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# Facile synthesis of benzyl β-D-galactofuranoside. A convenient intermediate for the synthesis of D-galactofuranose-containing molecules

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**Abstract**—Benzyl β-D-galactofuranoside was efficiently obtained from 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose, via benzyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranoside. Conditions for the O-debenzylation were investigated in order to evaluate the synthetic application of the benzyl group as an anomeric protector of a galactofuranose moiety in synthetic strategies involving galactofuranose.


*Keywords:* β-D-Galactofuranosides; Benzyl galactofuranoside; *exo*-β-D-Galactofuranosidase substrate; Raney nickel

The importance of galactofuranose as target for the development of chemotherapeutic agents has been well recognized.[1,2] Much effort had been directed to the synthesis of D-galactofuranoside derivatives[3] with different protecting groups as synthons in order to develop efficient sequences for the synthesis of substrates,[4,5] inhibitors,[6–8] and tools[9–12] for the characterization of enzymes involved in the glycobiology of galactofuranose.[13]

Benzyl glycosides are widely used in carbohydrate synthesis because of the mild conditions involved in the removal of the benzyl group by catalytic hydrogenolysis. Benzyl β-D-galactofuranoside (**3**) was first obtained as a mixture of the furanoside and pyranoside anomers by the diethyl dithioacetal method with benzyl alcohol in the presence of mercuric oxide and mercuric chloride.[14] The β-furanoside form was isolated after several fractional crystallizations in 12% yield. A crude mixture of benzyl galactosides was later used for the synthesis of the disaccharide, β-D-Gal*f*-(1→5)-D-Gal*f*.[15]

Our continuing interest in the development of methodologies for the synthesis of galactofuranose derivatives led us to look for an easier alternative for the synthesis of **3**. As starting material, penta-*O*-benzoyl-α,β-D-galactofuranose (**1**) was used.[16] This compound has the advantage of being easily obtained in one step by benzoylation of D-galactose in hot pyridine, with selective crystallization from ethanol. Other furanoside templates involve more steps and chromatographic purifications.[17]

Previous methods used to convert **1** to its glycoside using $SnCl_4$-promoted glycosylation,[18] or the Koenings–Knorr procedure[19] failed when the alcohol was benzyl alcohol. However, condensation of **1** with benzyl alcohol took place in the presence of $BF_3{\cdot}Et_2O$, and compound **2** was stereoselectively obtained (Scheme 1), although 3 days were necessary for quantitative conversion. Recently, we prepared heteroaryl 1-thio-β-D-galactofuranosides as potential inhibitors of β-D-galactofuranosidases, and we also observed that from among various thiols, 2-mercaptopyrimidine and 4,6-dimethyl-2-mercaptopyridine were reactive only with $BF_3{\cdot}Et_2O$.[7] The β anomeric configuration of **2** was confirmed on the basis of the small $J_{1,2}$ value (<0.5 Hz), and the chemical shifts observed for the anomeric carbon (105.1 ppm) and for C-2 and C-4 (82.1, 81.6 ppm) from the respective $^1H$ and $^{13}C$ NMR spectra, as usually observed for

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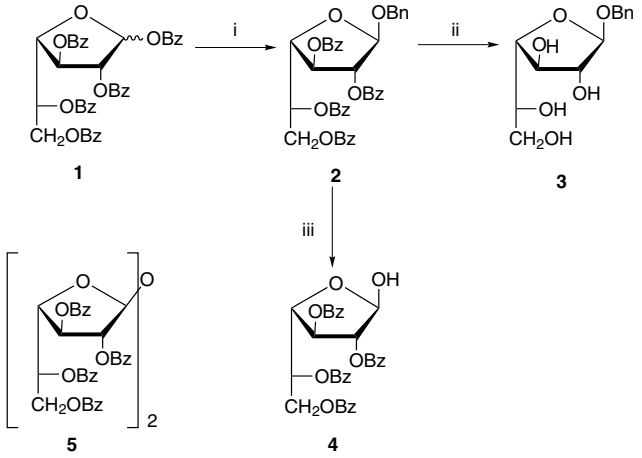


**Scheme 1.** Reagents and conditions: (i) $BF_3{\cdot}Et_2O$, $CH_2Cl_2$, BnOH (82%); (ii) NaOMe/MeOH, $CH_2Cl_2$ (94%); (iii) Raney Ni, EtOH, reflux (96%).

β-D-galactofuranosides.[4,16,18] Under longer reaction time (7 days), some α anomer was formed, as observed in the $^1$H NMR (β/α ratio 3:1) spectra. The α-anomer was probably formed by anomerization of **2** under the glycosylation conditions,[20] even though pure β-anomer **2** could be isolated in 70% yield by crystallization of the crude product from ethanol.

Debenzoylation of **2** with sodium methoxide–methanol afforded benzyl β-D-galactofuranoside (**3**, Scheme 1) in almost quantitative yield, which after crystallization showed physical constants identical to those reported.[14] The $^1$H NMR spectrum of **3** showed a $J_{1,2}$ value (1.4 Hz) indicative of a *trans* relationship for H-1 and H-2 (β configuration). Accordingly, the $^{13}$C NMR spectrum showed the characteristic[4] β-D-galactofuranose pattern of signals, with the anomeric signal at 107.6 ppm and those corresponding to C-2 and C-4 appearing over 80 ppm ($\delta$ 83.5, 82.1, C-4, and C-2, respectively).

Compound **3** could be used for strategies in which a modification in the sugar is introduced, like the deoxygenation of a specific position,[10] or the introduction of a radiolabel.[5,12] After these modifications, it could also be transformed into a glycosyl donor, or be transformed into a glycosyl acceptor by proper partial protection. To investigate the synthetic application of the benzyl group as an anomeric protector of a galactofuranose moiety, the cleavage of the benzyl group of **2** was attempted under a variety of conditions. Hydrogenolysis of **2** in the presence of either 10% Pd/C, $Pd(OH)_2$/C, $Rh/Al_2O_3$, or tris(triphenylphosphine)–RhCl, as heterogeneous catalysts,[21] was unsuccessful. Using $PtO_2$, only partial hydrogenolysis occurred after 12 h. Heterogenolytic catalytic-transfer hydrogenolysis[22] also failed. As expected, using $SnCl_4$[23] the 1,1-disaccharide **5** (Scheme 1)[19] was obtained. Although a more labile benzyl-type protecting group could be used,[24] we considered that it was highly desirable to find conditions for the hydrogenolysis of a simple *O*-benzyl group. Compound **2** could only be O-debenzylated using Raney nickel[25] in ethanol under reflux, affording 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranose[26] (**4**, Scheme 1). By contrast, free glycoside **3** was resistant under these conditions, but it was O-debenzylated by hydrogenation over 10% Pd/C at 20 psi, similarly as reported for benzyl β-D-Gal*f*-(1→5)-D-Gal*f*.[15]

Benzyl β-D-galactofuranoside (**3**) was evaluated as substrate of the *exo*-β-D-galactofuranosidase (E.C. 3.2.1.146) from *Penicillium fellutanum*, under conditions previously described.[5] The enzymatic reaction was followed by HPAEC–PAD analysis.

Apparent $K_m$ and $V_{max}$ values of 3.7 mM and 0.091 mmol $h^{-1}$ $mg^{-1}$, respectively, were determined for **3** (Fig. 1).

These kinetic parameters indicate that benzyl β-D-galactofuranoside has a lower affinity compared with 4-nitrophenyl β-D-galactofuranoside ($K_m$ 0.31 mM),[27] the usual substrate for detecting and measuring β-D-galactofuranosidase activity.[4,6] This lower affinity could be due to different interactions of the benzyl group in the active site of the enzyme. This structural dependence on the aglycon was also observed when benzyl 1-thio-β-D-galactofuranoside was compared with other thiogalactofuranosides as β-D-galactofuranosidase inhibitors.[6]

In conclusion, we have described an efficient, facile, and stereoselective procedure for the synthesis of crystalline benzyl β-D-galactofuranoside (**3**) via the perbenzoylated derivative **2** and the conditions for the removal of the 1-*O*-benzyl group. The simplicity of these procedures shows compound **3** as a useful intermediate for synthetic strategies involving the galactofuranose moiety.

## 1. Experimental

### 1.1. General procedures

Thin-layer chromatography (TLC) was performed on 0.2-mm Silica Gel 60 $F_{254}$ (E. Merck) aluminum-supported plates. Detection was effected by exposure to

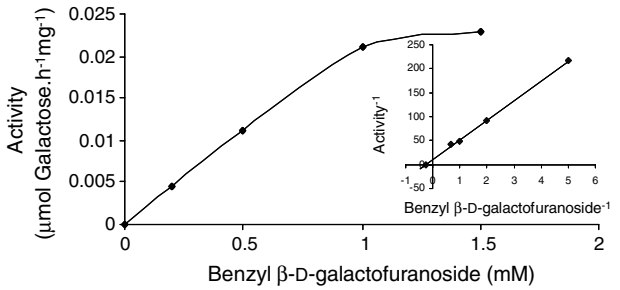


**Figure 1.** Plot of *Penicillium fellutanum exo*-β-D-galactofuranosidase activity, expressed in Gal μmol $h^{-1}$ $mg^{-1}$, versus benzyl β-D-galactofuranoside concentration (mM). Assay conditions are indicated in the Experimental. Inset: Lineweaver–Burk reciprocal plot.

UV light and by spraying with 10% (v/v) $H_2SO_4$ in EtOH and charring. Column chromatography was performed on Silica Gel 60 (200–400 mesh, E. Merck). NMR spectra were recorded with a Bruker AC 200 spectrometer at 200 MHz ($^1$H) and 50 MHz ($^{13}$C) or with a Bruker AM 500 spectrometer at 500 MHz ($^1$H) and 125 MHz ($^{13}$C). Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 343 polarimeter, with a path length of 1 dm.

### 1.2. Benzyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranoside (2)

To an externally cooled solution of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose[16] (**1**, 2.1 g, 3.0 mmol) in dry $CH_2Cl_2$ (10 mL), $BF_3{\cdot}Et_2O$ (0.75 mL, 5.87 mmol), and benzyl alcohol (0.9 mL, 8.7 mmol) were added. After 15 min of stirring at 0 °C, the solution was kept at room temperature for 3 days. After that time TLC examination showed complete conversion into a product with $R_f$ 0.63 (9:1 toluene–EtOAc), slightly faster-moving than the starting material. The mixture was diluted with $CH_2Cl_2$ and extracted with aq $NaHCO_3$. The organic phase was washed with water, dried ($MgSO_4$), and concentrated under vacuum. The syrup obtained was dissolved in hot EtOH and afforded crystalline compound **2** (1.73 g, 82%); mp 115–116 °C, $[\alpha]_D$ −25 (*c* 1, $CHCl_3$); $^1$H NMR ($CDCl_3$): δ 8.15–7.20 (m, 25H, aromatic), 6.04 (ddd, 1H, *J* 3.6, 5.3, 7.8 Hz, H-5), 5.65 (d, 1H, *J* 5.3 Hz, H-3), 5.55 (s, 1H, H-2), 5.40 (s, 1H, H-1), 4.71 (t, 1H, *J* 5.3 Hz, H-4), 4.81, 4.65 (2d, 2H, *J* 11.9 Hz, C*H*$_2$Ph), 4.66–4.63 (m, 2H, H-6a, H-6b); $^{13}$C NMR ($CDCl_3$): δ 166.1, 165.7, 165.6, 165.4 (4Ph*C*O), 138.2–127.5 (aromatic), 105.1 (C-1), 82.1 (C-2), 81.6 (C-4), 77.6 (C-3), 70.4, 69.1 (C-5, *C*H$_2$Ph), 63.5 (C-6). The assignments were supported by heteronuclear correlation spectroscopy experiments. Anal. Calcd for $C_{41}H_{34}O_{10}$: C, 71.71; H, 4.99. Found: C, 71.49; H, 5.21. From preparations of 7 days, the α-anomer showed, inter alia, signals at δ 99.9 (C-1), 79.1, 77.7 (C-2, C-4).

### 1.3. Preparation of benzyl β-D-galactofuranoside (3)

To a suspension of **1** (1.5 g, 2.18 mmol) in anhyd MeOH (12.0 mL) at 0 °C, 0.5 N NaOMe in MeOH (3 mL) was added. After 2 h of stirring at room temperature, the solution was passed through a column (1.5 × 5 cm) containing Amberlite IR-120 ($H^+$) resin. The solvent was evaporated, and the remaining methyl benzoate was eliminated by several co-evaporations with water to afford **2** (0.55 g, 94%), which crystallized in vacuo. After recrystallization from EtOAc the product gave mp 85–87 °C; lit.[14] 80–81 °C; $[\alpha]_D$ −99 (*c* 1, $H_2O$), lit.[14] −96; $R_f$ 0.58 (7:1:2 PrOH–$NH_3$–$H_2O$), 0.61 (4:1 EtOAc–MeOH); $^1$H NMR ($D_2O$): δ 7.38–7.35 (m, 5H, aromatic), 5.04 (d, 1H, *J* 1.4 Hz, H-1), 4.70, 4.58 (2d, 2H, $J_{gem}$ 11.9 Hz, PhC$H_2$), 4.03–4.00 (m, 2H, H-2, H-3), 3.91 (dd, 1H, *J* 5.9 Hz, *J* 4.1 Hz, H-4), 3.77 (ddd, 1H, *J* 4.1 Hz, *J* 4.5 Hz, *J* 7.5 Hz, H-5), 3.73 (dd, 1H, *J* 4.4 Hz, *J* 11.9 Hz, H-6a), 3.68 (dd, 1H, *J* 7.5 Hz, *J* 11.9 Hz, H-6b); $^{13}$C NMR ($D_2O$): δ 137.9–129.1 (aromatic), 107.6 (C-1), 83.5 (C-4), 82.1 (C-2), 77.5 (C-3), 71.5, 70.7 (C-5, *C*H$_2$Ph), 63.67 (C-6). The assignments were supported by heteronuclear correlation spectroscopy experiments. From the O-debenzoylation of an anomeric mixture, signals corresponding to benzyl α-D-galactofuranoside were assigned in the $^{13}$C NMR ($D_2O$) spectrum: δ 101.2 (C-1), 82.1, 77.3 (C-2, C-4), 75.3 (C-3), 73.6 (C-5), 63.0 (C-6).

### 1.4. O-Debenzylation of benzyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranoside (2)

A vigorously stirred solution of **2** (0.10 g, 0.14 mmol) in abs EtOH was heated under reflux for 4 h in the presence of freshly prepared Raney nickel[25] (0.30 g). The reaction was monitored by TLC, which showed complete conversion to 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranose[26] (**4**, $R_f$ 0.30, 9:1 toluene–EtOAc). After filtration of the catalyst over Celite, the solvent was evaporated and compound **4** was obtained as a syrup (0.80 g, 96%) with NMR spectra in agreement with reported data.[26]

### 1.5. Enzymatic assays

The enzymatic activity was assayed using the filtered medium of a stationary culture of *P. fellutanum* as the source of *exo*-β-D-galactofuranosidase (60 μL, 8 μg of protein),[6] benzyl β-D-galactofuranoside as substrate (0.25–1.5 mM), 15 μL of 66 mM NaOAc buffer (pH 4.0), in a final volume of 80 μL. After incubation overnight at 37 °C, the samples were centrifuged for 30 min at 10,000*g* through an Ultrafree-MC centrifugal filter (MW 5000). The amount of galactose released in the filtrates was analyzed by HPAEC–PAD. $K_m$ and $V_{max}$ values were determined by Lineweaver–Burk reciprocal plots.

### 1.6. HPAEC–PAD analysis

The analysis of the galactose released in enzymatic assays was performed by HPAEC–PAD using a Dionex DX-300 HPLC system with pulse amperometric detection (PAD), set at 30 nA and $E_1 = +0.05$ V, $E_2 = +0.60$ V, and $E_3 = -0.60$ V. The column used was a CarboPac-PA10 anion-exchange analytical column (4 × 250 mm), equipped with a PA-10 guard column (5 × 50 mm). The following program was used: 18 mM NaOH, isocratically, at a flow rate of 1 mL/min. The amounts of galactose released were estimated from the

peak areas using 2-deoxygalactose as the internal standard. The response for galactose and compound **3** with respect to 2-deoxygalactose was independently determined.

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