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The glycosyl-aldonolactone approach for the synthesis of β-D-Gal*f*-(1→3)-D-Man*p* and 3-deoxy-β-D-*xylo*-hexofuranosyl-(1→3)-D-Man*p*

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Abstract

A convenient synthesis of free β-D-Gal*f*-(1→3)-D-Man*p* (**8a**) is reported. The disaccharide is present as external unit in the lipopeptidophosphoglycan (LPPG) of *Trypanosoma cruzi* and internally in the lipophosphoglycan (LPG) of *Leishmania*. Condensation of 2,5,6-tri-*O*-benzoyl-D-mannono-1,4-lactone (**1**) with 1,2,3,5,6-penta-*O*-benzoyl-D-galactofuranose, promoted by $SnCl_4$, led to the β-glycosyl-lactone, a key intermediate for disaccharide **8a**, readily obtained by successive reduction of the lactone with diisoamylborane and debenzoylation. As in the LPG of *Leishmania* the HO-3 group of the galactofuranose is glycosylated by α-D-Gal*p*, we also synthesized 3-deoxy-β-D-*xylo*-hexofuranosyl-(1→3)-D-Man*p* (**8b**) and *p*-nitrophenyl 3-deoxy-β-D-*xylo*-hexofuranoside for studying the influence of HO-3 in the interaction with specific glycosidases. The disaccharide **8a**, and its corresponding alditol, were good substrates for the β-D-galactofuranosidase from *Penicillium fellutanum*, whereas the 3-deoxyglycosides were not hydrolyzed by the enzyme.

Keywords: β-D-Galactofuranose disaccharides; Glycosyl-lactone; Deoxy sugars; β-D-Galactofuranosidase; *Trypanosoma cruzi*; *Leishmania*

1. Introduction

In recent years we have been interested in the glycobiology of galactofuranose, particularly in regard to its presence in *Trypanosomatids* [1]. Parasites of this family, such as *Trypanosoma cruzi* and *Leishmania* species, are infectious to humans. It is interesting that in the glycoinositolphospholipid anchor-like structures, galactofuranose is present β-(1→3)-linked to α-mannose, either as a terminal non-reducing unit, as in the lipopeptidophosphoglycan (LPPG) of *T. cruzi* [2,3], or internal, as in the *Leishmania* lipophosphoglycan (LPG) [4]. As expected, since galactofuranose is absent in mammalian glycoconjugates, it is antigenic [5].

Mutant cells of *L. donovani*, which were defective in the biosynthetic step introducing the internal galactofuranose, were destroyed by macrophages, confirming the role of galactofuranose for intracellular survival of the parasite [6].

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Derivatives of β-D-Gal*f*-(1→3)-α-D-Man*p* have been synthesized [7–10]. However, deprotection to the free disaccharide was not reported in any of these instances.

Glycosyl-lactones were previously used in our laboratory as precursors of galactofuranose-containing disaccharides [11,12]. Selectively substituted aldonolactones, used as glycosylating agents for monosaccharides, are readily obtained from the corresponding aldonolactones [13,14]. The lactone can be chemoselectively reduced to the furanoid sugar. Complete deprotection results in isomerization of the reducing end to the more stable pyranose. Thus, in the present work, the disaccharide β-D-Gal*f*-(1→3)-D-Man*p* (**8a**) was prepared in few steps.

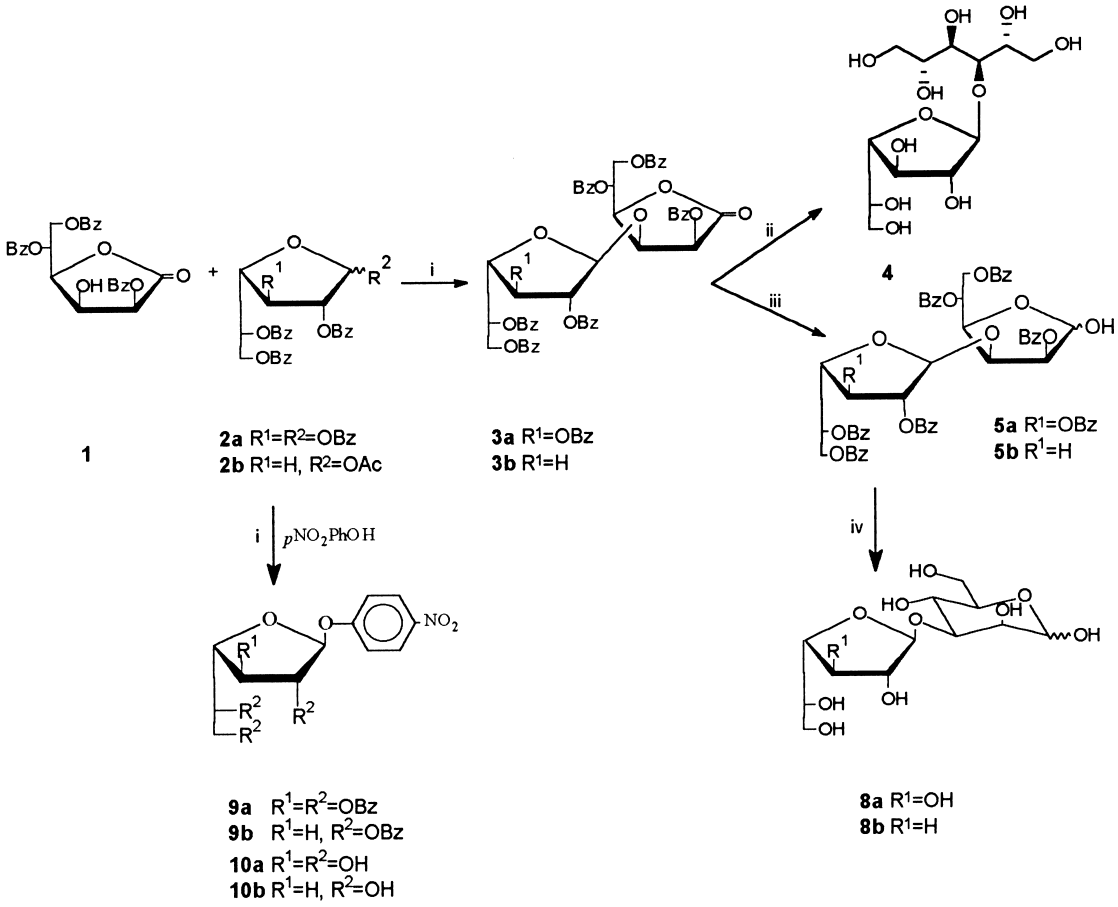
Taking in consideration that the Gal*f* is linked through its HO-3 to α-D-Gal*p* in the LPG of *Leishmania* [4], we also synthesized the 3-deoxy analogue **8b** which, lacking the HO-3, could be useful for studies on inhibition of the particular glycosyltransferase involved in the construction of the LPG glycan.

We have recently reported studies on the synthesis of inhibitors of the β-D-galactofuranosidase from *Penicillium fellutanum* [15]. It was interesting to explore the substrate specificity of this enzyme towards disaccharides **8a** and **8b**. As already described, deoxy sugar derivatives are very useful in defining the importance of each specific hydroxyl group for the interaction between carbohydrates and proteins [16].

2. Results and discussion

In previous work, we showed that benzoylation of D-mannono-1,4-lactone with 3.3 molar equivalents of benzoyl chloride in pyridine afforded tribenzoate **1** as the main product (60%), isolated by column chromatography [13]. We have now facilitated the isolation and improved the yield of **1** (70%) by crystallization from benzene of the compound from the crude benzoylation mixture.

Partially benzoylated aldonolactone **1** was a useful precursor for the target disaccharides **8a** and



Scheme 1. (i) $SnCl_4$, Cl_2CH_2; (ii) $NaBH_4$/MeOH, NaOMe/MeOH; (iii) DSB/THF; (iv) NaOMe/MeOH.

8b, as the free HO-3 could be readily glycosylated by using a Lewis acid catalyst, as previously described for the preparation of other glycosyl-lactones [11,12]. Thus, condensation of **1** with **2a** in the presence of $SnCl_4$ gave the glycosyl-lactone **3a** in 85% yield. The anomeric configuration of the glycosidic linkage was established as β by means of the ^{1}H NMR spectrum of **3a**, which showed H-1′ and H-2′ signals as broad singlets, in accordance with the 1,2-*trans* configuration. The ^{13}C NMR spectrum showed signals at δ 106.7 (C-1′), 83.9 (C-4′), and 81.3 (C-2′) corresponding to the β-galactofuranose unit, and the signals of the lactone carbons were clearly assigned by comparison with those in compound **1** [13]. In particular, the resonance of C-3 appeared shifted downfield (4.6 ppm), as expected because of the glycosylation of HO-3.

Reduction of **3a** with sodium borohydride, followed by *O*-deacylation with sodium methoxide, afforded crystalline 3-*O*-β-D-galactofuranosyl-D-mannitol (**4**). The ^{1}H NMR spectrum of **4** showed a broad singlet for H-1′ (δ 5.12, $J_{1',2'} < 1$ Hz), and in its ^{13}C NMR the anomeric signal (109.1 ppm), low-field signals for C-4′ and C-2′ (83.7 and 82.1 ppm), and three resonances for the CH_2OH groups, at 63.9, 63.6, and 63.4 ppm, were observed. The disaccharide alditol **4** has been previously isolated from the lichen *Peltigera horizontalis* by methanolic extraction [17], and this is the first synthesis and spectroscopic characterization of the compound.

Reduction of the lactone group of **3a** with diisoamylborane [18,19] gave **5a** in 88% yield. The anomeric region of the ^{13}C NMR spectrum of **5a** showed the resonances for *C*-1 Gal*f* (106.6 and 106.4 ppm) and *C*-1 Man*f* at δ 99.9 (β anomer) and 95.6 (α anomer). The α:β ratio was established as 1.4:1 from the relative signal intensities. Standard benzoylation of the anomeric HO of **5a** gave a mixture of the diastereoisomeric per-*O*-benzoyl derivatives having the α (**6**) and the β (**7**) configurations, which were separated by column chromatography and individually characterized. Thus, the anomeric proton signal in the ^{1}H NMR spectrum of **6**, centered at 6.77 ppm with $J_{1,2}$ 2.4 Hz indicates the α configuration for this compound. The ^{1}H NMR of **7** showed a similar chemical shift (6.82 ppm), but the $J_{1,2}$ value of 4.8 Hz indicates the β configuration at C-1. Furthermore, these data and also the chemical shifts observed for *C*-1 in the ^{13}C NMR spectra of **6** and **7**, are in good agreement with the values observed for the per-*O*-benzoylated derivatives of D-lyxofuranose, the analogous pentose [20,21].

O-Debenzoylation of **5a** with NaOMe gave the pure disaccharide **8a** in 70% overall yield from **2a**. The ^{1}H NMR spectrum showed in the anomeric region broad singlets for H-1′ (5.15), H-1α (5.10), and H-1β (4.86 ppm). This disaccharide constitutes the terminal unit of the glycosidic chains in the LPPG isolated from epimastigote forms of *Trypanosoma cruzi* [2,3]. The unit for Gal*f*-β(1→3)-Man*p* is also present in the core oligosaccharide of LPG of *Leishmania*, but in this case it is located in the middle of the chain [4]. Although some derivatives have been previously prepared, this is the first synthesis of free disaccharide **8a**. The earlier reports described the syntheses of the methyl glycoside [7], and the 8-methoxycarbonyloctyl [8] derivatives of **8a**, using the orthoester method. McAuliffe and Hindsgaul [9] described the synthesis of methyl 3-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside, starting from the peracetylated dithioacetal of D-galactose. The same protected disaccharide has also been prepared, using phenyl 1-selenogalactofuranoside as the glycosyl donor [10].

The same approach was used to synthesize the analogous 3-deoxy disaccharide **8b**. In this case, lactone **1** was condensed with 1-*O*-acetyl-3-deoxy-2,5,6-tri-*O*-benzoyl-β-D-*xylo*-hexofuranoside (**2b**) [22], in the presence of $SnCl_4$. The glycosyl-lactone **3b** was isolated from the reaction mixture by crystallization from ethanol (65% yield). The ^{13}C NMR spectrum of **3b** showed the distinctive resonance for the deoxy carbon (C-3, 31.7 ppm) and some upfield displacement for C-2′ and C-4′ with respect to the spectrum of **3a**.

Reduction of the lactone group of **3b** with diisoamylborane gave compound **5b** in 90% yield. Treatment of **5b** with NaOMe afforded the 3′-deoxy disaccharide **8b** in 92% yield. The spectroscopic behavior of compounds **5b** and **8b** was very similar to that of **5a** and **8a**, respectively. Thus, they showed similar chemical shifts for their anomeric signals and also the anomeric compositions were alike.

We have previously reported the synthesis of 4-nitrophenyl β-D-galactofuranoside (**10a**) [23] as a useful substrate for the detection of β-D-galactofuranosidase activity of *Penicillium fellutanum* [15]. Now, compound **10b**, the 3-deoxy analogue of **10a**, was prepared with the purpose of investigating the

specificity of the enzyme. Compound **2b** was condensed with 4-nitrophenol in the presence of $SnCl_4$, to give compound **9b** stereoselectively in 86% yield. The ^1H NMR spectrum of **9b** showed a broad singlet at 6.02 ppm for the anomeric proton and indicates the 1,2-*trans* configuration. The other signals were in good agreement with those reported for the 3-deoxy-D-*xylo*-hexofuranosides [22].

Standard debenzoylation of **9b** afforded compound **10b**, in 95% yield. Its ^{13}C NMR spectrum showed the signal of the anomeric carbon with a similar chemical shift as that observed in compound **10a**, and the signals corresponding to the other carbons of the ring shifted upfield because of the absence of OH-3.

Incubation of the enzyme with the analogue **10b** under the same conditions used with substrate **10a**, showed that the galactofuranosidase can not hydrolyze the glycosidic linkage within the pH range of 4–9 and incubation times of 1.5–5 h. Moreover, hydrolysis of substrate **10a** was not modified by the presence of **10b**, showing that the 3-deoxy-galactofuranoside did not interact with the enzyme.

The substrate specificity of β-D-galactofuranosidase could be monitored by high-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Compounds **4**, **8a**, and **8b** were incubated with the enzyme under the same conditions that compound **10a**, and the digests were analyzed by HPAEC-PAD after convenient dilution with water. Disaccharide **8a** ($t_r = 23.17$ min), was hydrolyzed to Gal ($t_r = 3.62$ min) and Man ($t_r = 4.02$). The alditol disaccharide **4** ($t_r = 9.45$ min) was also a good substrate for the enzyme, and gave galactose and mannitol. Incubation of **4** and **8a** during 18 h resulted in complete hydrolysis of the disaccharides.

In accord with the results obtained with glycoside **10b**, the 3-deoxy disaccharide **8b** ($t_r = 10.12$ min) was also found resistant to hydrolysis by β-D-galactofuranosidase. No new peaks appeared in the 'monosaccharide region' of the chromatogram, confirming that the enzyme does not hydrolyze the glycosidic linkage in absence of the OH-3 group in the nonreducing unit.

In summary, with this simple approach we efficiently synthesized β-D-Gal*f*-(1→3)-D-Man*p* (**8a**) and 3-deoxy-β-D-*xylo*-hexofuranosyl-(1→3)-D-Man*p* (**8b**). These disaccharides could be useful for biosynthetic studies of the *Leishmania* LPG. Moreover **8a**, which could be readily acylated, is a convenient synthon for the synthesis of higher oligosaccharides of the natural glycoconjugates. We also observed that HO-3 plays an important role in the interaction between the substrate and the β-D-galactofuranosidase.

3. Experimental

General methods.—Melting points were determined with a Thomas–Hoover apparatus. Optical rotations were measured with a Perkin–Elmer 343 polarimeter. NMR Spectra were recorded with a Bruker AC 200 spectrometer. Column chromatography was performed on Silica Gel 60 (200–400 mesh, Merck). Thin-layer chromatography (TLC) was carried out on precoated aluminium plates of Silica Gel $60F_{254}$ (Merck), using the following solvents: (*a*) 9:1 toluene–EtOAc, (*b*) 7:1:2 1-PrOH–NH_3–H_2O. The spots were visualized by exposure to UV light and by spraying the plates with 10% (v/v) H_2SO_4 in EtOH, followed by heating. 1,2,3,5,6-Penta-*O*-benzoyl-D-galactofuranose (**2a**) was prepared by benzoylation of D-galactose at 100 °C [11]. 1-*O*-Acetyl-2,5,6-tri-*O*-benzoyl-3-deoxy-β-D-*xylo*-hexofuranose (**2b**) was prepared as previously described [22].

2,5,6-Tri-O-*benzoyl*-D-*mannono-1,4-lactone* (**1**).—To a stirred solution of D-mannono-1,4-lactone (1.0 g, 5.62 mmol) in pyridine (7.5 mL), cooled in an ice-water bath, BzCl (2.0 mL, 17.04 mmol) was slowly added. The mixture was stirred for 1.5 h at 0 °C, and then poured into ice-water (200 mL). After 2 h the syrup was separated and dissolved in CH_2Cl_2. The organic solution was washed with aq $NaHCO_3$ (100 mL) and aq NaCl (2×100 mL), dried ($MgSO_4$) and the solvent evaporated to afford an amorphous solid. Addition of benzene (80 mL) to the solid led to crystalline compound **1** (1.93 g, 70%) which upon recrystallization from ethanol had mp 135–136 °C, lit: 136–138 °C [13].

2,5,6-Tri-O-*benzoyl-3*-O-*(2,3,5,6-tetra*-O-*benzoyl*-β-D-*galactofuranosyl)*-D-*mannono-1,4-lactone* (**3a**).—To a solution of 1,2,3,5,6-penta-*O*-benzoyl-D-galactofuranose (**2a**, 0.35 g, 0.50 mmol) in dry CH_2Cl_2 (10 mL) was added, $SnCl_4$ (0.08 mL, 0.66 mmol) and the solution was stirred for 10 min at 0 °C. Compound **1** (0.25 g, 0.50 mmol) was added, and the mixture was stirred overnight at room temperature. The solution was poured into aq $NaHCO_3$, extracted with CH_2Cl_2 and the organic extract was washed with water, dried

($MgSO_4$), filtered and evaporated. The residue was purified by column chromatography (19:1 toluene–EtOAc), and the fractions containing the product having R_f 0.62 (solvent a) were pooled and concentrated. The resulting crystalline mass was recrystallized from EtOH affording compound **3a** (0.45 g, 85%), mp 92–93 °C; $[\alpha]_D$ −42° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.25–7.05 (H-aromatic), 6.02 (d, $J_{2,3}$ 3.8 Hz, H-2), 5.86 (m, 2 H, H-5,5′), 5.74 (s, $J_{1',2'}$ < 1 Hz, H-2′), 5.54 (d, $J_{2',3'}$ 3.6 Hz, H-3′), 5.51 (s, H-1′), and 5.08–4.23 (m, 7 H, H-3,4,6a,6b,4′,6′a,6′b); ^{13}C NMR ($CDCl_3$) δ 169.0 (*C*-1), 165.6–164.6 (*C*=O benzoate), 133.9–128.3 (*C*-aromatic), 106.9 (C-1′), 83.9 (C-4′), 81.3 (C-2′), 77.6, 76.4 (C-4,3′), 73.1 (C-3), 70.9, 70.6 (C-2,5′), 68.2 (C-5), 63.6, 63.1 (C-6,6′). Anal. Calcd for $C_{61}H_{48}O_{18}$: C, 68.54; H, 4.53. Found: C, 68.76; H, 4.75.

β-D-*Galactofuranosyl-(1→3)*-D-*mannitol* (**4**).—Compound **3a** (0.32 g, 0.3 mmol) was suspended in MeOH (10 mL) and $NaBH_4$ (0.20 g, 5.3 mmol) was added. After stirring overnight, 0.5 M NaOMe in MeOH (5 mL) was added. The stirring was continued for 3 h, when TLC monitoring showed a single spot (R_f 0.28, R_{Gal} 1, solvent b). The solution was deionized with Dowex 50W (H^+) resin and then co-evaporated with water several times. The syrup was crystallized from *i*PrOH–EtOH affording **4** (0.089 g, 89%), which had mp 150–151 °C, $[\alpha]_D$ −55° (*c* 1, H_2O), lit: $[\alpha]_D$ −61° (*c* 2, H_2O) [17]; ^1H NMR (D_2O), anomeric region, δ 5.12 (H-1′, $J_{1',2'}$ 1.5 Hz); ^{13}C NMR (D_2O) δ 109.1 (C-1′), 83.7 (C-4′), 82.1 (C-2′),77.8, 76.9 (C-3,3′) 71.6, 71.5, 71.4, 70.3 (C-2,4,5,5′), 63.9, 63.6, 63.4 (C-1,6,6′). Anal. Calcd for $C_{12}H_{24}O_{11}$: C, 41.86, H, 7.03. Found: 41.69, H, 6.87.

2,5,6-Tri-O-*benzoyl-3*-O-*(2,3,5,6-tetra*-O-*benzoyl*-β-D-*galactofuranosyl)*-D-*mannofuranose* (**5a**).—To a solution of freshly prepared bis(3-methyl-2-butyl)borane (1.9 mmol) in anhydrous tetrahydrofuran (2.0 mL) a solution of compound **3a** (0.50 g, 0.47 mmol) in tetrahydrofuran (3 mL) was added. The solution was stirred for 16 h at room temperature and then processed as already described [18]. Boric acid was eliminated by co-evaporation with MeOH, and the syrup was purified by a short column chromatography (19:1 toluene–EtOAc). Fractions having R_f 0.22 (solvent a) were pooled and evaporated to give syrupy **5a** (0.44 g, 88%), $[\alpha]_D$ −35° (*c* 1, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 106.6 (C-1′β of the α anomer), 106.4 (C-1′β of the β anomer), 99.9 (C-1β), 95.6 (C-1α), 83.1, 82.2 (C-4′ α,β), 82.2, 81.7 (C-2′ α,β), 77.7 (C-3′ α,β), 75.1–69.8 (C-2,3,4,5, and 5′ for both anomers), and 63.7–63.4 (C-6,6′ for both anomers). Anal. Calcd for $C_{61}H_{50}O_{18}$: C, 68.40; H, 4.70. Found: C, 68.25; H, 4.48.

1,2,5,6-Tetra-O-*benzoyl-3*-O-*(2,3,5,6-tetra*-O-*benzoyl*-β-D-*galactofuranosyl)*-α-D-*mannofuranose* (**6**) *and 1,2,5,6-tetra*-O-*benzoyl-3*-O-*(2,3,5,6-tetra*-O-*benzoyl*-β-D-*galactofuranosyl)*-β-D-*mannofuranose* (**7**).—Compound **5a** (0.2 g, 0.19 mmol) was conventionally benzoylated with BzCl (1.0 mL) in pyridine (2.0 mL). After 2 h, TLC examination of the solution (solvent a) showed two main products which were separated by column chromatography (49:1 toluene–EtOAc) after conventional work up of the reaction mixture. From fractions having R_f 0.50 compound **6** (0.07 g, 37%) was isolated as a syrup, $[\alpha]_D$ +12° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) anomeric region δ: 6.77 ($J_{1,2}$ 2.4 Hz, H-1); ^{13}C NMR ($CDCl_3$) anomeric region δ: 106.4 (C-1′), 99.4 (C-1). Anal. Calcd for $C_{68}H_{54}O_{19}$: C, 69.50; H, 4.63. Found: C, 69.79 ; H, 4.61.

Evaporation of the solvent from slower-moving fractions (R_f 0.43) afforded syrupy **7** (0.09 g, 41%) which had $[\alpha]_D$ −59° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) anomeric region δ: 6.82 ($J_{1,2}$ 4.8 Hz, H-1); ^{13}C NMR ($CDCl_3$) anomeric region δ: 106.6 (C-1′), 93.2 (C-1). Anal. Calcd for $C_{68}H_{54}O_{19}$: C, 69.50; H, 4.63. Found: C, 69.31; H, 4.48.

3-O-β-D-*Galactofuranosyl*-D-*mannose* (**8a**).—Compound **5a** (0.40 g, 0.37 mmol) was suspended in a 0.5 M solution of NaOMe in MeOH (10.0 mL), and stirred until complete dissolution (1 h), when TLC showed a single spot (R_f 0.32, solvent b). The solution was made neutral with Dowex 50W (H^+) resin and concentrated to afford compound **8a** (0.12 g, 94%), which had $[\alpha]_D$ −47° (*c* 1, H_2O); ^1H NMR (D_2O) anomeric region δ: 5.16 (bs, H-1 α-Man*p*), 5.10 (bs, H-1 β-Gal*f*), 4.83 (bs, H-1 β-Man*p*); ^{13}C NMR (D_2O) δ: 105.7, 105.5 (C-1 Gal*f*), 94.8, 94.7 (C-1 Man*p*), 84.0, 83.9 (C-4′), 82.3 (C-2′), 77.9 (C-3′), 76.9–66.2 (C-2,3,4,5,5′ of both anomers) and 63.8, 61.8 (C-6,6′). Anal. Calcd for $C_{12}H_{22}O_{11}$: C, 42.11; H, 6.48. Found: C, 42.36; H, 6.61.

2,5,6-Tri-O-*benzoyl-3*-O-*(2,5,6-tri*-O-*benzoyl-3-deoxy*-β-D-xylo-*hexofuranosyl)*-D-*mannono-1,4-lactone* (**3b**).—To a solution of 1-*O*-acetyl-2,5,6-tri-*O*-benzoyl-3-deoxy-β-D-*xylo*-hexofuranose (**2b**, 0.57 g, 1.1 mmol) in dry CH_2Cl_2 (5 mL) cooled at 0 °C, was added $SnCl_4$ (0.16 mL, 1.3 mmol). After 15 min of stirring, a solution of lactone **1** (0.59 g,

1.2 mmol) in CH_2Cl_2 (2 mL) was added, and the mixture was stirred overnight at room temperature. The mixture was treated as described for **3a**. The glycosyl-lactone **3b** was crystallized from EtOH from the crude syrup (0.66 g, 65%). After recrystallization from the same solvent, compound **3b** had mp 152–153 °C; $[\alpha]_D$ −71° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.09–7.13 (H-aromatic), 5.99 (d, $J_{2,3}$ 4.2 Hz, H-2), 5.85 (m, H-5), 5.52 (d, $J_{2',3'a}$ 6.9 Hz, H-2′), 5.50 (bs, H-1′), 5.42 (m, H-5′), 5.13 (dd, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 12.6 Hz, H-6a), 5.03 (m, 2 H, H-3,4), 4.65 (dd, $J_{5,6b}$ 4.6 Hz, H-6b), 4.45 (m, H-4′), 4.26 (m, 2 H, H-6a,6b), 2.76 (m, $J_{3'a,4'}$ 8.2 Hz, $J_{3'a,3'b}$ 14.6 Hz′, H-3′a), and 2.08 (dd, $J_{3'b,4'}$ 5.1 Hz, H-3′b); ^{13}C NMR ($CDCl_3$) δ 169.3 (*C*-1), 165.7–164.9 (*C*=O benzoates), 133.8–127.7 (C-aromatics), 106.5 (C-1′), 78.4, 77.8 (C-4′,2′), 76.4 (C-4), 72.0 (C-3), 71.7 (C-5′), 70.9 (C-2), 68.1 (C-5), 63.7, 62.4 (C-6,6′), 31.7 (C-3′). Anal. Calcd for $C_{54}H_{44}O_{16}$: C, 68.34; H, 4.68. Found: C, 68.45; H, 4.89.

2,5,6-Tri-O-*benzoyl-3*-O-*(2,5,6-tri*-O-*benzoyl-3-deoxy*-β-D-xylo-*hexofuranosyl)*-D-*mannofuranose* (**5b**).—Compound **3b** (1.0 g, 1.05 mmol) was reduced with diisoamylborane (4.2 mmol) as just described for the preparation of **5a**. Syrupy compound **5b** (0.90 g, 90%) crystallized from EtOH; mp 148–149 °C, $[\alpha]_D$ −59° (*c* 0.8, $CHCl_3$); ^{13}C NMR ($CDCl_3$) δ 106.6, 106.1 (C-1′ β-Gal*f* for α and β anomers), 99.8 (C-1 β-Man*f*), 95.4 (C-1 α-Man*f*), 79.1, 78.2, 78.0, 77.5 (C-2′,4′), 74.2–69.7 (C-2,3,4,5 and 5′ for both anomers), 63.7 (C-6,6′) and 32.0, 31.8 (C-3′ for both anomers). Anal Calcd. for $C_{54}H_{46}O_{16}$: C, 68.20; H, 4.88. Found: C, 68.48; H, 5.00.

3-O-*(3-Deoxy*-β-D-xylo-*hexofuranosyl)*-D-*mannose* (**8b**).—Compound **5b** (0.50 g, 0.52 mmol) was stirred with a 0.5 M solution of NaMeO in MeOH (10.0 mL), until complete dissolution occurred. The solution was made neutral with Dowex 50W (H^+) resin and concentrated to afford compound **8b** (0.15 g, 89%), which had $[\alpha]_D$ −130° (*c* 1, H_2O); ^1H NMR (D_2O) anomeric region δ: 5.10 (bs, H-1 β-Gal*f* and H-1 α-Man*p*), 4.81 (bs, H-1 β-Man*p*); ^{13}C NMR (D_2O) δ: 105.9, 105.6 (C-1 β-Gal*f*), 94.9, 94.7 (C-1α,β-Man*p*), 79.3 (C-4′), 75.4–66.0 (C-2,2′,3,4,5,5′), 63.8, 61.8 (C-6,6′) and 34.8 (C-3′). Anal. Calcd for $C_{12}H_{22}O_{10}\cdot 2H_2O$: C, 39.78; H, 7.23. Found: C, 40.50; H, 7.18.

4-Nitrophenyl 2,5,6-tri-O-*benzoyl-3-deoxy*-β-D-xylo-*hexofuranoside* (**9b**).—To a solution of 1-*O*-acetyl-2,5,6-tri-*O*-benzoyl-3-deoxy-β-D-*xylo*-hexofuranose (**2b**, 0.57 g, 1.1 mmol) in dry CH_2Cl_2 (5 mL) cooled at 0 °C, $SnCl_4$ (0.16 mL, 1.3 mmol) was added. After 15 min, 4-nitrophenol (0.17 g, 1.2 mmol) was added, and the mixture was stirred overnight at room temperature. The mixture was processed as described for **3a**. From the crude syrup, compound **9b** (0.54 g, 86%) crystallized upon addition of EtOH. After recrystallization from the same solvent, it had mp 57–59 °C, $[\alpha]_D$ −80° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.19–7.10 (H-aromatic), 6.02 ($J_{1,2}$ < 0.5, H-1), 5.78 (m, H-5), 5.67 (d, $J_{2,3}$ 7.0, $J_{2,3'}$ 2.0 Hz, H-2), 4.78 ($J_{3,4}$ 8.2, $J_{3',4}$ 6.2 Hz, H-4), 4.71 ($J_{6,6'}$ 12.0 Hz, H-6), 4.66 (H-6′), 2.90 ($J_{3,3'}$ 14.5 Hz, H-3), 2.29 (H-3′); ^{13}C NMR ($CDCl_3$) δ: 166.1–165.7 (*C*=O benzoate), 161.0 (*C*-1, *p*-nitrophenyl), 142.5 (*C*-4, *p*-nitrophenyl), 133.5–128.4 (*C*-aromatic), 125.7 (C-2,6 *p*-nitrophenyl), 116.5 (C-3,5 *p*-nitrophenyl), 104.4 (C-1), 78.0 (C-4,2), 71.7 (C-5), 63.4 (C-6), 32.8 (C-3). Anal. Calcd for $C_{33}H_{27}NO_{10}$: C, 66.33; H, 4.55. Found: C, 66.06; H, 4.32.

4-Nitrophenyl-3-deoxy-β-D-xylo-*hexofuranoside* (**10b**).—Compound **9b** (0.60 g, 0.85 mmol) was stirred with a 0.5 M solution of NaOMe–MeOH, during 2 h. The mixture was made neutral by addition of Dowex 50W (H^+) resin, filtered, and the filtrate concentrated to afford syrupy compound **10b** (0.23 g, 95%); $[\alpha]_D$ −170° (*c* 1, H_2O); ^1H NMR (D_2O) δ: 5.81 (bs, H-1); ^1H NMR (Me_2SO-d_6) δ: 8.20 ($J_{2,3}$ 9.9 Hz, H-3 of *p*-nitrophenyl), 7.20 (H-2 of *p*-nitrophenyl), 5.70 ($J_{1,2}$ < 0.5 Hz, H-1), 4.30 (H-2,4), 3.45 (H-5,6,6′), 2.38 (m, H-3), 1.80 (m, $J_{2,3'}$ 2.6, $J_{3',4}$ 5.4, $J_{3,3'}$ 13.2 Hz, H-3′); ^{13}C NMR (Me_2SO-d_6) δ: 161.7 (C-1 of *p*-nitrophenyl), 141.3 (C-4 of *p*-nitrophenyl), 125.6 (C-2,6 of *p*-nitrophenyl), 116.5 (C-3,5 of *p*-nitrophenyl), 106.5 (C-1), 79.7 (C-4), 74.1 (C-2), 72.5 (C-5), 62.5 (C-6), 34.0 (C-3). Anal. Calcd for $C_{12}H_{15}NO_7$: C, 50.53; H, 5.30. Found: C, 50.62; H, 5.23.

Assays of β-D-*galactofuranosidase activity*.—The filtered medium of a stationary culture (20 days) of *Penicillium fellutanum* (previously *P. charlesii* G Smith NRRL 1987) was used as the enzyme source [24]. Protein was determined using bovine serum albumin as standard [25]. The enzymatic assays were made with 0.31 μmol of the tested substrates, 100 μL of 66 mM NaOAc buffer (pH 4) and 100 μL of the enzyme medium (20 μg protein), in a final volume of 500 μL. The enzymatic reactions were incubated for 1.5 h or as indicated, at 37 °C and stopped in two different ways. In the case of compounds **10a** and **10b**, they were stopped with

1 mL of sodium carbonate buffer (pH 9.0), and the 4-nitrophenol released was measured spectrophotometrically at 410 nm.

In the case of compounds **4**, **8a** and **8b**, 30 μL of the incubation mixture was heated for 2 min at 80 °C and conveniently diluted. Aliquots of 30 ng were analyzed by HPAEC.

HPAEC analysis of enzymatic digests.—Samples were analyzed using HPAEC-PAD, with a CarboPac PA1 column (4×25 mm). Eluants were 200 mM NaOH, water, and 250 mM NaOAc–150 mM NaOH, respectively. The column was equilibrated in 20 mM NaOH. After 10 min, a linear gradient of NaOAc, to a limit concentration of 125 mM over 30 min, was used. The pulsed amperometric detector sensitivity was set at 100 mA (attenuation full scale) and the pulse potentials were as follows: $E_1 = 0.05$ V, $t_1 = 480$ ms (Range 2, position 5); $E_2 = 0.6$ V, $t_2 = 120$ ms (Position 2); $E_3 = -0.6$ V, $t_3 = 60$ ms (Position 1). The time constant was set to 3 s.

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