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Synthesis of the (1→6)-linked thiodisaccharide of galactofuranose: Inhibitory activity against a β-galactofuranosidase

Evangelina Repetto, Carla Marino, Oscar Varela *

CIHIDECAR-CONICET-UBA, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires 1428, Argentina

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ABSTRACT

A new (1→6)-linked thiodisaccharide formed by two galactofuranosyl units has been synthesized. Methyl (methyl α,β-D-galactofuranosid)uronate was employed as the starting compound, which was per-O-silylated with TBSCl and reduced with $LiAlH_4$ to afford methyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-β-D-galactofuranoside (2β) as a key precursor for the preparation of methyl per-*O*-*tert*-butyldimethylsilyl-6-thio-β-D-galactofuranoside (**12**). The free thiol group of **12** was glycosylated and the product O-deprotected to afford the target β-D-Gal*f*-*S*-(1→6)-β-D-Gal*f*-*O*Me (**14**). The conformations of this thiodisaccharide were preliminarily studied using combined theoretical calculations and NMR data. Furthermore, the glycomimetic **14** showed to be a competitive inhibitor of the β-galactofuranosidase from *Penicillum fellutanum* (K_i = 3.62 mM).

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1. Introduction

Carbohydrate mimetics are usually designed and prepared introducing alterations in the core structure or modification of the O-glycosidic linkage with respect to their natural analogues. To overcome the problem of the lability of the glycosidic bond, stable mimetics have been synthesized.[1] The development of modified linkages between monosaccharide units has risen considerable interest due to the stability to enzyme degradation and the resulting potential for glycosidase inhibition.[2] The most common modification is the replacement of the anomeric oxygen atom by a sulfur, a nitrogen or a carbon atom to afford, respectively, S-, N-, or C-glycosides.

During the last years, we have been involved in the synthesis and biological evaluation of thiooligosaccharides and we have reported valuable procedures for the construction of the thioglycosidic linkage, that complement the approaches already described in the literature.[3,4] For example, the Michael addition[5,6] and the ring-opening reaction of epoxides[7,8] or thiirane derivatives[8,9] of sugars have been employed as key steps in the synthesis of thiooligosaccharides. However, the methodologies reported so far are mostly referred to the construction of thioglicosidic linkages between pyranose rings, while just a few examples of thiooligosaccharides constituted by furanose rings have been reported. We have described some straightforward strategies for the synthesis of thiodisaccharides that contain a 1-thiopentofuranose[10] or 1-thiohexofuranose[11] moiety as non reducing end. Furthermore, the synthesis of oligosaccharide analogues having the ring oxygen atom of Gal*f* replaced by sulfur has been reported.[12]

The interest in thiodisaccharides of furanose sugars, and particularly of Gal*f*, arises from the fact that this sugar is present in numerous structures considered to be essential for virulence in many pathogenic organisms.[13,14] Thus, the motif β-D-Gal*f*-(1→6)-Gal*f* occurs in polyfuranosides of pathogenic *Aspergillus*, *Cryphonectria parasitica*, *Renibacterium salmoninarum* and *Mycobacterium*, and in many other microorganisms.[13,14] For example, the arabinogalactan of the cell wall complex of mycobacteria and other *Actinomycetes*[15] comprises a linear chain of approximately 30 alternating β-(1→5) and β-(1→6)-linked D-Gal*f* residues. The inhibition of the enzymes involved in the metabolism of these polyfuranosides is expected to prevent the proliferation of mycobacteria, including the causative agent of tuberculosis, *Mycobacterium tuberculosis*.[16]

In addition, as such furanosyl residues are not found in mammals, these enzymes can be considered interesting targets for the design of new antituberculosis drugs. In fact, aryl and heteroaryl 1-thio-β-D-galactofuranosides have been evaluated as inhibitors of a β-galactofuranosidase[17] and 1-thioalkyl galactofuranosides and their sulfones have been tested as inhibitors of mycobacteria growth.[18] However, thiodisaccharides formed by two Gal*f* units have not been synthesized.

In view of the previous considerations we wish to report here the synthesis of the first dithiodisaccharide constituted by two furanose units. This compound is the glycomimetic of the already

* Corresponding author. Tel./fax: +54 11 45763352.
E-mail address: varela@qo.fcen.uba.ar (O. Varela).

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mentioned, natural and rather common β-Gal*f*-(1→6)-D-Gal*f* unit. The free thiodisaccharide has been evaluated as inhibitor of the β-galactosidase from *Penicillium fellutanum*.

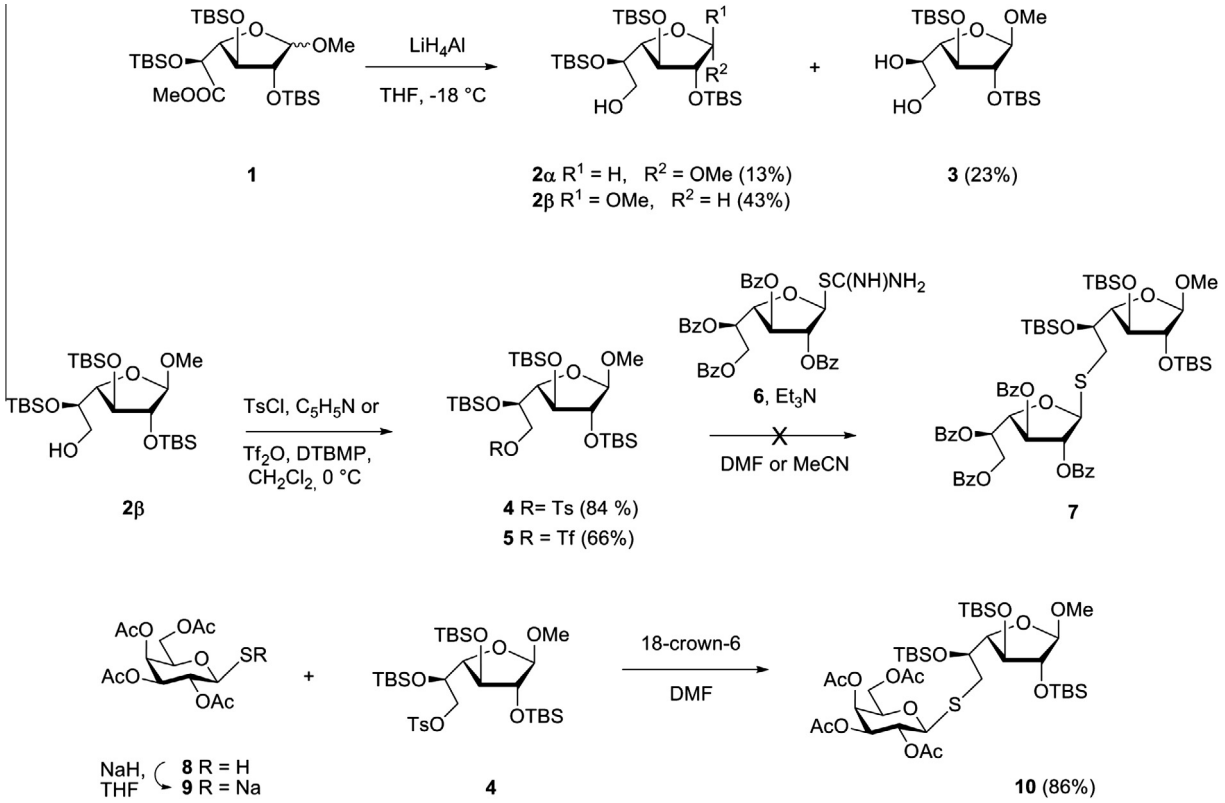
2. Results and discussion

2.1. Synthesis of β-Gal*f*-*S*-(1→6)-D-Gal*f*-*O*Me (14)

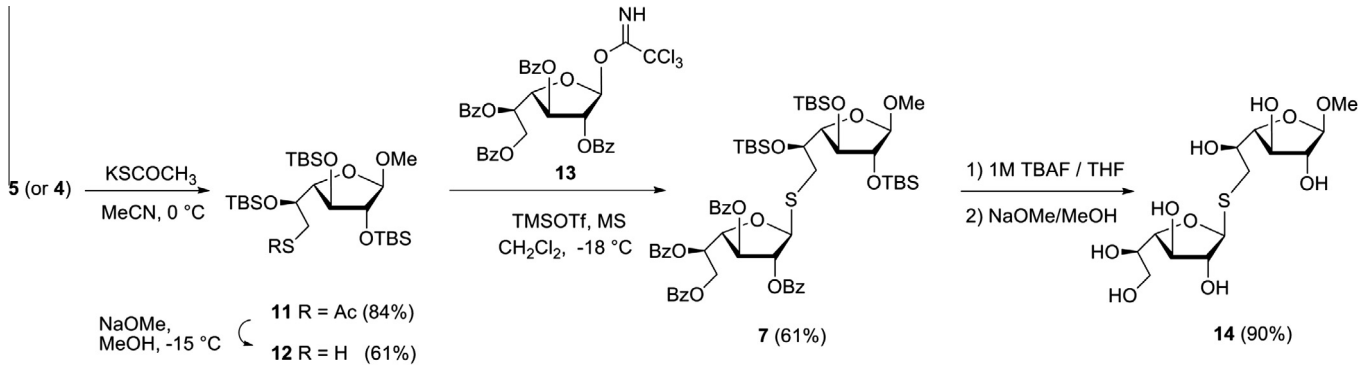
The preparation of the target thiodisaccharide constituted by two galactofuranosyl units via a displacement reaction (S_N2) required a fully protected Gal*f* derivative having a good leaving group at C-6. For the preparation of such a precursor we started from methyl (methyl per-*O*-TBS-α,β-D-galactofuranosid)uronate (**1α,β**), readily prepared from D-galacturonic acid[9] (Scheme 1). The reduction of **1α,β** with a slight excess of $LiAlH_4$ at −18 °C gave mainly **2α**, **2β** and **3**. The product distribution showed to be highly dependent on the reaction time. Thus, when the reduction was conducted for short times, the expected 2,3,5-tri-*O*-silyl derivatives **2α** and **2β** were obtained, being the latter the major isomer. This compound was conveniently protected for the activation of the OH-6 as sulfonate. Thus, treatment of **2β** with tosyl chloride or triflic anhydride afforded the 6-*O*-sulfonyl derivatives **4** or **5**, respectively. The ^{1}H NMR spectrum of these products showed the downfield shifting of the C-6 methylene group, vicinal to the sulfonate, while the chemical shift of the H-5 signal was much less affected. This result indicates that sulfonylation of OH-6 took place and excludes the possibility of 5→6 *O*-TBS migration, which we have observed at higher temperatures.[9]

Sulfonyl derivatives of carbohydrates have been widely used as glycosyl acceptors of 1-thioaldoses to form the thioglycosidic linkage in thiooligosaccharides.[3,4,19] This methodology takes advantage of the high nucleophilicity of the thiol group. Unfortunately the derivatives of 1-thio-Gal*f* are highly unstable compounds.[9–11] The per-*O*-TBS derivatives of 1-thio-D-Gal*f* has been recently prepared as a α,β mixture of anomers,[20] therefore this is not a convenient precursor for our purposes as, in the case that the reaction occurs, a mixture of α and β S-linked disaccharides is expected. In contrast, we have described that the isothiouronium salt of Gal*f* **6** releases, under alkaline conditions, the elusive 1-thio-Gal*f*, which was trapped in β-configuration by a sugar enone as Michael acceptor.[10] Furthermore, it has been reported that 1-thioglycopyranoses generated in situ from the corresponding isothiouronium salts reacted with halide or sulfonate derivatives to give thioglycosides.[21] For these reasons, the glycosyl isothiourea **6** was prepared, and the reaction with the sulfonates **4** and **5** was attempted under varied conditions, using Et_3N as catalyst and acetonitrile or DMF as solvents. In all instances, the reaction was unsuccessful. In view of these negative results, we decided to conduct an analogous reaction using as nucleophile the thiol **8**, the pyranose counterpart of 1-thio-D-Gal*f*. Under the standard conditions that failed for the furanose compound, the thiol **8** (via the sodium salt **9**) reacted with **4** to give the expected thiodisaccharide **10** in 86% yield. The results obtained seem to indicate that the concentration or nucleophilicity of the glycosyl thiol generated in situ from the isothiourea **6** were not sufficient for the substitution reaction. Therefore, an alternative approach was attempted, based in the glycosylation of a SH-6 group in a Gal*f* derivative, such as **12**.

The sulfonyl derivatives **4** and **5** were appropriate precursors for the synthesis of **12** (Scheme 2). Thus, nucleophilic displacement of the sulfonate group in **4** or **5** with potassium thioacetate led to good yields of the 6-*S*-acetyl derivative **11**. Treatment of **11** with NaOMe/MeOH produced the removal of the *S*-acetyl group to give the 6-thio-Gal*f* **12**. The incorporation of a sulfur atom in C-6 of Gal*f* was evidenced by the ^{1}H NMR spectrum, which showed an upfield shifting for the signals of C-6 methylene group of **12** with respect to the same signals in **2β**. The ^{13}C NMR spectrum of **12** showed also



Scheme 1. Synthesis of 6-*O*-sulfonyl-β-D-Gal*f*-*O*Me **4** and **5** as precursors of thiodisaccharides.



Scheme 2. Synthesis of β-D-Gal*f*-*S*-(1→6)-D-Gal*f*-*O*Me (**14**).

the presence of a carbon bonded to sulfur (27.9 ppm) and the signals of C-1 (109.2 ppm), C-2 and C-4 (>80 ppm) characteristic of alkyl β-D-galactofuranosides. Therefore, the β-furanosyl configuration for **12** was maintained, and ring expansion or rearrangements, rather common in thiofuranoses,[22] had not occurred.

The free thiol group of **12** was glycosylated using the trichloroacetimidate derivative **13**[23] as glycosyl donor to afford the target thiodisaccharide **7**. The structure of **7** was confirmed on the basis of the NMR spectra which showed diagnostic data for the O- ($\delta_{H\text{-}1}$ 4.72, $J_{1,2}$ 1.9 Hz, $\delta_{C\text{-}1}$ 109.1) and S-galactofuranosides ($\delta_{H\text{-}1'}$ 5.62, $J_{1',2'}$ 1.2 Hz, $\delta_{C\text{-}1'}$ 88.1).[9,10,20]

Removal of the protecting groups of **7** was accomplished by reaction with tetrabutylammonium fluoride (TBAF) for the O-desilylation, followed by O-debenzoylation with NaOMe/MeOH. The ^{13}C NMR spectrum of this product showed signals at 91.6 and 35.7 ppm, corresponding to C-1′ and C-6, respectively, being diagnostics for the *S*-(1→6) inter Gal*f* linkage.

2.2. Conformational analysis

The ^{1}H NMR spectrum of the free thiodisaccharide **14**, recorded in methanol-d_4, appeared quite well resolved and the conformation of the furanose rings could be evaluated according to the values of the coupling constants (*J*). The coupled protons in the non reducing end ring of **14** showed larger *J* values than those corresponding to the same couplings in the other ring. The observed magnitudes for *J* suggested a $^4E \rightleftharpoons {}^4T_3 \rightleftharpoons E_3$ conformation for the 1-thio Gal*f* ring, whereas the conformational equilibrium seems to be shifted towards the $E_0 \rightleftharpoons {}^1T_0 \rightleftharpoons {}^1E$ segment of the pseudorotational itinerary for the furanose ring of the reducing end. In this case, the anomeric substituent is quasiaxially oriented satisfying the anomeric effect, which is stronger in O- than in S-glycosides. A similar conformational behavior has been reported for O- and S- galactofuranosides,[17] and it was also observed by the theoretical calculations described below. The possible rotamers around the C4–C5 linkage were also examined and the small values for the corresponding coupled protons ($J_{4,5}$ = 1.7 and $J_{4',5'}$ = 3.1 Hz) suggested that they adopt a 'gauche' relationship. For simplification we have employed the notation used for the dihedral angles formed by rotation of the hydroxymethyl group in pyranosides.[24] Thus, according to the relative orientation of the endocyclic and exocyclic oxygen atoms three rotamers: gg, gt and tg are defined (Fig. 1). In the gg conformer H4–H5 are *trans* oriented, and this geometry does not justify the observed $J_{4,5}$ and $J_{4',5'}$ values. In the tg conformer C6 is directed towards the furanose ring and interacts with the ring substituents. The gg and tg rotamers present a repulsive gauche C5–C6 interaction which is absent in the gt form, which should be the preferred conformer. Furthermore, the NOESY spectrum of **14** showed an intramolecular NOE contact between H3 and H5 (and H3′, H5′), as expected for the proposed planar zigzag gt conformation for the C4–C6 segment of **14**. In addition to some intraresidual cross peaks, such as H1–H3, H1′–H3′, H2–H4 and H2′–H4′, other interresidue interactions were observed in the NOESY spectrum of **14**. These type of NOE contacts are usually interpreted as the result of conformations around of the glycosidic linkages that bring close in space protons belonging to the two moieties of the disaccharide.[25] Therefore we tried to find some conformations that could explain the observed interresidual NOE contacts. To explore preliminarily the conformational behavior of the thiodisaccharide **14** a combined theoretical calculations and NMR approach was employed.

From the conformational point of view, the substitution of the interglycosidic oxygen by sulfur in thiodisaccharides leds to populate different states compared to the natural counterparts.[12,25] This fact is the result of the differences in the C–S bond lengh (1.78 Å) and the C–S–C bond angle (99°) with respect to the values for C–O (1.41 Å) and C–O–C (116°) and the magnitude of stereoelectronic effects, in particular the anomeric effects. Thus, the conformation of many thiodisaccharides formed by pyranose units was studied using a combination of NMR spectroscopy and molecular mechanics and dynamics calculations.[26] The theoretical results were experimentally confirmed by the interresidue NOEs that unequivocally characterize the minimum energy conformations determined by calculations.[25] The same type of study applied to **14** is far more complex as the two furanose rings are linked through four bonds instead of the three bonds that link the pyranose rings in (1→6)-disaccharides. In fact, the determination of the conformation for this type of disaccharides is more complex than that in disaccharides involving glycosidic bonds to other positions of the pyranose (C-2 to C-4) as, because of the C5–C6 exocyclic bond, a third angle (ω) is needed to describe all the possible orientations between the pyranose rings.[27] For this preliminary study we performed molecular mechanics (MM+) and the minimum energy structures were refined using a semiempirical method (AM1). Fortunately, and in agreement with above mentioned analysis based on coupling constants, all the minimum energy structures found for **14** showed exclusively the presence of the gt rotamer for the C4–C5 bond. The other three torsion angles that involve the linkages between the furanose rings were defined as follows: Φ = H1′–C1′–S–C6, Ψ = C1′–S–C6–C5 and ω = S–C6–C5–C4. A

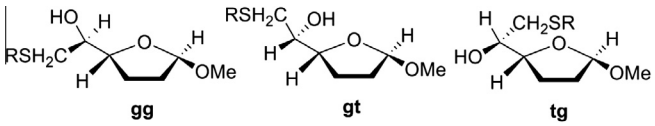


Figure 1. Notation for the dihedral angles around the C4–C5 bond.

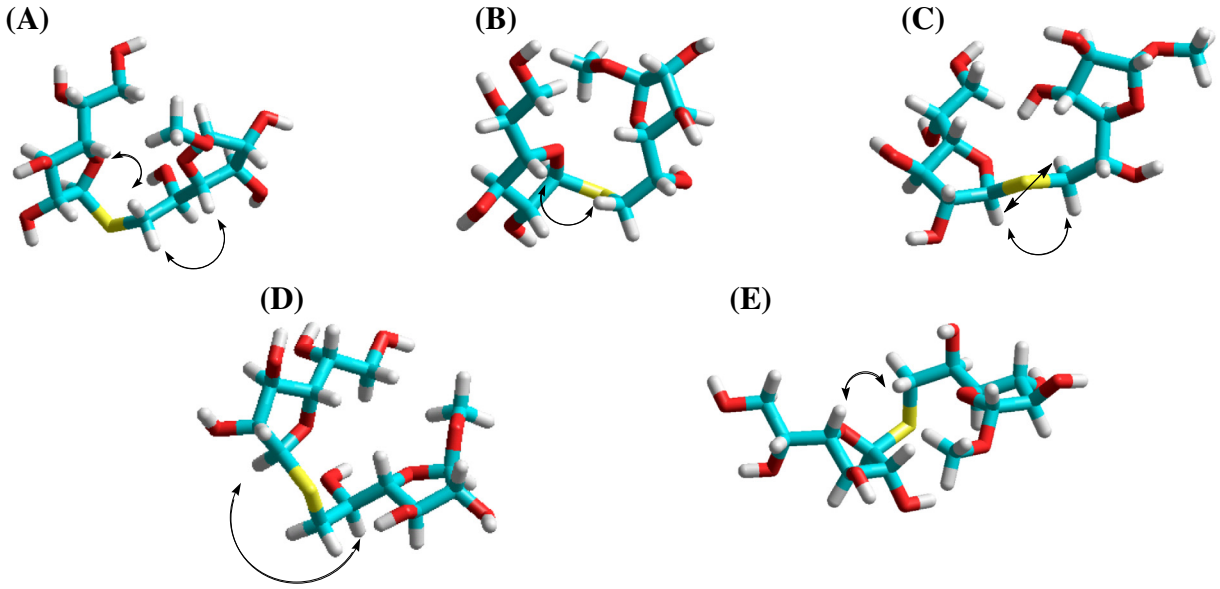


Figure 2. Depiction of the more stable conformers of **14**. Experimental NOE contacts are shown.

schematic representation of these angles has been included in Supplementary Figure S1. In addition, the sign of the rotation angles has been illustrated for ω.

Among the calculated low energy conformations we selected those that justify observed interresidue NOE contacts. Thus, for the conformation A (Fig. 2) was detected a characteristic NOE between H-4′ and pro(*R*) H-6 of the methylene at C-6. As an extension of the nomenclature employed for common disaccharides,[24] this lower energy arrangement was labeled as *anti* Φ/*syn* Ψ/*anti* ω. The *syn* and *anti* rotamers, formed by rotation of the S-glycosidic linkage, have been depicted in Fig. S2 of Supplementary data. A rotation around the C5–C6 bond leads to the following minimum energy conformer *anti* Φ/*syn* Ψ/*syn* ω (B), that should also exhibit the H4′–H6a,6b NOE cross-peak. The conformers C and D present a *syn* disposition for the torsion angles Φ and ω, whereas the aglyconic dihedral angle Ψ is found as *anti* Ψ in C and *syn* Ψ in D. These two conformations, C and D, seem to be confirmed by the respective observed NOE contacts H1′–H6a,6b and H1′–H5. The following low energy rotamer was E, which has an *anti* Φ arrangement, as that observed for A. However, opposite to this conformer, E exhibits *anti* Ψ/*syn* ω geometries. The presence of E in the conformational equilibrium is justified by the NOE contact between H-4′ and pro(*S*) H-6.

Similar to O- and S-glycosides, the conformations of A–D around Φ are stabilized by the exo-anomeric effect and also, as observed for thiodisaccharides, compound **14** exhibits *syn* Ψ and *anti* Ψ conformers.[25] The presence of a sulfur atom and a methylene group in the chain that links the two furanose moieties confers, as expected, a high degree of flexibility. This effect accounts for the average values observed for $J_{5,6a}$ and $J_{5,6b}$, which is a consequence of the relative orientation that H-6a and H-6b adopt with respect to H-5 by rotation of the C5–C6 bond. It is worth to mention that for flexible compounds one of the existing conformations in solution, or even a distorted form of it, could be selected and bound to the binding site of a protein without major energy conflicts.[25]

2.3. Evaluation of the inhibitory activity

As neither endo nor exo galactofuranosidases are commercially available, thiodisaccharide **14** was evaluated as inhibitor of the exo β-D-galactofuranosidase from *P. fellutanum*. This is a model enzyme frequently employed for galactofuranosidase activity, that we were able to isolate in our laboratory from the culture broth of the fungus.[17a] The natural substrate for this enzyme is the extracellular glycopeptide called peptidophosphogalactomannan (pPGM), containing terminal (1→5)-linked β-D-Gal*f* units, attached to an α-mannose core.[28] For the evaluation of thiodisaccharide **14** as in vitro inhibitor of the exo β-D-galactofuranosidase from *P. fellutanum* was followed the protocol usually employed.[11,17] 4-Nitrophenyl β-D-galactofuranoside was used as substrate and D-galactono-1,4-lactone (**15**) as the reference inhibitor. Compound **14** was subjected to the enzymatic reaction, in concentrations ranging from 0.6 to 2.4 mM. Releasing of 4-nitrophenol was employed as a measurement of galactofuranosidase activity. The effect of the concentration of **14** on the activity of the enzyme is shown in Figure 3.

The Lineweaver–Burk plot (Fig. 4) indicates that the thiodisaccharide **14** is a competitive inhibitor of the enzyme (K_i = 3.62 mM), weaker than D-galactono-1,4-lactone (K_i = 0.10 mM). The enzyme activity showed to be highly dependent on the glycone and also the aglycone structure, in agreement with previous work from this

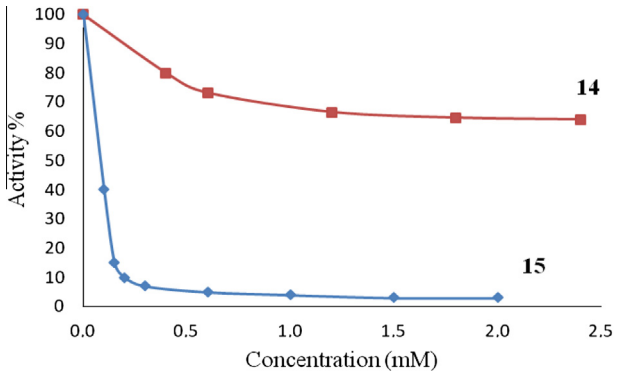


Figure 3. Effect of the concentration of thiodisaccharide **14** on the enzymatic activity of the exo β-D-galactofuranosidase from *Penicillium fellutanum*. 4-Nitropenyl β-D-galactofuranoside was used as substrate and D-galactono-1,4-lactone (**15**) as reference inhibitor. Each point is the mean obtained from three replicate experiments.

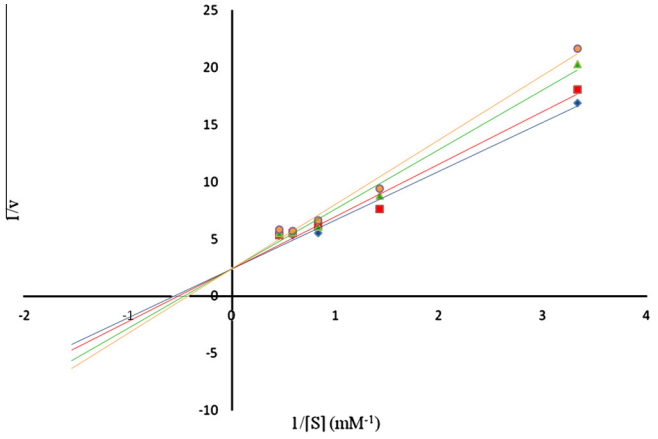


Fig. 4. Lineweaver–Burk reciprocal plot for the inhibition of the exo β-D-galactofuranosidase from *Penicillium fellutanum* by thiodisaccharide **14** at concentrations: (♦) 0.00, (■) 0.60, (▲) 1.00 and (●) 1.40 mM. Each point is the mean obtained from three replicate experiments.

laboratory on mimetics of Gal*f*, including thioglycosides,[11,17] which were tested as substrates or inhibitors of the enzyme. For example, while 4-nitrophenyl β-D-Gal*f* is an useful substrate for the measurement of the enzyme activity (K_m = 0.31 mM), the benzyl (K_m = 3.70 mM) and methyl (K_m = 2.60 mM) analogues are less effective.[17b]

On the other hand, 4-aminophenyl β-D-1-thio-Gal*f* (IC_{50} = 0.08 mM) is more effective as inhibitor than its precursor 4-nitrophenyl β-D-1-thio-Gal*f* (IC_{50} = 0.60 mM), suggesting hydrogen bonding donation in the active site of the enzyme.[17a] Nevertheless, the best inhibitor found for this enzyme, although moderate, is D-galactono-1,4-lactone (K_i = 0.10 mM), which resembles the transition state of the hydrolysis.[17a]

3. Conclusions

Thiodisaccharide Gal*f*-*S*-(1→6)-Gal*f*-*O*Me was synthesized starting from readily available galactofuanosyl precursors. The conformational behavior of this thiodisaccharide was studied preliminarily using combined theoretical calculations and NMR data. For the low energy conformations detected, the thioglycosidic linkage adopts the two rotamers that justify the exoanomeric effect and the C4−C6 segment is found predominantly in the planar zigzag conformation, while the S−C6 linkage is highly flexible. As far as we know, this is the first example of a thiodisaccharide constituted by two furanose units. The new glycomimetic proved to be a moderate competitive inhibitor (K_i = 3.62 mM) of the β-galactofuranosidase from *P. fellutanum*, an enzyme that has not been fully characterized. The gen encoding the enzyme has not been identified, nor has the amino acid sequence been determined. Therefore, the thiodisaccharide could be employed as a useful tool to study the active site of the β-galactofuranosidase. As inhibitor of this enzyme, Gal*f*-*S*-(1→6)-Gal*f*-*O*Me showed a similar activity to that determined for β-D-Gal*f*-*S*-(1→6)-D-Gal*p*-*O*Me. Therefore, the inhibitory activity seems to rely on the presence of the non-reducing Gal*f* unit, while the pyranose or furanose configuration for the reducing Gal residue has a negligible effect.

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 5% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Column chromatography was carried out with Silica Gel 60 (230–400 mesh, Merck). Optical rotations were measured with a Perkin–Elmer 343 digital polarimeter. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AC 200 or with a Bruker AMX 500 instruments. Assignments of ^1H and ^{13}C were assisted by 2D ^1H COSY and HSQC experiments. MM+ and AM1 calculations have been performed with Hyperchem Professional 8.0.3.

4.2. Synthesis

4.2.1. Methyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-α-D-galactofuranoside (2α), methyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-β-D-galactofuranoside (2β), and methyl 2,3-di-*O*-*tert*-butyldimethylsilyl-β-D-galactofuranoside (3)

A solution of uronate **1** (564 mg, 1.00 mmol)[9] in dry THF (15 mL) was cooled to −18 °C and $LiAlH_4$ (50 mg, 1.30 mmol) was added. The reaction mixture was stirred for 30 min, and then EtOAc (15 mL), MeOH (15 mL), and AcOH (to pH 7) were added in sequence, and finally the mixture was concentrated. The solid obtained was suspended in CH_2Cl_2 and centrifuged (4−5 times) to eliminate Li and Al salts. Evaporation of the solvent afforded a residue that showed by TLC (hexane/EtOAc, 10:1) spots corresponding to **2α** (R_f 0.47), **2β** (R_f 0.43), and **3** (R_f 0.00) that were isolated by column chromatography (toluene/EtOAc 60:1 → EtOAc). The following yields were obtained **2α** (70 mg, 13%), **2β** (230 mg, 43%), and **3** (97 mg, 23%) and the compounds exhibited physical and spectral data that were in accordance with those already reported.[9]

4.2.2. Methyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-6-*O*-tosyl-β-D-galactofuranoside (4)

To a solution of **2β** (110 mg, 0.20 mmol) in dry pyridine (2 mL) was added tosyl chloride (100 mg, 0.52 mmol) and the mixture was stirred at rt for 24 h. Addition of MeOH (5 mL) followed by concentration led to a syrup that was dissolved in CH_2Cl_2 (10 mL). The organic solution was washed with water (20 mL), dried ($MgSO_4$) and concentrated. Analysis by TLC (hexane/EtOAc, 10:1) revealed a major product of R_f 0.58, which was purified by column chromatography (hexane/EtOAc, 25:1) to afford **4** (116 mg, 84%); $[\alpha]_D^{25}$ −14.4 (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.78, 7.33 (2d, 4H, $J \sim 8.2$ Hz, H-aromatic), 4.65 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.12 (dd, 1H, $J_{5,6}$ = 3.2, $J_{6a,6b}$ = 9.3 Hz, H-6a), 4.01 (dd, 1H, $J_{2,3}$ = 2.7, $J_{3,4}$ = 5.7 Hz, H-3), 3.98 (ddd, 1H, $J_{4,5}$ = 3.7, $J_{5,6a}$ = 3.2, $J_{5,6b}$ = 7.2 Hz, H-5), 3.95 (dd, 1H, $J_{5,6b}$ = 7.2, $J_{6a,6b}$ = 9.3 Hz, H-6b), 3.94 (dd, 1H, $J_{1,2}$ = 1.5, $J_{2,3}$ = 2.7 Hz, H-2), 3.79 (dd, 1H, $J_{3,4}$ = 5.7, $J_{4,5}$ = 3.7 Hz, H-4), 3.28 (s, 3H, CH_3O), 2.44 (s, 3H, CH_3Ph), 0.87 (×2), 0.85 (3s, 27H, $(CH_3)_3CSiMe_2$), 0.08, 0.07 (×3), 0.05, 0.04 (6s, 18H, $(CH_3)_2SiBu^t$); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 144.7, 133.0, 129.8, 128.0 (C-aromatic), 109.3 (C-1), 84.4 (C-4), 84.1 (C-2), 79.4 (C-3), 71.3 (C-6), 70.5 (C-5), 54.9 (CH_3O), 25.9, 25.7 (×2) [$(CH_3)_3CSiMe_2$], 21.6 (CH_3Ph), 18.3, 17.9, 17.8 [$(CH_3)_3CSiMe_2$], −4.0, −4.1, −4.4, −4.6 (×2), −4.9 [$(CH_3)_2SiBu^t$]. HRMS (ESI) *m*/*z* $[M+Na]^+$ calcd for $C_{32}H_{62}O_8SSi_3$+Na 713.33709, found 713.33654.

4.2.3. Methyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-6-*O*-triflyl-β-D-galactofuranoside (5)

A solution of compound **2β** (130 mg, 0.24 mmol) in dry CH_2Cl_2 (10 mL) was cooled to 0 °C and 2,6-di-*tert*-butyl-4-methyl-pyridine (195 mg, 0.95 mmol) and triflic anhydride (80 μL, 0.47 mmol) were added under N_2 atmosphere. The mixture was stirred for 1 h and then was poured into ice/water and extracted with CH_2Cl_2. The organic extract was washed with water (10 mL), dried ($MgSO_4$) and concentrated. The residue obtained (R_f 0.78, hexane/EtOAc, 10:1),

was purified by column chromatography (hexane to hexane/EtOAc, 60:1) to afford **5** (105 mg, 66%); $[\alpha]_D^{25}$ −18.4 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.71 (br s, $J_{1,2}$ <1.0 Hz, 1H, H-1), 4.62 (dd, 1H, $J_{5,6}$ = 3.3, $J_{6a,6b}$ = 10.1 Hz, H-6a), 4.37 (dd, 1H, $J_{5,6b}$ = 7.7, $J_{6a,6b}$ = 10.1 Hz, H-6b), 4.14 (dddd, 1H, $J_{4,5} \sim 4.6$, $J_{5,6a}$ = 3.3, $J_{5,6b}$ = 7.7 Hz, H-5), 3.99 (m, 2H, H-2, H-3), 3.92 (T, 1H, $J_{3,4} = J_{4,5}$ = 4.6 Hz, H-4), 3.34 (s, 3H, CH_3O), 0.92, 0.89, 0.87 (3s, 27H, (CH_3)$_3$CSiMe$_2$), 0.13, 0.10, 0.08 (3s, 18H, (CH_3)$_2$SiBu^t); ^{13}C NMR ($CDCl_3$, 125.7 MHz,) 109.4 (C-1), 85.4 (C-4), 83.4 (C-2), 79.1 (C-3), 77.9 (C-6), 70.7 (C-5), 54.9 (CH_3O), 25.8, 25.7 (×2) [(CH_3)$_3$CSiMe$_2$], 18.2, 17.9, 17.8 [(CH_3)$_3$CSiMe$_2$], −4.0, −4.4, −4.5, −4.6, −4.7, −4.9 [(CH_3)$_2$SiBu^t]. HRMS (ESI) *m*/*z* [M+Na]$^+$ calcd for $C_{26}H_{55}F_3O_8SSi_3$+Na 691.2775, found 691.2770.

4.2.4. Methyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-6-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-thio-β-D-galactofuranoside (10)

2,3,4,6-Tetra-*O*-acetyl-1-thio-galactopyranose **8** (36 mg, 0.10 mmol) and NaH (5 mg, 0,21 mmol) dissolved in dry THF (5 mL) were stirred at rt for 30 min. The solvent was evaporated and the obtained salt was added to a solution of **4** (30 mg, 0.04 mmol) and 18-crown-6 (15 mg, 0.06 mmol) in DMF (2 mL). The reaction proceeded at rt for 26 h and was concentrated. The product (R_f 0.56, hexane/EtOAc, 2:1) was purified by column chromatography (hexane/EtOAc 20:1 → 5:1) to afford **10** (33 mg, 86%); $[\alpha]_D^{25}$ −35.2 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 5.44 (dd, 1H, $J_{3',4'}$ = 3.4, $J_{4',5'}$ = 0.9 Hz, H-4′), 5.24 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2′), 5.02 (dd, 1H, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.4 Hz, H-3′), 4.70 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 4.46 (d, 1H, $J_{1',2'}$ = 10.0 Hz, H-1′), 4.15 (dd, 1H, $J_{5',6'a}$ = 7.2, $J_{6'a,6'b}$ = 11.3 Hz, H-6′a), 4.11 (dd, 1H, $J_{5',6'b}$ = 6.4, $J_{6'a,6'b}$ = 11.3 Hz, H-6′b), 4.10 (dd, 1H, $J_{2,3}$ = 3.1, $J_{3,4}$ = 6.0 Hz, H-3), 4.02 (dd, 1H, $J_{3,4}$ = 6.0, $J_{4,5}$ = 2.9 Hz, H-4), 3.99 (dd, 1H, $J_{1,2}$ = 2.0, $J_{2,3}$ = 3.1 Hz, H-2), 3.92 (m, 2H, H-5, H-5′), 3.35 (s, 3H, CH_3O), 2.93 (dd, 1H, $J_{5,6a}$ = 7.8, $J_{6a,6b}$ = 13.2 Hz, H-6a), 2.87 (dd, 1H, $J_{5,6b}$ = 6.0, $J_{6a,6b}$ = 13.2 Hz, H-6b), 2.15, 2.05, 2.04, 1.99 (4s, 12H, CH_3CO), 0.92, 0.88 (×2) (2s, 27H, (CH_3)$_3$CSiMe$_2$), 0.16, 0.14, 0.10, 0.09 (×2), 0.07 (5s, 18H, (CH_3)$_2$SiBu^t); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 170.3, 170.2, 170.1, 169.4 (MeCO), 109.1 (C-1), 84.6 (C-2), 84.0 (C-4), 83.7 (C-1′), 79.6 (C-3), 74.5, 71.8, 71.7 (C-3′, 5, 5′). 67.2, 67.0 (C-2′, 4′), 61.2 (C-6′), 55.1 (CH_3O), 32.4 (C-6), 26.0, 25.7 (×2) [(CH_3)$_3$CSiMe$_2$), 20.7 (×2), 20.6 (×2) (CH_3CO), 18.2, 17.9 (×2) [(CH_3)$_3$CSiMe$_2$), −3.5, −4.0, −4.2, −4.3, −4.4, −4,9 [(CH_3)$_2$SiBu^t). HRMS (ESI) *m*/*z* [M+Na]$^+$ calcd for $C_{39}H_{74}O_{14}SSi_3$+Na 905.3999, found 905.4026.

4.2.5. Methyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-6-*S*-acetyl-6-thio-β-D-galactofuranoside (11)

(a) *Starting from methyl 2,3,5-tri-O-tert-butyldimethylsilyl-6-O-tosyl-β-D-galactofuranoside (**4**):* To a solution of compound **4** (100 mg, 0.15 mmol) in DMF (4 mL), potassium thioacetate was added (212 mg, 1.88 mmol). The mixture was stirred at 80 °C for 50 h when TLC (hexane/EtOAc, 10:1) showed a main spot of R_f 0.60. The solvent was evaporated and the dark residue was dissolved in CH_2Cl_2 (15 mL) and washed (2 × 15 mL). The organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (hexane/EtOAc, 40:1) to afford **11** (54 mg, 60%); $[\alpha]_D^{25}$ −28.0 (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.74 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.10 (dd, 1H, $J_{2,3}$ = 2.8, $J_{3,4}$ = 5.3 Hz, H-3), 4.00 (dd, 1H, $J_{1,2}$ = 1.8, $J_{2,3}$ = 2.8 Hz, H-2), 3.92 (dd, 1H, $J_{3,4}$ = 5.4, $J_{4,5}$ = 4.1 Hz, H-4), 3.83 (ddd, 1H, $J_{4,5}$ = 4.1 Hz, H-5), 3.37 (s, 3H, CH_3O), 3.15 (dd, 1H, $J_{5,6a}$ = 6.8, $J_{6a,6b}$ = 13.5 Hz, H-6a), 3.06 (dd, 1H, $J_{5,6b}$ = 6.1, $J_{6a,6b}$ = 13.5 Hz, H-6b), 2.35 (s, 3H, SCOCH_3), 0.94, 0.90, 0.88 (3s, 27H, (CH_3)$_3$CSiMe$_2$), 0.18, 0.17, 0.11 (×2), 0.10, 0.09 (6s, 18H, (CH_3)$_2$SiBu^t); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 195.3 (SCOMe), 109.3 (C-1), 85.5 (C-4), 84.2 (C-2), 79.8 (C-3), 71.7 (C-5), 55.0 (CH_3O), 32.6 (C-6), 30.4 (SCOCH_3), 26.0, 25.7 (×2) [(CH_3)$_3$CSiMe$_2$], 18.3, 17.9, 17.8 [(CH_3)$_3$CSiMe$_2$], −3.9, −4.0, −4.4, −4.5, −4.6, −4.9 [(CH_3)$_2$SiBu^t]. HRMS (ESI) *m*/*z* [M+Na]$^+$ calcd for $C_{27}H_{58}O_6SSi_3$+Na 613.3160, found 617.3154.

(b) *Starting from methyl 2,3,5-tri-O-tert-butyldimethylsilyl-6-O-triflyl-β-D-galactofuranoside (**5**):* To a solution compound **5** (100 mg, 0.15 mmol) in dry MeCN (10 mL), potassium thioacetate was added (75 mg, 0.66 mmol). The mixture was stirred at 0 °C for 3 h when TLC (hexane/EtOAc, 10:1) showed a main spot of R_f 0.60. The solvent was evaporated and the residue was purified by column chromatography (hexane/EtOAc, 90:1) to afford **11** (75 mg, 84%). Compound **11** showed the same physical and spectral properties as the product obtained according to the procedure (a).

4.2.6. Methyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-6-thio-β-D-galactofuranoside (12)

A solution of compound **11** (84 mg, 0.14 mmol) in CH_2Cl_2 (5 mL) was cooled to −15 °C and a 0.5 M NaOMe/MeOH solution (5 mL) was added. The mixture was stirred for 2 h, when TLC (hexane/EtOAc, 10:1) showed a main spot of R_f 0.82. The solvent was evaporated and the resulting residue was dissolved in CH_2Cl_2 (15 mL) and washed with water (2 × 15 mL), dried ($MgSO_4$) and concentrated to a syrup, which was purified by column chromatography (hexane/toluene 7:1 → 1:1) to afford **12** (70 mg, 61%); $[\alpha]_D^{25}$ −28.2 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 4.70 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.08 (dd, 1H, $J_{3,4}$ = 5.2, $J_{4,5}$ = 4.4 Hz, H-4), 4.01 (dd, 1H, $J_{2,3}$ = 2.5, $J_{3,4}$ = 5.2 Hz, H-3), 3.98 (dd, 1H, $J_{1,2}$ = 1.5, $J_{2,3}$ = 2.5 Hz, H-2), 3.80 (ddd, 1H, $J_{4,5}$ = 4.4 Hz, H-5), 3.35 (s, 3H, CH_3O), 2.79 (dddd, 1H, $J_{5,6a}$ = 6.4, $J_{6a,HS}$ = 8.6, $J_{6a,6b}$ = 13.6 Hz, H-6a), 2.58 (dddd, 1H, $J_{5,6b}$ = 5.8, $J_{6b,HS}$ = 8.6, $J_{6a,6b}$ = 13.6 Hz, H-6b), 1.48 (t, 1H, $J_{6a,HS} = J_{6b,HS}$ = 8.6 Hz, *HS*), 0.92, 0.89, 0.88 (3s, 27H, (CH_3)$_3$CSiMe$_2$), 0.14, 0.13, 0.10, 0.09, 0.08, 0.07 (6s, 18H, (CH_3)$_2$SiBu^t); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 109.2 (C-1), 85.0 (C-4), 84.2 (C-2), 79.8 (C-3), 74.4 (C-5), 54.9 (CH_3O), 27.9 (C-6), 26.0, 25.7 (×2) [(CH_3)$_3$CSiMe$_2$], 18.3 (×2), 17.8 [(CH_3)$_3$CSiMe$_2$], −3.9, −4.0, −4.1, −4.3, −4.4, −4.5, −4.8 [(CH_3)$_2$SiBu^t]. HRMS (ESI) *m*/*z* [M+Na]$^+$ calcd for $C_{25}H_{56}O_5SSi_3$+Na 575.3054, found 575.3049.

4.2.7. Methyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-6-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-6-thio-β-D-galactofuranoside (7)

A suspension of the thiol **12** (92 mg, 0.17 mmol), the trichloroacetimidate **13**[23] (246 mg, 0.33 mmol), and freshly activated powdered molecular sieves (4 Å) in dry CH_2Cl_2 (10 mL) was stirred at room temperature for 1.5 h. The mixture was cooled to −18 °C, and TMSOTf (22 μL, 0.13 mmol) was added. After 40 min Et_3N (5 μL, 0.03 mmol) was added and the mixture was concentrated. TLC (toluene/EtOAc, 15:1) showed a main product (R_f 0.65), which was purified by column chromatography (toluene/EtOAc, 60:1) to afford the thiodisaccharide **7** (118 mg, 61%); $[\alpha]_D^{25}$ −38.8 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.09–7.28 (H-aromatic), 6.09 (m, 1H, H-5′), 5.70 (dt, 1H, $J_{3',4'}$ = 4.3 Hz, H-3′), 5.62 (d, 1H, $J_{1',2'}$ = 1.2 Hz, H-1′), 5.52 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 1.2 Hz, H-2′), 4.83 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 4.3 Hz, H-4′), 4.75 (m, 2H, H-6′a, H-6′b), 4.72 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 4.11 (t, 2H, H-3, H-4), 4.00 (t, 2H, H-2, H-5), 3.34 (s, 3H, CH_3O), 2.98 (dd, 1H, $J_{5,6a}$ = 7.5, $J_{6a,6b}$ = 13.3 Hz, H-6a), 2.92 (dd, 1H, $J_{5,6b}$ = 6.0, $J_{6a,6b}$ = 13.3 Hz, H-6b), 0.89, 0.87, 0.84 (3s, 27H, (CH_3)$_3$CSiMe$_2$), 0.12, 0.11, 0.08 (×2), 0.07 (×2) (6s, 18H, (CH_3)$_2$SiBu^t); ^{13}C NMR ($CDCl_3$, 125.7 MHz) δ 166.0, 165.6, 165.4, 165.2 (PhCO), 133.5–128.3 (C-aromatic), 109.1 (C-1), 88.1 (C-1′), 84.5 (C-4), 84.4 (C-2), 82.6 (C-2′), 81.5 (C-4′), 79.7 (C-3), 77.8 (C-3′), 71.5 (C-5), 70.3 (C-5′), 63.5 (C-6′), 55.0 (CH_3O), 33.7 (C-6),

26.0, 25.7 (×2), [(CH_3)$_3$CSiMe$_2$], 18.2, 17.9, 17.8 [(CH_3)$_3$$C$SiMe$_2$], −3.7, −4.0, −4.3, −4.4 (×2), −4.9 [(CH_3)$_2$SiBu^t]. HRMS (ESI) *m*/*z* [M+Na]$^+$ calcd for $C_{59}H_{82}O_{14}SSi_3$+Na 1153.4625, found 1153.4656.

4.2.8. Methyl 6-*S*-(β-D-galactofuranosyl)-6-thio-β-D-galactofuranoside (14)

Removal of the *O*-silyl groups of **7** (65 mg, 0.06 mmol) was performed with 1 M TBAF (198 μL, 0.68 mmol) in THF (2 mL). The solution was stirred at 0 °C and monitoring by TLC (toluene/EtOAc, 1:1) showed the gradual conversion of the starting compound **7** (R_f 0.95) into a more polar product (R_f 0.75). After 2 h the mixture was concentrated and the yellow residue obtained was dissolved in CH_2Cl_2. The solution was washed with water, dried ($MgSO_4$) and concentrated. The resulting syrup was dissolved in a (5 mM) NaOMe/MeOH solution (30 mL) and the mixture was stirred at rt for 4 h. Monitoring by TLC (*n*-BuOH/EtOH/H_2O, 2.5:1:1) showed a product with R_f 0.67, which was UV inactive. The solvent was evaporated and the residue was purified by column chromatography (EtOAc → EtOAc/MeOH, 1:1) to afford **14** (20 mg, 90%); $[\alpha]_D^{25}$ −93.8 (*c* 1.1, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.13 (d, 1H, $J_{1',2'}$ = 4.4 Hz, H-1′), 4.76 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.07 (dd, 1H, $J_{2',3'}$ = 4.9, $J_{3',4'}$ = 7.4 Hz, H-3′), 4.00 (m, 2H, H-3, H-4), 3.98 (dd, 1H, $J_{3',4'}$ = 7.4, $J_{4',5'}$ = 3.1, Hz, H-4′), 3.93 (m, 2H, H-2, H-2′), 3.88 (ddd, 1H, $J_{4,5}$ = 1.7, $J_{5,6a}$ = 7.6, $J_{5,6b}$ = 5.8 Hz, H-5), 3.74 (ddd, 1H, $J_{4',5'}$ = 3.1, $J_{5',6'a}$ = $J_{5',6'b}$ = 6.1 Hz, H-5′), 3.63 (d, 2H, $J_{5',6'a}$ = $J_{5',6'b}$ = 6.1 Hz, H-6′a, H-6′b), 3.37 (s, 3H, CH_3O), 2.89 (dd, 1H, $J_{5,6a}$ = 7.6, $J_{6a,6b}$ = 13.9 Hz, H-6a), 2.84 (dd, 1H, $J_{5,6b}$ = 5.8, $J_{6a,6b}$ = 13.9 Hz, H-6b); ^{13}C NMR (CD_3OD, 125.7 MHz) δ 110.4 (C-1), 91.2 (C-1′), 85.5 (C-4), 83.9, 83.3 (C-2, 2′), 82.9 (C-4′), 78.8 (C-3), 78.4 (C-3′), 72.1 (C-5′), 71.7 (C-5), 64.4 (C-6′), 55.3 (CH_3O), 35.7 (C-6). HRMS (ESI) *m*/*z* [M+Na]$^+$ calcd for $C_{13}H_{24}O_{10}S$+Na 395.0988, found 395.0976.

4.3. Enzymatic assays

The enzymatic activity was assayed using the filtered medium of a stationary culture of *P. fellutanum* as source of exo β-D-galactofuranosidase and 4-nitrophenyl β-D-galactofuranoside as substrate.[17a] The standard assay was conducted with 50 μL of 66 mM NaOAc buffer (pH 4.6), 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. Compound **14** was incorporated in the amounts required to obtain a final concentration of 0.6 to 2.4 mM. The enzymatic reaction was stopped after 1.5 h of incubation at 37 °C by addition of 1 mL of 0.1 M Na_2CO_3 buffer (pH 9.0). The 4-nitrophenol released was measured spectrophotometrically at 410 nm. K_m and K_i values were determined by the Lineweaver–Burk plot.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.02.057. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. (a) Koester, D. C.; Holkenbrink, A.; Werz, D. B. *Synthesis* **2010**, *19*, 3217; (b) Sears, P.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1999**, *38*, 2300.
2. (a) Yuasa, H.; Izumi, M.; Hashimoto, H. *Curr. Top. Med. Chem.* **2009**, *9*, 76; (b) Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515.
3. Pachamuthu, K.; Schmidt, R. R. *Chem. Rev.* **2006**, *106*, 160.
4. Szilágyi, L.; Varela, O. *Curr. Org. Chem.* **2006**, *10*, 1745.
5. Uhrig, M. L.; Manzano, V. E.; Varela, O. *Eur. J. Org. Chem.* **2006**, 162.
6. Cagnoni, A. J.; Uhrig, M. L.; Varela, O. *Bioorg. Med. Chem.* **2009**, *17*, 6203.
7. Manzano, V. E.; Uhrig, M. L.; Varela, O. *J. Org. Chem.* **2008**, *73*, 7224.
8. Manzano, V. E.; Uhrig, M. L.; Varela, O. *Org. Biomol. Chem.* **2012**, *10*, 8884.
9. Repetto, E.; Manzano, V. E.; Uhrig, M. L.; Varela, O. *J. Org. Chem.* **2012**, *77*, 253.
10. Repetto, E.; Marino, C.; Uhrig, M. L.; Varela, O. *Eur. J. Org. Chem.* **2008**, 540.
11. Repetto, E.; Marino, C. M.; Uhrig, M. L.; Varela, O. *Bioorg. Med. Chem.* **2009**, *17*, 2703.
12. Randell, K. D.; Johnston, B. D.; Lee, E. E.; Pinto, B. M. *Tetrahedron: Asymmetry* **2000**, *11*, 207.
13. Peltier, P.; Euzen, R.; Daniellou, R.; Nugier-Chauvin, C.; Ferrieres, V. *Carbohydr. Res.* **2008**, *343*, 1897.
14. Marino, C.; Gallo-Rodriguez, C.; Lederkremer, R. M. Galactofuranosyl-Containing Glycans: Occurrence, Synthesis and Biochemistry. In *Glycans: Biochemistry, Characterization and Applications*; Mora-Montes, H. M., Ed.; Nova Science Publisher: New York, 2012; pp 201–268.
15. Besra, G. S.; Khoo, K. H.; McNeil, M. R.; Dell, A.; Morris, H. R.; Brennan, P. J. *Biochemistry* **1995**, *34*, 4257.
16. Barry, C. E. *Biochem. Pharmacol.* **1997**, *54*, 1165.
17. (a) Marino, C.; Mariño, K.; Miletti, L.; Manso Alves, M. J.; Colli, W.; Lederkremer, R. M. *Glycobiology* **1998**, *8*, 901; (b) Mariño, K.; Maino, C. *Arkivoc* **2005**, *XII*, 341; (c) Mariño, K.; Lima, C.; Maldonado, S.; Marino, C.; Lederkremer, R. M. *Carbohydr. Res.* **2002**, *337*, 891.
18. Davis, C. B.; Hartnell, R. D.; Madge, P. D.; Owen, D. J.; Thomson, R. J.; Chong, A. K. J.; Coppel, R. L.; von Itzstein, M. *Carbohydr. Res.* **2007**, *342*, 1773.
19. Driguez, H. *ChemBioChem* **2001**, *2*, 311.
20. Baldoni, L.; Marino, C. *Carbohydr. Res.* **2012**, *362*, 70.
21. (a) Ibatullin, F. M.; Selivanov, S. I.; Shavva, A. G. *Synthesis* **2001**, *3*, 419; (b) Ibatullin, F. M.; Shabalin, K. A.; Jänis, J. V.; Selivanov, S. I. *Tetrahedron Lett.* **2001**, *42*, 4565.
22. (a) Hughes, N. A.; Wood, C. J. *J. Chem. Soc., Perkin Trans. 1* **1986**, 695; (b) Hughes, N. A.; Munkombwe, N. M.; Todhunyer, N. D. *Carbohydr. Res.* **1991**, *216*, 119.
23. (a) Choudhury, A. K.; Roy, N. *Carbohydr. Res.* **1998**, *308*, 207; (b) Gallo-Rodriguez, C.; Gandolfi, L.; de Lederkremer, R. M. *Org. Lett.* **1999**, *1*, 245.
24. Yuasa, H.; Hashimoto, H. *Trends Glycosci. Glyc.* **2001**, *13*, 31.
25. (a) Montero, E.; García-Herrero, A.; Asensio, J. L.; Hirai, K.; Ogawa, S.; Santoyo-González, F.; Cañada, F. J.; Jiménez-Barbero, J. *Eur. J. Org. Chem.* **2000**, 1945; (b) García-Herrero, A.; Montero, E.; Muñoz, J. L.; Espinosa, J. F.; Vián, A.; García, J. L.; Asensio, J. L.; Cañada, F. J.; Jiménez-Barbero, J. *J. Am. Chem. Soc.* **2002**, *124*, 4804.
26. (a) Peters, T.; Pinto, B. M. *Curr. Opin. Struct. Biol.* **1998**, *6*, 710; (b) Imberty, A. *Curr. Opin. Struct. Biol.* **1997**, *7*, 617; (c) Jimenez-Barbero, J.; Asensio, J. L.; Cañana, J.; Poveda, A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 549.
27. (Bonas, G.; Vignon, M. R. *Carbohydr. Res.* **1991**, *211*, 191; (b) Javaroni, F.; Ferreira, A. B. B.; da Silva, C. O. *Carbohydr. Res.* **2009**, *344*, 1235; (c) Grachev, A. A.; Gerbst, A. G.; Gening, M. L.; Titov, D. V.; Yudina, O. N.; Tsetkov, Y. E.; Shashkov, A. S.; Pier, G. B.; Nifantiev, N. E. *Carbohydr. Res.* **2011**, *346*, 2499.
28. (a) Gander, J. E.; Jentoft, N. H.; Drewes, L. R.; Rick, P. D. *J. Biol. Chem.* **1974**, *249*, 2063; (b) Rietschel-Berst, M.; Jentoft, N. H.; Rick, P. D.; Pletcher, C.; Fang, F.; Gander, J. E. *J. Biol. Chem.* **1977**, *252*, 3219.