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Thiodisaccharide Sulfoxides: Absolute Configuration of the SO Sulfur Atom and Influence on the Biological Activity towards the β-Galactosidase from *E. coli*

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Benzyl 3-deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-β-D-*threo*-pentopyranoside (**3**) is a potent inhibitor of the β-galactosidase from *Escherichia coli* synthesized in our laboratory. The 2′,3′,4′,6′-tetra-*O*-acetyl derivative of this thiodisaccharide was oxidized with *m*-chloroperoxybenzoic acid to give a 2:1 diastereomeric mixture of sulfoxides **2*S*** and **2*R***. The absolute configurations of their sulfur stereocenters were determined by using NMR techniques and by taking into account the anisotropic effects of the S=O group in the major conformers. *O*-Deacetylation of **2*S*** and **2*R*** afforded the free thiodisaccharide *S*-oxides **4*S*** and **4*R***, which were evaluated as inhibitors of the above-mentioned enzyme. The kinetic studies showed that **4*S*** and **4*R*** are competitive inhibitors of the enzyme with K_i values of 0.19 and 0.45 mM, respectively. Further enzymatic reactions demonstrated that **4*S*** is also a substrate of the β-galactosidase, as it was diastereoselectively hydrolyzed, with **4*R*** remaining unchanged under the same conditions.

Introduction

Oligosaccharides are essential components of all living things as they display various important biological roles in the fields of biochemistry, medicine, and biotechnology. Among the many analogues of oligosaccharides described, the thiooligosaccharides show interesting biological properties and are useful tools in glycobiology for studying various metabolic processes.[1] In addition, thiosugars are being investigated as promising therapeutics for the treatment of various diseases.[2] The replacement of the interglycosidic oxygen atom by a sulfur atom provides stability to the thioglycosidic linkage towards glycosidases and, in many cases, increases the potential of such molecules to act as inhibitors of these enzymes.[3] We have reported the synthesis of several thio-bridged disaccharides that show interesting biological activities, mainly as inhibitors of specific glycosidases.[4]

The oxidation of the sulfur atom in sulfides with different substituents affords enantiomeric sulfoxides, and when thioglycosides are oxidized, diastereomeric mixtures of glycoside *S*-oxides may be obtained.[5] The first sulfoxide and sulfone derivatives of thiodisaccharides were prepared by Witczak et al.[6] These compounds have been shown to be inhibitors of the proliferation of selected murine and human tumor cell lines, but the configuration of the SO group has not been established. In contrast, an empirical rule based on ^{1}H and ^{13}C NMR spectroscopic data,[5] as well as a method that combines NMR and circular dichroism, have been employed to assign the absolute configuration of the sulfur atom in glycosyl sulfoxides.[7] The configuration of the sulfur in sulfoxides is of crucial importance as it plays a significant role in the biological activity of the molecule. For example, some thioglycoside sulfoxides display oral anti-thrombotic activity, which is related to the configuration of the sulfur. Furthermore, the synthesis of nonracemic sulfoxides is a subject of interest because numerous compounds with a specific configuration at the sulfur stereocenter are employed as pharmaceuticals (e.g., sulforaphane,[8] esomeprazole,[9] and armodafinil[10]). Additionally, it has been reported that diastereomeric sugar sulfoxides are hydrolyzed with different kinetics by acids and react in a completely diastereoselective manner with glycosidases.[11,12] Therefore, compared with the thiodisaccharide precursors, the sulfinyl analogues are expected not only to

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alter the lipophilicity of the molecule but also to vary the geometrical disposition of the component sugar moieties,[13] which should markedly affect the biological activity. For all these reasons, sugar sulfoxides are interesting compounds in their own right and the knowledge of their stereochemistry is an important topic.

In line with our previous work on the synthesis of 3-deoxy-*S*-(1→4)-disaccharide *S*-oxides and the determination of the configuration of the sulfur stereocenter,[14] we report herein on the oxidation of the sulfur atom of the strongest inhibitor of the β-galactosidase from *E. coli* synthesized in our laboratory, benzyl 3-deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-β-D-*threo*-pentopyranoside. The analysis of the conformation around the S=O group of the resulting sulfoxides, required for the assignment of the configuration of the sulfur, was facilitated by the presence in the pentopyranose ring of two methylene groups vicinal to the carbon that is bonded to the S atom (C-4). Furthermore, free thiodisaccharide *S*-oxides were prepared and the effect of the oxidation of the S atom and its configuration on the inhibitory activity and their ability to act as substrates of the β-galactosidase from *E. coli* were studied. The inhibition kinetics were also determined.

Results and Discussion

The thiodisaccharide **1** with a pentopyranose unit as the reducing end has been synthesized by employing the methodology previously described.[15] The oxidation of the sulfur atom of **1** with *m*-chloroperoxybenzoic acid (*m*CPBA) at 0 °C led to a diastereomeric mixture of thiodisaccharide *S*-oxides **2** (Scheme 1). The diastereoisomers were separated by column chromatography in yields of 55 and 26%, respectively. The absolute stereochemistry of the sulfur stereocenter was assigned as *S* and *R*, respectively, as described below. The stereoselective oxidation of chiral substrates, such as thiosugars, with *m*CPBA has been reported previously.[16]

To explain the preference for the formation of the *S* isomer in the oxidation of the thiodisaccharide **1**, a conformational analysis of this compound was conducted. The coupling constants (*J*) observed for the vicinal protons of the 4-thio-3-deoxy-pentopyranose ring of **1** suggest an equilibrium between the $^1C_4$ and $^4C_1$ conformations. The contribution of each chair was established by a quantitative comparison of the experimental coupling constants and those calculated for the optimized geometries by using the generalized Karplus equation proposed by Altona and implemented in the MSpin program, as already described.[17] The experimental and calculated *J* values are shown in Table 1. The calculations indicated contributions of both the $^4C_1$ (58%) and $^1C_4$ (42%) chairs to the equilibrium. The presence of the $^4C_1$ form was confirmed by characteristic nuclear Overhauser effect (NOE) cross-peaks observed between 1-H and 3_B-H and 5_B-H in the 2D NOESY spectrum, whereas the NOE contact between 3_A-H and 5_A-H (for $^1C_4$) was very weak.

Table 1. Experimental and calculated coupling constants for the chair conformations of the pentopyranoside ring of **1**.

$^3J_{H,H}$	$J_{calcd.}$ [Hz]		$J_{exp.}$ [Hz]
	$^1C_4$ chair	$^4C_1$ chair	
1-H, 2-H	3.2	1.6	2.1
2-H, 3_A-H	11.2	3.1	7.2
2-H, 3_B-H	4.5	33.0	3.8
3_A-H, 4-H	4.8	3.3	3.7
3_B-H, 4-H	2.0	12.3	7.5
4-H, 5_A-H	3.2	3.9	
4-H, 5_B-H	1.4	11.5	

A similar conformational analysis based on the observed *J* values and NOE patterns was performed on the pentopyranose rings of sulfoxides **2*R*** and **2*S*** (Figure 1). The experimental *J* values and those calculated by means of the MSpin program are shown in Table 2. It is worth mentioning that this type of analysis applied to the galactopyranose units of **1**, **2*R***, and **2*S*** indicate the almost exclusive presence of the $^4C_1$ chair, which was confirmed by the observed intra-residue NOE interactions between 1′-H and 3′-H, 1′-H and 5′-H, and 3′-H and 5′-H in the respective NOESY spectrum of each compound. In contrast, the pentopyranose rings of the sulfoxides **2*R*** and **2*S*** are found to adopt, similarly to that of thiodisaccharide **1**, a conformational equilibrium between the two chair forms. This was experimentally confirmed by the observation of key intra-residue NOEs between 1-H and 3_B-H and 5_B-H for the $^4C_1$ chair, and between 3_A-H and 5_A-H for the $^1C_4$ chair. A shift of the equilibrium toward the $^1C_4$ conformation was observed for both sulfoxides **2*R*** and **2*S*** in comparison with **1**. Thus, according to our calculations, the $^1C_4$ conformer

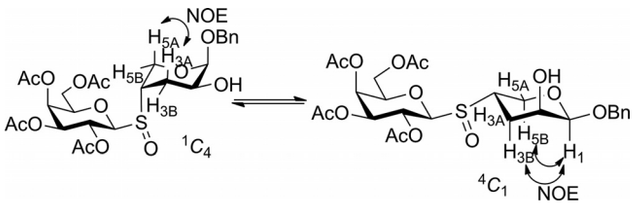
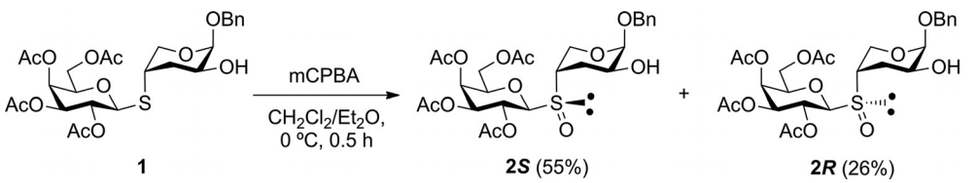


Figure 1. Conformational equilibrium of the pentopyranoside rings of compounds **2*S*** and **2*R***.



Scheme 1. Oxidation of thiodisaccharide **1** to afford the diasteromeric sulfoxides **2*S*** and **2*R***.

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contributes 65% to the conformational equilibrium of the major isomer **2*S***, with the proportion even higher for the **2*R*** isomer (84%). Thus, the conformation of the pentopyranoside ring is not only affected by the introduction of a polar SO group at C-4, but also by the configuration of this group.

Table 2. Experimental and calculated coupling constants for the chair conformations of the pentopyranoside rings of **2*S*** and **2*R***.

	2*S*			**2*R***		
	$J_{calcd.}$ [Hz]		$J_{exp.}$ [Hz]	$J_{calcd.}$ [Hz]		$J_{exp.}$ [Hz]
$^3J_{H,H}$	$^1C_4$	$^4C_1$		$^1C_4$	$^4C_1$	
1-H, 2-H	3.1	1.6	2.6	3.3	1.5	3.2
2-H, 3_A-H	11.1	3.2	8.9	11.1	3.4	10.2
2-H, 3_B-H	4.6	2.9	ca. 5.3	4.6	2.7	4.2
3_A-H, 4-H	5.2	3.8	4.6	4.7	3.7	4.7
3_B-H, 4-H	1.8	12.3	ca. 5.3	2.1	12.3	4.2
4-H, 5_A-H	3.7	4.3	ca. 4.0	3.1	4.5	3.1
4-H, 5_B-H	1.2	11.6	ca. 4.0	1.4	11.6	3.3

In the next step, we tried to elucidate the preferred conformations around the sulfur and sulfoxide linking groups. Many low-energy conformations are expected to be reached by rotation of the torsion angles ϕ(H1′–C1′–S–C4) and ψ(C1′–S–C4–H4). The presence of given rotamers in the equilibrium was confirmed by characteristic NOE cross-peaks in the NOESY spectrum of **1**. This methodology has previously been employed for the conformational analysis of the thioglycosidic linkage in thiodisaccharides.[17] The NOESY spectrum of thiodisaccharide **1** shows an intense NOE interaction between 1′-H and 4-H, which indicates a highly populated *syn* ϕ/*syn* ψ conformation of the interglycosidic linkage (Figure 2). The observed inter-residue NOE contact for 2′-H and 4-H suggests the presence of a minor *anti* ϕ/*syn* ψ conformer. It is important to mention that both rotamers are stabilized by the *exo*-anomeric effect in which one of the sulfur lone-pairs is disposed *anti* to the C1′–O1′ bond. The structures shown in Figure 2 are depicted with the pentopyranoside in the $^4C_1$ chair. However, the same NOE contacts are expected for this ring in the opposite $^1C_4$ form.

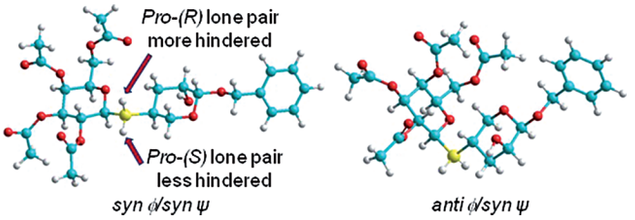

Figure 2. Conformations of thiodisaccharide **1** in accord with NOE interactions (the pentopyranose ring in the $^4C_1$ chair).

Assuming that the thiodisaccharide **1** is oxidized in the preferred *syn* ϕ/*syn* ψ and *anti* ϕ/*syn* ψ conformations, the latter does not show a preferential side of attack on the sulfur atom. However, for the more populated *syn* ϕ/*syn* ψ ($^4C_1$ or $^1C_4$) conformer of **1**, the delivery of oxygen from *m*CPBA should occur at the less hindered pro-(*S*) lone pair of electrons of sulfur. Therefore the *S* isomer is expected to be the major product of oxidation.

The configurational assignment of the sulfur atom requires the previous determination of the conformation of the sulfoxides. Therefore the ROESY and NOESY spectra were recorded to detect key inter-residue NOE cross-peaks. In the first instance, for the conformational analysis, the more populated $^4C_1$ form of the pentopyranose ring of **2** was considered. The spectra of the major isomeric sulfoxide (later assigned as **2*S***) show an intense inter-residue NOE contact exclusively between 1′-H and 4-H, which strongly suggests the existence of a highly populated *syn* ϕ/*syn* ψ conformer (Figure 3). The expected NOE between 1′-H and 5_B-H was also detected. Moreover, the existence of a weak NOE contact between 1′-H and 3_B-H, characteristic of the *syn* ϕ/*anti* ψ conformer, suggests a minor presence of this rotamer. Additionally, the detection of a medium NOE contact between 2′-H and 3_B-H and a weak NOE between 2′-H and 4-H also complies with the simultaneous occurrence of a minor *anti* ϕ/*syn* ψ conformation.

A similar analysis was conducted on the $^4C_1$ geometry of the pentopyranose unit. In this case the occurrence of NOE cross-peaks between 1′-H and 4-H, 1′-H and 3_B-H, and 2′-H and 3_B-H, characteristic of the respective *syn* ϕ/*syn* ψ,

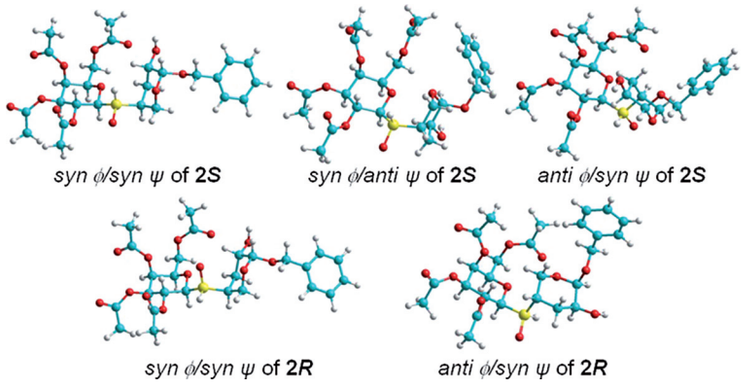

Figure 3. Conformations of sulfoxides **2*S*** and **2*R*** in accord with NOE interactions (the pentopyranose ring in the $^1C_4$ chair).

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syn ϕ/*anti* ψ, and *anti* ϕ/*syn* ψ conformers, should be expected. Therefore the previous analysis performed on the $^1C_4$ form is also valid for the $^4C_1$ conformer of the pentopyranoside ring. For both chairs, the *syn* ϕ/*syn* ψ rotamer of **2*S*** shows the polar C–O_{ring} and S=O linkages to be *anti* disposed. Such an arrangement should prevent the dipolar repulsion expected for a *gauche* relationship between the two polar bonds.

The conformation of the sulfoxide **2*R*** was analyzed in a similar manner, taking into account the major $^1C_4$ form of the pentopyranose ring. The intense NOE interactions between 1′-H and 4-H and between 1′-H and 5_B-H observed in the NOESY spectrum of **2*R*** again suggest the existence of a highly populated *syn* ϕ/*syn* ψ conformer (Figure 3). The interaction of 2′-H with 4-H is now very weak and the interaction of 2′-H with 3-H could not be detected, which indicates that the population of the *anti* ϕ/*syn* ψ conformer is rather low. As no NOE contact between 1′-H and 3-H could be observed, the proportion of the *syn* ϕ/*anti* ψ conformation should be very small and under the limit of detection. Therefore these data evidence the fact that compound **2*R*** displays a conformational equilibrium between a major (*syn* ϕ/*syn* ψ) and a minor (*anti* ϕ/*syn* ψ) conformer.

Once the conformation of sulfoxides **2*S*** and **2*R*** had been established, the absolute configurations of the sulfur stereocenters were assigned taking into account the shielding/deshielding effects produced by the anisotropy of the S=O group, according to the procedure recently described by our group.[14]

To evaluate the anisotropy effects we assume the S=O bond to be acetylenic, with axial symmetry, and with shielding cones oriented along the S=O bond.[18] In addition, hydrogen atoms that are α *anti* axial with respect to the lone-pair electrons of the sulfoxide group experience shielding. In contrast, significant deshielding is observed for protons that are in a *syn*-1,3-parallel arrangement with respect to the S=O bond.

The relative orientations of a given proton with respect to the S=O bond were established for each experimentally determined major conformer in the conformational equilibrium. Relevant chemical shifts for **2*S*** and **2*R***, and their differences with respect to those of substrate **1**, are summarized in Table 3. Negative Δδ values indicate deshielding, and positive values indicate shielding.

For both sulfoxides, the chemical shifts observed for some specific protons, for example, 3_B-H and 5_B-H, are diagnostic of the configuration of the sulfur stereocenter. Thus, for the major oxidation product, the configuration was established as *S* on the basis of the experimental Δδ values reported in Table 3. For the *syn* ϕ/*syn* ψ conformation with the pentopyranoside in the $^1C_4$ chair conformation, the most affected signal is the one corresponding to 5_B-H, which is equatorially disposed and located over the S=O bond in a region of maximum deshielding effect. The 2′-H proton has a similar relative orientation to 5_B-H, but the longer distance diminishes the intensity of the deshielding effect. The axially oriented 3_A-H and 5_A-H are almost unaffected by anisotropy because of the long distance to S=O. The other two conformers of **2*S*** (*syn* ϕ/*anti* ψ and *anti* ϕ/*syn* ψ) also show 5_B-H located in the S=O anisotropic deshielding region. Similarly, in the *syn* ϕ/*syn* ψ rotamer, but with the pentopyranoside in the $^4C_1$ conformation, the C2′–H2′ and C5–H5_B bonds are almost perpendicular to the direction of the S=O linkage, lying in the deshielding region.

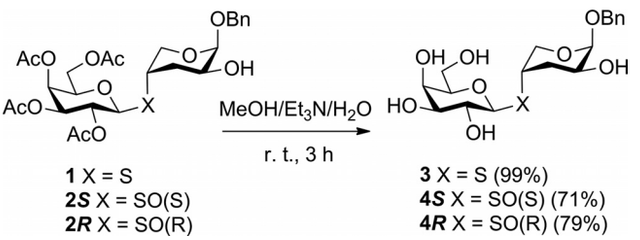
The **2*R*** isomer shows the 3_B-H signal strongly shifted downfield. This may be justified by taking into account the anisotropy effect of the S=O bond on 3_B-H, which in the *syn* ϕ/*syn* ψ arrangement is located in the deshielding cone. In contrast, 5_B-H (the least deshielded proton) is oriented in the direction of the S=O bond and closer to the sulfur. The downfield shift of 2′-H may be attributed to the 1,3-diaxial interaction between the C2′–H2′ bond and the S=O group. The axially disposed 3_A-H and 5_A-H are a long away from the S=O group and are practically unaffected by anisotropy. For the *anti* ϕ/*syn* ψ ($^1C_4$) rotamer, the 3_B-H signal is also strongly deshielded because it is located over the S=O group, whereas 2′-H is also unscreened as the C2′–H2′ bond is perpendicular to the S=O linkage.

In both diastereoisomers **2*S*** and **2*R***, the observed upfield shift of 1′-H, in comparison with 1′-H in **1**, may be justified by taking into account that for the major *syn* ϕ/*syn* ψ ($^1C_4$) conformer of **2*S***, 1′-H is *anti* to the sulfur lone pair, and in that of **2*R***, the C1′–H1′ bond is aligned with S=O, lying in the shielding region.

Once the configuration of the sulfur stereocenter in the sulfoxides had been established, they were *O*-deprotected to evaluate their biological activity. Removal of the acetyl protecting groups by hydrolysis with a mixture of MeOH/Et_3N/H_2O (4:1:5) afforded the free 3-deoxy-*S*-(1→4)-disaccharide sulfoxides **4*S*** and **4*R*** (Scheme 2).

Table 3. Chemical shifts of the protons of thiodisaccharide **1** and the sulfoxides **2*S*** and **2*R***, and their differences with respect to thiodisaccharide **1**.

	δ [ppm]								
	1-H	1′-H	2-H	2′-H	3_A-H	3_B-H	4-H	5_A-H	5_B-H
2*S*	4.76	4.36	3.97	5.55	2.23	2.11	3.47	4.12	4.02
2*R*	4.84	4.27	4.03	5.68	2.20	2.45	3.60	4.09	3.48
1	4.65	4.53	3.90	5.24	2.23	1.83	3.45	4.08	3.47
$\Delta\delta_{\mathbf{1}-\mathbf{2S}}$	–0.11	0.17	–0.07	–0.31	–0.03	–0.28	–0.02	–0.04	–0.55
$\Delta\delta_{\mathbf{1}-\mathbf{2R}}$	–0.19	0.26	–0.13	–0.44	0.00	–0.62	–0.15	–0.01	–0.01



Scheme 2. Deprotection of thiodisaccharide **1** and sulfoxides **2*S*** and **2*R***.

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Biological Evaluation

The inhibitory activity of the free 3-deoxy-*S*-(1→4)-disaccharide sulfoxides **4*S*** and **4*R*** was evaluated in vitro against the β-galactosidase from *E. coli*. This enzyme has been widely used by us and many other research groups as a model enzyme for kinetic studies. The β-galactosidase shows a high affinity for the β-galactopyranosyl unit at the nonreducing end, as in lactosides.[19] Moreover, 4-thiolactoside analogues, like **3**, have already been tested as inhibitors of this enzyme, and the interactions of the amino acids of its active site with this type of thiodisaccharide have been established.[17] It was mentioned above that the pentopyranose ring can be conformationally described as an equilibrium between the $^1C_4$ and $^4C_1$ chairs, in accordance with the fact that this ring has a higher flexibility and a lower conformational barrier than the hexopyranose ring. This flexibility is an important factor as we have demonstrated,[17] by using a combination of NMR spectroscopy and molecular modeling techniques, that thiodisaccharide **3** undergoes conformational distortion, by deformation of the pentopyranose ring or by rotation of the thioglycosidic linkage, to adapt to the enzymatic pocket. The flexibility of the pentopyranose ring may be modified by replacement of the glycosyl sulfide group at C-4 of **3** by a glycosyl sulfoxide group, as in **4*R*** or **4*S***, and the rotation of the thioglycosidic linkage could be restricted in the sulfoxides due to the presence of the additional S=O bond. To explore the effect of the oxidation of sulfur on the inhibitory activity of the enzyme, inhibition studies were conducted with sulfoxides **4*S*** and **4*R***. In the inhibition assays *o*-nitrophenyl β-D-galactopyranoside was employed as the substrate, and was incubated with increasing concentrations of the thiodisaccharide *S*-oxides. The released *o*-nitrophenol was quantified spectrophotometrically at λ = 410 nm; the dose-response curves (Figure 4) and Lineweaver–Burk plots (Figure 5) were subsequently constructed. The effect of increasing concentrations of glycomimetics **4*R*** and **4*S*** on the inhibition of the *E. coli* β-galactosidase indicate that both sulfoxides act as inhibitors of this enzyme. The kinetics of inhibition, determined on the basis of the Lineweaver–Burk plots, show that compounds **4*S*** and **4*R*** are competitive inhibitors of the enzyme with IC_{50} values of 0.44 and 0.61 mM and inhibition constants (K_i) of 0.19 and 0.45 mM, respectively.

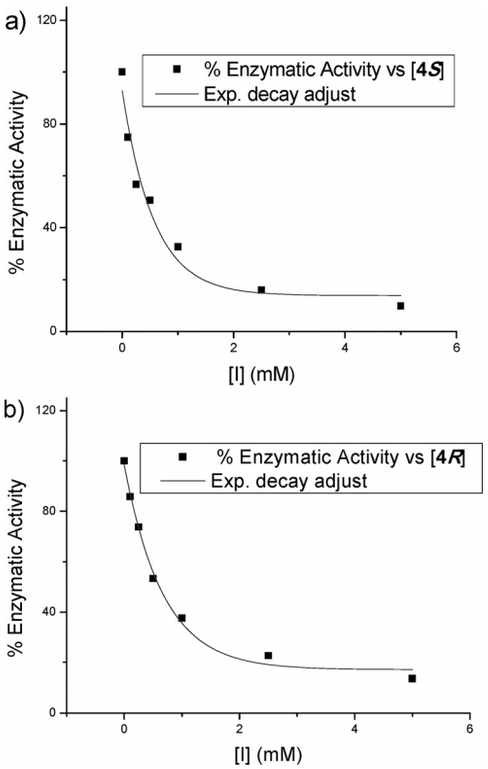

Figure 4. Effect of the concentration of thiodisaccharide *S*-oxides **4*S*** (a) and **4*R*** (b) on the enzymatic activity of the β-galactosidase from *E. coli*.

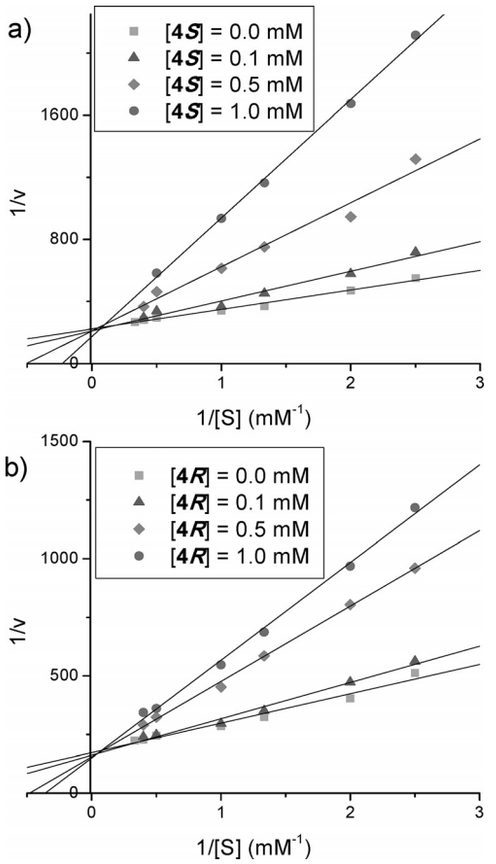

Figure 5. Lineweaver–Burk plots for the inhibition of the β-galactosidase from *E. coli* by thiodisaccharide *S*-oxides **4*S*** (a) and **4*R*** (b).

The inhibition constants indicate that **4*S*** is approximately twice as strong an inhibitor of the enzyme than **4*R***. However, comparison of the K_i values of the sulfoxides with that reported for the precursor thiodisaccharide **3** (K_i = 32 μM)[15] shows that this compound displays inhibitory activity that is 6- and 14-fold stronger than **4*S*** and **4*R***, respectively. This result can be explained by taking into account the conformational flexibility of the pentopyranose ring and the rotational freedom of the interglycosidic linkage,

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which allows the distorted conformations of the glycomimetic to be accommodated in the catalytic site of the enzyme. In the ground state, selected coupling constants for vicinal protons of the pentopyranoside ring of **4*S*** ($J_{2,3A}$ = 10.8 Hz) and **4*R*** ($J_{4,5A}$ = 2.8 Hz, $J_{4,5B}$ < 1 Hz) indicate the almost exclusive presence of this ring as the $^1C_4$ chair, whereas the thiodisaccharide **3** shows a 1:1 conformational equilibrium between the $^1C_4$ and $^4C_1$ forms.[17] Furthermore, the replacement of one sulfur lone pair in **3** by an oxygen atom, as in sulfoxides **4*S*** and **4*R***, is expected to restrict the rotation of the interglycosidic linkage as a result of electronic and steric changes around the sulfur atom. All this is the subject of further studies.

Finally, enzymatic reactions were carried out to determine whether the thiodisaccharide **3** and sulfoxides **4*S*** or **4*R*** are substrates of the β-galactosidase from *E. coli*. Thus, each individual compound was incubated at 28 °C for 48 h in the presence of the enzyme in sodium phosphate buffer at pH 7.3. After this time **4*S*** showed to be a substrate of the β-galactosidase, whereas **3** and **4*R*** remained unchanged under the same reaction conditions, which indicates that the stereoselective hydrolysis of **4*S*** takes place. This observation is in agreement with the results reported by Khiar et al.,[11] which showed that from the diastereomeric mixture of phenyl β-D-galactopyranosyl sulfoxides, the isomer with the *S* absolute configuration at the sulfur was diastereoselectively hydrolyzed by the β-galactosidase, whereas the *R* isomer remained unchanged. Both thiodisaccharide sulfoxides **4*R*** and **4*S*** bind to the active site of the enzyme and behave as inhibitors, but only **4*S*** acts as a substrate. It is tempting to speculate that **4*S*** would be able to adopt a conformation having the sulfinyl oxygen oriented towards the activating amino acid of the active site, in agreement with Heightman and Vasella's lateral protonation model for glycosidase enzymes.[20]

Conclusions

The *m*CPBA oxidation of the sulfur atom of benzyl 3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-β-D-*threo*-pentopyranoside (**1**) gave a mixture of diastereomeric sulfoxides that could be separated by column chromatography. The reaction was diastereoselective in favor of the *S*-sulfoxide **2*S***. This fact has been explained by taking into account the more populated conformations of the thiodisaccharide, which showed that the *pro*-(*S*) lone pair of the sulfur is sterically less hindered.

The configuration of the sulfur stereocenter of the two sulfoxides was assigned on the basis of the shifts of NMR signals of specific protons in comparison with the corresponding signals of the precursor thiodisaccharide.[14] The method proved to be general and particularly useful for (1→4)-sulfoxides with a 3-deoxy-pentopyranose unit as the reducing end that possesses two methylene groups vicinal to the carbon bonded to the S=O interglycosidic group. The shifts were interpreted in terms of the shielding/deshielding effects generated by the anisotropy of the S=O functionality in the preferred conformation of the thiodisaccharide sulfoxides. Thus, for the major diastereoisomer, 5_B-H is the most deshielded signal, in agreement with the fact that only in the *S* sulfoxide does 5_B-H have a suitable relative orientation to experience a strong deshielding by the S=O anisotropy in the experimentally determined conformations of the molecule (*syn* ϕ/*syn* ψ, *syn* ϕ/*anti* ψ, and *anti* ϕ/*syn* ψ with the pentopyranose ring in the $^1C_4$ or $^4C_1$ chair). For the other diastereoisomer, 3_B-H is strongly shifted downfield with respect to the same resonance in the thiodisaccharide. A similar analysis revealed that, according to the established conformations, 3_B-H is suitably oriented for deshielding if the configuration of the sulfoxide is *R*. In agreement with the fact that the anomeric carbon in *R* sulfoxides is more shielded than in the *S* isomer,[5] the C-1′ resonance of the compound assigned as *R* is shifted upfield by 4.9 ppm with respect to that of the S counterpart.

The thiodisaccharide sulfoxides were *O*-deacetylated under mild conditions and the products **4*S*** and **4*R*** were evaluated as inhibitors of the β-galactosidase from *E. coli*. Although both sulfoxides behave as competitive inhibitors, they display a weaker inhibitory activity than the parent thiodisaccharide **3**. Furthermore, the *S*-sulfoxide **4*S*** showed to be a substrate of the β-galactosidase, whereas the thiodisaccharide **3** and the *R*-sulfoxide **4*R*** remained unchanged under the same reaction conditions. Therefore the *S* isomer can be diastereoselectively hydrolyzed by the enzyme. To explain all these facts, studies on the interactions between the glycomimetics and the β-galactosidase are being conducted.

Experimental Section

General: Column chromatography was carried out with silica gel 60 (230–400 mesh). Analytical TLC was performed on silica gel 60 F254 aluminium-supported plates (layer thickness 0.2 mm). The spots were visualized by exposure to UV light and by charring with a solution of 5% (v/v) sulfuric acid in EtOH containing 0.5% *p*-anisaldehyde or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (26.5 g/L), $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10.5 g/L), and 6% (v/v) H_2SO_4 in H_2O followed by charring at around 140 °C. Optical rotations were measured at 25 °C in a 1 dm cell in the solvent indicated. NMR spectra were recorded at 500 (^{1}H) or 125.7 MHz (^{13}C); chemical shifts are given relative to tetramethylsilane or a residual solvent peak ($CHCl_3$: ^{1}H: δ = 7.26 ppm; ^{13}C: δ = 77.2 ppm; H_2O: ^{1}H: δ = 4.79 ppm). The assignment of ^{1}H and ^{13}C chemical shifts was assisted by 2D ^{1}H COSY and 2D ^{1}H-^{13}C HSQC experiments. HRMS were recorded by using the electrospray ionization (ESI) technique and Q-TOF detection.

Oxidation of the Sulfur Atom of Thiodisaccharide 1 − Synthesis of Sulfoxides 2*S* and 2*R*: A solution of 80% *m*CPBA (81 mg, 0.375 mmol in 3.6 mL of CH_2Cl_2) was added to a solution of thiodisaccharide **1** (104 mg, 0.182 mmol) in diethyl ether (3.6 mL) cooled to 0 °C. The reaction mixture was stirred for 30 min at 0 °C until complete conversion of the starting material [R_f = 0.62, toluene/EtOAc (1:2)] into two products (R_f = 0.25 and 0.12). The mixture was diluted with EtOAc (50 mL) and then stirred for 30 min with satd. aq. $NaHSO_3$ (20 mL). The organic layer was separated and stirred for an additional 30 min with satd. aq. $NaHCO_3$

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(20 mL). Finally, the organic extract was washed with satd. aq. $NaHCO_3$ (20 mL) and brine (20 mL), dried ($MgSO_4$), and filtered. Evaporation of the solvent followed by co-evaporation with toluene/EtOH (1:1, 5× 20 mL) gave an oily residue. Column chromatography using toluene/EtOAc (1:1→1:4) as eluent allowed the separation of sulfoxides **2*S*** and **2*R***.

Benzyl 3-Deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-β-D-*threo*-pentopyranoside (*S*)-*S*-Oxide (2*S*): From the first fractions of the column was isolated syrupy **2*S*** (59 mg, 55%); R_f = 0.25 (toluene/EtOAc, 1:2). $[\alpha]_D^{25}$ = –55.1 (*c* = 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz): δ = 7.37–7.29 (m, 5 H, *Ph*CH_2O), 5.55 (t, $J_{1',2'} = J_{2',3'}$ = 10.1 Hz, 1 H, 2′-H), 5.45 (d, $J_{3',4'}$ = 3.2 Hz, 1 H, 4′-H), 5.11 (dd, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.3 Hz, 1 H, 3′-H), 4.82, 4.58 (2 d, *J* = 11.7 Hz, 1 H each, PhCH_2O), 4.76 (d, $J_{1,2}$ = 2.8 Hz, 1 H, 1-H), 4.36 (d, $J_{1',2'}$ = 10.1 Hz, 1 H, 1′-H), 4.17–4.06 (m, 3 H, 5_A-H, 6′a-H, 6′b-H), 4.04–4.00 (m, 2 H, 5_B-H, 5′-H), 3.98–3.95 (m, 1 H, 2-H), 3.48–3.45 (m, 1 H, 4-H), 2.23 (ddd, $J_{3A,3B}$ = 13.7, $J_{3A,2}$ = 8.9, $J_{3A,4}$ = 4.6 Hz, 1 H, 3_A-H), 2.15, 2.05, 2.04, 1.98 (4 s, 12 H, CH_3CO), 2.13–2.08 (m, 1 H, 3_B-H) ppm. ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ = 170.5, 170.1 (×2), 169.8 (*C*OCH_3), 137.0, 128.7, 128.4, 128.3 (C-aromatic), 97.5 (C-1), 92.1 (C-1′), 75.9 (C-5′), 71.5 (C-3′), 70.0 (*C*H_2OBn), 67.0 (C-4′), 66.1 (C-2′), 64.9 (C-2), 61.4 (C-6), 57.8 (C-5), 53.3 (C-4), 28.4 (C-3), 20.9, 20.8 (×2), 20.7, (CO*C*H_3) ppm. HRMS (ESI): calcd. for $C_{26}H_{34}NaO_{13}S$ 609.1612 $[M + Na]^+$; found 609.1624.

Benzyl 3-Deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-β-D-*threo*-pentopyranoside (*R*)-*S*-Oxide (2*R*): The second product isolated from the column was syrupy **2*R*** (28 mg, 26%); R_f = 0.12 (toluene/EtOAc, 1:2). $[\alpha]_D^{25}$ = –61.1 (*c* = 0.7, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz): δ = 7.38–7.30 (m, 5 H, *Ph*CH_2O), 5.68 (t, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, 1 H, 2′-H), 5.46 (dd, $J_{3',4'}$ = 3.4, $J_{4',5'}$ = 1.0 Hz, 1 H, 4′-H), 5.19 (dd, $J_{2',3'}$ = 10.1, $J_{3',4'}$ = 3.4 Hz, 1 H, 3′-H), 4.84 (d, $J_{1,2}$ = 3.2 Hz, 1 H, 1-H), 4.79, 4.60 (2 d, *J* = 11.7 Hz, 1 H each, PhCH_2O), 4.27 (d, $J_{1',2'}$ = 9.9 Hz, 1 H, 1′-H), 4.19 (dd, $J_{6'a,6'b}$ = 11.5, $J_{6'a,5'}$ = 7.0 Hz, 1 H, 6′a-H), 4.13 (dd, $J_{6'a,6'b}$ = 11.5, $J_{6'b,5'}$ = 5.9 Hz, 1 H, 6′b-H), 4.09 (dd, $J_{5A,5B}$ = 13.0, $J_{5A,4}$ = 3.1 Hz, 1 H, 5_A-H), 4.05–4.00 (m, 2 H, 5′-H, 2-H), 3.61–3.58 (m, 1 H, 4-H), 3.48 (ddd, $J_{5A,5B}$ = 13.0, $J_{5B,4}$ = 3.3, $J_{5B,3B}$ = 1.2 Hz, 1 H, 5_B-H), 2.45 (dt, $J_{3B,3A}$ = 14.4, $J_{3B,2} = J_{3B,4}$ = 4.2 Hz, 1 H, 3_B-H), 2.20 (ddd, $J_{3A,3B}$ = 14.5, $J_{3A,2}$ = 10.1, $J_{3A,4}$ = 4.7 Hz, 1 H, 3_A-H), 2.16, 2.08, 2.03, 1.99 (4 s, 12 H, CH_3CO) ppm. ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ = 170.4 (×2), 170.3, 168.8 (*C*OCH_3), 137.0, 128.7, 128.3, 128.2 (C-aromatic), 97.8 (C-1), 87.2 (C-1′), 76.0 (C-5′), 72.0 (C-3′), 70.2 (*C*H_2OBn), 66.9 (C-4′), 64.6 (C-2), 64.1 (C-2′), 61.5 (C-6), 58.7 (C-5), 52.8 (C-4), 26.0 (C-3), 20.8 (×2), 20.7 (×2) (CO*C*H_3) ppm. HRMS (ESI): calcd. for $C_{26}H_{34}NaO_{13}S$ 609.1612 $[M + Na]^+$; found 609.1603.

General Procedure for the *O*-Deacetylation of Thiodisaccharide Sulfoxides 2*S* and 2*R*: A suspension of thiodisaccharide *S*-oxide **2*S*** or **2*R*** (0.1 mmol) in a mixture of MeOH/Et_3N/H_2O (4:1:5, 0.30 mL) was stirred at room temperature. The solid progressively dissolved and TLC (hexane/EtOAc, 1:1 or 1:1.5) showed complete consumption of the starting material after 3 h. The solution was concentrated and the residue 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_2O, 2.5:1:1). The respective R_f values are given in each individual case.

Benzyl 3-Deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-β-D-*threo*-pentopyranoside (*S*)-*S*-Oxide (4*S*): The general procedure for the *O*-deacetylation was applied to **2*S*** (59 mg, 0.1 mmol) to obtain syrupy **4*S*** (30 mg, 71%). R_f = 0.67 (*n*BuOH/EtOH/H_2O, 2.5:1:1). $[\alpha]_D^{25}$ = –69.4 (*c* = 1.2, MeOH). ^1H NMR (D_2O, 500 MHz): δ = 7.49–7.40 (m, 5 H, *Ph*CH_2O), 4.92 (d, $J_{1,2}$ = 3.1 Hz, 1 H, 1-H), 4.82, 4.65 (2 d, *J* = 11.8 Hz, 1 H each, PhCH_2O), 4.60 (d, $J_{1',2'}$ = 9.7 Hz, 1 H, 1′-H), 4.18–4.15 (m, 1 H, 5_B-H), 4.10–4.01 (m, 3 H, 2, 2′-H, 4′-H), 3.97–3.94 (m, 2 H, 4-H, 5_A-H), 3.86–3.72 (m, 4 H, 5′-H, 3′-H, 6′a-H, 6′b-H), 2.31 (ddd, $J_{3A,3B}$ = 14.5, $J_{3A,2}$ = 10.8, $J_{3A,4}$ = 4.0 Hz, 1 H, 3_A-H), 2.02 (m, $J_{3B,3A}$ = 14.5 Hz, 1 H, 3_B-H) ppm. ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ = 136.9, 128.8, 128.5, 128.4 (C-aromatic), 97.3 (C-1), 91.8 (C-1′), 80.3 (C-5′), 73.9 (C-3′), 69.9 (*C*H_2OBn), 68.5 (C-4′), 66.2 (C-2′), 63.7 (C-2), 61.2 (C-6′), 56.2 (C-5), 52.2 (C-4), 26.3 (C-3) ppm. HRMS (ESI): calcd. for $C_{18}H_{26}NaO_9S$ 441.1190 $[M + Na]^+$; found 441.1183.

Benzyl 3-Deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-β-D-*threo*-pentopyranoside (*R*)-*S*-Oxide (4*R*): Removal of the *O*-acetyl groups of **2*R*** (25 mg, 0.043 mmol) gave **4*R*** as a colorless syrup (14 mg, 79%). R_f = 0.59 (*n*BuOH/EtOH/H_2O, 2.5:1:1). $[\alpha]_D^{25}$ = –99.6 (*c* = 0.9, MeOH). ^1H NMR (D_2O, 500 MHz): δ = 7.49–7.40 (m, 5 H, *Ph*CH_2O), 4.97 (d, $J_{1,2}$ = 2.8 Hz, 1 H, 1-H), 4.81, 4.68 (2 d, *J* = 11.9 Hz, 1 H each, CH_2OBn), 4.34 (d, $J_{1',2'}$ = 9.9 Hz, 1 H, 1′-H), 4.16 (dd, $J_{5A,5B}$ = 13.6, $J_{5A,4}$ = 2.8 Hz, 1 H, 5_A-H), 4.11–4.07 (m, 1 H, 2-H), 4.04 (t, $J_{1',2'} = J_{2',3'}$ = 9.7 Hz, 1 H, 2′-H), 4.02 (d, $J_{5',6'b}$ = 2.3 Hz, 1 H, 5′-H), 3.92–3.89 (m, 1 H, 4-H), 3.86 (br. d, $J_{3',4'}$ = 3.4, $J_{4',5'}$ < 1 Hz, 1 H, 4′-H), 3.85 (d, $J_{6'a,6'b}$ = 11.0 Hz, 1 H, 6′a-H), 3.81 (dd, $J_{2',3'}$ = 9.7, $J_{3',4'}$ = 3.4 Hz, 1 H, 3′-H), 3.76 (dd, $J_{6'a,6'b}$ = 11.0, $J_{6'b,5'}$ = 2.9 Hz, 1 H, 6′b-H), 3.61 (br. d, $J_{5A,5B}$ = 13.6, $J_{4,5B}$ < 1 Hz, 1 H, 5_B-H), 2.33–2.23 (m, 2 H, 3_B-H, 3_A-H) ppm. ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ = 136.9, 128.7, 128.5, 128.4 (C-aromatic), 97.4 (C-1), 88.1 (C-1′), 80.1 (C-4′), 73.9 (C-3′), 70.0 (*C*H_2OBn), 68.8 (C-5′), 64.9 (C-2′), 63.7 (C-2), 60.9 (C-6′), 57.4 (C-5), 51.5 (C-4), 24.5 (C-3) ppm. HRMS (ESI): calcd. for $C_{18}H_{26}NaO_9S$ 441.1190 $[M + Na]^+$; found 441.1199.

Enzymatic Assays

Inhibition of β-Galactosidase: Compounds **4*S*** and **4*R*** were tested as inhibitors of the β-galactosidase from *E. coli* (grade VIII, Sigma, EC 3.2.1.23, 589 U/mg). The enzyme (0.075 U; 1 U = 1 enzyme unit hydrolyses 1 μmol of *o*-nitrophenyl galactopyranoside per minute) was incubated with a solution of *o*-nitrophenyl β-D-galactopyranoside (concentration range: 0.4–3.0 mM) in sodium phosphate buffer (100 mM, pH 7.3, $MgCl_2$ 1.26 mM, 2-mercaptoethanol 31.8 mM) in the absence or presence of sulfoxide **4*S*** (0.1–1.0 mM) or **4*R*** (0.1–1.0 mM); the final volume was adjusted to 0.5 mL. After 10 min at 37 °C, the reaction was quenched by the addition of sodium borate buffer (0.05 M, 4.0 mL, pH 10). The concentration of the *o*-nitrophenol released was measured by visible absorption spectroscopy at 410 nm. The absorbance in the presence of **4*S*** or **4*R*** was significantly lower than that of the control experiments, which shows inhibition of the enzyme activity. The IC_{50} values were determined from the dose-response curve (Figure 4) and the K_i values were determined from Lineweaver–Burk plots (Figure 5).

Evaluation of 3, 4*S*,and 4*R* as Substrates of β-Galactosidase from *E. coli*: Compounds **3** (36 mM), **4*S*** (33 mM), or **4*R*** (32 mM), dissolved in sodium phosphate buffer (100 mM) at pH 7.3 containing $MgCl_2$ (1 mM) and 2-mercaptoethanol (5 mM), were incubated at 28 °C for 48 h in the presence of the β-galactosidase from *E. coli* (0.05 mg/mL). After this time, the solvent was evaporated and the resulting mixture was dissolved in D_2O and analyzed by ^{13}C NMR spectroscopy. The releasing of D-galactose from **3**, **4*S***, or **4*R*** is indicative of which compound is a substrate of the β-galactosidase from *E. coli*.

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