviously, greater amounts of the compound, required for biochemical studies, could be produced by chemical synthesis. In this paper we report the first chemical synthesis of the nucleotide sugar **1**.



Scheme 1 *Reagents*: i, 1,1′-carbonyldiimidazole, DMF; ii, DMF as solvent.



Scheme 2 *Reagents*: i, (a) PhCOCl, pyridine; (b) H_2SO_4, Ac_2O; ii, HBr, AcOH–CH_2Cl_2; iii, $(BnO)_2PO_2H$, Et_3N, toluene; iv, (a) H_2, $Pd(OH)_2/C$, ethyl acetate–Et_3N; (b) Et_3N–MeOH–water.

Results and discussion

UDP-Gal*f* **1** was prepared from α-D-galactofuranosyl phosphate **2** and uridine 5′-monophosphate **3** (Scheme 1). Compound **2**, in turn, was synthesized starting from methyl α,β-D-galactofuranoside **5**,[10] which was converted to the known[11] galactofuranosyl dibenzyl phosphate **9** (Scheme 2) by consecutive benzoylation, acetolysis (→ **6**), 1-bromination (→ **8**) and glycosylation of dibenzyl hydrogen phosphate in the presence of triethylamine. This approach appeared to be more reliable in our hands than the preparation of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose **7**,[12,13] as a starting substance, by direct benzoylation of D-galactose. Two-step deprotection of the phosphotriester **9** was performed as described[11] to produce the glycosyl phosphate **2** (88%) isolated as a triethylammonium salt.

The phospho-imidazolidate activation[14–16] was chosen for the preparation of the pyrophosphate **1**. This method was reported[16] to be equally effective as the phospho-morpholidate approach for *O*-deprotected derivatives. Before the condensation, the nucleotide **3** (triethylammonium salt) was converted to the activated UMP-imidazolide **4** by the reaction with 1,1′-carbonyldiimidazole. The reaction of compounds **2** and **4** in dimethylformamide (DMF) was monitored by ^{31}P NMR, which revealed the greatest proportion of the product **1** (corresponding to 45–50% yield) in the mixture at 18–19 h. Basic degradation of UDP-Gal*f* **1** (δ_P −9.9, d and −10.7, d, $J_{P,P}$ 20.9 Hz) seemed to be the major side reaction, decreasing the yield and resulting in α-D-galactofuranose 1,2-cyclophosphate

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(δ_P 17.2)† and UMP **3** (δ_P 1.6). The nucleotide **3** then reacted with the imidazolide **4** (δ_P −8.7), which is in excess, to give symmetrical diuridine diphosphate (δ_P −9.6, d, $J_{P,P}$ 21.5 Hz). After the work-up, UDP-Gal*f* **1** (a dipotassium salt) was isolated in 23% yield using gel-chromatography on a column of Sephadex G-15 followed by reversed-phase C-18 HPLC.

It is noteworthy that coupling of the galactosyl phosphate **2** and UMP-morpholidate in pyridine in the presence of 1*H*-tetrazole, which was reported[18] to be an efficient catalyst in phospho-morpholidate condensations, produced the nucleotide sugar **1** in 12% yield. The highest proportion of the product (corresponding to 25–30% yield) in the reaction mixture was observed at 15–17 h using ^{31}P NMR spectroscopy.

The structure of compound **1** was unambiguously confirmed by NMR and mass spectrometry. The ^{31}P NMR spectrum exhibited two doublet signals (δ_P −10.1, d, P^{α} and −11.4, d, P^{β}, $J_{P,P}$ 20.9 Hz), which are characteristic for nucleoside diphosphosugars.[9,14–16,18] The presence of the (1→5)-pyrophosphate bridge was confirmed by the C-1, C-2, H-1 and H-2 signals of the D-galactofuranose residue and C-4 and C-5 signals of the D-ribofuranose residue (see Experimental section). These signals were shifted as a result of the α- and β-effects of phosphorylation and coupled with phosphorus. The α-configuration of the D-galactofuranosyl phosphate fragment was evident from the value of $J_{H1,H2}$ 4.2 Hz, which was close to that published[11] for the α-phosphate **2**. The coupling constant value for the corresponding isomeric β-phosphate was reported[11] to be about 1.7 Hz. In general, our ^{1}H, ^{13}C and ^{13}P NMR data were in agreement with those published[9] for UDP-Gal*f* **1**, apart from distinctive assignments of signals for C-2, C-3 and C-4 of the D-ribofuranose residue, which were amended in the light of the ^{13}C NMR data of uridine 5′-monophosphate **3**.[19] The molecular mass of compound **1** was confirmed by electrospray mass spectrometry. The signals in the mass spectrum (see Experimental section) corresponded to the pseudo-molecular ions for the uridine diphosphohexose (*M*, 566.055 for the free acid **1**).

It should be noted that UDP-Gal*f* **1** is rather unstable: it was hydrolysed (≈95% extent), forming D-galactose and uridine 5′-diphosphate in 0.015 mol dm^{-3} aq. solution after 24 h at 22 °C. Nevertheless, a frozen solution of the compound was stored at −80 °C for several months without detectable degradation. A purity control could be properly performed using reversed-phase C-18 HPLC.

The nucleotide sugar **1** is currently being tested as a substrate for the *Leishmania* β-D-galactofuranosyl transferase and the results will be published in due course.

Experimental

General procedures

Mps were determined on a Reichter hot-plate apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter; $[a]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra (^{1}H at 300 MHz, ^{13}C at 75 MHz, and ^{31}P at 121 MHz) were recorded with a Bruker DPX-300 spectrometer for solutions in deuterium oxide, unless otherwise indicated. Chemical shifts (δ in ppm) are given relative to those for Me_4Si (for ^{1}H and ^{13}C) and external aq. 85% H_3PO_4 (for ^{31}P); *J*-values are given in Hz. ES mass spectra were recorded with a Micromass Quattro system (Micromass Biotech, UK). Flash-column chromatography (FCC) was performed on Kieselgel 60 (0.040–0.063 mm) (Merck). Gel-filtration chromatography was performed on a column (95 × 2.5 cm) of Sephadex G-15 in water, flow rate 1.5 cm^3 min^{-1}. Reversed-phase HPLC was done on a semipreparative C-18 column (Chromsphere 5, 25 × 1 cm) using 0.02 mol dm^{-3} aq. KH_2PO_4 as eluent at 3 cm^3 min^{-1} with UV-monitoring at 270 nm. Anhydrous pyridine and DMF were purchased from Aldrich. Dichloromethane, toluene and pyridine were freshly distilled from CaH_2. All the solutions containing UDP-Gal*f* **1** were concentrated *in vacuo* (oil pump, ≈0.01 mmHg) at 18–20 °C.

Uridine 5′-monophosphate disodium salt (Sigma) was converted to the triethylammonium salt **3** (containing 1.42 equiv. of Et_3N, detected by ^{1}H NMR spectrometry) by passing its aq. solution through a column (15 × 2.5 cm) of Dowex 50W × 8 (triethylammonium form); the column was washed with water, then appropriate fractions were pooled and freeze-dried.

Dibenzyl 2,3,5,6-tetra-*O*-benzoyl-α-D-galactofuranosyl phosphate 9

1-*O*-Acetyl-2,3,5,6-tetra-*O*-benzoyl-α,β-D-galactofuranose **6** {$[a]_D^{25}$ +9.5 (*c* 1, $CHCl_3$); δ_H ($CDCl_3$) 2.10 (s, Ac^{α}), 2.22 (s, Ac^{β}), 4.69–4.86 (3 H, m, 4-H and 6-H_2), 5.64 (d, $J_{2,3}$ 1.4, 2-H^{β}), 5.75 (dd, $J_{3,4}$ 4.8, 3-H^{β}), 5.79 (dd, $J_{2,3}$ 7.1, 2-H^{α}), 5.91 (dt, $J_{4,5}$ = $J_{5,6a}$ = 6.0, $J_{5,6b}$ 3.8, 5-H^{α}), 6.12 (dt, $J_{4,5}$ = $J_{5,6a}$ = 4.0, $J_{5,6b}$ 7.0, 5-H^{β}), 6.27 (dd, $J_{3,4}$ 6.3, 3-H^{α}), 6.55 (s, 1-H^{β}), 6.70 (d, $J_{1,2}$ 4.8, 1-H^{α}) and 7.15–8.20 (20 H, m, 4 × Ph); α : β = 1 : 3.7} was prepared from methyl α,β-D-galactofuranoside **5**[10] by conventional benzoylation with benzoyl chloride in pyridine followed by acetolysis with 1.4% H_2SO_4 in Ac_2O (v/v).

To a stirred solution of the tetrabenzoate **6** (3.13 g, 4.9 mmol) in CH_2Cl_2 (30 cm^3) cooled to 0 °C was added a 33% solution (10 cm^3) of HBr in AcOH. After storage for 2 h at 0 °C and an additional 2 h at rt, the mixture was concentrated to dryness and the residue was azeotroped with toluene (3 × 30 cm^3) to give the galactofuranosyl bromide **8**.

To a stirred mixture of dibenzyl hydrogen phosphate (1.91 g, 6.86 mmol) and Et_3N (0.97 cm^3, 6.95 mmol) in toluene (10 cm^3) was added a solution of the prepared bromide **8** in the same solvent (25 cm^3) and the stirring was continued for a further 16 h. The solid (Et_3NHBr) was filtered off and the solvent was removed under reduced pressure. FCC (toluene–ethyl acetate, 9 : 1 v/v) of the residue produced the protected α-D-galactofuranosyl phosphate **9** (2.48 g, 59%) as a solid, mp 110–111 °C; $[a]_D^{26}$ +51.3 (*c* 1, $CHCl_3$); δ_P −2.35 {lit.,[11] mp 112–113 °C; $[a]_D$ +54.6 (*c* 1, $CHCl_3$); δ_P −3.66}. The ^{1}H and ^{13}C NMR data were virtually identical to those published.[11]

α-D-Galactofuranosyl bis(triethylammonium) phosphate 2

The galactosyl phosphate **9** (1.82 g, 2.13 mmol) was deprotected by successive hydrogenation over palladium hydroxide (20 wt% Pd on carbon, contains ≈50% of water; 1 g, ≈0.94 mmol Pd) in ethyl acetate–triethylamine (10 : 1) and debenzoylation with MeOH–water–triethylamine (5 : 2 : 1 v/v/v) following the published[11] procedure. The resulting syrup was dissolved in water and applied to a column (12 × 2.5 cm) of Amberlite IRA-420 (HCO_3^--form) resin. The column was first washed with water and then with a gradient of 0.1→0.4 mol dm^{-3} aq. triethylammonium hydrogen carbonate. Appropriate fractions were freeze-dried to give the phosphate **2** (0.865 g, 88%) as a hygroscopic amorphous solid, $[a]_D^{26}$ +44.5 (*c* 1, water); δ_P 0.43 {lit.,[11] for the bis(cyclohexylammonium) salt $[a]_D$ +41 (*c* 1, water); δ_P 2.50}. The ^{1}H NMR data were nearly identical to those published.[11]

Uridine 5′-(α-D-galactofuranosyl diphosphate), dipotassium salt 1

To a solution of uridine 5′-monophosphate **3** (93 mg, 0.20 mmol of the triethylammonium salt containing 1.42 equiv. of Et_3N) in DMF (1.5 cm^3) was added 1,1′-carbonyldiimidazole (97 mg, 0.60 mmol) and the mixture was kept for 3 h at rt.

† The ^{13}P NMR chemical-shift value is characteristic for similar cyclic phosphates:[17] for uridine 2′,3′-cyclophosphate, δ_P 17.6; for 4-nitrophenyl α-D-galactopyranoside 3,4-cyclophosphate, δ_P 16.8.

Methanol (0.05 cm^3) was added to destroy the excess of reagent and the reaction mixture was concentrated. The residue was azeotroped with pyridine (3 × 2 cm^3), then dried *in vacuo* for 15–20 min to produce the imidazolide **4**. α-D-Galactofuranosyl phosphate **2** (58 mg, 0.126 mmol) was dried by evaporation of pyridine (3 × 2 cm^3). A solution of the imidazolide **4** in pyridine (2 cm^3) was added and the resulting mixture was taken to dryness again. The residue was dissolved in DMF (1 cm^3) and the solution was kept under argon at rt while the reaction was monitored by ^{31}P NMR spectroscopy. After 19 h, the reaction mixture was diluted with water, washed with chloroform and the aqueous phase was concentrated. Gel chromatography of the residue (with monitoring by analytical HPLC on C-18 column) provided UDP-Gal*f*-containing fractions, which were pooled, concentrated and repurified using preparative HPLC to separate the product **1** from UMP **3** and uridine 5′-diphosphate (the retention times were 4.50 min, 5.20 min and 4.15 min, respectively). The appropriate fractions were desalted twice by gel filtration to produce UDP-Gal*f* **1** (18.4 mg, 22.7%) as an amorphous solid, $[a]_D^{22}$ +18.5 (*c* 0.75, water); δ_H 3.49 (1 H, dd, $J_{5,6a}$ 7.0, $J_{6a,6b}$ 11.6, 6-H^a, Gal), 3.58 (1 H, dd, $J_{5,6b}$ 4.1, 6-H^b, Gal), 3.64 (1 H, ddd, 5-H, Gal), 3.69 (1 H, dd, $J_{4,5}$ 5.1, 4-H, Gal), 4.03 (1 H, ddd, $J_{2,3}$ 8.5, $J_{2,P}$ 2.2, 2-H, Gal), 4.07–4.17 (3 H, m, 4-H and 5-H_2, Rib), 4.11 (1 H, dd, $J_{3,4}$ 7.1, 3-H, Gal), 4.24 (2 H, m, 2- and 3-H, Rib), 5.51 (1 H, dd, $J_{1,2}$ 4.2, $J_{1,P}$ 5.5, 1-H, Gal), 5.83 (1 H, d, $J_{5,6}$ 8.7, 6-H, uracil), 5.85 (1 H, d, $J_{1,2}$ 4.9, 1-H, Rib) and 7.83 (1 H, d, 5-H, uracil); δ_C 62.6 (C-6, Gal), 65.3 (d, $J_{5,P}$ 5.1, C-5, Rib), 70.1 (C-3, Rib), 72.5 (C-5, Gal), 73.9 (C-3, Gal), 74.2 (C-2, Rib), 77.0 (d, $J_{2,P}$ 8.0, C-2, Gal), 82.2 (C-4, Gal), 83.6 (d, $J_{4,P}$ 9.5, C-4, Rib), 88.7 (C-1, Rib), 98.0 (d, $J_{1,P}$ 5.5, C-1, Gal), 103.1 (C-6, uracil) and 142.0 (C-5, uracil); δ_P −10.1 (d, $J_{P,P}$ 20.9, P^α) and −11.4 (d, P^β); ESMS(−) *m*/*z* 564.61 (65%, $[M - H]^-$), 586.66 (100, $[M - 2H + Na]^-$) and 602.60 (27, $[M - 2H + K]^-$) (free acid $C_{15}H_{24}N_2O_{17}P_2$ requires M, 566.055) {lit.,[9] δ_P −10.5 (d, $J_{P,P}$ 20.2, P^α) and −11.8 (d, P^β); ESMS(−) *m*/*z* 565.1 (100%, $[M - H]^-$), 602.9 (20, $[M - 2H + K]^-$) and 640.8 (10, $[M - 3H + 2K]^-$)}.

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