Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 343 (2008) 1863–1869
Carbohydrate RESEARCH

Note

Facile synthesis of methyl α- and β-D-[6-^{3}H]galactofuranosides from D-galacturonic acid. Substrates for the detection of galactofuranosidases

Andrea Bordoni, Carlos Lima, Karina Mariño, Rosa M. de Lederkremer and Carla Marino*

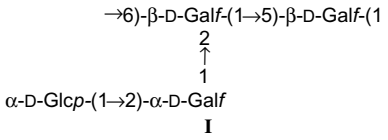
CIHIDECAR (CONICET), Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón II, Ciudad Universitaria, 1428 Buenos Aires, Argentina

Received 15 January 2008; received in revised form 19 February 2008; accepted 23 February 2008
Available online 29 February 2008

Abstract—Galactofuranose metabolism is a good target for the development of novel chemotherapeutic agents for the treatment of some microbial infections. A simple procedure for the synthesis of methyl (methyl α,β-D-galactopyranosid)uronate followed by NaB^3H_4 reduction gave a straightforward access to radiolabeled substrates for galactofuranosidases.

Keywords: D-Galacturonic acid; Galactofuranosides; Galactofuranosidases; *Penicillium* varians; Methyl glycofuranosides

Galactofuranosyl (Gal*f*) residues are present in glycoconjugates of many pathogenic bacteria, fungi, and protozoan parasites, and are highly immunogenic.[1–3] The fact that Gal*f* is essential for the survival or virulence of various pathogenic bacteria[3,4] but is absent in higher eukaryotes has generated great interest in its biosynthetic pathways. Labeled substrates are important as they are sensitive tools for the characterization of enzymes. Although the metabolism of β-D-galactofuranosides has been well characterized,[3–9] the α-D-Gal*f* biosynthetic and metabolic machinery still remain unclear. The description of the enzymes involved in its metabolism is important considering that glycoconjugates containing α-D-Gal*f* residues have been described in several pathogenic microorganisms including bacteria, (*Escherichia coli*,[10] *Streptococcus pneumoniae*,[11] and *Hafnia alvei*[12]) and fungi (*Paracoccidioides brasiliensis*, causative agent of paracoccidioidomycosis.[13]) *Penicillium varians*, a nonpathogenic fungus synthesizes varianose, an extracellular (cell wall) polysaccharide with the repeating unit **I**.[14,15]



I

The role of varianose during fungal growth is still uncertain but in *Penicillium fellutanum,* another filamentous fungus, the extracellular glycopeptide peptidophosphogalactomannan (pPGM) has been described as a reserve source of phosphate, choline, and/or carbohydrates.[16] These nutrients are released by enzyme-catalyzed depolymerization and, among other hydrolases, a β-D-galactofuranosidase is involved in this degradation.[17] By analogy with the physiological role of pPGM in *P. fellutanum*, the presence of enzymes with the specific role of hydrolyzing varianose could be proposed in *P. varians*. β-D-Galactofuranosidase activity has been detected in *Penicillium funiculosum*,[18] then called *P. varians*.[19] Degradation of varianose would require the presence of an α-D-galactofuranosidase and an α-D-glucosidase. The synthesis of methyl α-D-[6-^{3}H]galactofuranoside (**5α***) reported in the present study affords a sensitive substrate for the detection of α-D-galactofuranosidases.

* Corresponding author. Tel./fax: +54 11 4576 3352; e-mail: cmarino@qo.fcen.uba.ar

0008-6215/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.02.019

As part of our ongoing project on the development of synthetic tools for the characterization of the enzymes involved in the galactofuranosyl metabolism,[20–24] we have previously developed a synthetic sequence for the introduction of a tritium label at the 6-position of galactofuranose derivatives. The key step was the oxidation of the C-6 hydroxyl group of a conveniently prepared derivative with pyridinium chlorochromate, followed by the reduction with NaB^3H_4. Using this sequence, methyl β-D-[6-^{3}H]galactofuranoside (**5β***)[21] and UDP-β-D-[6-^{3}H]galactofuranoside (UDP-Gal*f**)[23] were obtained. We have shown the usefulness of **5β*** for the detection of the known exo-β-D-galactofuranosidase from *P. fellutanum*[21] and combined with other synthetic tools, the compound also allowed us to detect, for the first time, *exo*-β-D-galactofuranosidase activity in *Trypanosoma cruzi*. In the latter case, the availability of the radioactive material was essential for the detection because the colored biological material, in addition to the low level of enzyme activity, precluded the use of colorimetric assays.[22]

We envisioned using D-galacturonic acid (**1**) as a convenient starting material for the preparation of **5α***. It was previously described that by heating **1** with methanol under reflux in the presence of a cation exchange resin (H^+ form), methyl (methyl α-D-galactopyranosid)uronate (**4**) was obtained. However, the same reaction at room temperature for 8 h afforded methyl D-galacturonate **2**[25] (Scheme 1).

In our case, the treatment of **1** with methanol in the presence of Amberlite IR-120H for 8 h at 35 °C in an orbital shaker afforded the furanosidic isomer **3** as an α:β mixture in a 3:1 ratio, but a significant proportion of the nonglycosylated product, **2**, was still present. After keeping the reaction for 48 h, the NMR spectra showed that the furanosidic derivative **3** was the only product, in a 2.6:1 β:α ratio as deduced by the integration of the well-resolved H-5 doublets (Table 1, entry 1). The pyranose glycosides were not detected by NMR spectroscopy.

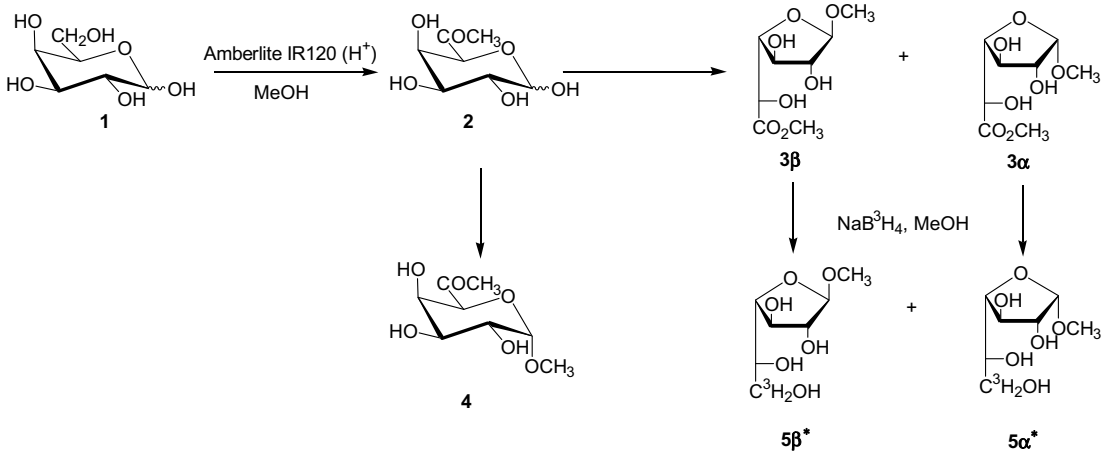
These results suggest that esterification of the carboxylic group, which proceeds faster than glycosylation,[26] favors the formation of the furanosidic ring. For comparison, we performed the same reaction with D-galactose. We observed by NMR spectroscopy that the crude product was composed mainly of the methyl β-D-galactofuranoside (**5β**), and minor amounts of the α-furanosidic and the α,β-pyranosidic isomers (Table 1, entry 2). Using column chromatography, compound **5β** was isolated in 60% yield. The preference of galactose to afford the furanosidic form by acid catalysis in methanol is well known. By the traditional Fisher glycosylation, 53% of methyl β-D-Gal*f* was obtained after chromatographic purification.[27] By comparison, the procedure using resin described here has the advantage of an easier work-up. As methyl β-D-Gal*f* is widely used as a starting material in the synthesis of galactofuranose containing molecules,[28] we consider this to be a valuable method.

By treating glucose with methanol in the presence of Amberlite IR-120H, a mixture composed by the four glycosides was obtained, but the reaction was not

Table 1. Methyl glycosides formed by the [H^+]-resin catalyzed procedure

Entry	Saccharide	Product distribution (%)			
		β-*f*	α-*f*	β-*p*	α-*p*
1	D-Galacturonic acid	71[a]	29[a]	—	—
		62[b]	28[b]		
2	D-Galactose	68[a]	17[a]	6[a]	9[a]
		60[b]			
3	D-Glucose[c]	27[a]	29[a,d]		10[a]
4	D-Mannose	11[a]	3[a]	20[a]	66[a]

[a] Determined by NMR spectroscopy.
[b] Isolated pure products after column chromatography.
[c] 34% of unreacted glucose was detected.
[d] Corresponds to the integration of the signals of the α-*f* and β-*p* glycosides, which were not resolved.



Scheme 1.

complete after 48 h (Table 1, entry 3). As expected, mannose led to methyl α-D-Man*p* as the major product (Table 1, entry 4). For the synthesis of D-glycofuranosiduronic acids and D-glycofuranosides of long chain alcohols, a stereospecific procedure involving the complexation of the C-5 and C-6 hydroxyl groups with alkaline-earth cations was described previously.[29] Our results confirm that the configuration at C-4 of galactose

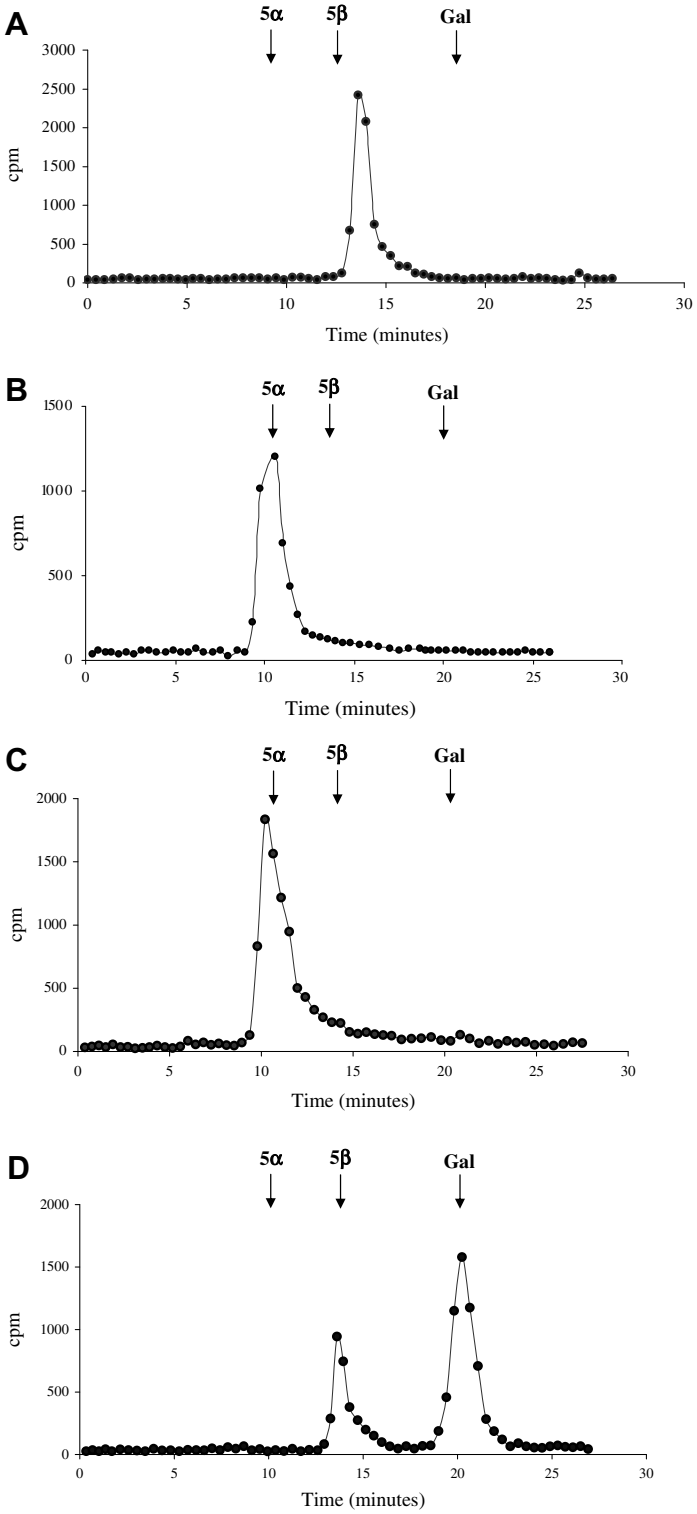


Figure 1. HPAEC-PAD analysis of [6-^{3}H]methyl-β-D-galactofuranoside (**5β***) and [6-^{3}H]methyl-α-D-galactofuranoside (**5α***) and enzymatic assays. (A) **5β***, (B) **5α***, (C) enzymatic assay on *P. varians* medium cultures of 30 days, using **5α*** as a substrate, (D) enzymatic assay on *P. varians* medium cultures of 30 days, using **5β*** as a substrate. A carboPac MA-1 column with the conditions indicated in Section 1 was used. Authentic samples of methyl β-D-Gal*f* (**5β**), methyl α-D-Gal*f* (**5β**) and galactose were used.

is responsible for the preference for the furanosidic forms, and that no complexation of the C-5/C-6 diol is necessary, at least in methanol.

The simplicity of the procedure for the preparation of **3**, followed by the reduction of the ester group constitutes a direct strategy for accessing [^{3}H]-labeled galactofuranosides for the studies of galactofuranosidases. It is well known that the reduction of carboxylic esters with $NaBH_4$ is usually slow, but that the presence of an heteroatom at the vicinal position favors the reaction as it has been observed in aldonolactones.[30] In the case of **3**, we observed that complete reduction occurred by the use of an excess of $NaBH_4$ (3 equiv) in methanol at room temperature for 3 h. The influence of the hydroxyl group at the α-position is evidenced by the fact that the 5-deoxy analogue of **3β** could not be reduced under similar conditions, and the reactivity of the $NaBH_4$ had to be enhanced by the addition of I_2, as we have recently reported.[24] Compounds **5α** and **5β** were analyzed by high performance anion-exchange chromatography with pulse amperometric detection (HPAEC-PAD), and conditions for good resolution were established (Fig. 1, see Section 1).

Reduction of **3β** by using NaB^3H_4, afforded **5β*** in a significantly shorter way (Fig. 1, panel A). Although the stereoselectivity in favor of **3α** is poor, the efficiency of the chromatographic separation from **3β** and the simplicity of the route for introducing the tritium label favor its use. Next, compound **3α** was reduced in a methanolic solution of NaB^3H_4, affording **5α***, which showed the same chromatographic properties as nonradioactive **5α** (Fig. 1, panel B).

With compound **5α*** in hand as a new substrate for α-D-galactofuranosidase detection, and using other substrates previously developed,[20,31,32] we investigated enzyme activities in *P. varians* cultures. The culture medium of different ages was filtered, dialyzed, lyophilized, and used for the assays. The presence of β-D-galactofuranosidase activity was evaluated using 4-nitrophenyl β-D-galactofuranoside as a substrate.[20,31] The activity detected was in accordance with the results previously reported.[18] Using 4-nitrophenyl α-D-glucopyranoside as a substrate,[33] no α-glucosidase activity was detected. The radiolabeled substrates **5β*** and **5α*** were used under the conditions described for the detection of β-D-galactofuranosidase activity in *P. fellutanum*[21] and the activity was followed by HPAEC-PAD. No α-D-galactofuranosidase activity was detected under the conditions used in this study (Fig. 1, panel C). However, the effectiveness of the radiolabeled substrates was demonstrated by the detection of β-galactofuranosidase in a culture medium, by the release of radioactive galactose from **5β** (Fig. 1, panel D). It cannot be ruled out that other conditions (pH, temperature) are required for the activity of an α-galactofuranosidase or if an *endo*-enzyme is acting in *P. varians*.

In summary, conditions to afford furanosidic derivatives of D-galacturonic acid and D-galactose in high yield by the reaction of the free monosaccharides with methanol in the presence of Amberlite IR-120H have been described. This is an easy methodology, which combined with the reduction of the carboxylate ester group of **3** with NaB^3H_4 constitutes a direct strategy for accessing to [^{3}H]-labeled galactofuranosides.

1. Experimental

1.1. General methods

Thin-layer chromatography (TLC) was performed on 0.2 mm Silica Gel 60 F_{254} (Merck) aluminum supported plates. Detection was effected by exposure to UV light and by spraying with 10% (v/v) H_2SO_4 in EtOH and charring. Column chromatography was performed on Silica Gel 60 (200–400 mesh, Merck). NMR spectra were recorded with a Bruker AC 200 spectrometer at 200 MHz (^{1}H) and 50 MHz (^{13}C) or with a Bruker AM 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C). Melting points were determined with a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 343 polarimeter, with a path length of 1 dm.

1.2. General procedure for methylation promoted by cation exchange resin

A suspension of the substrate (5.55 mmol) in MeOH (20.0 mL) with 1.0 g of Amberlite IR-120H was stirred at 35 °C in an orbital shaker at 120 rpm for 48 h. After filtration, the solvent was removed under diminished pressure. The following products were thus obtained:

1.2.1. Methyl (methyl β-D-galactofuranosid)uronate (3β). TLC examination of the crude product from D-galacturonic acid (1.00 g) showed a main component with $R_f = 0.41$ (EtOAc, twice developed) and a lower moving product ($R_f = 0.36$). NMR analysis showed the presence of **3** in a β/α ratio of 2.6:1 as indicated by the integration of the H-5 signals. The syrup (1.11 g) was purified by column chromatography (49:1 EtOAc–toluene). Fractions of $R_f = 0.48$ afforded syrupy **3β** (0.71 g, 62%), $[\alpha]_D$ −125 (*c* 1, MeOH); lit.[34] $[\alpha]_D$ −112 (*c* 1.38, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 4.89 (d, $J = 1.9$ Hz, 1H, H-1), 4.50 (d, $J = 2.7$ Hz, 1H, H-5), 4.29 (dd, $J = 2.7$, 6.5 Hz, 1 H, H-4), 4.18 (dd, $J = 3.9$, 6.5 Hz, 1H, H-3), 4.03 (dd, $J = 1.9$, 3.9 Hz, 1H, H-2), 3.81 (s, 3 H, OCH_3), 3.38 (s, 3H, CO_2CH_3); ^{13}C NMR (50.3 MHz, D_2O): δ 174.2 (C-6), 109.0 (C-1), 84.1, 81.0 (C-2,C-4), 76.3 (C-3), 70.0 (C-5), 55.6 ($COOCH_3$), 53.5 (OCH_3).

1.2.2. Methyl (methyl α-D-galactofuranosid)uronate (3α). Fractions of $R_f = 0.36$ afforded methyl (methyl α-D-galactofuranosid)uronate (**3α**, 0.31 g, 28%). Recrystallized from 1:1 hexane–EtOAc it gave mp 63–64 °C, $[\alpha]_D$ +95 (*c* 1, MeOH); lit.[34] mp 64–65 °C, $[\alpha]_D$ +93 (*c* 1.17, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 4.85 (d, $J = 4.8$ Hz, 1H, H-1), 4.41 (d, $J = 3.8$ Hz, 1H, H-5), 4.18 (apparent t, $J = 9.5$ Hz, 1H, H-3), 4.14 (dd, $J = 4.7$, 8.4 Hz, 1H, H-2), 4.12 (dd, $J = 3.8$, 7.5 Hz, 1H, H-4), 3.80 (s, 3H, OCH_3), 3.41 (s, 3H, CO_2CH_3); ^{13}C NMR (125.8 MHz, D_2O): δ 174.4 (C-6), 102.6 (C-1), 82.3 (C-4), 76.5 (C-2), 73.8 (C-3), 70.9 (C-5), 56.1 ($COOCH_3$), 53.6 (OCH_3).

1.2.3. Other glycosylation reactions.

1.2.3.1. D-Galactose. TLC analysis of the crude product showed four spots, which were identified as methyl β-D-Gal*f*, methyl α-D-Gal*f*, and methyl α,β-D-Gal*p*. The mixture was purified by column chromatography (100:7→100:12, EtOAc–MeOH). Fractions of $R_f = 0.59$ (14:1:1 *n*PrOH–NH_3–H_2O) afforded methyl β-D-galactofuranoside (0.64 g, 60%), $[\alpha]_D$ +110 (*c* 1, water); in agreement with data from the literature.[27,36] Fractions of $R_f = 0.55$ corresponded to methyl α-D-Gal*f*,[35] which could not be well separated from the pyranose forms.

1.2.3.2. D-Glucose and D-mannose. The crude syrups were analyzed by NMR spectroscopy[36] (Table 1, entries 3 and 4).

1.3. Reduction of methyl (methyl D-galactofuranosid)uronates (3) with $NaBH_4$

To a suspension of the substrate (0.92 g, 4.17 mmol) in MeOH (15 mL), $NaBH_4$ (0.41 g, 12.69 mmol) was added in 4 portions (4 × 0.12 g) every 0.5 h. After stirring for 3 h, TLC analysis showed complete consumption of the starting material (14:1:1 *n*PrOH–NH_3–H_2O). The solution was deionized through a column of Amberlite IR-120H resin by elution with MeOH. Evaporation of the solution under reduced pressure and several co-evaporations with MeOH afforded a syrup, which was purified by column chromatography (100:7 EtOAc–MeOH). The following compounds were thus obtained:

Methyl β-D-galactofuranoside (**5β**), 0.66 g, 82%, with the same chromatographic and spectroscopic properties as an authentic sample of **5β**.[35,36]

Methyl α-D-galactofuranoside (**5α**), 0.63 g, 78%, with the same chromatographic and spectroscopic properties as those reported.[27,36]

1.4. Reduction methyl (methyl D-galactofuranosid)uronates (3) with NaB^3H_4

The substrate (2.2 mg, 9.9 μmol) was reduced in MeOH (0.3 mL) with 10 mCi of NaB^3H_4 in 0.1 M KOH. The mixture was left overnight at room temperature, and then solid $NaBH_4$ (2 mg, 0.05 mmol) was added. After 4 h, the solution was decationized as described in Section 1.3 and concentrated under reduced pressure. The material was further deionized through a IWT TMD-8 (mixed form) resin column, and the solvent was evaporated.

1.5. 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:

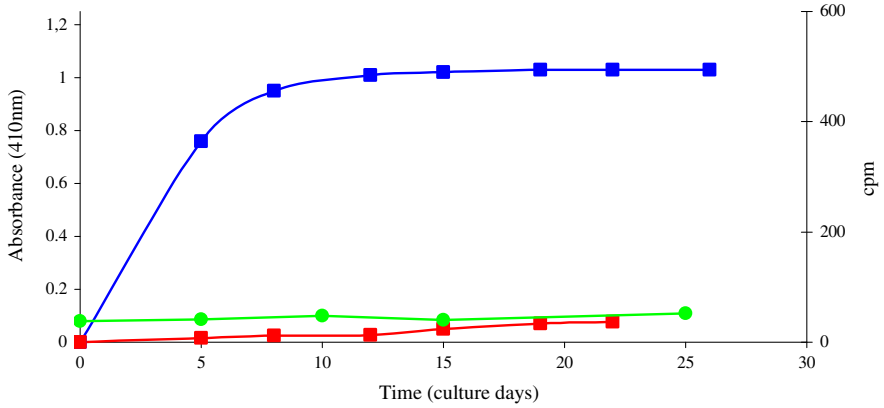


Figure 2. Enzymatic activity in filtered and lyophilized culture media from *Penicillium varians*. ■—■ β-D-galactofuranosidase: determined using 4-nitrophenyl β-D-galactofuranoside as a substrate. ●—● α-D-glucosidase: monitored using 4-nitrophenyl α-D-glucopyranoside. ■—■ α-D-galactofuranosidase: monitored using [6-^{3}H]methyl-α-D-galactofuranoside (**5α***) as a substrate. Each point is the mean obtained from three replicate experiments.

600 mM NaOH, isocratically, at a flow rate of 0.4 mL/min. Radioactivity in the fractions was determined in a Rack-beta Wallack liquid scintillation counter using a scintillation cocktail (Optiphase 'Hisafe' 3, LKB). $t_{R\,5\alpha} = 9.9$ min, $t_{R\,5\beta} = 13.6$ min, $t_{R\,Man} = 16.5$ min, $t_{R\,Gal} = 20.2$ min, $t_{R\,Glc} = 18.2$ min.

1.6. Culture of *P. varians*

An inoculum of *P. varians* (CBS 386.48) was grown under the conditions previously described[14] with glucose (5 g/L) as a carbon source. The filtered medium of different ages was dialyzed (membrane tubing MWCO 8000), lyophilized, and used for the enzymatic assays (Fig. 2).

1.7. Enzyme assays

Exo-β-D-galactofuranosidase activity was measured by using 4-nitrophenyl β-D-galactofuranoside[31] or [6-^{3}H]methyl β-D-galactofuranoside,[21] and α-D-glucosidase activity was monitored by using 4-nitrophenyl α-D-glucoside as a substrate.[33] α-D-Galactofuranosidase activity was tested by incubating the culture medium (20 μL, 50 mg total protein) with **5α*** (40,000 cpm) as a substrate, 12 μL of 66 mM sodium acetate buffer (pH 5) in a final volume of 15 μL, and incubated overnight at 37 °C. The sample was centrifuged for 40 min at 10,000*g* through an Ultrafree-MC centrifugal filter (MW 5000). The filtrate was analyzed by HPAEC-PAD.

Acknowledgments

We thank the Universidad de Buenos Aires, ANPCyT, and CONICET for financial support. R.M.L. and C.M. are research members of CONICET, and A.B. was supported by a fellowship from CONICET.

References

1. Lederkremer, R. M.; Colli, W. *Glycobiology* **1995**, *5*, 547–552.
2. Houseknecht, J. B.; Lowary, T. L. *Curr. Opin. Chem. Biol.* **2003**, *7*, 677–682.
3. Pedersen, L. L.; Turco, S. J. *Cell Mol. Life Sci.* **2003**, *60*, 259–266.
4. Kremer, L.; Dover, L. G.; Morehouse, C.; Hitchin, P.; Everett, M.; Morris, H. R.; Dell, A.; Brennan, J.; MacNeil, M. R.; Flaherty, C.; Duncan, K.; Besra, G. S. *J. Biol. Chem.* **2001**, *276*, 26430–26440.
5. Nassau, P. M.; Martin, S. L.; Brown, R. E.; Weston, A.; Monsey, D.; MacNeil, M.; Duncan, K. *J. Bacteriol.* **1996**, *178*, 1047–1052.
6. Sanders, D. A. R.; Staines, A. G.; MacMahon, S. A.; MacNeil, M. R.; Whitfield, C.; Naismith, J. H. *Nat. Struct. Biol.* **2001**, *8*, 858–863.
7. Fullerton, S. W. B.; Daff, S.; Sanders, D. A. R.; Ingledew, W. J.; Whitfield, C.; Chapman, S. K.; Naismith, J. H. *Biochemistry* **2003**, *42*, 2104–2109.
8. Rose, N. L.; Completo, G. C.; Lin, S.-J.; McNeil, M.; Palcic, M. M.; Lowary, T. L. *J. Am. Chem. Soc.* **2006**, *128*, 6721–6729.
9. Beláňová, M.; Dianišková, P.; Brennan, P. J.; Completo, G. C.; Rose, N. L.; Lowary, T. L.; Mikušová, K. *J. Bacteriol.* **2008**, *190*, 1141–1145.
10. Linnerborg, M.; Wollin, R.; Widmalm, G. *Eur. J. Biochem.* **1997**, *246*, 565–573.
11. Richards, J. C.; Perry, M. B.; Kniskern, P. J. *Can. J. Chem.* **1989**, *67*, 1038–1050.
12. Petersson, C.; Jachymek, W.; Kenne, L.; Niedziela, T.; Lugowski, C. *Carbohydr. Res.* **1997**, *298*, 219–227.
13. Ahrazem, O.; Prieto, A.; San-Blas, G.; Leal, J. A.; Jiménez-Barbero, J.; Bernabé, M. *Glycobiology* **2003**, *13*, 743–747.
14. Haworth, W. N.; Raistrick, H.; Stacey, M. *Biochem. J.* **1935**, *29*, 2668–2678.
15. Jansson, P.-E.; Lindberg, B. *Carbohydr. Res.* **1980**, *82*, 97–102.
16. Gander, J. E.; Jentoft, N. H.; Drewes, L. H.; Rick, P. D. *J. Biol. Chem.* **1974**, *249*, 2063–2072.
17. Rietschel-Berst, M.; Jentoft, N. H.; Rick, P. D.; Pletcher, C.; Fang, F.; Gander, J. E. *J. Biol. Chem.* **1977**, *252*, 3219–3226.
18. Cousin, M. A.; Notermans, S.; Hoogerhout, P.; Van Boom, J. H. *J. Appl. Bacteriol.* **1989**, *66*, 311–317.
19. Species fungorum. Global Species database. http://www.speciesfungorum.org.
20. Miletti, L.; Marino, C.; Mariño, K.; Lederkremer, R. M.; Colli, W.; Manso Alves, J. M. *Carbohydr. Res.* **1999**, *320*, 176–182.
21. Mariño, K.; Marino, C.; Lederkremer, R. M. *Anal. Biochem.* **2002**, *301*, 325–328.
22. Miletti, L.; Mariño, K.; Marino, C.; Colli, W.; Manso Alves, J. M.; Lederkremer, R. M. *Mol. Biochem. Parasitol.* **2003**, *127*, 85–88.
23. Mariño, K.; Marino, C.; Lima, C.; Baldoni, L.; Lederkremer, R. M. *Eur. J. Org. Chem.* **2005**, 2958–2964.
24. Bordoni, A.; Lederkremer, R. M.; Marino, C. *Tetrahedron* **2008**, *64*, 1703–1710 and references cited therein.
25. Cadotte, J. E.; Smith, F.; Spriestersbach, D. *J. Am. Chem. Soc.* **1952**, *74*, 1501–1504.
26. (a) Jansen, E. F.; Jang, R. *J. Am. Chem. Soc.* **1946**, *68*, 1475–1477; (b) Larsson, K.; Petersson, G. *Carbohydr. Res.* **1974**, *34*, 323–329; (c) Vlahov, J.; Snatzke, G. *Liebigs Ann. Chem.* **1983**, 570–574.
27. Augestad, I.; Berner, E. *Acta Chem. Scand.* **1954**, *8*, 251–256.
28. (a) Bai, Y.; Lowary, T. L. *J. Org. Chem.* **2006**, *71*, 9672–9680; (b) Kovensky, J.; McNeil, M.; Sinay, P. *J. Org. Chem.* **1999**, *64*, 6202–6205; (c) Bai, Y.; Lowary, T. L. *J. Org. Chem.* **2006**, *71*, 9658–9671; (d) Choudhury, K. A.; Roy, N. *Carbohydr. Res.* **1998**, *308*, 207–211; (e) Pathak, A. K.; Pathak, V.; Seitz, L.; Maddry, J. A.; Gurcha, S. S.; Besra, G. S.; Suling, W. J.; Reynolds, R. C. *Bioorg. Med. Chem.* **2001**, *12*, 3129–3143.
29. (a) Bertho, J. N.; Ferrières, V.; Plusquellec, D. *J. Chem. Soc., Chem. Commun.* **1995**, 1391–1393; (b) Ferrières, V.; Bertho, J.-N.; Plusquellec, D. *Carbohydr. Res.* **1998**, *311*, 25–35.

30. Banfi, L.; Narisano, E.; Riva, R. In *Handbook of Reagents for Organic Synthesis. Oxidizing and Reducing Agents*; Burke, S. D., Danheiser, R. L., Eds.; Wiley & Sons: Chichester, UK, 1999; pp 394–400.
31. Varela, O.; Marino, C.; Lederkrekremer, R. M. *Carbohydr. Res.* **1986**, *155*, 247–251.
32. Marino, C.; Mariño, K.; Miletti, L.; Manso Alves, M. J.; Colli, W.; Lederkremer, R. M. *Glycobiology* **1998**, *8*, 901–904.
33. Oliveira, D. E.; Santos Nieto, A. L. C.; Panek, A. D. *Anal. Biochem.* **1981**, *113*, 188–192.
34. Matsuhiro, B.; Zanlungo, A. B.; Dutton, G. G. S. *Carbohydr. Res.* **1981**, *97*, 11–18.
35. Marino, C.; Varela, O.; Lederkremer, R. M. *Carbohydr. Res.* **1989**, *190*, 65–76.
36. Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.