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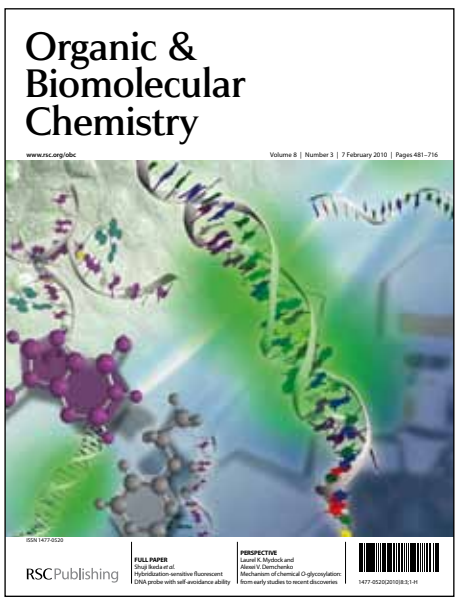

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Convenient synthesis of 4-thiolactose, 3,4-dithiolactose and related thiooligosaccharides and disulfides. Inhibitory activity of the glycomimetics against a β-galactosidase

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The ring-opening reaction of sugar 3,4-epoxides by 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose (**7**) as nucleophile, led to (1→3)- and (1→4)-thiodisaccharides. High regio and diastereoselectivities were achieved in the synthesis of the per-*O*-acetyl derivative of the β-D-Gal*p*-*S*-(1→4)-4-thio-α-D-Glc*p*-*O*-iPr (**10**). Analogues of the 4-thiolactoside **10** have been prepared, with the β-D-Gal*p* non-reducing end *S*-linked to D-Glc*p*, D-Gul*p* and D-Ido*p*. Similar regioselective attack of **7** to C-4 of 2-propyl 3,6-di-*O*-acetyl-3,4-epithio-α-D-galactopyranoside (**6**) led to 2-propyl 3,4-dithiolactoside derivative **15**. During this reaction the free 3-SH group of **15** underwent oxidative dimerization or oxidative coupling with the SH function of **7** to give the respective disulfides. Glycosylation of the thiol group of **15** using trichloroacetimidate derivatives of β-D-Gal*p* or β-D-Gal*f* afforded the corresponding branched dithiotrisaccharides. The free compounds were evaluated as inhibitors of the *E. coli* β-galactoside. The bis(2-propyl 3,4-dithiolactosid-3-yl)-disulfide, obtained from **15**, displayed the strongest inhibitory activity in these series of glycomimetics and proved to be a non-competitive inhibitor (*K*i = 95 μM).

Introduction

In recent years, the development of glycomimetics has received considerable attention as these compounds display interesting biological activities and may be involved in many metabolic pathways. The search for new glycomimetics has led to analogues of carbohydrates in which one or more oxygen atoms have been replaced by sulfur[1] or other heteroatoms.[2] In particular, thiooligosaccharides are sugar mimetics with at least one interglycosidic linkage mediated by sulfur. They are usually resistant to metabolic processes and are frequently employed as useful tools for glycobiology.[3] The understanding of enzyme-inhibitor interactions is essential to provide insight into binding and recognition events, and also for the rational design of inhibitors of glycosidases.[4] Inhibition of these enzymes may disrupt the biosynthesis and catabolism of glycoconjugates, thus interfering vital processes. Therefore, glycosidase inhibitors have shown promise as therapeutics in the treatment of diabetes,[5] lysosomal storage diseases[6] and viral infections,[7] including influenza[8] and HIV.[9] Indeed several glycosidase inhibitors are already used clinically for treating some of these diseases.[10,11] Enzyme inhibition also constitutes an innovative approach for drug development in cancer therapies.[12]

Because of all these biologically relevant issues, the synthesis of thiooligosaccharides is a subject of intensive current research, and convenient approaches have been reported.[3,13,14] Thioglycosidic linkages have also been constructed using enzymes to promote the coupling of appropriate carbohydrate moieties.[14,15] In our laboratory, we have developed regio and diastereoselective strategies for the construction of the thioglycosidic linkage of thiodisaccharides. Thus, the Michael addition of 1-thioaldoses to hexose-[16] or pentose-derived sugar enones[17] was the key step in the synthesis of 3-deoxy-4-thio analogues of natural or non-natural (1→4)-disaccharides; and thiodisaccharides with a thiofuranose as non-reducing end have also been prepared.[18,19] Similarly, 4,6′-thioether-linked disaccharides have been obtained as nonglycosidic, hydrolytically stable glycomimetics.[20] Moreover, we have described a straightforward procedure for the synthesis of (1→3)- or (1→4)-thiodisaccharides based on the ring-opening of sugar epoxides with 1-thioaldoses.[21] In addition, the controlled ring-opening reaction of sugar thiiranes by 1-thiopyranoses was successfully accomplished to afford, regio and stereoselectively, β-*S*-(1→4)-3,4-dithiodisaccharides that were employed as precursors of branched dithiotrisaccharides.[22]

The thiodisaccharides obtained by some of these methodologies have been evaluated as inhibitors of glycosidases, and many of them were active against the β-galactosidase from *Escherichia coli*.[16,17] This enzyme has been extensively studied,[23] and it has shown to be highly specific for β-galactopyranosyl units linked to a wide variety of aglycons. Among the

thioglycomimetics, simple thiogalactopyranosides[24] (such as isopropyl or phenylethyl) are good inhibitors and thiodisaccharides like 4-thiolactose[25] and analogues[16,17] display also inhibitory activity against the enzyme.

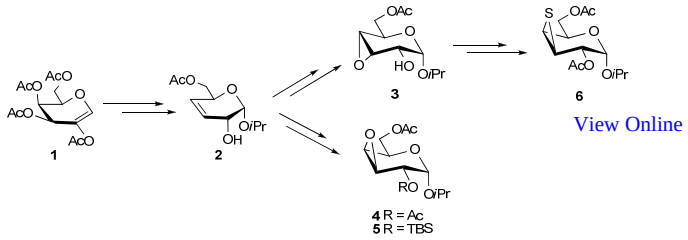
As continuation of this previous work we report here convenient procedures for the synthesis of thiooligosaccharides and disulfides with a basic structure of 4-thiolactose. The ring-opening of the epoxide or thiirane derivatives of sugars was employed as key reaction to control the regio and stereoselectivity in the construction of the thioglycosidic linkage. The resulting products have been evaluated as inhibitors against the β-galactosidase from *E. coli*.

Results and discussion

Synthesis of thioglycomimetics

The oxirane and episulfide derivatives **3-6** were prepared as thioglycosyl acceptors. These compounds possess the convenient configuration at C-3 and C-4 to provide, *via* a ring-opening reaction, a glucopyranose unit as reducing end of the resulting thiodisaccharides. Thus, the starting 2,3,4,6-tetra-*O*-acetyl-D-galactal (**1**) was converted into the allylic alchohol **2**, which upon oxidation, afforded the epoxides derivatives **3-5**[21] (Scheme 1). The thiirane derivative **6** was also synthesized using the sugar epoxide **3** as precursor.[22]

As we wanted to have a galactopyranose unit as non-reducing end of the ring-opening products, 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose (**7**) was employed as nucleophilic thioglycosyl donor (Scheme 2). The ring-opening reaction was performed in methanol containing LiOMe to convert the thiol group of **7** into the more nucleophilic thiolate. As the basic medium promotes *O*-deacetylation, when the reaction was finished, the base was neutralized and the mixture was reacetylated. This procedure applied for the ring-opening reaction of the epoxide group of **3** by the 1-thioaldose **7**, gave the expected thiodisaccharide derivatives **8** and **9** in an excellent overall yield (95%). The stereochemistry of **8** and **9** was deduced from their NMR-spectra (1D/2D). The site of attack of the thiol was readily established because of the shielding effect of the sulfur atom, which induces an upfield shifting of the signal of the *S*-linked carbon and that of the corresponding proton. As the latter appears in a clean region of the spectrum (2.80–3.50 ppm) the coupling constants (*J*) could be accurately measured. For example, H-3 (δ = 3.27) in **8** gave large values for the $J_{2,3}$ (11.7 Hz) and $J_{3,4}$ (11.3 Hz) indicating a *trans*-diaxial disposition for the coupled protons, and hence a *gluco* configuration for the reducing end of **8**. In contrast, the H-4 signal (δ = 3.28) in **9** showed small values for $J_{3,4}$ and $J_{4,5}$ (both 2.7 Hz) suggesting a *gulo* configuration for the sulfur-containing moiety of **9**. In the ^{13}C NMR spectra, the signals of the carbons bonded to sulfur, C-3 (in **8**) and C-4 (in **9**) appear, respectively, at 46.7 and 44.8 ppm, whereas the anomeric carbons linked to sulfur (C-1') resonate at higher field (83.8 and 83.0 ppm) than the *O*-glycosidic anomeric carbons (C-1, 93.7 and 94.4 ppm).



Scheme 1 Synthesis of epoxides **3-5** and thiirane **6**

Furthermore, the large value for the coupling constant between H-1' and H-2' (~ 10 Hz) in both **8** and **9** is indicative that the β-configuration of the 1-thioaldose **7** is maintained during the reaction and remains in the final product.

Thiodisaccharide **9** is the expected product of *trans*-diaxial opening of the oxirane ring of **3**, according to the Fürst-Plattner rule. However, no regioselectivity was found as **9** and the *trans*-diequatorial product **8** were produced in similar amounts. The poor regioselectivity observed in the formation of **8** and **9** could be explained, as reported for analogous compounds,[26] according to stabilizing or repulsive effects that operate during the ring-opening reaction. Thus, formation of the *trans*-diaxial product **9** by attack of thiolate **7** to the C-4 of **3** is expected to produce a slight distortion of the conformation of the pyranose ring, but a 1,3-diaxial interaction between the substituent at C-3 and the anomeric isopropyl group is generated during the opening of the epoxide. In contrast, the transition state that leads to **8** is free of such an a respulsive interaction and, in addition, **8** is an stable product as all the substituents of the chair, from C-2 to C-5, are equatorially oriented.

To obtain a 4-thiolactose derivative, we conducted the reaction of 1-thioaldose **7** with the epoxide **4**, which has opposite stereochemistry at C-3 and C-4 compared to that of **3**. This reaction afforded the expected thiodisaccharides **10** and **11**, together with a product which was isolated and identified as the thiodisaccharide **12**. Formation of **12** could be the result of 3,4→2,3 isomerization in *O*-deacetylated **4** by epoxide migration,[21,26] followed by diaxial ring-opening by attack of the thiol group of **7** to C-2. The stereochemistry of the reducing-end of **10**-**12** was established from the NMR spectra, as already described for the related thiodisaccharides **8** and **9**. It is worth to mention that the spectral data of **10** were coincident with those reported for the analogue methyl 4-thiolactoside.[27] Unfortunately, the reaction between **4** and **7** afforded a low yield (21%) of the desired per-*O*-acetyl derivative of 4-thiolactoside **10**. However, we have previously reported that the regioselectivity in the ring-opening of 3,4-epoxides like **4** could be improved by installing a bulky substituent on OH-2.[21] For example, a bulky TBS group at HO-2 is expected to hinder the attack of the nucleophile to the vicinal C-3. Furthermore, if the HO-2 remains protected during the reaction, the epoxide migration should be prevented. For these reasons, the epoxide **5** was treated with 1-thiogalactose **7** as described for the previous ring-opening reactions. After the usual work-up, two products were isolated by column chromatography, the expected thiodisaccharide **13** (51%) and the second component that was identified as **10**. The latter seems to be formed by *O*-desilylation once that **13** has been obtained, since **12** has the same 4-thio-D-*gluco* configuration for the reducing-end as **13**.

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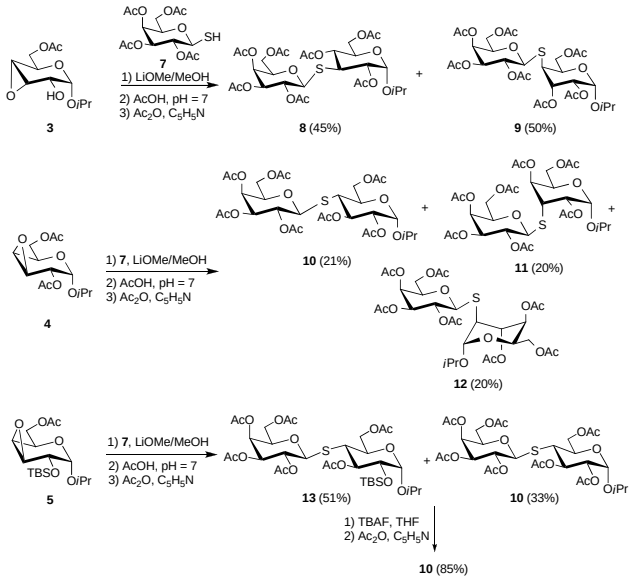
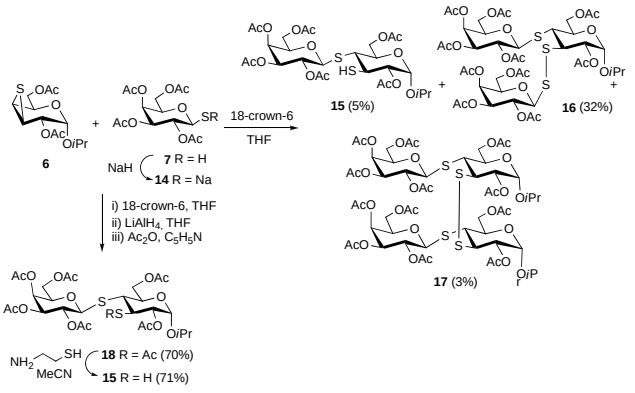


Scheme 2 Synthesis of thiodisaccharides from epoxides **3**-**5**

To avoid the separation of **10** and **13** by chromatography, in further preparations the crude mixture of reaction was treated with TBAF for the *O*-desilylation prior to reacetylation. This way compound **13** was converted into **10**, which was isolated in 85% overall yield.

Our next target was the 3,4-dithiolactoside derivative **15** (Scheme 3), an analogue of **10** having the HO-3 group replaced by thiol. Compound **15** was prepared by ring-opening of the 3,4-thiirane **6** with the thiolate **14**. In these reactions the expected ring-opening products are accompanied by (1→3)-disulfides, formed by oxidative coupling of the thiol groups present in the starting 1-thioaldose or in the resulting dithiodisaccharides.[21] However, the reduction of disulfides in the reaction mixture with $LiAlH_4$, led to the major expected product. Thus, the 3,4-dithiodisaccharide **18** (70%) was obtained after $LiAlH_4$ reduction of the crude mixture of reaction of **6** with **14**, followed by acetylation. The thiol group was released from its acetyl derivative by treatment of **18** with 2-aminoethanethiol (cysteamine) to give **15**. We have now identified all the products formed by reaction of **6** with **14**. These products were isolated by careful column chromatography and repurification of the fraction that contained disulfides **16** and **17**.

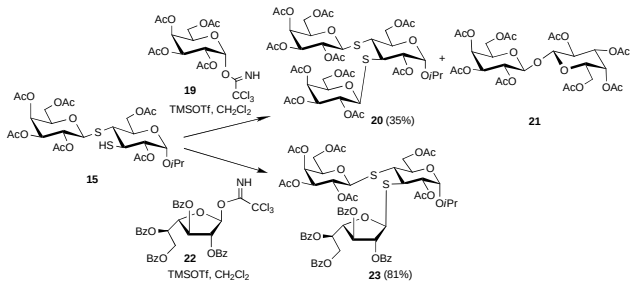
Once isolated, the structure of **16** and **17** was readily established by NMR (1D/2D). The signal of C-3, linked to the disulfide group, was shifted downfield (48.9 in **16** and 50.9 ppm in **17**) with respect to the C-3 signal in **15** (40.2 ppm). As previously reported,[22] these relative shiftings may be used as diagnostic of disulfide formation. Similarly, the signal of the anomeric carbon (C-1') linked to the disulfide in **16** appeared at a δ value (88.5 ppm) intermediate between that of *O*- and *S*-glycosides (94.2 and 81.5 ppm, respectively).



Scheme 3 Products obtained by ring-opening of thiirane **6**

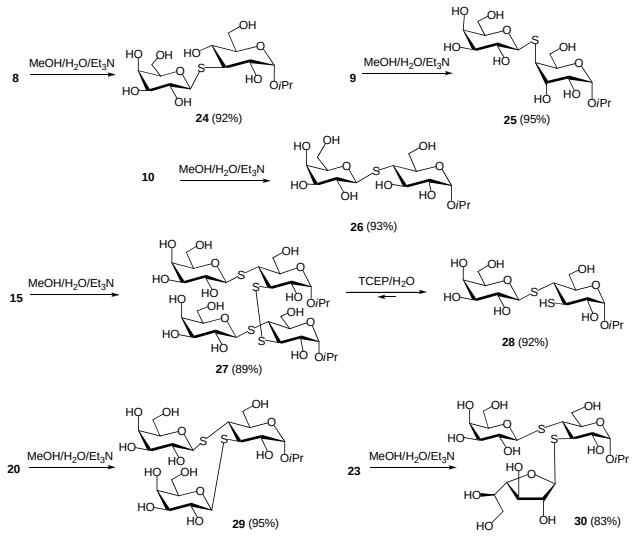
The free thiol group of the dithiodisaccharide **15**, obtained under the optimized conditions described above, was subjected to glycosylation (Scheme 4). This reaction was conducted, in first instance, using an excess of the trichloroacetimidate **19** as glycosyl donor. Reaction of **15** and **19** under catalysis with trimethylsilyl triflate (TMSOTf), and in the presence of 4 Å molecular sieves, led to the dithiotrisaccharide **20** in a moderate yield (35%). The yield could not be improved even though a number of different conditions were employed. The resistance of the 3-thiol group of **15** to glycosylation may be attributed to the sterical hindrance of the vicinal *gauche*-disposed thiopyranose moiety at C-4 and the acetoxy group at C-2. Probably due to the low reactivity of the thiol group, part of the trichloroacetimidate **19** is converted into the Gal*p*-(1→1)-Gal*p* derivative **21**.[28] The sterical hindrance on the SH group of **15** justifies why disulfides **16** and **17** are formed during the ring-opening reaction of thiirane **6**. The longer and more flexible *S*-*S* bond, compared to the 3-thioglycosidic linkage in **20**, would alleviate the crowd in the C-3 region of the 3,4-dithioglucopyanose moiety. In agreement with this behaviour, glycosylation of **15** with the galactofuranose trichloroacetimidate **22**, as less bulkier analogue of **19**, led to the dithiotrisaccharide **23** in a very good yield (81%).

The per-*O*-acetyl derivatives of thiodisaccharides **8**-**10** and dithiotrisaccharides **20** and **23** were fully deprotected by a smooth hydrolysis of the acetyl esters with an aqueous methanol solution of triethylamine at room temperature (Scheme 5). The resulting free products were purified by elution of their respective solutions in water through a column filled with a mixed-bed ion-exchange resin and then through a reverse phase minicolumn. The NMR spectra of thiodisaccharides **24** -**26** exhibited the expected values for the signals of the anomeric protons (H-1 at ~5.0 ppm, $J_{1,2}$ = 3.5–4.0 Hz and H-1' at ~4.5 ppm, $J_{1',2'}$ = 9.6–9.8 Hz); whereas the anomeric carbon of the thioglycoside appeared shifted upfield (~12 ppm) with respect to that of the *O*-glycoside. The dithiotrisaccharides **29** and **30** showed the additional signals due to the respective *S*-linked Gal*p* or Gal*f* units.



Scheme 4 Synthesis of dithiotrisaccharides **20** and **23**

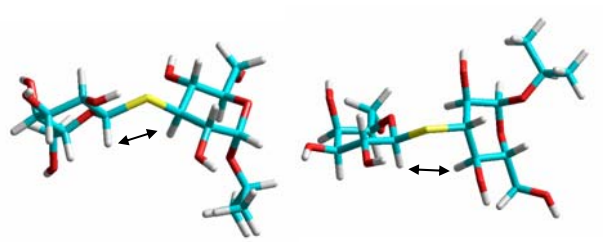
Interestingly, the deprotection of **15** afforded a single product with spectral properties that were in agreement with those expected for the resulting dithiodisaccharides. However, the R_f value was lower than those measured for the analogue thiodisaccharide **24**-**26**. Finally, the MS of the compound revealed that we were dealing with a dimeric structure, assigned as the disulfide **27**. The structure of **27** was confirmed by treatment of the compound with tris(2-carboxyethyl)phosphine hydrochloride (TCEP). This reagent is employed for the reductive cleavage of the disulfide linkages in water and polar solvents.[29] As expected, TLC examination of the reaction showed the conversion of the disulfide into the faster moving dithiodisaccharide **28**. The NMR spectra of **27** and **28** were quite similar, but a careful analysis showed some differences in the chemical shifts of the signals of the carbon atoms (C-3 and C-4) linked to sulfur, and also for those of H-3 and H-4. For example, H-3 and H-4 signals appeared in **27** overlapped as a multiplet at 3.13-3.02 ppm; whereas in **28** they exhibited well resolved triplets at 3.29 and 2.85 ppm, respectively. We have also observed that **28** in solution is rapidly oxidized to **27**, even under an inert Ar atmosphere.



Scheme 5 *O*-Deacetylation of thioglycomimetics **8**-**10**, **15, 20** and **23**

The conformation of the pyranose rings of dithiodisaccharides **24**, **25** and **26** was established by ^{1}H-NMR. As expected, the Gal*p* and Glc*p* rings of **24** and **26** adopt the $^4C_1$ conformation according to the ^3J coupling constants. Also, the Gal*p* ring of **25** adopts the same conformation as indicated by the small value for $J_{3,4}$ (3.6 Hz). Similarly, the large values for $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ in **28** are indicative of the $^4C_1$ chair for the 3,4-dithioGlc*p* ring. In this case, the *J* values are even larger (0.6–1.4 Hz) than those found for the respective coupled protons in **26**, due to the replacement of OH by SH and being the sulfur atom less electronegative than oxygen. In the field of thiooligosaccharides, is always interesting to evaluate how the substitution of the interglycosidic oxygen by sulfur modifies the conformational behavior of the analogues with respect to their natural counterparts. Differences should be expected as the C-S bond length (1.78 Å) and the C-S-C bond angle (99°) differ from the values for C-O (1.41 Å) and C-O-C (116°) and the size of stereoelectronic effects, in particular the anomeric effects, should be different as well. In fact, the conformation of methyl 4-thiolactoside was studied using a combination of NMR spectroscopy and molecular mechanics and dynamics calculations.[25a] The theoretical results were experimentally confirmed by the interresidue NOEs that unequivocally characterize the minimum energy regions of the conformational maps. Thus, according with the torsional angles ϕ (H1'-C1'-S-C4) and ψ (C1'-S-C4-H4), three main conformations were found for methyl 4-thiolactoside: *syn* ϕ/*anti* ψ, *syn* ϕ/*syn* ψ and *anti* ϕ/*syn* ψ, which were confirmed by the respective interresidue observed NOE contacts H1'-H3, H1'-H4, and H2'-H4. To determine the NOE correlations that suggest the presence of given conformers, the NOESY spectrum of the thiodisaccharides were recorded and analyzed. Thus, in the NOESY spectrum of compound **26**, the isopropyl glycoside analogue of methyl 4-thiolactoside, the same NOEs were detected suggesting a similar conformational behavior for the thioglycosidic linkage of both compounds. In contrast, for methyl α-lactoside was predicted and experimentally proven,[30] the almost exclusive contribution of the *syn* ϕ/*syn* ψ conformation. The variation in bond length and angles may explain the higher flexibility of **26** versus its natural *O*-analogue.

Preliminary conformational information was also obtained from the NOESY spectra of thiodisaccharides **24** and **25**. The interresidue NOE contacts between H1'-H3 and H1'-H4 observed for **24** suggested the presence of the respective *syn* ϕ/*syn* ψ and *syn* ϕ/*anti* ψ conformations around the thioglycosidic bond (Figure 1). On the other hand, the NOESY spectrum of **25** exhibited characteristic interresidue cross peaks between H1'-H3, H2'-H4 and H1'-H4 that justify, respectively, the presence of the *syn* ϕ/*anti* ψ, *anti* ϕ/*syn* ψ and *syn* ϕ/*syn* ψ conformations. It is worth to mention that all the conformations found for the dithiodisaccharides are stabilized by the exo-anomeric effect. Therefore, similar to the *O*-glycosides, the thiodisaccharides adopt exo-anomeric conformations around ϕ, but the orientations around the aglyconic bond ψ are rather different for *S*- and *O*-glycosides. The major conformer is always centered at the *syn* ψ region,[25] but *anti* ψ conformers are also found for the thioglycosides. For the binding of a flexible compound to a protein, one of the existing conformations could be selected and bound to the binding site without major energy conflicts.[25b]



syn Φ/ syn Ψ syn Φ/ anti Ψ

Figure 1. Conformations proposed for **24** according to NOE contacts.

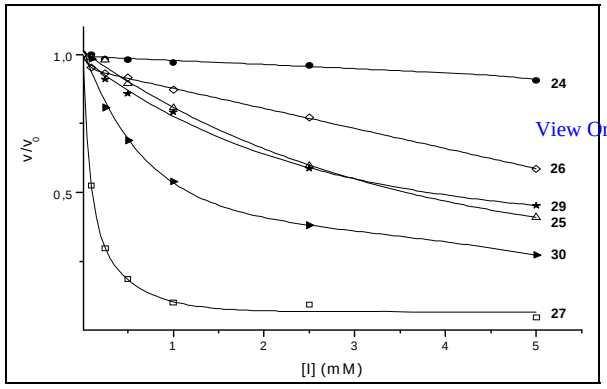


Figure 2. Effect of concentration of thioglycomimetics on the enzymatic activity of the β-galactosidase from *E. coli*. Each point is the mean obtained from three replicate experiments.

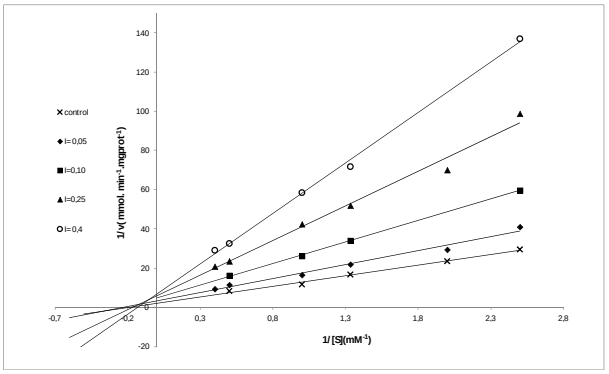


Figure 3. Lineweaver–Burk plot for inhibition of *E. coli* β-galactosidase by disulfide **27**

The free compounds **25**-**30** were evaluated as inhibitors of the β-galactosidase from *E. coli*. Thiodisaccharides **24** and **25** are useful to establish the influence of structural changes on the reducing end, in comparison with the 4-thiolactoside **26**; whereas **27**, **29** and **30** will show the effect of the glycosylation or disulfide formation involving the thiol group at C-3 of **28**. Unfortunately, this compound could not be evaluated as underwent rapidly oxidative dimerization during the isolation and incubation.

Evaluation of the inhibitory activity

For the evaluation of the inhibitory activity of thiodisaccharides **24**-**26**, disulfide **27** and dithiotrisaccharides **29** and **30** against the *E. coli* β-galactosidase a standard protocol was followed.[16] The substrate employed was *o*-nitrophenyl β-D-galactopyranoside, and the release of *o*-nitrophenol was measured spectrophotometrically. The enzymatic reaction was performed in the presence of the potential inhibitor at concentrations ranging from 0.05 to 5.0 mM, and the release of *o*-nitrophenol is referred to the control (Figure 2).

The isopropyl 4-thiolactoside **26** showed only a weak inhibitory activity. Similarly, methyl 4-thiolactoside was determined to be a weak competitive inhibitor of the enzyme (*K*i = 7.7 mM).[25] The thiodisaccharide **24** was not active in the range of concentrations employed. Therefore, the change of position of the thiogalactopyranosyl unit from C-4 to C-3 of the glucopyranose residue has a negative effect on the inhibition. In contrast, compound **25**, with a D-*gulo*-hexopyranoside as reducing end, showed an increased activity with respect to **26**. Interestingly, the dithiotrisaccharides that contain a 3-S-Gal*p* (**29**) or 3-S-Gal*f* (**30**) unit were stronger inhibitors of *E. coli* β-galactosidase than **26**. The IC_{50} values determined for **25** (3.53 ± 0.02 mM), **29** (3.79 ± 0.02 mM) and **30** (1.16 ± 0.06 mM) allows the comparison of their relative activity. The best inhibitor of these series of compounds was the symmetric disulfide **27** (IC_{50} = 0.111 ± 0.005 mM). The kinetics of inhibition of **27** was determined on the basis of Lineweaver-Burk plot (Figure 3), which indicated that **27** in a non-competitive inhibitor with *K*i = 95 μM (*K*m = 1.15 mM). On the other hand, the dithiotrisaccharide **30** is a mixted-type inhibitor, while the analogue **25**, as methyl 4-thiolactoside,[25] is a competitive inhibitor.

These results suggest that small molecules, like the S-disaccharides, structurally similar to the *O*-glycoside substrate, compete with this compound for binding to the active site of the β-galactosidase. Contrarily, a bulky molecule, such as **27**, is probably not able to interact with the catalytic site, but decreases the efficiency of the hydrolysis of the *O*-glycoside by interacting with some other sites of the enzyme.

Conclusions

The ring–opening reaction of 3,4-epoxide or 3,4-thiirane derivatives of sugars by the nucleophilic 1-thiogalactose was successfully applied for the respective synthesis of 4-thiolactosides and 3,4-dithiolactosides. Under optimized conditions, the formation of the thioglycosidic linkage took place with high regio and diastereoselectivities to give *S*-(1→4)-linked disaccharides having the 4-thio- or 3,4-dithio-D-Glc*p* as reducing end.

The 2-propyl per-*O*-acetyl-3,4-dithiolactoside possesses a thiol group free that can be glycosylated or converted into disulfides, molecules with potential biological activity.[31,32] Thiols can also act as antioxidants[33] and have an essential role in many biochemical redox reactions.[34]

One of the synthetic mimetics, the symmetric bis(2-propyl 3,4-

dithiolactosid-3-yl)-disulfide (**27**), showed to be a good non-competitive inhibitor of the *E. coli* β-galactosidase. From the inhibition studies we can conclude that the activity of the thiodisaccharides depends on the site of *S*-bonding (1→3 or 1→4) of Gal*p*SH to the hexose at the reducing-end, and the configuration of this unit. Thus, the *S*-(1→3) linked disaccharide **24**, is the less active compound. The inhibitory activity increases for the 4-thiolactoside **26**, the analogue of a natural substrate of the enzyme. The *S*-disaccharide **25**, which has the C-3 and C-4 stereocenters with opposite configuration than those in **26**, is more active than this compound. Interestingly, the 3-deoxy analogue of **25**,[16] is an even more potent inhibitor (*K*i = 0.16 mM) than **25** (*K*i = 1.24 mM). Therefore, the change of the configuration at C-4 and the deoxygenation at C-3 of the reducing end of these series of *S*-(1→4) linked analogues of 4-thiolactoside provide more potent inhibitors.

The thiolactosides obtained are relevant not only as analogues of the naturally occurring lactose, but also because the motif Gal*p*-β-(1→4)-Glc*p* is found in complex molecules. For example, such a moiety is a component of gangliosides of marine organisms,[35] and also of the GM_3 ganglioside, a characteristic antigen of murine B16 melanoma.[36] A trimeric β-lactosyl cluster has been synthesized as a GM_3 analogue that was an effective acceptor of a sialyltransferase.[37] The exopolysaccharide from *Lactobacillus delbrueckii* contains a lactosyl residue as a lateral chain.[38] With regard to the branched dithiotrisaccharides, a pattern of a Glc*p* core with glycosyl residues at HO-3 and HO-4 is found in the sialyl X Lewis epitope[39] and related compounds;[40] whereas the motif β-D-Gal*f*(1→3)Glc*p* was found in oligosaccharides structures from *Streptococcus thermophilus*.[35]

Experimental

General Methods

Column chromatography was carried out with Silica Gel 60 (230-400 mesh). Analytical thin layer chromatography (TLC) was performed on Silica Gel 60 F254 aluminium supported plates (layer thickness 0.2 mm). Visualization of the spots was effected by exposure to UV light and by charring with a solution of 5 % (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Optical rotations were measured at 25 °C in a 1 dm cell in the solvent indicated. The nuclear magnetic resonance (NMR) spectra were recorded at 500 MHz (^{1}H) or 125.7 MHz (^{13}C); chemical shifts are relative to tetramethylsilane or a residual solvent peak ($CHCl_3$: ^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.2 ppm). Assignments of ^{1}H and ^{13}C were assisted by 2D ^{1}H–COSY and 2D ^{1}H–^{13}C HSQC experiments. In the description of the NMR spectra of dithiotrisaccharides or the asymmetric disulfide, the H and C atoms of the *S*-glycosyl substituent at C-3 of the pyranose core have been labelled as H' and C'; whereas those of C-4 of the pyranose, as H'' and C''. High resolution mass spectra (HRMS) were obtained by Electrospray Ionization (ESI) and Q-TOF detection.

2-Propyl 2,4,6-tri-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3-thio-α-D-glucopyranoside (8) and 2-propyl 2,3,6-tri-*O*-acetyl-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-gulopyranoside (9)

2-Propyl 6-*O*-acetyl-3,4-anhydro-α-D-allopyranoside[21] (**3**, 0.25 g, 0.87 mmol) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose (**7**, 0.45 g, 1.2 mmol) were dissolved in 2 M LiOMe/MeOH (3.1 mL) and the mixture was stirred in a screw-cap tube under Ar at 65 °C for 24 h. The solution was neutralized with acetic acid, concentrated and the residue was acetylated with pyridine (1 mL) and Ac_2O (1 mL) at room temperature for 15 h and concentrated. Monitoring of the mixture by TLC (1:1 toluene/EtOAc) showed two main products (R_f 0.37 and 0.46), which were separated by column chromatography (3:1→2:1 hexane/EtOAc). The first compound eluted from the column was identified as the thiodisaccharide **8** (0.31 g, 45%); $[\alpha]_D^{25}$ +62.7 (*c* 1.1 in $CHCl_3$); ^{1}H RMN ($CDCl_3$, 500 MHz): δ 5.42 (d, 1H, $J_{3',4'}$ = 3.1 Hz, H-4'), 5.14 (t, 1H, $J_{1',2'}$ = $J_{2',3'}$ = 9.9 Hz, H-2'), 5.10 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1), 5.03 (dd, 1H, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 3.1 Hz, H-3'), 4.92 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 11.3 Hz, H-4), 4.82 (dd, 1H, $J_{1,2}$ = 3.4, $J_{2,3}$ =11.3 Hz, H-2), 4.77 (d, 1H, $J_{1',2'}$ = 9.9 Hz, H-1'), 4,20 (dd, 1H, $J_{5,6a}$ = 4.9, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.17 (dd, 1H, $J_{5',6'a}$ = 6.7, $J_{6'a,6'b}$ = 11.1 Hz, H-6'a), 4,08 (dd, 1H, $J_{5,6b}$ = 2.3, $J_{6a,6b}$ = 12.2 Hz, H-6b), 4.05 (m, 1H, H-5), 4.03 (dd, 1H, $J_{5',6'b}$ = 6.7, $J_{6'a,6'b}$ = 11.1 Hz, H-6'b), 3,98 (t, 1H, $J_{5',6'a}$ = $J_{5',6'b}$ = 6.7 Hz, H-5'), 3.89 (m, 1H, *J* = 6.2 Hz, Me_2CHO), 3.27 (dd, 1H, $J_{2,3}$ = 11.7, $J_{3,4}$ = 11.3 Hz, H-3), 2.15, 2.13, 2.10, 2.09, 2.06, 2.02, 1.97 (7 s, 21H, CH_3CO), 1.25, 1.14 (2 d, 3H each, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C RMN ($CDCl_3$, 125.7 MHz): δ 170.7, 170.4, 170.2, 170.0, 169.8, 169.4, 169.3 (CH_3CO), 93.7 (C-1), 83.8 (C-1'), 74.0 (C-5'), 72.5 (C-2), 71.8 (C-3'), 71.1 (Me_2CHO), 68.4 (C-5), 67.5 (C-2'), 67.0 (C-4'), 66.8 (C-4), 62.5 (C-6), 61.1 (C-6'), 46.7 (C-3), 23.0, 21.6 [$(CH_3)_2CHO$], 20.8 – 20.6 (CH_3CO). Found: C, 50.52; H, 6.19; S, 4.40. Calc. for $C_{29}H_{42}O_{17}S$: C, 50.14; H, 6.09; S, 4.62%.

From further fractions from the column compound was isolated syrupy **9** (0.35 g, 50%); $[\alpha]_D^{25}$ +37.5 (*c* 1.0 in $CHCl_3$); ^{1}H RMN ($CDCl_3$, 500 MHz): δ 5.43 (dd, 1H, $J_{3',4'}$ = 3.2, $J_{4',5'}$ < 1.0 Hz, H-4'), 5.35 (m, 1H, H-3), 5.34 (d, 1H, $J_{1,2}$ = 4.2 Hz, H-1), 5.24 (t, 1H, $J_{1',2'}$ = $J_{2',3'}$ = 10.0 Hz, H-2'), 5.06 (dd, 1H, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.2 Hz, H-3'), 5.03 (d, 1H, $J_{1,2}$ = 4.2 Hz, H-2), 4.68 (d, 1H, $J_{1',2'}$ = 10.0 Hz, H-1'), 4.65 (m, 1H, H-5), 4.22 – 4.08 (m, 4H, H-6a, 6b, 6'a, 6'b), 3.95 (dd, 1H, $J_{5',6'a}$ = 6.7, *J* = 6.5 Hz, H-5'), 3.87 (m, 1H, *J* = 6.2 Hz, Me_2CHO), 3.28 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 2.7 Hz, H-4), 2.16, 2.14, 2.10, 2.07, 2.06, 2.04, 1.98 (7 s, 3H each, CH_3CO), 1.25, 1.13 (2 d, 3H each, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C-RMN ($CDCl_3$, 50.3 MHz) δ 170.6, 170.5, 170.3 (×2), 170.0, 169.9, 169.8 (CH_3CO), 94.4 (C-1), 83.0 (C-1'), 74.7, 71.8, 70.6 (C-3, 3', 5'), 70.4 (Me_2CHO), 67.1, 66.9, 65.4, 65.1 (C-2, 5, 2', 4'), 63.8 (C-6), 61.2 (C-6'), 44.8 (C-4), 23.0, 21.4 [$(CH_3)_2CHO$], 21.1 – 20.5 (CH_3CO). Found: C, 50.22; H, 6.21; S, 4.57. Calc. for $C_{29}H_{42}O_{17}S$: C, 50.14; H, 6.09; S, 4.62%.

2-Propyl 2,4,6-tri-*O*-acetyl-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-glucopyranoside (10), 2-propyl 2,4,6-tri-*O*-acetyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3-thio-α-D-gulopyranoside (11) and 2-propyl 2,4,6-tri-*O*-acetyl-2-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-

galactopyranosyl)-2-thio-α-D-idopyranoside (12).

2-Propyl 2,6-di-*O*-acetyl-3,4-anhydro-α-D-galactopyranoside[21] (**4**, 0.25 g, 0.87 mmol) and the 1-thioaldose **7** (0.38 g, 1.04 mmol) were dissolved in 2M LiOMe in MeOH (2.6 mL) and the mixture was stirred under Ar at 65 ºC for 24 h, and then neutralized, concentrated and acetylated as described in the previous item. Monitoring by TLC (3:1 CH_2Cl_2/EtOAc) reavealed two main spots (R_f 0.64 and 0.47). The residue was subjected to column chromatography (9:1 CH_2Cl_2/EtOAc) to afford first the less polar product, which was identified as **10** (125 mg, 21%); $[\alpha]_D^{25}$ +46.9 (*c* 1.0 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.44 (dd, 1H, $J_{2,3}$ = 9.9, $J_{3,4}$ = 11.1 Hz, H-3), 5.42 (dd, 1H, $J_{3',4'}$ = 3.3 Hz, H-4'), 5.15 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 5.11 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 9.9 Hz, H-2'), 5.02 (dd, 1H, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 3.3 Hz, H-3'), 4.77 (dd, 1H, $J_{1,2}$ = 3.7, $J_{2,3}$ = 9.9 Hz, H-2), 4.71 (d, 1H, $J_{1',2'}$ = 9.9 Hz, H-1'), 4.50 (dd, 1H, $J_{5,6a}$ = 4.1, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.45 (dd, 1H, $J_{5,6b}$ = 2.1, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.20 (ddd, 1H, $J_{4,5}$ = 11.2, $J_{5,6a}$ = 4.1, $J_{5,6b}$ = 2.1 Hz, H-5), 4.10–4.04 (m, 2H, $J_{5',6'a} = J_{5',6'b}$ = 6.7, $J_{6'a,6'b}$ = 12.5 Hz, H-6'a, 6'b), 3.89 (dt, 1H, $J_{4',5'}$ = 0.8, $J_{5',6'a} = J_{5',6'b}$ = 6.7 Hz, H-5'), 3.89 (m, 1H, *J* = 6.2 Hz, Me_2CHO), 2.91 (t, 1H, $J_{3,4} = J_{4,5}$ = 11.1 Hz, H-4), 2.15, 2.09, 2.04 (× 4), 1.96 (6 s, 21H, CH_3CO), 1.23, 1.12 (2 d, 3H each, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ 170.5, 170.3, 170.2, 170.1, 169.8, 169.6, 169.5 (CH_3CO), 94.2 (C-1), 82.6 (C-1'), 74.0 (C-5'), 72.4 (C-2), 71.8 (C-4'), 71.0 (Me_2CHO), 68.8 (C-5), 67.6 (C-3), 66.9, 66.8 (C-2', 3'), 63.4 (C-6), 61.3 (C-6'), 46.5 (C-4), 23.1, 21.4 [$(CH_3)_2CHO$], 20.8 – 20.5 (CH_3CO). HRMS (ESI+): *m/z* found 717.2039 ([M+Na]$^+$); calc. for $C_{29}H_{42}NaO_{17}S$ 717,2035.

From further fractions of the column was isolated the component of R_f 0.47, which although chromatographically homogenous, was in fact a 1:1 mixture of two thiodisaccharides **11** and **12**. These two products were partially separated by column chromatography using 9:1 CH_2Cl_2/EtOAc as eluent. The first product isolated from the column was the thiodisaccharide **12** (117 mg, 20%); $[\alpha]_D$ +31.5 (*c* 1.7 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.40 (dd, 1H, $J_{3',4'}$ = 3.3, $J_{4',5'}$ = 0.9 Hz, H-4'), 5.25 (dd, 1H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2'), 5.15 (t, 1H, $J_{2,3} \approx J_{3,4}$ = 4.1 Hz, H-3), 5.05 (d, 1H, $J_{1,2}$ = 2.0, H-1), 5.02 (dd, 1H, $J_{2',3'}$ =10.0, $J_{3',4'}$ = 3.3 Hz, H-3'), 4.84 (dd, 1H, $J_{3,4}$ = 3.8, $J_{4,5}$ = 2.4 Hz, H-4), 4.65 (d, 1H, $J_{1',2'}$ = 10.0 Hz, H-1'), 4.46 (ddd, 1H, $J_{4,5}$ = 2.5, $J_{5,6a}$ = 7.5, $J_{5,6b}$ = 5.4 Hz, H-5), 4.18 (dd, 1H, $J_{5,6a}$ = 7.5, $J_{6a,6b}$ = 11.5 Hz, H-6a), 4.14 (dd, 1H, $J_{5,6a}$ = 5.4, $J_{6a,6b}$ = 11.5 Hz, H-6a), 4.11–4.05 (m, 2H, H-6'a, 6'b), 3.96 (dt, 1H, $J_{4',5'}$ = 0.9, $J_{5',6'a} = J_{5',6'b}$ = 6.5 Hz, H-5'), 3.89 (m, 1H, *J* = 6.2 Hz, Me_2CHO), 3.28 (dd, 1H, $J_{1,2}$ = 2.0, $J_{2,3}$ = 4.1 Hz, H-2), 2.14, 2.09, 2.06, 2,05, 2,03, 2,02, 1,97 (7 s, 21H, CH_3CO), 1.21, 1.15 (2d, each 3H, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ 170.5, 170.3, 170.2, 170.1, 169.9, 169.6, 169.2 (CH_3CO), 98.8 (C-1), 84.3 (C-1'), 74.4 (C-5'), 71.9 (C-3'), 69.8 (Me_2CHO), 68.9 (C-3), 67.6 (C-4), 67.4 (C-2'), 67.2 (C-4'), 64.1 (C-5), 62.5 (C-6), 61.2 (C-6'), 43.2 (C-2), 23.2, 21.5 [$(CH_3)_2CHO$], 20.9, 20.8, 20.7 (×2), 20.6, 20.5 (CH_3CO). Found: C, 48.20; H, 6.08. Calc. for $C_{29}H_{42}O_{17}S+H_2O$: C, 48.87; H, 6.22%. HRMS (ESI+): *m/z* found 717.2064 ([M+Na]$^+$); calc. for $C_{29}H_{42}NaO_{17}S$ 717.2035.

Further fractions from the column led to **11** (116 mg, 20%); $[\alpha]_D^{25}$ +37.6 (*c* 1.0 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.40 (dd, 1H, $J_{3',4'}$ = 3.4, $J_{4',5'}$ = 1.0 Hz, H-4'), 5.22 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2'), 5.19 (dd, 1H, $J_{1,2}$ = 3.3, $J_{2,3}$ = 5.5 Hz, H-2), 5.06 (t, 1H, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.4 Hz, H-3'), 5.02 (br dd, 1H, $J_{3,4}$ = 3.1, $J_{4,5}$ = 1.3 Hz, H-4), 4.95 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 4.59 (ddd, 1H, $J_{4,5}$ = 1.3, $J_{5,6a}$ = 5.5, $J_{5,6b}$ = 7.0 Hz, H-5), 4.53 (d, 1H, $J_{1',2'}$ = 10.0 Hz, H-1'), 4.15 (dd, 1H, $J_{5,6a}$ = 5.5, $J_{6a,6b}$ = 11.4 Hz, H-6a), 4.14–4.05 (m, 3H, H-6b, 6'a, 6'b), 3.91 (dt, 1H, $J_{4',5'}$ = 1.0, $J_{5',6'a} = J_{5',6'b}$ = 6.6 Hz, H-5'), 3.89 (m, 1H, *J* = 6.2 Hz, Me_2CHO), 3.58 (dd, 1H, $J_{2,3}$ = 5.4, $J_{3,4}$ = 3.0 Hz, H-3), 2.13, 2.12, 2.10, 2.06, 2.05, 2.03, 1.97 (7 s, each 3H, CH_3CO), 1.23, 1.13 (2 d, each 3H, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ 170.5, 170.4, 170.3, 170,2, 170.1, 170.0, 169.2 (CH_3CO), 93.9 (C-1), 84.0 (C-1'), 74.6 (C-5'), 72.7 (C-4), 72.0 (C-3'), 70.5 (Me_2CHO), 67.3 (C-4'), 66.9 (C-2'), 66.3 (C-2), 62.9 (C-5), 62,8 (C-6), 61.2 (C-6'), 42.5 (C-3), 23.0, 21.3 [$(CH_3)_2CHO$], 21.0, 20.9, 20.7 (×2), 20.6 (×3) (CH_3CO). HRMS (ESI+): *m/z* found 717.2060 ([M+Na]$^+$); calc. for $C_{29}H_{42}NaO_{17}S$ 717.2035;.

2-Propyl 3,6-di-*O*-acetyl-2-*O*-*tert*-butyldimethylsilyl-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-glucopyranoside (13) and its conversion into 10.

Epoxide **5** (0.25 g, 0.70 mmol) and the 1-thioaldose **7** (0.30 g, 0.83 mmol) were dissolved in 0.8M LiOMe in MeOH (2.1 mL) and stirred under Ar at 60 ºC for 24 h. The reaction mixture was processed and acetylated as described above for the analogous reactions starting from **3** or **4**. The resulting crude product showed by TLC (1:1 hexane/EtOAc) two main spots having R_f 0.45 (major) and R_f 0.29. Purification by column chromatography (5.6:1→2.5:1 hexane/EtOAc) gave first the major product **13** as a syrup (0.27 g, 51%); $[\alpha]_D^{25}$ +21.3 (*c* 1.0 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.43 (dd, 1H, $J_{3',4'}$ = 3.2, $J_{4',5'}$ = 0.8 Hz, H-4'), 5.35 (dd, 1H, $J_{2,3}$ = 9.3, $J_{3,4}$ = 11.2 Hz, H-3), 5.11 (dd, 1H, $J_{1',2'} = J_{2',3'}$ = 9.9 Hz, H-2'), 5.04 (dd, 1H, $J_{2',3'}$ = 9.9, $J_{3',4'}$ = 3.3 Hz, H-3'), 4.87 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.79 (d, 1H, $J_{1',2'}$ = 9.9 Hz, H-1'), 4.54 (dd, 1H, $J_{5,6a}$ = 6.5, $J_{6a,6b}$ = 12.9 Hz, H-6a), 4.42 (dd, 1H, $J_{5,6b}$ = 1.7, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.22 (m, 1H, $J_{4,5}$ = 11.2, $J_{5,6a}$ = 6.5, $J_{5,6b}$ = 1.7 Hz, H-5), 4.09–4.04 (m, 2H, H-6'a, 6'b), 3.91 (dt, 1H, $J_{4',5'}$ = 0.8, $J_{5',6'a} = J_{5',6'b}$ = 6.6 Hz, H-5'), 3.85 (m, 1H, *J* = 6.2 Hz, Me_2CHO), 3.68 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 9.3 Hz, H-2), 2.79 (t, 1H, $J_{3,4} = J_{4,5}$ = 11.2 Hz, H-4), 2.16, 2.10, 2.06 (×2), 2.05, 1.96 (6 s, 18H, CH_3CO), 1.25, 1.18 (2 d, 3H each, *J* = 6.2 Hz, $(CH_3)_2CHO$); 0.87 (s, 9H, $(CH_3)_3SiCMe_2$), 0.06, 0.05 (2 s, 3H each, $(CH_3)_2SiBu^t$); ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ 170.6, 170.2, 169.8, 169.6, 169,5 (×2) (CH_3CO), 97.5 (C-1), 82.4 (C-1'), 73.8 (C-5'), 72.5 (C-2), 71.8 (C-3'), 70.9 (Me_2CHO), 70.2 (C-3), 68.8 (C-5), 66.9 (C-4'), 66.7 (C-3'), 63.9 (C-6), 61.2 (C-6'), 46.7 (C-4), 25.4 [$(CH_3)_3CSiMe_2$], 23.3, 21.6 [$(CH_3)_2CHO$], 20.7–20.5 (CH_3CO), 18.4 [$(CH_3)_3CSiMe_2$], –4.5, –5.0 [$(CH_3)_2SiBu^t$]. HRMS (ESI+): *m/z* found 789.2769 ([M+Na]$^+$); calc. for $C_{33}H_{54}NaO_{16}SSi$ 789.2794.

The minor product (R_f 0.29) was also obtained as a syrup and identified as **10** (160 mg, 33%), which showed the same properties as the product obtained from the oxirane **4**.

Alternatively, reaction of **5** (60 mg, 0.19 mmol) and **7** (83 mg, 0.23 mmol), under the conditions employed above, led to a crude mixture that was subjected to *O*-desilylation with 1 M TBAF in THF (0.25 mL), followed by acetylation. Purification by column

chromatography (3:1→2:1 hexane/EtOAc) led to thiodisaccharides **10** (110 mg, 85%) as the only product isolated.

2-Propyl 2,6-di-*O*-acetyl-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3,4-dithio-α-D-glucopyranoside (15), 2-propyl 2,6-di-*O*-acetyl-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)- 3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-disulfide-3,4-dithio-α-D-glucopyranoside (16) and bis[2-propyl 2,6-di-*O*-acetyl-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3,4-dithio-α-D-glucopyranosid-3-yl]-disulfide (17)

To a solution of **7** (175 mg, 0.49 mmol) in anhydrous THF (20 mL) was added NaH (24 mg, 0.72 mmol) under N_2. The mixture was stirred at room temperature until evolution of gases ceased (~1 h). The solvent was evaporated and the resulting salt **14** was dissolved in THF (5 mL). To this solution, cooled at –18 °C (ice/salt bath) a solution of 2-propyl 3,6-di-*O*-acetyl-3,4-epithio-α-D-galactopyranoside[21] (**6**, 100 mg, 0.32 mmol) in THF (1 mL) was added. The mixture was stirred at –18 °C for 10 minutes and, upon addition of 18-crown-6 (10 mg, 0.038 mmol), Ar was bubbled into the solution and the stirring was continued for 30 min, when an additional portion of 18-crown-6 (10 mg) was added. The solution was allowed to reach room temperature until monitoring by TLC (1:1 toluene/EtOAc) revealed the complete consumption of the starting **6** (R_f 0.72). The mixture was concentrated and the residue was subjected to column chromatography (hexane/EtOAc 4:1→1.5:1), to afford the thiodisaccharide **15** (11 mg, 5%).[22]

Further fractions of the column afforded a mixture of compounds **16** and **17** (0.12 g). The less polar product was the major component of the mixture, which was identified as **16** (106 mg, 32%); $[\alpha]_D^{25}$ +11.3 (*c* 1.0 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.46 (dd, 1H, $J_{3'',4''}$ = 3.4, $J_{3'',5''}$ = 1.1 Hz, H-4''), 5.45 (dd, 1H, $J_{3',4'}$ = 3.5, $J_{4',5'}$ = 1.8 Hz, 4'), 5.17 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2'), 5.16 (t, 1H, $J_{1'',2''} = J_{2'',3''}$ = 10.1 Hz, H-2''), 5.10 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 5.09 (dd, 1H, $J_{2'',3''}$ = 10.0, $J_{4'',3''}$ = 3.3 Hz, H-3''), 5.07 (dd, 1H, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.4 Hz, H-3'), 4.90 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 10.5 Hz, H-2), 4.62 (dd, 1H, $J_{5,6a}$ = 2.1, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.60 (d, 1H, $J_{1',2'}$ = 10.0 Hz, H-1'), 4.57 (d, 1H, $J_{1'',2''}$ = 10.2 Hz, H-1''), 4.53 (dd, 1H, $J_{5,6b}$ = 4.2, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.24 (m, 2H, H-5,6'a), 4.12 (m, 4H, H-6'a, 6'b, 6''a, 6''b), 3.98 (ddd, 1H, $J_{4',5'}$ = 2.1, $J_{5',6'a}$ = 5.6 , $J_{5',6'b}$ = 8.4 Hz, H-5'), 3.92 (m, 1H, *J* = 6.2 Hz, Me_2CHO), 3.88 (dt, 1H, $J_{4'',5''}$ = 1.0, $J_{5'',6''a} = J_{5'',6''b}$ = 6.1 Hz, H-5''), 3.32 (t, 1H, $J_{3,4} = J_{4,5}$ = 11.1 Hz, H-4), 3.24 (t, 1H, $J_{2,3} = J_{3,4}$ = 11.6 Hz, H-3), 2.21, 2.20, 2.16, 2.15, 2.12, 2.10, 2.08, 2.07, 2.02, 2.01, 2.00 (11 s, 33H, CH_3CO), 1.28, 1.18 (2 d, 3H each, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ 170.2 – 169.3 (CH_3CO), 94.2 (C-1), 88.5 (C-1''), 81.5 (C-1'), 74.5, 74.3 (C-5', 5''), 71.8, 71.6 (C-3', 3''), 70.7 (Me_2CHO), 69.9 (C-2), 69.1 (C-5), 67.6, 67.0, 66.9, 66.7 (C-2', 2'', 4', 4''), 63.8 (C-6), 61.5, 60.1 (C-6', 6''), 48.9 (C-3), 44.6 (C-4), 23.2, 21.6 [$(CH_3)_2CHO$], 21.0 – 20.6 (CH_3CO). HRMS (ESI+): *m/z* found 1053.2381 ($[M+Na]^+$); calc. for $C_{41}H_{58}NaO_{24}S_3$ 1053.2378.

The other component isolated from the mixture was **17** (14 mg, 3%); $[\alpha]_D^{25}$ 19,3 (*c* 0,5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.37 (dd, 1H, $J_{3',4'}$ = 3.4, $J_{4',5'}$ = 1.0 Hz, H-4'), 5.10 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2'), 5.02 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.00 (dd, 1H, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.4 Hz, H-3'), 4.83 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 10.8 Hz, H-2), 4.61 (d, 1H, $J_{1',2'}$ = 10.0 Hz, H-1'), 4.51 (dd, 1H, $J_{5,6a}$ = 1.9, $J_{6a,6b}$ = 11.8 Hz, H-6a), 4.34 (dd, 1H, $J_{5,6b}$ = 5.5, $J_{6a,6b}$ = 11.8 Hz, H-6b), 4.13 (ddd, $J_{4,5}$ = 10.8, $J_{5,6a}$ = 1.9, $J_{5,6b}$ = 5.5 Hz, H-5), 4.06 (dd, 1H, $J_{5',6'a}$ = 6.4, $J_{6'a,6'b}$ = 11.2 Hz, H-6'a), 4.01 (dd, 1H, $J_{5',6'b}$ = 7.2, $J_{6'a,6'b}$ = 11.2 Hz, H-6'b), 3.67 (ddd, 1H, $J_{4',5'}$ = 1.0, $J_{5',6'a}$ = 6.4, $J_{5',6'b}$ = 7.2 Hz, H-5'), 3.87 (m, 1H, *J* = 6.2 Hz, Me_2CHO), 3.15 (dd, 1H, $J_{2,3}$ = 10.8, $J_{3,4}$ = 11.3 Hz, H-3), 2.90 (dd, 1H, $J_{3,4}$ = 11.3, $J_{4,5}$ = 10.8 Hz, H-4), 2.10, 2.09, 2.06, 2.04, 1.98, 1.91 (6 s, 18H, CH_3CO), 1.19, 1.07 (2 d, each 3H, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 170.5–169.5 (CH_3CO), 93.9 (C-1), 82.7 (C-1'), 74.2 (C-5'), 74.8 (C-3'), 74.3 (C-2), 70.8 (Me_2CHO), 69.7 (C-5), 67.1 (C-2'), 66.9 (C-4'), 64.3 (C-6), 61.1 (C-6'), 50.9 (C-3), 46.8 (C-4), 23.2, 21.6 [$(CH_3)_2CHO$], 20,8 – 20,6 (CH_3CO); Found: C, 48.32; H, 5.87. Calc. for $C_{54}H_{78}O_{30}S_4$: C, 48.57; H, 5.89%. HRMS (ESI+): *m/z* found 1357.3355 ($[M+Na]^+$); calc. for $C_{54}H_{78}NaO_{30}S_4$ 1357.3353.

2-Propyl 2,6-di-*O*-acetyl-3,4-di-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3,4-dithio-α-D-glucopyranoside (20) and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (21)

Compound **15** (36 mg, 0.054 mmol) and the trichloroacetimidate **19**[41] (53 mg, 0.107 mmol) were mixed with recently activated molecular sieves (4 Å) in CH_2Cl_2 (1.3 mL). The mixture was stirred at room temperature for 90 min. Then, the suspension was cooled to –18 °C and TMSOTf (2.5 µL, 13.5 µmol) was added and stirring was continued until TLC (CH_2Cl_2/EtOAc 3:1) showed the conversion of **15** (R_f 0.71) and **19** (R_f 0.63) into two lower moving products (R_f 0.37 y 0.26). After addition of Et_3N, the solution was concentrated and the residue was purified by column chromatography (CH_2Cl_2/EtOAc 9:1 → 4:1). The first product which eluted from the column was identified as 2,3,4,6-tetra-*O*-acetyl-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1-β-D-galactopyranoside (**21**, 20 mg), which showed identical spectroscopic and physical data as those described in the literature.[27]

The more polar compound was characterized as the dithiotrisaccharide **20** (20 mg, 35%); $[\alpha]_D^{25}$ +9.5 (*c* 1.0 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.43 (dd, 1H, $J_{3'',4''}$ = 3.4, $J_{4'',5''}$ = 1.0 Hz, H-4''), 5.42 (dd, 1H, $J_{3',4'}$ = 3.4, $J_{4',5'}$ = 1.0 Hz, H-4'), 5.16 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2'), 5.12 (t, 1H, $J_{1'',2''} = J_{2'',3''}$ = 10.1 Hz, H-2''), 5.11 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.07 (dd, 1H, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.4 Hz, H-3'), 5.04 (dd, 1H, $J_{2'',3''}$ = 10.1, $J_{3'',4''}$ = 3.4 Hz, H-3''), 4.81 (dd, 1H, $J_{1,2}$ =11.1, $J_{2,3}$ = 3.6 Hz, H-2), 4.73 (d, 1H, $J_{1'',2''}$ = 10.1 Hz, H-1''), 4.69 (d, 1H, $J_{1',2'}$ = 10.0 Hz, H-1'), 4.60 (dd, 1H, $J_{5,6a}$ = 4.4, $J_{6a,6b}$ = 11.9 Hz, H-6a), 4.47 (dd, 1H, $J_{5,6b}$ = 2.0, $J_{6a,6b}$ = 11.9 Hz, H-6b), 4.22 (ddd, 1H, $J_{4,5}$ = 10.7, $J_{5,6a}$ = 4.4, $J_{5,6b}$ = 2.0 Hz, H-5), 4.16 (dd, 1H, $J_{5',6'a}$ = 7.2, $J_{6'a,6'b}$ = 11.2 Hz, H-6'a), 4.15-4.05 (m, 3H, H-6'b, H-6''a, H-6''b), 3.97 (ddd, $J_{4',5'}$ = 1.0, $J_{5',6'a} = J_{5',6'b}$ = 7.2 Hz, H-5'), 3.89 (m, 2H, H-5'', Me_2CHO), 3.39 (dd, 1H, $J_{2,3}$ =11.0, $J_{3,4}$= 12.0Hz, H-3), 2.81 (dd, 1H, $J_{3,4}$ =12.0, $J_{4,5}$ = 10.7 Hz, H-4), 2.18, 2.15, 2.14, 2.13, 2.12, 2.07, 2.06, 2.05, 1.98, 1.97 (10 s, 30H, CH_3CO), 1.26, 1.15 (2 d, 3H each, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ 170.4 – 169.5 (CH_3CO), 93.8 (C-

1), 82.4 (C-1''), 81.1 (C-1'), 74.4 (C-5'), 74.1 (C-5''), 73.4 (C-2), 71.7, 71.6 (C-3', 3''), 70.8 (Me_2CHO), 69.5 (C-5), 67.0, 66.9, 66.6 (C-2'', 4', 4''), 66.6 (C-2'), 64.2 (C-6), 61.4, 61.0 (C-6', 6''), 45.3 (C-4), 44.1 (C-3), 23.2, 21.5 [$(CH_3)_2CHO$], 20.9 – 20.5 (CH_3CO). Found: C, 49.29; H, 5.79. Calc. for $C_{41}H_{58}O_{24}S_2$: C, 49.29; H, 5.85%. HRMS (ESI+): *m/z* found 1021.2693 ($[M+Na]^+$); calc. for $C_{41}H_{58}NaO_{24}S_2$ 1021.2651.

General procedure for the *O*-deacylation of the thiooligosaccharides

The *O*-acylated thiodisaccharides **8**, **9**, **10** or **15** or the dithiotrisaccharides **20** and **23** were suspended in MeOH/H_2O/Et_3N 4:5:1 (10 mL) was stirred at room temperature for 2 h. The mixture was concentrated and the residue, dissolved in water (1 mL) was eluted through a column filled with Dowex MR-3C mixed bed ion-exchange resin. The deionized solutions were concentrated and the free thiooligosaccharides were purified by dissolution in water (1 mL) and filtration through an octadecyl C18 minicolumn (Amprep, Amersham Biosciences). Evaporation of the solvent afforded the fully deprotected thiooligosaccharides, which monitored by TLC (2,5:1:1 *n*-BuOH/EtOH/H_2O) exhibited a single spot (R_f values are reported for each compound).

2-Propyl 3-*S*-(β-D-galactopyranosyl)-3-thio-α-D-glucopyranoside (24)

The general procedure for the *O*-deacylation applied to compound **8** (0.36 g, 0.52 mmol) afforded **24** (192 mg, 92%); R_f 0.58; $[\alpha]_D^{25}$ +80.7 (*c* 1.04 in H_2O); ^1H RMN (D_2O, 500 MHz): δ 4.92 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.51 (d, 1H $J_{1',2'}$ = 9.6 Hz, H-1'), 3.89 (m, 1H, *J* = 6.2 Hz, Me_2CHO), 3.86 (d, 1H, $J_{3',4'}$ = 3.0 Hz, H-4'), 3.75 (dd, 1H, $J_{6a,6b}$ = 10.3 Hz, H-6a), 3.69 – 3.59 (m, 5H, H-5, 5', 6b, 6'a, 6'b), 3.56 (dd, 1H, $J_{1,2}$ = 3.5, $J_{2,3}$ = 10.7 Hz, H-2), 3.54 (dd, 1H, $J_{2',3'}$ = 9.4, $J_{3',4'}$ = 3.0 Hz, H-3'), 3.53 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 9.5 Hz, H-2'), 3.32 (t, 1H, $J_{3,4} = J_{4,5}$ = 8.9 Hz, H-4), 3,01 (t, 1H, $J_{2,3} = J_{3,4}$ = 10.7 Hz, H-3), 1.16, 1.08 (2 d, 3H each, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C RMN (D_2O, 125.7 MHz): δ 95.5 (C-1), 85.4 (C-1'), 79.1 (C-5 ó 5'), 74.2 (C-3'), 72.8 (C-5 ó 5'), 70.3 (C-2), 70.2 (Me_2CHO), 70.0 (C-2'), 68.9 (C-4'), 67.7 (C-4), 61.1 × 2 (C-6, C-6'), 51.9 (C-3), 22.3, 20.4 [$(CH_3)_2CHO$]. Found: C, 44.98; H, 7.29. Calc. for $C_{15}H_{28}O_{10}S$: C, 44.99%; H, 7.05. HRMS (ESI+): *m/z* found 423.1301 ($[M+Na]^+$); calc. for $C_{15}H_{28}NaO_{10}S$ 423.1295.

2-Propyl 4-*S*-(β-D-galactopyranosyl)-4-thio-α-D-gulopyranoside (25)

Deacetylation of **9** (0.13 g, 0.19 mmol) gave syrupy **25** (72 mg, 95%); R_f 0,55; $[\alpha]_D^{25}$ +51.7 (*c* 1.1 in H_2O); ^1H RMN (D_2O, 500 MHz): δ 4.88 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 4.41 (d, 1H, $J_{1',2'}$ = 9.8 Hz, H-1'), 4.36 (ddd, 1H, $J_{4,5}$ = 2.1, $J_{5,6a}$ = 5.1, $J_{5,6b}$ = 7.1 Hz , H-5), 4.01 (t, 1H, $J_{2,3} = J_{3,4}$ = 3.6 Hz, H-3), 3.95 (t, 1H, $J_{1,2} = J_{2,3}$ = 3.8 Hz, H-2), 3.82 (d, 1H, $J_{3',4'}$ = 2.8 Hz, H-4'), 3.82 (m, 1H, *J* = 6.3 Hz, Me_2CHO), 3,69 (dd, 1H, $J_{5,6a}$ = 5.1, $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.64 (dd, 1H, $J_{5,6b}$ = 7.1, $J_{6a,6b}$ = 12.1 Hz , H-6b), 3.59 – 3.54 (m, 3H, H-5', 6'a, 6'b), 3.49 (dd, 1H, $J_{2',3'}$ = 9.5, $J_{3',4'}$ = 3.3 Hz, H-3'), 3.40 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 9.6, H-2'), 3.21 (dd, 1H, $J_{3,4}$ = 3.0, $J_{4,5}$ = 2.6 Hz, H-4), 1.11, 1.04 (2 d, 3H each, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C RMN (D_2O, 125.7 MHz): δ 97.2 (C-1), 85.6 (C-1'), 79.1 (C-5'), 73.8 (C-3'), 72.0 (Me_2CHO), 71.8 (C-3), 69.7 (C-2'), 68.7 (C-4), 66.3 (C-5), 64.4 (C-2), 62.2 (C-6), 61.1 (C-6'), 47.2 (C-4), 22.4, 20.6 [$(CH_3)_2CHO$]. Found: C, 44.87; H, 7.38. Calc. for $C_{15}H_{28}O_{10}S$: C, 44.99; H, 7.05%. HRMS (ESI+): *m/z* found 423.1297 ($[M+Na]^+$); calc. for $C_{15}H_{28}NaO_{10}S$ 423.1295.

2-Propyl 4-*S*-(β-D-galactopyranosyl)-4-thio-α-D-glucopyranoside (26)

Deacetylation of **10** (50 mg, 0.072 mmol) was performed in the same conditions describe above, to afford **26** (27 mg, 93%); R_f 0,57; $[\alpha]_D^{25}$ +59.5 (*c* 0.8 in H_2O); ^1H RMN (D_2O, 500 MHz): δ 5.07 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1), 4.58 (d, 1H, $J_{1',2'}$ = 9.7 Hz, H-1'), 4.10 – 3.90 (m, 4H, H-5, 6'a, 6´b, Me_2CHO), 3.77 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3), 3.75 – 3.62 (m, 6H, H-3, 6a, 6b, 3', 4', 5'), 3.57 (dd, $J_{1,2}$ = 3.9, $J_{2,3}$ = 9.7 Hz, H-2), 3.56 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 9.7, H-2'), 2.84 (t, 1H, $J_{3,4} = J_{4,5}$ = 10.8 Hz, H-4), 1.23, 1.17 (2 d, 3H each, *J* = 6.3 Hz, $(CH_3)_2CHO$); ^{13}C RMN (D_2O, 125.7 MHz): δ 96.3 (C-1), 84.2 (C-1'), 79.1, 73.7, 72.3, 72.0, 70.7, 69.8, 69.7, 68.7 (C-2, 3, 5, 2', 3', 4', 5', Me_2CHO), 61.4 (C-6), 61.2 (C-6'), 47.4 (C-4), 22.4, 20.4 [$(CH_3)_2CHO$]. HRMS (ESI+): *m/z* found 423.1303 ($[M+Na]^+$); calc. for $C_{15}H_{28}NaO_{10}S$ 423.1295.

bis[2-Propyl 4-*S*-(β-D-galactopyranosyl)-3,4-dithio-α-D-galactopyranosid-3-yl]-disulfide (27)

Deacetylation of **15** (50 mg, 0.07 mmol) gave **27** (28 mg, 89%); R_f 0.48; $[\alpha]_D^{25}$ +14.1 (*c* 1.7 in MeOH); ^1H NMR (D_2O, 500 MHz): δ 5.01 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.57 (d, 1H, $J_{1',2'}$ = 9.8 Hz, H-1'), 4.05 – 3.84 (m, 6H, H-5, 6a, 6b, Me_2CHO, 4', 5'), 3.73 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 10.1 Hz, H-2), 3.70 – 3.54 (m, 3H, H-3', 6'a, 6'b), 3.47 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 9.6 Hz, H-2'), 3.13 – 3.02 (m, 2H, H-3, 4), 1.16, 1.11 (2 d, 3H each, *J* = 6.2 Hz, $(CH_3)_2CHO$); ^{13}C NMR (D_2O, 125.7 MHz): δ 96.2 (C-1), 83.8 (C-1'), 78.9, 73.8, 70.9, 69.8, 69.6, 69.58, 68.5 (C-2, 5, 2', 3', 4', 5', Me_2CHO), 62.0 (C-6), 60.9 (C-6'), 46.6, 45.2 (C-3, 4), 22.5, 20.6 [$(CH_3)_2CHO$]. HRMS (ESI+): *m/z* found 853.2112 ($[M+Na]^+$); calc. for $C_{30}H_{54}NaO_{18}S_4$ 853.2091.

2-Propyl 4-*S*-(β-D-galactopyranosyl)-3,4-dithio-α-D-glucopyranoside (28)

Compound **27** (85 mg, 0.10 mmol) was treated with tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 300 mg, 1.0 mmol) in H_2O (4.3 mL) at rt for 1 h, when TLC analysis (2.5:1:1 *n*-BuOH/EtOH/H_2O) showed the complete conversion of **27** into a faster moving spot of R_f 0.64. The solution was passed through a column filled with Dowex MR-3C mixed bed ion-exchange resin and the resulting deionized solution was concentrated. The residue was dissolved in water (1 mL) and eluted through an octadecyl C18 minicolumn. Upon evaporation of water was obtained **28** (38 mg, 92%); ^1H NMR (D_2O, 500 MHz): δ 4.96 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.50 (d, 1H, $J_{1',2'}$ = 9.8 Hz, H-1'), 3.98 – 3.58 (m, 9H, H-5, 6a, 6b, Me_2CHO, 3', 4', 5', 6'a, 6'b), 3.56 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 10.6 Hz, H-2), 3.49 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 9.6 Hz, H-2'), 3.29 (t, 1H, $J_{2,3} = J_{3,4}$ = 11.1 Hz, H-3), 2.85 (t, 1H, $J_{3,4}$

= $J_{4,5}$ = 11.1 Hz, H-4), 1.17, 1.11 (2 d, 3H each, J = 6.2 Hz, $(CH_3)_2$CHO); ^{13}C NMR (D_2O, 125.7 MHz): δ 95.9 (C-1), 83.7 (C-1'), 78.9, 73.8, 73.1, 72.5, 70.8, 69.6, 68.6 (C-2, 5, 2', 3', 4', 5', Me_2CHO), 61.9 (C-6), 61.0 (C-6'), 48.8 (C-4), 43.0 (C-3), 22.4, 20.6 [$(CH_3)_2$CHO]. HRMS (ESI+): *m/z* found 439.1059 ([M+Na]$^+$); calc. for $C_{15}H_{28}NaO_9S_2$ 439.1067.

2-Propyl 3,4-di-*S*-(β-D-galactopyranosyl)-3,4-dithio-α-D-glucopyranoside (29)

Dithiotrisaccharide **20** was deacetylated under the conditions described above, to afford free **29** (22 mg, 95%), R_f 0.45; $[\alpha]_D^{25}$ +18.1 (*c* 0.8 in H_2O); ^{1}H NMR (D_2O, 500 MHz): δ 4.98 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.62 (d, 1H, $J_{1',2'}$ = 9.6 Hz, H-1'), 4.51 (d, 1H, $J_{1'',2''}$ = 9.6 Hz, H-1''), 4.01 – 3.84 y 3.67 – 3.53 (m, 12H, H-5, 6a, 6b, 3', 4', 5', 6'a, 6'b, 3'', 4'', 5'', 6''a, 6''b), 3.75 (dd, 1H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 10.6 Hz, H-2), 3.51 (t, 1H, $J_{1',2'}$ = $J_{2',3'}$ = 9.6, H-2'), 3.50 (t, 1H, $J_{1'',2''}$ = $J_{2'',3''}$ = 9.6, H-2''), 3.19 (dd, 1H, $J_{2,3}$ = 10.7, $J_{3,4}$ = 11.8 Hz, H-3), 2.93 (dd, 1H, $J_{3,4}$ = 11.7, $J_{4,5}$ = 11.2 Hz, H-4), 1.15, 1.09 (2 d, 3H each, J = 6.2 Hz, $(CH_3)_2$CHO); ^{13}C NMR (D_2O, 125.7 MHz): δ 95.7 (C-1), 84.0, 83.6 (C-1',1''), 78.9, 78.8, 73.7, 72.7, 71.8, 70.6, 69.9, 69.7, 68.7, 68.6 (C-2, 5, 2', 3', 4', 5', 2'', 3'', 4'', 5'', Me_2CHO), 62.0 (C-6) , 61.1, 61.0 (C-6', 6''), 47.9, 44.0 (C-3, 4) 22.4, 20.4 [$(CH_3)_2$CHO]. HRMS (ESI+): *m/z* found 601.1580 ([M+Na]$^+$); calc. for $C_{21}H_{38}NaO_{14}S_2$ 601.1595.

2-Propyl 3-*S*-(β-D-galactofuranosyl)-4-*S*-(β-D-galactopyranosyl)-3,4-dithio-α-D-glucopyranoside (30)

Compound **23** (100 mg, 0.08 mmol) was deacetylated following the general procedure to afford **30** (40 mg, 83%); R_f 0.57 (*n*-BuOH/EtOH/H_2O 2.5:1:1); $[\alpha]_D^{25}$ +12.1 (*c* 1.4 in MeOH); ^{1}H NMR (D_2O, 500 MHz): δ 5.34 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1'), 4.95 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.48 (d, 1H, $J_{1'',2''}$ = 9.6 Hz, H-1''), 4.00–3.48 (m, 17H, H-2, 5, 6a, 6b, Me_2CHO, 2', 3', 4', 5', 6'a, 6'b, 2'', 3'', 4'', 5'', 6''a, 6''b), 3.05 (dd, 1H, $J_{2,3}$ = 10.5, $J_{3,4}$ = 12.0 Hz, H-3), 2.96 (dd, 1H, $J_{3,4}$ = 12.0, $J_{4,5}$ = 10.6 Hz, H-4), 1.11, 1.07 (2 d, 3H each, J = 6.3 Hz, $(CH_3)_2$CHO); ^{13}C NMR (D_2O, 125.7 MHz): δ 95.7 (C-1), 87.5 (C-1'), 83.9 (C-1''), 81.8, 81.7 (C-2',4'), 79.0 (C-3'), 76.5 (C-5'), 73.8, 73.0, 72.2, 70.6, 70.5, 69.7, 68.7 (C-2, 5, 2'', 3'', 4'', 5'', Me_2CHO), 62.8 (C-6), 62.1, 61.0 (C-6', 6''), 48.5 (C-3), 43.4 (C-4), 22.4, 20.6 [$(CH_3)_2$CHO]. Found: C, 43.21; H, 6.42. Calc. for $C_{21}H_{38}O_{14}S_2$: C, 43.59; H, 6.62%. HRMS (ESI+): *m/z* found 601.1589 ([M+Na]$^+$); calc. for $C_{21}H_{38}NaO_{14}S_2$ 601.1595.

Enzymatic Assay. Inhibition of the β-galactosidase from *E. coli*

The *E. coli* β-galactosidase (EC 3.2.1.23, grade VIII, >500 U/mg protein) was purchased from Sigma. The enzyme (0.3 U; 1U = 1 enzyme unit hydrolyses 1 μmol of the nitrophenyl glycoside per minute) was incubated with *o*-nitrophenyl β-D-galactopyranoside (concentration range: 0.4 to 2.5 mM) in sodium phosphate buffer (100 mM, pH 7.3, $MgCl_2$ 1.2 mM, 2-mercaptoethanol 100 mM) in the absence (control) or presence of the thioglycomimetics (concentrations from 0.05 to 5.0 mM); the final volume was 0.50 mL. After 10 min at 37 ºC, the reaction was quenched by adding 0.2 M aqueous sodium borate buffer (4.0 mL, pH 10.0). The concentration of the released *o*-nitrophenol (ε 21300) was measured by visible absorption spectroscopy at 410 nm. The *K*i and *K*m values were determined from de Lineweaver-Burk plot.

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Notes and references

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† Electronic Supplementary Information (ESI) available: ^{1}H and ^{13}C NMR spectra for the thiodisaccharides **8**-**13**, **24**-**26**, **28**; disulfides **16**, **17**, **27** and dithiotrisaccharides **20**, **29**, **30**. See DOI: 10.1039/b000000x/

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