Bioorganic & Medicinal Chemistry 18 (2010) 5339–5345

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of 5-deoxy-β-D-galactofuranosides as tools for the characterization of β-D-galactofuranosidases

Andrea Bordoni, Rosa M. de Lederkremer, Carla Marino *

CIHIDECAR-CONICET, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Pabellón II, Ciudad Universitaria, 1428 Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 13 April 2010
Revised 13 May 2010
Accepted 14 May 2010
Available online 20 May 2010

Keywords:
5-Deoxy-D-galactofuranosides
PET deoxygenation
Galactofuranosidase substrates
Galactofuranosidase inhibition

ABSTRACT

Derivatives of 5-deoxy-β-D-galactofuranose (5-deoxy-α-L-*arabino*-hexofuranose) have been synthesized starting from D-galacturonic acid. The synthesis of methyl 5-deoxy-α-L-*arabino*-hexofuranoside (**14α**) was achieved by an efficient strategy previously optimized, involving a photoinduced electron transfer (PET) deoxygenation. Compound **14α** was converted into per-*O*-acetyl-5-deoxy-α,β-L-*arabino*-hexofuranoside (**16**), an activated precursor for glycosylation reactions. The $SnCl_4$-promoted glycosylation of **16** led to 4-nitrophenyl (**19α**), and 4-methylthiophenyl 5-deoxy-α-L-*arabino*-hexofuranosides (**20α**). The oxygenated analog 4-methylphenyl 1-thio-β-D-galactofuranoside (**23β**) was also prepared. The 5-deoxy galactofuranosides were evaluated as inhibitors or substrates of the *exo*-β-D-galactofuranosidase from *Penicillium fellutanum*, showing that the absence of HO-5 drastically diminishes the affinity for the protein.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

The glycobiology of galactofuranose is a field of great interest, since β-D-galactofuranosyl residues are constituents of infectious microorganisms, such as the bacteria *Mycobacterium tuberculosis* and *Mycobacterium leprae*,[1,2] trypanosomatids like *Trypanosoma cruzi* and *Leishmania*,[3–7] and fungi like *Paracoccidioides brasilensis*,[8] but are absent in mammalian cells. Therefore, the metabolic pathways involved in the biosynthesis and degradation of these microbial glycoconjugates, are attractive targets for the development of therapeutic agents. The enzymes involved in the biosynthesis of Gal*f*-containing molecules are the UDP-galactopyranosylmutase (EC 5.4.99.9),[9] which catalyzes the interconversion of UDP-Gal*p* into the donor of Gal*f* residues UDP-Gal*f*, and the galactofuranosyltransferases, which are responsible for the incorporation of the sugar into the glycoconjugates.[10] The galactofuranosyl content of these glycoconjugates may vary. For example, the presence of β-D-Gal*f* units in the mucins of the parasite *T. cruzi* depends on the strain.[11] Also in *T. cruzi*, one of the differences in the surface carbohydrates between the epimastigote form and the infective trypomastigote form, is the Gal*f* content of the glycoinositolphospholipids (GIPLs), the most abundant cell-surface molecules in epimastigotes.[12] These structural variations could be attributed to the action of β-D-galactofuranosidases.

*exo*β-D-Galactofuranosidases (EC 3.2.1.146) were characterized in *Penicillium* and *Aspergillius* species[13] and in *Helminthosporium sacchari*.[14] The best known is the specific *exo*-β-D-galactofuranosidase first purified from the culture medium of *Penicillium fellutanum*.[15] The natural substrate for the enzyme is the extracellular glycopeptide peptidophosphogalactomannan (pPGM), containing terminal 1→5 linked β-D-Gal*f* units. This glycoconjugate is a reserve source of phosphate, choline, and/or carbohydrates, which are released by enzyme-catalyzed depolymerization.[16] Among other hydrolases, the *exo*β-D-galactofuranosidase is involved in this degradation. To date, the gene encoding this enzyme has not been identified, nor has the aminoacid sequence been determined.

There have been significant efforts directed towards the development of galactofuranosyl containing molecules as tools for the Gal*f*-processing enzymes.[17] Our laboratory has also been involved in studies on the glycobiology of D-Gal*f*, particularly for the *exo*β-D-galactofuranosidase from *P. fellutanum*, which has become a model enzyme. We have described the synthesis of substrates for these enzymes (Fig. 1),[18–23] inhibitors (Fig. 2),[25–28] and deoxygenated analogs of the substrates (Fig. 3).[28–32] We have contributed to the synthesis of galactofuranosyl precursors[32,33] and the development of and new glycosylation methodologies.[28,33] We have also synthesized radioactive substrates (**4*** and **5***) which were used for studies on the galactofuranosidase[21,23] and on the galactofuranosyltransferase.[22]

The activity of the *exo*β-D-galactofuranosidase is dependent on the aglycone structure, both in substrates and inhibitors. For example, while compound **8** is a moderate inhibitor (IC_{50} 0.08 mM), compound **7**, its synthetic precursor, is much less effective (IC_{50} 0.60 mM). We demonstrated that lactone **6** is an efficient inhibitor of the enzyme (IC_{50} 0.02 mM),[24] and we developed an affinity

* Corresponding author. Fax: +54 11 4576 3346.
E-mail address: cmarino@qo.fcen.uba.ar (C. Marino).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.05.038

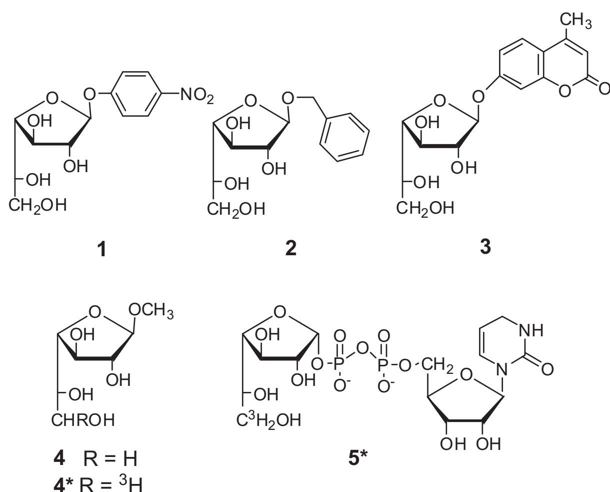


Figure 1. Synthetic substrates for the characterization of β-D-Gal*f*-processing enzymes.

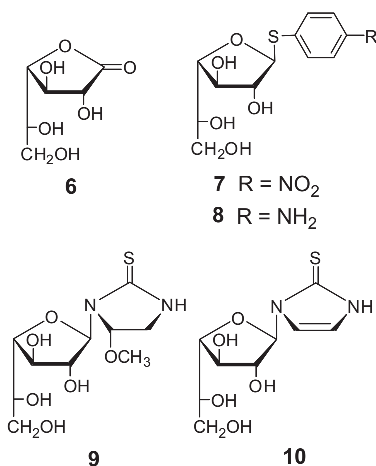


Figure 2. Inhibitors of the *exo*-β-D-galactofuranosidase from *Penicillium fellutanum*.

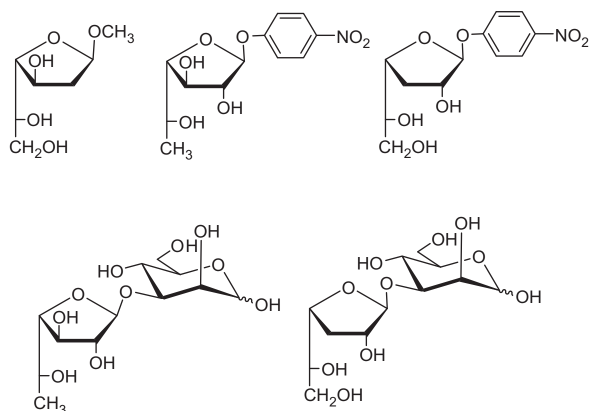


Figure 3. Synthetic 2,3 and 6-deoxygenated analogs of substrates for *exo*-β-D-galactofuranosidase.

chromatographic system for the purification of galactofuranosidases by immobilization of **8**, using lactone **6** for the elution of the enzyme.[34] By this approach, galactofuranosidase activity was detected in *T. cruzi* for the first time.[35]

The glycone specificity of the *exo*β-D-galactofuranosidase is also high and it was first evidenced by the fact that α-L-arabinofuranosides, the pentosyl homologs, were not hydrolyzed by the enzyme.[15] Deoxy sugars, analogs of the substrates, are useful in defining the importance of each specific hydroxyl group for the interaction between carbohydrates and proteins. They also play a significant role in many active compounds, such as antibiotics, antiviral drugs, and glycosylation inhibitors. The development of strategies for the synthesis of deoxy sugars as glycobiological tools has become an important field of research, and they have been synthesized as inhibitors of glycosidases, and glycosyltransferases.[36] In the field of glycofuranoses, deoxygenated D-arabinofuranose derivatives were synthesized as biochemical probes to study substrate specificity of arabinosyltranferases of *M. tuberculosis*,[37] and deoxygenated L-arabinofuranosides were prepared to investigate their potential as acceptors in biocatalytic methods.[38] An interesting strategy to afford 2-deoxy furanosides starting from 2,3-anhydro-furanosyl thioglycosides has been developed.[39]

As part of our project aimed to the characterization of galactofuranose related enzymes, we developed the synthesis of galactofuranosyl analogs deoxygenated at C-2, -3, or -6 (Fig. 3).[28–32] We found that they were resistant to the hydrolytic activity of the *exo*β-D-galactofuranosidase, indicating that the hydroxyl groups at these positions are essential for the enzyme recognition. Frequently, glycopyranosidases have a more relaxed specificity. For example, jack bean and almond α-mannosidases both hydrolyzed 4-nitrophenyl α-D-rhamnopyranoside at a rate approaching that for the α-D-mannopyranoside.[40]

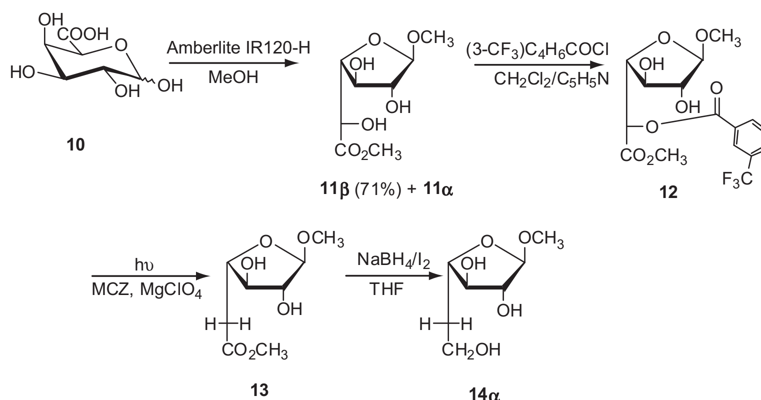
In order to further characterize the substrate specificity of this enzyme we developed a strategy for deoxygenation at C-5 of glycofuranosides. We reported the synthesis of methyl 5-deoxy-β-D-galactofuranoside (methyl 5-deoxy-α-L-*arabino*-hexofuranoside, **14α**), by a photoinduced electron transfer (PET) deoxygenation of a derivative of the methyl (methyl-D-galactofuranosid)uronate (**11**) as the key step. The advantage of the reported synthesis relies on the easy access to the furanosic derivative **11**, obtained in one step from D-galacturonic acid (**10**), the high regioselectivity for the introduction of the photoreductible 3-(trifluormethyl)benzoyl group, and the effectiveness of the PET deoxygenation of **12**, assisted by the carboxymethyl ester group at the vicinal position (Scheme 1).[32]

Having compound **14α** in hand, we have now studied its properties as inhibitor and substrate for the *exo*β-D-galactofuranosidase of *P. fellutanum*. Nevertheless, compound **14α** is not a convenient substrate, because no chromophore is released by action of the enzyme. Thus, considering the high affinity of the chromogenic derivative **1**, we developed the synthesis of its 5-deoxy analog **19α** (Scheme 2). The synthesis and biological evaluation of thioglycoside **23β** (Scheme 3) and its deoxygenated analog **20α** is also reported (Scheme 2).

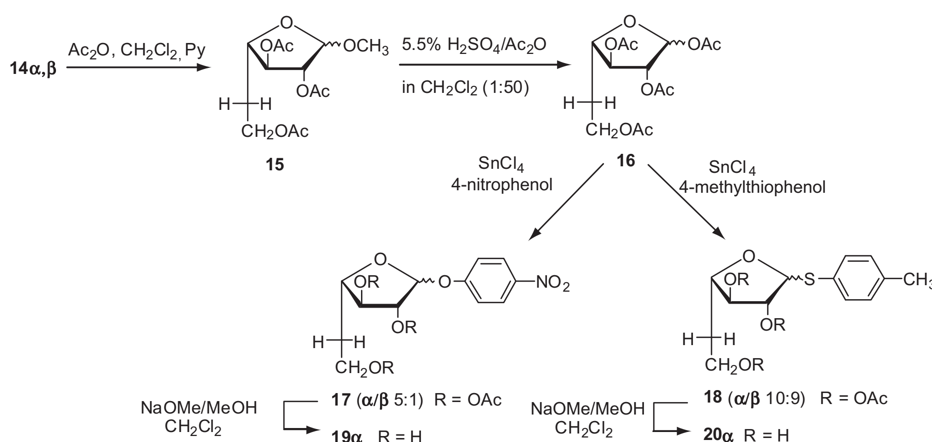
2. Results and discussion

2.1. Synthesis and chemical characterization

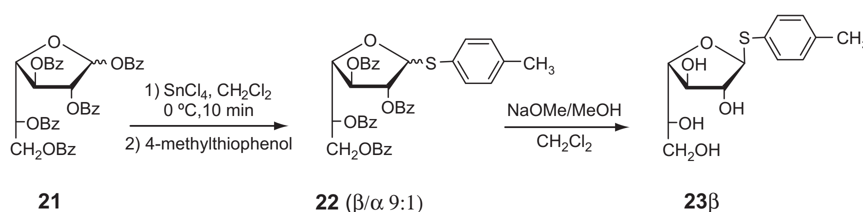
For the synthesis of **19α** we envisioned a route starting from **14**, which has been prepared in our laboratory by a sequence succinctly shown in Scheme 1. However, in the present case and in contrast to the original synthesis of **14α**,[32] it was not necessary to separate the mixture of anomers of the methyl glycoside **14**, obtained from D-galacturonic acid, since the anomeric configuration is lost during the followings steps (Scheme 2). In fact, we chose acetates as protective groups of **14** because during the acetolysis of benzoylated derivatives, transesterification was observed.[22] Starting from **15**, the conditions for the acetolysis reaction were optimized. In order to avoid the drastic conditions



Scheme 1. Synthetic route to methyl 5-deoxy-α-L-*arabino*-hexofuranoside (**14α**, Ref. 32).



Scheme 2. Synthesis of 4-nitrophenyl 5-deoxy-α-L-*arabino*-hexofuranoside (**19α**) and 4-methylphenyl 5-deoxy-1-thio-α-L-*arabino*-hexofuranoside (**20α**).



Scheme 3. Synthesis of 4-methylphenyl 1-thio-β-D-galactofuranoside (**23β**).

of the conventional acetolysis the removal of the anomeric *O*-methyl group has been attempted under milder conditions. Thus, trityl tetrafluorborate (Ph_3CBF_4) has been described as a chemoselective reagent for the hydrolysis of several methyl glycosides, including pentofuranosides.[41] However, in our hands this method was not effective, as even under longer reaction times or higher concentrations of the reagent, the hydrolysis was incomplete. Similarly, the use of $SnCl_4$ for the substitution of a benzoyloxy anomeric substituent by an acetyloxy group,[27] was also unsuccessful for **15**, probably due to the poorer capacity of the methoxide compared to an acetyloxy as leaving group. Also, treatment of **15** with acetic anhydride and trifluoroacetic acid[42] afforded **16** in low yield.

Finally, the acetolysis was satisfactorily performed using acetic anhydride and H_2SO_4 diluted in a CH_2Cl_2 solution,[22,43] affording compound **16** in 80% yield. The ^{1}H NMR spectrum of crude **16** indicated that the 1,2-*trans* anomer (α-anomer) was the major component. Integration of the area of the H-1 signal (α-anomer: 6.10 ppm, $J_{1,2}$ < 0.5 Hz; β-anomer: 6.32 ppm, $J_{1,2}$ = 4.0 Hz) indicated a 3:1 α/β ratio. The signals corresponding to H-5 and 5′ were overlapped with those of the methyl groups of the acetates. A similar anomeric relationship was determined from the ^{13}C NMR spectrum, which showed in the anomeric region the resonance of C-1 at 99.0 ppm (α-anomer) and 93.7 ppm (β-anomer), and signals at 33.7 ppm (β-anomer) and 32.0 ppm (α-anomer) corresponding to the deoxygenated C-5 of both anomers.

$SnCl_4$ was efficiently used in this laboratory as glycosylation promoter for the synthesis of D-Gal*f* derivatives, such as simple glycofuranosides,[44] thioglycosides,[24] disaccharides,[44] thiodisaccharides,[27] and oligosaccharides.[45] Thus, treatment of **16** with $SnCl_4$ and subsequent addition of 4-nitrophenol or 4-methylthiophenol led to **17** and **18**, respectively. The ^{1}H NMR spectrum of **17** showed a 5:1 ratio of 1,2-*trans* and 1,2-*cis* anomeric signals at δ 5.71 ($J_{1,2}$ < 0.5 Hz) and 5.93 ($J_{1,2}$ = 4.5 Hz). The ^{13}C NMR spectrum

showed, in the anomeric region, resonances at 103.5 and 98.1 ppm, shifted upfield in comparison to signals of *O*-alkylglycoside **14**,[32] as expected for *O*-aryl glycosides.[46] The ^{1}H NMR spectrum of **18** showed the resonances of H-1 at 5.39 ppm ($J_{1,2}$ = 2.0 Hz) and 5.53 ppm ($J_{1,2}$ = 4.9 Hz), corresponding to the 1,2-*trans* and 1,2-*cis* diastereomers, respectively, in similar amount. The ^{13}C NMR spectrum of **18** showed the C-1 signals at 90.8 ppm (α-anomer) and 89.5 ppm (β-anomer), in agreement with values observed for other thiogalactofuranosides.[24,26] The diastereocontrol of the $SnCl_4$-promoted procedure for the per-*O*-acetyl derivative **16** was less effective than for the benzoylated derivatives of D-galactofuranose,[24,44] as result of the less efficient anchimeric effect.

Compounds **17** and **18** were O-deacetylated with NaMeO/MeOH/CH_2Cl_2 to afford good yields of **19** and **20**, respectively. Recrystallization of compound **19** from water afforded the 1,2-*trans* glycoside **19α** as a single diastereoisomer, as observed in the ^{1}H NMR spectrum, which showed the anomeric signal as a singlet at 5.64 ppm ($J_{1,2}$ < 1.0 Hz). The signals corresponding to the diastereotopic hydrogens at C-5 appeared at high fields (1.87 and 1.96 ppm). The ^{13}C NMR spectrum showed the resonance of C-1 and C-5 at 108.5 and 38.2 ppm, respectively. The pure anomer **20α** was obtained by recrystallization from hexane/EtOAc (3:2). The ^{1}H NMR spectrum showed the anomeric proton as a doublet at δ 5.19 ($J_{1,2}$ = 4.5 Hz) and H-5a and H-5b as a dddd at 1.84 and 1.99 ppm, respectively. The larger $J_{1,2}$ for free 1,2-*trans* 1-thio-galactofuranosides, compared with *O*-galactofuranosides, was previously observed, and it was attributed to a conformational shift toward conformations with the anomeric sulfur atom in a quasiequatorial position,[24] reflecting the weaker anomeric effect of this atom.[47]

With the aim of evaluating the influence of HO-5 on the inhibitory activity of thioglycosides,[24,26] the oxygenated analog **23**, was synthesized. Following the described procedure for 1-thiogalactofuranosides,[24] $SnCl_4$-promoted glycosylation of perbenzoylated derivative **21**[48] with 4-methylthiophenol afforded **22**, and subsequent deprotection with NaMeO/MeOH in CH_2Cl_2, led to thioglycoside **23β** in 80% overall yield. NMR spectra of **23β** showed the presence of the 1,2-*trans* thioglycoside as the only product, and they were in agreement with those previously reported for galactofuranosyl thiogalactofuranosides.[24,26]

2.2. Biological evaluation

Previous studies with simple β-D-galactofuranosides as substrates for the *exo*β-D-galactofuranosidase activity have shown a high aglycone specificity. 4-Nitrophenyl β-D-Gal*f* (**1**) is a good substrate (K_m 0.31 mM) and proved to be useful for the measurement of the enzymatic activity,[18,34] whereas benzyl β-D-Gal*f* (**2**, K_m 3.7 mM)[20] is less effective. On the other hand, the 4-methylumbelliferyl analog **3**, considerably bulkier than **1**, is not hydrolyzed by the enzyme. The methyl glycoside **4** (K_m 2.60 mM) has also been kinetically characterized.[49] To assess the ability of the 5-deoxy derivatives as substrates for the *exo*-β-D-galactofuranosidase, compound **14α** was incubated with the enzyme under the conditions employed for substrate **1**. The reaction was monitored by HPAEC-PAD and no release of 5-deoxy galactose (t_R 24.12 min) from **14α** (t_R 13.68 min) was detected.

A colorimetric assay was also performed, using compound **19α** as substrate and monitoring the reaction by measurement of the 4-nitrophenol released. A control reaction under the test conditions (NaOAc buffer, pH 5.5, 37 °C, 1.5 h),[24] showed some chemical hydrolysis of **19α**, in contrast with the oxygenated analog **1**, which is stable. This different behavior is not surprising considering the lability of the glycosidic linkage in deoxy sugars compared to the oxygenated analogs.[50] On the other hand, enzymatic hydrolysis of **19α** was also observed. The kinetic analysis, considering the chemical hydrolysis at each point, gave a K_m value of 1.29 mM (Fig. 4). The difference in the affinity of **14α**, which is not hydrolyzed, and **19α**, a substrate with low affinity, correlates with the differences observed between **1** (K_m 0.31 mM)[18,34] and **4** (K_m 2.60 mM).[49]

Inhibition of the galactofuranosidase activity also depends on the structure of the aglycone. Thus far, the best inhibitor we have tested was lactone **6**. As above exemplified for thioglycosides **7** and **8**, also for nucleoside a high sensitivity of the enzyme was observed. Thus, compound **9** (IC_{50} 0.10 mM) was ten times more active than the analog **10** (IC_{50} 1.05 mM), suggesting that the change in the flexibility of the imidazolidine ring facilitates the interaction with the active site of the enzyme.[25]

For the evaluation of the new compounds as in vitro inhibitors of the *exo*β-D-galactofuranosidase from *P. fellutanum*, the protocol previously developed was followed. 4-Nitrophenyl β-D-galactofuranoside (**1**)[18] was used as substrate and lactone **6** as the reference inhibitor (IC_{50} 0.02 mM).[24] Releasing of 4-nitrophenol from **1** was employed as a measurement of galactofuranosidase activity. Compounds **11β**, **13**, **14α**, **19α**, **20α**, and **23β** were subjected to the enzymatic reaction, in concentrations ranging from 0.2 to 2.5 mM. Compounds **11β**, **13**, and **14α** did not show inhibitory activity (not shown). Thus, the presence of a carboxymethyl ester at C-6, the absence of HO-5, or both modifications prevent the interaction with the enzyme. The thioglycoside **23β** showed a low inhibitory activity, with 30% inhibition at 0.25 mM, in accordance with the activity of thiogalactofuranosides with similar structures (Fig. 5).[24] The 5-deoxy analog **20α**, instead, was not active, and no inhibition was observed at concentrations up to 2.5 mM. These results indicate the importance of HO-5 in the interaction with the protein, so its absence abolishes the inhibitory activity due to the presence of –S observed for thioglycoside **23β**.

3. Conclusion

The synthesis of derivatives of β-D-Gal*f* deoxygenated at C-5 starting from D-galacturonic acid, was successfully accomplished. The derivatives were evaluated as substrate and/or inhibitors of the *exo*β-D-galactofuranosidase from *P. fellutanum*. When evaluated as inhibitors, they showed no activity. Indeed, deoxygenation at C-5 in an inhibitor suppressed its activity. As substrate, compound **14α** showed no activity, indicating the importance of HO-5 for the sugar–enzyme interaction as previously observed for HO-2, -3, and -6.[28–30] However, an increase in the interaction of the glycone 5-deoxy Gal*f* with the enzyme was observed by introducing the 4-nitrophenyl group at the anomeric position, as previously observed for the oxygenated analogs.[24]

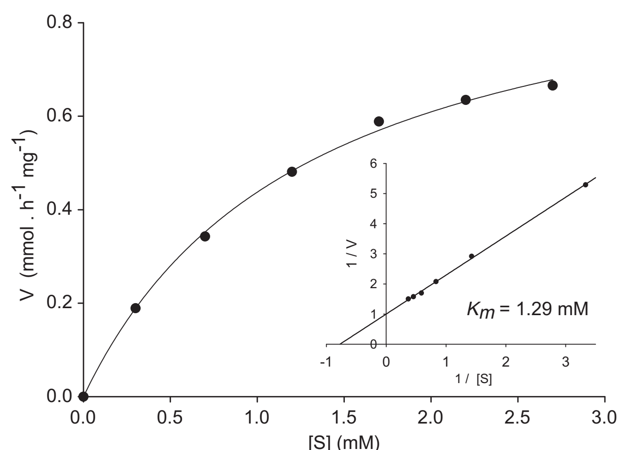


Figure 4. Kinetic analysis of substrate **19α**. Each point is the mean obtained from three replicate experiments. Inset: Lineweaver–Burk reciprocal plot.

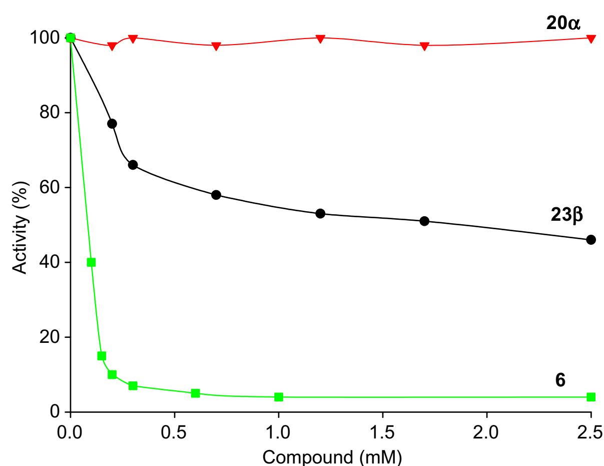


Figure 5. Effect of the concentration of added compounds on the enzymatic hydrolysis of **1** by the *exo*-β-D-galactofuranosidase from *Penicillium fellutanum*. The numbers indicate the compound added. Each point is the mean obtained from three replicate experiments.

These results reinforce the concept of the high selectivity of the β-D-galactofuranosidase, which requires all the hydroxyls of the galactofuranosyl residue in order to produce an efficient sugar–protein interaction.

On the other hand, the presence of (1→5)-linked β-D-galactofuranosides is a common feature of biologically important glycoconjugates.[1–7] In *Penicillium* and *Aspergillius* species side chains of (1→5)-interlinked β-D-Gal*f* units are inmunodominant.[13] Alternating β-(1→5) and β-(1→6) galactofuranosyl residues construct the arabinogalactan of mycobacteria and a bifunctional galactofuranosyl transferases, GlfT2, thought to be responsible for the polymerization.[2a] The deoxy galactofuranosides described in the present paper, lacking the HO-5, could be also useful for studies on the inhibition and identification of the galactofuranosyltransferase activities.

4. Experimental

4.1. General methods

Analytical thin layer chromatography (TLC) was performed on Silica Gel 60 F254 (Merck) aluminum supported plates (layer thickness 0.2 mm) with solvent systems given in the text. Visualization of the spots was effected by exposure to UV light and charring with a solution of 10% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Column chromatography was carried out with Silica Gel 60 (230–400 mesh, Merck). Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 343 digital polarimeter. For anomeric mixtures, the optical rotations were not measured. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AMX 500 spectrometer. Assignments of ^{1}H and ^{13}C were assisted by 2D ^{1}H-COSY and HSQC experiments. High resolution mass spectra (HRMS ESI+) were recorded in a Bruker micrOTOF-Q II spectrometer.

4.2. Synthesis

4.2.1. Methyl 2,3,6-tri-*O*-acetyl-5-deoxy-α,β-L-*arabino*-hexofuranoside (15)

To a solution of methyl 5-deoxy-α,β-L-*arabino*-hexofuranoside (**14**, 0.41 g, 2.30 mmol)[32] in anhydrous CH_2Cl_2 (30.0 mL) cooled to 0 °C, pyridine (1.6 mL, 19.8 mmol) and acetic anhydride (1.7 mL, 18.0 mmol) were added. The mixture was stirred at room temperature during 8 h and then was kept at 4 °C for 16 h. TLC examination showed the conversion of the starting material into a single product (R_f = 0.75, toluene/EtOAc 4:1). The mixture was evaporated under reduced pressure and co-evaporated with toluene. Compound **15** (1.33 g, 97%) was used without further purification. For the major 1,2-*trans* anomer ^{1}H NMR (CDCl$_3$, 500 MHz): δ 5.01 (d, 1H, J = 1.5 Hz, H-2), 4.88 (dd, 1H, J = 5.8, 1.5 Hz, H-3), 4.84 (s, 1H, H-1), 4.27–4.22 (m, 1H, H-6a), 4.18–4.13 (m, 1H, H-6b), 4.10 (ddd, 1H, J = 8.5, 5.5, 1.5 Hz, H-4), 3.36 (s, 3H, OCH_3), 2.15–2.10 (m, 1H, H-5a), 2.09 (s, 6H, 2 COCH_3), 2.04 (s, 3H, COCH_3), 2.03–1.96 (m, 1H, H-5b). ^{13}C NMR (CDCl$_3$, 125.8 MHz): δ 170.9, 170.3, 169.8 (*C*OCH$_3$), 106.3 (C-1), 81.8 (C-2), 80.1 (C-3), 78.8 (C-4), 61.0 (C-6), 54.7 (O*C*H$_3$), 32.1 (C-5), 20.9, 20.8, 20.7 (CO*C*H$_3$). HRMS (ESI+) calcd for $C_{13}H_{20}O_8Na^+$ [M+Na]$^+$ 327.10504, found 327.10526.

4.2.2. 1,2,3,6-Tetra-*O*-acetyl-5-deoxy-α,β-L-*arabino*-hexofuranose (16)

To a solution of crude compound **15** in dry CH_2Cl_2 (18 mL) cooled to 0 °C, acetic anhydride (0.33 mL, 3.5 mmol) and H_2SO_4 (21 μL, 0.38 mmol) were added. The mixture was stirred at room temperature during 8 h and then was kept at 4 °C for 16 h. TLC monitoring showed consumption of the starting material and the formation of a main product of R_f 0.39 (hexane/EtOAc 3:2). The reaction was stopped by dilution with CH_2Cl_2 (75 mL) and addition of cold water (5 mL). After stirring for 15 min, the organic layer was washed with satd aq $NaHCO_3$ (3× 50 mL) and brine. The organic solution was dried (Na_2SO_4), concentrated, and the residue was purified by column chromatography (hexane/EtOAc 85:15) to give compound **16** (0.26 g, 80%) as α/β mixture in a 6:1 ratio. For the major product (α-anomer): ^{1}H NMR (CDCl$_3$, 500 MHz) δ 6.14 (s, 1H, H-1), 5.18 (dd, 1H, J = 1.9, 0.5 Hz, H-2), 4.98 (ddd, 1H, J = 2.4, 1.9, 0.5 Hz, H-3), 4.29–4.22 (m, 2H, H-4, H-6a), 4.16–4.11 (m, 1H, H-6b), 2.13–2.05 (m, 14H, H-5a, H-5b, 4 COCH_3); ^{13}C NMR (CDCl$_3$, 125.8 MHz): δ 170.8, 169.3, 169.2 (*C*OCH$_3$), 99.0 (C-1), 81.3 (C-4), 80.8 (C-2), 79.4 (C-3), 60.6 (C-6), 32.0 (C-5), 21.0, 20.8, 20.7, 20.6 (CO*C*H$_3$). For the β-anomer: ^{1}H NMR (CDCl$_3$, 500 MHz): δ 6.36 (d, 1H, J = 4.0 Hz, H-1), 5.33–5.19 (m, 2H, H-2, H-3), 4.29–4.22 (m, 1H, H-6), 4.16–4.11 (m, 2H, H-4, H-6), 2.13–2.05 (m, 14H, H-5a, H-5b, 4 COCH_3); ^{13}C NMR (CDCl$_3$, 125.8 MHz): δ 170.3, 169.9, 169.4 (*C*OCH$_3$), 93.8 (C-1), 79.2 (C-4), 77.8, 75.5 (C-2, C-3), 60.6 (C-6), 33.7 (C-5), 20.9, 20.8, 20.7, 20.5 (CO*C*H$_3$). HRMS (ESI+) calcd for $C_{14}H_{20}O_9Na^+$ [M+Na]$^+$ 355.09995, found 355.10041.

4.2.3. General procedure for $SnCl_4$-promoted glycosylation

The procedure previously described was followed.[44] To a solution of **16** or **21**[48] (0.25 mmol) in anhydrous CH_2Cl_2 (2.0 mL) cooled at 0 °C, $SnCl_4$ (0.03 mL, 0.4 mmol) was added. After 10 min of stirring, 4-nitrophenol or 4-methylthiophenol (1.2 equiv) was added and the solution was stirred at room temperature for 1.5–3.5 h. The reaction mixture was washed with satd aq $NaHCO_3$ and then with brine. The organic solution was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography with the solvent specified in each individual case.

4.2.3.1. 4-Nitrophenyl 2,3,6-tri-*O*-acetyl-5-deoxy-α,β-L-*arabino*-hexofuranoside (17). The syrup obtained by $SnCl_4$-promoted glycosylation of **16** with 4-nitrophenol was purified by column chromatography (hexane/EtOAc 7:3), and fractions of R_f = 0.63 (hexane/EtOAc, 1:1) afforded an amorphous solid (0.09 g, 84%) characterized as an α/β-anomeric mixture of **17** in a 5:1 ratio. For the α-anomer: ^{1}H NMR (CDCl$_3$, 500 MHz) δ 8.18 (d, 2H, J = 9.2 Hz, H-aromatic), 7.11 (d, 2H, J = 9.2 Hz, H-aromatic), 5.71 (s, 1H, H-1), 5.34 (d, 1H, J = 1.8 Hz, H-2), 5.03 (dd, 1H, J = 5.1, 1.8 Hz, H-3), 4.28–4.25 (m, 1H, H-4), 4.24–4.20 (m, 1H, H-6a), 4.15–4.10 (m, 1H, H-6b), 2.14–1.98 (m,

11H, H-5a, H-5b, 3 COCH_3); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.7, 170.07, 169.6 (COCH$_3$); 160.9 (C-1′), 142.6 (C-4′), 125.7 (C-3′), 116.5 (C-2′), 103.9 (C-1), 81.4 (C-2), 80.9 (C-4), 78.5 (C-3), 60.6 (C-6), 32.0 (C-5), 20.8, 20.7, 20.6 (COCH$_3$). For the β-anomer: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.18 (d, 2H, J = 9.2 Hz, H-3′), 7.11 (d, 2H, J = 9.2 Hz, H-2́), 5.94 (d, 1H, J = 4.5 Hz, H-1), 5.45 (dd, 1H, J = 6.7, 5.4 Hz, H-3), 5.22 (dd, 1H, J = 4.5, 6,7 Hz, H-2), 4.15–4.10 (m, 1H, H-4), 4.09–4.04 (m, 1H, H-6a), 4.01–3.96 (m, 1H, H-6b), 2.14–1.98 (m, 10H, H-5a, 3 COCH_3), 1.94–1.86 (m, 1H, H-5b); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.3, 170.16, 170.02 (COCH$_3$), 161.1 (C-1′), 142.7 (C-4′), 125.8 (C-3′), 116.6 (C-2′), 98.1 (C-1), 79.3 (C-4), 78.1 (C-3), 76.65 (C-2), 60.5 (C-6), 34.1 (C-5), 20.8, 20.7, 20.6 (COCH$_3$). HRMS (ESI+) calcd for $C_{18}H_{21}NNaO_{10}$ $[M+Na]^+$ 434.10632, found: 434.10584.

4.2.3.2. 4-Methylphenyl 2,3,6-tri-*O*-acetyl-5-deoxy-1-thio-α,β-L-*arabino*-hexofuranoside (18). Compound **18** was obtained from **16** according to the general procedure of $SnCl_4$-promoted glycosylation with 4-methylthiophenol. After purification by column chromatography (hexane/EtOAc, 85:15) amorphous solid compound **18** (0.16 g, 87%) was obtained as an inseparable anomeric mixture (α/β 10:9), R_f = 0.53 (hexane/EtOAc 6:4). For the α-anomer: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40 (d, 2H, J = 9.2 Hz, H-aromatic), 7.38 (d, 2H, J = 9.2 Hz, H-aromatic), 5.39 (dd, 1H, ^3J = 2.0 Hz, ^4J = 0.6 Hz, H-1), 5.22 (t, 1H, J = 2.2 Hz, H-2), 4.96 (ddd, 1H, J = 5.9; 2.4 Hz, ^4J = 0.6 Hz, H-3), 4.38 (ddd, 1H, J = 8.5, 5.8, 4.6 Hz, H-4), 4.23–4.19 (m, 1H, H-6a), 4.17–4.14 (m, 1H, H-6b), 2.33 (s, 3H, CH_3Ar), 2.17, 2.12, 2.04 (3s, 9H, COCH_3), 2.19–2.00 (m, 2H, H-5a, H-5b); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.9, 170.2, 169.7 (COCH$_3$), 138.1, 132.8, 130.1, 129.9 (C-aromatic), 90.8 (C-1), 82.0 (C-2), 80.0 (C-3), 78.5 (C-4), 60.9 (C-6), 32.7 (C-5), 21.1, 21.0, 20.8, 20.7 (3 COCH$_3$, CH$_3$Ar). For the β-anomer: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40 (d, 2H, J = 9.2 Hz, H-aromatic), 7.11 (d, 2H, J = 9.2 Hz, H-aromatic), 5.53 (d, 1H, J = 4.9 Hz, H-1), 5.46 (dd, 1H, J = 4.9, 3.0 Hz, H-2), 5.09 (dd, 1H, J = 3.7, 3.0 Hz, H-3), 4.29–4.25 (m, 1H, H-6a), 4.23–4.19 (m, 1H, H-6b), 3.99 (ddd, 1H, J = 8.0, 5.0, 3.9 Hz, H-4), 2.33 (s, 3H, COCH_3), 2.09, 2.07, 2.03 (3s, 9H, COCH_3), 2.10–2.00 (m, 2H, H-5a, H-5b). ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 170.9, 170.2, 169.7 (COCH$_3$), 138.1, 132.8, 130.1, 129.9 (C-aromatic), 89.5 (C-1), 80.5 (C-4), 80.0 (C-3), 77.6 (C-2), 61.1 (C-6), 31.4 (C-5), 21.1, 21.0, 20.84, 20.83 (3 COCH$_3$, CH$_3$Ar). HRMS (ESI+) calcd for $C_{19}H_{24}O_7SNa^+$ $[M+Na]^+$: 419.11409, found: 419.11349.

4.2.3.3. 4-Methylphenyl 2,3,5,6-tetra-*O*-benzoyl-1-thio-α,β-D-galactofuranoside (22). The $SnCl_4$-promoted procedure was followed, starting from **21**[48] and 4-methylthiophenol. Column chromatography (toluene) afforded thioglycoside **22** (0.60 g, 83%), R_f = 0.72 (toluene/EtOAc 9:1), as an inseparable anomeric mixture (β/α 9:1). For the β-anomer: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.08–7.06 (m, 20H, H-aromatic), 6.11–6.09 (m, 1H, H-5), 5.78 (s, 1H, H-1), 5.69 (d, 1H, J = 5.0 Hz, H-3), 5.65 (s, 1H, H-2), 4.96 (t, 1H, J = 4.4 Hz, H-4), 4.76 (dd, 1H, J = 4.5, 11.8 Hz, H-6a), 4.71 (dd, 1H, J = 6.9, 11.8 Hz, H-6b), 2.35 (s, 3H, CH_3Ar); ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 166.0, 165.7, 165.3, 165.2 (COPh), 138.2–125.3 (C-aromatic), 91.6 (C-1), 82.3 (C-2), 81.4 (C-4), 77.9 (C-3), 70.3 (C-5), 63.4 (C-6), 21.1 (CH$_3$Ph). For the α-anomer: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.08–7.06 (m, 20H, H-aromatic), 6.04–5.99 (m, 1H, H-5), 5.93–5.92 (m, 2H, H-2, H-3), 5.78–5.77 (m, 1H, H-1), 4.86 (dd, 1H, J = 4.1, 12.0 Hz, H-6a), 4.78–4.74 (m, 1H, H-6b), 4.54 (t, 1H, J = 4.8 Hz, H-4), 2.37 (s, 3H, CH_3Ar). ^{13}C NMR ($CDCl_3$, 125.8 MHz): δ 166.0, 165.5, 165.3, 165.2 (COPh), 138.2–125.3 (C-aromatic), 90.0 (C-1), 81.6 (C-4), 78.1 (C-2), 77.0 (C-3), 70.6 (C-5), 63.4 (C-6), 21.2 (CH$_3$Ar). Anal. Calcd for $C_{41}H_{34}O_9S$: C, 70.07; H, 4.88. Found: C, 69.80; H, 4.78.

4.2.4. General procedure for O-deacylation

Compounds **17**, **18**, or **22** (0.1 mmol) were dissolved in anhydrous CH_2Cl_2 (3 mL) and cooled to 0 °C. Then, a solution 0.35 M NaOMe/MeOH (1.0 mL) was added and after 30 min of stirring it was diluted with methanol and concentrated under reduced pressure to two thirds of the volume. The solution was deionized by elution with MeOH through a column with Amberlite IR-120 plus cation-exchange resin (H^+) and purified as indicated in each case.

4.2.4.1. 4-Nitrophenyl 5-deoxy-α-L-*arabino*-hexofuranoside (4-nitrophenyl 5-deoxy-β-D-galactofuranoside, 19α). Compound **17** (0.04 g, 0,098 mmol) was O-deacetylated with NaOMe (0.35 M, 1.0 mL) in CH_2Cl_2 (3 mL) as described above. Column chromatography (toluene/EtOAc 95:5) afforded a compound **19** (19.2 mg, 67%), R_f = 0.33 (EtOAc), which crystallized from water gave pure α-anomer (89%), mp 90–92 °C, $[\alpha]_D$ −105 (c 0.3, MeOH). ^{1}H NMR (CD_3OD, 500 MHz): δ 8.21 (ddd, 2H, J = 10.4, 5.8, 3.3 Hz, 1H, H-3′), 7.19 (ddd, 2H, J = 10.4, 5.8, 3.3 Hz, 1H, H-2′), 5.64 (s, 1H, H-1), 4.58 (dd, J = 4.3, 1,8 Hz, 1H, H-2), 4.08 (ddd, 1H, J = 8.1, 7.0, 4.7 Hz, 1H, H-4), 3.82 (dd, J = 7.0, 4.3 Hz, 1H, H-3), 3.71 (ddd, J = 10.8, 6.9, 5.2 Hz, 1H, H-6a), 3.64 (ddd, J = 10.8, 7.9, 6.2 Hz, 1H, H-6b), 1.96 (dddd, J = 14.0, 7.9, 6.9, 4.7 Hz, 1H, H-5a), 1,87 (dddd, J = 14.0, 8.1, 6.2, 5.2 Hz, 1H, H-5b); ^{13}C NMR (CD_3OD, 125.8 MHz): δ 164.4 (C-1′), 144.4 (C-4′), 127. 5 (C-3′), 118.5 (C-2′), 108.5 (C-1), 84.9 (C-2), 83.6 (C-4), 83.5 (C-3), 60.5 (C-6), 38.2 (C-5). HRMS (ESI+) calcd for $C_{12}H_{15}NO_7Na^+$ $[M+Na]^+$: 308.07407, found: 308.07459.

4.2.4.2. 4-Methylphenyl 5-deoxy-1-thio-α-L-*arabino*-hexofuranoside (4-methylphenyl 5-deoxy-1-thio-β-D-galactofuranoside, 20α). A solution of **18** (0.14 g, 0.35 mmol) in anhydrous CH_2Cl_2 (10 mL) was O-deacetylated with 1.4 M NaOMe/MeOH (0.75 mL) according to the general procedure. Column chromatography (toluene/EtOAc 15:85) afforded compound **20α** (0.08 g, 85%), R_f = 0.48 (EtOAc), which was recrystallized (92%) from hexane/EtOAc (3:2), mp 89–91 °C, $[\alpha]_D$ −312 (c 1, MeOH). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.41 (d, 2H, J = 8.6 Hz, H-aromatic); 7.15 (d, 2H, J = 8.6 Hz, H-aromatic), 5.19 (d, 1H, J = 4.5 Hz, H-1), 4.01 (ddd, 1H, J = 12.5, 8.1, 5.4 Hz, H-4), 3.99 (dd, 1H, J = 5.2, 4.5 Hz, H-2), 3.76 (ddd, 1H, J = 12.2, 10.8, 4.3 Hz, H-6a), 3.72 (dd, 1H, J = 8.8, 5.4 Hz, H-3), 3.72 (ddd, 1H, J = 10.8, 7.9, 6.2 Hz, H-6b), 2.34 (s, 3H, CH_3Ar), 1.99 (dddd, 1H, J = 13.8, 12.2, 7.7, 4.5 Hz, H-5a), 1.84 (dddd, 1H, J = 13.8, 8.2, 6.4, 5.2 Hz, H-5b); ^{13}C NMR (CD_3OD, 125.8 MHz): δ 139.5, 134.2, 133.3, 131.4 (C-aromatic), 93.9 (C-1), 84.5 (C-2), 83.2 (C-3), 80.9 (C-4), 60.7 (C-6), 37.6 (C-5), 22.0 (CH$_3$Ar). HRMS (ESI+) calcd for $C_{13}H_{19}O_4SNa^+$ $[M+Na]^+$: 293.08267; found: 293.08180.

4.2.4.3. 4-Methylphenyl 1-thio-β-D-galactofuranoside (23β). Deprotection of **22** (0.39 g, 0.55 mmol) in anhydrous CH_2Cl_2 (15 mL) with 1.4 M NaMeO (1.25 mL) according to the general procedure, led to compound **23** (0.15 g, 98%) which recrystallized from hexane/EtOAc (1:1) gave pure β-anomer, mp 124–126 °C, $[\alpha]_D$ −253 (c 1.0, MeOH). ^{1}H NMR (D_2O, 500 MHz): δ 7.46 (d, 2H, J = 8.8 Hz, H-aromatic), 7.27 (d, 2H, J = 8.8 Hz, H-aromatic), 5.21 (d, 1H, J = 5.9 Hz, H-1), 4.13 (dd, 1H, J = 7.8, 6.0 Hz, H-3), 4.02 (t, 1H, J = 5.9 Hz, H-2), 3.88 (dd, 1H, J = 7.8, 3.3 Hz, H-4), 3.80 (ddd, 1H, J = 7.6, 4.7, 3.3 Hz, H-5), 3.67 (dd, 1H, J = 11.7, 4.7 Hz, H-6a), 3.62 (dd, 1H, J = 11.7, 7.6 Hz, H-6b), 2.34 (s, 3H, CH_3Ar); ^{13}C NMR (D_2O, 125.8 MHz): δ 140.0, 134.0, 130.7, 128.7 (C-aromatic), 90.9 (C-1), 82.0 (C-4), 80.3 (C-2), 76.1 (C-3), 70.9 (C-5), 63.4 (C-6), 20.9 (CH$_3$Ar). Anal. Calcd for $C_{13}H_{18}O_5S$: C, 54.53; H, 6.34. Found: C, 54.47; H, 6.21.

4.3. Enzymatic assays

The enzymatic activity was assayed using the filtered medium of a stationary culture of *P. fellutanum* as source of β-D-galactofuranosidase and 4-nitrophenyl β-D-galactofuranoside (**1**) as substrate[18,24] The standard assay was conducted with 50 μL of 66 mM NaOAc

buffer (pH 4.9), 15 μL of a 5 mM solution of 4-nitrophenyl β-D-galactofuranoside, and 20 μL (4 μg protein) of the enzyme medium, in a final volume of 250 μL. For the evaluation as inhibitors, compounds **11β**, **13**, **14α**, **20α**, and **23β** were incorporated in the amounts required to obtain a final concentration of 0.2–2.5 mM. The enzymatic reaction was stopped after 1.5 h of incubation at 37 °C by addition of 0.5 mL of 0.1 M Na_2CO_3 buffer (pH 9.0). The 4-nitrophenol released was measured spectrophotometrically at 410 nm.

For the evaluation of **14α** and **19α** as substrates, they were used in replacement of 4-nitrophenyl β-D-galactofuranoside in the standard assay in the amount needed for a final concentration of 0.3–2.7 mM.[24] For compound **14α**, release of the 5-deoxy galactose was monitored by HPAEC-PAD analysis. For **19α**, the 4-nitrophenol released was spectrophotometrically measured. One unit of activity was defined as the amount of 4-nitrophenol formed per hour per microgram of protein at 37 °C. K_m and V_m values were determined by Lineweaver–Burk plot. Controls without enzyme were incubated in parallel.

4.4. HPAEC-PAD analysis

Analysis by HPAEC-PAD was performed with a Dionex ICS-3000 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 MA-10 anion-exchange analytical column (4 × 250 mm), equipped with a MA-10 guard column (5 × 50 mm). The following program was used: 600 mM NaOH, isocratically, at a flow rate of 0.4 mL/min: t_R Gal = 20.2 min, t_R 5-deoxy galactose = 24.12 min, and t_R **14α** = 13.68 min.

Acknowledgments

The authors acknowledge financial support from the University of Buenos Aires (Project X-130), the National Research Council of Argentina (CONICET, Project PIP 1683), and the National Agency for Promotion of Science and Technology (ANPCyT, Project 1107). R.M.L. and C.M. are Research Members of CONICET, and A.B. was supported by a fellowship from CONICET.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.05.038.

References and notes

1. Crick, D. C.; Mahaprata, S.; Brennan, P. J. *Glycobiology* **2001**, *11*, 107.
2. (a) Besra, G. S.; Khoo, K. H.; McNeil, M. R.; Dell, A.; Morris, H. R.; Brennan, P. J. *Biochemistry* **1995**, *34*, 4257; (b) Bhamidi, S.; Scherman, M. S.; Rithner, C. D.; Prenni, J. E.; Chatterjee, D.; Khoo, K.-H.; McNeil, M. R. *J. Biol. Chem.* **2008**, *283*, 12992.
3. Lederkremer, R. M.; Colli, W. *Glycobiology* **1995**, *5*, 547.
4. McConville, M. J.; Collidge, T. A.; Ferguson, M. A.; Schneider, P. *J. Biol. Chem.* **1993**, *268*, 15595.
5. Turco, S. J.; Descoteaux, A. *Annu. Rev. Microbiol.* **1992**, *46*, 65.
6. Peltier, P.; Euzen, R.; Daniellou, R.; Nugier-Chauvin, C.; Ferrières, V. *Carbohydr. Res.* **2008**, *343*, 1897.
7. (a) Lowary, T. L. *Curr. Opin. Chem. Biol.* **2003**, *7*, 749; (b) Richards, M. R.; Lowary, T. L. *ChemBioChem* **2009**, *10*, 1.
8. Ahrazem, O.; Prieto, A.; San-Blas, G.; Leal, J. A.; Jiménez-Barbero, J.; Bernabé, M. *Glycobiology* **2003**, *13*, 743.
9. (a) Sanders, D. A. R.; Staines, A. G.; MacMahon, S. A.; McNeil, M. R.; Whitfield, C.; Naismith, J. H. *Nat. Struct. Biol.* **2001**, *8*, 858; (b) Beis, K.; Srikannathasan, V.; Liu, H.; Fullerton, S.; Bamford, V. A.; Sanders, D. A. R.; Whitfield, C.; McNeil, M. R.; Naismith, J. H. *J. Mol. Biol.* **2005**, *348*, 971; (c) Beverley, S. M.; Owens, K. L.; Showalter, M.; Griffith, C. L.; Doering, T. L.; Jones, V. C.; McNeil, M. R. *Eukaryot. Cell* **2005**, *4*, 1147; (d) Dykhuizen, E. C.; May, J. F.; Tongpenyai, A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2008**, *130*, 6706.
10. (a) Kremer, L.; Dover, L. G.; Morehouse, C.; Hitchin, P.; Everett, M.; Morris, H. R.; Dell, A.; Brennan, J.; McNeil, M. R.; Flaherty, C.; Duncan, K.; Besra, G. S. *J. Biol. Chem.* **2001**, *276*, 26430; (b) Beláňová, M.; Dianišková, P.; Brennan, P. J.; Completo, G. C.; Rose, N. L.; Lowary, T. L.; Mikušová, K. *J. Bacteriol.* **2008**, *190*, 1141; (c) Rose, N. L.; Completo, G. C.; Lin, S. J.; McNeil, M.; Palcic, M. M.; Lowary, T. L. *J. Am. Chem. Soc.* **2006**, *128*, 6721; (d) Mikusová, K.; Yagi, T.; Stern, R.; McNeil, M. R.; Besra, G. S.; Crick, D. C.; Brennan, P. J. *J. Biol. Chem.* **2000**, *275*, 33890.
11. Lederkremer, R. M.; Agusti, R. *Adv. Carbohydr. Chem. Biochem.* **2009**, *62*, 311.
12. Golgher, D. B.; Colli, W.; Souto-Padron, T.; Zingales, B. *Mol. Biochem. Parasitol.* **1993**, *60*, 249.
13. Cousin, M. A.; Notermans, S.; Hoogerhout, P.; Van Boom, J. H. *J. Appl. Bacteriol.* **1989**, *66*, 311.
14. Daley, L. S.; Ströbel, G. A. *Plant Sci. Lett.* **1983**, *30*, 145.
15. Rietschel-Berst, M.; Jentoft, N. H.; Rick, P. D.; Pletcher, C.; Fang, F.; Gander, J. E. *J. Biol. Chem.* **1977**, *252*, 3219.
16. Gander, J. E.; Jentoft, N. H.; Drewes, L. H.; Rick, P. D. *J. Biol. Chem.* **1974**, *249*, 2063.
17. (a) Completo, G. C.; Lowary, T. L. *J. Org. Chem.* **2008**, *73*, 4513; (b) Gandolfi-Donadío, L.; Gallo-Rodriguez, C.; Lederkremer, R. M. *Carbohydr. Res.* **2008**, *343*, 1870; (c) Riordan, J. M.; Reynolds, R. C. *Bioorg. Med. Chem.* **2007**, *15*, 5629; (d) Pathak, A. K.; Pathak, V.; Seitz, L.; Gurcha, S. S.; Besra, G. S.; Riordan, J. M.; Reynolds, R. C. *Bioorg. Med. Chem.* **2007**, *15*, 5629; (e) Bohn, M. L.; Colombo, M. I.; Pisano, P. L.; Stortz, C. A.; Rúveda, E. A. *Carbohydr. Res.* **2007**, *342*, 2522; (f) Euzen, R.; Ferrières, V.; Plusquellec, D. *Carbohydr. Res.* **2006**, *341*, 2759; (g) Lee, Y. J.; Lee, B.-Y.; Jeon, H. B.; Kim, K. S. *Org. Lett.* **2006**, *8*, 3971; (h) Wen, X.; Crick, D. C.; Brennan, P. J.; Hultin, P. G. *Bioorg. Med. Chem.* **2003**, *11*, 3579; (i) Gandolfi-Donadio, L.; Gallo-Rodriguez, C.; Lederkremer, R. M. *J. Org. Chem.* **2002**, *67*, 4430; (j) Pathak, A. K.; Besra, G. S.; Crick, D.; Maddry, J. A.; Morehouse, C. B.; Suling, W. J.; Reynolds, R. C. *Bioorg. Med. Chem.* **1999**, *7*, 2407; (k) McAuliffe, J. C.; Hindsgaul, O. *J. Org. Chem.* **1997**, *62*, 1234.
18. Varela, O.; Marino, C.; Lederkremer, R. M. *Carbohydr. Res.* **1986**, *155*, 247.
19. Marino, C.; Cancio, M. J.; Varela, O.; Lederekremer, R. M. *Carbohydr. Res.* **1995**, *276*, 209.
20. Mariño, K.; Baldoni, L.; Marino, C. *Carbohydr. Res.* **2006**, *341*, 2886.
21. Mariño, K.; Marino, C.; Lederkremer, R. M. *Anal. Biochem.* **2002**, *301*, 325.
22. Mariño, K.; Marino, C.; Lima, C.; Baldoni, L.; Lederkremer, R. M. *Eur. J. Org. Chem.* **2005**, 2958.
23. Bordoni, A.; Lima, C.; Mariño, K.; Lederkremer, R. M.; Marino, C. *Carbohydr. Res.* **2008**, *343*, 1863.
24. Marino, C.; Mariño, K.; Miletti, L.; Manso Alves, M. J.; Colli, W.; Lederkremer, R. M. *Glycobiology* **1998**, *8*, 901.
25. Marino, C.; Herczegh, P.; Lederkremer, R. M. *Carbohydr. Res.* **2001**, *333*, 123.
26. Mariño, K.; Marino, C. *Arkivoc* **2005**, *XII*, 341.
27. Repetto, E.; Marino, C.; Uhrig, M. L.; Varela, O. *Bioorg. Med. Chem.* **2009**, *17*, 2703.
28. Marino, C.; Chiocconi, A.; Varela, O.; de Lederkremer, R. M. *Carbohydr. Res.* **1998**, *311*, 183.
29. Chiocconi, A.; Marino, C.; de Lederkremer, R. M. *Carbohydr. Res.* **2000**, *323*, 7.
30. Chiocconi, A.; Marino, C.; Otal, E.; de Lederkremer, R. M. *Carbohydr. Res.* **2002**, *337*, 2119.
31. Bordoni, A.; Lederkremer, R. M.; Marino, C. *Carbohydr. Res.* **2006**, *341*, 2286.
32. Bordoni, A.; Lederkremer, R. M.; Marino, C. *Tetrahedron* **2008**, *64*, 1703.
33. Baldoni, L.; Marino, C. *J. Org. Chem.* **2009**, *74*, 1994.
34. Miletti, L. C.; Marino, C.; Mariño, K.; Lederkremer, R. M.; Colli, W.; Alves, M. J. M. *Carbohydr. Res.* **1999**, *320*, 176.
35. Miletti, L. C.; Mariño, K.; Marino, C.; Colli, W.; Alves, M. J. M.; Lederkremer, R. M. *Mol. Biochem. Parasitol.* **2003**, *127*, 85.
36. (a) Kirschning, A.; Jesberger, M.; Schöning, K. U. *Synthesis* **2001**, 507; (b) Lederkremer, R. M.; Marino, C. *Adv. Carbohydr. Chem. Biochem.* **2007**, *61*, 143; (c) Hou, D.; Lowary, T. L. *Carbohydr. Res.* **2009**, *344*, 1911.
37. Pathak, A. K.; Pathak, V.; Suling, W. J.; Riordan, J. R.; Gurcha, S. S.; Besra, G. S.; Reynolds, R. C. *Bioorg. Med. Chem.* **2009**, *17*, 872.
38. Lopez, G.; Nugier-Chauvin, C.; Rémond, C.; O'Donohue, M. *Carbohydr. Res.* **2007**, *342*, 2202.
39. Lowary, T.; Hou, D. *Org. Lett.* **2007**, *9*, 4487.
40. Nishio, T.; Miyake, Y.; Tsujii, H.; Hakamata, W.; Kadokura, K.; Oku, T. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 2038.
41. Kumar, A.; Doddi, V. R.; Vankar, Y. D. *J. Org. Chem.* **2008**, *73*, 5993.
42. Witczak, Z. J.; Chhabra, R.; Chen, H.; Xie, X.-Q. *Carbohydr. Res.* **1997**, *301*, 167.
43. Gelin, M.; Ferrières, V.; Plusquellec, D. *Eur. J. Org. Chem.* **2000**, 1423.
44. (a) Lederkremer, R. M.; Marino, C.; Varela, O. *Carbohydr. Res.* **1990**, *200*, 227; (b) Marino, C.; Varela, O.; Lederkremer, R. M. *Carbohydr. Res.* **1989**, *190*, 65.
45. (a) Gandolfi-Donadío, L.; Gallo-Rodriguez, C.; Lederkremer, R. M. *J. Org. Chem.* **2003**, *68*, 6928; (b) Mendoza, V. M.; Kashiwagi, G. A.; Lederkremer, R. M.; Gallo-Rodriguez, C. *Carbohydr. Res.* **2010**, *345*, 385.
46. Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem.* **1983**, *41*, 177.
47. Zunszain, P.; Varela, O. *J. Chem. Res.* **1995**, (S) *486*, (M) 2910.
48. Marino, C.; Gandolfi-Donadío, L.; Gallo-Rodriguez, C.; Bai, Y.; Lederkremer, R. M. In *Carbohydrate Chemistry: Proven Methods*, Kovac, P., Ed.; CRC Press, accepted for publication.
49. Tuekam, B. A.; Park, Y.-I.; Unkefer, C. J.; Gander, J. E. *Appl. Environ. Microbiol.* **2001**, *67*, 4648.
50. BeMiller, J. N. *Adv. Carbohydr. Chem.* **1967**, *22*, 25.