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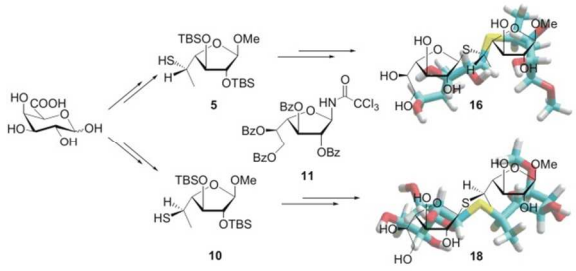

Description of the synthesis, molecular modeling and inhibitory properties of furanosyl thiodisaccharides that are mimetics of the motif β-D-Gal*f*-(1→5)-D-Gal*f,* found in glycoconjugates of pathogenic microorganisms.

Synthesis of galactofuranosyl-(1→5)-thiodisaccharide glycomimetics as inhibitors of a β-D-galactofuranosidase§

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The first synthesis of methyl β-D-galactofuranosyl-(1→5)-thiofuranosides is reported. These molecules, which have the 6-deoxy-5-thio derivative of L-altrofuranose (**16**) or D-galactofuranose (**18**) as reducing end, are mimetics of the motif β-D-Gal*f*-(1→5)-D-Gal*f* found in glycoconjugates of many pathogenic microorganisms. The conformational preferences of **16** and **18** in solution were assessed by means of molecular modeling and NMR techniques. These thiodisaccharides have been evaluated as inhibitors of the β-D-galactofuranosidase from *Penicillium fellutanum*. The kinetics of the inhibition showed that they behave as competitive inhibitors. As expected, compound **18** (*K*i = 0.15 mM), with the same configuration for the reducing end as the natural substrate of the enzyme, was a stronger inhibitor than **16** (*K*i = 2.23 mM).

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§ Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of compounds **5-7**, **10**, **12-18** and NOESY spectra of compound **16** and **18**. See DOI:……

Introduction

Carbohydrate mimetics are usually prepared by replacing oxygen atoms in a sugar with carbon atoms or other heteroatoms. The modified molecules generally show altered binding properties or increased stability toward enzyme degradation, with respect to their natural counterparts.[1] Moreover, glycomimetics are useful tools for glycobiology, as they are employed to investigate recognition events that initiate immunological responses to bacterial and viral infections and in signaling processes that occur in inflammation and cancer metastasis.[2] The replacement of interglycosidic oxygen atoms in oligosaccharides by sulfur atoms leads to thiooligosaccharides. This structural modification usually induces resistance to enzymatic hydrolysis and, in many cases, inhibition of the activity of such enzymes, with the advantage that thiooligosaccharides are tolerated by most biological systems.[3] The varied applications of thiooligosaccharides has stimulated investigations about their synthesis.[3,4] In this regard, we have contributed to the design and development of straightforward and stereoselective synthesis of thiooligosaccharides, and many of them have been evaluated as enzyme inhibitors.[5-7] The thioglycosidic linkage has been regio- and diastereoselective constructed using as key reactions the Michael addition[5] and the epoxide[6] or thiirane ring-opening.[6b,7] Among other topics we have focused our attention on the synthesis of thiooligosaccharides that possess a furanose sugar as constituent, since most methodologies reported are referred to the synthesis of thiooligosaccharides formed by pyranose units. Thus, we have described successful approaches to prepare *S*-disaccharides of 1-thiopentofuranose[8] or 1-thiohexofuranose[9] as non-reducing end; and the (1→6)-linked thiodisaccharide of galactofuranose (Gal*f*) has also been prepared (Figure 1).[10] In addition, other researchers have reported the synthesis of analogues with the ring oxygen atom of Gal*f* replaced by sulfur (4-thiohexofuranose, Figure 1).[11]

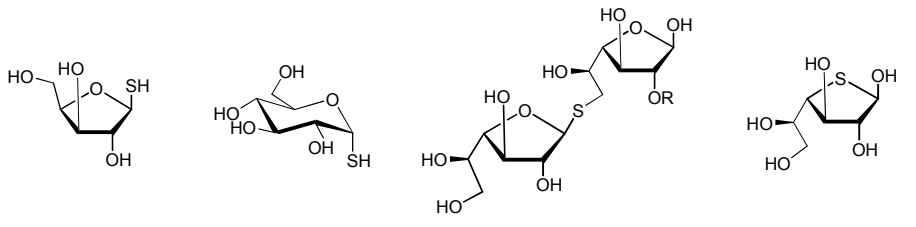


Figure 1 Examples of sulfur-containing glycomimetics.

The interest in furanose-containing molecules arises from the fact that furanoses are widespread in nature, and particularly Gal*f* occurs in glycoconjugates of bacteria, fungi, and protozoan parasites.[12] For example, the motif β-D-Gal*f*-(1→5)-D-Gal*f* is found in polyfuranosides of many pathogenic microorganisms, including *Aspergillus* and *Mycobacterium*.[12d,e] The fact that Gal*f* is present in structures that are considered to be essential for the survival or virulence of such microorganisms,[12,13] but is absent in higher eukaryotes, has attracted increasing interest on the biosynthetic pathways that involve this sugar,[13,14] the inhibition of the related enzymes as well as the synthesis of D-Gal*f* containing molecules as biological probes.[12b,d,e,15]

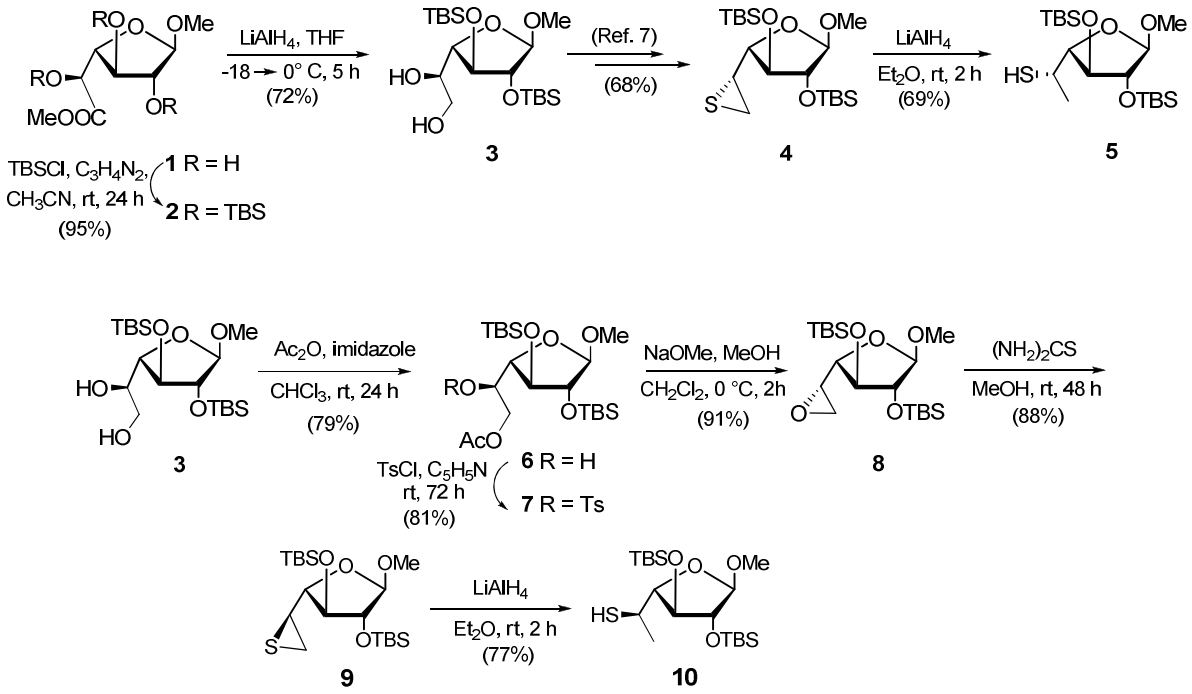
Degradation of Gal*f* containing glycoconjugates is promoted in some microorganisms by a β-D-galactofuranosidase. Thus, a specific *exo* β-D-galactofuranosidase has been isolated from the culture medium of *Penicillium fellutanum*[16] and later described in *Helminthosporium sacchari*[17] and *Penicillium* and *Apergillus* species.[18] This enzyme in *P. fellutanum* is responsible for the release of galactose by depolymerization of an extracellular glycopeptide that contains (1→5)-linked β-D-Gal*f* units.[16] In addition, the amount of Gal*f* containing glycoconjugates is dramatically diminished during differentiation of *Trypanosoma cruzi* from the invasive to the infective stages[19] and we have detected for the first time β-D-galactofuranosidase activity in this protozoo.[20] The inhibition of the enzymes involved in the metabolism of the polyfuranosides is expected to prevent the proliferation of *T. cruzi*, the agent of Chagas disease[20] and mycobacteria, including *Mycobacterium tuberculosis*, the agent of tuberculosis.[18b] Furthermore, as to date the amino acid sequence for the catalytic site of the enzyme has not been identified, nor has the interactions with the substrate been determined, the development of new inhibitors can serve as tools for studying Gal*f* processing enzymes.

We report here the synthesis of the methyl glycoside of the 6-deoxy-5-thio analogue of β-D-Gal*f*-(1→5)-D-Gal*f*, a rather common motif in polyfuranosides of pathogenic microorganisms. This thiodisaccharide was evaluated as inhibitor of the β-D-galactofuranosidase from *P. fellutanum.* The analogous thiodisaccharide which has opposite configuration for the stereocenter that carries the sulfur atom in the hexofuranose reducing end was also synthesized, in order to assess the influence of such a stereocenter on the inhibitory activity of the same enzyme.

Results and Discussion

Synthesis of methyl 5-*S*-(β-D-galactofuranosyl)-6-deoxy-5-thio-α-L-altrofuranoside (16) and methyl 5-*S*-(β-D-galactofuranosyl)-6-deoxy-5-thio-β-D-galactofuranoside (18)

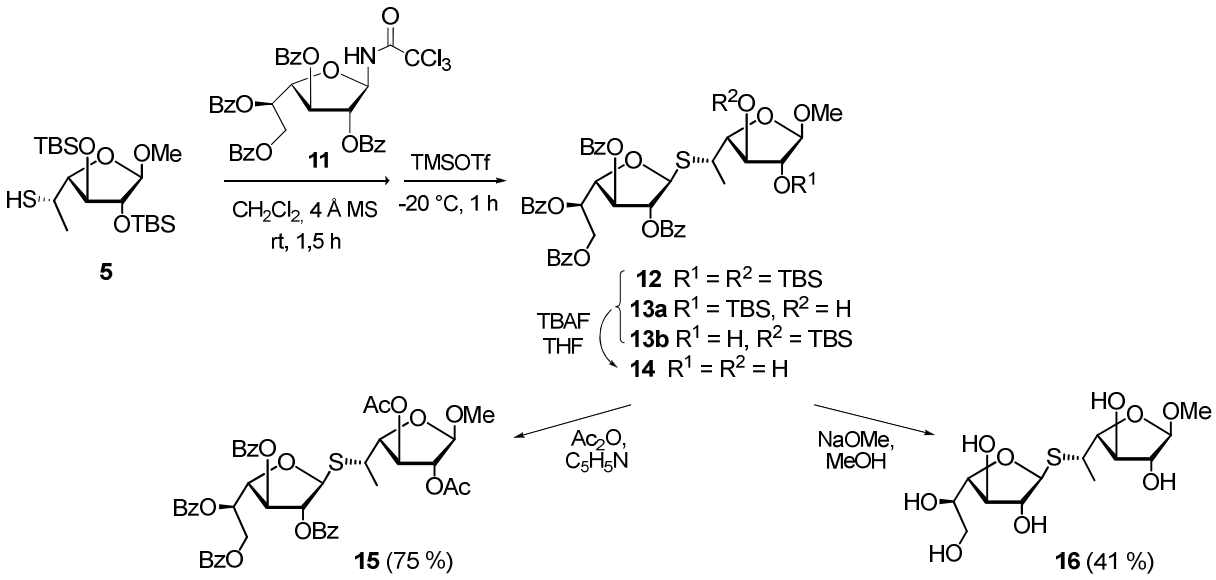
The target thiodisaccharides were designed using the 5-thiol derivatives **5** and **10** as key precursors. The synthetic route employed for the preparation of these two glycomimetics is depicted in Scheme 1. In the design of the target molecules we took into account that the deoxygenation at C-6 of galactofuranosyl derivatives increases the resistance to the hydrolytic activity of the β-D-galactofuranosidase from *P. fellutanum*.[21] In addition, thiols **5** and **10** are expected to be readily prepared by reduction of the thiirane precursors **4** and **9**, respectively, which have been previously synthesized in our laboratory starting from the α,β anomeric mixture of the uronate **1** via the diol **3**.[7] However, in this instance, we started from the anomerically pure glycoside **1**, which was obtained from methyl (methyl β-D-galactofuranosid)uronate, the product of methanolysis of D-galacturonic acid.[22] Compound **1** was silylated with an excess of *tert*-butyldimethylsilyl chloride (TBSCl) to give **2**, which was treated with an excess of $LiAlH_4$ at –18 ºC for 5 h and then the temperature was slowly increased until 0 ºC. Under these conditions, the reduction of the methyl ester and the removal of the silyl ether at C-5 took place to give the diol **3** in 72 % isolated yield. The regioselective tosylation of the primary hydroxyl group of **3**, followed by epoxide formation and conversion of the epoxide into the thiirane group led to the 5,6-epithio-α-L-altrofuranoside derivative **4**[7] in 68 % overall yield from **1**. The ring-opening of the thiirane was accomplished by reduction of **4** with $LiAlH_4$ to afford the 5-thio derivative **5** (69 %).



Scheme 1 Synthesis of 5-thiols **5** and **10** as glycosyl acceptors for the synthesis of the thiodisaccharides.

For the synthesis of the thiol **10**, which has opposite configuration at C-5 with respect to **5**, a double inversion of the configuration of the C-5 stereocenter of the starting compound **3** was required. For this reason, the tosylate leaving group was installed in the secondary hydroxyl group of **3**. This was accomplished by regioselective acetylation of HO-6 to give **6** (79 % yield) and subsequent tosylation of HO-5, to afford **7** (81 %). Treatment of **7** with NaOMe/MeOH produced the *O*-deacetylation and nucleophilic attack of the resulting alcoxyde to C-5 to afford the epoxide **8** (91 %) by displacement of the tosylate leaving group and the inversion of the configuration at C-5. The second configurational inversion of this stereocenter was achieved on treatment of epoxide **8** with thiourea, to give the thiirane **9** (88 %), which belongs to the D-*galacto* series.

The reduction of the thiirane group of **4** and **9** with $LiAlH_4$ to the respective 5-thiol derivatives **5** and **10** was confirmed using the ^{1}H NMR spectra. The diagnostic H-5 signal appeared at high field because it was geminal to the thiol group, and showed coupling with the vicinal protons of SH, C-4 and the C-6 methyl group, which appeared as a doublet in the region of 1.4 ppm. The ^{13}C NMR spectra of **5** and **10** were also in agreement with the proposed structures.

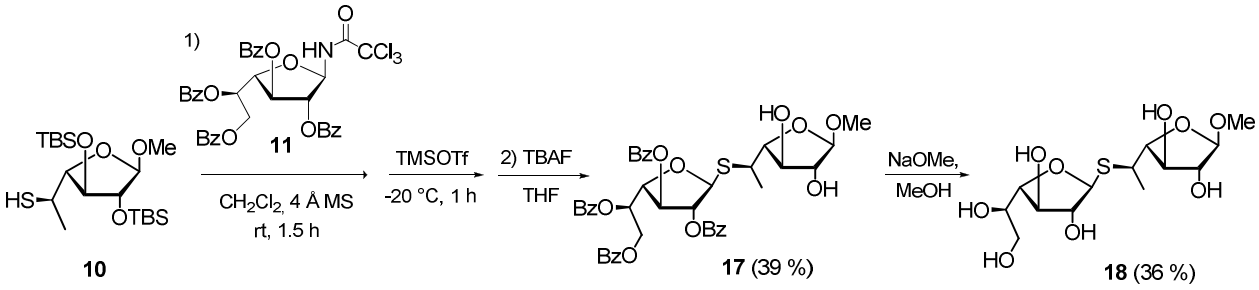


Scheme 2 Synthesis of methyl 5-*S*-(β-D-galactofuranosyl)-6-deoxy-5-thio-α-L-altrofuranoside (**16**).

Having the thiols **5** and **10** in hand, the next step was the synthesis of the target glycomimetics **16** and **18**. We have reported that the thioglycosidic bond can be constructed from the sugar thiols, with exclusive diastereoselectivity in favour of the β anomer of galactofuranosides, using as glycosyl donor the per-*O*-benzoyl-β-D-galactofuranosyl trichloroacetimidate (**11**),[7,10] which can be readily prepared from per-*O*-benzoyl-α,β-D-galactofuranose.[23] The glycosylation of thiol **5** (Scheme 2) was attempted using **11** as glycosylating agent and trimethylsilyl triflate (TMSOTf) as catalyst. Under the usual reaction conditions,[7,10] a rather complex mixture was obtained, and their components were isolated by column chromatography. The less polar product was the expected fully substituted thiodisaccharide **12**, but the following fractions from the column afforded partially protected thiodisaccharides. Thus, an inseparable mixture of the 2-*O*-TBS and 3-*O*-TBS derivatives of the thiodisaccharide (**13 a,b**) was obtained together with the fully *O*-desilylated product **14**. The location of the TBS substituent in **13a** and **13b** was tentatively assigned by comparison of the ^{13}CNMR spectrum of the mixture with respect to that of **14**, according to the carbon signal (C-2 or C-3) that showed a stronger downfield shifting on monosylilation of **14**.[24] On this basis, the ratio of **13a**:**13b** was determined approximately as 1:3. The structure of **14** was also confirmed by 2,3-di-*O*-acetylation to give **15**, which was fully characterized.

Treatment of compounds **12** or **13a,b** with tetrabutylammonium fluoride (TBAF) in THF produced the complete removal of the TBS groups to afford the thiodisaccharide **14**. To avoid the isolation of the silyl derivatives **12**, **13a,b** by column chromatography, the crude mixture of thioglycosylation was subjected directly to de-*O*-silylation by treatment with TBAF/THF to afford **14**, which was purified by filtration through a short column of silica gel. The removal of the benzoyl protecting groups of **14** was performed using sodium methoxide in methanol, to give the free thiodisaccharide **16** in 41 % yield from **5**, after three steps.

The same route was applied for the synthesis of the thiodisaccharide **18** formed by two furanosyl units of *galacto* configuration *S*-(1→5) linked (Scheme 3). The glycomimetic **18** was isolated in 36 % yield from **10**.



Scheme 3 Synthesis of methyl 5-*S*-(β-D-galactofuranosyl)-6-deoxy-5-thio-β-D-galactofuranoside (**18**).

Conformational analysis

The structure of the thiodisaccharides **16** and **18** and their synthetic precursors and intermediates was confirmed on the basis of their NMR spectra, which were fully assigned with assistance of 2D-experiments. Thus, the ^{13}C NMR spectra of compounds **12-18** showed the signal of the anomeric carbon of the reducing *O*-furanosyl unit shifted downfield (107.6−109.6 ppm) with respect to the same signals of the 1-thiofuranosyl moiety (96.9−89.3 ppm) due to the shielding effect of the sulfur atom over the vicinal magnetic nucleus. Furthermore, the ^{1}H NMR spectra (recorded in $CDCl_3$) of the thiodisaccharide derivatives **12-15** and **17** showed small coupling constant values for the signal of the protons of both furanose rings as they possess the same relative configuration. This behavior is characteristic of β-galactofuranosides.[7,9,10] The small *J*

values suggests a conformation of both the *O*- and *S*-furanoside rings centered in the ${}^1T_0 \rightleftharpoons {}^1E \rightleftharpoons {}^1T_2$ segment of the pseudorotational ring. Such a conformation is stabilized by the quasiaxial disposition of the anomeric substituent (anomeric effect) and the quasiequatorial orientation of the bulky lateral chain at C-4. However, the ^{1}H NMR spectra of the free thiodisaccharide **16** and **18**, recorded in D_2O, showed a significant increment of the magnitude of the coupling constants for the thiofuranoside ring protons, while those of the *O*-furanosides remind similar to the one measured for **12-15** and **17** (Table 1). The larger *J* values suggest a tendency for all the *trans* protons of the Gal*f* rings of **16** and **18** to occupy quasiaxial orientations. Hence, the conformational equilibrium seems to be shifted towards the ${}^4T_3 \rightleftharpoons E_3 \rightleftharpoons {}^2T_3$ segment. The conformational change may be attributed to the strength of the anomeric effect, which is less intense for *S*- than for *O*-glycosides,[25] and it should be even more weakened as the spectra were recorded in D_2O.[26] In the resulting conformation all the substituents of the ring are quasiequatorially disposed. Other *O*- and *S*-galactofuranosyl derivatives have shown a similar behavior.[27]

Table 1 Coupling constant (*J*) values for the ring protons of selected compounds (**12**, **15**, **16** and **18**).

	$J_{1,2}$ (Hz)	$J_{2,3}$ (Hz)	$J_{3,4}$ (Hz)	$J_{1',2'}$ (Hz)	$J_{2',3'}$ (Hz)	$J_{3',4'}$ (Hz)
12	1.2	1.2	5.2	<1	1.2	5.2
15	<1	1.1	5.6	<1	<1	4.3
16	1.4	2.6	-	5.4	5.4	7.5
18	1.7	3.2	6.3	4.9	5.3	7.4

It was also interesting to determine the conformation of the thiodisaccharides **16** and **18**, as the thioglycosidic linkage is located in a flexible region of the molecule. The conformation of many thiodisaccharides constituted by two pyranosyl residues has been studied using NMR methods complemented with theoretical calculations.[28] Interresidue NOE interactions have been employed to characterize the minimum energy conformations determined by molecular modelling.[28d] However, as there are practically no examples of

thiodisaccharides formed by furanose units, no conformational studies, apart from our own previous report,[10] are available on this type of molecules. Such a conformational analysis is rather difficult as three bonds (C1'–S–C5–C4) are required to link the two furanose rings. This situation resembles that of the pyranosyl (1→6)-linked disaccharides, which also involves three bonds, and is more complex than that in disaccharides with glycosidic linkages to other positions of the pyranose (C2 to C4). Because of the additional C5–C6 exocyclic bond, a third angle (ω) is needed to describe all the possible orientations between the pyranose rings.[29] Similarly, we have defined the three torsion angles around the S-glycosidic linkage, which are the following: Φ = H1'–C1'–S–C5, Ψ = C1'–S–C5–C4 and ω = S–C5–C4–H4. The usual convention[10] has been employed to establish the sign of the rotation of the dihedral angles.[30] Also for this preliminary study we performed molecular mechanics (MM+) and selected structures which showed minimum energy values were refined using a semiempirical method (AM1).[10] The contribution to the conformational equilibrium of such low energy structures was experimentally confirmed by the detection of interresidue NOE contacts characteristic of a given conformer. The magnitude of the $J_{4,5}$ value, which is a function of the angle ω, was also considered.

For the thiodisaccharide **16**, a minimum energy conformation was found for the *syn* Φ/*syn* Ψ/*syn* ω arrangement (**A**), which is characterized by the H1'–H4 NOE contact (Figure 2). Since in the ^{1}H NMR spectrum of **16** the signals of H3 and H4 are overlapped, the observed NOE was attributed to the interaction of H1'–H4 as no low energy structures showing the H1'–H3 NOE have been found. The less intense NOE interaction H1'–H6, shown by this conformer, was also detected in the NOESY spectrum of **16**. The presence in the conformational equilibrium of the *syn* Φ/*anti* Ψ/*anti* ω form (**B**) was confirmed by the intense H1'–H5 NOE. This interaction should also be detected for the low energy arrangement *syn* Φ/*syn* Ψ/*anti* ω (**C**), which should be present in the conformational equilibrium.

The average value for $J_{4,5}$ (4.5 Hz) for **16** is in agreement with the relative disposition of H-4 and H5, which are *anti* in **A**, but *syn*-oriented in **B** and **C**. In addition, the experimentally detected intraresidue NOE cross peak between H4 and H6 protons of the terminal methyl group is in accordance with the proposed structures.

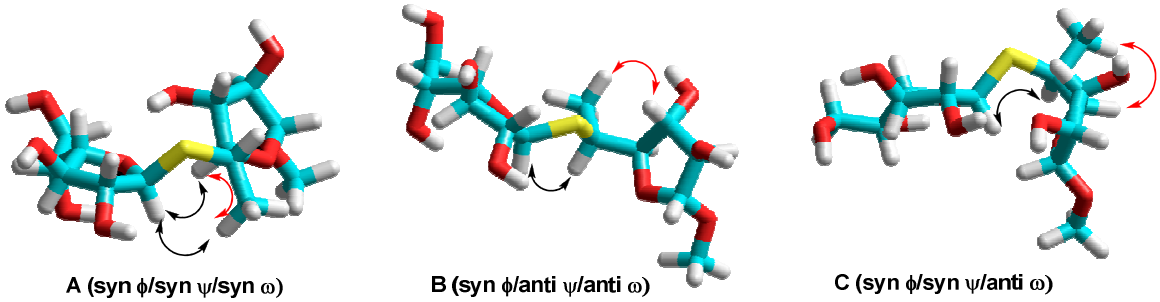


Figure 2 Low energy conformations of **16**, according to theoretical calculations and experimental NOE contacts (interresidue in black, intraresidue in red).

A similar conformational analysis was applied to the thiodisaccharide **18** (Figure 3). As for the analogue **16**, the *syn* Φ/*syn* Ψ/*syn* ω (**D**) was the lowest energy arrangement, according to theoretical calculations. The observed NOE contacts between H1'–H4 and H1´–H5 support experimentally the contribution of **D** to the conformational equilibrium. The other low energy structure *syn* Φ/*syn* Ψ/*anti* ω (**E**), which was also confirmed by the detected NOE interactions of H1' with H5 and with the CH_3–6 protons. The third low energy rotamer **F** (*syn* Φ/*anti*Ψ/*syn* ω) arises by rotation around of the S–C5 linkage (Ψangle) from **D**. The arrangement **F** is also characterized by the intense H1´–H5 NOE cross peak.

The averaged value for the H4–H5 coupling constant ($J_{4,5}$ 4.6 Hz, H4 and H5 are *gauche* in **D** and **E**, and *anti* in **F**) and the observed intraresidue NOE cross peaks H3–H6 (in **D**) and H4-H6 (in **E** and **F**) are in agreement with the conformations proposed.

Interestingly, no conformations other than the *syn* Φ, with Φ having a positive value, have been experimentally confirmed. Similar to other thiodisaccharides, all the conformations around the Φ angle theoretically found, and experimentally confirmed, are stabilized by the *exo* anomeric effect. These results are in agreement with previous reports[31] that indicated that the conformational preferences of the glycosidic fragments are governed by steric interactions as well as stereoelectronic effects, such as the anomeric and *exo* anomeric effects.

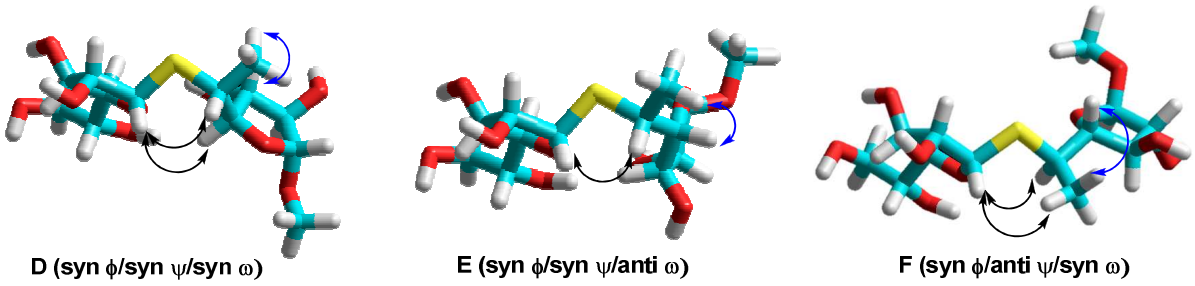


Figure 3 Low energy conformations of **18**, according to theoretical calculations and experimental NOE contacts (interresidue in black, intraresidue in blue)

Evaluation of the inhibitory activity

The natural substrate for the *exo* β-D-galactofuranosidase is the peptide phosphogalactomannan (pPGM), an extracellular glycopeptide from *P. fellutanum* containing terminal (1→5)-linked β-D-Gal*f* units, attached to an α-mannose core.[16] The enzyme, which is not commercially available, has been isolated in our laboratory from the culture growth of the fungus.[15] Interestingly, the enzyme activity showed to be highly dependent on both the glycone and the aglycone structures.[22]

As thiodisaccharides **16** and **18** are mimetics of the natural terminal disaccharide of the pPGM, they have been evaluated as inhibitors of the enzyme. For the inhibition studies, 4-nitrophenyl β-D-galactofuranoside was employed as substrate and the protocol previously established in our laboratory was followed.[10,25] The inhibitory profile was compared with those of the known inhibitor aldono-1,4-lactone (**19,** *K*i = 0.10 mM) and the parent thiodisaccharide D-Gal*f*-(1→6)-1-*S*-D-Gal*f* (**20**, *K*i = 3.62 mM).[10] Compounds **16** and **18-20** were subjected to the enzymatic reaction, in concentrations ranging from 0.1 to 1.6 mM. Releasing of 4-nitrophenol was employed as a measurement of galactofuranosidase activity. The effect of the concentration of such compounds on the activity of the enzyme is shown in Figure 4. In addition, the Lineweaver–Burk plots indicated that **16** (*K*i = 2.23 mM) and **18** (*K*i = 0.15 mM) are competitive inhibitors, as shown in Figures 5 and 6. Compound **18** (IC_{50} 0.15 mM), with the same configuration as the natural substrate of the enzyme, is about 15-fold a stronger inhibitor of the β-D-galactofuranosidase than the thiodisaccharide **16**, with a L-*altro* configuration for the reducing end, and even more potent (24-fold) than the *S*-(1→6) linked disaccharide **20**. These results demonstrate that the configuration of the aglycone moiety (D-Gal*f* versus L-Alt*f*) of the thiodisaccharide and the site of linkage (*S*-(1→5) versus *S*-(1→6)) have an strong influence on the inhibitory activity.

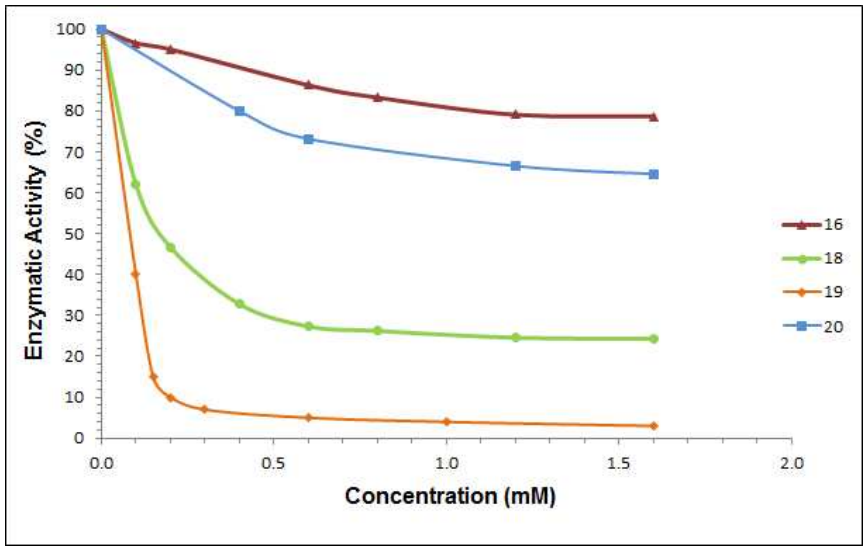


Figure 4 Effect of the concentration of thiodisaccharides **16** and **18** on the enzymatic activity of the *exo* β-D-galactofuranosidase from *Penicillium fellutanum*. 4-Nitrophenyl β-D-galactofuranoside was used as substrate and D-galactono-1,4-lactone (**19**) as reference inhibitor. For comparison, the inhibition by thiodisaccharide **20**[10] was also included. Each point is the mean of three replicate experiments.

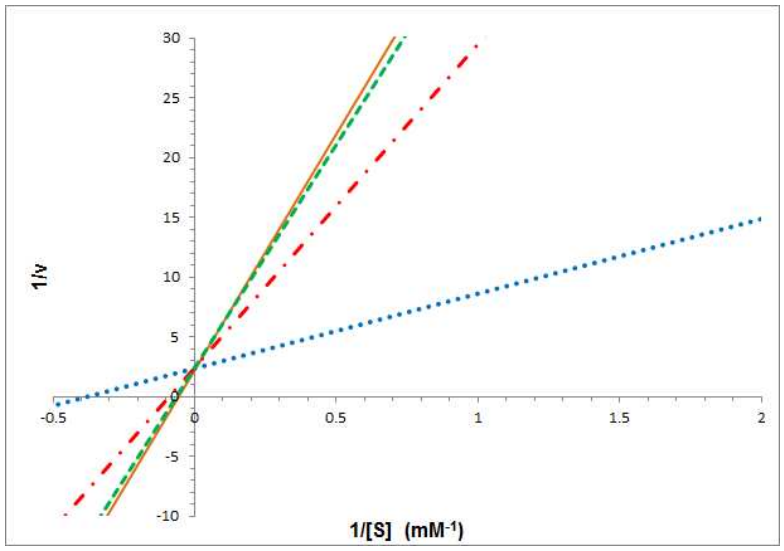


Figure 5 Lineweaver-Burk reciprocal plot for the inhibition of the *exo* β-D-galactofuranosidase from *P. fellutanum* by thiodisaccharide **16** at concentrations: (●●●) 0.00, (—●—) 0.25, (—) 0.50 and (— —) 1.00 mM. Each point is the mean obtained from three replicate experiments.

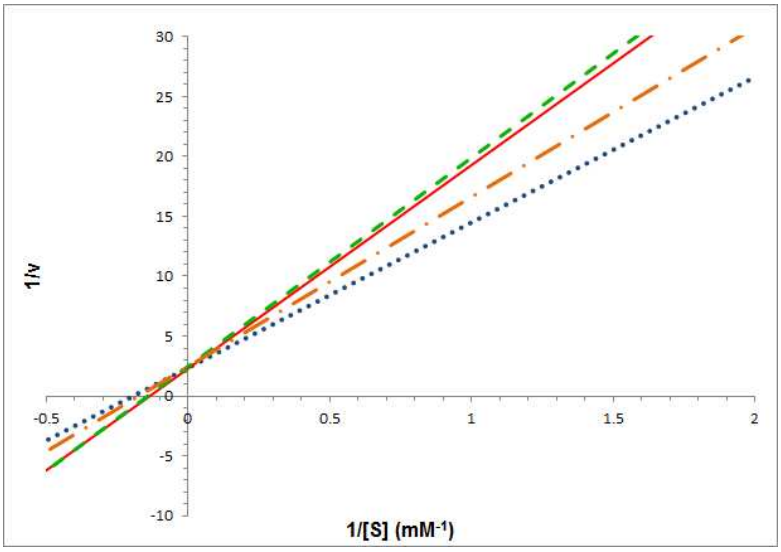


Figure 6 Lineweaver-Burk reciprocal plot for the inhibition of the *exo* β-D-galactofuranosidase from *P. fellutanum* by thiodisaccharide **18** at concentrations: (●●●) 0.00, (—●—) 0.20, (— —) 0.40 and (—) 0.60 mM. Each point is the mean obtained from three replicate experiments.

Conclusions

The regioselective reduction of the methyl glycosides of 5,6-epithio-α-L-altro- (**4**) or β-D-galacto-furanoside (**9**) afforded the corresponding 6-deoxy-5-thio- derivatives **5** and **10** with very good yields. The thiol group of these two key intermediates was glycosylated using the per-*O*-benzoyl-Gal*f* trichloroacetimidate (**11**) as glycosyl donor to afford, after removal of the protective groups of the hydroxyl functions, the glycomimetics of β-D-Gal*f*-(1→5)-D-Gal*f*. This motif is found in many pathogenic microorganisms, including bacteria, fungi and protozoan parasites.

The conformation in solution of the thiodisaccharides was studied using theoretical calculations and confirmation of the minimum energy structures by the presence of characteristic NOE contacts. The *exo*-anomeric effect seems to play an important role in stabilizing the conformation of the thioglycosidic linkage of the 1-thio-β-D-Gal*f*, as all the conformers found satisfied such an effect.

In the design of the thiodisaccharides we took into account that the presence of the non-reducing Gal*f* unit is required for the recognition of the molecule by the β-galactofuranosidase of *P. fellutanum*, a model enzyme which has been isolated and

purified in our laboratory. Therefore, the glycomimetics were evaluated as inhibitors of the enzyme. The kinetics of the inhibition indicated that they are competitive inhibitors. In addition, the inhibitory activity showed to be affected by the inversion of the configuration of the stereocenter linked to sulfur in the reducing end of the thiodisaccharides, being the one that contains the 5-thio-D-Gal*f* unit (the same configuration as the natural substrate) a stronger inhibitor than that with the 5-thio-L-Alt*f* moiety.

Experimental

General methods

The solvents used were distilled, dried and stored according to standard procedures. 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. Chemical shifts (δ) are reported in ppm, with residual chloroform (δ 7.27 for ^{1}H and δ 77.1 for ^{13}C) or acetone (δ 2.16 for ^{1}H and δ 29.8 for ^{13}C) as internal references. Assignments of ^{1}H and ^{13}C NMR spectra were assisted by 2D ^{1}H COSY and HSQC experiments. High resolution mass spectra (HRMS) were obtained by Electrospray Ionization (ESI) and Q-TOF detection. Molecular mechanics (MM+) and semiempirical quantum chemical (AM1) calculations have been performed with Hyperchem Professional 8.0.

Methyl 2,3-di-*O*-*tert*-butyldimethylsilyl-β-D-galactofuranoside (3)

A solution of **2**[7] (603 mg, 1.07 mmol) in dry THF (25 mL) was cooled to −18 °C and $LiAlH_4$ (81 mg, 2.14 mmol) was added. The reaction mixture was stirred at −18 °C for 5 h and the temperature was gradually increased to 0 °C. Analysis by TLC (hexane/EtOAc 7:3) showed a single spot of R_f 0.30. To the mixture were sequentially added EtOAc (15 mL), MeOH (15 mL), and AcOH (to pH 7), and finally the mixture was concentrated. The residue was purified by column chromatography (hexane/EtOAc 3:2) to afford **3** (325 mg, 72%). Physical and spectroscopic data were in agreement with those previously reported.[7]

Methyl 6-deoxy-2,3-di-*O-tert*-butyldimethylsilyl-6-deoxy-5-thio-α-L-altrofuranoside (5)

To a solution of thiirane **4**[7] (214 mg, 0.509 mmol) in dry ethyl ether (10 mL) was added $LiAlH_4$ (32 mg, 0.865 mmol) and the mixture was stirred at room temperature for 3 h. Monitoring by TLC (hexane/EtOAc 97:3) revealed a major spot of R*f* 0.36. After sequential addition of EtOAc (10 mL) and MeOH (10 mL) the mixture was concentrated. The resultant solid was purified by column chromatography (hexane/EtOAc 99:1) to afford **5** (148 mg, 69%); $[\alpha]_D^{25}$ –39.9 (c 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 4.71 (br s, 1H, H-1), 4.00 (dd, $J_{2,3}$ = 1.9, $J_{3,4}$ = 5.2 Hz, 1H, H-3), 3.96 (dd, $J_{1,2}$ = 0.9, $J_{2,3}$ = 1.9 Hz, 1H, H-2), 3.88 (t, $J_{3,4}$ = $J_{4,5}$ = 5,2 Hz, 1H, H-4), 3.35 (s, 3H, CH_3O), 3.11 (ddq, $J_{4,5}$ = 5,2, $J_{5,SH}$ = 7.3, $J_{5,6}$ = 6.9 Hz, 1H, H-5), 1.71 (d, $J_{5,SH}$ = 7.3 Hz, 1H, SH), 1.37 (d, $J_{5,6}$ = 6.9 Hz, 3H, H-6), 0.89, 0.88 [2s, 18 H, $(CH_3)_3CSiMe_2$], 0.10 (x2), 0.09, 0.08 [4s, 12 H, $(CH_3)_2SiBu^t$]; ^{13}C NMR (125.7 MHz, $CDCl_3$) *δ* 109.7 (C-1), 89.8 (C-4), 83.9 (C-2), 80.7 (C-3), 54.8 (CH_3O), 36.5 (C-5), 25.9 [x2, $(CH_3)_3CSiMe_2$], 20.6 (C-6), 18.0 (x2, Me_3CSiMe_2), –3.9, –4.4 (x2), –4.7 [$(CH_3)_2SiBu^t$]; HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{19}H_{42}NaO_4SSi_2$ 445.2235, found 445.2243.

Methyl 6-*O*-acetyl-2,3-di-*O-tert*-butyldimethylsilyl-β-D-galactofuranoside (6)

To a solution of the diol **3** (262 mg, 0.623 mmol) in dry CH_2Cl_2 (5 mL) was added imidazole (53 mg, 0,807 mmol) and acetic anhydride (64 µL, 0.681 mmol). The mixture was stirred at room temperature for 24 h. TLC analysis (hexane/EtOAc 7:3) showed a main spot of R*f* 0.57. The mixture was poured into ice/water (10 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The extract was dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (hexane/EtOAc 9:1) to afford **6** (228 mg, 79%), $[\alpha]^{25}_D$ –28.6 (c 0.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$ with a drop of D_2O) *δ* 4.71 (br s, 1H, H-1), 4.22 (dd, $J_{5,6a}$ = 7.4, $J_{6a,6b}$ = 11.3 Hz, 1H, H-6a), 4.12 (dd, $J_{5,6b}$ = 5.0, $J_{6a,6b}$ = 11.3 Hz, 1H, H-6b), 4.09 (dd, $J_{2,3}$ = 2.9, $J_{3,4}$ = 5.3 Hz, 1H, H-3), 3.99 (dd, $J_{1,2}$ = 1.1, $J_{2,3}$ = 2.9 Hz, 1H, H-2), 3.92 (dd, $J_{3,4}$ = 5.3, $J_{4,5}$= 1.9 Hz, 1H, H-4), 3.87 (ddd, $J_{4,5}$~ 1.9, $J_{5,6a}$ = 7.4, $J_{5,6b}$ = 5.0 Hz, 1H, H-5), 3.34 (s, 3H, CH_3O), 2.09 (s, 3H, CH_3CO), 0.89, 087 [2s, 18 H, $(CH_3)_3CSiMe_2$], 0.10, 0.09, 0.07 [3s, 12 H, $(CH_3)_2SiBu^t$]; ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 171.2 (CO), 109.8 (C-1), 83.9 (C-4), 83.2 (C-2), 79.4 (C-3), 68.2 (C-5), 66.4 (C-6), 55.1 (CH_3O), 25.9, 25.8 [$(CH_3)_3CSiMe_2$], 21.1 (CH_3CO), 18.0 (x2, Me_3CSiMe_2), –4.2, –4.5 (x2), –4.8 [$(CH_3)_2SiBu^t$]; HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{21}H_{44}NaO_7Si_2$ 487.2518, found 487.2528.

Methyl 6-*O*-acetyl-2,3-di-*O*-*tert*-butyldimethylsilyl-5-*O*-tosyl-β-D-galactofuranoside (7)

To a solution of **6** (196 mg, 0.422 mmol) in dry pyridine (10 mL) was added tosyl chloride (646 mg, 3.4 mmol). The reaction mixture was stirred at room temperature for 3 days. Analysis by TLC (hexane/EtOAc 4:1) showed a major product of R*f* 0.54. The mixture was diluted with MeOH (15 mL) and the syrup obtained after evaporation of the solvent was dissolved in CH_2Cl_2 (15 mL). The organic layer was washed with water (20 mL), dried ($MgSO_4$), and concentrated. The resulting residue was purified by column chromatography (hexane/EtOAc 9:1) to afford **7** (211 mg, 81%), $[\alpha]^{25}_D$–12.0 (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.82 (d, *J* = 8.2 Hz, 2H, H-aromatic), 7.31 (d, *J* = 8.2 Hz, 2H, H-aromatic), 4.93 (dt, $J_{5,6a}$ = 3.7, $J_{5,6b}$ = 7.5 Hz, 1H, H-5), 4.57 (d, $J_{1,2}$ = 1.1 Hz, 1H, H-1), 4.31 (dd, $J_{5,6a}$ = 3.7, $J_{6a,6b}$ = 12.3 Hz, 1H, H-6a), 4.16 (dd, $J_{5,6b}$ = 7.5, $J_{6a,6b}$ = 12.3 Hz, 1H, H-6b), 4.06–4.02 (m, 2H, H-3,4), 3.94 (dd, $J_{1,2}$ = 1.1, $J_{2,3}$ = 1.8 Hz, 1H, H-2), 3.26 (s, 3H, CH_3O), 2.43 (s, 3H, C*H*$_3$Ar), 1.94 (s, 3H, CH_3CO), 0.87 [2s, 18 H, (C*H*$_3$)$_3$CSiMe$_2$], 0.10, 0.09, 0.08, 0.06 [4s, 13 H, (C*H*$_3$)$_2$SiBu^t]; ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 170.5 (CO), 144.7, 134.4, 129.7, 128.2 (C-aromatic), 109.7 (C-1), 83.9 (C-2), 83.2 (C-4), 79.5 (C-3), 78.0 (C-5), 63.1 (C-6), 55.1 (*C*H$_3$O), 25.9, 25.8 [(*C*H$_3$)$_3$CSiMe$_2$], 21.75 (*C*H$_3$Ar), 20.75 (*C*H$_3$CO), 18.0, 17.9 (Me$_3$*C*SiMe$_2$), –4.20, –4.4, –4.7, –4.8 [(*C*H$_3$)$_2$SiBu^t]; HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{28}H_{50}NaO_9SSi_2$ 641.2606, found 641.2591.

Methyl 5,6-anhydro-2,3-di-*O*-*tert*-butyldimethylsilyl-α-L-altrofuranoside (8)

A solution of **7** (211 mg, 0.341 mmol) in CH_2Cl_2 (8 mL) was cooled at 0 °C, and 2 M NaOMe/MeOH (1.9 mL) solution was added. The mixture was stirred at 0 °C for 2 h, when TLC analysis (hexane/EtOAc, 9:1) showed a main product of R*f* 0.72. The mixture was diluted with CH_2Cl_2 (15 mL) and washed with water (2 × 20 mL). The organic extract was dried ($MgSO_4$) and concentrated and the residue was purified by column chromatography (hexane/EtOAc 95:5) to afford **8** (125 mg, 91%). Physical and spectroscopic data were in agreement with those previously reported.[7]

Methyl 6-deoxy-2,3-di-*O*-*tert*-butyldimethylsilyl-6-deoxy-5-thio-β-D-galactofuranoside (10)

The thiirane **9**[7] (113 mg, 0.269 mmol) was reduced as already described for **4**, to afford **10** (87 mg, 77%), R*f* 0.38 (hexane/EtOAc 97:3), $[\alpha]_D^{25}$–64.0 (c 1, $CHCl_3$); ^{1}H NMR (500 MHz,

$CDCl_3$) δ 4.68 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.04 (dd, $J_{2,3}$ = 3.7, $J_{3,4}$ = 6.7 Hz, 1H, H-3), 3.99 (dd, $J_{1,2}$ = 1.7, $J_{2,3}$ = 3.7 Hz, 1H, H-2), 3.80 (dd, $J_{3,4}$ = 6.7, $J_{4,5}$ = 3.8 Hz, 1H, H-4), 3.35 (s, 3H, CH_3O), 3.04 (ddq, $J_{4,5}$ = 3.8, $J_{5,SH}$ = 8.1, $J_{5,6}$ = 7.0 Hz, 1H, H-5), 1.74 (d, $J_{5,SH}$ = 8.1 Hz, 1H, SH), 1.43 (d, $J_{5,6}$ = 7.0 Hz, 3H, H-6), 0.89, 0.87 [2s, 18 H, $(CH_3)_3CSiMe_2$], 0.10, 0.09, 0.08 (x2) [4s, 12 H, $(CH_3)_2SiBu^t$]; ^{13}C NMR (125.7 MHz, $CDCl_3$) *δ* 109.2 (C-1), 86.4 (C-4), 84.4 (C-2), 80.8 (C-3), 55.0 (CH_3O), 36.5 (C-5), 26.0, 25.9 [$(CH_3)_3CSiMe_2$], 23.3 (C-6), 18.0 (x 2, Me_3CSiMe_2), –3.9, –4.3, –4.4, –4.7 [$(CH_3)_2SiBu^t$]; HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{19}H_{42}NaO_4SSi_2$ 445.2235, found 445.2252.

Methyl 2,3-di-*O*-*tert*-butyldimethilsilyl-5-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-6-deoxy-5-thio-α-L-altrofuranoside (12), methyl 2-*O*-*tert*-butyldimethilsilyl-5-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-6-deoxy-5-thio-α-L-altrofuranoside (13a), methyl 3-*O*-*tert*-butyldimethilsilyl-5-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-6-deoxy-5-thio-α-L-altrofuranoside (13b) and methyl 5-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-6-deoxy-5-thio-α-L-altrofuranoside (14)

A suspension of the thiol **5** (66 mg, 0.16 mmol), the trichloroacetimidate **11**[23] (134 mg, 0.19 mmol), and freshly activated powdered molecular sieves (4 Å) in dry CH_2Cl_2 (7 mL) was stirred at room temperature for 1.5 h. The mixture was then cooled to –20 °C, and TMSOTf (12 µL, 0.07 mmol) was added. After 1 h, TLC analysis (toluene/EtOAc 93:7) showed complete consumption of the starting thiol **5** and the presence of three spots having R_f 0.60, 0.26 and 0.00. The latter exhibited R_f 0.58 in a more polar solvent (hexane/EtOAc 3:7). The Lewis acid was neutralized upon addition of Et_3N (5 µL) and the mixture was poured into water (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic extract was dried ($MgSO_4$), concentrated, and the residue was purified by column chromatography (toluene/EtOAc 60:1) to afford the following thiodisaccharides:

Compound **12** was isolated as a syrup (6 mg, 4%); $[\alpha]_D^{25}$ –53.1 (c 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) *δ* 8.10–7.87 (H-aromatic), 7.60–7.27 (H-aromatic), 6.10 (dt, $J_{4',5'} = J_{5',6'a} \sim 4.5$, $J_{5',6'b}$ = 7.0 Hz, 1H, H-5'), 5.80 (br s, 1H, H-1'), 5.64 (dd, $J_{2',3'}$ = 1.2, $J_{3',4'}$ = 5.2 Hz, 1H, H-3'), 5.52 (t, $J_{1',2'} = J_{2',3'}$ = 1.2, 1H, H-2'), 4.87 (dd, $J_{3',4'}$ = 5.2, $J_{4',5'}$ = 4.5 Hz, 1H, H-4'), 4.74 (dd, $J_{5',6'a}$ = 4.8, $J_{6'a,6'b}$ = 11.8 Hz, 1H, H-6'a), 4.72 (br s, 1H, H-1), 4.71 (dd, $J_{5',6'b}$ = 7.0, $J_{6'a,6'b}$ = 11.8 Hz, 1H, H-6'b), 4.09 (dd, $J_{3,4}$ = 5.6, $J_{4,5}$= 4.3 Hz, 1H, H-4), 4.01 (dd, $J_{2,3}$ = 2.6, $J_{3,4}$ = 5.6 Hz, 1H, H-3), 3.99 (dd, $J_{1,2}$ = 1.1, $J_{2,3}$ = 2.6 Hz, 1H, H-2), 3.34 (s, 3H, CH_3O), 3.31 (m,

1H, H-5), 1.38 (d, $J_{5,6}$ = 7.1 Hz, 3H, H-6), 0.87, 0.87 [2s, 19 H, $(CH_3)_3CSiMe_2$], 0.1, 0.09, 0.08, 0.07 [4s, 14 H, $(CH_3)_2SiBu^t$]; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.2, 165.8, 165.7, 165.3 (PhCO), 133.6–128.5 (C-aromatic), 109.6 (C-1), 89.1 (C-1'), 86.7 (C-4), 84.3 (C-2), 83.1 (C-2'), 81.2 (C-4'), 80.8 (C-3), 78.1 (C-3'), 70.4 (C-5'), 63.6 (C-6'), 54.9 (CH_3O), 42.0 (C-5), 25.9 [×2, $(CH_3)_3CSiMe_2$], 18.0 (Me_3CSiMe_2), 17.1 (C-6), –4.0, –4.4 (×2), –4.7 [$(CH_3)_2SiBu^t$]; HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{53}H_{68}NaO_{13}SSi_2$ 1023.3811, found 1023.3808.

Compound **13a** and **13b** were isolated as an inseparable mixture (27 mg, 20%, ratio **13a**:**13b** ~1:3); they showed the following ^{1}H and ^{13}C NMR spectra (only sugar signals are reported).

Compound **13a**: ^{1}H NMR (500 MHz, $CDCl_3$), the following signals have been identified: δ 5.69 (d, $J_{3',4'}$ = 5.6 Hz, 1H, H-3'), 5.37 (t, $J_{1',2'} = J_{2',3'}$ = 1.3 Hz, 1H, H-2'), 4.14 (dd, 1H, $J_{3,4}$ = 5.3, $J_{4,5}$ = 2.9 Hz, 1H, H-4), 4.19 (dd, $J_{2,3}$ = 1.7, $J_{3,4}$ = 5.3 Hz, 1H, H-3), 3.98 (d, $J_{2,3}$ = 1.7, 1H, H-2), 3.53 (m, 1H, H-5), 3.30 (s, 3H, CH_3O), 1.49 (d, $J_{5,6}$ = 7.5 Hz, 3H, H-6); ^{13}C NMR (126 MHz, $CDCl_3$) δ 109.1 (C-1), 90.0 (C-1'), 89.1 (C-4), 84.0 (C-2'), 83.1 (C-2), 80.8 (C-4'), 80.1 (C-3), 77.5 (C-3'), 70.2 (C-5'), 63.8 (C-6'), 54.8 (CH_3O), 41.2 (C-5), 18.1 (C-6).

Compound **13b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.11 (m, 1H, H-5), 5.94 (s, 1H, H-1'), 5.65 (d, $J_{3,4}$ = 5.2 Hz, 1H, H-3'), 5.52 (t, $J_{1',2'} = J_{2',3'}$ = 1.3 Hz, 1H, H-2'), 4.90 (dd, 1H, $J_{3',4'}$ = 4.8, $J_{4',5'}$ = 3.9 Hz, H-4'), 4.81-4.77 (m, 2H, H-1, H-6'), 4.71 (dd, $J_{6a,6b}$ = 11.9, $J_{5,6a}$ = 7.3 Hz, 1H, H-6a), 4.10 (m, 1H, H-3), 4.06-4.03 (m, 2H, H-2, H-4), 3.34 (s, 3H, CH_3O), 3.16 (dq, $J_{4,5}$ = 8.6, $J_{5,6}$ = 7.0 Hz, 1H, H-5), 1.49 (d, $J_{5,6}$ = 7.0 Hz, 3H, H-6); ^{13}C NMR (126 MHz, $CDCl_3$) δ 109.4 (C-1), 89.6 (C-4), 87.7 (C-1'), 83.2 (C-2'), 81.6 (C-2), 81.4 (C-4'), 80.2 (C-3), 78.0 (C-3'), 70.4 (C-5'), 63.8 (C-6'), 54.9 (CH_3O), 43.5 (C-5), 18.3 (C-6).

Compound **14** was isolated as a syrup (22 mg, 18%); $[\alpha]_D^{25}$ –61.1 (c 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04–7.84 (H-aromatic), 7.61–7.25 (H-aromatic), 6.11 (ddd, $J_{4',5'}$= 3.5, $J_{5',6'a}$ = 4.7, $J_{5',6'b}$ = 7.0 Hz, Hz, 1H, H-5'), 5.99 (br s, 1H, H-1'), 5.69 (d, $J_{3',4'}$ = 5.4 Hz, 1H, H-3'), 5.36 (d, $J_{1',2'}$ = 1.1 Hz, 1H, H-2'), 4.88 (br s, 1H, H-1), 4.86 (m, 1H, H-4'), 4.79 (dd, $J_{5',6'a}$ = 4.7, $J_{6'a,6'b}$ = 11.8 Hz, 1H, H-6'a), 4.74 (dd, $J_{5',6'b}$ = 7.0, $J_{6'a,6'b}$ = 11.8 Hz, 1H, H-6'b), 4.09 (dd, $J_{3,4}$ = 3.7, $J_{4,5}$ = 4.7 Hz, 1H, H-4), 4.06 (br d, $J_{2,3}$ = 1.0 Hz, 1H, H-2), 4.03 (dd, $J_{2,3}$ = 1.0, $J_{3,4}$ = 3.7 Hz, 1H, H3), 3.44 (dq, $J_{4,5}$ = 4.7, $J_{5,6}$ = 7.4 Hz, 1H, H-5), 3.34 (s, 3H, CH_3O), 1.37 (d, $J_{5,6}$ = 7.4 Hz, 3H, H-6); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.3, 165.9, 165,9, 165.7 (PhCO), 133.8–128.5 (C-aromatic), 108.6 (C-1), 90.2 (C-4), 89.3 (C-1'), 83.5 (C-2'), 81.2 (C-2), 80.9 (C-4'), 78.8 (C-3), 77.6 (C-3'), 70.2 (C-5'), 63.5 (C-6'), 54.8 (CH_3O), 41.3

(C-5), 18.3 (C-6); HRMS (ESI) m/z [M + Na]+ calcd for $C_{41}H_{40}NaO_{13}S$ 795.2082, found 795.2092.

*An alternative procedure for the synthesis of **14**:* The crude mixture of **12**, **13a,b** and **14**, obtained as described in the previous item, starting from the thiol **5** (66 mg, 0.156 mmol) and the trichloroacetimidate **11** (134 mg, 0.187 mmol), was dissolved in THF (6 mL) and TBAF (300 mg, 1.1 mmol) was added. The mixture was stirred overnight at room temperature. Monitoring by TLC (hexane/EtOAc 3:7) showed a main spot of R_f 0.58. The mixture was concentrated and the residue was dissolved in CH_2Cl_2; the resulting solution was washed with water, dried ($MgSO_4$) and the residue was purified by column chromatography (hexane/EtOAc 3:2) to afford syrupy **14** (54 mg, 45% from **5**). The thiodisaccharide **14** showed the same physical and spectroscopic as the compound previously described.

Methyl 2,3-di-*O*-acetyl-5-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-6-deoxy-5-thio-α-L-altrofuranoside (15)

To a solution of the thiodisaccharide **14** (60 mg, 0.078 mmol) in dry pyridine (0.5 mL) was added acetic anhydride (0.5 mL). The mixture was stirred at room temperature for 18 h, and then diluted with MeOH (5 mL) and, after stirring for 1 h, it was concentrated. The syrup obtained was dissolved in CH_2Cl_2 (5 mL), washed with water (10 mL), dried ($MgSO_4$) and concentrated. The resulting residue was purified by column chromatography (toluene/EtOAc 9:1) to afford **15** (49 mg, 75%); R_f 0.54 (toluene/EtOAc 85:15); $[\alpha]_D^{25}$ –84.7 (c 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ8.10–7.87 (m, H-aromatic),7.61–7.27 (m, H-aromatic), 6.11 (dt, $J_{4',5'}$ = $J_{5',6'a}$~ 4.3, $J_{5',6'b}$ = 7.1Hz, 1H, H-5'), 5.90 (s, 1H, H-1'), 5.65 (d, $J_{3',4'}$ = 4.3 Hz, 1H, H-3'), 5.53 (br s, 1H, H-2'), 5.21 (dd, $J_{2,3}$ = 1.1, $J_{3,4}$ = 5.6 Hz, 1H, H-3), 4.98 (d, $J_{2,3}$ = 1.1 Hz, 1H, H-2), 4.88 (s, 2H, H-1), 4.85 (t, $J_{3',4'}$ = $J_{4',5'}$ = 4.3 Hz, 1H, H-4'), 4.78 (dd, $J_{6'a,6'b}$ = 11.8, $J_{5',6'a}$ = 4.4 Hz, 1H, H-6'a), 4.73 (dd, $J_{6'a,6'b}$ = 11.8, $J_{5',6'b}$ = 7.1 Hz, 1H, H-6'b), 4.29 (dd, $J_{3,4}$ = 5.6, $J_{4,5}$ = 4.5 Hz, 1H, H-4), 3.47 (qd, $J_{4,5}$ = 4.5, $J_{5,6}$ = 7.2 Hz, 1H, H-5), 3.35 (s, 3H, CH_3O), 2.08 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 1.33 (d, $J_{5,6}$ = 7.2 Hz, 3H, H-6); ^{13}C NMR (126 MHz, $CDCl_3$) δ170.2, 170.1 (*C*H_3CO), 166.2, 165.9, 165.6, 165.3 (PhCO), 133.8–128.5 (C-aromatic), 106.4 (C-1), 89.2 (C-1'), 85.0 (C-4), 83.0 (C-2'), 82.2 (C-2), 81.5 (C-4'), 78.0 (C-3'), 77.7 (C-3), 70.3 (C-5'), 63.6 (C-6'), 54.9 (*C*H_3O), 41.6 (C-5), 21.0, 20.9 (*C*H_3CO), 17.3 (C-6); HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{45}H_{44}NaO_{15}S$ 879.2293, found 879.2293.

Methyl 5-*S*-(β-D-galactofuranosyl)-6-deoxy-5-thio-α-L-altrofuranoside (16)

Compound **14** (54 mg, 0.07 mmol) was dissolved in CH_2Cl_2 (12 mL) and 10 mM NaOMe/MeOH solution (6 mL) was added. The mixture was stirred at room temperature for 45 min, when TLC (*n*BuOH/EtOH/H_2O 2.5:1:1) showed a main spot of R_f 0.63. The reaction mixture was concentrated and the residue was dissolved in H_2O (1 mL), was passed through a column filled with Dowex 50X, which washed with water. The eluate was concentrated, dissolved in H_2O (1 mL) and filtered through an octadecyl C18 minicolumn, to afford **16** (23 mg, 41% from **5**); $[\alpha]_D^{25}$–182.7 (c 1, CH_3OH); ^{1}H NMR (500 MHz, D_2O) *δ* 5.28 (d, $J_{1',2'}$ = 5.4 Hz, 1H, H-1'), 4.93 (d, $J_{1,2}$ = 1.4 Hz, 1H, H-1), 4.12 (dd, $J_{2',3'}$ = 5.4, $J_{3',4'}$ = 7.5 Hz, 1H, H-3'); 4.10–4.07 (m, 2H, H-3, H-4), 4.05 (dd, $J_{1,2}$ = 1.4, $J_{2,3}$ = 2.6 Hz, 1H, H-2), 4.02 (t, $J_{1',2'}$ = $J_{2',3'}$ = 5.4 Hz, 1H, H-2'), 3.99 (dd, $J_{3',4'}$ = 7.5, $J_{4',5'}$ = 3.5 Hz, 1H, H-4'), 3.83 (ddd, $J_{4',5'}$ = 3.5, $J_{5',6'a}$ = 4.7, $J_{5',6'b}$ = 7.5 Hz, 1H, H-5'), 3.68 (dd, $J_{5',6'a}$ = 4.7, $J_{6'a,6'b}$ = 11.7 Hz, 1H, H-6'a), 3.64 (dd, $J_{5',6'b}$ = 7.5, $J_{6'a,6'b}$ = 11.7 Hz, 1H, H-6'b), 3.40 (s, 3H, CH_3O), 3.34 (dq, $J_{4,5}$ = 4.5, $J_{5,6}$ = 7.2 Hz, 1H, H-5), 1.37 (d, $J_{5,6}$ = 7.2 Hz, 3H, H-6); ^{13}C NMR (126 MHz, D_2O) *δ* 107.7 (C-1), 87.6 (C-1'), 86.3 (C-4), 80.9 (C-4'), 80.7 (C-2'), 80.5 (C-2), 77.2 (C-3), 75.5 (C-3'), 70.0 (C-5'), 62.3 (C-6'), 54.4 (CH_3O), 41.2 (C-5), 16.5 (C-6); HRMS (ESI) m/z [M + Na]$^+$ calcd for $C_{13}H_{24}NaO_9S$ 379.1033, found 379.1041.

Methyl 5-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-6-deoxy-5-thio-β-D-galactofuranoside (17)

The glycosylation of the thiol **10** (60 mg, 0.14 mmol) with the trichloroacetimidate **11** (120 mg, 0.17 mmol) was conducted as already described for the preparation of **15**. The crude mixture containing the analogues of **12**-**14** was treated with TBAF (300 mg, 1.1 mmol) in THF (6 mL). The usual workup and purification afforded **17** (48 mg, 39% from **10**); $[\alpha]_D^{25}$–87.8 (c 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 8.13–7.82 (H-aromatic), 7.60–7.17 (H-aromatic), 6.06 (ddd, $J_{4',5'}$= 3.6, $J_{5',6'a}$ = 4.5, $J_{5',6'b}$ = 6.4 Hz, 1H, H-5'), 5.82 (br s, 1H, H-1'), 5.67 (dd, $J_{2',3'}$ = 1.3, $J_{3',4'}$ = 5.2 Hz, 1H, H-3'), 5.51 (t, $J_{1',2'}$ = $J_{2',3'}$ = 1.3 Hz, 1H, H-2'), 4.90 (dd, $J_{3',4'}$ = 5.2, $J_{4',5'}$ = 3.6 Hz, 1H, H-4'), 4.85 (br s, 1H, H-1), 4.81 (dd, $J_{5',6'a}$ = 4.5, $J_{6'a,6'b}$ = 11.9 Hz, 1H, H-6'a), 4.74 (dd, $J_{5',6'b}$ = 6.4, $J_{6'a,6'b}$ = 11.9 Hz, 1H, H-6'b), 4.07 (dd, $J_{3,4}$ = 4.4, $J_{4,5}$ = 3.2 Hz, 1H, H-4), 4.03 (br s, 1H, H-3), 3.99 (br s, 1H, H-2), 3.36 (s, 3H, CH_3O), 3.31 (dq, $J_{4,5}$ = 3.2, $J_{5,6}$ = 7.1 Hz, 1H, H-5), 1.54 (d, $J_{5,6}$ = 7.1 Hz, 3H, H-6); ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 166.6, 165.9, 165,7, 165.7 (Ph*C*O), 133.9–128.5 (C-aromatic),

108.9 (C-1), 89.1 (C-4), 88.1 (C-1'), 83.1 (C-2'), 81.7 (C-4'), 81.4 (C-2), 80.3 (C-3), 77.9 (C-3'), 70.5 (C-5'), 63.6 (C-6'), 55.0 (CH_3O), 43.3 (C-5), 20.3 (C-6); HRMS (ESI) m/z [M + Na]$^+$ calcd for $C_{41}H_{40}NaO_{13}S$ 795.2081, found 795.2092.

Methyl 5-*S*-(β-D-galactofuranosyl)-6-deoxy-5-thio-β-D-galactofuranoside (18)

The thiol **10** (50 mg, 0.12 mmol) was subjected to glycosylation with the trichloroacetimidate **11** (107 mg, 0.145 mmol) and subsequent deprotection reactions, as already described for **16**, to afford **18** (15 mg, 36%); R_f 0.60 (*n*BuOH/EtOH/H_2O 2.5:1:1); $[\alpha]_D^{25}$ –104.8 (c 1.0, CH_3OH); ^{1}H NMR (500 MHz, D_2O) δ 5.26 (d, $J_{1',2'}$ = 4.9 Hz, 1H, H-1'), 4.90 (d, $J_{1,2}$ = 1.7 Hz, 1H, H-1), 4.12 (dd, $J_{2',3'}$ = 5.3, $J_{3',4'}$ = 7.4 Hz, 1H, H-3'), 4.10 (dd, $J_{2,3}$ = 3.2, $J_{3,4}$ = 6.3 Hz, 1H, H-3), 4.06-4.03 (m, H-2, H-2'), 4.03 (dd, $J_{3,4}$ = 6.3, $J_{4,5}$ = 4.6 Hz, 1H, H-4), 4.00 (dd, $J_{3',4'}$ = 7.4, $J_{4',5'}$ = 3.5 Hz, 1H, H-4'), 3.85 (ddd, $J_{4',5'}$ = 3.5, $J_{5',6'a}$ = 4.6, $J_{5',6'b}$ = 7.5 Hz, 1H, H-5'), 3.69 (dd, $J_{5',6'a}$ = 4.6, $J_{6'a,6'b}$ = 11.6 Hz, 1H, H-6'a), 3.64 (dd, $J_{5',6'b}$ = 7.5, $J_{6'a,6'b}$ = 11.6 Hz, 1H, H-6'b), 3.41 (s, 3H, CH_3O), 3.25 (dq, $J_{4,5}$ = 4.6, $J_{5,6}$ = 7.1 Hz, 1H, H-5), 1.46 (d, $J_{5,6}$ = 7.1 Hz, 3H, H-6); ^{13}C NMR (126 MHz, D_2O) δ 107.6 (C-1), 86.9 (C-1'), 85.6 (C-4), 81.1 (C-2), 80.8 (C-4'), 80.6 (C-2'), 77.5 (C-3), 75.8 (C-3'), 69.9 (C-5'), 62.3 (C-6'), 54.5 (CH_3O), 41.4 (C-5), 18.1 (C-6); HRMS (ESI) m/z [M + Na]$^+$ calcd for $C_{13}H_{24}NaO_9S$ 379.1033, found 379.1034.

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.[25] The standard assay was conducted with 50 μL of 66 mM NaOAc buffer (pH4.6), 20 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 **16** and **18** were incorporated in the amounts required to obtain a final concentration of 0.1 to 1.6 mM. The enzymatic reaction was stopped after 1.5 h of incubation at 37 ºC by addition of 1mL 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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