Synthesis of heteroaryl 1-thio-β-D-galactofuranosides and evaluation of their inhibitory activity towards a β-D-galactofuranosidase

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Dedicated to Professor Rosa M. de Lederkremer on the occasion of her 70th anniversary

Abstract

Heteroaryl 1-thio-β-D-galactofuranosides have been synthesized and evaluated as inhibitors of the exo-β-D-galactofuranosidase from *Penicillium fellutanum*. 2-Pyridinethiol, 4-pyridinethiol, 1-methylimidazole-2-thiol, 5-methyl-1,3,4-thiadiazole-2-thiol, 2-pyrimidinethiol and 4,6-dimethyl-2-pyrimidinethiol were employed as thiols, as such heteroaromatic aglycons are expected to display particular interactions with the active site of the enzyme. These thiols were condensed with per-*O*-benzoyl-D-galactofuranose, in the presence of $SnCl_4$ or $BF_3.OEt_2$, followed by *O*-debenzoylation under mild conditions to afford the heteroaryl 1-thio-β-D-galactofuranosides in high yields. The enzymatic assays showed that 4,6-dimethyl-2-pyrimidyl 1-thio-β-D-galactofuranoside was the best inhibitor (IC_{50} 135 μM), considerable more potent than the analogue lacking the methyl groups in the aglycon moiety.

Keywords: Galactofuranose, galactofuranosidase inhibitors, heteroaryl 1-thio-β-D-galactofuranosides, glycobiology

Introduction

The synthesis of thioglycosides has received considerable attention because of their activity as inhibitors[1] and inducers of glycosidases,[2] and also because of their use as glycosyl donors in the convergent synthesis of oligosaccharides.[3]

Our laboratory has long been interested in the enzymes related to galactofuranose glycobiology, as galactofuranosyl residues are found in glycoconjugates of pathogenic microorganisms, such as the bacteria *Mycobacterium tuberculosis* and *M. leprae*,[4,5] trypanosomatids like *Trypanosoma cruzi* and *Leishmania*,[6-8] and fungi like *Paracoccidioides*

brasilensis,[9] but it is 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 antimicrobial agents.

The enzymes responsible for the incorporation of galactofuranosyl units are the UDP-galactopyranosylmutase (EC 5.4.99.9),[10-12] which converts UDP-Gal*p* into the donor UDP-Gal*f*, and a UDP-galactofuranosyltransferase, which is responsible for the incorporation of the sugar into the glycoconjugates.[13] The galactofuranosyl content of these glycoconjugates varies *in vivo*. For example the presence of β-D-Gal*f* units in the mucins of the parasite *T. cruzi* depends on the strain.[14] Also in *T. cruzi*, the main difference between the epimastigote form (the divisible form in the midgut of the insect host) and the trypomastigote form (the invasive form) is the Gal*f* content in the glycoinositolphospholipids (GIPLs), the most abundant cell-surface molecules.[15] The degradation of the GIPLs could be involved in the parasite differentiation, what suggests that the inhibition of this metabolic pathway could prevent the evolution of the parasite to the infective form. These structural variations are attributed to the action of β-D-galactofuranosidases.

Penicillium fellutanum is a non pathogenic fungus which produces an extracellular peptidophosphogalactomannan (pPGM) containing terminal β-D-Gal*f* units. The percentage of this sugar decreases with the age of the culture as result of the action of an exo-β-D-galactofuranosidase.[16,17] The enzymes of this fungus related to galactofuranose glycoconjugates, have become model enzymes for us. We developed the synthesis of galactofuranose derivatives, as tools for their characterization, which once optimized could be used for the study of analogue enzymes in other microorganisms. Thus, we have synthesized chromogenic[18,19] and radioactive substrates,[20,21] and deoxygenated analogues of galactofuranosides.[22-24] We reported the synthesis of the alkyl, benzyl and aryl 1-thio-β-D-galactofuranosides **1-6**[25] and nucleosides **7-8**[26] (Figure 1), and their activities as exo-β-D-galactofuranosidase inhibitors have been proved. We also demonstrated that D-galactono-1,4-lactone (**9**) is and efficient inhibitor of such enzyme,[25] and we developed an affinity chromatography system for the purification of β-D-galactofuranosidases by immobilization of **6**, using lactone **9** for the elution of the enzyme.[27] We studied the influence of the inhibitors on the culture of *Penicillium fellutanum*,[28] which produces a galactofuranosidase.[17] The development of such tools allowed us to detect β-D-galactofuranosidase activity in *T. cruzi* for the first time.[29]

The growing interest in galactofuranose glycobiology prompted us to explore the inhibitory activities of new thioglycosides, such as heteroaryl 1-thio-β-D-galactofuranosides.

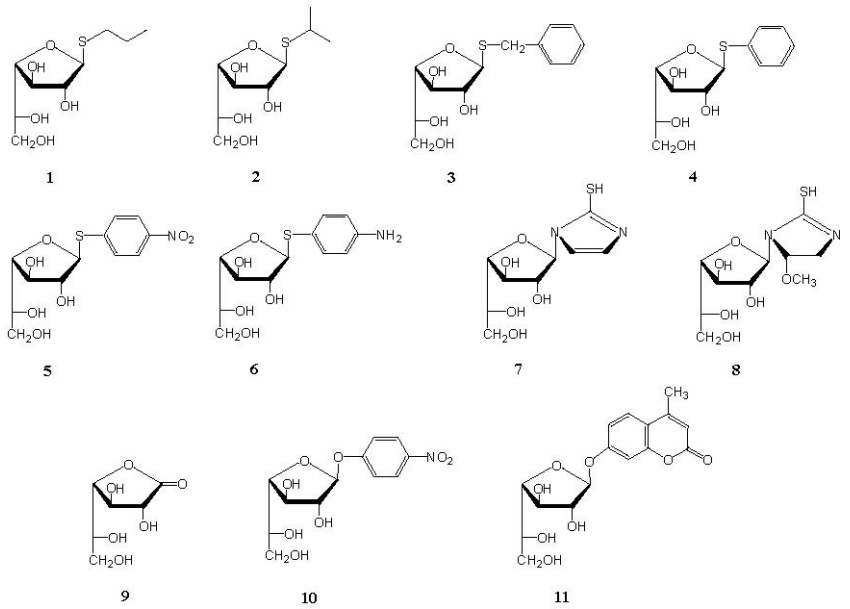


Figure 1. Inhibitors and substrates of exo-β-D-galactofuranosidase from *Penicillium fellutanum*.

We expect that these compounds could present an increased inhibitory activity as result of different interactions of the aglycon heteroatoms with the active site of the enzyme. Heteroaryl 1-thio-β-D-glycofuranosides were also synthesized by Plusquellec[30,31] and they were used as donors for the synthesis of D-glycofuranosyl 1-phosphates, according to a procedure based on the "remote activation concept".[32] We now report the synthesis of heteroaryl 1-thio-β-D-galactofuranosides and their activity as inhibitors of the exo-β-D-galactofuranosidase from *P. fellutanum*.

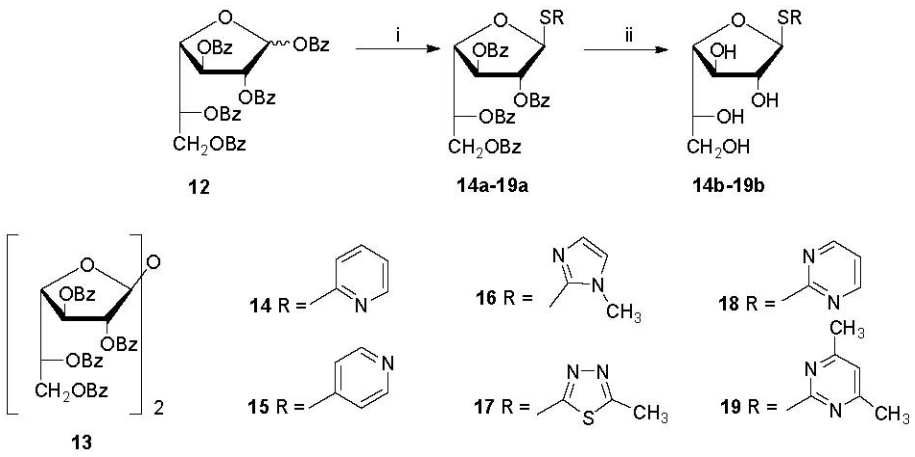
Results and Discussion

In contrast to glycosyltransferases, glycosidases are characterized for a broad substrate specificity. Compounds with considerable structural differences with respect to the natural substrates, can be hydrolysed with a characteristic kinetics,[33] or act as inhibitors of the glycosidase.[1] However, the exo-β-D-galactofuranosidase from *P. fellutanum* is highly specific, and its activity strongly depends on the size and polarity of the aglycons, as well as the glycon structure. For example, 4-nitrophenyl β-D-galactofuranoside (**10**, Figure 1)[18] is a good substrate for this enzyme (K_M 0.31 mM),[27] whereas 4-methylcoumarin-7-yl β-D-galactofuranoside (**11**) [19], with similar polarity but considerably bulkier, was not hydrolyzed by the enzyme.[34] On the other hand, compound **6** was more potent than **5** as a galactofuranosidase inhibitor,[25] proving the polarity dependence. The nucleoside **8** (IC_{50} 0.10 mM) was ten times more active than the analogue **7**, suggesting that the change in the flexibility of the imidazolidine ring facilitates the interaction with the active site of the enzyme.[26] The importance of the glycon structure was first

evidenced by the fact that α-L-arabinofuranosides, the pentosyl homologues, were not hydrolyzed by the enzyme,[17] and later we found that galactofuranosyl analogues deoxygenated at positions 2, 3 or 6 were resistant to the hydrolytic activity of the exo-β-D-galactofuranosidase, indicating that the hydroxyl groups at these positions are essential for the interaction with the enzyme.[22-24]

Considering the specificity of this enzyme, we selected thiols with certain size for the synthesis of new thiogalactofuranosides as potential inhibitors. Thus, glycosylations of 2-pyridinethiol, 4-pyridinethiol, 1-methylimidazole-2-thiol, 5-methyl-1,3,4-thiadiazole-2-thiol, 2-pyrimidinethiol and 4,6-dimethyl-2-pyrimidinethiol were conducted.

We have previously described the preparation of alkyl, benzyl and aryl 1-thio-β-D-galactofuranosides starting from 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose (**12**) and $SnCl_4$ as promoter of the glycosylation.[25] However, attempted condensation of the selected thiols with **12,** under the same conditions, led to a single crystalline product spectroscopically identified as 1,1'-disacharide **13** (Scheme 1).[35] This fact was attributed to the low solubility of the thiols in the reaction solvent (CH_2Cl_2). Therefore, the reaction was conducted in CH_3CN, in which the thiols employed were completely soluble. In this case an excess of the Lewis acid was required, as the promoter underwent partial complexation by the aglycons.[30-32] Under these conditions, 2-pyridinethiol, 4-pyridinethiol, 1-methylimidazole-2-thiol and 5-methyl-1,3,4-thiadiazole-2-thiol led to thioglycosides **14a-17a** highly stereoselectively and in good yields. As 2-pyrimidinethiol and 4,6-dimethyl-2-pyrimidinethiol were not reactive under these conditions, $BF_3.OEt_2$ was employed to obtain thioglycosides **18a** and **19a**, although longer reaction times were required.



Scheme 1. Synthesis of heteroaryl 1-thio-β-D-galactofuranosides. i. $SnCl_4$ or $BF_3.OEt_2$, CH_3CN, RSH; ii. NaOMe/MeOH, CH_2Cl_2.

Compounds **14a-16a**, **18a** and **19a** were successfully debenzoylated by treatment with NaOMe in CH_2Cl_2/MeOH, leading to thioglycosides **14b-16b**, **18b** and **19b**, in almost quantitative yield. Compound **18b** was previously prepared *via* the octylglycoside as

intermediate and was evaluated as galactofuranosyl donor in the synthesis of free hexoglycofuranosyl 1-phosphates,[30,31] but the sequence involved more steps than the one we described. Compound **17a** was base sensitive, and by debenzoylation gave methyl β-D-galactofuranoside as the only product. Similarly, treatment of **17a** with MeOH-TEA-H_2O (5:2:1) also gave the methyl glycoside.

The ^{13}C NMR spectra of compounds **14a-19a**, **14b-16b**, **18b** and **19b** (Table 1), showed the characteristic β-D-galactofuranosyl pattern of signals, with those corresponding to C-2 and C-4 appearing over 80 ppm, and the anomeric signals shifted upfield in comparison with the analogue *O*-glycofuranosides, due to the shielding effect of sulfur atom.[36]

Table 1. ^{13}C NMR chemical shifts of compounds **14a-19a**, **14b-16b**, **18b** and **19b** (125 MHz)

Comp.	C-1	C-2	C-3	C-4	C-5	C-6	Others
14[a]a	87.6	82.1	77.8	82.4	70.2	63.4	156.0, 149.9, 136.5, 123.4, 121. 2
15aa	88.2	81.9	77.3	82.8	70.0	62.9	
16aa	91.3	81.9	77.5	82.3	70.0	63.3	33.7
17aa	90.1	81.8	77.5	83.0	69.9	63.3	15.6
18aa	87.9	81.8	77.6	82.9	70.3	63.5	170.0, 157.5, 117.5
19aa	87.9	82.0	77.8	83.0	70.4	63.5	23.7 (x2)
14bb	88.7	80.8	76.3	82.7	71.0	63.4	156.7, 149.9, 139.2, 125.4, 122.8
15bb	88.6	81.1	76.4	82.8	71.0	63.4	149.2 (x2), 123.7 (x2), 121.4
16bb	91.9	81.3	76.4	82.7	70.8	63.3	34.6
18bb	87.9	80.3	76.2	83.3	71.1	63.4	169.7, 158.9, 119.1
19bb	88.2	80.6	76.3	83.2	71.1	63.4	169.4, 168.4, 118.6, 23.4

[a]CDCl3. [b]D2O

The ^1H NMR spectra of **14a** and **16a-18a** (Table 2) were similar to those previously described for per-*O*-benzoylated 1-thio-β-D-galactofuranosides,[25] but with the anomerical signals significantly shielded downfield as effect of the heteroaryl aglycons. Compounds **14a** and **16a-18a**, showed vicinal coupling constants characteristic of conformations of the ^1E-$^1T_0$-E_0 segment of the pseudorotational itinerary, having the anomeric substituent in a quasi-axial position.[37] In contrast, compounds **15a, 19a** and the free thioglycosides **14b-16b**, **18b** and **19b**, showed vicinal coupling constants suggesting a conformational shift towards the ^3E-$^2T_3$-E_3 region of the circle, with the anomeric sulfur atom in a quasi equatorial disposition.

Table 2. 1H NMR chemical shifts for compounds **14a-19a**, **14b-16b**, **18b** and **19b** (500 MHz)

	δ (ppm) *J* (Hz)							
Comp.	H-1	H-2	H-3	H-4	H-5	H-6	H-6'	Others
	$JB_{1,2B}$	$JB_{2,3B}$	$JB_{3,4B}$	$JB_{4,5B}$	$JB_{5,6B}$	$JB_{6,6'B}$	$JB_{5,6'B}$	
14a[a]	6.72	5.79	5.77	4.85	6.12	4.75	4.72	8.47 (H-6')
	(< 0.5)	(< 0.5)	(4.5)	(4.5)	(4.6)	(12.0)	(6.7)	
15a[a]	6.84	5.89	6.29	4.75	5.87	4.79	4.68	
	(4.9)	(7.4)	(6.9)	(4.6)	(4.3)	(12.2)	(6.3)	
16a[a]	6.01	5.73	5.70	4.93	6.06	4.72	4.64	
	(< 0.5)	(< 0.5)	(4.9)	(4.1)	(4.3)	(1.9)	(7.1)	
17a[a]	6.30	5.71	5.76	4.92	6.12	4.75	4.71	2.69 (C*H*B_{3B})
	(< 0.5)	(1.2)	(4.6)	(4.1)	(4.5)	(11.9)	(6.9)	
18a[a]	6.83	5.28*		4.84	6.12	4.77	4.74	8.55 (H-4',6'), 7.06 (H-5')
	(< 0.5)		(4.3)	(4.5)	(11.9)	(6.5)		
19a[a]	6.84	5.89*	6.29	4.78	5.89*	4.75	4.68	
	(4.6)	(6.0)	(6.7)	(4.7)	(4.3)	(12.0)	(6.4)	
14b[b]	5.59	4.08*		3.90	3.71	3.51	3.47	8.25 (H-6'), 7.63 (H-4'), 7.38 (H-3'),
	(5.1)		(7.2)	(3.2)	(5.0)	(11.6)	(6.4)	7.14 (H-5')
15b[b]	5.52	4.10*		3.91	3.72	3.50*		8.21(H-2'), 7.32 (H-3')
	(5.5)		(7.0)	(3.2)				
16b[b]	5.13	4.02	3.96	3.74	3.65	3.50	3.45	7.16, 6.98 (H-1',2')
	(4.8)	(5.41)	(7.5)	(3.5)	(4.8)	(11.6)	(7.3)	
18b[b]	5.84	4.18	4.10	3.92	3.71	3.51	3.47	
	(5.6)	(5.6)	(7.0)	(3.4)	(4.8)	(11.6)	(6.9)	
19b[b]	5.89	4.20	4.09	3.94	3.72	3.52	3.48	6.90 (H-5')
	(5.2)	(5.5)	(7.1)	(3.5)	(4.6)	(11.7)	(7.4)	

[a]CDCl3. [b]D2O. *center of a complex multiplet

Previous protocols for the evaluation of the thioglycosides as inhibitors of β-D-galactofuranosidase, were followed. 4-Nitrophenyl β-D-galactofuranoside (**10**) was used as substrate, and lactone **9** was tested as a reference inhibitor (IC_{50} 0.10 mM).[25] The enzymatic reaction was performed in the presence of thioglycosides **14b-16b**, **18b** and **19b** at

concentrations ranging from 0.15 to 1.25mM. We found that compounds **14b**-**16b**, **18b** are weak inhibitors (Figure 2), whereas **19b** is a stronger inhibitor (IC_{50} 135 μM). Interestingly, the structural difference between pyrimidyl derivatives **18b** and **19b** is the presence of the 4'- and 6'-methyl groups in the latter. These subtituents should favor particular interactions in the active site of the enzyme, resulting in an enhancement of the inhibitory activity. Although the IC_{50} value is moderate, there are not reports about more potent inhibitors of this enzyme.

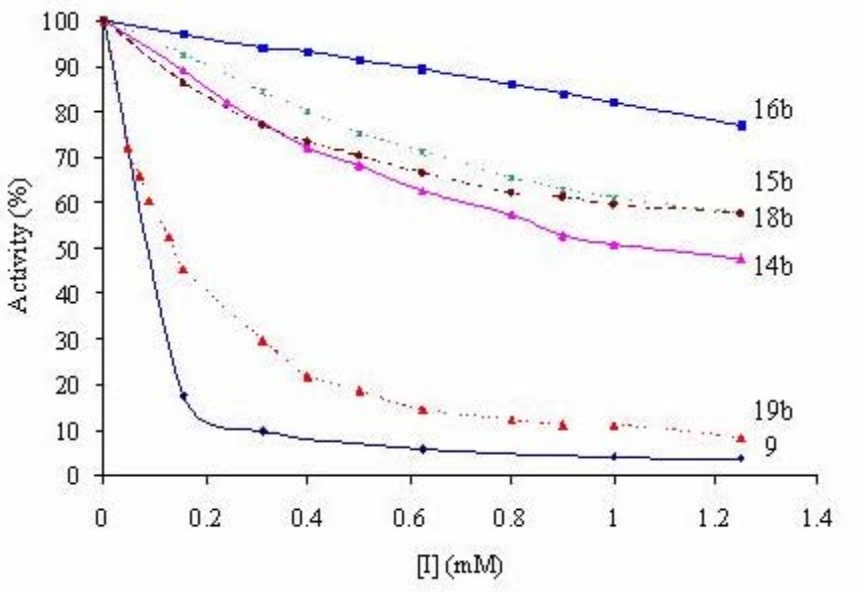


Figure 2. Effect of concentration of heteroaryl 1-thio-β-D-galactofuranosides on the enzymatic activity of β-D-galactofuranosidase from *Penicillium fellutanum*. Incubations were performed as described in the Experimental Section. The amount of 4-nitrophenol released from 4-nitrophenyl β-D-galactofuranoside was determined as a measure of galactofuranosidase activity. The numbers indicate the heteroaryl 1-thio-β-D-galactofuranoside added.

Recent progresses in the tridimensional analysis of glycosidases and their crystalline structures have contributed to the rational design of enzyme inhibitors. Unfortunately, none of the β-D-galactofuranosidases have been yet sequenced nor crystallized. Therefore, the synthesis and evaluation of 1-thio-β-D-galactofuranosides as those described here can contribute to the understanding of how the enzyme works. Furthermore, evaluation of these compounds as inhibitors of galactofuranosyltransferases, and of their benzoylated precursors as glycosyl donors, are in progress.

Experimental Section

General Procedures. 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; concentrations are given in g/100 mL. Microanalyses were performed by Atlantic Microlab Inc. Thin layer chromatography (TLC) was performed on 0.2 mm Silica Gel 60 F_{254} (Merck) aluminium supported plates, using the following solvents: (a) 2:1 hexane-EtOAc, (b) 4:1 toluene-EtOAc, (c) 5:1 toluene-EtOAc, (d) 7:1:2 nPrOH-NH_3-H_2O. 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). 1,2,3,4,6-penta-*O*-benzoyl-α,β-D-galactofuranose (**12**) was synthesized as previously described.[38]

Thioglycosylation of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose

Method A. To a solution of **12** (0.70 g, 1.00 mmol) in dry CH_3CN (15.0 mL) cooled to 0 ºC, $SnCl_4$ (0.48 mL, 4.0 mmol) was added. After 10 min of stirring, the corresponding thiol (RSH, 3.0 mmol) was added, and the solution was stirred until TLC analysis showed total compsumption of **12** (2-3 h). The solution was diluted with CH_2Cl_2, extracted with sat. aq. $NaHCO_3$, and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure.

The following galactofuranosyl thioglycosides were obtained:

2-Pyridyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (14a). 0.57 g, 92 %, R_F 0.43 (solvent a). An analytical sample was obtained by column chromatography purification (solvent 8:2 hexane-EtOAc) as a syrup which gave $[\alpha]_D$ –62º (*c* 1, $CHCl_3$). Anal. Calcd. For $C_{39}H_{31}N_1O_9S$ (689.73): C, 67.91; H, 4.53; N, 2.03, S, 4.65. Found: C, 67.87; H, 4.73; N, 2.13, S, 4.59.

4-Pyridyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (15a). 0.61 g, 89 %, R_F 0.51 (solvent a). Recrystallized from EtOH gave $[\alpha]_D$ –32º (*c* 1, $CHCl_3$), and mp 172-174 ºC. Anal. Calcd. For $C_{39}H_{31}N_1O_9S$ (689.73): C, 67.91; H, 4.53; N, 2.03, S, 4.65. Found: C, 67.80; H, 4.59; N, 2.15, S, 4.73.

2-(1-Methylimidazoyl) 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (16a). 0.66 g, 96 %, R_F 0.27 (solvent c). An analytical sample was obtained by column chromatography purification (solvent 95:5 hexane-EtOAc) as a syrup which gave $[\alpha]_D$ –83º (*c* 1, $CHCl_3$). Anal. Calcd. For $C_{37}H_{30}N_2O_9S_2$ (710.14): C, 65.88; H, 4.66; N, 4.04, S, 4.63. Found: C, 66.01; H, 4.60; N, 3.94, S, 4.46.

2-(5-Methyl-1,3,4-thiadiazolyl) 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (17a). 0.63 g, 90 %, R_F 0.34 (solvent b). An analytical sample was obtained by column chromatography purification (solvent 2:1 to 1,5:1 hexane-EtOAc) as a crystalline product which recrystallized from methanol gave $[\alpha]_D$ –85º (*c* 1, $CHCl_3$), mp 125-126 ºC. Anal. Calcd. For $C_{37}H_{30}N_2O_9S_2$ (710.14): C, 62.52; H, 4.25; N, 3.94, S, 9.02. Found: C, 62.54; H, 4.18; N, 3.92, S, 8.93.

Method B. To a solution of **12** (0.70 g, 1.00 mmol) and RSH (3.0 mmol) in dry CH_3CN (15.0 mL) cooled to 0 ºC, $BF_3.OEt_2$ (4.0 mmol) was added. The solution was stirred at room temperature until TLC analysis showed total compsumption of **12** (12-18 h). Then, the solution was diluted with CH_2Cl_2 treated as in method A. The following galactofuranosyl thioglycosides were obtained.

2-Pyrimidyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (18a). 0.45 g, 90 %, R_F 0.28 (solvent a). An analytical sample was obtained by column chromatography purification as a pail yellow syrup which gave $[\alpha]_D$ –32º (*c* 1, $CHCl_3$). Anal. Calcd. For $C_{38}H_{30}N_2O_9S$ (690.72): C, 66.08; H, 4.38; N, 4.06, S, 4.64. Found: C, 65.99; H, 4.42; N, 3.90, S, 4.39.

4,6-Dimethyl-2-pyrimidyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactofuranoside (19a). 0.63 g, 88 %, R_F 0.55 (solvent c). An analytical sample was obtained by column chromatography purification (sv. 95:5 toluene-EtOAc) as a colourless syrup which gave $[\alpha]_D$ –13º (*c* 1, $CHCl_3$). Anal. Calcd. For $C_{40}H_{34}N_2O_9S$ (718.20): C, 66.84; H, 4.77; N, 3.90, S, 4.46. Found: C, 66.80; H, 4.71; N, 3.81, S, 4.30.

De-*O*-benzoylation. General procedure

To a solution of **14a-16a**, **18a** or **19a** (0.7 mmol) in dried CH_2Cl_2 (10.0 mL) at 0 ºC, 0.5 N NaOMe/MeOH (5.0 mL) was added. The solution was stirred under argon during 30 min. Then, the solution was diluted with methanol, concentrated under vacuum in order to eliminate the CH_2Cl_2, passed through a column of Dowex 50W (H^+) and eluted with methanol. The solvent was removed under vacuum and the remaining methyl benzoate was eliminated by several coevaporations with water.The following compounds were obtained.

2-Pyridyl 1-thio-β-D-galactofuranoside (14b). 0.17 g, 90 %, R_F 0.68 (solvent d). After column chromatography purification (solvent EtOAc) and crystallization from ethanol gave $[\alpha]_D$ –283º (*c* 1, CH_3OH), mp 97-99 ºC. Anal. Calcd. For $C_{11}H_{15}NO_5S$ (273.07): C, 48.34; H, 5.53; N, 5.12, S, 11.73. Found: C, 48.30; H, 5.63; N, 5.12, S, 11.25.

4-Pyridyl 1-thio-β-D-galactofuranoside (15b). 0.16 g, 88 %, R_F 0.65 (solvent d). After recrystallization from ethanol gave $[\alpha]_D$ –283º (*c* 1, CH_3OH), mp 173-174 ºC. Anal. Calcd. For $C_{11}H_{15}NO_5S$ (273.07): C, 48.34; H, 5.53; N, 5.12, S, 11.73. Found: C, 48.43; H, 5.54; N, 5.18, S, 11.46.

2-(1-Methylimidazole) 1-thio-β-D-galactofuranoside (16b). 0.15 g, 89 %, R_F 0.61 (solvent d). After column chromatography purification (solvent EtOAc) and recrystallization from methanol gave $[\alpha]_D$ –296° (*c* 0.5, H_2O), mp 174-175 ºC. Anal. Calcd. For $C_{10}H_{16}N_2O_5S$ (302.25): C, 43.47; H, 5.84; N, 10.14, S, 11.61. Found: C, 43.37; H, 5.79; N, 10.05, S, 11.39.

2-Pyrimidyl 1-thio-β-D-galactofuranoside (18b). 0.17 g, 92 %, R_F 0.63 (solvent d). An analytical sample was obtained by column chromatography purification (solvent EtOAc) as yellow syrup which gave $[\alpha]_D$ – 255º (*c* 1, CH_3OH), lit. – 260º,[31] and NMR spectra (Tables 1 and 2) in agreement with reported data.[31]

4,6-Dimethyl-2-pyrimidyl 1-thio-β-D-galactofuranoside (19b). 0.20 g, 95 %, R_F 0.66 (solvent d). An analytical sample was obtained by column chromatography purification (solvent EtOAc)

and crystallization from EtOAc. $[\alpha]_D$ –240º (*c* 1, CH_3OH), mp 149-150 ºC. Anal. Calcd. For $C_{12}H_{18}N_2O_5S$ (302.25): C, 47.67; H, 6.00; N, 9.27, S, 10.61. Found: C, 47.44; H, 5.90; N, 9.37, S, 10.48.

Enzymatic Assays

The enzymatic activity was assayed using the filtered medium of a stationary culture of *P. fellutanum* as the enzyme source, and 4-nitrophenyl β-D-galactofuranoside (**10**) as substrate.[17] The standard assay was done with 100 μL of 66 mM NaOAc buffer (pH 4), 62 μL of a 5 mM solution of substrate **10** and 100 μL (20 μg protein) of the enzyme medium, in a final volume of 500 μL. The inhibitors were incorporated in the amounts needed to obtain a final concentration from 0.05 to 1.25 mM. The enzymatic reaction was stopped after 1.5 h of incubation at 37 ºC, by addition of 1 mL of 0.1 M Na_2CO_3 buffer (pH 9). The 4-nitrophenol released was measured spectrophotometrically at 410 nm.

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