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Synthesis of β-D-galactofuranosyl nucleoside analogues. A new type of β-D-galactofuranosidase inhibitor

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Abstract

The development of β-D-galactofuranosidase inhibitors provides a good chemotherapeutic target for treatment of major human diseases, because β-D-galactofuranose is a constituent of important pathogen microorganisms but is absent in mammals. With this purpose we have prepared β-D-galactofuranosyl nucleoside analogues, derived by the addition of nucleophiles to perbenzoylated β-D-galactofuranosyl isothiocyanate, a compound previously prepared in this laboratory. *N*-β-D-Galactofuranosyl-*O*-ethylthiourethane, *N*-β-D-galactofuranosyl-4-oxoimidazolidine-2-thione, *N*-β-D-galactofuranosyl-4-imidazoline-2-thione, and *N*-β-D-galactofuranosyl-4-methoxyimidazolidine-2-thione, were prepared. The biological assays showed that imidazoline and imidazolidine-2-thione derivatives act as a new type of exo β-D-galactofuranosidase inhibitor.

Keywords: Galactofuranosidase inhibitors; Isothiocyanate; Thiohydantoin; 4-Imidazoline; Imidazolidine-2-thion derivatives

1. Introduction

The development of specific inhibitors of glycosidases is one of the primary objectives of glycobiology. β-D-Galactofuranose is a constituent of important microorganism glycoconjugates. The absence of galactofuranose in mammal glycoconjugates suggests that the enzymes involved in the metabolism of this sugar in bacteria, fungi and protozoa would be a good target for the design of drugs.[1] An exo β-D-galactofuranosidase has been detected in only a few species: *Penicillium fellutanum*,[2] *Helminthosporium sacchari*,[3] and *Aspergillius*.[4] Terminal nonreducing residues of Gal*f* are found in the lipopeptidophosphoglycan (LPPG) of *Trypanosoma cruzi*.[1] Variations in the galactofuranosyl content of these glycoconjugates could be due to the action of an exo β-D-galactofuranosidase.

We have previously developed the synthesis of 1-thio-β-D-galactofuranosides, and they have been evaluated as inhibitors of the enzyme from *P. fellutanum*.[5] Taking into account their biological activity, we have developed an affinity chromatographic system for the isolation of the enzyme[6] and studied the influence of the inhibitors in cultures of the fungi (unpublished).

We also found that D-galactono-1,4-lactone inhibits the enzyme,[5] and in general, this characteristic of the aldonolactones has been attributed to their structural similarity with the glycosyloxocarbonium ion intermediate of the

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enzymatic glycoside cleavage.[7] The *N*-thiocarbonyl glycosyl derivatives also provide planar resonance structures, similar to the hydrolytic reaction intermediate. Thus, these type of compounds, usually obtained from the corresponding isothiocyanates, are convenient precursors for the design and synthesis of glycosidase inhibitors.[8,9] Furthermore, thiohydantoin derivatives have previously been shown to act as inhibitors of carbohydrate processing enzymes.[10,11]

The synthesis of 2,3,5,6-tetra-benzoyl-β-D-galactofuranosyl isothiocyanate (**1**) was previously described as a simple and efficient one-step procedure from 1,2,3,5,6-penta-*O*-benzoyl-β-D-galactofuranose.[12,13] We also described its reaction with several alcohols, amines, and amino acids, which led to a variety of *N*-glycosyl derivatives of β-D-galactofuranose.

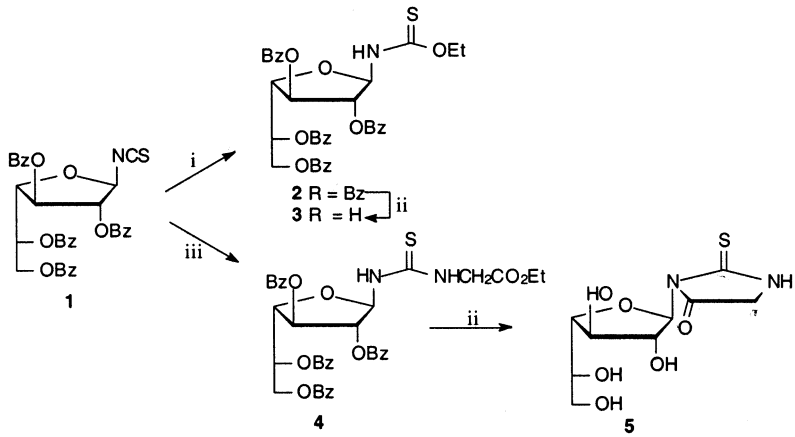
We now describe the preparation of 1,3-diaza-1-β-D-galactofuranosyl-2-thione heterocycles, starting from compound **1**, and their evaluation as inhibitors of exo β-D-galactofuranosidase from *P. fellutanum*.

2. Results and discussion

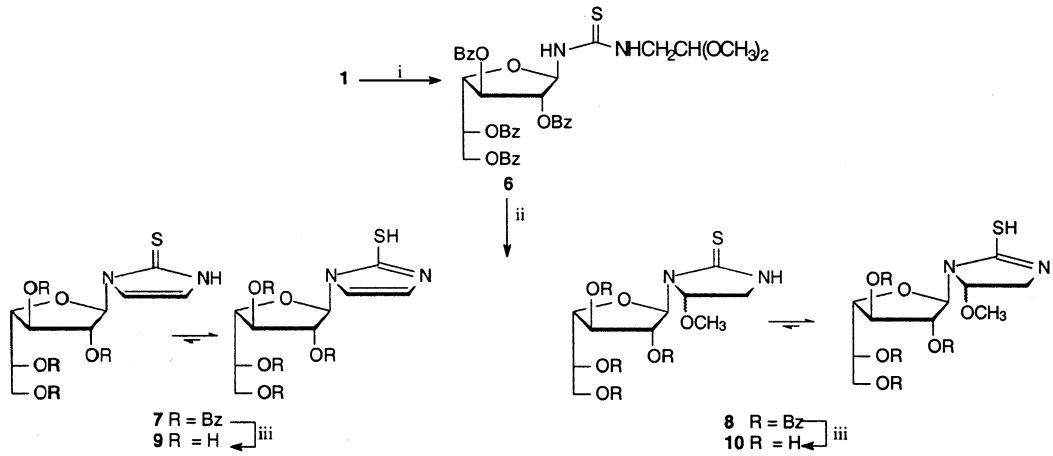
Debenzoylation of compound **2**, derived from the addition of ethanol to **1**,[13] led to fully unprotected compound **3** (Scheme 1). Similar treatment of **4**, obtained by condensation of **1** and L-glycine ethyl ester[12] led, by cyclization, to the glycosyl thiohydantoin **5**. This structure was assigned on the basis of the ^{1}H and ^{13}C NMR spectra. The ^{13}C NMR spectrum of **5** showed signals at 179.1 and 168.2 ppm, which were assigned to the thiocarbonyl and the carbonyl groups of the thiohydantoin moiety, respectively, and a signal at δ 46.8 for C-5 of the same unit. Signals for the sugar carbon atoms were similar to those observed for other β-D-galactofuranosyl derivatives, with a higher field signal for C-1′ (δ 88.5), because of the higher shielding effect of nitrogen, in comparison with the oxygenated analogues (C-1, δ 105).[14]

Nucleophilic addition of aminoacetaldehyde dimethyl acetal to **1** led almost quantitatively, after 2 h of reaction, to the thiourea derivative **6** (92%, Scheme 2). The ^{1}H NMR spectrum of **6** showed the characteristic galactofuranosyl pattern, with a signal at 6.08 ppm for the anomeric proton as observed for analogous β-D-galactofuranosyl nucleosides.[12] The ^{13}C NMR spectrum showed the resonance for the thiocarbonyl group at 183.8 ppm and signals at 46.8 (C-4), 102.2 (C-5) and 54.4 ppm (OCH_3), as expected for the aglycon moiety. For the β-D-galactofuranosyl unit, the expected signals were observed.

Compound **6** was treated with 0.1 N HCl in DMF in order to hydrolyze the acetal group and lead to cyclization by condensation of the generated aldehyde with the glycosylic nitrogen. A similar reaction was earlier communicated for the D-ribofuranose analogue of **6**, which led to the imidazole-2-thione derivative in 29% yield. This structure was assigned on the basis of the ^{1}H NMR spectrum. The reaction time was not specified and no other



Scheme 1. (i) EtOH, 60 °C; (ii) NaOMe–MeOH; (iii) $H_2NCH_2CO_2Et{\cdot}HCl$, toluene, EDPA.



Scheme 2. (i) Dimethylaminoacetaldehyde, toluene; (ii) HCl 0.1 M–DMF; (iii) NaOMe–MeOH.

product was isolated.[15] In our case, we observed that, at room temperature, the reaction proceeded very slowly and after 30 h, two products (**7** and **8**) with similar chromatographic behavior were formed, although starting compound **6** was still present. Isolation by column chromatography (4:1 toluene–ethyl acetate) afforded **7** (52%), **8** (35%), along with some unreacted **6** (8%). When the reaction was performed by heating in a boiling-water bath, the starting material was completely transformed into compound **7** (69%) in 2 h, and no compound **8** was detected. The isolated compound **8** could also be transformed into **7** by heating under reflux with 0.1 N HCl in DMF.

The ^{1}H NMR spectrum of **7** showed a doublet at 6.70 ppm for the anomeric proton with $J_{1,2} \sim 2.5$ Hz, characteristic of the β-D-Gal*f* configuration. For the 4-imidazoline-2-thione ring, the vinylic protons showed overlapped signals at 6.98 ppm with $J_{4,5}$ 4.8 Hz, in agreement with the values reported for the ribofuranosyl analogue.[15] The ^{13}C NMR spectrum showed a signal at 162.9 ppm for C-2, indicating that the tautomeric equilibrium of the imidazole moiety was mainly displaced to the thioenol form, and vinylic signals (C-4,5) were observed at δ 114.6 and 114.9.

The ^{13}C NMR spectrum of **8** clearly showed that one of the methoxyl groups was still present in this compound, and the 2-thione function was present as the thioenolic form. No vinylic carbons were detected, and signals at δ 64.9 and 93.5 were observed instead. The ^{1}H NMR spectrum of **8** showed the presence of two epimeric compounds in similar amounts, displaying the pattern of a methylene group with no equivalent hydrogens as two double doublets centered at 3.96 and 4.14 ppm (H-5a,5b), with a large J_{gem} (14.2 Hz) coupling, each of them coupled with a deprotected proton (H-4). This pattern indicated the imidazolidine structure **8**. Both epimers showed distinguishable signals for the aglycon moiety and the anomeric proton, but the sugar signals were overlapped.

Compounds **7** and **8** were debenzoylated (NaOMe–MeOH), affording compounds **9** and **10**. For compound **9**, the^{1}H NMR spectrum showed vinylic signals at 7.21 and 6.88 ppm with $J_{4,5}$ 2.7 Hz, and the galactofuranosyl signals with $J_{1',2'}$ 5.0 Hz, larger than for **7**, suggesting that a change in the conformation occurred, as previously observed for analogous compounds.[8,12] In the ^{13}C NMR spectrum of **9** the vinylic carbons were observed at 115.6 and 115.2 ppm, and the signal at δ 162 showed that compound **9** was in the thioenolic form.

Compound **10** showed similar signals to **9** for the sugar unit, and to **8** for the aglycon moiety, indicating unequivocally the presence of the methoxyl group, and the thioenolic form. On being kept, the epimeric mixture of **10** partly decomposed to **9**, and only one of the epimers was evident by NMR. NOE ex-

periments did not afford information on the configuration of C-4, due to the conformational flexibility of the molecule. Attempts to crystallize compounds **8** and **10** for the determination of the absolute configuration were not successful.

With the purpose of evaluating compounds **3**, **5**, **9**, and **10** as inhibitors of exo β-D-galactofuranosidase, we performed typical experiments for the measurement of the enzymatic activity, in the presence of different amounts of these compounds (Fig. 1). 4-Nitrophenyl β-D-galactofuranoside[16] was used as substrate (Fig. 1), and D-galactono-1,4-lactone was tested as a reference inhibitor (IC_{50} 0.03 mM).[6]

We observed that **3** and **5** were not biologically active (data not shown). Compound **9** was found to be a weak inhibitor, with IC_{50} 1.05 mM. Compound **10** was ten times more active (IC_{50} 0.10 mM), suggesting that a change in the flexibility of the imidazolidine ring facilitates the interaction with the active site of the enzyme.

In summary, we found a new type of β-D-galactofuranosidase inhibitor, the imidazole-2-thione derivatives, and observed that the activity is dependent on the structure of the imidazole ring. This fact opens the possibility of continuing with the design of more potent inhibitors by modifications of this structure. Furthermore, the comparative activity of these compounds should provide information about the chemical topography of the active site of the enzyme.

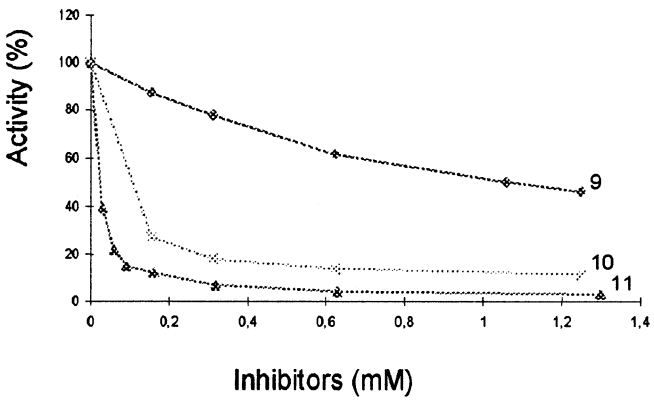


Fig. 1. Effect of concentration of inhibitors on the enzymatic activity of β-D-galactofuranosidase from *P. fellutanum*. The amount of 4-nitrophenol released from 4-nitrophenyl β-D-galactofuranoside was determined as a measure of galactofuranosidase activity.

3. Experimental

General methods.—Optical rotations were measured with a Perkin–Elmer 343 polarimeter. NMR spectra were recorded with Bruker AC 200 or AM 500 spectrometers. TLC examination was performed on precoated plates (E. Merck, Silica 60 F_{254}). Column chromatography was performed on Silica Gel 60 (200–400 mesh, E. Merck). Compounds **2**[13] and **4**[12] were prepared as previously described.

N-*(2,3,5,6-Tetra*-O-*benzoyl-β*-D-*galacto-furanosyl)*-N′-*(2,2-dimethoxyethyl) thiourea* (**6**).—To a solution of 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl isothiocyanate (**1**, 0.70 g, 1.01 mmol)[12,13] in anhyd toluene (15.0 mL), aminoacetaldehyde dimethyl acetal (0.16 mL, 1.5 mmol) was added. The solution was kept at rt until complete reaction occurred (2 h). TLC examination (2:1 toluene–EtOAc) showed a slower-moving component (R_f 0.35) than **1** (R_f 0.80). The solution was poured over 5% HCl, extracted (CH_2Cl_2), and dried ($MgSO_4$). After evaporation of the solvent, compound **6** (0.69 g, 92%) was obtained as a syrup having $[\alpha]_D$ −25° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$), δ 7.00 (s, 1 H, NH), 6.08 (m, 2 H, H-1,5), 5.71, (m, 2 H, H-2,3), 4.77 (m, 3 H, H-4,6a,b), 4.52 (t, 1 H, $J_{4',5'}$ 5.1 Hz, H-5′), 3.73 (d, 2 H, H-4′), 3.36 (2 s, 6 H, OCH_3); ^{13}C NMR (25.3 MHz, $CDCl_3$), δ 183.8 (C-2′), 102.2 (C-5′), 88.3 (C-1), 81.6 (C-4), 80.7 (C-2), 78.1 (C-3), 71.1 (C-5), 63.3 (C-6), 46.8 (C-4′). Anal. Calcd for $C_{39}H_{38}N_2O_{11}S$: C, 63.06; H, 5.16. Found: C, 63.19, H, 5.10.

N-*(2,3,5,6-Tetra*-O-*benzoyl-β*-D-*galacto-furanosyl)-4-imidazoline-2-thione* (**7**) *and* N-*(2,3,5,6-tetra*-O*-benzoyl-β*-D-*galactofuranosyl)-4-methoxyimidazolidine-2-thione* (**8**).—Compound **6** was dissolved in 0.1 N HCl–DMF and stirred during 48 h at rt. TLC analysis showed two products (R_f 0.26 and 0.18) and some starting material. Complete reaction did not occurred, even after several days. Column chromatography (5:1 toluene–EtOAc) of the mixture gave compound **7** (0.28 g, 52%), **8** (0.20 g, 35%), and **6** (0.04 g, 8%).

When the reaction was performed in a boiling-water bath, the starting material was completely transformed after 1 h, and only

compound **7** (0.37, 69%) was obtained. Compound **7** had $[\alpha]_D$ −19° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$), δ 6.98 (d, 2 H, $J_{4,5}$ 4.8 Hz, H-4,5), 6.70 (d, 1 H, $J_{1',2'}$ 2.5 Hz, H-1′), 6.09 (m, 1 H, $J_{5',6'a}$ 3.5, $J_{5',6'b}$ 4.6 Hz, H-5′), 5.98 (dd, 1 H, $J_{2',3'}$ 2.5, $J_{3',4'}$ 3.3 Hz, H-3′), 5.73, (dd, 1 H, H-2′), 4.88 (dd, 1 H, $J_{4',5'}$ 3.3 Hz, H-4′), 4.82 (dd, 1 H, $J_{6'a,6'b}$ 11.9 Hz, H-6′a), 4.72 (dd, 1 H, H-6′b); ^{13}C NMR (25.3 MHz, $CDCl_3$), δ 162.9 (C-2), 114.9, 114.6 (C-5,4), 89.8 (C-1′), 84.0 (C-4′), 80.6 (C-2′), 78.9 (C-3′), 71.7 (C-5′), 63.2 (C-6′). Anal. Calcd for $C_{37}H_{30}N_2O_9S$: C, 65.48; H, 4.45. Found: C, 65.53, H, 4.59.

Compound **8** had $[\alpha]_D$ −7° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$), selected data: δ 5.84 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1′), 5.55 (dd, 1 H, $J_{4,5a}$ 5.1, $J_{4,5b} \sim 1.0$ Hz, H-4), 4.15, 3.96 (2 dd, 2 H, $J_{5a,5b}$ 14.2 Hz, H-5a,b). For the other epimer, δ 5.89 (d, 1 H, $J_{1',2'}$ 2.0 Hz, H-1′), 5.48 (dd, 1 H, $J_{4,5a}$ 4.8, $J_{4,5b} \sim 1.0$ Hz, H-4), 4.10, 3.87 (2 dd, 2 H, $J_{5a,5b}$ 14.2 Hz, H-5a,b). ^{13}C NMR (25.3 MHz, $CDCl_3$), δ 160.2 (C-2), 93.5, 93.1 (C-4 both epimers), 90.7 (C-1′), 81.1 (C-2′,4′), 78.3 (C-3′), 71.3 (C-5′), 64.9, 64.6 (C-5 both epimers), 63.6 (C-6′), 55.9 (OCH_3). Anal. Calcd for $C_{38}H_{34}N_2O_{10}S$: C, 64.22; H, 4.82. Found: C, 64.00, H, 4.69.

Debenzoylation: general procedure.—Compounds **2**,[13] **4**,[12] **7** and **8** were suspended in 0.5 N NaOMe–MeOH, and stirred at rt during 2 h. The solution was passed through a column (1.5 × 5.0 cm) containing Dowex 50 W(H^+) resin. The solvent was removed under vacuum and the remaining MeOBz was eliminated by several coevaporations with water. The following compounds were obtained.

N-*β*-D-*Galactofuranosyl*-O-*ethylthiourethane* (**3**).—Yield 95%, $[\alpha]_D$ −15° (*c* 1, water); ^1H NMR (200 MHz, D_2O), selected data: δ 5.58 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1); ^{13}C NMR (25.3 MHz, D_2O), δ 183.8 (*C*=S), 88.9 (C-1), 81.7 (C-4), 79.9 (C-2), 75.5 (C-3), 71.7 (C-5), 63.1 (C-6), 61.6 (O*C*H_3). Anal. Calcd for $C_9H_{17}NO_6S$: C, 40.44; H, 6.41. Found: C, 40.29, H, 6.33.

N-*β*-D-*Galactofuranosyl*-*5*-*oxo*-*imidazolidine*-*2*-*thione* (**5**).—Yield 83%, $[\alpha]_D$ −25° (*c* 1, $CHCl_3$); ^1H NMR (D_2O, 200 MHz), selected data, δ 5.98 (d, 1 H, $J_{1',2'}$ 6.7 Hz, H-1′), 5.06 (dd, 1 H, $J_{2',3'}$ 5.1 Hz, H-2′), 4.15 (s, 2 H, H-5); ^{13}C NMR (D_2O, 25.3 MHz), δ 179.1 (C-2), 168.2 (C-4), 88.5 (C-1′), 84.8 (C-4′), 80.3 (C-2′), 75.1 (C-3′), 71.8 (C-5′), 62.9 (C-6′), 49.7 (C-5). Anal. Calcd for $C_9H_{14}N_2O_6S$: C, 38.84, H, 5.07. Found: C, 39.02, H, 5.10.

N-*β*-D-*Galactofuranosyl*-*4*-*imidazoline*-*2*-*thione* (**9**).—Yield 92%, $[\alpha]_D$ −22° (*c* 1, water); ^1H NMR (500 MHz, Me_2SO-d_6), δ 7.21 (d, 1 H, $J_{4,5}$ 2.7 Hz, H-4), 6.88 (d, 1 H, H-5), 6.06 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1′), 4.07 (m, 2 H, $J_{2',3'}$ 2.5, $J_{3',4'}$ 3.3 Hz, H-2′,3′), 4.04 (dd, 1 H, $J_{4',5'}$ 3.3 Hz, H-4′), 3.39 (m, 2 H, H-6′a,b), 3.03 (m, 1 H, H-5′); ^{13}C NMR (25.3 MHz, Me_2SO-d_6), δ 162.0 (C-2), 115.6, 115.2 (C-5,4), 89.2 (C-1′), 83.5 (C-4′), 80.7 (C-2′), 75.3 (C-3′), 70.5 (C-5′), 62.3 (C-6′). Anal. Calcd for $C_9H_{14}N_2O_5S$: C, 41.22; H, 5.38. Found: C, 41.35, H, 5.26.

N-*β*-D-*Galactofuranosyl*-*4*-*methoxyimidazolidine*-*2*-*thione* (**10**).—Yield 93%, $[\alpha]_D$ −64° (*c* 1, water); ^1H NMR (500 MHz, Me_2SO-d_6), selected data: δ 5.63 (d, 1 H, $J_{1',2'}$ 5.2 Hz, H-1′, both epimers), 5.23, 5.18 (2 d, 2 H, $J_{4,5}$ 7.0 Hz, H-4 both epimers), 4.21 (dd, 1 H, H-5 one epimer), 4.03 (m, 2 H, H-2′,3′), 3.95 (dd, 1 H, H-4′), 3.10 (m, 1 H, H-5′), 3.45 (m, 2 H, H-6′a,b); ^{13}C NMR (25.3 MHz, Me_2SO-d_6), δ 167.5 (C-2), 93.6 (C-4), 88.3 (C-1′), 81.5 (C-4′), 79.7 (C-2′), 75.6 (C-3′), 71.5 (C-5′), 65.1 (C-5), 63.4 (C-6′), 56.4 (O*C*H_3). Anal. Calcd for $C_{10}H_{18}N_2O_6S$: C, 40.80; H, 6.16. Found: C, 40.63, H, 6.24.

Alternatively the mixture of **7** and **8** was debenzoylated and compounds **9** and **10** were separated by column chromatography with 4:1 AcOEt–*i*PrOH.

Assays of β-D-galactofuranosidase inhibition.—The filtered medium of a stationary culture of *P. fellutanum* was used as the enzyme source.[5] The standard assay (100% of activity) was performed with 100 μm of 66 mM NaOAc buffer (pH 4.0), 62 μL of a 5 mM solution of the substrate (4-nitrophenyl β-D-galactofuranosidase) and 100 μL (20 μg protein) of the enzyme medium, in a final volume of 500 μL. The inhibitors were incorporated in various amounts to give a final concentration range of 0.05–1.30 mM. The enzymatic reaction was started with the addition of the enzyme, and after 1.5 h at 37 °C it was stopped with 1 mL of 0.1 M Na_2CO_3

buffer (pH 9). The 4-nitrophenol released was measured spectrophotometrically at 410 nm.

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