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Stereoselective Synthesis of 3-Deoxy-4-*S*-(1→4)-Thiodisaccharides and Their Inhibitory Activities Towards β-Glycoside Hydrolases

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The sulfur linkage of β-(1→4)-thiodisaccharides was constructed with excellent diastereoselectivity by Michael addition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactose (**2**) or its β-D-glucose isomer (**3**) to sugar-derived (2*S*, 6*S*)-6-acetoxymethyl-2-(2-propyloxy)-2*H*-pyran-3(6*H*)-one (**1**). These reactions led to the per-*O*-acetyl glycosides of 3-deoxy-4-*S*-glycopyranosyl-4-thiohexopyranosid-2-ulose (**4** and **5**, respectively). Similar conjugated addition to the enone **1** of the isothiouronium salts **6** or **7**, precursors in the synthesis of **2** or **3**, also afforded the thiodisaccharides **4** or **5**, respectively, with exclusive formation of the isomer that has an *R* configuration for the C-4 stereocenter of the reducing-end. The carbonyl function of **4** and **5** was reduced, and the resulting products were *O*-deacetylated to give the free 4-*S*(1→4)-thiodisaccharides **10**, **11**, **14**, and **15**, which have a deoxy functionality adjacent to the thio group. These compounds were tested as inhibitors of glycoside hydrolases. Thus **11**, the 3-deoxy-4-thiomimetic of Galβ(1→4)Gal, proved to be a competitive inhibitor of the β-galactosidase from *E. coli* (K_i = 0.16 mM).

Introduction

Thioglycosides are much more stable towards the action of glycoside hydrolases than the *O*-glycosidic analogs, and they can thus inhibit enzymatic hydrolysis.[1] owing to their stability to hydrolysis and their close similarity to the natural *O*-linked counterparts, thioglycosides are seen as potential precursors of promising carbohydrate-based therapeutics.[2] Thiooligosaccharides in which at least one oxygen atom of the interglycosidic linkage has been replaced by a sulfur atom also act as enzyme inhibitors. They are valuable in structural biology as they provide insight into binding, recognition, and mechanism of action of hydrolytic enzymes.[3,4]

Considerable attention has been focused on the synthesis of thiooligosaccharides, and chemical[3–6] and chemoenzymatic[7,8] approaches have been reported. Most thiooligosaccharide syntheses are based on the enhanced nucleophilicity of sulfur relative to that of oxygen; thus, S_N2 displacement of an electrophilic leaving group by a 1-thioaldose derivative or glycosylation of a thiosugar acceptor with an activated glycosyl donor are procedures that are frequently employed.[3–6] Witczak and coworkers[9] studied the Michael addition of anomeric thiolates to levoglucosenone and isolevoglucosenone, and Thiem et al. described analogous additions of per-*O*-acetyl-1-thioglucose to levoglucosenone and other sugar enones.[10] Similarly, we have explored the conjugated addition of common thiols to the α,β-unsaturated carbonyl system of dihydropyran-2-ones,[11,12] which were readily prepared from hexoses[13] or pentoses,[14,15] and proved to be versatile building blocks for the synthesis of varied molecules.[16–20] The Michael addition of thiols to dihydropyranones led to alkyl 3-deoxy-4-thiohexopyranosid-2-uloses in high yield.[11,12] In this work we describe the coupling reaction of 1-thioaldose derivatives to a sugar enone to afford the corresponding 3-deoxy-4-*S*-glycosyl-4-thiohexopyranosid-2-uloses and their conversion, by reduction of the carbonyl group, into the corresponding thiodisaccharides derivatives. The inhibitory activity of the free thiodisaccharides towards glycoside hydrolases was also studied.

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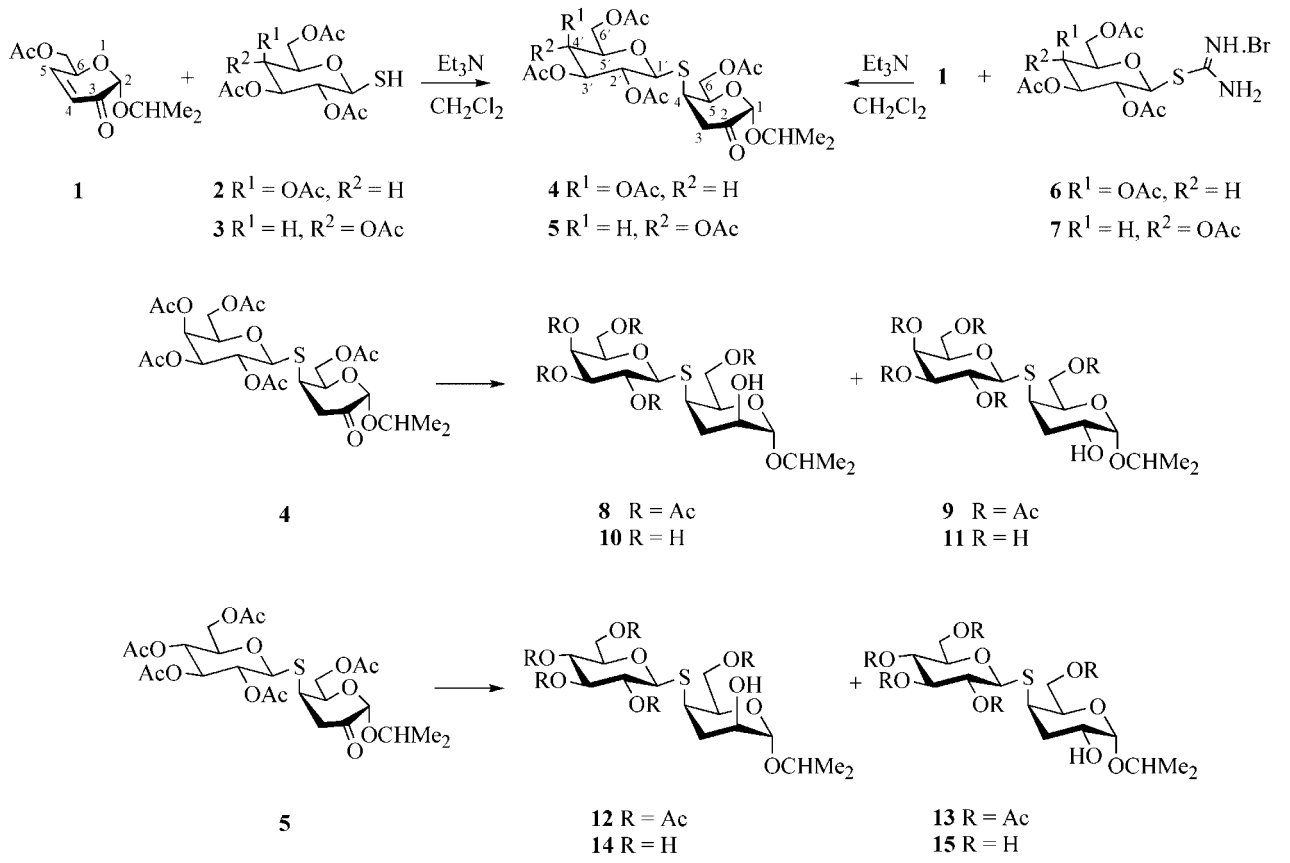
Results and Discussion

(2*S*,6*S*)-6-Acetoxymethyl-2-(2-propyloxy)-2*H*-pyran-3(6*H*)-one (**1**), readily prepared from D-galactose via 2-acetoxy-3,4,6-tri-*O*-acetyl-D-galactal,[13] was employed as a Michael acceptor of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranose[21] (**2**). The addition of **2** to **1** proceeded rapidly at 0 °C (30 min) in a CH_2Cl_2 solution containing a catalytic amount of triethylamine (Et_3N). The ^1H NMR spectrum of the conjugated addition product **4** is in agreement with an *R* configuration for the new stereocenter generated at C-4 (Scheme 1), as the small coupling constants values ($J_{3a,4}$ = 5.0 Hz, $J_{3b,4}$ = 2.7 Hz, and $J_{4,5}$ = 2.0 Hz) indicate that 4-H is equatorially oriented (gauche to 5-H) and that the C-4–4-H bond bisects the angle formed by the methylene protons



Scheme 1.

(3a,3b-H). The high diastereofacial selectivity in the addition reaction in favor of the D-*threo* isomer may be attributed to the stereocontrol exerted by the axially oriented isopropoxy group in the preferred $^0H_5$ conformation of the dihydropyranone,[14,22] which is stabilized by the anomeric effect, that is intensified by the presence of the carbonyl group vicinal to the anomeric center.[23] We have observed a similar selectivity in cycloadditions[14,17] and additions of common thiols to **1**.[11] Furthermore, the configuration of the C-4 stereocenter of the thiodisaccharides is opposite to that produced by similar reactions applied to levoglucosenone.[9,10]

The Michael addition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose[21] (**3**) to **1** was also studied. Under the reaction conditions employed previously, coupling of **1** with **3** led to the thiodisaccharide **5** in 81% yield. The coupling constants in the ^{1}H NMR spectrum of **5** ($J_{3a,4}$ = 4.9 Hz and $J_{3b,4}$ = 1.5 Hz) are indicative of a D-*threo* configuration for the ulose moiety.

Recently, Ibatullin and coworkers[24,25] described a facile procedure for the conversion of isothiouronium salts of sugars into thioglycosides, thiooligosaccharides, and glycosylthioesters. For the synthesis of thiodisaccharides or thiooligosaccharides,[25] the triethylamine-promoted reaction of the glycosyl isothiouronium bromide with a conveniently protected triflate derivative of a sugar was employed. As the Michael additions described above are also promoted by triethylamine, we explored the in situ coupling of the glycosyl isothiouronium salts **6** and **7** with the Michael acceptor **1**. Compounds **6** and **7** were readily prepared by reaction of tetra-*O*-acetyl-α-D-galactopyranosyl bromide, or the respective gluco isomer, with thiourea.[21] The NMR spectra of the crude isothiouronium salts **6** and **7** reaveal that these products are obtained with a high degree of purity. The signal for the anomeric proton of **6** and **7** shows a large value for $J_{1,2}$ (≈ 9.8 Hz), which indicates a β configuration for the anomeric center. The ^{13}C NMR spectra of **6** and **7** show, as expected,[26] the resonances for the anomeric and isothiouronium carbon atoms at 80.1 and 166.4 ppm (for **6**), and 80.1 and 166.7 ppm (for **7**), respectively. The enone **1** (1.0 molar equiv.) and Et_3N (1.2 molar equiv.) were sequentially added to a suspension of crude **6** or **7** (1.5 molar equiv.) in CH_2Cl_2. Monitoring of the reaction mixture by TLC showed rapid conversion of **1** into the thiodisaccharides **4** or **5**, which were isolated by column chromatography in 81% yield.

Both reactions of **1** with **2** and **3**, or with **6** and **7**, afforded **4** and **5**, respectively, in yields higher than 80%; however, we observed that during the chromatographic purification, β-elimination of the glycosylthiolate from **4** or **5** with concomitant formation of **1** (the retro-Michael reaction) occurred to some extent. Therefore, the crude ulosides **4** and **5** were subjected to sodium borohydride reduction to afford the corresponding 3-deoxy-4-*S*-glycosyl-4-thiohexopyranosides in overall yields higher than 90% from **1**. For example, reduction of **4** led to the isomers that have the 3-

deoxy-4-thio-α-D-*lyxo* (**8**) and α-D-*xylo* (**9**) configurations for the reducing-end in 23% and 72% isolated yields, respectively (overall yield 95%). The structure of the reduction products can readily be established from the ^{1}H NMR spectra, as well as from 2D COSY experiments for full assignments. Thus, the ^{1}H spectrum (500 MHz) of **8** shows the signal for 1-H as a broad singlet, which suggests a diequatorial orientation for 1-H and 2-H, and an *S* configuration for the new stereocenter at C-2. This is confirmed by the small values for $J_{2,3a}$ (3.8 Hz) and $J_{2,3b}$ (3.1 Hz), which indicate that 2-H is equatorially disposed. Furthermore, the small coupling constant values measured for $J_{3a,4}$ (3.8 Hz), $J_{3b,4}$ (3.1 Hz), and $J_{4,5}$ (3.0 Hz) confirm, again, the *R* configuration for the sulfur-containing stereocenter (C-4), and hence, the 3-deoxy-4-thio-α-D-*lyxo* configuration for the reducing-end of **8**. Also, the $J_{1,2}$ (4.0 Hz) and $J_{2,3ax}$ (11.7 Hz) values confirm the *R* configuration for the C-2 stereocenter of **9**.

As reverse selectivity in the reduction of uloses with L-Selectride® has been reported;[9] we performed the reduction of the carbonyl function of **4** with such a reducing agent. However, in this case, in a manner similar to the reduction of **4** with sodium borohydride, compound **9** was obtained as the major isomer (ratio **9/8** = 3.7:1.0, estimated by NMR spectroscopy). The isolated yield of **9** was lower than that in the previous reduction, as partial deacylation in the L-Selectride reaction could not be prevented. The acetylation of the crude mixture gave the per-*O*-acetyl derivatives (including 2-OH) of **8** and **9**; in contrast to **8** and **9** these products could not be separated by column chromatography because of their similar mobility in a number of solvents.

Sodium borohydride reduction of crude **5** afforded the 3-deoxy-4-*S*-β-D-glucopyranosyl-4-thio-α-D-*lyxo*- (**12**) and α-D-*xylo*-hexopyranoside (**13**) derivatives in 19% and 71% isolated yields from **1**, respectively. The configuration of the reducing-end was assigned on the basis of the NMR spectra, which were also in full agreement with the spectroscopic data of the corresponding thiodisaccharide analogs **8** and **9**. The reduction of the carbonyl function of **4** or **5** was expected to be governed by the relative steric contribution of the axial thioglycosyl substituent at C-4 as well as by the axial anomeric substituent. The fact that the reduction of **4** or **5** led to the α-D-*xylo* isomers **9** and **13**, respectively, as major products suggests that the axial isopropyl group at the carbon atom vicinal to the carbonyl group induces the attack of the hydride from the opposite face of the sugar ring. Therefore, the anomeric substituent seems to determine the steric course of both the conjugated addition of 1-thiolate to **1** and the further reduction of the carbonyl group.

To obtain the free thiodisaccharides, the penta-*O*-acetyl derivatives **8**, **9**, **12**, and **13** were treated with an aqueous methanol solution of triethylamine at room temperature for 2 h. The resulting thiodisaccharides **10**, **11**, **14**, and **15** were purified by elution of their respective solution in water through a column filled with a mixed-bed ion-exchange resin and then through a reverse phase minicolumn. The *S*-disaccharides were obtained as crystalline products in 92–93% yield.

The methodology described here is useful for the synthesis of (1→4)-linked thiodisaccharides that have a deoxy functionality adjacent to the thio group. The resulting products **10**, **11**, **14**, and **15** constitute novel structures, which involve galactose or glucose at the non-reducing end. D-Galactose (D-Gal) is an important constituent of glycoconjugates involved in varied biological recognition events. For example, terminal residues of D-Gal are found in the sugar moieties of asialoglycoproteins.[27] Thiodisaccharide **11** is the 3-deoxy-4-thio analog of βGal(1→4)Gal, a repeating unit of pectic galactans isolated from plants, which are susceptible to hydrolysis by a β-(1→4)-endogalactanase.[28] Furthermore, the galabiose [Galα(1→4)Gal] moiety was found in globoseries of glycolipids on uroepithelial cells and erythrocytes, and β-thioglycosides of galabiose have been synthesized and have been shown to be inhibitors of adhesins of bacteria.[29,30] The route described here for the synthesis of **11** is highly efficient as this product was obtained in 66% overall yield from **1**.

With regard to the inhibition of glycoside hydrolases by thiooligosaccharides, the most complete studies on *S*(1→4)

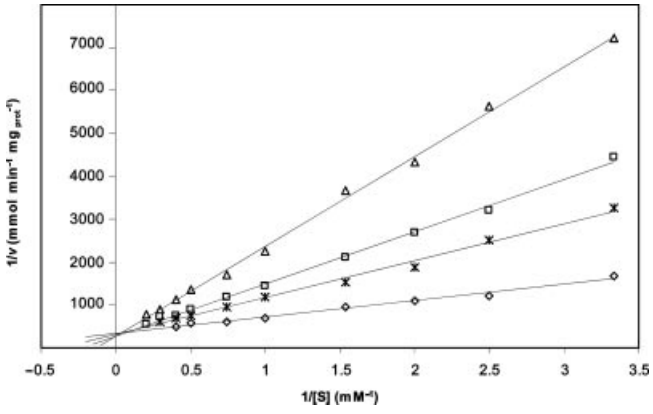


Figure 1. Lineweaver–Burk plot for inhibition of *E. coli* β-galactosidase by thiodisaccharide **11** at concentrations: diamonds: 0.00, stars: 0.10, squares: 0.25 and triangles: 0.50 mM of inhibitor.

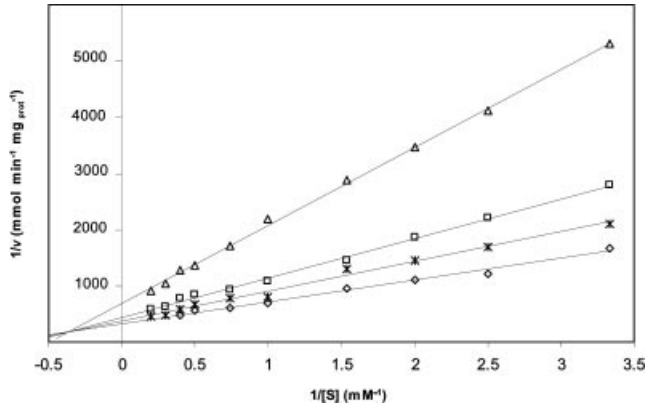


Figure 2. Lineweaver–Burk plot for inhibition of *E. coli* β-galactosidase by thiodisaccharide **10** at concentrations: diamonds: 0.00, stars: 0.10, squares: 0.25 and triangles: 0.50 mM of inhibitor.

derivatives were achieved in the cellobiose and maltose series with cellulases and amylases of various origins.[3] Many *S*-disaccharides usually act as competitive inhibitors of glycoside hydrolases,[1] but mixed-type inhibition also occurs frequently.[1,31]

Thiogalactosides **10** and **11** were tested as inhibitors of β-galactosidase from *E. coli* under the classical conditions described for this enzyme. The substrate employed was *o*-nitrophenyl-β-D-galactopyranoside, and the released *o*-nitrophenol was measured spectrophotometrically. On the basis of the Lineweaver–Burk plot, compound **11** is a competitive inhibitor, with an inhibitory activity of K_i = 0.16 mM and K_m = 1.17 mM (Figure 1). Compound **10** is a mixed-type inhibitor with K_i = 0.12 mM (Figure 2).

Thiodisaccharides **14** and **15**, having glucose at the nonreducing end, were tested as inhibitors of the β-glucosidase from almonds. Although this β-D-glucoside hydrolase has been shown to have a broad specificity,[32] compounds **14** and **15** did not exhibit an appreciable inhibition.

Experimental Section

General: Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Analytical thin layer chromatography (TLC) was performed on Silica Gel 60 F254 (Merck) aluminum-supported plates (layer thickness 0.2 mm) with solvent systems that are given in the text. Visualization of the spots was effected by exposure to UV light and charring with a solution of 5% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Column chromatography was carried out with Silica Gel 60 (230–400 mesh, Merck). Optical rotations were measured with a Perkin–Elmer 343 digital polarimeter, for solutions in $CHCl_3$ or water. Nuclear magnetic resonance (NMR) spectra were recorded with Bruker AC 200 or Bruker AMX 500 instruments. For solutions in $CDCl_3$, tetramethylsilane was used as an internal standard. Assignments of ^1H and ^{13}C were assisted by 2D ^1H COSY and 2D ^1H–^{13}C CORR experiments.

2-Propyl 6-*O*-Acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-*threo*-hexopyranosid-2-ulose (4)

a) Starting from 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-galactopyranose (2): (2*S*, 6*S*)-6-Acetoxymethyl-2-(2-propyloxy)-2*H*-pyran-3(6*H*)-one[13] (**1**, 63 mg, 0.27 mmol) and compound **2** (100 mg, 0.27 mmol) were dissolved in anhydrous CH_2Cl_2 (0.5 mL). The reactive vial was flushed with a stream of nitrogen, sealed, and cooled to 0 °C. A 10% solution of NEt_3 in CH_2Cl_2 (0.2 mL) was added, and the mixture was stirred at 0 °C for 30 min. TLC (EtOAc/hexane, 1.5:1) showed complete conversion of the starting materials into a major spot with R_f = 0.50. The reaction mixture was concentrated, and the residue purified by flash chromatography (hexane/EtOAc, 1.5:1) to give crystalline thiodisaccharide **4** (136 mg, 83%), which was recrystallized from EtOH. M.p. 126 °C. $[\alpha]_D^{25}$ = +26.6 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 5.42 (dd, 1 H, $J_{3',4'}$ = 3.3, $J_{4'5'}$ = 1.0 Hz, 4′-H), 5.18 (dd, 1 H, $J_{1',2'} \approx J_{2',3'}$ = 10.0 Hz, 2′-H), 5.03 (dd, 1 H, 3′-H), 4.79 (ddd, $J_{4,5}$ = 2.0, $J_{5,6a}$ = 4.3, $J_{5,6b}$ = 7.5 Hz, 1 H, 5-H), 4.74 (br. s, 1 H, 1-H), 4.57 (d, 1 H, 1′-H), 4.29 (dd, 1 H, $J_{6a,6b}$ = 11.9 Hz, 6a-H), 4.25 (dd, 1 H, 6b-H), 4.17 (dd, 1 H, $J_{5',6'a}$ = 6.4, $J_{6'a,6'b}$ = 11.2 Hz, 6′a-H), 4.10 (dd, 1 H, $J_{5',6'b}$ = 7.0 Hz, 6′b-H), 4.00 (m, *J* = 6.3 Hz, 1 H, Me_2CH), 3.88 (ddd, 1 H, 5′-H), 3.68 (m, 1 H, 4-H), 3.18 (dd, 1 H, $J_{3a,4}$ = 5.0, $J_{3a,3b}$ = 15.1 Hz, 3a-H), 2.78 (ddd, 1 H, $J_{3b,4}$ = 2.7, $J_{3b,5}$ = 1.0 Hz, 3b-H), 2.16, 2.08, 2.07, 2.05, 1.98 (5 s, 3 H each, CH_3CO), 1.27, 1.18 [2 d, 6 H, *J* = 6.3 Hz, $(CH_3)_2C$] ppm. ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 198.8 (C-2), 170.6, 170.4, 170.2, 170.0, 169.7 (5 CH_3CO), 97.8 (C-1), 82.7 (C-1′), 74.5 (C-5′), 71.8 (×2, C-3′, Me_2CH), 68.6 (C-5), 67.0 (×2, C-2′, -4′), 64.9 (C-6), 61.3 (C-6′), 44.6 (C-4), 43.4 (C-3), 23.2, 21.7 [$(CH_3)_2C$], 20.7, 20.6 (×3), 20.5 (5 CH_3CO) ppm. $C_{25}H_{36}O_{14}S$ (592.61): calcd. C 50.67, H 6.12, S 5.41; found C 50.68, H 6.21, S 5.39.

b) Starting from 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosylisothiouronium Bromide (6): Tetra-*O*-acetyl-α-D-galactopyranosyl bromide was treated with thiourea to give the corresponding isothiouronium salt **6**.[21] The ^1H and ^{13}C NMR spectra ([D$_6$]DMSO) were consistent with those already described.[26] Crude **6** (0.32 g, 0.66 mmol) was suspended in dry CH_2Cl_2 (1 mL) and the enone **1** (0.10 g, 0.44 mmol) was added. After addition of NEt_3 (73 μL, 0.52 mmol), under nitrogen, the mixture was stirred for 30 min at room temperature. The solvent was evaporated, and the residue chromatographed as described above, to afford the crystalline thiodisaccharide **4** (0.21 g, 81%). Compound **4** showed the same properties as those of the product reported in part a).

2-Propyl 6-*O*-Acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-thio-α-D-*threo*-hexopyranosid-2-ulose (5)

a) Starting from 2,3,4,6-Tetra-*O*-hexopyranosid-1-thio-β-D-glucopyranose: Enone **1** (63 mg, 0.27 mmol) reacted with 1-thiosugar **3**[21] (100 mg, 0.27 mmol) under the conditions used for the preparation of **4** to give crystalline **5** (131 mg, 80%). M.p. 154–155 °C (from EtOH). $[\alpha]_D^{25}$ = +12.3 (*c* = 0.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 5.20 (dd, 1 H, $J_{2',3'}$ = 9.0, $J_{3',4'}$ = 9.9 Hz, 3′-H), 5.08 (dd, 1 H, $J_{4',5'}$ = 9.2 Hz, 4′-H), 4.99 (dd, 1 H, $J_{1',2'}$ = 10.1 Hz, 2′-H), 4.78 (m, 1 H, 5-H), 4.73 (s, 1 H, 1-H), 4.60 (d, 1 H, 1′-H), 4.27–4.14 (m, 3 H, 6a-, 6b-, 6′a-H), 4.15 (dd, 1 H, $J_{5',6'b}$ = 2.0, $J_{6'a,6'b}$ = 12.3 Hz, 6′b-H), 4.01 (m, *J* = 6.2 Hz, 1 H, Me_2CH), 3.67–3.63 (m, 2 H, 4, 5′-H), 3.16 (dd, 1 H, $J_{3a,4}$ = 4.9, $J_{3a,3b}$ = 15.2 Hz, 3a-H), 2.80 (dd, 1 H, $J_{3b,4}$ = 1.5 Hz, 3b-H), 2.09, 2.08, 2.07, 2.02, 2.00 (5 s, 3 H each, 5 CH_3CO), 1.27, 1.18 [2 d, 3 H each, *J* = 6.2 Hz, $(CH_3)_2CH$] ppm. ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 198.8 (C-2), 170.6, 170.5, 170.1, 169.5, 169.3 (5 CH_3CO), 97.8 (C-1), 82.0 (C-1′), 75.8 (C-5′), 73.7 (C-4′), 71.8 (Me_2CH), 69.7, 68.5, 68.0 (C-2′, -3′, -5), 64.7 (C-6), 61.7 (C-6′), 44.5 (C-4), 43.5 (C-3), 23.2, 21.7 [$(CH_3)_2CH$], 20.7–20.5 (CH_3CO) ppm. $C_{25}H_{36}O_{14}S$ (592.61): calcd. C 50.67, H 6.12, S 5.41; found C 50.84, H 6.10, S 5.37.

b) Starting from 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl Isothiouronium Bromide (7): Compound **7**, prepared from acetobromoglucose,[21] showed the following spectroscopic data. ^1H NMR (200 MHz, [D$_6$]DMSO): δ = 9.27, 9.11 (2 s, 2 H each, 2 NH_2) 5.73 (d, $J_{1,2}$ = 9.9 Hz, 1 H, 1-H), 5.32 (t, 1 H, $J_{3,4} \approx J_{4,5} \approx$ 9.3 Hz, 4-H), 5.10 (t, 2 H, 2,3-H), 4.23–4.05 (m, 3 H, 5, 6, 6′-H), 2.06, 2.02, 2.00, 1.98 (4 s, 3 H each, 4 CH_3CO) ppm. ^{13}C NMR (50.3 MHz, [D$_6$]DMSO): δ = 170.6, 170.0, 169.9, 169.8 (CH_3CO), 166.7 [$SC(NH_2)_2$], 80.1 (C-1), 75.7, 72.8, 69.1, 67.7 (C-2, -3, -4, -5), 62.0 (C-6), 21.0, 20.8, 20.7, 20.6 (CH_3CO) ppm.

Crude **7** (0.32 g, 0.66 mmol) reacted with enone **1** (0.10 g, 0.44 mmol), as described in the previous synthesis of **4**, to give the disaccharide **5** (0.21 g, 81%). Compound **5** exhibited the same properties as those of the product reported in part a).

2-Propyl 6-*O*-Acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4-thio-α-D-*lyxo*- and α-D-*xylo*-Hexopyranosides (8 and 9, respectively)

a) Sodium Borohydride Reduction of 4: Thiodisaccharide **4** from a crude preparation from enone **1** (0.15 g, 0.66 mmol) was dissolved in dry MeOH (3 mL) and treated with sodium borohydride (25 mg,

0.66 mmol) at 0 °C for 30 min. The mixture was stirred in batch with Dowex 50W(H^+) resin, filtered, and concentrated. Column chromatography of the residue with hexane/EtOAc (1.5:1) afforded the less polar product (R_f = 0.48, hexane/EtOAc, 1:2) first, which was identified as **8** (90 mg, 23%). The disaccharide **8** crystallized upon standing. M.p. 52 °C. $[\alpha]_D^{25}$ = +35.0 (c = 0.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 5.43 (dd, 1 H, $J_{3',4'}$ = 3.2, $J_{4',5'}$ = 1.0 Hz, 4′-H), 5.24 (t, 1 H, $J_{1',2'} \approx J_{2',3'}$ = 9.9 Hz, 2′-H), 5.06 (dd, 1 H, 3′-H), 4.86 (br. s, 1 H, 1-H), 4.65 (d, 1 H, 1′-H), 4.43 (ddd, $J_{4,5}$ = 3.0, $J_{5,6a}$ = 7.2, $J_{5,6b}$ = 4.2 Hz, 1 H, 5-H), 4.25 (dd, $J_{5,6a}$ = 7.6, $J_{6a,6b}$ = 11.8 Hz, 1 H, 6a-H), 4.21 (dd, 1 H, $J_{5,6b}$ = 4.4 Hz, 6b-H), 4.16 (dd, 1 H, $J_{5',6'a}$ = 6.6, $J_{6'a,6'b}$ = 11.3 Hz, 6′a-H), 4.09 (dd, 1 H, $J_{5',6'b}$ = 6.8 Hz, 6′b-H), 3.95 (m, J = 6.2 Hz, 1 H, Me_2CH), 3.92 (ddd, 1 H, 5′-H), 3.59 (m, 1 H, 2-H), 3.47 (d, $J_{2,OH}$ = 9.7 Hz, 1 H, O*H*), 3.33 (m, 1 H, 4-H), 2.28 (dt, 1 H, $J_{2,3a} \approx J_{3a,4}$ = 3.8, $J_{3a,3b}$ = 14.9 Hz, 3a-H), 2.23 (dt, 1 H, $J_{2,3b} \approx J_{3b,4}$ = 3.1 Hz, 3b-H), 2.17, 2.07, 2.06, 2.05, 1.98 (5 s, 3 H each, 5 CH_3CO), 1.21, 1.16 [2 d, 3 H each, J = 6.2 Hz, $(CH_3)_2CH$] ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 170.6, 170.3, 170.1, 169.9, 169.5 (CH_3CO), 98.9 (C-1), 82.7 (C-1′), 74.7, 71.9, 69.5, 68.2 67.7, 67.1 (×2) (C-2′, -3′, -4′, -5′, -2, -5, Me_2CH), 65.1 (C-6), 61.3 (C-6′), 39.3 (C-4), 30.4 (C-3), 23.1, 21.5 [$(CH_3)_2CH$], 20.7, 20.6 (×3), 20.5 (5 CH_3CO) ppm. $C_{25}H_{38}O_{14}S$ (594.63): calcd. C 50.50, H 6.44, S 5.39; found C 50,62, H 6.66, S 5.31.

Further fractions from the column (R_f = 0.38) afforded syrupy **9** (282 mg, 72%), which crystallized upon standing. M. p. 56 °C. $[\alpha]_D^{25}$ = +37.0 (c = 1.4, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$ + D_2O): δ = 5.42 (dd, 1 H, $J_{3',4'}$ = 3.4, $J_{4',5'}$ = 1.0 Hz, 4′-H), 5.20 (t, 1 H, $J_{1',2'} \approx J_{2',3'}$ = 9.9 Hz, 2′-H), 5.04 (dd, 1 H, 3′-H), 4.89 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1-H), 4.56 (d, 1 H, 1′-H), 4.28 (ddd, $J_{4,5}$ = 2.3, $J_{5,6a}$ = 3.7, $J_{5,6b}$ = 7.8 Hz, 1 H, 5-H), 4.21 (dd, 1 H, $J_{6a,6b}$ = 11.8 Hz, 6a-H), 4.17 (dd, 1 H, $J_{5',6'a}$ = 6.3, $J_{6'a,6'b}$ = 11.5 Hz, 6′a-H), 4.16 (dd, 1 H, 6b-H), 4.09 (dd, 1 H, $J_{5',6'b}$ = 6.5 Hz, 6′b-H), 3.99 (ddt, 1 H, $J_{1,2} \approx J_{2,3a}$ = 4.0, $J_{2,3b}$ = 11.7, $J_{2,OH}$ = 10.9 Hz, 2-H), 3.95 (m, J = 6.2 Hz, 1 H, Me_2CH), 3.89 (ddd, 1 H, 5′-H), 3.30 (m, 1 H, 4-H), 2.16, 2.07, 2.05 (×2), 1.98 (4 s overlapped with m, 17 H, 5 CH_3CO + 3-, 3′-H), 1.26, 1.19 [2 d, 6 H, J = 6.2 Hz, $(CH_3)_2C$] ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 170.6, 170.4, 170.1, 169.9, 169.4 (CH_3CO), 96.7 (C-1), 83.7 (C-1′), 74.5, 71.9, 70.7, 68.1, 67.3, 67.2, 65.5 (C-2′, -3′, 4′, -5′, -2, -5, Me_2CH), 64.0 (C-6), 61.4 (C-6′), 43.4 (C-4), 34.7 (C-3), 23.1, 21.9 [$(CH_3)_2CH$], 20.7, 20.6 (×3), 20.5 (CH_3CO) ppm. $C_{25}H_{38}O_{14}S$ (594.63): calcd. C 50.50, H 6.44, S 5.39; found C 50.59, H 6.31, S 5.34.

b) L-Selectride Reduction of 4: A stirred solution of **4** (88 mg, 0.149 mmol) in anhydrous THF (2 mL) was cooled to –78 °C, and L-Selectride (1 M in THF, 0.25 mL) was added under argon. The mixture was stirred for 6 h at –78 °C, and then glacial acetic acid was added dropwise (pH = 6). The mixture was concentrated, the residue dissolved in MeOH (30 mL), and the solvent evaporated. The resulting syrup was dissolved in CH_2Cl_2 (50 mL) and washed with water (2 × 20 mL). The organic extract was dried ($MgSO_4$) and concentrated. The NMR spectra of the crude product showed the almost exclusive formation of **9** and **8** (3.7:1 ratio). Column chromatography of the mixture gave compound **8** (12.9 mg, 14.5%) and then **9** (43 mg, 48.6%).

2-Propyl 6-*O*-Acetyl-3-deoxy-4-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4-thio-α-D-*lyxo*- and α-D-*xylo*-hexopyranosides (12 and 13, respectively): Thiodisaccharide **5** from a crude preparation from enone **1** (0.228 g, 1.0 mmol) was reduced with sodium borohydride as described for the analogous reduction of **4**. Column chromatography (hexane/EtOAc, 1.5:1) led first to the crystalline thiodisaccharide **12** (112 mg, 19%). R_f = 0.41, (hexane/EtOAc, 1:2). M.p. 162–163 °C (from MeOH). $[\alpha]_D^{25}$ = +20.6 (c = 0.9, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 5.29 (t, 1 H, $J_{2',3'} = J_{3',4'}$ = 9.3 Hz, 3′-H), 5.14 (dd, 1 H, $J_{4',5'}$ = 10.0 Hz, 4′-H), 5.09 (dd, 1 H, $J_{1',2'}$ = 10.2 Hz, 2′-H), 4.91 (s, 1 H, 1-H), 4.73 (d, 1 H, 1′-H), 4.48 (ddd, $J_{4,5}$ = 2.9, $J_{5,6a}$ = 7.4, $J_{5,6b}$ = 4.4 Hz, 1 H, 5-H), 4.31 (dd, 1 H, $J_{5',6'a}$ = 5.1, $J_{6'a,6'b}$ = 12.4 Hz, 6′a-H), 4.30 (dd, 1 H, $J_{6a,6b}$ = 11.7 Hz, 6a-H), 4.26 (dd, 1 H, 6b-H), 4.21 (dd, 1 H, $J_{5',6'b}$ = 2.4 Hz, 6′b-H), 4.00 (m, J = 6.2 Hz, 1 H, Me_2CH), 3.76 (ddd, 1 H, 5-H), 3.65 (m, 1 H, 2-H), 3.49 (d, 1 H, $J_{H-2,OH}$ = 10.0 Hz, *H*O), 3.36 (m, 1 H, 4-H) 2.33 (dt, 1 H, $J_{2,3a} \approx J_{3a,4}$ = 4.0, $J_{3a,3b}$ = 14.8 Hz, 3a-H), 2.27 (dt, 1 H, $J_{2,3b} \approx J_{3b,4}$ = 3.0 Hz, 3b-H), 2.14, 2.11 (×2), 2.08, 2.06 (5 s, 3 H each, 5 CH_3CO), 1.27, 1.21 [2 d, 3 H each, J = 6.2 Hz, $(CH_3)_2CH$] ppm. ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 170.6, 170.5, 170.1, 169.4 (CH_3CO), 98.9 (C-1), 82.2 (C-1′), 76.1, 73.8, 69.9, 69.6, 68.2, 68.1, 67.5 (C-2′, -3′, -4′, -5′, -2, -5, Me_2CH), 65.0 (C-6), 61.9 (C-6′), 39.3 (C-4), 30.6 (C-3), 23.1, 21.5 [$(CH_3)_2CH$], 20.7, 20.6, 20.5 (CH_3CO) ppm. $C_{25}H_{38}O_{14}S$ (594.63): calcd. C 50.50, H 6.44, S 5.39; found C 50.42, H 6.33, S 5.38.

From the next fractions of the column (R_f = 0.31), crystalline **13** was isolated (0.42 g, 71%). M.p. 159–160 °C (from MeOH). $[\alpha]_D^{25}$ = +28.1 (c = 1.0, $CHCl_3$). ^1H NMR (500 MHz, Cl_3CD): δ = 5.21 (t, 1 H, $J_{2',3'} \approx J_{3',4'}$ = 9.3 Hz, 3′-H), 5.08 (dd, 1 H, $J_{4',5'}$ = 10.0 Hz, 4′-H), 5.00 (dd, 1 H, $J_{1',2'}$ = 10.2 Hz, 2′-H), 4.89 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1-H), 4.59 (d, 1 H, 1′-H), 4.27 (ddd, $J_{4,5}$ = 2.4, $J_{5,6a}$ = 3.5, $J_{5,6b}$ = 8.0 Hz, 1 H, 5-H), 4.22 (dd, 1 H, $J_{5',6'a}$ = 5.1, $J_{6'a,6'b}$ = 12.4 Hz, 6′a-H), 4.19 (dd, 1 H, $J_{6a,6b}$ = 11.8 Hz, 6a-H), 4.16 (dd, 1 H, $J_{5',6'b}$ = 2.4 Hz, 6′b-H), 4.14 (dd, 1 H, 6b-H), 3.98 (dt, 1 H, $J_{1,2} = J_{2,3a}$ = 4.0, $J_{2,3b}$ = 11.5 Hz, 2-H), 3.95 (m, J = 6.2 Hz, 1 H, Me_2CH), 3.67 (ddd, 1 H, 5′-H), 3.29 (m, 1 H, 4-H), 2.16 (ddd, 1 H, $J_{3a,4}$ = 3.3, $J_{3a,3b}$ = 13.1 Hz, 3a-H), 2.09, 2.07, 2.04, 2.03, 2.00 (5 s, 3 H each + 1 H, 5 CH_3CO + 3b-H), 1.25, 1.19 [2 d, 3 H each, J = 6.2 Hz, $(CH_3)_2CH$] ppm. ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 170.7–169.4 (CH_3CO), 96.7 (C-1), 83.1 (C-1′), 75.9, 73.8, 70.8, 70.0, 68.2, 68.0, 65.5 (C-2′, -3′, -4′, -5′, -2, -5, Me_2CH), 64.0 (C-6), 62.0 (C-6′), 43.2 (C-4), 34.8 (C-3), 23.2, 21.9 [$(CH_3)_2CH$], 20.8, 20.7, 20.6, 20.5 (CH_3CO) ppm. $C_{25}H_{38}O_{14}S \cdot 0.5H_2O$ (603.63): calcd. C 49.75, H 6.47, S 5.34; found C 49.62, H 6.46, S, 5.33.

General Procedure for the *O*-Deacetylation of Thiodisaccharides 8, 9, 12, and 13: A solution of the acetylated thiodisaccharides **8**, **9**, **12**, or **13** (0.10 mmol) in MeOH/Et_3N/H_2O (4:1:5, 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 a Dowex MR-3C mixed-bed ion-exchange resin. The deionized solutions were concentrated, and the free thiodisaccharides were purified by dissolution in water (1 mL) and filtration through an octadecyl C18 minicolumn (Amprep, Amersham Biosciences). Evaporation of the solvent afforded the crystalline free thiodisaccharides.

2-Propyl 3-Deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-α-D-*lyxo*-hexopyranoside (10): Deacetylation of **8** (101 mg, 0.17 mmol) gave **10** (61 mg, 93%). M.p. 88–90 °C. $[\alpha]_D^{25}$ = +48.1 (c = 0.6, H_2O). ^1H NMR (500 MHz, D_2O): δ = 4.68 (d, $J_{1,2}$ = 2.5 Hz, 1 H, 1-H), 4.32 (d, 1 H, $J_{1',2'}$ = 9.8 Hz, 1′-H), 4.07 (ddd, $J_{4,5}$ = 3.2, $J_{5,6a}$ = 4.8, $J_{5,6b}$ = 7.7 Hz, 1 H, 5-H), 3.91 (m, J = 6.2 Hz, 1 H, Me_2CH), 3.79 (br. d, 1 H, $J_{3',4'}$ = 3.2, $J_{4',5'} < 1$ Hz, 4′-H), 3.72 (dd, $J_{5,6a}$ = 4.8, $J_{6a,6b}$ = 11.9 Hz, 1 H, 6a-H), 3.66 (dd, 1 H, $J_{5,6b}$ = 7.7 Hz, 6b-H), 3.59 (dd, 1 H, $J_{5',6'a}$ = 7.6, $J_{6'a,6'b}$ = 11.4 Hz, 6′a-H), 3.54 (dd, 1 H, $J_{5',6'b}$ = 4.1 Hz, 6′b-H), 3.53–3.50 (m, 2 H, 2, 5′-H), 3.48 (dd, 1 H, $J_{2',3'}$ = 9.5, $J_{3',4'}$ = 3.2 Hz, 3′-H), 3.35 (t, 1 H, 2′-H), 3.18 (m, 1 H, 4-H), 2.21 (dt, 1 H, $J_{2,3a} \approx J_{3a,4}$ = 4.3, $J_{3a,3b}$ = 14.8 Hz, 3a-H), 1.97 (dt, 1 H, $J_{2,3b} \approx J_{3b,4}$ = 5.0 Hz, 3b-H), 1.08, 1.02 [2 d, 3 H each, J = 6.2 Hz, $(CH_3)_2C$] ppm. ^{13}C NMR (50.3 MHz, D_2O):

δ = 98.1 (C-1), 87.9 (C-1′), 79.7, 74.6, 72.6, 71.2, 70.7, 69.4, 67.3 (C-2′, -3′, -4′, -5′, -2, -5, Me_2CH), 62.4, 61.9 (C-6′, -6), 41.3 (C-4), 32.8 (C-3), 23.0, 21.2 [$(CH_3)_2C$] ppm. $C_{15}H_{28}O_9S.H_2O$ (402.44): calcd. C 44.78, H 7.46, S 7.96; found C 44.40, H 7.09, S 7.96.

2-Propyl 3-Deoxy-4-*S*-(β-D-galactopyranosyl)-4-thio-α-D-*xylo*-hexopyranoside (11): The general procedure for the *O*-deacetylation was applied to **9** (221 mg, 0.372 mmol) and this led to **11** (131 mg, 92%). M.p. 156 °C. $[\alpha]_D^{25}$ = +40.2 (*c* = 0.9, H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 4.85 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 4.41 (d, 1 H, $J_{1',2'}$ = 9.8 Hz, 1′-H), 4.12 (ddd, $J_{4,5}$ = 1.9, $J_{5,6a}$ = 5.0, $J_{5,6b}$ = 7.1 Hz, 1 H, 5-H), 3.99 (ddd, 1 H, $J_{2,3a}$ = 11.6, $J_{2,3b}$ = 4.5, 2-H), 3.88 (m, *J* = 6.2 Hz, 1 H, Me_2CH), 3.84 (d, 1 H, $J_{3',4'}$ = 3.4, $J_{4',5'}$ < 1 Hz, 4′-H), 3.66–3.54 (m, 5 H, 5′, 6a, 6b, 6′a, 6′b-H), 3.52 (dd, 1 H, $J_{2',3'}$ = 9.3, $J_{3',4'}$ = 3.4 Hz, 3′-H), 3.42 (t, 1 H, 2′-H), 3.40 (m, 1 H, 4-H), 2.05 (ddd, 1 H, $J_{3a,4}$ = 3.6, $J_{3a,3b}$ = 13.0 Hz, 3a-H), 1.99 (ddd, 1 H, $J_{3b,4}$ = 4.5 Hz, 3b-H), 1.13, 1.06 [2 d, 3 H each, *J* = 6.2 Hz, $(CH_3)_2CH$] ppm. ^{13}C NMR (50.3 MHz, D_2O): δ = 96.9 (C-1), 86.2 (C-1′), 79.8, 74.7, 71.3, 71.1, 70.6, 69.5, 64.8 (C-2′, -3′, -4′, -5′, -2, -5, Me_2CH), 63.4 (C-6), 61.9 (C-6′), 43.8 (C-4), 33.3 (C-3), 23.2, 21.3 [$(CH_3)_2CH$] ppm. $C_{15}H_{28}O_9S$ (384.44): calcd. C 46.86, H 7.34, S 8.34; found C 46.70, H 7.40, S 8.09.

2-Propyl 3-Deoxy-4-*S*-(β-D-glucopyranosyl)-4-thio-α-D-*lyxo*-hexopyranoside (14): The thiodisaccharide **12** (118 mg, 0.198 mmol) was deacetylated as described above to afford **14** (70 mg, 92%). M.p. 73 °C. $[\alpha]_D^{25}$ = +21.6 (*c* = 0.5, H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 4.70 (d, $J_{1,2}$ = 2.5 Hz, 1 H, 1-H), 4.41 (d, 1 H, $J_{1',2'}$ = 9.8 Hz, 1′-H), 4.08 (ddd, $J_{4,5}$ = 3.2, $J_{5,6a}$ = 4.8, $J_{5,6b}$ = 7.7 Hz, 1 H, 5-H), 3.91 (m, *J* = 6.2 Hz, 1 H, Me_2CH), 3.73 (dd, 1 H, $J_{5',6'a}$ = 2.0, $J_{6'a,6'b}$ = 12.5 Hz, 6′a-H), 3.68 (m, 2 H, 6a, 6b-H), 3.54 (dd, 1 H, $J_{5',6'b}$ = 5.7 Hz, 6′b-H), 3.52 (m, 1 H, 2-H), 3.32 (t, 1 H, $J_{2',3'} \approx J_{3',4'}$ = 8.9 Hz, 3′-H), 3.28 (ddd, 1 H, $J_{4',5'}$ = 9.8 Hz, 5′-H), 3.23 (dd, 1 H, 4′-H), 3.17 (m, 1 H, 4-H), 3.12 (dd, 1 H, 2′-H), 2.22 (dt, 1 H, $J_{2,3a} \approx J_{3a,4}$ = 4.0, $J_{3a,3b}$ = 14.6 Hz, 3a-H), 1.96 (dt, 1 H, $J_{2,3b} \approx J_{3b,4}$ = 4.6 Hz, 3b-H), 1.08, 1.03 [2 d, 3 H each, *J* = 6.2 Hz, $(CH_3)C$] ppm. ^{13}C NMR (125.7 MHz, D_2O): δ = 98.3 (C-1), 87.4 (C-1′), 80.7, 78.1, 75.5, 72.8, 71.4, 70.3, 67.5 (C-2′, -3′, -4′, -5′, -2, -5, Me_2CH), 62.6, 61.7 (C-6, -6′), 41.4 (C-4), 32.9 (C-3), 23.2, 21.3 [$(CH_3)_2C$] ppm. $C_{15}H_{28}O_9S.H_2O$ (402.44): calcd. C 44.78, H 7.46, S 7.96; found C 44.39, H 7.06, S 7.57.

2-Propyl 3-Deoxy-4-*S*-(β-D-glucopyranosyl)-4-thio-α-D-*xylo*-hexopyranoside (15): Deacetylation of **13** (123 mg, 0.207 mmol) gave **15** (74 mg, 93%). M.p. 174–175 °C. $[\alpha]_D^{25}$ = +19.4 (*c* = 0.6, H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 4.92 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1-H), 4.54 (d, 1 H, $J_{1',2'}$ = 9.8 Hz, 1′-H), 4.20 (ddd, $J_{4,5}$ = 2.1, $J_{5,6a}$ = 4.6, $J_{5,6b}$ = 7.1 Hz, 1 H, 5-H), 4.06 (dt, $J_{2,3a}$ = 11.6, $J_{2,3b}$ = 4.4 Hz, 1 H, 2-H), 3.95 (m, *J* = 6.2 Hz, 1 H, Me_2CH), 3.84 (dd, 1 H, $J_{5',6'a}$ = 2.0, $J_{6'a,6'b}$ = 12.3 Hz, 6′a-H), 3.69 (dd, $J_{5,6a}$ = 4.6, $J_{6a,6b}$ = 11.8 Hz, 1 H, 6a-H), 3.66–3.61 (m, 2 H, 6b-, 6′b-H), 3.47 (m, 1 H, 4-H), 3.44 (t, 1 H, $J_{2',3'}$ = 9.1, $J_{3',4'}$ = 8.7 Hz, 3′-H), 3.40 (ddd, 1 H, $J_{4',5'}$ = 9.8, $J_{5'6'a}$ = 2.0, $J_{5',6'b}$ = 5.4 Hz, 5′-H), 3.35 (dt, 1 H, 4′-H), 3.25 (dt, 1 H, 4′-H), 2.13 (ddd, 1 H, $J_{3a,4}$ = 3.4, $J_{3a,3b}$ = 13.0 Hz, 3a-H), 2.05 (ddd, 1 H, $J_{3b,4}$ = 3.2 Hz, 3b-H), 1.20, 1.13 [2 d, 3 H each, *J* = 6.2 Hz, $(CH_3)_2C$] ppm. ^{13}C NMR (125.7 MHz, D_2O): δ = 97.0 (C-1), 85.7 (C-1′), 80.8, 78.1, 73.3, 71.4, 71.2, 70.4, 64.9 (C-2′, -3′, -4′, -5′, -2, -5, Me_2CH), 63.6, 61.7 (C-6, -6′), 43.8 (C-4), 33.4 (C-3), 23.2, 21.4 [$(CH_3)_2C$] ppm. $C_{15}H_{28}O_9S$ (384.44): calcd. C 46.86, H 7.34, S 8.34; found C 46.65, H 7.58, S 7.94.

Enzymatic Assay

Inhibition of β-Galactosidase: The inhibitory activity of compounds **10** and **11** towards *E. coli* β-galactosidase (grade VIII, Sigma, EC 3.2.1.23, 117 U/mg) was determined under the following conditions: The enzyme (0.3 U; 1 U = 1 enzyme unit hydrolyzes 1 μmol of the appropriate nitrophenyl glycoside per minute) was incubated with *o*-nitrophenyl-β-D-galactopyranoside (concentration range: 0.3–5.0 mM) in sodium phosphate buffer (100 mM, pH 7.3, $MgCl_2$: 1.2 mM, 2-mercaptoethanol: 100 mM) in the absence or presence of compounds **10** or **11** (concentrations used: 0.10, 0.25 and 0.50 mM); the final volume was 0.50 mL. After 20 min at 37 °C, the reaction was terminated by adding sodium borate buffer 0.2 M (2.0 mL, pH 10.0). The concentration of the released *o*-nitrophenol was measured by visible absorption spectroscopy at 410 nm. The K_i and K_m values were determined from the Lineweaver–Burk plot (Figure 1 and Figure 2).

Inhibition of β-Glucosidase: Inhibitory activity studies of compounds **14** and **15** towards β-glucosidase from almonds (Biochemika, EC 3.2.1.21, 12.4 U/mg) were conducted. The enzyme (0.01 U) was incubated with *p*-nitrophenyl-β-D-glucopyranoside (concentration range: 0.4 to 2.0 mM) in sodium acetate buffer (50 mM, pH 5.6) in the absence or presence of compounds **14** or **15** (concentrations used: 0.3 to 2.4 mM); the final volume was 0.50 mL. After 30 min at 37 °C, the reaction was terminated by adding sodium borate buffer (0.2 M, pH 10.0, 2 mL), and the solution was analyzed by visible absorption spectroscopy at 400 nm. As the absorbance values in the presence or absence of **14** or **15** were similar, an inhibition control was performed by using the same substrate at a concentration of 0.65 mM and δ-gluconolactone as inhibitor (concentration range: 0.2 to 3.0 mM).[32] As expected, a decrease in the absorbance was observed for increasing concentrations of δ-gluconolactone.

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[1] a) C. Marino, K. Mariño, L. Miletti, M. J. Manso-Alves, W. Colli, R. M. Lederkremer, *Glycobiology* **1998**, *8*, 901–904; b) Z. J. Witczak, D. Boryczewski, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3265–3268.
[2] Z. J. Witczak, *Curr. Med. Chem.* **1999**, *6*, 165–178.
[3] H. Driguez, *Top. Curr. Chem.* **1997**, *187*, 85–116.
[4] H. Driguez, *ChemBioChem* **2001**, *2*, 311–318.
[5] J. Defaye, J. Gelas, *Studies in Natural Products Chemistry* (Ed.: Atta-ur-Rahman), Elsevier, Amsterdam, **1991**, pp. 315–357.
[6] L. Szilagyi, O. Varela, *Curr. Org. Chem.* **2005**, in press.
[7] M. Jahn, J. Marles, R. A. J. Warren, S. G. Withers, *Angew. Chem. Int. Ed.* **2003**, *42*, 352–354.
[8] J. R. Rich, A. Szpacenko, M. M. Palcic, D. R. Bundle, *Angew. Chem. Int. Ed.* **2004**, *43*, 613–615.
[9] a) Z. J. Witczak, J. Sun, R. Mielguj, *Bioorg. Med. Chem. Soc.* **1995**, *5*, 2169–2174; b) Z. J. Witczak, R. Chhabra, H. Chen, X.-Q. Xie, *Carbohydr. Res.* **1997**, *301*, 167–175; c) Z. J. Witczak, H. Chen, P. Kaplon, *Tetrahedron: Asymmetry* **2000**, *11*, 519–532; d) Z. J. Witczak, P. Kaplon, P. M. Dey, *Carbohydr. Res.* **2003**, *338*, 11–18.
[10] B. Becker, J. Thimm, J. Thiem, *J. Carbohydr. Chem.* **1996**, *15*, 1179–1181.
[11] M. L. Uhrig, O. Varela, *Austr. J. Chem.* **2002**, *55*, 155–160.
[12] M. L. Uhrig, O. Varela, *Carbohydr. Res.* **2002**, *337*, 2069–2076.
[13] G. Defina, O. Varela, R. M. Lederkremer, *Synthesis* **1988**, 891–893.
[14] C. Iriarte Capaccio, O. Varela, *J. Org. Chem.* **2001**, *66*, 8859–8866.
[15] M. L. Uhrig, O. Varela, *Synthesis* **2005**, *6*, 893–898.

[16] C. Iriarte Capaccio, O. Varela, *Tetrahedron: Asymmetry* **2000**, *11*, 4945–4954.
[17] C. Iriarte Capaccio, O. Varela, *J. Org. Chem.* **2002**, *67*, 7839–7846.
[18] C. Iriarte Capaccio, O. Varela, *Tetrahedron Lett.* **2003**, *44*, 4023–4026.
[19] C. Iriarte Capaccio, O. Varela, *Carbohydr. Res.* **2004**, *339*, 1207–1213.
[20] C. Iriarte Capaccio, O. Varela, *Tetrahedron: Asymmetry* **2004**, *15*, 3023–3028.
[21] a) D. Horton, *Methods Carbohydr. Chem.* **1963**, *2*, 433–437; b) D. Horton, M. L. Wolfrom, *J. Org. Chem.* **1962**, *27*, 1794–1800.
[22] W. G. Dauben, B. A. Kowalczyk, F. W. Lichtenthaler, *J. Org. Chem.* **1990**, *55*, 2391–2398.
[23] E. L. Eliel, S. H. Wilen, *Stereochemistry of Organic Compounds*, Wiley Interscience, New York, **1994**, pp. 731–733.
[24] F. M. Ibatullin, S. I. Selivanov, A. G. Shavva, *Synthesis* **2001**, *3*, 419–422.
[25] F. M. Ibatullin, K. A. Shabalin, J. V. Jänis, S. I. Selivanov, *Tetrahedron Lett.* **2001**, *42*, 4565–4567.
[26] F. M. Ibatullin, K. A. Shabalin, J. V. Jänis, A. G. Shavva, *Tetrahedron Lett.* **2003**, *44*, 7961–7964.
[27] a) R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683–720; b) H. Lis, N. Sharon, *Eur. J. Biochem.* **1993**, *218*, 1–27.
[28] P. M. Dey, K. Brinson, *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 265–382.
[29] J. Ohlsson, A. Larsson, S. Haataja, J. Alajääski, P. Stenlund, J. S. Pinkner, S. J. Hultgren, J. Finne, J. Kihlberg, U. J. Nilsson, *Org. Biomol. Chem.* **2005**, *3*, 886–900.
[30] J. Ohlsson, A. Sundin, U. J. Nilsson, *Chem. Commun.* **2003**, 384–385.
[31] H. Hashimoto, K. Shimada, S. Horito, *Tetrahedron Lett.* **1993**, *34*, 4953–4956.
[32] a) M. P. Dale, H. E. Ensley, K. Cern, K. A. R. Sastry, L. D. Byers, *Biochemistry* **1985**, *24*, 3530–3539; b) A. Lohse, T. Hardkei, A. Jensen, I. W. Plesner, M. Bols, *Biochem. J.* **2000**, *349*, 211–215.

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