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Synthesis of Sulfur Analogues of Methyl and Allyl Kojibiosides and Methyl Isomaltoside and Conformational Analysis of the Kojibiosides

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Abstract: The synthesis of methyl and allyl 5'-thio-α-D-kojibiosides and methyl 5'-thio-α-D-isomaltoside is described. The phenylselenoglycoside and trichloroacetimidate of 2,3,4,6-tetra-*O*-acetyl-5-thioglucose have been employed as glycosyl donors to glycosylate glucopyranosyl acceptors with 2-OH and 6-OH positions free. The disaccharides thus obtained are potential glucosidase inhibitors. The conformational preferences of allyl 5'-thiokojibioside (**34**) were studied by comparison of experimental NOE curves with the theoretical counterparts for the corresponding methyl glycoside **25**, derived from a Boltzmann-averaged grid search using the program PIMM91. Very good agreement of experimental NOE curves derived from selective NOE measurments with the theoretical curves is found. The data are consistent with the population of a global minimum structure (Φ=-43, Ψ=-39 degrees) to the extent of 90%, and a second local minimum (Φ=-36, Ψ=-173 degrees) to the extent of 6%. An X-ray crystal structure of **34** at 190 K (R=4.2%) indicates a conformation (Φ=-46, Ψ=-23 degrees) that is similar to that of the global minimum.

The application of glycosidase inhibitors in biochemical studies as well as in medicinal chemistry has placed considerable emphasis on their synthesis. Glycosidase inhibitors have been used as probes for the determination of the topography of the active sites of enzymes and to provide a better understanding of the mechanism of enzyme action.[1] Chemical modifications of the natural substrates have been performed to afford analogues that possess good affinity towards the enzyme and yet remain chemically inert towards hydrolysis. These inhibitors were then used to probe structure-function relationships. The compounds can also inhibit the processing of *N*-linked oligosaccharides. By targeting the enzymes involved in glycoprocessing, aberrant glycans can be produced that lead, in turn, to modified glycoproteins. The effects of such a modification of the oligosaccharide on the properties of the glycoprotein can then be investigated and provide an insight into the role of oligosaccharides in glycoprotein function. A variety of inhibitors of these trimming enzymes is now established.[2] For example, 1-Deoxynojirimycin, *N*-alkylated-1-deoxynojirimycin, and castanospermine are effective Glucosidase I inhibitors with K_i values in the micromolar range. 1-Deoxymannojirimycin and swainsonine are known to inhibit the Mannosidases I and II, respectively.[3] Therapeutically, glycosidase inhibitors have potential as anti viral agents, for example, in HIV treatment,[4] as antibacterial[5] and anticancer agents,[6] and in the treatment of metabolic disorders such as diabetes.[3]

Acquired immunodeficiency syndrome (AIDS) causes the destruction of T4 lymphocyte cells, thereby interfering with the body's immune defenses and leaving it more susceptible to infections. The viral infection is initiated by the association of the glycoprotein gp120 on the human immunodeficiency virus (HIV) envelope with CD4 receptors expressed on the surface of the T4 cells. Thus, interference of this primary interaction could provide a potential anti-viral therapy.[4] Viral glycoprotein gp120 is heavily *N*-glycosylated.[7] The viral infectivity is strongly dependent on the nature of the oligosaccharides of gp120. Their biosynthesis occurs via a

precursor Glc_3-Man_9-$GlcNAc_2$, and requires the trimming enzyme Glucosidase I, which is involved in the cleavage of the terminal α-1,2-linked glucose residue from the precursor.[8] Altering the carbohydrate structure of gp120 results in a lower affinity of gp120 for CD4, thereby interfering with viral infectivity.[9] Inhibitors of glycoprotein processing enzymes have been shown to possess anti-HIV activity[10] and are candidates as anti-HIV therapeutic agents. The search for other glucosidase inhibitors has led us to investigate sugar analogues containing sulfur and selenium.[11] The resistance to enzyme hydrolysis of sulfur-based compounds makes them promising targets as glucosidase inhibitors.[2a,12]

Some of the biologically active thio-sugars with sulfur in the ring include 5-thio-α-D-glucopyranose[2b] and 5-thio-α-L-fucopyranose[13], inhibitors of α-glucosidases and α-fucosidases, respectively, with K_i values in the millimolar range. Recently, the fucose derivative has been reported to have excellent inhibitory activity against the enzyme α-L-fucosidase (30 μM).[14]

We report here the chemical synthesis of the sulfur heteroanalogue related to the disaccharide α-Glc-(1-2)-α-Glc, in which the endocyclic oxygen atom of the nonreducing sugar residue has been replaced by sulfur, as a potential inhibitor of Glucosidase I. We have also applied the methodology to the synthesis of a 5'-thioisomaltose derivative for evaluation as an inhibitor of glucoamylase and other glucosidases.

In addition, we report the conformational analysis of one of the candidate inhibitors, namely the 5'-thiokojibioside by a combined NMR spectroscopic and molecular mechanics protocol. The X-ray crystal structure of this compound is also described.

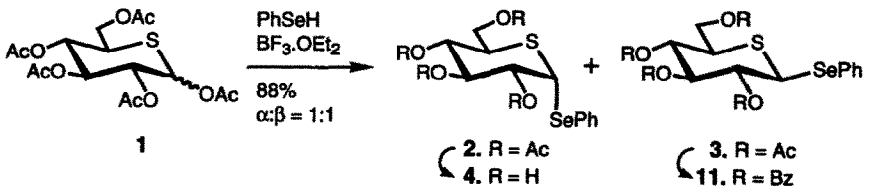
RESULTS AND DISCUSSION

Synthesis

Our initial efforts focused on the use of phenyl selenoglycosides of 2,3,4,6-tetra-O-acetyl-5-thioglucopyranose as the glycosyl donor. The synthesis of the phenyl selenoglycosides was attempted by a modification of the procedure described for the phenyl selenoglycosides of glucose.[15] A solution of the peracetylated 5-thio-α,β-D-glucopyranosides (**1**)[16] and phenyl selenol was treated with boron trifluoride etherate at -78° C followed by a gradual increase of the temperature. The reaction required 36 h for completion and afforded a 1:1 α/β mixture of the selenoglycosides **2** and **3** (Scheme 1) in 88% yield. Deacetylation of compound **2** under Zemplen conditions afforded the deprotected selenoglycoside **4**.

Glycosylations of the primary hydroxyl acceptor **5**[17] with the phenyl selenoglycoside **2** were attempted with nitrosyl tetrafluoroborate ($NOBF_4$) as the promoter. $NOBF_4$ is an established promoter for thioglycosides[18] and in order to avoid complications due to the endocyclic sulfur, one molar equivalent of $NOBF_4$ was employed at -78° C. After 2 h at -78° C and another 2 h at ambient temperature the α-disaccharide **6** was isolated in 35% yield and no β-disaccharide was detected (Table 1, entry 1). The low yield

of this reaction was due to transesterification, and the acetylated acceptor **7** was isolated (50%). There is precedent for this type of acetyl group migration from a glycosyl donor to the free hydroxyl group of a reactive acceptor in the presence of a Lewis acid.[19] It is of interest to note the extraordinary α-stereoselectivity of this glycosylation reaction, in spite of a participating acetate substituent at the C-2 position of the glycosyl donor. This was an unexpected, yet encouraging result. As more glycosylations with 5-thioglucopyranosyl donors were conducted, the preferential formation of the α-product emerged as a common feature.



Scheme 1

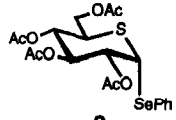
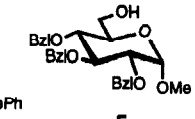
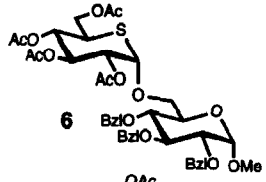
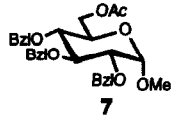
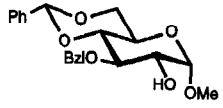
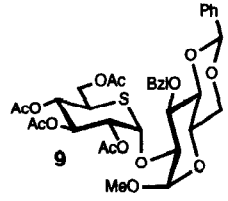
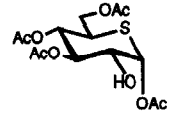
$NOBF_4$-mediated glycosylations of the acceptor **8**[20] with the selenoglycoside **2** afforded only the α-disaccharide **9** in a yield of 22% (43% based on reacted acceptor) (Table 1, entry 2). The recovered acceptor (50%) and compound **10** were also isolated.

To overcome the impediments associated with the peracetylated selenoglycosides, the perbenzoylated counterpart **11** was synthesized in 96% yield by deacetylation of **3** with ammonia/methanol followed by benzoylation in the presence of benzoyl chloride and pyridine to afford **11**. Preliminary glycosylations with this donor were poor yielding due to excessive elimination, a substantial amount of the corresponding glucal being obtained.

The trichloroacetimidate of 2,3,4,6-tetra-O-acetyl-5-thioglucopyranose[11] was examined next as the glycosyl donor. Retrosynthetic analysis of the disaccharides **6** and **9** indicated that the formation of an α-glycosidic bond may be effected with a β-trichloroacetimidate **12** (Scheme 2). This assumption was based on the results of Schmidt et al.[21] who reported that the glycosylations of glycosyl trichloroacetimidate donors with non-participating substituents at C-2 occur with inversion of configuration at the anomeric centre. However, our trichloroacetimidate had an acetate substituent at the C-2 position and whether or not it offered anchimeric assistance remained to be examined.

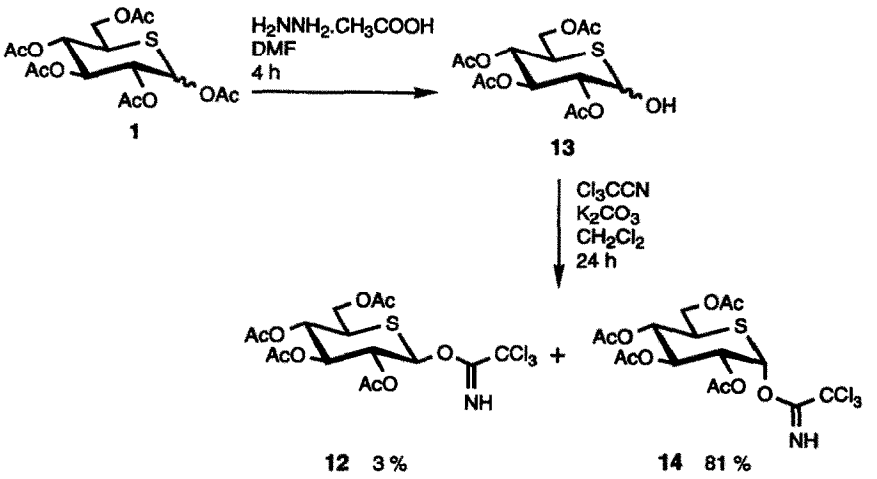
The selective 1-O-deacetylation of the peracetylated sugar was effected by the method of Excoffier et al.[22] with hydrazine acetate (Scheme 2). The mixture of hemiacetals **13** thus formed consisted predominantly of the α-anomer due to a strong anomeric effect associated with 5-thioglucose.[23] This mixture was treated with trichloroacetonitrile and potassium carbonate in anticipation of the formation of the β-trichloroacetimidate, as reported by Schmidt et al.[21] for the corresponding reaction with the glucose analogue. In contrast to

Table 1. Glycosylations with the Selenoglycoside of 5-Thioglucose

Entry	Donor	Acceptor	Promoter	Molar Ratio[a]	Reaction Conditions	Product	Yield (%)
1	 **2**	 **5**	$NOBF_4$	1 : 1 : 1	-78°C for 2 h RT for 2 h	 **6**	35%
						 **7**	50%
2	**2**	 **8**	$NOBF_4$	1 : 1 : 1	-30°C to 0°C in 2.5 h	 **9**	22%
						8	45%
						 **10**	33%

[a]donor : acceptor : promoter.

Schmidt's results, compound **13** reacted to afford predominantly the α-isomer **14** (81%) and a minor amount (3%) of the β-isomer **12**. We suggest that this is the direct result of the α-preference of the hemiacetal **13**.



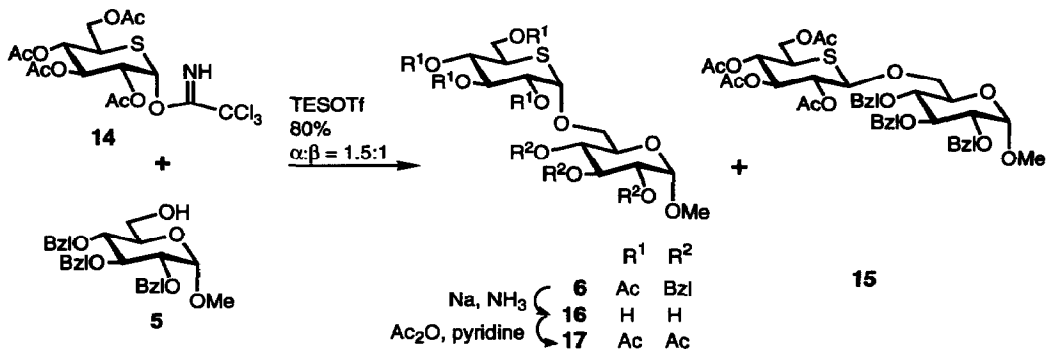
Scheme 2

Glycosylation of the 6-hydroxy acceptor **5** with the glycosyl trichloroacetimidate **14** and triethylsilyl triflate (TESOTf) as the promoter led to an improvement in the yield (80%) as compared to the $NOBF_4$-mediated glycosylations with the selenoglycoside of thioglucose. Although the α-selectivity was not conserved, an α-preference was maintained and the α- and β-disaccharides **6**, **15** were obtained in a 1.5:1 ratio. (Scheme 3).

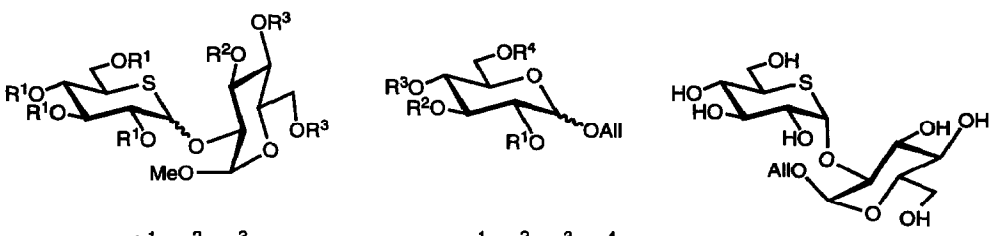
Debenzylation of the disaccharide **6** with H_2 in the presence of Pd/C proved to be unsuccessful due to the poisoning of the catalyst. A one step deacetylation and debenzylation was effected with sodium in liquid ammonia (Scheme 3). Compound **16** was purified as its peracetylated derivative **17** and then deacetylated under Zemplen conditions in an overall yield of 60% with respect to the blocked disaccharide **6**.

Although earlier attempts at the synthesis of the disaccharide **9** from the selenoglycoside of thioglucose were stereoselective, they were unsuccessful in terms of yields. An alternative synthesis using the trichloroacetimidate donor **14** was investigated. Glycosylation of the acceptor **8** with the glycosyl trichloroacetimidate **14** at -78° C and with the sequential addition of two aliquots of 0.1 equivalent of triethylsilyl triflate afforded the α- and β- disaccharides **9** and **18** in a 3.5:1 ratio (Table 2, entry 1). A minor amount of the orthoester **19** was also isolated. In another experiment with 2 aliquots of 0.7 equivalent of TESOTf, a significant proportion of the orthoester **19** was formed (40%) (Table 2, entry 2) and the α to β ratio of the disaccharides also obtained was 10:1 (40%). The separation of the mixture of α- and β-disaccharides

proved to be difficult by chromatography and required chemical manipulation. The hydrolysis of the benzylidene acetal was effected with 80% aqueous acetic acid. The mixture of diols **20** and **21** was then acetylated to give **22** and **23** which were separated by column chromatography. The α-anomer **22** was deacetylated with ammonia in methanol to afford **24**. Subsequent debenzylation with H_2 in the presence of Pd/C required 72 h and a large excess of Pd/C. The disaccharide **25** was thus obtained in a yield of 60%.



Scheme 3

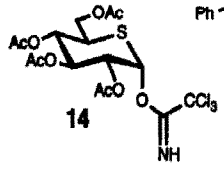
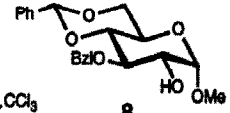
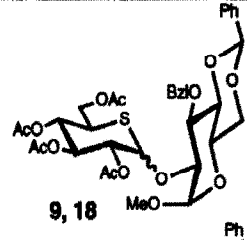
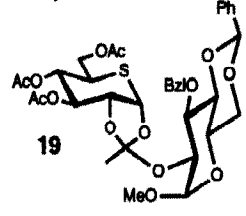


		R1	R2	R3
20	α	Ac	Bzl	H
21	β	Ac	Bzl	H
22	α	Ac	Bzl	Ac
23	β	Ac	Bzl	Ac
24	α	H	Bzl	H
25	α	H	H	H

		R1	R2	R3	R4
26	α	H	Bz	CHPh	
27	α/β	H	H	H	H
28	α	H	H	CHPh	
29	β	H	H	CHPh	
30	α	Bz	Bz	CHPh	

The difficulties experienced in debenzylation of the target disaccharides prompted us to examine the use of the acceptor **26** instead of **8**. This block possessed a benzoyl substituent at C-3 rather than a benzyl group

Table 2. Synthesis of Methyl 3-*O*-Benzyl-4,6-*O*-Benzylidene-2-*O*-(2,3,4,6-Tetra-*O*-Acetyl-5'-Thio-(α,β)-D-Glucopyranosyl)-α-D-Glucopyranoside and the Corresponding Orthoester.

Entry	Donor	Acceptor	Molar Ratio[a]	Reaction Conditions	Product	Yield (%)
1	 **14**	 **8**	1 : 1.3 : 0.1 +0.1	-78°C for 4 h	 **9, 18**	90% α:β=3.5:1
					 **19**	3%
2	**14**	**8**	1 : 0.8 : 0.07 +0.07	-78°C for 2 h	**9, 18**	40% α:β=10:1
					19	40%

[a]donor : acceptor : promoter.

and was synthesized as its allyl glycoside since it would then have the potential of being elaborated to the trisaccharide.

The acceptor **26** was synthesized by conventional methods. A Fischer allylation of glucose afforded an α/β-mixture of allyl glycosides **27** that was selectively protected at the 4 and 6 positions with a benzylidene acetal to give compounds **28** and **29**. The α- and β-isomers were separated at this stage and the α-isomer **28** was benzoylated to give compound **30**. The selective removal of the benzoyl substituent at C-2 was effected with hydrazine hydrate by the method of Ishido et al.[24] to provide compound **26**.

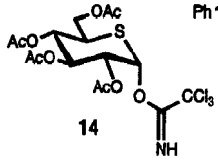
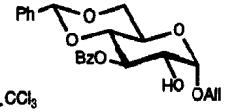
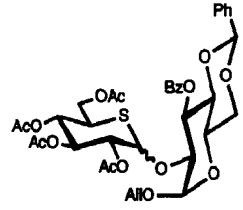
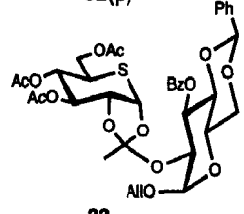
The acceptor **26** was glycosylated with the trichloroacetimidate **14** in the presence of 0.1 equivalent of TESOTf to afford an anomeric mixture of the disaccharides **31** and **32** in a ratio of 9:1 (Table 3, entry 1). The improved α-selectivity of this reaction may be due to the lower reactivity of acceptor **26** as compared to **8** due to the replacement of the activating benzyl substituent with a benzoate group. When an analogous reaction was conducted at the lower temperature of -78° C, the orthoester **33** was obtained (Table 3, entry 2). An additional 0.1 equivalent of triethylsilyl triflate was required for a complete reaction. A comparison of entries 2 and 3 of Table 3 shows the results of two reactions performed at different promoter concentrations, other reaction conditions being kept constant. Similar yields, and orthoester to disaccharide ratios suggest that the product dependence of the reactions is a function of temperature rather than acid concentrations.

The α- and β-disaccharides were again inseparable by chromatography. However, the pure α-isomer was fractionally crystallized from the mixture. The disaccharide **31** was deprotected by the initial hydrolysis of the benzylidene acetal with 80% CH_3COOH followed by deesterification under Zemplen conditions to afford the target disaccharide **34**.

The formation of the orthoesters **19** and **33** was confirmed by the use of ^1H and ^{13}C NMR spectra. The singlet corresponding to the protons of the C-methyl group was observed at a higher field than the signals attributed to the other acetoxy methyl groups.[25] Moreover, as is usually the case in orthoesters, the ^{13}C chemical shift of the C-methyl was downfield from the signals of the acetoxy methyls. This was further confirmed by the ^{13}C-^1H chemical-shift correlated spectrum in which a correlation was observed between the peaks attributed to the protons and the carbon of the C-methyl group. A ^{13}C signal in the region of 120 ppm (due to the orthoester carbon) was observed in the ^{13}C spectra of compounds **19** (122.4 ppm) and **33** (121.9 ppm) and did not correlate with any peak in the ^1H spectra.

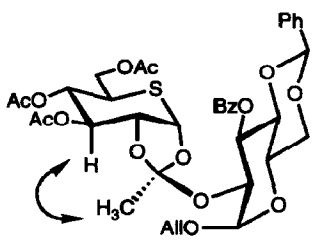
We speculated that the α-disaccharides might arise from the rearrangement of the orthoesters. To verify this postulate, the orthoester **33**, that was isolated at temperatures below -50° C, was reintroduced into the same initial reaction conditions and the reaction mixture was then warmed to room temperature. The orthoester **33** rearranged to afford a mixture of the α- and β-disaccharides **31** and **32** in a ratio of 7:1. Thus, the results of the rearrangement of the orthoesters is reflective of results obtained in reactions that were conducted without their isolation. We suggest that the preferential α-disaccharide formation is preceded by

Table 3. Synthesis of Allyl 3-*O*-Benzoyl-4,6-*O*-Benzylidene-2-*O*-(2,3,4,6-Tetra-*O*-acetyl-5'-Thio-(α,β)-D-Glucopyranosyl)-α-D-Glucopyranoside and the Corresponding Orthoester.

Entry	Donor	Acceptor	Molar Ratio[a]	Reaction Conditions	Product	Yield (%)
1	 **14**	 **26**	1 : 2 : 0.1	-78°C for 1 h RT for 1 h	 **31**(α) **32**(β)	80% α:β = 9:1
2	**14**	**26**	1 : 2 : 0.1 +0.1	-78°C for 3 h	 **33**	54%
					31 (**31:33** = 1:3.5)	16%
3	**14**	**26**	1 : 2 : 0.07 +0.07	-78°C for 3 h	**31**	19%
					33 (**31:33** = 1:3)	56%

[a]donor : acceptor : promoter.

orthoester formation, a result that has limited precedent.[26,27] In general, the rearrangement of orthoesters is an established method for the preparation of 1,2-trans glycosides.[28] These orthoesters are in the *exo*-configuration. We thought that our orthoester may possess an *endo*-configuration which rearranged to the unexpected 1,2-cis glucoside. This possibility was examined by 2D NOE (NOESY) experiments. The NOESY contacts of the C-methyl group were observed with the H-3 of the nonreducing sugar residue (Figure 1). This disagreed with our premise and indicated an *exo*-configuration of the orthoesters.



33

Figure 1. NOE contact indicating *exo* configuration of the orthoester **33**

*Conformational analysis of the kojibiosides **25** and **34***

A detailed understanding of the binding between protein and carbohydrate requires knowledge of the topographies presented by the different oligosaccharides. Such information could be utilized in the design of superior inhibitors. An appreciation of the conformational features of possible importance can be gained by detailed NMR (NOE) experiments, in conjunction with molecular mechanics, molecular dynamics or Monte Carlo calculations.[29-31] One recognizes that these theories suffer from being based on the conformations of the free ligands. Although it is logical that low-energy conformations bind to the active sites of enzymes, it is nevertheless possible that the energy required to induce conformational changes will be supplied by the free energy of binding. The direct examination of ligand conformations when bound to the enzymes by transferred-NOE experiments [32] is, therefore, preferable. As a prelude to the analysis of the conformation of the kojibioside **34** when bound to glucosidase enzymes, we present here the analysis of the free ligand conformation by NOE measurements, in conjunction with molecular mechanics calculations.

The assignment of all ^{1}H NMR resonances of allyl 5'-thiokojibioside (**34**) was performed with the use of a phase-sensitive 2D COSY experiment and was confirmed with a 2D NOESY and 1D transient NOE experiments. Vicinal coupling constants $^3J_{H,H}$ confirmed that both pyranose rings in **34** adopt the $^4C_1$

conformation. Assignments of the signals of the prochiral protons H6-*pro*-R and H6-*pro*-S were made by analogy to those in D-glucose[33] and vicinal coupling constants $J_{5,6}$ were derived from 1D ^{1}H NMR spectra or 1D transient NOE experiments. With different parameterizations of the Karplus equation, the ratios of gg, gt and tg (*gauche gauche*, *gauche trans* and *trans gauche*) conformers of the two C5-C6 linkages in **34** were calculated. The ratios are gg:gt:tg = 58:38:4[34], gg:gt:tg = 66:44:0[35] and gg:gt:tg = 59:50:-9[36] for the reducing glucose unit and gg:gt:tg = 51:36:13[34], gg:gt:tg = 53:37:10[35] and gg:gt:tg = 50:47:3[36] for the 5'-thioglucose unit, respectively. According to the Boltzmann-averaging of all conformations calculated during the contour search, the theoretical ratio of gg:gt:tg is 44:46:10 for the glucose residue and 52:47:1 for the 5'-thioglucose residue. Although the different parametrizations of the Karplus equation gave slightly different results for the experimental rotational distribution at the C5-C6 linkage in **34**, they are all close to a distribution of gg:gt:tg = 60:40:0, with somewhat higher gg-values for the reducing glucose unit. Together with the $^3J_{H,H}$ coupling constants, these values suggest, that the substitution of oxygen with sulfur in the pyranose ring of the 5'-thioglucose unit does not change the ring conformation or the rotational distribution about the C5-C6 linkage appreciably.

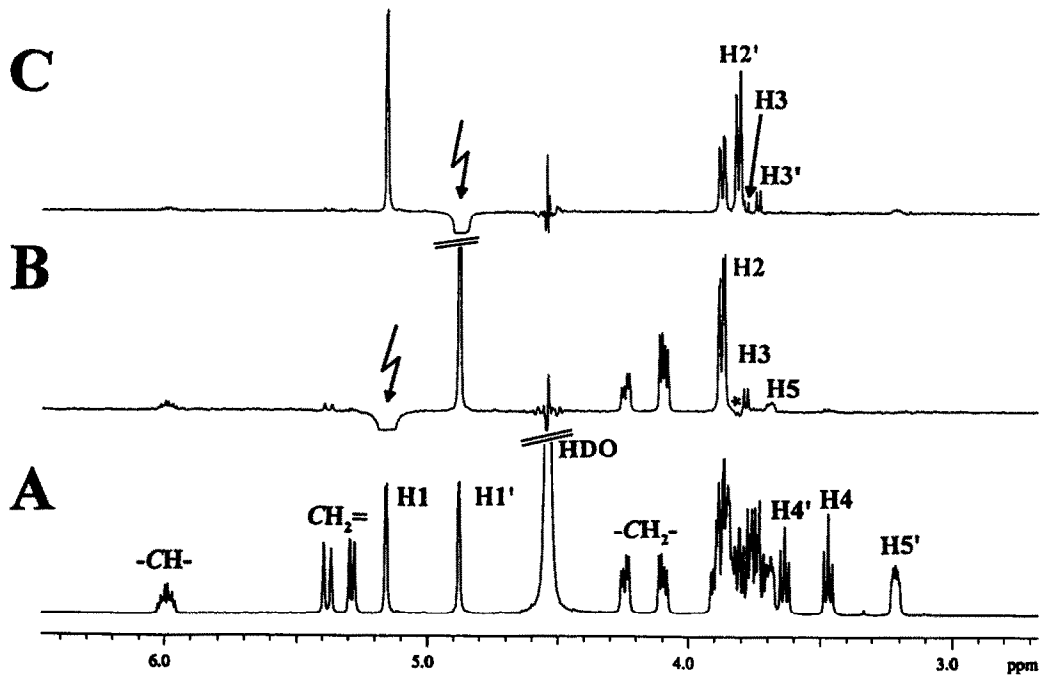


Figure 2. ^{1}H NMR spectra of **34**. **A**: normal 1D spectrum. **B** and **C**: 1D transient NOE difference spectra after selective inversion of H1 and H1', respectively (mixing time 1340 ms). The three spin effect H2'{H1} is marked with an asterisk.

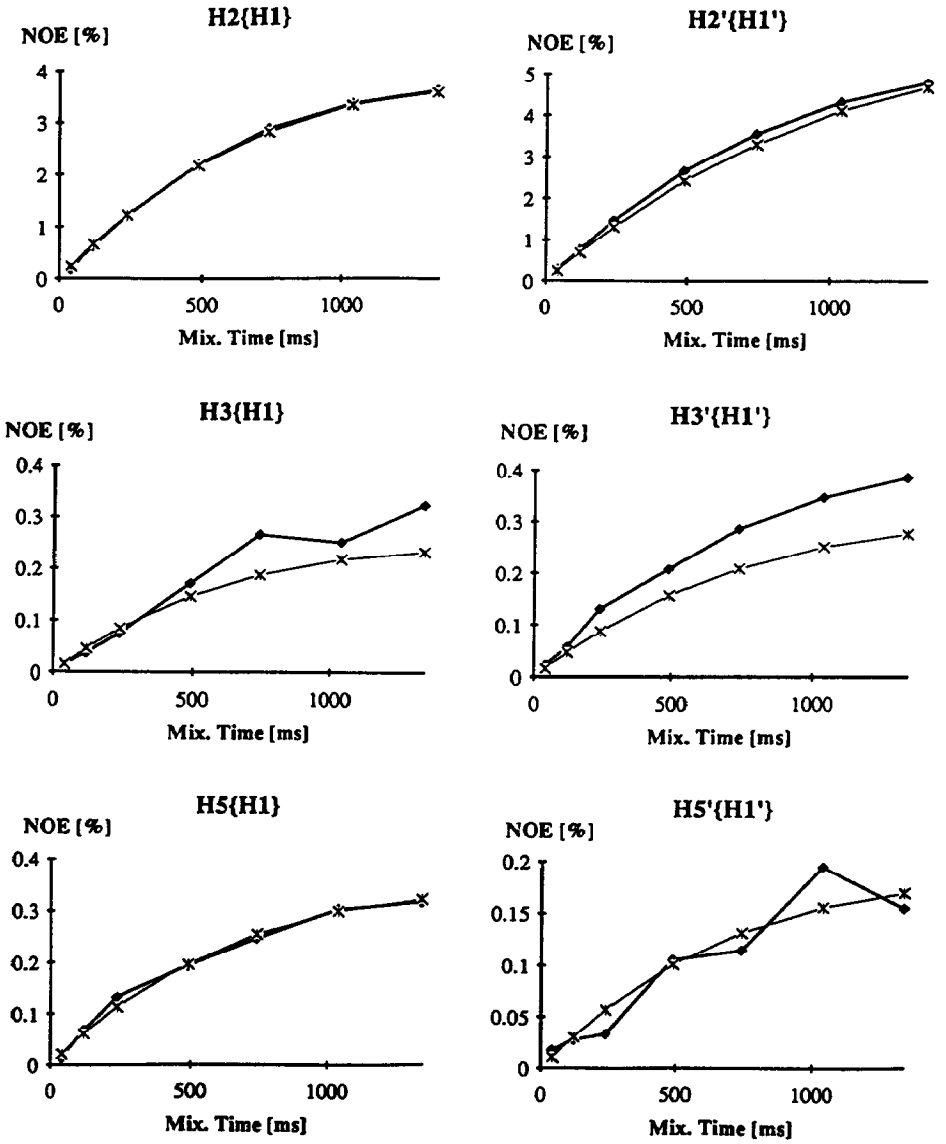


Figure 3. Comparison of experimental and theoretical intraglycosidic NOE curves. Experimental NOEs are marked with ◆ and bold lines; theoretical NOEs with * and fine lines.

Figure 2 shows selected 1D transient NOE difference spectra for **34**. The NOE curves are shown in Figures 3 and 4. These indicate that the NOEs for **34** are small and positive at 316 K and that the product $\omega_0\tau_c$ is close to 1. To identify all interglycosidic NOE contacts which are suitable for a quantitative treatment, 1D transient NOE experiments with a mixing time of 840 ms were performed for all resonances sufficiently resolved (H1, H1', H4', H4 and H5', see Figure 2). Additionally, a 2D NOESY spectrum with a mixing time of 800 ms was acquired to confirm that all accessible interglycosidic NOE contacts were found with the selective NOE experiments. The following interglycosidic NOEs were found: H1'{H1}, H1{H1'}, H2{H1'}, H3{H1'} and H2{H5'}. In addition to these direct NOEs, an indirect dipolar interaction or three spin effect[37] was observed. After selective inversion of H1 a very small *negative* enhancement of H2' is visible in the NOE difference spectrum at long mixing times (see Figure 2). The interglycosidic NOE H2{H5'} is buried in a multiplet together with the strong intraglycosidic enhancements H6'-*pro*-R{H5'} and H6'-*pro*-S{H5'} so that a quantitative treatment of this NOE is not possible. The interglycosidic NOEs H1'{H1}, H1{H1'}, H2{H1'} are sufficiently resolved to assess their integrals directly or by line deconvolution H3{H1'}. Therefore, 1D transient NOE experiments with different mixing times were performed for the resonances of H1 and H1'. Altogether, these four interglycosidic and six intraglycosidic (H2{H1}, H3{H1}, H5{H1}, H1'{H2'}, H1'{H3'} and H1'{H5'}) NOE curves form the basis for a quantitative treatment of NOE effects in **34**.

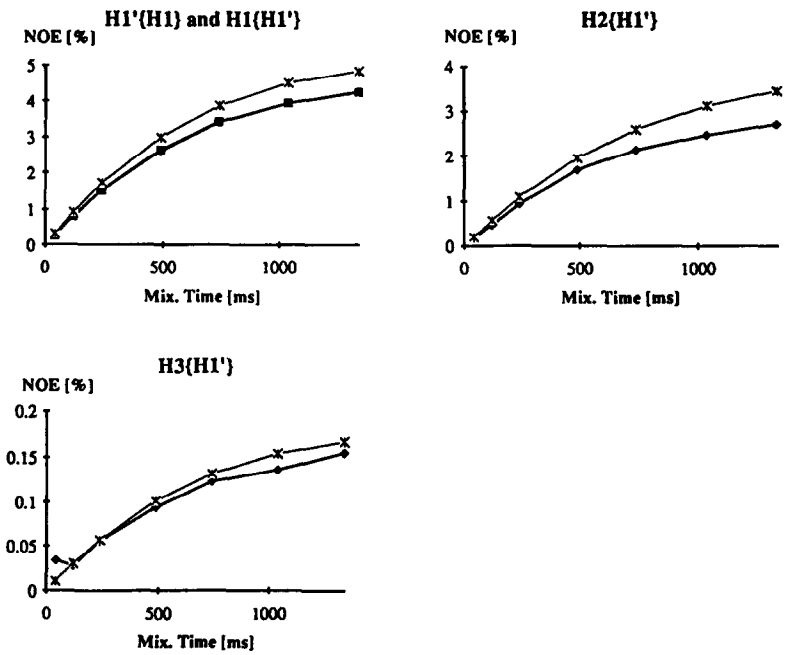


Figure 4. Comparison of experimental and theoretical interglycosidic NOE curves. Experimental NOEs are marked with ◆ or ■ (for the NOE H1{H1'}) and bold lines; theoretical NOEs with ✱ and fine lines.

The contour map shown in Figure 5 is based on 21393 structures of methyl 5'-thiokojibioside (**25**) and reveals four conformations that are low in energy, which are designated as **A**, **B**, **C** and **D** and which coincide well with the MM3 minima described previously for the case of kojibiose.[38] The corresponding energies, percentages and torsion angles are summarized in Table 4. Conformations in the areas **A**, **B** and **D** account for about 99% of all populated structures of methyl 5'-thiokojibioside (**25**) and are all in the *-gauche* range of the Φ angle. The Ψ angle can adopt the *-gauche*, *+gauche* and the *trans* conformation. Structure **A**, of lowest energy, displays a *-gauche* Ψ angle and coincides with the X-ray structure[39] (see Figure 6). Selected structural features of **A** are compiled in Table 5 together with the data from the X-ray structure and those for conformer **D**. The crystal structure of **34** indicates that, in accord with expectations based on the anomeric effect, the S-C1'-O1' bond angle is greater than tetrahedral[40] and that the torsional preference about the Φ angle is consistent with expectations based on the exo-anomeric effect.[41] The S-C5'-C6'-O6 and O5-C5-C6-O6 torsion angles indicate a gt orientation about both C5-C6 linkages.

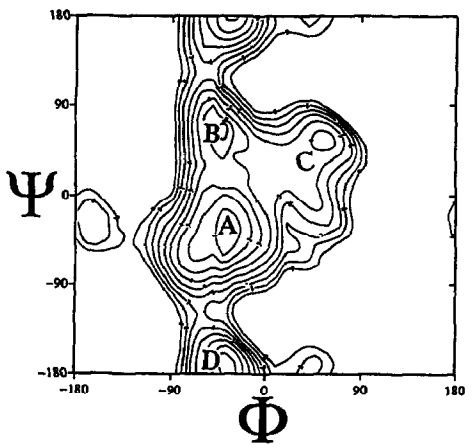


Figure 5. Φ / Ψ contour map of **25** created from structures obtained from grid searches starting from different combinations of ω angles. Energies are given in kcal/mol

Table 4. Φ and Ψ Angles of the Low Energy Structures **A**-**D** of Methyl 5'-Thiokojibioside (**25**), Energies of the Fully Optimized Structures, and Percentages Determined by Boltzmann-Averaging Based on the Contour Search.

Structure:	Φ (°)	Ψ (°)	Energy (kcal/mol)	Percentage (%)
A	-43	-39	-417.0	89.0
B	-41	72	-414.4	4.0
C	56	54	-408.8	0.5
D	-36	-173	-415.5	6.0

Table 5. Selected Structural Features of 5'-Thiokojibiosides.

		X-ray[a]	A[b]	D[b]
Φ (°)	H1'-C1'-O1'-C2	-46	-43	-36
	C2'-C1'-O1'-C2	-157	-161	-154
	S-C1'-O1'-C2	79	77	86
Ψ (°)	C1'-O1'-C2-H2	-23	-39	-173
	C1'-O1'-C2-C3	-141	-157	74
	C1'-O1'-C2-C1	95	81	-58
ω1 (°)	S-C5'-C6'-O6'	-69	-61	-62
ω2 (°)	O5-C5-C6-O6	-59	-62	59
bond-angles (°)	C6'-C5'-S	111	109	110
	C5'-S-C1'	96	102	102
	S-C1'-O1'	114	111	112
	C1'-O1'-C2	114	115	120
bond-lengths (Å)	C5'-S	1.82	1.81	1.81
	S-C1'	1.81	1.81	1.83
	C1'-O1'	1.44	1.43	1.43
	O1'-C2	1.43	1.43	1.44

[a] Data for allyl 5'-thiokojibioside (**34**)[39]
[b] Data for methyl 5'-thiokojibioside (**25**)

In contrast to the X-ray structure which displays only one energy minimum of **34**, NOE measurements represent the average of all conformations populated in aqueous solution. To identify different conformations on the basis of NOE data, the various NOE effects found for **34** have to be evaluated and assigned to the different minimum energy conformations. The calculations show that minimum **A** is the major conformation. The strong NOEs H1'{H1}, H1{H1'} and H2{H1'} (Figure 7) represent a rather short distance in this area of conformational space, so that small contributions of other structures would not change these values much. As a result, they do not contain much information about minimum energy structures other than the global minimum. The NOE H2{H5'} is buried in a multiplet and cannot be quantified. In addition, it is very small, so that it is not useful for the quantitation of relative amounts of conformations. Only the NOE H3{H1'} can be used for this purpose as will be shown in the following. This NOE is sufficiently resolved to determine its magnitude by line deconvolution (Figures 2 and 4). The distance between H3 and H1' for the various minimum energy conformations is: **A** = 4.5 Å, **B** = 3.7 Å, **C** = 2.7 Å, **D** = 2.4 Å. The distances in minima **A** and **B** are too large to contribute to this experimental NOE. Due to a relatively high potential energy, the calculations show only a population of 0.5% for conformations in the area of minimum **C**, so that these conformations also do not contribute significantly to this NOE. Only conformations of the minimum energy structure **D** show a population which is high enough, together with a relatively short H3-H1' distance, to justify this NOE.

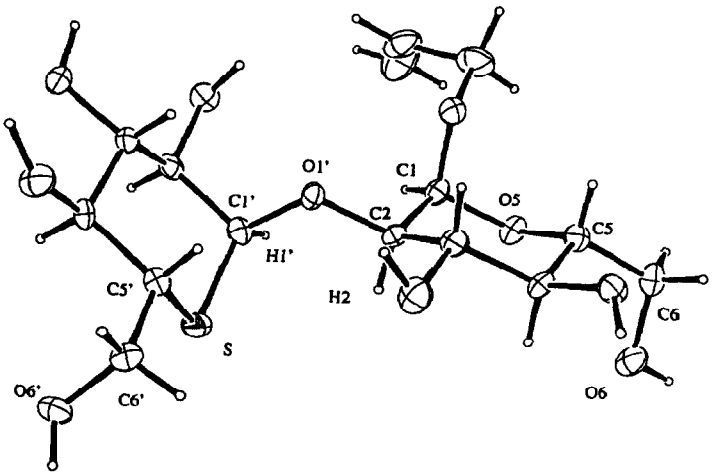


Figure 6. X-ray and molecular structure of **34**.[39]

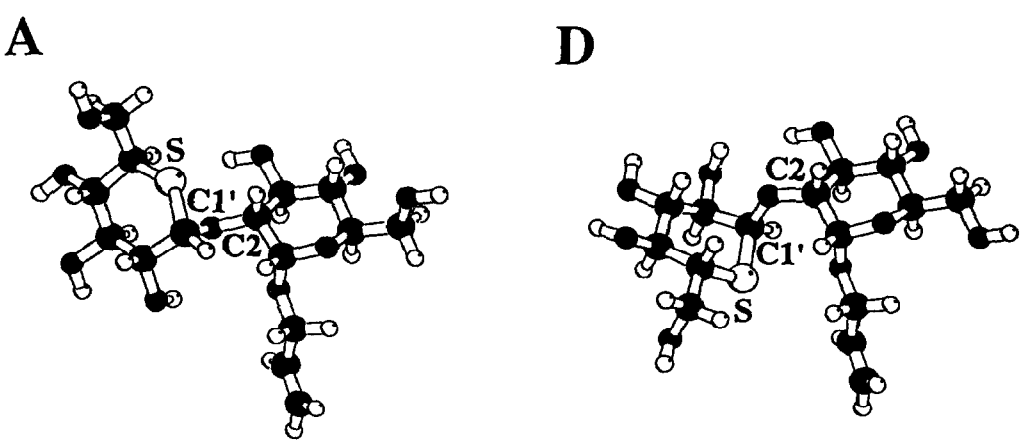


Figure 7. Ball and stick models of the minimum energy structures **A** and **D** of **34**. Plots were produced with MOLSCRIPT.[42]

The intraglycosidic NOEs H2{H1}, H3{H1}, H5{H1}, H1'{H2'}, H1'{H3'} and H1'{H5'} should be relatively independent of different orientations about the glycosidic linkage. Therefore, they can be used to determine the overall correlation time τ_c of **34** by a fitting procedure. A τ_c value of 50 ps in combination with a leakage rate of 0.05 Hz[43] gave good agreement of theoretical and experimental intraglycosidic NOE data (Figure 3). This correlation time also gave good agreement for all theoretical and experimental interglycosidic NOE curves (Figure 4). Although some of the experimental NOE data are very small (the maximum NOE for H3{H1}, H5{H1}, H1'{H3'}, H1'{H5'} and H3{H1'} is always smaller than 0.5 %), they fit those calculated very well, many of them being almost identical. This is especially important for the NOE H3{H1'} which determines the percentage of molecules with conformations in the area of the minimum energy conformation **D**; structure **D** is ~1.5 kcal/mol higher in energy than the global energy minimum **A** and its population shows the flexibility of this glycosidic linkage. The maximum NOE H2{H5'} was calculated to be 0.3% at a mixing time of 1340 ms which would be a reasonable value if the experimental integral of this NOE could be assessed accurately. In addition, even the very small indirect NOE effect (three spin effect) is predicted (maximum theoretical NOE -0.13% at 1340 ms mixing time).

In summary, experimental $^3J_{H,H}$ coupling constants for **34** show, that the monosaccharide units in this compound display very similar behaviour in terms of ring conformation and rotational distribution at the C5-C6 linkages as compared to D-glucose. The substitution of oxygen with sulfur in the 5'-thioglucose unit does not seem to influence this feature to any appreciable extent. The conformational preferences at the glycosidic linkage were studied by comparing six intraglycosidic and four interglycosidic experimental NOE curves with their theoretical counterparts derived from a Boltzmann-averaged grid search using the program PIMM91.[44] Very good agreement of experimental NOE curves derived from selective NOE-measurements with the theoretical curves is found. The NOEs H1'{H1}, H1{H1'} and H2{H1'} can be observed in conformations close to the global minimum energy structure (Φ = -43, Ψ = -39 degrees) which are the major conformers (~90%) in aqueous solution. In addition to conformations close to this global minimum structure, conformations of a minimum energy structure with a Ψ-angle of ~180° are populated to the extent of ~6%. This conclusion derives from a comparison of the experimental NOE curve H3{H1'}, which is only consistent with region **D** of conformational space, with the theoretical NOE curve. In addition, the calculations also show the NOE H2{H5'} which could not be integrated due to severe overlap in the NMR spectra and a very small indirect NOE- or three spin effect which is observed in the experimental NOE spectra. Therefore, it can be concluded that the theoretical model used in this study accurately reflects the conformational distribution of the kojibioside in aqueous solution.

EXPERIMENTAL

General Methods

Melting points were determined on a Fisher-Johns melting-point apparatus and are uncorrected. Optical rotations were measured with a Rudolph Research Autopol II automatic polarimeter. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Merck silica gel 60F-254 as the adsorbent. The developed plates were air-dried, exposed to UV light and/or sprayed with 5% sulfuric acid in ethanol, and heated at 150° C. All new compounds were characterized by either microanalysis or electrospray mass spectrometry. All compounds were purified by medium-pressure column chromatography on Kieselgel 60 (230-400 mesh). Solvents were distilled before use and were dried, as necessary, by literature procedures. Solvents were evaporated under reduced pressure and below 40° C. Reactions performed under nitrogen were also carried out in deoxygenated solvents. Transfers under nitrogen were effected by means of standard Schlenk-tube techniques.

General NMR experiments

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AMX-400 (400.13 MHz for ^{1}H, 100.6 MHz for ^{13}C) and AMX-600 (600.13 MHz for ^{1}H) NMR spectrometers, equipped with X32 computers. The spectra were recorded in deuterochloroform or deuterium oxide. Chemical shifts are given in ppm downfield from tetramethylsilane (TMS) for those spectra measured in deuterochloroform, and 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) for those spectra measured in deuterium oxide. Chemical shifts (δ) and coupling constants (J) were obtained from a first-order analysis of the spectra. All new compounds were fully characterized by the use of routine ^{1}H, ^{13}C, ^{1}H-homonuclear and ^{1}H-^{13}C correlated inverse-detected NMR spectra. The ^{1}H-homonuclear chemical-shift correlated (COSY) spectra[45] were acquired using a pulse sequence d1-90°-d0-45°-FID with quadrature detection in both dimensions. The initial data sets for COSY and NOESY spectra (1024 x 512 data points) were zero-filled once in the F_1 direction to give a final data set of 1024 x 1024 real data points. For the inverse detection experiments, a four-pulse sequence was used for the $^{1}H\{^{13}C\}$-^{13}C correlation.[46] The data sets of 2048 x 512 data points were zero-filled once in the F_1-direction, to give a final data set of 1024 x 1024 real data points.

NOE experiments with compound 34

Compound **34** (10 mg) was lyophilized twice from 1.0 ml D_2O (99.9%, ISOTEC INC.) and then dissolved in 0.5 ml of D_2O (99.9%, ISOTEC INC.). The sample was degassed by repeated evacuation and inflation with argon and was sealed under argon. NOE spectra were recorded non spinning at 600 MHz, at 316 K,with a spectral width of 3.8 ppm. One dimensional spectra were collected with 8 K data points and were zero-filled to 16 K prior to Fourier transformation. A 2D NOESY experiment was acquired in phase-sensitive

mode using TPPI[46c] with 48 scans per increment preceded by 32 dummy scans, a relaxation delay of 3.5 s and a mixing time of 800 ms. Zero filling of the acquired data (512 t_1 values and 2 K data points in t_2) led to a final data matrix of 1 K x 2 K (F_1 x F_2) data points. 1D transient NOE experiments and data treatment were performed as described.[47] 80 ms Gaussian-shaped[48] 180° pulses with 2 K data points and a truncation level of 1% were used for selective inversion of specific resonances. For each 1D transient NOE spectrum, 256 scans preceded by 32 dummy scans were acquired. The relaxation delay was 4.3 s. Corrected mixing times were 41, 120, 240, 490, 700, 1040 and 1340 ms for all experiments. Processing of spectra and user-defined line deconvolutions were performed with standard Uxnmr (Bruker) software.

Computations on the methyl glycoside 25

All calculations were performed on SGI Personal Iris 4D/35 and SGI 380 Power series computers. The molecular mechanics program PIMM91[44] was used. The hydrogen bond search routine was disabled for all computations to eliminate artificial stabilizations due to hydrogen bonding that would not be observed in an aqueous environment. First, a grid search was performed by permuting both of the ω angles and all of the hydroxyl group torsion angles to 60, 180 and 300 degrees to give 2187 fully optimized structures. Contour searches were performed in which the intersaccharidic torsion angles Φ and Ψ, i.e., H1'-C1'-O1'-C2 and C1'-O1'-C2-H2, were permuted starting from 0 as well as from 360 degrees, with an increment or decrement of 10 degrees. These two angles were fixed to a particular value while the rest of the molecule was allowed to relax fully. For each of the nine possible conformations of the two ω angles, i.e., O(S)5-C5-C6-O6, the contour search was performed independently. The starting orientation of the hydroxyl groups was chosen according to the predominant conformation, i.e., highest percentage distribution according to a Boltzmann averaging. Thus, 21393 structures were calculated, and for each Φ/Ψ combination, the one lowest in energy was selected for the contour plot, independent of the corresponding orientation of the hydroxymethyl group. The population was determined for each of these conformations according to a Boltzmann averaging and those structures were selected that exceeded 0.01%. This resulted in 531 structures which were used to calculate averaged theoretical proton-proton distances on the basis of $\langle r^{-3} \rangle$,[49] with weighting of each conformer according to its percentage contribution. The program CROSREL[50] which uses a full relaxation matrix approach[51] was used to calculate theoretical NOE values, assuming isotropic motion and neglecting effects of strong scalar coupling[52] and cross-correlation,[53] with an overall correlation time τ_c of 50 ps and a leakage rate of 0.05 Hz.[43] A qualitative comparison of theoretical with experimental NOE data was made by visual inspection of the curves.

Synthesis

Phenyl 2,3,4,6-Tetra-*O*-acetyl-1-seleno-5-thio-α,β-D-glucopyranoside (2,3). A mixture of diphenyl diselenide (1.9 g, 6.1 mmol) and 50% hypophosphorous acid (19 mL) was refluxed under N_2 with vigorous stirring until the mixture was colourless (4 h). The reaction mixture was cooled and anhydrous CH_2Cl_2 (10 mL) was added. The solution of phenylselenol in CH_2Cl_2 was transferred under N_2 into a round bottom flask containing water by means of a syringe. The flask was rinsed with additional portions of CH_2Cl_2 (2x5 mL) which were transferred as above. After shaking the organic layer with water, it was syringed into a round bottom flask containing magnesium sulfate, under N_2. The water layer was re-extracted with CH_2Cl_2 (10 mL) and the CH_2Cl_2 layer transferred as above. The dried phenylselenol solution was then added to a mixture of 1,2,3,4,6-penta-*O*-acetyl-5-thio-α,β-D-glucopyranoside (**1**)[2] (1.7 g, 4.2 mmol) with a syringe. The magnesium sulfate was washed with CH_2Cl_2 (2x5 mL) and the washings were transferred to the reaction mixture that was cooled to -78° C. $BF_3.OEt_2$ (0.55 mL, 4.5 mmol) was added slowly and the mixture was allowed to warm to room temperature. After 36 h it was neutralized with Et_3N and washed with water (2x15 mL) and sat aq $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated to give a syrup which was chromatographed with hexane-ethyl acetate (2:1) as eluant [R_f α-isomer (**2**) = 0.3, β-isomer (**3**) = 0.28]. The products were crystallized from ethanol (1.8 g, 85%). (α : 0.93 g, 43%; β : 0.87 g, 42%). α-isomer (**2**) mp = 117° C, $[\alpha]_D^{21}$ = 288 (*c* 1.0 in CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 20.5 (4COCH$_3$), 41.2 (C-5), 45.8 (C-6), 61.2 (C-1), 71.9 (C-2), 72.3 (C-4), 75.3 (C-3), 128.0-135.8 (Ar), 169.5, 169.8, 170.4 (4*C*OCH$_3$); ^{1}H NMR ($CDCl_3$): δ 1.80, 2.00, 2.01, 2.05 (12H, 4s, 4COC*H*$_3$), 3.70 (1H, m, H-5), 4.06 (1H, dd, $J_{5,6a}$ = 3.1 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.34 (1H, dd, $J_{5,6b}$ = 5.7 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b), 4.85 (1H, d, $J_{1,2}$ = 4.6 Hz, H-1), 5.18 (1H, dd, $J_{1,2}$ = 4.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 5.18 (1H, dd, $J_{3,4}$ = 9.4 Hz, $J_{4,5}$ = 10.8 Hz, H-4), 5.53 (1H, t, $J_{2,3+3,4}$ = 19.5 Hz , H-3), 7.2-7.5 (5H, m, Ar); Anal. Calcd for $C_{20}H_{24}O_9SSe$: C, 47.72; H, 4.81. Found: C, 47.60; H, 4.89. β-isomer (**3**) $[\alpha]_D^{22}$ = 10 (*c* 1.0 in CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 20.4, 20.5, 20.6 (4COCH$_3$), 42.4 (C-5), 45.9 (C-1), 61.2 (C-6), 71.8 (C-4), 74.2 (C-2), 74.6 (C-3), 126.4-135.7 (Ar), 169.2, 169.3, 169.6, 170.4 (4*C*OCH$_3$); ^{1}H NMR ($CDCl_3$): δ 1.98, 2.00, 2.04, 2.06 (12H, 4s, 4COC*H*$_3$), 3.20 (1H, ddd, $J_{4,5}$ = 10.8 Hz, $J_{5,6a}$ = 3.4 Hz, $J_{5,6b}$ = 5.2 Hz, H-5), 4.01 (1H, d, $J_{1,2}$ = 10.8 Hz, $J_{1,Se}$ = 13.6 Hz, H-1), 4.06 (1H, dd, $J_{5,6a}$ = 3.4 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.18 (1H, dd, $J_{5,6b}$ = 5.2 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b), 5.01 (1H, t, $J_{2,3+3,4}$ = 19.1 Hz, H-3), 5.16 (1H, dd, $J_{1,2}$ = 10.8 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 5.22 (1H, dd, $J_{3,4}$ = 9.6 Hz, $J_{4,5}$ = 10.8 Hz, H-4), 7.1-7.6 (5H, m, Ar). Anal. Calcd for $C_{20}H_{24}O_9SSe$: C, 47.72; H, 4.81. Found: C, 47.62; H, 4.77.

Phenyl 1-Seleno-5-thio-α-D-glucopyranoside (4). A freshly prepared solution of sodium methoxide in MeOH (0.2N, 1.5 mL) was added to **2** (60 mg, 1.19 mmol) and the mixture was stirred under N_2 for 3 h. The solution was acidified to a pH 3 with Rexyn (H^+) resin, and filtered. The filtrate was neutralized with Amberlite basic ion exchange resin, filtered and concentrated. The residue was purified by column chromatography (silica gel) with hexane-dichloromethane-methanol (3:1:1) as eluant [R_f = 0.4]. The *title compound* was obtained as a powder (34 mg, 85%) and was crystallized from ethanol. ^{13}C NMR (acetone d_6): δ 46.3 (C-5), 51.8 (C-1), 61.5 (C-6), 74.6 (C-4), 75.7 (C-2), 76.1 (C-3). ^{1}H NMR (D_2O): 3.27 (1H, dt, $J_{4',5'}$ = 10.3 Hz, $J_{5',6a'}$ = 3.1 Hz, $J_{5',6b'}$ = 5.6 Hz, H-5), 3.54 (1H, t, $J_{2,3+3,4}$ = 18.1 Hz, H-3), 3.61 (1H, t,

$J_{3,4+4,5}$ = 19.2 Hz, H-4), 3.83 (1H, dd, $J_{5,6a}$ = 3.1 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 3.90 (1H, dd, $J_{5,6b}$ = 5.6 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b), 3.78 (1H, dd, $J_{1,2}$ = 4.5 Hz, $J_{2,3}$ = 9.3 Hz, H-2), 4.7 (1H, d, $J_{1,2}$ = 4.5 Hz, H-1). 7.3-7.7 (5H, Ar). ES-MS Calcd. for $C_{12}H_{16}O_4SSe$: M^+ 335; Found: 358 $(M+Na)^+$.

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (6).

1. Preparation from the selenoglycoside (2). A mixture of phenyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-5-thio-β-D-glucopyranoside (**2**) (0.05 g, 0.1 mmol), methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**5**)[17] (0.05 g, 0.1 mmol) and dry 4 Å molecular sieves in anhydrous CH_2Cl_2 (1 mL) was stirred under nitrogen for 1 h. The reaction mixture was cooled to -78° C and nitrosyl tetrafluoroborate (0.01 g, 0.1 mmol) was added. After 2 h at -78° C the reaction mixture was warmed to room temperature. A TLC indicated that the reaction was almost complete. The mixture was filtered through celite, concentrated and chromatographed with hexane-ethyl acetate (1.75:1) as eluant [R_f = 0.26]. The *title compound* was isolated as a syrup (0.1 g, 46%). Also isolated was methyl 2,3,4-tri-*O*-benzyl-6-*O*-acetyl-α-D-glucopyranoside (**7**) (23 mg, 45%). α-disaccharide (**6**): $[\alpha]_D^{20}$ 150.9 (*c* 0.53 in CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 20.5, 20.53, 20.7 ($4COCH_3$), 38.3 (C-5'), 55.2 (OCH_3), 61.3 (C-6'), 66.5 (C-6), 70.4 (C-5), 70.8 (C-3'), 72.3 (C-4'), 73.1 ($CH_2C_6H_5$), 74.9 (C-2'), 75.0, 75.7 ($2CH_2C_6H_5$), 77.9 (C-4), 79.9, 80.0 (C-1', C-2, C-5'), 82.0 (C-3), 97.8 (C-1), 121.5-138.8 (Ar), 169.4, 169.6, 169.9, 170.5 ($4COCH_3$). ^1H NMR ($CDCl_3$): δ 1.93, 1.99, 2.01, 2.03 (12H, 4s, $4COCH_3$), 3.35-3.41 (1H, m, H-5), 3.38 (3H, s, OCH_3), 3.41 (1H, dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.52 (1H, t, $J_{3,4+4,5}$ = 18.8 Hz, H-4), 3.72 (1H, dd, $J_{5,6a}$ = 1.2 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6a), 3.76 (1H, ddd, $J_{4,5}$ = 10.0 Hz, $J_{5,6a}$ = 1.2 Hz, $J_{5,6b}$ = 4.5 Hz, H-5), 3.86 (1H, dd, $J_{5,6b}$ = 4.5 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6b), 3.98 (1H, t, $J_{2,3+3,4}$ = 18.7 Hz, H-3), 3.99 (1H, dd, $J_{5',6a'}$ = 3.0 Hz, $J_{6a',6b'}$ = 12.1 Hz, H-6a'), 4.32 (1H, dd, $J_{5',6b'}$ = 4.5 Hz, $J_{6a',6b'}$ = 12.1 Hz, H-6b'), 4.57 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 4.65 (1H, d, J = 11.0 Hz, $CHHC_6H_5$), 4.68 (1H, d, J = 12.0 Hz, $CHHC_6H_5$), 4.76 (1H, d, *J* = 12.0 Hz, $CHHC_6H_5$), 4.82 (1H, d, *J* = 11.0 Hz, $CHHC_6H_5$), 4.91 (1H, d, *J* = 11.0 Hz, $CHHC_6H_5$), 4.98 (1H, d, *J* = 11.0 Hz, $CHHC_6H_5$), 5.00 (1H, d, $J_{1',2'}$ = 3.0 Hz, H-1'), 5.11 (1H, dd, $J_{1',2'}$ = 3.0 Hz, $J_{2',3'}$ = 10.1 Hz, H-2'), 5.27 (1H, dd, $J_{3',4'+4',5'}$ = 20.5 Hz, H-4'), 5.49 (1H, t, $J_{2',3'+3',4'}$ = 19.6 Hz, H-3'), 7.25-7.40 (15H, m, Ar). Anal. Calcd for $C_{42}H_{50}O_{14}S$: C, 62.20; H, 6.22. Found: C, 62.31; H, 6.31.

2. Preparation from the trichloroacetimidate (14). A mixture of *O*-(2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl) trichloroacetimidate (**14**) (0.18 g, 0.35 mmol), methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**5**) (0.13 g, 0.27 mmol) and dry 4 Å molecular sieves in anhydrous CH_2Cl_2 (2 mL) was stirred under nitrogen for 1 h. The reaction mixture was cooled to -78° C and triethylsilyl triflate (0.01 mL, 0.04 mmol) was added. After 2.5 h at -78° C additional TESOTf (0.005 mL, 0.2 mmol) was added and the reaction mixture was warmed to room temperature. The reaction was quenched with triethylamine and the mixture was filtered through celite and concentrated. Chromatography of the residue with hexane-ethyl acetate (1.75:1) as eluant [R_f α-disaccharide (**6**)= 0.26; β-disaccharide (**15**)= 0.20]. (α-isomer)**6**): 0.1 g, 46%, β-isomer (**15**): 0.07 g, 34%).

Methyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (9).

1. Preparation from the selenoglycoside (2). A mixture of phenyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-5-thio-α-D-glucopyranoside (**2**) (56 mg, 0.1 mmol), methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**8**)[20] (43 mg, 0.1 mmol) and dry 4 Å molecular sieves in anhydrous CH_2Cl_2 (1 mL) was stirred under N_2 for 1 h. The reaction mixture was cooled to -30° C and nitrosyl tetrafluoroborate (14 mg, 0.1 mmol) was added. The reaction was allowed to warm to 0° C in 2.5 h. A TLC indicated that the selenoglycoside **2** had completely reacted although acceptor **8** was still present. The mixture was filtered through celite, concentrated and chromatographed with hexane-ethyl acetate (1.25:1) as eluant [R_f = 0.34]. The *title compound* **9** was isolated as a syrup (18 mg, 22%). Also recovered was unreacted acceptor **8** (23 mg, 45%) and 1,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranoside (**10**) (14 mg, 33%). α-disaccharide (**9**): ^{13}C NMR ($CDCl_3$): δ 20.5, (4CO*C*H$_3$), 37.9 (C-5'), 55.1 (O*C*H$_3$), 60.6 (C-6'), 62.3 (C-5), 69.0 (C-6), 71.0 (C-3'), 71.7 (C-4'), 75.0 (C-2), 75.4, 75.5 (C-2', *C*H$_2$C$_6$H$_5$), 76.3 (C-1'), 76.7 (C-3), 82.5 (C-4), 96.7 (C-1), 101.4 (O*C*HC$_6$H$_5$), 126.0-138.3 (Ar), 169.4, 170.3 (4*C*OCH$_3$). ^{1}H NMR ($CDCl_3$): δ 1.99, 2.01, 2.02, 2.09 (12H, 4s, 4COC*H*$_3$), 3.38 (3H, s, OC*H*$_3$), 3.44 (1H, m, H-5'), 3.60 (1H, dd, $J_{5',6a'}$ = 2.3 Hz, $J_{6a',6b'}$ = 12.3 Hz, H-6a'), 3.71 (1H, t, $J_{3,4+4,5}$ = 18.4 Hz, H-4), 3.76 (1H, t, $J_{5,6a + 6a,6b}$ = 20.5 Hz, H-6a), 3.84 (1H, dt, $J_{5,6b}$ = 4.3 Hz, $J_{4,5 + 5,6a}$ = 19.5 Hz, H-5), 3.96 (1H, dd, $J_{5',6b'}$ = 3.7 Hz, $J_{6a',6b'}$ = 12.3 Hz, H-6b'), 4.01 (1H, dd, $J_{1,2}$ = 3.2 Hz, $J_{2,3}$ = 10.1 Hz, H-2), 4.05 (1H, t, $J_{2,3+3,4}$ = 18.2 Hz, H-3), 4.29 (1H, dd, $J_{5,6b}$ = 4.3 Hz, $J_{6a,6b}$ = 9.5 Hz, H-6b), 4.72 (1H, d, J = 11.8 Hz, C*H*HC$_6$H$_5$), 4.77 (1H, d, $J_{1,2}$ = 3.2 Hz, H-1), 4.97 (1H, d, *J* = 11.8 Hz, CH*H*C$_6$H$_5$), 5.01 (1H, dd, $J_{1',2'}$ = 2.9 Hz, $J_{2',3'}$ = 10.0 Hz, H-2'), 5.05 (1H, d, $J_{1',2'}$ = 2.8 Hz, H-1'), 5.24 (1H, dd, $J_{3',4'+4',5'}$ = 20.5 Hz, H-4'), 5.54 (1H, dd, $J_{2',3'+3',4'}$ = 19.6 Hz, H-3'), 5.6 (OC*H*C$_6$H$_5$), 7.2-7.5 (10H, m, Ar). Anal. Calcd for $C_{35}H_{42}O_{14}S$: C, 58.49; H, 5.89. Found: C, 58.21; H, 5.90.

2. Preparation from the trichloroacetimidate (14). A mixture of *O*-(2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl) trichloroacetimidate (**14**) (0.23 g, 0.4 mmol), methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**8**) (0.19 g, 0.5 mmol) and dry 4 Å molecular sieves in anhydrous CH_2Cl_2 (4 mL) was stirred under nitrogen for 1 h. The reaction mixture was cooled to -78° C and triethylsilyl triflate (0.01 mL, 0.04 mmol) was added. After 2.5 h at -78° C additional TESOTf (0.01 mL, 0.40 mmol) was added to the reaction mixture that was stirred for an additional 4 h at -78° C. The reaction was quenched with triethylamine and the mixture was warmed to room temperature, filtered through celite, and concentrated to give a foam that was chromatographed with toluene-ethyl acetate (3.5:1) as eluant [R_f disaccharide (**9**)= 0.28; orthoester (**19**)=0.35]. A mixture of the α and β disaccharides was obtained (0.29 g, 90%, α-isomer (**9**): 0.22 g, 68%, β-isomer (**18**) 0.07 g, 23%). Also isolated was the orthoester **19** (0.01 g, 3%). **18** ^{1}H NMR ($CDCl_3$): δ 4.82 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 5.03 (1H, d, $J_{1,2}$ = 9.1 Hz, H-1), 5.06 (1H, dd, $J_{1,2+2,3}$ = 16.3 Hz, H-2'), 5.30 (1H, dd, $J_{3,4+4,5}$ = 19.5 Hz, H-4'), 5.40 (1H, t, $J_{2,3+3,4}$ = 17.0 Hz , H-3'), 5.55 (1H, s, OC*H*C$_6$H$_5$).

Phenyl 2,3,4,6-Tetra-*O*-benzoyl-1-seleno-5-thio-β-D-glucopyranoside (11). Phenyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-5-thio-β-D-glucopyranoside (**3**) (0.35 g, 0.7 mmol) was dissolved in methanol (5 mL) and ammonia gas was bubbled through the solution periodically, until deacetylation was complete. The solvent was evaporated and the dry deacetylated sugar was dissolved in pyridine (5 mL). The solution was cooled to 0°C

and benzoyl chloride (0.8 mL, 6.9 mmol) was added dropwise. The reaction mixture was warmed to room temperature. After 3 h the excess benzoyl chloride was destroyed with methanol and the solvents were evaporated. The residue was dissolved in dichloromethane, and washed successively with HCl (2 N) and $NaHCO_3$. The organic extracts were dried over $MgSO_4$ and concentrated to give a foam that was chromatographed with hexane-ethyl acetate (3:1) as eluant [R_f = 0.4]. The *title compound* was obtained as a white solid that was crystallized from ethanol (100%). (0.52 g, 96%). mp = 143° C, $[\alpha]_D^{21}$ = 52 (*c* 0.5 in CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 42.5 (C-1), 46.2 (C-5), 61.8 (C-6), 72.6 (C-2, C-3), 74.5 (C-4). ^1H NMR ($CDCl_3$): δ 3.68 (1H, m, H-5), 4.34 (1H, d, $J_{1,2}$ = 10.3 Hz, H-1), 4.41 (1H, dd, $J_{5,6a}$ = 5.4 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.56 (1H, dd, $J_{5,6b}$ = 4.0 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6b), 5.66 (1H, t, $J_{2,3+3,4}$ = 19.0 Hz , H-3), 5.72 (1H, t, $J_{1,2+2,3}$ = 19.4 Hz , H-2), 5.84 (1H, t, $J_{3,4+4,5}$ = 19.8 Hz , H-4), 7.2-8.1 (20 H, m, Ar). Anal. Calcd for $C_{40}H_{32}O_8SSe$: C, 63.91; H, 4.29. Found: C, 63.80; H, 4.10.

***O*-(2,3,4,6-Tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl) trichloroacetimidate (14).** A mixture of α and β peracetylated 5-thioglucose (**1**)[2] (1.21 g, 3.0 mmol) was dissolved in *N,N*-dimethylformamide (30 mL). Hydrazine acetate (0.4 g, 3.9 mmol) was added and the reaction mixture was stirred under N_2 for 3 h. Ethyl acetate (50 mL) was added and the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with aq NaCl (5%, 2x30 mL). The organic extracts were dried ($MgSO_4$), filtered and concentrated and the syrup was purified by column chromatography with hexane-ethyl acetate (1:1.2) as eluant [R_f = 0.34]. A mixture of 2,3,4,6-tetra-*O*-acetyl-5-thio-α/β-D-glucopyranosides (**13**) was obtained as a foam (1.0 g, 91%). The anomeric mixture **13** (2.0 g, 5.5 mmol) was dissolved in CH_2Cl_2 (24 mL), trichloroacetonitrile (5.5 mL, 55 mmol) and potassium carbonate (7.5 g, 54.2 mmol) were added and the reaction was stirred under N_2 for 24 h. The mixture was diluted with diethyl ether, filtered through celite and concentrated. The resulting brown foam was chromatographed with hexane-ethyl acetate (2:1) as eluant [R_f α-isomer = 0.35; β-isomer = 0.25]. The *title compound* was obtained as a foam (α-isomer (**14**): 2.2 g, 81%, β-isomer (**12**): 0.09 g, 3%). **14** $[\alpha]_D^{20}$ +217 (*c* 1.0 in CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 20.4, ($4COCH_3$), 40.1 (C-5), 60.8 (C-6), 70.6 (C-3), 71.7 (C-4), 73.5 (C-2), 75.8 (C-1), 90.7 (CCl_3), 160.7 (*C*=N), 169.4, 169.6, 170.3 ($4COCH_3$). ^1H NMR ($CDCl_3$): δ 1.99, 2.02, 2.05, 2.07 (12H, 4s, $4COCH_3$), 3.63 (1H, ddd, $J_{4,5}$ = 10.9 Hz, $J_{5,6a}$ = 3 Hz, $J_{5,6b}$ = 4.8 Hz, H-5), 4.07 (1H, dd, $J_{5,6a}$ = 3.0 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.39 (1H, dd, $J_{5,6b}$ = 4.8 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6b), 5.30 (1H, dd, $J_{1,2}$ = 3.2 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 5.37 (1H, dd, $J_{3,4}$ = 10.0 Hz, $J_{4,5}$ = 10.8 Hz, H-4), 5.56 (1H, t, $J_{2,3+3,4}$ = 19.7 Hz, H-3), 6.34 (1H, d, $J_{1,2}$ = 3.2 Hz, H-1), 8.68 (1H, s, N*H*CCl_3). Anal. Calcd. for $C_{16}H_{20}O_9NCl_3S$: C, 37.76; H, 3.96; N, 2.75. Found: C, 37.80; H, 4.01; N, 2.55. β-isomer (**12**) ^{13}C NMR ($CDCl_3$): δ 41.0 (C-5), 62.2 (C-6), 70.8 (C-4), 72.4 (C-4, C-2), 76.2 (C-1). ^1H NMR ($CDCl_3$): δ 2.02, 2.04, 2.05, 2.08 (12H, 4s, $4COCH_3$), 3.37 (1H, m, H-5), 4.21 (1H, dd, $J_{5,6a}$ = 4.3 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 4.33 (1H, dd, $J_{5,6b}$ = 5.4 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6b), 5.15 (1H, t, $J_{2,3+3,4}$ = 16.3 Hz, H-3), 5.37 (1H, t, $J_{3,4+4,5}$ = 18.4 Hz, H-4), 5.51 (1H, t, $J_{1,2+2,3}$ = 15.0 Hz, H-2), 6.07 (1H, d, $J_{1,2}$ = 7.6 Hz, H-1), 8.71 (1H, s, N*H*CCl_3). Anal. Calcd. for $C_{16}H_{20}O_9NCl_3S$: C, 37.76; H, 3.96; N, 2.75. Found: C, 38.08; H, 4.12; N, 2.51.

Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-5'-thio-β-D-glucopyranosyl)-α-D-glucopyranoside (15). This compound was obtained in the reaction of *O*-(2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl) trichloroacetimidate (**14**) and methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**5**) along with the α-isomer (**6**). $[\alpha]_D^{20}$+27.8 (*c* 1.08 in CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 20.4, 20.5, 20.6 ($4COCH_3$), 41.0 (C-5'), 55.2 (OCH_3), 62.4 (C-6'), 69.2 (C-6), 69.9 (C-5), 71.4 (C-4'), 73.1 (C-3'), 73.3 ($CH_2C_6H_5$), 74.7 (C-2'), 74.9, 75.6 ($2CH_2C_6H_5$), 77.7 (C-4), 80.2 (C-2), 81.9 (C-3, C-1'), 97.9 (C-1), 127.5-138.8 (Ar), 169.1, 169.2, 169.7, 170.4 ($4COCH_3$). ^1H NMR ($CDCl_3$): δ 1.96, 1.99, 2.02, 2.06 (12H, 4s, $4COCH_3$), 3.10 (1H, m, H-5'), 3.37 (3H, s, OCH_3), 3.41 (1H, t, $J_{3,4+4,5}$ = 19.0 Hz, H-4), 3.50 (1H, dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 3.63 (1H, dd, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6a), 3.73 (1H, m, H-5), 3.94-3.99 (2H, m, H-3, H-6b), 4.13 (1H, dd, $J_{5',6a'}$ = 4.0 Hz, $J_{6a',6b'}$ = 11.8 Hz, H-6a'), 4.27 (1H, dd, $J_{5',6b'}$ = 5.5 Hz, $J_{6a',6b'}$ = 11.8 Hz, H-6b'), 4.55 (1H, d, J = 11.6 Hz, $CHHC_6H_5$), 4.58 (1H, d, $J_{1',2'}$ = 8.5 Hz, H-1'), 4.59 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 4.66 (1H, d, *J* = 12.0 Hz, $CHHC_6H_5$), 4.78 (1H, d, *J* = 12.0 Hz, $CHHC_6H_5$), 4.79 (1H, d, *J* = 11.0 Hz, $CHHC_6H_5$), 4.87 (1H, d, *J* = 11.0 Hz, $CHHC_6H_5$), 4.97 (1H, d, *J* = 11.6 Hz, $CHHC_6H_5$), 5.04 (1H, t, $J_{2',3'+3',4'}$ = 18.3 Hz, H-3'), 5.30 (1H, t, $J_{3',4'+4',5'}$ = 19.4 Hz, H-4'), 5.31 (1H, t, $J_{1',2'+2',3'}$ = 16.0 Hz, H-2'), 7.20-7.50 (15H, m, Ar). Anal. Calcd for $C_{42}H_{50}O_{14}S$: C, 62.2 H, 6.22. Found: C, 62.08; H, 6.19.

Methyl 6-*O*-(5'-Thio-α-D-glucopyranosyl)-α-D-glucopyranoside (16). A freshly prepared solution of sodium methoxide in methanol (0.2 N, 5 mL) was added to methyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (**17**) (0.50 g, 0.08 mmol) and the mixture was stirred under N_2 for 6 h. The solution was acidified to a pH of 3 with Rexyn (H^+) resin, filtered and concentrated. The residue was purified by column chromatography with ethyl acetate-methanol-water (6:3:1) as eluant [R_f = 0.34]. Further purification by Sephadex LH20 filtration yielded the *title compound* **16** (24 mg, 82%). $[\alpha]_D^{20}$ +319.0° (*c* 1 in CH_3OH); ^{13}C NMR (D_2O; 150.23 MHz): δ 45.7 (C-5'), 57.8 (OCH_3), 62.7 (C-6'), 68.9 (C-6), 72.1 (C-4), 72.6 (C-5), 73.8 (C-2), 76.3, 76.6 (C-3', C-3, C-4'), 78.0 (C-2'), 84.5 (C-1'), 102.1 (C-1). ^1H NMR (D_2O; 600.14 MHz): δ 3.20 (1H, dt, $J_{4',5'}$ = 10.0 Hz, $J_{5',6a'}$ = 3.2 Hz, $J_{5',6b'}$ = 5.6 Hz, H-5'), 3.37 (3H, s, OCH_3), 3.44 (1H, t, $J_{3,4+4,5}$ = 19.4 Hz, H-4), 3.51 (1H, dd, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 3.54-3.68 (4H, m, H-4', H-3, H-3', H-6a), 3.76-3.80 (2H, m, H-5, H-2'), 3.81 (1H, dd, $J_{5',6a'}$ = 3.2 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6a'), 3.87 (1H, dd, $J_{5',6b'}$ = 5.6 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6b'), 4.10 (1H, dd, $J_{5,6b}$ = 4.4 Hz, $J_{6a,6b}$ = 11.1 Hz, H-6b), 4.72 (1H, d, $J_{1',2'}$ = 2.9 Hz, H-1'), 4.76 (1H, d, $J_{1,2}$ = 3.7 Hz, H-1). ES-MS Calcd. for $C_{13}H_{24}O_{10}S$: M^+ 372. Found: 395 $(M+Na)^+$.

Methyl 2,3,4-Tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (17). Methyl 2,3,4-tri-*O*-benzyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-5'-thio-α-D-glucopyranosyl-α-D-glucopyranoside (**6**) (0.21 g, 0.26 mmol) was dissolved in THF (5 mL) and cooled to -78° C. Ammonia was condensed into the flask (10 mL) and sodium was added until the reaction was complete, as determined by TLC (approx. 150 mg). At this time a persistent blue colour was observed. The reaction was quenched with MeOH and allowed to warm to room temperature. The ammonia was evaporated with a slow stream of air and the reaction mixture was neutralized with Amberlyst (H^+) ion exchange resin. The residue obtained after concentration was dissolved in acetic anhydride (4 mL) and pyridine (4 mL) and stirred for 24 h. The solution

was washed successively with water, HCl (2 N), aq sat $NaHCO_3$ and dried over $MgSO_4$. After concentration, the residue was chromatographed with hexane-ethyl acetate (1:2) as eluant [R_f = 0.34]. The *title compound* **17** was obtained as a syrup (0.11 g, 67%). ^{13}C NMR ($CDCl_3$): δ 20.5, 20.6, (7COCH$_3$), 38.5 (C-5'), 55.4 (OCH$_3$), 61.2 (C-6'), 67.1 (C-6), 67.9 (C-5), 69.4 (C-4), 70.3 (C-2), 70.8, 70.9 (C-3, C-3'), 72.2 (C-4'), 74.7 (C-2'), 79.9 (C-1'), 96.6 (C-1), 169.5, 170.0, 170.1, 170.5 (7COCH$_3$). ^1H NMR ($CDCl_3$): δ 1.99, 2.00, 2.01, 2.04, 2.06, 2.07, 2.08 (21H, 7s, 7COCH$_3$), 3.42 (3H, s, OCH$_3$), 3.44-3.52 (2H, m, H-5', H-6a), 3.88 (1H, dd, $J_{5,6b}$ = 5.9 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6b), 3.97 (1H, m, H-5), 4.03 (1H, dd, $J_{5',6a'}$ = 2.7 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6a'), 4.35 (1H, dd, $J_{5',6b'}$ = 4.8 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6b'), 4.81-4.88 (2H, m, H-1', H-2), 4.91 (1H, d, $J_{1,2}$ = 3.5 Hz, H-1), 5.03 (1H, t, $J_{3,4+4,5}$ = 19.6 Hz, H-4), 5.14 (1H, t, $J_{1',2'}$ = 2.6, $J_{2',3'}$ = 10.0 Hz, H-2'), 5.28 (1H, t, $J_{3',4'+4',5'}$ = 20.4 Hz, H-4'), 5.5 (2H, H-3, H-3'). Anal. Calcd for $C_{22}H_{38}O_{17}S$: C, 48.65; H, 5.75. Found: C, 48.52; H, 5.76.

3,4,6-Tri-*O*-acetyl-1,2-(methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranos-2-yl)-α-D-5'-thio-α-D-glucopyranose orthoacetate (19). A mixture of *O*-(2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl) trichloroacetimidate (**14**) (0.15 g, 0.3 mmol), methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**8**) (0.1 g, 0.24 mmol) and dry 4 Å molecular sieves in anhydrous CH_2Cl_2 (3 mL) was stirred under N_2 for 1 h. The reaction mixture was cooled to -78° C and triethylsilyl triflate (0.005 mL, 0.02 mmol) was added. After 1 h at -78° C more TESOTf (0.005 mL, 0.02 mmol) was added and the mixture was stirred for an additional hour at -78° C. The reaction was quenched with triethylamine and the mixture was then warmed to room temperature, filtered through celite, concentrated, and chromatographed with toluene-ethyl acetate (3.5:1) as eluant [R_f orthoester (**19**)= 0.30; disaccharide (**9**)= 0.28]. The *title compound* **19** was obtained as a powder (0.07 g, 40%). Also isolated was the anomeric mixture of the disaccharides **9** and **18** (0.07 g, 40%, α:β = 10:1). Orthoester **19** : ^{13}C NMR ($CDCl_3$): δ 20.6 (3COCH$_3$), 22.9 (CCH3), 37.9 (C-5'), 55.2 (OCH$_3$), 61.3 (C-6), 62.2 (C-2'), 70.3 (C-6'), 73.3, 74.3 (C-3, C-4), 74.8 (C-5'), 75.5 ($CH_2C_5H_5$), 76.7 (C-2), 82.2 (C-4'), 99.8 (C-1'), 101.3 (OCOC$_6$H$_5$), 121.9 (CCH$_3$), 126.0-138.5 (Ar), 169.4 (3COC$_6$H$_5$). ^1H NMR ($CDCl_3$): δ 1.52 (3H, s, CCH$_3$), 2.03 (3H, s, COCH$_3$), 2.07 (6H, s, 2COCH$_3$), 3.40 (3H, s, OCH$_3$), 3.49 (1H, m, H-5'), 3.64 (1H, t, $J_{3,4+4,5}$ = 18.3 Hz, H-4), 3.74 (1H, t, $J_{5,6a+6a,6b}$ = 20.1 Hz, H-6a), 3.77-3.83 (2H, m, H-2, H-5), 3.90 (1H, t, $J_{2,3+3,4}$ = 18.5 Hz, H-3), 4.10 (1H, dd, $J_{5',6a'}$ = 3.1 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6a'), 4.25-4.36 (3H, m, H-6b, H-2', H-6b'), 4.71 (1H, d, J = 11.0 Hz, CHHC$_6$H$_5$), 4.80 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1), 4.85 (1H, d, *J* = 11.0 Hz, CHHC$_6$H$_5$), 5.03-5.13 (2H, m, H-4', H-3'), 5.35 (1H, d, $J_{1',2'}$ = 5.5 Hz, H-1'), 5.56 (OCHC$_6$H$_5$), 7.2-7.5 (10H, m, Ar). Anal. Calcd for $C_{35}H_{42}O_{14}S$: C, 58.49; H, 5.89. Found: C, 58.23; H, 5.88.

Methyl 2-*O*-(5'-Thio-α-D-glucopyranosyl)-α-D-glucopyranoside (25). Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-acetyl-5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (**9**) (47 mg, 0.7 mmol) was treated with 80% aq AcOH (4 mL) and heated at 50° C for 4 h. The acetic acid was removed *in vacuo* and the residue was dissolved in methanol (4 mL) and treated with ammonia until deacetylation was complete, as determined by TLC. The solution was concentrated and the residue was dissolved in ethanol (3 mL). Ethyl acetate (2 mL), acetic acid (7 mL) and Pd/C were added and the reaction mixture was stirred under

an atmosphere of hydrogen at 52 psi overnight. A TLC indicated that no reaction had occurred. More Pd/C was added, the reaction mixture was stirred for another 48 h under H_2 and filtered through celite. Solvents were evaporated and the residue was purified by column chromatography with ethyl acetate-methanol-water as eluant [R_f = 0.3] to afford the *title compound* **25** (24 mg, 60%). ^{13}C NMR (D_2O): δ 45.7 (C-5'), 57.8 (OCH_3), 62.9 (C-6'), 63.5 (C-6), 72.5 (C-4), 74.1 (C-3/3'), 74.4 (C-4'), 76.3 (C-5), 76.7 (C- 3/3'), 77.9, 78.1 (C-2, C-2'), 82.7 (C-1'), 98.9 (C-1), ^1H NMR (D_2O): δ 3.20 (1H, ddd, $J_{4',5'}$ = 10.5 Hz, $J_{5',6a'}$ = 3.1 Hz, $J_{5',6b'}$ = 5.4 Hz, H-5'), 3.40 (3H, s, OCH_3), 3.43 (1H, t, $J_{3,4+3,4}$ = 19.0 Hz, H-4), 3.58-3.66 (2H, m, H-4', H-5), 3.66-3.77 (4H, m, H-6b, H-6a, H-3, H-3'), 3.77-3.80 (3H, m, H-6a', H-2', H-2), 3.90 (1H, t, $J_{5',6b'}$ = 5.4, $J_{6a',6b'}$ = 11.8 Hz, H-6b'), 4.86 (1H, d, $J_{1,2}$ = 3.0 Hz, H-1'), 4.98 (1H, d, $J_{1',2'}$ = 3.2 Hz, H-1). ES-MS Calcd. for $C_{13}H_{24}O_{10}S$: M^+ 372. Found: 395 $(M+Na)^+$. An α/β mixture of the disaccharides **9** and **18** was treated with aqueous acetic acid to remove the benzylidene acetal, the residue was dried over magnesium sulfate, concentrated and treated with acetic anhydride and pyridine. After 24 h the solution was concentrated by cold distillation and purified by column chromatography with hexane-ethyl acetate 2:1 as eluant [R_f α-isomer (**22**)= 0.3, β-isomer (**23**)= 0.28]. Compound **22** was deprotected as above to afford **25**.

Allyl 3-*O*-Benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (26). Allyl alcohol (83 mL) containing trifluoromethanesulfonic acid (0.82 mL, 9.3 mmol) and D-glucose (10 g, 55.5 mmol) was refluxed for 3.5 h. The reaction mixture was cooled, neutralized with triethylamine, concentrated and the residue was dried over P_2O_5 under vacuum, overnight. The crude mixture of α/β-allyl glucopyranosides (**27**) (12.25 g, 55.5 mmol) was dissolved in dry *N,N*-dimethylformamide (80 mL), and α,α-dimethoxytoluene (17 mL) and *p*-toluenesulfonic acid (1 g, 5.6 mmol) were added. The reaction mixture was heated under N_2 at 50° C for 2 h, cooled to room temperature, diluted with CH_2Cl_2 and washed successively with aq $NaHCO_3$ and water. The organic extracts were dried ($MgSO_4$) and concentrated to give a syrup. Column chromatography with hexane-ethyl acetate (1:2) as eluant gave the mixture of compounds **28** and **29** [R_f = 0.4]. Crystallization from ethanol afforded allyl 4,6-O-benzylidene-α-D-glucopyranoside **28** (77%). Compound **28** (3.48 g, 11.3 mmol) was dissolved in pyridine (26 mL), the solution was cooled to 0° C and benzoyl chloride (8 mL, 68.9 mmol) was added dropwise and the reaction mixture was warmed to room temperature. After 2 h the starting material had completely reacted, as determined by TLC. The reaction was quenched with ice and the solvents were evaporated *in vacuo*, below 20° C. A solution of the residue in CH_2Cl_2 was washed successively with HCl (2N), aq $NaHCO_3$, and water. The organic extracts were dried ($MgSO_4$), concentrated, and allyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**30**) was crystallized from ethyl acetate-hexane (4.9 g, 85%). Hydrazine hydrate (1 mL, 20.7 mmol) was added to a solution of compound **30** (4.27 g, 8.27 mmol) in pyridine (40 mL). The reaction mixture was stirred for 24 h, quenched with acetone and solvents were evaporated *in vacuo*, below 20° C. The residue was purified by column chromatography with hexane-ethyl acetate (2:1) as eluant [R_f = 0.35]. Crystallization from ethanol afforded the *title compound* **26** (3.37 g, 42%). $[\alpha]_D^{20}$ +54.6 (*c* 0.85 in $CHCl_3$), mp = 176° C, ^{13}C NMR ($CDCl_3$): δ 63.1, 68.9, 69.1, 72.0, 73.1, 78.9 (C-2, C-3, C-4, C-5, C-6, OCH_2CHCH_2); 98.5 (C-1); 101.5 ($OCHC_6H_5$); 118.4 (OCH_2CHCH_2); 166.7 (*CO*). ^1H NMR ($CDCl_3$): δ 2.32 (1H, d, *OH*), 3.78 (dd, 1H, $J_{5,6a+6a,6b}$ = 19.0 Hz, H-6a), 3.80 (1H, dd, $J_{3,4+4,5}$ = 19.0 Hz, H-4), 4.83 (1H, ddd, $J_{1,2}$ = 4.0 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.95 (1H, m, H-5), 4.20 (2H, m, OCH_2CHCH_2), 4.32 (1H,

dd, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 9.0 Hz, H-6b), 5.01 (1H, d, $J_{1,2}$ = 4.0 Hz, H-1), 5.3 (2H, m, OCH_2CHCH_2), 5.52 (1H, s, $OCHC_6H_5$), 5.61 (1H, dd, $J_{2,3+3,4}$ = 19.0 Hz, H-3), 5.95 (1H, m, OCH_2CHCH_2), 7.3-8.1 (10H, m, Ar). Anal. Calcd for $C_{23}H_{24}O_7$: C, 66.98; H, 5.91. Found: C, 67.12; H, 5.91.

Allyl 3-O-Benzoyl-4,6-O-benzylidene-2-O-(2,3,4,6-tetra-*O*-acetyl-5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (31). A mixture of *O*-(2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl) trichloroacetimidate (**14**) (0.3 g, 0.6 mmol), allyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**26**) (0.49 g, 1.2 mmol) and dry 4 Å molecular sieves in anhydrous CH_2Cl_2 (6 mL) was stirred under N_2 for 1 h. The reaction mixture was cooled to -78° C and triethylsilyl triflate (0.014 mL, 0.06 mmol) was added. The mixture was stirred at -78° C for 1 h, allowed to reach room temperature and stirred for another 1 h. It was then cooled to -70° C, quenched with collidine and warmed to room temperature. The mixture was washed successively with hydrochloric acid (2 N) and sodium hydrogen carbonate, dried over magnesium sulfate and concentrated. The residue was chromatographed with hexane-ethyl acetate (1.3:1) as eluant [R_f = 0.32]. A mixture of the α-and β-disaccharides (9:1) was obtained (360 mg, 80%, α-isomer (**31**): 324 mg, 72%, β-isomer (**32**): 36 mg, 8%). The α-disaccharide **31** was crystallized from ethanol: mp 185° C; $[\alpha]_D^{20}$ 206 (*c* 1.0 in CH_2Cl_2); ^{13}C NMR ($CDCl_3$): δ 20.3, 20.5, 20.51, 20.8 ($4COCH_3$), 38.2 (C-5'), 60.5 (C-6'), 68.8 (OCH_2CHCH_2) 68.9 (C-6), 70.7 (C-3), 71.45 (C-3'), 71.51 (C-4'), 75.0 (C-2'), 75.6 (C-2), 77.5 (C-1'), 79.5 (C-4), 95.1 (C-1), 101.6 ($OCHC_6H_5$), 118.4 (OCH_2CHCH_2), 126.1-130.1 (Ar), 169.0, 169.3, 170.4 ($4COCH_3$). ^1H NMR ($CDCl_3$): δ 1.77, 1.94, 1.99, 2.06 (12H, 4s, $4COCH_3$), 3.05 (1H, m, H-5'), 3.56 (1H, dd, $J_{5',6a'}$ = 3.5 Hz, $J_{6a',6b'}$ = 12.8 Hz, H-6a'), 3.75-3.85 (3H, m, H-6b', H-6a, H-4), 3.98-4.05 (1H, $OCHHCHCH_2$), 4.14 (1H, dd, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 4.22-4.26 (1H, $OCHHCHCH_2$), 4.32 (1H, dd, $J_{5,6b}$ = 5.0 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6b), 4.96 (1H, d, $J_{1',2'}$ = 3.0 Hz, H-1'), 4.99 (1H, dd, $J_{1',2'}$ = 3.0 Hz, $J_{2',3'}$ = 9.6 Hz, H-2'), 5.01 (1H, d, $J_{1,2}$ = 3.0 Hz, H-1), 5.1 (1H, dd, $J_{3',4'+4',5'}$ = 21.2 Hz, H-4'), 5.22-5.29 (1H, OCH_2CHCH_2), 5.28 (1H, dd, $J_{2',3'+3',4'}$ = 20.5 Hz, H-3'), 5.36-5.41 (1H, OCH_2CHCH_2), 5.53 ($OCHC_6H_5$), 5.91 (1H, t, $J_{2,3+3,4}$ = 20.2 Hz, H-3), 5.88-5.98 (1H, OCH_2CHCH_2), 7.2-8.2 (10H, m, Ar). Anal. Calcd for $C_{37}H_{42}O_{16}S$: C, 57.36; H, 5.46. Found: C, 57.11; H, 5.57.

3,4,6-Tri-*O*-acetyl-1,2-(allyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranos-2-yl)-α-D-5'-thio-α-D-glucopyranose orthoacetate (33). A mixture of *O*-(2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-glucopyranosyl) trichloroacetimidate (**14**). (0.15 g, 0.3 mmol), allyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**26**) (0.24 g, 0.6 mmol) and dry 4 Å molecular sieves in anhydrous CH_2Cl_2 (3 mL) was stirred under nitrogen for 1 h. The reaction mixture was cooled to -78° C and triethylsilyl triflate (0.007 mL, 0.03 mmol) was added. The mixture was stirred at -78° C for 1 h and more TESOTf (0.007 mL, 0.03 mmol) was added. After another 2 h at -78° C the reaction was quenched with triethylamine and the mixture was warmed to room temperature, filtered through celite and concentrated. The residue was chromatographed with hexane-ethyl acetate (1.5:1) as eluant [R_f orthoester (**33**)= 0.32; disaccharide (**31**)= 0.28]. The *title compound* **33** was obtained as a powder (0.124 g, 54%). Also isolated was the α-disaccharide **31** (36 mg, 16%). Orthoester **33** : ^{13}C NMR ($CDCl_3$): δ 20.6, 20.7, 20.8 ($3COCH_3$), 22.5 ($CCH3$), 39.9 (C-5'), 61.4 (C-6'), 62.5 (C-5), 68.7 (OCH_2CHCH_2), 69.0 (C-6), 70.3 (C-4'), 70.5 (C-3), 73.0 (C-2), 73.2 (C-3'), 78.7 (C-2'), 79.8 (C-4), 97.1 (C-

1), 101.6 ($OCHC_6H_5$), 118.6 (OCH_2CHCH_2), 121.9 (CCH_3), 126.2-137.0 (Ar), 165.3 (COC_6H_5), 169.5, 169.6, 170.5 ($3COCH_3$). ^{1}H NMR ($CDCl_3$): δ 1.64 (3H, s, CCH_3), 2.0 (3H, s, $COCH_3$), 2.06 (6H, s, $2COCH_3$), 3.43 (1H, m, H-5'), 3.72-3.80 (2H, m, H-4, H-6a), 3.95-4.04 (3H, m, H-2, H-5, $OCHHCHCH_2$), 4.06 (1H, dd, $J_{5',6a'}$ = 3.2 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6a'), 4.22-4.32 (4H, m, H-6b', H-2', H-6b, $OCHHCHCH_2$), 4.98-5.06 (2H, m, H-3', H-4'), 5.08 (1H, d, $J_{1,2}$ = 3.8 Hz, H-1), 5.24-5.42 (2H, m, OCH_2CHCH_2), 5.34 (1H, d, $J_{1',2'}$ = 5.5 Hz, H-1'), 5.49 ($OCHC_6H_5$), 5.74 (1H, t, $J_{2,3+3,4}$ = 19.4 Hz, H-3), 5.94 (1H, m, OCH_2CHCH_2), 7.2-8.2 (10 H, m, Ar). Anal. Calcd for $C_{37}H_{42}O_{16}S$: C, 57.36; H, 5.46. Found: C, 57.21; H, 5.74.

Allyl 2-O-(5'-Thio-α-D-glucopyranosyl)-α-D-glucopyranoside (34). Allyl 3-O-benzyl-4,6-O-benzylidene-2-O-(2,3,4,6-tetra-*O*-acetyl-5'-thio-α-D-glucopyranosyl)-α-D-glucopyranoside (**31**) (125 mg, 0.16 mmol) was treated with 80% aqueous acetic acid (4 mL) and heated at 50° C for 4 h. The acetic acid was removed in vacuo, the residue dissolved in CH_2Cl_2 was dried ($MgSO_4$) and concentrated. A freshly prepared solution of sodium methoxide in methanol (0.2 N, 2.0 mL) was added and the mixture was stirred under N_2 for 3 h. The solution was acidified to a pH of 3 with Rexyn (H^+) resin, and filtered. The *title compound* **34** was crystallized from the reaction mixture with hexane-dichloromethane-methanol (45 mg, 75%), mp 178-179° C; $[\alpha]_D^{20}$ +260 (*c* 1 in CH_3OH); ^{13}C NMR (D_2O): δ 50.5 (C-5'), 67.8 (C-6'), 68.5 (C-6), 76.3 (OCH_2CHCH_2), 77.4 (C-4), 81.1 (C-4'), 68.2, 79.1, 79.6, 81.6, 82.7, 82.9 (C-2, 3, 4, 2', 3', OCH_2CHCH_2), 87.5 (C-1'), 101.8 (C-1), 127.1 (OCH_2CHCH_2); ^{1}H NMR, 600 MHz, 316 K, (D_2O): δ 3.22 (1H, ddd, $J_{4',5'}$ = 9.1 Hz, $J_{5',6\text{-}pro\text{-}S'}$ = 3.3 Hz, $J_{5',6\text{-}pro\text{-}R'}$ = 5.6 Hz, H-5'), 3.47 (1H, dd, $J_{3,4}$ = 9.2 Hz, $J_{4,5}$ = 9.9 Hz, H-4), 3.63 (1H, dd, $J_{3',4'}$ = 9.1 Hz, $J_{4',5'}$ = 9.1 Hz, H-4'), 3.7 (1H, ddd, $J_{5,4}$ = 9.9 Hz, $J_{5,6\text{-}pro\text{-}S}$ = 2.2 Hz, $J_{5,6\text{-}pro\text{-}R}$ = 5.4 Hz, H-5), 3.73 (1H, dd, $J_{2',3'}$ = 9.1 Hz, $J_{3',4'}$ = 9.1 Hz, H-3'), 3.75 (1H, dd, $J_{5,6\text{-}pro\text{-}R}$ = 5.4 Hz, $J_{6\text{-}pro\text{-}R,6\text{-}pro\text{-}S}$= 12.4 Hz, H-6-*pro*-R), 3.775 (1H, dd, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 3.84 (1H, dd, $J_{1',2'}$ = 3.1 Hz, $J_{2',3'}$ = 9.6 Hz, H-2'), 3.84 (1H, dd, $J_{5',6'\text{-}pro\text{-}S}$ = 3.2 Hz, $J_{6'\text{-}pro\text{-}S,6'\text{-}pro\text{-}R}$ = 11.9 Hz, H-6'-*pro*-S), 3.85 (1H, dd, $J_{5,6\text{-}pro\text{-}S}$ = 2.2 Hz, $J_{6\text{-}pro\text{-}R,6\text{-}pro\text{-}S}$= 12.4 Hz, H-6-*pro*-S), 3.875 (1H, dd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 9.9 Hz, H-2), 3.895 (1H, dd, $J_{5',6'\text{-}pro\text{-}R}$ = 5.6 Hz, $J_{6'\text{-}pro\text{-}R,6'\text{-}pro\text{-}S'}$ = 11.9 Hz, H-6'-*pro*-R), 3.63 (1H, dd, $J_{3',4'}$ = 9.1 Hz, $J_{4',5'}$ = 9.1 Hz, H-4'), 4.1 (1H, t, $OCHHCHCH_2$), 4.25 (1H, t, $OCHHCHCH_2$), 4.88 (1H, d, $J_{1',2'}$ = 3.1 Hz, H-1'), 5.16 (1H, d, $J_{1,2}$ = 3.6 Hz, H-1) 5.28-5.38 (2H, m, OCH_2CHCH_2), 5.99 (1H, m, OCH_2CHCH_2). Anal. Calcd for $C_{15}H_{26}O_{10}S$: C, 45.22; H, 6.53. Found: C, 45.04; H, 6.85.

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