Synthesis of α-D-Galactofuranosyl Phosphate

Rosa M. de Lederkremer,* Verónica B. Nahmad, and Oscar Varela

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina

Received September 1, 1993

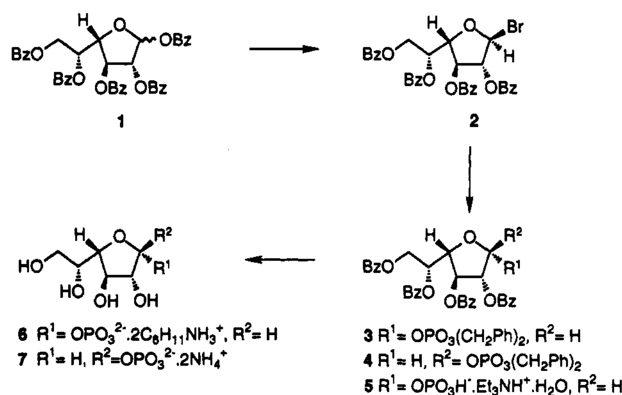
D-Galactose is extensively distributed in nature as a constituent of oligosaccharides, polysaccharides, and glycoconjugates. However, its presence in the furanoic configuration is apparently restricted to bacteria,[1–3] protozoa,[4–6] and fungi.[7–13] In particular, galactofuranose is modifying the oligosaccharide core of glycoinositol phospholipids of *Trypanosoma cruzi*,[14,15] the agent of Chagas' disease (South American trypanosomiasis). It was also reported[16] that polyclonal antisera to glycoproteins of *T. cruzi* recognize β-D-galactofuranosyl epitopes. The difference in the configuration of galactose in mammals and in the parasites makes galactofuranose a good target for the inhibition of the biosynthesis of the glycoconjugates of *T. cruzi*. Studies were reported[17] on the biosynthesis of the galactofuranosyl units in the exocellular polysaccharide of *Penicillium charlesii*. It was described that UDP-α-D-galactofuranose (UDP-Gal*f*) is the substrate for the galactofuranosyltransferase, and that the galactofuranosyl nucleotide would be formed from UDP-Glc*p* or UDP-Gal*p*. According to the authors, the UDP-Gal*f* could be formed by ring contraction of the hexose residue, while still attached to the nucleotide. In the case of the biosynthesis of the T_1 antigen from some variants of *Salmonella* the authors also conclude[18] that UDP-galactopyranose was the source of Gal*f*. However, attempts to incorporate Gal*f* in glycoproteins of *Crithidia fasciculata* by *in vitro* incubation with either UDP-Glc*p* or UDP-Gal*p* were unsuccessful.[5]

No chemical synthesis of UDP-Gal*f* or α-D-Gal*f*1-phosphate was described. The formation of the galactofuranosyl nucleotide in cell-free extracts of *P. charlesii* was inferred on the basis of its lability and the results of periodate oxidation.[17]

In this paper we report the synthesis of α-D-Gal*f*1-phosphate, a first step for the synthesis of the nucleotide which is required for the study of the biosynthesis of galactofuranosyl units in parasite glycoconjugates. The synthesis was planned taking into account the exceptional lability of a furanose 1-phosphate.

Results and Discussion

Benzoylation[19] of D-galactose with benzoyl chloride–pyridine at 100 °C afforded a crystalline mixture of per-*O*-benzoylated α- and β-D-galactofuranose (1). On treatment with trimethylsilyl bromide[20] the mixture 1 rendered, in almost quantitative yield, the corresponding tetra-*O*-benzoyl-D-galactofuranosyl bromide (2) having the β configuration, as determined by its ^{1}H NMR spectrum, which showed a broad singlet ($J_{1,2} < 1$ Hz) in the anomeric region (6.60 ppm) indicating[19,21] a *trans* relationship for H-1 and H-2. The ^{13}C NMR spectrum of 2 showed the anomeric signal at 88.5 ppm; C-2 and C-4 resonate at much lower fields (85.7 and 84.9 ppm) than C-5 (69.7 ppm), as observed for other furanoid derivatives of galactose.[19,22]



Treatment of 2 with 1.5 equiv of triethylammonium dibenzyl phosphate in toluene afforded a mixture (TLC: R_f 0.24 and 0.16) which was chromatographed on a silica gel column to give two main fractions. The lower migrating product (R_f 0.16) was obtained crystalline; its ^{1}H NMR spectrum showed the H-1 signal as a double doublet ($J_{1,2}$ = 4.8 and $J_{H-1,P}$ = 5.8 Hz). The $J_{1,2}$ value indicates[21] a *cis* relationship for H-1 and H-2 and hence an α configuration for the anomeric center. The ^{13}C NMR spectrum of the product showed the resonances for C-1 (δ_c 97.6) and C-2 (δ_c 76.7) as doublets, due to their respective couplings with ^{31}P. The ^{31}P NMR spectrum of the compound gave a signal centered at −3.7 ppm. The product was therefore characterized as dibenzyl 2,3,5,6-tetra-*O*-benzoyl-α-D-galactofuranosyl phosphate (3).

The fastest migrating material isolated from the column was determined by ^{13}C NMR spectroscopy to be a mixture containing three components. The anomeric region of the spectrum showed a doublet for C-1 at 103.3 ppm ($J_{C-1,P}$ = 5.5 Hz), indicating a glycosyl phosphate derivative, and

(1) Daffe, M.; Brennan, P. J.; Mc Neil, M. *J. Biol. Chem.* **1990**, *265*, 6734.
(2) Richards, J. C.; Perry, M. B.; Carlo, D. T. *Can. J. Biochem. Cell. Biol.* **1983**, *61*, 178.
(3) Gerwig, G. J.; Kamerling, J. P.; Vliegenthart, J. F. G.; Morag, E.; Lamed, R.; Bayer, E. A. *Eur. J. Biochem.* **1992**, *205*, 799.
(4) Lederkremer, R. M.; Casal, O. L.; Alves, M. J. M.; Colli, W. *FEBS Lett.* **1980**, *116*, 25.
(5) Mendelzon, D. H.; Parodi, A. J. *J. Biol. Chem.* **1986**, *261*, 2129.
(6) Moraes, C. T.; Bosch, M.; Parodi, A. J. *Biochemistry* **1988**, *27*, 1543.
(7) Unkefer, C. J.; Gander, J. E. *J. Biol. Chem.* **1990**, *265*, 685.
(8) Notermans, S.; Veeneman, G. H.; Van Zuylen, C. W. E. M.; Hoogerhout, P.; Van Boom, J. H. *Mol. Immunol.* **1988**, *25*, 975.
(9) Groisman, J. F.; Lederkremer, R. M. *Eur. J. Biochem.* **1987**, *165*, 327.
(10) Nakajima, T.; Yoshida, M.; Hiura, N.; Matsuda, K. *J. Biochem.* **1984**, *96*, 1005.
(11) Mendonça-Previato, L.; Gorin, P. A. J.; Travassos, L. R. *Infect. Immun.* **1980**, *29*, 934.
(12) Barreto-Bergter, E. M.; Travassos, L. R.; Gorin, P. A. J. *Carbohydr. Res.* **1980**, *86*, 273.
(13) Lee, W.-L.; Lloyd, K. O. *Arch. Biochem. Biophys.* **1975**, *171*, 613.
(14) Lederkremer, R. M.; Lima, C.; Ramirez, M. I.; Ferguson, M. A. J.; Homans, S. W.; Thomas-Oates, J. *J. Biol. Chem.* **1991**, *266*, 23670.
(15) Lederkremer, R. M.; Lima, C. E.; Ramirez, M. I.; Gonçalves, M. F.; Colli, W. *Eur. J. Biochem.*, in press.
(16) De Arruda, M. V.; Colli, W.; Zingales, B. *Eur. J. Biochem.* **1989**, *182*, 413.
(17) García Trejo, A.; Haddock, J. W.; Chittenden, G. J. F.; Baddiley, J. *Biochem. J.* **1971**, *122*, 49.
(18) Sarvas, M.; Nikaido, H. *J. Bacteriol.* **1971**, *105*, 1063.
(19) Varela, O.; Marino, M. C.; Lederkremer, R. M. *Carbohydr. Res.* **1986**, *155*, 247.
(20) Gillard, J. W.; Israel, M. *Tetrahedron Lett.* **1981**, *22*, 513.
(21) Bundle, D. R.; Lemieux, R. U. *Methods Carbohydr. Chem.* **1976**, *7*, 79.
(22) Marino, M. C.; Varela, O.; Lederkremer, R. M. *Carbohydr. Res.* **1989**, *189*, 65.

0022-3263/94/1959-0690$04.50/0 © 1994 American Chemical Society

two singlets at 101.1 and 96.0 ppm due, respectively, to the β and α anomers of tetra-*O*-benzoyl-D-galactofuranose.[23] The relative intensity of the anomeric signals indicated that the glycosyl phosphate was a minor component (~25%) of the mixture. The ^{1}H NMR spectrum of this material showed the resonance for H-1 of the dibenzyl phosphate derivative at 6.13 ppm, as a doublet ($J_{H-1,P}$ = 4.9 Hz). The fact that $J_{1,2}$ was not observed ($J_{1,2}$ < 1 Hz) dictates a *trans* relationship[21] for H-1 and H-2 and hence a β configuration for the dibenzyl tetra-*O*-benzoyl-D-galactofuranosyl phosphate (4).

Compound 4 was rather unstable, and rapidly decomposed on storage or when chromatographic purifications were attempted. Furthermore, on heating (50 °C) the syrupy mixture of 4 with the galactofuranose tetrabenzoates, the proportion of 4 dramatically decreases, by conversion to the latter products and also by isomerization to its α anomer (3). The instability of a phosphate or a protected phosphoryl group at the anomeric center of a furanose is well documented,[24,25] but compound 4 seems to be much less stable than its α analog 3. The instability of 4 could be the result of the presence of a *trans*-disposed benzoyl group on C-2, which may participate anchimerically[26] in the expulsion of the good leaving group located on C-1, to form a stable acyloxonium phosphate ion pair, precursor of the galactofuranose tetrabenzoates.

Hydrogenolysis of the benzyl protecting groups of 3 (Pd/C) in the presence of triethylamine afforded, according to the elemental analysis and the integral from the ^{1}H NMR spectrum, tetra-*O*-benzoyl-α-D-galactofuranosyl phosphate triethylammonium salt monohydrate (5). Niggemann and Thiem[27] also described the formation of analogous products on hydrogenolysis of dibenzyl glycopyranosyl phosphates in the presence of triethylamine.

Debenzoylation of 5 took place under mild conditions with 5:2:1 methanol–water–triethylamine to give, after purification by ion-exchange column chromatography, crystalline α-D-galactofuranosyl phosphate bis(cyclohexylammonium salt) (6). The ^{1}H NMR spectrum of 6 showed a double doublet due to H-1 (5.42 ppm) with $J_{H-1,P}$ = 4.9 Hz and $J_{1,2}$ = 4.5 Hz. The anomeric carbon signal of 6 appeared as a doublet at 96.9 ppm ($J_{C-1,P}$ = 5.3 Hz). The furanosidic nature of this product was confirmed by regioselective periodate oxidation of the exocyclic glycol system of 6, followed by borohydride reduction of the resulting aldehyde. As expected, the product obtained showed the same chromatographic mobility as arabinofuranosyl phosphate,[28] and it released arabinose on mild acid hydrolysis.

For comparative purposes, the isolation of free β-D-galactofuranosyl phosphate (7) was attempted. Therefore, the mixture which contained compound 4 was subjected to hydrogenolysis followed by debenzoylation in the conditions described for the preparation of 6. Compound 7 was separated from the large amount of contaminating D-galactose by ion-exchange column chromatography. From the column a few milligrams of the ammonium salt of 7 were obtained. The spectral data for the anomeric region of 7: H-1 (5.32 ppm), $J_{H-1,P}$ = 6.4 Hz and $J_{1,2}$ = 1.7 Hz, and C-1 (103.3 ppm) confirmed the β configuration for the product.

Chittenden in 1972, reported[29] the preparation of D-galactofuranosyl phosphate by different procedures, including the phosphorylation of tetra-*O*-acetyl-D-galactofuranosyl halides with 1 equiv of dibenzyl phosphate. However, the intermediate products were neither isolated nor characterized, and the anomeric configuration was assigned as β on the basis of its optical rotation. As we have used ^{13}C and ^{1}H NMR to arrive at unambiguous anomer assignments for 6 and 7, and the intermediate products, the $[\alpha]_D$ value (+16°) reported for the product formulated as β-D-galactofuranosyl phosphate, would indicate a mixture of this with its α anomer. Maryanoff *et al.*[25,30] have also described the formation of α,β mixtures of dibenzyl glycosyl phosphates by reaction of 2-acylglycofuranosyl halides with triethylammonium dibenzyl phosphate.

In summary, a new sugar phosphate (6), a chemical precursor of UDP-Gal*f*, has been prepared by a direct route, in a moderate overall yield (32%) from 1.

Experimental Section

General. ^{31}P Chemical shifts are given relative to external H_3PO_4 (δ 0). The following solvent systems were used for analytical thin-layer chromatography (TLC): (A) 9:1 toluene–EtOAc, (B) 5:3:1 EtOH–28% aqueous ammonia–water, (C) 13:2:1:1 MeCN–EtOH–H_2O–AcOH. Detection was effected by charring after spraying with 5% (v/v) H_2SO_4 in EtOH. Paper chromatography was performed on Whatman No. 1 with 3:3:1:3 1-butanol–pyridine–28% aqueous ammonia–0.01% EDTA as solvent. Sugar phosphates were detected by the ammonium molybdate–$HClO_4$ reagent.[31] Column chromatography was performed on silica gel 60 (230–400 mesh, Merck).

Dibenzyl 2,3,5,6-Tetra-*O*-benzoyl-α-D-galactofuranosyl Phosphate (3). To a solution of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose[19] (1, 1.95 g, 2.79 mmol) in anhydrous CH_2Cl_2 (15 mL) was added bromotrimethylsilane (4 mL, 30.3 mmol) dropwise, with external cooling (0 °C). The mixture was stirred for 24 h at room temperature, when TLC examination showed a single spot (R_f 0.32, solvent A), more polar than the starting material (R_f 0.52). The solution was concentrated *in vacuo*, and then toluene was added and evaporated in order to remove the excess reagent. The resulting 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl bromide (2, 1.84 g, quantitative yield), showed to be essentially pure according to its ^{1}H (Table 1) and ^{13}C NMR spectra (Table 2), and it was used for the next step without further purification.

Crude 2 (1.84 g, 2.79 mmol) was dissolved in anhydrous toluene (5 mL) and treated with 1.5 equiv of triethylammonium dibenzyl phosphate [triethylamine (0.43 g), dibenzyl phosphate (1.17 g)]. The mixture was stirred for 3 h at room temperature and filtered to remove the triethylamine hydrobromide. The filtrate was concentrated *in vacuo* at 35 °C. The residue, which showed two main spots by TLC (R_f 0.24 and 0.16, solvent A), was chromatographed on a silica gel column, with 15:1 toluene–EtOAc as eluent.

From fractions which showed by TLC the spot of R_f 0.24 a syrup (1.07 g) was obtained. The ^{13}C NMR spectrum of this material showed it to contain three main components: α and β 2,3,5,6-tetra-*O*-benzoyl-D-galactofuranose, and dibenzyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl phosphate (4).

Upon concentration of the fractions containing the product of R_f 0.16, dibenzyl 2,3,5,6-tetra-*O*-benzoyl-α-D-galactofuranosyl phosphate (3, 0.98 g, 41%) was obtained as a solid: mp 112–113

(23) Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27.
(24) Bunton, C. A.; Humeres, E. *J. Org. Chem.* **1969**, *34*, 572.
(25) Maryanoff, B. E.; Reitz, A. B.; Tutwiler, G. F.; Benkovic, S. J.; Bencovik, P. A.; Pilkis, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 7851.
(26) Paulsen, H. *Adv. Carbohydr. Chem. Biochem.* **1971**, *26*, 127.
(27) Niggemann, J.; Thiem, J. *Liebigs Ann. Chem.* **1992**, 535.
(28) Aspinall, G. O.; Cottrell, W. J.; Matheson, N. K. *Can J. Biochem.* **1972**, *50*, 574.
(29) Chittenden, G. J. F. *Carbohydr. Res.* **1972**, *25*, 35.
(30) Maryanoff, B. E.; Reitz, A. B.; Nortey, S. O. *Tetrahedron* **1988**, *44*, 3093.
(31) Skipski, V. P.; Barclay, M. In *Methods in Enzymology*; Colowick, S. P., Kaplan, N. O., Eds.; *Lipids*; Lowenstein, J. M., Ed.; Academic Press: New York, 1969; Vol. XIV, p 530.

Table 1. ^{1}H-NMR Data for Compounds 2–7

	δ (ppm)						J (Hz)	
compd	H-1	H-2	H-3	H-4	H-5	H-6,6′	$J_{1,2}$	$J_{H-1,P}$
2[a]	6.60	5.85	5.65	4.92	6.14	4.68	<1	
3[a]	6.34	5.73	6.15	4.72	5.83	4.75,4.62	4.5	5.8
4[a]	6.13	5.53	5.63	4.87–4.63	6.05	4.87–4.63	<1	4.9
5[a]	6.12	5.60	6.12	4.53	5.82	4.82,4.68	4.5	7.2
6[b]	5.42	4.00	4.17		3.73–3.56		4.5	4.9
7[b]	5.32	4.03	3.99–3.91		3.68	3.59,3.53	1.7	6.4

[a] 200 MHz, $CDCl_3$. [b] 500 MHz, D_2O.

Table 2. ^{13}C NMR Data for Compounds 2–7

	$δ^{13}$C (ppm), J (Hz)							
compd	C-1 ($J_{C-1,P}$)	C-2 ($J_{C-2,P}$)	C-3	C-4	C-5	C-6	aromatic C	PhCO
2	88.5	84.9	76.6	85.7	69.7	63.5	133.9–128.3	166.1,165.7 165.6,165.3
3[a]	97.6 (4.4)	76.7 (6.0)	73.3	79.9	70.7	62.7	135.4–127.6	165.8,165.6 165.5,165.4
4[b]	103.3 (5.5)	82.1	77.1	84.1	70.2	63.6	133.5–127.6	165.6–165.3
5[c]	96.0 (4.0)	76.8 (6.7)	74.3	78.9	71.7	63.1	133.3–128.0	166.1,165.9 165.8,165.5
6[d]	96.9 (5.3)	77.9 (7.6)	75.3	82.1	72.6	63.4		
7	103.3	82.5 (7.0)	77.4	84.1	71.6	63.2		

[a] δ $PhCH_2$ 69.4, 69.2 ($J_{C,H}$ 5.0 Hz). [b] δ $PhCH_2$ 69.8, 69.6. [c] δ CH_3CH_2N 45.5 and 8.4. [d] δ $C_6H_{11}NH_3^+$ 51.0, 31.7, 25.2, and 24.7.

°C, $[\alpha]_D$ +54.6° (c 1.0, $CHCl_3$); ^{31}P NMR ($CDCl_3$) δ −3.67. Anal. Calcd for $C_{48}H_{41}O_{13}P$: C, 67.29; H, 4.82. Found: C, 67.11; H, 4.86.

2,3,5,6-Tetra-*O*-benzoyl-α-D-galactofuranosyl Phosphate Triethylammonium Salt Hydrate (5). Compound 3 (0.81 g, 0.95 mmol) was dissolved in EtOAc (10 mL) containing triethylamine (0.8 mL) and hydrogenated with 10% Pd/charcoal (80 mg) as catalyst, at atmospheric pressure (15 psi) and room temperature. After 24 h, the catalyst was removed by filtration, and the filtrate was concentrated to afford 5 (0.74 g, 97%) as a white solid. Upon crystallization from ethanol–ether it gave mp 89–90 °c; $[\alpha]_D$ +68° (c 1.0; $CHCl_3$). Anal. Calcd for $C_{34}H_{28}O_{13}P \cdot (C_2H_5)_3NH \cdot H_2O$: C, 60.37; H, 5.83; N, 1.76. Found: C, 59.90; H, 5.90; N, 1.76.

α-D-Galactofuranosyl Phosphate Bis(cyclohexylammonium salt) (6). A solution of 5 (0.64 g, 0.72 mmol) in 5:2:1 MeOH–water–triethylamine (35 mL) was stirred at 30 °C for 25 h, when a single spot (R_f 0.46, solvent B) was detected by TLC. The mixture was concentrated and the triethylamine and methyl benzoate were removed by coevaporation with water, under vacuum at 35 °C. The resulting syrup was dissolved in water and applied to a column of AG 3-X4 resin. The column was first eluted with water and then with a gradient of 0.3–1 M aqueous cyclohexylamine. Fractions which gave a positive test for phosphorus[31] were collected and freeze-dried to afford 6 (0.29 g, 80%). Compound 6 was obtained crystalline from methanol–ether; it gave mp 137–138 °C; $[\alpha]_D$ +41° (c 1.0, water); paper chromatography, $R_{H_3PO_4}$ 1.44; ^{31}P NMR (D_2O) δ +2.5. Anal. Calcd for $C_6H_{11}O_9P \cdot 2C_6H_{14}N$, C, 47.15; H, 8.57; N, 6.11. Found: C, 46.86; H, 8.72; N, 6.34.

In order to confirm its furanose structure, compound 6 (10 mg) was oxidized with aqueous $NaIO_4$ (0.05M, 2 mL), adjusting the pH to 9 with aqueous ammonia. After 20 min of stirring in the dark, ethylene glycol (0.05 mL) was added and the mixture was kept for 30 min at room temperature. Aqueous ammonia (1 M, 0.05 mL) and sodium borohydride (20 mg) were added, and the solution was stirred for 2 h and then neutralized with acetic acid. The mixture was freeze-dried, and the residue was examined by paper chromatography, showing a single spot having $R_{H_3PO_4}$ 1.79, which developed a blue color with the reagent for phosphorus.[31] In the same solvent arabinofuranosyl phosphate[28] has $R_{H_3PO_4}$ 1.83.

Hydrolysis of a sample was performed by dissolution in 0.1 M HCl and heating for 30 min at 100 °C. Paper chromatography gave a single spot having the same mobility as arabinose.

β-D-Galactofuranosyl Phosphate Ammonium Salt (7). The crude mixture (0.07 g) previously obtained (see preparation of 3), which contained compound 4, was subjected to hydrogenation followed by debenzoylation with 5:2:1 $MeOH-H_2O-Et_3N$ (10 mL). TLC examination of the material obtained upon evaporation of the solvent showed a minor component (R_f 0.08, solvent C), and a large amount of galactose (R_f 0.38). This mixture was dissolved in water and applied to an AG 3-X4 (HO^- form) column, which was first eluted with water until no galactose was detected (TLC) in the eluate and then with 1 M aqueous ammonia. Fractions which gave a positive phosphorus test[31] were collected and freeze-dried, affording 7 (10 mg) as an amorphous solid: $[\alpha]_D$ −3° (c 0.2, water); ^{31}P NMR (D_2O) δ +1.1.

Acknowledgment. We thank the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR), CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), and the University of Buenos Aires for financial support, and UMYMFORM (CONICET-FCEN) for the microanalyses. We are also grateful to Dr. Juan A. Galbis Pérez (Universidad de Sevilla, Spain) for recording the ^{31}P NMR spectra. R.M.L. and O.V. are Research Members of the National Research Council of Argentina.