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Synthesis of 4-nitrophenyl β-D-fucofuranoside and β-D-fucofuranosyl-(1 → 3)-D-mannopyranose: modified substrates for studies on catalytic requirements of β-D-galactofuranosidase

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Abstract

Syntheses of 4-nitrophenyl β-D-fucofuranoside (**6**) and β-D-fucofuranosyl-(1 → 3)-D-mannopyranose (**10**) are reported. These compounds, as analogues of galactofuranosides, were used for studying the influence of the hydroxyl group at C-6 in the interaction of the substrate with β-D-galactofuranosidase. For the synthesis of the fucofuranosides, 2,3,5-tri-*O*-benzoyl-6-bromo-6-deoxy-D-galactono-1,4-lactone (**1**) was the key intermediate, which upon reduction of the lactone group with diisoamylborane, acetylation of the anomeric hydroxyl group, and catalytic hydrogenolysis of the bromine at C-6, led to 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-α,β-D-fucofuranose (**4**), a convenient derivative for the preparation of fucofuranosides. Compound **4** was glycosylated in the presence of $SnCl_4$, either with 4-nitrophenol for the preparation of **6**, or with 2,5,6-tri-*O*-benzoyl-D-mannono-1,4-lactone (**7**), for the synthesis of disaccharide **10**, via the glycosyl-aldonolactone approach. The synthetic route developed for the β-D-fucofuranosides is simple and efficient. Compound **6** was not hydrolyzed by incubation with the exo β-D-galactofuranosidase from *Penicillium fellutanum*, showing that HO-6 is essential for interaction of the substrate with the enzyme.

Keywords: Deoxy sugars; β-D-Fucofuranosides; exo β-D-Galactofuranosidase

1. Introduction

In connection with our studies on the substrate specificity of the exo β-D-galactofuranosidase from *Penicillium fellutanum*, we are synthesizing deoxy analogues of galactofuranosides. A limited number of substrates have been previously tested with the enzyme, namely, ethyl and methyl galactofuranosides, arabinofuranosides, some galactofuranosyl disaccharides, and galactopyranosides [1]. A natural substrate for the enzyme is the peptidophosphogalactomannan from *P. fellutanum* [1,2]. Variations in the content of galactofuranosyl units were attributed to the action of the β-D-galactofuranosidase. Galactofuranose is also an important component of parasite glycoconjugates [3] present as terminal units in the lipopeptidophosphoglycan of *Trypanosoma cruzi* [4,5], or internally in the *Leishmania* lipophosphoglycan [6]. The deoxy analogues of the galactofuranosides could also

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be useful for studies on the interaction with the glycosyltransferase involved in the construction of the lipophosphoglycan.

In this context, we have previously reported the synthesis of β-D-Gal*f*-(1→3)-mannopyranose and the 3-deoxy analogues, 3-deoxy-β-D-*xylo*-hexofuranosyl-(1→3)-mannopyranose and 4-nitrophenyl 3-deoxy-β-D-*xylo*-hexofuranose, and have shown that whereas the enzyme hydrolyzes the former, the absence of OH-3 prevents the interaction [7]. The aim of the present work was the synthesis of 6-deoxy analogues of the substrate, that is the β-D-fucofuranosides, and the evaluation of the influence of the primary hydroxyl group in the interaction with the enzyme.

L-Fucose is the more abundant enantiomer in nature, and has only been found in the pyranose configuration. The less abundant D-fucose also occurs as the furanoside. The α anomer is present in the cell-wall antigen from *Eubacterium saburreum* L 452 [8], and in the O-antigen from different strains of *Pseudomonas syringae* [9,10]. β-D-Fucofuranose was found as a sulfated steroidal glycoside in the starfish *Dermaterias imbricata* [11].

The β-D- and β-L-fucofuranosides of secondary and tertiary alcohols, prepared from the peracetylated fucofuranoses, have been used for the determination of the absolute configuration of the alcohols by NMR techniques [12,13]. The method we now describe is a good alternative for the synthesis of the fucofuranosides.

2. Results and discussion

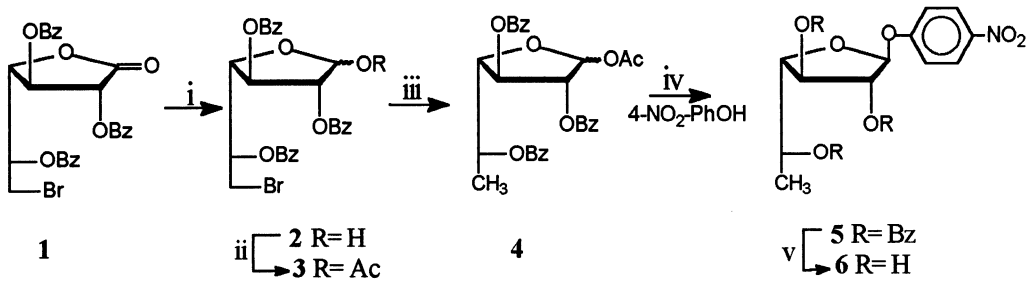
During recent years, considerable progress on the synthesis of L-fucopyranosides (oligosaccharides) has been made, but few reports describe the preparation of fucofuranosides [12,13].

The synthesis of glycofuranosides implies the availability of a precursor with the appropriate ring size. Gardiner and Percival [14] described the reaction of L-fucose with 0.8% methanolic hydrogen chloride, at room temperature. This glycosylation led to an anomeric mixture of the furanosic (64%) and pyranosic forms, which were isolated by silica gel column chromatography. The same reaction at high temperature gave higher proportions of the pyranosides [14].

We have previously obtained crystalline per-*O*-benzoyl-α,β-D-galactofuranose by direct benzoylation of D-galactose at high temperature [15,16]. The mixture of the furanosic benzoates was used for the preparation of galactofuranosyl disaccharides [16,17]. The simplicity of the procedure led us to try direct benzoylation of the analogous D-fucose for the synthesis of furanosic benzoates. When benzoylation was performed at 60 °C, the pyranosic forms were the main products, according to the relative signal intensities in the ^{1}H NMR spectrum. The α,β-furanosic forms were obtained in only 12% yield. Benzoylation at room temperature increased the yield of the α-pyranosic derivative, as usual, and the furanosic forms were obtained in 22% yield. The proportions of the furanose benzoates were similar to those reported for the acetylation of fucose with acetic anhydride–sodium acetate [18]. Although the mixtures were resolved by column chromatography, the procedure was not convenient for the preparation of a starting material. Signals for H-1 were assigned by comparison with those for perbenzoyl derivatives of D-galactose [15], and were 6.89 ppm (α-*p*, $J_{1,2}$ 3.6 Hz), 6.86 ppm (α-*f*, $J_{1,2}$ 4.8 Hz), 6.81 (β-*f*, $J_{1,2} < 1$ Hz), and 6.33 ppm (β-*p*, $J_{1,2}$ 8.3 Hz).

We previously showed the convenience of aldono-1,4-lactones as precursors for the synthesis of glycofuranosides [19], deoxy sugars [7], and furanosic disaccharides [16,17]. The lactone moiety can be selectively substituted [17], or deoxygenated via β-elimination reactions [20], with the anomeric center 'protected' as a lactone group. Following controlled reduction of this group by diisoamylborane [21,22], the free HO-1 is obtained, which can be differentially derivatized or activated for a glycosylation reaction.

2,3,5-Tri-*O*-benzoyl-6-bromo-6-deoxy-D-galactono-1,4-lactone (**1**) has been prepared in our laboratory in 90% yield as an intermediate for the synthesis of abequose (3,6-dideoxy-D-*xylo*-hexose) and its glycofuranosides [23]. We now used compound **1** as a convenient precursor for the synthesis of furanose derivatives of



Scheme 1. (i) DSBH, THF; (ii) Ac_2O, Py; (iii) H_2, Pd–C, Et_3N; (iv) $SnCl_4$, CH_2Cl_2; (v) NaOMe, MeOH.

D-fucose. Thus, reduction of **1** with diisoamylborane [21,22] afforded an anomeric mixture of 2,3,5-tri-*O*-benzoyl-6-bromo-6-deoxy-D-galactofuranoses (**2**) in 95% yield (Scheme 1). The ^{13}C NMR spectrum of **2** showed two signals in the anomeric region at 100.0 and 95.6 ppm, assigned to the β and α anomers, respectively (ratio β/α ~2.2). The signals at δ 29.6 and 29.2 were assigned to the C-6 of both anomers, and the other signals (Table 1) resemble those of the galactofuranosides previously described [16,17].

Compound **2** was acetylated with the purpose of having an appropriate leaving group for the glycosylation reactions. Thus, compound **3** was obtained (95%) in a β/α ratio ~3.3, according to the relative signal intensities from the ^1H and ^{13}C NMR spectra. The anomeric region of the ^1H NMR spectrum (Table 2) showed a broad singlet at δ 6.51, which was assigned to the H-1 of the β anomer. A doublet at 6.64 ppm was attributed to H-1 of the α anomer.

Catalytic hydrogenolysis of **3** in ethyl acetate afforded the 6-deoxy derivative **4** in 95% yield. The removal of the bromine at C-6 was evidenced by the upfield shift of the C-6 signal (15.7 ppm β, 16.1 ppm α) in the ^{13}C NMR spectrum of **4** (Table 1), as compared with the same signal in **3** (29.6 ppm).

Thus, the acylated fucofuranoses could be obtained in a total yield of 81% starting from D-galactono-1,4-lactone. This starting material compares very favorably in price with D-fucose, previously used for the preparation of D-fucofuranose derivatives [12,13].

Glycosylation of **4** was performed using $SnCl_4$ as catalyst, on the basis of our experience on the stereoselective synthesis of *O*- and *S*-glycosides of β-D-galactofuranose [16,17,24]. Condensation of **4** with 4-nitrophenol in the presence of the Lewis acid gave the β-glycoside **5**, as established by NMR spectroscopy. NMR data for **5** resemble those for the β-D-galactofuranoside derivatives [16] with the expected differences. ^1H NMR spectrum (Table 2) showed H-1 and H-2 signals as broad singlets in accordance with the 1,2-trans configuration, and signals for H-5 and H-6 upfield shielded because of the absence of oxygen at C-6. The characteristic pattern of β-D-galactofuranosides was observed in the ^{13}C NMR spectrum, with signals for C-1 at 104.0 ppm and for C-2 and C-4 over 80 ppm (Table 1).

By debenzoylation of **5** with sodium methoxide, glycoside **6** was obtained in 51% yield from **1**. The ^{13}C NMR chemical shifts of **6** (Table 1) were in good agreement with those reported by Kobayashi for other β-D-fucofuranosides [12,13]. The ^1H NMR spectrum of 4-nitrophenyl β-D-fucofuranoside (**6**, Table 2) was completely assigned.

For the preparation of disaccharide **10**, we used the glycosyl-aldonolactone approach previously developed in this laboratory [7]. Compound **4** and the partially benzoylated mannono-1,4-lactone **7** [7] were convenient

Table 1
^{13}C NMR (50.3 MHz) chemical shifts of compounds **1–6**

Compound	C-1	C-2	C-3	C-4	C-5	C-6
2β [a]	100.0	51.8	77.6	82.9	72.1	29.2
3β [a]	99.3	81.8	77.7	83.6	71.8	29.2
4β [a]	99.3	81.7	77.0	85.7	69.2	15.7
5 [a]	104.0	82.4	77.0	85.5	69.3	16.3
6 [b]	106.2	82.1	77.9	89.5	68.1	19.0

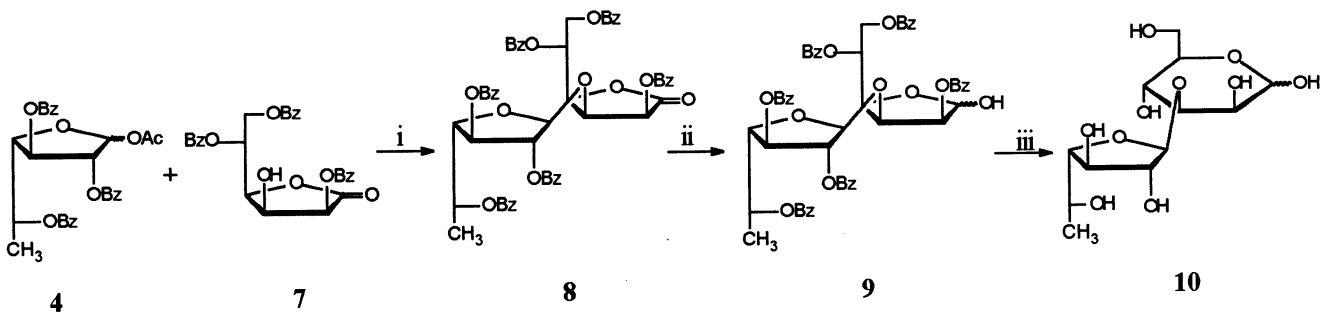
[a] The chemical shifts are expressed for a solution in $CDCl_3$, relative to internal Me_4Si.

[b] In D_2O.

Table 2
^{1}H NMR (200.1 MHz) chemical shifts of compounds **1–6**

Compound	δ (ppm), J (Hz)					
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$)	H-6,6′ ($J_{6,6'}$)
1 [a]		6.10 (6.0)	5.80	5.20 (6.0)	5.80 (2.5)	3.73
2β [a]	5.72 (<0.5)	5.52	5.52	4.95 (4.3)	4.45 (6.2)	3.78 (8.4)
3β [a]	6.51 (<0.5)	5.61	5.61 (3.2)	4.88 (4.6)	5.83	3.77
4β [a]	6.50 (<0.5)	5.60 (<1)	5.64 (4.6)	4.61 (4.5)	5.55 (6.5)	1.55
5 [a]	6.05 (<0.5)	5.76 (<1)	5.68 [b]	4.60 (4.8)	5.68 [b]	1.55
6 [c]	5.71 (1.4)	4.30 (2.9)	4.00 (5.2)	3.85 [b]	3.85 (6.2)	1.16

[a] The chemical shifts are expressed for a solution in $CDCl_3$, relative to internal Me_4Si.
[b] Center of a complex multiplet.
[c] For a solution in D_2O, relative to HOD as internal reference.



Scheme 2. (i) $SnCl_4$, CH_2Cl_2; (ii) DSBH, THF; (iii) NaOMe, MeOH.

precursors for disaccharide **10** (Scheme 2). Thus, condensation of **4** with **7** promoted by $SnCl_4$ led to the glycosyl-lactone derivative **8** as a crystalline product in 65% yield. ^{1}H and ^{13}C NMR spectra were fully assigned by comparison with the spectra of the corresponding monosaccharides. The β-D-configuration of the glycosidic linkage was confirmed by the small $J_{1,2}$ value (< 0.5 Hz) [25]. The ^{13}C NMR spectrum showed the signals of the galactofuranosyl unit (105.9, 85.5 and 81.5 ppm for C-1′, 4′ and 2′, respectively), similar to those of compound **5**, and the signals for the lactone moiety similar to the signals of lactone **7**.

Reduction of **8** with diisoamylborane led to disaccharide **9** with HO-1 free, in the α/β ratio ~ 2. Deacylation of **9** with sodium methoxide afforded disaccharide **10** in 49% overall yield from D-galactono-1,4-lactone. The tautomeric expansion of the reducing unit was evidenced in the ^{1}H NMR spectrum. The H-1′ chemical shift for the β-D-Fuc*f* unit was influenced by the anomeric configuration of the mannopyranose unit, and appeared as two close doublets at δ 5.14 ($J_{1',2'}$ 1.2 Hz) and 5.12 ($J_{1',2'}$ 1.5 Hz). The mannopyranose signals appeared at 5.18 and 4.87 ppm, in good agreement with those reported for the disaccharide β-D-Gal*f*-(1 → 3)-D-mannopyranose [7]. The α/β ratio was ~ 1.7 from the relative signal intensities. The ^{13}C NMR spectrum of **10** showed signals at 94.9 and 94.7 ppm for C-1 of the α and β anomers, respectively, in accordance with values for mannopyranose, and signals at 105.1 and 104.8 ppm for C-1 of the β-D-fucofuranosyl unit. The absence of a substituent at C-6′ elicits a downfield shift of the C-4′ signal, whereas the resonance corresponding to C-5′ is shifted upfield. The same effect was observed on C-6 deoxygenation of D-galactopyranose [26].

The synthetic route developed for the synthesis of β-D-fucofuranosides is particularly efficient, because no chromatographic separation of pyranosic and furanosic forms is required. The methodology could be extended to the synthesis of the L enantiomeric glycofuranosides, and is likewise convenient, since

the corresponding starting material, L-galactono-1,4-lactone, is less expensive than L-fucose.

The exo β-D-galactofuranosidase from *P. fellutanum* was incubated with fucofuranoside **6**, under the conditions previously described as the optimum for the incubation of 4-nitrophenyl β-D-galactofuranoside [24]. No hydrolysis of **6** was observed, even after 24 h of incubation. Some reports showed that analogues of substrates of some enzymes could act as inhibitors of the enzymes [27]. Thus, we incubated the enzyme in the presence of both the substrate and compound **6.** We observed that the presence of **6** did not modify the activity of the enzyme, even if preincubation with **6** was performed prior to addition of the substrate.

The lack of hydrolytic activity on the deoxy structure indicates a high specificity of the β-D-galactofuranosidase with respect to the glycone moiety, with both HO-3 and HO-6 of the substrates being essential for interaction with the enzyme.

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, E. Merck). 2,3,5-Tri-*O*-benzoyl-6-bromo-6-*deoxy*-D-galactono-1,4-lactone (**1**) [23] and 2,5,6-tri-*O*-benzoyl-D-mannono-1,4-lactone (**7**) [7] were prepared as previously described. Enzymatic assays were performed as reported [24].

Benzoylation of D-fucose

At high temperature. The procedure previously described for the preparation of penta-*O*-benzoyl-α,β-D-galactofuranose [15] was adapted for D-fucose. A soln of D-fucose (0.5 g, 2.77 mmol) in anhyd pyridine (7.0 mL) was stirred for 2 h at 100 °C. The temperature was lowered to 60 °C, and BzCl was added dropwise. The reaction was performed for 1.5 h at 60 °C. The mixture was then poured into ice water, and the syrup was dissolved in CH_2Cl_2 and successively washed with 5% HCl, satd aq $NaHCO_3$, and then dried (Na_2SO_4). The syrup obtained after evaporation of the solvent showed, in the ^1H NMR ($CDCl_3$) anomeric region: δ 6.89 ppm (α-*p*, $J_{1,2}$ 3.6 Hz, 5%), 6.86 ppm (α-*f*, $J_{1,2}$ 4.8 Hz, 6%), 6.81 (β-*f*, $J_{1,2} < 1$ Hz, 6%), 6.33 ppm (β-*p*, $J_{1,2}$ 8.3 Hz, 83%).

At room temperature (rt). To a soln of D-fucose (0.5 g, 2.77 mmol) in pyridine (7.0 mL), BzCl (2.0 mL) was added, and the mixture was stirred for 2 h. The soln was poured into ice water and processed as already described. ^1H NMR ($CDCl_3$), anomeric region: δ 6.89 ppm (α-*p*, $J_{1,2}$ 3.6 Hz, 46%), 6.86 ppm (α-*f*, $J_{1,2}$ 4.8 Hz, 14%), 6.81 (β-*f*, $J_{1,2} < 1$ Hz, 8%), 6.33 ppm (β-*p*, $J_{1,2}$ 8.3 Hz, 32%).

*2,3,5-Tri-*O*-benzoyl-6-bromo-6-deoxy-*D*-galactofuranose* (**2**).—To a soln of freshly prepared bis(3-methyl-2-butyl)borane (36.8 mmol) in anhyd THF (2.0 mL) [21,22], compound **1** (2.55 g, 4.6 mmol) was added. The soln was stirred for 16 h at rt and then processed as already described [21], affording **2** (2.42 g, 95%) as an amorphous solid; $[\alpha]_D$ −19° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{27}H_{23}BrO_8$: C, 58.39; H, 4.17. Found: C, 58.83; H, 4.38.

*1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-6-bromo-6-deoxy-*D*-galactofuranose* (**3**).—Compound **2** (2.0 g, 3.61 mmol) was conventionally acetylated (1:1 Ac_2O–pyridine, 7.0 mL) to afford compound **3** (2.07 g, 95%), which upon crystallization from EtOH had a mp of 135–136 °C; $[\alpha]_D$ −10° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{29}H_{23}BrO_8$: C, 58.30; H, 4.22. Found: C, 58.10; H, 4.21.

*1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-α,β-*D*-furanose* (**4**).—A soln of compound **3** (1.0 g, 1.68 mmol) in EtOAc (10 mL) containing Et_3N (1 mL) was hydrogenated with Pd–C (0.15 g) at 45 psi (3 atm) for 3 h. The catalyst was filtered and the filtrate was successively washed with 5% HCl, satd aq $NaHCO_3$, and then dried (Na_2SO_4). After evaporation of the solvent, the syrup obtained (0.83 g, 95%) was recrystallized from EtOH and had a mp of 99–100 °C; $[\alpha]_D$ −19° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{29}H_{26}O_{19}$: C, 67.18; H, 5.05. Found: C, 67.06; H, 5.14.

*4-Nitrophenyl 2,3,5-tri-*O*-benzoyl-β-*D*-furanoside* (**5**).—To a soln of **4** (1.0 g, 2.0 mmol) in dry CH_2Cl_2 (10.0 mL), $SnCl_4$ (0.25 mL, 2.0 mmol) was added, and the soln was stirred for 10 min at 0 °C. Then, 4-nitrophenol

(0.30 g, 2.4 mmol) was added and the mixture was stirred for 3 h at rt. The mixture was poured into satd 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) to obtain compound **5** (0.70 g, 60%) as a syrup; $[\alpha]_D$ −97° (*c* 1, $CHCl_3$). Anal. Calcd for $C_{33}H_{27}O_{11}$: C, 66.33; H, 4.55. Found: C, 66.52; H, 4.70.

*4-Nitrophenyl β-*D*-fucofuranoside* (**6**).—Compound **5** (0.35 g, 0.6 mmol) was suspended in a 0.5 M soln of NaOMe in MeOH (10.0 mL), and stirred for 2 h. The soln was neutralized with Dowex 50W (H^+) resin, concd and water was evaporated from the residue several times, affording compound **6** (0.16 g, 94%); $[\alpha]_D$ −145° (*c* 1.4, CH_3OH). Anal. Calcd for $C_{12}H_{15}NO_7$: C, 50.53; H, 5.30. Found: C, 50.33; H, 5.47.

*2,5,6-Tri-*O*-benzoyl-3-*O*-(2,3,5-tri-*O*-benzoyl-β-*D*-fucofuranosyl)-*D*-mannono-1,4-lactone* (**8**).—To a soln of **4** (0.57 g, 1.1 mmol) in dry CH_2Cl_2 (10 mL), $SnCl_4$ (0.14 mL, 1.21 mmol) was added, and the mixture was stirred for 10 min at 0 °C. Compound **7** (0.58 g, 1.2 mmol) was added, and stirring was continued for 3 h at rt. The soln was processed as described for **5**. The glycosyl-lactone obtained (**8**, 0.68 g, 65%) was crystallized from EtOH, and after recrystallization from the same solvent, had a mp of 182–183 °C; $[\alpha]_D$ −47° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.09–7.13 (H-aromatic), 6.05 (d, H-2, $J_{2,3}$ 3.8 Hz), 5.85 (m, H-5′), 5.72 (d, H-2′, $J_{2',3'}$ 0.8 Hz), 5.53 (bs, H-1′, $J_{1',2'} < 0.5$ Hz), 5.45 (dd, H-3′, $J_{3',4'}$ 3.3 Hz), 5.31 (m, H-5), 5.12–5.01 (H-3,4,6a), 4.65 (dd, H-6b, $J_{5,6b}$ 3.0 Hz, $J_{6a,6b}$ 12.6 Hz), 4.39 (d, H-4′, $J_{4',5'}$ 4.5 Hz), 1.13 (d, 3 H-6′, $J_{5',6'}$ 6.4 Hz); ^{13}C NMR ($CDCl_3$): δ 168.7 (*C*-1), 165.7–164.9 (*C*=O benzoates), 133.9–125.6 (*C*-aromatics), 105.9 (C-1′), 85.5 (C-4′), 81.5 (C-2′), 77.0, 76.1 (C-4,3′), 71.8 (C-3), 70.6 (C-5′), 69.2 (C-2), 67.9 (C-5), 61.8 (C-6), 15.5 (C-6′). Anal. Calcd for $C_{54}H_{44}O_{16}$: C, 68.35; H, 4.67. Found: C, 68.64; H, 4.45.

*2,5,6-Tri-*O*-benzoyl-3-*O*-(2,3,5-tri-*O*-benzoyl-β-*D*-fucofuranosyl)-*D*-mannofuranose* (**9**).—Compound **8** (0.60 g, 0.63 mmol) was reduced with bis(3-methyl-2-butyl)borane (2.6 mmol) in THF (5 mL), as described for compound **1**. The syrup obtained (**8**, 0.57 g, 95%) was purified by chromatography on a short column (19:1 toluene–EtOAc); $[\alpha]_D$ −75° (*c* 1, $CHCl_3$); ^{13}C NMR ($CDCl_3$) inter alia: δ 106.0 (C-1′); 99.9, 95.5 (C-1, both anomers), 15.9, 15.4 (C-6′ both anomers). Anal. Calcd for $C_{54}H_{46}O_{16}$: C, 68.20; H, 4.82. Found: C, 68.41; H, 4.99.

*3-*O*-β-*D*-Fucofuranosyl-*D*-mannose* (**10**).—Compound **9** (0.30 g, 0.32 mmol) was suspended in a 0.5 M soln of NaOMe in MeOH (10.0 mL) and stirred until complete dissolution occurred (2 h). The soln was made neutral with Dowex 50W (H^+), and concd. Methyl benzoate was eliminated by repeated evaporation with water. Compound **10** (0.10 g, 97%) was obtained as a syrup; $[\alpha]_D$ −13° (*c* 0.9, H_2O); ^1H NMR (D_2O) inter alia: δ 5.18 (d, H-1 α anomer, $J_{1,2}$1.8 Hz), 5.14 (d, H-1′ β anomer, $J_{1',2'}$ 1.2 Hz), 5.12 (d, H-1′ β anomer, $J_{1',2'}$ 1.5 Hz), 4.87 (d, H-1 β anomer, $J_{1,2}$ 1.0 Hz), 1.22 (d, H-6′ α anomer, $J_{5',6'}$ 6.1 Hz), 1.21 (d, H-6′ β anomer, $J_{5',6'}$ 6.6 Hz);^{13}C NMR (D_2O) δ: 105.1, 104.8 (C-1′), 94.9, 94.7 (C-1), 88.1 (C-4′), 82.4 (C-2′), 78.3 (C-3′), 75.9–66.0 (C-2,3,4,5), 68.3 (C-5′), 61.6 (C-6), 19.1, 16.4 (C-6′). Anal. Calcd for $C_{12}H_{22}O_{10}$: C, 44.17; H, 6.80. Found: C, 44.41; H, 7.04.

*Assays for specificity of exo β-*D*-galactofuranosidase from* P. fellutanum.—The enzyme was incubated with 4-nitrophenyl β-D-galactofuranoside as a control reaction, with 4-nitrophenyl β-D-fucofuranoside (**6**) for studying the specificity of the enzyme, or with both, in order to determine if compound **6** inhibited the enzyme. The assays were done by incubating 100 μL of the culture medium containing the enzyme (20 μg of protein), with 60 μL of a 5 mM soln of the substrate in 100 μL of 66 mM NaOAc buffer (pH 4.0), in a final volume of 500 μL. The enzymatic reaction was started by the addition of the enzyme, and after 1.5 h at 37 °C it was stopped with 1 mL of 0.1 M Na_2CO_3 buffer (pH 9.0). The released 4-nitrophenol was measured spectrophotometrically at 410 nm.

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