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Thiodisaccharides with galactofuranose or arabinofuranose as terminal units: Synthesis and inhibitory activity of an *exo* β-D-galactofuranosidase

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ABSTRACT

Thiodisaccharides having β-D-Gal*f* or α-L-Ara*f* units as non-reducing end have been synthesized by the $SnCl_4$- or MoO_2Cl_2-promoted thioglycosylation of per-*O*-benzoyl-D-galactofuranose (**1**), its 1-*O*-acetyl analogue **4**, or per-*O*-acetyl-α-L-arabinofuranose (**16**) with 6-thioglucose or 6-thiogalactose derivatives. After convenient removal of the protecting groups, the free thiodisaccharides having the basic structure β-D-Gal*f*(1→6)-6-thio-α-D-Glc*p*-OMe (**5**) or β-D-Gal*f*(1→6)-6-thio-α-D-Gal*p*-OMe (**15**) were obtained. The respective α-L-Ara*f* analogues **18** and **20** were prepared similarly from **16**. Alternatively, β-D-Gal*f*(1→4)-4-thio-3-deoxy-α-L-Xyl*p*-OiPr was synthesized by Michael addition to a sugar enone of 1-thio-β-D-Gal*f* derivative, generated in situ from the glycosyl isothiourea derivative of **1**. The free *S*-linked disaccharides were evaluated as inhibitors of the β-galactofuranosidase from *Penicillium fellutanum*, being **15** and **20** the more active inhibitors against this enzyme.

1. Introduction

Carbohydrates play important roles in recognition events that initiate immunological responses to bacterial and viral infections and in signaling processes that occur in inflammation and cancer metastasis.[1] Carbohydrate mimetics, which are useful to investigate these processes,[2] are usually prepared by replacing oxygen atoms in a sugar with carbon atoms or other heteroatoms. These modified molecules generally show altered binding properties or increased stability toward enzyme degradation.[3]

Oligosaccharide analogues that are resistant toward enzymatic hydrolysis may act as glycosidase inhibitors. In this regard, thioglycosides are particularly valuable, as they are tolerated by most biological systems and they are usually resistant to metabolic processes.[4] The potential of thiooligosaccharides as therapeutics prompted the investigation about their synthesis and the study of their biological activities.[4,5] In recent years, we have directed our efforts toward the synthesis and inhibitory activities of this type of molecules,[6] in particular thiodisaccharides that possess a furanose unit as non-reducing end. Thus, we have recently reported successful approaches to prepare *S*-disaccharides of 1-thiopentofuranoses.[7]

Among the furanoses, arabinofuranose and galactofuranose are widespread in nature. Polysaccharides containing galactofuranosyl and arabinofuranosyl residues are key components of many microorganisms. Arabinofuranose is found in soil bacteria, fungi and plants,[8] and galactofuranose occurs in glycoconjugates of many pathogenic bacteria, fungi, and protozoan parasites and are highly immunogenic.[9] Both sugars are present in the mycolyc acid-arabinogalactan-peptidoglycan (mAGP) complex from *Mycobacterium tuberculosis*.[10] The fact that Gal*f* is essential for the survival or virulence of various pathogenic bacteria,[9c,11] but is absent in higher eukaryotes, has attracted increasing interest in its biosynthetic pathways.[11,12] Degradation of Gal*f* containing glycoconjugates is promoted in some microorganisms by a β-D-galactofuranosidase. The best known is the specific exo β-D-galactofuranosidase first purified from the culture medium of *Penicillium fellutanum*[13] and later described in *Helminthosporium sacchari*[14] and *Penicillium* and *Aspergillius* species.[15,16] The role of this enzyme in *P. fellutanum* is the release of galactose as carbon source by depolymerization of an extracellular glycopeptide during the fungus growth.[13]

The β-D-galactofuranosidase is an interesting target considering that during differentiation of *Trypanosoma cruzi* from the invasive stage to the infective one, the amount of glycoconjugates containing Gal*f* units is dramatically diminished.[17] We have previously described the synthesis of substrates,[18] inhibitors[19] and an affinity purification method[20] of the exo β-D-galactofuranosidase from *P. fellutanum*. These developments, in combination with other tools, allowed the detection for the first time of β-D-galactofuranosidase activity in *T. cruzi*.[21] As inhibitors we have synthesized aryl and heteroaryl 1-thio-β-D-galactofuranosides.[19] Alkyl 1-thio-β-D-galactofuranosides and the corresponding sulfones were also prepared and tested as inhibitors of mycobacteria growth.[22] Furthermore,

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in *P. fellutanum*, 4-aminophenyl thiogalactofuranoside proved to induce dramatic modifications during the mycelia growth.[23]

In line with these studies, we decided to investigate about the synthesis and potential inhibitory activity of thiodisaccharides containing a Gal*f* unit, as such compounds have not been previously prepared. Therefore, we report here the synthesis of thiodisaccharides having terminal β-D-Gal*f* or α-L-Ara*f* units. These compounds have been evaluated as inhibitors of the β-galactofuranosidase from *P. fellutanum*.

2. Results and discussion

2.1. Synthesis and chemical characterization

The per-*O*-benzoyl derivative of Gal*f* (**1**), readily prepared by high temperature benzoylation of D-galactose,[24] was employed as precursor of the galactofuranosyl non-reducing end of the thiodisaccharides. The thiosugar component used as thiol donor was, in first instance, methyl 2,3,4-tri-*O*-benzoyl 6-thio-α-D-glucopyranoside (**2**).[25] The thioglycosylation of **1** with the thiosugar **2**, using MoO_2Cl_2 as neutral catalyst, led to low yields of the expected thiodisaccharide **3** (Scheme 1). This type of reaction has been successfully applied for analogous thioglycosylations of 1-*O*-acetyl pyranoses[26] or 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose.[7] The failure in the MoO_2Cl_2-catalyzed reaction between **1** and **2** was attributed to the fact that **1** possesses a benzoyloxy group as anomeric substituent, instead of an acetyloxy, as in the cited examples. Therefore, the synthesis of the 1-*O*-acetyl-per-*O*-benzoyl-Gal*f* from **1** was attempted. For the activation of the anomeric center of **1**, tin(IV) chloride was employed. This Lewis acid proved to be adequate for the in situ anomeric activation of per-*O*-acyl furanoses, which underwent further glycosylation with conveniently protected aldonolactones,[18a,27] or with aryl or heteroaryl thiols.[19] Thus, compound **1** was treated with $SnCl_4$ and, upon addition of acetic anhydride, the expected 1-*O*-acetyl derivative **4** was obtained (~80% yield). The NMR spectra of the crude product indicated that the β anomer was the major component. Integration of the area of the H-1 signal (β anomer: 6.52 ppm, $J_{1,2}$ < 1.0 Hz; α anomer: 6.66 ppm, $J_{1,2}$ = 4.8 Hz) gave a 5:1 β:α ratio. A similar relationship was determined from the ^{13}C NMR spectrum, which showed in the anomeric region the resonance of C-1 at 99.4 ppm (β anomer) and 93.4 ppm (α anomer). Recrystallization of the crude product from EtOH afforded the β anomer as a single diastereoisomer.

With the Gal*f* derivative **4** in hand, the reaction with **2** was conducted using MoO_2Cl_2 as catalyst. In this case, the thiodisaccharide **3** was obtained in a good yield. As previously observed,[26] the MoO_2Cl_2-promoted thioglycosylation took place under diastereocontrol with selective formation of the 1,2-*trans*-thioglycosidic linkage, as established by the NMR data of **3**. Thus, the small coupling constant value between H-1 and H-2 ($J_{1,2}$ < 1.0 Hz) and the low field resonance of C-1,[18] confirmed the β anomeric configuration for the furanose moiety of **3**.

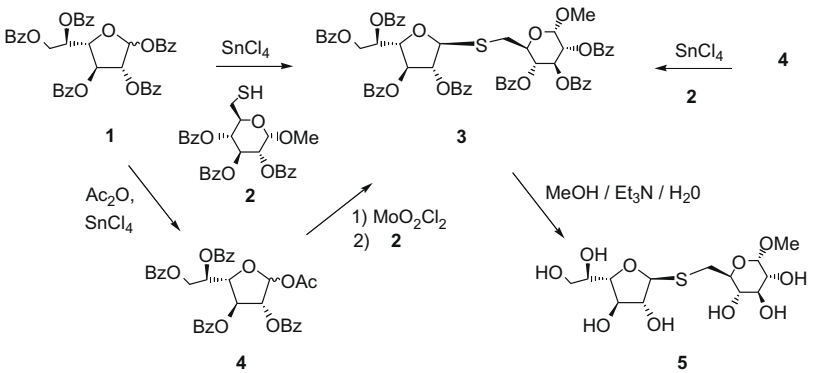
As $SnCl_4$ promotes successfully glycosylations[18a,19,27] or acetolysis of **1**, this Lewis acid was employed for thioglycosylations as an alternative to the neutral species MoO_2Cl_2. As expected, reaction **1** with **2**, in the presence of $SnCl_4$, led to the thiodisaccharide **3**, in a good yield. Similarly, the $SnCl_4$-promoted condensation of the glycosyl donor **4** with **2** led to **3** in a yield comparable to that of the previous reaction. Because of the anchimeric assistance of the benzoyloxy group at C-2 of **1** or **4**, the reaction was highly diasteroselective in favor of the β anomeric configuration for the *S*-linkage in **3**.

The per-*O*-benzoyl-*S*-linked disaccharide **3** was *O*-debenzoylated with a mixture of MeOH/Et_3N/H_2O (3:1:3) to give the free thiodisaccharide **5**. This is the thioanalogue of the motif β-D-Gal*f*(1→6)-D-Glc*p* found in the lipopolysaccharide O-antigen of *Escherichia coli* K-12.[9d,28] The Gal*f* containing glycoconjugates appeared to be essential for virulence in pathogenic Gram-negative bacteria.[9d]

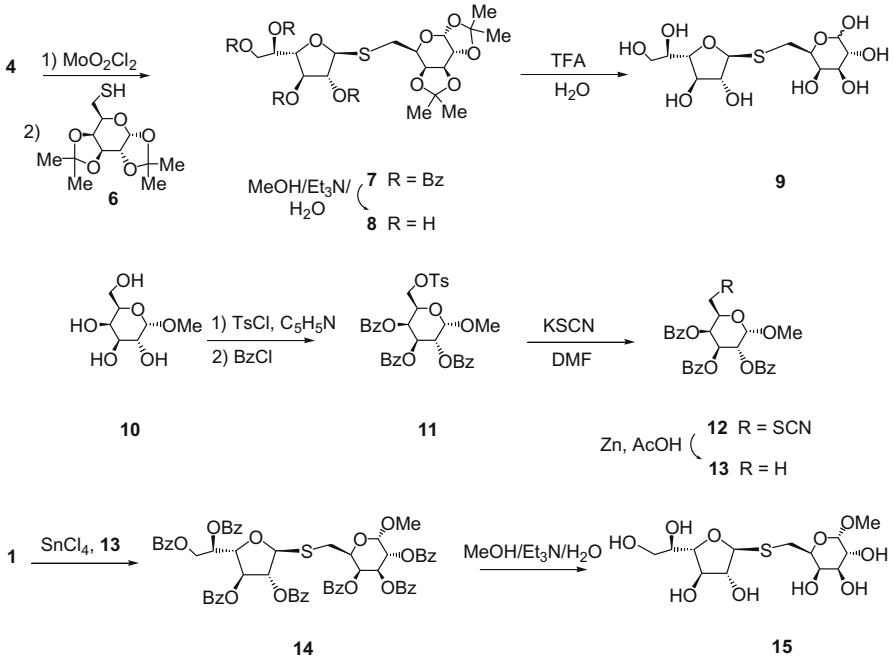
The thiodisaccharide β-D-Gal*f*-*S*-(1→6)-6-thio-D-Gal*p* (**9**) was interesting as the β-D-Gal*f*(1→6)-D-Gal*p* unit was found in nature as a constituent of the exopolysaccharide of *Lactobacillus rhamnosus*[29] and of *Streptococcus thermophilus*.[30] This disaccharide has also been obtained by partial hydrolysis of immunological polysaccharides from *Mycoplasma mycoides*[31] and *M. tuberculosis*.[32] Furthermore, the thiodisaccharide **9** was useful to investigate the influence of the change of configuration of the reducing end, with respect to **5**, on the inhibition of the β-galactofuranosidase.

For the synthesis of **9** (Scheme 2) the Gal*f* derivative **4** was allowed to react with methyl 2,3:4,5-di-*O*-isopropylidene-6-thio-α-D-galactose (**6**).[7] The MoO_2Cl_2-catalyzed reaction between **4** and **6** led to the stereoselective formation of the thioglycosidic bond of β configuration to produce **7**. In this case $SnCl_4$ could not be employed, as Lewis acids promote the removal of the isopropylidene protecting groups of **6**.

The thiodisaccharide **7** was *O*-debenzoylated to afford the partially protected derivative **8**, which was treated with TFA for the hydrolysis of the acetal groups. However, this deprotection could not be successfully accomplished because the acidic conditions required for the hydrolysis of the isopropylidene functions produced partial degradation of the interglycosidic *S*-linkage. This hydrolysis could not be prevented, even under smooth conditions, such as acetic acid/THF/water 5:1:1 at 65 °C, or under acetolysis reaction conditions in which thioglycosidic bonds between pyranose units were stable.[33] It is known that furanosides are much more labile to acids than their pyranoside analogues.[34] As the purification of **9** from the hydrolysis products was rather difficult and low yielding, a thiosugar with protecting groups labile to alkali was used.



Scheme 1. Synthesis of thiodisaccharide **5**.

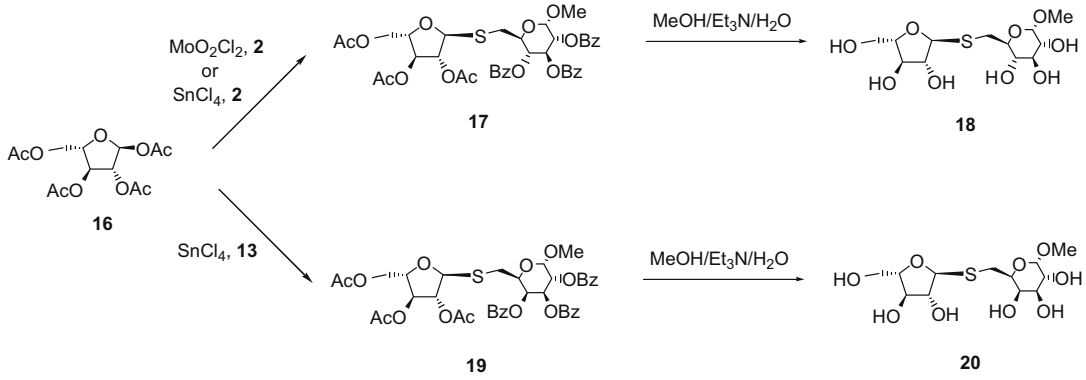


Scheme 2. Synthesis of thiodisaccharides **8** and **15**.

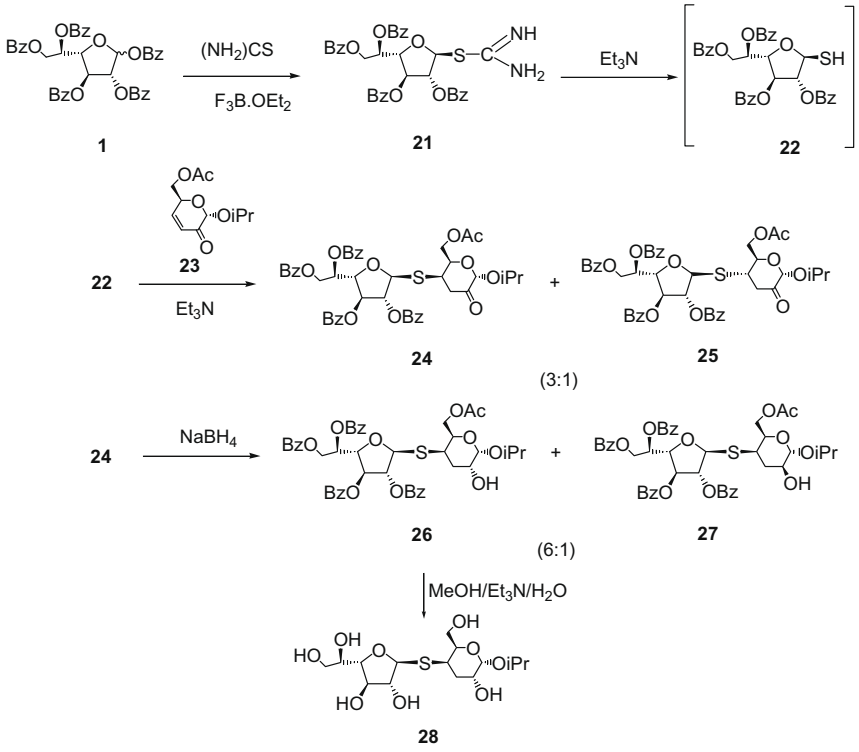
In view of the successful results in the synthesis of **5**, the thiosugar **13** (the *galacto* analogue of **2**) was selected as thiol donor. As compound **9** has not been previously described, it was prepared by a short route starting from methyl α-D-galactopyranoside (**10**). Selective tosylation of the primary hydroxyl group of **10**,[35] followed by benzoylation, afforded the tosyl derivative **11**. Treatment of **11** with potassium thiocyanate in DMF at 120 °C led to the thiocyano derivative **12** (55%) and unreacted **11** (24%). The low reactivity of this tosylate towards substitution may be attributed to the fact that the incoming nucleophile is hindered by the C-4 substituent in an axial disposition, an effect commonly observed for 6-*O*-sulfonates of galactosides.[36] Reduction of **12** with Zn in acetic acid gave the 6-thiohexopyranoside **13**. Reaction of **4** with **13**, in the presence of $SnCl_4$, led to the thiodisaccharide **14**. As previously observed, the glycosylation took place with exclusive diasterocontrol in favor of the β anomer. Debenzoylation of **14** with MeOH/Et_3N/H_2O (3:1:3) gave the target thiodisaccharide β-D-Gal*f*-*S*-(1→6)-6-thio-α-D-Gal*p*-OMe (**15**).

The synthesis of thiodisaccharides containing α-L-Ara*f* as non reducing end was carried out, since this sugar has the same stereochemistry for the substituents of the five-membered ring as β-D-Gal*f*. Thiosugars **2** and **13** (Scheme 3) were employed as thiol donors in the reaction with commercially available tetra-*O*-acetyl-α-L-Ara*f* (**16**). Thus, activation of **16** with $SnCl_4$ or MoO_2Cl_2 followed by addition of thiosugar **2** afforded the thiodisaccharide **17**. Analogous coupling of **16** with the 6-thiosugar derivative **13**, in the presence of $SnCl_4$, led to the thiodisaccharide **19**. The configuration for the anomeric center of the Ara*f* unit in compounds **17** and **19** was established as α, since their ^{1}H NMR spectra showed, similar to the β-D-Gal*f* analogues, small $J_{1',2'}$ values ($J_{1',2'} < 1$ Hz), characteristic of 1,2-*trans* thiofuranosides. Removal of the protecting groups of **17** and **19** with MeOH/Et_3N/H_2O (3:1:3) afforded the free thiodisaccharides **18** and **20**.

Starting from the per-*O*-benzoyl Gal*f* derivative **1**, a different methodology for the construction of the thioglycosidic linkage was employed. This methodology involves the 1-thioGal*f* derivative **22** as an intermediate (Scheme 4). However, we have observed that the procedures that are successful for the synthesis of 1-thiopyranoses derivatives usually fail for furanoses, which undergo self-condensation to the disulfide and other side reactions.[7] To overcome this difficulty 1-thiosugars like **22**, were generated from the respective *S*-glycosyl isothiourea derivatives and reacted in situ with a sugar enone to yield the corresponding thiodisaccharides. Thus, the *S*-glycosyl isothiourea **21** was synthesized by reaction



Scheme 3. Synthesis of thiodisaccharides 1**8** and **20**.



Scheme 4. Synthesis of thiodisaccharide 2**8**.

of **1** with thiourea in the presence of $F_3B{\cdot}OEt_2$. Crude compound **21** was treated with Et_3N to promote the conversion of the isothiourea into the thiosugar **22**. Michael addition of **22** with the hex-3-eno-pyranosid-2-ulose **23** gave the thiodisaccharide **24** as major product, accompanied with the diastereoisomeric *S*-linked disaccharide **25**. The addition took place diasteroselectively from the face of the enone opposite to the anomeric substituent (ratio **24**:**25** > 3:1). Thiodisaccharides **24** and **25** were readily separated by column chromatography. However, they were slightly contaminated by the starting enone **23**, as the retro-Michael reaction seems to take place during the chromatographic purification, as we have previously observed.[7] The configuration for the C-4 stereocenter was established as *R* (the thio group axially oriented), since the ^1H NMR spectrum of **24** showed only small coupling constant values of H-4 with the adjacent methylene protons ($J_{3ax,4}$ = 5.0, $J_{3eq,4}$ = 2.4 Hz) and with H-5 ($J_{4,5}$ = 2.4 Hz). Furthermore, the small value for the coupling between H-1′ and H-2′ ($J_{1',2'}$ < 1 Hz) was indicative of the β-D-Gal*f* configuration, as described for the analogous thiodisaccharides reported here.

The carbonyl group of compound **24** was reduced with sodium borohydride to afford the mixture of epimeric thiodisaccharides **26** and **27** (~6:1 ratio). The selectivity observed suggests that the approach of the hydride is controlled by the anomeric substituent adjacent to the carbonyl. The configuration for the C-2 and C-4 stereocenters of the reducing-end was readily established on the basis of the ^1H NMR data. Thus, the major diasteroisomer **26** has the α-D-*xylo* configuration, as H-2 showed small coupling constant values with H-1 ($J_{1,2}$ = 3.7 Hz) and H-3eq ($J_{2,3eq}$ = 4.3 Hz), whereas the $J_{2,3ax}$ value was large (11.5 Hz), indicating that the HO-2 is equatorially disposed. The fact that the H-4 signal appeared as a broad singlet, with small coupling constants with H-3ax, H-3eq and H-5 confirms the configuration already assigned for C-4 in **24**. A similar analysis for **27**, which showed only small coupling constant values for H-2 and H-4 with the respective coupled protons, indicated the α-D-*lyxo* configuration for the reducing end. Removal of the acetyl and benzoyl protecting groups of **26** by hydrolysis with MeOH/Et_3N/H_2O (3:1:3) afforded the free thiodisaccharide **28**.

2.2. Evaluation of the inhibitory activity

The inhibitory activity of thiodisaccharides **5**, **8**, **15**, **18**, **20** and **28** against the exo β-D-galactofuranosidase from *P. fellutanum* was evaluated in vitro following a previous protocol.[19a] 4-Nitrophenyl β-D-galactofuranoside[18d] was used as substrate and D-galactono-1,4-lactone (**29**) as a reference inhibitor (IC_{50} 0.02 mM).[19a] The enzymatic reaction was performed in the presence of thiodisaccharides **5**, **8**, **15**, **18** **20** and **28** at concentrations ranging from 0.2 to 1.0 mM. The amount of 4-nitrophenol released from 4-nitrophenyl β-D-galactofuranoside was determined as a measure of galactofuranosidase activity (Fig. 1).

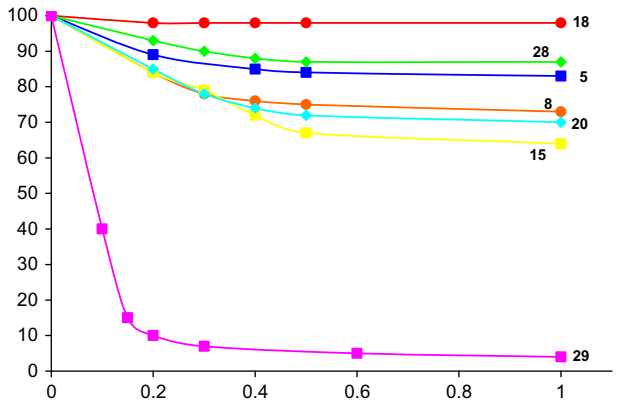


Figure 1. Effect of concentration of thiodisaccharides on the enzymatic activity of exo β-D-galactofuranosidase from *Penicillium fellutanum*. Each point is the mean obtained from three replicate experiments.

The thiodisaccharide **18** was not active, and compounds **8** and **15**, although the more active thiodisaccharides, exhibited only a weak inhibitory activity (IC > 1 mM) compared with lactone **29** (IC_{50} 0.02 mM). Thus, the thiodisaccharides constituted by β-D-Gal*f* *S*-linked to galactose in the pyranose form (**15**) or even substituted by isopropylidene groups (**8**) are able to interact with the enzyme. Probably for this reason, the thiodisaccharide α-L-Ara*f*(1→6)-6-thio-α-D-Gal*p*-OMe (**20**), structurally related to **15**, exhibits a similar inhibitory activity as **15** and **8**. In contrast, thiodisaccharide **18**, the analogue of **20** having a Glc*p* unit replacing Gal*p* in the reducing end, was not active.

Thiodisaccharide **5**, with a structure similar to that of **15** and **18**, showed an inhibitory activity intermediate between that of **15** and **18**. Therefore, the presence of a Glc*p* unit as reducing-end decreases the inhibitory activity of these molecules. Compound **28**, in which Gal*f* is *S*-linked to 3-deoxy-4-thio-D-*xylo*-pyranoside is also a weak inhibitor of the enzyme. These results are in accordance with the high specificity previously observed for the enzyme.[13]

3. Conclusion

The synthesis of thiodisaccharides constituted by Gal*f* or Ara*f* as non-reducing end has been successfully accomplished. The 1-*O*-acetyl-per-*O*-benzoyl derivative of Gal*f* (**4**) was synthesized and employed for the $SnCl_4$ and MoO_2Cl_2-promoted thioglycosylations, and showed to be more reactive than the per-*O*-benzoyl analogue **1**, in particular for reactions catalyzed by MoO_2Cl_2. This catalyst was very useful when the thiosugar precursor contained acid labile protecting groups. Additionally, the glycosyl isothiourea derivative of Gal*f* was prepared and used to generate in situ the 1-thio-D-Gal*f*, which was coupled to a sugar enone via a Michael addition reaction.

The free thiodisaccharides have been evaluated as inhibitors of the exo β-D-galactofuranosidase from *P. fellutanum*. Those thiodisaccharides constituted by β-D-Gal*f* or α-L-Ara*f* *S*-linked to Gal*p* displayed higher inhibitory activity than the others. Thiodisaccharides formed by two D-Gal*f* units linked through sulfur are expected to be active inhibitors and, in addition, will provide more information about the galactofuranosidase specificity. The synthesis of this type of compounds is under way.

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. Assignements of ^1H and ^{13}C were assisted by 2D ^1H-COSY and HSQC experiments.

4.2. Synthesis

4.2.1. 1-*O*-Acetyl-2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranoside (4)

1,2,3,5,6-Penta-*O*-benzoyl-α,β-D-galactofuranoside[24] (**1**, 0.46 g, 0.66 mmol) was dissolved in anhydrous Cl_2CH_2 (11 mL), cooled to 0 °C and $SnCl_4$ (92 μL, 0.77 mmol) was added. After 10 min acetic anhydride (136 μL, 1.44 mmol) was added and the mixture was stirred at 0 °C for 6 h. TLC showed the conversion of the starting material into **4** (R_f = 0.48, toluene/EtOAc, 9:1). The solution was diluted with Cl_2CH_2, extracted with satd aq $NaHCO_3$ (3 × 20 mL) and with brine. The extract was dried ($MgSO_4$) and concentrated, and the residue was purified by flash chromatography (toluene/EtOAc, 98:2) to give compound **4** as a colorless oil (0.29 g, 78%). It was recrystallized from EtOH, mp 115 °C; $[\alpha]_D^{20}$ −5.7 (*c* 0.9, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 8.07–7.28 (m, 20H, H-aromatic), 6.52 (br s, 1H, H-1), 6.08 (ddd, 1H, $J_{4,5}$ = 3.6, $J_{5,6}$ = 4.0, $J_{5,6'}$ = 7.3 Hz, H-5), 5.71 (dd, 1H, $J_{2,3}$ = 1.0, $J_{3,4}$ = 4.4 Hz, H-3), 5.60 (d, 1H, $J_{2,3}$ = 1.0 Hz, H-2), 4.80 (dd, 1H, $J_{5,6}$ = 4.0, $J_{6,6'}$ = 12.0 Hz, H-6), 4.79 (dd, 1H, $J_{4,5}$ = 3.6, $J_{3,4}$ = 4.4 Hz, H-4), 4.72 (dd, 1H, $J_{5,6'}$ = 7.3, $J_{6,6'}$ = 12.0 Hz, H-6′), 2.19 (s, 3H, C*H*$_3$CO); ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.0 (MeCO), 166.0, 165.7, 165.5, 165.2 (PhCO), 133.7–128.2 (C-aromatic), 99.4 (C-1), 83.6 (C-4), 81.3 (C-2), 77.4 (C-3), 76.1 (C-5), 63.5 (C-6), 21.0 (*C*H$_3$CO). Anal. Calcd for $C_{36}H_{30}O_{11}$: C, 67.71; H, 4.73. Found: C, 67.78; H, 4.55.

4.2.2. General procedure for the thioglycosylation of Gal*f* derivatives

4.2.2.1. Method A: $SnCl_4$-Promoted thioglycosylation of 1 or 4. 1,2,3,5,6-Penta-*O*-benzoyl-α,β-D-galactofuranoside (**1**, 0.10 mmol) or 1-*O*-acetyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranoside (**4**, 0.10 mmol) dissolved in anhydrous CH_2Cl_2 (∼1 mL), and cooled externally to 0 °C, was treated with $SnCl_4$ (0.11–0.12 mmol). After 10 min, the corresponding thiosugar (0.12 mmol) was added and the mixture was stirred at 0 °C for 4 h. The reaction mixture was extracted with satd aq $NaHCO_3$ and then with brine. The organic solution was dried ($MgSO_4$) and concentrated. The residue was subjected to column chromatography with solvent specified in each individual case.

4.2.2.2. Method B: MoO_2Cl_2-Catalyzed thioglycosylation of 4. To a solution of MoO_2Cl_2 (0.007 mmol) in anhydrous CH_2Cl_2 (0.2 mL) was added under Argon, a solution of compound **4** (0.10 mmol) in CH_2Cl_2 (0.5 mL). The mixture was stirred at room temperature (rt) for 10 min and a solution of the thiosugar (0.05–0.06 mmol) in CH_2Cl_2 (0.5 mL) was added. The mixture turned blue and gradually changed to yellowish brown. After stirring at rt for 24 h, the solution was diluted with CH_2Cl_2 (20 mL) and washed with satd aq $NaHCO_3$ and then with brine. The organic extract was dried ($MgSO_4$) and concentrated, and the resulting residue purified by column chromatography with the solvent indicated in each case.

4.2.3. Methyl 2,3,4-tri-*O*-benzoyl-6-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-6-thio-α-D-glucopyranoside (3)

The $SnCl_4$-promoted procedure (Method A) was followed starting from **1** (60 mg, 0.086 mmol) in anhydrous CH_2Cl_2 (1 mL) and $SnCl_4$ (12 μL, 0.10 mmol). After the addition of methyl 2,3,4-tri-*O*-benzoyl-6-thio-α-D-glucopyranoside **2**[25] (54 mg, 0.103 mmol) the mixture was stirred at 0 °C for 4 h and then processed as indicated above. Purification by column chromatography (toluene/EtOAc, 98:2) afforded thiodisaccharide **3** (58 mg, 61%); $[\alpha]_D^{20}$ −14.7 (*c* 1.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 8.12–7.27 (m, 35H, H-aromatic), 6.12 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 6.08 (ddd, 1H, $J_{4',5'} \sim J_{5',6'a}$ = 4.0, $J_{5',6'b}$ = 6.9 Hz, H-5′), 5.93 (br s, 1H, $J_{1',2'}$ < 1 Hz, H-1′), 5.68 (dt, 1H, $J_{2',3'}$ = 1.1, $J_{3',4'}$ = 5.0 Hz, H-3′), 5.46 (d, 1H, $J_{2',3'}$ = 1.1 Hz, H-2′), 5.44 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 5.22 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 9.8 Hz, H-2), 5.19 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.82 (dd, 1H, $J_{4',5'}$ = 4.0, $J_{3',4'}$ = 5.0 Hz, H-4′), 4.77 (dd, 1H, $J_{5',6'a}$ = 4.4, $J_{6'a,6'b}$ = 11.9 Hz, H-6′a), 4.74 (dd, 1H, $J_{5',6'b}$ = 6.9, $J_{6'a,6'b}$ = 11.9 Hz, H-6′b), 4.31 (ddd, 1H, $J_{5,6b}$ = 2.2, $J_{4,5}$ = $J_{5,6a}$ = 9.8 Hz, H-5), 3.49 (s, 3H, C*H*$_3$O), 3.07 (dd, 1H, $J_{5,6a}$ = 9.8, $J_{6a,6b}$ = 14.6 Hz, H-6a), 2.85 (dd, 1H, $J_{5,6b}$ = 2.2, $J_{6a,6b}$ = 14.6 Hz, H-6b); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 165.8, 165.7, 165.5 (PhCO), 133.6–128.3 (C-aromatic), 96.6 (C-1), 89.2 (C-1′), 82.6 (C-2′), 81.3 (C-4′), 77.8

(C-3′), 72.3 (C-4), 72.1 (C-2), 71.5 (C-5), 70.2 (×2, C-3,5′), 63.4 (C-6′), 55.5 (CH_3O), 31.3 (C-6). Anal. Calcd for $C_{62}H_{52}O_{17}S$: C, 67.61; H, 4.76; S, 2.91. Found: C, 67.27; H, 4.83; S, 2.55.

The same procedure (method A) was applied for the reaction of 1-*O*-acetyl-2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranose (**4**, 60 mg, 0.094 mmol) with **2** (59 mg, 0113 mmol), to produce **3** (61 mg, 59%).

Alternatively, thiodisaccharide **3** was synthesized using MoO_2Cl_2 as catalyst (Method B). To a solution of MoO_2Cl_2 (1.2 mg, 0.006 mmol) in anhydrous CH_2Cl_2 (0.2 mL), was added under Argon a solution of compound **4** (55 mg, 0.086 mmol) in CH_2Cl_2 (0.5 mL). The mixture was stirred for 10 min at rt and methyl 2,3,4-tri-*O*-benzoyl-6-thio-α-D-glucopyranoside[25] (**2**, 24 mg, 0.046 mmol) in CH_2Cl_2 (0.5 mL) was added. After the usual work-up and purification procedure, the thiodisaccharide **3** (28 mg, 56%) was obtained (R_f = 0.59, toluene/EtOAC, 10:1). This product was identical to the one previously described.

4.2.4. 1,2:3,4-di-*O*-isopropylidene-6-*S*-(2,3,5,6-tetra-*O*-benzoyl–D-galactofuranosyl)-6-thio-α-D-galactopyranose (7)

The MoO_2Cl_2-catalyzed procedure (Method B) was followed, starting from **4** (160 mg, 0.251 mmol), MoO_2Cl_2 (2.7 mg, 0.014 mmol) and **6**[7,37] (35 mg, 0.127 mmol). After 24 h of stirring at rt, TLC showed a main spot of R_f = 0.48 (toluene/EtOAc, 7:1). Thiodisaccharide **7** (40 mg, 37%) was purified by column chromatography using (toluene/EtOAc, 97:3); $[\alpha]_D^{20}$ −73.5 (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10–7.10 (m, 15H, H-aromatic), 6.09 (ddd, 1H, $J_{5',6'a}$ = 4.2, $J_{4',5'}$ = 4.8, $J_{5',6'b}$ = 7.3 Hz, H-5′), 5.75 (t, 1H, $J_{1',3'}$ = 0.8, $J_{1',2'}$ = 1.5 Hz, H-1′), 5.67 (dddd, 1H, $J_{1',3'}$ = 0.8, $J_{2',3'}$ = 1.5, $J_{3',4'}$ = 4.8 Hz, H-3′), 5.55 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 1.5 Hz, H-2′), 5.52 (d, 1H, $J_{1,2}$ = 5.0 Hz, H-1), 4.81 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 4.8 Hz, H-4′), 4.78 (dd, 1H, $J_{5',6'a}$ = 4.2, $J_{6'a,6'b}$ = 11.9 Hz, H-6′a), 4.72 (dd, 1H, $J_{5',6'b}$ = 7.3, $J_{6'a,6'b}$ = 11.9 Hz, H-6′b), 4.60 (dd, $J_{2,3}$ = 2.4, $J_{3,4}$ = 8.0 Hz, H-3), 4.30 (m, 2H, H-2,4), 3.97 (ddd, 1H, $J_{4,5}$ = 1.7, $J_{5,6b}$ = 6.3, $J_{5,6a}$ = 7.5 Hz, H-5), 3.06 (dd, 1H, $J_{5,6a}$ = 7.5, $J_{6a,6b}$ = 13.7 Hz, H-6a), 2.89 (dd, 1H, $J_{5,6b}$ = 6.3, $J_{6a,6b}$ = 13.7 Hz, H-6b), 4.49, 1.44, 1.32, 1.29 (4s, 12H, 2 × $(CH_3)_2$C); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 166.0, 165.7, 165.5, 165.3 (PhCO), 133.5–128.3 (C-aromatic), 109.4, 108.6 (Me_2C), 96.6 (C-1), 88.4 (C-1′), 82.6 (C-2′), 81.5 (C-4′), 77.9 (C-3′), 71.7, 70.5 (C-2,4), 70.8 (C-3), 70.3 (C-5′), 68.8 (C-5), 63.5 (C-6′), 30.5 (C-6), 26.0, 25.9, 24.9, 24.5 [2 × $(CH_3)_2$C]. Anal. Calcd for $C_{46}H_{46}O_{14}S$: C, 64.61; H, 5.43. Found: C, 64.94; H, 5.33.

4.2.5. Methyl 2,3,4-tri-*O*-benzoyl-6-*O*-tosyl-α-D-galactopyranoside (11)

To a suspension of methyl α-D-galactopyranoside (**10**, 1.50 g, 7.73 mmol) in anhydrous pyridine (15 mL) was added tosyl chloride (2.2 g, 11.54 mmol) and the mixture was stirred for 4 days at rt. Then, benzoyl chloride (4.5 mL, 38.7 mmol) was added and the stirring was maintained at rt for 18 h. Then, the mixture was poured into ice/water. Analysis by TLC (hexane/EtOAc, 2:1) showed a major product of R_f = 0.44, together with a minor one of R_f = 0.59. These products were isolated by column chromatography (hexane/EtOAc, 9:1–3:1). The less polar component was spectroscopically identified as methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranoside[38] (1.05 g, 22.3%). From the following fractions from the column was isolated **11** (2.00 g, 39.2%); $[\alpha]_D^{20}$ +164.1 (*c* 0.3, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.00–7.10 (m, 19H, H-aromatic), 5.90 (dd, 1H, $J_{3,4}$ = 3.5, $J_{2,3}$ = 10.3 Hz, H-3), 5.85 (dd, 1H, $J_{4,5}$ = 1.1, $J_{3,4}$ = 3.5 Hz, H-4), 5.57 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 10.3 Hz, H-2), 5.24 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.49 (br t, 1H, $J_{4,5}$ = 1.1, $J_{5,6'}$ = 5.7, $J_{5,6}$ = 7.0 Hz, H-5), 4.24 (dd, 1H, $J_{5,6}$ = 7.0, $J_{6,6'}$ = 10.3 Hz, H-6), 4.09 (dd, 1H, $J_{6,6'}$ = 5.7, $J_{5,6'}$ = 7.0, $J_{6,6'}$ = 10.3 Hz, H-6′), 3.45 (s, 3H, CH_3O), 2.33 (s, 3H, CH_3Ar); ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.0, 165.8, 165.3 (PhCO), 127.9 (C-aromatic), 97.5 (C-1), 69.0, 68.7, 68.1, 67.3, 66.6 (C-2,3,4,5,6), 55.8 (CH_3O), 21.6 (CH_3Ar). Anal. Calcd for $C_{35}H_{32}O_{10}S$: S, 4.97. Found: S, 4.90.

4.2.6. Methyl 2,3,4-tri-*O*-benzoyl-6-deoxy-6-thiocyano-α-D-galactopyranoside (12)

Tosylate **11** (0.34 g, 0.51 mmol) was dissolved in anhydrous DMF (4 mL) and heated with KSCN (0.29 g, 3.00 mmol) at 120 °C for 48 h. TLC showed formation of a major product of R_f = 0.44 (toluene/EtOAc, 17:1) and some starting material remaining (R_f = 0.40). Column chromatography (toluene/EtOAc, 99:1) afforded **12** (0.16 g, 55.2%) and **11** (0.08 g, 24%). Compound **12**: $[\alpha]_D^{20}$ +182.5 (*c* 1.2, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.11–7.20 (m, 15H, H-aromatic), 5.97 (dd, 1H, $J_{3,4}$ = 3.4, $J_{2,3}$ = 10.6 Hz, H-3), 5.89 (dd, 1H, $J_{4,5}$ = 0.9, $J_{3,4}$ = 3.4 Hz, H-4), 5.67 (dd, 1H, $J_{1,2}$ = 3.5, $J_{2,3}$ = 10.6 Hz, H-2), 5.26 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.24 (br t, 1H, $J_{5,6} \sim J_{5,6'} \sim$ 7.1 Hz, H-5), 3.48 (s, 3H, CH_3O), 3.13 (d, 2H, $J_{5,6} \sim J_{5,6'} \sim$ 7.1 Hz, H-6,6′); ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.1, 165.8, 165.4 (PhCO), 133.9–128.3 (C-aromatic), 112.0 (SCN), 97.7 (C-1), 70.3, 68.9, 68.1, 67.9 (C-2,3,4,5), 56.1 (CH_3O), 34.6 (C-6). Anal. Calcd for $C_{29}H_{25}O_8SN.1.5H_2O$: C, 60.62; H, 4.91; S, 5.58. Found: C, 60.48; H, 4.90; S, 5.40.

4.2.7. Methyl 2,3,4-tri-*O*-benzoyl-6-thio-α-D-galactopyranoside (13)

The thiocyano derivative **12** (0.70 g, 1.28 mmol) was heated under reflux with Zn (1.67 g, 25.6 mmol) in glacial acetic acid (25 mL) for 24 h. The mixture was poured into water, and extracted with CH_2Cl_2. The organic phase was washed successively with $NaCO_3H$, and brine, dried and concentrated. The resulting syrup showed by TLC a main spot of R_f = 0.49 (toluene/EtOAc, 17:1). Purification by column chromatography (toluene/EtOAc, 99:1) afforded **13** (0.52 g, 78.3%); $[\alpha]_D^{20}$ +240.7 (*c* 1.1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.10–7.15 (m, 15H, H-aromatic), 5.99–5.93 (m, 2H, H-3,4), 5.64 (dd, 1H, $J_{1,2}$ = 3.7, $J_{2,3}$ = 10.7 Hz, H-2), 5.28 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.26 (dd, 1H, $J_{5,6'}$ = 5.8, $J_{5,6}$ = 7.9 Hz, H-5), 3.52 (s, 3H, CH_3O), 2.81 (ddd, 1H, $J_{5,6} \sim J_{6,SH}$ = 7.9, $J_{6,6'}$ = 14.0 Hz, H-6), 2.64 (ddd, 1H, $J_{5,6'}$ = 5.8, $J_{6',SH}$ = 9.5, $J_{6,6'}$ = 14.0 Hz, H-6′), 1.76 (dd, 1H, $J_{6,SH}$ = 7.9, $J_{6',SH}$ = 9.5 Hz, S*H*); ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.1, 165.8, 165.5 (PhCO), 133.6–128.3 (C-aromatic), 97.6 (C-1), 71.0, 70.1, 69.4, 68.6 (C-2,3,4,5), 55.8 (s, 3H, CH_3O), 24.8 (C-6). Anal. Calcd for $C_{28}H_{26}O_8S$: C, 64.36; H, 5.01; S, 6.13. Found: C, 64.41; H, 5.10; S, 6.18.

4.2.8. Methyl 2,3,4-tri-*O*-benzoyl-6-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-6-thio-α-D-galactopyranoside (14)

The $SnCl_4$-promoted thioglycosylation (Method A) was employed, starting from **1** (79 mg, 0.11 mmol), $SnCl_4$ (16 μL, 0.13 mmol) and **13** (71 mg, 0.135 mmol). Column chromatography (toluene/EtOAc, 98:2) afforded thiodisaccharide **14** (65 mg, 52.5%); R_f = 0.52 (toluene/EtOAc, 10:1); $[\alpha]_D^{20}$ +65.0 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.13–7.22 (m, 35H, H-aromatic), 6.03 (ddd, 1H, $J_{4',5'} = J_{5',6'a}$ = 4.3, $J_{5',6'b}$ = 6.8 Hz, H-5′), 5.95 (dd, 1H, $J_{3,4}$ = 3.4, $J_{2,3}$ = 10.7 Hz, H-3), 5.89 (br d, 1H, $J_{4,5}$ < 1, $J_{3,4}$ = 3.4 Hz, H-4), 5.89 (br s, 1H, $J_{1',2'}$ < 1 Hz, H-1′), 5.70 (d, 1H, $J_{3',4'}$ = 5.0 Hz, H-3′), 5.62 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 10.6 Hz, H-2), 5.48 (br s, 1H, $J_{1',2'} \sim J_{2',3'}$ < 1.0 Hz, H-2′), 5.27 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.79 (t, 1H, $J_{4',5'}$ = 4.3, $J_{3',4'}$ = 5.0 Hz, H-4′), 4.71 (dd, 1H, $J_{5',6'a}$ = 4.5, $J_{6'a,6'b}$ = 11.8 Hz, H-6′a), 4.67 (dd, 1H, $J_{5',6'b}$ = 7.0, $J_{6'a,6'b}$ = 11.8 Hz, H-6′b), 4.46 (dd, 1H, $J_{5,6b}$ = 3.5, $J_{5,6a}$ = 9.5 Hz, H-5), 3.50 (s, 3H, CH_3O), 3.12 (dd, 1H, $J_{5,6a}$ = 9.5, $J_{6a,6b}$ = 14.3 Hz, H-6a), 2.76 (dd, 1H, $J_{5,6b}$ = 3.8, $J_{6a,6b}$ = 14.3 Hz, H-6b); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 166.1, 165.9, 165.7 (×2), 165.5 (×2), 165.3 (PhCO), 134.5–128.2 (C-aromatic), 97.4 (C-1), 89.0 (C-1′), 82.6 (C-2′), 81.3 (C-4′), 77.7 (C-3′), 70.8 (C-4), 70.4 (C-5), 70.2 (C-5′), 69.3 (C-2), 68.5 (C-3), 63.2 (C-6′), 55.7 (CH_3O), 30.7 (C-6). Anal. Calcd for $C_{62}H_{52}O_{17}S$: C, 67.61; H, 4.76. Found: C, 67.36; H, 4.76.

4.2.9. Methyl 2,3,4-tri-*O*-benzoyl-6-*S*-(2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl)-6-thio-α-D-glucopyranoside (17)

Tetra-*O*-acetyl-α-L-arabinofuranose (**16**, 50 mg, 0.16 mmol) and **2** (99 mg, 0.19 mmol) reacted in the presence of $SnCl_4$ (20 μL,

0.17 mmol) as described above (Method A) to give **17** (R_f = 0.34, toluene/EtOAc, 5:1). Column chromatography (toluene/EtOAc, 95:5) afforded thiodisaccharide **17** (61 mg, 50%) as a yellowish syrup; $[\alpha]_D^{20}$ −29.3 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00–7.28 (m, 15H, H-aromatic), 6.12 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 5.61 (s, 1H, $J_{1',2'} < 1$ Hz, H-1′), 5.68 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 5.23 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 9.5 Hz, H-2), 5.22 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.08 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 1.7 Hz, H-2′), 5.02 (dd, 1H, $J_{2',3'}$ = 1.7, $J_{3',4'}$ = 5.1 Hz, H-3′), 4.39 (m, 2H, H-4′,5′a), 4.27 (m, 2H, H-5,5′b), 3.52 (s, 3H, CH_3O), 3.00 (dd, 1H, $J_{5,6a}$ = 9.7, $J_{6a,6b}$ = 14.5 Hz, H-6a), 2.81 (dd, 1H, $J_{5,6b}$ = 2.3, $J_{6a,6b}$ = 14.5 Hz, H-6b), 2.11, 2.09 (2s, 9H, CH_3CO); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 170.6, 170.0, 169.5 (MeCO), 165.8, 165.7, 165.5 (PhCO), 133.5–128.3 (C-aromatic), 96.7 (C-1), 88.9 (C-1′), 81.8 (C-2′), 79.7 (C-4′), 77.6 (C-3′), 72.4 (C-4), 72.1 (C-2), 71.2 (C-5), 70.1 (C-3), 62.9 (C-5′), 55.5 (CH_3O), 31.7 (C-6), 20.8, 20.7 (CH_3CO). Anal. Calcd for $C_{39}H_{40}O_{15}S$: C, 59.97; H, 5.17. Found: C, 60.07; H, 5.44.

Alternatively, **17** (94 mg, 65%) was obtained by catalysis of MoO_2Cl_2 (Method B), starting from **16** (100 mg, 0.31 mmol), **2** (97 mg, 0.19 mmol) and MoO_2Cl_2 (3.8 mg, 0.02 mmol).

4.2.10. Methyl 2,3,4-tri-*O*-benzoyl-6-*S*-(2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl)-6-thio-α-D-galactoyranoside (19)

Tetra-*O*-acetyl-α-L-arabinofuranose (**16**, 50 mg, 0.157 mmol) and **13** (99 mg, 0.190 mmol) reacted under the presence of $SnCl_4$ (20 μL, 0.17 mmol) as described above (Method A). Purification by column chromatography (toluene/EtOAc, 95:5) afforded syrupy thiodisaccharide **19** (75 mg, 61%); R_f = 0.33 (toluene/EtOAc, 5:1); $[\alpha]_D^{20}$ +67.5 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10–7.15 (m, 15H, H-aromatic), 5.94 (dd, 1H, $J_{3,4}$ = 3.5, $J_{2,3}$ = 10.6 Hz, H-3), 5.90 (dd, 1H, $J_{4,5}$ = 0.7, $J_{3,4}$ = 3.5 Hz, H-4), 5.62 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 10.6 Hz, H-2), 5.52 (br s, 1H, H-1′), 5.28 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.09 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 1.6 Hz, H-2′), 5.03 (dd, 1H, $J_{2',3'}$ = 1.6, $J_{3',4'}$ = 5.4 Hz, H-3′), 4.41 (ddd, 1H, $J_{4,5}$ = 0.7, $J_{5,6b}$ = 4.2, $J_{5,6a}$ = 9.2 Hz, H-5), 4.34 (ddd, 1H, $J_{4',5'a}$ = 3.4, $J_{3',4'}$ = 5.4, $J_{4',5'b}$ = 6.1 Hz, H-4′), 4.32 (dd, 1H, $J_{4',5'a}$ = 3.4, $J_{5'a,5'b}$ = 12.6 Hz, H-5′a), 4.24 (dd, 1H, $J_{4',5'b}$ = 6.1, $J_{5'a,5'b}$ = 12.6 Hz, H-5′b), 3.04 (dd, 1H, $J_{5,6a}$ = 9.2, $J_{6a,6b}$ = 14.1 Hz, H-6a), 2.74 (dd, 1H, $J_{5,6b}$ = 4.2, $J_{6a,6b}$ = 14.1 Hz, H-6b); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 170.5, 170.0, 169.5 (MeCO), 166.1, 165.7, 165.5 (PhCO), 133.5–128.2 (C-aromatic), 97.4 (C-1), 88.7 (C-1′), 81.7 (C-2′), 79.7 (C-4′), 77.2 (C-3′), 70.7 (C-4), 70.0 (C-5), 69.3 (C-2), 68.4 (C-3), 62.7 (C-5′), 55.7 (CH_3O), 31.1 (C-6), 20.7 (CH_3CO). Anal. Calcd for $C_{39}H_{40}O_{15}S$: C, 59.97; H, 5.17. Found: C, 60.10; H, 5.19.

4.2.11. 2-Propyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-4-thio-α-D-*threo*-hexopyranosid-2-ulose (24) and 2-propyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-4-thio-α-D-*erythro*-hexopyranosid-2-ulose (25)

To a solution of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranose[24] (**1**, 280 mg, 40 mmol) in MeCN (anhyd, 8 mL), was added thiourea (34 mg, 0.45 mmol), followed by $F_3B{\cdot}OEt_2$ (260 μL, 2.07 mmol). The mixture was heated under reflux for 2.5 h, when TLC (toluene/EtOAc, 9:1) showed complete consumption of the starting material. Evaporation of the solvent gave the β-D-galactofuranosyl isothiourea (**21**) as a syrup; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15–7.20 (m, H-aromatic + NH_2), 6.36 (s, 1H, H-1), 6.09 (m, 1H, H-5), 5.80 (d, 1H, $J_{3,4}$ = 3.6 Hz, H-3), 5.55 (s, 1H, H-2), 4.95 (dd, 1H, $J_{3,4}$ = 3.6, $J_{4,5}$ = 4.1 Hz, H-4), 4.89 (dd, 1H, $J_{5,6}$ = 4.7, $J_{6,6'}$ = 11.9 Hz, H-6), 4.66 (dd, 1H, $J_{5,6'}$ = 6.5, $J_{6,6'}$ = 11.9 Hz, H-6′); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.8, (SC(NH_2)$_2$), 166.2, 165.7, 165.6, 165.3 (PhCO), 133.4–128.3 (C-aromatic), 88.2 (C-1), 84.2, 81.7 (C-2,4), 77.0 (C-3), 69.8 (C-5), 63.0 (C-6).

To the original mixture, cooled to 0 °C, 2-propyl 6-*O*-acetyl-2,3-dideoxy-α-D-*glycero*-hex-3-enopyranosid-2-ulose (**23**, 76 mg, 0.33 mmol) and Et_3N (518 μl, 3.72 mmol) were added. The mixture was stirred at 0 °C for 3 h. TLC (toluene/EtOAc, 9:1) showed two main products of R_f = 0.34 (major) and 0.16, respectively. After concentration and purification by flash chromatography (toluene/EtOAc, 96:4), thiodissacharide **24** (145 mg, 52%) was obtained. This product was slightly contaminated by the enone **23**. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10–7.16 (m, 20H, H-aromatic), 6.10 (dt, 1H, $J_{4',5'} \sim J_{5',6'a}$ = 4.3, $J_{5',6'b}$ = 6.4 Hz, H-5′), 5.70 (br d, 1H, $J_{2',3'} < 1.0$, $J_{3',4'}$ = 4.8 Hz, H-3′), 5.69 (br s, 1H, H-1′), 5.50 (br s, 1H, H-2′), 4.80–4.75 (m, 3H, H-4′,5,6′a), 4.79 (br s, 1H, $J_{1,2} < 1.0$ Hz, H-1), 4.74 (dd, 1H, $J_{5',6'a}$ = 6.4, $J_{6'a,6'b}$ = 12.0 Hz, H-6′b), 4.37 (dd, 1H, $J_{5,6a}$ = 7.3, $J_{6a,6b}$ = 11.6 Hz, H-6a), 4.28 (dd, 1H, $J_{5,6b}$ = 4.9, $J_{6a,6b}$ = 11.6 Hz, H-6b), 4.02 (m, 1H, *J* = 6.2 Hz, Me$_2$C*H*O), 3.70 (dt, $J_{3eq,4} = J_{4,5}$ = 2.4, $J_{3ax,4}$ = 5.0 Hz, H-4), 3.17 (dd, 1H, $J_{3ax,4}$ = 5.0, $J_{3ax,3eq}$ = 15.2 Hz, H-3ax), 2.82 (dd, 1H, $J_{3eq,4}$ = 2.4, $J_{3ax,3\text{-}eq}$ = 15.2 Hz, H-3eq), 2.05 (s, 3H, CH_3CO$_2$), 1.28, 1.18 (2d, 6H, *J* = 6.2 Hz, (CH_3)$_2$CHO); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 198.7 (C-2), 170.4 (MeCO), 166.1, 165.7, 165.5, 165.2 (PhCO), 133.6–128.4 (C-aromatic), 98.0 (C-1), 86.6 (C-1′), 82.6, 82.1 (C-2′,4′), 77.8 (C-3′), 71.8, 70.2, 68.4 (Me$_2$CHO, C-5,5′), 64.6, 63.4 (C-6,6′), 45.1 (C-4), 42.6 (C-3), 23.3, 21.8 [(CH_3)$_2$CHO], 20.7 (CH_3CO).

Further fractions from the column afforded syrupy **25** (45 mg, 16%); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.09–7.28 (m, 20H, H-aromatic), 6.08 (dt, 1H, $J_{4',5'} = J_{5',6'a}$ = 4.3, $J_{5',6'b}$ = 7.0 Hz, H-5′), 5.72 (br s, 1H, $J_{1',2'} < 1.0$ Hz, H-1′), 5.70 (br d, 1H, $J_{2',3'} < 1.0$, $J_{3',4'}$ = 4.3 Hz, H-3′), 5.47 (br s, 1H, H-2′), 4.85 (t, 1H, $J_{3',4'} = J_{4',5'}$ = 4.3 Hz, H-4′), 4.79 (br s, 1H, $J_{1,2} < 1.0$ Hz, H-1), 4.78 (dd, 1H, $J_{5',6'a}$ = 4.3, $J_{6'a,6'b}$ = 11.8 Hz, H-6′a), 4.72 (dd, 1H, $J_{5',6'b}$ = 7.0, $J_{6'a,6'b}$ = 11.8 Hz, H-6′b), 4.59 (dd, 1H, $J_{5,6a}$ = 1.4, $J_{6a,6b}$ = 11.9 Hz, H-6a), 4.47 (dd, 1H, $J_{5,6b}$ = 5.0, $J_{6a,6b}$ = 11.9 Hz, H-6b), 4.43 (ddd, 1H, $J_{5,6a}$ = 1.4, $J_{5,6b}$ = 5.0, $J_{4,5}$ = 10.8 Hz, H-5), 3.98 (m, 1H, *J* = 6.2 Hz, Me$_2$C*H*O), 3.42 (ddd, 1H, $J_{3eq,4}$ = 5.0, $J_{3ax,4}$ = 12.8 Hz, H-4), 3.02 (dd, 1H, $J_{3ax,4}$ = 12.8, $J_{3ax,3eq}$ = 14.6 Hz, H-3ax), 2.86 (dd, 1H, $J_{3eq,4}$ = 5.0, $J_{3ax,3eq}$ = 14.6 Hz, H-3eq), 2.06 (s, 3H, CH_3CO$_2$), 1.25, 1.17 (2d, 6H, *J* = 6.2 Hz, (CH_3)$_2$CHO); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 199.0 (C-2), 170.6 (MeCO), 166.0, 165.6, 165.4, 165.3 (PhCO), 133.7–128.3 (C-aromatic), 97.7 (C-1), 87.9 (C-1′), 82.7 (C-2′), 81.8 (C-4′), 77.6 (C-3′), 71.8, 70.3, 70.2 (Me$_2$CHO, C-5,5′), 63.6, 63.2 (C-6,6′), 42.9, 42.7 (C-3,4), 23.2, 21.7 [(CH_3)$_2$CHO], 20.7 (CH_3CO).

4.2.12. 2-Propyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-4-thio-α-D-*xylo*-hexopyranoside (26) and 2-propyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranosyl)-4-thio-α-D-*lyxo*-hexopyranoside (27)

Compound **24** (163 mg, 0.19 mmol) was dissolved in THF (5 mL) and treated with $NaBH_4$ (50 mg, 1.31 mmol) at −18 °C. The mixture was stirred for 30 min, neutralized with Dowex 50 (H^+) resin, filtered and concentrated, and evaporated with MeOH, the syrup showed by TLC (hexane/EtOAc, 7:3) two products of R_f 0.60 (minor) and 0.53 (major). Column chromatography (hexane/EtOAc, 69:31) afforded first the less polar product **27** (10 mg, 6%) as a syrup and the major thiodisaccharide **26** (65 mg, 41%) as a colorless oil. Compound **26**: $[\alpha]_D^{20}$ −24.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.17–7.23 (m, 20H, H-aromatic), 6.09 (br q, 1H, $J_{4',5'}$ = 4.2, $J_{5',6'a} = J_{5',6'b}$ = 5.0 Hz, H-5′), 5.69 (br d, 1H, $J_{2',3'} < 1.0$, $J_{3',4'}$ = 5.0 Hz, H-3′), 5.65 (br s, 1H, $J_{1',2'} < 1.0$, H-1′), 5.50 (br s, 1H, H-2′), 4.93 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.79 (br t, 1H, $J_{3',4'} \sim J_{4',5'}$ = 4.5 Hz, H-4′), 4.74 (m, 2H, H-6′a,6′b), 4.28 (m, 2H, H-5,6a), 4.16 (dd, 1H, $J_{5,6b}$ = 7.5, $J_{6a,6b}$ = 14.7 Hz, H-6b), 4.10 (tt, 1H, $J_{1,2}$ = 3.7, $J_{2,3eq}$ = 4.3, $J_{2,3ax} = J_{2,OH}$ = 11.5 Hz, H-2) 3.95 (m, 1H, *J* = 6.2 Hz, Me$_2$C*H*O), 3.33 (br s, 1H, H-4), 2.25 (ddd, 1H, $J_{3eq,4}$ = 3.5, $J_{2,3eq}$ = 4.3, $J_{3ax,3eq}$ = 13.0 Hz, H-3eq), 2.04 (ddd, 1H, $J_{3ax,4}$ = 5.1, $J_{2,3ax}$ = 11.5, $J_{3ax,3eq}$ = 13.0 Hz, H-3ax), 2.03 (s, 3H, CH_3CO$_2$), 1.85 (d, 1H, $J_{2,OH}$ = 11.5 Hz, *H*O), 1.27, 1.19 (2 d, 6H, *J* = 6.2 Hz, (CH_3)$_2$CHO); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 170.5 (MeCO), 166.1, 165.7, 165.5, 165.4 (PhCO), 133.7–128.4 (C-aromatic), 96.9 (C-1), 87.6 (C-1′), 82.8 (C-2′), 81.7 (C-4′), 77.8 (C-3′), 70.9 (Me$_2$CHO), 70.2 (C-5′), 67.8 (C-2), 65.4 (C-6), 64.3 (C-5),

63.5 (C-6′), 43.5 (C-4), 34.0 (C-3), 23.2, 22.0 [(*C*H$_3$)$_2$CHO], 20.8 (*C*H$_3$CO). Anal. Calcd for $C_{45}H_{46}O_{14}S$: C, 64.12; H, 5.50. Found: C, 64.19; H, 5.78.

Compound **27**: $[\alpha]_D^{20}$ −32.3 (*c* 0.9, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ 8.08–7.23 (m, 20H, H-aromatic), 6.08 (ddd, 1H, $J_{4',5'} \sim J_{5',6'a}$ = 4.2, $J_{5',6'b}$ = 6.4 Hz, H-5′), 5.77 (br s, 1H, $J_{1',2'}$ < 1.0 Hz, H-1′), 5.71 (brd, 1H, $J_{2',3'}$ < 1.0, $J_{3',4'}$ = 5.0 Hz, H-3′), 5.50 (br s, 1H, H-2′), 4.92 (br s, 1H, $J_{1,2}$ < 1 Hz, H-1), 4.79 (m, 3H, H-4′,5,6′a), 4.76 (dd, 1H, $J_{6'a,6'b}$ = 11.9 Hz, H-6′b), 4.45 (m, 1H, H-2), 4.38 (dd, 1H, $J_{5,6a}$ = 8.0, $J_{6a,6b}$ = 11.4 Hz, H-6a), 4.23 (dd, 1H, $J_{5,6b}$ = 4.0 Hz, H-6b), 3.99 (m, 1H, *J* = 6.2 Hz, Me$_2$C*H*O), 3.28 (br s, 1H, H-4), 2.31 (m, 2H, H-3ax,3eq), 2.05 (s, 3H, C*H*$_3$CO$_2$), 1.26, 1.20 (2d, 6H, *J* = 6.2 Hz, (C*H*$_3$)$_2$CHO); ^{13}C NMR (125.7 MHz, CDCl$_3$): δ 170.5 (Me*C*O), 166.0, 165.7, 165.5, 165.4 (Ph*C*O), 133.7–128.4 (C-aromatic), 99.0 (C-1), 86.8 (C-1′), 82.8, 82.0 (C-2′,4′), 77.8 (C-3′), 70.2, 69.5, 67.9, 67.5 (C-2,5,5′,Me$_2$*C*HO), 65.2, 63.4 (C-6,6′), 39.4 (C-4), 29.6 (C-3), 23.2, 21.5 [(*C*H$_3$)$_2$CHO], 20.8 (*C*H$_3$CO). Anal. Calcd for $C_{45}H_{46}O_{14}S$: C, 64.12; H, 5.50. Found: C, 63.98; H, 5.51.

4.3. General procedure for the *O*-deacylation of thiodisaccharides 3, 7, 14, 17, 19 and 26

The *O*-acylated thiodisaccharide (0.10 mmol) was suspended in MeOH/Et$_3$N/H$_2$O, 3:1:3 (16 mL) and the mixture was stirred at 40 °C for 25–30 h. When TLC showed complete consumption of the starting material, the mixture was concentrated and the residue, dissolved in 1 mL of MeOH/water 1:1 (or in the case of compound **8** in MeOH/acetone 1:1), was passed through a column filled with Dowex MR-3C mixed bed ion-exchange resin. The eluate was concentrated, dissolved in water, or MeOH/water 1:1, and purified in an octadecyl C18 minicolumn. The pure fractions according with TLC (in *n*-BuOH/EtOH/H$_2$O, 2.5:1:1) or MeOH were collected and concentrated to afford the free thiodisaccharide.

4.3.1. Methyl 6-*S*-(β-D-galactofuranosyl)-6-thio-α-D-glucopyranoside (5)

The O-deacetylation procedure applied to **3** led to the free thiodisaccharide **5** (67%), R_f = 0.44 (*n*-BuOH/EtOH/H$_2$O, 2.5:1:1); $[\alpha]_D^{20}$ −31.8 (*c* 0.5, H$_2$O); ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.15 (d, 1H, $J_{1',2'}$ = 5.3 Hz, H-1′), 4.70 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.03 (dd, 1H, $J_{2',3'}$ = 5.4, $J_{3',4'}$ = 7.5 Hz, H-3′), 3.94 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 5.3 Hz, H-2′), 3.88 (dd, 1H, $J_{4',5'}$ = 3.5, $J_{3',4'}$ = 7.5 Hz, H-4′), 3.75 (ddd, 1H, $J_{4',5'}$ = 3.5, $J_{5',6'a}$ = 4.7, $J_{5',6'b}$ = 7.7 Hz, H-5′), 3.70 (ddd, 1H, $J_{5,6a}$ = 2.2, $J_{5,6b}$ = 8.8, $J_{4,5}$ = 9.0 Hz, H-5), 3.60 (dd, 1H, $J_{5',6'a}$ = 4.7, $J_{6'a,6'b}$ = 11.6 Hz, H-6′a), 3.56 (dd, 1H, $J_{5',6'b}$ = 7.7, $J_{6'a,6''b}$ = 11.6 Hz, H-6′b), 3.54 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.8 Hz, H-3), 3.49 (dd, 1H, $J_{1,2}$ = 3.7, $J_{2,3}$ = 9.8 Hz, H-2), 3.36 (s, 3H, C*H*$_3$O), 3.23 (dd, 1H, $J_{4,5}$ = 9.0, $J_{3,4}$ = 9.8 Hz, H-4), 3.07 (dd, 1H, $J_{5,6a}$ = 2.2, $J_{6a,6b}$ = 14.3 Hz, H-6a), 2.78 (dd, 1H, $J_{5,6b}$ = 8.8, $J_{6a,6b}$ = 14.3 Hz, H-6b); ^{13}C NMR (125.7 MHz, CDCl$_3$): δ 99.9 (C-1), 89.4 (C-1′), 81.3 (C-4′), 81.1 (C-2′), 76.0 (C-3′), 72.9 (C-3), 72.6 (C-4), 71.9 (C-5), 71.2 (C-2), 70.4 (C-5′), 62.8 (C-6′), 55.2 (*C*H$_3$O), 32.4 (C-6). Anal. Calcd for $C_{13}H_{24}O_{10}S.0.5\,H_2O$: C, 40.92; H, 6.61. Found: C, 40.82; H, 6.85.

4.3.2. 1,2:3,4-di-*O*-isopropylidene-6-*S*-(-D-galactofuranosyl)-6-thio-α-D-galactopyranose (8)

Deprotection of **7** afforded **8** (91%); R_f = 0.64 (MeOH); $[\alpha]_D^{20}$ −150.8 (*c* 1, H$_2$O); ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.46 (d, 1H, $J_{1,2}$ = 5.0 Hz, H-1), 5.12 (d, 1H, $J_{1',2'}$ = 4.3 Hz, H-1′), 4.63 (dd, 1H, $J_{2,3}$ = 2.4, $J_{3,4}$ = 7.9 Hz, H-3), 4.38 (dd, 1H, $J_{4,5}$ = 1.8, $J_{3,4}$ = 7.9 Hz, H-4), 4.34 (dd, 1H, $J_{2,3}$ = 2.4, $J_{1,2}$ = 5.0 Hz, H-2), 4.05 (dd, 1H, $J_{2',3'}$ = 4.8, $J_{3',4'}$ = 7.4 Hz, H-3′), 3.96 (ddd, 1H, $J_{4,5}$ = 1.6, $J_{5,6b}$ = 6.7, $J_{5,6a}$ = 7.0 Hz, H-5), 3.94 (dd, 1H, $J_{4',5'}$ = 3.1, $J_{3',4'}$ = 7.4 Hz, H-4′), 3.90 (t, 1H, $J_{1',2'} = J_{2',3'} \sim$5.0 Hz, H-2′), 3.73 (ddd, 1H, $J_{4',5'}$ = 3.1, $J_{5',6'a} = J_{5',6'b}$ = 6.8 Hz, H-5′), 3.60 (m, 2H, H-6′a,6′b), 2.87 (dd, 1H, $J_{5,6a}$ = 7.0, $J_{6a,6b}$ = 13.7 Hz, H-6a), 2.77 (dd, 1H, $J_{5,6b}$ = 6.8, $J_{6a,6b}$ = 13.7 Hz, H-6b), 1.51, 1.40, 1.34, 1.33 (4s, 12H, 2 (C*H*$_3$)$_2$C); ^{13}C NMR (125.7 MHz, CDCl$_3$): δ 110.3, 109.9 (Me$_2$*C*), 97.9 (C-1), 90.7 (C-1′), 83.9 (C-2′), 83.2 (C-4′), 78.5 (C-3′), 72.8 (C-4), 72.3 (C-5′), 72.2 (C-3), 71.9 (C-2), 69.8 (C-5), 64.6 (C-6′), 31.3 (C-6), 26.4, 26.3, 25.1, 24.6 [(*C*H$_3$)$_2$C]. Anal. Calcd for $C_{18}H_{30}O_{10}S$: C, 49.30; H, 6.90. Found: C, 50.00; H, 7.39.

4.3.3. Methyl 6-*S*-(β-D-galactofuranosyl)-6-thio-α-D-galactopyranoside (15)

O-Debenzoylation of **14** led to **15** (67%); R_f = 0.68 (MeOH); $[\alpha]_D^{20}$ −13.5 (*c* 0.9, H$_2$O); ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.12 (d, 1H, $J_{1',2'}$ = 5.3 Hz, H-1′), 4.71 (d, 1H, H-1), 4.03 (dd, 1H, $J_{2',3'}$ = 5.3, $J_{3',4'}$ = 7.4 Hz, H-3′), 3.93 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 5.3 Hz, H-2′), 3.92–3.86 (m, 2H, H-4,5), 3.88 (dd, 1H, $J_{4',5'}$ = 3.5, $J_{3',4'}$ = 7.5 Hz, H-4′), 3.76 (ddd, 1H, $J_{4',5'}$ = 3.5, $J_{5',6'a}$ = 4.6, $J_{5',6'b}$ = 7.6 Hz, H-5′), 3.71 (m, 2H, H-2,3), 3.60 (dd, 1H, $J_{5',6'a}$ = 4.6, $J_{6'a,6'b}$ = 11.6 Hz, H-6′a), 3.55 (dd, 1H, $J_{5',6'b}$ = 7.6, $J_{6'a,6'b}$ = 11.6 Hz, H-6′b), 3.35 (s, 3H, C*H*$_3$O), 2.90 (dd, 1H, $J_{5,6a}$ = 8.9, $J_{6a,6b}$ = 14.0 Hz, H-6a), 2.74 (dd, 1H, $J_{5,6b}$ = 4.8, $J_{6a,6b}$ = 14.0 Hz, H-6b); ^{13}C NMR (125.7 MHz, CDCl$_3$): δ 99.5 (C-1), 89.1 (C-1′), 81.3 (C-4′), 81.0 (C-2′), 76.0 (C-3′), 70.9, 70.3, 70.1 (C-4,5,5′), 69.5, 68.0 (C-2,3), 62.7 (C-6′), 55.2 (*C*H$_3$O), 31.5 (C-6). Anal. Calcd for $C_{13}H_{24}O_{10}S$: C, 41.91; H, 6.50. Found: C, 42.26; H, 6.70.

4.3.4. Methyl 6-*S*-(α-L-arabinofuranosyl)-6-thio-α-D-glucopyranoside (18)

Removal of the ester protecting groups from **17** led to the free thiodisaccharide **18** (68%); R_f = 0.57 (*n*-BuOH/EtOH/H$_2$O, 2.5:1:1); $[\alpha]_D^{20}[\alpha]_D^{20}$ −13.2 (*c* 1, H$_2$O); ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.17 (d, 1H, $J_{1',2'}$ = 4.8 Hz, H-1′), 4.70 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 3.96 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 4.8 Hz, H-2′), 3.95 (m, 1H, H-4′), 3.89 (dd, 1H, $J_{2',3'}$ = 4.8, $J_{3',4'}$ = = 7.2 Hz, H-3′), 3.73 (dd, 1H, $J_{4',5'a}$ = 2.9, $J_{5'a,5'b}$ = 12.4 Hz, H-5′a), 3.71 (m, 1H, H-5), 3.63 (dd, 1H, $J_{4',5'b}$ = 5.3, $J_{5'a,5'b}$ = 12.5 Hz, H-5′b), 3.53 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 3.48 (dd, 1H, $J_{1,2}$ = 3.7, $J_{2,3}$ = 9.5 Hz, H-2), 3.36 (s, 3H, C*H*$_3$O), 3.23 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.08 (dd, 1H, $J_{5,6a}$ = 2.0, $J_{6a,6b}$ = 14.2 Hz, H-6a), 2.78 (dd, 1H, $J_{5,6b}$ = 8.8, $J_{6a,6b}$ = 14.2 Hz, H-6b); ^{13}C NMR (125.7 MHz, CDCl$_3$): δ 99.2 (C-1), 89.4 (C-1′), 82.2, 81.4 (C-2′,4′), 75.8 (C-3′), 72.9 (C-3), 72.7 (C-4), 71.8 (C-5), 71.2 (C-2), 60.6 (C-5′), 55.1 (*C*H$_3$O), 32.9 (C-6). Anal. Calcd for $C_{12}H_{22}O_9S.0.5H_2O$: C, 41.00; H, 6.60. Found: C, 41.36; H, 6.86.

4.3.5. Methyl 6-*S*-(α-L-arabinofuranosyl)-6-thio-α-D-galactopyranoside (20)

O-Deacylation of **19** gave thiodisaccharide **20** (77%); R_f = 0.79 (MeOH); $[\alpha]_D^{20}$ −44.4 (*c* 0.9, MeOH); ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.16 (d, 1H, $J_{1',2'}$ = 4.8 Hz, H-1′), 4.72 (d, 1H, H-1), 3.97 (ddd, 1H, $J_{4',5'a}$ = 3.0, $J_{4',5'b}$ = 5.3, $J_{3',4'}$ = 7.3 Hz, H-4′), 3.96 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 4.8 Hz, H-2′), 3.93 (br dd, 1H, $J_{5,6b}$ = 4.5, $J_{5,6a}$ = 9.0 Hz, H-5), 3.91 (br s, 1H, H-4), 3.90 (dd, 1H, $J_{2',3'}$ = 4.8, $J_{3',4'}$ = 7.3 Hz, H-3′), 3.73 (dd, 1H, $J_{4',5'a}$ = 3.0, $J_{5'a,5'b}$ = 12.6 Hz, H-5′a), 3.72 (m, 2H, H-2,3), 3.64 (dd, 1H, $J_{4',5'b}$ = 5.3, $J_{5'a,5'b}$ = 12.6 Hz, H-5′b), 3.35 (s, 3H, C*H*$_3$O), 2.92 (dd, 1H, $J_{5,6a}$ = 9.0, $J_{6a,6b}$ = 14.0 Hz, H-6a), 2.76 (dd, 1H, $J_{5,6b}$ = 4.5, $J_{6a,6b}$ = 14.0 Hz, H-6b); ^{13}C NMR (125.7 MHz, CDCl$_3$): δ 99.4 (C-1), 89.2 (C-1′), 82.2, 81.3 (C-2′,4′), 75.8 (C-3′), 70.7, 70.2 (C-4,5), 69.4, 68.0 (C-2,3), 60.6 (C-5′), 55.2 (*C*H$_3$O), 31.4 (C-6). Anal. Calcd for $C_{12}H_{22}O_9S$: C, 42.08; H, 6.48. Found: C, 41.58; H, 6.67.

4.3.6. 2-Propyl 3-deoxy-4-*S*-(β-D-galactofuranosyl)-4-thio-α-D-*xylo*-hexopyranoside (28)

O-Deacylation of **26** afforded syrupy **28** (92%); R_f = 0.58 (*n*-BuOH/EtOH/H$_2$O, 2.5:1:1); $[\alpha]_D^{20}$ −83.3 (*c* 0.8, MeOH); ^{1}H NMR (500 MHz, CDCl$_3$): δ 5.12 (d, 1H, $J_{1',2'}$ = 4.7 Hz, H-1′), 4.90 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.16 (m, 1H, H-5), 4.03 (dd, 1H, $J_{2',3'}$ = 4.7, $J_{3',4'}$ = 7.4 Hz, H-3′), 4.02 (dt, 1H, $J_{1,2}$ = 3.7, $J_{2,3eq}$ = 4.6,

$J_{2,3ax}$ = 12.0 Hz, H-2), 3.97 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 4.7 Hz, H-2′), 3.94 (m, 1H, J = 6.2 Hz, Me_2CHO), 3.90 (dd, 1H, $J_{4',5'}$ = 3.6, $J_{3',4'}$ = 7.4 Hz, H-4′), 3.78 (ddd, 1H, $J_{4',5'}$ = 3.6, $J_{5',6'a}$ = 4.6, $J_{5',6'b}$ = 7.5 Hz, H-5′), 3.62 (m, 2H, H-6a,6b), 3.61 (dd, 1H, $J_{5',6'a}$ = 4.6, $J_{6'a,6'b}$ = 11.6 Hz, H-6′a), 3.56 (dd, 1H, $J_{5',6'b}$ = 7.5, $J_{6'a,6'b}$ = 11.6 Hz, H-6′b), 3.30 (m, 1H, H-4), 2.12 (ddd, 1H, $J_{3ax,4}$ = 3.7, $J_{2,3ax}$ = 12.0, $J_{3ax,3eq}$ = 13.2 Hz, H-3ax), 2.03 (dddd, 1H, $J_{3eq,4}$ = 3.5, $J_{2,3eq}$ = 4.6, $J_{3ax,3eq}$ = 13.2 Hz, H-3eq), 1.18, 1.11 (2d, 6H, J = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 96.9 (C-1), 88.8 (C-1′), 82.4, 82.2 (C-2′,4′), 77.1 (C-3′), 71.3, 71.2, 71.1 (C-5,5′,Me_2CHO), 64.9 (C-2), 63.8, 63.5 (C-6,6′), 44.3 (C-4), 33.4 (C-3), 23.2, 21.3 [$(CH_3)_2CHO$]. Anal. Calcd for $C_{15}H_{28}O_9S.H_2O$: C, 44.76; H, 7.51; S, 7.97. Found: C, 45.25; H, 7.92; S, 7.78.

4.4. Enzymatic assays

The enzymatic activity was assayed using the filtered medium of a stationary culture of *P. fellutanum* as source of β-D-galactofuranosidase and 4-nitrophenyl β-D-galactofuranoside as substrate[19a] The standard assay was conducted with 50 μL of 66 mM NaOAc buffer (pH 4.9), 15 μL of a 5 mM solution of 4-nitrophenyl β-D-galactofuranoside and 25 μL (10 μg protein) of the enzyme medium, in a final volume of 250 μL. The thiodisaccharides were incorporated in the amounts needed to obtain a final concentration from 0.2 to 1.0 mM. The enzymatic reaction was stopped after 1.5 h of incubation at 37 °C by addition of 0.5 mL of 0.1 M Na_2CO_3 buffer (pH 9.0). The 4-nitrophenol released was measured spectrophotometrically at 410 nm.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.02.045.

References and notes

1. (a) Varky, A. *Glycobiology* **1993**, *3*, 97; (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683; (c) Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Disc.* **2005**, *4*, 477.
2. (a) Sears, P.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1999**, *38*, 2300; (b) Marcaurelle, L. A.; Bertozzi, C. R. *Chem. Eur. J.* **1999**, *5*, 1384.
3. (a) Robina, I.; Vogel, P. *Synthesis* **2005**, *5*, 675; (b) Renaudet, O.; Dumy, P. *Tetrahedron* **2008**, *58*, 2127; (c) Yuan, X.; Linhardt, R. J. *Curr. Top. Med. Chem.* **2005**, *5*, 1393; (d) Arjona, O.; Gómez, A. M.; López, J. C.; Plumet, J. *Chem. Rev.* **2007**, *107*, 1919.
4. (a) Driguez, H. *ChemBioChem* **2001**, *2*, 311; (b) Driguez, H. *Top. Curr. Chem.* **1997**, *187*, 85; (c) Defaye, J.; Gelas, J.. In *Studies in Natural Product Chemistry*; Attaur-Rahman, Ed.; Elsevier: Amsterdam, 1991; Vol. 8, pp 315–357.
5. (a) Szilágyi, L.; Varela, O. *Curr. Org. Chem.* **2006**, *10*, 1745; (b) Pachamuthu, K.; Schmidt, R. R. *Chem. Rev.* **2006**, *106*, 160.
6. (a) Manzano, V. E.; Uhrig, M. L.; Varela, O. *J. Org. Chem.* **2008**, *73*, 7224; (b) Uhrig, M. L.; Szilágyi, L.; Kovér, K. E.; Varela, O. *Carbohydr. Res.* **2007**, *342*, 1841; (c) Uhrig, M. L.; Manzano, V. E.; Varela, O. *Eur. J. Chem.* **2006**, 162.
7. Repetto, E.; Marino, C.; Uhrig, M. L.; Varela, O. *Eur. J. Org. Chem.* **2008**, 540.
8. Minic, Z.; Do, C. T.; Rihouey, C.; Morin, H.; Lerouge, P.; Jounin, L. *J. Exp. Bot.* **2006**, *57*, 2339.
9. (a) Lederkremer, R. M.; Colli, W. *Glycobiology* **1995**, *5*, 547; (b) Houseknecht, J. B.; Lowary, T. L. *Curr. Opin. Chem. Biol.* **2003**, *7*, 677; (c) Pedersen, L. L.; Turco, S. J. *Cell. Mol. Life Sci.* **2003**, *60*, 259; (d) Peltier, P.; Euzen, R.; Daniellou, R.; Nugier-Chauvin, C.; Ferrières, V. *Carbohydr. Res.* **2008**, *343*, 1897.
10. Crick, D. C.; Mahaprata, S.; Brennan, P. J. *Glycobiology* **2001**, *11*, 107.
11. Kremer, L.; Dover, L. G.; Morehouse, C.; Hitchin, P.; Everett, M.; Morris, H. R.; Dell, A.; Brennan, J.; MacNeil, M. R.; Flaherty, C.; Duncan, K.; Besra, G. S. *J. Biol. Chem.* **2001**, *276*, 26430.
12. (a) Nassau, P. M.; Martin, S. L.; Brown, R. E.; Weston, A.; Monsey, D.; McNeil, M. R.; Duncan, K. *J. Bacteriol.* **1996**, *178*, 1047; (b) Sanders, D. A. R.; Staines, A. G.; McMahon, S. A.; McNeil, M. R.; Whitfield, C.; Naismith, J. H. *Nat. Struct. Biol.* **2001**, *8*, 858.
13. Rietschel-Berst, M.; Jentoft, N. H.; Rick, P. D.; Pletcher, C.; Fang, F.; Gander, J. E. *J. Biol. Chem.* **1977**, *252*, 3219.
14. Daley, L. S.; Ströbel, G. A. *Plant Sci. Lett.* **1983**, *30*, 145.
15. Cousin, M. A.; Notermans, S.; Hoogerhout, P.; Van Boom, J. H. *J. Appl. Bacteriol.* **1989**, *66*, 311.
16. Wallis, G.-L.; Hemming, F. W.; Pederby, J. F. *Biochim. Biophys. Acta* **2001**, *1525*, 19.
17. Golgher, D. B.; Colli, W.; Souto-Padron, T.; Zingales, B. *Mol. Biochem. Parasitol.* **1993**, *60*, 249.
18. (a) Marino, C.; Varela, O.; Lederkremer, R. M. *Carbohydr. Res.* **1989**, *190*, 65; (b) Marino, C.; Cancio, M. J.; Varela, O.; Lederekremer, R. M. *Carbohydr. Res.* **1995**, *276*, 209; (c) Mariño, K.; Marino, C.; Lederkremer, R. M. *Anal. Biochem.* **2002**, *301*, 325; (d) Varela, O.; Marino, C.; Lederkremer, R. M. *Carbohydr. Res.* **1986**, *155*, 247.
19. (a) Marino, C.; Mariño, K.; Miletti, L.; Manso Alves, M. J.; Colli, W.; Lederkremer, R. M. *Glycobiology* **1998**, *8*, 901; (b) Mariño, K.; Marino, C. *Arkivoc* **2005**, *XII*, 341.
20. Miletti, L. C.; Marino, C.; Mariño, K.; Lederkremer, R. M.; Colli, W.; Alves, M. J. M. *Carbohydr. Res.* **1999**, *320*, 176.
21. Miletti, L. C.; Mariño, K.; Marino, C.; Colli, W.; Alves, M. J. M.; Lederkremer, R. M. *Mol. Biochem. Parasitol.* **2003**, *127*, 85.
22. 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.
23. Mariño, K.; Lima, C.; Maldonado, S.; Marino, C.; Lederkremer, R. M. *Carbohydr. Res.* **2002**, *337*, 891.
24. D'Accorso, N. B.; Thiel, I. M. E.; Schüller, M. *Carbohydr. Res.* **1983**, *124*, 177.
25. Sherry, B. D.; Loy, R. N.; Toste, F. D. *J. Am. Chem. Soc.* **2004**, *126*, 4510.
26. Weng, S.-S.; Lin, Y.-D.; Chen, C.-T. *Org. Lett.* **2006**, *8*, 5633.
27. du Mortier, C.; Varela, O.; Lederkremer, R. M. *Carbohydr. Res.* **1989**, *189*, 79.
28. Stevenson, G.; Neal, B.; Liu, D.; Hobbs, M.; Packer, N. H.; Batley, M.; Redmond, J. W.; Lindquist, L.; Reeves, P. *J. Bacteriol.* **1994**, *176*, 4144.
29. Lipinski, T.; Jones, C.; Lemercinier, X.; Korzeniowska-Kowal, A.; Strus, M.; Rybka, J.; Gamian, A.; Heczko, P. B. *Carbohydr. Res.* **2003**, *338*, 605.
30. Faber, E. J.; van den Haak, M. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Carbohydr. Res.* **2001**, *331*, 173.
31. Plackett, P.; Buttery, S. H. *Biochem. J.* **1964**, *90*, 201.
32. Vilkas, E.; Amar, C.; Markovits, J.; Vliegenthart, J. F. G.; Kamerling, J. P. *Biochim. Biophys. Acta* **1973**, *297*, 423.
33. Witczak, Z. J.; Chhabra, R.; Chen, H.; Xie, X.-Q. *Carbohydr. Res.* **1997**, *301*, 167.
34. (a) Robyt, J. F. In *Essentials of Carbohydrate Chemistry*; Cantor, C. R., Ed.; Springer Verlag: New York, 1998; pp 179–180; (b) Green, J. W. *Adv. Carbohydr. Chem.* **1966**, *21*, 95.
35. Lewis, B. A.; Smith, F.; Stephen, A. M. *Methods Carbohydr. Chem.* **1963**, *2*, 172.
36. Boons, G.-J.; Hale, K. J. In *Organic Synthesis with Carbohydrates*; Sheffield Academic Press: Sheffield, 2000; pp 60–61.
37. Martins Alho, M. A.; D'Accorso, N. B.; Thiel, I. M. E. *J. Heterocycl. Chem.* **1996**, *33*, 1339.
38. Constantino, V.; Fattorusso, E.; Imperatore, C.; Mangoni, A. *J. Org. Chem.* **2004**, *69*, 1174.