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Synthesis of pentopyranosyl-containing thiodisaccharides. Inhibitory activity against β-glycosidases

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ABSTRACT

β-(1→4)-Thiodisaccharides formed by a pentopyranose unit as reducing or non reducing end have been synthesized using a sugar enone derived from a hexose or pentose as Michael acceptor of a 1-thiopentopyranose or 1-thiohexopyranose derivatives. Thus, 2-propyl per-*O*-acetyl-3-deoxy-4-*S*-(β-D-Xyl*p*)-4-thiohexopyranosid-2-ulose (**3**) and benzyl per-*O*-acetyl-3-deoxy-4-*S*-(β-D-Gal*p*)-4-thiopentopyranosid-2-ulose (**11**) were obtained in almost quantitative yields. The carbonyl function of these uloses was reduced with $NaBH_4$ or K-Selectride, and the stereochemical course of the reduction was highly dependent on the reaction temperature, reducing agent and solvent. Unexpectedly, reduction of **3** with $NaBH_4$–THF at 0 °C gave a 3-deoxy-4-*S*-(β-D-Xyl*p*)-4-thio-α-D-*ribo*-hexopyranoside derivative (**6**) as major product (74% yield), with isomerization of the sulfur-substituted C-4 stereocenter of the pyranone. Reduction of **11** gave always as major product the benzyl 3-deoxy-4-*S*-(Gal*p*)-4-thio-β-D-*threo*-pentopyranoside derivative **14**, which was the only product isolated (80% yield) in the reduction with K-Selectride in THF at −78 °C. Deprotection of **14** and its epimer at C-2 (**13**) afforded, respectively the free thiodisaccharides **19** and **18**. They displayed strong inhibitory activity against the β-galactosidase from *Escherichia coli*. Thus, compound **18** proved to be a non-competitive inhibitor of the enzyme (K_i = 0.80 mM), whereas **19** was a mixed-type inhibitor (K_i = 32 μM).

1. Introduction

Glycosidases are vital for living systems as they are involved in varied biological processes, including metabolic disorders and diseases.[1] Inhibition of glycosidases may prevent the proliferation of pathogenic microorganisms and hence glycosidase inhibitors are seen as potential therapeutic agents. In fact, they are already used or tested in the treatment of diabetes and HIV infection and as antifungal compounds.[2] Also, enzyme inhibition constitutes an innovative approach for drug development in cancer therapies.[3]

Sugar mimetics in which oxygen atoms have been replaced by other heteroatoms present altered binding properties or increased stability toward enzyme degradation, compared with the natural analogues.[4] Among the sugar mimetics, thiooligosaccharides are interesting compounds as, in many cases, they act as enzyme inhibitors,[5] and they are usually tolerated by biological systems and resistant to metabolic processes.[6] Because of the potential of thiooligosaccharides as enzyme inhibitors and therapeutics, the investigation about their synthesis and the study of their biological activities are topics of current research.[6,7]

In recent years, we have reported diastereoselective procedures for the construction of the sulfur mediated interglycosidic linkage of thiodisaccharides. Thus, we have recently described the synthesis of 3,4-epoxides and their use as acceptors of 1-thioaldoses to give, by ring-opening, (1→3)- or (1→4)-thiodisaccharides.[8] Alternatively, the S-linkage has been generated by Michael addition of 1-thioaldoses to hexose-derived sugar enones.[9] This methodology led to 3-deoxy-4-*S*-(1→4)-thiodisaccharides, which proved to be inhibitors of β-glycosidases. Also thiodisaccharides with a thiofuranose as non-reducing end have been prepared[10,11] and evaluated as inhibitors of a β-D-galactofuranosidase.[11] The procedure based on the conjugated addition has been employed by Witczak and coworkers for the thioglycosylation of levoglucosenone[5b,d,12] or isolevoglucosenone.[13] In our case, hexose-derived sugar enones have also been used as Michael acceptors of 1-thiohexoses to generate the S-linkage between two hexopyranose units.[9] However, the procedure has not been applied for the synthesis of thiodisaccharides constituted by a pentopyranose moiety. Furthermore, we considered that the presence of a pentopyranose as reducing end of the thiodisaccharide was a relevant issue, as an increased flexibility at the reducing end of the thiodisaccharide could facilitate its accommodation in the active site of the enzyme that demands distortion of both substrates and inhibitors from their more stable ground-state conformation.[14] Therefore, we report herein the syn-

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thesis of thiodisaccharides with a pentopyranose as reducing or non-reducing unit. The new products have been evaluated as inhibitors of glycosidases.

2. Results and discussion

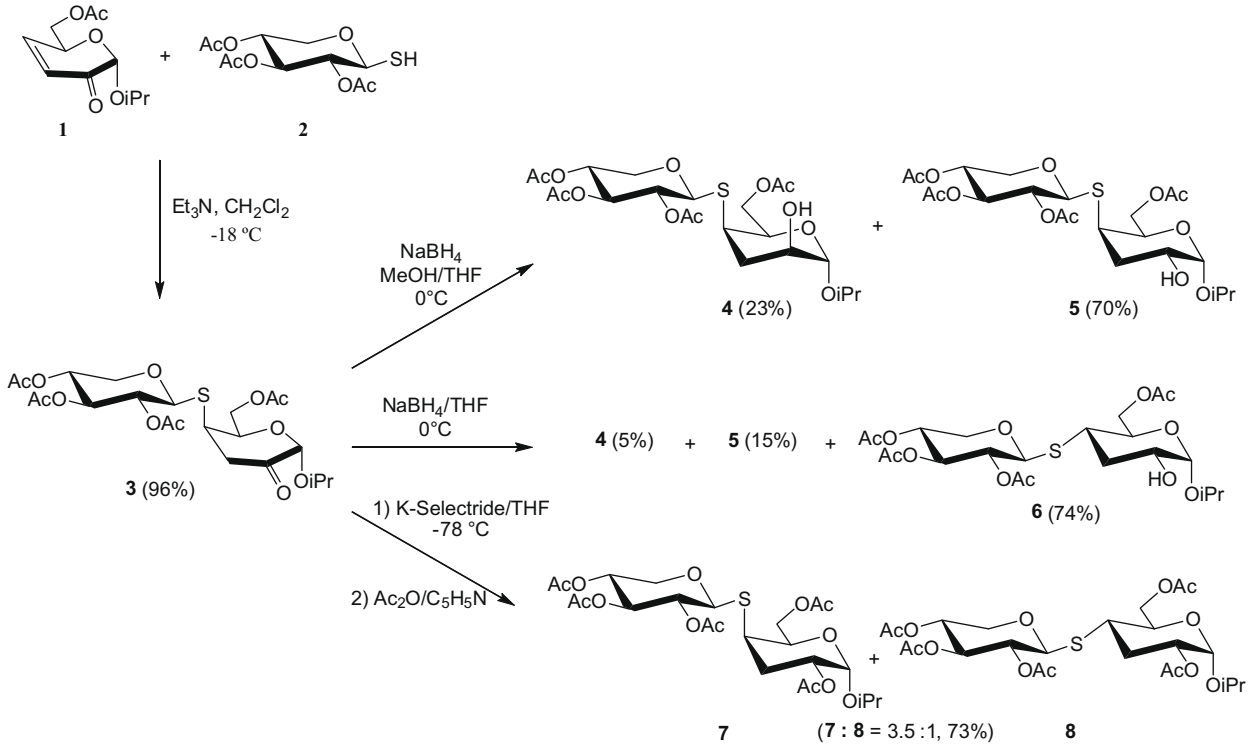
2.1. Synthesis and chemical characterization

The synthesis of the S-linked disaccharide with xylopyranose as non-reducing end was conducted by Michael addition of 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranose[15] (**2**) to the α,β-unsaturated system of 2-propyl 6-*O*-acetyl-3,4-dideoxy-α-D-*glycero*-hex-3-enopyranosid-2-ulose[16] (**1**). Under optimized conditions (CH_2Cl_2 at −18 °C for 30 min in the presence of triethylamine (TEA) as a catalyst) this reaction led to the thiodisaccharide **3** in 96% yield (Scheme 1). The addition was highly diastereoselective as **3** was the only isomer isolated. The *R* configuration for the new stereocenter at C-4 in **3** was established on the basis of the ^{1}H NMR spectrum. Thus, in accordance with the equatorial orientation of H-4, the ^{1}H NMR spectrum of **3** showed small coupling constant values of H-4 with the methylene protons at C-3 ($J_{3a,4}$ = 4.9 Hz, $J_{3b,4}$ = 1.6 Hz) and with H-5 ($J_{4,5}$ = 1.8 Hz). Similar to previous results in our laboratory, the stereocontrol was provided by the axially oriented anomeric substituent of α configuration.[9] When the reaction was carried out at room temperature the stereoselectivity was partially lost, which was attributed to the higher reactivity of the 1-thiopentopyranose, in comparison with the analogous 1-thiohexopyranoses previously studied. The increased nucleophilicity of **2** may be due to the lack of the electron-withdrawing acetoxymethyl substituent on C-5 of the 1-thiopyranose, which diminishes the electron density on the ring oxygen atom vicinal to the sulfur-containing anomeric center.[17]

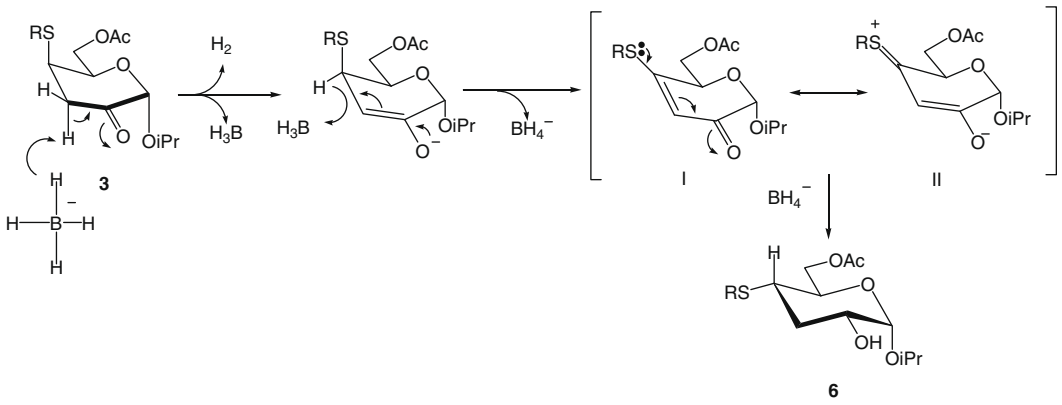
The reduction of **3** with $NaBH_4$ was performed in MeOH/THF or THF, as the ulose was partially soluble in MeOH at 0 °C. In THF–MeOH the reduction proceeded rapidly at 0 °C to afford two products which were separated by column chromatography. The resulting 3-deoxy-4-*S*-glycosyl-4-thiohexopyranosides, having the 4-thio-α-D-*lyxo* (**4**) and α-D-*xylo* (**5**) configuration for the reducing end, were isolated in 23% and 70% yield, respectively. A similar stereoselectivity was observed in previous studies,[9,18] suggesting that the axial thioglycosyl group, β to the carbonyl, has a minor contribution in the steric hindrance than the vicinal axial anomeric isopropyl group.

The structures of **4** and **5** were confirmed by NMR spectroscopy. Compound **4** showed the signal of H-1 as a broad singlet, indicating a diequatorial disposition for H-1 and H-2. In contrast, the H-1 signal in **5** appeared as a doublet with $J_{1,2}$ = 3.6 Hz, which is in agreement with an equatorial–axial disposition for H-1 and H-2. The H-4 signal in **4**, as well as in **5**, appeared protected by the proximity of sulfur, as multiplets at ~3.27 ppm, with small coupling constants with their vicinal H-3ax, H-3eq and H-5, confirming that H-4 is equatorially disposed. The resonances of the β-1-thioxylosyl protons in **3**, **4** and **5** were in accordance with those reported for other thioxylopyranosides.[19]

Reduction of **3** with an excess of $NaBH_4$ in anhydrous THF led to a mixture of three reduction products, upon careful separation by column chromatography. The first fractions afforded **4** and **5** in 5% and 15% yield, respectively. The more polar product (74%) was identified as the thiodisaccharide **6**. Its structure was confirmed by ^{1}H and ^{13}C NMR spectroscopy, assisted by 2D ^{1}H COSY and ^{1}H–^{13}C HSQC experiments. The large coupling constant values of H-4 with H-5 (11.0 Hz) and H-3ax (13.0 Hz), and the small one with H-3eq (4.2 Hz) indicated that **6** had reverse configuration at C-4 with respect to **4** and **5**. This result was rather unexpected and suggested the isomerization of the C-4 stereocenter in the starting **3** mediated by the species BH_4^- and BH_3. To explain this isomerization–reduction we propose the hypothetical mechanism depicted in Scheme 2. Initial removal of the hydrogen α to the carbonyl (H-3) in **3** by the basic BH_4^- leads to enolate formation and releasing of borane. The BH_3 would promote the elimination of the



Scheme 1. Synthesis of thiodisaccharides **3**–**8**.



Scheme 2. Proposed mechanism for the isomerization of C-4 during the $NaBH_4$/THF reduction of **3**.

allylic hydride in the enolate, giving rise to **I**, stabilized by resonance of the non shared electron pair of sulfur that is conjugated with the α,β-unsaturated carbonyl system. Reduction of the sulfonium ion and the carbonyl group of **II**, gives rise to **6**. This reduction follows the previous tendency observed, with approach of hydride from the less hindered β face, resulting in an *S* configuration for the C-4 stereocenter. This approach allows the bulky 1-thioxylopyranose residue at C-4 to adopt an equatorial disposition in the final product **6**. The isomerization seems to be inhibited in the presence of a protic solvent such as MeOH.

The carbonyl group of **3** was also reduced with K-Selectride in THF at −78 °C. After the usual work-up, the reaction mixture showed by TLC some spots of lower mobility than those corresponding to thiodisaccharides **4**–**6**, suggesting partial O-deacetylation. Therefore, the mixture was acetylated under standard conditions to afford, after purification, a chromatographically homogenous product that revealed to be a 3.5:1 mixture of **7**:**8**. The identity of **7** was confirmed by acetylation of its precursor **5**. The ^{1}H NMR spectrum of **8** in the mixture showed, similar to that of **6**, large coupling constant values for H-4 with H-3ax and H-5, indicating isomerization of C-4 during the reduction. The fact that the extent of isomerization is diminished respect to the analogous $NaBH_4$/THF reduction of **3** may be attributed to the axially oriented thioglycosyl and isopropoxy groups, which hinder both faces of the molecule for the approach of the bulky K-Selectride to remove H-3 (Scheme 2).

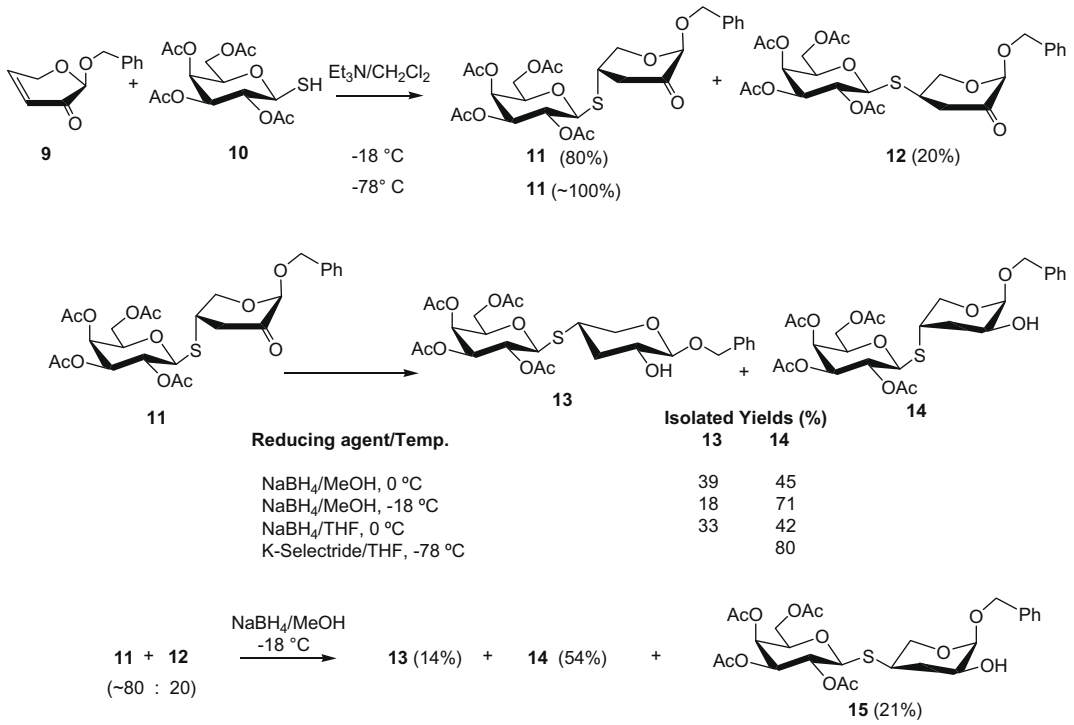
To go further into the study of structure–activity relationship in thiodisaccharides, we explored the use of a pyranone derived form a pentose as Michael acceptor or 1-thiogalactopyranose (Scheme 3). The target thiodisaccharides are expected to act as inhibitors of the β-galactosidase from *Escherichia coli*, since analogous *S*-(1→4) linked disaccharides of Gal*p* bonded to a 3-deoxy-4-thiohexopyranose were inhibitors of this enzyme.[9] Also, the thiodisaccharides were designed taking into account some stereoelectronic and conformational requirements of carbohydrate derived inhibitors to fit in the binding site of the enzyme, as explained later (see Section 2.2). Thus, 2-(*S*)-benzyloxy-2*H*-pyran-3(6*H*)-one (**9**) was prepared in enantiomerically pure form from benzyl β-D-arabinopyranoside, following the procedure previously described by us.[20]

Michael addition of per-*O*-acetyl-β-D-1-thiogalactopyranose[9] (**10**) to the enone **9** in CH_2Cl_2 was carried out at −18 °C, in the presence of TEA as catalyst. Under these conditions, a 4:1 mixture of **11**:**12** was formed. Separation of these stereoisomers by column chromatography was difficult, although in some cases partial separation was possible by adding TEA to the eluting solvent. The ^{1}H NMR spectrum of **11** admitted a first order analysis and the assignments were fully confirmed by 2D NMR techniques. Consistent with the equatorial disposition of the substituent at C-4, the H-4 signal appeared as a multiplet that exhibited relatively small coupling constants with the vicinal H-3a, H-3b, H-5a, H-5b (5.5, 4.3, 3.0, 3.8 Hz, respectively). Furthermore, the long-range coupling constant $J_{3b,5b}$ (1.8 Hz), also observed in pyranoses,[21] was in accordance with the respective quasy-equatorial/equatorial disposition of H-3b and H-5b in **11**. The configuration of C-4 in this compound indicates the approach of the 1-thioaldose to the enone system of **9** from the β face, opposite to the anomeric substituent. Lowering the temperature of the Michael addition resulted in an increase in the diastereoselectivity. Thus, the reaction of **9** with **10** performed at −78 °C afforded only **11** in almost quantitative yield.

Reduction of **11** with $NaBH_4$ in MeOH at 0 °C gave the thiodisaccharides **13** and **14** in 39% and 45% yield, respectively. They possess the β-D-*erythro* (**13**) and β-D-*threo* (**14**) configuration for the reducing end, according to their ^{1}H-NMR data. Furthermore, the large coupling constants observed for the trans-diaxial protons of **13** ($J_{1,2}$ = 7.0 Hz, $J_{2,3ax}$ = 11.5 Hz and $J_{4,5ax}$ = 10.7 Hz) indicated that the 4-thio-3-deoxy unit adopts mainly the $^4C_1$ conformation, with all substituents (at C-1, C-2 and C-4) in an equatorial disposition. On the other hand, the coupling constant values for the major product **14** ($J_{2,3a}$ = 7.2 Hz, $J_{2,3b}$ = 3.8 Hz, $J_{3a,4}$ = 3.7 Hz and $J_{3b,4}$ = 7.5 Hz) suggests contribution of the $^1C_4$ conformer to the conformational equilibrium. These results are in accordance with the behavior of similar compounds having small *S*-linked residues at C-4.[18]

Reduction of **11** with K-Selectride in THF at −78 °C produced **14** as a single product in 80% yield. It is interesting to note that according to this and previous studies,[18] K-Selectride is useful to reduce 4-thio-2-uloses derived from pentoses with high stereoselectivity, no matter the size of the sulfur substituent. Furthermore, in contrast with the analogous reduction of **3** with $NaBH_4$/THF no isomerization was observed for the reduction of **11**. This fact may be explained on the basis of the mechanism proposed for the isomerization (Scheme 2). The electron-withdrawing effect of the acetoxymethyl group in the non-reducing end of **11** would diminish the electron donation of sulfur, affecting the stabilization of the intermediate species. In contrast, the 1-thioxylopyranoside in **3** lacks of the acetoxymethyl substituent. Furthermore, the conformational flexibility of the pentopyranoside ring may contribute to relieve the instability caused by the axial 4-thioglycopyranosyl substituent in **14**.

The reduction of the original 4:1 mixture of **11** and **12** (obtained as described above by coupling of **9** and **10** at −18 °C) was conducted with $NaBH_4$ in MeOH at −18 °C. In this case, three isomeric thiodisaccharides were obtained, which were purified by column chromatography. The previously described **13** and **14** were isolated



Scheme 3. Synthesis of thiodisaccharides **11**–**15**.

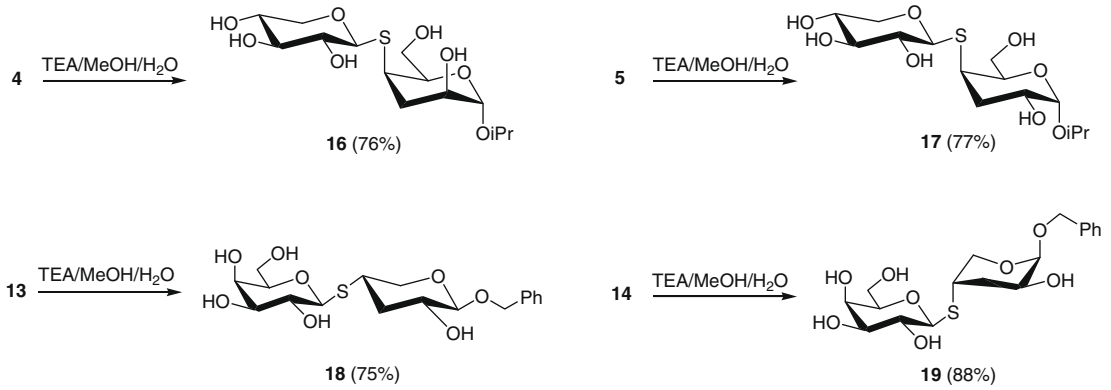
first in 14% and 54% yield, respectively. The more polar thiodisaccharide **15** was then obtained in 21% yield. It was clear that this last compound was the only reduction product of **12**, as the configuration of the C-2 and C-4 stereocenters was established by ^{1}H NMR as *S* and *R*, respectively. The $J_{1,2}$ value (3.6 Hz) was consistent with an equatorial-axial disposition for H-1 and H-2, and the large *J* values ($J_{2,3ax} = J_{3ax,4} = 12.2$ Hz, $J_{4,5ax} = 11.5$ Hz) indicated that the coupled protons were axially disposed. A long-range coupling constant $J_{3eq,5eq}$ (1.8 Hz) was measured and the value is characteristic of protons equatorially oriented and connected through four bonds.[21]

Thiodisaccharides **4** and **5** were O-deacetylated by treatment with MeOH/Et_3N/H_2O (Scheme 4). The free isopropyl 3-deoxy-4-*S*-(β-D-xylopyranosyl)-4-thio-α-D-*lyxo*- and α-D-*xylo*-hexopyranosides (**16** and **17**, respectively) obtained were purified by ion-exchange chromatography with Dowex MR-3C resin followed by elution through a reverse-phase column. Similar O-deacetylation of **13** and **14** afforded, respectively the free thiodisaccharides **18** and **19**.

Thiodisaccharide **17** is an analogue of the structure found in agarans from the red seaweed *Georgiella confluens*.[22] The agarans isolated from red seaweeds are usually galactans constituted by a regular backbone of alternating (1→3) β-D-Gal*p* and (1→4) α-L-Gal*p* units. A variety of substituents (sulfate, methyl, pyruvate or even β-D-xylopyranose residues) appeared linked to this regular chain. However, polysaccharides having the motif β-D-xylopyranosyl(1→4)-D-galactopyranose are rather unusual in nature. The thiodisaccharide **18** is structurally related to the fragment β-D-Gal(1→4)-D-Xyl present in the linkage region between the protein and the polysaccharide chain of heparin and other animal proteoglycans.[23]

2.2. Evaluation of the inhibitory activity

Specific glycosidases are involved in the hydrolysis of xylose residues in polysaccharides. Thus, xylanase and β-xylosidase are key enzymes for the degradation of the plant polysaccharide xylan.[24] The β-xylosidase (or xylobiases, or *exo*-β-1,4-xylanase, EC



Scheme 4. Synthesis of thiodisaccharides **16**–**19**.

3.2.1.37) is responsible for the complete degradation of xylan, since it cleaves short chain xylooligosaccharides from the reducing end, liberating D-xylose as the only product of hydrolysis.[24] The best substrate of xylosidase is xylobiose and some thioxylobiosides have been synthesized and tested as inducers and inhibitors of this enzyme.[25] Also, imidazolo[1,2-*a*]piperidinoses derived from D- and L-treose and D- and L-erythrose have been synthesized and their inhibitory activity against β-xylosidase from *Aspergillus niger* has been determined.[26] Recently, simple amino alcohols have been described as inhibitors of β-xylosidase from *Selenomonas ruminantium*.[27]

The inhibitory activity of thiodisaccharides **16** and **17** against the β-xylosidase from *A. niger* was studied following the methodology described in detail in Section 4.3.1. No inhibition of this enzyme was detected even at large concentrations of thiodisaccharides. They neither display any inhibitory activity towards the β-glucosidase from almonds nor to the β-galactosidase from *E. coli*.

The free thiodisaccharides **18** and **19** were also evaluated as inhibitors of the β-galactosidase from *E. coli*. This enzyme has been extensively studied,[28] and it has shown to be highly specific for the β-galactopyranosyl non-reducing end of the substrate, which may be linked to a wide variety of aglycons. Enzyme–substrate interactions have been studied by labeling of specific amino acids located at the active site,[29] and also by X-ray[30] and NMR[14,31a] experiments. Among the thiosugars, simple thiogalactopyranosides, such as isopropyl β-D-thiogalactoside are good inhibitors.[31] We have reported[9] that thiodisaccharides having a β-D-1-thiogalactose S-bonded (1→4) to a 3-deoxy-4-thiohexopyranose moiety display inhibitory activity against the β-galactosidase from *E. coli*. Jiménez-Barbero and coworkers[14] demonstrated that 4-thiolactose competes with lactose for the same binding site of the enzyme and they showed, by NMR and ab initio quantum mechanical studies, that the 3D-shapes of the ligand-inhibitor within the enzyme binding site depend on the relative size of the stereoelectronic barriers for the chair conformation and for the rotation of the interglycosidic linkage. Therefore, as mentioned above, in the design of the inhibitor we took into account the higher flexibility of a pentopyranose ring compared to that of a hexopyranose ring. Also, as the presence of a hydrophobic group distal to the thiogalactose residue enhances the inhibitory activity[32] (for example, phenylethyl β-D-thiogalactopyranoside behaves as a strong inhibitor[31b]) we judged convenient to increase the hydrophobicity of the reducing end of the thiodisaccharide by introducing a benzyl 3-deoxy pentopyranoside unit. For all these reasons we selected benzyl pent-2-ulo-3-enopyranoside **9** as Michael acceptor.

In accordance with our expectations, thiodisaccharides **18** and **19** displayed inhibitory activity against the β-galactosidase from *E. coli*. The inhibition was evaluated using *o*-nitrophenyl β-D-galactopyranoside as substrate, which was incubated with increasing concentrations of the thiodisaccharides. The released *o*-nitrophenol was quantified spectrophotometrically and the Lineweaver–Burk plots (Figs. 1 and 2) were constructed. Thiodisaccharide **18** proved to be a non-competitive inhibitor of the enzyme, with K_i = 0.80 mM, whereas **19** was a strong mixed-type inhibitor with K_i = 32 μM.

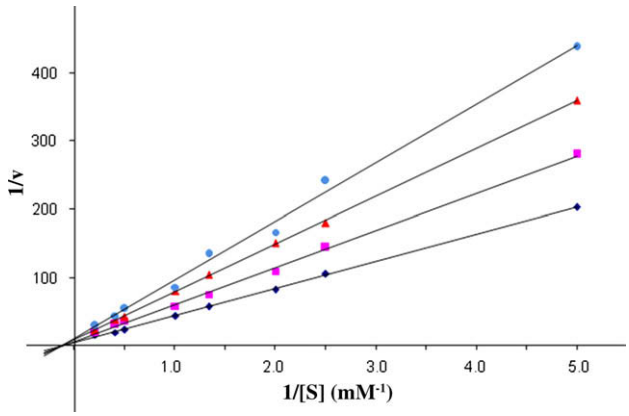


Figure 1. Lineweaver–Burk plot for inhibition of *E. coli* β-galactosidase by thiodisaccharide **18** at concentration: ◇: 0.00, □: 0.40, △: 0.80 and ○: 1.20 mM of inhibitor.

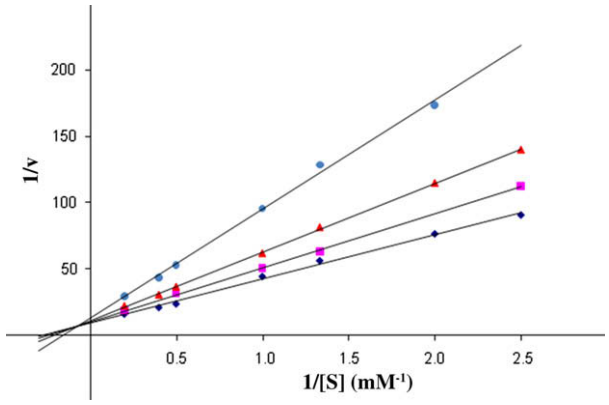


Figure 2. Lineweaver–Burk plot for inhibition of *E. coli* β-galactosidase by thiodisaccharide **19** at concentration: ◇: 0.00, □: 0.01, △: 0.02 and ○: 0.05 mM of inhibitor.

3. Conclusion

The Michael addition showed to be a very useful reaction for the construction of the sulfur mediated interglycosidic bond between a pentopyranose *S*-(1→4)-linked to a hexopyranose and viceversa. In these reactions sugar enones (dihydropyranones) derived from pentoses or hexoses were useful Michael acceptors of 1-thioaldoses to give, with very high diastereoselectivity, the corresponding 3-deoxy-4-*S*-(β-D-glycopyranosyl)-4-thiopyranosyd-2-ulose in almost quantitative yields. The stereochemical course of the carbonyl reduction in the ulose thiodisaccharides was highly sensitive to the reducing agent and the reduction conditions employed. In general, a higher diastereoselectivity was observed at lower temperatures. In some particular cases (with $NaBH_4$ or K-Selectride in anhydrous THF), the reduction took place with inversion of the C-4 stereocenter. A distinct structural feature of this unexpected isomerization is that it seems to take place only when the substituent at C-4 is a 1-thiopentopyranosyl derivative, as it was not observed for similar reductions of 4-thiohexopyranosyl analogues studied in the present and previous reports.[9]

Among the new thiodisaccharides synthesized, benzyl 3-deoxy-4-*S*-(β-D-Gal*p*)-4-thio-β-D-*threo*-pentopyranoside (K_i = 32 μM) was stronger inhibitor of the β-galactosidase from *E. coli* compared to benzyl 3-deoxy-4-*S*-(β-D-Gal*p*)-4-thio-β-D-*erythro*-pentopyranoside (K_i = 0.80 mM), which indicates that the configuration of the C-2 stereocenter of the pentose is relevant for the activity. The former thiodisaccharide was also a better inhibitor than the analogues of Gal*p*-*S*-(1→4) linked to 3-deoxy-4-thiohexopyranose unit having the D-*lyxo* or D-*xylo* configurations (K_i = 0.12 mM and 0.16 mM, respectively). The increment of the inhibitory activity seems to correlate with the higher conformational flexibility of the 3-deoxy-4-thio-β-D-*threo*-pentopyranose ring with respect to that of the hexopyranoses, which could facilitate the accommodation of the inhibitor in the binding site of the enzyme. Moreover, the inhibition may be additionally increased by the higher hydro-

phobicity of the benzyl 3-deoxypentopyranoside regarding the 3-deoxyhexopyranoside.

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

4.2. Synthesis

4.2.1. 2-Propyl per-*O*-acetyl-3-deoxy-4-*S*-(β-D-Xyl*p*)-4-thiohexopyranosid-2-ulose (3)

2-Propyl 6-*O*-acetyl-3,4-dideoxy-α-D-*glycero*-hex-3-enopyranosid-2-ulose[16] (**1**, 323 mg, 1.42 mmol) and 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranose[15] (**2**, 410 mg, 1.42 mmol) were dissolved in anhydrous CH_2Cl_2 (6 mL). The reaction mixture was cooled to −18 °C and Et_3N (10 μL) was added. After 30 min of stirring at −18 °C, TLC showed complete consumption of the starting materials, and a single spot of R_f = 0.49 (hexane/EtOAc 1:1). The mixture was evaporated to dryness and the residue was purified by column chromatography using hexane/EtOAc 2.5:1 to afford **3** (710 mg, 96%) as a white solid, which was recrystallized from EtOH. Mp 152 °C (EtOH); $[\alpha]_D^{20}$ −18.9 (*c* 0.6, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.16 (t, 1H, $J_{2',3'} \approx J_{3',4'}$ = 8.3 Hz, H-3′), 4.93 (ddd, 1H, $J_{4',5'eq}$ = 5.0, $J_{4',3'}$ = 8.3, $J_{4',5'ax}$ = 8.9 Hz, H-4′), 4.91 (t, 1 H, $J_{1',2'}$ = 8.5, $J_{2',3'}$ = 8.3 Hz, H-2′), 4.78 (ddd, 1H, $J_{4,5}$ = 1.8, $J_{5,6a}$ = 4.6, $J_{5,6b}$ = 7.4 Hz, H-5), 4.73 (br s, 1H, H-1), 4.63 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1′), 4.27 (dd, 1H, $J_{5,6a}$ = 4.6, $J_{6a,6b}$ = 11.7 Hz, H-6a), 4.23 (dd, 1H, $J_{5,6b}$ = 7.4, $J_{6a,6b}$ = 11.7 Hz, H-6b), 4.20 (dd, 1H, $J_{4',5'eq}$ = 5.0, $J_{5'ax,5'eq}$ = 11.7 Hz, H-5′eq), 4.00 (m, 1H, *J* = 6.2 Hz, C*H*$(CH_3)_2$), 3.63 (ddd, 1H, $J_{3ax,4}$ = 1.6, $J_{4,5}$ = 1.8, $J_{3eq,4}$ = 4.9 Hz, H-4), 3.33 (dd, 1H, $J_{4',5'ax}$ = 8.9, $J_{5'eq,5'ax}$ = 11.7 Hz, H-5′ax), 3.16 (dd, 1H, $J_{3a,4}$ = 4.9, $J_{3a,3b}$ = 15.2 Hz, H-3a), 2.77 (dd, 1H, $J_{3b,4}$ = 1.6, $J_{3a,3b}$ = 15.2 Hz, H-3b), 2.08, 2.07, 2.05, 2.04 (4s, 12H, 4CH_3CO), 1.27, 1.18 (2 d, 6H, CH(CH_3)$_2$). ^{13}C NMR (50.28 MHz, $CDCl_3$) δ = 198.8 (C-2), 170.6, 169.8, 169.7, 169.6 (*C*OCH$_3$), 97.9 (C-1), 82.3 (C-1′), 71.9, 71.8, 69.7, 68.6, 68.4, 65.3, 64.7 (C-5, C-6, C-2′, C-3′, C-4′, C-5′, *C*H$(CH_3)_2$), 44.7, 43.6 (C-3, C-4), 23.3, 21.8 [(CH_3)$_2$CH], 20.8 (×2), 20.7 (×2) (*C*H$_3$CO). Anal. Calcd for $C_{22}H_{32}O_{12}S$: C, 50.76; H, 6.20; S, 6.16. Found: C, 50.60; H, 6.12; S, 6.10.

4.2.2. Reduction of 3

4.2.2.1. Reduction with $NaBH_4$ in MeOH/THF 1:1. Synthesis of 2-propyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-4-thio-α-D-*lyxo*-hexopyranoside (4) and 2-propyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-4-thio-α-D-*xylo*-hexopyranoside (5). Compound **3** (150 mg, 0.29 mmol) was dissolved in a mixture of MeOH/THF 1:1 (5 mL) and $NaBH_4$ (11 mg, 0.29 mmol) was added at 0 °C with stirring. After 30 min two main products were detected by TLC (R_f = 0.50 and R_f = 0.43, hexane/EtOAc 1:1.5). The mixture was made neutral by addition of Dowex 50 H^+ resin, the solvent was evaporated and purification was performed by column chromatography (hexane/EtOAc 1.5:1). From the first fractions of the column, was isolated crystalline **4** (35 mg, 23%), mp 113 °C (MeOH), $[\alpha]_D^{20}$ −3.7 ((*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.20 (t, 1H, $J_{2',3'} \approx J_{3',4'}$ = 8.2 Hz, H-3′), 4.97 (t, 1H, $J_{1',2'} \approx J_{2',3'}$ = 8.3 Hz, H-2′), 4.96 (ddd, 1H, $J_{4',5'eq}$ = 4.9, $J_{3',4'}$ = 8.2, $J_{4',5'ax}$ = 8.7 Hz, H-4′), 4.89 (br s, 1H, H-1), 4.76 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1′), 4.43 (ddd, 1H, $J_{4,5}$ = 2.7, $J_{5,6b}$ = 4.3, $J_{5,6a}$ = 7.3 Hz, H-5), 4.25 (dd, 1H, $J_{5,6a}$ = 7.3, $J_{6a,6b}$ = 11.7 Hz, H-6a), 4.24 (dd, 1H, $J_{4',5'eq}$ = 4.9, $J_{5'ax,5'eq}$ = 11.8 Hz, H-5′eq), 4.21 (dd, 1H, $J_{5,6b}$ = 4.3, $J_{6a,6b}$ = 11.7 Hz, H-6b), 3.96 (m, 1H, *J* = 6.2 Hz, C*H*$(CH_3)_2$), 3.61 (m, 2H, H-2 + HO), 3.41 (dd, 1H, $J_{4',5'ax}$ = 8.7, $J_{5'ax,5'eq}$ = 11.8 Hz, H-5′ax), 3.28 (br q, 1H, $J_{3b,4} \approx J_{4,5}$ = 2.7, $J_{3a,4}$ = 3.6 Hz, H-4), 2.29 (ddd, 1H, $J_{2,3a} \approx J_{3a,4}$ = 3.6, $J_{3a,3b}$ = 15.0 Hz, H-3a), 2.24 (ddd, 1H, $J_{2,3a} \approx J_{3a,4}$ = 2.6, $J_{3a,3b}$ = 15.0 Hz, H-3b), 2.08, 2.06 (×2), 2.05 (s, 3H each, CH_3CO), 1.22, 1.16 (2d, 6H, CH(CH_3)$_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 170.6, 169.8, 169.7, 169.5 (*C*OCH$_3$), 99.0 (C-1), 82.1 (C-1′), 71.7 (C-3′), 69.7 (C-2′), 69.5 [O*C*H$(CH_3)_2$], 68.4 (C-4′), 68.0 (C-5), 67.7 (C-2), 65.3 (C-5′), 65.1 (C-6), 39.2 (C-4), 30.4 (C-3), 23.2, 21.5 [(CH_3)$_2$CH], 20.8 (×2), 20.7 (×2) (*C*H$_3$CO). Anal. Calcd for $C_{22}H_{34}O_{12}S$: C, 50.57; H, 6.56; S, 6.14. Found: C, 50.45; H, 6.63; S, 6.04.

Further elution of the column afforded crystalline **5** (105 mg, 70%), mp 135 °C (MeOH), $[\alpha]_D^{20}$ −7.8 (*c* 0.5, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.15 (t, 1H, $J_{2',3'} \approx J_{3',4'}$ = 7.8 Hz, H-3′), 4.93 (dd, 1H, $J_{2',3'}$ = 7.8, $J_{1',2'}$ = 8.0 Hz, H-2′), 4.92 (ddd, 1H, $J_{4',5'eq}$ = 5.1, $J_{3',4'}$ = 7.8, $J_{4',5'ax}$ = 8.6 Hz, H-4′), 4.92 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.65 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.28–4.26 (m, 1H, H-5), 4.23 (dd, 1H, $J_{4',5'eq}$ = 5.1, $J_{5'eq,5'ax}$ = 11.5 Hz, H-5′eq), 4.19 (dd, 1H, $J_{5,6a}$ = 4.6, $J_{6a,6b}$ = 11.9 Hz, H-6a), 4.14 (dd, 1H, $J_{5,6b}$ = 8.0, $J_{6a,6b}$ = 11.9 Hz, H-6b), 4.02–3.99 (m, 1H, H-2), 3.94 (m, 1H, *J* = 6.0 Hz, C*H*$(CH_3)_2$), 3.38 (dd, 1H, $J_{4',5'ax}$ = 8.6, $J_{5'ax,5'eq}$ = 11.5 Hz, H-5′ax), 3.26 (m, 1H, H-4), 2.20 (m, 1H, H-3a), 2.08, 2.06 (×2), 2.05 (4 s, 3H each, CH_3CO), 1.22, 1.16 (2 d, 6H, CH(CH_3)$_2$), 2.00 (m, 1H, H-3b). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 169.8, 169.7 (×2), 169.4 (*C*OCH$_3$), 96.8 (C-1), 83.1 (C-1′), 71.6 (C-3′), 70.9 [O*C*H$(CH_3)_2$], 69.9, 68.4 (C-2′, C-4′), 67.9 (C-5), 65.4 (C-6), 64.9 (C-5′), 64.1 (C-2), 43.4 (C-4), 34.6 (C-3), 23.2, 22.0 [(CH_3)$_2$CH], 20.8 (×2), 20.7 (×2) (*C*H$_3$CO). Anal. Calcd for $C_{22}H_{34}O_{12}S$: C, 50.57; H, 6.56; S, 6.14. Found: C, 50.74; H, 6.60; S, 5.84.

4.2.2.2. Reduction with $NaBH_4$/THF. Synthesis of 2-propyl 6-*O*-acetyl-3-deoxy-4-*S*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-4-thio-α-D-*ribo*-hexopyranoside (6). To a solution of **3** (430 mg, 0.83 mmol) in anhydrous THF (5 mL) was added $NaBH_4$ (78.2 mg, 2.08 mmol) and stirred at 0 °C. After 30 min, TLC showed no starting material remaining and one major product of R_f = 0.38 (hexane/EtOAc 1:1.5). Fainter spots of R_f = 0.50 and 0.43, comigrating respectively with **4** and **5**, were also observed. The mixture was diluted with MeOH and treated with Dowex 50 H^+, then concentrated and subjected to column chromatography (hexane/EtOAc 1.5:1). Thiodisaccharides **4** (20 mg, 5%) and **5** (66 mg, 15%) were isolated from the first fraction of the column and they exhibited the same properties reported above. The less polar, major product was obtained crystalline and identified as **6** (320 mg, 74%), mp 132 °C (*i*PrOH), $[\alpha]_D^{20}$ +23.8 ((*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 5.17 (t, 1H, $J_{3',4'} \approx J_{2',3'}$ = 8.3 Hz), 4.94 (ddd, 1H, $J_{4',5'a}$ = 5.0, $J_{3',4'}$ = 8.3 Hz, $J_{4',5'b}$ = 8.8 Hz, H-4′), 4.92 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.91 (t, 1H, $J_{1',2'} \approx J_{2',3'}$ = 8.3 Hz, H-2′), 4.70 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1′), 4.44 (dd, 1H, $J_{5,6a}$ = 2.0, $J_{6a,6b}$ = 11.9 Hz, H-6a), 4.34 (dd, 1H, $J_{5,6b}$ = 5.0, $J_{6a,6b}$ = 11.9 Hz, H-6b), 4.23 (dd, 1H, $J_{4',5'a}$ = 5.0, $J_{5'a,5'b}$ = 11.8 Hz, H-5′a), 3.95 (m, 1H, *J* = 6.2 Hz, C*H*$(CH_3)_2$), 3.86 (ddd, 1 H, $J_{5,6a}$ = 2.0, $J_{5,6b}$ = 5.0, $J_{4,5}$ = 11.0 Hz, H-5), 3.71 (dddd, 1H, $J_{1,2}$ = 3.7, $J_{2,3eq}$ = 4.6, $J_{2,OH}$ = 11.4, $J_{2,3ax}$ = 12.2Hz, H-2), 3.38 (dd, 1H, $J_{4',5'b}$ = 8.8, $J_{5'a,5'b}$ = 11.8 Hz, H-5′b), 2.89 (ddd, 1H, $J_{3eq,4}$ = 4.2, $J_{4,5}$ = 11.0, $J_{3ax,4}$ = 13.0 Hz, H-4), 2.24 (ddd, 1H, $J_{3eq,4}$ = 4.2, $J_{2,3eq}$ = 4.6, $J_{3eq,3ax}$ = 12.2 Hz, H-3 eq), 2.11, 2.08, 2.07, 2.06 (4s, 12H, CH_3CO), 1.90 (d, 1H, $J_{2,OH}$ = 11.4 Hz, HO–), 1.83 (ddd, 1H, $J_{2,3ax} = J_{3eq,3ax}$ = 12.2, $J_{3ax,4}$ = 13.0 Hz, H-3ax), 1.26, 1.20 (2d, 6H, CH(CH_3)$_2$). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 170.6, 169.8, 169.7, 169.4 (*C*OCH$_3$), 96.2 (C-1), 83.0 (C-1′), 71.8 (C-3′), 70.6 (O*C*H$(CH_3)_2$),

70.0 (C-2′), 69.9 (C-5′), 68.4 (C-4′), 67.4 (C-2), 65.0 (C-5′), 64.0 (C-6), 39.8 (C-4), 35.7 (C-3), 23.2, 21.9 ($CH(CH_3)_2$), 20.8, 20.7 (×2) (CH_3CO). Anal. Calcd for $C_{22}H_{34}O_{12}S$: C, 50.57; H, 6.56; S, 6.14. Found: C, 50.35; H, 6.68; S, 6.04.

4.2.2.3. Reduction with K-Selectride/THF. Synthesis of 2-propyl 2,6-di-*O*-acetyl-3-deoxy-4-*S*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-4-thio-α-D-*xylo*-hexopyranoside (7) and 2-propyl 2,6-di-*O*-acetyl-3-deoxy-4-*S*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-4-thio-α-D-*ribo*-hexopyranoside (8). To a solution of compound **3** (70 mg, 0.15 mmol) in THF (1.5 mL), cooled at −78 °C, was added K-Selectride (0.23 mL, 0.23 mmol) with constant stirring. After 3 h the mixture was made neutral by addition of Dowex 50 H^+ resin and the solvent was evaporated. The residue showed by TLC some spots of low mobility, suggesting partially O-deacetylated products. Therefore, the mixture was treated with 1:1 acetic anhydride-pyridine (1 mL). Upon acetylation, TLC (hexane/AcOEt 1:1) exhibited a major product (R_f = 0.41), which was purified by column chromatography (hexane/EtOAc 2.5:1). Although the product (56 mg, 73%) was chromatographically homogeneous, the NMR analysis revealed the presence of two components (3.5:1 ratio). The mixture could not be separated with all the solvent systems attempted. The major component of the mixture was identified as **7**, and its structure was confirmed as this product showed identical properties to that obtained by acetylation of **5**. Compound **7**: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.16 (t, 1H, $J_{2',3'} \approx J_{3',4'}$ = 8.0 Hz, H-3′), 5.13 (dt, 1H, $J_{1,2}$ = 3.7, $J_{2,3eq}$ = 4.6, $J_{2,3ax}$ = 12.0, H-2), 5.07 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.93 (dd, 1H, $J_{2',3'}$ = 7.8, $J_{1',2'}$ = 8.0 Hz, H-2′), 4.92 (ddd, 1H, $J_{4',5'eq}$ = 5.1, $J_{3',4'}$ = 7.8, $J_{4',5'ax}$ = 8.6 Hz, H-4′), 4.68 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.37 (ddd, 1H, $J_{4,5}$ = 2.3, $J_{5,6a}$ = 4.1, $J_{5,6b}$ = H-5), 4.23 (dd, 1H, $J_{4',5'eq}$ = 4.8, $J_{5'eq,5'ax}$ = 11.8 Hz, H-5′eq), 4.19 (dd, 1H, $J_{5,6a}$ = 4.1, $J_{6a,6b}$ = 11.7 Hz, H-6a), 4.14 (dd, 1H, $J_{5,6b}$ = 7.8, $J_{6a,6b}$ = 11.7 Hz, H-6b), 3.89 (m, 1H, J = 6.2 Hz, $CH(CH_3)_2$), 3.38 (dd, 1H, $J_{4',5'ax}$ = 8.4, $J_{5'ax,5'eq}$ = 11.8 Hz, H-5′ax), 3.26 (m, 1H, $J_{4,5}$ = 2.3, $J_{3eq,4}$ = 3.0, $J_{3ax,4}$ = 3.7, H-4), 2.29 (td, 1H, $J_{3ax,4}$ = 3.7, $J_{2,3ax}$ = 12.0, $J_{3ax,3eq}$ = 12.6, H-3ax), 2.07 (m, 16H, 3H each + 1H, CH_3CO + H-3 eq), 1.24, 1.13 (2d, 6H, J = 6.2, $CH(CH_3)_2$), 2.00 (m, 1H, H-3 eq). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 170.5, 170.2, 169.8, 169.3, 169.1 ($COCH_3$), 94.3 (C-1), 82.9 (C-1′), 71.5 (C-3′), 70.5 [$OCH(CH_3)_2$], 69.7, 68.3 (C-2′, C-4′), 67.6 (C-5), 65.4, 64.9, 64.1 (C-2, C-5′, C-6), 43.2 (C-4), 30.6 (C-3), 23.1, 21.7 [$(CH_3)_2CH$], 20.8 (×2), 20.7 (×2), 20.6 (CH_3CO).

Compound **8** showed the following diagnostic signals: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.03 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.79 (ddd, 1H, $J_{1,2}$ = 3.5, $J_{2,3eq}$ = 4.6, $J_{2,3ax}$ = 11.8 Hz, H-2), 4.69 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 3.94 (ddd, 1H, $J_{4,5}$ = 11.0, $J_{5,6a}$ = 2.0, $J_{5,6b}$ = 4.7 Hz, H-5), 2.96 (m, 1H, $J_{3eq,4}$ = 4.6, $J_{4,5}$ = 11.0, $J_{3ax,4}$ = 12.6 Hz, H-4); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 93.7 (C-1), 83.0 (C-1′), 39.8 (C-4), 31.0 (C-3).

4.2.3. Benzyl 3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-β-D-*glycero*-pentopyranosid-2-ulose (11)

A solution of (2*S*)-2-benzyloxy-2*H*-pyran-3(6*H*)-one[20] (**9**, 414 mg, 2.03 mmol) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose[9] (**10**, 739 mg, 2.03 mmol) in dry CH_2Cl_2 (2.5 mL) was cooled to −78 °C and Et_3N (13 μL) was added. After stirring at −78 °C for 3 h a very major product was detected by TLC (R_f = 0.22, hexane/EtOAc 1:1). The solvent was evaporated to dryness to afford **11** (1.15 g, ~100%) as a white foam. This crude product was pure enough to be used as starting material in the following reactions. Purification of a portion of this material by column chromatography (hexane/EtOAc 2.5:1) gave an analytical sample of **11**. $[\alpha]_D^{20}$ −69.8 ((c 0.9, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.38–7.30 (m, 5H, H-aromatic), 5.43 (dd, 1H, $J_{4',5'}$ = 0.8, $J_{3',4'}$ = 3.3, H-4′), 5.23 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2′), 5.05 (dd, 1H, $J_{3',4'}$ = 3.4, $J_{2',3'}$ = 10.0 Hz, H-3′), 4.81 (d, 1H, J = 11.7 Hz, PhCH_2), 4.75 (br s, 1H, $J_{1,3eq}$ <1 Hz, H-1), 4.63 (d, 1H, J = 11.7, PhCH_2), 4.57 (d, 1H, $J_{1',2'}$ = 9.9 Hz, H-1′), 4.42 (dd, 1H, $J_{4,5a}$ = 3.0, $J_{5a,5b}$ = 12.0 Hz, H-5a), 4.15 (dd, 1H, $J_{5',6'a}$ = 7.0, $J_{6'a,6'b}$ = 11.4 Hz, H-6′a), 4.10 (dd, 1H, $J_{5',6'b}$ = 6.3, $J_{6'a,6'b}$ = 11.4 Hz, H-6′b), 3.93 (br t, 1H, $J_{4',5'}$ = 0.8, $J_{5',6'b}$ = 6.3, $J_{5',6'a}$ = 7.0 Hz, H-5′), 3.80 (ddd, 1H, $J_{3b,5b}$ = 1.8, $J_{4,5b}$ = 3.8, $J_{5a,5b}$ = 12.0 Hz, H-5b), 3.74 (m, 1H, H-4), 3.14 (dd, 1H, $J_{3a,4}$ = 5.5, $J_{3a,3b}$ = 15.5 Hz, H-3a), 2.61 (br ddd, 1H, $J_{3b,5b}$ = 1.8, $J_{3b,4}$ = 4.3, $J_{3a,3b}$ = 15.5 Hz, H-3b), 2.15, 2.06, 2.05, 1.98 (4s, 12H, CH_3CO). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 199.1 (C-2), 170.5, 170.2, 170.0, 169.6 ($COCH_3$), 136.4, 128.3, 128.2 (C-aromatic), 98.4 (C-1), 83.7 (C-1′), 74.7 (C-5′), 71.7 (C-3′), 70.1 (CH_2Ph), 67.2 (C-4′), 67.0 (C-2′), 63.4 (C-5), 61.5 (C-6′), 42.5 (C-3, C-4), 20.8, 20.7 (×2), 20.6 (CH_3CO). Anal. Calcd for $C_{26}H_{32}O_{12}S$: C, 54.92; H, 5.67; S, 5.64. Found: C, 54.80; H, 5.82; S, 5.79.

When the reaction was conducted at −18 °C, an inseparable mixture of **11** and its α-L-*glycero* isomer **12** was obtained (4:1 ratio, estimated by NMR). Compound **12** partial ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 97.7 (C-1), 84.4 (C-1′), 63.2 (C-5), 42.9, 42.7 (C-3, C-4).

4.2.4. Reduction of 11

4.2.4.1. Reduction with $NaBH_4$/MeOH. Synthesis of benzyl 3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-β-D-*erythro*-pentopyranoside (13) and benzyl 3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-β-D-*threo*-pentopyranoside (14). To a solution of **11** (751 mg, 1.32 mmol) in MeOH (7.0 mL), cooled to 0 °C, $NaBH_4$ (55 mg, 1.48 mmol) was added under stirring. After 30 min, TLC (hexane/EtOAc 1:1.5) showed the complete conversion of the starting material into two compounds of R_f = 0.40 and 0.33. The mixture was treated with Dowex 40 (H^+) resin and concentrated, and the residue was purified by column chromatography (hexane/EtOAc 2.5:1). From the first fractions of the column was isolated syrupy **13** (290 mg, 39%); $[\alpha]_D^{20}$ −64.5 (c 0.4, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.38–7.30 (m, 5H, H-aromatic), 5.43 (br d, 1H, $J_{4',5'}$ <1, $J_{3',4'}$ = 3.0, H-4′), 5.25 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2′), 5.05 (dd, 1H, $J_{3',4'}$ = 3.0, $J_{2',3'}$ = 10.0 Hz, H-3′), 4.90, 4.58 (2 d, 1H each, J = 11.8 Hz, PhCH_2), 4.49 (d, 1H, $J_{1',2'}$ = 9.9 Hz, H-1′), 4.27 (d, 1H, $J_{1,2}$ = 7.0 Hz, H-1), 4.15 (m, 1H, H-5 eq), 4.14 (dd, 1 H, $J_{5',6'a}$ = 7.0, $J_{6'a,6'b}$ = 11.5 Hz, H-6′a), 4.10 (dd, 1H, $J_{5',6'b}$ = 6.3, $J_{6'a,6'b}$ = 11.5 Hz, H-6′b), 3.92 (br t, 1H, $J_{4',5'}$ <1, $J_{5',6'a}$ = 6.3, $J_{5',6'b}$ = 7.0 Hz, H-5′), 3.58 (ddd, 1H, $J_{2,3eq}$ = 4.8, $J_{1,2}$ = 7.0, $J_{2,3ax}$ = 11.5 Hz, H-2), 3.35 (dd, 1H, $J_{4,5ax}$ = 10.7, $J_{5ax,5eq}$ = 11.4 Hz, H-5ax), 3.21 (dddd, 1H, $J_{3eq,4}$ = 4.3, $J_{4,5eq}$ = 4.5, $J_{4,5ax}$ = 10.7, $J_{3ax,4}$ = 11.6 Hz, H-4), 2.45 (br s, 1H, HO), 2.34 (dddd, 1H, $J_{3eq,5eq}$ = 1.8, $J_{3eq,4}$ = 4.3, $J_{2,3eq}$ = 4.8, $J_{3a,3b}$ = 13.0 Hz, H-3eq), 2.17, 2.07 (×2), 1.99 (4s, 3H each, CH_3CO), 1.49 (ddd, 1H, $J_{2,3ax}$ = 11.5, $J_{3ax,4}$ = 11.6, $J_{3ax,3eq}$ = 13.0 Hz, H-3ax). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 170.2 (×2), 170.0, 169.5 ($COCH_3$), 137.1, 128.6, 128.1 (×2) (C-aromatic), 103.7 (C-1), 82.9 (C-1′), 74.8 (C-5′), 71.8 (C-3′), 70.8 (CH_2Ph), 69.9 (C-5), 69.2 (C-2), 67.2 (C-4′), 66.9 (C-2′), 61.5 (C-6′), 37.5 (C-4), 35.7 (C-3), 20.8, 20.7, 20.6, 20.5 (CH_3CO). Anal. Calcd for $C_{26}H_{34}O_{12}S$: C, 54.73; H, 6.01; S, 5.62. Found: C, 54.55; H, 6.12; S, 5.94.

Further fractions from the column afforded **14** (342 mg, 45%) as a syrup; $[\alpha]_D^{20}$ −70.2 (c 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.35–7.29 (m, 5H, H-aromatic), 5.43 (d, 1H, $J_{4',5'}$ <1, $J_{3',4'}$ = 3.4, H-4′), 5.24 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2′), 5.05 (dd, 1H, $J_{3',4'}$ = 3.4, $J_{2',3'}$ = 10.0 Hz, H-3′), 4.85, 4.60 (2 d, 1H each, J = 11.8Hz, PhCH_2), 4.65 (d, 1H, $J_{1,2}$ = 2.2 Hz, H-1), 4.54 (d, 1H, $J_{1',2'}$ = 10.0 Hz, H-1′), 4.15 (dd, 1H, $J_{5',6'a}$ = 6.9, $J_{6'a,6'b}$ = 11.3 Hz, H-6′a), 4.12–4.07 (m, 2H, H-5a + H-6′b), 3.93 (br t, 1H, $J_{4',5'}$ <1, $J_{5',6'a} \approx J_{5',6'b}$ = 6.9 Hz, H-5′), 3.90 (ddd, 1H, $J_{1,2}$ = 2.1, $J_{2,3b}$ = 3.8, $J_{2,3a}$ = 7.2 Hz, H-2), 3.48–3.43 (m, 2H, H-4 + H-5b), 2.23 (ddd, 1H, $J_{3a,4}$ = 3.7, $J_{2,3a}$ = 7.2, $J_{3a,3b}$ = 12.2 Hz, H-3a), 2.17, 2.07, 2.05, 1.98 (4s, 12H, CH_3CO), 1.83 (ddd, 1 H, $J_{2,3b}$ = 3.8, $J_{3b,4}$ = 7.5, $J_{3a,3b}$ = 12.2 Hz, H-3b). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 170.5, 170.3, 170.0, 169.5 ($COCH_3$), 137.1, 129.2, 128.0 (C-aromatic), 98.3 (C-1), 83.8 (C-1′), 74.6 (C-5′), 71.8 (C-3′), 69.9 (CH_2Ph), 67.2

(C-4′), 67.2 (C-2′), 66.6 (C-5), 65.8 (C-2), 61.5 (C-6′), 38.0 (C-4), 34.7 (C-3), 20.8, 20.7 (×2), 20.6 (*C*H$_3$CO). Anal. Calcd for $C_{26}H_{34}O_{12}S$: C, 54.73; H, 6.01; S, 5.62. Found: C, 54.52; H, 6.24; S, 5.61.

When the reaction was carried out at −18 °C, and treated as described above, compounds **13** and **14** were obtained in 18% and 71% yield, respectively.

4.2.4.2. Reduction of 11 with NaBH$_4$/THF or with K-Selectride/THF. Synthesis of 13 and 14. To a solution of **11** (100 mg, 0.18 mmol) in anhydrous THF (7.4 mL), cooled at 0 °C, NaBH$_4$ (9 mg, 0.23 mmol) was added under stirring. After 20 min the solution was neutralized with Dowex 50 W (H$^+$) resin, filtered and concentrated. The residue was diluted with MeOH, concentrated and then purified by column chromatography (hexane/EtOAc 2:1). From the first fractions of the column was isolated **13** (32 mg, 33%) and further fractions from the column afforded **14** (42 mg, 42%).

The reduction of **11** was also performed with K-Selectride/THF as follows. To a solution of **11** (400 mg, 0.70 mmol) in anhydrous THF (7.4 mL), cooled to −78 °C was added 1 M solution of K-Selectride in THF (1.32 mL, 1.32 mmol) under nitrogen. The mixture was stirred at −78 °C for 3 h and then the temperature was raised to −20 °C. After the usual work-up and purification by column chromatography (hexane/EtOAc 2.5:1) was isolated **14** (322 mg, 80%) as a single product.

4.2.4.3. Reduction of the mixture 11 + 12. Synthesis of benzyl 3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-L-*erythro*-pentopyranoside (15). A mixture of **11** and **12** (~4:1, 200 mg, 0.35 mmol) obtained by coupling of **9** and **10** at −18 °C, was reduced with NaBH$_4$ (13.4 mg, 0.35 mmol) in MeOH (2 mL). The reaction mixture was stirred at −18 °C for 30 min, and then it was treated with Dowex 50 W (H$^+$) resin as described above. TLC (hexane/EtOAc 1: 1.5) analysis showed a major spot of R_f = 0.40, together with two minor components of R_f = 0.33 and 0.22. The spots of R_f = 0.40 and 0.33 comigrated with samples of **13** and **14**, respectively. Careful purification by column chromatography (hexane/EtOAc 2.5:1) led to the previously described **13** (28 mg, 14%) and **14** (108 mg, 54%). The third more polar thiodisaccharide eluted was identified as **15** (42 mg, 21%); mp 131 °C; $[\alpha]_D^{20}$ −37.7 (*c* 0.8, CHCl$_3$). ^{1}H NMR (500 MHz, CDCl$_3$) δ = 7.42–7.36 (m, 5H, H-aromatic), 5.45 (br d, 1H, $J_{4',5'}$ <1, $J_{3',4'}$ = 3.4, H-4′), 5.19 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2′), 5.05 (dd, 1H, $J_{3',4'}$ = 3.4, $J_{2',3'}$ = 10.0 Hz, H-3′), 4.87 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.80 (d, 1H, *J* = 11.6, PhC*H*$_2$), 4.62 (d, 1H, $J_{1',2'}$ = 10.0 Hz,H-1′), 4.52 (d, 1H, *J* = 11.6, PhC*H*$_2$), 4.17 (dd, 1H, $J_{5',6'a}$ = 7.2, $J_{6'a,6'b}$ = 11.4 Hz, H-6′a), 4.11 (dd, 1H, $J_{5',6'b}$ = 6.0, $J_{6'a,6'b}$ = 11.4 Hz, H-6′b), 3.94 (br t, 1H, $J_{4',5'}$ <1, $J_{5',6'b}$ = 6.0, $J_{5',6'b}$ = 7.2 Hz, H-5′), 3.79 (ddd, 1H, $J_{3eq,5eq} \approx$ 1.6, $J_{4,5eq}$ = 4.8, $J_{5eq,5ax}$ = 11.2 Hz, H-5 eq), 3.71 (dddd, 1H, $J_{1,2}$ = 3.6, $J_{2,3eq}$ = 4.2, $J_{2,OH}$ = 11.4 Hz, $J_{2,3ax}$ = 12.2 Hz, H-2), 3.60 (t, 1H, $J_{4,5ax} \approx J_{5ax,5eq}$ = 11.5 Hz, H-5ax), 3.04 (dddd, 1H, $J_{3eq,4}$ = 4.3, $J_{4,5eq}$ = 4.8, $J_{5ax,4}$ = 11.5, $J_{3ax,4}$ = 12.2 Hz, H-4), 2.21 (br ddd, 1H, $J_{3eq,5eq} \approx$ 1.6, $J_{2,3eq}$ = 4.2, $J_{3eq,4}$ = 4.3, $J_{3eq,3ax}$ = 12.2 Hz, H-3 eq), 2.15, 2.08 (×2), 2.00 (4s, 3H each, C*H*$_3$CO), 2.03 (d, 1H, $J_{2,OH}$ = 11.4, OH), 1.70 (ddd, 1H, $J_{2,3ax} = J_{3ax,3eq} = J_{3ax,4}$ = 12.2 Hz, H-3ax). ^{13}C NMR (50.3 MHz, CDCl$_3$) δ = 170.3, 170.2, 170.0, 169.5 (*C*OCH$_3$), 137.5, 128.6, 128.0 (C-aromatic), 96.2 (C-1), 84.9 (C-1′), 74.5, 71.8, 69.4, 67.9, 67.5, 67.2, 63.4, 61.7 (C-2′, C-3′, C-4′, C-5́, C-6′, C-5, C-2, OCH$_2$Ph), 40.4 (C-4), 34.5 (C-3), 20.8, 20.7, 20.6 (×2) (*C*H$_3$CO). Anal. Calcd for $C_{26}H_{34}O_{12}S$: C, 54.73; H, 6.01; S, 5.62. Found: C, 54.99; H, 6.01; S, 5.76.

4.2.5. General procedure for the O-deacetylation of thiodisaccharides 4, 5, 13 and 14

A suspension of thiodisaccharides **4**, **5**, **13** or **14** (0.10 mmol) in a mixture of MeOH/Et$_3$N/H$_2$O 4:1:5 (10 mL) was stirred at room temperature. The solid was progressively dissolving and after 2 h TLC (hexane/EtOAc 1:1 or 1:1.5) showed complete consumption of the starting material. The solution was concentrated and the residue was dissolved in water (1 mL) and eluted through a column filled with Dowex MR-3C mixed bed ion-exchange resin. The eluate was concentrated and additionally purified by filtration through an octadecyl C18 minicolumn (Amprep, Amersham Biosciences). Evaporation of the solvent afforded the free thiodisaccharide, which showed a single spot by TLC (*n*-BuOH/EtOH/H$_2$O 2.5:1:1). The respective R_f values are given in each individual case.

4.2.5.1. 2-Propyl 3-deoxy-4-*S*-(β-D-xylopyranosyl)-4-thio-α-D-*lyxo*-hexopyranoside (16). O-Deacetylation of **4** gave crystalline **16** (76%, R_f = 0.56); mp 187–188 °C; $[\alpha]_D^{20}$ +40.3 (*c* 0.5, H$_2$O). ^{1}H NMR (D$_2$O, 500 MHz) δ = 4.70 (d, 1H, $J_{1,2}$ = 2.8 Hz, H-1), 4.34 (d, 1H, $J_{1',2'}$ = 9.8 Hz, H-1′), 4.08 (ddd, 1H, $J_{4,5}$ = 3.7, $J_{5,6b}$ = 4.1, $J_{5,6a}$ = 8.1 Hz, H-5), 3.92 (m, 1H, *J* = 6.2Hz, C*H*(CH$_3$)$_2$), 3.84 (dd, 1H, $J_{4',5'a}$ = 5.4, $J_{5'a,5'b}$ = 11.4 Hz, H-5′a), 3.68 (dd, 1H, $J_{5,6a}$ = 8.1, $J_{6a,6b}$ = 12.1 Hz, H-6a), 3.61 (dd, 1H, $J_{5,6b}$ = 4.1, $J_{6a,6b}$ = 12.1 Hz, H-6b), 3.53 (ddd, 1H, $J_{1,2}$ = 2.8, $J_{2,3a}$ = 4.3, $J_{2,3b}$ = 4.9 Hz, H-2), 3.46 (ddd, 1H, $J_{4',5'a}$ = 5.4, $J_{3',4'}$ = 9.0, $J_{4',5'b}$ = 10.6 Hz, H-4′), 3.27 (t, 1H, $J_{2',3'} \approx J_{3',4'}$ = 9.0 Hz, H-3′), 3.16 (br t, 1H, $J_{4',5'b}$ = 10.6, $J_{5'a,5'b}$ = 11.4 Hz, H-5′b), 3.15 (m, 1H, H-4), 3.11 (dd, 1H, $J_{2',3'}$ = 9.0, $J_{1',2'}$ = 9.7 Hz, H-2′), 2.21 (ddd, 1H, $J_{2,3a} \approx J_{3a,4}$ = 4.3, $J_{3a,3b}$ = 14.8 Hz, H-3a), 1.96 (ddd, $J_{2,3b} \approx J_{3b,4}$ = 4.9 Hz, $J_{3a,3b}$ = 14.8 Hz, H-3b), 1.09, 1.03 (2d, 3H each, *J* = 6.2, CH(C*H*$_3$)$_2$). ^{13}C NMR (D$_2$O, 127.5 MHz) δ = 97.4 (C-1), 87.1 (C-1′), 77.1 (C-3′), 72.5 (C-2′), 72.0 (C-5), 70.5 [*C*H(CH$_3$)$_2$], 69.0 (C-4′), 68.8 (C-5′), 66.6 (C-2), 61.9 (C-6), 40.2 (C-4), 32.0 (C-3), 22.3, 20.4 [CH(*C*H$_3$)$_2$]. Anal. Calcd for $C_{14}H_{26}O_8S$: C, 47.44; H, 7.39; S, 9.05. Found: C, 47.41; H, 7.64; S, 9.14.

4.2.5.2. 2-Propyl 3-deoxy-4-*S*-(β-D-xylopyranosyl)-4-thio-α-D-*xylo*-hexopyranoside (17). Standard deprotection of **5** gave **17** white crystals (77%, R_f = 0.66); mp 148–150 °C; $[\alpha]_D^{20}$ +18.3 (*c* 1.2, H$_2$O). ^{1}H NMR (D$_2$O, 500 MHz) δ = 4.88 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1), 4.43 (d, 1H, $J_{1',2'}$ = 9.7 Hz, H-1′), 4.16 (ddd, 1H, $J_{4,5}$ = 2.2, $J_{5,6a} = J_{5,6b}$ = 6.0 Hz, H-5), 4.04 (ddd, 1 H, $J_{1,2}$ = 3.9, $J_{2,3eq}$ = 4.8, $J_{2,3ax}$ = 11.8 Hz, H-2), 3.91 (m, 1H, *J* = 6.3 Hz, C*H*(CH$_3$)$_2$), 3.89 (dd, 1H, $J_{4',5'a}$ = 5.4, $J_{5'a,5'b}$ = 11.3 Hz, H-5′a), 3.59 (d, 2H, $J_{5,6a} \approx J_{5,6b} \approx$ 6.0 Hz, H-6a + H-6b), 3.53 (ddd, 1H, $J_{4',5'a}$ = 5.4, $J_{3',4'}$ = 9.0, $J_{4',5'b}$ = 10.7 Hz, H-4′), 3.36 (ddd, 1H, $J_{4,5}$ = 2.2, $J_{3eq,4}$ = 3.3, $J_{3ax,4}$ = 3.6 Hz, H-4), 3.34 (t, 1H, $J_{2',3'} \approx J_{3',4'}$ = 9.0 Hz, H-3′), 3.22 (dd, 1H, $J_{4',5'b}$ = 10.7, $J_{5'a,5'b}$ = 11.3 Hz, H-5′b), 3.19 (dd, 1H, $J_{2',3'}$ = 9.0, $J_{1',2'}$ = 9.7 Hz, H-2′), 2.06 (ddd, 1H, $J_{3ax,4}$ = 3.6, $J_{2,3ax}$ = 11.8, $J_{3ax,3eq}$ = 13.2 Hz, H-3ax), 1.98 (ddd, $J_{3eq,4}$ = 3.3 $J_{2,3eq}$ = 4.8, $J_{3ax,3eq}$ = 13.2 Hz, H-3 eq), 1.16, 1.08 (2d, 3H each, *J* = 6.3, CH(C*H*$_3$)$_2$)). ^{13}C NMR (D$_2$O, 127.5 MHz) δ = 96.1 (C-1), 85.5 (C-1′), 77.1 (C-3′), 72.3 (C-2′), 70.5, 70.4 (C-5, *C*H(CH$_3$)$_2$), 69.1 (C-4′), 68.8 (C-5′), 63.9 (C-2), 62.9 (C-6), 42.7 (C-4), 32.4 (C-3), 22.4, 20.5 [CH(*C*H$_3$)$_2$]. Anal. Calcd for $C_{14}H_{26}O_8S$: C, 47.44; H, 7.39; S, 9.05. Found: C, 47.16; H, 7.35; S, 9.14.

4.2.5.3. Benzyl 3-deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-β-D-*erythro*-pentopyranoside (18). O-Deacetylation of **13** gave crystalline **18** (75%, R_f = 0.64); mp 90 °C; $[\alpha]_D^{20}$ -43.3 (*c* 0.7, H$_2$O). ^{1}H NMR (500 MHz, CDCl$_3$) δ = 7.23–7.29 (m, 5H, H-aromatic), 4.71, 4.54 (2d, 1H each, *J* = 11.6 Hz, PhC*H*$_2$), 4.38 (d, 1H, $J_{1',2'}$ = 9.8 Hz, H-1′), 4.28 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1), 3.95 (ddd, 1H, $J_{3eq,5eq}$ = 2.2, $J_{4,5eq}$ = 4.4, $J_{5ax,5eq}$ = 11.6 Hz, H-5eq), 3.80 (br d, 1H, $J_{4',5'}$ <1, $J_{3',4'}$ = 3.2, H-4′), 3.59 (dd, 1H, $J_{5',6'a}$ = 8.9, $J_{6'a,6'b}$ = 12.1 Hz, H-6′a), 3.53 (m, 2H, H-5′ + H-6′b), 3.47 (dd, 1H, $J_{3',4'}$ = 3.4, $J_{2',3'}$ = 9.5 Hz, H-3′), 3.39 (ddd, 1H, $J_{2,3eq}$ = 4.8, $J_{1,2}$ = 7.4, $J_{2,3ax}$ = 10.7 Hz, H-2), 3.36 (br t, 1H, $J_{2',3'}$ = 9.5, $J_{1',2'}$ = 9.8 Hz, H-2′), 3.32 (dd, 1H, $J_{4,5ax}$ = 10.9, $J_{5ax,5eq}$ = 11.7 Hz, H-5ax), 3.08 (dddd, 1H, $J_{3eq,4} \approx J_{4,5eq}$ = 4.4, $J_{4,5ax}$ = 10.7, $J_{3ax,4}$ = 12.0 Hz, H-4), 2.21 (dddd, 1H, $J_{3eq,5eq}$ = 2.2, $J_{3eq,4}$ = 4.4, $J_{2,3eq}$ = 4.8, $J_{3a,3b}$ = 12.5 Hz, H-3 eq), 1.40 (ddd, 1H, $J_{2,3ax}$ = 10.7, $J_{3ax,4}$ = 12.0, $J_{3ax,3eq}$ = 12.5 Hz, H-3ax). ^{13}C NMR (125.7 MHz, D$_2$O)

δ = 136.6, 128.7 (×2), 128.4 (C-aromatic), 103.1 (C-1), 84.4 (C-1′), 78.9 (C-5′), 73.8 (C-3′), 71.1 (*C*H$_2$Ph), 68.7 (C-2′), 69.1 (C-5), 68.7 (C-4), 68.3 (C-2), 61.0 (C-6′), 37.1 (C-4), 36.3 (C-3). Anal. Calcd for $C_{18}H_{26}O_8S.H_2O$: C, 51.42; H, 6.71; S, 7.63. Found: C, 51.23; H, 6.63; S, 8.00.

4.2.5.4. Benzyl 3-deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-β-D-*threo*-pentopyranoside (19). Removal of the *O*-acetyl groups of **14** gave **19** as white crystals (88%, R_f = 0.68); mp 120 °C; $[\alpha]_D^{20}$ −92.7 (*c* 0.6, H_2O). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.30–7.22 (m, 5H, H-aromatic), 4.65 (m, 2H, PhC*H*$_2$ + H-1, superimposed with HDO signal), 4.44 (d, 1H, *J* = 11.8 Hz, PhC*H*$_2$), 4.38 (d, 1H, $J_{1',2'}$ = 9.8 Hz, H-1′), 3.94 (dd, 1H, $J_{4,5a}$ = 3.2, $J_{5a,5b}$ = 12.0 Hz, H-5a), 3.84 (ddd, 1H, $J_{1,2}$ = 2.8, $J_{2,3b}$ = 3.5, $J_{2,3a}$ = 8.4 Hz, H-2), 3.80 (br d, 1H, $J_{4',5'}$ <1, $J_{3',4'}$ = 3.3, H-4′), 3.58 (dd, 1H, $J_{5',6'a}$ = 8.8, $J_{6'a,6'b}$ = 12.3 Hz, H-6′a), 3.55–3.51 (m, 2H, $J_{5',6'b}$ = 4.0, $J_{6'a,6'b}$ = 12.3, H-5′ + H-6′b), 3.47 (dd, 1H, $J_{3',4'}$ = 3.3, $J_{2',3'}$ = 9.5 Hz, H-3′), 3.42 (dd, 1H, $J_{4,5b}$ = 5.6, $J_{5a,5b}$ = 12.0 Hz, H-5b), 3.38 (br t, 1H, $J_{2',3'}$ = 9.5, $J_{1',2'}$ = 9.8 Hz, H-2′), 3.33 (dddd, 1H, $J_{4,5eq}$ = 3.2, $J_{3a,4}$ = 4.2, $J_{4,5b}$ = 5.6, $J_{3b,4}$ = 7.2 Hz, H-4), 2.05 (ddd, 1H, $J_{3a,4}$ = 4.2, $J_{2,3a}$ = 8.6, $J_{3a,3b}$ = 13.5 Hz, H-3a), 1.77 (ddd, 1H, $J_{2,3b}$ = 3.5, $J_{3b,4}$ = 7.2, $J_{3a,3b}$ = 13.5 Hz, H-3b). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ = 137.0, 128.7, 128.5, 128.3 (C-aromatic), 98.2 (C-1), 85.3 (C-1′), 78.9 (C-5′), 73.9 (C-2′), 70.0 (*C*H$_2$Ph), 69.6 (C-3′), 68.7 (C-4′), 65.4, 65.0 (C-5, C-2), 61.1 (C-6′), 38.2 (C-4), 33.2 (C-3). Anal. Calcd for $C_{18}H_{26}O_8S$: C, 53.72; H, 6.51; S, 7.97. Found: C, 53.38; H, 6.74; S, 8.06.

4.3. Enzymatic assays

4.3.1. Inhibition of β-xylosidase

Inhibitory activity studies of compounds **16** and **17** towards *A. niger* β-xylosidase (Sigma–Aldrich, EC 3.2.1.37, 5.8 U/mg) were conducted. The enzyme (0.04 U) was incubated with *p*-nitrophenyl β-D-xylopyranoside (concentration range: 0.5–10.0 mM) in sodium acetate buffer (50 mM, pH 5.0) in the absence or presence of compounds **17** (concentrations used: 5.0–15.0 mM) and **16** (concentrations used: 2.0–10.0 mM). The final volume was adjusted to 250 μL for **17** and 500 μL for **16**. After 15 min at 25 °C, the reaction was stopped by adding sodium borate buffer (0.2 M, pH 10.0, 1.00–2.00 mL) and the solution was measured by visible absorption spectroscopy at 400 nm. The absorbance in the presence or absence of **16** or **17** was similar, indicating that no inhibition of the enzyme was achieved.

4.3.2. Inhibition of β-glucosidase

Inhibitory activity studies of compound **17** towards β-glucosidase from almonds (Biochemika, EC 3.2.1.21, 12.4 U/mg) were conducted. The enzyme (2.0 mU) was incubated with *p*-nitrophenyl β-D-glucopyranoside (concentration range: 0.5–10.0 mM) in sodium acetate buffer (50 mM, pH 5.6) in the absence or presence of compound **17** (concentrations used: 4.0–6.0 mM) and the final volume was 250 μL. After 15 min at 37 °C, the reaction was terminated as described in Section 4.4.1. The absorbance at 400 nm in the presence or absence of **17** was similar, indicating that inhibition of the enzyme did not take place.

4.3.3. Inhibition of β-galactosidase

The inhibitory activity of compounds **16**, **17**, **18** and **19** towards *E. coli* β-galactosidase (grade VIII, Sigma, EC 3.2.1.23, 117 U/mg) was determined under the following conditions: The enzyme (0.3 U; 1U = 1 enzyme unit hydrolyses 1 μmol of *o*-nitrophenyl galactopyranoside per minute) was incubated with *o*-nitrophenyl-β-D-galactopyranoside (concentration range: 0.2–5.0 mM) in sodium phosphate buffer (100 mM, pH 7.3, $MgCl_2$ 1.2 mM, 2-mercaptoethanol 100 mM) in the absence or presence of compounds **16** (0.6–4.0 mM), **17** (0.6–4.0 mM), **18** (0.4–1.2 mM) and **19** (0.01–0.05 mM); the final volume was adjusted to 0.50 mL. After 10 min at 37 °C, the reaction was terminated by adding sodium borate buffer 0.2 M (4.0 mL, pH 10.0). The concentration of the released *o*-nitrophenol was measured by visible absorption spectroscopy at 410 nm. The absorbance in the presence or absence of **16** or **17** was similar, indicating no inhibition of the enzyme. However, the absorbance in the presence of **18** or **19** was significantly lower than the absorbance in control experiments. The K_i and K_m values were determined from Lineweaver–Burk plots (Fig. 1).

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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.2009.07.055.

References and notes

1. (a) Davies, G. J.; Gloster, T. M.; Henrissat, B. *Curr. Opin. Struct. Biol.* **2005**, *15*, 637; (b) Zechel, D. L.; Withers, S. G. *Acc. Chem. Res.* **2000**, *33*, 11; (c) Davies, G. J.; Henrissat, B. *Structure* **1995**, *3*, 853; (d) McCarter, J. D.; Withers, S. G. *Curr. Opin. Struct. Biol.* **1994**, *4*, 885.
2. (a) Cipolla, L.; La Ferla, B.; Gregori, M. *Comb. Chem. High Throughput Screening* **2006**, *9*, 571; (b) Asano, N. *Glycobiology* **2003**, *13*, 93; (c) Greimel, P.; Spreitz, J.; Stütz, A. E.; Wrodnigg, T. M. *Curr. Top. Med. Chem.* **2003**, *3*, 513; (d) Lohse, A.; Hardlei, T.; Jensen, A.; Plesner, I. W.; Bols, M. *Biochem. J.* **2000**, *349*, 211; (e) Ratner, L.; Heyden, N. V.; Dedera, D. *Virology* **1991**, *181*, 180.
3. (a) Gerber-Lemaire, S.; Juillerat-Jeanneret, L. *Mini-Rev. Med. Chem.* **2006**, *6*, 1043; (b) Paulsen, H.; Brockhausen, I. *Glycoconjugate J.* **2001**, *18*, 867; (c) Goss, P. E.; Baker, M. A.; Caarver, J. P.; Dennis, J. W. *Clin. Cancer Res.* **1995**, *1*, 935.
4. (a) Witczak, Z. J.; Culhane, J. M. *Appl. Microbiol. Biotechnol.* **2005**, *69*, 237; (b) Witczak, Z. J. *Curr. Med. Chem.* **1999**, *6*, 165; (c) Becker, B.; Thimm, J.; Thiem, J. *J. Carbohydr. Chem.* **1996**, *15*, 1179.
5. (a) Wen, X.; Yuan, Y.; Kuntz, D. A.; Rose, D. R.; Pinto, B. M. *Biochemistry* **2005**, *44*, 6729; (b) Witczak, Z. J.; Kaplon, P.; Dey, P. M. *Carbohydr. Res.* **2003**, *338*, 11; (c) Kiefel, M. J.; von Itzstein, M. *Chem. Rev.* **2002**, *102*, 471; (d) Witczak, Z. J.; Sun, J.; Mielguj, R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2169.
6. (a) Driguez, H. *ChemBiochem.* **2001**, *2*, 311; (b) Driguez, H. *Top. Curr. Chem.* **1997**, *187*, 85.
7. (a) Szilágyi, L.; Varela, O. *Curr. Org. Chem.* **2006**, *10*, 1745; (b) Pachamuthu, K.; Schmidt, R. R. *Chem. Rev.* **2006**, *106*, 160.
8. Manzano, V. E.; Uhrig, M. L.; Varela, O. *J. Org. Chem.* **2008**, *73*, 7224.
9. Uhrig, M. L.; Manzano, V. E.; Varela, O. *Eur. J. Org. Chem.* **2006**, 162.
10. Repetto, E.; Marino, C.; Uhrig, M. L.; Varela, O. *Eur. J. Org. Chem.* **2008**, *3*, 540.
11. Repetto, E.; Marino, C.; Uhrig, M. L.; Varela, O. *Bioorg. Med. Chem.* **2009**, *3*, 540.
12. Witczak, Z. J.; Chhabra, R.; Chen, H.; Xie, X.-Q. *Carbohydr. Res.* **1997**, *301*, 167.
13. Witczak, Z. J.; Lorchak, D.; Nguyen, N. *Carbohydr. Res.* **2007**, *342*, 1929.
14. García-Herrero, A.; Montero, E.; Muñoz, J. L.; Espinosa, J. F.; Vián, A.; García, J. L.; Asensio, J. L.; Cañada, F. J.; Jiménez-Barbero, J. *J. Am. Chem. Soc.* **2002**, *124*, 4804.
15. Stanek, J.; Šindlerova, M.; Černý, M. *Collect. Czech. Chem. Commun.* **1965**, *30*, 297.
16. De Fina, G.; Varela, O.; Lederkremer, R. M. *Synthesis* **1988**, 891.
17. Horton, D.; Priebe, W.; Varela, O. *J. Org. Chem.* **1986**, *51*, 3479.
18. (a) Uhrig, M. L.; Varela, O. *Aust. J. Chem.* **2002**, *55*, 155; (b) Uhrig, M. L.; Varela, O. *Carbohydr. Res.* **2002**, , *337*, 2069.
19. Henrissat, B.; Hamer, G. K.; Taylor, M. G.; Marchessault, R. H. *Can. J. Chem.* **2002**, *80*, 1162.
20. Uhrig, M. L.; Varela, O. *Synthesis* **2005**, 893.
21. Durette, P. L.; Horton, D. *J. Org. Chem.* **1971**, *36*, 2658.
22. Kolender, A. A.; Matulewicz, M. C. *Carbohydr. Res.* **2002**, *337*, 57.
23. Rivera-Sagredo, A.; Fernández-Mayoralas, A.; Jiménez-Barbero, J.; Martín-Lomas, M. *Carbohydr. Res.* **1992**, 129. and references cited therein.
24. Biely, P. In *Handbook of Food Enzymology*; Whitaker, J. R., Voragen, A. G. J., Wong, D. W. S., Eds.; Marcel Dekker Inc.: New York, 2003; p 879.
25. (a) Jänis, J.; Hakanpää, J.; Hakulinen, N.; Ibatullin, F. M.; Hoxha, A.; Derrick, P. J.; Rouvinen, J.; Vainiotalo, P. *FEBS J.* **2005**, *272*, 2317; (b) Ibatullin, F. M.; Shabalin,

K. A.; Jänis, J. V.; Shavva, A. G. *Tetrahedron Lett.* **2003**, *44*, 7961; (c) Ibatullin, F. M.; Shabalin, K. A.; Jänis, J. V.; Selivanov, S. I. *Tetrahedron Lett.* **2001**, *42*, 4565; (d) Defaye, J.; Guillot, J.-M.; Biely, P.; Vršanská, M. *Carbohydr. Res.* **1992**, *228*, 47.
26. Dubost, E.; Le Nouën, D.; Streith, J.; Tarnus, C.; Tschamber, T. *Eur. J. Org. Chem.* **2006**, 610.
27. Jordan, D. B.; Mertens, J. A.; Braker, J. D. *Biochim. Biophys. Acta* **2009**, *1794*, 144.
28. (a) Richard, J. P. *Biochemistry* **1998**, *37*, 4305; (b) Sinnot, M. L. *Chem. Rev.* **1990**, *90*, 1171; (c) Griesser, H. W.; Müller-Hill, B.; Overath, P. *Eur. J. Biochem.* **1983**, *137*, 567; (d) Matthews, B. W. *C.R. Biol.* **2005**, *328*, 549.
29. (a) Huber, R. E.; Hlede, I. Y.; Roth, N. J.; McKenzie, K. C.; Ghumman, K. K. *Biochem. Cell. Biol.* **2001**, *79*, 183; (b) Gebler, J. C.; Aebersold, R.; Withers, S. G. *J. Biol. Chem.* **1992**, *267*, 11126; (c) Cupples, C. G.; Miller, J. H.; Huber, R. E. *J. Biol. Chem.* **1990**, *265*, 5512.
30. (a) Juers, D. H.; Heightman, T. D.; Vasella, A.; McCarter, J. D.; Mackenzie, L.; Withers, S. G.; Matthews, B. W. *Biochemistry* **2001**, *40*, 14781; (b) Jacobson, R. H.; Zhang, X. J.; DuBose, R. F.; Matthews, B. W. *Nature* **1994**, *369*, 761.
31. (a) Sutendra, G.; Wong, S.; Fraser, M. E.; Huber, R. E. *Biochem. Biophys. Res. Commun.* **2007**, *352*, 566; (b) Espinosa, J. F.; Montero, E.; Vian, A.; García, J. L.; Dietrich, H.; Schmidt, R. R.; Martín-Lomas, M.; Imberty, A.; Cañada, F. J.; Jiménez-Barbero, J. *J. Am. Chem. Soc.* **1998**, *120*, 1309.
32. Steiner, A. J.; Schitter, G.; Stütz, A. E.; Wrodnigg, T. M.; Tarling, C. A.; Withers, S. G.; Fantur, K.; Mahuran, D.; Paschke, E.; Tropak, M. *Bioorg. Med. Chem.* **2008**, *16*, 10216.